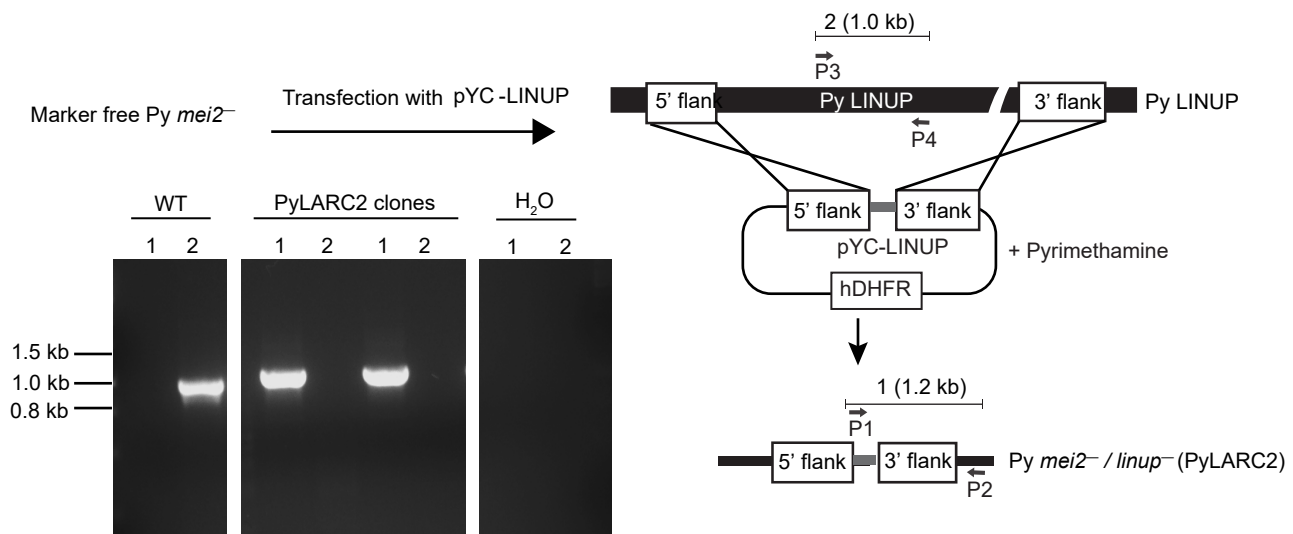


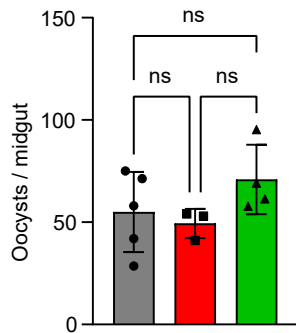
## Appendix Figures and Tables

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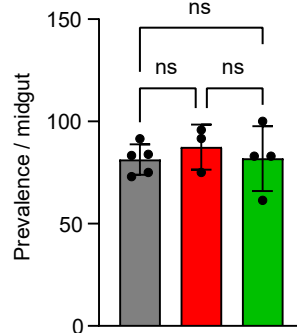
A



