IMMUNOMODULATORY EFFECTS OF LL-37 IN THE EPITHELIA

by

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B.Sc.(H), Queen's University, 2006

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Microbiology and Immunology)

THE UNIVERSITY OF BRITISH COLUMBIA
Vancouver

June 2008

ABSTRACT

The cationic host defence peptide LL-37 is an immunomodulatory agent that plays an important role in epithelial innate immunity. Previously, concentrations of LL-37 thought to represent levels present during inflammation have been shown to elicit the production of cytokines and chemokines by epithelial cells. To investigate the potential of lower concentrations of LL-37 to alter epithelial cell responses, normal primary keratinocytes and bronchial epithelial cells were treated with pro-inflammatory stimuli in the presence or absence of 1 – 3 μg/ml LL-37. Low, physiologically relevant concentrations of LL-37 synergistically increased IL-8 production by both proliferating and differentiated keratinocytes in response to IL-1β and the TLR5 agonist flagellin, and synergistically increased IL-8 production by bronchial epithelial cells in response to IL-1β, flagellin, and the TLR2/1 agonist PAM3CSK4. Treatment of bronchial epithelial cells with LL-37 and the TLR3 agonist poly(I:C) resulted in synergistic increases in IL-8 release and cytotoxicity. The synergistic increase in IL-8 production observed when keratinocytes were co-stimulated with flagellin and LL-37 was suppressed by pretreatment with inhibitors of Src-family kinase signalling and NF-κB translocation. These data suggest that low concentrations of LL-37 may alter epithelial responses to microbes *in vivo*. Microarray analysis of keratinocyte transcriptional responses after LL-37 treatment suggest that LL-37 may alter the expression of growth factors and a number of genes important to innate immune responses. LL-37 may thus play a more important role than previously suspected in the regulation of epithelial inflammation; an improved understanding of the mechanisms by which LL-37 alters chemokine responses could lead to the development of novel anti-infective and anti-inflammatory therapeutics.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

First and foremost I would like to acknowledge the kindness and support of my supervisor, Dr. Bob Hancock, and my committee members, Dr. Pauline Johnson and Dr. Mike Gold. Without their help it would have been impossible to complete this thesis on the timeline I have followed.

I would like to thank the entire Hancock lab for their help over the last two years- particularly, I am indebted to Sheena Tam for her help with ELISAs, to Yue Xin Li for having helped me find my feet, and to Jelena Pistolic for her constant guidance. Shaan Gellatly, Aaron Wyatt, and Laurence Madera have been great co-workers and even better friends.

Outside of the lab, I would like to thank the friends who have become my family in Vancouver. They know who they are, but I would like to single out Peter and Terry Herd, who helped me make this city home, and Shawn Northwood, who did everything from lending me his car at midnight to handing me a beer when I just needed a break.

DEDICATION

This thesis is dedicated to my grandmother, Dr. A. A. Crowder,
because I still know the difference between a grass and a sedge.

CO-AUTHORSHIP STATEMENT

The research program from which these results were generated was collaboratively designed by Dr. R. E. W. Hancock and N. C. J. Filewod. Experiments were designed, performed, and analysed by NCJF. The manuscript was written by NCJF and REWH.

A version of Chapter II will be submitted for publication as follows:

Filewod, N. C. J., Pistolic, J. and R. E. W. Hancock. Low physiological doses of the human host defence peptide LL-37 alter the responses of keratinocytes and bronchial epithelial cells to pro-inflammatory stimuli.

A version of Chapter III will eventually be submitted for publication as follows:

Filewod, N. C. J., Falsafi, R., Gardy, J., and R. E. W. Hancock. Title to be determined.

CHAPTER I

INTRODUCTION

The innate immune system forms the basis of the body's defence against invasive microorganisms. Due to the frequency with which microbes are encountered, innate immunity is, through necessity, both highly reactive and highly regulated; inflammation is a necessary response to infection and injury, but unnecessary or uncontrolled inflammation can have extremely deleterious effects. The regulation of differential responses to both harmless and pathogenic microbes is accordingly a subject of great interest, as an improved understanding of the processes governing innate immune responses might lead to novel therapeutics to combat infection (1).

The epithelia are one of the frontlines of this complex defence network. Surfaces such as the skin and the bronchial epithelium are not merely physical barriers, but play roles as sentinels of the innate immune system, activating further host defences upon infection or injury. Both keratinocytes (the major cell type present in the skin) and bronchial epithelial cells release chemokines that attract other immune cells after exposure to pathogen-associated signature molecules such as flagellin, the protein monomer that is the basic unit of bacterial flagella (2, 3), while keratinocytes have been suggested to both directly attack extracellular bacteria via the production of antimicrobials (4) and to destroy internalized bacteria via autophagy (5). Hence, the skin and bronchial epithelium are capable of rapid and effective responses to pathogens.

One such response is the production of host defence peptides, a diverse array of innate immune molecules that play a role in both the initial inflammatory response to infection and injury and the eventual restoration of tissue homeostasis via wound-healing

activities. This chapter reviews the role of host defence peptides in the skin and bronchial epithelium, and discusses how their immunomodulatory properties render them fundamentally important to epithelial innate immunity.

HOST DEFENCE PEPTIDES

Host defence peptides are an evolutionarily ancient component of innate immunity. The number and structural diversity of described peptides (6) suggests that they may be ubiquitous to complex life. As a class, however, animal host defence peptides share common characteristics: being generally short (12 – 50 amino acids long) and positively charged (7). Their cationic and amphipathic nature enables host defence peptides to interact with negatively charged lipids integral to all biological membranes. This enables many peptides to either destroy the permeability barrier or attack anionic cytoplasmic targets and exert direct antimicrobial activity against both Gram-negative and Gram-positive bacteria and fungi (8). Considerable research activity has been devoted to the development of modified host defence peptides as 'natural antibiotics' (9). More recently, however, many host defence peptides have been shown to modulate systemic immune responses and provoke wound healing in epithelial tissues (10-12), suggesting that their *in vivo* role may extend beyond the direct killing of pathogens.

Several classes of host defence peptides are present in human epithelia, of which the defensin family and the cathelicidin LL-37 are the most important. Defensins are characterized by a three-stranded β-sheet (the 'defensin fold') and the presence of three internal disulphide bonds, the arrangement of which distinguishes α- and β-defensins (13). (A third group, the θ-defensins, are cyclic molecules that are not expressed in humans (14)). Of the α-defensins, the Human Neutrophil Peptides 1 – 3 are employed by neutrophils in the killing of microbes, while Human Defensins 5 and 6 are expressed by

the Paneth cells of the small intestine (15) and in the epithelia of the female reproductive tract (16). The four well-characterized β-defensins play an important role in epithelial defence. Human β-defensin-1 (hBD1) is constitutively expressed in a variety of epithelial tissues, including bronchial epithelium and skin (15, 17), while hBD2 – 4 show inducible expression in both tissues (18-23).

The host defence peptide LL-37 is the sole member of the cathelicidin family expressed in humans. LL-37, which is an amphipathic α-helical peptide, is a cleavage product of a larger protein, the 18-kDa Human Cathelidin Protein (hCAP18) (24). hCAP18 was originally isolated from the specific granules of neutrophils, where it has been proposed that LL-37 plays a similar role to the Human Neutrophil Peptides in the direct killing of phagocytosed bacteria (25), although there is little evidence of large amounts of the cleaved product LL-37 inside cells and hCAP18 has no direct antimicrobial activity. In addition to neutrophils, LL-37 is expressed by monocytes/macrophages, NK cells, γδ T cells, B cells (26), and mast cells (27). Expression patterns vary between epithelial tissues; LL-37 is constitutively expressed by lung epithelial cells, and secreted into airway fluids (28), and constitutively expressed by the non-keratized squamous epithelia of the buccal mucosa, tongue, esophagus, cervix and vagina (29). Keratinocytes, however, have been suggested to express LL-37 only following induction by pro-inflammatory compounds or during disease states (30).

The value of the expression of host defence peptides by epithelial cells was originally thought to rely upon the ability of both defensins and LL-37 to act as 'natural antibiotics' by impairing the integrity of lipid membranes, a non-specific antimicrobial activity that allows them to be effective against both Gram-negative and Gram-positive bacteria, fungi, and enveloped viruses (8, 18, 19, 31, 32). This antimicrobial activity, however, is extremely salt-sensitive, and some controversy exists over its *in vivo*

relevance; a convincing body of work suggests that defensins and LL-37 are unlikely to show significant antimicrobial activity in the presence of physiological concentrations of mono- and divalent cations (10, 33), except at very high concentrations (e.g. α-defensins in the phagolysosomes of neutrophils or β-defensins in the crypts of the intestine). However some authors maintain that these host defence peptides do in fact retain antimicrobial activity in tissue culture medium (4). While the defensins and LL-37 might show direct antimicrobial activity at epithelial surfaces at areas of high local concentrations (e.g. immediately following release from the granules of neutrophils), it seems unlikely that they would show significant antimicrobial activity in the epithelia itself. Instead, I hypothesize that their primary importance is dependent on their ability to mediate responses to infection and wounding.

INDUCED EXPRESSION OF HOST DEFENCE PEPTIDES

The ability of epithelial cells to produce host defence peptides in response to the presence of pathogens relies on their expression of conserved families of receptor molecules specific for pathogen 'signature' molecules (also termed Pathogen Associated Molecular Patterns); that is, molecules present, and loosely conserved, in the structure of bacteria and viruses that do not normally appear in eukaryotic cells. Toll-like and Nod-like Receptors (TLRs and NLRs, respectively) both fulfill this function, as do RIG-1-like RNA helicases (RLRs). TLRs are membrane-associated receptors (34), while NLRs and RLRs are cytosolic (35, 36). Although they use different mechanisms of signal transduction, the stimulation of either TLRs or NLRs can result in increased host defence peptide expression in epithelial cells (37, 38). As the work in this thesis concerns TLR signalling, the topic will be discussed briefly here.

Since the recognition of the importance of TLRs to innate immunity (39), much has been discovered about their specificities and mechanisms of signal transduction [reviewed in (34) and (40)]. The TLRs can be broken down into groups based on their ligand specificity. Various lipid-based microbial molecules are recognized by TLR4 (as a homodimer) or TLR2 (as a heterodimer with TLR1 or TLR6), while TLR3 homodimers and various homo- and heterodimers of TLR7, TLR8 and TLR9 recognize nucleic-acids and related molecules. TLR5 is a receptor for the bacterial protein flagellin. TLRs are presumed to form low-affinity dimers, which undergo a change in configuration when their extramembrane domains interact with ligand. This conformational shift allows the recruitment of specific adaptors, which bind the cytosolic face of the TLR complex via the interaction of Toll/IL-1 Receptor (TIR) domains. Five such adaptors have been characterized (MyD88, MAL, TRIF, TRAM, and SARM); they are differentially recruited to various TLR complexes, allowing for variation in downstream responses. With the exception of TLR3 signalling and some TLR4 responses, however, the involvement of MyD88 in the signalling complex leads to the recruitment of IRAK4 and, through a signal transduction cascade, the eventual activation of the transcription factor NF-κB (among others) or the activation of the MAP kinases p38 and c-Jun amino-terminal kinase (JNK). In the epithelia, these responses are fundamentally important to the mobilization of innate immune defences and the activation of adaptive immunity (41-43), and play a central role in the control of the inducible expression of hDB2-4.

Wounding can also increase the expression of host defence peptides in infected epithelial tissues. After skin is wounded, vitamin D3 signalling results in increased expression of hCAP18 (44, 45); similarly, the promotor region of hCAP18 contains several elements responsive to IL-6, a cytokine produced by epithelial cells in response to both damaged cell parts and infection (29). The expression of hBD2 is also increased

in wounded skin and chronic wounds (46), although it exhibits decreased expression in burned tissue (47). Both LL-37 and hBD2 are thus inducible by wounding.

Both wounding and the presence of pathogen signature molecules can increase the expression of host defence peptides by epithelial cells; the quantification of actual concentrations of peptide *in vivo*, however, is technically difficult. Some data is available on systemic concentrations of the hBDs: hBD2 is present in plasma at concentrations of about 8.3 fmol/ml, which increase almost 4-fold during bacterial pneumonia (48), while hBD3 has been observed to have a normal serum concentration of about 140 pg/ml, which rises to about 250 pg/ml during bacterial pneumonia (49). The relevance of these findings to the epithelia is uncertain. Human β-defensin 2, however, has been described to increase from barely detectable levels to almost 100 pg/ml in the bronchoalveolar lavage of patients with diffuse panbronchiolitis, a condition characterized by inflammation and recurrent infection by *Pseudomonas aeruginosa* (50). The *in vivo* concentrations of LL-37 are better characterized. Tracheal aspirates from healthy neonates contain concentrations of approximately 5 μg/ml and concentrations increase 2- to 3-fold during systemic or pulmonary infections (51). Bronchoalveolar lavage fluids from adult cystic fibrosis patients contain concentrations of LL-37 ranging from 0 – 16 μg/ml (52). In the skin, concentrations can range widely; keratinocytes do not usually express LL-37 (30), however LL-37 is present in sweat at concentrations averaging about 5 μg/ml (53), and has been suggested to be present in concentrations as high as 1 mg/ml in psoriatic scales (54). Taken together, these findings suggest that inflammation and wounding can induce increases in the expression of host defence peptides, creating localized areas of high concentration where they can act to promote immune responses, and, eventually, tissue repair.

PRO-INFLAMMATORY EFFECTS OF HOST DEFENCE PEPTIDES

The β-defensins and LL-37 possess many immunomodulatory effects, allowing them to recruit immune cells to the site of epithelial invasion, stimulate the release of chemical messengers by epithelial cells, and activate immune cells to better respond to the presence of pathogens. Various β-defensins are chemoattractants for monocytes, neutrophils, T cells, immature dendritic cells, and mast cells (55-58), while LL-37 can recruit T cells, neutrophils, monocytes (59), and mast cells (27). The expression of host defence peptides in wounded or infected tissue can therefore help facilitate an immediate immune response.

Host defence peptides can also elicit the release of a variety of cytokines by epithelial cells, thereby inducing a number of secondary effects on the innate immune response. In *in vitro* experiments, hBD2-4 and LL-37 stimulate the release of IL-8, IL-18, IL-6, IL-10, IP-10, MCP-1, MIP-3α, and RANTES by keratinocytes (11, 60). Similarly, LL-37 induces the production of IL-8 by bronchial epithelial cells (61, 62), and airway smooth muscle cells (63). The secretion of this plethora of chemical messengers aids in the recruitment of immune cells and may allow other pleiotropic effects.

In addition, both β-defensins and LL-37 can alter the responses of immune cells, allowing them to modulate the immune response in ways that may facilitate the resolution of infection. A prototypic example of these effects is the ability of LL-37 injection to block sepsis in both rat and mouse models (61, 64); intriguingly, there is a detectable protective effect even when the peptide is administered 12 h prior to the induction of sepsis (65). In humans, LL-37 can dramatically reduce the production of TNF-α by peripheral blood mononuclear cells (PBMCs) treated with LPS (66). While this response may not always be relevant to epithelial infection, it is interesting that LL-

37 and the human β-defensins alter mast cell behaviour. Exposure to host defence peptides results in increased expression of TLRs and production of proinflammatory cytokines (67), and can also provoke mast cell degranulation (27, 68), accordingly increasing the permeability of skin vasculature (69). Both hBD3 and LL-37 can also antagonize neutrophil apoptosis, which has been suggested to enable a more prolonged response to invading pathogens (70 , 71). The expression of inducible host defence peptides thus appears to be an excellent alarm signal, resulting in the rapid mobilization of immune cells and provoking local inflammation.

Unfortunately no single mechanism can explain these effects. For instance, β-defensins recruit dendritic and T cells using the CCR6 receptor (57), but CCR6 is not required for the chemotaxis of macrophages and mast cells (72). Similarly, the receptor FPRL-1 mediates LL-37-induced chemotaxis of T cells, neutrophils and monocytes (59), but is not involved in mast cell chemotaxis (27). Nor is there a clear consensus on how host defence peptides elicit cytokine production by epithelial cells. A number of candidate receptors have been suggested, but the fact that a synthetic form of LL-37 composed entirely of D amino acids can also provoke IL-8 release from keratinocytes argues against a specific conventional receptor (73). The observation of differential responses to LL-37 treatment has led some authors to suggest that host defence peptides possess a complex mode of action with multiple points of intervention (66).

Regardless, the ability of host defence peptides to mobilize epithelial defences is of considerable importance *in vivo*. Overexpression of HBD2 in rat lung tissues is protective against *Pseudomonas aeruginosa* infection and sepsis-induced lung injury (74). Similarly, mice inoculated with cancerous cells that overexpress HBD2 are more resistant to bacterial infections (75). The creation of a total β-defensin knockout mouse has not yet been reported, but a knockout of the murine homologue of HBD1 has been

created. The resultant phenotype is mild, perhaps due to the diversity of murine defensins, which may create considerable redundancy in function, but is associated with delayed clearance of pulmonary *Haemophilus influenzae* infection (76) and increased proclivity to colonization of the bladder by Staphylococcal bacteria (77). Similarly, mice deficient in the murine homolog of LL-37, CRAMP, are more prone to skin infections (78). These effects have been suggested to be the result of direct antimicrobial action by the host defence peptide in question, but could equally well result from improved or impaired activation of the immune response associated with altered peptide expression.

The importance of host defence peptides to epithelial homeostasis is also strongly suggested by their association with human disease states. For instance, dysregulation of defensin expression coincides with Crohn's disease, an inflammatory bowel syndrome (79), whereas increased genomic β-defensin copy number is associated with the incidence of psoriasis, an inflammatory disorder of the skin (80). The LL-37 precursor hCAP18 is selectively induced in keratinocytes in a variety of inflammatory skin disorders, including psoriasis, subacute lupus erythematosis, nickel allergy challenge, and atopic dermatitis (30). LL-37 is also induced in verruca vulgaris, a condition caused by Human Papilloma Virus (HPV) infection (81), and in fungal infections of the skin (82) Multiple lines of evidence reaffirm the role of host defence peptides as pluripotent effectors of innate immunity in both skin and lung tissues, able to activate responses to pathogens and mediate an eventual return to homeostasis.

WOUND-HEALING ACTIVITIES OF HOST DEFENCE PEPTIDES

As might be expected of molecules secreted by damaged tissue, host defence peptides have the ability to stimulate the migration and proliferation of epithelial cells, and the formation of new blood vessels, a process known as angiogenesis. The

importance of this activity *in vivo* is demonstrated by the association of improperly regulated host defence peptide expression with disease states involving chronic infection and hyper-proliferation. In addition to eliciting inflammatory responses to pathogens, host defence peptides facilitate the eventual return to tissue homeostasis.

Relatively little work has been done to characterize the wound-healing abilities of β-defensins, although keratinocytes treated with the growth factors insulin-like growth factor 1 and TGF-α have been shown to increase their expression of hBD3 (83), and hBD2-4 are known to stimulate keratinocyte proliferation and migration (60). LL-37, in contrast, has been extensively studied as a growth-stimulating agent. LL-37 induces cell migration, cell proliferation, and the healing of mechanically induced wounds in cultured bronchial epithelial cells at a concentration of 1 μg/ml (84) and acts as a growth factor for lung cancer cells at concentrations as low as 5 ng/ml (85). The peptide also induces migration and proliferation in primary keratinocytes (60, 86) and keratinocyte cell lines (87). A number of studies have implicated EGFR signalling in the ability of LL-37 to stimulate cell proliferation and migration (84-86). Activation of EGFR leads to signal transduction by STAT1 and STAT3 (60, 86) and, in the HaCaT keratinocyte cell line, results in the induction of the transcription factors Snail and Slug, which control proliferative responses, and the activation of matrix metalloproteases, which play roles in tissue remodelling (87). Wound repair also requires the formation of new blood vessels; LL-37 has been shown to stimulate angiogenesis in a rabbit hind limb model, via an FPRL-1 receptor dependent mechanism (88). LL-37 is thus an important factor in epithelial wound repair.

The *in vivo* relevance of LL-37 stimulated wound healing has been demonstrated in a number of studies. Adenoviral gene transfer of hCAP18 stimulates wound healing in diabetic mice (87) and it has been observed that LL-37 is lacking in chronic ulcers in

human skin (44). Furthermore, treatment of excised wounded keratinocytes with an anti-LL-37 antibody inhibits cell proliferation (44). Psoriasis, which in addition to being an inflammatory disorder is also marked by increased keratinocyte proliferation, is also associated with increased expression of hBD2 and LL-37 (54, 89), although it is unclear whether this is a result of the disorder or a contributing factor to its etiology. In conclusion, there is good evidence that LL-37 (and possibly also hBD2-4) act as growth factors for epithelial cells both *in vitro* and *in vivo*.

THEME AND HYPOTHESIS

In the skin and lung epithelia, LL-37 plays an important role in the regulation of tissue homeostasis; at high concentrations it mediates a pro-inflammatory response that combats infection, whereas at lower concentrations it stimulates wound healing. Systemically, LL-37 exhibits potent immunodulatory activity, but LL-37-mediated immunomodulation has not been observed in epithelial tissues. Taken together, these observations have led me to address the question: what role do low, physiologically relevant concentrations of LL-37 exert on epithelial innate immune responses? Although LL-37 accumulates to high concentration during inflammation, its role in the initiation of inflammation, when concentrations are presumably much lower, is poorly understood. As LL-37 may be involved in the etiology of psoriasis (90), a medical condition that involves dysregulated epithelial inflammation, the results of these studies might have medical relevance.

I hypothesized that low, physiologically relevant concentrations of LL-37, such as would be expected to be present in epithelial tissues prior to inflammation, would alter TLR-mediated IL-8 responses in epithelial cells, and that the altered response would be reliant on MAPK signalling. This hypothesis was investigated here using cultured

primary keratinocytes and bronchial epithelial cells by exposing these cells to pro-inflammatory stimuli in the presence or absence of low concentrations of LL-37, and initially assessing IL-8 production will be assayed by ELISA. This research thus aimed to increase our understanding of the role of host defence peptides in the regulation of inflammation in the epithelia.

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CHAPTER II[1]

Low physiological doses of the human host defence peptide LL-37 alter the responses of keratinocytes and bronchial epithelial cells to pro-inflammatory stimuli

INTRODUCTION

The cationic host defence (antimicrobial) peptide LL-37, the sole member of the cathelicidin family expressed in humans (1), is important for innate immune responses in both the skin and the lung epithelium. LL-37 is present in low concentrations at the surface of both tissues; bronchial epithelial cells constitutively express LL-37 and secrete it into the airway surfactant (2), and while it is thought that keratinocytes do not normally express LL-37 (1), the peptide is detectable in human sweat at concentrations of about 5 μg/ml (3). LL-37 is a cleavage product of the 18-kDa human cathelicidin protein (hCAP18) (4); expression of this precursor has been shown to increase in both keratinocytes and bronchial epithelium in response to pro-inflammatory stimuli and wounding (1, 5-8). This enhanced expression underlies the increased concentration of LL-37 observed in epithelial infections. For instance, the peptide is present at concentrations of about 5 μg/ml in tracheal aspirates from healthy neonates but increases 2- to 3-fold in concentration as a result of systemic or pulmonary infection (9). Similarly, concentrations of 0-16 μg/ml were observed in bronchoalveolar lavage from adults with cystic fibrosis; increased expression of LL-37 correlated with the severity of degenerative lung disease (10).

[1] A version of this chapter will be submitted for publication. Filewod, N. C. J., Pistolic, J. and R. E. W. Hancock. Low physiological doses of the human host defence peptide LL-37 alter the responses of keratinocytes and bronchial epithelial cells to pro-inflammatory stimuli.

The value of this inducible expression of LL-37 has been previously attributed to the ability of LL-37 to act as a 'natural antibiotic' with direct killing activity against a variety of pathogens (2, 11). It has been convincingly demonstrated, however, that LL-37, even at concentrations exceeding those observed at most epithelial surfaces, lacks direct antimicrobial activity at physiological salt concentrations (12), indicating the possibility that this peptide might play some other role in the restoration of tissue homeostasis. Indeed, at concentrations of around 25-40 μg/ml, LL-37 can elicit the release of cytokines and chemokines by epithelial cells, act as a growth factor, and alter the function of other immune cells to allow more effective responses to pathogens. LL-37 stimulates the production of IL-8, IL-18, IL-6, IL-10, IP-10, MCP-1, MIP-3α and RANTES by keratinocytes (13, 14) and IL-8 by bronchial epithelial cells (15, 16). It promotes keratinocyte proliferation and migration (14, 17, 18), wound healing responses by bronchial epithelial cells (19), proliferation of lung cancer cells (20), and angiogenesis in a rabbit hind limb model (21). Treatment of excised skin samples with an antibody directed against LL-37 impairs wound healing *ex vivo* (7). LL-37 can also alter the function of other cell types important for epithelial defence. The peptide promotes cellular recruitment at low concentrations (1-5 μg/ml), being both directly chemotactic for T cells, neutrophils, monocytes (22), and mast cells (23). Higher concentrations of LL-37 (15-40 μg/ml) stimulate the production of chemokines in monocytic cells (15, 16) and neutrophils (24 , 25). LL-37 increases the longevity of neutrophils by antagonizing apoptosis, which would help to increase the ability of the epithelium to resist infectious agents (24 , 25). It also activates mast cells, increasing their expression of Toll-like receptors and their production of proinflammatory cytokines (26), and provoking degranulation (23, 27), which would increase the permeability of the skin vasculature to

enable an influx of immune effector cells. LL-37 is accordingly a pluripotent effector of innate immunity in the epithelia.

Toll-like receptor (TLR) signalling is a fundamental mechanism by which epithelial cells recognize the presence of microbes. The TLR family are so-called pattern recognition receptors for conserved 'signature molecules' (sometimes referred to as pathogen associated molecular patterns or PAMPs) that occur in microbes but not in mammalian cells. TLRs are transmembrane receptors, variously located on the cell surface or contained within cytosolic vacuoles, and form homo- and hetero- dimers that undergo a conformational change when they bind ligand, stimulating signal transduction through an assortment of adaptor molecules (28) resulting, typically, in the activation of the transcription factor NF-κB, among others, and the mitogen activated protein kinases (MAPK) p38, Erk1/2 and JNK (29). Various TLRs recognize different classes of signature molecules. For example, TLR5 recognizes the bacterial protein flagellin (30), while a heterodimer of TLR2/1 recognizes bacterial lipoprotein (31), and TLR3 homodimerizes within cytosolic vacuoles to bind viral dsRNA (32). Along with other conserved pattern receptors such as NOD-like receptors and RIG-I-like RNA helicases, TLRs constitute a mainstay of epithelial innate immunity.

In other cell types, LL-37 can profoundly alter cytokine responses to TLR ligands. LL-37 treatment suppresses production of the quintessential proinflammatory cytokine TNF-α by PBMCs in response to lipopolysaccharide (LPS) (33), and the peptide is protective against endotoxemia in rat and mouse models (15, 34). LL-37 can also increase cytokine responses. For instance, peripheral blood mononuclear cells treated with either IL-1β or

GM-CSF show synergistic increases in IL-8 production when co-stimulated with LL-37 (35). LL-37 is thus able to alter responses important to the regulation of innate immunity. LL-37 plays an important anti-infective role in the epithelia. Mice deficient in CRAMP, the murine homolog of LL-37, show increased morbidity following streptococcal skin infection (36). Furthermore, human skin disorders such as psoriasis, which is associated with increased LL-37 expression, are typically associated with a decreased frequency of skin infections, while those in which LL-37 expression is reduced (such as atopic dermatitis) show an increased frequency of infection (37, 38). These findings are intriguing, as LL-37 does not appear to possess direct antimicrobial activity at physiological salt concentrations (12). It was thus of interest to determine whether LL-37 might activate innate immunity in some other manner. Accordingly, we investigated the possibility that LL-37 might alter epithelial responses to TLR ligands.

Herein we report that low, physiologically-relevant concentrations of LL-37 profoundly alter chemokine production by normal human keratinocytes and bronchial epithelial cells in response to pro-inflammatory stimuli. The addition of LL-37 at 1-3 μg/ml led to a synergistic increase in IL-8 production in response to IL-1β and agonists of TLR5, TLR3, and TLR2/1, but not GM-CSF. This synergistic response was suppressed by pre-treatment of keratinocytes with the Src kinase inhibitors PP2 and Su6656. Treatment of keratinocytes with flagellin and peptide increased the phosphorylation of the transcription factor CREB. We hypothesize that the collaboration of LL-37 with endogenous and exogenous immune stimulants might represent a novel mechanism for the regulation of inflammatory responses in the epithelia.

MATERIALS AND METHODS

Cell cultivation: Normal primary adult keratinocytes were obtained from Cascade Biologics (Portland, OR) and maintained in their proprietary Epilife medium with the addition of a Human Keratinocyte Growth Supplement that contained bovine pituitary extract, bovine insulin, hydrocortisone, bovine transferrin, and human epidermal growth factor. Unsupplemented Epilife contained 0.65 μM calcium. The medium was changed every two days and cells were passaged prior to confluence to avoid differentiation. Cultures were only used for a maximum of six passages.

Normal primary adult bronchial epithelial cells were obtained from Cambrex BioScience Inc. (Walkersville, MD) and cultivated in their proprietary BEGM basal medium with the addition of 'Singlequot' growth supplements, comprising human epidermal growth factor, triiodothyronine, bovine pituitary extract, epinephrine, transferrin, insulin, hydrocortisone, gentamycin/amphotericin, and retinoic acid. The cells were maintained for up to 6 passages as described for the keratinocyte cultures. All cells were cultivated in a 37° C incubator containing 5% CO_2.

Reagents:

Human LL-37 peptide (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES) was synthesized at the Nucleic acid/Protein synthesis unit at the University of British Columbia, using F-Moc chemistry. The synthesized peptide was re-suspended in endotoxin-free water (Sigma-Aldrich, Oakville, ON) and stored at -20°C until further use.

Cell stimulation: Keratinocytes were seeded into tissue-culture-treated 24-well plates (Corning Inc. Life Sciences, Acton, MA) at a density of 7000 cells/cm^2 and cultivated in supplemented medium until they attained the desired level of confluence. Subconfluent keratinocytes were used at about 70% confluence, while calcium-differentiated keratinocytes were grown to confluence and cultivated for 2 days in unsupplemented Epilife medium containing 1.35 mM calcium. Bronchial epithelial cells were seeded into tissue-culture-treated 24-well plates at a density of 10,000 cells/cm^2 and cultivated in supplemented medium until confluence was attained.

Once cells had reached the appropriate level of confluence or had been differentiated, the medium was replaced with fresh unsupplemented medium (1 ml/well). After a two-hour rest, the cells were treated with LL-37 and/or pro-inflammatory stimuli. *Salmonella typhimurium* flagellin, poly(I:C), and PAM3CSK4 were obtained from InvivoGen (San Diego, CA), while recombinant IL-1β and GM-CSF were purchased from Research Diagnostics (Flanders, NJ). Twenty-four hours later, supernatants were collected. Supernatants were stored at 4°C until assayed for LDH activity, and then frozen until assayed for IL-8 concentration.

Assays: Cytotoxicity was monitored using a Cytotoxicity Detection Kit (Roche, ON), which measures LDH activity in collected supernatants. Results were normalized using a negative control (untreated cells) and a positive control (cells treated with unsupplemented media containing 2% Triton-X), according to the following formula: % cytotoxicity = (sample – negative control)/(positive control – negative control) x 100. IL-8 concentrations in supernatants were assessed by Enzyme Linked Immunosorbent Assay

(ELISA), as per the manufacturer's instructions (Biosource International, Camarillo, CA).

Apoptosis was detected by the terminal uridine deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) assay (Promega, Madison, WI). Bronchial epithelial cells were seeded at 10,000 cell/cm^2 into Lab-Tek 8-chambered slides (Nalge Nunc Interational, Rochester, NY) and allowed to grow to confluence before being rested in unsupplemented medium for two hours and treated with peptide and poly(I:C). TUNEL was performed at 6 and 24 hours. Slides were visualized using an Eclipse 7E2000-S fluorescence microscope (Nikon, ON) equipped with a Photometric Coolsnap ES camera (Roper Scientific, AZ). Images were processed using the Image-Pro Plus software package (Media Cybernetics Inc., MD).

Inhibitor studies: Experiments involving inhibitors were performed as previously described, with the addition of the appropriate inhibitor to the fresh unsupplemented medium that the cells received two hours prior to treatment with peptide and/or pro-inflammatory stimuli. PP2 and SU6656 were purchased from Biosource and used at concentrations of 10 and 5 μM, respectively. Bay11-7085 was purchased from Calbiochem (Mississauga, ON) and used at a concentration of 2 μM. As a control, some cells received unsupplemented medium containing concentrations of DMSO at levels equivalent to or exceeding those present in the inhibitor solutions.

Western blotting: Keratinocytes were seeded into 60 mm tissue-culture-treated Petri dishes (Corning Life Science) at a density of 7000 cells/cm^2, and cultivated as previously described until they reached around 70% confluency. The cells received fresh

unsupplemented medium and were rested two hours prior to stimulation with peptide and/or flagellin. Thirty minutes after treatment, the cells were washed with ice-cold PBS containing 1 mM sodium orthovanadate and lysed with NP-40 lysis buffer (1% NP-40, 20 mM Tris-HCl pH 8, 137 mM NaCl, 10% glycerol, 2 mM EDTA) supplemented with protease and phosphatase inhibitor cocktails (Sigma-Aldrich). Lysis continued for 30 min on ice, following which the cells were scraped and the lysates were centrifuged for 10 min at 15,000 rpm at 4 °C. Protein concentration in the lysates was assayed using a BCA assay (Pierce, Rockford, IL), after which the lysates were denatured by addition of SDS-PAGE sample buffer and heating at 95° C for 5 minutes and then resolved on a 12% acrylamide gel. Protein was then transferred to a PVDF Immuno-blot membrane (Bio-Rad, Hercules, CA) via the application of a 100 V potential for 1 hour. The membrane was rendered hydrophobic by drying three times with methanol, and then incubated with the appropriate primary antibody in 5% non-fat milk TBST solution for 1 h. The membrane was then washed four times for ten minutes each time in TBST and incubated with the appropriate secondary antibody in 5% non-fat milk TBST solution for thirty minutes, then washed a further four times in TBST. The blot was then visualized using an ECL chemiluminescence kit (Bio-Rad); images were exposed on X-ray film (Kodak, Rochester, NY). The monoclonal anti-phospho-CREB antibody and the monoclonal horse radish peroxidase-conjugated anti-rabbit-IgG antibody (Cell Signaling Technology, Danvers, MA) were used at concentrations of 1:1000 and 1:5000, respectively. The monoclonal anti-GAPDH antibody (Fitzgerald, Concord, MA) and monoclonal anti-mouse-IgG antibody (Amersham, Piscataway, NJ) were used at concentrations of 1:1000 and 1:5000, respectively.

RESULTS:

LL-37 increased IL-8 production by subconfluent keratinocytes in response to pro-inflammatory stimuli.

LL-37 is a potent immunomodulatory agent, although many of these effects have only been demonstrated at pathological concentrations (25 μg/ml). Even at low physiological concentrations (<5 μg/ml), however, LL-37 almost completely inhibits TNF-α production by LPS-stimulated PBMCs (33), and synergistically increases the production of IL-1β-induced cytokines by PBMCs (35). LL-37-mediated immunomodulation has not, however, been previously demonstrated in epithelial cells at low physiological concentrations. To investigate the possibility that such immunomodulatory effects would only be observed in synergy with other agents, we treated subconfluent primary keratinocytes with LL-37 in combination with IL-1β, GM-CSF, and a variety of TLR agonists that were chosen based on the relative expression of TLRs in epithelial cells (39). These studies employed LL-37 at concentrations of <3 μg/ml; these low concentrations of LL-37 did not, in isolation, markedly increase IL-8 production over a twenty-four hour time period (Fig. 2.1-2.3). However when the keratinocytes were co-stimulated with LL-37 and pro-inflammatory stimuli, the presence of 3 μg/ml of LL-37 resulted in a 2-fold increase in IL-8 production in response to flagellin (Fig. 2.1) and a 2.6-fold change in IL-8 production in response to IL-1β (Fig. 2.2), but did not significantly alter IL-8 production in response to PAM3CSK4 or GM-CSF (data not shown). After background subtraction, it was revealed that the increase in IL-8 due to these combinations was greater than the sum of the individual treatments, implying that co-stimulation of the cells with LL-37 and either flagellin or IL-1β resulted in a synergistic increase in IL-8 production (Fig. 2.3).

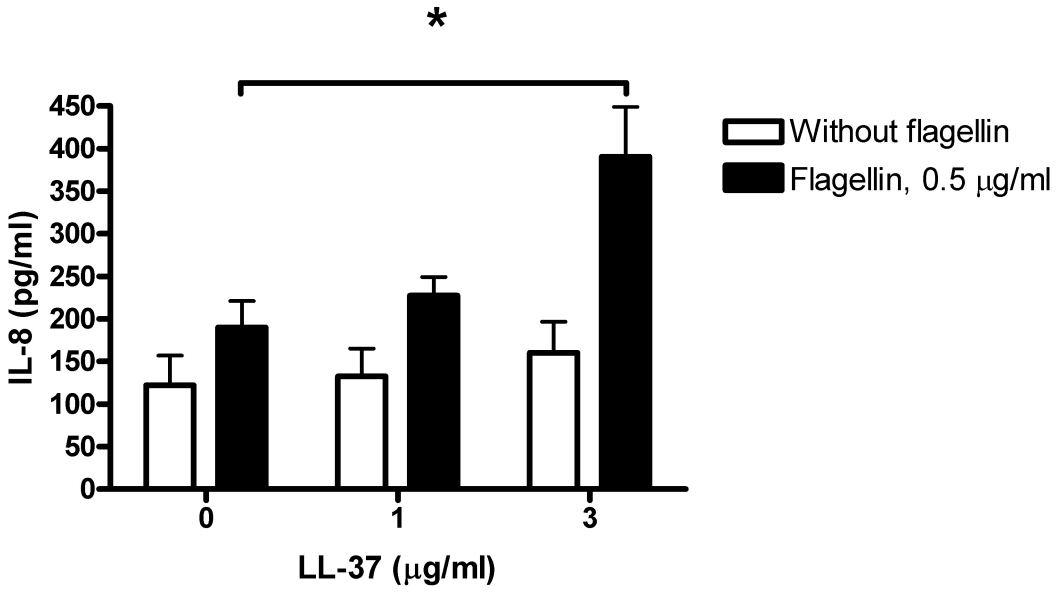

Figure 2.1: LL-37 alters IL-8 production by subconfluent keratinocytes in response to flagellin. Supernatants were collected at 24 hours. Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test.

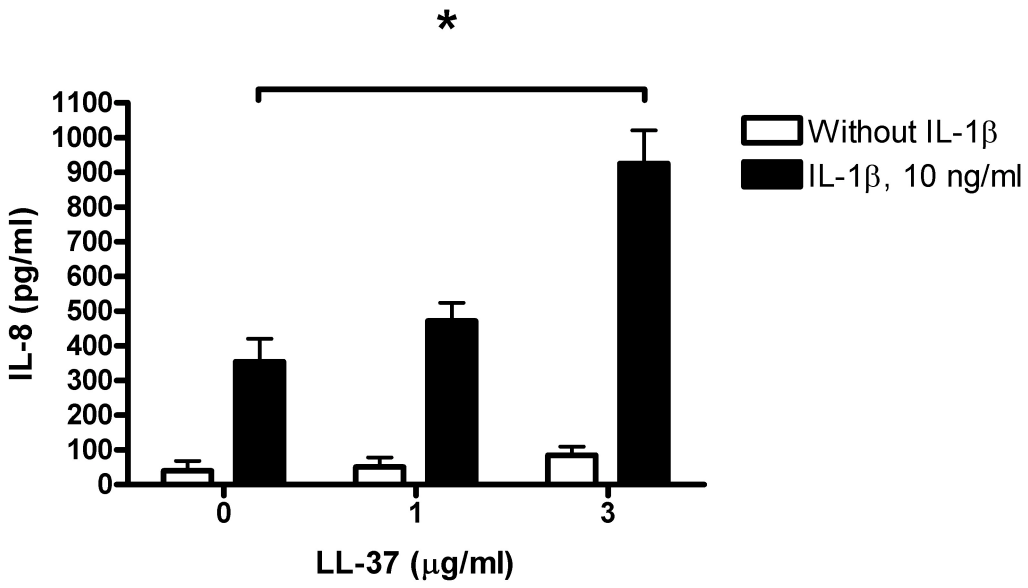

Figure 2.2: LL-37 alters of IL-8 production by subconfluent keratinocytes in response to IL-1β. Supernatants were collected at 24 hours. Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test.

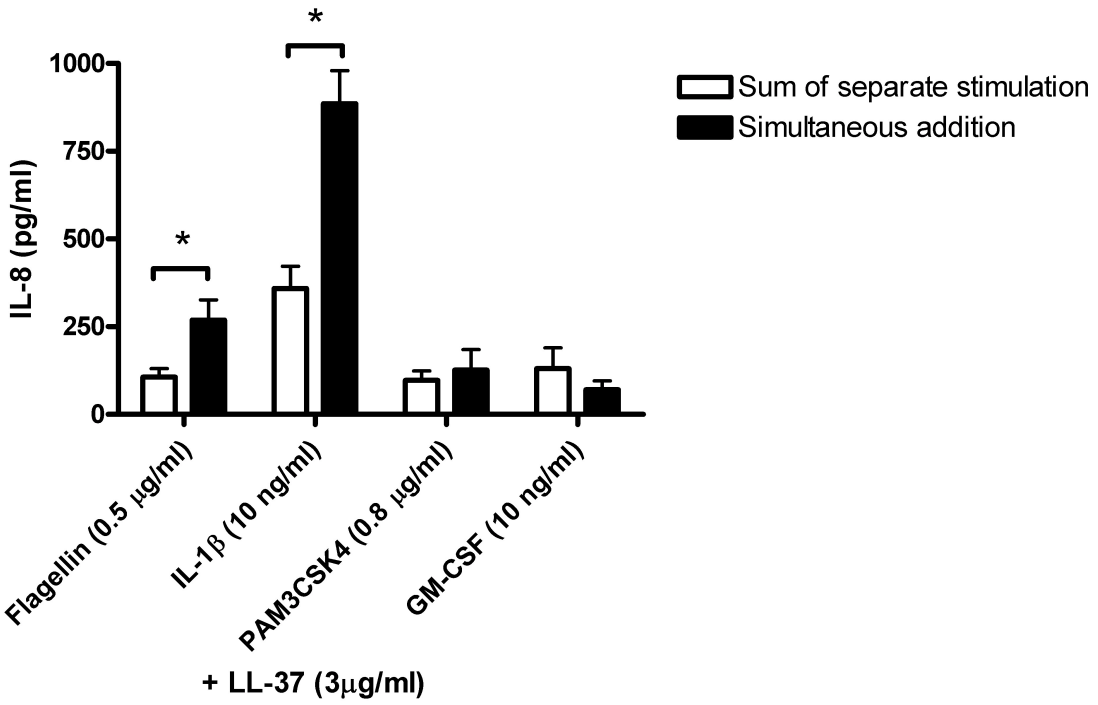

Figure 2.3: Low doses of LL-37 synergistically increase IL-8 production by subconfluent keratinocytes in response to flagellin and IL-1β. Supernatants were collected at 24 hours. Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test; * indicates $p<0.05$. A background subtraction was performed on the data.

