NATÁLIA FRANCISCO SCARAMELE

Modulação gênica de neutrófilos humanos após infecção *in vitro* com *Leishmania infantum*

Araçatuba – SP

2023

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Tese apresentada à Faculdade de Medicina Veterinária de Araçatuba da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para obtenção do título de Doutora em Ciência Animal (Medicina Veterinária Preventiva e Produção Animal)

Orientadora: Pesquisadora Flávia Lombardi Lopes

Araçatuba – SP

2023

S285m

Scaramele, Natalia Francisco
Modulação gênica de neutrófilos humanos após infecção in vitro com Leishmania infantum / Natalia Francisco Scaramele. -- Araçatuba, 2023
142 f.

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Medicina Veterinária, Araçatuba
Orientadora: Flavia Lombardi Lopes

1. Leishmaniose. 2. RNA Mensageiro. 3. MicroRNAs. 4. RNA Longo não Codificante. 5. Neutrófilos. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Medicina Veterinária, Araçatuba. Dados fornecidos pelo autor(a).

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Araçatuba, 19 de junho de 2023

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gov.br SANDRA MARCIA MUXEL

Araçatuba, 19 de junho de 2023.

Aos meus pais, Osvaldo Scaramele
e Lázara Francisco Scaramele.

AGRADECIMENTOS

Aos meus pais (*in memoriam*), que sempre incentivaram meus estudos e muito se esforçaram para que eu chegasse até aqui.

Ao amor da minha vida, Guilherme Bruno da Silva, por toda serenidade, e por sua paciência em me manter firme durante nossos anos juntos.

As amigas que a faculdade e a ciência me deram: Amanda, Beatriz, Daniela, Jéssica, Juliana, Maria Fernanda, Maria Sara, Mariana. Obrigada pelas discussões científicas, por toda a ajuda laboratorial, e pela amizade que espero perdurar para além da universidade.

A todos os colegas que colaboraram direta e indiretamente para a realização deste trabalho, em especial ao pessoal do laboratório de Imunologia da FMVA, por toda paciência e ajuda, sem as quais este trabalho não seria realizado.

A minha orientadora, Flávia Lombardi Lopes, que me aguentou por mais quatro anos de doutorado no melhor laboratório, apelidado recentemente de EpiHome, pela confiança e companheirismo construídos ao longo destes anos, e por todo conhecimento que me proporcionou experimentar junto ao mundo científico.

À UNESP – Universidade Estadual Paulista “Júlio de Mesquita Filho”, em especial ao Programa de Pós-Graduação em Ciência Animal da FMVA (Faculdade de Medicina Veterinária de Araçatuba) pela oportunidade de realização do doutorado.

A todos os funcionários e colaboradores, sempre solícitos e dispostos.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

SCARAMELE, N. F. **Modulação gênica de neutrófilos humanos após infecção *in vitro* com *Leishmania infantum*.** 2023. 139 f. Tese (Doutorado) – Faculdade de Medicina Veterinária, Universidade Estadual Paulista, Araçatuba, 2023.

RESUMO

A leishmaniose é uma zoonose tropical negligenciada com altas taxas de morbidade e mortalidade. É causada por protozoários do gênero *Leishmania* e transmitida durante o repasto sanguíneo das fêmeas de *Lutzomyia longipalpis*. Uma das manifestações clínicas da doença é a leishmaniose visceral, causada principalmente pela espécie *L. infantum* (syn. *chagasi*) nas Américas. Os sintomas da leishmaniose visceral incluem febre, anorexia, perda de peso, distensão abdominal, fraqueza e um aumento abdominal significativo, decorrente de hepato e esplenomegalia. Apesar da disponibilidade de medicamentos para tratar a doença, nenhuma vacina está comercialmente disponível para humanos. O papel dos neutrófilos como parte da resposta imune inata à *Leishmania* spp. infecção é dubio e varia de acordo com a espécie causadora da infecção. Ainda, a expressão global de RNAs codificantes, microRNAs e RNAs longos não codificantes muda como parte da resposta imune contra patógenos. No presente trabalho buscamos entender os padrões de expressão gênica de transcritos (codificantes e não codificantes) em neutrófilos humanos infectados *in vitro* com *L. infantum*. Isolamos neutrófilos de sangue total de doadores saudáveis do sexo masculino (n=5) e dividimos em grupos: 1) infectados com *L. infantum* (MOI = 5:1) e 2) controles não infectados. No grupo infectado as promastigotas não internalizadas foram removidas após 3 horas e, ao tempo de 20 horas pós-infecção o RNA total foi extraído das células dos grupos controle e infectado. Posteriormente foram realizadas duas técnicas: 1) sequenciamento de RNA total, e 2) microarranjo de microRNA. Observamos mudanças na expressão de 212 transcritos (log2(FC)±0,58, FDR≤0,05), sendo classificados: 202 RNAs mensageiros, seis RNAs longos não-codificantes, um RNA “miscellaneous”, um RNA nuclear pequeno, um pseudogene não processado e um pseudogene processado. E na expressão de 289 microRNAs (FC±2, FDR≤0,01). Com o auxílio de ferramentas bioinformáticas, pudemos ainda predizer funções biológicas aos nossos achados, e com isso inferimos que as mudanças observadas tanto na expressão de RNAs codificadores como dos não codificadores de neutrófilos estão associadas ao prejuízo na fagocitose, produção de óxido nítrico e de citocinas pró-inflamatórias após a infecção com *L. infantum*, favorecendo a persistência do parasito. Nosso trabalho lança luz sobre vários mecanismos utilizados pela *L. infantum* para controlar a resposta imune mediada por neutrófilos e identifica vários alvos para futuros estudos funcionais, visando o desenvolvimento de tratamentos preventivos ou curativos para esta prevalente zoonose.

Palavras-chave: Leishmaniose. RNA Mensageiro. MicroRNAs. RNA Longo não Codificante. Neutrófilos. Imunidade Inata.

SCARAMELE, N. F. **Gene modulation of human neutrophils after *in vitro* infection with *Leishmania infantum*.** 2023. 139 f. Tese (Doutorado) – Faculdade de Medicina Veterinária, Universidade Estadual Paulista, Araçatuba, 2023.

ABSTRACT

Leishmaniasis is a neglected tropical zoonosis with high morbidity and mortality rates. It is caused by protozoa of the genus *Leishmania* and transmitted during the blood meal of female *Lutzomyia longipalpis*. One of the clinical manifestations of the disease is visceral leishmaniasis, caused mainly by the species *L. infantum* (syn. *chagasi*) in the Americas. Symptoms of visceral leishmaniasis include fever, anorexia, weight loss, abdominal distention, weakness, and significant abdominal enlargement due to hepatomegaly and splenomegaly. Despite the availability of drugs to treat the disease, no vaccine is commercially available for humans. The role of neutrophils as part of the innate immune response to *Leishmania* spp. infection is dubious and varies according to the species causing the infection. Furthermore, the global expression of coding RNAs, microRNAs and long non-coding RNAs changes as part of the immune response against pathogens. In the present work, we aimed to understand the gene expression patterns of coding and non-coding transcripts of human neutrophils infected *in vitro* with *L. infantum*. We isolated neutrophils from whole blood of healthy male donors (n=5) and divided them into groups: 1) infected with *L. infantum* (MOI = 5:1) and 2) uninfected controls. In the infected group, the non-internalized promastigotes were removed after 3 hours and at the time of 20 hours post-infection, total RNA was extracted from control and infected neutrophils. Subsequently, two techniques were performed: 1) total RNA sequencing, and 2) microRNA microarray. We observed changes in the expression of 212 transcripts ($\log2(FC)\pm0.58$, $FDR\leq0.05$), being classified as: 202 messenger RNAs, six long non-coding RNAs, one miscellaneous RNA, one small nuclear RNA, one unprocessed pseudogene and one processed pseudogene. We also observed changes in the expression of 289 microRNAs ($FC\pm2$, $FDR\leq0.01$). With the aid of bioinformatics tools, we were also able to predict biological functions to our findings, inferring that the changes observed in the expression of both coding and non-coding RNAs are associated with impaired phagocytosis, production of nitric oxide and pro-inflammatory cytokines expression after neutrophils infection with *L. infantum*, favoring the persistence of the parasite. Our work sheds light on several mechanisms used by *L. infantum* to control the neutrophil-mediated immune response and identifies several targets for future functional studies, aiming at the development of preventive or curative treatments for this prevalent zoonosis.

Keywords: Leishmaniasis. RNA, Messenger. MicroRNAs. RNA, Long Noncoding. Neutrophils. Immunity, Innate.

SUMÁRIO

1 INTRODUÇÃO GERAL

A leishmaniose é uma doença tropical negligenciada com a segunda maior taxa de mortalidade e terceira maior taxa de morbidade entre as enfermidades parasitárias.[1] É causada por protozoários do gênero *Leishmania,*[2] pertencentes à ordem Kinetoplastida, com dois subgêneros (*Leishmania* e *Viannia*) e mais de 20 espécies.[1] O ciclo do protozoário inclui duas formas morfológicas: uma promastigota extracelular flagelada encontrada no vetor – fêmeas de flebotomíneos da família *Psychodidae* – e uma amastigota intracelular obrigatória que se multiplica nos fagócitos do hospedeiro vertebrado até romperem a célula e infectarem outros tecidos.[3,4] Ao realizar o repasto sanguíneo, o vetor ingere o protozoário, que retorna à sua forma flagelada e pode infectar novos hospedeiros.[5]

De acordo com a Organização Mundial da Saúde a doença possui três principais apresentações clínicas: leishmaniose cutânea (LC), leishmaniose mucocutânea (LMC) e leishmaniose visceral (LV). A LC é a forma mais comum da doença, causa feridas e lesões ulcerosas em partes expostas do corpo, podendo deixar cicatrizes. Os casos de LC se distribuem majoritariamente entre as Américas, bacia do Mediterrâneo, Oriente Médio e Asia Central.[6] Ainda 20% dos casos de LC evoluem para LMC, onde as lesões podem causar uma destruição parcial ou total das membranas mucosas,[6,7] mais de 90% dos casos de LMC incidem sobre Bolívia, Brasil, Etiópia e Peru.[6] As espécies *L. aethiopica, L. amazonensis L. braziliensis* e *L. major* podem causar ambas as formas da doença, enquanto *L. Mexicana* e *L. venezuelensis* causam exclusivamente LC, e *L. guyanensis* e *L. panamensis* podem causar apenas LCM.[7] Estimativas atuais mostram uma incidência anual global de 0.7-1.2 milhões de casos cutâneos por ano.[8]

Enquanto a LV, também chamada de calazar ou “kala-azar”, é a manifestação mais severa da doença, sendo potencialmente fatal sem tratamento e é causada principalmente por *L. donovani* e *L. infantum* no velho mundo, e por *L. infantum*, também chamada de *L. chagasi* nas Américas.[9,10] Com a disseminação do parasito no organismo do hospedeiro ocorre aumento no número de células fagocíticas, principalmente no fígado e baço, levando hepato- e esplenomegalia, e na medula óssea, os sintomas incluem febre, anorexia, perda de peso, distensão abdominal e fraqueza.[1,3,9] No período entre 2001 e 2021 foram reportados 69.665 casos humanos

de LV nas Américas, com uma média anual de 2.488 casos, sendo 93,5% deles concentrados no Brasil, onde se destacam as regiões Nordeste, Sudeste e Centro-Oeste.[11]

Hoje, mais de 1 bilhão de pessoas vivem em áreas endêmicas para leishmaniose e estima-se que 30.000 novos casos de LV ocorram anualmente tornando esta zoonose um problema de saúde pública. A OMS e a Organização Pan-Americana de Saúde vêm desenvolvendo ações para eliminar essa e outras doenças negligenciadas. Em parceria com outras agências foram desenvolvidas recomendações para o tratamento das leishmanioses nas Américas usando a metodologia de classificação da análise, desenvolvimento e avaliação de recomendações (GRADE). O enfoque nas recomendações terapêuticas resultou nas diretrizes para o tratamento das leishmanioses nas Américas.[12] Apesar dos antimoniais pentavalentes terem sido anteriormente considerados padrão de tratamento[9] a principal droga recomendada hoje, para pacientes imunocompetentes, é a anfotericina B lipossomal, seguida por antimoniais pentavalentes ou desoxicolato de anfotericina B. Quando falamos de pacientes imunocomprometidos o uso de antimoniais pentavalentes não é recomendado.[12]

Uma alternativa para o controle no número de casos em humanos é diminuir a população de cães infectados, já que a alta prevalência em cães representa um aumento no risco de casos humanos, uma vez que os cães são o principal reservatório de *L. infantum* no Brasil. Na espécie canina o tratamento também é medicamentoso, mas estratégias de prevenção como uso de coleiras antiparasitárias e produtos do tipo “spot-on” são aplicadas para diminuir o contato com o vetor,[13] esses métodos repelentes junto ao tratamento profilático com domperidona reduz a infecção de *L. infantum* em cães.[14] Atualmente algumas vacinas estão comercialmente disponíveis para LV canina no mundo.[15,16,17] Embora as vacinas sejam a forma mais eficaz de prevenir doenças, ainda não há vacina licenciada para uso clínico da LV humana.[18,19] Apesar dos muitos testes em modelos animais[20] a variação antigênica e polimorfismos gênicos do parasito[21] fazem parte dos problemas enfrentados no desenvolvimento da vacina para humanos e de acordo com o Ministério da Saúde[22] não existem estudos que comprovem que a aplicação da única vacina comercializável no Brasil reduza a incidência da leishmaniose visceral em seres humanos, sendo assim seu uso está restrito à proteção individual dos cães, tendo em torno de 70% de eficácia, de acordo com o fabricante.

Além disso, existe uma lacuna de entendimento na interação patógeno/hospedeiro relacionada a resposta imune inata. A resposta imune inata do hospedeiro parece ser importante para determinar o desenvolvimento de uma resposta adaptativa permissiva ou protetiva contra a LV.[23] Neutrófilos são as células imunes mais abundantes no sangue, provenientes de linhagem mieloide, são granulócitos do tipo polimorfonuclear.[24] São rapidamente recrutados frente a injurias ou infecções, incluindo aquelas causadas por protozoários,[24,25] sendo as principais células efetoras na inflamação aguda.[26] Isso se deve a sua ampla função microbicida, observada através dos processos de liberação de grânulos, chamado de degranulação; da produção de espécies reativas de oxigênio; da liberação das armadilhas celulares de neutrófilos (NETs), e da fagocitose.[24]

Os neutrófilos são observados em modelos de leishmaniose pouco após a inoculação do parasito.[27] Num primeiro momento, apenas como resposta a injuria tecidual, mas posteriormente de forma proporcional a infectividade da cepa inuoculada[28], outro fator capaz de modular a resposta neutrofílica é a saliva do flebotomíneo.[29,30] O reconhecimento do parasito tem início graças a presença de receptores na membrana celular deste leucócito de núcleo multilobulado, como os *Toll-Like Receptors* (TLR), responsáveis pelo reconhecimento de Padrões Moleculares Associados a Patógenos, que usam proteínas adaptadoras para desencadear a produção de citocinas pró-infamatórias;[31] a presença do parasito também é capaz de ativar a via alternativa do sistema complemento, promovendo a fagocitose e culminando na lise do patógeno.[32]

Após a fagocitose da *Leishmania*, os grânulos citoplasmáticos do neutrófilo são liberados dentro do vacúolo que contêm o parasito, chamado de vacúolo parasitóforo, o que ativa o complexo NADPH oxidase liberando superóxido, que contribui para a eliminação do parasito.[33] Os grânulos dos neutrófilos também compõe as NETs, onde são encontradas elastase, catepsina G, mieloperoxidase, lactoferrina, gelatinase, histonas e, sobretudo, DNA.[34] Essas armadilhas apresentam um potencial microbicida significativo e contribuem para a eliminação de parasitas extracelulares.[33] Apesar das atividades microbicidas destacadas, a *Leishmania* possui habilidade de evadir e atenuar a função microbicida do fagócito manipulando a sua via de ativação, a fim de favorecer sua sobrevivência e proliferação.[35,36] Um exemplo disso é o mecanismo conhecido como “cavalo de Tróia”, quando neutrófilos apoptóticos liberam quimiocinas capazes de recrutar macrófagos que, por sua vez, fagocitam os neutrófilos infectados

antes da liberação da *Leishmania*, impedindo a ativação de mecanismos efetores nos macrófagos.[37,38] Algumas espécies de *Leishmania* apesar de promoverem essa sinalização, ainda são liberadas pelos neutrófilos, antes desses serem fagocitados, e desta forma são facilmente fagocitadas por macrófagos, a esse mecanismo de favorecimento foi dado o nome de “coelho de Tróia” .[37,39]

De maneira geral, o papel dos neutrófilos na leishmaniose é ambíguo e parece variar de acordo com a espécie causadora da infecção.[40] Neste trabalho propomos que mudanças na expressão gênica do hospedeiro poderiam explicar essa ambiguidade, uma vez que a resposta imune pode ser regulada por processos dinâmicos nomeados mecanismos epigenéticos, que agem concomitante ou separadamente, permitindo a rápida repressão ou ativação de um gene específico, de um conjunto de genes ou mesmo de proteínas. Para que um indivíduo mantenha seu estado de saúde é necessária uma regulação temporal e tecidual da expressão gênica, alcançada, entre outros, por modificações na expressão de RNAs não-codificadores (ncRNAs).[41]

Um exemplo de ncRNA são os microRNAs (miRNAs); formados por uma sequência curta e endógena de nucleotídeos (~22 nt) na sua fase madura.[42] Sua biogênese tem início no núcleo, onde miRNAs primários ou pri-miRNAs são transcritos pela RNA polimerase II a partir, principalmente, de regiões intergênicas ou antisenso de genes anotados[43] e então clivados por um complexo microprocessador, formado pela ribonuclease Drosha e seu cofator DGCR8, originando um precursor em forma de haste-alça (do inglês “hairpin”).[44] Esse precursor, também chamado de pré-miRNA é exportado do núcleo para o citoplasma, onde a enzima Dicer fragmenta a parte da alça, originando um RNA complementar em dupla fita. A partir da separação desse duplex, uma fita simples associada a Dicer se vincula à proteína de ligação ao RNA responsiva à transativação (TRBP) e a proteína Argonauta, especificamente AGO2 formando o Complexo de Silenciamento Induzido por miRNA (miRISC, sigla do inglês "miRNA Induced Silencing Complex").[44,45] Uma vez ativado, esse complexo é capaz de se ligar a RNAs mensageiros (mRNA) de diferentes modos, alguns estudos observaram que miRNAs podem se ligar a regiões promotoras, e estimular a transcrição. Entretanto os miRNAs são conhecidos principalmente por sua função repressora, que ocorre ao se ligarem em regiões 3’ UTR (do inglês “untranslated region”). Outro mecanismo, menos comum, é a ligação à região 5’ UTR do alvo.[46] Os primeiros (2-8 nt) nucleotídeos do miRNA maduro, região chamada de “seed”, são os

responsáveis por essa ligação e consequente inibição do processo de tradução de proteínas, que pode ocorrer de formas distintas: impedindo a ligação do fator de inibição eucariótico as suas subunidades, e consequentemente a associação do mRNA ao complexo ribossômico; bloqueando a associação da unidade 60 S com a 40 S do ribossomo; inibindo a ativação de polissomos após o início da tradução ou desestabilizando e degradando o mRNA complementar, todas resultando na regulação da expressão gênica.[47,48]

Os miRNAs e sua maquinaria associada são altamente conservados entre espécies, e sua expressão varia em diferentes tecidos no decorrer do tempo.[44] Predições bioinformáticas mostram que 60% de genes codificadores de proteína em humanos, e 30% em mamíferos no geral, são regulados por miRNAs, ressaltando a importância desse pequeno e notável RNA.[49,50] Além disso, estudos demonstram que a regulação de miRNAs do hospedeiro está relacionada a resistência ou susceptibilidade às infecções.[48] Falando especificamente de infecções causadas por *Leishmania*, sabemos que diferentes hospedeiros (humanos, cobaias e cães) sofrem alteração na expressão de miRNAs relacionados ao controle da resposta imune, entretanto tais estudos são conduzidos majoritariamente em células mononucleares,[48] e até o presente momento não foram investigadas em neutrófilos.

Outro tipo de ncRNA que abordaremos neste trabalho são os RNAs longos não-codificadores (lncRNAs), transcritos com mais de 200 nucleotídeos que, assim como os miRNAs, tem sua transcrição iniciada com a enzima RNA polimerase II, e podem ter origem intergênica, intrônica, antisenso, bidirecional ou em “enhancers”.[51] Mesmo sendo primariamente transcritos como os mRNAs, parte dos lncRNAs não são poliadenilados, ou seja, não possuem cauda polyA.[52] A transcrição de lncRNAs intrônicos, conhecidos como lincRNAs parece ser iniciada pelos mesmos fatores de transcrição que transcrevem mRNAs, uma vez que a região promotora de ambos é similar,[53] mas em alguns casos a transcrição dos lncRNAs parece depender de um proto-oncogene, o fator de transcrição cMyc, e da enzima Dicer,[54] a mesma responsável por fragmentar a alça do miRNA “hairpin”. Depois de transcritos, os lncRNAs podem ser encontrados em diferentes locais dentro das células, conforme revisado por Fernandes et al.[51]

Ainda segundo a revisão de Fernandes et al.[51] eles desempenham diversas funções, podendo se associar a cromatina, as histonas, fatores de transcrição ou mesmo ao DNA, e ainda interagir com mRNAs e miRNAs, além de serem exportados

da célula em vesículas chamadas exossomas. Tais funções podem ser presumíveis de acordo com a localização, os lncRNAs localizados no núcleo geralmente exercem uma regulação transcricional, e atuam na cromatina próxima de onde foram transcritos e, quando seus efeitos são restritos ao locus cromossomal do qual são transcritos, são classificados como de ação *cis*.[55,56] Nesse caso eles podem interferir positiva ou negativamente com a transcrição, servindo como ancoras para modificadores de cromatina,[57] como co-ativadores para fatores de transcrição[58] ou bloqueando a transcrição ao interagir com a RNA polimerase II.[56] Ainda, o processo de transcrição de lncRNAs podem impactar na expressão de genes vizinhos, como resultado do remodelamento da cromatina e do nucleossomo.[59] Ao afetar genes expressos em outros cromossomos, ou distantes de seu locus de transcrição, os lncRNAs são classificados como de ação *trans*.[56] Ademais, quando situados no citoplasma celular, agindo pós-transcricionalmente, os lncRNAs podem interagir com miRNAs de diferentes modos. Na forma conhecida como “esponja” os lncRNAs se ligam aos miRNAs por complementariedade, impedindo que esses exerçam sua função repressora nos mRNAs, entretanto os miRNAs também são capazes de promover a degradação do lncRNA, com o complexo miRISC, e esses dois ncRNAs podem competir pelo mesmo mRNA alvo. Os lncRNAs podem estar envolvidos no processo de splicing e geração de isoformas, e na estabilidade de mRNAs, ao se associar a proteínas de ligação ao RNA (RBP, do inglês *RNA binding protein*) que induzem deadenilação e/ou decapagem do mRNA maduro, levando a degradação do mRNA por exonucleases, favorecendo seu decaimento.[60,61] Ainda, o lncRNA por si só pode recrutar repressores de tradução, agindo como regulador negativo na expressão de proteínas.[51]

O repertório de expressão de RNAs não-codificadores, no geral, parece estar positivamente correlacionado à complexidade do organismo que o expressa[55] e apesar de lncRNAs já terem sido investigados durante infecções com *L. infantum*,[62,63] nenhum estudo ainda investigou a expressão de lncRNAs ou de miRNAs em neutrófilos humanos expostos ao parasita *Leishmania*.

1.2 Objetivos

1.2.1 Objetivo Geral

Determinar os padrões de expressão de RNAs codificadores e não codificadores como parte da interação hospedeiro/parasita, em um modelo *in vitro* de infecção aguda por *L. infantum* em neutrófilos humanos.

1.2.2 Objetivos Específicos

1) Determinar a expressão dos mRNAs em neutrófilos humanos infectados *in vitro* com *L. infantum*;

2) Determinar a expressão dos miRNAs em neutrófilos humanos infectados *in vitro* com *L. infantum*;

3) Determinar a expressão dos lncRNAs em neutrófilos humanos infectados *in vitro* com *L. infantum*

4) Associar mudanças na expressão de não-codificadores com o controle da expressão gênica, visando compreender o diálogo hospedeiro/microrganismo.

2 CAPÍTULO 1 - *LEISHMANIA INFANTUM* INFECTION MODULATES MESSENGER RNA, MICRORNA AND LONG NON-CODING RNA EXPRESSION IN HUMAN NEUTROPHILS *IN VITRO*

L. INFANTUM INFECTION MODULATES NEUTROPHILS (MICRO)TRANSCRIPTOME

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2.1 Resumo

Nas Américas, L. infantum (sin. chagasi) é a principal causa de leishmaniose visceral humana. O papel dos neutrófilos como parte da resposta inata à Leishmania spp. infecção é duvidosa e varia de acordo com a espécie causadora da infecção. A expressão global de RNAs codificantes, microRNAs e RNAs longos não codificantes muda como parte da resposta imune contra patógenos. Alterações na expressão do mRNA e do RNA não codificante decorrentes da infecção por Leishmania spp. são amplamente estudados em macrófagos, mas escassos em neutrófilos, a primeira célula a encontrar o tripanosomatídeo, principalmente após a infecção por L. infantum. Aqui, objetivamos entender os padrões de expressão de transcritos codificantes e não codificantes durante a infecção aguda in vitro de neutrófilos humanos por L. infantum. Nós isolamos neutrófilos do sangue total de doadores saudáveis do sexo masculino (n=5) e dividimos em grupos: 1) infectados com L. infantum (MOI = 5:1) e 2) controles não infectados. Após 3 horas de exposição do grupo infectado aos promastigotas de L. infantum, seguidas de 17 horas de incubação, o RNA total foi extraído e o RNA-Seq total e o miRNA microarray foram realizados. Um total de 212 genes foram diferencialmente expressos em neutrófilos após análise de RNA-Seq (log2(FC)±0,58, FDR≤0,05). A infecção in vitro com L. infantum aumentou a expressão de 197 e reduziu a expressão de 92 miRNAs em neutrófilos humanos (FC±2, FDR≤0,01). Por fim, 5 genes regulados negativamente foram classificados como lncRNA, e dos 10 genes regulados positivamente, havia apenas 1 lncRNA. Outras análises bioinformáticas indicaram que alterações no transcriptoma e microtranscriptoma de neutrófilos, após infecção in vitro com L. infantum, podem prejudicar a fagocitose, apoptose e diminuir a produção de óxido nítrico. Nosso trabalho lança luz sobre vários mecanismos utilizados por L. infantum para controlar a resposta imune mediada por neutrófilos e identifica vários alvos para futuros estudos funcionais, visando o desenvolvimento de tratamentos preventivos ou curativos para esta zoonose prevalente.

2.2 Abstract

In the Americas, *L. infantum* (syn. *chagasi*) is the main cause of human visceral leishmaniasis. The role of neutrophils as part of the innate response to *Leishmania* spp. infection is dubious and varies according to the species causing the infection. Global expression of coding RNAs, microRNAs and long non-coding RNAs changes as part of the immune response against pathogens. Changes in mRNA and non-coding RNA expression resulting from infection by *Leishmania* spp. are widely studied in macrophages, but scarce in neutrophils, the first cell to encounter the trypanosomatid, especially following infection by *L. infantum*. Herein, we aimed to understand the expression patterns of coding and non-coding transcripts during acute *in vitro* infection of human neutrophils by *L. infantum*. We isolated neutrophils from whole blood of healthy male donors (n=5) and split into groups: 1) infected with *L. infantum* (MOI = 5:1), and 2) uninfected controls. After 3 hours of exposure of infected group to promastigotes of *L. infantum*, followed by 17 hours of incubation, total RNA was extracted and total RNA-Seq and miRNA microarray were performed. A total of 212 genes were differentially expressed in neutrophils following RNA-Seq analysis (log2(FC)±0.58, FDR≤0.05). *In vitro* infection with *L. infantum* upregulated the expression of 197 and reduced the expression of 92 miRNAs in human neutrophils (FC±2, FDR≤0.01). Lastly, 5 downregulated genes were classified as lncRNA, and of the 10 upregulated genes, there was only 1 lncRNA. Further bioinformatic analyzes indicated that changes in the transcriptome and microtranscriptome of neutrophils, following *in vitro* infection with *L. infantum*, may impair phagocytosis, apoptosis and decrease nitric oxide production. Our work sheds light on several mechanisms used by *L. infantum* to control neutrophil-mediated immune response and identifies several targets for future functional studies, aiming at the development of preventive or curative treatments for this prevalent zoonosis.

2.3 Author Summary

Visceral leishmaniasis is a neglected tropical disease that causes fever, weight loss, anemia and swelling of liver and spleen. About 2500 cases are reported annually in the Americas, with a high mortality rate. Understanding how the immune system of people with visceral leishmaniasis responds to this parasite is essential for the development of preventive and curative methods. In order to understand how gene expression is modulated during visceral leishmaniasis, we infected *in vitro* cultured human neutrophils, the first immune cells to be recruited in this infection, with *Leishmania infantum*, the protozoan that causes visceral leishmaniasis in the Americas. Next, we measured the expression of coding RNAs, responsible for the production of proteins required for an effective immune response, and of non-coding RNAs, able to control these coding RNAs, thus helping or hindering the host response to infection. Analysis of all RNAs affected by infection points to an attempt of the parasite to control the host cells in order to remain safe within neutrophils, contributing to disease persistence. Our work identifies several targets for future functional studies, aiming at the development of preventive or curative treatments for this prevalent zoonosis.

2.1 Introduction

Leishmania genus cause the neglected tropical disease leishmaniasis, some *Leishmania* spp. cause cutaneous injury (e.g. *L. major*, *L. mexicana*, *L. amazonensis*, *L. braziliensis*) while other species can cause damage to internal organs resulting in visceral leishmaniasis (VL) (e.g. *L. donovani*, *L. infantum*) [1]. In the Americas, *L. infantum* (syn. *chagasi*) is the main cause of VL in mammals, especially humans (69,665 cases from 2001 to 2021) [2]. In 2021, 93.5% of human VL cases reported in the American continent occurred in Brazil, leading to the highest mortality rate since 2012 [2].

Immune response is paramount to the establishment of defense against infections [3]. In innate immunity, macrophages are already established as the main *Leishmania* host cells and, through some mechanisms such as the formation of NLRP3 inflammasome, these cells are able to mobilize neutrophils to the site [4]. Neutrophils also have known functions in the early stages of *Leishmania* infection, such as the formation of neutrophil extracellular traps and cytokine production [5]. Notwithstanding, authors suggest that neutrophils serve as shelter for *Leishmania* prior to the infection of macrophages [6–8], and that *Leishmania* is able to attenuate neutrophil action [9], while others defend the importance of neutrophil recruitment and its protective role [10]. It is consensus that neutrophil action seems to vary according to the infecting *Leishmania* species [11]. Whether this duality susceptibility *vs.* resistance in neutrophils is due to transcriptome and epigenetic changes remains to be elucidated.

Changes in global expression of messenger RNAs (mRNAs) are able to clarify the response to metabolic disorders, i.e. obesity [12], autoimmunity, cancer immunity, as well as infectious immune response against a plethora of pathogens [reviewed [13,14]]. Transcriptomic studies were conducted in dogs, mice and humans infected with several *Leishmania* species [15–20]. In 2022 Maruyama *et al.* [21] used whole blood mRNA-Seq analysis to investigate co-expression between mRNA and long non-coding RNAs (lncRNAs) during *L. infantum* natural infection and, most recently, Fernandes *et al.* [22] investigated the influence of three *Leishmania* species, including *L. infantum*, on lncRNA and mRNA expression in human macrophages.

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LncRNAs are a type of non-coding RNA (ncRNA) that, despite its lower level of expression and poor conservation among species [23], are known to regulate numerous physiological and pathological processes, including host response to infectious diseases [24,25]. Its action can be better defined depending on its location within a cell, presence in the nucleus would indicate direct transcriptional control over DNA, whilst presence in the cytoplasm could indicate post-transcriptional action via binding to mRNAs or small ncRNAs, named microRNAs (miRNAs) [25].

MiRNAs are a short sequence (~22 nucleotides) ncRNA with post-transcriptional regulatory function [26,27], mediated by binding of the seed region (6~8 first nucleotides) of a mature miRNA [28] to the 3' UTR end of mRNAs, blocking translation, or breaking complementary mRNAs [29]. LncRNAs may bind with the seed regions of miRNAs and reduce the availability of these miRNAs, in yet another ncRNA regulatory process termed competitive-endogenous RNA [30]. Also, miRNA expression is temporally regulated, is varied in different tissues [31], and has already been implicated in host immune response [32–35]. In response to VL-causing species (*L. donovani* and *L. infantum*), miRNAs have been shown to be modulated in different hosts [36–41], and seem to regulate important immune mechanisms [42,43]. Literature also suggests that miRNAs produced by *Leishmania* may influence its pathogenicity [44].

Changes in mRNA and ncRNA expression occurring post *Leishmania* spp. infection are widely studied in macrophages, as previously reviewed [45,46]; however, studies are scarce regarding *Leishmania* infection in neutrophils [47], and absent in this cell type when we target *L. infantum* infection, the most relevant species for VL in the Americas. Thus, our aim was to analyze the co-expression of coding and non-coding transcripts in an acute infection of neutrophils by *L. infantum*, employing an in vitro model of acute neutrophilic response to this prevalent infection.

2.2 Results

2.2.1 Transcriptome expression profile is modulated during *in vitro* infection of human neutrophils by *L. infantum*

After exposure of neutrophils to *L. infantum*, our RNA-Seq analysis revealed 212 DEGs (log2(FC)±0.58, FDR≤0.05) (S1 Table). According to BioMart annotations, 202

DEGs were classified as mRNA, 6 were classified as lncRNA and 4 had other classifications (S2 Table). Furthermore, 202 of the total transcripts were downregulated by infection with *L. infantum*, while 10 were upregulated in the same comparison (Fig 1).

