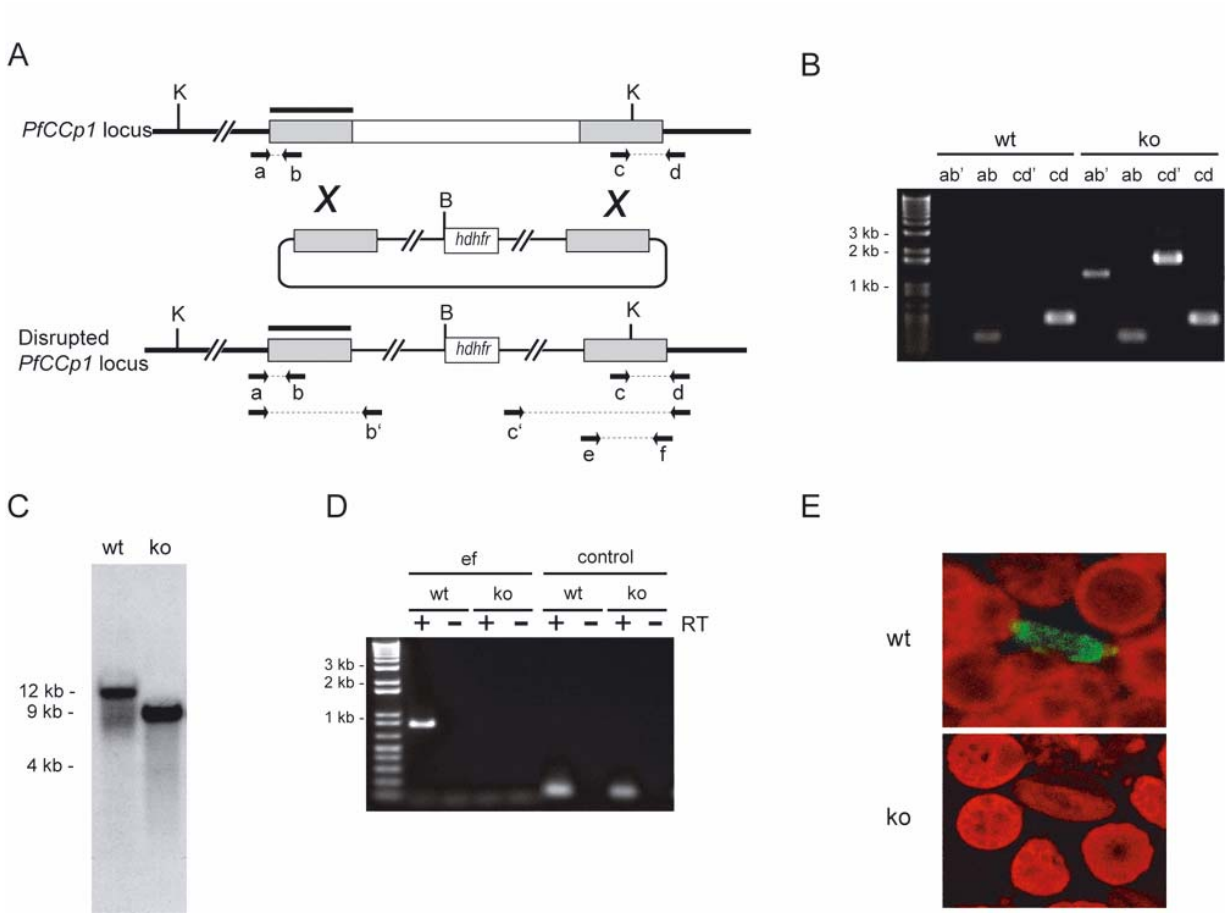
