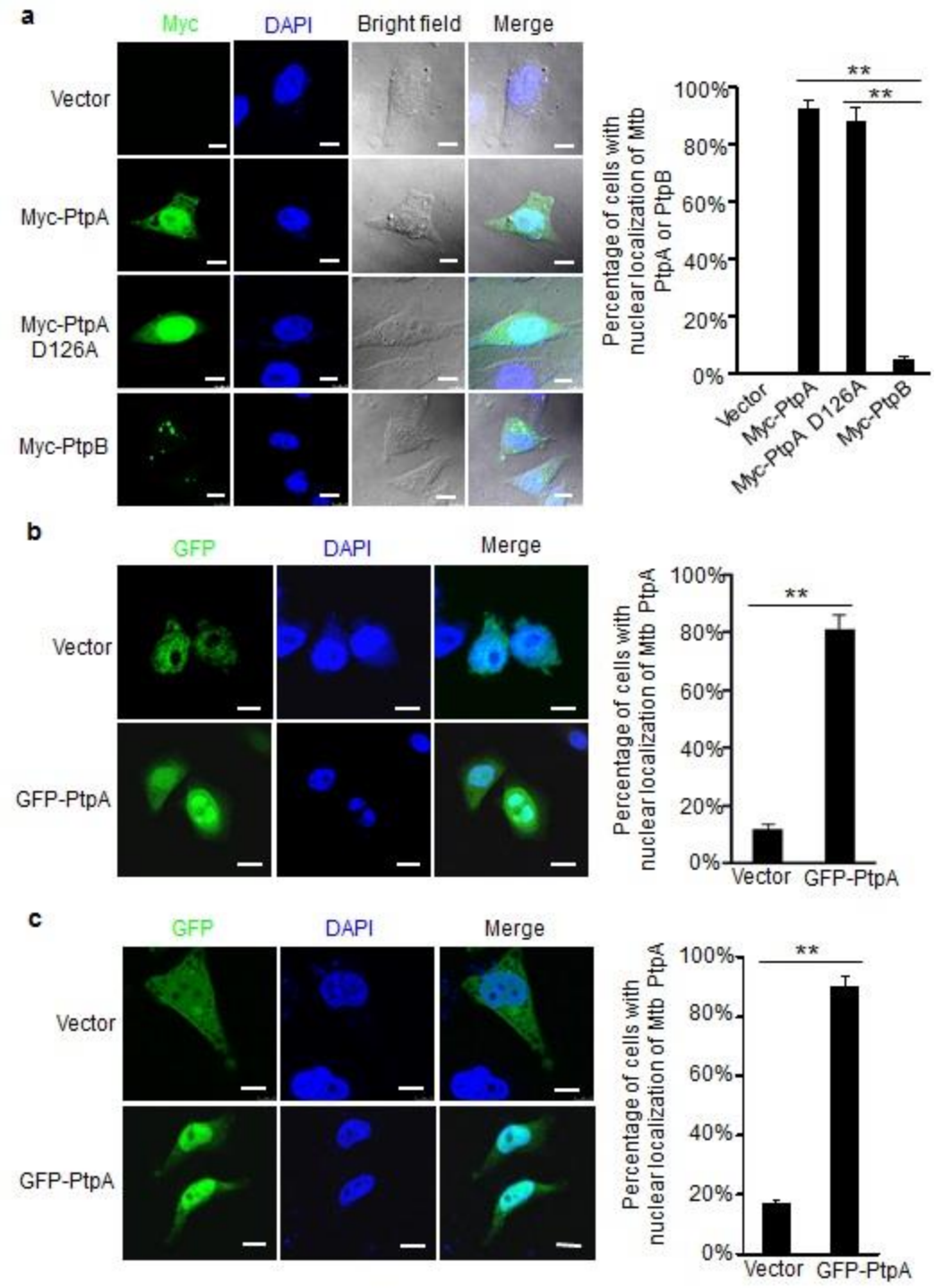
File name: Supplementary Information
Description: Supplementary Figures and Supplementary Table

File name: Supplementary Data 1
Description: The list of 3351 candidate Mtb PtpA-specific ChIP-seq signals.