The ability of low doses of LL-37 to dramatically increase IL-8 production in response to low concentrations of pro-inflammatory stimuli demonstrated that LL-37 had immunomodulatory activity. As not all keratinocytes in the skin are in a proliferative state, however, but differentiate as they move upwards through the skin strata, we wished to repeat the experiment in a system that modelled terminal differentiation.

LL-37 increased IL-8 production by calcium-differentiated keratinocytes in response to pro-inflammatory stimuli.

Increased extracellular calcium concentrations provoke differentiation and stratification in cultured keratinocytes (40). To better model upper skin strata, we grew keratinocytes to confluence and then maintained them for two days in growth-factor-free media containing 1.35 mM Ca^{2+}. The cells were co-stimulated with LL-37 and pro-inflammatory stimuli; the presence of 3 μg/ml LL-37 resulted in a 2-fold increase in IL-8 production in response to flagellin (Fig. 2.4) and a 1.6-fold increase in IL-8 production in response to IL-1β (Fig. 2.5), a less pronounced effect than was observed in subconfluent cells. LL-37 did not significantly alter IL-8 production by differentiated cells in response to PAM3CSK4 or GM-CSF (data not shown). Co-stimulation of the cells with LL-37 and either flagellin or IL-1β resulted in a synergistic increase in IL-8 production (Fig. 2.6).

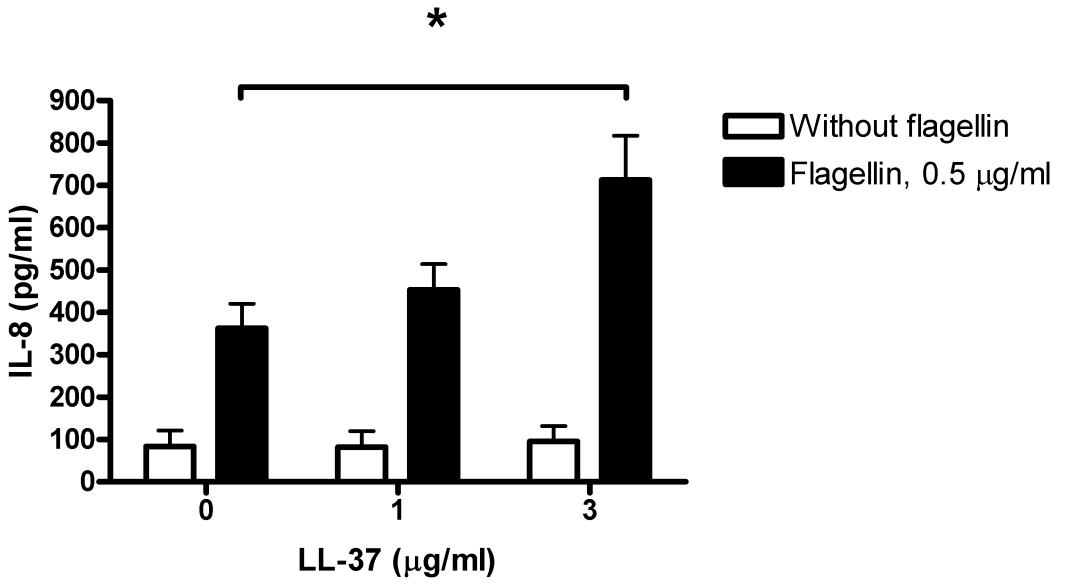

Figure 2.4: LL-37 alters IL-8 production by differentiated keratinocytes in response to flagellin. Supernatants were collected at 24 hours. Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test.

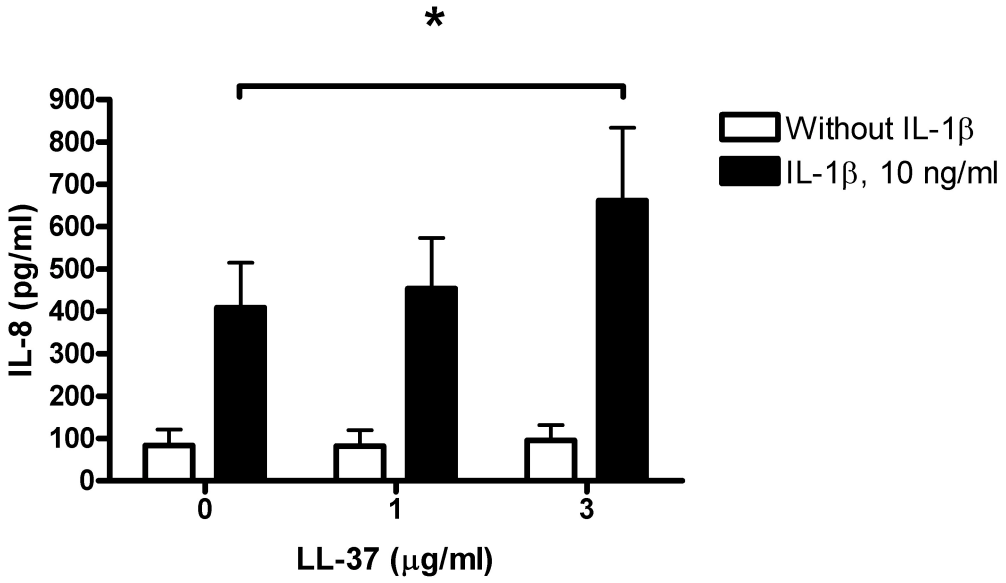

Figure 2.5: LL-37 alters IL-8 production by differentiated keratinocytes in response to IL-1β. Supernatants were collected at 24 hours. Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test.

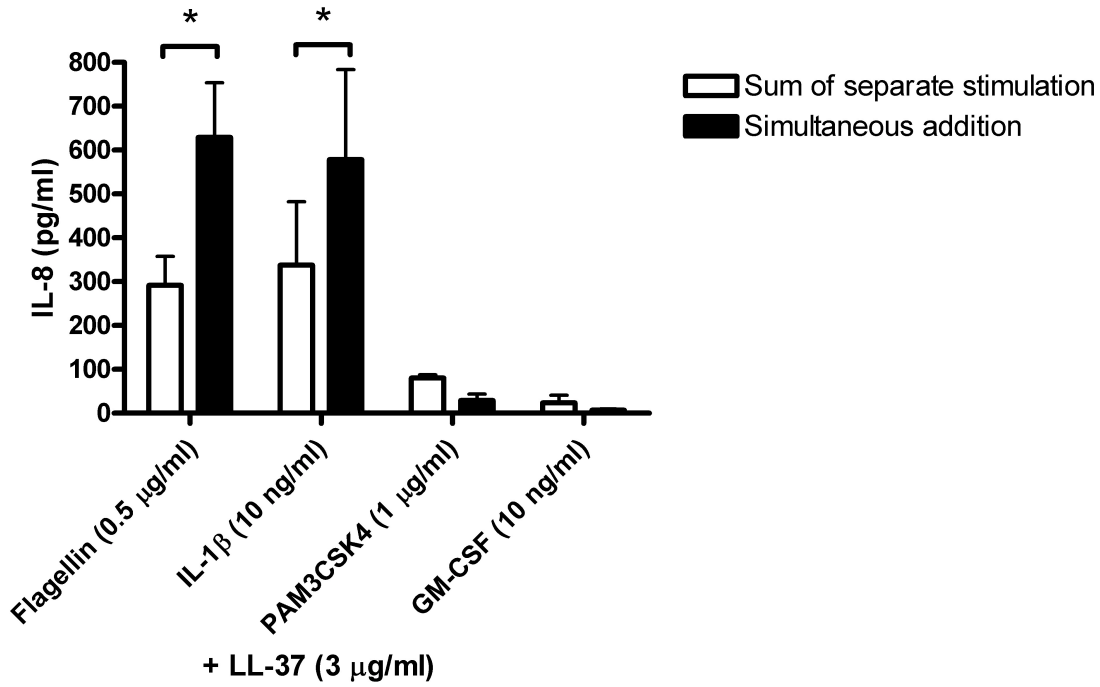

Figure 2.6: Low doses of LL-37 synergistically increase IL-8 production by differentiated keratinocytes in response to flagellin and IL-1β. Supernatants were collected at 24 hours.

Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test; * indicates $p<0.05$. A background subtraction was performed on the data.

These findings indicated that LL-37 was able to alter innate immune responses in both proliferating and differentiated keratinocytes. This was intriguing since LL-37 is only produced by keratinocytes following infection and injury (1, 7). Therefore, it was of interest to see if similar effects would be observed in a cell type that consistently encounters LL-37.

LL-37 increased IL-8 production by bronchial epithelial cells in response to pro-inflammatory stimuli.

Bronchial epithelial cells constitutively produce LL-37 and secrete it into the airway surfactant (2). Accordingly, we tested the ability of LL-37 to alter IL-8 release in this cell type. The presence of 3 μg/ml LL-37 resulted in a 3-fold increase in IL-8 production in response to flagellin (Fig. 2.7), a 4-fold increase in IL-8 production in response to IL-1β (Fig. 2.8), and a 2.2-fold increase in IL-8 release in response to PAM3CSK4 (Fig. 2.9), stronger responses than those observed in keratinocytes. Co-stimulation of the cells with LL-37 and either flagellin or IL-1β resulted in a synergistic increase in IL-8 production (Fig. 2.10).

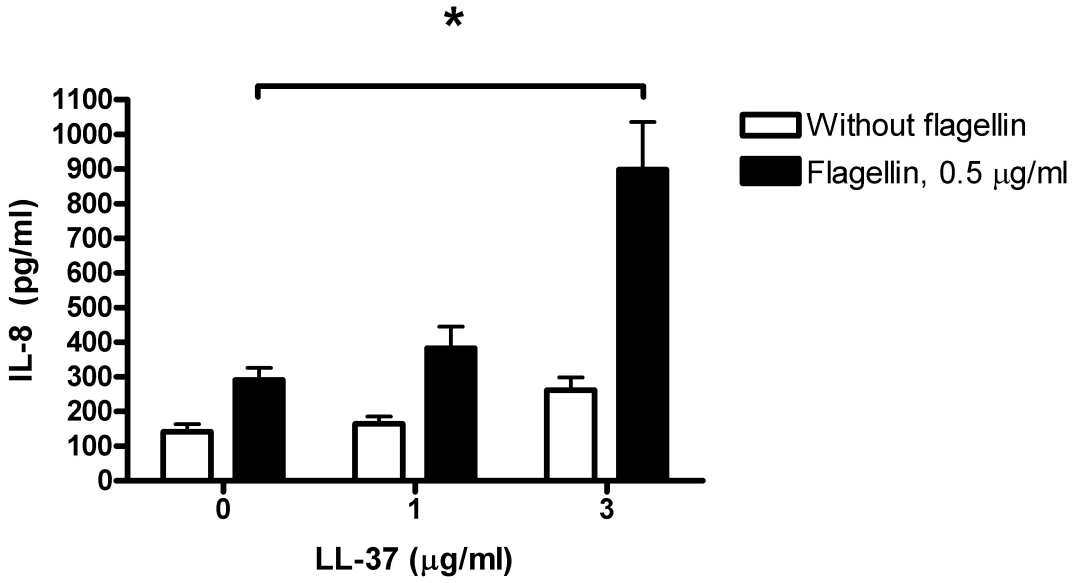

Figure 2.7: LL-37 alters IL-8 production by bronchial epithelial cells in response to flagellin. Supernatants were collected at 24 hours. Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test.

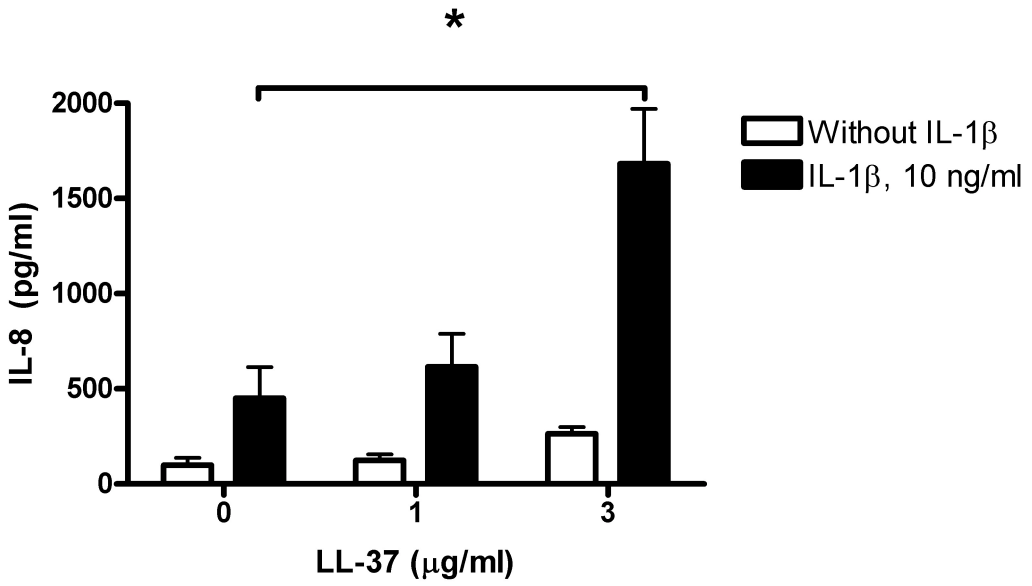

Figure 2.8: LL-37 alters IL-8 production by bronchial epithelial cells in response to IL-1β. Supernatants were collected at 24 hours. Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test.

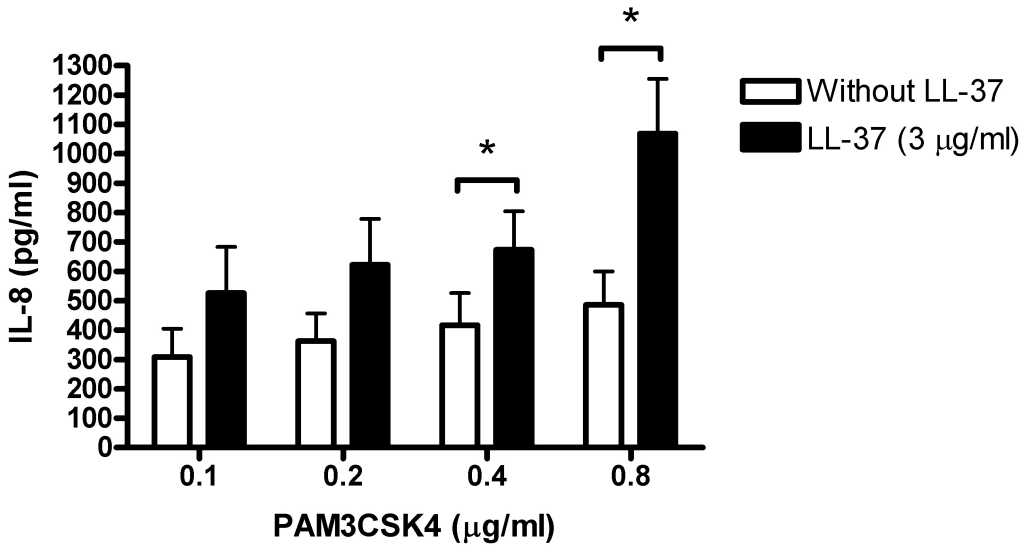

Figure 2.9: LL-37 alters IL-8 production by bronchial epithelial cells in response to PAM3CSK4. Supernatants were collected at 24 hours. Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test; * indicates $p<0.05$.

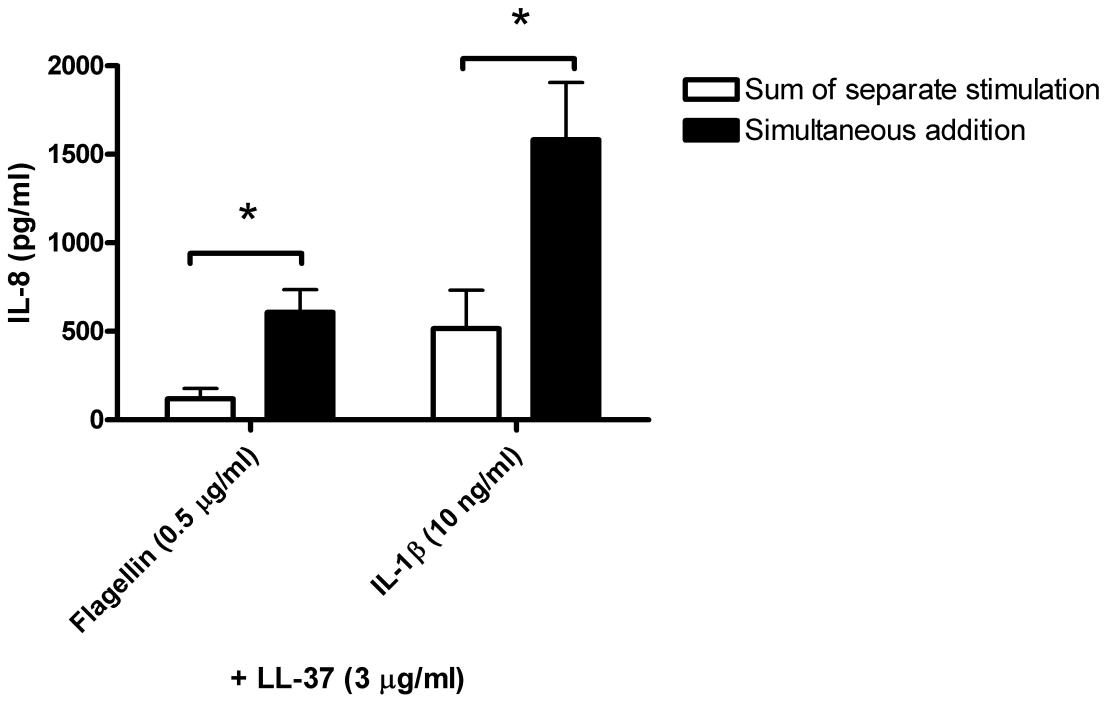

Figure 2.10: Low doses of LL-37 synergistically increase IL-8 production by bronchial epithelial cells in response to flagellin and IL-1β. Supernatants were collected at 24 hours. Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test; * indicates $p<0.05$. A background subtraction was performed on the data.

Co-stimulation of bronchial epithelial cells with LL-37 and poly(I:C) elicited a rapid IL-8 response and delayed cytotoxicity.

In addition to the above agonists of surface TLRs, potential synergy with an intracellular TLR was investigated. Bronchial epithelial cells encounter infectious viral particles in vivo and are accordingly responsive to the presence of virus-associated signature molecules such as dsRNA (Guillot 2005) that interact with intracellular TLRs. To investigate whether LL-37 would show synergy with a viral signature molecule, bronchial epithelial cells were stimulated with the TLR3 agonist poly(I:C) in the presence or absence of 3 μg/ml LL-37, and supernatants were collected at 3, 6, and 24

hours. Co-stimulation with poly(I:C) and peptide elicited a strong, rapid IL-8 response (Fig. 2.11), followed by substantial cytotoxicity (Fig. 2.12). LL-37 did not induce notable cytotoxicity in the absence of poly(I:C), and poly(I:C) was minimally cytotoxic at early timepoints. However co-stimulation with poly(I:C) and peptide resulted in a synergistic increase in both IL-8 release (Fig. 2.13) and cytotoxicity (Fig. 2.14). TUNEL assays performed on bronchial epithelial cells treated with both poly(I:C) and peptide did not detect any increase in apoptosis at 6 h or 24 h (data not shown). As the IL-8 response preceded the cytotoxicity, it was concluded that the increased IL-8 production in these cells was a result of LL-37-mediated immunomodulation.

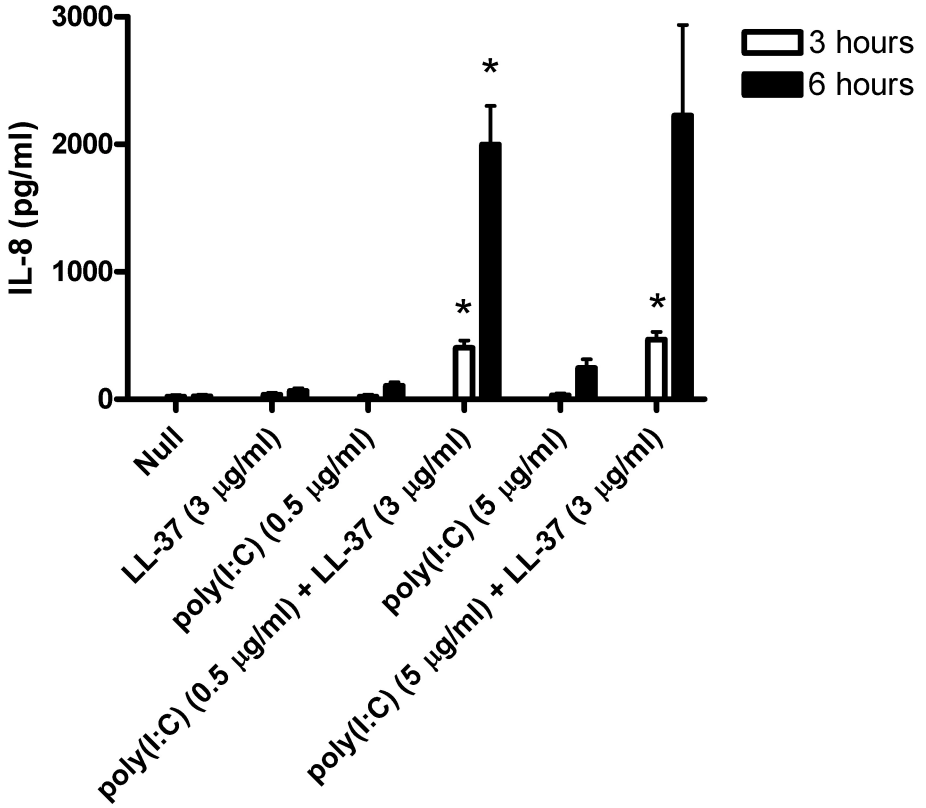

Figure 2.11: Co-stimulation of bronchial epithelial cells with LL-37 and poly(I:C) elicits a rapid IL-8 response. Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test; * indicates $p<0.05$ when compared to null.

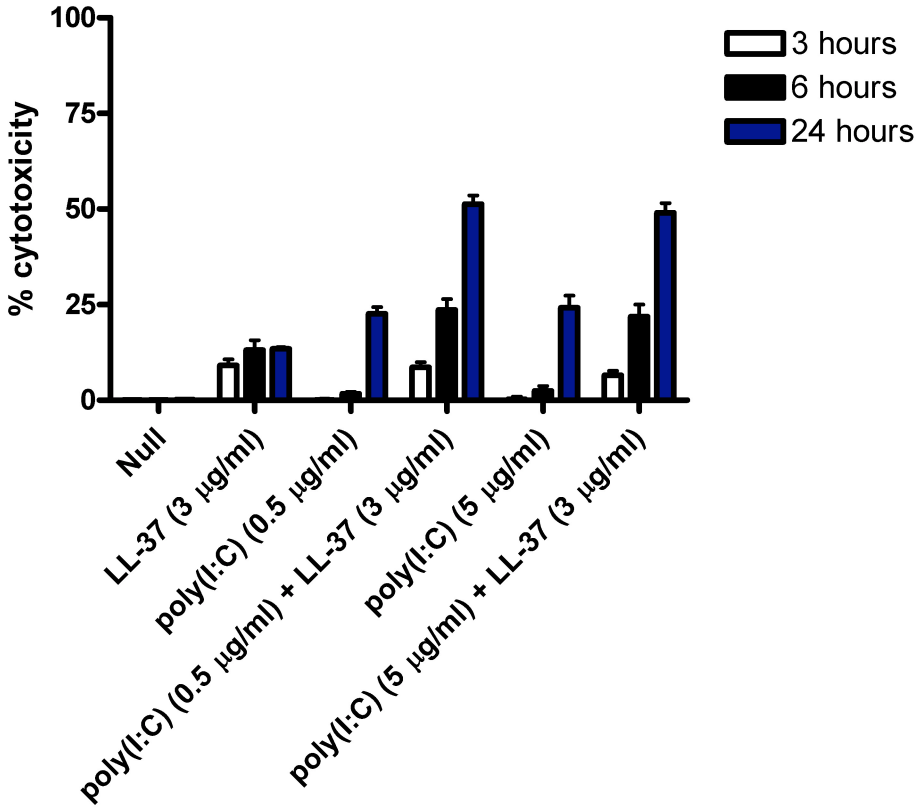

Figure 2.12: Co-stimulation of bronchial epithelial cells with LL-37 and poly(I:C) results in rapid and pronounced cytotoxicity as measured by LDH release. Error bars show S.E.M. of at least three independent experiments. For clarity, the results of statistical analysis are omitted.

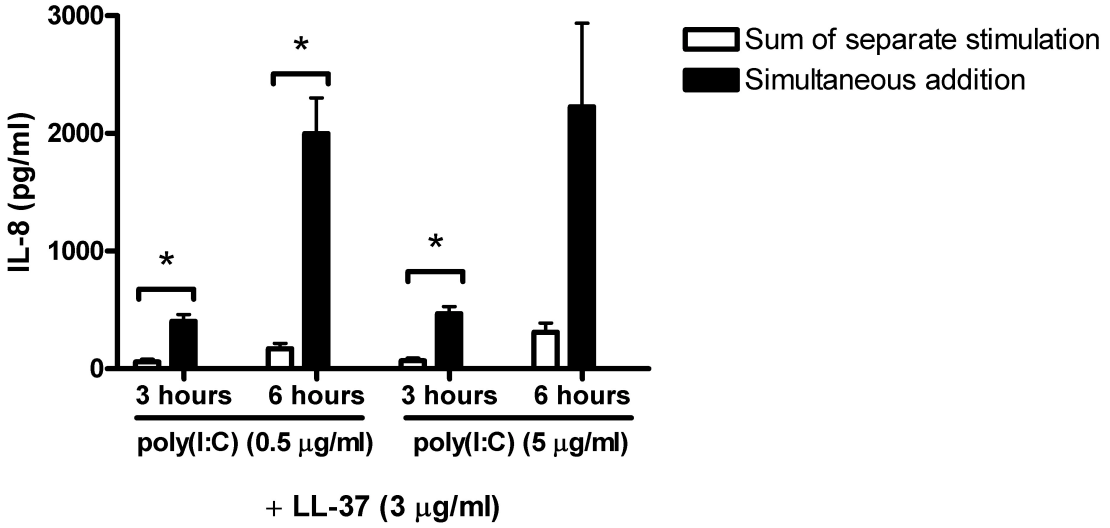

Figure 2.13: Low doses of LL-37 synergistically increase IL-8 production by bronchial epithelial cells in response to poly(I:C). Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test; * indicates $p<0.05$.

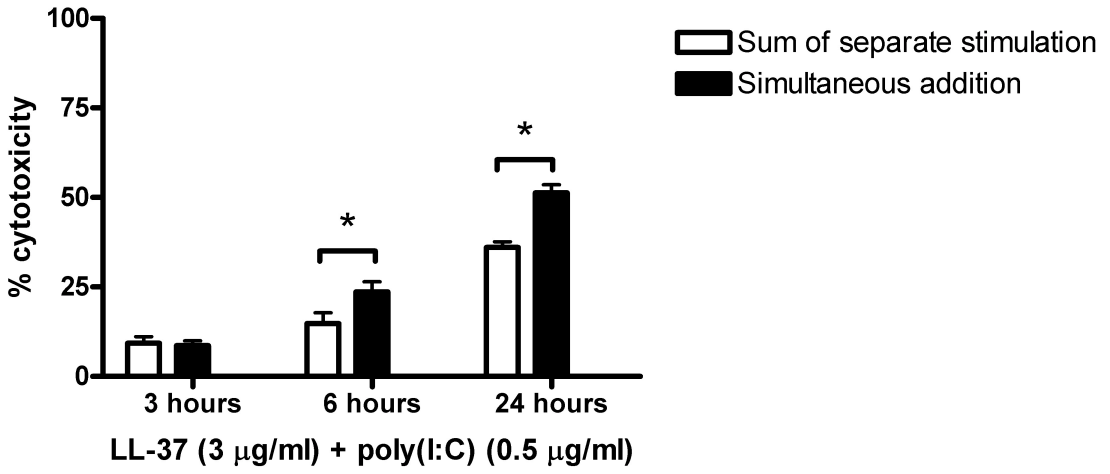

Figure 2.14: Low doses of LL-37 synergistically increase cytotoxicity subsequent to treatment with poly(I:C). Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test; * indicates $p<0.05$.

Inhibition of the synergistic increase in IL-8 production by inhibitors of Src-family kinase signalling or NF-κB translocation

In bronchial epithelial cells, Src-family kinases can regulate the activation of p38 and ERK1/2, and the subsequence release of IL-8 (41). Similarly, the inhibition of Src-family kinases inhibits LL-37-induced IL-8 release in airway smooth muscle cells (42). Additionally, the production of IL-8 by bronchial epithelial cells in response to TLR ligands has been shown to be dependent upon EGFR signalling (43); in keratinocytes, SRC family kinases are activated downstream of EGFR (44). To investigate the possibility that the observed synergistic response was dependent on Src-family kinase activity, keratinocytes were treated with the inhibitors PP2 and SU6656. The presence of either inhibitor resulted in a 70% decrease in IL-8 release relative to that typically observed when cells were treated with flagellin and LL-37 (Fig. 2.15).

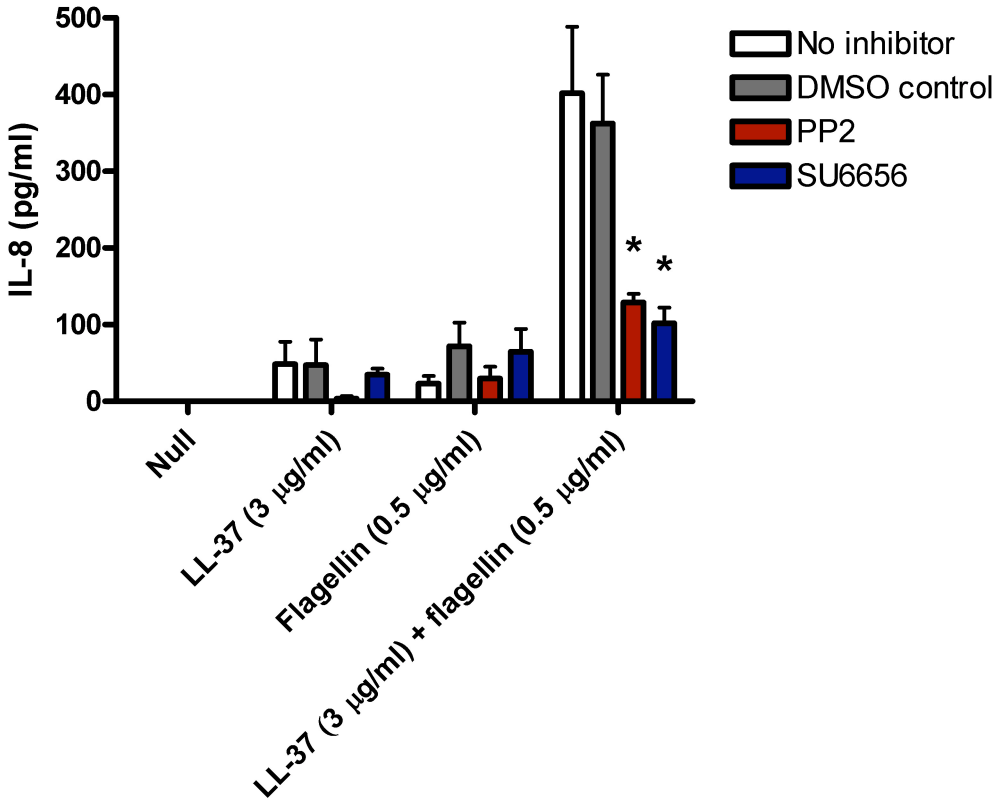

Figure 2.15: The Src-family kinase inhibitors PP2 and SU6656 suppress IL-8 production by keratinocytes in response to co-stimulation with LL-37 and flagellin. Cells were pretreated for 2 hours with 10 μM PP2 or 5 μM SU6656. Error bars show S.E.M. of at least three independent experiments. Statistical comparisons were performed using a 2-tailed Student's T-test; * indicates $p<0.05$.

As the translocation of NF-κB is required for LL-37-mediated cytokine production in PBMCs (33, 35), we investigated the effects of the IκB inhibitor Bay11 on IL-8 responses to LL-37/flagellin co-stimulation. Keratinocytes pretreated with Bay11 exhibited an 80% reduction in IL-8 production after flagellin/LL-37 co-stimulation (Fig. 2.16).

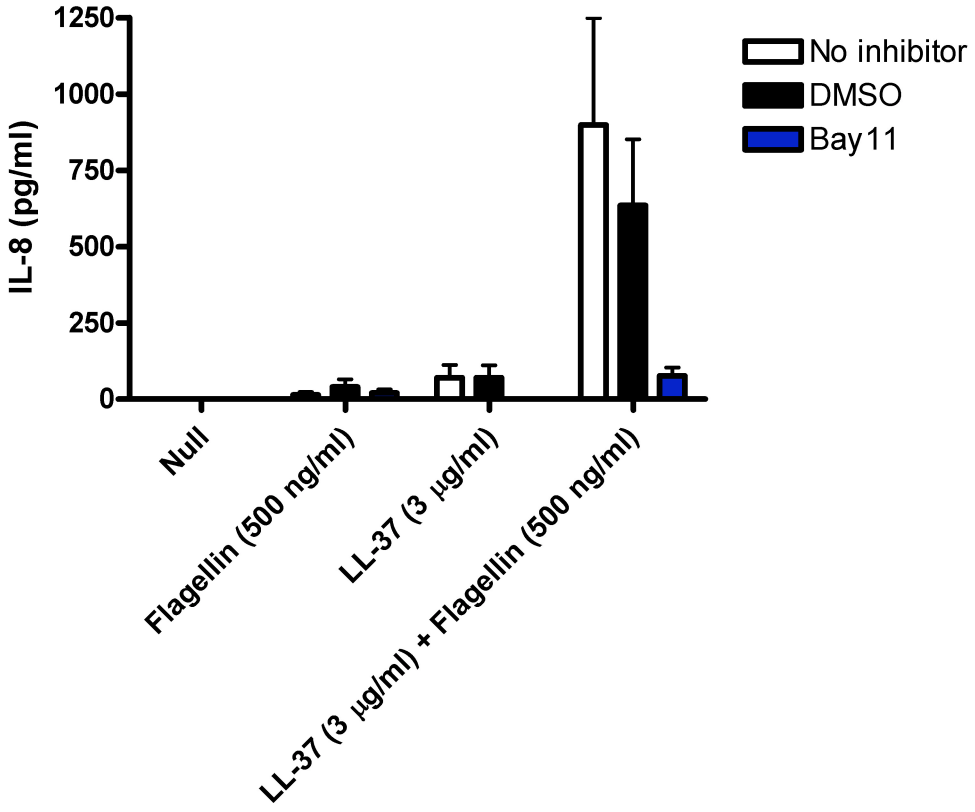

Figure 2.16: The NF-κB inhibitor Bay11 suppresses IL-8 production by keratinocytes in response to co-stimulation with LL-37 and flagellin. Error bars show S.E.M. of at least three independent experiments.

Stimulation of keratinocytes with LL-37 and flagellin resulted in a strong increase in the phosphorylation of the transcription factor CREB.

Having determined that the observed synergistic responses were dependent upon the activation of Src-family kinases and NF-κB, the impact of TLR stimulation on known effects of LL-37 treatment was investigated. In PBMC, LL-37 stimulation results in increased phosphorylation of the MAP kinase ERK-1(45), which is known to phosphorylate the transcription factor CREB, stimulating its translocation into the nucleus (46). Interestingly, co-stimulation of PBMC with LL-37 and IL-1β led to

stronger increases in the phosphorylation of CREB than were detectable following stimulation with either alone (35). To determine if a similar effect would result from co-stimulation of keratinocytes with flagellin and LL-37, cells were treated with LL-37 and/or flagellin and lysed after 30 min; the low doses of LL-37 used in the experiment did not markedly increase CREB phosphorylation either in isolation or when cells were co-stimulated with flagellin (Fig. 2.17).

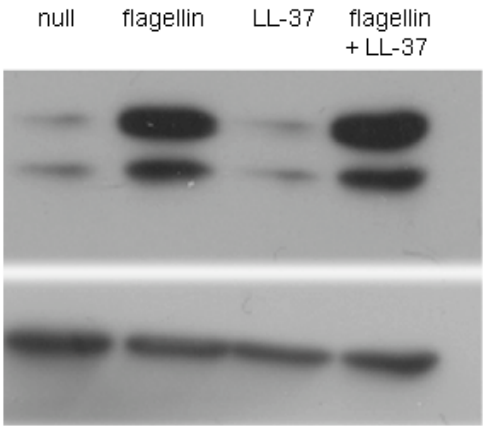

Figure 2.17: Low doses of LL-37 do not alter the increased phosphorylation of CREB observed after keratinocytes are stimulated with flagellin. Cells were lysed 30 minutes post-stimulation. Bands are, from top to bottom: phospho-CREB, phospho-ATF-1, and GAPDH. The blot shown is representative of three independent experiments.

DISCUSSION

We have demonstrated here a novel immunomodulatory role for LL-37 in its interactions with epithelial cells. Low doses of LL-37 that were insufficient to elicit strong increases in IL-8 production on their own were able to synergistically increase IL-8 production by keratinocytes and bronchial epithelial cells in response to pro-inflammatory stimuli.

The exact mechanism underlying these effects is not completely clear. Previous studies have shown that the inhibition of Src-family kinases can reduce IL-8 production in response to LL-37 treatment of smooth muscle cells (42); similarly, the ability of LL-37 to alter epithelial cell responses was shown here to be suppressed by pre-treatment with Src-family kinase inhibitors. It seems possible that this effect is a consequence of the ability of Src-family kinases to regulate the phophorylation state of the MAP kinases p38 and ERK1/2 (41), the activation of which is necessary for LL-37-stimulated IL-18 production in keratinocytes (13) and a variety of peptide-mediated effects in other cell types (16, 45, 47). The translocation of the transcription factor NF-κB is also necessary for IL-8 production by keratinocytes (48). Thus as expected, an inhibitor of NF- κB translocation blocked cellular IL-8 production in response to co-stimulation by LL-37 and flagellin. These results indicate that the ability of LL-37 to alter epithelial cell responses to pro-inflammatory stimuli may be dependent upon altered MAPK signalling and NF-κB activation.

The ability of LL-37 to induce synergistic increases in IL-8 production and cytotoxicity in cells treated with the TLR3 agonist poly(I:C) was intriguing. Rapid secretion of IL-8, followed by a widespread loss of membrane integrity, was observed in cells that were treated with poly(I:C) and LL-37 and although cells treated with poly(I:C) alone also showed increases in cytotoxicity, the effect was not as rapid nor as pronounced. The mechanism underlying this effect is unclear, but it is presumed that the cells underwent necrotic cell death as we were unable to detect increased levels of apoptosis using a TUNEL assay. As poly(I:C) is anionic and LL-37 is cationic, their ability to induce strong responses in epithelial cells might result in part from complex formation. LL-37 has been previously shown to form complexes with human DNA, allowing a strong and

immunologically unusual activation of plasmacytoid dendritic cells (49). The potential ability of LL-37 to form complexes with nucleic acids and alter TLR signalling is an intriguing topic for further investigation. As dsRNA like poly(I:C) is thought to reflect a viral activating signal for TLR3, it seems possible that the induction of cell death might actually represent a viral defence signal, as indeed may the induction of IL-8 that would recruit neutrophils to the vicinity of the LL-37 stimulated epithelium.

As the effects described here are mediated by low concentrations of LL-37, equivalent to those normally present in sweat and airway surfactant (3, 10), we postulate that LL-37-mediated increases in IL-8 release may be important to the regulation of epithelial inflammation *in vivo*. Both keratinocytes and bronchial epithelial cells show increased expression of LL-37 as a consequence of wounding (7, 8) and inflammation (50), suggesting that local increases in LL-37 concentrations at the epithelial surface, together with an appropriate additional endogenous or exogenous signal, may serve as an alarm signal, resulting in increased IL-8 production in response to pro-inflammatory stimuli and a concomitant increase in immune cell recruitment. The ability of LL-37 to alter epithelial cell responses to pro-inflammatory stimuli also suggests new possibilities in the etiology of psoriasis, an auto-immune disorder that results in localized hyper-proliferative areas of skin inflammation. Although classically regarded as a disorder of the adaptive immune system, it has been suggested that psoriasis might result from the abnormal over-expression of host defence peptides in the epithelium (51). As LL-37 is expressed at high concentrations in psoriatic plaques (37), the ability of LL-37 to increase keratinocyte IL-8 responses may be important to the pathophysiology of psoriasis.

The complex defences of innate immunity are vital to the maintenance of epithelial homeostasis. The observation here that modest concentrations of LL-37 can alter epithelial IL-8 production indicates that LL-37 plays a broader role than previously suspected in the regulation of the inflammatory response. An improved understanding of the mechanisms underlying these responses might facilitate the development of novel anti-infective therapeutics.

ACKNOWLEDGEMENTS:

We gratefully acknowledge the invaluable guidance of Jelena Pistolic and the technical assistance of Sheena Tam. NCJF was the recipient of an NSERC CGS M award and a MSFHR Junior Trainee Award. We gratefully acknowledge the support of Genome BC and Genome Prairie for the 'Pathogenomics of Innate Immunity' research program.

