

SUPPLEMENTARY INFORMATION

Control of *Mycobacterium tuberculosis* protein secretion by ESX-4 and the outer membrane EsxUT-EsxEF complex

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Supplementary Figures

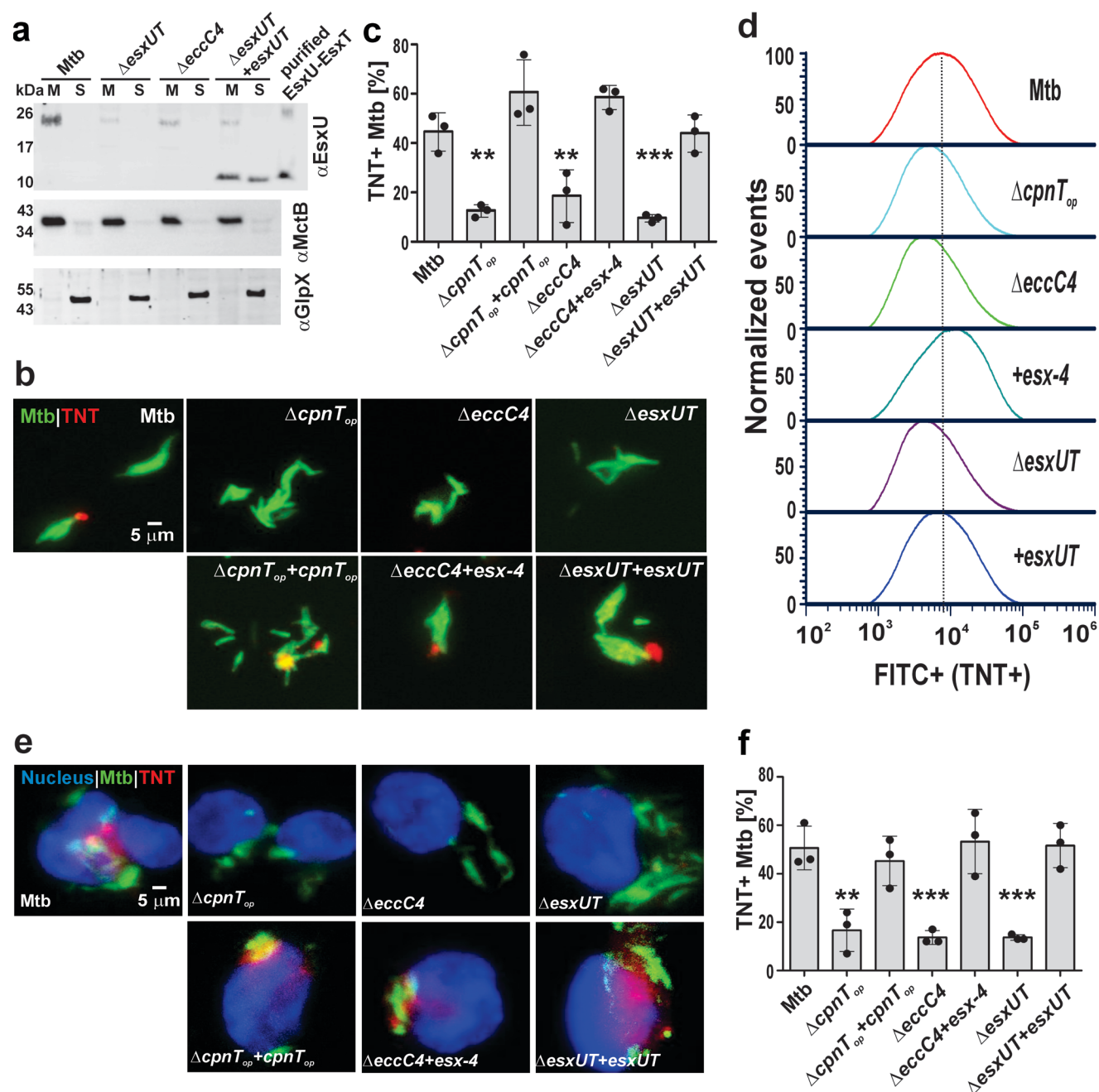


Figure S1. EsxUT is required for TNT surface accessibility on the surface of *M. tuberculosis* and its secretion into the cytosol of infected macrophages.

a. Subcellular localization of EsxU in the indicated Mtb strains using an anti-EsxU antiserum. A band corresponding to ~ 26 kDa is observed in Mtb, which correlates to a heterotrimer consisting of EsxU (10 kDa), EsxT (6kDa) and EsxU or an EsxU homolog. The Mtb strain Δ eccC4 lacks the EccC4 ATPase, which is essential for the activity of the ESX-4 system. MctB and GlpX were used as marker proteins for the membrane and cytosolic fractions, respectively. 100 ng purified EsxU-EsxT were loaded. M: membrane fraction; S: soluble fraction. **b.** Detection of surface accessible TNT in Mtb by fluorescence microscopy. The indicated Mtb strains were metabolically labeled with DMN-Trehalose (green) and stained with an anti-TNT antiserum (red). Scale bar = 5 μ m. **c.** Quantification of surface-

accessible TNT in Mtb from images shown in Fig. 1b. Strains were scored positive for TNT relative to Mtb $\Delta cpnT_{op}$ (n=3 independent experiments). **d.** Surface accessibility of TNT in 50,000 Mtb cells by flow cytometry using anti-TNT antiserum. The peak fluorescence for wt Mtb is indicated by a dashed line. **e.** Analysis of TNT secretion into the cytosol of THP-1 macrophages infected with DMN-Trehalose labelled Mtb strains (green) at an MOI of 10:1. The cells were fixed, permeabilized with 0.2% Triton X-100 and stained using anti-TNT antiserum (red). The nuclei of the macrophages were stained with DAPI (blue). Scale bar = 5 μ m. **f.** Quantification of TNT secretion into the cytosol of infected macrophages in images shown in Fig. 1e, relative to Mtb $\Delta cpnT_{op}$ (n=3 independent experiments). TNT-positive *M. tuberculosis* cells (%) are quantified in the plots. All quantitative data (c, f) are represented as mean $\pm$ standard deviation (SD). Asterisks indicate significant differences (**p value ≤ 0.01 , ***p value ≤ 0.001 using one-way ANOVA with Dunnett's correction) compared to wt Mtb. Representative images/blots of three independent experiments are shown (n=3). Source data are provided in the Source Data file.

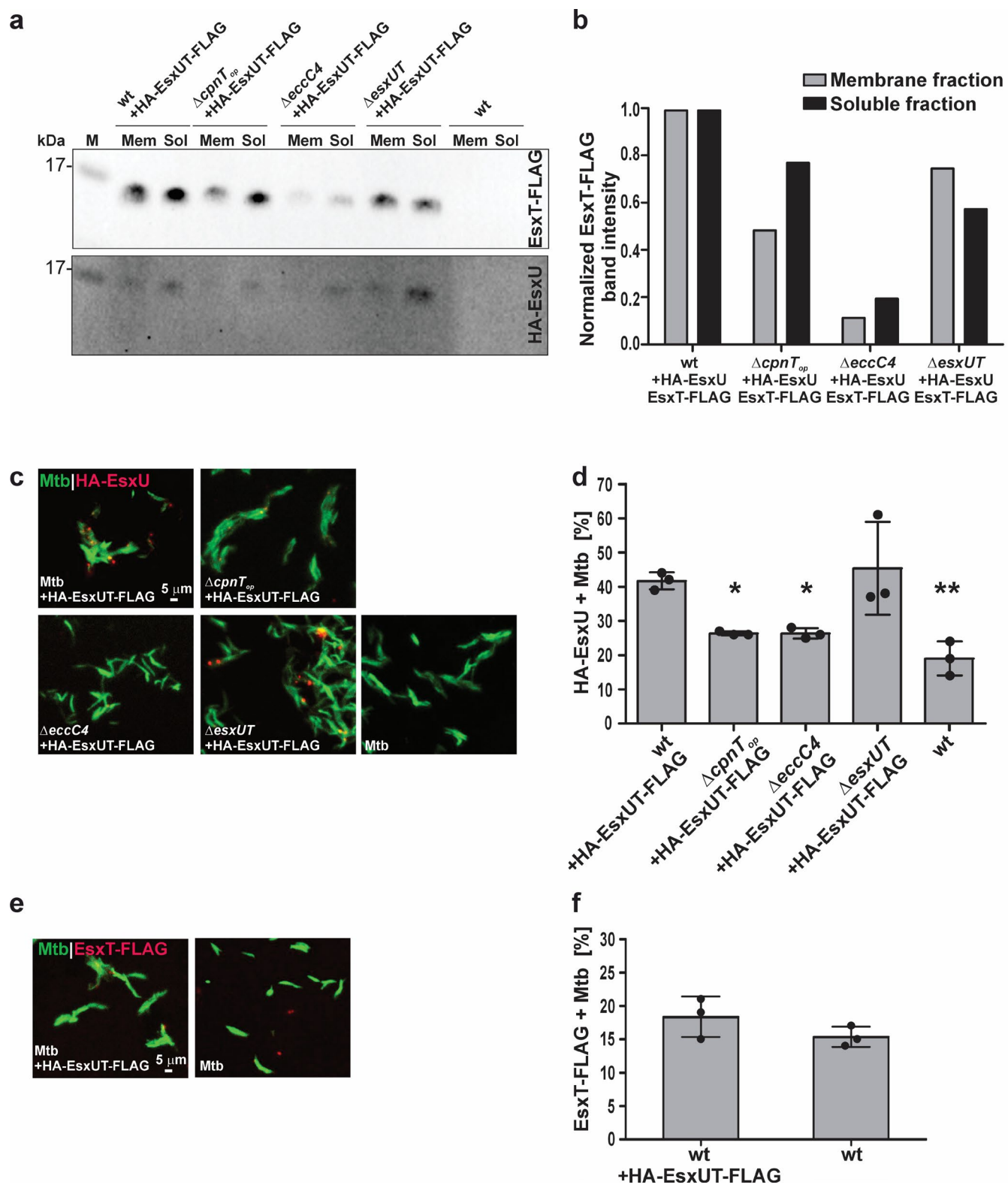


Fig. S2: EsxEF is required for the membrane localization and surface-accessibility of EsxUT in *M. tuberculosis*.

a. The cell lysates of Mtb strains producing $_{HA}EsxU/EsxT_{FLAG}$ were subjected to ultracentrifugation to obtain water-soluble (Sol) and membrane (Mem) fractions. The localization of $_{HA}EsxU$ and $EsxT_{FLAG}$ was detected using a monoclonal HA antibody and a polyclonal FLAG antibody, respectively. The gel was loaded with 10 μ g of each fraction for the detection of the tagged EsxU and EsxT. **b.** Quantification of the EsxT-FLAG bands from the immunoblot in **a** using ImageJ. **c, e.** Surface accessibility of (**c**) HA-tagged EsxU and (**e**) FLAG-tagged EsxT in Mtb strains producing $_{HA}EsxU/EsxT_{FLAG}$. The Mtb strains were metabolically labeled with DMN-trehalose (green) and stained with a monoclonal HA antibody or polyclonal FLAG antibody which detects the tagged EsxU or EsxT, respectively (red). The surface exposure of the tagged EsxU or EsxT was analyzed using a fluorescence microscope. **d, f.** Quantification of fluorescence images to determine *M. tuberculosis* cells (%) with surface accessible HA-tagged EsxU and FLAG-tagged EsxT, scored relative to untagged wt. Data are represented as mean $\pm$ SD of three independent experiments (n=3) and representative images are shown. Asterisks indicate significant differences (*p value $\leq$ 0.05, **p value $\leq$ 0.01; one-way ANOVA with Dunnett's correction) in comparison to wt *M. tuberculosis* producing $_{HA}EsxU/EsxT_{FLAG}$ protein. Source data are provided in the Source Data file.