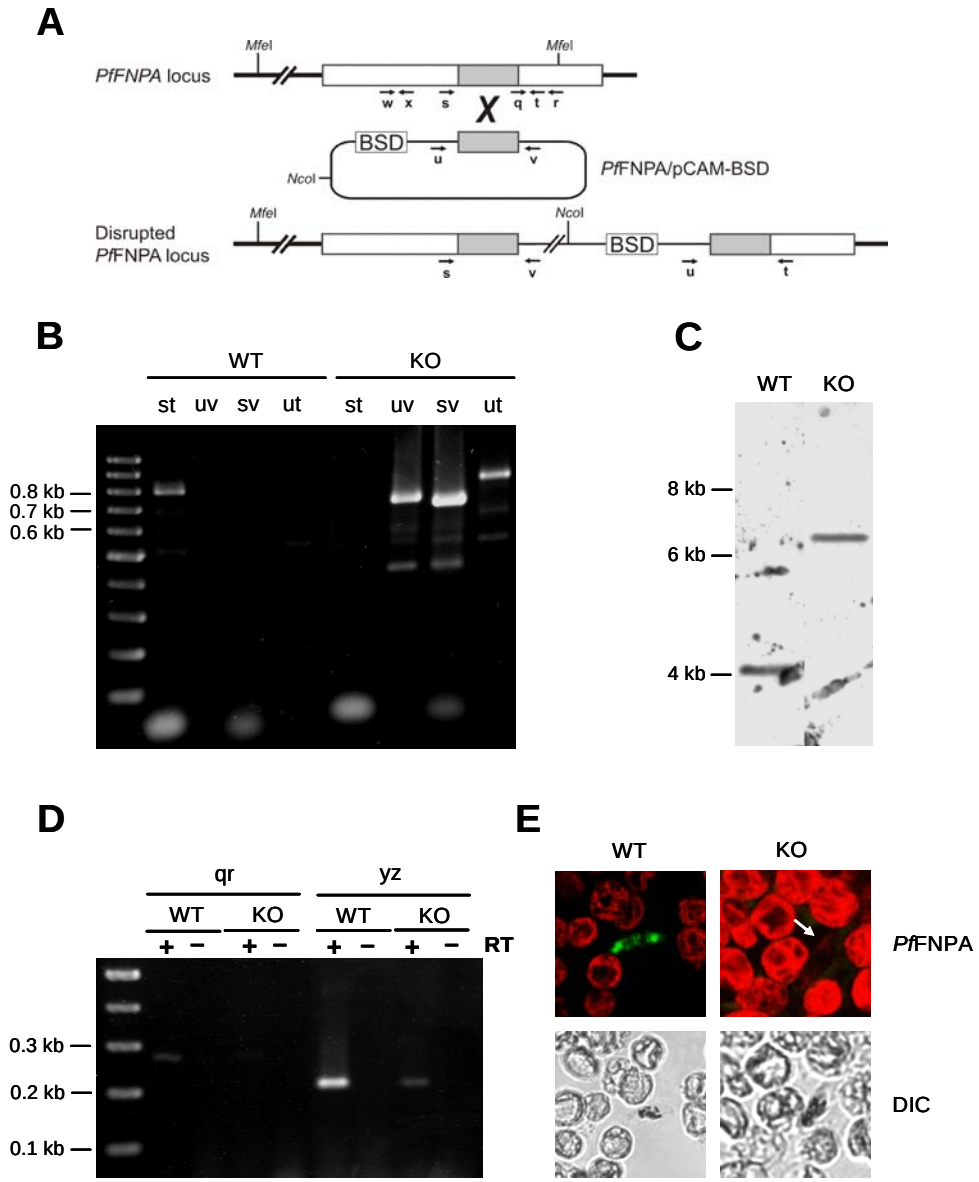
Supplemental Figure 1



