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Supporting information for article:

Active-site residues and salt bridges at the subdomain interfaces of the adenylation domain of *Mycobacterium tuberculosis* NAD^+-dependent DNA ligase A are important for the initial steps of nick-sealing activity

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Table S1 List of primers used to generate mutants in AdD domain and MtbLigA

H236Y	Forward	5’- ATC TGC TAT GGG CTG GGC CAC GTG GAG-3’
	Reverse	5’- CAG CCC ATA GCA GAT CAT CCG CAG CCG-3’
H236A	Forward	5’-ATC TGC GCA GGA CTG GGC CAC GTG GAG -3’
	Reverse	5’-CAG CCC TGC GCA GAT CAT CCG TAGCCG -3’
K123A	Forward	5’-GAG CTA GCA ATC GAC GGC GTC GCG CTG-3’
	Reverse	5’-GTC GAT TGC GAG CTC ACA CAG GTA ATG-3’
K123R	Forward	5’-GAG CTC AGA ATC GAC GGA GTA GCG CTG-3’
	Reverse	5’- GTC GAT TCT GAG CTC ACA CAG GTA ATG-3’
E22A	Forward	5’- CTG GCC GCA GAG GTA CGT GAG CAC CAG-3’
	Reverse	5’- CAC CTC TGC GGC CAG AGC CTG CCA CTG-3’
E26A	Forward	5’-GTG CGT GCA CAC CAG TTC CGT TAT-3’
	Reverse	5’-CTG GTG TGC ACG TAC CTC CTC GGC-3’
E87A	Forward	5’-CAT CTC GCG CGA ATG CTC AGC CTA GAC-3’
	Reverse	5’-CAT TCG CGC GAG ATG GTC GAC GGG-3’

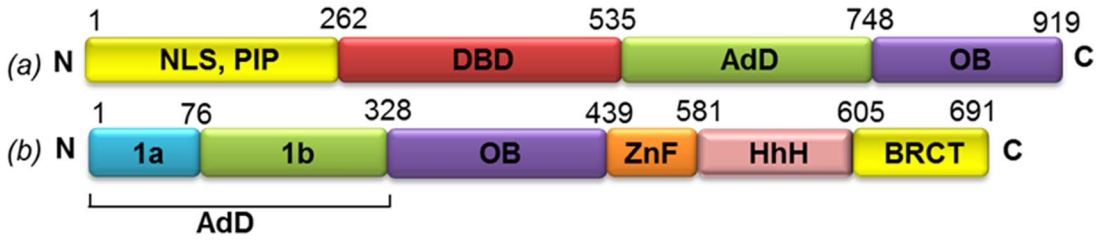

Figure S1 Architectural difference in eukaryotic and prokaryotic DNA ligase. Domain organization of *(a)* human LigI and *(b)* MtbLigA. Eukaryotic and mycobacterial DNA ligases have functionally similar domains, but they are jumbled in their linear sequences with other minor differences. AdD is adenylation domain (which is further subdivided in to 1a and 1b subdomain in case of MtbLigA), OB fold is oligomer binding fold, ZnF stands for Zinc finger, HhH represents helix hairpin helix, BRCT denotes BRCA1 C terminal like domain. NLS stands for nuclear localization sequence and PIP stands for protein interaction peptide motif that mediates interaction with other proteins.

