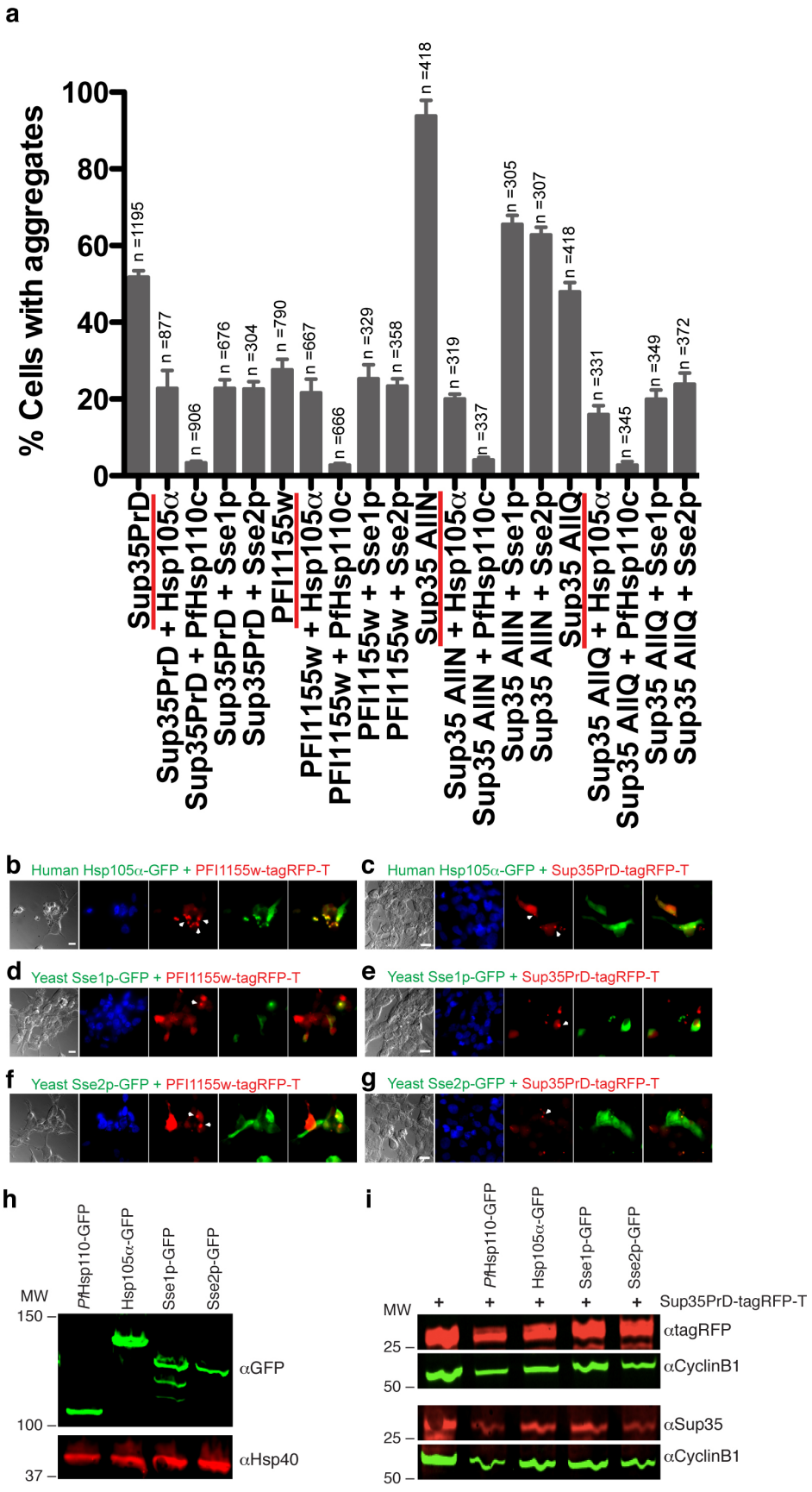
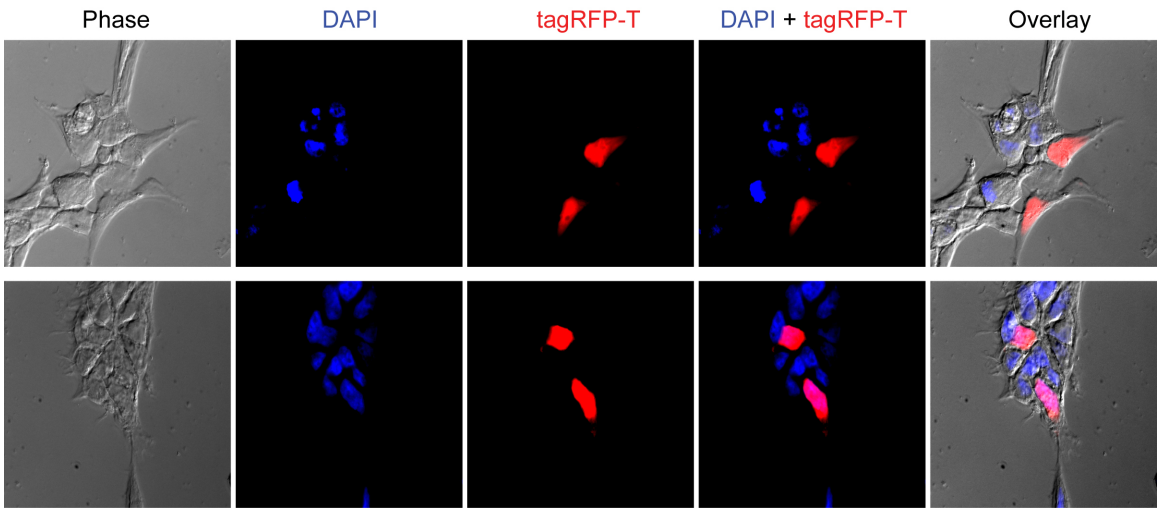
