RESEARCH ARTICLE

Leishmania regulates host YY1: Comparative proteomic analysis identifies infection modulated YY1 dependent proteins

Harsimran Kaur Brar[1], Eleanor Chen[1☯], Fabian Chang[1☯], Shawna Angel Lu[1], Dilraj Kaur Longowal[1], Kyung-Mee Moon [iD][2], Leonard J. Foster[2], Neil Reiner[1], Devki Nandan [iD][1]*

1 Division of Infectious Diseases, Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada, **2** Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada

☯ These authors contributed equally to this work.
* dnandan@mail.ubc.ca

Abstract

The protein Yin-Yang 1 (YY1) is a ubiquitous multifunctional transcription factor. Interestingly, there are several cellular functions controlled by YY1 that could play a role in *Leishmania* pathogenesis. Leishmaniasis is a human disease caused by protozoan parasites of the genus *Leishmania.* This study examined the potential role of macrophage YY1 in promoting *Leishmania* intracellular survival. Deliberate knockdown of YY1 resulted in attenuated survival of *Leishmania* in infected macrophages, suggesting a role of YY1 in *Leishmania* persistence. Biochemical fractionation studies revealed *Leishmania* infection caused redistribution of YY1 to the cytoplasm from the nucleus where it is primarily located. Inhibition of nuclear transport by leptomycin B attenuates infection-mediated YY1 redistribution and reduces *Leishmania* survival. This suggests that *Leishmania* induces the translocation of YY1 from the nucleus to the cytoplasm of infected cells, where it may regulate host molecules to favour parasite survival. A label-free quantitative whole proteome approach showed that the expression of a large number of macrophage proteins was dependent on the YY1 level. Interestingly, several of these proteins were modulated in *Leishmania*-infected cells, revealing YY1-dependent host response and suggesting their potential role in *Leishmania* pathogenesis. Together, this study identifies YY1 as a novel virulence factor that promotes *Leishmania* survival inside host macrophages.

Introduction

Leishmaniasis is a vector-borne disease caused by the intracellular protozoan parasite of the genus *Leishmania* [1,2]. The life cycle of *Leishmania* alternates between two major different cell morphologies. The motile promastigote morphology exists in

the lumen of the sandfly where as non-motile amastigote morphology resides in the phagolysosome of mammalian mononuclear phagocytes [3]. In humans, leishmaniasis has two main clinical forms, cutaneous leishmaniasis and visceral leishmaniasis. Visceral leishmaniasis, the more severe form of the disease, is mainly caused by infection with *Leishmania donovani* [1] and is most likely fatal if left untreated. According to WHO, an estimated 50,000–90,000 new cases of visceral leishmaniasis occur worldwide each year [4]. Unfortunately, no vaccines are available against any forms of leishmaniasis in humans, only a few approved vaccines are registered for canine leishmaniasis [5]. Additionally, current therapies are toxic to healthy cells and have limited efficacy [6]. More in-depth knowledge regarding *Leishmania*-host macrophage interactions may lead to the development of new innovative therapies to cure and control leishmaniasis.

Yin-Yang 1 (YY1) is a ubiquitous, multifunctional, zinc-finger transcription factor that regulates genes directly by binding to gene promoters or indirectly by interacting with other transcription factors [7,8]. Interestingly, through transcriptomic profiling, YY1 has been identified as one of three transcriptional regulators activated by *Leishmania* amastigotes in bone marrow derived macrophages [9]. This may serve as an initial hint towards YY1's possible involvement in promoting *Leishmania* intracellular survival specifically. Moreover, YY1 has been implicated in the control of various cellular mechanisms that likely benefit *Leishmania* pathogenesis. For example, YY1 has been linked to anti-inflammatory M2 macrophage polarization [10,11]. This would create a pro-parasitic, immunosuppressive environment that would enable *Leishmania* survival [12,13]. Additionally, YY1 has been shown to interact with and activate cell signaling protein Akt [14] in breast cancer cells. Interestingly, *Leishmania* infection has also been shown to activate Akt, which enhances parasite survival in host macrophages by inhibiting apoptosis and pro-inflammatory responses. In fact, Akt inhibition directly resulted in a marked decrease in parasite survival [15].

Although initially discovered as a nuclear transcription factor [16], YY1 has since been found to be present in the cytoplasm of several cultured cell lines [17–19] and tissues [19,20], which may suggest alternative, non-transcriptional roles of YY1. In this context, it was interesting to note that vaccinia virus induces YY1 translocation from the nucleus to the cytoplasm of infected human macrophages, where it seems to take part in pathogenesis [21,22]. This raised the possibility that *Leishmania* could induce translocation of YY1 to the cytoplasm to engage YY1 interaction with cytoplasmic factor in infected cells to its favor.

In this study, we show that host macrophage YY1 plays an important role in the persistence of *Leishmania* in infected cells. The deliberate knockdown of YY1 significantly attenuated the survival of *Leishmania* in human macrophages, demonstrating that YY1 is essential for *Leishmania* persistence. Strikingly, *Leishmania* redirects YY1 from its primary location of the nucleus to the cytoplasm during infection. Treatment with leptomycin B, an inhibitor of the Crm1 transport system, blocked YY1 transport to the cytoplasm and attenuated *Leishmania* survival. Comparative quantitative proteomic analysis revealed that the expression of a large number of macrophage proteins (537 out of 6559 proteins) was dependent on the level of YY1. Interestingly,

the expression of many YY1-dependent proteins was affected in *Leishmania*-infected cells, suggesting the role of YY1 in *Leishmania* pathogenesis. Taken together, this study identifies host macrophage YY1 as a novel virulence factor that *Leishmania* regulates to promote its survival in infected cells.

Materials and methods

Ethics statement

Buffy coats from healthy donors were purchased from the Canadian Blood Services Network Centre for Applied Development Ethics (Institutional Ethics Certificate: H23-00636). The source of human blood cells did not disclose the identity of the human donors, thereby anonymizing all data to the investigators. This study abides by the principles of the Declaration of Helsinki. All work with animals in this study was reviewed and approved by the University of British Columbia Animal Care Committee (protocol license number A14-0218). The animal care and use of protocol adhered to the standards and regulations provided by the Canadian Council on Animal Care in Science.

Reagents and antibodies

The human monocytic cell line, THP- 1 (TIB-202TM), was purchased from American Type Culture Collection (ATCC). RPMI 1640 media and Fetal bovine serum (FBS) were obtained from Gibco. HEPES, penicillin/streptomycin, L-glutamine, Hanks' balanced salt solution (HBSS), M199 medium, folic acid, hemin, adenosine, and Phorbol 12-myristate 13-acetate (PMA) were purchased from Millipore Sigma. Anti-Lamin A/C (#2032), anti-YY1 (D5D9Z), and leptomycin B (#9676) were purchased from Cell Signaling. Anti-actin (SC-47778) was obtained from Santa Cruz biotechnology. Anti-GAPDH (G041) was obtained from Applied Biological Materials Inc.

Culturing and differentiation of THP-1 cells

THP-1 cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 10 mM HEPES, 100 U/mL penicillin/streptomycin, and 2 mM L-glutamine at 37^{0}C with 5% CO_2. Cells were passaged every 2–3 days. To induce differentiation, THP-1 cells were treated with 10 ng/mL PMA for 16–18 hours. Adhered differentiated THP-1 (dTHP-1) cells were then subjected to washings with HBSS and provided with fresh media lacking PMA. The cells were then rested for 24 hours before any subsequent treatment.

Parasite culture and infection of dTHP-1 cells

Leishmania donovani Sudan strain S2 promastigotes were cultured in M199 medium supplemented with 10% heat-inactivated FBS, 20 mM HEPES, 10 μg/mL folic acid, 3 μg/mL hemin, 2 mM L-glutamine, 100 U/mL penicillin/streptomycin, and 100 μM adenosine at 26°C. Virulence was maintained by regular passages in Syrian Golden hamsters from which fresh amastigotes were obtained from heavily infected hamaster spleen tissue essentially as described [23] and *in vitro* transformed into promastigotes as previously described [24]. For *in vitro* infection, stationary phase promastigotes were incubated with dTHP-1 cells at a MOI (*Leishmania*: THP-1) of 20:1 unless otherwise indicated. Infection rate determination was performed as previously described [24].

Purification of human monocytes and *in vitro* differentiation to macrophages

Buffy coats from healthy donors were purchased from Canadian Blood Services Network Centre and diluted 1:2 in PBS supplemented with EDTA. Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coat using density gradient centrifugation on Ficoll-Paque PLUS (Cytiva) at room temperature. After isolation, cells were washed with PBS to remove platelets. The cells were then allowed to adhere in a 5% CO_2 incubator at 37°C for 1 hour. Non-adherent cells were removed by thorough washings with RPMI 1640. Adherent cells were collected using a cell scraper, and monocytes

were counted using Tuerk's solution. The cells were plated at a density of 0.75×10^6 cells/mL in multiwell plates in RPMI medium supplemented with 10% FBS, 2 mM L-glutamine, and 100 U/mL penicillin/streptomycin. For monocyte derived macrophage (hMDM) differentiation, purified cells were plated in cell culture dishes in complete maturation media (RPMI 1640 with 10% FCS, 100 U/mL penicillin/streptomycin, and 10 ng/mL granulocytes-macrophage colony-stimulating factor (GM-CSF) (Peprotech, Stockholm, Sweden). After 3 days, the cells were washed with HBSS and given fresh supplemented RPMI medium containing GM-CSF. The cells were washed again on day 6, rested in supplemented RPMI medium without GM–CSF, and used for experiments on day 7.

siRNA transfection

3 unique YY1 (human) siRNA duplexes and one non-specific (scrambled) negative control were obtained from OriGene (SR322232). THP-1 and hMDMs Macrophages were transfected with 100 nM of siRNA using the HiPerfect (Qiagen) transfection reagent according to the manufacturer's protocol. The plates were gently mixed to ensure uniform distribution of the transfection complex. THP-1 cells were treated with siRNA 24 hours prior to PMA-mediated differentiation and hMDMs were treated with siRNA for 24 hours after GM-CSF-mediated differentiation. To confirm YY1 knockdown, whole cell lysates of transfected cells were analysed for YY1 levels by Western blotting 24 hours post transfection. YY1 deficient cells were subsequently incubated with or without *Leishmania* and assessed in downstream assays.

Western blotting

Cells were washed with HBSS and lysed in ice-cold cell lysis buffer (20 mM Tris-HCl, pH 7.4, 1% Triton X-100, 1 mM EDTA, 0.15 M NaCl, 1 mM sodium orthovanadate, 5 mM NaF, 5 μg/mL aprotinin, 5 μg/mL leupeptin, and 2 mM PMSF) on ice. For *L. donovani* whole cell lysates, parasites were washed with HBSS and lysed in ice-cold cell lysis buffer with double the aprotinin, leupeptin, and PMSF concentration. Lysates were boiled in equal volumes of 4X Laemmli sample loading buffer for 7 min, separated by SDS-PAGE and transferred to nitrocellulose membrane using a semi-dry transfer apparatus. Transferred proteins were probed with appropriate antibodies, according to the manufacturer's instructions. Protein bands were captured on Blue X-ray film (Carestream) using ECL Select. Densitometry analysis was performed using ImageJ.

Parasite rescue assay

dTHP-1 cells or hMDMs were infected with stationary phase *L. donovani* at a MOI of 20:1. After 24 hours of infection, cells were extensively washed to remove non-internalized *Leishmania*. Infected cells were then lysed using 0.01% SDS, followed by the transformation of live, rescued *Leishmania* amastigotes to promastigotes in M199 media by incubating plates at 26°C for 48 hours. The evaluation of their growth was performed by manual counting of transformed promastigotes using a hemocytometer as described previously [24–26].

Biochemical subcellular fractionation

Subcellular fractionation was performed as previously described [27] with minor modifications. Briefly, THP-1 cells (1.5 x 10^6 cells per well) were differentiated in a six-well plate with PMA. Cells were lysed in 900 μL of digitonin lysis buffer (50 mM HEPES pH 7.4, 150 mM NaCl, 20 μg/mL digitonin, 1 mM PMSF, 5 μg/mL aprotinin, 5 μg/mL leupeptin). Lysates were incubated on ice for 10 minutes with intermittent mixing followed by centrifugation at 2000 x g for 4 minutes at 4°C. The supernatant was carefully removed and the remaining cell pellet was extracted in 2X SDS-PAGE sample loading buffer. Extracted proteins from each fraction were analyzed by Western blot using an anti-GAPDH antibody as a marker of the cytosol and an anti-Lamin A/C as markers of the nucleus.

Liquid chromatography – tandem mass spectrometry and protein identification

Cell pellets were lysed in 50% (v/v) 2,2,2-trifluoroethanol, reduced and alkylated, and digested with trypsin [28]. The peptides were cleaned with C-18 STop And Go Extraction (STAGE) tips [29] and eluted using 40% (v/v) acetonitrile in 0.1% (v/v) formic acid. The peptides were resuspended in Buffer A consisting of 0.5% acetonitrile, 0.1% formic acid in MS grade water and peptide concentration was estimated by UV absorbance at 205nm using a NanoDropOne (Thermo Scientific, A205nm scopes). Approximately 100 ng of peptides were loaded onto timsTOF Pro2 (Bruker Daltonics) coupled to NanoElute UHPLC (Bruker Daltonics) using Aurora Series Gen3 25 cm analytical column (IonOpticks) using a DIA-PASEF method. Instrument parameters follow paper [30] with following method changes. The PASEF ramps contained 25 isolation windows from 299.5 m/z to 1200.5 m/z with ion mobility range of 0.7 - 1.3 $V \cdot s \cdot cm^{-2}$ and collision energy ramped from 20.0 eV at 0.6 $V \cdot s \cdot cm^{-2}$ to 65.0 eV at 1.6 $V \cdot s \cdot cm^{-2}$. Sample injection order was randomized.

