Supplemental Material

Characterization of mycobacterial PolD2 and PolD1 as RNA/DNA polymerases homologous to the POL domain of bacterial DNA ligase D

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Supplemental Tables S1 and S2

Supplemental Figures S1, S2, and S3

Table S1

Prevalence of PolD1 and PolD2 in mycobacterial proteomes

Species	PolD1 (aa)	PolD2 (aa)
Mycobacterium abscessus	342	409
Mycobacterium avium	342	426
Mycobacterium colombiense	343	422
Mycobacterium gilvum	349	412
*Mycobacterium intracell*ulare	343	422
Mycobacterium kansasii	345	416
Mycobacterium marinum	346	420
Mycobacterium parascrofulaceum	343	416
Mycobacterium phlei	349	411
Mycobacterium rhodesiae	349	411
Mycobacterium smegmatis	350	426
Mycobacterium thermoresistibile	344	410
Mycobacterium tuberculosis	346	397
Mycobacterium tusciae	349	428
Mycobacterium ulcerans	346	423
Mycobacterium vanbaalenii	349	412
Mycobacterium xenopi	358	413

Table S2

Mycobacterium smegmatis strains used in this study

Strain	Genotype	Source
mc^2155 "wild-type"	*ept1*	Ref. 30
MGM 801	mc^2155 *ligD-(D136A-D138A)*	Ref. 21
MGM 843	Δ*polD1*	This study
MGM 2027	*mc^2155* Δ*polD2::loxP-hyg-loxP*	This study
MGM 2029	*mc^2155* Δ*polD2::loxP*	This study
MGM 845	MGM801 Δ*polD1*	This study
MGM 2028	MGM801 Δ*polD2*::*loxP-hyg-loxP*	This study
MGM 2030	MGM801 Δ*polD2*::*loxP*	This study
MGM 846	MGM 2030 Δ*polD1*	This study

