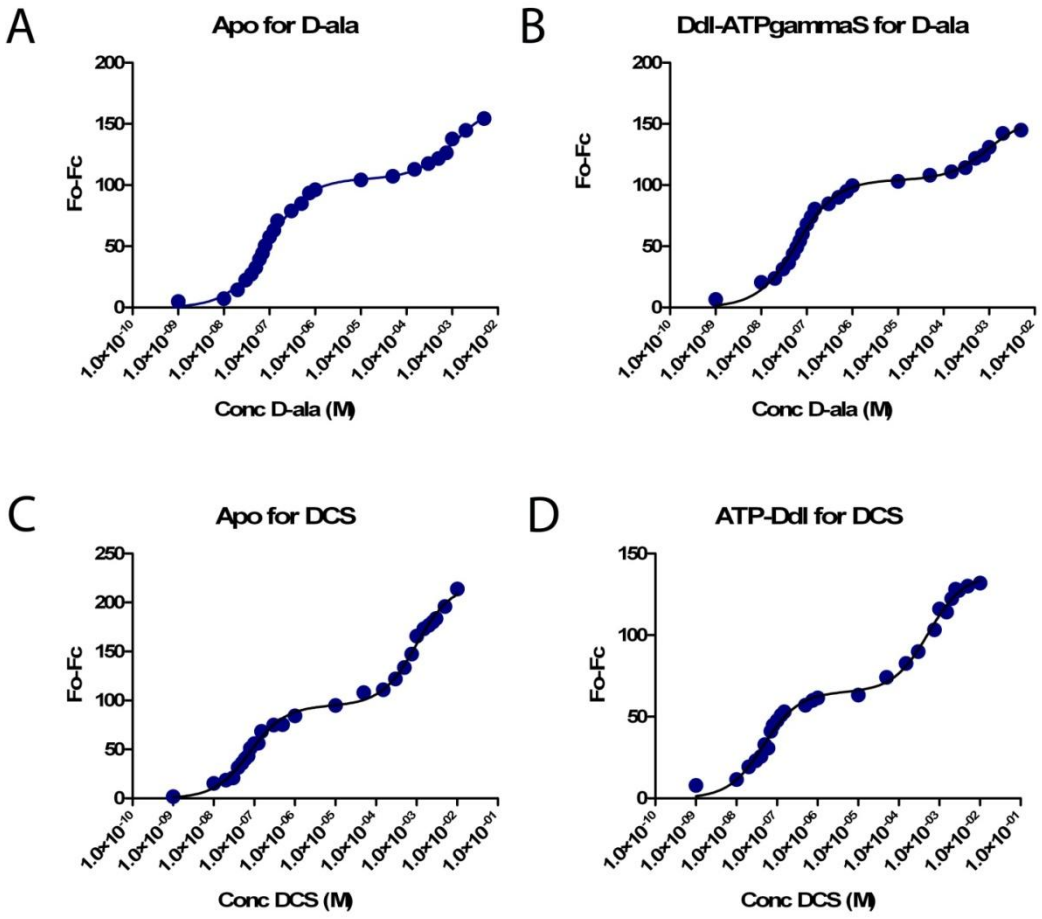
Supplemental Table I. *Mtb* Ddl sequence identity to organisms with structures deposited in the protein data bank.

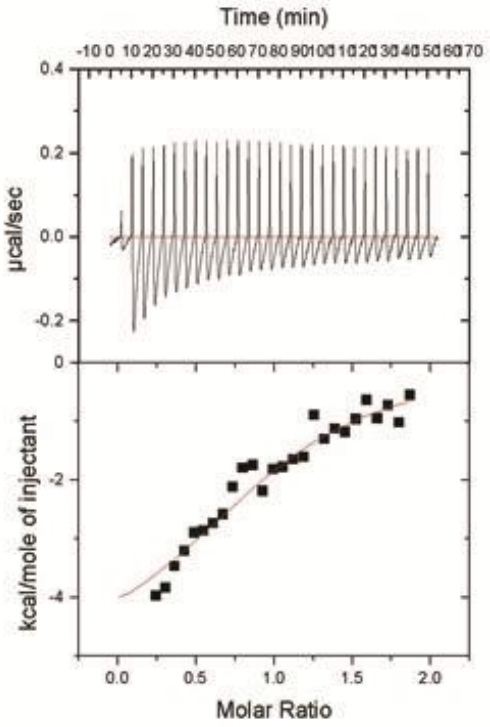
Organism	Percent identity to TB Ddl[1]
E. coli Ddla	43.0%
E.coli Ddlb	36.0%
T. caldophilus	41.0%
H. pylori	26.0%
T. thermophilus	42.0%
S. aureus	36.0%

