

Supplementary Figures

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PfPIMMS01      M---KLLNNRFVVLCPPIIILFFFLNSVVLGNNNRNNINFHETENAAKAMRKLLSGEINSI 57
PbPIMMS01      MMHFPKYSYTLICIILIFALLF----TIASSYNNEDNKNFNGTENAATAKAIKLLNGEIDTI 56
PyPIMMS01      M-YFPKYSYTLICIILISLLF----TIASSYNNENGNKNFNETENAAKAIKLLNGEIDTI 55
PcPIMMS01      MARFPKYSYILCIILIFALLF----TTAFSYNNGDNKNFNETENAAKAIKLLNGEIDTI 56
PkPIMMS01      M-----YFLHNIICPVRSYCYTGRKEHEQSRICETENAAAIRKLLNGEIDTI 48
PvPIMMS01      M---KLLSASPCTLYIT-LLFVLLVHVTVGGKHMNNQDFAETEQAARAIKLLNGEIDTI 56
Consensus          :::          . :. : : **:**:*:****.***:*

PfPIMMS01      KLDNGDELKIKLNDEKHKDSTKWD--KSYSFISNLLEEKYSQTDLFRKKQEINEANTKII 115
PbPIMMS01      KLENGDELKTKSSNIQGD-----YSWKNNRRL----- 85
PyPIMMS01      KLENGDELKIKSSNIQED-----YSWKNNRQL----- 84
PcPIMMS01      KLENGDELKIRSDSSIQDD-----YSWQNNRRL----- 85
PkPIMMS01      KLENGNELKIRPRDEKYDAHKRYNDETNYSFIDNSEEEN-VQTDLLRKN-ESVEVGRKI- 105
PvPIMMS01      KLENGNKLKIRSDSEKDDAHKRHNDKTNYSFIDNSEEEN-VQTNLLRKN-ETVDAGSKIV 114
Consensus          **:***:** : ..          **: .* .

PfPIMMS01      EDRQEFYILNNDIEIENIATRFVLENNFDELYIQSFKQSLIDI IQSLNN 163
PbPIMMS01      ----- 85
PyPIMMS01      ----- 84
PcPIMMS01      ----- 85
PkPIMMS01      DEQENFHTLDNESTESHKQISHGNKFHESETEAFAQNLKDIIRSLNN 153
PvPIMMS01      EDQEDFYILDNESIEAIANKISMENKFHEFEAEFAQSLKDIIRSLNN 162
Consensus

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Figure S1. Multiple sequence alignment of *Plasmodium* PIMMS01 orthologues

Sequence alignment of PIMMS01 orthologs in six *Plasmodium* species: *P. falciparum* (PfPIMMS01; PF3D7_0112100), *P. berghei* (PbPIMMS01; PBANKA_0201700), *P. yoelii* (PyPIMMS01; PYYM_0204600), *P. chabaudi* (PcPIMMS01; PCHAS_0200100), *P. knowlesi* (PkPIMMS01; PKNH_0201500) and *P. vivax* (PvPIMMS01; PVX_081200). In the consensus sequence, dots and colons mark conserved amino acid residues with weakly and strongly similar properties, respectively, and asterisks mark amino acid residues that are identical between orthologues. Predicted signal peptides are highlighted in grey.

PfPIIMMS57 M-GKCDFFIFL-PIFFFVLIVYVHVVLGQPSDVPNVRDLIKRFENSNEVSNNSNNIFNRRN 58
PbPIIMMS57 MTRNLFFFIYY-HAVIFMIFWKFNGKGGDEQKRNVKDLIRRYESINNSN----- 49
PyPIIMMS57 MKRNLFFFIYY-HAAIFMVFWKFNGKGGNEKKNVKDLIRRYESINNSNDLNNSSSSSS 59
PcPIIMMS57 MKRNLFFFIYC-HAAICIVFWKFNGHNGNEKKNVKDLIRRYETINNPDLNNSSSTSA 59
PkPIIMMS57 MLPKCILSIYIISCVLLLLLWIKIGDVNGQLPRRGVNEELIRRFENQSTEDRQQRDSNEN- 59
PvPIIMMS57 MVPKCIFSIYATFCALLLLSWNIGDVKGQPPKRGVRNDLIKWFHGGSEDTQRDSNEN- 59
Consensus : : * : : : * . . . * : : * .

PfPIIMMS57 NQLERNIHYDPNNLYNIRQN--PRHENIAGRSYVSRNPNEFSSD-----IMIN 104
PbPIIMMS57 ---DSNYE-DRDNAPNRRQNV-AFDDQKPSTSYANRNDKQYGRMMKKNANNDGNNMAN 104
PyPIIMMS57 DLTDNYE-DRDNAPNRRQNV-AFDDQPPSTSYANRNNNGQYGRMMKGNANNDGNNMIN 117
PcPIIMMS57 ESTGAPYE-NRDP I PYRGQNA-PIDDDQPPSTSYANRSNESQYMRMDMPN----- 106
PkPIIMMS57 KSFESGSQ-GIEHPLNKGQNTTIYEEPPQPGQSYEPARRS-SSF-----PTQKSSLN 108
PvPIIMMS57 KSFQSGSQ-GWEQPLNRGQNTSIYDEQQPGTSGYGPVRRSSASL-----PTEKRSFS 109
Consensus . . : ** . : . **

PfPIIMMS57 RNNVHDEQRVRD-----EGRE-----NN 122
PbPIIMMS57 DEDYDDD-NSKSEKFNYHEPSNN---YRGNNNYMYSNTQIP-----MYSNKSPLY 152
PyPIIMMS57 DEDYDDDDSDSEEFNYQEPSNN---YRGNNNYMHNSNSQTP-----MYANKSYLY 166
PcPIIMMS57 TEDYDD-DDSDSDEFYSYSQISNN---NRGNNNYMYSNSQVP-----RHGNKSSLS 154
PkPIIMMS57 VDDYSDVSDDEDNDYAS-NTRNDYKRDHRIGQQHQ-----RG 144
PvPIIMMS57 VDDYSEDDSDDEDNDYGG-NLGDYPRDYRNDQQHQYGSSHQMRQTYDQAEEDDRDSIKHG 168
Consensus :: : . . .

