Fig. S1

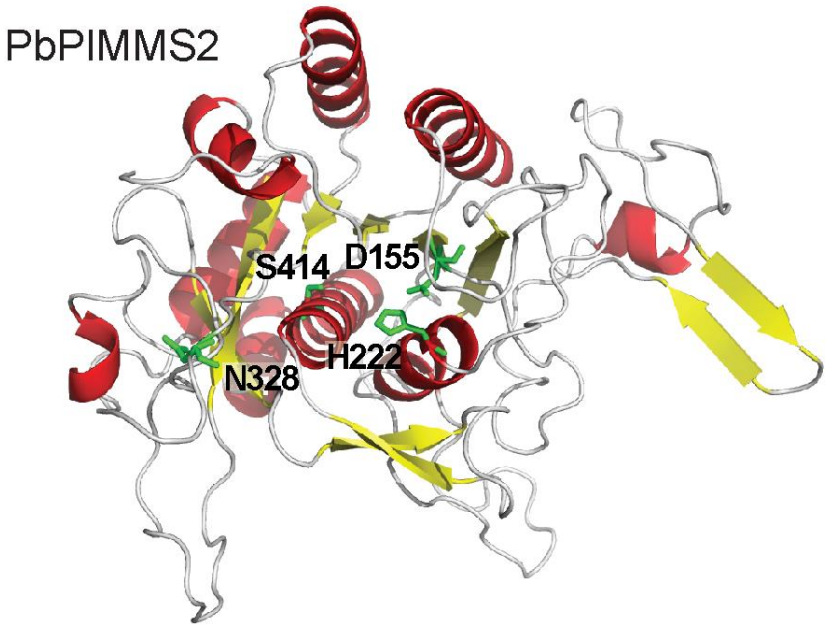


FIG S1 PIMMS2 3D homology model. A 3D homology model of the subtilisin-like domain of PbPIMMS2 based on the known structure of PvSUB1. Conserved amino acid residues of PbPIMMS2/PvSUB1 D155/316, H222/372, N328/453 and S414/538 are shown in stick form.

