Supplemental Data

Mycobacterium tuberculosis virulence is mediated by PtpA dephosphorylation of Human Vacuolar Protein Sorting 33B

Supplemental Experimental Procedures

Materials and Methods

Oligonucleotides, expression vectors, and protein production

Oligonucleotide sequences used in this work are available upon request. *ptp*A and *sap*M were amplified from *M. tuberculosis* genomic DNA by PCR. Oligonucleotides were designed including *Afl*II (forward) and *Cla*I (reverse) restriction sites. The amplified gene was cloned into pALACE vector and used for protein overexpression (Bach et al., 2006). *VPS*33B driven by T7 promoter and containing a N'-histidine tag was purchased from Genecopoeia Inc., MD.

Site-directed mutagenesis Site-directed mutagenesis was carried out by PCR overlapping. Oligonucleotides were designed with 15 bases flanking both sides of the mutated codons. The products obtained after PCR amplification were digested with *Dpn*I and chemically transformed into *E. coli* strain DH5α. Point mutations were verified by sequencing (NAPS, UBC, Canada).

Phosphoaminoacid analysis 12 mg of VPS33B was autophosphorylated in a kinase buffer (20mM Tris-HCl, 5mM $MgCl_2$, 2mM $MnCl_2$, 10μCi γ^{32}P-ATP, 1mM DTT, pH 7.0) for 30 min at 37^{o}C. The protein was hydrolyzed after addition of 200 µl of 6N HCl and boiling at 110^{o}C for 2h. The hydrolysate was dried in a Speed-Vac concentrator and stopped when the reaction volume reached about 20 μl. The hydrolysate along P-Ser, P-Thr and P-Tyr standards (Sigma) were analyzed by ascending thin-layer chromatography (flexible cellulose plate, Merck) using a solvent containing isobutyric acid: 0.5M NH_4OH (5:3, v/v). After drying the plate at room temperature, it was exposed to a screen overnight and then scanned in a PhosphorImager. Following the radiolabeling analysis, the phosphoamino acid markers (P-Ser, P-Thr and P-Tyr) were visualized by spraying the plate with an acetonic solution of 0.25% ninhydrin.

***In vitro* dephosphorylation of VPS33B by PtpA** Two separated reactions containing 1 μg of VPS33B were autophosphorylated in a kinase buffer as described in the previous paragraph. After 30 min, the reaction in the first tube was stopped after addition of sample buffer. The second tube was supplemented with 0.34 μg of PtpA for 15 min at 37^{o}C, and then the reaction was stopped by the addition of sample buffer. The same volume of PtpA in the same kinase buffer under the same phosphorylation conditions was used as negative control.

Autophosphorylation of mutated VPS33B Autophosphorylated proteins were resolved in a 10% SDS-gel and exposed to a screen overnight after drying the gel. After image scanning (PhosphorImager) the correspondent spots were excised from the dried gel and the cpm of each sample was measured in a scintillation counter.

Western immunoblot of "trapping" experiment Proteins resolved in SDS-PAGE were transferred to a nitrocellulose membrane. The membrane was blocked with 3% milk/PBS overnight and then exposed to rabbit-αPtpA antibodies for 2h at room temperature. After washing with PBST (PBS/0.05% Tween-20) three times for 15 min each, the membrane was exposed to goat-αrabbit conjugated to HRP (Bio-Rad) for 1h. After washing the membrane as stated above, bands were visualized using the Supersignal West Pico kit (Pierce). The same membrane was stripped using 0.5N NaCl for 5 min at room temperature and re-blocked with 3% milk/PBS overnight. Next day, the membrane was exposed first to sheep αVPS33B antibodies and then to donkey αsheep conjugated to HRP (Jackson) using the same scheme described previously.

Transfection of THP-1 cells 20-40 μg of *malE-sc-007* (αPtpA), *malE-sc-060* (αSapM) or *malE-sc-αubi* (αubi) cloned into pDSRed2-N1 vector were incubated with 70 μl 2M $CaCl_2$ and 450 ml of 0.2 x buffer A (3 mM Sodium citrate, 30 mM NaCl, pH 7.2) and 500 μl buffer B containing per litre: 0.38 g $NaHPO_4$, 0.76 g KCl, 10 g HEPES, 16 g NaCl and 2 g glucose, pH 7.5 for 30 min at room temperature. The mixture was added to 10^6 THP-1 cells and incubated for 1h at 37°C. Cells were rinsed with warmed RPMI 1640 (three times) and glycerol shocked using buffer B supplemented with 15% glycerol for 5 min at room temperature. The transfected cells were washed with warmed RPMI 1640 medium (three times) and resuspended in warmed RPMI 1640 supplemented with 5% FCS, glutamine, and antibiotics as described in Methods. 100 μg/ml neomycin was supplemented to the medium in order to select for positive transfected cells.

Calculation of expressed single-chain antibodies in macrophages The gene coding for DS-red protein was amplified from pDSRed2-N1and subcloned into pMAL (NEB). A calibration curve was plotted using recombinant MBP/DS-red protein in serial dilutions using a 96-well black plate. After counting the cells, serial dilutions of THP-1 monocytes stable-transfected with sc-007 as well as non-transfected THP-1 cells (for background subtraction) were prepared after cell lysis using a hypotonic buffer (20mM Tris-HCl, 1mM DTT, 5% glycerol, 0.1% NP-40, pH 7.2). The fluorescence was measured after elimination of debris by centrifugation at 13000 rpm for 30 min. Fluorescence from non-transfected THP-1 cells was subtracted from sc-007 stable-transfected THP-1 cells.

Determination of PtpA and sc-007 Kd Protein-protein interaction was measured by a Fusion® αHT AlphaScreen apparatus (Perkin Elmer) using the AlphaScreen Histidine Detection kit. This technology requires the utilization of two different tags in the proteins to analyze. Recombinant PtpA fused to GST was biotinylated (Pierce) and used as the donor beads, while His-sc-007 was used as the acceptor beads. The assay was performed according to the instructions of the manufacturer. The obtained results were processed with GraphPad 4.0 software for Kd determination.

Coating Latex Beads 50 μl of surfactant-free white aldehyde/sulfate latex beads (4 mm) (Invitrogen Molecular probes) was washed (three times) with ice-cold 25 mM Mes buffer pH 5.5. Washed beads were mixed with 100 μg of protein in the same buffer at room temperature overnight. Next day, the beads were washed with ice-cold Hank's buffer (three times) and resuspended in 1 ml Hank's buffer supplemented with 0.1% glycine pH7.2. The coating of proteins was verified by SDS-PAGE (data not shown).

Infection of macrophages with latex beads for confocal microscopy Adherent macrophages (on cover slips) were infected with 5 μl protein-coated beads for 3h. The cells were washed with Hank's buffer (Sigma) (three times) and then 2.5% paraformaldehyde was added for 20 min at room temperature. Samples were permeabilized (Bach et al., 2006) with sheep αVPS33B antibodies and then with donkey αsheep antibodies conjugated to Alexa 633 (Jackson).

Macrophages infection Macrophages were infected with opsonized bacteria at a M.O.I of 1:10 for 2h and placed at 37^{o}C and 5% CO_2 after washing away non-internalized bacteria. 100 μg/ml gentamicin or 10 μg/ml amikacin was added to the medium to kill non-washed bacteria. Infected macrophages were collected at different time points, washed with warmed PBS (three times), and plated onto 7H10 plates supplemented with 10% OADC. In the co-infection experiment, plates were supplemented with 20 μg/ml kanamycin for the selection of *Δptp*A mutant.

Gene complementation of Δ*ptp*A mutant strain The *Δptp*A mutant strain was complemented with *ptpA* cloned into pALACE. Electroporated bacteria were plated on 7H10 supplemented with 10% OADC, 20 μg/ml kanamycin and 20 μg/ml hygromycin. A single colony was picked up and grew in rolling bottles containing 7H9 supplemented with 10% OADC, 0.05% Tween-80, 20 μg/ml kanamycin and 20 μg/ml hygromycin. Cells were washed extensively before macrophage infection.