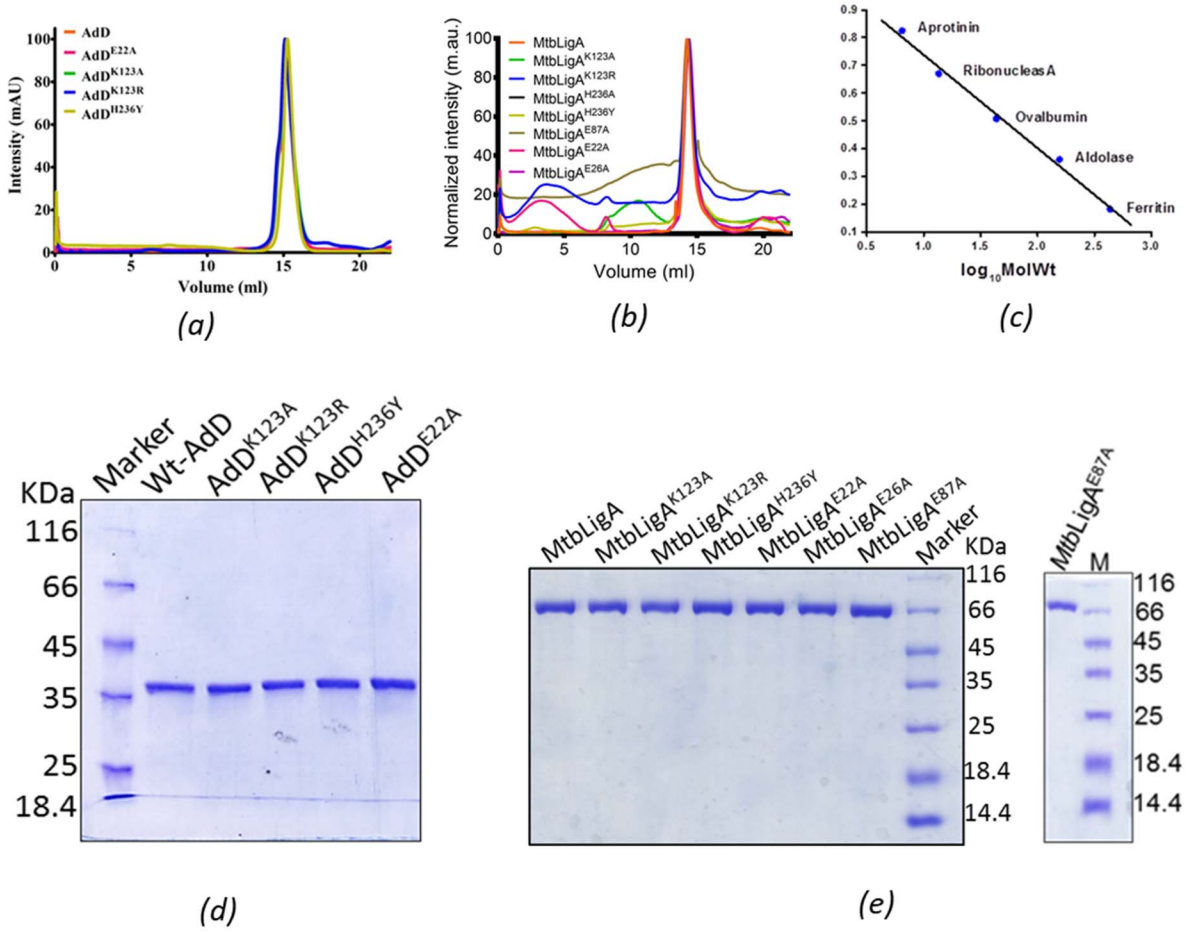

Figure S2 Purification of proteins. *(a and b)* Size exclusion chromatographic (SEC) analysis of proteins used in the study using Superdex 200 10/30 increase column (GE Healthcare). *(c)* The standard curve proteins is marked as blue dots in the chromatogram as 1: Ferritin (440 kDa), 2: Aldolase (158 kDa), 3: Ovalbumin (44 kDa), 4: Ribonuclease A (13.7 kDa) and 5: Aprotinin (6.5 kDa) for Superdex 200 10/30 increase column. *(d and e)* SDS-PAGE analysis of SEC purified recombinant proteins used in the present study. Electrophoresis was performed using 12% polyacrylamide gel, followed by Coomassie Blue staining.