Fig. S2

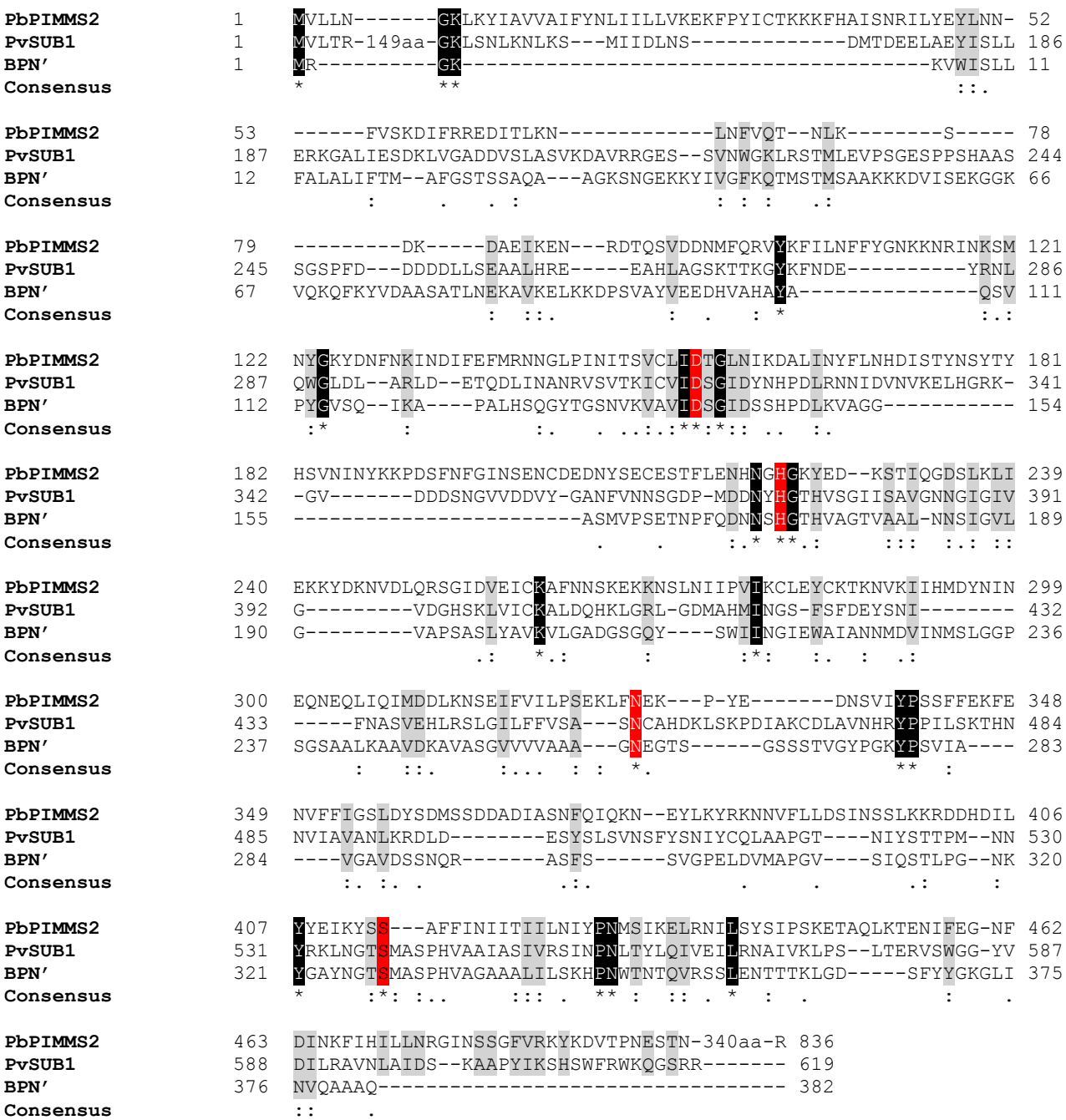