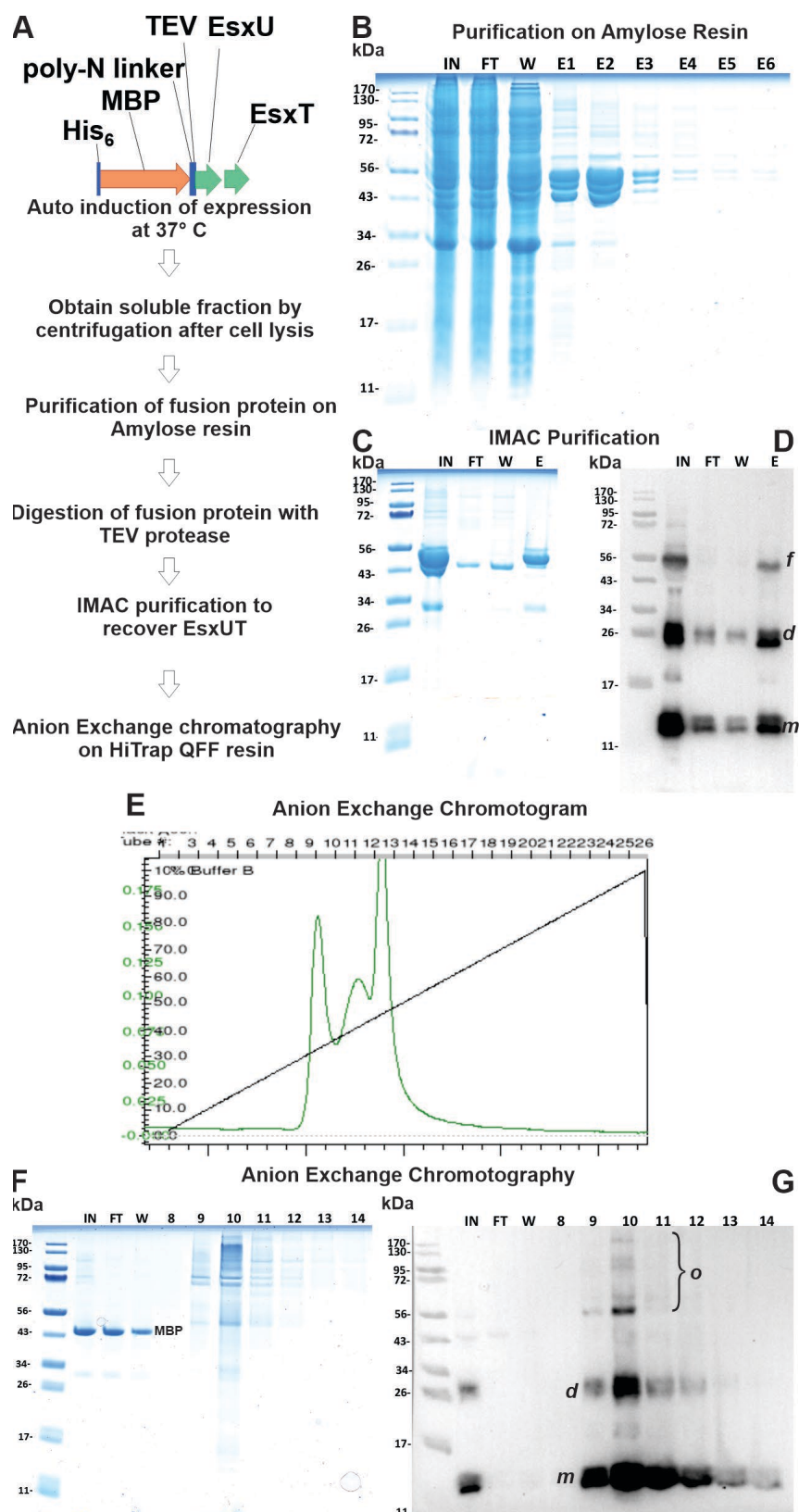


Figure S3. Purification of the *M. tuberculosis* EsxUT complex from *E. coli*.
A. Schemes of MBP-EsxUT fusion protein construct and purification steps.

B. SDS-PAGE of purification of MBP-EsxUT fusion protein on Amylose resin. Lanes: IN – input, soluble fraction after cell lysis and centrifugation at 50 k g; FT – flow-through; W – wash with column buffer without D-maltose; E1-E6 – elutions with 20 mM D-maltose buffer. 5 µg of protein was loaded per lane. Molecular weight protein standard in kDa is on the left.

C and D. IMAC purification of EsxUT after TEV protease treatment. Samples were analyzed on polyacrylamide gel (C) and Western blot (D) stained with EsxU antiserum. Lanes: IN – input, sample after incubation with TEV protease; FT – flow-through; W – wash with 20 mM imidazole; E – elution with 500 mM imidazole. Cleaved EsxUT (marked **d** for dimer, and **m** for monomer) was collected from FT and W fractions of the purification step. Elution fraction was discarded since it contains His₆-MBP-EsxUT fusion protein (marked **f**). 5 µg of protein was loaded per lane.

E. Chromatogram of anion exchange purification of EsxUT on HiTrap QFF resin. EsxUT was eluted by applying 150 mM to 2 M NaCl gradient (black line). Green line is Absorbance at 280 nm. EsxUT elutes at 570 mM NaCl.

Fractions after anion exchange were loaded on polyacrylamide gel stained with Coomassie blue (**F**) and Western blot (**G**) stained with EsxU antiserum. Lanes: IN – input, sample after IMAC; FT – flow-through collected during column loading; W – column wash with 150 mM NaCl before elution with 0.15 – 2 M NaCl gradient; numbers 8-14 – elution fractions corresponding to peaks in **E**. MBP –is marked on the gel and elutes in the flow-through and washing steps at 150 mM NaCl. EsxU monomers (**m**), dimers (**d**), and high molecular weight oligomers (**o**) are visible on the Western. Same protein amount was loaded in each lane. Molecular weight protein standard in kDa is on the left.

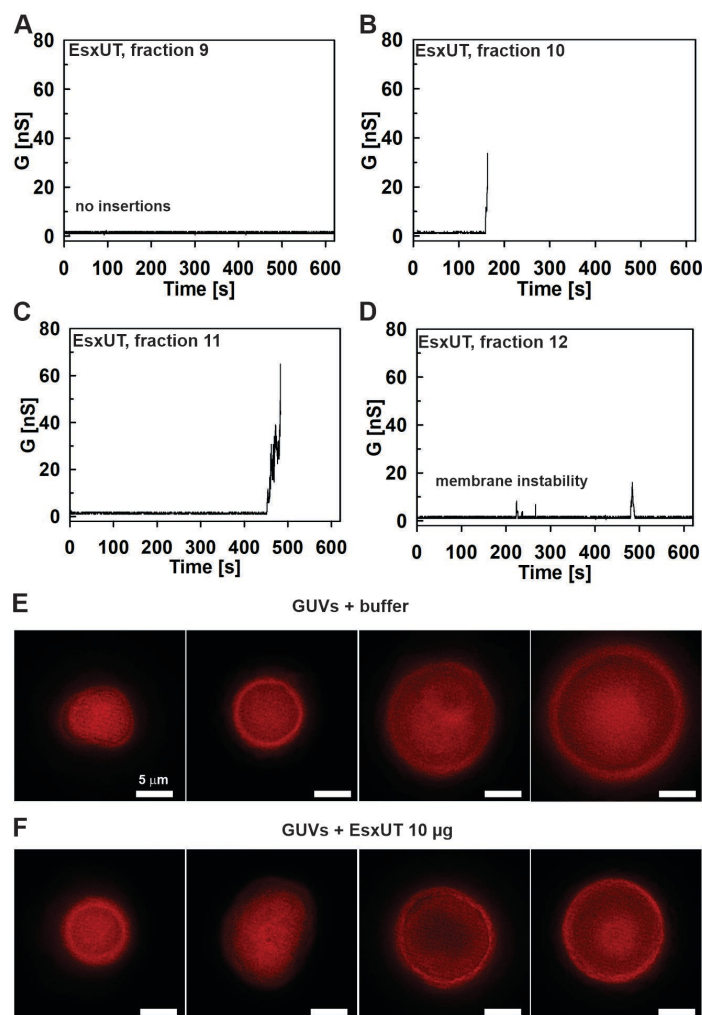


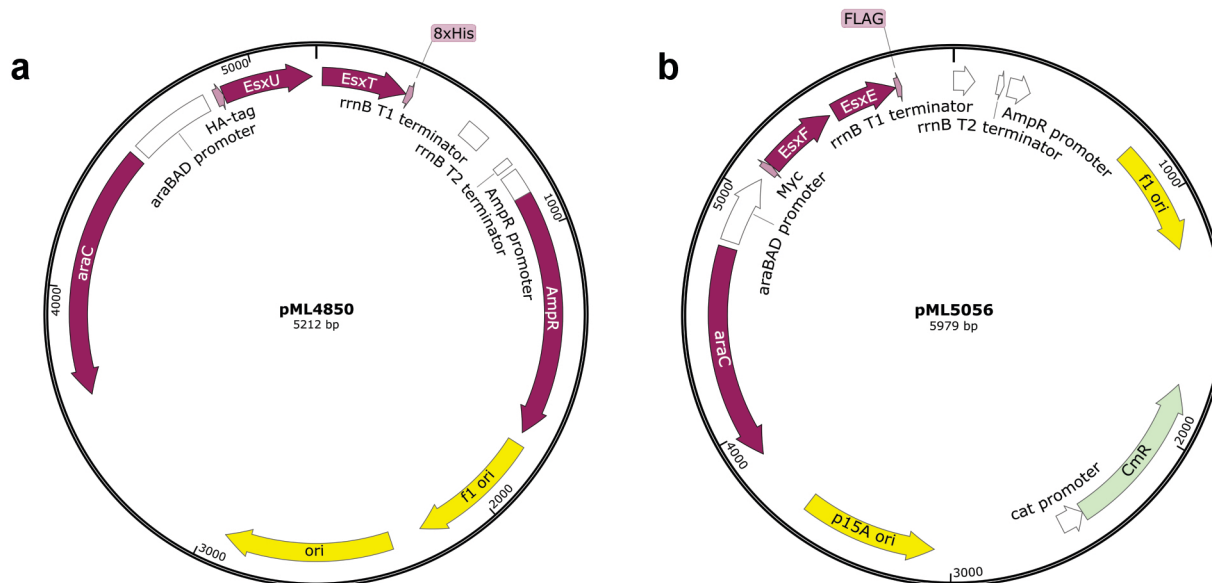
Figure S4. Characterization of purified *M. tuberculosis* EsxUT.

A - D. Lipid bilayer experiments with EsxUT from Anion Exchange purification, Fig. SX. Current traces of individual fractions of Anion Exchange in diphytanoyl phosphatidylcholine (DPhPC) membranes are shown. The experiments were performed in 1M KCl, 25 mM sodium phosphate, pH 7.4 at 10 mV applied potential. A total of 7 membranes with fraction 9 (**A**) was recorded. No insertions were observed when 1 – 10 µg of protein was present. Five membranes with fraction 10 (**B**) was recorded with 0.88 – 15 µg of protein present. In only one membrane several fast insertions were observed and the membrane broke immediately after insertions (**B**). Similar result was obtained with fraction 11 (**C**) where one membrane out of seven resulted in fast insertions followed by membrane breakage (1 – 10 µg of protein was present). Panel **D** shows experiment with fraction 12 (1 – 10 µg of protein) where one membrane out of 7 exhibited instability.

E. Giant unilamellar vesicles (GUVs) treated with FM464X dye to label dimyristoyl phosphocholine (DMPC) lipid membrane. GUVs were incubated for 30 min in buffer containing 25 mM sodium phosphate, 150 mM NaCl, pH 7.0. Representative micrographs are shown. The white bar is 5 µm.

F. Giant unilamellar vesicles (GUVs) stained with FM464X dye. GUVs were incubated for 30 min with 10 µg of purified EsxUT in a buffer containing 25 mM sodium phosphate, 150 mM NaCl, pH 7.0. Representative micrographs are shown. The scale bar is 5 µm.

