

UCLA

UCLA Electronic Theses and Dissertations

Title

Mycobacterium tuberculosis tRNA triggers TLR8 to induce a pathway for Th1 cell instruction

Permalink

<https://escholarship.org/uc/item/5mn9477s>

Author

Keegan, Caroline

Publication Date

2016

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Mycobacterium tuberculosis tRNA triggers TLR8
to induce a pathway for Th1 cell instruction

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy
in Microbiology, Immunology and Molecular Genetics

by

Caroline Keegan

2016

© Copyright by

Caroline Keegan

2016

ABSTRACT OF THE DISSERTATION

Mycobacterium tuberculosis tRNA triggers TLR8
to induce a pathway for Th1 cell instruction

by

Caroline Keegan

Doctor of Philosophy in Microbiology, Immunology and Molecular Genetics

University of California, Los Angeles, 2016

Professor Robert L. Modlin, Chair

Mycobacterium tuberculosis, the etiologic agent of tuberculosis, has infected one third of the world's population and is one of the leading global infectious disease threats. Treatment is challenging, lasting upwards of six months even for drug sensitive infections, and the proliferation of multi and extensively drug resistant forms of tuberculosis underscore the urgent need for knowledge of the mechanisms driving pathogenesis of this disease. The ability of the innate immune system to combat infection involves activation of pattern recognition receptors (PRRs) that detect evolutionarily conserved pathogen-associated molecular patterns, including nucleic acids and lipoproteins. Activation of PRRs, including Toll-like receptors (TLRs), induces secretion of inflammatory and immunomodulatory cytokines that instruct adaptive

immune pathways for T cell differentiation. For intracellular pathogens like *M. tuberculosis*, a Th1 response is required, whereas a Th2 response is beneficial for the control of extracellular parasites, but is associated with active tuberculosis disease. This dissertation seeks to understand modulators of the initial immune response to *M. tuberculosis* through characterization of innate immune pathways induced by distinct bacterial ligands. The first study explores a mechanism by which Th1 or Th2 cytokines alter the vitamin D-dependent antimicrobial pathway in response to *M. tuberculosis* 19kD lipoprotein, and illustrates the importance of the Th1 cytokine IFN- γ in guiding an antimicrobial response. Next, is a comparison of the immune response induced by the *M. tuberculosis*-derived ligands: 19kD lipoprotein and purified tRNA. *M. tuberculosis* tRNA was found to induce a gene network inducing secretion of key Th1 cytokines including IL-12p70 and IFN γ , which are necessary for host defense against TB.

The dissertation of Caroline Keegan is approved.

Linda Baum

Genhong Cheng

Stephen Smale

Robert L. Modlin, Committee Chair

University of California, Los Angeles

2016

TABLE OF CONTENTS

List of Figures and Tables	vi
Acknowledgements	viii
Vita	x
Chapter 1	Introduction – Innate and adaptive immune response to <i>Mycobacterium tuberculosis</i>
	1
	References
	26
Chapter 2	T-cell cytokines differentially control human monocyte antimicrobial responses by regulating vitamin D metabolism
	41
	References
	46
Chapter 3	<i>Mycobacterium tuberculosis</i> tRNA triggers $\alpha\beta\gamma\delta$ [$\gamma\delta$] instruction of Th1 cells
	49
	References
	98

LIST OF FIGURES AND TABLES

FIGURES

CHAPTER 1

Figure 1-1	IL-12 / IFN- γ feedback loop during <i>M. tuberculosis</i> infection	23
Figure 1-2	Human Toll-like receptors and ligands	24
Figure 1-3	Cell wall structure of mycobacteria	25

CHAPTER 2

Figure 1	T-cell cytokines differentially influence TLR2/1-induced expression of the antimicrobial peptides cathelicidin and DEFB4.	43
Figure 2	IFN- γ and IL-4 upregulate vitamin D pathway genes in TLR2/1-activated monocytes	43
Figure 3	Effects of IL-4 on 1,25D ₃ responsiveness	44
Figure 4	IFN- γ and IL-4 differentially regulate vitamin D metabolism	44
Figure 5	Regulation of 24-hydroxylase activity by IL-4	45
Figure S1	IL-4 and 1,25D ₃ similarly regulate vitamin D metabolism	48
Figure S2	mRNA expression levels of cytochrome p450 enzymes in TLR2/1 activated monocytes with or without IL-4	48

CHAPTER 3

Figure 3-1	Comparison of gene expression patterns induced by <i>M. tuberculosis</i> lipoprotein and tRNA	80
Figure 3-2	Network analysis of pathways and functions	81
Figure 3-3	Integrated model of central pathways and functions	82
Figure 3-4	Analysis of Th1 genes and validation in PBMC	83
Figure 3-5	Role of IL-18 and NK cells	84
Figure 3-6	Role for TLR8	85
Figure 3-7	Comparison of cytokine secretion by nucleic acid PAMP and synergy between TLR8 and TLR3	86
Figure 3-8	IFN- γ expression in PBMC	87

TABLES

CHAPTER 3

Table 1-1	Top significant differentially expressed genes	88
-----------	------------------------------------------------	----

ACKNOWLEDGEMENTS

I would like to thank my mentor, Robert Modlin, for giving me the opportunity to join his lab, for his constant encouragement, and for helping me find the story in my data. I would also like to thank my committee members, Linda Baum, Genhong Cheng and Steve Smale, for their guidance and insightful feedback. I am grateful to the past and present members of the Modlin Lab, especially Stephan Krutzik, who began this project, and Mirjam Schenk; for their help with experiments and discussions of results. I was fortunate to share this journey with my fellow ModLab PhDs – Susan, Manali and Angeline; grad school would have been nowhere near as fun without their friendship. Finally, I could not have succeeded without the love and support of my parents, Jim and Michele; my sister, Leah; and my fiancé, Yasser; who kept me going through the challenging times and who are a constant source of happiness in my life.

Chapter 1 contains reprints of figures used with permission. Figure 1-1 is adapted with permission from Nature Publishing Group (License #3960000587574). Figure 1-2 is adapted with permission from Elsevier (License #3960431065745). Figure 1-3 is adapted with permission from Nature Publishing Group (License #3960421067287).

Chapter 2 is a reprint of the full citation Edfeldt, K., Liu, P. T., Chun, R., Fabri, M., Schenk, M., Wheelwright, M., Keegan, C., Krutzik, S. R., Adams, J. S., Hewison, M., and Modlin, R. L. 2010. T-cell cytokines differentially control human monocyte

antimicrobial responses by regulating vitamin D metabolism. Proc Natl Acad Sci U S A 107:22593-22598.

Chapter 3 is a version of a manuscript in preparation for publication.

Contributors include Stephan Krutzik, Mirjam Schenk, Jing Lu, Matteo Pellegrini, Barry Bloom, John Belisle, Sarah Fortune, Peter Dedon, and all work was performed with direction by Robert Modlin.

VITA

- 2009 Bachelor of Science, Microbiology, Immunology and Molecular Genetics / Biology
University of California, Los Angeles
- 2011-2012 Teaching Assistant
Department of Microbiology, Immunology and Molecular Genetics
University of California, Los Angeles
- 2009-2016 Graduate Student Researcher
Department of Microbiology, Immunology and Molecular Genetics
University of California, Los Angeles

PUBLICATIONS

- Keegan, C., Krutzik, S., Schenk, M., Lu, J., Pellegrini, M., Bloom, B. R., Belisle, J. T., Fortune, S., Dedon, P., and Modlin, R. L. Mycobacterium tuberculosis tRNA triggers a gene network for instruction of Th1 cells. *In preparation for submission.*
- Edfeldt, K., P.T. Liu, R. Chun, M. Fabri, M. Schenk, M. Wheelwright, C. Keegan, S.R. Krutzik, J.S. Adams, M. Hewison, and R.L. Modlin. 2010. T-cell cytokines differentially control human monocyte antimicrobial responses by regulating vitamin D metabolism. *Proc Natl Acad Sci U S A* 107:22593-22598.
- McHardy, I., C. Keegan, J.H. Sim, W. Shi, and R. Lux. 2010. Transcriptional profiles of *Treponema denticola* in response to environmental conditions. *PLoS One* 5:e13655.

CHAPTER 1

Introduction

Innate and adaptive immune response to

Mycobacterium tuberculosis

Tuberculosis

Mycobacterium tuberculosis, the etiologic agent of tuberculosis, has been a plague on humanity since ancient times. The genomes of ancestral strains of *M. tuberculosis* have been identified and sequenced from Neolithic human remains (HersHKovitz et al., 2008), and the disease was common in the 5th century BCE where Hippocrates reported, "Consumption was the most considerable of the diseases which then prevailed, and the only one which proved fatal to many persons" (Hippocrates and Adams, 1938). Currently, one third of the world's population is infected, and tuberculosis remains one of the leading global infectious disease threats (WHO, 2015).

The most common form of disease is pulmonary tuberculosis, an affliction of the lungs characterized by fever, chest pain, persistent cough, coughing up blood and weight loss. Extrapulmonary tuberculosis, disease occurring outside of the lungs, may take several forms including Pott's disease (infection of the spine), scrofula (infection of the lymph nodes), and miliary tuberculosis (extensive disseminated infection) (Frith, 2014). For much of history, tuberculosis was thought to be an inherited condition. However, in 1882, Robert Koch presented definitive proof that tuberculosis was a transmissible bacterial infection. He demonstrated the presence of bacilli in infected tissues which he isolated and grew in culture, then used the cultured bacteria to infect guinea pigs and reconstitute disease. For this discovery, he was awarded the Nobel Prize in 1905. Still, there was no effective treatment for tuberculosis until the 1940's

when treatment with the antibiotic streptomycin was found to be successful (Zumla et al., 2013), although antibiotic resistance swiftly developed.

At this time, the treatment regimen for susceptible strains involves a six month course of a combination of antibiotics, isoniazid and rifampicin, which have toxic side effects. Premature discontinuation of treatment has fueled the rise of resistant strains (Cohen et al., 2015). The emergence of multi- and extensively-drug resistant tuberculosis is especially pronounced in developing regions where populations are crowded and lack reliable access to health care. The primary preventative measure against tuberculosis has been in use since 1921: inoculation with live *Bacillus Calmette–Guérin* (BCG), an attenuated strain of *Mycobacterium bovis*. However, this vaccine confers only modest protection. A recent meta-analysis determined that infection was reduced by 27%, and progression from latent to active disease was reduced by 58% (Roy et al., 2014). Clearly, there is a need for new methods of tuberculosis prevention and treatment, and for that a thorough understanding of the interaction between host and pathogen is required.

Pathogenesis of tuberculosis

Tuberculosis is transmitted by small droplets containing bacilli which are aerosolized when a person with active pulmonary infection coughs, sneezes or spits. If these particles are inhaled, bacteria are delivered deep into the lungs of the new host. The infectious dose is very low; less than ten bacilli are required to establish infection. In the lungs, *M. tuberculosis* first encounters resident alveolar macrophages which

phagocytose the invading bacteria; engulfing the pathogen and sequestering it in an internal compartment called a phagosome. Neutrophils are rapidly recruited to the site of infection, where they may assist in killing the pathogen as well as secreting chemoattractants to recruit T cells and dendritic cells. Usually, phagocytosed pathogens are destroyed as the phagosome fuses with lysosomes, which contain antimicrobial peptides reactive oxygen species, reactive nitrogen intermediates, and an acidic pH to degrade the contents of the phagolysosome. The degraded products may then be displayed by antigen presenting cells, which thereby instructs the adaptive response. However, *M. tuberculosis* is able to subvert this process, preventing lysosomal fusion and creating a comfortable environment where it may grow (Pethe et al., 2004; Romagnoli et al., 2012). There are also reports that *M. tuberculosis* can escape from the phagosome and replicate within the cytosol (Rahman et al., 2014; Simeone et al., 2012; Srinivasan et al., 2016; van der Wel et al., 2007). It can further attenuate the host immune response through secretion of virulence factors (Houben et al., 2012; Lee et al., 2013; Master et al., 2008; Romagnoli et al., 2012; Sun et al., 2013).

Despite the many strategies employed by *M. tuberculosis* to undermine the host immune response, the majority of humans infected with *M. tuberculosis* are able to control the pathogen and develop a latent infection in which the bacteria is contained within granulomas in the lungs and becomes dormant. However, 5-10% of these latent infections will progress to active disease within the host's lifetime. Individuals with weakened immune systems are especially at risk; 25% of tuberculosis deaths are

associated with HIV co-infection. Considering the staggeringly high number of latently infected individuals, it is important to understand the differences in immune response between those that maintain latent infection and those that suffer disease progression.

Th1 vs. Th2 polarization during M. tuberculosis infection

Although it is not fully clear which immune mechanisms are required for protective immunity against tuberculosis in humans, there is strong correlative evidence to suggest that induction of an appropriate adaptive T cell response contributes to host defense. For control of intracellular pathogens like *M. tuberculosis*, a T helper 1 (Th1) response is required, whereas a T helper 2 (Th2) response is beneficial for the control of extracellular parasites, but is associated with active tuberculosis (Geffner et al., 2009). The Th1 response, associated with containment of *M. tuberculosis* infection, is dominated by the cytokines IFN- γ , IL-12p70, and TNF- α (da Silva et al., 2015). The defining cytokine of Th1-mediated immunity, IFN- γ , is produced by NK and activated T cells, and drives many pathways for defense against *M. tuberculosis*. In mice, IFN- γ is required for induction of nitric oxide (NO) and control of tuberculosis infection. In humans, IFN- γ is critical for phagosome maturation and production of the antimicrobial peptides cathelicidin and beta defensin 2 as part of the vitamin D-dependent antimicrobial pathway (Fabri et al., 2011; Klug-Micu et al., 2013). In both mouse and human systems, IFN- γ can also help overcome the *M. tuberculosis*-induced arrest of phagosome maturation by inducing autophagy, “self-eating”, a process in which the infected cell induces its own destruction, killing invading pathogens in the process

(Alonso et al., 2007; Deretic et al., 2006; Gutierrez et al., 2004). Defects in IFN- γ production and signaling pathways lead to susceptibility to mycobacterial disease (Boisson-Dupuis et al., 2015).

In contrast, a Th2 response is associated with disease progression (Harris et al., 2007) and is associated with production of IL-4, IL-10 and type I IFN. IL-10 is an anti-inflammatory cytokine, known to suppress secretion of IL-12, a Th1 cytokine critical for control of *M. tuberculosis* infection. IL-10 has also been demonstrated to suppress phagosome maturation, a key method employed by phagocytes to destroy pathogens, in *M. tuberculosis*-infected macrophages (O'Leary et al., 2011). Studies have linked abundance of type I interferon with active tuberculosis (Berry et al., 2010; Teles et al., 2013), and hypervirulent strains induce more type I interferon (Manca et al., 2005). The T helper response can be skewed to Th1 or Th2 by cytokines produced as part of the innate response, so it is important to identify how the immune system initially recognizes and responds to *M. tuberculosis* and how this regulates T cell polarizing cytokine response patterns.

Modulation of adaptive immunity by the IL-12 cytokine family

The bioactive form of IL-12, IL-12p70 is one of four members of the IL-12 family of heterodimeric cytokines, two proinflammatory, and two inhibitory. Each member is made from a combination of two independently regulated subunits which are sometimes shared between cytokines, and signal through dimeric receptors which are similarly shared. Despite their similarities in composition, each cytokine induces distinct

responses. Bioactive IL-12 is a combination of IL-12p35 and IL-12p40 and is produced by monocytes, macrophages, dendritic cells and B cells (Gubler et al., 1991). It is known to synergize with IL-18 to induce IFN- γ from natural killer cells and T cells, and is the canonical impetus for Th1 polarization (Micallef et al., 1996). IL-23, a combination of IL-12p19 and IL-12p40, indirectly supports the formation of T helper 17 (Th17) cells by enhancing transcription of *IL17* (Hunter, 2005). IL-27 is composed of Epstein-Barr virus-induced gene 3 (EBI3) protein, and IL-27p28. In mice, IL-27 aids Th1 polarization; acting in synergy with IL-2 and IL-12 to enhance IFN- γ secretion by NK cells (Pflanz et al., 2002), and suppressing the differentiation of naïve T cells into Th17 or inducible T regulatory (iTreg) populations (Neufert et al., 2007). The most recently discovered member of the IL-12 family is IL-35, a combination of EBI3 protein and IL-12p35. In a mouse model, IL-35 suppresses proliferation of both Th1 and Th2 cells, instead converting naïve T cells into a regulatory population designated iTr35 cells (Collison et al., 2012; Collison et al., 2007).

IL-12 mediated response during bacterial infection

IL-12 plays an essential role in the immune response of *M. tuberculosis* by inducing secretion of IFN- γ and promoting Th1 polarization. Diminished IL-12p70 signaling is associated with active tuberculosis, as illustrated by (Song et al., 2000) who found reduced IL-12p70 response to *M. tuberculosis* 30kDa protein in blood from patients with active tuberculosis vs PPD-reactive donors. Deficiencies in members of the IFN- γ and IL-12 signaling pathways, specifically mutations in their receptors

IL12RB1 and IFNGR1, account for the majority of known etiologies for the genetic disorder Mendelian susceptibility to mycobacterial disease (MSMD) (Figure 1-1), which renders the affected susceptible to infection from typically nonpathogenic mycobacteria such as the vaccine strain *M. bovis* BCG (Bustamante et al., 2014). The known causes center around IFN- γ -mediated immunity; ultimately resulting in impaired IFN- γ production or inability to respond to IFN- γ , but defects in IL-12 signaling are among the most common causes of MSMD. For example, a loss of function mutation in IL-12RB1, a component of the IL-12 receptor, produces cells that are unable to respond to IL-12 or IL-23; ultimately resulting in very low IFN- γ production. Other genetic causes of decreased IL-12 signaling are loss of function mutations in IL-12p40 or in NF- κ B essential modulator (NEMO), a kinase which, as its name implies, is required for NF- κ B activation (Ben-Mustapha et al., 2014; Braue et al., 2015). *IL12B* knockout mice were susceptible to death from tuberculosis infection, and exhibited low IFN- γ production. *IL12A* knockout mice survived longer than the *IL12B* knockouts, and produced a little more IFN- γ , however still died early. IL-23 was able to compensate for lack of IL-12p35 for a short time early in the infection; however, the mice eventually succumbed to infection (Cooper et al., 2002; Khader et al., 2005).

IL-12p70 protein regulation

Although IL-12 induces Th1 cells that produce IFN- γ , there is also evidence that IFN- γ signaling can augment IL-12 induction, mediated through an interferon-stimulated response element (ISRE) in the promoter of both *IL12A* and *IL12B*. Priming by IFN- γ

can lead to IL-12 induction through both IRF1 which induces *IL12A* transcription (Kollet and Petro, 2006), and IRF8 which induces both *IL12A* and *IL12B*. IRF3, which is activated downstream of signaling through the TLR3 and TLR4 adaptor protein TRIF, also binds to the p35 promoter (Goriely et al., 2006), and may explain why synergistic activation of Toll-like receptors induces high IL-12p70 secretion (Bekeredjian-Ding et al., 2006).

IL12B transcriptional regulation

Regulation of the IL-12p40 subunit has been more extensively studied. *IL12B* is transcribed only in immune cells (D'Andrea et al., 1992) following recognition of microbial stimuli. TLR engagement results in freeing of the NF- κ B transcription factor complex from an inhibitory protein, allowing the transcription factor to translocate from the cytosol to the nucleus, binding to *IL12B* promoter (Plevy et al., 1997; Sanjabi et al., 2000; Sanjabi et al., 2005). Nucleosome remodeling is also critical for exposing a transcription enhancer element in the *IL12B* promoter (Zhou et al., 2007). IFN- γ further enhances production of IL-12p40 (Murphy et al., 1995; Wang et al., 2000). The IL-12p40 subunit may also be secreted as a monomer or homodimer which can antagonize bioactive IL-12p70 by competitively binding to the IL-12 receptor, but not strongly enough to trigger signaling (Ling et al., 1995).

IL12A transcriptional regulation

The IL-12p35 subunit is encoded by *IL12A*, and, in contrast to *IL12B*, is constitutively expressed at low levels in many cell types, including ones that would not produce bioactive IL-12. The IL-12p35 subunit is not secreted unless as part of the IL-12p70 or IL-35 heterodimer. There are several mechanisms by which *IL12A* transcription is enhanced in immune cells. Similar to *IL12B*, NF- κ B activation is required for transcription of *IL12A* (Grumont et al., 2001), and is enhanced by IFN- γ (Hayes et al., 1995) through binding of IRF1 to an ISRE in the *IL12A* promoter (Liu et al., 2003; Liu et al., 2004). IFN- γ signaling further enhances *IL12A* transcription by driving nucleosome remodeling and exposing G/C rich regions that are binding sites for transcription factor sp1 (SP1) (Goriely et al., 2003). Gene expression is often used as an indicator for eventual protein production, but assessment of IL-12p70 production is not so straightforward, especially in a mixed cell population. In addition to *IL12A* transcription in non-immune cells, the mRNA product produced by unstimulated cells contains additional ATG sites in the 5' untranslated region, which interfere with translation. Following perturbation by microbial stimuli, translation begins from an alternate start codon, allowing protein production (Babik et al., 1999).

Human interferons

Interferons are a class of small signaling molecules that trigger the production of a broad range of effector molecules associated with interfering with viral replication and triggering an immune state to combat viral infection. Of the three interferon families, the

type I interferon family is the most diverse, comprising thirteen isoforms of IFN- α , and IFN- β which are the basis for most studies on the effects of type I interferon, as well as lesser known members IFN- ϵ , IFN- κ and IFN- ω which are still under investigation. All type I interferons signal through the interferon- α/β receptor (IFNAR), a heterodimeric protein composed of the subunits IFNAR1 and IFNAR2.

Type II interferon consists solely of IFN- γ , which signals through the interferon-gamma receptor (IFNGR), a heterodimer formed by IFNGR1 and IFNGR2. Type I and type II interferon signaling pathways are self-reinforcing and antagonistic to the other. Type III interferon contains three isoforms of IFN- λ which signal through a receptor composed of IFN- λ -R1 and IL-10RB, a subunit which is shared with the IL-10 receptor. While it has not yet been thoroughly studied, modulation of the immune response by type III interferon appears nearest in effect to type I interferon (Jordan et al., 2007). Type II interferon is critical for immune control of *M. tuberculosis* infection (Flynn et al., 1993), but both positive and negative effects have been attributed to type I interferon.

Divergent effects of type I interferon during bacterial infection

Though essential for control of many viral infections, type I interferons are generally detrimental in cases of bacterial infection, including infection by mycobacteria. A type I interferon signature was found in the blood of patients with active tuberculosis (Berry et al., 2010; Maertzdorf et al., 2011; Ottenhoff et al., 2012), and type I interferons were shown to suppress production of immune-protective cytokines in both *M. leprae* (Teles et al., 2013) and *M. tuberculosis* infections (McNab et al., 2014). In mice,

hypervirulent strains of *M. tuberculosis* induce more type I interferon, and IFNAR knockout mice survive infection longer (Manca et al., 2005), pointing to a role for type I interferon in driving disease progression. Similarly, a virulence plasmid that stimulates production of type I and type III interferons is associated with increased pathogenesis of *Borrelia burgdorferi*, the causative agent of Lyme disease. (Krupna-Gaylord et al., 2014)

Effect of type I interferon on the IL-12 pathway

Studies have shown both constructive and detrimental effects of type I interferon on the induction of Th1 cytokines including IL-12. High levels of type I interferon were shown to inhibit production of IL-12p70 in both human and mouse cells. Addition of IFN- α or IFN- β inhibited IL-12p70 secretion in response to SAC (fixed *S. aureus* Cowan strain, a potent inducer of IL-12) in mouse spleen cells (Cousens et al., 1997) and in primary human monocytes (Byrnes et al., 2001). In a contrasting study, type I interferon was found to enhance IL-12p70 secretion in response to SAC (Hermann et al., 1998). Furthermore, IL-12p70 secretion in response to combinations of TLR ligands was suppressed in bone marrow-derived macrophages (BMDM) derived from IFNAR knockout mice. A similar result was achieved in human monocyte-derived dendritic cells (mDC) by blocking IFNAR with neutralizing antibodies (Gautier et al., 2005). The discrepancy in results may be explained by a divergent role for type I interferon signaling in different cell types, or stages of immune response. Type I interferon appears to exert a direct effect on IL12B transcription by inhibiting binding of transcription factors to an enhancer element in the IL12B promoter (Byrnes et al., 2001).

However, the main method for type I interferon suppression of IL-12 seems to be indirect, via induction of IL-10 which is a potent inhibitor of both IL12A and IL12B transcription. Thus, the positive effect of type I interferon can be detected in experiments carried out in the presence of IL-10 neutralizing antibodies. Type I interferon was also shown to suppress IL-10 in monocytes and macrophages, while enhancing IL-10 production in T cells, indicating different roles for type I interferon in early vs. late immune response (Feng et al., 2002).

Modulation of IFN- γ signaling by type I interferon

Studies also show conflicting results as to whether type I interferon enhances (Hunter et al., 1997) or inhibits (McRae et al., 1998) secretion of IFN- γ . These paradoxical effects of type I interferon may be explained by the timing of target cell exposure to interferon (Taro et al., 2003). IFN- α enhanced secretion of IL-12p70 in immature human dendritic cells, but repressed secretion from mature dendritic cells (Heystek et al., 2003). Furthermore, TLR stimulation of immature human dendritic cells induced IFN- γ production in $\gamma\delta$ T cells, an innate-like subset of T cells (Devilder et al., 2009). This pathway for IFN- γ secretion was shown to be mediated by type I interferon, and was not reprised using mature dendritic cells, consistent with the idea that type I interferon induces different pathways during the initial and sustained immune response.

Type I interferon has also been shown to synergize with IL-18 (Matikainen et al., 2001) or IL-12 (Duluc et al., 2009) to stimulate IFN- γ secretion and Th1 immunity in the context of viral infection (Matikainen et al., 1998) (Malmgaard and Paludan, 2003).

IFN- α/β also induces production of ISG15, an “interferon stimulated gene” recently discovered as a necessary component for IFN- γ production during mycobacterial infection (Bogunovic et al., 2012). In addition to acting in synergy with IL-12 to induce IFN- γ secretion, ISG15 attenuates IFN- α/β production (Zhang et al., 2015). However, IFN- α and IFN- β were both shown to inhibit the ability of monocytes to mount a Th1 response to bacteria stimuli (de Paus et al., 2013). Still, it is possible that a small amount of type I interferon produced during the beginning of the innate immune response may synergize with signals from additional microbial stimuli to initiate Th1 polarization and cytokine production.

Innate recognition of M. tuberculosis cell wall components

The ability of the innate immune system to combat infection involves activation of pattern recognition receptors (PRRs) that detect evolutionarily conserved pathogen-associated molecular patterns (PAMPs), including nucleic acids and lipoproteins (Figure 1-2). Activation of PRRs, including Toll-like receptors (TLRs), induces secretion of inflammatory and immunomodulatory cytokines that instruct adaptive immune pathways for T cell differentiation. The cell wall of *M. tuberculosis* contains numerous PAMPs that can be recognized by cell surface PRR (Figure 1-3). The mycobacterial plasma membrane is interspersed with a variety of lipoproteins which are surrounded by layers of peptidoglycan and arabinogalactan, followed by a coating of mycolic acids from which the bacterium derives its waxy appearance and acid fastness. Many of these components are recognized by innate immune receptors, and modulate the host

immune response. Trehalose dimycolate (TDM), the most abundant glycolipid on *M. tuberculosis* cell surface has been demonstrated to inhibit fusion of liposomes (Spargo et al., 1991) and delay phagosomal acidification (Axelrod et al., 2008). However, studies also show that TDM is a potent immune activator detected by the carbohydrate-binding C-type lectin receptors in both murine and human cells (Ishikawa et al., 2009; Ostrop et al., 2015).

TLR2 can form a heterodimeric receptor in combination with TLR1 or TLR4, and recognizes a wide variety of lipoproteins and glycoproteins; however, whether this leads to an immune state beneficial or detrimental to host survival is still contested. Lipoproteins extracted from the cell wall of virulent *M. tuberculosis* H37Rv activated both human and mouse macrophages in a TLR2 dependent manner (Brightbill et al., 1999). Further studies employed the *M. tuberculosis* 19kD lipoprotein to activate TLR2 in macrophages infected with the avirulent strain *M. tuberculosis* H37Ra, which led to decreased bacterial survival (Thoma-Uszynski et al., 2001). Later, it was found that, in human primary monocytes cultured in vitamin D sufficient serum, TLR2 induces production of the antimicrobial peptides cathelicidin and beta defensin 2, which directly kill mycobacteria (Liu et al., 2007b). However, despite the ability of TLR2 to activate production of inducible nitric oxide synthase (iNOS), which produces nitric oxide (NO), a critical antimicrobial response in mouse macrophages, some studies have found TLR2 to be dispensable for *in vivo* control of *M. tuberculosis* infection in mice (Holscher et al., 2008). TLR2 activation was shown to suppress IFN- γ mediated response to *M.*

tuberculosis in human macrophages, inhibiting antigen presentation (Gehring et al., 2003).

The cytoplasmic PRR, NOD2, which detects bacterial peptidoglycan (PGN), has also been demonstrated to contribute to anti-mycobacterial immunity. Activation of NOD2 with muramyl dipeptide (MDP), the minimal immunostimulatory motif from PGN, induced production of cathelicidin in human alveolar macrophages, and led to increased control of *M. tuberculosis* H37Rv infection (Juarez et al., 2012).

Detection of M. tuberculosis by endosomal PRR

Following phagocytosis by a host cell, *M. tuberculosis* is contained within a phagocytic vacuole where it may encounter endosomal TLRs. TLR3, TLR7, TLR8 and TLR9 all detect nucleic acids, and are sequestered in a subcellular compartment to avoid detection of self-RNA/DNA in the cytosol. TLR3 detects double stranded RNA (dsRNA); TLR7 and TLR8 sense single stranded RNA (ssRNA), although they are preferentially activated by different nucleotide sequences, and TLR9 recognizes unmethylated CpG-DNA. These TLRs are differentially expressed by subsets of immune cells. TLR3 is predominately expressed in myeloid dendritic cells (mDC), NK cells and T cells; TLR7 and TLR9 are expressed by B cells and plasmacytoid dendritic cells (pDC); and TLR8 is primarily expressed by monocytes (Hornung et al., 2002; Kadowaki et al., 2001).

Innate recognition of bacterial DNA

The potential for bacterial nucleic acids to trigger the innate immune response is beginning to be elucidated (Yamashiro et al., 2014). Within the past 10 years, *M. tuberculosis* DNA has been established as a ligand for the endosomally located TLR9, which detects the unmethylated GC motifs common in bacterial DNA. In mice, detection of mycobacterial DNA by TLR9 was demonstrated to synergize with TLR2 to control high-dose infection *in vivo* (Bafica et al., 2005); although a later study found that double (TLR2/9) and triple (TLR2/4/9) knockout mice were able to control infection (Holscher et al., 2008). In human macrophages, BCG-derived DNA induced TNF- α , which was dependent on phagosomal acidification.

Mycobacterial DNA also activates cytosolic DNA receptors. In mice, DNA from virulent *M. tuberculosis* activated the AIM2 inflammasome, leading to activation of caspase-1 and secretion of proinflammatory IL-1 β . (Saiga et al., 2012) (Yang et al., 2013). The AIM2 inflammasome is critical for control of other intracellular bacteria such as *Francisella tularensis* (Fernandes-Alnemri et al., 2010) and *Streptococcus pneumoniae* (Fang et al., 2011). Recently, STING (stimulator of interferon genes) has been identified as a receptor for extracellular (Watson et al., 2012) and cytosolic (Collins et al., 2015) *M. tuberculosis* DNA. STING is critical for production of type I interferon (Wassermann et al., 2015) in response to *M. tuberculosis*.

Innate recognition of bacterial RNA

There are several indications that *M. tuberculosis* RNA also activates an innate immune response. Extracellular RNA fragments isolated from *M. tuberculosis* broth culture were found to induce apoptosis in human monocytes. Protein kinase-R (PKR), an interferon-inducible dsRNA detector, was shown to be critical for secretion of proinflammatory cytokines in BCG-infected primary human monocytes. A detrimental effect of bacterial RNA was demonstrated in a murine model, where transfection of BCG-RNA induced secretion of IL-10 and suppressed Th1 polarization, an effect that was dependent on the endosomal dsRNA receptor TLR3 (Bai et al., 2014).

Studies of other intracellular pathogens have detailed more extensive modulation of the immune response through recognition of bacterial RNA. *E.coli* RNA activated the NLRP3 inflammasome in human and mouse macrophages, leading to secretion of IL-18 and IL-1 β (Sha et al., 2014). *Borrelia burgdorferi* RNA activated TLR8 in human monocytes, inducing secretion of IFN- β , and synergizing with pathogen-bound TLR2 to control infection (Cervantes et al., 2011; Cervantes et al., 2013). TLR8 was also found to detect RNA from *Staphylococcus aureus* (Bergstrom et al., 2015) and *Streptococcus pyogenes* (Eigenbrod et al., 2015) and induce secretion of IFN- β and inflammatory cytokines.

Role of IL-18

IL-18 is a proinflammatory cytokine that can modulate induction of both Th1 and Th2 immunity, though it is more commonly associated with production of Th1 cytokines

(Nakanishi et al., 2001). Many cells types express *IL 18* mRNA, but the protein is most commonly secreted by monocytes, macrophages, granulocytes and keratinocytes. IL-18 is initially produced in a non-functional precursor form, pro-IL-18, that must undergo proteolytic processing to yield mature IL-18 protein. Like the related cytokine IL-1 β , IL-18 can be processed by the proteases caspase-1 or caspase-4, which themselves exist as inactive precursors until recruited during inflammasome assembly (Akita et al., 1997; Kajiwara et al., 2014), which is initiated in response to a variety of toxins, cell stress signals, and microbial ligands. Secretion of IL-18 and IL-1 β typically requires two signals: one to activate NF- κ b, as in a microbial ligand binding a TLR, leading to precursor protein production, and another to signal cell damage and trigger inflammasome assembly. Fresh monocytes, however, are particularly proficient producers of IL-18 since they already contain active caspase-1, due to inflammasome activation by endogenously generated ATP (Netea et al., 2009).

When originally discovered, IL-18 was described as interferon gamma inducing factor (IGIF) (Okamura et al., 1995). True to its name, IL-18 acts in synergy with a variety of cytokines for the purpose of inducing IFN- γ secretion by NK and T cells. It is considered a characteristic inducer of Th1 cell polarization, but some studies have found the opposite effect within a different cytokine milieu. In mice, in the absence of IL-12, IL-18 can synergize with IL-2 to induce secretion of IL-13 and promote Th2 proliferation (Hoshino et al., 1999). Consistent with this observation, it was found that the presence of IL-12 or IL-4 determined whether addition of IL-18 would lead to promotion of Th1 or Th2 proliferation in a murine model (Smeltz et al., 2001).

Evidence points to a positive role for IL-18 in the control of mycobacterial infection in both humans and mice. IL-18 has been demonstrated to promote production of Th1 cytokines by *M. leprae*-infected human cells (Garcia et al., 1999), and IL18 knockout mice succumbed rapidly to *M. tuberculosis* infection (Schneider et al., 2010).

Role of TLR8

Genome-wide association studies (GWAS) compare genetic variations between large groups of individuals with the intent of discovering variants connected to a trait of interest, such as susceptibility to a disease. These studies have identified polymorphisms in TLR8 that are associated with altering progression of multiple diseases, including HIV, HCV, and tuberculosis (Davila et al., 2008; Oh et al., 2008; Wang et al., 2014). Of particular interest is the single-nucleotide polymorphism (SNP) rs3764880, an A→G substitution that alters the TLR8 start codon, resulting in a truncation of the first three amino acids. This polymorphism was associated with the progression of HIV disease. Patients with the TLR8-G variant exhibited a more gradual decline in CD4⁺ T cell count, thus were slower to progress to AIDS (defined as having counts of less than 200 CD4⁺ T cells/μl), suggesting a protective effect for the TLR8-G variant (Oh et al., 2008).

Surprisingly, a GWAS comparing pulmonary tuberculosis patients to healthy controls found an association between the TLR8-G variant and resistance to developing active infection in Indonesian and Russian populations (Davila et al., 2008). Since then,

it has been correlated with a protective effect against tuberculosis in Pakistani, Turkish and South African populations (Bukhari et al., 2015; Dalgic et al., 2011; Salie et al., 2015). While studies found no association in South African or female Indonesian populations (Chimusa et al., 2014; Kobayashi et al., 2012), a meta-analysis found a correlation between TLR8-A and susceptibility to tuberculosis.

The possibility that TLR8 may play a role the response to tuberculosis is particularly interesting, since the nucleic acid-sensing, endosomal TLR have been primarily studied in the context of viral infection. TLR8 has long been established as a receptor for uridine-rich ssRNA, and a contributor to defense against viral infections, but how this might contribute during bacterial infection was unknown. In recent years, TLR8 has been demonstrated to recognize RNA during infection by the pathogens *Borrelia burgdorferi*, *Streptococcus pyogenes*, and *Staphylococcus aureus* (Bergstrom et al., 2015; Cervantes et al., 2013; Eigenbrod et al., 2015). TLR8 is upregulated in response to BCG infection of THP-1 macrophages (Tang et al., 2016).

It is possible that perturbation of TLR8 activity could influence the outcome of *M. tuberculosis* infection. Stimulation of HIV-infected human PBMC with the TLR8 agonist, ssPolyU RNA, has been shown to inhibit viral replication and induce production of antiviral molecules (Buitendijk et al., 2014). The synthetic TLR8 agonist VTX-1463 is currently being investigated as a potential treatment for allergic rhinitis (Horak, 2011); the mechanism of action appears to be due to shifting the balance of T cell activation to favor development of Th1 vs. Th2 response. Since new tuberculosis therapies involving drug delivery directly into the lungs of infected persons are being investigated (Man et

al., 2016), it may be possible to develop a treatment that modifies the immune response to *M. tuberculosis* locally in the lungs. Further research is required to determine if activation of TLR8 during *M. tuberculosis* infection will have a positive effect on disease outcome. Indeed, overproduction of Th1 cytokines such as IFN- γ during infection has been associated with immunopathology (Couper et al., 2008), so care must be taken. However, it is worth investigating if locally enhancing a Th1 response in the lungs will have an effect on clearance of *M. tuberculosis* infection.

TLR8 is unique, in that it is particularly disposed to induce secretion of IL-12p70 in the absence of signals from other receptors, whereas other TLRs require exogenous cytokine signals to induce IL-12p70 (Bekeredjian-Ding et al., 2006). Stable extracellular RNA has been detected in the supernatant of cultured *M. tuberculosis*, with tRNA being the most highly represented species of RNA (Obregon-Henao et al., 2012). Since tRNA contains regions of both single and double stranded RNA, we hypothesized that it may act as a ligand for TLR8. Also, TLR8 has been shown to be activated by products of RNA degradation (Tanji et al., 2015). TLR8 is present in the endosomal compartment of monocytes, macrophages and myeloid dendritic cells, the cell types most likely to encounter and phagocytose *M. tuberculosis* during initial infection. Thus it is possible that signaling through TLR8 could contribute to direction of the early innate immune response to *M. tuberculosis*. I wanted to explore the possible contribution of tRNA to the immune response to *M. tuberculosis* in contrast to the pathways induced by other TLR ligands.

Figure 1-1: IL-12/IFN- γ positive feedback loop between macrophages/dendritic cells and natural killer/T cells during *M. tuberculosis* infection. Adapted from Chapman and Hill, 2012.

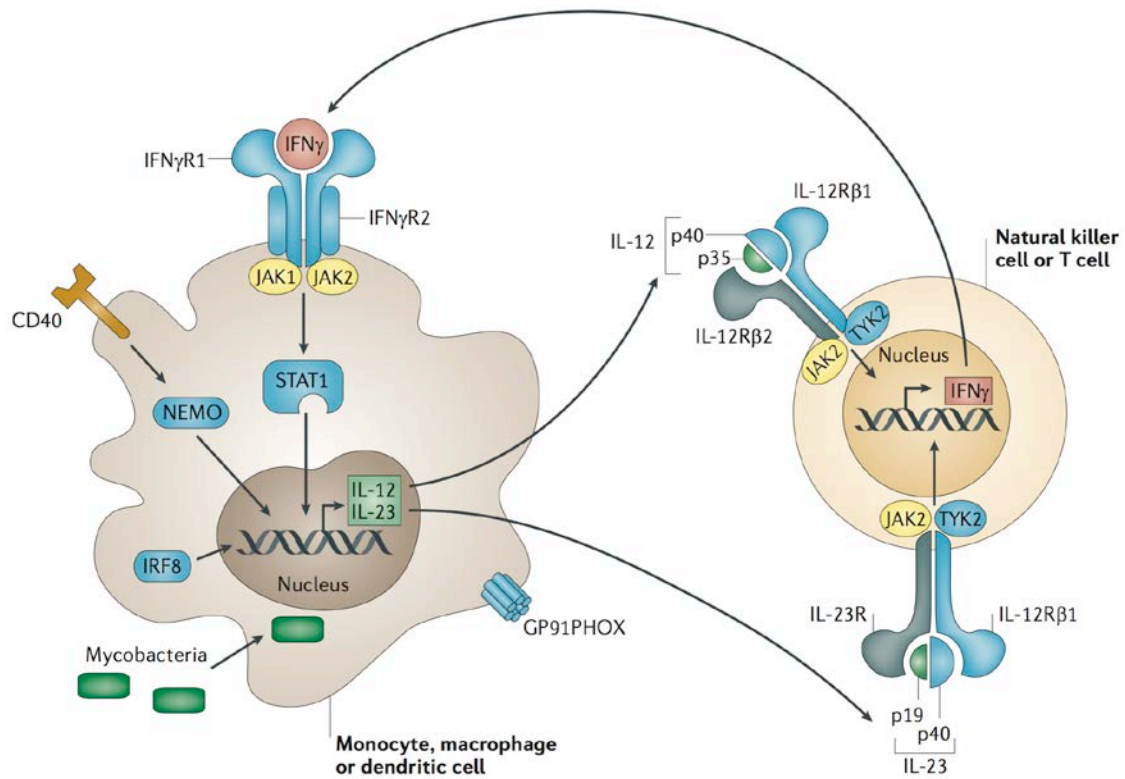
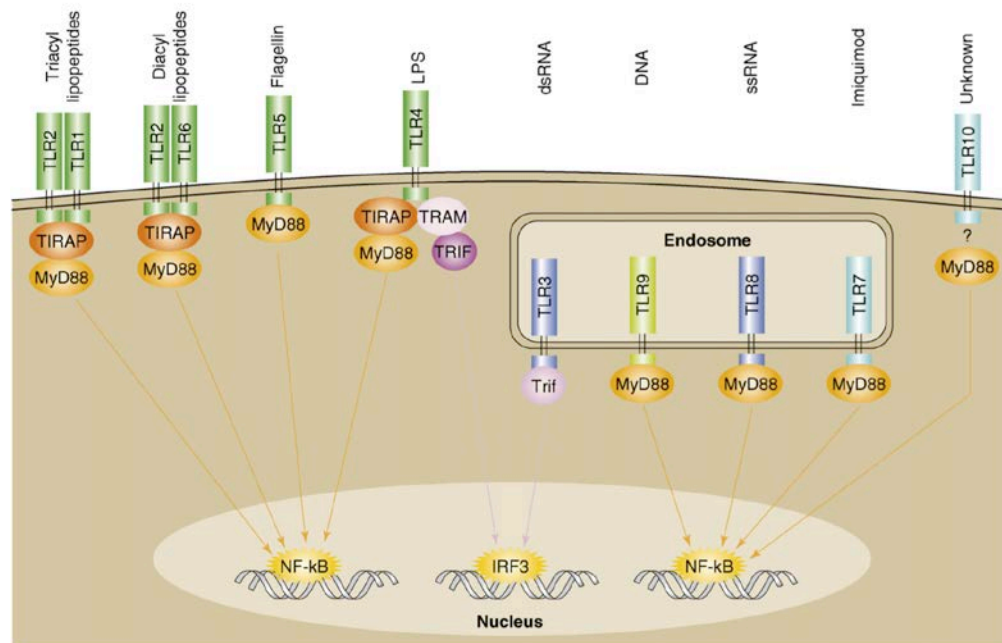
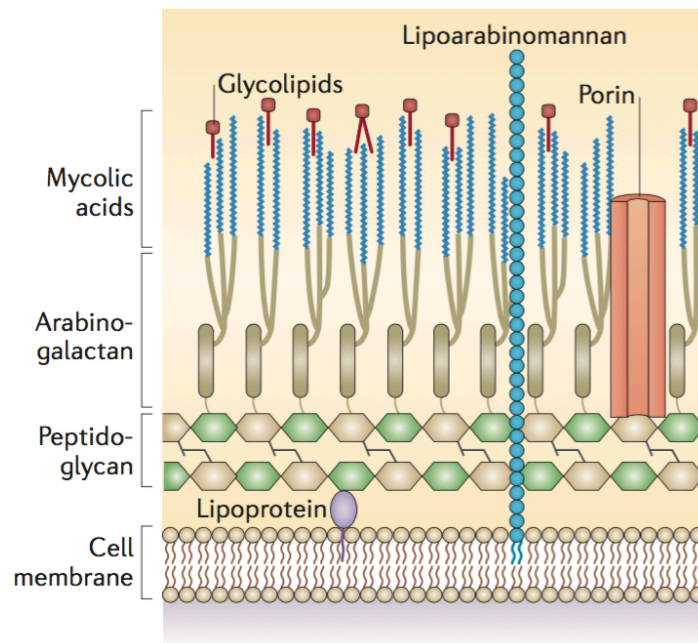


Figure 1-2: Human TLRs and their ligands. Adapted from Liu et al., 2007.