PfPIIMMS57 RRSSVPNNAHTSAKYGSDQEI MQNPKVNNLHLGKSLDDFYNNLGVSSDDFEDDEQVNYN 182
PbPIIMMS57 SNFRDPNENHSENYGD-----QYSPQNSPNYLDRSFDDFRNKLGLSSEDFSRRETETYSN 207
PyPIIMMS57 SNFRDPNENQPSSENYDD-----QYSPQNNPPYLGRSFDNFRNRLGLNTEDFSRGTETYSN 221
PcPIIMMS57 NNRRDPNENSPSGKYGD-----QDSSQNNAPYLGRSFDNFRNKLGLSSEDFSRDSEYYSN 209
PkPIIMMS57 NRDGFPDEEPPRREEGG-----RMT-PKGNTYLGNDFEHFNKRLGLSMDDYMSDTEDDSF 198
PvPIIMMS57 NRDGYPDEGPPRREEGG-----RMT-PGNVYLGKDFEHFNKRLGLSMDDYMSDKEDDSM 222
Consensus . * : : . . . : * . . . * : * : : :

PfPIIMMS57 END---AFSSFSHNNQNDTTLTYDEFYVDSNNYTRNGSSSSSNNNNNNNNIGNNGK 238
PbPIIMMS57 TTNKRNT-----GLDNDLNSFVTDDIQYTKYESTENP-----INSSNRDRDSGMR-- 252
PyPIIMMS57 TTNKRNT-----GLNNDSDSFTDDIQYTKYESSSEN-----INSSRQRHDSGMR-- 266
PcPIIMMS57 TNNRNT-----GLNDEFNTP IADDIQYTKYENSENS-----NNSTRNRDRDSGMR-- 254
PkPIIMMS57 DSSDNSDDLPRNKLNAHDTDSSSIYEDIQFVRHRDNRQP-----NDDVQFSRTDGRR-- 250
PvPIIMMS57 GSSNNSDDLRSNESNAYDADQSSIYEDIQFVKRRNRQP-----NKHVQLSKTDGRR-- 274
Consensus .. : : : : : *

PfPIIMMS57 ITYENENDVSYNDSNNGNSTLSNIHRDNKIKPNLS-----SLYGNKTFNNYSNST----- 287
PbPIIMMS57 ---QSPNNEYNDRNNNG--NRRNSNMYINPNEYDDTYTNSYGYNSQNKINSYVMRYG 307
PyPIIMMS57 ---QSPNNEYNDRNNN-S--NRRNSNMYINTDEDDDSYNSPYGYNQNGPNASQVMRYR 320
PcPIIMMS57 ---QSPSNEYNRRNNN-----NINSNTYNNPDEDDDSYFNPGYNSQSSSSSSQLMEHG 306
PkPIIMMS57 ---NAM---YNRSDNGAEESPPRESKRV--NNLSSDVYDYAYGRNGSNMSEPAN-LYSYP 301
PvPIIMMS57 ---NAI---YRSGSDEEAPHSESEIV--RDPSSVYDYAYGRNGPNRSAPLN-LYSYP 325
Consensus : * : ** : . .

PfPIIMMS57 FNDVSNYNRDDEITPFNNRNVYVPSEYSNPLD-AYVHPNNQFSTQYQYGNMMEEMNKL 346
PbPIIMMS57 LGGNDNIDQ-Q-----TIYSNP--IDYTSSNN-----NYSSSFGGNIKP 344
PyPIIMMS57 LNSDDSDIDQ-Q-----SIYSNP--IDDISTSD-----NYSLNFGGKKS 357
PcPIIMMS57 LNSGSGSIDQ-R-----SVYSNP--LEYMSPNV-----DYRPSFEGRIK 343
PkPIIMMS57 ---EDDISQ-RTIPPIENRK---SRNRSSPLQDDYSIPSD-----TYRNSYDSE--- 344
PvPIIMMS57 ---EEDFNR-RTSPPVGSKK---SMSSSSPLQDDYIKPLD-----SYSDSHDSM--- 368
Consensus . . . : . * . * . *

PfPIMMS57 RRVNF APRDIYTVNNSL-----DG---HSINNNTPLNND-AGPS 383
PbPIMMS57 -RNIQHIENDNSSILNSQRDYEDSDRNS-S--RY----GDNRSNRQNKSPIRNID----- 391
PyPIMMS57 -RSIQHIENDNSSILNSQRNYEDIDNNS-S--RN----GDNRSKIQNKPSIRIFD----- 404
PcPIMMS57 -RNIQPIEDDNYNMLNSPRHYEDIDSTS-S--GN----NSNRNSRNKPPIRSVD----- 390
PkJIMMS57 -----DHIYSVIPGEEQYEDINQRRTQGYSSLGNGARPDRNEILPVRGIKKSASS 396
PvPIMMS57 -----NHLYDVIPGEEQYEDINRRRTPRYNSFDNAVHLDRSELLPVRGVKKSASS 420
Consensus . : . . . : : ..

PfPIMMS57 YMTEGKKNVNKNININTNTNTNTNTNTNTNTNTNTNTNKSKKRSDSNFFSKNKNNNSA 443
PbPIMMS57 ---RGNKGDA-----QNRSKRVAHANSI 411
PyPIMMS57 ---RGNKGDA-----QNKSKRLSYSNSN 424
PcPIMMS57 ---RSGKGDA-----QNRSSFRMSRSNSD 410
PkJIMMS57 YHPEGRESKT-----DGSLKKVRKNNKE 419
PvPIMMS57 YQPEGGKGM-----DNFFNKFRKSKRV 443
Consensus . * : . . . : :

PfPIMMS57 STNE-----L-SRYKDVSKKIFKDNMYNRYENKNYPQSVYVFNEYQNNFF FNKILKRS- 495
PbPIMMS57 PI-----LHRKYDEERQ---PTAHSTITNKILASYIDIYTAQQRNIFYNKNTRVE- 458
PyPIMMS57 PI-----LNKNYE-PRQ---PITRNSTSNKILASYIDIYTAQQRNIFYNKNTRVE- 470
PcPIMMS57 PT-----VNKSYGEARQ---SAPRAQTNKILESIEYIETHQRNIFYNKIIRVE- 457
PkJIMMS57 D-----SSTVMHTQ---QGSKNDNRVSKPLSIITLFSTYQRNLFLNKYSKSSG 464
PvPIMMS57 DMPKGVSPASQGVNSAHTQ---RGPKDGRQGSKSPSVLTTFTTYQRDLFLRKYSKVGG 499
Consensus : . . : : * : * . * :

PfPIMMS57 -----SSNSIKQIFQKNTQHKNTNNVSVTSELQVATQHCISTNKNKLTKPVIL 544
PbPIMMS57 -----KSPIKNFFKMS---NSNDKII SVTEELLNSSKQCI IKNINKLNTNEVLL 503
PyPIMMS57 -----KSPIKFFKMN---NSNDKII SVTEELLNSSKQCIVNNINKLNTNDVLL 515
PcPIMMS57 -----RSPVKQFFKMR---NSNDKMISVTEELLNSSKQCIVKNINKLNRDVL 502
PkJIMMS57 KVGGNVNA--GRTGVKRLFVFSR--SGKDSGLSIMGELQTAVEQCIVKNSNKLRLVLM 520
PvPIMMS57 GSGGMGGSNASSRGVRL--FSR--SGKDDQGSITGELQTAAEQCIAKNRSKLSRPVLM 555
Consensus : : * : ** : : ** . * . * . : :

