IMC1b IS A PUTATIVE MEMBRANE SKELETON PROTEIN INVOLVED IN CELL SHAPE, MECHANICAL STRENGTH, MOTILITY AND INFECTIVITY OF MALARIA OOKINETES Annie Z Tremp¹, Emad I Khater², Johannes T Dessens¹

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. Step2: 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 subtilus*; 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 *Hind*III restriction sites (H), and expected *Hind*III restriction fragments (horizontal arrows) with sizes shown in kb. The sequences of the probes are indicated by thick lines. **B**: Southern blot analysis of *Hind*III-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.