TRENDS in Molecular Medicine

Figure 1-3: Cell wall structure of mycobacteria. Adapted from Brown et al., 2015.



References

- Akita, K., T. Ohtsuki, Y. Nukada, T. Tanimoto, M. Namba, T. Okura, R. Takakura-Yamamoto, K. Torigoe, Y. Gu, M.S. Su, M. Fujii, M. Satoh-Itoh, K. Yamamoto, K. Kohno, M. Ikeda, and M. Kurimoto. 1997. Involvement of caspase-1 and caspase-3 in the production and processing of mature human interleukin 18 in monocytic THP.1 cells. *J Biol Chem* 272:26595-26603.
- Alonso, S., K. Pethe, D.G. Russell, and G.E. Purdy. 2007. Lysosomal killing of Mycobacterium mediated by ubiquitin-derived peptides is enhanced by autophagy. *Proc Natl Acad Sci U S A* 104:6031-6036.
- Axelrod, S., H. Oschkinat, J. Enders, B. Schlegel, V. Brinkmann, S.H. Kaufmann, A. Haas, and U.E. Schaible. 2008. Delay of phagosome maturation by a mycobacterial lipid is reversed by nitric oxide. *Cell Microbiol* 10:1530-1545.
- Babik, J.M., E. Adams, Y. Tone, P.J. Fairchild, M. Tone, and H. Waldmann. 1999. Expression of murine IL-12 is regulated by translational control of the p35 subunit. *J Immunol* 162:4069-4078.
- Bafica, A., C.A. Scanga, C.G. Feng, C. Leifer, A. Cheever, and A. Sher. 2005. TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to Mycobacterium tuberculosis. *J Exp Med* 202:1715-1724.
- Bai, W., H. Liu, Q. Ji, Y. Zhou, L. Liang, R. Zheng, J. Chen, Z. Liu, H. Yang, P. Zhang, S.H. Kaufmann, and B. Ge. 2014. TLR3 regulates mycobacterial RNA-induced IL-10 production through the PI3K/AKT signaling pathway. *Cell Signal* 26:942-950.
- Bekeredjian-Ding, I., S.I. Roth, S. Gilles, T. Giese, A. Ablasser, V. Hornung, S. Endres, and G. Hartmann. 2006. T cell-independent, TLR-induced IL-12p70 production in primary human monocytes. *J Immunol* 176:7438-7446.
- Ben-Mustapha, I., M. Ben-Ali, N. Mekki, E. Patin, C. Harmant, J. Bouguila, H. Elloumi-Zghal, A. Harbi, M. Bejaoui, L. Boughammoura, J. Chemli, and M.R. Barbouche. 2014. A 1,100-year-old founder effect mutation in IL12B gene is responsible for Mendelian susceptibility to mycobacterial disease in Tunisian patients. *Immunogenetics* 66:67-71.
- Bergstrom, B., M.H. Aune, J.A. Awuh, J.F. Kojen, K.J. Blix, L. Ryan, T.H. Flo, T.E. Mollnes, T. Espevik, and J. Stenvik. 2015. TLR8 Senses Staphylococcus aureus RNA in Human Primary Monocytes and Macrophages and Induces IFN-beta Production via a TAK1-IKKbeta-IRF5 Signaling Pathway. *J Immunol* 195:1100-1111.

- Berry, M.P., C.M. Graham, F.W. McNab, Z. Xu, S.A. Bloch, T. Oni, K.A. Wilkinson, R. Banchereau, J. Skinner, R.J. Wilkinson, C. Quinn, D. Blankenship, R. Dhawan, J.J. Cush, A. Mejias, O. Ramilo, O.M. Kon, V. Pascual, J. Banchereau, D. Chaussabel, and A. O'Garra. 2010. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* 466:973-977.
- Bogunovic, D., M. Byun, L.A. Durfee, A. Abhyankar, O. Sanal, D. Mansouri, S. Salem, I. Radovanovic, A.V. Grant, P. Adimi, N. Mansouri, S. Okada, V.L. Bryant, X.F. Kong, A. Kreins, M.M. Velez, B. Boisson, S. Khalilzadeh, U. Ozcelik, I.A. Darazam, J.W. Schoggins, C.M. Rice, S. Al-Muhsen, M. Behr, G. Vogt, A. Puel, J. Bustamante, P. Gros, J.M. Huibregtse, L. Abel, S. Boisson-Dupuis, and J.L. Casanova. 2012. Mycobacterial disease and impaired IFN-gamma immunity in humans with inherited ISG15 deficiency. *Science* 337:1684-1688.
- Boisson-Dupuis, S., J. Bustamante, J. El-Baghdadi, Y. Camcioglu, N. Parvaneh, S. El Azbaoui, A. Agader, A. Hassani, N. El Hafidi, N.A. Mrani, Z. Jouhadi, F. Ailal, J. Najib, I. Reisli, A. Zamani, S. Yosunkaya, S. Gulle-Girit, A. Yildiran, F.E. Cipe, S.H. Torun, A. Metin, B.Y. Atikan, N. Hatipoglu, C. Aydogmus, S.S. Kilic, F. Dogu, N. Karaca, G. Aksu, N. Kutukculer, M. Keser-Emiroglu, A. Somer, G. Tanir, C. Aytekin, P. Adimi, S.A. Mahdavian, S. Mamishi, A. Bousfiha, O. Sanal, D. Mansouri, J.L. Casanova, and L. Abel. 2015. Inherited and acquired immunodeficiencies underlying tuberculosis in childhood. *Immunol Rev* 264:103-120.
- Braue, J., V. Murugesan, S. Holland, N. Patel, E. Naik, J. Leiding, A.T. Yacoub, C.N. Prieto-Granada, and J.N. Greene. 2015. NF-kappaB Essential Modulator Deficiency Leading to Disseminated Cutaneous Atypical Mycobacteria. *Mediterranean journal of hematology and infectious diseases* 7:e2015010.
- Brightbill, H.D., D.H. Libraty, S.R. Krutzik, R.B. Yang, J.T. Belisle, J.R. Bleharski, M. Maitland, M.V. Norgard, S.E. Plevy, S.T. Smale, P.J. Brennan, B.R. Bloom, P.J. Godowski, and R.L. Modlin. 1999. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science* 285:732-736.
- Brown, L., J.M. Wolf, R. Prados-Rosales, and A. Casadevall. 2015. Through the wall: extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. *Nat Rev Microbiol* 13:620-630.
- Buitendijk, M., S.K. Eszterhas, and A.L. Howell. 2014. Toll-like receptor agonists are potent inhibitors of human immunodeficiency virus-type 1 replication in peripheral blood mononuclear cells. *AIDS Res Hum Retroviruses* 30:457-467.
- Bukhari, M., M.A. Aslam, A. Khan, Q. Iram, A. Akbar, A.G. Naz, S. Ahmad, M.M. Ahmad, U.A. Ashfaq, H. Aziz, and M. Ali. 2015. TLR8 gene polymorphism and

- association in bacterial load in southern Punjab of Pakistan: an association study with pulmonary tuberculosis. *Int J Immunogenet* 42:46-51.
- Bustamante, J., S. Boisson-Dupuis, L. Abel, and J.L. Casanova. 2014. Mendelian susceptibility to mycobacterial disease: genetic, immunological, and clinical features of inborn errors of IFN-gamma immunity. *Semin Immunol* 26:454-470.
- Byrnes, A.A., X. Ma, P. Cuomo, K. Park, L. Wahl, S.F. Wolf, H. Zhou, G. Trinchieri, and C.L. Karp. 2001. Type I interferons and IL-12: convergence and cross-regulation among mediators of cellular immunity. *European Journal of Immunology* 31:2026-2034.
- Cervantes, J.L., S.M. Dunham-Ems, C.J. La Vake, M.M. Petzke, B. Sahay, T.J. Sellati, J.D. Radolf, and J.C. Salazar. 2011. Phagosomal signaling by *Borrelia burgdorferi* in human monocytes involves Toll-like receptor (TLR) 2 and TLR8 cooperativity and TLR8-mediated induction of IFN-beta. *Proc Natl Acad Sci U S A* 108:3683-3688.
- Cervantes, J.L., C.J. La Vake, B. Weinerman, S. Luu, C. O'Connell, P.H. Verardi, and J.C. Salazar. 2013. Human TLR8 is activated upon recognition of *Borrelia burgdorferi* RNA in the phagosome of human monocytes. *J Leukoc Biol* 94:1231-1241.
- Chapman, S.J., and A.V. Hill. 2012. Human genetic susceptibility to infectious disease. *Nat Rev Genet* 13:175-188.
- Chimusa, E.R., N. Zaitlen, M. Daya, M. Moller, P.D. van Helden, N.J. Mulder, A.L. Price, and E.G. Hoal. 2014. Genome-wide association study of ancestry-specific TB risk in the South African Coloured population. *Hum Mol Genet* 23:796-809.
- Cohen, K.A., T. Abeel, A. Manson McGuire, C.A. Desjardins, V. Munsamy, T.P. Shea, B.J. Walker, N. Bantubani, D.V. Almeida, L. Alvarado, S.B. Chapman, N.R. Mvelase, E.Y. Duffy, M.G. Fitzgerald, P. Govender, S. Gujja, S. Hamilton, C. Howarth, J.D. Larimer, K. Maharaj, M.D. Pearson, M.E. Priest, Q. Zeng, N. Padayatchi, J. Grosset, S.K. Young, J. Wortman, K.P. Mlisana, M.R. O'Donnell, B.W. Birren, W.R. Bishai, A.S. Pym, and A.M. Earl. 2015. Evolution of Extensively Drug-Resistant Tuberculosis over Four Decades: Whole Genome Sequencing and Dating Analysis of *Mycobacterium tuberculosis* Isolates from KwaZulu-Natal. *PLoS Med* 12:e1001880.
- Collins, A.C., H. Cai, T. Li, L.H. Franco, X.D. Li, V.R. Nair, C.R. Scharn, C.E. Stamm, B. Levine, Z.J. Chen, and M.U. Shiloh. 2015. Cyclic GMP-AMP Synthase Is an Innate Immune DNA Sensor for *Mycobacterium tuberculosis*. *Cell Host Microbe* 17:820-828.

- Collison, L.W., G.M. Delgoffe, C.S. Guy, K.M. Vignali, V. Chaturvedi, D. Fairweather, A.R. Satoskar, K.C. Garcia, C.A. Hunter, C.G. Drake, P.J. Murray, and D.A. Vignali. 2012. The composition and signaling of the IL-35 receptor are unconventional. *Nat Immunol* 13:290-299.
- Collison, L.W., C.J. Workman, T.T. Kuo, K. Boyd, Y. Wang, K.M. Vignali, R. Cross, D. Sehy, R.S. Blumberg, and D.A. Vignali. 2007. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 450:566-569.
- Cooper, A.M., A. Kipnis, J. Turner, J. Magram, J. Ferrante, and I.M. Orme. 2002. Mice Lacking Bioactive IL-12 Can Generate Protective, Antigen-Specific Cellular Responses to Mycobacterial Infection Only if the IL-12 p40 Subunit Is Present. *The Journal of Immunology* 168:1322-1327.
- Couper, K.N., D.G. Blount, and E.M. Riley. 2008. IL-10: the master regulator of immunity to infection. *J Immunol* 180:5771-5777.
- Cousens, L.P., J.S. Orange, H.C. Su, and C.A. Biron. 1997. Interferon-alpha/beta inhibition of interleukin 12 and interferon-gamma production in vitro and endogenously during viral infection. *Proc Natl Acad Sci U S A* 94:634-639.
- D'Andrea, A., M. Rengaraju, N.M. Valiante, J. Chehimi, M. Kubin, M. Aste, S.H. Chan, M. Kobayashi, D. Young, E. Nickbarg, and et al. 1992. Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. *J Exp Med* 176:1387-1398.
- da Silva, M.V., M.G. Tiburcio, J.R. Machado, D.A. Silva, D.B. Rodrigues, V. Rodrigues, and C.J. Oliveira. 2015. Complexity and Controversies over the Cytokine Profiles of T Helper Cell Subpopulations in Tuberculosis. *J Immunol Res* 2015:639107.
- Dalgic, N., D. Tekin, Z. Kayaalti, E. Cakir, T. Soylemezoglu, and M. Sancar. 2011. Relationship between toll-like receptor 8 gene polymorphisms and pediatric pulmonary tuberculosis. *Dis Markers* 31:33-38.
- Davila, S., M.L. Hibberd, R. Hari Dass, H.E. Wong, E. Sahiratmadja, C. Bonnard, B. Alisjahbana, J.S. Szeszko, Y. Balabanova, F. Drobniewski, R. van Crevel, E. van de Vosse, S. Nejentsev, T.H. Ottenhoff, and M. Seielstad. 2008. Genetic association and expression studies indicate a role of toll-like receptor 8 in pulmonary tuberculosis. *PLoS Genet* 4:e1000218.
- de Paus, R.A., A. van Wengen, I. Schmidt, M. Visser, E.M. Verdegaal, J.T. van Dissel, and E. van de Vosse. 2013. Inhibition of the type I immune responses of human monocytes by IFN-alpha and IFN-beta. *Cytokine* 61:645-655.

- Deretic, V., S. Singh, S. Master, J. Harris, E. Roberts, G. Kyei, A. Davis, S. de Haro, J. Naylor, H.H. Lee, and I. Vergne. 2006. Mycobacterium tuberculosis inhibition of phagolysosome biogenesis and autophagy as a host defence mechanism. *Cell Microbiol* 8:719-727.
- Devilder, M.C., S. Allain, C. Dousset, M. Bonneville, and E. Scotet. 2009. Early triggering of exclusive IFN-gamma responses of human Vgamma9Vdelta2 T cells by TLR-activated myeloid and plasmacytoid dendritic cells. *J Immunol* 183:3625-3633.
- Duluc, D., F. Tan, M. Scotet, S. Blanchard, I. Fremaux, E. Garo, B. Horvat, P. Eid, Y. Delneste, and P. Jeannin. 2009. PolyI:C plus IL-2 or IL-12 induce IFN-gamma production by human NK cells via autocrine IFN-beta. *Eur J Immunol* 39:2877-2884.
- Eigenbrod, T., K. Pelka, E. Latz, B. Kreikemeyer, and A.H. Dalpke. 2015. TLR8 Senses Bacterial RNA in Human Monocytes and Plays a Nonredundant Role for Recognition of Streptococcus pyogenes. *J Immunol* 195:1092-1099.
- Fabri, M., S. Stenger, D.M. Shin, J.M. Yuk, P.T. Liu, S. Realegeno, H.M. Lee, S.R. Krutzik, M. Schenk, P.A. Sieling, R. Teles, D. Montoya, S.S. Iyer, H. Bruns, D.M. Lewinsohn, B.W. Hollis, M. Hewison, J.S. Adams, A. Steinmeyer, U. Zugel, G. Cheng, E.K. Jo, B.R. Bloom, and R.L. Modlin. 2011. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. *Sci Transl Med* 3:104ra102.
- Fang, R., K. Tsuchiya, I. Kawamura, Y. Shen, H. Hara, S. Sakai, T. Yamamoto, T. Fernandes-Alnemri, R. Yang, E. Hernandez-Cuellar, S.R. Dewamitta, Y. Xu, H. Qu, E.S. Alnemri, and M. Mitsuyama. 2011. Critical roles of ASC inflammasomes in caspase-1 activation and host innate resistance to Streptococcus pneumoniae infection. *J Immunol* 187:4890-4899.
- Feng, X., D. Yau, C. Holbrook, and A.T. Reder. 2002. Type I interferons inhibit interleukin-10 production in activated human monocytes and stimulate IL-10 in T cells: implications for Th1-mediated diseases. *J Interferon Cytokine Res* 22:311-319.
- Fernandes-Alnemri, T., J.W. Yu, C. Juliana, L. Solorzano, S. Kang, J. Wu, P. Datta, M. McCormick, L. Huang, E. McDermott, L. Eisenlohr, C.P. Landel, and E.S. Alnemri. 2010. The AIM2 inflammasome is critical for innate immunity to Francisella tularensis. *Nat Immunol* 11:385-393.
- Flynn, J.L., J. Chan, K.J. Triebold, D.K. Dalton, T.A. Stewart, and B.R. Bloom. 1993. An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. *J Exp Med* 178:2249-2254.

- Frith, J. 2014. History of Tuberculosis. Part 1 - Phthisis, consumption and the White Plague. *J Mil Veterans Health* 22:
- Garcia, V.E., K. Uyemura, P.A. Sieling, M.T. Ochoa, C.T. Morita, H. Okamura, M. Kurimoto, T.H. Rea, and R.L. Modlin. 1999. IL-18 promotes type 1 cytokine production from NK cells and T cells in human intracellular infection. *J Immunol* 162:6114-6121.
- Gautier, G., M. Humbert, F. Deauvieau, M. Scuiller, J. Hiscott, E.E. Bates, G. Trinchieri, C. Caux, and P. Garrone. 2005. A type I interferon autocrine-paracrine loop is involved in Toll-like receptor-induced interleukin-12p70 secretion by dendritic cells. *J Exp Med* 201:1435-1446.
- Geffner, L., N. Yokobori, J. Basile, P. Schierloh, L. Balboa, M.M. Romero, V. Ritacco, M. Vescovo, P. Gonzalez Montaner, B. Lopez, L. Barrera, M. Aleman, E. Abatte, M.C. Sasiain, and S. de la Barrera. 2009. Patients with multidrug-resistant tuberculosis display impaired Th1 responses and enhanced regulatory T-cell levels in response to an outbreak of multidrug-resistant Mycobacterium tuberculosis M and Ra strains. *Infect Immun* 77:5025-5034.
- Gehring, A.J., R.E. Rojas, D.H. Canaday, D.L. Lakey, C.V. Harding, and W.H. Boom. 2003. The Mycobacterium tuberculosis 19-kilodalton lipoprotein inhibits gamma interferon-regulated HLA-DR and Fc gamma R1 on human macrophages through Toll-like receptor 2. *Infect Immun* 71:4487-4497.
- Goriely, S., D. Demonte, S. Nizet, D. De Wit, F. Willems, M. Goldman, and C. Van Lint. 2003. Human IL-12(p35) gene activation involves selective remodeling of a single nucleosome within a region of the promoter containing critical Sp1-binding sites. *Blood* 101:4894-4902.
- Goriely, S., C. Molle, M. Nguyen, V. Albarani, N.O. Haddou, R. Lin, D. De Wit, V. Flamand, F. Willems, and M. Goldman. 2006. Interferon regulatory factor 3 is involved in Toll-like receptor 4 (TLR4)- and TLR3-induced IL-12p35 gene activation. *Blood* 107:1078-1084.
- Grumont, R., H. Hochrein, M. O'Keeffe, R. Gugasyan, C. White, I. Caminschi, W. Cook, and S. Gerondakis. 2001. c-Rel regulates interleukin 12 p70 expression in CD8(+) dendritic cells by specifically inducing p35 gene transcription. *J Exp Med* 194:1021-1032.
- Gubler, U., A.O. Chua, D.S. Schoenhaut, C.M. Dwyer, W. McComas, R. Motyka, N. Nabavi, A.G. Wolitzky, P.M. Quinn, and P.C. Familletti. 1991. Coexpression of two distinct genes is required to generate secreted bioactive cytotoxic lymphocyte maturation factor. *Proceedings of the National Academy of Sciences* 88:4143-4147.

- Gutierrez, M.G., S.S. Master, S.B. Singh, G.A. Taylor, M.I. Colombo, and V. Deretic. 2004. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell* 119:753-766.
- Harris, J., S.A. De Haro, S.S. Master, J. Keane, E.A. Roberts, M. Delgado, and V. Deretic. 2007. T helper 2 cytokines inhibit autophagic control of intracellular Mycobacterium tuberculosis. *Immunity* 27:505-517.
- Hayes, M.P., J. Wang, and M.A. Norcross. 1995. Regulation of interleukin-12 expression in human monocytes: selective priming by interferon-gamma of lipopolysaccharide-inducible p35 and p40 genes. *Blood* 86:646-650.
- Hermann, P., M. Rubio, T. Nakajima, G. Delespesse, and M. Sarfati. 1998. IFN-alpha priming of human monocytes differentially regulates gram-positive and gram-negative bacteria-induced IL-10 release and selectively enhances IL-12p70, CD80, and MHC class I expression. *J Immunol* 161:2011-2018.
- Hershkovitz, I., H.D. Donoghue, D.E. Minnikin, G.S. Besra, O.Y. Lee, A.M. Gernaey, E. Galili, V. Eshed, C.L. Greenblatt, E. Lemma, G.K. Bar-Gal, and M. Spigelman. 2008. Detection and molecular characterization of 9,000-year-old Mycobacterium tuberculosis from a Neolithic settlement in the Eastern Mediterranean. *PLoS One* 3:e3426.
- Heystek, H.C., B. den Drijver, M.L. Kapsenberg, R.A. van Lier, and E.C. de Jong. 2003. Type I IFNs differentially modulate IL-12p70 production by human dendritic cells depending on the maturation status of the cells and counteract IFN-gamma-mediated signaling. *Clin Immunol* 107:170-177.
- Hippocrates, and F. Adams. 1938. Hippocrates, selected works. Edwards Brothers, Inc., Ann Arbor, Mich.,. 109 p. pp.
- Holscher, C., N. Reiling, U.E. Schaible, A. Holscher, C. Bathmann, D. Korbel, I. Lenz, T. Sonntag, S. Kroger, S. Akira, H. Mossmann, C.J. Kirschning, H. Wagner, M. Freudenberg, and S. Ehlers. 2008. Containment of aerogenic Mycobacterium tuberculosis infection in mice does not require MyD88 adaptor function for TLR2, -4 and -9. *Eur J Immunol* 38:680-694.
- Horak, F. 2011. VTX-1463, a novel TLR8 agonist for the treatment of allergic rhinitis. *Expert Opin Investig Drugs* 20:981-986.
- Hornung, V., S. Rothenfusser, S. Britsch, A. Krug, B. Jahrsdorfer, T. Giese, S. Endres, and G. Hartmann. 2002. Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J Immunol* 168:4531-4537.

- Hoshino, T., R.H. Wiltout, and H.A. Young. 1999. IL-18 is a potent coinducer of IL-13 in NK and T cells: a new potential role for IL-18 in modulating the immune response. *J Immunol* 162:5070-5077.
- Houben, D., C. Demangel, J. van Ingen, J. Perez, L. Baldeon, A.M. Abdallah, L. Caleechurn, D. Bottai, M. van Zon, K. de Punder, T. van der Laan, A. Kant, R. Bossers-de Vries, P. Willemsen, W. Bitter, D. van Soolingen, R. Brosch, N. van der Wel, and P.J. Peters. 2012. ESX-1-mediated translocation to the cytosol controls virulence of mycobacteria. *Cell Microbiol* 14:1287-1298.
- Hunter, C.A. 2005. New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. *Nat Rev Immunol* 5:521-531.
- Hunter, C.A., K.E. Gabriel, T. Radzanowski, L.E. Neyer, and J.S. Remington. 1997. Type I interferons enhance production of IFN-gamma by NK cells. *Immunol Lett* 59:1-5.
- Ishikawa, E., T. Ishikawa, Y.S. Morita, K. Toyonaga, H. Yamada, O. Takeuchi, T. Kinoshita, S. Akira, Y. Yoshikai, and S. Yamasaki. 2009. Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. *J Exp Med* 206:2879-2888.
- Jordan, W.J., J. Eskdale, M. Boniotto, M. Rodia, D. Kellner, and G. Gallagher. 2007. Modulation of the human cytokine response by interferon lambda-1 (IFN-lambda1/IL-29). *Genes Immun* 8:13-20.
- Juarez, E., C. Carranza, F. Hernandez-Sanchez, J.C. Leon-Contreras, R. Hernandez-Pando, D. Escobedo, M. Torres, and E. Sada. 2012. NOD2 enhances the innate response of alveolar macrophages to Mycobacterium tuberculosis in humans. *Eur J Immunol* 42:880-889.
- Kadowaki, N., S. Ho, S. Antonenko, R.W. Malefyt, R.A. Kastelein, F. Bazan, and Y.J. Liu. 2001. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med* 194:863-869.
- Kajiwara, Y., T. Schiff, G. Voloudakis, M.A. Gama Sosa, G. Elder, O. Bozdagi, and J.D. Buxbaum. 2014. A critical role for human caspase-4 in endotoxin sensitivity. *J Immunol* 193:335-343.
- Khader, S.A., J.E. Pearl, K. Sakamoto, L. Gilmartin, G.K. Bell, D.M. Jelley-Gibbs, N. Ghilardi, F. deSavauge, and A.M. Cooper. 2005. IL-23 Compensates for the Absence of IL-12p70 and Is Essential for the IL-17 Response during Tuberculosis but Is Dispensable for Protection and Antigen-Specific IFN- Responses if IL-12p70 Is Available. *The Journal of Immunology* 175:788-795.

- Klug-Micu, G.M., S. Stenger, A. Sommer, P.T. Liu, S.R. Krutzik, R.L. Modlin, and M. Fabri. 2013. CD40 ligand and interferon-gamma induce an antimicrobial response against *Mycobacterium tuberculosis* in human monocytes. *Immunology* 139:121-128.
- Kobayashi, K., R. Yuliwulandari, H. Yanai, I. Naka, L.T. Lien, N.T. Hang, M. Hijikata, N. Keicho, and K. Tokunaga. 2012. Association of TLR polymorphisms with development of tuberculosis in Indonesian females. *Tissue Antigens* 79:190-197.
- Kollet, J.I., and T.M. Petro. 2006. IRF-1 and NF-kappaB p50/cRel bind to distinct regions of the proximal murine IL-12 p35 promoter during costimulation with IFN-gamma and LPS. *Mol Immunol* 43:623-633.
- Krupna-Gaylord, M.A., D. Liveris, A.C. Love, G.P. Wormser, I. Schwartz, and M.M. Petzke. 2014. Induction of type I and type III interferons by *Borrelia burgdorferi* correlates with pathogenesis and requires linear plasmid 36. *PLoS One* 9:e100174.
- Lee, E.J., M.H. Pontes, and E.A. Groisman. 2013. A bacterial virulence protein promotes pathogenicity by inhibiting the bacterium's own F1Fo ATP synthase. *Cell* 154:146-156.
- Ling, P., M.K. Gately, U. Gubler, A.S. Stern, P. Lin, K. Hollfelder, C. Su, Y.C. Pan, and J. Hakimi. 1995. Human IL-12 p40 homodimer binds to the IL-12 receptor but does not mediate biologic activity. *J Immunol* 154:116-127.
- Liu, J., S. Cao, L.M. Herman, and X. Ma. 2003. Differential regulation of interleukin (IL)-12 p35 and p40 gene expression and interferon (IFN)-gamma-primed IL-12 production by IFN regulatory factor 1. *J Exp Med* 198:1265-1276.
- Liu, J., X. Guan, T. Tamura, K. Ozato, and X. Ma. 2004. Synergistic activation of interleukin-12 p35 gene transcription by interferon regulatory factor-1 and interferon consensus sequence-binding protein. *J Biol Chem* 279:55609-55617.
- Liu, P.T., S.R. Krutzik, and R.L. Modlin. 2007a. Therapeutic implications of the TLR and VDR partnership. *Trends Mol Med* 13:117-124.
- Liu, P.T., S. Stenger, D.H. Tang, and R.L. Modlin. 2007b. Cutting edge: vitamin D-mediated human antimicrobial activity against *Mycobacterium tuberculosis* is dependent on the induction of cathelicidin. *J Immunol* 179:2060-2063.
- Maertzdorf, J., D. Reipsilber, S.K. Parida, K. Stanley, T. Roberts, G. Black, G. Walzl, and S.H. Kaufmann. 2011. Human gene expression profiles of susceptibility and resistance in tuberculosis. *Genes Immun* 12:15-22.

- Malmgaard, L., and S.R. Paludan. 2003. Interferon (IFN)-alpha/beta, interleukin (IL)-12 and IL-18 coordinately induce production of IFN-gamma during infection with herpes simplex virus type 2. *J Gen Virol* 84:2497-2500.
- Man, D.K., M.Y. Chow, L. Casettari, M. Gonzalez-Juarrero, and J.K. Lam. 2016. Potential and development of inhaled RNAi therapeutics for the treatment of pulmonary tuberculosis. *Adv Drug Deliv Rev* 102:21-32.
- Manca, C., L. Tsenova, S. Freeman, A.K. Barczak, M. Tovey, P.J. Murray, C. Barry, and G. Kaplan. 2005. Hypervirulent M. tuberculosis W/Beijing strains upregulate type I IFNs and increase expression of negative regulators of the Jak-Stat pathway. *J Interferon Cytokine Res* 25:694-701.
- Master, S.S., S.K. Rampini, A.S. Davis, C. Keller, S. Ehlers, B. Springer, G.S. Timmins, P. Sander, and V. Deretic. 2008. Mycobacterium tuberculosis prevents inflammasome activation. *Cell Host Microbe* 3:224-232.
- Matikainen, S., A. Lehtonen, T. Sareneva, and I. Julkunen. 1998. Regulation of IRF and STAT gene expression by retinoic acid. *Leuk Lymphoma* 30:63-71.
- Matikainen, S., A. Paananen, M. Miettinen, M. Kurimoto, T. Timonen, I. Julkunen, and T. Sareneva. 2001. IFN-alpha and IL-18 synergistically enhance IFN-gamma production in human NK cells: differential regulation of Stat4 activation and IFN-gamma gene expression by IFN-alpha and IL-12. *Eur J Immunol* 31:2236-2245.
- McNab, F.W., J. Ewbank, A. Howes, L. Moreira-Teixeira, A. Martirosyan, N. Ghilardi, M. Saraiva, and A. O'Garra. 2014. Type I IFN induces IL-10 production in an IL-27-independent manner and blocks responsiveness to IFN-gamma for production of IL-12 and bacterial killing in Mycobacterium tuberculosis-infected macrophages. *J Immunol* 193:3600-3612.
- McRae, B.L., R.T. Semnani, M.P. Hayes, and G.A. van Seventer. 1998. Type I IFNs inhibit human dendritic cell IL-12 production and Th1 cell development. *J Immunol* 160:4298-4304.
- Micallef, M.J., T. Ohtsuki, K. Kohno, F. Tanabe, S. Ushio, M. Namba, T. Tanimoto, K. Torigoe, M. Fujii, M. Ikeda, S. Fukuda, and M. Kurimoto. 1996. Interferon-gamma-inducing factor enhances T helper 1 cytokine production by stimulated human T cells: synergism with interleukin-12 for interferon-gamma production. *Eur J Immunol* 26:1647-1651.
- Murphy, T.L., M.G. Cleveland, P. Kulesza, J. Magram, and K.M. Murphy. 1995. Regulation of interleukin 12 p40 expression through an NF-kappa B half-site. *Molecular and Cellular Biology* 15:5258-5267.

- Nakanishi, K., T. Yoshimoto, H. Tsutsui, and H. Okamura. 2001. Interleukin-18 regulates both Th1 and Th2 responses. *Annu Rev Immunol* 19:423-474.
- Netea, M.G., C.A. Nold-Petry, M.F. Nold, L.A. Joosten, B. Opitz, J.H. van der Meer, F.L. van de Veerdonk, G. Ferwerda, B. Heinhuis, I. Devesa, C.J. Funk, R.J. Mason, B.J. Kullberg, A. Rubartelli, J.W. van der Meer, and C.A. Dinarello. 2009. Differential requirement for the activation of the inflammasome for processing and release of IL-1 β in monocytes and macrophages. *Blood* 113:2324-2335.
- Neufert, C., C. Becker, S. Wirtz, M.C. Fantini, B. Weigmann, P.R. Galle, and M.F. Neurath. 2007. IL-27 controls the development of inducible regulatory T cells and Th17 cells via differential effects on STAT1. *Eur J Immunol* 37:1809-1816.
- O'Leary, S., M.P. O'Sullivan, and J. Keane. 2011. IL-10 blocks phagosome maturation in mycobacterium tuberculosis-infected human macrophages. *Am J Respir Cell Mol Biol* 45:172-180.
- Obregon-Henao, A., M.A. Duque-Correa, M. Rojas, L.F. Garcia, P.J. Brennan, B.L. Ortiz, and J.T. Belisle. 2012. Stable extracellular RNA fragments of Mycobacterium tuberculosis induce early apoptosis in human monocytes via a caspase-8 dependent mechanism. *PLoS One* 7:e29970.
- Oh, D.Y., S. Taube, O. Hamouda, C. Kucherer, G. Poggensee, H. Jessen, J.K. Eckert, K. Neumann, A. Storek, M. Pouliot, P. Borgeat, N. Oh, E. Schreier, A. Pruss, K. Hattermann, and R.R. Schumann. 2008. A functional toll-like receptor 8 variant is associated with HIV disease restriction. *J Infect Dis* 198:701-709.
- Okamura, H., K. Nagata, T. Komatsu, T. Tanimoto, Y. Nukata, F. Tanabe, K. Akita, K. Torigoe, T. Okura, and S. Fukuda. 1995. A novel costimulatory factor for gamma interferon induction found in the livers of mice causes endotoxic shock. *Infection and immunity* 63:3966-3972.
- Ostrop, J., K. Jozefowski, S. Zimmermann, K. Hofmann, E. Strasser, B. Lepenies, and R. Lang. 2015. Contribution of MINCLE-SYK Signaling to Activation of Primary Human APCs by Mycobacterial Cord Factor and the Novel Adjuvant TDB. *J Immunol* 195:2417-2428.
- Ottenhoff, T.H., R.H. Dass, N. Yang, M.M. Zhang, H.E. Wong, E. Sahiratmadja, C.C. Khor, B. Alisjahbana, R. van Crevel, S. Marzuki, M. Seielstad, E. van de Vosse, and M.L. Hibberd. 2012. Genome-wide expression profiling identifies type 1 interferon response pathways in active tuberculosis. *PLoS One* 7:e45839.
- Pethe, K., D.L. Swenson, S. Alonso, J. Anderson, C. Wang, and D.G. Russell. 2004. Isolation of Mycobacterium tuberculosis mutants defective in the arrest of phagosome maturation. *Proc Natl Acad Sci U S A* 101:13642-13647.

- Pflanz, S., J.C. Timans, J. Cheung, R. Rosales, H. Kanzler, J. Gilbert, L. Hibbert, T. Churakova, M. Travis, E. Vaisberg, W.M. Blumenschein, J.D. Mattson, J.L. Wagner, W. To, S. Zurawski, T.K. McClanahan, D.M. Gorman, J.F. Bazan, R. de Waal Malefyt, D. Rennick, and R.A. Kastelein. 2002. IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4+ T cells. *Immunity* 16:779-790.
- Plevy, S.E., J.H. Gemberling, S. Hsu, A.J. Dorner, and S.T. Smale. 1997. Multiple control elements mediate activation of the murine and human interleukin 12 p40 promoters: evidence of functional synergy between C/EBP and Rel proteins. *Molecular and Cellular Biology* 17:4572-4588.
- Rahman, A., P. Sobia, N. Gupta, L.V. Kaer, and G. Das. 2014. Mycobacterium tuberculosis subverts the TLR-2-MyD88 pathway to facilitate its translocation into the cytosol. *PLoS One* 9:e86886.
- Romagnoli, A., M.P. Etna, E. Giacomini, M. Pardini, M.E. Remoli, M. Corazzari, L. Falasca, D. Goletti, V. Gafa, R. Simeone, G. Delogu, M. Piacentini, R. Brosch, G.M. Fimia, and E.M. Coccia. 2012. ESX-1 dependent impairment of autophagic flux by Mycobacterium tuberculosis in human dendritic cells. *Autophagy* 8:1357-1370.
- Roy, A., M. Eisenhut, R.J. Harris, L.C. Rodrigues, S. Sridhar, S. Habermann, L. Snell, P. Mangtani, I. Adetifa, A. Lalvani, and I. Abubakar. 2014. Effect of BCG vaccination against Mycobacterium tuberculosis infection in children: systematic review and meta-analysis. *BMJ* 349:g4643.
- Saiga, H., S. Kitada, Y. Shimada, N. Kamiyama, M. Okuyama, M. Makino, M. Yamamoto, and K. Takeda. 2012. Critical role of AIM2 in Mycobacterium tuberculosis infection. *Int Immunol* 24:637-644.
- Salie, M., M. Daya, L.A. Lucas, R.M. Warren, G.D. van der Spuy, P.D. van Helden, E.G. Hoal, and M. Moller. 2015. Association of toll-like receptors with susceptibility to tuberculosis suggests sex-specific effects of TLR8 polymorphisms. *Infect Genet Evol* 34:221-229.
- Sanjabi, S., A. Hoffmann, H.C. Liou, D. Baltimore, and S.T. Smale. 2000. Selective requirement for c-Rel during IL-12 P40 gene induction in macrophages. *Proc Natl Acad Sci U S A* 97:12705-12710.
- Sanjabi, S., K.J. Williams, S. Sacconi, L. Zhou, A. Hoffmann, G. Ghosh, S. Gerondakis, G. Natoli, and S.T. Smale. 2005. A c-Rel subdomain responsible for enhanced DNA-binding affinity and selective gene activation. *Genes Dev* 19:2138-2151.

- Schneider, B.E., D. Korbel, K. Hagens, M. Koch, B. Raupach, J. Enders, S.H. Kaufmann, H.W. Mittrucker, and U.E. Schaible. 2010. A role for IL-18 in protective immunity against *Mycobacterium tuberculosis*. *Eur J Immunol* 40:396-405.
- Sha, W., H. Mitoma, S. Hanabuchi, M. Bao, L. Weng, N. Sugimoto, Y. Liu, Z. Zhang, J. Zhong, B. Sun, and Y.J. Liu. 2014. Human NLRP3 inflammasome senses multiple types of bacterial RNAs. *Proc Natl Acad Sci U S A* 111:16059-16064.
- Simeone, R., A. Bobard, J. Lippmann, W. Bitter, L. Majlessi, R. Brosch, and J. Enninga. 2012. Phagosomal rupture by *Mycobacterium tuberculosis* results in toxicity and host cell death. *PLoS Pathog* 8:e1002507.
- Smeltz, R.B., J. Chen, J. Hu-Li, and E.M. Shevach. 2001. Regulation of interleukin (IL)-18 receptor alpha chain expression on CD4(+) T cells during T helper (Th)1/Th2 differentiation. Critical downregulatory role of IL-4. *J Exp Med* 194:143-153.
- Song, C.H., H.J. Kim, J.K. Park, J.H. Lim, U.O. Kim, J.S. Kim, T.H. Paik, K.J. Kim, J.W. Suhr, and E.K. Jo. 2000. Depressed Interleukin-12 (IL-12), but not IL-18, Production in Response to a 30- or 32-Kilodalton *Mycobacterial* Antigen in Patients with Active Pulmonary Tuberculosis. *Infection and Immunity* 68:4477-4484.
- Spargo, B.J., L.M. Crowe, T. Itoneda, B.L. Beaman, and J.H. Crowe. 1991. Cord factor (alpha,alpha-trehalose 6,6'-dimycolate) inhibits fusion between phospholipid vesicles. *Proc Natl Acad Sci U S A* 88:737-740.
- Srinivasan, L., S.A. Gurses, B.E. Hurley, J.L. Miller, P.C. Karakousis, and V. Briken. 2016. Identification of a Transcription Factor That Regulates Host Cell Exit and Virulence of *Mycobacterium tuberculosis*. *PLoS Pathog* 12:e1005652.
- Sun, J., V. Singh, A. Lau, R.W. Stokes, A. Obregon-Henao, I.M. Orme, D. Wong, Y. Av-Gay, and Z. Hmama. 2013. *Mycobacterium tuberculosis* nucleoside diphosphate kinase inactivates small GTPases leading to evasion of innate immunity. *PLoS Pathog* 9:e1003499.
- Tang, J., L. Zhan, and C. Qin. 2016. Inhibition of TLR8 mediated signaling promotes BCG induced apoptosis in THP-1 cells. *Microb Pathog* 93:78-82.
- Tanji, H., U. Ohto, T. Shibata, M. Taoka, Y. Yamauchi, T. Isobe, K. Miyake, and T. Shimizu. 2015. Toll-like receptor 8 senses degradation products of single-stranded RNA. *Nat Struct Mol Biol* 22:109-115.
- Taro, N., D. Odile, F.M. Thomas, L.P. David, S. Jean Maguire van, and A.v.S. Gijs. 2003. Timing of IFN-beta exposure during human dendritic cell maturation and

- naive Th cell stimulation has contrasting effects on Th1 subset generation: a role for IFN-beta-mediated regulation of IL-12 family cytokines and IL-18 in naive Th cell differentiation. *Journal of immunology (Baltimore, Md. : 1950)* 171:5233-5243.
- Teles, R.M., T.G. Graeber, S.R. Krutzik, D. Montoya, M. Schenk, D.J. Lee, E. Komisopoulou, K. Kelly-Scumpia, R. Chun, S.S. Iyer, E.N. Sarno, T.H. Rea, M. Hewison, J.S. Adams, S.J. Popper, D.A. Relman, S. Stenger, B.R. Bloom, G. Cheng, and R.L. Modlin. 2013. Type I interferon suppresses type II interferon-triggered human anti-mycobacterial responses. *Science* 339:1448-1453.
- Thoma-Uszynski, S., S. Stenger, O. Takeuchi, M.T. Ochoa, M. Engele, P.A. Sieling, P.F. Barnes, M. Rollinghoff, P.L. Bolcskei, M. Wagner, S. Akira, M.V. Norgard, J.T. Belisle, P.J. Godowski, B.R. Bloom, and R.L. Modlin. 2001. Induction of direct antimicrobial activity through mammalian toll-like receptors. *Science* 291:1544-1547.
- van der Wel, N., D. Hava, D. Houben, D. Fluitsma, M. van Zon, J. Pierson, M. Brenner, and P.J. Peters. 2007. M. tuberculosis and M. leprae translocate from the phagolysosome to the cytosol in myeloid cells. *Cell* 129:1287-1298.
- Wang, C.H., H.L. Eng, K.H. Lin, H.C. Liu, C.H. Chang, and T.M. Lin. 2014. Functional polymorphisms of TLR8 are associated with hepatitis C virus infection. *Immunology* 141:540-548.
- Wang, I.M., C. Contursi, A. Masumi, X. Ma, G. Trinchieri, and K. Ozato. 2000. An IFN-gamma-inducible transcription factor, IFN consensus sequence binding protein (ICSBP), stimulates IL-12 p40 expression in macrophages. *J Immunol* 165:271-279.
- Wassermann, R., M.F. Gulen, C. Sala, S.G. Perin, Y. Lou, J. Rybniker, J.L. Schmid-Burgk, T. Schmidt, V. Hornung, S.T. Cole, and A. Ablasser. 2015. Mycobacterium tuberculosis Differentially Activates cGAS- and Inflammasome-Dependent Intracellular Immune Responses through ESX-1. *Cell Host Microbe* 17:799-810.
- Watson, R.O., P.S. Manzanillo, and J.S. Cox. 2012. Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. *Cell* 150:803-815.
- WHO. 2015. Global Tuberculosis Report. In World Health Organization, Geneva.
- Yamashiro, L.H., S.C. Oliveira, and A. Bafica. 2014. Innate immune sensing of nucleic acids from mycobacteria. *Microbes Infect* 16:991-997.

