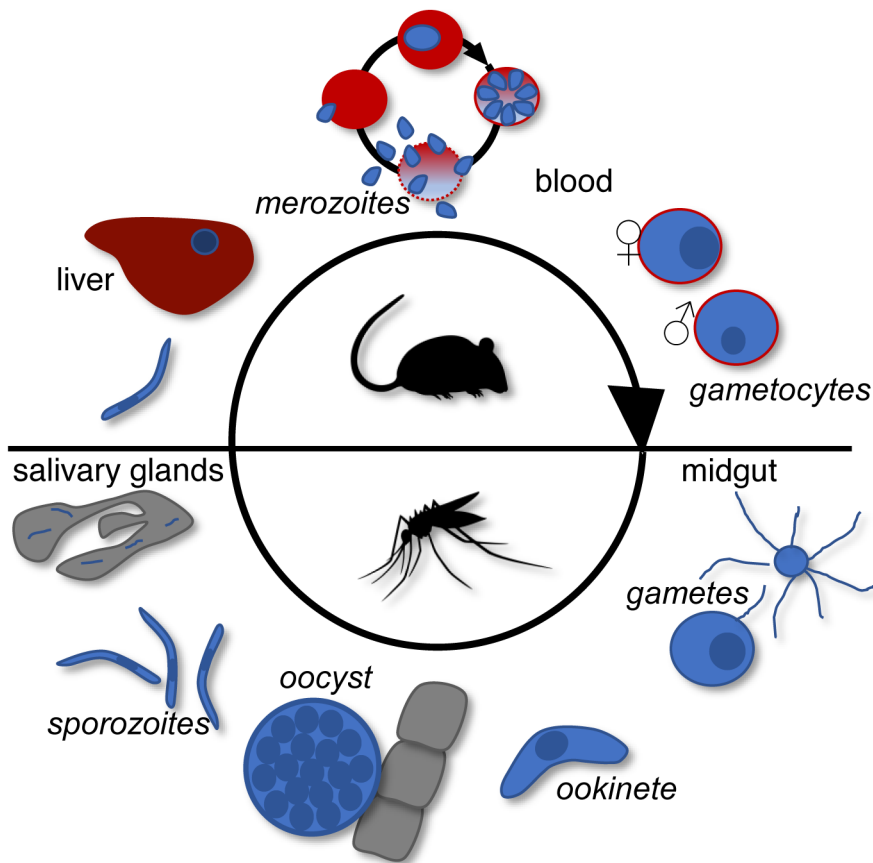


Expanded View Figures

Figure EV1. Life cycle of malaria parasites in mouse and mosquito.

Parasites are depicted blue, mosquito tissues grey, liver dark red, and red blood cells red.



