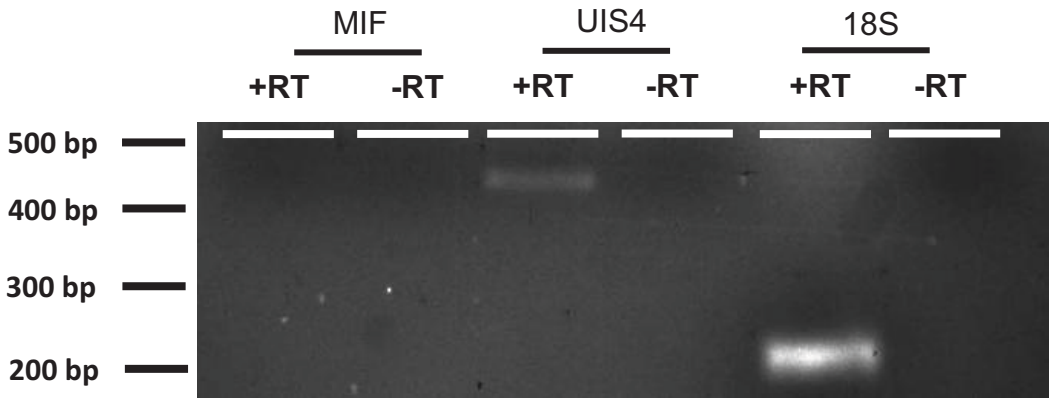
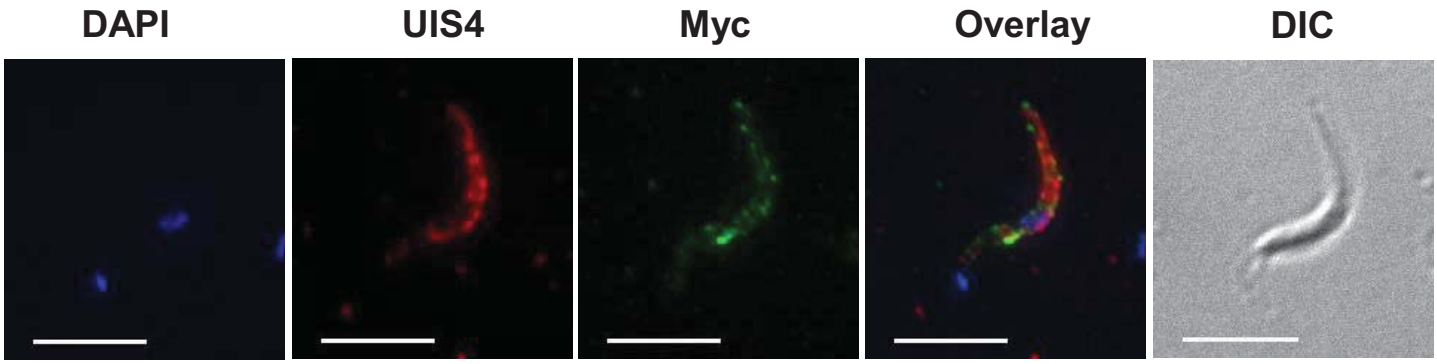
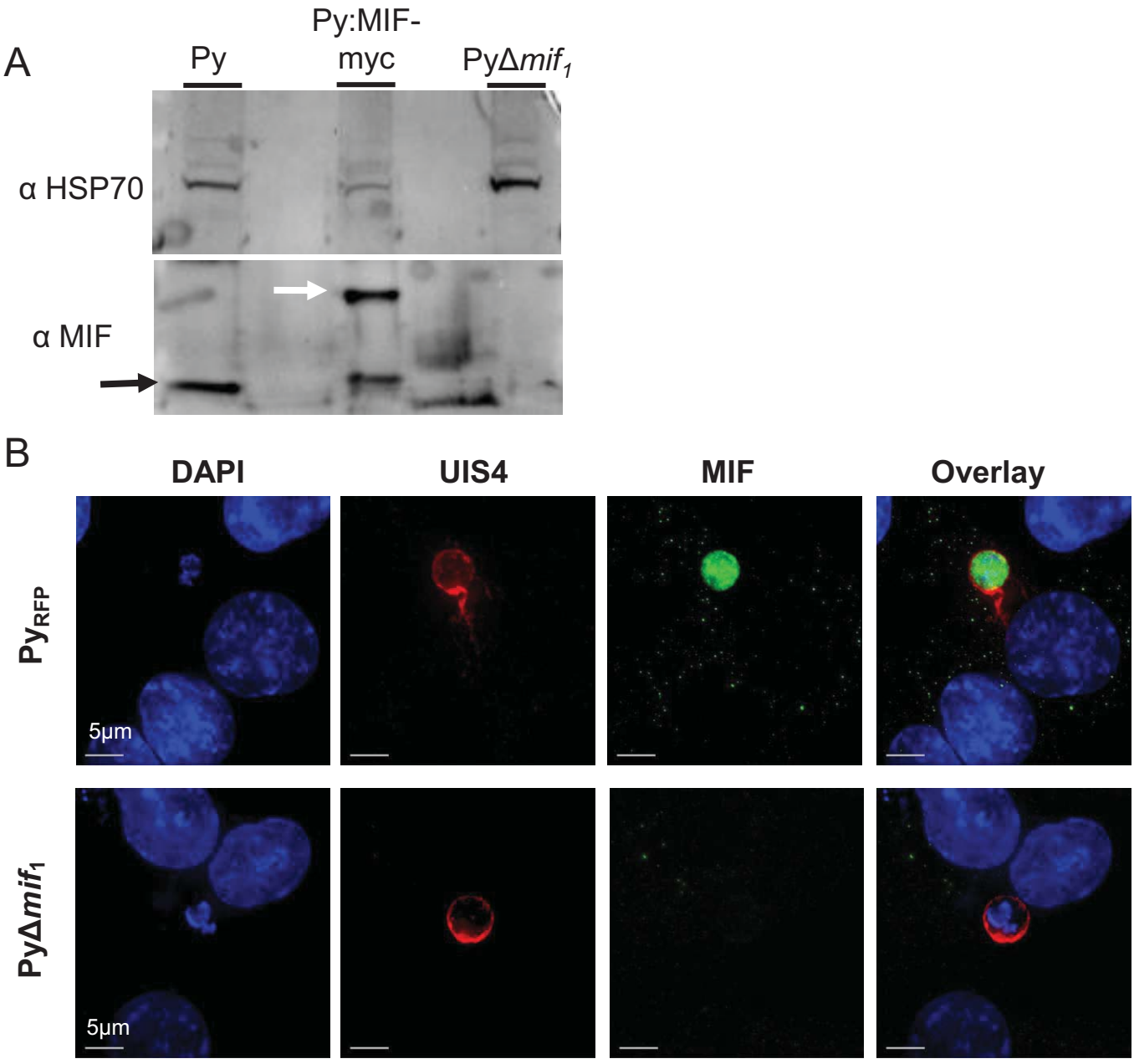