- Yang, Y., X. Zhou, M. Kouadir, F. Shi, T. Ding, C. Liu, J. Liu, M. Wang, L. Yang, X. Yin, and D. Zhao. 2013. the AIM2 inflammasome is involved in macrophage activation during infection with virulent *Mycobacterium bovis* strain. *J Infect Dis* 208:1849-1858.
- Zhang, X., D. Bogunovic, B. Payelle-Brogard, V. Francois-Newton, S.D. Speer, C. Yuan, S. Volpi, Z. Li, O. Sanal, D. Mansouri, I. Tezcan, G.I. Rice, C. Chen, N. Mansouri, S.A. Mahdavian, Y. Itan, B. Boisson, S. Okada, L. Zeng, X. Wang, H. Jiang, W. Liu, T. Han, D. Liu, T. Ma, B. Wang, M. Liu, J.Y. Liu, Q.K. Wang, D. Yalnizoglu, L. Radoshevich, G. Uze, P. Gros, F. Rozenberg, S.Y. Zhang, E. Jouanguy, J. Bustamante, A. Garcia-Sastre, L. Abel, P. Lebon, L.D. Notarangelo, Y.J. Crow, S. Boisson-Dupuis, J.L. Casanova, and S. Pellegrini. 2015. Human intracellular ISG15 prevents interferon-alpha/beta over-amplification and auto-inflammation. *Nature* 517:89-93.
- Zhou, L., A.A. Nazarian, J. Xu, D. Tantin, L.M. Corcoran, and S.T. Smale. 2007. An inducible enhancer required for Il12b promoter activity in an insulated chromatin environment. *Mol Cell Biol* 27:2698-2712.
- Zumla, A., P. Nahid, and S.T. Cole. 2013. Advances in the development of new tuberculosis drugs and treatment regimens. *Nat Rev Drug Discov* 12:388-404.

CHAPTER 2

**T-cell cytokines differentially control human monocyte
antimicrobial responses by regulating vitamin D metabolism**

T-cell cytokines differentially control human monocyte antimicrobial responses by regulating vitamin D metabolism

Kristina Edfeldt^{a,1}, Philip T. Liu^{a,b,1}, Rene Chun^b, Mario Fabri^a, Mirjam Schenk^a, Matthew Wheelwright^c, Caroline Keegan^c, Stephan R. Krutzik^a, John S. Adams^b, Martin Hewison^b, and Robert L. Modlin^{a,c,2}

^aDivision of Dermatology, Department of Medicine; ^bOrthopaedic Hospital, Department of Orthopaedic Surgery; and ^cDepartment of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine at University of California, Los Angeles, CA 90095

Edited* by Diane E. Griffin, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, and approved November 17, 2010 (received for review August 6, 2010)

We investigated the mechanisms by which T-cell cytokines are able to influence the Toll-like receptor (TLR)-induced, vitamin D-dependent antimicrobial pathway in human monocytes. T-cell cytokines differentially influenced TLR2/1-induced expression of the antimicrobial peptides cathelicidin and DEF4, being up-regulated by IFN- γ , down-regulated by IL-4, and unaffected by IL-17. The Th1 cytokine IFN- γ up-regulated TLR2/1 induction of 25-hydroxyvitamin D-1 α -hydroxylase (i.e., CYP27B1), leading to enhanced bioconversion of 25-hydroxyvitamin D₃ (25D₃) to its active metabolite 1,25D₃. In contrast, the Th2 cytokine IL-4, by itself and in combination with the TLR2/1 ligand, induced catabolism of 25D₃ to the inactive metabolite 24,25D₃, and was dependent on expression of vitamin D-24-hydroxylase (i.e., CYP24A1). Therefore, the ability of T-cell cytokines to differentially control monocyte vitamin D metabolism represents a mechanism by which cell-mediated immune responses can regulate innate immune mechanisms to defend against microbial pathogens.

innate immune response | interferon- γ | interleukin-4 | *Mycobacterium tuberculosis*

The ability of Toll-like receptors (TLRs) to trigger a direct antimicrobial activity is a key aspect of their role in innate immunity. In mouse monocytes, activation of the TLR2/1 heterodimer by microbial lipoproteins (1–3), induces an antimicrobial activity against *Mycobacterium tuberculosis* that is nitric oxide (NO)-dependent, but in human monocytes is NO-independent (4). Instead, a key antimicrobial mechanism for TLR-activated human monocytes involves induction of the 25-hydroxyvitamin D-1 α -hydroxylase (i.e., CYP27B1), which enzymatically converts the major circulating form of vitamin D, 25-hydroxyvitamin D₃ (25D₃) into the active form of vitamin D, 1,25D₃. Parallel TLR-mediated up-regulation of the vitamin D receptor (VDR) and activation of this receptor by 1,25D₃ leads to downstream induction of the genes encoding the antimicrobial peptides cathelicidin and DEF4 (5–10). Here, we tested the hypothesis that adaptive T-cell cytokines, including key cytokines of the Th1, Th2, and Th17 pattern, regulate the TLR2/1-induced, vitamin D-dependent antimicrobial pathway.

Results

Effect of T-Cell Cytokines on TLR2/1 Induction of Cathelicidin and DEF4. To determine the role of individual cytokines on the TLR-triggered vitamin D-dependent induction of antimicrobial peptides, monocytes were treated with TLR2/1L with or without a specific T-cell cytokine, and cathelicidin and DEF4 mRNAs measured at 24 h. IFN- γ by itself up-regulated cathelicidin and DEF4 mRNA levels by twofold (Fig. 1A; $P < 0.05$ and $P < 0.001$). Consistent with previous findings, TLR2/1L induced both cathelicidin and DEF4 mRNAs (8, 10). However, whereas IFN- γ augmented TLR2/1L-triggered induction of cathelicidin by 4.1-fold ($P < 0.01$), it had no effect on TLR2/1L-mediated

induction of DEF4 (Fig. 1A). The addition of IL-17 had no effect on induction of antimicrobial peptide gene expression in the presence or absence of TLR2/1L (Fig. 1B).

Whereas IFN- γ augmented TLR2/1 induction of cathelicidin, IL-4 had the opposite effect. IL-4 inhibited TLR2/1 induction of both cathelicidin and DEF4 mRNA by greater than 90% (Fig. 1C; $P < 0.05$). IL-4 also affected baseline expression of both cathelicidin and DEF4 in the absence of TLR2/1 induction, reducing mRNA levels by 20% to 40% (Fig. 1C; $P < 0.001$ and $P < 0.05$). Together, these data indicate that IFN- γ and IL-4 differentially modulate TLR2/1-induced expression of cathelicidin and DEF4.

Effect of T-Cell Cytokines on TLR2/1 Induction of CYP27B1 and the VDR. To explore the mechanism by which the T-cell cytokines IFN- γ and IL-4 differentially regulated TLR2/1-induced antimicrobial peptide gene expression, we investigated the mRNA levels for CYP27B1 and the VDR. TLR2/1 activation of human monocytes is known to up-regulate both CYP27B1 and the VDR, the activity of both being required for induction of cathelicidin expression (8). IFN- γ induced by 2.4-fold the expression of CYP27B1 in human monocytes, but synergized with TLR2/1L to induce CYP27B1 mRNA levels to 6.9-fold over media control ($P < 0.01$) and 2.5-fold over cells treated with TLR2/1L alone ($P < 0.05$; Fig. 2A). In addition, IFN- γ increased TLR2/1L up-regulation of VDR expression, although the effect was less pronounced (2.1-fold; $P < 0.05$).

Surprisingly, the effect of IL-4 on CYP27B1 and VDR expression was similar to that of IFN- γ . IL-4 augmented TLR2/1-induced CYP27B1 (sixfold; $P < 0.05$) and VDR (threefold; $P < 0.05$) mRNA expression in human monocytes (Fig. 2B). Therefore, although IFN- γ and IL-4 differentially regulated TLR2/1L induction of antimicrobial peptide gene expression, they had identical effects on TLR2/1-induced expression of key vitamin D pathway genes, CYP27B1, and the VDR.

Effect of IL-4 on Monocyte Vitamin D Response. To explore the possible mechanisms by which IL-4 inhibited TLR2/1L-induced antimicrobial peptide expression despite increasing CYP27B1 and VDR, the effect of IL-4 on direct VDR activation was in-

Author contributions: K.E., P.T.L., and R.L.M. designed research; K.E., P.T.L., R.C., M.F., M.S., M.W., and C.K. performed research; K.E., P.T.L., R.C., M.F., S.R.K., J.S.A., M.H., and R.L.M. analyzed data; and K.E., P.T.L., and R.L.M. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Freely available online through the PNAS open access option.

¹K.E. and P.T.L. contributed equally to this work.

²To whom correspondence should be addressed. E-mail: rmodlin@mednet.ucla.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1011624108/-DCSupplemental.

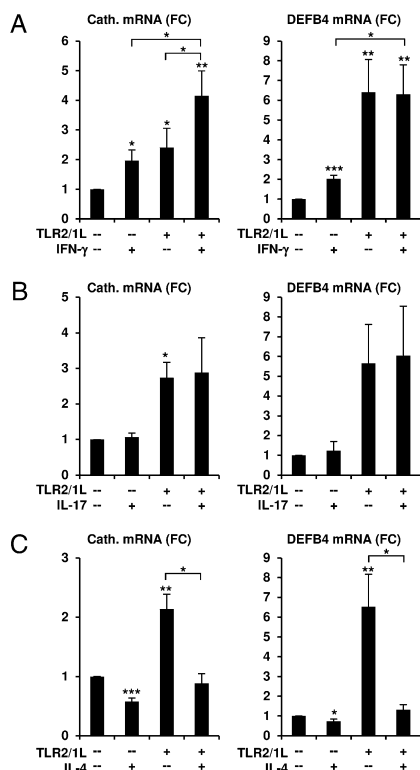


Fig. 1. T-cell cytokines differentially influence TLR2/1-induced expression of the antimicrobial peptides cathelicidin and DEFB4. Primary human monocytes were stimulated with TLR2/1L (10 μ g/mL) with or without the T-cell cytokines (A) IFN- γ (1 ng/mL), (B) IL-17A (10 μ g/mL), or (C) IL-4 (10³ U/mL) for 24 h in vitamin D sufficient serum. mRNA levels of cathelicidin and DEFB4 were determined by qPCR and fold change (FC) was calculated. Data represent mean values \pm SEM from three to eight independent experiments (* P < 0.05, ** P < 0.01, *** P < 0.001).

investigated. The simultaneous addition of IL-4 with 1,25D₃ to monocytes inhibited cathelicidin expression (Fig. 3A). Similarly, when monocytes were pretreated for 6 h with IL-4, there was inhibition of 1,25D₃-induced cathelicidin expression (Fig. 3B). In these experiments, the addition of exogenous 1,25D₃ was limited to 10⁻⁸ M, after which the amount of 1,25D₃ affected cellular viability. However, in both cases, increasing 1,25D₃ to concentrations higher than 10⁻⁸ M did not overcome the IL-4 inhibition of cathelicidin expression. This is most likely a result of the well recognized ability of 1,25D₃ to self-induce expression and activity of the catabolic enzyme CYP24A1, leading to the rapid inactivation of bioactive vitamin D (11).

The vitamin D host defense against mycobacteria requires the up-regulation of cathelicidin as well as the induction of autophagy, a critical cellular process for inducing phagosome maturation (7, 12). Given that IL-4 has been shown to inhibit starvation and IFN- γ -induced autophagy (13), we next examined whether IL-4 could inhibit 1,25D₃-induced autophagy. Primary human monocytes incubated with 1,25D₃ showed a 3.8-fold enhancement of autophagy (P < 0.01) as measured by an increase in the percentage of LC3 punctate cells (Fig. 3C and D). Preincubation of monocytes with IL-4 for 6 h before the addition of 1,25D₃ resulted in a marked

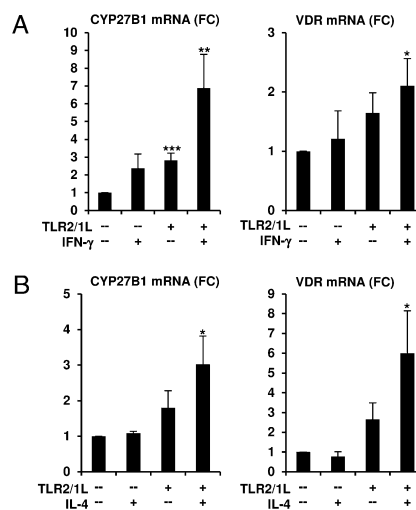


Fig. 2. IFN- γ and IL-4 up-regulate vitamin D pathway genes in TLR2/1-activated monocytes. Primary monocytes were stimulated with TLR2/1L (10 μ g/mL) with or without the T-cell cytokines (A) IFN- γ (1 ng/mL) or (B) IL-4 (10³ U/mL) for 24 h. mRNA levels of CYP27B1 and the VDR were subsequently determined by qPCR and fold change (FC) was calculated. Data represent mean values \pm SEM from four to seven independent experiments (* P < 0.05, ** P < 0.01, *** P < 0.001).

decrease in LC3 punctate cells, reaching levels lower than media-treated background levels (P < 0.001). Therefore, IL-4 inhibits vitamin D-induced autophagy in primary human monocytes.

Effect of T-Cell Cytokines on Monocyte Vitamin D Metabolism. The differential ability of IFN- γ and IL-4 to affect TLR2/1-induced cathelicidin expression, as well as the effects of IL-4 on 1,25D₃-induced host responses, suggested that these cytokines achieve at least some of their effects by regulating monocyte vitamin D metabolism. Therefore, we next examined the bioconversion of 25D₃ to its active metabolite 1,25D₃ as well as the inactive metabolite 24,25D₃. This was accomplished by adding ³H-25D₃ to TLR2/1L and/or T-cell cytokine treated monocytes and measuring conversion to the resulting ³H-vitamin D metabolites by HPLC. TLR2/1L-treated monocytes converted 25D₃ to 1,25D₃ at a slightly higher rate (9.5 fmol/h per million cells) compared with media control cells (8.7 fmol/h per million cells), both shown as a summary of five separate experiments (Fig. 4A; P < 0.05). Treatment of monocytes with IFN- γ alone induced a similar conversion of 25D₃ to 1,25D₃ as observed in cells treated with TLR2/1L (11.4 fmol/h per million cells; P < 0.05; Fig. 4A). However, IFN- γ in combination with TLR2/1L strongly induced bioconversion of 25D₃ to active 1,25D₃ (45.4 fmol/h per million cells), to levels sevenfold higher than media control cells (Fig. 4A; P < 0.05). The Th2 cytokine IL-4 alone had no effect on CYP27B1 activity (6.4 fmol/h per million cells; Fig. 4A). However, in combination with TLR2/1L, IL-4 induced bioconversion of 25D₃ to 1,25D₃ (14.6 fmol/h per million cells; P < 0.05; Fig. 4A). Together, these data indicated that IFN- γ potentiation of TLR2/1-induced antimicrobial peptides is associated with the synergistic effects of IFN- γ plus TLR2/1 on CYP27B1 activity leading to enhanced bioconversion of 25D₃ to 1,25D₃.

Given that IL-4 inhibited TLR2/1-induced antimicrobial peptide expression but also enhanced CYP27B1 activity, we next examined the effects of IFN- γ and IL-4 on vitamin D catabolism,

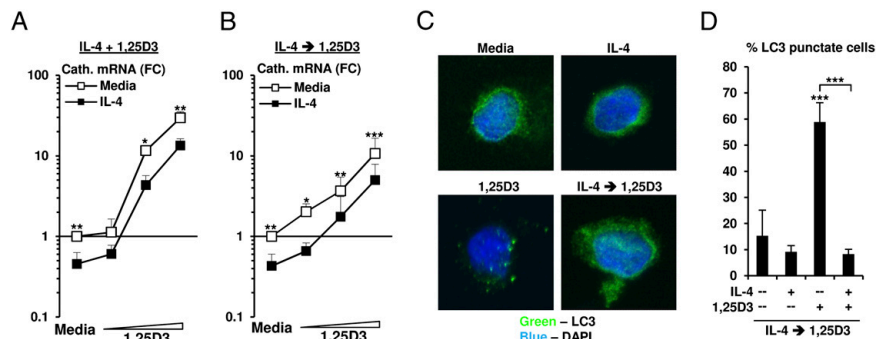


Fig. 3. Effects on IL-4 on 1,25D₃ responsiveness. (A) Primary monocytes were cotreated with IL-4 (10³ U/mL) and 1,25D₃ (10⁻¹⁰ to 10⁻⁸ M) for 18 h or (B) pretreated with IL-4 (10³ U/mL) for 6 h and then stimulated with 1,25D₃ (2.5 × 10⁻¹⁰ to 10⁻⁸ M) for 18 h. mRNA levels of cathelicidin were subsequently determined by qPCR and fold change (FC) was calculated as ratio to media control cells. Data represent mean values ± SEM from four to seven independent experiments. Primary monocytes were treated as in B and immunolabeled for intracellular LC3 expression (green) and cellular nuclei (blue). Cells were visualized using confocal microscopy (C) and enumerated (D) for the percentage of cells positive for LC3-punctate formation. Data displayed are the average percentage of cells positive for LC3-punctate formation per field of view from three independent donors ± SEM (n > 7; *P < 0.05, **P < 0.01, ***P < 0.001).

measuring conversion of 25D₃ to the inactive metabolite 24,25D₃. TLR2/1 activation had no statistically significant effect on vitamin D catabolism in monocytes (Fig. 4B). Similarly, IFN-γ alone or together with TLR2/1L had no effect on the conversion of 25D₃ to 24,25D₃, which was similar to media control monocytes. Strikingly, treatment of monocytes with the Th2 cytokine IL-4 alone strongly induced 24-hydroxylase activity, increasing the production of 24,25D₃ by fivefold over media control monocytes to a rate of 51.0 fmol/h per million cells compared with 10.6 ± fmol/h per million cells in media control cells (Fig. 4B; P < 0.05). IL-4 had a comparable effect in TLR2/1L-treated monocytes, inducing catabolism of 25D₃ to 24,25D₃ at a rate of 40.7 fmol/h per million cells compared with 10.6 fmol/h per million cells in control-treated cells (Fig. 4B; P < 0.05). These data suggest that up-regulation of vitamin D catabolism (i.e., conversion of 25D₃ to 24,25D₃) provides a mechanism to explain the IL-4-mediated inhibition of TLR2/1-induced antimicrobial protein expression. Although IFN-γ

and IL-4 both up-regulated TLR-induced CYP27B1 mRNA expression by approximately twofold, the TLR-induced conversion of 25D₃ to 1,25D₃ was threefold greater in the presence of IFN-γ versus IL-4. Interestingly, treatment of monocytes with 1,25D₃ alone (10⁻⁸ M) induced 24-hydroxylase activity to a level (65.8 fmol/h per million cells) similar to that observed for treatment with IL-4, providing a potential explanation for the inability of exogenously added 1,25D₃ to overcome the cathelicidin-suppressive effects of IL-4 (Fig. S1).

IL-4-Induced Catabolism of Vitamin D Is Dependent on CYP24. To investigate the mechanism by which IL-4 up-regulates 24-hydroxylation of 25D₃, further studies were carried out to characterize expression of CYP24A1, the primary enzyme involved in catalyzing 24-hydroxylation of vitamin D metabolites (11). Intriguingly, IL-4 inhibited the baseline expression of CYP24A1 mRNA (35%; P < 0.01), and also inhibited the TLR2/1-induced up-regulation of CYP24A1 expression (84%; P < 0.05; Fig. 5A). We were also unable to detect any significant changes in CYP24A1 protein expression or alterations in mRNA encoding the CYP24A1 splice variant (14) (Fig. 5B). A number of other cytochrome p450 enzymes (CYP3A1, CYP2J2, CYP27A1) have been suggested to exhibit potential 24-hydroxylase activity; however, none of these were found to be up-regulated in monocytes following treatment with IL-4 (Fig. S2).

To directly measure the contribution of CYP24A1 to the 24-hydroxylase activity in monocytes treated with IL-4, monocytes were transfected with siRNA oligos specific for human CYP24A1 or a nonspecific control (siCTRL) and then treated with IL-4. Knockdown of CYP24A1 expression inhibited the conversion of 25D₃ to 24,25D₃ by 58% relative to the control siRNA (P < 0.05; Fig. 5C), whereas conversion of 25D₃ to 1,25D₃ increased by 235% (P = 0.059; Fig. 5D). The effect of CYP24A1 knockdown on IL-4 suppression of TLR2/1 responses was next investigated. In siCTRL transfected monocytes, IL-4 significantly inhibited TLR2/1-induced cathelicidin (P < 0.05; Fig. 5E) and DEFB4 mRNA expression (P < 0.05; Fig. 5F). The effect of IL-4 was reversed in siCYP24A1 transfected monocytes, restoring TLR2/1-induced responses. It was also noted that TLR2/1L-induced cathelicidin trended to be increased in CYP24A1 knockdown monocytes, suggesting a regulatory role for CYP24A1 even in the absence of IL-4.

Given that IL-4 inhibited 1,25D₃-induced autophagy, we next determined whether the effect of IL-4 was dependent on CYP24A1 expression. In monocytes transfected with siCYP24A1,

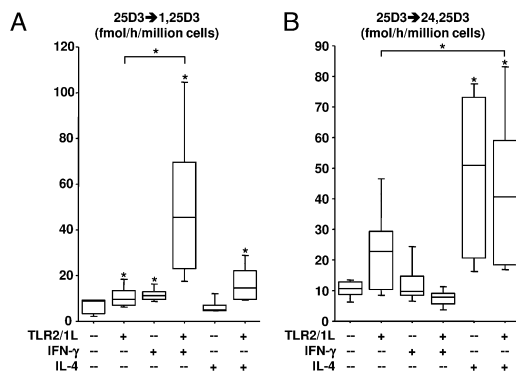


Fig. 4. IFN-γ and IL-4 differentially regulate vitamin D metabolism. Primary monocytes treated with TLR2/1L with or without IFN-γ (100 ng/mL) or IL-4 (10³ U/mL) for 48 h and the ability to convert [³H]-25D₃ to [³H]-1,25D₃ and [³H]-24,25D₃ was measured by HPLC. The rate of conversion of (A) [³H]-25D₃ to [³H]-1,25D₃ and (B) [³H]-24,25D₃ was calculated (n = 5) and presented in box-and-whisker plots depicting minimum value, lower quartile, median, upper quartile, and maximum value (*P < 0.05).

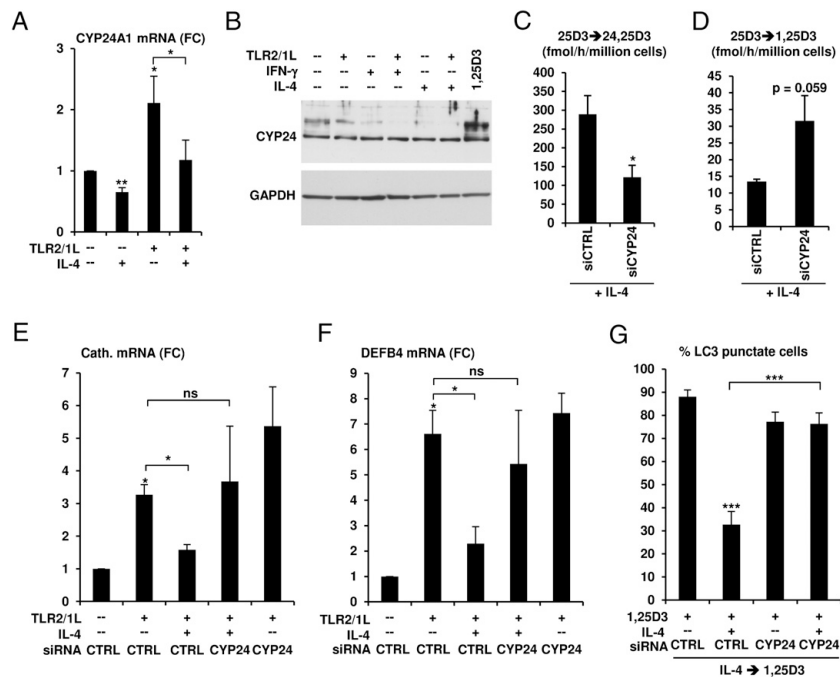


Fig. 5. Regulation of 24-hydroxylase activity by IL-4. (A) Primary human monocytes were stimulated with TLR2/1L (10 μ g/mL) with or without IL-4 (10³ U/mL) for 24 h in vitamin D sufficient serum. mRNA levels of CYP24A1 were subsequently determined by qPCR and fold change (FC) was calculated. Data represent mean values \pm SEM from five independent experiments. (B) Western blot was performed on total cell lysates from primary human monocytes stimulated with TLR2/1L (10 μ g/mL) with or without IFN- γ (100 ng/mL) or IL-4 (10³ U/mL) for 48 h. 1,25D₃ (10⁻⁸ M) was included as a positive control. (C) Primary human monocytes were transfected with siRNA oligos specific for CYP24A1 (siCYP24) or nonspecific (siCTRL), then treated with IL-4 (10³ U/mL) for 40 h, followed by 25D₃ bioconversion as measured by HPLC. The rate of conversion of [³H]-25D₃ to [³H]-24,25D₃ and [³H]-1,25D₃ was calculated ($n = 3$). Primary human monocytes transfected with siCTRL or siCYP24 were stimulated with TLR2/1L and IL-4 as detailed in A. (E) Cathelicidin and (F) DEFB4 mRNA levels were measured by using qPCR. Data represent mean values \pm SEM from five independent donors. (G) Primary monocytes transfected with siCYP24 or siCTRL, then treated with IL-4 (10³ U/mL) for 6 h followed by the addition of 1,25D₃ (10⁻⁸ M) for 18 h. The cells were immunolabeled for intracellular LC3 expression and cellular nuclei. Cells were visualized by using confocal microscopy and enumerated for the percentage of cells positive for LC3-punctate formation. Data displayed are the average percentage of cells positive for LC3-punctate formation per field of view from two independent donors \pm SEM ($n > 23$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ns, not significant).

IL-4 treatment had no effect on 1,25D₃-induced autophagy (Fig. 5G). In contrast, in monocytes transfected with siCTRL, IL-4 inhibited 1,25D₃-induced autophagy by 62% ($P < 0.001$; Fig. 5G). These observations confirmed that although IL-4 had no effect on monocyte expression or activity of CYP24A1, the enzyme mediated, at least in part, IL-4-induced 24-hydroxylase activity as well as IL-4 inhibition of TLR2/1-induced antimicrobial peptide expression and autophagy.

Discussion

Although a key function of the innate immune response is to instruct the acquired T-cell response, it has become increasingly clear that T-cell cytokines regulate the innate immune response. We therefore investigated whether specific T-cell cytokines regulate the TLR2/1-induced, vitamin D-dependent antimicrobial pathway in human monocytes. The striking result was that IFN- γ and IL-4 differentially affected TLR2/1 induction of the antimicrobial peptides cathelicidin and DEFB4 by different mechanisms. TLR2/1 and IFN- γ together up-regulated expression of CYP24A1 and monocyte conversion of 25D₃ to the active metabolite 1,25D₃, resulting in activation of the VDR and downstream induction of cathelicidin and DEFB4. In contrast, IL-4 induced CYP24A1-dependent conversion of 25D₃ to the

inactive metabolite 24,25D₃, inhibiting TLR2/1 induction of antimicrobial peptide expression and autophagy. Therefore, Th1 and Th2 cytokine responses differentially affect innate antimicrobial pathways through regulation of opposing facets of monocyte vitamin D metabolism.

A key finding of the present study was that IL-4 was found to have a previously unrecognized and dramatic effect on monocyte vitamin D metabolism. IL-4, alone or in the presence of TLR2/1L, enhanced conversion of 25D₃ to the inactive catabolic product 24,25D₃. However, there was no detectable up-regulation, as measured by quantitative PCR (qPCR) or Western blot, of cytochrome P450 enzymes with known or potential 24-hydroxylase activity in IL-4-treated monocytes. Nevertheless, we were able to demonstrate by siRNA knockdown a role for the established 24-hydroxylase CYP24A1 in mediating the conversion of 25D₃ to 24,25D₃. Knockdown of CYP24A1 also reversed the ability of IL-4 to inhibit TLR2/1-induced antimicrobial peptide expression as well as 1,25D₃-mediated autophagy. There is evidence to suggest that increases in 24-hydroxylase activity can occur in the absence of elevated enzyme levels, but may instead be a result of preferential delivery of the substrate to a mitochondrial microenvironment containing the enzyme (15). Although our data indicate that one mechanism by which IL-4 inhibits TLR2/1-

induced and 1,25D₃-triggered responses is via up-regulation of 24-hydroxylase activity, we cannot exclude that IL-4 inhibits 1,25D₃ induction of antimicrobial peptide expression and autophagy via other mechanisms. In addition, it will be of interest to determine the effect of IL-4 on vitamin D metabolism in other cell types, including macrophages. Nevertheless, inhibition of CYP24A1 expression by siRNA also led to increased conversion of 25D₃ to 1,25D₃, presumably by (i) maintaining the pool of substrate 25D₃ available to the CYP27B1-hydroxylase and (ii) inhibiting the conversion of active metabolite 1,25D₃ to inactive, 24-hydroxylated metabolites. The present findings are clinically relevant, as increased IL-4 responses have been observed in patients with tuberculosis in developing countries (16–18) and associated with the development of tuberculosis in health care workers in a developed country (19).

In contrast to the action of IL-4 on vitamin D metabolism, IFN- γ enhanced TLR2/1L induction of the 1- α hydroxylase CYP27B1, potentiating the conversion of 25D₃ to 1,25D₃. IFN- γ has been shown to up-regulate conversion of 25D₃ to 1,25D₃ in activated macrophages from patients with tuberculosis and sarcoidosis (20, 21) and was later shown to induce CYP27B1 directly (22, 23). In addition, local IFN- γ production was seen in disease lesions of patients with localized mycobacterial infection, including tuberculosis pleuritis (24).

We previously reported that Th1 and Th2 cytokines differentially affect TLR2-induced proinflammatory cytokine release, specifically IL-12p40 and TNF- α (25). This was related in part to the ability of IFN- γ to up-regulate TLR1 expression and IL-4 to inhibit TLR2 expression. However, both of these cytokines up-regulated expression of the mRNAs encoding for CYP27B1 and CYP24A1, yet differentially affected monocyte vitamin D metabolism and subsequent expression of antimicrobial peptide mRNAs, providing a unique mechanism by which these cytokines affect host immune responses. These cytokines influence a number of other key host defense pathways, for example autophagy, which facilitates endosomal maturation, required for inhibiting the growth of intracellular *M. tuberculosis* (12, 26). IFN- γ has been shown to induce autophagy (27), whereas IL-4 can block starvation-induced or IFN- γ -induced autophagy and autophagic control of intracellular *M. tuberculosis* growth (13). In data presented here, vitamin D-induced autophagy (12) was also blocked by treatment with IL-4. More recently, the TLR2/1L-induced, vitamin D-dependent antimicrobial pathway has been shown to involve autophagy (28). It is therefore reasonable to hypothesize that the ability of IFN- γ and IL-4 to differentially regulate monocyte vitamin D metabolism controls the monocyte autophagy program.

In summary, our data point to a key and differential role for Th1 versus Th2 cytokines in regulating TLR-induced antimicrobial responses through their ability to trigger distinct monocyte vitamin D metabolic pathways, providing a unique mechanism by which IL-4 regulates innate immune responses.

Materials and Methods

Reagents. TLR2/1L is a triacylated lipopeptide of the *M. tuberculosis* 19-kDa antigen (EMC Microcollections). Recombinant human cytokines used were IFN- γ (BD Biosciences), IL-4 (PeproTech), and IL-17A (eBioscience). Pre-designed siRNA oligos were purchased (Thermo Scientific) and used as recommended by the manufacturer. Radiolabeled ³H-25D₃ (specific activity, 155 Ci/mmol; Perkin-Elmer) and unlabeled 1,25D₃ (Biomol) were purchased. Antibodies used for Western blot were rabbit anti-human CYP24 (Santa Cruz Biotechnology), mouse anti-human GAPDH (BioVision), and HRP-conjugated goat anti-mouse and goat anti-rabbit (Thermo Scientific). Antibodies used for confocal were mouse anti-human LC3 antibodies (MBL International) and Alexa 488-labeled goat anti-mouse IgG1 secondary antibody (Invitrogen).

Cell Culture. All donors were healthy and provided written informed consent, with approval by the institutional review board of the University of California, Los Angeles, for the collection of peripheral blood and subsequent analysis. Human monocytes were purified from peripheral blood mononuclear cells through plastic adherence, and cultured in 10% FCS or 10% vitamin D sufficient human serum as reported (8). For transfections, Percoll-enriched monocytes were transfected with 100 pmol of siRNA oligos by using the Amaxa Nucleofection System and the Human Monocyte Kit (Lonza) as previously described (10). For measurement of autophagy, monocytes were adhered onto glass chamber slides and then treated as noted. The cells were then immunolabeled for LC3 as previously described (12) and visualized by using confocal microscopy.

Real-Time qPCR. Following stimulation, RNA was isolated, cDNA synthesized, and qPCR performed as previously described (8). Reactions use SYBR Green PCR Master Mix and were run on a DNA Engine Opticon II (Bio-Rad). The relative quantities of the gene tested per sample were calculated against 36B4 using the delta delta cycle threshold formula as previously described (29). The data were normalized by fold change to media control samples.

Measurement of 25D₃ Bioconversion. Cells were treated for 48 h, followed by incubation with radiolabeled ³H-25D₃ for 5 h in serum-free media. Measurement of 25D₃ bioconversion to 1,25D₃ or 24,25D₃ was carried out as previously described (9).

Western Blot Analysis. Monocytes were treated with for 48 h and total cell extracts prepared using M-PER reagent with protease inhibitors (Thermo Scientific) according to the manufacturer's recommendations. Equal amount of protein were loaded onto 12% Bis-Tris NuPage minigels (Invitrogen) and electrophoresed for 1 h at 125 V. Western blotting was performed by standard methods. Briefly, resolved proteins were transblotted onto nitrocellulose membrane for 1 h at 95 V, then blocked for 1 h followed by overnight incubation at 4 °C with primary antibody. The appropriate secondary antibody was incubated for 1 h at room temperature and specific protein detected by using SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific).

ACKNOWLEDGMENTS. We thank Dr. Schibler and the University of California, Los Angeles, California NanoSystems Institute Advanced Light Microscopy Core Facility for their assistance with the confocal studies. K.E. is supported by the Wenner-Gren Foundation. This work was supported by National Institutes of Health Grants AI 022553, AI 047868, and AI 073539.

- Brightbill HD, et al. (1999) Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science* 285:732–736.
- Aliprantis AO, et al. (1999) Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. *Science* 285:736–739.
- Takeuchi O, et al. (2002) Role of TLR1 in mediating immune response to microbial lipoproteins. *J Immunol* 169:10–14.
- Thoma-Uzyski S, et al. (2001) Induction of direct antimicrobial activity through mammalian toll-like receptors. *Science* 291:1544–1547.
- Wang TT, et al. (2004) Cutting edge: 1,25-dihydroxyvitamin D₃ is a direct inducer of antimicrobial peptide gene expression. *J Immunol* 173:2909–2912.
- Martineau AR, et al. (2007) IFN- γ - and TNF-independent vitamin D-inducible human suppression of mycobacteria: The role of cathelicidin LL-37. *J Immunol* 178:7190–7198.
- Liu PT, Stenger S, Tang DH, Modlin RL (2007) Cutting edge: Vitamin D-mediated human antimicrobial activity against *Mycobacterium tuberculosis* is dependent on the induction of cathelicidin. *J Immunol* 179:2060–2063.
- Liu PT, et al. (2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 311:1770–1773.
- Krutzik SR, et al. (2008) IL-15 links TLR2/1-induced macrophage differentiation to the vitamin D-dependent antimicrobial pathway. *J Immunol* 181:7115–7120.
- Liu PT, et al. (2009) Convergence of IL-1 β and VDR activation pathways in human TLR2/1-induced antimicrobial responses. *PLoS ONE* 4:e5810.
- Tanaka Y, DeLuca HF (1974) Stimulation of 24,25-dihydroxyvitamin D₃ production by 1,25-dihydroxyvitamin D₃. *Science* 183:1198–1200.
- Yuk JM, et al. (2009) Vitamin D₃ induces autophagy in human monocytes/macrophages via cathelicidin. *Cell Host Microbe* 6:231–243.
- Harris J, et al. (2007) T helper 2 cytokines inhibit autophagic control of intracellular *Mycobacterium tuberculosis*. *Immunity* 27:505–517.
- Ren S, et al. (2005) Alternative splicing of vitamin D-24-hydroxylase: A novel mechanism for the regulation of extrarenal 1,25-dihydroxyvitamin D synthesis. *J Biol Chem* 280:20604–20611.
- Adams JS, et al. (2004) Response element binding proteins and intracellular vitamin D binding proteins: Novel regulators of vitamin D trafficking, action and metabolism. *J Steroid Biochem Mol Biol* 89:461–465.
- Bhattacharya S, Singla R, Dey AB, Prasad HK (1999) Dichotomy of cytokine profiles in patients and high-risk healthy subjects exposed to tuberculosis. *Infect Immun* 67:5597–5603.
- van Crevel R, et al. (2000) Increased production of interleukin 4 by CD4⁺ and CD8⁺ T cells from patients with tuberculosis is related to the presence of pulmonary cavities. *J Infect Dis* 181:1194–1197.

18. Smith SM, et al. (2002) Decreased IFN-gamma and increased IL-4 production by human CD8(+) T cells in response to Mycobacterium tuberculosis in tuberculosis patients. *Tuberculosis (Edinb)* 82:7–13.
19. Ordway DJ, et al. (2004) Increased interleukin-4 production by CD8 and gamma delta T cells in health-care workers is associated with the subsequent development of active tuberculosis. *J Infect Dis* 190:756–766.
20. Adams JS, Modlin RL, Diz MM, Barnes PF (1989) Potentiation of the macrophage 25-hydroxyvitamin D-1-hydroxylation reaction by human tuberculous pleural effusion fluid. *J Clin Endocrinol Metab* 69:457–460.
21. Adams JS, Gacad MA (1985) Characterization of 1 alpha-hydroxylation of vitamin D₃ sterols by cultured alveolar macrophages from patients with sarcoidosis. *J Exp Med* 161:755–765.
22. Overbergh L, et al. (2004) Regulation of 25-hydroxyvitamin d-1alpha-hydroxylase by IFN-gamma in human monocytic THP1 cells. *J Steroid Biochem Mol Biol* 89:90: 453–455.
23. Stoffels K, et al. (2006) Immune regulation of 25-hydroxyvitamin-D3-1alpha-hydroxylase in human monocytes. *J Bone Miner Res* 21:37–47.
24. Barnes PF, et al. (1989) Compartmentalization of a CD4+ T lymphocyte subpopulation in tuberculous pleuritis. *J Immunol* 142:1114–1119.
25. Krutzik SR, et al. (2003) Activation and regulation of Toll-like receptors 2 and 1 in human leprosy. *Nat Med* 9:525–532.
26. Gutierrez MG, et al. (2004) Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell* 119:753–766.
27. Kabeya Y, et al. (2000) LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J* 19:5720–5728.
28. Shin DM, et al. (2010) Mycobacterial lipoprotein activates autophagy via TLR2/1/CD14 and a functional vitamin D receptor signaling. *Cell Microbiol* 12:1648–1665.
29. Monney L, et al. (2002) Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. *Nature* 415:536–541.

CHAPTER 3

***Mycobacterium tuberculosis* tRNA triggers a
gene network for instruction of Th1 cells**

Abstract

The ability of the innate immune system to utilize specific pattern recognition receptors (PRRs) to recognize distinct microbial components termed pathogen associated molecular patterns (PAMPs) triggers common and unique host response pathways. These PRRs are located in specific cellular compartments to facilitate recognition of the pathogen. Yet for *Mycobacterium tuberculosis*, which resides in endosomes, there is little information about how innate recognition of bacterial components might contribute to host defense. We therefore compared the gene expression profiles of *M. tuberculosis* tRNA, a stable component of the culture filtrate, with triacylated lipopeptide, which is recognized by Toll-like receptor 2/1 (TLR2/1) located on the cell surface. Integrated bioinformatics analysis of RNA-seq data from stimulated peripheral blood mononuclear cells (PBMC) identified a tRNA-induced pathway for induction of Th1 cells involving NK cell activation as well as IL-18, IFN- γ and IL-12. Functional studies demonstrated that *M. tuberculosis* tRNA specifically induced IL-18, which was required for upregulation of IFN- γ in NK cells, with both required for optimal induction of IL-12p70. Finally, the ability of *M. tuberculosis* tRNA to trigger this pathway was TLR8 dependent, and could be reconstituted by the co-addition of TLR3 and TLR8 specific ligands. In summary, *M. tuberculosis* tRNA triggers the endosomal PRR TLR8, leading to the IL-18 and IFN- γ dependent upregulation of IL-12p70, relevant for induction of a Th1 response.

Introduction

To defend the host against infection, the innate immune system utilizes distinct 'pathogen recognition receptors' (PRRs) to recognize a diverse array of microbial determinants called 'pathogen associated molecular patterns' (PAMPs). In some cases, these PRRs are redundant, triggering nearly identical immune responses, but different PAMPs located in distinct cellular compartments, can also induce distinct immunologic pathways. For example, in mycobacteria, muramyl dipeptide triggers the cytoplasmic receptor NOD2 in monocytes/macrophages to induce a specific DC pathway not induced by triacylated lipopeptide which triggers the cell surface receptor TLR2/1 (Schenk et al., 2012). Furthermore, DNA from mycobacteria, which reside in the host phagosome, gains access to the cytoplasm to trigger nucleotide sensors such as STING, leading to a type I interferon response (Collins et al., 2015; Manzanillo et al., 2012; Wassermann et al., 2015; Watson et al., 2012). As such, the host has deployed PRRs in a variety of subcellular locations to recognize distinct microbial ligands and mount both common and specific immune effector responses.

In addition to proteins and DNA, *M. tuberculosis* releases RNA into the culture filtrate. Examination of the RNA present in *M. tuberculosis* culture filtrate revealed that tRNA was the most abundant form (Obregon-Henao et al., 2012). Treatment of human monocytes with *M. tuberculosis* tRNA induced apoptosis, which may contribute to disease pathogenesis. However, the extent of the immune response triggered by *M. tuberculosis* tRNA is unknown, and it is unclear whether tRNA induces common or specific immune responses in comparison to other mycobacterial ligands. Also, the

PRR which recognizes *M. tuberculosis* is not defined, but we expect that one possibility is that the ligand is recognized in the endosomal pathway, such that TLR8 is a likely candidate. Therefore, we undertook to investigate the innate immune response to *M. tuberculosis* tRNA with the hypothesis that *M. tuberculosis* tRNA, via activation of TLR8, triggered distinct immune responses as compared to mycobacterial lipopeptide, which activates a TLR2/1 heterodimer.

Results

M. tuberculosis tRNA and bacterial lipopeptide induce distinct gene expression patterns.

To test the hypothesis that *M. tuberculosis* tRNA, via activation of TLR8, triggered distinct immune responses as compared to mycobacterial lipopeptide, which activates a TLR2/1 heterodimer, we compared the response of human peripheral blood mononuclear cells (PBMC). The gene expression profiles induced by *M. tuberculosis* tRNA were derived by performing RNA-seq on stimulated PBMC. *M. tuberculosis* tRNA was prepared from strain as described (Chan et al., 2015). A ssRNA 30-mer derived from an HIV-1 sequence served as a positive control. In addition, we compared the response to a mycobacterial 19-kDa triacylated lipopeptide, which activates cell surface TLR2/1 (TLR2/1 ligand, TLR2/1L). The optimal concentrations of these ligands was determined by dose titration or used as previously established in the lab.

