

Supplementary Materials for
**Cytoplasmic isoleucyl tRNA synthetase as an attractive multistage
antimalarial drug target**

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Other Supplementary Material for this manuscript includes the following:

Data file S1
Tables S2, S5, and S6
MDAR reproducibility Checklist

SUPPLEMENTAL TABLES

<i>Compound Name</i>	<i>Alternative Name</i>	<i>Canonical string</i>	<i>SMILES</i>	<i>Source</i>	<i>Tag</i>	<i>Ref.</i>
<i>MMV007938</i>	GNF-Pf3888	C1Cc2sc3ncnc(Sc4nc5cccc5[nH]4)c3c2C1		Malaria Box		
<i>MMV019266</i>	TCMDC-123835	Cc1sc2ncnc(Sc3nc4cccc4[nH]3)c2c1C		Malaria Box	CAS-421578-49-6	(12)
<i>MMV019837</i>	TCMDC-124514	CN1C(SC2=C3C=CSC3=N C=N2)=NC2=C1C=CC=C2		Charles River library	CAS-565210-12-0	
<i>MMV019869</i>	TCMDC-124553	CCc1cc2c(Sc3nc4cccc4[nH]3)ncnc2s1		Charles River library	CAS-496969-34-7	(12)
<i>MMV019904</i>	TCMDC-124602	S(C1=NC=C2C=CC=CN12)C1=C2C=CSC2=NC=N1		Malaria Box	CAS-931613-95-5	(12)
<i>MMV020525</i>	TCMDC-125258	COCCN1C(SC2=C3C=CS C3=NC=N2)=NC2=C1C=CC=C2		Charles River library	CAS-878079-46-0	(11)
<i>MMV062850</i>		CN1C=CN=C1SC1=C2C=CSC2=NC=N1		Charles River library	CAS-571918-58-6	(11)
<i>MMV1081413</i>		CC1=C(CC(N)=O)SC(SC2=C3SC=CC3=NC=N2)=N1		Charles River library		(11)
<i>MMV1091186</i>		CC1=C(CC(N)=O)SC(SC2=C3C4=C(CCC4)SC3=NC=N2)=N1		Charles River library		(11)
<i>MMV1328428</i>		C1CC2=C(C1)C1=C(S2)N=CN=C1SC1=NC2=C(C=CC=C2)N1C1=CC=CC=C1		Charles River library	CAS-853351-17-4	(11)
<i>Thiasioleucine</i>		CSC(C)C(N)C(O)=O			CAS-443-80-1	
<i>Reveromycin A</i>		CCCC[C@]1(CC[C@]2(C[C@@H]([C@H](O2)C/C=C(\C)/C=C/[C@@H]([C@@H](C)/C=C/C(=O)O)O)C)O[C@H]1/C=C/C(=C/C(=O)O)/C)OC(=O)CCC(=O)O			CAS 134615-37-5	
<i>Epoxomicin</i>		CC(C)C(C(=O)NC(C(C)O)C(=O)NC(CC(C)C)C(=O)C1(CO1)C)NC(=O)C(C(C)CC)N(C)C(=O)C			CAS 134381-21-8	

Table S1. Compounds used in this study.

Table S2. Attached Excel File. EC₅₀s for select compounds against standard lines (Dd2, 3D7, HepG2, PbLuc)

Clone Name	cIRS Mutation	Genetic Background	Compounds	Ref.
3D7-A10	--	3D7	MMV1091186, Epoxomicin	(93)
Dd2-B2	--	Dd2	MMV1081413, MMV019869, MMV1091186, MMV019266, MMV019904, MMV007938, MMV1328428, MMV020525, MMV019837, MMV062850, Reveromycin A, Thiaisoleuine	(93)
Dd2-B2-EW	--	Dd2	--	(94)
HepG2-A16-CD81 ^{EGFP26}	--	HepG2	MMV1081413, MMV019869, MMV1091186, MMV019266, MMV019837, MMV062850	(15)
<i>P. berghei</i> ANKA GFP-Luc-SM _{con}	--	<i>P. berghei</i> , HepG2	MMV019869, MMV1091186, MMV019266, MMV062850	(15, 68)
PfDEG-cIRS-HR-L810F-GFP-3D7-6G7	L810F	3D7	MMV1091186, MMV019266, MMV019904, MMV007938, MMV1328428, MMV020525, Thiaisoleuine	(9)
PfDEG-cIRS-HR-Wt-GFP-3D7-1A3	--	3D7	MMV1091186, MMV019266, MMV019904, MMV007938, MMV1328428, MMV020525, Thiaisoleuine	(9)
PfMDA-apiIRS-KD-Dd2-aTc	--	Dd2	MMV019869, MMV019266	
PfMDA-apiIRS-KD-Dd2+aTc	--	Dd2	MMV019869, MMV019266	
PfMDA-cIRS-E180D-E-Dd2-A6	E180D	Dd2	MMV019869, MMV1091186, Reveromycin A	
PfMDA-cIRS-KD-Dd2-aTc	--	Dd2	MMV019869, MMV019266, Reveromycin A	
PfMDA-cIRS-KD-Dd2+aTc	--	Dd2	MMV019869, MMV019266, Reveromycin A	
PfMDA-cIRS-L810F-E-Dd2-A2	L810F	Dd2	MMV019869	
PfMDA-cIRS-V500A-E-Dd2-G6	V500A	Dd2	MMV019869, MMV1091186, Reveromycin A	
PfMDA-cIRS-V500Si-E-Dd2-D1	V500Si	Dd2	MMV019869	
PfMDA-cIRS-V500Si-E-Dd2-D2	V500Si	Dd2	MMV1091186, Reveromycin A	
PfMDA-MMV019869-Ev-Dd2-S1-A5	E180D	Dd2	MMV019869	
PfMDA-MMV019869-Ev-Dd2-S1-C3	E180D	Dd2	MMV019869	
PfMDA-MMV019869-Ev-Dd2-S1-D1	E180D	Dd2	MMV019869	
PfMDA-MMV019869-Ev-Dd2-S1-G12	E180D	Dd2	MMV019869	
PfMDA-MMV019869-Ev-Dd2-S2-A9	C502Y	Dd2	MMV019869	
PfMDA-MMV019869-Ev-Dd2-S2-E2	C502Y	Dd2	MMV019869	
PfMDA-MMV019869-Ev-Dd2-S2-G7	V500A	Dd2	MMV019869, MMV019266	
PfMDA-MMV019869-Ev-Dd2-S2-H4	W395L	Dd2	MMV019869	
PfMDA-MMV019869-Ev-Dd2-S3-A1	S288I	Dd2	MMV019869	