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CHAPTER III[2]

INTRODUCTION

Although often considered as two separate processes, the inflammatory and wound-healing responses of the skin are intimately interlinked (1). The cationic host defence peptide LL-37 is a pleiotropic effector molecule of innate immunity that exerts both pro-inflammatory and growth-stimulating effects upon keratinocytes, suggesting that it might be important in the restoration of tissue homeostasis after wounding and infection. The expression by keratinocytes of both the LL-37 precursor hCAP18 and LL-37 itself is increased during inflammation. Indeed, even though keratinocytes are thought to not normally express LL-37, increased concentrations of the peptide are detectable in inflamed skin both as a result of atopic and autoimmune responses (2, 3), and as a consequence of fungal and viral infections (4, 5). The treatment of cultivated keratinocytes with pathological doses of LL-37 elicits the production of IL-8 (2), IL-6, IL-10, IP-10, MCP-1, MIP-3α, RANTES (7), and IL-18 (8). Furthermore, LL-37 is chemotactic for a variety of immune cells, including monocytes, T cells and mast cells (9, 10). The importance *in vivo* of these activities is suggested by the phenotype of mice deficient in the murine LL-37 homolog CRAMP, which exhibit moderate increased susceptibility to skin infection by Group A Streptococcal bacteria (3).

Low doses of LL-37, however, stimulate wound healing. LL-37 provokes keratinocyte migration and proliferation (7, 12). These activities involve activation of the Epidermal Growth Factor Receptor (EGFR), downstream signalling through the Stat3 pathway, and

[2] A version of this chapter will be submitted for publication. Filewod, N. C. J., Falsafi, R., Gardy, J., and R. E. W. Hancock. Title to be determined.

the activation of the transcription factors Snail and Slug (12, 13). Similarly, the treatment of excised skin samples with an anti-LL-37 blocking antibody has been shown to inhibit re-epithelialisation in a concentration-dependent manner (4). LL-37 is also an angiogenic factor, able to stimulate the development of capillaries in a rabbit hind limb model (15) and elicit the secretion of vascular endothelial growth factor by keratinocytes (5). LL-37 is thus thought to be involved in both the initial responses to infection and eventual tissue repair.

It is increasingly apparent that Toll-like receptor (TLR) signalling plays an important role in the maintenance of epithelial tissue homeostasis and integrity. MyD88-dependent signalling induced by commensal microbes has been shown to be necessary for appropriate responses to epithelial injury in a murine model of colitis (6); similarly, the stimulation of TLR2 and TLR5 provokes growth and reduces apoptosis in bronchial epithelial cells (7). These effects have been suggested to be dependent upon EGFR signalling and independent of the production of pro-inflammatory cytokines (7). While the relevance of these findings to the skin has yet to be confirmed, the possibility of a common mechanism in TLR- and LL-37-mediated growth responses aroused my curiosity. LL-37 is able to alter responses to TLR stimulation in a variety of cell types (19-22), including keratinocytes (Chapter II). Accordingly, I hypothesized that the presence of TLR2 and TLR5 ligands might alter keratinocyte growth responses to LL-37 treatment *in vitro*. In this Chapter, preliminary evidence is presented that indicates that the ability of LL-37 to stimulate keratinocyte proliferation relies upon the secondary production of growth factors by peptide-stimulated cells. Furthermore, genes were identified that showed altered regulation after LL-37 treatment. If continued as suggested

in the discussion, these studies may improve our understanding of the interplay of innate immunity and commensal microflora in the repair of epithelial injury.

MATERIALS AND METHODS

Cell cultivation: Normal primary adult keratinocytes were obtained from Cascade Biologics (Portland, OR) and maintained in their proprietary Epilife medium with the addition of a Human Keratinocyte Growth Supplement that contained bovine pituitary extract, bovine insulin, hydrocortisone, bovine transferrin, and human epidermal growth factor. Unsupplemented Epilife contained 0.65 μM calcium. The medium was changed every two days and cells were passaged prior to confluence to avoid differentiation. Cultures were only used for a maximum of six passages. The cells were cultivated in a 37° C incubator containing 5% CO_2.

Reagents: Human peptide LL-37 (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPR-TES) was synthesized at the Nucleic acid/Protein synthesis unit at the University of British Columbia, using F-Moc chemistry. The synthesized peptide was re-suspended in endotoxin-free water (Sigma-Aldrich, Oakville, ON) and stored at -20°C until further use.

RNA isolation: Keratinocytes were seeded into tissue-culture-treated 6-cm Petri dishes (Corning Inc. Life Sciences, Acton, MA) at a density of 7000 cells/cm^2 and cultivated in supplemented medium until they reached about 70% confluence. The medium was then replaced with unsupplemented Epilife (Cascade Biologics) and the cells were rested for 2 hours prior to stimulation with 3 μg/ml LL-37 or a vehicle control. RNA was isolated at

1, 2 and 4 hours using an RNeasy Mini-kit (Qiagen, Mississauga, ON). Eluted RNA was treated with an RNAse inhibitor (Ambion, Austin, TX) and stored at -80° until use.

Microarray analysis: RNA purity and integrity were assessed using an Agilent 2100 Bioanalyzer using RNA 6000 Nano kits (Agilent Technologies, Santa Clara, CA). The RNA was amplified using an amPULSE RNA Amplification kit from Kreatech (Amsterdam, The Netherlands), as per the manufacturer's instructions. Briefly, the RNA was reverse transcribed to make sCDNA, from which dsCDNA was synthesized. The dsCDNA was then amplified via In Vitro Transcription (IVT), yielding aRNA. This aRNA was labeled with Cyanine 3 and Cyanine 5, and the labeled product was cleaned using the columns provided. Yield and fluorophore incorporation were measured using a NanoDrop 1000 fluorometer/spectrophotometer (Nanodrop, Wilmington, DE). Microarray slides were printed using the human genome 21K Array-Ready Oligo Set (Qiagen) at the Jack Bell Research Centre (Vancouver, BC). The slides were prehybridised for 45 minutes at 48° C in prehybridization buffer containing 5 x SSC (Ambion), 0.1% (w/v) SDS, and 0.2% (w/v) BSA. Equivalent (20 pmol) cyanine-labelled samples from control and treated cells were then mixed and hybridized on the array slides, in Ambion SlideHyb buffer no. 2 (Ambion) for 18 hours at 50°C in a hybridization oven. Following hybridization, the slides were washed twice in 2x SSC/0.2% SDS for 15 minutes at 65°C, and then once in 2x SSC for 15 minutes at 42°C, followed by washing in 0.2% SSC for 15 minutes at room temperature. Slides were then centrifuged for 2 minutes at 2000 x g, dried, and scanned using a ScanArray Express software/scanner (PerkinElmer). The images were quantified using Imagene software (BioDiscovery, El Segundo, CA).

Bioinformatic analysis: Assessment of slide image quality, data normalization, detection of differential gene expression, and statistical analysis were conducted using the ArrayPipe (www.pathogenomics.ca/arraypipe) web-based software package (8). The following steps were applied: markers were flagged and excluded, background correction was applied using the Limma 'normexp' method, spot intensities within each subgrid were normalized using the Limma Loess method, a Limma eBayes modified t-test was performed, and an annotated list of genes and fold changes was obtained. A list of differentially expressed genes was obtained for each timepoint using a cutoff p-value of 0.05 and a minimal fold change calculated by ArrayPipe to allow a 40% chance of false discovery based on an analysis of variation between the biological repeats (this high false discovery rate was chosen so as not to miss any of the important dysregulated genes, reasoning that downstream analyses will eventually decipher which of the results are most reliable; in typical confirmatory experiments from the Hancock lab usually more than 70% of genes can be confirmed and this number rises as the extent of differential expression rises above 2-fold). Transcription factor binding site over-representation analysis was performed using the oPossum web-based software package (http://burgundy.cmmt.ubc.ca/oPOSSUM) (9) using the default settings. Pathway overrepresentation analysis was performed using the InnateDB database and web-based software package using a p-value cutoff of 0.05 and a fold change cutoff of 1.5 (http://innatedb.ca) (publication in press); pathway diagrams were produced using the Cerebral Cytoscape plug-in (http://innatedb.ca/resources.jsp) (10). Gene ontology overrepresentation analysis was performed using the GOTree machine web-based software package (http://genereg.ornl.gov/gotm/) (11).

RESULTS:

Treatment with low doses of LL-37 resulted in altered gene expression.

As LL-37 is known to provoke keratinocyte migration and proliferation (7, 12), we wished to investigate altered gene expression in response to low dose LL-37 stimulation. Keratinocytes were grown to about 70% confluence and stimulated with 3 μg/ml of LL-37; RNA was collected 1, 2 and 4 hours post-stimulation. Microarray analysis revealed differences in gene expression between LL-37-treated and control cells at all timepoints. Two hundred and sixty-five genes showed significant differential expression at the 1-hour timepoint, 150 at the 2 hour timepoint, and 592 at 4 hours. Selected results of relevance to wound healing and innate immunity are presented in Table 3.1 (1 hour timepoint), Table 3.2 (2 hour timepoint), and Table 3.3 (4 hour timepoint); complete results are presented in Supplementary Tables 7-9, respectively.

Table 3.1: Selected genes showing differential expression in keratinocytes 1 hour post-treatment with 3 μg/ml LL-37. Genes were excluded if they showed <2.5-fold change, and gene lists were manually curated for potential relevance to wound healing and innate immune responses.

Gene Name	Gene Description	Fold Change	p Value
SEM4A	Inhibits axonal extension by providing local signals to specify territories inaccessible for growing axons	4.70	0.0023
E2F2	Transcription activator that binds DNA cooperatively with DP proteins through the E2 recognition site.	4.33	0.0029
CAD19	Cadherins are calcium dependent cell adhesion proteins.	3.55	0.005
ITA4	Integrins alpha-4/beta-1 (VLA-4) and alpha-4/beta-7 are receptors for fibronectin.	3.44	0.0056
AKT2	General protein kinase capable of phosphorylating several known proteins	3.35	0.006
PLCB4	The production of the second messenger molecules diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) is mediated by activated phosphatidylinositol-specific phospholipase C enzymes.	2.83	0.0106
ONEC2	Transcriptional activator. Activates the transcription of a number of liver genes such as HNF3B	2.77	0.0114
FGD1	Activates CDC42, a member of the Ras-like family of Rho-and Rac proteins, by exchanging bound GDP for free GTP. Plays a role in regulating the actin cytoskeleton and cell shape	2.76	0.0115
ATG4D	Cysteine protease required for autophagy.	2.53	0.0157
CLTR1	Receptor for cysteinyl leukotrienes mediating bronchoconstriction of individuals with and without asthma.	-2.50	0.0166
BKRB2	Receptor for bradykinin.	-2.53	0.0157

Gene Name	Gene Description	Fold Change	p Value
CUL5	Component of E3 ubiquitin ligase complexes, which mediate the ubiquitination and subsequent proteasomal degradation of target proteins. Seems to be involved poteosomal degradation of p53/TP53 stimulated by adenovirus E1B-55 kDa protein. May form a cell surface vasopressin receptor	-2.66	0.0012
NP060218.1	Histone H5	-2.67	0.0129
PO2F3	Transcription factor that binds to the octamer motif (5'-ATTTGCAT-3').	-2.76	0.0115
NFASC	Cell adhesion, ankyrin-binding protein which may be involved in neurite extension, axonal guidance, synaptogenesis, myelination and neuron-glial cell interactions.	-2.80	0.0109
DLX5	Homeobox protein DLX-5	-2.87	0.01
PIAS1	Plays a crucial role as a transcriptional coregulation in various cellular pathways, including the STAT pathway, the p53 pathway and the steroid hormone signaling pathway.	-2.90	0.0097
CASR	Senses changes in the extracellular concentration of calcium ions.	-2.93	0.0094
TLE4	Transcriptional corepressor that binds to a number of transcription factors.	-2.95	0.0091
CELR1	Receptor that may have an important role in cell/cell signaling during nervous system formation	-3.25	0.0067
VNN2	Probable hydrolase. Involved in the thymus homing of bone marrow cells. May regulate beta-2 integrin-mediated cell adhesion, migration and motility of neutrophil	-3.43	0.0056
PLOD2	Forms hydroxylysine residues in -Xaa-Lys-Gly- sequences in collagens.	-3.73	0.0044
SL9A8	Involved in pH regulation. Plays an important role in signal transduction	-3.75	0.0043
RTC1	Catalyzes the conversion of 3'-phosphate to a 2',3'-cyclic phosphodiester at the end of RNA.	-3.81	0.0003
TIGD6	Tigger transposable element-derived protein 6	-3.89	0.0039
F261	Synthesis and degradation of fructose 2,6-bisphosphate	-3.93	0.0002
K22E	Probably contributes to terminal cornification. Associated with keratinocyte activation, proliferation and keratinization	-4.52	0.0026
DB118	Has antibacterial activity	-4.56	0.0025
RNAS1	Endonuclease that catalyzes the cleavage of RNA on the 3' side of pyrimidine nucleotides. Acts on single stranded and double stranded RNA	-4.90	0.0021
ZN134	May be involved in transcriptional regulation	-5.67	0.0015
FGF2	The heparin-binding growth factors are angiogenic agents in vivo and are potent mitogens for a variety of cell types in vitro.	-7.71	0.0008
ATRX	Could be a global transcriptional regulator.	-9.95	0.0005
ZHANG	Strongly activates transcription when bound to HCFC1.	-12.16	0.0003

Table 3.2: Selected genes showing differential expression in keratinocytes 2 hours post-treatment with 3 µg/ml LL-37. Genes were excluded if they showed <2.5-fold change, and gene lists were manually curated for potential relevance to wound healing and innate immune responses.

Gene Name	Gene Description	Fold Change	p Value
TRI16	May play a role in the regulation of keratinocyte differentiation	7.67	0.004
MIF; MMP11	May play an important role in the progression of epithelial malignancies; The expression of MIF at sites of inflammation suggest a role for the mediator in regulating the function of macrophage in host defense.	6.36	0.0054
FOS	Nuclear phosphoprotein which forms a tight but non-covalently linked complex with the JUN/AP-1 transcription factor.	4.57	0.0099
TIE2	This protein is a protein tyrosine-kinase transmembrane receptor for angiopoietin 1.	4.35	0.011
GBG8	Guanine nucleotide-binding proteins (G proteins) are involved as a modulator or transducer in various transmembrane signaling systems.	4.24	0.0116
RT12	28S ribosomal protein S12, mitochondrial precursor; S12mt; MRP-S12; MT-RPS12	4.20	0.0118
KS6A2	Serine/threonine kinase that may play a role in mediating the growth-factor and stress induced activation of the transcription factor CREB	3.92	0.0137
NPT2B	May be involved in actively transporting phosphate into cells via Na(+) cotransport. May have a role in the synthesis of surfactant in lungs' alveoli.	3.13	0.0162
ATE1	Involved in the posttranslational conjugation of arginine to the N-terminal aspartate or glutamate of a protein. This arginylation is required for degradation of the protein via the ubiquitin pathway.	3.06	0.0244
TRIO	Promotes the exchange of GDP by GTP. Could play a role in coordinating cell-matrix and cytoskeletal rearrangements necessary for cell migration and cell growth.	3.06	0.0244
NP115589.2	Engulfment and cell motility.	3.05	0.0247
PPIL2	PPIases accelerate the folding of proteins. It catalyzes the cis-trans isomerization of proline imidic peptide bonds in oligopeptides	3.02	0.0252
NGLY1	Specifically deglycosylates the denatured form of N-linked glycoproteins in the cytoplasm and assists their proteasome-mediated degradation.	2.99	0.0258
RET7	Intracellular transport of retinol	2.96	0.0264
ABI2	May be involved in cytoskeletal reorganization.	2.66	0.0352
HSH2D	May be a modulator of the apoptotic response through its ability to affect mitochondrial stability (By similarity). Adapter protein involved in tyrosine kinase and CD28 signaling. Seems to affect CD28-mediated activation of the RE/AP element of the interleukin-2 promoter	2.53	0.0406
RND1	Controls rearrangements of the actin cytoskeleton.	2.52	0.041
TRIC	May play a role in the formation of the epithelial barrier	-2.60	0.0374
PVRL3	Plays a role in cell-cell adhesion. Also involved in the formation of cell-cell junctions, including adherens junctions and synapses. Induces endocytosis-mediated down-regulation of PVR from the cell surface, resulting in reduction of cell movement and proliferation.	-2.64	0.0449
SIRPG	Probable immunoglobulin-like cell surface receptor. On binding with CD47, mediates cell-cell adhesion.	-2.70	0.0339

Gene Name	Gene Description	Fold Change	p Value
SG1D1	May bind androgens and other steroids, may also bind estramustine, a chemotherapeutic agent used for prostate cancer.	-2.72	0.0332
SMURF2	E3 ubiquitin-protein ligase which accepts ubiquitin from an E2 ubiquitin-conjugating enzyme in the form of a thioester and then directly transfers the ubiquitin to targeted substrates. I	-2.80	0.0307
DLG1	Essential multidomain scaffolding protein required for normal development (By similarity). Recruits channels, receptors and signaling molecules to discrete plasma membrane domains in polarized cells. May play a role in adherens junction assembly, signal transduction, cell proliferation, synaptogenesis and lymphocyte activation	-2.87	0.0287
RNF34	Has E3 ubiquitin-protein ligase activity. Regulates the levels of CASP8 and CASP10 by targeting them for proteasomal degradation. Protects cells against apoptosis induced by TNF. Binds phosphatidylinositol-5-phosphate and phosphatidylinositol-3-phosphate	-2.89	0.0283
CUL5	Component of E3 ubiquitin ligase complexes, which mediate the ubiquitination and subsequent proteasomal degradation of target proteins. Seems to be involved poteosomal degradation of p53/TP53 stimulated by adenovirus E1B-55 kDa protein. May form a cell surface vasopressin receptor	-3.36	0.0067
LAIR1	Functions as an inhibitory receptor that plays a constitutive negative regulatory role on cytolytic function of natural killer (NK) cells, B-cells and T-cellsModulates cytokine production in CD4+ T-cells, downregulating IL2 and IFNG production while inducing secretion of transforming growth factor beta. Down-regulates also IgG and IgE production in B-cells as well as IL8, IL10 and TNF secretion. Inhibits proliferation and induces apoptosis in myeloid leukemia cell lines as well as prevents nuclear translocation of NF-kappa-B p65 subunit/RELA and phosphorylation of I-kappa-B alpha/CHUK in these cells.	-3.48	0.0178
PLOD2	Forms hydroxylysine residues in -Xaa-Lys-Gly- sequences in collagens. These hydroxylysines serve as sites of attachment for carbohydrate units and are essential for the stability of the intermolecular collagen cross-links	-3.94	0.0135
CELR3	Has an important role in stress fiber formation induced by active diaphanous protein homolog 1 (DRF1). Induces microspike formation, in vivo (By similarity). In vitro, stimulates N-WASP-induced ARP2/3 complex activation in the absence of CDC42 (By similarity). May play an important role in the maintenance of sarcomeres and/or in the assembly of myofibrils into sarcomeres. Implicated in regulation of actin polymerization and cell adhesion	-5.25	0.0446
CX04A	May have an important role of cell protection in inflammation reaction	-5.66	0.0067
BPAEA	Cytoskeletal linker protein. Anchors keratin-containing intermediate filaments to the inner plaque of hemidesmosomes. The proteins may self-aggregate to form filaments or a two-dimensional mesh	-6.43	0.0053
ENSG00-000117114	Brain-specific angiogenesis inhibitor.	-6.58	0.0051
RRP5	Involved in the biogenesis of rRNA.	-6.63	0.0051

Gene Name	Gene Description	Fold Change	p Value
FGF2	The heparin-binding growth factors are angiogenic agents in vivo and are potent mitogens for a variety of cell types in vitro. There are differences in the tissue distribution and concentration of these 2 growth factors	-8.32	0.0011
MUC16	Thought to provide a protective, lubricating barrier against particles and infectious agents at mucosal surfaces	-8.92	0.0032

Table 3.3: Selected genes showing differential expression in keratinocytes 4 hours post-treatment with 3 µg/ml LL-37. Genes were excluded if they showed <2.5-fold change, and gene lists were manually curated for potential relevance to wound healing and innate immune responses.

Gene Name	Gene Description	Fold Change	p Value
EGF	EGF stimulates the growth of various epidermal and epithelial tissues in vivo and in vitro and of some fibroblasts in cell culture	7.03	0.0004
DUS9	Inactivates MAP kinases. Has a specificity for the ERK family; Required for the uptake of creatine in muscles and brain	5.99	0.0006
IFNA1	Produced by macrophages, IFN-alpha have antiviral activities. Interferon stimulates the production of two enzymes: a protein kinase and an oligoadenylate synthetase	5.52	0.0008
SMAD6	Antagonist of signaling by TGF-beta (transforming growth factor) type 1 receptor superfamily members; has been shown to inhibit selectively BMP (bone morphogenetic proteins) signaling.	5.07	0.001
INHBC	Inhibins and activins inhibit and activate, respectively, the secretion of follitropin by the pituitary gland. Inhibins/activins are involved in regulating a number of diverse functions such as hypothalamic and pituitary hormone secretion, gonadal hormone secretion, germ cell development and maturation, erythroid differentiation, insulin secretion, nerve cell survival, embryonic axial development or bone growth, depending on their subunit composition. Inhibins appear to oppose the functions of activins	4.99	0.0011
CARD9	Activates NF-kappa-B via BCL10	4.60	0.0014
DACH2	Transcription factor that is involved in regulation of organogenesis.	3.63	0.0034
IGF1R	This receptor binds insulin-like growth factor 1 (IGF1) with a high affinity and IGF2 with a lower affinity.	3.63	0.0035
SULF1	Exhibits arylsulfatase activity and highly specific endoglucosamine-6-sulfatase activity. It can remove sulfate from the C-6 position of glucosamine within specific subregions of intact heparin. Diminishes HSPG (heparan sulfate proteoglycans) sulfation, inhibits signaling by heparin-dependent growth factors, diminishes proliferation, and facilitates apoptosis in response to exogenous stimulation	3.07	0.0068
KCC1D	Calcium/calmodulin-dependent protein kinase belonging to a proposed calcium-triggered signaling cascade. May regulate calcium-mediated granulocyte function. May play a role in apoptosis of erythroleukemia cells. Activates MAP	2.99	0.0076

Gene Name	Gene Description	Fold Change	p Value
	kinase MAPK3 (By similarity). In vitro, phosphorylates transcription factor CREM isoform Beta and probably CREB1		
ZN174	Transcriptional repressor	2.99	0.0077
HXD11	Sequence-specific transcription factor which is part of a developmental regulatory system that provides cells with specific positional identities on the anterior-posterior axis	2.97	0.0079
HMGB3	Binds preferentially single-stranded DNA and unwinds double stranded DNA	2.80	0.0433
ISCU	Involved in the assembly or repair of the [Fe-S] clusters present in iron-sulfur proteins. Binds iron	2.79	0.0103
SIM2	Transcription factor that may be a master gene of CNS development in cooperation with Arnt. It may have pleiotropic effects in the tissues expressed during development	2.73	0.0114
LECT1	Bifunctional growth regulator that stimulates the growth of cultured chondrocytes in the presence of basic fibroblast growth factor (FGF) but inhibits the growth of cultured vascular endothelial cells. May contribute to the rapid growth of cartilage and vascular invasion prior to the replacement of cartilage by bone during endochondral bone development	2.68	0.0123
PCOC1	Binds to the C-terminal propeptide of type I procollagen and enhances procollagen C-proteinase activity	2.64	0.0132
HSPA1	In cooperation with other chaperones, Hsp70s stabilize preexistent proteins against aggregation and mediate the folding of newly translated polypeptides in the cytosol as well as within organelles. These chaperones participate in all these processes through their ability to recognize nonnative conformations of other proteins. They bind extended peptide segments with a net hydrophobic character exposed by polypeptides during translation and membrane translocation, or following stress-induced damage	2.60	0.0141
TGFB3	Involved in embryogenesis and cell differentiation	2.50	0.0433
CLC4E	May play a role in the response to inflammatory stimuli in peritoneal macrophages. May be involved in immune surveillance processes under transcriptional control of CEBPB	-2.51	0.0165
TLR5	Participates in the innate immune response to microbial agents. Mediates detection of bacterial flagellins. Acts via MyD88 and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response.	-2.56	0.0153
TNR19	Can mediate activation of JNK and NF-kappa-B. May promote caspase-independent cell death	-2.64	0.0131
CBX8	Component of the Polycomb group (PcG) multiprotein PRC1 complex, a complex required to maintain the transcriptionally repressive state of many genes, including Hox genes, throughout development. PcG PRC1 complex acts via chromatin remodeling and modification of histones; it mediates monoubiquitination of histone H2A 'Lys-119', rendering chromatin heritably changed in its expressibility	-2.69	0.0068
E2F3	Transcription activator.	-2.72	0.0116

Gene Name	Gene Description	Fold Change	p Value
KU70	Single stranded DNA-dependent ATP-dependent helicase.	-2.73	0.0114
NDRF	Appears to mediate neuronal differentiation	-2.73	0.0113
RABP2	Cytosolic CRABPs may regulate the access of retinoic acid to the nuclear retinoic acid receptors. CRABP2 may participate in a regulatory feedback mechanism to control the action of retinoic acid on cell differentiation	-2.77	0.001
IKKB	Phosphorylates inhibitors of NF-kappa-B thus leading to the dissociation of the inhibitor/NF-kappa-B complex and ultimately the degradation of the inhibitor. Also phosphorylates NCOA3	-2.78	0.0104
DUSP4	Regulates mitogenic signal transduction by dephosphorylating both Thr and Tyr residues on MAP kinases ERK1 and ERK2	-2.82	1.71E-05
SMAD7	Antagonist of signaling by TGF-beta (transforming growth factor) type 1 receptor superfamily members; has been shown to inhibit TGF-beta (Transforming growth factor) and activin signaling by associating with their receptors thus preventing SMAD2 access.	-2.84	0.0095
DDFL1	Promotes cell proliferation	-2.87	0.009
TCF21	Involved in epithelial-mesenchymal interactions in kidney and lung morphogenesis that include epithelial differentiation and branching morphogenesis. May play a role in the specification or differentiation of one or more subsets of epicardial cell types	-2.88	0.009
TENS1	May be involved in cell migration, cartilage development and in linking signal transduction pathways to the cytoskeleton	-2.93	0.0083
PO3F2	Transcription factor.	-2.94	0.0081
EPO	Erythropoietin is the principal hormone involved in the regulation of erythrocyte differentiation and the maintenance of a physiological level of circulating erythrocyte mass	-2.97	0.0124
AN32A	Implicated in a number of cellular processes, including proliferation, differentiation, caspase-dependent and caspase-independent apoptosis, suppression of transformation (tumor suppressor), inhibition of protein phosphatase 2A, regulation of mRNA trafficking and stability in association with ELAVL1, and inhibition of acetyltransferases as part of the INHAT (inhibitor of histone acetyltransferases) complex	-2.97	0.0039
NFKB2	Appears to have dual functions such as cytoplasmic retention of attached NF-kappa-B proteins and generation of p52 by a cotranslational processing. The proteasome-mediated process ensures the production of both p52 and p100 and preserves their independent function. p52 binds to the kappa-B consensus sequence 5'-GGRNNYYCC-3', located in the enhancer region of genes involved in immune response and acute phase reactions.	-3.02	0.0073
STYK1	Probable tyrosine protein-kinase, which has strong transforming capabilities on a variety of cell lines. When overexpressed, it can also induce tumor cell invasion as well as metastasis in distant organs. May act by activating both MAP kinase and phosphatidylinositol 3'-kinases (PI3K) pathways	-3.10	0.0065
CLD5	Plays a major role in tight junction-specific obliteration of the intercellular space	-3.13	4.38E-05

Gene Name	Gene Description	Fold Change	p Value
FOSL2N	Fos-related antigen 2	-3.13	0.0063
PGM5	Component of adherens-type cell-cell and cell-matrix junctions. Lacks phosphoglucomutase activity	-3.14	0.0062
CIDEA	Activates apoptosis	-3.24	0.0055
ZN703	May function as a transcriptional repressor	-3.26	0.0053
SEM3D	Induces the collapse and paralysis of neuronal growth cones. Could potentially act as repulsive cues toward specific neuronal populations. Binds to neuropilin	-3.27	0.0053
LTBP3	May be involved in the assembly, secretion and targeting of TGFB1 to sites at which it is stored and/or activated. May play critical roles in controlling and directing the activity of TGFB1. May have a structural role in the extra cellular matrix (ECM)	-3.42	4.30E-03
HS2ST	Heparan sulfate 2-O-sulfotransferase	-3.43	0.0043
TRAF1	Adapter protein and signal transducer that links members of the tumor necrosis factor receptor family to different signaling pathways by association with the receptor cytoplasmic domain and kinases. Mediates activation of NF-kappa-B and JNK and is involved in apoptosis. The TRAF1/TRAF2 complex recruits the apoptotic suppressors BIRC2 and BIRC3 to TNFRSF1B/TNFR2	-3.70	0.0032
DOCK4	Involved in regulation of adherens junction between cells. Functions as a guanine nucleotide exchange factor (GEF), which activates Rap1 small GTPase by exchanging bound GDP for free GTP	-4.03	0.0023
PLOD2	Forms hydroxylysine residues in -Xaa-Lys-Gly- sequences in collagens. These hydroxylysines serve as sites of attachment for carbohydrate units and are essential for the stability of the intermolecular collagen cross-links	-4.05	0.0021
DOK3	Docking proteins interact with receptor tyrosine kinases and mediate particular biological responses. DOK3 is a negative regulator of JNK signaling in B-cells through interaction with INPP5D/SHIP. May modulate Abl function	-4.07	0.0022
FAN	Couples the p55 TNF-receptor (TNF-R55 / TNFR1) to neutral sphingomyelinase (N-SMASE). Specifically binds to the N-smase activation domain of TNF-R55. May regulate ceramide production by N-SMASE	-4.81	0.0012
CAD12	Cadherins are calcium dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types	-5.12	0.001
FGF2	The heparin-binding growth factors are angiogenic agents in vivo and are potent mitogens for a variety of cell types in vitro. There are differences in the tissue distribution and concentration of these 2 growth factors	-5.27	0.0008
CAN11	Calcium-regulated non-lysosomal thiol-protease which catalyze limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction	-5.41	0.0008
PSD12	Acts as a regulatory subunit of the 26S proteasome which is involved in the ATP-dependent degradation of ubiquitinated proteins	-6.44	0.0005
NP004849.2	HCO3- transporter, cytoplasmic; HCO3- transporter, eukaryote; HCO3-transporter, c-terminal; Na+/HCO3-transporter	-6.60	0.0004
DEF	Regulates the p53 pathway to control the expansion	-6.98	0.0103

Gene Name	Gene Description	Fold Change	p Value
	growth of digestive organs		
MFSD4	Major facilitator superfamily domain-containing protein 4	-7.32	0.0003
CDC6	Involved in the initiation of DNA replication. Also participates in checkpoint controls that ensure DNA replication is completed before mitosis is initiated	-7.58	0.0003
PLAL1	Shows weak transcriptional activatory activity. Transcriptional regulator of the type 1 receptor for pituitary adenylate cyclase-activating polypeptide	-7.76	0.0079
EYA4	Thought to play a role in transcription regulation during organogenesis through its intrinsic protein phosphatase activity.	-118.41	1.61E-06

The differentially expressed genes might have been co-regulated by a set of common transcription factors.

As a large number of genes showed altered expression, I was curious to determine if some of them might be potentially co-regulated. Accordingly, a transcription factor binding site overrepresentation analysis was performed (Table 3.4). In this analysis, the frequency with which predicted transcription factor binding sites appear within the promoter regions of differentially expressed genes is compared with the frequency expected due to chance alone; a high Z score and low Fisher score suggest that the transcription factor binding site appears more often than can be explained by chance. This analysis revealed several transcription factors that might mediate downstream effects of LL-37 stimulation. Transcription factors that were implicated at multiple timepoints are presented in bold and may merit further investigation; these include Foxd1, Foxd3, FoxI1, HMG-IY, Nkx2-5, Prrx2, and SRY. Foxd1 and HMG-1Y are known to alter NF-kB responses (12, 13), making them expecially interesting targets.

Table 3.4: Transcription factor binding sites overrepresented in the promoter regions of genes showing differential expression after stimulation with 3 µg/ml LL-37.

	Transcription factor	TF class	Z-score	Fisher score
1 hour	ATHB5	HOMEO-ZIP	6.091	2.36E-03
	ELF5	ETS	3.067	2.03E-01
	EMBP1	bZIP	6.34	2.47E-02

	Transcription factor	TF class	Z-score	Fisher score
	FOXD1	FORKHEAD	3.657	4.06E-02
	Foxd3	FORKHEAD	3.994	9.94E-02
	HMG-IY	HMG	4.124	3.52E-02
	MYC-MAX	bHLH-ZIP	5.283	1.73E-02
	NFYA	CAAT-BOX	5.244	2.43E-02
	NR3C1	NUCLEAR RECEPTOR	6.561	5.27E-02
	TLX1-NFIC	HOMEO/CAAT	3.419	7.94E-02
2 hours	Athb-1	HOMEO-ZIP	11.91	8.51E-03
	Broad-complex_1	ZN-FINGER, C2H2	11.33	3.74E-06
	Broad-complex_3	ZN-FINGER, C2H2	12.12	4.42E-03
	FOXD1	FORKHEAD	10.17	2.89E-03
	FOXI1	FORKHEAD	10.94	2.58E-02
	HMG-IY	HMG	12.74	6.82E-03
	Nkx2-5	HOMEO	12.76	5.47E-01
	Prrx2	HOMEO	11.51	2.79E-01
	SOX9	HMG	11.39	1.44E-03
	SRY	HMG	15.73	1.90E-01
4 hours	Broad-complex_4	ZN-FINGER, C2H2	25.37	8.78E-06
	Foxa2	FORKHEAD	15.98	2.53E-03
	Foxd3	FORKHEAD	18.62	1.79E-03
	FOXI1	FORKHEAD	21.85	2.39E-03
	Hunchback	ZN-FINGER, C2H2	21.07	3.89E-04
	IRF1	TRP-CLUSTER	16.29	9.90E-04
	Nkx2-5	HOMEO	19.1	4.70E-02
	Prrx2	HOMEO	21.26	1.47E-03
	Sox5	HMG	22.33	3.43E-06
	SRY	HMG	21.54	4.43E-03

Pathway and gene ontology over-representation analysis suggested that LL-37 selectively activates genes involved in protein synthesis, tissue remodelling, and innate immune responses.

Having identified genes showing altered expression after LL-37 treatment, as well as a number of transcription factors that might be putative effectors of LL-37-mediated downstream effects, I wished to gain insight into the potential biological consequences of altered gene expression after LL-37 treatment. Accordingly, I performed a pathway over-representation analysis (Table 3.5), which is intended to identify signalling pathways

whose component proteins show non-random changes in expression as a result of the experimental treatment. Such a test generates two sets of statistics: a p-value, representing the probability that the number of genes found to shown altered reguation amongst the pathway components occurred by chance alone, and a second p-value corrected for multiple testing, representing the probabilility that the first statistical test resulted from chance alone, given the number of tests being performed. No pathways were found to be significantly overrepresented amongst the differentially regulated genes at any timepoint when results were corrected for multiple testing; accordingly, a corrected p-value of 0.3 was chosen as a cutoff for further analysis. This corrected p-value represents a 30% probability that the obtained results were due to chance alone. No pathways showed downregulation with a corrected p value of less than 0.3. The up-regulated pathways were predominantly involved in mRNA translation and protein synthesis. Notably, almost all results were found to originate from the 4 hour dataset, perhaps as a result of the larger number of differentially expressed genes detected at that timepoint.

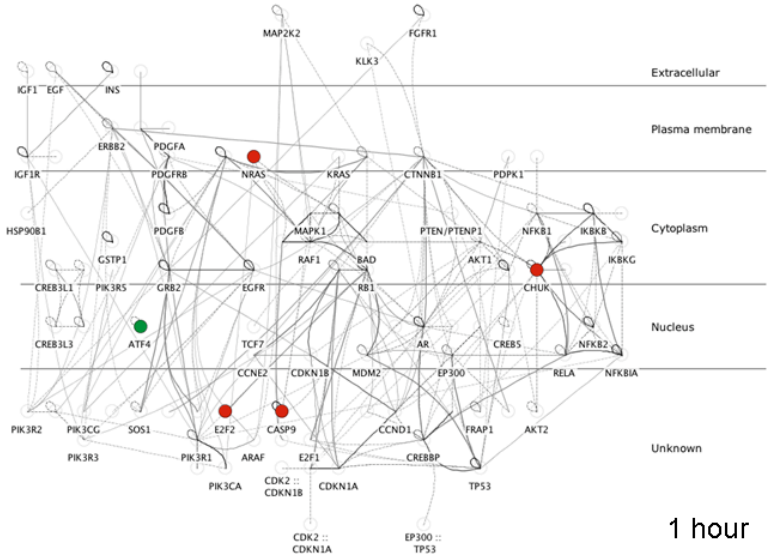
Table 3.5: Results of pathway over-representation analysis.

Condition	InnateDB Pathway ID	Pathway	Genes Up-regulated	p-value	p-Value, corrected for multiple testing
LL37_4hr	1958	L13a-mediated translational silencing of Ceruloplasmin expression	6	0.001	0.158
LL37_4hr	1710	Processing of Intronless Pre-mRNAs	7	0.001	0.167
LL37_4hr	1376	Formation of the Editosome	7	0.003	0.168
LL37_4hr	1319	mRNA Editing: C to U Conversion	7	0.003	0.168
LL37_4hr	1373	Eukaryotic Translation Elongation	5	0.003	0.172
LL37_4hr	1248	Peptide chain elongation	5	0.003	0.172
LL37_4hr	1464	mRNA 3`-end processing	7	0.003	0.175
LL37_4hr	1535	SLBP Dependent Processing of Replication-Dependent Histone	7	0.001	0.178

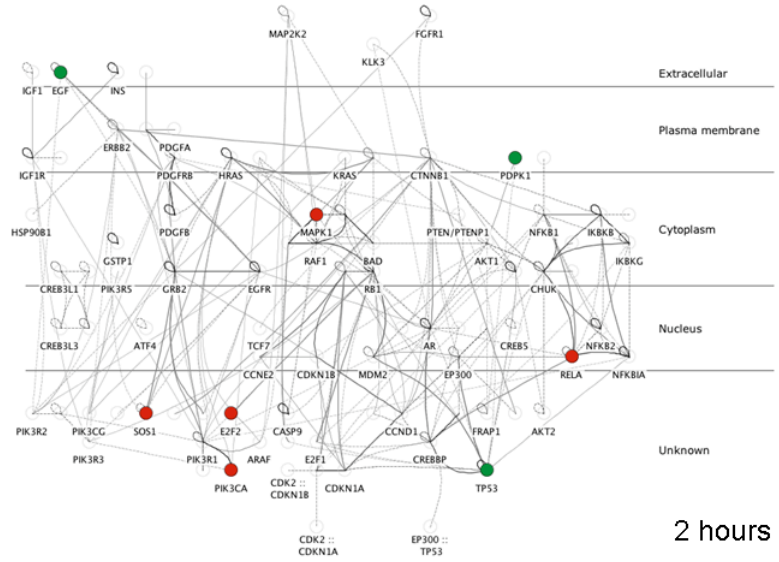
Condition	InnateDB Pathway ID	Pathway	Genes Up-regulated	p-value	p-Value, corrected for multiple testing
		Pre-mRNAs			
LL37_4hr	1630	Cap-dependent Translation Initiation	6	0.002	0.181
LL37_4hr	1923	Formation of a pool of free 40S subunits	6	0.002	0.181
LL37_4hr	1919	GTP hydrolysis and joining of the 60S ribosomal subunit	6	0.002	0.181
LL37_4hr	1527	Ribosomal scanning and start codon recognition	6	0.002	0.181
LL37_4hr	1251	Activation of the mRNA upon binding of the cap-binding complex and eIFs, and subsequent binding to 43S	6	0.002	0.183
LL37_4hr	1921	Viral mRNA Translation	5	0.002	0.184
LL37_4hr	1354	SLBP independent Processing of Histone Pre-mRNAs	7	0.001	0.193
LL37_4hr	1505	Transport of Mature mRNA Derived from an Intronless Transcript	7	0.001	0.198
LL37_4hr	1361	Transport of Mature mRNA derived from an Intron-Containing Transcript	7	0.002	0.201
LL37_4hr	1706	Eukaryotic Translation Termination	5	0.002	0.206
LL37_4hr	474	Ribosome	5	0.001	0.209
LL37_4hr	1655	Cleavage of Growing Transcript in the Termination Region	7	0.004	0.235
LL37_1hr	1980	Formation of PAPS	2	0	0.243
LL37_4hr	1611	Transport of the SLBP Dependant Mature mRNA	7	0.001	0.243
LL37_4hr	973	Skeletal muscle hypertrophy is regulated via akt-mtor pathway	3	0.005	0.278
LL37_4hr	1685	Translation initiation complex formation	4	0.005	0.282
LL37_4hr	1088	Noncanonical Wnt signaling pathway	3	0	0.296
LL37_4hr	544	Prostate cancer	5	0.006	0.299

The results of this analysis suggested that LL-37 treatment might upregulate a number of genes involved in transcription, translation and growth responses. To identify specific genes that might be important to the mode of action of LL-37, some of these over-represented pathways were visualized using Cerebral. The ‘prostate cancer’ pathway aroused my interest; although I am not interested in prostate biology, I hypothesized that

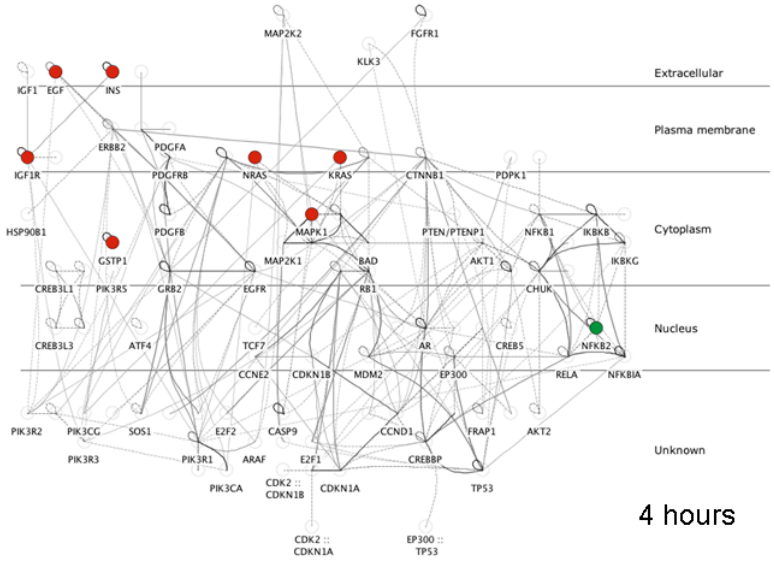
genes important to oncogenesis might also be involved in growth responses and wound repair. Indeed, visualization of the ‘Prostate cancer’ pathway revealed that LL-37 treatment alters keratinocyte expression of growth factors and growth factor receptors (Fig. 3.1), including EFG and insulin. In combination with the apparent upregulation of pathways involved in protein synthesis, this suggested LL-37 might selectively upregulate genes involved in tissue remodelling.