PBMC from three donors were stimulated with the various ligands, and the cells collected after 1, 6 and 24 h. The mRNAs were isolated, libraries prepared and gene expression profiles obtained by RNA-seq. Principal component analysis of the DESeq2 normalized counts was used to first identify samples displaying similar trends in gene expression (Fig. 3-1A) (5). At 6 and 24 hour timepoints, PCA analysis indicated that gene expression for *M. tuberculosis* tRNA and ssRNA treated PBMC were similar, while gene expression for lipopeptide treated and untreated PBMC formed distinct groups. PCA analysis of gene expression data at 1h formed a single group, with all three ligands inducing similar profiles.

A second unsupervised analysis, hierarchical clustering, was performed to identify the specific relationships between samples (Fig. 3-1B). Consistent with the PCA, gene expression profiles for both tRNA and ssRNA at the 6 and 24h timepoints clustered together. The samples for TLR2/1L at 6h formed their own group, as did the samples for the untreated controls at 6 and 24h. All the 1h samples, regardless of the stimulus, clustered on a separate branch from the 6 and 24h samples, likely indicating that there was little difference in the transcriptional response by the various stimuli at this early timepoint.

To determine the gene signatures induced in PBMC by each ligand, differential gene expression was calculated with DEseq2 (Love et al., 2014), using a threshold of $FC > 2$ as compared to unstimulated cells. To study the gene signatures, a $FDR < 0.05$ was used to filter significantly regulated genes for use in downstream analyses. We calculated the gene signature for each ligand by calculating the maximum induction of a given gene over all timepoints for a given ligand. Comparison of the overall gene signatures showed a striking overlap of the tRNA and ssRNA signatures, with 1,465 common genes out of a total of 1,692 genes for tRNA and 1,791 for ssRNA (Fig. 3-1C). Although there was some overlap with the genes induced by TLR2/1L, there were 1,069 common genes that were exclusively induced by both tRNA and ssRNA, contributing to the common canonical pathways and biologic functions observed for the two ligands. In contrast, TLR2/1L induced a distinct signature, with 720 significantly induced genes, of which 215 were unique to TLR2/1 activation and 324 were distinct from the tRNA/ssRNA common signature.

A series of supervised analyses were performed to investigate the immune pathways induced by each ligand (Fig 3-1D). This included parallel bioinformatics analyses of gene signatures using the canonical pathways and biofunction tools in Ingenuity, as well as analysis of key modules derived by Weighted Gene Correlation Network Analysis (WGCNA). Ingenuity analysis of the biologic functions in Ingenuity showed overlap between tRNA and ssRNA gene signatures, identifying roles for NK cells, DC, monocytes, T cells including Th1 cells. In contrast, the biologic functions of the TLR2/1L induced gene profile indicated monocytes, macrophages and neutrophil associated pathways. Ingenuity analysis of the ligand-induced signatures identified several canonical pathways that were more significantly induced by tRNA as compared to TLR2/1L (Fig. 3-1E). These include DC maturation, NK-DC crosstalk, IFN signaling, PRR recognition of bacteria and viruses, communication between innate and adaptive immune cells. In comparison to the large differences in pathway significance for the tRNA and TLR2/1L induced gene signatures, the significance for the enrichment of pathway-associated genes was relatively similar for the tRNA and ssRNA signatures.

Identification of gene expression networks induced by M. tuberculosis tRNA.

To identify modules of highly interconnected genes, we used a systems biology approach: weighted gene co-expression network analysis (WGCNA), an unbiased approach that defines modules of highly interconnected genes based on pairwise correlations (Langfelder and Horvath, 2008b). WGCNA uses correlations to group genes into modules, similar to traditional clustering analysis, but unlike other

approaches, raises each correlation to a power, thus lending more weight to stronger, more reliable correlations. As we were interested in identifying gene modules correlated with the individual stimuli, we utilized the data from 6 and 24 hour timepoints was used for network construction. To determine which modules were associated with each ligand, we performed module eigengene correlation to combined 6 and 24h data for each ligand and the media control. WGCNA identified 31 gene modules, of which nine were positively correlated with at least one condition (Fig. 3-2A). Of these, the significantly correlated modules associated with tRNA treatment of PBMC were designated MEturquoise ($P = 0.03$), and MEbrown4 ($P = 0.04$). MEturquoise was also associated with ssRNA, as were MEmagenta4 and MEcoral3. TLR2/1L activation of PBMC was associated with MEantiquewhite4 and MElightyellow. Several modules were associated with the media treated cells.

Gene ontology analysis (Bindea et al., 2009) of the antiquewhite4 module associated with TLR2/1L revealed significant enrichment of terms including 'regulation of macroautophagy' and 'IL-1 signaling pathway' (Fig. 3-2B). The lightyellow module was enriched for genes associated with 'chemotaxis' and 'chemokine signaling pathway'. Analysis of the turquoise module, which correlated with tRNA and ssRNA, revealed significant enrichment of terms including 'immune system', 'IFN gamma signaling', 'TLR signaling pathway' and 'defense response'. We noted the presence of IL18, IFNG, and IL12A consistent with the biologic function analysis of the tRNA induced genes that identified a Th1 signature.

The turquoise module, which contained 2239 genes, was filtered using the 'immune system' GO term, identifying 339 genes and was inclusive of the key Th1-associated genes. A heatmap showing the fold-change induction of these genes in each samples relative to all samples showed that all genes were more robustly induced by tRNA and ssRNA, although the magnitude of the response was variable. This clearly illustrated that these genes were differentially induction by tRNA and ssRNA as compared to the TLR2/1L and media, although the level of induction varied (Fig. 3-2C). Therefore, we performed further filtering for significant induction by tRNA at either timepoint, which identified 241 genes. The correlation of these individual genes as calculated by WGCNA was visualized by a connectivity map (Hu et al., 2004) (Fig. 3-2D). We filtered by edge weight of 0.2, removing genes not connected to IL18, IFNG and IL12A, which yielded 120 genes. This analysis identified that IL-18, IFNG and IL12A were connected to each other and were multiply connected to other genes in the module. These included IL18R1, IL18RAP and IL12RB2, which encode relevant cytokine receptors, as well as STAT1, STAT2, STAT3 and STAT5A, which encode proteins involved in signaling pathways related to the induction or downstream effects of the identified cytokines. A number of Type I interferon genes were identified as well as IFN-induced genes. We noted the expression of CASP4, TLR3 and ISG15.

Integration of the bioinformatics analysis of the gene expression profiles for the tRNA treatment of PBMC and the WGCNA turquoise module was performed to link individual cell types with specific genes and functional pathways (Fig. 3-3E). This analysis identified that *M. tuberculosis* tRNA directly or indirectly triggered pathways

associated with monocytes, NK, DC and T cells, including Th1 cells, to induce a set of genes including IL18, IL18R1, IFNG, JAK2, STAT1, IL12A, IL12B, IL12RB2, and STAT4. These genes are contained within functional pathways including 'Role of PRR recognition of Bacteria and Viruses', 'TLR signaling', 'IFN- γ signaling', 'DC maturation', 'T helper differentiation' and 'defense response'. Together, these data suggest a model in which tRNA activates specific cell types that interact to establish a host-defense gene network involved in the innate instruction of an adaptive Th1 response.

Validation of M. tuberculosis tRNA gene expression profiles.

The key genes identified by the integrative informatics analysis as part of the *M. tuberculosis*-tRNA induced Th1 pathway were visualized using log₂ normalized counts at the different timepoints (Fig 3-4A). Both tRNA and ssRNA, but not TLR2/1L, significantly induced expression of IL18 mRNA (IL18: tRNA FC = 2.7 vs. media, $P = 0.005$) and its receptor heterodimer (IL18R1: tRNA FC = 2.8, $P = 0.0001$, IL18RAP: tRNA FC = 3.2, $P = 1.6 \times 10^{-15}$). IFNG mRNA was strikingly upregulated by both RNA treatments at 6t (IFNG: tRNA FC = 426, $P = 7.7 \times 10^{-52}$), while no significant change was observed in response to TLR2/1L. IL12A, which encodes the IL-12p35 component of the biologically active IL-12p70 heterodimer, was induced by 8-12 fold only in response to tRNA and ssRNA. IL12B, which encodes the p40 subunit of the IL-12p70 heterodimer, was significantly induced by all three ligands, although expression was 5-6 times greater in response to tRNA and ssRNA than TLR2/1L. IL23A, which encodes IL-23p19 and combines with IL-12p40 to form active IL-23, was not significantly induced

by any of the treatments. IL6 mRNA was strongly induced by all three ligands. This analysis revealed that additional genes connected to the central genes in the Th1 pathway, including TLR3, CASP4, and ISG15, were also upregulated, but again only in response to tRNA and ssRNA, not TLR2/1L.

To determine whether the induction of the cytokine mRNAs leads to protein production, we stimulated PBMC with *M. tuberculosis* tRNA, ssRNA40, and TLR2/1L, collected supernatants at 24h and measured cytokines by ELISA or CBA. Consistent with the gene expression data, PBMC secreted IL-18, IFN- γ and IL-12p70 in response to tRNA (IL-18: 59 ± 6 pg/ml, IFN- γ : 14366 ± 2275 pg/ml, IL-12p70: 433 ± 85 pg/ml, $P = 0.0001$ Fig 3-4B) and ssRNA, but not media or TLR2/1L. IL-12p40 was secreted in response to all treatments, although tRNA and ssRNA induced approximately seven times more IL-12p40 protein than TLR2/1L. IL-6 secretion was induced for all treatments. Low levels of IL-23 were detected in supernatants of all three treatments. The differential production of IL-12p70 and IL-23 is consistent with the informatics analysis indicating a gene network for instruction of a Th1 immune response.

To determine the sequence of cytokine induction in response to the *M. tuberculosis* tRNA, the timecourse of IL-18, IFN- γ and IL-12p70 protein production was measured (Fig. 3-4C). The earliest detected response was the production of IL-18 at 6h in response to tRNA and ssRNA (tRNA: 45 ± 5 pg/ml vs. media 11 ± 3 pg/ml, FC = 4.1, $P = 0.0001$; ssRNA: 50 ± 7 pg/ml, FC = 4.5, $P = 0.0001$). IL-18 protein increased twofold by 24h (76 ± 2 pg/ml vs. media 15 ± 8 pg/ml, FC = 5.0, $P = 0.0001$). In contrast for IFN- γ and IL-12p70, little protein was measured at 1h or 6h, but there was significant induction by

24h (IFN- γ : 15715 \pm 4287 pg/ml, FC = 1048, P = 0.0001 vs. media 14 \pm 9 pg/ml;; IL-12p70: 139 \pm 22 pg/ml P = 0.0001 vs. media n.d.). The temporal pattern of IL-6 secretion was similar to that of IL-18, protein was detected by 6h and concentration doubled by 24h. These data indicate that IL-18 secretion is triggered early, followed by secretion of IFN- γ and IL-12p70.

Interdependence of tRNA induced cytokines in the induction of IL-12p70

Given that IL-18 was secreted earliest, we determined its role on downstream cytokine secretion by pre-treating PBMC with IL-18 neutralizing antibodies prior to addition of the microbial ligands. Pre-treatment of PBMC with anti-IL-18 dramatically reduced tRNA-induced secretion of both IFN- γ (93% reduction, P =0.0003) and IL-12p70 (70% reduction, P <0.0001) (Fig 3-5A). As a control, we measured induction of IL-6, which was not affected by anti-IL-18 treatment.

Since IL-18 is known to enhance secretion of IFN- γ (Garcia et al., 1999; Lee et al., 2011; Ushio et al., 1996) and IFN- γ primes IL12A transcription (Liu et al., 2004), we hypothesized that IFN- γ contributed to the production of IL-12p70. The pre-treatment of PBMC with neutralizing anti-IFN- γ antibodies reduced the *M. tuberculosis* tRNA-induced secretion of IL-12p70 as compared to media (62% reduction, P < 0.0001) as well as the isotype control (Fig 3-5B). Secretion of IL-18 was unaffected by anti-IFN- γ treatment, which is expected given the earlier induction of IL-18. Again, IL-6 production was not diminished by the addition of anti-IFN- γ antibodies.

Previous studies have shown that CD56⁺ cells secrete IFN- γ in response to a variety of TLR stimuli including TLR7/8 agonists (Gorski et al., 2006). In order to determine if CD56⁺ cells were required for *M. tuberculosis* induction of IFN- γ , we depleted from PBMC and measured IFN- γ release. In PBMC depleted of CD56⁺ cells, *M. tuberculosis* tRNA induced IFN- γ was almost entirely eliminated (tRNA: 141 \pm 118 pg/ml CD56 depleted vs. 4557 \pm 4157 pg/ml PBMC, 97% reduction, $P=0.0215$; ssRNA: 313 \pm 244 pg/ml CD56 depleted vs. 12730 \pm 12005 pg/ml PBMC, also 97% reduction, $P=0.0097$) (Fig 3-5C). We further demonstrated that CD56⁺ cells were the major source of IFN- γ by flow cytometry (Fig 3-8). PBMC were stimulated as above, then stained with antibodies for IFN- γ , CD3 and CD56 to measure intracellular IFN- γ and differentiate NK, NKT and T cell populations. IFN- γ was detectable above background in mTB tRNA and ssRNA-treated PBMC, and of IFN- γ positive lymphocytes, the majority were CD56⁺CD3⁻ (mTB tRNA: 62 \pm 9%; ssRNA 69 \pm 9%) followed by CD56⁻CD3⁺ (mTB tRNA: 28 \pm 10%; ssRNA: 22 \pm 8%), and a small number of CD56⁺CD3⁺ (mTB tRNA: 2.8 \pm 1.5%; ssRNA: 2.4 \pm 1%) (Fig 3-5D). Since the frequency of NK cells in PBMC is much lower than that of T cells (~10% vs. ~75%) this indicated that the populations were differentially predisposed to produce IFN- γ in response to RNA stimuli. To quantify this, the frequency of IFN- γ positive cells within each lymphocyte subpopulation was calculated. NK cells had the highest frequency of IFN- γ -producing cells (mTB tRNA: 10 \pm 5%; ssRNA 40 \pm 13%) followed by NKT cells (mTB tRNA: 3.1 \pm 2%; ssRNA: 12 \pm 6%), and only a small fraction of T cells were IFN- γ ⁺ (mTB tRNA: 0.3 \pm 0.1%; ssRNA:

1.2±0.4%). Although T cells are major producers of IFN- γ during the adaptive immune response, this early timepoint measures the innate immune response, in which NK cells produce the majority of IFN- γ production.

tRNA induces cytokine responses via TLR8

The secondary structure of tRNA includes both single and double stranded RNA regions. Given that we detected induction of both TLR8 (a PRR for single stranded RNA) and TLR3 (a PRR for double stranded RNA) mRNAs by *M. tuberculosis* tRNA, we explore the role of these PRRs in triggering the Th1 cytokine network.

In order to test the requirement of TLR8 signaling for tRNA induction of the Th1 pathway, we utilized a specific TLR8 antagonist. A dose titration was performed to determine the optimal concentration for the antagonist and preclude off-target effects. PBMC were pre-treated with the TLR8-antagonist VTX-3119 or control molecule VTX-764, and stimulated with TL8-506, a synthetic TLR8-specific agonist (Lu et al., 2012). Pre-treatment with the TLR8 antagonist suppressed cytokine response to the TLR8 agonist vs. pre-treatment with control compound (IL-18: 60% reduction $P<0.0001$; IFN- γ : 67% reduction $P=0.024$; IL-12p70: 47% reduction $P=0.0004$). (Fig 3-6A).

Next, PBMC were pre-incubated with the TLR8 antagonist or control compound, then stimulated with *M. tuberculosis* tRNA, ssRNA or TLR2/1L. Treatment with the TLR8 antagonist reduced secretion of IL-18 by 64% for activation by mTB tRNA (mTB tRNA: 12±6 pg/ml vs. 35±16 pg/ml, $P<0.0001$) and 54% for ssRNA. IFN- γ secretion was 90% lower for (mTB tRNA: 695±224 pg/ml vs. 7536±2788 pg/ml $P<0.0001$) and 46% for

ssRNA. Secretion of IL-12p70 was diminished by 82% for mTB tRNA (mTB tRNA: 42 ± 15 pg/ml vs. 232 ± 57 pg/ml, $P < 0.0001$) and 67% for ssRNA (Fig 3-6B). IL-6 secretion was not significantly affected for any treatment.

To further define the role of TLR8 in the induction of IL-12p70, we surveyed the ability of various oligomers and base analogues targeting endosomal and cytosolic nucleic acid PRR for ability to induce secretion of this cytokine in PBMC (Fig 3-7C). As before, mTB tRNA and ssRNA induced IL-18, and induction also seen for synthetic TLR7 and TLR8 agonists. IFN- γ was strongly induced by ssRNA as well as, to a lesser extent, mTB tRNA and TLR8-specific ligand. High levels of IL-12p70 were secreted in response to ssRNA and mTB tRNA. Despite their induction of IL-18 and IFN- γ , only low amounts of IL-12p70 were produced in response to synthetic agonists for R848, 3M-002 (TLR7/8), and TL8-506 (TLR8). By itself, poly I:C (TLR3) was a weak inducer of IL-18, IFN- γ and IL-12p70. Even lower levels of these cytokines were observed in response to Imiquimod (TLR7), CpG DNA (TLR9), cyclic-di-GMP (STING), dA:dT (STING/CDS) (Ablasser et al., 2009; Ma et al., 2015; Zhang et al., 2011). These data suggest that TLR8 activation alone was not sufficient to explain the strong induction of IL-12p70 by *M. tuberculosis* tRNA.

We next addressed whether a combination of a specific TLR8 ligand plus a second ligand activating a different PRR could lead to IL-12p70 secretion. Given that tRNA has both single and double stranded RNA regions, and previously tRNA has been shown to induce type I IFN via activation of TLR3 (Wang et al., 2006), it was logical to ask whether activation of both TLR8 and TLR3 could induce IL-12p70. We found that

the TLR3 agonist weakly induced IL-18, that induction was greater with the TLR8 agonist, and when combined, the induction of IL-18 by the TLR3 and TLR8 ligands was additive. In contrast, the TLR3 and TLR8 ligands by themselves weakly induced IFN- γ and IL-12p70, but the combination of the two ligands resulted in the synergistic induction of these cytokines (Fig 3-7D).

Discussion

The ability of the immune system to mount a Th1 response is critical for host defense against many intracellular bacteria including *M. tuberculosis*. To generate a Th1 response, the innate immune system must recognize the microbial invader and instruct the adaptive T cell response, yet the classic pathogen associated molecular patterns (PAMPs) of mycobacteria that trigger the innate response, lipoproteins which activate TLR2/1 and muramyl dipeptide, which activates NOD2, are weak inducers of Th1 cells. Here, we found that *M. tuberculosis* tRNA, found in the culture filtrate, induces IL-12p70, a potent trigger of Th1 cells. Exploring tRNA induced gene expression profiles in PBMC, we identified a pathway for the induction of Th1 responses, beginning with the production of IL-18, which led to NK cell secretion of IFN- γ , and the subsequent induction of IL-12p70. The ability of *M. tuberculosis* tRNA to trigger this Th1 gene network was dependent on TLR8 and upregulated by TLR3 ligand, suggesting a role for nucleotide receptors in mounting a cell-mediated immune response against mycobacteria.

Our data provide a mechanism by which *M. tuberculosis* induces NK cell production of IFN- γ , involving bacterial tRNA triggering of TLR8 to induce the production of IL-18. This provides a pathway for induction of IFN- γ by the innate immune system independent of the adaptive T cell response. The role of NK cells in tuberculosis was suggested by studies in mouse models of disease, in which NK cells are recruited to the lung as early as 7 days after infection (Junqueira-Kipnis et al., 2003), with a role in preventing tissue damage (Feng et al., 2006). In human tuberculosis, NK cells have

been identified at the site of infection in patients, both in the pleural fluid (Schierloh et al., 2005) and in granulomas in pulmonary lesions (Portevin et al., 2012).

In addition to, and dependent upon the induction of IFN- γ from NK cells, *M. tuberculosis* tRNA was a potent inducer of IL-12p70, a key cytokine of the innate immune system which leads to the instruction of Th1 cells. As such, the tRNA \rightarrow IL-18 \rightarrow IFN- γ \rightarrow IL-12p70 pathway, by induction of Th1 cells, would lead to sustained induction of IFN- γ , a cytokine essential to host defense against mycobacterial infection. Mice lacking the gene for IFN- γ died from *M. tuberculosis* challenge 2-3 weeks from iv challenge and within a month from aerosol challenge (Flynn et al., 1993). Activation of mouse macrophages by IFN- γ and TNF- α induced killing of intracellular bacteria through the induction of nitric oxide (Chan et al., 1995; MacMicking et al., 1997). A key role for IFN- γ in the human immune response to *M. tuberculosis* was indicated by studies finding that individuals with genetic disorders leading to the decreased production of, or response to IFN- γ , are highly susceptible to tuberculosis and other mycobacterial diseases (Altare et al., 1998; Jouanguy et al., 1999; Newport et al., 1996). One mechanism by which IFN- γ contributes to host defense against *M. tuberculosis* in humans is through activation of macrophage antimicrobial activity via the vitamin-D dependent induction of the antimicrobial peptides cathelicidin and DEFB4 (Fabri et al., 2011).

Both *M. tuberculosis* tRNA and ssRNA induced a gene network, determined by using WGCNA to detect correlated genes, that was associated with innate instruction of an adaptive T cell response. In addition to IL18, IL12A, IL12B, and IFNG, which are

known to polarize towards a Th1 adaptive response, we found upregulation of multiple T-cell costimulatory molecules, including CD40 and CD80 (Hermann et al., 1998; Klug-Micu et al., 2013). Also of note were chemoattractants, such as CXCL10, which recruit T cells, NK cells, DC, and monocytes to the site of infection (Lande et al., 2003), and ISG15 which enhances the IFN- γ response to mycobacterial infection (Fan and Zhang, 2013).

In addition to the induction of IFN- γ , *M. tuberculosis* tRNA and ssRNA strongly induced type I interferon mRNAs and protein. Although in general, type I interferon antagonizes cell-mediated immunity in bacterial infection (O'Garra et al., 2013; Teles et al., 2013), type I interferon may also enhance a pathway for induction of IL-12p70 and IFN- γ in the same context. Type I interferon was required for induction of IL-18 during *Streptococcus pneumoniae* infection in a mouse model (Fang et al., 2014). The augmented induction of IL-18 by type I interferon may involve IRF1 signaling and inflammasome activation. IFN- α has been shown to synergize with IL-18 to induce secretion of IFN- γ from human NK cells (Matikainen et al., 2001). IFN- α , at low levels has been shown to augment the ability of TLR ligands to trigger IL-12p70 production in both monocytes and DC (Gautier et al., 2005; Hermann et al., 1998). However, in situations where type I interferon levels are high, there may be inhibition of IL-12p70 (Guarda et al., 2011; Manca et al., 2005) resulting in pathogenesis (Orme et al., 2015). An early burst of type I interferon may enhance production of cytokines that initiate Th1 polarization, while sustained type 1 interferon signaling enhances release of IL-10 and suppresses the Th1 pathway.

Transfer RNA was identified as an abundant RNA in *M. tuberculosis* culture filtrate (Obregon-Henao et al., 2012), perhaps accumulating as stable products, likely through bacterial lysis. In considering how the innate immune system recognizes this tRNA, we considered that *M. tuberculosis* resides primarily in the endosomal pathway, which is surveyed by nucleotide-sensing TLRs including TLR3 (dsRNA), TLR7 (ssRNA), TLR8 (ssRNA) and TLR9 (dsDNA). Human monocytes express primarily TLR8 (Kadowaki et al., 2001) and is localized to endosomes/phagosomes, providing the innate immune system to distinguish self RNA (cytoplasm) vs non-self RNA (endosome/phagosome). Our data indicate that this tRNA induces the Th1 pathway via TLR8, as a specific antagonist blocked cytokine production. This is consistent with TLR8 recognition of ssRNA, including single stranded regions present in stem loops sequences which are present in tRNA, for example in the anti-codon sequence (Chan and Lowe, 2009). Finally, a TLR8 gain of function polymorphism (TLR8 A1G), correlates with increased resistance to tuberculosis in humans (Bukhari et al., 2015; Davila et al., 2008; Daya et al., 2014; Lai et al., 2016; Salie et al., 2015; Sun et al., 2015; Wu et al., 2015). Clearly, it will be important to link TLR8 to recognition of *M. tuberculosis* in infected macrophages, which requires an understanding of the kinetics of tRNA release in cells and the dissociation of other pathways that trigger IL-18 induction. Another issue is that mouse TLR8 is a weak sensor of RNA, precluding studies in knockout animals. Nevertheless, TLR8 recognizes *Borrelia burgdorferi* RNA in infected human macrophages (Cervantes et al., 2013)

Although *M. tuberculosis* was a potent inducer of IL-12p70, and did so in a TLR8-dependent fashion, a TLR8 agonist alone was not sufficient to upregulate IL-12p70 production. The structure of tRNA, containing both ssRNA and dsRNA regions, led us to a set of experiments looking at the response to a combination of ligands including a synthetic ligand which activates TLR8 and dsRNA which activates TLR3. We found that although the combination of TLR3 and TLR8 ligands induced IL-18 in an additive manner, but were synergistic in the induction of IFN- γ and IL-12p70. The ability of the TLR3 ligand to act synergistically with the TLR8 ligand may relate to a direct activation of NK cells (Schmidt et al., 2004) or induction of other cytokines such as IL-15 (Zhou et al., 2007) that enhance IFN- γ and IL-12p70 production. The concept that tRNA induces a unique innate response via induction of both TLR8 and TLR3 is consistent with studies demonstrating that this ligand activates both TLR8 (Sha et al., 2014) and TLR3 (Wang et al., 2006) individually. Therefore, the ability of a single microbial ligand to induce multiple PRRs thereby induces a distinct innate immune response.

Materials and Methods

Cell purification and culture

Whole blood was obtained from healthy donors who provided written informed consent (UCLA Institutional Review Board). PBMC were isolated by Ficoll-hpyaque (GE Healthcare) density gradient centrifugation and cultured in RPMI (Gibco) supplemented with 10% FCS (Hyclone) and 1 % Pen/Strep glutamine (Gibco) at a density of 2×10^6 / ml in 96-well flat bottomed plates (Corning) at 37°C with 4% CO₂.

Reagents for Cell Stimulation

TLR2/1L, a synthetic lipopeptide derived from the 19kDa mycobacterial lipoprotein was obtained from EMC Microcollections and used at 10 µg/ml. PolyI:C (HMW) and TLR-506 were from Invivogen and used at 2 µg/ml and 500 nM respectively. ssRNA40 (phosphothioate backbone, HPLC purified) was synthesized by IDT and used at 500 ng/ml. Purified tRNA from *M. tuberculosis* H37Rv, prepared as described(Chan et al., 2015), was gift from Peter Dedon and used at 1 µg/ml. Nucleic acid ligands were complexed with Dotap (Roche) according to manufacturer's instructions to facilitate delivery to the endosome. Reagents were determined to be endotoxin-free by Limulus amoebocyte lysate assay (Lonza).

Cytokine Quantification

Cell supernatants were harvested at 24h unless otherwise noted. Cytokines measured by sandwich ELISA using antibody pairs were as follows: IL-18 (MBL Intl.), IFN-γ (BD),

IL-6 and IL-12p40 (Invitrogen). IFN- α , IL-1b, IL-10, IL-12p70 and TNF- α were measured by CBA (BD, Flex Sets).

Blocking Antibodies and TLR inhibitors

PBMC were treated with the following monoclonal neutralizing antibodies compounds for 30 min before stimulation. IL-18 10 μ g/ml (MBL Intl), IFN- γ 10 μ g/ml (BD), IgG1 10 μ g/ml from corresponding manufacturer was used as a control. The specific TLR8-inhibitor VTX-3119 and related control compound VTX-764 were gifts from VentiRx Pharmaceuticals and were used at a concentration of 100 nM which was determined by dose titration to provide optimal inhibition without off-target effects.

CD56 depletion

PBMC were depleted of CD56+ cells using CD56 MicroBeads (Miltenyi Biotech) as directed by manufacturer's protocol. Depletion was confirmed at >99% purity by flow cytometry. CD56 depleted PBMC were cultured at 1.8×10^6 /ml to reflect the loss of CD56+ cells, estimated to comprise 10% of PBMC.

Flow cytometry

PBMC were labeled with antibodies to CD3 (CD3-FITC Invitrogen) and CD56 (CD56-PE eBioscience), or isotype control. For intracellular detection of IFN-g, PBMC were treated with GolgiPlug (BD) 4h prior to harvest. Following surface staining and fixation, cells were treated with Perm/Wash Buffer (BD) and stained with IFN γ -APC (Invitrogen)

or isotype control. Flow cytometry was performed on a LSRII (BD Biosciences) in the UCLA Jonsson Comprehensive Cancer Center (JCCC) and Center for AIDS Research Flow Cytometry Core Facility that is supported by National Institutes of Health awards P30 CA016042 and 5P30 AI028697. Analysis was performed in FlowJo (Tree Star Inc.).

RNA sequencing

PBMC were stimulated as described. RNA was harvested at 1, 6 and 24h and isolated with RNeasy micro kit (Qiagen) according to manufacturer's directions, including on-column DNase digestion. RNA was quantified by Nanodrop and quality assessed by Agilent 2100 Bioanalyzer. Libraries were created from high quality RNA using TruSeq RNA Library Prep Kit v2 (Illumina). Library construction was performed by Marco Morselli and Arturo Rinaldi. Libraries were quantified by Qubit, pooled by donor in groups of 12, and sequenced in duplicate on a HiSeq 2500 Sequencing System (Illumina) at the UCLA Clinical Microarray Core.

Differential Expression Analysis

Sequenced reads were aligned to the human reference genome (build hg19 UCSC) using TopHat and Bowtie2. Raw counts were calculated with HTseq using the hg19 Ensembl annotation. Normalization to a negative binomial distribution and differential expression analysis was performed using the DESeq2 package for R. FDR was controlled by applying the Benjamin-Hochberg correction to P-values. Sequence

alignment, mapping and DEG analysis was performed by Jing Lu. Differentially expressed genes were identified using the cutoffs $FC > 2$ vs Media and adjusted P-value < 0.05 . Hierarchical clustering was performed with “hclust”, principle component analysis via “prcomp” and heatmaps using “pheatmap” in R (version 3.2.4). Weighted Gene Correlation Network Analysis was performed using the WGCNA package as described (Langfelder and Horvath, 2008a). A network of relevant gene relationships as calculated by WGCNA was visualized using visANT.

Functional Analysis

Functional analysis of differentially expressed genes was performed using Ingenuity Pathway Analysis (Qiagen). Gene ontology term analysis was performed using the ClueGO plugin (version 2.2.5) for Cytoscape (version 3.3.0) using the GO term database files from 08.06.2016. Significantly enriched terms were identified by right-sided hypergeometric test with Benjamini-Hochberg P-value correction using a cutoff of $P < 0.05$. A network connecting canonical pathways and biological functions from IPA, GO terms, and differentially expressed genes was visualized using Gephi (beta version 0.9.1).

Statistics

Results are shown as mean \pm SEM. Statistical analysis was performed using GraphPad Prism 7. Cytokine data was transformed using $\log_{10}(x+1)$, and parametric distribution assessed using the Shapiro-Wilk test of normality. One-way ANOVA was

performed for comparisons between three or more groups followed by Tukey post-test.

Two-way repeated measures ANOVA was used to assess significance in experiments with multiple factors, followed by Tukey post test for multiple comparisons.

Fig 3-1. Network analysis. (A) Principle component analysis of correlation of gene expression from RNAseq on rLog() matrix output from DESeq2. Ellipse denotes 95% confidence interval from kmeans clustering of PC1 and PC2 variance. (B) Hierarchical clustering of rLog() transformed counts. Euclidean distance, complete clustering. (C) Overlap of significantly induced genes by mTB tRNA, ssRNA and TLR2/1L, defined by $FC > 2$ over Media and $FDR < 0.05$. (D) Top Bio Functions identified by Ingenuity Pathway Analysis of significantly induced genes for mTB tRNA, ssRNA or TLR2/1L. (E) Comparison of significantly induced functional pathways induced by mTB tRNA to ssRNA or TLR2/1L.

Fig 3-2. Identification of RNA – correlated modules and associated functional analysis. (A) WGCNA eigengene modules correlated to at least one treatment condition $P < 0.05$. Red indicates positive correlation and green inverse correlation. (B) Heatmap of median-centered $r\text{Log}()$ normalized counts for genes in MEturquoise module. (C) Top hits for functional term annotation of WGCNA modules positively correlated with mTB tRNA and ssRNA or TLR2/1L signatures. (D) Visualization of top correlated genes to select genes associated with Th1 response to mTB infection.

Fig 3-3. (E) Integrated network of gene expression and functional analysis terms.

Fig 3-4. Analysis of Th1 associated genes and validation in PBMC. (A) rLog normalized counts for select genes at 6h and 24h. (B) PBMC were stimulated with mTB tRNA, ssRNA or TLR2/1L and cytokine secretion measured at 24h. (n>=20) (C) Cytokine secretion at 1h, 6h and 24h from PBMC stimulated as above. (n>=7). Data are represented as mean \pm SEM.

Fig 3-5. Role of IL-18 and NK cells. (A) PBMC were pre-treated with monoclonal anti-IL-18 neutralizing antibody or IgG1 isotype control for 30m before stimulation with TLR2/1L, ssRNA or mTB tRNA. Supernatants were collected at 24h and cytokine secretion measured (n=5). Statistical significance calculated by two-way repeated measures ANOVA and multiple comparisons by Tukey post-hoc test. * $P < 0.05$, ** $P < 0.01$. (B) PBMC were pre-treated with monoclonal anti-IFN- γ neutralizing antibody for 30m before stimulation as previous. Cytokine secretion was measured at 24h (n=3). Statistics were calculated as above. (C) PMBC were depleted of CD56⁺ cells, stimulated as shown and cytokine secretion measured at 24h. (n=3) Statistical significance calculated by one-way repeated measures ANOVA and Šidák correction applied to comparison between PBMC and CD56 depleted populations. (D) PBMC were stimulated, and BrefeldinA added at 20h to arrest cytokine secretion. Cells were collected at 24h, stained with CD3-FITC, CD56-PE and IFN- γ -APC and measured by Flow cytometry. IFN- γ ⁺ lymphocytes were divided into subpopulations determined by CD3/CD56 staining and shown as percentage of parent (IFN- γ ⁺) population or (E) divided first by CD3/CD56 markers then percent IFN- γ ⁺ cells calculated. Data are represented as mean \pm SEM.

Fig 3-6. Role of TLR8 and synergy with TLR3. (A) Inhibition of cytokine response to ultra-selective TLR8 agonist TL8-506 by selective TLR8 antagonist VTX-3119. (B) PBMC were pre-treated with TLR8 antagonist or control for 30m before treatment with TLR2/1L, ssRNA and mTB tRNA. Cytokine secretion was measured at 24h. (IL-18: n=3; IL-12p70 and IFN- γ (n=5). Statistical significance calculated by two-way repeated measures ANOVA and multiple comparisons by Tukey post-hoc test. * P < 0.05, ** P < 0.01.

Fig 3-7. (C) PBMC were stimulated with nucleotide oligomers complexed with Dotap (endosomal PRR) or Lipofectamine2000 (cytosolic PRR) or synthetic agonists for 24h (n>=4). (D) PBMC were stimulated with poly I:C, TL8-506 or combination and cytokine secretion measured at 24h (n=3). Statistical significance calculated by one-way repeated measures ANOVA and multiple comparisons by Tukey post-test. Data are represented as mean \pm SEM.

Fig 3-8. Gating strategy and representative Flow staining of PBMC stimulated with TLR ligands.

Figure 3-1: ssRNA and mTB tRNA induce similar expression patterns

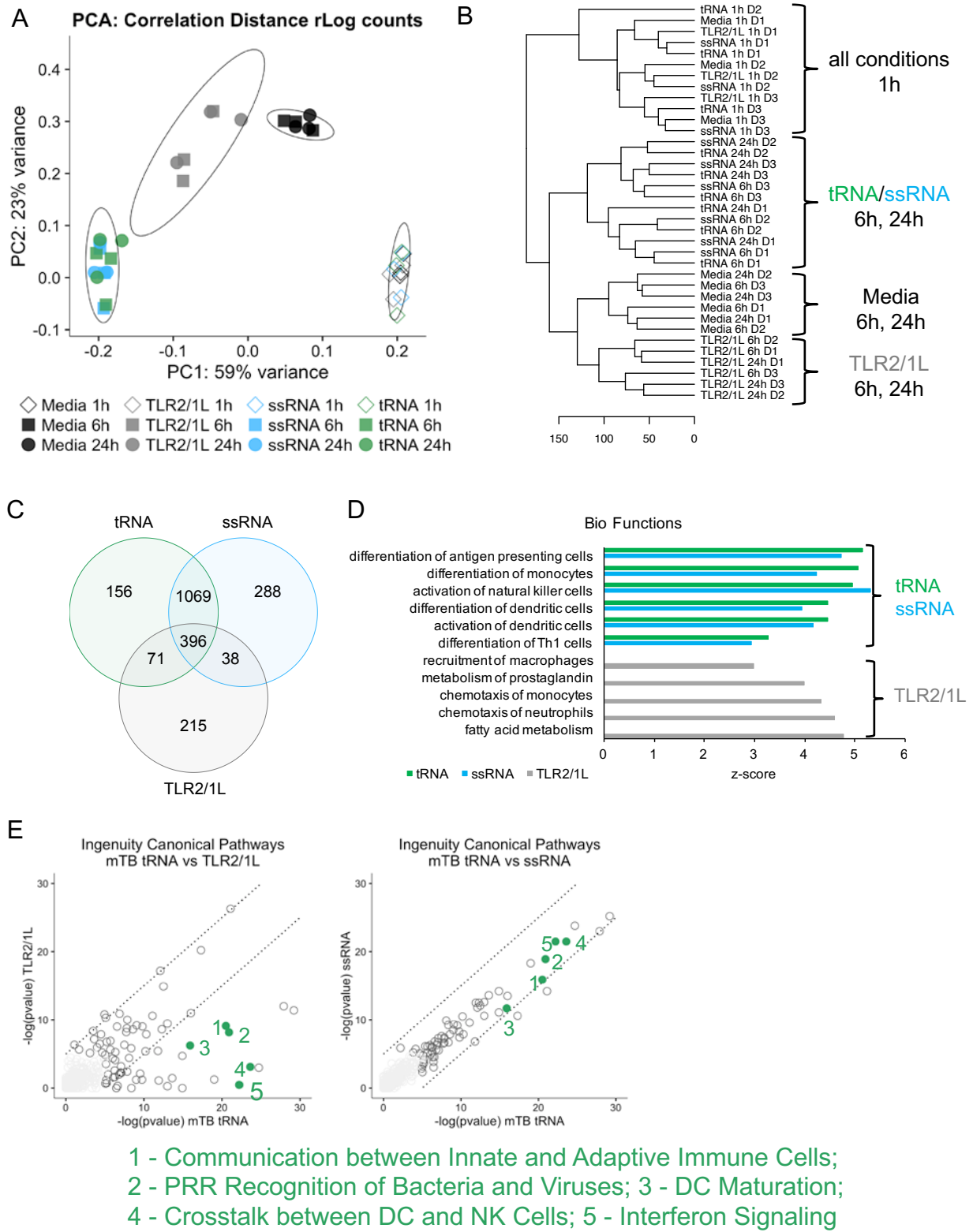


Figure 3-2: Network analysis of pathways and functions activated by tRNA/ssRNA or TLR2/1L

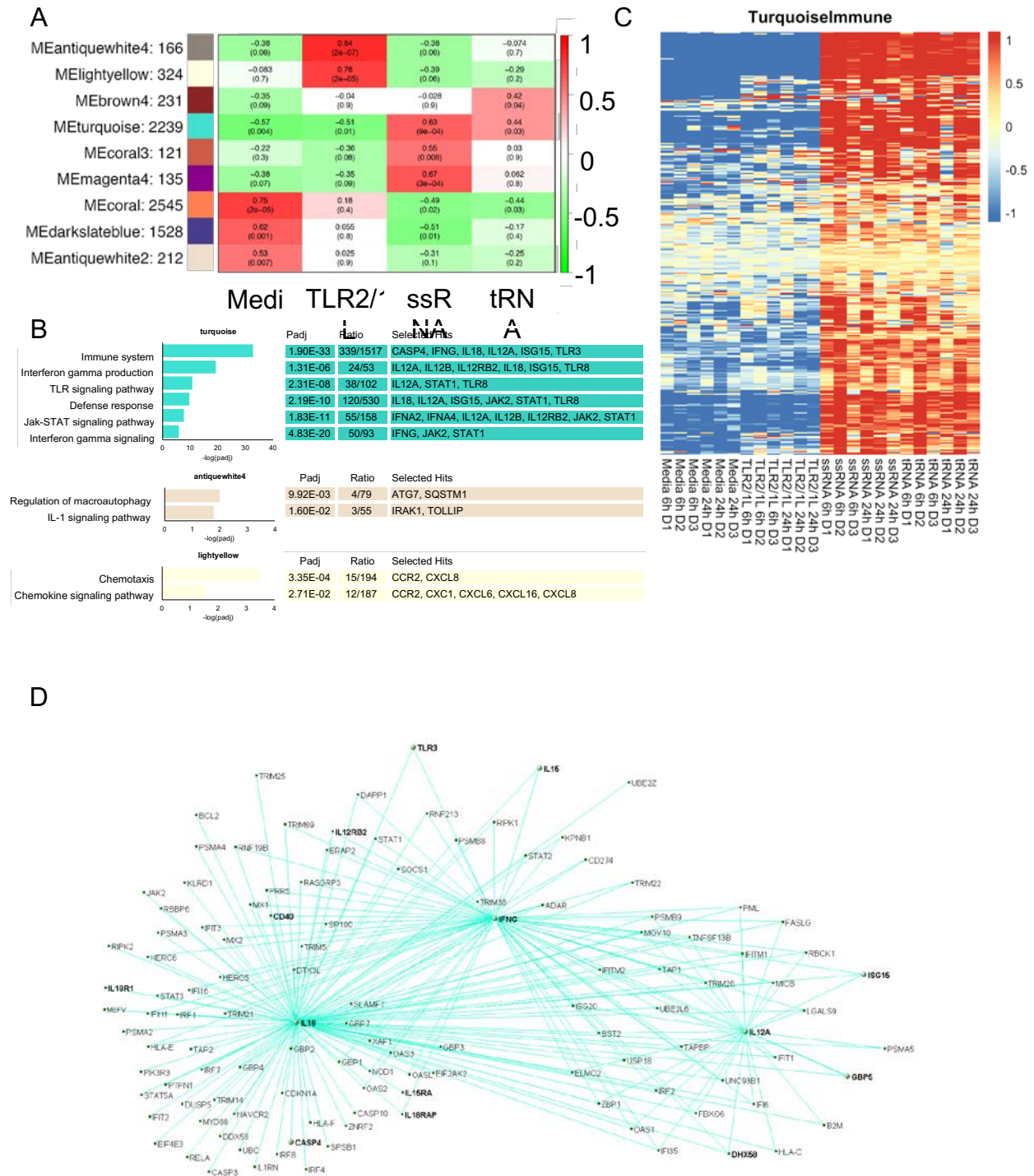


Figure 3-3: Network analysis of pathways and functions activated by tRNA/ssRNA or TLR2/1L

