Supporting information

Table S1

Data collection	
Microscope	Titan KRIOS with K2-detector
Voltage	300 kV
Pixel size (Å)	1.058
Micrographs collected (#)	2655
Refinement	
Particles (#)	168565
Resolution (Å) at FSC = 0.143	3.37
Cc_mask (CC_volume)	0.762 (0.741)
RMS deviations	
Bonds (Å)	0.008
Angles (°)	1.198
Chirality (°)	0.073
Planarity (°)	0.009
Validation	
Clash score	2.33
Favoured rotamers (%)	96.9
Ramachandran favoured (%)	99.16
Ramachandran allowed (%)	0.84
Ramachandran outliers (%)	0.00

Table S1. Results of CryoEM Data collection / refinement

Fig S1

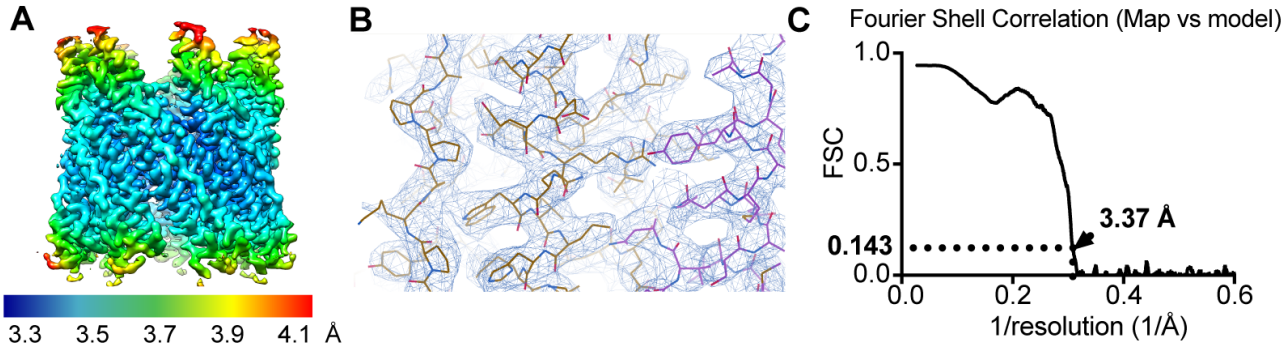

Fig S1. High-resolution structure of M. tuberculosis heptameric form of EspB. . A) Final EspB map using C7 symmetry with colors based on the local resolution estimation by Bsoft. B) Close-up view of a detailed region showing the quality of the electronic potential map. C) The Fourier shell correlation curves for the final cryoEM maps using C7 symmetry.

