Supporting Online Material

Inhibitory mechanism of the Qβ lysis protein A_2

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Supplementary Methods

***Plasmid construction*.** For yeast-two-hybrid analyses, plasmids pGBKT7-A_2 and pGADT7-murA/murAL^{138Q} were amplified in XL-1 Blue. pGBKT7-A_2 was cloned from a cDNA copy of the Qβ RNA reverse transcribed using the M-MLV Reverse Transcriptase according to manufacturer's instructions (Ambion). *A_2* was amplified by PCR using A_2KpnI-NdeI-For (GTATAAGAGGTACCACATATGCCTAAATTACC) and A_2Rev-BamHI (GCAGCCGGATCCAGTTTCA) primers. The PCR product was inserted into pGBKT7 (Clontech) at the BamHI and an NdeI restriction site that was introduced upstream of the *cmyc* epitope tag by site-directed mutagenesis, described previously (Smith et al., 1998), with the following primers: pGBKT7-NdeI-up-FOR (GGGCGAGCCGCCCATATGGAGGAGC) and pGBKT7-NdeI-up-Rev (CTGCTCCTCCATATGGGCGGCTCGCC). pGADT7-murA/murA^{L138Q} was similarly constructed by inserting *murA/murA^{L138Q}* amplified from pZE12-murA/murA^{L138Q} by PCR with the primers NcoI-murA-FOR (TTGGTTCCATGGATAAATTTCGTG) and BamHI-murA-REV (TATTATTCTAGAGGATCCGCTCTCAGACGATTAACCAC) into the NcoI and BamHI sites of pGADT7 (Clontech). Construction of pZE12-murA has been previously described (Bernhardt *et al.*, 2001). Alleles of *murA** used in the MurA bioassay were constructed by site-directed mutagenesis (Smith *et al.*, 1998) into pZE12-murA with primers listed in Supplementary Table S2. For purification, an oligo-histidine tag ($G_2H_6G_2$) was adjoined to the C-terminus of *murA* by site-directed PCR mutagenesis. To generate pZA31-murA^{Bs}, *murAA* (*murA^{Bs}*) was cloned out of *Bacillus subtilis* W23 with the following primers: KpnI-NdeI-BsMurA-For ATATATGGTACCCATATGGAAAAAAT and XbaI-BamHI-BsMurA-Rev GAGTGGTCTAGAGGATCCTTATGCAT and ligated into pZA31 (Lutz & Bujard, 1997) at the KpnI and XbaI restriction sites. This plasmid has a $P_{Ltet0-1}$ promoter which is constitutively on in a *tetR$^-$* background. The MBP-A_2 fusion vector, pETMBP-A_2, was constructed by amplifying the *A_2* gene via PCR, as described above, using KpnI-NdeI-For GTATAAGAGGTACCACATATGCCTAAATTACC and A_2Rev-BamHI GCAGCCGGATCCAGTTTCA primers. The PCR product was digested with NdeI and BamHI and ligated into p202 (pET28b-MBP vector with a TEV protease cleavage site, kindly provided by Dr. Sacchettini, TAMU) similarly digested.

***MurA purification*.** Purification of MurA bound with UDP-NAM and PEP was performed according to the purification protocol described Experimental proceedures section of the main text except the ammonium sulfate precipitation steps were removed.

Substrate analysis. Residual nucleotide binding of purified MurA was tested by denaturation and deproteinization of the sample according to the method previously described (Mizyed *et al.*, 2005). Absorbance of the sample at 260 nm was assessed. No residual nucleotide binding was observed for MurA purified with succissive ammonium sulfate precipitation steps (data not shown).

Supplementary Tables and Figures

Table S1. MurA*^{D305A} Qβ protection assay.

Protects No plaques	Does not Protect Plaques
WT (D305A)	L138Q
L111R	V87R
S127R	K88E
E130A	A119R
Q131R	P121R
G133R	V122R
T135R	I126R
K137D	E130R
L138P	I136R
E139A	L138M
E139R	L138R
E140R	V143R
G141R	I156R
K152R	N330R
H155R	F332R
V157R	M333R
D159R	P336R
K160R	M366R
V335R	T368R
R391R	H394R

Table S2. Primers for construction of MurA* alleles.

