A *Plasmodium* Mei2-like RNA Binding Protein is essential for completion of liver stage schizogony

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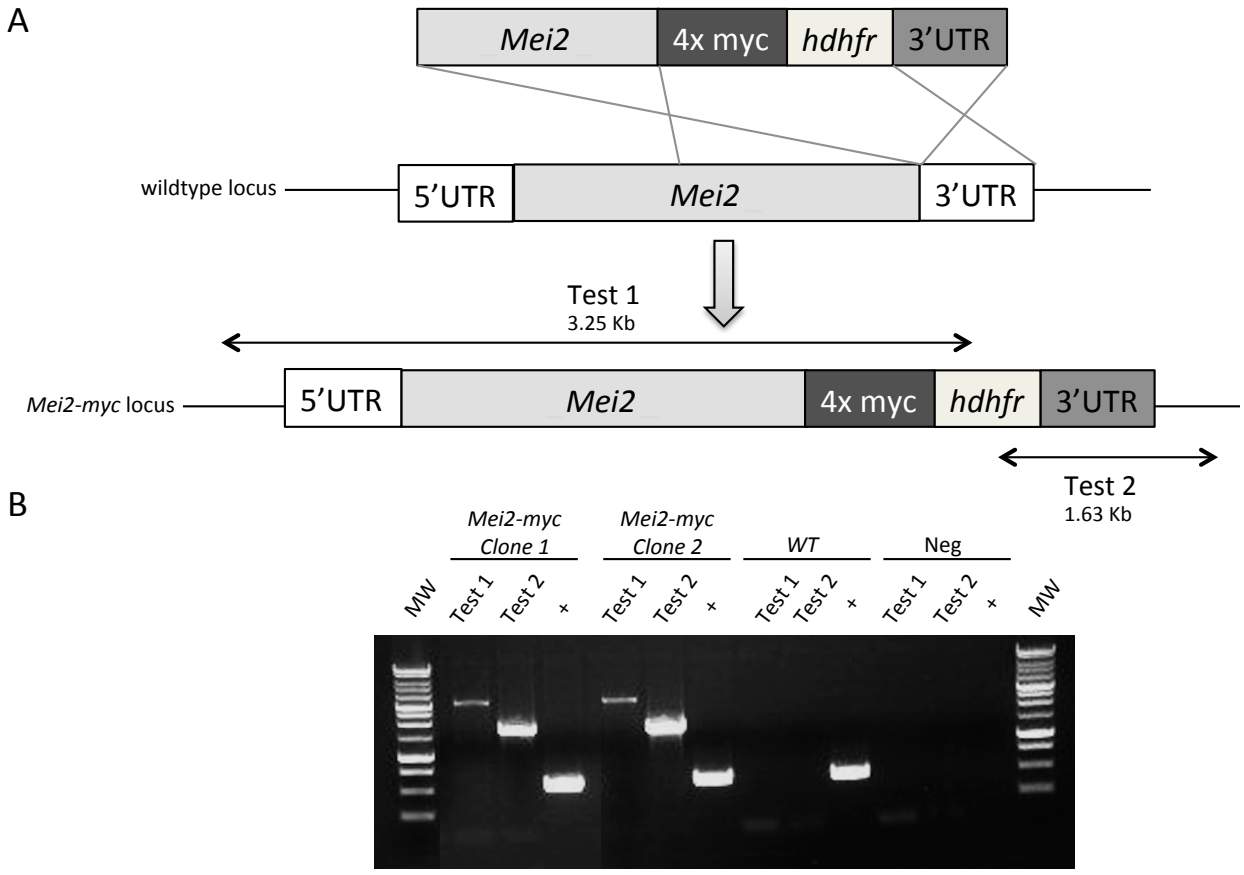
Supplementary Tables and Figures

Supplementary Table 1. Oligonucleotide primers used in this study.