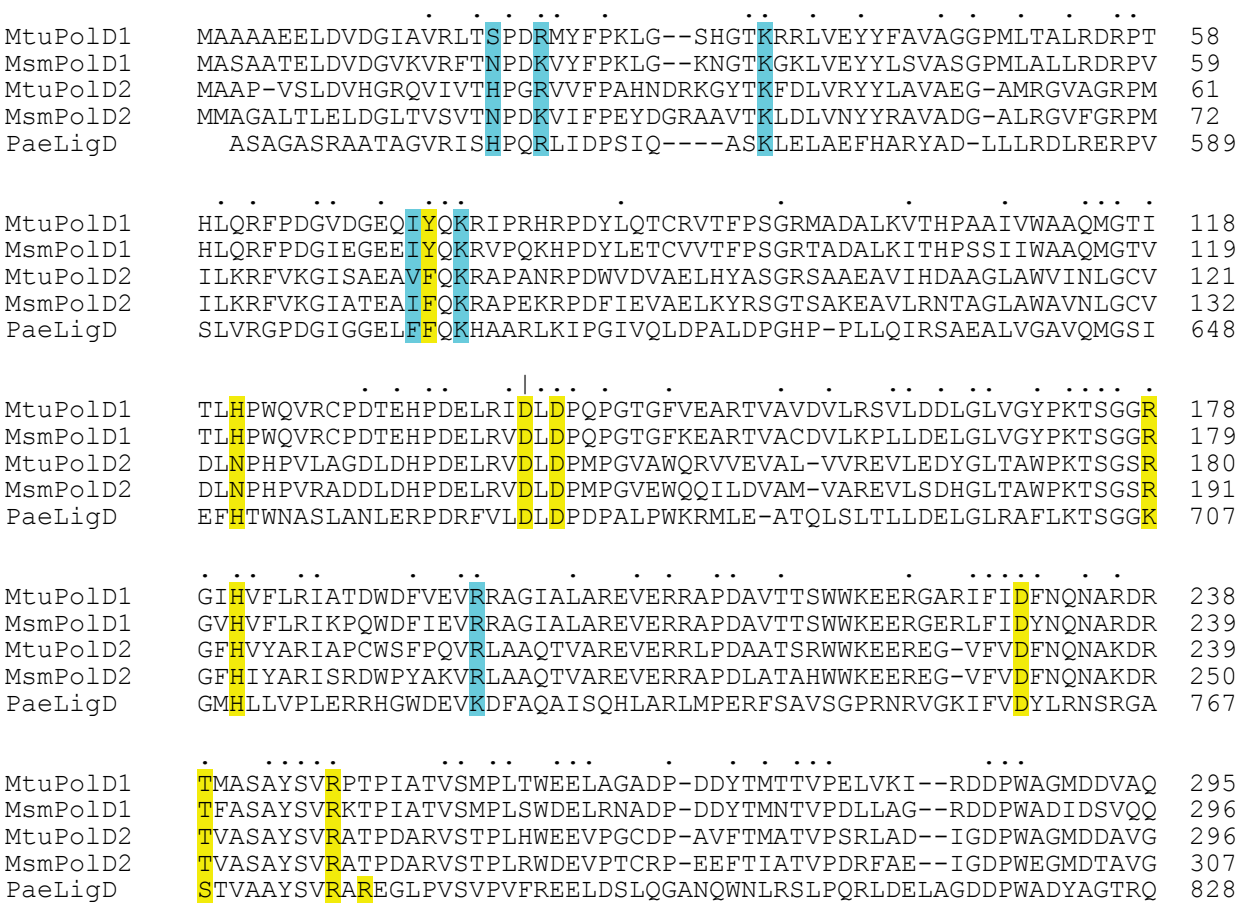

Figure S1. **Mycobacterial PolD1 and PolD2 are homologous to the LigD POL domain**. The amino acid sequences of MtuPolD1, MsmPolD1, MtuPolD2 and MsmPolD2 are aligned to the homologous segment of the POL domain of *Pseudomonas aeruginosa* (Pae) LigD. Gaps in the alignment are denoted by –. Positions of side chain identity/similarity in all five proteins are denoted by ●. The active site aspartate that was mutated to alanine in MsmPolD1 and MsmPolD2 is indicated by | above the alignment. Counterpart of amino acids in bacterial LigD that contact manganese or ATP are highlighted in yellow; amino acids that contact DNA are highlighted in cyan.

