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**IMC1b IS A PUTATIVE MEMBRANE SKELETON PROTEIN INVOLVED IN CELL SHAPE, MECHANICAL STRENGTH, MOTILITY AND INFECTIVITY OF MALARIA OOKINETES**

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**SUPPLEMENTARY MATERIAL**

**Fig. S1.** A genetic tool for generating genetically modified parasites expressing GFP-tagged IMC1b. Step 1: the *imc1b* coding sequence plus its 5'UTR is cloned upstream of, and in-frame with, *egfp* in plasmid pDNR-EGFP. Step 2: 3'UTR of *imc1b* is cloned into plasmid pLP-DHFR2. Step 3: The *imc1b*-specific sequences of the above plasmids are combined with the selectable marker cassette in plasmid pLP-IMC1b/EGFP by Cre-*loxP* recombination. *LoxP* sites are indicated by black arrows; noncoding sequences are indicated in white; coding sequences are indicated in light gray; *imc1b*-specific sequences are indicated in dark gray; Amp r: ampicillin resistance gene; Cm r: chloramphenicol resistance gene; SacB: sucrase gene from *Bacillus subtilis*; egfp: enhanced green fluorescent protein; UTR: untranslated region; pro: bacterial promoter sequence.

**Fig. S2.** Generation and molecular analysis of genetically modified parasite lines IMC1b/GFP (GFP) and IMC1b-KO (KO). A: Schematic diagram of wild-type (WT) and genetically modified *imc1b* loci on genomic DNA. Indicated are positions of the *HindIII* restriction sites (H), and expected *HindIII* restriction fragments (horizontal arrows) with sizes shown in kb. The sequences of the probes are indicated by thick lines. B: Southern blot analysis of *HindIII*-digested parasite genomic DNA. C: Reverse transcription-PCR analysis of ookinete samples. D: Western blot analysis of ookinete samples using anti-GFP antibodies. Apparent sizes of the bands are shown in kDa.
Fig. S1

1. pDONR-EGFP (6053 bp)

2. pLP-DHFR2 (7441 bp)

3. pLP-IMC1b/EGFP (12478 bp)
Fig. S2

A

WT

GFP

KO

WT

KO

GFP

imc1b probe
tgdhfr probe

B

WT GFP KO WT GFP KO

imc1b probe tgdhfr probe

C

WT KO

imc1b

WTKO

imc1b

D

GFP KO WT

-100 -30