Allele (*murA**)	Sequence
L138Q	FOR: ACCATCAAACTGGAAGAAGGT
	REV: ACCTTCTTCCAGTTTGATGGT
L138M	FOR: ACCATCAAAATGGAAGAAGGT
	REV: ACCTCCTTCCATTTTGATGGT
L138P	FOR: ACCATCAAACCGGAAGAAGGT
	REV: ACCTCCTTCCGGTTTGATGGT
L138R	FOR: ACCATCAAACGGGAAGAAGGT
	REV: ACCTTCTTGCCGTTTGATGGT
A119R	FOR: GTACGATCGGTCGTCGTCCGGTTG
	REV: CAACCGGACGACGACCGATCGTAC
P121R	FOR: CGGTGCGCGTCGTGTTGATCTAC
	REV: GTAGATCAACACGACGCGCACCG
V122R	FOR: GTGCGCGTCCGCGTGATCTACACATT
	REV: AATGTGTAGATCACGCGGACGCGCAC
D123R	FOR: GCGCGTCCGGTTCGTCTACACATTTC
	REV: GAAATGTGTAGACGAACCGGACGCGC
I126R	FOR: GATCTACACCGTTCTGGCCTCGAAC
	REV: GTTCGAGGCCAGAACGGTGTAGATC
S127R	FOR: GATCTACACATTCGTGGCCTCGAAC
	REV: GTTCGAGGCCACGAATGTGTAGATC
E130A	FOR: TCTGGCCTCGCACAATTAGGC
	REV: GCCTAATTGTGCGAGGCCAGA
E130R	FOR: CTGGCCTCCGTCAATTAGGCGCG
	REV: CGCGCCTAATTGACGGAGGCCAG
Q131R	FOR: CTGGCCTCGAACGTTTAGGCGCGAC
	REV: GTCGCGCCTAAACGTTCGAGGCCAG
G133R	FOR: CCTCGAACAATTACGTGCGACCATCAA
	REV: TTGATGGTCGCACGTAATTGTTCGAGG
A134R	FOR: AACAATTAGGCCGTACCATCAAAC
	REV: GTTTGATGGTACGGCCTAATTGTT
T135R	FOR: AATTAGGCGCGCGTATCAAACTGGA
	REV: TCCAGTTTGATACGCGCGCCTAATT
I136R	FOR: TAGGCGCGACCCGTAAACTGGAAGA
	REV: TCTTCCAGTTTACGGGTCGCGCCTA
E139A	FOR: ATCAAACTGGCAGAAGGTTAC
	REV: GTAACCTTCTGCCAGTTTGAT
E139R	FOR: GCGACCATCAAACTGCGTGAAGGTTA
	REV: CGTAACCTTCACCGAGTTTGATGGTC

K137D	FOR: GCGACCATCGATCTGGAAGAAGG
	REV: CCTTCTTCCAGATCGATGGTCGC
G141R	FOR: AACTGGAAGAACGTTACGTTAAA
	REV: TTTAACGTAACGTTCTTCCAGTT
V143R	FOR: GAAGAAGGTTACCGTAAAGCTTCCG
	REV: CGGAAGCTTTACGGTAACCTTCTTC
K152R	FOR: GATGGTCGTTTGCGTGGTGCACATATC
	REV: GATATGTGCACCACGCAAACGACCATC
H155R	FOR: GAAAGGTGCACGTATCGTGATGG
	REV: CCATCACGATACGTGCACCTTTC
D159R	FOR: ATCGTGATGCGTAAAGTCAGCG
	REV: CGCTGACTTTACGCATCAC
K160E	FOR: GTGATGGATGAAGTCAGCGTT
	REV: AACGCTGACTTCATCCATCAC
D305A	FOR: GCATTCCCGACCGCTATGCAGGCC
	REV: GGCCTGCATAGCGGTCGGGAATGC
E329R	FOR: GAAACGGTCTTTCGTAACCGCTTTATGC
	REV: GCATAAAGCGGTTACGAAAGACCGTTTC
N330R	FOR: CGGTCTTTGAACGTCGCTTTATGC
	REV: GCATAAAGCGACGTTCAAAGACCG
E332R	FOR: CTTTGAAAACCGCCGTATGCATGTGCC
	REV: GGCACATGCATACGGCGGTTTTCAAAG
M333R	FOR: GAAAACCGCTTTCGTCATGTGCCAGAG
	REV: CTCTGGCACATGACGAAAGCGGTTTTC
V335R	FOR: CGCTTTATGCATCGTCCAGAGCTG
	REV: CAGCTCTGGACGATGCATAAAGCG
P336R	FOR: GCTTTATGCATGTGCGTGAGCTGAGCC
	REV: GGCTCAGCTCACGCACATGCATAAAGC
E337R	FOR: GCATGTGCCACGTCTGAGCCGTATGGC
	REV: GCCATACGGCTCAGACGTGGCACATGC
R340E	FOR: GAGCTGAGCGAAATGGGCGCGCACGCC
	REV: GGCGTGCGCGCCCATTTCGCTCAGCTC
M366R	FOR: CGCACAGGTTCGTGCAACCGATCTGCG
	REV: CGCAGATCGGTTGCACGAACCTGTGCG
T368R	FOR: GGTTATGGCACGTGATCTGCGTGC
	REV: GCACGCAGATCACGTGCCATAACC
R391E	FOR: CGGTGGTTGATGAAATTTATCACATCG
	REV: CGATGTGATAAATTTCATCAACCACCG
H394R	FOR: GATCGTATTTATCGTATCGATCGTGGC
	REV: GCCACGATCGATACGATAAATACGATC

