Supplemental Information

Structural Basis for Specific Inhibition of tRNA Synthetase by an ATP Competitive Inhibitor

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This file contains:

Figures S1 to S4

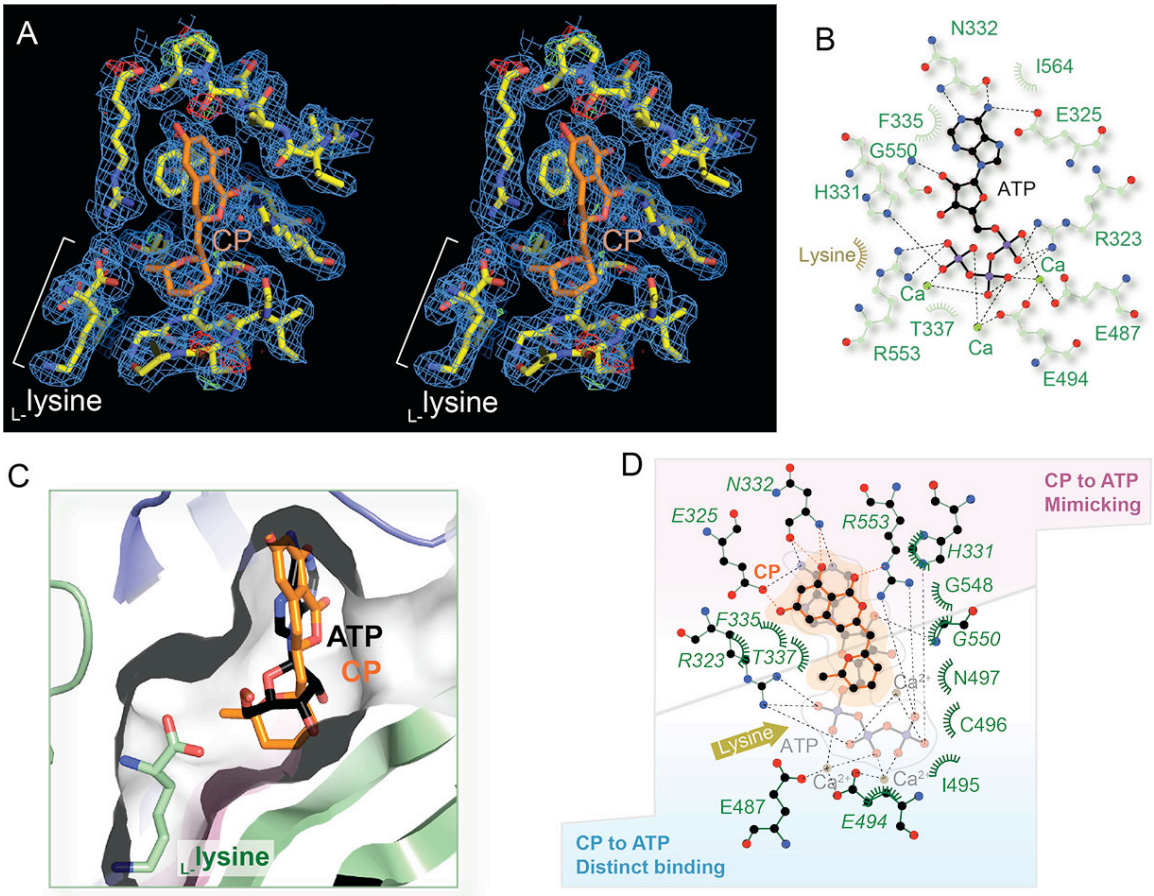


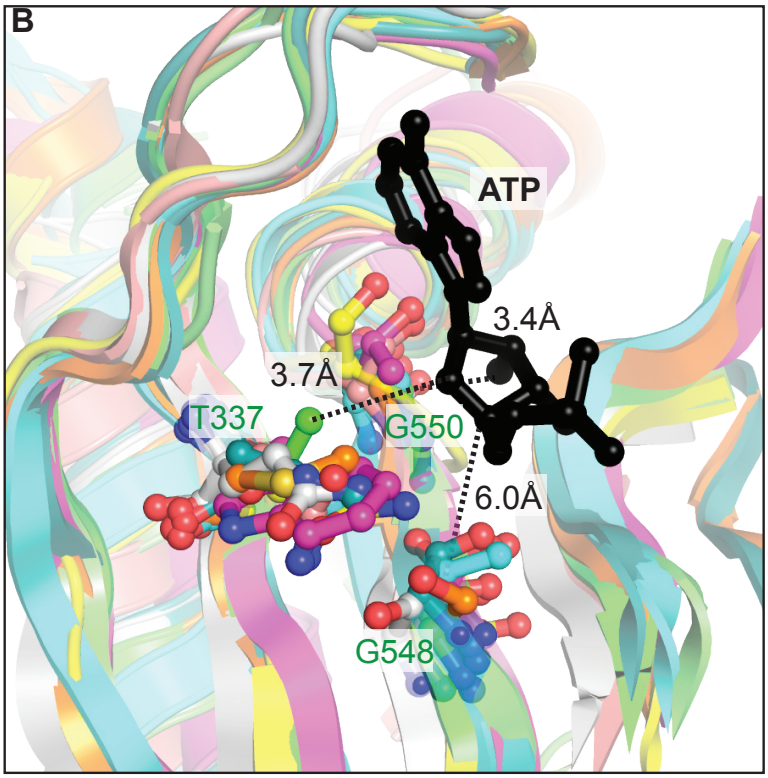
Figure S1. Cladosporin Occupies ATP Binding Site in Human LysRS, Related to Figure 1.

A. Stereo view of the cladosporin (CP) binding site in human LysRS (*Hs*LysRS). LysRS residues within 5 Å of CP are shown in yellow. CP is shown in orange. 2Fo-Fc electron density is colored in blue at 1.0 σ. Fo-Fc electron density is shown at +/- 3.0 σ, and colored in green and red for positive and negative peaks, respectively. **B**. Schematic presentation of ATP binding in *Hs*LysRS. ATP and hydrogen-bonding residues are shown in stick representations, and other residues within 4.5 Å of ATP are shown in light green. **C**. Superimposition of CP and ATP bound complex structures. CP is shown as orange sticks. ATP is shown as black sticks. The enzymatic pocket of *Hs*LysRS is shown as white surface, and the protein chain is shown as cartoon. **D**. Overlapped schematic presentations of CP and ATP binding in *Hs*LysRS. Shared interacting residues are shown as italic. Half of CP is mimicking ATP, the other half is recognized by interactions distinct from ATP. For **B, C** and **D**, the *Hs*LysRS-ATP structure is from pdb: 3BJU.

A

Class I	Class II
ArgRS	SerRS
MetRS	ProRS
LeuRS	ThrRS
IleRS	HisRS
ValRS	GlyRSα_2
CysRS	AlaRS
GluRS	AspRS
GlnRS	AsnRS
LysRS-I	LysRS-II
TrpRS	PheRS
TyrRS	GlyRS$\alpha\beta$
	SepRS
	PylRS

