

Supplemental Table 1. Depletion of *PySAP1* does not affect sporozoite development in *Anopheles* mosquitoes.

Experiment	Genotype	Mean ooSPZs/ mosquito¹	Mean sgSPZs/ mosquito²
1	<i>Pysap1(-)</i>	79,000	17,000
	<i>PyWT</i>	85,000	18,000
2	<i>Pysap1(-)</i>	96,000	34,000
	<i>PyWT</i>	102,000	27,000
3	<i>Pysap1(-)</i>	60,000	15,000
	<i>PyWT</i>	65,000	14,000
4	<i>Pysap1(-)</i>	63,000	17,500
	<i>PyWT</i>	ND	20,000

¹Mean number of oocyst sporozoites per mosquito midgut dissected at day 10 post blood meal infection from ≥ 20 female mosquitoes.

²Mean number of salivary gland sporozoites per mosquito dissected at day 14/15 post blood meal infection from ≥ 50 female mosquitoes.

ND: not done

Supplemental Table 2. Sequences of primers used in this study, restriction endonucleases sites are underlined.

Primer	Sequence
PySAP1rep1F	GGGGT <u>ACCGT</u> GCAATGTGAAAATGATAATGCTCGATAAG
PySAP1rep2R	GCCCA <u>AGCTTTTT</u> CTTTCTTAAATACAAAAAATAATTTAT
PySAP1rep3F	GG <u>ACTAGT</u> CCAGCTATAAACTCCGAAACATCGAATTATGT
PySAP1rep4R	TCC <u>CCGCGG</u> GCCATCGCGTTGATGCTTTTGGGAATTATTGA
PySAP1TestF	CTCTTTTTGGGAGTCAAAAACGGTATGC
PySAP1TestR	CACCCTTATAACCATCATTATCTACTTTTCC
TgF	GGCTACGTCCCGCACGGACGAATCCAGATGG
TgR	CGCATTATATGAGTTCATTTTACACAATCC
PySAP1orfF	GGTAAACCACGGCACGTTCCCTATGTTT
PySAP1orfR	CTTGATTTATCAGCATTGTTAATATGCCC
PyCSP F	AAGAAGTGTACCATTTTAGTTGTAGCGTCAC
PyCSP R	CACTACTGGTTGATTCAATTTATTTTGAGCCTC
PyTRAP F	CCAAGCAATCTACCAGAAAATCCATCTGACTCA
PyTRAP R	CCAGTCATTATCTTCAGGTAATTTAAACTGTTC
PyUIS3 F	TCATCAGGATTAGTGGCAAGTGTTATTGGGGTAC
PyUIS3 R	TGGTTGATATTGTTCTTTAAGAAAATGCTCCAC
PyUIS4 F	CACCCTGAAGTCCGAGAAAAATTTGGAATTAGA
PyUIS4R	TATGTATGGGTCAAATGGTTTATCATTCTACT
PyUIS2 F	GAAGAAGTCCCAGATGAATCAAAAGAAGATGAC
PyUIS2 R	ATCGTAGAAAGCTAGTTTTGTTCCGACTTTTCC

PyUIS28 F	TGGGTAGTAGTTTTGTTGAGAGAGTATGT
PyUIS28 R	CCAAGGATTGGGTACAAAATTGGAAGTTTCC
PyP52 F	AGTTGTGCATGCAAAAGAGATGAATATATCGGC
PyP52 R	GCCAAAATGTTCTTTTCTTTCTCAAAGGTCCATTG
PyS4 F	TGTTTGAGAGGCAAAAATGGATCAGAAATGTCG
PyS4 R	GTCATATTCTTCATCACCCACCTTCATATTCCCA
PySPECT1 F	CCATTTTAGTCCTATTTATCATTTTAAAATGTG
PySPECT1 R	CAGTTTCACAACATCATCAGAAGTATCTTCA
PySPECT2F	GAATGTGGAACACAAGCTATGCCATTTTCTGAT
PySPECT2 R	TCCTATTGATTGGCCTGAACCATGTACTIONGAGCA
PyHSP70 F	CCCGATGAAGCAGTAGCATTAGGTGCAGCT
PyHSP70 R	TGGAACACCTCTTGGGGCTGGTGGTATACC
PfSAP1orfF	GAAAAATGAAGAAGATAAATCCGATGGAGATG
PfSAP1orfR	CGTCCTCCTTTTTATTTTCGATCCTCCGTTGTAC
PfCSP F	GAGAAAATTAGCTATTTTATCTGTTTCTTCC
PfCSP R	ATCAGGATTACCATCCGCTGGTTGCTTTAA
PfHSP70 F	CCTGACGAAGCTGTCGCTTTAGGTGCTGCT
PfHSP70 R	TACACCTCTTGGTGCTGGTGGAAATTCCAAC