1 hour



2 hours



4 hours

Figure 3.1: Genes within the 'Prostate cancer' pathway showing altered regulation after stimulation with 3 μg/ml LL-37. Red indicates upregulation; green, downregulation. Results were excluded unless the absolute value of the fold change was >1.5 and the result was statistically significant ($p \leq 0.05$).

To further clarify the biological consequences of LL-37 treatment of keratinocytes, I performed a gene ontology over-representation analysis. In this analysis the observed number of genes associated with a specific gene ontology term is compared with the expected number (based on the size of the dataset) in order to identify ontological categories that appear more often than can be explained by chance alone. The results of this analysis (Table 3.6) provided a chronology of the cellular response: at one hour post-stimulation, a variety of genes involved in the determination of tissue morphology were upregulated, as were genes having transcription co-activator activity. At two hours post-stimulation, genes involved in intracellular signalling and cytoskeletal organization were selectively upregulated. Four hours post-stimulation, genes involved in cell differentiation and the innate immune response showed selective enrichment. Overall, these results support a role for low doses of LL-37 in wound repair and the regulation of innate immunity.

Table 3.6: Over-represented gene ontology terms associated with differentially expressed genes at 1, 2, and 4 hours after stimulation with 3 μg/ml LL-37. Notable findings are presented in bold font.

	Biological Process	Observed	Expected	Relative enrichment	p Value
1 hour timepoint	microspike biogenesis	2	0.11	18.18	0.0051
	filopodium formation	2	0.08	25	0.0028
	mitotic recombination	2	0.08	25	0.0028
	detection of chemical stimulus	2	0.13	15.38	0.0065
	detection of calcium ion	2	0.1	20	0.0039
	amine transport	5	0.65	7.69	0.0005
	neutral amino acid transport	2	0.15	13.33	0.0097
	monoamine transport	2	0.08	25	0.0028
	morphogenesis of a branching structure	2	0.04	50	0.0006
	mammary gland development	2	0.04	50	0.0006

	Biological Process	Observed	Expected	Relative enrichment	p Value
	tube morphogenesis	2	0.08	25	0.0028
	gland development	2	0.11	18.18	0.0051
	tube development	2	0.15	13.33	0.0097
	embryonic pattern specification	2	0.15	13.33	0.0097
	regulation of Ras GTPase activity	2	0.1	20	0.0039
	regulation of Rho GTPase activity	2	0.1	20	0.0039
	regulation of Cdc42 GTPase activity	2	0.1	20	0.0039
	Molecular Function				
	transcription coactivator activity	7	2.2	3.18	0.0066
	phospholipase C activity	3	0.37	8.11	0.0059
	phosphoinositide phospholipase C activity	3	0.33	9.09	0.0042
	inositol or phosphatidylinositol phosphodiesterase activity	3	0.33	9.09	0.0042
	amine transporter activity	5	0.73	6.85	0.0008
	monoamine transporter activity	2	0.07	28.57	0.0018
	calcium channel activity	4	0.76	5.26	0.0069
	calcium-activated potassium channel activity	2	0.15	13.33	0.0096
	Cellular Component				
	ruffle	3	0.33	9.09	0.0040
	integral to plasma membrane	31	16.75	1.85	0.0005
	intrinsic to plasma membrane	31	16.89	1.84	0.0006
	plasma membrane part	35	20.34	1.72	0.0009
	plasma membrane	36	24.2	1.49	0.0085
2 hour timepoint	***Biological Process***	Observed	Expected	Relative enrichment	p Value
	signal transduction	35	24.23	1.44	0.0097
	intracellular signaling cascade	17	8.82	1.93	0.0062
	small GTPase mediated signal transduction	9	2.63	3.42	0.0013
	ARF protein signal transduction	2	0.13	15.38	0.0069
	regulation of ARF protein signal transduction	2	0.13	15.38	0.0069
	regulation of small GTPase mediated signal transduction	6	0.95	6.32	0.0004
	regulation of Rho protein signal transduction	4	0.54	7.41	0.0021
	Rho protein signal transduction	4	0.69	5.8	0.0051
	regulation of signal transduction	8	2.57	3.11	0.0041
	cell maturation	2	0.15	13.33	0.0096
	neuron maturation	2	0.06	33.33	0.0013
	neuron remodeling	2	0.02	100	0.0001
	neurogenesis	4	0.69	5.8	0.0049
	generation of neurons	4	0.68	5.88	0.0047
	neuron differentiation	4	0.63	6.35	0.0036

	Biological Process	Observed	Expected	Relative enrichment	p Value
	neuron development	4	0.53	7.55	0.0019
	retrograde vesicle-mediated transport\, Golgi to ER	2	0.11	18.18	0.0053
	cytoskeleton organization and biogenesis	9	2.93	3.07	0.0026
	actin filament-based process	6	1.31	4.58	0.0020
	actin cytoskeleton organization and biogenesis	6	1.18	5.08	0.0012
	actin filament organization	3	0.19	15.79	0.0008
	protein amino acid acylation	2	0.15	13.33	0.0096
	protein amino acid acetylation	2	0.13	15.38	0.0077
	developmental maturation	2	0.15	13.33	0.0096
	Molecular Function				
	potassium channel regulator activity	2	0.12	16.67	0.0067
	cytoskeletal adaptor activity	2	0.05	40	0.0011
	GTPase regulator activity	7	2.13	3.29	0.0055
	guanyl-nucleotide exchange factor activity	7	0.99	7.07	0.0001
	ARF guanyl-nucleotide exchange factor activity	2	0.12	16.67	0.0067
	Ras guanyl-nucleotide exchange factor activity	4	0.56	7.14	0.0024
	Rho guanyl-nucleotide exchange factor activity	4	0.49	8.16	0.0014
	small GTPase regulator activity	6	1.12	5.36	0.0009
	channel or pore class transporter activity	8	2.77	2.89	0.0065
	alpha-type channel activity	8	2.68	2.99	0.0054
	ion channel activity	8	2.46	3.25	0.0032
	anion channel activity	3	0.42	7.14	0.0087
	chloride channel activity	3	0.37	8.11	0.0061
	ion transporter activity	11	4.66	2.36	0.0068
	Cellular Component				
	cell projection	4	0.82	4.88	0.0093
	lamellipodium	3	0.16	18.75	0.0005
	leading edge	3	0.28	10.71	0.0026
	basolateral plasma membrane	3	0.2	15	0.0010
	cell junction	5	1.05	4.76	0.0039
4 hour timepoint	***Biological Process***	Observed	Expected	Relative enrichment	p Value
	response to nutrient	3	0.39	7.69	0.0063
	cell differentiation	24	13.83	1.74	0.0062
	intracellular protein transport	15	7.43	2.02	0.0078
	fructose 2,6-bisphosphate metabolism	2	0.1	20	0.0034
	peptide hormone processing	2	0.1	20	0.0034
	ion transport	28	16.53	1.69	0.0044
	anion transport	10	4.11	2.43	0.0084

	Biological Process	Observed	Expected	Relative enrichment	p Value
	inorganic anion transport	9	3.38	2.66	0.0069
	chloride transport	5	1.02	4.9	0.0034
	localization	77	58.11	1.33	0.0042
	establishment of localization	77	57.55	1.34	0.0032
	innate immune response	6	1.8	3.33	0.0092
	Molecular Function				
	anion binding	5	1.23	4.07	0.0076
	chloride ion binding	5	1.23	4.07	0.0076
	protein homodimerization activity	7	2.14	3.27	0.0056
	fructose-2,6-bisphosphate 2-phosphatase activity	2	0.14	14.29	0.0076
	intramolecular transferase activity	3	0.39	7.69	0.0066
	intramolecular transferase activity\, phosphotransferases	3	0.3	10	0.0030
	6-phosphofructo-2-kinase activity	2	0.09	22.22	0.0031
	phosphotransferase activity\, alcohol group as acceptor	26	15.47	1.68	0.0068
	nitric-oxide synthase regulator activity	2	0.09	22.22	0.0031
	receptor signaling protein activity	10	3.25	3.08	0.0016
	receptor signaling protein serine/threonine kinase signaling protein activity	2	0.12	16.67	0.0051
	transmembrane receptor protein serine/threonine kinase signaling protein activity	2	0.14	14.29	0.0076
	transforming growth factor beta receptor\, cytoplasmic mediator activity	2	0.12	16.67	0.0051
	transforming growth factor beta receptor\, inhibitory cytoplasmic mediator activity	2	0.05	40	0.0005
	chloride channel activity	5	1.18	4.24	0.0064
	voltage-gated chloride channel activity	3	0.44	6.82	0.0091
	ion transporter activity	25	14.82	1.69	0.0075
	anion transporter activity	10	2.76	3.62	0.0005
	iodide transporter activity	2	0.05	40	0.0005
	Cellular Component				
	No terms enriched				

DISCUSSION:

To date, only one microarray experiment has previously been performed on LL-37-stimulated primary keratinocytes and in that experiment a much higher concentration of LL-37 was used (about 50 μg/ml) (2). While the complete results of that experiment are

not publicly available, the published findings suggest that such concentrations result in a marked upregulation of a variety of pro-inflammatory cytokines. In contrast, in our experiment, low concentrations of LL-37 altered the expression of a variety of genes apparently involved in growth responses, tissue remodelling, and RNA and protein processing, indicating a very different response. While these results have yet to be confirmed, they suggest a number of interesting directions for future work.

The results of this microarray should be confirmed by an independent experiment. Ideally this experiment would confirm some of the more interesting results using the more accurate technique qRT-PCR and examine the effects of TLR stimulation, LL-37 stimulation, and combinatorial TLR and LL-37 stimulation, upon genes of interest that showed differential expression in the microarray. This would allow some insight into potential interplay between TLR signalling and LL-37-mediated growth responses. The observation that LL-37 appears to upregulate the expression of a number of growth factors, including EGF and insulin, merits further investigation, as was the exciting observation of effects on genes of innate immunity at 4 hours (DUS9, IFNA1, SMAD6, CARD9, HMGB3, HSPA1, TGFB3, *CLC4E, TLR5,TNR19, RABP2, IKKB, DUSP4, SMAD7, TCF21, NFKB2, STYK1, TRAF1, DOCK4, DOK3, FAN,* although many of these represented a down regulation of innate immunity - genes in italics). Should these effects be confirmed, it would be interesting to investigate their relevance to LL-37-mediated growth and pro- and anti-inflammatory responses.

The results of the transcription factor binding site over-representation analysis also suggest new targets for investigation. Many of the transcription factors identified by the transcription factor binding site overrepresentation analysis have been previously

identified as effectors of wound healing or inflammation. For instance, Foxd1 regulates the activity of NF-AT and NF-κB in T cells (12), while Foxd3 has been shown to be induced by Snail in *Xenopus* embryos (14). HMG-1/Y helps NF-κB to activate transcription (13), and NKx2-5 has been implicated in the regulation of genes involved in bladder responses to wounding and infection (15). Prrx2 plays an important role in the regulation of wound repair by fetal fibroblasts (16), and SRY has been identified as a potential downstream effector of p38 in a previous keratinocyte microarray experiment (17). The potential role of these transcription factors in the mechanism of action of LL-37 merits further investigation.

To complement these studies, this microarray experiment should be repeated in a model of the bronchial epithelium. LL-37 has been shown to stimulate wound-healing responses in bronchial epithelial cells via EGFR activation (18). A comparative analysis of genes showing altered regulation in response to LL-37 in both keratinocytes and bronchial epithelial cells might reveal a conserved set of 'wound response genes' and facilitate the identification of downstream effectors of LL-37-mediated effects. These studies, when complete, should increase our understanding of epithelial wound responses and the interplay between TLR and LL-37-mediated responses in the maintenance of epithelial homeostasis.

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CHAPTER IV

In this thesis, I have demonstrated that low, physiologically relevant doses of the host defence peptide LL-37 can substantially increase the amount of IL-8 released by epithelial cells exposed to pro-inflammatory stimuli. As epithelial concentrations of LL-37 increase in response to infection or wounding (1-5), the ability of LL-37 to boost innate immune responses suggests a potential regulatory role for LL-37 in the epithelial inflammatory response.

Prior to this work, considerable evidence identified LL-37 as both an immunomodulatory agent and an important component of epithelial defence. Administration of LL-37 rescues rats and mice from systemic endotoxaemia (6, 7), suppresses TNF-α production by peripheral blood mononuclear cells in response to lipopolysaccharide (8), and increases cytokine and chemokine production by peripheral blood mononuclear cells in response to IL-1β and GM-CSF (9). In the epithelia, LL-37 has been shown to have a generally pro-inflammatory effect, eliciting the production of IL-8, IL-18, IL-6, IL-10, IP-10, MCP-1, MIP-3α, and RANTES by keratinocytes (10, 11) and the production of IL-8 (6, 12) and IL-6 (Pistolic and Hancock, unpublished data) by bronchial epithelial cells. Studies involving the effects of LL-37 treatment upon epithelial cells, however, have generally sought to replicate *in vivo* concentrations of LL-37 during infection through the use of 20 – 50 μg/ml concentrations of peptide; in inflammation, concentrations of LL-37 have been suggested to increase to as much as 50 μg/ml in the lung (13) and mg/ml concentrations in the skin (14). The work presented in this thesis is novel as it demonstrates that LL-37 can alter epithelial cell responses at concentrations far lower than those thought to be present during inflammation. Indeed, concentrations of

LL-37 exceeding those used in these studies are thought to be present in normal human sweat (15) and airway surfactant (13). This finding has some interesting implications.

One of the great challenges of epithelial immunology is to better understand the mechanisms that regulate differential responses to commensal and pathogenic microbes. Toll-like receptor (TLR) ligands, for instance, are often referred to as Pathogen Associated Molecular Patterns (PAMPs), a name that overlooks the fact that the same molecules are found in a variety of microbial species that do not elicit inflammation. It would be an error to overlook the influence of microbial manipulation of host defence in the maintenance of such immunological cease-fires (16), but the mechanisms used by the body to distinguish friend from foe are of primary interest due to their potential to reveal new targets for therapeutic intervention. My thesis advances this field by revealing a novel mechanism that might allow differential responses to microbes based on epithelial integrity; areas in which the epithelial layer is compromised would be expected to exhibit increased local concentrations of LL-37, which would strongly increase the pro-inflammatory responses of epithelial cells and facilitate a more rapid return to homeostasis.

Accordingly, these findings suggest several avenues of investigation. One goal of future work should be to confirm that LL-37 alters epithelial cell responses *in vivo*. While cultured primary cells are a useful model system, it would be interesting to expand these studies into *ex vivo* and *in vivo* experiments. One potential model system would be the collection of skin samples from volunteers using a punch biopsy; excised skin could then be maintained in tissue culture medium and stimulated with TLR ligands or live bacteria in the presence and absence of LL-37. Transcriptional profiling of the stimulated cells

should provide additional evidence that LL-37 regulates epithelial inflammation *in vivo*, and might also provide some evidence as to the mechanisms underlying those effects. Complementary studies could be pursued in a mouse model. Mice deficient in the murine LL-37 homologue CRAMP are susceptible to skin infection by Group A Streptococci (17), a finding which was attributed to the purported 'direct killing' ability of host defence peptides. As LL-37 is thought to lack direct antimicrobial activity at physiological salt concentrations (18), it would be exceedingly interesting to study the CRAMP knockout mouse in order to determine the actual mechanism underlying its propensity to skin infection. For instance, do increased concentrations of CRAMP alter the responses of murine keratinocytes to bacteria? If so, it would be interesting to examine potential differences in cytokine responses elicited from wild-type and CRAMP-deficient epithelial cells by pro-inflammatory stimuli. One might also study the ability of exogenous CRAMP or LL-37 to alter the innate immune responses of the knockout mouse. For instance, could topical application of a host defence peptide increase immune cell recruitment and inflammation in infected epithelial tissues? The results of such studies would dramatically expand our understanding of the role of host defence peptides in epithelial inflammation.

Future studies should also address the mechanism by which LL-37 modulates its immunomodulatory effects in epithelial cells. While it would be ideal to identify a specific receptor for LL-37, a number of lines of evidence suggest that such a receptor may not exist. For instance, in the lung epithelial cell line A549 LL-37 is rapidly taken up and translocated to the perinuclear region (19), suggesting that interaction with a cell-surface receptor may be less relevant. Similarly, the ability of a D-amino-acid form of LL-37 to elicit IL-8 release from keratinocytes (20) suggests that LL-37 does not mediate

its effects via a traditional 'lock and key' receptor. Two lines of investigation accordingly suggest themselves. First, I am interested in the possibility that LL-37 mediates its wide variety of effects via the general activation of cellular stress response pathways. LL-37 is cytotoxic to human cells when present in high concentrations, and I hypothesize that it causes local alterations in membrane chemistry even when at tolerated concentrations. Local disruptions in membrane integrity might activate diverse stress response pathways, which would then mediate downstream responses such as cell proliferation, migration and angiogenesis by provoking the compensatory production of growth factors. Transitory alteration of membrane integrity might also serve as a 'danger signal'; the release of cytoplasmic contents into the extracellular environment might alter the responses of neighbouring cells to pro-inflammatory stimuli. This hypothesis would be difficult to test; however transcriptional profiling of epithelial cell responses to LL-37 might reveal stress-response pathways that could be further investigated as host defence peptide targets. A second line of investigation would address the possibility that LL-37 can mediate its immunomodulatory effects by altering the binding of TLR ligands to TLR receptors. I am intrigued by the ability of LL-37/poly(I:C) co-stimulation to provoke rapid IL-8 and extreme cytotoxicity in bronchial epithelial cells, a phenomenon which merits further investigation as a potential anti-viral defence mechanism *in vivo*. The activation of TLR3 allows innate immune responses to a number of viral infections (21); as LL-37 concentrations are expected to increase at sites of infection, the increases in IL-8 release and cytotoxicity observed when bronchial epithelial cells were co-stimulated with LL-37 and poly(I:C) might represent an adaptation to limit viral replication and spread. It would be interesting to investigate these responses are in fact TLR-dependent, or instead rely on other pathways, such as the RIG-1-like helicases, which also detect dsRNA. Similarly, as LL-37 is able to form complexes with human

DNA and activate plasmacytoid dendritic cells (22), it would be interesting to investigate whether the presence of LL-37 could allow human DNA to elicit an inappropriate inflammatory response in the epithelia—were this to prove the case, it might have profound implications for the etiology of autoimmune conditions of the skin such as psoriasis. An improved understanding of the dramatic response observed to dsRNA and peptide might inform the design of future immunostimulants, or be potentially relevant to the design of vaccine adjuvants.

As low doses of peptide, in conjunction with appropriate additional stimuli, can alter cytokine production by epithelial cells, it would also be interesting to investigate the ability of host defense peptides to alter TLR-mediated growth responses. In the murine colonic epithelium, TLR stimulation is necessary for the maintenance of epithelial repair and homeostasis; mice deficient in TLR signaling show dramatically worsened symptoms in a colitis model (23). As LL-37 is a known growth factor for a variety of epithelial cell types (10, 24, 25), it would be interesting to investigate potential interactions between LL-37 and TLR ligands in the alteration of epithelial growth responses. Previously, a synthetic peptide based on the sequence of LL-37 has been shown to alter the proliferative responses of bronchial epithelial cells to LPS and LTA (26). Accordingly, I suggest that the microarray analysis presented in Chapter III be continued, as it might allow the identification of proliferative pathways activated in epithelial cells by LL-37 treatment. Once genes of interest have been identified, qPCR could be used to determine if they showed altered expression in cells that were stimulated with both LL-37 and a TLR ligand. These studies might increase our understanding of how host defense peptides facilitate epithelial growth responses and speed the return to tissue homeostasis.

Ultimately, the goal of these studies is the identification of targets for therapeutic intervention. An improved understanding of host defense peptide-mediated epithelial immunomodulation might eventually translate into the development of both novel immunostimulants and anti-inflammatories. For instance, if LL-37 can complex with nucleic acids and provoke a strong immune response, perhaps syntheic peptide/nucleic acid complexes might be useful therapeutic agents for papillomaviral skin infection, which typically does not trigger inflammation. Alternately, understanding the mechanism of action by which LL-37 alters pro-inflammatory responses in the epithelia might allow for the design of therapies to block those processes in autoimmune conditions such as psoriasis. In order to proceed, however, a better understanding of the interaction between LL-37 and other regulators of inflammation is required. For instance, 1'25'-dihydroxy Vitamin D (calcitriol), an important signaling molecule in the epithelia, strongly induces increased expression of the LL-37 precursor hCAP-18 in keratinocytes (27), yet Vitamin D3 analogues are typically used as psoriasis therapies, where they reduce inappropriate inflammation (28). Given these apparently contradictory indications, it would be interesting to investigate the effects of Vitamin D3 treatment on the ability of LL-37 to alter epithelial cell responses to pro-inflammatory stimuli. Such studies might improve our understanding of the regulation of epithelial inflammation.

To conclude, the skin and bronchial epithelium are complex immune organs. It is to be hoped that an improved understanding of the role of host defense peptides in epithelial innate immunity will someday allow the design of new strategies both to boost innate immunity in order to resolve infections, and to restrain it when it goes awry.

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APPENDIX

SUPPLEMENTARY TABLES:

Supplementary Table 7: Differentially expressed genes 1 hour post-stimulation with 3 μg/ml LL-37.

Gene Name	Gene Description	Fold Change	p Value
SEM4A	Inhibits axonal extension by providing local signals to specify territories inaccessible for growing axons	4.70	0.0023
E2F2	Transcription activator that binds DNA cooperatively with DP proteins through the E2 recognition site, 5'-TTTC[CG]CGC-3' found in the promoter region of a number of genes whose products are involved in cell cycle regulation or in DNA replication. The DRTF1/E2F complex functions in the control of cell-cycle progression from g1 to s phase. E2F-2 binds specifically to RB1 protein, in a cell-cycle dependent manner	4.33	0.0029
KCE1L	Potassium voltage-gated channel subfamily E member 1-like protein; AMME syndrome candidate gene 2 protein; AMMECR2 protein	3.94	0.0037
NP_073590.2	Acc:NP_073590]; S100P binding protein isoform a [Source:RefSeq_peptide	3.91	0.0038
CAD19	Cadherins are calcium dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types	3.55	0.005
NP_659002.1	Antifreeze protein, type I; Pollen allergen Poa pIX/Phl pVI, C-terminal; Zinc finger, CCCH-type	3.48	0.0054
ITA4	Integrins alpha-4/beta-1 (VLA-4) and alpha-4/beta-7 are receptors for fibronectin. They recognize one or more domains within the alternatively spliced CS-1 and CS-5 regions of fibronectin. They are also receptors for VCAM1. Integrin alpha-4/beta-1 recognizes the sequence Q-I-D-S in VCAM1. Integrin alpha-4/beta-7 is also a receptor for MADCAM1. It recognizes the sequence L-D-T in MADCAM1. On activated endothelial cells integrin VLA-4 triggers homotypic aggregation for most VLA-4-positive leukocyte cell lines. It may also participate in cytolytic T-cell interactions with target cells	3.44	0.0056
SCUB2	Signal peptide, CUB and EGF-like domain-containing protein 2 precursor; Protein CEGP1	3.36	0.006
AKT2	General protein kinase capable of phosphorylating several known proteins	3.35	0.006
ZN566	May be involved in transcriptional regulation	3.03	0.0203

Gene Name	Gene Description	Fold Change	p Value
KCMB1	Regulatory subunit of the calcium activated potassium KCNMA1 (maxiK) channel. Modulates the calcium sensitivity and gating kinetics of KCNMA1, thereby contributing to KCNMA1 channel diversity. Increases the apparent Ca(2+)/voltage sensitivity of the KCNMA1 channel. It also modifies KCNMA1 channel kinetics and alters its pharmacological properties. It slows down the activation and the deactivation kinetics of the channel. Acts as a negative regulator of smooth muscle contraction by enhancing the calcium sensitivity to KCNMA1. Its presence is also a requirement for internal binding of the KCNMA1 channel opener dehydrosoyasaponin I (DHS-1) triterpene glycoside and for external binding of the agonist hormone 17-beta-estradiol (E2). Increases the binding activity of charybdotoxin (CTX) toxin to KCNMA1 peptide blocker by increasing the CTX association rate and decreasing the dissociation rate	3.00	0.0087
RNF40	E3 ubiquitin ligase protein that mediates monoubiquitination of 'Lys-120' of histone H2B. H2B 'Lys-120' ubiquitination gives a specific tag for epigenetic transcriptional activation and is also prerequisite for histone H3 'Lys-4' and 'Lys-79' methylation. Forms a ubiquitin ligase complex in cooperation with the E2 enzyme UBE2E1/UBCH6. It thereby plays a central role in histone code and gene regulation. Required for transcriptional activation of Hox genes	2.95	0.0091
Q8NHH4	Pistil-specific extensin-like protein; Proline-rich region	2.95	0.0092
IRK15	Inward rectifier potassium channels are characterized by a greater tendency to allow potassium to flow into the cell rather than out of it. Their voltage dependence is regulated by the concentration of extracellular potassium; as external potassium is raised, the voltage range of the channel opening shifts to more positive voltages. The inward rectification is mainly due to the blockage of outward current by internal magnesium	2.86	0.0102
NP_000924.2	The production of the second messenger molecules diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) is mediated by activated phosphatidylinositol-specific phospholipase C enzymes. This form has a role in retina signal transduction	2.83	0.0106
CASP	May be involved in intra-Golgi retrograde transport	2.78	0.0064
ZN214	May be involved in transcriptional regulation	2.78	0.0112
TTBK1	Serine/threonine kinase which is able to phosphorylate TAU on serine, threonine and tyrosine residues. Induces aggregation of TAU	2.77	0.0113
ONEC2	Transcriptional activator. Activates the transcription of a number of liver genes such as HNF3B	2.77	0.0114
FGD1	Activates CDC42, a member of the Ras-like family of Rho-and Rac proteins, by exchanging bound GDP for free GTP. Plays a role in regulating the actin cytoskeleton and cell shape	2.76	0.0115
K1199	May be involved in hearing	2.75	0.0024

Gene Name	Gene Description	Fold Change	p Value
TDGF1	Could play a role in the determination of the epiblastic cells that subsequently give rise to the mesoderm	2.71	0.0123
PARC	Cytoplasmic anchor protein in p53-associated protein complex. Regulates the subcellular localization of p53 and subsequent function	2.67	0.013
BSN	Is thought to be involved in the organization of the cytomatrix at the nerve terminals active zone (CAZ) which regulates neurotransmitter release. Seems to act through binding to ERC2/CAST1. Essential in regulated neurotransmitter release from a subset of brain glutamatergic synapses. Involved in the formation of the retinal photoreceptor ribbon synapses	2.66	0.013
EST1A	Component of the telomerase ribonucleoprotein (RNP) complex that is essential for the replication of chromosome termini. May have a general role in telomere regulation. Promotes in vitro the ability of TERT to elongate telomeres. Overexpression induces telomere uncapping, chromosomal end-to-end fusions (telomeric DNA persists at the fusion points) and did not perturb TRF2 telomeric localization. Dephosphorylates RENT1. Plays a role in nonsense-mediated mRNA decay. May function as endonuclease. Degrades single-stranded RNA (ssRNA), but not ssDNA or dsRNA	2.65	0.0132
NP_064614.2	PR domain-containing protein 11	2.61	0.0141
SRR	Catalyzes the synthesis of D-serine from L-serine	2.60	0.0142
O15420	Acc:O15420]; CAGH1 alternate open reading frame. [Source:Uniprot/SPTREMBL	2.57	0.0149
B3GN2	Catalyzes the initiation and elongation of poly-N-acetyllactosamine chains	2.54	0.0155
ATG4D	Cysteine protease required for autophagy, which cleaves the C-terminal part of either MAP1LC3, GABARAPL2 or GABARAP, allowing the liberation of form I. A subpopulation of form I is subsequently converted to a smaller form (form II). Form II, with a revealed C-terminal glycine, is considered to be the phosphatidylethanolamine (PE)-conjugated form, and has the capacity for the binding to autophagosomes	2.53	0.0157
TMPS5	Transmembrane protease, serine 5; Spinesin	2.52	0.016
NP_060105.3	SET	2.45	0.0179
S10A6	Protein S100-A6; S100 calcium-binding protein A6; Calcyclin; Prolactin receptor-associated protein; PRA; Growth factor-inducible protein 2A9; MLN 4	2.45	0.0237
RASN	Ras proteins bind GDP/GTP and possess intrinsic GTPase activity	2.45	0.0183
MAST1	Appears to link the dystrophin/utrophin network with microtubule filaments via the syntrophins. Phosphorylation of DMD or UTRN may modulate their affinities for associated proteins	2.43	0.0183

Gene Name	Gene Description	Fold Change	p Value
HEY1	Downstream effector of Notch signaling which may be required for cardiovascular development. Transcriptional repressor which binds preferentially to the canonical E box sequence 5'-CACGTG-3'. Represses transcription by the cardiac transcriptional activators GATA4 and GATA6	2.43	0.0185
NPBW2	Interacts specifically with a number of opioid ligands. Receptor for neuropeptides B and W, which may be involved in neuroendocrine system regulation, food intake and the organization of other signals	2.42	0.0187
NP_001018068.1	Basic helix-loop-helix dimerisation region bHLH	2.42	0.0187
SEL1L	May play a role in Notch signaling (By similarity). May be involved in the endoplasmic reticulum quality control (ERQC) system also called ER-associated degradation (ERAD) involved in ubiquitin-dependent degradation of misfolded endoplasmic reticulum proteins	2.41	0.019
TCRG1	Transcription factor that binds RNA polymerase II and inhibits the elongation of transcripts from target promoters. Regulates transcription elongation in a TATA box-dependent manner. Necessary for TAT-dependent activation of the human immunodeficiency virus type 1 (HIV-1) promoter	2.40	0.0192
CCG4	Thought to stabilize the calcium channel in an inactivated (closed) state	2.39	0.0196
ZN598	Zinc finger protein 598	2.39	0.0097
ELN	Major structural protein of tissues such as aorta and nuchal ligament, which must expand rapidly and recover completely. Molecular determinant of the late arterial morphogenesis, stabilizing arterial structure by regulating proliferation and organization of vascular smooth muscle	2.37	0.0201
NEO1	May be involved as a regulatory protein in the transition of undifferentiated proliferating cells to their differentiated state. May also function as a cell adhesion molecule in a broad spectrum of embryonic and adult tissues	2.36	0.0207
ADDB	Membrane-cytoskeleton-associated protein that promotes the assembly of the spectrin-actin network. Binds to calmodulin. Calmodulin binds preferentially to the beta subunit	2.35	0.0208
ACE2	Carboxypeptidase which converts angiotensin I to angiotensin 1-9, a peptide of unknown function, and angiotensin II to angiotensin 1-7, a vasodilator. Also able to hydrolyze apelin-13 and dynorphin-13 with high efficiency. May be an important regulator of heart function. In case of human coronaviruses SARS and HCoV-NL63 infections, serve as functional receptor for the spike glycoprotein of both coronaviruses	2.35	0.0211
NM_014069.1	Proline-rich region	2.34	0.0214
ABHD9	Abhydrolase domain-containing protein 9 precursor	2.33	0.0217
ZN225	May be involved in transcriptional regulation	2.33	0.0217
NP_056368.1	Pleckstrin-like	2.33	0.0218

Gene Name	Gene Description	Fold Change	p Value
AICDA	RNA-editing deaminase involved in somatic hypermutation, gene conversion, and class-switch recombination. Required for several crucial steps of B-cell terminal differentiation necessary for efficient antibody responses	2.31	0.0223
NP_079093.2	ADAM-TS Spacer 1	2.31	0.0224
DPOLQ	Could be involved in the repair of interstrand cross-links	2.31	0.0226
TLX3	T-cell leukemia homeobox protein 3; Homeobox protein Hox-11L2	2.30	0.0228
JAD1C	Histone demethylase that specifically demethylates 'Lys-4' of histone H3, thereby playing a central role in histone code. Does not demethylate histone H3 'Lys-9', H3 'Lys-27', H3 'Lys-36', H3 'Lys-79' or H4 'Lys-20'. Demethylates trimethylated and dimethylated but not monomethylated H3 'Lys-4'. Participates in transcriptional repression of neuronal genes by recruiting histone deacetylases and REST at neuron-restrictive silencer elements	2.29	0.0232
MKL2	Acts as a transcriptional coactivator of serum response factor (SRF). Required for skeletal myogenic differentiation	2.29	0.0233
NP_060478.2	Zinc finger, C2H2-subtype; Zinc finger, C2H2-type	2.29	0.0233
CRSP3	Plays a role in transcriptional coactivation	2.27	0.0149
Q96MH6-2	Acc:NP_689630]; transmembrane protein 68 [Source:RefSeq_peptide	2.26	0.032
ASB3	Ankyrin repeat and SOCS box protein 3; ASB-3	2.26	0.0245
ARNT	Required for activity of the Ah (dioxin) receptor. This protein is required for the ligand-binding subunit to translocate from the cytosol to the nucleus after ligand binding. The complex then initiates transcription of genes involved in the activation of PAH procarcinogens. The heterodimer with HIF1A or EPAS1/HIF2A functions as a transcriptional regulator of the adaptive response to hypoxia	2.26	0.0033
TMG2	Transmembrane gamma-carboxyglutamic acid protein 2 precursor; Proline-rich Gla protein 2; Proline-rich gamma-carboxyglutamic acid protein 2	2.25	0.0248
Q8IYI7	Component of the COPII coat, that covers ER-derived vesicles involved in transport from the endoplasmic reticulum to the Golgi apparatus. COPII acts in the cytoplasm to promote the transport of secretory, plasma membrane, and vacuolar proteins from the endoplasmic reticulum to the Golgi complex	2.25	0.0251
GSTT1	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles. Acts on 1,2-epoxy-3-(4-nitrophenoxy)propane, phenethylisothiocyanate 4-nitrobenzyl chloride and 4-nitrophenethyl bromide. Displays glutathione peroxidase activity with cumene hydroperoxide	2.23	0.0258
ENSG00000211744	Immunoglobulin V-set; Immunoglobulin-like	2.23	0.0259

Gene Name	Gene Description	Fold Change	p Value
NDN	Growth suppressor that facilitates the entry of the cell into cell cycle arrest. Functionally similar to the retinoblastoma protein it binds to and represses the activity of cell-cycle-promoting proteins such as SV40 large T antigen, adenovirus E1A, and the transcription factor E2F. Necdin also interacts with p53 and works in an additive manner to inhibit cell growth. Functions also as transcription factor and binds directly to specific guanosine-rich DNA sequences	2.23	0.003
SLC14A1	Specialized low-affinity urea transporter. Mediates urea transport in erythrocytes	2.22	0.0265
SGCD	Component of the sarcoglycan complex, a subcomplex of the dystrophin-glycoprotein complex which forms a link between the F-actin cytoskeleton and the extracellular matrix	2.20	0.0273
NP_940905.2	Blood group Rhesus C/E and D polypeptide; Calcium-activated BK potassium channel, alpha subunit; EAG/ELK/ERG potassium channel; Ion transport 2	2.20	0.0275
NP_001546.2	Immunoglobulin; Immunoglobulin V-set; Immunoglobulin-like; Interleukin-1 receptor, type I and type II; Vascular endothelial growth factor receptor, VEGFR, N-terminal	2.19	0.028
Q6NUT1	Eukaryotic translation initiation factor 4E (eIF-4E)	2.18	0.0282
YTDC2	YTH domain-containing protein 2	2.18	0.0285
LIPG	Gastric triacylglycerol lipase precursor; Gastric lipase; GL	2.18	0.0285
KCNB1	Mediates the voltage-dependent potassium ion permeability of excitable membranes. Channels open or close in response to the voltage difference across the membrane, letting potassium ions pass in accordance with their electrochemical gradient	2.17	0.0083
Q5VYN8	Regulator of G protein signalling	2.17	0.0289
GLCI1	Glucocorticoid-induced transcript 1 protein	2.17	0.029
FGD2	May activate CDC42, a member of the Ras-like family of Rho- and Rac proteins, by exchanging bound GDP for free GTP. May play a role in regulating the actin cytoskeleton and cell shape	2.16	0.0297
ST5	May be involved in cytoskeletal organization and tumorgenicity. Isoform 1 seems to be involved in a signaling transduction pathway leading to activation of MAPK1/ERK2. Isoform 3 may block ERK2 activation stimulated by ABL1. Isoform 3 may alter cell morphology and cell growth	2.15	0.03
GLI4	Zinc finger protein GLI4; Krueppel-related zinc finger protein 4; Protein HKR4	2.15	0.0302
PHF20	Possible transcription factor	2.15	0.0303
HNRPM	Pre-mRNA binding protein in vivo, binds avidly to poly(G) and poly(U) RNA homopolymers in vitro. Involved in splicing. Acts as a receptor for carcinoembryonic antigen in Kupffer cells, may initiate a series of signaling events leading to tyrosine phosphorylation of proteins and induction of IL-1 alpha, IL-6, IL-10 and tumor necrosis factor alpha cytokines	2.14	0.0309

Gene Name	Gene Description	Fold Change	p Value
Q6ZS65	Acc:NP_001009909]; leucine zipper protein 2 [Source:RefSeq_peptide	2.14	0.0309
Q96MM7-2	Heparan sulphate 6-sulfotransferase	2.13	0.031
VPP4	Part of the proton channel of the V-ATPase that is involved in normal vectorial acid transport into the urine by the kidney	2.11	0.0129
CCNF	Likely to be involved in the control of the cell cycle during S phase and G2	2.11	0.0325
ZP3	The mammalian zona pellucida, which mediates species-specific sperm binding, induction of the acrosome reaction and prevents post-fertilization polyspermy, is composed of three to four glycoproteins, ZP1, ZP2, ZP3, and ZP4. ZP3 is essential for sperm binding and zona matrix formation	2.11	0.0328
KREM1	Receptor for Dickkopf protein. Cooperates with Dickkopf to block Wnt/beta-catenin signaling	2.09	0.0342
EXOC5	Component of the exocyst complex involved in the docking of exocystic vesicles with fusion sites on the plasma membrane	2.08	0.0101
Q5VZE3	Acc:Q8NBJ4]; Golgi phosphoprotein 2 (Golgi membrane protein GP73). [Source:Uniprot/SWISSPROT	2.07	0.0351
SMAD6	Antagonist of signaling by TGF-beta (transforming growth factor) type 1 receptor superfamily members; has been shown to inhibit selectively BMP (bone morphogenetic proteins) signaling by competing with the co-SMAD SMAD4 for receptor-activated SMAD1. SMAD6 is an inhibitory SMAD (I-SMAD) or antagonistic SMAD. Binds to regulatory elements in target promoter regions	2.07	0.0355
RAD50	Component of the MRN complex, which plays a central role in double-strand break (DSB) repair, DNA recombination, maintenance of telomere integrity and meiosis. The complex possesses single-strand endonuclease activity and double-strand-specific 3'-5' exonuclease activity, which are provided by MRE11A. RAD50 may be required to bind DNA ends and hold them in close proximity. This could facilitate searches for short or long regions of sequence homology in the recombining DNA templates, and may also stimulate the activity of DNA ligases and/or restrict the nuclease activity of MRE11A to prevent nucleolytic degradation past a given point. The complex may also be required for DNA damage signaling via activation of the ATM kinase. In telomeres the MRN complex may modulate t-loop formation	2.06	0.0112
TAAR8	Orphan receptor. Could be a receptor for trace amines. Trace amines are biogenic amines present in very low levels in mammalian tissues. Although some trace amines have clearly defined roles as neurotransmitters in invertebrates, the extent to which they function as true neurotransmitters in vertebrates has remained speculative. Trace amines are likely to be involved in a variety of physiological functions that have yet to be fully understood	2.06	0.0349

Gene Name	Gene Description	Fold Change	p Value
FKB1B	Associates with the ryanodine receptor (RYR-2) in cardiac muscle sarcoplasmic reticulum and may play a unique physiological role in excitation-contraction coupling in cardiac muscle. There are four molecules of FKBP12.6 per heart muscle RYR. Has the potential to contribute to the immunosuppressive and toxic effects of FK506 and rapamycin. PPIases accelerate the folding of proteins. It catalyzes the cis-trans isomerization of proline imidic peptide bonds in oligopeptides	2.06	0.0364
SMC2	Central component of the condensin complex, a complex required for conversion of interphase chromatin into mitotic-like condense chromosomes. The condensin complex probably introduces positive supercoils into relaxed DNA in the presence of type I topoisomerases and converts nicked DNA into positive knotted forms in the presence of type II topoisomerases	2.06	0.0364
CTBP2	Corepressor targeting diverse transcription regulators	2.05	0.037
RAP2B	Ras-related protein Rap-2b precursor	2.05	0.0371
NP_061889.1	Eggshell protein; Intermediate filament protein; Keratin, type I	2.04	0.0375
F104B	Protein FAM104B	2.04	0.0377
Q9Y2I9	Peptidase M14, carboxypeptidase A; RabGAP/TBC	2.03	0.0384
PI2R	Receptor for prostacyclin (prostaglandin I2 or PGI2). The activity of this receptor is mediated by G(s) proteins which activate adenylate cyclase	2.03	0.0387
Q9BTA9-2	WW domain-containing adapter protein with coiled-coil	2.00	0.0055
PSYR	Receptor for the glycosphingolipid psychosine (PSY) and several related glycosphingolipids. May have a role in activation-induced cell death or differentiation of T-cells	1.99	0.0416
ASPX	Acrosomal protein SP-10 precursor; Acrosomal vesicle protein 1	1.99	0.0417
VMAT1	Involved in the vesicular transport of biogenic amines	1.99	0.0423
NP_001010984.1	Peptidase M, neutral zinc metallopeptidases, zinc-binding site	1.98	0.0425
EPDR1	Mammalian ependymin-related protein 1 precursor; MERP-1; UCC1 protein	1.98	0.0483
IFT74	Intraflagellar transport 74 homolog; Coiled-coil domain-containing protein 2; Capillary morphogenesis protein 1; CMG-1	1.98	0.0098
RAE1L	Binds mRNA. May function in nucleocytoplasmic transport and in directly or indirectly attaching cytoplasmic mRNPs to the cytoskeleton	1.97	0.0033
CCDC13	Coiled-coil domain-containing protein 13	1.96	0.045
GRP75	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone	1.95	0.0454
ZN434	May be involved in transcriptional regulation	1.95	0.0454
FRGL	Putative FRG1-like protein C20orf80	1.95	0.0455