PfPIMMS57 KYIALNKKYLNKYEYAMSIIHFTCNKNKNNKSNMCLDIKPMLYENDKNI SADMINRLPN 604
PbPIMMS57 KNLALNNTNMIKNEYQAVSIVVFNKIKN---REHLDIKSVPYNYSEAYVENS SVILPN 560
PyPIMMS57 KNLALNNTNMIKNEYQAVSIVVFNKIKN---KESHLDIKSVPYKYSELHVENSS TILPN 572
PcPIMMS57 KNLALNNTNMIKNEYQTVSIVVNSKYKN---TELHLNIKVPYKYSEVHVENSS TMLPN 559
PkJIMMS57 KNLAFNDSKLLENYEVAVSIRNKNNG---SATCLDIRPMVYREDDPDASSIVTSLPN 576
PvPIMMS57 KNLAFNDPKLLKNYEVAVSISDNCKNG---NAACLDIRPMIYREDDPDASIVTSLPN 611
Consensus * : * : : : : : * : * : : : * : : * : * :

PfPIMMS57 VYILNNTIEYILTDPKTSYKSLIKNNIKNHLLTVTDIVLILSNNYFKN-INSILVNHYI 663
PbPIMMS57 MYILNTYEFMLSNQRICGKFRTI IKNKKRDNNLKPVDVVLMLSLFDYIKN-MNPILVSNLM 619
PyPIMMS57 MYILNTYEFMLSNQRICGFRTI IKNKKRDNNLKPVDVVLMLSLFDYIKN-MNPILVSNLI 631
PcPIMMS57 MYILNTYEFMLSNQRMCCKFRTI IKNKKRDNNLKPVDVVLMLSLFDYIKN-VSPRFVSNLM 618
PkJIMMS57 IYILSTYEFLLTNRMCGLPTMVKNRVKENKLTPTDIVLLLSGAYFKSWMNNTLVKHLI 636
PvPIMMS57 IYILSTYEFLLTNRMCGLPTMVKNRVKENKLTPTDIVLLLSGAYFKSWMNNTLVKHLI 671
Consensus : * * . * * : : : : : : : : : : * . * * : : : : * : : :

PfPIMMS57 SFLNTHKNETYLLKYLSALISVFPFKPAMKIYYDQR-YINLSLYTLNLEIAKVFNGLLN 722
PbPIMMS57 QFIKTKQSIYMKKYFNIMVALIPFKPALKIYYGEKIYKDLTTYKIQSEIKNIFDELLL 679
PyPIMMS57 KFIKTKQSIYMKKYFNTMVALIPFKPALKIYYGESIYKDLTTYKMQSEIKNIFDELLL 691
PcPIMMS57 QFIKTKQSIYMKKYFDTMVALIPFKPALKIYYGEKMYNDLTTYKIQSEIKSIFDELLL 678
PkJIMMS57 SFLKTRVYLYEKYFLAVLISLSSFIPALKIYFGER-YAKVSAHNLDAEVNKMIPEMLG 695
PvPIMMS57 AFLTTKRVIHLEKYFLAVLISFAPFKPALKMYFGER-YAKLSAYSLDVEVNKMVGEMLG 730
Consensus * : * * . : : * : : : : * * * : * : : : : * : . . : *

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PfPIMMS57      VILKWSQAFKNEKHNKHTNQIVIQMYKNLSSFSHNRIPNLGHMFKSLEVILRYQRIYNDIK 782
PbPIMMS57      ISIKWAQAFKDKYSATDNKVIDLDMHRTISEFSTSKTRTLSTKTFVSLLENILYKELITSDMI 739
PyPIMMS57      ISIKWAQAFKDKYSANDNKIILDMHQTIKSFSTSKTRTLSTKTFVSLLENILYKELITNDIV 751
PcPIMMS57      ISLKWAQAFKDKYSSADNKIILDMHRTISEFSTSKTKTLSTTFVSLLENILYKELISNDMI 738
PkPIMMS57      AALRWTQAFQEKFSDESNRILLNVHKTSLASYSKERNRGLSRKFRLEALLLRQKVASDVV 755
PvPIMMS57      VALRWTQAFQEKFSDESNRVLLQVHRRLLAAYSRSRNGGLTGKFRTLQELLRQKVASDVT 790
Consensus      :*:***::: . *:::~::~: :~:~* :~: * * :~:~* :~: :~:~*~:

PfPIMMS57      KGKEDYEYEVMIQYFTKYVRNVYAKTITS----- 810
PbPIMMS57      DNKDDNQKVLISYIVKCFRNIYYTTFYMMSNKRTY 774
PyPIMMS57      DNKDDNQKVLISYIVKCFRNIYYSAFYLMGNKRTY 786
PcPIMMS57      NNKDENQKVLISYIMKCFRNIYYSAFYIMNNKRN- 772
PkPIMMS57      SNQDEGYRHLIQQILKYVRNVHSTVFE----- 782
PvPIMMS57      SNQDQGYRLLIQHVLKYVQNVHLLTVLE----- 817
Consensus      ..::: . :~* . * ..*~:~: .::

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Figure S2. Multiple sequence alignment of *Plasmodium PIMMS57* orthologues

Sequence alignment of PIMMS57 orthologs in six *Plasmodium* species: *P. falciparum* (PfPIMMS57; PF3D7_1244500), *P. berghei* (PbPIMMS57; PBANKA_1457700), *P. yoelii* (PyPIMMS57; PYYM_1461700), *P. chabaudi* (PcPIMMS57; PCHAS_1460000), *P. knowlesi* (PkPIMMS57; PKNH_1463700) and *P. vivax* (PvPIMMS57; PVX_101120). In the consensus sequence, dots and colons mark conserved amino acid residues with weakly and strongly similar properties, respectively, and asterisks mark amino acid residues that are identical between orthologues. Predicted signal peptides are highlighted in grey and predicted transmembrane domains are shown with red fonts, respectively. Predicted *P. falciparum* serine/threonine protein phosphatase type I catalytic (PfPP1c) binding motifs are shown in boxes.

