Supplemental Material

Structure and mechanism of *Mycobacterium smegmatis* polynucleotide phosphorylase

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Supplemental Figures S1, S2, S3

Supplemental Tables S1, S2

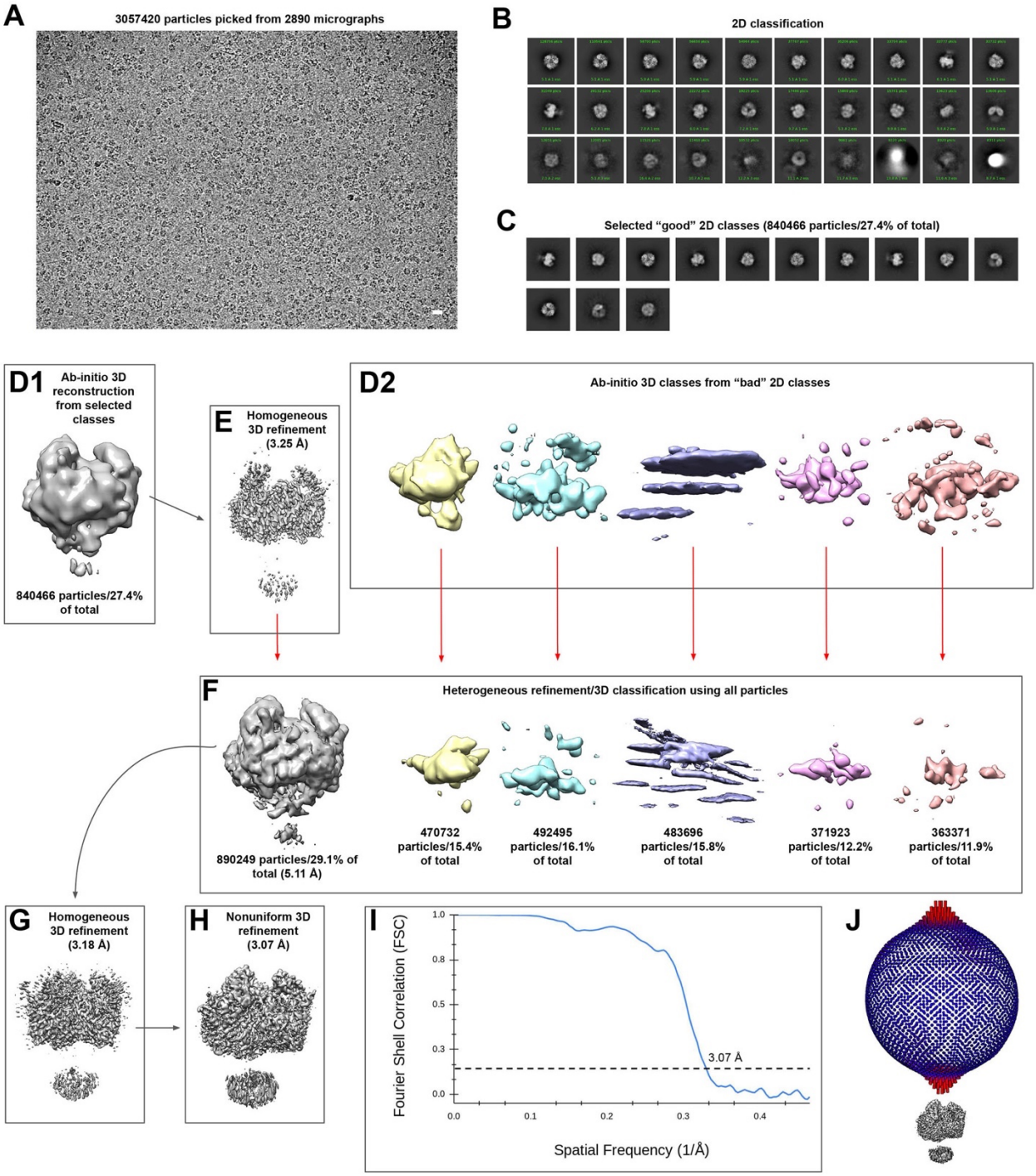

Figure S1. Cryo-EM analysis workflow for MsmPNPase. (A) Representative micrograph (scale bar, 150 Å). (B) Two-dimensional class averages (box size, 319.2 Å). (C) Selected 2D classes. (D1) 3D initial model generated from selected 2D classes in panel C. (D2) Ab-initio 3D models generated from "bad" particles (unselected 2D classes) from panel B. (E) Homogeneous 3D refinement of particles in panel D1. (F) Heterogeneous 3D refinement for filtering "good" particles from the total particle dataset via 3D classification. (G) Homogeneous 3D refinement of the "good" particle class from panel F. (H) Nonuniform 3D refinement for final structure. (I) Fourier Shell Correlation curve of the final map measured via cryoSPARC (dotted line, 0.143 Å cutoff). (J) Angular distribution of all particle orientations used in the final 3D reconstruction of the map.