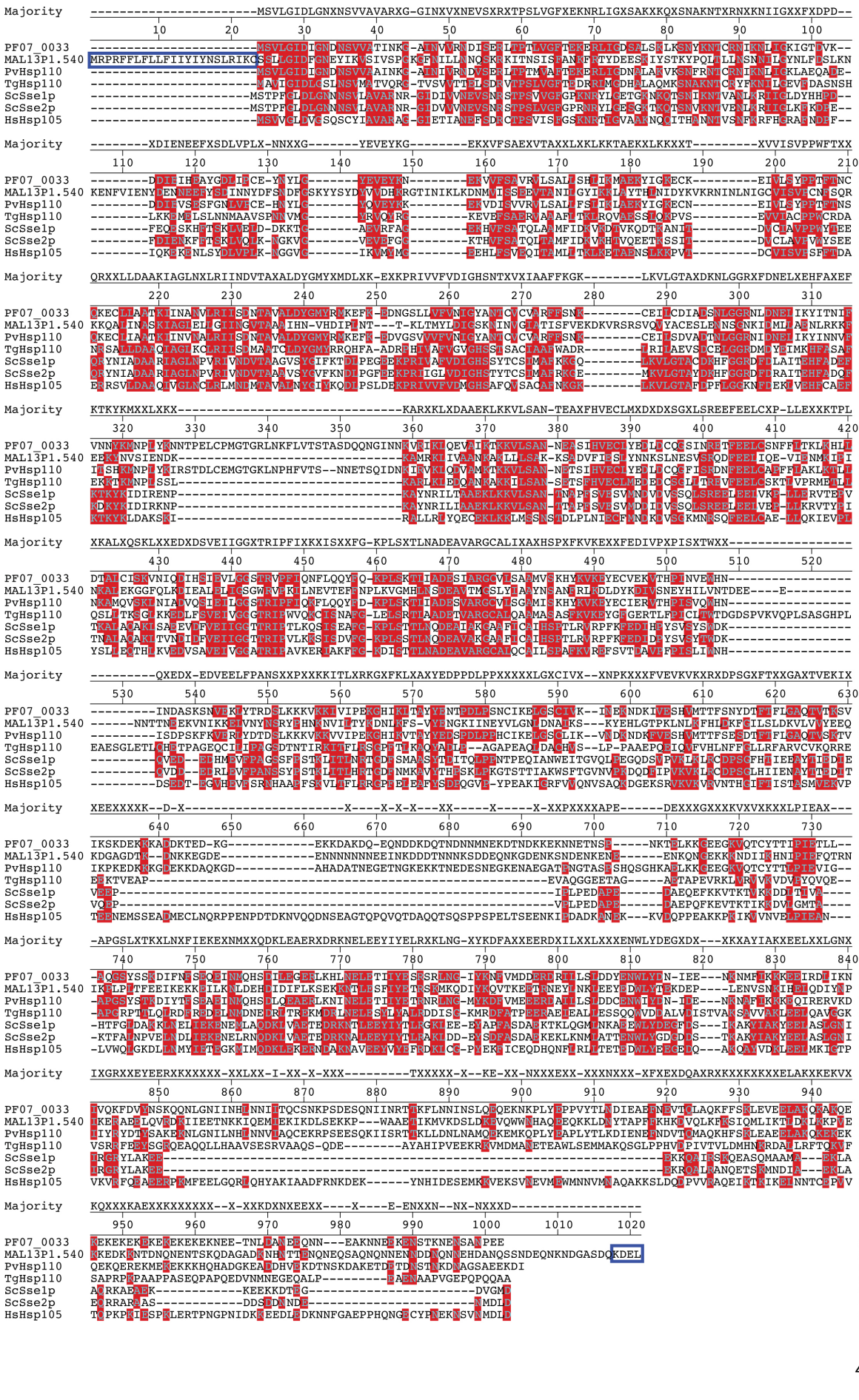
SUPPLEMENTARY INFORMATION