PfPIMMS22 MTNII ECTFKT PPDNAKTPDNAV IWNQFYQCDEK GWYSLSNHDEIALRPTTFNDKRIKFL 60
PbPIMMS22 MTNVVECTFKT P PETAKAPDNAV IWNRFQYQCDEK GWYSLSNHDEITLRPTIFNDGRIKFL 60
PyPIMMS22 MTNVVECTFKAP PETAKAPDNAV IWNRFQYQCDEK GWYSLSNHDEITLRPTIFNDGRIKFL 60
PcPIMMS22 MTNVVECTFKI P PETAKAPDNAV IWNRFQYQCDEK GWYSLSNHDEITLRPTIFNDDRIKFL 60
PkPIMMS22 MTNVVECTFKT P PETAKAPENAV IWNRFQYQCDEK GWYSLTNHDEIMLRPTAFSDGRIKFL 60
PvPIMMS22 MTNVVECTFKT P PETAKAPDNA IWNRFQYQCDEK GWYSLTNHDEIMLRPTAFSDGRIKFL 60
Consensus ***.:***** *.:.**.:**.* ** *****:*.** ***** *. * *****

PfPIMMS22 VQLPEIPSEFES ILSGRYDAKAWGKEDCVVIEG EKDVHIRLPGFKEKINYNHTRFPTF 120
PbPIMMS22 PQLDTIPEEFESVLCGKYDAKAWGKDDCNLVIEG EKDVHISLPLGKKEKINYNHKERFPTF 120
PyPIMMS22 PQLDTIPEEFESVLCGKYDAKAWGKDDCNVIEG EKDVHISLPLGKKEKINYNHKERFPTF 120
PcPIMMS22 PQLDTIPEEFESVLCGKYDAKAWGKDDCNVIEG EKDVHISLPGIKEKINYNHKERFPTF 120
PkPIMMS22 PQLDKIPEEFESVLCGKYDAKAWGKDDCNVIEG EKDVHISLPLGQEKINYNHRRERFPTF 120
PvPIMMS22 PQLDKIPEEFESVLCGKYDAKAWGKDDCNVIEG EKDVHISLPLGQEKINYNHRRERFPTF 120
Consensus ** *.**.*.:.**.:**.:**.* ** *****:*.** ***** **.:***** *****

PfPIMMS22 LKNWKIIVSILNEHVTLIRINAETALIININEKKNVTVKSVDFNNGFLCVNPHTNLAIAY 180
PbPIMMS22 LKNSKIIVSLLNENLTVIRINLETALLICINEKKSIVKVINFNNGFACVNPYSNLAIAY 180
PyPIMMS22 LKNSKIIVSLLNENLTVIRINIETGLLISINEKKSIVKVINFNNGFACVNPYSNLAIAY 180
PcPIMMS22 LKNSKIIVSLLNENITVIRINIETALLICINEKKNVIVKVINFNKGFACVNPYSNLAIAY 180
PkPIMMS22 LKNWKIIVGMLNEHITVIRIN TETAI IISISEKKNVTVKCVDFNNGFLCVNPHTNLAIAY 180
PvPIMMS22 LKNWKIIVGMLNEHITVIRIN TETAI IIVSINEKSNVTVKCVDFNNGFLCVNPHTNLAIAY 180
Consensus *** **.:**.:**.:**.* ** .:..* **..* **.:**.* *****:*****.*

PfPIMMS22 GDFALSSLKKCELIQNI PHEGGKWGFFTHLFWGHI IIPKELEIKLPSPGLKLI GKKIDT 240
PbPIMMS22 GGFAFNDLKKCEIVPTI THSGCEWAFFVHLFWGHI IIPKDLELKI PSSGLKLI GKKVDT 240
PyPIMMS22 GGFAFNDLKKCEIVPTI THSGCEWAFFVHLFWGHI IIPKDLELKI PSSGLKLI GKKVDT 240
PcPIMMS22 GGFAFNDLKKCEVVP SITHSGCEWAFFVHLFWGHI IIPKDIELKI PSPGLKLI GKRVD 240
PkPIMMS22 GGFALSELKKCELVPSI THEGAEWGFFVHLFWGHI IIPKDIEIKLPSPGLKLI GKKIDT 240
PvPIMMS22 GDFALSELKKCELVPN I THEGAEWGFFVHLFWGHI IIPKDIEIKLPSPGLKLI GKKIDT 240
Consensus *.**.:..*****:..* **.* :*.**.******:*****:*.**.* *****:..**

PfPIMMS22 LAIVSIPPNIHIVKIDGPKCIRKLEYGQDYNITAIKSSSEDVDIYILFDGHL LKYEFSF 300
PbPIMMS22 IAIVSLPPNIQIHVKIDGPKCIRKVEYGQDYNITAIKSSSEDIDIYVLF DQGQLLYEFSY 300
PyPIMMS22 IAII SLPPNIQIHVKIDGPKCIRKVEYGQDYNITAIKSSSEDIDIYVLF DQGQLLYEFSY 300
PcPIMMS22 IAII SLPPNIQIHVKIDGPKCVRKVEYGQDYNITAIKSSSEDIDIYVLF DQGQLLYEFSY 300
PkPIMMS22 VAIISLPPNIYI HVKIDGPKCIRKLEYGQDYSITAIKSSSEDIDIYVLF DQQLIKYEFSF 300
PvPIMMS22 VAIISLPPNIYI HVKIDGPKCIRKLEYGQDYSITAIKSSSEDIDIYLLFDGQLIKYEFSF 300
Consensus :**.:**.* *****:*****:*.**.* ** *****:***:***:*.**.* **

PfPIMMS22 DIRLNKPEKGRSLHSAKLKCKIN SKSVTSFIFQETKNCKILLG SNCPSDNLGHLLNSQTI 360
PbPIMMS22 DTRLNKEGKGKS IHNAKLKCTSKSKEVSTFIFQESPNCKVLLG SNCPTDNLGHMLCNQTI 360
PyPIMMS22 DTRLNKEGKGKS IHNAKLKCKISKSKEVSTFVFQESQNCVLLG SNCPTDNLGHMLCNQTI 360
PcPIMMS22 DTRLNKEGKGKS IHNAKLKCKISKSKEVSTFVFQESPNCKVLLG SNCPTDNLGHMLCNQTI 360
PkPIMMS22 DTRLNKVKGGRS INCAKLKCTNKSKEVTSFIFQPTANSKLLLD SNCPTDNMGHLLCNQTM 360
PvPIMMS22 DTRLNKVKGGRS INYAKLKCTNKSKEVTSFVFQATANSKLLLD SNCPTDNMGHLLCNQTI 360
Consensus * ***** **.:**.: ***** .*****:*.**.* :*.**.* *****:***.* **.