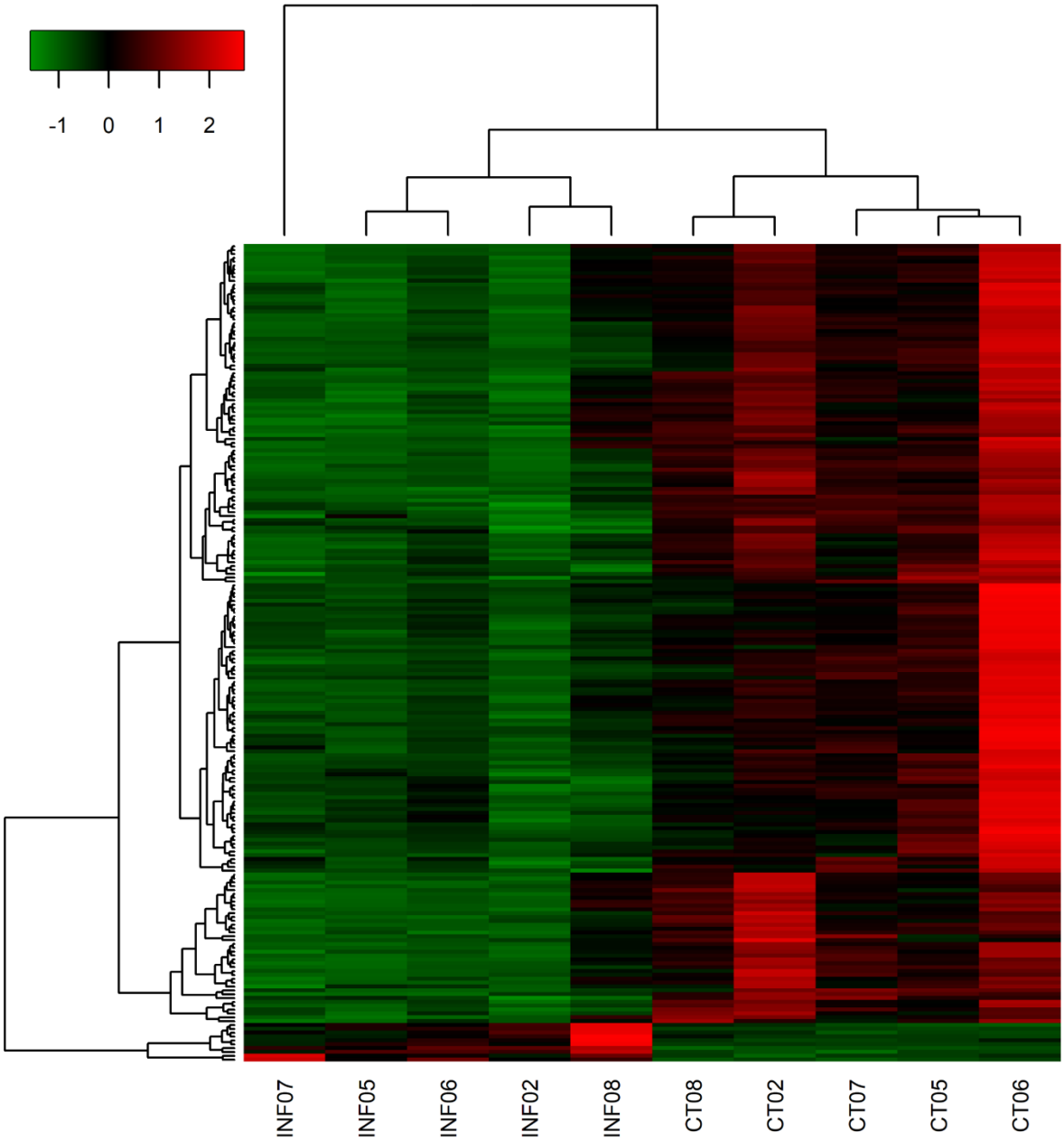

Fig 1. Heatmap of transcripts modulated by *L. infantum* in human neutrophils. Transcripts were grouped by hierarchical clustering. In vitro infection by *L. infantum* decreased expression of 202 transcripts and increased expression of 10 transcripts in human neutrophils. Heatmap shows normalized values for 212 differentially expressed transcripts (log2(FC)±0.58; FDR≤0.05), also found in S1 Table. This analysis was performed using heatmap3 package in RStudio and color scale represents z-score.

Functional enrichment analysis resulted in 131 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, including different pathways related to immune response, mostly innate immune response (S3 Table). The pathway with the highest percentage of DEGs was Leishmaniasis, as can be seen in Table 1, which shows the top 10 KEGG pathways regulated by DEGs after infection of neutrophils with *L. infantum*.

Table 1. Pathway enrichment analysis for differentially expressed genes following *in vitro* infection of human neutrophils by *L. infantum*.

Pathway	% DEG	p-adj	DEG/Total	DEGs
Leishmaniasis	21.13	3.60E-21	15/71	*IFNGR1, CYBA, NCF4, NFKBIA, NFKB1, PTPN6, TLR4, TLR2, NCF1, IFNGR2, CYBB, ITGAM, MYD88, RELA, HLA-DRA*
Legionellosis	16.67	3.0E-12	09/54	*NFKB2, NFKBIA, NFKB1, TLR4, TLR2, ITGAM, CD14, MYD88, RELA*
Antigen processing and presentation	14.93	4.7E-13	10/67	*CTSS, CTSB, B2M, TAP1, HLA-DRA, HLA-C, HLA-E, HLA-F, TAPBP, HLA-B*
Allograft rejection	14.71	4.6E-07	05/34	*HLA-DRA, HLA-C, HLA-E, HLA-F, HLA-B*
PD-L1 expression and PD-1 checkpoint pathway in cancer	13.64	5.6E-15	12/88	*IFNGR1, HIF1A, NFKBIA, NFKB1,*

				PTPN6, CD274, TLR4, TLR2, NFKBIE, IFNGR2, MYD88, RELA
Graft-versus-host disease	13.51	6.8E-07	05/37	*HLA-DRA, HLA-C, HLA-E, HLA-F, HLA-B*
Phagosome	13.14	2.2E-21	18/137	*CYBA, NCF4, RAB5C, TLR4, TLR2, RAB5A, NCF1, CTSS, CYBB, TAP1, ITGAM, CD14, FCAR, HLA-DRA, HLA-C, HLA-E, HLA-F, HLA-B*
Ferroptosis	12.82	8.7E-07	05/39	*GCLC, HMOX1, CYBB, FTH1, NCOA4*
NF-kappa B signaling pathway	12.75	1.1E-15	13/102	*CFLAR, NFKB2, ICAM1, NFKBIA, NFKB1, TNFAIP3, TLR4, BCL2A1, CD14, MYD88, RELA, LYN, CCL4*
Viral myocarditis	12.73	4.9E-09	07/55	*BID, ICAM1, HLA-DRA, HLA-C, HLA-E, HLA-F, HLA-B*

Table shows the top 10 pathways, organized according to the percentage of differentially expressed genes identified in the pathway, and contains the name of the pathway (Pathway), the percentage of differentially expressed genes (%DEGs), FDR value (p-adj) and ratio between identified DEGs in pathway and total number of genes in pathway, name of DEGs is also presented for each pathway.

2.2.2 Microtranscriptome expression profile is modulated during *in vitro* infection of human neutrophils by *L. infantum*

From 6027 probes representing the human species in Affymetrix® miRNA 4.1 Array strips, 446 were DE (FC±2, FDR≤0.01) in our experiment (S4 Table). Details regarding probe classification can be seen in S1 Fig.

We evaluate differential expression of mature miRNAs, since they can associate with seed region of expressed transcripts, preventing mRNA translation [48]. Thus, target prediction (see Methods) was carried out for 289 DE probes, representing mature miRNAs. From 289 miRNAs, 197 were increased in neutrophils post *L. infantum* infection, while 92 were decreased post-infection (p.i.) (Fig 2). In general, the action of miRNAs is inhibitory, impeding translation of mRNAs to proteins, so we considered as potential targets of upregulated miRNAs, all downregulated mRNA transcripts in the infected group of our RNA-Seq, whereas potential targets of downregulated miRNAs included the upregulated mRNAs in the same comparison.

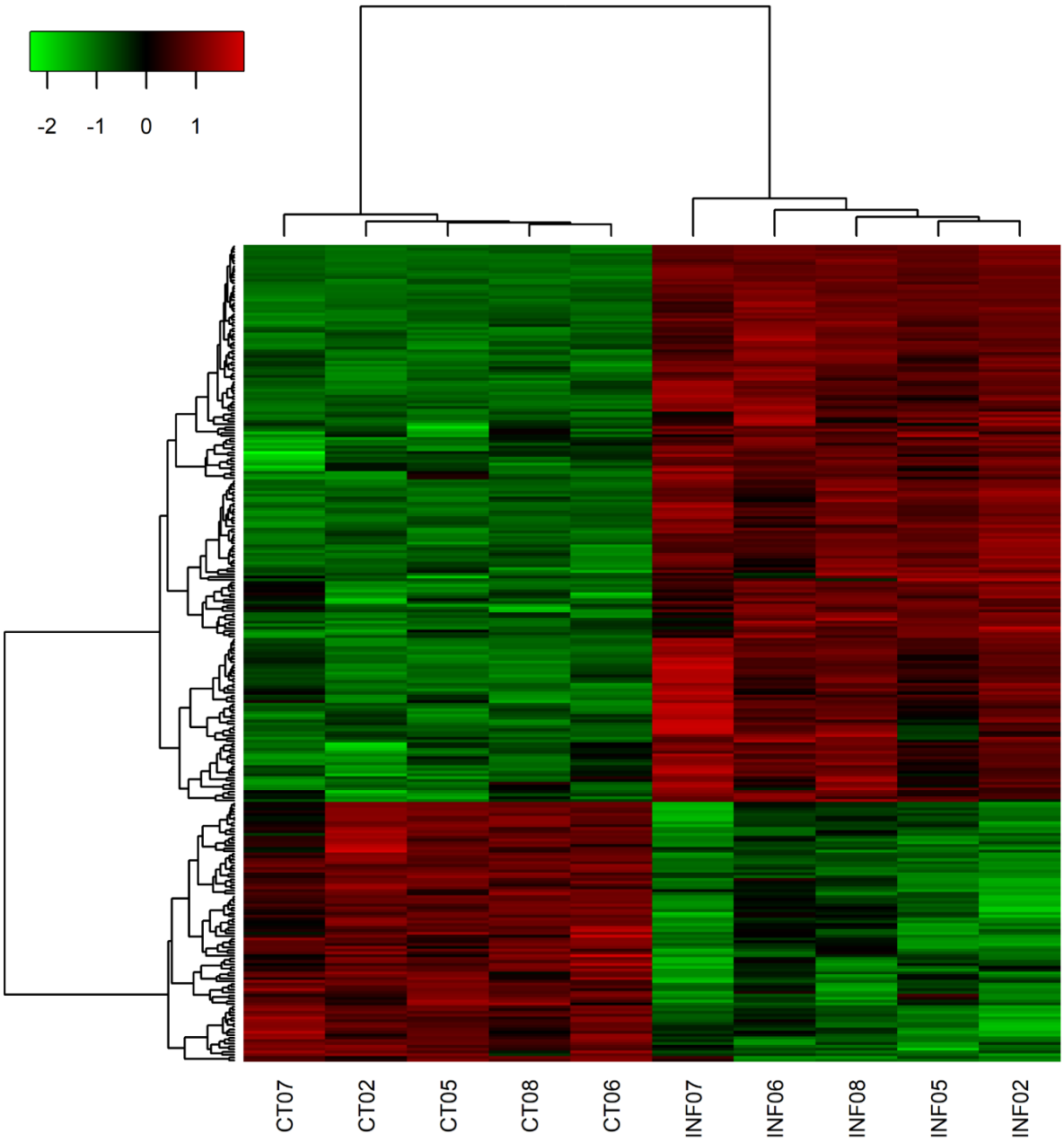

Fig 2. Heatmap of miRNAs modulated by *L. infantum* in human neutrophils. MiRNAs were grouped by hierarchical clustering. A total of 197 miRNAs were increased and 92 were decreased following *in vitro* infection of neutrophils with *L. infantum*. Heatmap shows corrected signal for 289 differentially expressed miRNAs (FC±2; FDR≤0.01). Analysis was performed using heatmap3 package in RStudio and color scale represents z-score.

For functional enrichment analysis, we compared targets predicted by TarBase, and added those predicted simultaneously in both, microT-CDS and TargetScan, with those DE coding transcripts in our data (Fig 3A). Thus, we found 556 downregulated transcripts, potential gene targets of the 197 upregulated miRNAs, while 193 upregulated transcripts have potential as targets for 92 downregulated miRNAs (Fig 3B, Fig 3C).

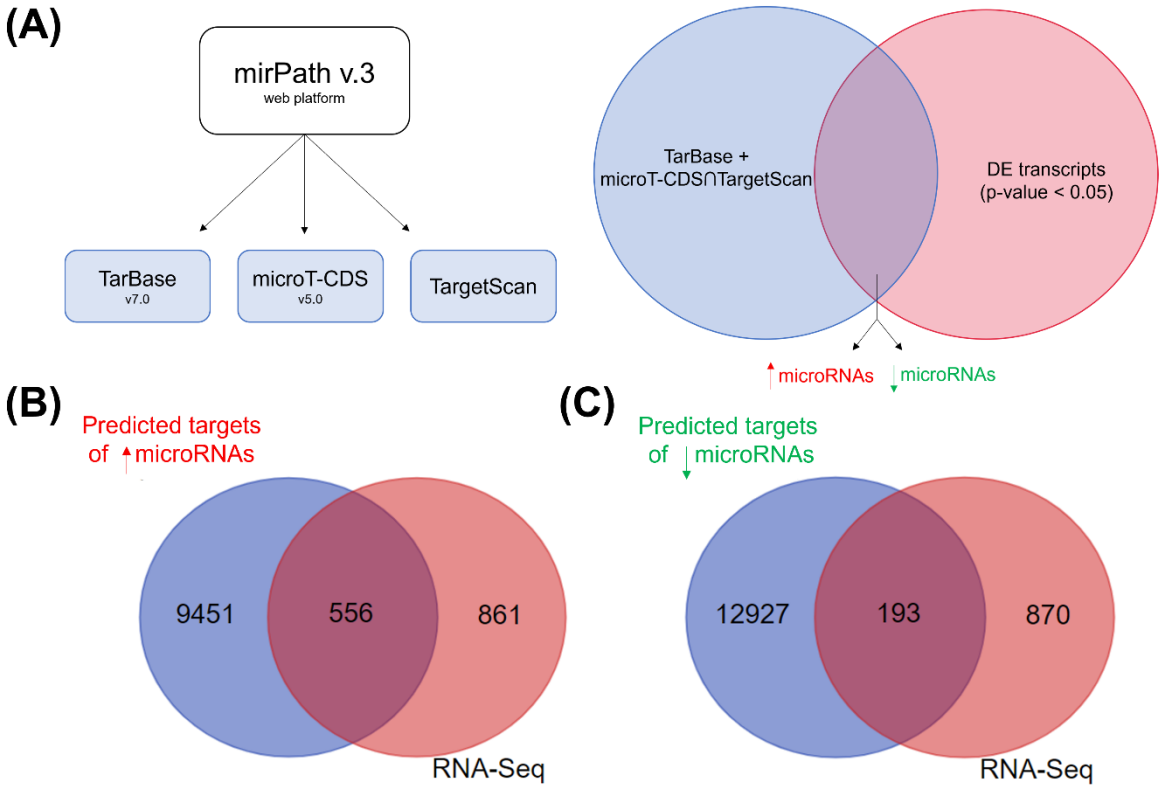

Figure 3. Target prediction of upregulated and downregulated miRNAs following *in vitro* infection of human neutrophils by *L. infantum*. (A) Differentially expressed miRNAs were divided according to expression patterns (up- or downregulated). DIANA-miRPath v3.0(https://dianalab.ece.uth.gr/html/mirpathv3/index.php?r=mirpath) web platform was used to access three target prediction databases (TarBase, microT-CDS and TargetScan) for both, and then TarBase plus microT-CDS∩TargetScan predicted targets were compared to transcripts in our RNA-Seq analysis (p-value≤0.05). (B) 556 downregulated transcripts (p-value≤0.05) were found as potential targets of upregulated miRNAs. (C) 193 upregulated transcripts (p-value≤0.05) were found as potential targets of downregulated miRNAs.

2.2.3 Changes in microtranscriptome expression profile following *L. infantum* infection of neutrophils affects immunological pathways

We analyzed up and downregulated miRNA target genes with the g:GOSt tool, from gProfiler, resulting in functional enrichment analysis using KEGG database. For those downregulated transcripts in infected neutrophils, potential target genes of upregulated miRNAs, 181 pathways were enriched (FDR≤0.05), highlighting the Leishmaniasis Pathway, and several other inflammatory pathways such as Antigen processing and presentation, Toll-like receptor signaling pathway, NF-kappa B signaling pathway, JAK-STAT signaling pathway, among others. Top 10 pathways, organized according to the percentage of DEGs identified in the pathway, can be seen

below (Table 2). Meanwhile, 29 pathways had FDR≤0.05 when using the upregulated transcripts in infected cells, potential targets of downregulated miRNAs. Among them, we predominantly observed the presence of physiological and constitutive cellular processes. All pathways regulated by both, upregulated and downregulated target transcripts, can be found in S5 Table.

Table 2. Pathway enrichment analysis for downregulated transcripts, targets of upregulated miRNAs, following *in vitro* infection of human neutrophils by *L. infantum*.

Pathway	% DEG	p-adj	DEG/Total	DEGs
Antigen processing and presentation	20.90	1.40E-14	14/67	*B2M*, *CD74*, *CTSS*, *HLA-A*, *HLA-B*, *HLA-C*, *HLA-E*, *HSPA5*, *PSME1*, *PSME2*, *RFX5*, *TAP1*, *TAP2*, *TAPBP*
Primary immunodeficiency	18.92	1.35E-07	07/37	*ADA*, *IL2RG*, *ORAI1*, *PTPRC*, *RFX5*, *TAP1*, *TAP2*
B cell receptor signaling pathway	18.42	7.93E-14	14/76	*GRB2*, *IKBKB*, *INPPL1*, *LILRB2*, *LILRB3*, *LYN*, *NFKB1*, *NFKBIA*, *NFKBIB*, *PIK3AP1*, *PIK3CD*, *RAC1*, *RAC2*, *RELA*
Viral myocarditis	18.18	3.67E-10	10/55	*ACTB*, *BID*, *EIF4G1*, *HLA-A*, *HLA-B*, *HLA-C*,

				HLA-E, *ICAM1*, *RAC1*, *RAC2*
Prolactin signaling pathway	17.91	6.73E-12	12/67	*CCND2*, *CISH*, *GRB2*, *IRF1*, *NFKB1*, *PIK3CD*, *RELA*, *SHC1*, *SOCS3*, *STAT1*, *STAT3*, *STAT5A*
Chronic myeloid leukemia	17.81	9.57E-13	13/73	*BCL2L1*, *E2F3*, *GAB2*, *GRB2*, *IKBKB*, *MDM2*, *NFKB1*, *NFKBIA*, *PIK3CD*, *RELA*, *SHC1*, *SMAD3*, *STAT5A*
Pancreatic cancer	17.57	1.12E-12	13/74	*BCL2L1*, *E2F3*, *IKBKB*, *NFKB1*, *PIK3CD*, *RAC1*, *RAC2*, *RALB*, *RALGDS*, *RELA*, *SMAD3*, *STAT1*, *STAT3*
PD-L1 expression and PD-1 checkpoint pathway in cancer	17.05	3.15E-14	15/88	*EML4*, *HIF1A*, *IFNGR1*, *IFNGR2*, *IKBKB*, *MAP2K3*, *MYD88*, *NFKB1*, *NFKBIA*, *NFKBIB*, *PIK3CD*, *RELA*, *STAT1*, *STAT3*, *TICAM1*
Fc gamma R-mediated phagocytosis	17.02	4.48E-15	16/94	*ACTR3*, *ARPC5*, *CFL1*,

				GAB2, GSN, HCK, INPPL1, LIMK2, LYN, MARCKS, PIK3CD, PTPRC, RAC1, RAC2, VASP, WAS
Leishmaniasis	16.90	1.33E-11	12/71	*C3, IFNGR1, IFNGR2, ITGAM, MYD88, NCF4, NFKB1, NFKBIA, NFKBIB, RELA, STAT1, TAB2*

Table shows the top 10 pathways, organized according to the percentage of differentially expressed genes identified in the pathway, and contains the name of the pathway (Pathway), the percentage of differentially expressed genes (%DEGs), FDR value (p-adj) and ratio between identified DEGs in pathway and total number of genes in pathway, name of DEGs is also presented for each pathway.

2.2.4 Long non-coding RNAs expressed in neutrophils, following *in vitro* infection with *L. infantum*, have *cis* and *trans* action

LncRNAs exert transcriptional and post-transcriptional regulation of gene expression [49]. After acute *in vitro* exposure of neutrophils to *L. infantum*, we observed a total of six DE lncRNAs (log2(FC)±0.58; FDR≤0.05). *HIF1A-AS3* was upregulated, while *PELATON, SLC39A13-AS1, SERPINB9P1, MIR3945HG* and *LINC01093* were reduced in neutrophils p.i. (Fig 4).

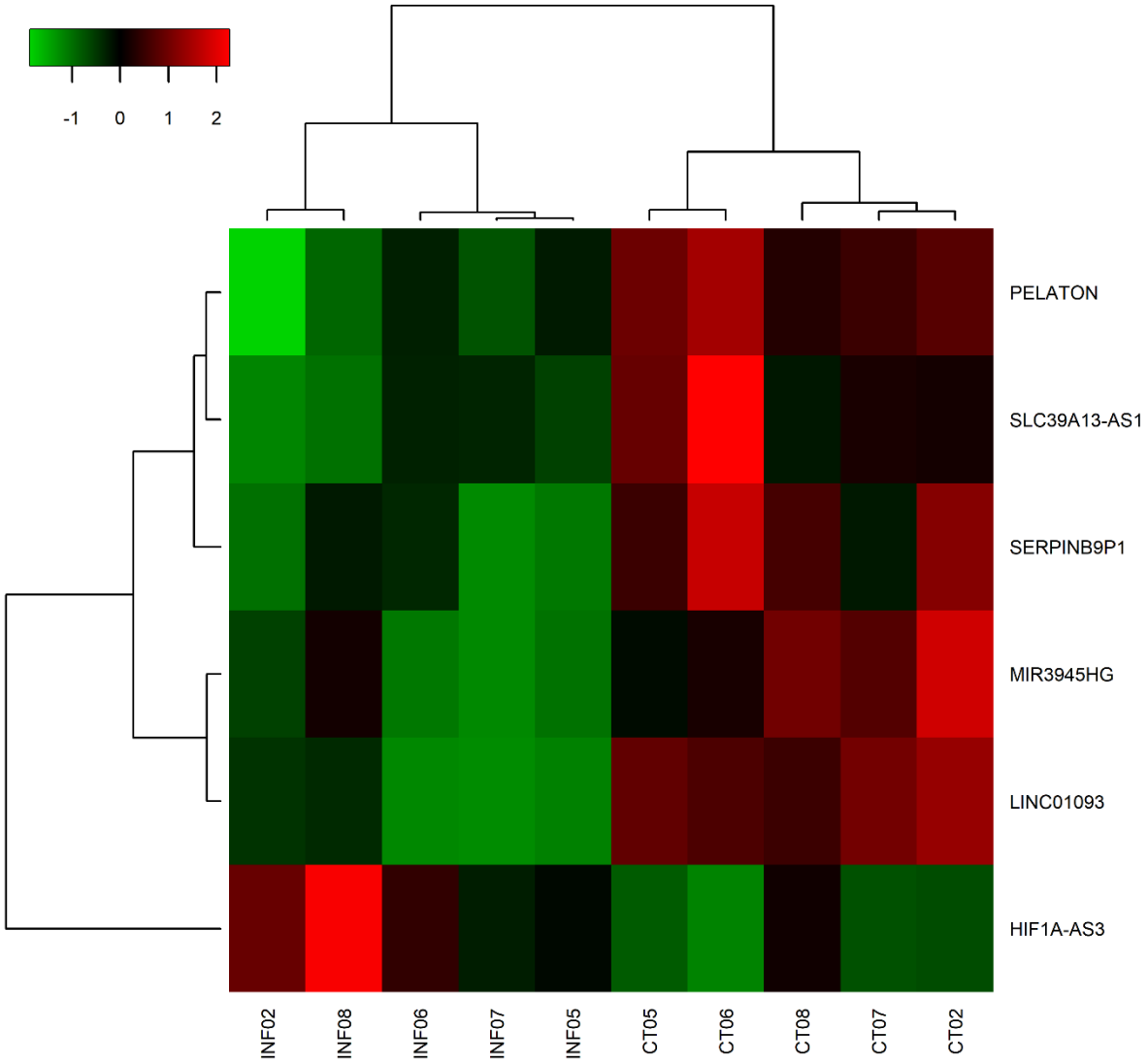

Figure 4. Heatmap of lncRNAs modulated by *L. infantum* following *in vitro* infection of human neutrophils. LncRNAs were grouped by hierarchical clustering. One lncRNA (*HIF1A-AS3*) was up regulated after *in vitro* infection with *L. infantum*, while five (*PELATON, SLC39A13-AS1, SERPINB9P1, MIR3945HG* and *LINC01093*) were down regulated. Heatmap shows normalized values for six differentially expressed transcripts (log_2(FC)±0.58; FDR≤0.05), classified as lncRNAs, using BioMart documentation (release 103). Analysis was performed using heatmap3 package in RStudio and color scale represents z-score.

We classified lncRNAs in *cis* (lncRNA and target in chromosomal proximity) or *trans* (lncRNA and target distant from one another). After applying Pearson's correlation on mRNA/lncRNA pairs (|r|≥0.8; p-value≤0.05), we observed 342 significant interactions. Pair *SPI1/SLC39A13-AS1* (r=0.96; p-value<0.01) has been classified as *cis*-acting by the FEELnc algorithm. And 175 pairs, including all six lncRNAs DE, with ndG≤-0.10, according to the LncTar tool, were predicted to have a distant action (*trans*-targets) (S6 Table).

2.2.5 Network analysis of DE mRNAs, DE lncRNAs and enriched pathways

For a broad view between co-expression of mRNAs/lncRNAs and their relationship with enriched pathways, we performed network analysis, using g:GOSt tool, from gProfiler platform (https://biit.cs.ut.ee/gprofiler/gost) and Cytoscape software (version 3.9.0) (https://cytoscape.org/). The trans-acting *SERPINB9P1* lncRNA and its positively correlated ($|r| \geq 0.8$; p-value≤ 0.05) mRNAs had the highest binding potential (ndG$\leq$-0.20) and is presented in Fig 5, highlighting once again the Leishmaniasis pathway and others closely related to immune response processes.

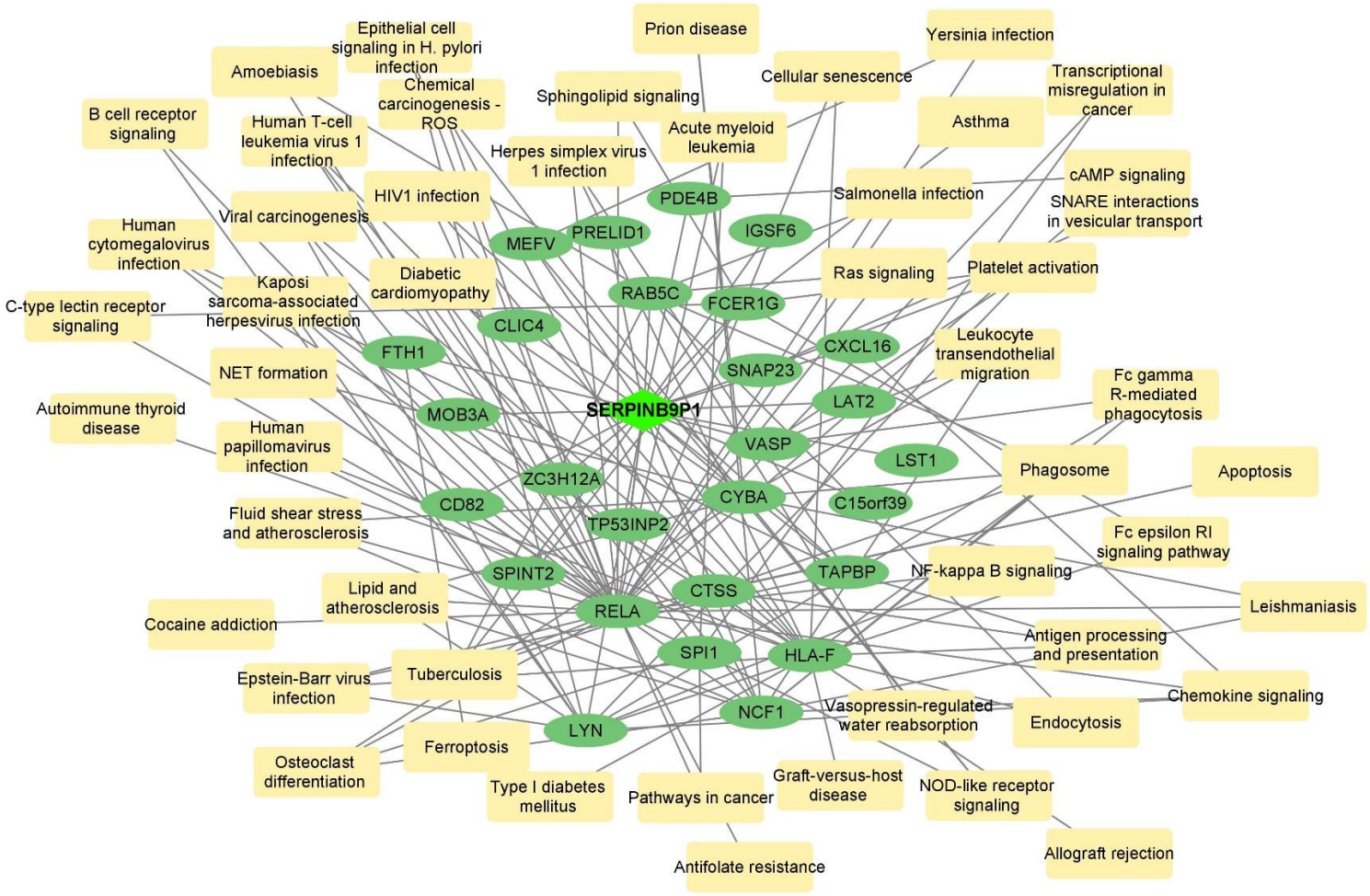

Figure 5. Network of co-expressed mRNAs/lncRNA and their enriched pathways, following *in vitro* infection of human neutrophils by *L. infantum*. LncRNA *SERPINB9P1* and partner mRNAs, all positively correlated (|r|≥0.8; p-value≤0.05 and ndG≤-0.20), are shown. Pathways obtained after enrichment analysis of co-expressed transcripts are also represented. Diamond shape corresponds to lncRNA, ellipses correspond to mRNAs and rectangles to pathways. Analysis was performed using Cytoscape software (version 3.9.0).

2.2.6 Leishmaniasis pathway is regulated by mRNAs, miRNAs and lncRNAs

Of the 71 genes that compose the Leishmaniasis pathway, 15 (*CYBA, CYBB, HLA-DRA, IFNGR1, IFNGR2, ITGAM, MYD88, NCF1, NCF4, NFKB1, NFKBIA, PTPN6, RELA, TLR2* and *TLR4*) are DEGs downregulated (log2FC≤-0.58; FDR≤0.05), and four (*C3, NFKBIB, STAT1* and *TAB2*) downregulated (p-value≤0.05) are miRNA targets, after *in vitro* infection of neutrophils by *L. infantum*. Of the 19, 12 are targets of p.i. upregulated miRNAs (Table 3). Also, DE mRNAs are positively correlated pairs of 5 DE lncRNAs (Fig 6). In summary, our DEGs regulate inflammatory signaling, phagocytosis and apoptosis in the Leishmaniasis pathway (Fig 7).

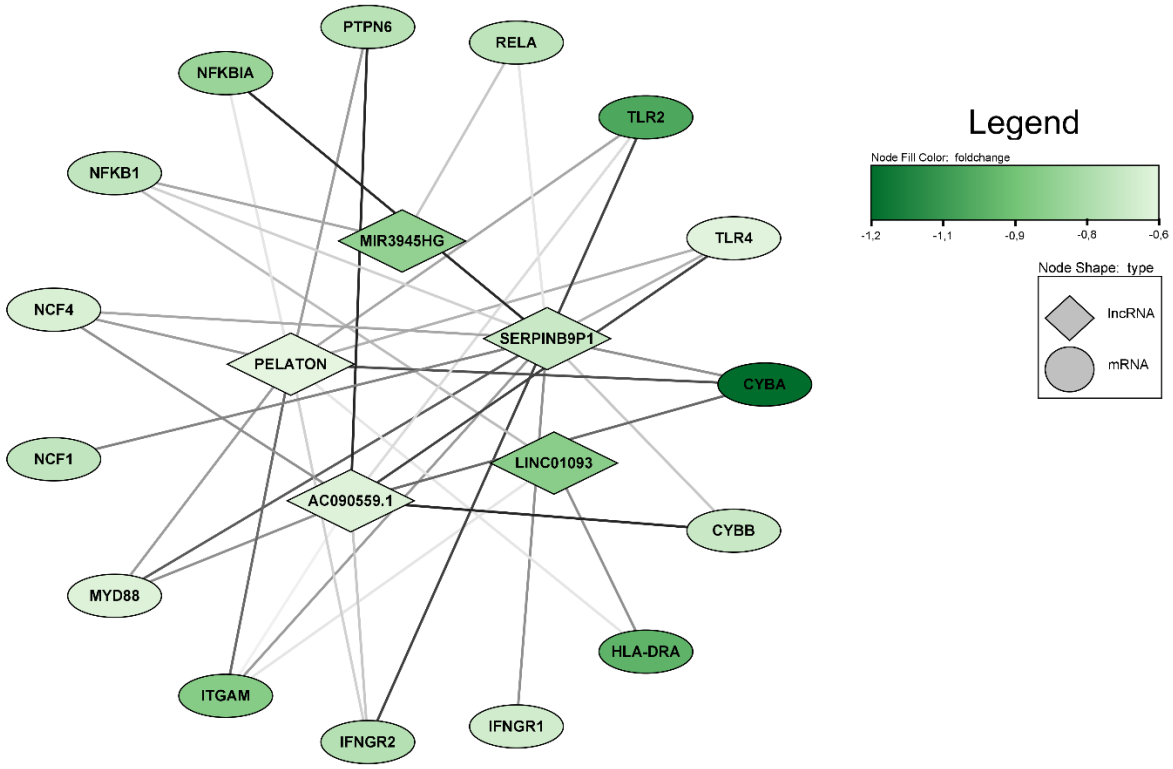

Figure 6. Network of co-expressed mRNAs/lncRNA present in Leishmaniasis Pathway. mRNA/lncRNA pairs, positively correlated (|r|≥0.8; p-value≤0.05 and ndG≤-0.10) enriching the Leishmaniasis pathway are show, color of the lines varies according to correlation value. The higher |r|, darker the line. Diamond shape corresponds to lncRNA and ellipses correspond to mRNAs. Analysis was performed using Cytoscape software (version 3.9.0).