<i>PfMDA-MMV019869-Ev-Dd2-S3-D8</i>	<i>S288I</i>	<i>Dd2</i>	MMV019869
<i>PfMDA-MMV019869-Ev-Dd2-S3-E11</i>	<i>S288I</i>	<i>Dd2</i>	MMV019869
<i>PfMDA-MMV019869-Ev-Dd2-S3-E3</i>	<i>S288I</i>	<i>Dd2</i>	MMV019869
<i>PfMDA-MMV1081413-Ev-Dd2-S1-A3</i>	<i>W395L</i>	<i>Dd2</i>	MMV1081413
<i>PfMDA-MMV1081413-Ev-Dd2-S1-E8</i>	<i>W395L</i>	<i>Dd2</i>	MMV1081413
<i>PfMDA-MMV1081413-Ev-Dd2-S1-H4</i>	<i>W395L</i>	<i>Dd2</i>	MMV1081413, MMV019869, MMV1091186, MMV019266, MMV019904, MMV007938, MMV1328428, MMV020525, MMV019837, MMV062850, Thiaisoleuine
<i>PfMDA-MMV1091186-Ev-3D7-S1-C12</i>	<i>N269K</i>	<i>3D7</i>	MMV1091186, Epoxomicin
<i>PfMDA-MMV1091186-Ev-3D7-S2-A9</i>	<i>N269K</i>	<i>3D7</i>	MMV1091186, Epoxomicin
<i>PfMDA-MMV1091186-Ev-3D7-S3-H11</i>	<i>E180Q</i>	<i>3D7</i>	MMV1091186, Epoxomicin

Table S3. Names of strains and clones used in this study. Pf (*P. falciparum*). *P. berghei* (*Plasmodium berghei*). MDA (MALDA). DEG (Daniel E. Goldberg Laboratory). MMV (Medicines for Malaria Venture). Ev (Evolved under pressure of the indicated compound in the clone name)). E (CRISPR Edited). +aTc (with anhydrous tetracycline; target gene is expressed). -aTc (without anhydrous tetracycline; target gene is repressed). KD (knockdown). HR (homologous recombination). Dd2 (*P. falciparum* background that is chloroquine resistant). 3D7 (*P. falciparum* background that is chloroquine sensitive). HepG2 (human hepatoma nontumorigenic cell line). Unless referenced, all strains were constructed for this study.

<i>Sample Name</i>	<i>Aligned Reads</i>	<i>Percent Reads Aligned to Reference</i>	<i>Mean Coverage</i>	<i>Percent Bases Covered by ≥5 Reads</i>
<i>PfMDA-MMV1081413-Ev-Dd2-S1-A3</i>	30944369	98.6	107.88	96.1
<i>PfMDA-MMV1081413-Ev-Dd2-S1-E8</i>	34033176	98.6	118.86	96.9
<i>PfMDA-MMV1081413-Ev-Dd2-S1-H4</i>	31266492	98.6	109.99	96.6
<i>Dd2-B2</i>	27645387	93.7	98.94	96.8
<i>PfMDA-MMV1091186-Ev-3D7-S1-C12</i>	18740811	99.9	69.33	98.2
<i>PfMDA-MMV1091186-Ev-3D7-S1-A9</i>	16939868	99.9	62.75	98
<i>PfMDA-MMV1091186-Ev-3D7-S1-H11</i>	16557150	99.9	61.73	97.1
<i>3D7-A10</i>	21798488	92.4	68.16	97.5
<i>PfMDA-MMV019869-Ev-Dd2-S1-A5</i>	19874051	93.5	70.03	96.6
<i>PfMDA-MMV019869-Ev-Dd2-S1-C3</i>	12871785	96.7	47.42	94.6
<i>PfMDA-MMV019869-Ev-Dd2-S1-D1</i>	11321050	97.6	42.39	95.8
<i>PfMDA-MMV019869-Ev-Dd2-S1-G12</i>	12512137	96.5	45.55	95.7
<i>PfMDA-MMV019869-Ev-Dd2-S2-A9</i>	13883123	93.7	50.27	95.4
<i>PfMDA-MMV019869-Ev-Dd2-S2-E2</i>	13170282	97.1	47.47	95
<i>PfMDA-MMV019869-Ev-Dd2-S2-G7</i>	13559258	97.5	49.13	95.9
<i>PfMDA-MMV019869-Ev-Dd2-S2-H4</i>	17183160	97.6	62.04	96.3
<i>PfMDA-MMV019869-Ev-Dd2-S3-A7</i>	16942000	97.5	61.09	96.4
<i>PfMDA-MMV019869-Ev-Dd2-S3-D8</i>	11395916	97.7	42.2	95.7
<i>PfMDA-MMV019869-Ev-Dd2-S3-E3</i>	12660472	97.7	46.68	95.6
<i>PfMDA-MMV019869-Ev-Dd2-S3-E11</i>	12095161	97.7	44.78	96.2
<i>Dd2-B2-EW</i>	18654880	98.1	67.98	96.6

