Supplemental Table 1

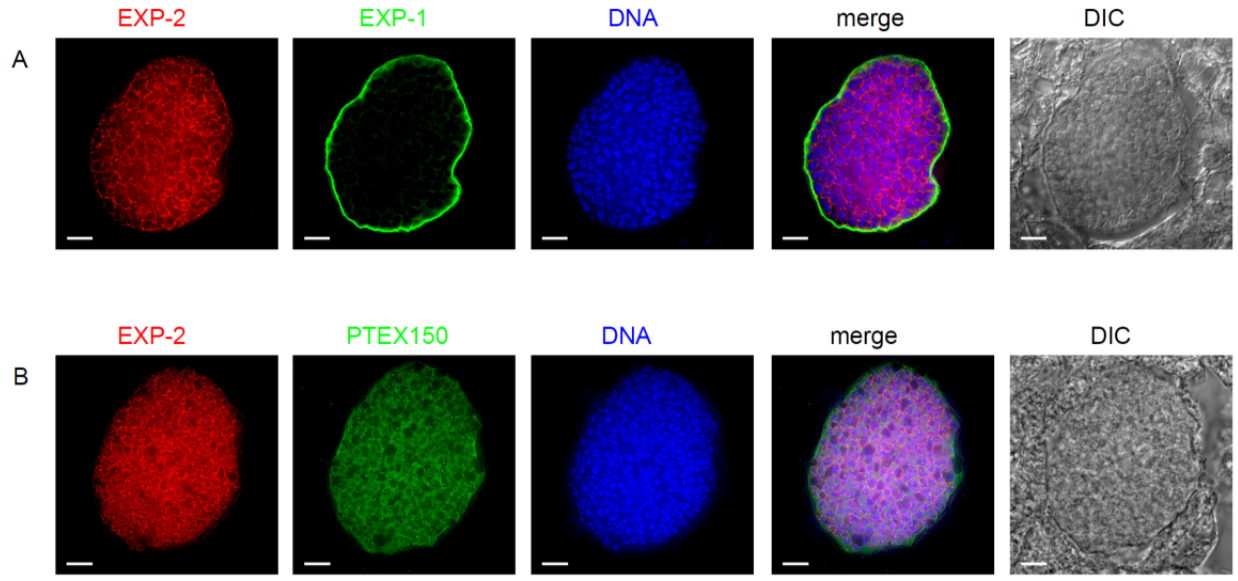
Oligonucleotide primers used in the study

Primer	DNA sequence (5' to 3')
Plasmodium falciparum 18s forward	AATCTTGAACGAGGATGCC
Plasmodium falciparum 18s reverse	GGAAACCTTGTTACGACTTCTCC
Plasmodium falciparum MSP1 forward	GAAGAAATTACTACAAAAGGTGCAAGTG
Plasmodium falciparum MSP1 reverse	CGTCTAATTCATTTGCACGAAT
Plasmodium falciparum AMA-1 forward	TTTGCTTTTCCTCCAACAGA
Plasmodium falciparum AMA-1 reverse	GATCCGAAGCACTCAATTCAA
Plasmodium falciparum EBA-175 forward	ATAAAAGATGGAAGAGTTATGGAACTCC
Plasmodium falciparum EBA-175 reverse	CCCATAGCAAGATGTCCATAATCTAAAA
Human apolipoprotein AI forward	AGCGTGACCTCCACCTTCAG
Human apolipoprotein AI reverse	CCTTCACCTCCTCCAGATCCTT
Mouse glyceraldehyde-3-phosphate dehydrogenase forward	AGGTCGGTGTGAACGGATTTG
Mouse glyceraldehyde-3-phosphate dehydrogenase reverse	TGTAGACCATGTAGTTGAGGTCA
Plasmodium falciparum Pf47 T1F	GGAGGGAAAAAATAATCGTACA
Plasmodium falciparum Pf47 T1R	TCAACCAAGTCATTCTGAGA
Plasmodium falciparum Pf47 T2F	GTAATTTATGGGATAGCGAT
Plasmodium falciparum Pf47 T2R	GTTTTTCATCGAAATGCGTA