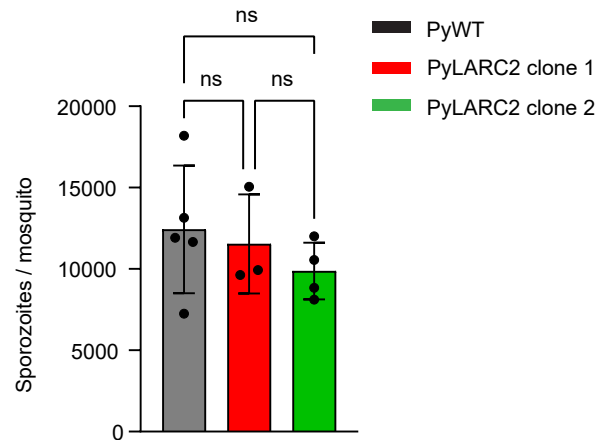
B



C



D

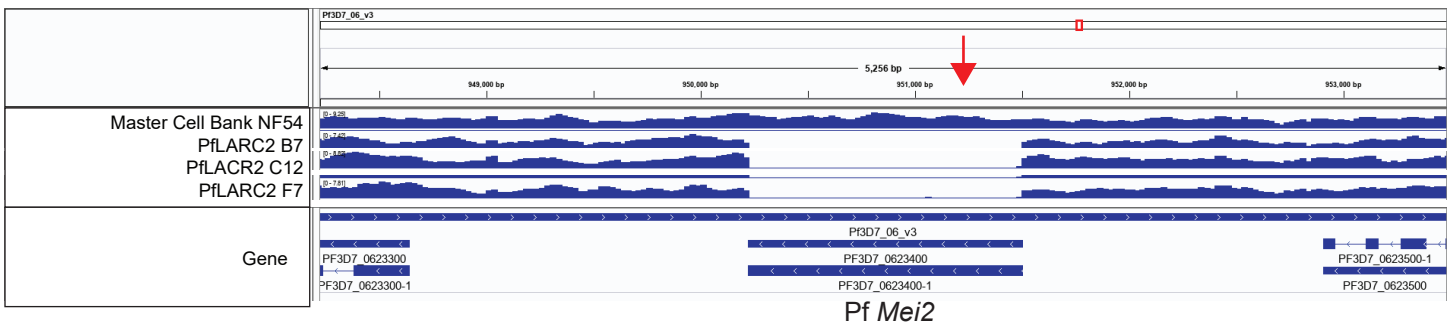


### Appendix Figure S1: PyLARC2 generation and analysis of mosquito stage development. A.

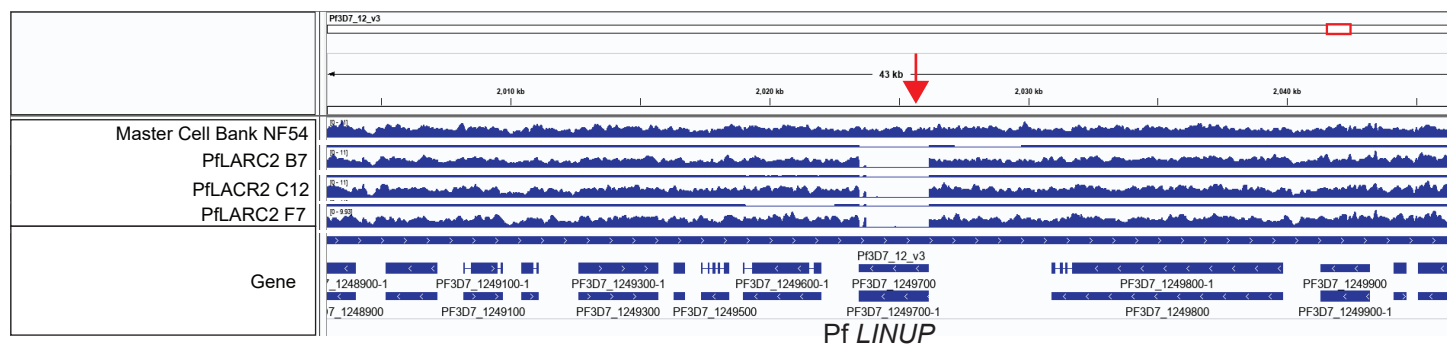
The schematic depicts the generation of the PyLARC2 parasite using CRISPR/Cas9-mediated gene editing. Blood stage schizonts of marker free *Py mei2*<sup>-</sup> were transfected with pYC-LINUP plasmid, followed by positive selection with pyrimethamine to select for *Py mei2*<sup>-</sup>/*linup*<sup>-</sup> (PyLARC2) parasites. Clonal populations were derived by limiting dilution cloning. Agarose gel electrophoresis shows the PCR products corresponding to the gene deletion of *Py LINUP* in clones 1 and 2 of PyLARC2. Primers used to verify the gene deletion are indicated and the sizes of the PCR amplicons are shown in kilobases. B. PyLARC2 clones 1 and 2 did not have defects in mosquito infectivity as counts for B., oocysts/midgut, C., oocyst prevalence and D., salivary gland sporozoites/mosquito were comparable between PyLARC2 clones 1 (red) and 2 (green) to PyWT (black). Data is represented as mean  $\pm$  SD, for at least  $n = 3$  biological replicates. Statistical analysis was carried out using one way ANOVA using Tukey's multiple comparison test.  $P > 0.05$  is taken as not significant.

Appendix Figure S2

**A**



**B**



Appendix Figure S2: Gene deletion of *Pf Mei2* and *Pf LINUP* in PflARC2 was confirmed by whole genome sequencing. A. The absence of *Pf Mei2* (PF3D7\_0623400) in chromosome 6 and B. *Pf LINUP* (PF3D7\_1249700) in chromosome 12 in PflARC2 clones F7, C12 and B7 was confirmed by whole genome sequencing when compared to PfNF54-WT.