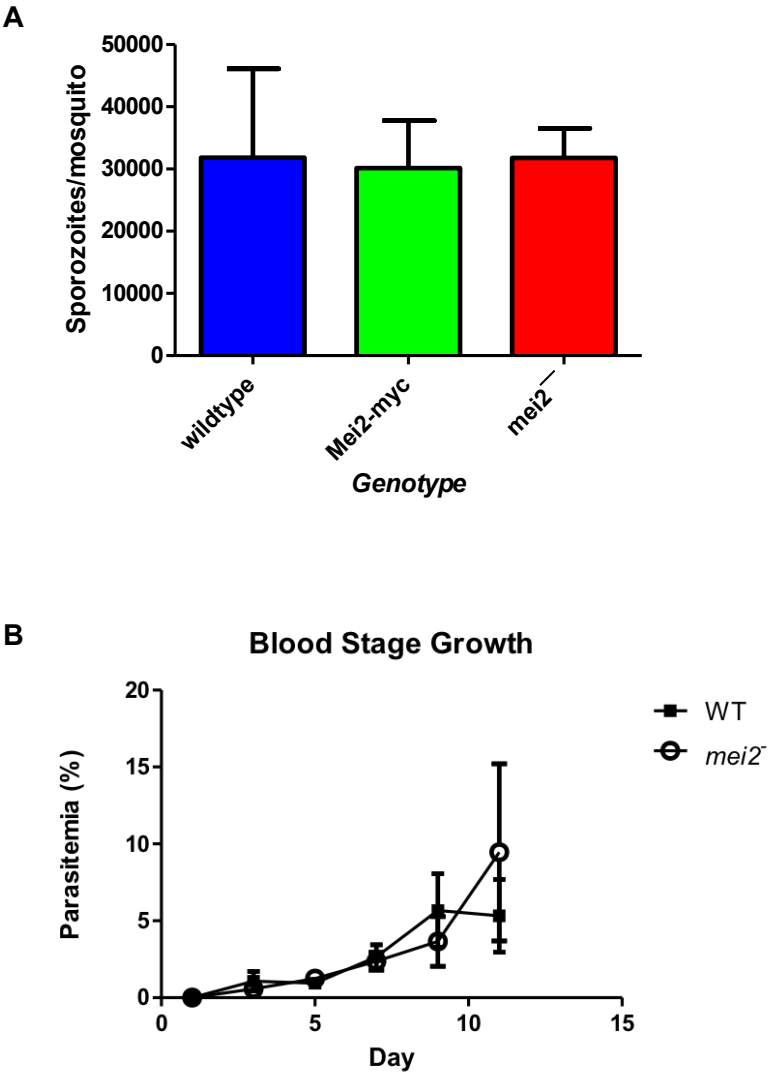
Oligonucleotide Primer Name	**Oligonucleotide Primer Sequence**
For knockout of *PlasMei2*	
Py *Mei2* 5 UTR F	5' ATAAGCTTCTACCTGTAATGGAAATATCGAC 3'
Py *Mei2* 5 UTR R	5' ATCCGCGGTTTTGTCCGTCCTTATTTATATTTAC 3'
Py *Mei2* 3 UTR F	5' ATGGTACCCTGCAGTATTGTTTATAAAATTATAGAATTTTACAAC 3'
Py *Mei2* 3 UTR R	5' ATGCGGCCGCTTAACAAATGTAAGCATTATATATACAAC 3'
Py *Mei2* Test F	5' TGGTTCCATGTATGTATGTCAG 3'
Py *Mei2* Test R	5' TTGCCATTTATCTCCTTCACAG 3'
Py *Mei2* qF	5' TCTGATTATGAAAGTGATAAAGAC 3'
Py *Mei2* qR	5' CCAAGTGGTATAGATTCTTCAC 3'
pL0034 Test F	5' AAGCACAATATCTAGGATACTAC 3'
pL0034 Test R	5' TGATTAGCATAGTTAAATAAAAAAAG 3'
For epitope tagging of *PlasMei2*	
Py *Mei2myc* ORF F	5' GCTTACATTTGTTAAGGGCCCAAATAGAGGATACTTTATTAAGTCC 3'
Py *Mei2myc* ORF R	5' ATCACTAGTATATTTATGTGATCGAAGTTTTATAG 3'
Py *Mei2myc* 3 UTR F	5' TACCGCGGTATTGTTTATAAAATTATAGAATTTTACAAC 3'
Py *Mei2myc* 3 UTR R	5' AAGTATCCTCTATTTGGGCCCTTAACAAATGTAAGCATTATATATACAAC 3'
pDEFmyc Test F	5' CAAATTTGAAGTATATGAGAAGAATG 3'
pDEFmyc Test R	5' CATCAGAGCAGATTGTACTGAG 3'
Py SAP1 qF	5' ATTCTACCCCCATTTATTCCAG 3'
Py SAP1 qR	5' ATCGTTATTACTTATGGGATTGC 3'
For RT-PCR to detect *PlasMei2* and *18S* RNA	
Py 18S F	5' GGATTGGTTTTGACGTTTTTGCGGTCATACTGCTTAATC 3'
Py 18S R	5' CCTCTAAGAAGCATTAAATAAAGCGAATACATCCTTATCAGAAGAGAGG 3'
Py *Mei2* qF	5' TCTGATTATGAAAGTGATAAAGAC 3'
Py *Mei2* qR	5' CCAAGTGGTATAGATTCTTCAC 3'