E

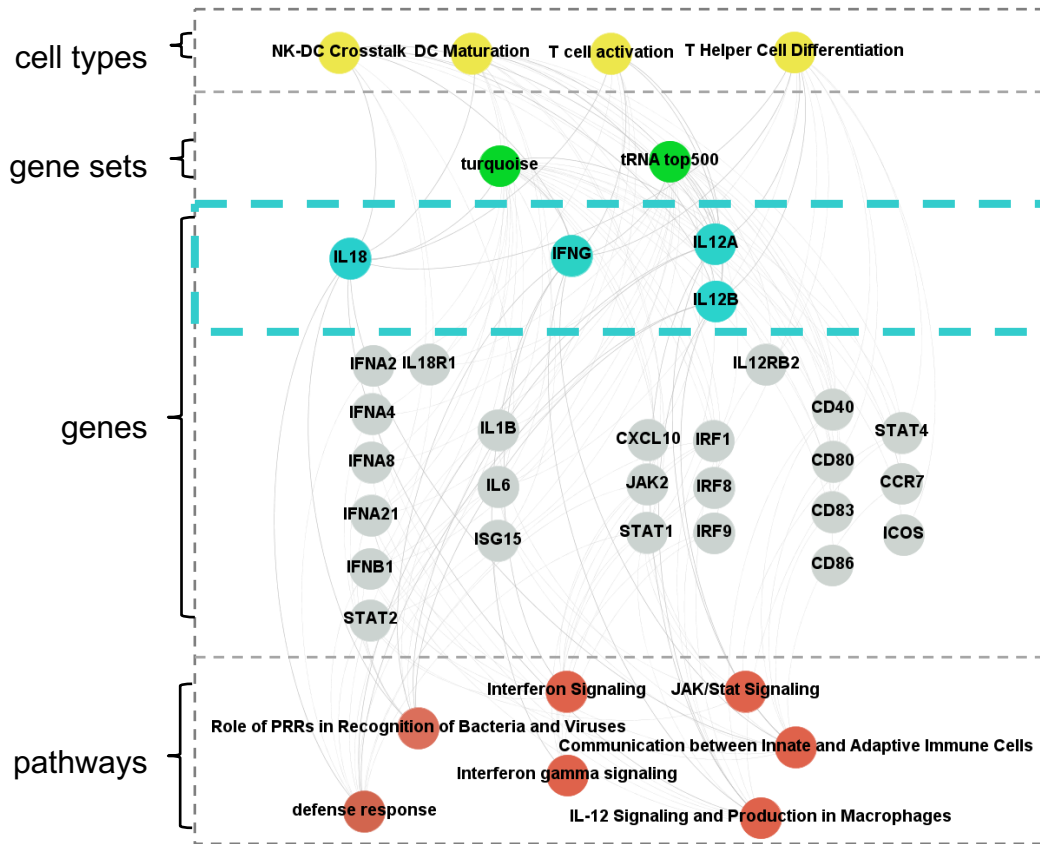
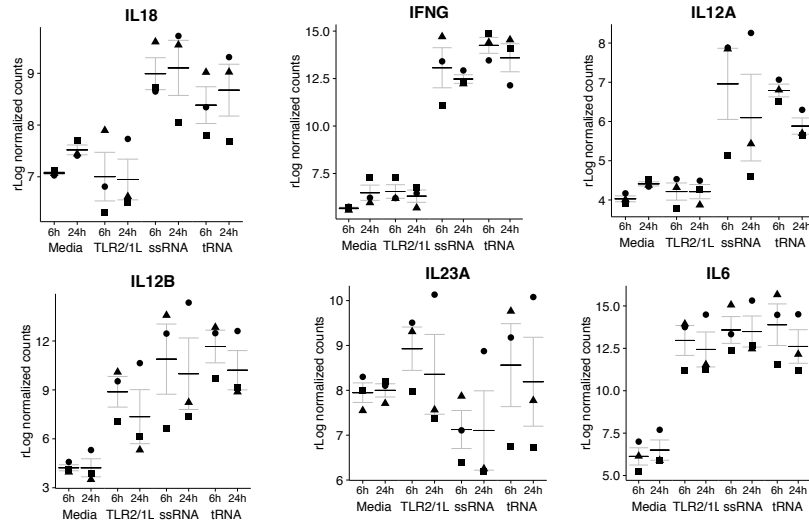
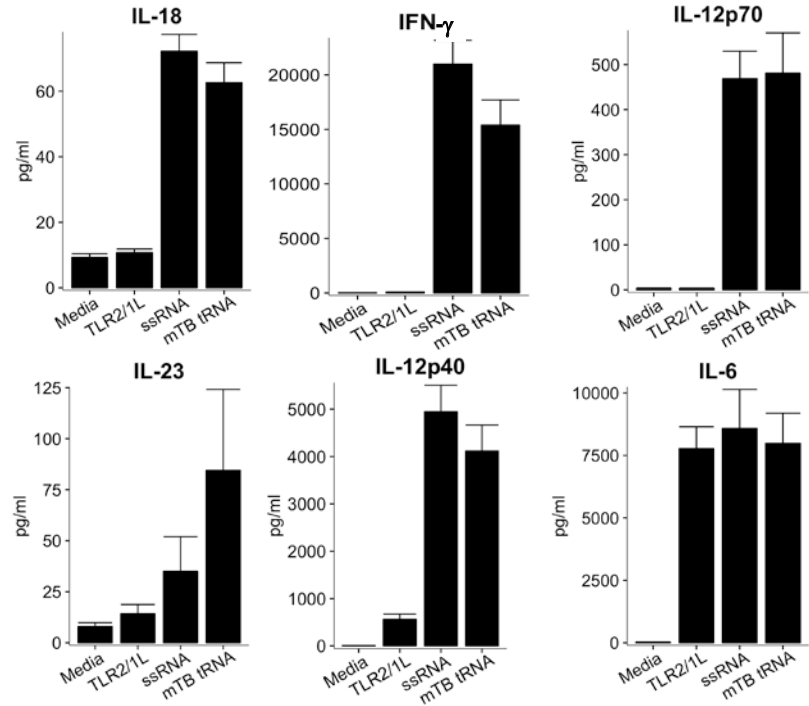


Figure 3-4: Gene expression and cytokine secretion by PBMC

A



B



C

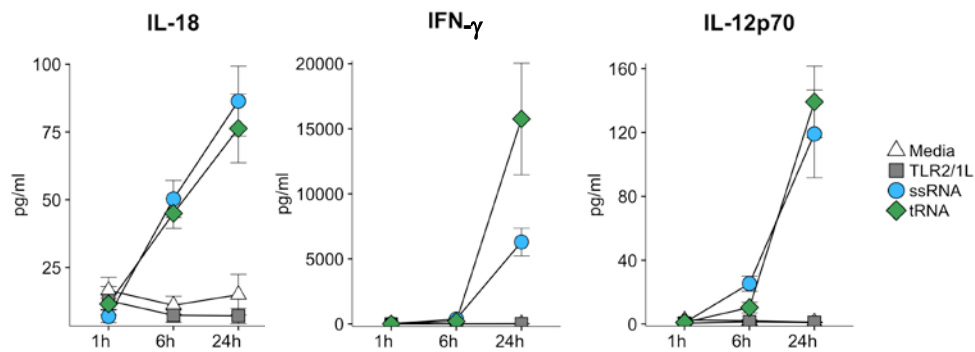


Figure 3-5: Role for IL-18 and NK cells

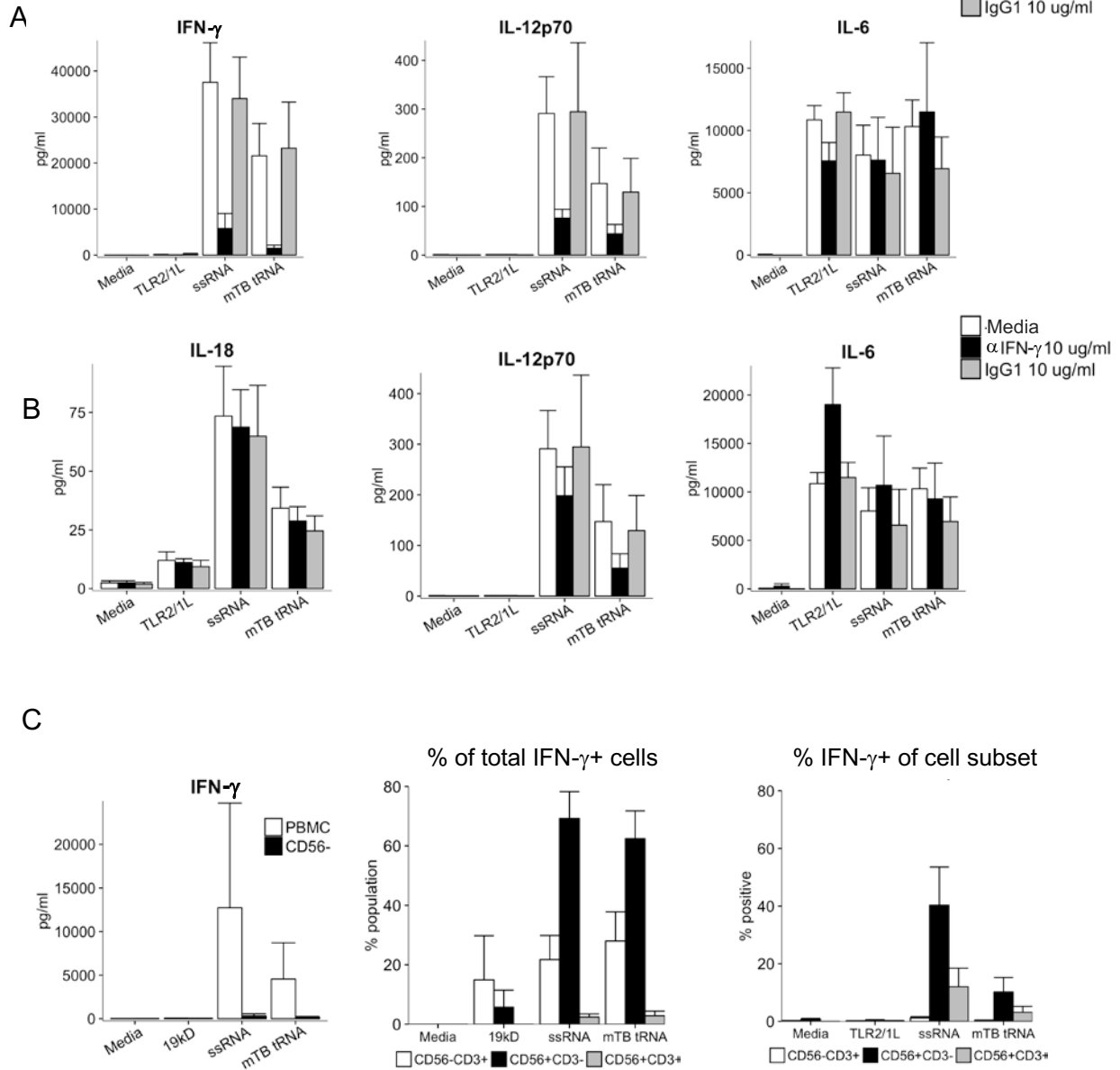


Figure 3-6: Role of TLR8

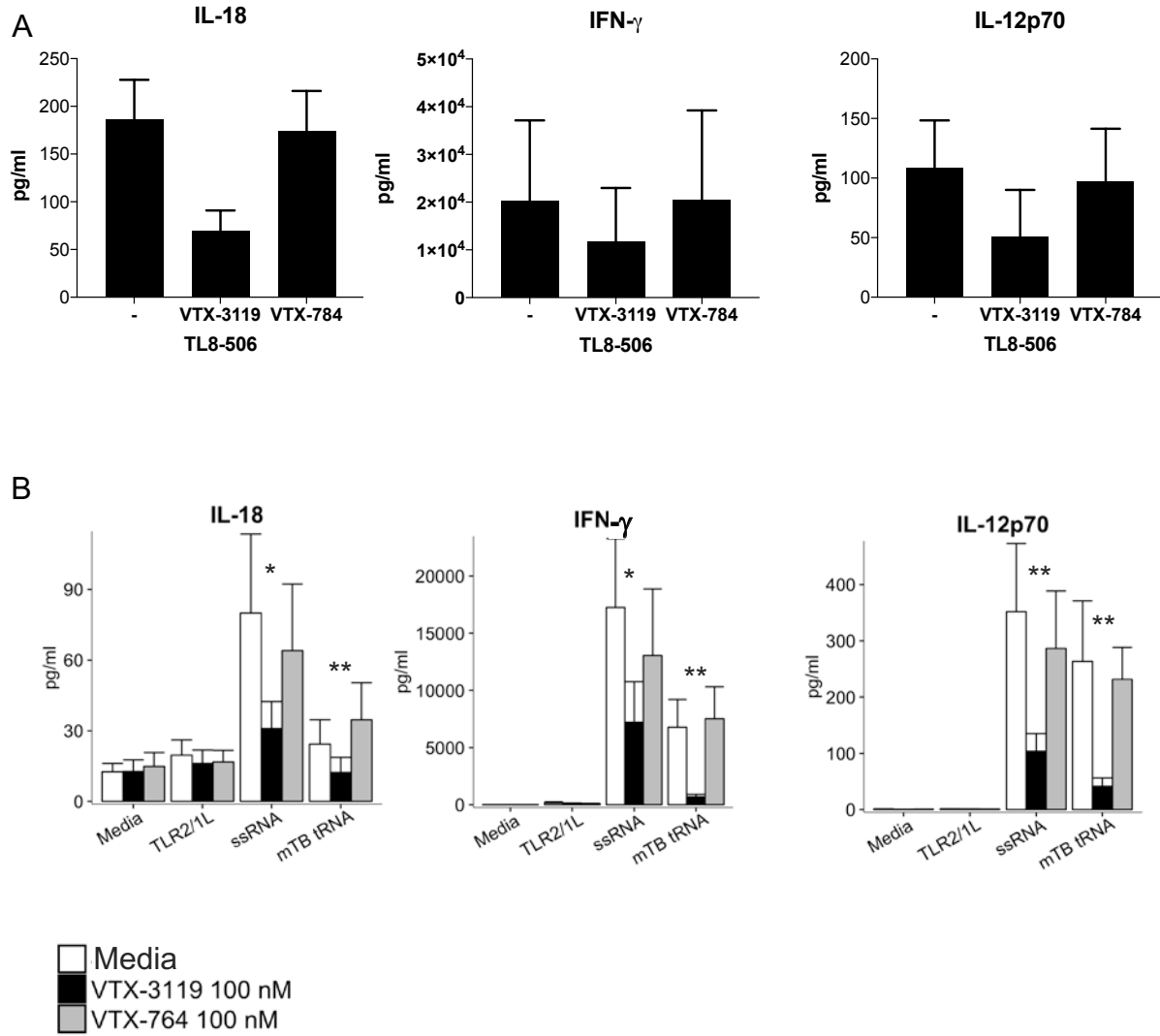
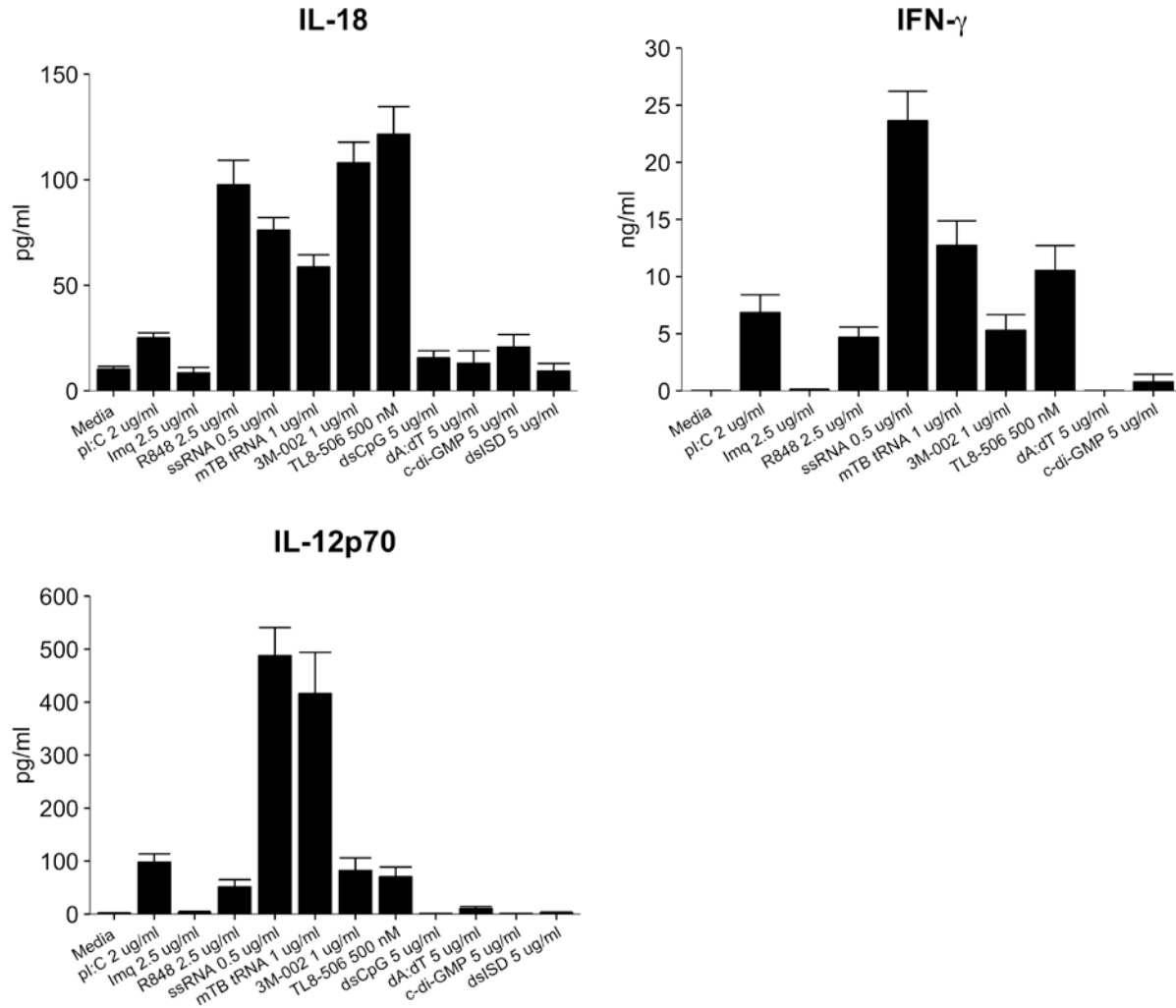


Figure 3-7: Cytokine secretion by nucleic acid PAMP; synergy with TLR3

C



D

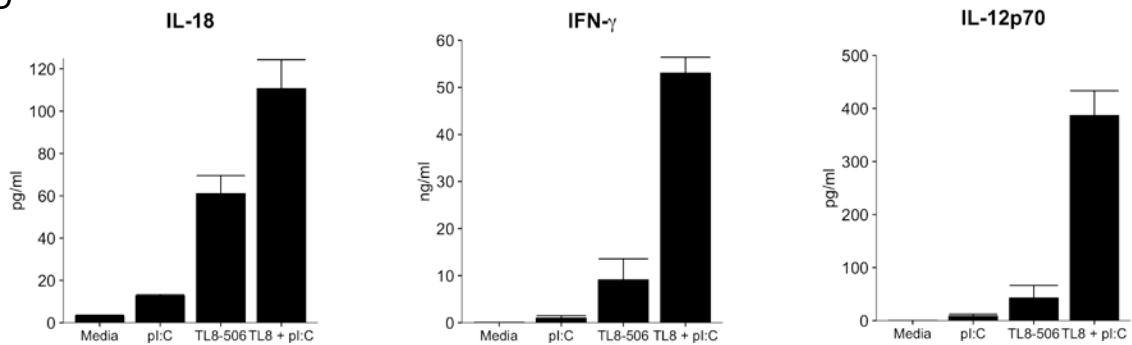


Figure 3-8: IFN- γ expression in PBMC

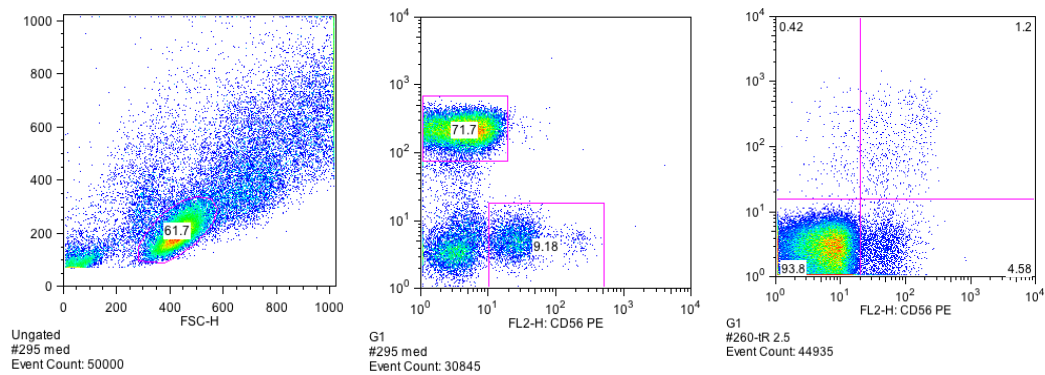


Table 1-1: Top 250 upregulated/downregulated genes. FC > 2, padj < 0.05

gene	<i>M. tuberculosis</i> 19kD lipopeptide			<i>M. tuberculosis</i> tRNA			ssRNA40			gene description
	log2FC	padj	timepoint	log2FC	padj	timepoint	log2FC	padj	timepoint	
A2M	-2.88	5.61E-04	24h	-3.06	4.80E-05	6h	-3.36	5.58E-06	24h	alpha-2-macroglobulin
ABCA1	2.30	5.58E-04	24h	1.40	3.63E-02	24h	1.94	1.51E-03	24h	ATP-binding cassette, sub-family A (ABC1), member 1
ABCB5	2.52	1.50E-04	6h	1.98	2.30E-03	24h	0.71	3.58E-01	24h	ATP-binding cassette, sub-family B (MDR/TAP), member 5
ABCC3	0.03	1.00E+00	1h	-2.40	1.15E-03	24h	-3.37	1.11E-06	24h	ATP-binding cassette, sub-family C (CFTR/MRP), member 3
ABTB2	2.81	5.64E-07	6h	4.24	6.07E-17	24h	4.89	1.14E-22	24h	ankyrin repeat and BTB (POZ) domain containing 2
AC021860.1	0.21	1.00E+00	1h	-2.12	1.30E-03	24h	-1.33	4.51E-02	6h	Uncharacterized protein
ACER1	-2.00	1.78E-03	24h	-1.69	1.53E-03	6h	-1.72	2.29E-03	24h	alkaline ceramidase 1
ADAM15	-1.59	3.94E-08	24h	-1.81	2.25E-11	6h	-1.97	2.31E-13	24h	ADAM metalloproteinase domain 15
ADAM19	0.73	4.05E-01	24h	3.29	9.93E-12	24h	3.30	5.85E-12	24h	ADAM metalloproteinase domain 19
ADAM23	-1.41	1.29E-02	24h	-1.35	4.26E-03	6h	-1.61	9.49E-04	24h	ADAM metalloproteinase domain 23
ADAM28	-1.61	1.07E-03	24h	-1.70	1.41E-04	24h	-1.76	5.44E-05	24h	ADAM metalloproteinase domain 28
ADAMTS10	-0.90	3.03E-01	24h	-1.67	3.59E-03	6h	-2.31	1.97E-05	6h	ADAM metalloproteinase with thrombospondin type 1 motif, 10
ADAMTS14	3.09	7.79E-04	24h	2.20	1.38E-02	24h	1.66	6.73E-02	24h	ADAM metalloproteinase with thrombospondin type 1 motif, 14
ADORA2A	3.20	2.93E-10	6h	3.12	9.73E-11	6h	2.90	1.93E-09	6h	adenosine A2a receptor
ADORA3	-1.88	7.74E-03	24h	-4.27	5.66E-13	6h	-4.19	5.05E-13	24h	adenosine A3 receptor
ADRA2A	0.31	1.00E+00	1h	-1.63	3.63E-02	24h	-2.44	8.93E-04	24h	adrenoceptor alpha 2A
ADRA2B	2.67	1.96E-04	1h	0.82	1.00E+00	1h	0.31	7.48E-01	6h	adrenoceptor alpha 2B
ADRB1	1.98	2.39E-02	1h	-2.11	3.82E-03	6h	-1.69	3.73E-02	24h	adrenoceptor beta 1
ADTRP	2.87	2.01E-09	24h	1.97	4.39E-05	24h	1.77	2.08E-04	24h	androgen-dependent TRPV-regulating protein
AIFM3	-1.45	6.68E-03	24h	-1.63	5.16E-04	6h	-1.80	1.01E-04	24h	apoptosis-inducing factor, mitochondrial-associated, 3
AIM2	-0.25	8.11E-01	24h	3.05	7.29E-16	24h	3.52	2.04E-21	24h	absent in melanoma 2
AK4	3.86	2.02E-08	6h	3.31	5.47E-07	6h	2.78	3.12E-05	6h	adenylate kinase 4
AK5	-2.00	2.69E-07	6h	-1.55	1.63E-05	6h	-1.85	3.46E-08	24h	adenylate kinase 5
ALDH1A1	-1.52	1.20E-02	24h	-1.52	5.11E-03	24h	-1.78	4.65E-04	6h	aldehyde dehydrogenase 1 family, member A1
ALDH2	-1.44	5.97E-03	6h	-1.68	1.53E-04	6h	-1.57	3.90E-04	6h	aldehyde dehydrogenase 2 family (mitochondrial)
ALDH7A1	-1.52	4.10E-02	6h	-1.47	1.54E-02	6h	-1.78	2.84E-03	6h	aldehyde dehydrogenase 7 family, member A1
ALOX15B	2.74	3.86E-04	24h	2.68	1.94E-04	24h	2.24	1.79E-03	24h	arachidonate 15-lipoxygenase, type B
ALOXE3	0.12	1.00E+00	1h	-2.62	9.03E-04	24h	-2.29	5.14E-03	6h	arachidonate lipoxygenase 3
ALS2CL	-1.78	4.98E-07	24h	-2.30	1.06E-11	24h	-2.49	2.46E-14	24h	ALS2 C-terminal like
AMICA1	-1.55	4.83E-04	6h	-3.03	4.32E-16	6h	-3.60	1.13E-22	6h	adhesion molecule, interacts with CXADR antigen 1
AMOTL2	2.43	1.83E-03	24h	6.29	1.59E-24	6h	6.43	1.03E-25	6h	angiomotin like 2
ANG	-1.92	1.00E-02	6h	-2.19	5.12E-04	6h	-1.89	3.86E-03	24h	angiogenin, ribonuclease, RNase A family, 5
ANGPT1	-1.64	3.45E-02	6h	-1.74	5.57E-03	6h	0.42	1.00E+00	1h	angiotensinogen
ANKRD1	2.94	2.76E-03	24h	4.76	1.09E-08	24h	3.17	2.26E-04	24h	ankyrin repeat domain 1 (cardiac muscle)
ANKRD22	0.18	1.00E+00	1h	3.66	1.54E-07	24h	4.06	2.38E-09	6h	ankyrin repeat domain 22
ANKRD55	-1.51	2.39E-02	24h	-1.72	3.15E-03	24h	-2.05	1.86E-04	24h	ankyrin repeat domain 55
ANTXR1	0.53	1.00E+00	1h	-2.42	9.75E-03	24h	-2.43	7.16E-03	24h	anthrax toxin receptor 1
ANXA9	-1.14	1.13E-02	24h	-2.37	8.40E-09	24h	-2.64	4.41E-11	6h	annexin A9
AP003774.4	-2.62	3.83E-05	24h	-1.82	3.61E-03	24h	-1.40	1.83E-02	24h	HCG1652096, isoform CRA_a; Uncharacterized protein
APCDD1L	2.95	1.22E-03	6h	2.53	5.47E-03	24h	0.97	3.64E-01	24h	adenomatous polyposis coli down-regulated 1-like
APOBEC3A	-1.65	2.73E-02	6h	5.69	8.09E-26	24h	6.10	1.06E-29	24h	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A
APOBEC3B	0.09	1.00E+00	1h	5.60	3.75E-23	24h	5.71	3.72E-24	24h	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B
APOC1	-2.11	1.51E-02	6h	-2.91	4.18E-05	6h	-3.17	6.30E-06	6h	apolipoprotein C-I
APOE	-1.99	2.23E-02	6h	-2.96	1.97E-05	6h	-3.03	9.80E-06	24h	apolipoprotein E
APOL1	0.33	7.44E-01	6h	3.32	1.34E-21	6h	3.49	5.50E-24	6h	apolipoprotein L, 1
APOL6	0.07	1.00E+00	1h	3.39	3.53E-17	6h	3.70	1.39E-20	6h	apolipoprotein L, 6
AQP9	3.05	2.52E-05	24h	3.12	3.41E-06	6h	3.31	6.08E-07	24h	aquaporin 9
ARHGAP23	0.64	4.90E-01	6h	2.81	8.88E-11	6h	3.34	1.51E-14	24h	Rho GTPase activating protein 23
ARHGAP6	0.20	1.00E+00	1h	-2.15	3.05E-04	24h	-2.84	5.79E-07	24h	Rho GTPase activating protein 6
ARN2	3.37	3.17E-05	24h	4.76	6.19E-11	24h	5.38	4.93E-14	24h	aryl-hydrocarbon receptor nuclear translocator 2
ASB9	-2.34	3.81E-03	6h	-1.65	1.51E-02	6h	-1.74	1.29E-02	24h	ankyrin repeat and SOCS box containing 9
ASGR1	-2.23	7.42E-03	6h	-3.79	2.39E-08	6h	-3.87	1.15E-08	24h	asialoglycoprotein receptor 1
ASGR2	-2.38	1.59E-02	6h	-3.42	2.21E-05	24h	-3.14	8.55E-05	24h	asialoglycoprotein receptor 2
ASIC1	-0.41	7.92E-01	24h	-2.64	2.29E-04	24h	-2.58	1.43E-04	24h	acid-sensing (proton-gated) ion channel 1
ATF3	1.44	2.73E-01	1h	3.50	4.75E-11	6h	3.54	2.01E-11	6h	activating transcription factor 3
ATP13A3	3.18	1.25E-08	24h	2.16	9.39E-05	6h	2.03	2.32E-04	6h	ATPase type 13A3
ATP6V0D2	0.32	1.00E+00	1h	-2.55	2.37E-03	24h	-2.39	2.80E-03	24h	ATPase, H ⁺ transporting, lysosomal 38kDa, V0 subunit d2
AVP1	0.35	7.11E-01	24h	-1.83	1.10E-05	6h	-3.10	3.75E-14	6h	arginine vasopressin-induced 1
AVPR2	-1.11	2.82E-01	6h	-2.04	2.64E-03	6h	-2.37	4.96E-04	6h	arginine vasopressin receptor 2
AXL	-2.65	7.18E-07	24h	2.25	2.77E-06	6h	2.34	7.34E-07	6h	AXL receptor tyrosine kinase
B4GALNT1	2.32	3.36E-02	6h	3.00	6.27E-04	24h	3.15	1.71E-04	24h	beta-1,4-N-acetyl-galactosaminyl transferase 1
BAALC	2.50	1.01E-05	6h	3.15	1.19E-09	24h	2.27	1.88E-05	24h	brain and acute leukemia, cytoplasmic
BASP1	2.38	8.69E-10	24h	2.04	6.87E-08	24h	2.74	4.20E-14	24h	brain abundant, membrane attached signal protein 1
BATF	2.48	5.21E-06	6h	2.99	1.53E-09	6h	3.93	2.86E-16	6h	basic leucine zipper transcription factor, ATF-like
BATF2	0.27	1.00E+00	1h	3.87	2.56E-07	6h	3.88	1.83E-07	6h	basic leucine zipper transcription factor, ATF-like 2
BATF3	1.55	1.51E-02	6h	3.83	4.88E-15	24h	3.75	7.62E-15	6h	basic leucine zipper transcription factor, ATF-like 3
BCAT1	2.70	7.24E-11	24h	1.78	1.60E-05	6h	1.58	1.54E-04	24h	branched chain amino-acid transaminase 1, cytosolic
BCL2A1	2.29	2.02E-04	6h	3.50	5.13E-11	6h	3.94	6.38E-14	6h	BCL2-related protein A1
BCL2L14	1.35	2.20E-02	6h	6.54	7.57E-55	24h	6.98	9.39E-63	24h	BCL2-like 14 (apoptosis facilitator)
BHLHE22	2.56	1.11E-02	1h	4.77	4.38E-14	6h	6.08	2.42E-22	24h	basic helix-loop-helix family, member e22
BMF	-1.11	1.10E-03	6h	-2.17	1.15E-14	6h	-1.62	1.74E-08	24h	Bcl2 modifying factor
BNC2	-0.92	3.26E-01	24h	-2.21	7.11E-04	24h	-2.05	7.94E-04	24h	basophilin 2
C10orf10	1.99	2.64E-04	6h	2.91	3.73E-10	6h	4.93	5.23E-29	6h	chromosome 10 open reading frame 10
C10orf105	-3.06	7.94E-05	6h	-3.60	4.59E-07	6h	-3.46	1.35E-06	6h	chromosome 10 open reading frame 105
C10orf54	0.11	1.00E+00	1h	-2.38	2.37E-06	6h	-1.85	3.20E-04	6h	chromosome 10 open reading frame 54
C11orf21	-1.42	2.81E-07	24h	-1.95	1.62E-14	6h	-1.98	5.56E-15	6h	chromosome 11 open reading frame 21
C11orf45	0.46	1.00E+00	1h	-2.42	2.25E-03	24h	-3.29	1.15E-05	24h	chromosome 11 open reading frame 45
C11orf96	3.60	4.01E-05	6h	3.26	6.30E-05	6h	2.17	1.13E-02	24h	chromosome 11 open reading frame 96
C14orf132	0.09	1.00E+00	1h	-2.63	2.97E-06	24h	-2.98	2.84E-08	24h	chromosome 14 open reading frame 132
C15orf38	0.52	1.00E+00	1h	-2.33	7.22E-04	6h	-2.50	2.11E-04	6h	chromosome 15 open reading frame 38
C16orf74	-1.43	4.58E-02	24h	-2.50	7.31E-06	6h	-3.30	1.36E-09	24h	chromosome 16 open reading frame 74
C19orf35	-0.92	3.21E-01	6h	-2.30	2.45E-05	6h	-2.52	2.47E-06	6h	chromosome 19 open reading frame 35
C1orf127	-2.84	6.32E-04	24h	-3.56	5.90E-06	24h	-3.49	3.94E-06	24h	chromosome 1 open reading frame 127
C1orf162	-1.20	2.88E-02	6h	-2.15	3.31E-07	6h	-2.33	2.23E-08	24h	chromosome 1 open reading frame 162
C1orf173	0.90	1.00E+00	1h	5.48	1.14E-20	6h	6.03	4.38E-25	6h	chromosome 1 open reading frame 173
C1orf204	-0.80	5.48E-01	6h	0.14	1.00E+00	1h	-2.28	7.48E-04	24h	chromosome 1 open reading frame 204
C1orf233	-0.25	8.35E-01	24h	-2.24	7.42E-07	6h	-1.82	7.04E-05	6h	chromosome 1 open reading frame 233
C1QTNF1	6.80	5.01E-33	6h	6.05	4.19E-27	6h	3.79	3.36E-11	24h	C1q and tumor necrosis factor related protein 1
C20orf197	-1.37	1.99E-01	24h	-2.00	6.84E-03	6h	-2.62	4.23E-04	6h	chromosome 20 open reading frame 197
C22orf42	2.67	1.29E-03	6h	3.29	8.47E-06	24h	2.43	1.05E-03	6h	chromosome 22 open reading frame 42
C2CD4A	3.20	1.80E-06	6h	2.70	2.55E-05	6h	2.75	1.43E-05	6h	C2 calcium-dependent domain containing 4A
C2CD4B	3.51	1.78E-04	6h	2.49	5.36E-03	6h	3.35	7.44E-05	6h	C2 calcium-dependent domain containing 4B
C2orf40	-1.60	1.49E-02	24h	-2.82	5.54E-06	24h	-3.75	1.88E-09	24h	chromosome 2 open reading frame 40
C2orf66	0.04	9.91E-01	6h	3.49	3.87E-06	6h	3.48	3.56E-06	6h	chromosome 2 open reading frame 66
C5AR2	-1.29	5.56E-02	6h	-2.43	1.01E-06	6h	-3.10	1.38E-10	6h	complement component 5a receptor 2
C8orf56	2.82	5.45E-03	6h	3.70	1.48E-05	24h	2.73	1.79E-03	24h	chromosome 8 open reading frame 56

C9orf139	-1.58	3.69E-02	6h	-3.04	2.16E-07	6h	-2.69	4.42E-06	6h	chromosome 9 open reading frame 139
C9orf173	0.69	1.00E+00	1h	-2.74	2.59E-04	24h	-1.73	1.40E-02	24h	chromosome 9 open reading frame 173
CA12	5.64	1.92E-11	24h	3.05	4.74E-04	24h	3.04	3.61E-04	24h	carbonic anhydrase XII
CA4	-1.96	6.31E-02	24h	0.62	1.00E+00	1h	-2.02	1.82E-02	24h	carbonic anhydrase IV
CACNA1A	0.20	9.09E-01	6h	4.51	5.97E-25	6h	4.73	1.25E-27	6h	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit
CACNA1I	-1.79	2.07E-03	24h	-2.10	4.93E-05	24h	-2.50	5.15E-07	24h	calcium channel, voltage-dependent, T type, alpha 1I subunit
CALHM2	-1.32	6.26E-04	6h	-2.32	9.20E-13	6h	-2.32	1.14E-12	24h	calcium homeostasis modulator 2
CALY	-1.84	3.04E-02	24h	-1.75	2.25E-02	24h	-2.87	6.18E-05	24h	calcyon neuron-specific vesicular protein
CAMK1	0.46	1.00E+00	1h	-3.31	4.22E-06	24h	-3.14	7.70E-06	24h	calcium/calmodulin-dependent protein kinase I
CAMK1G	3.74	2.02E-08	6h	5.17	1.14E-17	6h	5.41	1.86E-19	6h	calcium/calmodulin-dependent protein kinase IG
CARD9	-0.49	7.90E-01	6h	-3.19	5.01E-07	6h	-3.39	6.80E-08	24h	caspase recruitment domain family, member 9
CASP5	2.35	6.14E-04	24h	1.96	2.28E-03	6h	3.03	3.85E-07	6h	caspase 5, apoptosis-related cysteine peptidase
CATSPER1	-1.25	5.32E-02	24h	-2.19	2.41E-05	24h	-3.07	4.82E-10	24h	cation channel, sperm associated 1
CAV1	2.32	8.89E-04	24h	1.03	1.53E-01	6h	0.44	6.15E-01	6h	caveolin 1, caveolae protein, 22kDa
CCDC65	-1.51	3.90E-06	24h	-2.30	2.03E-14	6h	-2.58	6.76E-16	24h	coiled-coil domain containing 65
CCDC68	-2.01	3.83E-02	6h	-1.80	2.39E-02	6h	0.56	1.00E+00	1h	coiled-coil domain containing 68
CCDC80	2.20	8.30E-03	24h	2.13	5.11E-03	24h	3.38	6.38E-07	24h	coiled-coil domain containing 80
CCL1	5.40	7.20E-10	6h	3.69	2.44E-05	24h	2.85	1.37E-03	24h	chemokine (C-C motif) ligand 1
CCL13	2.91	5.12E-03	6h	2.43	9.09E-03	6h	2.37	9.88E-03	6h	chemokine (C-C motif) ligand 13
CCL15	0.50	8.48E-01	6h	3.33	8.28E-05	24h	2.32	7.92E-03	24h	chemokine (C-C motif) ligand 15
CCL17	3.06	1.44E-03	6h	2.91	1.08E-03	24h	2.02	2.79E-02	24h	chemokine (C-C motif) ligand 17
CCL18	3.59	5.32E-05	6h	3.78	2.88E-06	6h	3.33	4.16E-05	24h	chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)
CCL19	1.41	3.75E-01	6h	4.68	6.88E-08	24h	5.15	1.37E-09	24h	chemokine (C-C motif) ligand 19
CCL2	5.27	1.95E-21	24h	6.10	2.92E-30	6h	6.13	1.42E-30	6h	chemokine (C-C motif) ligand 2
CCL20	3.22	7.95E-03	1h	2.96	1.14E-03	6h	1.59	1.13E-01	6h	chemokine (C-C motif) ligand 20
CCL23	2.88	3.44E-05	6h	2.56	7.70E-05	6h	1.84	5.90E-03	24h	chemokine (C-C motif) ligand 23
CCL25	1.09	1.00E+00	1h	3.35	1.14E-04	24h	2.66	2.40E-03	24h	chemokine (C-C motif) ligand 25
CCL3	3.80	7.64E-07	1h	4.90	7.78E-14	6h	4.68	9.24E-13	6h	chemokine (C-C motif) ligand 3
CCL3L1	3.00	7.87E-03	1h	3.94	9.29E-07	6h	3.44	2.13E-05	6h	chemokine (C-C motif) ligand 3-like 1
CCL3L3	2.93	1.29E-02	1h	3.02	4.07E-04	24h	3.31	6.23E-05	24h	chemokine (C-C motif) ligand 3-like 3
CCL4	3.22	1.32E-04	1h	4.35	1.59E-10	6h	4.33	1.56E-10	6h	chemokine (C-C motif) ligand 4
CCL4L1	3.33	2.03E-05	1h	4.37	1.39E-11	6h	4.21	6.30E-11	6h	chemokine (C-C motif) ligand 4-like 1
CCL4L2	3.51	1.43E-05	1h	2.99	1.86E-05	6h	3.31	7.90E-04	1h	chemokine (C-C motif) ligand 4-like 2
CCL7	4.90	4.06E-12	24h	6.27	2.33E-21	6h	6.13	1.67E-20	6h	chemokine (C-C motif) ligand 7
CCL8	1.56	8.04E-02	6h	7.55	3.02E-37	6h	7.61	6.05E-38	6h	chemokine (C-C motif) ligand 8
CCM2L	3.75	2.96E-05	24h	2.06	2.55E-02	6h	3.68	9.47E-06	6h	cerebral cavernous malformation 2-like
CCNA1	1.69	1.76E-02	6h	4.31	1.01E-15	24h	3.50	1.09E-10	24h	cyclin A1
CCR2	-2.26	8.82E-04	6h	-4.49	2.16E-15	6h	-4.39	8.56E-15	24h	chemokine (C-C motif) receptor 2
CCSER1	-1.71	3.74E-02	6h	0.89	2.20E-01	6h	1.13	9.78E-02	24h	coiled-coil serine-rich protein 1
CD101	-2.15	3.88E-03	6h	-3.24	2.07E-07	24h	-3.55	5.47E-09	24h	CD101 molecule
CD163L1	-1.98	2.14E-02	24h	-3.51	7.16E-07	24h	-1.89	1.05E-02	24h	CD163 molecule-like 1
CD180	-1.99	2.25E-05	24h	-2.48	1.20E-08	24h	-2.39	2.14E-08	24h	CD180 molecule
CD1B	0.50	8.58E-01	6h	-2.11	2.91E-02	6h	-2.44	8.54E-03	24h	CD1b molecule
CD1C	-2.25	7.33E-03	24h	-2.19	3.28E-03	6h	-1.94	9.83E-03	6h	CD1c molecule
CD1D	-2.36	7.95E-04	24h	-1.73	9.61E-03	6h	0.09	1.00E+00	1h	CD1d molecule
CD1E	-2.79	6.62E-03	24h	-3.41	1.15E-04	6h	-3.45	7.73E-05	6h	CD1e molecule
CD248	-0.75	4.10E-01	24h	-2.50	2.04E-06	6h	-2.73	1.62E-07	6h	CD248 molecule, endosialin
CD27	-1.13	7.80E-04	24h	-1.94	2.39E-11	6h	-2.40	4.23E-17	24h	CD27 molecule
CD274	2.79	2.94E-07	6h	4.88	1.06E-23	6h	5.18	8.42E-27	6h	CD274 molecule
CD300LB	0.02	1.00E+00	1h	-2.11	1.44E-02	6h	-2.28	6.46E-03	6h	CD300 molecule-like family member b
CD300LD	0.48	1.00E+00	1h	-2.11	1.41E-02	24h	0.73	1.00E+00	1h	CD300 molecule-like family member d
CD302	-1.75	4.71E-04	6h	-2.36	1.46E-06	24h	-2.47	2.76E-08	6h	CD302 molecule
CD36	-2.97	8.51E-07	6h	-3.56	2.23E-10	24h	-1.87	1.58E-03	24h	CD36 molecule (thrombospondin receptor)
CD38	0.39	7.57E-01	6h	4.24	2.31E-23	24h	4.46	6.24E-26	24h	CD38 molecule
CD4	-1.91	4.85E-08	24h	-2.28	3.62E-12	24h	-2.33	6.79E-13	24h	CD4 molecule
CD40	1.01	2.26E-01	6h	3.88	7.16E-15	24h	4.15	4.23E-17	24h	CD40 molecule, TNF receptor superfamily member 5
CD69	0.86	1.00E+00	1h	3.78	4.72E-10	6h	3.66	1.47E-09	6h	CD69 molecule
CD80	2.44	1.31E-03	24h	5.05	1.78E-15	24h	5.29	3.72E-17	24h	CD80 molecule
CD9	-2.24	2.27E-03	24h	-2.75	2.18E-05	24h	-2.99	2.19E-06	24h	CD9 molecule
CDC42EP5	4.79	1.94E-10	24h	2.30	2.05E-03	6h	3.95	6.58E-08	24h	CDC42 effector protein (Rho GTPase binding) 5
CDH23	-1.51	8.58E-05	24h	-1.27	6.92E-04	24h	-1.26	4.76E-04	24h	cadherin-related 23
CDH26	-1.40	1.08E-02	6h	-1.62	1.88E-03	24h	-2.20	3.85E-06	6h	cadherin 26
CDKN1C	-1.95	3.56E-02	6h	1.63	4.06E-02	24h	1.32	9.95E-02	24h	cyclin-dependent kinase inhibitor 1C (p57, Kip2)
CEBPA	-2.43	3.94E-04	24h	-3.37	1.47E-07	24h	-3.50	1.31E-08	24h	CCAAT/enhancer binding protein (C/EBP), alpha
CECR1	-1.59	1.62E-10	24h	0.36	1.00E+00	1h	0.98	7.64E-05	6h	cat eye syndrome chromosome region, candidate 1
CFB	1.55	2.28E-01	6h	3.81	2.12E-06	6h	4.34	3.34E-08	24h	complement factor B
CH25H	1.56	9.26E-01	1h	3.73	2.52E-07	24h	3.71	2.07E-07	24h	cholesterol 25-hydroxylase
CHRD	-1.95	2.34E-02	6h	0.10	1.00E+00	1h	-1.39	4.98E-02	24h	chordin
CHRNA6	3.10	2.42E-07	24h	3.29	4.69E-10	6h	3.61	4.06E-12	6h	cholinergic receptor, nicotinic, alpha 6 (neuronal)
CHST13	-2.81	3.57E-05	24h	-3.73	1.21E-08	24h	-3.75	2.61E-09	24h	carbohydrate (chondroitin 4) sulfotransferase 13
CHST3	1.21	3.44E-01	24h	3.45	5.50E-06	24h	2.43	1.92E-03	24h	carbohydrate (chondroitin 6) sulfotransferase 3
CKB	2.42	2.00E-04	24h	3.25	2.22E-08	24h	2.96	3.27E-07	24h	creatine kinase, brain
CLCN4	-1.26	3.20E-03	24h	-2.32	9.51E-09	24h	-2.30	1.22E-09	24h	chloride channel, voltage-sensitive 4
CLEC10A	-3.73	3.42E-05	24h	-4.06	1.21E-06	24h	-4.26	2.05E-07	24h	C-type lectin domain family 10, member A
CLEC12B	-1.59	2.84E-02	6h	-1.03	1.05E-01	6h	-1.54	9.79E-03	6h	C-type lectin domain family 12, member B
CLEC4A	0.50	7.05E-01	24h	-1.77	3.59E-03	6h	-2.74	1.63E-06	24h	C-type lectin domain family 4, member A
CLEC4F	-2.36	1.62E-03	24h	1.37	5.20E-02	24h	0.09	1.00E+00	1h	C-type lectin domain family 4, member F
CLEC9A	-2.18	1.23E-02	24h	-1.83	1.53E-02	6h	-2.14	4.44E-03	6h	C-type lectin domain family 9, member A
CLGN	2.97	3.43E-04	6h	1.28	1.51E-01	6h	0.84	3.91E-01	6h	calmagin
CLTCL1	-1.19	2.39E-02	24h	-2.38	1.03E-07	24h	-2.61	9.39E-10	24h	clathrin, heavy chain-like 1
CMPK2	0.18	1.00E+00	1h	5.39	7.38E-43	6h	5.66	2.73E-47	24h	cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial
CNTNAP2	-1.28	3.48E-02	24h	-2.62	6.44E-07	24h	-2.48	1.85E-07	6h	contactin associated protein-like 2
COL13A1	0.41	8.41E-01	6h	-2.05	7.16E-03	24h	-2.31	1.25E-03	24h	collagen, type XIII, alpha 1
COL23A1	0.14	9.56E-01	24h	-3.16	1.64E-04	24h	-2.91	4.38E-04	24h	collagen, type XXIII, alpha 1
COL26A1	-1.47	4.41E-02	24h	-1.99	2.23E-03	24h	-2.96	3.16E-06	24h	collagen, type XXVI, alpha 1
COL5A1	0.55	1.00E+00	1h	-2.22	8.99E-05	24h	-1.21	2.11E-02	24h	collagen, type V, alpha 1
COL6A3	0.12	1.00E+00	1h	-2.16	4.25E-04	24h	-2.07	4.63E-04	24h	collagen, type VI, alpha 3
COL8A2	-2.12	1.45E-03	24h	-1.53	1.48E-02	6h	0.40	1.00E+00	1h	collagen, type VIII, alpha 2
COLEC12	-1.81	4.67E-02	24h	-1.95	1.19E-02	24h	-2.24	2.17E-03	24h	collectin sub-family member 12
CPED1	-2.43	3.94E-03	6h	-2.57	6.25E-04	24h	0.63	1.00E+00	1h	cadherin-like and PC-esterase domain containing 1
CPM	1.78	8.80E-04	6h	2.40	1.90E-07	6h	3.44	7.94E-15	6h	carboxypeptidase M
CPNE7	-0.66	1.00E+00	1h	-1.89	7.61E-05	6h	-2.33	5.72E-07	6h	copine VII
CREB5	-1.81	1.71E-05	24h	-0.88	3.77E-02	6h	-1.06	9.63E-03	6h	cAMP responsive element binding protein 5
CRIP3	-1.19	7.69E-02	24h	-2.29	6.49E-06	6h	-2.51	1.30E-06	6h	cysteine-rich protein 3
CRYBB1	-1.80	4.47E-02	24h	-2.51	1.14E-03	24h	-3.01	4.71E-05	24h	crystallin, beta B1
CSF1	3.16	2.98E-06	6h	2.56	9.20E-05	24h	2.36	2.61E-04	24h	colony stimulating factor 1 (macrophage)
CSF1R	0.16	9.33E-01	24h	-2.29	1.57E-04	24h	-2.69	3.93E-06	24h	colony stimulating factor 1 receptor
CSF2	3.97	1.64E-09	6h	4.19	1.39E-11	6h	3.43	4.86E-08	6h	colony stimulating factor 2 (granulocyte-macrophage)

