

**Supplemental Information**  
Cell Host & Microbe, Volume 8

**The Systematic Functional Analysis of *Plasmodium* Protein Kinases Identifies Essential Regulators of Mosquito Transmission**

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**SUPPLEMENTAL EXPERIMENTAL PROCEDURES**

**Generation of Gene Knockout Parasites**

Oligonucleotides used to amplify targeting sequences from *P. berghei* genomic DNA are described in “Table oligonucleotides”. Depending on construct design, restriction digests with KpnI/SacII or Apal/XbaI were used to release the linear targeting vector from the cloning plasmid. Electroporated parasites were mixed immediately with 100 µl of reticulocyte-rich blood from a phenylhydrazine treated, naive mouse and incubated at 37°C for 15 minutes. Re-invaded parasites were injected intraperitoneally into naive mice. From day 1 post infection pyrimethamine was applied in the drinking water for four days. Mice were monitored for 15 days to allow any slow growing mutants to reach patency. Drug selection was repeated after passage to a second mouse, and resistant parasites were used for cloning by limiting dilution, and genotyping.

**Genotype Analysis of Mutants.**

Chromosomes of wild type and knockout parasites were separated by pulsed field gel electrophoresis (PFGE) on an LKB 2015 Pulsaphor system or CHEF DR III using a linear ramp of 60–500 s for 72 hr at 4V/cm. Gels were blotted and hybridized with a probe recognizing both the resistance cassette in the targeting vector and, more weakly, the 3'UTR of the *P. berghei* *dhfr-ts* locus on chromosome 7. Two diagnostic PCR reactions were used as illustrated in Fig. 2A. In one reaction primers 1 and 2 were used to determine correct integration of the selectable marker at the target locus. Primers 3 and 4 (Fig. 2A) verified deletion of the target gene. Sequences for the locus-specific primers (P1, P3, and P4) are given in Table S4. The generic primer P2 was either ol248 (5'-GATGTGTTATGTGATTAAATTACACAC-3') or ol539 (5'-CAATGATTCAAAATAGTTGGACTTG-3'), depending on whether the 5' or 3' end of the *tgdhfr-ts* cassette was used to verify integration. Having confirmed integration on one end by diagnostic PCR, we usually used Southern blot analysis to probe integration on the opposite end of the construct. Genomic DNA from wild type and mutant parasites was digested with diagnostic restriction enzymes cutting once within the knockout cassette, as illustrated in Fig. 2A. The fragments were separated on a 0.8% agarose gel, blotted onto a nylon membrane,

and probed with a PCR fragment homologous to the *P. berghei* genomic DNA just outside of the targeted region.

### Phenotype Analysis

Infections for phenotype screens were initiated by the intraperitoneal injection of infected blood containing  $5 \times 10^6$  parasites into mice that had been pretreated with 0.2 ml of 6 mg/ml phenylhydrazine in PBS intraperitoneally to induce reticulocytosis. Asexual stages and gametocyte production were monitored on Giemsa-stained blood films.

Exflagellation was examined on days 3-4 post infection. 5  $\mu$ l of gametocyte-infected blood were obtained from the tail with a heparinized pipette tip and mixed immediately with 40  $\mu$ l of ookinete culture medium (RPMI1640 containing 25 mM HEPES, 25% fetal bovine serum, 10 mM sodium bicarbonate, 50  $\mu$ M xanthurenic acid at pH 7.6). The mixture was placed under a Vaseline-coated cover slip and 10-20 min later exflagellation centers were counted by phase contrast microscopy in 12-15 fields of view using a 63x objective and 10x ocular lens.

Ookinete formation was assessed on the same day. 10  $\mu$ l of infected tail blood were obtained as above, mixed immediately with 40  $\mu$ l ookinete culture medium, and incubated for 1 h at 19°C to allow completion of gametogenesis and fertilisation. Each culture was then diluted with 0.4 ml of ookinete medium and transferred to a 24-well plate, which was incubated at 20°C for a further 21–24 h to allow ookinete differentiation. Cultures were pelleted for 2 min at 5000 rpm and then incubating for 5min on ice with 50  $\mu$ l of ookinete medium containing the DNA dye Hoechst 33342 to a final concentration of 5  $\mu$ g/ml and a Cy3-conjugated mouse monoclonal antibody 13.1, which recognizes the P28 protein on the surface of ookinetes and any undifferentiated macrogametes or zygotes. Labeled cultures were placed on a microscope slide under a Vaseline-rimmed cover slip and P28-positive cells counted with a Leica DMR fluorescence microscope. Ookinete conversion was expressed as the percentage of P28 positive parasites that had differentiated into ookinetes. *In vitro* cross fertilisation experiments with the *srpk* mutant were carried out by mixing 5  $\mu$ l volumes of tail blood containing equal number of either *srpk*, *cdpk4* or *nek-4* mutant gametocytes (Liu et al., 2008). Ookinete conversion was then determined as above.

For mosquito transmission experiments 50-100 *Anopheles stephensi* SD500 mosquito were allowed to feed for 20-30 min on anaesthetized mice that had been infected with wild type or mutant parasites 4-5 days earlier, had reached an asexual parasitaemia ~5-7% and were carrying comparable numbers of gametocytes as determined on Giemsa stained blood films. Unfed mosquitoes were removed the following day. On day 14 post feeding approximately 20 mosquitoes were dissected and oocysts on their midguts counted. DNA replication in the cysts was examined by staining with Hoechst 33342 for 10-15

minutes before guts were washed and mounted under Vaseline-rimmed cover slips. Images were recorded using a 63x oil immersion objective on a Leica DMR fluorescence microscope equipped with a Leica DC500 digital camera. On day 21 post feeding another 20 mosquitoes were dissected and their guts and salivary glands crushed separately in a loosely fitting homogenizer to release sporozoites, which were then quantified using a haemocytometer.

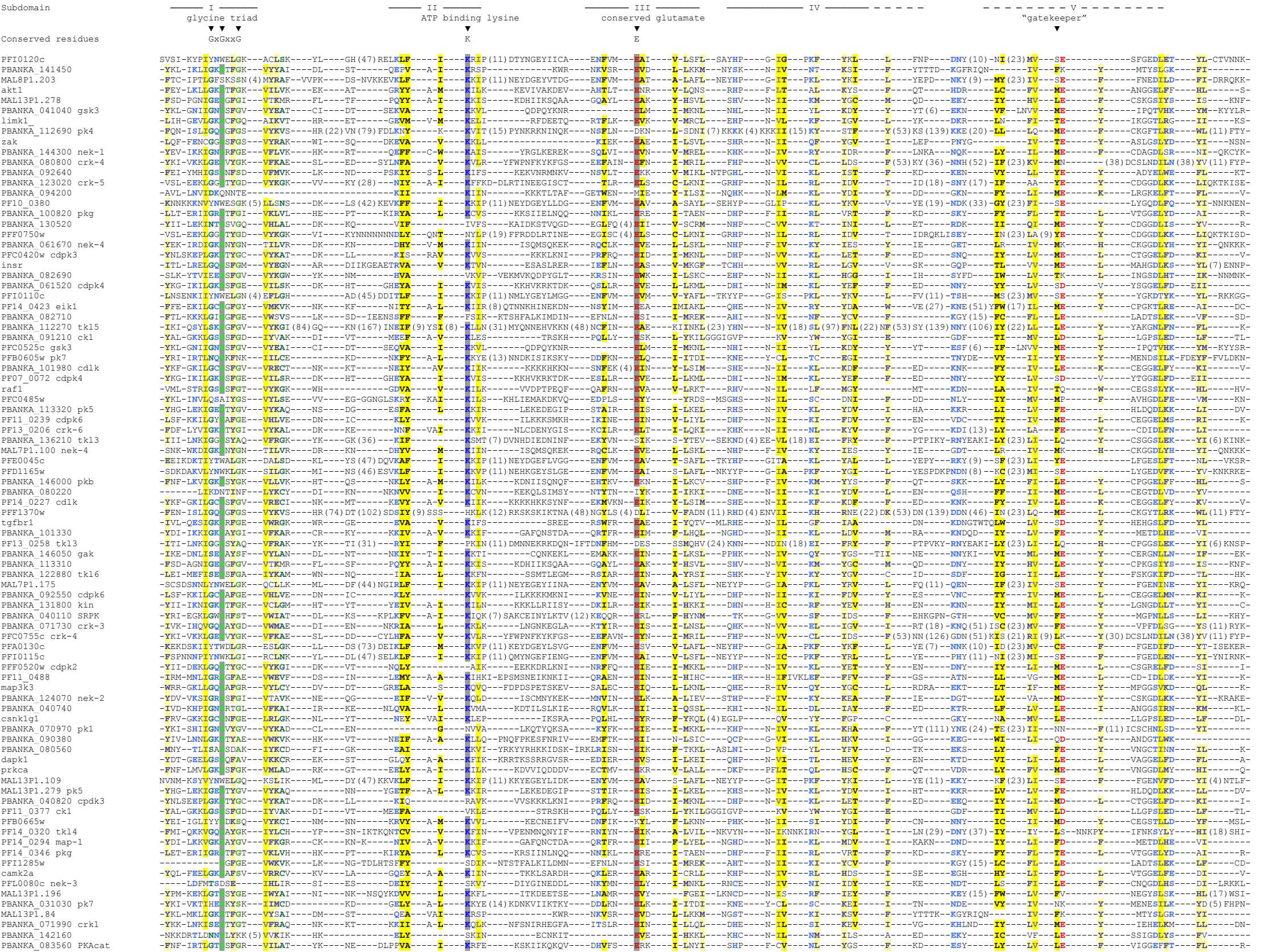
Due to day-to-day variations in transmission levels, all data were normalized to a matching number of wild type controls analyzed on the same day. Average total infection levels for wild type were as follows:  $67.5 \pm 7.0$  % of female gametes transformed into ookinetes within 24 h *in vitro* ( $n = 25$  cultures);  $97.8 \pm 55.4$  wt oocysts were counted per infected mosquito midgut on day 14 p.i. ( $n = 43$  experiments);  $31,000 \pm 7,400$  sporozoites were obtained per mosquito midgut on day 21 p.i. ( $n = 34$  experiments);  $6,600 \pm 2,800$  sporozoites were recovered from salivary gland per mosquito on day 21 p.i. ( $n = 37$  experiments).

All mutants with salivary gland infections were analysed for the infectivity of their sporozoites. 30-50 infected mosquitoes were allowed to feed for 20 min on 3 anesthetized naïve C57Bl/6 mice, which are highly susceptible to sporozoite challenge. Mice were monitored for blood stage infections from day 3 and until about day 15 post feeding.