Electron microscopy Adherent macrophages infected with either bacteria or beads were collected by gently scraping after washing with PBS (three times). Cells were pelleted by centrifugation and resuspended in 1.5 ml 2% paraformaldehyde containing 0.1% glutaraldehyde, both EM grade. After 30 min fixation, samples were washed with PBS (three times) for 15 min. Samples were then embedded in 4% low melting point agarose and dehydrated using a graded series of 30%, 50%, 70% ethanol for 10 min each. 90% ethanol was used as the last step of dehydration (three times) for 10 min. Samples were infiltrated in LR Gold (Cedarlane Laboratories) followed by polymerization using 1.5% benzoyl peroxide for 24h on ice. Sample sections of 55 nm thickness were cut using a Leica EM UC6 Ultracut microtome and placed on coated nickel grids. Immunostaining was performed on the grids after blocking (1% BSA, 0.1% Tween-20 in PBS buffer) for 30 min. The grids were immunolabeled with rabbit αPtpA (1:10) and sheep αVPS33B (1:20) antibodies for 1h at room temperature. The grids were washed (0.1% Tween-20 in PBS) and incubated with the same blocking solution followed by incubation with 18 nm colloidal gold affinity pure donkey αsheep (1:10) IgG (H+L) (Jackson) and 10 nm gold particles goat αrabbit IgG (BB International) for 1h at room temperature. The immunolabeling was carried out according to well-established protocols (Jaunin et al., 1998). In order to immobilize

immunogold complexes, sections were fixed in 1% glutaraldehyde for 5 min, and then stained with 2% uranyl acetate. Images were analyzed in a Tecnai 12 electron microscope (FEI Company) at James Hogg iCapture Centre (Saint Paul Hospital, Vancouver, BC, Canada).

Cytosol/membrane fractionation of infected macrophages. Cytosolic and membrane fractions of macrophages infected with the WT, Δ*ptp*A or Δ*ptp*A+p*ptp*A strains were extracted with the ProteoExtract Subcellular Proteome extraction kit (Calbiochem) according to the instruction of the manufacturer.

Co-localization analysis Co-localization analyses were performed using the co-localization analysis plugin of ImageJ program. This plugin is able to generate Pearson's and Mander's coefficients as well as the Intensity Correlation Quotient (ICQ) (http://www.macbiophotonics.ca/ imageJ/colour analysis.htm). Pearson's correlation coefficient (Rr) ranges from -1 to +1. A value of -1 indicates perfect exclusion, zero represents random localization, while +1 indicates perfect correlation. Mander's overlap coefficient (R) ranges from 0 to +1. A value of zero indicates a low co-localization, while +1 represents high co-localization. The ICQ parameter ranges between -0.5 to +0.5. A value between $-0.5 \leq ICQ < 0$ indicates a segregated staining, ICQ~0 represents a random staining, while $0 < ICQ \leq +0.5$ indicates dependent staining. Co-localization analyses were performed by comparing two channels simultaneously.

The co-localization analyses of the confocal microscopy presented throughout this article is shown in Supplemental Table S1.

siRNA and protein complementation Endogenous VPS33B was silenced by transfecting two different siRNA sequences in two independent experiments using the transfection reagent Dharmafect #2. The two sequences corresponding to catalog numbers ON-TARGET plus duplex J-007261-09-0005 and J-007261-10-0005 were purchased from Dharmacon (Lafayette, CO) and used according to the instruction of the manufacturer. Adherent macrophages in 6-well plates were transfected with siRNA for 48h. After washing with Hank's buffer (three times), 1 μg of the quadruple mutated VPS33B (Figure S8) was delivered intracellularly for 2h using Profect P2 (Targeting Systems) and according to the instruction of the manufacturer. WT-VPS33B at same concentration was used as positive control. 1 μg/well FITC-dextran was then supplemented overnight. Next day, cells were washed with Hank's buffer (three times) and delivered intracellularly again following the same protocol as mentioned before. Cells were infected with 25 μl PtpA-, Arg17Ala- or BSA-coated beads for 2h, washed with Hank's buffer (three times) and fixed with paraformaldehyde (Hmama et al., 2004). Samples were processed using a FACS machine (Hmama et al., 2004).

Supplemental Figures

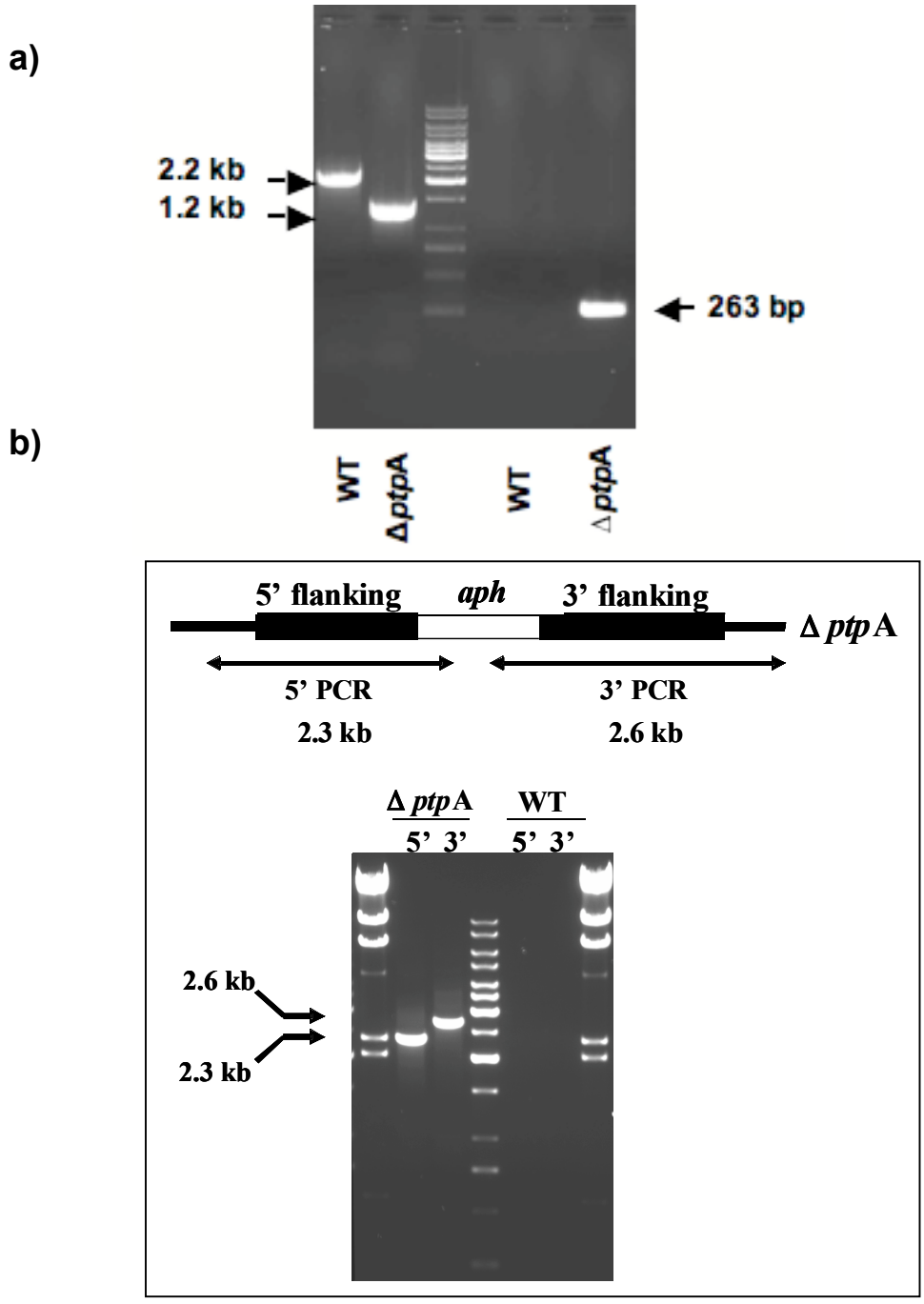


Figure S1. Construction of the *ptpA*-targeting vector.