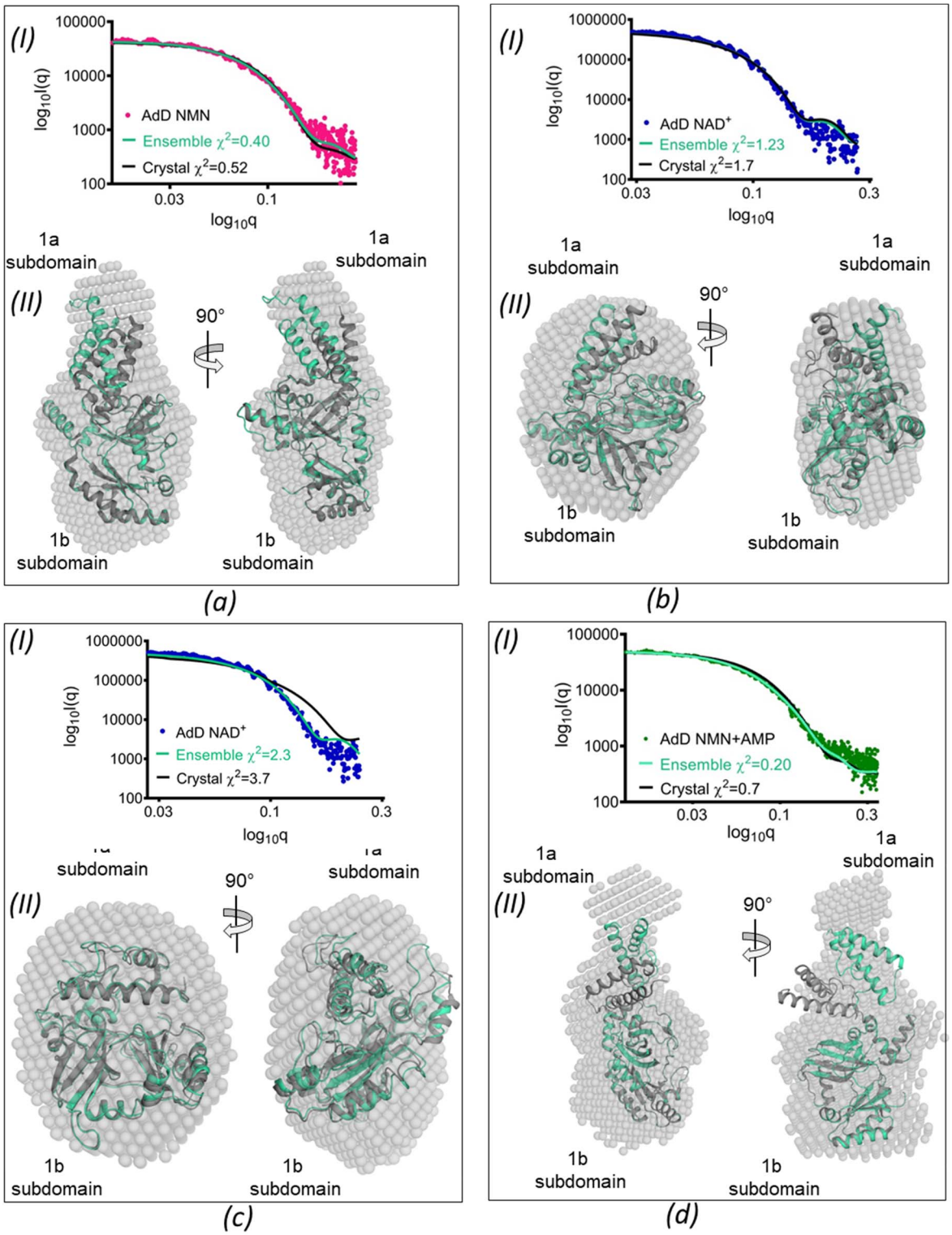

Figure S3 SAXS data validation and fitting. *(a)* I) *CRYSOL* fitting of NMN bound crystal structure (PDB entry 1ta8) (black line) of AdD domain and ensemble (cyan line) into the experimental scattering curve. II) The superimposition of *DAMMIF* generated bead model and high-resolution crystal structure (dark gray color) and ensemble (cyan color) using *SUPCOMB*. *(b)* I) In the presence of NAD^+ the AdD domain adapts a closed compact structure and theoretical scattering was fitted on

the experimental scattering using *CRYSOL* shows fitting of crystal structure (PDB entry 1tae) (black line) and comparatively good fitting of ensemble (cyan color). II)The high-resolution models were superimposed on the *DAMMIF* generated bead model and crystal structure (dark gray color) and ensemble (cyan color) are shown. *(c)* I) After the transfer of AMP to the active site lysine the 1a subdomain adapts a partially closed conformation (PDB entry 3sgi) (black line) and ensemble (cyan line). II) The high-resolution models were superimposed on the *DAMMIF* generated bead model and crystal structure (dark gray color) and ensemble (cyan color) are shown. *(d)* I) The incubation of AMP and NMN with the AdD domain adapts a more extended conformation (cyan line) in solution as compare to crystals structure (PDB entry 6kjm) (black line). II) The high-resolution models were superimposed on the *DAMMIF* generated bead model and crystal structure (dark gray color) and ensemble (cyan color) are shown.