### Supplemental References

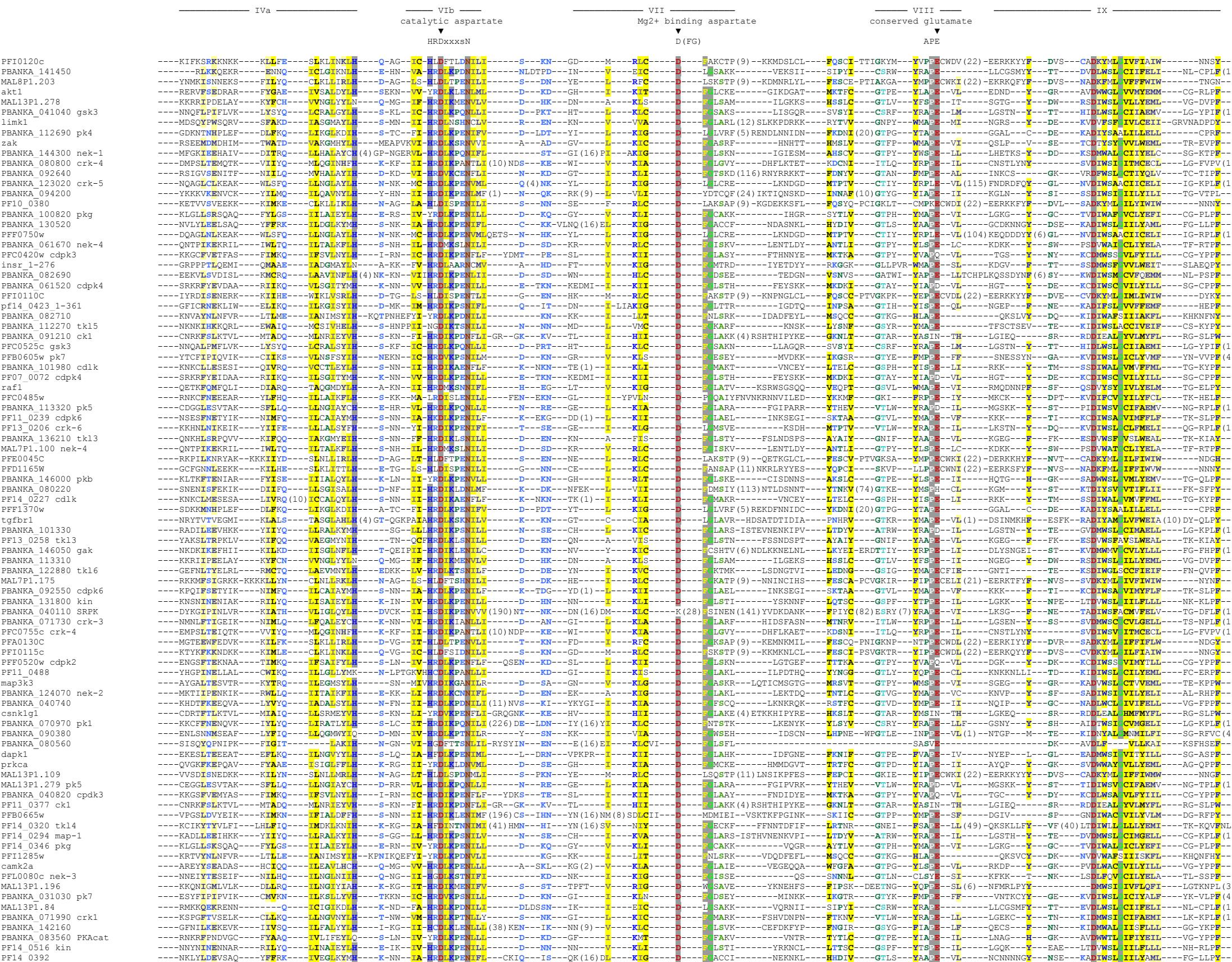
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Tewari et al., Figure S1 A (top)



Tewari et al., Figure S1 A (cont.)

Tewari et al., Figure S1 A (cont.)



Tewari et al., Figure S1 A (cont.)

PFB0520w tk11  
 PFL1370w nek-1  
 PBANKA\_101090 tk14  
 PFI1280c  
 cdk2  
 PBANKA\_135090 crk-6  
 PFI1415w  
 PBANKA\_130920 lammer  
 PFB015w cdpk1  
 mapk1\_1-289  
 PF11\_0242 cdpk7  
 PBANKA\_030850 tk11  
 PF08\_0044 pk1  
 PBANKA\_082960  
 MAL7P1.144  
 PBANKA\_093300 prk4  
 PBANKA\_130690  
 PF11\_0060  
 PFI1\_0141 mrk  
 gsk3b  
 map4k1  
 PFI1\_0147 map-2  
 PF13\_0085 pk9  
 MAL7P1.91 est  
 PFI0125C  
 PFI1\_0464  
 PBANKA\_092700 tk12  
 clk1  
 PFC0385c  
 mapkapk2  
 csnk1  
 pfc0105w srpk  
 pfa0380w eik2  
 PFI1685w PCACat  
 PBANKA\_061580 est  
 PF11\_0156 prk4  
 PF14\_0476  
 PBANKA\_060060 nek-3  
 MAL7P1.26  
 PBANKA\_031140  
 PFI0105cpw  
 PBANKA\_093860 ck2  
 PBANKA\_031420 cdpk1  
 PBANKA\_010410  
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 PFB0150c  
 PFL1885c pk2  
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 prkaca  
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 PBANKA\_040940  
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 nek7  
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 PFL2280w gak  
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 PFI1\_0227  
 PFF0260  
 PBANKA\_141360 pk9  
 PFI1145c tk15  
 PFI1\_0510  
 PFI1\_0220 tk12  
 rock1  
 src  
 PFC0060C  
 PBANKA\_122500 FIKK  
 pak1  
 PF14\_0431 lammer  
 PF14\_0408  
 PFL2250c pkb  
 egfr  
 PFD0740w crk-3  
 MAL7P1.73  
 PBANKA\_121280 mrk  
 map2k1  
 PFD0865c crk-1  
 PF14\_0733  
 PFI0100C  
 PBANKA\_135260  
 PBANKA\_130840 eik1  
 PF11\_0096 ck2  
 PBANKA\_131370  
 Consensus/80%

-----YKINMKLQDII-----KISKD-----ITAFMSFLH-----N-KG-----IM-HCDLKLSSNILLI-----S---IT---RD-----T-----KIC-----FOLSVF-----NKYNKPK-----YLGV-----GTYQ-----WTAP-----VL-----RSEG-----Y-----TK-----EAD1YSF0VILWEMI-----HR-KIPF-----  
 -----MFGKIEBHAII-----DITQ-----LLHALAYCH(4)GP-NGERVL-HRDLKPONIPL-----ST-----GI(16)P-----AKG-----FOLSKN-----IGIESM-----HSCV-----GTPY-----YNSP-----LL-----LHETKS-----Y-----DD-----KSDMWAL-CIILYELC-----SG-KTPF-----  
 -----NRIYIJKIQLK(5)LNIFIQ-----IIEQIYVH-----K-KK-----II-HFDINTCNILLI(93)MKK-----KD-----CQ(6)-----VIS-----FESKF-----FLRDTDFIF-----FRKNR-----GNEI-----LAAP-----LL(77)-DKIRILR(6)KS(40)KSDIWLS-SLLYEMI-----TN-ESLF-----  
 -----DEKVIGIDISL-----KMCKQ-----LAEVINFLL(4)NK-KN-----VII-HRDIKPENILL-----D-----SD-----WN-----T-----HIC-----FOLSEVE-----KSDN-----MTPSV-----VTIW-----YRSPE-----LL-----LQNNT-----Y-----DQ-----K1DIWSL-CLFVELI-----TG-RPLF(8)-----  
 -----ALTGIPPLPLIK-----SYLFQ-----LLGIALFCH-----S-HR-----VL-HRDLKPONIPL-----N-----TE-----GA-----T-----KIC-----FOLALAR-----FGVPVRT-----YTHEV-----YRPE-----IL-----LGCK-----Y-----ST-----AVDIWSL-CIFAEVM-----TR-RALF(11)-----  
 -----KLYNLN1KEVK-----YIILFE-----LLLA1CYLH-----S-NN-----YL-HRDLKPENILL-----N-----SK-----GE-----T-----KIC-----FOLLSVE-----KSDN-----MTPSV-----VTIW-----YRPE-----IL-----LQNNT-----Y-----DQ-----K1DIWSL-CLFVELI-----TG-RPLF(8)-----  
 -----NNRRKKEYKK(11)EILRQ-----LLNVCLYIY-----R-NK-----IF-HSDIKPNSNIVI-----KN-----IH(16)Y(13)KJL-----FOLCATF-----KTDY-----HGSII-----NTRQ-----YRPE-----VI-----LNIG-----W-----DV-----SSDMWS-CILALELY-----TG-NLLF(10)-----  
 -----DYNGFHIEDIK-----LYCIE-----ILKA1HYLR-----K-LK-----LT-HTDLKPENILL-----D-----DP-----HF(16)Q(7)KJL-----FOLCATF-----KTDY-----HGSII-----NTRQ-----YRPE-----VI-----LNIG-----W-----DV-----SSDMWS-CILALELY-----TG-NLLF(10)-----  
 -----NRHKFEDCAA-----NIMKQ-----ILSGICYLH-----K-HN-----IV-HRDLKPENILL-----E-NK-----S(2)-----T-----KIC-----FOLLSF-----FSKDNK-----LDRDL-----GTAY-----YRPE-----VL-----RKK-----Y-----NE-----KCDVMS-CVILYILL-----CG-YPPF-----  
 -----KTQHLSDNDHIC-----YFLYQ-----LLRG1KYH-----S-AN-----VL-HRDLKPONIPL-----N-----TT-----CD-----T-----KIC-----FOLALAR-----ADPDHDHTGF-----YTHEV-----ATRW-----YRPE-----IM-----LNSKG-----Y-----TK-----SID1WSV-CILALERM-----SN-RPIF(11)-----  
 -----ABTRLSIHSIAN-----K1ITQ-----LIKTVAYLH-----R-CG-----II-HRDIKPENILL-----TDKS-----RD-----AQ-----T-----KIC-----FOLSLTL-----CAPNEL-----LREP-----GTLA-----YVAP-----VI-----TLOG-----Y-----NH-----KVDAMSI-IIILYILL-----SG-KLPF-----  
 -----PBANKA\_030850 tk11-----KMKD-----ITFSMSFLH-----N-KG-----IL-HCDLKPONIPL-----S-----ES-----GE-----T-----KIC-----FOLSIQ-----NFNDNP-----YLGIV-----GTYQ-----WTAP-----IL-----DFKNKN-----Y-----AL-----PKLWMI-GITIFILL-----TG-THPF-----  
 -----PF08\_0044 pk1-----KKSFLNENQK-----IYLYQ-----LLRATLYH-----S-LC-----II-HRDIKPONIPL(245)ND-----NT-----DX-----T(85)KLC-----FOLSIK-----LLENK-----YFSV-----CSRY-----YRPE-----LL-----LFGNSNT-----SQ-----AID1WSI-CVMGELL-----LG-KPLF(11)-----  
 -----KTYVVPPEYMA-----EILFQ-----LLRACFVYI-----E-NQ-----IV-HSDIKPNSNIV-----KN-----IK(16)Y(13)KJL-----FOLCATF-----EYCSQ-----CYGE-----EANV-----FPLS-----FPKSL-----YRPE-----NF-----RKKKN-----Y-----DQ-----K1DIWSL-CLFVELI-----TG-THPF-----  
 -----KTFQHLSDNDHIC-----YFLYQ-----LLRG1KYH-----S-AN-----VL-HRDLKPONIPL-----N-----TT-----CD-----T-----KIC-----FOLAR-----ADPDHDHTGF-----YTHEV-----ATRW-----YRPE-----IM-----LNSKG-----Y-----TK-----SID1WSV-CILALERM-----SN-RPIF(11)-----  
 -----PF11\_0242 cdpk7-----K1ITQ-----LIKTVAYLH-----R-CG-----II-HRDIKPENILL-----TDKS-----RD-----AQ-----T-----KIC-----FOLSLTL-----CAPNEL-----LREP-----GTLA-----YVAP-----VI-----TLOG-----Y-----NH-----KVDAMSI-IIILYILL-----SG-KLPF-----  
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 -----PF08\_0044 pk1-----KKSFLNENQK-----IYLYQ-----LLRATLYH-----S-LC-----II-HRDIKPONIPL(245)ND-----NT-----DX-----T(85)KLC-----FOLSIK-----LLENK-----YFSV-----CSRY-----YRPE-----LL-----LFGNSNT-----SQ-----AID1WSI-CVMGELL-----LG-KPLF(11)-----  
 -----KTYVVPPEYMA-----EILFQ-----LLRACFVYI-----E-NQ-----IV-HSDIKPNSNIV-----KN-----IK(16)Y(13)KJL-----FOLCATF-----EYCSQ-----CYGE-----EANV-----FPLS-----FPKSL-----YRPE-----NF-----RKKKN-----Y-----DQ-----K1DIWSL-CLFVELI-----TG-THPF-----  
 -----KTFQHLSDNDHIC-----YFLYQ-----LLRG1KYH-----S-AN-----VL-HRDLKPONIPL-----N-----TT-----CD-----T-----KIC-----FOLAR-----ADPDHDHTGF-----YTHEV-----ATRW-----YRPE-----IM-----LNSKG-----Y-----TK-----SID1WSV-CILALERM-----SN-R