**Appendix Table S1: Oligonucleotide primers used in the study.**

| <b>Primers for Py LARC2 generation and genotyping</b>     | <b>Sequence</b>   |
|---|---|
| PY17X_1465200<br>5UTR F                                   | 5' - ATAAGCTTGTTTGTATCTTGGATTTTGAAATGGTTG - 3'                                |
| PY17X_1465200<br>5UTR R                                   | 5' -<br>AATAGCATTGACCAAGCTAGCTATAGGCGCGCCTTTTGGATCAA<br>ACGTGGGGTATATATG - 3' |
| PY17X_1465200<br>3UTR F                                   | 5' -<br>TCCAAAAGGCGCGCCTATAGCTAGCTTGGTCAATGCTATTATAT<br>ATTTTATTACTTCCC - 3'  |
| PY17X_1465200<br>3UTR R                                   | 5' - TAGCGGCCGCTAACCATTACATATTCGATAATGTAAACAG -<br>3'                         |
| PY17X_1465200<br>guide F                                  | 5' - TATTCGATGGAGTTGTCTCTGCTT - 3'  |
| PY17X_1465200<br>guide R                                  | 5' - AAACAAGCAGAGACAACCTCCATCG - 3'   |
| P1  | 5' - AGGCGCGCCTATACCCGGGTTGG - 3'   |
| P2  | 5' - GCAATAAAATAAGCAGAATGCGTATGC - 3'   |
| P3  | 5' - ACAATAATACTCCAACCATCTCTTC - 3'   |
| P4  | 5' - TTTCTCAAAGTTTAAAATAGTATCTCC - 3'   |
|   |   |
| <b>Primers for the Pf LARC2 generation and genotyping</b> |   |
| PF3D7_1249700<br>_Guide 3F                                | 5' - TATTGTTGTTGTTGTTTGCCTTGG - 3'  |
| PF3D7_1249700<br>_Guide 3R                                | 5' - AAACCCAAGGCAAACAACAACAAC - 3'  |
| PF3D7_1249700<br>_Guide 5F                                | 5' - TATTATATGTGCTACTCCATATAC - 3'  |
| PF3D7_1249700<br>_Guide 5R                                | 5' - AAACGTATATGGAGTAGCACATAT - 3'  |
| PF3D7_1249700<br>_5UTR F                                  | 5' - ATACTAGTTTTTATTTTTTATATGAACCATTTAAGAC - 3'                               |
| PF3D7_1249700<br>_5UTR R                                  | 5' -<br>TACTTTTCCCAACCCGGGTATAGGCGCGCCTCTTTCAACAAA<br>ATAATATATGTGTAC - 3'    |
| PF3D7_1249700<br>_3UTR F                                  | 5' -<br>TTGTTGAAAGAGGCGCGCCTATACCCGGGTTGGGGAAAAGTAA<br>AATTCGCGATAAAAATC - 3' |

|                          |  |
|--------------------------|--|
| PF3D7_1249700<br>_3UTR R | 5' - TAGCGGCCGCAATATATATGTGATTTTATTAAGTACGG - 3' |
| A                        | 5' - TAAAATGATATATGGAATACAATTATG - 3'            |
| B                        | 5' - TTCTTCCTCTTTGATCTTTTACC - 3'                |
| C                        | 5' - GAAGGCCCATTAATAAATATAGAC - 3'               |
| D                        | 5' - CCAAGTAACAGAACAGACTTTGTTAC - 3'             |
| E                        | 5' - GGC GCGCCCTTAAGGCCTGCAGG - 3'               |
| F                        | 5' - TAAATGAGGGATAAAACCTCAAAAAG - 3'             |
| G                        | 5' - AATATGTTTCAAACGGTTAAATGTAAG - 3'            |
| H                        | 5' - CTTAACAACCATCTTCTTGACAATAGGG - 3'           |
| I                        | 5' - GTTATGTGATTAACCTTCGCTTGATAC - 3'            |
| J                        | 5' - AGGCGCGCCTATACCCGGGTTGG - 3'                |
| K                        | 5' - CCCAGAAACGCTGGTGAAAG - 3'                   |
| L                        | 5' - CGCCTCCATCCAGTCTATTAATTG - 3'               |
| M                        | 5' - CGGAGCCCTGCTGTTTCGACAGC - 3'                |
| N                        | 5' - GCCATTCTTCTTCTCGCCGGGCAGCT - 3'             |
| O                        | 5' - CGAATTTAGATATTTCCAGAG - 3'                  |
| P                        | 5' - CTTAATGCCTTTCTCCTCCTGG - 3'                 |

**Appendix Table S2: PfSPZ-LARC2 liver stages do not transition to productive blood stage infection after sporozoite infection in FRG huHep mice.**

| Mice          | Parasite equivalents/ml (Day 7) | Copies of Pf 18s rRNA/ml (Day 7) | Parasite equivalents/ml (Day 14) | Copies of Pf 18s rRNA/ml (Day 14) |
|---------------|---------------------------------|----------------------------------|----------------------------------|-----------------------------------|
| PfSPZ_1       | 11,518                          | 85,231,282                       | 90372128                         | 668,753,745,913                   |
| PfSPZ_2       | 1,101                           | 8,147,908                        | 159362886                        | 1,179,285,358,246                 |
| PfSPZ_3       | 506,919                         | 3,751,204,288                    | 1045652350                       | 7,737,827,387,488                 |
| PfSPZ_4       | 1,248                           | 9,236,016                        | 734301163                        | 5,433,828,607,469                 |
| PfSPZ-LARC2_1 | 0                               | 1,199                            | 0                                | 0                                 |
| PfSPZ-LARC2_2 | 197                             | 1,455,700                        | 10                               | 76,290                            |
| PfSPZ-LARC2_3 | 27                              | 198,041                          | 51                               | 378,030                           |
| PfSPZ-LARC2_4 | 4                               | 32,655                           | 19                               | 141,478                           |
| PfSPZ-LARC2_5 | 11                              | 81,921                           | 11                               | 79,393                            |
| PfSPZ-LARC2_6 | 2,689                           | 19,900,587                       | 58                               | 427,541                           |