



An intracellular membrane protein GEP1 regulates xanthurenic acid induced gametogenesis of malaria parasites

Yuanyuan Jiang^{1,4}, Jun Wei^{1,4}, Huiting Cui^{1,4}, Chuanyuan Liu¹, Yuan Zhi¹, ZhengZheng Jiang¹, Zhenkui Li¹, Shaoneng Li¹, Zhenke Yang¹, Xu Wang¹, Pengge Qian¹, Cui Zhang¹, Chuanqi Zhong¹, Xin-zhuan Su³ & Jing Yuan^{1,2}  

Gametocytes differentiation to gametes (gametogenesis) within mosquitos is essential for malaria parasite transmission. Both reduction in temperature and mosquito-derived XA or elevated pH are required for triggering cGMP/PKG dependent gametogenesis. However, the parasite molecule for sensing or transducing these environmental signals to initiate gametogenesis remains unknown. Here we perform a CRISPR/Cas9-based functional screening of 59 membrane proteins expressed in the gametocytes of *Plasmodium yoelii* and identify that GEP1 is required for XA-stimulated gametogenesis. GEP1 disruption abolishes XA-stimulated cGMP synthesis and the subsequent signaling and cellular events, such as Ca²⁺ mobilization, gamete formation, and gametes egress out of erythrocytes. GEP1 interacts with GC α , a cGMP synthesizing enzyme in gametocytes. Both GEP1 and GC α are expressed in cytoplasmic puncta of both male and female gametocytes. Depletion of GC α impairs XA-stimulated gametogenesis, mimicking the defect of GEP1 disruption. The identification of GEP1 being essential for gametogenesis provides a potential new target for intervention of parasite transmission.

¹State Key Laboratory of Cellular Stress Biology, Innovation Center for Cell Signaling Network, School of Life Sciences, Xiamen University, 361102 Xiamen, Fujian, China. ²Lingnan Guangdong Laboratory of Modern Agriculture, 510642 Guangzhou, China. ³Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA. ⁴These authors contributed equally: Yuanyuan Jiang, Jun Wei, Huiting Cui. ✉email: yuanjing@xmu.edu.cn

Male and female gametocytes are sexual precursor cells essential for malaria parasite transmission. Within 10–15 min after being taken up by a mosquito, gametocytes differentiate into gametes in mosquito midgut, a process known as gametogenesis. A female gametocyte forms a rounded female gamete, whereas a male gametocyte undergoes three mitotic divisions, assembles eight intracytoplasmic axonemes, and produces eight flagellated male gametes¹. Both male and female gametes egress from their residing erythrocytes via an inside-out mechanism, during which the parasitophorous vacuole membrane (PVM) ruptures prior to the opening of the erythrocyte membrane (EM)². After the release from erythrocytes, the male and female gametes fertilize to produce zygotes and then the motile ookinetes that penetrate mosquito midgut wall to develop into oocysts each containing thousands of sporozoites. The sporozoites then migrate to mosquito salivary glands and are injected into a new host when the mosquito bites again.

Gametogenesis is triggered by two stimuli, a drop in temperature of approximately 5 °C^{3,4} and the presence of xanthurenic acid (XA) that is a metabolite of tryptophan from mosquito^{5,6}. An additional signal reported to induce gametogenesis is an increase in pH from 7.4 to 8⁴. Since the groundbreaking discovery of XA as a trigger for *Plasmodium* gametogenesis in mosquitoes, studies have shown that XA can enhance parasite guanylyl cyclase (GC) activity on gametocyte membrane fraction, leading to increased level of second messenger 3′–5′-cyclic guanosine monophosphate (cGMP)⁷. Two integral membrane GC proteins (GC α and GC β) are found in *Plasmodium* parasites. GC α has been implicated to be responsible for cGMP synthesis during gametogenesis because disruption of GC β has no effect on XA-induced gametogenesis^{8–10}. The increased level of cGMP activates cGMP-dependent protein kinase G (PKG) that functions as a master regulator of the downstream signaling events during gametogenesis¹¹. Inhibition of PKG using Compound 2 (C2) prevented gametocytes rounding up, gamete formation of both sexes, and gametes egress from erythrocytes in *P. falciparum* and *P. berghei*^{11,12}. PKG-dependent Ca²⁺ mobilization was also observed in the cytosol of *P. falciparum* and *P. berghei* gametocytes 10–15 s after addition of XA^{13,14}. PKG activates the synthesis of inositol (1,4,5)-trisphosphate (IP3) via phosphoinositide metabolism and triggers cytosolic mobilization of Ca²⁺ that likely originates from the endoplasmic reticulum¹⁵. Unfortunately, the molecule(s) responsible for sensing XA or transducing the XA-stimulated signal to activate the cGMP-PKG signaling remain unknown.

Membrane proteins are known to play critical roles in sensing, transporting, and/or transducing environmental signals to initiate cellular responses. To identify potential molecules involved in sensing or transducing XA signal during gametogenesis, we perform CRISPR/Cas9-mediated genetic deletion screens of 59 candidate genes encoding integral membrane proteins expressed in gametocytes of the rodent malaria parasite *P. yoelii*. We identify a multiple-spanning membrane protein GEP1 (gametogenesis essential protein 1) that was essential for XA-stimulated gametogenesis. Disruption of GEP1 completely abolishes XA-stimulated gametogenesis of both sexes. Parasites deficient of GEP1 show no synthesis of XA-stimulated cGMP and no downstream cellular and signaling events such as Ca²⁺ mobilization, parasite egress out of PVM and EM, genome replication and axoneme assembly in male gametocytes, and release of translational repression in female gametocytes. GEP1 interacts with GC α in gametocytes, and GC α depletion also impairs XA-stimulated gametogenesis, mimicking the effects of GEP1 disruption. This study identifies a molecule essential for the initiation of gametogenesis and a potential target for blocking parasite transmission.

Results

GEP1 is essential for XA-stimulated gametogenesis. To identify membrane proteins critical in sensing XA or transducing XA-induced signal during gametogenesis, we identified 59 *P. yoelii* genes that are expressed in gametocytes and encode proteins with 1 to 22 predicted transmembrane domains (TMs) from the PlasmoDB database (Supplementary Table 1). We designed single guide RNA (sgRNA) to disrupt each of these genes using CRISPR/Cas9 methods^{16,17} and were able to successfully knock-out (KO) 45 (76%) of the genes in the *P. yoelii* 17XNL strain, obtaining at least two cloned lines for each mutant (Supplementary Fig. 1a, c, d, i). The remaining 14 genes (24%) were refractory to repeated deletion attempts using three independent sgRNA sequences, suggesting their essential roles for asexual blood-stage growth.

The 45 gene deletion mutants proliferated asexually in mouse blood normally and were able to produce both male and female gametocytes although the gametocytemia level varied among these mutants (Supplementary Fig. 2, Supplementary Fig. 3a). Next we measured the gametogenesis of male gametocyte by counting exflagellation centers (ECs) formed in vitro after stimulation with 50 μ M XA at 22 °C. Only one mutant (PY17X_1116300 disruption) showed complete deficiency in EC formation and male gamete release (Fig. 1a–c). The PY17X_1116300 gene contains four exons (Fig. 1d) encoding a putative amino acid transporter protein that is essential for gametogenesis; we therefore name the gene *gep1* for gametogenesis essential protein 1. As controls, disruption of *P. yoelii* *cdpk4* or *map2* also caused defect in EC formation (Fig. 1a), confirming the phenotypes observed in *P. berghei*^{13,18}. Consequently, the Δ *gep1* mutant parasite produced no ookinete in in vitro culture (Supplementary Fig. 3b), oocyst in *Anopheles stephensi* midgut (Fig. 1f), or sporozoite in mosquito salivary gland (Supplementary Fig. 3c).

To further confirm the phenotype of Δ *gep1*, we generated three additional *gep1* mutant parasites (Δ *gep1n*, Δ *gep1fl*, and Δ *gep1mScarlet*) (Fig. 1d, Supplementary Fig. 1c–e). The Δ *gep1n* parasite had a 464 bp deletion at the 5′ coding region, causing a frameshift for the remaining coding region. The Δ *gep1fl* parasite had the whole *gep1* coding region deleted, and the Δ *gep1mScarlet* parasite had its *gep1* coding regions replaced with a gene encoding red fluorescent protein mScarlet. These mutations were confirmed by PCR and DNA sequencing (Supplementary Fig. 1j, k), and the mutant parasites displayed developmental phenotypes similar to those of Δ *gep1* in both mouse and mosquito stages (Fig. 1e, f, Supplementary Fig. 3a–c). We also reintroduced the 558 bp deleted segment plus a sextuple HA epitope (6HA) into the Δ *gep1* parasite to rescue the gene function using Cas9-mediated homologous replacement (Fig. 1d, Supplementary Fig. 1b, j). Two clones of the rescued parasite (Δ *gep1/gep1::6HA*1 and Δ *gep1/gep1::6HA*2) showed expression of the GEP1::6HA protein in both Western blotting and immunofluorescence analysis (IFA) (Supplementary Fig. 3d, e). Importantly, both clones produced wild type (WT) levels of EC in vitro (Fig. 1e) and midgut oocyst in mosquitoes (Fig. 1f). The GEP1 protein is well-conserved among *P. yoelii*, *P. berghei*, and the human *P. falciparum* parasites (Supplementary Fig. 4), suggesting conserved function. Deletion of *P. berghei* *gep1* gene (PBANKA_1115100) resulted in parasite clones that failed to form XA-stimulated ECs in vitro and midgut oocyst in mosquitoes (Supplementary Fig. 1l, m, Supplementary Fig. 3f–h). Together, these results demonstrate that GEP1 depletion completely block male gametogenesis and mosquito transmission of malaria parasites.

GEP1 is expressed in cytosol puncta of gametocytes. GEP1 is a *Plasmodium*-specific protein with 905 residues and 14 predicted

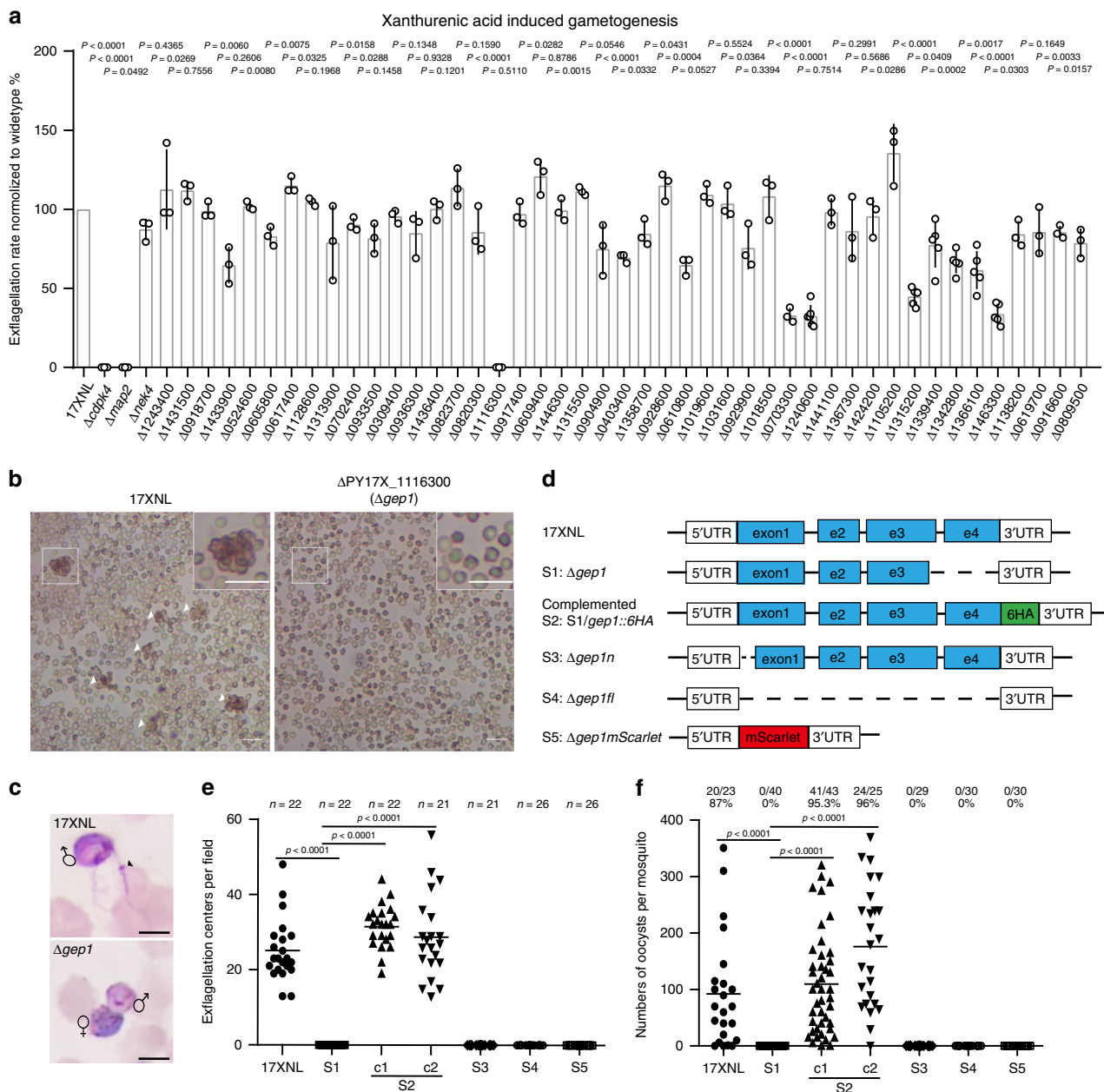


Fig. 1 Membrane proteins screening identified *gep1* essential for gametogenesis. **a** In vitro XA stimulated exflagellation rates for *P. yoelii* 17XNL wild type (WT) and 45 mutant strains each with a specific gene disruption. The exflagellation rate of each mutant was normalized with that of WT parallelly tested each time. The numbers for the gene name are the gene IDs derived in PlasmoDB. Data are shown as mean \pm SD from $n = 3$ independent experiments for strains except $n = 5$ for Δ 1315200, Δ 1339400, Δ 1342800, Δ 1366100 and Δ 1463300, and $n = 6$ for Δ 1240600. **b** Representative images of XA stimulated exflagellation centers (ECs, white arrows) under light microscope (10 \times). Scale bar = 20 μ m. **c** Images of the exflagellated male gametes (Black arrow) after Giemsa staining under light microscope (100 \times). Scale bar = 5 μ m. **d** Diagrams of WT *gep1* gene structure and various mutants: S1 (Δ gep1), deletion in C-terminus; S2 (Δ gep1/gep1::6HA), reconstructed *gep1* with a 6HA tag; S3 (Δ gep1n), deletion in N-terminus; S4 (Δ gep1fl), deletion of the full coding region; S5 (Δ gep1mScarlet), coding region replaced with *mScarlet* gene. **e** XA-stimulated EC counts from WT and the *gep1* mutants. c1 and c2 are two clones of S2 parasite. n is the numbers of microscopic fields counted (40 \times). **f** Oocyst counts from WT and the *gep1* mutants. Oocysts are counted from the mosquito midguts 7 days post blood feeding. x/y on the top is the number of mosquito containing oocyst/the number of mosquito dissected; the percentage number is the mosquito infection prevalence. Experiments were independently repeated six times in **b**, and three times in **c**, **e**, and **f**. Two-tailed unpaired Student's *t* test was applied in **a**, **e**, and **f**. Source data of **a**, **e**, and **f** are provided as a Source Data file.

TMs (Fig. 2a). Previous transcriptomic study indicated the *gep1* gene is transcribed in gametocytes and ookinetes, but not asexual blood stages of *P. falciparum* and *P. berghei*^{19,20}. To investigate protein expression and localization, we tagged the endogenous *GEP1* with 6HA at N-terminus (Supplementary Fig. 1g, j), generating 6HA::*gep1* parasite that had normal development throughout the life cycle (Supplementary Fig. 5a). The *GEP1*

protein is expressed in gametocytes and ookinetes, but not in asexual blood stages and other mosquito stages of the 6HA::*gep1* parasite (Fig. 2b, c). We also tagged the *GEP1* protein with quadruple Myc (4Myc) (Supplementary Fig. 1j, Supplementary Fig. 5b) and observed similar expression pattern in the 4Myc::*gep1* parasite (Fig. 2d). In addition, mScarlet fluorescent signals driven by the endogenous *gep1* promoter were detected only in

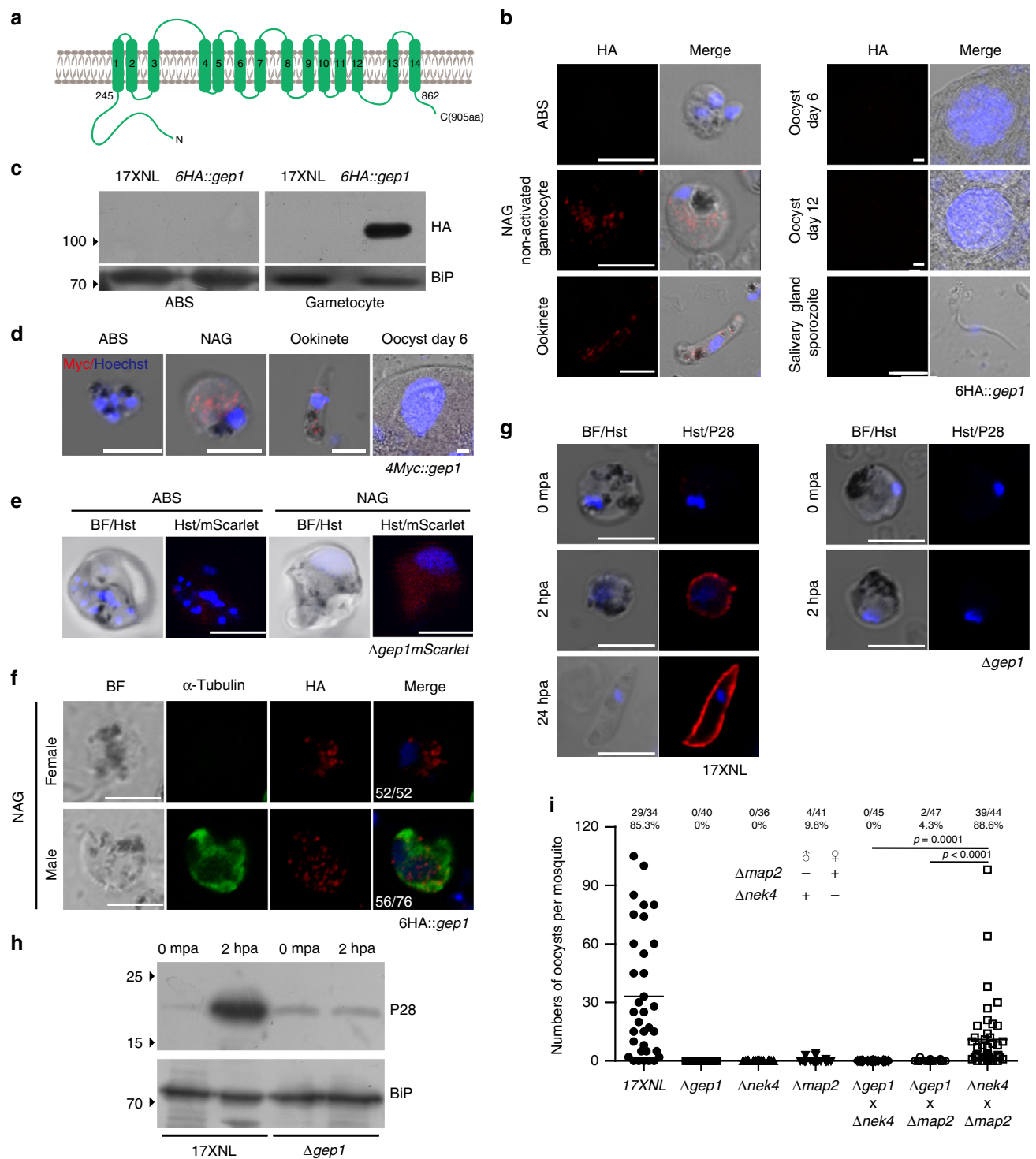


Fig. 2 GEPI is essential for gametogenesis of both sexes. **a** Predicted GEPI protein structure with 14 TM domains (green bar) and cytoplasmic N-termini and C-termini. **b** IFA analysis of GEPI expression in asexual blood stages (ABS), gametocytes, ookinetes, oocysts, and sporozoites of the *6HA::gep1* parasite using anti-HA antibody. Hoechst 33342 (Blue) is used for nuclear acid stain for all images in this figure. **c** Western blot analysis of GEPI in ABS and gametocytes of the *6HA::gep1* parasite. BiP as loading control. **d** IFA analysis of GEPI in the *4Myc::gep1* parasite using anti-Myc antibody. **e** mScarlet fluorescence protein expression driven by the endogenous *gep1* promoter in ABS and gametocytes of the *Δgep1mScarlet* parasite. **f** Co-staining of GEPI and α -Tubulin (male gametocyte specific) in the non-activated (NAG) *6HA::gep1* gametocytes. *x/y* in the figure is the number of cell displaying signal/the number of cell tested. **g** and **h**, P28 expression during in vitro gametocyte to ookinete differentiation. P28 expression is detected in female gametes, fertilized zygotes, and ookinetes in IFA (**g**) and western blot (**h**). mpa: minute post activation; hpa, hour post activation. **i** Day 7 midgut oocyst counts from mosquitoes infected with parasites, including 17XNL, *Δgep1*, *Δnek4*, or *Δmap2* parasite alone, as well as mixtures of *Δgep1/Δnek4*, *Δgep1/Δmap2*, or *Δmap2/Δnek4* parasites. *Δnek4* and *Δmap2* are female and male gamete-defect parasites, respectively. *x/y* on the top is the number of mosquito containing oocyst/the number of mosquito dissected; Mosquito infection prevalence is shown above. Scale bar = 5 μ m for all images in this figure. Experiments were independently repeated three times in **b**, **c**, **d**, **e**, **f**, **g**, and two times in **i**. Two-tailed unpaired Student's *t* test in **i**.

gametocytes, but not in asexual blood stages of the *Δgep1mScarlet* parasite (Fig. 2e). Co-staining 6HA::*gep1* gametocytes with anti- α -Tubulin (male gametocyte specific) and anti-HA antibody showed that GEP1 was expressed in both male and female gametocytes (Fig. 2f). Interestingly, GEP1 is not expressed in plasma membrane, but in punctate dots in the cytoplasm of gametocytes and ookinetes (Fig. 2b, d, f).

GEP1 regulates both male and female gametogenesis. Because GEP1 is expressed in both male and female gametocytes, we asked whether GEP1 also regulates the gametogenesis of female gametocytes. P28 protein, a marker for female gamete²¹, is expressed in female gametes, fertilized zygotes, and ookinetes of 17XNL parasite, but not in the *Δgep1* parasite 2 h after XA-stimulation (Fig. 2g, h), indicating that GEP1 depletion also cause defect in female gametogenesis. We next performed genetic crosses between *Δgep1* and *Δmap2* (male gamete-deficient) or *Δnek4* (female gamete-deficient) parasites^{22,23} (Supplementary Fig. 1j, k). No midgut oocyst was observed in mosquitoes from the *Δgep1* × *Δmap2* or *Δgep1* × *Δnek4* cross day 7 post infection (pi), whereas the *Δmap2* × *Δnek4* cross produced slightly fewer oocysts than the WT parasite (Fig. 2i), suggesting no functional male and female gametes in the *Δgep1* parasite. Together, these results demonstrate that GEP1 is essential for both male and female gametogenesis.

The purified *Δgep1* gametocytes had morphology indistinguishable from that of WT 17XNL parasite (Supplementary Fig. 6a); however, whether GEP1 depletion causes gametocyte death or affects the fitness of gametocytes remains to be determined. We analyzed cell viability by Trypan blue exclusion assay. No gametocyte of WT or *Δgep1* parasites were stained by Trypan blue (Supplementary Fig. 6b). As a control, both gametocytes were stained after heating the parasites at 60 °C for 5 min. In addition, staining with propidium iodide (PI) also indicated that the *Δgep1* gametocytes are viable (Supplementary Fig. 6c). To further confirm the observations, we disrupted the endogenous *gep1* in a *P. yoelii* reporter strain *DFsc7* that expressed GFP and mCherry in male and female gametocytes, respectively²⁴ (Supplementary Fig. 1j, Supplementary Fig. 6d, e). The expressions of fluorescent proteins in both male and female gametocytes were comparable with those of the parental parasite (Supplementary Fig. 6f, g). These results suggest that GEP1-depleted gametocytes are viable, but lost the ability to produce functional male and female gametes.

GEP1 depletion blocks PKG-mediated signaling. Upon stimulation, male gametocytes undergo tubulin polymerization into microtubules and three rounds of genome replication, resulting in release of eight flagellated gametes within 10–15 min²⁵. The lack of exflagellation suggests defect in either axoneme assembly or egress from erythrocyte of the *Δgep1* male gametes. Typical cytosolic distribution of α -Tubulin was observed in male gametocytes of WT, *Δgep1*, and *Δmap2* parasites before XA stimulation (Fig. 3a). Assembled axonemes were formed and coiled around the nucleus of WT and *Δmap2* gametocytes 8 min post XA stimulation, but axoneme formation was not observed in the *Δgep1* parasite (Fig. 3a). By 15 min, WT gametocytes released flagellated male gametes, but not *Δmap2* and *Δgep1* gametocytes (Fig. 3a). Strikingly, α -Tubulin remained in cytosol of the *Δgep1* male gametocytes (Fig. 3a). We also analyzed the genome replication in stimulated male gametocytes. Flow cytometry analysis of DNA content in Hoechst-stained gametocytes showed that fluorescence increased (from 8.4% to 28.5%) in WT, but not in the *Δgep1* parasites (from 8.4% to 7.6%) after XA stimulation (Fig. 3b). As reported for *P. berghei*^{13,22}, no genome replication

occurs in the *Δcdpk4* parasite (Fig. 3b, Supplementary Fig. 1j, k). These results show no axoneme assembly or mitotic division in the stimulated *Δgep1* male gametocytes.

Differentiation of male and female gametes result in sequential rupture of PVM and EM for escaping from erythrocytes^{2,26}. TER119 is a plasma membrane protein of mouse erythrocytes^{27,28}, and anti-TER119 antibody showed no EM staining for stimulated WT male and female gametocytes (Fig. 3c). In contrast, intact EM was observed for the *Δgep1* gametocytes 30 min post stimulation (Fig. 3c), indicating that GEP1 depletion affects EM lysis.

XA triggers a cytosolic Ca²⁺ mobilization event within 10–15 s post stimulation of gametocytes¹³, which is essential for gametes formation and EM rupture^{11,13}. We next examined XA-stimulated Ca²⁺ mobilization in the *Δgep1* gametocytes using Fluo-8 probe as described^{29–31}. Fluo-8 did not affect the gametogenesis since WT gametocytes pre-loaded with Fluo-8 could form XA-stimulated ECs (Supplementary Fig. 7a) and responded to A23187, a Ca²⁺ ionophore¹³, in a dose-dependent manner using flow cytometry (Supplementary Fig. 7b). As expected, XA triggered a sharp increase in cytosolic Ca²⁺ signal in WT gametocytes, reaching maximal levels 10–15 s post stimulation, which resembled the observations in *P. berghei* using luminescence-based GFP::Aequorin sensor^{13,15}. However, no Ca²⁺ response was detected in XA stimulated *Δgep1* gametocytes (Fig. 3d). Ca²⁺ mobilization occurred in the *Δmap2* gametocytes as MAP2 functions downstream of Ca²⁺ signal^{18,22} (Fig. 3d).

Different from Ca²⁺-dependent EM rupture, PVM rupture is controlled by a Ca²⁺-independent mechanism². To study PVM lysis, a parasite line *sep1::4Myc* was generated by C-terminally tagging a PVM protein SEP1 with 4Myc^{27,28} (Supplementary Fig. 1j). This parasite line developed normally throughout the life cycle (Supplementary Fig. 5e), indicating intact protein function of SEP1::4Myc. We next deleted the *gep1* gene in the *sep1::4Myc* parasite, generating *sep1::4Myc/Δgep1* mutant (Supplementary Fig. 1j). IFA showed lysis of Sep1::4Myc-labeled PVM in the *sep1::4Myc* gametocytes (Fig. 3e), while intact PVM was maintained in the *sep1::4Myc/Δgep1* gametocytes 8 min post XA stimulation (Fig. 3e), indicating no PVM lysis in stimulated *Δgep1* gametocytes. Together, these results suggest that GEP1 functions upstream of PKG in XA-stimulated signaling cascade (Fig. 3f).

Impaired cGMP synthesis in GEP1 deficient parasite. Because cGMP is the direct upstream signal activating PKG in XA-stimulated gametogenesis^{7,11,13,15}, we examined intracellular cGMP synthesis during gametogenesis. Purified gametocytes were stimulated with XA for 2 min, and cGMP levels were measured using an enzyme immunoassay^{7,32}. Strikingly, XA induced a significant increase in cGMP level in WT gametocytes (Fig. 4a), consistent with previous observation in *P. falciparum*⁷. In contrast, the *Δgep1* gametocytes failed to increase cGMP in response to XA stimulation (Fig. 4a). As a control, cGMP response occurred in *Δmap2* gametocytes because MAP2 functions downstream of both cGMP and Ca²⁺ signaling^{18,22}. These results indicate that GEP1 regulates cGMP level, the most upstream intracellular signal known in *Plasmodium* gametogenesis.

cGMP level is tightly regulated by the opposing actions of cGMP-synthesizing GC and cGMP-hydrolyzing phosphodiesterase (PDE)^{10,11,33}. Inhibition of PDE activity by specific inhibitor Zaprinast (Zap) has been shown to trigger *P. falciparum* gametogenesis in the absence of XA^{11,33}. Indeed, treatment of WT gametocytes with 100 μ M Zap also induced EC counts comparable to those induced by 50 μ M XA (Fig. 4b), and gametogenesis stimulated by either XA or Zap could be blocked

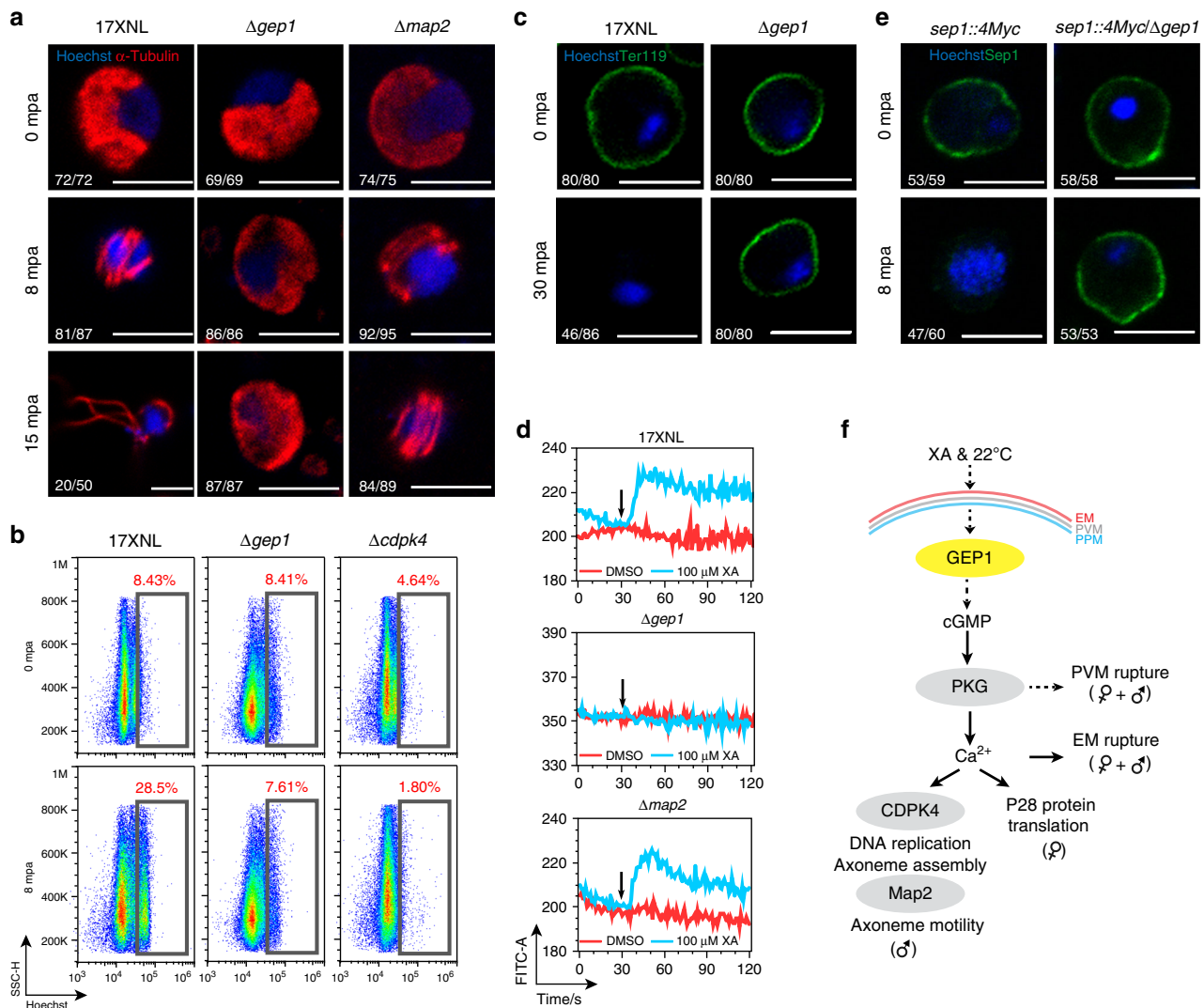


Fig. 3 GEP1 acts upstream of PKG in the cGMP-PKG-Ca²⁺ signaling cascade. **a** α -Tubulin expression and distribution in differentiating male gametocytes from 17XNL, $\Delta gep1$ and $\Delta map2$ parasites after XA stimulation. mpa: minute post XA activation. **b** Flow cytometry analysis of genomic DNA content in XA-stimulated male gametocytes of 17XNL, $\Delta gep1$ and $\Delta cdkp4$ parasites. The parasites were fixed with 4% paraformaldehyde at indicated time and stained with Hoechst. **c** Representative images of gametocytes stained by anti-mouse TER119 antibody 0 and 30 min post XA stimulation (mpa). **d** Flow cytometry detection of cytosolic Ca²⁺ in gametocytes using Fluo-8 probe. Purified gametocytes were preloaded with Fluo-8, and signals were collected 30 s before addition of XA or DMSO. Black arrows indicate the time for DMSO or XA addition. **e** Representative IFA images of the *sep1::4Myc* and *sep1::4Myc/Δgep1* gametocytes stained by anti-Myc antibody. **f** Proposed location of GEP1 in the XA-PKG-Ca²⁺ signal cascade of gametogenesis. GEP1 depletion causes defect in both Ca²⁺-dependent and Ca²⁺-independent cellular events of gametogenesis. EM: erythrocyte membrane, PVM: parasitophorus vacuole membrane, PPM: parasite plasma membrane. *x/y* in **a**, **c**, and **e** are the number of cell displaying representative signal/the number of cell analyzed. Scale bar = 5 μ m for all images in this figure. All experiments in this figure were repeated three times independently with similar results.

by a *Plasmodium* PKG protein inhibitor C2 (Fig. 4b), consistent with the established cGMP-PKG signal cascade of gametogenesis^{14,15}. In contrast, the $\Delta gep1$ gametocytes failed to form ECs after treatment with Zap (Fig. 4b). No EC were observed in the control $\Delta map2$ gametocytes treated in either XA or Zap (Fig. 4b). Consistently, we examined the intracellular cGMP level in gametocytes treated with Zap for 2 min and detected significant increase in both WT and $\Delta map2$ gametocytes, but not in the $\Delta gep1$ gametocytes (Fig. 4c). Together, these results suggest that the GC activity for cGMP synthesis is impaired, and therefore no elevation of cGMP in the $\Delta gep1$ gametocytes after XA stimulation or Zap inhibition of PDE activity. In addition to XA and Zap, increasing pH from 7.4 to 8.0 has been reported to induce gametogenesis although the underlying mechanism is not clear^{2,4}. Treating WT gametocytes with pH 8.0 at 22 °C indeed

induced comparable number of ECs to those induced by XA or Zap (Fig. 4b), and gametogenesis could be blocked by C2 treatment (Fig. 4b)¹⁵, indicating that the signaling stimulated by pH 8.0 is also cGMP/PKG-dependent. However, pH 8.0 treatment could not induce gametogenesis of the $\Delta gep1$ gametocytes, further suggesting impaired activity of cGMP synthesis in GEP1 deficient parasite (Fig. 4d).