Approximately 1.9 kb DNA fragments containing the upstream and the downstream regions of *ptpA* gene were amplified by PCR using *Pfu* DNA polymerase (Fermentas) and cloned sequentially into a non-replicating vector pBS-PacI. The 3' flanking region included the last 123 bp of the *ptpA* gene. The *aph* gene from

pUC4K conferring kanamycin resistance was then inserted into the unique *Bam*HI site located between the 5' and 3' flanking regions. The *aph* gene replaced a large segment of the *ptpA* gene (369 bp deletion corresponding to the genomic locations 2507151 to 2507519). A gene encoding gentamicin resistance obtained from pUC-Gm was cloned upstream of the 5' flanking region at the unique *Eco*RI site, and the *Pac*I-cassette of pGOAL17 containing the *lacZ-sacB* genes (Parish and Stoker, 2000) was cloned at the unique *Pac*I site located downstream of the 3' flanking region to make the final *ptpA*-knockout construct (Figure 1A I). This plasmid carried the *aph* gene (located between the *ptpA* flanking regions) for positive selection and the *B. subtilis sacB* gene to facilitate counterselection on sucrose (Pelicic et al., 1996). In addition, the gentamicin resistance cassette and the *lacZ* marker located in the vector served to screen the loss of the vector following homologous recombination.

Isolation of *M. tuberculosis* Δ*ptp*A. The *ptpA* deletion mutant was isolated by a sequential two-step selection protocol involving positive selection for kanamycin resistance and counterselection on medium containing 2% sucrose. The *ptpA*-targeting vector was electroporated into *M. tuberculosis* H37Rv strain and transformants were selected on 7H11+Kan plates. Colonies displaying the expected phenotype for single crossovers (KanR colonies displaying expression of the *lacZ* and *gm* genes) were grown further and counterselected on 7H11 + Kan + 2% sucrose plates. Putative recombinants with the desired phenotypes (Suc^R, Kan^R, Gm^S, $LacZ^-$) were further screened by PCR using pairs of oligonucleotides (located on either side of the deleted region). This PCR was expected to amplify a 1.2 kb product in the wild type and a 2.2 kb product in Δ*ptpA* strain (Figure S1a). In another PCR analysis, a set of oligonucleotide pairs located within the deleted region was used, and as expected, the specific 263 bp product was amplified only from the wild type DNA and not from the *ΔptpA* DNA (Figure S1a).

Furthermore, PCR analyses using pairs of oligonucleotides, one located in the *aph* gene and the other on the genomic DNA external to the cloned flanking regions amplified the expected size 5' and 3' PCR products from the Δ*ptpA* DNA (Figure S1b).

For Southern hybridization analysis, a 547 bp probe corresponding to the genomic location 2505977-2506524 was amplified by PCR and radioactively labeled with α-^{32}P-dCTP using the MegaPrime DNA labeling system (GE Healthcare). The Southern analysis revealed the expected *Xho*I restriction digestion pattern for the wild type and the Δ*ptpA* mutant strain (Figure 1A II).

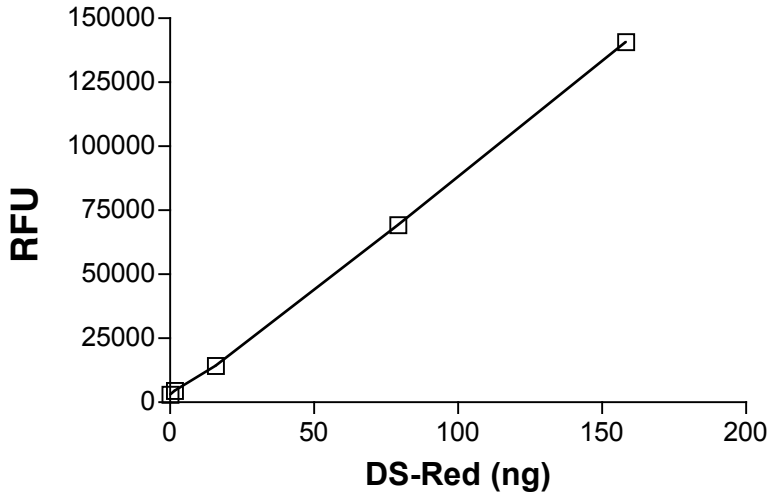
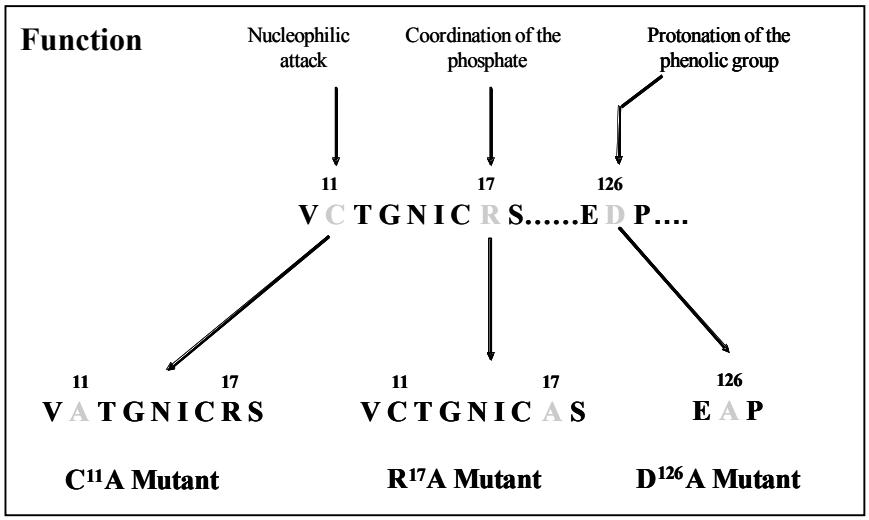


Figure S2. Calibration curve for DS-Red protein to calculate intracellular-expressed single-chain antibodies.

Recombinant DS-red protein was over-expressed using pMAL vector. Purified protein was used for preparing serial dilutions. Samples were measured in a Fusion® αHT AlphaScreen apparatus (Perkin Elmer) using 480 nm excitation and 580 nm emission filters. Each point represents an average of three consecutive measuring by the apparatus.

A



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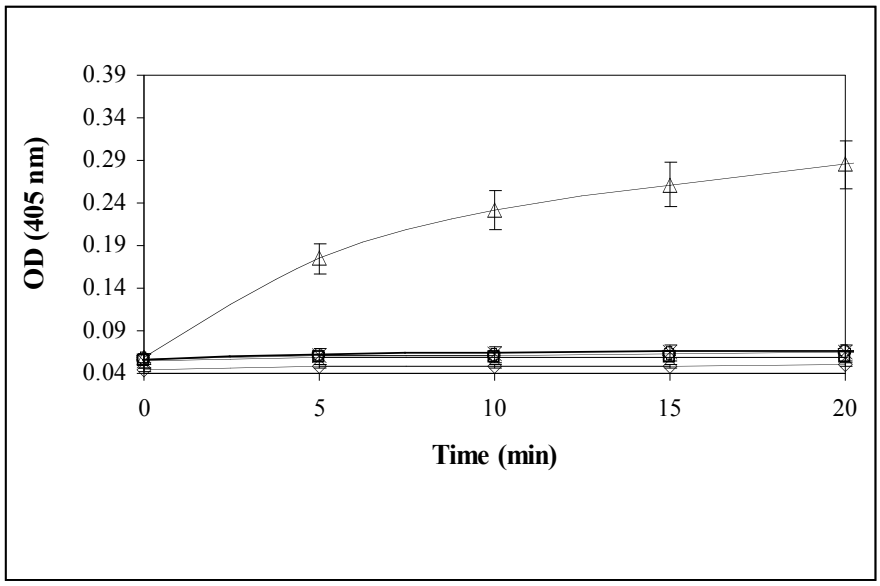


Figure S3. (A) Scheme describing the residue functions and the site-directed mutations made in *ptp*A. (B) PtpA catalytic activity. The activity of recombinant active and defective PtpA proteins was followed by cleavage of *p*-nitrophenyl phosphate (Cowley et al., 2002). The expression of PtpA was carried out according to (Bach et al., 2006). WT- PtpA (open triangles), Asp126Ala (open circles), Cys11Ala (X), Arg17Ala (open rhombuses), BSA (open squares). Results are expressed as mean ± SD.