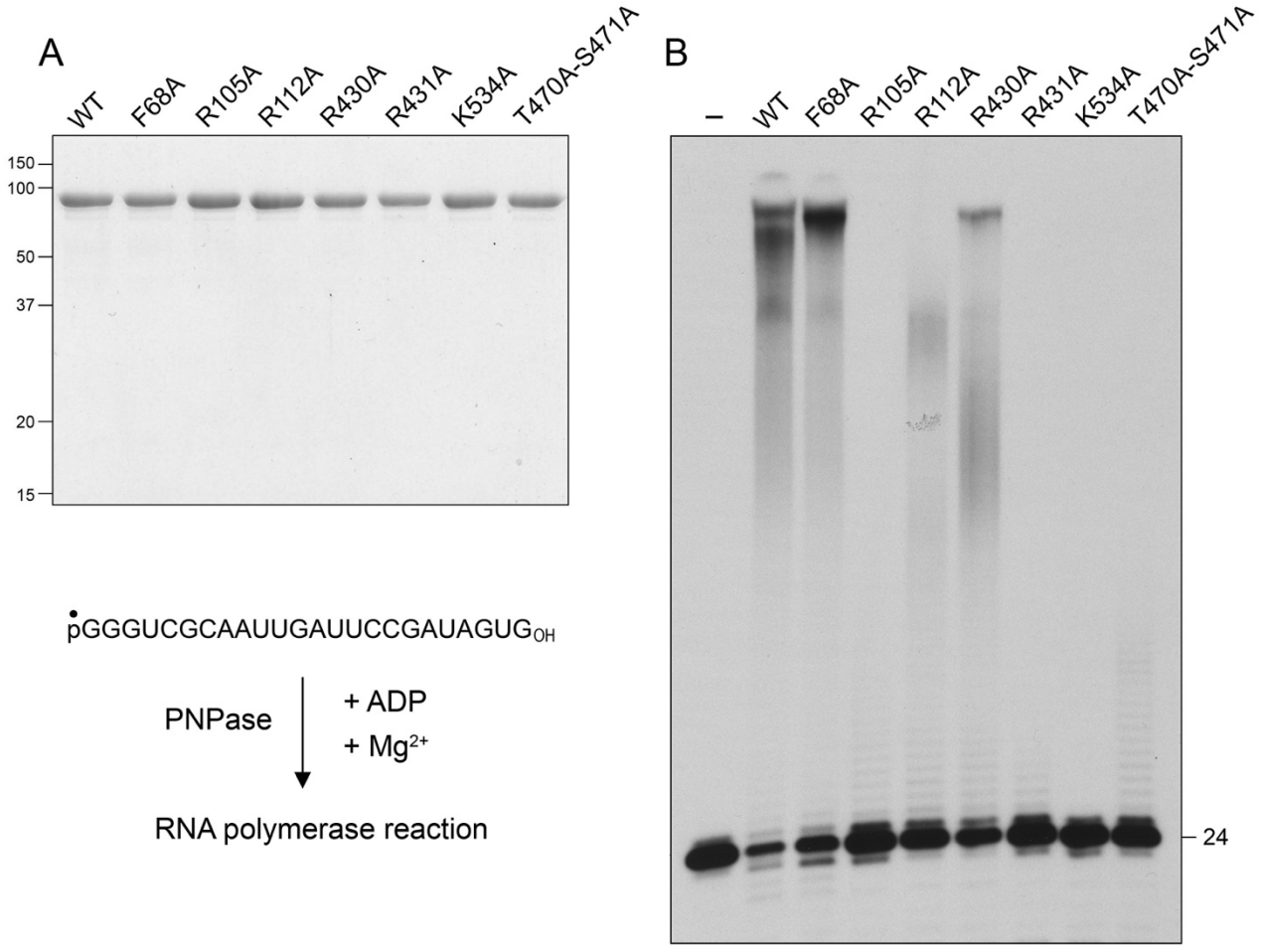

Figure S2. Effects of alanine mutations on MsmPNPase RNA polymerase activity. (A) Aliquots (5 µg) of wild-type and mutant MsmPNPase homotrimer preparations were analyzed by SDS-PAGE. A scan of the Coomassie blue-stained gel is shown. The positions and sizes (kDa) of marker polypeptides are indicated on the left. (B) As depicted below panel A, RNA polymerase activity was assayed by ADP/Mg^{+}-dependent elongation of a 5' ^{32}P-labeled 24-mer RNA primer. Polymerase reaction mixtures (10 µl) containing 20 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 2 mM ADP, 1 pmol (0.1 µM) 5' ^{32}P-labeled 24-mer RNA primer, and 10 pmol (1 µM) MsmPNPase homotrimer as specified above the lanes were incubated at 37°C for 15 min. The products were analyzed by urea-PAGE and visualized by autoradiography.