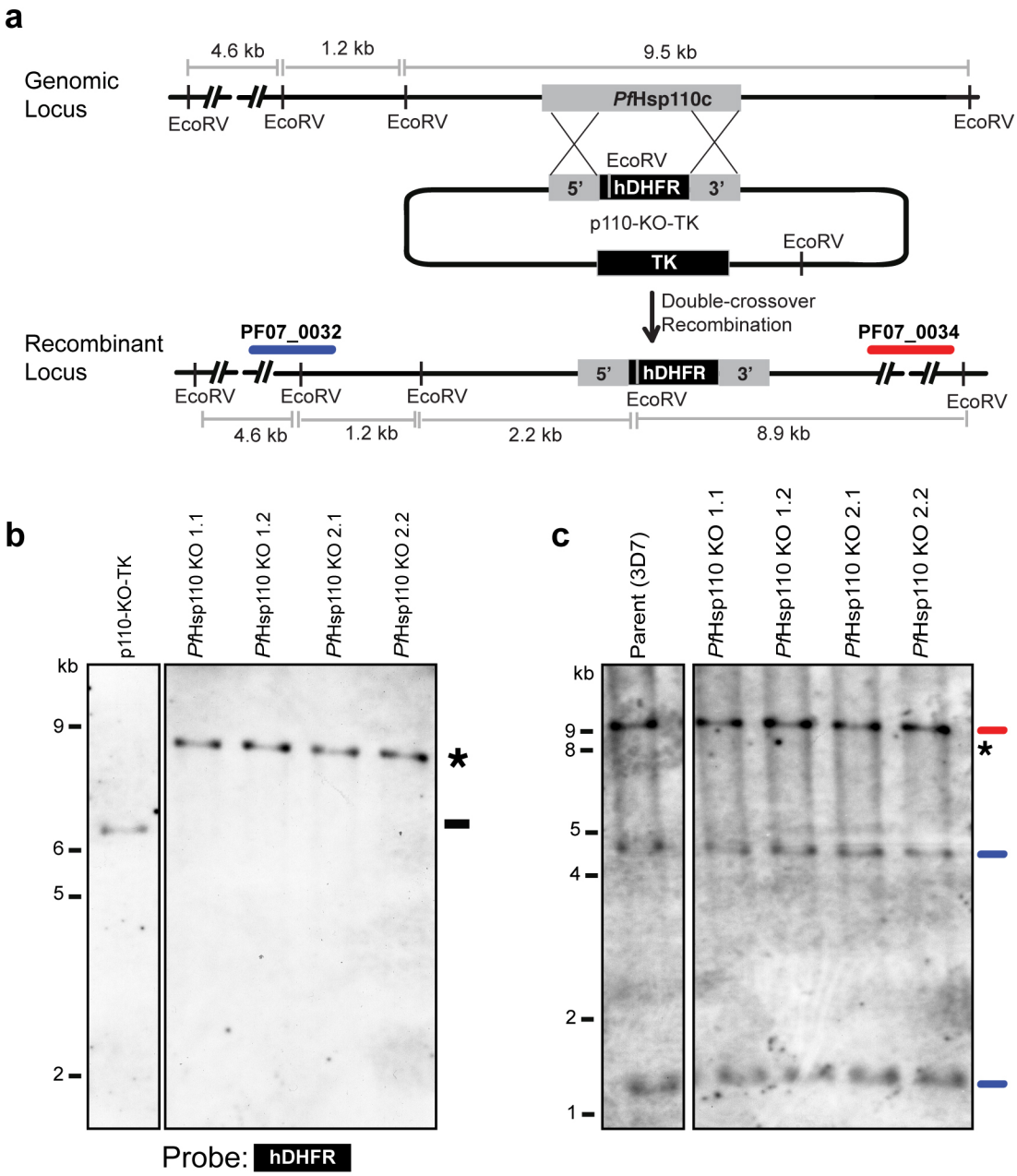
Supplementary Figure S1. Proportion of HEK293T transfectants showing fluorescent foci after heat sho
Blinded slides were counted for the appearance of fluorescent foci of tagRFP-T fusions (underlined in red) transfected transiently into HEK293T cells that were incubated at 40°C for 6 hours prior to fixation. In cells that were also co-transfected with Hsp110 homologs fused to GFP, only cells expressing both GFP and tagRFP-T fusions were counted. Data is shown as the percent of total cells counted where fluorescent foci of tagRFP-T fusions were observed and the total number of cells counted is indicated above the columns. Each transfection was performed and counted a minimum of three times and at least 100 cells were counted for each transfection. Data is shown as mean ± S.E.M. (**b-g**) Hsp110 orthologs from human: Hsp105α (**b,c**) and yeast: Sse1p (**d,e**) and Sse2p (**f,g**) were fused to GFP and co-expressed with PFI1155w-tagRFP-T (**b,d,f**) or Sup35PrD-tagRFP-T (**c,e,g**) in HEK293T cells. The cells were fixed and observed by fluorescence microscopy. The arrowheads point to fluorescent foci of tagRFP-T fusion protein, indicating aggregation. Images from left to right are phase, DAPI, RFP, GFP and RFP-GFP merge. Scale bar represents 10 μm. (**h**) Western blot of total cell lysates from HEK293T cells expressing Hsp110 homologs (indicated at top). The Hsp110-GFP fusions were detected by immunoblotting whole cell lysates with anti-GFP antibodies (JL8). After incubation with the transfection reagent for 40 hours, the cells were heat shocked for 6 hours. Hsp40 (Ydj1) was the loading control. (**i**) Western blot of total cell lysates from HEK293T cells expressing Sup35PrD-tagRFP-T alone or with Hsp110 homologs (indicated at top). Sup35PrD-tagRFP-T was detected by immunoblotting whole cell lysates with anti-tagRFP antibodies (top panel) or with anti-Sup35 antibodies (bottom panel). After incubation with the transfection reagent for 40 hours, the cells were heat shocked at 40°C for 6 hours. CyclinB1 was the loading control.



