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ORIGINAL ARTICLE

Comparative Analysis of Host-Pathogen Protein-Protein Interactions between Human and Various Strains of *Mycobacterium Tuberculosis*

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SUMMARY

Background: **Tuberculosis remains a substantial health threat globally, despite decades having elapsed since the identification of its causative agent, *Mycobacterium tuberculosis.* Approximately 35% of the global population is sub-clinically infected, leading as one of the primary causes of human mortality. The increased prevalence of drug-resistant strains of Mtb necessitates identification of important drug targets. Therefore, the aim of the study was to comparatively analyze the protein-protein interactions between the host and the pathogen (*Mycobacterium tuberculosis*) to uncover the conserved molecular mechanisms of infection, providing insight into strain-specific variations.**

Methods: **One of the major problems is the diverse spectrum of diseases caused by different Mtb. To date, most research has their attention on a specific pathogenic strain. Therefore, to screen common and effective drug targets of different strains, we compared the protein-protein interactions of four virulent strains (H37Rv, CDC1551, CAS/NITR204, and Erdman) and one a virulent strain (H37Ra) of Mtb with its human host. Here, the interolog method was adopted to identify the biomolecular-interactions between Mtb and its human host.**

Results: **As a result, an interaction network has been developed, and the target has been screened through multiple parameters, such as the highest interacting partners, virulent factors, subcellular localization, and predicted protein interactions.**

Conclusions: **This study substantially resulted in the identification of potential drug targets, ATP synthase subunit alpha and gamma, and chaperone proteins DNAK and HTPG.**

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Supplementary Data

The details of the method for comparative host-pathogen interactions (HPIs) between the human and the infectious proteins of different strains of *Mycobacterium tuberculosis*

Mycobacterium tuberculosis (*Mtb*) is a clever pathogen. It adopts several strategies for its survival and pathogenicity within the host. The study of molecular interaction helps in deciphering the proteins which play an essential role in establishing a disease. The amount of experimentally validated data about host-pathogen (Human-*Mtb*) interactions (HPIs) is very limited [1]. The study also suggests that different strains of tuberculosis show different patterns of infection [2]. To date, there have been no such studies performed on discussing host-pathogen protein-protein interactions between different strains of *Mtb* and the human host.

An extensive study is required to understand the pattern of infection and the factors responsible for the virulence of the pathogen *Mtb*. *In vivo* and *in vitro* studies are tedious and expensive; therefore, computational methods are effective techniques to identify a broader set of HPIs [3]. Template sets of known inter- or intra-species protein-protein interactions (PPIs) across different organisms are used as a foundation of computational methods for HPI prediction, assuming the function and interaction pattern of the orthologous protein similar or conserved [4].

A host-pathogen interaction can be inferred when the two proteins constitute a potential pair of HPIs that bear a significant structural or sequence homology with the corresponding pair of interacting proteins from the template PPI set [3,5]. Earlier various studies on host-pathogen interactions were carried out through a homology structure- or sequence-based approach. In the present study, a sequence-based homology protein mapping or interologs method was used for identifying common protein-protein interactions of different strains of *Mtb* with its human host.

In this study, Bianna Interlog Prediction Server (BIPS) has been used for acquiring the data on HPIs between *Mtb* and the human host. A comprehensive library of HPIs consisting of a total 3,198 HPIs were constructed, which were then screened, refined, and validated through various parameters like virulence factors, cellular and subcellular localization, functional feature, and the biological process. Common interactors were identified and are listed in Table S1. The Common HPIs were identified among all the studied strains and the common HPIs among the virulent strains are listed in Table S2.

Highest interacting proteins

The highly-connected node or the hub protein has great importance for every pathogen in establishing a disease through protein-protein interactions with the host. The host proteins that are involved in the specific pathways (especially proteins associated with immunity and defense mechanisms) are major targets of the pathogens. The fitness of pathogen and establishment of disease relies on the successful interaction of pathogen proteins with the human host [6]. The highest interacting proteins of the pathogen are listed in Table S3.

