SUPPORTING INFORMATION FILE

STRUCTURAL BASIS FOR NUCLEOTIDE BINDING AND REACTION CATALYSIS IN MEVALONATE DIPHOSPHATE DECARBOXYLASE †

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Running Title: Crystal Structures of WT and mutant MDD ternary complexes

SUPPLEMENTAL FIGURE LEGENDS

SFigure 1. F_o-F_c map of ligands bound to MDD. (A) F_o-F_c map (*green mesh* at 2.0σ contour) of the refined WT MDD-FMVAPP-ATPγS structure (gray) in the absence of modeled ligand. (B) F_o-F_c map (*green mesh* at 2.0σ contour) of the refined S192A MDD-FMVAPP-ATPγS structure (cyan) in the absence of modeled ligand. (C) F_o-F_c map (*green mesh* at 2.0σ contour) of the refined D283A MDD-FMVAPP-ATPγS structure (red) in the absence of modeled ligand. (D) F_o-F_c map (*green mesh* at 3.0σ contour) of the refined WT MDD-MVAPP structure (gray) in the absence of modeled ligand. (E) F_o-F_c map (*green mesh* at 3.0σ contour) of the refined S192A MDD-DPGP structure (cyan) in the absence of modeled ligand. (F) F_o-F_c map (*green mesh* at 3.0σ contour) of the refined D283A MDD-DPGP structure (red) in the absence of modeled ligand.

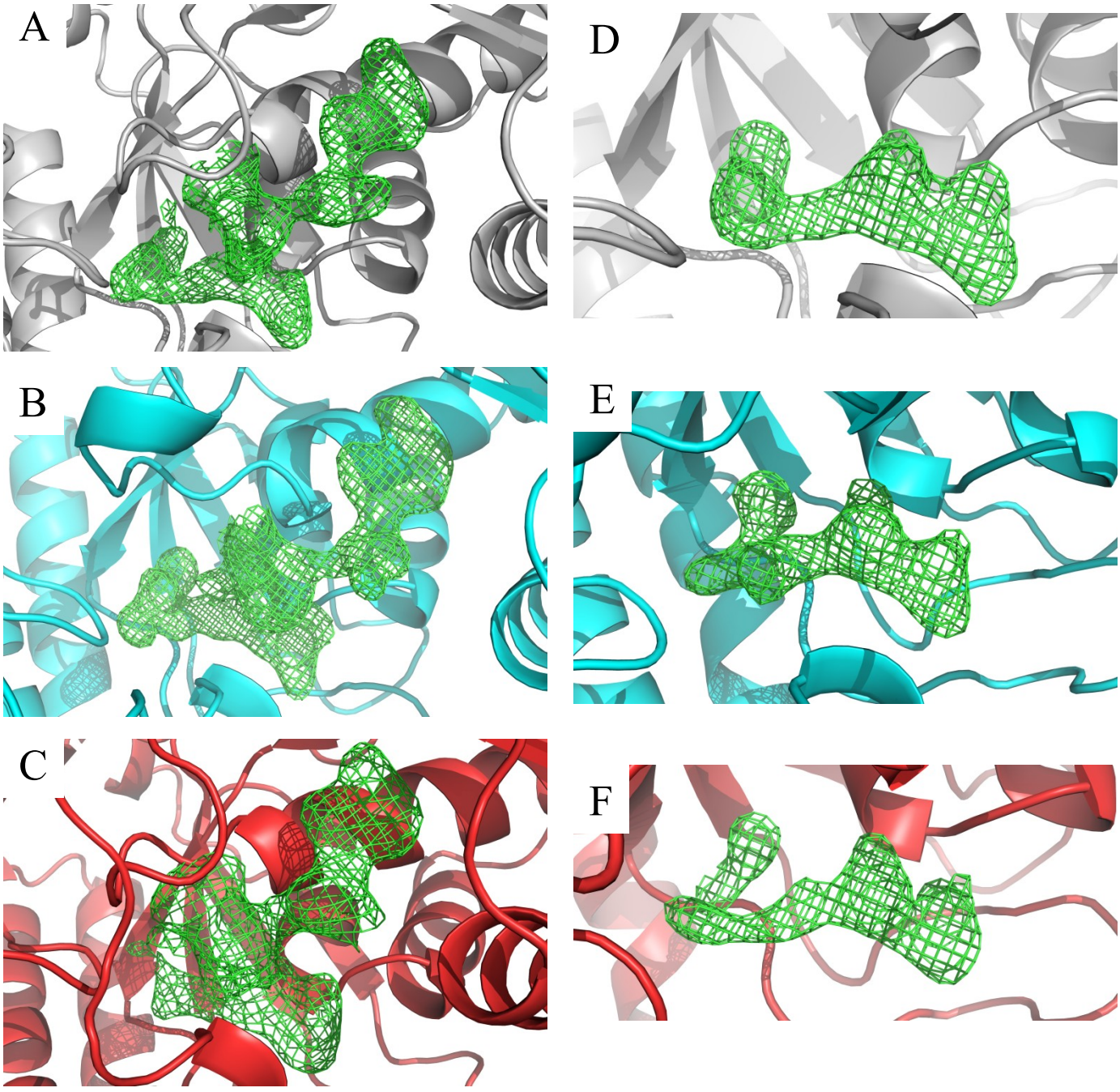
SFigure 2. $2F_o$-F_c map of ligands bound to MDD. (A) $2F_o$-F_c map (*blue mesh* at 1.0σ contour) of the refined WT MDD-FMVAPP-ATPγS structure (gray) with one molecule each of FMVAPP and ATPγS (yellow, ball and stick) modeled in a single active site. (B) $2F_o$-F_c map (*blue mesh* at 1.0σ contour) of the

