

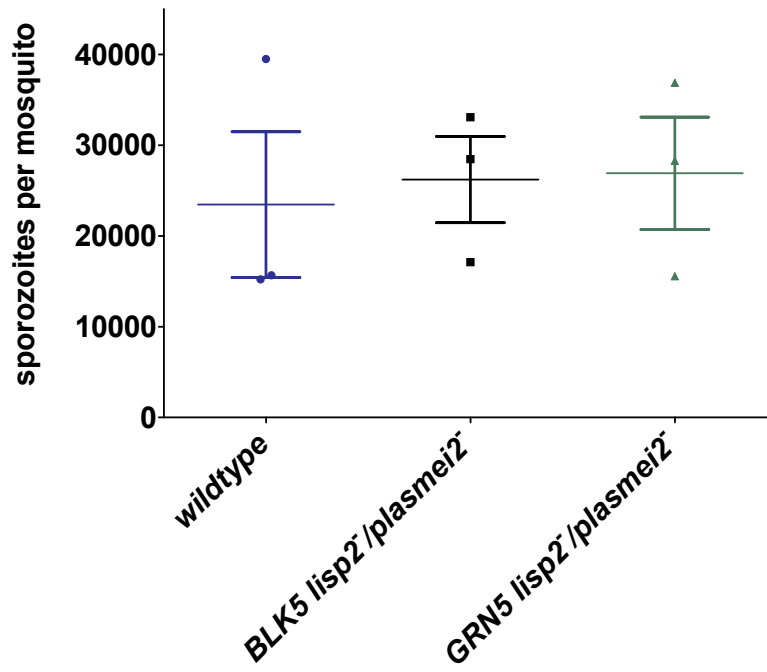
SUPPLEMENTAL TABLE 1

Oligonucleotide primers used in the study

Oligonucleotide Primer Name	Oligonucleotide Primer Sequence
For the knockout of <i>LISP2</i>	
Py <i>LISP2</i> 5 UTR F	5' <i>ataagctt</i> GGTACATCGACATTCACC 3'
Py <i>LISP2</i> 5 UTR R	5' CTTT TAGGTTTTCTGGGCCCTTTTATGTGTAAAAAAGTAAATGATTATAATAAAAAG 3'
Py <i>LISP2</i> 3 UTR F	5' TTTTTTACACATAAAAAGGGCCAGAAAAACCTAAAAGAGGTAATAC 3'
Py <i>LISP2</i> 3 UTR R	5' <i>ataagctt</i> GGAAATAACTTCAAATTAATACTACAAAATATC 3'
Py <i>LISP2</i> guide F	5' <i>tatt</i> CATATTGAAGATATTGCCCC 3'
Py <i>LISP2</i> guide R	5' <i>aac</i> GGGGCAATATCTTCAATATG 3'
Py <i>LISP2</i> Test F	5' TTTTTTAACGATGTAACAGTGTG 3'
Py <i>LISP2</i> Test R	5' AATGCGTAACAAAGAACCAGG 3'
Py <i>LISP2</i> qF	5' AGCAGCCTGAATACGTTGTG 3'
Py <i>LISP2</i> qR	5' TTATGGTTTGGTGAAGACGGG 3'
For the knockout of <i>PlasMei2</i>	
Py <i>PlasMei2</i> 5 UTR F	5' <i>ataagctt</i> CTACCTGTAATGGAAATATCGAC 3'
Py <i>PlasMei2</i> 5 UTR R	5' <i>atccg</i> TTTTGTCCGTCCTTATTTATATTTAC 3'
Py <i>PlasMei2</i> 3 UTR F	5' <i>atggtacc</i> CTGCAGTATTGTTATAAAATTATAGAAATTTACAAC 3'
Py <i>PlasMei2</i> 3 UTR R	5' <i>atcggccgc</i> TAAACAAATGTAAGCATTATATACAAC 3'
Py <i>PlasMei2</i> Test F	5' TGGTTCCATGTATGTATGTCAG 3'
Py <i>PlasMei2</i> Test R	5' TTGCCATTTATCTCCTTCACAG 3'
Py <i>PlasMei2</i> qF	5' TCTGATTATGAAAGTGATAAAGAC 3'
Py <i>PlasMei2</i> qR	5' CCAAGTGGTATAGATTCTTCAC 3'
pL0034 Test F	5' AAGCACAATATCTAGGATACTAC 3'
pL0034 Test R	5' TGATTAGCATAGTTAAATAAAAAAAG 3'
For the knockout of <i>FabB/F</i>	
Py <i>FabB/F</i> 5 UTR F	5' <i>ataagctt</i> GCTATTTATAAGAGTTTGAGAGG 3'
Py <i>FabB/F</i> 5 UTR R	5' <i>atccg</i> TTTTCGTTAAATAATTTATAATACAAG3'
Py <i>FabB/F</i> 3 UTR F	5' <i>atggcatt</i> CTGCAGATTTATTAGTTGATATTATCATTATTAG 3'
Py <i>FabB/F</i> 3 UTR R	5' <i>ataagctt</i> ATATATATATTAATGCGTTTCCATTC 3'
Py <i>Fabb/F</i> Test F	5' AAGTATAGAAACATGAAATGAACC 3'
Py <i>Fabb/F</i> Test R	5' CGAATGCTTAATTGTATAGTCTC 3'
Py <i>Fabb/F</i> qF	5' CATATGGGAATATAGGACATGTG 3'
Py <i>Fabb/F</i> qR	5' CCTGTGCATACAACCTCTGGAAG 3'
pL0034 Test F	5' AAGCACAATATCTAGGATACTAC 3'
pL0034 Test R	5' TGATTAGCATAGTTAAATAAAAAAAG 3'

SUPPLEMENTAL FIGURE 1

A



B

Parasite	Mice injected	Dose	Patent
wildtype	5	10,000	5/5
BLK5	12	200,000	0/12
GRN5	17	200,000	0/17

SUPPLEMENTAL FIGURE 1 LEGEND

Independent clones of *P. yoelii lisp2⁻/plasmei2⁻* produce salivary gland sporozoites and are attenuated at the liver stage of development. A. Three

independent infectious blood meal infections of *Anopheles stephensi* mosquitoes were carried out to compare wildtype, *P. yoelii lisp2⁻/plasmei2⁻* clone GRN5 and clone BLK5. Average salivary gland sporozoite numbers were enumerated at day 14 after the infectious blood meal from at least 50 individual mosquitoes. There are no significant differences in sporozoite numbers between the three parasites.

B. Salivary gland sporozoites were injected IV into susceptible BALB/cByJ mice and blood stage patency was evaluated for 21 days after infection. All mice that received wildtype sporozoites (50,000) became patent whilst none of the mice that received *P. yoelii lisp2⁻/plasmei2⁻* clone GRN5 and clone BLK5 sporozoites (200,000) became patent.