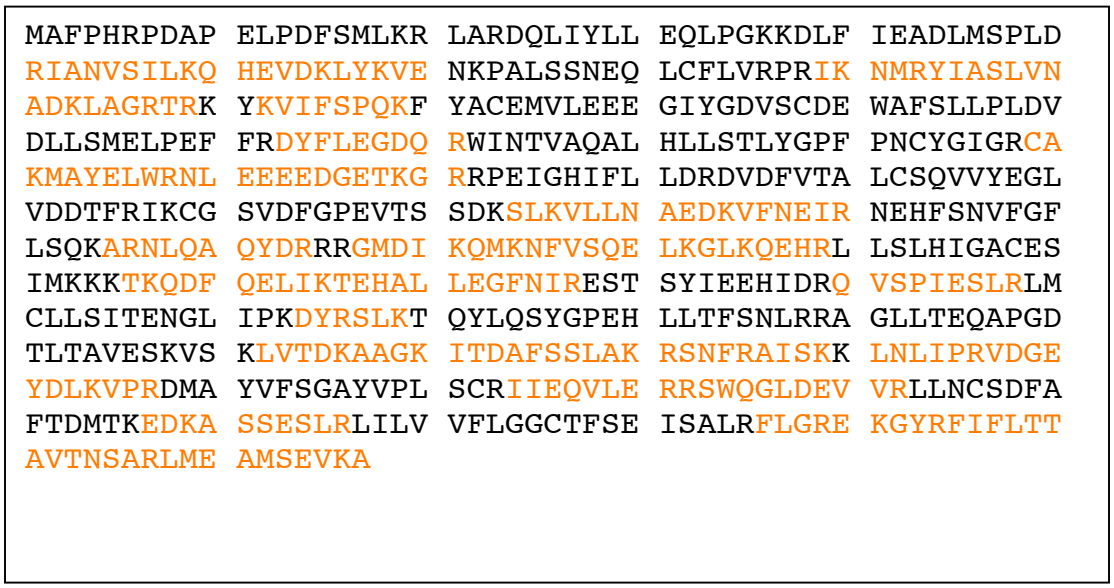
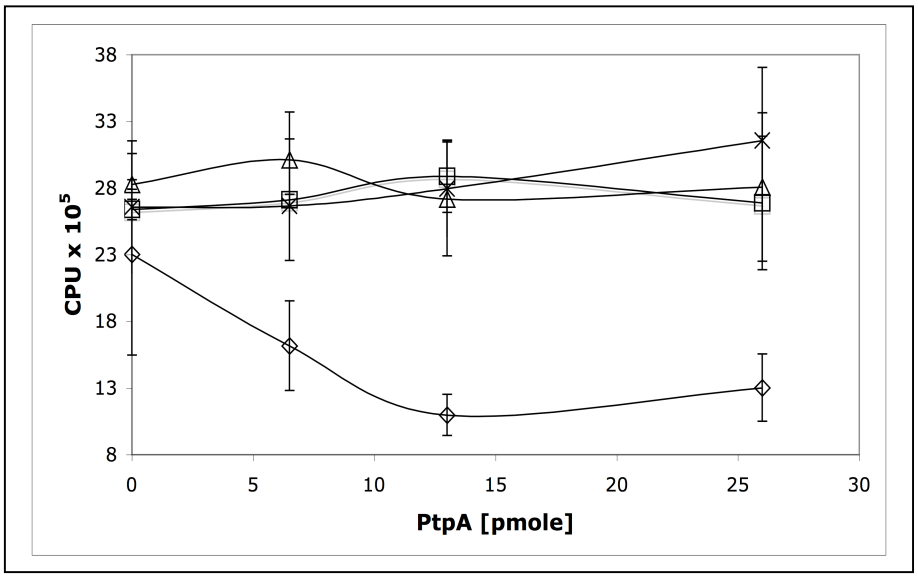


Figure S4. Identification of VPS33B.

Peptides corresponding to VPS33B were identified by ESI MS/MS (in red).

A



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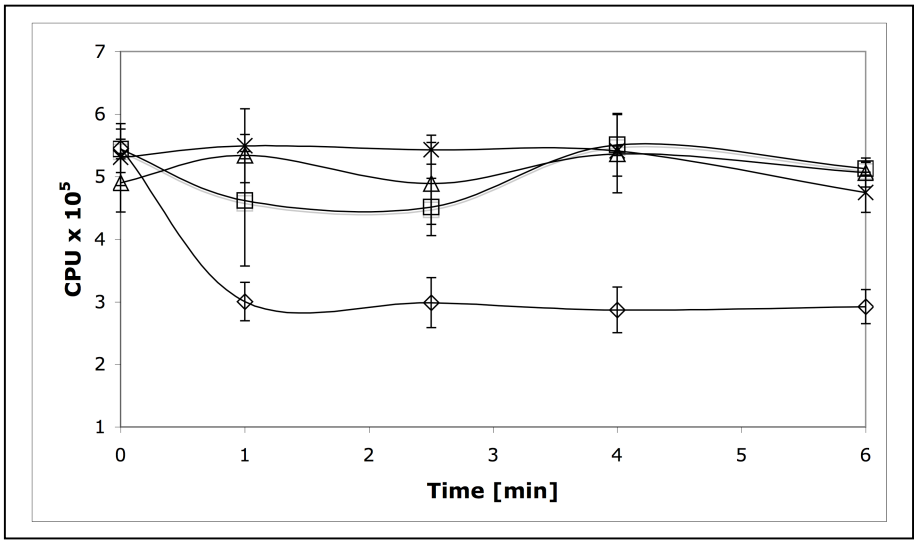

Figure S5. Time- and dose-dependent dephosphorylation of VSP33B by active and non-active PtpA.

Recombinant VPS33B was autophosphorylated in a reaction containing kinase buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, 2 mM $MnCl_2$, 1 mM DTT, pH 7.5) for 30 min at 30^{o}C. Reaction mixtures were stopped by addition of 10% phosphoric acid and spotted on phosphocellulose paper. After washing the papers extensively with 1% phosphoric acid (three times), radioactivity levels were read in a scintillation counter. The dephosphorylation by active PtpA was followed in a dose (A)- and time (B) -dependent manner. Wt-PtpA = open rhombuses, $C^{11}A$ = open squares, $D^{126}A$ = open triangles, and $R^{17}A$ = X. Each reading point represents the mean ± SD of three independent experiments.