refined S192A MDD-FMVAPP-ATPγS structure (green) with one molecule each of FMVAPP and ATPγS (yellow, ball and stick) modeled per enzyme active site. (C) $2F_o$-F_c map (*blue mesh* at 1.0σ contour) of the refined D283A MDD-FMVAPP-ATPγS structure (red) with one molecule each of MVAPP and ATPγS (yellow, ball and stick) modeled per enzyme active site. (D) $2F_o$-F_c map (*blue mesh* at 1.0σ contour) of the refined WT MDD-MVAPP structure (gray) with one molecule of MVAPP (yellow, ball and stick) modeled per enzyme active site. (E) $2F_o$-F_c map (*blue mesh* at 1.0σ contour) of the refined S192A MDD-DPGP structure (green) with one molecule of DPGP (yellow, ball and stick) modeled per enzyme active site. (F) $2F_o$-F_c map (*blue mesh* at 1.0σ contour) of a representative model-to-map region of the refined S192A apo-MDD structure (green). (G) $2F_o$-F_c map (*blue mesh* at 1.0σ contour) of the refined D283A MDD-DPGP structure (red) with one molecule of DPGP (yellow, ball and stick) modeled per enzyme active site.

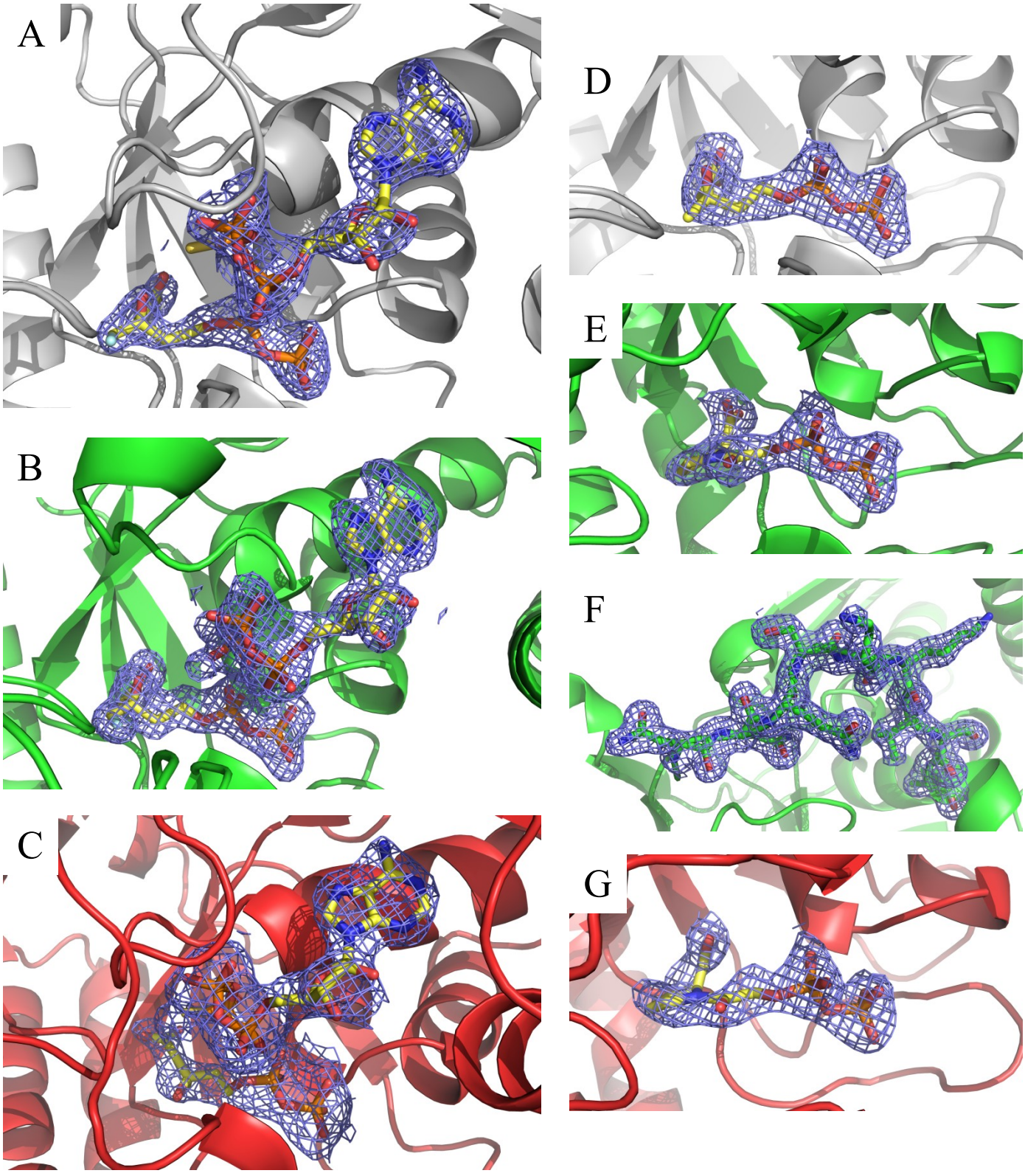