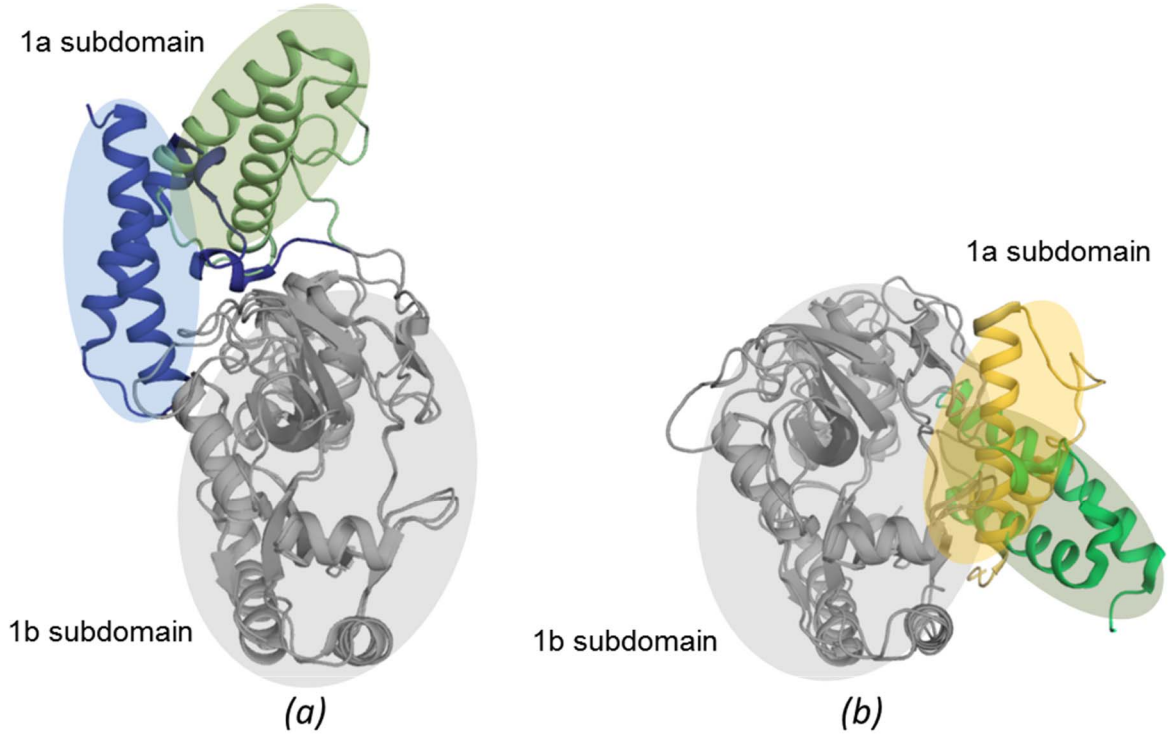

Figure S4 1a subdomain rotation based on SAXS analysis. (a) Superimposition of the crystal structure of *E. faecalis* LigA bound with NMN (PDB entry 1ta8, blue color 1a subdomain) and *M. tuberculosis* AdD domain bound with NMN and AMP (PDB entry 6kjm, forest green color 1a subdomain). The 1b subdomain (gray color) of both crystal structures was superimposed (r.m.s.d = 0.946) to observe the 1a subdomain in an open extended conformation. (b) Superimposition of crystal structure of *E. faecalis* LigA bound with NAD^+ (PDB entry 1tae, green color 1a subdomain) and *M. tuberculosis* BRCT deleted LigA bound with AMP (PDB entry 3sgi). The 1b subdomain (gray color) of both crystal structure was superimposed (r.m.s.d = 1.288) to observe the 1a subdomain rotation in the closed conformation.