Gene Name	Gene Description	Fold Change	p Value
ENP1	In the nervous system, could hydrolyze ATP and other nucleotides to regulate purinergic neurotransmission. Could also be implicated in the prevention of platelet aggregation. Hydrolyzes ATP and ADP equally well	1.95	0.0456
TRI65	Tripartite motif-containing protein 65	1.95	0.0462
EST1A	Component of the telomerase ribonucleoprotein (RNP) complex that is essential for the replication of chromosome termini. May have a general role in telomere regulation. Promotes in vitro the ability of TERT to elongate telomeres. Overexpression induces telomere uncapping, chromosomal end-to-end fusions (telomeric DNA persists at the fusion points) and did not perturb TRF2 telomeric localization. Dephosphorylates RENT1. Plays a role in nonsense-mediated mRNA decay. May function as endonuclease. Degrades single-stranded RNA (ssRNA), but not ssDNA or dsRNA	1.94	0.0465
PELI1	Scaffold protein involved in the IL-1 signaling pathway via its interaction with the complex containing IRAK kinases and TRAF6. Required for NF-kappa-B activation and IL-8 gene expression in response to IL-1	1.94	0.0469
PSN2	Probable catalytic subunit of the gamma-secretase complex, an endoprotease complex that catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors and APP (beta-amyloid precursor protein). Requires the other members of the gamma-secretase complex to have a protease activity. May play a role in intracellular signaling and gene expression or in linking chromatin to the nuclear membrane. May function in the cytoplasmic partitioning of proteins	1.93	0.0479
PIGZ	Mannosyltransferase involved in glycosylphosphatidylinositol-anchor biosynthesis. Transfers a fourth mannose to some trimannosyl-GPIs during GPI precursor assembly. The presence of a fourth mannose in GPI is facultative and only scarcely detected, suggesting that it only exists in some tissues	1.93	0.0217
NP_653217.1	Proline-rich region	1.93	0.0274
USP6	Has an ATP-independent isopeptidase activity, cleaving at the C-terminus of the ubiquitin moiety. In vitro, isoform 2, but not isoform 3, shows deubiquitinating activity	1.92	0.0487
SYNP2	Has an actin-binding and actin-bundling activity	1.92	0.0489
PP1RA	Inhibitor of PPP1CA and PPP1CC phosphatase activities. Has inhibitory activity on PPP1CA only when phosphorylated. Binds to mRNA, single-stranded DNA (ssDNA), poly(A) and poly(G) homopolymers	1.92	0.049
NP_002411.3	Ion transport	1.91	0.0191
NP_056993.2	Ribosomal protein S14	1.90	0.0087
DNPEP	Likely to play an important role in intracellular protein and peptide metabolism	1.90	0.0086

Gene Name	Gene Description	Fold Change	p Value
BCAT2	Catalyzes the first reaction in the catabolism of the essential branched chain amino acids leucine, isoleucine, and valine. May also function as a transporter of branched chain alpha-keto acids	1.90	0.0316
H2B2E	Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling	1.90	0.0215
TBCA	Tubulin-folding protein; involved in the early step of the tubulin folding pathway	1.89	0.0084
Q8NBT7	Acc:Q8NBT7]; CDNA FLJ90757 fis, clone SKNMC1000014 (FLJ90757 protein). [Source:Uniprot/SPTREMBL	-1.92	0.0099
S12A5	Mediates electroneutral potassium-chloride cotransport in mature neurons. Transport occurs under isotonic conditions, but is activated 20-fold by cell swelling. Important for Cl(-) homeostasis in neurons	-1.93	0.0486
ITBA1	Transmembrane protein 187; Protein ITBA1	-1.93	0.0228
NP_060170.1	DH; cAMP/cGMP-dependent protein kinase	-1.94	0.0471
CA021	Uncharacterized protein C1orf21; Cell proliferation-inducing gene 13 protein	-1.94	0.0471
PTN18	Differentially dephosphorylate autophosphorylated tyrosine kinases which are known to be overexpressed in tumor tissues	-1.94	0.0469
CG034	Uncharacterized protein C7orf34 precursor; MSSP-binding protein CTM-1	-1.94	0.0203
MKRN3	Makorin-3; Zinc finger protein 127; RING finger protein 63	-1.94	0.0464
OCRL	Converts phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 4-phosphate. Also converts inositol 1,4,5-trisphosphate to inositol 1,4-bisphosphate and inositol 1,3,4,5-tetrakisphosphate to inositol 1,3,4-trisphosphate. May function in lysosomal membrane trafficking by regulating the specific pool of phosphatidylinositol 4,5-bisphosphate that is associated with lysosomes	-1.94	0.0414
IBP5	IGF-binding proteins prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth promoting effects of the IGFs on cell culture. They alter the interaction of IGFs with their cell surface receptors	-1.95	0.046
GATA1	Transcriptional activator which probably serves as a general switch factor for erythroid development. It binds to DNA sites with the consensus sequence [AT]GATA[AG] within regulatory regions of globin genes and of other genes expressed in erythroid cells	-1.95	0.043
DUSP4	Regulates mitogenic signal transduction by dephosphorylating both Thr and Tyr residues on MAP kinases ERK1 and ERK2	-1.95	0.0392

Gene Name	Gene Description	Fold Change	p Value
GNS	N-acetylglucosamine-6-sulfatase precursor; G6S; Glucosamine-6-sulfatase	-1.95	0.0457
GRIK2	Receptor for glutamate. L-glutamate acts as an excitatory neurotransmitter at many synapses in the central nervous system. The postsynaptic actions of Glu are mediated by a variety of receptors that are named according to their selective agonists. May be involved in the transmission of light information from the retina to the hypothalamus. This receptor binds domoate > kainate > quisqualate > 6-cyano-7-nitroquinoxaline-2,3-dione > L-glutamate = 6,7-dinitroquinoxaline-2,3-dione > dihydrokainate	-1.96	0.0452
ENSG00000171914	Adenosine/AMP deaminase active site; Band 4.1; I/LWEQ	-1.96	0.0236
M3K8	Able to activate NF-kappa-B 1 by stimulating proteasome-mediated proteolysis of NF-kappa-B 1/p105. Plays a role in the cell cycle. The longer form of cot has some transforming activity, although it is much weaker than the activated cot oncoprotein	-1.97	0.0434
NP_006537.3	KH, type 1; RNA-binding region RNP-1 (RNA recognition motif)	-1.98	0.043
ALKB1	Alkylated DNA repair protein alkB homolog 1	-1.98	0.0377
Q86V25-2	Angiogenesis inhibitor. Inhibits network formation by endothelial cells	-1.99	0.0422
ENSG00000132463	RNA-binding region RNP-1 (RNA recognition motif)	-1.99	0.013
O75800-2	Zinc finger MYND domain-containing protein 10; BLu protein	-1.99	0.0417
NP_659453.2	Aralkyl acyl-CoA:amino acid N-acyltransferase; Aralkyl acyl-CoA:amino acid N-acyltransferase, C-terminal	-2.00	0.0413
GPSM3	Functions as a receptor for membrane-bound ligands Jagged1, Jagged2 and Delta1 to regulate cell-fate determination. Upon ligand activation through the released notch intracellular domain (NICD) it forms a transcriptional activator complex with RBP-J kappa and activates genes of the enhancer of split locus. Affects the implementation of differentiation, proliferation and apoptotic programs. May regulate branching morphogenesis in the developing vascular system	-2.00	0.0412
Q6P578	Tetratricopeptide repeat protein 3; TPR repeat protein 3; TPR repeat protein D; RING finger protein 105	-2.00	0.0019
NP_872354.1	Plays a role in mediating Ca(2+) influx following depletion of intracellular Ca(2+) stores. Acts as Ca(2+) sensor in the endoplasmic reticulum via its EF-hand domain. Upon Ca(2+) depletion, translocates from the endoplasmic reticulum to the plasma membrane where it activates the Ca(2+) release-activated Ca(2+) (CRAC) channel subunit, TMEM142A/ORAI1	-2.01	0.0276
SCF	Stimulates the proliferation of mast cells. Able to augment the proliferation of both myeloid and lymphoid hematopoietic progenitors in bone marrow culture. Mediates also cell-cell adhesion. Acts synergistically with other cytokines, probably interleukins	-2.01	0.0045

Gene Name	Gene Description	Fold Change	p Value
NP_056477.1	WD-40 repeat	-2.02	0.0392
Q8TAC0	Acc:Q8TAC0]; MGC27345 protein. [Source:Uniprot/SPTREMBL	-2.02	0.0057
DNAS1	Among other functions, seems to be involved in cell death by apoptosis. Binds specifically to G-actin and blocks actin polymerization	-2.03	0.0339
NP_064618.3	Metallophosphoesterase	-2.03	0.0387
SERC3	May be involved in cellular transformation	-2.03	0.0384
TF2H4	Component of the core-TFIIH basal transcription factor involved in nucleotide excision repair (NER) of DNA and, when complexed to CAK, in RNA transcription by RNA polymerase II	-2.03	0.0382
PDE4C	cAMP-specific 3',5'-cyclic phosphodiesterase 4C; DPDE1; PDE21	-2.04	0.0326
G3P	Glyceraldehyde-3-phosphate dehydrogenase; GAPDH	-2.04	0.0123
RBL2	Key regulator of entry into cell division. Directly involved in heterochromatin formation by maintaining overall chromatin structure and, in particular, that of constitutive heterochromatin by stabilizing histone methylation. Recruits and targets histone methyltransferases SUV420H1 and SUV420H2, leading to epigenetic transcriptional repression. Controls histone H4 'Lys-20' trimethylation. Probably acts as a transcription repressor by recruiting chromatin-modifying enzymes to promoters. Potent inhibitor of E2F-mediated trans-activation, associates preferentially with E2F5. Binds to cyclins A and E. Binds to and may be involved in the transforming capacity of the adenovirus E1A protein. May act as a tumor suppressor	-2.05	0.0112
NP_872339.2	Acc:Q6ZRT9]; CDNA FLJ46112 fis, clone TESTI2035962 (Novel protein). [Source:Uniprot/SPTREMBL	-2.06	0.0361
NP_001005472.1	Ribosomal protein S2	-2.06	0.0358
NP_078886.2	Transcription factor jumonji	-2.07	0.0356
CACB3	The beta subunit of voltage-dependent calcium channels contributes to the function of the calcium channel by increasing peak calcium current, shifting the voltage dependencies of activation and inactivation, modulating G protein inhibition and controlling the alpha-1 subunit membrane targeting	-2.08	0.0347
ECHD1	Enoyl-CoA hydratase domain-containing protein 1	-2.09	0.0339
MPIP2	Tyrosine protein phosphatase which functions as a dosage-dependent inducer of mitotic progression. Directly dephosphorylates CDC2 and stimulates its kinase activity. The three isoforms seem to have a different level of activity	-2.10	0.0333
SYFB	Phenylalanyl-tRNA synthetase beta chain; Phenylalanine--tRNA ligase beta chain; PheRS	-2.10	0.0333
CU045	Uncharacterized protein C21orf45; FAPP1-associated protein 1	-2.10	0.0488
K1279	May play a role in both peripheral and central nervous system	-2.10	0.0207
TMM58	Transmembrane protein 58 precursor	-2.11	0.0044

Gene Name	Gene Description	Fold Change	p Value
PYGL	Phosphorylase is an important allosteric enzyme in carbohydrate metabolism. Enzymes from different sources differ in their regulatory mechanisms and in their natural substrates. However, all known phosphorylases share catalytic and structural properties	-2.12	0.0245
MDM4	Inhibits p53- and p73-mediated cell cycle arrest and apoptosis by binding its transcriptional activation domain. Inhibits degradation of MDM2. Can reverse MDM2-targeted degradation of p53 while maintaining suppression of p53 transactivation and apoptotic functions	-2.13	0.031
LRP4	Potential cell surface endocytic receptor, which binds and internalizes extracellular ligands for degradation by lysosomes	-2.14	0.0304
RIPK1	Promotes apoptosis and activation of NF-kappa-B. Required for TNFRSF1A mediated activation of NF-kappa-B	-2.15	0.0302
NP_073742.1	Acc:Q5VW35]; Novel protein (FLJ12806). [Source:Uniprot/SPTREMBL	-2.15	0.0062
MA2C1	Alpha-mannosidase 2C1; Alpha-D-mannoside mannohydrolase; Mannosidase alpha class 2C member 1; Alpha mannosidase 6A8B	-2.16	0.0297
DSG2	Component of intercellular desmosome junctions. Involved in the interaction of plaque proteins and intermediate filaments mediating cell-cell adhesion	-2.16	0.0294
ORAI2	Key regulator or component of store-operated Ca(2+) channel and transcription factor NFAT nuclear import	-2.20	0.0276
SLIK1	Enhances neuronal dendrite outgrowth	-2.21	0.0271
G3P	Glyceraldehyde-3-phosphate dehydrogenase; GAPDH	-2.22	0.0012
SOX30	Transcriptional activator. Binds to the DNA sequence 5'-ACAAT-3' and shows a preference for guanine residues surrounding this core motif	-2.23	0.0134
LAT3	Sodium-independent, high affinity transport of large neutral amino acids. Has narrower substrate selectivity compared to SLC7A5 and SLC7A8 and mainly transports branched-chain amino acids and phenylalanine. Plays a role in the development of human prostate cancer, from prostatic intraepithelial neoplasia to invasive prostate cancer	-2.24	0.0254
Q6UX34	ASCL830 (UNQ830), mRNA [Source:RefSeq_dna; Acc:NM_206895]	-2.26	0.0247
PRP39	Involved in pre-mRNA splicing	-2.26	0.0247
Q96NM2	Acc:NM_001004325]; keratin associated protein 5-2 (KRTAP5-2), mRNA [Source:RefSeq_dna	-2.28	0.0238
Q9HAJ0	Acc:Q9HAJ0]; CDNA FLJ11556 fis, clone HEMBA1003079. [Source:Uniprot/SPTREMBL	-2.28	0.0237
S38A3	Sodium-dependent amino acid/proton antiporter. Mediates electrogenic cotransport of glutamine and sodium ions in exchange for protons. Also recognizes histidine, asparagine and alanine. May mediate amino acid transport in either direction under physiological conditions. May play a role in nitrogen metabolism and synaptic transmission	-2.30	0.023

Gene Name	Gene Description	Fold Change	p Value
SSXT	SSXT protein; Synovial sarcoma, translocated to X chromosome; SYT protein	-2.31	0.0226
GBRG2	GABA, the major inhibitory neurotransmitter in the vertebrate brain, mediates neuronal inhibition by binding to the GABA/benzodiazepine receptor and opening an integral chloride channel	-2.31	0.0223
NP_001073963.1	Tetratricopeptide TPR_2	-2.32	0.0329
Q6ZNA8	May participate in a common DNA damage response pathway associated with the activation of homologous recombination and double-strand break repair. Binds to single and double stranded DNA and exhibits DNA-dependent ATPase activity. Underwinds duplex DNA and forms helical nucleoprotein filaments	-2.32	0.0221
Q9H4F8-2	Calcium-binding EF-hand; Protease inhibitor, Kazal-type; Proteinase inhibitor I1, Kazal; Thyroglobulin type-1	-2.33	0.0216
OBSCN	Immunoglobulin; Immunoglobulin C1-set; Immunoglobulin I-set; Immunoglobulin V-set; Immunoglobulin-like; Proline-rich region; Protein kinase; Tyrosine protein kinase, active site	-2.37	0.0202
ARSB	Arylsulfatase B precursor; ASB; N-acetylgalactosamine-4-sulfatase; G4S	-2.38	0.0188
NP_071425.2	Type II fibronectin, collagen-binding	-2.39	0.0197
MSRE	Membrane glycoproteins implicated in the pathologic deposition of cholesterol in arterial walls during atherogenesis. Two types of receptor subunits exist. These receptors mediate the endocytosis of a diverse group of macromolecules, including modified low density lipoproteins (LDL)	-2.39	0.0197
L2HDH	L-2-hydroxyglutarate dehydrogenase, mitochondrial precursor; Duranin	-2.41	0.019
DNM3B	Required for genome wide de novo methylation and is essential for development. DNA methylation is coordinated with methylation of histones. Isoforms 4 and 5 are probably not functional due to the deletion of two conserved methyltransferase motifs	-2.44	0.0181
Q9H9G5	Shows growth cone collapsing activity on dorsal root ganglion (DRG) neurons in vitro. May be a stop signal for the DRG neurons in their target areas, and possibly also for other neurons. May also be involved in the maintenance and remodeling of neuronal connections	-2.44	0.0181
SAM14	Sterile alpha motif domain-containing protein 14	-2.45	0.0179
RSU1	Potentially plays a role in the Ras signal transduction pathway. Capable of suppressing v-Ras transformation in vitro	-2.47	0.0173
NP_001007793.1	Required for high-affinity binding to nerve growth factor (NGF), neurotrophin-3 and neurotrophin-4/5 but not brain-derived neurotrophic factor (BDNF). Known substrates for the Trk receptors are SHC1, PI 3-kinase, and PLC-gamma-1. Has a crucial role in the development and function of the nociceptive reception system as well as establishment of thermal regulation via sweating. Activates ERK1 by either SHC1- or PLC-gamma-1-dependent signaling pathway	-2.49	0.0104

Gene Name	Gene Description	Fold Change	p Value
CLTR1	Receptor for cysteinyl leukotrienes mediating bronchoconstriction of individuals with and without asthma. Stimulation by LTD4 results in the contraction and proliferation of smooth muscle, edema, eosinophil migration and damage to the mucus layer in the lung. This response is mediated via a G-protein that activates a phosphatidylinositol-calcium second messenger system. The rank order of affinities for the leukotrienes is LTD4 >> LTE4 = LTC4 >> LTB4	-2.50	0.0166
C43BP	Phosphorylates on Ser and Thr residues the Goodpasture autoantigen (in vitro). Isoform 2 seems to be less active	-2.52	0.0161
WDR55	WD repeat protein 55	-2.52	0.016
BKRB2	Receptor for bradykinin. It is associated with G proteins that activate a phosphatidylinositol-calcium second messenger system	-2.53	0.0157
NP_055654.2	Phosphoesterase, PA-phosphatase related	-2.53	0.0157
ACSL5	Activation of long-chain fatty acids for both synthesis of cellular lipids, and degradation via beta-oxidation. Utilizes a wide range of saturated fatty acids with a preference for C16-C18 unsaturated fatty acids	-2.54	0.0154
LR37A	Leucine-rich repeat-containing protein 37A; Leucine-rich repeat-containing protein 37B precursor; C66 SLIT-like testicular protein	-2.59	0.0002
Q9NSC5-3	EVH1	-2.61	0.0003
CUL5	Component of E3 ubiquitin ligase complexes, which mediate the ubiquitination and subsequent proteasomal degradation of target proteins. Seems to be involved poteosomal degradation of p53/TP53 stimulated by adenovirus E1B-55 kDa protein. May form a cell surface vasopressin receptor	-2.66	0.0012
NP_060218.1	Histone H5	-2.67	0.0129
TRIPB	Binds the ligand binding domain of the thyroid receptor (THRB) in the presence of triiodothyronine and enhances THRB-modulated transcription. Golgi auto-antigen; probably involved in maintaining cis-Golgi structure	-2.69	0.0126
INHBC	Inhibins and activins inhibit and activate, respectively, the secretion of follitropin by the pituitary gland. Inhibins/activins are involved in regulating a number of diverse functions such as hypothalamic and pituitary hormone secretion, gonadal hormone secretion, germ cell development and maturation, erythroid differentiation, insulin secretion, nerve cell survival, embryonic axial development or bone growth, depending on their subunit composition. Inhibins appear to oppose the functions of activins	-2.71	0.0122
METTL6	Probable methyltransferase	-2.75	0.0116
PO2F3	Transcription factor that binds to the octamer motif (5'-ATTTGCAT-3'). Regulated the expression of a number of genes such as SPRR2A or placental lactogen	-2.76	0.0115

Gene Name	Gene Description	Fold Change	p Value
GBF1	Promotes guanine-nucleotide exchange on ARF5. Promotes the activation of ARF5 through replacement of GDP with GTP	-2.79	0.0111
NFASC	Cell adhesion, ankyrin-binding protein which may be involved in neurite extension, axonal guidance, synaptogenesis, myelination and neuron-glial cell interactions	-2.80	0.0109
CT121	May act as a protein that binds a hydrophobic ligand	-2.82	0.0485
DAAM1	Binds to disheveled (Dvl) and Rho, and mediates Wnt-induced Dvl-Rho complex formation. May play a role as a scaffolding protein to recruit Rho-GDP and Rho-GEF, thereby enhancing Rho-GTP formation	-2.86	0.0343
DLX5	Homeobox protein DLX-5	-2.87	0.01
ZNF19	May be involved in transcriptional regulation	-2.89	0.0039
PIAS1	Functions as an E3-type small ubiquitin-like modifier (SUMO) ligase, stabilizing the interaction between UBE2I and the substrate, and as a SUMO-tethering factor. Plays a crucial role as a transcriptional coregulation in various cellular pathways, including the STAT pathway, the p53 pathway and the steroid hormone signaling pathway. The effects of this transcriptional coregulation, transactivation or silencing, may vary depending upon the biological context	-2.90	0.0097
CASR	Senses changes in the extracellular concentration of calcium ions. The activity of this receptor is mediated by a G-protein that activates a phosphatidylinositol-calcium second messenger system	-2.93	0.0094
TLE4	Transcriptional corepressor that binds to a number of transcription factors. Inhibits the transcriptional activation mediated by PAX5, and by CTNNB1 and TCF family members in Wnt signaling. The effects of full-length TLE family members may be modulated by association with dominant-negative AES	-2.95	0.0091
Q8NBC0	Orphan nuclear receptor, HMR type	-3.00	0.0442
T2R16	Gustducin-coupled receptor implicated in the perception of bitter compounds in the oral cavity and the gastrointestinal tract. Signals through PLCB2 and the calcium-regulated cation channel TRPM5	-3.05	0.0082
FBXL7	Probably recognizes and binds to some phosphorylated proteins and promotes their ubiquitination and degradation	-3.05	0.0081
CATG	Cathepsin G precursor; CG	-3.15	0.0073
ASAHL	Degrades bioactive fatty acid amides to their corresponding acids, with the following preference: N-palmitoylethanolamine > N-myristoylethanolamine > N-lauroylethanolamine = N-stearoylethanolamine > N-arachidonoylethanolamine > N-oleoylethanolamine. Also exhibits weak hydrolytic activity against the ceramides N-lauroylsphingosine and N-palmitoylsphingosine	-3.16	0.0073
SC6A3	Amine transporter. Terminates the action of dopamine by its high affinity sodium-dependent reuptake into presynaptic terminals	-3.20	0.007

Gene Name	Gene Description	Fold Change	p Value
MBOA5	Membrane-bound O-acyltransferase domain-containing protein 5; O-acyltransferase domain-containing protein 5	-3.24	0.0006
CELR1	Receptor that may have an important role in cell/cell signaling during nervous system formation	-3.25	0.0067
F102A	May play a role in estrogen action	-3.30	0.0064
VNN2	Probable hydrolase. Involved in the thymus homing of bone marrow cells. May regulate beta-2 integrin-mediated cell adhesion, migration and motility of neutrophil	-3.43	0.0056
NP_659462.1	Chaperonin clpA/B; Disease resistance protein	-3.56	0.0003
NP_116250.2	Acc:NP_116250]; serine active site containing 1 [Source:RefSeq_peptide	-3.65	0.0047
EVI2B	EVI2B protein precursor; Ecotropic viral integration site 2B protein homolog; EVI-2B	-3.70	0.0045
AGPAT5	Converts lysophosphatidic acid (LPA) into phosphatidic acid by incorporating an acyl moiety at the sn-2 position of the glycerol backbone	-3.72	0.0044
PLOD2	Forms hydroxylysine residues in -Xaa-Lys-Gly- sequences in collagens. These hydroxylysines serve as sites of attachment for carbohydrate units and are essential for the stability of the intermolecular collagen cross-links	-3.73	0.0044
SL9A8	Involved in pH regulation to eliminate acids generated by active metabolism or to counter adverse environmental conditions. Major proton extruding system driven by the inward sodium ion chemical gradient. Plays an important role in signal transduction	-3.75	0.0043
RTC1	Catalyzes the conversion of 3'-phosphate to a 2',3'-cyclic phosphodiester at the end of RNA. The mechanism of action of the enzyme occurs in 3 steps: (A) adenylation of the enzyme by ATP; (B) the enzyme acts on RNA-N3'P to produce RNA-N3'PP5'A; (C) a non catalytic nucleophilic attack by the adjacent 2'hydroxyl on the phosphorus in the diester linkage to produce the cyclic end product. The biological role of this enzyme is unknown but it is likely to function in some aspects of cellular RNA processing	-3.81	0.0003
TIGD6	Tigger transposable element-derived protein 6	-3.89	0.0039
F261	Synthesis and degradation of fructose 2,6-bisphosphate	-3.93	0.0002
AAT1	Isoform 4 may play a role in spermatogenesis	-4.18	0.0032
ATP7B	Involved in the export of copper out of the cells, such as the efflux of hepatic copper into the bile	-4.40	0.0028
K22E	Probably contributes to terminal cornification. Associated with keratinocyte activation, proliferation and keratinization	-4.52	0.0026
NP_115510.1	Acc:NP_115510]; glutamine rich 2 [Source:RefSeq_peptide	-4.53	0.0026
DB118	Has antibacterial activity	-4.56	0.0025
BCL7A	B-cell CLL/lymphoma 7 protein family member A	-4.62	0.0024
MAGBA	Melanoma-associated antigen B10; MAGE-B10 antigen	-4.66	0.0024

Gene Name	Gene Description	Fold Change	p Value
Q7Z3S7	Cache; VWA N-terminal; von Willebrand factor, type A	-4.69	0.0023
RNAS1	Endonuclease that catalyzes the cleavage of RNA on the 3' side of pyrimidine nucleotides. Acts on single stranded and double stranded RNA	-4.90	0.0021
GABARAPL3	Gamma-aminobutyric acid receptor-associated protein-like 3; GABA(A) receptor-associated protein-like 3	-5.16	0.0018
ENSG00000184956	Orphan nuclear receptor, HMR type	-5.44	0.0016
ZN134	May be involved in transcriptional regulation	-5.67	0.0015
OR2S1	Putative odorant receptor	-6.58	0.0011
FGF2	The heparin-binding growth factors are angiogenic agents in vivo and are potent mitogens for a variety of cell types in vitro. There are differences in the tissue distribution and concentration of these 2 growth factors	-7.71	0.0008
ATRX	Could be a global transcriptional regulator. Modifies gene expression by affecting chromatin. May be involved in brain development and facial morphogenesis	-9.95	0.0005
ZHANG	Strongly activates transcription when bound to HCFC1. Suppresses the expression of HSV proteins in cells infected with the virus in a HCFC1-dependent manner. Also suppresses the HCFC1-dependent transcriptional activation by CREB3 and reduces the amount of CREB3 in the cell. Able to down-regulate expression of some cellular genes in CREBZF-expressing cells	-12.16	0.0003
ENSG00000199437	Acc:RF00416]; Small nucleolar RNA ACA43 [Source:RFAM	-13.06	0.0003
ZNF613	May be involved in transcriptional regulation	-47.09	5.4E-05

Supplementary Table 8: Differentially expressed genes 2 hours post-stimulation with 3 µg/ml LL-37.

Gene Name	Gene Description	Fold Change	p Value
NP_001032407.1	May play a role in the regulation of keratinocyte	7.67	0.004
TMEM33	Transmembrane protein 33; DB83 protein	7.24	0.0044
MIF	May play an important role in the progression of epithelial malignancies; The expression of MIF at sites of inflammation suggest a role for the mediator in regulating the function of macrophage in host defense. Also acts as a phenylpyruvate tautomerase	6.36	0.0054
TSN13	Tetraspanin-13; Tspan-13; Transmembrane 4 superfamily member 13; Tetraspan NET-6	5.47	0.0071
ENSG00000203581	Acc:NR_002169]; olfactory receptor, family 1, subfamily F, member 2 (OR1F2) on chromosome 16 [Source:RefSeq_dna	5.19	0.0078

Gene Name	Gene Description	Fold Change	p Value
KCNH2	Pore-forming (alpha) subunit of voltage-gated inwardly rectifying potassium channel. Channel properties are modulated by cAMP and subunit assembly. Mediates the rapidly activating component of the delayed rectifying potassium current in heart (IKr). Isoform 3 has no channel activity by itself, but modulates channel characteristics when associated with isoform 1	4.82	0.009
FOS	Nuclear phosphoprotein which forms a tight but non-covalently linked complex with the JUN/AP-1 transcription factor. In the heterodimer, c-fos and JUN/AP-1 basic regions each seems to interact with symmetrical DNA half sites. Has a critical function in regulating the development of cells destined to form and maintain the skeleton. It is thought to have an important role in signal transduction, cell proliferation and differentiation	4.57	0.0099
TIE2	This protein is a protein tyrosine-kinase transmembrane receptor for angiopoietin 1. It may constitute the earliest mammalian endothelial cell lineage marker. Probably regulates endothelial cell proliferation, differentiation and guides the proper patterning of endothelial cells during blood vessel formation	4.35	0.011
GBG8	Guanine nucleotide-binding proteins (G proteins) are involved as a modulator or transducer in various transmembrane signaling systems. The beta and gamma chains are required for the GTPase activity, for replacement of GDP by GTP, and for G protein-effector interaction	4.24	0.0116
RT12	28S ribosomal protein S12, mitochondrial precursor; S12mt; MRP-S12; MT-RPS12	4.20	0.0118
FGD5	May activate CDC42, a member of the Ras-like family of Rho- and Rac proteins, by exchanging bound GDP for free GTP. May play a role in regulating the actin cytoskeleton and cell shape	3.93	0.0135
KS6A2	Serine/threonine kinase that may play a role in mediating the growth-factor and stress induced activation of the transcription factor CREB	3.92	0.0137
IDH2	Plays a role in intermediary metabolism and energy production. It may tightly associate or interact with the pyruvate dehydrogenase complex	3.72	0.0153
Q9BSF7	Acc:Q9BSF7]; MGC13008 protein. [Source:Uniprot/SPTREMBL	3.65	0.016
Q6I9Y3	Chromo; Post-SET zinc-binding region; Pre-SET zinc-binding region; SET	3.58	0.0167
SP4	Binds to GT and GC boxes promoters elements. Probable transcriptional activator	3.52	0.0174
NPT2B	May be involved in actively transporting phosphate into cells via Na(+) cotransport. It may be the main phosphate transport protein in the intestinal brush border membrane. May have a role in the synthesis of surfactant in lungs' alveoli	3.13	0.0162
NP_116256.2	Proline-rich region; Tropomyosin	3.10	0.0235
BT2A1	Butyrophilin subfamily 2 member A1 precursor; Butyrophilin subfamily 2 member A3 precursor	3.07	0.0242

Gene Name	Gene Description	Fold Change	p Value
ATE1	Involved in the posttranslational conjugation of arginine to the N-terminal aspartate or glutamate of a protein. This arginylation is required for degradation of the protein via the ubiquitin pathway. Does not arginylate cysteine residues	3.06	0.0244
TRIO	Promotes the exchange of GDP by GTP. Together with leukocyte antigen-related (LAR) protein, it could play a role in coordinating cell-matrix and cytoskeletal rearrangements necessary for cell migration and cell growth	3.06	0.0244
NP_115589.2	Engulfment and cell motility, ELM	3.05	0.0247
PPIL2	PPIases accelerate the folding of proteins. It catalyzes the cis-trans isomerization of proline imidic peptide bonds in oligopeptides	3.02	0.0252
NGLY1	Specifically deglycosylates the denatured form of N-linked glycoproteins in the cytoplasm and assists their proteasome-mediated degradation. Cleaves the beta-aspartyl-glucosamine (GlcNAc) of the glycan and the amide side chain of Asn, converting Asn to Asp. Prefers proteins containing high-mannose over those bearing complex type oligosaccharides. Can recognize misfolded proteins in the endoplasmic reticulun that are exported in the cytosol to be destroyed and deglycosylate them, while it has no activity toward native proteins. Deglycosylation is prerequisite for subsequent proteasome-mediated degradation of some, but not all, misfolded glycoproteins	2.99	0.0258
RET7	Intracellular transport of retinol	2.96	0.0264
NP_997646.1	AT-rich interaction region	2.94	0.027
BAALC	May play a synaptic role at the postsynaptic lipid rafts by interacting with CAMK2A	2.87	0.0287
LDHC	Possible role in sperm motility	2.83	0.0298
ABI2	May act in regulation of cell growth and transformation by interacting with nonreceptor tyrosine kinases ABL1 and/or ABL2. May be involved in cytoskeletal reorganization. Regulates ABL1/c-Abl-mediated phosphorylation of MENA	2.66	0.0352
NP_006537.3	KH, type 1; RNA-binding region RNP-1 (RNA recognition motif)	2.66	0.0353
NP_079174.2	Adenovirus fibre protein; DENN; dDENN; uDENN	2.63	0.0363
GPX7	Glutathione peroxidase 7 precursor; CL683	2.62	0.0368
ZN485	May be involved in transcriptional regulation	2.61	0.0371
CAH3	Reversible hydration of carbon dioxide	2.61	0.0373
ARHG4	DH; Guanine-nucleotide dissociation stimulator, CDC24; Pleckstrin-like; Src homology-3; Variant SH3	2.56	0.0391
CHST6	Catalyzes the transfer of sulfate to position 6 of non-reducing N-acetylglucosamine (GlcNAc) residues of keratan. Mediates sulfation of keratan in cornea. Keratan sulfate plays a central role in maintaining corneal transparency. Acts on the nonreducing terminal GlcNAc of short and long carbohydrate substrates that have poly-N-acetyllactosamine structures	2.56	0.0393

Gene Name	Gene Description	Fold Change	p Value
SNX23	May be involved in several stages of intracellular trafficking. Probable microtubule-dependent motor protein	2.54	0.04
P48067-2	Terminates the action of glycine by its high affinity sodium-dependent reuptake into presynaptic terminals. May play a role in regulation of glycine levels in NMDA receptor-mediated neurotransmission	2.54	0.0401
HSH2D	May be a modulator of the apoptotic response through its ability to affect mitochondrial stability (By similarity). Adapter protein involved in tyrosine kinase and CD28 signaling. Seems to affect CD28-mediated activation of the RE/AP element of the interleukin-2 promoter	2.53	0.0406
RND1	Lacks intrinsic GTPase activity. Has a low affinity for GDP, and constitutively binds GTP. Controls rearrangements of the actin cytoskeleton. Induces the Rac-dependent neuritic process formation in part by disruption of the cortical actin filaments. Causes the formation of many neuritic processes from the cell body with disruption of the cortical actin filaments	2.52	0.041
RFIP2	A Rab11 effector protein acting in the regulation of the transport of vesicles from the endosomal recycling compartment (ERC) to the plasma membrane. Also involved in receptor-mediated endocytosis and membrane trafficking of recycling endosomes, probably originating from clathrin-coated vesicles. Binds preferentially to phosphatidylinositol 3,4,5-trisphosphate (PtdInsP3) and phosphatidic acid (PA)	2.51	0.0417
PDZD2	PDZ domain-containing protein 2; PDZ domain-containing protein 3; Activated in prostate cancer protein	2.50	0.042
BIN3	Involved in cytokinesis and septation where it has a role in the localization of F-actin	2.45	0.0445
KCMB1	Regulatory subunit of the calcium activated potassium KCNMA1 (maxiK) channel. Modulates the calcium sensitivity and gating kinetics of KCNMA1, thereby contributing to KCNMA1 channel diversity. Increases the apparent Ca(2+)/voltage sensitivity of the KCNMA1 channel. It also modifies KCNMA1 channel kinetics and alters its pharmacological properties. It slows down the activation and the deactivation kinetics of the channel. Acts as a negative regulator of smooth muscle contraction by enhancing the calcium sensitivity to KCNMA1. Its presence is also a requirement for internal binding of the KCNMA1 channel opener dehydrosoyasaponin I (DHS-1) triterpene glycoside and for external binding of the agonist hormone 17-beta-estradiol (E2). Increases the binding activity of charybdotoxin (CTX) toxin to KCNMA1 peptide blocker by increasing the CTX association rate and decreasing the dissociation rate	2.42	0.0462

Gene Name	Gene Description	Fold Change	p Value
NP_079038.2	KRAB box; Zinc finger, C2H2-subtype; Zinc finger, C2H2-type	2.39	0.0476
MCR	Receptor for both mineralocorticoids (MC) such as aldosterone and glucocorticoids (GC) such as corticosterone or cortisol. Binds to mineralocorticoid response elements (MRE) and transactivates target genes. The effect of MC is to increase ion and water transport and thus raise extracellular fluid volume and blood pressure and lower potassium levels	2.39	0.0477
FARP2	Rho-guanine nucleotide exchange factor that activates RAC1. Plays a role in the response to class 3 semaphorins and remodeling of the actin cytoskeleton	2.39	0.0479
NP_060156.1	Appr-1-p processing	2.38	0.0485
CPN2	Involved in DNA repair and mitotic recombination. May play an active role in recombination processes in concert with other members of the RAD52 epistasis group; May function in chaperone-mediated protein folding; The 83 kDa subunit binds and stabilizes the catalytic subunit at 37 degrees Celsius and keeps it in circulation. Under some circumstances it may be an allosteric modifier of the catalytic subunit	2.38	0.0486
TNFC	Cytokine that binds to LTBR/TNFRSF3. May play a specific role in immune response regulation. Provides the membrane anchor for the attachment of the heterotrimeric complex to the cell surface. Isoform 2 is probably non-functional	2.38	0.0487
ABD12	PPIases accelerate the folding of proteins. It catalyzes the cis-trans isomerization of proline imidic peptide bonds in oligopeptides; This is a calcium-activated, phospholipid-dependent, serine- and threonine-specific enzyme. May play a role in cell motility by phosphorylating CSPG4	2.37	0.0026
MYST3	Histone acetyltransferase which may act as a transcriptional coactivator for RUNX1 and RUNX2	2.36	0.0496
NP1L2	Acidic protein which may be involved in interactions with other proteins or DNA	2.30	0.0081
VWF	Important in the maintenance of hemostasis, it promotes adhesion of platelets to the sites of vascular injury by forming a molecular bridge between sub-endothelial collagen matrix and platelet-surface receptor complex GPIb-IX-V. Also acts as a chaperone for coagulation factor VIII, delivering it to the site of injury, stabilizing its heterodimeric structure and protecting it from premature clearance from plasma	2.28	0.0092
AMAC1L1	Protein AMAC1; Transmembrane protein 21A; Protein AMAC1L2	2.17	0.0114
RMD5B	RMD5 homolog B	-2.19	0.0148
DHB3	Favors the reduction of androstenedione to testosterone. Uses NADPH while the two other EDH17B enzymes use NADH	-2.29	0.0476
Q5T6R2	Could be involved in signal transduction	-2.34	0.0257

Gene Name	Gene Description	Fold Change	p Value
Q96GD3-2	Component of the Polycomb group (PcG) multiprotein PRC1 complex, a complex required to maintain the transcriptionally repressive state of many genes, including Hox genes, throughout development. PcG PRC1 complex acts via chromatin remodeling and modification of histones; it mediates monoubiquitination of histone H2A 'Lys-119', rendering chromatin heritably changed in its expressibility	-2.36	0.0497
PRB4S	Basic salivary proline-rich protein 4 allele S precursor; Salivary proline-rich protein Po; Parotid o protein; Protein N1; Glycosylated protein A	-2.38	0.0487
UBAP1	Ubiquitin-associated protein 1; UBAP	-2.38	0.0485
COPE	The coatomer is a cytosolic protein complex that binds to dilysine motifs and reversibly associates with Golgi non-clathrin-coated vesicles, which further mediate biosynthetic protein transport from the ER, via the Golgi up to the trans Golgi network. Coatomer complex is required for budding from Golgi membranes, and is essential for the retrograde Golgi-to-ER transport of dilysine-tagged proteins. In mammals, the coatomer can only be recruited by membranes associated to ADP-ribosylation factors (ARFs), which are small GTP-binding proteins; the complex also influences the Golgi structural integrity, as well as the processing, activity, and endocytic recycling of LDL receptors	-2.40	0.0473
Q75VX8	Proline-rich region	-2.40	0.0471
Q5K675	Acc:Q5K675]; Osteoligament factor. [Source:Uniprot/SPTREMBL	-2.41	0.0466
MYPN	Component of the sarcomere that tethers together nebulin (skeletal muscle) and nebulette (cardiac muscle) to alpha-actinin, at the Z lines	-2.42	0.0461
NALDL	NAALADase-like activity unknown. Has no NAAG hydrolyzing activity. Exhibits a dipeptidyl-peptidase IV type activity. In vitro, cleaves Gly-Pro-AMC	-2.43	0.0457
CCG6	Thought to stabilize the calcium channel in an inactivated (closed) state	-2.45	0.0446
NP_277045.1	Sterol-sensing 5TM box	-2.45	0.0443