Data were searched against Uniprot's human and *Leishmania donovani* proteomes (UP000005640 and UP000008980) with a custom list of contaminants using DIA-NN version 1.9.1 [31]. Library-free search mode was enabled, using trypsin/P protease specificity and 1 missed cleavages. Other search parameters include 1 maximum number of variable modification, N-terminal M excision, carbamidomethylation of C, and oxidation of M. Peptide length ranged 7–30, precursor charge ranged 2–4, precursor and fragment m/z ranged 200–1700. Precursor FDR was set to 1%, with 0 for settings 'mass accuracy', 'MS1 accuracy' and 'scan window'. Settings 'heuristic protein inference', 'match between run (MBR)', and 'no shared spectra' were all enabled. 'Protein name from FASTA' was chosen for protein inference parameter along with 'double-pass mode' for neural network classifier. 'QuantUMS (high precision)' was used for quantification strategy, 'RT-dependent' mode for cross-run normalisation, and 'IDs, RT & IM profiling' mode for library generation.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [32] partner repository with the dataset identifier PXD059968.

Quantitative analysis of liquid chromatography-tandem mass spectrometry data

All downstream analysis was carried out in Microsoft Excel and R software (http://www.r-project.org). From the total number of proteins detected by liquid chromatography-tandem mass spectrometry (LC-MS/MS), potential contaminants and *L. donovani* proteins were filtered out. Only proteins that had raw intensity values in all three replicates were retained for further analysis. All downstream analyses were performed on log2-transformed ratios (fold changes). A two-sample, two-tailed T-test was conducted to compare the log2-transformed normalized ratios. A p-value of less than 0.05 was considered statistically significant. Benjamini-Hochberg procedure was used to further calculate adjusted p-values [33,34]. No minimum fold change cut-off was set for a protein to be considered modulated. Volcano plot, comparing average log_2 values with -log10(p-value) was also plotted using R software for better visualization.

Uniprot Princeton GO Mapper (https://go.princeton.edu/cgi-bin/GOTermMapper) was used for Gene Ontology (GO) annotation with the following considerations: Organism annotation set to "Homo sapiens (GOA @EBI + Ensembl)" and Ontology set to "Generic slim." The protein annotation involved three biological aspects: Biological process ("process"), Molecular function ("function"), and Cellular component ("component"). GO terms with three or more protein members were kept. Additionally, broad terms like "Organelle" spanning several subcategories were omitted; for identical GO terms, only one term was retained (e.g., "Extracellular space" was kept, while "Extracellular region" was removed; "Extracellular matrix" was retained while "External encapsulating structure" was omitted; "Carbohydrate derivative metabolic process" was kept and "Carbohydrate metabolic process" was omitted; "Mitotic nuclear division" was omitted while keeping "Mitotic cell cycle"; "Catalytic activity" was kept, while "Catalytic activity acting on proteins or DNA" was omitted).

Results

YY1 protein is required for the survival of *Leishmania* in infected dTHP-1 cells

To investigate the possible role of host macrophage YY1 protein in the perseverance of *Leishmania*, we downregulated YY1 in THP-1 cells using siRNA mediated gene silencing and assessed parasite survival. Effectiveness of YY1 knockdown in THP-1 cells was determined by Western blot analysis on whole cell lysates (Fig 1A and 1B). Two of the three YY1 siRNAs used were successful in significantly knocking down YY1 protein levels compared to the control siRNA and were selected for use in downstream experiments. To analyze the effects of YY1 knockdown on *Leishmania* survival, control and YY1 knockdown cells were differentiated with PMA for 24 hours post siRNA transfection, infected with *L. donovani* for 24 hours, then assessed using a parasite rescue assay as described in "Materials and methods". The results of the parasite rescue assay, presented in Fig 1C, show a clear reduction of *Leishmania* survival in YY1 knockdown cells. Together, our data strongly suggest that macrophage YY1 could play an important role in the perseverance of *Leishmania* in infected host cells.
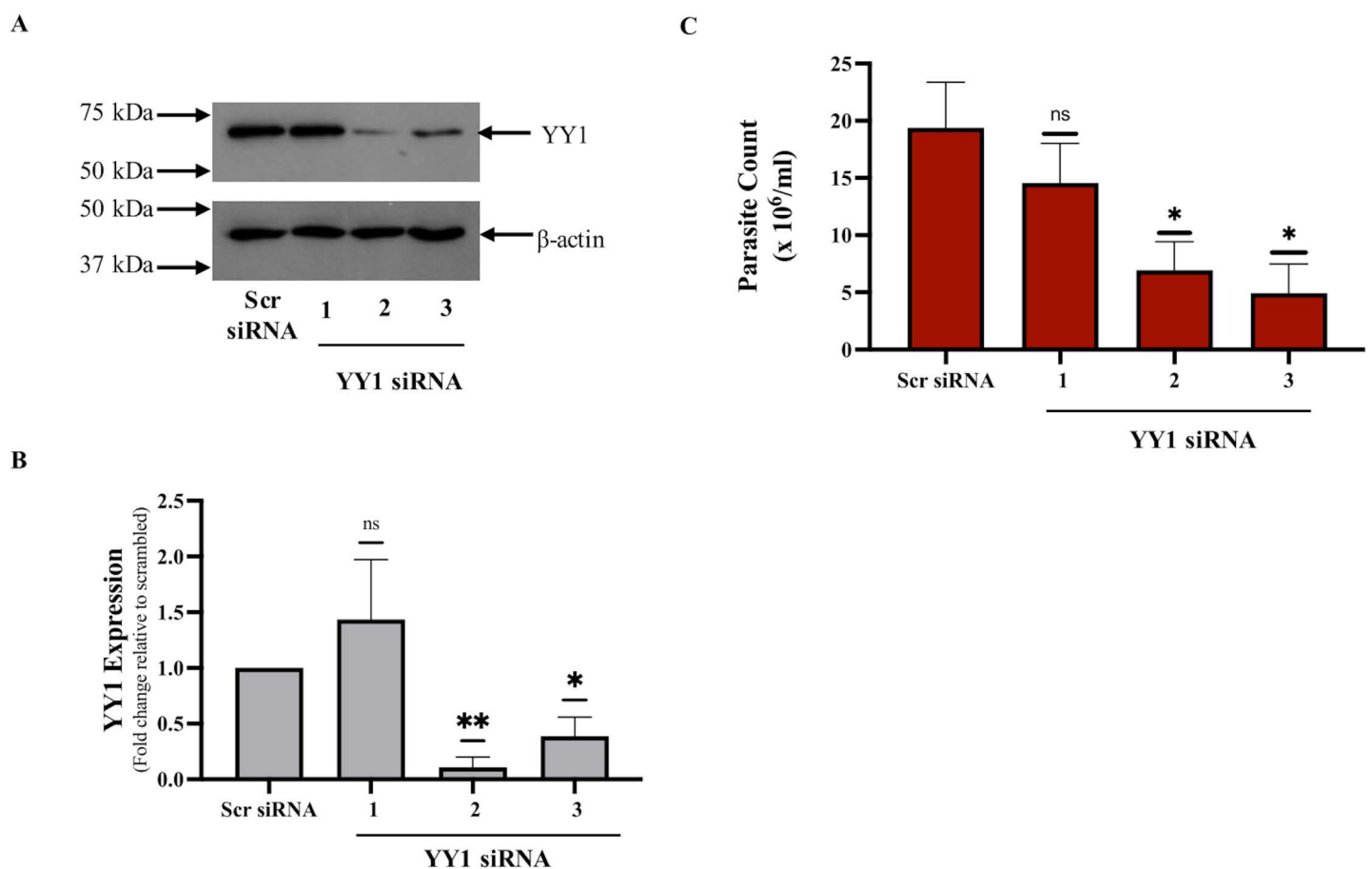
Fig 1. YY1 knockdown inhibits *L. donovani* survival in dTHP-1 cells. (A) THP-1 cells were transfected with three unique YY1 siRNAs or a scrambled siRNA (scr siRNA) for 24 hours followed by differentiation with PMA, and subsequently infection with *L. donovani* at a 20:1 MOI for 24 hours. Cells were washed extensively and Western blotting was performed using the specified antibodies on whole-cell lysates of infected dTHP-1 cells. (B) Histogram representing densitometric analysis of YY1 knockdown normalized to Actin levels. Bars represent mean±SD of three independent experiments. (C) Parasite rescue assay was performed in parallel as described in "Materials and methods". Bars represent the mean±SD of four independent experiments. Statistical significance was determined using two-sample two-tailed T-test (ns: not significant, *: $p < 0.05$, **: $p < 0.01$).

YY1 is critical for *Leishmania* survival in primary human macrophages

The results presented above in Fig 1 showed that YY1 is required for the survival of *Leishmania* in infected dTHP-1 cells. While THP-1 cells are widely used in leishmaniasis related studies [35–38], it was of interest to validate results presented in Fig 1 in primary human macrophages. Therefore, peripheral human blood monocytes were isolated from healthy donors and differentiated into macrophages (hMDMs) to investigate the role of YY1 in *Leishmania* survival in these cells. For this investigation, YY1 was downregulated in hMDMs by transfecting with two different siRNAs against YY1. A scrambled siRNA was used as a control. After 24 hours of transfection, cells were infected with *L.donovani*. 24 hours post infection, cells were lysed and processed for a parasite rescue assay as described in "Materials and methods". In parallel, cells were also processed for Western Blot analysis to ensure effectiveness of siRNAs to knockdown YY1 (Fig 2B). The results
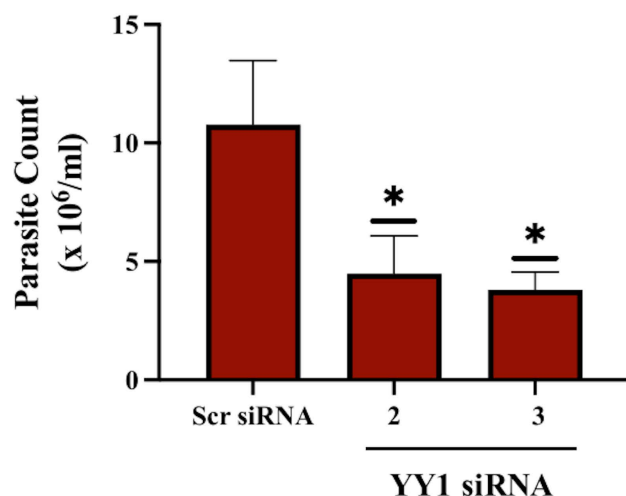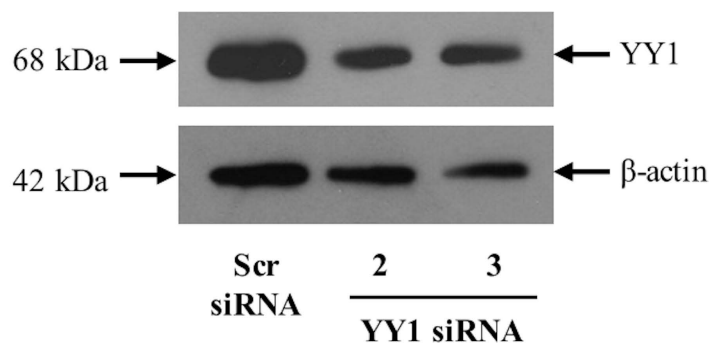
Fig 2. YY1 knockdown reduces *L. donovani* survival in hMDMs. (A) Peripheral human blood monocytes were isolated and differentiated to macrophages as described in "Materials and methods". hMDMs were transfected with two unique YY1 siRNAs or a scrambled siRNA (scr siRNA) for 24 h followed by infection with *L. donovani* at a 20:1 MOI for 24 h. The parasite rescue assay was then performed as described in "Materials and methods". Bars represent mean ± SD of three independent experiments. Statistical significance was determined using two-sample two-tailed T-test (*: $p < 0.05$). (B) YY1 knockdown was verified by Western blot using the indicated antibodies on whole cell lysates of infected hMDMs.

https://doi.org/10.1371/journal.pone.0323227.g002

shown in Fig 2A clearly demonstrate that YY1 is important for the survival of *Leishmania* in hMDMs thus validating the results obtained using dTHP-1 cells.

Leishmania mediates cytoplasmic translocation of YY1 in infected host cells

Working with human primary human monocytes/macrophages, in contrast to macrophage/monocytic cell lines, has limitations such as limited yield from a single donor, inter donor variability, shorter lifespan, limited expansion capacity, and notoriously difficult transfection. To counter these limitations, rest of the study presented here was performed in PMA diffentiated THP-1 cells.

Despite YY1 being a transcription factor, it has been found in both the nucleus and the cytoplasm of cultured cell lines [18,19]. This raises the possibility that cytoplasmic YY1 may have functions unrelated to its primary role as a transcription factor. In the context of host-pathogen interactions, a previous study showed that vaccinia virus mediates Crm 1-dependent cytoplasmic translocation of YY1 in infected cells [22]. However, viral infection did not alter YY1 levels at the whole cell level [22]. Given that *Leishmania* survival requires YY1 expression in host macrophages, *Leishmania* infection may modulate YY1 expression. To investigate whether *Leishmania* alters the abundance of host YY1, dTHP-1 cells were infected with *L. donovani* (MOI 10:1 and 20:1) and YY1 protein levels were monitored by Western blotting over 48 hours. As shown in supplementary Fig S1, expression of YY1 over 48 hours of infection at both MOIs was not significantly affected relative to non-infected control cells.

Since we didn't see any significant changes in YY1 expression at whole cell level, it was of interest to investigate whether like vaccinia virus, *Leishmania* infection also induces cytoplasmic translocation of host macrophage YY1 from its primary location in the nucleus. The intracellular localization of YY1 during *Leishmania* infection in THP-1 was investigated by performing biochemical cell fractionation as described in "Material and methods". Cells were gently lysed using digitonin and nuclei were pelleted by centrifugation. Lamin A/C and GAPDH were used as specific markers of the nuclear and the cytoplasmic fractions, respectively. As shown in Fig 3A and 3C, GAPDH and Lamin A/C were highly enriched in cytoplasmic and nuclear fractions, respectively. Cytoplasmic fractions were also tested for the possible contamination of Lamin A/C (Fig S2). The results, presented in Fig 3, show that *Leishmania* causes a substantial increase in cytoplasmic YY1 levels, while reducing nuclear YY1 levels in infected THP-1 cells. This suggests that *Leishmania* induces YY1 translocation to the cytoplasm from the nucleus during infection.