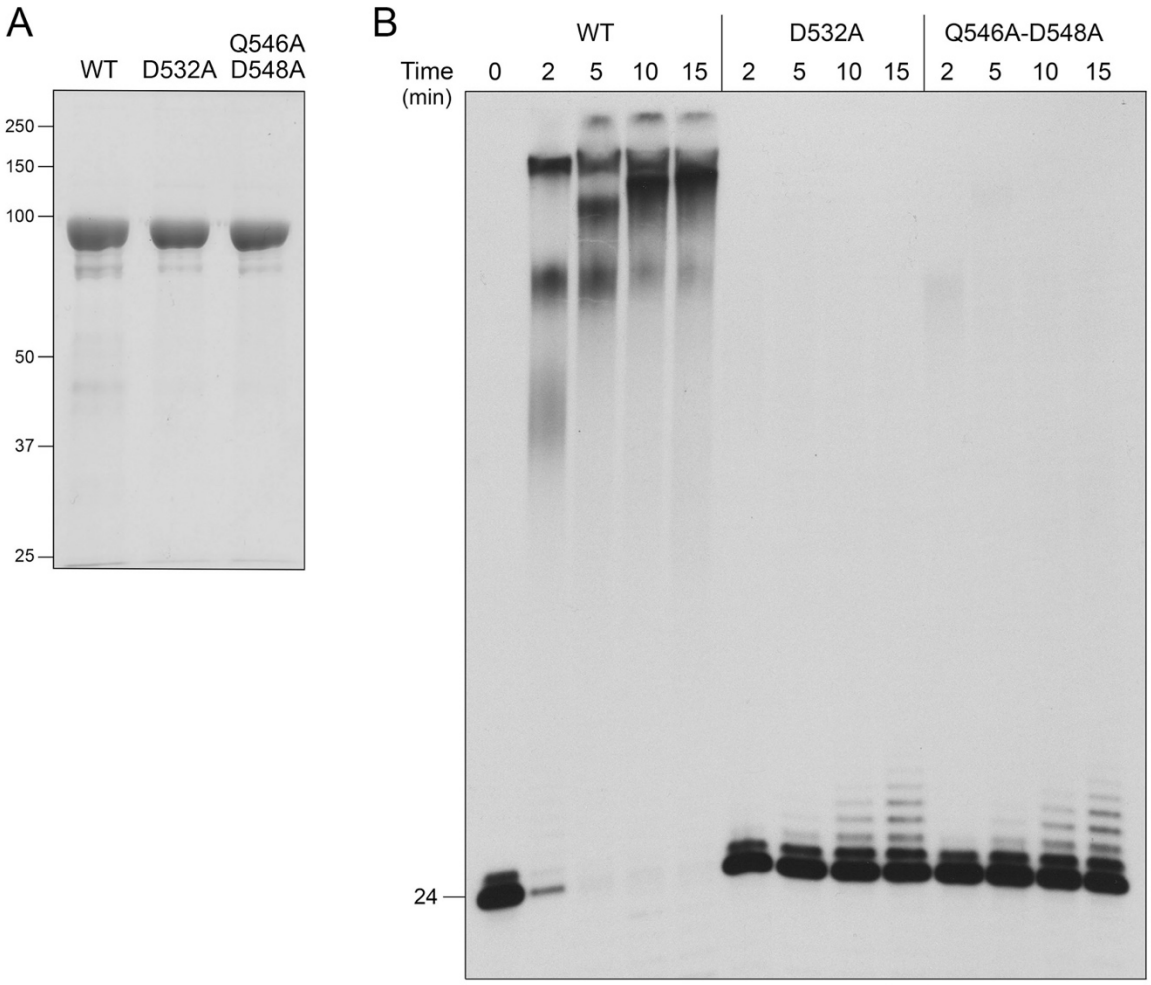

Figure S3. Effect of metal ligand mutations on MsmPNPase RNA polymerase activity. (A) Aliquots (5 µg) of wild-type and mutant MsmPNPase homotrimer preparations were analyzed by SDS-PAGE. A scan of the Coomassie blue-stained gel is shown. The positions and sizes (kDa) of marker polypeptides are indicated on the left. (B) Polymerase reaction mixtures (70 µl) containing 20 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 2 mM ADP, 0.1 µM 5' ^{32}P-labeled 24-mer RNA primer, and 1 µM wild-type or mutant MsmPNPase homotrimer as specified were incubated at 37°C. Aliquots (10 µl) were withdrawn at the times specified and quenched with formamide/EDTA. The products were analyzed by urea-PAGE and visualized by autoradiography.

Table S1. Statistics of cryo-EM data collection and model refinement

Data collection and processing	
Microscope	Titan Krios G2
Voltage (kV)	300
Nominal magnification (×)	22,500
Detector	Gatan K3
Automation software	SerialEM
Defocus range (μm)	−0.8 to −2
Pixel size (Å)	1.064
Exposure rate (e− $pixel^{-1}$ s^{-1})	20
Electron exposure (e− $Å^{-2}$)	53
Frames per exposure (no.)	60
Micrographs (no.)	2,890
Total extracted particles (no.)	3,057,420