Table 3. Leishmaniasis pathway.

Pathway	% DEG	p-adj	DEG/Total	DEGs			miRNAs		
				Name	log2FC	p-adj	Name	FC	p-adj
Leishmaniasis	21.13	3.60E-21	15/71	*C3*	-0.61	5.34E-02	miR-642b-3p	10.08	1.27E-06
				CYBA	-1.20	7.59E-08		N/A	
				CYBB	-0.70	2.15E-02		N/A	
				HLA-DRA	-0.97	5.30E-06		N/A	
				IFNGR1	-0.68	2.37E-02	miR-675-5p	4.14	1.00E-04
				IFNGR2	-0.75	6.08E-03	miR-4443	10.34	1.27E-06
				ITGAM	-0.87	5.72E-07	miR-8064	3.64	8.80E-06
							miR-6867-5p	2.88	5.60E-03
				MYD88	-0.65	4.79E-03	miR-7152-3p	4.81	1.30E-03
							miR-6867-5p	2.88	5.60E-03
				NCF1	-0.72	1.53E-02		N/A	
				NCF4	-0.66	3.34E-03	miR-3944-5p	2.68	3.40E-03
				NFKB1	-0.72	4.34E-03	miR-675-5p	4.14	1.00E-04
				NFKBIA	-0.82	3.07E-03	let-7g-3p	3.78	4.90E-03
				NFKBIB	-0.35	3.41E-01	miR-6882-3p	2.63	3.60E-03
				PTPN6	-0.74	6.22E-03		N/A	
				RELA	-0.73	8.58E-04	miR-765	6.39	5.76E-05
							miR-564	3.98	1.50E-03
							miR-4437	3.42	6.00E-03
				STAT1	-0.33	4.80E-01	miR-198	5.75	3.00E-04
				TAB2	-0.44	2.32E-01	miR-4484	2.18	2.00E-03
				TLR2	-1.01	2.11E-05		N/A	
				TLR4	-0.64	2.01E-02		N/A	

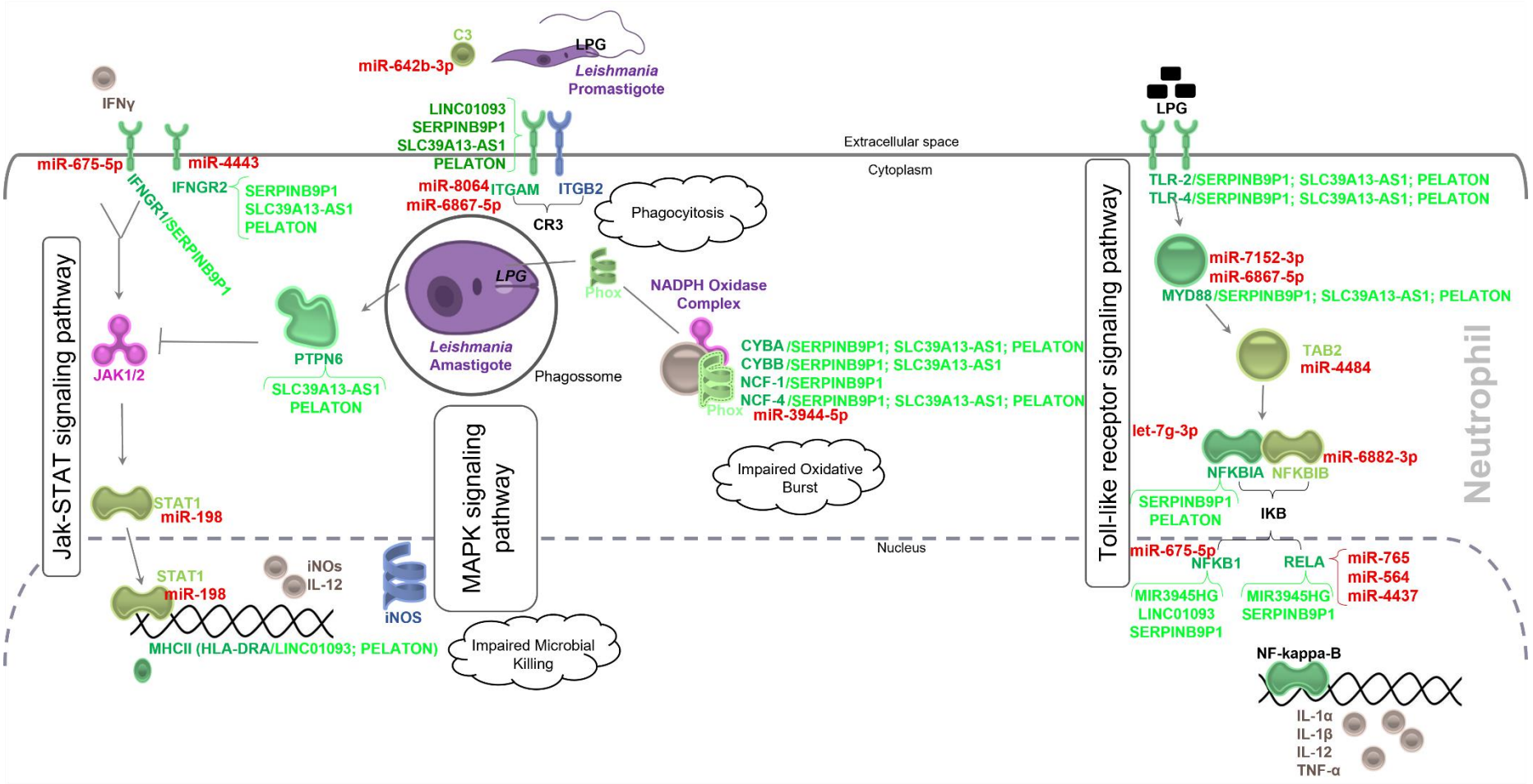

Figure 7. Regulation of the Leishmaniasis pathway following *in vitro* infection of human neutrophils by *L. infantum*. *L. infantum* infection impairs multiple immune signaling pathways, negatively affecting processes such as phagocytosis, apoptosis, and NO production, and can also impair pro-inflammatory signaling in human neutrophils. This suggests that *L. infantum* can regulate host response through increased expression of miRNAs. Green color represents downregulation of mRNA and correlated lncRNAs, red color indicates upregulation of miRNAs, molecule shape was based on the online tool https://targetexplorer.ingenuity.com/, and track functionality was based on KEGG database.

2.3 Discussion

Our results have shown that exposure of human neutrophils to *L. infantum* leads to significant changes in the expression of coding and non-coding transcripts. These transcripts act in several immune related pathways, and the Leishmaniasis pathway was enriched with the highest percentage of DEGs (21.13%), all downregulated after *L. infantum* infection, yet more than half of DEGs are targets of p.i. upregulated miRNAs.

As part of the Leishmaniasis pathway, both units of interferon-gamma (IFNγ) receptor were decreased in our neutrophils following *L. infantum* infection. Interestingly, Interferon Gamma Receptor 1 (*IFNGR1*) (log_2(FC)=-0.68, FDR=0.02) is a predicted target of the p.i. upregulated miR-675-5p (FC=4.14, FDR<0.01) and is positively correlated to *SERPINB9P1* (log_2(FC)=-0.70, FDR<0.01) lncRNA. In addition, Interferon Gamma Receptor 2 (*IFNGR2*) (log_2(FC)=-0.75, FDR<0.01) is a predicted target of the upregulated miR-4443 (FC=10.34, FDR<0.01), besides being correlated to three lncRNAs (*SERPINB9P1, SLC39A13-AS1* and *PELATON*). In macrophages, recognition of IFNγ increases nitric oxide synthase (iNOS) production and therefore the production of nitric oxide, favoring the elimination of *Leishmania* amastigotes [48]. Likewise in our neutrophils, we propose that IFNγ signal transduction is impaired due to downregulation of its receptors, likely mediated by upregulated ncRNAs. Interference with Jak-STAT signaling pathway and decrease of *STAT1*, by upregulation of miR-198 (FC=5.75, FDR<0.01), further supports a failure in transcriptional activation of iNOS, thus suggesting a possible impairment of NO production in infected neutrophils.

An axis of the Leishmaniasis pathway mediates *Leishmania* phagocytosis and involves some of our DE transcripts, namely Complement receptor 3 (*CR3* or *CD11*) and the complement factor *C3*. We observed *C3* downregulation p.i. (log_2(FC)=-0.61, FDR=0.05), and propose that this could be mediated by upregulation of miR-642b-3p (FC=10.08, FDR<0.01). In mice, lack of *C3* complement impairs progression of skin lesions caused by *L. major*, decreasing the presence of neutrophils during infection [49]. *CR3* is formed by two subunits: Integrin Subunit Alpha M (*ITGAM*), e Integrin Subunit Beta 2 (*ITGB2*). *ITGAM*, also called *CR3a*, is decreased p.i. in our experiment (log_2(FC)=-0.87, FDR<0.01), and is a target of the upregulated miR-8064 and miR-6867-5p. This subunit is also positively correlated with *LINC01093* (log_2(FC)=-0.86,

FDR<0.01), *SERPINB9P1* (log_2(FC)=-0.70, FDR<0.01), *SLC39A13-AS1* (log_2(FC)=-0.65, FDR<0.05), and *PELATON* (log_2(FC)=-0.63, FDR<0.01), and works as a neutrophil priming marker [50]. Neutrophil priming, first described by McPhail and Snyderman [51], regulates the intensity of neutrophil response at the site of inflammation [52] and enhances superoxide production. This process can occur in two ways: 1) rapid, with granule release or 2) delayed, activating transcription factors and stimulating cytokine production [52]. The increase of miR-8064 and miR-6867-5p could affect phagocytosis in neutrophils by decreasing *CD11*, following initial parasite internalization. After internalization of *Leishmania,* lipophosphoglycan present in phagocytosed amastigotes can interfering with NADPH Oxidase Complex formation by preventing *CYBA/CYBB* and *NCF1/NCF2/NCF4* binding, which leads to impaired oxidative burst and *Leishmania* resistance against host immune response [53]. Our results demonstrate that four components (*CYBA, CYBB, NCF1* and *NCF4* mRNAs) of the NADPH Oxidase Complex were decreased following *L. infantum* infection. Thus, downregulation of these transcripts and an increase of miR-3944-5p, accounting directly for the decrease seen in its target, *NCF4* mRNA, could represent a mechanism of survival elicited by infection.

Within the Leishmaniasis pathway, members of the Toll-like receptor signaling pathway (*TLR2, TLR4, MYD88, NFKBIA, NFKB1* and *RELA*) were all downregulated in neutrophils following *L. infantum* infection, and co-expressed with our five downregulated lncRNAs *(LINC01093, MIR3945HG, SERPINB9P1, SLC39A13-AS1* and *PELATON*). Interestingly, we observe several upregulated miRNAs (let-7g-3p, miR-4437, miR-564, miR-675-5p, miR-6867-5p, miR-6882-3p, miR-7152-3p and miR-765) predicted to target and, therefore, negatively regulate the aforementioned transcripts of the Toll-like receptor signaling pathway, likely impairing production of several pro-inflammatory cytokines, in our model. Effective killing of *Leishmania* and consequent control of infection appears to depend on correct downstream TLR signaling in other innate immune system cells [54–56]. In our experiment, members of the Toll-like receptor signaling pathway are targets of upregulated miRNAs, suggesting that the presence of the parasite affects immune response, at least partly, through modulation of miRNA expression in neutrophils.

We found 289 miRNAs DE after *L. infantum* infection in neutrophils, 197 up and 92 downregulated p.i.. Previously described as players in the pathogenicity of *Leishmania* [44] miRNAs seem to greatly influence the enriched pathways in our model. Functional

enrichment of upregulated miRNA targets resulted in 181 pathways, predominantly related to immune response. We emphasize that 122 pathways are common to those enriched by all DEGs, despite being targets or not of miRNAs. The Leishmaniasis pathway, our focus herein, has 7% (14/197) of all upregulated miRNAs controlling the decrease of genes that participate in this pathway.

Our top five miRNAs with the highest fold change were miR-3167, miR-4688, miR-7155-5p, miR-552-3p and miR-642a-3p. So far, none of these miRNAs have been linked to *Leishmania* infection. But the enrichment of their 19 targets is related to glycosaminoglycan biosynthesis. In macrophages infected with *L. major*, degradation of glycosaminoglycans, and consequent carbon availability, is favorable for parasite survival. As our upregulated miRNAs seem to try to prevent the production of glycosaminoglycans, the unavailability of this molecule may be unfavorable to the presence of *Leishmania* parasites [57], indicating that our top five upregulated miRNAs act protectively against *Leishmania* infection.

Expression increases for miRNAs following infection ranges from two to 261-fold. Among them, with the highest fold-change, miR-3167 had 19 predicted targets (microT-CDS∩TargetScan) and has never been reported in leishmaniasis. From all 19 predicted targets, only Metal Regulatory Transcription Factor 1 (*MTF1*) was DE ($\log_2$(FC) = -0.74; FDR<0.01) in our experiment. *MTF1* encodes a transcription factor that induces expression of metallothioneins, important low molecular weight proteins, responsible for carrying metals throughout the organism [58]. To date, only an unpublished study has associated *MTF1* to leishmaniasis, where knock-down of *MTF1* increases *L. panamensis* survival in THP-1 monocytes exposed to pentavalent antimonial drugs [59]. In macrophages, metallothioneins stimulate cytokine production [60], and since *MTF1* is required for their transcription, upregulation of miR-3167 could, in turn, negatively affect cytokine production.

In regards to the other type of ncRNA investigated in our study, lncRNAs are recently being studied in the context of *Leishmania* infection, these molecules with diverse regulatory functions seem to play a fundamental role in mounting immune response of the infected host [21,22]. In our neutrophil model, we found six lncRNAs DE, which represents less than 3% of DEGs, contrary to the findings by Fernandes *et al* who observed in macrophages infected with different species of *Leishmania* the presence of 24% of lncRNAs [22], a hypothesis for such a different finding would be, not only the use of different cells, but mainly the time of infection used, while the

macrophages were exposed, among others, to *L. infantum* for 24 hours, our neutrophils were processed after 20 hours of exposure to the parasites, which happened for only 3 hours.

We identified only one upregulated lncRNA – *HIF1A-AS3* (log_2(FC)=0.72, FDR=0.02) – during acute *L. infantum* infection. This lncRNA was recently cited as the third identified antisense RNA of Hypoxia-Inducible Factor 1-Alpha (*HIF1A*) gene [61], which in turn is differentially downregulated in our study (log_2(FC)=-1.36, FDR<0.01). We found no significant correlation between *HIF1A/HIF1A-AS3* pair. *HIF1A* gene limits the microbicidal ability of myelocyte-derived cells [62] and, in macrophages, *HIF1A* seems to play a fundamental role in survival of *L. donovani* at late phase (30 hours p.i.), but not at an earlier stage (6 hours p.i.) of infection [63]. Increase in *HIF1-AS3* appears to drive a positive feedback for the increase in *HIF1A* in human tumoral cells, through promoter transactivation [64]. Whether the *L. infantum* induced increase in *HIF1-AS3* lncRNA in our neutrophils could lead to an increase in *HIF1A* at later times, as part of the aforementioned positive feedback loop, remains to be investigated.

Our study is the first to investigate the transcriptome and microtranscriptome of human neutrophils following in vitro infection with *L. infantum*. Observed changes in mRNA and ncRNA expression may impair phagocytosis, apoptosis and decrease nitric oxide production, favoring pathogen survival, and may decrease neutrophil priming in the first 20 hours of infection. Our work sheds light on several mechanisms used by *L. infantum* to control neutrophil-mediated immune response and identifies several targets for future functional studies, aiming at the development of preventive or curative treatments for this prevalent zoonosis.

2.4 Materials and Methods

All procedures were approved by local Research Ethics Committee (protocol number 3.926.267). Informed-consent documents were signed by all blood donors.

2.4.1 Maintenance of parasites

L. infantum promastigotes were isolated from a dog diagnosed with VL and further characterized by Sanger sequencing. Strain was maintained at 26ºC in a complete Schneider medium (Sigma-Aldrich Co.), supplemented with 10% of inactivated bovine

fetal serum, 2% sterile male urine and 1% of penicillin-streptomycin. Promastigotes were tested for viability and absence of contamination and washed with phosphate buffered saline (PBS) (pH = 7.4) before infection of neutrophils.

2.4.2 Neutrophil purification

Peripheral blood was collected from five healthy male donors (range 28-44 age) in heparinized vacuum tubes. Whole blood was immediately separated using a discontinuous density gradient [65] Histopaque®-1119 (Sigma-Aldrich Co.). Briefly, heparinized blood was layered onto a double gradient Histopaque-1077/1119, and centrifuge at 700 × g for 30 minutes at room temperature, forming visible layers. Granulocyte layer was collected in a new tube, and any remnants red blood cells were hemolyzed with diluted (1:10) commercial solution Hemalise-RBC 10x (LGC Biotecnologia Ltda.). Cells were then PBS (pH = 7.4) washed, pelleted, and neutrophils (>97% following $CD45^+$ and $CD16^+$ flow cytometry analysis) were resuspended in commercial RPMI-1640/Hepes medium (LGC Biotecnologia Ltda.), supplemented with 10% of inactivated bovine fetal serum and 1% of penicillin-streptomycin.

Total number of neutrophils was counted in Neubauer chamber and viability (>98%) was estimated using Trypan Blue solution 0.4% (Life Technologies™).

2.4.3 *In vitro* infection

For each subject neutrophils (10^6/well) were separated into control and infected groups. Those of infected group were exposed to promastigotes at a ratio of five *L. infantum* for each neutrophil (Multiplicity of Infection - MOI = 5:1). After 3 hours at 37°C and 5% CO_2, neutrophils from the infected and control groups were washed with PBS (pH = 7.4) – in the infected group, PBS wash was used to remove non-internalized promastigotes, while in the control group same procedure was performed for sample pairing. At this point, cytocentrifugation technique was performed to confirm internalization of *L. infantum* amastigotes in the infected group (average internalization rate of 2 amastigotes/neutrophil). Following the wash, neutrophils were resuspended in RPMI supplemented medium, and replated, maintaining the infected and control groups. After 20 hours total, viable neutrophils (>97%) were washed and resuspended in PBS (pH = 7.4) for immediate total RNA extraction.

2.4.4 Total RNA extraction and quantification

Total RNA was extracted from $2x10^6$ neutrophils for each subject in both groups (n = 5 / group) using the miRVana kit (Thermo Fisher Scientific Co.) to preserve miRNAs, according to the manufacturer's instructions. Immediately after extraction, samples concentration was measured on a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific Co.). Total RNA was kept at -80ºC until further experiments were carried out.

2.4.5 RNA Sequencing (RNA-Seq) and functional enrichment analysis of differentially expressed transcripts

Total RNA-Seq libraries were constructed from 150 ng of total RNA, using Zymo-Seq RiboFree Total RNA Library Kit (Zymo Research Co.), according to manufacturer's instructions. Briefly, complementary DNA was synthesized using reverse transcription, ribosomal RNA was depleted, and adapters were attached before index library was amplified by Polymerase Chain Reaction. RNA-Seq libraries were sequenced on an Illumina NovaSeq sequencer to a sequencing depth of at least 30 million read pairs (150 bp paired-end sequencing) per sample, resulting in fastq files.

Quality control of raw reads (fastq files) was carried out using FastQC (Galaxy Version 0.73+galaxy0), available at the Galaxy web platform (www.usegalaxy.org). Illumina adapters were removed using Cutadapt (Galaxy Version 4.0+galaxy0) tool [66], and trimmed reads were aligned to human reference genome (assembly GRCh38.p13-v.34) with HISAT2 (Galaxy Version 2.2.1+galaxy0) [67]. Reads overlapping with exons were assigned to genes using FeatureCounts (Galaxy Version 2.0.1+galaxy2) [68]. Differential gene expression analysis was completed using DESeq2 (Galaxy Version 2.11.40.7+galaxy1) [69]. Genes with log2FoldChange (log2(FC))±0.58 and FDR≤0.05 were considered differentially expressed (DE).

Differentially expressed genes (DEGs) are subsequently used for functional enrichment analysis, performed using g:GOSt tool, from g:Profiler (version e108_eg55_p17_9f356ae) with Benjamini-Hochberg FDR multiple testing correction method applying significance threshold of 0.05.

2.4.6 Microtranscriptome array, target prediction and functional enrichment analysis

Total RNA (500 ng/sample) was labeled using the FlashTag™ Biotin HSR RNA Labeling Kit (Thermo Fisher Scientific Co.), according to the manufacturer's instructions, including enzyme-linked oligosorbent assay as quality control. Hybridization to the Affymetrix® miRNA 4.1 Array strips was carried out at 48ºC for 20 hours. Subsequently, strips were processed and scanned using the GeneAtlas® System (Affymetrix). Raw intensity values were background corrected, $\log_2$ transformed and then quantile normalized by the software Transcriptome Analysis Console (TAC) 4.0.1 (Thermo Fisher Scientific Co.) using the Robust Multi-array Average (RMA) algorithm. Statistical analysis was also performed in the TAC software 4.0.1 (Thermo Fisher Scientific Co.) by one-way ANOVA, comparing infected *vs.* control group.

DE miRNAs (FC±2, FDR≤0.01) were separated into increased or decreased expression following *L. infantum* infection. All transcripts' targets, available in the TarBase v7.0 database – a platform for previously experimentally observed targets – were considered [70]. Additionally, predicted transcripts in both microT-CDS v5.0 and TargetScan databases, simultaneously, were also considered [71,72]. DIANA-miRPath v3.0 (https://dianalab.e-ce.uth.gr/html/mirpathv3/index.php?r=mirpath) web platform was used to access all databases [73]. To improve our target prediction, we compared all predicted targets with our transcripts resulting from RNA-Seq analysis (p-value≤0.05), transcripts present in both were used as input for functional enrichment analysis with g:GOSt tool, from gProfiler platform (version e108_eg55_p17_9f356ae; https://biit.cs.ut.ee/gprofiler/gost) applying the Benjamini-Hochberg FDR multiple testing correction method set at 0.05.

2.4.7. Long non-coding RNA classification, mRNA/lncRNA co-expression and network interaction

All DEGs ($\log_2$(FC)±0.58; FDR≤0.05) were submitted to biotype classification with the BioMart tool (release 103; www.ensembl.org/biomart/martview/) [74]. According to annotations relied on Ensembl's coding/non-coding classification, transcripts biotyped as "lncRNA" were considered as lncRNAs, while those biotyped as "protein_coding" were considered as mRNAs, other classifications were not used for further analyses.

Pearson correlation coefficient was employed to calculate possible correlations between mRNA/lncRNA pairs (|r|≥0.8 and p-value≤0.05). Classifier Module of Flexible Extraction of Long Non-coding RNAs (FEELnc) tool [75] was utilized to predict nearby partner genes (those up to 100kbps distance), also called *cis*-targets. Further investigation with LncTar tool (http://www.cuilab.cn/lnctar), which estimates normalized free energy (ndG≤-0.10), was applied to predicted the potential of ligation between lncRNAs and probable mRNA targets [76]; mRNA/lncRNA pairs with matching potential binding were assumed to be *trans*-targets, i.e., with distant action. Gene co-expression networks were built using the Cytoscape software (version 3.9.0) (https://cytoscape.org/), with DE lncRNAs and their corresponding *cis* and *trans* target genes (|r|≥0.8; p-value≤0.05 and ndG≤-0.10). g:GOSt tool, from gProfiler platform (https://biit.cs.ut.ee/gprofiler/gost) was used to perform functional enrichment analysis using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways database, and also combined an integrated co-expression regulatory network.

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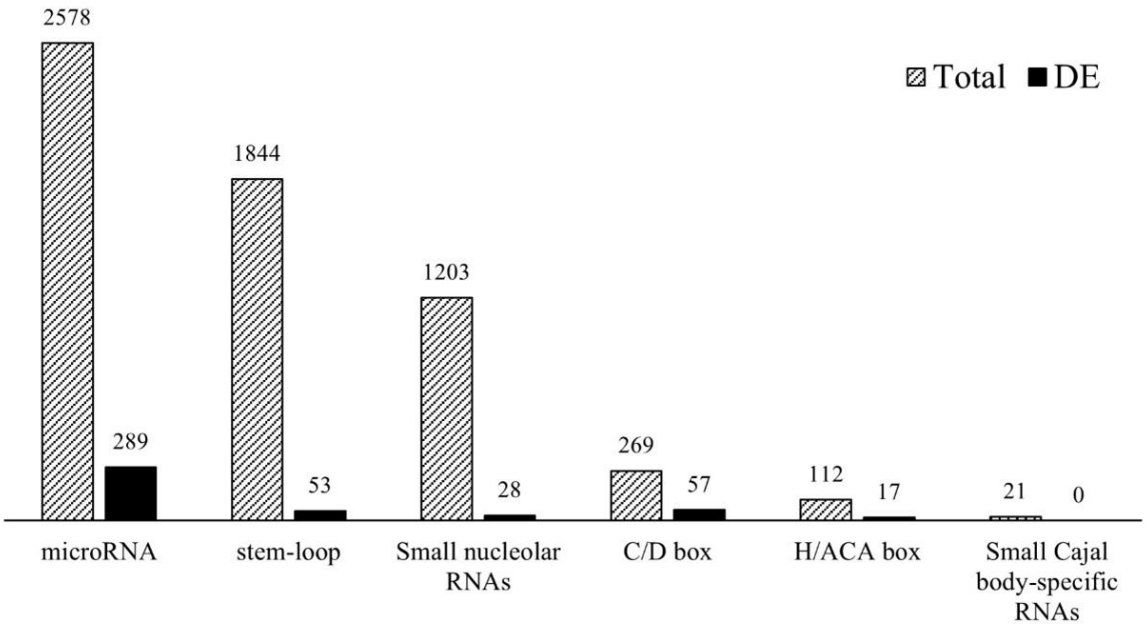
2.6 Supporting information



S1 Fig. Probe distribution of small RNAs according to Affymetrix classification. Column plot represents distribution of total probes and differentially expressed probes contained in Affymetrix miRNA 4.1 Array strips. There are six possible classifications: mature microRNAs, stem-loop miRNAs, Small nucleolar RNAs, C/D box, H/ACA box, Small Cajal body-specific RNAs.

S1 Table. Differentially expressed genes (DEGs), resulting from RNA-Seq analyzes. A total of 212 DEGs (log2(FC)±0.58 and FDR≤0.05), being 202 with decreased expression after *L. infantum* infection, and 10 with increased expression post-infection.

GeneID	Gene name	Gene type	log2(FC)	P-value	P-adj	Base mean	StdErr	Wald-Stats
ENSG00000197249	SERPINA1	protein_coding	-1.51	4.30E-15	8.30E-12	1097.63	0.19	-7.85
ENSG00000132965	ALOX5AP	protein_coding	-1.47	1.61E-16	5.44E-13	377.74	0.18	-8.25
ENSG00000169403	PTAFR	protein_coding	-1.46	1.19E-14	1.80E-11	674.08	0.19	-7.72
ENSG00000171051	FPR1	protein_coding	-1.42	2.70E-13	2.80E-10	292.87	0.19	-7.31
ENSG00000140749	IGSF6	protein_coding	-1.42	3.84E-17	2.59E-13	367.43	0.17	-8.42
ENSG00000169504	CLIC4	protein_coding	-1.41	2.53E-16	6.83E-13	221.96	0.17	-8.19
ENSG00000126262	FFAR2	protein_coding	-1.36	7.72E-13	6.52E-10	271.12	0.19	-7.17
ENSG00000100644	HIF1A	protein_coding	-1.36	8.01E-14	9.84E-11	612.80	0.18	-7.47
ENSG00000123700	KCNJ2	protein_coding	-1.34	2.75E-15	6.19E-12	303.16	0.17	-7.90
ENSG00000120217	CD274	protein_coding	-1.32	1.36E-16	5.44E-13	186.26	0.16	-8.27
ENSG00000137312	FLOT1	protein_coding	-1.31	1.49E-13	1.67E-10	1163.61	0.18	-7.39
ENSG00000077150	NFKB2	protein_coding	-1.30	4.49E-13	4.34E-10	1047.64	0.18	-7.24
ENSG00000100368	CSF2RB	protein_coding	-1.30	5.14E-13	4.63E-10	921.03	0.18	-7.22
ENSG00000150977	RILPL2	protein_coding	-1.24	1.24E-10	7.59E-08	653.17	0.19	-6.43
ENSG00000254087	LYN	protein_coding	-1.22	3.44E-10	1.73E-07	1061.95	0.19	-6.28
ENSG00000178719	GRINA	protein_coding	-1.21	4.68E-10	2.26E-07	270.68	0.19	-6.23
ENSG00000051523	CYBA	protein_coding	-1.20	1.29E-10	7.59E-08	536.33	0.19	-6.43
ENSG00000102096	PIM2	protein_coding	-1.20	5.56E-10	2.59E-07	320.42	0.19	-6.20
ENSG00000101336	HCK	protein_coding	-1.17	8.65E-11	5.92E-08	626.27	0.18	-6.49
ENSG00000101190	TCFL5	protein_coding	-1.16	1.20E-14	1.80E-11	161.12	0.15	-7.72
ENSG00000170458	CD14	protein_coding	-1.16	2.49E-09	1.02E-06	288.96	0.19	-5.96
ENSG00000125733	TRIP10	protein_coding	-1.13	3.35E-19	4.52E-15	259.78	0.13	-8.96
ENSG00000116514	RNF19B	protein_coding	-1.12	3.39E-09	1.31E-06	452.31	0.19	-5.91
ENSG00000137393	RNF144B	protein_coding	-1.12	3.15E-09	1.25E-06	357.40	0.19	-5.92

ENSG00000161921	CXCL16	protein_coding	-1.11	2.36E-09	9.96E-07	265.80	0.19	-5.97
ENSG00000104093	DMXL2	protein_coding	-1.10	2.09E-09	9.09E-07	907.44	0.18	-5.99
ENSG00000133048	CHI3L1	protein_coding	-1.08	5.79E-09	2.12E-06	648.98	0.19	-5.82
ENSG00000144802	NFKBIZ	protein_coding	-1.04	4.10E-08	1.18E-05	638.52	0.19	-5.49
ENSG00000116679	IVNS1ABP	protein_coding	-1.03	2.91E-10	1.57E-07	976.24	0.16	-6.30
ENSG00000127954	STEAP4	protein_coding	-1.03	1.56E-08	5.02E-06	342.37	0.18	-5.65
ENSG00000171049	FPR2	protein_coding	-1.03	2.70E-10	1.52E-07	224.60	0.16	-6.32
ENSG00000124102	PI3	protein_coding	-1.01	1.42E-07	3.56E-05	1635.09	0.19	-5.26
ENSG00000137462	TLR2	protein_coding	-1.01	7.80E-08	2.11E-05	379.39	0.19	-5.37
ENSG00000184557	SOCS3	protein_coding	-1.00	3.80E-07	8.56E-05	285.50	0.20	-5.08
ENSG00000102921	N4BP1	protein_coding	-0.99	1.91E-08	5.73E-06	641.59	0.18	-5.62
ENSG00000085514	PILRA	protein_coding	-0.99	8.58E-09	2.97E-06	172.02	0.17	-5.76
ENSG00000275302	CCL4	protein_coding	-0.98	7.30E-07	1.39E-04	394.65	0.20	-4.95
ENSG00000135842	NIBAN1	protein_coding	-0.98	5.03E-07	1.01E-04	948.07	0.19	-5.03
ENSG00000175857	GAPT	protein_coding	-0.97	9.12E-07	1.66E-04	217.56	0.20	-4.91
ENSG00000204287	HLA-DRA	protein_coding	-0.97	1.69E-08	5.30E-06	167.93	0.17	-5.64
ENSG00000011600	TYROBP	protein_coding	-0.96	4.35E-07	9.30E-05	203.34	0.19	-5.05
ENSG00000126561	STAT5A	protein_coding	-0.95	9.40E-13	7.47E-10	494.59	0.13	-7.14
ENSG00000143119	CD53	protein_coding	-0.95	9.09E-08	2.41E-05	470.50	0.18	-5.34
ENSG00000198814	GK	protein_coding	-0.95	1.23E-06	2.12E-04	363.74	0.19	-4.85
ENSG00000161791	FMNL3	protein_coding	-0.94	8.03E-09	2.86E-06	1175.46	0.16	-5.77
ENSG00000103569	AQP9	protein_coding	-0.94	1.88E-06	2.96E-04	1003.20	0.20	-4.77
ENSG00000185215	TNFAIP2	protein_coding	-0.94	1.24E-06	2.12E-04	1650.28	0.19	-4.85
ENSG00000066336	SPI1	protein_coding	-0.94	5.08E-07	1.01E-04	343.08	0.19	-5.02
ENSG00000155629	PIK3AP1	protein_coding	-0.94	6.57E-08	1.85E-05	764.02	0.17	-5.40
ENSG00000139572	GPR84	protein_coding	-0.94	4.75E-07	9.72E-05	152.99	0.19	-5.04
ENSG00000173442	EHBP1L1	protein_coding	-0.93	1.54E-06	2.58E-04	647.55	0.19	-4.81
ENSG00000114784	EIF1B	protein_coding	-0.93	1.15E-07	3.00E-05	111.51	0.17	-5.30
ENSG00000145901	TNIP1	protein_coding	-0.93	2.62E-07	6.11E-05	1258.28	0.18	-5.15