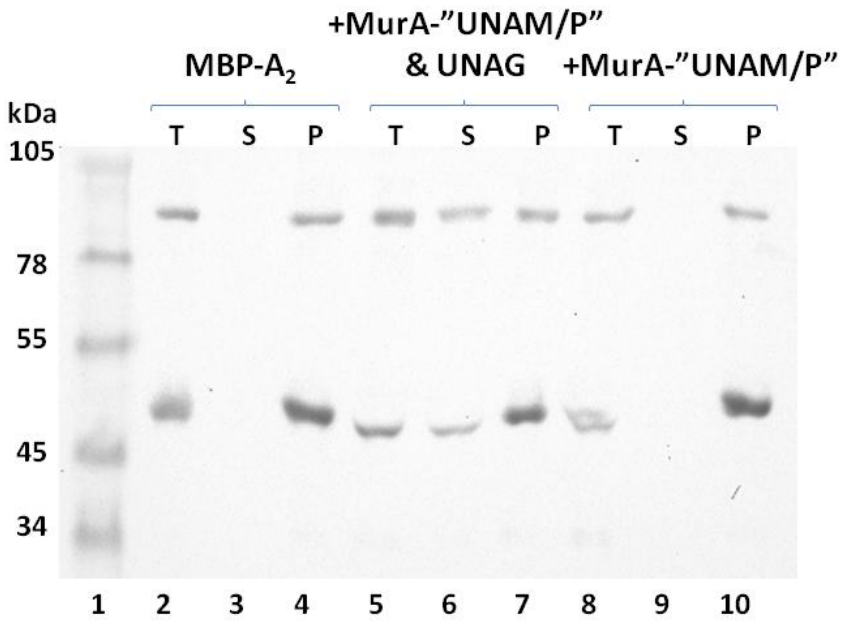

Fig. S1. A_2 does not bind the dormant complex of MurA. Fusion cleavage analysis of A_2-MurA binding in the presence of MurA liganded to UDP-NAM (UNAM) and PEP (P). MBP-A_2 was cleaved with TEV protease. UDP-NAG (UNAG, 1 mM) was included in a reaction. Binding was assessed as A_2 solubility after centrifugation: Total fraction (T), supernatant after centrifugation (S), and pellet fraction (P). Samples were resolved on SDS-PAGE and immunoblotted with the α-A_2 antibody. MBP-A_2 has an apparent MW of 100 kDa and cleaved A_2 ~50 kDa. UNAM/P was included in quotation marks since it was previously determined that a small fraction of MurA co-purifies with these two substrates (Mizyed *et al.*, 2005, Zhu *et al.*, 2012).