Leishmania mediated cytoplasmic transport of host YY1 protein involves Crm 1 transport system

The observed increase in cytoplasmic YY1 level may be due to increased YY1 nuclear export or decreased nuclear import. To differentiate between the two mechanisms, we selectively inhibited the nuclear export of proteins. Classic nuclear export occurs through the nuclear pore complexes involving exportins (also known as chromosomal maintenance 1, Crm1) [39]. In the absence of a characterized nuclear export signal of YY1, we used leptomycin B to inhibit Crm1 mediated nuclear export. Leptomycin B, initially used as an anti-fungal agent, was the first specific inhibitor of the Crm1 system reported [40]. For this study, dTHP-1 cells were pretreated with leptomycin B then washed and followed by *Leishmania* infection for 24 hours. Cytosolic fractions from non-infected and infected cells were analyzed for the presence of YY1 by Western blotting. As shown in Fig 4A and 4B, treatment of cells with leptomycin B resulted in decreased cytoplasmic YY1 level in both control and *Leishmania* infection conditions. This suggests that the main origin of cytoplasmic YY1 is from the export of nuclear YY1; *Leishmania* likely increases YY1 nuclear export via the Crm1 export system rather than increasing cytoplasmic retention of de novo cytoplasmic YY1 or decreasing nuclear import. In parallel, to confirm that cytoplasmic YY1 is important for *Leishmania* survival, we performed a parasite rescue assay in leptomycin B treated cells. As expected, blockage of nuclear export by leptomycin B significantly attenuated survival of *Leishmania* in infected cells as compared to vehicle alone treated infected cells (Fig 4C).

A

B

C

D
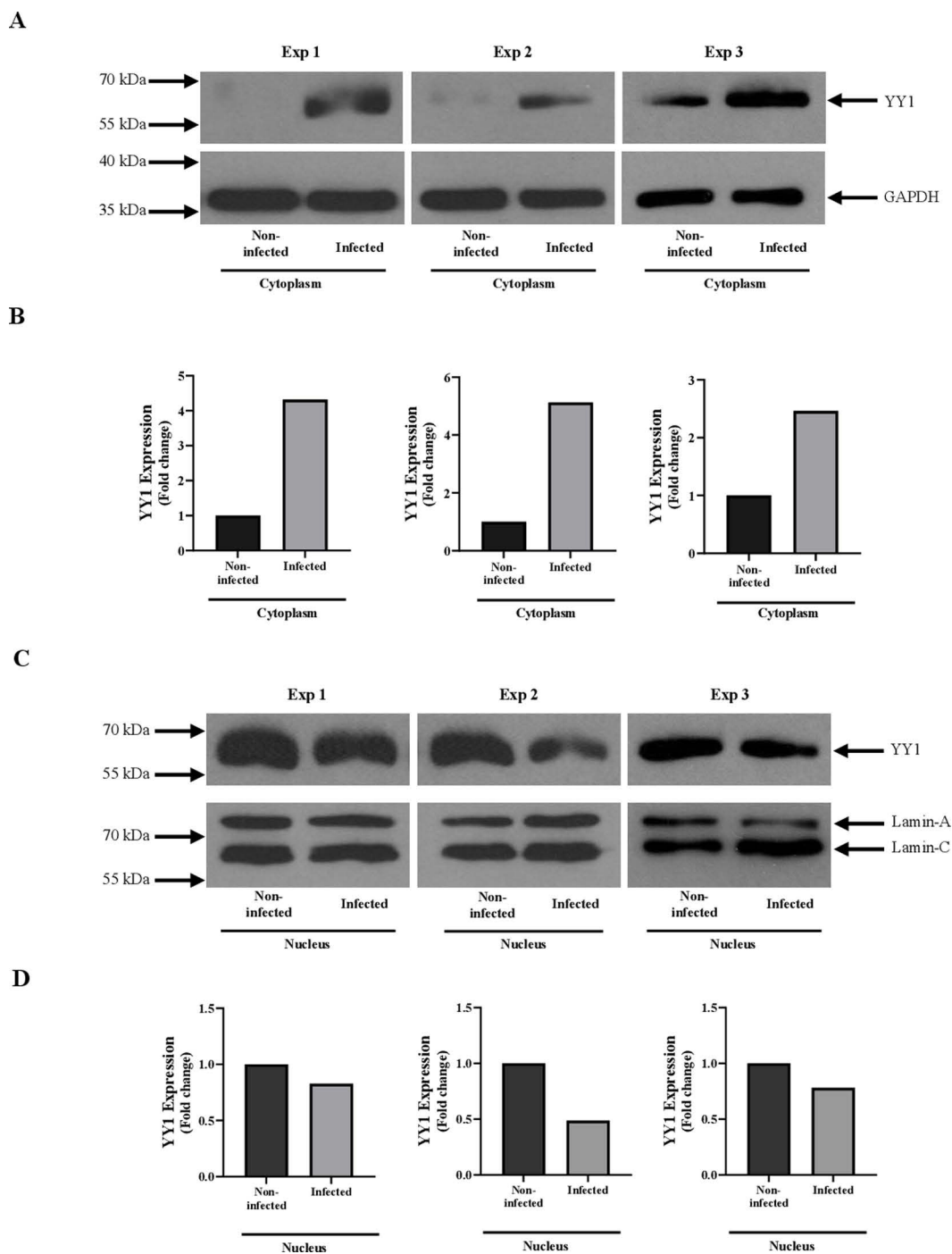
Fig 3. ***L. donovani* infection induces cytoplasmic translocation of YY1 in dTHP-1 cells.** dTHP-1 cells were infected with *L. donovani* at 20:1 MOI for 24 hours and cellular fractionation was performed as described in "Materials and methods". (A) The cytoplasmic fractions of non-infected and infected cells were then Western blotted using the indicated antibodies. (B) Histograms representing densitometric analysis of Western blots of cytoplasmic fractions from dTHP-1 cells. (C) The nuclear fractions of non-infected and infected cells were Western blotted using the indicated antibodies. (D) Histograms representing densitometric analysis of western blots of nuclear fractions from dTHP-1 cells. The data shown are from three independent experiments.
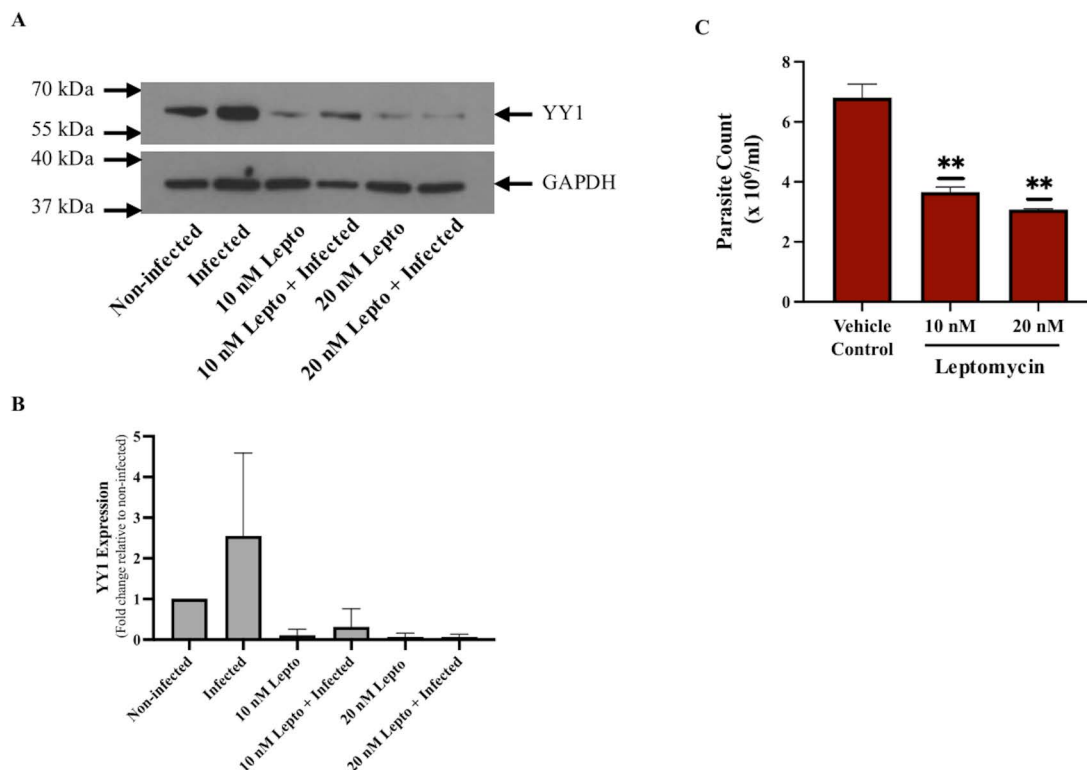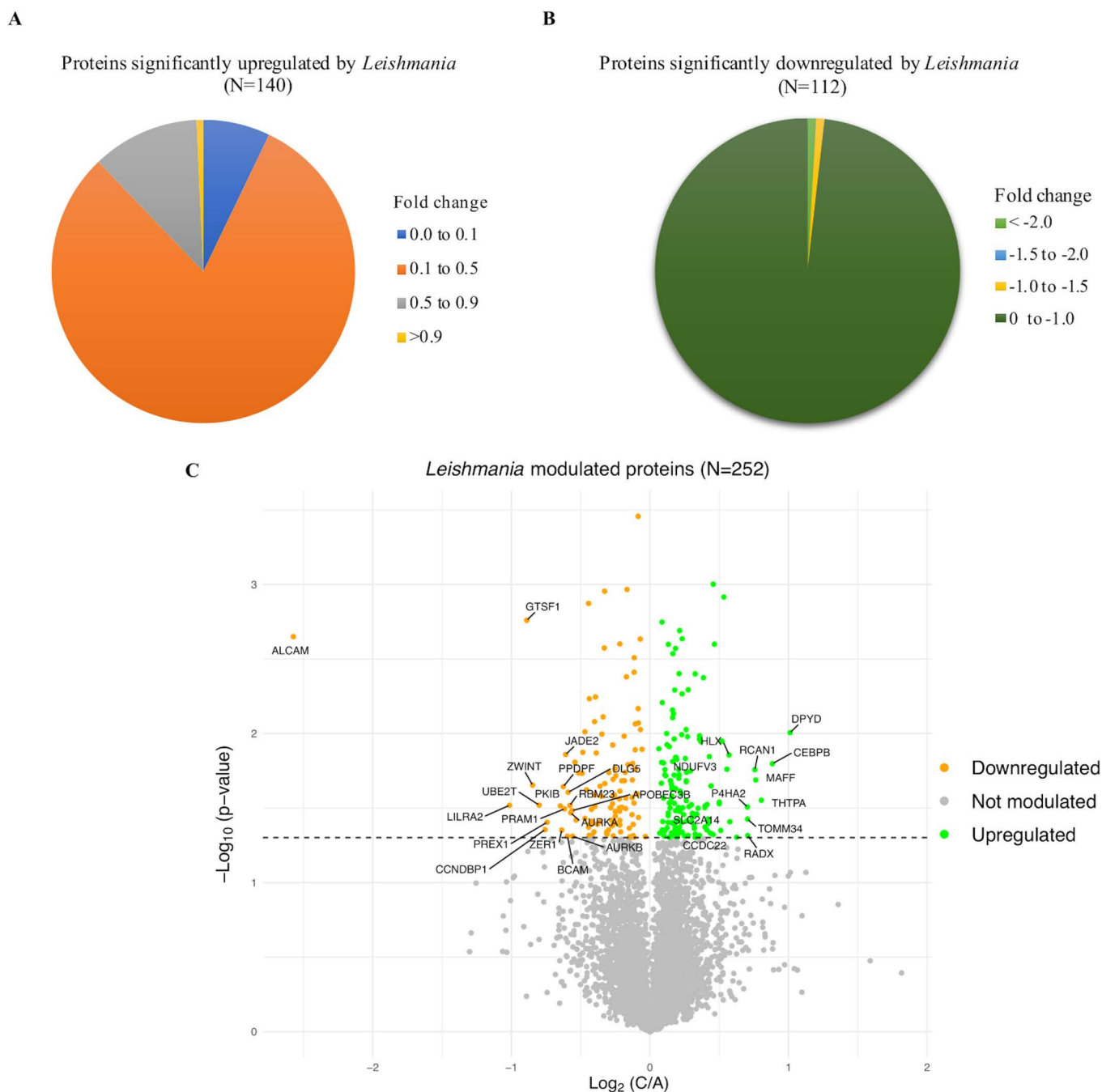
Fig 4. Leptomycin B inhibits cytoplasmic translocation of YY1 in dTHP-1 cells. (A) dTHP-1 cells were either untreated or treated with leptomycin B (Lepto) at a concentration of 10 nM or 20 nM for 3 hours prior to infection with *L. donovani* at a MOI of 20:1. After 24 hours, cells were fractionated as described in "Materials and methods". YY1 protein levels in cytoplasmic fractions were verified by Western blotting with the indicated antibodies. (B) Histogram representing densitometric analysis of YY1 expression in Western blot of cytoplasmic fractions from dTHP-1 cells. (C) **Leptomycin B attenuates *L. donovani* survival in dTHP-1 cells**. In parallel, parasite rescue assay was performed as described in "Materials and methods". For this assay, dTHP-1 cells were either treated with vehicle alone, or treated with leptomycin B at a concentration of 10 nM or 20 nM for 3 hours, followed by infection with *L. donovani* at a MOI of 20:1 for 24 hours. Bars represent the mean ± SD of three independent experiments. Statistical significance was determined using two-sample two-tailed T-test (**: $p < 0.01$).

https://doi.org/10.1371/journal.pone.0323227.g004

The results thus far show that YY1 is translocated to cytoplasm during *Leishmania* infection, and host macrophage YY1 is important for optimal intracellular survival of *Leishmania*. These interesting findings prompted us to further study the role of YY1 in the pathogenesis of *Leishmania* infection. To this end, we used a comprehensive, unbiased label free quantitative proteomic analysis of *Leishmania*-infected cells in an YY1-deficient condition.