Cytoscape visualization of protein-protein interactions

The HP-PPIs were visualized through the Cytoscape 3.7.1 [7] (Shannon et al., 2003). The data of protein interactions were analyzed and visualized by applying various parameters, tools, and filters of Cytoscape. For the layout, various algorithms such as prefuse force-directed, edge-weighted spring embedded, and circular layout were used for refining the visualization of the interactions. Human proteins and Mycobacterium proteins can be distinguished by the shape of the nodes, i.e oval for human proteins and diamond shape for Mycobacterium proteins (Figure S1).

References:

1. Rapanoel HA, Mazandu GK, Mulder NJ. Predicting and analyzing interactions between Mtb and its human host. PLoS One 2013;8(7):e67472. https://doi.org/10.1371/journal.pone.0067472

2. Manabe YC, Dannenberg AM, Tyagi SK, et al. Different strains of Mycobacterium tuberculosis cause various spectrums of disease in the rabbit model of tuberculosis. Infect Immun 2003; 71(10):6004-11. https://doi.org/10.1128/iai.71.10.6004-6011.2003

3. Zhou H, Jin J, Wong L. Progress in computational studies of host-pathogen interactions. J Bioinform Comput Biol 2013; 11(02):1230001. https://doi.org/10.1142/S0219720012300018

4. Walhout AJ, Sordella R, Lu X, et al. Protein interaction mapping in C. elegans using proteins involved in vulval development. Science 2000;287(5450):116-22. https://www.doi.org/10.1126/science.287.5450.116

5. Zhou H, Gao S, Nguyen NN, et al. Stringent homology-based prediction of H. sapiens-M. tuberculosis H37Rv protein-protein interactions. Biol Direct 2014;9:5. https://doi.org/10.1186%2F1745-6150-9-5

6. Asensio NC, Giner EM, De Groot NS, Burgas MT. Centrality in the host–pathogen interactome is associated with pathogen fitness during infection. Nat Commun 2017;8:14092. https://doi.org/10.1038/ncomms14092

7. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13(11):2498-504. https://doi.org/10.1101/gr.1239303

Table S1. List of HPIs common among all the studied strains that show interaction with human hosts (description obtained from UniProt database).

Mtb protein	Description
Adenylosuccinate synthetase (purA)	Plays an important role in the de novo pathway of purine nucleotide biosynthesis. Catalyzes the first committed step in the biosynthesis of AMP from IMP.
Glucose-6-phosphate isomerase (pgi)	Is involved in step 2 of the subpathway that synthesizes D-glyceraldehyde 3-phosphate and glycerone phosphate from D-glucose.
Phosphoglycerate kinase (pgk)	Is involved in step 2 of the subpathway that synthesizes pyruvate from D-glyceraldehyde 3-phosphate.
Malate dehydrogenase (mdh)	Catalyzes the reversible oxidation of malate to oxaloacetate.
ATP synthase alpha (AtpA)	Produces ATP from ADP in the presence of a proton gradient across the membrane. The alpha chain is a regulatory subunit.
ATP synthase gamma chain (AtpG)	Produces ATP from ADP in the presence of a proton gradient across the membrane. The gamma chain is believed to be important in regulating ATPase activity and the flow of protons through the CF0 complex.
Glyceraldehyde-3-phosphate dehydrogenase	Plays an essential role in glycolysis cycle.
60 kDa chaperonin (groL)	Prevents misfolding and promotes the refolding and proper assembly of unfolded polypeptides generated under stress conditions.
50S ribosomal protein L2 (rplB)	One of the primary rRNA binding proteins. Required for association of the 30S and 50S subunits to form the 70S ribosome and for tRNA binding and peptide bond formation. It has been suggested to have peptidyltransferase activity; this is somewhat controversial. Makes several contacts with the 16S rRNA in the 70S ribosome.
Cytochrome c oxidase subunit 1 (J113_21205)	Cytochrome c oxidase is the component of the respiratory chain that catalyzes the reduction of oxygen to water. Subunits 1 - 3 form the functional core of the enzyme complex. CO I is the catalytic subunit of the enzyme. Electrons originating in cytochrome c are transferred via the copper A center of subunit 2 and heme A of subunit 1 to the bimetallic center formed by heme A3 and copper B.
Chaperone protein DNAK	Acts as a chaperone. It is a recombinant extracellular protein that activates the expression of NF-kappa-B in immortalized human dermal endothelial cells in a TLR2- and TLR4-dependent manner. Activation occurs via MYD88-dependent and -
Chaperone protein HTPG	Molecular chaperone. Has ATPase activity.