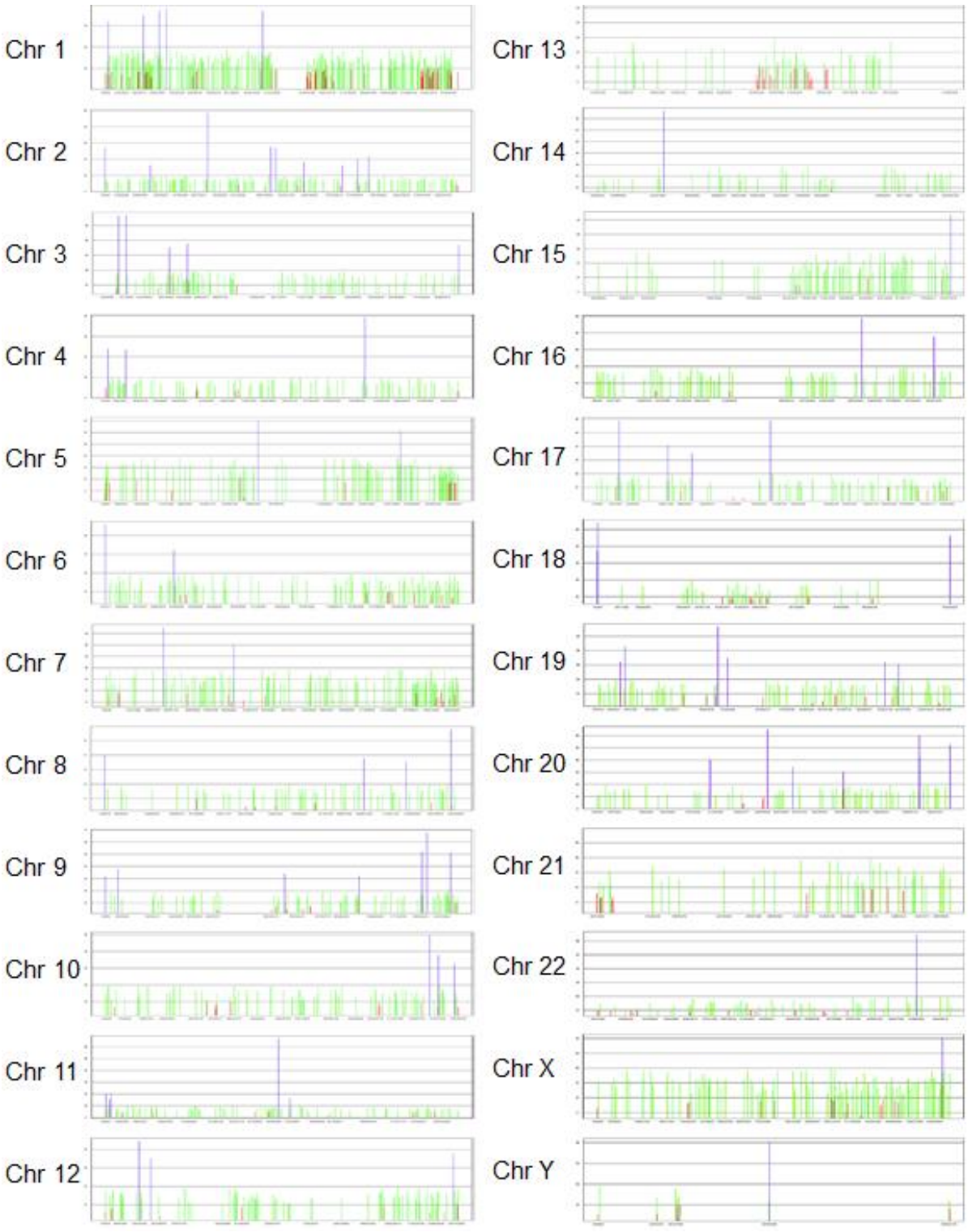
File name: Supplementary Data 2
Description: The list of 280 candidate Mtb PtpA-regulated protein-coding genes.

File name: Peer Review File
Description:

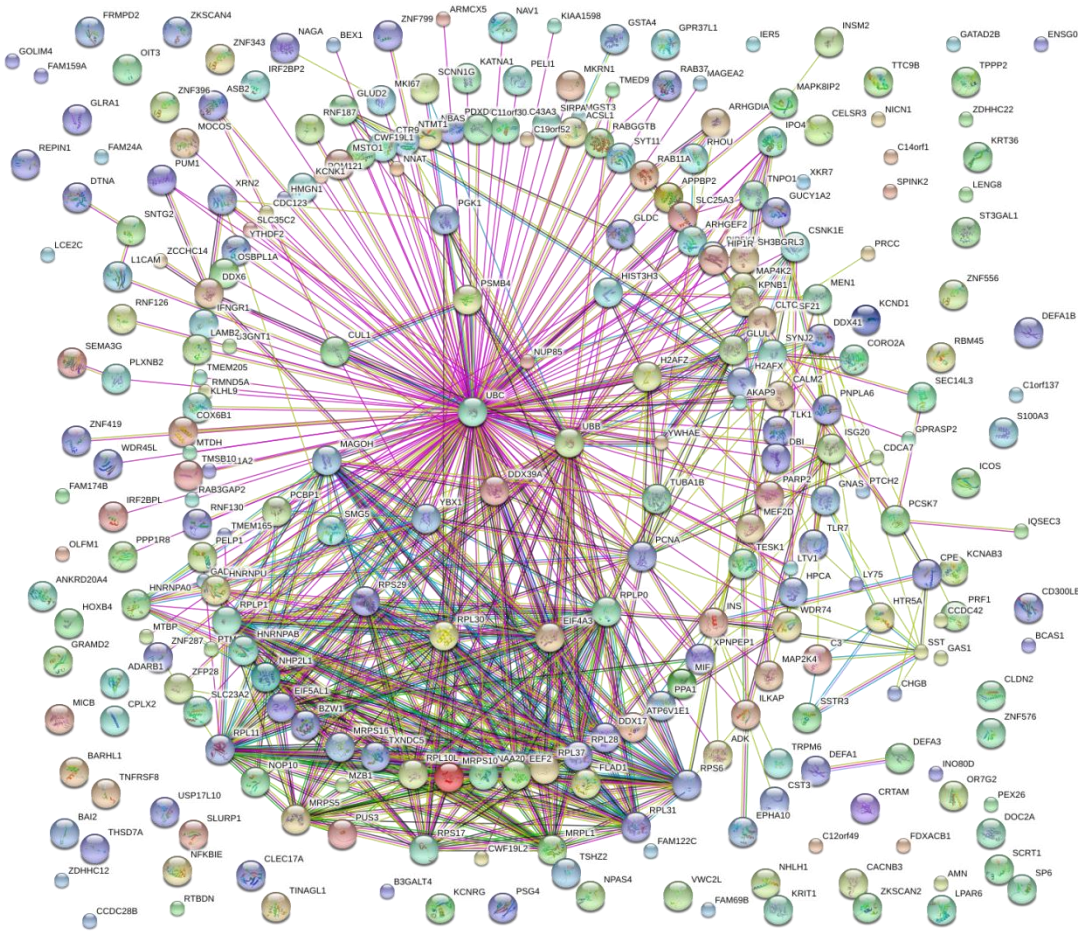


Supplementary Figure 1: *M. tuberculosis* PtpA is present both in the cytoplasm and nucleus of host cells. (**a**) U937 cells were transfected with an empty vector or a vector encoding Myc-tagged wild-type PtpA (Myc-PtpA, green), or PtpA D126A (Myc-PtpA D126A, green), or PtpB (Myc-PtpB, green), and then examined by confocal microscopy at 24 h post-transfection. (**b, c**) U937 cells (**b**) or A549 cells (**c**) were transfected with an empty

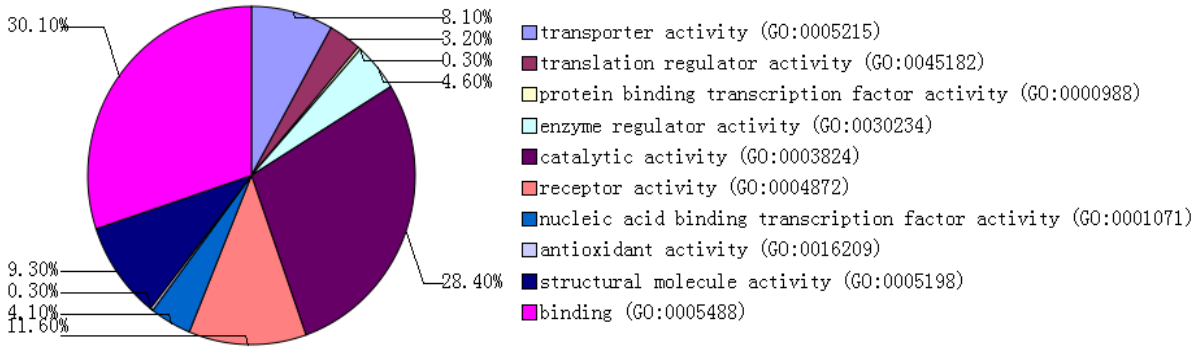
vector encoding GFP (green) alone or GFP-PtpA (green), and then examined by confocal microscopy at 24 h post-transfection. Nuclei (blue) were stained with DNA-binding dye (DAPI). Scale bars, 10 μm. Right, percentage of cells with nuclear localization of *M. tuberculosis* (Mtb) PtpA or PtpB. About 200 cells were counted. *$P < 0.05$ and ** $P < 0.01$ (unpaired two-tailed Student's *t* test). Data are representative of one experiment with at least three independent biological replicates (**a-c**; mean and s.e.m., n = 3).



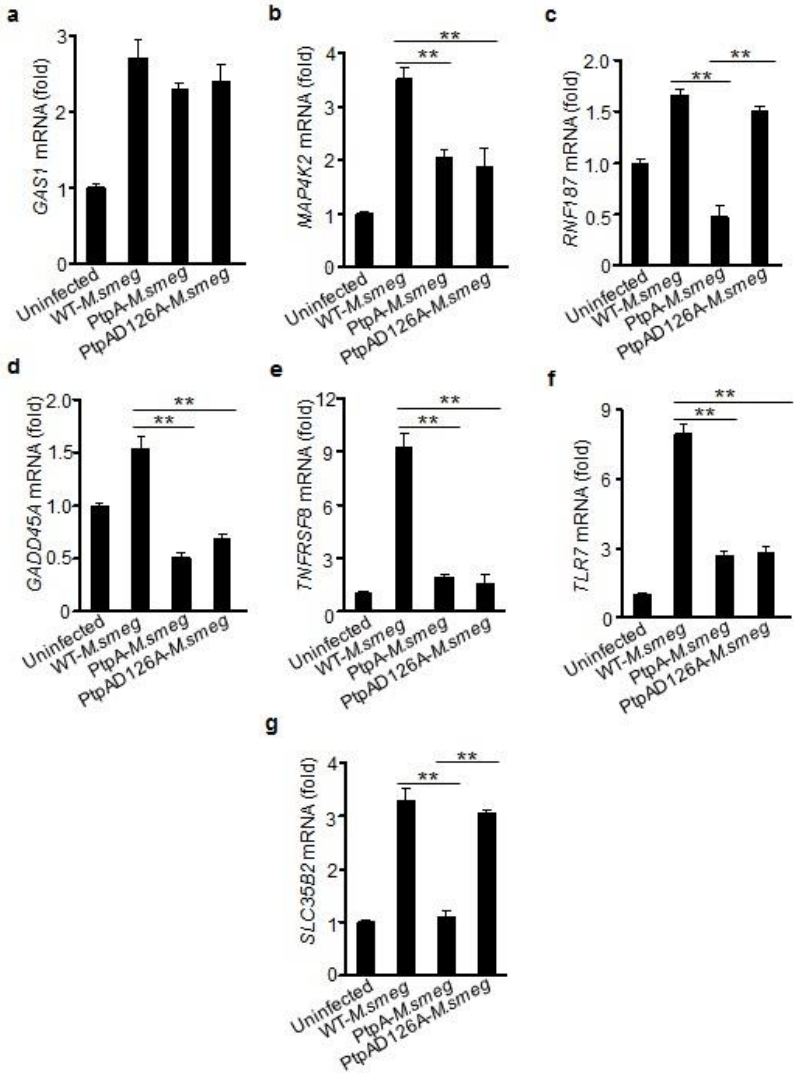
Supplementary Figure 2: Distribution of the 3351 PtpA ChIP-seq signals in chromosomes. Red: fold-enrichment < 10; Green: 10 < fold-enrichment < 20; Purple: fold-enrichment > 20.