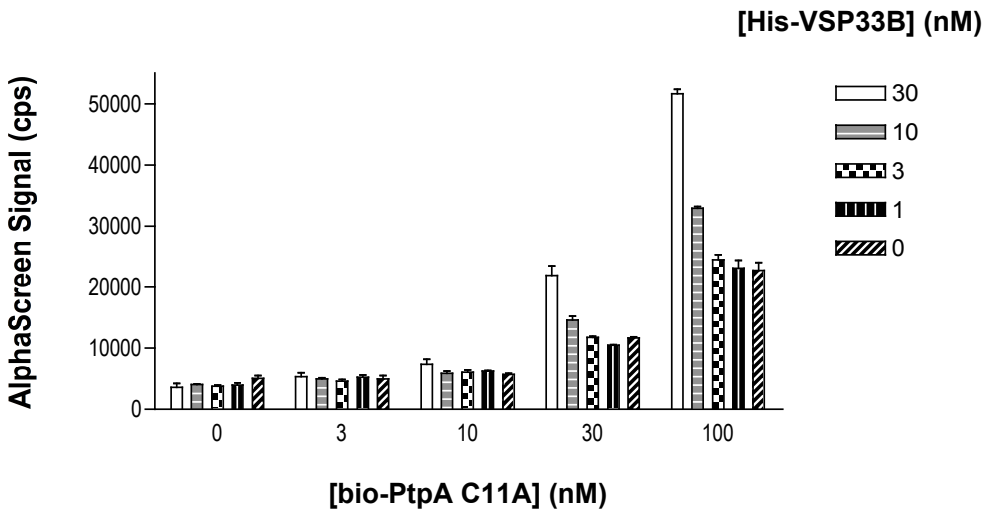

Figure S6. VPS33B and PtpA interaction using Amplified Luminescent Proximity Homogeneous Assay (AlphaScreen Technology).
AlphaScreen Histidine (Nickel Chelate) detection kit was used for determination of protein-protein interactions. VSP33B was immobilized to beads by his tag, while Cys11Ala mutant was immobilized by biotinylation according to the instruction of the manufacturer (Pierce, IL). The assay buffer contained 25 mM Hepes, 100 mM NaCl, and 0.1% Tween-20, pH 7.4. The interaction was performed overnight and the results represent the mean ± SD of three independent experiments. Wild-type PtpA and Asp126Ala mutant failed to interact with VPS33B as a result of the redox state of the reaction (data not shown). However, the Cys11Ala mutant showed a Kd of 2.119 nM. This result was expected as it has been reported that low molecular weight protein tyrosine phosphatases protect the catalytic site by the formation of a S-S bridge between Cys11 and Cys16 in PtpA as a response to the changing redox states (see reference in the main text). Then, we suggest that as a result of the absence of reducing agents in the reaction and as well as one of the cysteine partners (Cys11Ala), the active site, although inactive, was still sufficient to interact with VPS33B.

Tyrosine predictions

Name	Pos	Context	Score	Pred
		v		
VPS33B	28	DQLIYLLEQ	0.042	.
VPS33B	67	VDKLYKVEN	0.161	.
VPS33B	94	KNMRYIASL	0.177	.
VPS33B	111	RTRKYKVIF	0.119	.
VPS33B	121	PQKFYACEM	0.035	.
VPS33B	133	EEGIYGDVS	0.985	*Y*
VPS33B	164	FFRDYFLEG	0.052	.
VPS33B	187	LSTLYGPFP	0.082	.
VPS33B	194	FPNCYGIGR	0.064	.
VPS33B	204	AKMAYELWR	0.064	.
VPS33B	247	SQVVYEGLV	0.141	.
VPS33B	312	LQAQYDRRR	0.022	.
VPS33B	382	ESTSYIEEH	0.903	*Y*
VPS33B	415	IPKDYRSLK	0.078	.
VPS33B	422	LKTQYLQSY	0.577	*Y*
VPS33B	426	YLQSYGPEH	0.014	.
VPS33B	501	VDGEYDLKV	0.215	.
VPS33B	511	RDMAYVFSG	0.860	*Y*
VPS33B	517	FSGAYVPLS	0.625	*Y*
VPS33B	593	REKGYRFIF	0.331	.
		^		

Figure S7. Prediction of phosphorylation sites in VPS33B and site-directed mutagenesis.

Potential tyrosine phosphorylation sites were predicted using Netphos 2.0 (Blom et al., 1999) (http://www.cbs.dtu.dk/services/NetPhos/). Selected Tyr residues (in yellow) were consecutively mutated to Glu in the same original VPS33B plasmid starting at Y133 and using the PCR overlapping method described previously in page 1. Double, triple and quadruple mutated proteins were over-expressed, purified and the kinase activity of each mutation was performed as described previously (Supplemental Experimental Procedures).

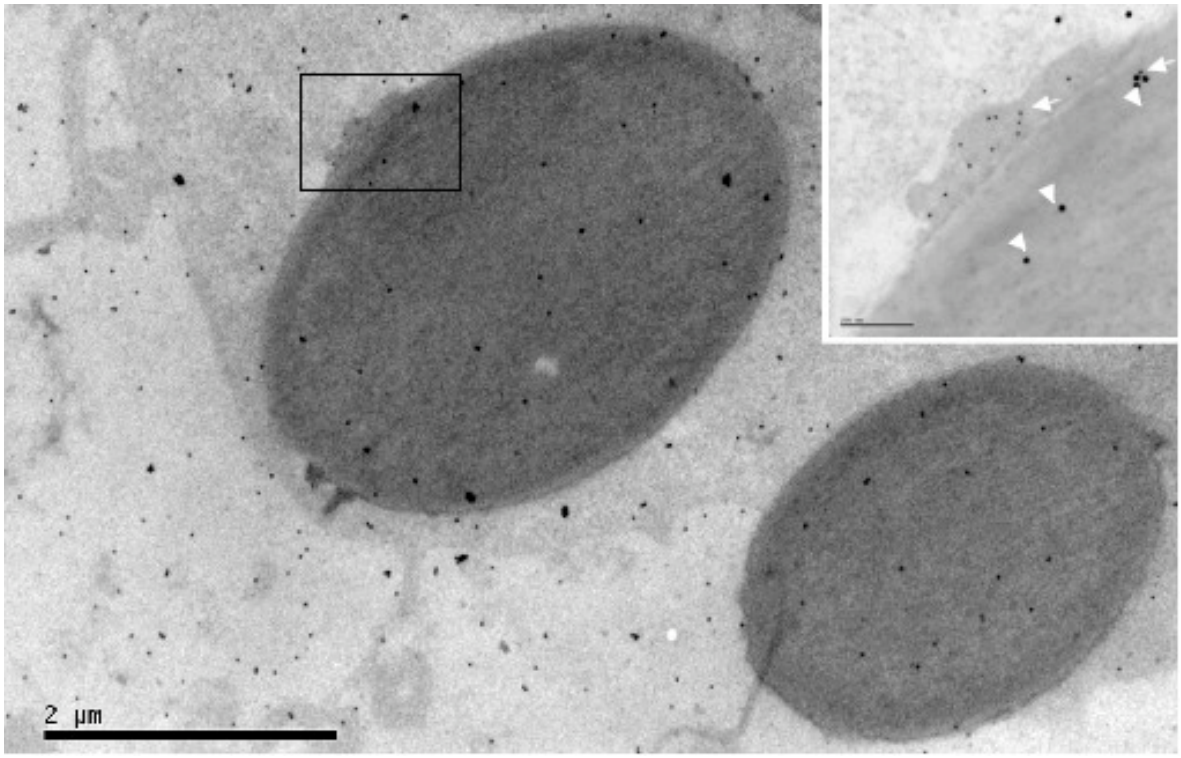

Figure S8. Electron microscopy of macrophages infected with PtpA-coated latex beads. Samples were processed as described in Supplemental Experimental Procedures. White arrows point to immuno-labeling of PtpA (10 nanogold conjugated antibodies) while arrowheads indicate VPS33B (18 nanogold conjugated antibodies). Resolution is 9700X, Insert: 97000X. Bars in insert is 0.2 µm.

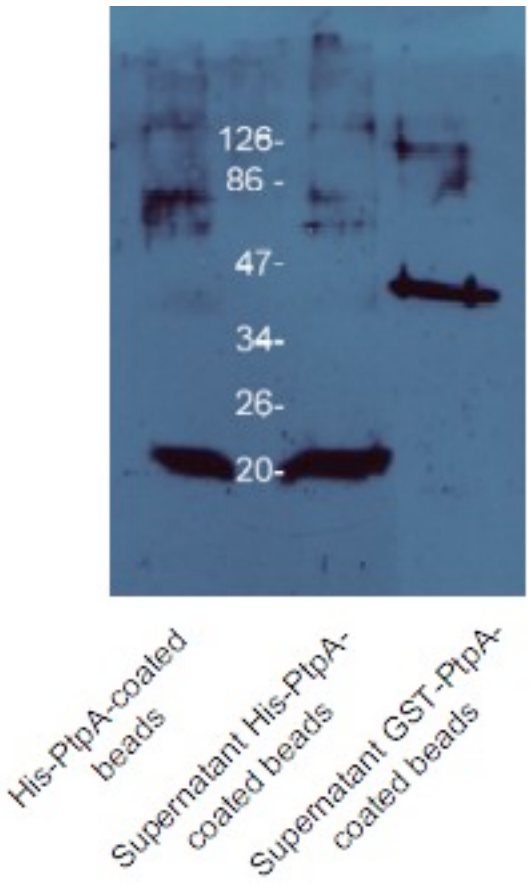

Figure S9. Detachment of PtpA from latex beads.
Beads were kept at 4°C overnight in Hank's buffer. Next day, beads were separated by centrifugation (10000 rpm for 5min, three times) and 1 volume of supernatant was diluted in 5 volumes of cold acetone, placed at -20°C for 2h. Precipitated proteins were centrifuged at 13000 rpm for 30 min, resuspended in sample buffer and resolved on a 12% SDS-PAGE, blotted onto a nitrocellulose membrane and blocked with 3% milk in PBS overnight. Polyclonal rabbit αPtpA antibodies (1:5000) were used as primary antibody while goat αrabbit antibodies (1:5000) (Bio Rad) were used as secondary antibodies.