Comparative quantitative proteomic analysis of *Leishmania*-infected macrophages

The aim of the study was to investigate YY1 dependent host proteome during *L. donovani* infection. Therefore, it was important to first determine how *L. donovani* alters the proteome of dTHP-1 cells. For this, quantitative proteomic analysis was done for non-infected and infected dTHP-1 cells. We first filtered out the common contaminants and *L. donovani* proteins from initial list of 14220 proteins identified. This resulted in total of 10487 proteins. For downstream analysis, only those proteins that had raw intensity values in all three biological replicates in both uninfected and infected cells were used (6585 proteins) (Table S1). 252/6585 proteins were found to be significantly modulated (adjusted p-value < 0.05) by *L. donovani*, out of which 140 proteins were upregulated and 112 were downregulated (Table S1) (Fig 5A and 5B). Volcano plot which illustrates the *Leishmania* modulated proteins (upregulated/downregulated) has also been plotted (Fig 5C) for better visualization of the data.

A Proteins significantly upregulated by *Leishmania*
(N=140)

Fold change
- ■ 0.0 to 0.1
- ■ 0.1 to 0.5
- ■ 0.5 to 0.9
- ■ >0.9

B Proteins significantly downregulated by *Leishmania*
(N=112)

Fold change
- ■ < -2.0
- ■ -1.5 to -2.0
- ■ -1.0 to -1.5
- ■ 0 to -1.0

C *Leishmania* modulated proteins (N=252)

- ● Downregulated
- ● Not modulated
- ● Upregulated

Fig 5. Comparative analysis of global proteome of control and *L. donovani* infected cells. dTHP-1 cells were either infected with *L. donovani* or not. Cells were then dislodged in cell dissociation buffer (without enzyme) and mass spectrometric analysis was perfomed as described in "Materials and methods". (A) Pie-chart depicting the distribution of proteins significantly upregulated by *L. donovani* infection, categorized based on the average of log2 values from three independent replicates (fold change). (B) Pie-chart depicting the distribution of proteins significantly downregulated by *L. donovani* infection, categorizd based on the average of log2 values from three independent experiments. Two sample, two tailed T-test was used to measure statistical significance with p-value <0.05 considered significant. (C) Volcano plot of proteins showing significant fold changes in *L. donovani* infected cells as compared to non-infected cells. Proteins significantly upregulated by *L. donovani* infection are shown in green dots, those that were downregulated, as orange dots and not modulated as grey dots. Data is from three independent experiments. The horizontal dashed line represents the p-value cut off of 0.05 or -log10(p-value) of 1.301. **C** denotes infected; **A** denotes non-infected.

Identification of host macrophage proteins modulated by *Leishmania* and dependent on YY1

For this study, we performed a quantitative proteomic analysis of *L.donovani*-infected cells in a YY1-deficient condition and compared it to proteome of *L.donovani*-infected normal cells. We chose those proteins that have raw intensity values in all replicates of control and *L. donovani* infected and YY1 knockdown and *L. donovani* infected cells, which resulted in a total of 245 proteins (Table S2). Next, we choose (i) proteins upregulated in *L. donovani* infected cells (average log2(C/A) > 0), and that were recovered closer to non-infected abundance, (average log2 (D/C) < 0) by YY1 knockdown, and (ii) proteins downregulated by *L. donovani* infection (average log2 (C/A) < 0) and were recovered by YY1 knockdown (average log2 (D/C) > 0) (Table 1). In average log2 analysis, A represents protein level in control and uninfected cells; C represents

Table 1. YY1-dependent *L. donovani*-modulated host proteins in dTHP-1 cells.

YY1-dependent L. donovani- upregulated host proteins in dTHP-1 cells

Protein description	Gene name	Uniprot ID	Fold change (Average Log$_2$ C/A > 0)	Fold change (Average Log$_2$ D/C < 0)
Tubulin polyglutamylase complex subunit 1 (PGs1)	TPGS1 C19orf20	Q6ZTW0	0.429287	-1.022591511
Pleckstrin (Platelet 47 kDa protein) (p47)	PLEK P47	P08567	0.193316	-0.02834847
FERM domain-containing protein 4A	FRMD4A FRMD4 KIAA1294	Q9P2Q2	0.264011	-0.550649734
Dihydropyrimidinase-related protein 2 (DRP-2) (Collapsin response mediator protein 2) (CRMP-2) (N2A3) (Unc-33-like phosphoprotein 2) (ULIP-2)	DPYSL2 CRMP2 ULIP2	Q16555	0.224368	-0.406896493
Cytochrome c oxidase assembly protein COX19 (hCOX19)	COX19	Q49B96	0.20478	-0.567578851
Golgi integral membrane protein 4 (Golgi integral membrane protein, cis) (GIMPc) (Golgi phosphoprotein 4) (Golgi-localized phosphoprotein of 130 kDa) (Golgi phosphoprotein of 130 kDa)	GOLIM4 GIMPC GOLPH4 GPP130	O00461	0.145495	-0.227740876
Ankyrin repeat domain-containing protein 17 (Gene trap ankyrin repeat protein) (Serologically defined breast cancer antigen NY-BR-16)	ANKRD17 GTAR KIAA0697	O75179	0.192595	-0.211788204
NADP-dependent malic enzyme (NADP-ME) (EC 1.1.1.40) (Malic enzyme 1)	ME1	P48163	0.181688	-0.367545834

YY1-dependent L. donovani- downregulated host proteins in dTHP-1 cells

Protein description	Gene names	Uniprot ID	Fold change (Average Log$_2$ C/A < 0)	Fold change (Average Log$_2$ D/C > 0)
Schlafen family member 5	SLFN5	Q08AF3	-0.10537	0.487793
tRNA (34–2'-O)-methyltransferase regulator WDR6 (WD repeat-containing protein 6)	WDR6	Q9NNW5	-0.34629	0.391072
Tripartite motif-containing protein 2 (EC 2.3.2.27) (E3 ubiquitin-protein ligase TRIM2) (RING finger protein 86) (RING-type E3 ubiquitin transferase TRIM2)	TRIM2 KIAA0517 RNF86	Q9C040	-0.26802	0.408278
Afadin (ALL1-fused gene from chromosome 6 protein) (Protein AF-6) (Afadin adherens junction formation factor)	AFDN AF6 MLLT4	P55196	-0.10618	0.458748
ER degradation-enhancing alpha-mannosidase-like protein 3 (EC 3.2.1.113) (Alpha-1,2-mannosidase EDEM3)	EDEM3 C1orf22	Q9BZQ6	-0.10487	0.300969
Disks large homolog 5 (Discs large protein P-dlg) (Placenta and prostate DLG)	DLG5 KIAA0583 PDLG	Q8TDM6	-0.59043	0.272367
Cytoplasmic FMR1-interacting protein 2 (p53-inducible protein 121)	CYFIP2 KIAA1168 PIR121	Q96F07	-0.22064	0.184068
NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 6 (Complex I-B17) (CI-B17) (NADH-ubiquinone oxidoreductase B17 subunit)	NDUFB6	O95139	-0.43758	0.313633

protein level in control and *L. donovani* infected cells and D represents protein level in YY1 knockdown and *L. donovani* infected cells. 31/245 proteins were observed to be significantly modulated by YY1 knockdown in *L. donovani*-infected cells. Of these, 16 proteins were recovered (8 proteins upregulated, 8 proteins downregulated) by YY1 knockdown (Table 1; Table S2). The *L. donovani* infection sensitive and YY1 dependent host proteins were also classified by GO terms in three groups: biological process, molecular function, and cellular component (Fig 6A–6C).

Global proteome analysis of YY1 knockdown THP-1 cells and control THP-1 cells

It is becoming increasingly clear that YY1 is a ubiquitous protein that serves many important and diverse biological roles [7,41]. Expectedly, our proteomic results presented in Table S3 revealed that a large number of macrophage proteins (approximately 5% of identified proteins) are sensitive to abundance of YY1. An extensive literature search revealed no published global protein profiling of YY1 dependent proteins in THP-1 cells or other macrophages in humans or murine models. Thus, it was of interest to analyse YY1-sensitive macrophage proteins in detail. For this analysis, proteins that showed raw intensity values in all three replicates in both control and YY1 knockdown macrophages were chosen (6559 proteins) (Table S3). Out of the 6559 proteins, 537 showed significant changes (adjusted p-value < 0.05) in abundance in the YY1 knockdown cells. Of the 537 proteins significantly modulated, expression level of 291 proteins increased (Fig 7A) while expression of 246 decreased in the YY1 knockdown THP-1 cells (Fig 7B) (Table S3). As seen in Table 3, YY1 is the
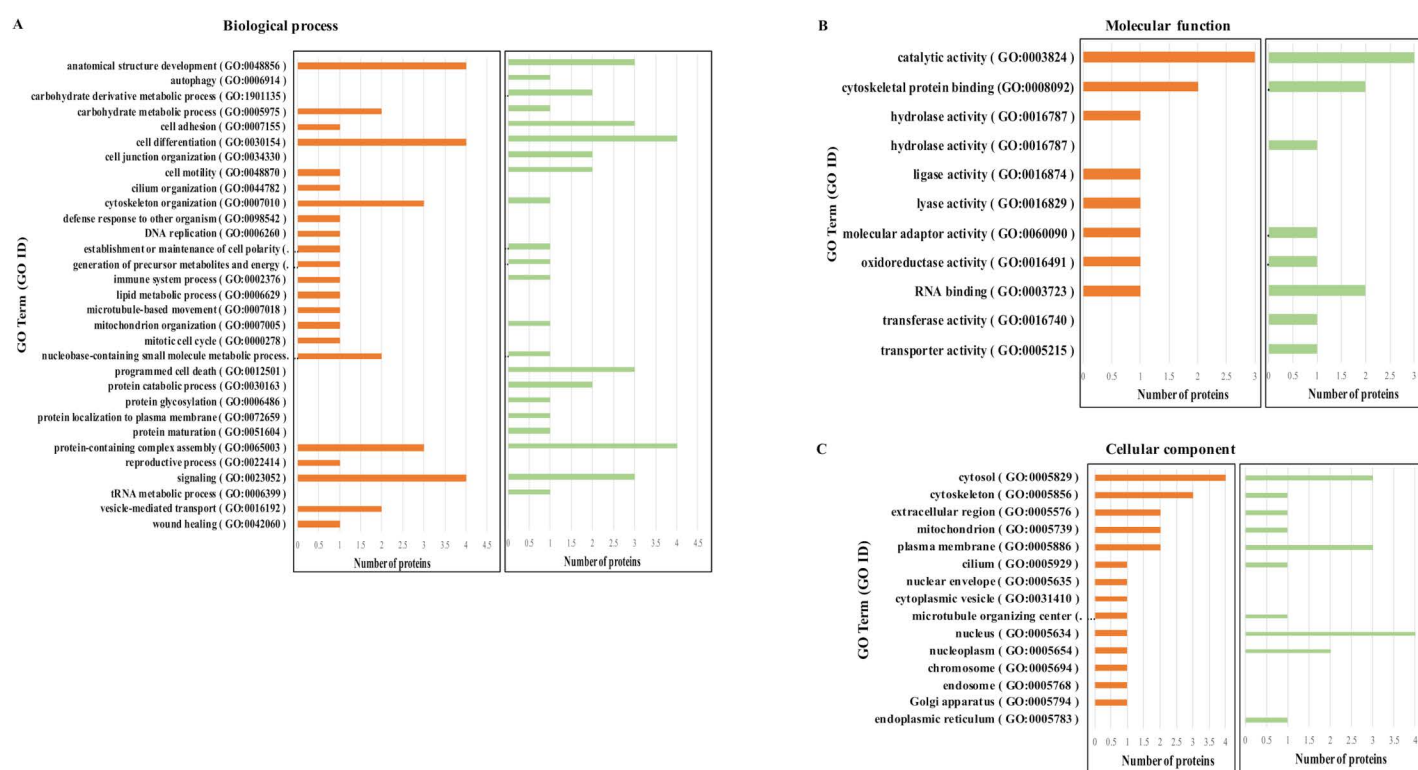
Fig 6. Gene ontology analysis of YY1-dependent *L. donovani* modulated proteins in THP-1 cells. Gene ontology (GO) enrichment analysis was performed on significantly modulated proteins by *L. donovani* (upregulated or downregulated) and recovered by YY1 (downregulated and upregulated, respectively) knockdown. Proteins were categorized into: (A) Biological process, (B) Molecular Function, and (C) Cellular component. Orange represents the proteins significantly upregulated by *L. donovani* and recovered by YY1 and green represents the proteins significantly downregulated by *L. donovani* and recovered by YY1.