Tewari et al., Figure S1 A (cont.)

— X ————— XI —————

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PFI0120c
PBANKA_141450
MAL8P1.203
Hs akt1
MAL13P1.278
PBANKA_041040 gsk3
Hs limk1
PBANKA_112690 pk4
Hs zak
PBANKA_144300 nek-1
PBANKA_080800 crk-4
PBANKA_092640
PBANKA_123020 crk-5
PBANKA_094200
PF10_0380
PBANKA_100820 pkg
PBANKA_130520
PFF0750w
PBANKA_061670 nek-4
PFC0420w cdpk3
insr_1-276
PBANKA_082690
PBANKA_061520 cdpk4
PFI010C
pf14_0423_1-361
PBANKA_082710
PBANKA_112270 tk15
PBANKA_091210 ck1
PFC0525c gsk3
PFB0605w pk7
PBANKA_101980 cdk1
PF07_0072 cdpk4
raf1
PFC0485w
PBANKA_113320 pk5
PF11_0239 cdpk6
PF13_0206 crk-6
PBANKA_136210 tk13
MAL7P1.100 nek-4
PFE0045C
PFD1165W
PBANKA_146000 pkb
PBANKA_080220
PF14_0227 cdk1
PFF1370w
tgef1
PBANKA_101330
PF13_0258 tk13
PBANKA_146050 gak
PBANKA_113310
PBANKA_122880 tk16
MAL7P1.175
PBANKA_092550 cdpk6
PBANKA_131800 kin
PBANKA_040110 SRPK
PBANKA_071730 crk-3
PFC0755c crk-4
PFA0130C
PFI0115c
PFF0520w cdpk2
PF11_0488
map3k3
PBANKA_124070 nek-2
PBANKA_040740
csnk1gl
PBANKA_070970 pk1
PBANKA_090380
PBANKA_080560
dap1
prkca
MAL13P1.109
MAL13P1.279 pk5
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PFB0665w
PF14_0320 tk14
PF14_0294 map-1
PF14_0346 pkg
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camk2a
PFL0080c nek-3
MAL13P1.196
PBANKA_031030 pk7
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PBANKA_083560 PKAcat
PF14_0516 kin
PF14_0392
PFB0520w tk11