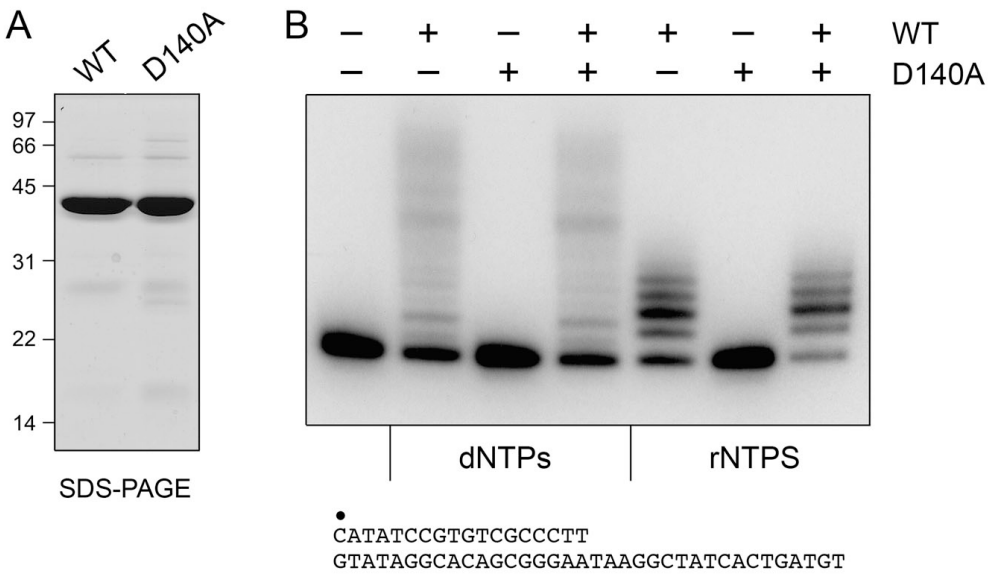

Figure S2. **Polymerase activity of recombinant MsmPolD1**. (A) Aliquots (5 μg) of the phosphocellulose preparations of wild-type MsmPolD1 and mutant D140A were analyzed by SDS-PAGE. The Coomassie Blue-stained gel is shown. The positions and sizes (kDa) of marker polypeptides are indicated on the *left*. (B) Polymerase reaction mixtures (20 μl) containing 50 mM Tris-HCl (pH 7.5), 1.25 mM $CoCl_2$, 1 pmol of ^{32}P-labeled primer-template DNA substrate, 100 μM each of dATP, dGTP, dCTP and dTTP (dNTPs) or 100 μM each of ATP, GTP, CTP and UTP (rNTPs), and 400 ng of MsmPolD1 (WT or D140A, where indicated by +) were incubated for 10 min at 37°C. The products were resolved by PAGE and visualized by autoradiography.

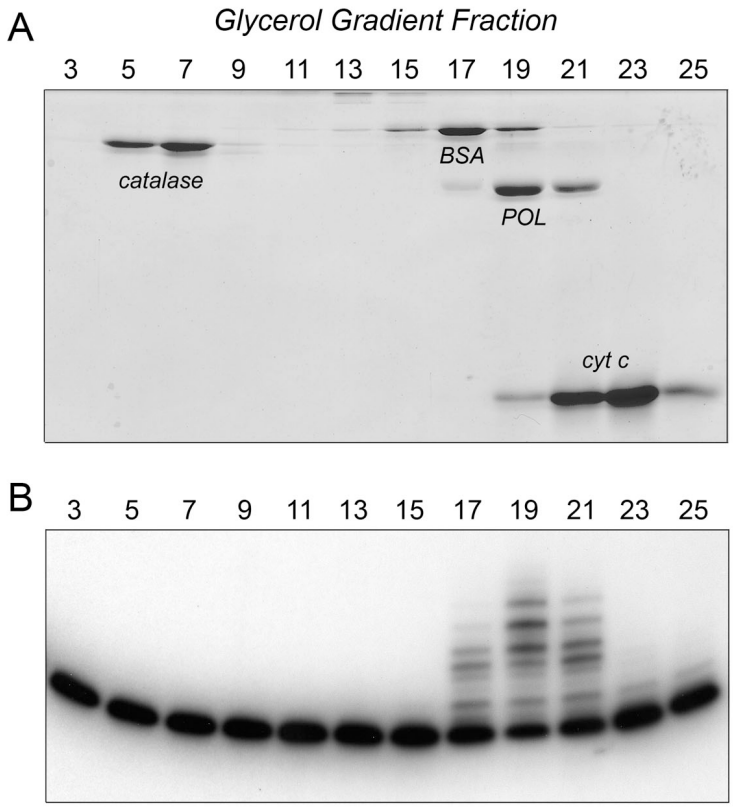

Figure S3**. MsmPolD1 sediments as a monomer**. Glycerol gradient sedimentation of a mixture of MsmPolD1, catalase, BSA and cytochrome *c* was performed as described under Experimental Procedures. (A) Aliquots (15 µl) of the odd-numbered gradient fractions were analyzed by SDS-PAGE. The Coomassie Blue-stained gel is shown. (B) Polymerase reaction mixtures (20 µl) containing 50 mM Tris-HCl (7.5), 1.25 mM $CoCl_2$, 1 pmol of ^{32}P-labeled primer-template DNA substrate, 100 µM each of dATP, dGTP, dCTP and dTTP, and 2 µl of the odd-numbered gradient fractions were incubated for 10 min at 37°C. The reaction products were resolved by PAGE and visualized by autoradiography.