ENSG00000166523	CLEC4E	protein_coding	-0.92	3.27E-06	4.92E-04	398.25	0.20	-4.65
ENSG00000186431	FCAR	protein_coding	-0.91	1.36E-06	2.29E-04	133.94	0.19	-4.83
ENSG00000198053	SIRPA	protein_coding	-0.90	2.39E-07	5.68E-05	658.87	0.17	-5.17
ENSG00000107968	MAP3K8	protein_coding	-0.90	8.56E-07	1.58E-04	244.83	0.18	-4.92
ENSG00000229644	NAMPTP1	processed_pseudogene	-0.90	1.83E-06	2.91E-04	102.69	0.19	-4.77
ENSG00000137478	FCHSD2	protein_coding	-0.89	5.77E-09	2.12E-06	365.97	0.15	-5.82
ENSG00000182541	LIMK2	protein_coding	-0.88	7.10E-08	1.96E-05	609.00	0.16	-5.39
ENSG00000162783	IER5	protein_coding	-0.88	5.65E-06	7.71E-04	657.56	0.19	-4.54
ENSG00000142512	SIGLEC10	protein_coding	-0.88	9.40E-06	1.19E-03	359.85	0.20	-4.43
ENSG00000169896	ITGAM	protein_coding	-0.87	1.27E-09	5.72E-07	523.89	0.14	-6.07
ENSG00000234745	HLA-B	protein_coding	-0.86	2.89E-06	4.39E-04	2762.36	0.18	-4.68
ENSG00000167642	SPINT2	protein_coding	-0.86	1.11E-08	3.74E-06	249.66	0.15	-5.71
ENSG00000249173	LINC01093	lncRNA	-0.86	3.46E-10	1.73E-07	158.42	0.14	-6.28
ENSG00000146232	NFKBIE	protein_coding	-0.86	1.57E-06	2.59E-04	202.79	0.18	-4.80
ENSG00000155307	SAMSN1	protein_coding	-0.86	1.21E-06	2.12E-04	311.60	0.18	-4.85
ENSG00000140379	BCL2A1	protein_coding	-0.86	1.46E-05	1.68E-03	436.73	0.20	-4.34
ENSG00000142634	EFHD2	protein_coding	-0.86	6.44E-06	8.62E-04	444.05	0.19	-4.51
ENSG00000163874	ZC3H12A	protein_coding	-0.86	4.03E-06	5.86E-04	349.65	0.19	-4.61
ENSG00000125430	HS3ST3B1	protein_coding	-0.85	6.95E-07	1.34E-04	158.99	0.17	-4.96
ENSG00000110446	SLC15A3	protein_coding	-0.84	1.55E-07	3.82E-05	461.73	0.16	-5.25
ENSG00000251230	MIR3945HG	lncRNA	-0.84	1.74E-05	1.94E-03	218.71	0.20	-4.30
ENSG00000146112	PPP1R18	protein_coding	-0.84	1.68E-06	2.71E-04	732.86	0.18	-4.79
ENSG00000091317	CMTM6	protein_coding	-0.84	9.22E-06	1.17E-03	301.85	0.19	-4.43
ENSG00000197329	PELI1	protein_coding	-0.83	1.52E-05	1.74E-03	264.34	0.19	-4.33
ENSG00000157593	SLC35B2	protein_coding	-0.83	8.01E-07	1.50E-04	133.48	0.17	-4.94
ENSG00000204525	HLA-C	protein_coding	-0.83	1.98E-05	2.14E-03	1685.27	0.19	-4.27
ENSG00000155465	SLC7A7	protein_coding	-0.83	6.43E-12	4.83E-09	320.28	0.12	-6.87
ENSG00000140350	ANP32A	protein_coding	-0.82	1.26E-07	3.20E-05	621.40	0.16	-5.29
ENSG00000100906	NFKBIA	protein_coding	-0.82	3.00E-05	3.07E-03	1100.03	0.20	-4.17

ENSG00000266412	NCOA4	protein_coding	-0.82	1.81E-05	2.00E-03	496.74	0.19	-4.29
ENSG00000105967	TFEC	protein_coding	-0.82	2.60E-05	2.70E-03	215.11	0.20	-4.21
ENSG00000184588	PDE4B	protein_coding	-0.82	1.93E-07	4.65E-05	445.34	0.16	-5.21
ENSG00000104081	BMF	protein_coding	-0.82	9.45E-11	6.08E-08	354.87	0.13	-6.48
ENSG00000268500	SIGLEC5	protein_coding	-0.81	1.14E-06	2.02E-04	197.95	0.17	-4.87
ENSG00000115828	QPCT	protein_coding	-0.81	2.08E-05	2.23E-03	204.04	0.19	-4.26
ENSG00000184602	SNN	protein_coding	-0.80	3.07E-05	3.10E-03	507.91	0.19	-4.17
ENSG00000126432	PRDX5	protein_coding	-0.80	1.01E-05	1.26E-03	179.08	0.18	-4.42
ENSG00000130724	CHMP2A	protein_coding	-0.80	1.05E-05	1.29E-03	195.20	0.18	-4.41
ENSG00000089351	GRAMD1A	protein_coding	-0.80	4.48E-07	9.31E-05	488.53	0.16	-5.05
ENSG00000141968	VAV1	protein_coding	-0.80	1.10E-06	1.98E-04	691.47	0.16	-4.87
ENSG00000229314	ORM1	protein_coding	-0.80	6.03E-05	5.29E-03	173.12	0.20	-4.01
ENSG00000175471	MCTP1	protein_coding	-0.79	1.14E-08	3.74E-06	340.03	0.14	-5.71
ENSG00000101236	RNF24	protein_coding	-0.79	3.16E-07	7.24E-05	321.42	0.16	-5.11
ENSG00000092531	SNAP23	protein_coding	-0.79	5.19E-06	7.38E-04	254.20	0.17	-4.56
ENSG00000090339	ICAM1	protein_coding	-0.79	1.35E-05	1.57E-03	469.87	0.18	-4.35
ENSG00000143321	HDGF	protein_coding	-0.79	1.30E-05	1.52E-03	365.89	0.18	-4.36
ENSG00000231925	TAPBP	protein_coding	-0.78	5.55E-06	7.65E-04	459.22	0.17	-4.54
ENSG00000163131	CTSS	protein_coding	-0.78	3.73E-05	3.60E-03	481.11	0.19	-4.12
ENSG00000112303	VNN2	protein_coding	-0.78	4.49E-05	4.24E-03	136.63	0.19	-4.08
ENSG00000277443	MARCKS	protein_coding	-0.78	5.24E-05	4.72E-03	851.93	0.19	-4.04
ENSG00000103313	MEFV	protein_coding	-0.78	3.42E-06	5.08E-04	270.39	0.17	-4.64
ENSG00000205542	TMSB4X	protein_coding	-0.77	7.34E-05	6.13E-03	2023.53	0.20	-3.96
ENSG00000201098	RNY1	misc_RNA	-0.77	1.85E-05	2.03E-03	305.75	0.18	-4.28
ENSG00000202538	RNU4-2	snRNA	-0.77	1.89E-05	2.06E-03	196.66	0.18	-4.28
ENSG00000184730	APOBR	protein_coding	-0.77	1.24E-05	1.48E-03	675.90	0.18	-4.37
ENSG00000167996	FTH1	protein_coding	-0.77	8.09E-05	6.62E-03	7689.90	0.20	-3.94
ENSG00000108774	RAB5C	protein_coding	-0.77	5.21E-05	4.72E-03	104.04	0.19	-4.05
ENSG00000114737	CISH	protein_coding	-0.77	2.19E-06	3.41E-04	215.86	0.16	-4.73

ENSG00000086300	SNX10	protein_coding	-0.77	5.49E-07	1.08E-04	267.68	0.15	-5.01
ENSG00000183019	MCEMP1	protein_coding	-0.76	2.46E-05	2.57E-03	146.70	0.18	-4.22
ENSG00000159388	BTG2	protein_coding	-0.76	4.57E-05	4.26E-03	387.19	0.19	-4.08
ENSG00000166710	B2M	protein_coding	-0.76	6.54E-05	5.66E-03	4208.94	0.19	-3.99
ENSG00000159128	IFNGR2	protein_coding	-0.75	7.24E-05	6.08E-03	256.55	0.19	-3.97
ENSG00000166920	C15orf48	protein_coding	-0.75	9.54E-05	7.72E-03	420.43	0.19	-3.90
ENSG00000275023	MLLT6	protein_coding	-0.75	3.03E-05	3.08E-03	1190.39	0.18	-4.17
ENSG00000184384	MAML2	protein_coding	-0.74	1.58E-05	1.79E-03	351.56	0.17	-4.32
ENSG00000108518	PFN1	protein_coding	-0.74	5.29E-05	4.73E-03	346.37	0.18	-4.04
ENSG00000188786	MTF1	protein_coding	-0.74	5.44E-06	7.57E-04	362.54	0.16	-4.55
ENSG00000111679	PTPN6	protein_coding	-0.74	7.50E-05	6.22E-03	543.25	0.19	-3.96
ENSG00000113916	BCL6	protein_coding	-0.74	3.64E-06	5.34E-04	416.31	0.16	-4.63
ENSG00000140030	GPR65	protein_coding	-0.73	5.03E-05	4.59E-03	126.67	0.18	-4.05
ENSG00000204592	HLA-E	protein_coding	-0.73	7.12E-05	6.01E-03	2016.43	0.18	-3.97
ENSG00000173039	RELA	protein_coding	-0.73	6.35E-06	8.58E-04	252.81	0.16	-4.51
ENSG00000125753	VASP	protein_coding	-0.73	4.56E-05	4.26E-03	584.09	0.18	-4.08
ENSG00000123610	TNFAIP6	protein_coding	-0.73	3.46E-05	3.41E-03	927.93	0.18	-4.14
ENSG00000177105	RHOG	protein_coding	-0.73	3.52E-05	3.44E-03	157.35	0.18	-4.14
ENSG00000105698	USF2	protein_coding	-0.72	3.59E-05	3.49E-03	359.85	0.18	-4.13
ENSG00000109320	NFKB1	protein_coding	-0.72	4.69E-05	4.34E-03	666.01	0.18	-4.07
ENSG00000158517	NCF1	protein_coding	-0.72	2.06E-04	1.53E-02	180.93	0.19	-3.71
ENSG00000118503	TNFAIP3	protein_coding	-0.72	1.75E-04	1.34E-02	1239.42	0.19	-3.75
ENSG00000266094	RASSF5	protein_coding	-0.72	6.94E-06	9.10E-04	637.51	0.16	-4.50
ENSG00000197622	CDC42SE1	protein_coding	-0.71	4.85E-05	4.45E-03	596.63	0.18	-4.06
ENSG00000168394	TAP1	protein_coding	-0.71	4.40E-07	9.30E-05	414.62	0.14	-5.05
ENSG00000068308	OTUD5	protein_coding	-0.71	6.60E-06	8.75E-04	142.99	0.16	-4.51
ENSG00000204642	HLA-F	protein_coding	-0.70	3.22E-05	3.23E-03	396.66	0.17	-4.16
ENSG00000170006	TMEM154	protein_coding	-0.70	2.96E-04	1.98E-02	434.54	0.19	-3.62
ENSG00000204160	ZDHHC18	protein_coding	-0.70	1.01E-04	8.14E-03	379.54	0.18	-3.89

ENSG00000085117	CD82	protein_coding	-0.70	5.28E-06	7.43E-04	663.07	0.15	-4.55
ENSG00000131669	NINJ1	protein_coding	-0.70	2.11E-04	1.55E-02	261.98	0.19	-3.71
ENSG00000165168	CYBB	protein_coding	-0.70	3.27E-04	2.15E-02	784.09	0.19	-3.59
ENSG00000230438	SERPINB9P1	lncRNA	-0.70	2.64E-05	2.73E-03	253.16	0.17	-4.20
ENSG00000136603	SKIL	protein_coding	-0.69	2.78E-04	1.91E-02	528.00	0.19	-3.63
ENSG00000134070	IRAK2	protein_coding	-0.69	2.29E-04	1.63E-02	546.52	0.19	-3.69
ENSG00000213654	GPSM3	protein_coding	-0.69	3.16E-04	2.10E-02	190.96	0.19	-3.60
ENSG00000015475	BID	protein_coding	-0.69	3.92E-05	3.75E-03	308.49	0.17	-4.11
ENSG00000123146	ADGRE5	protein_coding	-0.69	4.04E-05	3.84E-03	407.95	0.17	-4.11
ENSG00000204482	LST1	protein_coding	-0.68	2.23E-04	1.62E-02	203.90	0.19	-3.69
ENSG00000162511	LAPTM5	protein_coding	-0.68	3.63E-04	2.32E-02	1143.54	0.19	-3.57
ENSG00000167173	C15orf39	protein_coding	-0.68	2.49E-04	1.75E-02	345.29	0.19	-3.66
ENSG00000027697	IFNGR1	protein_coding	-0.68	3.77E-04	2.37E-02	540.89	0.19	-3.56
ENSG00000197122	SRC	protein_coding	-0.68	1.02E-05	1.26E-03	485.49	0.15	-4.41
ENSG00000108819	PPP1R9B	protein_coding	-0.67	2.58E-04	1.81E-02	474.68	0.18	-3.65
ENSG00000081237	PTPRC	protein_coding	-0.67	6.68E-04	3.69E-02	1503.37	0.20	-3.40
ENSG00000204264	PSMB8	protein_coding	-0.67	4.35E-07	9.30E-05	215.32	0.13	-5.05
ENSG00000106348	IMPDH1	protein_coding	-0.67	1.51E-04	1.17E-02	209.63	0.18	-3.79
ENSG00000124731	TREM1	protein_coding	-0.67	3.34E-04	2.16E-02	429.18	0.19	-3.59
ENSG00000177885	GRB2	protein_coding	-0.67	1.82E-04	1.39E-02	542.98	0.18	-3.74
ENSG00000115271	GCA	protein_coding	-0.66	2.28E-04	1.63E-02	135.67	0.18	-3.69
ENSG00000265972	TXNIP	protein_coding	-0.66	5.64E-04	3.24E-02	904.84	0.19	-3.45
ENSG00000187764	SEMA4D	protein_coding	-0.66	1.97E-08	5.78E-06	833.84	0.12	-5.61
ENSG00000110931	CAMKK2	protein_coding	-0.66	2.77E-06	4.25E-04	465.61	0.14	-4.69
ENSG00000100365	NCF4	protein_coding	-0.66	3.36E-05	3.34E-03	303.63	0.16	-4.15
ENSG00000093134	VNN3P	transcribed_unitary_pseudogene	-0.66	3.98E-07	8.81E-05	174.80	0.13	-5.07
ENSG00000196189	SEMA4A	protein_coding	-0.66	2.49E-04	1.75E-02	458.79	0.18	-3.66
ENSG00000146278	PNRC1	protein_coding	-0.65	4.75E-04	2.82E-02	678.61	0.19	-3.49
ENSG00000080371	RAB21	protein_coding	-0.65	1.33E-04	1.04E-02	448.48	0.17	-3.82

ENSG00000255197	SLC39A13-AS1	lncRNA	-0.65	6.90E-04	3.77E-02	106.50	0.19	-3.39
ENSG00000129450	SIGLEC9	protein_coding	-0.65	1.21E-04	9.47E-03	164.60	0.17	-3.85
ENSG00000183484	GPR132	protein_coding	-0.65	2.28E-05	2.42E-03	187.59	0.15	-4.24
ENSG00000172936	MYD88	protein_coding	-0.65	5.39E-05	4.79E-03	249.79	0.16	-4.04
ENSG00000116017	ARID3A	protein_coding	-0.65	2.15E-04	1.57E-02	274.06	0.18	-3.70
ENSG00000078804	TP53INP2	protein_coding	-0.65	7.57E-05	6.24E-03	156.02	0.16	-3.96
ENSG00000075420	FNDC3B	protein_coding	-0.65	5.81E-04	3.32E-02	796.81	0.19	-3.44
ENSG00000196923	PDLIM7	protein_coding	-0.64	5.18E-04	3.02E-02	284.72	0.19	-3.47
ENSG00000136869	TLR4	protein_coding	-0.64	3.03E-04	2.01E-02	349.80	0.18	-3.61
ENSG00000121966	CXCR4	protein_coding	-0.64	9.24E-04	4.71E-02	921.11	0.19	-3.31
ENSG00000141480	ARRB2	protein_coding	-0.64	2.74E-04	1.89E-02	385.53	0.17	-3.64
ENSG00000186174	BCL9L	protein_coding	-0.63	4.54E-04	2.73E-02	556.40	0.18	-3.51
ENSG00000123728	RAP2C	protein_coding	-0.63	6.49E-04	3.59E-02	143.14	0.19	-3.41
ENSG00000102908	NFAT5	protein_coding	-0.63	2.87E-04	1.95E-02	500.09	0.17	-3.63
ENSG00000144566	RAB5A	protein_coding	-0.63	1.15E-04	9.05E-03	232.13	0.16	-3.86
ENSG00000235568	NFAM1	protein_coding	-0.63	9.33E-04	4.74E-02	369.53	0.19	-3.31
ENSG00000224397	PELATON	lncRNA	-0.63	1.07E-05	1.30E-03	444.11	0.14	-4.40
ENSG00000198604	BAZ1A	protein_coding	-0.63	4.98E-04	2.92E-02	449.85	0.18	-3.48
ENSG00000174837	ADGRE1	protein_coding	-0.63	1.67E-04	1.29E-02	215.73	0.17	-3.77
ENSG00000168389	MFSD2A	protein_coding	-0.62	7.45E-06	9.68E-04	357.99	0.14	-4.48
ENSG00000158470	B4GALT5	protein_coding	-0.62	3.35E-04	2.16E-02	366.12	0.17	-3.59
ENSG00000158869	FCER1G	protein_coding	-0.62	5.83E-04	3.33E-02	240.41	0.18	-3.44
ENSG00000086730	LAT2	protein_coding	-0.62	7.52E-04	4.01E-02	313.64	0.18	-3.37
ENSG00000148926	ADM	protein_coding	-0.61	2.90E-04	1.95E-02	212.42	0.17	-3.62
ENSG00000003402	CFLAR	protein_coding	-0.61	9.16E-05	7.46E-03	1238.71	0.16	-3.91
ENSG00000186635	ARAP1	protein_coding	-0.61	7.25E-04	3.93E-02	1603.15	0.18	-3.38
ENSG00000146094	DOK3	protein_coding	-0.60	5.04E-04	2.95E-02	344.76	0.17	-3.48
ENSG00000204304	PBX2	protein_coding	-0.60	7.31E-04	3.95E-02	237.56	0.18	-3.38
ENSG00000178385	PLEKHM3	protein_coding	-0.59	2.05E-04	1.53E-02	469.27	0.16	-3.71

ENSG00000186469	GNG2	protein_coding	-0.59	6.31E-04	3.52E-02	419.07	0.17	-3.42
ENSG00000134802	SLC43A3	protein_coding	-0.59	5.34E-04	3.08E-02	344.91	0.17	-3.46
ENSG00000169230	PRELID1	protein_coding	-0.58	6.12E-05	5.34E-03	140.16	0.15	-4.01
ENSG00000172081	MOB3A	protein_coding	-0.58	8.69E-04	4.51E-02	285.36	0.18	-3.33
ENSG00000001084	GCLC	protein_coding	0.60	4.73E-04	2.82E-02	267.74	0.17	3.50
ENSG00000110721	CHKA	protein_coding	0.62	7.73E-06	9.95E-04	168.55	0.14	4.47
ENSG00000164733	CTSB	protein_coding	0.62	2.00E-04	1.52E-02	787.39	0.17	3.72
ENSG00000170385	SLC30A1	protein_coding	0.68	4.13E-04	2.55E-02	132.09	0.19	3.53
ENSG00000136235	GPNMB	protein_coding	0.69	1.62E-06	2.64E-04	207.06	0.14	4.80
ENSG00000258667	HIF1A-AS3	lncRNA	0.72	2.74E-04	1.89E-02	146.03	0.20	3.64
ENSG00000052795	FNIP2	protein_coding	0.78	1.28E-05	1.52E-03	481.22	0.18	4.36
ENSG00000100292	HMOX1	protein_coding	1.08	1.87E-08	5.73E-06	388.45	0.19	5.62
ENSG00000147872	PLIN2	protein_coding	1.11	5.53E-14	7.47E-11	266.44	0.15	7.52
ENSG00000125148	MT2A	protein_coding	1.29	8.77E-11	5.92E-08	113.23	0.20	6.49

S2 Table. Distribution of DE transcripts (RNA-Seq) according to ENSEMBL classification. DE transcripts (FDR≤0.05 and log2(FC)±0.58) were classified according to biotype in biomart (ENSEMBL).

Biotype	Classification	No of elements	% of elements
protein_coding	Gene/transcipt that contains an open reading frame (ORF)	202	95.28
lncRNA	A non-coding gene/transcript >200bp in length	6	2.83
misc_RNA	Miscellaneous RNA. A non-coding RNA that cannot be classified	1	0.47
processed_pseudogene	Pseudogene that lack introns and is thought to arise from reverse transcription of mRNA followed by reinsertion of DNA into the genome	1	0.47
snRNA	Small RNA molecules that are found in the cell nucleus and are involved in the processing of pre messenger RNAs	1	0.47
transcribed_unitary_pseudogene	A species specific unprocessed pseudogene without a parent gene, as it has an active orthologue in another species	1	0.47
TOTAL		212	100.00

S3 Table. Pathways enriched by differentially expressed genes (DEGs). Differentially expressed genes (FDR≤0.05 and log2(FC)±0.58) were inserted on gProfiler and resulted in 131 enriched pathways, most of them related to the immune system.

Term name	Adjusted p-value	Negative log10 of adjusted p-value	Term size	Intersection size	% of DEGs in Pathway	Intersections
Leishmaniasis	3.6E-21	20.44	71	15	21.13	ENSG00000027697,ENSG00000051523, ENSG00000100365,ENSG00000100906, ENSG00000109320,ENSG00000111679, ENSG00000136869,ENSG00000137462, ENSG00000158517,ENSG00000159128, ENSG00000165168,ENSG00000169896, ENSG00000172936,ENSG00000173039, ENSG00000204287
Legionellosis	3.0E-12	11.52	54	9	16.67	ENSG00000077150,ENSG00000100906, ENSG00000109320,ENSG00000136869, ENSG00000137462,ENSG00000169896, ENSG00000170458,ENSG00000172936, ENSG00000173039
Antigen processing and presentation	4.7E-13	12.33	67	10	14.93	ENSG00000163131,ENSG00000164733, ENSG00000166710,ENSG00000168394, ENSG00000204287,ENSG00000204525, ENSG00000204592,ENSG00000204642, ENSG00000231925,ENSG00000234745
Allograft rejection	4.6E-07	6.34	34	5	14.71	ENSG00000204287,ENSG00000204525, ENSG00000204592,ENSG00000204642, ENSG00000234745
PD-L1 expression and PD-1 checkpoint pathway in cancer	5.6E-15	14.25	88	12	13.64	ENSG00000027697,ENSG00000100644, ENSG00000100906,ENSG00000109320, ENSG00000111679,ENSG00000120217, ENSG00000136869,ENSG00000137462, ENSG00000146232,ENSG00000159128, ENSG00000172936,ENSG00000173039
Graft-versus-host disease	6.8E-07	6.17	37	5	13.51	ENSG00000204287,ENSG00000204525, ENSG00000204592,ENSG00000204642, ENSG00000234745
Phagosome	2.2E-21	20.65	137	18	13.14	ENSG00000051523,ENSG00000100365, ENSG00000108774,ENSG00000136869,

						ENSG00000137462,ENSG00000144566, ENSG00000158517,ENSG00000163131, ENSG00000165168,ENSG00000168394, ENSG00000169896,ENSG00000170458, ENSG00000186431,ENSG00000204287, ENSG00000204525,ENSG00000204592, ENSG00000204642,ENSG00000234745
Ferroptosis	8.7E-07	6.06	39	5	12.82	ENSG00000001084,ENSG00000100292, ENSG00000165168,ENSG00000167996, ENSG00000266412
NF-kappa B signaling pathway	1.1E-15	14.97	102	13	12.75	ENSG00000003402,ENSG00000077150, ENSG00000090339,ENSG00000100906, ENSG00000109320,ENSG00000118503, ENSG00000136869,ENSG00000140379, ENSG00000170458,ENSG00000172936, ENSG00000173039,ENSG00000254087, ENSG00000275302
Viral myocarditis	4.9E-09	8.31	55	7	12.73	ENSG00000015475,ENSG00000090339, ENSG00000204287,ENSG00000204525, ENSG00000204592,ENSG00000204642, ENSG00000234745
Type I diabetes mellitus	9.4E-07	6.03	40	5	12.50	ENSG00000204287,ENSG00000204525, ENSG00000204592,ENSG00000204642, ENSG00000234745
Acute myeloid leukemia	5.2E-10	9.29	65	8	12.31	ENSG00000066336,ENSG00000109320, ENSG00000126561,ENSG00000140379, ENSG00000169896,ENSG00000170458, ENSG00000173039,ENSG00000177885
B cell receptor signaling pathway	5.9E-11	10.23	76	9	11.84	ENSG00000100906,ENSG00000109320, ENSG00000111679,ENSG00000141968, ENSG00000146232,ENSG00000155629, ENSG00000173039,ENSG00000177885, ENSG00000254087
Inflammatory bowel disease	9.1E-09	8.04	61	7	11.48	ENSG00000027697,ENSG00000109320, ENSG00000136869,ENSG00000137462, ENSG00000159128,ENSG00000173039, ENSG00000204287
Osteoclast differentiation	4.8E-16	15.31	124	14	11.29	ENSG00000011600,ENSG00000027697,

						ENSG00000051523,ENSG00000066336, ENSG00000077150,ENSG00000100365, ENSG00000100906,ENSG00000109320, ENSG00000158517,ENSG00000159128, ENSG00000173039,ENSG00000177885, ENSG00000184557,ENSG00000198053
Th1 and Th2 cell differentiation	1.7E-10	9.77	86	9	10.47	ENSG00000027697,ENSG00000100906, ENSG00000109320,ENSG00000126561, ENSG00000146232,ENSG00000159128, ENSG00000173039,ENSG00000184384, ENSG00000204287
Prolactin signaling pathway	1.7E-08	7.77	67	7	10.45	ENSG00000109320,ENSG00000114737, ENSG00000126561,ENSG00000173039, ENSG00000177885,ENSG00000184557, ENSG00000197122
Tuberculosis	6.9E-20	19.16	173	18	10.40	ENSG00000015475,ENSG00000027697, ENSG00000108774,ENSG00000109320, ENSG00000134070,ENSG00000136869, ENSG00000137462,ENSG00000144566, ENSG00000158869,ENSG00000159128, ENSG00000163131,ENSG00000166523, ENSG00000169896,ENSG00000170458, ENSG00000172936,ENSG00000173039, ENSG00000197122,ENSG00000204287
Natural killer cell mediated cytotoxicity	1.4E-13	12.84	117	12	10.26	ENSG00000011600,ENSG00000015475, ENSG00000027697,ENSG00000090339, ENSG00000111679,ENSG00000141968, ENSG00000158869,ENSG00000159128, ENSG00000177885,ENSG00000204525, ENSG00000204592,ENSG00000234745
Autoimmune thyroid disease	2.5E-06	5.60	49	5	10.20	ENSG00000204287,ENSG00000204525, ENSG00000204592,ENSG00000204642, ENSG00000234745
Epstein-Barr virus infection	1.5E-20	19.82	191	19	9.95	ENSG00000015475,ENSG00000077150, ENSG00000090339,ENSG00000100906, ENSG00000109320,ENSG00000118503, ENSG00000137462,ENSG00000146232, ENSG00000166710,ENSG00000168394,

						ENSG00000172936,ENSG00000173039, ENSG00000204287,ENSG00000204525, ENSG00000204592,ENSG00000204642, ENSG00000231925,ENSG00000234745, ENSG00000254087
Chagas disease	5.0E-10	9.30	98	9	9.18	ENSG00000003402,ENSG00000027697, ENSG00000100906,ENSG00000109320, ENSG00000136869,ENSG00000137462, ENSG00000159128,ENSG00000172936, ENSG00000173039
Leukocyte transendothelial migration	5.9E-11	10.23	110	10	9.09	ENSG00000051523,ENSG00000090339, ENSG00000100365,ENSG00000121966, ENSG00000125753,ENSG00000141968, ENSG00000158517,ENSG00000165168, ENSG00000169896,ENSG00000266094
Toll-like receptor signaling pathway	5.6E-10	9.25	100	9	9.00	ENSG00000100906,ENSG00000107968, ENSG00000109320,ENSG00000136869, ENSG00000137462,ENSG00000170458, ENSG00000172936,ENSG00000173039, ENSG00000275302
T cell receptor signaling pathway	6.5E-10	9.19	102	9	8.82	ENSG00000081237,ENSG00000100906, ENSG00000107968,ENSG00000109320, ENSG00000111679,ENSG00000141968, ENSG00000146232,ENSG00000173039, ENSG00000177885
Adipocytokine signaling pathway	5.9E-07	6.23	69	6	8.70	ENSG00000100906,ENSG00000109320, ENSG00000110931,ENSG00000146232, ENSG00000173039,ENSG00000184557
Th17 cell differentiation	7.5E-10	9.12	104	9	8.65	ENSG00000027697,ENSG00000100644, ENSG00000100906,ENSG00000109320, ENSG00000126561,ENSG00000146232, ENSG00000159128,ENSG00000173039, ENSG00000204287
Fc gamma R-mediated phagocytosis	7.4E-09	8.13	94	8	8.51	ENSG00000081237,ENSG00000101336, ENSG00000125753,ENSG00000141968, ENSG00000158517,ENSG00000182541, ENSG00000254087,ENSG00000277443
Toxoplasmosis	9.5E-10	9.02	107	9	8.41	ENSG00000027697,ENSG00000100906,

						ENSG00000109320,ENSG00000136869, ENSG00000137462,ENSG00000159128, ENSG00000172936,ENSG00000173039, ENSG00000204287
Staphylococcus aureus infection	7.9E-08	7.10	84	7	8.33	ENSG00000090339,ENSG00000169403, ENSG00000169896,ENSG00000171049, ENSG00000171051,ENSG00000186431, ENSG00000204287
Amoebiasis	9.1E-09	8.04	97	8	8.25	ENSG00000108774,ENSG00000109320, ENSG00000136869,ENSG00000137462, ENSG00000144566,ENSG00000169896, ENSG00000170458,ENSG00000173039
Human immunodeficiency virus 1 infection	4.5E-17	16.34	207	17	8.21	ENSG00000015475,ENSG00000100906, ENSG00000109320,ENSG00000121966, ENSG00000136869,ENSG00000137462, ENSG00000166710,ENSG00000168394, ENSG00000172936,ENSG00000173039, ENSG00000182541,ENSG00000186469, ENSG00000204525,ENSG00000204592, ENSG00000204642,ENSG00000231925, ENSG00000234745
Malaria	6.6E-05	4.18	49	4	8.16	ENSG00000090339,ENSG00000136869, ENSG00000137462,ENSG00000172936
Pertussis	9.4E-07	6.03	76	6	7.89	ENSG00000109320,ENSG00000136869, ENSG00000169896,ENSG00000170458, ENSG00000172936,ENSG00000173039
Kaposi sarcoma-associated herpesvirus infection	5.4E-15	14.27	191	15	7.85	ENSG00000015475,ENSG00000027697, ENSG00000090339,ENSG00000100644, ENSG00000100906,ENSG00000101336, ENSG00000109320,ENSG00000173039, ENSG00000186469,ENSG00000197122, ENSG00000204525,ENSG00000204592, ENSG00000204642,ENSG00000234745, ENSG00000254087
Chemokine signaling pathway	4.8E-14	13.32	179	14	7.82	ENSG00000100906,ENSG00000101336, ENSG00000109320,ENSG00000121966, ENSG00000141480,ENSG00000141968, ENSG00000158517,ENSG00000161921,

						ENSG00000173039,ENSG00000177885, ENSG00000186469,ENSG00000197122, ENSG00000254087,ENSG00000275302
Lipid and atherosclerosis	1.1E-15	14.97	209	16	7.66	ENSG00000015475,ENSG00000051523, ENSG00000090339,ENSG00000100365, ENSG00000100906,ENSG00000109320, ENSG00000136869,ENSG00000137462, ENSG00000141968,ENSG00000158517, ENSG00000165168,ENSG00000170458, ENSG00000172936,ENSG00000173039, ENSG00000197122,ENSG00000254087
Neutrophil extracellular trap formation	6.6E-14	13.18	184	14	7.61	ENSG00000051523,ENSG00000100365, ENSG00000103569,ENSG00000109320, ENSG00000129450,ENSG00000136869, ENSG00000137462,ENSG00000158517, ENSG00000165168,ENSG00000169896, ENSG00000171049,ENSG00000171051, ENSG00000173039,ENSG00000197122
Human cytomegalovirus infection	1.4E-15	14.86	215	16	7.44	ENSG00000015475,ENSG00000100906, ENSG00000109320,ENSG00000121966, ENSG00000166710,ENSG00000168394, ENSG00000173039,ENSG00000177885, ENSG00000186469,ENSG00000197122, ENSG00000204525,ENSG00000204592, ENSG00000204642,ENSG00000231925, ENSG00000234745,ENSG00000275302
HIF-1 signaling pathway	2.0E-08	7.70	108	8	7.41	ENSG00000027697,ENSG00000100292, ENSG00000100644,ENSG00000109320, ENSG00000136869,ENSG00000159128, ENSG00000165168,ENSG00000173039
Asthma	7.5E-03	2.12	27	2	7.41	ENSG00000158869,ENSG00000204287
TNF signaling pathway	2.3E-08	7.65	110	8	7.27	ENSG00000003402,ENSG00000090339, ENSG00000100906,ENSG00000107968, ENSG00000109320,ENSG00000118503, ENSG00000173039,ENSG00000184557
Epithelial cell signaling in Helicobacter pylori infection	1.3E-05	4.89	69	5	7.25	ENSG00000100906,ENSG00000109320, ENSG00000173039,ENSG00000197122, ENSG00000254087