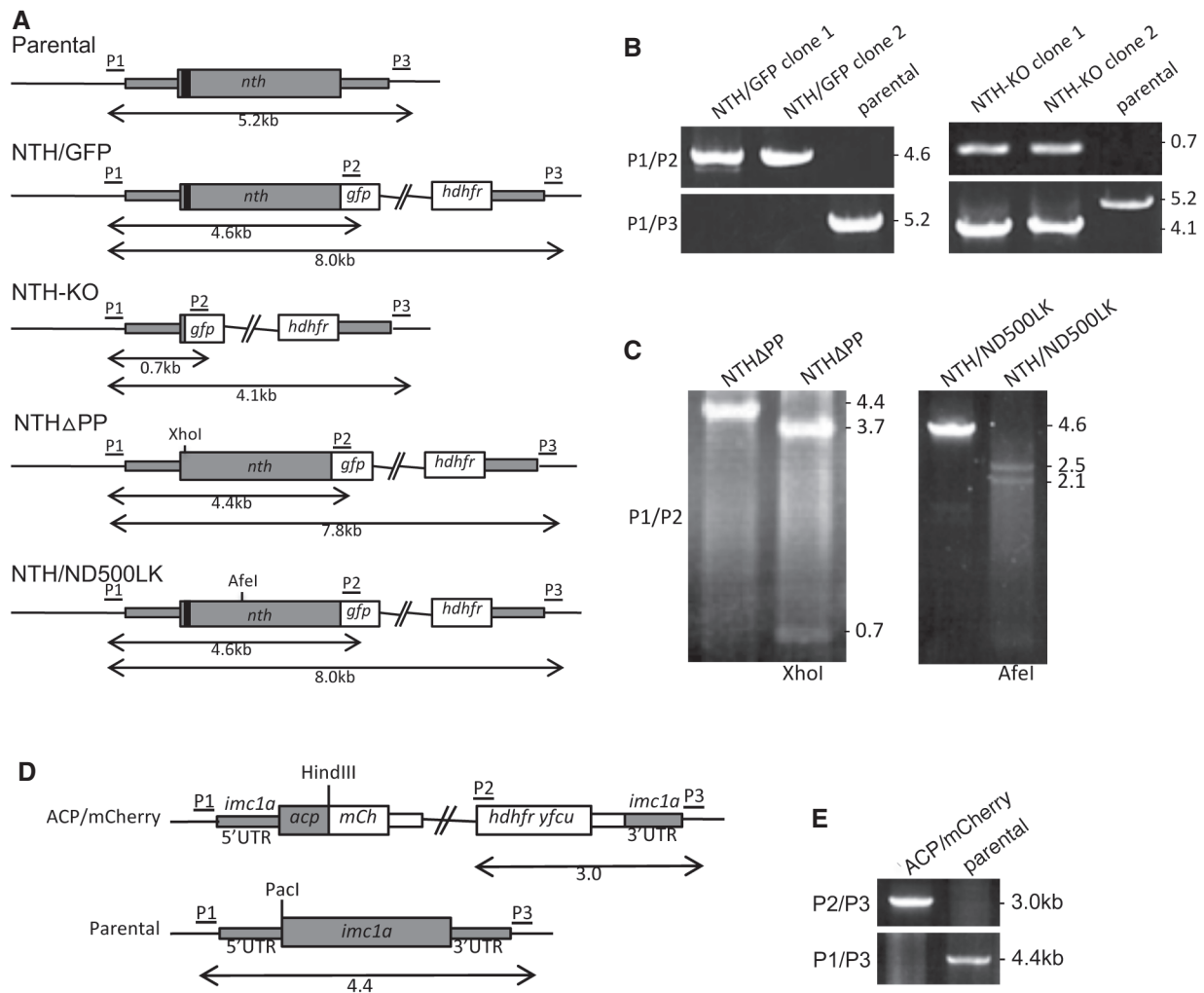


Figure EV2. Generation of genetically modified parasite lines.