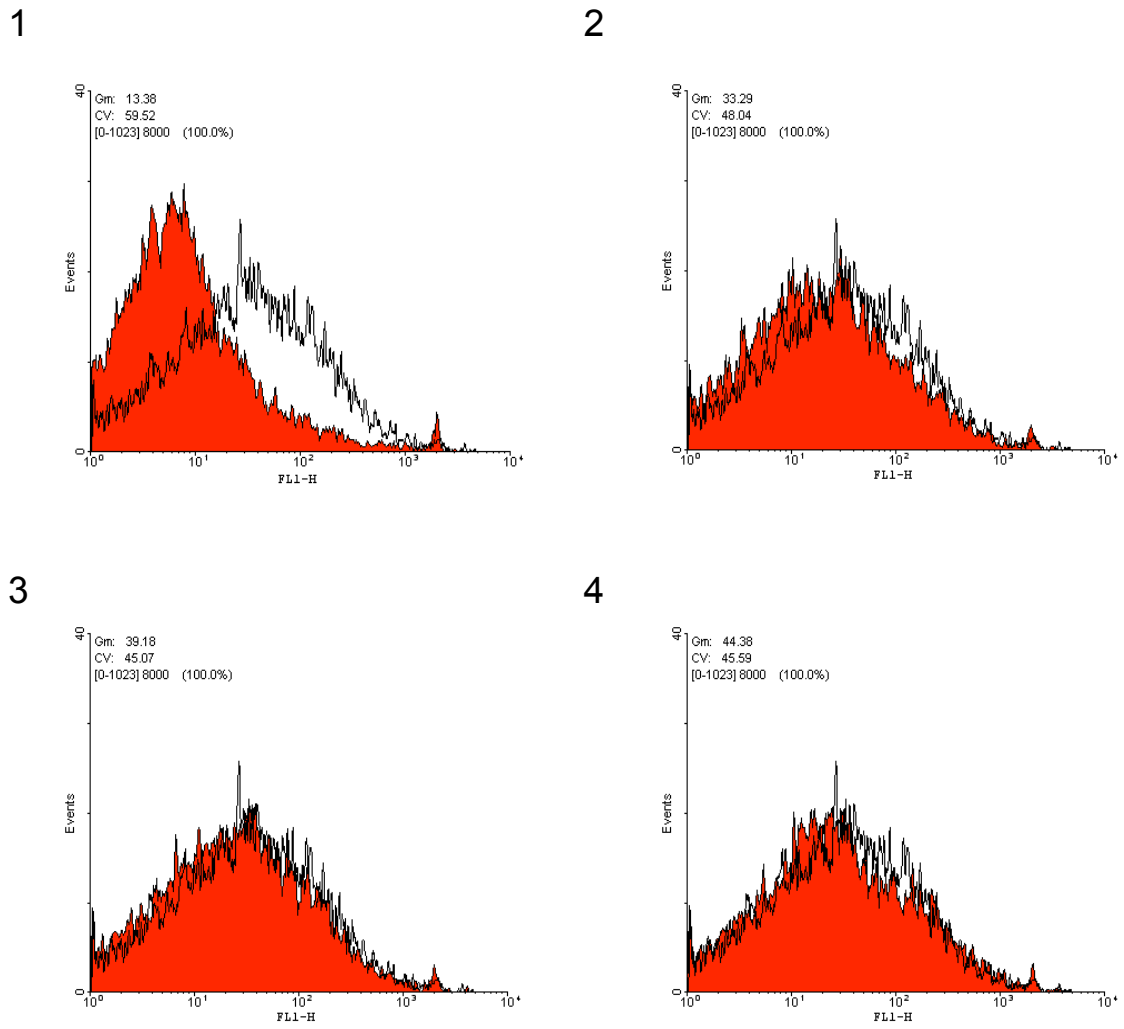

Figure S10. Inhibition of phagosome-lysosome fusion.
Inhibition of phagosome-lyososome fusion by latex beads coated with recombinant PtpA and catalytic-defective PtpA using FACS analysis. Phagosome-lysosome fusion was quantified using a flow cytometry based system based on the co-localization of fluorescent FITC-dextran labeled lysosome and coated beads (Hmama et al., 2004). **1**. Wt-PtpA **2**. $D^{126}A$-PtpA **3**. $C^{11}A$-PtpA **4**. Wt-PtpA incubated with sc-007 prior to macrophage infection. Graphs were overlaid with BSA-coated bead graph used as control (in black). One representative experiment is shown.

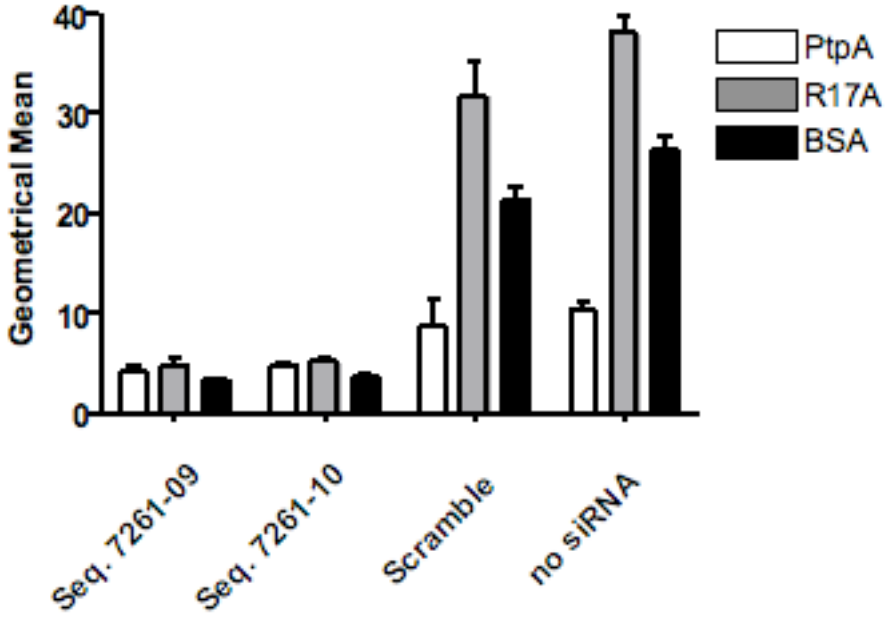

Figure S11. Infection of siRNA-VPS33B silenced macrophages with coated beads.

Inhibition of phagolysosome fusion in cells treated with siRNA sequences J007261-9, J007261-10 and J-007261 (scramble). Silenced THP-1 cells were infected with protein-coated beads and processed as described in Supplemental Experimental procedures. Results are expressed as mean ± SD. Geometrical mean is the distribution parameter given by the FACS machine and is obtained by multiplaying the "n" individual values of a cluster together and getting the n^{th} root of this product.