Supplementary Figure S2. Expression of tagRFP-T in HEK293T cells. HEK293T cells were tran transfected with pcDNA3.1 vector that expresses tagRFP-T alone. Prior to fixation, transfected cells were heat shocked at 40°C for 6 hours. The fluorescence of tagRFP-T was uniformly distributed throughout the cytoplasm of transfected cells and foci of tagRFP-T fluorescence were never observed. Images from left to right are phase, DAPI, tagRFP-T, DAPI-tagRFP-T merge and overlay of all images.

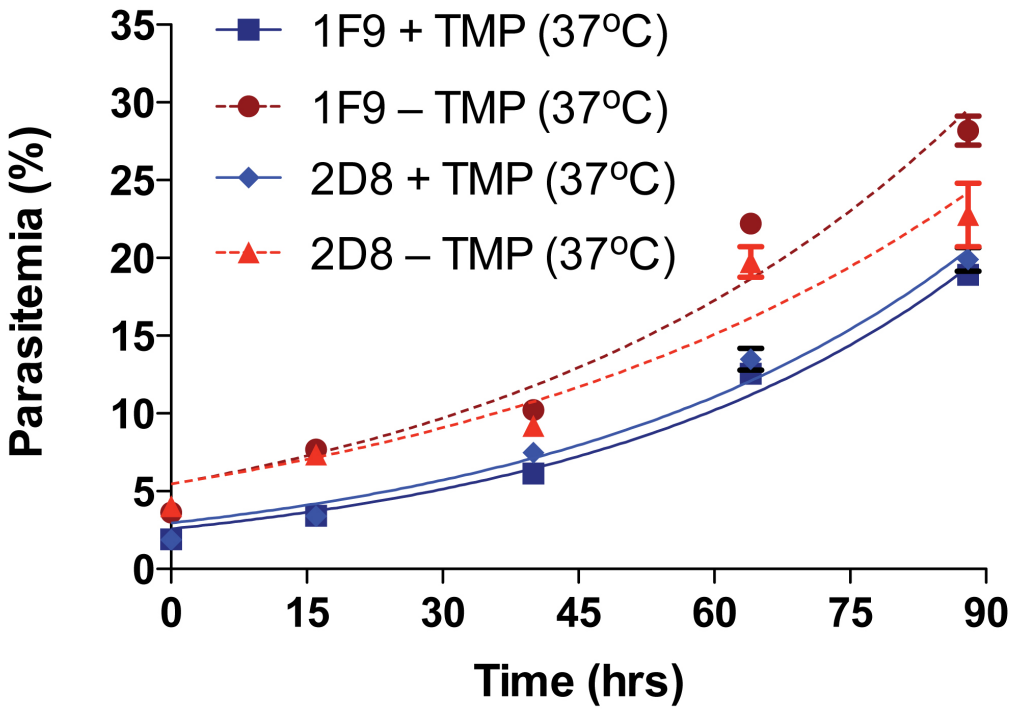


Supplementary Figure S3. Sequence homology of Hsp110. Sequence alignment of the two *Plasm falciparum* Hsp110 proteins (PF07_0033 and MAL13P1.540) with homologs from *Plasmodium vivax* (Pv), *Toxoplasma gondii* (Tg), *Saccharomyces cerevisae* (Sc) and *Homo sapiens* (Hs). The predicted signal peptide and ER-retention signal in the ER-targeted *Pf*Hsp110 are boxed in blue. The alignment was generated using ClustalW and the conserved residues are highlighted in red.