Table S4. Sequencing statistics for Whole Genome Sequencing

Table S5. Attached Excel file. Alleles identified in this study

Table S6. Attached Excel file. Classification and metabolomic data

<i>Line</i>	<i>Primer Sequence (5'->3')</i>
Sanger sequencing	
<i>5'ATG-cIRS</i>	<i>ATGTTAAGATTTGTCAATGAATCTTTTTTG</i>
<i>E180D-edited</i>	<i>CTCTTTATCTATTTTCATATTCTACGGGTAG</i>
<i>E180D-WT</i>	<i>CAATCCACCTACCAATACGTTGAACG</i>
<i>V500A</i>	<i>TGTGACACGTCCAATGAAAAACATGC</i>
<i>V500A-edited</i>	<i>CATATGAAGGTGCGCAATGAGCGATT</i>
<i>V500A-WT</i>	<i>CTTACACGTACAAACCAAGCTGGGATAG</i>
<i>3'TAA-cIRS</i>	<i>TTATTGGTTGGTAAAAATGACGAGTACCTC</i>
CRISPR gRNA (#)	
<i>E180D (1)</i>	<i>AGGTTTGGATGGGATTGCCA</i>
<i>V500A (3)</i>	<i>GTAAGTGATGATGCTGGTAC</i>
<i>V500 silent (3)</i>	<i>GTAAGTGATGATGCTGGTAC</i>

Table S7. Primers used in this study

<i>Compound</i>	<i>Compound Binding Site Residues</i>	<i>Distance from Allosteric Site (Å)</i>	<i>Distance from Editing Site (Å)</i>	<i>Compound point of measurement</i>
<i>Reveromycin A</i>	<i>W449, W456, W529, Y571, R454, R460, D527, F48, V89, P90, F191, W529, R462</i>	<i>15-24</i>	<i>33-38</i>	<i>1,7-dioxaspiro[5.5]undecane of bound reveromycin A</i>
<i>Ile-AMP</i>	<i>F48, H54, H56, D85, W529, E561, M603, K605</i>	<i>21-31</i>	<i>39-43</i>	<i>adenosine of the intermediate</i>
<i>Thiaisoleucine (L810)</i>	<i>L810</i>	<i>33-43</i>	<i>47-50</i>	<i>L810</i>
<i>Mupirocin</i>	<i>F48, H54, H56, D85, R601, K602, M603, S604, K605, Q565, W569, N669</i>	<i>24-32</i>	<i>40-44</i>	<i>oxane of mupirocin</i>