Co-expression of HA-EsxU/EsxT-8xHis and Myc-EsxF/EsxE-FLAG



Co-expression of 8xHis-EsxF/EsxE-FLAG and HA-EsxU/EsxT-Myc

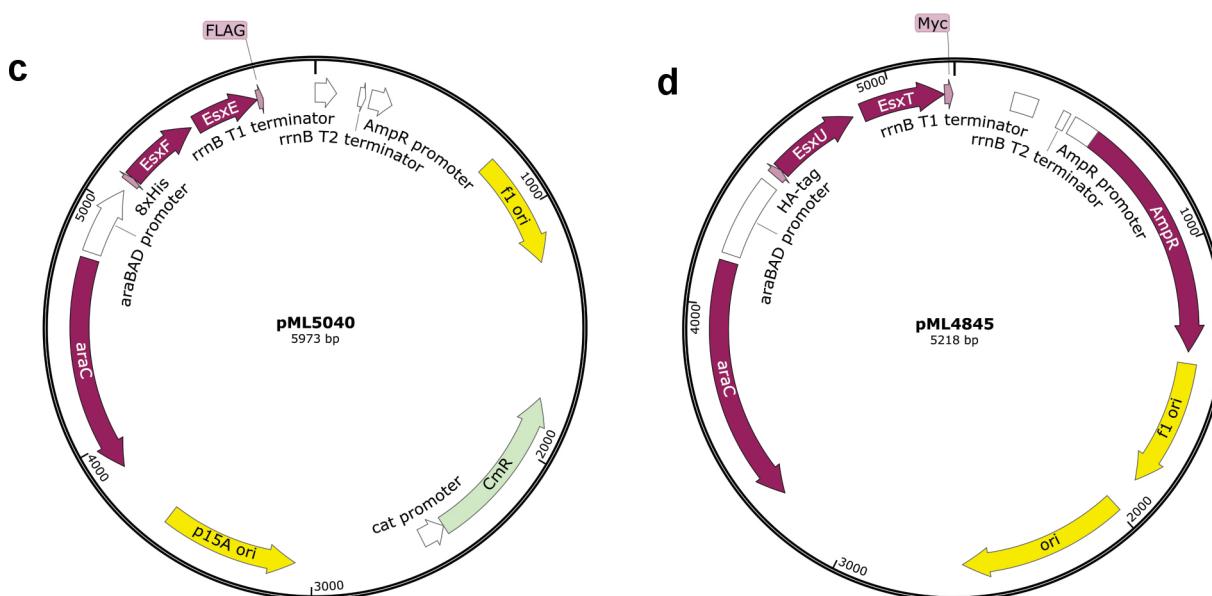


Fig. S5. Co-expression vectors to examine the interactions of small Esx proteins in *E. coli*.

The genomic regions of EsxU-EsxT and EsxF-EsxE were amplified from Mtb wt using specific primers containing the 1 kDa tag sequences (Table S4). The tagged sequences were inserted in the respective vectors to obtain: **a.** pML4850 expressing HA-EsxU-EsxT-8xHis; **b.** pML5056 expressing Myc-EsxF-EsxE-FLAG; **c.** pML5040 expressing 8xHis-EsxF-EsxE-FLAG; and **d.** pML4845 expressing HA-EsxU-EsxT-Myc.

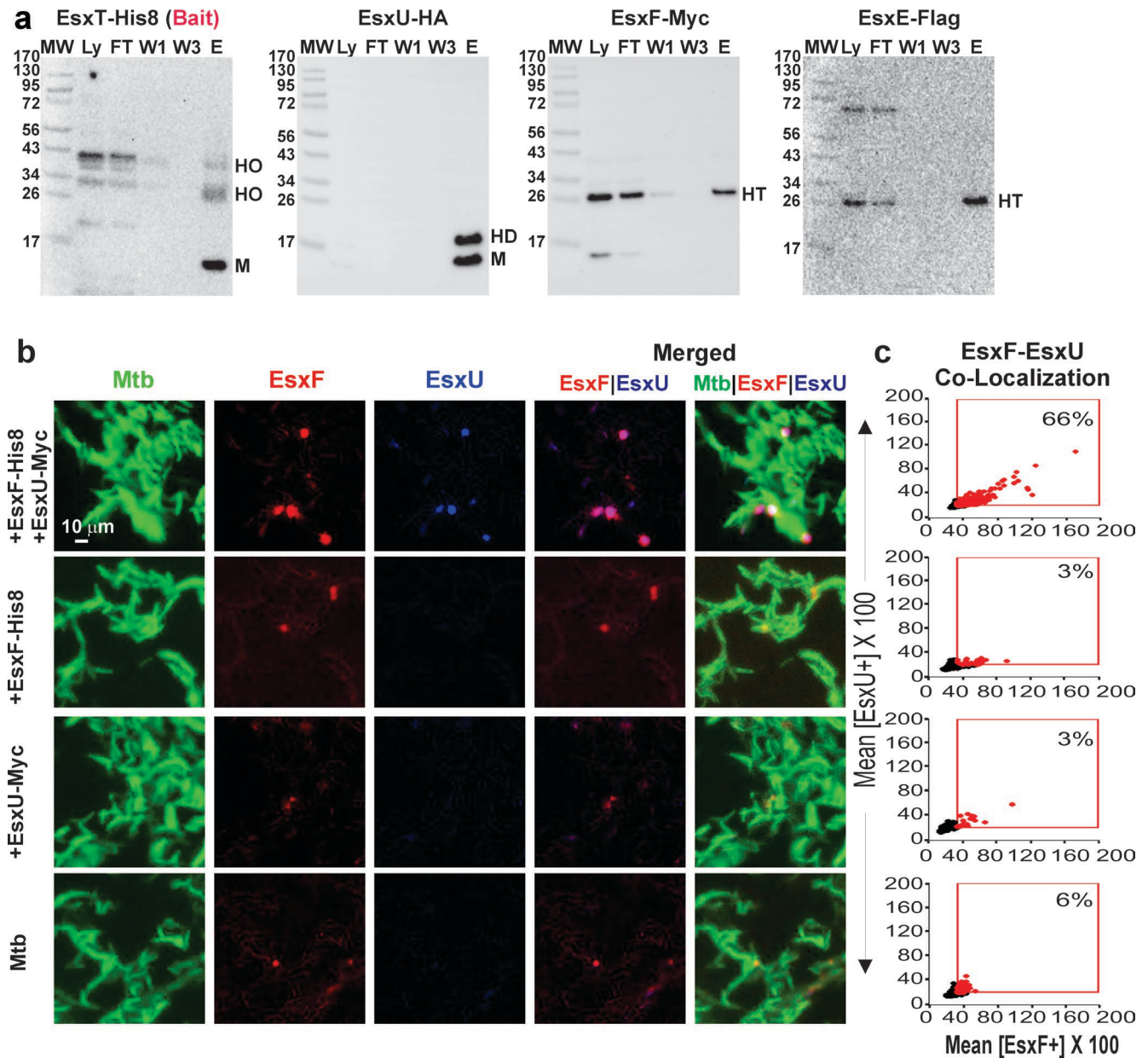


Fig. S6. EsxEF and EsxUT interact and co-localize on the cell surface of *M. tuberculosis*.

a. Pull-down assay: The bait protein EsxT_{His8} in a lysate (Ly) of *E. coli* producing _{HA}EsxU/EsxT_{His8} and _{Myc}EsxF/EsxE_{FLAG} was captured on a Ni(II) affinity column. The 10-fold diluted cell lysate (Ly), the flow through (FT), buffers of wash one (W1) and wash three (W3) and the sample eluted with imidazol (E) were analyzed in Western blots using tag-specific antibodies. M- monomer, HD- heterodimer, HT- heterotrimer, HO- hetero-oligomer. **b.** The surface accessibility of tagged EsxF and EsxU proteins in Mtb strains producing either _{His8}EsxF-EsxE or _{Myc}EsxU-EsxT or both Esx pairs together. Strains were scored positive for tagged EsxF or EsxU relative to untagged wt Mtb. The indicated Mtb strains were metabolically labeled with DMN-trehalose (green) and stained with anti-His (red) and anti-Myc (blue) Alexa fluor-conjugated antibodies. Co-localization of EsxF and EsxU in these Mtb strains was visualized by fluorescence microscopy showing the merged images and quantified as a percentage of positive cells **(c)**. Representative images/blots of three independent experiments are shown (n=3).

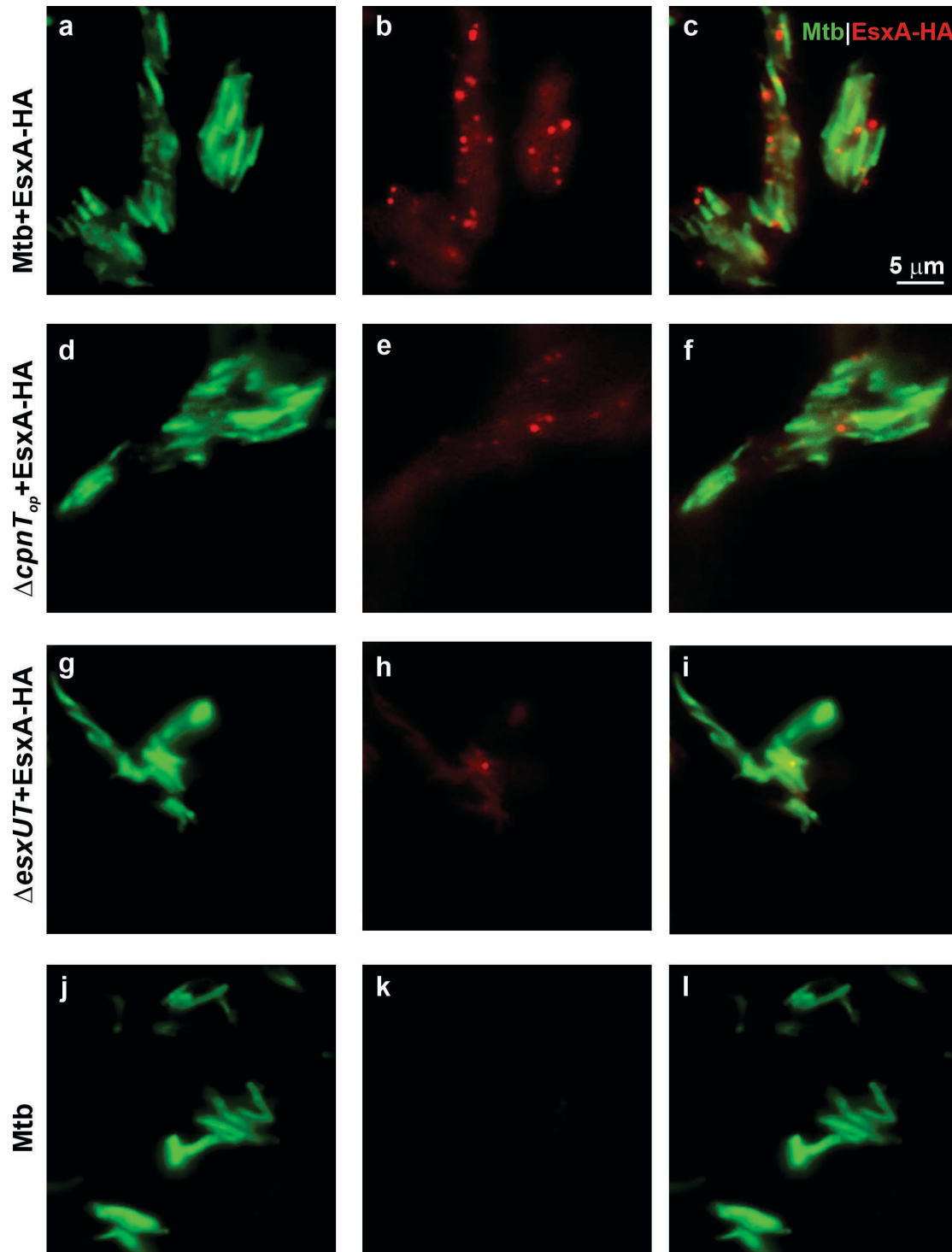


Fig. S7: Role of EsxEF-EsxUT in surface exposure of EsxA in *M. tuberculosis*.

These high resolution images of the experiment shown in Fig. 4A were acquired using a fluorescence microscope with a 100X oil objective phase. The parent strain *M. tuberculosis* mc²6206 (Mtb) and derivative strains were metabolically labeled with DMN-trehalose (green) and were probed for HA-tagged EsxA using a monoclonal HA-tag antibody and an Alexa fluor 594-conjugated secondary antibody (red). HA tagged EsxA levels in: **a-c.** Mtb mc²6206, **d-f.** Mtb $\Delta cpnT_{op}$, and **g-i.** Mtb $\Delta esxUT$. The fluorescence of these strains was normalized compared to Mtb mc²6206 (**j-l**), which does not produce an HA-tagged protein (**k**).