B



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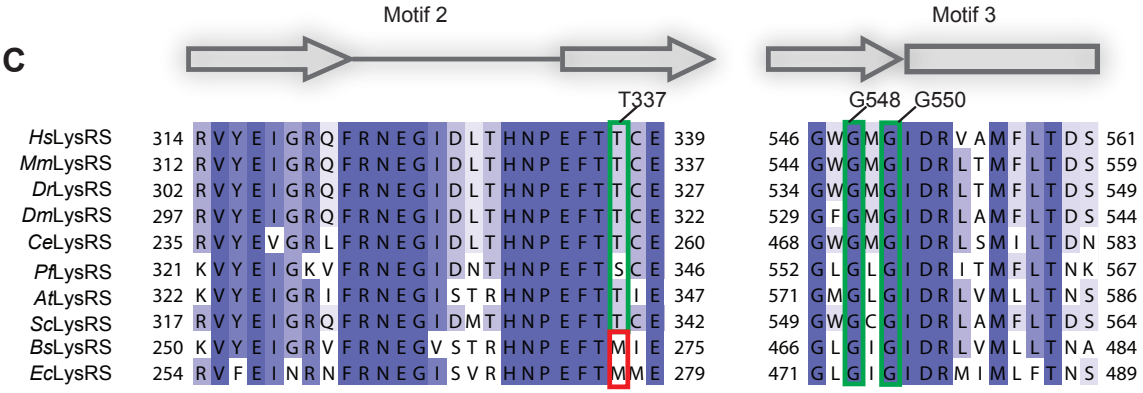

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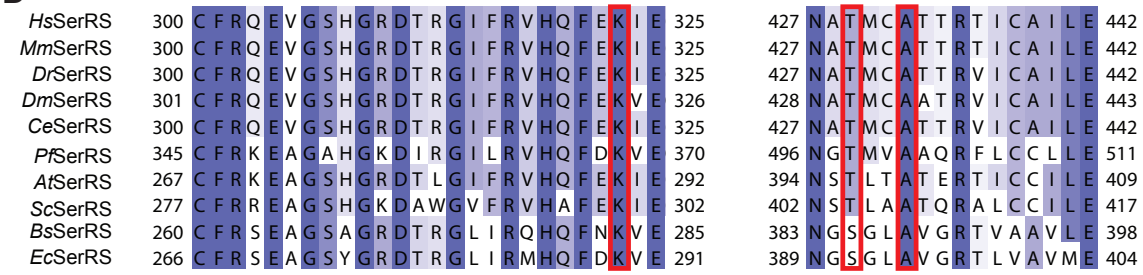

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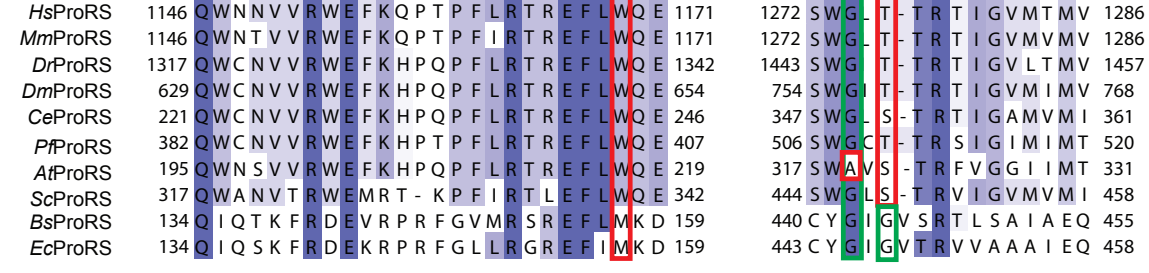