Table S8. Distances from PfcIRS mutations to known cIRS inhibitors

SUPPLEMENTAL FIGURES

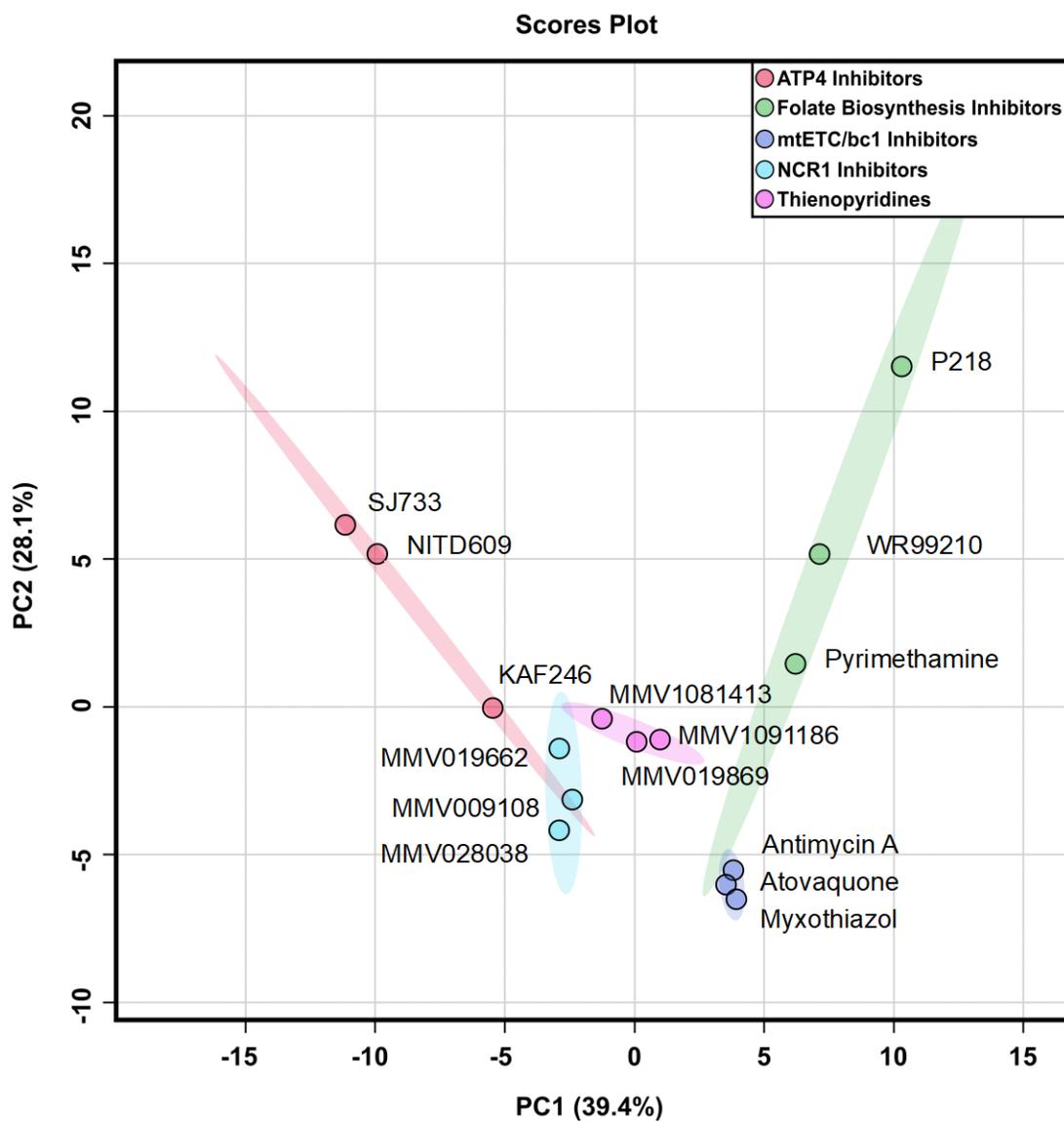


Fig. S1. Metabolomics principal component analysis showing the thienopyrimidines cluster together and apart from other known *P. falciparum* inhibitor classes when comparing PC1 vs PC2.

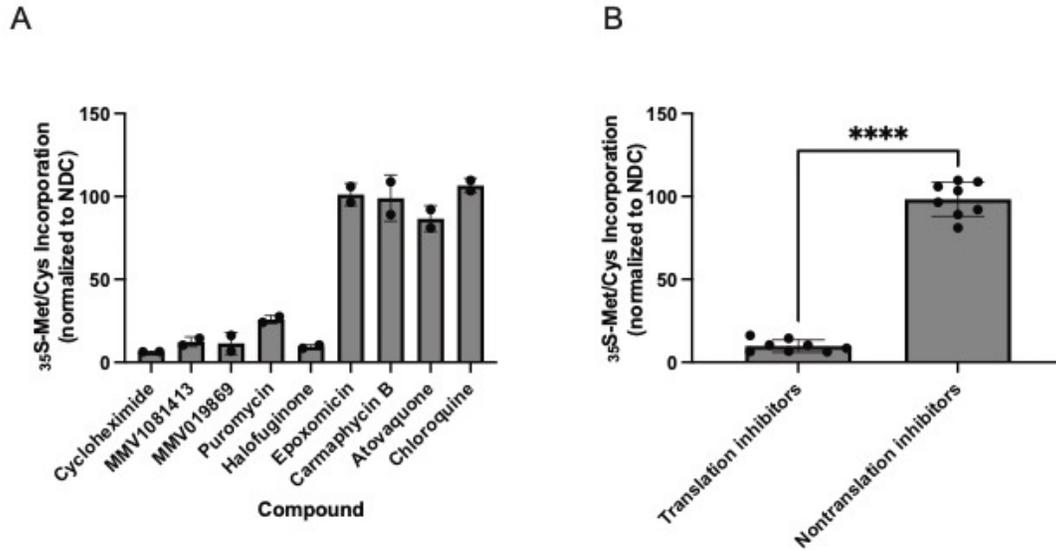


Fig. S2. *Translation inhibition assay.* Synchronous parasites were treated with compounds at 100× the EC₅₀ (see Methods) for one hour and ³⁵S methionine. Incorporation rates were normalized to the no drug control (NDC). A) ³⁵S-Met/Cys incorporation per compound showing two biological replicates for each compound. B) Aggregate data for cycloheximide, halofuginone, MMV1081413 and MMV019869 (translation inhibitors) compared to epoxomicin, carmaphycin B, atovaquone and chloroquine (non-translation inhibitors) using a non-paired t test. **** p < 0.0001.

*HaeI*RS 501 ... E S V D H L T I P S R C G K . . G S L H R I . . . S E V F D C W F E S G S M P Y A Q V H Y P F E N - K 545
*SceI*RS 503 ... D V I D K L T I P S K Q G K . . G D L K R I . . . E E V F D C W F E S G S M P Y A S Q H Y P F E N - T 547
*PfiI*RS 681 ... H Y I D N I E I K N P K G D T Y P K L K R I . . . P E V F D C W F E S G S M P Y A K V H Y P F S T E T 728
*PvuI*RS 619 ... H F I D H I E I E N P K G K D H P K L K R I . . . P E V F D C W F E S G S M P Y A K V H Y P F S T E K 666
*SalI*RS 499 ... E A K D L L P E G F T H P G S P N G T F T K E . . . T D I M D V W F D S G S H R G V L E 540
*Pfal*RS 1005 L S F N I Y D V Y D Q I M N H D E Y N I R S M E N K D N I L L K N I L Q K K K I F D T S K M Y K W I N K Q I Q K G Q K K Y Y S F Q W N S I K D I V F S K N N K K 1085

*HaeI*RS 546 R E F E D A F P A D F I A E G I D Q T R G W F Y T L L V L A T 576
*SceI*RS 548 E K F D E R V P A N F I S E G L D Q T R G W F Y T L A V L G T 578
*PfiI*RS 729 N D F H K I F P A D F I A E G L D Q T R G W F Y T L L V I S T 759
*PvuI*RS 667 E N F H K I F P A D F I A E G L D Q T R G W F Y T L L V I S T 667
*SalI*RS 541 T R E L S F A D M Y L E G S D Y R G W F N S S I T T S V 571
*Pfal*RS 1086 E F F K K H F N I F L C C E G I D G I R G W F Q S F F V F F C L N W I N Q R K K T R S Q L L K N T D K I I D K G Q V V I K R E Q I K N N I K H Y T L N N N N N 1186