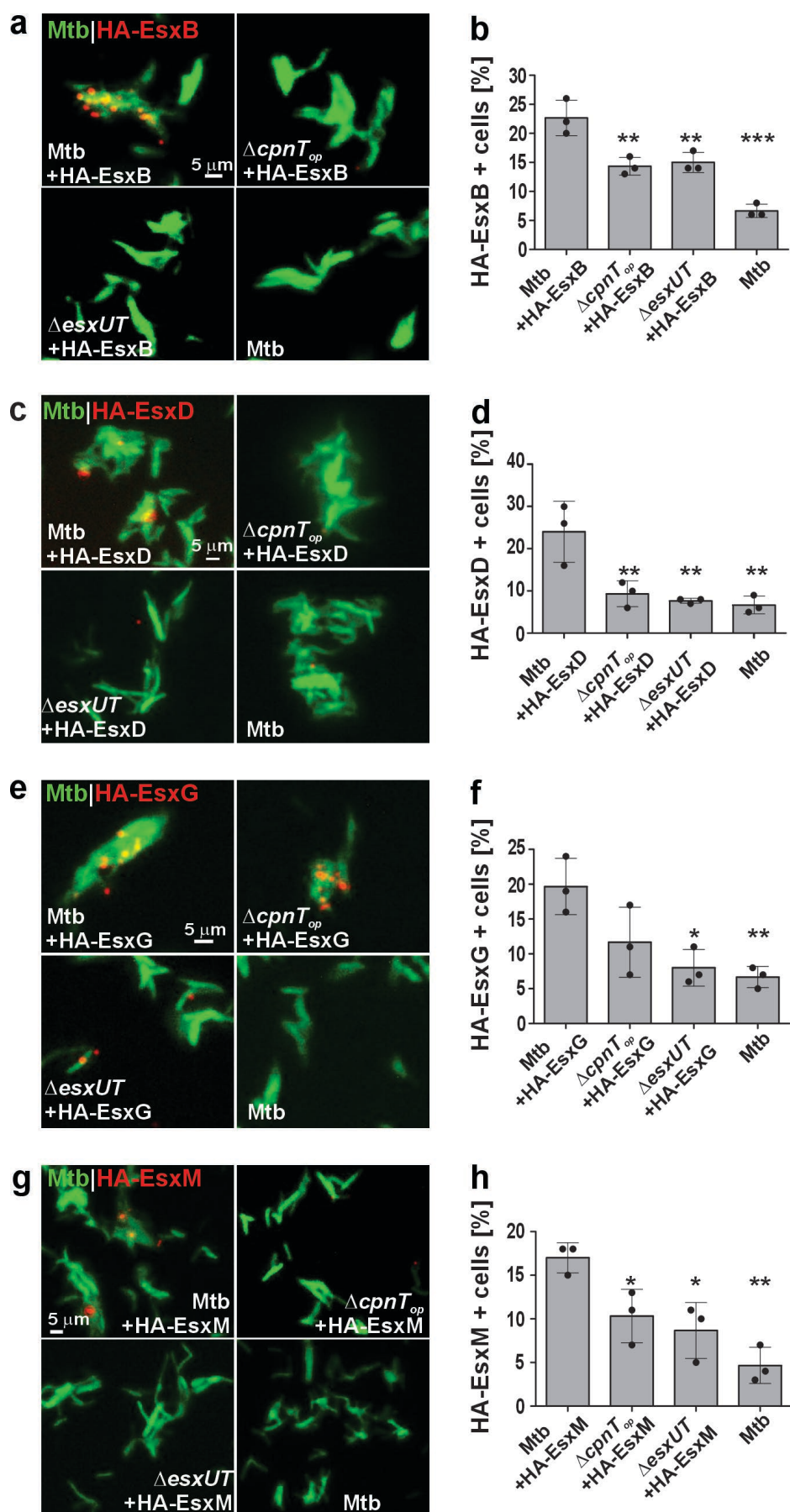


Fig. S8: Role of EsxEF-EsxUT in surface exposure of EsxB and ESX-associated EsxB homologs in *M. tuberculosis*.

Fluorescence microscope images of surface accessible HA-tagged: **a.** EsxB, **c.** EsxD, **e.** EsxG, and **g.** EsxM in the parent strain *M. tuberculosis* mc²6206 (Mtb) and derivative strains. DMN-Trehalose stained Mtb strains (green) were probed for the HA-tagged small Esx proteins (red). Scale bar = 5 μ m. Quantification of surface exposed HA tagged: **b.** EsxB, **d.** EsxD, **f.** EsxG, and **h.** EsxM in comparison to the untagged WT cells, determined from Cytation 5 analysis. Data are represented as mean $\pm$ SD of three independent experiments (n=3) and asterisks indicate significant differences (*p value $\leq$ 0.05, **p value $\leq$ 0.01, ***p value $\leq$ 0.001 calculated using the one-way ANOVA with Dunnett's correction) in comparison to the tagged WT strain. Representative images of three independent experiments are shown (n=3). Source data are provided in the Source Data file.

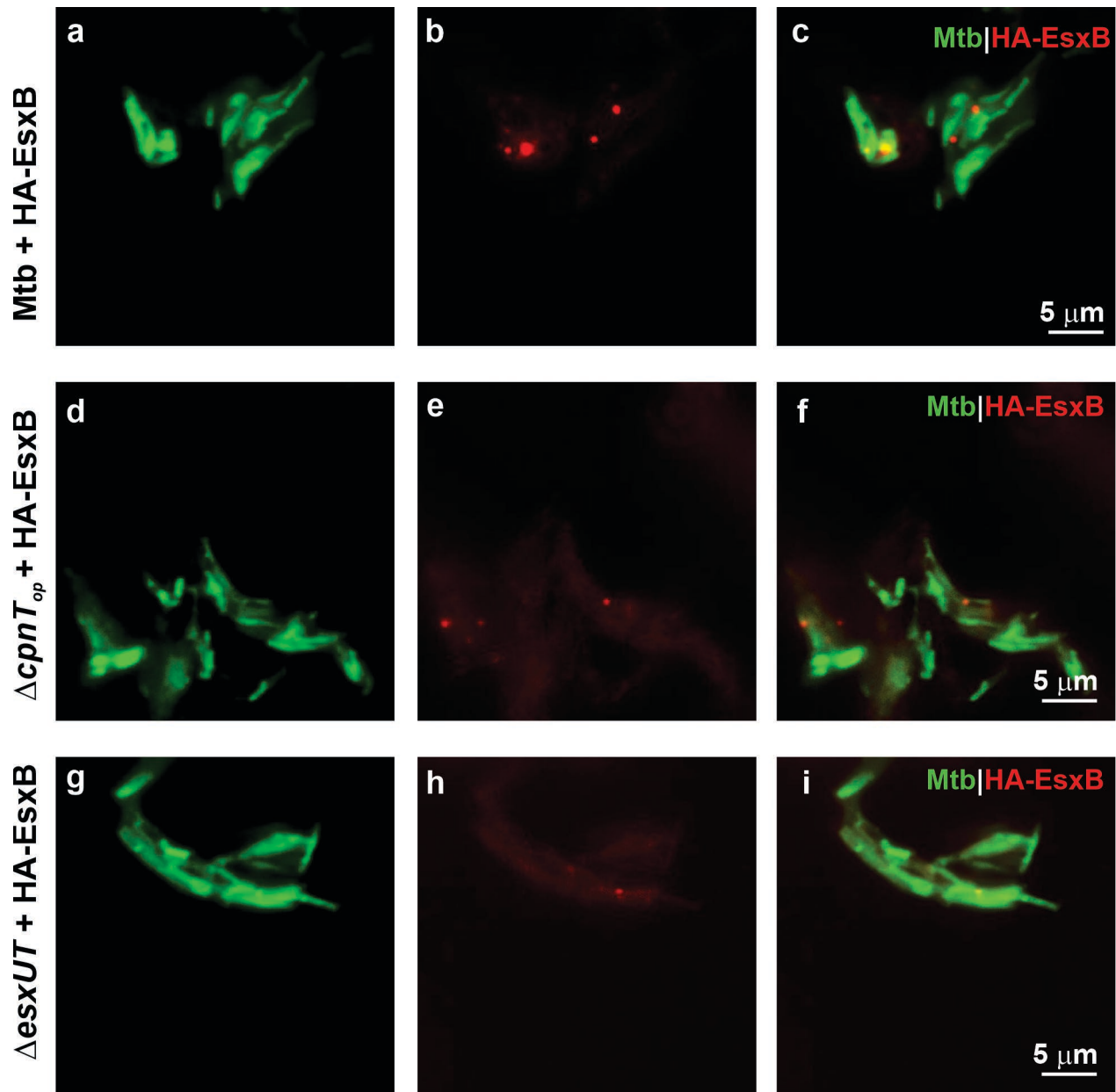


Fig. S9: Role of EsxEF-EsxUT in surface exposure of EsxB in *M. tuberculosis*.

These high resolution images of the experiment shown in Fig. S8A were acquired using a fluorescence microscope with a 100X oil objective phase. DMN-Trehalose stained Mtb strains (green) were probed for HA-tagged EsxB (red) in the parent strain *M. tuberculosis* mc²6206 (Mtb) and derivative strains. HA tagged EsxB levels in: **a-c.** Mtb mc²6206, **d-f.** Mtb $\Delta cpnTop$, and **g-i.** Mtb $\Delta esxUT$. The HA fluorescence levels in the strains were normalized compared to the untagged Mtb mc²6206 strain (Fig. S7k).

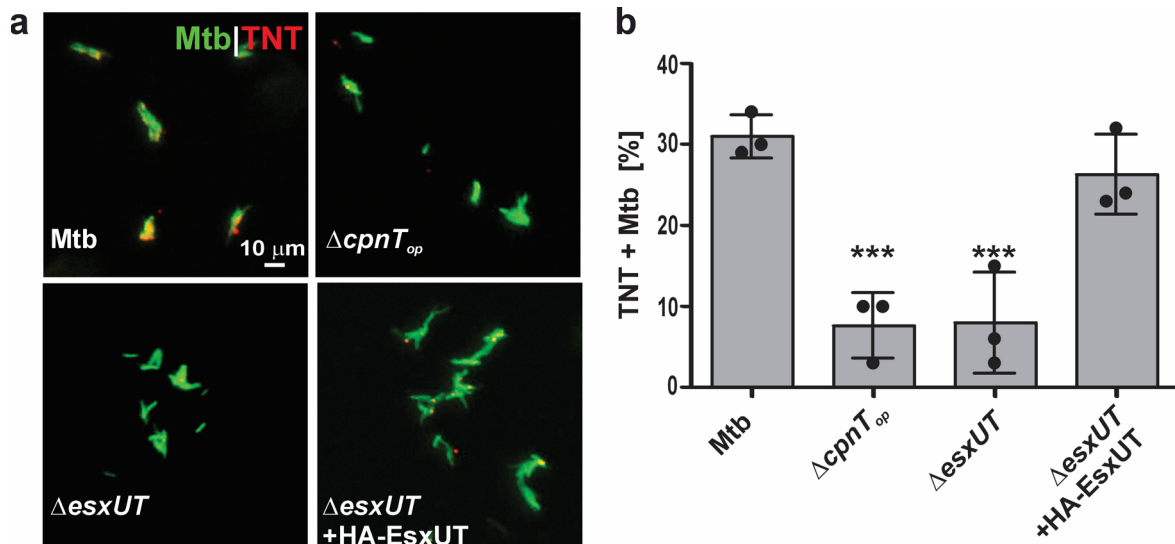


Fig. S10: A C-terminal HA tag does not alter the function of EsxUT in TNT export in *M. tuberculosis*.

a. Surface accessibility of TNT in Mtb strains producing HA-tagged EsxUT. The Mtb strains were metabolically labeled with DMN-trehalose (green) and stained with anti-TNT antiserum (red). The surface exposure of the TNT was analyzed using a fluorescence microscope. **b.** Quantification of fluorescence images to determine *M. tuberculosis* cells (%) with surface accessible TNT, scored relative to *cpnT_{op}* deletion mutant. Data are represented as mean $\pm$ SD of three independent experiments (n=3) and representative images are shown. Asterisks indicate significant differences (***) p value $\leq$ 0.001; one-way ANOVA with Dunnett's correction) in comparison to wt Mtb. Source data are provided in the Source Data file.