CSF3	5.60	7.19E-12	6h	5.12	5.74E-11	6h	2.42	4.48E-03	24h	colony stimulating factor 3 (granulocyte)
CSF3R	-1.43	4.69E-02	6h	-1.20	4.76E-02	6h	-1.18	4.77E-02	24h	colony stimulating factor 3 receptor (granulocyte)
CSPG4	-2.05	4.51E-02	24h	-2.59	2.82E-03	24h	0.74	1.00E+00	1h	chondroitin sulfate proteoglycan 4
CSRP2	1.37	1.48E-01	6h	3.50	3.66E-09	6h	4.30	9.32E-14	6h	cysteine and glycine-rich protein 2
CST3	-2.27	3.07E-06	24h	0.28	1.00E+00	1h	-1.04	3.90E-02	24h	cystatin C
CST6	-3.83	1.21E-05	24h	-1.88	4.06E-02	24h	-2.53	2.82E-03	24h	cystatin E/M
CTHRC1	2.78	2.94E-03	24h	1.94	3.62E-02	6h	1.77	5.23E-02	24h	collagen triple helix repeat containing 1
CTNND2	2.71	1.53E-03	6h	1.97	1.69E-02	24h	0.57	5.67E-01	24h	catenin (cadherin-associated protein), delta 2
CTSF	-0.91	8.53E-02	24h	-1.96	6.16E-07	24h	-2.79	1.13E-13	24h	cathepsin F
CTSL	3.21	6.03E-08	6h	3.66	3.60E-11	24h	3.19	9.34E-09	24h	cathepsin L
CTTNBP2	-2.57	8.45E-03	6h	-3.74	4.94E-06	24h	-2.39	4.00E-03	24h	cortactin binding protein 2
CUX2	-1.46	1.19E-02	24h	-0.86	9.79E-02	6h	-1.04	3.85E-02	6h	cut-like homeobox 2
CX3CR1	-1.81	1.21E-03	6h	-2.57	1.40E-07	24h	-2.28	2.59E-06	24h	chemokine (C-X3-C motif) receptor 1
CXCL1	5.90	6.38E-21	24h	5.19	3.85E-17	6h	3.46	5.84E-08	24h	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
CXCL10	-2.58	3.20E-03	24h	5.36	7.59E-14	6h	5.40	3.18E-14	6h	chemokine (C-X-C motif) ligand 10
CXCL11	-2.05	3.74E-02	24h	6.34	1.46E-18	6h	6.43	3.36E-19	6h	chemokine (C-X-C motif) ligand 11
CXCL13	2.77	3.91E-03	24h	3.67	1.02E-05	24h	5.19	5.08E-11	24h	chemokine (C-X-C motif) ligand 13
CXCL2	3.95	1.38E-08	24h	2.89	2.32E-05	6h	2.35	1.34E-01	1h	chemokine (C-X-C motif) ligand 2
CXCL3	4.28	3.05E-08	24h	2.89	1.58E-04	6h	1.77	1.00E+00	1h	chemokine (C-X-C motif) ligand 3
CXCL5	6.67	2.17E-17	24h	4.07	3.30E-07	6h	1.80	4.26E-02	6h	chemokine (C-X-C motif) ligand 5
CXCL6	4.59	4.07E-08	6h	3.43	2.68E-05	6h	1.84	3.85E-02	6h	chemokine (C-X-C motif) ligand 6
CXCL9	-2.38	1.31E-02	24h	4.25	3.78E-08	6h	4.20	4.62E-08	6h	chemokine (C-X-C motif) ligand 9
CXCR1	-1.59	4.97E-02	6h	-1.76	6.43E-03	6h	-1.77	5.44E-03	6h	chemokine (C-X-C motif) receptor 1
CXCR2	-2.32	2.22E-08	6h	-3.13	1.14E-15	6h	-3.00	2.03E-14	6h	chemokine (C-X-C motif) receptor 2
CXXC4	0.25	1.00E+00	1h	-2.12	2.54E-03	6h	-1.97	5.35E-03	6h	CXXC finger protein 4
CYB5R2	2.94	6.24E-05	24h	2.90	2.48E-05	24h	3.26	1.03E-06	24h	cytochrome b5 reductase 2
CYBRD1	-1.94	4.54E-05	6h	-2.45	7.73E-09	6h	-2.28	8.39E-08	6h	cytochrome b reductase 1
CYP19A1	1.29	3.02E-01	6h	6.17	5.24E-20	6h	7.35	2.15E-28	6h	cytochrome P450, family 19, subfamily A, polypeptide 1
CYP11A1	-1.44	3.23E-01	6h	-1.92	4.19E-02	6h	-2.31	1.01E-02	6h	cytochrome P450, family 1, subfamily A, polypeptide 1
CYP24A1	0.61	7.76E-01	24h	-2.10	2.31E-02	6h	-2.47	5.71E-03	6h	cytochrome P450, family 24, subfamily A, polypeptide 1
CYP27B1	3.62	1.69E-06	24h	5.11	9.96E-14	24h	4.98	3.13E-13	24h	cytochrome P450, family 27, subfamily B, polypeptide 1
CYP2J2	0.85	4.16E-01	6h	4.89	2.58E-23	6h	4.98	2.97E-24	6h	cytochrome P450, family 2, subfamily J, polypeptide 2
CYP2S1	0.04	1.00E+00	1h	-2.07	5.48E-03	6h	-3.12	7.64E-06	6h	cytochrome P450, family 2, subfamily S, polypeptide 1
CYP3A5	2.42	1.69E-04	6h	1.71	4.34E-01	1h	0.17	8.50E-01	24h	cytochrome P450, family 3, subfamily A, polypeptide 5
CYP3A7	1.51	1.80E-01	24h	3.47	4.01E-06	24h	3.02	5.39E-05	24h	cytochrome P450, family 3, subfamily A, polypeptide 7
CYP4F12	0.15	1.00E+00	1h	-2.37	2.32E-03	24h	-1.90	9.82E-03	24h	cytochrome P450, family 4, subfamily F, polypeptide 12
CYP7B1	1.44	1.70E-01	24h	4.21	3.92E-10	24h	5.44	1.77E-17	24h	cytochrome P450, family 7, subfamily B, polypeptide 1
CYTL1	-1.69	3.81E-02	6h	-2.53	1.87E-04	6h	-3.29	6.38E-07	24h	cytokine-like 1
DAB2	0.27	8.79E-01	6h	-2.62	2.10E-06	24h	-3.06	1.05E-08	24h	Dab, mitogen-responsive phosphoprotein, homolog 2 (Drosophila)
DBNDD1	-1.62	5.82E-03	24h	-2.62	1.32E-07	6h	-2.69	7.90E-07	24h	dysbindin (dystrobrevin binding protein 1) domain containing 1
DBP	-1.24	2.30E-02	24h	-2.51	4.47E-09	6h	-2.49	5.12E-09	6h	D site of albumin promoter (albumin D-box) binding protein
DDX58	0.23	1.00E+00	1h	4.06	9.95E-33	6h	4.27	3.33E-36	6h	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58
DDX80	0.32	1.00E+00	1h	3.73	3.51E-22	6h	3.92	1.34E-24	6h	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60
DDX60L	0.58	3.95E-01	24h	3.30	4.15E-19	6h	3.48	3.47E-21	24h	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60-like
DEFB1	-1.33	3.21E-01	6h	6.24	6.74E-18	24h	5.68	5.56E-15	24h	defensin, beta 1
DEPDC7	0.01	1.00E+00	1h	-1.53	9.19E-04	6h	-2.52	1.57E-07	6h	DEP domain containing 7
DEPTOR	-2.17	4.52E-04	24h	-3.47	6.60E-09	24h	-3.03	6.66E-08	24h	DEP domain containing MTOR-interacting protein
DFNA5	2.86	3.10E-08	24h	1.86	3.90E-04	24h	0.75	2.10E-01	24h	deafness, autosomal dominant 5
DGKI	2.93	3.06E-03	24h	1.40	1.00E+00	1h	0.24	8.63E-01	6h	diacylglycerol kinase, iota
DHRS9	-3.53	1.52E-08	6h	-1.51	2.08E-02	6h	-2.10	5.85E-04	6h	dehydrogenase/reductase (SDR family) member 9
DHX58	-0.31	5.41E-01	24h	3.51	4.76E-49	24h	3.43	5.82E-47	24h	DEXH (Asp-Glu-X-His) box polypeptide 58
DLL1	3.43	6.69E-11	24h	2.97	3.90E-09	6h	2.99	2.46E-09	6h	delta-like 1 (Drosophila)
DLL4	2.78	6.61E-03	6h	4.00	2.55E-06	24h	4.90	2.14E-09	24h	delta-like 4 (Drosophila)
DNAAF1	2.90	1.96E-04	1h	4.24	9.04E-12	6h	4.59	7.57E-14	6h	dynein, axonemal, assembly factor 1
DNAJB13	-1.38	5.74E-02	24h	-2.37	2.13E-04	24h	-3.24	2.76E-07	24h	DnaJ (Hsp40) homolog, subfamily B, member 13
DNER	2.76	6.62E-03	24h	0.79	5.04E-01	6h	0.41	7.51E-01	6h	delta/notch-like EGF repeat containing
DOK2	-1.03	1.62E-01	6h	-2.13	1.32E-05	6h	-2.23	4.15E-06	24h	docking protein 2, 56kDa
DPEP2	-1.50	3.69E-07	24h	-2.66	1.06E-23	6h	-3.00	5.14E-30	24h	dipeptidase 2
DPEP3	-1.15	9.44E-03	6h	-2.14	1.44E-08	6h	-2.52	5.83E-11	24h	dipeptidase 3
DRAM1	1.93	1.32E-04	6h	3.20	1.68E-13	6h	3.48	5.77E-16	6h	DNA-damage regulated autophagy modulator 1
DSG1	-1.89	2.16E-03	24h	-2.25	1.26E-04	24h	-2.60	2.72E-06	24h	desmocollin 1
DUOXA2	0.69	1.00E+00	1h	3.73	2.21E-06	24h	3.22	4.46E-05	24h	dual oxidase maturation factor 2
DUSP5	1.99	2.43E-05	6h	3.73	5.39E-20	6h	4.08	7.51E-24	24h	dual specificity phosphatase 5
DYRK3	2.40	1.71E-05	24h	1.73	2.37E-03	24h	1.61	1.64E-03	6h	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3
EBI3	3.60	1.04E-08	6h	3.86	6.88E-11	24h	4.31	1.22E-13	24h	Epstein-Barr virus induced 3
EDN1	1.72	1.12E-01	1h	3.76	2.55E-11	24h	4.19	3.23E-14	24h	endothelin 1
EDN3	0.22	1.00E+00	1h	-2.43	2.55E-05	6h	-1.96	5.74E-04	6h	endothelin 3
EDNRB	0.27	9.01E-01	24h	-2.07	1.47E-02	24h	-2.69	7.01E-04	24h	endothelin receptor type B
EEP01	-0.41	7.24E-01	6h	-2.49	2.02E-08	24h	-3.01	2.60E-12	24h	endonuclease/exonuclease/phosphatase family domain containing 1
EFNA1	-0.78	3.41E-02	24h	-2.32	5.19E-13	6h	-2.91	6.02E-16	6h	ephrin-A1
EGR1	2.79	1.32E-04	1h	1.93	4.10E-01	1h	2.55	1.23E-02	1h	early growth response 1
EGR2	2.34	6.11E-04	1h	0.91	1.69E-01	6h	1.12	1.00E+00	1h	early growth response 2
EGR4	2.34	3.98E-02	1h	0.13	1.00E+00	1h	0.24	1.00E+00	1h	early growth response 4
EHF	2.65	1.05E-03	6h	1.17	1.67E-01	6h	1.49	5.24E-02	24h	ets homologous factor
EIF2AK2	0.27	1.00E+00	1h	3.64	8.18E-36	6h	3.82	3.43E-39	24h	eukaryotic translation initiation factor 2-alpha kinase 2
ELFN2	-1.13	1.25E-01	6h	-1.55	1.16E-02	24h	-2.36	2.50E-05	6h	extracellular leucine-rich repeat and fibronectin type III domain containing 2
ELOVL7	3.92	1.14E-08	6h	4.06	3.29E-10	6h	3.66	2.41E-08	24h	ELOVL fatty acid elongase 7
EMP1	2.31	1.79E-04	24h	2.59	4.47E-06	24h	2.23	7.85E-05	6h	epithelial membrane protein 1
EMR1	2.43	4.99E-05	6h	2.58	2.07E-06	6h	3.39	1.06E-10	6h	egf-like module containing, mucin-like, hormone receptor-like 1
ENHO	-2.36	4.50E-03	24h	-2.36	1.39E-03	6h	-3.19	1.87E-05	6h	energy homeostasis associated
ENPP2	1.13	2.59E-01	24h	3.21	4.71E-07	24h	5.26	2.06E-18	24h	ectonucleotide pyrophosphatase/phosphodiesterase 2
ENTHD1	2.29	2.40E-02	24h	3.18	1.58E-04	24h	3.76	2.55E-06	24h	ENTH domain containing 1
EPHA1	-1.08	3.90E-02	24h	-2.11	1.90E-07	6h	-2.15	9.63E-08	6h	EPH receptor A1
EPHB2	-3.12	4.00E-06	24h	0.30	1.00E+00	1h	0.10	1.00E+00	1h	EPH receptor B2
EPHB3	-2.55	2.50E-03	6h	-2.89	8.31E-05	6h	-2.36	1.47E-03	6h	EPH receptor B3
EPHK1	-1.14	5.43E-02	24h	-2.78	7.70E-09	24h	-3.23	1.64E-11	6h	epioplakin 1
EPS8	-2.17	6.05E-06	24h	-1.59	8.35E-04	24h	-1.51	9.44E-04	24h	epidermal growth factor receptor pathway substrate 8
EPST11	0.15	1.00E+00	1h	3.77	5.81E-28	6h	3.93	1.57E-30	6h	epithelial stromal interaction 1 (breast)
ERBB3	0.13	1.00E+00	1h	-2.43	6.13E-09	6h	-3.03	5.11E-13	24h	erb-b2 avian erythroblastic leukemia viral oncogene homolog 3
EREG	3.62	7.12E-07	1h	2.23	9.97E-04	6h	1.11	1.00E+00	1h	epiregulin
ETV3L	2.52	9.27E-03	6h	1.39	1.37E-01	6h	2.90	3.33E-04	6h	ets variant 3-like
ETV5	2.60	2.66E-05	24h	0.66	3.82E-01	6h	2.69	2.31E-06	6h	ets variant 5
ETV7	-1.02	3.55E-01	24h	4.75	2.69E-14	6h	4.92	1.96E-15	6h	ets variant 7
EVPL	-0.94	2.07E-01	24h	-2.46	1.72E-05	24h	-2.62	1.14E-05	6h	envoplakin
EXOC3L4	1.02	1.00E+00	1h	3.46	2.78E-05	24h	3.27	5.71E-05	24h	exocyst complex component 3-like 4
EXT1	0.45	1.00E+00	1h	2.36	2.82E-05	6h	3.45	1.04E-10	6h	exostosin glycosyltransferase 1
F13A1	-2.26	2.58E-02	24h	-2.23	1.17E-02	24h	-2.09	1.58E-02	24h	coagulation factor XIII, A1 polypeptide
F3	3.58	4.88E-04	1h	3.39	6.37E-05	6h	1.40	1.53E-01	6h	coagulation factor III (thromboplastin, tissue factor)

FABP3	-1.47	1.04E-01	24h	-1.95	7.91E-03	24h	-2.31	8.25E-04	6h	fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor)
FABP4	-4.04	2.20E-05	6h	-3.18	4.70E-04	6h	-4.01	3.58E-06	6h	fatty acid binding protein 4, adipocyte
FADS1	0.30	1.00E+00	1h	-2.11	6.43E-06	24h	-2.01	1.12E-05	6h	fatty acid desaturase 1
FAM124A	3.26	3.48E-06	6h	2.21	2.31E-03	24h	0.85	3.10E-01	24h	family with sequence similarity 124A
FAM13A	-1.42	3.81E-02	24h	-1.22	4.60E-02	24h	0.09	1.00E+00	1h	family with sequence similarity 13, member A
FAM153C	-1.53	4.84E-02	24h	-1.79	8.90E-03	24h	-1.96	2.11E-03	24h	family with sequence similarity 153, member C
FAM167B	-0.95	2.93E-01	24h	-1.89	1.88E-03	6h	-2.84	1.13E-05	24h	family with sequence similarity 167, member B
FAM198B	0.49	1.00E+00	1h	-2.84	9.73E-05	24h	-3.20	5.54E-06	24h	family with sequence similarity 198, member B
FAM26F	-1.70	1.38E-01	24h	3.56	1.13E-05	24h	2.96	2.88E-04	24h	family with sequence similarity 26, member F
FAM3D	-2.85	4.76E-03	24h	-3.02	7.23E-04	24h	-3.15	3.20E-04	24h	family with sequence similarity 3, member D
FBN2	-1.89	9.06E-03	6h	-2.66	1.53E-05	24h	-1.85	2.78E-03	6h	fibrillin 2
FBXL16	-1.30	3.80E-03	24h	-2.38	2.50E-10	6h	-2.65	1.95E-12	24h	F-box and leucine-rich repeat protein 16
FBX06	0.36	6.22E-01	6h	3.27	3.09E-29	6h	3.43	2.60E-32	6h	F-box protein 6
FCER1A	-2.36	9.79E-05	6h	-2.24	5.69E-05	6h	-2.30	2.75E-05	6h	Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide
FCGR2B	-1.63	1.62E-02	24h	-1.62	7.19E-03	24h	-1.69	3.62E-03	24h	Fc fragment of IgG, low affinity IIB, receptor (CD32)
FCGR2C	-1.87	2.41E-02	24h	-3.46	3.41E-07	24h	-2.00	4.19E-03	24h	Fc fragment of IgG, low affinity IIC, receptor for (CD32) (gene/pseudogene)
FCGR3B	-1.59	6.04E-03	6h	0.51	1.00E+00	1h	0.56	1.00E+00	1h	Fc fragment of IgG, low affinity IIIB, receptor (CD16b)
FCRL4	3.08	1.01E-04	24h	2.92	1.16E-04	24h	3.85	4.69E-08	24h	Fc receptor-like 4
FERMT2	3.03	2.47E-08	6h	3.03	3.25E-09	6h	2.17	4.57E-05	6h	fermitin family member 2
FFAR2	2.57	3.39E-04	1h	3.29	1.15E-08	6h	4.21	5.03E-14	6h	free fatty acid receptor 2
FFAR3	1.54	1.35E-01	24h	2.47	8.46E-04	6h	4.05	3.93E-09	6h	free fatty acid receptor 3
FFAR4	-2.06	1.86E-02	24h	-1.82	2.39E-02	24h	-2.03	7.88E-03	6h	free fatty acid receptor 4
FGD4	0.42	1.00E+00	1h	-1.73	1.65E-04	24h	-2.28	1.39E-07	6h	FYVE, RhoGEF and PH domain containing 4
FGF2	2.85	2.61E-03	6h	1.43	1.00E+00	1h	1.07	3.02E-01	6h	fibroblast growth factor 2 (basic)
FGFBP2	-1.48	5.34E-02	24h	-2.49	3.04E-05	24h	-3.13	5.03E-08	24h	fibroblast growth factor binding protein 2
FGL2	-2.40	3.54E-04	6h	0.20	1.00E+00	1h	0.47	1.00E+00	1h	fibrinogen-like 2
FILIP1L	0.62	1.00E+00	1h	-2.19	4.05E-06	6h	-2.58	5.24E-08	6h	filamin A interacting protein 1-like
FJX1	3.26	1.73E-06	6h	4.48	2.73E-13	6h	6.32	2.82E-26	6h	four jointed box 1 (Drosophila)
FLRT2	2.50	2.30E-02	24h	0.83	1.00E+00	1h	0.28	1.00E+00	1h	fibronectin leucine rich transmembrane protein 2
FLT1	2.61	3.64E-03	6h	2.42	2.41E-03	6h	2.42	2.13E-03	6h	frms-related tyrosine kinase 1
FN1	-4.87	3.51E-08	24h	-4.20	8.93E-07	24h	-3.27	1.64E-04	24h	fibronectin 1
FNDCA	3.12	1.01E-03	6h	1.16	2.68E-01	6h	1.04	3.21E-01	6h	fibronectin type III domain containing 4
FOLR2	-1.99	2.15E-02	24h	0.25	1.00E+00	1h	-1.71	2.26E-02	24h	folate receptor 2 (fetal)
FOS	0.42	1.00E+00	1h	-3.19	6.22E-06	6h	-3.27	2.69E-06	6h	FBJ murine osteosarcoma viral oncogene homolog
FOXRED2	-1.46	3.86E-02	24h	-2.08	3.64E-04	24h	-1.62	5.06E-03	24h	FAD-dependent oxidoreductase domain containing 2
FRP2	3.03	3.71E-05	24h	2.36	8.54E-04	6h	3.67	2.95E-08	6h	formyl peptide receptor 2
FRMD3	0.46	1.00E+00	1h	3.12	4.45E-18	6h	3.96	3.98E-29	6h	FERM domain containing 3
FRMD7	4.44	1.50E-07	1h	2.39	6.83E-03	24h	3.02	1.63E-04	24h	FERM domain containing 7
FSD1L	2.46	9.56E-06	24h	4.05	6.69E-17	6h	5.49	7.20E-31	6h	fibronectin type III and SPRY domain containing 1-like
FUCA1	-1.82	1.98E-02	6h	-3.68	8.57E-10	6h	-4.25	6.12E-13	24h	glucosidase, alpha-L-1, tissue
FXYP6	0.09	1.00E+00	1h	2.52	1.40E-03	6h	3.41	5.23E-06	6h	FXYP domain containing ion transport regulator 6
G0S2	3.16	3.66E-04	6h	3.39	2.12E-05	6h	3.69	2.14E-06	6h	G0/G1switch 2
GAL3ST2	3.39	6.83E-05	24h	2.87	3.04E-04	6h	4.00	1.77E-07	24h	galactose-3-O-sulfotransferase 2
GAL3ST4	-1.23	1.94E-01	6h	-1.91	2.96E-03	6h	-2.37	1.21E-04	6h	galactose-3-O-sulfotransferase 4
GALNT14	0.26	9.02E-01	6h	-2.26	9.40E-04	24h	-1.09	1.03E-01	24h	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylglucosaminyltransferase 14
GALNT9	-0.94	4.24E-01	24h	-2.45	1.49E-03	24h	-1.72	1.95E-02	24h	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylglucosaminyltransferase 9
GAPT	0.63	1.00E+00	1h	-2.25	7.67E-05	24h	-2.84	1.99E-07	24h	GRB2-binding adaptor protein, transmembrane
GATM	-1.71	2.57E-02	24h	-1.81	7.27E-03	24h	-1.36	4.27E-02	24h	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
GBA3	3.77	2.17E-05	6h	1.93	3.65E-02	6h	2.58	2.78E-03	6h	glucosidase, beta, acid 3 (gene/pseudogene)
GBP1	1.28	6.20E-01	1h	4.59	7.56E-18	6h	4.87	3.43E-20	6h	guanylate binding protein 1, interferon-inducible
GBP4	0.33	7.90E-01	6h	3.82	1.00E-20	6h	4.07	1.62E-23	6h	guanylate binding protein 4
GBP5	0.34	8.11E-01	6h	4.30	7.34E-22	6h	4.63	2.05E-25	6h	guanylate binding protein 5
GBP6	0.82	4.45E-01	24h	3.36	3.44E-09	24h	3.00	1.09E-07	24h	guanylate binding protein family, member 6
GBP7	1.01	3.61E-01	6h	3.89	1.85E-11	6h	4.20	2.36E-13	6h	guanylate binding protein 7
GCGR	3.22	7.65E-04	24h	2.15	2.47E-02	24h	4.00	1.71E-06	24h	glucagon receptor
GCH1	1.53	1.09E-02	6h	3.77	3.44E-16	6h	3.85	6.62E-17	6h	GTP cyclohydrolase 1
GCKR	4.03	1.14E-06	6h	2.22	8.90E-03	6h	2.11	1.40E-02	24h	glucokinase (hexokinase 4) regulator
GCNT1	-1.82	8.49E-05	24h	0.25	6.93E-01	6h	0.42	4.53E-01	6h	glucosaminyl (N-acetyl) transferase 1, core 2
GEM	2.42	5.84E-04	6h	2.74	1.04E-05	6h	2.13	1.37E-03	24h	GTP binding protein overexpressed in skeletal muscle
GFR2A	-2.09	2.05E-02	24h	-3.01	8.86E-05	24h	-3.40	3.75E-06	24h	GDNF family receptor alpha 2
GGT5	2.94	1.48E-03	24h	4.33	6.23E-08	24h	2.78	8.19E-04	24h	gamma-glutamyltransferase 5
GJA3	3.54	3.14E-06	6h	3.69	1.66E-07	6h	2.46	8.40E-04	6h	gap junction protein, alpha 3, 46kDa
GJA4	0.69	7.75E-01	6h	5.11	7.45E-10	24h	4.76	8.03E-09	24h	gap junction protein, alpha 4, 37kDa
GJB2	5.08	6.52E-11	24h	4.36	5.73E-09	6h	3.11	6.13E-05	24h	gap junction protein, beta 2, 26kDa
GJB6	-0.84	3.12E-01	6h	-2.10	4.67E-05	6h	-3.38	1.77E-09	24h	gap junction protein, beta 6, 30kDa
GLIS3	2.65	8.19E-04	24h	0.91	2.96E-01	6h	0.19	1.00E+00	1h	GLIS family zinc finger 3
GMPP	-0.84	2.04E-01	6h	4.95	3.53E-37	24h	4.52	3.42E-31	24h	guanosine monophosphate reductase
GOLGA7B	0.17	1.00E+00	1h	-2.23	9.12E-04	24h	-0.97	1.43E-01	24h	Golgin subfamily A member 7B; cDNA FLJ43465, clone OCBBF2036476
GPBAR1	-1.76	6.04E-03	6h	-1.41	1.68E-02	24h	-1.61	3.46E-03	24h	G protein-coupled bile acid receptor 1
GPC4	-2.65	5.79E-04	24h	-2.03	2.91E-03	6h	-2.19	1.12E-03	6h	glypican 4
GNPMB	-0.26	9.26E-01	6h	-3.10	4.46E-05	24h	-3.85	1.31E-07	24h	glycoprotein (transmembrane) nmb
GPR133	0.33	1.00E+00	1h	-2.56	6.52E-04	24h	-2.46	4.58E-04	6h	G protein-coupled receptor 133
GPR146	-1.54	7.17E-03	24h	-1.70	1.64E-03	24h	-1.91	1.34E-04	24h	G protein-coupled receptor 146
GPR162	0.24	1.00E+00	1h	-3.05	1.47E-05	6h	-2.79	7.51E-05	6h	G protein-coupled receptor 162
GPR31	1.91	3.81E-02	6h	3.63	1.26E-07	6h	1.98	7.59E-03	6h	G protein-coupled receptor 31
GPR34	-2.54	1.86E-04	6h	-2.79	1.78E-05	24h	-2.62	2.61E-05	24h	G protein-coupled receptor 34
GPR42	1.51	2.15E-01	24h	2.57	2.59E-03	24h	3.48	8.60E-06	24h	G protein-coupled receptor 42 (gene/pseudogene)
GUCY2D	0.12	1.00E+00	1h	-2.26	9.79E-03	24h	-2.13	1.10E-02	24h	guanylate cyclase 2D, membrane (retina-specific)
HAL	-1.46	2.37E-03	6h	-1.77	1.83E-05	6h	-2.13	2.64E-07	6h	histidine ammonia-lyase
HAMP	2.99	1.91E-05	6h	1.96	4.14E-03	6h	3.69	3.07E-09	6h	hepcidin antimicrobial peptide
HAS1	4.27	1.45E-06	6h	3.05	4.19E-04	6h	2.99	4.73E-04	6h	hyaluronan synthase 1
HAS2	1.33	3.26E-01	24h	3.12	1.30E-04	6h	3.53	7.99E-06	6h	hyaluronan synthase 2
HBEGF	2.38	4.01E-05	6h	1.22	8.22E-01	1h	-1.78	1.23E-03	6h	heparin-binding EGF-like growth factor
HEL22	0.30	8.03E-01	6h	4.08	6.48E-27	6h	4.62	2.00E-34	24h	helicase with zinc finger 2, transcriptional coactivator
HERC5	0.21	8.18E-01	24h	5.02	1.96E-54	24h	5.32	2.02E-61	24h	HECT and RLD domain containing E3 ubiquitin protein ligase 5
HERC6	0.26	5.92E-01	24h	4.48	2.25E-96	24h	4.85	7.82E-113	24h	HECT and RLD domain containing E3 ubiquitin protein ligase family member 6
HES1	3.41	1.15E-07	6h	2.53	5.91E-05	6h	2.44	1.34E-04	24h	hes family bHLH transcription factor 1
HES4	0.85	5.18E-01	24h	5.13	3.54E-15	24h	4.40	2.04E-11	24h	hes family bHLH transcription factor 4
HESX1	-0.99	2.68E-01	24h	5.81	9.05E-40	6h	6.08	1.25E-43	6h	HESX homeobox 1
HEY1	4.13	1.64E-09	6h	1.16	1.84E-01	24h	0.78	3.78E-01	24h	hes-related family bHLH transcription factor with YRPW motif 1
HGF	-3.07	1.63E-12	6h	-1.55	4.19E-04	24h	-1.70	3.70E-05	24h	hepatocyte growth factor (hepatoietin A; scatter factor)
HKDC1	-1.68	3.18E-04	24h	-2.06	8.18E-06	24h	-2.39	4.34E-08	24h	hexokinase domain containing 1
HLA-DMA	-1.98	8.15E-05	24h	-1.22	1.46E-02	6h	-1.61	6.78E-04	24h	major histocompatibility complex, class II, DM alpha
HLA-DMB	-1.98	2.72E-03	24h	-1.63	7.50E-03	6h	-1.94	9.12E-04	6h	major histocompatibility complex, class II, DM beta
HLA-DPA1	-1.53	2.78E-03	24h	0.26	6.87E-01	24h	0.22	1.00E+00	1h	major histocompatibility complex, class II, DP alpha 1
HLA-DPB1	-1.60	4.20E-04	24h	0.22	1.00E+00	1h	-0.99	2.47E-02	24h	major histocompatibility complex, class II, DP beta 1
HLA-DQB1	-1.44	3.23E-03	24h	-0.36	5.46E-01	6h	-0.92	4.84E-02	24h	major histocompatibility complex, class II, DQ beta 1
HLA-DQB2	-1.60	4.51E-03	24h	-0.50	4.34E-01	6h	0.10	1.00E+00	1h	major histocompatibility complex, class II, DQ beta 2