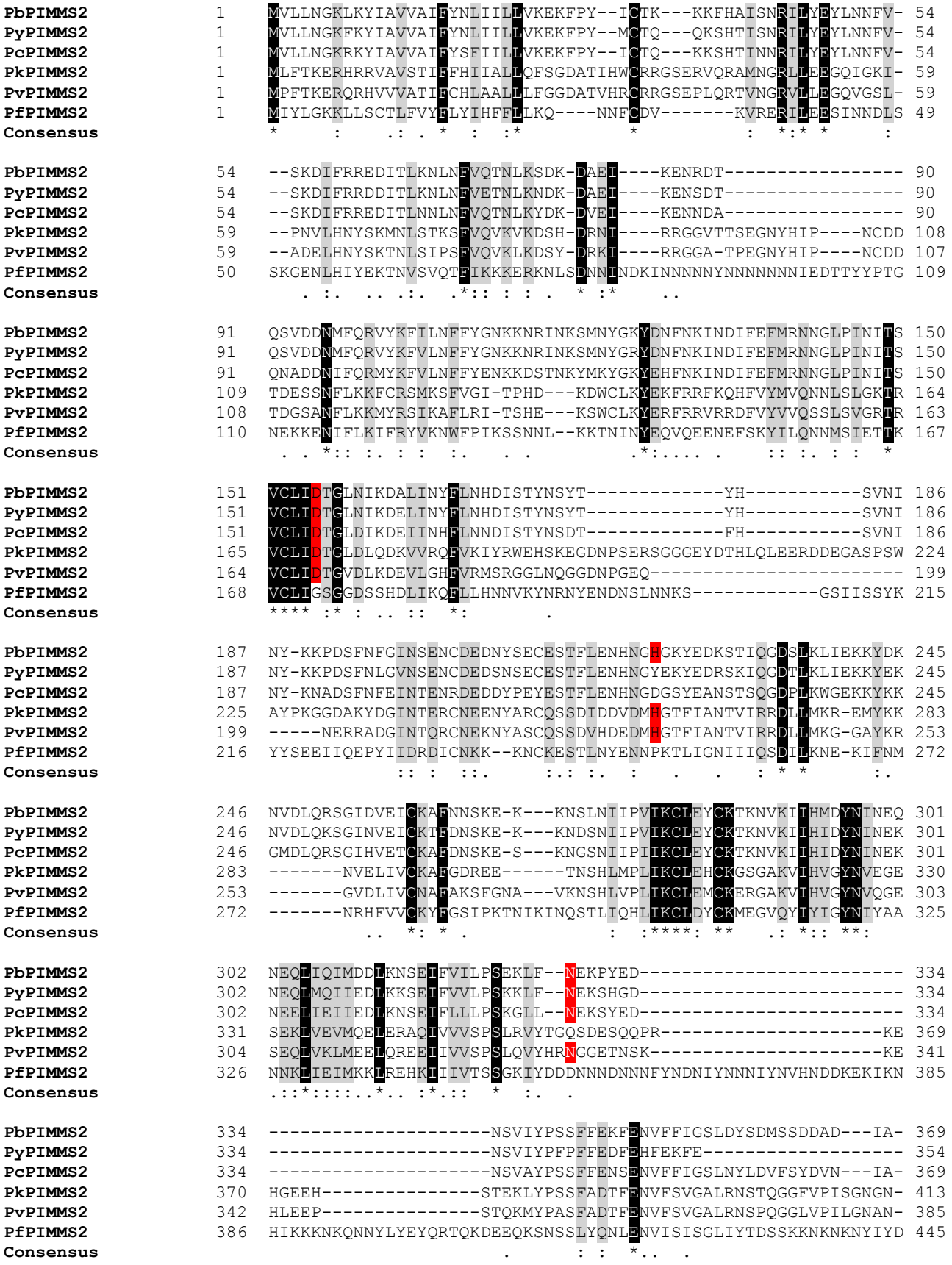
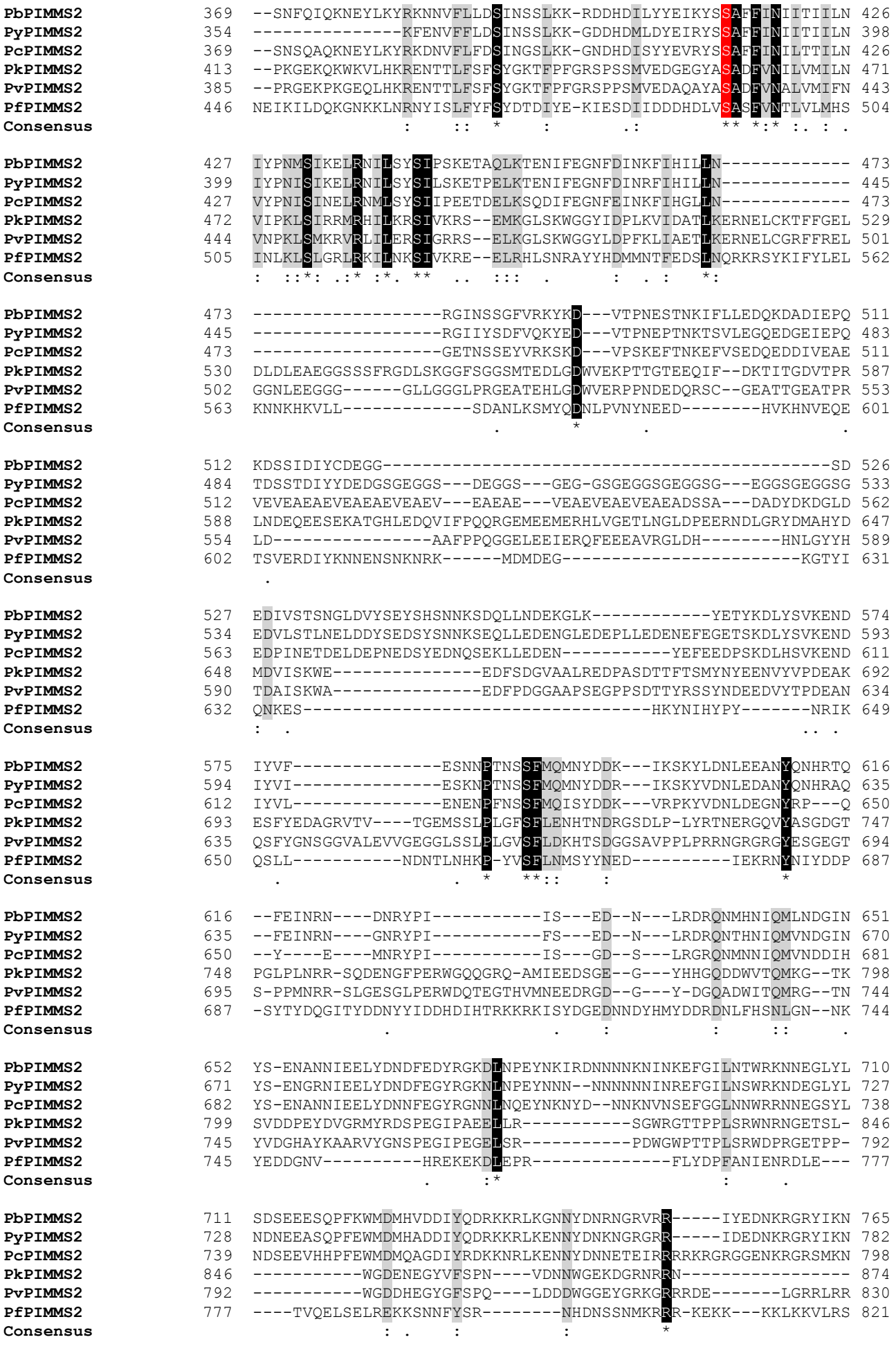