https://doi.org/10.1371/journal.pone.0323227.g006
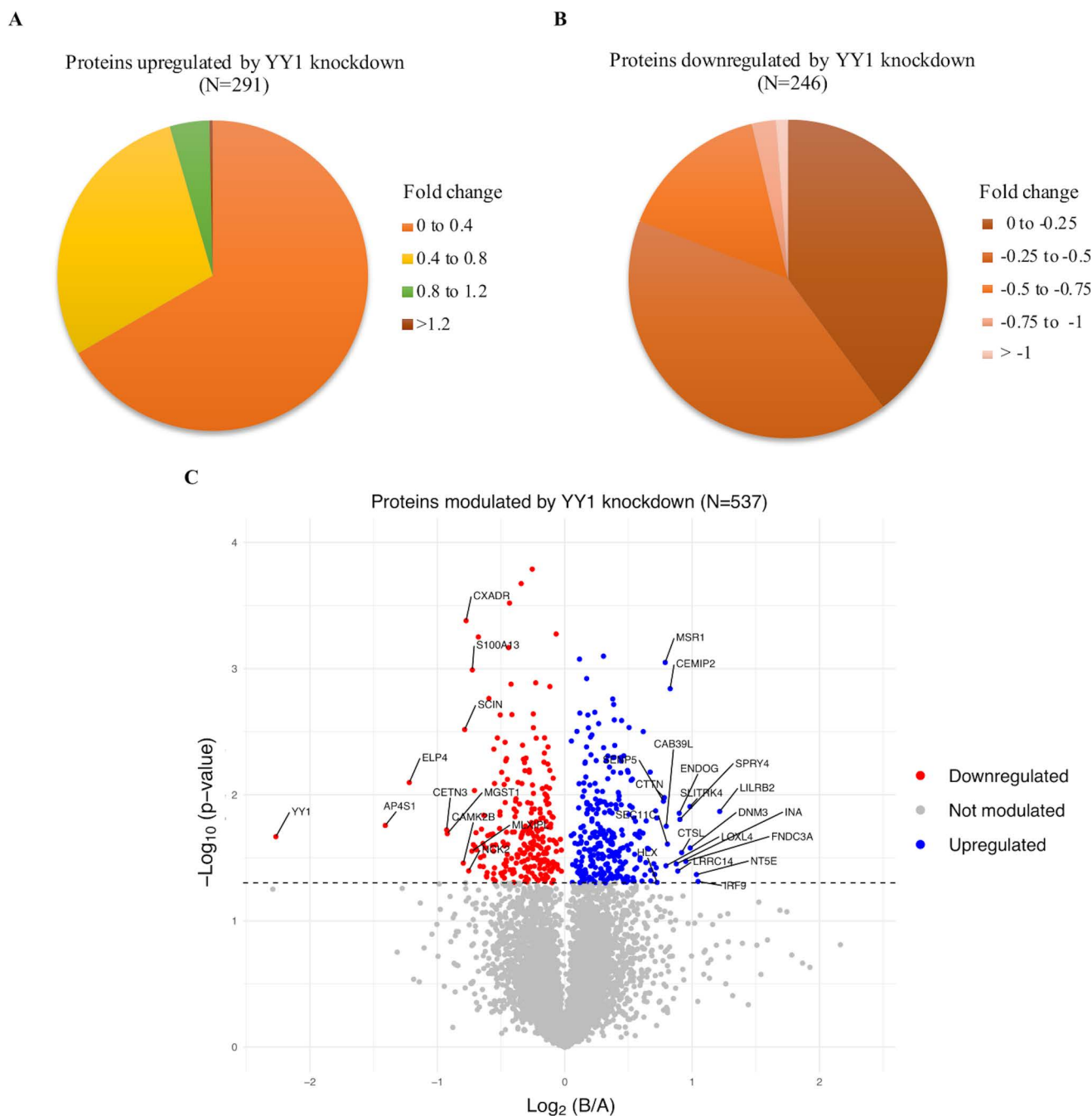
Fig 7. Comparative analysis of global proteome of control and YY1 knockdown cells. THP-1 cells were transfected with equal mix of two different siRNAs (50 nM each) or a scrambled siRNA for 24 hours, followed by differentiation. Cells were then dislodged in cell dissociation solution (without enzyme) and mass spectrometric analysis was perfomed as described in "Materials and methods". (A) Pie-chart illustrating the distribution of proteins significantly upregulated by YY1 knockdown, categorized based on average of log2 values. (B) Pie-chart demonstrating the distribution of proteins significantly downregulated by YY1 knockdown, categorized based on average of log2 values. Two sample, two tailed T-test was used to measure statistical significance with p-value <0.05 considered significant. (C) Volcano plot of proteins showing significant fold changes in YY1 knockdown cells as compared to control cells. Proteins significantly upregulated by YY1 knockdown are shown as blue dots, those that were downregulated, as red dots and those that are not modulated as grey dots. Data is from three independent experiments. The p-value cut off of 0.05 or - log10(p-value) of 1.301 is represented by the horizontal dashed line. **B** denotes YY1 knockdown macrophages; **A** denotes control macrophages.

most significantly downregulated protein which validates our knockdown experiment. The global proteome changes, in YY1 knockdown THP-1 cells were also visualized in a volcano plot as shown in Fig 7C.

Gene ontology analysis of YY1 sensitive macrophage proteins

To further analyze YY1 sensitive macrophage proteins regarding their biological importance, all significantly modulated proteins were organized into three categories by GO terms: biological process, molecular function and cellular component (Tables **S4–S6** in File S1). However, the top 30 proteins upregulated or downregulated significantly by YY1 knockdown are presented in Table 2 and 3 along with their GO terms in three categories: biological process, molecular function and cellular component (Fig 8).

Regarding biological functions associated with altered proteins in YY1 knockdown cells, the highest number of proteins upregulated and downregulated by YY1 knockdown were associated with anatomical structural development, cell differentiation, and signaling (Fig 8A). A high number of proteins downregulated by YY1 knockdown were also seen involved in the regulation of DNA-templated transcription. Proteins affected by YY1 knockdown were also involved in programmed cell death (Fig 8A). In the case of molecular function, both upregulated and downregulated proteins were mostly involved in catalytic activity (Fig 8B). In the case of cellular localization, most of the proteins downregulated or upregulated were seen to be present in both the nucleus and cytoplasm (Fig 8C), but highest in nucleus as can be expected from YY1 being a transcriptional factor. In the case of upregulated proteins, a high number of proteins were also seen to be part of the plasma membrane.

It was intriguing to investigate the significance of YY1-dependent macrophage proteins beyond leishmaniasis, particularly in the context of infection and immunity. For this purpose, we reviewed the literature related to infection and immunity, primarily utilizing PubMed, Scopus, and Google Scholar biological databases. In this search, we excluded Covid-19. Of the 537 YY1-dependent proteins, 54 have previously been reported in infections and 50 in immunity-related studies. Interestingly, proteins that are upregulated by YY1 knockdown are more involved in infection and immunity (Table S3).

Discussion

In the current study, we identified YY1 as a novel virulence factor promoting *Leishmania* survival in our macrophage-cell model dTHP-1 and primary human monocyte derived macrophages. Thus depleting endogenous YY1 resulted in significantly reduced survival of *Leishmania* in infected cells. YY1 is a transcriptional factor ubiquitously expressed in humans and is known to have a fundamental role in several normal biological processes via its ability to initiate, activate, or repress transcription depending upon the context in which it binds [16,73,74]. Interestingly, some of the known functions of YY1 such as being antiapoptotic [75–77] and interaction with cell signaling proteins Akt [14] are relevant to the pathogenesis of *Leishmania* infection [15,78]. The above properties of YY1 were the basis of our hypothesis that *Leishmania* regulates host YY1 to promote its survival in infected host cells, leading us to initiate the current study. Based on this presumptive role of YY1 in *Leishmania* survival, we expected that the knockdown of YY1 in macrophages would impact *Leishmania* survival. In the present study, siRNAs against YY1 successfully knocked down expression of YY1 in both dTHP-1 and hMDMs, as confirmed by Western blot analysis (Figs 1A and 2B). Control cells and YY1 knockdown cells were infected for parasite rescue assay. Here, we observed a significant decrease in the survival of *Leishmania* as compared to infected control cells, demonstrating that YY1 plays an important role in *Leishmania* survival within host cells (Figs 1C and 2A).

In addition to YY1 presence in the nucleus, its localization in the cytoplasm has also been observed. The presence of YY1 in both the nucleus and the cytoplasm [17–20] suggests a role of YY1 other than as a transcription factor [74]. Although the transcriptional activities and gene regulatory functions of nuclear YY1 is well described, the information regarding the function of YY1 in the cytoplasm is very limited. The redirection of YY1 localization from the nucleus to the cytoplasm has been shown in BSC-40 cells and human blood adherent monocytes in response to vaccinia virus infection [21,22] suggesting that vaccinia virus has evolved to recruit cellular YY1 for viral gene expression. The virus mediated translocation of YY1 seems to be dependent on Crm1 transport system. In fact, the Crm1-dependent export system is

Table 2. Top 30 proteins upregulated by YY1 knockdown in dTHP-1 cells.

Protein description	Uniprot ID	Gene name	Fold change (Average log$_2$)	p-value	Adjusted p-value	Known roles related to pathogens	Known roles in Immunity
Leukocyte immunoglobulin-like receptor subfamily B member 2	Q8N423	LILRB2	1.21638239	0.013567783	0.013780261	Inhibitory receptor LILRB2 is targeted for immune evasion of *P. falciparum* [42] Infection with *Salmonella typhimurium* led to an upregulation of LILRB2 [43]	Transmit inhibitory signals in macrophages and other myeloid cells [44]
Interferon regulatory factor 9	Q00978	IRF9	1.046978197	0.04869277	0.048696462	NA	IRF9, a transcription factor, facilitates the expression of interferon-stimulated genes through JAK-STAT pathway [45]
5'-nucleotidase	P21589	NT5E	1.034390315	0.042954619	0.043389199	NA	NT5E performs numerous homeostatic functions in healthy organs and tissues [46]
Dynamin-3	Q9UQ16	DNM3	0.984937977	0.02642286	0.027560521	NA	NA
SLIT and NTRK-like protein 4	Q8IW52	SLITRK4	0.983317362	0.012409657	0.012569832	NA	NA
Fibronectin type-III domain-containing protein 3A	Q9Y2H6	FNDC3A	0.950278077	0.033766848	0.034450652	NA	NA
Procathepsin L	P07711	CTSL	0.918165432	0.028752389	0.030167598	Ctsl helps contain mycoplasma infection by supporting host immune response to infection [47]	NA
Protein sprouty homolog 4	Q9C004	SPRY4	0.90391386	0.015701084	0.016014898	NA	NA
Endonuclease G, mitochondrial	Q14249	ENDOG	0.89919109	0.014025527	0.014711359	*Leptospira interrogans* induces apoptosis in macrophages by mediating mitochondrial release of AIF and/or EndoG via Bid [48] Herpes simplex virus (HSV) uses host UL12.5 to deploy host cellular proteins, including ENDOG, to destroy mtDNA for their survival [49]	EndoG, a mitochondrion-specific nuclease is involved in caspase-independent apoptotic pathway [50]
Leucine-rich repeat-containing protein 14	Q15048	LRRC14	0.887431751	0.040263633	0.040689013	NA	LRRC14 is a negative regulator of TLR signaling, which is important for innate immunity [51]
Alpha-internexin	Q16352	INA	0.875746072	0.035352176	0.03594041	NA	NA
Cell surface hyaluronidase	Q9UHN6	CEMIP2	0.827603099	0.001443178	0.001489758	NA	NA
Signal peptidase complex catalytic subunit SEC11C	Q9BY50	SEC11C	0.806515055	0.024626403	0.025325885	NA	NA
Calcium-binding protein 39-like	Q9H9S4	CAB39L	0.796717849	0.01777717	0.017225326	NA	NA

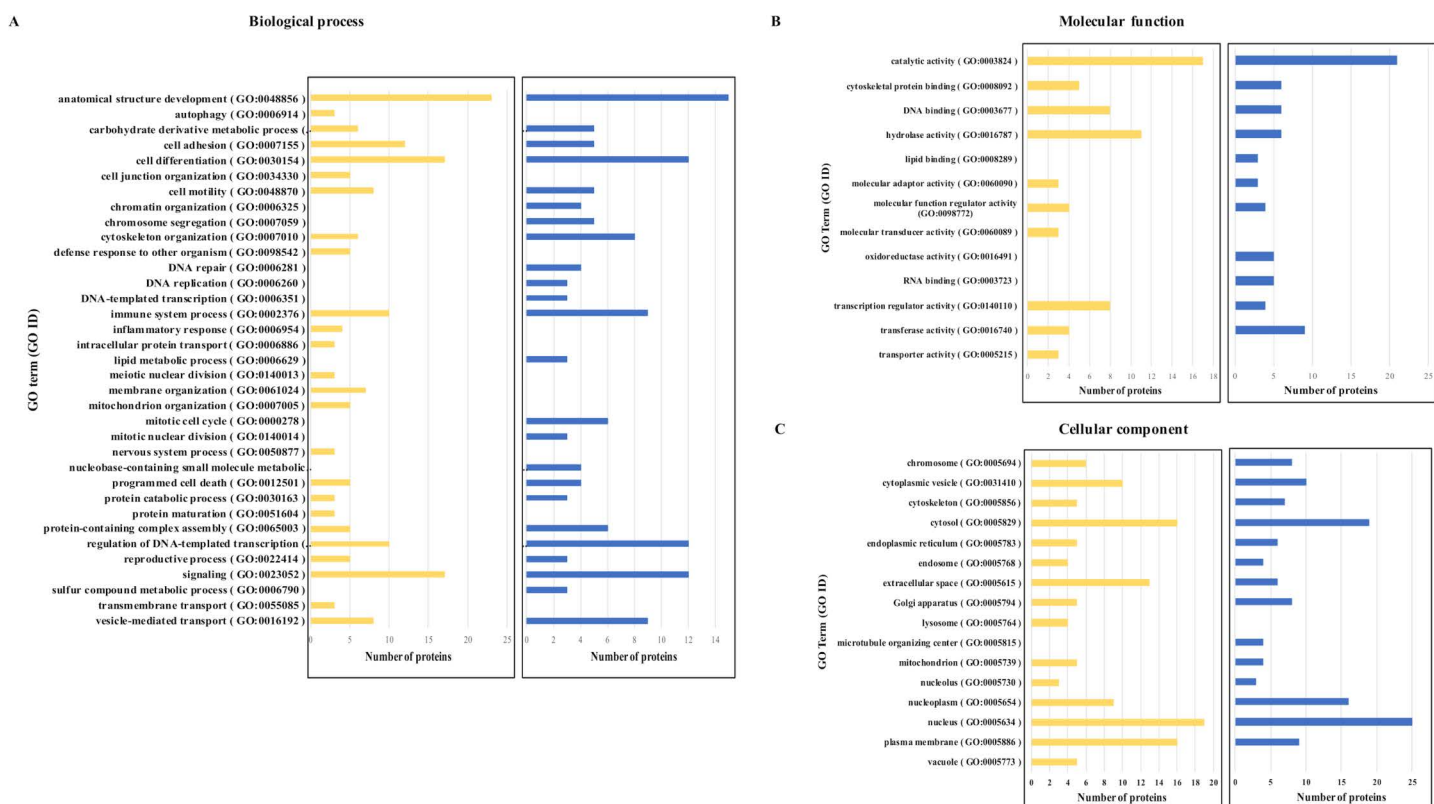*(Continued)*

Table 2. (Continued)