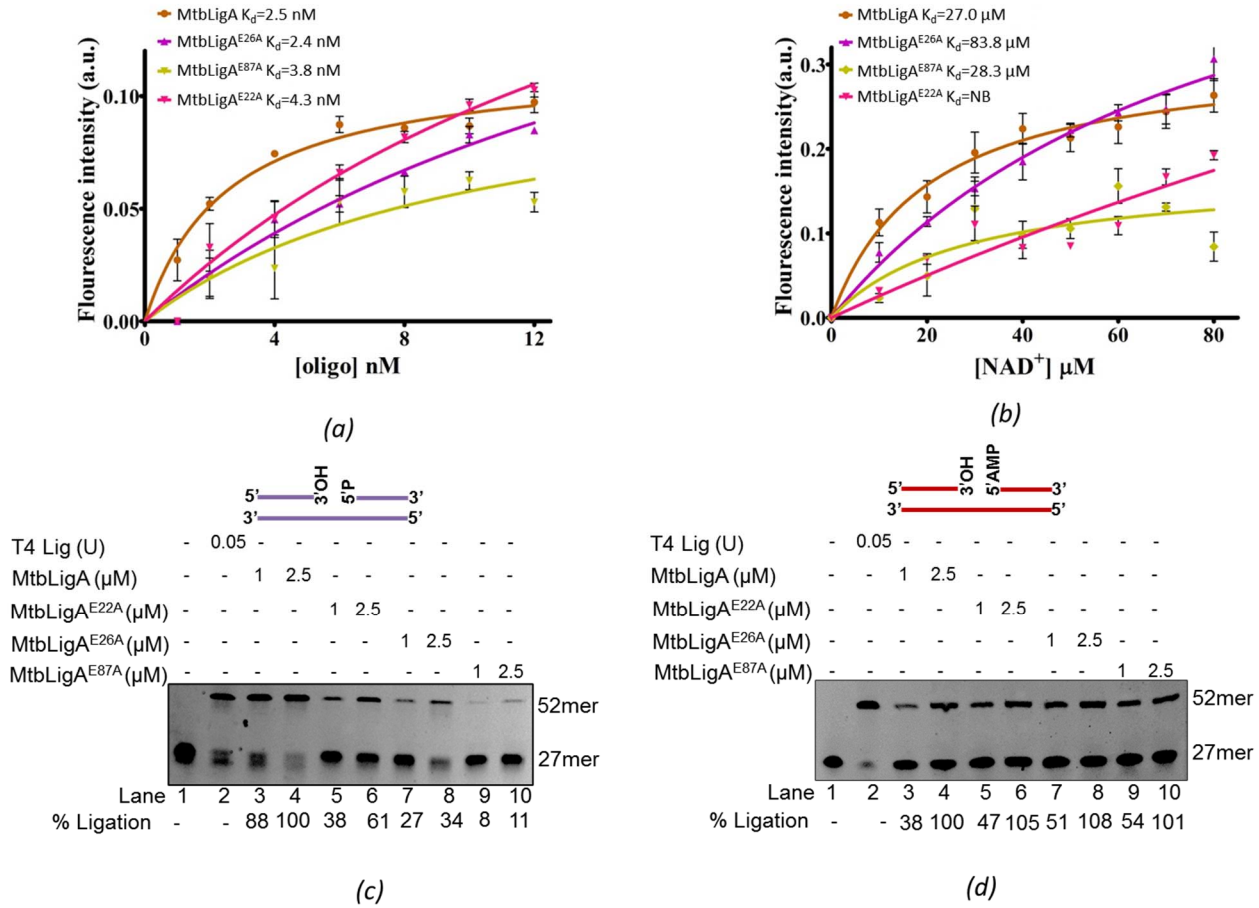

Figure S5 Binding affinity of wt-MtbLigA and mutants and effect of mutations on in vitro ligation activity. *(a)* Comparison of binding affinity (K_d) of wt-MtbLigA and 1a subdomain mutants (MtbLigA^{E22A}, MtbLigA^{E26A} and MtbLigA^{E87A}) with 5′-dephosphorylated nicked DNA substrate. *(b)* Comparison of binding affinity of wt-MtbLigA and 1a subdomain mutants (MtbLigA^{E22A}, MtbLigA^{E26A} and MtbLigA^{E87A}) with co-factor NAD^+, where NB stands for no binding. Comparison of *in vitro* ligation activity of wt-MtbLigA and 1a subdomain mutants (MtbLigA^{E22A}, MtbLigA^{E26A} and MtbLigA^{E87A}) on *(c)* 5'-phosphorylated nicked ds-DNA and *(d)* 5′-adenylated nicked ds-DNA. DNA substrate (10 nM) was incubated with T4 DNA Ligase (0.05U, lane 2), MtbLigA (1 µM and 2.5 µM, lane 3 and 4) and 1a subdomain mutants MtbLigA^{E22A} (1 µM and 2.5 µM, lane 5 and 6), MtbLigA^{E26A} (1 µM and 2.5 µM, lane 7 and 8), MtbLigA^{E87A} (1 µM and 2.5 µM, lane 9 and 10) under standard conditions. Lane 0 is control without any protein. Reaction products were analysed on 8 M urea-12% polyacrylamide gels. Intensity of the fluorescent bands corresponding to the products of respective activities were scanned and quantified by using ImageQuant LAS 4000 and ImageQuantTL 8.1 software (GE Healthcare). The images are single representative image of experiments carried out in duplicate. A standard deviation of ± 2.5 was obtained for the % DNA ligated. The position of the ligated product at 52mer and unligated substrate 27mer is indicated.

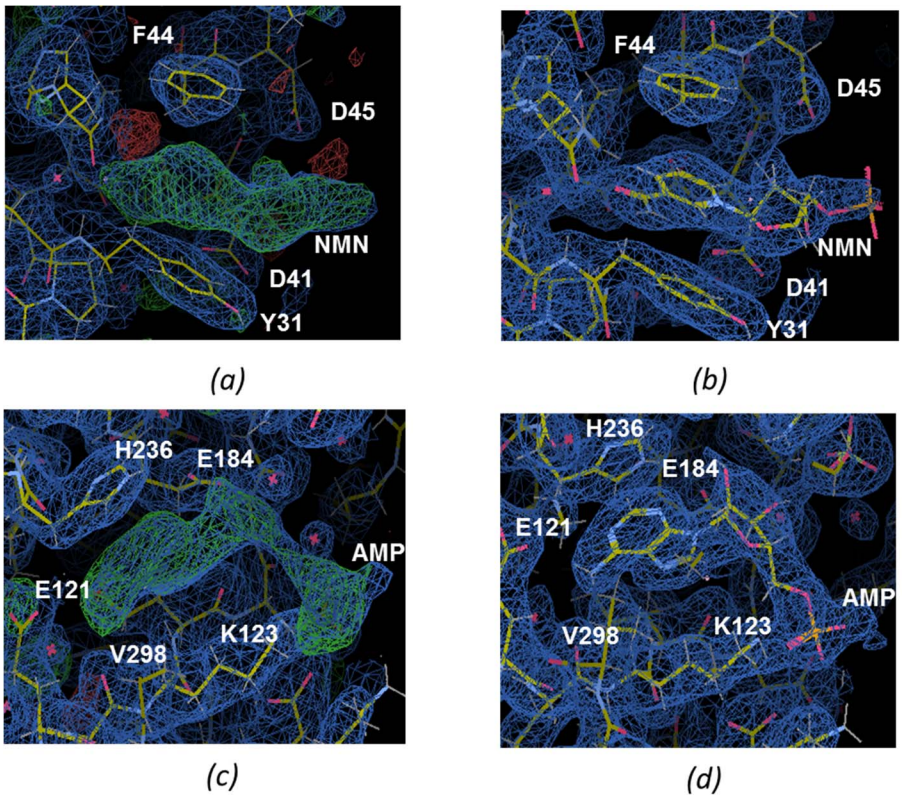

Figure S6 The electron density map of AdD^{E22A} mutant shows the binding of NMN and AMP in their respective binding pocket. *(a)* The SigmaA-weighted 2Fo-Fc map (blue, contoured at 1.0) and Fo-Fc (Green/red contoured at 3.0) is shown in the absence of NMN. *(b)* The following refinement in the presence of NMN is shown at same contour level. *(c)* The SigmaA-weighted 2Fo-Fc map (blue, contoured at 1.0) and Fo-Fc (Green/red contoured at 3.0) is shown in the absence of AMP. *(d)* The following refinement in the presence of AMP is shown at same contour level.