SFigure 3. Co-crystal structures of mutant MDD bound to the substrate DPGP. (A) 2.15 Å co-crystal structure of *S. epidermidis* S192A MDD in cartoon format (green). Substrate DPGP is represented as ball and stick (yellow). Active site side chains within interaction distance of DPGP are depicted in ball and stick (purple). (B) 2.10 Å co-crystal structure of *S. epidermidis* D283A MDD in cartoon format (red). Substrate DPGP is represented as ball and stick (yellow). Active site side chains within interaction distance of DPGP are depicted in ball and stick (cyan). Hydrogen bonding distances can be found in Supplemental Table 5.

SFigure 4. Structural alignment of MDD crystal structures. (A) Alignment of WT MDD crystal structures by Local-Global Alignment (*28*); apo- (red), FMVAPP-bound (green), DPGP-bound (blue), MVAPP-bound (orange), FMVAPP/ATPγS-bound (gray) (*28*). (B) Alignment of S192A MDD crystal structures by Local-Global Alignment; apo- (red), FMVAPP-bound (green), DPGP-bound (blue), FMVAPP/ATPγS-bound (gray) (*28*). (C) Alignment of D283A MDD crystal structures by Local-Global Alignment; DPGP-bound (blue), MVAPP/ATPγS-bound (gray) (*28*). Alignment statistics can be found in Supplemental Table 1.