Table S2. List of HPIs common among the studied virulent strains that show interaction with human hosts (data obtained from HPPPI database and experimental results).

Proteins present in virulent strains of Mycobacterium tuberculosis	Interactors in human host	UniProt ID of nteractors in human host	Literature/Source DB
8. PE-PGRS family protein PE_PGRS33	Toll-like receptor 2 (toll/interleukin-1 receptor-like protein 4) (CD antigen CD282)	TLR2_HUMAN	PMID:17095513 PMID:26978522
9. PE-PGRS family protein PE_PGRS33	Toll-like receptor	A0A0S2Z4S4_HUMAN	PMID:17095513
10. PE-PGRS family protein PE_PGRS33	Toll-like receptor 2	D2JYJ4_HUMAN	PMID:26978522
11. PE-PGRS family protein PE_PGRS33	Fibronectin	FINC_HUMAN	PMID: 17095513
12. PE-PGRS family protein PE_PGRS61	Toll-like receptor 2	TLR2_HUMAN	PMID:27483162
13. PE-PGRS family protein PE_PGRS61	Toll-like receptor 2	D2JYJ4_HUMAN	PMID:27483162
14. PE-PGRS family protein PE_PGRS61	Toll-like receptor 2	D2JYJ0_HUMAN	PMID:27483162

Table S2. List of HPIs common among the studied virulent strains that show interaction with human hosts (data obtained from HPPPI database and experimental results) (continued).