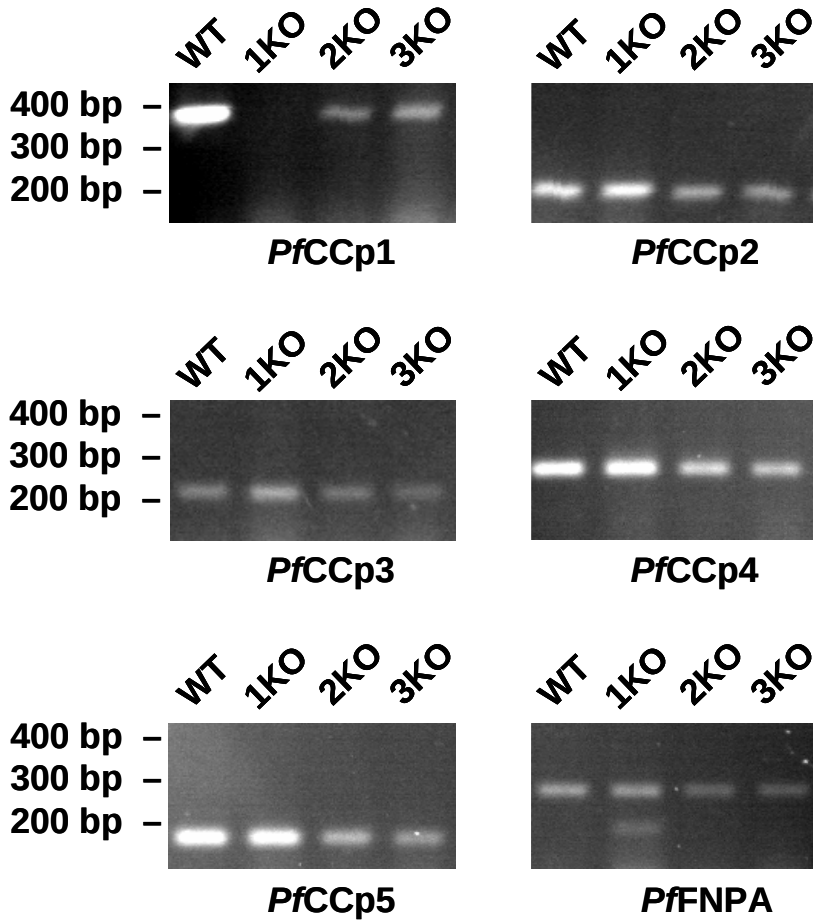
Supplemental Fig. 1. Targeted gene disruption of *PfCCp1* via double homologous recombination. *A.* Schematic of the *PfCCp1* locus and the locations of PCR primers that yield products diagnostic of wild type and disrupted loci. Not shown is the *thymidine kinase* gene expression cassette that is used to force the second recombination event via negative selection with ganciclovir drug pressure. *B.* Ethidium bromide-stained agarose gel electrophoresis of PCR products diagnostic of integration events, using primer pairs indicated above the respective lanes. *C.* Southern blot of KpnI plus BamHI cut genomic DNA from wild type and gene knockout line showing complete disruption of the *PfCCp1* gene locus. The probe used to screen the Southern blot is indicated in panel A as a solid horizontal bar above the 5' coding region of the *PfCCp1* gene. *D.* RT-PCR of *PfCCp1* transcripts with set of primers "e f" shows loss of expression in knockout clone. Reverse transcriptase and sham controls are indicated above the lanes by "+" and "-", respectively. The housekeeping gene, *arginyl-tRNA synthetase* (PFL0900c), was used as a loading control. *E.* Indirect immunofluorescence assay demonstrated that *Pf*CCp1 is absent in *PfCCp1*-KO gametocytes. *Pf*CCp1 was stained with anti-*Pf*CCp1 mouse antibodies followed by goat anti-mouse secondary antibodies conjugated with Alexa Fluor 488 (green). Erythrocytes were counterstained with Evans Blue (red). wt, wild type *P. falciparum* NF54; ko, *PfCCp1* knockout clone.