GEP1 interacts and co-localizes with GCa. We next carried out immunoprecipitation and mass spectrometry experiments to identify molecules that may interact with GEP1 in gametocytes. By comparison of peptide signals (hits) between WT and *6HA::gep1* gametocyte samples from three biological replicates, we obtained 308 proteins that might interact with GEP1 (Supplementary Table 2), including GCa protein that is the enzyme

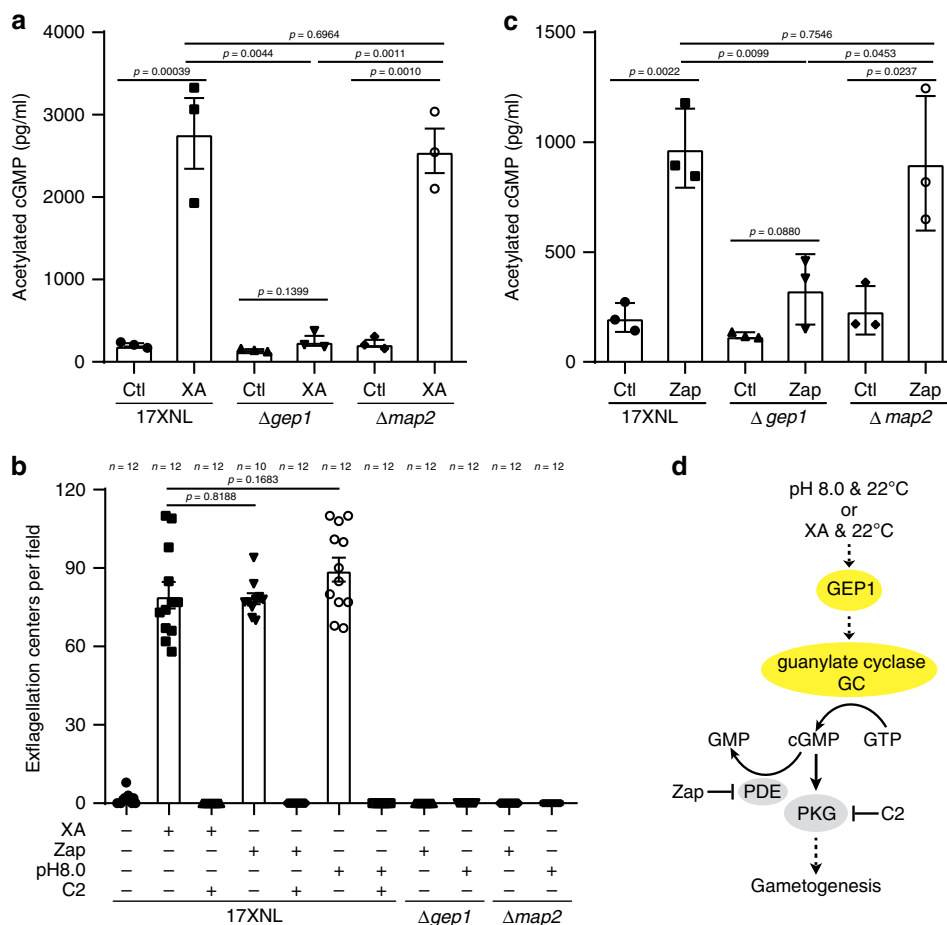


Fig. 4 Impaired activity of cGMP synthesis in GEP1 deficient gametocytes. **a** Enzyme immunoassay detecting intracellular cGMP level in XA-stimulated gametocytes of the 17XNL, $\Delta gep1$, and $\Delta map2$ parasites. Cells were incubated with 100 μ M XA at 22 °C for 2 min before assay. Ctl are control groups without XA stimulation. **b** Exflagellation center counts of 17XNL, $\Delta gep1$, and $\Delta map2$ parasites after treatment with XA (100 μ M), Zaprinast (Zap, 100 μ M), or pH 8.0 alone at 22 °C, or at the presence of compound 2 (C2, 5 μ M). *n* is the numbers of microscopic fields counted (40 \times). **c** Enzyme immunoassay detecting intracellular cGMP level in Zap-treated gametocytes of the 17XNL, $\Delta gep1$, and $\Delta map2$ parasites. Cells were incubated with 100 μ M Zap at 22 °C for 2 min before assay. Ctl are control groups without Zap stimulation. **d** Proposed role of GEP1 in regulating cGMP synthesis activity of guanylyl cyclase in gametogenesis. All source data are provided as a Source Data file. Experiments in **a**, **b**, and **c** were repeated three times independently. Data are shown as mean \pm SD; two-tailed unpaired Student's *t* test.

presumably responsible for cGMP synthesis during gametogenesis (Fig. 5a, b)^{8–10}. The *P. yoelii* GC α is a large protein (3850 amino acids) with 22 TMs distributed in an N-terminal P4-ATPase-like domain (ALD) and a C-terminal guanylate cyclase domain (GCD)^{34,35}. To study the expression of GC α in gametocytes, we generated two parasite lines (*gca::6HA* and *gca::4Myc*) with endogenous GC α C-terminally tagged with 6HA and 4Myc, respectively (Supplementary Fig. 1j). These parasites developed normally in mouse and mosquito hosts (Supplementary Fig. 5c, d). Similar to GEP1, GC α was also expressed as cytoplasmic puncta in both male and female gametocytes of the *gca::6HA* and *gca::4Myc* parasites (Supplementary Fig. 8a). To further confirm the interaction between GEP1 and GC α , we generated a doubly tagged parasite line, *4Myc::gep1/gca::6HA* (*DTS1*), by tagging the endogenous GEP1 with 4Myc in the *gca::6HA* parasite (Supplementary Fig. 1j, Supplementary Fig. 5f–h). Results from immunoprecipitation using anti-Myc antibody indicated that GC α interacted with GEP1 in cell lysate of the *DTS1* gametocytes (Fig. 5c). We next generated another independent doubly tagged parasite, *6HA::gep1/gca::4Myc* (*DTS2*) by tagging GC α with 4Myc in the *6HA::gep1* parasite (Supplementary Fig. 1j, Supplementary Fig. 5f–h) and detected similar interaction between GEP1 and GC α (Fig. 5d). As a

control, no interaction between GEP1 and GC β was detected in gametocytes of the *4Myc::gep1/gcb::6HA* (*DTS3*) parasite (Supplementary Fig. 8b). These data demonstrate that GEP1 interacts with GC α in gametocytes. In addition, IFA results from the *DTS1* parasite showed that GEP1 and GC α are co-localized at cytosolic puncta in non-activated gametocytes (Fig. 5e, f). Together, these data suggest that GEP1 co-localizes and binds to GC α in gametocytes.

GC α depletion causes defect in XA-stimulated gametogenesis. GC α has been implicated in cGMP synthesis during gametogenesis^{8–10}; however, there has been no direct evidence to support the speculation. We attempted to disrupt the *gca* gene but failed to obtain a GC α mutant parasite, indicating an essential function in asexual blood stage development, as reported in *P. falciparum* and *P. berghei* previously¹⁰. We used a promoter swap method described previously³⁶ to replace 1322 bp of endogenous *gca* promoter region with that (1626 bp) of *sera1* gene (PY17X_0305700) (Fig. 6a, Supplementary Fig. 1h), whose transcripts are expressed in asexual stages, but absent in gametocytes and mosquito stages³⁷. In this editing, a 6HA tag was inserted in frame at the N-terminus of the GC α coding sequence. Correct modification in two parasite clones of the resulting mutant

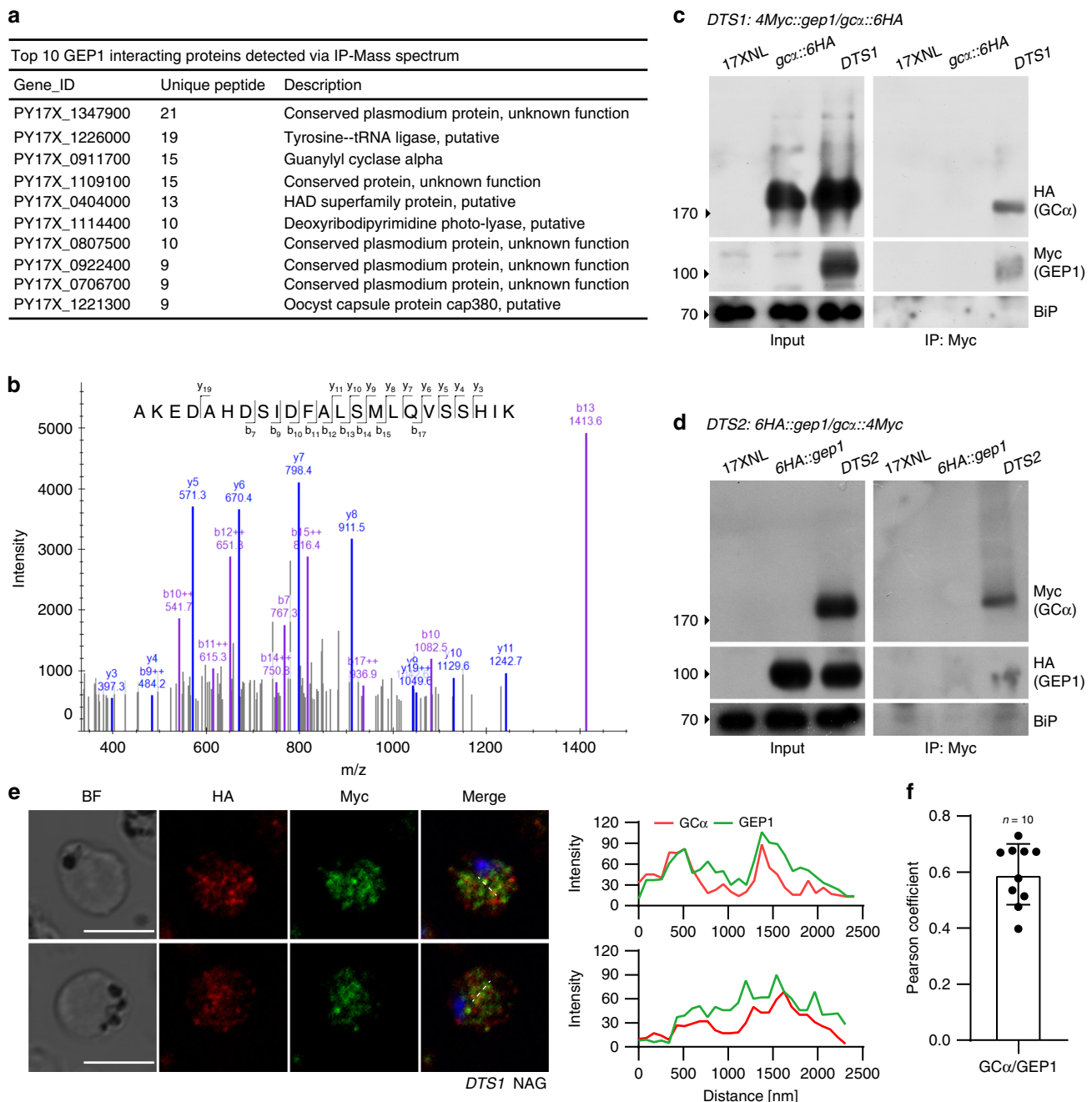


Fig. 5 GEP1 interacts with GC α in gametocytes. a Top 10 GEP1 interacting proteins in the gametocytes of the *6HA::gep1* parasite detected by immunoprecipitation and mass spectrometry (MS), including guanylyl cyclase α (GC α) with 15 peptides detected. **b** MS2 spectrum of a representative peptide of the GC α protein. **c** Co-immunoprecipitation of Myc::GEP1 and GC α ::HA proteins in gametocytes of the double tagged parasite *4Myc::gep1/gc α ::6HA* (*DTS1*). IP-Myc, anti-Myc antibody was used. **d** Co-immunoprecipitation of HA::GEP1 and GC α ::Myc proteins in gametocytes of the double tagged parasite *6HA::gep1/gc α ::4Myc* (*DTS2*). IP-Myc, anti-Myc antibody was used. **e** Two-colored IFA of GEP1 and GC α proteins in the *DTS1* gametocytes using anti-HA (GC α) and anti-Myc (GEP1) antibodies (left panel). Cross sections (white dash line) of the cells show the co-localization of GEP1 and GC α (right panel). Scale bar = 5 μ m. **f** Pearson coefficient analysis for GEP1 and GC α co-localization shown in **e**, data are shown as mean \pm SD from $n = 10$ cells measured. Experiments in **c**, **d**, and **e** were repeated three times independently with similar results.

parasite *gcakd* was confirmed by PCR (Supplementary Fig. 1j). The promoter replacement allowed expression of the GC α protein in asexual blood stages at a level comparable with that of another parallelly modified parasite *6HA::gc α* (Supplementary Fig. 1j), but significantly reduced GC α protein expression in gametocytes (Fig. 6b, c). Notably, the *gcakd* parasite completely lost the ability to synthesize cGMP and form ECs after XA stimulation in vitro (Fig. 6d, e). In mosquitos fed with *gcakd* parasite-infected mouse

blood, no oocyst was detected in mosquito midgut (Fig. 6f). These results support that GC α is the GC responsible for XA-stimulated cGMP synthesis in gametogenesis (Fig. 6g). In addition, the phenotype caused by GC α knockdown in gametocytes resembles that of GEP1 defect.

Compared to the expression of GC α in both male and female gametocytes, GC β expression was detected in *gc β ::6HA* female gametocytes only⁸ (Supplementary Fig. 8a, lower panel). In

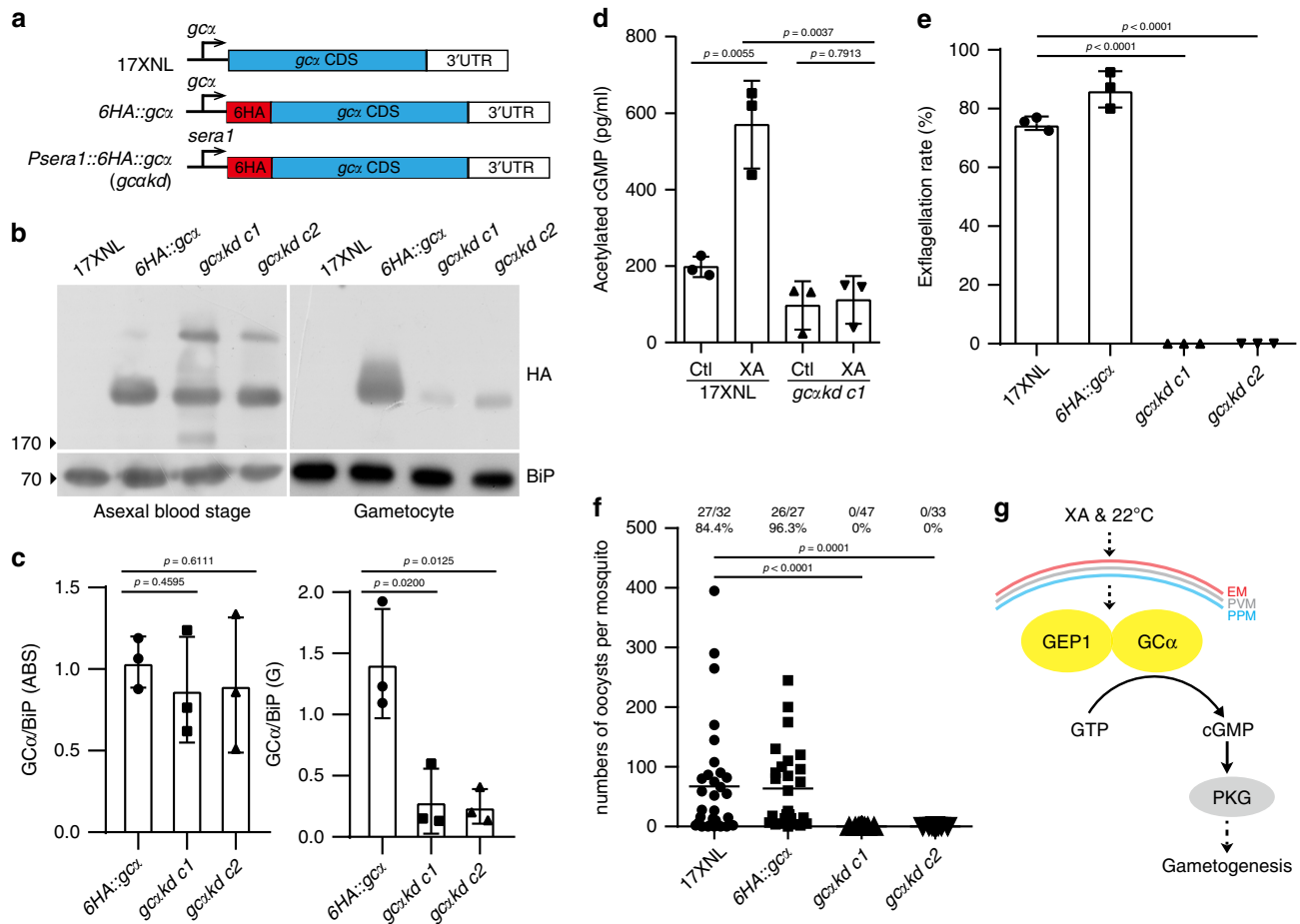


Fig. 6 GCα knockdown in gametocytes results in gametogenesis defect. **a** Diagram showing a promoter swap strategy to knockdown *gcα* expression in gametocytes, generating HA-tagged *gcαkd* mutant with endogenous *gcα* promoter replaced with the *sera1* promoter. **b** Western blotting of GCα expression in asexual blood stages and gametocytes of the *gcαkd* parasite. The 6HA::*gcα* as a control. **c** Quantitative analysis of GCα protein expression in **b**. **d** Intracellular cGMP level in XA-stimulated gametocytes of the 17XNL and *gcαkd* parasites. Cells were incubated with 100 μM XA at 22 °C for 2 min before assay. Ctl are control groups without XA stimulation. **e** In vitro exflagellation rates for 17XNL, 6HA::*gcα*, and two clones of the *gcαkd* parasite after XA stimulation. **f** Day 7 midgut oocyst counts in mosquitoes infected with 17XNL, 6HA::*gcα*, and two clones of the *gcαkd* parasites. Mosquito infection prevalence is shown above. **g** A proposed model of GEPI/GCα interaction essential for XA-stimulated cGMP synthesis and gametogenesis. Experiments were independently repeated three times in **b**, **d**, **e**, and **f**. Data are shown as mean ± SD in **c**, **d**, **e**, and **f**. Two-tailed unpaired Student's *t* test in **c**, **d**, **e**, and **f**. Source data of **c**, **d**, **e**, and **f** are provided as a Source Data file.

addition, GCβ depletion had no effect on XA-stimulated elevation of cGMP (Supplementary Fig. 8c) and in vitro EC formation (Supplementary Fig. 8d) in gametocytes of the Δ*Gcβ* parasite⁸, in agreement with previous reports in *P. falciparum* and *P. berghei*^{9,33}. These results exclude the involvement of GCβ in XA-stimulated cGMP signaling and gametogenesis.

GEPI depletion has no effect on GCα expression and localization. As GCα and GEPI interacted with each other and functioned upstream of cGMP signaling, we investigated whether GEPI depletion would affect the expression and cellular localization of GCα in gametocytes. We deleted *gep1* gene in the *gcα*::6HA parasite, generating a *gcα*::6HA/Δ*gep1* mutant parasite (Supplementary Fig. 1j, Supplementary Fig. 5i, j). GEPI depletion had no effect on *gcα* mRNA level or GCα protein abundance in gametocytes of the *gcα*::6HA/Δ*gep1* parasite compared to the parental parasite (Fig. 7a, b). As a control, depletion of CDPK4 had no effect on both mRNA and protein level of GCα either because CDPK4 functions downstream of cGMP signal (Fig. 7a, b). In addition, XA stimulation had no effect on protein abundance of both GEPI and GCα in gametocytes of the *DTS1* parasite (Fig. 7c).

Next, we investigated the effect of XA stimulation in cellular localization of GEPI and GCα proteins in gametocytes of the 6HA::*gep1* or *gcα*::6HA parasite, respectively. Two minutes post XA stimulation, both GEPI and GCα were expressed as cytoplasmic puncta in activated female gametocytes (Fig. 7d, e). Even 8 min post XA stimulation, both GEPI and GCα still maintained in cytoplasmic puncta in activated female gametocytes (Supplementary Fig. 9a, b). Strikingly, both proteins were redistributed from cytoplasm to the cell periphery of activated male gametocytes 2 min post XA stimulation (Fig. 7d, e). We further investigated the localization of both GEPI and GCα in activated gametocytes of the *DTS1* parasite. Two color IFA results indicate that GEPI and GCα were co-localized in cytoplasm of activated female gametocytes but in cell periphery of activated male gametocytes 2 min post XA stimulation (Supplementary Fig. 9c, d), repeating the results from single color IFA. In activated male gametocytes, eight axonemes are assembled in the cytoplasm and coiled around the enlarged nucleus containing octaploid genome, likely pushing the cytosolic puncta to cell periphery. However, no redistribution of GCα was detected from cytoplasm to cell periphery in the stimulated *gcα*::6HA/Δ*gep1* male gametocytes (Fig. 7e), which could be explained by no

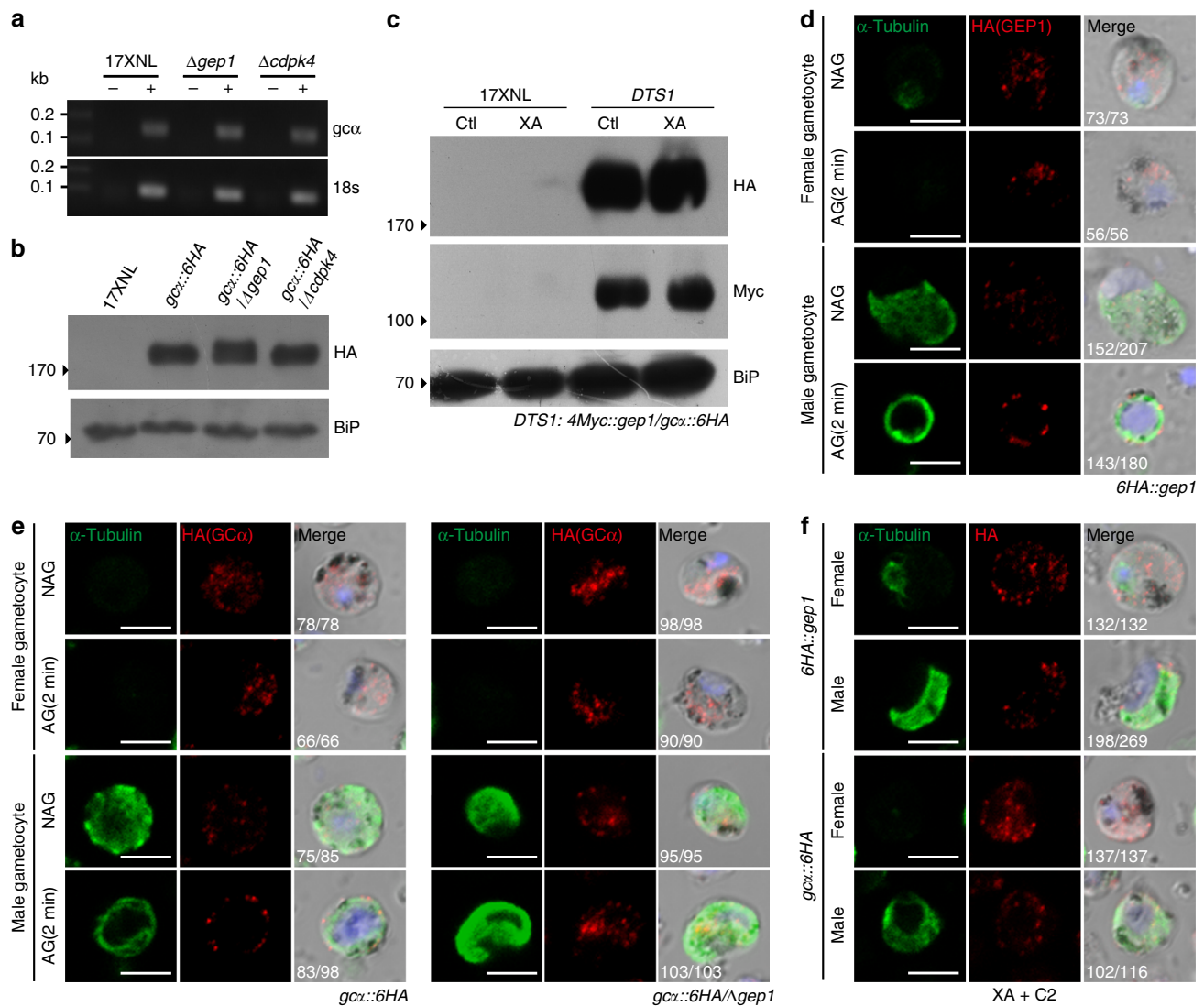


Fig. 7 GC α expression and localization in the GEPI1-depleted gametocytes. **a** RT-PCR analysis of *gca* transcript in gametocytes of the 17XNL, $\Delta gep1$, and $\Delta cdpk4$ parasites. **b** Western blotting detecting GC α protein in gametocytes of the 17XNL, *gca::6HA*, *gca::6HA/Δgep1*, and *gca::6HA/Δcdpk4* parasites. **c** Western blotting detecting GEP1 (Myc) and GC α (HA) proteins expression in gametocytes of *DTS1* parasite 2 min post XA stimulation. Ctl are control groups without XA stimulation. **d** Co-staining of GEP1 and α -Tubulin expressions in gametocytes of the *6HA::gep1* parasite 2 min post XA stimulation. NAG: non-activated, AG: XA stimulation. **e** Co-staining of GC α and α -Tubulin expressions in the *gca::6HA* and *6HA::gca/Δgep1* gametocytes 2 min post XA stimulation. NAG: non-activated, AG: XA stimulation. **f** Co-staining of α -Tubulin and HA-tagged GEP1 or GC α expressions in the *6HA::gep1* (upper panel) and *gca::6HA* (lower panel) gametocytes 2 min post XA stimulation plus C2 treatment. x/y in **d**, **e**, and **f** are the number of cell displaying representative signal/the number of cell analyzed. Scale bar = 5 μ m for all images in this figure. All experiments in this figure were repeated three times independently.

initiation of gametogenesis caused by GEP1 depletion. To further confirm the observations above, we treated the gametocytes with PKG inhibitor C2 to block the initiation of XA-stimulated gametogenesis. Indeed, no redistribution of either GEP1 or GC α was observed from cytoplasm to the cell periphery in the stimulated male gametocytes of the *6HA::gep1* and *gca::6HA* parasite respectively (Fig. 7f). Together, these results indicate that GEP1 does not regulate the expression level and localization of GC α in non-activated male and female gametocytes, but affects the localizations of GC α in XA activated male gametocytes.

XA stimulation likely enhances the GEP1/GC α interaction.

Lastly we asked whether XA stimulation could enhance the interaction between GEP1 and GC α in gametocytes. Proximity Ligation Assay (PLA) is a homogeneous immunohistochemical tool that couples the specificity of ELISA with the sensitivity of

PCR, which allows in situ detection of endogenous proteins interaction with high specificity and sensitivity^{38,39}. We performed the PLA to investigate the protein interaction in both non-activated gametocytes and activated gametocytes 2 min post XA stimulation. Robust PLA signals were detected in cytoplasm of the non-activated gametocytes of *DTS1* parasite when both anti-Myc and anti-HA primary antibodies were present (Fig. 8a), indicative of GEP1 and GC α interaction. As a control, no PLA signal was detected in gametocytes of the single tagged *gca::6HA* parasite. 2 min post XA stimulation, the PLA signals were detected in cytoplasm of activated female gametocytes but in cell periphery of activated male gametocytes (Fig. 8a), which is consistent with the protein localization in IFA analysis (Fig. 7d, e, Supplementary Fig. 9c). Quantifying the number of PLA signal dots in each cells of gametocytes showed no difference between non-activated and activated gametocytes (Fig. 8b). However, the fluorescence intensity of PLA signal in the XA-activated

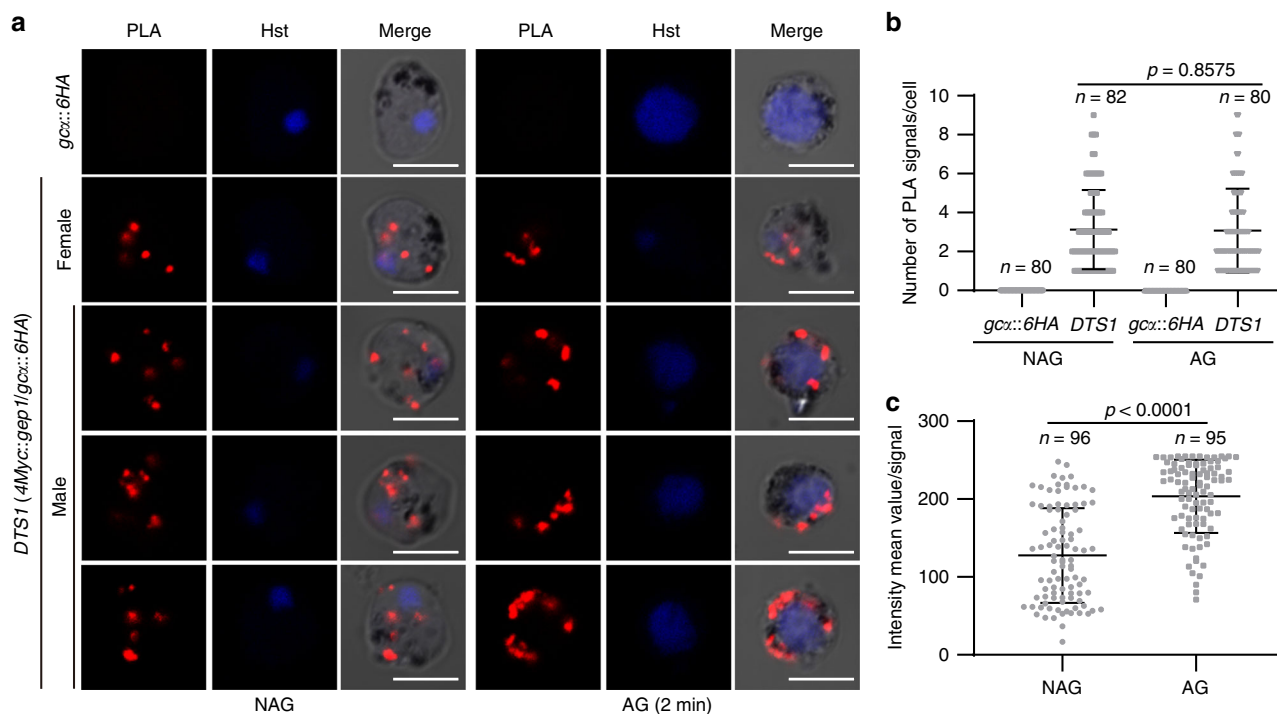


Fig. 8 XA stimulation likely enhances the interaction between GEP1 and GC α . **a** Proximity Ligation Assay (PLA) detecting protein interaction between GEP1 and GC α in *DTS1* gametocytes. NAG: non-activated, AG: 2 min after XA stimulation. Activated male gametocytes were observed with enlarged nucleus containing replicated genome. Scale bar = 5 μ m. **b** Number of PLA signal dot in each cell shown in **a**, *n* is the number of cells counted. **c** Fluorescence intensity value for each PLA signal dot shown in **a**, *n* is the number of PLA signal dot measured. Source data are provided as a Source Data file. Experiment was repeated three times independently. Data are shown as mean \pm SD; two-tailed unpaired Student's *t* test.

gametocytes is significantly higher than that of the non-activated gametocytes (Fig. 8c), suggesting possible enhanced interaction between GEP1 and GC α in gametocytes after XA stimulation. We performed the PLA experiment in another independent doubly tagged parasite *DTS2* and observed the same results (Supplementary Fig. 10a–c).

Discussion

It has been well-established that the XA-cGMP-PKG-Ca²⁺ signaling drives gametogenesis of *Plasmodium* parasites^{7,11,13} since the discovery of mosquito-derived XA as an inducer for gametogenesis more than two decades ago^{5,6}. However, how the parasite senses external stimuli such as XA and reduction in environmental temperature to activate the cGMP signaling pathway remains unknown. In this study, we identified a membrane protein (GEP1) that responds to XA stimulation and binds to GC α , leading to activation of cGMP-PKG-Ca²⁺ signaling pathway and gametogenesis after functional screening 59 genes encoding integral membrane proteins expressed in gametocytes. Using CRISPR/Cas9 method, we successfully obtained gene deletion mutant parasites for 45 out of 59 candidate genes. To the best of our knowledge, our study is the first CRISPR/Cas9-based gene functional screening performed in malaria parasites, and the results from our CRISPR/Cas9-based screen largely matched the outcomes of a recent gene disruption screening using conventional homologous recombination in *P. berghei*⁴⁰. Of the 45 genes, 25 orthologs of *P. berghei* were shown to be dispensable for asexual blood stage proliferation, 8 orthologs were resistant for disruption, and 12 orthologs were not tested in the screening of *P. berghei* (Supplementary Table 1)⁴⁰. For the 14 disruption-resistant genes in our hands, all of the *P. berghei* orthologs also failed deletion attempts⁴⁰.

After establishing the causative relationship of GEP1 deletion and gametogenesis defect, we investigated the position where GEP1 exerts its function in the XA-stimulated signaling cascade during gametogenesis. Previous studies have shown that cGMP enhances exflagellation of *P. berghei* and *P. falciparum*^{41,42}. In addition, XA was shown to increase cGMP synthesis by GC from isolated membrane preparations of *P. falciparum* gametocytes⁷, suggesting that XA-stimulated gametogenesis is mediated by elevated GC activity and cGMP synthesis. Consistent with these observations, we detected significant increases in cytosolic cGMP level in WT gametocytes 2 min after XA stimulation, but not in Δ *gep1* gametocytes. GEP1 depletion resulted in impaired cGMP production in response to XA, indicating that GEP1 locates upstream of cGMP in the XA-cGMP-PKG-Ca²⁺ cascade. Compared with the 10–15 min required for whole process of gametogenesis, XA rapidly triggers a cytosolic Ca²⁺ mobilization within 10–15 s post stimulation, which was also observed in other studies¹³. These results suggest that GEP1 functions at an early or initiating step of gametogenesis. Consistently, disruption of *gep1* causes defects in all PKG-downstream cellular and signaling events during gametogenesis, including Tubulin polymerization for axoneme assembly, genome replication in male gametocytes, release of P28 translational repression in female gametocytes, PVM and EM rupture for egressing of both male and female gametes from erythrocytes, and Ca²⁺ mobilization. These results suggest that GEP1 functions upstream of cGMP-PKG-Ca²⁺ cascade in XA-stimulated gametogenesis.