Apoptosis	4.8E-09	8.32	130	9	6.92	ENSG00000003402,ENSG00000015475, ENSG00000100368,ENSG00000100906, ENSG00000109320,ENSG00000140379, ENSG00000163131,ENSG00000164733, ENSG00000173039
Antifolate resistance	8.4E-03	2.08	29	2	6.90	ENSG00000109320,ENSG00000173039
C-type lectin receptor signaling pathway	2.9E-07	6.53	102	7	6.86	ENSG00000077150,ENSG00000100906, ENSG00000109320,ENSG00000158869, ENSG00000166523,ENSG00000173039, ENSG00000197122
Chronic myeloid leukemia	1.7E-05	4.77	73	5	6.85	ENSG00000100906,ENSG00000109320, ENSG00000126561,ENSG00000173039, ENSG00000177885
Yersinia infection	5.3E-09	8.28	132	9	6.82	ENSG00000100906,ENSG00000103313, ENSG00000109320,ENSG00000136869, ENSG00000141968,ENSG00000172936, ENSG00000173039,ENSG00000177105, ENSG00000197122
Influenza A	1.3E-10	9.89	163	11	6.75	ENSG00000015475,ENSG00000027697, ENSG00000090339,ENSG00000100906, ENSG00000109320,ENSG00000136869, ENSG00000159128,ENSG00000172936, ENSG00000173039,ENSG00000184557, ENSG00000204287
Measles	6.3E-09	8.20	135	9	6.67	ENSG00000015475,ENSG00000100906, ENSG00000109320,ENSG00000118503, ENSG00000126561,ENSG00000136869, ENSG00000137462,ENSG00000172936, ENSG00000173039
Cytosolic DNA-sensing pathway	1.4E-04	3.84	60	4	6.67	ENSG00000100906,ENSG00000109320, ENSG00000173039,ENSG00000275302
Necroptosis	9.9E-10	9.00	152	10	6.58	ENSG00000003402,ENSG00000015475, ENSG00000027697,ENSG00000118503, ENSG00000126561,ENSG00000130724, ENSG00000136869,ENSG00000159128, ENSG00000165168,ENSG00000167996
Cell adhesion molecules	9.5E-09	8.02	143	9	6.29	ENSG00000081237,ENSG00000090339, ENSG00000120217,ENSG00000169896,

						ENSG00000204287,ENSG00000204525, ENSG00000204592,ENSG00000204642, ENSG00000234745
Hepatitis B	1.5E-09	8.82	159	10	6.29	ENSG00000015475,ENSG00000100906, ENSG00000109320,ENSG00000126561, ENSG00000136869,ENSG00000137462, ENSG00000172936,ENSG00000173039, ENSG00000177885,ENSG00000197122
NOD-like receptor signaling pathway	2.5E-10	9.59	175	11	6.29	ENSG00000051523,ENSG00000100906, ENSG00000103313,ENSG00000109320, ENSG00000118503,ENSG00000136869, ENSG00000164733,ENSG00000165168, ENSG00000172936,ENSG00000173039, ENSG00000265972
Fc epsilon RI signaling pathway	1.8E-04	3.74	64	4	6.25	ENSG00000141968,ENSG00000158869, ENSG00000177885,ENSG00000254087
Viral carcinogenesis	4.8E-11	10.32	194	12	6.19	ENSG00000077150,ENSG00000100906, ENSG00000109320,ENSG00000126561, ENSG00000173039,ENSG00000177885, ENSG00000197122,ENSG00000204525, ENSG00000204592,ENSG00000204642, ENSG00000234745,ENSG00000254087
Human T-cell leukemia virus 1 infection	7.0E-12	11.15	211	13	6.16	ENSG00000066336,ENSG00000077150, ENSG00000090339,ENSG00000100906, ENSG00000109320,ENSG00000126561, ENSG00000166710,ENSG00000173039, ENSG00000204287,ENSG00000204525, ENSG00000204592,ENSG00000204642, ENSG00000234745
RIG-I-like receptor signaling pathway	2.3E-04	3.65	68	4	5.88	ENSG00000068308,ENSG00000100906, ENSG00000109320,ENSG00000173039
African trypanosomiasis	1.2E-02	1.92	35	2	5.71	ENSG00000090339,ENSG00000172936
Relaxin signaling pathway	9.3E-07	6.03	123	7	5.69	ENSG00000100906,ENSG00000109320, ENSG00000141480,ENSG00000173039, ENSG00000177885,ENSG00000186469, ENSG00000197122
Primary immunodeficiency	1.3E-02	1.88	37	2	5.41	ENSG00000081237,ENSG00000168394

Fluid shear stress and atherosclerosis	1.6E-06	5.81	134	7	5.22	ENSG00000051523,ENSG00000090339, ENSG00000100292,ENSG00000109320, ENSG00000158517,ENSG00000173039, ENSG00000197122
Neurotrophin signaling pathway	9.8E-06	5.01	115	6	5.22	ENSG00000100906,ENSG00000109320, ENSG00000134070,ENSG00000146232, ENSG00000173039,ENSG00000177885
Salmonella infection	3.7E-10	9.44	236	12	5.08	ENSG00000081237,ENSG00000100906, ENSG00000108518,ENSG00000108774, ENSG00000109320,ENSG00000136869, ENSG00000137462,ENSG00000144566, ENSG00000170458,ENSG00000172936, ENSG00000173039,ENSG00000177105
AGE-RAGE signaling pathway in diabetic complications	6.6E-05	4.18	99	5	5.05	ENSG00000090339,ENSG00000109320, ENSG00000126561,ENSG00000165168, ENSG00000173039
JAK-STAT signaling pathway	3.7E-07	6.43	159	8	5.03	ENSG00000027697,ENSG00000100368, ENSG00000111679,ENSG00000114737, ENSG00000126561,ENSG00000159128, ENSG00000177885,ENSG00000184557
Alcoholic liver disease	2.2E-06	5.66	141	7	4.96	ENSG00000100906,ENSG00000109320, ENSG00000110931,ENSG00000136869, ENSG00000170458,ENSG00000172936, ENSG00000173039
Vasopressin-regulated water reabsorption	1.7E-02	1.78	42	2	4.76	ENSG00000108774,ENSG00000144566
Rheumatoid arthritis	5.3E-04	3.27	86	4	4.65	ENSG00000090339,ENSG00000136869, ENSG00000137462,ENSG00000204287
Endocytosis	4.7E-09	8.33	237	11	4.64	ENSG00000108774,ENSG00000121966, ENSG00000130724,ENSG00000141480, ENSG00000144566,ENSG00000186635, ENSG00000197122,ENSG00000204525, ENSG00000204592,ENSG00000204642, ENSG00000234745
Cellular senescence	3.4E-06	5.46	152	7	4.61	ENSG00000109320,ENSG00000173039, ENSG00000204525,ENSG00000204592, ENSG00000204642,ENSG00000234745, ENSG00000266094

Hepatitis C	3.4E-06	5.46	152	7	4.61	ENSG00000003402,ENSG00000015475, ENSG00000100906,ENSG00000109320, ENSG00000173039,ENSG00000177885, ENSG00000184557
Intestinal immune network for IgA production	1.8E-02	1.75	44	2	4.55	ENSG00000121966, ENSG00000204287
Transcriptional misregulation in cancer	9.3E-07	6.03	182	8	4.40	ENSG00000066336,ENSG00000109320, ENSG00000113916,ENSG00000140379, ENSG00000144802,ENSG00000169896, ENSG00000170458,ENSG00000173039
IL-17 signaling pathway	6.6E-04	3.18	91	4	4.40	ENSG00000100906,ENSG00000109320, ENSG00000118503,ENSG00000173039
Chemical carcinogenesis - reactive oxygen species	2.0E-07	6.69	206	9	4.37	ENSG00000051523,ENSG00000100292, ENSG00000100644,ENSG00000100906, ENSG00000109320,ENSG00000158517, ENSG00000173039,ENSG00000177885, ENSG00000197122
Prostate cancer	7.0E-04	3.15	93	4	4.30	ENSG00000100906,ENSG00000109320, ENSG00000173039,ENSG00000177885
Mitophagy - animal	3.8E-03	2.42	70	3	4.29	ENSG00000100644,ENSG00000173039, ENSG00000197122
Non-small cell lung cancer	3.9E-03	2.40	71	3	4.23	ENSG00000126561,ENSG00000177885, ENSG00000266094
Platelet activation	1.6E-04	3.80	120	5	4.17	ENSG00000092531,ENSG00000125753, ENSG00000158869,ENSG00000197122, ENSG00000254087
Cocaine addiction	2.1E-02	1.68	48	2	4.17	ENSG00000109320,ENSG00000173039
Herpes simplex virus 1 infection	1.1E-13	12.97	477	19	3.98	ENSG00000015475,ENSG00000027697, ENSG00000085514,ENSG00000100906, ENSG00000109320,ENSG00000137462, ENSG00000159128,ENSG00000166710, ENSG00000168394,ENSG00000172936, ENSG00000173039,ENSG00000184557, ENSG00000197122,ENSG00000204287, ENSG00000204525,ENSG00000204592, ENSG00000204642,ENSG00000231925, ENSG00000234745
Insulin resistance	1.0E-03	3.00	103	4	3.88	ENSG00000100906,ENSG00000109320,

						ENSG00000173039,ENSG00000184557
Pathogenic Escherichia coli infection	1.1E-05	4.94	183	7	3.83	ENSG00000100906,ENSG00000109320, ENSG00000111679,ENSG00000136869, ENSG00000172936,ENSG00000173039, ENSG00000197122
Shigellosis	6.6E-07	6.18	239	9	3.77	ENSG00000100906,ENSG00000108518, ENSG00000109320,ENSG00000136869, ENSG00000145901,ENSG00000170458, ENSG00000172936,ENSG00000173039, ENSG00000197122
Morphine addiction	5.5E-03	2.26	80	3	3.75	ENSG00000141480,ENSG00000184588, ENSG00000186469
Autophagy - animal	2.6E-04	3.58	135	5	3.70	ENSG00000003402,ENSG00000078804, ENSG00000100644,ENSG00000110931, ENSG00000164733
ErbB signaling pathway	5.6E-03	2.25	81	3	3.70	ENSG00000126561,ENSG00000177885, ENSG00000197122
Sphingolipid signaling pathway	1.5E-03	2.84	114	4	3.51	ENSG00000015475,ENSG00000109320, ENSG00000158869,ENSG00000173039
Mineral absorption	2.8E-02	1.55	57	2	3.51	ENSG00000100292,ENSG00000167996
Proteoglycans in cancer	2.0E-05	4.71	200	7	3.50	ENSG00000100644,ENSG00000111679, ENSG00000136869,ENSG00000137462, ENSG00000141968,ENSG00000177885, ENSG00000197122
Longevity regulating pathway	6.6E-03	2.18	86	3	3.49	ENSG00000109320,ENSG00000110931, ENSG00000173039
Chemical carcinogenesis - receptor activation	2.1E-05	4.69	202	7	3.47	ENSG00000109320,ENSG00000113916, ENSG00000126561,ENSG00000141480, ENSG00000173039,ENSG00000177885, ENSG00000197122
Rap1 signaling pathway	2.1E-05	4.68	203	7	3.45	ENSG00000108518,ENSG00000125753, ENSG00000141968,ENSG00000169896, ENSG00000171051,ENSG00000197122, ENSG00000266094
Coronavirus disease - COVID-19	2.3E-05	4.64	207	7	3.38	ENSG00000100906,ENSG00000109320, ENSG00000136869,ENSG00000137462, ENSG00000165168,ENSG00000172936, ENSG00000173039

Small cell lung cancer	7.4E-03	2.13	90	3	3.33	ENSG00000100906,ENSG00000109320, ENSG00000173039
Hematopoietic cell lineage	7.5E-03	2.12	91	3	3.30	ENSG00000169896,ENSG00000170458, ENSG00000204287
Pathways in cancer	4.6E-11	10.34	517	17	3.29	ENSG00000015475,ENSG00000027697, ENSG00000066336,ENSG00000077150, ENSG00000100292,ENSG00000100368, ENSG00000100644,ENSG00000100906, ENSG00000109320,ENSG00000121966, ENSG00000126561,ENSG00000159128, ENSG00000173039,ENSG00000177885, ENSG00000186469,ENSG00000266094, ENSG00000266412
Diabetic cardiomyopathy	1.3E-04	3.89	187	6	3.21	ENSG00000051523,ENSG00000100365, ENSG00000109320,ENSG00000158517, ENSG00000165168,ENSG00000173039
Choline metabolism in cancer	8.2E-03	2.09	94	3	3.19	ENSG00000100644,ENSG00000110721, ENSG00000177885
Lysosome	2.1E-03	2.68	126	4	3.17	ENSG00000104093,ENSG00000162511, ENSG00000163131,ENSG00000164733
Ras signaling pathway	3.9E-05	4.41	225	7	3.11	ENSG00000108774,ENSG00000109320, ENSG00000144566,ENSG00000173039, ENSG00000177885,ENSG00000186469, ENSG00000266094
Renal cell carcinoma	3.5E-02	1.45	65	2	3.08	ENSG00000100644,ENSG00000177885
Insulin signaling pathway	2.4E-03	2.62	131	4	3.05	ENSG00000125733,ENSG00000137312, ENSG00000177885,ENSG00000184557
Renin secretion	3.8E-02	1.42	68	2	2.94	ENSG00000123700,ENSG00000164733
Regulation of actin cytoskeleton	2.2E-04	3.66	207	6	2.90	ENSG00000108518,ENSG00000121966, ENSG00000141968,ENSG00000169896, ENSG00000182541,ENSG00000197122
Adherens junction	3.9E-02	1.41	69	2	2.90	ENSG00000111679,ENSG00000197122
Axon guidance	8.0E-04	3.10	174	5	2.87	ENSG00000121966,ENSG00000182541, ENSG00000187764,ENSG00000196189, ENSG00000197122
cAMP signaling pathway	2.3E-04	3.64	210	6	2.86	ENSG00000100906,ENSG00000109320, ENSG00000126262,ENSG00000141968,

						ENSG00000173039,ENSG00000184588
Non-alcoholic fatty liver disease	3.2E-03	2.50	142	4	2.82	ENSG00000015475,ENSG00000109320, ENSG00000173039,ENSG00000184557
MAPK signaling pathway	2.1E-05	4.67	286	8	2.80	ENSG00000077150,ENSG00000107968, ENSG00000109320,ENSG00000141480, ENSG00000170458,ENSG00000172936, ENSG00000173039,ENSG00000177885
Bacterial invasion of epithelial cells	4.1E-02	1.39	72	2	2.78	ENSG00000177105,ENSG00000197122
p53 signaling pathway	4.1E-02	1.39	72	2	2.78	ENSG00000015475,ENSG00000085117
PPAR signaling pathway	4.3E-02	1.37	74	2	2.70	ENSG00000147872,ENSG00000198814
Pancreatic cancer	4.3E-02	1.37	74	2	2.70	ENSG00000109320,ENSG00000173039
Growth hormone synthesis, secretion and action	1.3E-02	1.89	113	3	2.65	ENSG00000126561,ENSG00000177885, ENSG00000184557
EGFR tyrosine kinase inhibitor resistance	4.4E-02	1.35	76	2	2.63	ENSG00000177885,ENSG00000197122
Human papillomavirus infection	4.1E-05	4.39	315	8	2.54	ENSG00000109320,ENSG00000173039, ENSG00000177885,ENSG00000184384, ENSG00000204525,ENSG00000204592, ENSG00000204642,ENSG00000234745
Gap junction	4.8E-02	1.32	80	2	2.50	ENSG00000177885,ENSG00000197122
GABAergic synapse	5.0E-02	1.30	82	2	2.44	ENSG00000186469,ENSG00000197122
Cytokine-cytokine receptor interaction	1.0E-03	3.00	281	6	2.14	ENSG00000027697,ENSG00000100368, ENSG00000121966,ENSG00000159128, ENSG00000161921,ENSG00000275302
Focal adhesion	8.4E-03	2.08	192	4	2.08	ENSG00000125753,ENSG00000141968, ENSG00000177885,ENSG00000197122
PI3K-Akt signaling pathway	4.7E-04	3.33	342	7	2.05	ENSG00000109320,ENSG00000136869, ENSG00000137462,ENSG00000155629, ENSG00000173039,ENSG00000177885, ENSG00000186469
Oxytocin signaling pathway	2.5E-02	1.59	148	3	2.03	ENSG00000110931,ENSG00000123700, ENSG00000197122
Tight junction	3.0E-02	1.52	159	3	1.89	ENSG00000123728,ENSG00000125753, ENSG00000197122
Alcoholism	3.9E-02	1.41	177	3	1.69	ENSG00000110931,ENSG00000177885,

						ENSG00000186469
Prion disease	1.8E-02	1.75	243	4	1.65	ENSG00000051523,ENSG00000100365, ENSG00000158517,ENSG00000165168
MicroRNAs in cancer	8.2E-03	2.09	306	5	1.63	ENSG00000100292,ENSG00000104081, ENSG00000109320,ENSG00000177885, ENSG00000277443
Neuroactive ligand-receptor interaction	4.6E-02	1.34	336	4	1.19	ENSG00000148926,ENSG00000169403, ENSG00000171049,ENSG00000171051
Pathways of neurodegeneration - multiple diseases	3.2E-02	1.50	442	5	1.13	ENSG00000015475,ENSG00000109320, ENSG00000144566,ENSG00000165168, ENSG00000173039

S4 Table. Differentially expressed miRNAs from microarray analyzes. A total of 446 probes were differentially expressed (FC±2 and FDR≤0.01) after *in vitro L. infantum* infection, 289 were classified as miRNAs.

Accession	Transcript ID	Type	Fold Change	p-val	FDR
MIMAT0015042	hsa-miR-3167	miRNA	261.7	4.73E-11	4.94E-08
MIMAT0019777	hsa-miR-4688	miRNA	167.5	2.09E-15	1.39E-11
MIMAT0028220	hsa-miR-7155-5p	miRNA	85.6	4.94E-12	7.81E-09
MIMAT0003215	hsa-miR-552-3p	miRNA	71.7	5.96E-11	4.94E-08
MIMAT0020924	hsa-miR-642a-3p	miRNA	59.6	1.35E-10	9.97E-08
U70F	U70F	HAcaBox	39.03	2.01E-08	4.09E-06
ENSG00000252380	ENSG00000252380	snoRNA	25.6	9.52E-08	1.07E-05
MIMAT0002824	hsa-miR-498	miRNA	23.0	3.36E-12	7.81E-09
MIMAT0027580	hsa-miR-6839-5p	miRNA	22.4	9.82E-08	1.09E-05
MIMAT0003315	hsa-miR-645	miRNA	21.5	1.98E-08	4.09E-06
MIMAT0014997	hsa-miR-3132	miRNA	18.7	5.89E-12	7.81E-09
U70F	U70F	HAcaBox	18.57	4.40E-06	2.00E-04
MIMAT0019725	hsa-miR-4658	miRNA	18.2	4.11E-12	7.81E-09
MIMAT0018089	hsa-miR-3667-5p	miRNA	17.68	5.23E-11	4.94E-08
MIMAT0031007	hsa-miR-8080	miRNA	17.62	1.97E-07	1.84E-05
MIMAT0004778	hsa-miR-508-5p	miRNA	14.72	1.41E-09	5.52E-07
MIMAT0017987	hsa-miR-3610	miRNA	14.14	8.66E-07	5.32E-05
MIMAT0019827	hsa-miR-4716-3p	miRNA	13.94	3.44E-08	5.07E-06
MIMAT0019026	hsa-miR-4491	miRNA	13.91	6.92E-07	4.68E-05
MIMAT0027644	hsa-miR-6872-5p	miRNA	12.6	5.64E-05	1.10E-03
MI0006428	hsa-mir-1281	Stem-loop	12.39	1.28E-07	1.34E-05
ENSG00000252505	ENSG00000252505	snoRNA	12.19	3.07E-08	4.74E-06
MIMAT0015005	hsa-miR-3137	miRNA	11.96	2.08E-09	7.25E-07
MIMAT0005795	hsa-miR-1323	miRNA	11.89	4.46E-08	6.17E-06

ENSG00000252505	ENSG00000252505	snoRNA	11.87	1.02E-08	2.25E-06
MIMAT0019776	hsa-miR-1343-3p	miRNA	11.75	1.37E-09	5.52E-07
MIMAT0003330	hsa-miR-654-5p	miRNA	11.56	4.56E-08	6.17E-06
MIMAT0018961	hsa-miR-4443	miRNA	10.34	4.61E-09	1.27E-06
MIMAT0003269	hsa-miR-601	miRNA	10.21	9.46E-08	1.07E-05
MIMAT0018444	hsa-miR-642b-3p	miRNA	10.08	4.21E-09	1.27E-06
MIMAT0020958	hsa-miR-4482-3p	miRNA	9.56	3.99E-08	5.63E-06
MIMAT0018001	hsa-miR-3620-3p	miRNA	9.23	2.29E-10	1.46E-07
MIMAT0003309	hsa-miR-639	miRNA	8.87	1.96E-07	1.84E-05
MI0022227	hsa-mir-6515	Stem-loop	8.79	5.07E-08	6.62E-06
MIMAT0026474	hsa-miR-208a-5p	miRNA	8.65	2.40E-08	4.24E-06
MIMAT0005873	hsa-miR-1208	miRNA	8.51	2.86E-07	2.43E-05
MIMAT0027416	hsa-miR-6758-5p	miRNA	7.96	2.19E-05	6.00E-04
MIMAT0015029	hsa-miR-3155a	miRNA	7.5	2.58E-07	2.25E-05
MIMAT0001627	hsa-miR-433-3p	miRNA	7.26	7.51E-06	3.00E-04
MIMAT0019853	hsa-miR-4731-5p	miRNA	7.04	1.01E-08	2.25E-06
MI0014186	hsa-mir-3158-1	Stem-loop	6.89	1.10E-07	1.18E-05
MI0014187	hsa-mir-3158-2	Stem-loop	6.89	1.10E-07	1.18E-05
MIMAT0003221	hsa-miR-557	miRNA	6.87	3.46E-10	1.76E-07
U70D	U70D	HAcaBox	6.76	6.00E-08	7.62E-06
MIMAT0019211	hsa-miR-3158-5p	miRNA	6.76	5.48E-06	2.00E-04
MIMAT0004566	hsa-miR-218-2-3p	miRNA	6.54	3.24E-08	4.88E-06
MIMAT0003945	hsa-miR-765	miRNA	6.39	9.65E-07	5.76E-05
MIMAT0019881	hsa-miR-4746-3p	miRNA	6.28	5.77E-06	2.00E-04
MIMAT0018959	hsa-miR-4441	miRNA	6.21	8.07E-07	5.15E-05
MI0022678	hsa-mir-6833	Stem-loop	6.18	4.63E-05	9.00E-04
MIMAT0004673	hsa-miR-29c-5p	miRNA	6.11	1.89E-05	5.00E-04
ENSG00000200377	ENSG00000200377	snoRNA	5.96	3.42E-06	1.00E-04
MIMAT0016882	hsa-miR-4253	miRNA	5.93	1.67E-07	1.68E-05

MIMAT0013802	hsa-miR-2861	miRNA	5.91	2.43E-10	1.46E-07
MIMAT0018997	hsa-miR-4470	miRNA	5.84	6.88E-06	2.00E-04
ENSG00000238767	ENSG00000238767	snoRNA	5.81	1.99E-09	7.25E-07
MIMAT0019867	hsa-miR-4738-3p	miRNA	5.76	1.89E-05	5.00E-04
MIMAT0000228	hsa-miR-198	miRNA	5.75	9.92E-06	3.00E-04
MIMAT0015089	hsa-miR-3202	miRNA	5.75	1.53E-05	4.00E-04
MI0015852	hsa-mir-4321	Stem-loop	5.75	4.00E-05	8.00E-04
MIMAT0014996	hsa-miR-3131	miRNA	5.68	4.47E-09	1.27E-06
MIMAT0004761	hsa-miR-483-5p	miRNA	5.55	3.41E-10	1.76E-07
MIMAT0027618	hsa-miR-6859-5p	miRNA	5.55	8.28E-06	3.00E-04
MIMAT0027566	hsa-miR-6833-5p	miRNA	5.36	8.67E-07	5.32E-05
MIMAT0027434	hsa-miR-6767-5p	miRNA	5.33	5.37E-09	1.37E-06
MIMAT0027678	hsa-miR-6889-5p	miRNA	5.33	2.46E-06	1.00E-04
MI0022625	hsa-mir-6780a	Stem-loop	5.32	2.03E-08	4.09E-06
MIMAT0027476	hsa-miR-6788-5p	miRNA	5.32	1.66E-05	5.00E-04
MIMAT0027363	hsa-miR-6731-5p	miRNA	5.29	2.98E-08	4.71E-06
MI0014252	hsa-mir-3202-1	Stem-loop	5.28	2.59E-06	1.00E-04
MI0014253	hsa-mir-3202-2	Stem-loop	5.28	2.59E-06	1.00E-04
ENSG00000238769	ENSG00000238769	snoRNA	5.24	5.00E-09	1.33E-06
MIMAT0016907	hsa-miR-4281	miRNA	5.24	2.35E-08	4.24E-06
MIMAT0027502	hsa-miR-6801-5p	miRNA	5.14	4.83E-06	2.00E-04
MIMAT0018185	hsa-miR-3911	miRNA	5.12	1.11E-06	6.38E-05
MIMAT0025477	hsa-miR-6510-3p	miRNA	5.12	5.00E-04	4.80E-03
ENSG00000268369	ENSG00000268369	snoRNA	5.03	1.91E-05	5.00E-04
SNORA11D	SNORA11D	HAcaBox	5.03	1.91E-05	5.00E-04
SNORA11E	SNORA11E	HAcaBox	5.03	1.91E-05	5.00E-04
spike_in-control-44	spike_in-control-44	Spike-in controls	5.01	1.88E-06	9.38E-05
MIMAT0018201	hsa-miR-3926	miRNA	4.99	2.46E-05	6.00E-04
MIMAT0018940	hsa-miR-4425	miRNA	4.97	1.77E-05	5.00E-04

MIMAT0019894	hsa-miR-4754	miRNA	4.96	2.28E-07	2.05E-05
MIMAT0015088	hsa-miR-514b-3p	miRNA	4.92	1.87E-06	9.38E-05
MIMAT0016849	hsa-miR-4294	miRNA	4.91	3.51E-05	7.00E-04
MIMAT0028219	hsa-miR-7154-3p	miRNA	4.9	4.79E-07	3.46E-05
MIMAT0017996	hsa-miR-3616-3p	miRNA	4.88	1.72E-07	1.70E-05
MIMAT0027432	hsa-miR-6766-5p	miRNA	4.85	5.11E-05	1.00E-03
MIMAT0027628	hsa-miR-6864-5p	miRNA	4.83	4.92E-07	3.51E-05
MIMAT0028215	hsa-miR-7152-3p	miRNA	4.81	7.49E-05	1.30E-03
MIMAT0030421	hsa-miR-7846-3p	miRNA	4.79	2.21E-08	4.24E-06
MI0017344	hsa-mir-4710	Stem-loop	4.79	3.00E-04	3.40E-03
MIMAT0019692	hsa-miR-4635	miRNA	4.76	1.00E-03	8.70E-03
MIMAT0027530	hsa-miR-6815-5p	miRNA	4.72	7.32E-07	4.81E-05
14qII-27	14qII-27	CDBox	4.67	1.31E-06	7.03E-05
MIMAT0026639	hsa-miR-1301-5p	miRNA	4.64	6.00E-04	5.60E-03
MIMAT0002806	hsa-miR-490-3p	miRNA	4.57	1.04E-05	3.00E-04
MIMAT0027468	hsa-miR-6784-5p	miRNA	4.55	2.73E-08	4.53E-06
MI0016768	hsa-mir-4429	Stem-loop	4.55	1.59E-06	8.22E-05
MIMAT0019836	hsa-miR-4722-5p	miRNA	4.54	1.40E-05	4.00E-04
MI0016778	hsa-mir-4437	Stem-loop	4.48	5.05E-06	2.00E-04
MI0014177	hsa-mir-3150a	Stem-loop	4.41	9.64E-09	2.25E-06
MIMAT0018080	hsa-miR-3659	miRNA	4.36	2.85E-05	7.00E-04
MIMAT0026637	hsa-miR-1296-3p	miRNA	4.35	2.23E-06	1.00E-04
MIMAT0004685	hsa-miR-302d-5p	miRNA	4.29	6.00E-04	5.60E-03
MIMAT0023705	hsa-miR-6080	miRNA	4.27	7.80E-08	9.41E-06
MIMAT0027619	hsa-miR-6859-3p	miRNA	4.26	8.45E-05	1.40E-03
MI0003624	hsa-mir-611	Stem-loop	4.22	2.43E-08	4.24E-06
MIMAT0030979	hsa-miR-8052	miRNA	4.17	4.37E-07	3.22E-05
MIMAT0016919	hsa-miR-4292	miRNA	4.17	7.70E-07	4.96E-05
MIMAT0030422	hsa-miR-7847-3p	miRNA	4.14	3.18E-07	2.54E-05

MIMAT0004284	hsa-miR-675-5p	miRNA	4.14	2.75E-06	1.00E-04
MI0017404	hsa-mir-4763	Stem-loop	4.12	4.93E-05	1.00E-03
spike_in-control-48	spike_in-control-48	Spike-in controls	4.07	3.09E-05	7.00E-04
MIMAT0027623	hsa-miR-6861-5p	miRNA	4.04	9.70E-10	4.59E-07
MIMAT0019699	hsa-miR-4640-5p	miRNA	4	2.30E-08	4.24E-06
MIMAT0003228	hsa-miR-564	miRNA	3.98	9.67E-05	1.50E-03
MIMAT0018164	hsa-miR-3713	miRNA	3.96	2.28E-07	2.05E-05
MI0019297	hsa-mir-5692a-1	Stem-loop	3.96	5.00E-04	5.10E-03
MI0019298	hsa-mir-5692a-2	Stem-loop	3.96	5.00E-04	5.10E-03
MIMAT0017392	hsa-miR-3200-5p	miRNA	3.92	7.75E-06	3.00E-04
MIMAT0027426	hsa-miR-6763-5p	miRNA	3.91	9.19E-08	1.07E-05
MIMAT0019958	hsa-miR-4788	miRNA	3.91	5.39E-06	2.00E-04
MIMAT0019773	hsa-miR-4686	miRNA	3.85	2.13E-05	6.00E-04
MIMAT0004584	hsa-let-7g-3p	miRNA	3.78	5.00E-04	4.90E-03
U70D	U70D	HAcaBox	3.73	2.89E-08	4.67E-06
MIMAT0027583	hsa-miR-6840-3p	miRNA	3.72	1.01E-06	5.99E-05
MIMAT0030991	hsa-miR-8064	miRNA	3.64	7.17E-08	8.80E-06
MIMAT0018931	hsa-miR-4419a	miRNA	3.61	9.44E-09	2.25E-06
MIMAT0027652	hsa-miR-6876-5p	miRNA	3.61	5.00E-04	5.20E-03
MIMAT0026475	hsa-miR-210-5p	miRNA	3.55	3.23E-05	7.00E-04
MIMAT0027662	hsa-miR-6881-5p	miRNA	3.46	3.98E-05	8.00E-04
MIMAT0004979	hsa-miR-936	miRNA	3.43	5.33E-05	1.00E-03
MI0014190	hsa-mir-3160-2	Stem-loop	3.42	1.82E-05	5.00E-04
MIMAT0015087	hsa-miR-514b-5p	miRNA	3.42	4.00E-04	4.40E-03
MIMAT0018953	hsa-miR-4437	miRNA	3.42	6.00E-04	6.00E-03
MIMAT0014999	hsa-miR-378b	miRNA	3.39	5.39E-05	1.00E-03
14qII-27	14qII-27	CDBox	3.34	4.00E-05	8.00E-04
MIMAT0027472	hsa-miR-6786-5p	miRNA	3.32	1.29E-09	5.52E-07
MIMAT0005593	hsa-miR-1238-3p	miRNA	3.29	3.15E-07	2.54E-05

MIMAT0027538	hsa-miR-6819-5p	miRNA	3.29	4.51E-07	3.29E-05
MIMAT0014984	hsa-miR-3122	miRNA	3.29	1.22E-05	4.00E-04
MIMAT0030987	hsa-miR-8060	miRNA	3.28	2.36E-05	6.00E-04
MIMAT0018962	hsa-miR-4444	miRNA	3.27	4.00E-04	4.20E-03
MIMAT0015048	hsa-miR-3173-3p	miRNA	3.21	2.00E-04	2.80E-03
MI0017320	hsa-mir-1343	Stem-loop	3.08	8.40E-07	5.31E-05
MIMAT0026917	hsa-miR-1910-3p	miRNA	3.08	6.64E-05	1.20E-03
MIMAT0022721	hsa-miR-1247-3p	miRNA	3.07	1.68E-05	5.00E-04
MIMAT0023706	hsa-miR-6081	miRNA	3.07	2.44E-05	6.00E-04
MIMAT0018971	hsa-miR-4450	miRNA	3.07	9.00E-04	7.90E-03
MIMAT0003282	hsa-miR-614	miRNA	3.05	3.56E-07	2.73E-05
MI0003557	hsa-mir-552	Stem-loop	3.05	2.76E-06	1.00E-04
MI0014177	hsa-mir-3150a	Stem-loop	3.04	1.17E-06	6.65E-05
MI0016899	hsa-mir-4532	Stem-loop	3.04	9.37E-05	1.50E-03
MIMAT0019883	hsa-miR-4747-3p	miRNA	2.98	4.42E-05	9.00E-04
MIMAT0019006	hsa-miR-4478	miRNA	2.97	3.31E-05	7.00E-04
MI0016898	hsa-mir-4531	Stem-loop	2.97	4.00E-04	4.70E-03
MIMAT0015078	hsa-miR-3194-5p	miRNA	2.96	8.00E-04	7.50E-03
MIMAT0019361	hsa-miR-3976	miRNA	2.93	7.00E-04	6.50E-03
MI0016810	hsa-mir-4462	Stem-loop	2.91	1.15E-05	4.00E-04
MI0022715	hsa-mir-6868	Stem-loop	2.89	8.15E-05	1.30E-03
MIMAT0027684	hsa-miR-6892-5p	miRNA	2.89	1.10E-03	8.90E-03
MIMAT0019903	hsa-miR-4758-5p	miRNA	2.88	3.75E-08	5.41E-06
MIMAT0019922	hsa-miR-4769-5p	miRNA	2.88	2.45E-06	1.00E-04
MIMAT0027634	hsa-miR-6867-5p	miRNA	2.88	6.00E-04	5.60E-03
MIMAT0019743	hsa-miR-4667-5p	miRNA	2.84	4.44E-05	9.00E-04
MIMAT0019778	hsa-miR-4689	miRNA	2.81	1.28E-06	6.94E-05
MIMAT0019723	hsa-miR-4656	miRNA	2.8	2.45E-06	1.00E-04
MIMAT0018191	hsa-miR-3917	miRNA	2.78	1.99E-05	5.00E-04