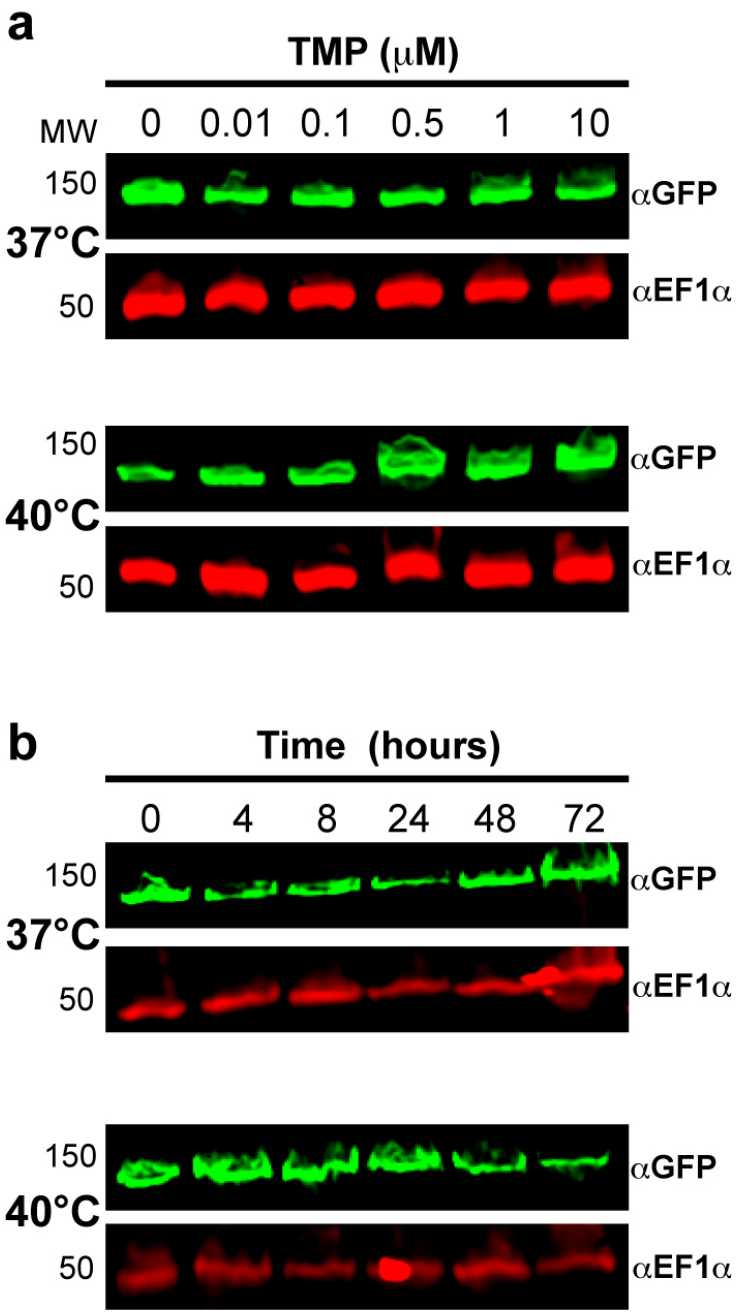


Supplementary Figure S4. Gene duplication in the *Pf*Hsp110c locus. (**a**) Scheme showing the strategy to knock out the *Pf*Hsp110 gene via double crossover homologous recombination. Plasmid (p110-KO) containing a positive drug selection marker, human dihydrofolate reductase (hDHFR) and a negative drug selection marker, thymidine kinase (TK) was transfected into the parent strain, 3D7. EcoRV restriction sites used to detect integration along with the expected sizes are indicated. The probes used for detection in the Southern blot, the genes 5' (PF07_0032, blue bar) and 3' (PF07_0034, red bar) of the *Pf*Hsp110c gene, are indicated(**b**) Southern blot of genomic and plasmid DNA digested with EcoRV and detected with the hDHFR probe. Bands expected from a double crossover integration of the hDHFR drug selection marker into the *Pf*Hsp110c gene were observed in two clones each isolated from the two independent transfection pools (indicated by *). The band expected for the digested plasmid (—) was not seen in any of the clones. (**c**) Southern blot of genomic and plasmid DNA

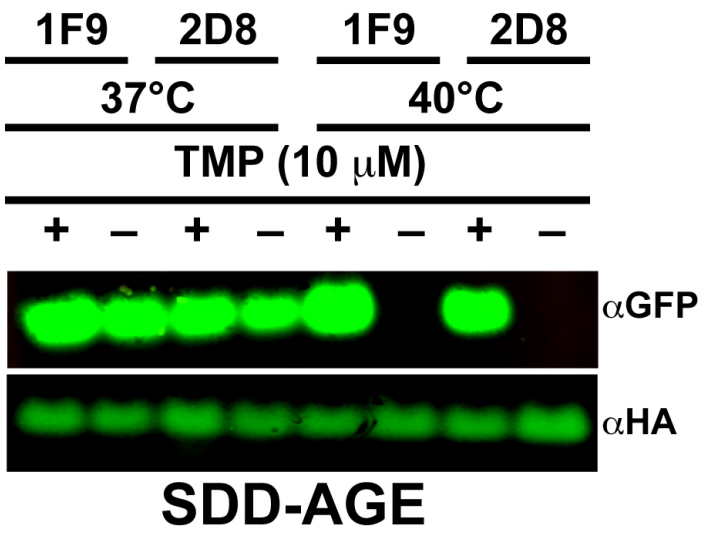
digested with EcoRV. Bands expected from a double crossover integration (indicated by *) into the *Pf*Hsp110c gene were not observed in two clones when probed against the PF07_0034 gene (—). The band expected for the PF07_0032 (—) was seen in all of the clones. If the duplication event had encompassed both genes, then we should have observed two bands, one for the integration copy and the other for the wild type gene, for PF0_0034.