*HaeI*RS 577 A L F G Q P P F K N V I V N G L V L A S D G Q K M S R K K N Y P D V S I I 615
*SceI*RS 579 H L F G S V P Y K N V I V S G I V L A A D G R K M S K L K N Y P D P S I V L 617
*PfiI*RS 760 L L F N K A P F K N L I C N G L V L A S D G K K M S R L K N Y P D P L Y I L 798
*PvuI*RS 698 L L F D R A P F K N L I C N G L V L A S D G K K M S R L K N Y P D P L Y I L 736
*SalI*RS 572 A T R G V S P Y K F L L S H G F V M D G E G K K M S K S L G N V I V P D Q V V 610
*Pfal*RS 1167 N D N N N N N N S I F S D D S L C F N N T S S I E T N S K R L P S H S Y L P I K N V I V H N Y V V D S N N I K M S K S L N V I S R E L F F E K E K D D I P T 1247

*HaeI*RS 616 Q K Y G A D A L R L Y L I N S P V V R A E N L R F 640
*SceI*RS 618 N K Y G A D A L R L Y L I N S P V L K A E S L K F 642
*PfiI*RS 799 N K Y G A D S L R L Y L I N S V A V R A E N L K F 823
*PvuI*RS 737 D K Y G A D S L R L Y L I N S V A V R A E N L K F 761
*SalI*RS 611 K K G A D I A R L W V S S T D Y L A D V . . . R I 633
*Pfal*RS 1248 T S K R K D T Q K P D D L N K N T D I N N K G N I N N V N N N N D I L M K T G K Q I D L I K K N K N K D L N K R F N A D I V R L W V C C Y N F V - N R N I S I 1327

*HaeI*RS 641 K E E G V R D V L K D V L L P W Y N A Y R F L I Q N V L R L Q K E E E I E F L Y N E N T V R E S P . N I T D R W I L S F M Q S L I G F F E T E M A A Y R L Y T V V 720
*SceI*RS 643 K E E G V K E V V S K V L L P W W N S F K F L D G Q I A L L K K M S N I D F Q Y D D S V . . K S D . N V M D R W I L A S M Q S L V Q I I H E M G Q Y K L Y T V V 720
*PfiI*RS 824 Q E K G V N E I V K S F I L P Y Y H S F R F F S Q E V T R Y E T T N K M Q F L F N T D Y I Y K N D . N I M D Q W I F S S L Q S L I N S V H T E M K A Y K L Y N V L 903
*PvuI*RS 762 Q E K G V N E V V K S F I L P F Y H S F R F F S Q E V T R Y E C L K K K K F L Q D E V I Y K N D . N I M D K W I F S S V M L T K L V H L E M Q A Y K L Y N V L 841
*SalI*RS 634 S D E I L K Q T . S D D Y R K I R N T L R F M L G N I N D F N P D T D S I P E S E L L E V D R Y L L N R L R E F T A S T I N N Y E N F D Y L N I Y 705
*Pfal*RS 1328 S Y E I L E N I N K Y I X L K L Y N T F K F I M N N I Y D L N F D G T K D N M K I K W D N I Q M I D K F I L Y K K D N L I K H C T K A K N F Q L Q L L I 1404

*HaeI*RS 721 P R L V K F V D . I L T N W Y V R M N R R L K G E N G M E D C V M A L . E T L F S V L L S L C R L M A P Y T P F L T E L M Y Q N L K V L I D P V S 792
*SceI*RS 721 P K L L N F I D . E L T N W Y I R F N R R L K G E N G V E D C L K A L . N S L F D A L F T F V R A M A P F T P F L S E S I Y L R L K E Y I P E A V L 793
*PfiI*RS 904 P K L L N F I E . N L T N W Y I R L N R D R M R G M L G K E N T H Q S L . N V L C R T L Y L F T I I M A P F T P F I S E Y I Y Q F L K N I K Y T N N Q N M E H N E 982
*PvuI*RS 842 P K L L Q F I E . N L T N W Y I R L N R D R M R G S L G E E D C L R A L . C T Y T R T L H L F T V L M A P F T P F I T E Y I Y Q L R R V V S G G T A V S G A I . 919
*SalI*RS 706 Q E V Q N F I N V E L S N F Y L D Y G K D I L Y I E Q R D S H I R R S M Q T V L Y Q I L V D M T K L L A P I L V H T A E E W S H T P H V 774
*Pfal*RS 1405 K Y I M N F I Y . T D L A I Y I D Y S K D R L Y I H E K N S L N R T N Q Q I L Y K I L R D L I I L L G P I V P H L S E D I Y N L Q L L K N K T K R K Y M T T H 1484

*HaeI*RS 793 V Q D K D T L S I H Y L M L P R V R E . . . E L I D - K K T E S A V S Q M Q S V I E L G R V I R D R K T I P I K 844
*SceI*RS 794 A K Y G K D G R S V H F L S Y P V V K K . . . E Y F D - E A I E T A V S R M Q S V I D L G R N I R E K K T I S L K 846
*PfiI*RS 983 N N E T T N I K E E D L N R N D I H K S V H F I M L P Q V D D . . K Y I I K Y E F I E L I E H M K D V I L L G R N L R E K R K T P N K 1047
*PvuI*RS 920 S G V T D G G A A N G G V S H Q S V H F R M L P Q V D D . . H Y S I D Y G I E L I E K M K T V I L L G R V L R E R R K V A S K 981
*SalI*RS 775 K E E S V H L A D M P K V V E V D Q A L D K W R T F M N L R D D V N R A E T A R N E K V I G K S L E A K V T I A S N 834
*Pfal*RS 1485 N N N N N N I S I N E G K I K S L F L R P F P K F K N Y K Q V N L D T L F L I K Y I H K Q I S P Y F S N S L Q A I V Y I F S N 1548