[1]Output from BlastP, NCBI website (http://www.ncbi.nlm.nih.gov). Version 2.2.24 with default algorithm parameters.

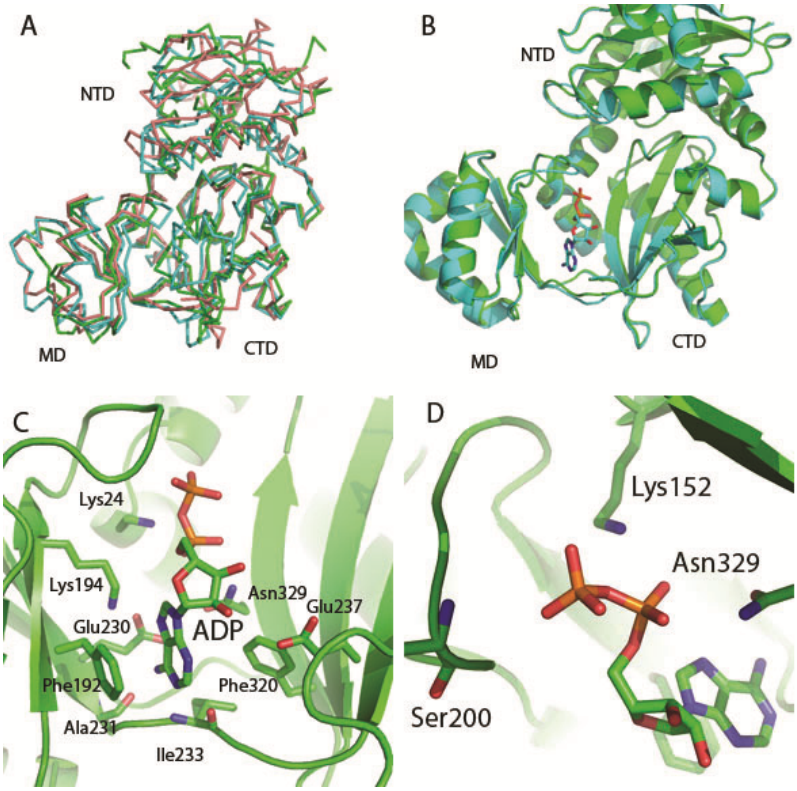
Supplemental Figure 1. Intrinsic fluorescence quenching of Ddl. The y-axis denotes the difference in fluorescence upon ligand titration with the x-axis denoting ligand concentration. Data points were recorded ranging from 1 nM to 10 mM for DCS and 1 nM to 5 mM for D-ala. The curve was fit to model two distinct binding events. A, Apo Ddl affinity for D-ala. B, ATPγS saturated Ddl affinity for D-ala. C, Apo Ddl affinity for DCS. D, ATP saturated affinity for DCS.



<u>Supplemental Figure 2.</u> Isothermal titration calorimetry of Apo Ddl for ATP. A total of 30 titrations of ATP were injected into apo Ddl. Data was fit using one binding site per monomer. The data produced a dissociation constant of 14 μM.