MIMAT0019749	hsa-miR-4669	miRNA	2.77	4.69E-05	9.00E-04
MIMAT0015070	hsa-miR-3188	miRNA	2.76	1.68E-05	5.00E-04
MI0015896	hsa-mir-4288	Stem-loop	2.75	1.00E-03	8.30E-03
ENSG00000200891	ENSG00000200891	snoRNA	2.74	8.00E-04	7.20E-03
ENSG00000238629	ENSG00000238629	snoRNA	2.73	2.43E-05	6.00E-04
MI0023619	hsa-mir-7161	Stem-loop	2.73	2.82E-05	7.00E-04
MIMAT0019231	hsa-miR-3944-5p	miRNA	2.68	3.00E-04	3.40E-03
MIMAT0019859	hsa-miR-4734	miRNA	2.65	5.39E-07	3.72E-05
MIMAT0019953	hsa-miR-2467-3p	miRNA	2.65	2.08E-06	9.99E-05
MIMAT0027377	hsa-miR-6738-5p	miRNA	2.64	3.00E-04	3.70E-03
MIMAT0026608	hsa-miR-181d-3p	miRNA	2.64	1.10E-03	8.80E-03
MIMAT0027665	hsa-miR-6882-3p	miRNA	2.63	3.00E-04	3.60E-03
MIMAT0031012	hsa-miR-8085	miRNA	2.62	9.00E-04	8.10E-03
MIMAT0019868	hsa-miR-4739	miRNA	2.6	3.72E-09	1.23E-06
MIMAT0027596	hsa-miR-6848-5p	miRNA	2.6	2.13E-06	1.00E-04
MI0014182	hsa-mir-3154	Stem-loop	2.58	3.64E-05	8.00E-04
MIMAT0022271	hsa-miR-664b-5p	miRNA	2.56	1.00E-04	2.00E-03
MIMAT0016874	hsa-miR-4321	miRNA	2.54	1.30E-06	6.98E-05
MIMAT0027670	hsa-miR-6885-5p	miRNA	2.53	5.32E-05	1.00E-03
MIMAT0015064	hsa-miR-3184-5p	miRNA	2.5	3.47E-06	1.00E-04
MIMAT0018929	hsa-miR-4417	miRNA	2.49	3.67E-06	2.00E-04
MIMAT0018987	hsa-miR-4463	miRNA	2.48	1.95E-07	1.84E-05
MI0016088	hsa-mir-3687	Stem-loop	2.48	3.00E-04	3.20E-03
MIMAT0016881	hsa-miR-4260	miRNA	2.48	3.00E-04	3.20E-03
ENSG00000238608	ENSG00000238608	snoRNA	2.47	1.10E-03	9.10E-03
MIMAT0027660	hsa-miR-6880-5p	miRNA	2.46	1.20E-06	6.65E-05
MIMAT0015080	hsa-miR-3196	miRNA	2.46	4.24E-05	9.00E-04
MIMAT0005889	hsa-miR-548l	miRNA	2.46	3.00E-04	3.50E-03
MIMAT0004951	hsa-miR-887-3p	miRNA	2.44	5.00E-04	4.90E-03

ENSG00000252617	ENSG00000252617	snoRNA	2.44	7.00E-04	6.90E-03
MIMAT0018958	hsa-miR-4440	miRNA	2.42	1.20E-06	6.65E-05
MIMAT0003306	hsa-miR-636	miRNA	2.41	7.88E-05	1.30E-03
MIMAT0024597	hsa-miR-6124	miRNA	2.4	3.45E-05	7.00E-04
MIMAT0027404	hsa-miR-6752-5p	miRNA	2.39	5.09E-08	6.62E-06
MIMAT0018981	hsa-miR-4459	miRNA	2.39	4.27E-07	3.18E-05
MI0000474	hsa-mir-134	Stem-loop	2.39	3.00E-04	3.20E-03
MIMAT0019896	hsa-miR-4755-3p	miRNA	2.37	6.09E-08	7.62E-06
MIMAT0027486	hsa-miR-6793-5p	miRNA	2.37	1.74E-06	8.87E-05
MIMAT0016852	hsa-miR-4298	miRNA	2.37	2.90E-05	7.00E-04
MIMAT0022947	hsa-miR-1238-5p	miRNA	2.36	1.15E-05	4.00E-04
MI0022595	hsa-mir-6750	Stem-loop	2.36	3.00E-04	3.30E-03
MIMAT0027398	hsa-miR-6749-5p	miRNA	2.35	2.11E-06	1.00E-04
MIMAT0027412	hsa-miR-6756-5p	miRNA	2.33	2.68E-08	4.53E-06
MIMAT0027448	hsa-miR-6774-5p	miRNA	2.33	1.58E-05	5.00E-04
MIMAT0027038	hsa-miR-1343-5p	miRNA	2.32	3.49E-05	7.00E-04
ENSG00000238629	ENSG00000238629	snoRNA	2.3	3.25E-05	7.00E-04
MIMAT0002847	hsa-miR-518c-5p	miRNA	2.3	8.30E-05	1.40E-03
ACA67	ACA67	HAcaBox	2.3	2.00E-04	2.70E-03
MIMAT0027385	hsa-miR-6742-5p	miRNA	2.28	5.00E-04	5.00E-03
MIMAT0028228	hsa-miR-7159-5p	miRNA	2.28	8.00E-04	7.00E-03
MIMAT0019849	hsa-miR-4728-5p	miRNA	2.27	8.54E-07	5.32E-05
MIMAT0027418	hsa-miR-6759-5p	miRNA	2.27	1.20E-06	6.65E-05
MIMAT0003280	hsa-miR-612	miRNA	2.25	2.00E-04	2.50E-03
MI0008335	hsa-mir-1914	Stem-loop	2.25	3.00E-04	3.60E-03
MIMAT0025476	hsa-miR-6510-5p	miRNA	2.24	1.53E-05	4.00E-04
MIMAT0004920	hsa-miR-541-3p	miRNA	2.23	2.00E-04	2.80E-03
MI0017374	hsa-mir-4737	Stem-loop	2.23	3.00E-04	3.60E-03
MI0025908	hsa-mir-8072	Stem-loop	2.22	7.49E-05	1.30E-03

MIMAT0018960	hsa-miR-4442	miRNA	2.22	3.00E-04	3.40E-03
MIMAT0016901	hsa-miR-4271	miRNA	2.22	9.00E-04	7.90E-03
MIMAT0025854	hsa-miR-6722-3p	miRNA	2.21	2.29E-06	1.00E-04
MIMAT0018076	hsa-miR-3656	miRNA	2.2	1.95E-06	9.53E-05
MI0015858	hsa-mir-4259	Stem-loop	2.2	3.00E-04	3.50E-03
MIMAT0027392	hsa-miR-6746-5p	miRNA	2.19	3.62E-07	2.73E-05
MIMAT0015010	hsa-miR-3141	miRNA	2.19	8.73E-06	3.00E-04
MIMAT0027576	hsa-miR-6837-5p	miRNA	2.19	6.42E-05	1.10E-03
MIMAT0027381	hsa-miR-6740-5p	miRNA	2.19	8.00E-04	7.00E-03
MIMAT0019018	hsa-miR-4484	miRNA	2.18	1.00E-04	2.00E-03
MIMAT0004901	hsa-miR-298	miRNA	2.18	1.10E-03	9.20E-03
MIMAT0027640	hsa-miR-6870-5p	miRNA	2.17	6.00E-04	5.60E-03
MIMAT0019031	hsa-miR-4496	miRNA	2.17	1.20E-03	9.40E-03
MI0018166	hsa-mir-5187	Stem-loop	2.15	5.32E-06	2.00E-04
ENSG00000252440	ENSG00000252440	snoRNA	2.15	4.00E-04	4.00E-03
MI0016872	hsa-mir-4508	Stem-loop	2.14	6.00E-04	6.10E-03
MIMAT0018084	hsa-miR-3663-5p	miRNA	2.13	6.40E-06	2.00E-04
MIMAT0016873	hsa-miR-4322	miRNA	2.12	9.51E-06	3.00E-04
MI0022559	hsa-mir-6724	Stem-loop	2.12	3.00E-04	3.80E-03
MIMAT0000267	hsa-miR-210-3p	miRNA	2.12	4.00E-04	4.60E-03
MI0022707	hsa-mir-6860	Stem-loop	2.11	8.18E-08	9.68E-06
MIMAT0022941	hsa-miR-1227-5p	miRNA	2.11	1.95E-06	9.53E-05
MIMAT0026605	hsa-miR-489-5p	miRNA	2.11	5.00E-04	4.80E-03
MIMAT0024598	hsa-miR-6125	miRNA	2.1	4.13E-06	2.00E-04
MIMAT0007890	hsa-miR-1914-3p	miRNA	2.1	1.00E-04	1.90E-03
MI0018001	hsa-mir-5095	Stem-loop	2.09	2.16E-05	6.00E-04
ENSG00000238309	ENSG00000238309	snoRNA	2.09	1.00E-03	8.10E-03
MI0017371	hsa-mir-4734	Stem-loop	2.08	6.82E-06	2.00E-04
MIMAT0027460	hsa-miR-6780a-5p	miRNA	2.08	1.91E-05	5.00E-04

MI0000826	hsa-mir-346	Stem-loop	2.07	2.00E-04	2.30E-03
MI0017374	hsa-mir-4737	Stem-loop	2.05	5.00E-04	5.30E-03
MI0023615	hsa-mir-7155	Stem-loop	2.04	2.00E-04	2.10E-03
MIMAT0004696	hsa-miR-323a-5p	miRNA	2.04	2.00E-04	2.20E-03
MIMAT0027556	hsa-miR-6828-5p	miRNA	2.03	4.05E-05	8.00E-04
MIMAT0026486	hsa-miR-328-5p	miRNA	2.02	3.60E-07	2.73E-05
MIMAT0028109	hsa-miR-7106-5p	miRNA	2.02	9.05E-06	3.00E-04
MI0015843	hsa-mir-4313	Stem-loop	2.02	1.56E-05	5.00E-04
MIMAT0019072	hsa-miR-4533	miRNA	2.02	1.00E-04	2.00E-03
MI0003670	hsa-mir-662	Stem-loop	2.02	2.00E-04	2.70E-03
MIMAT0019050	hsa-miR-4513	miRNA	2.02	2.00E-04	2.90E-03
MI0017350	hsa-mir-4716	Stem-loop	2.01	2.00E-04	2.70E-03
MIMAT0005922	hsa-miR-1268a	miRNA	2	7.46E-07	4.85E-05
U35A	U35A	CDBox	-2	4.45E-05	9.00E-04
MIMAT0003339	hsa-miR-421	miRNA	-2	1.00E-04	2.00E-03
MI0000078	hsa-mir-22	Stem-loop	-2	3.00E-04	3.80E-03
MI0003662	hsa-mir-647	Stem-loop	-2.01	3.89E-06	2.00E-04
U82	U82	CDBox	-2.01	1.00E-04	1.90E-03
MIMAT0000232	hsa-miR-199a-3p	miRNA	-2.01	4.00E-04	4.40E-03
MIMAT0004563	hsa-miR-199b-3p	miRNA	-2.01	4.00E-04	4.40E-03
MIMAT0000256	hsa-miR-181a-5p	miRNA	-2.02	3.88E-05	8.00E-04
U32A	U32A	CDBox	-2.02	3.00E-04	3.40E-03
MIMAT0000101	hsa-miR-103a-3p	miRNA	-2.03	2.00E-04	2.80E-03
U14B	U14B	CDBox	-2.03	1.20E-03	9.80E-03
HBII-142	HBII-142	CDBox	-2.04	1.28E-05	4.00E-04
MIMAT0003888	hsa-miR-766-3p	miRNA	-2.04	1.00E-04	1.80E-03
MIMAT0003261	hsa-miR-593-5p	miRNA	-2.04	2.00E-04	2.40E-03
MIMAT0025482	hsa-miR-6513-5p	miRNA	-2.05	9.41E-05	1.50E-03
MIMAT0019217	hsa-miR-3189-5p	miRNA	-2.05	4.00E-04	3.90E-03

MIMAT0004558	hsa-miR-181a-2-3p	miRNA	-2.05	4.00E-04	4.20E-03
ENSG00000238525	ENSG00000238525	snoRNA	-2.05	6.00E-04	6.20E-03
MI0001446	hsa-mir-424	Stem-loop	-2.05	8.00E-04	7.00E-03
MI0017357	hsa-mir-4722	Stem-loop	-2.06	1.00E-04	1.90E-03
U38A	U38A	CDBox	-2.07	2.20E-05	6.00E-04
MIMAT0000100	hsa-miR-29b-3p	miRNA	-2.07	4.00E-04	4.40E-03
HBII-142	HBII-142	CDBox	-2.08	1.40E-05	4.00E-04
mgh28S-2409	mgh28S-2409	CDBox	-2.08	1.50E-05	4.00E-04
MIMAT0019772	hsa-miR-4685-3p	miRNA	-2.08	1.00E-04	2.00E-03
MIMAT0000104	hsa-miR-107	miRNA	-2.08	2.00E-04	2.50E-03
U54	U54	CDBox	-2.09	4.82E-06	2.00E-04
ACA28	ACA28	HAcaBox	-2.09	9.00E-04	7.70E-03
MIMAT0016898	hsa-miR-4263	miRNA	-2.1	2.53E-05	6.00E-04
MIMAT0004784	hsa-miR-455-3p	miRNA	-2.1	6.47E-05	1.10E-03
MIMAT0004672	hsa-miR-106b-3p	miRNA	-2.11	2.03E-07	1.87E-05
MIMAT0000222	hsa-miR-192-5p	miRNA	-2.11	2.34E-05	6.00E-04
ACA44	ACA44	HAcaBox	-2.11	8.12E-05	1.30E-03
ENSG00000200969	ENSG00000200969	snoRNA	-2.11	4.00E-04	4.60E-03
MIMAT0021084	hsa-miR-5092	miRNA	-2.11	5.00E-04	5.00E-03
U58B	U58B	CDBox	-2.12	2.60E-06	1.00E-04
ENSG00000265941	ENSG00000265941	snoRNA	-2.12	1.00E-04	2.00E-03
U36B	U36B	CDBox	-2.12	1.00E-04	2.00E-03
ACA44	ACA44	HAcaBox	-2.12	5.00E-04	4.90E-03
ENSG00000252840	ENSG00000252840	snoRNA	-2.12	5.00E-04	4.90E-03
MIMAT0030416	hsa-miR-1273h-3p	miRNA	-2.12	8.00E-04	7.30E-03
MI0016805	hsa-mir-4459	Stem-loop	-2.12	8.00E-04	7.40E-03
ENSG00000200969	ENSG00000200969	snoRNA	-2.12	8.00E-04	7.60E-03
MIMAT0001340	hsa-miR-423-3p	miRNA	-2.13	2.74E-05	6.00E-04
SNORD119	SNORD119	CDBox	-2.13	3.00E-04	3.80E-03

snR38C	snR38C	CDBox	-2.13	5.00E-04	5.00E-03
MIMAT0000460	hsa-miR-194-5p	miRNA	-2.14	6.08E-05	1.10E-03
ACA48	ACA48	HAcaBox	-2.15	7.08E-07	4.72E-05
MIMAT0000419	hsa-miR-27b-3p	miRNA	-2.15	2.00E-04	2.80E-03
MIMAT0005577	hsa-miR-1226-3p	miRNA	-2.15	3.00E-04	3.10E-03
MIMAT0015044	hsa-miR-3169	miRNA	-2.15	4.00E-04	4.00E-03
MIMAT0009196	hsa-miR-103a-2-5p	miRNA	-2.15	5.00E-04	5.40E-03
Z17B	Z17B	CDBox	-2.16	2.00E-04	2.10E-03
MIMAT0002888	hsa-miR-532-5p	miRNA	-2.16	9.00E-04	7.80E-03
HBII-429	HBII-429	CDBox	-2.16	1.20E-03	9.40E-03
U17b	U17b	HAcaBox	-2.17	7.93E-05	1.30E-03
MIMAT0004675	hsa-miR-219a-2-3p	miRNA	-2.17	2.00E-04	2.30E-03
HBII-85-2	HBII-85-2	CDBox	-2.17	2.00E-04	2.70E-03
MIMAT0025463	hsa-miR-6503-3p	miRNA	-2.19	4.78E-06	2.00E-04
MIMAT0000760	hsa-miR-331-3p	miRNA	-2.2	1.00E-04	1.90E-03
MIMAT0004749	hsa-miR-424-3p	miRNA	-2.21	6.76E-06	2.00E-04
ENSG00000202252	ENSG00000202252	snoRNA	-2.24	1.12E-05	4.00E-04
MIMAT0019895	hsa-miR-4755-5p	miRNA	-2.24	3.52E-05	7.00E-04
MIMAT0000252	hsa-miR-7-5p	miRNA	-2.24	2.00E-04	2.10E-03
MIMAT0027088	hsa-miR-5189-3p	miRNA	-2.25	1.20E-03	9.60E-03
U71a	U71a	HAcaBox	-2.26	3.17E-05	7.00E-04
U79	U79	CDBox	-2.26	3.00E-04	3.70E-03
MIMAT0000087	hsa-miR-30a-5p	miRNA	-2.27	3.81E-06	2.00E-04
mgh28S-2411	mgh28S-2411	CDBox	-2.27	9.52E-06	3.00E-04
MIMAT0002816	hsa-miR-494-3p	miRNA	-2.27	5.00E-04	5.00E-03
MIMAT0002174	hsa-miR-484	miRNA	-2.27	8.00E-04	7.50E-03
MI0009983	hsa-mir-1973	Stem-loop	-2.27	9.00E-04	7.70E-03
MIMAT0000617	hsa-miR-200c-3p	miRNA	-2.28	1.10E-03	9.20E-03
MIMAT0018085	hsa-miR-3663-3p	miRNA	-2.29	9.00E-05	1.40E-03

U25	U25	CDBox	-2.3	1.50E-06	7.83E-05
U49A	U49A	CDBox	-2.32	1.25E-05	4.00E-04
U28	U28	CDBox	-2.35	2.25E-05	6.00E-04
U49A	U49A	CDBox	-2.36	1.34E-05	4.00E-04
HBII-438A	HBII-438A	CDBox	-2.36	6.07E-05	1.10E-03
HBII-438B	HBII-438B	CDBox	-2.36	6.07E-05	1.10E-03
HBII-289	HBII-289	CDBox	-2.36	2.00E-04	2.90E-03
MIMAT0004597	hsa-miR-140-3p	miRNA	-2.37	2.04E-06	9.87E-05
ENSG00000238525	ENSG00000238525	snoRNA	-2.37	8.89E-05	1.40E-03
MIMAT0000461	hsa-miR-195-5p	miRNA	-2.37	1.00E-04	2.00E-03
MIMAT0015041	hsa-miR-1260b	miRNA	-2.38	6.62E-06	2.00E-04
ACA20	ACA20	HAcaBox	-2.38	2.00E-04	2.60E-03
MIMAT0021024	hsa-miR-5002-3p	miRNA	-2.39	9.00E-04	7.60E-03
MIMAT0000278	hsa-miR-221-3p	miRNA	-2.4	4.31E-05	9.00E-04
MIMAT0003322	hsa-miR-652-3p	miRNA	-2.4	5.88E-05	1.10E-03
MIMAT0011777	hsa-miR-2277-3p	miRNA	-2.4	1.00E-04	2.00E-03
MIMAT0028222	hsa-miR-7156-5p	miRNA	-2.4	1.20E-03	9.60E-03
MIMAT0004553	hsa-miR-7-1-3p	miRNA	-2.42	7.12E-07	4.72E-05
U28	U28	CDBox	-2.42	3.24E-06	1.00E-04
MIMAT0017984	hsa-miR-3607-5p	miRNA	-2.42	1.00E-04	2.00E-03
MIMAT0000245	hsa-miR-30d-5p	miRNA	-2.44	1.83E-07	1.78E-05
HBII-180A	HBII-180A	CDBox	-2.45	5.62E-06	2.00E-04
ENSG00000222345	ENSG00000222345	snoRNA	-2.45	9.42E-05	1.50E-03
MIMAT0004515	hsa-miR-29b-2-5p	miRNA	-2.46	1.10E-06	6.38E-05
ENSG00000201199	ENSG00000201199	snoRNA	-2.46	8.09E-05	1.30E-03
ENSG00000201592	ENSG00000201592	snoRNA	-2.46	8.09E-05	1.30E-03
MIMAT0004955	hsa-miR-374b-5p	miRNA	-2.48	3.08E-05	7.00E-04
MIMAT0016916	hsa-miR-4286	miRNA	-2.48	8.06E-05	1.30E-03
MIMAT0000424	hsa-miR-128-3p	miRNA	-2.49	4.12E-05	8.00E-04

MIMAT0004682	hsa-miR-361-3p	miRNA	-2.5	3.29E-05	7.00E-04
MIMAT0000759	hsa-miR-148b-3p	miRNA	-2.51	7.00E-04	6.50E-03
U27	U27	CDBox	-2.52	3.50E-05	7.00E-04
U60	U60	CDBox	-2.53	6.00E-06	2.00E-04
HBII-239	HBII-239	CDBox	-2.53	1.42E-05	4.00E-04
ACA3-2	ACA3-2	HAcaBox	-2.53	1.65E-05	5.00E-04
MIMAT0000085	hsa-miR-28-5p	miRNA	-2.55	9.32E-07	5.65E-05
U76	U76	CDBox	-2.58	3.45E-05	7.00E-04
MIMAT0022726	hsa-miR-1306-5p	miRNA	-2.58	7.00E-04	6.50E-03
MIMAT0004692	hsa-miR-340-5p	miRNA	-2.58	1.10E-03	9.20E-03
MIMAT0003218	hsa-miR-92b-3p	miRNA	-2.59	2.50E-05	6.00E-04
U42B	U42B	CDBox	-2.59	3.07E-05	7.00E-04
MIMAT0000693	hsa-miR-30e-3p	miRNA	-2.6	5.07E-05	1.00E-03
MIMAT0025458	hsa-miR-6501-5p	miRNA	-2.6	3.00E-04	3.90E-03
MIMAT0000727	hsa-miR-374a-5p	miRNA	-2.62	2.00E-04	2.70E-03
U42B	U42B	CDBox	-2.64	6.08E-05	1.10E-03
MIMAT0027687	hsa-miR-6893-3p	miRNA	-2.66	4.00E-04	4.00E-03
U55	U55	CDBox	-2.67	8.75E-05	1.40E-03
U49A	U49A	CDBox	-2.68	2.50E-05	6.00E-04
U49B	U49B	CDBox	-2.68	2.50E-05	6.00E-04
MIMAT0016872	hsa-miR-4317	miRNA	-2.68	1.00E-04	2.00E-03
MIMAT0000765	hsa-miR-335-5p	miRNA	-2.71	4.82E-05	9.00E-04
ACA24	ACA24	HAcaBox	-2.75	3.00E-04	3.60E-03
ENSG00000206903	ENSG00000206903	snoRNA	-2.75	3.00E-04	3.60E-03
ENSG00000207130	ENSG00000207130	snoRNA	-2.75	3.00E-04	3.60E-03
U57	U57	CDBox	-2.76	1.51E-07	1.56E-05
MIMAT0004502	hsa-miR-28-3p	miRNA	-2.76	8.58E-06	3.00E-04
MIMAT0000072	hsa-miR-18a-5p	miRNA	-2.76	1.22E-05	4.00E-04
HBII-276	HBII-276	CDBox	-2.76	6.91E-05	1.20E-03

ENSG00000212615	ENSG00000212615	snoRNA	-2.79	5.00E-06	2.00E-04
MIMAT0003885	hsa-miR-454-3p	miRNA	-2.8	5.82E-05	1.10E-03
MIMAT0022259	hsa-miR-5100	miRNA	-2.82	3.28E-06	1.00E-04
HBII-202	HBII-202	CDBox	-2.86	4.83E-05	9.00E-04
MIMAT0004501	hsa-miR-27a-5p	miRNA	-2.88	4.88E-05	1.00E-03
MIMAT0009448	hsa-miR-1973	miRNA	-2.88	5.00E-04	5.20E-03
MIMAT0019877	hsa-miR-3591-3p	miRNA	-2.91	1.20E-03	9.60E-03
U27	U27	CDBox	-2.92	1.28E-05	4.00E-04
MIMAT0000691	hsa-miR-130b-3p	miRNA	-2.96	3.24E-05	7.00E-04
MIMAT0019817	hsa-miR-4711-3p	miRNA	-3	1.46E-05	4.00E-04
MIMAT0018443	hsa-miR-374c-5p	miRNA	-3.01	6.00E-04	6.00E-03
MIMAT0000318	hsa-miR-200b-3p	miRNA	-3.04	1.50E-05	4.00E-04
MIMAT0018000	hsa-miR-23c	miRNA	-3.07	6.47E-05	1.10E-03
MIMAT0000750	hsa-miR-340-3p	miRNA	-3.08	2.61E-05	6.00E-04
MIMAT0005941	hsa-miR-1284	miRNA	-3.09	2.40E-05	6.00E-04
U80	U80	CDBox	-3.13	4.96E-06	2.00E-04
U33	U33	CDBox	-3.17	5.25E-06	2.00E-04
MIMAT0000425	hsa-miR-130a-3p	miRNA	-3.19	6.81E-05	1.20E-03
U103	U103	CDBox	-3.25	3.44E-07	2.68E-05
U103B	U103B	CDBox	-3.25	3.44E-07	2.68E-05
MIMAT0031180	hsa-miR-7977	miRNA	-3.25	2.41E-06	1.00E-04
HBII-99	HBII-99	CDBox	-3.28	3.80E-05	8.00E-04
U58A	U58A	CDBox	-3.3	1.22E-06	6.68E-05
ACA20	ACA20	HAcaBox	-3.36	1.00E-04	1.70E-03
MIMAT0027507	hsa-miR-6803-3p	miRNA	-3.37	8.89E-06	3.00E-04
MIMAT0004484	hsa-let-7d-3p	miRNA	-3.4	1.60E-07	1.63E-05
MIMAT0000227	hsa-miR-197-3p	miRNA	-3.43	4.00E-09	1.26E-06
MIMAT0017991	hsa-miR-3613-3p	miRNA	-3.5	3.00E-04	3.70E-03
U59B	U59B	CDBox	-3.53	1.13E-06	6.44E-05

MIMAT0017982	hsa-miR-3605-3p	miRNA	-3.58	4.67E-06	2.00E-04
MIMAT0017986	hsa-miR-3609	miRNA	-3.6	1.67E-05	5.00E-04
U41	U41	CDBox	-3.7	1.00E-04	1.80E-03
U59A	U59A	CDBox	-3.73	1.95E-06	9.53E-05
U58C	U58C	CDBox	-3.74	2.34E-07	2.07E-05
U58C	U58C	CDBox	-3.8	3.04E-07	2.49E-05
MIMAT0004780	hsa-miR-532-3p	miRNA	-3.87	2.00E-04	2.40E-03
MIMAT0003298	hsa-miR-629-3p	miRNA	-4.38	2.81E-06	1.00E-04
MIMAT0019837	hsa-miR-4722-3p	miRNA	-4.45	6.78E-05	1.20E-03
MIMAT0002807	hsa-miR-491-5p	miRNA	-4.45	7.00E-04	6.50E-03
U78	U78	CDBox	-4.93	9.37E-07	5.65E-05
ENSG00000212378	ENSG00000212378	snoRNA	-5.23	2.99E-07	2.48E-05
U78	U78	CDBox	-5.23	2.99E-07	2.48E-05
MIMAT0000443	hsa-miR-125a-5p	miRNA	-5.36	6.22E-05	1.10E-03
HBII-55	HBII-55	CDBox	-5.37	2.79E-07	2.40E-05
U47	U47	CDBox	-8.04	5.38E-07	3.72E-05

S5a Table. Pathways enriched by upregulated miRNA targets.

Term name	Adjusted p-value	Negative log10 of adjusted p-value	Term size	Intersection size	% of DE in Pathway	Intersections
Steroid biosynthesis	1.2E-02	1.92	19	2	10.53	ENSG00000057252,ENSG00000107798
Glycolysis / Gluconeogenesis	3.4E-06	5.47	64	6	9.38	ENSG00000134333,ENSG00000150768, ENSG00000111669,ENSG00000107789, ENSG00000074800,ENSG00000111640
Cholesterol metabolism	5.0E-04	3.30	50	4	8.00	ENSG00000057252,ENSG00000141458, ENSG00000107798,ENSG00000213585
Glutathione metabolism	6.2E-04	3.21	56	4	7.14	ENSG00000104687,ENSG00000008394, ENSG00000148834,ENSG00000001084
Cysteine and methionine metabolism	4.4E-03	2.36	49	3	6.12	ENSG00000134333,ENSG00000060982, ENSG00000001084
Antigen processing and presentation	1.0E-03	2.99	67	4	5.97	ENSG00000164733,ENSG00000216490, ENSG00000204388,ENSG00000135047
Biosynthesis of amino acids	1.3E-03	2.89	74	4	5.41	ENSG00000111669,ENSG00000060982, ENSG00000074800,ENSG00000111640
Mineral absorption	6.5E-03	2.18	57	3	5.26	ENSG00000187193,ENSG00000125144, ENSG00000100292
Ferroptosis	4.2E-02	1.38	39	2	5.13	ENSG00000001084,ENSG00000100292
Chemical carcinogenesis - DNA adducts	8.4E-03	2.08	63	3	4.76	ENSG00000008394,ENSG00000148834, ENSG00000138061
Rheumatoid arthritis	1.9E-03	2.72	86	4	4.65	ENSG00000112715,ENSG00000149968, ENSG00000135047,ENSG00000100554
HIF-1 signaling pathway	5.6E-04	3.25	108	5	4.63	ENSG00000134333,ENSG00000112715, ENSG00000100292,ENSG00000074800, ENSG00000111640
Fluid shear stress and atherosclerosis	1.6E-04	3.78	134	6	4.48	ENSG00000169439,ENSG00000008394, ENSG00000148834,ENSG00000112715, ENSG00000100292,ENSG00000135047
Metabolism of xenobiotics by cytochrome P450	1.0E-02	2.00	68	3	4.41	ENSG00000008394,ENSG00000148834, ENSG00000138061
Lysosome	9.4E-04	3.03	126	5	3.97	ENSG00000141458,ENSG00000164733, ENSG00000119899,ENSG00000107798, ENSG00000135047
ECM-receptor interaction	1.6E-02	1.80	82	3	3.66	ENSG00000118785,ENSG00000188153, ENSG00000072571

Carbon metabolism	4.2E-03	2.37	112	4	3.57	ENSG00000150768,ENSG00000111669, ENSG00000074800,ENSG00000111640
Chemical carcinogenesis - reactive oxygen species	1.6E-04	3.78	206	7	3.40	ENSG00000008394,ENSG00000148834, ENSG00000106546,ENSG00000112715, ENSG00000100292,ENSG00000138061, ENSG00000213585
mTOR signaling pathway	1.6E-03	2.80	151	5	3.31	ENSG00000052795,ENSG00000104290, ENSG00000085415,ENSG00000102547, ENSG00000100554
Hepatocellular carcinoma	1.9E-03	2.72	162	5	3.09	ENSG00000198431,ENSG00000008394, ENSG00000148834,ENSG00000104290, ENSG00000100292
Proteoglycans in cancer	4.2E-03	2.37	200	5	2.50	ENSG00000169439,ENSG00000137710, ENSG00000112715,ENSG00000104290, ENSG00000135047
Cell cycle	4.2E-02	1.38	120	3	2.50	ENSG00000006634,ENSG00000169679, ENSG00000076003
Chemical carcinogenesis - receptor activation	4.2E-03	2.37	202	5	2.48	ENSG00000008394,ENSG00000148834, ENSG00000106546,ENSG00000112715, ENSG00000138061
Human papillomavirus infection	1.0E-03	2.99	315	7	2.22	ENSG00000185825,ENSG00000118785, ENSG00000188153,ENSG00000112715, ENSG00000055332,ENSG00000104290, ENSG00000100554
MicroRNAs in cancer	4.2E-03	2.37	306	6	1.96	ENSG00000137710,ENSG00000085563, ENSG00000112715,ENSG00000104290, ENSG00000100292,ENSG00000138061
Pathways in cancer	1.9E-04	3.72	517	10	1.93	ENSG00000198431,ENSG00000008394, ENSG00000148834,ENSG00000188153, ENSG00000112715,ENSG00000085276, ENSG00000104290,ENSG00000187098, ENSG00000100292,ENSG00000183230
Endocytosis	4.2E-02	1.38	237	4	1.69	ENSG00000039319,ENSG00000204388, ENSG00000205302,ENSG00000136631
Metabolic pathways	3.8E-09	8.41	1457	24	1.65	ENSG00000104687,ENSG00000114480, ENSG00000134333,ENSG00000136267, ENSG00000150768,ENSG00000111669, ENSG00000008394,ENSG00000144362,

						ENSG00000156110,ENSG00000148834, ENSG00000060982,ENSG00000087299, ENSG00000136010,ENSG00000001084, ENSG00000107789,ENSG00000085276, ENSG00000165092,ENSG00000136169, ENSG00000128050,ENSG00000100292, ENSG00000146070,ENSG00000074800, ENSG00000111640,ENSG00000100554
Alzheimer disease	3.6E-02	1.44	353	5	1.42	ENSG00000055332,ENSG00000104290, ENSG00000159593,ENSG00000111640, ENSG00000213585

S5b Table. Pathways enriched by downregulated miRNA targets.