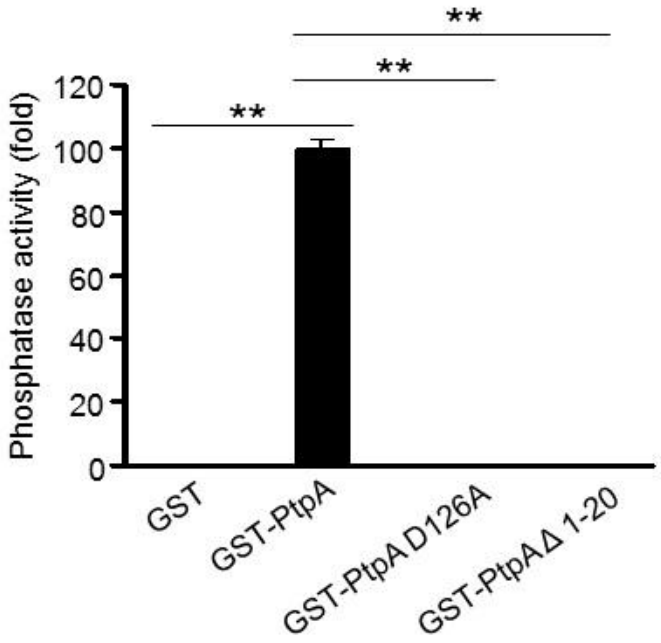
Supplementary Figure 3: The network of proteins encoded by 280 potential PtpA target genes. The protein-protein interaction network was visualized by the STRING 10.0.



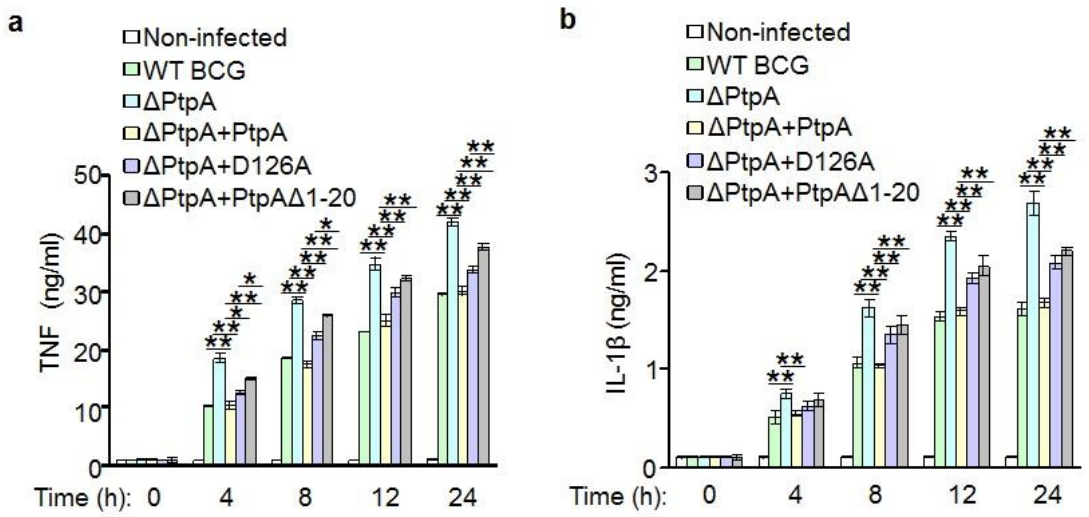
Supplementary Figure 4: Gene ontology enrichment analysis results. The pie chart presents the specific ontology (GO) terms for proteins encoded by 280 potential PtpA target genes.