The cytosolic cGMP level is balanced by the activities of cGMP-synthesizing GC and cGMP-hydrolyzing PDE^{10,11,33}. That inhibition of PDE activity by inhibitor Zap could trigger gametogenesis in the absence of XA suggests the existence of low and sub-threshold endogenous cGMP level precluding PKG activation in gametocytes^{11,33}. Strikingly, the Δ *gep1* gametocytes not only

failed to initiate XA-stimulated gametogenesis, but also could not undergo Zap-induced gametogenesis. Consistently, we detected no significant Zap-induced elevation of cytosolic cGMP level in the *Δgep1* gametocytes as seen in WT gametocytes. These results suggest that GEP1 is an essential component of the GC synthesis machinery, and its depletion completely impairs parasite ability to synthesize cGMP, resulting in no accumulation of basal level cGMP in gametocytes.

Two large guanylyl cyclases (GCα and GCβ) for cGMP synthesis are found in *Plasmodium* parasites³⁴. GCα and GCβ in *P. yoelii* consist of 3850 and 3015 amino acids, respectively, and both proteins are predicted to have 22 TMs distributed in an N-terminal P4-ATPase-like domain (ALD) and a C-terminal guanylate cyclase domain (GCD). GC enzymes possessing the ALD/GCD structure are observed in many protozoan species^{34,43}. Whereas the GCD is responsible for cGMP synthesis, the function of the ALD is still obscure. Both *P. berghei* and *P. falciparum* parasites without GCβ can produce functional male gametes^{9,10}. Consistent with these reports, our study also showed deletion of *gcβ* did not affect XA-stimulated cGMP elevation and male gamete formation, confirming that GCβ is not the enzyme for cGMP synthesis during gametogenesis. Using unbiased immunoprecipitation and mass spectrometry analysis, we found that GEP1 interacted with GCα and this interaction was confirmed by co-immunoprecipitation and co-localization analyses. Furthermore, we attempted to disrupt the *gca* gene, but were not able to obtain a viable mutant parasite, consistent with previous reports in other *Plasmodium* species¹⁰. Alternatively, we generated a mutant parasite with decreased GCα expression in gametocytes. Specific knockdown of GCα in gametocytes blocked XA-stimulated cGMP elevation and the consequent gametogenesis, mimicking the defect of GEP1 disruption. These results indicate that GCα is the enzyme for cGMP synthesis in gametogenesis.

Interestingly, GEP1 and GCα proteins were expressed as cytoplasmic puncta in female gametocytes either before or after XA stimulation. In the contrast, both proteins were redistributed from cytoplasm to the cell periphery of male gametocytes post XA stimulation. Once gametogenesis is initiated after XA stimulation, eight axonemes are assembled and coiled around the enlarged nucleus containing octaploid genome^{18,22}, possibly occupying most cytoplasmic space and pushing cytoplasmic vesicles, including the GEP1/GCα residing puncta or possible membrane vesicle, to the periphery of the stimulated male gametocytes. Consistent with our observations, Carucci et al. also revealed that GCα displayed a peripheral localization in the *P. falciparum* stimulated gametocytes using immunoelectron microscopy³⁴. In addition, these results also suggest that GEP1 likely exerts its function in controlling cGMP synthesis by directly binding GCα and regulating GCα conformation because GEP1 depletion had no effect in the expression and cellular localization of GCα in gametocytes.

GEP1 possesses 14 predicted TM domains, encoding a possible sodium-neurotransmitter symporter or amino acid transporter family protein. Three independent studies recently revealed that the *Toxoplasma gondii*, another Apicomplexan parasite, regulates natural egress of tachyzoites from host cell via a guanylate cyclase receptor platform^{44–46}. Similar to *Plasmodium* GCα and GCβ, *T. gondii* guanylate cyclase (TgGC) also possesses the atypical ALD/GCD structure. By crosslinking experiment coupled to immunoprecipitation and mass spectrometry, 55 TgGC-interacting proteins were identified⁴⁴, including a top 5th hit (TGGT1_208420) encoding a putative sodium-neurotransmitter symporter family protein. Notably, TGGT1_208420 displays some similarity in protein sequence with GEP1. These results suggest the interaction between GC and sodium-neurotransmitter symporter family protein is conserved in *Plasmodium* and *T.*

gondii. Similar to *P. yoelii* GEP1, depletion of this protein does not cause tachyzoite growth defect⁴⁴, suggesting a dispensable role in asexual lytic cycle of *T. gondii* although its function in sexual cycle is unknown. In addition, these studies also identified another *T. gondii* GC-interacting protein UGO that is believed to act as a chaperone⁴⁴. Whether the *Plasmodium* UGO ortholog protein (PY17X_1204500) plays a similar role in the GC machinery remains to be determined.

Based on our results, we proposed a model for GEP1/GCα mediated cGMP signaling in XA-stimulated gametogenesis. The membrane protein GEP1 acts as a binding partner of GCα. In the absence of XA, GEP1 supports a functional conformation of GCα that maintains its basal catalytic activity and synthesizes low and sub-threshold endogenous cGMP level precluding PKG activation. In the presence of XA, the stimulation enhances the interaction of GEP1/GCα, leading to enhanced GC activity of GCα and increased cGMP level for PKG activation. In the GEP1-deficient gametocytes, GCα loses catalytic activity of cGMP synthesis and therefore fails to elevate cGMP level in response to XA, Zap treatment, or environmental pH. Currently, we could not exclude the possibility that there is an unknown molecule as the XA sensor residing in cytoplasm or plasma membrane and functioning upstream of GEP1/GCα complex. XA-stimulated gametocyte to gamete differentiation in the midgut is the first and essential step for mosquito transmission of malaria parasites, and elucidating the mechanisms involved may facilitate development of measures to block disease transmission.

Methods

Animal usage and ethics statement. Animal experiments were performed in accordance with the approved protocols (XMULAC20140004) by the Committee for Care and Use of Laboratory Animals of Xiamen University. ICR mice (female, 5 to 6 weeks old) were purchased and housed in the Animal Care Center of Xiamen University and kept at room temperature under a 12 h light/dark cycle at a constant relative humidity of 45%.

Mosquito maintenance. The *Anopheles stephensi* mosquito (strain Hor) was reared at 28 °C, 80% relative humidity and at a 12 h light/dark cycle. Mosquitoes were fed on a 10% sucrose solution.

Plasmid construction and parasite transfection. CRISPR/Cas9 plasmid pYcM was used for all the genetic modifications. For gene deleting, 5'-genomic and 3'-genomic segments (400 to 700 bp) of the target genes were amplified as left and right homologous arms, respectively, using gene specific primers (Supplementary Table 3). The PCR products were digested with appropriate restriction enzymes, and the digested products were inserted into matched restriction sites of pYcM. Oligonucleotides for sgRNAs were annealed and ligated into pYcM¹⁷. For each deletion modification, two sgRNAs were designed to disrupt the coding region of a target gene (Supplementary Table 3) using the online program ZiFit⁴⁷. For gene tagging, a 400 to 800 bp segment from N-terminal or C-terminal of the coding region and 400 to 800 bp sequences from 5'UTR or 3'UTR of a target gene were amplified and fused with a DNA fragment encoding 6HA or 4Myc in frame at N-terminal or C-terminal of the gene. For each tagging modification, two sgRNAs were designed to target sites close to the C-terminal or N-terminal of the gene coding region. Infected red blood cells (iRBC) were electroporated with 5 μg circular plasmid DNA using Lonza Nucleofector. Transfected parasites were immediately injected *i.v.* into a naive mouse and treated with pyrimethamine (6 μg/ml) in drinking water. Parasites with transfected plasmids usually appear 5 to 7 days post drug selection.

Genotype analysis of transgenic parasites. All transgenic parasites were generated from *P. yoelii* 17XNL strain or *P. berghei* ANKA strain. The schematic for different genetic modifications and the results of parasite transfection, single cloning and genetic verification of modified strains are summarized in Supplementary Fig. 1. Blood samples from infected mice were collected from the orbital sinus, and blood cells were lysed using 1% saponin in PBS. Parasite genomic DNAs were isolated from blood stage parasites using DNeasy Blood kits (QIAGEN). For each parasite, both 5' and 3' homologous recombination events were detected using specific PCR primers (Supplementary Fig. 1). PCR products from some modified parasites were DNA sequenced. All the primers used in this study are listed in Supplementary Table 3. Parasite clones with targeted modifications were obtained after limiting dilution. At least two clones for each gene-modified parasite were

used for phenotype analysis. Parasite growth characteristics in mouse and in mosquito for the modified parasite strains are shown in Supplementary Fig. 5.

Negative selection with 5-fluorouracil. Parasites subjected to sequential modifications were negatively selected with 5-Fluorouracil (5FC, Sigma, F6627) to remove episomal plasmid. 5FC (2 mg/ml) in drinking water was provided to mice in a dark bottle for 8 days with a change of drug on day 4. Clearance of episomal plasmid in parasites after negative selection was confirmed by checking the parasite survival after reapplying pyrimethamine pressure (6 µg/ml) in new infected mice.

Gametocyte induction. ICR mice were treated with phenylhydrazine (80 µg/g mouse body weight) through intraperitoneal injection. Three days post treatment, the mice were infected with 3.0×10^6 parasites through tail vein injection. Gametocytemia usually peaks at day 3 post infection. Male and female gametocytes were counted via Giemsa staining of thin blood smears. Gametocytemia was calculated as the ratio of male or female gametocyte over parasitized erythrocytes. All experiments were repeated three times independently.

Male gametocyte exflagellation assay. Two and a half microliters of mouse tail blood with 4–6% gametocytemia were added to 100 µl exflagellation medium (RPMI 1640 supplemented with 10% fetal calf serum and 50 µM XA, pH 7.4) containing 1 µl of 200 units/ml heparin. After 10 min of incubation at 22 °C, the numbers of EC and RBC were counted in a hemocytometer under a light microscope. The percentage of RBCs containing male gametocytes was calculated from Giemsa-stained smears, and the number of ECs per 100 male gametocytes was then calculated as exflagellation rate. Compound 2 (5 µM) and Zaprinast (100 µM) were added to exflagellation medium with or without XA (for Zaprinast) to evaluate their effects on exflagellation.

In vitro ookinete differentiation. In vitro culture for ookinete differentiation was prepared as described previously¹³. Briefly, mouse blood with 4–6% gametocytemia was collected in heparin tubes and immediately added to ookinete culture medium (RPMI 1640 medium containing 25 mM HEPES, 10% fetal calf serum, 100 µM XA, and pH 8.0) in a blood/medium volume ratio of 1:10. The cultures were incubated at 22 °C for 12 h to allow gametogenesis, fertilization, and ookinete differentiation. Ookinete formation was monitored by Giemsa-staining of culture smears. Ookinete conversion rate was calculated as the number of ookinetes (including mature and immature) per 100 female gametocytes.

Mosquito feeding and transmission assay. Thirty female mosquitoes were allowed to feed on an anaesthetized mouse with 4–6% gametocytemia for 30 min. Mosquito midguts were dissected on day 7 post blood-feeding and stained with 0.1% mercurochrome for detection of oocyst. Salivary glands from 20–30 mosquitoes were dissected on day 14 post blood-feeding, and the number of sporozoites per mosquito was calculated.

Parasite genetic cross. Genetic crosses between two different parasite lines were performed by infecting phenylhydrazine pre-treated mice with equal numbers of both parasites. Day 3 pi, 30 female mosquitoes were allowed to feed on mice carrying gametocytes for 30 min. Mosquito midguts were dissected on day 7 post blood-feeding and stained with 0.1% mercurochrome for oocyst counting.

Gametocyte purification. Gametocytes were purified using the method described previously⁴⁸. Briefly, mice were treated with phenylhydrazine 3 days before parasite infection. From day 3 pi, infected mouse were treated with sulfadiazine at 20 mg/l in drinking water to eliminate asexual blood stage parasites. After 48 h treatment with sulfadiazine, mouse blood containing gametocytes was collected from orbital sinus into a heparin tube. Gametocytes were separated from the uninfected erythrocyte by centrifugation using 48% Nycodenz solution (27.6% w/v Nycodenz in 5 mM Tris-HCl, 3 mM KCl, 0.3 mM EDTA, pH 7.2) and prepared in gametocyte maintenance buffer (GMB, 137 mM NaCl, 4 mM KCl, 1 mM CaCl₂, 20 mM glucose, 20 mM HEPES, 4 mM NaHCO₃, pH 7.24–7.29, 0.1% BSA)⁴⁸. Gametocytes were harvested from the interphase and washed three times in the GMB buffer. All the operations were performed at 19–22 °C.

Trypan blue staining. Purified gametocytes were prepared in PBS and mixed with 0.4% trypan blue solution at a 1:9 volume ratio. The mixtures were incubated at room temperature for 5 min and examined under a light microscope.

Propidium iodide staining. Purified gametocytes were prepared in PBS and stained with Propidium iodide (PI) at a final concentration of 50 µg/ml. The mixtures were incubated at room temperature for 10 min, washed with PBS twice, and then examined under a fluorescence microscope.

Flow cytometry analysis. For measuring DNA content in gametocytes, half of purified gametocytes were immediately fixed and half were transferred to

exflagellation medium for gametogenesis for 8 min before fixation. Cells were fixed in 4% paraformaldehyde (PFA) for 20 min, washed in PBS and stained with Hoechst 33342 (0.5 µg/ml) for 30 min. Hoechst fluorescence signal of gametocytes was collected using Novocyte 3130 flow cytometer. For detecting GFP and mCherry in gametocytes, the gametocytes were stained with Hoechst 33342 and washed with PBS twice, GFP and mCherry fluorescence signal of gametocytes was collected using BD LSR Fortessa flow cytometer. Cell gating strategies are provided in Supplementary Fig. 11.

Ca²⁺ mobilization assay using flow cytometry. Purified gametocytes were washed three times with Ca²⁺ free buffer (CFB, 137 mM NaCl, 4 mM KCl, 20 mM glucose, 20 mM HEPES, 4 mM NaHCO₃, pH 7.2–7.3, 0.1% BSA) and then incubated in CFB containing 5 µM Fluo-8 at 37 °C for 20 min. Fluo-8 loaded gametocytes were washed twice with CFB and suspended in RPMI 1640 for flow cytometer analysis. Fluo-8 fluorescence signal reflecting cellular Ca²⁺ content in gametocytes were collected using BD LSR Fortessa flow cytometer. Signals were consecutively collected at 30 s before until 90 s post addition of XA (100 µM) or A23187 (0.1 and 1 µM). Cell gating strategies are provided in Supplementary Fig. 11.

Detection of cellular cGMP. The assay for measuring cGMP levels in gametocytes was performed using a cyclic cGMP enzyme immunoassay kit (Cayman Chemical, #581021). For each test, more than 1.5×10^7 gametocytes were collected and maintained in GMB buffer on ice. After treatment with 100 µM XA or 100 µM Zap for 2 min, cells were immediately lysed by 0.2 M cold hydrochloric acid on ice for 10 min, vortexed, and passed through a 22-gauge needle. For each replicate, three equal volumes of cell extract from each parasite preparation were parallel tested according to manufacturer's instructions.

Antibodies and antiserum. The primary antibodies used were: rabbit anti-HA (Western blot, 1:1000 dilution, IFA, 1:500 dilution) and rabbit anti-Myc (Western blot, 1:1000 dilution, IFA, 1:500 dilution) from Cell Signaling Technology; mouse anti-HA (IFA, 1:200) and mouse anti-Myc (IFA, 1:200) from Santa Cruz; mouse anti- α -Tubulin II from Sigma-Aldrich (IFA, 1:1000). The secondary antibodies used were: goat anti-rabbit IgG HRP-conjugated and goat anti-mouse IgG HRP-conjugated secondary antibodies from Abcam (1:5000); the Alexa 555 labeled goat anti-rabbit IgG, Alexa 555 labeled goat anti-mouse IgG, and Alexa 488 labeled goat anti-mouse IgG secondary antibodies from Thermo Fisher Scientific (1:500); Alexa 488 labeled anti-mouse TER-119 IgG antibody from BioLegend (IFA, 1:1000), biotinylated anti-rabbit IgG (H+L) antibody from Cell Signaling Technology (IFA, 1:1000); Streptavidin-ACP from Bioscience (IFA, 1:500). The anti-sera, including rabbit anti-Hep17 (Western blot, 1:1000), rabbit anti-P28 (Western blot, 1:1000, IFA, 1:1000), rabbit anti-BiP (Western blot, 1:1000) were prepared by immunization of synthetic peptides or recombinant protein as described previously⁸.

Immunofluorescence assays. Purified parasites or chemical-treated parasites were fixed in 4% PFA and transferred onto a poly-L-Lysine pre-treated coverslip. The fixed cells were permeabilized with 0.1% Triton X-100 PBS solution for 7 min, blocked in 5% BSA solution for 60 min at room temperature or 4 °C overnight, and incubated with the primary antibodies diluted in PBS with 3% BSA at 4 °C for 12 h. The coverslip was incubated with fluorescently conjugated secondary antibodies. Cells were stained with Hoechst 33342, mounted in 90% glycerol solution, and sealed with nail polish. All images were captured and processed using identical settings on a Zeiss LSM 780 confocal microscope.

Proximity ligatain assay. The PLA assay detecting in situ protein interaction was performed using the kit (Sigma-Aldrich: DUO92008, DUO92001, DUO92005, and DUO82049). Non-activated and activated gametocytes were fixed with 4% PFA for 30 min, permeabilized with 0.1% Triton X-100 for 10 min, and blocked with a blocking solution overnight at 4 °C. The primary antibodies were diluted in the Duolink Antibody Diluent, added to the cells and then incubated in a humidity chamber overnight at 4 °C. The primary antibodies were removed and the slides were washed with Wash Buffer A twice. The PLUS and MINUS PLA probe were diluted in Duolink Antibody Diluent, added to the cells and incubated in a pre-heated humidity chamber for 1 h at 37 °C. Next, cells were washed with Wash Buffer A and incubated with the ligation solution for 30 min at 37 °C. Then, cells were washed with Wash Buffer A twice and incubated with the amplification solution for 100 min at 37 °C in the dark. Cells were washed with 1× Wash Buffer B twice and 0.01× Wash Buffer B once. Finally, cells were incubated with Hoechst 33342 and washed with PBS. Images were captured and processed using identical settings on a Zeiss LSM 780 confocal microscope.

Protein extraction and western blotting. Proteins were extracted from asexual blood parasites and gametocytes using buffer A (0.1% SDS, 1 mM DTT, 50 mM NaCl, 20 mM Tris-HCl, pH 8.0) containing protease inhibitor cocktail and PMSF. After ultrasonication, the protein solution was kept on ice for 15 min before centrifugation at $14,000 \times g$ for 10 min at 4 °C. The supernatant was lysed in Laemmli sample buffer. GEPI protein was separated in 9% SDS-PAGE and transferred to PVDF membrane (Millipore, IPVH00010). GC α and GC β proteins were separated in 4.5% SDS-PAGE.

The membrane was blocked with TBST buffer (0.3 M NaCl, 20 mM Tris-HCl, 0.1% Tween 20, pH 8.0) containing 5% skim milk and incubated with primary antibodies. After incubation, the membrane was washed three times with TBST and incubated with HRP-conjugated secondary antibodies. The membrane was washed five times in TBST before enhanced chemiluminescence detection.

Immunoprecipitation. For immunoprecipitation analysis, 6.0×10^7 gametocytes were lysed in 1 ml protein extraction buffer A plus (0.01% SDS, 1 mM DTT, 50 mM NaCl, 20 mM Tris-HCl; pH8.0). After ultrasonication, the protein solution was incubated on ice for 15 min before centrifugation at $14,000 \times g$ at 4 °C for 10 min. Rabbit anti-Myc antibody (1 µg, CST, #2272 s) or Rabbit anti-HA antibody (1 µg, CST, #3724 s) was added to the supernatant, and the solution was incubated on a vertical mixer at 4 °C for 15 h. After incubation, 20 µl buffer A plus pre-balanced protein A/G beads (Pierce, #20423) was added and incubated for 5 h. The beads were washed three times with buffer A plus before elution with Laemmli buffer.

Mass spectrometry. After immunoprecipitation as described above, proteins were eluted twice with 0.3% SDS in 20 mM Tris-HCl (pH 8.0). Eluted proteins were precipitated using 20% trichloroacetic acid (TCA), washed twice with 1 ml cold acetone, and dried in centrifugation vacuum. The protein pellets were dissolved in buffer containing 1% SDC, 10 mM TCEP, 40 mM CAA, Tris-HCl pH 8.5 and were digested with trypsin (1:100 ratio) at 37 °C for 12–16 h after dilution with water to reduce SDS content to 0.5%. Peptides were desalted using SDB-RPS StageTips. For timsTOF Pro, an ultra-high pressure nano-flow chromatography system (Elute UHPLC, Bruker) was coupled. Liquid chromatography was performed on a reversed-phase column (40 cm \times 75 µm i.d.) at 50 °C packed with Magic C18 AQ 3-µm 200-Å resin with a pulled emitter tip. The timsTOF Pro was operated in PASEF mode⁴⁹. Bruker.tdf raw files were converted to mgf files with the vendor provided software. The mgf files were searched against *P. yoelii* 17X genome database (downloaded from Uniprot) using PEAKS Studio X (BSI, Canada). Candidate peptides of targeted proteins were systematically validated by manual inspection of spectra.

Bioinformatics analysis and tools. The genomic sequences of *Plasmodium* genes were downloaded from the *Plasmodium* database of PlasmoDB (<http://plasmodb.org>). Transmembrane domains of proteins were identified using the TMHMM Server (<http://www.cbs.dtu.dk/services/TMHMM/>). Multiple sequence alignments were performed by ClustalW in MEGA7.0 [41]. Flow cytometry data were analyzed using FlowJo v10.

Quantification and statistical analysis. Statistical analysis was performed using GraphPad Software 8.0. Two-tailed Student's *t*-test or Whiney Mann test was used to compare differences between treated groups. *P*-value in each statistical analysis was indicated within the figures.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data supporting the findings of this study are available within the paper and its Supplementary Information files or are available from the corresponding author on reasonable request. The source data underlying Figs. 1a, e, f, 2i, 4a–c, 5e–f, 6c–f, 8b–c and Supplementary Figs. 2a, 3a–c, f, 5a–j, 6d–e, 8c–d, 9c–d, and 10b–c are provided as a Source Data file.

Received: 1 August 2019; Accepted: 14 March 2020;
Published online: 09 April 2020

References

- Guttery, D. S., Roques, M., Holder, A. A. & Tewari, R. Commit and transmit: molecular players in *Plasmodium* sexual development and zygote differentiation. *Trends Parasitol.* **31**, 676–685 (2015).
- Sologub, L. et al. Malaria proteases mediate inside-out egress of gametocytes from red blood cells following parasite transmission to the mosquito. *Cell Microbiol.* **13**, 897–912 (2011).
- Sinden, R. E. & Croll, N. A. Cytology and kinetics of microgametogenesis and fertilization in *Plasmodium yoelii nigeriensis*. *Parasitology* **70**, 53–65 (1975).
- Sinden, R. E. Sexual development of malarial parasites. *Adv. Parasitol.* **22**, 153–216 (1983).
- Billker, O. et al. Identification of xanthurenic acid as the putative inducer of malaria development in the mosquito. *Nature* **392**, 289–292 (1998).
- Garcia, G. E., Wirtz, R. A., Barr, J. R., Woolfitt, A. & Rosenberg, R. Xanthurenic acid induces gametogenesis in *Plasmodium*, the malaria parasite. *J. Biol. Chem.* **273**, 12003–12005 (1998).
- Muhia, D. K., Swales, C. A., Deng, W., Kelly, J. M. & Baker, D. A. The gametocyte-activating factor xanthurenic acid stimulates an increase in membrane-associated guanylyl cyclase activity in the human malaria parasite *Plasmodium falciparum*. *Mol. Microbiol.* **42**, 553–560 (2001).
- Gao, H. et al. ISPI-anchored polarization of Gβbeta/CDC50A complex initiates malaria ookinete gliding motility. *Curr. Biol.* **28**, 2763–2776 e2766 (2018).
- Hirai, M., Arai, M., Kawai, S. & Matsuoka, H. PbgCbeta is essential for *Plasmodium* ookinete motility to invade midgut cell and for successful completion of parasite life cycle in mosquitoes. *J. Biochem.* **140**, 747–757 (2006).
- Moon, R. W. et al. A cyclic GMP signalling module that regulates gliding motility in a malaria parasite. *PLoS Pathog.* **5**, e1000599 (2009).
- McRobert, L. et al. Gametogenesis in malaria parasites is mediated by the cGMP-dependent protein kinase. *PLoS Biol.* **6**, e139 (2008).
- Taylor, H. M. et al. The malaria parasite cyclic GMP-dependent protein kinase plays a central role in blood-stage schizogony. *Eukaryot. Cell* **9**, 37–45 (2010).
- Billker, O. et al. Calcium and a calcium-dependent protein kinase regulate gamete formation and mosquito transmission in a malaria parasite. *Cell* **117**, 503–514 (2004).
- Bennink, S., Kiesow, M. J. & Pradel, G. The development of malaria parasites in the mosquito midgut. *Cell Microbiol.* **18**, 905–918 (2016).
- Brochet, M. et al. Phosphoinositide metabolism links cGMP-dependent protein kinase G to essential Ca(2+)(+) signals at key decision points in the life cycle of malaria parasites. *PLoS Biol.* **12**, e1001806 (2014).
- Zhang, C. et al. Efficient editing of malaria parasite genome using the CRISPR/Cas9 system. *MBio* **5**, e01414–e01414 (2014).
- Zhang C., et al. CRISPR/Cas9 mediated sequential editing of genes critical for ookinete motility in *Plasmodium yoelii*. *Mol. Biochem. Parasitol.* (2016).
- Rangarajan, R. et al. A mitogen-activated protein kinase regulates male gametogenesis and transmission of the malaria parasite *Plasmodium berghei*. *EMBO Rep.* **6**, 464–469 (2005).
- Lopez-Barragan, M. J. et al. Directional gene expression and antisense transcripts in sexual and asexual stages of *Plasmodium falciparum*. *BMC Genomics* **12**, 587 (2011).
- Yeoh, L. M., Goodman, C. D., Mollard, V., McFadden, G. I. & Ralph, S. A. Comparative transcriptomics of female and male gametocytes in *Plasmodium berghei* and the evolution of sex in alveolates. *BMC Genomics* **18**, 734 (2017).
- Grotendorst, C. A., Kumar, N., Carter, R. & Kaushal, D. C. A surface protein expressed during the transformation of zygotes of *Plasmodium gallinaceum* is a target of transmission-blocking antibodies. *Infect. Immun.* **45**, 775–777 (1984).
- Tewari, R., Dorin, D., Moon, R., Doerig, C. & Billker, O. An atypical mitogen-activated protein kinase controls cytokinesis and flagellar motility during male gamete formation in a malaria parasite. *Mol. Microbiol.* **58**, 1253–1263 (2005).
- Reininger, L. et al. A NIMA-related protein kinase is essential for completion of the sexual cycle of malaria parasites. *J. Biol. Chem.* **280**, 31957–31964 (2005).
- Liu, C., Li, Z., Jiang, Y., Cui, H. & Yuan, J. Generation of *Plasmodium yoelii* malaria parasite carrying double fluorescence reporters in gametocytes. *Mol. Biochem. Parasitol.* **224**, 37–43 (2018).
- Sinden, R. E., Butcher, G. A., Billker, O. & Fleck, S. L. Regulation of infectivity of *Plasmodium* to the mosquito vector. *Adv. Parasitol.* **38**, 53–117 (1996).
- Rupp, I. et al. Malaria parasites form filamentous cell-to-cell connections during reproduction in the mosquito midgut. *Cell Res.* **21**, 683–696 (2011).
- Deligianni, E. et al. A perforin-like protein mediates disruption of the erythrocyte membrane during egress of *Plasmodium berghei* male gametocytes. *Cell Microbiol.* **15**, 1438–1455 (2013).
- Kehrer, J. et al. A putative small solute transporter is responsible for the secretion of G377 and TRAP-containing secretory vesicles during *Plasmodium* Gamete Egress and sporozoite motility. *PLoS Pathog.* **12**, e1005734 (2016).
- Zipperer, E. M., Neggess, M., Kushwaha, A., Rayavara, K. & Desai, S. A. A kinetic fluorescence assay reveals unusual features of Ca(+)(+) uptake in *Plasmodium falciparum*-infected erythrocytes. *Malar. J.* **13**, 184 (2014).
- Gao, X., Gunalan, K., Yap, S. S. & Preiser, P. R. Triggers of key calcium signals during erythrocyte invasion by *Plasmodium falciparum*. *Nat. Commun.* **4**, 2862 (2013).
- Singh, S., Alam, M. M., Pal-Bhowmick, I., Brzostowski, J. A. & Chitnis, C. E. Distinct external signals trigger sequential release of apical organelles during erythrocyte invasion by malaria parasites. *PLoS Pathog.* **6**, e1000746 (2010).
- Lakshmanan, V. et al. Cyclic GMP balance is critical for malaria parasite transmission from the mosquito to the mammalian host. *MBio* **6**, e02330 (2015).
- Taylor, C. J., McRobert, L. & Baker, D. A. Disruption of a *Plasmodium falciparum* cyclic nucleotide phosphodiesterase gene causes aberrant gametogenesis. *Mol. Microbiol.* **69**, 110–118 (2008).

34. Carucci, D. J. et al. Guanylyl cyclase activity associated with putative bifunctional integral membrane proteins in *Plasmodium falciparum*. *J. Biol. Chem.* **275**, 22147–22156 (2000).
35. Baker, D. A. et al. Cyclic nucleotide signalling in malaria parasites. *Open Biol.* **7**, 331–339 (2017).
36. Sebastian, S. et al. A *Plasmodium* calcium-dependent protein kinase controls zygote development and transmission by translationally activating repressed mRNAs. *Cell Host Microbe* **12**, 9–19 (2012).
37. Qian, P. et al. A Cas9 transgenic *Plasmodium yoelii* parasite for efficient gene editing. *Mol. Biochem. Parasitol.* **222**, 21–28 (2018).
38. Bagchi, S., Fredriksson, R. & Wallen-Mackenzie, A. In Situ Proximity Ligation Assay (PLA). *Methods Mol. Biol.* **1318**, 149–159 (2015).
39. Alam, M. S. Proximity Ligation Assay (PLA). *Curr. Protoc. Immunol.* **123**, e58 (2018).
40. Bushell, E. et al. Functional profiling of a *Plasmodium* genome reveals an abundance of essential genes. *Cell* **170**, 260–272 e268 (2017).
41. Kawamoto, F., Alejo-Blanco, R., Fleck, S. L., Kawamoto, Y. & Sinden, R. E. Possible roles of Ca²⁺ and cGMP as mediators of the exflagellation of *Plasmodium berghei* and *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* **42**, 101–108 (1990).
42. Kawamoto, F. et al. The roles of Ca²⁺/calmodulin- and cGMP-dependent pathways in gametogenesis of a rodent malaria parasite, *Plasmodium berghei*. *Eur. J. Cell Biol.* **60**, 101–107 (1993).
43. Linder, J. U. et al. Guanylyl cyclases with the topology of mammalian adenylyl cyclases and an N-terminal P-type ATPase-like domain in paramecium, tetrahymena and plasmodium. *EMBO J.* **18**, 4222–4232 (1999).
44. Bisio, H., Lunghi, M., Brochet, M. & Soldati-Favre, D. Phosphatidic acid governs natural egress in *Toxoplasma gondii* via a guanylate cyclase receptor platform. *Nat. Microbiol.* **4**, 420–428 (2019).
45. Brown, K. M. & Sibley, L. D. Essential cGMP signaling in toxoplasma is initiated by a Hybrid P-type ATPase-guanylate cyclase. *Cell Host Microbe* **24**, 804–816 e806 (2018).
46. Yang, L. et al. An apically located hybrid guanylate cyclase-ATPase is critical for the initiation of Ca(2+) signaling and motility in *Toxoplasma gondii*. *J. Biol. Chem.* **294**, 8959–8972 (2019).
47. Wright, D. A. et al. Standardized reagents and protocols for engineering zinc finger nucleases by modular assembly. *Nat. Protoc.* **1**, 1637–1652 (2006).
48. Raabe, A. C., Wengelnik, K., Billker, O. & Vial, H. J. Multiple roles for *Plasmodium berghei* phosphoinositide-specific phospholipase C in regulating gametocyte activation and differentiation. *Cell Microbiol.* **13**, 955–966 (2011).
49. Meier, F. et al. Online parallel accumulation-serial fragmentation (PASEF) with a novel trapped ion mobility mass spectrometer. *Mol. Cell Proteom.* **17**, 2534–2545 (2018).

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31772443, 31872214, 31970387), the Natural Science Foundation of Fujian Province

(2019J05010), the 111 Project sponsored by the State Bureau of Foreign Experts and Ministry of Education of China (BP2018017), and the Division of Intramural Research, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH, X.-z.S.). The authors thank Bradley Otterson (NIH Library Writing Center) for manuscript editing assistance.

Author contributions

J.Y.Y., C.H.T., W.J., L.C.Y., Z.Y., J.Z.Z., L.Z.K., L.S.N., Y.Z.K., W.X., and Q.P.G. generated the modified parasites, J.Y.Y. conducted the phenotype analysis, IFA assay, image analysis, mosquito experiments, and performed the biochemical experiments. Z.C. performed the Ca²⁺ mobilization, Z.C.Q. analyzed the MS results, J.Y.Y., C.H.T., and Y.J. analyzed the data. Y.J. and C.H.T. supervised the work. X.-z.S. and Y.J. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41467-020-15479-3>.

Correspondence and requests for materials should be addressed to J.Y.