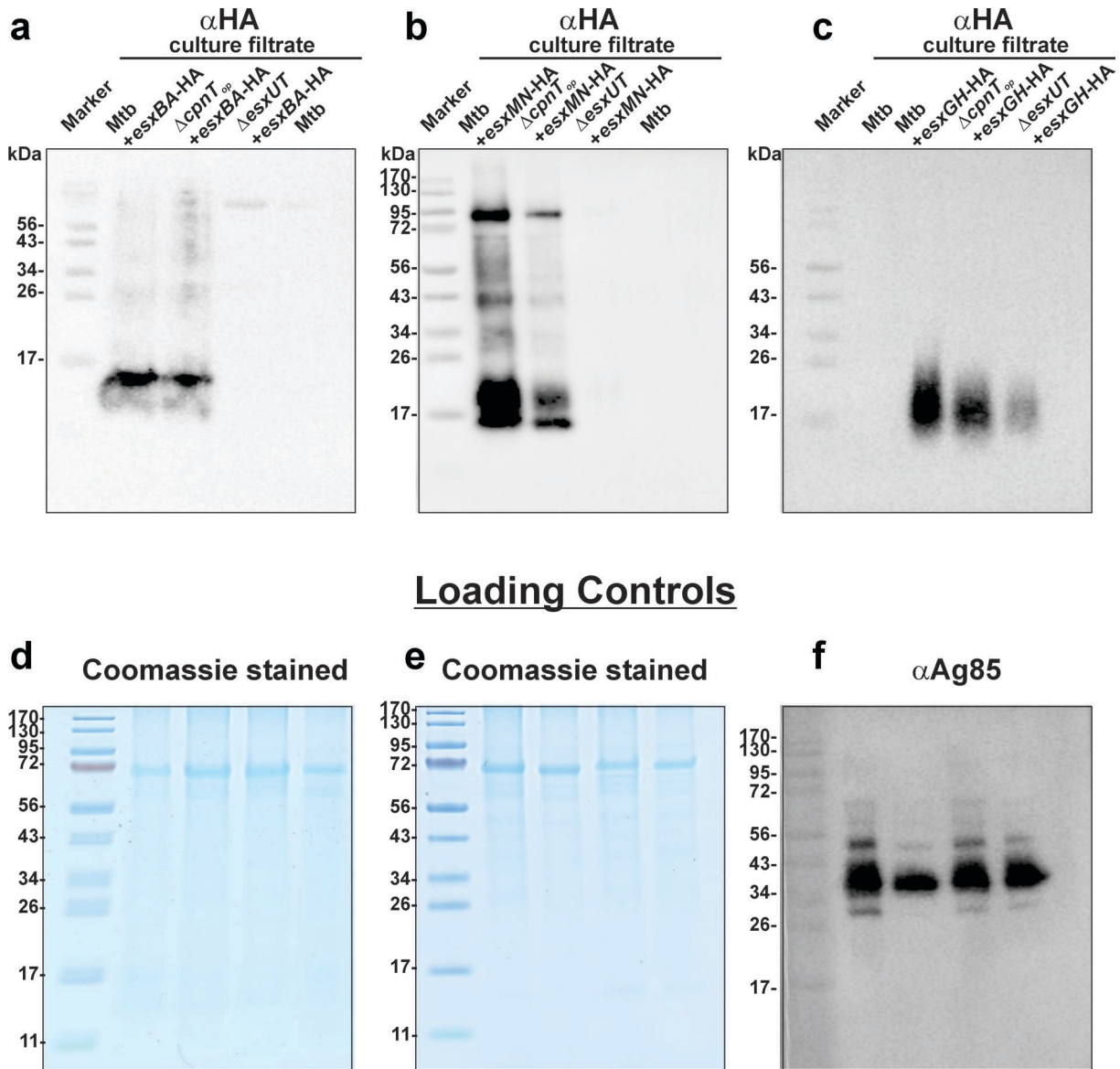


Fig. S11: Analysis of HA-tagged Esx proteins in the culture filtrates of *M. tuberculosis*.

Culture filtrates of the indicated *M. tuberculosis* strains were separated on an SDS-polyacrylamide gel and blotted on a PVDF membrane. The uncropped versions of the blots presented in Fig. 5 are shown. **(a-c)** Uncropped blots probed for HA-tagged **(a)** EsxA, **(b)** EsxN and **(c)** EsxH in the culture filtrates of the indicated Mtb strains. **(d-f)** The loading controls for the HA-tagged Esx proteins in culture filtrate: **(d)** EsxA, **(e)** EsxN and **(f)** EsxH.

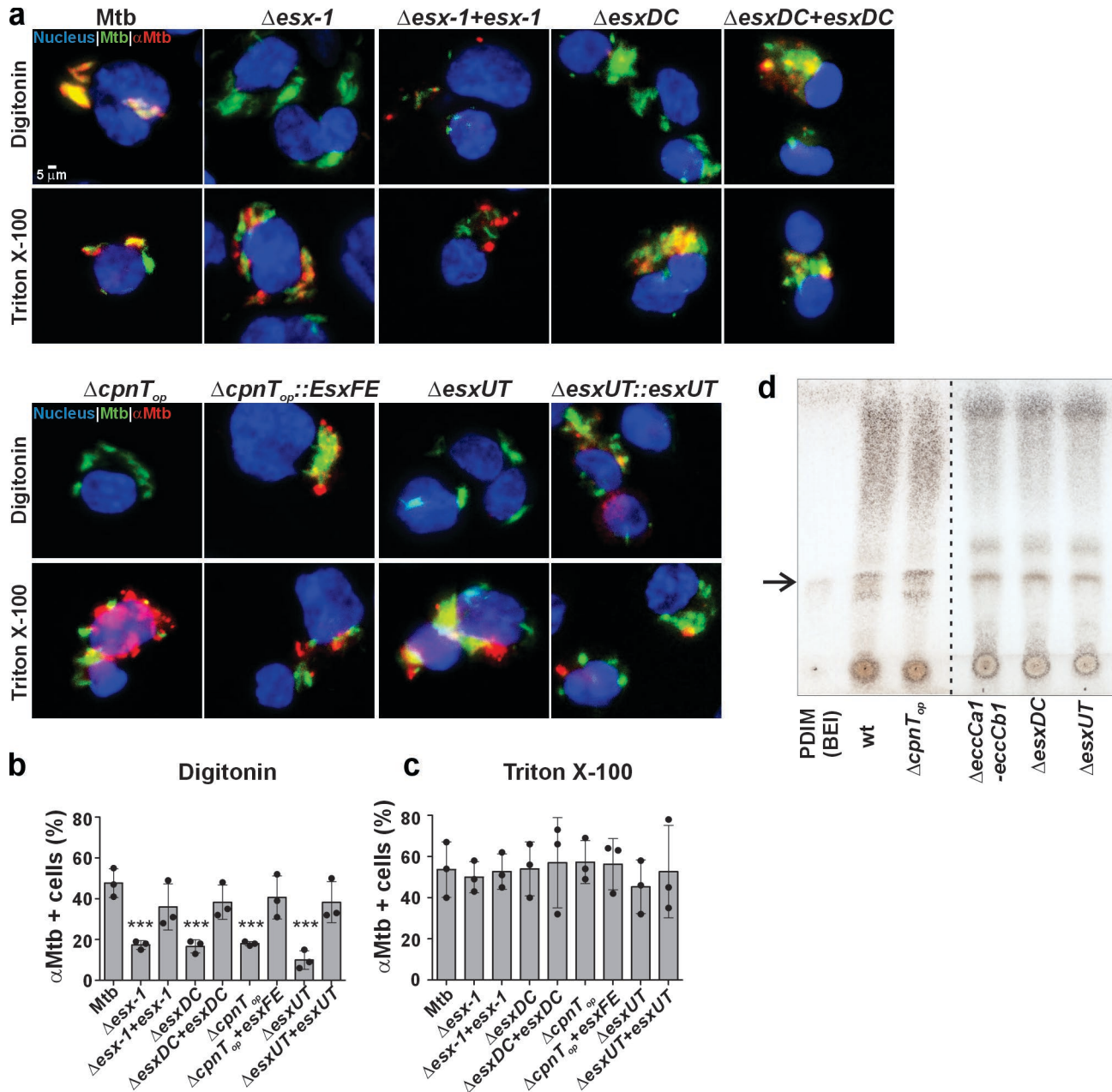


Fig. S12: EsxEF and the Esx effector proteins of the ESX-1, ESX-2 and ESX-4 systems are required for phagosomal permeabilization by *M. tuberculosis*.

a. Determination of phagosomal permeabilization in macrophages using Mtb specific antibodies. The DMN-trehalose labeled parent strain *M. tuberculosis* mc²6206 (Mtb) and derivative strains were used to infect the THP-1 macrophages at an MOI of 10:1. The macrophages were fixed after 48 h of infection, permeabilized either with digitonin which facilitates the access of antibodies in an antiserum against the tuberculin purified protein derivative (PPD) (anti-Mtb antibody) to the cytosol of macrophages or with Triton X-100 to enable the access of antibodies to the phagosomal compartment¹. Cells were incubated with an anti-Mtb antibody (α Mtb) and an Alexa Fluor-594 secondary antibody (red). The nuclei of the infected macrophages were stained with DAPI and analyzed using Cytation 5. Scale bar = 5 μ m. **b, c.** Quantification of infected macrophages positive for Mtb proteins in comparison to the *eccCa1-eccCb1* deletion mutant when permeabilized with: **b.**

Digitonin, and **c.** Triton X-100. Data are represented as mean $\pm$ SD of three independent experiments (n=3) and asterisks indicate significant differences (***) p value ≤ 0.001 calculated using the one-way ANOVA with Dunnett's correction) in comparison to Mtb mc²6206 strain. Representative images of three independent experiments are shown (n=3). **d.** Analysis of phthiocerol dimycocerosates (PDIM) in Mtb mc²6206 (Mtb) and derivative strains. TLC of the lipids extracted from the different Mtb strains indicating the presence of PDIM in relation to the control PDIM obtained from BEI Resources. Source data are provided in the Source Data file.

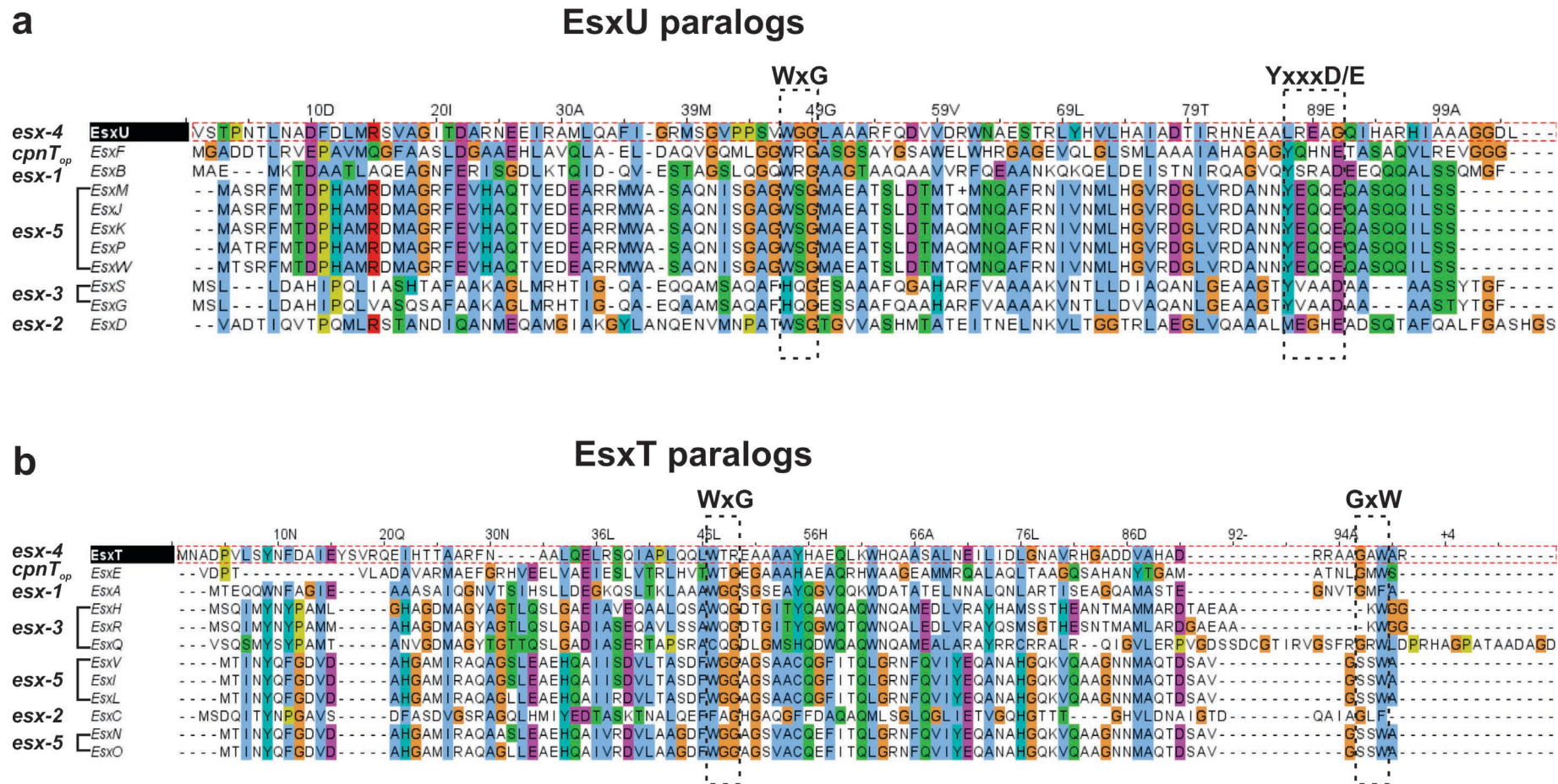


Fig. S13: Sequence alignment of EsxU and EsxT paralogs

Multiple sequence alignments of EsxU (a) and EsxT (b) paralogs were generated using Clustal Omega. The sequences were obtained from Mycobrowser (Release 5), aligned in Clustal Omega and visualized using the Jalview software. The associations of the Esx proteins with their cognate type VII secretion systems is indicated on the left. The locations of the type VII secretion signal YxxxD/E and of the WxG and GxW motifs are shown in sequence alignments.