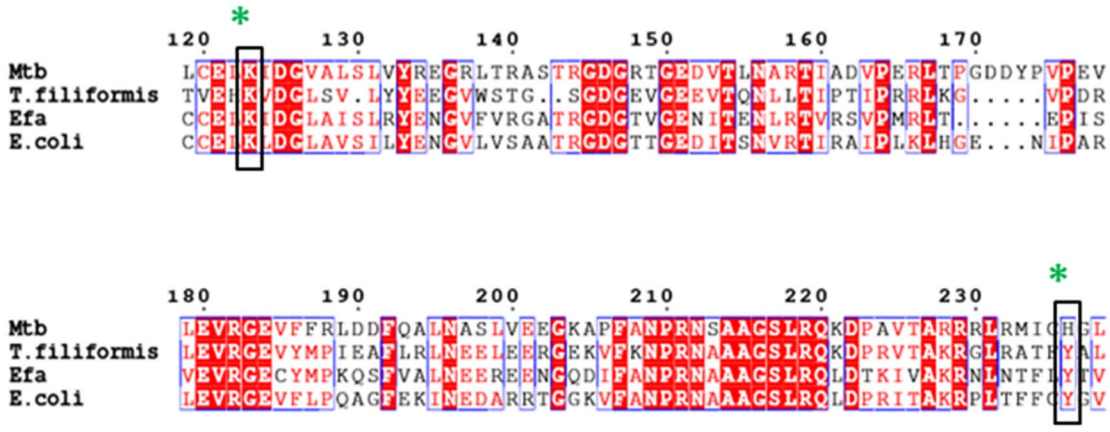

Figure S7 Multiple sequence alignment of AdD domain of LigA homologs. The highly conserved residues are boxed in black. The amino acid sequence of LigA homologs were aligned from *M. tuberculosis, T. filiformis, E. faecalis and E.coli.* The highly conserved active site lysine K123 and H236 in case of *M. tuberculosis* is marked with green asterisk at the top of sequence.