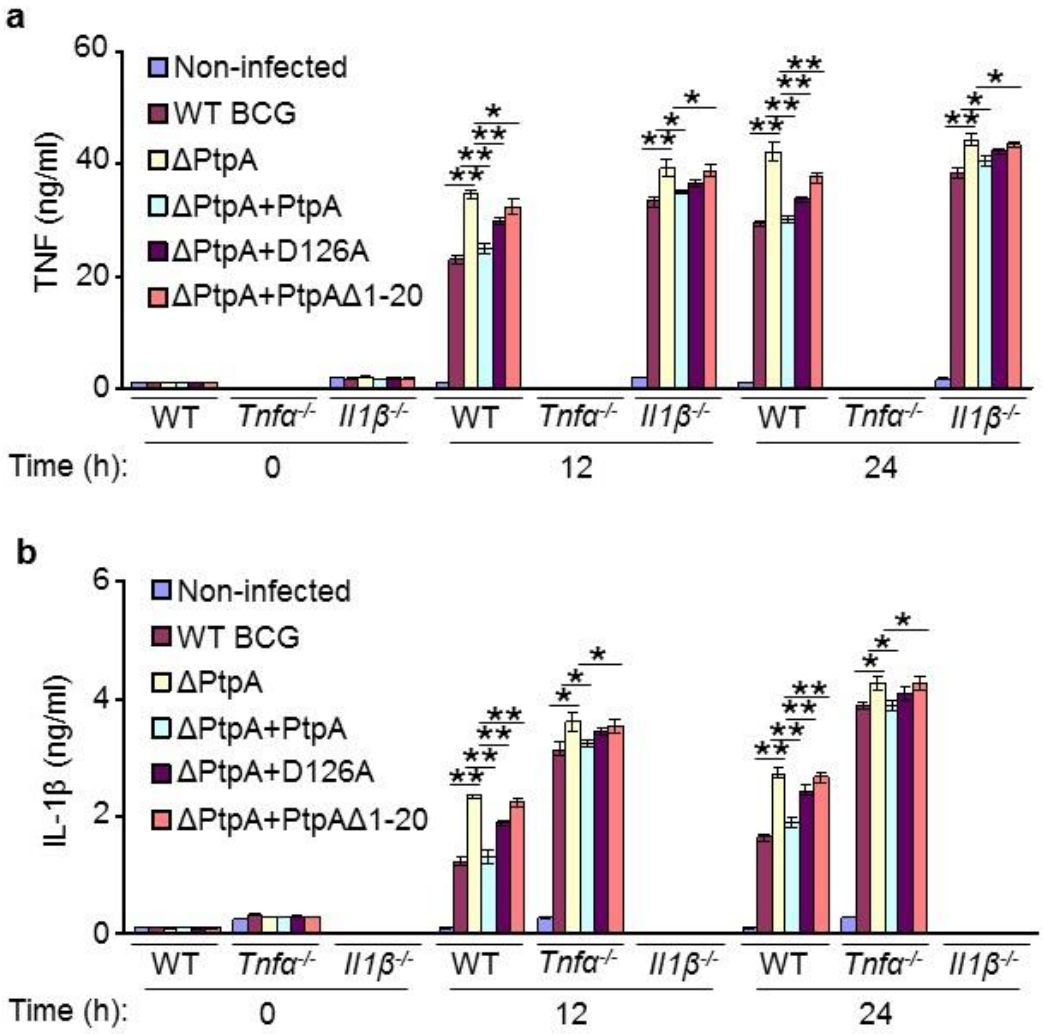
Supplementary Figure 5: Transcriptional regulation of potential target genes by PtpA. Quantitative PCR analysis of *GAS1, MAP4K2, RNF187, GADD45A, TNFRSF8, TLR7* and *SLC35B2* mRNAs were performed using U937 cells infected with wild-type *M. smegmatis* (WT-*M. smeg*), or Mtb PtpA-overexpressing *M. smegmatis* (PtpA-*M. smeg*), or PtpA D126A-overexpressing *M. smegmatis* (PtpA D126A-*M. smeg*) for 12 h. Analysis of each gene was normalized to the gene *Gapdh*. *$P < 0.05$ and ** $P < 0.01$ (unpaired two-tailed Student's *t* test). Data are representative of one experiment with at least three independent biological replicates (**a-g**; mean and s.e.m., n = 3).



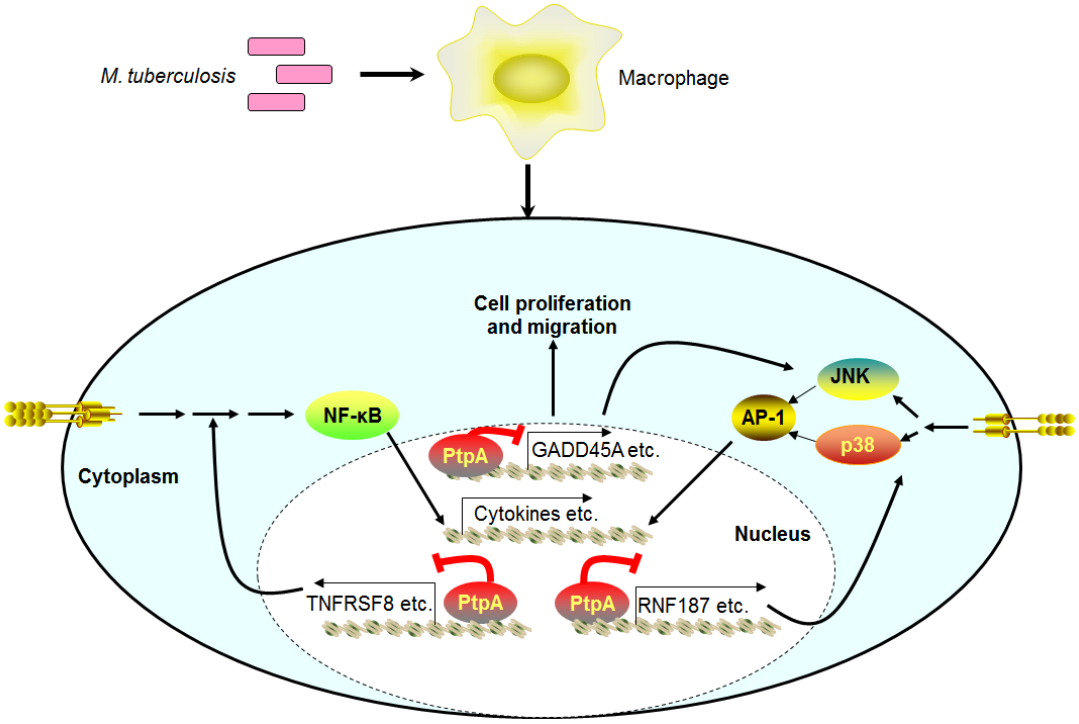
Supplementary Figure 6: The DNA-binding region of PtpA is required for its phosphatase activity. Para-nitrophenyl phosphate phosphatase assay. Purified GST, GST-PtpA, GST-PtpA D126A, or GST-PtpA Δ 1-20 proteins (2.5 μM) were incubated with 10 mM para-nitrophenyl phosphate (pNPP) at 37℃ for 30 min. OD value was read at 405 nm. *P < 0.05 and ** P < 0.01 (unpaired two-tailed Student's t test). Data are representative of one experiment with at least three independent biological replicates (mean and s.e.m., n = 3).