- A** Schematic diagram of the unmodified (parental) and modified *nth* alleles in parasite lines NTH/GFP, NTH-KO, NTH Δ PP, and NTH/ND500LK. The *nth* gene is indicated with coding sequence (wide grey bars, introns not shown) and 5' and 3' untranslated regions (UTRs; narrow grey bars). Also indicated are the relative positions of the putative apicoplast targeting signal (black box), GFP module (*gfp*), the human DHFR selectable marker gene cassette (*hdhfr*); the XhoI and AfeI diagnostic restriction sites; and primers used for diagnostic PCR amplification (P1–P4). Sizes of PCR products are also indicated.
- B** Diagnostic PCR of parasite lines NTH/GFP and NTH-KO. Integration of the modified GFP-tagged alleles into the target locus across the 5' integration site was carried out with primers P1 (ATATTTCCCCTAATTTCCCTT) and P2 (GTGCCCATTAACATCACC), amplifying expected products of ~ 4.6 kb in NTH/GFP and 0.7 kb in NTH-KO. Absence of the unmodified *nth* allele was carried out with primers P1 (ATATTTCCCCTAATTTCCCTT) and P3 (ATTCAACTACTCGAATTTATGTTATCG), amplifying expected products of ~ 5.2 kb from the parental parasite and 4.1 kb in NTH-KO parasites.
- C** Diagnostic PCR and restriction enzyme digests of parasite lines NTH Δ PP and NTH/ND500LK. Primers P1 and P2 amplify a 4.4 kb product in NTH Δ PP that is digested by XhoI into the expected two fragments of ~ 3.7 kb and 0.7 kb, confirming presence of the mutation. Primers P1 and P2 amplify a 4.6 kb product in NTH/ND500LK that is digested by AfeI into the expected two fragments of ~ 2.5 kb and 2.1 kb, confirming presence of the mutation.
- D** Schematic diagram of the modified *acp* allele (PBANKA_0305600) in parasite line ACP/mCherry. The *acp* gene is indicated with coding sequence (wide grey bars, introns not shown) and *imc1a* 5' and 3' untranslated regions (UTRs; narrow grey bars). Also indicated are the relative positions of the mCherry module (*mCh*); the human DHFR::yFCU selectable marker gene cassette (*hdhfr yfcu*); and primers used for diagnostic PCR amplification (P1–P3). Sizes of PCR products are also indicated.
- E** Diagnostic PCR for integration into the target *imc1a* locus with primers P2 and P3, giving rise to a 3.0 kb product (top panel). Diagnostic PCR with primer pair P1 and P3 amplified an ~ 4.4 kb fragment from the parental (wild-type) parasites, and no product in the ACP/mCherry parasites, confirming absence of the unmodified *imc1a* allele in the clonal transgenic parasite line (bottom panel).