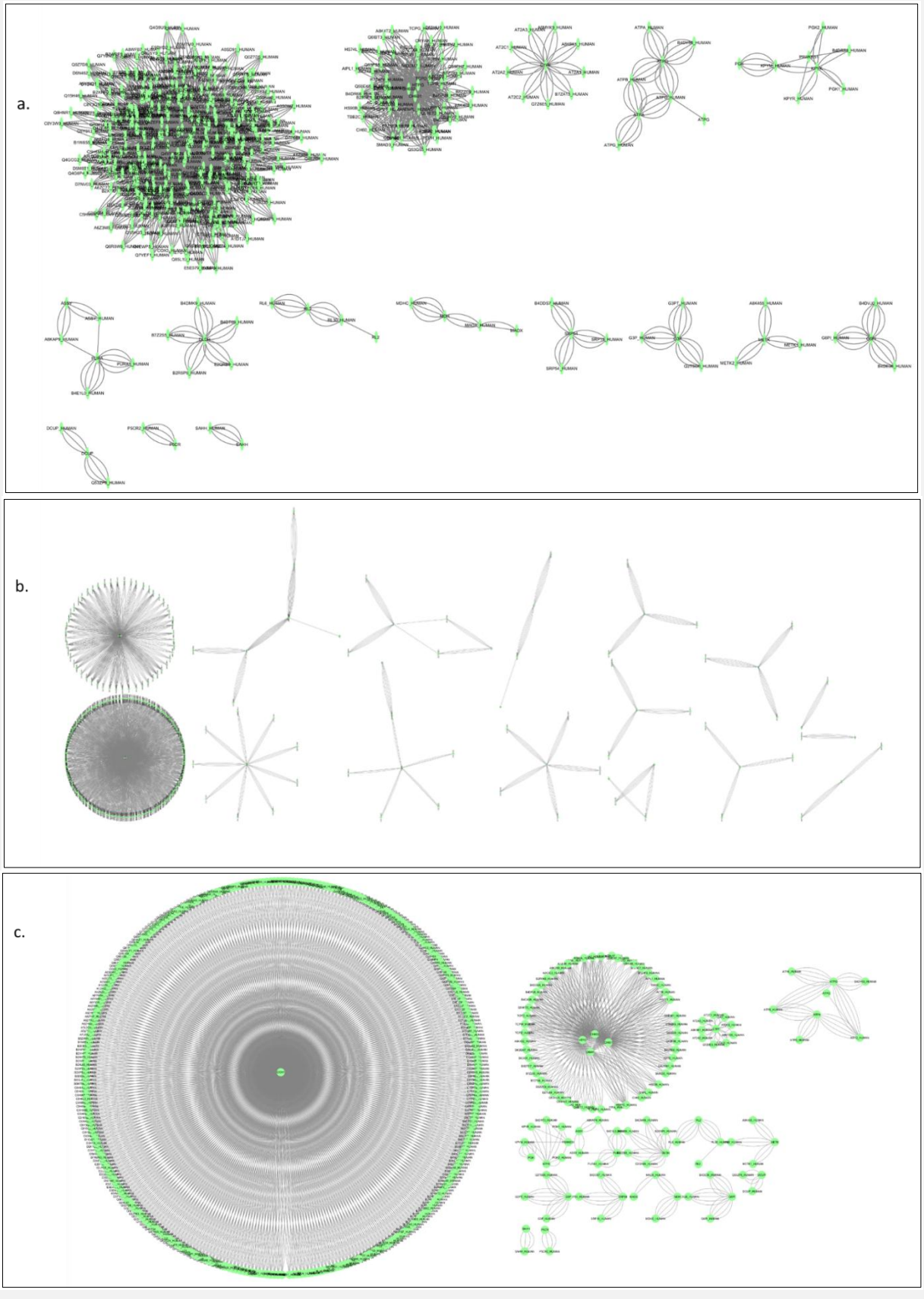
Proteins present in virulent strains of Mycobacterium tuberculosis	Interactors in human host	UniProt ID of nteractors in human host	Literature/Source DB
15. PE-PGRS family protein PE_PGRS61	Toll-like receptor 2	D2JYJ2_HUMAN	PMID:27483162
16. PE-PGRS family protein PE_PGRS61	Toll-like receptor 2	D2JYJ1_HUMAN	PMID:27483162
17. PE-PGRS family protein PE_PGRS61	Toll-like receptor 2	D2JYJ3_HUMAN	PMID:27483162
18. PE family immunomodulator PE35	Toll-like receptor 2	D2JYJ3_HUMAN	PMID: 24467650
19. PE family immunomodulator PE35	Toll-like receptor 2	D2JYJ2_HUMAN	PMID: 24467650
20. PE family immunomodulator PE35	Toll-like receptor 2	TLR4_HUMAN	mint, PATRIC
21. DNA-binding protein HU homolog	Complement C3 precursor	CO3_HUMAN	mtbbase
22. DNA-binding protein HU homolog	Alpha-2-HS-glycoprotein	FETUA_HUMAN	PMID: 18482453
23. DNA-binding protein HU homolog	Laminin subunit gamma-1	LAMC1_HUMAN	PMID: 15919224
24. DNA-binding protein HU homolog	Heparan sulfate	NDST2_HUMAN	PMID: 15919224
25. Methionine synthase (MS)	Fibronectin	FINC_HUMAN	PMID: 16677310
26. Methionine synthase (MS)	Laminin subunit gamma-1	LAMC1_HUMAN	PMID: 16677310
27. Sulfatase family protein/Formylglycine-generating enzyme	Interleukin-8 precursor	IL8_HUMAN	Agbase, PATRIC
28. Alpha-ketoglutarate-dependent sulfate ester	Interleukin-8 precursor	IL8_HUMAN	hpidb; agbase
29. PE-PGRS family protein PE_PGRS5	Toll-like receptor 2 (toll/interleukin-1 receptor-like protein 4) (CD antigen CD282)	TLR2_HUMAN	PMID: 29921671
30. PE-PGRS family protein PE_PGRS11	Toll-like receptor 2 (toll/interleukin-1 receptor-like protein 4) (CD antigen CD282)	TLR2_HUMAN	PMID: 20176745
31. PE-PGRS family protein PE_PGRS11	Fibronectin	FINC_HUMAN	Unpublished
32. PPE family protein, MPTR subgroup => PPE34	Toll-like receptor 2 (toll/interleukin-1 receptor-like protein 4) (CD antigen CD282)	TLR2_HUMAN	biogrid, PATRIC
33. PPE family protein => PPE68, component of type VII secretion system ESX-1	Interacts with PE35. PE35/PPE68 complex interacts with human TLR2	TLR2_HUMAN	mint, PATRIC, PMID: 24467650
34. Phosphate-binding protein PstS 1 (p38)	Toll-like receptor 2	TLR2_HUMAN	PMID: 24708540
35. 6 kDa early secretory antigenic target, esxA	beta-2-microglobulin (B2M)	F5H6I0_HUMAN	PMID: 25356553
36. Uncharacterized oxidoreductase Rv1144	ATP-dependent RNA helicase DDX24	H2AX_HUMAN	PMID: 25356553
37. Probable low molecular weight protein-tyrosine-phosphatase, PTPase, EC 3.1.3.48	Scm-like with four MBT domains protein 1, hSFMBT (renal ubiquitous protein 1)	SMBT1_HUMAN	PMID: 25356553