*HaeI*RS 845 - Y P L K E I V V I H Q D P E A L K D I K S L E K Y . I I E E L N V R K V T L S T D K N K Y G I R L R A E P D H M V L G K R L K G A F . . K A V M T S I K 917
*SceI*RS 847 - T P L K T L V I L H S D E S Y L K D V E A L K N Y . I I E E L N V R D V V I T S D E A K Y G V E Y K A V A D W P V L G K K L K D A . . K K V K D A L P 919
*PfiI*RS 1048 - K P L K S L T I L H K N E S F F K H F D R I S N Y . I K E E L N I L N V E Y S N D I S . . C L N F S A I P N F K K L G V K L G Y N I . . K N I Q N K I K 1118
*PvuI*RS 982 - K P L K R I T I L H P S K E Y F Q N F E Q I V L Y . I K E E L N V L H V D C S D D T S . . C V D F S A V P N Y K T L G V K L G A D L . . K K V Q N K I K 1052
*SalI*RS 835 - D K F N A S E F L T S F D A L H Q L F I V S V K V V D K . . L D D Q A C W N Y S E D L G A V D E L T H L C P R C Q Q V V 868
*Pfal*RS 1549 N D N I I N L I K S F L R T P D P L E Q F N N Y D D L R F L F N V S N I F I C D N I C Q V E E K D K N Y K T Y K I P L P N I E K N Q I K N K N K 1621

*HaeI*RS 918 Q L S S E E L E Q F Q K T G T I V V E G H E L H D E D I R L M Y T F D Q A T G G T A Q F E A H S D A Q A L V L L D V T P D Q S M V D E G M A R E V I N R I Q K L R 998
*SceI*RS 920 S V T S E Q V R E Y L E S G K L E V A G I E L V K G D L N A I R G L P E S A V Q A G . Q E T R T D D D V L I I M D T N I Y S E L K S L E H H H H H 992
*PfiI*RS 1119 N M N A E D I K L F Q N K Q I I I D N I L L E Q D D I I I Q M N . . . H N I Q N D N T E A I S N N Y I T I L M D F T A D Q L E N M A S A R E I C N H I Q K I R 1106
*PvuI*RS 1053 S L D S H S V R K Y A E G K I T L E G V T L E G D D I I V Q M K . . . P T F Q N E N T D I I S N D S V T L L M D F T T D Q L E N M A N A R E L C N H I Q K M R 1130
*SalI*RS 869 T . A Y E H G D I V I E H A D G E K C E R C W N Y S E D L G A V D E L T H L C P R C Q Q V V 913
*Pfal*RS 1622 N K K K N L N D F Q P N Q K P I V D H M L N F D E N I E H A I I S I G I N K T E S K R C S R C W M Y G T V Y S . . F E G E Y F C P R G L N V I 1691

*HaeI*RS 999 K K C N L V P T D E I T V Y Y K A K S E G T Y L N S V I E S H T E F I F T T I K A P L K P Y P V S P S D K V L I Q E K T Q L K G S E L I T L T R G S S L 1075
*SceI*RS
*PfiI*RS 1197 K N L S L N Q N S P V L M H I Y I Y D - Q T - F K N H M L N E N E Y I K K C L R R N L N I I D L E K D V Q N L T D K F F E E K I T V N E K E V L V I F T N Q . . . 1272
*PvuI*RS 1131 K N L S L T Q N S P V K M L V Y I A D - D A - L R T N M V S E M A Y I R K L R R E L H V L P S Q E D Y N A L A D K M H D E E I L L A G C P V R L V F A P A . . . 1206
*SalI*RS 914 K S L V 917
*Pfal*RS 1692 K S H Y N 1696

*HaeI*RS 1076 P G P A C A Y V N L N I C A N G S E Q G G V L L L E N P K G D N R L D L L K L K S V V T S I F G V K N T E L A V F H D E T E I Q N Q T D L L S L S G K T L C V T A 1156
*SceI*RS
*PfiI*RS
*PvuI*RS
*SalI*RS
*Pfal*RS

*HaeI*RS 1157 G S A P S L I N S S T L L C Q Y I N L Q L L N A K P Q E C L M G T V G T L L L E N P L G Q N G L T H Q G L L Y E A A K V F G L R S R K L K L F L N E T Q T Q E I 1237
*SceI*RS
*PfiI*RS
*PvuI*RS
*SalI*RS
*Pfal*RS

*HaeI*RS 1238 T E D I P V K T L N M K T V Y V S V L P T T A D F 1262
*SceI*RS
*PfiI*RS
*PvuI*RS
*SalI*RS
*Pfal*RS

Fig. S3. Multiple sequence alignment of IRS enzymes. HscIRS (*Homo sapiens* cytoplasmic IRS). SccIRS (*Saccharomyces cerevisiae* cytoplasmic IRS). PfcIRS (*Plasmodium falciparum* cytoplasmic IRS). PvcIRS (*Plasmodium falciparum* cytoplasmic IRS). SaIRS (*Staphylococcus aureus* IRS). PfaIRS (*Plasmodium falciparum* apicoplast IRS). ClustalX coloring with shading intensity modified by the conservation index at each position. The higher the percent conservation, the higher the intensity of the displayed color. Blue = hydrophobic residue. Red = positive charge residue. Magenta = negative charge residue. Green = polar residue. Pink = cysteine. Orange = glycine. Yellow = proline. Cyan = aromatic residue. White = unconserved. *Denotes PfcIRS mutations (E180D/Q, N269K, S288I, W395L, V500A, C502Y).