Gene Name	Gene Description	Fold Change	p Value
O94813-3	Thought to act as molecular guidance cue in cellular migration, and function appears to be mediated by interaction with roundabout homolog receptors. During neural development involved in axonal navigation at the ventral midline of the neural tube and projection of axons to different regions. SLIT1 and SLIT2 seem to be essential for midline guidance in the forebrain by acting as repulsive signal preventing inappropriate midline crossing by axons projecting from the olfactory bulb. In spinal chord development may play a role in guiding commissural axons once they reached the floor plate by modulating the response to netrin. In vitro, silences the attractive effect of NTN1 but not its growth-stimulatory effect and silencing requires the formation of a ROBO1-DCC complex. May be implicated in spinal chord midline post-crossing axon repulsion. In vitro, only commissural axons that crossed the midline responded to SLIT2. In the developing visual system appears to function as repellent for retinal ganglion axons by providing a repulsion that directs these axons along their appropriate paths prior to, and after passage through, the optic chiasm. In vitro, collapses and repels retinal ganglion cell growth cones. Seems to play a role in branching and arborization of CNS sensory axons, and in neuronal cell migration. In vitro, Slit homolog 2 protein N-product, but not Slit homolog 2 protein C-product, repels olfactory bulb (OB) but not dorsal root ganglia (DRG) axons, induces OB growth cones collapse and induces branching of DRG axons. Seems to be involved in regulating leukocyte migration	-2.49	0.0425
XPA	Involved in DNA excision repair. Initiates repair by binding to damaged sites with various affinities, depending on the photoproduct and the transcriptional state of the region	-2.50	0.0419
Q8NHQ8-2	Ras association domain-containing protein 8; Carcinoma-associated protein HOJ-1	-2.53	0.0406
PDE4C	cAMP-specific 3',5'-cyclic phosphodiesterase 4C; DPDE1; PDE21	-2.54	0.0399
CP2J2	This enzyme metabolizes arachidonic acid predominantly via a NADPH-dependent olefin epoxidation to all four regioisomeric cis-epoxyeicosatrienoic acids. One of the predominant enzymes responsible for the epoxidation of endogenous cardiac arachidonic acid pools	-2.55	0.0397
KIF5C	Kinesin is a microtubule-associated force-producing protein that may play a role in organelle transport	-2.57	0.0389
SF04	Plays a role in premRNA splicing	-2.58	0.0384
S26A2	Sulfate transporter. May play a role in endochondral bone formation	-2.58	0.0384
MARVELD2	May play a role in the formation of the epithelial barrier	-2.60	0.0374
RHGXX	GTPase activator for the Rho-type GTPases by converting them to an inactive GDP-bound state	-2.62	0.0369

Gene Name	Gene Description	Fold Change	p Value
ADCY6	Membrane-bound, calcium-inhibitable adenylyl cyclase	-2.62	0.0368
ARMET	Protein ARMET precursor; Arginine-rich protein	-2.64	0.0359
PVRL3	Plays a role in cell-cell adhesion through heterophilic trans-interactions with nectin-like proteins or nectins, such as trans-interaction with PVRL2/nectin-2 at Sertoli-spermatid junctions. Trans-interaction with PVR induces activation of CDC42 and RAC small G proteins through common signaling molecules such as SRC and RAP1. Also involved in the formation of cell-cell junctions, including adherens junctions and synapses. Induces endocytosis-mediated down-regulation of PVR from the cell surface, resulting in reduction of cell movement and proliferation. Plays a role in the morphology of the ciliary body	-2.64	0.0449
GDE5	Putative glycerophosphodiester phosphodiesterase 5	-2.65	0.0356
BHLH2	May function as a transcriptional factor to modulate chondrogenesis in response to the cAMP pathway	-2.66	0.0351
DOCK8	Potential guanine nucleotide exchange factor (GEF). GEF proteins activate some small GTPases by exchanging bound GDP for free GTP	-2.67	0.0347
FASP1	Uncharacterized protein C21orf45; FAPP1-associated protein 1	-2.68	0.0345
TMUB1	Transmembrane and ubiquitin-like domain-containing protein 1; Ubiquitin-like protein SB144	-2.68	0.0344
SIRPG	Probable immunoglobulin-like cell surface receptor. On binding with CD47, mediates cell-cell adhesion. Engagement on T-cells by CD47 on antigen-presenting cells results in enhanced antigen-specific T-cell proliferation and co-stimulates T-cell activation	-2.70	0.0339
SG1D1	May bind androgens and other steroids, may also bind estramustine, a chemotherapeutic agent used for prostate cancer. May be under transcriptional regulation of steroid hormones	-2.72	0.0332
METTL6	Probable methyltransferase	-2.73	0.0329
SMURF2	E3 ubiquitin-protein ligase which accepts ubiquitin from an E2 ubiquitin-conjugating enzyme in the form of a thioester and then directly transfers the ubiquitin to targeted substrates. Interacts with SMAD1, SMAD2 and SMAD7 in order to trigger their ubiquitination and proteasome-dependent degradation. Enhances the inhibitory activity of SMAD7 and reduces the transcriptional activity of SMAD2. Coexpression of SMURF2 with SMAD1 results in considerable decrease in steady-state level of SMAD1 protein and a smaller decrease of SMAD2 level	-2.80	0.0307
KCNK7	Probable potassium channel subunit. No channel activity observed in vitro as protein remains in the endoplasmic reticulum. May need to associate with an as yet unknown partner in order to reach the plasma membrane	-2.81	0.0303

Gene Name	Gene Description	Fold Change	p Value
ZDH11	Probable palmitoyltransferase ZDHHC11; Zinc finger DHHC domain-containing protein 11; DHHC-11; Zinc finger protein 399	-2.84	0.0296
MULK	Lipid kinase that can phosphorylate both monoacylglycerol and diacylglycerol to form lysophosphatidic acid (LPA) and phosphatidic acid (PA), respectively. Does not phosphorylate sphingosine. Overexpression increases the formation and secretion of LPA, resulting in transactivation of EGFR and activation of the downstream MAPK signaling pathway, leading to increased cell growth	-2.86	0.029
DLG1	Essential multidomain scaffolding protein required for normal development (By similarity). Recruits channels, receptors and signaling molecules to discrete plasma membrane domains in polarized cells. May play a role in adherens junction assembly, signal transduction, cell proliferation, synaptogenesis and lymphocyte activation	-2.87	0.0287
RNF34	Has E3 ubiquitin-protein ligase activity. Regulates the levels of CASP8 and CASP10 by targeting them for proteasomal degradation. Protects cells against apoptosis induced by TNF. Binds phosphatidylinositol-5-phosphate and phosphatidylinositol-3-phosphate	-2.89	0.0283
RSU1	Potentially plays a role in the Ras signal transduction pathway. Capable of suppressing v-Ras transformation in vitro	-2.90	0.0279
ENP5	Likely to promote reglycosylation reactions involved in glycoproteins folding and quality control in the endoplasmic reticulum. Hydrolyzes UDP, GDP and IDP but not any other nucleoside di-, mono- or triphosphates, nor thiamine pyrophosphate	-2.93	0.0271
CYH4	Promotes guanine-nucleotide exchange on ARF1 and ARF5. Promotes the activation of ARF through replacement of GDP with GTP	-2.95	0.0028
Q9NWT9	Calcium-binding EF-hand	-2.96	0.0265
LRC8B	Leucine-rich repeat-containing protein 8B; T-cell activation leucine repeat-rich protein	-3.01	0.0255
SFXN2	Potential iron transporter	-3.03	0.0108
GBF1	Promotes guanine-nucleotide exchange on ARF5. Promotes the activation of ARF5 through replacement of GDP with GTP	-3.04	0.0248
GUAD	Catalyzes the hydrolytic deamination of guanine, producing xanthine and ammonia	-3.04	0.0247
Q9H6U4	Glycoside hydrolase, family 85	-3.06	0.0243
HHEX	Recognizes the DNA sequence 5'-ATTAA-3'. May play a role in hematopoietic differentiation	-3.09	0.0238
Q495Z4	Adrenergic receptor, beta 1; Proline-rich region	-3.14	0.0228
NP_079280.1	Acc:NP_079280]; coiled-coil domain containing 15 [Source:RefSeq_peptide	-3.15	0.0227
MAGB1	Melanoma-associated antigen B1; MAGE-B1 antigen; MAGE-XP antigen; DSS-AHC critical interval MAGE superfamily 10; DAM10	-3.16	0.0224

Gene Name	Gene Description	Fold Change	p Value
CUL5	Component of E3 ubiquitin ligase complexes, which mediate the ubiquitination and subsequent proteasomal degradation of target proteins. Seems to be involved poteosomal degradation of p53/TP53 stimulated by adenovirus E1B-55 kDa protein. May form a cell surface vasopressin receptor	-3.36	0.0067
FGF3	Could be involved in ear development	-3.39	0.0189
LAIR1	Functions as an inhibitory receptor that plays a constitutive negative regulatory role on cytolytic function of natural killer (NK) cells, B-cells and T-cells. Activation by Tyr phosphorylation results in recruitment and activation of the phosphatases PTPN6 and PTPN11. It also reduces the increase of intracellular calcium evoked by B-cell receptor ligation. May also play its inhibitory role independently of SH2-containing phosphatases. Modulates cytokine production in CD4+ T-cells, downregulating IL2 and IFNG production while inducing secretion of transforming growth factor beta. Down-regulates also IgG and IgE production in B-cells as well as IL8, IL10 and TNF secretion. Inhibits proliferation and induces apoptosis in myeloid leukemia cell lines as well as prevents nuclear translocation of NF-kappa-B p65 subunit/RELA and phosphorylation of I-kappa-B alpha/CHUK in these cells. Inhibits the differentiation of peripheral blood precursors towards dendritic cells	-3.48	0.0178
FA49A	Protein FAM49A	-3.53	0.0173
Q9NSC5-3	EVH1	-3.58	0.0132
F261	Synthesis and degradation of fructose 2,6-bisphosphate	-3.64	0.0161
FA53C	Protein FAM53C	-3.65	0.0163
SEM6A	Can act as repulsive axon guidance cues. May play a role in channeling sympathetic axons into the sympathetic chains and controlling the temporal sequence of sympathetic target innervation	-3.72	0.0154
DPYL1	Dihydropyrimidinase-related protein 1; DRP-1; Collapsin response mediator protein 1; CRMP-1	-3.79	0.0147
PLOD2	Forms hydroxylysine residues in -Xaa-Lys-Gly- sequences in collagens. These hydroxylysines serve as sites of attachment for carbohydrate units and are essential for the stability of the intermolecular collagen cross-links	-3.94	0.0135
NARGL	May belong to a complex displaying N-terminal acetyltransferase activity	-3.97	0.0133
C43BP	Phosphorylates on Ser and Thr residues the Goodpasture autoantigen (in vitro). Isoform 2 seems to be less active	-3.98	0.0156
TRFM	Involved in iron cellular uptake. Seems to be internalized and then recycled back to the cell membrane. Binds a single atom of iron per subunit. Could also bind zinc	-4.03	0.0128
RB11B	Possesses GTPase activity	-4.08	0.0125
SPAG1	Plays a role in fertilization. Binds GTP and has GTPase activity	-4.20	0.0118

Gene Name	Gene Description	Fold Change	p Value
ANR11	May recruit HDACs to the p160 coactivators/nuclear receptor complex to inhibit ligand-dependent transactivation	-4.23	0.0116
HRSL1	HRAS-like suppressor	-4.52	0.0101
Q69YY3	DKFZ	-4.55	0.01
OVOL1	Putative transcription factor. Involved in hair formation and spermatogenesis. May function in the differentiation and/or maintenance of the urogenital system	-4.66	0.0096
CLCN4	Voltage-gated chloride channel. Chloride channels have several functions including the regulation of cell volume; membrane potential stabilization, signal transduction and transepithelial transport	-4.72	0.0093
BSND	Functions as a beta-subunit for CLCNKA and CLCNKB chloride channels. In the kidney CLCNK/BSND heteromers mediate chloride reabsorption by facilitating its basolateral efflux. In the stria, CLCNK/BSND channels drive potassium secretion by recycling chloride for the basolateral SLC12A2 cotransporter	-4.77	0.0091
PHF14	PHD finger protein 14	-4.99	0.0084
TRBV6-1	Immunoglobulin V-set; Immunoglobulin-like	-5.20	0.0078
CELR3	Has an important role in stress fiber formation induced by active diaphanous protein homolog 1 (DRF1). Induces microspike formation, in vivo (By similarity). In vitro, stimulates N-WASP-induced ARP2/3 complex activation in the absence of CDC42 (By similarity). May play an important role in the maintenance of sarcomeres and/or in the assembly of myofibrils into sarcomeres. Implicated in regulation of actin polymerization and cell adhesion	-5.25	0.0446
TULP4	Tubby-related protein 4; Tubby-like protein 4; Tubby superfamily protein	-5.56	0.0069
CX04A	May have an important role of cell protection in inflammation reaction	-5.66	0.0067
APOB	Apolipoprotein B is a major protein constituent of chylomicrons (apo B-48), LDL (apo B-100) and VLDL (apo B-100). Apo B-100 functions as a recognition signal for the cellular binding and internalization of LDL particles by the apoB/E receptor	-5.72	0.0065
ELAV4	May play a role in neuron-specific RNA processing. Protects CDKN1A mRNA from decay by binding to its 3'-UTR	-6.31	0.0055
BPAEA	Cytoskeletal linker protein. Anchors keratin-containing intermediate filaments to the inner plaque of hemidesmosomes. The proteins may self-aggregate to form filaments or a two-dimensional mesh	-6.43	0.0053
KPBB	Phosphorylase b kinase catalyzes the phosphorylation of serine in certain substrates, including troponin I. The beta chain acts as a regulatory unit and modulates the activity of the holoenzyme in response to phosphorylation	-6.43	0.0053

Gene Name	Gene Description	Fold Change	p Value
ENSG00000117114	Brain-specific angiogenesis inhibitor; CD97 antigen; D-galactoside/L-rhamnose binding SUEL lectin; EMR1 hormone receptor; GPCR, family 2, secretin-like; GPS; Hormone receptor, extracellular; Latrophilin receptor; Latrophilin, C-terminal; Olfactomedin-like	-6.58	0.0051
RRP5	Involved in the biogenesis of rRNA	-6.63	0.0051
TMG1	Transmembrane gamma-carboxyglutamic acid protein 1 precursor; Proline-rich Gla protein 1; Proline-rich gamma-carboxyglutamic acid protein 1	-6.82	0.0049
ENSG00000086200	Importin-beta, N-terminal	-6.86	0.0048
FGF2	The heparin-binding growth factors are angiogenic agents in vivo and are potent mitogens for a variety of cell types in vitro. There are differences in the tissue distribution and concentration of these 2 growth factors	-8.32	0.0011
GLRA2	The glycine receptor is a neurotransmitter-gated ion channel. Binding of glycine to its receptor increases the chloride conductance and thus produces hyperpolarization (inhibition of neuronal firing)	-8.57	0.0034
MUC16	Thought to provide a protective, lubricating barrier against particles and infectious agents at mucosal surfaces	-8.92	0.0032
LIPA2	Alters PTPRF cellular localization and induces PTPRF clustering. May regulate the disassembly of focal adhesions. May localize receptor-like tyrosine phosphatases type 2A at specific sites on the plasma membrane, possibly regulating their interaction with the extracellular environment and their association with substrates	-9.95	0.0028
IGS21	Immunoglobulin superfamily member 21 precursor	-10.15	0.0027
NP_079354.2	Cystinosin/ERS1p repeat	-10.21	0.0027
NP_036401.1	EGF-like region; Glycoside hydrolase, family 56; Glycoside hydrolase, family 56, sperm surface protein PH20; Multicopper oxidase, copper-binding site	-14.31	0.0017
ENSG00000199437	Acc:RF00416]; Small nucleolar RNA ACA43 [Source:RFAM	-20.72	0.0011

Supplementary Table 9: Differentially expressed genes 4 hours post-stimulation with 3 μg/ml LL-37.

Gene Name	Gene Description	Fold Change	p Value
Q9H6U4	Glycoside hydrolase, family 85	10.07	0.0001
RBP17	May function as a nuclear transport receptor	9.32	0.0002

Gene Name	Gene Description	Fold Change	p Value
EGF	EGF stimulates the growth of various epidermal and epithelial tissues in vivo and in vitro and of some fibroblasts in cell culture	7.03	0.0004
CLCN5	Voltage-gated chloride channel. Chloride channels have several functions including the regulation of cell volume; membrane potential stabilization, signal transduction and transepithelial transport. May play an important role in renal tubular function	6.26	0.0065
DUS9	Inactivates MAP kinases. Has a specificity for the ERK family; Required for the uptake of creatine in muscles and brain	5.99	0.0006
ENPP5	May play a role in neuronal cell communication. Lacks nucleotide pyrophosphatase and lysopholipase D activity	5.93	0.0006
IFNA1	Produced by macrophages, IFN-alpha have antiviral activities. Interferon stimulates the production of two enzymes: a protein kinase and an oligoadenylate synthetase	5.52	0.0008
Q96NK6	Peptidase S1 and S6, chymotrypsin/Hap	5.49	0.0008
GPR25	Orphan receptor	5.21	0.0009
SMAD6	Antagonist of signaling by TGF-beta (transforming growth factor) type 1 receptor superfamily members; has been shown to inhibit selectively BMP (bone morphogenetic proteins) signaling by competing with the co-SMAD SMAD4 for receptor-activated SMAD1. SMAD6 is an inhibitory SMAD (I-SMAD) or antagonistic SMAD. Binds to regulatory elements in target promoter regions	5.07	0.001
NP_001025226.1	GTPase-activating protein for Rho family members. May play a role in the reduction of the p21rasGTPase-activating potential of p120GAP	4.99	0.0011
INHBC	Inhibins and activins inhibit and activate, respectively, the secretion of follitropin by the pituitary gland. Inhibins/activins are involved in regulating a number of diverse functions such as hypothalamic and pituitary hormone secretion, gonadal hormone secretion, germ cell development and maturation, erythroid differentiation, insulin secretion, nerve cell survival, embryonic axial development or bone growth, depending on their subunit composition. Inhibins appear to oppose the functions of activins	4.99	0.0011
CCG4	Thought to stabilize the calcium channel in an inactivated (closed) state	4.79	0.0012
Q8N370	Major facilitator superfamily MFS_1	4.79	0.0012
NP_060845.2	Sodium:dicarboxylate symporter; Zinc/iron permease	4.72	0.0013
DAB1	Adapter molecule functioning in neural development. May regulate SIAH1 activity	4.63	0.0014
CARD9	Activates NF-kappa-B via BCL10	4.60	0.0014
ADCK1	The function of this protein is not yet clear. It is not known if it has protein kinase activity and what type of substrate it would phosphorylate (Ser, Thr or Tyr)	4.54	0.0015
GIPR	This is a receptor for GIP. The activity of this receptor is mediated by G proteins which activate adenylyl cyclase	4.53	0.0015

Gene Name	Gene Description	Fold Change	p Value
DIAP2	Absolutely required for simian virus 40 DNA replication in vitro. It participates in a very early step in initiation. RP-A is a single-stranded DNA-binding protein; Could be involved in oogenesis. Involved in the regulation of endosome dynamics. Implicated in a novel signal transduction pathway, in which isoform 3 and CSK are sequentially activated by RHOD to regulate the motility of early endosomes through interactions with the actin cytoskeleton	4.29	0.0018
NP_001032671.1	Fatty acid desaturase; Fatty acid desaturase, type 1	4.25	0.0019
FOXP1	Transcriptional repressor that plays an important role in the specification and differentiation of lung epithelium. Can act with CTBP1 to synergistically repress transcription but CTPBP1 is not essential. Essential transcriptional regulator of B cell development	4.16	0.0021
ZN566	May be involved in transcriptional regulation	4.05	0.0195
NP_076955.1	Acc:XR_019463]; similar to CG31855-PA (LOC401770), mRNA [Source:RefSeq_dna	3.99	0.0024
AP3B1	Subunit of non-clathrin- and clathrin-associated adaptor protein complex 3 that plays a role in protein sorting in the late-Golgi/trans-Golgi network (TGN) and/or endosomes. The AP complexes mediate both the recruitment of clathrin to membranes and the recognition of sorting signals within the cytosolic tails of transmembrane cargo molecules. AP-3 appears to be involved in the sorting of a subset of transmembrane proteins targeted to lysosomes and lysosome-related organelles	3.86	0.0108
TPBG	Trophoblast glycoprotein precursor; 5T4 oncofetal trophoblast glycoprotein; 5T4 oncotrophoblast glycoprotein; 5T4 oncofetal antigen; M6P1	3.73	0.0004
Q502X4	Enzyme which catalyzes the acetylation of polyamines. Substrate specificity: norspermidine > spermidine = spermine >> N(1)acetylspermine = putrescine	3.70	0.0032
AP3S2	Part of the AP-3 complex, an adapter-related complex which is not clathrin-associated. The complex is associated with the Golgi region as well as more peripheral structures. It facilitates the budding of vesicles from the Golgi membrane and may be directly involved in trafficking to lysosomes	3.68	0.0033
GRPEL1	Essential component of the PAM complex, a complex required for the translocation of transit peptide-containing proteins from the inner membrane into the mitochondrial matrix in an ATP-dependent manner. Seems to control the nucleotide-dependent binding of mitochondrial HSP70 to substrate proteins	3.68	0.0033
NP_005178.4	EDG-8 sphingosine 1-phosphate receptor; Proline-rich region; TAFH/NHR1; Treacher Collins syndrome protein Treacle; Zinc finger, MYND-type	3.65	0.0034

Gene Name	Gene Description	Fold Change	p Value
DACH2	Transcription factor that is involved in regulation of organogenesis. Seems to be a regulator for SIX1 and SIX6. Seems to act as a corepressor of SIX6 in regulating proliferation by directly repressing cyclin-dependent kinase inhibitors, including the p27Kip1 promoter. Is recruited with SIX6 to the p27Kip1 promoter in embryonal retina. SIX6 corepression seems also to involve NCOR1, TBL1, HDAC1 and HDAC3. May be involved together with PAX3, SIX1, and EYA2 in regulation of myogenesis. In the developing somite, expression of DACH2 and PAX3 is regulated by the overlying ectoderm, and DACH2 and PAX3 positively regulate each other's expression (By similarity). Probably binds to DNA via its DACHbox-N domain	3.63	0.0034
IGF1R	This receptor binds insulin-like growth factor 1 (IGF1) with a high affinity and IGF2 with a lower affinity. It has a tyrosine-protein kinase activity, which is necessary for the activation of the IGF1-stimulated downstream signaling cascade	3.63	0.0035
AT131	Probable cation-transporting ATPase 13A1	3.59	0.0036
NP_001480.3	Nuclear hormone receptor, DNA-binding; Nuclear hormone receptor, ligand-binding; Retinoid X receptor; Steroid hormone receptor; Vitamin D receptor	3.53	0.0038
N4BP2	Has 5'-polynucleotide kinase and nicking endonuclease activity. May play a role in DNA repair or recombination	3.52	0.0039
NET4	May play an important role in neural, kidney and vascular development. Promotes neurite elongation from olfactory bulb explants	3.49	0.0415
GP126	Orphan receptor	3.45	0.0042
CYB5	Cytochrome b5 is a membrane bound hemoprotein which function as an electron carrier for several membrane bound oxygenases	3.44	0.0134
M10L1	Putative RNA helicase. Isoform 1 may play a role in male germ cell development	3.38	0.0207
RBM16	DH; Guanine-nucleotide dissociation stimulator, CDC24; PDZ/DHR/GLGF; Pleckstrin-like; Raf-like Ras-binding; Spectrin/pleckstrin-like	3.33	0.0048
DNS2A	Hydrolyzes DNA under acidic conditions with a preference for double-stranded DNA. Plays a major role in the degradation of nuclear DNA in cellular apoptosis during development. Necessary for proper fetal development and for definitive erythropoiesis in fetal liver, where it degrades nuclear DNA expelled from erythroid precursor cells	3.23	0.0055
Q9H9G5	Plexin; Semaphorin/CD100 antigen	3.22	0.0056

Gene Name	Gene Description	Fold Change	p Value
PZP	Is able to inhibit all four classes of proteinases by a unique 'trapping' mechanism. This protein has a peptide stretch, called the 'bait region' which contains specific cleavage sites for different proteinases. When a proteinase cleaves the bait region, a conformational change is induced in the protein which traps the proteinase. The entrapped enzyme remains active against low molecular weight substrates (activity against high molecular weight substrates is greatly reduced). Following cleavage in the bait region a thioester bond is hydrolyzed and mediates the covalent binding of the protein to the proteinase	3.22	0.0056
NIPBL	Probably plays a structural role in chromatin. Involved in sister chromatid cohesion, possibly by interacting with the cohesin complex	3.18	0.0058
Q5JSH9	Antifreeze protein, type I; Orphan nuclear receptor, NOR1 type; Proline-rich region; Ribosomal protein P2; Salmonella/Shigella invasin protein C; Zinc finger, MIZ-type	3.17	0.006
NP_036256.1	CHORD; CS	3.17	0.006
TLX3	T-cell leukemia homeobox protein 3; Homeobox protein Hox-11L2	3.12	0.0064
RAI3	Unknown. This G-protein coupled receptor could be involved in modulating differentiation and maintaining homeostasis of epithelial cells. The comparable expression level in fetal lung and kidney with adult tissues suggests a possible role in embryonic development and maturation of these organs. This retinoic acid-inducible GPCR provide evidence for a possible interaction between retinoid and G-protein signaling pathways	3.09	0.0067
SFRIP	Plays a role in pre-mRNA alternative splicing by regulating spliceosome assembly	3.09	0.0067
SULF1	Exhibits arylsulfatase activity and highly specific endoglucosamine-6-sulfatase activity. It can remove sulfate from the C-6 position of glucosamine within specific subregions of intact heparin. Diminishes HSPG (heparan sulfate proteoglycans) sulfation, inhibits signaling by heparin-dependent growth factors, diminishes proliferation, and facilitates apoptosis in response to exogenous stimulation	3.07	0.0068
NP_114158.2	Acc:NP_114158]; spermatogenesis associated 9 isoform a [Source:RefSeq_peptide	3.03	0.0072
KLDC2	Kelch domain-containing protein 2; Hepatocellular carcinoma-associated antigen 33; Host cell factor homolog LCP	3.02	0.0073
TRI50	Tripartite motif-containing protein 50	3.01	0.0075
KCC1D	Calcium/calmodulin-dependent protein kinase belonging to a proposed calcium-triggered signaling cascade. May regulate calcium-mediated granulocyte function. May play a role in apoptosis of erythroleukemia cells. Activates MAP kinase MAPK3 (By similarity). In vitro, phosphorylates transcription factor CREM isoform Beta and probably CREB1	2.99	0.0076
ZN174	Transcriptional repressor	2.99	0.0077

Gene Name	Gene Description	Fold Change	p Value
HXD11	Sequence-specific transcription factor which is part of a developmental regulatory system that provides cells with specific positional identities on the anterior-posterior axis	2.97	0.0079
PKCB1	Protein kinase C-binding protein 1; Rack7; Cutaneous T-cell lymphoma-associated antigen se14-3; CTCL tumor antigen se14-3; Zinc finger MYND domain-containing protein 8	2.93	0.0083
ZFP95	May be involved in transcriptional regulation	2.91	0.0085
MAP4	Non-neuronal microtubule-associated protein. Promotes microtubule assembly	2.88	0.0089
MAGA5	Not known, though may play a role tumor transformation or progression	2.88	0.0089
GABARAPL3	Gamma-aminobutyric acid receptor-associated protein-like 3; GABA(A) receptor-associated protein-like 3	2.88	0.0033
GUF1	GTP-binding protein GUF1 homolog	2.86	0.0092
AOAH	Removes the secondary (acyloxyacyl-linked) fatty acyl chains from the lipid A region of bacterial lipopolysaccharides	2.84	0.0095
ASPM	Probable role in mitotic spindle regulation and coordination of mitotic processes (By similarity). May have a preferential role in regulating neurogenesis	2.83	0.0096
NP_064614.2	PR domain-containing protein 11	2.82	0.0098
NP_062536.2	Proline-rich region	2.82	0.0099
HMGB3	Binds preferentially single-stranded DNA and unwinds double stranded DNA	2.80	0.0433
ISCU	Involved in the assembly or repair of the [Fe-S] clusters present in iron-sulfur proteins. Binds iron	2.79	0.0103
Q9BSC4-2	NUC153	2.78	0.0105
PAPOA	Polymerase that creates the 3' poly(A) tail of mRNA's. Also required for the endoribonucleolytic cleavage reaction at some polyadenylation sites. May acquire specificity through interaction with a cleavage and polyadenylation specificity factor (CPSF) at its C-terminus	-2.76	0.0109
GBRD	GABA, the major inhibitory neurotransmitter in the vertebrate brain, mediates neuronal inhibition by binding to the GABA/benzodiazepine receptor and opening an integral chloride channel	-2.76	0.0108
RABP2	Cytosolic CRABPs may regulate the access of retinoic acid to the nuclear retinoic acid receptors. CRABP2 may participate in a regulatory feedback mechanism to control the action of retinoic acid on cell differentiation	-2.77	0.001
GBRG3	GABA, the major inhibitory neurotransmitter in the vertebrate brain, mediates neuronal inhibition by binding to the GABA/benzodiazepine receptor and opening an integral chloride channel	-2.77	0.0105
FRMD5	FERM domain-containing protein 5	-2.78	0.0105
IKKB	Phosphorylates inhibitors of NF-kappa-B thus leading to the dissociation of the inhibitor/NF-kappa-B complex and ultimately the degradation of the inhibitor. Also phosphorylates NCOA3	-2.78	0.0104

Gene Name	Gene Description	Fold Change	p Value
DOK5	Docking proteins interact with receptor tyrosine kinases and mediate particular biological responses. DOK5 functions in RET-mediated neurite outgrowth and plays a positive role in activation of the MAP kinase pathway. Putative link with downstream effectors of RET in neuronal differentiation	-2.81	0.01
CLCN3	Voltage-gated chloride channel. Chloride channels have several functions including the regulation of cell volume; membrane potential stabilization, signal transduction and transepithelial transport. May play an important role in neuronal cell function through regulation of membrane excitability by protein kinase C. It could help neuronal cells to establish short-term memory	-2.82	0.0098
DUSP4	Regulates mitogenic signal transduction by dephosphorylating both Thr and Tyr residues on MAP kinases ERK1 and ERK2	-2.82	1.71E-05
ZN659	Zinc finger protein 659	-2.83	0.0097
SMAD7	Antagonist of signaling by TGF-beta (transforming growth factor) type 1 receptor superfamily members; has been shown to inhibit TGF-beta (Transforming growth factor) and activin signaling by associating with their receptors thus preventing SMAD2 access. Functions as an adaptor to recruit SMURF2 to the TGF-beta receptor complex. SMAD7 is an inhibitory SMAD (I-SMAD) or antagonistic SMAD whose inhibitory activity is enhanced by SMURF2	-2.84	0.0095
G7D	Involved in meiotic recombination. Facilitate crossovers between homologs during meiosis	-2.84	0.0095
C1QA	C1q associates with the proenzymes C1r and C1s to yield C1, the first component of the serum complement system. The collagen-like regions of C1q interact with the Ca(2+)-dependent C1r(2)C1s(2) proenzyme complex, and efficient activation of C1 takes place on interaction of the globular heads of C1q with the Fc regions of IgG or IgM antibody present in immune complexes	-2.86	0.0093
SP1	Binds to GC box promoters elements and selectively activates mRNA synthesis from genes that contain functional recognition sites. Can interact with G/C-rich motifs from serotonin receptor promoter	-2.87	0.0091
GLE1	Required for the export of mRNAs containing poly(A) tails from the nucleus into the cytoplasm. May be involved in the terminal step of the mRNA transport through the nuclear pore complex (NPC)	-2.87	0.0091
DDFL1	Promotes cell proliferation	-2.87	0.009
TCF21	Involved in epithelial-mesenchymal interactions in kidney and lung morphogenesis that include epithelial differentiation and branching morphogenesis. May play a role in the specification or differentiation of one or more subsets of epicardial cell types	-2.88	0.009
LYZL6	Lysozyme-like protein 6 precursor	-2.88	0.009

Gene Name	Gene Description	Fold Change	p Value
IMA4	Functions in nuclear protein import as an adapter protein for nuclear receptor KPNB1. Binds specifically and directly to substrates containing either a simple or bipartite NLS motif. Docking of the importin/substrate complex to the nuclear pore complex (NPC) is mediated by KPNB1 through binding to nucleoporin FxFG repeats and the complex is subsequently translocated through the pore by an energy requiring, Ran-dependent mechanism. At the nucleoplasmic side of the NPC, Ran binds to importin-beta and the three components separate and importin-alpha and -beta are re-exported from the nucleus to the cytoplasm where GTP hydrolysis releases Ran from importin. The directionality of nuclear import is thought to be conferred by an asymmetric distribution of the GTP- and GDP-bound forms of Ran between the cytoplasm and nucleus. In vitro, mediates the nuclear import of human cytomegalovirus UL84 by recognizing a non-classical NLS. In vitro, mediates the nuclear import of human cytomegalovirus UL84 by recognizing a nonclassical NLS	-2.88	0.0089
O95394-2	Phosphoglucomutase/phosphomannomutase; Phosphoglucomutase/phosphomannomutase C-terminal; Phosphoglucomutase/phosphomannomutase alpha/beta/alpha domain I	-2.89	0.0089
Q96FJ0-2	Mov34/MPN/PAD-1	-2.89	0.0088
Q59GS4	IQ calmodulin-binding region; Myosin head, motor region	-2.91	0.0117
NP_653185.1	Acc:Q5T8I8]; Novel protein (FLJ30525) (Fragment). [Source:Uniprot/SPTREMBL	-2.91	0.0086
NP_055772.2	DENN; WD-40 repeat; dDENN	-2.92	0.0084
NOX1	NOH-1S is a voltage-gated proton channel that mediates the H(+) currents of resting phagocytes and other tissues. It participates in the regulation of cellular pH and is blocked by zinc. NOH-1L is a pyridine nucleotide-dependent oxidoreductase that generates superoxide and might conduct H(+) ions as part of its electron transport mechanism, whereas NOH-1S does not contain an electron transport chain	-2.92	0.0241
TENS1	May be involved in cell migration, cartilage development and in linking signal transduction pathways to the cytoskeleton	-2.93	0.0083
RM40	39S ribosomal protein L40, mitochondrial precursor; L40mt; MRP-40; Nuclear localization signal-containing protein deleted in velocardiofacial syndrome; Up-regulated in metastasis	-2.93	0.0083
PO3F2	Transcription factor that binds preferentially to the recognition sequence which consists of two distinct half-sites, ('GCAT') and ('TAAT'), separated by a nonconserved spacer region of 0, 2, or 3 nucleotides. Positively regulates the genes under the control of corticotropin-releasing hormone (CRH) and CRH II promoters	-2.94	0.0081

Gene Name	Gene Description	Fold Change	p Value
RPIA	Ribose-5-phosphate isomerase; Phosphoriboisomerase	-2.95	0.0081
EPO	Erythropoietin is the principal hormone involved in the regulation of erythrocyte differentiation and the maintenance of a physiological level of circulating erythrocyte mass	-2.97	0.0124
NP_079038.2	KRAB box; Zinc finger, C2H2-subtype; Zinc finger, C2H2-type	-2.97	0.0079
O60696	Zinc finger, PHD-type	-2.97	0.0079
RMD5B	RMD5 homolog B	-2.97	0.0189
AN32A	Implicated in a number of cellular processes, including proliferation, differentiation, caspase-dependent and caspase-independent apoptosis, suppression of transformation (tumor suppressor), inhibition of protein phosphatase 2A, regulation of mRNA trafficking and stability in association with ELAVL1, and inhibition of acetyltransferases as part of the INHAT (inhibitor of histone acetyltransferases) complex	-2.97	0.0039
ATN1	Atrophin-1; Dentatorubral-pallidoluysian atrophy protein	-2.97	0.0016
OR5V1	Putative odorant receptor	-2.99	0.0077
PI51B	Mediates RAC1-dependent reorganization of actin filaments (By similarity). Participates in the biosynthesis of phosphatidylinositol-4,5-bisphosphate	-3.00	0.0076
PDZD1	A scaffold protein that connects plasma membrane proteins and regulatory components, regulating their surface expression in epithelial cells apical domains. May be involved in the coordination of a diverse range of regulatory processes for ion transport and second messenger cascades. In complex with SLC9A3R1, may cluster proteins that are functionally dependent in a mutual fashion and modulate the trafficking and the activity of the associated membrane proteins. May play a role in the cellular mechanisms associated with multidrug resistance through its interaction with ABCC2 and PDZK1IP1. May potentiate the CFTR chloride channel activity. May function to connect SCARB1 with the cellular machineries for intracellular cholesterol transport and/or metabolism. May be involved in the regulation of proximal tubular Na(+)-dependent inorganic phosphate cotransport therefore playing an important role in tubule function	-3.00	0.0075
TPD52	Tumor protein D52; Protein N8	-3.01	0.0074

Gene Name	Gene Description	Fold Change	p Value
NFKB2	Appears to have dual functions such as cytoplasmic retention of attached NF-kappa-B proteins and generation of p52 by a cotranslational processing. The proteasome-mediated process ensures the production of both p52 and p100 and preserves their independent function. p52 binds to the kappa-B consensus sequence 5'-GGRNNYYCC-3', located in the enhancer region of genes involved in immune response and acute phase reactions. p52 and p100 are respectively the minor and major form; the processing of p100 being relatively poor. Isoform p49 is a subunit of the NF-kappa-B protein complex, which stimulates the HIV enhancer in synergy with p65	-3.02	0.0073
Q5TF39	Cytochrome c-type biogenesis protein CcbS; Major facilitator superfamily MFS_1	-3.03	0.0072
NP_640332.1	Ankyrin; Proline-rich region	-3.04	0.0071
DUSP7	Regulates the activity of the MAP kinase family in response to changes in the cellular environment. PYST2-S may act as a negative regulator of PYST2-L although it is unclear whether this is by competing for transcription, translation or activation factors	-3.04	0.0019
STB5L	May play a role in vesicle trafficking and exocytosis	-3.04	0.0071
OR2H1	Putative odorant receptor	-3.05	0.007
MAP7	Microtubule-stabilizing protein that may play an important role during reorganization of microtubules during polarization and differentiation of epithelial cells. Associates with microtubules in a dynamic manner. May play a role in the formation of intercellular contacts. Colocalization with TRPV4 results in the redistribution of TRPV4 toward the membrane and may link cytoskeletal microfilaments	-3.06	0.0069
WD51A	WD repeat protein 51A	-3.07	0.0069
NP_060554.3	Tropomyosin	-3.07	3.2E-05
ZC12B	Protein ZC3H12B	-3.07	0.0068
ENSG00000180150	High mobility group protein HMG14 and HMG17; Histone H5	-3.07	0.0068
LOC197350	2Fe-2S ferredoxin, iron-sulfur binding site; Caspase, p20 subunit; Peptidase C14, caspase catalytic; Peptidase C14, caspase non-catalytic subunit p10; Peptidase C14, caspase precursor p45	-3.09	0.0218
ENSG00000117707	Homeobox prospero-like	-3.10	0.0066
STYK1	Probable tyrosine protein-kinase, which has strong transforming capabilities on a variety of cell lines. When overexpressed, it can also induce tumor cell invasion as well as metastasis in distant organs. May act by activating both MAP kinase and phosphatidylinositol 3'-kinases (PI3K) pathways	-3.10	0.0065
GAGE10	Antigen, recognized on melanoma by autologous cytolytic T-lymphocytes; Antigen, recognized on melanoma by autologous cytolytic T-lymphocytes. Completely silent in normal adult tissues, except testis	-3.11	0.0065

Gene Name	Gene Description	Fold Change	p Value
VATG3	Catalytic subunit of the peripheral V1 complex of vacuolar ATPase (V-ATPase). V-ATPase is responsible for acidifying a variety of intracellular compartments in eukaryotic cells	-3.11	0.0065
SNG4	Synaptogyrin-4	-3.11	0.0003
Q9BT26	Acc:Q9BT26]; MGC10981 protein. [Source:Uniprot/SPTREMBL	-3.12	0.0064
KBTB3	Kelch repeat and BTB domain-containing protein 3; BTB and kelch domain-containing protein 3	-3.13	0.0063
FOSL2	Fos-related antigen 2	-3.13	0.0063
CLD5	Plays a major role in tight junction-specific obliteration of the intercellular space	-3.13	4.38E-05
PGM5	Component of adherens-type cell-cell and cell-matrix junctions. Lacks phosphoglucomutase activity	-3.14	0.0062
ENSG00000174672	Proline-rich region; Protein kinase; Serine/threonine protein kinase, active site; Tyrosine protein kinase	-3.15	0.0003
NP_001276.2	ATPase, F1 complex, gamma subunit; Chloride channel calcium-activated; von Willebrand factor, type A	-3.21	0.0057
GOG8A	Adapter protein that may provide indirect link between the endocytic membrane traffic and the actin assembly machinery. May regulate the formation of clathrin-coated vesicles; May be involved in maintaining Golgi structure	-3.22	0.0056
F261	Synthesis and degradation of fructose 2,6-bisphosphate	-3.24	0.0003
ABCA2	Probable transporter, its natural substrate has not been found yet. May have a role in macrophage lipid metabolism and neural development	-3.24	0.0044
CIDEA	Activates apoptosis	-3.24	0.0055
ACV1B	On ligand binding, forms a receptor complex consisting of two type II and two type I transmembrane serine/threonine kinases. Type II receptors phosphorylate and activate type I receptors which autophosphorylate, then bind and activate SMAD transcriptional regulators	-3.25	0.0054
LPP3	Catalyzes the conversion of phosphatidic acid (PA) to diacylglycerol (DG). In addition it hydrolyzes lysophosphatidic acid (LPA), ceramide-1-phosphate (C-1-P) and sphingosine-1-phosphate (S-1-P). The relative catalytic efficiency is LPA = PA > C-1-P > S-1-P. May be involved in cell adhesion and in cell-cell interactions	-3.25	0.0054
ZN703	May function as a transcriptional repressor	-3.26	0.0053
SEM3D	Induces the collapse and paralysis of neuronal growth cones. Could potentially act as repulsive cues toward specific neuronal populations. Binds to neuropilin	-3.27	0.0053
Q4LDG9	Leucine-rich repeat	-3.27	0.0052
NP_112579.2	GSG1-like; Voltage-dependent calcium channel gamma	-3.30	0.0051
RNAS1	Endonuclease that catalyzes the cleavage of RNA on the 3' side of pyrimidine nucleotides. Acts on single stranded and double stranded RNA	-3.33	0.0403