Supplementary Figure 1. *Plasmodium* species Mei2 sequence alignment. The RNA recognition motif 2 (RRM_2) (underlined in green) at the C-terminus of *Plasmodium* PlasMei2 is highly conserved as is a short span of amino acids at the N-terminus (underlined in cyan), which could play a role in spatial expression.

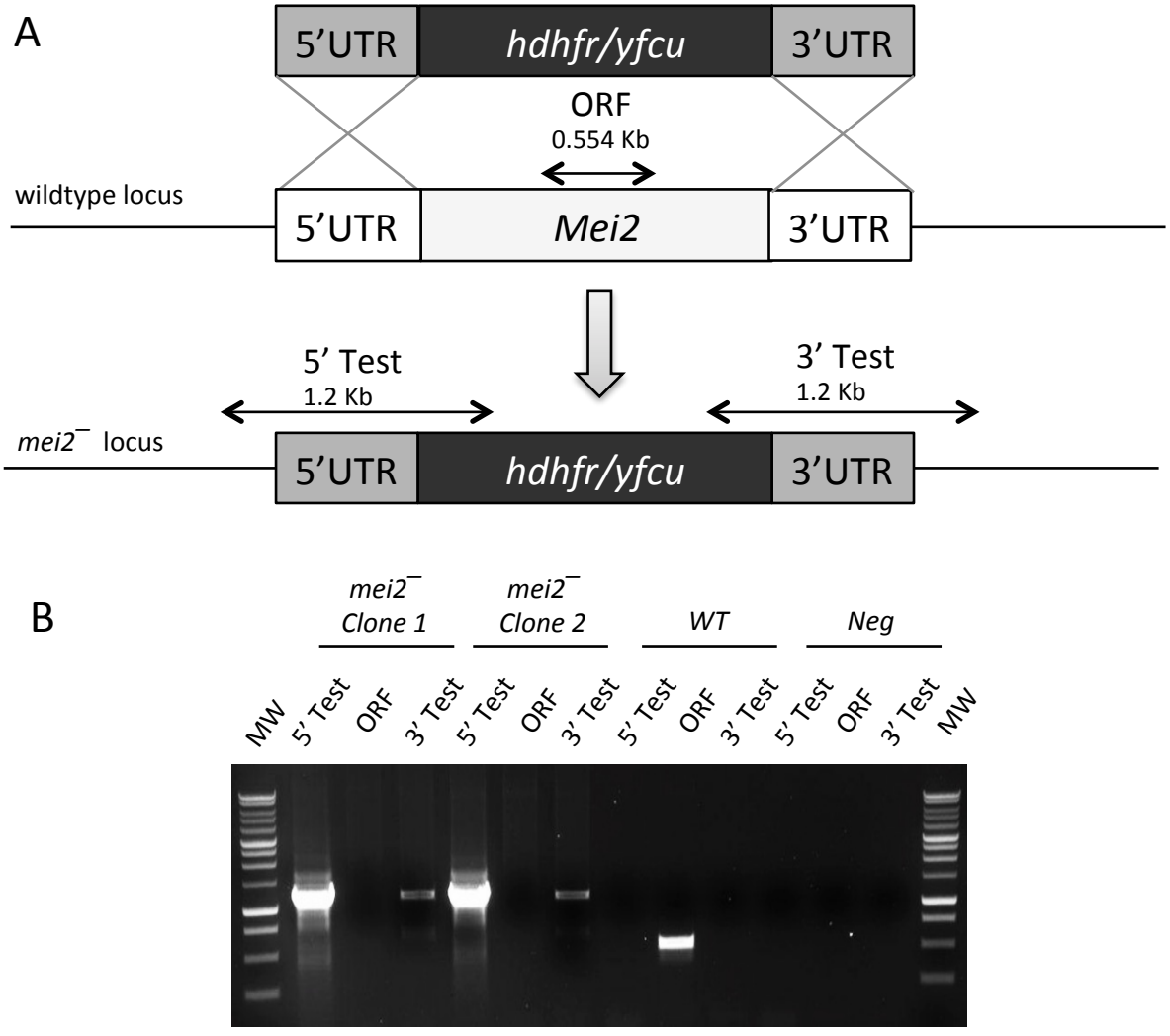
Supplemental Figure 2: Creation of *P. yoelii PlasMei2-myc.* A. Schematic of genomic locus of wildtype and transgenic parasite and the test primers pairs (double arrowed lines) used for subsequent genotyping PCR. Integration of the myc tag occurred by double-crossover homologous recombination (crossed lines) using regions of similarity from the *P. yoelii PlasMei2* open reading frame and 3'UTR region. B. The genotyping PCR gel provides evidence of recombination based on the successful integration of the myc tag (test 1) as well as the 3'UTR (test 2) in two independent clones (*P. yoelii PlasMei2-myc* clone 1 and clone 2). The positive control (to ensure that the DNA was sound), denoted as (+), was a 0.53 kb region of the *P. yoelii SAP1* gene. The molecular weight marker used was the 1 kb DNA Ladder from New England Biolabs.