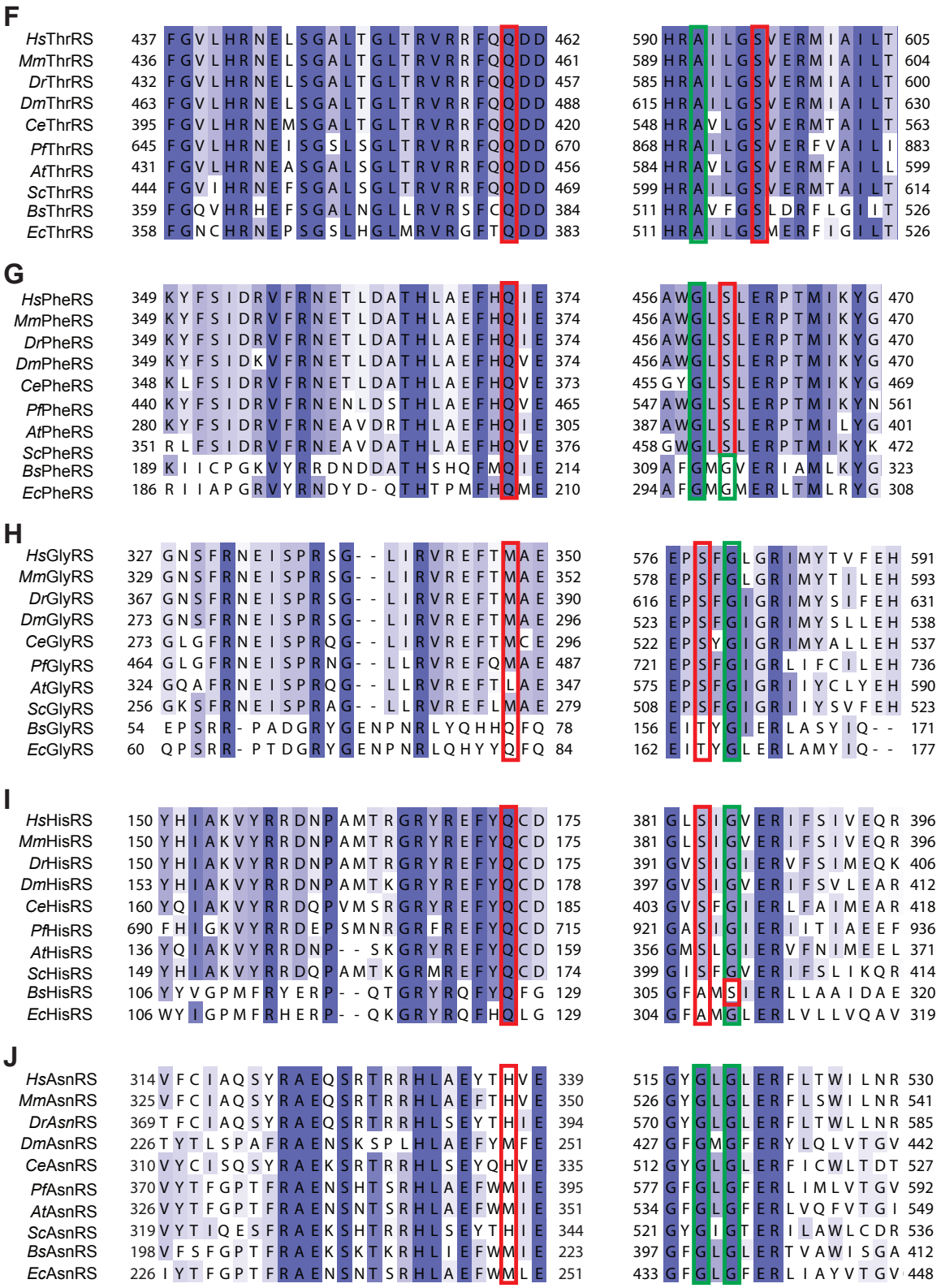
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MmThrRS 436 FGVLHRNELSGALTGLTRVRRFQQDD 461 589 HRAILGSVERMIAILT 604
DrThrRS 432 FGVLHRNELSGALTGLTRVRRFQQDD 457 585 HRAILGSVERMIAILT 600
DmThrRS 463 FGVLHRNELSGALTGLTRVRRFQQDD 488 615 HRAILGSVERMIAILT 630
CeThrRS 395 FGVLHRNEMSGALTGLTRVRRFQQDD 420 548 HRAVLGSVERMTAILT 563
PfThrRS 645 FGVLHRNEISGSLSGLTRVRRFQQDD 670 868 HRAILGSVERFVAILI 883
AtThrRS 431 FGVLHRNEASGALSGLTRVRRFQQDD 456 584 HRAVLGSVERMFAILL 599
ScThrRS 444 FGVIHRNEFSGALSGLTRVRRFQQDD 469 599 HRAILGSVERMTAILT 614
BsThrRS 359 FGQVHRHEFSGALNGLLRVRSFCQDD 384 511 HRAVFGSLDRFLGIIT 526
EcThrRS 358 FGNCHRNEPSGSLHGLMRVRGFTQDD 383 511 HRAILGSMERFIGILT 526
G
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PfPheRS 440 KYFSIDRVFRNENLDSTHLAEFHQVE 465 547 AWGLSLERPTMIKYN 561
AtPheRS 280 KYFSIDRVFRNEAVDRTHLAEFHQIE 305 387 AWGLSLERPTMILYG 401
ScPheRS 351 RLFSIDRVFRNEAVDATHLAEFHQVE 376 458 GWGLSLERPTMIKYK 472
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EcPheRS 186 RIIAPGRVYRNDYD-QTHTPMFHQME 210 294 AFGMGMERLTMLRYG 308
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DmGlyRS 273 GNSFRNEISPRSG--LIRVREFTMAE 296 523 EPSFGIGRIMYSLLEH 538
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PfGlyRS 464 GLGFRNEISPRNG--LLRVREFQMAE 487 721 EPSFGIGRLIFCILEH 736
AtGlyRS 324 GQAFRNEISPRQG--LLRVREFTLAE 347 575 EPSFGIGRIIYCLYEH 590
ScGlyRS 256 GKSFRNEISPRAG--LLRVREFLMAE 279 508 EPSFGIGRIIYSVFEH 523