The color codes are:



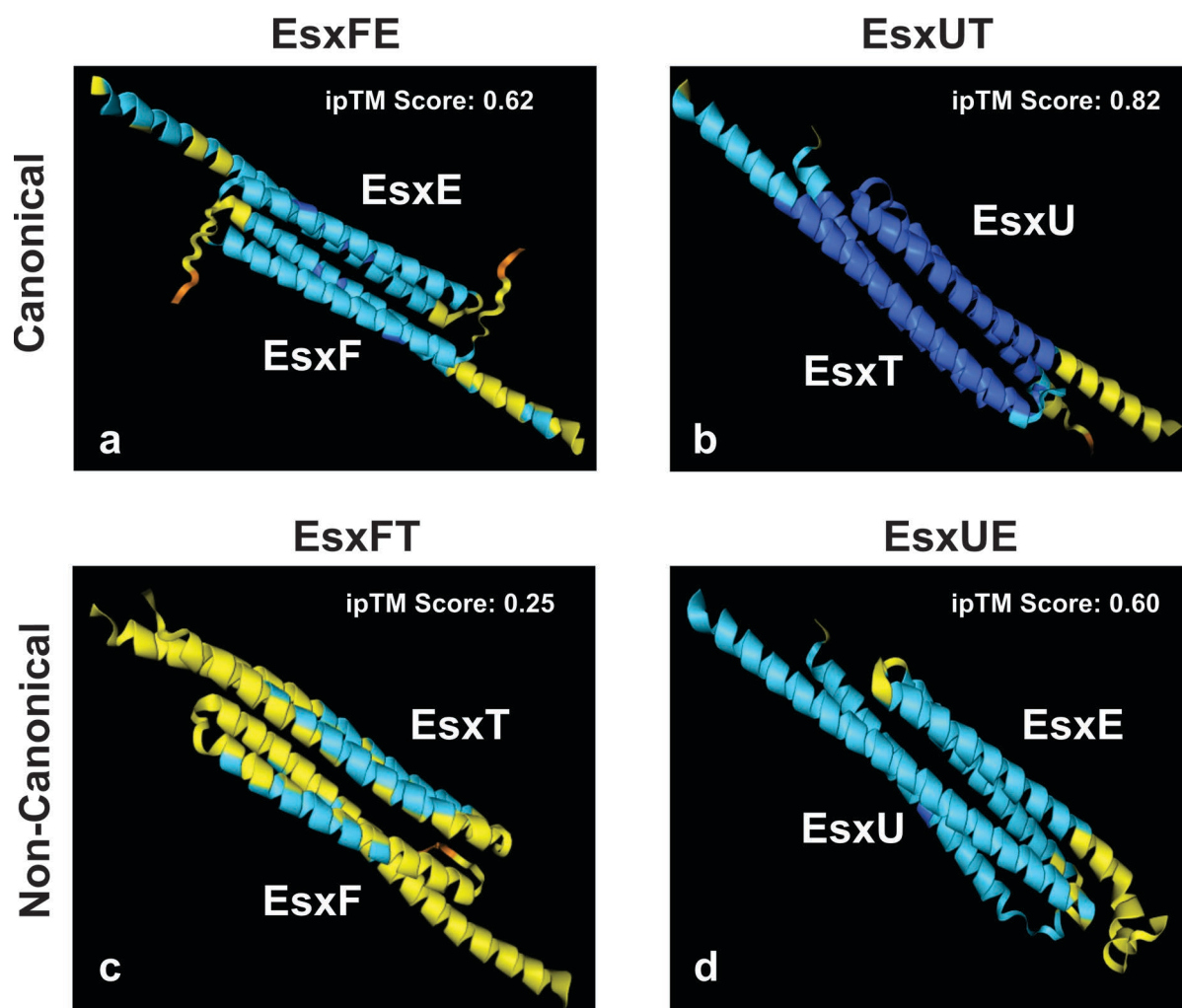


Fig. S14: AlphaFold models for the interactions of EsxEF and EsxUT of *M. tuberculosis*.

Structural models of Esx protein dimers as predicted by AlphaFold 3² show the probable interactions between canonical heterodimers EsxFE (a) and EsxUT (b), and non-canonical heterodimers EsxFT (c) and EsxUE (d). The “interface predicted template modelling” (ipTM) score is a confidence metric used by AlphaFold to assess the accuracy of predicted protein-protein interaction interfaces and is indicated for each protein pair. Table S6 contains a complete list of the ipTM scores for the predicted Esx dimers and the DOI of the models.

Supplementary Tables

Peptide	Sequence	Position (number of amino acids)	Source
EsxC	DQITYNPGAVSD	3-14 (12 aa)	ProMab
EsxF	DDTLRVEPAVMQG	4-16 (13 aa)	Genscript
EsxU	TLNADFDLMRSVAGI	6-20 (15 aa)	Genscript

Table S1. Peptide sequences selected for antibody generation

Strain name	Relevant genotypes and description	Source or Reference
<i>M. tuberculosis</i> mc ² 6206	H37Rv derivative; $\Delta leuCD \Delta panCD$; avirulent <i>M. tuberculosis</i> (BSL-2)	3
<i>M. tuberculosis</i> ML2016	mc ² 6206 derivative; $\Delta esxF-esxE-cpnT-ift::loxP$ (referred to as $\Delta cpnT$ operon)	4
<i>M. tuberculosis</i> ML2648	ML2016 derivative; L5 attB::pML3964	1
<i>M. tuberculosis</i> ML2690	ML2769 derivative; $\Delta eccC4::loxP$	1
<i>M. tuberculosis</i> ML2696	ML2690/cosmid I60 (contains <i>esx-4</i> operon)	1
<i>M. tuberculosis</i> ML3006	mc ² 6206 derivative; $\Delta esxUT::hyg^R$	This study
<i>M. tuberculosis</i> ML3002	ML3006 derivative; $\Delta esxUT::loxP$	This study
<i>M. tuberculosis</i> ML3003	ML3002 derivative; L5 attB::pML4328	This study
<i>M. tuberculosis</i> ML2636	ML2016 derivative; pML3938; L5 attB::pML3954	4
<i>M. tuberculosis</i> ML2640	ML2016 derivative; pML3938; L5 attB::pML3958	4
<i>M. tuberculosis</i> mc ² 6206; attB (Ms6)::pML5084	mc ² 6206; $P_{smyc}::8xHis-esxF-esxE$; hyg^R ; integrative	This study
<i>M. tuberculosis</i> mc ² 6206; attB (L5)::pML4862	mc ² 6206; $P_{smyc}::Myc-esxU-esxT$; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> mc ² 6206; attB (Ms6)::pML5084; attB (L5)::pML4862	mc ² 6206; $P_{smyc}::8xHis-esxF-esxE$; hyg^R ; integrative; $P_{smyc}::Myc-esxU-esxT$; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> mc ² 6206; attB (L5)::pML4863	mc ² 6206; $P_{smyc}::HA-esxB-esxA$; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML2016; attB (L5)::pML4863	ML2016; $P_{smyc}::HA-esxB-esxA$; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML3002; attB (L5)::pML4863	ML3002; $P_{smyc}::HA-esxB-esxA$; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> mc ² 6206; attB (L5)::pML4864	mc ² 6206; $P_{smyc}::esxB-esxA-HA$; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML2016; attB (L5)::pML4864	ML2016; $P_{smyc}::esxB-esxA-HA$; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML3002; attB (L5)::pML4864	ML3002; $P_{smyc}::esxB-esxA-HA$; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> mc ² 6206; attB (L5)::pML4854	mc ² 6206; $P_{smyc}::HA-esxD-esxC$; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML2016; attB (L5)::pML4854	ML2016; $P_{smyc}::HA-esxD-esxC$; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML3002; attB (L5)::pML4854	ML3002; $P_{smyc}::HA-esxD-esxC$; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> mc ² 6206; attB (L5)::pML4855	mc ² 6206; $P_{smyc}::esxD-esxC-HA$; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML2016; attB (L5)::pML4855	ML2016; $P_{smyc}::esxD-esxC-HA$; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML3002; attB (L5)::pML4855	ML3002; $P_{smyc}::esxD-esxC-HA$; <i>aph</i> ; integrative	This study

Table S2. Strains used in this study

Strain name	Relevant genotypes and description	Source or Reference
<i>M. tuberculosis</i> mc ² 6206; attB (L5)::pML4860	mc ² 6206; <i>P_{smyc}::HA-esxG-esxH</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML2016; attB (L5)::pML4860	ML2016; <i>P_{smyc}::HA-esxG-esxH</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML3002; attB (L5)::pML4860	ML3002; <i>P_{smyc}::HA-esxG-esxH</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> mc ² 6206; attB (L5)::pML4861	mc ² 6206; <i>P_{smyc}::esxG-esxH-HA</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML2016; attB (L5)::pML4861	ML2016; <i>P_{smyc}::esxG-esxH-HA</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML3002; attB (L5)::pML4861	ML3002; <i>P_{smyc}::esxG-esxH-HA</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> mc ² 6206; attB (L5)::pML4869	mc ² 6206; <i>P_{smyc}::HA-esxM-esxN</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML2016; attB (L5)::pML4869	ML2016; <i>P_{smyc}::HA-esxM-esxN</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML3002; attB (L5)::pML4869	ML3002; <i>P_{smyc}::HA-esxM-esxN</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> mc ² 6206; attB (L5)::pML4870	mc ² 6206; <i>P_{smyc}::esxM-esxN-HA</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML2016; attB (L5)::pML4870	ML2016; <i>P_{smyc}::esxM-esxN-HA</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML3002; attB (L5)::pML4870	ML3002; <i>P_{smyc}::esxM-esxN-HA</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML3007	mc ² 6206 derivative; Δ <i>eccCa1-eccCb1::hyg^R</i>	This study
<i>M. tuberculosis</i> ML3001	ML3007 derivative; Δ <i>eccCa1-eccCb1::loxP</i>	This study
<i>M. tuberculosis</i> ML3001; cosmid 2F9	ML3001; <i>bla</i> ; <i>hyg</i> ; ~4337-4369 kb contains the <i>esx-1</i> locus of <i>M. tuberculosis</i> H37Rv	This study
<i>M. tuberculosis</i> ML3008	mc ² 6206 derivative; Δ <i>esxDC::hyg^R</i>	This study
<i>M. tuberculosis</i> ML3000	ML3008 derivative; Δ <i>esxDC::loxP</i>	This study
<i>M. tuberculosis</i> ML3000; attB (L5)::pML4852	ML3000; <i>P_{smyc}::esxD-esxC</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML3000; cosmid I106	ML3000; <i>bla</i> ; <i>hyg</i> ; ~4349-4385 kb contains the <i>esx-2</i> locus of <i>M. tuberculosis</i> H37Rv	This study
<i>M. tuberculosis</i> mc ² 6206; attB (L5)::pML4875	mc ² 6206; <i>P_{smyc}::HA-espB</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML2016; attB (L5)::pML4875	ML2016; <i>P_{smyc}::HA-espB</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> ML3002; attB (L5)::pML4875	ML3002; <i>P_{smyc}::HA-espB</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> mc ² 6206; attB (Ms6)::pML4886	mc ² 6206; <i>P_{smyc}::HA-esxU-esxT-FLAG</i> ; <i>hyg^R</i> ; integrative	This study
<i>M. tuberculosis</i> ML2016; attB (Ms6)::pML4886	ML2016; <i>P_{smyc}::HA-esxU-esxT-FLAG</i> ; <i>hyg^R</i> ; integrative	This study
<i>M. tuberculosis</i> ML3002; attB (Ms6)::pML4886	ML3002; <i>P_{smyc}::HA-esxU-esxT-FLAG</i> ; <i>hyg^R</i> ; integrative	This study
<i>M. tuberculosis</i> ML2690; attB (Ms6)::pML4886	ML2690; <i>P_{smyc}::HA-esxU-esxT-FLAG</i> ; <i>hyg^R</i> ; integrative	This study
<i>M. tuberculosis</i> mc ² 6206; attB (L5)::pML4881	mc ² 6206; <i>P_{smyc}::HA-esxU-esxT</i> ; <i>aph</i> ; integrative	This study
<i>M. tuberculosis</i> <i>eccD5</i> Tet-OFF	Mtb Erdman derivative; <i>eccD5</i> conditional mutant; pMV-ppe62HA	This study
<i>M. tuberculosis</i> ML2409	mc ² 6206 derivative; pML3732	²
<i>M. tuberculosis</i> ML2016; pML3732	ML2016; oriM origin; <i>P_{imyc}::ppe62-HA-6xHis</i> ; <i>hyg^R</i> ; replicative	This study
<i>M. tuberculosis</i> ML3002; pML3732	ML3002; oriM origin; <i>P_{imyc}::ppe62-HA-6xHis</i> ; <i>hyg^R</i> ; replicative	This study