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1

Indirect immunofluorescence assay of *P. falciparum* late liver stages (LS) in the FRG KO huHep mouse infections. Liver sections were incubated with antibodies specific to the parasitophorous vacuole (PV) translocon associated proteins EXP-2 and PTEX150 and the PV membrane (PVM) associated protein EXP-1. DNA was visualized with 4', 6-diamidino-2-phenylindole (DAPI) and differential interference contrast microscopy (DIC) images of the liver sections were captured. (A) The developing seven day LS is delineated by the circumferential localization of the PVM marker EXP-1 whereas the PV marker EXP-2 is localized diffusely within the LS, indicating that it is dissociated from the PVM in late LS development. (B) A similar pattern seen in (A) is observed for the translocon component PTEX150 in day seven LS. Scale bar: 10 μm.

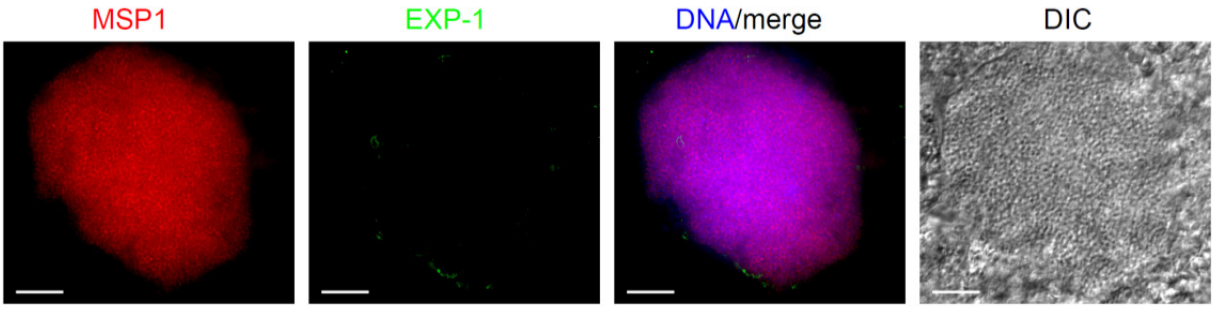


Supplemental Figure 2

Three-dimensional projections of *P. falciparum* merosome and merozoite aggregates in the infected FRG KO huHep mouse liver. Liver sections were incubated with antibodies specific to MSP-1 (red) and DNA was visualized with 4', 6-diamidino-2-phenylindole (DAPI) (blue). (A) Images of a merosome taken with DeltaVision deconvolution microscopy were compiled to represent the partial volume of the merosome. (B) Images of merozoite aggregates and individuated merozoites were compiled to represent the volume of the aggregate. The movies show rotating 3D representations of the data, rotating around the Y-axis. Scale bar: 10 μm.