HLA-DRB1	-1.51	4.57E-03	24h	0.45	1.00E+00	1h	0.13	1.00E+00	1h	major histocompatibility complex, class II, DR beta 1
HLA-DRB5	-1.59	4.28E-03	24h	-0.33	6.35E-01	6h	0.19	1.00E+00	1h	major histocompatibility complex, class II, DR beta 5
HNF1B	0.43	1.00E+00	1h	2.17	2.18E-02	6h	3.77	1.07E-05	6h	HNF1 homeobox B
HR	-2.02	9.38E-03	6h	-2.42	2.22E-04	6h	-3.47	1.44E-07	6h	hair growth associated
HRH1	2.54	2.99E-05	6h	1.51	1.27E-02	6h	0.61	3.99E-01	6h	histamine receptor H1
HS3ST2	0.77	1.00E+00	1h	-3.74	1.34E-05	24h	-4.46	8.33E-08	24h	heparan sulfate (glucosamine) 3-O-sulfotransferase 2
HS3ST3B1	2.47	2.96E-07	6h	2.40	1.20E-07	6h	1.47	2.18E-03	6h	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1
HSD11B1	1.26	3.98E-01	6h	3.09	2.39E-04	24h	4.87	4.83E-10	24h	hydroxysteroid (11-beta) dehydrogenase 1
HSD17B3	-1.60	3.63E-02	6h	-2.06	2.44E-03	24h	-2.21	5.48E-04	24h	hydroxysteroid (17-beta) dehydrogenase 3
HSPG2	-1.25	1.06E-01	6h	-2.28	6.30E-05	24h	-1.90	7.47E-04	6h	heparan sulfate proteoglycan 2
HTRA1	-1.03	5.75E-01	6h	-2.61	3.27E-03	6h	-2.50	4.49E-03	24h	HtrA serine peptidase 1
IDO1	2.07	5.02E-04	24h	5.71	7.43E-31	6h	5.60	6.88E-30	6h	indoleamine 2,3-dioxygenase 1
IER3	2.52	2.07E-04	6h	1.96	2.19E-03	6h	1.13	1.04E-01	6h	immediate early response 3
IER5L	-1.44	1.30E-01	1h	-3.18	1.05E-09	24h	-2.99	2.57E-09	24h	immediate early response 5-like
IFI27	0.35	1.00E+00	1h	6.71	2.84E-24	24h	6.19	8.74E-21	24h	interferon, alpha-inducible protein 27
IFI30	-1.80	1.23E-03	1h	-0.63	2.45E-01	6h	-0.40	4.73E-01	24h	interferon, gamma-inducible protein 30
IFI35	0.08	1.00E+00	1h	3.64	1.71E-24	24h	3.74	4.55E-26	6h	interferon-induced protein 35
IFI44	0.02	1.00E+00	1h	4.60	1.79E-28	24h	4.67	2.06E-29	24h	interferon-induced protein 44
IFI44L	0.34	8.13E-01	24h	5.55	2.22E-27	24h	5.85	1.75E-30	24h	interferon-induced protein 44-like
IFI6	0.09	1.00E+00	1h	5.53	1.93E-40	24h	5.25	1.81E-36	24h	interferon, alpha-inducible protein 6
IFIH1	0.42	1.00E+00	1h	3.94	4.13E-37	6h	4.12	2.12E-40	24h	interferon induced with helicase C domain 1
IFIT1	0.41	1.00E+00	1h	6.06	2.66E-33	24h	6.13	4.56E-34	24h	interferon-induced protein with tetratricopeptide repeats 1
IFIT1B	0.23	9.35E-01	24h	4.19	2.98E-08	24h	4.24	7.73E-09	24h	interferon-induced protein with tetratricopeptide repeats 1B
IFIT2	0.19	1.00E+00	1h	5.21	1.29E-30	6h	5.69	1.90E-36	24h	interferon-induced protein with tetratricopeptide repeats 2
IFIT3	0.36	1.00E+00	1h	5.44	3.31E-32	24h	5.64	1.28E-34	24h	interferon-induced protein with tetratricopeptide repeats 3
IFIT5	-0.18	8.79E-01	6h	3.39	1.63E-24	6h	3.62	6.60E-28	24h	interferon-induced protein with tetratricopeptide repeats 5
IFITM1	0.48	4.44E-01	6h	4.04	3.12E-41	24h	3.55	4.89E-32	24h	interferon induced transmembrane protein 1
IFITM3	0.08	1.00E+00	1h	4.58	4.17E-32	24h	4.42	5.34E-30	6h	interferon induced transmembrane protein 3
IFNA1	0.10	1.00E+00	1h	6.15	1.61E-16	6h	5.08	2.24E-11	6h	interferon, alpha 1
IFNA10	0.53	1.00E+00	1h	5.26	5.66E-11	6h	4.84	1.97E-09	6h	interferon, alpha 10
IFNA13	-0.32	1.00E+00	1h	5.05	1.56E-10	6h	4.28	9.30E-08	6h	interferon, alpha 13
IFNA14	0.15	9.66E-01	6h	5.70	5.16E-12	6h	5.74	2.71E-12	6h	interferon, alpha 14
IFNA16	0.95	1.00E+00	1h	6.62	7.97E-21	6h	5.72	1.43E-15	6h	interferon, alpha 16
IFNA17	-0.17	1.00E+00	1h	6.14	5.85E-16	6h	5.28	6.12E-12	6h	interferon, alpha 17
IFNA2	0.28	9.11E-01	24h	5.76	1.15E-14	6h	5.32	1.08E-12	6h	interferon, alpha 2
IFNA21	0.00	1.00E+00	6h	6.35	4.73E-17	6h	5.58	2.52E-13	6h	interferon, alpha 21
IFNA4	0.10	1.00E+00	1h	5.93	7.83E-16	6h	5.51	9.21E-14	6h	interferon, alpha 4
IFNA5	0.31	1.00E+00	1h	5.45	7.28E-16	6h	4.95	3.49E-13	6h	interferon, alpha 5
IFNA6	0.05	1.00E+00	1h	5.88	1.63E-13	6h	5.15	1.36E-10	6h	interferon, alpha 6
IFNA7	0.55	8.33E-01	6h	6.26	6.45E-17	6h	5.77	1.71E-14	6h	interferon, alpha 7
IFNA8	-0.39	8.73E-01	24h	6.93	1.13E-21	6h	6.19	2.17E-17	6h	interferon, alpha 8
IFNB1	0.45	1.00E+00	1h	6.58	3.38E-21	6h	6.69	5.49E-22	6h	interferon, beta 1, fibroblast
IFNG	1.01	3.71E-01	6h	8.74	7.69E-52	6h	8.16	2.29E-45	6h	interferon, gamma
IFNL1	0.23	9.27E-01	24h	3.52	3.86E-06	6h	2.64	8.14E-04	6h	interferon, lambda 1
IFNW1	0.15	9.55E-01	24h	6.26	4.14E-19	6h	6.09	3.79E-18	6h	interferon, omega 1
IGF2	1.94	9.08E-02	6h	2.11	1.66E-02	6h	5.23	1.02E-12	6h	insulin-like growth factor 2 (somatomedin A)
IGFBP4	0.11	9.54E-01	6h	3.62	2.47E-16	24h	3.44	5.68E-15	24h	insulin-like growth factor binding protein 4
IGFBP6	-1.39	1.85E-01	24h	0.40	1.00E+00	1h	-1.98	1.17E-02	6h	insulin-like growth factor binding protein 6
IGFN1	4.75	2.67E-09	24h	2.53	2.10E-03	6h	0.19	8.82E-01	24h	immunoglobulin-like and fibronectin type III domain containing 1
IGHV1OR15	-1.57	1.64E-03	24h	-1.36	2.55E-03	6h	-1.84	2.61E-05	24h	immunoglobulin heavy variable 1/OR15-1 (non-functional)
IGLON5	3.18	4.46E-07	6h	2.43	7.51E-05	6h	1.94	2.41E-03	6h	IgLON family member 5
IGSF22	-1.45	3.98E-02	6h	-0.99	9.17E-02	6h	-2.02	6.03E-04	6h	immunoglobulin superfamily, member 22
IL10	2.89	3.74E-09	6h	2.82	1.34E-09	6h	5.03	5.92E-30	6h	interleukin 10
IL12A	0.22	9.27E-01	6h	3.00	2.63E-06	6h	3.58	8.47E-09	6h	interleukin 12A (natural killer cell stimulatory factor 1)
IL12B	2.82	5.99E-03	6h	5.14	4.50E-10	6h	5.44	2.64E-11	6h	interleukin 12B (natural killer cell stimulatory factor 2)
IL12RB2	0.56	4.83E-01	6h	3.54	2.01E-21	6h	3.63	1.70E-22	24h	interleukin 12 receptor, beta 2
IL13	3.04	2.03E-04	24h	3.00	4.66E-05	6h	2.82	1.88E-04	24h	interleukin 13
IL15RA	1.70	6.05E-04	6h	4.68	7.87E-31	6h	4.43	7.85E-28	6h	interleukin 15 receptor, alpha
IL17A	3.58	1.60E-04	6h	1.85	6.13E-02	6h	1.64	1.01E-01	6h	interleukin 17A
IL17F	4.14	3.23E-06	24h	3.29	1.31E-04	6h	2.57	3.67E-03	6h	interleukin 17F
IL19	3.83	3.20E-05	6h	2.64	3.07E-03	6h	2.74	1.75E-03	6h	interleukin 19
IL1A	3.21	1.33E-03	6h	3.39	1.30E-04	6h	1.99	3.69E-02	6h	interleukin 1, alpha
IL1B	2.55	2.27E-02	6h	2.61	5.20E-03	6h	2.17	2.25E-02	6h	interleukin 1, beta
IL1RN	3.00	3.11E-06	6h	5.40	1.59E-21	24h	5.85	2.52E-25	24h	interleukin 1 receptor antagonist
IL22	3.52	7.17E-05	6h	3.73	6.61E-06	24h	2.99	3.52E-04	24h	interleukin 22
IL26	0.51	8.20E-01	6h	4.62	3.35E-11	24h	2.94	5.71E-05	24h	interleukin 26
IL27	0.59	7.11E-01	6h	5.83	4.15E-24	6h	6.39	4.90E-29	6h	interleukin 27
IL2RA	2.77	2.11E-06	24h	4.54	3.21E-18	24h	4.61	7.33E-19	24h	interleukin 2 receptor, alpha
IL31RA	0.74	1.00E+00	1h	3.21	2.01E-05	6h	2.92	1.11E-04	6h	interleukin 31 receptor A
IL36B	2.82	3.84E-03	6h	2.88	1.10E-03	24h	0.46	7.14E-01	6h	interleukin 36, beta
IL36G	4.37	1.27E-06	6h	4.36	2.16E-07	6h	3.63	2.03E-05	6h	interleukin 36, gamma
IL36RN	5.13	2.68E-11	24h	5.53	4.11E-14	24h	3.03	1.05E-04	24h	interleukin 36 receptor antagonist
IL5RA	0.23	1.00E+00	1h	-2.78	1.00E-04	24h	-2.14	1.02E-03	24h	interleukin 5 receptor, alpha
IL6	6.56	1.20E-23	6h	7.78	9.53E-35	6h	7.34	5.08E-31	6h	interleukin 6 (interferon, beta 2)
IL7	2.74	1.22E-06	24h	3.41	8.26E-12	6h	4.54	1.08E-19	24h	interleukin 7
IL8	4.08	2.96E-07	24h	3.52	3.92E-06	6h	1.50	1.00E+00	1h	interleukin 8
INHBA	4.19	4.06E-10	6h	5.23	3.98E-17	6h	3.55	3.64E-08	24h	inhibin, beta A
INSM1	2.91	1.51E-03	6h	2.23	8.97E-03	6h	3.38	1.92E-05	6h	insulinoma-associated 1
INSR	0.37	1.00E+00	1h	-2.15	2.35E-05	24h	-1.22	2.07E-02	6h	insulin receptor
IQCD	0.36	1.00E+00	1h	-2.84	3.00E-04	24h	-3.09	3.87E-05	24h	IQ motif containing D
IRAK2	2.31	1.30E-04	6h	2.58	1.88E-06	6h	2.09	1.46E-04	6h	interleukin-1 receptor-associated kinase 2
IRF7	0.04	9.82E-01	6h	3.75	1.43E-20	24h	3.44	1.55E-17	6h	interferon regulatory factor 7
IRG1	3.70	2.23E-07	6h	7.71	4.01E-34	6h	7.82	3.01E-35	6h	immunoresponsive 1 homolog (mouse)
ISG15	0.04	1.00E+00	1h	6.11	1.74E-32	24h	5.55	5.47E-27	24h	ISG15 ubiquitin-like modifier
ISG20	-0.36	6.36E-01	24h	4.37	1.18E-39	24h	3.93	3.54E-32	24h	interferon stimulated exonuclease gene 20KDa
ISLR	1.15	5.27E-01	6h	3.61	4.86E-05	24h	4.17	9.87E-07	24h	immunoglobulin superfamily containing leucine-rich repeat
ISM1	-1.99	6.19E-03	24h	-1.79	9.30E-03	24h	-1.68	7.90E-03	24h	isthmin 1, angiogenesis inhibitor
ITGA11	-3.26	6.02E-04	24h	-2.86	1.41E-03	24h	-2.87	8.94E-04	24h	integrin, alpha 11
ITGB5	-1.18	2.42E-01	24h	-2.80	3.49E-05	24h	-3.41	1.39E-07	24h	integrin, beta 5
ITGB8	5.50	5.78E-36	24h	5.11	1.13E-32	6h	5.13	2.80E-32	24h	integrin, beta 8
KANK1	2.93	1.21E-03	6h	2.06	1.61E-02	6h	1.18	2.12E-01	6h	KN motif and ankyrin repeat domains 1
KCNC3	0.16	1.00E+00	1h	-2.33	1.97E-06	24h	-1.77	2.95E-04	6h	potassium voltage-gated channel, Shaw-related subfamily, member 3
KCNE1L	3.47	3.50E-07	24h	6.39	6.33E-26	24h	4.92	1.34E-15	24h	KCNE1-like
KCNE3	-1.05	1.20E-01	6h	-3.06	1.55E-11	6h	-3.76	8.01E-17	6h	potassium voltage-gated channel, Isk-related family, member 3
KCNG1	0.06	1.00E+00	1h	-1.98	3.70E-10	6h	-2.29	2.03E-12	6h	potassium voltage-gated channel, subfamily G, member 1
KCNH3	-1.26	3.86E-04	6h	-1.96	2.99E-10	6h	-2.46	1.58E-14	6h	potassium voltage-gated channel, subfamily H (eag-related), member 3
KCNJ2	3.55	6.68E-09	1h	3.95	7.59E-14	6h	4.51	4.88E-18	6h	potassium inwardly-rectifying channel, subfamily J, member 2
KCNJ5	-1.96	3.02E-02	24h	-2.06	9.33E-03	24h	-2.84	1.22E-04	24h	potassium inwardly-rectifying channel, subfamily J, member 5

KCNK17	0.28	1.00E+00	1h	-2.57	7.00E-05	6h	-3.13	4.29E-06	6h	potassium channel, subfamily K, member 17
KCNQ1	-1.68	3.29E-03	6h	-1.82	3.09E-04	24h	-2.25	2.42E-06	24h	potassium voltage-gated channel, KQT-like subfamily, member 1
KCTD14	0.42	1.00E+00	1h	4.24	3.92E-09	24h	4.47	3.10E-10	6h	potassium channel tetramerization domain containing 14
KIAA0319	2.87	7.06E-04	24h	0.93	3.60E-01	24h	0.50	6.34E-01	24h	KIAA0319
KIAA1199	2.80	2.92E-03	24h	4.11	3.38E-07	24h	5.36	5.12E-12	24h	KIAA1199
KIAA1211	3.39	5.40E-12	6h	2.17	1.18E-05	6h	3.27	1.68E-12	24h	KIAA1211
KIAA1217	1.03	1.00E+00	1h	3.36	1.75E-07	6h	3.62	1.22E-08	6h	KIAA1217
KIAA1549L	1.47	1.00E+00	1h	1.42	1.83E-01	6h	3.88	9.11E-06	6h	KIAA1549-like
KIAA1644	1.80	4.77E-02	24h	2.55	5.00E-04	24h	4.49	6.15E-12	24h	KIAA1644
KLHL3	-1.43	2.26E-03	24h	-2.18	1.45E-07	24h	-1.87	3.59E-06	24h	kelch-like family member 3
KREMEN1	2.41	4.01E-05	6h	2.22	4.60E-05	6h	2.64	5.44E-07	6h	kringle containing transmembrane protein 1
KRT2	-1.50	1.51E-02	6h	-2.84	1.68E-07	6h	-3.56	1.70E-09	6h	keratin 2
KRT72	-1.67	4.18E-02	24h	-2.24	8.35E-04	24h	-3.02	1.79E-06	24h	keratin 72
KRT73	-1.53	3.64E-02	24h	-2.28	1.38E-04	6h	-2.88	1.16E-06	6h	keratin 73
LAG3	0.67	2.16E-01	6h	4.82	4.13E-53	24h	4.54	3.44E-47	24h	lymphocyte-activation gene 3
LAMA3	0.47	1.00E+00	1h	4.36	6.61E-07	24h	3.37	1.59E-04	24h	laminin, alpha 3
LAMB2	0.22	1.00E+00	1h	-2.44	1.35E-03	6h	-2.49	9.04E-04	6h	laminin, beta 2 (laminin S)
LAMB3	2.80	7.61E-11	6h	2.66	7.79E-11	6h	3.04	3.68E-14	6h	laminin, beta 3
LAMC2	2.94	3.91E-06	24h	0.98	2.22E-01	24h	0.72	3.62E-01	24h	laminin, gamma 2
LAMP3	1.98	3.47E-08	24h	4.39	7.38E-43	6h	4.69	4.16E-49	24h	lysosomal-associated membrane protein 3
LCN8	-1.70	1.36E-01	24h	-3.10	1.91E-04	6h	-3.25	8.12E-05	6h	lipocalin 8
LCNL1	-1.65	5.58E-02	6h	-1.24	5.33E-02	24h	-1.81	3.02E-03	24h	lipocalin-like 1
LDHAL6B	2.52	8.22E-03	24h	2.27	7.59E-03	6h	1.68	6.40E-02	24h	lactate dehydrogenase A-like 6B
LDLRAD3	1.20	8.69E-02	24h	1.12	4.94E-02	6h	3.43	1.68E-12	24h	low density lipoprotein receptor class A domain containing 3
LDLRAP1	-1.10	8.63E-05	24h	-2.01	2.27E-16	6h	-2.45	2.94E-24	24h	low density lipoprotein receptor adaptor protein 1
LEPREL2	-0.76	3.12E-01	24h	-1.69	5.37E-04	6h	-2.47	7.02E-07	24h	leprecan-like 2
LGALS3BP	0.13	1.00E+00	1h	3.26	1.06E-32	24h	2.98	1.29E-27	6h	lectin, galactoside-binding, soluble, 3 binding protein
LGR6	-0.69	1.25E-01	24h	-1.76	1.08E-07	24h	-2.69	3.89E-17	24h	leucine-rich repeat containing G protein-coupled receptor 6
LIF	2.69	1.75E-05	1h	2.87	1.00E-07	6h	2.93	4.20E-08	6h	leukemia inhibitory factor
LIFR	0.58	1.00E+00	1h	3.06	2.46E-04	24h	3.38	2.33E-05	24h	leukemia inhibitory factor receptor alpha
LILRA3	2.46	5.60E-04	24h	2.45	1.67E-04	6h	3.66	2.55E-09	6h	leukocyte immunoglobulin-like receptor, subfamily A (without TM domain), member 3
LILRA5	2.19	9.11E-05	24h	2.96	4.11E-09	24h	4.00	1.80E-16	24h	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 5
LINC00346	4.17	5.00E-07	1h	3.00	2.47E-04	24h	2.00	1.00E+00	1h	long intergenic non-protein coding RNA 346
LINGO3	-0.74	2.96E-01	24h	-2.48	2.45E-08	24h	-2.50	8.83E-09	6h	leucine rich repeat and Ig domain containing 3
LIPA	-1.44	3.32E-02	24h	-0.91	1.56E-01	24h	0.29	1.00E+00	1h	lipase A, lysosomal acid, cholesterol esterase
LIPG	0.51	8.24E-01	24h	0.94	4.17E-01	24h	3.47	7.46E-05	24h	lipase, endothelial
LIPM	0.07	1.00E+00	1h	2.73	1.14E-03	24h	3.35	2.82E-05	6h	lipase, family member M
LIPAR6	-1.64	6.88E-05	6h	-1.19	2.30E-03	6h	-1.54	4.00E-05	24h	lysophosphatidic acid receptor 6
LPPR3	0.22	1.00E+00	1h	-2.27	6.65E-03	24h	-1.75	3.24E-02	24h	hsa-mir-3187
LRP1	0.11	1.00E+00	1h	-2.98	8.35E-06	24h	-2.34	5.69E-04	6h	low density lipoprotein receptor-related protein 1
LRP5	-1.82	5.66E-05	24h	-2.54	3.80E-09	24h	-2.00	1.47E-06	24h	low density lipoprotein receptor-related protein 5
LRRRC26	0.25	1.00E+00	1h	0.71	1.00E+00	1h	-2.50	1.96E-03	6h	leucine rich repeat containing 26
LRRRC31	2.43	2.35E-02	24h	0.83	1.00E+00	1h	0.21	1.00E+00	1h	leucine rich repeat containing 31
LRRRC38	4.45	4.63E-09	24h	2.10	1.42E-02	24h	0.37	1.00E+00	1h	leucine rich repeat containing 38
LRRRC4	-1.02	3.06E-01	6h	-2.27	1.68E-04	6h	-2.11	4.53E-04	24h	leucine rich repeat containing 4
LRRRC6	-1.45	2.00E-02	6h	-1.13	3.17E-02	6h	-1.62	1.92E-03	6h	leucine rich repeat containing 6
LTPB2	0.58	7.13E-01	24h	-2.92	3.41E-05	24h	-2.80	5.06E-05	24h	latent transforming growth factor beta binding protein 2
LY6E	0.03	1.00E+00	1h	4.28	1.43E-23	24h	3.85	3.38E-19	24h	lymphocyte antigen 6 complex, locus E
LY86	-1.93	4.31E-03	24h	0.35	1.00E+00	1h	-1.61	8.15E-03	24h	lymphocyte antigen 86
LYNX1	-1.91	3.23E-06	24h	0.21	1.00E+00	1h	0.14	1.00E+00	1h	Ly6/neurotoxin 1
LYZ	0.49	1.00E+00	1h	-2.44	1.95E-03	24h	-2.60	6.49E-04	24h	lysozyme
M1AP	-1.90	6.21E-03	24h	-2.56	8.00E-05	24h	-3.56	3.38E-08	24h	meiosis 1 associated protein
MACC1	2.00	1.66E-04	24h	2.92	9.85E-11	6h	3.89	4.97E-18	24h	metastasis associated in colon cancer 1
MAMLD1	3.05	2.66E-08	24h	2.99	3.68E-09	6h	2.26	2.34E-05	24h	mastermind-like domain containing 1
MAP1LC3A	2.79	3.82E-05	24h	1.85	6.16E-03	24h	1.56	2.09E-02	24h	microtubule-associated protein 1 light chain 3 alpha
MARCI	-1.82	1.76E-02	6h	-2.05	1.32E-03	24h	-2.25	1.81E-04	24h	mitochondrial amidoxime reducing component 1
MARCH1	-1.40	9.41E-03	6h	-1.41	2.39E-03	6h	0.42	4.66E-01	6h	membrane-associated ring finger (C3HC4) 1, E3 ubiquitin protein ligase
MARVELD1	-2.15	2.83E-05	24h	-3.39	2.26E-12	24h	-3.49	6.86E-14	24h	MARVEL domain containing 1
MAS1	4.72	2.09E-08	24h	3.71	9.60E-06	24h	3.70	6.29E-06	24h	MAS1 oncogene
MDK	-1.02	3.07E-01	24h	3.22	5.82E-08	24h	2.83	1.86E-06	24h	midkine (neurtin growth-promoting factor 2)
MDS2	-1.85	6.78E-05	24h	-1.32	3.38E-03	6h	-1.56	2.94E-04	24h	myelodysplastic syndrome 2 translocation associated
MEF2C	-1.57	4.07E-08	6h	-1.38	9.92E-07	24h	-1.50	2.28E-08	6h	myocyte enhancer factor 2C
MET	5.25	5.89E-16	24h	3.08	3.15E-06	6h	1.01	2.01E-01	6h	met proto-oncogene
METTL7A	-1.62	6.77E-07	6h	-2.90	1.57E-21	24h	-2.29	2.04E-14	24h	methyltransferase like 7A
METTL7B	2.46	1.11E-02	24h	1.36	1.70E-01	24h	0.49	6.59E-01	24h	methyltransferase like 7B
MGST2	-1.64	1.53E-03	24h	-2.07	7.40E-06	6h	-2.50	2.81E-08	24h	microsomal glutathione S-transferase 2
MIF	0.31	1.00E+00	1h	0.10	1.00E+00	1h	-2.39	3.32E-06	6h	microphthalmia-associated transcription factor
MMP1	4.36	1.93E-06	24h	1.87	5.92E-02	6h	0.66	5.88E-01	6h	matrix metalloproteinase 1 (interstitial collagenase)
MMP10	5.12	9.89E-10	6h	2.81	1.12E-03	6h	0.51	6.85E-01	6h	matrix metalloproteinase 10 (stromelysin 2)
MMP14	4.65	2.60E-48	6h	3.51	2.98E-28	6h	2.69	8.85E-17	6h	matrix metalloproteinase 14 (membrane-inserted)
MMP19	3.76	3.27E-15	24h	2.74	8.89E-09	24h	1.55	2.10E-03	24h	matrix metalloproteinase 19
MMP2	-1.68	9.54E-02	6h	-2.19	3.85E-03	6h	-2.32	1.78E-03	6h	matrix metalloproteinase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)
MMP28	0.16	1.00E+00	1h	-3.35	2.32E-06	24h	-3.98	5.89E-09	24h	matrix metalloproteinase 28
MMP7	4.72	7.11E-15	6h	3.48	1.06E-08	24h	1.80	5.30E-03	6h	matrix metalloproteinase 7 (matrilysin, uterine)
MMP9	2.41	2.16E-02	24h	0.37	1.00E+00	1h	-2.30	1.03E-02	24h	matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase)
MNDA	-3.07	1.52E-10	6h	0.04	9.60E-01	24h	0.50	4.00E-01	24h	myeloid cell nuclear differentiation antigen
MPZL2	-1.73	7.99E-03	24h	-2.15	3.33E-04	24h	-1.54	7.63E-03	24h	myelin protein zero-like 2
MRC1L1	1.01	6.22E-01	6h	-2.87	2.14E-03	24h	-3.20	3.84E-04	24h	cDNA FLJ56855, highly similar to Macrophage mannose receptor 1
MRC2	-1.64	3.84E-03	24h	-2.24	6.50E-06	24h	-2.64	3.50E-08	24h	mannose receptor, C type 2
MS4A12	0.42	1.00E+00	1h	4.16	3.49E-07	6h	4.10	4.53E-07	6h	membrane-spanning 4-domains, subfamily A, member 12
MS4A2	-2.05	1.23E-02	6h	-1.45	3.65E-02	6h	-2.23	1.56E-03	6h	membrane-spanning 4-domains, subfamily A, member 2
MS4A6A	-3.18	5.58E-10	24h	-2.80	1.25E-08	6h	-3.01	6.81E-10	24h	membrane-spanning 4-domains, subfamily A, member 6A
MSANTD3	2.52	8.79E-13	24h	1.67	3.18E-06	24h	0.39	3.77E-01	24h	Myb/SANT-like DNA-binding domain containing 3
MSC	2.44	2.13E-03	6h	2.30	1.38E-03	24h	1.47	5.06E-02	24h	musculin
MSR1	-3.06	3.47E-08	24h	1.94	4.41E-04	24h	2.09	9.30E-05	6h	macrophage scavenger receptor 1
MSX2	0.10	1.00E+00	1h	-1.78	1.21E-02	24h	-2.66	1.00E-04	24h	msh homeobox 2
MT1M	1.79	1.63E-01	24h	3.56	8.78E-05	24h	2.86	1.79E-03	24h	metallothionein 1M
MUC1	0.26	8.99E-01	6h	3.38	1.99E-09	24h	3.16	1.77E-08	24h	mucin 1, cell surface associated
MUC8	-1.95	2.94E-02	24h	-2.63	9.71E-04	24h	-2.65	5.03E-04	24h	mucin 8
MUCL1	1.72	1.52E-01	24h	3.28	1.02E-04	24h	1.81	4.81E-02	24h	mucin-like 1
MX1	0.05	1.00E+00	1h	5.51	1.46E-50	24h	5.54	6.09E-51	24h	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78
MX2	0.01	1.00E+00	1h	4.33	2.38E-75	24h	4.53	1.30E-82	24h	myxovirus (influenza virus) resistance 2
MYBPH	3.23	1.01E-04	24h	0.45	7.09E-01	24h	1.77	3.30E-02	6h	myosin binding protein H
MYCL	-2.05	1.01E-03	6h	-3.75	2.98E-12	24h	-4.06	7.16E-15	6h	v-myc avian myelocytomatosis viral oncogene lung carcinoma derived homolog
MYO1B	3.86	1.12E-07	24h	3.38	1.66E-06	24h	3.00	1.95E-05	24h	myosin IB
MYO7A	-0.63	6.31E-01	24h	-2.31	4.97E-04	24h	0.08	9.43E-01	6h	myosin VIIA
NAMPT	2.89	2.67E-06	24h	2.32	8.60E-05	6h	2.85	6.14E-07	6h	nicotinamide phosphoribosyltransferase
NAMPTL	2.65	7.21E-04	24h	2.10	4.48E-03	6h	2.65	1.71E-04	6h	nicotinamide phosphoribosyltransferase-like

NDP	5.78	1.28E-12	6h	3.79	2.96E-06	6h	2.56	2.94E-03	6h	Norrie disease (pseudoglioma)
NEFH	2.77	2.05E-03	24h	2.66	1.23E-03	24h	2.09	1.17E-02	24h	neurofilament, heavy polypeptide
NEXN	0.22	1.00E+00	1h	5.17	1.70E-33	24h	5.47	9.36E-38	24h	nexlin (F actin binding protein)
NFAM1	-1.50	3.56E-02	6h	-1.93	6.24E-04	6h	-2.24	4.18E-05	6h	NFAT activating protein with ITAM motif 1
NFKBIA	2.52	2.20E-05	1h	1.98	1.70E-04	6h	1.97	1.51E-04	6h	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
NFKBIZ	2.66	1.49E-04	1h	2.27	1.54E-04	6h	2.91	4.40E-07	6h	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta
NFXL1	-2.07	1.28E-04	6h	-3.16	3.64E-11	24h	-2.69	1.50E-08	24h	nuclear transcription factor, X-box binding-like 1
NGF	2.35	4.41E-02	6h	1.90	5.74E-02	24h	1.25	2.30E-01	24h	nerve growth factor (beta polypeptide)
NHS	-1.82	3.02E-02	6h	-2.28	1.35E-03	24h	-2.01	3.48E-03	6h	Nance-Horan syndrome (congenital cataracts and dental anomalies)
NHSL2	-1.29	1.62E-01	6h	-2.35	2.71E-04	24h	-2.00	1.64E-03	24h	NHS-like 2
NIPAL1	-1.81	1.42E-02	24h	-1.77	8.90E-03	24h	-1.69	8.16E-03	24h	NIPA-like domain containing 1
NKAIN1	0.51	1.00E+00	1h	2.94	6.47E-04	6h	3.87	1.92E-06	6h	Na+/K+ transporting ATPase interacting 1
NLRP12	-0.73	4.44E-01	6h	-2.94	2.16E-09	6h	-3.19	5.86E-11	6h	NLR family, pyrin domain containing 12
NLRP3	2.90	3.39E-04	1h	1.83	1.10E-02	6h	1.88	7.47E-03	24h	NLR family, pyrin domain containing 3
NME8	-1.40	3.63E-03	24h	0.14	8.17E-01	6h	0.12	8.52E-01	6h	NME/NM23 family member 8
NMUR1	-2.00	9.15E-06	24h	-4.18	4.50E-18	24h	-4.06	2.77E-19	24h	neuromedin U receptor 1
NOG	-0.67	3.48E-01	24h	-2.65	2.00E-10	6h	-3.13	2.62E-14	6h	noggin
NOTCH3	3.62	1.42E-05	24h	0.04	1.00E+00	1h	-1.93	2.24E-02	6h	notch 3
NPTX1	-0.38	1.00E+00	1h	-2.53	2.66E-06	24h	-3.07	2.49E-09	6h	neuronal pentraxin I
NRCAM	-2.26	1.26E-02	6h	-3.31	9.68E-06	24h	-2.81	1.50E-04	6h	neuronal cell adhesion molecule
NRG1	0.27	1.00E+00	1h	-4.06	8.33E-08	6h	-4.00	1.40E-07	24h	neuregulin 1
NRIP3	2.54	1.29E-07	6h	1.73	3.32E-04	24h	1.24	1.21E-02	24h	nuclear receptor interacting protein 3
NRXN2	1.95	1.36E-03	6h	2.32	1.09E-05	6h	4.44	9.46E-20	6h	neurexin 2
NT5C3A	0.14	8.91E-01	24h	4.13	1.02E-40	6h	4.26	1.66E-43	6h	5'-nucleotidase, cytosolic IIIA
NT5DC2	1.01	2.67E-01	24h	-2.33	9.73E-05	24h	-3.22	1.28E-08	24h	5'-nucleotidase domain containing 2
OAS1	-1.13	1.49E-02	24h	4.77	4.31E-43	24h	4.58	8.67E-40	24h	2'-5'-oligoadenylate synthetase 1, 40/46kDa
OAS2	0.13	1.00E+00	1h	3.84	5.51E-31	24h	4.14	5.68E-36	24h	2'-5'-oligoadenylate synthetase 2, 69/71kDa
OAS3	0.22	1.00E+00	1h	4.83	5.68E-32	6h	5.32	1.00E-38	24h	2'-5'-oligoadenylate synthetase 3, 100kDa
OASL	-0.24	8.32E-01	24h	4.68	4.90E-31	24h	4.47	2.07E-28	24h	2'-5'-oligoadenylate synthetase-like
OCM	-1.51	3.81E-03	6h	-2.21	1.04E-06	6h	-2.46	3.16E-07	24h	oncomodulin
OCSTAMP	3.01	3.73E-03	6h	1.16	2.82E-01	24h	0.22	1.00E+00	1h	osteoclast stimulatory transmembrane protein
OLFMI	-2.96	1.05E-04	24h	-4.94	1.38E-12	24h	-6.48	5.86E-21	24h	olfactomedin 1
OLFMI2	-0.81	9.90E-04	24h	-2.29	5.61E-25	6h	-2.68	1.42E-30	6h	olfactomedin 2
OLFML3	-3.32	4.51E-05	24h	-2.22	4.86E-03	24h	-2.30	1.92E-03	24h	olfactomedin-like 3
OLIG2	3.06	1.15E-03	24h	2.25	1.17E-02	6h	0.76	4.90E-01	6h	oligodendrocyte lineage transcription factor 2
OR2B11	3.27	2.36E-03	1h	1.64	8.88E-02	6h	1.60	9.00E-02	6h	olfactory receptor, family 2, subfamily B, member 11
OR2W3	-1.56	4.67E-02	24h	-0.50	1.00E+00	1h	0.14	8.71E-01	6h	olfactory receptor, family 2, subfamily W, member 3
OSM	2.55	7.03E-05	6h	1.68	8.09E-03	24h	0.88	1.00E+00	1h	oncostatin M
OTOF	0.09	1.00E+00	1h	5.53	1.47E-20	24h	5.75	2.29E-22	24h	otoferlin
P2RX7	1.34	1.16E-01	6h	3.00	2.16E-07	6h	3.52	4.53E-10	6h	purinergic receptor P2X, ligand-gated ion channel, 7
PACSLIN1	-1.31	2.44E-04	24h	-2.35	2.06E-13	6h	-2.46	3.48E-14	6h	protein kinase C and casein kinase substrate in neurons 1
PALD1	-2.79	2.10E-06	24h	-3.28	4.09E-09	24h	-3.52	7.88E-11	24h	phosphatase domain containing, paladin 1
PAPLN	2.88	1.42E-05	24h	1.85	5.76E-03	24h	0.26	7.66E-01	24h	papilin, proteoglycan-like sulfated glycoprotein
PARP9	0.23	1.00E+00	1h	3.24	4.61E-28	6h	3.40	6.94E-31	24h	poly (ADP-ribose) polymerase family, member 9
PCOLCE	-0.90	1.49E-01	6h	-2.05	1.75E-06	6h	-2.38	3.25E-08	6h	procollagen C-endopeptidase enhancer
PDCD1LG2	0.87	5.54E-01	6h	2.73	8.90E-05	6h	3.80	1.04E-08	6h	programmed cell death 1 ligand 2
PDGFA	1.89	4.71E-03	6h	1.58	8.46E-03	6h	3.48	1.46E-10	24h	platelet-derived growth factor alpha polypeptide
PDGFC	0.34	1.00E+00	1h	-2.40	2.32E-05	24h	-2.37	1.48E-05	24h	platelet derived growth factor C
PDGFR1	1.77	4.53E-02	24h	7.06	3.15E-31	24h	7.27	2.54E-33	24h	platelet-derived growth factor receptor-like
PKA	-4.25	7.11E-10	6h	-3.75	8.94E-09	6h	-4.34	2.47E-11	6h	pyruvate dehydrogenase kinase, isozyme 4
PDPN	3.47	6.20E-09	6h	1.56	1.34E-02	6h	0.95	1.69E-01	6h	podoplanin
PFKFB3	2.32	1.38E-07	6h	2.41	5.33E-09	6h	2.87	1.30E-12	6h	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3
PGAP1	0.26	8.43E-01	24h	3.72	1.18E-17	6h	4.18	1.49E-21	24h	post-GPI attachment to proteins 1
PGBD5	-1.46	4.89E-02	6h	0.35	1.00E+00	1h	0.37	6.29E-01	24h	piggyBac transposable element derived 5
PGLYRP2	-0.12	9.47E-01	24h	-1.56	2.42E-02	6h	-2.33	7.26E-04	6h	peptidoglycan recognition protein 2
PGM5	-2.15	6.35E-04	6h	-1.37	9.06E-03	6h	-1.46	6.62E-03	6h	phosphoglucosyltransferase 5
PHLDA2	2.29	3.43E-03	6h	2.01	4.58E-03	24h	1.35	6.77E-02	24h	pleckstrin homology-like domain, family A, member 2
PHOSPHO1	-1.34	3.83E-02	24h	-3.12	8.23E-09	24h	-3.22	4.13E-10	24h	phosphatase, orphan 1
PI16	-1.34	1.77E-02	24h	-3.24	6.62E-12	6h	-3.21	2.47E-11	6h	peptidase inhibitor 16
PI3	3.49	1.48E-04	24h	2.19	1.78E-02	24h	1.21	2.26E-01	24h	peptidase inhibitor 3, skin-derived
PITPNM2	-1.46	1.12E-05	24h	-2.26	1.55E-13	24h	-2.28	2.85E-14	24h	phosphatidylinositol transfer protein, membrane-associated 2
PLA1A	0.76	1.00E+00	1h	4.45	6.52E-08	24h	4.25	1.94E-07	24h	phospholipase A1 member A
PLAC9	-0.81	3.58E-01	6h	-2.60	4.81E-05	24h	-2.04	4.20E-04	24h	placenta-specific 9
PLAT	2.48	1.74E-05	24h	2.59	1.58E-06	24h	2.39	8.24E-06	24h	plasminogen activator, tissue
PLAUR	2.62	7.35E-05	24h	2.19	4.97E-04	6h	1.68	9.19E-03	24h	plasminogen activator, urokinase receptor
PLB1	-1.70	2.13E-02	24h	-2.14	7.53E-04	24h	-2.09	6.29E-04	24h	phospholipase B1
PLD1	3.31	8.96E-18	24h	1.95	1.09E-06	24h	1.62	5.47E-05	24h	phospholipase D1, phosphatidylcholine-specific
PLEK2	0.34	8.64E-01	6h	-1.57	2.16E-02	6h	-2.56	5.98E-05	24h	pleckstrin 2
PLEKHA6	-1.46	4.42E-02	24h	0.31	6.80E-01	6h	0.76	2.16E-01	6h	pleckstrin homology domain containing, family A member 6
PLEKHG7	0.76	1.00E+00	1h	3.38	1.12E-10	6h	3.73	5.13E-13	6h	pleckstrin homology domain containing, family G (with RhoGef domain) member 7
PLP	-0.98	3.04E-02	24h	-3.23	3.48E-17	6h	-3.74	5.95E-18	24h	plasmolipin
PLSCR1	1.53	3.47E-08	24h	3.43	4.83E-44	6h	3.72	1.15E-51	24h	phospholipid scramblase 1
PLSCR4	0.63	7.52E-01	6h	4.32	4.43E-12	6h	4.78	7.36E-15	6h	phospholipid scramblase 4
PML	-0.49	2.27E-01	24h	3.19	3.05E-39	6h	3.38	2.69E-44	6h	promyelocytic leukemia
PMP22	2.19	2.55E-02	24h	0.21	1.00E+00	1h	-2.26	6.38E-03	6h	peripheral myelin protein 22
PNPLA1	3.09	4.06E-12	6h	2.09	2.10E-06	6h	1.17	1.47E-02	6h	patatin-like phospholipase domain containing 1
PNPT1	0.41	6.07E-01	24h	3.52	5.14E-23	6h	3.56	1.09E-23	6h	polyribonucleotide nucleotidyltransferase 1
PODN	-1.42	4.49E-02	24h	-1.72	7.16E-03	24h	-2.07	1.61E-04	6h	podocan
PP13439	2.46	3.72E-03	24h	1.24	1.90E-01	24h	1.35	1.20E-01	24h	
PPAP2B	3.01	4.98E-07	24h	1.01	1.44E-01	24h	0.64	3.87E-01	6h	phosphatidic acid phosphatase type 2B
PPBP	3.13	9.13E-04	6h	0.46	7.06E-01	6h	0.10	1.00E+00	1h	pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)
PPFIA4	-1.63	1.68E-02	24h	-3.21	1.68E-07	24h	-2.83	9.58E-07	24h	protein tyrosine phosphatase, receptor type, f polypeptide, interacting protein, alpha 4
PRAM1	-1.21	2.01E-01	6h	-2.58	2.51E-05	6h	-2.86	1.85E-06	6h	PML-RARA regulated adaptor molecule 1
PRLR	1.48	8.61E-02	24h	4.02	3.30E-11	24h	4.46	6.00E-14	24h	prolactin receptor
PROC	-3.03	1.12E-07	24h	-2.77	1.04E-06	24h	-3.43	6.24E-10	24h	protein C (inactivator of coagulation factors Va and VIIIa)
PRR16	2.57	1.57E-04	6h	2.06	1.24E-03	6h	1.83	4.30E-03	6h	proline rich 16
PRRT1	-0.92	9.44E-02	6h	-1.83	4.89E-06	6h	-2.48	8.43E-10	24h	proline-rich transmembrane protein 1
PRSS23	-1.66	1.57E-08	24h	-2.42	2.98E-16	24h	-2.50	4.64E-19	24h	protease, serine, 23
PRSS36	-1.22	1.42E-01	24h	-2.55	3.28E-05	24h	-3.14	7.70E-08	24h	protease, serine, 36
PRUNE2	-1.64	3.22E-03	24h	0.45	1.00E+00	1h	0.17	7.85E-01	24h	prune homolog 2 (Drosophila)
PSRC1	-1.41	3.95E-03	6h	-2.75	1.72E-09	24h	-3.31	2.91E-13	24h	proline/serine-rich coiled-coil 1
PTGDR	0.04	1.00E+00	1h	-2.17	1.28E-05	24h	-2.35	5.77E-07	24h	prostaglandin D2 receptor (DP)
PTGES	2.92	5.57E-05	24h	1.50	5.27E-02	24h	0.41	1.00E+00	1h	prostaglandin E synthase
PTGFRN	-2.61	1.54E-03	6h	-5.04	1.72E-13	6h	-5.21	1.78E-14	6h	prostaglandin F2 receptor inhibitor
PTGS2	3.84	1.88E-05	1h	3.66	2.26E-06	6h	3.40	1.13E-05	24h	prostaglandin-endoperoxide synthase 2
PTPRU	0.41	7.87E-01	24h	3.47	9.75E-10	24h	3.41	8.38E-10	24h	protein tyrosine phosphatase, receptor type, U
PTRF	-1.81	1.51E-02	6h	0.26	1.00E+00	1h	-0.88	2.12E-01	24h	polymerase I and transcript release factor
PTX3	2.71	5.97E-03	6h	2.95	4.96E-04	6h	5.22	2.91E-11	6h	pentraxin 3, long