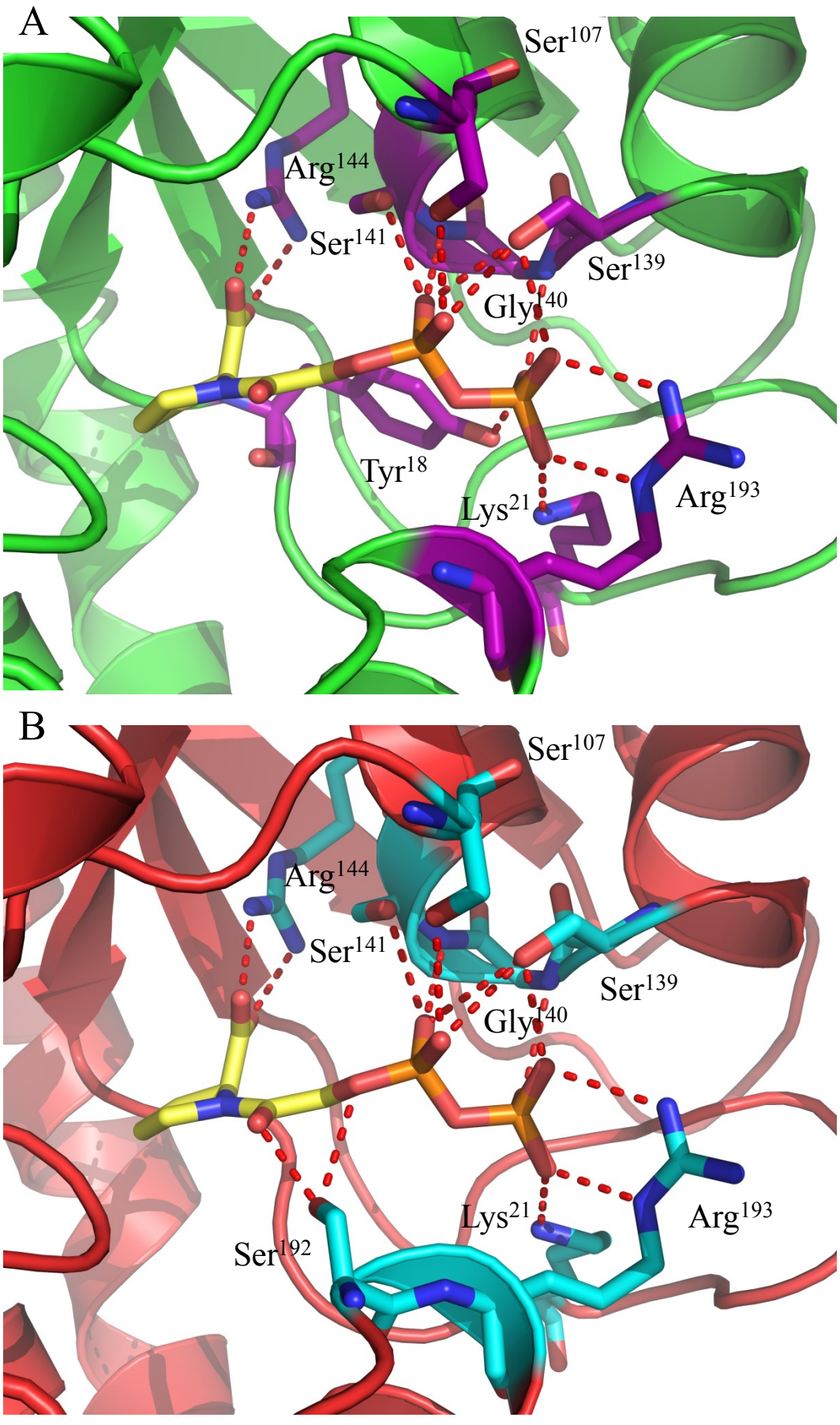
Supplemental Figure 1



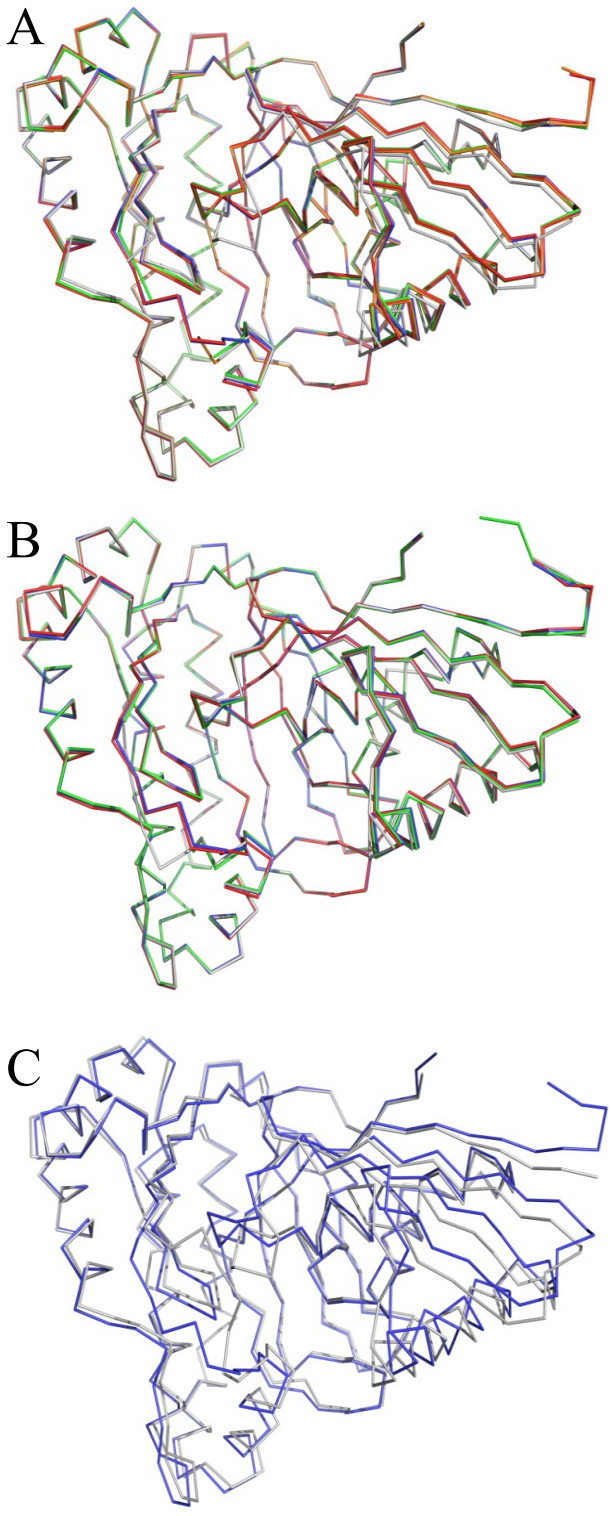
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Table 1

Supplemental Refinement Data

	S192A MDD	S192A MDD	S192A MDD	MDD	D283A MDD	MDD	D283A MDD
Protein	Native	Native	Native	Native	Native	Native	Native
Ligands		DPGP	FMVAPP AGS	FMVAPP AGS	MVAPP AGS	MVAPP	DPGP
Ligand Occupancy							
DPGP							
Chain 1		95.0					74.0
Chain 2		100.0					74.0
FMVAPP							
Chain 1			93.0	78.0			
Chain 2			100.0				
MVAPP							
Chain 1					96.0	94.0	
Chain 2					100.0	91.0	
Chain 3					92.0		
Chain 4					91.0		
Chain 5					88.0		
Chain 6					92.0		
Chain 7					96.0		
Chain 8					90.0		
ATPγS							
Chain 1			72.0	79.0	95.0		
Chain 2			81.0		96.0		