Supplemental Text 1: Annotation Correction and elucidation of the coding sequence of *PySAP1*

The annotated contig for *SAP1* gene **MALPY00932** (**AABL01000929**, gi:23491243) contains **9721** bps and encodes only a protein of **2694** amino acids. However, comparative bioinformatic analysis with *PfSAP1* DNA and protein sequence on sequenced chromosome11 (**MAL11**, **AE014843**, gi:23496404) revealed that the N-terminus was missing and the C-terminus was incorrectly terminated. The missing N-terminus with the 5'UTR of *PySAP1* was located on contig **MALPY01173** (**AABL01001169**, gi: 23478694) which contains **4359** bps and encodes a protein of **621** amino acids. These two *P. yoelii* predicted proteins overlapped identically between the first **141** amino acids of the **2694** amino acids protein (**MALPY00932**), and the amino acids **464** to **604** of the **621** amino acids protein (**MALPY01173**). We expect that a sequencing error occurred at the end of **contig MALPY01173** that caused the termination of the ORF and the splitting of the contig. In order to test this, we constructed sequencing primers where the forward sense primer (5'-TGGACTAAATAATTCTTTTCAAGTTATCGA-3') binds to a pre-overlap sequence in the **MALPY01173** contig, and the reverse antisense primer (5'-TGATAGCTATTTTGTTTAACATCTTGTTGT-3') binds to a post overlap sequence (even further downstream of the predicted stop codon of the **MALPY01173** contig). The expected size of the amplified fragment is **690 bps**. High-fidelity Pfu DNA polymerase amplification from *P. yoelii* genomic DNA and from cDNA (amplified from salivary gland sporozoites RNA) generated both ~ **690** bps DNA fragments that were sequenced. The fragments gave the same sequencing results and matched perfectly the predicted full length *PySAP1* coding sequence. Furthermore, we constructed pre-ATG sense forward primer (5'-GCTAACGCATATACCTATGCCTAAGGACGTATC-3') (that binds to 5'UTR region in the mature *SAP1* mRNA) and an antisense reverse primer (5'-GGGTTTTTCTTTTTTATGGTCAGATATGTCTTC-3') that binds in the coding sequence of the gene downstream the start codon. This sequence was amplified from salivary gland sporozoite cDNA with the expected fragment size of **574bps** and sequencing verified the matching of this sequence with the previously obtained data. For the verification of the C-terminus sequence and the intron - exon organization in this region, we constructed a forward sense primer (5'-CGAGGAAGACAAGTTCAACAATCTTTTAATCAC-3') that binds to a sequence before the end of the first exon, and an antisense primer (5'-ATGTAACGAGGTGTAGAAAAATGTCTAAA-3') that binds to a 3'UTR region in the

mature *SAP1* mRNA. Interestingly, the amplification from salivary gland sporozoite cDNA and from genomic DNA gave rise to two different fragments in size, 800 bps and 1041bps, respectively. Sequencing of both fragments revealed the correct missing exon 3 sequence, and the universal splicing signals gt and ag were detected in the fragment amplified from genomic DNA at the boundaries of the second intron separating the second exon from the third exon. Furthermore, using blast searches with the *P. yoelii* *SAP1* protein sequence, we retrieved the *P. berghei* **contig 4855 (11,703 bps)** from the Wellcome-trust Sanger institute blast server. In this contig we identified *PbSAP1* nucleotide sequence with the same exon-intron organization, the identical splicing signals and similar intron sequences to *PySAP1* nucleotide sequence. The predicted *PySAP1* and *PbSAP1* whole protein sequences shared up to 88% amino acid identity. Prior to the assembly of the new **contig 4855**, *PbSAP1* sequence was dispersed over 2 contigs with missing end and intermediate DNA sequences; genomic sequence **PB_RP1438 (CAAI01001256. gi: 56490746)** and genomic sequence **PB_RP0468 (CAAI01000407. gi:56493516)**.

***PySAP1* coding sequence:**

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ATGAGAAAAGGTAAACCACGGCAGTTCCTATGTTTTTGAGAAGCGAAAACAAAAAGATA
CTTTTTCTTTTTTCATATTTTATGTCCAAAATGATATACAATTTATTTTCGATCAT
TACGACATTGGTTTAATAGTTCTTACAAAATACATAACATACGAAAAAGATATGAAGAA
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TAA

PySAP1 amino acid sequence:

MRKGGKPRHVPMLRSENKILSFFSYFMSKNDIQFIFDHYDIGLIVLTKIHNIRKRYEE
DKNNLQHENNKNDLEDTESIDEKVRAFFLNYKINSNEDISDHKKEKPEKNKKGKGSVVK
EKSNETNEKFTAKNNYSIITRSKTEEIDPLKNDENDSPNSNNAHEENKPNEDITNTNS
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