PVRL4	2.93	5.58E-04	24h	1.04	2.82E-01	24h	0.14	9.04E-01	24h	poliovirus receptor-related 4
RAB37	-1.43	1.58E-03	24h	-2.04	2.61E-07	24h	-2.24	6.68E-09	24h	RAB37, member RAS oncogene family
RAB3A	0.35	1.00E+00	1h	-2.16	1.42E-07	6h	-1.87	8.41E-06	24h	RAB3A, member RAS oncogene family
RAB3C	0.05	1.00E+00	1h	-2.26	1.50E-02	24h	-2.30	1.02E-02	24h	RAB3C, member RAS oncogene family
RAB3D	0.18	8.78E-01	24h	-2.26	2.99E-08	6h	-2.14	1.52E-07	6h	RAB3D, member RAS oncogene family
RAB31L1	0.32	9.00E-01	6h	-2.38	2.09E-03	24h	-1.64	3.57E-02	24h	RAB3A interacting protein (rabin3)-like 1
RAB42	-0.29	9.09E-01	6h	-1.68	3.59E-02	24h	-2.41	9.76E-04	24h	RAB42, member RAS oncogene family
RAPGEF2	0.83	2.35E-01	24h	2.48	2.45E-08	6h	3.58	6.00E-17	24h	Rap guanine nucleotide exchange factor (GEF) 2
RASAL1	-1.15	3.81E-01	24h	-2.67	7.77E-04	6h	-3.16	3.97E-05	6h	RAS protein activator like 1 (GAP1 like)
RBMS2	-1.54	9.65E-03	6h	0.31	6.56E-01	24h	0.23	7.26E-01	24h	RNA binding motif, single stranded interacting protein 2
RBP7	-2.45	6.20E-04	6h	-2.85	6.91E-06	6h	-3.28	2.02E-07	6h	retinol binding protein 7, cellular
RCBTB2	-1.66	1.91E-04	6h	-2.07	1.61E-07	6h	-2.36	1.22E-09	6h	regulator of chromosome condensation and BTB domain containing protein 2
RCN3	-1.17	1.90E-01	6h	-2.87	6.30E-07	6h	-2.94	2.52E-07	6h	reticulocalbin 3, EF-hand calcium binding domain
REPS2	-1.18	5.23E-04	24h	-2.20	8.63E-12	24h	-1.91	1.18E-10	6h	RALBP1 associated Eps domain containing 2
RET	0.60	6.92E-01	6h	2.40	1.19E-04	24h	3.44	1.77E-09	24h	ret proto-oncogene
RGS1	1.25	4.02E-02	24h	4.29	3.89E-22	6h	4.18	5.05E-21	24h	regulator of G-protein signaling 1
RGS14	0.00	1.00E+00	1h	-2.44	2.43E-08	6h	-2.72	2.38E-10	6h	regulator of G-protein signaling 14
RGS16	2.84	4.07E-12	6h	3.04	3.12E-15	6h	3.38	7.78E-19	6h	regulator of G-protein signaling 16
RGS18	-1.47	2.61E-03	6h	-2.25	2.21E-07	24h	-1.75	4.20E-05	24h	regulator of G-protein signaling 18
RHBDL1	-1.40	1.22E-02	24h	-1.25	7.04E-03	6h	-1.20	1.37E-02	24h	rhomboid, veinlet-like 1 (Drosophila)
RHOBTB1	-1.91	3.81E-03	24h	-1.72	5.68E-03	24h	-2.01	5.61E-04	24h	Rho-related BTB domain containing 1
RIMBP2	-2.26	4.29E-02	24h	0.11	1.00E+00	1h	-2.23	1.58E-02	24h	RIMS binding protein 2
RIMBP3	-1.42	1.62E-02	24h	-2.15	1.51E-04	24h	-2.05	7.66E-05	24h	RIMS binding protein 3
RIN2	2.39	7.67E-05	6h	3.45	6.17E-11	6h	3.98	1.88E-14	6h	Ras and Rab interactor 2
RIPK2	1.22	4.97E-02	6h	3.29	1.16E-13	6h	2.96	2.99E-11	6h	receptor-interacting serine-threonine kinase 2
RNASE1	-3.98	1.81E-05	24h	-4.60	7.90E-08	24h	-5.06	1.59E-09	24h	ribonuclease, RNase A family, 1 (pancreatic)
RNASE6	-2.04	1.10E-03	6h	-3.58	1.08E-11	6h	-3.86	1.77E-13	24h	ribonuclease, RNase A family, k6
RNF152	0.23	1.00E+00	1h	3.91	4.64E-12	6h	4.18	7.06E-14	6h	ring finger protein 152
RPGRIP1	-1.48	3.49E-02	6h	-1.86	2.96E-03	24h	-2.08	2.64E-04	6h	retinitis pigmentosa GTPase regulator interacting protein 1
RPH3A	-2.90	3.53E-04	6h	-2.31	2.01E-03	6h	-2.26	2.38E-03	6h	rabphilin 3A homolog (mouse)
RSAD2	0.40	1.00E+00	1h	6.71	6.22E-54	24h	6.90	4.43E-57	24h	radical S-adenosyl methionine domain containing 2
RSP03	0.50	8.29E-01	24h	3.46	9.90E-05	6h	1.73	8.29E-02	6h	R-spondin 3
RTN4R	-1.02	1.02E-01	24h	-2.62	1.97E-08	6h	-2.48	1.09E-07	24h	reticulon 4 receptor
RTP4	0.04	9.77E-01	6h	3.63	9.30E-29	6h	3.72	3.43E-30	6h	receptor (chemosensory) transporter protein 4
RUFY4	-1.87	4.62E-02	24h	2.69	2.73E-04	24h	2.43	9.45E-04	24h	RUN and FYVE domain containing 4
RXFPI1	0.67	1.00E+00	1h	3.29	2.77E-04	24h	2.91	1.20E-03	24h	relaxin/insulin-like family peptide receptor 1
S100B	2.38	2.78E-03	24h	-0.22	1.00E+00	1h	-0.63	4.75E-01	24h	S100 calcium binding protein B
S100Z	-1.82	8.44E-03	6h	-2.39	2.79E-04	24h	-2.64	2.23E-05	6h	S100 calcium binding protein Z
SAMD4A	0.17	1.00E+00	1h	3.72	1.14E-16	6h	4.42	1.34E-23	6h	sterile alpha motif domain containing 4A
SAMD9	0.12	1.00E+00	1h	3.28	4.70E-20	6h	3.63	2.21E-24	24h	sterile alpha motif domain containing 9
SAMD9L	0.29	1.00E+00	1h	4.01	1.36E-30	6h	4.31	2.14E-35	6h	sterile alpha motif domain containing 9-like
SBSN	2.80	5.99E-03	6h	2.22	1.60E-02	6h	2.14	1.95E-02	6h	suprabasin
SCARF2	-1.43	2.93E-03	24h	-1.41	1.39E-03	6h	-1.97	2.88E-06	24h	scavenger receptor class F, member 2
SCG3	0.78	6.41E-01	24h	3.25	3.50E-05	24h	1.96	1.80E-02	24h	secretogranin III
SCG5	3.00	1.03E-04	6h	1.21	1.90E-01	24h	0.44	6.67E-01	24h	secretogranin V (7B2 protein)
SCNA4	-1.33	1.05E-01	24h	-2.29	9.61E-04	24h	-2.02	8.55E-04	6h	sodium channel, voltage-gated, type IV, alpha subunit
SCN4B	0.36	1.00E+00	1h	-2.21	5.50E-03	24h	-2.59	5.77E-04	24h	sodium channel, voltage-gated, type IV, beta subunit
SCNN1A	-1.87	1.37E-05	24h	-1.99	3.69E-06	24h	-2.20	5.48E-08	24h	sodium channel, non-voltage-gated 1 alpha subunit
SDC2	2.68	6.61E-08	24h	0.86	1.27E-01	6h	1.34	8.05E-03	6h	syndecan 2
SDK2	-0.93	4.64E-01	24h	-2.25	2.61E-03	24h	-1.86	1.25E-02	24h	sidekick cell adhesion molecule 2
SEMA3C	2.85	1.50E-08	24h	1.59	2.44E-03	24h	2.97	3.26E-10	24h	sema domain, Ig domain, short basic domain, secreted, (semaphorin) 3C
SEMA3F	2.46	1.70E-03	6h	1.96	6.62E-03	6h	1.02	2.47E-01	24h	sema domain, Ig domain, short basic domain, secreted, (semaphorin) 3F
SEMA4C	-0.37	6.91E-01	6h	-2.79	6.27E-15	24h	-2.97	3.83E-17	24h	sema domain, Ig domain, TM domain, and short cytoplasmic domain, (semaphorin) 4C
SEMA6C	-1.09	7.81E-03	24h	-2.68	1.28E-12	24h	-2.47	1.81E-12	24h	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6C
SEPP1	-3.75	9.99E-07	24h	-3.80	2.77E-07	24h	-3.17	1.12E-05	24h	selenoprotein P, plasma, 1
SEPT4	-1.45	8.25E-02	24h	2.41	5.07E-05	24h	1.68	5.32E-03	6h	septin 4
SERPINA9	2.95	2.36E-03	6h	1.49	1.00E+00	1h	-0.50	6.63E-01	24h	serpin peptidase inhibitor, clade A (alpha-1 antitrypsinase, antitrypsin), member 9
SERPINB2	3.53	1.96E-04	6h	3.35	1.08E-04	6h	2.11	2.13E-02	6h	serpin peptidase inhibitor, clade B (ovalbumin), member 2
SERPINB7	5.07	6.60E-10	24h	5.56	9.77E-13	24h	3.47	2.18E-05	24h	serpin peptidase inhibitor, clade B (ovalbumin), member 7
SERPINB9	2.41	1.06E-05	6h	3.08	3.48E-10	6h	3.18	6.35E-11	6h	serpin peptidase inhibitor, clade B (ovalbumin), member 9
SERPINE1	1.68	1.83E-01	6h	-1.43	1.48E-01	24h	-2.40	5.44E-03	24h	serpin peptidase inhibitor, clade E, member 1
SERPINF1	-2.52	6.38E-21	24h	-2.76	7.61E-25	24h	-3.14	4.47E-33	24h	serpin peptidase inhibitor, clade F, member 1
SERPINF2	-2.19	3.01E-05	24h	-2.86	8.54E-09	6h	-2.56	2.46E-07	6h	serpin peptidase inhibitor, clade F, member 2
SERPINF1	-2.17	4.12E-03	24h	4.50	2.62E-13	24h	4.05	5.40E-11	24h	serpin peptidase inhibitor, clade G (C1 inhibitor), member 1
SEZBL	-1.72	3.76E-03	24h	0.33	1.00E+00	1h	-1.96	4.41E-04	6h	seizure related 6 homolog (mouse)-like
SFRP5	-1.55	1.39E-03	24h	-3.16	4.34E-12	24h	-4.22	3.96E-20	24h	secreted frizzled-related protein 5
SGPP2	2.86	1.32E-03	6h	2.60	1.24E-03	6h	1.30	1.50E-01	6h	sphingosine-1-phosphate phosphatase 2
SH3BP4	-1.91	1.97E-05	24h	-1.96	5.52E-06	24h	-1.74	2.88E-05	24h	SH3-domain binding protein 4
SH3PXD2B	5.42	3.02E-17	24h	2.90	1.53E-05	24h	3.24	6.07E-07	24h	SH3 and PX domains 2B
SIAH3	0.32	1.00E+00	1h	-2.95	1.12E-05	24h	-2.73	4.72E-05	6h	siah E3 ubiquitin protein ligase family member 3
SIGIRR	-1.13	1.36E-02	24h	-2.09	1.23E-08	6h	-2.43	1.58E-11	24h	single immunoglobulin and toll-interleukin 1 receptor (TIR) domain
SIGLEC1	-3.84	1.82E-10	24h	3.14	5.36E-08	6h	3.59	2.13E-10	6h	sialic acid binding Ig-like lectin 1, sialoadhesin
SIGLEC11	0.05	1.00E+00	1h	-2.55	3.60E-03	24h	-2.52	2.82E-03	24h	sialic acid binding Ig-like lectin 11
SIGLEC7	0.66	6.29E-01	24h	-2.34	5.05E-04	6h	0.37	1.00E+00	1h	sialic acid binding Ig-like lectin 7
SIRPB2	0.29	8.98E-01	24h	-2.41	3.74E-03	6h	-2.48	2.38E-03	24h	signal-regulatory protein beta 2
SLAMF7	1.74	8.95E-04	6h	3.96	2.72E-20	6h	4.30	6.58E-24	6h	SLAM family member 7
SLAMF9	2.89	9.68E-04	24h	1.42	1.28E-01	24h	0.19	8.77E-01	24h	SLAM family member 9
SLC11A1	2.81	8.05E-04	24h	0.22	8.62E-01	24h	-0.68	4.94E-01	6h	solute carrier family 11 (proton-coupled divalent metal ion transporter), member 1
SLC16A10	3.26	6.75E-08	24h	2.01	8.82E-04	6h	1.73	4.68E-03	6h	solute carrier family 16 (aromatic amino acid transporter), member 10
SLC16A6	1.22	3.95E-02	24h	-1.08	3.64E-02	6h	-2.55	2.07E-08	6h	solute carrier family 16, member 6
SLC1A2	1.90	1.39E-02	24h	3.46	2.50E-08	24h	2.64	2.88E-05	24h	solute carrier family 1 (glial high affinity glutamate transporter), member 2
SLC1A7	-1.78	7.69E-02	24h	-1.93	1.40E-02	6h	-2.13	7.21E-03	24h	solute carrier family 2 (glutamate transporter), member 7
SLC2A12	0.35	1.00E+00	1h	3.86	5.55E-08	6h	3.97	1.73E-08	6h	solute carrier family 2 (facilitated glucose transporter), member 12
SLC2A4	-1.93	1.12E-02	6h	-1.60	2.88E-02	24h	0.80	1.00E+00	1h	solute carrier family 2 (facilitated glucose transporter), member 4
SLC2A9	-1.78	1.48E-03	24h	-1.31	1.63E-02	24h	-1.76	4.32E-04	24h	solute carrier family 2 (facilitated glucose transporter), member 9
SLC37A2	-1.65	4.08E-02	24h	-2.54	8.11E-05	24h	-2.26	4.32E-04	6h	solute carrier family 37 (glucose-6-phosphate transporter), member 2
SLC38A5	0.12	8.92E-01	6h	3.61	5.92E-54	24h	3.42	7.03E-49	6h	solute carrier family 38, member 5
SLC46A1	-1.88	6.82E-03	24h	-3.48	1.90E-08	24h	-3.25	3.71E-08	24h	solute carrier family 46 (folate transporter), member 1
SLC46A2	-2.28	3.77E-02	6h	-3.48	3.47E-05	6h	-2.37	6.64E-03	24h	solute carrier family 46, member 2
SLC4A3	-1.75	1.82E-02	24h	-2.11	1.90E-03	24h	-2.38	1.92E-04	24h	solute carrier family 4 (anion exchanger), member 3
SLC7A11	3.01	6.97E-07	24h	1.40	2.82E-02	6h	0.29	7.18E-01	24h	solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11
SLC7A8	0.31	1.00E+00	1h	-3.01	4.00E-04	24h	-3.82	2.20E-06	24h	solute carrier family 7 (amino acid transporter light chain, L system), member 8
SLCO2B1	0.88	6.88E-01	6h	-2.89	1.84E-03	24h	-3.57	5.01E-05	24h	solute carrier organic anion transporter family, member 2B1
SLCO4A1	2.90	3.83E-07	6h	2.43	8.65E-06	6h	4.07	2.59E-15	6h	solute carrier organic anion transporter family, member 4A1
SLITRK4	0.36	1.00E+00	1h	-2.13	3.38E-03	24h	0.36	1.00E+00	1h	SLIT and NTRK-like family, member 4
SLITRK5	0.04	1.00E+00	1h	-1.71	1.90E-02	6h	-2.65	4.04E-04	6h	SLIT and NTRK-like family, member 5
SMAD6	-1.65	4.45E-02	6h	-3.13	7.21E-07	6h	-3.21	2.81E-07	24h	SMAD family member 6
SMCO2	0.94	6.37E-01	6h	4.19	4.09E-07	24h	3.44	3.52E-05	24h	single-pass membrane protein with coiled-coil domains 2

SMPDL3A	3.31	1.39E-07	24h	1.77	6.31E-03	6h	3.00	4.24E-07	6h	sphingomyelin phosphodiesterase, acid-like 3A
SMTNL2	3.77	3.70E-06	24h	2.97	3.04E-04	24h	3.12	6.97E-05	24h	smoothenin-like 2
SNAI1	3.44	7.22E-08	6h	2.17	6.77E-04	6h	2.23	3.98E-04	6h	snail family zinc finger 1
SOAT2	0.08	1.00E+00	1h	-1.26	2.76E-02	6h	-2.57	1.78E-05	6h	sterol O-acetyltransferase 2
SOBP	0.73	4.45E-01	24h	4.56	2.27E-24	24h	4.14	1.74E-20	24h	sine oculis binding protein homolog (Drosophila)
SOC51	1.82	7.56E-05	6h	4.80	1.20E-35	24h	4.70	2.56E-34	6h	suppressor of cytokine signaling 1
SOC53	3.42	3.59E-11	6h	4.07	3.06E-17	6h	4.50	4.14E-21	6h	suppressor of cytokine signaling 3
SOD2	2.77	4.85E-08	6h	2.96	4.26E-10	6h	3.04	1.04E-10	6h	superoxide dismutase 2, mitochondrial
SORL1	-1.23	3.97E-02	6h	-2.18	1.46E-06	24h	-1.80	7.70E-05	6h	sortilin-related receptor, L(DLR class) A repeats containing
SOWAHD	-1.23	6.44E-01	1h	-2.59	1.82E-06	6h	-2.09	1.11E-04	24h	soyondowah ankyrin repeat domain family member D
SOX5	2.68	9.20E-03	24h	2.09	2.96E-02	24h	3.12	3.49E-04	24h	SRY (sex determining region Y)-box 5
SPAG6	0.92	1.00E+00	1h	3.72	2.25E-11	6h	3.76	1.32E-11	6h	sperm associated antigen 6
SPAG8	0.09	1.00E+00	1h	-1.94	2.24E-05	6h	-2.32	3.61E-07	24h	sperm associated antigen 8
SPATS2L	0.74	5.85E-02	6h	3.99	8.52E-52	6h	4.30	2.78E-60	6h	spermatogenesis associated, serine-rich 2-like
SPINK1	3.82	1.78E-09	24h	2.34	4.25E-04	24h	0.48	5.37E-01	6h	serine peptidase inhibitor, Kazal type 1
SP1	2.38	1.19E-02	6h	2.19	7.62E-03	6h	2.28	4.48E-03	6h	secreted phosphoprotein 1
SRPX	1.56	2.14E-01	24h	1.10	2.95E-01	6h	3.53	1.78E-05	6h	sushi-repeat containing protein, X-linked
SSTR2	1.95	7.59E-02	24h	6.07	3.34E-15	24h	5.34	5.30E-12	24h	somatostatin receptor 2
STAB1	-1.05	5.23E-01	6h	-3.65	6.03E-06	24h	-3.28	4.38E-05	24h	stabilin 1
STAC3	-1.61	3.76E-03	24h	0.43	1.00E+00	1h	0.18	1.00E+00	1h	SH3 and cysteine rich domain 3
STARD13	0.24	1.00E+00	1h	-2.20	6.86E-04	24h	-2.17	4.69E-04	24h	StAR-related lipid transfer (START) domain containing 13
STEAP1B	3.04	8.97E-04	24h	3.44	3.44E-05	24h	4.35	3.30E-08	24h	STEAP family member 1B
STEAP3	2.64	1.17E-06	6h	0.66	3.23E-01	24h	1.45	7.74E-03	6h	STEAP family member 3, metalloredutase
STON2	2.25	1.12E-02	24h	1.70	4.19E-02	24h	3.52	1.07E-06	24h	stonin 2
STXBP1	0.12	1.00E+00	1h	-1.91	3.50E-04	24h	-2.35	1.84E-06	6h	syntaxin binding protein 1
SUCNR1	2.27	6.80E-03	6h	3.18	4.85E-06	6h	5.39	1.80E-16	6h	succinate receptor 1
SVEP1	1.29	3.51E-01	24h	4.67	5.37E-09	24h	3.86	1.61E-06	24h	sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1
SYNPO2	1.96	4.05E-01	1h	3.09	1.85E-04	24h	4.07	2.09E-07	24h	synaptopodin 2
TBC1D30	2.44	3.70E-12	24h	1.35	1.54E-04	6h	1.52	1.50E-05	24h	TBC1 domain family, member 30
TBXAS1	0.29	1.00E+00	1h	-2.63	2.27E-05	24h	-3.01	5.07E-07	24h	thromboxane A synthase 1 (platelet)
TCEA3	-1.08	2.40E-02	24h	-2.29	2.88E-09	6h	-2.69	5.18E-12	6h	transcription elongation factor A (SII), 3
TCN2	-2.03	2.02E-03	24h	1.43	2.28E-02	24h	1.22	5.01E-02	24h	transcobalamin II
TDRD6	2.31	1.65E-07	24h	0.79	1.08E-01	6h	-0.25	6.92E-01	6h	tudor domain containing 6
TENM1	-1.42	8.81E-02	6h	-1.57	1.70E-02	24h	0.01	1.00E+00	1h	teneurin transmembrane protein 1
TET1	0.39	1.00E+00	1h	0.60	1.00E+00	1h	-2.73	8.88E-05	6h	tet methylcytosine dioxygenase 1
TFCP2L1	-1.18	4.63E-02	24h	-2.64	1.16E-06	24h	-2.87	7.45E-10	6h	transcription factor CP2-like 1
TFPI2	5.15	1.26E-14	6h	3.47	1.81E-07	6h	1.91	7.89E-03	6h	tissue factor pathway inhibitor 2
TFRC	1.77	1.91E-04	24h	1.70	1.13E-04	6h	3.46	1.57E-17	6h	transferrin receptor
TGFBI	0.11	1.00E+00	1h	-1.93	1.12E-02	24h	-2.44	6.61E-04	24h	transforming growth factor, beta-induced, 68kDa
TGM3	3.47	2.78E-05	6h	1.67	5.25E-02	6h	1.69	4.80E-02	24h	transglutaminase 3
TGM5	-2.86	1.26E-03	24h	-3.95	1.26E-06	24h	-4.46	2.13E-08	24h	transglutaminase 5
THAP2	2.40	6.86E-06	1h	0.96	7.32E-02	6h	0.87	1.06E-01	6h	THAP domain containing, apoptosis associated protein 2
TIMD4	-1.56	2.32E-03	24h	0.91	2.58E-02	6h	1.45	9.82E-05	6h	T-cell immunoglobulin and mucin domain containing 4
TJP1	0.43	8.70E-01	6h	3.04	1.51E-04	6h	4.69	4.45E-10	6h	tight junction protein 1
TLR5	0.25	1.00E+00	1h	-2.14	3.09E-06	24h	-1.47	1.10E-03	24h	toll-like receptor 5
TLR7	-3.07	1.21E-12	24h	1.73	5.45E-05	6h	2.07	6.31E-07	6h	toll-like receptor 7
TM4SF1	2.58	9.18E-03	6h	2.66	1.67E-03	6h	1.35	1.65E-01	6h	transmembrane 4 L six family member 1
TM4SF19	2.51	1.58E-06	24h	0.61	3.94E-01	24h	-1.08	5.61E-02	6h	transmembrane 4 L six family member 19
TM6SF1	-1.43	2.06E-03	6h	-2.61	6.08E-11	24h	-2.77	6.87E-13	24h	transmembrane 6 superfamily member 1
TMCR	-1.38	3.57E-06	24h	-1.92	1.09E-12	6h	-2.30	5.60E-18	6h	transmembrane channel-like 8
TMEM132A	2.38	1.90E-04	24h	1.94	1.61E-03	24h	0.64	3.80E-01	24h	transmembrane protein 132A
TMEM132C	-0.98	4.65E-01	24h	-1.91	3.00E-02	24h	-2.60	8.42E-04	6h	transmembrane protein 132C
TMEM132E	0.02	1.00E+00	1h	-2.94	3.70E-05	6h	-3.86	9.99E-08	24h	transmembrane protein 132E
TMEM144	-2.50	5.76E-07	6h	-2.04	1.05E-05	6h	-2.48	1.01E-07	6h	transmembrane protein 144
TMEM170B	-1.80	1.97E-04	6h	-2.44	7.73E-09	6h	-2.39	1.46E-08	6h	transmembrane protein 170B
TMEM171	-1.61	7.62E-02	24h	2.08	1.24E-03	24h	1.78	5.48E-03	6h	transmembrane protein 171
TMEM217	1.15	1.89E-01	24h	3.68	4.17E-13	6h	4.31	5.74E-16	24h	transmembrane protein 217
TMEM255A	1.93	9.08E-03	6h	3.52	1.02E-09	24h	3.19	2.93E-08	24h	transmembrane protein 255A
TMEM37	-1.74	1.74E-01	24h	-2.44	1.06E-02	24h	0.36	7.92E-01	6h	transmembrane protein 37
TMEM45B	-0.43	7.77E-01	6h	-2.14	9.53E-05	6h	-2.32	1.72E-05	6h	transmembrane protein 45B
TMEM52B	0.71	1.00E+00	1h	-2.05	2.87E-03	24h	-2.61	5.65E-05	24h	transmembrane protein 52B
TMEM54	1.78	6.06E-01	1h	3.08	2.90E-05	6h	4.37	1.48E-09	24h	transmembrane protein 54
TMEM63C	-1.40	6.12E-03	6h	-1.08	1.58E-02	6h	-1.42	1.38E-03	6h	transmembrane protein 63C
TMEM86A	-1.43	1.23E-02	6h	-2.53	6.31E-08	24h	-2.96	7.27E-11	6h	transmembrane protein 86A
TMEM92	-1.15	1.00E+00	1h	-1.59	3.28E-02	24h	-2.58	2.08E-04	24h	transmembrane protein 92
TMIE	-1.43	2.16E-02	24h	-1.72	2.83E-03	24h	-1.83	5.50E-04	6h	transmembrane inner ear
TNF	2.59	3.98E-02	1h	4.04	2.65E-07	6h	3.82	1.03E-06	6h	tumor necrosis factor
TNFAIP6	3.68	1.15E-07	6h	4.54	1.21E-12	6h	4.27	2.38E-11	6h	tumor necrosis factor, alpha-induced protein 6
TNFRSF10C	-1.45	3.76E-02	6h	-2.21	1.32E-04	24h	-1.65	3.08E-03	24h	tumor necrosis factor receptor superfamily, member 10c
TNFRSF11A	-2.66	1.27E-10	6h	-2.56	5.46E-11	6h	-3.26	5.48E-17	6h	tumor necrosis factor receptor superfamily, member 11a
TNFSF10	0.15	1.00E+00	1h	4.31	1.00E-24	6h	4.71	1.35E-29	6h	tumor necrosis factor (ligand) superfamily, member 10
TNFSF15	3.19	5.74E-08	6h	3.53	1.16E-10	6h	3.09	2.12E-08	6h	tumor necrosis factor (ligand) superfamily, member 15
TNFSF9	1.85	1.78E-03	24h	3.42	1.07E-12	6h	4.53	3.80E-22	6h	tumor necrosis factor (ligand) superfamily, member 9
TNIP3	6.17	4.33E-30	6h	6.34	1.02E-32	6h	7.88	1.16E-50	6h	TNFAIP3 interacting protein 3
TNNT1	0.09	1.00E+00	1h	-2.14	1.18E-03	6h	-2.71	1.65E-05	24h	troponin T type 1 (skeletal, slow)
TPBGL	0.52	7.52E-01	6h	-1.76	1.95E-02	6h	-2.67	2.60E-04	24h	trophoblast glycoprotein-like
TPCN1	0.16	1.00E+00	1h	-2.31	4.42E-08	24h	-2.49	1.83E-09	24h	two pore segment channel 1
TPM2	-1.77	1.94E-05	24h	-1.67	2.30E-05	24h	-2.29	9.53E-10	24h	tropomyosin 2 (beta)
TREM2	-2.68	1.09E-02	24h	-2.94	1.19E-03	6h	-3.13	4.42E-04	24h	triggering receptor expressed on myeloid cells 2
TRIM22	0.15	1.00E+00	1h	3.23	5.95E-22	6h	3.48	1.32E-25	6h	tripartite motif containing 22
TRPV4	0.29	1.00E+00	1h	-2.26	3.83E-03	24h	0.42	1.00E+00	1h	transient receptor potential cation channel, subfamily V, member 4
TSPAN32	-1.57	9.39E-04	24h	-2.04	1.07E-06	6h	-2.43	2.94E-09	6h	tetraspanin 32
TSPAN7	2.47	6.30E-03	24h	0.99	1.00E+00	1h	-0.59	1.00E+00	1h	tetraspanin 7
TTC16	-1.40	1.72E-02	24h	-1.41	8.42E-03	24h	-1.63	1.06E-03	24h	tetratricopeptide repeat domain 16
TXN	1.99	1.64E-03	24h	4.07	1.15E-14	24h	3.42	1.26E-10	24h	thioredoxin
UNC5B	0.35	1.00E+00	1h	-2.61	7.33E-05	24h	-2.54	5.86E-05	24h	unc-5 homolog B (C. elegans)
UNC5C	1.00	1.00E+00	1h	4.22	2.01E-08	6h	4.27	1.08E-08	6h	unc-5 homolog C (C. elegans)
UPB1	2.70	1.31E-07	6h	2.79	7.40E-09	24h	2.22	5.24E-06	24h	ureidopropionase, beta
USP18	0.41	1.00E+00	1h	6.26	1.94E-83	24h	6.28	9.82E-84	24h	ubiquitin specific peptidase 18
USP41	0.31	8.98E-01	24h	4.30	1.00E-08	24h	4.73	8.40E-11	24h	ubiquitin specific peptidase 41
VCAN	1.09	4.55E-01	24h	-2.27	9.28E-03	6h	-2.21	1.04E-02	6h	versican
VIPR1	-1.97	4.46E-07	24h	-2.76	2.55E-14	24h	-3.33	4.94E-21	24h	vasoactive intestinal peptide receptor 1
VNN3	2.41	3.50E-03	24h	1.53	5.50E-02	6h	2.09	5.06E-03	24h	vanin 3
VSIG1	-0.84	7.55E-02	24h	-2.06	1.12E-08	24h	-2.51	2.85E-13	6h	V-set and immunoglobulin domain containing 1
VSIG4	0.21	1.00E+00	1h	-2.90	1.95E-03	24h	-2.43	9.36E-03	24h	V-set and immunoglobulin domain containing 4
WARS	0.45	6.00E-01	6h	3.27	3.24E-20	6h	3.33	5.84E-21	6h	tryptophanyl-tRNA synthetase
WNK2	2.34	3.15E-06	24h	1.88	1.24E-04	6h	2.25	1.79E-06	24h	WNK lysine deficient protein kinase 2
WNT5A	3.62	9.74E-08	6h	3.40	1.26E-07	6h	1.34	6.75E-02	24h	wingless-type MMTV integration site family, member 5A

WNT7A	-1.70	2.24E-05	24h	-3.14	4.21E-18	6h	-4.23	4.97E-24	24h	wingless-type MMTV integration site family, member 7A
XAF1	0.13	1.00E+00	1h	3.97	1.65E-27	24h	4.18	1.10E-30	24h	XIAP associated factor 1
XIRP1	1.43	1.00E+00	1h	3.28	5.27E-05	6h	3.63	4.70E-06	6h	xin actin-binding repeat containing 1
YPEL4	-1.60	1.60E-06	6h	-2.51	1.09E-16	6h	-3.73	1.34E-32	6h	yippee-like 4 (Drosophila)
ZBP1	-0.69	4.95E-04	24h	3.31	1.54E-99	24h	3.00	1.86E-82	24h	Z-DNA binding protein 1
ZBTB32	0.28	8.03E-01	24h	3.37	6.88E-17	24h	2.77	1.04E-11	24h	zinc finger and BTB domain containing 32
ZC3H12C	2.30	3.67E-05	24h	2.55	5.67E-07	6h	2.65	1.68E-07	24h	zinc finger CCCH-type containing 12C
ZNF467	-1.98	2.13E-02	6h	-2.07	3.92E-03	6h	-1.74	1.71E-02	6h	zinc finger protein 467
ZNF503	-1.90	7.79E-04	6h	-1.96	1.02E-04	6h	-1.17	2.97E-02	6h	zinc finger protein 503

References

- Ablasser, A., F. Bauernfeind, G. Hartmann, E. Latz, K.A. Fitzgerald, and V. Hornung. 2009. RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nat Immunol* 10:1065-1072.
- Altare, F., A. Durandy, D. Lammas, J.F. Emile, S. Lamhamedi, F. Le Deist, P. Drysdale, E. Jouanguy, R. Doffinger, F. Bernaudin, O. Jeppsson, J.A. Gollob, E. Meinel, A.W. Segal, A. Fischer, D. Kumararatne, and J.-L. Casanova. 1998. Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. *Science* 280:1432-1435.
- Bindea, G., B. Mlecnik, H. Hackl, P. Charoentong, M. Tosolini, A. Kirilovsky, W.H. Fridman, F. Pages, Z. Trajanoski, and J. Galon. 2009. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 25:1091-1093.
- Bukhari, M., M.A. Aslam, A. Khan, Q. Iram, A. Akbar, A.G. Naz, S. Ahmad, M.M. Ahmad, U.A. Ashfaq, H. Aziz, and M. Ali. 2015. TLR8 gene polymorphism and association in bacterial load in southern Punjab of Pakistan: an association study with pulmonary tuberculosis. *Int J Immunogenet* 42:46-51.
- Cervantes, J.L., C.J. La Vake, B. Weinerman, S. Luu, C. O'Connell, P.H. Verardi, and J.C. Salazar. 2013. Human TLR8 is activated upon recognition of *Borrelia burgdorferi* RNA in the phagosome of human monocytes. *J Leukoc Biol* 94:1231-1241.
- Chan, C.T., W. Deng, F. Li, M.S. DeMott, I.R. Babu, T.J. Begley, and P.C. Dedon. 2015. Highly Predictive Reprogramming of tRNA Modifications Is Linked to Selective Expression of Codon-Biased Genes. *Chem Res Toxicol* 28:978-988.
- Chan, J., K. Tanaka, D. Carroll, J. Flynn, and B.R. Bloom. 1995. Effects of nitric oxide synthase inhibitors on murine infection with *Mycobacterium tuberculosis*. *Infect Immun* 63:736-740.
- Chan, P.P., and T.M. Lowe. 2009. GtRNAdb: a database of transfer RNA genes detected in genomic sequence. *Nucleic Acids Res* 37:D93-97.
- Collins, A.C., H. Cai, T. Li, L.H. Franco, X.D. Li, V.R. Nair, C.R. Scharn, C.E. Stamm, B. Levine, Z.J. Chen, and M.U. Shiloh. 2015. Cyclic GMP-AMP Synthase Is an Innate Immune DNA Sensor for *Mycobacterium tuberculosis*. *Cell Host Microbe* 17:820-828.

- Davila, S., M.L. Hibberd, D.R. Hari, H.E. Wong, E. Sahiratmadja, C. Bonnard, B. Alisjahbana, J.S. Szeszko, Y. Balabanova, F. Drobniewski, C.R. van, d. van, V, S. Nejentsev, T.H. Ottenhoff, and M. Seielstad. 2008. Genetic association and expression studies indicate a role of toll-like receptor 8 in pulmonary tuberculosis. *PLoS Genet* 4:e1000218.
- Daya, M., L. van der Merwe, P.D. van Helden, M. Moller, and E.G. Hoal. 2014. Investigating the Role of Gene-Gene Interactions in TB Susceptibility. *PLoS One* 10:e0123970.
- Fabri, M., S. Stenger, D.M. Shin, J.M. Yuk, P.T. Liu, S. Realegeno, H.M. Lee, S.R. Krutzik, M. Schenk, P.A. Sieling, R. Teles, D. Montoya, S.S. Iyer, H. Bruns, D.M. Lewinsohn, B.W. Hollis, M. Hewison, J.S. Adams, A. Steinmeyer, U. Zugel, G.H. Cheng, E.K. Jo, B.R. Bloom, and R.L. Modlin. 2011. Vitamin D Is Required for IFN-gamma-Mediated Antimicrobial Activity of Human Macrophages. *Sci. Transl. Med.* 3:104ra102.
- Fan, J.B., and D.E. Zhang. 2013. ISG15 regulates IFN-gamma immunity in human mycobacterial disease. *Cell Res* 23:173-175.
- Fang, R., H. Hara, S. Sakai, E. Hernandez-Cuellar, M. Mitsuyama, I. Kawamura, and K. Tsuchiya. 2014. Type I interferon signaling regulates activation of the absent in melanoma 2 inflammasome during *Streptococcus pneumoniae* infection. *Infect Immun* 82:2310-2317.
- Feng, C.G., M. Kaviratne, A.G. Rothfuchs, A. Cheever, S. Hieny, H.A. Young, T.A. Wynn, and A. Sher. 2006. NK cell-derived IFN-gamma differentially regulates innate resistance and neutrophil response in T cell-deficient hosts infected with *Mycobacterium tuberculosis*. *J. Immunol.* 177:7086-7093.
- Flynn, J.L., J. Chan, K.J. Triebold, D.K. Dalton, T.A. Stewart, and B.R. Bloom. 1993. An essential role for interferon-gamma in resistance to *Mycobacterium tuberculosis* infection. *J. Exp. Med.* 178:2249-2254.
- Garcia, V.E., K. Uyemura, P.A. Sieling, M.T. Ochoa, C.T. Morita, H. Okamura, M. Kurimoto, T.H. Rea, and R.L. Modlin. 1999. IL-18 promotes type 1 cytokine production from NK cells and T cells in human intracellular infection. *J Immunol* 162:6114-6121.
- Gautier, G., M. Humbert, F. Deauvieau, M. Scuiller, J. Hiscott, E.E. Bates, G. Trinchieri, C. Caux, and P. Garrone. 2005. A type I interferon autocrine-paracrine loop is involved in Toll-like receptor-induced interleukin-12p70 secretion by dendritic cells. *J Exp Med* 201:1435-1446.

- Gorski, K.S., E.L. Waller, J. Bjornton-Severson, J.A. Hanten, C.L. Riter, W.C. Kieper, K.B. Gorden, J.S. Miller, J.P. Vasilakos, M.A. Tomai, and S.S. Alkan. 2006. Distinct indirect pathways govern human NK-cell activation by TLR-7 and TLR-8 agonists. *Int Immunol* 18:1115-1126.
- Guarda, G., M. Braun, F. Staehli, A. Tardivel, C. Mattmann, I. Forster, M. Farlik, T. Decker, R.A. Du Pasquier, P. Romero, and J. Tschopp. 2011. Type I interferon inhibits interleukin-1 production and inflammasome activation. *Immunity* 34:213-223.
- Hermann, P., M. Rubio, T. Nakajima, G. Delespesse, and M. Sarfati. 1998. IFN-alpha priming of human monocytes differentially regulates gram-positive and gram-negative bacteria-induced IL-10 release and selectively enhances IL-12p70, CD80, and MHC class I expression. *J Immunol* 161:2011-2018.
- Hu, Z., J. Mellor, J. Wu, and C. DeLisi. 2004. VisANT: an online visualization and analysis tool for biological interaction data. *BMC Bioinformatics* 5:17.
- Jouanguy, E., S. Lamhamedi-Cherradi, D. Lammas, S.E. Dorman, M.C. Fondaneche, S. Dupuis, R. Doffinger, F. Altare, J. Girdlestone, J.F. Emile, H. Ducoulombier, D. Edgar, J. Clarke, V.A. Oxelius, M. Brai, V. Novelli, K. Heyne, A. Fischer, S.M. Holland, D.S. Kumararatne, R.D. Schreiber, and J.L. Casanova. 1999. A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. *Nat. Genet.* 21:370-378.
- Junqueira-Kipnis, A.P., A. Kipnis, A. Jamieson, M.G. Juarrero, A. Diefenbach, D.H. Raulet, J. Turner, and I.M. Orme. 2003. NK cells respond to pulmonary infection with Mycobacterium tuberculosis, but play a minimal role in protection. *J Immunol* 171:6039-6045.
- Kadowaki, N., S. Ho, S. Antonenko, R.W. Malefyt, R.A. Kastelein, F. Bazan, and Y.J. Liu. 2001. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J. Exp. Med.* 194:863-869.
- Klug-Micu, G.M., S. Stenger, A. Sommer, P.T. Liu, S.R. Krutzik, R.L. Modlin, and M. Fabri. 2013. CD40 ligand and interferon-gamma induce an antimicrobial response against Mycobacterium tuberculosis in human monocytes. *Immunology* 139:121-128.
- Lai, Y.F., T.M. Lin, C.H. Wang, P.Y. Su, J.T. Wu, M.C. Lin, and H.L. Eng. 2016. Functional polymorphisms of the TLR7 and TLR8 genes contribute to Mycobacterium tuberculosis infection. *Tuberculosis (Edinb)* 98:125-131.
- Lande, R., E. Giacomini, T. Grassi, M.E. Remoli, E. Iona, M. Miettinen, I. Julkunen, and E.M. Coccia. 2003. IFN- Released by Mycobacterium tuberculosis-Infected

- Human Dendritic Cells Induces the Expression of CXCL10: Selective Recruitment of NK and Activated T Cells. *The Journal of Immunology* 170:1174-1182.
- Langfelder, P., and S. Horvath. 2008a. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 9:559.
- Langfelder, P., and S. Horvath. 2008b. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 9:559.
- Lee, H.R., S.Y. Yoon, S.B. Song, Y. Park, T.S. Kim, S. Kim, D.Y. Hur, H.K. Song, H. Park, and D. Cho. 2011. Interleukin-18-mediated interferon-gamma secretion is regulated by thymosin beta 4 in human NK cells. *Immunobiology* 216:1155-1162.
- Liu, J., X. Guan, T. Tamura, K. Ozato, and X. Ma. 2004. Synergistic activation of interleukin-12 p35 gene transcription by interferon regulatory factor-1 and interferon consensus sequence-binding protein. *J Biol Chem* 279:55609-55617.
- Love, M.I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15:550.
- Lu, H., G.N. Dietsch, M.A. Matthews, Y. Yang, S. Ghanekar, M. Inokuma, M. Suni, V.C. Maino, K.E. Henderson, J.J. Howbert, M.L. Disis, and R.M. Hersherberg. 2012. VTX-2337 is a novel TLR8 agonist that activates NK cells and augments ADCC. *Clin Cancer Res* 18:499-509.
- Ma, F., B. Li, S.Y. Liu, S.S. Iyer, Y. Yu, A. Wu, and G. Cheng. 2015. Positive feedback regulation of type I IFN production by the IFN-inducible DNA sensor cGAS. *J Immunol* 194:1545-1554.
- MacMicking, J.D., R.J. North, R. LaCourse, J.S. Mudgett, S.K. Shah, and C.F. Nathan. 1997. Identification of nitric oxide synthase as a protective locus against tuberculosis. *Proc. Natl. Acad. Sci. U. S. A.* 94:5243-5248.
- Manca, C., L. Tsenova, S. Freeman, A.K. Barczak, M. Tovey, P.J. Murray, C. Barry, and G. Kaplan. 2005. Hypervirulent M. tuberculosis W/Beijing strains upregulate type I IFNs and increase expression of negative regulators of the Jak-Stat pathway. *J Interferon Cytokine Res* 25:694-701.
- Manzanillo, P.S., M.U. Shiloh, D.A. Portnoy, and J.S. Cox. 2012. Mycobacterium tuberculosis activates the DNA-dependent cytosolic surveillance pathway within macrophages. *Cell Host Microbe* 11:469-480.
- Matikainen, S., A. Paananen, M. Miettinen, M. Kurimoto, T. Timonen, I. Julkunen, and T. Sareneva. 2001. IFN-alpha and IL-18 synergistically enhance IFN-gamma

- production in human NK cells: differential regulation of Stat4 activation and IFN-gamma gene expression by IFN-alpha and IL-12. *Eur J Immunol* 31:2236-2245.
- Newport, M.J., C.M. Huxley, S. Huston, C.M. Hawrylowicz, B.A. Oostra, R. Williamso, and M. Levin. 1996. A mutation in the interferon-(gamma)-receptor gene and susceptibility to mycobacterial infection. *N. Engl. J. Med.* 335:1941-1949.
- O'Garra, A., P.S. Redford, F.W. McNab, C.I. Bloom, R.J. Wilkinson, and M.P. Berry. 2013. The immune response in tuberculosis. *Annu Rev Immunol* 31:475-527.
- Obregon-Henao, A., M.A. Duque-Correa, M. Rojas, L.F. Garcia, P.J. Brennan, B.L. Ortiz, and J.T. Belisle. 2012. Stable extracellular RNA fragments of Mycobacterium tuberculosis induce early apoptosis in human monocytes via a caspase-8 dependent mechanism. *PLoS One* 7:e29970.
- Orme, I.M., R.T. Robinson, and A.M. Cooper. 2015. The balance between protective and pathogenic immune responses in the TB-infected lung. *Nat Immunol* 16:57-63.
- Portevin, D., L.E. Via, S. Eum, and D. Young. 2012. Natural killer cells are recruited during pulmonary tuberculosis and their ex vivo responses to mycobacteria vary between healthy human donors in association with KIR haplotype. *Cell Microbiol* 14:1734-1744.
- Salie, M., M. Daya, L.A. Lucas, R.M. Warren, G.D. van der Spuy, P.D. van Helden, E.G. Hoal, and M. Moller. 2015. Association of toll-like receptors with susceptibility to tuberculosis suggests sex-specific effects of TLR8 polymorphisms. *Infect Genet Evol* 34:221-229.
- Schenk, M., S.R. Krutzik, P.A. Sieling, D.J. Lee, R.M. Teles, M.T. Ochoa, E. Komisopoulou, E.N. Sarno, T.H. Rea, T.G. Graeber, S. Kim, G. Cheng, and R.L. Modlin. 2012. NOD2 triggers an interleukin-32-dependent human dendritic cell program in leprosy. *Nat Med* 18:555-563.
- Schierloh, P., N. Yokobori, M. Aleman, R.M. Musella, M. Beigier-Bompadre, M.A. Saab, L. Alves, E. Abbate, S.S. de la Barrera, and M.C. Sasiain. 2005. Increased susceptibility to apoptosis of CD56dimCD16+ NK cells induces the enrichment of IFN-gamma-producing CD56bright cells in tuberculous pleurisy. *J. Immunol.* 175:6852-6860.
- Schmidt, K.N., B. Leung, M. Kwong, K.A. Zarembek, S. Satyal, T.A. Navas, F. Wang, and P.J. Godowski. 2004. APC-independent activation of NK cells by the Toll-like receptor 3 agonist double-stranded RNA. *J. Immunol.* 172:138-143.

- Sha, W., H. Mitoma, S. Hanabuchi, M. Bao, L. Weng, N. Sugimoto, Y. Liu, Z. Zhang, J. Zhong, B. Sun, and Y.J. Liu. 2014. Human NLRP3 inflammasome senses multiple types of bacterial RNAs. *Proc Natl Acad Sci U S A* 111:16059-16064.
- Sun, Q., Q. Zhang, H.P. Xiao, and C. Bai. 2015. Toll-like receptor polymorphisms and tuberculosis susceptibility: A comprehensive meta-analysis. *J Huazhong Univ Sci Technolog Med Sci* 35:157-168.
- Teles, R.M., T.G. Graeber, S.R. Krutzik, D. Montoya, M. Schenk, D.J. Lee, E. Komisopoulou, K. Kelly-Scumpia, R. Chun, S.S. Iyer, E.N. Sarno, T.H. Rea, M. Hewison, J.S. Adams, S.J. Popper, D.A. Relman, S. Stenger, B.R. Bloom, G. Cheng, and R.L. Modlin. 2013. Type I interferon suppresses type II interferon-triggered human anti-mycobacterial responses. *Science* 339:1448-1453.
- Ushio, S., M. Namba, T. Okura, K. Hattori, Y. Nukada, K. Akita, F. Tanabe, K. Konishi, M. Micallef, M. Fujii, K. Torigoe, T. Tanimoto, S. Fukuda, M. Ikeda, H. Okamura, and M. Kurimoto. 1996. Cloning of the cDNA for human IFN-gamma-inducing factor, expression in Escherichia coli, and studies on the biologic activities of the protein. *J. Immunol.* 156:4274-4279.
- Wang, Z., L. Xiang, J. Shao, and Z. Yuan. 2006. The 3' CCACCA sequence of tRNAAla(UGC) is the motif that is important in inducing Th1-like immune response, and this motif can be recognized by Toll-like receptor 3. *Clin Vaccine Immunol* 13:733-739.
- Wassermann, R., M.F. Gulen, C. Sala, S.G. Perin, Y. Lou, J. Rybniker, J.L. Schmid-Burgk, T. Schmidt, V. Hornung, S.T. Cole, and A. Ablasser. 2015. Mycobacterium tuberculosis Differentially Activates cGAS- and Inflammasome-Dependent Intracellular Immune Responses through ESX-1. *Cell Host Microbe* 17:799-810.
- Watson, R.O., P.S. Manzanillo, and J.S. Cox. 2012. Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. *Cell* 150:803-815.
- Wu, L., Y. Hu, D. Li, W. Jiang, and B. Xu. 2015. Screening toll-like receptor markers to predict latent tuberculosis infection and subsequent tuberculosis disease in a Chinese population. *BMC Med. Genet.* 16:19.
- Zhang, Z., B. Yuan, M. Bao, N. Lu, T. Kim, and Y.J. Liu. 2011. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat Immunol* 12:959-965.
- Zhou, R., H. Wei, R. Sun, and Z. Tian. 2007. Recognition of double-stranded RNA by TLR3 induces severe small intestinal injury in mice. *J. Immunol.* 178:4548-4556.