Chain 3					91.0		
Chain 4					97.0		
Chain 5					98.0		
Chain 6					99.0		
Chain 7					96.0		
Chain 8					95.0		

Supplemental Table 2

WT MDD + ATPgS Contacts

Enzyme		Distance (Å)	Inhibitor	
Amino Acid	**Atom**	**ATPgS**	**Atom**	**Group**
Glu69	OE1	2.75	O2'	Ribose hydroxyl
Ser94	OG	2.64	N6	Purine ring
Asn96	ND2	2.98	N7	Purine ring
Asn96	OD1	3.40	N6	Purine ring
Ala105	N	2.92	O2B	β-phosphoryl
Ser106	N	2.86	O1B	α-phosphoryl
Ser106	OG	2.61	O1B	α-phosphoryl
Ser107	N	3.33	O1B	α-phosphoryl
Ser107	N	3.22	O2G	β-phosphoryl
Ser107	N	3.20	O3B	γ-phosphoryl
Ser107	OG	2.83	O2G	β-phosphoryl
Ser107	OG	2.74	O3B	γ-phosphoryl
Ala108	N	3.01	O2G	β-phosphoryl
Ser141	OG	3.13	O3G	γ-phosphoryl
Ser192	N	3.24	O1A	α-phosphoryl
Ser192	OG	3.00	O1A	α-phosphoryl