Supplemental Figure 2



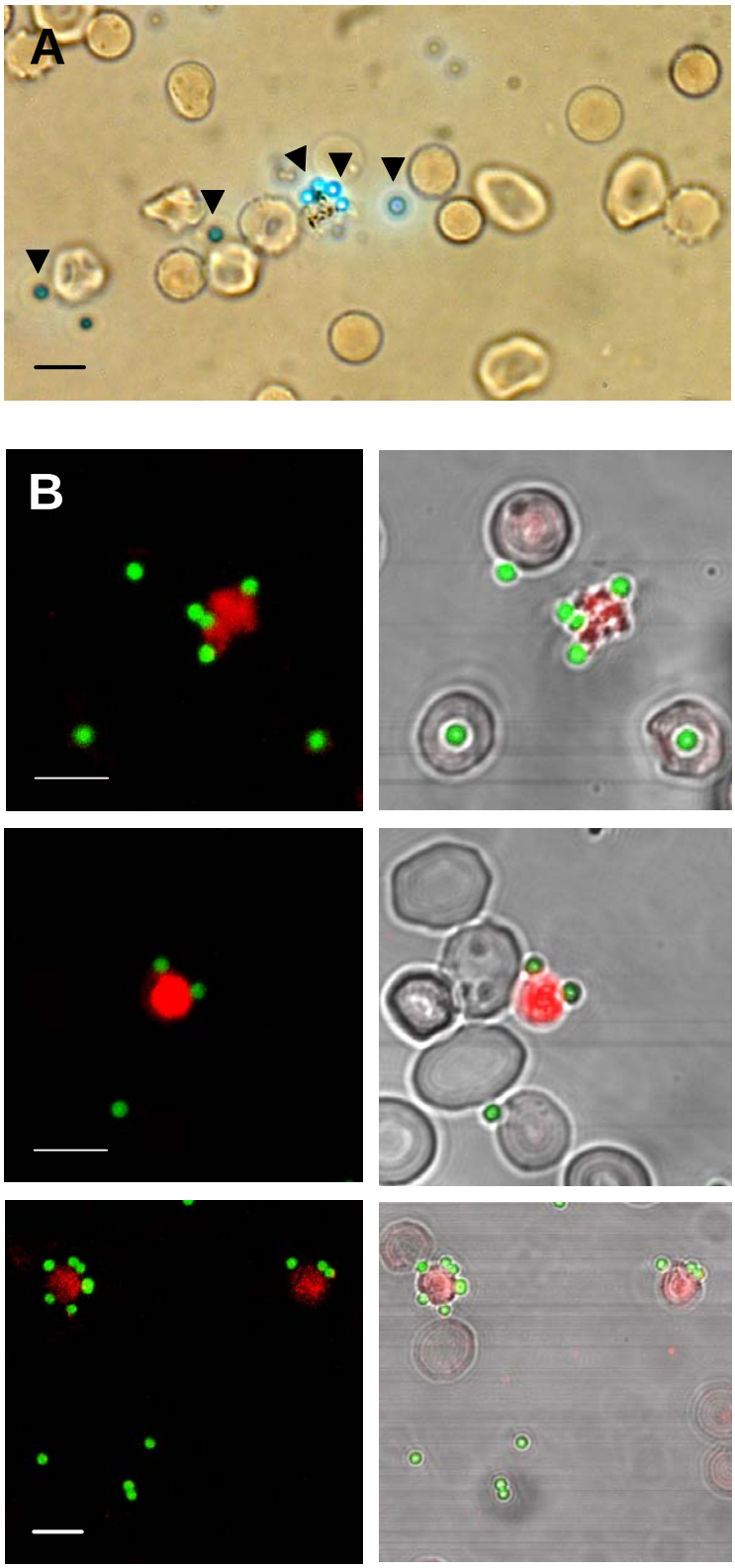
Supplemental Fig. 2. Targeted gene disruption of *PfFNPA* via single crossover homologous recombination. *A.* Schematic of the *PfFNPA* locus and the locations of PCR primers that yield products diagnostic of wild type and disrupted loci as well as oligonucleotides used for Southern blot and RT-PCR. *B.* Ethidium bromide-stained agarose gel electrophoresis of PCR products diagnostic of integration events, using primer pairs indicated above the respective lanes. *C.* Southern blot of MfeI plus NcoI cut genomic DNA from wild type and gene knockout line showing complete disruption of the *PfFNPA* gene locus. The probe used to screen the Southern blot was generated with oligonucleotides indicated in panel A as "w" and "x". *D.* RT-PCR of *PfFNPA* transcripts with set of primers "q r" shows decreased transcript for the disrupted gene: low transcript expression is due to unspecific promoter activity of the integrated pCAM-BSD vector. Reverse transcriptase (RT) and sham controls are indicated above the lanes by "+" and "-", respectively. Primers against *Pf39* were used as a loading control. *E.* Indirect immunofluorescence assay demonstrated that *Pf*FNPA is absent in *Pf*FNPA-KO gametocytes. *Pf*FNPA was stained with anti-*Pf*FNPA mouse antibodies followed by goat anti-mouse secondary antibodies conjugated with Alexa Fluor 488 (green). Erythrocytes were counterstained with Evans Blue (red). DIC, Differential interference contrast; WT, wild type *P. falciparum* NF54; KO, *Pf*FNPA knockout clone.

Supplemental Figure 3



Supplemental Fig. 3. *Pf*CCp transcript expression in *Pf*CCp gene-disruptant parasites. RT-PCR assay of gene-specific transcript expression using cDNA prepared from gametocytes reveals *PfCCp* transcript in the *Pf*CCp1-KO, *Pf*CCp2-KO, and *Pf*CCp3-KO lines, which are comparable to mRNA levels in the wild type. For *Pf*CCp2-KO and *Pf*CCp3-KO, transcript is also present for the respective disrupted genes, which might be due to unspecific promoter activity of the integrated pDT-*Tg*23 vector. Length of PCR products: *Pf*CCp1, 371 bp; *Pf*CCp2, 198 bp; *Pf*CCp3, 215 bp; *Pf*CCp4, 246 bp; *Pf*CCp5, 177 bp; *Pf*FNPA, 266 bp. 1KO, *Pf*CCp1-KO; 2KO, *Pf*CCp2-KO; 3KO, *Pf*CCp3-KO; WT, wild type.

Supplemental Figure 4



Supplemental Fig. 4. Binding of *Pf*CCp-coated latex beads to macrogametes. *A.* Image of fluorescent latex beads coated with the recombinant protein *Pf*CCp1rp1-6His/SUMO (arrowheads), which bind to a macrogamete in paraformaldehyde-fixed exflagellating parasite cultures 15 min post-activation. *B.* Binding of latex beads (green) to macrogametes was verified by immunofluorescence assay, using antibodies directed against *Pf*s25 (red). Left panel, confocal image; right panel, DIC overlay. Bar, 5 µm.