Gene Name	Gene Description	Fold Change	p Value
Q8TEE9	Proline-rich region	-3.39	0.001
ZN445	May be involved in transcriptional regulation	-3.41	7.32E-06
NP_006324.1	Exosome-associated	-3.42	0.0044
LTBP3	May be involved in the assembly, secretion and targeting of TGFB1 to sites at which it is stored and/or activated. May play critical roles in controlling and directing the activity of TGFB1. May have a structural role in the extra cellular matrix (ECM)	-3.42	4.30E-03
HS2ST	Heparan sulfate 2-O-sulfotransferase	-3.43	0.0043
Q5T7M9	Acc:Q5T7M9]; Novel protein possible ortholog of mouse RIKEN cDNA 2900024C23 gene. [Source:Uniprot/SPTREMBL	-3.44	0.0042
WIF1	Binds to WNT proteins and inhibits their activities. May be involved in mesoderm segmentation	-3.45	0.0042
LRIG1	Act as a feedback negative regulator of signaling by receptor tyrosine kinases, through a mechanism that involves enhancement of receptor ubiquitination and accelerated intracellular degradation	-3.45	0.0042
M4K3	May play a role in the response to environmental stress. Appears to act upstream of the JUN N-terminal pathway	-3.45	0.0042
MCHL1	Pro-MCH-like protein 1; Pro-melanin-concentrating hormone-like protein 1; Pro-MCH variant; Pro-MCH-like protein 2; Pro-melanin-concentrating hormone-like protein 2	-3.51	0.0039
NP_203131.1	Inhibits signal transduction by increasing the GTPase activity of G protein alpha subunits thereby driving them into their inactive GDP-bound form. Preferentially binds to G(o)-alpha and G(i)-alpha-3	-3.55	0.0038
TMM98	Transmembrane protein 98; Protein TADA1	-3.57	0.0037
PAK1	The activated kinase acts on a variety of targets. Likely to be the GTPase effector that links the Rho-related GTPases to the JNK MAP kinase pathway. Activated by CDC42 and RAC1. Involved in dissolution of stress fibers and reorganization of focal complexes. Involved in regulation of microtubule biogenesis through phosphorylation of TBCB. Activity is inhibited in cells undergoing apoptosis, potentially due to binding of CDC2L1 and CDC2L2	-3.57	0.0003
NP_733936.1	Succinate semialdehyde dehydrogenase, mitochondrial precursor; NAD(+)-dependent succinic semialdehyde dehydrogenase	-3.57	0.0037
NNAT	May participate in the maintenance of segment identity in the hindbrain and pituitary development, and maturation or maintenance of the overall structure of the nervous system. May function as a regulatory subunit of ion channels	-3.57	0.0002
MAMC2	MAM domain-containing protein 2 precursor	-3.59	0.0036
ENSG00000110077	CD20/IgE Fc receptor beta subunit	-3.59	0.0036
SOX18	Binds to the consensus sequence 5'-AACAAAG-3' and is able to trans-activate transcription via this site	-3.62	0.0002

Gene Name	Gene Description	Fold Change	p Value
UBP37	Ubiquitin carboxyl-terminal hydrolase 37; Ubiquitin thioesterase 37; Ubiquitin-specific-processing protease 37; Deubiquitinating enzyme 37	-3.68	0.0033
TRAF1	Adapter protein and signal transducer that links members of the tumor necrosis factor receptor family to different signaling pathways by association with the receptor cytoplasmic domain and kinases. Mediates activation of NF-kappa-B and JNK and is involved in apoptosis. The TRAF1/TRAF2 complex recruits the apoptotic suppressors BIRC2 and BIRC3 to TNFRSF1B/TNFR2	-3.70	0.0032
ODFP	Component of the outer dense fibers (ODF) of spermatozoa. ODF are filamentous structures located on the outside of the axoneme in the midpiece and principal piece of the mammalian sperm tail and may help to maintain the passive elastic structures and elastic recoil of the sperm tail	-3.74	0.0031
C43BP	Phosphorylates on Ser and Thr residues the Goodpasture autoantigen (in vitro). Isoform 2 seems to be less active	-3.75	0.0002
Q9NSC5-3	EVH1	-3.75	0.0025
Q8WVI0	Acc:Q8WVI0]; SNHG8 protein (Fragment). [Source:Uniprot/SPTREMBL	-3.76	0.003
SYLC	Leucyl-tRNA synthetase, cytoplasmic; Leucine--tRNA ligase; LeuRS	-3.83	0.0221
TWST1	Probable transcription factor, which seems to be involved in the negative regulation of cellular determination and in the differentiation of several lineages including myogenesis, osteogenesis, and neurogenesis. Inhibits myogenesis by sequestrating E proteins, inhibiting trans-activation by MEF2, and inhibiting DNA-binding by MYOD1 through physical interaction. This interaction probably involves the basic domains of both proteins (By similarity). Also represses expression of proinflammatory cytokines such as TNFA and IL1B	-3.83	0.0028
Q53RY2	Acc:P15336]; Cyclic AMP-dependent transcription factor ATF-2 (Activating transcription factor 2) (cAMP response element-binding protein CRE- BP1) (HB16). [Source:Uniprot/SWISSPROT	-3.84	0.0028
NP_659415.1	Acc:Q96M89]; CDNA FLJ32745 fis, clone TESTI2001511, weakly similar to MYOSIN II HEAVY CHAIN, NON MUSCLE. [Source:Uniprot/SPTREMBL	-3.88	0.0027
NP_116277.2	Collagen triple helix repeat; Dopamine D4 receptor; Fibrillar collagen, C-terminal; Pistil-specific extensin-like protein; Proline-rich region	-3.94	0.0025
AN30A	Ankyrin repeat domain-containing protein 30A; Serologically defined breast cancer antigen NY-BR-1	-3.95	0.0025
PLPL	Uncharacterized protein C14orf162; Myelin proteolipid protein-like protein	-3.98	0.0024
ENSG00000166965	Regulator of chromosome condensation, RCC1	-4.00	0.0024

Gene Name	Gene Description	Fold Change	p Value
5HT1E	This is one of the several different receptors for 5-hydroxytryptamine (serotonin), a biogenic hormone that functions as a neurotransmitter, a hormone, and a mitogen. The activity of this receptor is mediated by G proteins that inhibit adenylate cyclase activity	-4.00	0.0024
DOCK4	Involved in regulation of adherens junction between cells. Functions as a guanine nucleotide exchange factor (GEF), which activates Rap1 small GTPase by exchanging bound GDP for free GTP	-4.03	0.0023
PLOD2	Forms hydroxylysine residues in -Xaa-Lys-Gly- sequences in collagens. These hydroxylysines serve as sites of attachment for carbohydrate units and are essential for the stability of the intermolecular collagen cross-links	-4.05	0.0021
DOK3	Docking proteins interact with receptor tyrosine kinases and mediate particular biological responses. DOK3 is a negative regulator of JNK signaling in B-cells through interaction with INPP5D/SHIP. May modulate Abl function	-4.07	0.0022
ACCN3	Cation channel with high affinity for sodium, which is gated by extracellular protons and inhibited by the diuretic amiloride. Generates a biphasic current with a fast inactivating and a slow sustained phase. In sensory neurons is proposed to mediate the pain induced by acidosis that occurs in ischemic, damaged or inflamed tissue. May be involved in hyperalgesia. May play a role in mechanoreception. Heteromeric channel assembly seems to modulate channel properties	-4.14	0.0021
DCLK1	Probable kinase that may be involved in a calcium-signaling pathway controlling neuronal migration in the developing brain. May also participate in functions of the mature nervous system	-4.28	0.0019
ZCHC4	May be a methyltransferase	-4.34	0.0018
GRID2	Receptor for glutamate. L-glutamate acts as an excitatory neurotransmitter at many synapses in the central nervous system. The postsynaptic actions of Glu are mediated by a variety of receptors that are named according to their selective agonists	-4.35	0.0018
NP_055483.2	Acc:NP_149081]; GREB1 protein isoform b [Source:RefSeq_peptide	-4.36	0.0025
ENSG00000162105	Ankyrin; PDZ/DHR/GLGF; Src homology-3; Variant SH3	-4.37	0.0017
SMO	G protein-coupled receptor that probably associates with the patched protein (PTCH) to transduce the hedgehog's proteins signal. Binding of sonic hedgehog (SHH) to its receptor patched is thought to prevent normal inhibition by patched of smoothened (SMO)	-4.37	0.0017
ENSG00000196229	HMG-I and HMG-Y, DNA-binding; Molluscan rhodopsin C-terminal tail; Proline-rich region	-4.43	0.0016

Gene Name	Gene Description	Fold Change	p Value
SSR1	Receptor for somatostatin with higher affinity for somatostatin-14 than -28. This receptor is coupled via pertussis toxin sensitive G proteins to inhibition of adenylyl cyclase. In addition it stimulates phosphotyrosine phosphatase and Na(+)/H(+) exchanger via pertussis toxin insensitive G proteins	-4.47	0.0016
Q5VTT3	Tyrosine-protein kinase receptor which may be involved in the early formation of the chondrocytes. It seems to be required for cartilage and growth plate development	-4.55	0.0015
ABHD4	Lysophospholipase selective for N-acyl phosphatidylethanolamine (NAPE). Contributes to the biosynthesis of N-acyl ethanolamines, including the endocannabinoid anandamide by hydrolyzing the sn-1 and sn-2 acyl chains from N-acyl phosphatidylethanolamine (NAPE) generating glycerophospho-N-acyl ethanolamine (GP-NAE), an intermediate for N-acyl ethanolamine biosynthesis. Hydrolyzes substrates bearing saturated, monounsaturated, polyunsaturated N-acyl chains. Shows no significant activity towards other lysophospholipids, including lysophosphatidylcholine, lysophosphatidylethanolamine and lysophosphatidylserine	-4.56	0.0015
IGHV3-11	Immunoglobulin; Immunoglobulin V-set; Immunoglobulin-like	-4.76	0.0013
SUCR1	Receptor for succinate	-4.81	0.0012
FAN	Couples the p55 TNF-receptor (TNF-R55 / TNFR1) to neutral sphingomyelinase (N-SMASE). Specifically binds to the N-smase activation domain of TNF-R55. May regulate ceramide production by N-SMASE	-4.81	0.0012
NP_001020026.1	May be involved in transcriptional regulation	-5.04	0.001
CAD12	Cadherins are calcium dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types	-5.12	0.001
FGF2	The heparin-binding growth factors are angiogenic agents in vivo and are potent mitogens for a variety of cell types in vitro. There are differences in the tissue distribution and concentration of these 2 growth factors	-5.27	0.0008
PNMA1	Paraneoplastic antigen Ma1; Neuron- and testis-specific protein 1; 37 kDa neuronal protein	-5.28	0.0009
Q8NBC0	Orphan nuclear receptor, HMR type	-5.36	3.25E-05
NP_996803.2	Low density lipoprotein-receptor, class A; MAM	-5.37	0.0008
Q6ZN87	KRAB box	-5.40	0.0008
CAN11	Calcium-regulated non-lysosomal thiol-protease which catalyze limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction	-5.41	0.0008

Gene Name	Gene Description	Fold Change	p Value
NP_001025186.1	Photoreceptor required for regulation of circadian rhythm. Contributes to pupillar reflex and other non-image forming responses to light. May be able to isomerize covalently bound all-trans retinal back to 11-cis retinal	-5.43	0.0008
FXYD6	FXYD domain-containing ion transport regulator 6 precursor	-5.59	0.0007
CLGN	Probably plays an important role in spermatogenesis. Binds calcium ions	-5.78	0.0007
ADCK3	May be a chaperone-like protein essential for the proper conformation and functioning of protein complexes in the respiratory chain	-5.98	0.0006
PSD12	Acts as a regulatory subunit of the 26S proteasome which is involved in the ATP-dependent degradation of ubiquitinated proteins	-6.44	0.0005
NP_004849.2	HCO3- transporter, cytoplasmic; HCO3- transporter, eukaryote; HCO3-transporter, c-terminal; Na+/HCO3- transporter	-6.60	0.0004
Q6ICE7	Acc:Q6ICE7]; CN5H6.4 protein (OTTHUMP00000028648). [Source:Uniprot/SPTREMBL	-6.86	1.56E-05
TMG1	Transmembrane gamma-carboxyglutamic acid protein 1 precursor; Proline-rich Gla protein 1; Proline-rich gamma-carboxyglutamic acid protein 1	-6.87	0.0093
DEF	Regulates the p53 pathway to control the expansion growth of digestive organs	-6.98	0.0103
MFSD4	Major facilitator superfamily domain-containing protein 4	-7.32	0.0003
CDC6	Involved in the initiation of DNA replication. Also participates in checkpoint controls that ensure DNA replication is completed before mitosis is initiated	-7.58	0.0003
PLAL1	Shows weak transcriptional activatory activity. Transcriptional regulator of the type 1 receptor for pituitary adenylate cyclase-activating polypeptide	-7.76	0.0079
Q99490-2	Ankyrin; Arf GTPase activating protein; Miro-like; Pleckstrin-like; Ras; Ras GTPase	-8.10	0.0035
ENSG00000199437	Acc:RF00416]; Small nucleolar RNA ACA43 [Source:RFAM	-8.25	0.0163
Q86SZ9	WD repeat protein 23	-8.87	0.0002
GMDS	Conversion of GDP-D-mannose to GDP-4-keto-6-D-deoxymannose	-8.94	0.0002
TRBV6-1	Immunoglobulin V-set; Immunoglobulin-like	-9.27	0.0002
SC5A6	Transports pantothenate, biotin and lipoate in the presence of sodium	-12.33	8.34E-05
EYA4	Thought to play a role in transcription regulation during organogenesis through its intrinsic protein phosphatase activity. May be involved in development of the eye	-118.41	1.61E-06
ENP1	In the nervous system, could hydrolyze ATP and other nucleotides to regulate purinergic neurotransmission. Could also be implicated in the prevention of platelet aggregation. Hydrolyzes ATP and ADP equally well	2.74	0.0112
JHDM1D	JmjC domain-containing histone demethylation protein 1D	2.73	0.0113

Gene Name	Gene Description	Fold Change	p Value
SIM2	Transcription factor that may be a master gene of CNS development in cooperation with Arnt. It may have pleiotropic effects in the tissues expressed during development	2.73	0.0114
B3GN2	Catalyzes the initiation and elongation of poly-N-acetyllactosamine chains	2.71	0.0116
CCDC5	Regulator of spindle function and integrity during the metaphase-anaphase transition	2.71	0.0117
Q14591-2	Zinc finger, C2H2-subtype; Zinc finger, C2H2-type	2.70	0.0119
Q86V61	GAT; VHS	2.69	0.0121
LECT1	Bifunctional growth regulator that stimulates the growth of cultured chondrocytes in the presence of basic fibroblast growth factor (FGF) but inhibits the growth of cultured vascular endothelial cells. May contribute to the rapid growth of cartilage and vascular invasion prior to the replacement of cartilage by bone during endochondral bone development	2.68	0.0123
ENSG00000196547	Glycoside hydrolase, family 38; Glycosyl hydrolases 38, C-terminal	2.67	0.0125
DKKL1	Dickkopf-like protein 1 precursor; Soggy-1 protein; SGY-1	2.67	0.0126
CR2	Receptor for complement C3Dd, for the Epstein-Barr virus on human B-cells and T-cells and for HNRPU. Participates in B lymphocytes activation	2.67	0.0126
GBRG3	GABA, the major inhibitory neurotransmitter in the vertebrate brain, mediates neuronal inhibition by binding to the GABA/benzodiazepine receptor and opening an integral chloride channel	2.65	0.0129
ENSG00000186660	Antifreeze protein, type I; Zinc finger, C2H2-type	2.64	0.0131
ZNF490	May be involved in transcriptional regulation	2.64	0.0132
PCOC1	Binds to the C-terminal propeptide of type I procollagen and enhances procollagen C-proteinase activity	2.64	0.0132
ZNF506	May be involved in transcriptional regulation	2.62	0.0069
Q4ZG89	Major facilitator superfamily; Major facilitator superfamily MFS_1; Sugar transporter superfamily; Tetracycline resistance protein	2.61	0.0138
ATP7B	Involved in the export of copper out of the cells, such as the efflux of hepatic copper into the bile	2.61	0.0138
HSPA1A	In cooperation with other chaperones, Hsp70s stabilize preexistent proteins against aggregation and mediate the folding of newly translated polypeptides in the cytosol as well as within organelles. These chaperones participate in all these processes through their ability to recognize nonnative conformations of other proteins. They bind extended peptide segments with a net hydrophobic character exposed by polypeptides during translation and membrane translocation, or following stress-induced damage	2.60	0.0141

Gene Name	Gene Description	Fold Change	p Value
ANXA1	Calcium/phospholipid-binding protein which promotes membrane fusion and is involved in exocytosis. This protein regulates phospholipase A2 activity. It seems to bind from two to four calcium ions with high affinity	2.59	0.0247
IG2AS	Putative insulin-like growth factor 2 antisense gene protein; IGF2-AS; PEG8/IGF2AS protein	2.58	0.0099
CP4F2	Cytochromes P450 are a group of heme-thiolate monooxygenases. In liver microsomes, this enzyme is involved in an NADPH-dependent electron transport pathway. It oxidizes a variety of structurally unrelated compounds, including steroids, fatty acids, and xenobiotics; Cytochromes P450 are a group of heme-thiolate monooxygenases. This enzyme requires molecular oxygen and NADPH for the omega-hydroxylation of LTB4, a potent chemoattractant for polymorphonuclear leukocytes	2.56	0.0152
Q8IY89	Acc:Q9H7C2]; CDNA: FLJ21062 fis, clone CAS01044. [Source:Uniprot/SPTREMBL	2.54	0.0156
NP_116216.1	Basic helix-loop-helix dimerisation region bHLH; Proline-rich region	2.53	0.0001
S29A2	Mediates equilibrative transport of purine, pyrimidine nucleosides and the purine base hypoxanthine. Less sensitive than SLC29A1 to inhibition by nitrobenzylthioinosine (NBMPR), dipyridamole, dilazep and draflazine	2.53	0.016
PO4F1	Probable transcription factor which may play a role in the regulation of specific gene expression within a subset of neuronal lineages. May play a role in determining or maintaining the identities of a small subset of visual system neurons	2.53	0.0161
SCN4A	This protein mediates the voltage-dependent sodium ion permeability of excitable membranes. Assuming opened or closed conformations in response to the voltage difference across the membrane, the protein forms a sodium-selective channel through which Na(+) ions may pass in accordance with their electrochemical gradient. This sodium channel may be present in both denervated and innervated skeletal muscle	2.51	0.0167
TGFB3	Involved in embryogenesis and cell differentiation	2.50	0.0433
NP_071768.2	Ubiquitin	2.50	0.0293
EIF2AK4	Can phosphorylate the alpha subunit of EIF2 and may mediate translational control	2.49	0.0172
HXB5	Sequence-specific transcription factor which is part of a developmental regulatory system that provides cells with specific positional identities on the anterior-posterior axis	2.48	0.0175
NP_659002.1	Antifreeze protein, type I; Pollen allergen Poa pIX/Phl pVI, C-terminal; Zinc finger, CCCH-type	2.48	0.0176
MLZE	Melanoma-derived leucine zipper-containing extranuclear factor	2.47	0.0177
DB118	Has antibacterial activity	2.47	0.0177

Gene Name	Gene Description	Fold Change	p Value
PA24B	Calcium-dependent phospholipase A2 that selectively hydrolyzes glycerophospholipids in the sn-2 position with a preference for arachidonoyl phospholipids. Has a much weaker activity than PLA2G4A. Isoform 3 has calcium-dependent activity against palmitoyl-arachidonyl-phosphatidylethanolamine and low level lysophospholipase activity but no activity against phosphatidylcholine	2.46	0.0183
GPR17	Dual specificity receptor for uracil nucleotides and cysteinyl leukotrienes (CysLTs). Signals through G(i) and inhibition of adenylyl cyclase. May mediate brain damage by nucleotides and CysLTs following ischemia	2.45	0.0185
ARHGF	Specific GEF for RhoA activation and the regulation of vascular smooth muscle contractility	2.44	0.0471
NLK	Role in cell fate determination, required for differentiation of bone marrow stromal cells. Acts downstream of MAP3K7 and HIPK2 to negatively regulate the canonical Wnt/beta-catenin signaling pathway and the phosphorylation and destruction of the MYB transcription factor. May suppress a wide range of transcription factors by phosphorylation of the coactivator, CREBBP	2.44	0.0034
DUFFY	Non-specific receptor for many chemokines such as IL-8, GRO, RANTES, MCP-1 and TARC. It is also the receptor for the human malaria parasites Plasmodium vivax and Plasmodium knowlesi	2.43	0.0191
FBX24	Substrate-recognition component of the SCF (SKP1-CUL1-F-box protein)-type E3 ubiquitin ligase complex	2.42	0.0194
HEM4	Uroporphyrinogen-III synthase; UROS; Uroporphyrinogen-III cosynthetase; Hydroxymethylbilane hydrolyase [cyclizing]; UROIIIS	2.41	0.0198
CLC3A	C-type lectin domain family 3 member A precursor; C-type lectin superfamily member 1; Cartilage-derived C-type lectin	2.41	0.02
ADR2	Receptor for globular and full-length adiponectin (APM1), an essential hormone secreted by adipocytes that acts as an antidiabetic. Probably involved in metabolic pathways that regulate lipid metabolism such as fatty acid oxidation. Mediates increased AMPK, PPARA ligand activity, fatty acid oxidation and glucose uptake by adiponectin. Has some intermediate-affinity receptor activity for both globular and full-length adiponectin	2.40	0.0202
PO2F3	Transcription factor that binds to the octamer motif (5'-ATTTGCAT-3'). Regulated the expression of a number of genes such as SPRR2A or placental lactogen	2.40	0.0204

Gene Name	Gene Description	Fold Change	p Value
P2RX1	Ligand gated ion channel with relatively high calcium permeability. Binding to ATP mediates synaptic transmission between neurons and from neurons to smooth muscle. Seems to be linked to apoptosis, by increasing the intracellular concentration of calcium in the presence of ATP, leading to programmed cell death	2.40	0.0205
GNS	N-acetylglucosamine-6-sulfatase precursor; G6S; Glucosamine-6-sulfatase	2.38	0.0211
Q99919	Glycoside hydrolase family 2, TIM barrel	2.38	0.0212
MINK1	Serine/threonine kinase that may play a role in the response to environmental stress. Appears to act upstream of the JUN N-terminal pathway. May play a role in the development of the brain	2.37	0.0214
Q9Y3K2	Glutathione S-transferase, C-terminal; Glutathione S-transferase, N-terminal	2.37	0.0215
TEAD1	Binds specifically and cooperatively to the SPH and GT-IIC "enhansons" (5'-GTGGAATGT-3') and activates transcription in vivo in a cell-specific manner. The activation function appears to be mediated by a limiting cell-specific transcriptional intermediary factor (TIF). Involved in cardiac development. Binds to the M-CAT motif	2.37	0.0215
MRCKB	May act as a downstream effector of CDC42 in cytoskeletal reorganization. Contributes to the actomyosin contractility required for cell invasion, through the regulation of MYPT1 and thus MLC2 phosphorylation	2.37	0.0217
ARL5A	Endoglycosidase which is a cell surface and extracellular matrix-degrading enzyme. Cleaves heparan sulfate proteoglycans (HSPGs) into heparan sulfate side chains and core proteoglycans. Also implicated in the extravasation of leukocytes and tumor cell lines. Due to its contribution to metastasis and angiogenesis, it is considered to be a potential target for anti-cancer therapies; Lacks ADP-ribosylation enhancing activity	2.36	0.0218
Q9NZS4	HECT	2.36	0.0222
PODO	Plays a role in the regulation of glomerular permeability, acting probably as a linker between the plasma membrane and the cytoskeleton	2.35	0.0225
ENSG00000135365	Proline-rich region; Zinc finger, PHD-type	2.34	0.0025
APOL1	May affect the movement of lipids in the cytoplasm or allow the binding of lipids to organelles; May play a role in lipid exchange and transport throughout the body. May participate in reverse cholesterol transport from peripheral cells to the liver	2.34	0.023
H11	Histones H1 are necessary for the condensation of nucleosome chains into higher order structures	2.34	0.023
S26A2	Sulfate transporter. May play a role in endochondral bone formation	2.34	0.0231
ENSG00000104915	Target SNARE coiled-coil region	2.33	0.0235

Gene Name	Gene Description	Fold Change	p Value
BSN	Is thought to be involved in the organization of the cytomatrix at the nerve terminals active zone (CAZ) which regulates neurotransmitter release. Seems to act through binding to ERC2/CAST1. Essential in regulated neurotransmitter release from a subset of brain glutamatergic synapses. Involved in the formation of the retinal photoreceptor ribbon synapses	2.32	0.0236
CXA3	One gap junction consists of a cluster of closely packed pairs of transmembrane channels, the connexons, through which materials of low MW diffuse from one cell to a neighboring cell	2.32	0.0239
DARS2	Aspartyl-tRNA synthetase, mitochondrial precursor; Aspartate--tRNA ligase; AspRS	2.32	0.0241
NP_203752.1	Ankyrin; Tetratricopeptide TPR_1; Tetratricopeptide TPR_2; Tetratricopeptide region	2.31	0.0243
GNS	N-acetylglucosamine-6-sulfatase precursor; G6S; Glucosamine-6-sulfatase	2.31	0.0243
ZN501	May be involved in transcriptional regulation	2.30	0.0246
Q5VT18	Guanylate kinase; L27; PDZ/DHR/GLGF; Protein kinase; Src homology-3; Variant SH3	2.29	0.004
NP_612390.1	Ferric reductase, NAD binding; Flavoprotein pyridine nucleotide cytochrome reductase; NADH:cytochrome b5 reductase (CBR); Oxidoreductase FAD/NAD(P)-binding; Phenol hydroxylase reductase	2.29	0.0256
NP_057423.1	Guanine-nucleotide dissociation stimulator CDC25; RA	2.28	0.0258
MTL5	May have a role in spermatogenesis	2.28	0.026
TBCE	Tubulin-folding protein; involved in the second step of the tubulin folding pathway. Seems to be implicated in the maintenance of the neuronal microtubule network. Involved in regulation of tubulin heterodimer dissociation	2.27	0.0262
NP_079136.1	SET	2.27	0.0263
NP_055148.2	CD80-like, immunoglobulin C2-set; Immunoglobulin; Immunoglobulin I-set; Immunoglobulin V-set; Immunoglobulin-like; Vascular cell adhesion molecule-1	2.27	0.0264
CLK3	Phosphorylates serine- and arginine-rich (SR) proteins of the spliceosomal complex. May be a constituent of a network of regulatory mechanisms that enable SR proteins to control RNA splicing. Phosphorylates serines, threonines and tyrosines	2.27	0.0264
TMPS6	May play a specialized role in matrix remodeling processes in liver	2.27	0.0266
TXD13	Thioredoxin domain-containing protein 13 precursor	2.27	0.0267
NP_113634.3	Acc:Q6GW03]; HBV X-transactivated protein 13. [Source:Uniprot/SPTREMBL	2.26	0.0268
Q9H252-2	WD repeat protein 68; WD repeat protein An11 homolog	2.26	0.027

Gene Name	Gene Description	Fold Change	p Value
PELI1	Scaffold protein involved in the IL-1 signaling pathway via its interaction with the complex containing IRAK kinases and TRAF6. Required for NF-kappa-B activation and IL-8 gene expression in response to IL-1	2.26	0.027
Q9NXB7	Peptidase M14, carboxypeptidase A	2.26	0.0271
NP_001034976.1	May be involved in several stages of intracellular trafficking. Could play an important role in the regulation of glucose transport by insulin. May act as a downstream effector of RHOQ/TC10 in the regulation of insulin-stimulated glucose transport	2.25	0.0277
CEAM3	Major granulocyte receptor mediating recognition and efficient opsonin-independent phagocytosis of CEACAM-binding microorganisms, including Neissiria, Moxarella and Haemophilus species, thus playing an important role in the clearance of pathogens by the innate immune system. Responsible for RAC1 stimulation in the course of pathogen phagocytosis	2.25	0.0277
CM35A	CMRF35-A antigen precursor; CMRF-35; CD300c antigen	2.25	0.0278
NP_057732.2	Lupus La protein; RNA-binding protein Lupus La; RNA-binding region RNP-1 (RNA recognition motif)	2.25	0.0278
ATG4A	Cysteine protease required for autophagy, which cleaves the C-terminal part of either MAP1LC3, GABARAPL2 or GABARAP, allowing the liberation of form I. A subpopulation of form I is subsequently converted to a smaller form (form II). Form II, with a revealed C-terminal glycine, is considered to be the phosphatidylethanolamine (PE)-conjugated form, and has the capacity for the binding to autophagosomes. Preferred substrate is GABARAPL2 followed by MAP1LC3A and GABARAP	2.24	0.028
EGR1	Transcriptional regulator. Recognizes and binds to the DNA sequence 5'-CGCCCCCGC-3'(EGR-site). Activates the transcription of target genes whose products are required for mitogenesis and differentiation	2.24	0.0282
ABI2	May act in regulation of cell growth and transformation by interacting with nonreceptor tyrosine kinases ABL1 and/or ABL2. May be involved in cytoskeletal reorganization. Regulates ABL1/c-Abl-mediated phosphorylation of MENA	2.23	0.0286
Q8TC23	Negatively modulates RNA polymerase II function by binding to RPB5	2.21	0.0304
O60729-3	Dual specificity protein phosphatase; Tyrosine specific protein phosphatase and dual specificity protein phosphatase	2.20	0.0305
MFGM	Specific ligand for the alpha-v/beta-3 and alpha-v/beta-5 receptors. Also binds to phosphatidylserine-enriched cell surfaces in a receptor-independent manner. Zona pellucida-binding protein which may play a role in gamete interaction. Binds specifically to rotavirus and inhibits its replication	2.20	0.0305

Gene Name	Gene Description	Fold Change	p Value
KCD12	BTB/POZ domain-containing protein KCTD12; Pfetin; Predominantly fetal expressed T1 domain	2.20	0.0308
GP116	May have a role in the regulation of acid-base balance	2.20	0.0308
Q5TCU6	Band 4.1; I/LWEQ; Proline-rich region; Small proline-rich; Vinculin/alpha-catenin	2.20	0.031
S12A2	Electrically silent transporter system. Mediates sodium and chloride reabsorption. Plays a vital role in the regulation of ionic balance and cell volume	2.20	0.031
HDAC5	Responsible for the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3 and H4). Histone deacetylation gives a tag for epigenetic repression and plays an important role in transcriptional regulation, cell cycle progression and developmental events. Histone deacetylases act via the formation of large multiprotein complexes. Involved in muscle maturation by repressing transcription of myocyte enhancer MEF2C. During muscle differentiation, it shuttles into the cytoplasm, allowing the expression of myocyte enhancer factors	2.19	0.0314
ZN746	May be involved in transcriptional regulation	2.18	0.0321
KCP2	Keratinocytes-associated protein 2; KCP-2	2.18	0.0324
NP_071769.1	Guanylate-binding protein	2.17	0.0328
NP_005194.3	Collagen triple helix repeat; Galanin 3 receptor; Proline-rich region	2.17	0.0331
G137B	Integral membrane protein GPR137B; Transmembrane 7 superfamily member 1	2.16	0.0336
MESD2	Mesoderm development candidate 2; Renal carcinoma antigen NY-REN-61	2.16	0.034
CP2C9	Cytochromes P450 are a group of heme-thiolate monooxygenases. In liver microsomes, this enzyme is involved in an NADPH-dependent electron transport pathway. It oxidizes a variety of structurally unrelated compounds, including steroids, fatty acids, and xenobiotics. This enzyme contributes to the wide pharmacokinetics variability of the metabolism of drugs such as S-warfarin, diclofenac, phenytoin, tolbutamide and losartan	2.15	0.034
SO2B1	Mediates the Na(+)-independent transport of organic anions such as taurocholate, the prostaglandins PGD2, PGE1, PGE2, leukotriene C4, thromboxane B2 and iloprost	2.15	0.0344
ENSA	Endogenous ligand for sulfonylurea receptor. By inhibiting sulfonylurea from binding to the receptor, it reduces K(ATP) channel currents and thereby stimulates insulin secretion	2.14	0.0348
Q9NZ71-2	Decoy receptor for the cytotoxic ligands TNFS14/LIGHT and TNFSF6/FASL. Protects against apoptosis; Probable ATP-dependent DNA helicase	2.14	0.0348
FCHO2	FCH domain only protein 2	2.14	0.0348
DBC1	Inhibits cell proliferation by negative regulation of the G1/S transition. Mediates cell death which is not of the classical apoptotic type and regulates expression of components of the plasminogen pathway	2.13	0.0356

Gene Name	Gene Description	Fold Change	p Value
HMOX1	Heme oxygenase cleaves the heme ring at the alpha methene bridge to form biliverdin. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. Under physiological conditions, the activity of heme oxygenase is highest in the spleen, where senescent erythrocytes are sequestrated and destroyed	2.13	0.036
NUD16	NUDIX hydrolase	2.13	0.0362
GTR14	Facilitative glucose transporter (By similarity). May have a specific function related to spermatogenesis; Facilitative glucose transporter. Probably a neuronal glucose transporter	2.12	0.0365
GLPE	This protein is a minor sialoglycoprotein in human erythrocyte membranes	2.12	0.037
GCNT1	Forms critical branches in O-glycans	2.12	0.0371
CTDP1	Processively dephosphorylates 'Ser-2' and 'Ser-5' of the heptad repeats YSPTSPS in the C-terminal domain of the largest RNA polymerase II subunit. This promotes the activity of RNA polymerase II	2.11	0.038
Q8N8K0	Myb, DNA-binding	2.10	0.0385
NP_003311.2	Functions in signal transduction from heterotrimeric G protein-coupled receptors. Could be involved in the hypothalamic regulation of body weight	2.10	0.0388
U2AFL	U2 small nuclear ribonucleoprotein auxiliary factor 35 kDa subunit-related protein 1; U2(RNU2) small nuclear RNA auxiliary factor 1-like 1; CCCH type zinc finger, RNA-binding motif and serine/arginine rich protein 1	2.09	0.0397
LRC56	Leucine-rich repeat-containing protein 56	2.09	0.0397
STXB2	Involved in the protein trafficking from the Golgi apparatus to the plasma membrane	2.08	0.0399
GTF2E1	Recruits TFIIH to the initiation complex and stimulates the RNA polymerase II C-terminal domain kinase and DNA-dependent ATPase activities of TFIIH. Both TFIIH and TFIIE are required for promoter clearance by RNA polymerase	2.08	0.0399
NP_037422.2	Tat binding protein 1-interacting	2.08	0.0088
H2B2E	Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling	2.08	0.04
AQP10	Forms a water channel. Not permeable to urea and glycerol. May contribute to water transport in the upper portion of small intestine	2.08	0.0405

Gene Name	Gene Description	Fold Change	p Value
H32	Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling	2.07	0.0415
NP_001028751.1	IQ calmodulin-binding region; Myosin head, motor region	2.07	0.0417
RNF34	Has E3 ubiquitin-protein ligase activity. Regulates the levels of CASP8 and CASP10 by targeting them for proteasomal degradation. Protects cells against apoptosis induced by TNF. Binds phosphatidylinositol-5-phosphate and phosphatidylinositol-3-phosphate	2.06	0.0422
HIPK1	May play a role as a corepressor for homeodomain transcription factors. Phosphorylates DAXX in response to stress, and mediates its translocation from the nucleus to the cytoplasm. May be involved in malignant squamous cell tumor formation	2.06	0.0424
UBCE7IP1	Isoform 1 acts as an E3 ubiquitin ligase, which accepts ubiquitin from specific E2 ubiquitin-conjugating enzymes, and then transfers it to substrates promoting their degradation by the proteasome. Promotes degradation of TLR4 amd TLR9. Isoform 3/ZIN inhibits TNF and IL-1 mediated activation of NF-kappa-B. Promotes TNF and RIP mediated apoptosis	2.06	0.0424
BCAS1	Breast carcinoma amplified sequence 1; Novel amplified in breast cancer 1; Amplified and overexpressed in breast cancer	2.06	0.0425
SLC2B	May act as Rab effector protein and play a role in vesicle trafficking	2.06	0.0427
MPRD	Transport of phosphorylated lysosomal enzymes from the Golgi complex and the cell surface to lysosomes. Lysosomal enzymes bearing phosphomannosyl residues bind specifically to mannose-6-phosphate receptors in the Golgi apparatus and the resulting receptor-ligand complex is transported to an acidic prelyosomal compartment where the low pH mediates the dissociation of the complex	2.05	0.0429
ANC4	Component of the anaphase promoting complex/cyclosome (APC/C), a cell cycle-regulated ubiquitin ligase that controls progression through mitosis and the G1 phase of the cell cycle	2.05	0.0429
NP_064533.2	Acc:NP_064533]; spire homolog 1 [Source:RefSeq_peptide	2.05	0.01
RBM6	Specifically binds poly(G) RNA homopolymers in vitro	2.05	0.0433
GLYC	Interconversion of serine and glycine	2.05	0.0433

Gene Name	Gene Description	Fold Change	p Value
UGCGL1	Recognizes glycoproteins with minor folding defects. Reglucosylates single N-glycans near the misfolded part of the protein, thus providing quality control for protein folding in the endoplasmic reticulum. Reglucosylated proteins are recognized by calreticulin for recycling to the endoplasmic reticulum and refolding or degradation	2.05	0.0436
RHBL2	Involved in regulated intramembrane proteolysis and the subsequent release of functional polypeptides from their membrane anchors. Known substrate: EFNB3	2.05	0.0437
NP_061889.1	Eggshell protein; Intermediate filament protein; Keratin, type I	2.05	0.0438
ADRBK2	Specifically phosphorylates the agonist-occupied form of the beta-adrenergic and closely related receptors	2.04	0.0442
NP_006693.3	Cyclic nucleotide-binding; Patatin; Protein of unknown function UPF0028; cAMP/cGMP-dependent protein kinase	2.04	0.0429
ATXN7	Involved in neurodegeneration	2.04	0.0445
OR2B6	Putative odorant receptor	2.03	0.0449
DEN1A	DENN domain-containing protein 1A	2.03	0.0449
HXA9	Sequence-specific transcription factor which is part of a developmental regulatory system that provides cells with specific positional identities on the anterior-posterior axis	2.03	0.0451
NP_612147.1	RUN	2.03	0.0454
RRS1	Involved in ribosome biogenesis	2.03	0.0458
PAK6	The activated kinase acts on a variety of targets	2.02	0.0461
FAM126A	May have a role in the beta-catenin/Lef signaling pathway. May have a role in the process of myelination of the central and peripheral nervous system	2.02	0.0462
NP_001020951.1	Phospholipase/Carboxylesterase	2.02	0.0463
MAFG	Since they lack a putative transactivation domain, the small Mafs behave as transcriptional repressors when they dimerize among themselves. However, they seem to serve as transcriptional activators by dimerizing with other (usually larger) basic-zipper proteins and recruiting them to specific DNA-binding sites. Small Maf proteins heterodimerize with Fos and may act as competitive repressors of the NF-E2 transcription factor. Transcription factor, component of erythroid-specific transcription factor NF-E2. Activates globin gene expression when associated with NF-E2	2.02	0.0467
S26A4	Sodium-independent transporter of chloride and iodide	2.02	0.027

Gene Name	Gene Description	Fold Change	p Value
IL1F5	Is a highly and a specific antagonist of the IL-1 receptor-related protein 2-mediated response to interleukin 1 family member 9 (IL1F9). Could constitute part of an independent signaling system analogous to interleukin-1 alpha (IL-1A), beta (IL-1B) receptor agonist and interleukin-1 receptor type I (IL-1R1), that is present in epithelial barriers and takes part in local inflammatory response	2.02	0.047
MTLR	Receptor for motilin	2.01	0.0473
K1HB	Keratin, type I cuticular Ha3-II; Hair keratin, type I Ha3-II	2.01	0.0475
Q9UJT2-2	May play a role in testicular physiology, most probably in the process of spermatogenesis or spermiogenesis	2.01	0.0475
CEP41	Centrosomal protein of 41 kDa; Protein Cep41; Testis-specific gene A14 protein	2.01	0.0477
RNF39	May play a role in prolonged long term-potentiation (LTP) maintenance	2.00	0.0484
SC5A5	Mediates iodide uptake in the thyroid gland	2.00	0.0485
ANGL2	Induces sprouting in endothelial cells through an autocrine and paracrine action	-2.01	0.0175
CN159	UPF0317 protein C14orf159, mitochondrial precursor	-2.01	0.048
ESR1	Nuclear hormone receptor. The steroid hormones and their receptors are involved in the regulation of eukaryotic gene expression and affect cellular proliferation and differentiation in target tissues	-2.01	0.0476
NP_055721.3	Component of the ESCRT-II complex, which is required for multivesicular bodies (MVBs) formation and sorting of endosomal cargo proteins into MVBs. The MVB pathway mediates delivery of transmembrane proteins into the lumen of the lysosome for degradation. The ESCRT-II complex is probably involved in the recruitment of the ESCRT-III complex. Its ability to bind ubiquitin probably plays a role in endosomal sorting of ubiquitinated cargo proteins by ESCRT complexes. The ESCRT-II complex may also play a role in transcription regulation, possibly via its interaction with ELL	-2.01	0.0003
Q6WBX8-3	Cell cycle checkpoint control protein RAD9B homolog; RAD9 homolog B; hRAD9B	-2.01	0.0472
BACE2	Beta-secretase 2 precursor; Beta-site APP-cleaving enzyme 2; Aspartyl protease 1; Asp 1; ASP1; Membrane-associated aspartic protease 1; Memapsin-1; Aspartic-like protease 56 kDa; Down region aspartic protease	-2.01	0.0472
PGAM2	Interconversion of 3- and 2-phosphoglycerate with 2,3-bisphosphoglycerate as the primer of the reaction. Can also catalyze the reaction of EC 5.4.2.4 (synthase) and EC 3.1.3.13 (phosphatase), but with a reduced activity	-2.02	0.031
DLL1	Acts as a ligand for Notch receptors. Blocks the differentiation of progenitor cells into the B-cell lineage while promoting the emergence of a population of cells with the characteristics of a T-cell/NK-cell precursor	-2.02	0.0468