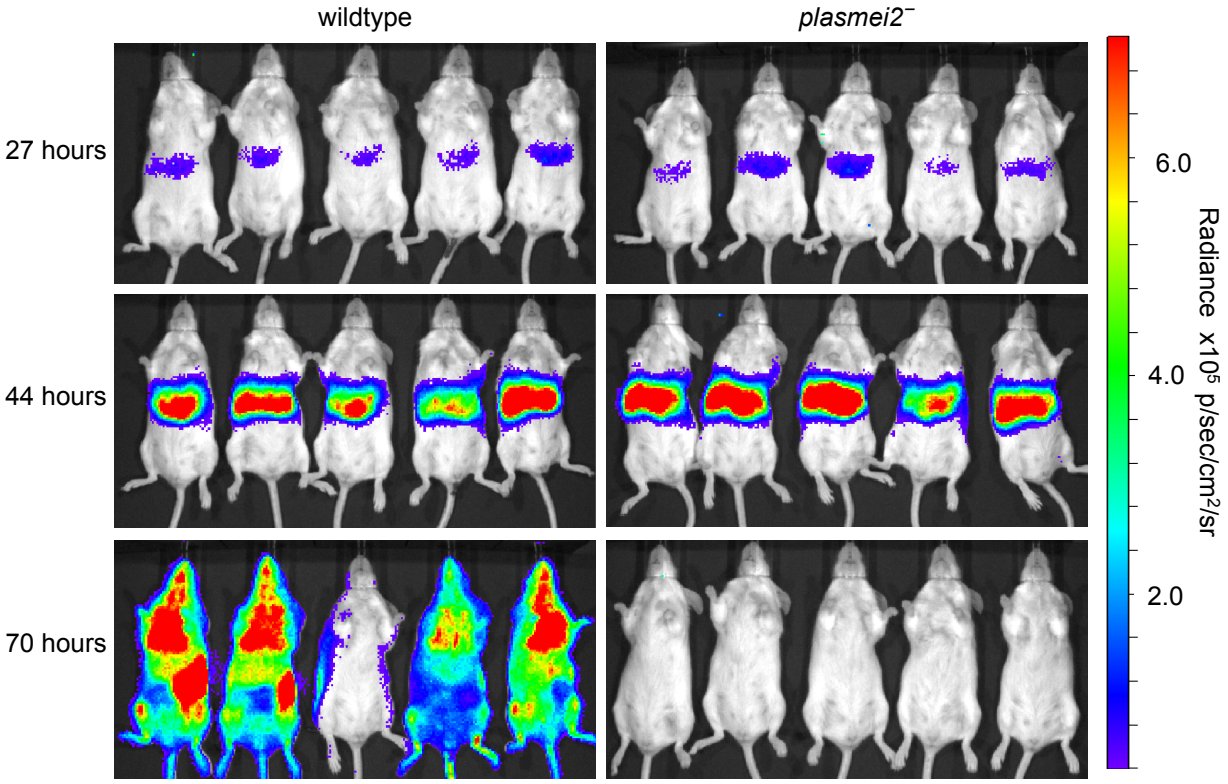
Supplementary Figure 3. Salivary gland sporozoites enumeration and blood stage growth. A. Salivary gland sporozoite numbers were enumerated from four separate mosquito feeds. For each feed, the average number of salivary gland sporozoites per mosquito for at least 100 mosquitoes was determined. This number was then averaged over the four feeds. There is no statistical difference between the three genotypes; *P. yoelii* wildtype, *P. yoelii PlasMei2-myc* and *P. yoelii plasmei2*$^{-}$. B. Blood stage growth was compared in groups of five Swiss Webster mice for wildtype *P. yoelii plasmei2*$^{-}$. 1 x 10^{6} infected red blood cells were injected intravenously into each mice and parasitemia measured every other day for eleven days. Growth rates were comparable suggesting PlasMei2 has no essential role in blood stage replication.



