Tremp, AZ; Khater, EI; Dessens, JT; (2008) IMC1b is a putative membrane skeleton protein involved in cell shape, mechanical strength, motility and infectivity of malaria ookinetes. The Journal of biological chemistry, 283 (41). pp. 27604-11. ISSN 0021-9258 DOI: https://doi.org/10.1074/jbc.M801302200

Downloaded from: http://researchonline.lshtm.ac.uk/7347/

DOI: https://doi.org/10.1074/jbc.M801302200

Usage Guidelines:

Please refer to usage guidelines at https://researchonline.lshtm.ac.uk/policies.html or alternatively contact researchonline@lshtm.ac.uk.

Available under license: http://creativecommons.org/licenses/by-nc-nd/2.5/
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 (6063 bp)
2. pLP-DHFR (7441 bp)
3. pDONR-IMC1b/EGFP (8220 bp)
4. pLP-DHFR/IMC1b (6182 bp)
5. pLP-IMC1b/EGFP (12478 bp)
Fig. S2

A

WT

GFP

KO

B

WT  GFP  KO  WT  GFP  KO

imc1b probe  tgdhfr probe

C

WT  KO

imc1b  tubulin

D

GFP  KO  WT

100  30