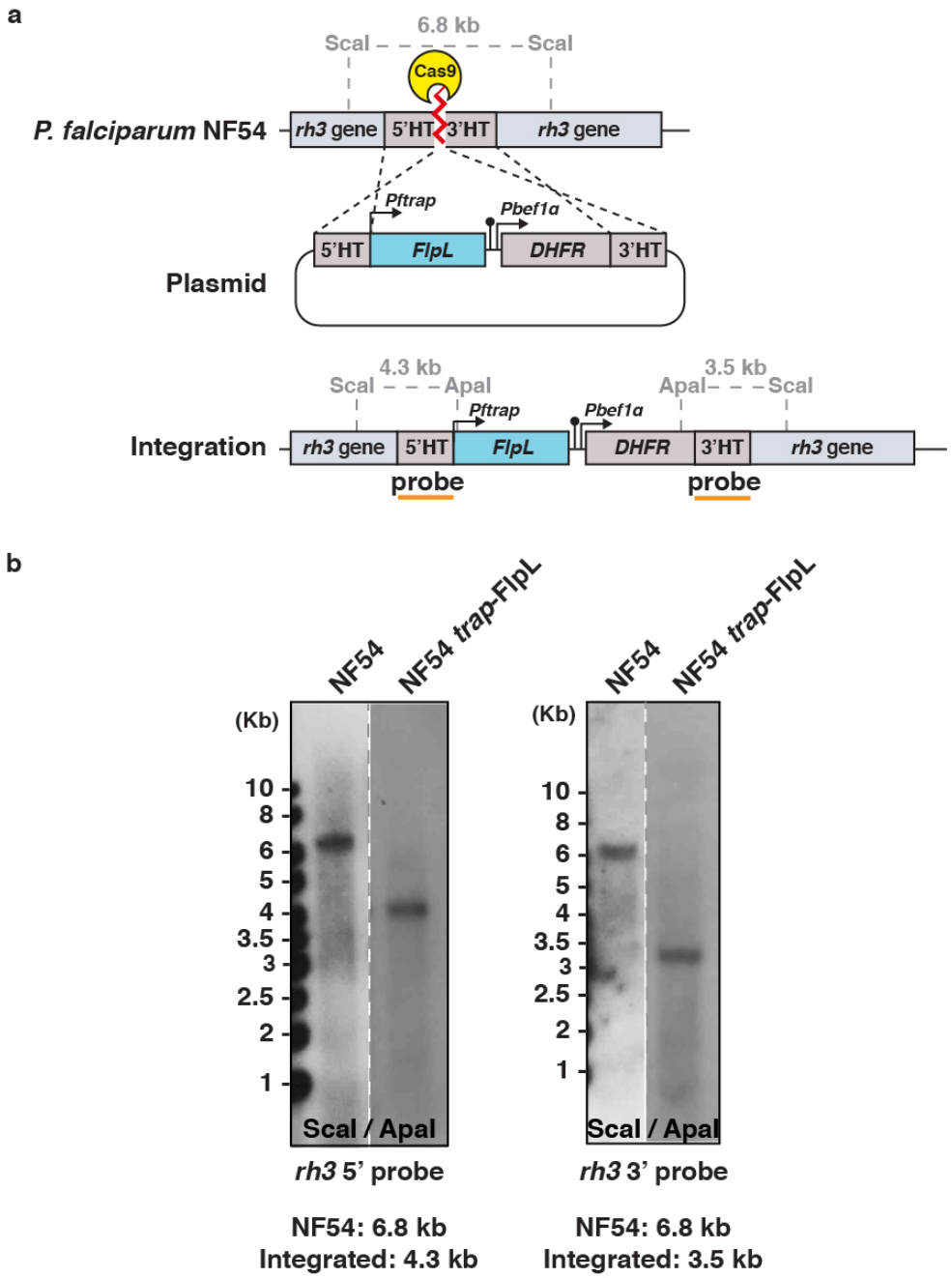
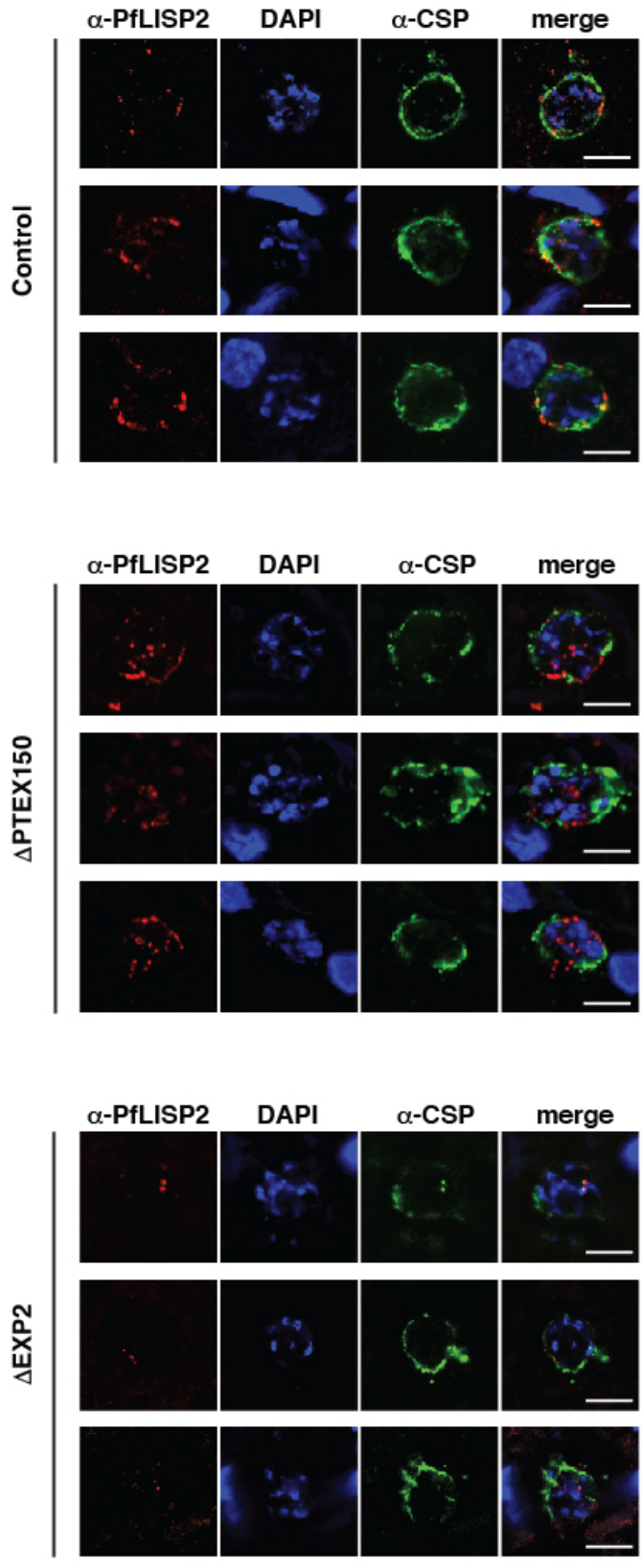
Supplementary information

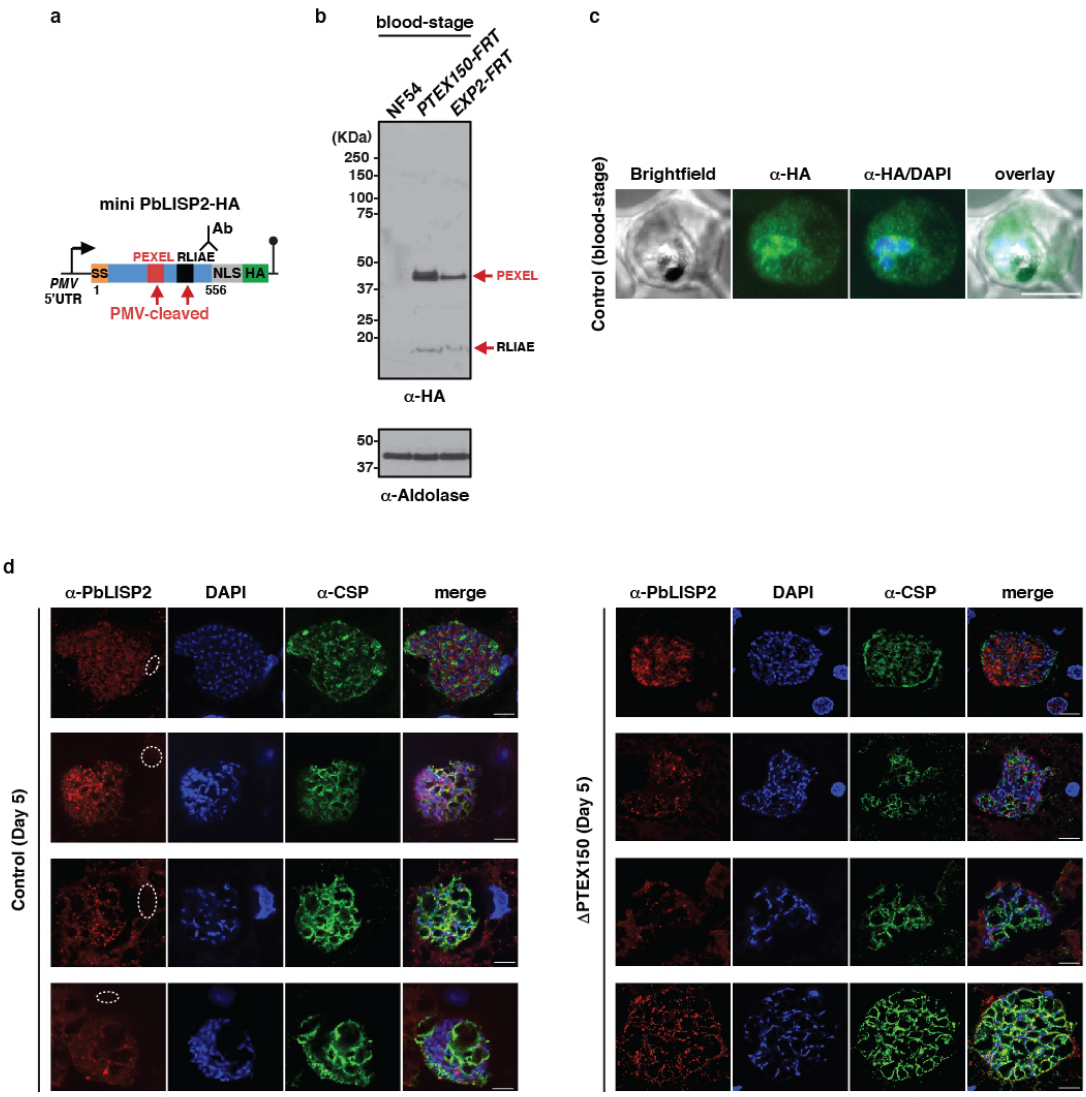
Plasmodium falciparum exoerythrocytic forms require the PTEX translocon for development in human hepatocytes



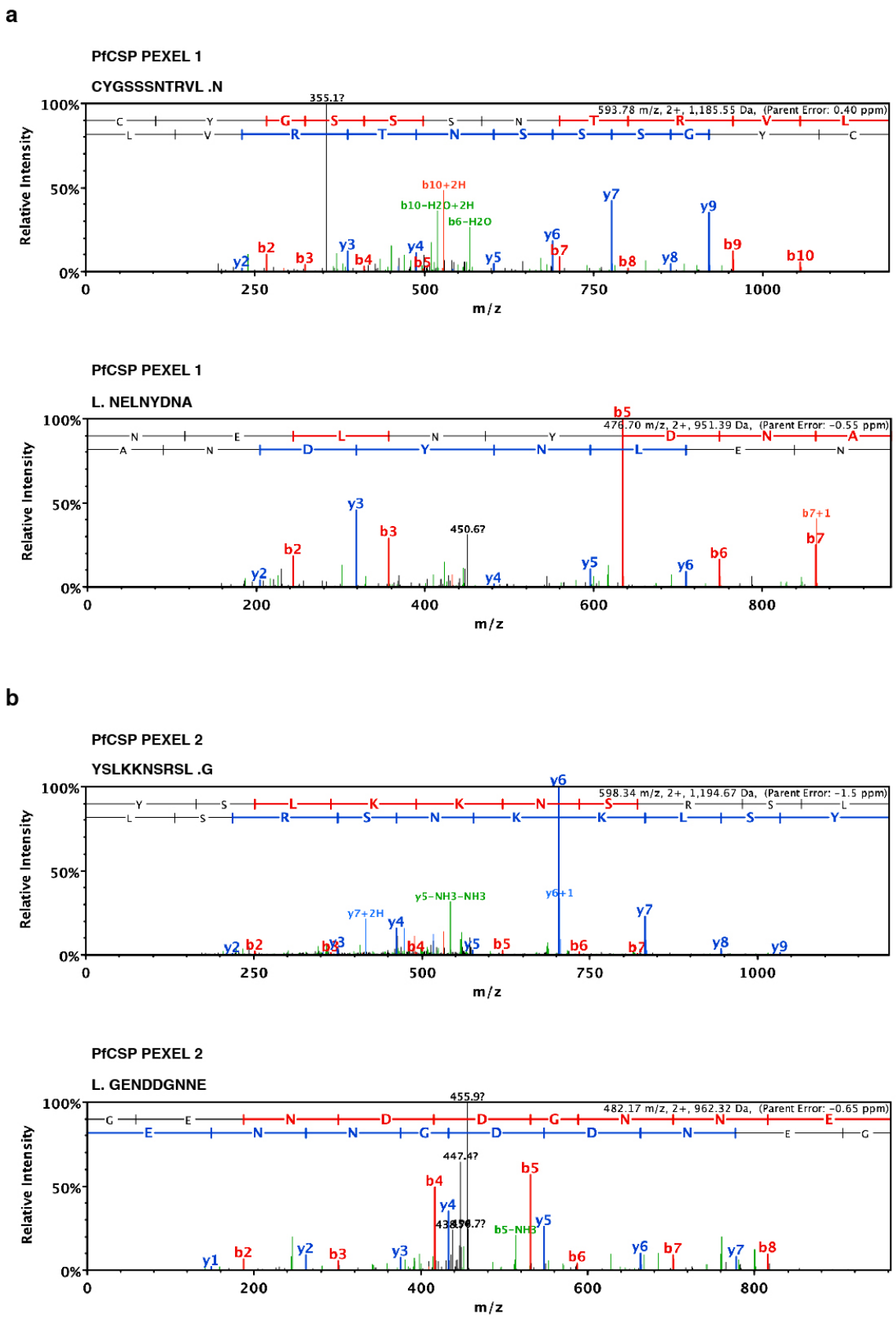
Supplementary Figure 1. Generation of *P. falciparum* NF54 *trap*-FlpL parasites. a Strategy for inserting a FlpL recombinase expression cassette (*Pftrap* gene promoter-FlpL recombinase-*Pftrap* gene terminator) into the *P. falciparum rh3* pseudogene locus by double cross-over homologous recombination. **b** Southern blot analysis of parental NF54 and NF54 *trap*-FlpL integrant parasites after digestion of genomic DNA with *Sca*I/*Apa*I. The probes used (orange bars) for genotyping and the expected fragment sizes after digestion are shown. Data represent n=1 experiment confirming integration.



Supplementary Figure 2. Expression and localization of *P. falciparum* LISP2 in infected hepatocytes. Immunofluorescence microscopy of fixed liver sections isolated from humanized mice reveals the expression and subcellular localization of PfLISP2 in *P. falciparum* exoerythrocytic forms on day 3 post-infection. (refer to Figures 5). Scale bar, 5 μm.