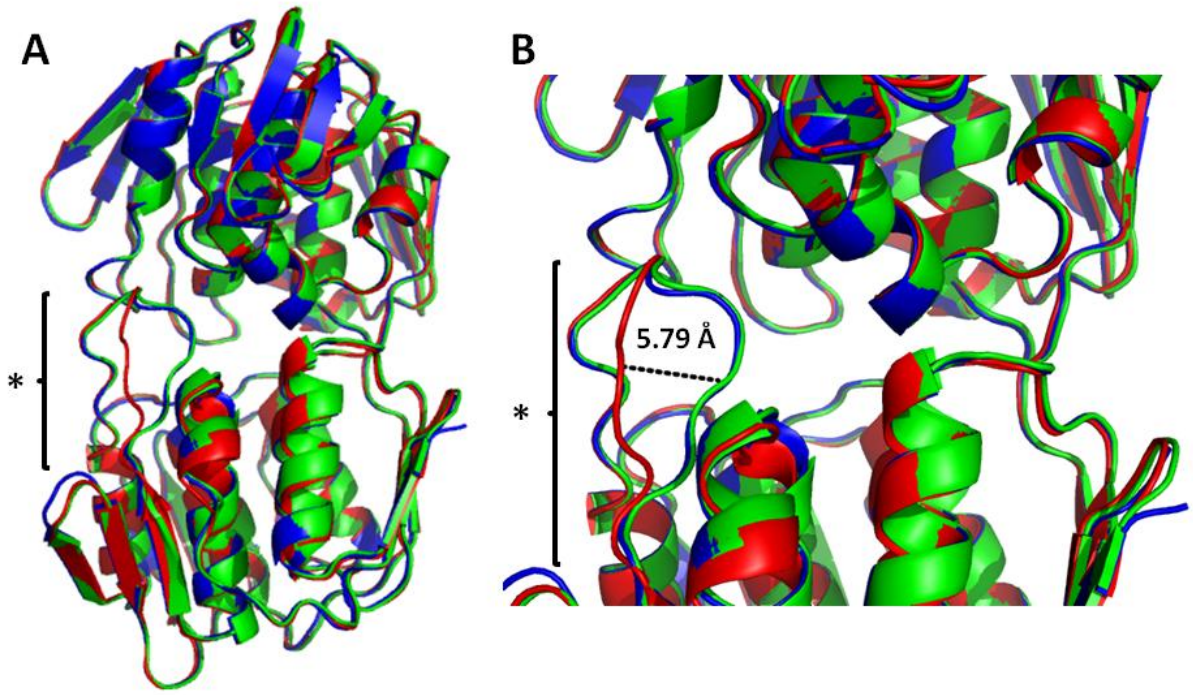

Fig. S2. Substrate-dependent analyses of MurA catalytic loop conformations. (A) Overlay of *E. coli* MurA bound with UDP-NAG (Han H., unpublished; PDB entry 3KQJ; blue), UDP-NAG/PEP (Skarzynski *et al*. 1998; PDB entry 1A2N; red), and UDP-NAG/fosfomycin (Skarzynski *et al*. 1996; PDB entry 1UAE; green); (B) Enlargement of structure active site/catalytic loop in part A. Distance between Gly114 residues is displayed. The catalytic loop is highlighted with an asterisk. The RMSD value of the catalytic loop region between the three structures is 1.477 Å. Figures were generated using PyMOL (Schrödinger, 2010).

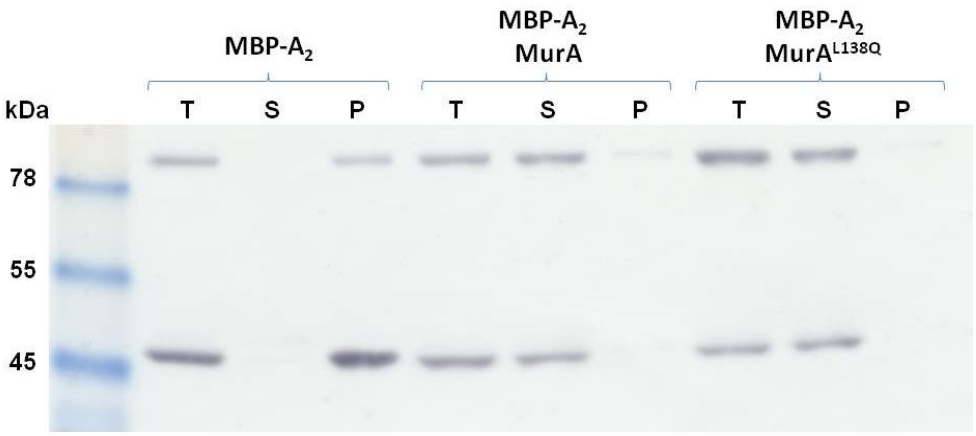

Fig. S3. A_2 binds $MurA^{L138Q}$. Fusion cleavage of MBP-A_2 was tested in the presence of UDP-NAG bound MurA and $MurA^{L138Q}$. Solubility of A_2 after centrifugation: Total fraction (T), supernatant after centrifugation (S), and pellet fraction (P) was assessed by Western blot that was probed with the α-A_2 antibody.