Fig S2

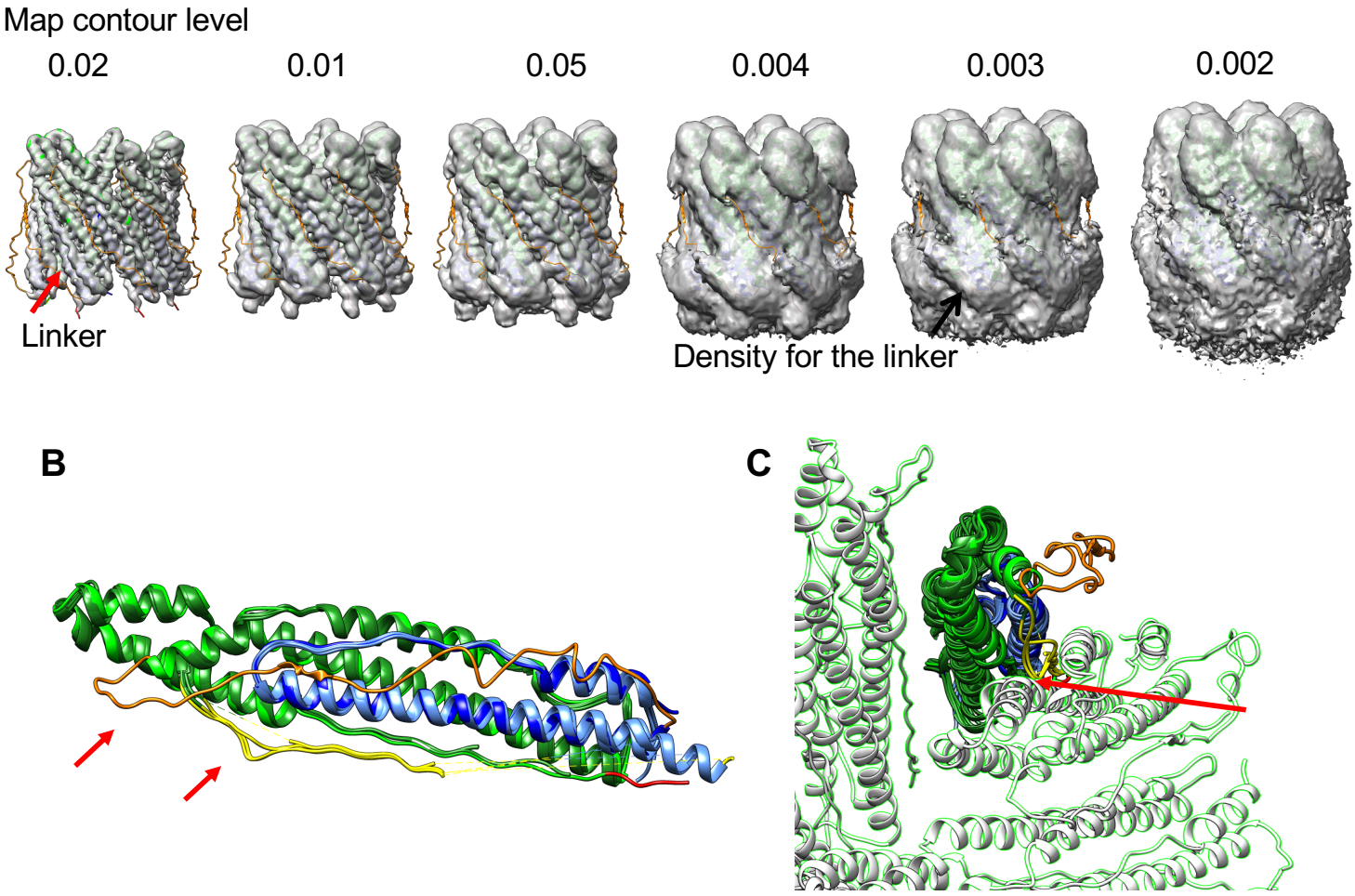

Fig S2. Conformational change of the disordered linker. **A)** Views of the non-masked map represented at different contour levels. The disordered linker was modeled to fit into the density visible only at low contour level in Chimera. The red arrow indicates the site of the disordered linker **B)** Superimposition of the structure of one EspB monomer from the cryoEM structure onto the X-ray structures of EspB with different space groups (PDB code: 4XXX, 4XXN, 4XY3, 4XWP). For each monomer, PE domains are colored in blue, PPE in green, and C-terminal in red. The linker of the monomer from the cryoEM structure is colored in orange whereas linkers from X-ray structure are in yellow. **C)** Superimposition of EspB X-ray monomer structure to a monomer from the cryoEM structure showing that linkers from X-ray structure (yellow) clash with the neighboring chain in the cryoEM structure.