-LWYISDPIIDINY (2)--YKCKNNNFNKIWTTLFW-----PKKIK-----RILR----QLLDL-DRRKNLN---LN--DLIN---DPWFTR-
)LIEVLGSPNINFY---ENIKDYTSTKNTSLIKEL-CELDIPLPSWD---HILG---NILEKEPDPNERLK---ID--EVLD---NSYF---
-LWKCSDPQLQDEF (2)--FKCDMNFDNFELTKNW-----PNELK---DIIK---QBLHV-EQRKCLN---LK--DLSA---HPWW---
-----YNQDH---EKLFLIELIMEIIRFPRT---LGPEAK---SLLS---GDLK---DPKGRLG(5)AK--EIMQ---HRRF---
-----DGTKTQZ---VNLAI---DLIK---KTUVV-DVNKRIR---LC--ELSS---DPWM-
)IIQILGTPTEQDM---KVMMNPYADVKFVNPKFD(5)PKGTPNNAI---NPVS---QFLKY-EPLKRLN---AI--EALA---DPFF---
-----LERTMDF---GLNVRGFLDRYCPNC---PPSF---PITV---RCCLD-DPEKRP8---FVKL---EWL-
-NVIMERYKTLNDT---RNYYTVPDVYKHLNPW-----YILM---LQMSKPNPADRPS---AA--DLYN---KI-
-----KGLEGLQV---AWLVEEKNERLTIPFSC---PRSFA---ELLR---CQWEA-DAKKRPS---FK--QIIS---IL-
-----HKANN---FSQLISELKGKGPFLPIK---GKSKELN---FLIK---NLLNL-SAKERPS---AL--QCLG---YQI-
)VFRGIPDQNFDNN---LKKELVGEPEKFVDR(13)RLNDKGL---DLID---QFLSY-DYKNRIT---AN--EALK---HEWF-
-----DGSTEW---FIYKNIKREINYPPII---PLDLV---DLIE---KLV1-NPECRIG(4)CE--DILQ---HPYF-
)IVNSL GKPNKNDL---EYFSDSRYFPKD(17)GKDELGT---DLIV---KMLY-NPDRIT---AA--DALS---HPWF-
)PMCFCMINYRNTDKI (2)--KKEKGKGINFNLLSFNNY---PLAK---DLCO---QFLQF-DPKKRIK(5)SY---HPWL-
-IWKRADPSKDKIF (2)--LLNNDINSIKLAEDW---PKGLK---NIIN---KLLDL-ESRMKIN---LD--DLVK---HPWWFY-
-----GNDQDQ---LEIFRDILTQGLTFPDY---VTDITDSI---NLIK---RLCR-LPQCRIG(5)FK--DIKE---NSPF-
-----DNEEKDI---KEAHNINVNKKIIFPKN---RINRCSNVX---NLLI---GMNL-NPINRLS---LD--QVIN---DPWV-
)IVNSL GKPNKNDL---EFFSNNSRFYPLKDF(17)GRIDEGL---DLV---KMLY-NPDRIT---AA--DALS---HPWF-
-----HSTKGIQ---QLCYNIRYAPIPDLPNI---YSKELN---NIYK---SMULR-EPNYRVT---VQ--QLLV---SDIV-
-----FGESDHE---ILSMVKKGYQFKGEW---NNIIEAK---DLIK---RCLTM-DADKRIC---AS--EALQ---HPWF-
-----QGLSNEQ---VLFKVMDDGGYLQDPDNC---PERV---DLMR---MCWF-NPKMRPT---FLEIVNLLKDDLHPSF-
-----QHYIISFEDSQQ---IYEKLVFTKVLPCF---HSKIDNSPKD---DLIK---ICLNY-DPDLRPT---AK--EIVD---LL-
-----NGSNEYD---ILKKVETGKTYTFDLFQF---KKISDKAK---DLIK---KMLY-TSAVVIS---AR--DALE---HEWI-
)LWNKADESLEDNDF (2)--FKENNMLDIFFTQTW---PEELK---YITS---QDLVLT-ETRKNLQ---FK--DLIN---HPWFS-
-----STSmersitlSN---LLKGIVPEYMKADNPKF---QFLS---SLLAI-NPQERCC-AV--NLLH---ESVL-
-----EGKNTQK---VDBEILNNINWKDNEF---SSLSEVAI---DLFLK---KLLER-NERKRLT---AF--EALN---HPWI-
-----NGSNEYD---ILKKVAGAKYTFDLFQF---KKISDKAK---DLIK---KMLY-TSAVVIS---AR--DALE---HEWI-
-----YDFNPKDILKHFEDIQDF---I---NLLI---VCVDE-SPFMRP8---FS--EIFR---LL-
-----YNSKNTK---IRHELINVRKTRPHIFSF---LPNSIK---KCIQ---KCFSF-NPEEPS---AY--EM---YKAL-
QGLKAISKKDKYDK---IMEKKISTSVEVLCRNT---SFEV---TYLN---TCRSL-RFEDRPTYTLR---RLLK---DLFI-
)IIVQLGTPTEQD---KEMNPYADIKFDPVSKD(5)PKGTPDEAI---NLIIT---QFLKY-EPLKRLN---PI--EALA---DPFF-
SIVELEPNNIRTKNI---EYPLDNRHFLYPLTNK(4)NNFLSNEDI---DFLK---FLRK-NPAERIT---SE--DALK---HEWL-
-----DGLNADL---IVDBEILNNINWKDNEF---SSLSEVAI---DLFLK---KLLER-NERKRLT---AF--EALN---HPWI-
-----EGKNTQK---VDBEILNNINWKDNEF---SSLSEVAI---DLFLK---KLLER-NERKRLT---AF--EALN---HPWI-
-----YDFNPKDILKHFEDIQDF---I---NLLI---VCVDE-SPFMRP8---FS--EIFR---LL-
-----SHINNRDQI (2)--VGGRYASPDLSKLYNCE---PRAMK---DCVKK-VKEERPL---FP--QILS---SIEL-
-----KCSLDKDI---YWMKMFKNKNYQKLLEK---KGLHLSKQV---DLIF---NCLIP-NENFIRYN---IN--EALN---HDWF-
)IFKILGTPNSONW-PDVFKLPKYDNPVYVNPLPWTIFKLQDTG---DLIS---KMLXL-DPNRPT---AK--QAIK---HPWF-
-----GGNTYDEVK---QSIFRDPDVKSLKSL---SQTAL---HMLK---LMQK-DYNKRP---AS--VLLH---HPWF-
)IYLLGDKDKLTT---VDKERKDMFMPYFEINML---KDAIDDEHTL---DLIS---KMLY-DPNYRIS---SK--EALK---HPCF-
-----DGLNADL---FCKISSLHLPFNDI---PRDL---SVLEY-DFTKRLPFDVIA---RKLK---HWEI-
-----DGTQKQI---QLCYNIRYAPIPDLPNI---YSKELN---NIYK---SMULR-EPYRAT---VQ--QLLV---SDIV-
-IWHCSDASTDENK (2)--FEKCDMSLDVFOILTST---PSGLK---NLLN---ELLI-EKRMV---LR--NLLS---YWPFTK-
-IWEKADSFNDKIF (2)--FVKCGMDLYNYESLTYNW---PDDK---DIIN---QBLHV-ENRAQLS---LK--ELCK---HPWWSN-
-----NGKSRLD---LFENIYKIKKIKISNR---SFEV---DLIK---KLLQK-NPQERLG(5)AS--EIKK---HPFF-
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-----TTIMERYKRNLNDT---RNYYTVPDVKHLNPW-----YILM---LOMSKPNPADRPS---AA--DV---YSKI-
-YDLVPSDFSVEME---KRVVCEQKLRPNIPNWR---QSCEA-RVMA-KIMR---ECWA-NGAARI(5)KTLSQLQ---QEGI-
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-----YGDTDNE---VLLKKVKGECFYENDW---GSISDAK---NLIT---KLLTY-NPNERCT---IE--EALN---HPWI-
-----QFNYLEKQKEL---VNKMKNLTYPKNQK---SNATL---SYTQF-DYELRPS---IS--EALS---YPLF-
-----AEYEA (2)--IFKIAQTQPTNPLPSHI---SEHGR---DFLR---RIFV-EARCRPS---AS--ELLT---HIFHA-
-----KSKNGNM (2)--IVQKVCEQPDPLPSIY---SKDLR---NLCY---WMMSK-CSEKRPT---VQ--DIIG---TEYF-
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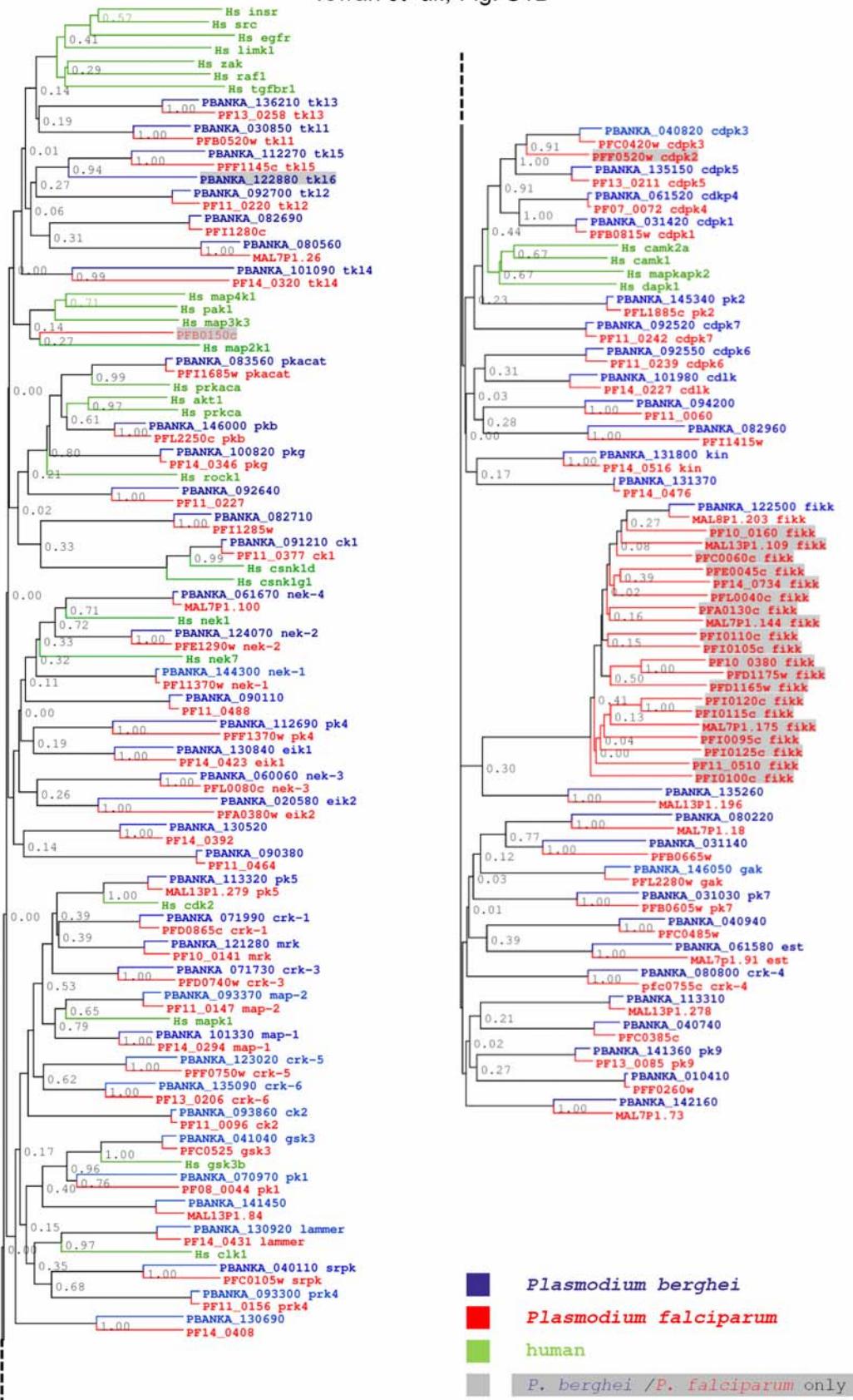
Tewari et al., Figure S1 A (cont.)

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Consensus/80%
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--QHYIITFDESDQ----IYEKLVDVTKLKPFCI--HSKINSPFA--DITR--ICLNY-DPNLRPT--AS-EIVQ---LL---
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LDLIYATLGNKTEK----AMNDIERYNSFPYEEHLIKSMSIDESVC----DLIS---KMLTY-DPYFRIS--SK-EALK---HSCF---
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)DYDIVSLQWGDE(227)FKMNVNQDMNMNPNKDY(38)LNYSDEYW---DLIT---NLLVH-VPSERLL--AC-EVLG---HDFF---
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----GSTNEERENV--IMMRIQDYHWSQLSFCEK(5)IDKLSPEK--DFLN--COLDK-NPKRPT--AS-SLIQ--HFFI---
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Tewari et al., Fig. S1B

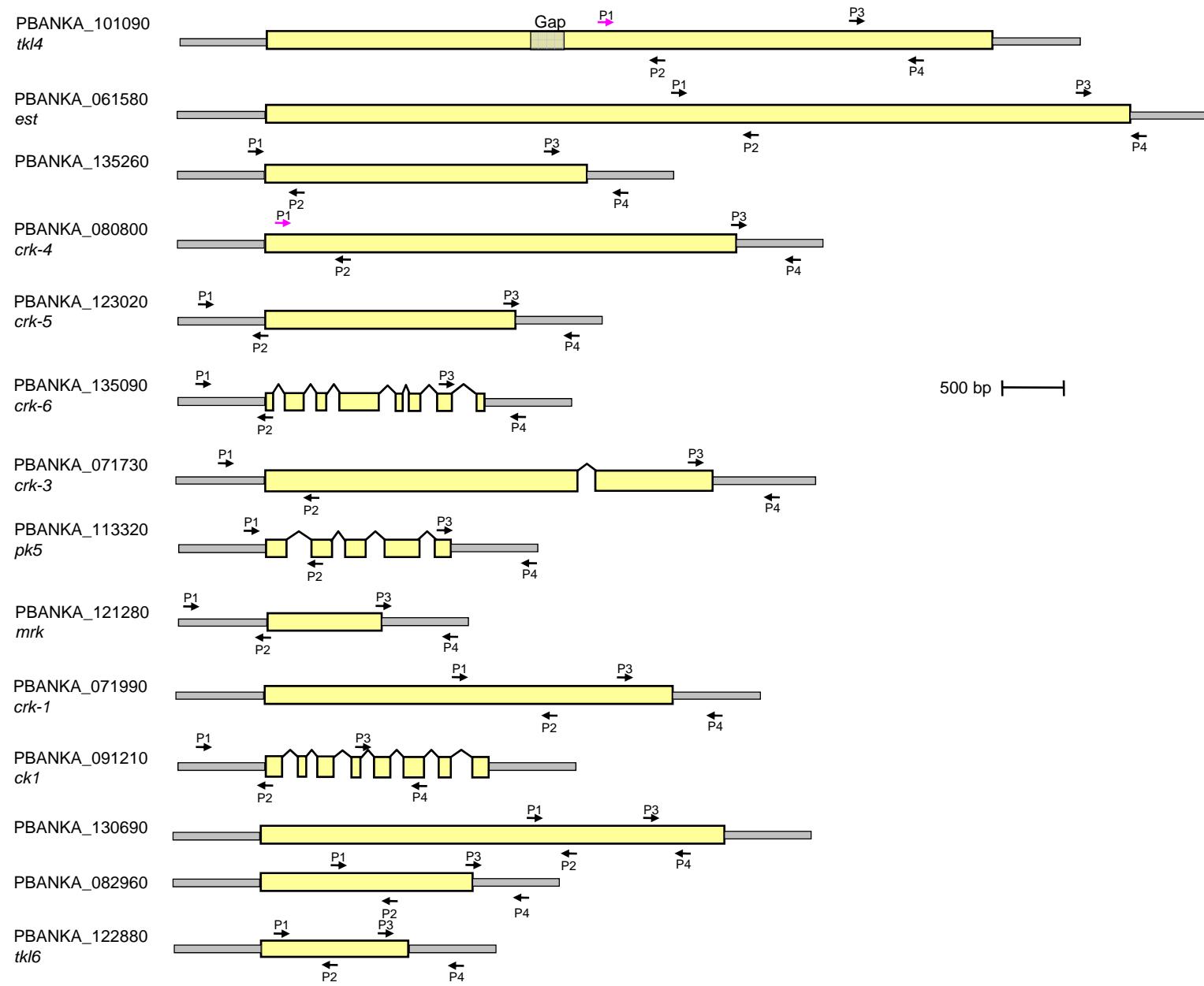


**Figure S1 (relates to Figure 1). Comparative Analysis of Kinase Domain Sequences from *P. berghei* and *P. falciparum***