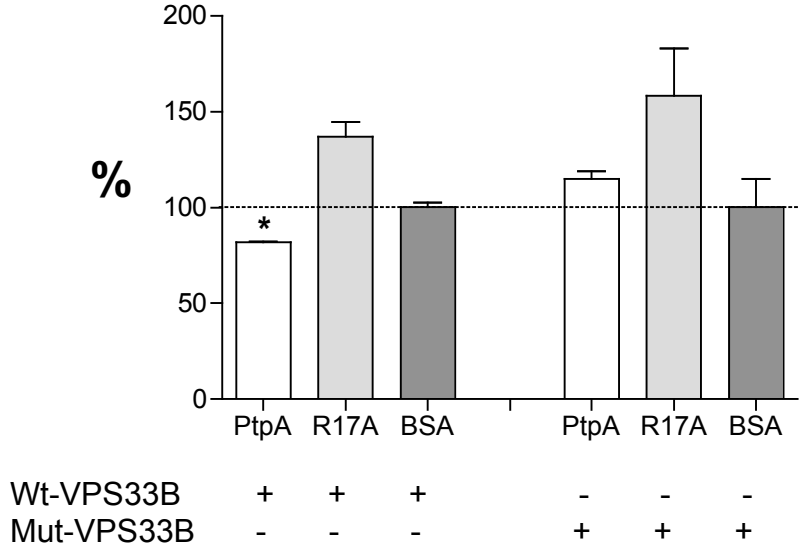

Figure S12. Infection of siRNA-VPS33B silenced macrophages with coated beads after complementation with VPS33B variants.

Inhibition of phagolysosome fusion in cells treated with siRNA-J007261-10. Silenced THP-1 cells were infected with protein-coated beads and complemented by delivery of either WT- VPS33B or VPS33B quadruple mutant according to the methods described in Supplementary Experimental Procedures. Results were normalized to 100% phagolysosome fusion of BSA. Asterisk represents a statistical mean difference using ANOVA one-way analysis. *P* value=0.0093. Results are expressed as mean ± SD.

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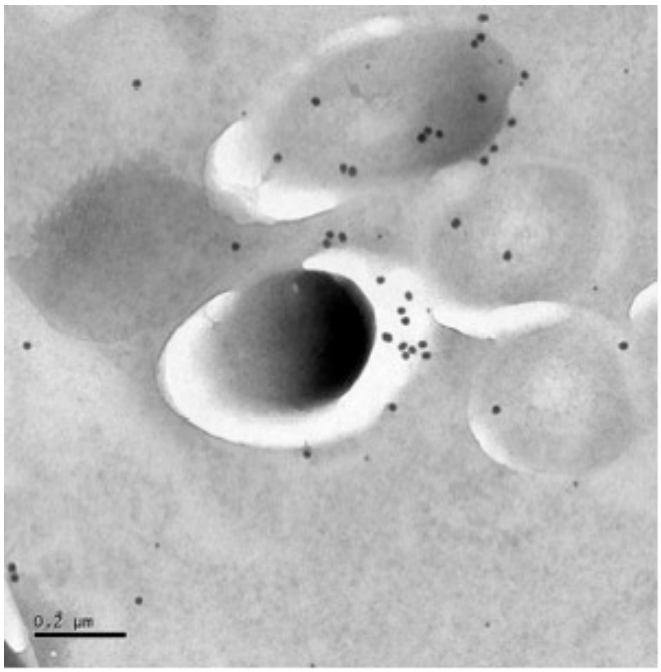

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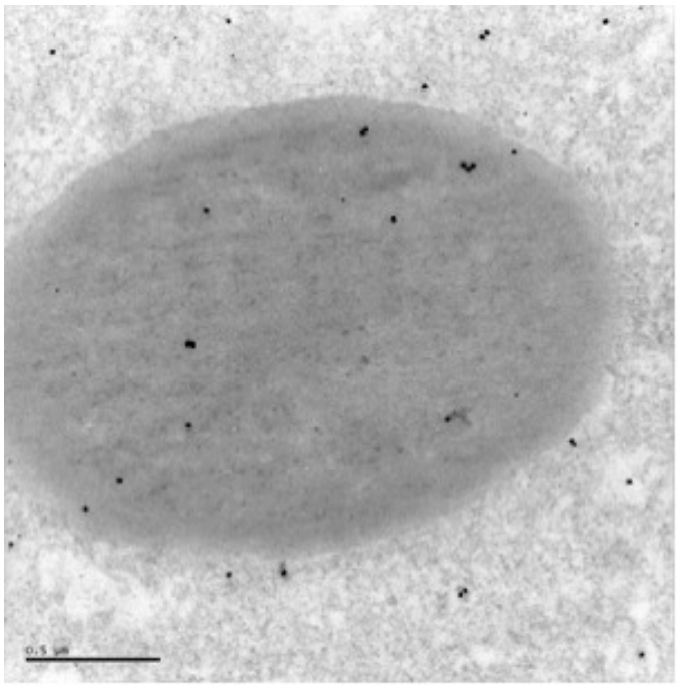


Figure S13. Electron microscopy of macrophages infected with Δ*ptp*A strain (A) and empty beads (B). Samples were processed as described in Supplemental Experimental Procedures. Resolution is 46000X in (A) and 37000X in (B).

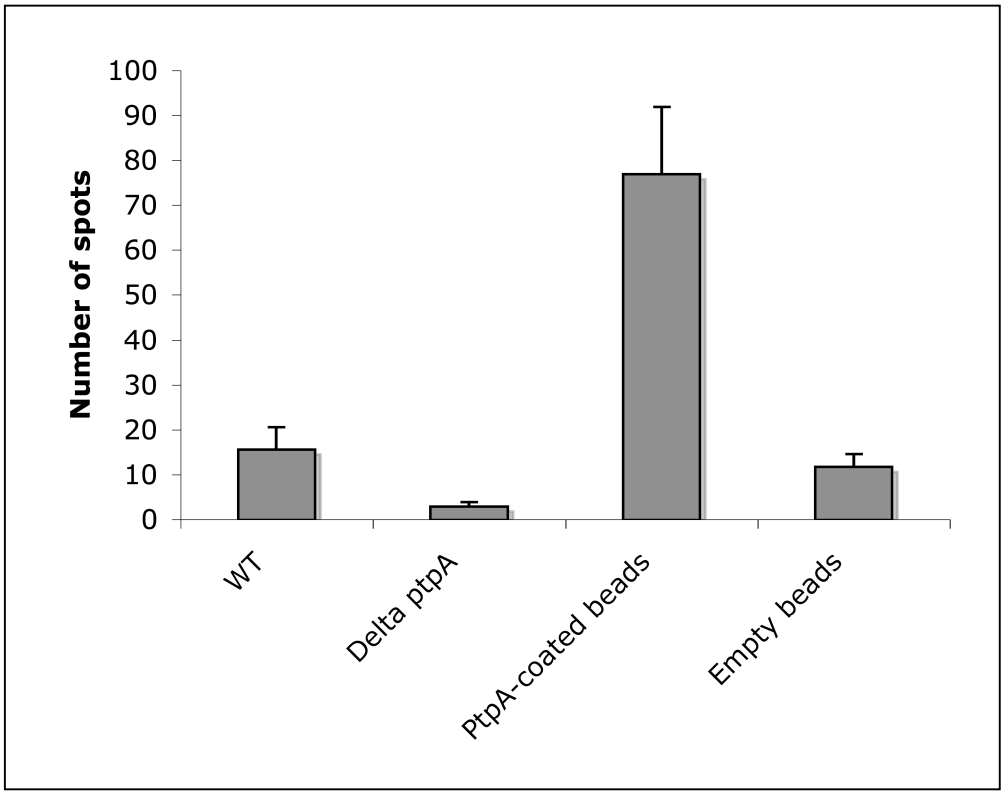


Figure S14. Statistical analysis of gold immunolabeling. Infected macrophages were gold immunolabeled according to Supplemental Experimental Procedures. 10 nanogold spots corresponding to PtpA were counted in a diameter of 0.5 μM around the bacterium- or bead-containing phagosome. Bars represent the mean ± SD of 20 and 10 different bacterium- or bead-infected macrophages.