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PfPIMMS22      AIFDAEIGEYLSHPQGLQLTSVFNTLSYPLDKE 393
PbPIMMS22      SIFDAEIGEYQSHPQGLLLTEAFEKLSYPVENA 393
PyPIMMS22      SIFDAEIGEYQSHPQGLLLTEVFEKLSYPVENA 393
PcPIMMS22      SIFDAEIGEYQSHPQGILLTEVFEKLSYPVENA 393
PkPIMMS22      SVFDAETGEYLSHPQGLKLTEVFNSLSYPPEE- 392
PvPIMMS22      SVFDAETGEYLSHPQGLQLTEVFNTLSYPPEKE 393
Consensus      ::*:*:*  ***  *****:  **..*:.*****  ::

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Figure S3. Multiple sequence alignment of *Plasmodium PIMMS22* orthologues

Sequence alignment of PIMMS22 orthologues in six *Plasmodium* species: *P. falciparum* (PfPIMMS22; PF3D7_0814600), *P. berghei* (PbPIMMS22; PBANKA_1422900), *P. yoelii* (PyPIMMS22; PY17X_1424900), *P. chabaudi* (PcPIMMS22; PCHAS_1424700), *P. knowlesi* (PkPIMMS22; PKNH_1425600) and *P. vivax* (PvPIMMS22; PVX_123040). In the consensus sequence, dots and colons mark conserved amino acid residues with weakly and strongly similar properties, respectively, and asterisks mark amino acid residues that are identical between orthologues.

PfPIMMS22 ---MTNIECTFKTPPDNAKTPDNAVWQFYCYDEKQWYSLSNHDEIALRPTTFNDKRI 57
PbPIMMS22 ---MTNVVECTFKTPPETAKAPDNAVWNRFYCYDEKQWYSLSNHDEITLRPTIFNDGRI 57
PyPIMMS22 ---MTNVVECTFKAPPETAKAPDNAVWNRFYCYDEKQWYSLSNHDEITLRPTIFNDGRI 57
PcPIMMS22 ---MTNVVECTFKIPPETAKAPDNAVWNRFYCYDEKQWYSLSNHDEITLRPTIFNDGRI 57
PkPIMMS22 ---MTNVVECTFKTPPETAKAPENAVWNAFYCYDEKQWYSLTNHDEIMLRPTAFSDGRI 57
PvPIMMS22 ---MTNVVECTFKTPPETAKAPDNAIWNSFYCYDEKQWYSLTNHDEIMLRPTAFSDGRI 57
T.gondii S15 ---MERQATCRYDPLVEVP-LPPGIVLWTPHQYYDGAGWLALPDREKLELKPTRWSDGRL 56
N.caninum S15 ---MERLSTCRYDPLTEVP-LPPGIVLWTPHQYYDGAGWLALPEHEKLMKPTRWADGRL 56
E.tenella S15 MEAVNKLCSCRFEPLVEVP-VPEKAVVWTSKNYDGSQWVGLADGEKLSLKPTLFDNRRL 59
Consensus : . * : . : . * : : * . : * * ** . * : : : : * * : * * :

PfPIMMS22 KFLVQLPEIPSEFESILSGRYDAKAWGKEDCYVVEIEGKDVHIRLPGFKEKINYNHTRERF 117
PbPIMMS22 KFLPQLDTIPEEFESVLCGKYDAKAWGKDDCNLVIEGKDVHISLPLGKEKINYNHKERF 117
PyPIMMS22 KFLPQLDTIPEEFESVLCGKYDAKAWGKDDCNVVEIEGKDVHISLPLGKEKINYNHKERF 117
PcPIMMS22 KFLPQLDTIPEEFESVLCGKYDAKAWGKDDCNVVEIEGKDVHISLPLGKEKINYNHKERF 117
PkPIMMS22 KFLPQLEKIPEEFESVLCGKYDAKAWGKDDCNVIEGKDVHISLPLQEKINYNHRERF 117
PvPIMMS22 KFLPQLEKIPEEFESVLCGKYDAKAWGKDDCNVIEGKDVHISLPLQEKINYNHRERF 117
T.gondii S15 RFLDPIDELPEPFKAVQSGKFDVKCWRGDCKLGIKDTVFLKSPISPDVAVYVHAERL 116
N.caninum S15 RFLDPIDELPEPFKAVQSGKFDVKCWRGDCKLGIKDTVFLKAPISPEVAVYVHADRL 116
E.tenella S15 LFLEPVEGVCEAFSSVQSGKYDVKCWSKLGCHLGIKDTVFLSTPKLKEVAVYSHPERL 119
Consensus * * : : . * : : * : : * : : * : : * : : * : : * : : * : : * : : * : :

PfPIMMS22 PTFLKNWKIIVSILNEHVTLIRINAETALIININEKKNVTVKSVDENNGFLCVNPHTNLA 177
PbPIMMS22 PTFLKNSKIIVSLLNENLTVIRINLETALLICINEKKSIVIVKSNFNNGFACVNPYSNLA 177
PyPIMMS22 PTFLKNSKIIVSLLNENLTVIRINLETALLICINEKKSIVIVKSNFNNGFACVNPYSNLA 177
PcPIMMS22 PTFLKNSKIIVSLLNENITVIRINLETALLICINEKKNVIVKSNFNNGFACVNPYSNLA 177
PkPIMMS22 PTFLKNWKIIVGMLNEHITVIRINTETAIISISEKKNVTVKSVDENNGFLCVNPHTNLA 177
PvPIMMS22 PTFLKNWKIIVGMLNEHITVIRINTETAIIVSINEKSNVTVKSVDENNGFLCVNPHTNLA 177
T.gondii S15 PTFPKSWKPLVFIILNQSLAMFRLTENLCLLVVAEKDKTMNISCVDYNGGFACHPSTNMV 176
N.caninum S15 PAFPKTWKPLVFIILNQSVAMFRLTENLCLLVVENDKTMNISCVDYNGGFACHPKTNMV 176
E.tenella S15 PAFPKSWKPLVFIILNQSVAMFRLTENLCLLVVENDKTMNISCVDYNGGFALSHPASDSA 179
Consensus * : * * . * : : * * . : : * : : * : : * : : * : : * : : * : : * : :

PfPIMMS22 IAYGDFALS---SLKKCELIQNIPEHGGKWFTHLFWKGHIIIPKELEIKLPS--PGLK 232
PbPIMMS22 IYGGFAFN---DLKKCEIVPTITHSGCEWAFFVHLFKWGHIVIPKDLELKIPS--SGLK 232
PyPIMMS22 IAYGGFAFN---DLKKCEIVPTITHSGCEWAFFVHLFKWGHIIIPKDLELKIPS--SGLK 232
PcPIMMS22 IAYGGFAFN---DLKKCEVVPSTHSGCEWAFFVHLFKWGHIIIPKDIEIKLIPS--PGLK 232
PkPIMMS22 IAYGGFALS---ELKKCELVPSITHEGAEWGFFVHLFKWGHIIIPKDIEIKLIPS--PGLK 232
PvPIMMS22 IAYGDFALS---ELKKCELVPSITHEGAEWGFFVHLFKWGHIIIPKDIEIKLIPS--PGLK 232
T.gondii S15 VAYGSYVLKNFEKLPSCQAIKMLTASGDWGFVQFYFPWGFVFPKSVELTRPQAVLGAV 236
N.caninum S15 VAYGSYIVKTFEKLNSCAAIPKMTASGDWGFVQFYFPWGFVFPKSVLELTRPQAVLGAV 236
E.tenella S15 LAYGSLAVKGFALPHCEVIVPRTSAAGEWGFVQLFQWGSFVVPKAVDLTRPSSILG-M 238
Consensus : : * * . * : : * : : * : : * : : * : : * : : * : : * : : * : :