FIG S2 Multiple sequence alignment of PbPIMMS2, PvSUB1 and bacterial BPN'. The conserved amino acid residues forming the putative catalytic triad (D155, H222 and S414) and stabilizing of the oxyanion hole (N328) are marked in red. Regions with no homology are shown as number of amino acids (aa). Black and gray shaded residues indicate conservation and similarity, respectively. BPN', Uniprot: P00782 and PvSUB1, PVX_097935.

Fig. S3


PbPIMMS2 1 MVLLNGKLKYIAVVAIFYNLIILLVKEKFPY--ICTK---KKFHAISNRILYEYLNNFV- 54
PyPIMMS2 1 MVLLNGKFKYIAVVAIFYNLIILLVKEKFPY--MCTQ---QKSHTISNRILYEYLNNFV- 54
PcPIMMS2 1 MVLLNGKRKYIAVVAIFYSFIILLVKEKFPY--ICTQ---KKSHTINNRILYEYLNNFV- 54
PkPIMMS2 1 MLFTKERHRRVAVSTIFFHIIALLQFSGDATIHWCRRGSERVQRAMNGRLLEEGQIGKI- 59
PvPIMMS2 1 MPFTKERQRHVVVATIFCHLAALLLFGGDATVHRCRRGSEPLQRTVNGRVLLEGQVGSL- 59
PfPIMMS2 1 MIYLGKKLLSCTLFVYFLYIHFFLLKQ----NNFCDV-------KVRERILEESINNDLS 49
Consensus
PbPIMMS2 54 --SKDIFRREDITLKNLNFVQTNLKSDK-DAEI----KENRDT---------- 90
PyPIMMS2 54 --SKDIFRRDDITLKNLNFVETNLKNDK-DAEI----KENSDT---------- 90
PcPIMMS2 54 --SKDIFRREDITLNNLNFVQTNLKYDK-DVEI----KENNDA---------- 90
PkPIMMS2 59 --PNVLHNYSKMNLSTKSFVQVKVKDSH-DRNI----RRGGVTTSEGNYHIP----NCDD 108
PvPIMMS2 59 --ADELHNYSKTNLSIPSFVQVKLKDSY-DRKI----RRGGA-TPEGNYHIP----NCDD 107
PfPIMMS2 50 SKGENLHIYEKTNVSVQTFIKKKERKNLSDNNINDKINNNNNNYNNNNNNNIEDTTYYPTG 109
Consensus
PbPIMMS2 91 QSVDDNMFQRVYKFILNFFYGNKKNRINKSMNYGKYDNFNKINDIFEFMRNNGLPINITS 150
PyPIMMS2 91 QSVDDNMFQRVYKFVLNFFYGNKKNRINKSMNYGRYDNFNKINDIFEFMRNNGLPINITS 150
PcPIMMS2 91 QNADDNIFQRMYKFVLNFFYENKKDSTNKYMKYGKYEHFNKINDIFEFMRNNGLPINITS 150
PkPIMMS2 109 TDESSNFLKKFCRSMKSFVGI-TPHD---KDWCLKYEKFRRFKQHFVYMVQNNLSLGKTR 164
PvPIMMS2 108 TDGSANFLKKMYRSIKAFLRI-TSHE---KSWCLKYERFRRVRRDFVYVVQSSLSVGRTR 163
PfPIMMS2 110 NEKKENIFLKIFRYVKNWFPIKSSNNL--KKTNINYEQVQEENEFSKYILQNNMSIETTK 167
Consensus
PbPIMMS2 151 VCLIDTGLNIKDALINYFLNHDISTYNSYT----------YH----------SVNI 186
PyPIMMS2 151 VCLIDTGLNIKDELINYFLNHDISTYNSYT----------YH----------SVNI 186
PcPIMMS2 151 VCLIDTGLDIKDEIINHFLNNDISTYNSDT----------FH----------SVNI 186
PkPIMMS2 165 VCLIDTGLDLQDKVVRQFVKIYRWEHSKEGDNPSERSGGGEYDTHLQLEERDDEGASPSW 224
PvPIMMS2 164 VCLIDTGVDLKDEVLGHFVRMSRGGLNQGGDNPGEQ---------- 199
PfPIMMS2 168 VCLIGSGGDSSHDLIKQFLLHNNVKYNRNYENDNSLNNKS----------GSIISSYK 215
Consensus
PbPIMMS2 187 NY-KKPDSFNFGINSENCDEDNYSECESTFLENHNGHGKYEDKSTIQGDSLKLIEKKYDK 245
PyPIMMS2 187 NY-KKPDSFNLGVNSENCDEDSNSECESTFLENHNGYEKYEDRSKIQGDTLKLIEKKYEK 245
PcPIMMS2 187 NY-KNADSFNFEINTENRDEDDYPEYESTFLENHNGDGSYEANSTSQGDPLKWGEKKYKK 245
PkPIMMS2 225 AYPKGGDAKYDGINTERCNEENYARCQSSDIDDVDMHGTFIANTVIRRDLLMKR-EMYKK 283
PvPIMMS2 199 -----NERRADGINTQRCNEKNYASCQSSDVHDEDMHGTFIANTVIRRDLLMKG-GAYKR 253
PfPIMMS2 216 YYSEEIIQEPYIIDRDICNKK--KNCKESTLNYENNPKTLIGNIIIQSDILKNE-KIFNM 272
Consensus
PbPIMMS2 246 NVDLQRSGIDVEICKAFNNSKE-K---KNSLNIIPVIKCLEYCKTKNVKIIHMDYNINEQ 301
PyPIMMS2 246 NVDLQKSGINVEICKTFDNSKE-K---KNDSNIIPVIKCLEYCKTKNVKIIHIDYNINEK 301
PcPIMMS2 246 GMDLQRSGIHVETCKAFDNSKE-S---KNGSNIIPIIKCLEYCKTKNVKIIHIDYNINEK 301
PkPIMMS2 283 -------NVELIVCKAFGDREE------TNSHLMPLIKCLEHCKGSGAKVIHVGYNVEGE 330
PvPIMMS2 253 -------GVDLIVCNAFAKSFGNA---VKNSHLVPLIKCLEMCKERGAKVIHVGYNVQGE 303
PfPIMMS2 272 -------NRHFVVCKYFGSIPKTNIKINQSTLIQHLIKCLDYCKMEGVQYIYIGYNIYAA 325
Consensus
PbPIMMS2 302 NEQLIQIMDDLKNSEIFVILPSEKLF--NEKPYED---------- 334
PyPIMMS2 302 NEQLMQIIEDLKKSEIFVVLPSKKLF--NEKSHGD---------- 334
PcPIMMS2 302 NEELIEIIEDLKNSEIFLLLPSKGLL--NEKSYED---------- 334
PkPIMMS2 331 SEKLVEVMQELERAQIVVVSPSLRVYTGQSDESQQPR----------KE 369
PvPIMMS2 304 SEQLVKLMEELQREEIIVVSPSLQVYHRNGGETNSK----------KE 341
PfPIMMS2 326 NNKLIEIMKKLREHKIIIVTSSGKIYDDDNNNDNNNFYNDNIYNNNIYNVHNDDKEKIKN 385
Consensus
PbPIMMS2 334 ----------NSVIYPSSFFEKFENVFFIGSLDYSDMSSDDAD---IA- 369
PyPIMMS2 334 ----------NSVIYPFPFFEDFEHFEKFE---------- 354
PcPIMMS2 334 ----------NSVAYPSSFFENSENVFFIGSLNYLDVFSYDVN---IA- 369
PkPIMMS2 370 HGEEH----------STEKLYPSSFADTFENVFSVGALRNSTQGGFVPISGNGN- 413
PvPIMMS2 342 HLEEP----------STQKMYPASFADTFENVFSVGALRNSPQGGLVPILGNAN- 385
PfPIMMS2 386 HIKKKNKQNNYLYEYQRTQKDEEQKSNSSLYQNLENVISISGLIYTDSSKKNKNKNYIYD 445
Consensus