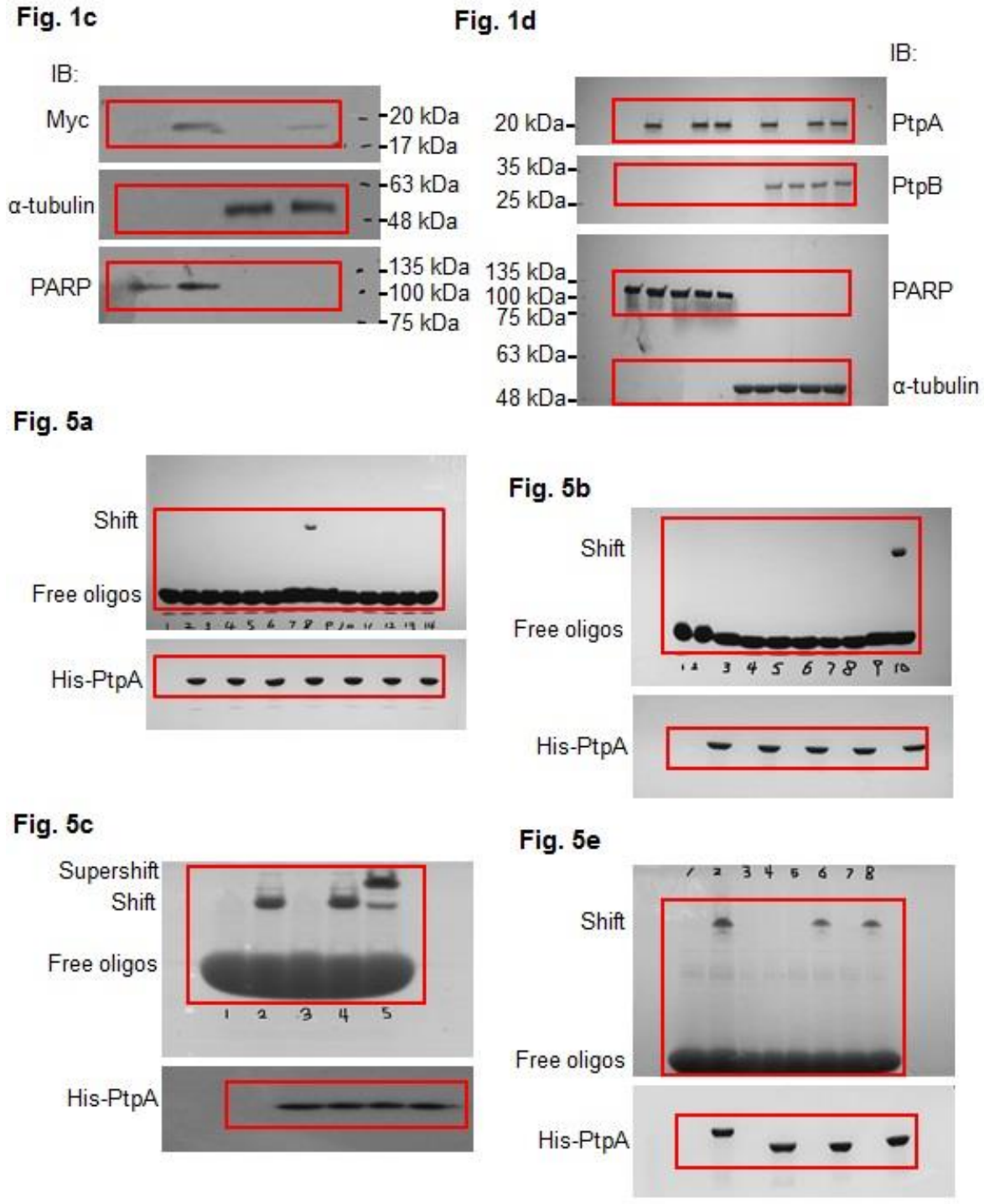
Supplementary Figure 7: Deletion of the DNA-binding region of PtpA abolishes its inhibitory effects on the production of TNF and IL-1β. Enzyme-linked immunosorbent assay (ELISA) of TNF (**a**) and IL-1β (**b**) in the medium of U937 cells infected with WT BCG, or PtpA-deleted BCG (BCG ΔPtpA), or BCG ΔPtpA complemented with WT PtpA (ΔPtpA + PtpA), or BCG ΔPtpA complemented with PtpA D126A (ΔPtpA + D126A), or BCG ΔPtpA complemented with PtpA Δ1-20 (ΔPtpA + PtpA Δ1-20) at a MOI of 10. *$P < 0.05$ and ** $P < 0.01$ (unpaired two-tailed Student's *t* test). Data are representative of one experiment with at lease three independent biological replicates (**a, b**; means and s.e.m. of n = 3 cultures).



Supplementary Figure 8: Nuclear PtpA-mediated immune suppression response is partially dependent on TNF and IL-1β. (a, b) ELISA of TNF (**a**) and IL-1β (**b**) in the medium of the bone marrow derived macrophages (BMDMs) from WT or *Tnfα*$^{-/-}$ or *Il1β*$^{-/-}$ mice. Cells were infected with WT BCG, or BCG ΔPtpA, or BCG (ΔPtpA + PtpA), or BCG (ΔPtpA + D126A), or BCG (ΔPtpA + PtpA Δ1-20) at a MOI of 10. *$P < 0.05$ and ** $P < 0.01$ (unpaired two-tailed Student's *t* test). Data are representative of one experiment with at lease three independent biological replicates (**a, b**; means and s.e.m. of n = 3 cultures).