PfPIMMS22 LIGKKIDTVAIIVSIPPNIIHVKIDGP-KCIRKLEYGQDYNITAIKSSSDVDIYVLFDFG 291
PbPIMMS22 LIGKKVDTIAIVSLPPNIQIHKIDGP-KCIRKVEYGQDYNITAIKSSSDIDIYVLFDFG 291
PyPIMMS22 LIGKKVDTIAIISLPPNIQIHKIDGP-KCIRKVEYGQDYNITAIKSSSDIDIYVLFDFG 291
PcPIMMS22 LIGKRVDTIAIISLPPNIQIHKIDGP-KCVRKVEYGQDYNITAIKSSSDIDIYVLFDFG 291
PkPIMMS22 LIGKKIDTVAIISLPPNIYIHKIDGP-KCIRKLEYGQDYSITAIKSSSDIDIYVLFDFG 291
PvPIMMS22 LIGKKIDTVAIISLPPNIYIHKIDGP-KCIRKLEYGQDYSITAIKSSSDIDIYVLFDFG 291
T.gondii S15 GMGKKVDTIGLVFHPNMFINVKLDIPAKTTRALQFGKDFQVTAKKTSETDIDVFLVIDG 296
N.caninum S15 GMGKKVDTIGLVFHPNMFINVKLDLPAKTTRALQFGKDFQVTAKKTSETDIEIFLVIDG 296
E.tenella S15 GLGKKVDCLGVLLLPNLLVVMVHLETP-KVQRSLQHGKDYLLTAVKTSSETDLDFLVMVDG 297
Consensus : * : : * : : * : : * : : * : : * : : * : : * : : * : : * : :

PfPIMMS22 HLLKYEFSDIRLNKPEKGRSLHSAKLCIN----KSKEVTSFIFQETKNCKILLGNSNCP 347
PbPIMMS22 QLLKYEFSDYDTRLNKEGKGSINHAKLKCTS----KSKEVSTFIFQESPNCKVLLGNSNCP 347
PyPIMMS22 QLLKYEFSDYDTRLNKEGKGSIHAKLKCSIS----KSKEVSTFVFQESQNCVLLGNSNCP 347
PcPIMMS22 QLLKYEFSDYDTRLNKEGKGSINHAKLKCSIS----KSKEVSTFVFQESPNCKVLLGNSNCP 347
PkPIMMS22 QLIKYEFSFDTRLNKVGKGRSINCAKLCNTN----KSKEVTSFIFQPTANSKLLLDNSNCP 347
PvPIMMS22 QLIKYEFSFDTRLNKVGKGRSINYAKLCNTN----KSKEVTSFVFQATANSKLLLDNSNCP 347
T.gondii S15 QLAKYNYSFDIRINKPERPKHTDNIHFKCSCDAEKKKPKPKFKLSACKDSVILLEQGCP 356
N.caninum S15 QLAKYNYSFDIRNNKPERPKHTDNIHFKCTDAEKKKPEPRFKLTACKDSVILLEQGCP 356
E.tenella S15 QLSVFNYSFDLRLNRPKNRHLDNAAFKGSLELDDKKK-CSRFIQNTKNSKVLVFPQGCP 356
Consensus : * : : * : : * : : * : : * : : * : : * : : * : : * : : * : :

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PfPIMMS22      SDNLG-HLLNSQTIAIFDAEIGEYLSHPQGLQLTSVFNTLSYPLDKE 393
PbPIMMS22      TDNLG-HMLCNQTISIFDAEIGEYQSHPOGLLLTEAFEKLSYPVENA 393
PyPIMMS22      TDNLG-HMLCNQTISIFDAEIGEYQSHPOGLLLTEVFEEKLSYPVENA 393
PcPIMMS22      TDNLG-HMLCNQTISIFDAEIGEYQSHPOGILLTEVFEEKLSYPVENA 393
PkPIMMS22      TDNMG-HLLCNQTMVFDVAETGEYLSHPQGLKLTVEFNLSYPPEE- 392
PvPIMMS22      TDNMG-HLLCNQTSVFDVAETGEYLSHPQGLQLTEVFNTLSYPPEKE 393
T.gondii S15    SGNPDEQLVSEQLIACFDAEVCLYTHPPALKLCDAFTDVAIRE--- 400
N.caninum S15  CGNPDEQLVSEQVIAFFDAEVCLYTHPPALKLCDAFTDVAIRE--- 400
E.tenella S15  LSEGD-HLVSSSLIAVFDAEICMYLTHPPALKLCSAFNTVALPVD-- 400
Consensus      .: . ::: .. :: ***** * :** .: * ..* ::

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Figure S4. Multiple sequence alignment of *Plasmodium* PIMMS22 and homologues in other apicomplexans

Sequence alignment of PIMMS22 orthologues in six *Plasmodium* species and homologs in other apicomplexan parasites. UniProt ID's- *T. gondii* S15 (CEL77905.1), *N. caninum* S15 (XP_003885565.1) and *E. tenella* S15 (XP_013230639.1). In the consensus sequence, dots and colons mark conserved amino acid residues with weakly and strongly similar properties, respectively, and asterisks mark amino acid residues that are identical between orthologues.