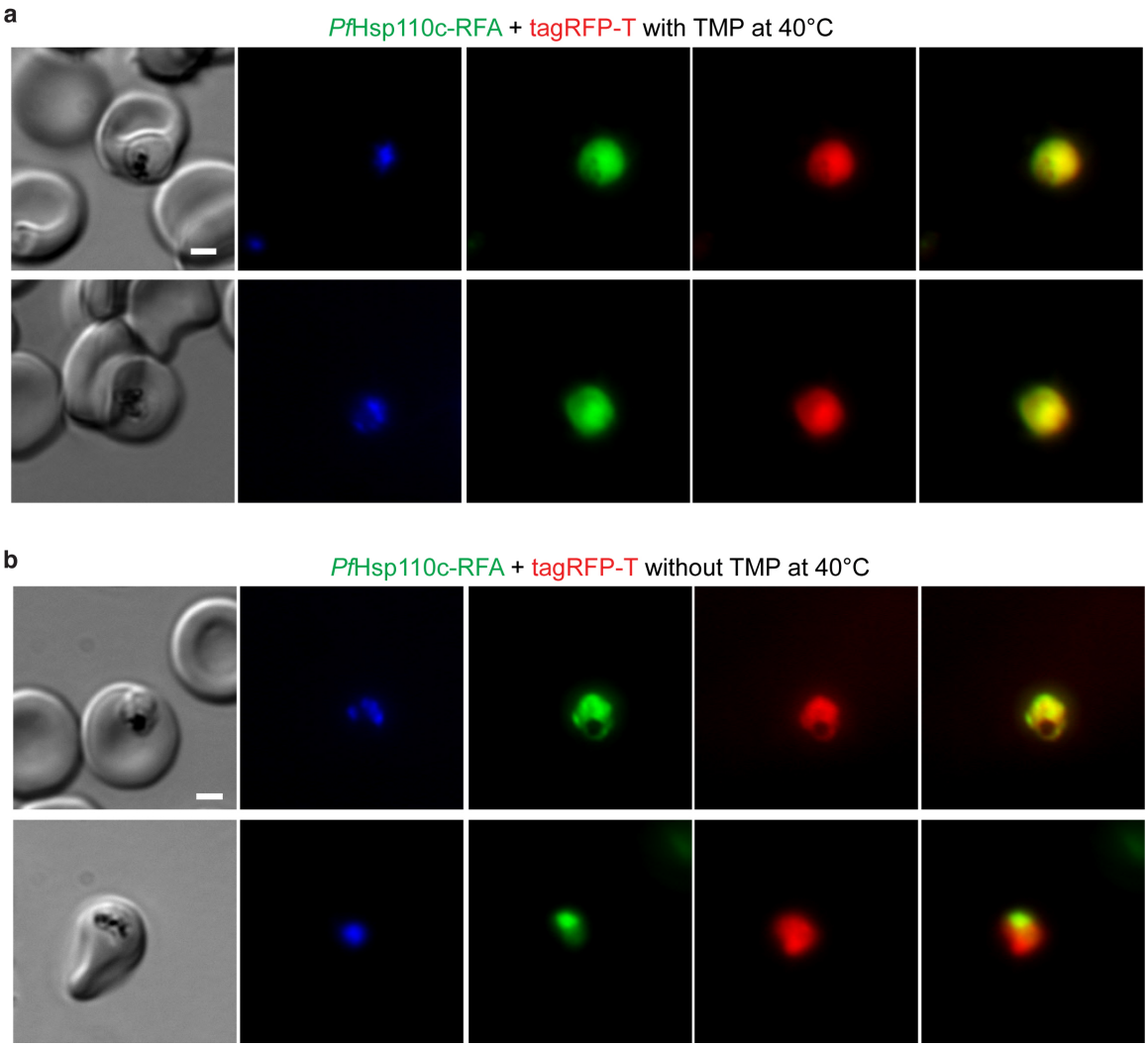
Supplementary Figure S5. *PfHsp110c-RFA* parasite lines briefly incubated with or without TMP at Asynchronous *PfHsp110c-RFA* parasite lines, 1F9 and 2D8, were incubated at 37°C for 6 hours with or without 10μM TMP. All parasites were transferred to fresh medium with 10 μM TMP and their growth was monitored over 5 days via flow cytometry. Data were fit to exponential growth curve equation and are represented as mean ± S.E.M. (n=3).



Supplementary Figure S6. *Pf*Hsp110c-RFA is not degraded in the absence of TMP**.** (**a, b**) *PfHsp110* parasites were incubated at 37°C or 40°C in different TMP concentrations for 24 hours (**a**) or without TMP for varying times (**b**). Degradation of *Pf*Hsp110c-RFA was monitored by immunoblotting parasite lysates (using monoclonal αGFP, JL8) after SDS-PAGE. EF1α is the loading control.