Supplementary Figure 1 *Py-mif* expression in Sporozoites. Py-*mif* transcripts were not detected in sporozoites after 45 amplification cycles. Expression of the upregulated in infectious sporozoite gene 4 (UIS4) and *18S* ribosomal RNA were used as positive controls.



Supplementary Figure 2. Localization of Py-MIF-myc *in vitro*. (A) *P. yoelii* sporozoites expressing a MIF-myc fusion protein were isolated from salivary glands of *Anopheles stephensi* mosquitoes and stained with polyclonal mouse antibody to Myc (green) and UIS4(red). DAPI (blue) was used to stain the parasite nucleus.



Supplementary Figure 3. Confirmation of Py*Δmif*. (A) Western blot of blood stage lysates from Py, an uncloned population of MIF-myc expressing Py (Py:MIF-myc), and the *mif* deletion clone 1 (PyΔmif_1). Blots were probed with either the anti-MIF antibody or an antibody against HSP70 (loading control). MIF-myc sample contains both endogenous MIF (black arrow) and the myc-tagged version (white arrow). (B) IFA on 20hr in vitro liver stages (HepG2 cells) from control (Py_{RFP}) or *mif* deficient parasites (PyΔmif_1). Parasites were stained with polyclonal mouse antibody to MIF (green) and UIS4 (red). DAPI (blue) was used to stain nuclei of the parasite and host cell.

TABLE S1. Description of primers utilized.