PbPIMMS2 369 --SNFQIQKNEYLKYRKNNVFLLDSINSSLKK-RDDHDILYYEIKYSSAFFINIITIILN 426
PyPIMMS2 354 ----------KFENVFFLDSINSSLKK-GDDHDMLDYEIRYSSAFFINIITIILN 398
PcPIMMS2 369 --SNSQAQKNEYLKYRKDNVFLFDSINGSLKK-GNDHDISYYEVRYSSAFFINILTTILN 426
PkPIMMS2 413 --PKGEKQKWKVLHKRENTTLFSFSYGKTFPFGRSPSSMVEDGEGYASADFVNILVMILN 471
PvPIMMS2 385 --PRGEKPKGEQLHKRENTTLFSFSYGKTFPFGRSPPSMVEDAQAYASADFVNALVMIFN 443
PfPIMMS2 446 NEIKILDQKGNKKLNRNYISLFYFSYDTDIYE-KIESDIIDDDHDLVSASFVNTLVLMHS 504
Consensus : :: * : .: ** *:* :. : .
PbPIMMS2 427 IYPNMSIKELRNILSYSIPSKETAQLKTENIFEGNFDINKFIHILLN---------- 473
PyPIMMS2 399 IYPNISIKELRNILSYSILSKETPELKTENIFEGNFDINRFIHILLN---------- 445
PcPIMMS2 427 VYPNISINELRNMLSYSIIPEETDELKSQDIFEGNFEINKFIHGLLN---------- 473
PkPIMMS2 472 VIPKLSIRRMRHILKRSIVKRS--EMKGLSKWGGYIDPLKVIDATLKERNELCKTFFGEL 529
PvPIMMS2 444 VNPKLSMKRVRLILERSIGRRS--ELKGLSKWGGYLDPFKLIAETLKERNELCGRFFREL 501
PfPIMMS2 505 INLKLSLGRLRKILNKSIVKRE--ELRHLSNRAYYHDMMNTFEDSLNQRKRSYKIFYLEL 562
Consensus : ::*: .:* :*. ** .. ::: . : . : *:
PbPIMMS2 473 ----------RGINSSGFVRKYKD---VTPNESTNKIFLLEDQKDADIEPQ 511
PyPIMMS2 445 ----------RGIIYSDFVQKYED---VTPNEPTNKTSVLEGQEDGEIEPQ 483
PcPIMMS2 473 ----------GETNSSEYVRKSKD---VPSKEFTNKEFVSEDQEDDIVEAE 511
PkPIMMS2 530 DLDLEAEGGSSSFRGDLSKGGFSGGSMTEDLGDWVEKPTTGTEEQIF--DKTITGDVTPR 587
PvPIMMS2 502 GGNLEEGGG------GLLGGGLPRGEATEHLGDWVERPPNDEDQRSC--GEATTGEATPR 553
PfPIMMS2 563 KNNKHKVLL----------SDANLKSMYQDNLPVNYNEED--------HVKHNVEQE 601
Consensus . * . .
PbPIMMS2 512 KDSSIDIYCDEGG--SD 526
PyPIMMS2 484 TDSSTDIYYDEDGSGEGGS---DEGGS---GEG-GSGEGGSGEGGSG---EGGSGEGGSG 533
PcPIMMS2 512 VEVEAEAEVEAEAEVEAEV---EAEAE---VEAEVEAEVEAEADSSA---DADYDKDGLD 562
PkPIMMS2 588 LNDEQEESEKATGHLEDQVIFPQQRGEMEEMERHLVGETLNGLDPEERNDLGRYDMAHYD 647
PvPIMMS2 554 LD----------AAFPPQGGELEEIERQFEEEAVRGLDH--------HNLGYYH 589
PfPIMMS2 602 TSVERDIYKNNENSNKNRK------MDMDEG----------KGTYI 631
Consensus .
PbPIMMS2 527 EDIVSTSNGLDVYSEYSHSNNKSDQLLNDEKGLK----------YETYKDLYSVKEND 574
PyPIMMS2 534 EDVLSTLNELDDYSEDSYSNNKSEQLLEDENGLEDEPLLEDENEFEGETSKDLYSVKEND 593
PcPIMMS2 563 EDPINETDELDEPNEDSYEDNQSEKLLEDEN----------YEFEEDPSKDLHSVKEND 611
PkPIMMS2 648 MDVISKWE----------EDFSDGVAALREDPASDTTFTSMYNYEENVYVPDEAK 692
PvPIMMS2 590 TDAISKWA----------EDFPDGGAAPSEGPPSDTTYRSSYNDEEDVYTPDEAN 634
PfPIMMS2 632 QNKES----------HKYNIHYPY-------NRIK 649
Consensus : . .. .
PbPIMMS2 575 IYVF----------ESNNPTNSSFMQMNYDDK---IKSKYLDNLEEANYQNHRTQ 616
PyPIMMS2 594 IYVI----------ESKNPTNSSFMQMNYDDR---IKSKYVDNLEDANYQNHRAQ 635
PcPIMMS2 612 IYVL----------ENENPFNSSFMQISYDDK---VRPKYVDNLDEGNYRP---Q 650
PkPIMMS2 693 ESFYEDAGRVTV----TGEMSSLPLGFSFLENHTNDRGSDLP-LYRTNERGQVYASGDGT 747
PvPIMMS2 635 QSFYGNSGGVALEVVGEGGLSSLPLGVSFLDKHTSDGGSAVPPLPRRNGRGRGYESGEGT 694
PfPIMMS2 650 QSLL----------NDNTLNHKP-YVSFLNMSYYNED----------IEKRNYNIYDDP 687
Consensus . . * **:: : *
PbPIMMS2 616 --FEINRN----DNRYPI----------IS---ED--N---LRDRQNMHNIQMLNDGIN 651
PyPIMMS2 635 --FEINRN----GNRYPI----------FS---ED--N---LRDRQNTHNIQMVNDGIN 670
PcPIMMS2 650 --Y----E----MNRYPI----------IS---GD--S---LRGRQNMNNIQMVNDDIH 681
PkPIMMS2 748 PGLPLNRR-SQDENGFPERWGQQGRQ-AMIEEDSGE--G---YHHGQDDWVTQMKG--TK 798
PvPIMMS2 695 S-PPMNRR-SLGESGLPERWDQTEGTHVMNEEDRGD--G---Y-DGQADWITQMRG--TN 744
PfPIMMS2 687 -SYTYDQGITYDDNYYIDDHDIHTRKKRKISYDGEDNNDYHMYDDRDNLFHSNLGN--NK 744
Consensus . . : : :: .
PbPIMMS2 652 YS-ENANNIEELYDNDFEDYRGKDLNPEYNKIRDNNNNKNINKEFGILNTWRKNNEGLYL 710
PyPIMMS2 671 YS-ENGRNIEELYDNDFEGYRGKNLNPEYNNN--NNNNNNINREFGILNSWRKNDEGLYL 727
PcPIMMS2 682 YS-ENANNIEELYDNNFEGYRGNNLNQEYNKNYD--NNKNVNSEFGGLNNWRRNNEGSYL 738
PkPIMMS2 799 SVDDPEYDVGRMYRDSPEGIPAEELLR----------SGWRGTTPPLSRWNRNGETSL- 846
PvPIMMS2 745 YVDGHAYKAARVYGNSPEGIPEGELSR----------PDWGWPTTPLSRWDPRGETPP- 792
PfPIMMS2 745 YEDDGNV----------HREKEKDLEPR----------FLYDPFANIENRDLE--- 777
Consensus . :* : .
PbPIMMS2 711 SDSEEESQPFKWMDMHVDDIYQDRKKRLKGNNYDNRNGRVRR-----IYEDNKRGRYIKN 765
PyPIMMS2 728 NDNEEASQPFEWMDMHADDIYQDRKKRLKENNYDNKNGRGRR-----IDEDNKRGRYIKN 782
PcPIMMS2 739 NDSEEVHHPFEWMDMQAGDIYRDKKNRLKENNYDNNETEIRRRRKRGRGGENKRGRSMKN 798
PkPIMMS2 846 ----------WGDENEGYVFSPN----VDNNWGEKDGRNRRN---------- 874
PvPIMMS2 792 ----------WGDDHEGYGFSPQ----LDDDWGGEYGRKGRRRDE-------LGRRLRR 830
PfPIMMS2 777 ----TVQELSELREKKSNNFYSR--------NHDNSSNMKRRR-KEKK---KKLKKVLRS 821
Consensus : . : : *