<u>Supplemental Figure 3.</u> The Ddl ligand binding sites and comparison to other species. A, Wire superimposition of *Mtb* Ddl, *E. coli* Ddl (accession code: 2DLN), and *S. aureus* Ddl (PDB accession code: 2I87) (15, 25). Only monomers (A chain) are depicted. The *Mtb* Ddl is shown in green, *E. coli* is shown in blue, and *S. aureus* is shown in pink. All species show divergence with the greatest being in the NTD. The *Mtb* monomer shows markedly higher global conservation to the *S. aureus* structure. B, Superimposition of the *S. aureus* apo (accession code: 2I87) and ligand bound forms of Ddl (accession code: 2I8C) (25). The global architecture shows very little divergence in fold. The apo monomer is shown in green and the ADP bound monomer is displayed in blue. C, ADP docked into the *Mtb* Ddl active site manually using PDB:2DLN as a guide (15). The adenine ring is held in place by stacking interactions with Phe192 and Phe320. Lys24, Asn329, Glu230, Ile233, Ala231, and Lys 194 all form hydrogen bonds with the ADP molecule. D, The same structure as depicted in panel C with electrostatic interactions with the phosphate groups highlighted



<u>Supplemental Figure 4.</u> Multiple Sequence Alignment for Ddl. Protein Sequences. Alignment of protein sequences from *M. tuberculosis* (labeled *Mtb*), *S. aureus* (labeled SA), and *E. coli* (labeled EC). The *Mtb* specific insertion is highlighted in yellow and conserved catalytic residues are highlighted in green. Results from ClustalW2 analysis (Version 2.0.12).


MTB VSANDRRDRRVRVAVVFGGRSNEHAISCVSAGSILRNLDSRRFDVIAVGITPAGSWVLTD 60
SA -------MTKENICIVFGGKSAEHEVSILTAQNVLNAIDKDKYHVDIIYITNDGDWRK-- 51
EC --------MTDKIAVLLGGTSAEREVSLNSGAAVLAGLREGGIDAYPVDPK--------- 43
MTB ANPDALTITNRELPQVKSGSGTELALPADPRRGGQLVSLPPGAGEVLESVDVVFPVLHGP 120
SA QNNITAEIKSTDELHLENGEALEISQLLKESSSG----------QPYDAVFPLLHGP 98
EC ---------EVDVTQLKSMG--------------------------FQKVFIALHGR 65
MTB YGEDGTIQGLLELAGVPYVGAGVLASAVGMDKEFTKKLLAADGLPVGAYA-VLRPP---- 175
SA NGEDGTIQGLFEVLDVPYVGNGVLSAASSMDKLVMKQLFEHRGLPQLPYISFLRSEYEKY 158
EC GGEDGTLQGMLELMGLPYTGSGVMASALSMDKLRSKLLWQGAGLPVAPWVALTRAEFEKG 125
MTB RSTLHRQECERLGLPVFVKPARGGSSIGVSRVSSWDQLPAAVARARRHDPKVIVEAAISG 235
SA EHNILKLVNDKLNYPVFVKPANLGSSVGISKCNNEAELKEGIKEAFQFDRKLVIEQGVNA 218
EC LSDKQLAEISALGLPVIVKPSREGSSVGMSKVVAENALQDALRLAFQHDEEVLIEKWLSG 185
MTB RELECGVLEMPDGTLEASTLGEIRVAGVRGREDSFYDFATKYLDDAAELDVPAKVDDQVA 295
SA REIEVAVLGN--DYPEATWPGEV------VKDVAFYDYKSKYKDGKVQLQIPADLDEDVQ 270
EC PEFTVAILGEEILPSIRIQPSGT----------FYDYEAKYLSDETQYFCPAGLEASQE 234
MTB EAIRQLAIRAFAAIDCRGLARVDFFLTDDG-PVINEINTMPGFTTISMYPRMWAASGVDY 354
SA LTLRNMALEAFKATDCSGLVRADFFVTEDNQIYINETNAMPGFTAFSMYPKLWENMGLSY 330
EC ANLQALVLKAWTTLGCKGWGRIDVMLDSDGQFYLLEANTSPGMTSHSLVPMAARQAGMSF 294
MTB PTLLATMIETTLARGVGLH------- 373
SA PELITKLIELAKERHQDKQKNKYKID 356
EC SQLVVRILELAD---------- 306