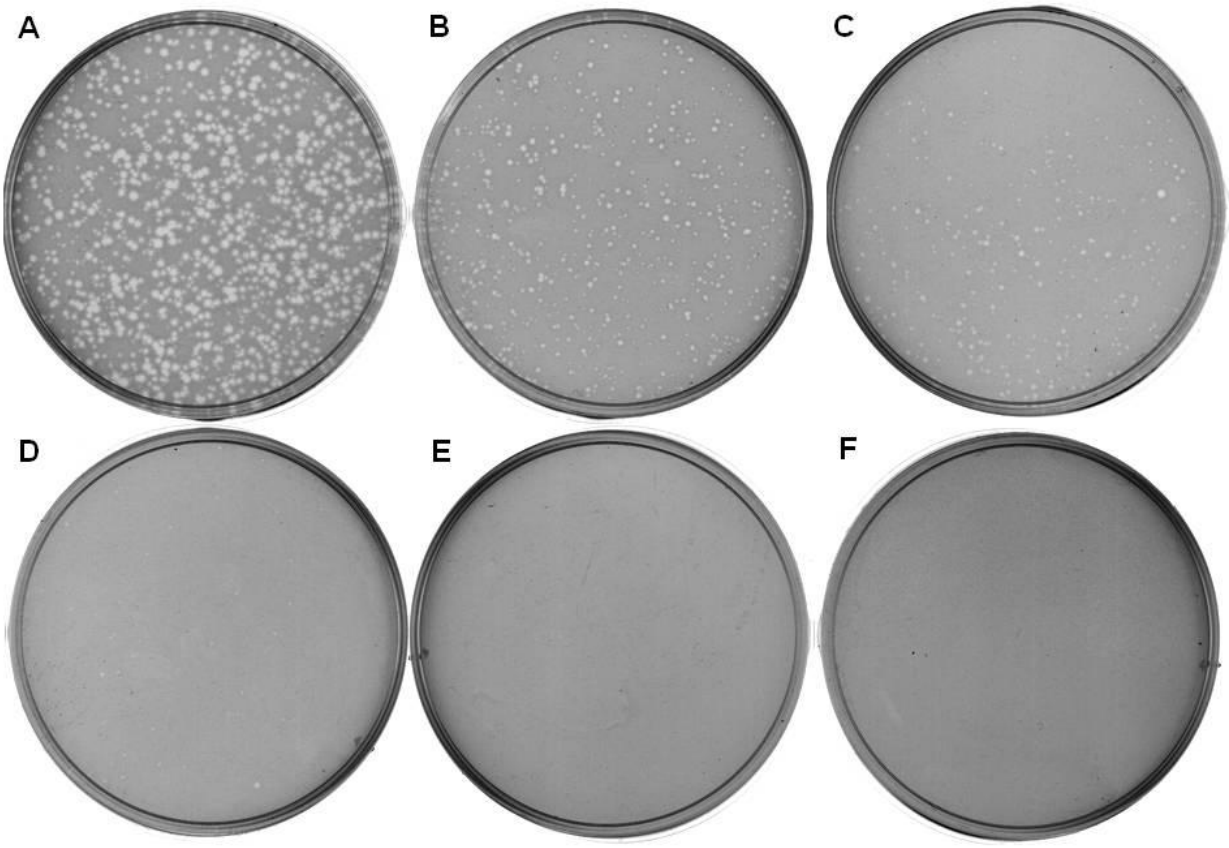

Fig. S4. MurA protection assay induction series. Protection from Qβ plating is observed on induction plates of 100 μM IPTG or greater. Qβ phage and bacteria are included in agar overlay on plates containing increasing amounts of IPTG (μM): (A) 0, (B) 12.5, (C) 25, (D) 50, (E) 100 and (F) 1000.