Gene Name	Gene Description	Fold Change	p Value
P25A	Promotes in vitro the polymerization of tubulin into double-walled tubules and polymorphic aggregates or bundled stabilized microtubules blocks. When overexpressed, inhibits mitotic spindle assembly and nuclear envelope breakdown, apparently without affecting other cellular events	-2.02	0.0464
P73	Participates in the apoptotic response to DNA damage. When overproduced, activates transcription from p53-responsive promoters and induces apoptosis. May be a tumor suppressor protein	-2.02	0.0323
CDN2D	Interacts strongly with CDK4 and CDK6	-2.02	0.0463
PROD	Converts proline to delta-1-pyrroline-5-carboxylate	-2.03	0.0165
RFX2	DNA-binding protein RFX2	-2.04	0.0194
KIF17	Transports vesicles containing N-methyl-D-aspartate (NMDA) receptor 2B along microtubules	-2.04	0.0446
RGS20	Inhibits signal transduction by increasing the GTPase activity of G protein alpha subunits thereby driving them into their inactive GDP-bound form. Binds selectively to G(z)-alpha and is inhibited by phosphorylation and palmitoylation of the G-protein	-2.04	0.0445
NLGN3	Neuronal cell surface protein thought to be involved in cell-cell-interactions by forming intercellular junctions through binding to beta-neurexins. May play a role in formation or maintenance of synaptic junctions. May also play a role in glia-glia or glia-neuron interactions in the developing peripheral nervous system	-2.04	0.0443
ADIPO	Important adipokine involved in the control of fat metabolism and insulin sensitivity, with direct anti-diabetic, anti-atherogenic and anti-inflammatory activities. Stimulates AMPK phosphorylation and activation in the liver and the skeletal muscle, enhancing glucose utilization and fatty-acid combustion. Antagonizes TNF-alpha by negatively regulating its expression in various tissues such as liver and macrophages, and also by counteracting its effects. Inhibits endothelial NF-kappa-B signaling through a cAMP-dependent pathway. May play a role in cell growth, angiogenesis and tissue remodeling by binding and sequestering various growth factors with distinct binding affinities, depending on the type of complex, LMW, MMW or HMW	-2.04	0.0442
EMAL2	May modify the assembly dynamics of microtubules, such that microtubules are slightly longer, but more dynamic	-2.04	0.0441
MMP12	May be involved in tissue injury and remodeling. Has significant elastolytic activity. Can accept large and small amino acids at the P1' site, but has a preference for leucine. Aromatic or hydrophobic residues are preferred at the P1 site, with small hydrophobic residues (preferably alanine) occupying P3	-2.04	0.0198
RBTN1	May be involved in gene regulation within neural lineage cells potentially by direct DNA binding or by binding to other transcription factors	-2.05	0.0318

Gene Name	Gene Description	Fold Change	p Value
HNF4G	Transcription factor. Has a lower transcription activation potential than HNF4-alpha	-2.05	0.0104
FTHFD	10-formyltetrahydrofolate dehydrogenase; 10-FTHFDH; Aldehyde dehydrogenase 1 family member L1	-2.05	0.0433
CENPN	Component of the CENPA-NAC (nucleosome-associated) complex, a complex that plays a central role in assembly of kinetochore proteins, mitotic progression and chromosome segregation. The CENPA-NAC complex recruits the CENPA-CAD (nucleosome distal) complex and may be involved in incorporation of newly synthesized CENPA into centromeres	-2.05	0.0405
FAM3C	Protein FAM3C precursor; Protein GS3786	-2.06	0.023
GBGT1	Catalyzes the formation of some glycolipid via the addition of N-acetylgalactosamine (GalNAc) in alpha-1,3-linkage to some substrate. Glycolipids probably serve for adherence of some pathogens	-2.06	0.0348
IFIH1	RNA helicase that, through its ATP-dependent unwinding of RNA, may function to promote message degradation by specific RNases. Seems to have growth suppressive properties. Involved in innate immune defense against viruses. Upon interaction with intracellular dsRNA produced during viral replication, triggers a transduction cascade involving MAVS/IPS1, which results in the activation of NF-kappa-B, IRF3 and IRF7 and the induction of the expression of antiviral cytokines such as IFN-beta and RANTES (CCL5). ATPase activity is specifically induced by dsRNA. Essential for the production of interferons in response to picornaviruses	-2.06	0.0421
Q8NI38	Ankyrin; Proline-rich region	-2.06	0.042
MOSC2	Catalytic component of the benzamidoxime prodrug-converting complex, a complex required to reduce N-hydroxylated structures, such as benzamidoxime prodrug. Benzamidoxime is an amidine prodrug produced by N-hydroxylation which is used to enhance bioavailability and increase intestinal absorption. It is then reduced into benzamidine, its active amidine, by the benzamidoxime prodrug-converting complex	-2.06	0.042
ACV1B	On ligand binding, forms a receptor complex consisting of two type II and two type I transmembrane serine/threonine kinases. Type II receptors phosphorylate and activate type I receptors which autophosphorylate, then bind and activate SMAD transcriptional regulators; On ligand binding, forms a receptor complex consisting of two type II and two type I transmembrane serine/threonine kinases. Type II receptors phosphorylate and activate type I receptors which autophosphorylate, then bind and activate SMAD transcriptional regulators. Receptor for TGF-beta. May bind activin as well	-2.06	0.042

Gene Name	Gene Description	Fold Change	p Value
NP_060340.2	Acc:NP_060340]; transmembrane protein 132A isoform a [Source:RefSeq_peptide	-2.06	0.0162
EP15	Involved in cell growth regulation. May be involved in the regulation of mitogenic signals and control of cell proliferation. Involved in the internalization of ligand-inducible receptors of the receptor tyrosine kinase (RTK) type, in particular EGFR	-2.07	0.0064
AIFM2	Oxidoreductase, which may play a role in mediating a TP53/p53-dependent apoptosis response. Probable oxidoreductase that acts as a caspase-independent mitochondrial effector of apoptotic cell death. Binds to DNA in a sequence-independent manner. May contribute to genotoxin-induced growth arrest	-2.07	0.0417
ABI2	May act in regulation of cell growth and transformation by interacting with nonreceptor tyrosine kinases ABL1 and/or ABL2. May be involved in cytoskeletal reorganization. Regulates ABL1/c-Abl-mediated phosphorylation of MENA	-2.07	0.0417
SIRT6	Mono-ADP-ribosyltransferase sirtuin-6; SIR2-like protein 6	-2.07	0.0057
ENSG00000168827	Protein synthesis factor, GTP-binding; Translation elongation factor EFG/EF2, C-terminal; Translation elongation factor EFG/EF2, domain IV; Translation elongation factor EFTu/EF1A, domain 2	-2.07	0.0415
68MP	6.8 kDa mitochondrial proteolipid	-2.07	0.0412
ENSG00000130224	Calponin-like actin-binding; Leucine-rich repeat	-2.07	0.0412
COF1	Controls reversibly actin polymerization and depolymerization in a pH-sensitive manner. It has the ability to bind G- and F-actin in a 1:1 ratio of cofilin to actin. It is the major component of intranuclear and cytoplasmic actin rods	-2.07	0.0076
UDA1	UDPGT is of major importance in the conjugation and subsequent elimination of potentially toxic xenobiotics and endogenous compounds. This isoform is active on odorants and seems to be involved in olfaction; it could help clear lipophilic odorant molecules from the sensory epithelium	-2.07	0.0411
NP_997208.1	Acc:NP_997208]; dpy-19-like 3 [Source:RefSeq_peptide	-2.07	0.0408
NP_067081.2	Pistil-specific extensin-like protein	-2.08	0.0408
OTOR	Otoraplin precursor; Fibrocyte-derived protein; Melanoma inhibitory activity-like protein	-2.08	0.0406
STON1	May be involved in the endocytic machinery; May function as a testis specific transcription factor. Binds DNA in conjunction with GTF2A2 and TBP (the TATA-binding protein) and together with GTF2A2, allows mRNA transcription	-2.08	0.0372
LRC17	Leucine-rich repeat-containing protein 17 precursor; p37NB	-2.08	0.0403

Gene Name	Gene Description	Fold Change	p Value
7B2	Acts as a molecular chaperone for PCSK2/PC2, preventing its premature activation in the regulated secretory pathway. Binds to inactive PCSK2 in the endoplasmic reticulum and facilitates its transport from there to later compartments of the secretory pathway where it is proteolytically matured and activated. Also required for cleavage of PCSK2 but does not appear to be involved in its folding. Plays a role in regulating pituitary hormone secretion. The C-terminal peptide inhibits PCSK2 in vitro	-2.08	0.0402
SORT	Functions as a sorting receptor in the Golgi compartment and as a clearance receptor on the cell surface. Required for protein transport from the Golgi apparatus to the lysosomes by a pathway that is independent of the mannose-6-phosphate receptor (M6PR). Also required for protein transport from the Golgi apparatus to the endosomes. Promotes neuronal apoptosis by mediating endocytosis of the proapoptotic precursor forms of BDNF (proBDNF) and NGFB (proNGFB). Also acts as a receptor for neurotensin. May promote mineralization of the extracellular matrix during osteogenic differentiation by scavenging extracellular LPL. Probably required in adipocytes for the formation of specialized storage vesicles containing the glucose transporter SLC2A4/GLUT4 (GLUT4 storage vesicles, or GSVs). These vesicles provide a stable pool of SLC2A4 and confer increased responsiveness to insulin. May also mediate transport from the endoplasmic reticulum to the Golgi	-2.08	0.0401
EGFR	Receptor for EGF, but also for other members of the EGF family, as TGF-alpha, amphiregulin, betacellulin, heparin-binding EGF-like growth factor, GP30 and vaccinia virus growth factor. Is involved in the control of cell growth and differentiation	-2.08	0.0399
FABD	Catalyzes the transfer of a malonyl moiety from malonyl-CoA to the free thiol group of the phosphopantetheine arm of the mitochondrial ACP protein (NDUFAB1). This suggests the existence of the biosynthesis of fatty acids in mitochondrias	-2.09	0.0396
MASP2	Serum protease that plays an important role in the activation of the complement system via mannose-binding lectin. After activation by auto-catalytic cleavage it cleaves C2 and C4, leading to their activation and to the formation of C3 convertase	-2.09	0.0395
NP_055451.1	Guanine-nucleotide dissociation stimulator CDC25; Pleckstrin-like	-2.09	0.0394
PAX9	Transcription factor required for normal development of thymus, parathyroid glands, ultimobranchial bodies, teeth, skeletal elements of skull and larynx as well as distal limbs	-2.09	0.0083
UT14C	Essential for spermatogenesis. May be required specifically for ribosome biogenesis and hence protein synthesis during male meiosis	-2.09	0.0367

Gene Name	Gene Description	Fold Change	p Value
H2B2E	Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling	-2.10	0.039
QSK	Serine/threonine-protein kinase QSK	-2.10	0.0388
MALT1	Enhances BCL10-induced activation of NF-kappa-B. Involved in nuclear export of BCL10. Binds to TRAF6, inducing TRAF6 oligomerization and activation of its ligase activity. Has ubiquitin ligase activity	-2.10	0.0159
CASB	Important role in determination of the surface properties of the casein micelles	-2.10	0.0386
NP_061959.2	Immunoglobulin; Immunoglobulin I-set; Immunoglobulin V-set; Immunoglobulin-like	-2.10	0.0385
BBC3	Essential mediator of p53-dependent and p53-independent apoptosis	-2.10	0.0089
Q13862	Acc:Q13862]; DNA-binding protein (Hypothetical protein SPBPBP). [Source:Uniprot/SPTREMBL	-2.10	0.0382
DDX23	Probably involved in pre-mRNA splicing	-2.10	0.0228
NP_065976.2	Cache; VWA N-terminal; von Willebrand factor, type A	-2.11	0.0376
INGR1	Receptor for interferon gamma. Two receptors bind one interferon gamma dimer	-2.11	0.0065
DYN1	Microtubule-associated force-producing protein involved in producing microtubule bundles and able to bind and hydrolyze GTP. Most probably involved in vesicular trafficking processes, in particular endocytosis	-2.11	0.0047
COPE	The coatomer is a cytosolic protein complex that binds to dilysine motifs and reversibly associates with Golgi non-clathrin-coated vesicles, which further mediate biosynthetic protein transport from the ER, via the Golgi up to the trans Golgi network. Coatomer complex is required for budding from Golgi membranes, and is essential for the retrograde Golgi-to-ER transport of dilysine-tagged proteins. In mammals, the coatomer can only be recruited by membranes associated to ADP-ribosylation factors (ARFs), which are small GTP-binding proteins; the complex also influences the Golgi structural integrity, as well as the processing, activity, and endocytic recycling of LDL receptors	-2.12	0.0371
MK	Has heparin binding activity, and growth promoting activity. Involved in neointima formation after arterial injury, possibly by mediating leukocyte recruitment. Also involved in early fetal adrenal gland development	-2.12	0.0046
ARRD3	Arrestin domain-containing protein 3	-2.12	0.0029
Q5T5W6	Myb, DNA-binding	-2.12	0.037
ORML3	ORM1-like protein 3	-2.12	0.0025

Gene Name	Gene Description	Fold Change	p Value
ARL3	Does not act as an allosteric activator of the cholera toxin catalytic subunit	-2.12	0.0365
CP39A	Involved in the bile acid metabolism. Has a preference for 24-hydroxycholesterol, and converts it into a 7-alpha-hydroxylated product	-2.12	0.0365
UBR1	E3 ubiquitin-protein ligase which is a component of the N-end rule pathway. Recognizes and binds to proteins bearing specific amino-terminal residues that are destabilizing according to the N-end rule, leading to their ubiquitination and subsequent degradation. May be involved in pancreatic homeostasis	-2.12	0.0365
FAT3	Aspartic acid and asparagine hydroxylation site; Cadherin; EGF-like; EGF-like calcium-binding; EGF-like region; EGF-like, laminin; EGF-like, type 2; EGF-like, type 3; Laminin G; Laminin G, subdomain 1; Laminin G, subdomain 2; Proline-rich region	-2.12	0.0364
OAT4	Mediates saturable uptake of estrone sulfate, dehydroepiandrosterone sulfate and related compounds	-2.13	0.0002
Q5VZI7	Cooperates with LY96 and CD14 to mediate the innate immune response to bacterial lipopolysaccharide (LPS). Acts via MyD88, TIRAP and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response	-2.13	0.0359
AGGF1	Promotes angiogenesis and the proliferation of endothelial cells. Able to bind to endothelial cells and promote cell proliferation, suggesting that it may act in an autocrine fashion	-2.13	0.0358
ENSG00000204124	Doublecortin	-2.13	0.0355
NP_071375.1	AAA ATPase, core; ATPase associated with various cellular activities, AAA-5; Proline-rich region	-2.14	0.0204
DNAL4	Force generating protein of respiratory cilia. Produces force towards the minus ends of microtubules. Dynein has ATPase activity	-2.14	0.0351
MLPH	Rab effector protein involved in melanosome transport. Serves as link between melanosome-bound RAB27A and the motor protein MYO5A	-2.15	0.0344
F262	Synthesis and degradation of fructose 2,6-bisphosphate	-2.15	0.0143
PLMN	Plasmin dissolves the fibrin of blood clots and acts as a proteolytic factor in a variety of other processes including embryonic development, tissue remodeling, tumor invasion, and inflammation; in ovulation it weakens the walls of the Graafian follicle. It activates the urokinase-type plasminogen activator, collagenases and several complement zymogens, such as C1 and C5. It cleaves fibrin, fibronectin, thrombospondin, laminin and von Willebrand factor. Its role in tissue remodeling and tumor invasion may be modulated by CSPG4	-2.16	0.0333

Gene Name	Gene Description	Fold Change	p Value
RIP	Mediates the import of RPA complex into the nucleus, possibly via some interaction with importin beta. Isoform 2 is sumoylated and mediates the localization of RPA complex into the PML body of the nucleus, thereby participating in RPA function in DNA metabolism	-2.17	0.0328
NP_478126.1	Exoribonuclease	-2.17	0.0265
GCYA2	Has guanylyl cyclase on binding to the beta-1 subunit. The alternatively spliced isoform alpha-2-I acts as a negative regulator of guanylyl cyclase activity as it forms non-functional heterodimers with the beta subunits	-2.17	0.0325
GNAO1	Guanine nucleotide-binding proteins (G proteins) are involved as modulators or transducers in various transmembrane signaling systems. The G(o) protein function is not clear	-2.17	0.0017
PADI2	Catalyzes the deimination of arginine residues of proteins	-2.19	0.0313
PREP	ATP-independent protease that degrades mitochondrial transit peptides after their cleavage. Also degrades other unstructured peptides. Specific for peptides in the range of 10 to 65 residues. Able to degrade amyloid beta A4 (APP) protein when it accumulates in mitochondrion, suggesting a link with Alzheimer disease. Shows a preference for cleavage after small polar residues and before basic residues, but without any positional preference	-2.19	0.0313
NRK2	Reduces laminin matrix deposition and cell adhesion to laminin, but not to fibronectin. Involved in the regulation of PXN at the protein level and of PXN tyrosine phosphorylation. May play a role in the regulation of terminal myogenesis. Catalyzes the synthesis of nicotinamide nucleotide (NMN) from nicotinamide riboside	-2.19	0.0312
ETV6	Transcriptional repressor; binds to the DNA sequence 5'-CCGGAAGT-3'	-2.19	0.0312
PRMT7	Probably methylates the guanidino nitrogens of arginyl residues in some proteins	-2.20	0.0309
IF	Promotes absorption of the essential vitamin cobalamin (Cbl) in the ileum by specific receptor-mediated endocytosis	-2.20	0.0004
MAGBI	Melanoma-associated antigen B18; MAGE-B18 antigen	-2.20	0.0005
TTC5	Tetratricopeptide repeat protein 5; TPR repeat protein 5	-2.20	0.0306
TRAIP	Inhibits activation of NF-kappa-B mediated by TNF	-2.20	0.0304
NP_001070249.1	Zinc finger, C2H2-subtype; Zinc finger, C2H2-type	-2.21	0.0301
NBEA	Binds to type II regulatory subunits of protein kinase A and anchors/targets them to the membrane. May anchor the kinase to cytoskeletal and/or organelle-associated proteins	-2.21	0.03

Gene Name	Gene Description	Fold Change	p Value
Q7Z5G2	Serine/threonine protein kinase involved in both mRNA surveillance and genotoxic stress response pathways. Recognizes the substrate consensus sequence [ST]-Q. Involved in nonsense-mediated decay (NMD) of mRNAs containing premature stop codons by phosphorylating UPF1/RENT1. Also acts as a genotoxic stress-activated protein kinase that displays some functional overlap with ATM. Can phosphorylate TP53/p53 and is required for optimal TP53/p53 activation after cellular exposure to genotoxic stress. Its depletion leads to spontaneous DNA damage and increased sensitivity to ionizing radiation (IR). May activate PRKCI but not PRKCZ	-2.21	0.0299
NP_116107.2	Major facilitator superfamily; Major facilitator superfamily MFS_1; Sugar transporter superfamily; Tetracycline resistance protein	-2.22	0.0296
Q53RC4	Fodrin, which seems to be involved in secretion, interacts with calmodulin in a calcium-dependent manner and is thus candidate for the calcium-dependent movement of the cytoskeleton at the membrane	-2.22	0.0161
ZN343	May be involved in transcriptional regulation	-2.22	0.0295
WBS14	Transcriptional repressor. Binds to the canonical and non-canonical E box sequences 5'-CACGTG-3'	-2.22	0.0292
IPP	May play a role in organizing the actin cytoskeleton	-2.22	0.0292
P04278-2	Functions as an androgen transport protein, but may also be involved in receptor mediated processes. Each dimer binds one molecule of steroid. Specific for 5-alpha-dihydrotestosterone, testosterone, and 17-beta-estradiol. Regulates the plasma metabolic clearance rate of steroid hormones by controlling their plasma concentration	-2.23	0.029
Q8N157-3	G-protein, beta subunit; Src homology-3; Variant SH3; WD-40 repeat	-2.23	0.0048
RB33A	Ras-related protein Rab-33A; Small GTP-binding protein S10	-2.23	0.0287
FPRL1	Low affinity receptor to N-formyl-methionyl peptides, which are powerful neutrophils chemotactic factors. Binding of FMLP to the receptor causes activation of neutrophils. This response is mediated via a G-protein that activates a phosphatidylinositol-calcium second messenger system. The activation of LXA4R could result in an anti-inflammatory outcome counteracting the actions of proinflammatory signals such as LTB4 (leukotriene B4)	-2.23	0.0287
NP_055873.1	ATPase associated with various cellular activities, AAA-5; von Willebrand factor, type A	-2.24	0.003
Q7Z5Q7	Acc:Q7Z5Q7]; Lung cancer oncogene 5. [Source:Uniprot/SPTREMBL	-2.24	0.0283
SEC62	Required for preprotein translocation	-2.24	0.0051
LMA2L	May be involved in the regulation of export from the endoplasmic reticulum of a subset of glycoproteins. May function as a regulator of ERGIC-53	-2.24	0.0042
KCD17	BTB/POZ domain-containing protein KCTD17	-2.24	0.0116

Gene Name	Gene Description	Fold Change	p Value
GALA	Contracts smooth muscle of the gastrointestinal and genitourinary tract, regulates growth hormone release, modulates insulin release, and may be involved in the control of adrenal secretion	-2.24	0.0281
Q9Y4E5-2	May be involved in transcriptional regulation. Coactivator for steroid receptors	-2.25	0.0277
ZN460	May be involved in transcriptional regulation	-2.25	0.0274
REXO1	Seems to have no detectable effect on transcription elongation in vitro	-2.26	0.0273
YETS2	YEATS domain-containing protein 2	-2.26	0.027
CAN5	Calpain-5; nCL-3; htra-3	-2.26	0.0269
NP_940905.2	Blood group Rhesus C/E and D polypeptide; Calcium-activated BK potassium channel, alpha subunit; EAG/ELK/ERG potassium channel; Ion transport 2	-2.26	0.0269
C8AP2	Participates in TNF-alpha-induced blockade of glucocorticoid receptor (GR) transactivation at the nuclear receptor coactivator level, upstream and independently of NF-kappa-B. Suppresses both NCOA2- and NCOA3-induced enhancement of GR transactivation. Involved in TNF-alpha-induced activation of NF-kappa-B via a TRAF2-dependent pathway. Acts as a downstream mediator for CASP8-induced activation of NF-kappa-B. Required for the activation of CASP8 in FAS-mediated apoptosis	-2.26	0.0269
CAN13	Probable non-lysosomal thiol-protease	-2.26	0.0269
MGST3	Also functions as a glutathione peroxidase	-2.26	0.0268
RHES	Binds to GTP and possesses intrinsic GTPase activity. May play a role in mediating signal transduction (By similarity). May be involved in mediating the insulin secretory response to efaroxan	-2.28	0.0262
PHF6	May play a role in transcriptional regulation	-2.28	0.0053
NAPSA	May be involved in processing of pneumocyte surfactant precursors	-2.29	0.0256
NP_057593.2	C-type lectin; Type II antifreeze protein	-2.29	0.0252
TM107	Transmembrane protein 107	-2.30	0.0251
ITF2	Transcription factor that binds to the immunoglobulin enchancer Mu-E5/KE5-motif. Binds to the E-box present in the somatostatin receptor 2 initiator element (SSTR2-INR) to activate transcription (By similarity). Preferentially binds to either 5'-ACANNTGT-3' or 5'-CCANNTGG-3'	-2.30	0.025
NP_065805.1	Proline-rich region; RhoGAP	-2.30	0.0248
ARP8	Actin-related protein 8	-2.30	0.0248

Gene Name	Gene Description	Fold Change	p Value
COA1	Can act on substrates such as myelin basic protein and histone 2A on serine and threonine residues; Catalyzes the rate-limiting reaction in the biogenesis of long-chain fatty acids. Carries out three functions: biotin carboxyl carrier protein, biotin carboxylase and carboxyltransferase; Essential ion channel and serine/threonine-protein kinase. Crucial for magnesium homeostasis. Has an important role in epithelial magnesium transport and in the active magnesium absorption in the gut and kidney. Isoforms of the type M6-kinase lack the ion channel region; F-box-like protein involved in the recruitment of the ubiquitin/19S proteasome complex to nuclear receptor-regulated transcription units. Plays an essential role in transcription activation mediated by nuclear receptors. Probably acts as integral component of the N-Cor corepressor complex that mediates the recruitment of the 19S proteasome complex, leading to the subsequent proteosomal degradation of N-Cor complex, thereby allowing cofactor exchange, and transcription activation; May be involved in the synthesis of gangliosides GD1c, GT1a, GQ1b and GT3 from GD1a, GT1b, GM1b and GD3 respectively; RNA-binding protein implicated in the regulation of several post-transcriptional events. Involved in pre-mRNA alternative splicing, mRNA translation and stability. Mediates exon inclusion and/or exclusion in pre-mRNA that are subject to tissue-specific and developmentally regulated alternative splicing. Specifically activates exon 5 inclusion of cardiac isoforms of TNNT2 during heart remodeling at the juvenile to adult transition. Acts as both an activator and repressor of a pair of coregulated exons: promotes inclusion of the smooth muscle (SM) exon but exclusion of the non-muscle (NM) exon in actinin pre-mRNAs. Activates SM exon 5 inclusion by antagonizing the repressive effect of PTB. Promotes exclusion of exon 11 of the INSR pre-mRNA. Increases translation and controls the choice of translation initiation codon of CEBPB mRNA. Increases mRNA translation of CEBPB in aging liver (By similarity). Increases translation of CDKN1A mRNA by antagonizing the repressive effect of CALR3. Mediates rapid cytoplasmic mRNA deadenylation. Recruits the deadenylase PARN to the poly(A) tail of EDEN-containing mRNAs to promote their deadenylation. Required for completion of spermatogenesis (By similarity). Binds to (CUG)n triplet repeats in the 3'-UTR of transcripts such as DMPK and to Bruno response elements (BREs). Binds to muscle-specific splicing enhancer (MSE) intronic sites flanking the alternative exon 5 of TNNT2 pre-mRNA. Binds to AU-rich sequences (AREs or EDEN-like) localized in the 3'-UTR of JUN and FOS mRNAs. Binds to the 5'-region of CDKN1A and CEBPB mRNAs. Binds with the 5'-region of CEBPB mRNA in aging liver	-2.30	0.0027

Gene Name	Gene Description	Fold Change	p Value
K1C19	Involved in the organization of myofibers. Together with KRT8, helps to link the contractile apparatus to dystrophin at the costameres of striated muscle	-2.31	0.0013
DEF4	This peptide has antibiotic and anti-fungi activity	-2.31	0.0243
CC28A	Coiled-coil domain-containing protein 28A; CCRL1AP	-2.32	0.0241
ITCH	E3 ubiquitin-protein ligase which accepts ubiquitin from an E2 ubiquitin-conjugating enzyme in the form of a thioester and then directly transfers the ubiquitin to targeted substrates. Regulates the transcriptional activity of several transcription factors, and probably plays an important role in the regulation of immune response	-2.32	0.0241
CCAR1	May be involved in apoptosis signaling in the presence of the reinoid CD437. Apoptosis induction involves sequestration of 14-3-3 protein(s) and mediated altered expression of multiple cell cycle regulatory genes including MYC, CCNB1 and CDKN1A. Plays a role in cell cycle progression and/or cell proliferation	-2.32	0.024
Q8TCQ1	Zinc finger, RING-type	-2.32	0.0136
KCNN3	Forms a voltage-independent potassium channel activated by intracellular calcium. Activation is followed by membrane hyperpolarization. Thought to regulate neuronal excitability by contributing to the slow component of synaptic afterhyperpolarization. The channel is blocked by apamin	-2.32	0.0142
NP_071746.1	Antibiotic biosynthesis monooxygenase; Calcium-binding EF-hand	-2.32	0.0239
NP_006171.2	Receptor for brain-derived neurotrophic factor (BDNF), neurotrophin-3 and neurotrophin-4/5 but not nerve growth factor (NGF). Involved in the development and/or maintenance of the nervous system. This is a tyrosine-protein kinase receptor. Known substrates for the TRK receptors are SHC1, PI-3 kinase, and PLC-gamma-1	-2.33	0.0052
ACHA2	After binding acetylcholine, the AChR responds by an extensive change in conformation that affects all subunits and leads to opening of an ion-conducting channel across the plasma membrane	-2.33	0.0234
GPR161	Orphan receptor	-2.33	0.0234
Q5TCU6	Probably involved in connections of major cytoskeletal structures to the plasma membrane. High molecular weight cytoskeletal protein concentrated at regions of cell-substratum contact and, in lymphocytes, at cell-cell contacts	-2.34	0.0008
GO45	Required for normal Golgi structure and for protein transport from the endoplasmic reticulum (ER) through the Golgi apparatus to the cell surface	-2.34	0.0229
SFRIP	Plays a role in pre-mRNA alternative splicing by regulating spliceosome assembly	-2.35	0.0132
NP_060340.2	Acc:NP_060340]; transmembrane protein 132A isoform a [Source:RefSeq_peptide	-2.35	0.0224

Gene Name	Gene Description	Fold Change	p Value
STK4	Stress-activated, pro-apoptotic kinase which, following caspase-cleavage, enters the nucleus and induces chromatin condensation followed by internucleosomal DNA fragmentation. Phosphorylates 'Ser-14' of histone H2B during apoptosis. Phosphorylates FOXO3 upon oxidative stress, which results in its nuclear translocation and cell death initiation	-2.35	0.0029
SIGIR	Acts as a negative regulator of the Toll-like and IL-1R receptor signaling pathways. Attenuates the recruitment of receptor-proximal signaling components to the TLR4 receptor, probably through an TIR-TIR domain interaction with TLR4. Through its extracellular domain interferes with the heterodimerization of Il1R1 and IL1RAP	-2.36	0.0219
NP_777577.1	AMP-dependent synthetase and ligase	-2.38	0.0213
STML1	Stomatin-like protein 1; SLP-1; Stomatin-related protein; STORP; EPB72-like 1; UNC24 homolog	-2.38	0.021
CRLD2	Cysteine-rich secretory protein LCCL domain-containing 2 precursor; LCCL domain-containing cysteine-rich secretory protein 2; Cysteine-rich secretory protein 11; CRISP-11	-2.40	0.0202
NP_689704.3	Peptidase M20; Peptidase M20, dimerisation	-2.40	0.0012
TNF18	Cytokine that binds to TNFRSF18/AITR/GITR. Important for interactions between activated T-lymphocytes and endothelial cells and may modulate T-lymphocyte survival in peripheral tissues	-2.41	0.0201
DCTN3	Together with dynein may be involved in spindle assembly and cytokinesis	-2.41	0.0198
NP_542398.2	Acc:NP_542398]; coiled-coil domain containing 104 [Source:RefSeq_peptide	-2.42	0.0004
ZN179	Zinc finger protein 179; Brain finger protein; RING finger protein 112	-2.42	0.0196
PER1	Component of the circadian clock mechanism which is essential for generating circadian rhythms. Negative element in the circadian transcriptional loop. Influences clock function by interacting with other circadian regulatory proteins and transporting them to the nucleus. Negatively regulates CLOCK|NPAS2-BMAL1|BMAL2-induced transactivation	-2.43	0.0435
NP_001073884.1	Lymphocyte-specific protein; Proline-rich region; RNA-binding region RNP-1 (RNA recognition motif); SWAP/Surp	-2.43	0.0313
GLT12	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor. Has activity toward non-glycosylated peptides such as Muc5AC, Muc1a and EA2, and no detectable activity with Muc2 and Muc7. Displays enzymatic activity toward the Gal-NAc-Muc5AC glycopeptide, but no detectable activity to mono-GalNAc-glycosylated Muc1a, Muc2, Muc7 and EA2. May play an important role in the initial step of mucin-type oligosaccharide biosynthesis in digestive organs	-2.44	0.0188

Gene Name	Gene Description	Fold Change	p Value
SFXN5	Transports citrate. Potential iron transporter	-2.44	0.0187
NP_006429.1	C-type lectin; Collagen triple helix repeat	-2.44	0.0187
SIP1	Transcriptional inhibitor that binds to DNA sequence 5'-CACCT-3' in different promoters. Represses transcription of E-cadherin	-2.45	0.0186
APBA2	Putative function in synaptic vesicle exocytosis by binding to STXBP1, an essential component of the synaptic vesicle exocytotic machinery. May modulate processing of the beta-amyloid precursor protein (APP) and hence formation of beta-APP	-2.45	0.0185
SHOC2	Leucine-rich repeat protein SHOC-2; Ras-binding protein Sur-8	-2.45	0.0184
MYL4	Regulatory light chain of myosin. Does not bind calcium	-2.46	0.018
OPCML	Binds opioids in the presence of acidic lipids; probably involved in cell contact	-2.47	0.0179
TCF3	Heterodimers between TCF3 and tissue-specific basic helix-loop-helix (bHLH) proteins play major roles in determining tissue-specific cell fate during embryogenesis, like muscle or early B-cell differentiation. Dimers bind DNA on E-box motifs: 5'-CANNTG-3'. Binds to the kappa-E2 site in the kappa immunoglobulin gene enhancer	-2.47	0.0179
MPDZ	Interacts with HTR2C and provokes its clustering at the cell surface (By similarity). Member of the NMDAR signaling complex that may play a role in control of AMPAR potentiation and synaptic plasticity in excitatory synapses	-2.48	0.0175
Q5VTQ0	Peptidase S26A, signal peptidase I; Tetratricopeptide TPR_2; Tetratricopeptide region	-2.48	0.0175
P66A	Transcriptional repressor	-2.48	0.0484
NTCP2	Plays a critical role in the sodium-dependent reabsorption of bile acids from the lumen of the small intestine. Plays a key role in cholesterol metabolism	-2.49	0.0171
CLC4E	May play a role in the response to inflammatory stimuli in peritoneal macrophages. May be involved in immune surveillance processes under transcriptional control of CEBPB	-2.51	0.0165
GBRL1	Gamma-aminobutyric acid receptor-associated protein-like 1; GABA(A) receptor-associated protein-like 1; Glandular epithelial cell protein 1; GEC-1; Early estrogen-regulated protein	-2.52	0.0003
NAPG	Required for vesicular transport between the endoplasmic reticulum and the Golgi apparatus	-2.52	0.0164

Gene Name	Gene Description	Fold Change	p Value
PACS2	Multifunctional sorting protein that controls the endoplasmic reticulum (ER)-mitochondria communication, including the apposition of mitochondria with the ER and ER homeostasis. In addition, in response to apoptic inducer, translocates BIB to mitochondria, which initiates a sequence of events including the formation of mitochondrial truncated BID, the release of cytochrome c, the activation of caspase-3 thereby causing cell death. May also involved in ion channel traficking, directing acidic cluster-containing ion channels to distict subcellular compartements	-2.52	0.0162
Q6ZN28	Variant SH3	-2.53	0.0016
LAT3	Sodium-independent, high affinity transport of large neutral amino acids. Has narrower substrate selectivity compared to SLC7A5 and SLC7A8 and mainly transports branched-chain amino acids and phenylalanine. Plays a role in the development of human prostate cancer, from prostatic intraepithelial neoplasia to invasive prostate cancer	-2.54	0.0157
TLR5	Participates in the innate immune response to microbial agents. Mediates detection of bacterial flagellins. Acts via MyD88 and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response	-2.56	0.0153
NP_001001552.3	Lamino-associated polypeptide 2/emerin	-2.57	0.0052
PRG3	Possesses similar cytotoxic and cytostimulatory activities to PRG2/MBP. In vitro, stimulates neutrophil superoxide production and IL8 release, and histamine and leukotriene C4 release from basophils	-2.57	0.0149
RHBT2	Rho-related BTB domain-containing protein 2; Deleted in breast cancer 2 gene protein; p83	-2.57	0.0149
Q6ZNV0	Ribosomal protein L22/L17	-2.57	0.0147
WDR55	WD repeat protein 55	-2.58	0.0147
DHX35	May be involved in pre-mRNA splicing	-2.58	0.0146
CDS1	Provides CDP-diacylglycerol an important precursor for the synthesis of phosphatidylinositol (PtdIns), phosphatidylglycerol, and cardiolipin. Overexpression may amplify cellular signaling responses from cytokines. May also play an important role in the signal transduction mechanism of retina and neural cells	-2.58	0.0145
Q6PJS5	Zinc finger, B-box; Zinc finger, RING-type	-2.59	0.027
TSN9	Tetraspanin-9; Tspan-9; Tetraspan NET-5	-2.60	0.0163
CLCN4	Voltage-gated chloride channel. Chloride channels have several functions including the regulation of cell volume; membrane potential stabilization, signal transduction and transepithelial transport	-2.60	0.0141
TMCC3	Transmembrane and coiled-coil domains protein 3	-2.60	0.005
NP_060615.1	Acc:NP_060615]; family with sequence similarity 82, member C [Source:RefSeq_peptide	-2.60	0.0039
NP_115510.1	Acc:NP_115510]; glutamine rich 2 [Source:RefSeq_peptide	-2.61	0.0018

Gene Name	Gene Description	Fold Change	p Value
Q9NSG0-5	GTPase activator for the Rho-type GTPases by converting them to an inactive GDP-bound state	-2.62	0.0257
ANK3	Membrane-cytoskeleton linker. The neural-specific isoforms may participate in the maintenance/targeting of ion channels and cell adhesion molecules at the nodes of Ranvier and axonal initial segments	-2.63	0.0135
CUL5	Component of E3 ubiquitin ligase complexes, which mediate the ubiquitination and subsequent proteasomal degradation of target proteins. Seems to be involved poteosomal degradation of p53/TP53 stimulated by adenovirus E1B-55 kDa protein. May form a cell surface vasopressin receptor	-2.63	0.0002
DOCK5	Protein phosphatase 2A regulatory subunit PR55	-2.64	0.0132
Q9NS68-2	Can mediate activation of JNK and NF-kappa-B. May promote caspase-independent cell death	-2.64	0.0131
ATP2C2	This magnesium-dependent enzyme catalyzes the hydrolysis of ATP coupled with the transport of calcium	-2.65	0.0129
COG2	Required for normal Golgi morphology and function	-2.65	0.0493
NP_659456.2	Acc:NM_145019]; family with sequence similarity 124A (FAM124A), mRNA [Source:RefSeq_dna	-2.66	0.0128
CT038	Binds Gram-positive and Gram-negative bacteria; DNA primase is the polymerase that synthesizes small RNA primers for the Okazaki fragments made during discontinuous DNA replication; Receptor for both mineralocorticoids (MC) such as aldosterone and glucocorticoids (GC) such as corticosterone or cortisol. Binds to mineralocorticoid response elements (MRE) and transactivates target genes. The effect of MC is to increase ion and water transport and thus raise extracellular fluid volume and blood pressure and lower potassium levels	-2.66	0.0127
PRND	Not known	-2.67	0.0126
NP_001074014.1	Protein phosphatase inhibitor, 1DARPP-32	-2.67	0.0018
GBRG2	GABA, the major inhibitory neurotransmitter in the vertebrate brain, mediates neuronal inhibition by binding to the GABA/benzodiazepine receptor and opening an integral chloride channel	-2.67	0.0124
SMYD4	SET and MYND domain-containing protein 4	-2.67	0.0124
NUP54	Component of the nuclear pore complex, a complex required for the trafficking across the nuclear membrane	-2.68	0.0122
PEVR2	Acts as receptor for porcine endogenous retrovirus subgroup A (PERV-A)	-2.69	0.012
CBX8	Component of the Polycomb group (PcG) multiprotein PRC1 complex, a complex required to maintain the transcriptionally repressive state of many genes, including Hox genes, throughout development. PcG PRC1 complex acts via chromatin remodeling and modification of histones; it mediates monoubiquitination of histone H2A 'Lys-119', rendering chromatin heritably changed in its expressibility	-2.69	0.0068

Gene Name	Gene Description	Fold Change	p Value
PTPRD	Receptor-type tyrosine-protein phosphatase delta precursor; Protein-tyrosine phosphatase delta; R-PTP-delta	-2.70	0.0118
NP_758438.1	Transcription factor AP-2, C-terminal	-2.71	0.0212
TESK2	Dual specificity protein kinase activity catalyzing autophosphorylation and phosphorylation of exogenous substrates on both serine/threonine and tyrosine residues. Phosphorylates cofilin at 'Ser-3'. May play an important role in spermatogenesis	-2.71	0.0116
E2F3	Transcription activator that binds DNA cooperatively with DP proteins through the E2 recognition site, 5'-TTTC[CG]CGC-3' found in the promoter region of a number of genes whose products are involved in cell cycle regulation or in DNA replication. The DRTF1/E2F complex functions in the control of cell-cycle progression from G1 to S phase. E2F-3 binds specifically to RB1 protein, in a cell-cycle dependent manner	-2.72	0.0116
SPG20	May be implicated in endosomal trafficking, or microtubule dynamics, or both	-2.73	0.0114
KU70	Single stranded DNA-dependent ATP-dependent helicase. Has a role in chromosome translocation. The DNA helicase II complex binds preferentially to fork-like ends of double-stranded DNA in a cell cycle-dependent manner. It works in the 3'-5' direction. Binding to DNA may be mediated by p70. Involved in DNA nonhomologous end joining (NHEJ) required for double-strand break repair and V(D)J recombination. The Ku p70/p86 dimer acts as regulatory subunit of the DNA-dependent protein kinase complex DNA-PK by increasing the affinity of the catalytic subunit PRKDC to DNA by 100-fold. The Ku p70/p86 dimer is probably involved in stabilizing broken DNA ends and bringing them together. The assembly of the DNA-PK complex to DNA ends is required for the NHEJ ligation step. Required for osteocalcin gene expression	-2.73	0.0114
NEUROD2	Appears to mediate neuronal differentiation	-2.73	0.0113
NP_112196.2	N2,N2-dimethylguanosine tRNA methyltransferase; Zinc finger, C2H2-type	-2.74	0.0112
TNR9	Receptor for TNFSF14/4-1BBL. Possibly active during T cell activation	-2.75	0.011
AIFM1	Probable oxidoreductase that acts as a caspase-independent mitochondrial effector of apoptotic cell death. Extramitochondrial AIF induces nuclear chromatin condensation and large scale DNA fragmentation (in vitro)	-2.75	0.011