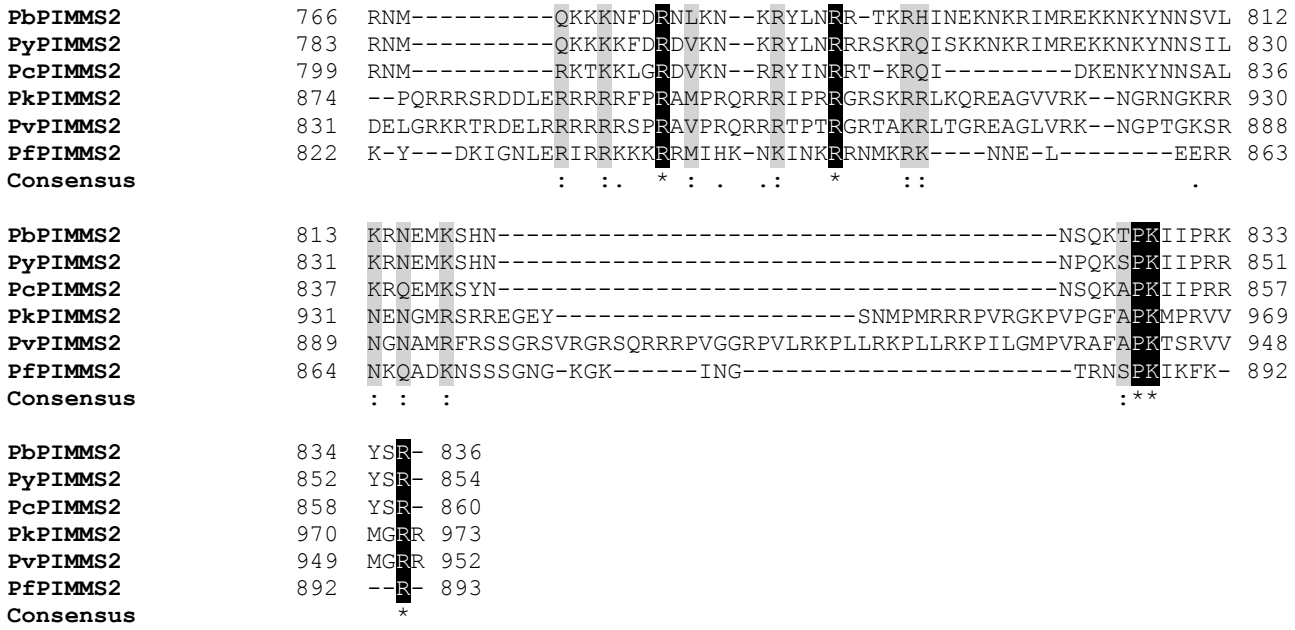


FIG S3 Multiple sequence alignment of *Plasmodium* PIMMS2 orthologs. Sequence alignment of PIMMS2 orthologs in six *Plasmodium* species: *P. berghei* (PbPIMMS2; PBANKA_1106900), *P. yoelii* (PyPIMMS2; PYYM_1109100), *P. chabaudi* (PcPIMMS2; PCHAS_1106600), *P. knowlesi* (PkPIMMS2; PKNH_1026300), *P. vivax* (PvPIMMS2; PVX_097925) and *P. falciparum* (PfPIMMS2; PF3D7_0507300). The residues forming the putative catalytic triad (Asp, His and Ser) and the oxyanion hole-stabilizing residue (Asn) are highlighted in red**.** Black and gray highlighted residues indicate identical and conserved amino acids, respectively.