Table S2. Strains used in this study (continued)

Strain name	Relevant genotypes and description	Source or Reference
<i>E. coli</i> DH5 α	F- Φ 80 Δ <i>lacZ</i> Δ M15 Δ (<i>lacZ</i> YA- <i>argF</i>) U169 <i>recA1 endA1 hsdR17</i> (rk-mk+) <i>phoA supE44 thi-1 gyrA96 relA1</i> λ -	Invitrogen
<i>E. coli</i> Mach1	F- Φ 80(<i>lacZ</i>) Δ M15 Δ <i>lacX74 hsdR</i> (rK-mK+) Δ <i>recA1398 endA1 tonA</i>	Invitrogen

Table S2. Strains used in this study (continued)

Plasmid	Properties	Reference
pML2424	pUC origin, pAL5000ts, <i>loxP</i> - <i>P_{smyc}::gfp_m²⁺-hyg-loxP</i> , <i>P_{wmyc}::sacR-sacB</i> ; <i>hyg^R</i> , vector for gene deletion in mycobacteria via homologous recombination	⁵
pML4806	pML2424-derivative; <i>hom_{up}-esxU-esxT- hom_{down}</i> ; <i>hyg^R</i> , <i>esxUT</i> deletion vector	This study
pML2714	pUC origin; pAL5000ts; <i>P_{hsp60}::cre</i> ; <i>P_{imyc}::pamcherry1m</i> ; <i>aph</i>	⁶
pML4328	ColE1 origin; <i>attP_{L5}</i> ; <i>P_{smyc}::esxU-esxT</i> ; <i>aph</i> ; integrative <i>esxU-esxT</i> expression vector	This study
pBAD24-sfGFPx1	pBR322 origin; <i>P_{BAD}::sfgfp_{x1}</i> ; <i>bla</i> ; <i>E.coli</i> replicative expression vector for Superfolder GFP	⁷
pML4850	pBAD24-sfGFPx1-derivative; <i>P_{BAD}::HA-esxU-esxT-8xHis</i> ; <i>bla</i> ; <i>E.coli</i> replicative tagged <i>esxU-esxT</i> expression vector	This study
pBAD33	pACYC184 origin; <i>P_{BAD}</i> ; <i>cat</i> ; <i>E.coli</i> replicative expression vector	⁸
pML5056	pBAD33-derivative; <i>P_{BAD}::Myc-esxF-esxE-FLAG</i> ; <i>cat</i> ; <i>E.coli</i> replicative tagged <i>esxF-esxE</i> expression vector	This study
pML5040	pBAD33-derivative; <i>P_{BAD}::8xHis-esxF-esxE-FLAG</i> ; <i>cat</i> ; <i>E.coli</i> replicative tagged <i>esxF-esxE</i> expression vector	This study
pML4845	pBAD24-sfGFPx1-derivative; <i>P_{BAD}::HA-esxU-esxT-Myc</i> ; <i>bla</i> ; <i>E.coli</i> replicative tagged <i>esxU-esxT</i> expression vector	This study
pML4862	ColE1 origin; <i>attP_{L5}</i> ; <i>P_{smyc}::Myc-esxU-esxT</i> ; <i>aph</i> ; integrative tagged <i>esxU-esxT</i> expression vector	This study
pML5084	ColE1 origin; <i>attP_{Ms6}</i> ; <i>P_{smyc}::8xHis-esxF-esxE</i> ; <i>hyg^R</i> ; integrative tagged <i>esxF-esxE</i> expression vector	This study
pML4863	ColE1 origin; <i>attP_{L5}</i> ; <i>P_{smyc}::HA-esxB-esxA</i> ; <i>aph</i> ; integrative tagged <i>esxB-esxA</i> expression vector	This study
pML4864	ColE1 origin; <i>attP_{L5}</i> ; <i>P_{smyc}::esxB-esxA-HA</i> ; <i>aph</i> ; integrative tagged <i>esxB-esxA</i> expression vector	This study
pML4854	ColE1 origin; <i>attP_{L5}</i> ; <i>P_{smyc}::HA-esxD-esxC</i> ; <i>aph</i> ; integrative tagged <i>esxD-esxC</i> expression vector	This study
pML4855	ColE1 origin; <i>attP_{L5}</i> ; <i>P_{smyc}::esxD-esxC-HA</i> ; <i>aph</i> ; integrative tagged <i>esxD-esxC</i> expression vector	This study
pML4860	ColE1 origin; <i>attP_{L5}</i> ; <i>P_{smyc}::HA-esxG-esxH</i> ; <i>aph</i> ; integrative tagged <i>esxG-esxH</i> expression vector	This study
pML4861	ColE1 origin; <i>attP_{L5}</i> ; <i>P_{smyc}::esxG-esxH-HA</i> ; <i>aph</i> ; integrative tagged <i>esxG-esxH</i> expression vector	This study
pML4869	ColE1 origin; <i>attP_{L5}</i> ; <i>P_{smyc}::HA-esxM-esxN</i> ; <i>aph</i> ; integrative tagged <i>esxM-esxN</i> expression vector	This study
pML4870	ColE1 origin; <i>attP_{L5}</i> ; <i>P_{smyc}::esxM-esxN-HA</i> ; <i>aph</i> ; integrative tagged <i>esxM-esxN</i> expression vector	This study
pML4805	pML2424-derivative; <i>hom_{up}-esxD-esxC- hom_{down}</i> ; <i>hyg^R</i> , <i>esxDC</i> deletion vector	This study
pML4852	ColE1 origin; <i>attP_{L5}</i> ; <i>P_{smyc}::esxD-esxC</i> ; <i>aph</i> ; integrative <i>esxDC</i> expression vector	This study
pML4803	pML2424-derivative; <i>hom_{up}-eccCa1-eccCb1- hom_{down}</i> ; <i>hyg^R</i> , <i>eccC1</i> deletion vector	This study

Table S3. Plasmids and cosmids used in this study

Plasmid	Properties	Reference
Cosmid 2F9	pYUB412 cosmid; <i>bla</i> ; <i>hyg</i> ; <i>int_{L5}</i> ; <i>attP_{L5}</i> ; coordinates of the integrated H37Rv DNA: ~4337-4369 kb contains the esx-1 locus of <i>M. tuberculosis</i> H37Rv	⁹
Cosmid I106	pYUB412 cosmid; <i>bla</i> ; <i>hyg</i> ; <i>int_{L5}</i> ; <i>attP_{L5}</i> ; coordinates of the integrated H37Rv DNA: ~4349-4385 kb contains the esx-2 locus of <i>M. tuberculosis</i> H37Rv	¹⁰
pML4875	ColE1 origin; <i>attP_{L5}</i> ; <i>P_{smyc}::HA-espB</i> ; <i>aph</i> ; integrative tagged <i>espB</i> expression vector	This study
pML4886	ColE1 origin; <i>attP_{Ms6}</i> ; <i>P_{smyc}::HA-esxU-esxT-FLAG</i> ; <i>hyg^R</i> ; integrative tagged <i>esxU-esxT</i> expression vector	This study
pML4881	ColE1 origin; <i>attP_{L5}</i> ; <i>P_{smyc}::HA-esxU-esxT</i> ; <i>aph</i> ; integrative tagged <i>esxU-esxT</i> expression vector	This study
pMV-ppe62HA	oriE origin; oriM origin; <i>P_{imyc}::ppe62-HA</i> ; <i>aph</i> ; replicative tagged PPE62 expression vector	This study
pML3732	ColE1 origin; oriM origin; <i>P_{imyc}::ppe62-HA-6xHis</i> ; <i>hyg^R</i> ; replicative tagged PPE62 expression vector	⁵

Table S3. Plasmids and cosmids used in this study (continued)

The origin of replication is denoted as “origin”. pAL5000ts denotes the temperature-sensitive origin of replication¹¹ of the pAL5000 plasmid¹². The *bla*, *hyg^R*, *cat* and *aph* genes confer resistance to ampicillin, hygromycin, chloramphenicol and kanamycin, respectively. The site specific integration of plasmids into the chromosomal *attB* site is mediated by the *attP_{L5}* site, facilitated by the mycobacteriophage L5 integrase encoding *int_{L5}* gene. The mycobacteriophage Ms6 integrase mediates the plasmid integration by the *attP_{Ms6}* site. The DNA fragments flanked by *loxP* sites are excised by Cre, the site-specific recombinase.