Primers for generating and genotyping PyΔmif	
Generation of PyΔmif construct [a, b]	
ccgcggTATCCAAAATATTTATTCCTATAGC	PyMIF-1 SacII
ccataatgtctcagggcccTCTCCATTTTCTATCTCTTCATTTT	PyMIF-2 ApaI
gatagaaaatggagagggcccTGAGACATTATGGTTCTTTCCTTTT	PyMIF-3 ApaI
gcggccgcGTTAAAACAATTCGAGAGATGAAAA	PyMIF-4 NotI
Mif ORF test	
ATGCCTTGCTGCGAATTAATAACAA	Sp/PyMIF
GCCAAATAGTGAACCACTAAAAGCA	Asp/PyMIF
PyΔmif 5' intergration test	
ATGAAAGTGAGGAGATAGACA	PyMIFtestF
TAATACGACTCACTATAGGG	T7F
PyΔmif 3' intergration test	
ATAGGTTTCATTATGCCATG	PyMIFtestR
GGCTACGTCCCGCACGGACGAATCCAGA	TgR
Generation of Py:mif-myc parasites	
ccgcggATGAATTATTTAAAATTGGTTAC	PyMIFmycF SacII
tctagaGCCAAATAGTGAACCACTAAAAGC	PyMIFmycRXbaI
Primers for RT- and QPCR	
Py-mif amplification	
ATGCCTTGCTGCGAATTAATAACAA	Sp/PyMIF
GCCAAATAGTGAACCACTAAAAGCA	Asp/PyMIF
Parasite 18S rRNA amplification	
GGGGATTGGTTTTGACGTTTTTGCG	18s F
AAGCATTAAATAAAGCGAATACATCCTTAT	18s R
Murine gapdh amplification	
CCTCAACTACATGGTTTACAT	gapdh F
GCTCCTGGAAGATGGTGATG	gapdh R

[a] Restriction enzyme sites are lowercase and underlined

[b] Over lapping sequences for SOE PCR are lowercase

Supplemental Methods:

Generation of Py:mif-myc parasites

As previously reported (48), we have generated a b3D-myc vector for epitope tagging genes of interest. This was made by integrating a quadruple myc tag sequence followed by a stop codon into the b3D.DT^H.^D vector (Catalog # MRA-80 in the MR4 Malaria Research and Reference Reagent Resource Center; http://www.mr4.org). To generate the *P. yoelii* mif-myc parasite line, approximately 700 bp of upstream sequence and the entire coding sequence of the *mif* gene (except for its stop codon) was amplified by PCR from *P. yoelii* 17XNL genomic DNA. This PCR product was cloned upstream in-frame with the quadruple myc tag in b3D_myc into the *SacII* and *XbaI* restriction sites (Table S1 for primers). The plasmid was subsequently linearized with *BstBI* and integrated into the *P. yoelii* 17XNL genome using standard procedures (28). This integration strategy created two functional copies of the *mif* gene, which were both under the control of the endogenous promoter. This parasite line was not cloned.

Immunofluoresence microscopy

Wild type Py or mif-myc sporozoites and liver stages were isolated and prepared as described in the manuscript. These samples were subsequently stained with a polyclonal UIS-4 antibody as previously described or with a monoclonal mouse anti-myc antibody at 1:500 dilution (Invitrogen, product # 13-2500). Samples were stained with secondary antibodies and visualized as described in the materials and methods.

Immunoblot

Parasites from approximately 1ml of blood from mice infected with Py, parasite strains were lysed using the

Western blot

Blood stage parasites from P. yoelii and PyΔ*mif*, or Mif-myc recombinant parasites were lysed in Mammalian Protein Extraction Reagent (M-PER (Thermo, Rockford, IL)) and the total protein concentration was measured at OD280 on a Nanodrop spectrophotometer (Thermo, Rockford, IL). One hundred ng of protein was boiled in SDS PAGE buffer containing 5% w/v beta mercaptoethanol for 10 min and was spun at 13,000 rpm for 1 min. The soluble fraction was then separated on a SDS-PAGE 4-20% gel (Thermo, Rockford, IL) and was transferred to a PVDF membrane. *P. yoelii* and PyΔ*mif*, or Mif-myc were labeled with Mif antisera and/or mouse monoclonal anti-myc antibody followed by goat anti-rabbit IgG IRDye 680 or goat anti-mouse IgG IRDye 800 (Ly-Core, Lincon, NE). Protein bands were detected by scanning the membrane on an Odyssey imaging system (LI-COR Biotechnology, Lincoln, NE).