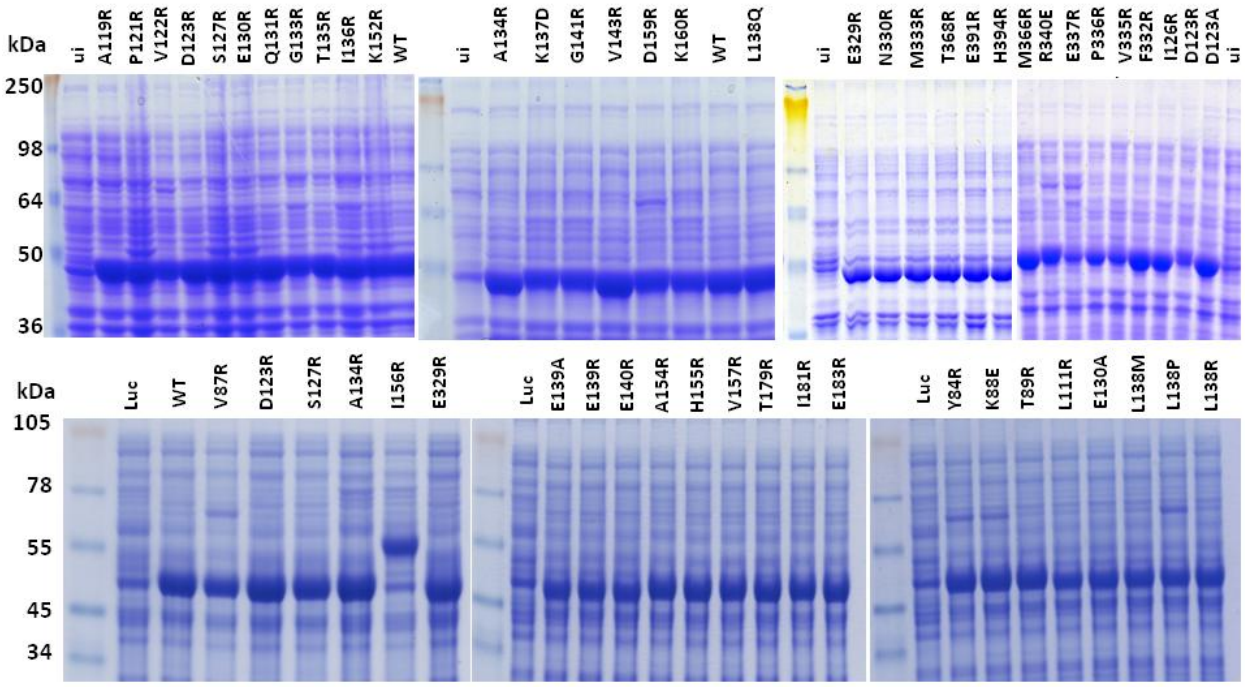

Fig. S5. MurA variants are expressed. TCA precipitated cells expressing MurA variants were run on SDS-PAGE. Alleles are shown above lanes. Uninduced control (ui) and Luciferase control (Luc)