Protein description	Uniprot ID	Gene name	Fold change (Average log$_2$)	p-value	Adjusted p-value	Known roles related to pathogens	Known roles in Immunity
Lysyl oxidase homolog 4	Q96JB6	LOXL4	0.794796243	0.036592403	0.037057728	*C. parvum* RNA enters host nuclei upon infection and causes suppression of the cadherin 3 (CDH3) gene in human intestinal epithelial cells which is involved in cell migration and adherence [52]	NA
Macrophage scavenger receptor types I and II	P21757	MSR1	0.788720284	0.000893981	0.000931099	MSR1 was required for TLR3-mediated recognition of the extracellular HCV dsRNA [53]	Involved in M2 macrophage polarization; component of other pathogen pattern recognition receptor signaling complexes [54]
Src substrate cortactin	Q14247	CTTN	0.782302917	0.010545141	0.009962756	Cortactin tyrosine phosphorylation is important for invasion of *C. parvum* in biliary epithelial cells [55]	NA
Sentrin-specific protease 5	Q96HI0	SENP5	0.774620406	0.011258233	0.010707635	NA	NA
H2.0-like homeobox protein	Q14774	HLX	0.72500883	0.049567595	0.049627561	NA	Functions in supporting normal hematopoietic cell proliferation [56] Drives maturation of Th1 and IFN-γ secretion in cooperation with T-bet [57]
Bcl-2-like protein 11	O43521	BCL2L11	0.722370217	0.015296611	0.015735568	Deletion of BCL2L11 in *L. major* infected mice significantly increased CD4 + T-cell responses and were resistant to persistent infection [58]	Promotes intrinsic pathway of apoptosis [59]. Its absence promotes autoimmunity.
Transcription factor Sp1	P08047	SP1	0.718280004	0.037904517	0.038081937	Host SP1 was involved in maintaining transcription of HSV-1 genes in fibroblasts [60]. SP1-mediates transcriptional regulation of *TINAGL1* expression in gastric epithelial cells (GECs) during *H. pylori* infection, ensuring *H. pylori* persistence and gastritis [61].	Basal transcription factor, involved in regulation of housekeeping genes [62] SP1 is also known to bind YY1 transcription factor [63]. They both have binding sites on promoter regions of various genes [64].
Zinc finger protein 561	Q8N587	ZNF561	0.713728075	0.013418262	0.013687151	NA	NA
Osteopontin	P10451	SPP1	0.706695852	0.04288259	0.043296089	*H. pylori* infection in gastric cancer cells induces SPP1 activation, leading to T cell inactivation [65]	Identified as a cytokine (Eta-1) which is produced by activated T cells [66]
Thymidine phosphorylase	P19971	TYMP	0.699170573	0.035217122	0.0358473		Plays an important role in the pyrimidine salvage pathway [67]

(Continued)

Table 2. (Continued)

Protein description	Uniprot ID	Gene name	Fold change (Average log$_2$)	p-value	Adjusted p-value	Known roles related to pathogens	Known roles in Immunity
F-box only protein 6	Q9NRD1	FBXO6	0.675798441	0.048262757	0.048044693	Impairs the survival of alveolar macrophages and antiviral immunity of the host in Inflenza A virus infection[68] Modulates IFN-I-mediated antiviral immune responses [69]	F-box family of proteins are the key components of SKP1-Cullin1-F-box (SCF) E3 ligase and controls cell cycle, cell proliferation and cell death [69]
Metal cation symporter ZIP14	Q15043	SLC39A14	0.674353222	0.039646534	0.040223464	NA	NA
Protein spinster homolog 1	Q9H2V7	SPNS1	0.670739943	0.006613296	0.00679702	NA	NA
RUN and FYVE domain-containing protein 2	Q8WXA3	RUFY2	0.658920147	0.026968881	0.028026071	NA	NA
Squalene monooxygenase	Q14534	SQLE	0.649560533	0.026601845	0.027746741	NA	NA
N-acetylglucosamine-6-sulfatase	P15586	GNS	0.639485243	0.016125673	0.016108007	NA	NA

https://doi.org/10.1371/journal.pone.0323227.t003

Fig 8. Gene ontology analysis of significantly modulated proteins in YY1 knockdown dTHP-1 cells. Gene ontology (GO) enrichment analysis was performed on top 30 most significantly upregulated and downregulated proteins in YY1 knockdown dTHP-1 cells and classified into three categories: (A) Biological process, (B) Molecular function, and (C) Cellular component. Yellow represents the proteins upregulated by YY1 knockdown and blue represents the proteins downregulated by YY1 knockdown.

https://doi.org/10.1371/journal.pone.0323227.g008

Table 3. Top 30 proteins downregulated by YY1 knockdown in dTHP-1 cells.

Protein description	Uniprot ID	Gene name	Fold change (Average log$_2$)	p-value	Adjusted p-value	Known roles related to pathogens	Known roles in Immunity
Transcriptional repressor protein YY1	P25490	YY1	-2.267320196	0.021510917	0.022346369	YY1 binds to the promoter of TLR4, promoting its transcription, and aggravating the proinflammatory response of macrophages infected with MTB [70]	NA
AP-4 complex subunit sigma-1	Q9Y587	AP4S1	-1.408672747	0.01752392	0.016945996	NA	NA
Elongator complex protein 4	Q96EB1	ELP4	-1.220185205	0.008009702	0.007914339	NA	NA
Centrin-3	O15182	CETN3	-0.926879521	0.019029529	0.018528864	NA	NA
Microsomal glutathione S-transferase 1	P10620	MGST1	-0.921717382	0.020366426	0.020577281	NA	NA
Calcium/calmodulin-dependent protein kinase type II subunit beta	Q13554	CAMK2B	-0.796009973	0.03487625	0.03556797	NA	NA
Scinderin	Q9Y6U3	SCIN	-0.785144146	0.003042603	0.002979516	NA	NA
Coxsackievirus and adenovirus receptor	P78310	CXADR	-0.774148618	0.00041697	0.000372439	NA	The deletion of the Macrophage-Specific Coxsackievirus and Adenovirus Receptor in mice enhances M1 polarity and myocarditis in Coxsackievirus B3 infection [71]
Cytoplasmic protein NCK2	O43639	NCK2	-0.754010333	0.040158869	0.040595903	NA	NA
Carbohydrate-responsive element-binding protein	Q9NP71	MLXIPL	-0.728503229	0.027999148	0.029329609	NA	NA
Protein S100-A13	Q99584	S100A13	-0.725199313	0.001024552	0.001024209	NA	NA
Ribonucleoside-diphosphate reductase large subunit	P23921	RRM1	-0.717153352	0.024875109	0.025698324	NA	NA
Synaptotagmin-like protein 1	Q8IYJ3	SYTL1	-0.708352904	0.009257719	0.009031657	NA	Synaptotagmin-like protein 1 has a role in exocytosis of secretory lysosomes from Cytotoxic T cells [72]
CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 4	Q11206	ST3GAL4	-0.702765513	0.025815532	0.026815642	NA	NA
C-type mannose receptor 2	Q9UBG0	MRC2	-0.696165189	0.020003412	0.020018622	NA	NA
Tubulin polyglutamylase complex subunit 1	Q6ZTW0	TPGS1	-0.691959047	0.027312439	0.02867784	NA	NA
12S rRNA N4-methylcytidine (m4C) methyltransferase	A6NJ78	METTL15	-0.67807795	0.000561304	0.000558659	NA	NA

(Continued)

Table 3. (Continued)

Protein description	Uniprot ID	Gene name	Fold change (Average log$_2$)	p-value	Adjusted p-value	Known roles related to pathogens	Known roles in Immunity
VPS35 endosomal protein-sorting factor-like	Q7Z3J2	VPS35L	-0.664751721	0.036921255	0.037430168	NA	NA
Calcineurin B homologous protein 3	Q96BS2	TESC	-0.661603623	0.031456749	0.032588454	NA	NA
Vacuolar protein sorting-associated protein 26C	O14972	VPS26C	-0.653308776	0.018758228	0.018063315	NA	NA
Core histone macro-H2A.2	Q9P0M6	MACROH2A2	-0.641820049	0.024358876	0.024767225	NA	NA
Esterase OVCA2	Q8WZ82	OVCA2	-0.638708916	0.037142379	0.037616387	NA	NA
Thiamin pyrophosphokinase 1	Q9H3S4	TPK1	-0.635451369	0.030548762	0.031843575	NA	NA
Mitotic spindle assembly checkpoint protein MAD2A	Q13257	MAD2L1	-0.635171686	0.014560012	0.015176909	NA	NA
60S ribosomal protein L35	P42766	RPL35	-0.622559748	0.026383389	0.027374302	NA	NA
Golgin subfamily A member 8O	F8WBI6; A6NCC3; H3BV12; I6L899	GOLGA8N;GOLGA8O;GOLGA8Q;GOLGA8R	-0.614271427	0.043977852	0.044227188	NA	NA
Leucine-rich repeat-containing protein 58	Q96CX6	LRRC58	-0.612591557	0.044858458	0.044972067	NA	NA
5'-nucleotidase domain-containing protein 1	Q5TFE4	NT5DC1	-0.608545779	0.041379186	0.041806331	NA	NA
Proteasome assembly chaperone 2	Q969U7	PSMG2	-0.603988015	0.021100345	0.02150838	NA	NA
Neuroserpin	Q99574	SERPINI1	-0.595052875	0.001725209	0.001582868	NA	NA

https://doi.org/10.1371/journal.pone.0323227.t002

used by many viral proteins: HIV-1 Rev protein, adenovirus E4 34 kDa protein and hepatitis B virus proteins [79]. Our result presented in this study shows that translocation of nuclear YY1 to the cytoplasm seems to be dependent on the Crm1 export system [22] since leptomycin B treatment greatly diminished the level of cytoplasmic YY1. As shown in Fig 3, *Leishmania*, like vaccinia virus, induced redirection of YY1 from the nucleus to the cytoplasm of infected macrophages using a Crm1-dependent export system. As expected, pre-treatment of cells with leptomycin B decreased survival of *Leishmania* in infected cells, strongly suggesting that transport of YY1 to the cytoplasm could be required for the optimal survival of *Leishmania.*

At present, we do not know the function of cytoplasmic YY1 in *Leishmania* infected cells. However, decreased survival of *Leishmania* found upon leptomycin B treatment suggests potential role of cytoplasmic YY1 in *Leishmania* survival. Nevertheless, our result suggests that in human macrophages infected with *Leishmania*, the Crm1 system is involved in YY1 transport to the cytoplasm. Identification of YY1 interacting factors in the cytoplasmic fraction of infected macrophages has potential to shed light on the cytoplasmic function of YY1 in pathogenesis of *Leishmania* infection. Perhaps, this translocation is required to induce interaction of YY1 to host factors to create a pro-parasitic environment suitable for *Leishmania* survival

To further elucidate the role of YY1 in *Leishmania* persistence, we performed comparative proteomic analysis of *Leishmania* infected YY1 knockdown cells. This analysis revealed differentially expressed proteins during *Leishmania* infection that are dependent on YY1. Interestingly, none of these YY1 dependent proteins has been implicated in *Leishmania* infection -related processes in previously published studies. However, further investigation of *Leishmania*-modulated YY1 dependent proteins is highly desirable. GO terms analysis of *Leishmania* upregulated YY1 dependent proteins revealed top GO terms for biological process as signaling (GO:0023052), cell differentiation (GO:0030154) anatomical structure development (GO:0048856). GO terms analysis of *Leishmania* downregulated YY1 dependent proteins revealed top GO terms for biological process as cell differentiation (GO:0030154) and protein-containing complex assembly (GO:0065003).

Given that YY1 knockdown results in broad effects on the host proteomic profile (approximately 5% of total identified proteins were affected), it was of interest to classify these YY1 sensitive proteins by GO terms in three categories: biological process, molecular function and cellular components. The top 10 GO terms for proteins that are upregulated by YY1 knockdown in the biological process category include anatomical structure development (GO:0048856), signaling (GO:0023052), cell differentiation (GO:0030154), regulation of DNA-templated transcription (GO:0006355), immune system process (GO:0002376), vesicle-mediated transport (GO:0016192), programmed cell death (GO:0012501), cell adhesion (GO:0007155), transmembrane transport (GO:0055085), protein-containing complex assembly (GO:0065003), and cytoskeleton organization (GO:0007010). This was not surprising as YY1 is known to participate in various biological functions including apoptosis, cell prolifertion, differentiation, replication and embryogenesis [7,74]. Our analysis also showed that top 10 GO terms in biological process category related to YY1 dependent downregulated proteins are anatomical structure development (GO:0048856), signaling (GO:0023052), cell differentiation (GO:0030154) regulation of DNA-templated transcription (GO:0006355), vesicle-mediated transport (GO:0016192), cytoskeleton organization (GO:0007010), mitotic cell cycle (GO:0000278), protein-containing complex assembly (GO:0065003), and programmed cell death (GO:0012501).