Fig. S4

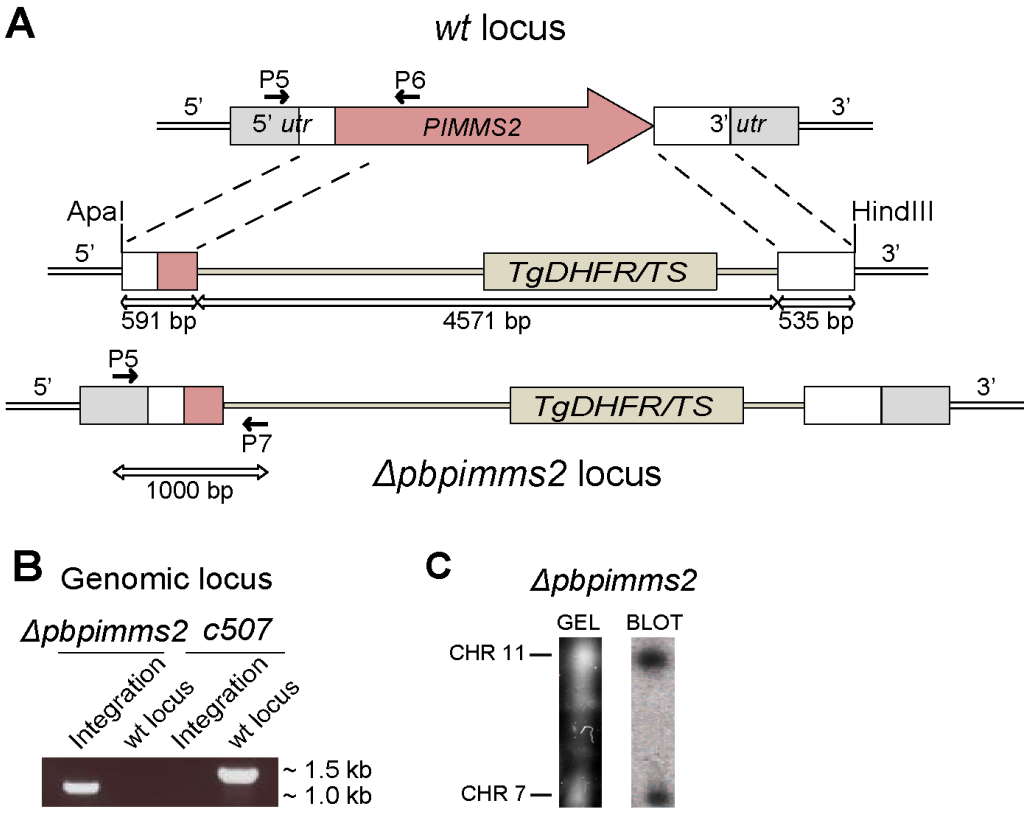


FIG S4 Generation and genotypic analysis of *ΔpbPIMMS2* mutant parasites. (A) Schematic representation of the native *PIMMS2* (*wt*) and the modified *Δpbpimms2* genetic loci. The disruption vector carries gene targeting sequences for *PIMMS2,* which flank the pyrimethamine based selectable marker *TgDHFR/TS*. The pBS-TgDHFR/TS gene disruption vector is presented in the middle. Integration of the ApaI/BamHI linearized vector results in replacement of *PIMMS2*. (B) Genotyping of *Δpbpimms2* mutant parasites by PCR based analysis of the genomic DNA from *c507* and cloned parasites show that the *Δpbpimms2* locus is only present in the knockout line. (C) Southern blot analysis on pulse field electrophoresis separated chromosomes from cloned *Δpbpimms2* parasites. The *dhfr/ts* cassette is detected at chromosome 11 corroborating integration of the disruption vector.

Table S1. Effect of *PIMMS2* knockout on oocyst development in *A. gambiae* and *A. stephensi*

Time	Parasite	Number of exp	Number of midguts	Prevalence (%)	Infection intensity		Infection range	P-value	Fold difference
					Arithmetic mean	Median			
Day 10	*A. gambiae* infections								
	wt	4	102	89.2	61.5	38	0-368		
	Δpbpimms2	4	117	82	5.8	3	0-38	<0.0001	-11 (-13)
Day 10	*A. stephensi* infections								
	wt	2	39	100	495.4	503	14-902		
	Δpbpimms2	2	60	98	101	93	0-281	<0.0001	-5 (-5)
Day 10	*A. gambiae L3-5* infections								
	wt	2	31	100	30.2	23	0-91		
	Δpbpimms2	2	42	57	5.2	2	0-29	<0.0001	-6 (-12)

The table reports results from *Δpbpimms2* or control *c507* (*wt*) parasite infections of *A.gambiae*. *Δpbpimms2* or *wt* infected midguts from independent biological replicates (indicated in the third column) were pooled for each group. The total number of midguts is indicated in the fourth column. Midguts with zero parasites were also considered for calculation of the arithmetic means of parasite densities. Prevalence shows the percentage of midguts with at least one oocyst. *P* values were calculated using the Mann-Whitney *U*-test. Fold differences between *Δpbpimms2* and *wt* oocyst densities were computed using both the arithmetic mean and the median (in parenthesis). In L3-5 infections, the density data refers to the number of melanised ookinetes.

Table S2. Effect of *PIMMS2* disruption on sporozoites development and mosquito-to-mouse transmission

Parasite	MG sporozoites		SG sporozoites		MG/SG	Infectivity to mice	
	Mean	SE	Mean	SE		Day 18	Day 21
A. stephensi							
wt	71,133	5,452	7,660	2,548	9.3	2/2	2/2
	57,612		16,930		3.4	2/2	2/2
	48,120		7,476		6.4	2/2	2/2
Δpbpimms2	8,854	570	2,057	389	4.3	2/2	2/2
	10,180		3,650		2.8	1/2	2/2
	11,270		3,229		3.5	2/2	2/2
A. gambiae							
wt	11,225	2,929	1,695	508	6.6	1/2	2/2
	23,216		1,897		12.2	1/2	2/2
	14,400		3,656		3.9	1/2	2/2
Δpbpimms2	1,800	340	325	190	5.5	0/2	0/2
	3,220		517		6.2	1/2	1/2
	2,728		1,100		2.5	1/2	1/2

The table outlines mean of *Δpbpimms2* or *wt* sporozoite densities in *A. stephensi* and *A. gambiae* midguts (MG) and salivary glands (SG). The mean was calculated by quantifying the number of sporozoites in suspensions from three pools of ten homogenised midguts or salivary glands, respectively, at day 21 post infection. SE represents standard error. The ratios of midgut to salivary gland sporozoites are shown. Infectivity to mice was assayed by allowing *Δpbpimms2* or *wt* infected mosquitoes to feed on two C57BL/6 mice (bite-back) at day 18 and 21 of infection, respectively. Mice were allowed to recover and parasitaemia was assessed at day 5 post-feeding and up to day 14 if infection was not detected.