Supplementary Figure 3. Expression, N-terminal processing and localization of a *P. berghei* LISP2 reporter during *P. falciparum* infection. a Schematic of mini *P. berghei* LISP2-HA reporter, comprising the first 556 residues of PbLISP2 (PBANKA_1003000) containing the ER-type signal sequence (SS), canonical PEXEL (RILAE) and a single copy of the non-canonical PEXEL RLIAE from the repeat region fused to a nuclear localization sequence (NLS) and triple hemagglutinin (HA) tag. Expression was driven from the *P. falciparum plasmepsin V* (*PMV*) gene promoter. The transgene was included in the *PTEX-FRT* plasmid (see Figure 2a). **b** Immunoblot with anti-HA antibodies shows specific expression of mini PbLISP2-HA in asexual blood-stages by *P. falciparum PTEX150-FRT* and *EXP2-FRT* parasites. Bands consistent with processing by PMV within the PEXEL and inefficiently within the RLIAE sequence are indicated. Aldolase was included as a loading control. **c** Immunofluorescence microscopy with anti-HA antibodies revealed the expression of mini PbLISP2-HA in *P. falciparum* asexual blood-stages (inefficient export). Scale bar, 5 μm. **d** Immunofluorescence microscopy with anti-PbLISP2 antibodies revealed the expression of mini PbLISP2-HA in *P. falciparum* liver-stages in humanized mice on day 5 post-infection. The protein was located within the parasite and was not specifically exported into the hepatocyte nucleus (see white hashed circles) or to the host cell cytoplasm. Scale bar, 10 μm.



Supplementary Figure 4. ***P. falciparum*** **CSP contains two PEXEL motifs that are cleaved by plasmepsin V. a** LC-MS/MS identification of CSP peptide 1 cleavage products following incubation with plasmepsin V. Peptide CYGSSSNT**RVLNE**LNYDNA was cleaved to CYGSSSNT**RVL**↓(top) and ↓**NE**LNYDNA (bottom). **b** LC-MS/MS identification of CSP peptide 2 cleavage products following incubation with plasmepsin V. Peptide YSLKKNS**RSLGE**NDDGNNE was cleaved to YSLKKNS**RSL**↓ (top) and ↓**GE**NDDGNNE (bottom). Refer to Fig. 6e.

Table S1. Oligonucleotide primers used in this study

Primer name	**Sequence**
FLP01	CCGCTCGAGTATTTTTACAATTTTAATAATAATACTAAAAC
FLP02	TCCCCGCGGCTTCTGAGGGAATTATTTTTTTTTTGGTAAATT
FLP03	TCCCCCCGGGATATAATACTTGAATAATATTTTATGTAATATAGTG
FLP04	AAACTGCAGGGAAAATTTTTTCTATATATTATTTTAAATATGTATG
FLP05	AAAGCGGCCGCGAAGATGTTGATTTTAGGTACTATGTC
FLP06	ACCTTAATTACCCATTTCTGTATTACCAATACGCCC
FLP07	ATCCCTAGGATGCGGAAGCAATAAATGTGGTTTTAGAAG
FLP08	ATCGGCGCCGGTTTCTTTCTCTTCTATAATATTTTTTATATTATTCATCC
qPCR primers	**Sequence**
WT_F	GGTAATACAGAAATGGTTAATCAATTCCGATGAC
WT_R	GGAACCTTTTTAATGCTTGCATTGCAGAATATC
PTEX150FRT_F	ATGTAATGCCCACCTGCAGCATGCCAGGGAACGATAGTGAT
PTEX150FRT_R	CTCCGGCTTGTTTCAGCAGAGAGAAGTTTGTTGCGCCG
EXP2FRT_F	ATATAATATTATTTACTTCTACATCCACTGAAAAA
EXP2FRT_R	GGGTCCTTGATGACTGTGGT
18S_F	GTAATTGGAATGATAGGAATTTACAGGT
18S_R	TCAACTACGAACGTTTTAACTGCAAC