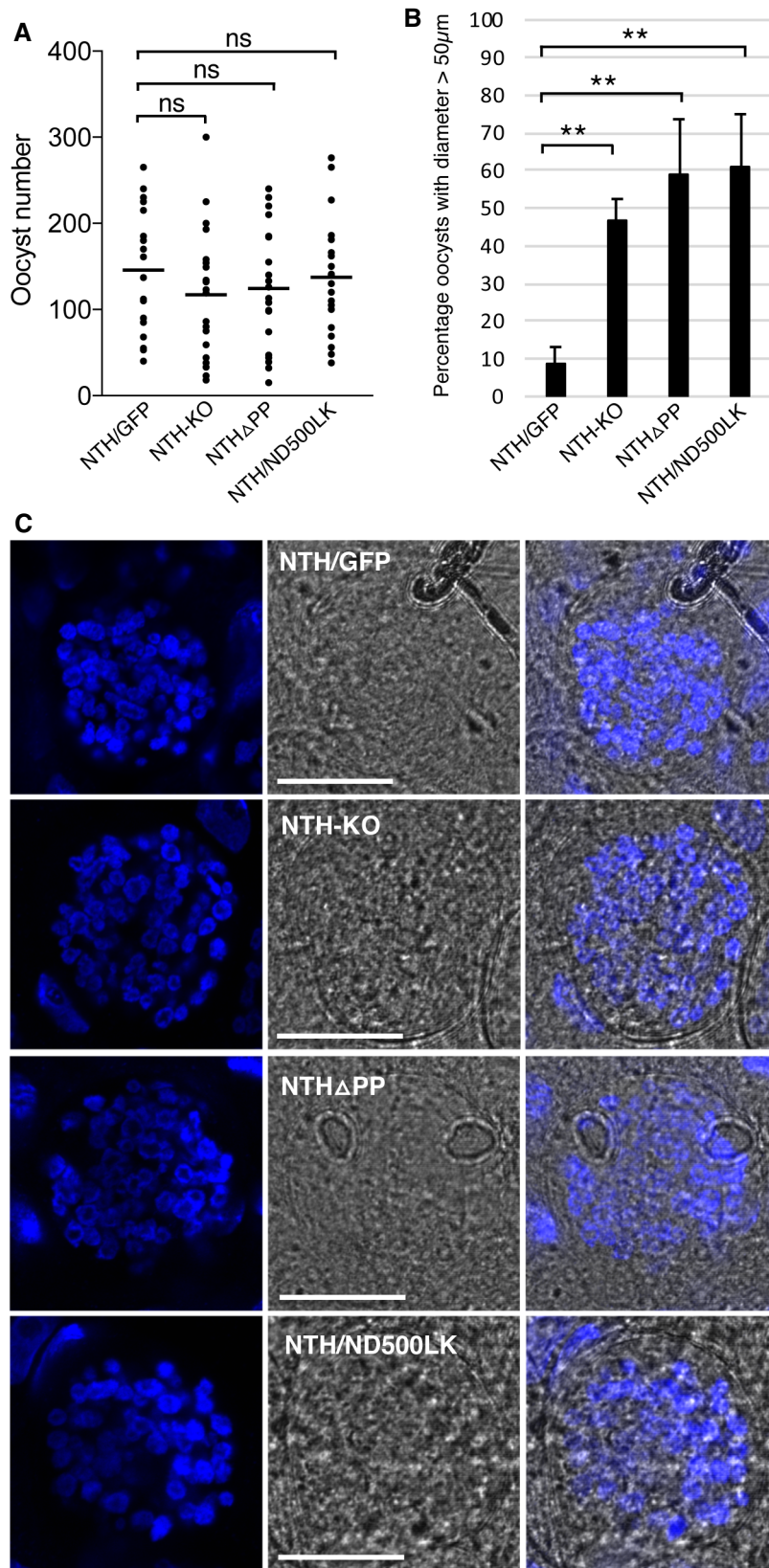


Figure EV3. Oocyst development in *Anopheles stephensi* infected with parasite lines NTH/GFP, NTH-KO, NTH Δ PP, and NTH/ND500LK.

- A** Aligned dot plot of oocyst numbers at 10 days post-infection. Horizontal lines mark mean values ($n = 20$); ns, not significantly different (Mann–Whitney).
- B** Bar chart showing percentage of oocyst with a diameter > 50 μ m at 15 days post-infection. Diameters were scored at 400 \times magnification using an eyepiece micrometre. Five infected mosquitoes and 100 oocysts per mosquito were sampled for each parasite line. Error bars show standard deviations ($n = 5$). ** mark statistically significant differences ($P < 0.001$, Mann–Whitney); ns, not significant.
- C** Brightfield and fluorescence images of oocysts pre-sporulation 11 days post-infection, stained with Hoechst DNA stain (blue), showing similar developmental progression between NTH/GFP and mutants. Scale bar = 20 μ m.

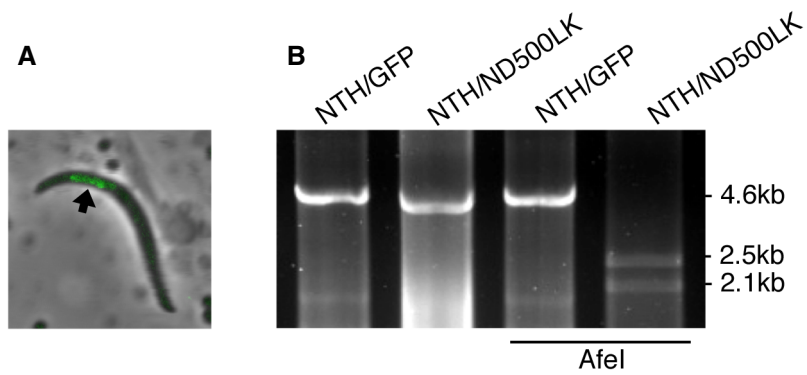


Figure EV4. NTH/ND500LK sporozoites can initiate intraerythrocytic infection in mice.

- A Merged brightfield and fluorescence image of a sporozoite of parasite line NTH/ND500LK showing localisation of NTH::GFP in the apicoplast (arrow).
- B PCR diagnostic for the presence of modified *nth* alleles in blood-stage infections obtained after sporozoite transmission of NTH/ND500LK or NTH/GFP sporozoites, amplifying an ~ 4.6 kb fragment. The NTH/ND500LK fragment contains an AfeI restriction site, diagnostic of the point mutation introduced into its *nth* allele (see also Fig EV2).