Supplementary Figure S7. SDD-AGE fractionated lysates of *Pf*Hsp110c-RFA parasites, incubated at 3
40°C with or without 10 μM TMP for 6 hours, were probed with JL8 monoclonal anti-GFP antibody as well as with anti-HA antibody since the RFA-tag has a HA sequence at the C-terminus. JL8 was unable to recognize its epitope within *Pf*Hsp110c-RFA in SDD-AGE-separated lysates of parasites that had been incubated at 40°C for 6 hours without TMP, even though the same antibody recognized the protein on denaturing SDS-PAGE (**Fig. 4a-c and Fig. S6**). The anti-HA antibody, however, was able to recognize *Pf*Hsp110c-RFA in all samples. In all cases, both 1F9 and 2D8 *PfHsp110-RFA* parasite clones behaved similarly. Thus, two monoclonal antibodies, 3E6 (**Fig. 4c**) and JL8 were unable to recognize GFP in destabilized *Pf*Hsp110-RFA unless the protein was fully denatured.



Supplementary Figure S8. Expression of tagRFP-T in *PfHsp110c-RFA* parasites. Live fluorescent ima *PfHsp110c-RFA* parasites expressing tagRFP-T under the control of the *hsp86* promoter. The parasites were heat shocked either with (**a**) or without (**b**) 10 μM TMP. Aggregation of the fluorescent tagRFP-T alone was never observed. Scale bar represents 2 μm. Images from left to right are phase, DAPI, GFP, RFP and GFP-RFP merge.

Supplementary Table S1. Primers used to generate constructs utilized in this study.

Primer Name	Sequence (5' to 3')	Restriction Site	PCR Purpose	Comments
5'Sac2-5'cg4KOinF	caatggccctttccgcggatgtcggtttaggtatagatataggaaatgacaattctgttgtagc	SacII	Forward primer for 5' homologous region of *Pf*Hsp110c	Used in making p110-KO-TK
3'Bgl2-5'cg4KOinF	tttccttataagatctccttgacaatctaaatcttcatataaacattcaacatgtatggacgc	BglII	Reverse primer for 5' homologous region of *Pf*Hsp110c	Used in making p110-KO-TK
5'Cla1-3'cg4KOinF	ctagaattcatatcgatcggaggataagggagaaaaaaaagatgcaaaagatcaagaac	ClaI	Forward primer for 3' homologous region of *Pf*Hsp110c	Used in making p110-KO-TK
3'Nco1-3'cg4KOinF	cctaggagttccatggttattcctctggattagctgaattttcatttttgttgagttctcc	NcoI	Reverse primer for 3' homologous region of *Pf*Hsp110c	Used in making p110-KO-TK
5'cg4-GFP-xho1	gcgcctcgagggtgcacagacagtaaccaagtctgttattaagtcc	XhoI	Forward primer for 1kb homologous region in the 3' end of *Pf*Hsp110c ORF	Used in making p110-GDB
3'cg4-GFP-avr2	gcgccctaggttcctctggattagctgaattttcatttttgttgag	AvrII	Reverse primer for 1kb homologous region in the 3' end of *Pf*Hsp110c ORF	Used in making p110-GDB
5'Avr2-tRT-inF	gaccggtatgcctaggatggtgtctaagggcgaagagctgattaaggagaacatgc	AvrII	Forward primer for tagRFP-T	Used in making episomal tagRFP-T parasite vectors
5' Xho1-tRT-inF	tttggaaaagctcgagatggtgtctaagggcgaagagctgattaaggagaacatgc	XhoI	Forward primer for tagRFP-T	Used in making episomal tagRFP-T alone parasite vector
3'Eag1-tRT-inF	taactcgacgcggccgttatttgtcgtcgtcgtctttgtagtctttgtcgtcgtcgtcttgtagtccttgtacagctcgtccatgccattaagtttgtgcc	EagI	Reverse primer for tagRFP-T	Used in making episomal tagRFP-T parasite vectors
5'Aat2-110prom-inF	gaaaagtgccacctgacgtccaacaataagttcttaataaggatatcaaataaatttcatgtatatatatcaatcc	AatII	Forward primer for *Pf*Hsp110c promoter	Used in making episomal parasite vectors with *Pf*Hsp110c promoter
3'Xho1-110prom-inF	gcataccggtctcgagcttttccaaaaatttgatatatatatatatataaatttatatatatg	XhoI	Reverse primer for *Pf*Hsp110c promoter	Used in making episomal parasite vectors with *Pf*Hsp110c promoter
5'Xho1-Cg4all-inF	acgatttttttctcgagatgtcggttttaggtatagatataggaaatgacaattctgttgtagc	XhoI	Forward primer for *Pf*Hsp110c ORF	Used in making episomal *Pf*Hsp110c ORF parasite vector
3'Avr2-Cg4all-inF	ctgcacctggcctaggttcctctggattagctgaattttcatttttgttgagttctcc	AvrII	Reverse primer for *Pf*Hsp110c ORF	Used in making episomal *Pf*Hsp110c ORF parasite vector
5'Xho1-Sse1p/2p-inF	tttggaaaagctcgagatgagtactccatttggtttagatttaggtaacaataactc	XhoI	Forward primer for Sse1p and Sse2p ORF	Used in making episomal Sse1p and Sse2p ORF parasite vector
3'Avr2-Sse1p-inF	tagacaccatcctagggtccatgtcaacatcaccttcagtgtccttcttttcttcc	AvrII	Reverse primer for Sse1p ORF	Used in making episomal Sse1p ORF parasite vector