Peer review information Nature Communications thanks David Baker, Gabriele Pradel and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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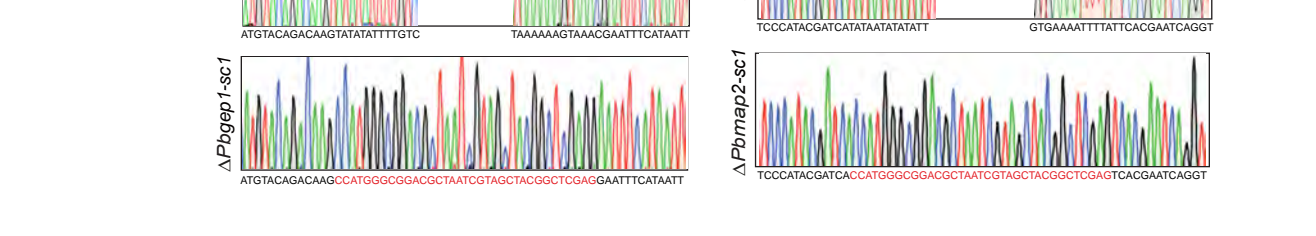
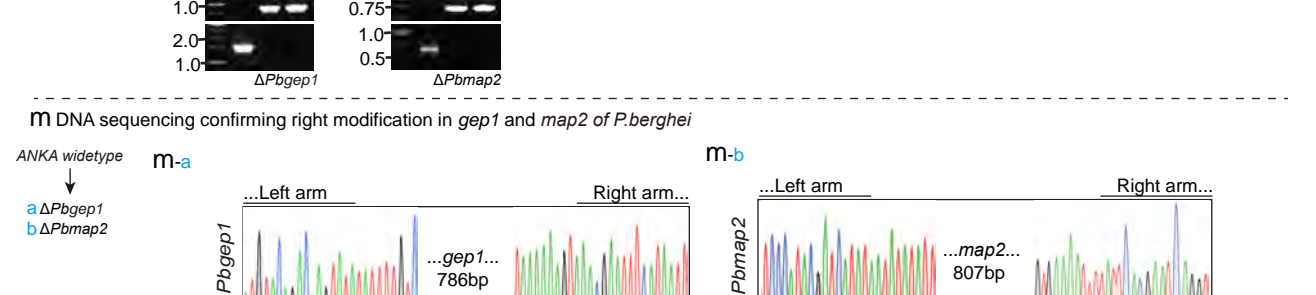
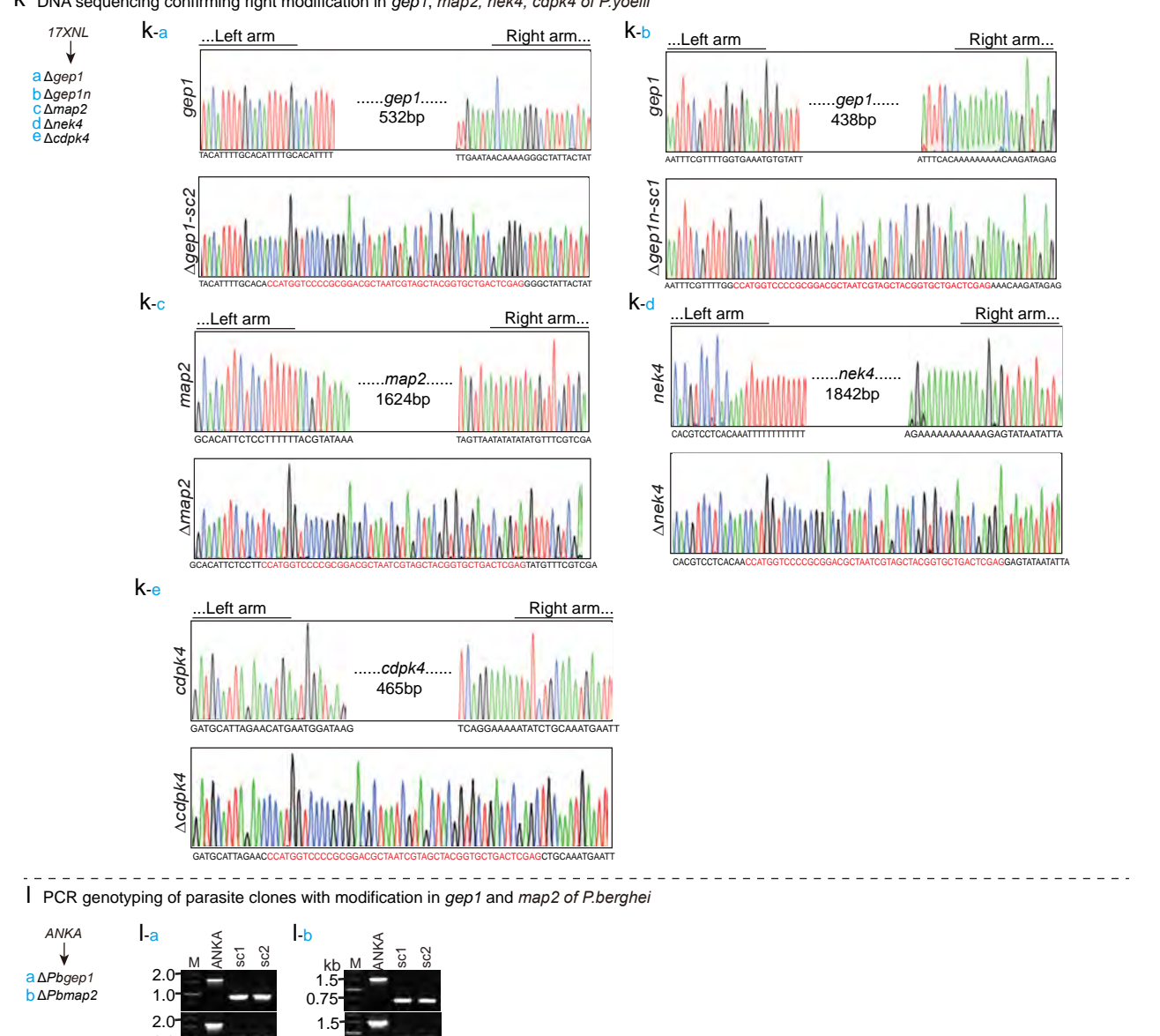
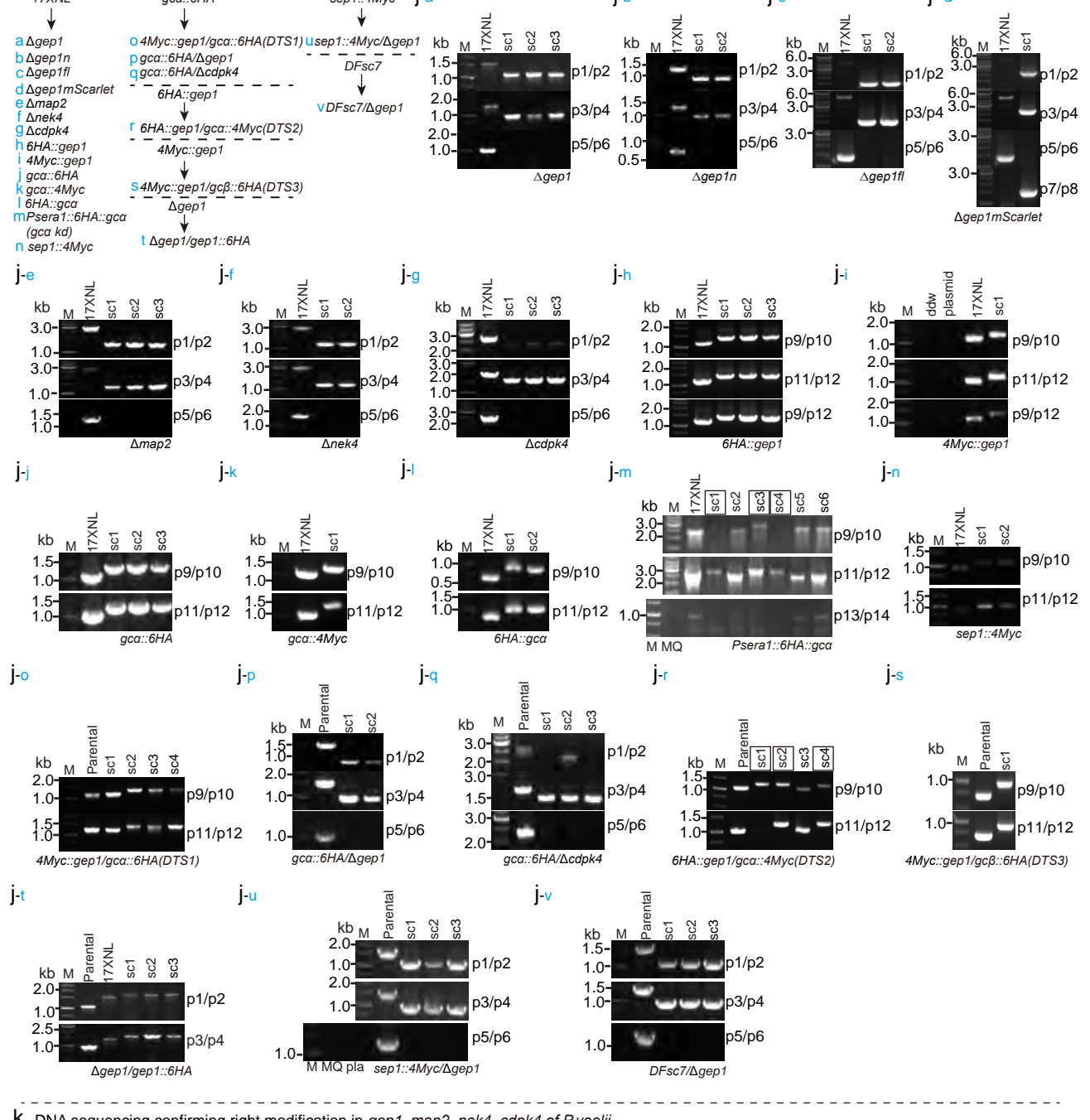
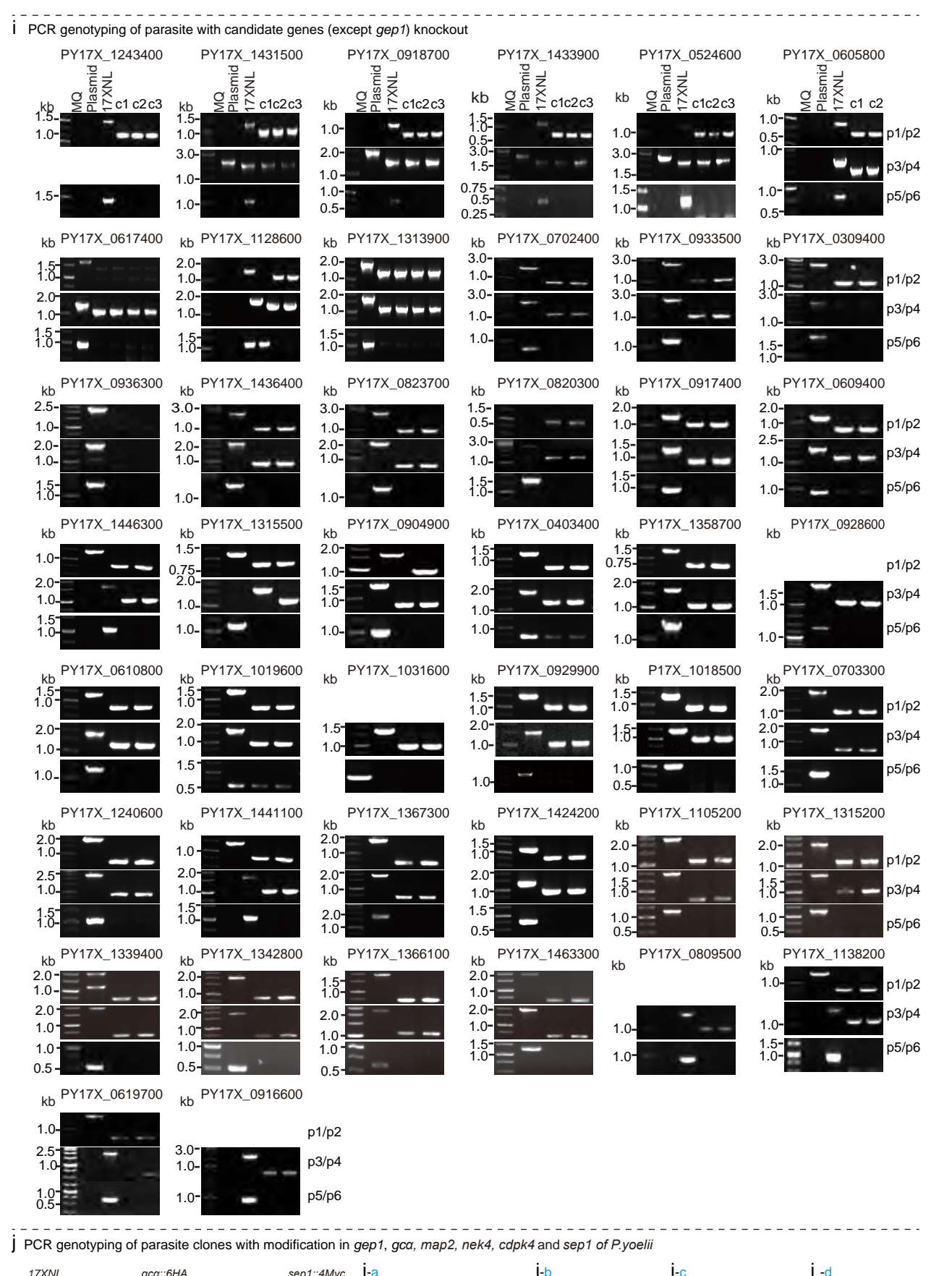
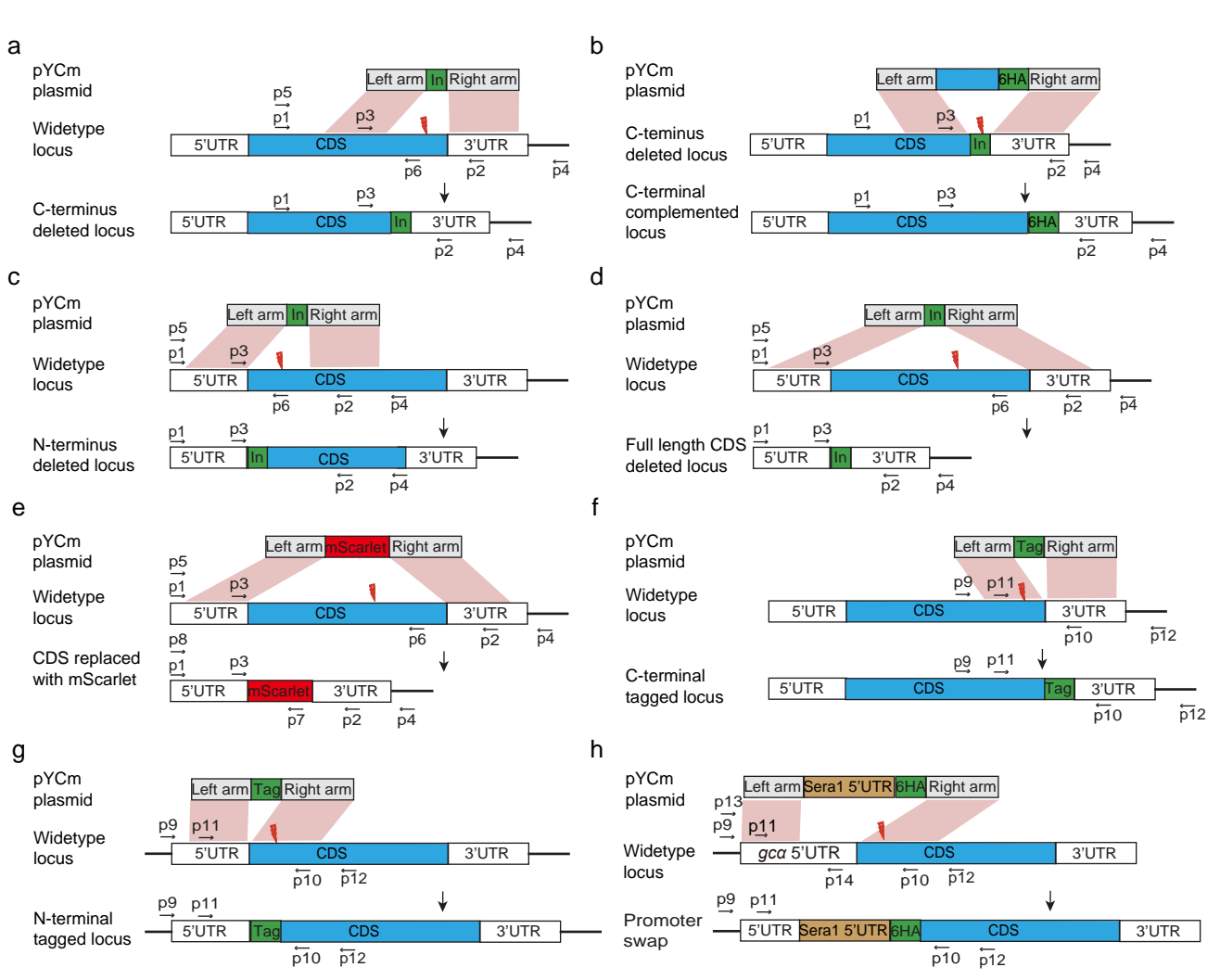
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Supplementary Information

An intracellular membrane protein GEP1 regulates xanthurenic acid induced gametogenesis of malaria parasites

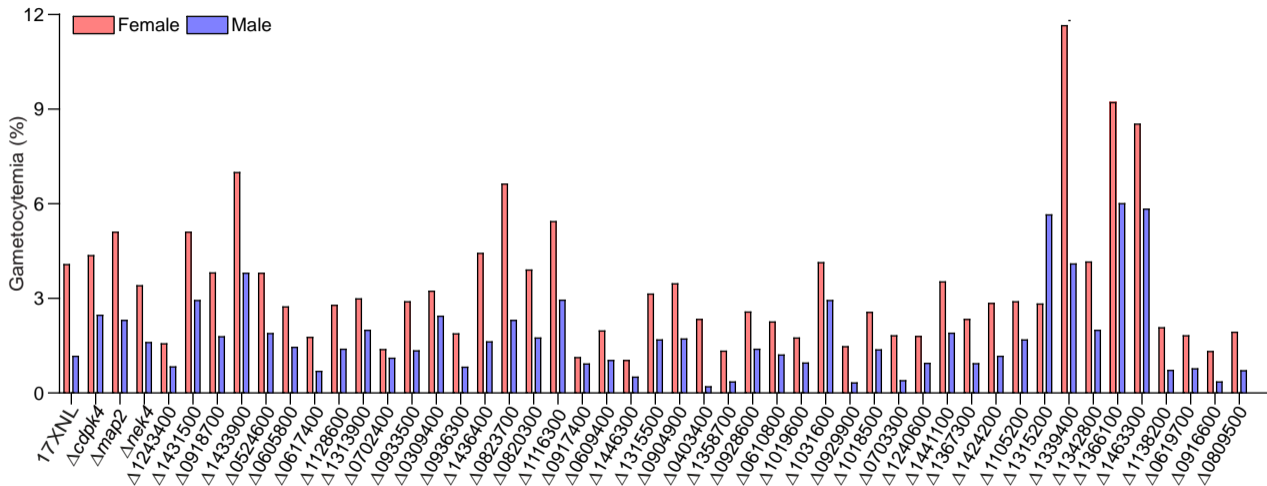
Jiang et al.

1. Supplementary Figures 1-11 and figure legends
2. Supplementary Table 1. List of candidate gene for screening in this study
3. Supplementary Table 2. GEP1 interacted proteins detected by Mass spectrum
4. Supplementary Table 3. Primers and oligonucleotides used in this study



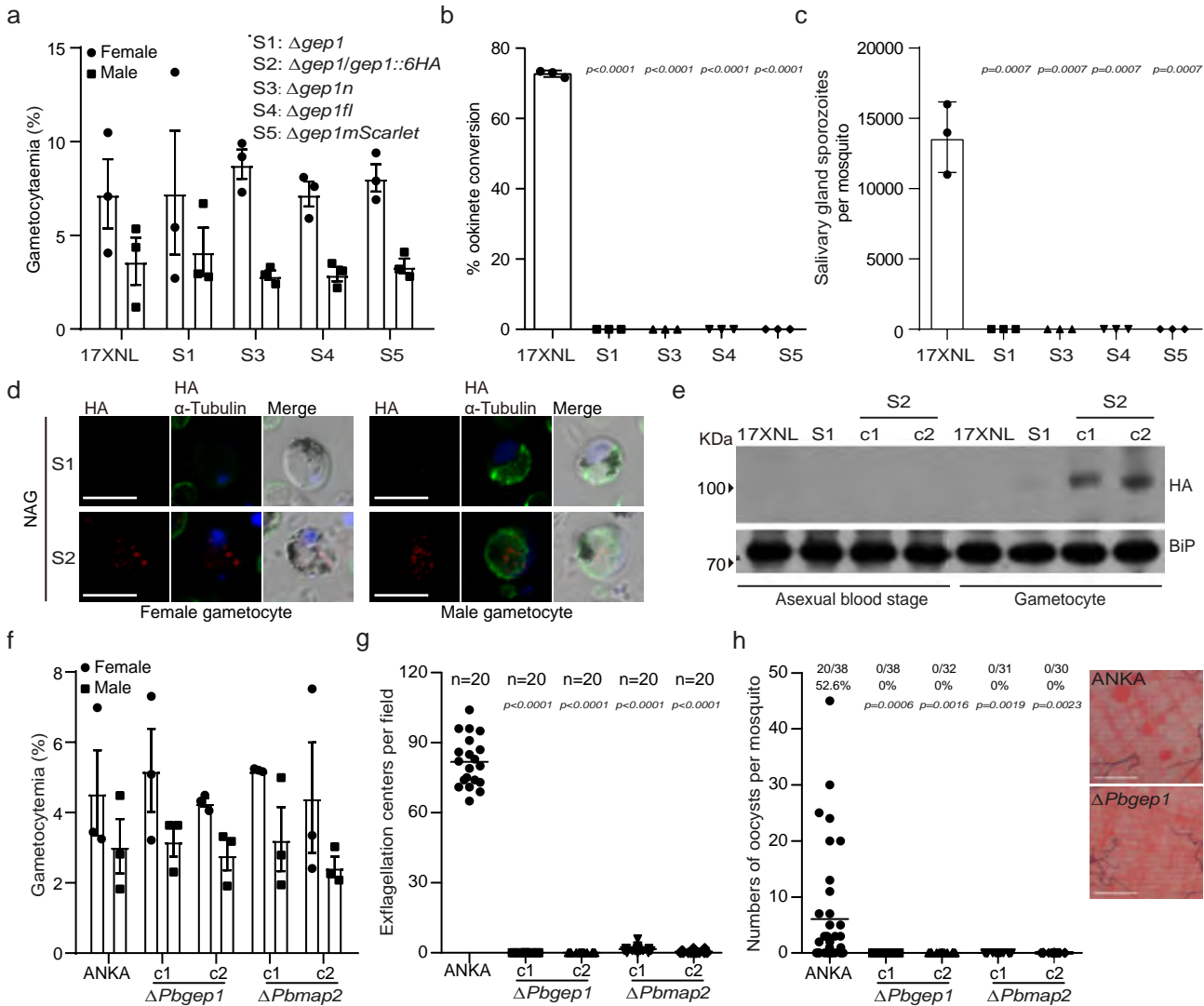
Supplementary Fig. 1. Genotyping and DNA sequencing of modified parasites.

(a to h) Schematic for CRISPR/Cas9-mediated gene modification, including gene deletion in the C-terminus **(a)**, gene re-constitution in the C-terminal **(b)**, gene deletion in the N-terminus **(c)**, deletion of full length CDS **(d)**, CDS replaced with mScarlet **(e)**, N-terminal **(f)** or C-terminal **(g)** tagging of genes with epitope tag, and promoter swap **(h)** via double cross homologous recombination. **(i to j and l)** For each modification, both 5' and 3' homologous recombination was detected using gene specific PCR pair ([Supplementary Table 3](#)) to confirm correct integration of the homologous template. At least two parasite clones (sc) for each modification were obtained after limiting dilution and phenotype analysis. **(k and m)** DNA sequencing to confirm correct modifications in some mutant parasites. Experiments in this figure were performed one time to ensure the correct genotype of the modified parasites.



Supplementary Fig. 2. Gametocyte formation in the mice.

The male and female gametocytemia in mice infected with 17XNL and 45 mutant parasites with candidate gene disrupted. The numbers shown in X axis are gene names (gene IDs) derived from the PlasmoDB database. Experiment was performed one time to ensure the formation of gametocyte in the mutants tested.



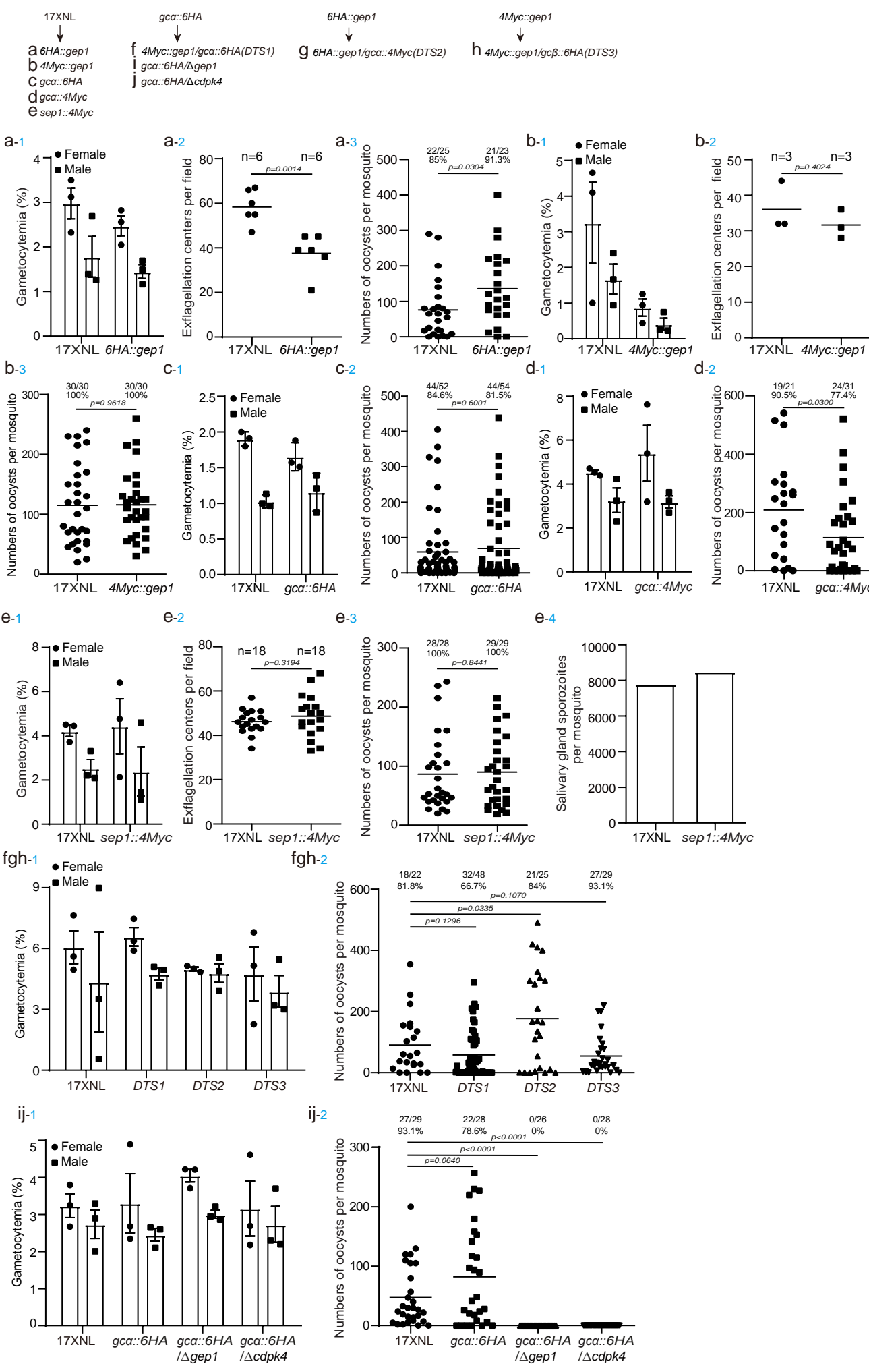
Supplementary Fig. 3. GEP1 disruption causes defect in gametogenesis.

a, Gametocytemia in mice infected with 17XNL or *gep1* mutant parasites: S1 ($\Delta gep1$), deletion in C-terminus; S3 ($\Delta gep1n$), deletion in N-terminus; S4 ($\Delta gep1fl$), deletion of the full coding region; S5 ($\Delta gep1mScarlet$), coding region replaced with *mScarlet* gene. **b**, *In vitro* ookinete conversion rates for 17XNL and the *gep1* mutants. **c**, Numbers of salivary gland sporozoites in mosquitoes 14 day after feeding on mice infected with the parasites. **d**, Co-staining of GEP1 and α -Tubulin (male gametocyte specific) in the non-activated gametocytes (NAG) of S1 and S2 parasites. Scale bar = 5 μ m. **e**, Western blot of GEP1 in ABS and gametocytes of S1 and S2 parasites. c1/c2: two independent clones of S2 parasite. **f**, Gametocytemia in mice infected with *P. berghei* ANKA and parasite with disrupted *Pbgep1* ($\Delta Pbgep1$) or *Pbmap2* ($\Delta Pbmap2$). **g**, Numbers of exflagellation centers (ECs) per microscopic field (40X) for the parasites in **f**. n is the numbers of microscopic fields counted (40X). **h**, Day 7 midgut oocyst counts in mosquitoes infected with the parasites in **f**. x/y on the top are the number of mosquito containing oocyst / the number of mosquito dissected; the percentage number indicates the mosquito infection prevalence. two-tailed unpaired Student's t test. Experiments were independently repeated three times in **a**, **b**, **c**, **d**, **f**, **g**, and two times in **e** and **h** with similar results. Data are shown as mean \pm SD; two-tailed unpaired Student's t test in **b**, **c**, **g** and **h**.