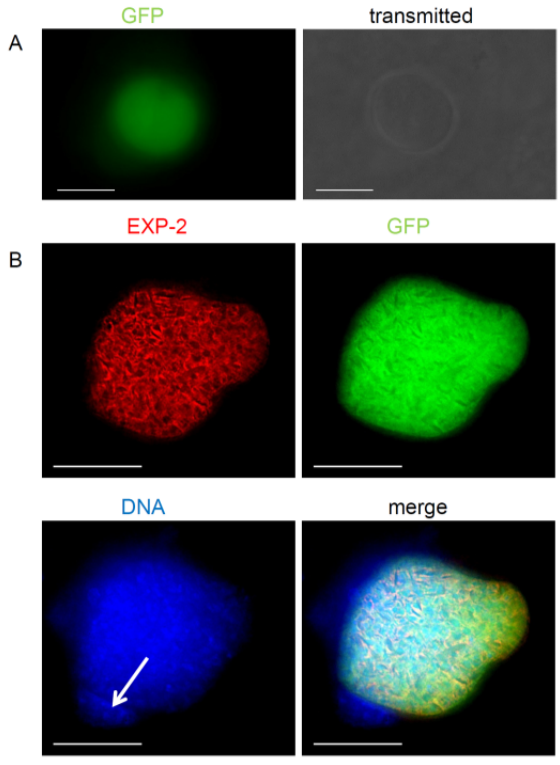
Supplemental Figure 3

Indirect Immunofluorescence assays of *P. falciparum*-infected FRG KO huHep mouse livers demonstrate PVM breakdown in late liver stages (LS). Liver sections were incubated with antibodies specific to merozoite surface protein 1 (MSP1) and the parasitophorous vacuole membrane (PVM) associated protein EXP-1. DNA was visualized with 4', 6-diamidino-2-phenylindole (DAPI) and differential interference contrast microscopy (DIC) images of the liver sections were captured. The PVM of the fully mature seven day LS, as expected, has broken down and little remains, based on EXP-1 expression. Individual merozoites are present in the mature LS parasite and this is evident in the DIC image. Scale bar: 10 μm.



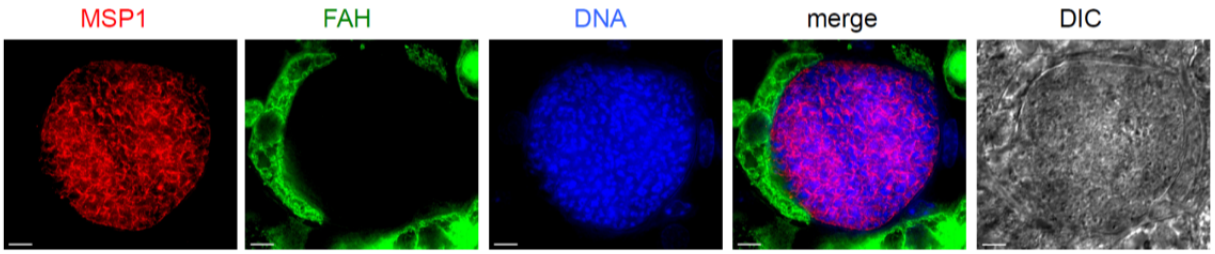
Supplemental Figure 4

Visualization of fluorescent liver stages (LS) in the infected FRG KO huHep mouse liver. A transgenic *P. falciparum* parasite (NF54HT-GFP) which expresses GFP throughout the life cycle (1), can be visualized by ex vivo fluorescence microscopy and by indirect immunofluorescence assay at six days of development. (A) Six day liver sections were examined *ex* vivo without fixation and GFP positive LS were visualized by fluorescence microscopy. Image shows a fluorescent LS and a transmitted light image. (B) Fixed liver sections from the same liver as (A) were examined by indirect immunofluorescence assay. LS parasites were positive for the parasitophorous vacuole associated protein EXP-2 and for GFP. DNA was visualized with 4', 6-diamidino-2-phenylindole (DAPI) and a differential interference contrast microscopy (DIC) image of the liver section was captured. The host cell nucleus is labeled with a white arrow. Scale bar: 20 μm.