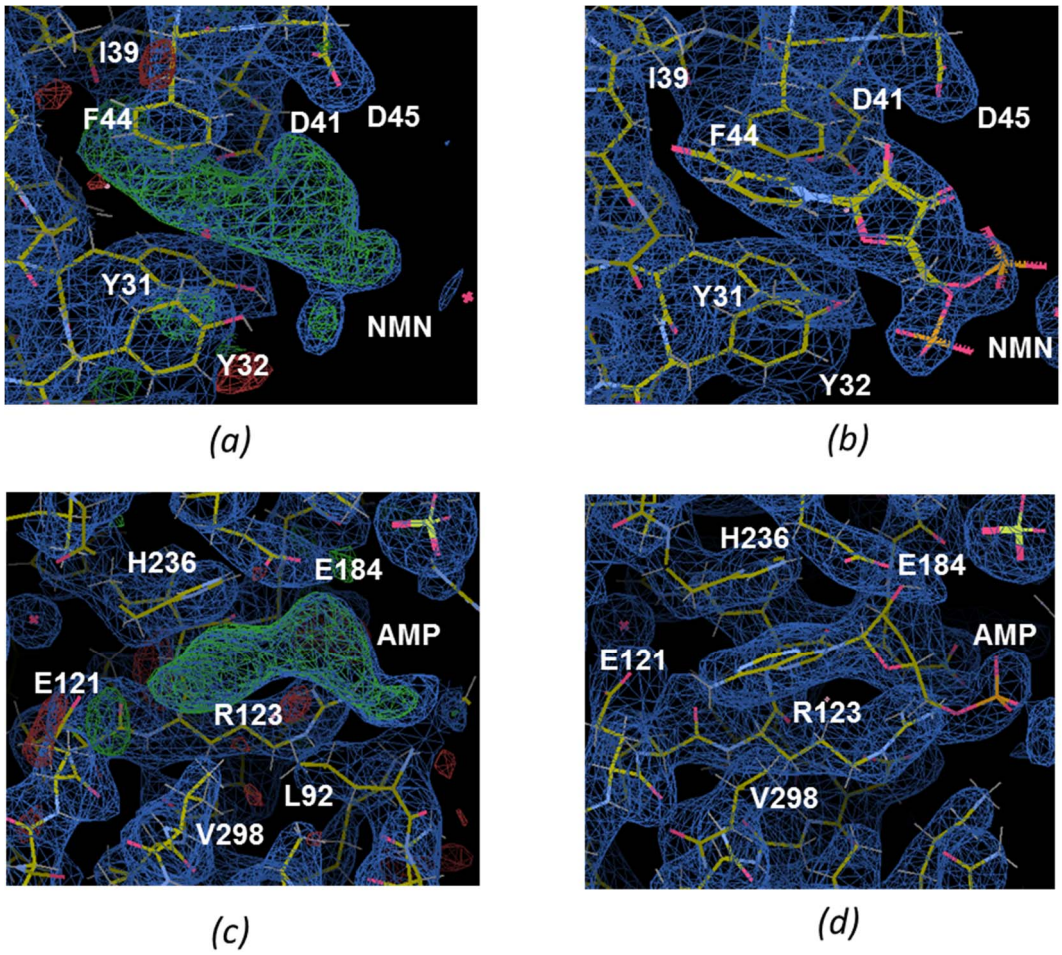

Figure S8 The electron density map of AdD^{K123A} mutant shows the binding of NMN in binding pocket. *(a)* The SigmaA-weighted 2Fo-Fc map (blue, contoured at 1.0) and Fo-Fc (Green/red contoured at 3.0) is shown in the absence of NMN. *(b)* The following refinement in the presence of NMN is shown at same contour level. The density map of AdD^{K123R} mutants shown *(c)* SigmaA-weighted 2Fo-Fc map (blue, contoured at 1.0) and Fo-Fc (Green/red contoured at 3.0) is shown in the absence of AMP. *(d)* The following refinement in the presence of AMP is shown at same contour level.