BsGlyRS 54 EPSRR-PADGRYGENPNRLYQHHQFQ 78 156 EITYGIERLASYIQ-- 171
EcGlyRS 60 QPSRR-PTDGRYGENPNRLQHYYQFQ 84 162 EITYGLERLAMYIQ-- 177
I
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MmHisRS 150 YHIAKVYRRDNPAMTRGRYREFYQCD 175 381 GLSIGVERIFSIVEQR 396
DrHisRS 150 YHIAKVYRRDNPAMTRGRYREFYQCD 175 391 GVSIGIERVFSIMEQK 406
DmHisRS 153 YHIAKVYRRDNPAMTKGRYREFYQCD 178 397 GVSIGVERIFSVLEAR 412
CeHisRS 160 YQIAKVYRRDQPVMSRGRYREFYQCD 185 403 GVSFGIERLFAIMEAR 418
PfHisRS 690 FHIGKVYRRDEPSMNRGRFREFYQCD 715 921 GASIGIERIITIAEEF 936
AtHisRS 136 YQIAKVYRRDNP--SKGRYREFYQCD 159 356 GMSLGIERVFNIMEEL 371
ScHisRS 149 YHIAKVYRRDQPAMTKGRMREFYQCD 174 399 GISFGVERIFSLIKQR 414
BsHisRS 106 YYVGPMFRYERP--QTGRYRQFYQFG 129 305 GFAMSIERLLAAIDAE 320
EcHisRS 106 WYIGPMFRHERP--QKGRYRQFHQLG 129 304 GFAMGLERLVLLVQAV 319
J
HsAsnRS 314 VFCIAQSYRAEQSRTRRHLAEYTHVE 339 515 GYGLGLERFLTWILNR 530
MmAsnRS 325 VFCIAQSYRAEQSRTRRHLAEFTHVE 350 526 GYGLGLERFLSWILNR 541
DrAsnRS 369 TFCIAQSYRAEQSRTRRHLSEYTHIE 394 570 GYGLGLERFLTWLLNR 585
DmAsnRS 226 TYTLSPAFRAENSKSPLHLAEFYMFE 251 427 GFGMGFERYLQLVTGV 442
CeAsnRS 310 VYCISQSYRAEKSRTRRHLSEYQHVE 335 512 GYGLGLERFICWLTDT 527
PfAsnRS 370 VYTFGPTFRAENSHTSRHLAEFWMIE 395 577 GFGLGFERLIMLVTGV 592
AtAsnRS 326 VYTFGPTFRAENSNTSRHLAEFWMIE 351 534 GFGLGFERLVQFVTGI 549
ScAsnRS 319 VYTIQESFRAEKSHTRRHLSEYTHIE 344 521 GYGIGTERILAWLCDR 536
BsAsnRS 198 VFSFGPTFRAEKSKTKRHLIEFWMIE 223 397 GFGLGLERTVAWISGA 412
EcAsnRS 226 IYTFGPTFRAENSNTSRHLAEFWMLE 251 433 GFGLGFERLIAYVTGV 448