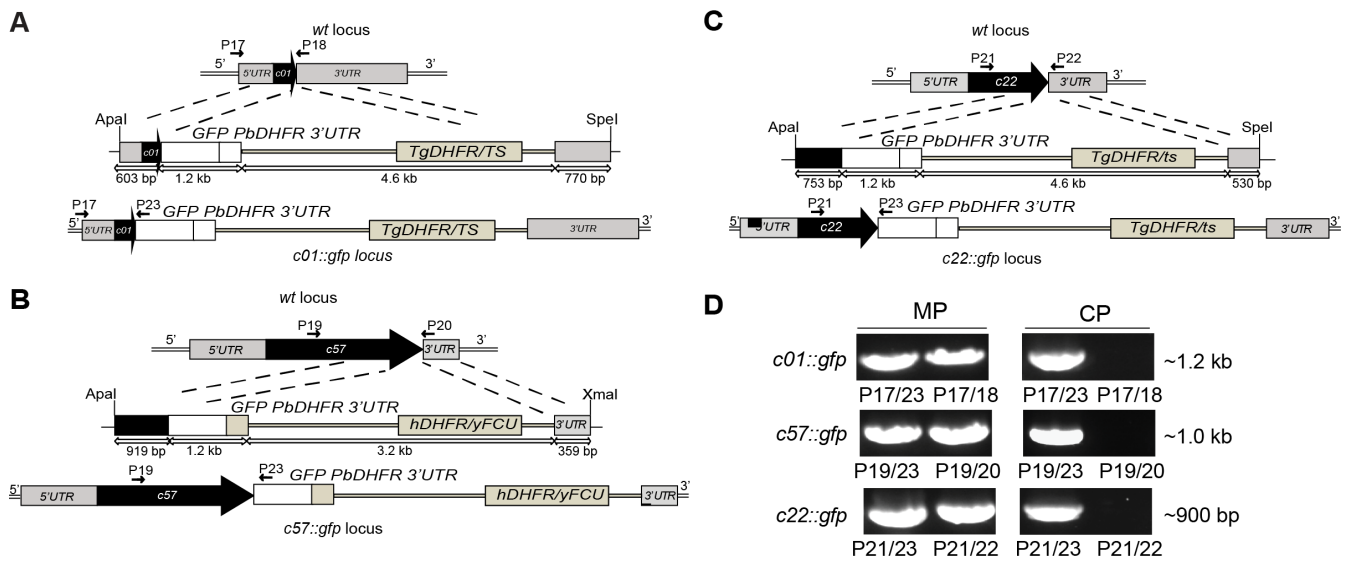


Figure S5. Generation of GFP-tagged *P. berghei* transgenic lines in the ANKA 2.34 reference line

Schematic representation of the *PIMMS01* (*c01::gfp*) (**A**), *PIMMS57* (*c57::gfp*) (**B**) and *PIMMS22* (*c22::gfp*) (**C**) GFP tagging by double crossover homologous recombination. Black arrows indicate binding sites for primers used in diagnostic PCR. (**D**) PCR genotypic analysis on mix parasite (MP) and clonal populations (CP) of the tagged transgenic parasites following transfection and dilution cloning. Used primer combinations are shown below each detected band.

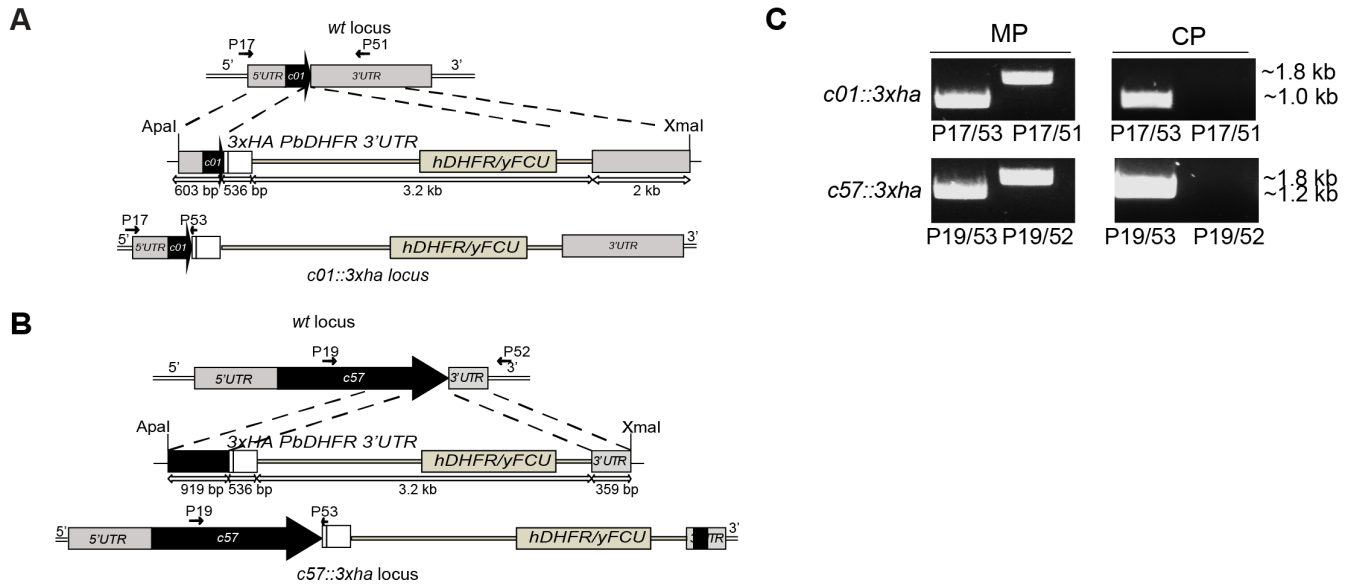


Figure S6. Generation of HA-tagged *P. berghei* transgenic lines in the ANKA 2.34 reference line

Schematic representation of the *PIMMS01* (*c01::3xha*) (**A**) and *PIMMS57* (*c57::3xha*) (**B**) HA tagging by double crossover homologous recombination. Black arrows indicate binding sites for primers used in diagnostic PCR. (**C**) PCR genotypic analysis on mix parasite (MP) and clonal populations (CP) of the tagged transgenic parasites following transfection and dilution cloning. Used primer combinations are shown below each detected band.

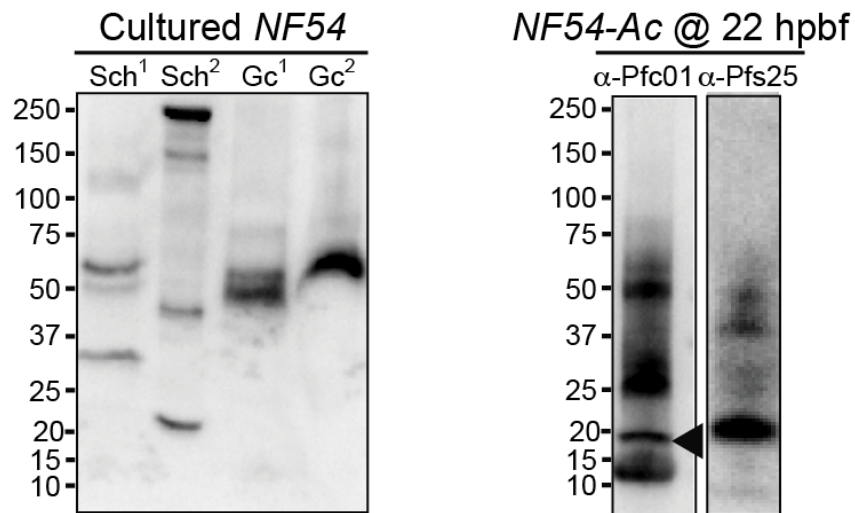


Figure S7. PfPIMMS01 expression in *in vitro* cultured schizonts and gametocytes

Western blot analysis using α -Pfc01 antibody on Triton soluble lysates (¹), Triton insoluble cell lysates (²) and whole cell lysates (³) of *P. falciparum* NF54 schizonts (Sch), gametocytes (Gc) and *A. coluzzii* midgut homogenates 22 hpbf. The PfPIMMS01 protein band is indicated with a black arrowhead. The antibody against Pfs25 (α -Pfs25) was used as a positive control in midgut homogenates.