Additionally, we analyzed the possible role of YY1 modulated proteins in infections other than leishmaniasis and in immunity. Our literature search relevant to infection and immunity revealed that out of 291 upregulated proteins, 43 have been implicated in infections, and 41 in immunity. Furthermore, of the 246 downregulated proteins, 11 are previously known to be associated with infection and 9 with immunity (Table S3). Together, the quantitative proteomic analysis of YY1 knockdown cells revealed that a large number of YY1 sensitive proteins are related to infection and immunity based on published reports. How many of the remaining YY1 sensitive macrophage proteins are related to infection and imunity remains to be investigated. We do note the limitations of our proteomic study, specifically the use of a single monocytic cell line, THP-1, and the absence of a broader and more extensive evaluation of the proteome data. Moreover, we understand that our data is limited to proteomic analysis, and it will be of interest to support these findings with relevant biological assays, warranting future studies. Nevertheless, proteomic data presented here can be a valuable resource for other researchers interested in YY1 functions and its role in infection and immunity.

In summary, the present study demonstrates that macrophage YY1 is required for the optimal survival of *Leishmania* in infected cells. It was also observed that *Leishmania* redirected nuclear YY1 to the cytoplasm during infection. Perhaps, this translocation is required to induce interaction with cytoplasmic factor to create a pro-parasitic environment suitable for *Leishmania* survival. Taken together, we have identified YY1 as a novel and essential protein that promotes *Leishmania* survival. To our knowledge, this is the first report showing the role of host YY1 in the pathogenesis of an intracellular parasitic infection and may have implications for other intracellular pathogens. In addition, to our knowledge, this is the first report providing global landscape of proteome-wide alterations that occur in human macrophages after knockdown of YY1. PMA-differentiated THP-1 cells have been extensively used as a model system to study macrophage-pathogen interactions involving intracellular pathogens such as *Leishmania* [35,36,80], *Mycobacterium tuberculosis* [81,82], *Toxoplasma gondii* [83] and HIV [84,85]. THP-1 has also been used as macrophage-cell model to study monocyte/macrophage functions [37,86]. Thus, YY1 sensitive proteome analysis of dTHP-1 is expected to provide valuable information regarding biological functions of human YY1 at least in macrophages.

Supporting information

Fig S1. *L. donovani* infection does not alter YY1 protein abundance in dTHP-1 cells. A) dTHP-1 cells were infected with *L. donovani* at a 10:1 or 20:1 MOI for 4, 10, 24, or 48 hours. The whole cell lysates from non-infected and *L. donovani*-infected cells were Western blotted for the indicated antibodies. B) Histogram of the densitometric analysis of whole cell lysates in Western blots. Bars represent the mean ± SD of three independent experiments. Statistical significance was determined using two-sample two tailed T-test; h.p.i refers to hours post-infection.
(TIF)

Fig S2. Lamin A/C are not detected in cytoplasmic fractions of non-infected and *L. donovani* infected dTHP-1 cells. A) The cytoplasmic fraction of non-infected and *Leishmania*-infected dTHP-1 cells and the nuclear fraction of non-infected dTHP-1 cells were analyzed by Western blot for the nuclear marker Lamin A/C (n = 3).
(TIFF)

Table S1. Proteins significantly modulated by *L. donovani* infection in dTHP-1 cells.
(XLSX)

Table S2. Proteins significantly modulated by *L. donovani* and recovered by YY1 knockdown in dTHP-1 cells.
(XLSX)

Table S3. Proteins significantly modulated by YY1 knockdown in dTHP-1 cells.
(XLSX)

File S1. Tables S4-S6. Gene ontology analysis of significantly modulated proteins in YY1 knockdown dTHP-1 cells. Table S4 represents biological process, Table S5 represents molecular function, and Table S6 represents cellular component.
(DOCX)

S1 File. Raw images.
(PDF)

Author contributions

Conceptualization: Neil Reiner, Devki Nandan.

Data curation: Dilraj Kaur Longowal, Kyung-Mee Moon.

Formal analysis: Harsimran Kaur Brar, Eleanor Chen, Shawna Angel Lu, Dilraj Kaur Longowal, Kyung-Mee Moon.

Funding acquisition: Leonard J Foster, Neil Reiner.

Investigation: Harsimran Kaur Brar, Eleanor Chen, Fabian Chang, Shawna Angel Lu, Devki Nandan.

Methodology: Eleanor Chen, Fabian Chang, Dilraj Kaur Longowal, Kyung-Mee Moon, Leonard J Foster.

Project administration: Neil Reiner, Devki Nandan.

Software: Kyung-Mee Moon, Leonard J Foster.

Supervision: Leonard J Foster, Neil Reiner, Devki Nandan.

Writing – original draft: Harsimran Kaur Brar.

References

1. Maxfield L, Crane JS. Leishmaniasis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [cited 2022 Jul 27]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK531456/

2. Steverding D. The history of leishmaniasis. Parasit Vectors. 2017;10(1):82. https://doi.org/10.1186/s13071-017-2028-5 PMID: 28202044

3. Serafim TD, Coutinho-Abreu IV, Dey R, Kissinger R, Valenzuela JG, Oliveira F, et al. Leishmaniasis: the act of transmission. Trends Parasitol. 2021;37(11):976–87. https://doi.org/10.1016/j.pt.2021.07.003 PMID: 34389215

4. Leishmaniasis [Internet]. [cited 2023 Oct 30]. Available from: https://www.who.int/news-room/fact-sheets/detail/leishmaniasis

5. Velez R, Gállego M. Commercially approved vaccines for canine leishmaniosis: a review of available data on their safety and efficacy. Trop Med Int Health. 2020;25(5):540–57. https://doi.org/10.1111/tmi.13382 PMID: 32034985

6. Dinc R. Leishmania vaccines: the current situation with its promising aspect for the future. Korean J Parasitol. 2022;60(6):379–91. https://doi.org/10.3347/kjp.2022.60.6.379 PMID: 36588414

7. Gordon S, Akopyan G, Garban H, Bonavida B. Transcription factor YY1: structure, function, and therapeutic implications in cancer biology. Oncogene. 2006;25(8):1125–42. https://doi.org/10.1038/sj.onc.1209080 PMID: 16314846

8. Khachigian LM. The Yin and Yang of YY1 in tumor growth and suppression. Int J Cancer. 2018;143(3):460–5. https://doi.org/10.1002/ijc.31255 PMID: 29322514

9. Chaparro V, Graber TE, Alain T, Jaramillo M. Transcriptional profiling of macrophages reveals distinct parasite stage-driven signatures during early infection by Leishmania donovani. Sci Rep. 2022;12(1):6369. https://doi.org/10.1038/s41598-022-10317-6 PMID: 35430587

10. Li H, Yang B, Huang J, Lin Y, Xiang T, Wan J. Cyclooxygenase-2 in tumor-associated macrophages promotes breast cancer cell survival by triggering a positive-feedback loop between macrophages and cancer cells. Oncotarget. 2015;6(30):29637–50.

11. Na Y-R, Yoon Y-N, Son D-I, Seok S-H. Cyclooxygenase-2 inhibition blocks M2 macrophage differentiation and suppresses metastasis in murine breast cancer model. PLoS One. 2013;8(5):e63451. https://doi.org/10.1371/journal.pone.0063451 PMID: 23667623

12. Tomiotto-Pellissier F, Bortoleti B da S, Assolini J, Gonçalves M, Carloto A, Miranda-Sapla M. Macrophage polarization in leishmaniasis: broadening horizons. Front Immunol. 2018;9:2529.

13. Emerson LE, Gioseffi A, Barker H, Sheppe A, Morrill JK, Edelmann MJ, et al. Leishmania infection-derived extracellular vesicles drive transcription of genes involved in M2 polarization. Front Cell Infect Microbiol. 2022;12:934611. https://doi.org/10.3389/fcimb.2022.934611 PMID: 36093197

14. Zhang Q, Wan M, Shi J, Horita DA, Miller LD, Kute TE, et al. Yin Yang 1 promotes mTORC2-mediated AKT phosphorylation. J Mol Cell Biol. 2016;8(3):232–43. https://doi.org/10.1093/jmcb/mjw002 PMID: 26762111

15. Thomas SA, Nandan D, Kass J, Reiner NE. Countervailing, time-dependent effects on host autophagy promotes intracellular survival of leishmania. J Biol Chem. 2018;293(7):2617–30.

16. Shi Y, Seto E, Chang LS, Shenk T. Transcriptional repression by YY1, a human GLI-Krüppel-related protein, and relief of repression by adenovirus E1A protein. Cell. 1991;67(2):377–88. https://doi.org/10.1016/0092-8674(91)90189-6 PMID: 1655281

17. Palko L, Bass H, Beyrouthy M, Hurt M. The yin yang-1 (yy1) protein undergoes a dna-replication-associated switch in localization from the cytoplasm to the nucleus at the onset of s phase. J Cell Sci. 2004;117(3):465–76.

18. Rizkallah R, Hurt MM. Regulation of the transcription factor YY1 in mitosis through phosphorylation of its DNA-binding domain. Mol Biol Cell. 2009;20(22):4766–76. https://doi.org/10.1091/mbc.e09-04-0264 PMID: 19793915

19. de Nigris F, Crudele V, Giovane A, Casamassimi A, Giordano A, Garban HJ, et al. CXCR4/YY1 inhibition impairs VEGF network and angiogenesis during malignancy. Proc Natl Acad Sci U S A. 2010;107(32):14484–9. https://doi.org/10.1073/pnas.1008256107 PMID: 20660740

20. Wan M, Huang W, Kute T, Miller L, Zhang Q, Hatcher H. Yin yang 1 plays an essential role in breast cancer and negatively regulates p27. Am J Pathol. 2012;180(5):2120–33.

21. Broyles SS, Liu X, Zhu M, Kremer M. Transcription factor YY1 is a vaccinia virus late promoter activator. J Biol Chem. 1999;274(50):35662–7.

22. Slezak K, Michalik M, Kowalczyk A, Rokita H. YY1 is recruited to the cytoplasm of vaccinia virus-infected human macrophages by the Crm1 system. Virus Res. 2004;102(2):177–84. https://doi.org/10.1016/j.virusres.2004.01.028 PMID: 15084399

23. Reiner NE. Host-parasite relationship in murine leishmaniasis: pathophysiological and immunological changes. Infect Immun. 1982;38(3):1223–30. https://doi.org/10.1128/iai.38.3.1223-1230.1982 PMID: 7152667

24. Moradimotlagh A, Chen S, Koohbor S, Moon K-M, Foster LJ, Reiner N, et al. Leishmania infection upregulates and engages host macrophage Argonaute 1, and system-wide proteomics reveals Argonaute 1-dependent host response. Front Immunol. 2023;14:1287539. https://doi.org/10.3389/fimmu.2023.1287539 PMID: 38098491

25. Colineau L, Lambertz U, Fornes O, Wasserman W, Reiner N. c-myc is a novel leishmania virulence factor by proxy that targets the host mirna system and is essential for survival in human macrophages. J Biol Chem. 2018;293(33):12805–19.

26. Jain S, Sahu R, Walker L, Tekwani B. A parasite rescue and transformation assay for antileishmanial screening against intracellular leishmania donovani amastigotes in thp1 human acute monocytic leukemia cell line. J Vis Exp. 2012;70:4054.

27. Holden P, Horton WA. Crude subcellular fractionation of cultured mammalian cell lines. BMC Res Notes. 2009;2:243. https://doi.org/10.1186/1756-0500-2-243 PMID: 20003239

28. Beck S, Michalski A, Raether O, Lubeck M, Kaspar S, Goedecke N, et al. The Impact II, a Very High-Resolution Quadrupole Time-of-Flight Instrument (QTOF) for Deep Shotgun Proteomics. Mol Cell Proteomics. 2015;14(7):2014–29. https://doi.org/10.1074/mcp.M114.047407 PMID: 25991688

29. Ishihama Y, Rappsilber J, Andersen J, Mann M. Microcolumns with self-assembled particle frits for proteomics. J Chromatogr A. 2002;979(1–2):233–9.

30. MacCarrone M, De Felici M, Bari M, Klinger F, Siracusa G, Finazzi-Agrò A. Down-regulation of anandamide hydrolase in mouse uterus by sex hormones. Eur J Biochem. 2000;267(10):2991–7.

31. Demichev V, Messner CB, Vernardis SI, Lilley KS, Ralser M. DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. Nat Methods. 2020;17(1):41–4. https://doi.org/10.1038/s41592-019-0638-x PMID: 31768060

32. Perez-Riverol Y, Bai J, Bandla C, García-Seisdedos D, Hewapathirana S, Kamatchinathan S. The pride database resources in 2022: a hub for mass spectrometry-based proteomics evidences. Nucleic Acids Res. 2022;50(D1):D543-52.

33. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Methodol. 1995;57(1):289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

34. Negrão F, Fernandez-Costa C, Zorgi N, Giorgio S, Nogueira Eberlin M, Yates J. Label-free proteomic analysis reveals parasite-specific protein alterations in macrophages following Leishmania amazonensis, Leishmania major, or Leishmania infantum infection. ACS Infect Dis. 2019;5(6):851–62.