Term name	Adjusted p-value	Negative log10 of adjusted p-value	Term size	Intersection size	% of DE in Pathway	Intersections
Antigen processing and presentation	1.4E-14	13.85	67	14	20.90	ENSG00000166710,ENSG00000204267, ENSG00000204525,ENSG00000206503, ENSG00000019582,ENSG00000100911, ENSG00000163131,ENSG00000168394, ENSG00000231925,ENSG00000092010, ENSG00000234745,ENSG00000143390, ENSG00000204592,ENSG00000044574
Primary immunodeficiency	1.3E-07	6.87	37	7	18.92	ENSG00000204267,ENSG00000147168, ENSG00000081237,ENSG00000196839, ENSG00000168394,ENSG00000276045, ENSG00000143390
B cell receptor signaling pathway	7.9E-14	13.10	76	14	18.42	ENSG00000177885,ENSG00000254087, ENSG00000155629,ENSG00000171608, ENSG00000165458,ENSG00000204577, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000100906, ENSG00000128340,ENSG00000173039, ENSG00000131042,ENSG00000104825
Viral myocarditis	3.7E-10	9.44	55	10	18.18	ENSG00000204525,ENSG00000206503, ENSG00000136238,ENSG00000090339, ENSG00000015475,ENSG00000128340, ENSG00000075624,ENSG00000234745, ENSG00000204592,ENSG00000114867
Prolactin signaling pathway	6.7E-12	11.17	67	12	17.91	ENSG00000177885,ENSG00000184557, ENSG00000168610,ENSG00000171608, ENSG00000115415,ENSG00000109320, ENSG00000118971,ENSG00000114737, ENSG00000126561,ENSG00000125347, ENSG00000173039,ENSG00000160691
Chronic myeloid leukemia	9.6E-13	12.02	73	13	17.81	ENSG00000177885,ENSG00000135679, ENSG00000166949,ENSG00000171608, ENSG00000104365,ENSG00000109320, ENSG00000033327,ENSG00000171552, ENSG00000126561,ENSG00000100906, ENSG00000173039,ENSG00000112242,

						ENSG00000160691
Pancreatic cancer	1.1E-12	11.95	74	13	17.57	ENSG00000166949,ENSG00000168610, ENSG00000171608,ENSG00000115415, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000160271, ENSG00000171552,ENSG00000144118, ENSG00000128340,ENSG00000173039, ENSG00000112242
PD-L1 expression and PD-1 checkpoint pathway in cancer	3.2E-14	13.50	88	15	17.05	ENSG00000168610,ENSG00000027697, ENSG00000171608,ENSG00000115415, ENSG00000104365,ENSG00000109320, ENSG00000172936,ENSG00000143924, ENSG00000100906,ENSG00000034152, ENSG00000159128,ENSG00000100644, ENSG00000173039,ENSG00000127666, ENSG00000104825
Fc gamma R-mediated phagocytosis	4.5E-15	14.35	94	16	17.02	ENSG00000115091,ENSG00000162704, ENSG00000015285,ENSG00000254087, ENSG00000171608,ENSG00000081237, ENSG00000165458,ENSG00000125753, ENSG00000136238,ENSG00000033327, ENSG00000182541,ENSG00000128340, ENSG00000148180,ENSG00000101336, ENSG00000172757,ENSG00000277443
Leishmaniasis	1.3E-11	10.88	71	12	16.90	ENSG00000055208,ENSG00000169896, ENSG00000027697,ENSG00000125730, ENSG00000115415,ENSG00000109320, ENSG00000172936,ENSG00000100365, ENSG00000100906,ENSG00000159128, ENSG00000173039,ENSG00000104825
Neurotrophin signaling pathway	2.3E-17	16.63	115	19	16.52	ENSG00000177885,ENSG00000171608, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000127314, ENSG00000162889,ENSG00000134070, ENSG00000100906,ENSG00000116473, ENSG00000173039,ENSG00000080815, ENSG00000160691,ENSG00000117676, ENSG00000067560,ENSG00000090376,

						ENSG00000111252,ENSG00000104825, ENSG00000160014
Chemokine signaling pathway	1.5E-25	24.82	179	29	16.20	ENSG00000172724,ENSG00000177885, ENSG00000180871,ENSG00000168610, ENSG00000015285,ENSG00000137486, ENSG00000254087,ENSG00000171608, ENSG00000115415,ENSG00000104365, ENSG00000109320,ENSG00000136238, ENSG00000114353,ENSG00000127314, ENSG00000105723,ENSG00000124126, ENSG00000078369,ENSG00000100906, ENSG00000116473,ENSG00000128340, ENSG00000173039,ENSG00000160691, ENSG00000141506,ENSG00000101336, ENSG00000275302,ENSG00000067560, ENSG00000108691,ENSG00000104825, ENSG00000161921
Osteoclast differentiation	5.5E-18	17.26	124	20	16.13	ENSG00000177885,ENSG00000055208, ENSG00000184557,ENSG00000027697, ENSG00000171608,ENSG00000115415, ENSG00000204577,ENSG00000104365, ENSG00000198053,ENSG00000109320, ENSG00000136238,ENSG00000033327, ENSG00000100365,ENSG00000067182, ENSG00000100906,ENSG00000159128, ENSG00000173039,ENSG00000131042, ENSG00000077150,ENSG00000171223
Adipocytokine signaling pathway	1.9E-10	9.73	69	11	15.94	ENSG00000184557,ENSG00000168610, ENSG00000028137,ENSG00000104365, ENSG00000109320,ENSG00000068366, ENSG00000067182,ENSG00000100906, ENSG00000173039,ENSG00000110931, ENSG00000104825
Yersinia infection	1.1E-18	17.95	132	21	15.91	ENSG00000055208,ENSG00000177105, ENSG00000115091,ENSG00000162704, ENSG00000015285,ENSG00000171608, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000172936, ENSG00000139436,ENSG00000103313,

						ENSG00000100906,ENSG00000034152, ENSG00000128340,ENSG00000173039, ENSG00000075624,ENSG00000117676, ENSG00000127666,ENSG00000067560, ENSG00000108691
Toxoplasmosis	2.1E-15	14.69	107	17	15.89	ENSG00000055208,ENSG00000110324, ENSG00000168610,ENSG00000027697, ENSG00000115415,ENSG00000104365, ENSG00000109320,ENSG00000114353, ENSG00000172936,ENSG00000171552, ENSG00000067182,ENSG00000100906, ENSG00000034152,ENSG00000159128, ENSG00000173039,ENSG00000141506, ENSG00000104825
Kaposi sarcoma-associated herpesvirus infection	7.4E-26	25.13	191	30	15.71	ENSG00000204525,ENSG00000206503, ENSG00000168610,ENSG00000254087, ENSG00000027697,ENSG00000125730, ENSG00000128016,ENSG00000005339, ENSG00000171608,ENSG00000115415, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000090339, ENSG00000162889,ENSG00000067182, ENSG00000124126,ENSG00000078369, ENSG00000100906,ENSG00000015475, ENSG00000100644,ENSG00000173039, ENSG00000234745,ENSG00000148737, ENSG00000112242,ENSG00000141506, ENSG00000101336,ENSG00000127666, ENSG00000204592,ENSG00000160014
NF-kappa B signaling pathway	1.5E-14	13.81	102	16	15.69	ENSG00000172724,ENSG00000055208, ENSG00000254087,ENSG00000104365, ENSG00000109320,ENSG00000172936, ENSG00000090339,ENSG00000171552, ENSG00000067182,ENSG00000118503, ENSG00000100906,ENSG00000173039, ENSG00000077150,ENSG00000275302, ENSG00000003402,ENSG00000127666
TNF signaling pathway	3.0E-15	14.52	110	17	15.45	ENSG00000055208,ENSG00000184557, ENSG00000028137,ENSG00000171608,

						ENSG00000104365,ENSG00000109320, ENSG00000090339,ENSG00000069399, ENSG00000067182,ENSG00000118503, ENSG00000100906,ENSG00000034152, ENSG00000125347,ENSG00000173039, ENSG00000003402,ENSG00000108691, ENSG00000171223
Acute myeloid leukemia	1.9E-09	8.72	65	10	15.38	ENSG00000177885,ENSG00000168610, ENSG00000169896,ENSG00000171608, ENSG00000104365,ENSG00000109320, ENSG00000126561,ENSG00000112033, ENSG00000173039,ENSG00000148737
Glycosaminoglycan biosynthesis - keratan sulfate	1.1E-02	1.98	13	2	15.38	ENSG00000086062,ENSG00000157350
Chagas disease	1.4E-13	12.85	98	15	15.31	ENSG00000027697,ENSG00000125730, ENSG00000171608,ENSG00000104365, ENSG00000109320,ENSG00000114353, ENSG00000172936,ENSG00000106366, ENSG00000067182,ENSG00000100906, ENSG00000159128,ENSG00000173039, ENSG00000003402,ENSG00000127666, ENSG00000108691
Epstein-Barr virus infection	6.3E-25	24.20	191	29	15.18	ENSG00000055208,ENSG00000166710, ENSG00000135679,ENSG00000204267, ENSG00000204525,ENSG00000206503, ENSG00000168610,ENSG00000254087, ENSG00000171608,ENSG00000115415, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000172936, ENSG00000090339,ENSG00000118971, ENSG00000026508,ENSG00000118503, ENSG00000100906,ENSG00000034152, ENSG00000015475,ENSG00000168394, ENSG00000231925,ENSG00000173039, ENSG00000234745,ENSG00000112242, ENSG00000077150,ENSG00000204592, ENSG00000104825
Legionellosis	1.1E-07	6.95	54	8	14.81	ENSG00000169896,ENSG00000125730,

						ENSG00000109320,ENSG00000172936, ENSG00000174903,ENSG00000100906, ENSG00000173039,ENSG00000077150
Epithelial cell signaling in Helicobacter pylori infection	3.4E-09	8.47	69	10	14.49	ENSG00000180871,ENSG00000254087, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000100906, ENSG00000173039,ENSG00000131100, ENSG00000033627,ENSG00000158769
Pertussis	5.2E-10	9.28	76	11	14.47	ENSG00000169896,ENSG00000125730, ENSG00000109320,ENSG00000114353, ENSG00000172936,ENSG00000125347, ENSG00000173039,ENSG00000172757, ENSG00000127666,ENSG00000067560, ENSG00000160014
Phagosome	5.2E-16	15.28	137	19	13.87	ENSG00000144566,ENSG00000204267, ENSG00000204525,ENSG00000206503, ENSG00000169896,ENSG00000125730, ENSG00000186431,ENSG00000136238, ENSG00000163131,ENSG00000108774, ENSG00000100365,ENSG00000173391, ENSG00000168394,ENSG00000075624, ENSG00000234745,ENSG00000111540, ENSG00000131100,ENSG00000033627, ENSG00000204592
Renal cell carcinoma	3.2E-08	7.49	65	9	13.85	ENSG00000177885,ENSG00000180370, ENSG00000005339,ENSG00000171608, ENSG00000136238,ENSG00000127314, ENSG00000068323,ENSG00000116473, ENSG00000100644
Leukocyte transendothelial migration	8.0E-13	12.10	110	15	13.64	ENSG00000147065,ENSG00000169896, ENSG00000171608,ENSG00000125753, ENSG00000136238,ENSG00000114353, ENSG00000127314,ENSG00000090339, ENSG00000100365,ENSG00000116473, ENSG00000128340,ENSG00000075624, ENSG00000266094,ENSG00000067560, ENSG00000158769

Human immunodeficiency virus 1 infection	9.2E-23	22.04	207	28	13.53	ENSG00000055208,ENSG00000166710, ENSG00000204267,ENSG00000204525, ENSG00000206503,ENSG00000028137, ENSG00000180370,ENSG00000171608, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000114353, ENSG00000182541,ENSG00000172936, ENSG00000171552,ENSG00000067182, ENSG00000078369,ENSG00000100906, ENSG00000034152,ENSG00000015475, ENSG00000128340,ENSG00000168394, ENSG00000231925,ENSG00000173039, ENSG00000234745,ENSG00000172757, ENSG00000204592,ENSG00000160014
Human cytomegalovirus infection	1.7E-23	22.76	215	29	13.49	ENSG00000177885,ENSG00000166710, ENSG00000135679,ENSG00000204267, ENSG00000110324,ENSG00000204525, ENSG00000180871,ENSG00000206503, ENSG00000168610,ENSG00000171608, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000114353, ENSG00000067182,ENSG00000078369, ENSG00000100906,ENSG00000015475, ENSG00000128340,ENSG00000168394, ENSG00000231925,ENSG00000173039, ENSG00000234745,ENSG00000112242, ENSG00000275302,ENSG00000204592, ENSG00000067560,ENSG00000108691, ENSG00000160014
Lipid and atherosclerosis	1.1E-22	21.98	209	28	13.40	ENSG00000112096,ENSG00000055208, ENSG00000168610,ENSG00000254087, ENSG00000171608,ENSG00000104365, ENSG00000109320,ENSG00000136238, ENSG00000172936,ENSG00000127314, ENSG00000090339,ENSG00000171552, ENSG00000100365,ENSG00000067182, ENSG00000173391,ENSG00000100906, ENSG00000034152,ENSG00000015475, ENSG00000116473,ENSG00000120889,

						ENSG00000173039,ENSG00000100219, ENSG00000028277,ENSG00000127666, ENSG00000067560,ENSG00000108691, ENSG00000044574,ENSG00000160014
Measles	5.5E-15	14.26	135	18	13.33	ENSG00000055208,ENSG00000160710, ENSG00000147168,ENSG00000147065, ENSG00000168610,ENSG00000171608, ENSG00000115415,ENSG00000104365, ENSG00000109320,ENSG00000172936, ENSG00000171552,ENSG00000118971, ENSG00000126561,ENSG00000118503, ENSG00000100906,ENSG00000015475, ENSG00000173039,ENSG00000104825
Tuberculosis	1.1E-18	17.95	173	23	13.29	ENSG00000144566,ENSG00000110324, ENSG00000169896,ENSG00000027697, ENSG00000125730,ENSG00000005339, ENSG00000115415,ENSG00000019582, ENSG00000109320,ENSG00000163131, ENSG00000172936,ENSG00000108774, ENSG00000067182,ENSG00000134070, ENSG00000015475,ENSG00000159128, ENSG00000111424,ENSG00000173039, ENSG00000111540,ENSG00000033627, ENSG00000143390,ENSG00000067560, ENSG00000160014
Inflammatory bowel disease	2.7E-07	6.56	61	8	13.11	ENSG00000147168,ENSG00000166949, ENSG00000168610,ENSG00000027697, ENSG00000115415,ENSG00000109320, ENSG00000159128,ENSG00000173039
Adherens junction	5.3E-08	7.28	69	9	13.04	ENSG00000166949,ENSG00000015285, ENSG00000196396,ENSG00000005339, ENSG00000136238,ENSG00000128340, ENSG00000075624,ENSG00000148737, ENSG00000067560
Toll-like receptor signaling pathway	4.9E-11	10.31	100	13	13.00	ENSG00000055208,ENSG00000101916, ENSG00000171608,ENSG00000115415, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000172936,

						ENSG00000100906,ENSG00000034152, ENSG00000173039,ENSG00000275302, ENSG00000127666
Influenza A	6.4E-17	16.19	163	21	12.88	ENSG00000184557,ENSG00000160710, ENSG00000027697,ENSG00000005339, ENSG00000171608,ENSG00000115415, ENSG00000104365,ENSG00000109320, ENSG00000172936,ENSG00000090339, ENSG00000067182,ENSG00000100836, ENSG00000100906,ENSG00000015475, ENSG00000159128,ENSG00000120889, ENSG00000173039,ENSG00000075624, ENSG00000127666,ENSG00000108691, ENSG00000104825
Ferroptosis	6.6E-05	4.18	39	5	12.82	ENSG00000197548,ENSG00000068366, ENSG00000110911,ENSG00000169564, ENSG00000266412
Th1 and Th2 cell differentiation	1.9E-09	8.71	86	11	12.79	ENSG00000147168,ENSG00000027697, ENSG00000115415,ENSG00000104365, ENSG00000109320,ENSG00000126561, ENSG00000100906,ENSG00000184384, ENSG00000159128,ENSG00000173039, ENSG00000104825
C-type lectin receptor signaling pathway	6.3E-11	10.20	102	13	12.75	ENSG00000135679,ENSG00000171608, ENSG00000115415,ENSG00000104365, ENSG00000109320,ENSG00000069399, ENSG00000162889,ENSG00000100906, ENSG00000125347,ENSG00000173039, ENSG00000077150,ENSG00000067560, ENSG00000160014
Th17 cell differentiation	7.9E-11	10.10	104	13	12.50	ENSG00000147168,ENSG00000166949, ENSG00000168610,ENSG00000027697, ENSG00000115415,ENSG00000104365, ENSG00000109320,ENSG00000126561, ENSG00000100906,ENSG00000159128, ENSG00000100644,ENSG00000173039, ENSG00000104825

Bacterial invasion of epithelial cells	7.3E-08	7.14	72	9	12.50	ENSG00000177105,ENSG00000115091, ENSG00000162704,ENSG00000171608, ENSG00000136238,ENSG00000166333, ENSG00000075624,ENSG00000160691, ENSG00000067560
Apoptosis - multiple species	4.5E-04	3.35	32	4	12.50	ENSG00000171552,ENSG00000115317, ENSG00000067182,ENSG00000015475
Viral carcinogenesis	1.1E-18	17.95	194	24	12.37	ENSG00000177885,ENSG00000135679, ENSG00000204525,ENSG00000206503, ENSG00000168610,ENSG00000254087, ENSG00000125730,ENSG00000005339, ENSG00000171608,ENSG00000109320, ENSG00000136238,ENSG00000118971, ENSG00000162889,ENSG00000126561, ENSG00000100906,ENSG00000148180, ENSG00000173039,ENSG00000234745, ENSG00000215301,ENSG00000077150, ENSG00000204592,ENSG00000067560, ENSG00000162924,ENSG00000112658
Natural killer cell mediated cytotoxicity	2.5E-11	10.60	117	14	11.97	ENSG00000177885,ENSG00000204525, ENSG00000206503,ENSG00000027697, ENSG00000171608,ENSG00000136238, ENSG00000090339,ENSG00000015475, ENSG00000128340,ENSG00000159128, ENSG00000120889,ENSG00000234745,E NSG00000160691, ENSG00000204592
Allograft rejection	5.6E-04	3.25	34	4	11.76	ENSG00000204525,ENSG00000206503, ENSG00000234745,ENSG00000204592
Cytosolic DNA-sensing pathway	3.4E-06	5.47	60	7	11.67	ENSG00000160710,ENSG00000104365, ENSG00000109320,ENSG00000100906, ENSG00000173039,ENSG00000275302, ENSG00000104825
Apoptosis	7.7E-12	11.11	130	15	11.54	ENSG00000171608,ENSG00000104365, ENSG00000109320,ENSG00000163131, ENSG00000171552,ENSG00000115317, ENSG00000067182,ENSG00000100906, ENSG00000015475,ENSG00000143384,

						ENSG00000120889,ENSG00000173039, ENSG00000075624,ENSG00000003402, ENSG00000100368
Human T-cell leukemia virus 1 infection	6.1E-18	17.21	211	24	11.37	ENSG00000166710,ENSG00000204525, ENSG00000147168,ENSG00000166949, ENSG00000206503,ENSG00000128016, ENSG00000005339,ENSG00000171608, ENSG00000104365,ENSG00000109320, ENSG00000090339,ENSG00000171552, ENSG00000118971,ENSG00000126561, ENSG00000067182,ENSG00000100906, ENSG00000173039,ENSG00000234745, ENSG00000112242,ENSG00000137076, ENSG00000077150,ENSG00000204592, ENSG00000160741,ENSG00000112658
JAK-STAT signaling pathway	8.1E-14	13.09	159	18	11.32	ENSG00000177885,ENSG00000184557, ENSG00000110324,ENSG00000147168, ENSG00000168610,ENSG00000027697, ENSG00000119535,ENSG00000005339, ENSG00000171608,ENSG00000115415, ENSG00000108342,ENSG00000171552, ENSG00000118971,ENSG00000114737, ENSG00000126561,ENSG00000159128, ENSG00000143384,ENSG00000100368
Hepatitis B	8.1E-14	13.09	159	18	11.32	ENSG00000177885,ENSG00000055208, ENSG00000166949,ENSG00000168610, ENSG00000005339,ENSG00000171608, ENSG00000115415,ENSG00000104365, ENSG00000109320,ENSG00000172936, ENSG00000126561,ENSG00000100906, ENSG00000034152,ENSG00000015475, ENSG00000173039,ENSG00000215301, ENSG00000112242,ENSG00000127666
Non-small cell lung cancer	8.4E-07	6.08	71	8	11.27	ENSG00000177885,ENSG00000168610, ENSG00000171608,ENSG00000101109, ENSG00000126561,ENSG00000143924, ENSG00000112242,ENSG00000266094

AGE-RAGE signaling pathway in diabetic complications	8.6E-09	8.07	99	11	11.11	ENSG00000166949,ENSG00000168610, ENSG00000171608,ENSG00000115415, ENSG00000109320,ENSG00000136238, ENSG00000090339,ENSG00000106366, ENSG00000126561,ENSG00000173039, ENSG00000108691
p53 signaling pathway	9.3E-07	6.03	72	8	11.11	ENSG00000085117,ENSG00000135679, ENSG00000171552,ENSG00000106366, ENSG00000118971,ENSG00000015475, ENSG00000120889,ENSG00000130766
Fc epsilon RI signaling pathway	5.2E-06	5.28	64	7	10.94	ENSG00000177885,ENSG00000254087, ENSG00000171608,ENSG00000136238, ENSG00000033327,ENSG00000034152, ENSG00000128340
Colorectal cancer	2.3E-07	6.63	83	9	10.84	ENSG00000177885,ENSG00000166949, ENSG00000171608,ENSG00000136238, ENSG00000160271,ENSG00000144118, ENSG00000128340,ENSG00000148737, ENSG00000067560
Graft-versus-host disease	7.7E-04	3.11	37	4	10.81	ENSG00000204525,ENSG00000206503, ENSG00000234745,ENSG00000204592
Prostate cancer	5.5E-08	7.26	93	10	10.75	ENSG00000177885,ENSG00000135679, ENSG00000005339,ENSG00000171608, ENSG00000104365,ENSG00000109320, ENSG00000100906,ENSG00000173039, ENSG00000148737,ENSG00000112242
Insulin resistance	1.3E-08	7.90	103	11	10.68	ENSG00000184557,ENSG00000168610, ENSG00000196396,ENSG00000171608, ENSG00000104365,ENSG00000109320, ENSG00000067182,ENSG00000100906, ENSG00000173039,ENSG00000117676, ENSG00000160741
Ras signaling pathway	2.4E-17	16.62	225	24	10.67	ENSG00000177885,ENSG00000144566, ENSG00000180370,ENSG00000171608, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000160271, ENSG00000033327,ENSG00000127314, ENSG00000171552,ENSG00000101109,

						ENSG00000108774,ENSG00000144118, ENSG00000078369,ENSG00000116473, ENSG00000128340,ENSG00000173039, ENSG00000160691,ENSG00000111540, ENSG00000266094,ENSG00000067560, ENSG00000160014,ENSG00000162924
Cellular senescence	6.1E-12	11.22	152	16	10.53	ENSG00000135679,ENSG00000204525, ENSG00000166949,ENSG00000206503, ENSG00000171608,ENSG00000109320, ENSG00000106366,ENSG00000118971, ENSG00000162889,ENSG00000034152, ENSG00000173039,ENSG00000234745, ENSG00000112242,ENSG00000266094, ENSG00000204592,ENSG00000160014
Fluid shear stress and atherosclerosis	1.5E-10	9.83	134	14	10.45	ENSG00000171608,ENSG00000104365, ENSG00000109320,ENSG00000136238, ENSG00000133433,ENSG00000090339, ENSG00000067182,ENSG00000128340, ENSG00000173039,ENSG00000075624, ENSG00000116584,ENSG00000067560, ENSG00000108691,ENSG00000160014
Antifolate resistance	4.4E-03	2.36	29	3	10.34	ENSG00000104365,ENSG00000109320, ENSG00000173039
RIG-I-like receptor signaling pathway	7.6E-06	5.12	68	7	10.29	ENSG00000104365,ENSG00000109320, ENSG00000100906,ENSG00000173039, ENSG00000068308,ENSG00000215301, ENSG00000104825
Proteasome	9.3E-04	3.03	39	4	10.26	ENSG00000204264,ENSG00000100911, ENSG00000092010,ENSG00000240065
Endocytosis	7.1E-17	16.15	237	24	10.13	ENSG00000144566,ENSG00000135679, ENSG00000004059,ENSG00000204525, ENSG00000147168,ENSG00000115091, ENSG00000166949,ENSG00000180871, ENSG00000162704,ENSG00000206503, ENSG00000137486,ENSG00000111737, ENSG00000100055,ENSG00000090487, ENSG00000108774,ENSG00000139436, ENSG00000151502,ENSG00000110047,

						ENSG00000234745,ENSG00000111540, ENSG00000077549,ENSG00000204592, ENSG00000067560,ENSG00000134287
Shigellosis	8.2E-17	16.09	239	24	10.04	ENSG00000055208,ENSG00000135679, ENSG00000115091,ENSG00000162704, ENSG00000125730,ENSG00000171608, ENSG00000100055,ENSG00000104365, ENSG00000109320,ENSG00000136238, ENSG00000172936,ENSG00000171552, ENSG00000108518,ENSG00000105723, ENSG00000026508,ENSG00000067182, ENSG00000100906,ENSG00000166333, ENSG00000173039,ENSG00000075624, ENSG00000137076,ENSG00000116584, ENSG00000067560,ENSG00000104825
Small cell lung cancer	4.6E-07	6.34	90	9	10.00	ENSG00000125952,ENSG00000076604, ENSG00000171608,ENSG00000104365, ENSG00000109320,ENSG00000171552, ENSG00000100906,ENSG00000173039, ENSG00000112242
Type I diabetes mellitus	1.0E-03	3.00	40	4	10.00	ENSG00000204525,ENSG00000206503, ENSG00000234745,ENSG00000204592
Pantothenate and CoA biosynthesis	2.3E-02	1.64	20	2	10.00	ENSG00000138621,ENSG00000112303
IL-17 signaling pathway	4.9E-07	6.31	91	9	9.89	ENSG00000055208,ENSG00000076604, ENSG00000104365,ENSG00000109320, ENSG00000108342,ENSG00000118503, ENSG00000100906,ENSG00000173039, ENSG00000108691
Glioma	9.9E-06	5.00	71	7	9.86	ENSG00000177885,ENSG00000135679, ENSG00000171608,ENSG00000008118, ENSG00000112242,ENSG00000160691, ENSG00000160014
Pathogenic Escherichia coli infection	8.7E-13	12.06	183	18	9.84	ENSG00000055208,ENSG00000115091, ENSG00000162704,ENSG00000180370, ENSG00000100055,ENSG00000104365, ENSG00000109320,ENSG00000136238, ENSG00000172936,ENSG00000067182,

						ENSG00000100906,ENSG00000120889, ENSG00000173039,ENSG00000075624, ENSG00000100345,ENSG00000116584, ENSG00000067560,ENSG00000104825
T cell receptor signaling pathway	1.2E-07	6.91	102	10	9.80	ENSG00000177885,ENSG00000180370, ENSG00000171608,ENSG00000081237, ENSG00000104365,ENSG00000109320, ENSG00000100906,ENSG00000173039, ENSG00000067560,ENSG00000104825
Salmonella infection	7.1E-16	15.15	236	23	9.75	ENSG00000055208,ENSG00000144566, ENSG00000177105,ENSG00000115091, ENSG00000162704,ENSG00000171608, ENSG00000100055,ENSG00000081237, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000172936, ENSG00000108774,ENSG00000108518, ENSG00000067182,ENSG00000100906, ENSG00000034152,ENSG00000120889, ENSG00000173039,ENSG00000075624, ENSG00000148737,ENSG00000111540, ENSG00000067560
Growth hormone synthesis, secretion and action	3.2E-08	7.49	113	11	9.73	ENSG00000177885,ENSG00000184557, ENSG00000168610,ENSG00000005339, ENSG00000171608,ENSG00000115415, ENSG00000114353,ENSG00000126561, ENSG00000034152,ENSG00000160691, ENSG00000171223
NOD-like receptor signaling pathway	4.6E-12	11.34	175	17	9.71	ENSG00000055208,ENSG00000265972, ENSG00000115415,ENSG00000104365, ENSG00000109320,ENSG00000170296, ENSG00000172936,ENSG00000171552, ENSG00000103313,ENSG00000118503, ENSG00000100906,ENSG00000173039, ENSG00000105835,ENSG00000127666, ENSG00000067560,ENSG00000108691, ENSG00000104825

Viral protein interaction with cytokine and cytokine receptor	5.8E-07	6.24	93	9	9.68	ENSG00000172724,ENSG00000110324, ENSG00000147168,ENSG00000180871, ENSG00000028137,ENSG00000067182, ENSG00000120889,ENSG00000275302, ENSG00000108691
SNARE interactions in vesicular transport	5.2E-03	2.28	31	3	9.68	ENSG00000135823,ENSG00000177951, ENSG00000135604
Sphingolipid signaling pathway	3.4E-08	7.47	114	11	9.65	ENSG00000171608,ENSG00000109320, ENSG00000136238,ENSG00000114353, ENSG00000033327,ENSG00000067182, ENSG00000015475,ENSG00000128340, ENSG00000163082,ENSG00000173039, ENSG00000067560
Tight junction	1.3E-10	9.89	159	15	9.43	ENSG00000115091,ENSG00000147065, ENSG00000162704,ENSG00000015285, ENSG00000125753,ENSG00000136238, ENSG00000137221,ENSG00000116473, ENSG00000075624,ENSG00000109756, ENSG00000123728,ENSG00000100345, ENSG00000116584,ENSG00000067560, ENSG00000158769
FoxO signaling pathway	1.1E-08	7.95	128	12	9.38	ENSG00000112096,ENSG00000177885, ENSG00000135679,ENSG00000166949, ENSG00000168610,ENSG00000005339, ENSG00000171608,ENSG00000104365, ENSG00000170296,ENSG00000101109, ENSG00000118971,ENSG00000113916
Hepatitis C	7.3E-10	9.14	152	14	9.21	ENSG00000177885,ENSG00000184557, ENSG00000168610,ENSG00000171608, ENSG00000115415,ENSG00000104365, ENSG00000109320,ENSG00000067182, ENSG00000100906,ENSG00000015475, ENSG00000173039,ENSG00000112242, ENSG00000003402,ENSG00000127666
Platelet activation	5.5E-08	7.26	120	11	9.17	ENSG00000254087,ENSG00000171608, ENSG00000125753,ENSG00000114353, ENSG00000127314,ENSG00000116473, ENSG00000276045,ENSG00000075624,

						ENSG00000137076,ENSG00000141506, ENSG00000067560
Rap1 signaling pathway	4.6E-12	11.34	203	18	8.87	ENSG00000169896,ENSG00000171608, ENSG00000125753,ENSG00000136238, ENSG00000114353,ENSG00000160271, ENSG00000127314,ENSG00000108518, ENSG00000144118,ENSG00000034152, ENSG00000116473,ENSG00000128340, ENSG00000075624,ENSG00000137076, ENSG00000266094,ENSG00000109756, ENSG00000067560,ENSG00000160014
VEGF signaling pathway	3.9E-04	3.41	57	5	8.77	ENSG00000114738,ENSG00000171608, ENSG00000136238,ENSG00000162889, ENSG00000128340
Mitophagy - animal	1.1E-04	3.98	70	6	8.57	ENSG00000170296,ENSG00000171552, ENSG00000068323,ENSG00000100644, ENSG00000173039,ENSG00000188554
Alcoholic liver disease	3.2E-08	7.50	141	12	8.51	ENSG00000055208,ENSG00000125730, ENSG00000104365,ENSG00000109320, ENSG00000172936,ENSG00000067182, ENSG00000100906,ENSG00000034152, ENSG00000173039,ENSG00000148737, ENSG00000110931,ENSG00000127666
Insulin signaling pathway	1.3E-07	6.90	131	11	8.40	ENSG00000177885,ENSG00000184557, ENSG00000132589,ENSG00000196396, ENSG00000171608,ENSG00000165458, ENSG00000104365,ENSG00000125733, ENSG00000160691,ENSG00000108946, ENSG00000160014
HIF-1 signaling pathway	1.9E-06	5.72	108	9	8.33	ENSG00000168610,ENSG00000027697, ENSG00000005339,ENSG00000171608, ENSG00000109320,ENSG00000106366, ENSG00000159128,ENSG00000100644, ENSG00000173039
Amoebiasis	8.0E-06	5.10	97	8	8.25	ENSG00000144566,ENSG00000169896, ENSG00000170542,ENSG00000171608, ENSG00000109320,ENSG00000108774, ENSG00000173039,ENSG00000111540

Malaria	2.1E-03	2.68	49	4	8.16	ENSG00000108342,ENSG00000172936, ENSG00000090339,ENSG00000108691
Autoimmune thyroid disease	2.1E-03	2.68	49	4	8.16	ENSG00000204525,ENSG00000206503, ENSG00000234745,ENSG00000204592
Pathways in cancer	5.0E-25	24.30	517	42	8.12	ENSG00000125952,ENSG00000177885, ENSG00000135679,ENSG00000147168, ENSG00000166949,ENSG00000168610, ENSG00000076604,ENSG00000027697, ENSG00000119535,ENSG00000005339, ENSG00000171608,ENSG00000115415, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000114353, ENSG00000181274,ENSG00000160271, ENSG00000133433,ENSG00000171552, ENSG00000101109,ENSG00000118971, ENSG00000126561,ENSG00000144118, ENSG00000112033,ENSG00000143924, ENSG00000078369,ENSG00000100906, ENSG00000015475,ENSG00000128340, ENSG00000155760,ENSG00000159128, ENSG00000100644,ENSG00000173039, ENSG00000148737,ENSG00000112242, ENSG00000077150,ENSG00000266094, ENSG00000067560,ENSG00000160014, ENSG00000266412,ENSG00000100368
MAPK signaling pathway	3.3E-14	13.49	286	23	8.04	ENSG00000125952,ENSG00000177885, ENSG00000055208,ENSG00000114738, ENSG00000137486,ENSG00000180370, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000172936, ENSG00000127314,ENSG00000101109, ENSG00000162889,ENSG00000067182, ENSG00000034152,ENSG00000116473, ENSG00000128340,ENSG00000173039, ENSG00000077150,ENSG00000109756, ENSG00000117676,ENSG00000173327, ENSG00000112658
Wnt signaling pathway	1.6E-08	7.81	162	13	8.02	ENSG00000166949,ENSG00000005339, ENSG00000136238,ENSG00000181274,