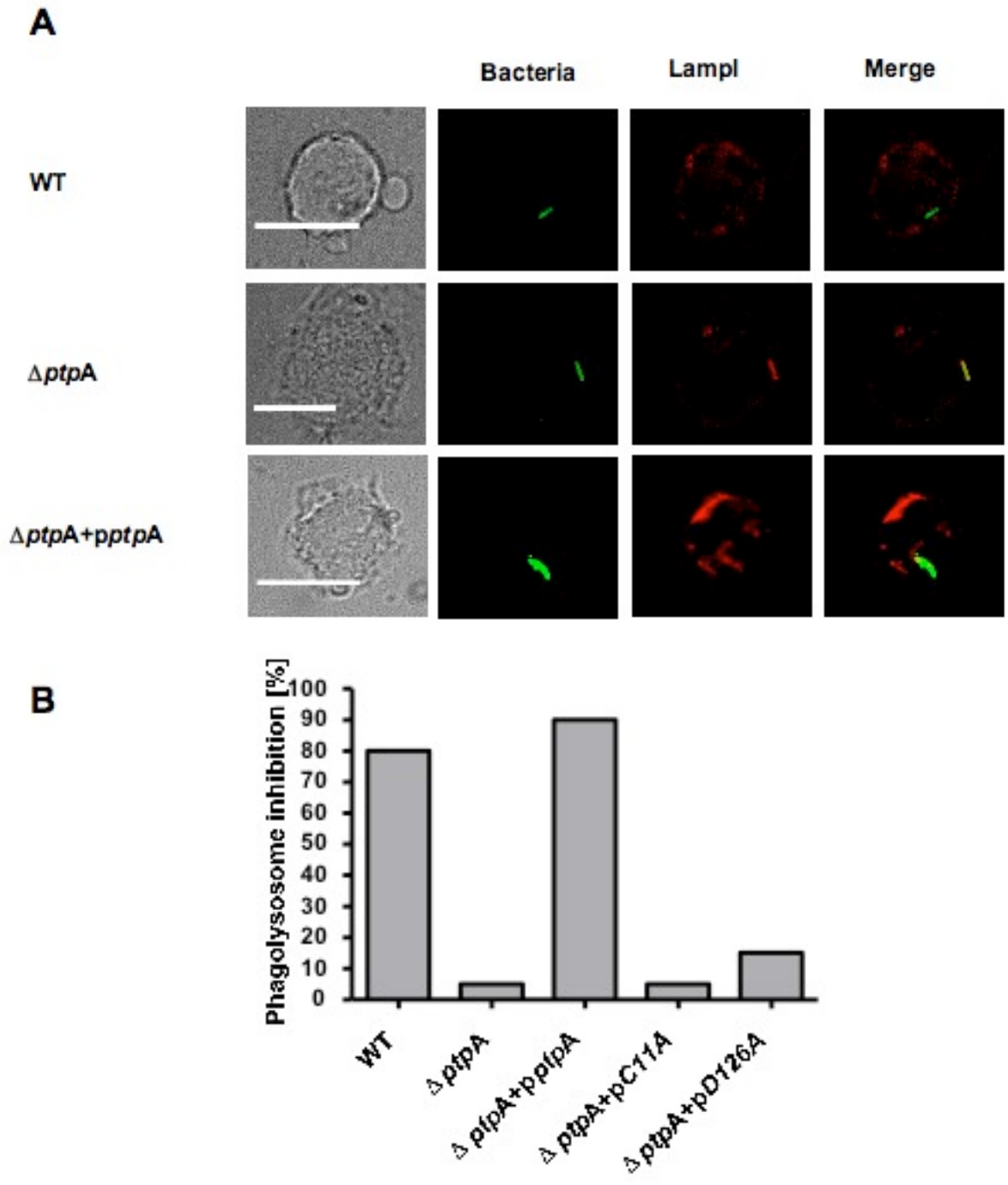


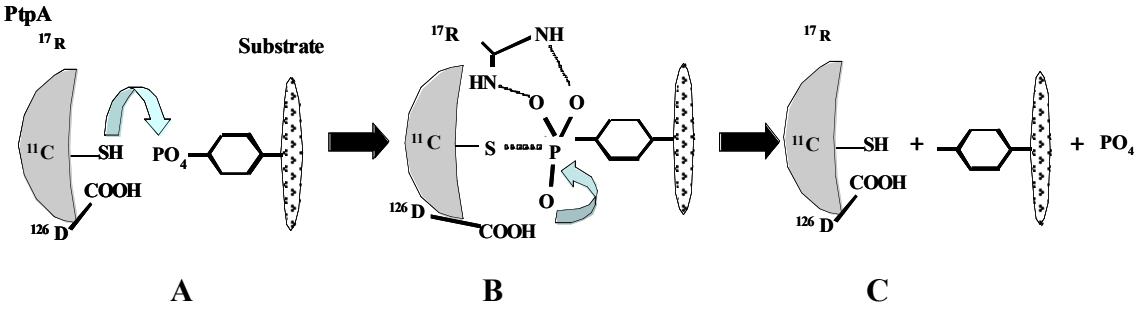
Figure S15. Co-localization of bacteria and lysosomes in infected macrophages

A) Macrophages were infected with live bacteria and immunostained as described in Supplemental Experimental Procedures. Goat-αmouse coupled to Texas Red was used to detect αLampI. Bacteria were stained with FITC. White bar is 20 µm. B) Percentage of phagolysosome inhibition of (A). Results are the observation of 20 independent infected macrophages. Phago-lysosome fusion is defined when the bacterium (in green) co-localized to LampI (in red), a marker for lysosomes.

Table S1 Co-localization analyses of the confocal microscopy images presented in this article.

Image	Rr	R	ICQ
Figure 3			
Live-WT	0.924	0.971	0.379
Live-KO	-	-	-0.5
Killed-WT	0.045	0.087	0.019
αVPS16/αVPS33B	0.95	0.967	0.425
bacterium/ αVPS33B	0.323	0.848	0.234

Notes



Scheme of PtpA catalytic activity

A. PtpA cysteine at position 11 attacks nucleophilically the phosphorylated tyrosine substrate. **B.** PtpA aspartate at position 126 protonates the phenolic oxygen and a cysteinyl-phosphate intermediate is formed facilitating the cleavage of the P-O bond. **C.** The transient intermediate is hydrolyzed by an activated water molecule.

Supplemental References

Bach, H., Sun, J., Hmama, Z., and Av-Gay, Y. (2006). *Mycobacterium avium* ssp *paratuberculosis* PtpA is an endogenous tyrosine phosphatase secreted during infection. Infect. Immun. *74*, 6540-6546.

Blom, N., Gammeltoft, S., and Brunak, S. (1999). Sequence and structure-based prediction of eukaryotic protein phosphorylation sites. J. Mol. Biol. *294*, 1351-1362.

Cowley, S. C., Babakaiff, R., and Av-Gay, Y. (2002). Expression and localization of the *Mycobacterium tuberculosis* protein tyrosine phosphatase PtpA. Res. Microbiol. *153*, 233-241.

Hmama, Z., Sendide, K., Talal, A., Garcia, R., Dobos, K., and Reiner, N. E. (2004). Quantitative analysis of phagolysosome fusion in intact cells: inhibition by mycobacterial lipoarabinomannan and rescue by an 1alpha, 25-dihydroxyvitamin D3-phosphoinositide 3-kinase pathway. J. Cell Sci. *117*, 2131-2140.

Jaunin, F., Visser, A. E., Cmarko, D., Aten, J. A., and Fakan, S. (1998). A new immunocytochemical technique for ultrastructural analysis of DNA replication in proliferating cells after application of two halogenated deoxyuridines. J. Histochem. Cytochem. *46*, 1203-1209.

Parish, T., and Stoker, N. G. (2000). Use of a flexible cassette method to generate a double unmarked *Mycobacterium tuberculosis* tlyA plcABC mutant by gene replacement. Microbiology *146 (Pt 8)*, 1969-1975.

Pelicic, V., Reyrat, J. M., and Gicquel, B. (1996). Expression of the *Bacillus subtilis sac*B gene confers sucrose sensitivity on mycobacteria. J. Bacteriol. *178*, 1197-1199.