S192A MDD + ATPgS Contacts

Enzyme		Distance (Å)	Inhibitor	
Amino Acid	**Atom**	**ATPgS**	**Atom**	**Group**
Glu69	OE2	3.01	O3'	Ribose hydroxyl
Glu69	OE2	3.32	O2'	Ribose hydroxyl
Ser94	OG	2.55	N6	Purine ring
Asn96	ND2	3.02	N7	Purine ring
Asn96	OD1	3.22	N6	Purine ring
Ser106	N	3.08	O1B	α-phosphoryl
Ser106	OG	3.33	O1B	α-phosphoryl
Ser107	N	2.96	O3B	γ-phosphoryl
Ser107	OG	2.58	S1G	γ-phosphoryl Sulfur
Ser107	OG	2.92	O3B	γ-phosphoryl
Ala108	N	3.01	O2G	β-phosphoryl
Ser141	OG	3.21	S1G	γ-phosphoryl Sulfur

D283A MDD + ATPgS Contacts

Enzyme		Distance (Å)	Inhibitor	
Amino Acid	**Atom**	**ATPgS**	**Atom**	**Group**
Glu69	OE1	2.68	O2'	Ribose hydroxyl
Glu69	OE2	3.04	O3'	Ribose hydroxyl
Ser94	OG	2.61	N6	Purine ring
Asn96	ND2	2.70	N7	Purine ring
Asn96	OD1	3.18	N6	Purine ring
Ala101	N	3.17	O1B	α-phosphoryl
Gly103	N	2.85	O2B	β-phosphoryl
Leu104	N	3.09	O2B	β-phosphoryl
Ala105	N	3.05	O2B	β-phosphoryl
Ser106	OG	2.67	O1B	α-phosphoryl
Ser107	N	3.35	O3B	γ-phosphoryl
Ser107	OG	2.60	O2G	β-phosphoryl
Ser107	OG	2.75	O3B	γ-phosphoryl
Ser107	OG	2.87	O3G	γ-phosphoryl
Ser141	OG	3.05	O2G	β-phosphoryl
Lys188	NZ	3.15	O3A	γ-phosphoryl
Ser192	N	3.37	O1A	α-phosphoryl
Ser192	OG	3.39	O2A	β-phosphoryl
Ser192	OG	3.37	O3G	γ-phosphoryl
Ser192	OG	3.25	O3A	γ-phosphoryl

Supplemental Table 3

	WT MDD							
Protein 1	**Protein 2**	**N1**	**N2**	**Distance (Å)**	**N**	**RMSD (Å2)**	**Seq_Id**	**LGA_S**
apo-MDD	FMVAPP	324	327	5.0	323	0.43	99.69	98.54
apo-MDD	DPGP	329	327	5.0	326	0.17	100.00	99.69
apo-MDD	MVAPP	325	327	5.0	324	0.58	99.38	98.73
apo-MDD	ternary complex	322	327	5.0	319	0.73	99.37	96.81
FMVAPP	DPGP	329	324	5.0	324	0.25	100.00	99.93
FMVAPP	MVAPP	325	324	5.0	322	0.40	99.38	99.17
FMVAPP	ternary complex	322	324	5.0	321	0.78	100.00	98.21
DPGP	MVAPP	325	329	5.0	324	0.33	100.00	98.32
DPGP	ternary complex	322	329	5.0	320	0.63	99.69	96.61
MVAPP	ternary complex	322	325	5.0	319	0.68	100.00	97.57

	S192A MDD							
Protein 1	**Protein 2**	**N1**	**N2**	**Distance (Å)**	**N**	**RMSD (Å2)**	**Seq_Id**	**LGA_S**
apo-MDD	FMVAPP	328	330	5.0	326	0.38	100.00	98.61
apo-MDD	DPGP	331	330	5.0	330	0.17	100.00	100.00
apo-MDD	ternary complex	330	330	5.0	330	0.49	100.00	99.67
FMVAPP	DPGP	331	328	5.0	326	0.32	100.00	99.25
FMVAPP	ternary complex	330	328	5.0	326	0.20	100.00	99.39
DPGP	ternary complex	330	331	5.0	330	0.47	100.00	99.40

	D283A MDD							
Protein 1	**Protein 2**	**N1**	**N2**	**Distance (Å)**	**N**	**RMSD (Å2)**	**Seq_Id**	**LGA_S**
DPGP	ternary complex	326	331	5.0	322	1.10	99.69	94.55