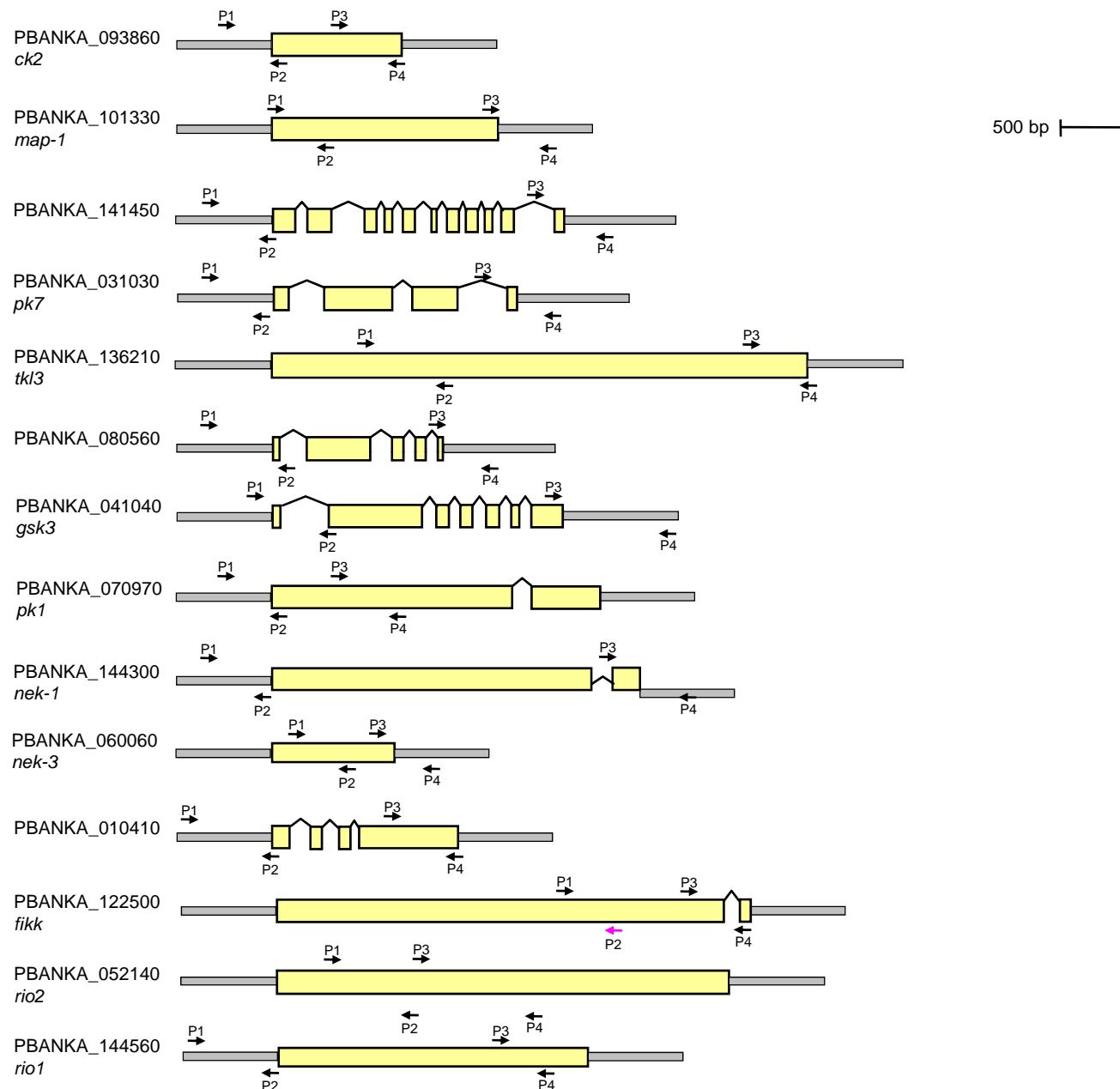
(A) Multiple sequence alignment of *Plasmodium* kinase domains. The alignment was generated using the MUSCLE software (Edgar, 2004) and coloured using the CHROMA software to highlight conserved residues (Goodstadt and Ponting, 2001). Kinase domain sequences for *P. falciparum* were taken from Ward et al., (2004), those from *P. berghei* are contained in a supplemental text file entitled “Pb kinase domain sequences”.

(B) Evolutionary relationships of *Plasmodium* and selected human kinase domains. The evolutionary history was inferred from the alignment in Figure S1 using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 72.77107574 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method (Zuckerkandl and Pauling, 1965) and are in the units of the number of amino acid substitutions per site. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). There were a total of 4009 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007).

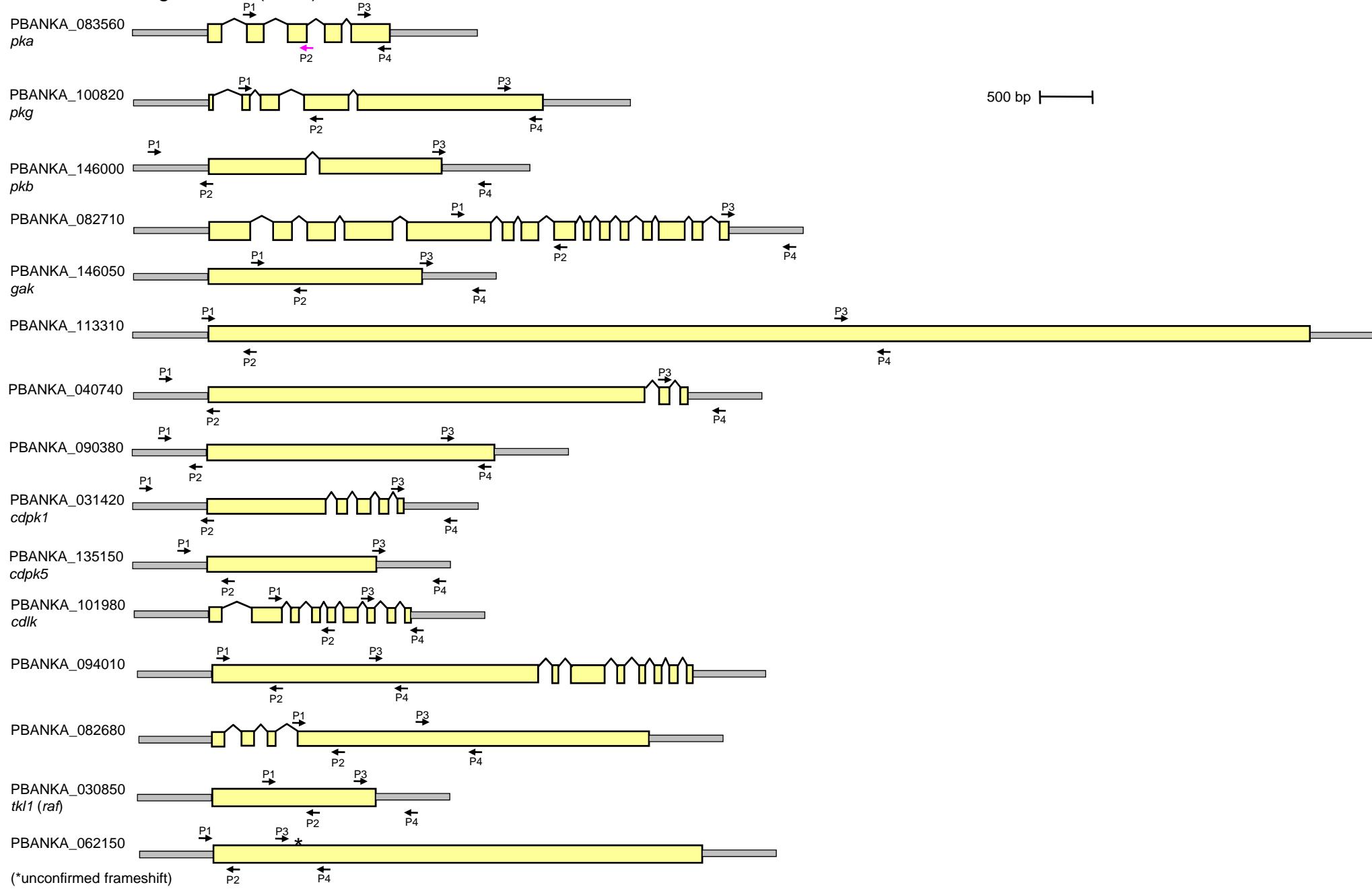
Tewari et al., Figure S2 A (top)



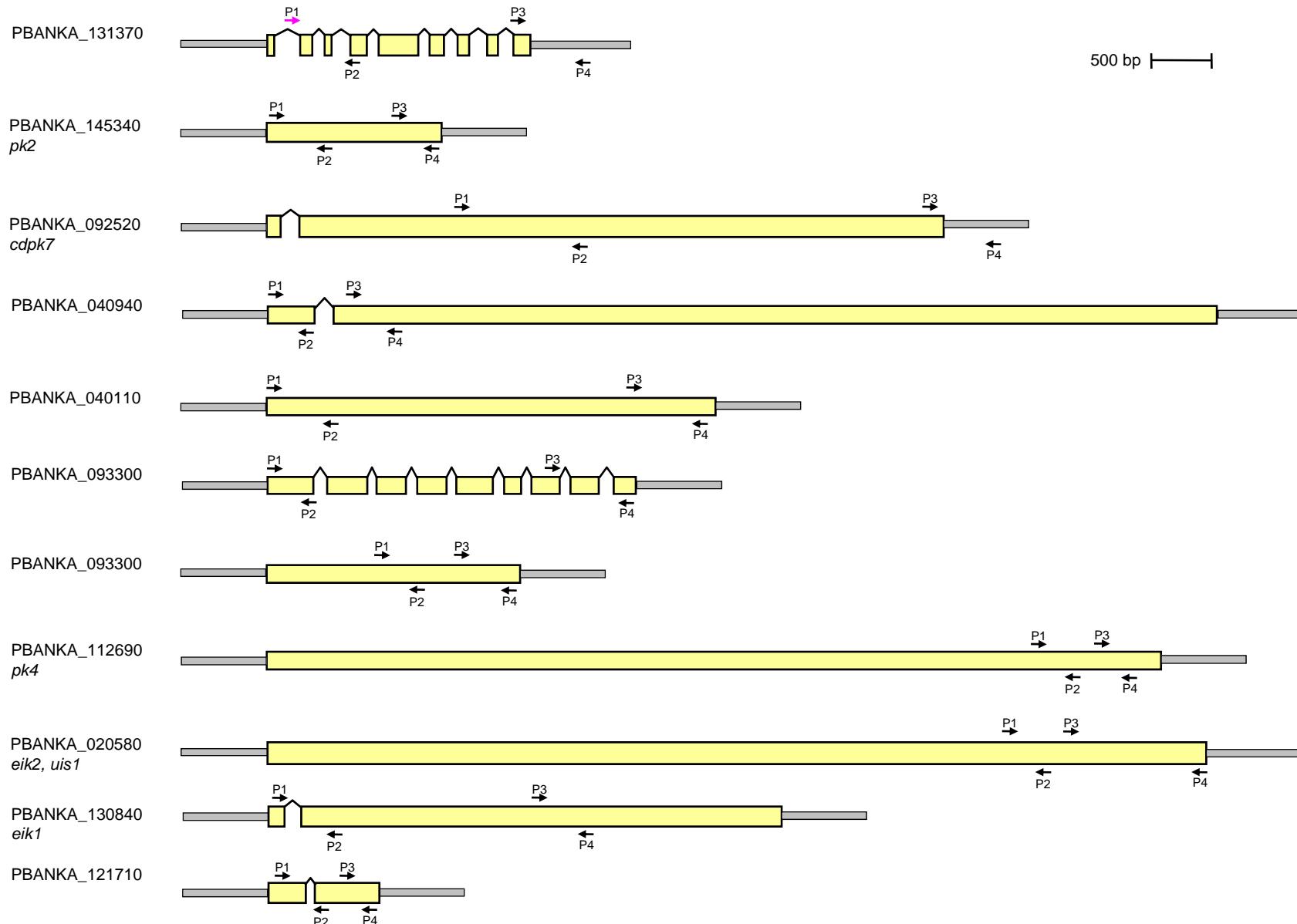
Tewari et al., Figure S2 A (cont.)