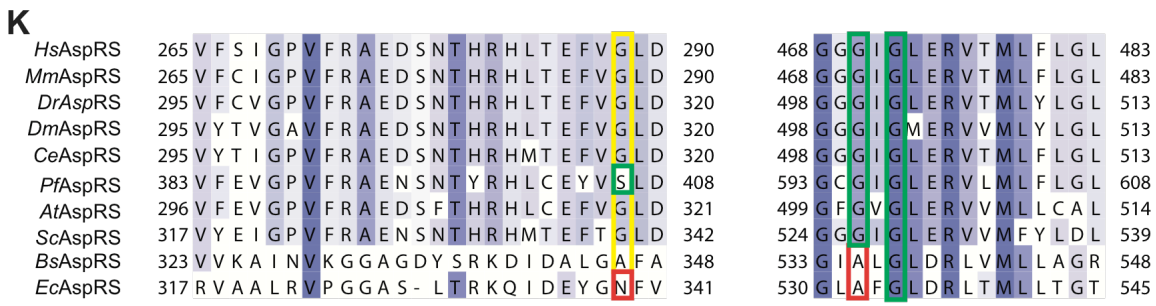

Figure S2. Family Specific Variation at the ATP Binding Pocket, Related to Figure 2.

A. The two classes of the 24 known aaRS families. **B.** Nine common class II aaRSs are superimposed with the bound ATP aligned. One representative ATP molecule is shown as black sticks. All proteins are shown as transparent cartoons. Three non-conserved residues at the bottom of ATP binding pocket are shown as sticks. Distances of ATP to *Hs*LysRS residues T337, G548, and G550 are indicated by black dashes. The structures are from pdbs: (LysRS: 3BJU; SerRS: 4L87; ProRS: 4HVC; GlyRS: 2ZT5; ThrRS: 4HWT; HisRS: 4G84; PheRS: 3L4G; AsnRS: 2XGT; AspRS: 4J15). **C-K.** Sequence alignments of motifs 2 and 3 of 9 class II tRNA synthetases. *Hs, Homo sapiens; Mm, Mus musculus; Dr, Danio rerio; Dm, Drosophila melanogaster; Ce, Caenorhabditis elegans; Pf, Plasmodium falciparum; At, Arabidopsis thaliana; Sc, Saccharomyces cerevisiae; Bs, Bacillus subtilis; Ec, Escherichia coli*. CP-binding compatible residues are indicated by green boxes; CP-repulsive residues are indicated by red boxes; and interaction-missing residues are indicated by yellow boxes.