Table S3. *PIMMS2* genetic complementation analysis

Experiment series	Parasite	Number of exp	Number of midguts	Prevalence (%)	Infection density		Parasite range	*P*-value
					Arithmetic mean	Median		
1 (Fig. 4D)	*Δpbpimms2*::*pimms2*^{wt}	2	50	82	26.2	14	0-146	
	Δpbpimms2	2	102	79	6.3	3	0-120	<0.0001
	wt	2	59	90	16.1	11	0-58	0.2409
2 (Fig. 4F)	*Δpbpimms2*::*pimms2*^{mut}	4	108	63	14	5	0-142	
	Δpbpimms2	4	127	71	8	3	0-120	0.0172
	Δpbpimms2::*pimms2*^{wt}	4	97	75	27	10	0-199	0.0031
	wt	4	89	73	40	13	0-253	0.0007

The table reports results of genetic complementation experiments reported in Figures 4D and 4F, respectively. For each experiment series, infected *A. gambiae* midguts from independent biological replicates (2 and 4, respectively) were pooled for each group. The numbers of midguts in each replicated are shown in the fourth column. Midguts with zero parasites were also considered for calculation of the arithmetic mean and median of infection intensities. The prevalence shows the percentage of midguts with at least one oocyst. *P* values were calculated using the Mann-Whitney *U*-test and presented for comparisons between *Δpbpimms2*::*pimms2*^{wt} and *Δpbpimms2 or wt* for the first series of infections and between *Δpbpimms2*::*pimms2*^{mut} and *Δpbpimms2* or *Δpbpimms2*::*pimms2*^{wt} or *wt* for the second series of infections. The *P* value of the comparison between *wt* and *Δpbpimms2*::*pimms2*^{wt} is 0.3474.

Table S4. Primers for RT-PCR, generation of *transgenic* parasites and protein expression.

Primer name	Sequence (5′ to 3′)	Description
PbPIMMS2 RT-PCR F	GACAATCCAAGGCGATTCAT	
PbPIMMS2 RT-PCR R	GCAATATCAGCGTCATCAGAA	
P28 RT-PCR F	AATGCACAGGTACAGGAGAAACTAAAT	
P28 RT-PCR R	CACACTCATAATGTTTTCCAGTCAATT	
AMA1 RT-PCR F	TATGGGTCCAAGATATTGTAGTAATAA	
AMA1 RT-PCR R	GAATTAGCTTTACCATAAATATCTGC	
CHT1 RT-PCR F	GCCCGCCCAGATGTAATTATA	
CHT1 RT-PCR R	TGCCAAATTCCTACACCATCG	
PbPIMMS2 a (P1)	TT-GGGCCC-CGTTGTAAAATTGTCCAAACAAA	Disruption upstream target *ApaI*
PbPIMMS2 b (P2)	CC-AAGCTT-ACACCCTTTGAAACATATTATCATCA	Disruption upstream target *HindIII*
*PbPIMMS2*c (P3)	T-GAATTC-ACTTTAAGATGGGGTTTAATTCAAAGA	Disruption downstream target *EcoRI*
*PbPIMMS2*d (P4)	TT-GGATCC-GTTTTCCGAACTAGGAAATTCATTAT	Disruption downstream target *BamHI*
PbPIMMS2 INT F (P5)	AGCGTCTAGTAGTTTGAGCTAGCTA	194 bp upstream of *PIMMS2*
PbPIMMS2 WT R (P6)	GCAATATCAGCGTCATCAGAA	802 bp downstream of *PIMMS2*
TgDHFR 5'UTR R (P7)	GATGTGTTATGTGATTAATTCATACAC	200 bp into the 5'UTR of *TgDHFR-TS*
PbPIMMS2 e (P8)	CC-AAGCTT-CGTTGTAAAATTGTCCAAACAAA	
PbPIMMS2 f (P9)	GG-CCGCGG-TTATCTAGAATATTTTCTTGGAATTA	
PbPIMMS2 g (P10)	TT-CTCGAG-ACTTTAAGATGGGGTTTAATTCAAAGA	
PbPIMMS2 h (P11)	TT-CCCGGG-GAAATTGTGTATTGTGTGAAAAATATA	
SUBO complement INT R (P12)	GAGGTTATATTAATTGGTAAACCATTG	
PbPIMMS2 D155A F (P13)	AACCTCAGTATGCTTAATAGCGACTGGATTGAATATTAAGGAT	
PbPIMMS2 H222A F (P14)	GAGAATCATAATGGGGCTGGAAAATATGAAGAT	
PbPIMMS2 S414A F (P15)	TATTATGAAATTAAATACAGTGCTGCATTTTTTATAAATATAA	
PbPIMMS2^{opt}CD F (P16)	GACAAGCTTGCGGCCGCACCCATCAACATCACCTCCGTGTG	
PbPIMMS2^{opt}CD R (P17)	TGCTCGAGTGCGGCCGCGATACCGCGGTTCAGCAGGATGTG	

Where appropriate, target restriction sites are shown as underlined italics and restriction site overhangs are also shown. The appropriate restriction enzyme is presented in the description column. F, forward; R, reverse. All primers are listed in a 5′ to 3′ direction.