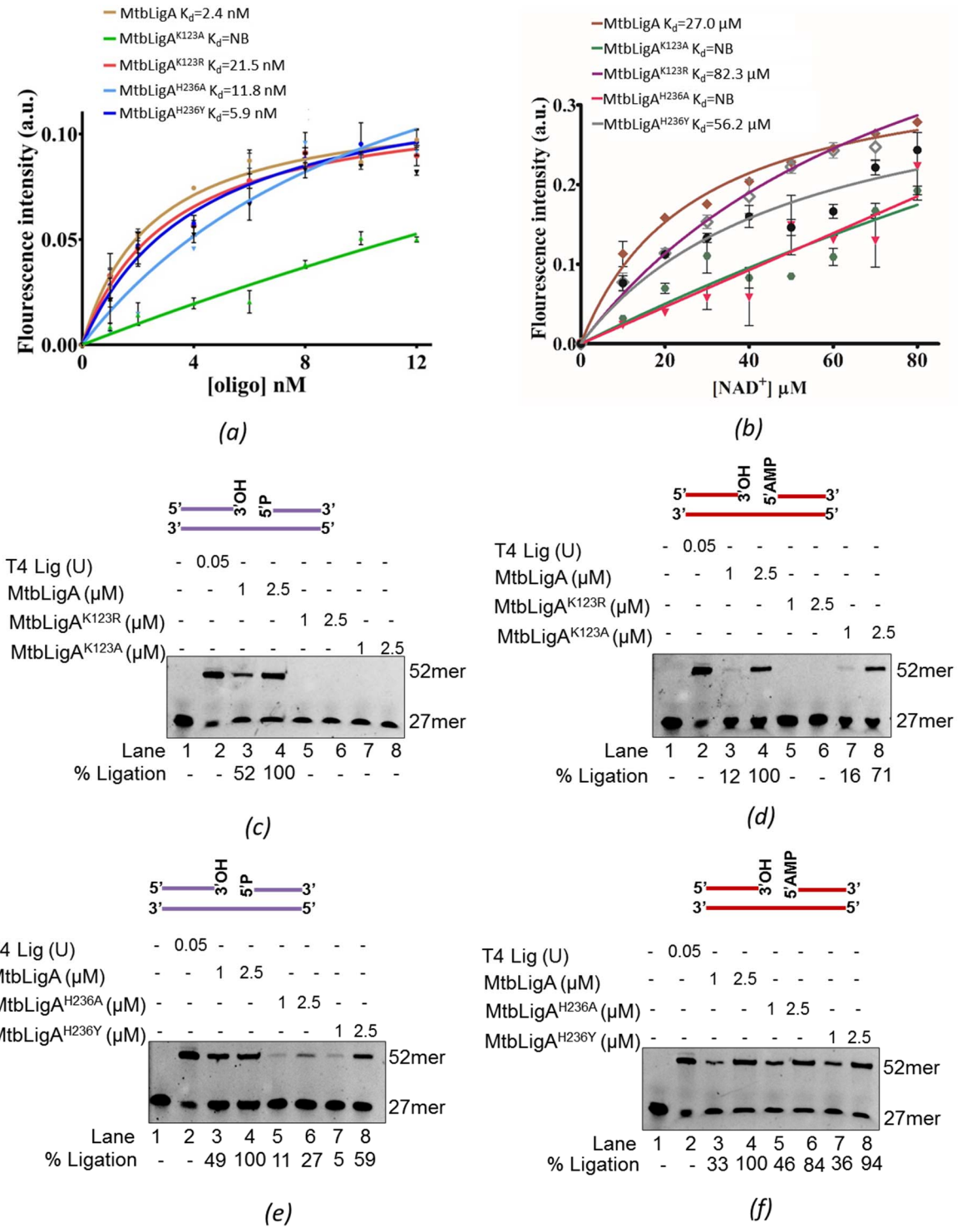

Figure S9 Determination of binding affinity and *in vitro* ligation activity of wt-MtbLigA and active site mutants. Determination of K_d value of MtbLigA, MtbLigA^{K123A}, MtbLigA^{K123R}, MtbLigA^{H236A} and MtbLigA^{H236Y} (blue) mutants with *(a)* 5′-dephosphorylated nicked DNA substrate and *(b)* with NAD^+ co-factor. NB stands for no binding. *(c)* Comparison of in vitro ligation activity of wt-MtbLigA and mutants MtbLigA^{K123A}, MtbLigA^{K123R}, MtbLigA^{H236A} and MtbLigA^{H236Y} on *(c, e)* 5′-phosphorylated nicked ds-DNA and *(d, f)* 5′-adenylated nicked ds-DNA.

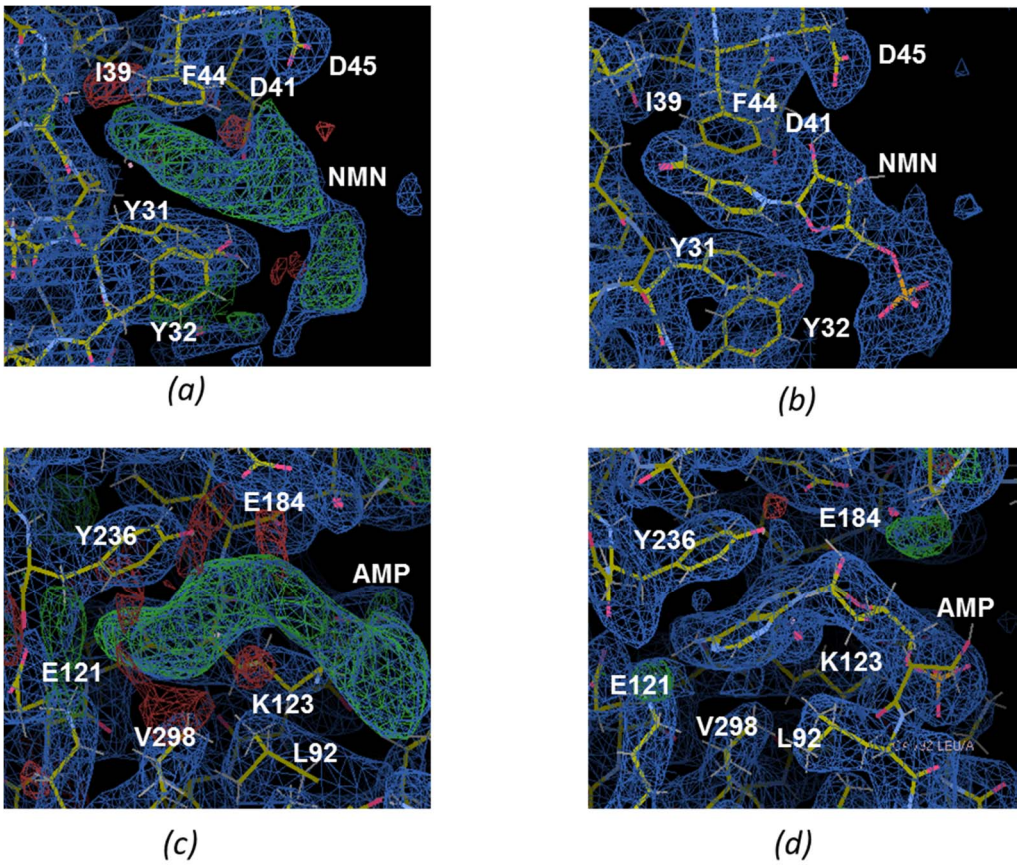

Figure S10 The electron density map of AdD^{H236Y} mutant shows the binding of NMN and AMP in their respective binding pocket. *(a)* The SigmaA-weighted 2Fo-Fc map (blue, contoured at 1.0) and Fo-Fc (Green/red contoured at 3.0) is shown in the absence of NMN. *(b)* The following refinement in the presence of NMN is shown at same contour level. *(c)* SigmaA-weighted 2Fo-Fc map (blue, contoured at 1.0) and Fo-Fc (Green/red contoured at 3.0) is shown in the absence of AMP. *(d)* The following refinement in the presence of AMP is shown at same contour level.