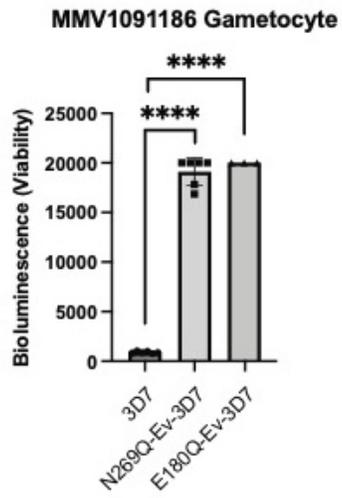
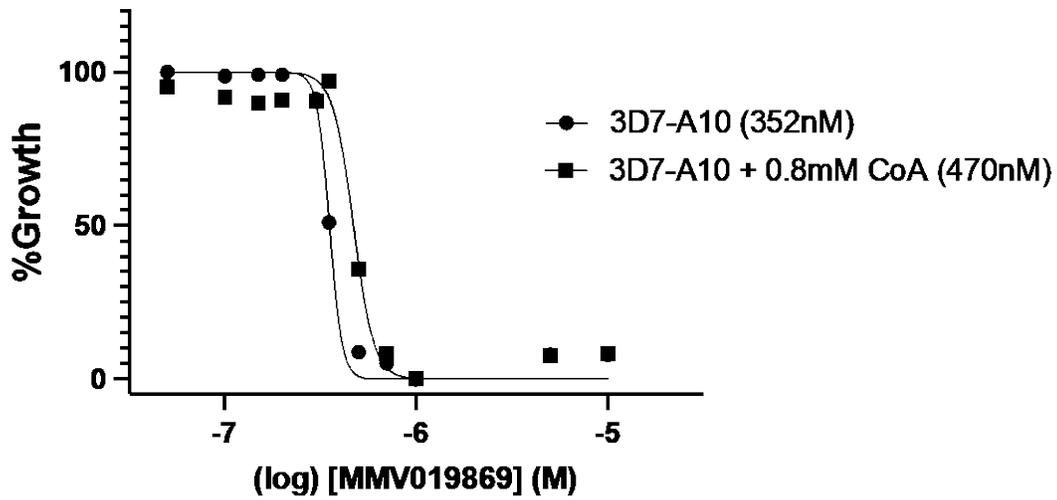


Fig. S4. Parasites are resistant to thienopyrimidines in the gametocyte stage

A



B

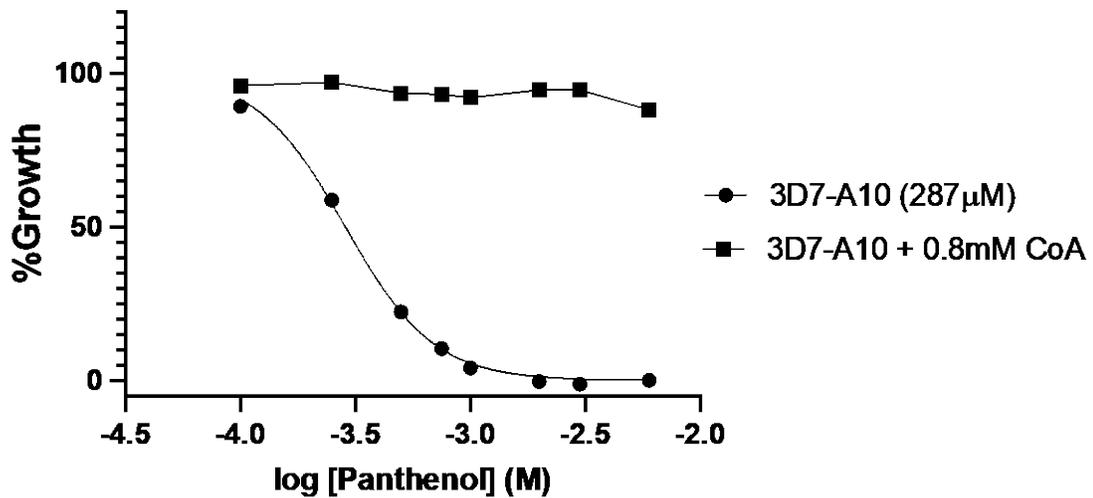


Fig. S5. Coenzyme A does not attenuate thienopyrimidine toxicity. A) Inclusion of 0.8 mM coenzyme A (square symbols) does not significantly decrease the toxicity of MMV019869 (dose response without coenzyme A in circles). B) The toxicity of the panthenol (a compound which targets pantothenate phosphorylation) was completely rescued with coenzyme A.