Supplemental Table 4

D283A MDD + MVAPP Contacts

Enzyme		Distance (Å)	Inhibitor	
Amino Acid	**Atom**	**MVAPP**	**Atom**	**Group**
Ala14	N	3.06	OAD	Carboxylate
Lys17	NZ	3.07	OAA	Carboxylate
Tyr18	O	3.23	OAE	C3-hydroxyl
Tyr18	OH	2.88	OAF	β-phosphoryl
Lys21	NZ	2.70	OAB	β-phosphoryl
Ser107	OG	2.99	OAH	α-phosphoryl
Ser139	OG	2.87	OAG	β-phosphoryl
Ser139	OG	2.73	OAH	α-phosphoryl
Gly140	N	2.66	OAF	β-phosphoryl
Ser141	N	2.99	OAC	α-phosphoryl
Ser141	OG	3.5	OAH	α-phosphoryl
Arg193	NE	2.59	OAB	β-phosphoryl
Arg193	NH2	3.03	OAB	β-phosphoryl
Arg193	NH2	2.70	OAG	β-phosphoryl

WT MDD + MVAPP Contacts

Enzyme		Distance (Å)	Inhibitor	
Amino Acid	**Atom**	**MVAPP**	**Atom**	**Group**
Tyr18	N	2.76	OAA	Carboxylate
Tyr18	OH	2.80	OAF	β-phosphoryl
Lys21	NZ	2.78	OAB	β-phosphoryl
Ser107	OG	3.39	OAH	α-phosphoryl
Ser139	OG	3.39	OAC	β-phosphoryl
Ser139	OG	2.54	OAG	α-phosphoryl
Gly140	N	2.80	OAF	β-phosphoryl
Ser141	N	3.01	OAC	α-phosphoryl
Ser141	OG	2.72	OAC	α-phosphoryl
Arg144	NH1	3.26	OAA	Carboxylate
Arg144	NH2	2.87	OAD	Carboxylate
Ser192	OG	2.95	OAH	α-phosphoryl
Arg193	NE	2.78	OAB	β-phosphoryl
Arg193	NH2	3.09	OAG	β-phosphoryl
Asp283	OD1	3.35	OAE	C3-hydroxyl

Supplemental Table 5

D283A MDD + DPGP Contacts

Enzyme		Distance (Å)	Inhibitor	
Amino Acid	**Atom**	**MVAPP**	**Atom**	**Group**
Lys21	NZ	2.73	OAF	β-phosphoryl
Lys21	NZ	3.41	OAG	β-phosphoryl
Ser107	OG	3.21	OAD	α-phosphoryl
Ser139	OG	2.73	OAC	β-phosphoryl
Ser139	OG	2.93	OAD	α-phosphoryl
Ser139	OG	3.32	OAH	α-phosphoryl
Gly140	N	3.07	OAG	β-phosphoryl
Ser141	N	3.01	OAH	α-phosphoryl
Ser141	OG	2.98	OAH	α-phosphoryl
Arg144	NH1	2.85	O	Carboxylate
Arg144	NH2	2.95	O	Carboxylate
Arg144	NH2	3.14	OXT	Carboxylate
Ser192	OG	3.25	OAM	α-phosphoryl
Ser192	OG	2.58	OAB	C2-hydroxyl
Arg193	NE	2.78	OAF	β-phosphoryl
Arg193	NH2	3.12	OAC	β-phosphoryl

S192A MDD + DPGP Contacts

Enzyme		Distance (Å)	Inhibitor	
Amino Acid	**Atom**	**MVAPP**	**Atom**	**Group**
Tyr18	OH	2.68	OAG	β-phosphoryl
Lys21	NZ	2.61	OAF	β-phosphoryl
Ser107	OG	2.84	OAD	α-phosphoryl
Ser139	OG	2.84	OAD	α-phosphoryl
Ser139	OG	2.95	OAC	β-phosphoryl
Ser139	OG	3.12	OAH	α-phosphoryl
Gly140	N	2.91	OAG	β-phosphoryl
Ser141	N	2.97	OAH	α-phosphoryl
Ser141	OG	2.75	OAH	α-phosphoryl
Arg144	NH1	3.12	O	Carboxylate
Arg144	NH2	2.96	O	Carboxylate
Arg144	NH2	3.13	OXT	Carboxylate
Arg193	NE	2.82	OAF	β-phosphoryl
Arg193	NH2	2.94	OAC	β-phosphoryl