<i>P.yoelii</i>	1	- - - - -	- - - - -	- - - - -	M	E T S N - - - K E I	I S S K L K - - - -	- D D K M I K N K K	23
<i>P.berghei</i>	1	- - - - -	- - - - -	- - - - -	M	E T T N - - - K E I	I S S K L K - - - -	- D D K M I K N K K	23
<i>P.chabaudi</i>	1	- - - - -	- - - - -	- - - - -	M	E I P N - - - K E I	I F S K L K - - - -	- D D K I I Q N K K	23
<i>P.vivax</i>	1	- - - - -	- - - - -	- - - - -	-	E D T P - - - - -	- - - - -	- - - - -	5
<i>P.falci-parum</i>	1	M S R E T S K D D F	Y K T N K Y G H S I	E E S K N I Y K E S	Y E S D L N N E L E	E K E N K M K N R K			50
<i>P.yoelii</i>	24	K Y L H K F S Q Y N	- - - - -	- - - - -	-	N N Y S K I F R	N K H T L G - - - -	- - - - F R S V G R	53
<i>P.berghei</i>	24	K Y L H K I S Q Y N	- - - - -	- - - - -	-	N N Y S K M F R	N K H T L G - - - -	- - - - F R S I G K	53
<i>P.chabaudi</i>	24	K P L H K I G Q Y H	- - - - -	- - - - -	-	N N Y S K I F R	N K H T L G - - - -	- - - - F R S I G R	53
<i>P.vivax</i>	5	- - - G A S A Q K S	- - - - -	- - - - -	-	- - - - -	- - - - -	- - - - K F F G R	17
<i>P.falci-parum</i>	51	R R N A S S S F H N	S F N R N N I S I N	K D N G Y N N I R K	I K N K Y G D D Y F	P F R F Y K N I N N			100
<i>P.yoelii</i>	54	T K S S I I K K N K	N K S - - - - L G	I I T K - - - - -	- - - - -	- - - - -	- - - - E N I N L I	79	
<i>P.berghei</i>	54	T K S S I V K K N K	N K S - - - - L G	I I T K - - - - -	- - - - -	- - - - -	- - - - E N I N L I	79	
<i>P.chabaudi</i>	54	T K S S I I K K N K	S K S - - - - L G	I I T K - - - - -	- - - - -	- - - - -	- - - - E N I N L I	79	
<i>P.vivax</i>	18	S A S P E R S K S T	Y E S - - - - -	- - - - -	- - - - -	- - - - -	- - - - V I G C V	35	
<i>P.falci-parum</i>	101	Y K C S N V N K Y N	Y K D K Y K Y E K G	Y T S K N T K I C N	F R N N R I K T Y K	N T Y H N I I N F I		150	
<i>P.yoelii</i>	80	R H N K S D V R K I	R S I L F L K A Y E	H S E L F H K K K Q	D R V E K L K L N -	- - - - K Y N K C V	124		
<i>P.berghei</i>	80	R H N K S D I R K I	R S I L F L K A Y E	H S E L F H K K K Q	D R V E K L K L N -	- - - - E Y N K C I	124		
<i>P.chabaudi</i>	80	R H N K S D V R K I	R S I L F L K A Y E	H S E L F H K K K Q	D R L E K L K L N -	- - - - K Y N K C I	124		
<i>P.vivax</i>	36	K E N K N D R R R I	R Y S L F L K A Y E	N A E L F Q M E R Q	N K R E K L K L R M	N E W S K W N T C I	85		
<i>P.falci-parum</i>	151	R E N R N D M K K V	R Y S L F L R A Y E	N S E L F V Q I N K Q	N K K E K H N L R -	- - - - K H N K C I	195		
<i>P.yoelii</i>	125	E I K D A K E K E R	L K L L K L L K S Y	N I K Y E G Y T N L	Y D L C D Y S I Y F	S E Q N Y K N G E E	174		
<i>P.berghei</i>	125	E I K D A K E K E R	L K L L K L L K S Y	N I K Y E G Y T N L	Y D L C D Y S I Y F	S E Q N Y K N G E E	174		
<i>P.chabaudi</i>	125	E V K D A K E K E R	L K L L K L L K S Y	N I K Y E G Y T N L	Y D L C D Y S I Y F	S E Q N N K N G K E	174		
<i>P.vivax</i>	86	H M K G A K E K Q R	L K L L N M L K N Y	Q I K Y Q T Y S N L	K D L C D Y S V Y F	D K P S - - - - -	129		
<i>P.falci-parum</i>	196	I K D A K E K E R	L K L L K L L K S Y	D I K Y E G Y T D L	Y T L C N Y S I Y Y	E D K - - - - -	238		
<i>P.yoelii</i>	175	I K D K Q L C K I N	E K N Q K K K Y D C	D N F L Y L V S I	G I S Y N D I I E M	A S V F E N M K Y L	224		
<i>P.berghei</i>	175	T K G N I C K I N	E K N Q K K K Y D C	D N F L Y L V S I	G I S Y N D I I E M	A S V F E N M K Y L	224		
<i>P.chabaudi</i>	175	V T D I Q M Y K I N	E K N Q K K K D D C	D N F L Y L V S I	G I S Y N D I I E M	A S V F E N M K Y L	224		
<i>P.vivax</i>	129	- - - I G T V P K E	S K S I L K E A Q R	D N F F F Y L V S M	G V S Y N D I I L V	S S V F Q N R E H L	176		
<i>P.falci-parum</i>	238	- - - - - K L V	E E L L N K Q - N C	N N F F F Y L V S I	G I S Y N D I I H M	A S V F E N M E Y L	280		
<i>P.yoelii</i>	225	K Y C N L Y M L P W	I Y K K V N E F H N	Y D V N T F V L Y C	V V Y S I T T L S S	S L Y L F I K Y K T	274		
<i>P.berghei</i>	225	K Y C N L Y M L P W	I Y K K V N E F H N	Y D V N T F V L Y C	V V Y S I T T L S S	S L Y L F I K Y K T	274		
<i>P.chabaudi</i>	225	K Y C N L Y M L P W	I Y K K V N E F H N	Y D V N T F V L H C	V V Y S I T T L S S	S L Y L F I K Y K T	274		
<i>P.vivax</i>	177	K N C N L F M L P W	I Y R K L N D F H S	M D V I T F L I H C	V G Y S L S T F P S	S L F L F I Q Y R S	226		
<i>P.falci-parum</i>	281	K Y G N L F M L P W	I Y K K L N D F Y N	F D V T F T I L H C	I I Y S I S T L N S	S L F L F I K Y K T	330		
<i>P.yoelii</i>	275	I A I I F P L F I T	Y I F F S A P F L L	Q E I N G G R F V L	D G C I S F F N S I	N T C H L P I A I S	324		
<i>P.berghei</i>	275	I A I I F P L F I T	Y V F F S A P F L L	Q E I N G G R F V L	D G C I S F F N S I	N T C H L P I A I S	324		
<i>P.chabaudi</i>	275	I A I I F P L F I T	Y M F F S A P F L L	Q E I N G G R F V L	D G C I S F F N S I	N S Y H L P I A I S	324		
<i>P.vivax</i>	227	F A I V F P L F F T	Y M C L L S T P F L F	Q E I N C G R F V L	D G C I S F F W S L	D Y Y H L P I G I I	276		
<i>P.falci-parum</i>	331	L A I I F P L F I T	Y L L L S T P L V L	Q E I N S G R F V L	D G C I S F F W S I	N N Y H L P I G I I	380		
<i>P.yoelii</i>	325	L I I S Y I L S I I	K C I D S I C L H L	H Y F S Y Y F L D K	N P W V Y K N L N N	K I C S K F N G N K	374		
<i>P.berghei</i>	325	L I I S Y I L S I I	K C I D S I C L H L	H Y F S Y Y F L E K	N P W V Y K N L N N	K I C S K F N G N K	374		
<i>P.chabaudi</i>	325	L I I S Y I L S I I	K C I D S I C L H L	H Y F S Y Y F I D K	N P W V Y K N L N N	K I C S K F N G N K	374		
<i>P.vivax</i>	277	L I I S Y V L S I I	N C V D I I C L Q V	I Y F S F Y L A P N	N P W V Y R N V D A	K I C S K F N A S R	326		
<i>P.falci-parum</i>	381	L I I S Y I L S I I	K C I D F I S L H L	L Y L S Y L F E N	N P W I Y K N I D S	K I C S K F N G S K	430		
<i>P.yoelii</i>	375	N I C N I S R N I C	S Y N I A D G K C E	I N T M K L S M K M	Y D T L L R K Y T E	P K T K N F G E D I	424		
<i>P.berghei</i>	375	N I C N I S R N I C	S Y N I A D G K C E	I N T M K L S M K M	Y D T L L R K Y T E	P K T K N F G G S I	424		
<i>P.chabaudi</i>	375	N I C N I G R N I C	S Y N I T D G K C E	I N T M K L S M K M	Y D T L L R K Y T E	P K T I N F G G N V	424		
<i>P.vivax</i>	327	N I C D F A R N I C	F Y N E A T A V C E	L N R V I K L G T K I	Y D T L L S K Y A E	P K S E K F A A S T	376		
<i>P.falci-parum</i>	431	N I C D F S R N I C	Y Y N D Q T N I C E	I N K I K L G T K I	Y D M L L N K Y I P	P K S E K F P I L V	480		
<i>P.yoelii</i>	425	V L Y S F L F L F I	Y N S F S K Y K S S	N K L I K L F T F L	I I F I F T I D I I	S L R D F S L I E L	474		
<i>P.berghei</i>	425	V L Y S F L F L F L	Y N S F S K Y K S S	N K L I K L F A F L	I I F I F T I D I I	S L R D F S L I E L	474		
<i>P.chabaudi</i>	425	V L Y S F L F L F L	Y N S F S K Y K S S	N K L I K L F T F L	I I F I F T I D I I	S L R D F S L I E L	474		
<i>P.vivax</i>	377	V L L S F G L L I L	Y N A F S K Y K T S	H K V L K I H L S I	L I L V F A S F I I	T M R D F S L L E F	426		
<i>P.falci-parum</i>	481	I L A S F L S L I I	Y N A F S K Y K T S	H K F L K I F L F L	L I L F L M N I I	T V W D F T L F E F	530		
<i>P.yoelii</i>	475	L S A D L N I S K I	F N I L S N H E V W	I S C M I H C I V N	M S F H S G I Y F Y	T S K G L R L G I N	524		
<i>P.berghei</i>	475	L S V D L N I S K I	F N I L S N H E V W	I S C M M H C I V N	M S F H S G I Y F Y	T S K G L R L G I N	524		
<i>P.chabaudi</i>	475	L S V D L N V S K I	F S I L S N H E V W	I S C M I H C I V N	M S F H S G I Y F Y	T S K G L R L G I N	524		
<i>P.vivax</i>	427	L L A D F G W E R V	R G V L L D H E V W	I A C M M H C A L S	M S L H S G M Y F Y	T A K G L R L G V D	476		
<i>P.falci-parum</i>	531	L L S D F N F N K I	V D I I L N Y E V W	I L C M L H C I V N	L S I H S G L Y F Y	T S K G L R L G I N	580		
<i>P.yoelii</i>	525	I I Y C T Y L I V M	C C F L F D I L F I	I T F S V I I G N N	L K N I E N N Y Y F	L L K L I K R N F Y	574		
<i>P.berghei</i>	525	I I Y C T Y L I V M	C C F L F D I L F I	I T F S V I I G N N	L K N I E N N Y Y F	L L K L I K R N F Y	574		
<i>P.chabaudi</i>	525	I I Y C T Y L I V M	C C F L F D I L F I	I T F S V I I G N N	L K N I E N N Y Y F	L L K L I K R N F Y	574		
<i>P.vivax</i>	477	V V R C A C T A L A	C C F L L D M L L F	V T F S N I I G T H	I K D I A K N Y A F	L V K L V K R N V F	526		
<i>P.falci-parum</i>	581	V V K S T Y I I T L	S C F L V D M L I F	V A F S N I I G K Y	L K D I N R N Y S F	L L K L I K K N I F	630		
<i>P.yoelii</i>	575	Y I L L P V A H N Y	Y N K F T L F L S I	I F A F I Y L T F M	L I S A S K R I D V	L F L S L N D I F H	624		
<i>P.berghei</i>	575	Y I L L P V A H N Y	Y N K F T L F L S I	I F A F I Y L N F M	L I S A S K R I D I	L F L S L N D I F H	624		
<i>P.chabaudi</i>	575	Y I L L P V A H N Y	Y N K F T L F L S I	I F A F I Y L T F M	L I S A S K R I D I	L F L S L N D I F H	624		
<i>P.vivax</i>	527	Y I L L P V G N N C	F S K S F F L G I	N I S V I Y L A F M	L L A S K R V E I	L F L S F D D V N F	576		
<i>P.falci-parum</i>	631	Y I L L I P V G N N L	Y N K F T L F L G I	Y L G I I F L T F L	L L S A S K R I D I	L F L S I N D M Y P	680		
<i>P.yoelii</i>	625	F R G N T Q N K I I	T L G W I F I F C L	Y Y I Y K S I N I N	Y L D I C I T Q L S	H I I I L L I L F Y	674		
<i>P.berghei</i>	625	F R G N T K K K I I	A L G W I F I F C L	Y Y I Y K S I N I N	Y L D I C I T Q L S	H I I I L L I L F Y	674		
<i>P.chabaudi</i>	625	F R G N T Q K M I I	T L G W I F I F C L	Y Y I Y R S I N I N	Y L D I C I T Q L S	H I I I L L I L F Y	674		
<i>P.vivax</i>	577	F K - - P K S R W F	D V R W V F L F L I	Y Y L Y S S V D T	F L D L F F T E M S	Q I V T L L I L F Y	624		
<i>P.falci-parum</i>	681	L N - - - S K K H I	T I V W V I F F I I	Y I N Y I L D N E	I R Y I L C Q Y V Y	Q L I T L L I L F Y	727		
<i>P.yoelii</i>	675	I N F N F F W I R G	I K K T A K K I G L	F P L S L N M I L I	F L N E F I F M Y C	E I R L K L Q N R V	724		
<i>P.berghei</i>	675	I N F N F F W I R G	I N K T T K K I G L	F P L A L N M I L I	F L N E F I F M Y F	E I L L K L Q N R V	724		
<i>P.chabaudi</i>	675	I N F N F F W I R G	I K K T A K K I G L	F P L A L N M I L I	F L N E F T F M Y F	E I F L K L Q N R V	724		
<i>P.vivax</i>	625	I N F N F F W V R G	F N K T M D K F G M	C P L L C Q V V L T	A L N L F L F F Y F	E I I F R L P N R V	674		
<i>P.falci-parum</i>	728	I N F N F F W L S G	I K E T V N K L G K	L P L I S K F F F T	F L N E F S L Y L L	E I I Y N V K Y R I	777		
<i>P.yoelii</i>	725	L L Y F I R Q F I N	I F I I P L F S I F	T Y S V F Q W I S C	R S P H S - - - L L	K E I T K - I Y L L	770		
<i>P.berghei</i>	725	L L Y F I R Q F I N	I F I I P L F S I F	I Y S T L Q W I S Y	R N S N S T L F L G	K E I A K - I N L L	773		
<i>P.chabaudi</i>	725	L L Y F I R Q F I N	I F I I P L C S I F	T Y S V F Q C I A C	R N S P G S A P F L G	K E M T K - S Y L L	773		
<i>P.vivax</i>	675	P L Y L V R Q L V N	I C V I P L V S V L	L S R C A S F G G P	K Q P R G G - - G L	Q R I L A D A Y E L	722		
<i>P.falci-parum</i>	778	S F F F I R Q F I N	I L I I P L S M L	I S S Y L S N M R K	K R K T K V K K G I	K Y I L Q N S Y S L	827		
<i>P.yoelii</i>	771	I I G N I D K S K H	I Q L E F N K N S K	Y I D W F N I Y L I	I F F R Y F A M D L	I F M C F L H L W N	820		
<i>P.berghei</i>	774	I I G N I D K S K H	I Q L E F N K N S K	Y M N W N I Y L I	I F F R Y F A M D L	I F M C F L H L W N	823		
<i>P.chabaudi</i>	774	I V G D I D K S K H	I Q L E F N K N S K	Y M K W F N I Y L I	I F F R Y F A M D L	I F M C F L H L W N	823		
<i>P.vivax</i>	723	A T E C A D K S K N	I Q L E P S Q T S	- - K W F N I Y M V	L F C K Y L G I D L	I F M C F V H V G S	769		
<i>P.falci-parum</i>	828	A I E Y T S K N K N	I Q L E Y I Q T M K	Y T K W F N I Y L I	F F L K Y I G L D I	I L M C I V Y V G N	877		
<i>P.yoelii</i>	821	E F L I K N E A F F	N L E N I I W G I D	P Y L F F F L L Y V	I Y V Y I C Y L H V	P L L I L I K K K N	870		
<i>P.berghei</i>	824	E F L I K N E A F F	N L K N I I W G I D	P Y F F F F L L Y V	I Y V Y I C Y L H V	P L L I L I K K K N	873		
<i>P.chabaudi</i>	824	E F L I K N E A F F	N L E N I I F G I D	P Y L F F F L L Y V	I Y V Y I C Y L H V	P L L I L I K K K K	873		
<i>P.vivax</i>	770	S I F S I K E N F F	K K K N V H L Q T D	P Y L L F F L L F L	C Y V Y V A Y V N V	P L L Q I K R R K	819		
<i>P.falci-parum</i>	878	K L C S Q N E I Y L	K K R N V N I L L S	Q Y - F L F I F L	L V Y V I S Y I N I	P L L H I K R K L	926		
<i>P.yoelii</i>	871	I F K I N N F N I L	D Y H I P F D K I K	R N Q K N S F Y G E	F S I R G - - - -	- 905			
<i>P.berghei</i>	874	I F K V N N F N I L	D Y H I P F D K I K	R N K K N S F Y G E	F S L R G - - - -	- 908			
<i>P.chabaudi</i>	874	I F K V N N F N I L	D Y P I P F D Q I R	Q N K K S S F Y G E	F S I R G - - - -	- 908			
<i>P.vivax</i>	820	L F P V N K F S I L	D Y P V C E P I K	R P R R G L L F E E	F K R G R L K K S A	S 860			
<i>P.falci-parum</i>	927	F F K P N N F N V L	D Y P V S F E K I K	H Q K K N S L F S E	F I N M - - - - -	- 960			

Supplementary Fig. 4. GEP1 sequence alignment among *Plasmodium* parasites.

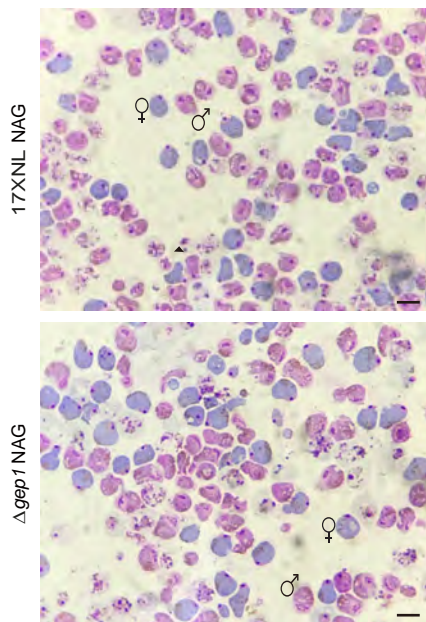
Aligned GEP1 amino acid sequences from *P. falciparum*, *P. vivax*, *P. chabaudi*, *P. berghei*, and *P. yoelii* (GeneID in PlasmoDB: PF3D7_0515500, PVP01_1018400, PCHAS_1114700, PBANKA_1115100, PY17X_1116300). Fourteen predicted transmembrane domains (TM1- TM14) are underlined.



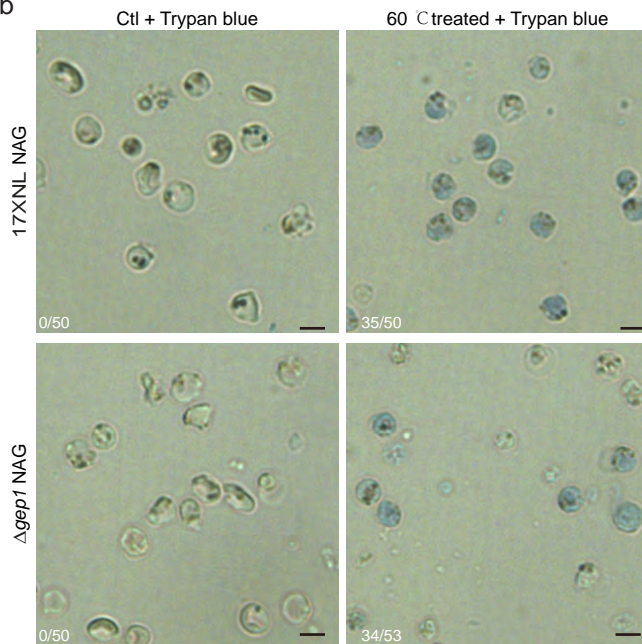
Supplementary Fig. 5. Parasite growth in mouse and mosquito of some strains.

Male and female gametocytes in mouse, *in vitro* exflagellation of male gametocytes, midgut oocyst formation and salivary gland sporozoite formation in mosquito of the modified parasites, including the *6HA::gеп1* (a), *4Myc::gеп1* (b), *gca::6HA* (c), *gca::4Myc* (d), *sep1::4Myc* (e), *DTS1* (f), *DTS2* (g), *DTS3* (h), and *gca::6HA/Δgеп1* (i, GEP1 disruption in the *gca::6HA*) and *gca::6HA/Δcdpk1* (j, CDPK1 disruption in the *gca::6HA*). Gametocytemia count was repeated three times, exflagellation and mosquito infection was performed one time to ensure the normal life cycle progression of these parasites. n in the exflagellation experiments is the numbers of microscopic fields counted (40X). x/y on the top of the oocyst count is the number of mosquito containing oocyst / the number of mosquito dissected; the percentage number is the mosquito infection prevalence. Gametocytemia data are shown as mean ± SEM. Two-tailed unpaired Student's t test was used in the exflagellation and oocyst counting.

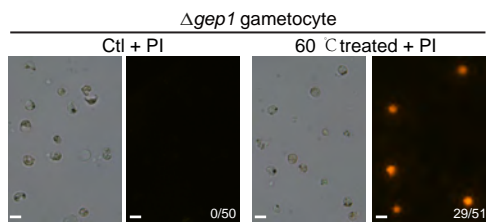
a



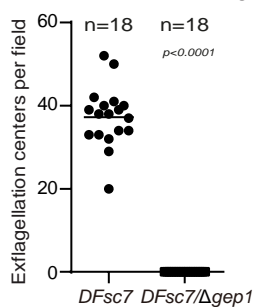
b



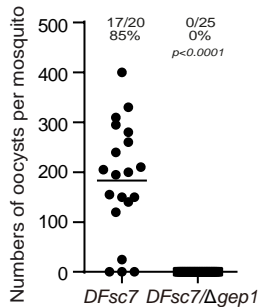
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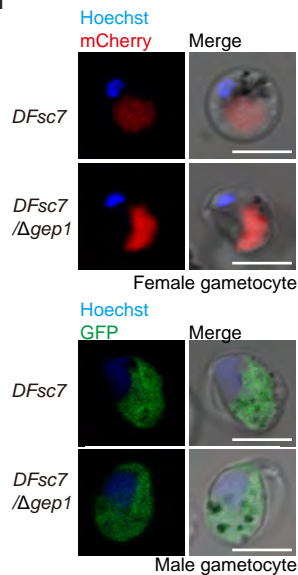
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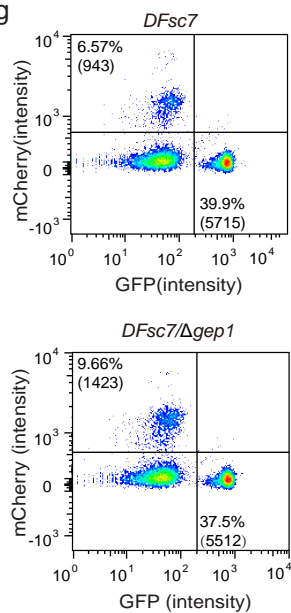
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f



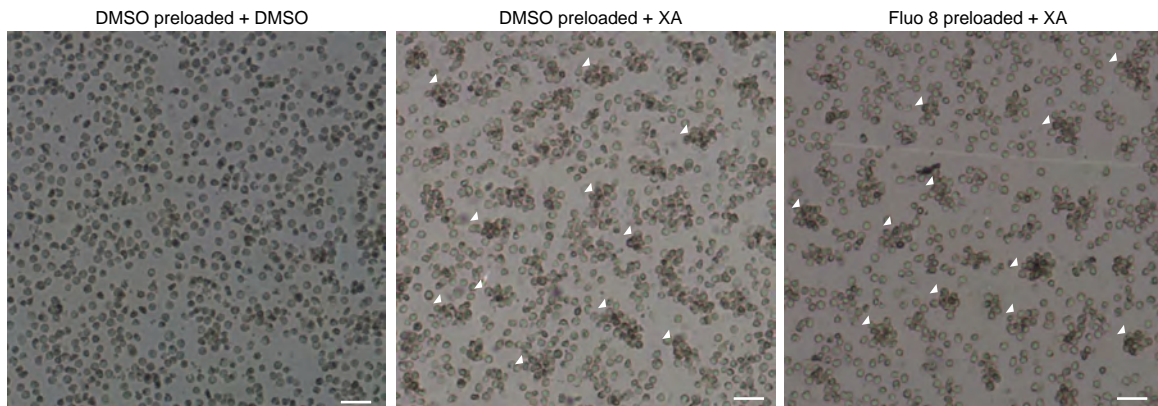
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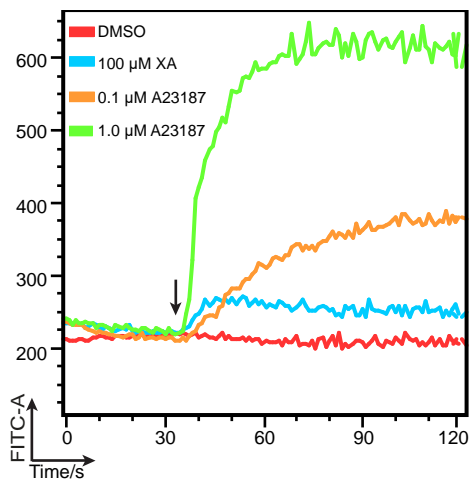
Supplementary Fig. 6. Gametocytes without GEP1 are viable.

a, Giemsa staining of the purified non-activated gametocytes (NAG). **b**, Cell viability analysis of the gametocytes by Trypan blue staining. **c**, Cell viability analysis of the $\Delta gep1$ gametocytes by propidium iodide (PI) staining. x/y in the **b** and **c** are the number of cell displaying signal / the number of cell counted. **d**, EC formation of the *DFsc7* and *DFsc7/ $\Delta gep1$* parasites after XA stimulation *in vitro*. n is the numbers of microscopic fields counted. **e**, Day 7 midgut oocysts counts from mosquitoes infected with *DFsc7* or *DFsc7/ $\Delta gep1$* parasites. Mosquito infection prevalence is shown above. **f**, GFP expression in male gametocytes and mCherry expression in female gametocytes of the *DFsc7* and *DFsc7/ $\Delta gep1$* parasites. **g**, Flow cytometry detection of GFP and mCherry fluorescence in male and female gametocytes of the *DFsc7* and *DFsc7/ $\Delta gep1$* parasites. Cell number and percentage are shown in the quadrants of the FACS plots. Scale bar = 5 μm for all images in this figure. Experiments in this figure were independently repeated three times with similar results. Two-tailed unpaired Student's t test in **d** and **e**.

a

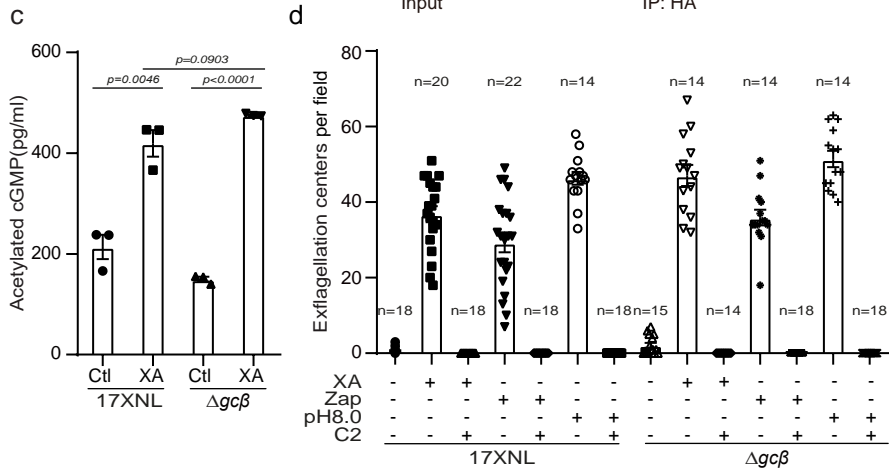
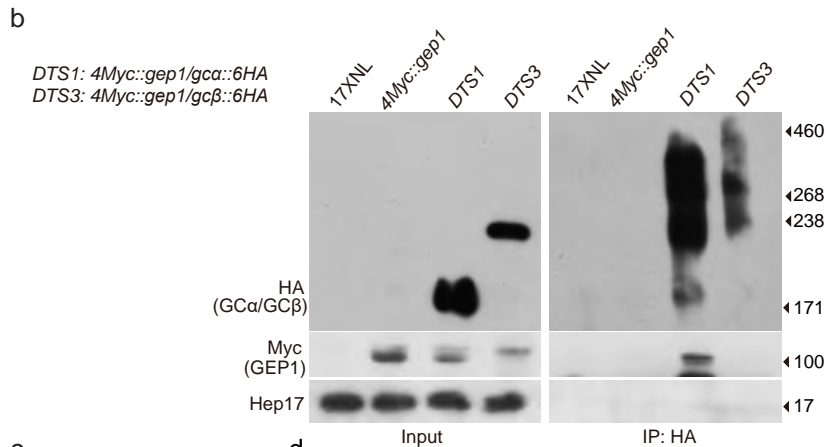
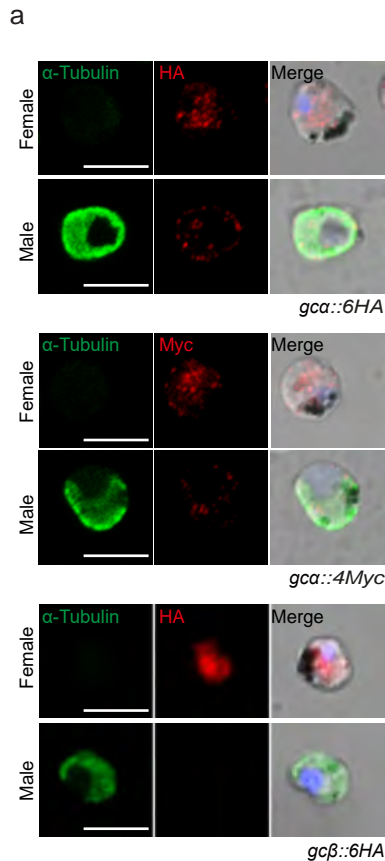


b



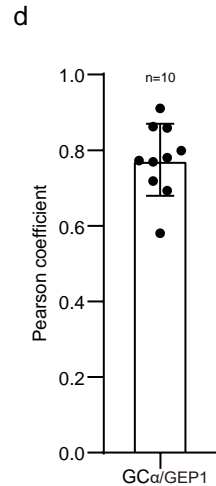
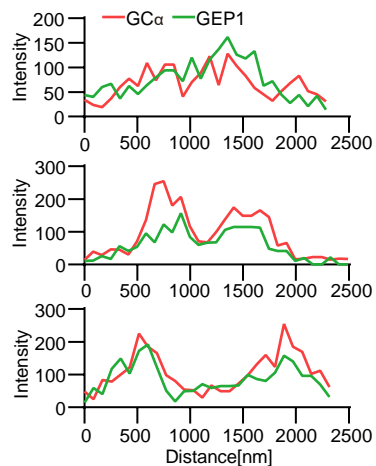
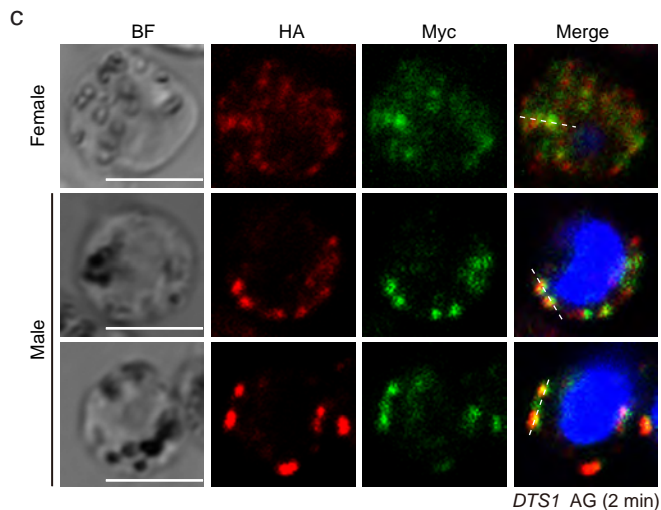
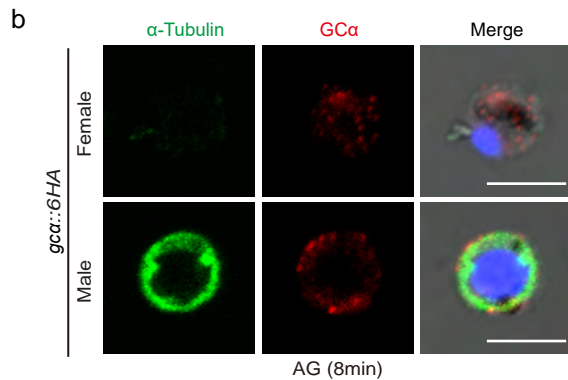
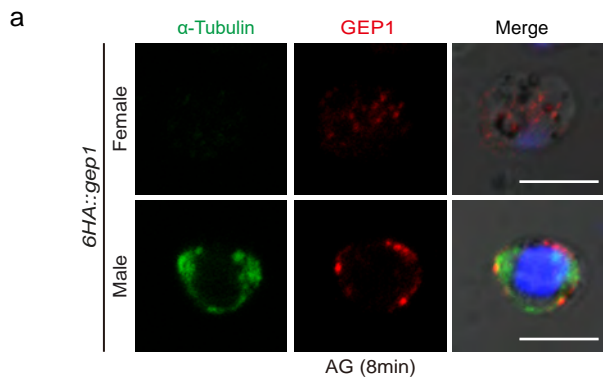
Supplementary Fig. 7. Cellular Ca²⁺ mobilization in activated gametocytes.

a, WT gametocytes pre-loaded with Fluo-8 are capable of forming XA-stimulated ECs (white arrows) *in vitro*. Scale bar = 20 μm . **b**, Cellular Ca²⁺ signals in Fluo-8 pre-loaded WT gametocytes in response to A23187 (Ca²⁺ ionophore) and XA stimulation using flow cytometry analysis. Purified gametocytes were preloaded with Ca²⁺ probe Fluo-8, and signals were collected 30 seconds before addition of A23187, XA, or DMSO. Black arrows indicate the time for chemical addition. Experiments in this figure were independently repeated three times with similar results.



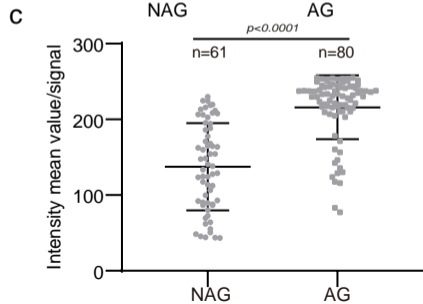
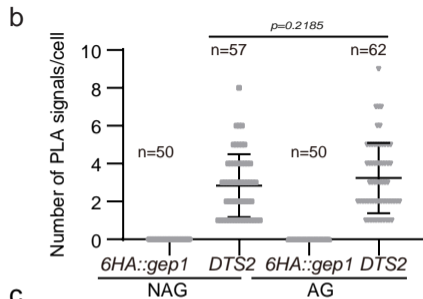
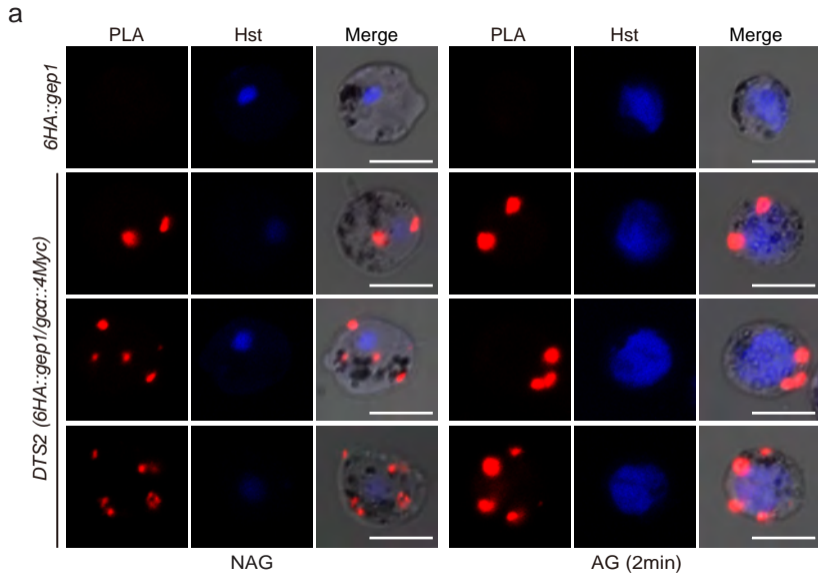
Supplementary Fig. 8. GC β is not involved in cGMP signaling of gametogenesis.

a, IFA of GC α and GC β expression in female and male gametocytes of the *gca::6HA*, *gca::4Myc*, and *gcb::6HA* parasites. α -Tubulin is a male gametocyte specific protein. Scale bar = 5 μ m. **b**, Co-immunoprecipitation of GEP1 and GC β in gametocyte extract of the *4Myc::gep1/gcb::6HA* parasites (Double Tagged Strain 3, *DTS3*). IP-HA, anti-HA antibody was used in pulldown. **c**, Enzyme immunoassay detecting intracellular cGMP level in XA-stimulated gametocytes of the 17XNL and Δ *gcb* parasites. Cells were incubated with 100 μ M XA at 22°C for 2 min before assay. Ctl is control group without XA stimulation. **d**, *In vitro* EC formation of the 17XNL and Δ *gcb* parasites after stimulation with XA (100 μ M), Zaprinast (Zap, 100 μ M), or pH 8.0 alone at 22°C, or conjugated with compound 2 (C2, 5 μ M). Experiments in this figure were independently repeated three times. Data are represented as mean \pm SEM in **c** and **d**, two-tailed unpaired Student's t test in **c**.



Supplementary Fig. 9. GEP1 co-localizes with GC α in activated gametocytes.

a, Co-staining of GEP1 and α -Tubulin expressions in the activated gametocytes (AG) of the *6HA::gep1* parasite 8 min post XA stimulation. **b**, Co-staining GC α and α -Tubulin in the *gca::6HA* gametocytes 8 min post XA stimulation. **c**, Co-staining of GEP1 and GC α in the *DTS1* gametocytes 2 min post XA stimulation using anti-HA (GC α) and anti-Myc (GEP1) antibodies (left panel). Activated male gametocytes were observed with enlarged nucleus. Cross sections (white dash line) of the cells show the co-localization of GEP1 and GC α (right panel). Scale bar = 5 μ m. **d**, Pearson coefficient analysis for GEP1 and GC α co-localization shown in **c**, data are shown as mean \pm SD from n=10 cells measured. Scale bar = 5 μ m for all image in this figure. Experiments in this figure were independently repeated three times with similar results.



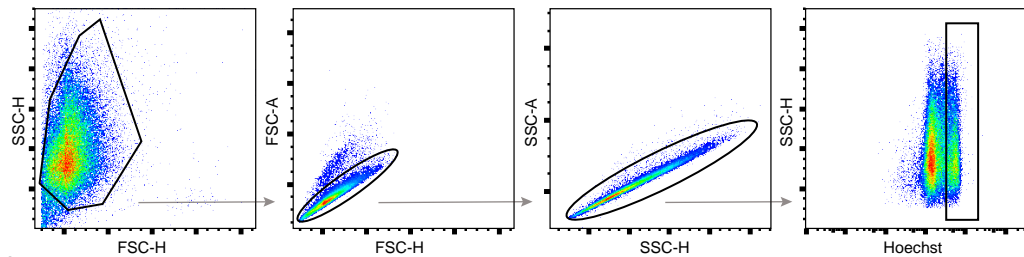
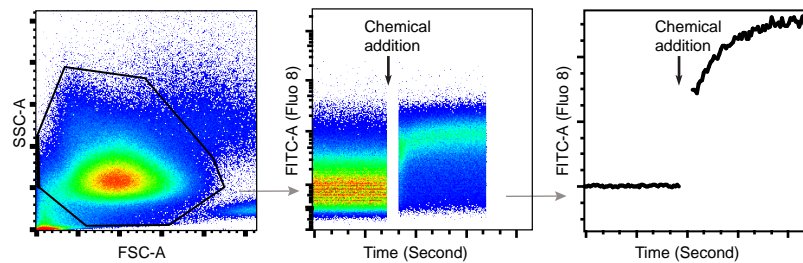
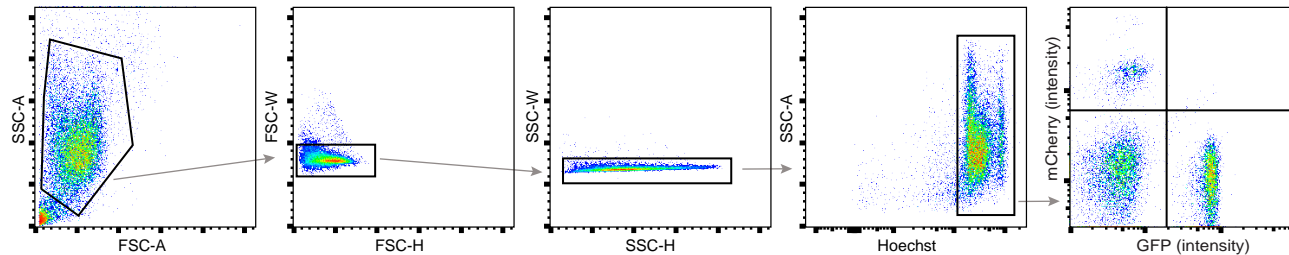
Supplementary Fig. 10. XA stimulation likely enhances GEP1/GC α interaction.

a, Proximity Ligation Assay (PLA) detecting protein interaction between GEP1 and GC α in *DTS2* gametocytes. NAG: non-activated, AG: 2 min after XA stimulation.

Activated male gametocytes were observed with enlarged nucleus. Scale bar = 5 μ m.

b, Number of PLA signal dot in each cell shown in **a**, n is the number of cells counted.

c, Fluorescence intensity value for each PLA signal dot shown in **a**. n is the number of PLA signal dot measured. Experiment was repeated three times independently with similar results. Data are represented as mean \pm SD; two-tailed unpaired Student's t test in **b** and **c**.

a**b****c**

1 **Supplementary Fig. 11. Gating strategies used for cell sorting in flow cytometry.**
2 **a**, Gating strategy to sort activated male gametocytes with rounds of genome
3 duplication from the purified gametocytes (including male and female) pre-stained with
4 Hoechst 33342 presented on Figure. 3b. **b**, Gating strategy to sort gametocyte preloaded
5 with Fluo-8 presented on Figure. 3d and Supplementary Figure. 7b. Black arrows
6 indicate the time for A23187, XA or DMSO addition. Signals were collected at 30 sec
7 before until 90 sec post addition. **c**, Gating strategy to sort male (GFP+) and female
8 (mCherry+) gametocytes from Hoechst 33342 pre-stained parasites containing
9 gametocytes and asexual blood stage parasites presented on Supplementary Figure. 6g.
10 Infected mice were phenylhydrazine treated for inducing gametocytes and sulfadiazine
11 treated to reduce asexual blood stage parasites. Parasites were further purified via
12 nycodenz centrifugation before flow cytometry analysis.
13

Supplementary Table 1. List of candidate gene for screening in this study.