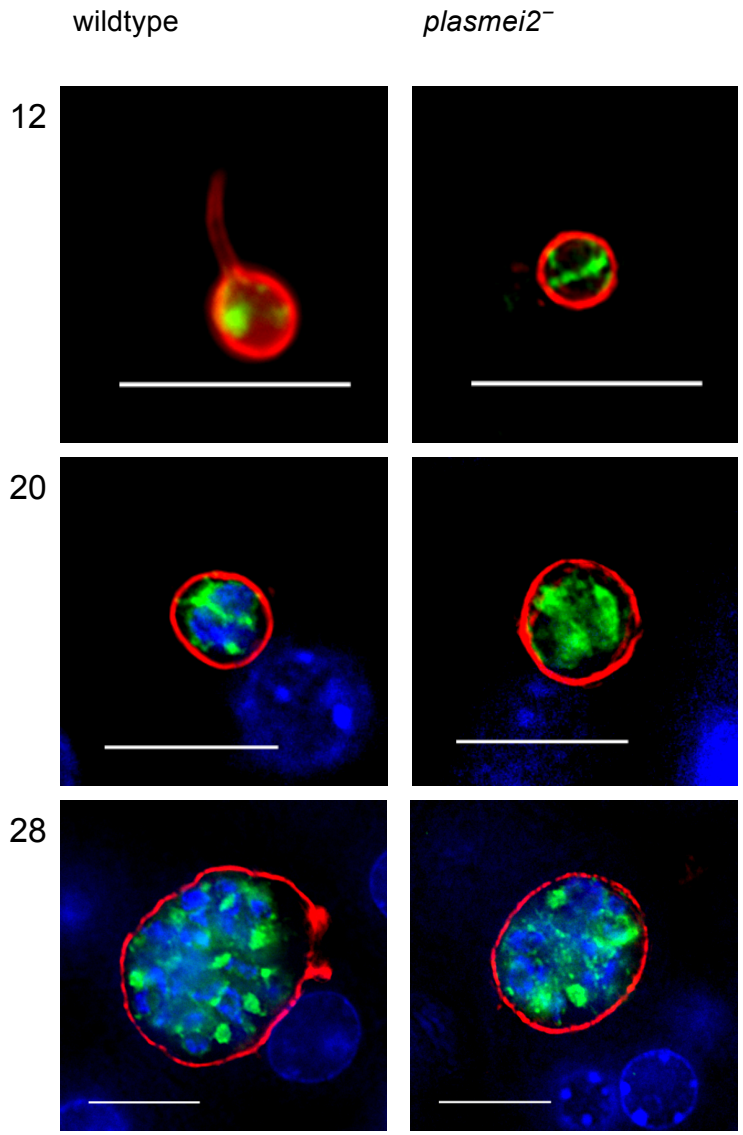
Supplementary Figure 4. Generation of *P. yoelii plasmei2¯*. A. Schematic of the *P. yoelii PlasMei2* genomic locus of wildtype and knockout parasites showing the test primers pairs (double arrowed lines) used for subsequent genotyping PCR. Using the Gene Insertion/Marker Out strategy (1), the *Py PlasMei2* gene was replaced with a selectable marker containing a fusion gene of mutant human dihydrofolate reductase (*hdhfr*) and yeast cytosine deaminase and uridyl phosphoribosyl transferase (*yfcu*) which facilitate positive and negative selection respectively. Gene replacement occurred by double-crossover homologous recombination (crossed lines) using the 5'UTR and 3'UTR regions of the *PlasMei2* gene. B. After transfection and subsequent cloning, genotyping by PCR was used to detect gene deletion. The agarose gel provides evidence of two individual clones that shoe successful gene knockout with the primer pairs used. The molecular weight marker used was the 1 kb DNA Ladder from New England Biolabs.