3'Avr2-Sse2p-inF	tagacaccatcctaggatcaaggtccatgttttcatcattgttgtcatcgc	XhoI	Reverse primer for Sse2p ORF	Used in making episomal Sse2p ORF parasite vector
5'Xho1-mHsp05-inF	tttggaaaagctcgagatgtcggtggtggggttggacgtgggctcgcagagc	XhoI	Forward primer for Hsp105α ORF	Used in making episomal Hsp105α ORF parasite vector
3'Avr2-mHsp105-inF	tagacaccatcctagggtccaagtccatattaacagaatttttctcattaggg	AvrII	Reverse primer for Hsp105α ORF	Used in making episomal Hsp105α ORF parasite vector
5'Xho1-PFI1155w-inF	acgatttttttctcgagatgaatgatcaaaaacgggcatcattaaacctatc	XhoI	Forward primer for PFI1155w	Used in making episomal parasite vector expressing PFI1155w
3'Avr2-PFI1155w-inF	ctgcacctggcctaggatgatttttttgcatatgaactgccattcttaatttatcctc	AvrII	Reverse primer for PFI1155w	Used in making episomal parasite vector expressing PFI1155w
5'Xho1-Sup35PrD-inF	acgatttttttctcgagatgtcggattcaaaccaaggcaacaatcagc	XhoI	Forward primer for Sup35PrD	Used in making episomal parasite vector expressing Sup35PrD
3'Avr2-Sup35PrD-inF	tagacaccatcctaggagacatacctttgagactgtggttggaaacc	AvrII	Reverse primer for Sup35PrD	Used in making episomal parasite vector expressing Sup35PrD
5'Hind-Sup35PrD-inF	agggagacccaagcttatgtcggattcaaaccaaggcaacaatcagc	HindIII	Forward primer for Sup35PrD	Used for making pcDNA3.1 mammalian vector expressing Sup35PrD-tagRFP-T
3'Bam-Sup35PrD-inF	agacaccatcggatcccagacataccttgagactgtggttggaaacc	BamHI	Reverse primer for Sup35PrD	Used for making pcDNA3.1 mammalian vector expressing Sup35PrD-tagRFP-T
5'Hind-83N-pcDNA	gtttaaacttaagcttatgaatgatcaaaaacgggcatcattaaacc	HindIII	Forward primer for PFI1155w	Used for making pcDNA3.1 mammalian vector expressing PFI1155w-tagRFP-T
3'Bam-83N-pcDNA	agacaccatcggatccccatgatttttttgcatatgaactgccattcttaatttatcc	BamHI	Reverse primer for PFI1155w	Used for making pcDNA3.1mammalian vector expressing PFI1155w-tagRFP-T
5'Not-pLex-Cg4GFP	ctcatcgatgcggccgcatgtcggtttaggtatagatataggaaatgacaattctg	NotI	Forward primer for *Pf*Hsp110c	Used for making pLexm mammalian vector expressing *Pf*Hsp110c-GFP
3'Xho-pLex-Cg4GFP	cgatactagtctcgagttatttttgtatagttcatccatgccatgtgtaatcccagc	XhoI	Reverse primer for *Pf*Hsp110c-GFP	Used for making pLexm mammalian vector expressing *Pf*Hsp110c-GFP
5'Not-mHsp105-pLex-inF	ctcatcgatgcggccgcatgtcggtggtggggttggacgtgggctcgcagagctgc	NotI	Forward primer for Hsp105α	Used for making pLexm mammalian vector expressing Hsp105α-GFP
3'Avr-mHsp105-pLex-inF	ctgcacctggcctagggtccaagtccatattaacagaatttttctcattaggg	AvrII	Reverse primer for Hsp105α	Used for making pLexm mammalian vector

				expressing Hsp105α-GFP
5'Not-Sse1-pLex-inF	ctcatcgatgcggccgcatgagtactccatttggtttagatttagg	NotI	Forward primer for Sse1p	Used for making pLexm mammalian vector expressing Sse1p-GFP
3'Avr-Sse1-pLex-inF	ctgcacctggcctagggtccatgtcaacatcaccttcagtgtcc	AvrII	Reverse primer for Sse1p	Used for making pLexm mammalian vector expressing Sse1p-GFP
5'Not-Sse2-pLex-inF	ctcatcgatgcggccgcatgagcactccatttggcttagatttagg	NotI	Forward primer for Sse2p	Used for making pLexm mammalian vector expressing Sse2p-GFP
3'Avr-Sse2-pLex-inF	ctgcacctggcctaggatcaaggtccatgttttcatcattgttgtcatcgc	AvrII	Reverse primer for Sse2p	Used for making pLexm mammalian vector expressing Sse2p-GFP