Supplementary Figure 9: Proposed model depicting the roles of PtpA plays in the nucleus of host cells during mycobacterial infection. PtpA inhibits the transcription of certain host genes such as *TNFRSF8* and *RNF187*, which might lead to inhibition of innate immune signaling pathways. Furthermore, PtpA promotes cell proliferation and migration of infected cells, partially through regulating the cell cycle checkpoint protein-coding gene *GADD45A*.



Supplementary Figure 10: Original uncropped scans of blots for Figure 1 and 5.

Supplementary Table 1. Plasmids, bacterial strains and oligonucleotides used in this study.

Name	Description	Reference
Plasmids		
pEGFP-N1	CMV promoter, for mammalian expression, GFP tag, Kan^R	Clontech
pEGFP-N1-PtpA	For expression of GFP-PtpA in mammalian cells	This study
p3xFlag-CMV14	CMV promoter, for mammalian expression, 3xFlag tag, Amp^R	Sigma
p3xFlag-CMV14-PtpA	For expression of Flag-PtpA in mammalian cells	This study
p3xFlag-CMV14-PtpA (1-20)	For expression of Flag-PtpA in mammalian cells	This study
pcDNA6A	T7 promoter, for mammalian expression, Myc tag, Amp^R	Invitrogen
pcDNA6A-PtpA	For expression of Myc-PtpA in mammalian cells	This study
pET30a	T7 promoter, for bacterial expression, 6xHis tag, Kan^R	Novagen
pET30a-PtpA	For expression of recombinant protein His_6-PtpA	This study
pET30a-PtpA (Δ1-20)	For expression of recombinant protein His_6-PtpA (Δ1-20)	This study
pET30a-PtpA (Δ41-49)	For expression of recombinant protein His_6-PtpA (Δ41-49)	This study
pET30a-PtpA (Δ65-75)	For expression of recombinant protein His_6-PtpA (Δ65-75)	This study
pRL-TK	Used in dual-luciferase assay for NF-κB and MAPK pathways	Feng Shao
pGL2-Basic	Promoterless vector for measuring the activity of promoter and enhancer sequences with a luciferase assay.	Promega
pGL2-Basic-GADD45A promoter	Used in dual-luciferase assay for measuring the activity of promoter	This study
pGL2-Basic-RNF187 promoter	Used in dual-luciferase assay for measuring the activity of promoter	This study
pGL2-Basic-GAS1 promoter	Used in dual-luciferase assay for measuring the activity of promoter	This study
pGL2-Basic-SLC35B2 promoter	Used in dual-luciferase assay for measuring the activity of promoter	This study
pGL2-Basic-TLK1 promoter	Used in dual-luciferase assay for measuring the activity of promoter	This study
pGL2-Basic-TLR7 promoter	Used in dual-luciferase assay for measuring the activity of promoter	This study
pGL2-Basic-MAP4K2 promoter	Used in dual-luciferase assay for measuring the activity of promoter	This study
pGL2-Basic-TNFRSF8 promoter	Used in dual-luciferase assay for measuring the activity of promoter	This study
Strains		
E. coli DH5α	F^- φ80*lac*ZΔM15 Δ (*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17 (r_k^-, m_k^+) *pho*A *sup*E44 λ^- *thi*-1 *gyr*A96 *rel*A1	Invitrogen
E.coli BL21 (DE3)	F^- *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm* (DE3)	Novagen
M. bovis BCG	Pasteur	ATCC 35734
M. smegmatis	mc^2 155	ATCC 700084
PtpA-*M. smegmatis*	Mtb PtpA-overexpressing *M. smegmatis*	This study
BCG ΔPtpA	BCG strain with deletion of PtpA	This study