Table S2. List of HPIs common among the studied virulent strains that show interaction with human hosts (data obtained from HPPPI database and experimental results) (continued).

Proteins present in virulent strains of Mycobacterium tuberculosis	Interactors in human host	UniProt ID of nteractors in human host	Literature/Source DB
38. Electron transfer flavoprotein subunit alpha, alpha-ETF (electron transfer flavoprotein large subunit, ETFLS)	TNF receptor-associated factor 6, EC 2.3.2.27 (E3 ubiquitin-protein ligase TRAF6) (interleukin-1 signal transducer) (RING finger protein 85) (RING-type E3 ubiquitin transferase TRAF6)	TRAF6_HUMAN	PMID: 25356553
39. Electron transfer flavoprotein subunit alpha, alpha-ETF (electron transfer flavoprotein large subunit, ETFLS)	DNA-3-methyladenine glycosylase, EC 3.2.2.21 (3-alkyladenine DNA glycosylase) (3-methyladenine DNA glycosidase) (ADPG) (N-methylpurine-DNA glycosylase)	3MG_HUMAN	PMID: 25356553
40. Electron transfer flavoprotein subunit alpha, alpha-ETF (electron transfer flavoprotein large subunit, ETFLS)	Histone deacetylase 5, HD5, EC 3.5.1.98 (antigen NY-CO-9)	HDAC5_HUMAN	PMID: 25356553
41. Electron transfer flavoprotein subunit alpha, alpha-ETF (electron transfer flavoprotein large subunit, ETFLS)	Electron transfer flavoprotein beta subunit lysine methyltransferase, EC 2.1.1.- (ETFB lysine methyltransferase, ETFB-KMT) (protein N-lysine methyltransferase METTL20)	ETKMT_HUMAN	PMID: 25356553

Table S3. List of highest interacting proteins (*Mtb* proteins showing interactions with human proteins).