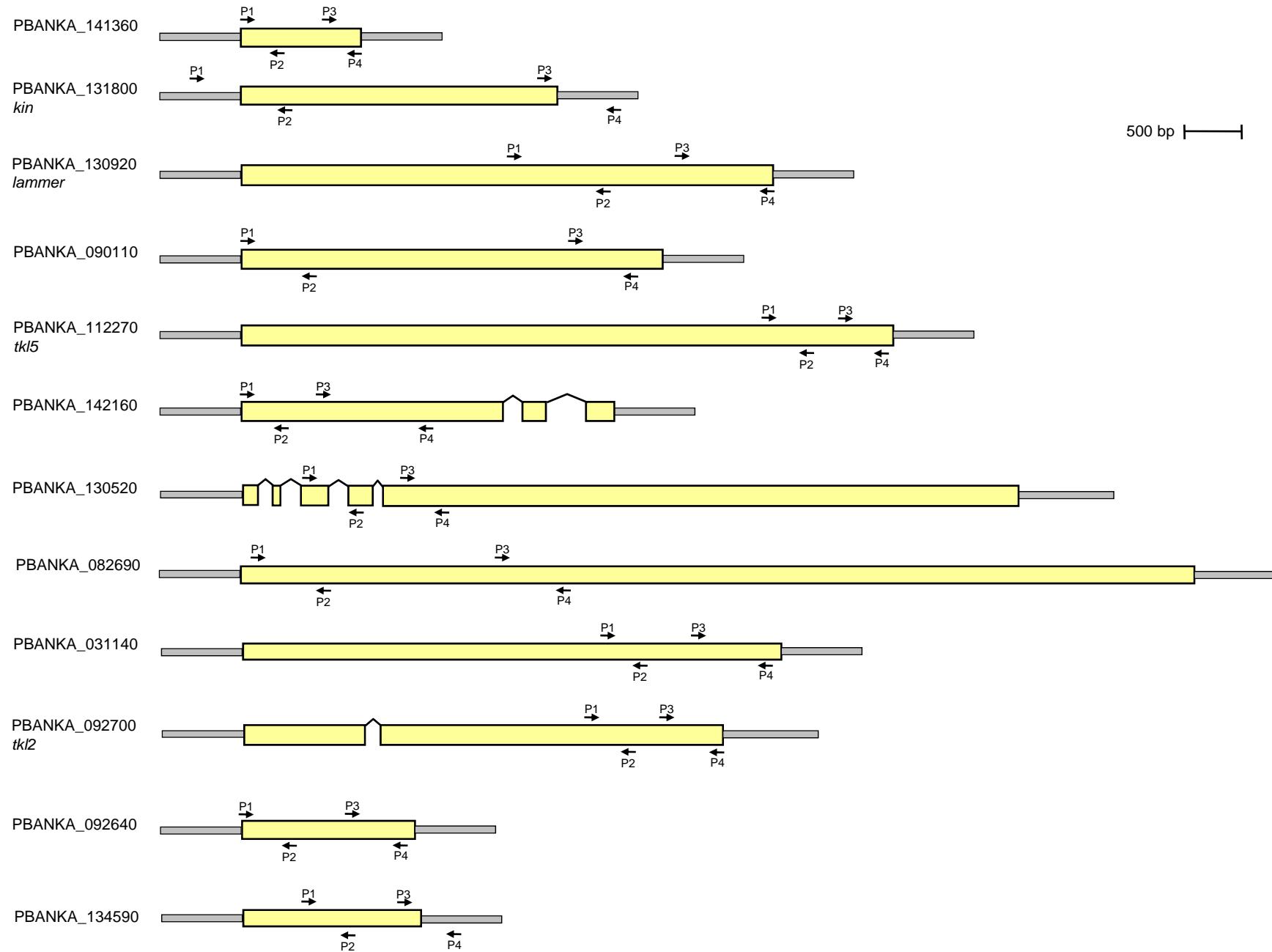
Tewari et al., Figure S2 A (cont.)



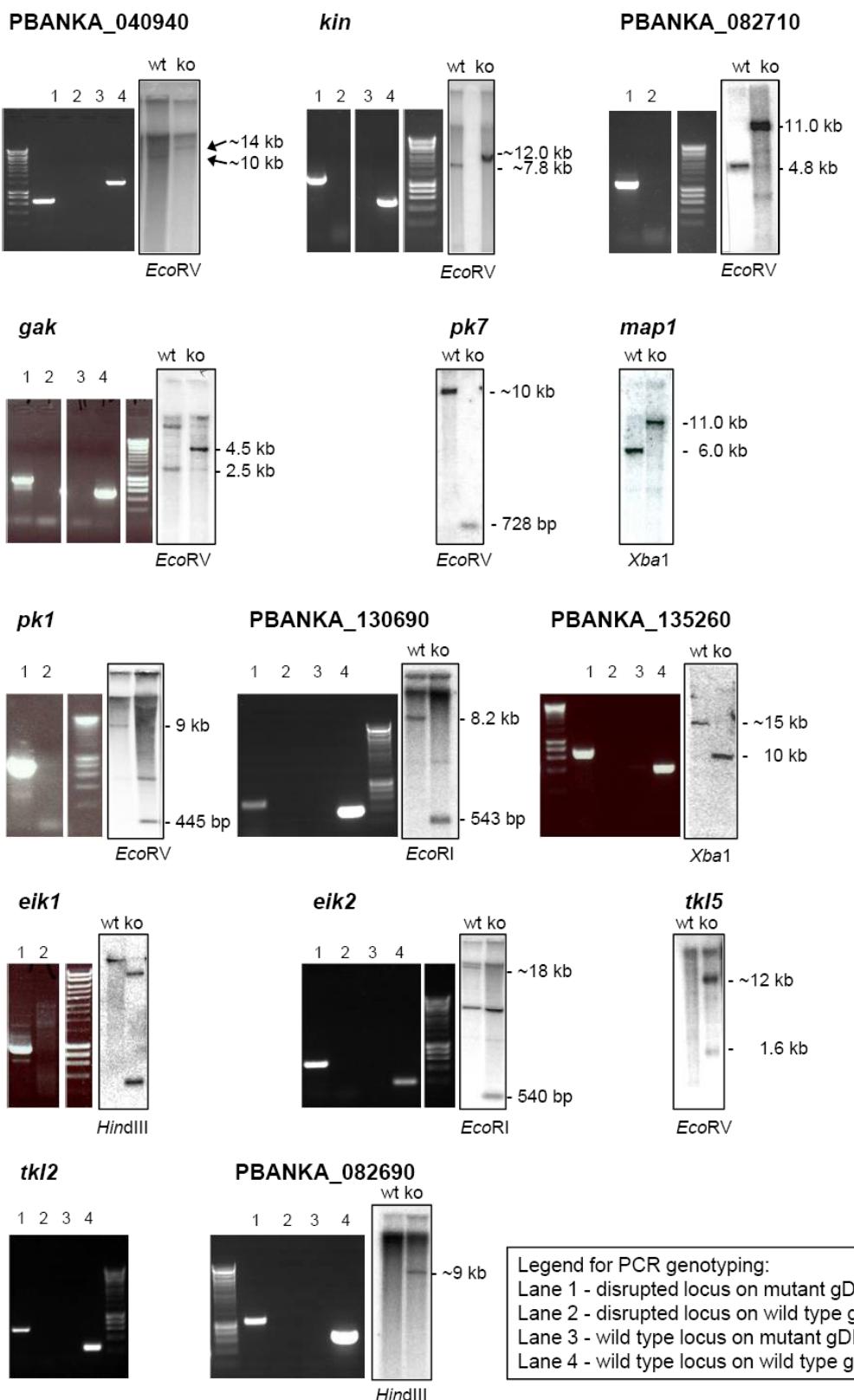
Tewari et al., Figure S2 A (cont.)



Tewari et al., Figure S2 A (cont.)



Tewari et al., Figure S2B



**Figure S2 (relates to Figure 2). Vector Designs and Genotyping Data**

(A) Description of gene targeting vectors. Gene models are shown as predicted in the May 2010 assembly of the *P. berghei* ANKA genome and have not been verified experimentally.

(B) PCR and Southern hybridisations confirming the disruption of 14 ePK genes. PCR genotyping (on the left) used the same rationale as illustrated in Figure 2. Southern blots (on the right) were routinely probed with gene-specific probes flanking the integration site. Diagnostic bands confirming correct integration are indicated by arrows. In a few cases evidence from only one method of genotyping was obtained. Additional details are available from the authors on request.