Fig S3

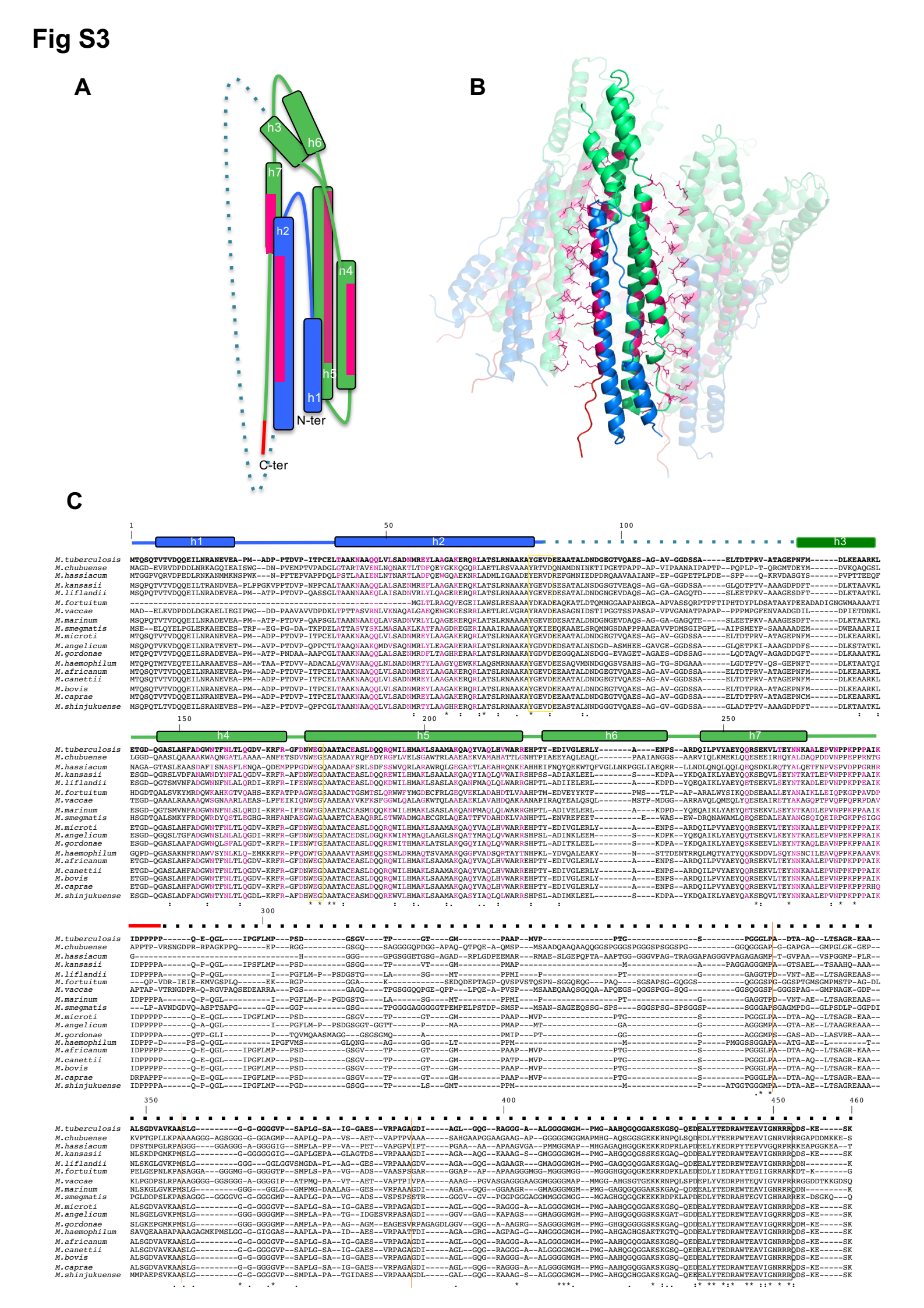
A
h3
h6
h7
h2
h4
h5
h1
N-ter
C-ter
B
C
h1
h2
h3
h4
h5
h6
h7
M.tuberculosis
M.chubuense
M.hassiacum
M.kansasii
M.liflandii
M.fortuitum
M.vaccae
M.marinum
M.smegmatis
M.microti
M.angelicum
M.gordonae
M.haemophilum
M.africanum
M.canettii
M.bovis
M.caprae
M.shinjukuense

Fig S3. Structure-based sequence alignment of EspB from different mycobacterial species. A) The topology of EspB is mapped and colored by domains (PE domain in blue, PPE in green). In pink are represented all the regions at the interface between the subunits. **B)** Side view of the monomeric structure in the context of heptamer. Residues located at the surface are shown in pink sticks **C)** Secondary structure elements of EspB are displayed above the sequences in the following color code: PE domain in blue, PPE domain in green, C-terminal domain in red. Dashed lines correspond to portions, which are not visible in the cryoEM map. In pink are represented all the residues at the interface between the subunits. The characteristic sequence motifs (YxxxD/E and WxG) of EspB are framed in yellow. An orange line indicates the MycP1 proteolytic cleavage sites after P332, A358 and A386 in *M. tuberculosis* EspB. Finally, the black frame denotes the conserved region at the C-terminus of EspB.