Mtb protein	Human protein (UniProt ID)
Cytochrome c oxidase subunit I (COX1)	COX 2, COX 3
Chaperone protein (DNAK)	HSP7C, HSP7C, HSP71, HSP71, ACTB, ACTB, ACTB, ACTB, HSP71, HSP71, HSP7C, HSP7C, CH60, ENPL, ENPL, HS90B, HS90A
Chaperone protein (HTPG)	TCPD, ACTB, TCPZ, HSP7C, TCPG, HS90A, TCPB, DNJC7, CRYAB, AIPL1, HS74L, PPIA, TCPH, TCPW
60 kDa chaperonin 1 (CH601)	TCPD, TCPZ, HSP7C, CH10, HS90A, TCPW
60 kDa chaperonin 2 (CH602)	TCPD, HS90B, CH60, ACTB, TCPZ, HSP7C, SMAD3, CH10, ENPL, HS90A, TCPW
ATP synthase subunit alpha (ATPA)	ATPG, ATPB, ATPB, ATPD
ATP synthase subunit gamma (ATPG)	B4DY56, ATPA, ATPB, ATPD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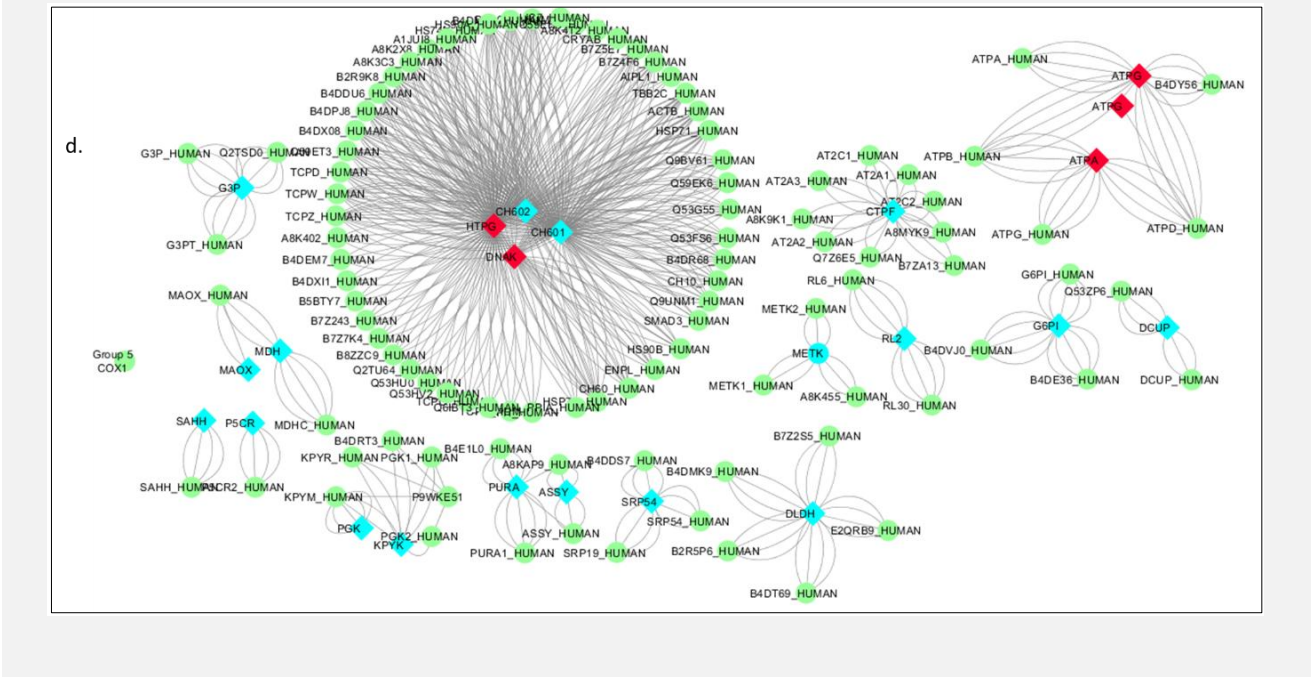

Figure S1. Visualization of protein interaction networks.

a - d) Cytoscape images of *Mtb* protein interaction data derived by BIPS analysis. a) Initial protein interaction network (> 3,000 proteins) laid out with a force-directed algorithm. b) The interaction of the proteins obtained through edge-weighted spring embedded layout. c) Circular layout of the interactions. d) The protein-protein interaction image: green oval shape is human proteins; cyan-colored diamond shape, denoting proteins of *Mtb*, and red diamond shape as a target protein.