BCG (Δ PtpA + PtpA)	created by introducing the integrative vector pMV306 carrying the ptpA gene and promoter into the BCG Δ PtpA strain	This study
BCG (Δ PtpA + D126A)	created by introducing the integrative vector pMV306 carrying the ptpA (D126A) gene and promoter into the BCG Δ PtpA strain	This study
BCG (Δ PtpA + PtpA Δ 1-20)	created by introducing the integrative vector pMV306 carrying the PtpA Δ 1-20 gene and promoter into the BCG Δ PtpA strain	This study
Oligonucleotides (5'-3')		
pEGFP-N1-PtpA-F	CCCAAGCTTGTGTCTGATCCGCTGCACGTC	This study
pEGFP-N1-PtpA-R	GCCTGCAGACTCGGTCCGTTCCGCGCGAGACGT	This study
p3xFlag-CMV14-PtpA-F	CCCAAGCTTGTGTCTGATCCGCTGCACGTC	This study
p3xFlag-CMV14-PtpA-R	GCTCTAGAACTCGGTCCGTTCCGCGCGAGACGT	This study
pcDNA6A-PtpA-F	CCCGAATTCGTGTCTGATCCGCTGCACGTC	This study
pcDNA6A-PtpA-R	GCCTCGAGACTCGGTCCGTTCCGCGCGAGACGT	This study
pET30a-PtpA-F	CCCGGATCCGTGTCTGATCCGCTGCACGTC	This study
pET30a-PtpA-R	GCAAGCTTCTCGGTCCGTTCCGCGCGAGACGT	This study
pET30a-PtpA-F (Δ1-20)	ACCTCGAGGCCGAGAAGATGTTCGCCCA	This study
pET30a-PtpA-R (Δ1-20)	TCTCGGCCTCGAGGTTAACGGATCCAGC	This study
pET30a-PtpA-F (Δ41-49)	TGCGAGTGGTAGGCAGTTGCGCCGACGA	This study
pET30a-PtpA-R (Δ41-49)	TGCCTACCACTCGCACCGCGTCACCCA	This study
pET30a-PtpA-F (Δ65-75)	GAGCCCACGTCGGCACCGAACACCTGGC	This study
pET30a-PtpA-R (Δ65-75)	GTGCCGACGAGGGCTCGCAACACCCCGG	This study
pGL2-Basic-*GADD45A* promoter-F	TCAGCTGGTGGGCGTCCAGAAGGAT	This study
pGL2-Basic-*GADD45A* promoter-R	ACTCACCTTTCGGTCTTCTGCTCTCC	This study
pGL2-Basic-*RNF187* promoter-F	TTAGCCGGGCGTCGTGGCAGGCGCC	This study
pGL2-Basic-*RNF187* promoter-R	CGCTGGCACAGGGCGCAGGCGGCCTC	This study
pGL2-Basic-*TNFRS*F8 promoter-F	CTTATGCATCCATCCATCCATCCAT	This study
pGL2-Basic-*TNFRSF8* promoter-R	CGTAGCGCCCCCAGGAACAGCAGTCCC	This study
pGL2-Basic-*GAS1* promoter-F	CGTCAGGCTCCCTAAGCCGTTCCTT	This study
pGL2-Basic-*GAS1* promoter-R	ACTGTCCCCCCGCGGGCCTCGCCGCC	This study
pGL2-Basic-*SLC35B2* promoter-F	TGGGGCAATAAACCAGCACAGTCTC	This study
pGL2-Basic-*SLC35B2* promoter-R	GTAAAGCGCTCACCCGGTGATGTGG	This study
pGL2-Basic-*TLR7* promoter-F-R	CAGGTGGACTAAGTAGATTAAAGAA	This study
pGL2-Basic-*TLR7* promoter-R	CAAAGATTACAGATAACACTTTTTA	This study
pGL2-Basic-*MAP4K2* promoter-F	AGCACAGGTCCCACCTCCAGCCCGG	This study
pGL2-Basic-*MAP4K2* promoter-R	TCGAAGCGGTCCCGCGGGTCCTGCAG	This study

GADD45A promoter (-700~-550bp)-F	TCAGCTGGTGGGCGTCCAGAAGGAT	This study
GADD45A promoter (-700~-550bp)-R	GGTTTCCCACCTGCCCGGTGTAACT	This study
GADD45A promoter (-550~-400bp)-F	GTTCTGCTTTCTGTGGAAAAGATTCT	This study
GADD45A promoter (-550~-400bp)-R	AGCAGTAGTATTATTTCGGTGCCCTG	This study
GADD45A promoter (-400~-250bp)-F	AATAAGCAGCTTCGCCTAGACTTAGA	This study
GADD45A promoter (-400~-250bp)-R	GGTGGCTATTAAAATCTATTTCCAGG	This study
*GADD45A*promoter (-250~-100bp)-F	CCTTAAAACAAAAGACATGAAAAGAT	This study
GADD45A promoter (-250~-100bp)-R	TTATCCGGTTGGCGGGGACCATTGG	This study
GADD45A promoter (-100~+50bp)-F	GAGTGCGCGCGGGACCCGCCTTCCC	This study
GADD45A promoter (-100~+50bp)-R	ACTCACCTTTCGGTCTTCTGCTCTCC	This study
GADD45A-QRT-F	ATCCTGCGCGTCAGCAACCC	This study
GADD45A-QRT-R	TGCACTGCGTGCTGGTGACG	This study
RNF187-QRT-F	CGGCGCCCGCGCGCGACGGC	This study
RNF187-QRT-R	CCCCGAGCCGCCCGAGTGGG	This study
GAS1-QRT-F	TGGGCTGCACCGAGGCCCGG	This study
GAS1-QRT-R	GCGCTGCACGGACGAATGCC	This study
TNFRSF8-QRT-F	TGTTCTCGAGACGACCTCGT	This study
TNFRSF8-QRT-R	GAGCCAGTACGACTCTGGCA	This study
Gapdh-QRT-F	GGAGCGAGATCCCTCCAAAAT	This study
Gapdh-QRT-R	GGCTGTTGTCATACTTCTCATGG	This study
MAP4K2-QRT-F	CTCCAGGGAGATGTCAAACT	This study
MAP4K2-QRT-R	CTCCCGAGGTGGCTGCTGTG	This study
SLC35B2-QRT-F	CTCTGATGAGGTTCCCCTGG	This study
SLC35B2-QRT-R	TCTTATCTGACTTGGGGTGT	This study
TLR7-QRT-F	GGATGGAAAC CAGCTACTAG	This study
TLR7-QRT-R	CTAACAGAACTGGCCAACAT	This study
Tnf-QRT-F	GAACTGGCAGAAGAGGCACT	This study
Tnf-QRT-R	CGTGGTGGCCCCTGCCACAAG	This study
Il1b-QRT-F	TGGGATGATG ATGATAACCT	This study
Il1b-QRT-R	GGTCCGACAGCACGAGGCTT	This study
Il12b-QRT-F	ACCCTGACCATCCAAGTCAAA	This study
Il12b-QRT-R	TTGGCCTCGCATCTTAGAAAG	This study