						ENSG00000118971,ENSG00000112033, ENSG00000140332,ENSG00000128340, ENSG00000155760,ENSG00000148737, ENSG00000080815,ENSG00000278224, ENSG00000067560
Non-alcoholic fatty liver disease	2.7E-07	6.56	142	11	7.75	ENSG00000184557,ENSG00000171608, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000105723, ENSG00000067182,ENSG00000015475, ENSG00000173039,ENSG00000100219, ENSG00000173327
Coronavirus disease - COVID-19	5.2E-10	9.28	207	16	7.73	ENSG00000055208,ENSG00000160710, ENSG00000101916,ENSG00000168610, ENSG00000125730,ENSG00000171608, ENSG00000115415,ENSG00000104365, ENSG00000109320,ENSG00000108342, ENSG00000172936,ENSG00000067182, ENSG00000100906,ENSG00000173039, ENSG00000108691,ENSG00000104825
Long-term potentiation	7.1E-04	3.15	65	5	7.69	ENSG00000005339,ENSG00000127314, ENSG00000116473,ENSG00000117676, ENSG00000160014
Collecting duct acid secretion	3.7E-02	1.43	26	2	7.69	ENSG00000131100,ENSG00000033627
Phototransduction	3.7E-02	1.43	26	2	7.69	ENSG00000078369,ENSG00000160014
Choline metabolism in cancer	6.2E-05	4.21	94	7	7.45	ENSG00000177885,ENSG00000015285, ENSG00000171608,ENSG00000136238, ENSG00000160271,ENSG00000128340, ENSG00000100644
Hepatocellular carcinoma	1.2E-07	6.90	162	12	7.41	ENSG00000177885,ENSG00000166949, ENSG00000171608,ENSG00000181274, ENSG00000133433,ENSG00000171552, ENSG00000155760,ENSG00000075624, ENSG00000148737,ENSG00000112242, ENSG00000160691,ENSG00000108604
Hippo signaling pathway - multiple species	3.9E-02	1.41	27	2	7.41	ENSG00000107551,ENSG00000101265
Relaxin signaling pathway	5.5E-06	5.26	123	9	7.32	ENSG00000177885,ENSG00000137486, ENSG00000171608,ENSG00000109320,

						ENSG00000114353,ENSG00000078369, ENSG00000100906,ENSG00000173039, ENSG00000160691
Focal adhesion	1.4E-08	7.86	192	14	7.29	ENSG00000177885,ENSG00000180370, ENSG00000171608,ENSG00000125753, ENSG00000136238,ENSG00000127314, ENSG00000118971,ENSG00000116473, ENSG00000128340,ENSG00000166333, ENSG00000075624,ENSG00000137076, ENSG00000160691,ENSG00000067560
mTOR signaling pathway	4.9E-07	6.31	151	11	7.28	ENSG00000177885,ENSG00000171608, ENSG00000104365,ENSG00000135362, ENSG00000135932,ENSG00000067182, ENSG00000155760,ENSG00000130766, ENSG00000131100,ENSG00000117676, ENSG00000067560
Notch signaling pathway	3.1E-03	2.51	55	4	7.27	ENSG00000005339,ENSG00000140332, ENSG00000184384,ENSG00000080815
Regulation of actin cytoskeleton	4.4E-09	8.36	207	15	7.25	ENSG00000115091,ENSG00000147065, ENSG00000162704,ENSG00000169896, ENSG00000180370,ENSG00000171608, ENSG00000136238,ENSG00000182541, ENSG00000108518,ENSG00000128340, ENSG00000148180,ENSG00000075624, ENSG00000172757,ENSG00000100345, ENSG00000067560
Necroptosis	5.2E-07	6.28	152	11	7.24	ENSG00000168610,ENSG00000027697, ENSG00000115415,ENSG00000126561, ENSG00000067182,ENSG00000118503, ENSG00000015475,ENSG00000159128, ENSG00000120889,ENSG00000003402, ENSG00000127666
Phospholipase D signaling pathway	1.9E-06	5.73	139	10	7.19	ENSG00000177885,ENSG00000180871, ENSG00000171608,ENSG00000100055, ENSG00000160271,ENSG00000033327, ENSG00000144118,ENSG00000160691, ENSG00000141506,ENSG00000067560
Lysosome	6.6E-06	5.18	126	9	7.14	ENSG00000166340,ENSG00000135677,

						ENSG00000163131,ENSG00000110911, ENSG00000104093,ENSG00000033627, ENSG00000189067,ENSG00000103066, ENSG00000162511
Platinum drug resistance	9.6E-04	3.02	70	5	7.14	ENSG00000135679,ENSG00000171608, ENSG00000133433,ENSG00000171552, ENSG00000015475
Vasopressin-regulated water reabsorption	1.1E-02	1.94	42	3	7.14	ENSG00000144566,ENSG00000108774, ENSG00000111540
Neutrophil extracellular trap formation	6.3E-08	7.20	184	13	7.07	ENSG00000101916,ENSG00000169896, ENSG00000125730,ENSG00000197548, ENSG00000171608,ENSG00000109320, ENSG00000136238,ENSG00000100365, ENSG00000103569,ENSG00000128340, ENSG00000173039,ENSG00000075624, ENSG00000129450
Endometrial cancer	3.5E-03	2.45	57	4	7.02	ENSG00000177885,ENSG00000171608, ENSG00000166333,ENSG00000148737
Human papillomavirus infection	1.8E-12	11.75	315	22	6.98	ENSG00000177885,ENSG00000135679, ENSG00000204525,ENSG00000206503, ENSG00000005339,ENSG00000171608, ENSG00000115415,ENSG00000104365, ENSG00000109320,ENSG00000118971, ENSG00000067182,ENSG00000125347, ENSG00000184384,ENSG00000155760, ENSG00000173039,ENSG00000234745, ENSG00000148737,ENSG00000080815, ENSG00000131100,ENSG00000033627, ENSG00000127666,ENSG00000204592
Longevity regulating pathway	3.2E-04	3.50	86	6	6.98	ENSG00000112096,ENSG00000171608, ENSG00000109320,ENSG00000173039, ENSG00000110931,ENSG00000130766
cAMP signaling pathway	4.0E-08	7.40	210	14	6.67	ENSG00000005339,ENSG00000171608, ENSG00000109320,ENSG00000136238, ENSG00000114353,ENSG00000127314, ENSG00000100906,ENSG00000116473, ENSG00000128340,ENSG00000276045, ENSG00000173039,ENSG00000126262,

						ENSG00000067560,ENSG00000160014
Autophagy - animal	1.1E-05	4.96	135	9	6.67	ENSG00000100330,ENSG00000197548, ENSG00000171608,ENSG00000170296, ENSG00000171552,ENSG00000078804, ENSG00000100644,ENSG00000110931, ENSG00000003402
Type II diabetes mellitus	1.3E-02	1.87	45	3	6.67	ENSG00000184557,ENSG00000171608, ENSG00000104365
Autophagy - other	4.6E-02	1.33	30	2	6.67	ENSG00000197548,ENSG00000170296
EGFR tyrosine kinase inhibitor resistance	1.4E-03	2.86	76	5	6.58	ENSG00000177885,ENSG00000168610, ENSG00000171608,ENSG00000171552, ENSG00000160691
Axon guidance	1.9E-06	5.73	174	11	6.32	ENSG00000128594,ENSG00000180370, ENSG00000171608,ENSG00000136238, ENSG00000114353,ENSG00000182541, ENSG00000128340,ENSG00000166333, ENSG00000172757,ENSG00000196189, ENSG00000067560
Cell adhesion molecules	1.8E-05	4.76	143	9	6.29	ENSG00000204525,ENSG00000128594, ENSG00000206503,ENSG00000169896, ENSG00000081237,ENSG00000090339, ENSG00000234745,ENSG00000204592, ENSG00000158769
Herpes simplex virus 1 infection	2.3E-15	14.64	477	30	6.29	ENSG00000055208,ENSG00000166710, ENSG00000184557,ENSG00000204267, ENSG00000204525,ENSG00000206503, ENSG00000027697,ENSG00000126759, ENSG00000125730,ENSG00000171608, ENSG00000115415,ENSG00000104365, ENSG00000019582,ENSG00000109320, ENSG00000172936,ENSG00000171552, ENSG00000067182,ENSG00000100906, ENSG00000015475,ENSG00000168394, ENSG00000167528,ENSG00000231925, ENSG00000159128,ENSG00000173039, ENSG00000234745,ENSG00000028277, ENSG00000169957,ENSG00000127666, ENSG00000204592,ENSG00000108691

Cocaine addiction	1.6E-02	1.80	48	3	6.25	ENSG00000109320,ENSG00000114353, ENSG00000173039
Vibrio cholerae infection	1.6E-02	1.80	48	3	6.25	ENSG00000075624,ENSG00000131100, ENSG00000033627
ErbB signaling pathway	1.8E-03	2.74	81	5	6.17	ENSG00000177885,ENSG00000180370, ENSG00000171608,ENSG00000126561, ENSG00000160691
Transcriptional misregulation in cancer	2.8E-06	5.55	182	11	6.04	ENSG00000125952,ENSG00000135679, ENSG00000138756,ENSG00000169896, ENSG00000109320,ENSG00000171552, ENSG00000118971,ENSG00000068323, ENSG00000173039,ENSG00000113916, ENSG00000162924
Staphylococcus aureus infection	2.1E-03	2.68	84	5	5.95	ENSG00000169896,ENSG00000125730, ENSG00000186431,ENSG00000090339, ENSG00000169403
Chemical carcinogenesis - receptor activation	1.2E-06	5.93	202	12	5.94	ENSG00000177885,ENSG00000168610, ENSG00000137486,ENSG00000171608, ENSG00000109320,ENSG00000114353, ENSG00000133433,ENSG00000126561, ENSG00000111424,ENSG00000173039, ENSG00000113916,ENSG00000117676
Renin secretion	6.4E-03	2.19	68	4	5.88	ENSG00000114353,ENSG00000276045, ENSG00000123700,ENSG00000160014
Breast cancer	1.3E-04	3.90	143	8	5.59	ENSG00000177885,ENSG00000171608, ENSG00000181274,ENSG00000155760, ENSG00000148737,ENSG00000112242, ENSG00000160691,ENSG00000077150
Gastric cancer	1.3E-04	3.90	143	8	5.59	ENSG00000177885,ENSG00000166949, ENSG00000171608,ENSG00000181274, ENSG00000155760,ENSG00000148737, ENSG00000112242,ENSG00000160691
Endocrine resistance	2.8E-03	2.55	90	5	5.56	ENSG00000177885,ENSG00000135679, ENSG00000171608,ENSG00000112242, ENSG00000160691
RNA degradation	7.8E-03	2.11	72	4	5.56	ENSG00000135473,ENSG00000204351, ENSG00000159388,ENSG00000088038
Proteoglycans in cancer	6.7E-06	5.17	200	11	5.50	ENSG00000177885,ENSG00000135679,

						ENSG00000147065,ENSG00000168610, ENSG00000171608,ENSG00000136238, ENSG00000026508,ENSG00000155760, ENSG00000100644,ENSG00000075624, ENSG00000067560
Hematopoietic cell lineage	2.9E-03	2.53	91	5	5.49	ENSG00000169896,ENSG00000119535, ENSG00000108342,ENSG00000166825, ENSG00000026508
Oxytocin signaling pathway	1.6E-04	3.80	148	8	5.41	ENSG00000114353,ENSG00000008118, ENSG00000075624,ENSG00000110931, ENSG00000123700,ENSG00000141506, ENSG00000067560,ENSG00000160014
PPAR signaling pathway	8.5E-03	2.07	74	4	5.41	ENSG00000068366,ENSG00000173391, ENSG00000112033,ENSG00000166333
Gastric acid secretion	8.5E-03	2.07	74	4	5.41	ENSG00000114353,ENSG00000075624, ENSG00000123700,ENSG00000160014
Chemical carcinogenesis - reactive oxygen species	8.7E-06	5.06	206	11	5.34	ENSG00000112096,ENSG00000177885, ENSG00000196396,ENSG00000171608, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000133433, ENSG00000100906,ENSG00000100644, ENSG00000173039
Cytokine-cytokine receptor interaction	2.0E-07	6.71	281	15	5.34	ENSG00000172724,ENSG00000110324, ENSG00000147168,ENSG00000180871, ENSG00000028137,ENSG00000027697, ENSG00000119535,ENSG00000108342, ENSG00000067182,ENSG00000159128, ENSG00000120889,ENSG00000275302, ENSG00000108691,ENSG00000161921, ENSG00000100368
Apelin signaling pathway	5.6E-04	3.26	135	7	5.19	ENSG00000166949,ENSG00000198336, ENSG00000114353,ENSG00000106366, ENSG00000078369,ENSG00000141506, ENSG00000160014
Thyroid hormone signaling pathway	1.6E-03	2.80	118	6	5.08	ENSG00000135679,ENSG00000005339, ENSG00000171608,ENSG00000115415, ENSG00000100644,ENSG00000075624
Melanogenesis	4.1E-03	2.38	99	5	5.05	ENSG00000005339,ENSG00000114353,

						ENSG00000155760,ENSG00000148737, ENSG00000160014
PI3K-Akt signaling pathway	8.0E-08	7.10	342	17	4.97	ENSG00000177885,ENSG00000135679, ENSG00000147168,ENSG00000119535, ENSG00000155629,ENSG00000171608, ENSG00000104365,ENSG00000109320, ENSG00000136238,ENSG00000108342, ENSG00000171552,ENSG00000118971, ENSG00000078369,ENSG00000143384, ENSG00000173039,ENSG00000141506, ENSG00000160741
Nucleotide metabolism	1.2E-02	1.92	83	4	4.82	ENSG00000100938,ENSG00000106348, ENSG00000196839,ENSG00000116337
Complement and coagulation cascades	1.2E-02	1.92	83	4	4.82	ENSG00000197249,ENSG00000169896, ENSG00000125730,ENSG00000106366
Cushing syndrome	9.3E-04	3.03	148	7	4.73	ENSG00000114353,ENSG00000127314, ENSG00000116473,ENSG00000155760, ENSG00000276045,ENSG00000148737, ENSG00000112242
Rheumatoid arthritis	1.3E-02	1.87	86	4	4.65	ENSG00000090339,ENSG00000131100, ENSG00000033627,ENSG00000108691
Cholinergic synapse	5.8E-03	2.23	108	5	4.63	ENSG00000171608,ENSG00000114353, ENSG00000078369,ENSG00000123700, ENSG00000141506
Amyotrophic lateral sclerosis	1.2E-06	5.92	327	15	4.59	ENSG00000144566,ENSG00000028137, ENSG00000136238,ENSG00000160803, ENSG00000171552,ENSG00000108518, ENSG00000153201,ENSG00000067182, ENSG00000168488,ENSG00000034152, ENSG00000015475,ENSG00000100219, ENSG00000075624,ENSG00000138279, ENSG00000044574
Signaling pathways regulating pluripotency of stem cells	2.6E-03	2.58	131	6	4.58	ENSG00000177885,ENSG00000166949, ENSG00000168610,ENSG00000171608, ENSG00000136603,ENSG00000155760
MicroRNAs in cancer	2.8E-06	5.55	306	14	4.58	ENSG00000177885,ENSG00000135679, ENSG00000168610,ENSG00000005339, ENSG00000171608,ENSG00000104365,

						ENSG00000109320,ENSG00000118971, ENSG00000026508,ENSG00000143384, ENSG00000112242,ENSG00000160691, ENSG00000067560,ENSG00000277443
Protein processing in endoplasmic reticulum	1.2E-03	2.94	154	7	4.55	ENSG00000135506,ENSG00000136026, ENSG00000160803,ENSG00000071537, ENSG00000100219,ENSG00000044574, ENSG00000148719
Phosphatidylinositol signaling system	1.6E-02	1.81	90	4	4.44	ENSG00000100330,ENSG00000171608, ENSG00000165458,ENSG00000160014
Melanoma	4.1E-02	1.39	70	3	4.29	ENSG00000135679,ENSG00000171608, ENSG00000112242
Inositol phosphate metabolism	4.2E-02	1.38	71	3	4.23	ENSG00000100330,ENSG00000171608, ENSG00000165458
Pancreatic secretion	1.8E-02	1.73	95	4	4.21	ENSG00000136238,ENSG00000127314, ENSG00000116473,ENSG00000067560
Cell cycle	8.8E-03	2.06	120	5	4.17	ENSG00000135679,ENSG00000166949, ENSG00000005339,ENSG00000118971, ENSG00000112242
Pathways of neurodegeneration - multiple diseases	5.2E-07	6.28	442	18	4.07	ENSG00000144566,ENSG00000028137, ENSG00000127946,ENSG00000109320, ENSG00000136238,ENSG00000181274, ENSG00000171552,ENSG00000115317, ENSG00000067182,ENSG00000168488, ENSG00000034152,ENSG00000015475, ENSG00000155760,ENSG00000173039, ENSG00000100219,ENSG00000080815, ENSG00000044574,ENSG00000160014
Hippo signaling pathway	4.6E-03	2.34	148	6	4.05	ENSG00000166949,ENSG00000106366, ENSG00000118971,ENSG00000155760, ENSG00000075624,ENSG00000148737
Estrogen signaling pathway	1.0E-02	1.99	125	5	4.00	ENSG00000177885,ENSG00000171608, ENSG00000114353,ENSG00000160691, ENSG00000160014
Dopaminergic synapse	1.1E-02	1.98	126	5	3.97	ENSG00000137486,ENSG00000114353, ENSG00000105723,ENSG00000078369, ENSG00000160014

Parathyroid hormone synthesis, secretion and action	2.3E-02	1.64	102	4	3.92	ENSG00000137486,ENSG00000114353, ENSG00000111424,ENSG00000067560
Glucagon signaling pathway	2.4E-02	1.61	104	4	3.85	ENSG00000005339,ENSG00000149923, ENSG00000160741,ENSG00000160014
Diabetic cardiomyopathy	3.3E-03	2.48	187	7	3.74	ENSG00000166949,ENSG00000171608, ENSG00000109320,ENSG00000136238, ENSG00000100365,ENSG00000128340, ENSG00000173039
cGMP-PKG signaling pathway	6.7E-03	2.17	161	6	3.73	ENSG00000125753,ENSG00000114353, ENSG00000141506,ENSG00000067560, ENSG00000160014,ENSG00000112658
AMPK signaling pathway	3.3E-02	1.48	114	4	3.51	ENSG00000171608,ENSG00000135932, ENSG00000110931,ENSG00000160741
Alzheimer disease	2.5E-04	3.60	353	12	3.40	ENSG00000171608,ENSG00000104365, ENSG00000109320,ENSG00000181274, ENSG00000110911,ENSG00000067182, ENSG00000015475,ENSG00000155760, ENSG00000173039,ENSG00000100219, ENSG00000080815,ENSG00000160014
Alcoholism	1.0E-02	1.99	177	6	3.39	ENSG00000177885,ENSG00000114353, ENSG00000078369,ENSG00000110931, ENSG00000160691,ENSG00000160014
Purine metabolism	3.8E-02	1.42	120	4	3.33	ENSG00000100938,ENSG00000106348, ENSG00000196839,ENSG00000116337
Spliceosome	4.1E-02	1.39	123	4	3.25	ENSG00000140829,ENSG00000092199, ENSG00000115524,ENSG00000169564
Vascular smooth muscle contraction	4.3E-02	1.36	126	4	3.17	ENSG00000148926,ENSG00000100345, ENSG00000067560,ENSG00000160014
Parkinson disease	1.1E-02	1.96	238	7	2.94	ENSG00000114353,ENSG00000110911, ENSG00000171552,ENSG00000115317, ENSG00000100219,ENSG00000044574, ENSG00000160014
Thermogenesis	2.0E-02	1.71	207	6	2.90	ENSG00000177885,ENSG00000068366, ENSG00000034152,ENSG00000075624, ENSG00000117676,ENSG00000108604
Metabolic pathways	1.9E-04	3.71	1457	29	1.99	ENSG00000066322,ENSG00000100938, ENSG00000070614,ENSG00000086062,

						ENSG00000100330,ENSG00000101365, ENSG00000111817,ENSG00000135677, ENSG00000171608,ENSG00000165458, ENSG00000106348,ENSG00000068366, ENSG00000196839,ENSG00000133433, ENSG00000166825,ENSG00000138621, ENSG00000116337,ENSG00000143811, ENSG00000163082,ENSG00000062282, ENSG00000124357,ENSG00000131100, ENSG00000105835,ENSG00000033627, ENSG00000159322,ENSG00000158470, ENSG00000112303,ENSG00000157350, ENSG00000101945

S6 Table. Co-expressed mRNAs/lncRNAs pairs with binding potential. Pairs of mRNAs/lncRNAs that exhibited a Pearson correlation coefficient of |r|≥0.8 and p-value≤0.05 were evaluated for their binding potential in LncTar (ndG≤-0.10).

LncRNA ID	LncRNA name	mRNA ID	mRNA name	dG	ndG	Start Position Query	End Position Query	Start Position Target	End Position Target
ENSG00000224397	PELATON	ENSG00000051523	CYBA	-35.9	-0.11	1	339	554	892
ENSG00000224397	PELATON	ENSG00000077150	NFKB2	-54.9	-0.11	232	733	1	502
ENSG00000224397	PELATON	ENSG00000078804	TP53INP2	-55.3	-0.11	1	539	369	907
ENSG00000224397	PELATON	ENSG00000085117	CD82	-106	-0.11	2531	3532	1	1002
ENSG00000224397	PELATON	ENSG00000085514	PILRA	-31.3	-0.11	1	292	259	550
ENSG00000224397	PELATON	ENSG00000086730	LAT2	-36	-0.11	1	339	204	542

APÊNDICE A – Referências da Introdução Geral

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ANEXO A – Normas da Revista PLOS Neglected Tropical Diseases

Submission Guidelines

About the Journal
PLOS Neglected Tropical Diseases publishes original research articles of importance to the NTDs community and the wider health community. We will consider manuscripts of any length; we encourage the submission of both substantial full-length bodies of work and shorter manuscripts that report novel findings that might be based on a more limited range of experiments. The writing style should be concise and accessible, avoiding jargon so that the paper is understandable for readers outside a specialty or those whose first language is not English. Editors will make suggestions for how to achieve this, as well as suggestions for cuts or additions that could be made to the article to strengthen the argument. Our aim is to make the editorial process rigorous and consistent, but not intrusive or overbearing. Authors are encouraged to use their own voice and to decide how best to present their ideas, results, and conclusions.

Style and Format
When you first submit to the journal, providing you include all the necessary information needed for editorial assessment and review, we will not ask you to make any formatting changes.

File format
Manuscript files can be in the following formats: DOC, DOCX, RTF or PDF.

Length
Manuscripts can be any length. There are no restrictions on word count, number of figures, or amount of supporting information.

Font
Use a standard font size and any standard font, except for the font named “Symbol”. To add symbols to the manuscript, use the Insert → Symbol function in your word processor or paste in the appropriate Unicode character.

Headings
Limit manuscript sections and sub-sections to 3 heading levels. Make sure heading levels are clearly indicated in the manuscript text.

Layout and spacing
Manuscript text should be double-spaced.

Page and line numbers
Include page numbers and line numbers in the manuscript file. Use continuous line numbers (do not restart the numbering on each page).

Tables
Insert tables immediately after the first paragraph in which they are cited.

Supporting Information
Upload Supporting Information (SI) files separately.

Language
Manuscripts must be submitted in English.

Abbreviations
Define abbreviations upon first appearance in the text.

Reference style
PLOS uses “Vancouver” style, as outlined in the ICMJE sample references.

Nomenclature
Use correct and established nomenclature wherever possible.

Use SI units. If you do not use these exclusively, provide the SI value in parentheses after each value. Read more about SI units.

Species names: Write in italics (e.g., Homo sapiens). Write out in full the genus and species, both in the title of the manuscript and at the first mention of an organism in a paper. After first mention, the first letter of the genus name followed by the full species name may be used (e.g., H. sapiens).

Genes, mutations, genotypes, and alleles: Write in italics. Use the recommended name by consulting the appropriate genetic nomenclature database (e.g., HUGO for human genes). It is sometimes advisable to indicate the synonyms for the gene the first time it appears in the text. Gene prefixes such as those used for oncogenes or cellular localization should be shown in roman typeface (e.g., v-fes, c-MYC).

Note that if your manuscript is accepted, PLOS will not perform a detailed copyediting step. Therefore, please carefully review your manuscript, paying special attention to spelling, punctuation, and grammar, as well as scientific content.

Manuscript Organization
Most manuscripts should be organized as follows. Instructions for each element appear below.
Title
Authors and Affiliations
Abstract
Author Summary
Introduction
Methods
Results
Discussion
Acknowledgments
References
Supporting information Captions

Parts of a Submission
Title: Include a full title and a short title for the manuscript.
Full title: 250 characters
Short title: 70 characters

Author list
Authorship requirements: All authors must meet the criteria for authorship as outlined in the authorship policy. Those who contributed to the work but do not meet the criteria for authorship can be mentioned in the Acknowledgments. Read more about Acknowledgments.

The corresponding author must provide an ORCID iD at the time of submission by entering it in the user profile in the submission system. Read more about ORCID.

Author names and affiliations
Enter author names on the title page of the manuscript and in the online submission system.

On the title page, write author names in the following order:
First name (or initials, if used)
Middle name (or initials, if used)
Last name (surname, family name)

Each author on the list must have an affiliation. The affiliation includes department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country. Authors have the option to include a current address in addition to the address of their affiliation at the time of the study. The current address should be listed in the byline and clearly labeled “current address.” At a minimum, the address must include the author’s current institution, city, and country.
If an author has multiple affiliations, enter all affiliations on the title page only. In the submission system, enter only the preferred or primary affiliation. Author affiliations will be listed in the typeset PDF article in the same order that authors are listed in the submission.

PLOS Neglected Tropical Diseases will contact all authors by email at submission to ensure that they are aware of the submission.

Cover letter

Title page: The title, authors, and affiliations should all be included on a title page as the first page of the manuscript file.

The Abstract should be succinct; it must not exceed 250–300 words.

The Abstract is conceptually divided into the following three sections with these headings: Background, Methodology/Principal Findings, and Conclusions/Significance.

Author Summary
We ask that all authors of research articles include a 150-200 word non-technical summary of the work, immediately following the Abstract. Subject to editorial review and author revision, this short text is published with all research articles as a highlighted text box.

Introduction
The introduction should put the focus of the manuscript into a broader context. As you compose the Introduction, think of readers who are not experts in this field. Include a brief review of the key literature. If there are relevant controversies or disagreements in the field, they should be mentioned so that a non-expert reader can delve into these issues further. The Introduction should conclude with a brief statement of the overall aim of the experiments and a comment about whether that aim was achieved.

Methods
This section should provide enough detail for reproduction of the findings. Protocols for new methods should be included, but well-established protocols may simply be referenced. Detailed methodology or supporting information relevant to the methodology can be published on our web site. This section should also include a section with descriptions of any statistical methods employed. These should conform to the criteria outlined by the Uniform Requirements.

Results
The Results section should include all relevant positive and negative findings. The section may be divided into subsections, each with a concise subheading. The Results section should be written in past tense.

Discussion
The Discussion should be concise and tightly argued. It should start with a brief summary of the main findings. It should include paragraphs on the generalizability, clinical relevance, strengths, and limitations of your study.

Do not include funding sources in the Acknowledgments or anywhere else in the manuscript file. Funding information should only be entered in the financial disclosure section of the submission system.

References
Any and all available works can be cited in the reference list. Acceptable sources include:
Published or accepted manuscripts
Manuscripts on preprint servers, providing the manuscript has a citable DOI or arXiv URL.
Do not cite the following sources in the reference list:
Unavailable and unpublished work, including manuscripts that have been submitted but not yet accepted (e.g., “unpublished work,” “data not shown”). Instead, include those data as supplementary material or deposit the data in a publicly available database.
Personal communications (these should be supported by a letter from the relevant authors but not included in the reference list)
Submitted research should not rely upon retracted research. You should avoid citing retracted articles unless you need to discuss retracted work to provide historical context for your submitted research. If it is necessary to discuss retracted work, state the article’s retracted status in your article’s text and reference list.
Ensure that your reference list includes full and current bibliography details for every cited work at the time of your article’s submission (and publication, if accepted). If cited work is corrected, retracted, or marked with an expression of concern before your article is published, and if you feel it is appropriate to cite the work even in light of the post-publication notice, include in your manuscript citations and full references for both the affected article and the post-publication notice. Email the journal office if you have questions.
References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., “We used the techniques developed by our colleagues [19] to analyze the data”). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Formatting references
Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the “Vancouver” style.

Supporting information
Authors can submit essential supporting files and multimedia files along with their manuscripts. All supporting information will be subject to peer review. All file types can be submitted, but files must be smaller than 20 MB in size.

Supporting information captions
List supporting information captions at the end of the manuscript file. Do not submit captions in a separate file.

The file number and name are required in a caption, and we highly recommend including a one-line title as well. You may also include a legend in your caption, but it is not required.

Figure captions
Insert figure captions in manuscript text, immediately following the paragraph where the figure is first cited (read order). Don't include captions as part of the figure files themselves or submit them in a separate document.

Tables
Cite tables in ascending numeric order upon first appearance in the manuscript file.

Read our policy on data availability.
Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (e.g., GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are provided and the data are at least as open as CC BY. Authors are encouraged to select repositories that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones.

Accession numbers
All appropriate data sets, images, and information should be deposited in an appropriate public repository.

Identifiers
As much as possible, please provide accession numbers or identifiers for all entities such as genes, proteins, mutants, diseases, etc., for which there is an entry in a public database, for example: Ensembl; Entrez Gene; FlyBase; InterPro; etc

Submission Criteria
Choose an image that represents the article in a striking and eye-catching way.
It can be derived from a figure or supporting information file from the paper, and it may be a cropped portion of an image or the entire image.
High resolution: between 300-600 dpi
The PLOS licenses and copyright policy also applies to striking images.
Additional Information Requested at Submission
Financial Disclosure Statement

This information should describe sources of funding that have supported the work. If your manuscript is published, your statement will appear in the Funding section of the article.

Include your statement in the Financial Disclosure section of the initial submission form.

Human and animal research
All research involving humans and animals must have been approved by the authors' institutional review board or equivalent committee(s), and that board must be named by the authors in the manuscript. For research involving human participants, informed consent must have been obtained (or the reason for lack of consent explained, e.g. the data were analyzed anonymously) and all clinical investigation must have been conducted according to the principles expressed in the Declaration of Helsinki. It must

be stated in the Methods section of the paper whether informed consent was written or oral. If informed consent was oral, it must be stated in the paper: (a) why written consent could not be obtained, (b) that the IRB approved the use of oral consent, and (c) how oral consent was documented.

ANEXO B – Parecer do Comitê de Ética em Pesquisa

UNESP - FACULDADE DE ODONTOLOGIA-CAMPUS DE ARAÇATUBA/ UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito da infecção de neutrófilos com Leishmania infantum na metilação do DNA em humanos

Pesquisador: FLAVIA LOMBARDI LOPES

Área Temática:

Versão: 3

CAAE: 26583319.3.0000.5420

Instituição Proponente: UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO

Patrocinador Principal: UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO

DADOS DO PARECER

Número do Parecer: 3.926.267

Apresentação do Projeto:

O presente estudo visa elucidar o diálogo hospedeiro/microorganismo no controle epigenético da resposta de neutrófilos humanos à infecção por L. (L.) infantum (syn. chagasi), bem como associar possíveis alterações nos padrões epigenéticos com o controle da expressão gênica deste tipo celular em um modelo de infecção in vitro.

Objetivo da Pesquisa:

Objetivo Primário:

Investigar, em neutrófilos humanos, os efeitos da infecção in vitro por L. (L.) infantum (syn.chagasi) nos padrões genômicos globais de metilação do DNA (RRBS-Seq), bem como associá-los a possíveis alterações na expressão gênica global (RNA-Seq).

Objetivo Secundário:

Identificar os alvos da modulação deste processo epigenético, consequente da invasão dos neutrófilos pelo principal agente causador da leishmaniose visceral no Brasil

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Telefone: (18)3636-3200 **Fax:** (18)3636-3332 **E-mail:** andrebertoz@foa.unesp.br

Avaliação dos Riscos e Benefícios:

Riscos:

Quanto a coleta por punção venosa, há risco de mal estar decorrente da coleta, indisposição do voluntário, edema no local da punção, roxidão no local da punção, sangramento local.Quanto ao uso de agentes patológicos no âmbito do cultivo, os riscos serão de toda forma minimizados pela correta ação de biossegurança.

Benefícios:

Auxiliar na identificação dos processos celulares, que possam favorecer ou prejudicar a resposta do hospedeiro frente a infecção por L. infantum.

Comentários e Considerações sobre a Pesquisa:

A metodologia proposta é capaz de atender os objetivos do estudo.

Considerações sobre os Termos de apresentação obrigatória:

O TCLE foi apresentado.

Recomendações:

Recomenda-se a aprovação do projeto.

Conclusões ou Pendências e Lista de Inadequações:

O CEP aprova o projeto.

Considerações Finais a critério do CEP:

Não havendo pendências, o CEP propõe a aprovação do projeto de pesquisa salientando que, de acordo com a Resolução 466 CNS de 12/12/2012 (título X, seção X.1., art. 3, item b, e, título XI, seção XI.2., item d), há necessidade de apresentação de relatórios semestrais, devendo o primeiro relatório ser enviado até 20/09/2020. O CEP reitera a necessidade de entrega de uma via (não cópia) do TCLE ao sujeito participante da pesquisa e solicita ao pesquisador responsável leitura da carta circular 003/2011 CONEP/CNS antes do início do projeto.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

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Continuação do Parecer: 3.926.267

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1408738.pdf	17/02/2020 14:20:29		Aceito
Projeto Detalhado / Brochura Investigador	projeto_detalhado.pdf	17/02/2020 14:20:03	FLAVIA LOMBARDI LOPES	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE2.pdf	17/12/2019 09:56:28	FLAVIA LOMBARDI LOPES	Aceito
Folha de Rosto	plataforma_brasil_flavia.pdf	19/11/2019 10:22:06	FLAVIA LOMBARDI LOPES	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

ARACATUBA, 20 de Março de 2020

Assinado por:
Aldiéris Alves Pesqueira
(Coordenador(a))

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