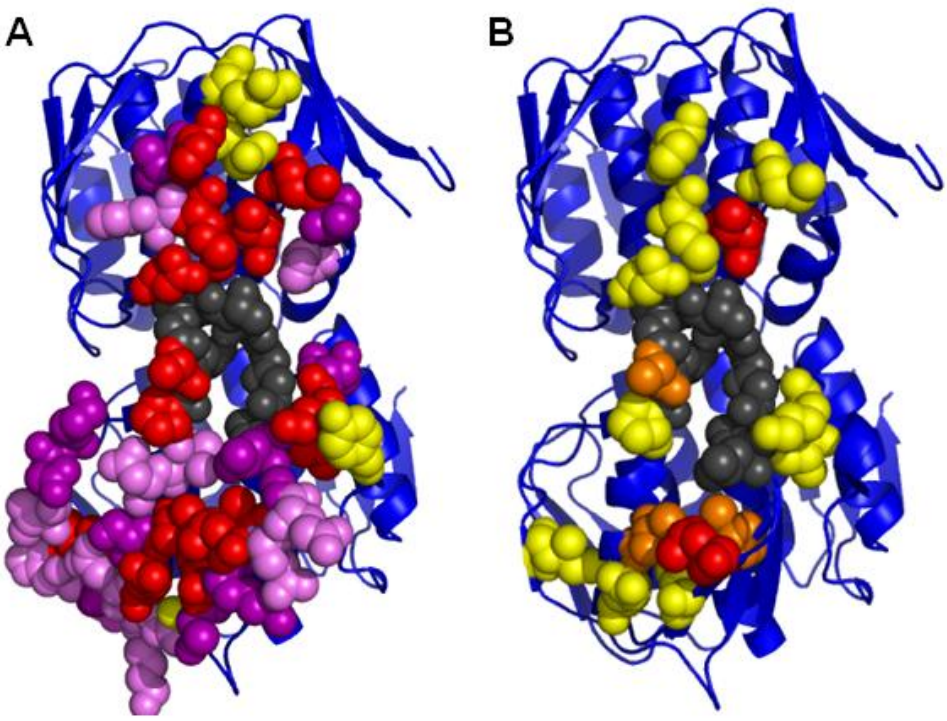

Fig. S6. MurA mutant surface maps. (A) MurA mutational data. Catalytic loop displayed as grey spheres. *Rats* are colored red. Residues providing protect ≥WT are shown in purple (weaker WT induction phenotype is lighter purple). Residues that are non-functional are shown as yellow spheres. (B) Differentiation of *rat* residues. Basal level protection *rats* are displayed as red spheres. Medium protecting *rats* are colored orange. Low protecting *rats* are shown in yellow. All residues were mapped onto the MurA UDP-NAG-bound state ("closed" conformation, front view) (Han H., unpublished; PDB entry 3KQJ). Figures were generated using PyMOL (Schrödinger, 2010).

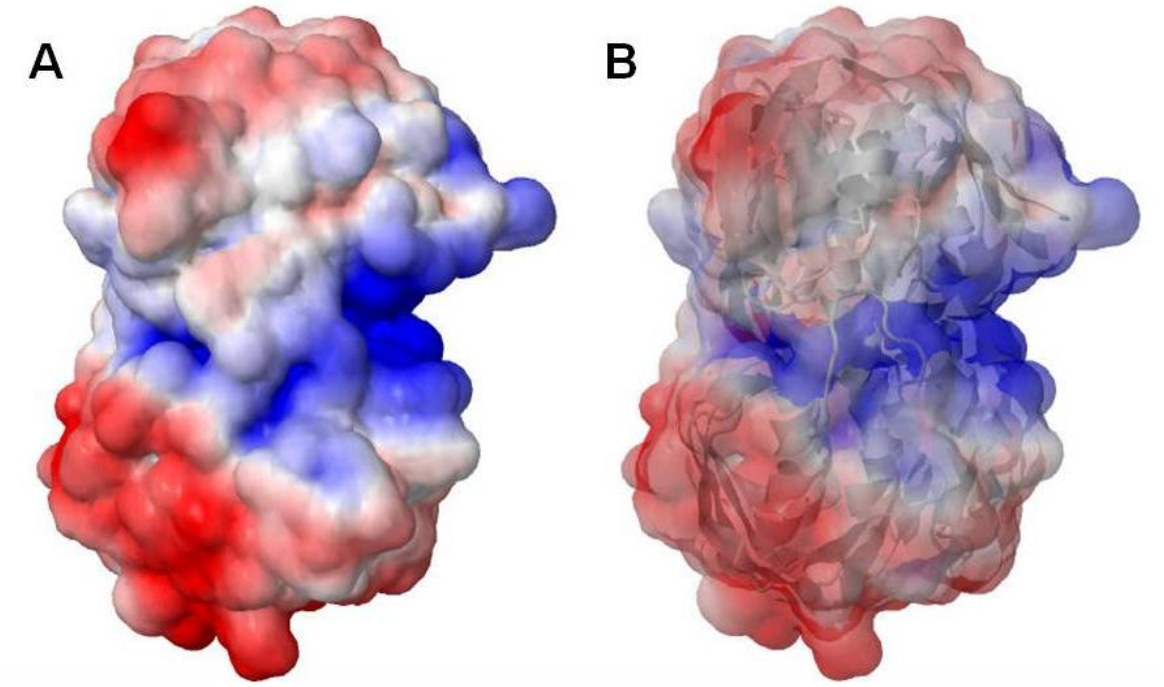

Fig. S7. The surface of MurA has negatively charged and hydrophobic characteristic. (Red) negatively charged residues, (blue) positively charged residues, and (white) hydrophobic residues. MurA tetrahedral intermediate (PDB entry 1A2N) (Skarzynski *et al.*, 1998) was used to generate figures with the online server: (http://kryptonite.nbcr.net/pdb2pqr/) (Dolinsky TJ, 2004). (A) surface view (B) transparent view

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