**Table S1 (relates to Fig.1): List of all *P. berghei* kinase-like genes and their *P. falciparum* orthologs.** Gatekeeper residues are taken from the multiple sequence alignment in Fig. S1, or from a manual alignment where indicated. Also shown are the total number of knock out attempts and how many of these led to deletion of the target as confirmed by genotype analysis. "p" gives an estimate for the likelihood of error for "possibly essential" genes. It is calculated from the average technical failure rate (0.248 per attempt for redundant genes in this study) to the power of n, where n is the total number deletion attempts for the gene. \* = genes for which no mutant clones were obtained despite positive genotyping

Gene name	<i>P. berghei</i> ID	<i>P. falciparum</i> ortholog	Group	"Gatekeeper"		Total	Successful	Outcome	p
					Knock out attempts				
<i>cdpk1</i>	PBANKA_031420	PFB0815w	CaMK	T		6	0	Possibly essential	0.00024
<i>cdpk2</i>	no ortholog	PFF0520w	CaMK					n/a	
<i>cdpk3</i>	PBANKA_040820	PFC0420w	CaMK	M		4	3	KO confirmed	
<i>cdpk4</i>	PBANKA_061520	PF07_0072	CaMK	S		7	7	KO confirmed	
<i>cdpk5</i>	PBANKA_135150	PF13_0211	CaMK	L		6	0	Possibly essential	0.00024
<i>cdpk6</i>	PBANKA_092550	PF11_0239	CaMK	M		16	12	KO confirmed	
<i>cdpk7</i>	PBANKA_092520	PF11_0242	CaMK	M		5	0	Possibly essential*	0.00095
<i>cdlk</i>	PBANKA_101980	PF14_0227	CaMK	L		11	8	KO confirmed	
<i>pk2</i>	PBANKA_145340	PFL1885c	CaMK	L		4	0	Possibly essential	0.00382
	PBANKA_040940	PFC0485w	CaMK	M		5	3	KO confirmed	
	PBANKA_094200	PF11_0060	CaMK	M				Not done	
	PBANKA_082960	PFI1415w	CaMK	?		5	0	Possibly essential	0.00095
	PBANKA_031140	PFB0665w	CaMK	M		5	0	Possibly essential	0.00095
	PBANKA_080220	MAL7P1.18	CaMK	M				Not done	
	PBANKA_131800	PF14_0516	CaMK, Snf1-like	L		4	3	KO confirmed	0.00382
	PBANKA_131370	PF14_0476	CaMK, Snf1-like	L		4	0	Possibly essential	0.00382
	PBANKA_141360	PF13_0085	CaMK, Snf1-like	M		9	0	Possibly essential	3.6E-06
<i>pka</i>	PBANKA_083560	PFI1685w	AGC	L		3	0	Possibly essential	0.01535
<i>pkg</i>	PBANKA_100820	PF14_0346	AGC	T		3	0	Possibly essential	0.01535
<i>pkb</i>	PBANKA_146000	PFL2250c	AGC	L		6	0	Possibly essential	0.00024
	PBANKA_092640	PF11_0227	AGC	M		8	0	Possibly essential	1.5E-05
	PBANKA_040740	PFC0385c	AGC	L		3	0	Possibly essential	0.01535
	PBANKA_082710	PFI1285w	AGC	L		3	3	KO confirmed	
	PBANKA_113310	MAL13P1.278	orphan	L		4	0	Possibly essential	0.00382
	PBANKA_090380	PF11_0464	orphan	Q		5	0	Possibly essential	0.00095
	PBANKA_010410	PFF0260w	orphan	M		8	0	Possibly essential	1.5E-05
<i>gak</i>	PBANKA_146050	PFL2280w	GAK	M		4	3	KO confirmed	
<i>pk7</i>	PBANKA_031030	PFB0605w	orphan	N		9	5	KO confirmed	
<i>est</i>	PBANKA_061580	MAL7P1.91	orphan	I (ma		4	0	Possibly essential	0.00382
<i>ck1</i>	PBANKA_091210	PF11_0377	CK1	L		4	0	Possibly essential	0.00382
<i>pk5</i>	PBANKA_113320	MAL13P1.279	CMGC, CRK	F		2	2	KO confirmed	
<i>mrk</i>	PBANKA_121280	PF10_0141	CMGC, CRK	M		6	0	Possibly essential	0.00024
<i>crk-1</i>	PBANKA_071990	PFD0865c	CMGC, CRK	M		3	0	Possibly essential	0.01535
<i>crk-3</i>	PBANKA_071730	PFD0740w	CMGC, CRK	F		4	0	Possibly essential	0.00382
<i>crk-5</i>	PBANKA_123020	PFF0750w	CMGC, CRK	Y		5	0	Possibly essential	0.00095
<i>crk-6; PK6</i>	PBANKA_135090	MAL13P1.185	CMGC, CRK	F		6	0	Possibly essential	0.00024
<i>map-1</i>	PBANKA_101330	PF14_0294	CMGC, MAPK	F		2	2	KO confirmed	
<i>map-2</i>	PBANKA_093370	PF11_0147	CMGC, MAPK	L		4	3	KO confirmed	
<i>ck2</i>	PBANKA_093860	PF11_0096	GMGC, CK2	F		4	0	Possibly essential	0.00382
<i>gsk-3</i>	PBANKA_041040	PFC0525c	CMGC, GSK3	M		9	0	Possibly essential	3.6E-06
<i>pk1</i>	PBANKA_070970	PF08_0044	CMGC, GSK3	M (ma		4	3	KO confirmed	
	PBANKA_141450	MAL13P1.84	CMGC, GSK3	F		5	0	Possibly essential	0.00095
<i>lammer</i>	PBANKA_130920	PF14_0431	CMGC, SRPK	F		3	0	Possibly essential	0.01535
<i>srpk</i>	PBANKA_040110	PFC0105w	CMGC, SRPK	F		5	4	KO confirmed	
<i>prk4</i>	PBANKA_093300	PF11_0156	CMGC, SRPK	F		4	0	Possibly essential	0.00382
	PBANKA_130690	PF14_0408	CMGC, SRPK	T		3	3	KO confirmed	
<i>crk-4</i>	PBANKA_080800	PFC0755c	CMGC	? (po		4	0	Possibly essential	0.00382
	no ortholog	PFB0150c	CMGC					n/a	
	PBANKA_135260	MAL13P1.196	CMGC	F		2	2	KO confirmed	
<i>nek-2</i>	PBANKA_124070	PFE1290w	CMGC, NEK	M		7	6	KO confirmed	

<i>nek-4</i>	PBANKA_061670	MAL7P1.100	CMGC, NEK	M	2	2	KO confirmed	
<i>nek-1</i>	PBANKA_144300	PFL1370w	CMGC, NEK	M	8	0	Possibly essential	1.5E-05
<i>nek-3</i>	PBANKA_060060	PFL0080c	CMGC, NEK	I	7	0	Possibly essential	5.9E-05
<i>eik1</i>	PBANKA_130840	PF14_0423	CMGC, ELK	M	4	2	KO confirmed	
<i>eik2; uis1</i>	PBANKA_020580	PFA0380w	CMGC, ELK	M	5	3	KO confirmed	
<i>pk4</i>	PBANKA_112690	PFF1370w	CMGC, ELK	M	3	0	Possibly essential	0.01535
	PBANKA_090110	PF11_0488	orphan	M	9	0	Possibly essential	3.6E-06
	PBANKA_130520	PF14_0392	orphan	M	6	0	Possibly essential*	0.00024
	PBANKA_142160	MAL7P1.73	orphan	L	3	0	Possibly essential	0.01535
<i>tkl6</i>	PBANKA_122880	no ortholog	TKL	L	5	0	Possibly essential	0.00095
<i>tkl5</i>	PBANKA_112270	PFF1145c	TKL	L	3	3	KO confirmed	
<i>tkl2</i>	PBANKA_092700	PF11_0220	TKL	Y	4	1	KO confirmed	
<i>tkl1 (raf)</i>	PBANKA_030850	PFB0520w	TKL	L	3	0	Possibly essential	0.01535
<i>tkl3</i>	PBANKA_136210	PF13_0258	TKL	L	5	0	Possibly essential*	0.00095
<i>tkl4</i>	PBANKA_101090	PF14_0320	TKL	M	3	0	Possibly essential	0.01535
	PBANKA_082690	PFI1280c	orphan	T	3	2	KO confirmed	
	PBANKA_080560	MAL7P1.26	orphan	F	5	0	Possibly essential	0.00095
	PBANKA_121710	PFC0945w	pseudokinase	n.d.	5	0	Possibly essential	0.00095
	PBANKA_094010	PF11_0079	pseudokinase	n.d.	4	0	Possibly essential	0.00382
	PBANKA_082680	PFI1275w	pseudokinase	n.d.	4	0	Possibly essential	0.00382
	PBANKA_134590	PF13_0166	pseudokinase	n.d.	3	0	Possibly essential	0.01535
	PBANKA_062150	MAL7P1.127	pseudokinase	n.d.	5	0	Possibly essential	0.00095
<i>rio1</i>	PBANKA_144560	PFL1490w	aPK, RIO		3	0	Possibly essential	0.01535
<i>rio2</i>	PBANKA_052140	PFD0975w	aPK, RIO		4	0	Possibly essential	0.00382
<i>fikk</i>	PBANKA_122500	MAL8P1.203	FIKK, ancestral	S	5	0	Possibly essential	0.00095
	no ortholog	MAL7P1.144	FIKK				n/a	
<i>R45</i>	no ortholog	PFD1175w	FIKK				n/a	
	no ortholog	PFI0095c	FIKK				n/a	
	no ortholog	PFI0100c	FIKK				n/a	
	no ortholog	PFI0105c	FIKK				n/a	
	no ortholog	PFI0110c	FIKK				n/a	
	no ortholog	PFI0115c	FIKK				n/a	
	no ortholog	PFI0120c	FIKK				n/a	
	no ortholog	PFI0125c	FIKK				n/a	
	no ortholog	PFL0040c	FIKK				n/a	
	no ortholog	PFD1165w	FIKK				n/a	
	no ortholog	PFE0045c	FIKK				n/a	
	no ortholog	PFC0060c	FIKK				n/a	
	no ortholog	PF14_0734	FIKK				n/a	
	no ortholog	PFA0130c	FIKK				n/a	
	no ortholog	PF11_0510	FIKK				n/a	
	no ortholog	PF10_0160	FIKK				n/a	
	no ortholog	MAL7P1.175	FIKK				n/a	
	no ortholog	PF10_0380	FIKK				n/a	
	no ortholog	MAL13P1.109	FIKK				n/a	

sum: 0.23139

**Table S2. Unique ePKs of *P. berghei*/*P. falciparum* and Their Distribution across Other *Plasmodium* Genomes**

	<i>P. berghei</i>	<i>P. yoelii</i>	<i>P. chabaudi</i>	<i>P. vivax</i>	<i>P. knowlesi</i>	<i>P. falciparum</i> 3D7	<i>P. reichenowi</i>	<i>P. gallinaceum</i>
<i>tkl6</i>	PBANKA_122880	<u>PY04849</u> <u>PY04848</u>	<u>PCAS_122950</u>	<u>PVX_093670</u>	<u>PKH_011700</u>	—	—	Pgal0325e01.p1k Pgal0616c03.p1k Pgal0616c03.q1k Pgal0489d11.p1k
<b>PFB0150c</b>	—	—	—	<u>PVX_003590</u>	<u>PKH_041680</u>	PFB0150c	3502696.c000217971. Contig1	gal28a.d0000062 93. Contig1
<i>cdpk2</i>	—	—	—	—	—	PFF0520W	3502696.c000218538. Contig1	gal28a.d0000019 39. Contig1
<b>Ancestral fikk</b>	PBANKA_122500	<u>PY03326</u>	<u>PCAS_122560</u>	<u>PVX_088265</u>	<u>PKH_011260</u>	MAL8P1.203	3502696.c000024654. Contig1	gal28a.d0000159 42. Contig1
<i>fikk expansion</i>	none	none	none	none	none	20	yes	none

**Table S3 (relates to Fig. 3). Data from phenotyping 23 mutants.** All data are given as percentage of wild type controls studied in parallel. SD = standard deviation. n = number of replicate experiments.