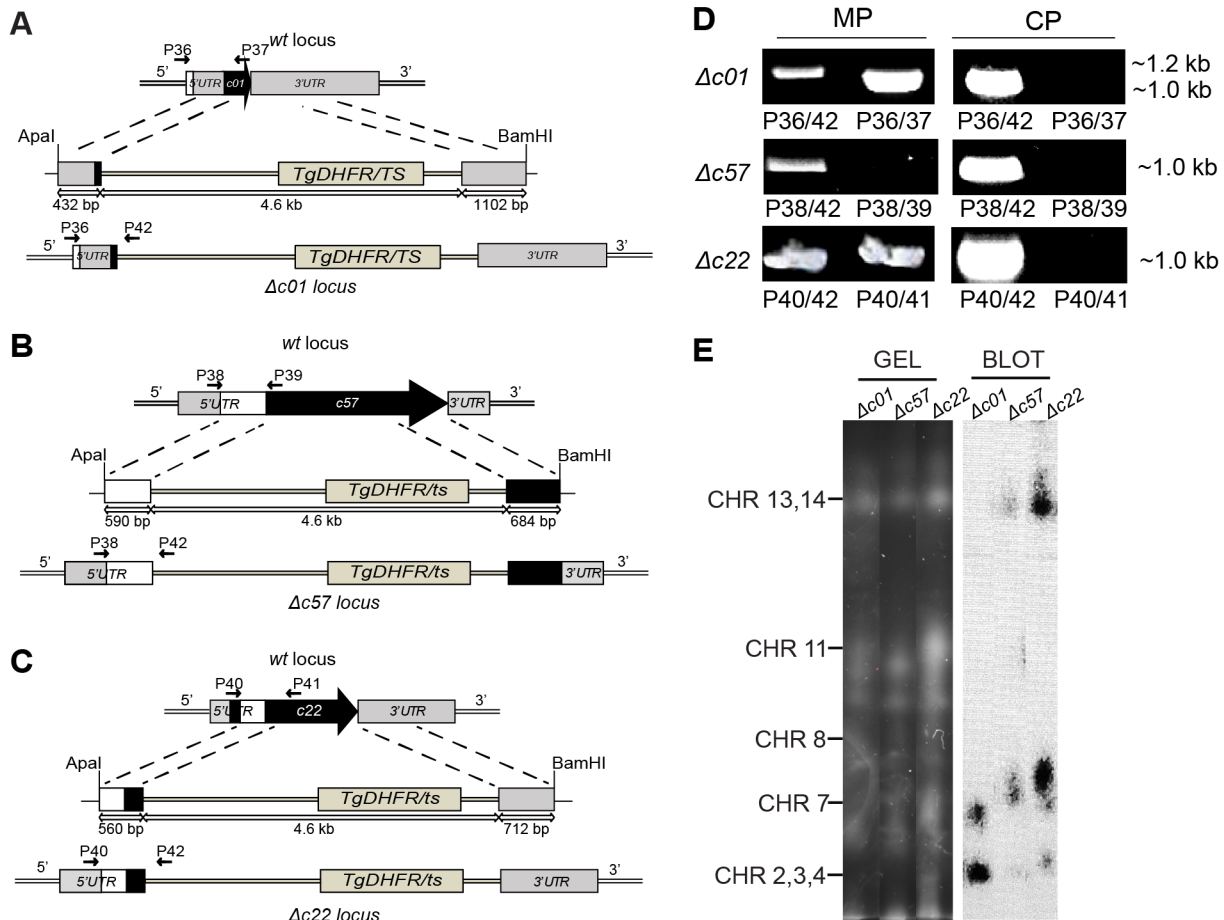


Figure S8. Generation of *P. berghei* $\Delta c01$, $\Delta c57$ and $\Delta c22$ knockout mutant lines

Schematic representation of *P. berghei* *PIMMS01* (A), *PIMMS57* (B) and *PIMMS22* (C) locus disruption by double crossover homologous recombination in the *c507* transgenic reference line. Black arrows indicate binding sites for primers used in diagnostic PCR. (D) PCR genotypic analysis on mix parasite (MP) and clonal populations (CP) of the knockout mutant parasites following transfection and dilution cloning. Used primer combinations are shown below each detected band. (E) Southern blot analysis on pulsed field gel electrophoresis separated transgenic chromosomes.

Legends of Supplementary Movies

Supplementary Movie 1. Gliding motility of c507 ookinetes.

In vitro cultured ookinetes were embedded in Matrigel and their movement was captured with time-lapse microscopy at 20 frames per sec for 2 min.

Supplementary Movie 2. Gliding motility of $\Delta c01$ ookinetes.

In vitro cultured ookinetes were embedded in Matrigel and their movement was captured with time-lapse microscopy at 20 frames per sec for 2 min.

Supplementary Movie 3. Gliding motility of $\Delta c01$ ookinetes.

In vitro cultured ookinetes were embedded in Matrigel and their movement was captured with time-lapse microscopy at 20 frames per sec for 2 min.

Supplementary Movie 4. Gliding motility of $\Delta c57$ ookinetes.

In vitro cultured ookinetes were embedded in Matrigel and their movement was captured with time-lapse microscopy at 20 frames per sec for 2 min.

Supplementary Movie 5. Gliding motility of $\Delta c57$ ookinetes.

In vitro cultured ookinetes were embedded in Matrigel and their movement was captured with time-lapse microscopy at 20 frames per sec for 2 min.

Supplementary Movie 6. Gliding motility of $\Delta c57$ ookinetes.

In vitro cultured ookinetes were embedded in Matrigel and their movement was captured with time-lapse microscopy at 20 frames per sec for 2 min.

Supplementary Movie 7. Gliding motility of $\Delta c22$ ookinetes.

In vitro cultured ookinetes were embedded in Matrigel and their movement was captured with time-lapse microscopy at 20 frames per sec for 2 min.

Supplementary Movie 8. Gliding motility of $\Delta c22$ ookinetes.

In vitro cultured ookinetes were embedded in Matrigel and their movement was captured with time-lapse microscopy at 20 frames per sec for 2 min.