Gene ID	Description	Ortholog in <i>P.falciparum</i>	Ortholog in <i>P.beighei</i>	Number of TM predicted	Blood-stage phenotype in <i>P.beighei</i> from <i>PlasmoGEM</i> database	Blood-stage phenotype in this study
PY17X_1243400	7-helix-1 protein, putative	PF3D7_0525400	PBANKA_1240200	3	Dispensable	Dispensable
PY17X_1431500	integral membrane protein GPR180, putative	PF3D7_1213500	PBANKA_1429300	7	Dispensable	Dispensable
PY17X_0918700	serpentine receptor, putative	PF3D7_1131100	PBANKA_0917100	7	Slow	Dispensable
PY17X_1433900	serpentine receptor, putative	PF3D7_1215900	PBANKA_1431600	8	Not tested	Dispensable
PY17X_0524600	serpentine receptor, putative	PF3D7_0422800	PBANKA_0523200	8	Dispensable	Dispensable
PY17X_1421700	GPCR-like receptor SR25, putative	PF3D7_0713400	PBANKA_1420000	8	Essential	Essential
PY17X_0605800	sexual stage-specific protein G37, putative	PF3D7_1204400	PBANKA_0603300	7	Not tested	Dispensable
PY17X_0617400	conserved Plasmodium membrane protein, unknown function	PF3D7_0717000	PBANKA_0614700	7	Not tested	Dispensable
PY17X_0914700	conserved Plasmodium membrane protein, unknown function	PF3D7_1135300	PBANKA_0913200	7	Essential	Essential
PY17X_1128600	protease, putative	PF3D7_0628400	PBANKA_1127100	8	Dispensable	Dispensable
PY17X_1313900	conserved Plasmodium protein, unknown function	PF3D7_1446300	PBANKA_1310000	8	Not tested	Dispensable
PY17X_0702400	folate transporter 1, putative	PF3D7_0828600	PBANKA_0702100	12	Dispensable	Dispensable
PY17X_0933500	folate transporter 2, putative	PF3D7_1116500	PBANKA_0931500	11	Dispensable	Dispensable
PY17X_0309400	GDP-fructose; GMP antiporter, putative	PF3D7_0212000	PBANKA_0308800	8	Not tested	Dispensable
PY17X_0936300	UDP-galactose transporter, putative	PF3D7_1113300	PBANKA_0934300	8	Essential	Dispensable
PY17X_1436400	phosphate translocator, putative	PF3D7_1218400	PBANKA_1434000	10	Dispensable	Dispensable
PY17X_0823700	major facilitator superfamily domain-containing protein, putative	PF3D7_0919500	PBANKA_0820400	12	Dispensable	Dispensable
PY17X_0820300	major facilitator superfamily domain-containing protein, putative	PF3D7_0916000	PBANKA_0817000	11	Dispensable	Dispensable
PY17X_1116300	amino acid transporter, putative	PF3D7_0515500	PBANKA_1115100	14	Dispensable	Dispensable
PY17X_0307300	transporter, putative	PF3D7_0209600	PBANKA_0306700	13	Essential	Essential
PY17X_0917400	amino acid transporter	PF3D7_1132500	PBANKA_0915900	15	Not tested	Dispensable
PY17X_0609400	amino acid transporter, putative	PF3D7_1208400	PBANKA_0606900	10	Dispensable	Dispensable
PY17X_1448600	amino acid transporter, putative	PF3D7_1231400	PBANKA_1446100	10	Essential	Essential
PY17X_1241000	multidrug resistance protein 1, putative	PF3D7_0523000	PBANKA_1237800	11	Essential	Essential
PY17X_1446300	multidrug resistance-associated protein 2, putative	PF3D7_1229100	PBANKA_1443800	11	Slow	Dispensable
PY17X_1315500	multidrug resistance protein 2, putative	PF3D7_1447900	PBANKA_1311700	10	Slow	Dispensable
PY17X_0904900	ABC transporter B family member 3, putative	PF3D7_1145500	PBANKA_0903500	6	Not tested	Dispensable
PY17X_0403400	ABC transporter B family member 4, putative	PF3D7_0302600	PBANKA_0401200	3	Dispensable	Dispensable
PY17X_1358700	ABC transporter B family member 5, putative	PF3D7_1339900	PBANKA_1353300	4	Slow	Dispensable
PY17X_0928600	protein GCN20, putative	PF3D7_1121700	PBANKA_0926600	0	Dispensable	Dispensable
PY17X_1370600	ABC transporter B family member 6, putative	PF3D7_1352100	PBANKA_1364800	4	Essential	Essential
PY17X_0610800	ABC transporter B family member 7, putative	PF3D7_1209900	PBANKA_0608300	6	Not tested	Dispensable
PY17X_1145400	ABC transporter E family member 1, putative	PF3D7_1368200	PBANKA_1144100	0	Essential	Essential
PY17X_1019600	ABC transporter G family member 2, putative	PF3D7_1426600	PBANKA_1018000	5	Slow	Dispensable
PY17X_1425800	ABC transporter F family member 1, putative	PF3D7_0813700	PBANKA_1423800	0	Essential	Essential
PY17X_1222000	ABC transporter I family member 1, putative	PF3D7_0319700	PBANKA_1218800	13	Essential	Essential
PY17X_1031600	FeS assembly ATPase SufC, putative	PF3D7_1413500	PBANKA_1029200	1	Essential	Dispensable
PY17X_0407300	ER membrane protein complex subunit 5, putative	PF3D7_0306700	PBANKA_0405100	2	Essential	Essential
PY17X_0929900	CorA-like Mg ²⁺ transporter protein, putative	PF3D7_1120300	PBANKA_0927900	2	Not tested	Dispensable
PY17X_1018500	CorA-like Mg ²⁺ transporter protein, putative	PF3D7_1427600	PBANKA_1017000	2	Dispensable	Dispensable
PY17X_0703300	magnesium transporter, putative	PF3D7_0827700	PBANKA_0703000	9	Not tested	Dispensable
PY17X_1240600	inner membrane complex protein, putative	PF3D7_0522600	PBANKA_1237300	8	Dispensable	Dispensable
PY17X_1441100	vacuolar iron transporter, putative	PF3D7_1223700	PBANKA_1438600	5	Dispensable	Dispensable
PY17X_1367300	E1-E2 ATPase, putative	PF3D7_1348800	PBANKA_1361600	10	Not tested	Dispensable
PY17X_0109300	zinc transporter ZIP1, putative	PF3D7_0609100	PBANKA_0107700	8	slow	Essential
PY17X_1424200	cation diffusion facilitator family protein, putative	PF3D7_0715900	PBANKA_1422200	4	Dispensable	Dispensable
PY17X_1105200	MOL01 domain-containing protein, putative	PF3D7_0504500	PBANKA_1104100	2	Dispensable	Dispensable
PY17X_1315200	conserved protein, unknown function	PF3D7_1447600	PBANKA_1311400	1	Dispensable	Dispensable
PY17X_1339400	transmembrane protein 43, putative	PF3D7_1471500	PBANKA_1334700	4	Dispensable	Dispensable
PY17X_1342800	conserved protein, unknown function	PF3D7_1322900	PBANKA_1338100	2	Dispensable	Dispensable
PY17X_1366100	conserved protein, unknown function	PF3D7_1347600	PBANKA_1360400	1	Dispensable	Dispensable
PY17X_1463300	dipeptidyl aminopeptidase 2, putative	PF3D7_1247800	PBANKA_1460700	1	Dispensable	Dispensable
PY17X_0911700	guanylyl cyclase, putative	PF3D7_1138400	PBANKA_0910300	19	slow	Essential
PY17X_1138200	guanylyl cyclase beta	PF3D7_1360500	PBANKA_1136700	22	slow	Dispensable
PY17X_0619700	LEM3/CDC50 family protein	PF3D7_0719500	PBANKA_0617000	2	Not tested	Dispensable
PY17X_0916600	LEM3/CDC50 family protein, putative	PF3D7_1133300	PBANKA_0915100	2	Dispensable	Dispensable
PY17X_0809500	P-type ATPase, putative	PF3D7_0319000	PBANKA_0806300	10	Dispensable	Dispensable
PY17X_1437200	aminophospholipid-transporting P-ATPase, putative	PF3D7_1219600	PBANKA_1434800	9	Essential	Essential
PY17X_1440800	phospholipid-ansporting ATPase, putative	PF3D7_1223400	PBANKA_1438300	10	slow	Essential

Supplementary Table 2. GEP1 interacted proteins detected by Mass spectrum.

Protein	Probability	Unique peptides	Gene_ID	Description	Protein size/aa
tr V7PTB0 V7PTB0_9APIC	1	21	PY17X_1347900	conserved Plasmodium protein, unknown function	2308
tr V7PT05 V7PT05_9APIC	1	19	PY17X_1226000	tyrosine-tRNA ligase, putative	372
tr V7PFK1 V7PFK1_9APIC	1	15	PY17X_0911700	guanylyl cyclase, putative	3850
tr V7PBU4 V7PBU4_9APIC	1	15	PY17X_1109100	conserved protein, unknown function	390
tr V7PHN7 V7PHN7_9APIC	1	13	PY17X_0404000	HAD superfamily protein, putative	1115
tr V7PBN0 V7PBN0_9APIC	1	10	PY17X_1114400	deoxyribodipyrimidine photo-lyase, putative	849
tr V7PNU6 V7PNU6_9APIC	1	10	PY17X_0807500	conserved Plasmodium protein, unknown function	1878
tr V7PDR5 V7PDR5_9APIC	1	9	PY17X_0922400	conserved Plasmodium protein, unknown function	1961
tr V7PGJ3 V7PGJ3_9APIC	1	9	PY17X_0706700	conserved Plasmodium protein, unknown function	1108
tr V7PVM5 V7PVM5_9APIC	1	9	PY17X_1221300	oocyst capsule protein Cap380, putative	3290
tr V7PV53 V7PV53_9APIC	1	8	PY17X_0113800	myosin E, putative	2403
tr V7PDP7 V7PDP7_9APIC	1	7	PY17X_0501900	reticulocyte binding protein, putative	2748
tr V7PGA5 V7PGA5_9APIC	1	6	PY17X_0623600	conserved Plasmodium protein, unknown function	1140
tr V7PJ59 V7PJ59_9APIC	1	6	PY17X_0520600	conserved Plasmodium protein, unknown function	225
tr V7PU86 V7PU86_9APIC	1	6	PY17X_1322800	conserved protein, unknown function	800
tr V7PYL5 V7PYL5_9APIC	1	6	PY17X_0103000	SAC3 domain-containing protein, putative	1203
tr V7PAS9 V7PAS9_9APIC	1	5	PY17X_0710300	2-oxoglutarate dehydrogenase E1 component, putative	1038
tr V7PBN8 V7PBN8_9APIC	1	5	PY17X_1370200	inner membrane complex protein 1f, putative	1122
tr V7PI71 V7PI71_9APIC	1	5	PY17X_0526500	reticulocyte binding protein, putative	2757
tr V7PN10 V7PN10_9APIC	1	5	PY17X_1016800	rRNA (adenosine-2'-O-)-methyltransferase, putative	2379
tr V7PNJ6 V7PNJ6_9APIC	1	5	PY17X_1135900	Sas10 domain-containing protein, putative	761
tr V7PP62 V7PP62_9APIC	1	5	PY17X_0216200	Plasmodium exported protein, unknown function	278
tr V7PRL1 V7PRL1_9APIC	1	5	PY17X_1223000	Cq2 protein, putative	2439
tr V7PDA4 V7PDA4_9APIC	1	4	PY17X_1102000	fam-a protein	609
tr V7PIB6 V7PIB6_9APIC	1	4	PY17X_0411100	eukaryotic translation initiation factor 3 subunit K,	235
tr V7PMC7 V7PMC7_9APIC	1	4	PY17X_1032900	mini-chromosome maintenance complex-binding protein, putative	910
tr V7PQH8 V7PQH8_9APIC	1	4	PY17X_1452400	CCR4-NOT transcription complex subunit 4, putative	1408
tr V7PSJ6 V7PSJ6_9APIC	1	4	PY17X_1430600	glycerol-3-phosphate 1-O-acyltransferase, putative	583
tr V7PXA8 V7PXA8_9APIC	1	4	PY17X_0103400	conserved Plasmodium protein, unknown function	499
tr V7PXF5 V7PXF5_9APIC	1	4	PY17X_0106800	conserved Plasmodium protein, unknown function	1921
tr V7PD97 V7PD97_9APIC	1	4	PY17X_0911300	WD repeat-containing protein, putative	1647
tr V7PJS9 V7PJS9_9APIC	1	4	PY17X_0417200	formate-nitrite transporter, putative	311
tr V7PKF4 V7PKF4_9APIC	1	4	PY17X_0419100	nucleoporin NUP221, putative	2003
tr V7PUE6 V7PUE6_9APIC	1	4	PY17X_0101221	reticulocyte binding protein, putative	2771
tr V7PBU9 V7PBU9_9APIC	1	3	PY17X_0301500	repetitive organellar protein, putative	1980
tr V7PDJ6 V7PDJ6_9APIC	1	3	PY17X_0915300	DNA-directed RNA polymerase I subunit RPA2, putative	1470
tr V7PDS4 V7PDS4_9APIC	1	3	PY17X_0309500	conserved Plasmodium protein, unknown function	987
tr V7PFR2 V7PFR2_9APIC	1	3	PY17X_0934500	histone-lysine N-methyltransferase SET7, putative	707
tr V7PI90 V7PI90_9APIC	1	3	PY17X_0419300	copper-transporting ATPase, putative	1981
tr V7PIU0 V7PIU0_9APIC	1	3	PY17X_0709200	protein transport protein SEC61 subunit beta, putative	79
tr V7PNL8 V7PNL8_9APIC	1	3	PY17X_0215800	AP2 domain transcription factor AP2-L, putative	1269
tr V7PP81 V7PP81_9APIC	1	3	PY17X_0832600	conserved Plasmodium protein, unknown function	131
tr V7PPU3 V7PPU3_9APIC	1	3	PY17X_1135500	U3 small nucleolar RNA-associated protein 21, putative	1246
tr V7PQK0 V7PQK0_9APIC	1	3	PY17X_0213300	cation transporting ATPase, putative	1682
tr V7PR31 V7PR31_9APIC	1	3	PY17X_1453700	p25-alpha family protein, putative	152
tr V7PRL8 V7PRL8_9APIC	1	3	PY17X_1136300	zinc finger protein, putative	571
tr V7PRR3 V7PRR3_9APIC	1	3	PY17X_1140100	conserved Plasmodium protein, unknown function	1415
tr V7PS22 V7PS22_9APIC	1	3	PY17X_1238000	EELM2 domain-containing protein, putative	825
tr V7PS95 V7PS95_9APIC	1	3	PY17X_1233600	ribonuclease, putative	2677
tr V7PXT9 V7PXT9_9APIC	1	3	PY17X_1340100	histone deacetylase, putative	1640
tr V7PU48 V7PU48_9APIC	1	3	PY17X_1331200	fam-a protein	387
tr V7PBE1 V7PBE1_9APIC	1	2	PY17X_1109300	conserved protein, unknown function	230
tr V7PE52 V7PE52_9APIC	1	2	PY17X_0923400	protein phosphatase, putative	288
tr V7PEX6 V7PEX6_9APIC	1	2	PY17X_1205600	WD repeat-containing protein, putative	2513
tr V7PEY8 V7PEY8_9APIC	1	2	PY17X_1105900	actin-like protein, putative	556
tr V7PFG6 V7PFG6_9APIC	1	2	PY17X_0910000	conserved Plasmodium protein, unknown function	166
tr V7PFI0 V7PFI0_9APIC	1	2	PY17X_1213100	conserved protein, unknown function	175
tr V7PG25 V7PG25_9APIC	1	2	PY17X_0944100	conserved Plasmodium protein, unknown function	708
tr V7PGV0 V7PGV0_9APIC	1	2	PY17X_0912800	CLPTM1 domain-containing protein, putative	644
tr V7PGX6 V7PGX6_9APIC	1	2	PY17X_1201900	conserved Plasmodium protein, unknown function	932
tr V7PI44 V7PI44_9APIC	1	2	PY17X_0404900	inner membrane complex protein 1e, putative	512
tr V7PI48 V7PI48_9APIC	1	2	PY17X_0405400	circumsporozoite (CS) protein	427
tr V7PIM7 V7PIM7_9APIC	1	2	PY17X_0704400	ubiquitin conjugation factor E4 B, putative	1240
tr V7PIX7 V7PIX7_9APIC	1	2	PY17X_0607700	DNA-directed RNA polymerase III subunit RPC2,	1414
tr V7PJT8 V7PJT8_9APIC	1	2	PY17X_0404100	DNA-directed RNA polymerases I, II, and III subunit RPABC2, putative	152
tr V7PQ08 V7PQ08_9APIC	1	2	PY17X_1437000	formin 2, putative	2823
tr V7PQ99 V7PQ99_9APIC	1	2	PY17X_1444900	conserved Plasmodium protein, unknown function	814
tr V7PRJ4 V7PRJ4_9APIC	1	2	PY17X_1464800	protein phosphatase PPM4, putative	734
tr V7PS26 V7PS26_9APIC	1	2	PY17X_1238300	protein phosphatase PPM9, putative	712
tr V7PTL0 V7PTL0_9APIC	1	2	PY17X_1341000	GTPase-activating protein, putative	616
tr V7PU13 V7PU13_9APIC	1	2	PY17X_0700600	fam-a protein	338
tr V7PUT1 V7PUT1_9APIC	1	2	PY17X_0109900	palmitoyltransferase DHHC2, putative	284
tr V7PUW8 V7PUW8_9APIC	1	2	PY17X_1456000	HD superfamily phosphohydrolase protein, putative	782
tr V7PWB5 V7PWB5_9APIC	1	2	PY17X_1240300	18S rRNA (guanine-N(7))-methyltransferase, putative	274
tr V7PYZ7 V7PYZ7_9APIC	1	2	PY17X_0111500	basal complex transmembrane protein 1, putative	634
tr V7PBB3 V7PBB3_9APIC	0.9999	3	PY17X_1111900	stearyl-CoA desaturase, putative	944
tr V7PI49 V7PI49_9APIC	0.9999	2	PY17X_0416400	zinc finger protein, putative	271
tr V7PJQ2 V7PJQ2_9APIC	0.9999	2	PY17X_1022100	peptidyl-prolyl cis-trans isomerase, putative	435
tr V7PPF6 V7PPF6_9APIC	0.9999	2	PY17X_1424900	conserved protein, unknown function	393
tr V7PR12 V7PR12_9APIC	0.9999	2	PY17X_1417200	U4/U6.U5 tri-snRNP-associated protein 2, putative	640

tr V7PVK6 V7PVK6_9APIC	0.9998	4	PY17X_1220100	ATP-dependent RNA helicase DDX42, putative	720
tr V7PKI5 V7PKI5_9APIC	0.9998	4	PY17X_0421700	reticulocyte binding protein, putative	2730
tr V7PQ59 V7PQ59_9APIC	0.9998	2	PY17X_1429100	protein phosphatase PPM7, putative	305
tr V7PTE3 V7PTE3_9APIC	0.9998	2	PY17X_1347700	ribose-phosphate pyrophosphokinase, putative	541
tr V7PAT4 V7PAT4_9APIC	0.9997	3	PY17X_0720800	RNA-binding protein NOB1, putative	491
tr V7PCA3 V7PCA3_9APIC	0.9997	3	PY17X_0717500	cdc2-related protein kinase 3	1263
tr V7PQS3 V7PQS3_9APIC	0.9997	3	PY17X_1445600	conserved Plasmodium protein, unknown function	1113
tr V7PBW3 V7PBW3_9APIC	0.9997	2	PY17X_1107500	transcription factor 25, putative	842
tr V7PIS2 V7PIS2_9APIC	0.9997	2	PY17X_0707800	RWD domain-containing protein, putative	228
tr V7PGU6 V7PGU6_9APIC	0.9995	2	PY17X_1215200	conserved Plasmodium protein, unknown function	2140
tr V7PH62 V7PH62_9APIC	0.9995	2	PY17X_1206500	U3 small nucleolar RNA-associated protein 25, putative	995
tr V7PWR8 V7PWR8_9APIC	0.9995	2	PY17X_1332700	serine/threonine protein phosphatase UIS2, putative	1339
tr V7PXB4 V7PXB4_9APIC	0.9995	2	PY17X_0103900	cation/H+ antiporter, putative	440
tr V7PHN2 V7PHN2_9APIC	0.9994	2	PY17X_0403900	CLP1 P-loop domain-containing protein, putative	1402
tr V7PF60 V7PF60_9APIC	0.9992	2	PY17X_1204500	conserved protein, unknown function	1075
tr V7PSZ8 V7PSZ8_9APIC	0.9992	2	PY17X_1354600	conserved oligomeric Golgi complex subunit 3, putative	1329
tr V7PDY2 V7PDY2_9APIC	0.9991	2	PY17X_0916800	conserved Plasmodium protein, unknown function	1073
tr V7PJX8 V7PJX8_9APIC	0.999	2	PY17X_1015000	large subunit GTPase 1, putative	763
tr V7PHA3 V7PHA3_9APIC	0.9989	2	PY17X_0511900	conserved Plasmodium protein, unknown function	1500
tr V7PR35 V7PR35_9APIC	0.9989	2	PY17X_0503500	serine/threonine protein phosphatase 8, putative	2124
tr V7PVS2 V7PVS2_9APIC	0.9988	2	PY17X_1356100	1-deoxy-D-xylulose 5-phosphate synthase, putative	1047
tr V7PSE4 V7PSE4_9APIC	0.9987	2	PY17X_1461300	conserved Plasmodium protein, unknown function	511
tr V7PT67 V7PT67_9APIC	0.9987	2	PY17X_1351300	conserved Plasmodium protein, unknown function	492
tr V7PMY2 V7PMY2_9APIC	0.9986	2	PY17X_1133200	histone-lysine N-methyltransferase, putative	511
tr V7PPG6 V7PPG6_9APIC	0.9986	2	PY17X_0211200	conserved protein, unknown function	713
tr V7PFE7 V7PFE7_9APIC	0.9985	2	PY17X_1209400	histone deacetylase 2, putative	2032
tr V7PGT8 V7PGT8_9APIC	0.9983	2	PY17X_0706000	conserved protein, unknown function	1006
tr V7PCP9 V7PCP9_9APIC	0.9982	2	PY17X_0312800	conserved Plasmodium protein, unknown function	354
tr V7PVZ3 V7PVZ3_9APIC	0.9981	2	PY17X_1231400	periodic tryptophan protein 2, putative	1040
tr V7PCA7 V7PCA7_9APIC	0.998	1	PY17X_0303800	DNA-directed RNA polymerase II 16 kDa subunit,	133
tr V7PCB5 V7PCB5_9APIC	0.998	1	PY17X_0718600	histone acetyltransferase, putative	1028
tr V7PDC0 V7PDC0_9APIC	0.998	1	PY17X_1120400	ATP synthase-associated protein, putative	128
tr V7PDN8 V7PDN8_9APIC	0.998	1	PY17X_1109600	conserved Plasmodium protein, unknown function	2719
tr V7PDY6 V7PDY6_9APIC	0.998	1	PY17X_0715000	ribosome assembly protein RRB1, putative	441
tr V7PEH6 V7PEH6_9APIC	0.998	1	PY17X_0933900	serine esterase, putative	1470
tr V7PEZ8 V7PEZ8_9APIC	0.998	1	PY17X_1105400	centrosomal protein CEP120, putative	525
tr V7PFL4 V7PFL4_9APIC	0.998	1	PY17X_0613900	pantothenate kinase 2	677
tr V7PH11 V7PH11_9APIC	0.998	1	PY17X_0610000	mago-binding protein, putative	150
tr V7PKB7 V7PKB7_9APIC	0.998	1	PY17X_1038600	RNA-binding protein 8A, putative	107
tr V7PL90 V7PL90_9APIC	0.998	1	PY17X_0815500	conserved protein, unknown function	856
tr V7PMQ8 V7PMQ8_9APIC	0.998	1	PY17X_0204600	bromodomain protein, putative	1337
tr V7PNQ7 V7PNQ7_9APIC	0.998	1	PY17X_1402800	ATP-dependent RNA helicase DHR1, putative	1557
tr V7PP89 V7PP89_9APIC	0.998	1	PY17X_0214900	ribosome biogenesis protein BRX1, putative	426
tr V7PPX4 V7PPX4_9APIC	0.998	1	PY17X_1434600	cell traversal protein for ookinetes and sporozoites	185
tr V7PPX5 V7PPX5_9APIC	0.998	1	PY17X_1138200	guanylyl cyclase beta	3015
tr V7PPZ0 V7PPZ0_9APIC	0.998	1	PY17X_0811500	E3 ubiquitin-protein ligase, putative	548
tr V7PQA0 V7PQA0_9APIC	0.998	1	PY17X_1146600	conserved Plasmodium protein, unknown function	2674
tr V7PQU3 V7PQU3_9APIC	0.998	1	PY17X_1447700	WD repeat-containing protein, putative	434
tr V7PR30 V7PR30_9APIC	0.998	1	PY17X_0503800	pre-mRNA-splicing factor RDS3, putative	111
tr V7PRG6 V7PRG6_9APIC	0.998	1	PY17X_1219600	mRNA-capping enzyme subunit beta, putative	613
tr V7PRY1 V7PRY1_9APIC	0.998	1	PY17X_1233800	zinc finger protein, putative	2333
tr V7PSC4 V7PSC4_9APIC	0.998	1	PY17X_1237800	zinc finger protein, putative	645
tr V7PSC6 V7PSC6_9APIC	0.998	1	PY17X_1369000	conserved Plasmodium protein, unknown function	864
tr V7PSF4 V7PSF4_9APIC	0.998	1	PY17X_1243200	structural maintenance of chromosomes protein 6, putative	1606
tr V7PT91 V7PT91_9APIC	0.998	1	PY17X_1450800	homocysteine S-methyltransferase, putative	507
tr V7PTT1 V7PTT1_9APIC	0.998	1	PY17X_1462800	conserved protein, unknown function	178
tr V7PUX3 V7PUX3_9APIC	0.998	1	PY17X_1456500	DNA gyrase subunit B, putative	930
tr V7PV18 V7PV18_9APIC	0.998	1	PY17X_1243000	zinc finger protein, putative	674
tr V7PVD4 V7PVD4_9APIC	0.998	1	PY17X_1368000	conserved Plasmodium protein, unknown function	2660
tr V7PX98 V7PX98_9APIC	0.998	1	PY17X_0102600	MYND-type zinc finger protein, putative	262
tr V7PEP9 V7PEP9_9APIC	0.9979	3	PY17X_0939500	conserved Plasmodium protein, unknown function	691
tr V7P9G4 V7P9G4_9APIC	0.9979	1	PY17X_1320000	syntaxin-6, putative	212
tr V7PBF4 V7PBF4_9APIC	0.9979	1	PY17X_1308100	zinc finger protein, putative	1223
tr V7PBN9 V7PBN9_9APIC	0.9979	1	PY17X_1113900	conserved protein, unknown function	251
tr V7PC70 V7PC70_9APIC	0.9979	1	PY17X_1304100	tubulin epsilon chain, putative	500
tr V7PCT4 V7PCT4_9APIC	0.9979	1	PY17X_0315400	SUZ domain-containing protein, putative	830
tr V7PCW5 V7PCW5_9APIC	0.9979	1	PY17X_1319100	LCCL domain-containing protein, putative	879
tr V7PFV2 V7PFV2_9APIC	0.9979	1	PY17X_0938000	endonuclease/exonuclease/phosphatase family protein, putative	653
tr V7PGI1 V7PGI1_9APIC	0.9979	1	PY17X_0705600	SAYSvFN domain-containing protein, putative	151
tr V7PNG3 V7PNG3_9APIC	0.9979	1	PY17X_1133600	splicing factor 3B subunit 5, putative	85
tr V7PNH1 V7PNH1_9APIC	0.9979	1	PY17X_1005300	gametocyte-specific protein, putative	177
tr V7PPK0 V7PPK0_9APIC	0.9979	1	PY17X_1412100	M1-family alanyl aminopeptidase, putative	1064
tr V7PQ92 V7PQ92_9APIC	0.9979	1	PY17X_1129800	amino acid transporter AAT1, putative	626
tr V7PQP2 V7PQP2_9APIC	0.9979	1	PY17X_1442900	small subunit rRNA processing factor, putative	418
tr V7PRI3 V7PRI3_9APIC	0.9979	1	PY17X_1463700	pre-mRNA-splicing factor RBM22, putative	393
tr V7PTE5 V7PTE5_9APIC	0.9979	1	PY17X_1454700	periodic tryptophan protein 1, putative	528
tr V7PUE5 V7PUE5_9APIC	0.9979	1	PY17X_1225800	AP-3 complex subunit delta, putative	1436
tr V7PH97 V7PH97_9APIC	0.9978	1	PY17X_0616200	conserved Plasmodium protein, unknown function	367
tr V7PLF5 V7PLF5_9APIC	0.9978	1	PY17X_0822900	conserved Plasmodium protein, unknown function	585
tr V7PNE8 V7PNE8_9APIC	0.9978	1	PY17X_1132200	phosphoinositide phosphatase SAC1, putative	814
tr V7PRS5 V7PRS5_9APIC	0.9978	1	PY17X_1229400	methionine aminopeptidase 1c, putative	680
tr V7PSD6 V7PSD6_9APIC	0.9978	1	PY17X_1459600	WD repeat-containing protein 82, putative	374
tr V7PLQ9 V7PLQ9_9APIC	0.9977	2	PY17X_1004000	6-cysteine protein	416
tr V7PM67 V7PM67_9APIC	0.9977	2	PY17X_0837300	conserved Plasmodium protein, unknown function	743
tr V7PQ77 V7PQ77_9APIC	0.9977	2	PY17X_1443000	conserved Plasmodium protein, unknown function	782

tr V7PE13 V7PE13_9APIC	0.9977	1	PY17X_0316800	Plasmodium exported protein, unknown function	248
tr V7PGY6 V7PGY6_9APIC	0.9977	1	PY17X_0521200	conserved Plasmodium protein, unknown function	710
tr V7PNC6 V7PNC6_9APIC	0.9977	1	PY17X_0805100	phosphopantetheine adenylyltransferase	1270
tr V7PNR0 V7PNR0_9APIC	0.9977	1	PY17X_0811600	conserved Plasmodium protein, unknown function	113
tr V7PPD4 V7PPD4_9APIC	0.9977	1	PY17X_0214200	alpha/beta hydrolase, putative	561
tr V7PQT0 V7PQT0_9APIC	0.9977	1	PY17X_1457700	ATP-dependent RNA helicase DBP9, putative	867
tr V7PWV2 V7PWV2_9APIC	0.9977	1	PY17X_1365100	conserved Plasmodium protein, unknown function	562
tr V7P9J2 V7P9J2_9APIC	0.9976	1	PY17X_1317600	signal recognition particle subunit SRP54, putative	500
tr V7PFN1 V7PFN1_9APIC	0.9976	1	PY17X_0931900	heat shock protein 90, putative	852
tr V7PFS2 V7PFS2_9APIC	0.9976	1	PY17X_0914700	conserved Plasmodium membrane protein, unknown function	416
tr V7PKJ1 V7PKJ1_9APIC	0.9976	1	PY17X_1033700	rhomboid protease ROM8, putative	637
tr V7PKU7 V7PKU7_9APIC	0.9976	1	PY17X_1027500	conserved Plasmodium protein, unknown function	4192
tr V7PNX9 V7PNX9_9APIC	0.9976	1	PY17X_1409500	protein phosphatase PPM6, putative	658
tr V7PQJ8 V7PQJ8_9APIC	0.9976	1	PY17X_1137900	ER membrane protein complex subunit 3, putative	256
tr V7PBS2 V7PBS2_9APIC	0.9975	1	PY17X_1317500	serine/threonine protein kinase, putative	375
tr V7PJE2 V7PJE2_9APIC	0.9975	1	PY17X_1028600	NADP-specific glutamate dehydrogenase, putative	470
tr V7PQY0 V7PQY0_9APIC	0.9975	1	PY17X_1414800	lipoate-protein ligase 1, putative	411
tr V7PRQ4 V7PRQ4_9APIC	0.9975	1	PY17X_1436000	thrombospondin-related apical membrane protein	349
tr V7PBZ0 V7PBZ0_9APIC	0.9974	1	PY17X_1312200	eukaryotic translation initiation factor 2-alpha kinase 1, putative	1496
tr V7PLV1 V7PLV1_9APIC	0.9974	1	PY17X_0812000	GTPase-activating protein, putative	891
tr V7PLV8 V7PLV8_9APIC	0.9974	1	PY17X_0215700	AP-4 complex subunit beta, putative	892
tr V7PNS6 V7PNS6_9APIC	0.9974	1	PY17X_0809800	cleavage and polyadenylation specificity factor, putative	954
tr V7PQ90 V7PQ90_9APIC	0.9974	1	PY17X_1145600	ribosomal protein L1, putative	312
tr V7PS98 V7PS98_9APIC	0.9974	1	PY17X_1234000	conserved oligomeric Golgi complex subunit 4, putative	1058
tr V7PTX9 V7PTX9_9APIC	0.9973	1	PY17X_1466200	transcription initiation factor IIA subunit 2, putative	177
tr V7PU03 V7PU03_9APIC	0.9973	1	PY17X_1330800	DNA-directed RNA polymerase III subunit RPC4, putative	339
tr V7PU30 V7PU30_9APIC	0.9973	1	PY17X_1328100	inner membrane complex sub-compartment protein 3, putative	150
tr V7PNX8 V7PNX8_9APIC	0.9972	2	PY17X_0804500	conserved Plasmodium protein, unknown function	3003
tr V7PDB9 V7PDB9_9APIC	0.9972	1	PY17X_0902700	membrane associated erythrocyte binding-like protein	1701
tr V7PI34 V7PI34_9APIC	0.9972	1	PY17X_0414600	vesicle transport v-SNARE protein, putative	197
tr V7PUK6 V7PUK6_9APIC	0.9972	1	PY17X_0102000	reticulocyte binding protein, putative	611
tr V7PLN3 V7PLN3_9APIC	0.9971	1	PY17X_1005700	conserved Plasmodium protein, unknown function	855
tr V7PN97 V7PN97_9APIC	0.9971	1	PY17X_0825400	conserved Plasmodium protein, unknown function	2161
tr V7PTS5 V7PTS5_9APIC	0.997	2	PY17X_1335900	rab GTPase activator, putative	1581
tr V7PK32 V7PK32_9APIC	0.997	1	PY17X_0408900	conserved Plasmodium protein, unknown function	280
tr V7PPE5 V7PPE5_9APIC	0.997	1	PY17X_1423900	GTP-binding translation elongation factor, putative	756
tr V7PER3 V7PER3_9APIC	0.9969	1	PY17X_1113000	CDK-activating kinase assembly factor MAT1, putative	260
tr V7PI60 V7PI60_9APIC	0.9969	1	PY17X_0406400	ubiquitin-conjugating enzyme E2, putative	139
tr V7PPU9 V7PPU9_9APIC	0.9969	1	PY17X_1136000	conserved Plasmodium protein, unknown function	1446
tr V7PUL6 V7PUL6_9APIC	0.9969	1	PY17X_1232200	nucleolar protein 10, putative	568
tr V7PGL4 V7PGL4_9APIC	0.9968	1	PY17X_0942000	DNA repair protein RAD51, putative	349
tr V7PPS8 V7PPS8_9APIC	0.9968	1	PY17X_0815800	conserved Plasmodium protein, unknown function	1219
tr V7PDK9 V7PDK9_9APIC	0.9966	2	PY17X_1112200	RNA pseudouridylylase synthase, putative	9786
tr V7PS28 V7PS28_9APIC	0.9966	1	PY17X_1224600	protein transport protein USE1, putative	320
tr V7PMC4 V7PMC4_9APIC	0.9965	2	PY17X_1033400	conserved Plasmodium protein, unknown function	980
tr V7PT14 V7PT14_9APIC	0.9965	1	PY17X_1356200	exoribonuclease, putative	812
tr V7PUP0 V7PUP0_9APIC	0.9965	1	PY17X_1233600	ribonuclease, putative	2677
tr V7PI88 V7PI88_9APIC	0.9964	1	PY17X_0524900	AP-4 complex subunit sigma, putative	146
tr V7PSK3 V7PSK3_9APIC	0.9963	1	PY17X_1431700	DNA-directed RNA polymerases I, II, and III subunit RPABC3, putative	143
tr V7PAT9 V7PAT9_9APIC	0.9962	1	PY17X_0711000	Snf2-related CBP activator, putative	1825
tr V7PGX8 V7PGX8_9APIC	0.9961	1	PY17X_0607900	conserved Plasmodium protein, unknown function	678
tr V7PPB9 V7PPB9_9APIC	0.996	1	PY17X_1406400	conserved protein, unknown function	512
tr V7PNF4 V7PNF4_9APIC	0.9959	1	PY17X_1006300	transcription factor, putative	373
tr V7PR83 V7PR83_9APIC	0.9959	1	PY17X_1457000	polyadenylation factor subunit 2, putative	481
tr V7PT86 V7PT86_9APIC	0.9959	1	PY17X_1450400	conserved Plasmodium protein, unknown function	1545
tr V7PK92 V7PK92_9APIC	0.9957	1	PY17X_1006900	AAA family ATPase, putative	625
tr V7PN06 V7PN06_9APIC	0.9957	1	PY17X_0503400	conserved Plasmodium protein, unknown function	252
tr V7PGS7 V7PGS7_9APIC	0.9956	1	PY17X_0523200	ribosome-recycling factor, putative	266
tr V7PS12 V7PS12_9APIC	0.9954	1	PY17X_1222800	cq1 protein, putative	997
tr V7PSD2 V7PSD2_9APIC	0.9953	1	PY17X_1238900	conserved protein, unknown function	906
tr V7PHT7 V7PHT7_9APIC	0.995	1	PY17X_0613500	trafficking protein particle complex subunit 5, putative	184
tr V7PQE1 V7PQE1_9APIC	0.995	1	PY17X_1434400	conserved Plasmodium protein, unknown function	727
tr V7PWG8 V7PWG8_9APIC	0.995	1	PY17X_0116400	PIR protein	632
tr V7PF24 V7PF24_9APIC	0.9949	1	PY17X_1209900	protein GPR89, putative	985
tr V7PHB0 V7PHB0_9APIC	0.9949	1	PY17X_0520000	conserved Plasmodium membrane protein, unknown function	442
tr V7PMT8 V7PMT8_9APIC	0.9949	1	PY17X_1129700	polyadenylate-binding protein 3, putative	535
tr V7PD44 V7PD44_9APIC	0.9946	1	PY17X_0604900	major facilitator superfamily domain-containing protein, putative	1040
tr V7PRY5 V7PRY5_9APIC	0.9941	1	PY17X_1220200	peptidase, putative	873
tr V7PAC6 V7PAC6_9APIC	0.9939	1	PY17X_1306300	conserved protein, unknown function	298
tr V7PDF5 V7PDF5_9APIC	0.9939	1	PY17X_0905900	SUMO-activating enzyme subunit 1, putative	369
tr V7PHN1 V7PHN1_9APIC	0.9939	1	PY17X_0511200	conserved protein, unknown function	1140
tr V7PJI1 V7PJI1_9APIC	0.9939	1	PY17X_0409600	dual specificity protein phosphatase, putative	471
tr V7PKU5 V7PKU5_9APIC	0.9939	1	PY17X_0829200	conserved Plasmodium protein, unknown function	362
tr V7PB62 V7PB62_9APIC	0.9938	1	PY17X_1116200	conserved protein, unknown function	779
tr V7PF12 V7PF12_9APIC	0.9938	1	PY17X_1104300	protein kinase, putative	684
tr V7PJV1 V7PJV1_9APIC	0.9938	1	PY17X_1017300	conserved Plasmodium protein, unknown function	1079
tr V7PTX5 V7PTX5_9APIC	0.9937	1	PY17X_1332300	zinc finger protein, putative	349
tr V7PF90 V7PF90_9APIC	0.9936	1	PY17X_1215600	GDP-L-fucose synthase, putative	343
tr V7PJR6 V7PJR6_9APIC	0.9936	1	PY17X_1020400	conserved Plasmodium protein, unknown function	365
tr V7PIZ6 V7PIZ6_9APIC	0.9926	1	PY17X_1031200	DnaJ protein, putative	381
tr V7PKQ1 V7PKQ1_9APIC	0.9926	1	PY17X_1029700	conserved Plasmodium protein, unknown function	1194