Gene name	<i>P. berghei</i> ID	Ookinete conversion			Oocyst numbers			Oocyst prevalence			Oocyst sporozoites			Salivary gland sporozoites			Transmission by mosquito bite
		% of control	SD	n	% of control	SD	n	% of control	SD	n	% of control	SD	n	% of control	SD	n	
<i>cdpk3</i>	PBANKA_040820	<b>94.65</b>	6.97	3	<b>3.30</b>	2.79	8	n.d.			n.d.			<b>0.00</b>	0.00	2	n.d.
<i>cdpk4</i>	PBANKA_061520	<b>0.00</b>	0.00	4	<b>0.00</b>	0.00	4	0.00	0.00	4	n.d.			<b>0.00</b>	0.00	4	n.d.
<i>cdpk6</i>	PBANKA_092550	<b>90.41</b>	11.70	3	<b>91.20</b>	11.94	3	<b>98.35</b>	5.79	3	<b>152.22</b>	19.18	3	<b>28.35</b>	44.69	3	transmitted (delayed)
<i>cdlk</i>	PBANKA_101980	<b>84.90</b>	7.63	6	<b>104.66</b>	17.48	3	<b>102.79</b>	13.03	3	<b>41.66</b>	18.50	3	<b>4.16</b>	1.70	3	transmitted
	PBANKA_040940	<b>70.59</b>	14.26	3	<b>49.04</b>	23.30	6	<b>97.36</b>	16.94	6	<b>132.61</b>	18.13	3	<b>9.98</b>	2.11	6	no transmission (0/3)
<i>kin</i>	PBANKA_131800	<b>89.00</b>	6.76	3	<b>105.28</b>	29.73	3	<b>103.85</b>	0.00	3	<b>148.56</b>	27.35	3	<b>4.65</b>	1.03	3	delayed to 15d
	PBANKA_082710	<b>97.11</b>	7.82	2	<b>91.58</b>	2.23	2	<b>95.56</b>	6.29	2	<b>52.93</b>	0.27	2	<b>51.24</b>	16.37	2	transmitted
<i>gak</i>	PBANKA_146050	<b>4.62</b>	1.54	3	<b>3.28</b>	3.60	6	<b>47.98</b>	32.20	6	<b>0.00</b>	0.00	3	<b>0.00</b>	0.00	3	n.d.
<i>pk7</i>	PBANKA_031030	<b>8.92</b>	3.70	8	<b>8.88</b>	5.59	4	<b>63.45</b>	8.63	4	<b>0.00</b>	0.00	7	<b>0.00</b>	0.00	3	n.d.
<i>pk5</i>	PBANKA_113320	<b>87.37</b>	14.24	3	<b>56.43</b>	2.28	3	<b>94.44</b>	9.62	3	<b>107.63</b>	18.95	3	<b>65.27</b>	16.06	3	transmitted
<i>map-1</i>	PBANKA_101330	<b>93.68</b>	15.82	3	<b>63.33</b>	23.43	3	<b>87.72</b>	12.15	3	<b>92.97</b>	18.31	3	<b>101.53</b>	23.25	3	transmitted
<i>map-2</i>	PBANKA_093370	<b>0.00</b>	0.00	3	<b>0.00</b>	0.00	3	<b>0.00</b>	0.00	3	n.d.			<b>0.00</b>	0.00	3	n.d.
<i>pk1</i>	PBANKA_070970	<b>98.40</b>	8.84	3	<b>87.36</b>	31.51	3	<b>104.00</b>	6.97	3	<b>98.82</b>	13.24	3	<b>104.79</b>	25.44	3	transmitted
<i>srpk</i>	PBANKA_040110	<b>0.00</b>	<b>0.00</b>	3	<b>0.00</b>	0.00	3	<b>0.00</b>	0.00	3	n.d.			n.d.		n.d.	n.d.
	PBANKA_130690	<b>98.50</b>	9.17	3	<b>106.15</b>	15.51	3	<b>100.00</b>	6.66	3	<b>128.00</b>	45.67	3	<b>124.94</b>	16.37	3	transmitted
	PBANKA_135260	<b>95.79</b>	10.15	3	<b>57.46</b>	9.47	3	<b>103.70</b>	8.49	3	<b>136.00</b>	28.09	3	<b>66.41</b>	6.58	3	transmitted
<i>nek-2</i>	PBANKA_124070	<b>0.00</b>	0.00	3	<b>0.00</b>	0.00	3	<b>0.00</b>	0.00	3	n.d.			n.d.		n.d.	n.d.
<i>nek-4</i>	PBANKA_061670	<b>0.00</b>	0.00	3	<b>0.00</b>	0.00	3	<b>0.00</b>	0.00	3	n.d.			n.d.		n.d.	n.d.
<i>eik1</i>	PBANKA_130840	<b>84.47</b>	11.65	3	<b>109.68</b>	55.03	3	<b>102.44</b>	26.38	3	<b>108.54</b>	11.61	3	<b>94.40</b>	26.16	3	transmitted
<i>eik2; uis1</i>	PBANKA_020580	<b>51.87</b>	19.40	3	<b>19.86</b>	15.46	3	<b>64.22</b>	44.57	3	n.d.			<b>14.89</b>	10.80	3	transmitted (4/4)
<i>tkl5</i>	PBANKA_112270	<b>83.08</b>	11.10	3	<b>77.46</b>	36.88	3	<b>100.00</b>	5.97	3	<b>144.73</b>	26.40	3	<b>102.34</b>	19.33	3	transmitted
<i>tkl2</i>	PBANKA_092700	<b>79.45</b>	13.07	3	<b>124.24</b>	15.96	3	<b>121.46</b>	2.53	3	<b>92.46</b>	5.29	3	<b>83.12</b>	10.89	3	transmitted
	PBANKA_082690	<b>83.11</b>	3.45	3	<b>84.21</b>	9.25	3	<b>94.83</b>	7.90	3	<b>84.55</b>	11.57	3	<b>81.98</b>	8.77	3	transmitted

**Table S4. *P. berghei* Protein Kinase Domain Sequences Used in This Study**

```
>PBANKA_101090 PbTKL4
ITFLKKINEHGNKGIFLCSYNIFNNKFFIILKIDIANNINENYIFKNIFNEVKCLLTQNSKKGICQMYSYGIIP
NCNNNGFTYLLMKYYEGNLKDLNYAHISYLKEIKKIRNNAKQFFLPIVCKKRTEEILNFKIAKITKKITKRC
KGNFIFCYKLCRYTNVYSCYTMILKNRIYIKIQLKFILFILNIFIQIIIEQIIVVHKKKIHFIDINTCNILINF
KKTYPLMSKKYKIKKNNYINNCNTKQTLKNIQSIVMRDDFVENERKIYLNFIPPSYQINNSIYKKQNKFYNMF
DTMASFHYRQYFKIIMKKDCQHIAIPSIVISDFGESKFFLRDTEFIFFRKRGNEILAPELLINKNKYIYSK
NKYKIEKKNGKEDTEIKSRKAIQDSFYFENIPQNIIFQNEQSEMYFYSTITCFENNCKFCLNTKNDKIRILY
NNINMKSLRKNSIVSLEFYKYLKKIKMLIKKKLGQNQKQLKKKINMSKSDIWLSGSLLYEMITNESLFNYYNFY
YIKIYEKKELLNELINKKIKTQFKELEYKFFFQFDLKKRKDVYEIYKESIKIYNFY

>PBANKA_061580 PbEST
LKLMHIIFGKKYKCYKNENDKMCIGLCYELKKIKIKLNNSVIEIKYPKFDIYDNIKAYNCGLTESECKFLFFQ
IVNGISFLQTCYQSNIRLTDIQLQNIVLFTDIYNVNPWKHL CISDFGCSAMEYATFYLENSKNYINAYKTLL
NQWQYQFKNQLSSYFQGTVYTMAGEGLCYDHMGNFRQSKYKLNLIYFYEQNFGNIEDRFQELQMPLVSIKNSINNF
NILVNNSSNFHGLEKDGKEMNNTYNDMDADKNGSEVCKGETKNSKKSTNKVNKA SANSNGNNNTSSKKK
GKVEENNDKKETSDKINLTEMNNKMNSSPEYLPFDVRSDSLGIILADLGKCGIGSYEYMISEKGNGNNNISKG
NIIDLNNIILNDLNILDDEENFYQYIMNYDILSIQWDNDEIENDQVNKNADRNNKKRDNSEMENKRNNNDNSNNN
GCTNNNYSKKQKNKSLENNNSKEDVPGNNCFVIDNANESISNEVSFCKDKPNAHNNYDIKEETNNKNAVLNQ
IKSTLNFDENKIKGNNDNSMKSLELEFTKYYKSFILKYKDYIKDICKIGYIKNEFVKYKKLYKHMMNENNIEILL
LLFLIIINNLTYNSCETQSFLKIELTIKNIGSGIFKFRNKKEKERYLQEKFANIQKDMYMTGNKEYWNSRLTNKY
LAVILKDVCYDNFANRKKGIKKCFNKFEYPLNYSDDYWNLADLLNYMPYERLLACEVIGHDF

>PBANKA_135260
YSIQNKLGAGAYGEIWYGINLNKNVPFQNVVLKKIFIKSVDESEQNLNDDEREKEYEIYAMREVYFGEIFKNCD
NISRYIEHFKESETSENNKEHITFIWIVFANEGYLSQHFLFETDKNNSGMIIPKLWWSIKKQNIGMLVIKDLHQ
LLNGINIAHKKHITHRDIKMENIFVSPNTPFTVRIGDWGSAVEYKNKSFFFFPSMEEETEGYQPPESLFGHMKKN
FMRPLPYDDMWGIGILFLQFVLGTKNPLEIKNKRNEMLKRIYSKSKDILKEVIFIQGLSDLCLIPWVQTPDRL
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