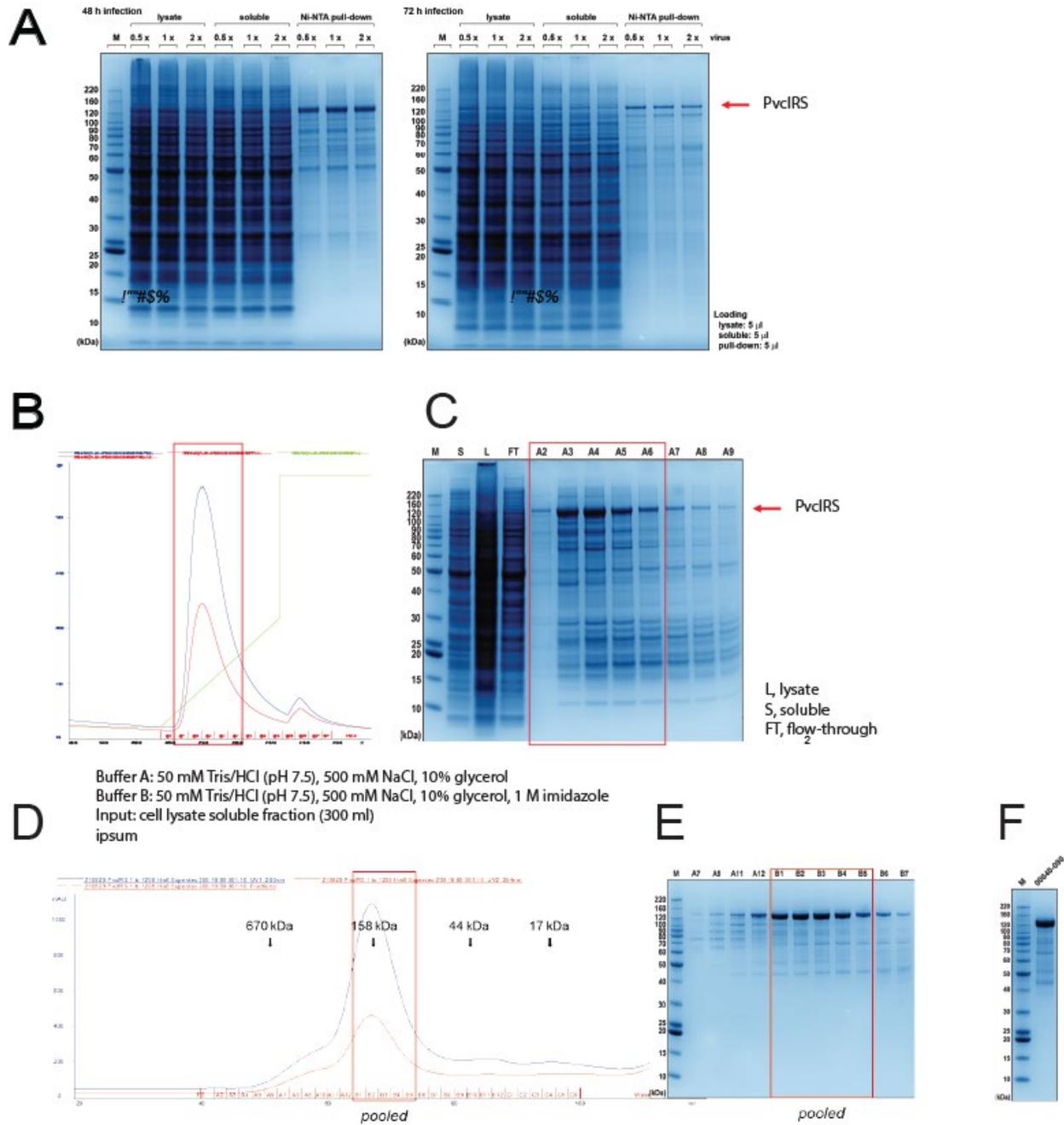
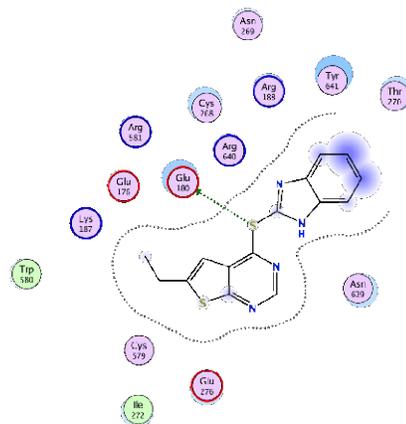
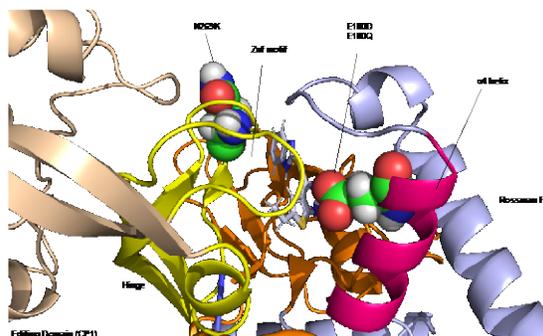


Fig. S6. Purification of recombinant PvcIRS. A) Purification of baculovirus expressed C-terminally hexa-His tagged PvcIRS over Ni NTA. 48 vs 72 h incubation shown on SDS-PAGE. B) Liquid chromatography fractionation of expressed PvcIRS. C) SDS-PAGE of collected fractions A2-6 containing PvcIRS. D) The recombinant protein is monomeric in solution as determined by size inclusion chromatography. E) Fractionation of pooled PvcIRS purifications. F) Purity of the protein used in subsequent biochemical assays.

A: MMV019869: 3.83Å from E180 to sulfide



- | | | | |
|---------------------|----------------------|---------------------|-----------------|
| ○ polar | → sidechain acceptor | ○ solvent residue | ⊗ arene-arene |
| ○ acidic | → sidechain donor | ○ metal complex | ⊗H arene-H |
| ○ basic | → backbone acceptor | ⋯ solvent contact | ⊗+ arene-cation |
| ○ greasy | → backbone donor | ⋯ metal/ion contact | |
| ○ proximity contour | ⊗ ligand exposure | ⊗ receptor exposure | |

Fig. S7. MMV019869 docked in the allosteric site adjacent to E180 mutation, Znf motif, and hinge region. Possible E180 and MMV019869 interactions depicted on the right.

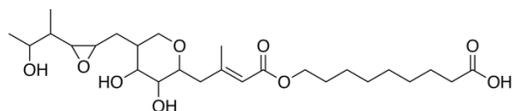


Fig. S8. Mupirocin inhibits SaIRS.