Particles used in map (no.)	890,249
Symmetry	C3
FSC threshold	0.143
Map resolution (Å)	3.07
Map sharpening B-factor ($Å^2$)	−166.9
Refinement	
Software	Phenix
FSC threshold	0.143
Model resolution (Å, masked/unmasked)	3.04/3.07
Map correlation coefficient	0.85
Model composition	
Nonhydrogen atoms	13,542
Protein residues	1761
Nucleotides	15
Magnesium	6
B-factor (mean, $Å^2$)	
Protein residues	23.7
Nucleotides (occupancy = 0.3)	16.5
Magnesium (occupancy = 1)	7.5
r.m.s. deviations	
Bond lengths (Å)	0.006
Bond angles (°)	0.654
Validation	
MolProbity score	2.38
Clashscore	8.65
Rotamer outliers (%)	6.5
Cβ outliers (%)	0.0
Ramachandran plot	
Favored (%)	95.7
Allowed (%)	4.3
Disallowed (%)	0.0

Table S2. MsmPNPase structural homology to other PNPase enzymes

Source	pdb ID	Z score	root mean square deviation	% identity
Streptomyces antibioticus	1E3H	54.8	1.2 Å at 580 Cα positions	73
Coxiella burnetii	4NBQ	50.7	2.6 Å at 546 Cα positions	44
Caulobacter crescentus	4AM3	48.7	2.5 Å at 548 Cα positions	45
Escherichia coli	3GCM	48.2	2.6 Å at 542 Cα positions	46
Acinetobacter baumannii	6D6K	47.7	2.2 Å at 548 Cα positions	43
Staphylococcus aureus	5XEX	45.1	2.6 Å at 511 Cα positions	45
Homo sapiens	3ULK	43.0	2.3 Å at 535 Cα positions	36