tr V7PMY3 V7PMY3_9APIC	0.9926	1	PY17X_1018300	exosome complex component RRP41, putative	246
tr V7PRM7 V7PRM7_9APIC	0.9926	1	PY17X_1224800	cell division cycle ATPase, putative	1162
tr V7PDF8 V7PDF8_9APIC	0.9925	1	PY17X_1117000	conserved Plasmodium protein, unknown function	198
tr V7PV90 V7PV90_9APIC	0.9925	1	PY17X_1248600	conserved Plasmodium protein, unknown function	96
tr V7PIV8 V7PIV8_9APIC	0.9921	1	PY17X_1215900	SPRY domain-containing protein, putative	2029
tr V7PLN0 V7PLN0_9APIC	0.9916	1	PY17X_0803600	conserved Plasmodium protein, unknown function	1255
tr V7PSS8 V7PSS8_9APIC	0.9915	2	PY17X_1220000	conserved Plasmodium protein, unknown function	1751
tr V7PK17 V7PK17_9APIC	0.9914	2	PY17X_1002800	pre-mRNA-splicing factor CLF1, putative	703
tr V7PI27 V7PI27_9APIC	0.9914	1	PY17X_0621000	Ham1-like protein, putative	196
tr V7PFL6 V7PFL6_9APIC	0.9908	1	PY17X_1215500	translation initiation factor eIF-2B subunit delta, putative	1039
tr V7PFS5 V7PFS5_9APIC	0.9908	1	PY17X_0935800	conserved Plasmodium protein, unknown function	700
tr V7PFZ3 V7PFZ3_9APIC	0.9904	1	PY17X_0624200	Rab GTPase activator and protein kinase, putative	1634
tr V7PQQ3 V7PQQ3_9APIC	0.9903	1	PY17X_1456800	vacuolar protein sorting-associated protein 16, putative	965
tr V7PQT1 V7PQT1_9APIC	0.9902	1	PY17X_1446300	multidrug resistance-associated protein 2, putative	1975
tr V7PTZ1 V7PTZ1_9APIC	0.99	1	PY17X_1430200	asparagine-rich antigen, putative	993
tr V7PGT1 V7PGT1_9APIC	0.9897	2	PY17X_0911400	conserved Plasmodium protein, unknown function	1744
tr V7PIW3 V7PIW3_9APIC	0.9892	1	PY17X_0510300	EF-hand calcium-binding domain-containing protein, putative	908
tr V7PNS0 V7PNS0_9APIC	0.9891	1	PY17X_0810300	stomatin-like protein, putative	398
tr V7PVP2 V7PVP2_9APIC	0.9891	1	PY17X_1358700	ABC transporter B family member 5, putative	820
tr V7PKR9 V7PKR9_9APIC	0.9888	1	PY17X_1028500	conserved Plasmodium protein, unknown function	2793
tr V7PNZ8 V7PNZ8_9APIC	0.9888	1	PY17X_1411000	RNA-binding protein, putative	154
tr V7PED3 V7PED3_9APIC	0.9882	1	PY17X_0942800	DEAD/DEAH box helicase, putative	1109
tr V7PNU2 V7PNU2_9APIC	0.9878	1	PY17X_0807900	mediator of RNA polymerase II transcription subunit 10, putative	257
tr V7PMM4 V7PMM4_9APIC	0.9876	1	PY17X_0827200	DNA-directed RNA polymerase II subunit RPB3, putative	335
tr V7PWC1 V7PWC1_9APIC	0.9873	2	PY17X_1240600	inner membrane complex protein, putative	486
tr V7PI62 V7PI62_9APIC	0.9873	1	PY17X_0416900	inorganic pyrophosphatase, putative	367
tr V7PUT0 V7PUT0_9APIC	0.9872	1	PY17X_1345400	mitochondrial fission 1 protein, putative	141
tr V7PKZ0 V7PKZ0_9APIC	0.9869	2	PY17X_0834700	peptidyl-prolyl cis-trans isomerase, putative	590
tr V7PD57 V7PD57_9APIC	0.9868	1	PY17X_1105200	MOLO1 domain-containing protein, putative	275
tr V7PU62 V7PU62_9APIC	0.9853	2	PY17X_1324900	conserved Plasmodium protein, unknown function	2979
tr V7PMY5 V7PMY5_9APIC	0.9847	1	PY17X_0833100	transcription initiation factor TFIID subunit 7, putative	389
tr V7PRP3 V7PRP3_9APIC	0.9845	1	PY17X_1138500	protein arginine N-methyltransferase 5, putative	729
tr V7PLY2 V7PLY2_9APIC	0.9832	1	PY17X_1011200	conserved protein, unknown function	374
tr V7PHJ7 V7PHJ7_9APIC	0.9823	2	PY17X_0607100	conserved protein, unknown function	1196
tr V7PQJ3 V7PQJ3_9APIC	0.9809	1	PY17X_1012000	conserved protein, unknown function	277
tr V7PFH9 V7PFH9_9APIC	0.9808	1	PY17X_0910600	conserved Plasmodium protein, unknown function	2069
tr V7PMS4 V7PMS4_9APIC	0.9806	1	PY17X_1128400	eukaryotic translation initiation factor 2-alpha kinase, putative	2580
tr V7PXK9 V7PXK9_9APIC	0.9775	1	PY17X_0109300	zinc transporter ZIP1, putative	344
tr V7PWK6 V7PWK6_9APIC	0.9761	1	PY17X_1248100	phosphoenolpyruvate/phosphate translocator, putative	518
tr V7PDX1 V7PDX1_9APIC	0.9756	1	PY17X_0927200	calcium-dependent protein kinase 7	1913
tr V7PRZ4 V7PRZ4_9APIC	0.9745	1	PY17X_1235000	AP2 domain transcription factor AP2-O2	2129
tr V7PBN5 V7PBN5_9APIC	0.9743	1	PY17X_1102800	conserved protein, unknown function	381
tr V7PFR0 V7PFR0_9APIC	0.9688	2	PY17X_0609100	conserved Plasmodium protein, unknown function	4285
tr V7PX28 V7PX28_9APIC	0.967	1	PY17X_1358100	conserved Plasmodium protein, unknown function	271
tr V7PEF2 V7PEF2_9APIC	0.9652	1	PY17X_0306900	exosome complex component MTR3, putative	271
tr V7PG51 V7PG51_9APIC	0.9651	1	PY17X_0926000	conserved protein, unknown function	492
tr V7PHH0 V7PHH0_9APIC	0.9632	2	PY17X_0928100	JmjC domain-containing protein, putative	447
tr V7PQI2 V7PQI2_9APIC	0.9557	1	PY17X_1403200	methyltransferase, putative	227
tr V7PTT3 V7PTT3_9APIC	0.9547	1	PY17X_1337600	mediator of RNA polymerase II transcription subunit 6, putative	205
tr V7PTD9 V7PTD9_9APIC	0.9531	1	PY17X_1415500	conserved protein, unknown function	524
tr V7PMS3 V7PMS3_9APIC	0.9452	1	PY17X_0823100	gamma-glutamylcysteine synthetase	999
tr V7PEM6 V7PEM6_9APIC	0.9447	1	PY17X_0312600	conserved Plasmodium protein, unknown function	830
tr V7PJ48 V7PJ48_9APIC	0.9413	1	PY17X_1035600	DNA damage-inducible protein 1, putative	385
tr V7PSD9 V7PSD9_9APIC	0.9392	3	PY17X_1240400	conserved Plasmodium protein, unknown function	8949
tr V7PDX8 V7PDX8_9APIC	0.937	1	PY17X_0313900	autophagy-related protein 11, putative	1423
tr V7PIS1 V7PIS1_9APIC	0.933	1	PY17X_0513200	methyltransferase, putative	577
tr V7PJE5 V7PJE5_9APIC	0.9298	1	PY17X_1020200	conserved Plasmodium protein, unknown function	364
tr V7PZB8 V7PZB8_9APIC	0.921	1	PY17X_1250700	PIR protein	316
tr V7PE58 V7PE58_9APIC	0.9198	2	PY17X_0935400	GTPase-activating protein, putative	372
tr V7PAK3 V7PAK3_9APIC	0.9149	1	PY17X_1371500	fam-c protein	94
tr V7PMS9 V7PMS9_9APIC	0.9094	1	PY17X_1128800	DNA methyltransferase 1-associated protein 1, putative	385
tr V7PJB0 V7PJB0_9APIC	0.9063	1	PY17X_1031000	zinc finger protein, putative	697

Supplementary Table 3. Primers and oligonucleotides used in this study.

Oligo sequence for candidate genes (except gpt1) knockout plasmid construction									
Gene ID	Gene name	Gene size (bp)/ deleted gene size (bp)	Left homologous arm		Right homologous arm		Target site of sgRNA		
			Forward primer	Reverse primer	Forward primer	Reverse primer	Oligo (Forward)	Oligo (Reverse)	
PY17X_1243400	7-helix-1 protein, putative	1410 / 756	CGGGGTACCTATATGTGTA ACTATTAGGG	CATGCCATGGAGCCCAAT GACCTTTTAT	CCGCTCGAGGGAAGTGC ACATGGGTATGC	CCCCTTAAGCAGGTCTA TCTGGAAATGTTA	TATTGCAAACTCAATCA TTATTG	AAACCAATAAATGATTGA TTTGG	
PY17X_1431500	integral membrane protein GPR180, putative	2080 / 327	CGGGGTACCTACATTAATG CTTGATATTG	CATGCCATGGCATATCTAT ATTGAATCC	CCGCTCGAGCACAAGC TATGGTATAACA	CCCCTTAAGTAAGCCTA TGAACATGCAC	TATTGGTATTCACAAA GGTAT	AAACCTACCAATTTTGTAA TAAAC	
PY17X_0918700	serpinine receptor, putative	1878 / 498	CGGGGTACCTTAATAGGA CCAATGTAA	CATGCCATGGACCTTGACT TGACGTATCC	CCGCTCGAGCTAGTTGG TGATGATAAA	CCCCTTAAGATAAAGAC AGACGATAT	TATTGGTAATACTATATC AACATA	AAACATGTTGATATAGTA TTACC	
PY17X_1433900	serpinine receptor, putative	1776 / 545	CGGGGTACCTTCATGTATT AACAAATGGA	CATGCCATGGATATATCA CGTCTGTG	CCGCTCGAGCAGAACAC TCAAGATTGGA	CCCCTTAAGCACAACCTA TATCCATACCT	TATTGTCACTCTTCAT AAGAAG	AAACCTCTTATGAAGAAG ATGAC	
PY17X_0524600	serpinine receptor, putative	2122 / 434	CGGGGTACCTTATATTGTAT GTGTGTACA	CATGCCATGGGATTACACC AATTTGTATG	CCGCTCGAGGATATAGA CAATCAATTAGA	CCCCTTAAGGTTTATGAT ATTCTGTATCTC	TATTGTATCATCAAAAGT AGTATA	AAACCTACTACTTTTGTG GATAC	
PY17X_1421700	GPCR-like receptor SR25, putative	1347 / 531	CGGGGTACCTACGACTTAC GTGTCTGATT	CATGCCATGGATTCATCA CCACTGTCTATC	CCGCTCGAGGCCATCTTA TTACAGATATGC	CCCCTTAAGTGACTTTT GATGGTAAAGAC	TATTGAAATTTGCTACTAT AGTATG	AAACCTACTATAGTAGCA AATTC	
PY17X_0605800	sexual stage-specific protein G37, putative	2513 / 459	CGGGGTACCTTAGTAACAC CTCAACTTATA	CATGCCATGGGTGGTATAT CCTTAAAGACC	CCGCTCGAGGCATGTAT CAGTAGGATATAG	CCCCTTAAGTGTATACCC CATAATGACGAT	TATTGTAATTTGCGCTC ATTGCTA	AAACCTAGCAATGAAGCGA AATTTAC	
PY17X_0617400	conserved Plasmodium membrane protein, unknown function	2400 / 415	CGGGGTACCTCTAATAATCC TTGTAGACCT	CATGCCATGGGATAGCAAT TTGTTAACTA	CCGCTCGAGGTATTTTA CAACTGTGCA	CCCCTTAAGGTTTGA TACTGGTATCTA	TATTGTAATTTGCTCTTA TTTAT	AAACCTAAGTAAAGCGAAA TTAAC	
PY17X_0914700	conserved Plasmodium membrane protein, unknown function	1251 / 343	CGGGGTACCTTACACGTGG CGAATGATAT	CATGCCATGGCGTAGTCTA CTAGTAATGA	CCGCTCGAGGCTTATTA TCAATTATACCT	CCCCTTAAGTATGATAT AGCTGTACTTATC	TATTGAATATAAAAGAAA GAGCTGG	AAACCGACTCTTCTTTTA TTCTC	
PY17X_1128800	protease, putative	2155 / 385	CGGGGTACCTTCCITCGCTA TATGTGCGAT	CATGCCATGGCAGATATA TGAATTTGCT	CCGCTCGAGCGTTTATCT TTATCTGTATTG	CCCCTTAAGTGTGACTTT CTGTGTATTACT	TATTGGTITTTCTTGATAT GCAAA	AAACCTTATCATCAAGAAA AAGCC	
PY17X_1313900	conserved Plasmodium protein, unknown function	3636 / 626	CGGGGTACCTCCGGCATATG GGATGTAGC	CATGCCATGGGCCCAACA ACAACAATCT	CCGCTCGAGAGGGTAGT GAGGAATGTTCAT	CCCCTTAAGCCATTTGT TACTATATATGG	TATTGTTTGAATAAATA GAATT	AAACCAATCTATATTTTCA AAAC	
PY17X_0702400	folate transporter 1, putative	1867 / 1102	CCCAAGCTTATCATGTTTCC GTTACTA	CATGCCATGGAGGGTATAA TATAATCGA	CCGCTCGAGGTTTGTAA AAAGGGAT	CCCCTTAAGTGTGAACTGA CCATTACTGC	TATTGATACATTCAGCT TCATCC	AAACGGATGAAGCTGATA AGTATC	
PY17X_0933500	folate transporter 2, putative	1362 / 1404	CCCAAGCTTGTATGTTCTTTA TGTATTTCC	CATGCCATGGATTTTGTGT CTTCTTCTC	CCGCTCGAGATGAGTGG CAACCCAAAAT	CCCCTTAAGTGTGGGTTT TCTCTCTCAAA	TATTGGTTCCGACCTTC TTTTAT	AAACATAAAGAAAGTCCG GAACCC	
PY17X_0309400	GDP-fructose: GMP antiporter, putative	1325 / 1325	CCCAAGCTTCCCCACACAT ACATATCTTT	CATGCCATGGTGTCTATTT GTTACGCTTA	CCGCTCGAGATGAACAA GCAGACAAGAC	CCCCTTAAGTCAACAAAT TTGTGAAGAAAC	TATTGGTAAATGTAAAA TAATAC	AAACCTATTATTTCAATTA CCAC	
PY17X_0936300	UDP-galactose transporter, putative	1044 / 1044	CCCAAGCTTAAATACACCT TAACAGC	CATGCCATGGTCTCCACAT AGTATCAAA	CCGCTCGAGCCTTTTGT TTCTGACTT	CCCCTTAAGCAGAACTG GAGGAGATGT	TATTGAAATGCATCAAA ACACT	AAACATGTGTTTGTATGC ATTTCC	
PY17X_1436400	phosphatase translocator, putative	2650 / 1462	CCCAAGCTTATACCTAAA GAGGAGAA	CATGCCATGGAAATTAACA GACTGTCTC	CCGCTCGAGTAAAGTCT CTCTGCTGT	CCCCTTAAGTGTATTTGT TGAATCTGA	TATTGATGTTTGTATCT TTAAA	AAACCTTTAAGTATCAAAA ACATC	
PY17X_0823700	major facilitator superfamily domain- containing protein, putative	1470 / 1470	CGGGGTACCTGTCTCTCT TCTCCATCTA	CATGCCATGGATTTTGTAA GTGACTCAT	CCGCTCGAGTTTACACAT TATTCATTTTG	CCCCTTAAGTGTGAGTG CGAGAGTAGAAA	TATTGGATATGATATAG CCGACA	AAACCTGGCCATATATCA TATCC	
PY17X_0820300	major facilitator superfamily domain- containing protein, putative	4033 / 1312	CCCAAGCTTACACAACAGCA CACATAACAG	CATGCCATGGATCTCTAGA ATCAGCTCAAT	CCGCTCGAGGAAGAGAG TAAACCCCAAT	CCCCTTAAGTGTGAGAA TATCATTGACGT	TATTGAAGCTATAAAAAC AGCAAA	AAACCTTGTGTTTATA GCTTC	
PY17X_0307300	transporter, putative	3986 / 363	CCCAAGCTTAAATGAAAGGA TAAGAGTGT	CATGCCATGGATTTGGGTA ATGACTGCTA	CCGCTCGAGAAATCGAAT ATACAGAAG	CCCCTTAAGTGAATATGT CCATTACCAG	TATTGAGCATATTTTCAAT TACTCA	AAACCTAGATAATGAAATA TGCTC	
PY17X_0917400	amino acid transporter	4068 / 444	CCCAAGCTTCTGTCTAATG TGCCCAATAA	CATGCCATGGGAATAACAC CTCCCTCTTT	CCGCTCGAGATTTTGAAT TAGAAGAG	CCCCTTAAGTATACATTT CAACAGATGG	TATTGTATCATGTTCATG CTCTTT	AAACCAAGAGCATGAACA TGATAC	
PY17X_0609400	amino acid transporter, putative	4545 / 370	CCCAAGCTTCGTGCTCATT TTGTGTTCC	CATGCCATGGGCTTCAAT TTGCTGTT	CCGCTCGAGAAACATCT GTTGGCTA	CCCCTTAAGTGCATTCAT TTTCTTTGCC	TATTGAGACATGTAAAGA GAAATGG	AAACCTATTTTCTTAAACA TGCTCC	
PY17X_1448600	amino acid transporter, putative	5556 / 388	CCCAAGCTTATTATGAGTAA CCTTCCG	CATGCCATGGACTTAAACA AACGCCAAT	CCGCTCGAGGAAATCT GTTTGTATGC	CCCCTTAAGTCTTTTAACT GTTTGGAC	TATTGTTTAAATGATGAA GTAGGA	AAACCTCTACTTCACTAAT TAAAC	
PY17X_1241000	multidrug resistance protein 1, putative	4266 / 684	CGGGGTACCGAAATCTACC GTTGAGTTGT	CATGCCATGGATACACCTT CTCCACAAAT	CCGCTCGAGTGGTTCAC ACTGTCCATGG	CCCCTTAAGGTTTCAACT ATTACACTTCC	TATTGTTGATAGAATCA AAATAA	AAACCTATTTGATTTCTAT CAAC	
PY17X_1446300	multidrug resistance- associated protein 2, putative	5928 / 665	CGGGGTACCTACATATCC ATGATGAATG	CATGCCATGGACACTATGC GTAACTATAT	CCGCTCGAGTCGATATG GAAGTAAGT	CCCCTTAAGGCATACCTT GATATGAATTCAC	TATTGATTTATGAATAT AATAG	AAACCTATTATTTCAATAT AATC	
PY17X_1315500	multidrug resistance protein 2, putative	2877 / 481	CGGGGTACCCACATAGTT GACATGATTGC	CATGCCATGGGTATATGGC ATATCTATTC	CCGCTCGAGGTTTACAG AATGACCAAGT	CCCCTTAAGAAATAGAT AATGACCAAC	TATTGATTTATGAATAT AATAG	AAACCTATTATTTCAATAT AATC	
PY17X_0904900	ABC transporter B family member 3, putative	2624 / 755	CGGGGTACCGAAATCGAA GTATAGACA	CATGCCATGGCTTAAAG ACACAACTCT	CCGCTCGAGTAGCACGT CTACACACACG	CCCCTTAAGTCCACTTGG GAAAGTGGATC	TATTGATTTATGAATAT AATAG	AAACCTATTATTTCAATAT AATC	
PY17X_0403400	ABC transporter B family member 4, putative	3876 / 577	CGGGGTACCGGAAATGCT GAAGATGTA	CATGCCATGGTGGCTAAA TGACTAATAT	CCGCTCGAGCACCACATT GTGACATATCC	CCCCTTAAGTGTAACTG TGTGTTGGCTAG	TATTGATTTATGAATAT AATAG	AAACCTATTATTTCAATAT AATC	
PY17X_1358700	ABC transporter B family member 5, putative	2463 / 679	CGGGGTACCGCTGTGGTTA GGAATGTTGG	CATGCCATGGCATATAAGG ACCTATTACG	CCGCTCGAGATGTTTCTAT ATCATCTACC	CCCCTTAAGAACACACA ATAAATCTGC	TATTGATTTATGAATAT AATAG	AAACCTATTATTTCAATAT AATC	
PY17X_0928600	protein GCN20, putative	2322 / 991	CGGGGTACCGAAGTATGG GTAATGAGAA	CATGCCATGGCCCATATA TCTATGTGTG	CCGCTCGAGCTTGGTTTT GACTCCAACCTC	CCCCTTAAGCTCTTAAT CTGATAAGCACAC	TATTGATTTATGAATAT AATAG	AAACCTATTATTTCAATAT AATC	
PY17X_1370600	ABC transporter B family member 6, putative	3087 / 552	CGGGGTACCGGCTACAT GTATAAATGC	CATGCCATGGTACAAGATA TGAGAGAGT	CCGCTCGAGCAATGAAC ATAGTAATGACG	CCCCTTAAGGTAGTAAA GACACTATCC	TATTGAACTGGCTGCATA ATTAAAG	AAACCTTAATATGACAGCC GATTC	
PY17X_0610800	ABC transporter B family member 7, putative	2418 / 605	CGGGGTACCGCATATATGG ATGATGGG	CCGCTCGAGCTACCTACC TTAACAATCC	CCGCTCGAGATCTATGAT ATGCTACTC	CCCCTTAAGGATTCACC ATTAAGGAATC	TATTGATTTATGAATAT AATAG	AAACCTATTATTTCAATAT AATC	
PY17X_1145400	ABC transporter E family member 1, putative	2055 / 690	CGGGGTACCCCGTTTATAT CAGTAAACCA	CATGCCATGGGCTGGTGC CTAAGCAATGG	CCGCTCGAGATGGGGA AAGGCAGGTGCA	CCCCTTAAGCTGTTCAC ACAAACAAAGCTG	TATTGAATGGCGAGATAT ATTACT	AAACGATAAATATCTCCG CATTC	
PY17X_1019600	ABC transporter G family member 2, putative	1971 / 769	CGGGGTACCGGATACATGA CAATGAATGATAGT	CATGCCATGGTCTAAGTTT CATTATCGACTG	CCGCTCGAGGAAGAAGA AGGATATATATGGA	CCCCTTAAGATTTGAGCT AATGCTTTGAGCA	TATTGATTTATGAATAT AATAG	AAACCTATTATTTCAATAT AATC	
PY17X_1425800	ABC transporter F family member 1, putative	3792 / 608	CGGGGTACCTATCATATGG TGATATAAG	CATGCCATGGGCCAATCTT CACACATATC	CCGCTCGAGCAATGGGT TGGAATAATCG	CCCCTTAAGTCACTTCT TTTATCACTCT	TATTGATGATGATGAATG TGATGTT	AAACCAATCATCATTATC ATTCATC	
PY17X_1222000	ABC transporter I family member 1, putative	8548 / 520	CGGGGTACCTTGAATGA ACTCAATGCTC	CATGCCATGGCATACAAGC AAGACTTATG	CCGCTCGAGCGACGATC GCACATATATG	CCCCTTAAGGTCTATG GAGTTTGTGCTAG	TATTGCCCCAACGTTTAA ACTATT	AAACATAGTTTAAAGCTT TGSSG	
PY17X_1031600	FeS assembly ATPase SuFC, putative	1083 / 809	CGGGGTACCGCTCACAGAT AAGGATTAGTA	CATGCCATGGATCATGCT TATTAGAAATTC	CCGCTCGAGGCACAATTT GTTGAGTAGATG	CCCCTTAAGCTCATTGA CTATTGTTAGTGT	TATTGATTTATGAATAT AATAG	AAACCTATTATTTCAATAT AATC	
PY17X_0407300	ER membrane protein complex subunit 5, putative	706 / 706	CCCAAGCTTGGGTTGTCCAC ATATTTTAT	CATGCCATGGCCCTTATTG TATTTCTCC	CCGCTCGAGGTTATCATT GCCCACTTTC	CCCCTTAAGGATTTTATT CCTTTAGCATTTTATG	TATTGCAGTTATGATAAC CTTGAT	AAACCTCAAGGTTATCATA ACTCCG	
PY17X_0929900	CorA-like Mg2+ transporter protein, putative	1466 / 538	CGGGGTACCAATAATACGT GGTTGCTTAC	CATGCCATGGTACAGAAAT CAACCAACATC	CCGCTCGAGATAAATACC CAACCAACATC	CCCCTTAAGTCTTCCAAA TCACTACAATCT	TATTGTTGTTGGTCTTA AACAATA	AAACCTGTTTAAAGCAACA CAACAC	
PY17X_1018500	CorA-like Mg2+ transporter protein, putative	1443 / 478	CCCAAGCTTGGAAAGATAAC TGTGTTCTGA	CATGCCATGGCGAATAACA TGTGTTTATGTT	CCGCTCGAGGTTCTAAG ATAAGTAACGGAG	CCCCTTAAGTCTATTCAG TAGCATTTCAGT	TATTGAACGAAAGTGATA GTGTAG	AAACCTACTATCATCTTT CGTTC	
PY17X_0703300	magnesium transporter, putative	1723 / 999	CCCAAGCTTATTTTACAAC GTTCCAGTC	CATGCCATGGCAACAGTGA TGTATAGACAG	CCGCTCGAGATGGTAATA CTAAAGGTGC	CCCCTTAAGTCTAGACTAC TAGGAAACAGAT	TATTGCAGTTGTTGCTCC GTTTGT	AAACCAAAACGGAGCAAC AGCTCC	
PY17X_1240600	inner membrane complex protein, putative	1461 / 1461	CCCAAGCTTCTCCATTTCAA ACCTACAG	CATGCCATGGTTTTCAATA TACTACACCT	CCGCTCGAGTAGCACCATT TTTCAATGTTT	CCCCTTAAGTATAGATGT GATGGGTGCTG	TATTGGGATAATTTTTCAG CGTAA	AAACCTACTGGCTCAAAAT ATCCG	
PY17X_1441100	vacuolar iron transporter, putative	981 / 981	CCCAAGCTTATGGGGTTT TGCTGTTG	CATGCCATGGATTTATGCC AGTCAAGTGA	CCGCTCGAGGCCGAAC ATAAGAAAC	CCCCTTAAGTCTTACAC ATCACCAATTT	TATTGCCAATTTTCCGG TTGTTG	AAACCAACCAACGGAAAC AATTCG	
PY17X_1367300	E1-E2 ATPase, putative	5705 / 1253	CCCAAGCTTAAAGCAATTA GAGATGAGT	CATGCCATGGCCCAATAGA CAATAGAAA	CCGCTCGAGAAATATGGA ATAAAGCTCGG	CCCCTTAAGTATATGAT CTTTAATCTTGA	TATTGTCGATGAGAAA ACGCT	AAACAAGGCTATTTCAT ACGAC	
PY17X_0109300	zinc transporter ZIP1, putative	2055 / 1462	CCCAAGCTTAAATAAAGG GCTGTATG	CATGCCATGGCGAGTCTGC TAAATAAATA	CCGCTCGAGGATTTTGGC CCCTTTTGT	CCCCTTAAGTGTGAAAG CTGATGTTGGAG	TATTGTTGCAATACCTT CACAC	AAACAATAAAGGATTTG GAGCT	
PY17X_1424200	cation diffusion facilitator family protein, putative	1557 / 452	CCCAAGCTTAAATTAACCT ACAGCGTAC	CATGCCATGGCTGGAGGTT ATATTTTATG	CCGCTCGAGATGTTTGT ACTGCTTTTGT	CCCCTTAAGCAAGAGCT ATCATTAACCTCT	TATTGCAGACCTCTAGT TGGAAA	AAACCTTCCAACTAGAGGT CTGTC	
PY17X_1138200	guanylyl cyclase beta	11179 / 864	CGGGGTACCTTAAATAC ACACACTTGTATG	CATGCCATGGACCTCGCT TTTATTTTATG	CCGCTCGAGTATCTGTT TAAATCGATGGAT	CCCCTTAAGTCAATA ATAATAGTTCAATCA	TATTGATGCAATTAGAT GGAAA	AAACCTTTCCCACTAATT GCTAC	
PY17X_0619700	LEM3/CDC50 family protein	1397 / 1397	CGGGGTACCTACGAAATAA ATACATGCTAAT	CATGCCATGGACTATGTAC ATTTTTTATGACCA	CATGCCATGGCTCCAAA AAAGGGGGGAAAG	CCCCTTAAGCAATTTT TATTTTATTTAAATATG	TATTGGAATTTTATTTA ATAT	AAACCTATTAAATATAAAA TTCC	
PY17X_0916600	LEM3/CDC50 family protein, putative	1122 / 1122	CGGGGTACCTACAGATCGA AGTGTAGCATT	CATGCCATGGATATATTT AGCATTTTACGAAAT	CATGCCATGGCAGAAATA AATTAACATAATA	CCCCTTAAGTCACTATG CAAAATGTACACTC	TATTGTAATGGGCTTG GAAATG	AAACCTACTTCCAGGCC ATTACC	
PY17X_0809500	P-type ATPase, putative	5295 / 962	CGGGGTACCAACACATATCG TTATCCCAATTA	CATGCCATGGTCTACATGAA AGCGAAAAGGA	CCGCTCGAGGGTAGAAA ATTTTTCACACAAAT	CCCCTTAAGTCTATGTT TCTATACAGCTGTG	TATTAAATAGAGATTTAA AATCT	AAACGATTTTAAATCT AATT	
PY17X_1437200	aminophospholipid- transporting P-ATPase, putative	4838 / 1011	CCGGAATTCATCAATATT TAAAAAATCTGA	CCGCTCGAGCTTTATGTG TTCGGGCTAT	CATGCCATGGATCTGAAG GATCAGCCCATAGA	CCCCTTAAGCTCATGTA CAATTAACAAATAGG	TATTGAACACAAATAGC CATACA	AAACCTGATGCGGATTTG GTTTC	