Supplementary Figure 5. *P. yoelii plasmei2⁻* and wildtype liver stages show similar growth rates as measured by luciferase activity but *plasmei2⁻* liver stages fail to transition to blood stages. Groups of five BALB/cByJ mice were infected intravenously with 50,000 wildtype or *plasmei2⁻* luciferase-expressing sporozoites. After 27, 44, and 70 hours mice were injected with luciferin and luciferase activity was measured using an *in vivo* imaging system (IVIS). Rainbow images of luminescence are presented as radiance (p/s/cm^2/sr) and maximum and minimum color scale limits were consistent between all groups. At 27 and 44 hours, luminescence is similar between the two groups but at 70 hours wildtype parasites transition to blood stage infection whereas *plasmei2⁻* parasites are eliminated and thus fail to express luciferase. At 70 hours all wildtype-infected mice were blood smear positive based on Giemsa-stained thin smear and all *plasmei2⁻*-infected mice were blood stage negative.



Supplementary Figure 6. *P. yoelii plasmei2*$^{-}$ and wildtype liver stages are indistinguishable at early time points. Liver sections from infected mice were subjected to immunofluorescence assay to study parasite phenotype. At 12 hours after sporozoite injection, almost (wildtype, 12 hours, top left panel) and completely (*P. yoelii plasmei2*$^{-}$, top right panel) dedifferentiated parasites were seen for both wildtype and *plasmei2*$^{-}$. Growth continued and was analyzed at 20 and 28 hours of infection (middle and bottom panels respectively). Parasites were visualized with antibodies to circumsporozoite protein (CSP), red (top panel), the parasitophorous membrane vacuole marker Hep17 (red, middle and bottom panels) and the endoplasmic reticulum marker BiP (green). Scale bar: 10 microns.



REFERENCES

1. **Lin JW, Annoura T, Sajid M, Chevalley-Maurel S, Ramesar J, Klop O, Franke-Fayard BM, Janse CJ, Khan SM.** 2011. A Novel 'Gene Insertion/Marker Out' (GIMO) Method for Transgene Expression and Gene Complementation in Rodent Malaria Parasites. PLoS One **6:**e29289.