35. Perea-Martínez A, García-Hernández R, Manzano JI, Gamarro F. Transcriptomic analysis in human macrophages infected with therapeutic failure clinical isolates of Leishmania INFANTUM. ACS Infect Dis. 2022;8(4):800–10. https://doi.org/10.1021/acsinfecdis.1c00513 PMID: 35352952

36. Singh AK, Pandey RK, Siqueira-Neto JL, Kwon Y-J, Freitas-Junior LH, Shaha C, et al. Proteomic-based approach to gain insight into reprogramming of THP-1 cells exposed to Leishmania donovani over an early temporal window. Infect Immun. 2015;83(5):1853–68. https://doi.org/10.1128/IAI.02833-14 PMID: 25690103

37. Chanput W, Mes JJ, Wichers HJ. THP-1 cell line: an in vitro cell model for immune modulation approach. Int Immunopharmacol. 2014;23(1):37–45. https://doi.org/10.1016/j.intimp.2014.08.002 PMID: 25130606

38. Nandan D, Chen E, Chang F, Moon K-M, Foster LJ, Reiner N. Comprehensive proteomic analysis of autophagosomes derived from Leishmania-infected macrophages. PLoS One. 2023;18(4):e0284026. https://doi.org/10.1371/journal.pone.0284026 PMID: 37027358

39. Azmi AS, Uddin MH, Mohammad RM. The nuclear export protein XPO1 - from biology to targeted therapy. Nat Rev Clin Oncol. 2021;18(3):152–69. https://doi.org/10.1038/s41571-020-00442-4 PMID: 33173198

40. Sun Q, Carrasco Y, Hu Y, Guo X, Mirzaei H, Macmillan J. Nuclear export inhibition through covalent conjugation and hydrolysis of leptomycin b by crm1. Proc Natl Acad Sci U S A. 2013;110(4):1303–8.

41. Sarvagalla S, Kolapalli SP, Vallabhapurapu S. The two sides of YY1 in cancer: a friend and a Foe. Front Oncol. 2019;9:1230. https://doi.org/10.3389/fonc.2019.01230 PMID: 31824839

42. Sakoguchi A, Saito F, Hirayasu K, Shida K, Matsuoka S, Itagaki S. Plasmodium falciparum rifin is a novel ligand for inhibitory immune receptor lilrb2. Biochem Biophys Res Commun. 2021;548:167–73.

43. Brown DP, Jones DC, Anderson KJ, Lapaque N, Buerki RA, Trowsdale J. The inhibitory receptor LILRB4 (ILT3) modulates antigen presenting cell phenotype and, along with LILRB2 (ILT4), is upregulated in response to salmonella infection. BMC Immunol. 2009;10:56.

44. Umiker B, Hashambhoy-Ramsay Y, Smith J, Rahman T, Mueller A, Davidson R. Inhibition of LILRB2 by a novel blocking antibody designed to reprogram immunosuppressive macrophages to drive T-cell activation in tumors. Mol Cancer Ther. 2023;22(4):471–84.

45. Paul A, Ismail MN, Tang TH, Ng SK. Phosphorylation of interferon regulatory factor 9 (IRF9). Mol Biol Rep. 2023;50(4):3909–17. https://doi.org/10.1007/s11033-023-08253-3 PMID: 36662450

46. Kordaß T, Osen W, Eichmüller SB. Controlling the immune suppressor: transcription factors and MicroRNAs regulating CD73/NT5E. Front Immunol. 2018;9:813. https://doi.org/10.3389/fimmu.2018.00813 PMID: 29720980

47. Xu X, Greenland J, Baluk P, Adams A, Bose O, McDonald DM, et al. Cathepsin L protects mice from mycoplasmal infection and is essential for airway lymphangiogenesis. Am J Respir Cell Mol Biol. 2013;49(3):437–44. https://doi.org/10.1165/rcmb.2013-0016OC PMID: 23600672

48. Hu W-L, Dong H-Y, Li Y, Ojcius DM, Li S-J, Yan J. Bid-Induced Release of AIF/EndoG from Mitochondria Causes Apoptosis of Macrophages during Infection with Leptospira interrogans. Front Cell Infect Microbiol. 2017;7:471. https://doi.org/10.3389/fcimb.2017.00471 PMID: 29184851

49. Duguay BA, Smiley JR. Mitochondrial nucleases ENDOG and EXOG participate in mitochondrial DNA depletion initiated by herpes simplex virus 1 UL12.5. J Virol. 2013;87(21):11787–97. https://doi.org/10.1128/JVI.02306-13 PMID: 23986585

50. Azami M, Ranjkesh Adermanabadi V, Khanahmad H, Mohaghegh MA, Zaherinejad E, Aghaei M, et al. Immunology and genetic of Leishmania infantum: the role of endonuclease G in the apoptosis. J Res Med Sci Off J Isfahan Univ Med Sci. 2018;23:36.

51. Wu C, Yang Y, Ou J, Zhu L, Zhao W, Cui J. Lrrc14 attenuates toll-like receptor-mediated nf-κb signaling through disruption of ikk complex. Exp Cell Res. 2016;347(1):65–73.

52. Ming Z, Gong A, Wang Y, Zhang X, Li M, Li Y. Trans-suppression of host cdh3 and loxl4 genes during cryptosporidium parvum infection involves nuclear delivery of parasite cdg7_flc_1000 RNA. Int J Parasitol. 2018;48(6):423–31.

53. Dansako H, Kato N. Recognition mechanism of hepatitis C viral infection. Nihon Rinsho. 2015;73(2):229–33. PMID: 25764675

54. Yang D, Lin T, Li C, Harrison AG, Geng T, Wang P. A critical role for MSR1 in vesicular stomatitis virus infection of the central nervous system. iScience. 2021;24(6):102678. https://doi.org/10.1016/j.isci.2021.102678 PMID: 34169243

55. Chen X-M, Huang BQ, Splinter PL, Cao H, Zhu G, McNiven MA, et al. Cryptosporidium parvum invasion of biliary epithelia requires host cell tyrosine phosphorylation of cortactin via c-Src. Gastroenterology. 2003;125(1):216–28. https://doi.org/10.1016/s0016-5085(03)00662-0 PMID: 12851885

56. Zhu X-Y, Guo Q-Y, Zhu M, Chen B-G, Wang L-Y, Zhang D-Q, et al. HLX affects cell cycle and proliferation in AML cells via the JAK/STAT signaling pathway. Oncol Lett. 2020;20(2):1888–96. https://doi.org/10.3892/ol.2020.11718 PMID: 32724432

57. Shotorbani S, He Z, Yang H, Sun Q, Xu Y, Su Z. Over-expression of hlx homeobox gene in dc2.4 dendritic cell enhances its maturation and antigen presentation. Cell Immunol. 2012;275(1–2):61–8.

58. Reckling S, Divanovic S, Karp CL, Wojciechowski S, Belkaid Y, Hildeman D. Proapoptotic Bcl-2 family member Bim promotes persistent infection and limits protective immunity. Infect Immun. 2008;76(3):1179–85. https://doi.org/10.1128/IAI.01093-06 PMID: 18086806

59. Sionov RV, Vlahopoulos SA, Granot Z. Regulation of bim in health and disease. Oncotarget. 2015;6(27):23058–134.

60. Sodroski CN, Oh HS, Chou S-F, Knipe DM. Sp1 facilitates continued HSV-1 gene expression in the absence of key viral transactivators. mBio. 2024;15(3):e0347923. https://doi.org/10.1128/mbio.03479-23 PMID: 38349188

61. Teng Y, Xie R, Xu J, Wang P, Chen W, Shan Z, et al. Tubulointerstitial nephritis antigen-like 1 is a novel matricellular protein that promotes gastric bacterial colonization and gastritis in the setting of Helicobacter pylori infection. Cell Mol Immunol. 2023;20(8):924–40. https://doi.org/10.1038/s41423-023-01055-4 PMID: 37336990

62. Beishline K, Azizkhan-Clifford J. Sp1 and the "hallmarks of cancer". FEBS J. 2015;282(2):224–58. https://doi.org/10.1111/febs.13148 PMID: 25393971

63. Li H, Liu H, Wang Z, Liu X, Guo L, Huang L. The role of transcription factors Sp1 and YY1 in proximal promoter region in initiation of transcription of the mu opioid receptor gene in human lymphocytes. J Cell Biochem. 2008;104(1):237–50.

64. Bai J, Chen D-B. Enhanced Sp1/YY1 expression directs CBS transcription to mediate VEGF-stimulated pregnancy-dependent H2S production in human uterine artery endothelial cells. Hypertension. 2021;78(6):1902–13. https://doi.org/10.1161/HYPERTENSIONAHA.121.18190 PMID: 34657441

65. Song H, Yao X, Zheng Y, Zhou L. Helicobacter pylori infection induces POU5F1 upregulation and SPP1 activation to promote chemoresistance and T cell inactivation in gastric cancer cells. Biochem Pharmacol. 2024;225:116253. https://doi.org/10.1016/j.bcp.2024.116253 PMID: 38701869

66. Wang KX, Denhardt DT. Osteopontin: role in immune regulation and stress responses. Cytokine Growth Factor Rev. 2008;19(5–6):333–45. https://doi.org/10.1016/j.cytogfr.2008.08.001 PMID: 18952487

67. Li W, Yue H. Thymidine phosphorylase is increased in COVID-19 patients in an acuity-dependent manner. Front Med. 2021;8:653773.

68. Cen M, Ouyang W, Lin X, Du X, Hu H, Lu H, et al. FBXO6 regulates the antiviral immune responses via mediating alveolar macrophages survival. J Med Virol. 2023;95(1):e28203. https://doi.org/10.1002/jmv.28203 PMID: 36217277

69. Du X, Meng F, Peng D, Wang Z, Ouyang W, Han Y. Noncanonical role of fbxo6 in regulating antiviral immunity. J Immunol. 2019;203(4):1012–20.

70. Yang X, Chen Y, Pu B, Yuan X, Wang J, Chen C. Yy1 contributes to the inflammatory responses of mycobacterium tuberculosis-infected macrophages through transcription activation-mediated upregulation tlr4. Mol Biotechnol. 2025;67(2):778–89.

71. Shin H, Jeon E, Lim B. Macrophage-specific coxsackievirus and adenovirus receptor deletion enhances macrophage M1 polarity in CVB3-induced myocarditis. Int J Mol Sci. 2023;24(6):5309.

72. Holt O, Kanno E, Bossi G, Booth S, Daniele T, Santoro A, et al. Slp1 and Slp2-a localize to the plasma membrane of CTL and contribute to secretion from the immunological synapse. Traffic. 2008;9(4):446–57. https://doi.org/10.1111/j.1600-0854.2008.00714.x PMID: 18266782

73. Hervieu GJ. Further insights into the neurobiology of melanin-concentrating hormone in energy and mood balances. Expert Opin Ther Targets. 2006;10(2):211–29. https://doi.org/10.1517/14728222.10.2.211 PMID: 16548771

74. Verheul TCJ, van Hijfte L, Perenthaler E, Barakat TS. The why of YY1: mechanisms of transcriptional regulation by Yin Yang 1. Front Cell Dev Biol. 2020;8:592164. https://doi.org/10.3389/fcell.2020.592164 PMID: 33102493

75. Sui G, Affar E, Shi Y, Brignone C, Wall N, Yin P. Yin yang 1 is a negative regulator of p53. Cell. 2004;117(7):859–72.

76. Feng Y, Wang X, Xu L, Pan H, Zhu S, Liang Q, et al. The transcription factor ZBP-89 suppresses p16 expression through a histone modification mechanism to affect cell senescence. FEBS J. 2009;276(15):4197–206. https://doi.org/10.1111/j.1742-4658.2009.07128.x PMID: 19583777

77. Noguera N, Travaglini S, Scalea S, Catalanotto C, Reale A, Zampieri M. Yy1 knockdown relieves the differentiation block and restores apoptosis in aml cells. Cancers. 2023;15(15):4010.

78. Bhardwaj N, Rosas LE, Lafuse WP, Satoskar AR. Leishmania inhibits STAT1-mediated IFN-gamma signaling in macrophages: increased tyrosine phosphorylation of dominant negative STAT1beta by Leishmania mexicana. Int J Parasitol. 2005;35(1):75–82. https://doi.org/10.1016/j.ijpara.2004.10.018 PMID: 15619518

79. Forgues M, Marrogi AJ, Spillare EA, Wu CG, Yang Q, Yoshida M, et al. Interaction of the hepatitis B virus X protein with the Crm1-dependent nuclear export pathway. J Biol Chem. 2001;276(25):22797–803. https://doi.org/10.1074/jbc.M101259200 PMID: 11287420

80. Brar HK, Roy G, Kanojia A, Madan E, Madhubala R, Muthuswami R. Chromatin-remodeling factor BRG1 is a negative modulator of L. donovani in IFNγ stimulated and infected THP-1 cells. Front Cell Infect Microbiol. 2022;12:860058. https://doi.org/10.3389/fcimb.2022.860058 PMID: 35433496

81. Rankine-Wilson L, Rens C, Sahile H, Av-Gay Y. Mycobacterium tuberculosis infection of THP-1 cells: a model for high content analysis of intracellular growth and drug susceptibility. Methods Mol Biol. 2022;2427:73–82.

82. Theus SA, Cave MD, Eisenach KD. Activated THP-1 cells: an attractive model for the assessment of intracellular growth rates of Mycobacterium tuberculosis isolates. Infect Immun. 2004;72(2):1169–73. https://doi.org/10.1128/IAI.72.2.1169-1173.2004 PMID: 14742569

83. Pandori WJ, Lima TS, Mallya S, Kao TH, Gov L, Lodoen MB. Toxoplasma gondii activates a Syk-CARD9-NF-κB signaling axis and gasdermin D-independent release of IL-1β during infection of primary human monocytes. PLoS Pathog. 2019;15(8):e1007923. https://doi.org/10.1371/journal.ppat.1007923 PMID: 31449558

84. Kisaka JK, Rauch D, Griffith M, Kyei GB. A macrophage-cell model of HIV latency reveals the unusual importance of the bromodomain axis. Virol J. 2024;21(1):80.

85. Guo Y, Xu W-W, Song J, Deng W, Liu D-Q, Zhang H-T. Intracellular overexpression of HIV-1 Nef impairs differentiation and maturation of monocytic precursors towards dendritic cells. PLoS One. 2012;7(7):e40179. https://doi.org/10.1371/journal.pone.0040179 PMID: 22808111

86. Bosshart H, Heinzelmann M. THP-1 cells as a model for human monocytes. Ann Transl Med. 2016;4(21):438. https://doi.org/10.21037/atm.2016.08.53 PMID: 27942529