PY17X_1440800	phospholipid-transporting ATPase, putative	5978 / 1030	CGGGGTACCACATTATTA GATAATCTGTAGG	CATGCCATGGCTTCAATC CGTAAAAATTACA	CCGCTCGAGTGATGGAG AAACTGATTTGA	CCGGAATTCGTTCCAT TCCATCTCCTA	TATTGCTTACGTAAAG AAAAA	AAACTTTTTGCTAAAGT AAAGC
PY17X_0911700	guanylyl cyclase, putative	11553 / 994	CGGGGTACCCTTCTGCAT TCTAATAATTAAC	CATGCCATGGCTTATTTTA TTTAAATGGTT	CCGCTCGAGTGATATG GTAATAAAGAAATT	CCGGAATTCCTTTACA AGTITATCAAAATGTC	TATTGTCGAAACATCGCT AGTAAA	AAACTTACTAGCGATGTT TCGAC
PY17X_1105200	MOL1 domain-containing protein, putative	1594 / 948	CCCAAGCTTGTGGTATATA TCTTTCCCA	CATGCCATGGTGAAGGGCT TTTAAATGGTT	CCGCTCGAGTGCTGAGC TATAGTACCAGA	CCGTTAAAGCGTGTGG TCAGTCTGTAA	TATTGCTTCTTATTTTGT TTGCT	AAAGCATTCTTATTTTGT TTGCT
PY17X_1315200	conserved protein, unknown function	765 / 765	CGGGGTACCCTGGAAGTTC AATTTATCC	CATGCCATGGAGTGCCTTA ACAGAGAGCT	CCGCTCGAGGAAACCAA ACAGAGAGCA	CCGGAATTCCTCAAAG ACATATACTAC	TATTGATGTGGAAATTC ATCTAC	AAAGCTAGATGAATTTCCA CAATC
PY17X_1339400	transmembrane protein 43, putative	1869 / 1412	CCCAAGCTTITTAATGTCT TATGCTCTG	CATGCCATGGTATAAAGC CTAAACCATAT	CCGCTCGAGCAAGAAAGC AAGATTACTAAA	CCGGAATTCCTCAATATC TACTTCTGTTAT	TATTGCTTATAGTAGATC TAAATG	AAACCCCACTGATACATT CTCAAC
PY17X_1342800	conserved protein, unknown function	2358 / 1213	CGGGGTACCCTGATGCC GAAGAAATTTT	CATGCCATGGTATAAATG TTTAAATGGTT	CCGCTCGAGTAAATATTG CATGAACAGCT	CCGGAATTCGAAAGTGT GTTGTAACATA	TATTGCTAGAAGACAC CTAAGAG	AAACTCTTAGTGTCTTT CTAAG
PY17X_1386100	conserved protein, unknown function	2603 / 1347	CCCAAGCTTCTTACAGT TTATATTAAGC	CATGCCATGGGATAACAA AAGAGAGATA	CCGCTCGAGTAAAGGACA GCCATATTAAG	CCGGAATTCGAAATTTG AACCTGGTACA	TATTGCTGCAAAATG TTGCTC	AAAGCAGCAATCAATT GCTCC
PY17X_1463300	dipeptidyl aminopeptidase 43, putative	2800 / 1367	CCCAAGCTTGTATTTTGGT TGTAGAGTG	CATGCCATGGTATAAATG CCACTGTCTCAG	CCGCTCGAGCAGAGTGC TATAAAACAGAT	CCGGAATTCGATAGTCT ATCTTTGTCGAT	TATTGACTTCGGAACA TTGTGG	AAACCCCAATGTTCCGA AGTATC
Primers for PCR-genotyping parasite with candidate genes (except gap1) knockout								
Gene name	Gene ID	P1	P2	P3	P4	P5	P6	
PY17X_1243400	7-helix-1 protein, putative	ATAACATTAAACAGATA GGCG	CAGGCTATCTGGAATGTTA	GAGAATAAAAGGTCATTGT GTC	CTATTAACCTGCATAAC CTTC	GAGAAACATGCAAAATCA ATCAA	CTATTAACCTGCATAAC CTTC	
PY17X_1431500	integral membrane protein GPR180, putative	TACAAATAACGAAGGTA TAGCA	CTATGTAATTTATGCTACGT GG	TACATTAATGCTTGATATTG G	CACAATTCATATTACGCA A	TACAAATAACGAAGGTA TAGCA	ACATACAACCACTACGT GCTT	
PY17X_0918700	serpentine receptor, putative	ATATGCCAAATAACAT GCCAA	ATAAAGACAGCAGATAT	TATGAACATATAAGAACGCC AGG	CTCTCGTAAATAATTACGT AGG	GTGTGTTACCAAAATGA TAGCA	CTCTCGTAAATAATTACG TAGG	
PY17X_1433900	serpentine receptor, putative	TCATTATGATTTATCC CAGCG	TCCTCCAAATCTGAGTGTTC TG	TTTCATGTAATAACAATGGA A	TGAAATATCTCTAAATGAA T	TCATTATGATTTATCC AGCG	CTCTCAACAAAATATATG GCGC	
PY17X_0524600	serpentine receptor, putative	GATGAGGTGTAAGACT TCGGT	CTTCACTACCTGCTCTCAC AT	TATATGTTATGTGTGATCA A	ATTATTACACTGAAGCGTT C	GATGAGGTGTAAGACT TCGGT	CACCTGAAAATAACG GATCC	
PY17X_1421700	GPCR-like receptor SR25, putative	TGAAGTGAAGTAGTTAG TTTTACT	ATGCATAAACAGAAAATAG AATAATAAC	CGAGTTTGGTAAATGAA GAGAT	ATGTGCACATATATATA GTGC	TGAAGTGAAGTAGTTAG TTTTACT	AGCAAACTTCTGAATTC TAATGC	
PY17X_0605800	sexual stage-specific protein G37, putative	ATCAAGATAGATAGTGT GGTCC	CTCTATACCTACTGATACAT GC	TTTAAACACCTCAACTTAT A	TTTAACTGCTACTACCA GTGG	ATCAAGATAGATAGTGT GGTCC	TGCTATGGAACAATCGG GACAA	
PY17X_0617400	conserved Plasmodium membrane protein, unknown function	ATGTATGTCTAGTATAC ATACA	AAAGTTGACTGGTATCTA A	GTTACCATAGTCAAGCTC TTA	GCCATACAAAATGCAAAA CCCA	GCTAGCCAAATAACTAA ACGAA	GCCATACAAAATGCAAAA ACCCA	
PY17X_0914700	conserved Plasmodium membrane protein, unknown function	GTTATTTGGAGTTACAT AAATGT	ATCTGTGCCCAAACTCTA AAAAG	GTTATGCGCAATTAATAAT G	AGGGCACCAATGAAAAGA GAA	GTTATTTGGAGTTACAT AAATGT	ATTGCCATGGACTATTTT GTTGCTAC	
PY17X_1128600	protease, putative	TTGATTCCTCACATTA AACTA	GAATCTATAATGAAAACGC AG	TCCTTCGCTATATGTGCGAT A	AATATAACCTGCTGAAG AGCA	TTGGATTCCTCACATTA AACTA	GAATATCATGAATGCAC ACCG	
PY17X_1313900	conserved Plasmodium protein, unknown function	TTTATTCGATGGCAITTC CAT	CCATTGTTACTATATATG G	AAGATGTTGTTGTTGGG CTT	TTGTTTGCATGTTTATG TCC	ATGATACCCCAAGAAAT GAGAG	TTGTTGCTATGTTTATG GTCC	
PY17X_0702400	folate transporter 1, putative	AAAACCTGTAATGCTCT T	ACTAGATGGTGTGACAT	TTTACATTCCACCCGTT	TCATGTCCATTTCTGTG	TTTACATTCCACCCGTT	GCAAAAGCACTCAACTC C	
PY17X_0933500	folate transporter 2, putative	AAGAAAAATCAAGAA T	CGTGCCTTAGTACGTGAT	ATACATGAGCTGCACCTT A	GATTCATCCTAATAGT TC	ATACATGAGCTGCACCT CTA	GATTTCTGTAATGGCTT CA	
PY17X_0309400	GDP-fructose, GMP antiporter, putative	ATAATGCCGAAAGGTTA A	AAATCCTGATCTCATCAT	GTGCAAACTAACTAAAATA A	ATCATACTTTTTCACACAA ACAAACTG	ATAATGCCGAAAGGTTA A	CATCTCCCTTCTCTCT C	
PY17X_0936300	UDP-galactose transporter, putative	CATATCCATGTTTAGGT T	TCACCATTGAAACTAGAA A	CAGCAAGTTTAGTAGAGCA AGT	ATTGACAATGATGCAAA ATCCAAAGC	CATATCCATGTTTAGGT T	TTATCTGTCTTGTGCTCT C	
PY17X_1436400	phosphate translocator, putative	CAAACCCAAAGGAAGA AC	ATTGATACGAGCAGSAGAG	TAGATCTATATACACATCC TGTGAT	ATCCACAGCCAGAGAGAT ACAAAGCC	CAAACCCAAAGGAAGA AC	GCTTCCCTGTTAGGCTT G	
PY17X_0823700	major facilitator superfamily domain-containing protein, putative	TATTTGTGTGTGCAGTC T	AAAAATGGGCAAACTACA C	TTTTACAAAATGATGTACC T	ATTGAATAGAAGAAATTA CACTATG	TATTTGTGTGTGCAGTC T	TAAAGGGGTATCCATAG T	
PY17X_0820300	major facilitator superfamily domain-containing protein, putative	GGGTGTATGTATATG TGTAGTACTT	ACCCTAAATGTATGCATGG CTCAC	TAATAGTATGAGGGCAAA T	TGTAATAAACAACAGG T	ACTGCAACAGTACGAA CT	TGTAATAAACAACAG GT	
PY17X_0307300	transporter, putative	TTCCGATGTTTAGTAT CT	CTGTTCATAACTTCTGTGA A	TTTTGTATATTGTTAGA	GATATTTGTTCCAGGTGA T	TTCCGATGTTTAGTAT CT	TCTGTTTTCTCAAATCTG TA	
PY17X_0917400	amino acid transporter	AGAAAAAAGTAAAGTGGT GTG	GTTTTCTATCTGTTGTCTCA A	CCAAATAAAGAAAGAGGG GAGGTGT	ATTCTATCTAATATTGGCT CCC	AGAAAAAAGTAAAGTGGT GTG	ATGTTCCAGAAATTTACCT TA	
PY17X_0609400	amino acid transporter, putative	CCTCGTGTGTACACATG GCACCT	ATTGGTATCATTGTCTAG ATCG	TTGTGTTCTATCCACCCGT A	TCATATGAGGGTAAAGCG GT	TTGTGTTCTATCCACCT CGT	CCCTTATGCCATCACTT TT	
PY17X_1448600	amino acid transporter, putative	TCGTTTTATCGAGCATG T	GCTCAGTGTGCTTTTTT	TTGCTGCAATCTATACATA A	TCTTAGAACAACTTAACG CT	TTGCTGCAATCTATAC ATA	TCAATAAGCCTACAGAC CAA	
PY17X_1241000	multidrug resistance protein 1, putative	GTGATAATAAAGAAT AATA	CACATAGTGTATTTCTCTTA A	GAACAAGTAAATGACAGGAA AAC	TATCCATATTTGATCAGT AAC	GTTGATAGAATAAATA TAAC	TATCCATATTTGATCAG TAAC	
PY17X_1446300	multidrug resistance-associated protein 2, putative	CTATGTATGCAACAAT TTGCAATGTT	ATTGCTGTGATATGGGCTTA A	GTAATTCATGGAATTCATG A	CCGAATTAACATCAATAT GATC	GGACTGATTCGCGCAAT GGCCTAT	CCGAATTAACATCAATAT GATC	
PY17X_1315500	multidrug resistance protein 2, putative	CTTATAGCGACATTTTT GGATGTT	ACATGTGAATATAATACACC CG	GCTGTGTTAATAACATATA CCT	CAATAGTAGAATAAATG TCC	GATTTAGGTAAAAGT GGA	CAATAGTAGAATAAATG TTCC	
PY17X_0904900	ABC transporter B family member 3, putative	GTGTGTAAAACACTTAA GGA	CACCACATGTATAGACATG A	GAAATATAAGTTAGGGTTC TGCTT	AAAGCMAAATAAAGGGCT AG	GTGTGTAAAACACTTAA GGA	GTTTGTATCAATAATA TC	
PY17X_0403400	ABC transporter B family member 4, putative	GAACAACAATATTCACG GATTCAGAAAT	ATTGCTTCTATTAGTGATA TGTCAC	CTGACACATACAGATAACAA CAG	CTATCTAACACAGCAATTT TGG	TATACATGGATCTCTTA GTC	CTATCTAACACAGCAATTT TTGG	
PY17X_1358700	ABC transporter B family member 5, putative	CGAATAACACATGAGACA GCTTGTATCT	ACCCTAGAATGAAAAGGT AGATG	GTCGAAAATCGACCATAT GG	GTCCAGGCTTACTTGTGTT CGCC	GATTAGACGATGAACATA AAGG	GTCCAGGCTTACTTGTGTT CGCC	
PY17X_0928800	protein GCN20, putative	GATATAGTGACAAAATG GCTGTTGTATCAT	ATTCTCACTCCCTACCTAG AGAGTTTAC	TGCGGCTTACATAGCTTA A	CTCGAGTCCATATCTACT TC	GTTAAGTTTATTTAAAGA AT	CTCGAGTCCATATCTACT TC	
PY17X_1370600	ABC transporter B family member 6, putative	TATGGCTGCATAAAAAT TGG	GTTATATACAATTTGAATAT A	TTGCGGAAATGCTTTTATCA AAGT	GTAITTTGTTCTGACTGTG TCAT	AGTATACAATCGACATA TCC	GTAITTTGTTCTGACTGTG TTTCAT	
PY17X_0610800	ABC transporter B family member 7, putative	GGTACTATATATTCCTAT GTAGT	AGTGTTCCTCTTTTCTCC ATAC	TGCTGAAGTACAGTATAGA AG	TCCTACTATAGCAACAGAA TT	CGAGATTCGAGTTTCCG ATG	TCCTACTATAGCAACAGAA TT	
PY17X_1145400	ABC transporter E family member 1, putative	GTAGTCTTGAATTTAGG CA	GGTAAAAGAGTTCAAATGTTT A	CTCGGATACAGTATATGAT A	CATACCTGCTACTAATGT TTGTG	GAATGGCGAGATATATT ATC	CATACCTGCTACTAATGT TTGTG	
PY17X_1019600	ABC transporter G family member 2, putative	GTATATATGATATATA AAGG	ATTTCACTATCATGATCAAT ATGAATTTATG	ATGCATCATACCACCTCT A	GAATGATCCATATAATGC TA	GCATTTAATAGAGGATAT AAG	GAATGATCCATATAATGC TA	
PY17X_1425800	ABC transporter F family member 1, putative	CATATATAAATCAAAACA TAT	CAACTTTTCAAAATCAGAAA A	GGCGCAATAGACGTTAACT A	ATTCTTCTTCCCTACAGAA A	GATGAGAATGATGATGA GAATG	ATTCTTCTTCCCTACAG AA	
PY17X_1222000	ABC transporter I family member 1, putative	GATAATATGATTTTGT TCTC	CATTTGCACTGAGATTGTG C	ACTCACATATACAGATAT GA	CTGTAACTATGCTACTACT CTG	GCATTTATGCTGAATAGC ATA	CTGTAACTATGCTACTACT CTG	
PY17X_1031600	FeS assembly ATPase SufC, putative	GAGTAAAACGACACAAA TGTGAAT	ATTGTGAACCCCTCATATTT GTACATGTT	GGCTAGCTGTGCTACATA T	CGCATATTTCTCGCAAT GCTC	GTTAATCATGATCATTA TG	CGCATATTTCTCGCAAT GCTC	
PY17X_0407300	ER membrane protein complex subunit 5, putative	CCTAACAATGTTGCACA CAGTAATACT	AGCCATAAACAATGTTTCC GATTGATG	TAGTATGCTCTTTTGGCG A	ATAGGCTGTCTCTCTTTC C	ATGACTCAACTTTTGTCT CTA	ATAGGCTGTCTCTCTTTC C	
PY17X_0929900	CorA-like Mg2+ transporter protein, putative	GTCTGAAGCGCTTCC CCCTCAACT	ATCTTTTCTGATTTAAATGTT TGGTGTGG	TTTGAAGTTTGTGATATTCG A	GGACCATACAGAAACAAA A	GTAGCTGATGATGGAAA TG	GGACCATACAGAAACAAA AA	
PY17X_1018500	CorA-like Mg2+ transporter protein, putative	GCTAGCTATATATGTA ATATGTTT	GTAAGAATAATGAACATTT TCTCCGTTAC	AAACTAAACCATTTGATTCG A	GCTAATGTCCAAAAGATG TA	TGTAATGAAAAGAACCC CA	GCTAATGTCCAAAAGATG TA	
PY17X_0703300	magnesium transporter, putative	GCTAAATGCTGTAACAT ATCGAAT	ATCTCTCTTTTATCATCTT CTTTTGTTCGTC	ACGCTGCTCTACATCACT A	AAATAGAATCTGCGAAG AT	AGTGTGTCTCCGTTT T	AAATAGAATCTGCGAAG AT	
PY17X_1240600	inner membrane complex protein, putative	CATTGAAAATTTGTA GTTGAAGCATTTG	ATGCACATGCTGCAATAG AGCATATGTGAAC	AGGGTGATATATTGAAAAC A	TTTTCTTACACTGAAAC A	CTCATATAGGAGATCAC CAT	TTTTCTTACACTGAAAC A	
PY17X_1441100	vacuolar iron transporter, putative	GAAGAAGTGGCACTACT AATTAACAAT	AGGTTGGTAACTAAAATGTT AAAAACGCA	TAACCTGACTGGCAATAAT A	GTAAGACGATAAAATGTG AA	GTTAGCAGAAAGACAAA GAG	GTAAGACGATAAAATGT GAA	
PY17X_1367300	E1-E2 ATPase, putative	GTAATGAAAATTTGAC AAATTTGATATACAT	ACCTGAATTTGATTAACCC GAGTTTATCC	TTTTTCTATGCTATTTG C	CATGTTTCTCTATATTT C	CTAATTTCCATACGACTC TA	CATGTTTCTCTATATTT C	

PY17X_0109300	zinc transporter ZIP1, putative	GTGTGATGCTGTTCTATA TTCATT	ATCTTGAGATTTTGGCTCTG AAAAAAGAGTGCAC	GTAATAAATAACACGGTCA	GATAAATGTGCTCTCC CT	TTTCTAAAGTCTATCAAG GC	GATAAATGTGCTCTCC CT
PY17X_1424200	cation diffusion facilitator family protein, putative	CTTATTTTCATTGATAAAA ACCTGCGCT	ACAAAAGCGATAAATATATT TCGTAGTG	GAGCGTACACGCTACATGA CCAAT	ATTGGATCTGGTATTGAA TATTTAGGGTTATACC	CAAGTTGAGCAAGTTGA TGGAT	ATTGGATCTGGTATTGAA TATTTAGGGTTATACC
PY17X_1138200	guanylyl cyclase beta	GCTCACACCTGACTGGA ACATA	ATGCAATAATAATAGTTC AATCA	ATGAGAGGCTTAATATTGGG T	TAATTCCTTAATGATATAA AAGTATAGACA	GCTCACACCTGACTGGA CATA	TCCGTCGAGTATGATCA AC
PY17X_0619700	LEM3/CDC50 family protein	GGTCATTCTAATGTTTAA AAGA	GAAATGTTATGTCATATCCA CC	CAAGACCATTCCCTTATATG TATGC	TCTACATAATAAAAAGCATC GC	CACATTGTGCTTATTITTA CAACC	TCTACATAATAAAAAGCAT CGC
PY17X_0916600	LEM3/CDC50 family protein, putative	AATTTCCCTTTGGGGTTT TCAC	ACATGTTAATATTTATCGA ATGGA	TGTTGATTATACAATTGCT TATGA	GGAATAATTTACAACAACA TAGTG	GGAATAATTTACAACAACA ATAGTG	TGGATGATACCATCACC ATTTAG
PY17X_0809500	P-type ATPase, putative	GGAAAAGTGCATATATC CACA	TGTTTITACAAAGTATGATC CTCT	ACTTAATGCATACCTTCCG T	ATACATATATACGCGGTA TATTGTG	GGAAAAGTGCATATATC CACA	FAGTTGTAACCGCGATAT CGA
PY17X_1437200	aminophospholipid-transporting P-ATPase, putative	TCCTCCACCCTTATAAAC CATA	TAGGTGGTAATAATATCACT G	TGAGTATAAAGCATACTCAC AAAG	GGTAACTTAACATATTTAT CATCAT	CATGAATATGTGTA AAAA GGACGA	TGAGTATAAAGCATACTC ACAAAG
PY17X_1440800	phospholipid-transporting ATPase, putative	TCATCGAAGAAACAAT GAGTA	ACCATATTGATTGATTTAA ACAT	CACCTGCACAAAATTTATC ATAC	GAAAGATTGTAATTTTAA A	TCATCGAAGAAACAAT GAGTA	TCTTCATCTTACTTAAAT TTCGT
PY17X_0911700	guanylyl cyclase, putative	ACACACCCAGCAGACACA TATTAG	ATGATTTAAATAAACCAATT GTAGC	TGATGTTTATGTTGGTTT TG	CTTGGCTTACCTTATTACT GCA	ACACACCCAGCAGACACA TATTAG	TCCCAATATGGTTGATTA TTIG
PY17X_1105200	MOL1 domain-containing protein, putative	GTTATATCTACACATAT GCATG	CGTGTGGTCCAGTCTGTA A	ATGTTGCTTTATCGTCTT	ACAGCCAAACAGTAGTCA	GCATTCTTATTTTGTG CT	CGTGTGGTCCAGTCTG TAA
PY17X_1315200	conserved protein, unknown function	ACTAACTCGTATTAG CCT	CTCAAAGGACATATACTTAC	TATACGCGATGTCATCTT	TTCACTGCAAAATTATGTT	GATTGTGAAAATTCATC TAC	CTCAAAGGACATATACTT AC
PY17X_1339400	transmembrane protein 43, putative	CATACCATATATGCATA TATAAAGC	CATCTTTATTCATGATATTA A	AATATTTTGGGGTGGTGTG TTTA	CGTAGAAGATAATTGTAC AG	GCAACATCGCTTACTAT AATCC	CATCTTTATTCATGATAT AACTG
PY17X_1342800	conserved protein, unknown function	TGCCATTTTCCCCACC CTTTTTGTAGG	TAAACATAATGGGAATACC ATATATGGG	GACGGTTTACAACCTTATT	GTAATGATGGCTGTTCTA	GGTAGAAGAACACCTTAA GAG	ACTTCCGCCATCAAAITA TC
PY17X_1386100	conserved protein, unknown function	GTGTAACATAAAAGGAA AAT	CTAAATAAACAAATGGACGT	GGITTAATAACCTTGAT	CTAACTCGATTTATAGGG	GGAATATTTACAATATTT TA	CTTAAATATGGCTGTCCT TA
PY17X_1463300	dipeptidyl aminopeptidase 2, putative	TAGTTTGGTACTATTTA GCG	CATAAAATATCCATCCATA T	ACACCCTTTTGGCTGAA	TCAACTGTTTCCGAAGGA	TACAAAACCAATGTCTA ACC	AGAGATAGGCATCTTTG TAA

Oligo sequence for gep1 (PY17X_1116300) knockout plasmid construction

Gene ID	KO	Gene size (bp)/ deleted gene size (bp)	Left homologous arm		Right homologous arm		Target site of sgRNA	
			Forward primer	Reverse primer	Forward primer	Reverse primer	Oligo (Forward)	Oligo (Reverse)
PY17X_1116300	gep1 N-terminal KO	3333 / 464	CCCAAGCTTTTGGTAAAGCA AATGTAT	CATGCCATGGCCAAAACGA AATTAATAC	CCGCTCGAGAAACAAGAT AGAGTAGAA	CCGGAATTCAGATGAAC TTAATTTGT	TATTGGTTAGTAAACAT AAATAAA	AAACTTTATTTGTTACT AACAC
PY17X_1116300	gep1 C-terminal KO	3333 / 558	CGGGGTACCCTTTATGCT TATATCAGCA	CATGCCATGGCTGCAAAA TGTACACATGC	CCGCTCGAGGGGCTACTT ACTATTATTGCA	CCCTTAAAGGTAGTGTG TCCCAATTTGAA		
PY17X_1116300	gep1 full length KO	3333 / 3333	CGGGGTACCACAACCTCTA CAAATAACA	CATGCCATGGCTTTTTTCA AACITACAAA	CCGCTCGAGACAACCAT TTTTCATATTT	CCCTTAAAGGTAGTGTG TCCCAATTTGAA	TATTGTAAACAGCTATA TATATT	AAACAATATAGACTGGT TTAAC
PY17X_1116300	gep1 full length replaced with mScarlet							

Primers for PCR-genotyping parasite with gep1 (PY17X_1116300) knockout

Gene ID	KO	P1	P2	P3	P4	P5	P6	P7
PY17X_1116300	gep1 N-terminal KO	ACGCACATTGGCCATAA ACATG	CAAAAGACACTTGCATTTCA AT	GCTTGGCTAAGCAAAATGTA TCA	TCTTGCAATAAAAAATGGA GCCG	TATTGACATTAGGATTTG GAAGTGT	TCTTGCAATAAAAAATGGA GCCG	
PY17X_1116300	gep1 C-terminal KO	ATATACTGCACATACCT GATTG	GGCACATACACATACAT ATA	ATATTCGCTTTCAATGG	CTTAAAGTAGCAGCATAT ATAGACAAGA	ATATACTGCACATACCT GATTG	GAATTCAGTGGATAT GCTTT	
PY17X_1116300	gep1 full length KO	ACGCACATTGGCCATAA ACATG	CCCTTAAAGGTAGTGTG CCAATTTGAA	CGGGGTACCACAACCTCTA CAAATAACA	CTTAAAGTAGCAGCATAT ATAGACAAGA	ACGCACATTGGCCATAA ACATG	TCTTGCAATAAAAAATGGA GCCG	
PY17X_1116300	gep1 full length replaced with mScarlet							

Oligo sequence for gene knockout plasmid construction

Gene ID	Gene name	Gene size (bp)/ deleted gene size (bp)	Left homologous arm		Right homologous arm		Target site of sgRNA	
			Forward primer	Reverse primer	Forward primer	Reverse primer	Oligo (Forward)	Oligo (Reverse)
PY17X_0619400	nek4	1709/1709	CCCAAGCTTTGTAAGCAAG GTTAAATAC	CATGCCATGGCTGTGAGGA CGTGTATAATA	CCGCTCGAGAGTATAAT ATTAGCTGCA	CCGGAATTCGAATGGT ACACCTGTTCT	TATTGAAACATGATGAAA AATAAA	AAACTTTATTTTTCATACTT GTTC
PY17X_0935700	map2	1587/1587	CCCAAGCTTGTCTCCCAA TTTTCTGTG	CATGCCATGGGAAAGGAAT GTGCGTATCTA	CCGCTCGAGTATGTTTCG TCGGAAAGGT	CCGGAATTCGGAATATG GCAACT	TATTGAAAAATTTATTA CCAACT	AAACGATTCGTGAATAAAT TTTTC
PY17X_0617900	odpk4	1792/465	CGGGGTACCAGAGTAGATC CAGTAGAATA	CATGCCATGGTCTTAATG CATCTCTGCTG	CCGCTCGAGCTGCAAA TGAATAGCTCAAT	CCCTTAAAGTGTGTATA TTCACGATATTT	TATTGACAGAAATGAAT AATAAT	AAACATTATTAATCATTTC TGTC

Primers for PCR-genotyping parasite with gene knockout

Gene ID	Gene name	P1	P2	P3	P4	P5	P6
PY17X_0619400	nek4	CTTTTAAAAACCGATAA AAGTGT	CCGGAATTCGAAATGGTAC ACCCTGTTCT	CCCAAGCTTGTGAAGCAAG GTTAAATAC	TCACACCGTATCATATTG TTGTC	GAGTAAGATTGTGTGAT TTTGG	TCACACCGTATCATATTG TTGTC
PY17X_0935700	map2	GGGAAACTGATAATAA GCGACAA	CCGGAATTCGGAATAT GAGCATTCTG	CCCAAGCTTGTCTCCCAA TTTTCTGTG	AGACACACTCACATTACG ATTG	GATATATGGCTACTGG TTGTA	AGACACACTCACATTAC GATTG
PY17X_0617900	odpk4	GCTCTTCCCTTGGACAT AGTTA	GGCGTGAACCAAAATAAG TAT	GAAGTAGATGCACTAGAA TA	AGACGGATAAAATGTCCG AT	AGTACACAAAAGTTAGC ACAAG	CCTTTTCTAATCTCTCAG TTGA

Oligo sequence for gene knockout in P.berghel ANKA plasmid construction

Gene ID	Gene name	Gene size (bp)/ deleted gene size (bp)	Left homologous arm		Right homologous arm		Target site of sgRNA	
			Forward primer	Reverse primer	Forward primer	Reverse primer	Oligo (Forward)	Oligo (Reverse)
PBANKA_1115100	gep1	3169/785	CCCAAGCTTTCACATTGAT CATAAAGCTT	CATGCCATGGCTGTGCTGT ACATGGACAAA	CCGCTCGAGTTCTAATTT CATATTGATG	CCGGAATTCATACATC CTTATTGATATA	TATTGTATTAGTTTCCG ATAG	AAACCCCATATTTATATTA TAAC
PBANKA_0933700	map2	1572/807	CCCAAGCTTGTCTCCCAAT TTTTATGTT	CATGCCATGGTGTGATCGTAT GGGAAAACGCT	CCGCTCGAGTACGAAAT CAGTATTATCC	CCGGAATTCATCTGAA ATAGATGGGTAT	TATTGCAATGAAGAAGAT AAAGT	AAACCTTTTATCTTCTTC ATTCC

Primers for PCR-genotyping parasite with gene knockout in ANKA

Gene ID	Gene name	P1	P2	P3	P4	P5	P6
PBANKA_1115100	gep1	GACACACAAAACCTCTTA TCGGCATAC	ACAAATCTCCCTCCATTAT TTTCTTG	CGAGATTGTCCATGTACA G	CTCAATAAGGGAGAATC ACGT	GGAAACGACAAAATAAG AAA	CATTATGATATACCAA TAG
PBANKA_0933700	map2	GCTATTATGAAAAAGG AGCTAACAT	TTATGTGGCCCTGGTCTT CATITTC	CGAATTTGTCAATCCATA TTT	GCCTGGTCAATTGTGATT CGTT	GATGATAGCACATATAT AGAT	GTCGGAATCGGCTATTT CTA

Oligo sequences for gene tagging plasmid construction

Gene name	Tag	Gene ID	Left homologous arm		Right homologous arm		Target site of sgRNA	
			Forward primer	Reverse primer	Forward primer	Reverse primer	Oligo (Forward)	Oligo (Reverse)
sep1	C-terminal 4Myc	PY17X_0526200	CCCAAGCTTTTGCCTTTTAA TTGGCCATA	CATGCCATGGCTGTGATGC TGGTGGATAA	CCGCTCGAGTTCTAATTT CTGATGGTTAT	CCCTTAAAGCACGGTAC TACTTACAATAA	TATTGTATTAGTTTCCG TCCGGC	AAACCCCGCAGCAAAAC TAATAC
gep1	N-terminal 6HA	PY17X_1116300	CATGCCATGGCACAACCTC TACAATAACA	CCGGATATCTCTTTTTCAA ACTTACAATA	CCGCTCGAGGAAACATC AAATAAAGAAAT	CCCTTAAAGTATACCAA TAGATACAAGAT	TATTGAGCTATTTTATGA GAATAA	AAACTTTTCTCATAAATA GCTC
gca	C-terminal 6HA	PY17X_0911700	CGGGGTACCCTGTATACCA AATTTAAATATGAGA	CATGCCATGGGCAAAATG AACCTGTGCTTTTGG	CCGCTCGAGTACTTTTATG AACCTTCTAATTTATG	CCGGAATTCATCATGTAC TGTGAACATATCA	TATTAATCGTCATATAAA ATATTT	AAACAATATTTTATATGA CGATT
gca	C-terminal 4Myc	PY17X_0911700	CCCAAGCTTCTTTCTGCTAT TTTTATGTT	CATGCCATGGCTTTTATATC TTTATCTGTTATTTTATG TTG	CCGCTCGAGATGCGAGAC GAAAAGGAAATGAAA AAGT	CCGGAATTCAGAAGTTG ATCCATCTAGTATGTA CATAATC	TATTCAATAGTAATATGA AAAATC	AAACGATTTTCTATATTCT ATTG

Primers for PCR-genotyping with gene tagging

Gene name	Gene name	Gene ID	P9	P10	P11	P12
sep1	C-terminal 4Myc	PY17X_0526200	CATGTGCAAAAATGAAAITA GCAAAAAGC	CCCCTTAAAGCACGGTACTA CTTACAATAA	GCTAGCGCCGGTTCTCG TGTGATAA	GTAATGGTATTGAGGG T
gep1	N-terminal 6HA	PY17X_1116300	GCTTGGCTAAGCAAAATGTA TCA	CCCCTTAAAGTATACCAATG ATACAAGAT	CATGCCATGGCACAACCTC TACAATAACA	CAAAGACACTTGCCATT TCAAT
gep1	N-terminal 4Myc	PY17X_1116300	CATGCCATGGCACAACCTC TACAATAACA	CCCCTTAAAGTATACCAATG ATACAAGAT	CCCCTTAAAGTATACCAATG ATACAAGAT	CAAAGACACTTGCCATT TCAAT
gca	C-terminal 6HA	PY17X_0911700	TTAGAAATGGCATATTTCAAT G	CCATGTACTGTGAACATATC A	GTGTATACCAAAATTTAAAT ATGAGA	ATGAAATTAATTTCCAAA ATGGA
gca	C-terminal 4Myc	PY17X_0911700	CACACACCCAGCACACATA TTTAGAT	ATCCAAAGCAGTCTCTGCTG TTG	CCTTGTCTTACCTCTATTAC TGCACCATGAT	ATCTCGAGGTACTTGCT CATTTTCTACTAAGCG

Oligo sequences for gca promoter swap plasmid construction

Gene name	Tag	Gene ID	Left homologous arm		Right homologous arm		Target site of sgRNA	
			Forward primer	Reverse primer	Forward primer	Reverse primer	Oligo (Forward)	Oligo (Reverse)
gca	N-terminal 6HA & promoter swap	PY17X_0911700	CCC AAGCTTCTCAATATGG TTGCATATAT	CATGCCATGGAAGAAGATA ATGTGCATACAC	CCGCTCGAGCAGACGAA AAAAGGAAATGA	CCGGAATCGAAGTTGA TCCATCTATGAT	TATTCATAGTAATATGA AAAATC	AAACGATTTTCATATTACT ATTG
Primers for PCR-genotyping with gca promoter swap								
Gene name	Gene name	Gene ID	P9	P10	P11	P12	P13	P14
gca	N-terminal 6HA & promoter swap	PY17X_0911700	TCATCCAGGTGTAGACTAA AAT	CCGGAATCGAAGTTGATC CATCTATGAT	CCCAAGCTTCTCAATATG GTTGCATATAT	AGCGTATAACTACTACCT ACAGT	AAACACAGACATAACTC CTTTAGA	AGCGTATAACTACTACTAC AGT
Primers for RT-PCR								
Gene name	Gene ID	Forward Primer	Reverse Primer					
18s Rna	PY17X_0522400	GGTTTTATAATTGGAAT GATGGGAAT	ACGCTATTGGAGCTGGAAT TACC					
gca	PY17X_0911700	GTTGTAATCATAGTGAT GGTTC	TCACATGTTATTCTCCTCT AA					
Primers for gene in situ complementation								
Gene name	Tag	Gene ID	TS		Left homologous arm		Right homologous arm	
			Oligo (Forward)	Oligo (Reverse)	Forward primer	Reverse primer	Forward primer	Reverse primer
Pygep1	C-terminal 6HA	PY17X_1116300	TATTGGGACGCTAATCGT AGCTA	AAACTAGCTACGATTAGCG TCCGC	CGGGGTACCCTTTATGC TTATATCAGCA	CATGCCATGGACCCCTT ATTGAAAATTCAC	CCGCTCGAGACAAACAT TTTTCATATTTT	CCCCTTAAGGTAGTGTG TCCAATTTGAA