Supplemental Figure 5

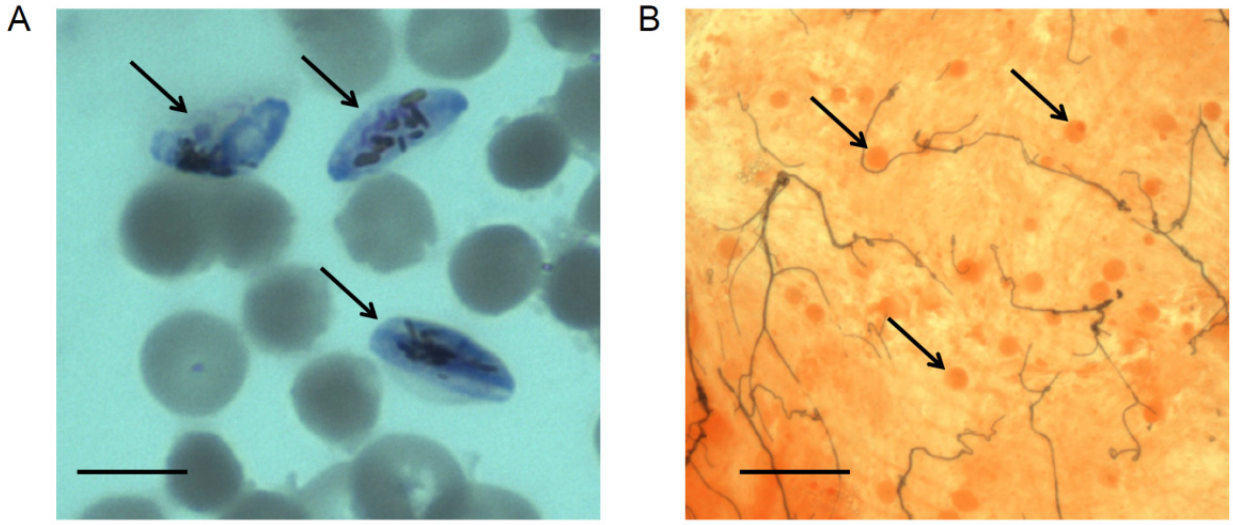
Indirect immunofluorescence assays of *P. falciparum*-infected FRG KO NOD huHep mouse livers show LS development. Liver sections were incubated with antibodies specific to merozoite surface protein 1 (MSP1) and human fumarylacetoacetate hydrolase (FAH). DNA was visualized with 4', 6-diamidino-2-phenylindole (DAPI) and a differential interference contrast microscopy (DIC) image of the liver section was captured. The FRG KO NOD huHep mouse is also able to support the development of the *P. falciparum* LS. Scale bar: 10 μm.



Supplemental Figure 6

Liver stage to blood stage transition in the FRG KO NOD huHep mouse yields blood stage parasites capable of sexual maturation in vitro and subsequent oocyst production in *A. stephensi* mosquito infections.

(A) *P. falciparum* NF54 blood stage cultures from the FRG KO NOD huHep mouse-transitioned blood stages were used for mature gametocyte production, as seen in the Giemsa-stained thin blood smear. Arrows point to mature gametocytes. (B) Midgut oocyst production was observed ten days after *A. stephensi* mosquitoes were fed on mature gametocyte cultures. The midguts were dissected and *stained in* 1.0% Mercurochrome to visualize the *P. falciparum* oocysts. Arrows point to representative oocysts. Scale bar for (A): 10 μm and for (B): 250 μm.



Supplemental Figure 7 Legend

Creation of *P. falciparum* NF54HT-GFP. *P. falciparum* NF54HT-GFP expresses GFP throughout the *P falciparum* life cycle under the constitutive EF1α promoter. (A) Cartoon showing the single cross-over integration of the GFP cassette into the dispensable *Pf47* locus. Primer pairs used for confirmatory PCR of the selected resistant population and clonal populations are shown with their expected size in base pairs (bp). (B) PCR demonstrating the integration of the GFP cassette into the *Pf47* locus using primer pairs that recognize the wild type locus and the recombinant locus. WT: wild type, P1: parental population 1, C1: clone C1 from limiting dilution of P1. Note that C1 is recombinant whilst P1 has some WT contamination. Size of DNA ladder is shown to the left in bp. Note: the PCR products are of the expected size.

REFERENCE:

1. Talman, A.M., Blagborough, A.M., and Sinden, R.E. 2010. A Plasmodium falciparum strain expressing GFP throughout the parasite's life-cycle. *PLoS One* 5:e9156.

Supplemental Figure 7