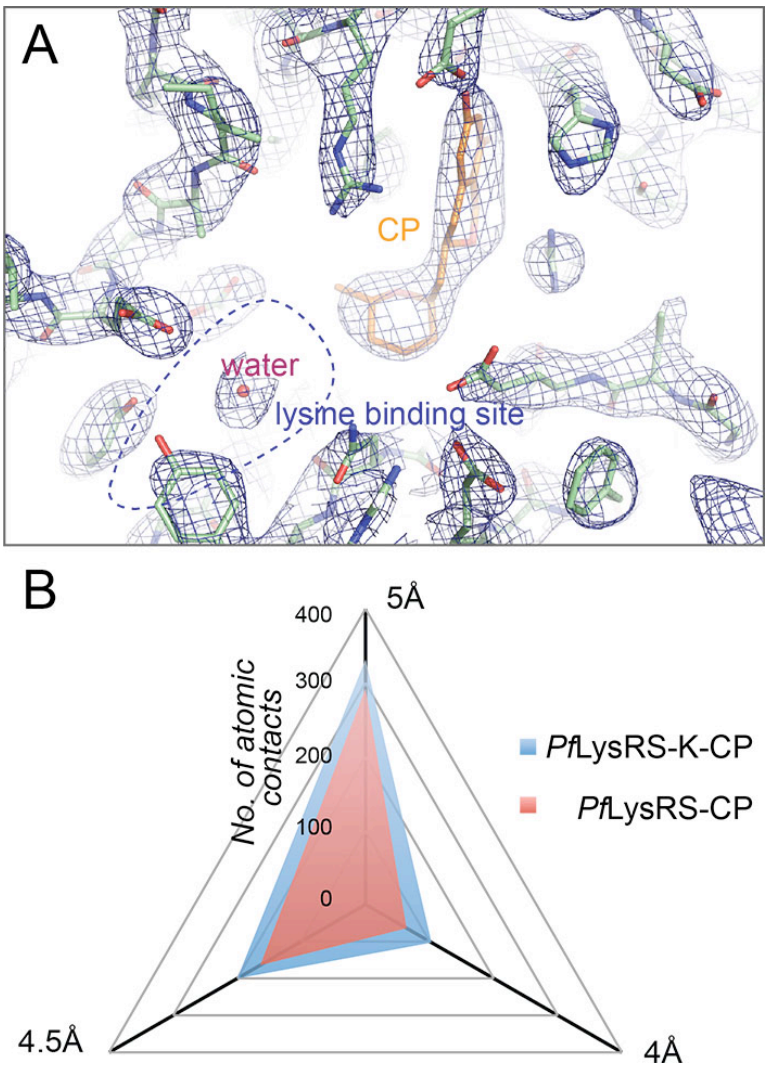

Figure S3. Cooperative Binding of CP and Lysine to *Pf*LysRS, Related to Figure 4. **A**. 2Fo-Fc electron density of the CP binding site in *Pf*LysRS is shown as blue meshes with 1.5σ. CP molecule is shown as orange sticks. Protein is shown as light green sticks. A water molecule is shown as a red ball. The lysine-binding site is indicated by dashed circle. **B**. Radar plot of total number of atomic contacts between *Pf*LysRS and CP with (blue) or without (red) lysine. Three different resolution cut-offs are used. CP binds to *Pf*LysRS with ~20% decreased atomic contacts in the absence of lysine.