Oligonucleotide	Sequence (5' to 3')	Purpose
SpeI-Up-esxU	TG <u>ACTAGT</u> CAGATCGCGGAGCGACTGGTGTG	pML4806
EsxUT-2	ATT <u>TAAATC</u> CTCGTCTGGTTCCCTCCGA	
EsxUT-3	CTTAATTA <u>ACGGCT</u> GAGCTTGGGCACGC	
NsiI-Dn-esxT	TGCATGCATGCAGTGCCTTGATCAACTGCTGGT	
Pac-SD-EsxU_1	GCTTAATTAACAGAAAGGAGGTAAATAGTGAGCACACCGAACACGCTGA	pML4328
EsxT_HindIII	CGAAGCTTCCTAGCGTGCCCAAGCTCCAGCCGCCCGC	
NheI-HA-EsxU-F	CTAGCTAGCAGGAGGAATTCGCCAATGTACCCGTACGACGTGCCGGACTACG CCGTGAGCACACCGAACACGCT	pML4850
XmaI-EsxU-R	CTAGCCCGGGCTGATCCTCGGTCTATAGGTTCGC	
XmaI-EsxT-F	CTAGCCCGGGGAGGATCAGCCTCGACTATGAACGCAGA	
HindIII-EsxT-8xHis-R	CATAAGCTTCTAGTGATGGTGATGGTGATGGTGATGGCGTGCCCAAGCTCCA GCC	
NdeI_Myc_esxF_F	GA <u>ACTACATAT</u> GGAACAAAACTCATCTCAGAAGAGGATCTGATGGGTGCCGA AC	pML5056
XmaI-EsxF-R	CTAGCCCGGGTCAGCCACCGCCACCTCACGA	
XmaI-EsxE-F	CTAGCCCGGGGAGGAGGAATTCGCCAGTGGATCCGACCGTGTTGG	
HindIII-EsxE-FLAG-R	CATAAGCTTTCATTTATCATCATCATCTTTATAATCCGACCACATACCCAAA TTCGT	
NdeI-His8-EsxF-F	GA <u>ACTACATAT</u> GCATCACCATCACCATCACCATCAC	pML5040
HindIII-EsxT-Myc-R	CATAAGCTTCTACAGATCCTCTTCTGAGATGAGTTTTTGTTCGCGTGCCCAA GCTCCAGC	pML4845
PacI-SD-Myc-F	GTCATTAATTAACAGAAAGGAGGTAAATAATGGAGCAGAAGCTGATCTCGGA AGAGGAC	pML4862
Myc- S(G)4-EsxU-F	ATGGAGCAGAAGCTGATCTCGGAAGAGGACCTGTCCGGCGGTGGCGGTGTGA GCACACCG	
S(G)4-EsxU-F	TCCGGCGGTGGCGGTGTGAGCACACCGAACACGCT	
EsxT-HindIII-Rev1	CATAAGCTTCTAGCGTGCCCAAGCTCCAGCCGCC	
PacI-SD-His8-F	GTCATTAATTAACAGAAAGGAGGTAAATAATGCATCACCATCACCATCACCA TCAC	pML5084
His8-S(G)4-EsxF-F	ATGCATCACCATCACCATCACCATCACTCCGGCGGTGGCGGTATGGGTGCCG ACGAC	
S(G)4-EsxF-F	TCCGGCGGTGGCGGTATGGGTGCCGACGACACGCT	
HindIII-EsxE-R1	CATAAGCTTTCACGACCACATACCCAAATTCGTGGCCATCGC	
PacI-SD-HA-F	GTCATTAATTAACAGAAAGGAGGTAAATAATGTACCCGTACGACGTGCCGGA CTACGCC	pML4863
HA-linker_i_EsxB_FP	GTACGACGTGCCGGACTACGCCTCCGGCGGTGGCGGTATGGCAGAGATGAAG ACC	
EsxA-HindIII-RP	GCTAGCAAGCTTATGCGAACATCCCAGTG	

Table S4. Oligonucleotides used in this study

The underlined sequences denote the restriction sites.

Oligonucleotide	Sequence (5' to 3')	Purpose
Pac-SD-EsxBF	GTCATCTTAATTAACAGAAAGGAGGTTAATAATGGCAGAGATGAAG	pML4864
HindIII-HA-linker-EsxARP	GTAAAGCTTAGGCGTAGTCCGGCACGTCGTACGGGTAAACCGCCACCGCCGGA TGCGAACATCCCAGT	
PacI-SD-HA-F	GTCATTAATTAACAGAAAGGAGGTTAATAATGTACCCGTACGACGTGCCGGA CTACGCC	pML4854
HA- S(G)4-EsxD-F	ATGTACCCGTACGACGTGCCGGACTACGCCTCCGGCGGTGGCGGTGTGGCAG ACACAATT	
S(G)4-EsxD-F	TCCGGCGGTGGCGGTGTGGCAGACACAATTCAGGTAACAC	
HindIII-EsxC-R-1	CATAAGCTTAGAACAAGCCCGCGATGGCCTGG	
PacI-SD-EsxD-F	GTCATTAATTAACAGAAAGGAGGTTAATAGTGGCAGACACAATTCAGGTAAC AC	pML4855
S(G)4-EsxC-R	ACCGCCACCGCCGGAGAACAAGCCCGCGATGGCCT	
HindIII-HA-S(G)4-R	CATAAGCTTAGGCGTAGTCCGGCACGTCGTACGGGTAAACCGCCACCGCCGGA	
PacI-SD-HA-F	GTCATTAATTAACAGAAAGGAGGTTAATAATGTACCCGTACGACGTGCCGGA CTACGCC	pML4860
HA- S(G)4-EsxG-F	ATGTACCCGTACGACGTGCCGGACTACGCCTCCGGCGGTGGCGGTATGAGCC TTTTGGAT	
S(G)4-EsxG-F	TCCGGCGGTGGCGGTATGAGCCTTTTGGATGCTCATATCCCAC	
EsxH-HindIII-R	CATAAGCTTCTAGCCGCCCCATTTGGC	
PacI-SD-EsxG-F	GTCATTAATTAACAGAAAGGAGGTTAATAATGAGCCTTTTGGATGCTCATAT CCCAC	pML4861
S(G)4-EsxH-R	ACCGCCACCGCCGGAGCCGCCCCATTTGGC	
HindIII-HA-S(G)4-R	CATAAGCTTAGGCGTAGTCCGGCACGTCGTACGGGTAAACCGCCACCGCCGGA	
EsxDC-1	TGACTAGTCCGTGGTATCGACCGCGATC	pML4805
Swal-esxDC-2-in-frame	ATTTAAATTGTTACCTGAATTGTGTCTGCCAC	
PacI-esxDC-3-in-frame	CTTAATTAAATCGGAACCGACCAGGCCAT	
EsxDC-4	TGCATGCATGGTGTGCGGATACCGAATCCC	
PacI-SD-EsxD-F	GTCATTAATTAACAGAAAGGAGGTTAATAGTGGCAGACACAATTCAGGTAAC AC	pML4852
HindIII-EsxC-R	CATAAGCTTAGAACAAGCCCGCGATGG	
SpeI-Up-eccCa1	TGACTAGTCACAGTCACACGAAGCGGTGCTGGT	pML4803
Swal-Up-eccCa1	ATTTAAATGCAGTGCGGCATCTTTCGACAGCA	
PacI-Dn-eccCb1	CTTAATTAAAGCCCAAGACCAGCTCCACCGTG	
NsiI-Dn-eccCb1	TGCATGCATGCAAACAACGACGTCACCTGCTGCA	

Table S4. Oligonucleotides used in this study (continued)

The underlined sequences denote the restriction sites.

Oligonucleotide	Sequence (5' to 3')	Purpose
PacI-SD-HA-F	GTCATTAATTAACAGAAAGGAGGTTAATAATGTACCCGTACGACGTGCCGGA CTACGCC	pML4875
HA-SG4-espB-F	GTACGACGTGCCGGACTACGCCTCCGGCGGTGGCGGTATGACGCAGTCGCAG ACCGTGAC	
HindIII-espB-R	CATAAGCTTCACTTCGACTCCTTACTGTCCT	
Overlap-HA-F	GGATCCGCATGCTTAATTAAGGGAGAACATGTACCCGTACGACGTGCCGGAC TACGCC	pML4886
EsxT-SG4-R	ACCGCCACCGCCGGAGCGTGCCCAAGCTCCAG	
EsxT-SG4-FLAG-R	CGTAAGCTTCACTTGTTCATCGTCATCCTTGTAATCACCGCCACCGCCGGAGC GTGCCCAA	
PacI-SD-HA-F	GTCATTAATTAACAGAAAGGAGGTTAATAATGTACCCGTACGACGTGCCGGA CTACGCC	pML4881
HA-SG4-EsxU	ATGTACCCGTACGACGTGCCGGACTACGCCTCCGGCGGTGGCGGTGTGAGCA CACCGAAC	
EsxT-HindIII-Rev1	CATAAGCTTCTAGCGTGCCCAAGCTCCAGCCGCC	
ppe62F	ACTCGGGCCTCACTAACAC	pMV- ppe62HA Confirmation
pMV261 Rev	GCAGTCGATCGTACGCTAGT	

Table S4. Oligonucleotides used in this study (continued)

The underlined sequences denote the restriction sites.

Antibody	Source	Identifier
Rabbit polyclonal anti-TNT	ProMab Biotechnologies, negatively purified antiserum was used	This study
Rabbit polyclonal anti-EsxU	GenScript	This study
Rabbit polyclonal anti-GlpX	UAB Hybridoma core	13
Mouse monoclonal anti-MctB	UAB Hybridoma core	13
Rabbit polyclonal anti-EsxF	GenScript	4
Mouse monoclonal anti His-Tag	ABclonal	AE003
Mouse monoclonal anti HA-Tag	Invitrogen	PI26183
Mouse monoclonal anti Myc-Tag	Invitrogen	MA121316
Rabbit polyclonal anti-FLAG	Millipore	F7425
Mouse monoclonal anti-Galectin-3 (clone A3A12)	Abcam	ab2785
Rabbit polyclonal anti- <i>Mycobacterium tuberculosis</i>	Abcam	ab905
Rabbit polyclonal anti-ESAT6	Abcam	ab45073
Rabbit polyclonal anti-EsxC	Promab	This study
Rabbit polyclonal anti-Antigen-85 complex	BEI Resources	NR-13800
Donkey anti-rabbit IgG (H+L)-Alexa Fluor-594 secondary	Invitrogen	A-21207
Goat anti-mouse IgG (H+L)-Alexa Fluor-594 secondary	Invitrogen	A-11005
Donkey anti-rabbit IgG (H+L)-Alexa Fluor-488	Invitrogen	A-21206
IRDye 680RD Donkey anti-rabbit IgG secondary	LI-COR	926-68073
Goat Anti-rabbit IgG-HRP	Sigma	A6154
Anti-Mouse IgG (H+L), HRP Conjugate	Promega	W4021
6x-His Tag Monoclonal Antibody (HIS.H8), Alexa Fluor 555	Invitrogen	MA121315A55
c-Myc Monoclonal Antibody (9E10), Alexa Fluor 647	Invitrogen	MA1980A647

Table S5. Antibodies used in this study

Canonical heterodimers	ipTM score	DOI	Date (YYYY-MM-DD)
EsxUT	0.82	https://doi.org/10.6084/m9.figshare.30182620.v1	2024-09-18
EsxBA	0.77	https://doi.org/10.6084/m9.figshare.30182824.v2	2024-09-30
EsxDC	0.76	https://doi.org/10.6084/m9.figshare.30182830.v1	2024-09-30
EsxFE	0.62	https://doi.org/10.6084/m9.figshare.30182827.v1	2024-09-13
EsxGH	0.56	https://doi.org/10.6084/m9.figshare.30182803.v1	2024-09-30
Non-Canonical heterodimers			
EsxFH	0.65	https://doi.org/10.6084/m9.figshare.30182788.v1	2024-09-26
EsxUE	0.60	https://doi.org/10.6084/m9.figshare.30182800.v1	2024-09-26
EsxDE	0.50	https://doi.org/10.6084/m9.figshare.30182797.v1	2024-09-26
EsxBE	0.47	https://doi.org/10.6084/m9.figshare.30182782.v1	2024-09-26
EsxGT	0.44	https://doi.org/10.6084/m9.figshare.30182767.v1	2024-09-26
EsxDA	0.43	https://doi.org/10.6084/m9.figshare.30182773.v1	2024-09-26
EsxDT	0.42	https://doi.org/10.6084/m9.figshare.30182785.v1	2024-09-30
EsxFA	0.42	https://doi.org/10.6084/m9.figshare.30182812.v1	2024-09-30
EsxGC	0.41	https://doi.org/10.6084/m9.figshare.30182806.v1	2024-09-30
EsxUA	0.40	https://doi.org/10.6084/m9.figshare.30182821.v1	2024-09-26
EsxBC	0.36	https://doi.org/10.6084/m9.figshare.30182815.v1	2024-09-26
EsxGA	0.36	https://doi.org/10.6084/m9.figshare.30182818.v1	2024-09-30
EsxUH	0.34	https://doi.org/10.6084/m9.figshare.30182779.v1	2024-09-26
EsxBH	0.33	https://doi.org/10.6084/m9.figshare.30182794.v1	2024-09-26
EsxBT	0.33	https://doi.org/10.6084/m9.figshare.30182761.v1	2024-09-26
EsxUC	0.30	https://doi.org/10.6084/m9.figshare.30182764.v1	2024-09-26
EsxGE	0.26	https://doi.org/10.6084/m9.figshare.30182791.v1	2024-09-30
EsxFT	0.25	https://doi.org/10.6084/m9.figshare.30182758.v1	2024-09-26
EsxFC	0.22	https://doi.org/10.6084/m9.figshare.30182776.v1	2024-09-30
EsxDH	0.18	https://doi.org/10.6084/m9.figshare.30182770.v1	2024-09-30

Table S6. The ipTM scores of the Esx heterodimers indicate the accuracy of each protein complex predicted by AlphaFold 3². The pdb files were uploaded to Figshare and the DOI of the models is listed. The models were predicted by Alphafold at the indicated dates.

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