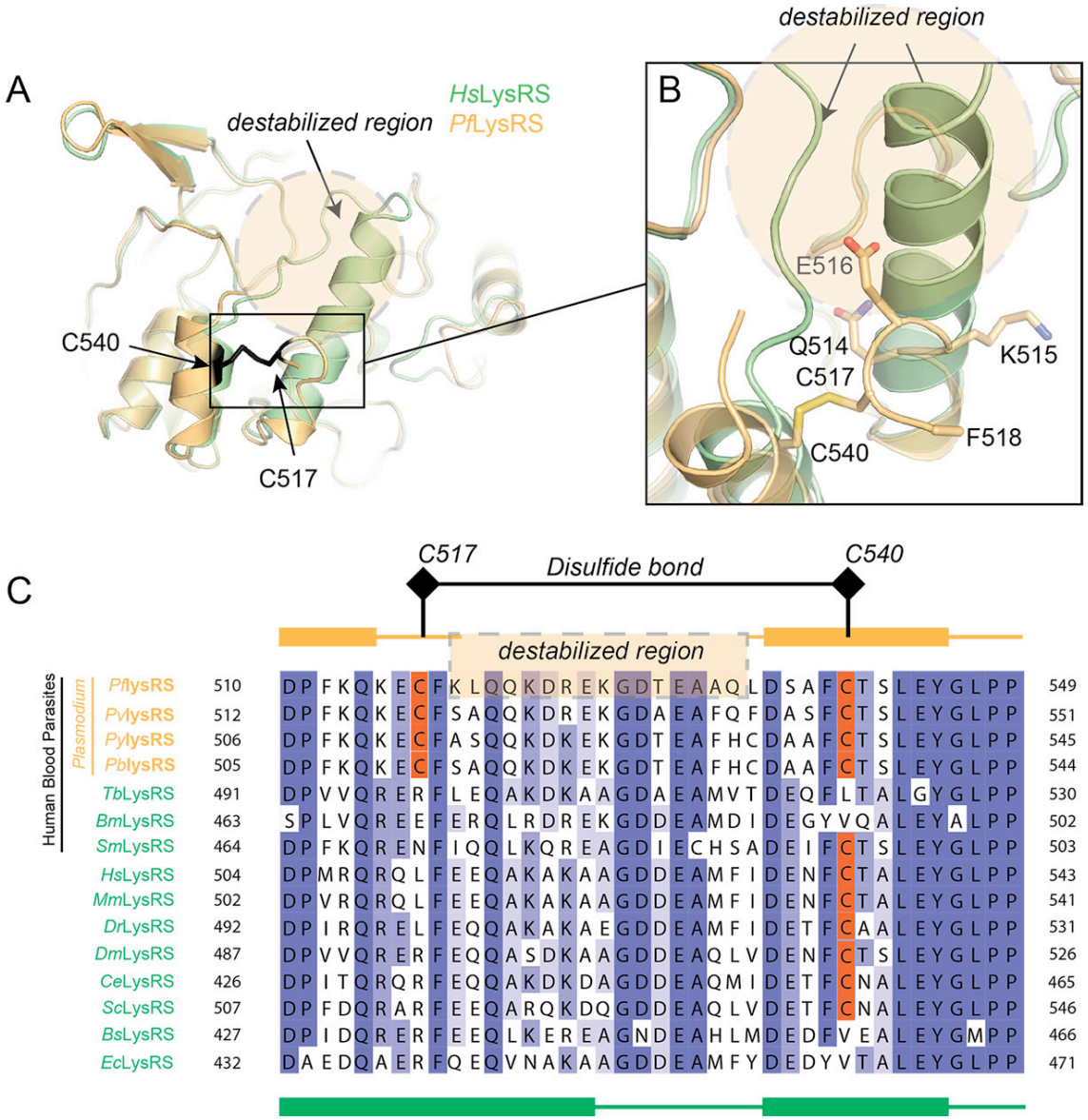

Figure S4. *Pf*LysRS Specific Disulfide Bond Destabilizes the Structure, Related to Figure 4.

A. Superimposition of *Pf*LysRS (light orange) and *Hs*LysRS (light green). The *Pf*LysRS specific disulfide bond is shown as black sticks. The destabilized region is cycled by dashes. **B**. Zoom-in view of the *Plasmodium* specific C517-C540 disulfide bond. The enclosed region of 518-535 is completely disordered in the *Pf*LysRS structure. Nearby residues are also shown as sticks. **C.** Sequence alignment of LysRSs in the region enclosed by the *Plasmodium* specific disulfide bond. *Pf, Plasmodium falciparum; Pv, Plasmodium vivax; Py, Plasmodium yoelii; Pb, Plasmodium berghei; Bm, Babesia microti; Tb, Trypanosoma brucei; Sm, Schistosoma mansoni; Hs, Homo sapiens; Mm,*

Mus musculus; Dr, Danio rerio; Dm, Drosophila melanogaster; Ce, Caenorhabditis elegans; Sc, Saccharomyces cerevisiae; Bs, Bacillus subtilis; Ec, Escherichia coli.