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Supplemental Information

Steroid Hormone Function Controls Non-competitive

Plasmodium Development in *Anopheles*

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Supplemental Tables:

Supplementary Table 1: Estimation of sporozoites produced per oocyst. Related to Figure 5.

In both *dsEcR*- and *dsGFP*-injected females, median sporozoites counted 18 days (d) pIBM from dissected salivary glands were divided by median oocysts counted 7–8 d pIBM from stained midguts.

The estimated number of sporozoites produced per oocyst was similar between *dsGFP* (Cntrl) and *dsEcR* (EcR) groups. n corresponds to sample size.

	Median #oocysts at 7–8 d pIBM	Median #sprz at 18 d pIBM	Sprz/Oocyst
Cntrl	36 (n = 191)	103,950 (n = 25)	2,888
EcR	21 (n = 194)	56,400 (n = 21)	2,686

Supplementary Table 2: qRT-PCR primer sequences. Related to Figure 3, Figure 6, Figure 7, Figure S5, and Figure S7.

Gene	Forward primer	Reverse primer	Size
<i>EcR</i>	CCACTTCCGGCACATAACG	TGGAGCAGGCCTTTAGTAAC	128 bp
<i>USP</i>	AGAAGGAGAAACCGATGCTG	AAATGTCCGGCTTCAGGTC	159 bp
<i>Vg</i>	CCGACTACGACCAGGACTTC	CTTCCGGCGTAGTAGACGAA	118 bp
<i>Lp</i>	CAGCCAGGATGGTGAGCTTAA	CACCAGCACCTTGGC GTT	127 bp
<i>TEP1</i>	AAAGCTACGAATTTGTTGCGTCA	TTCTCCCACACACCAAACGAA	83 bp
<i>TL2</i>	ATCAGGAGAATGAGTTCAAG	ATAAAGCAATCTCAACCATC	93 bp
<i>AGAP000210</i>	CCAGTACAATACCCCTTAAT	GACTCCAAATTTACTTCC	86 bp
<i>AGAP007672</i>	AGAACAAGTACCCAGTAGA	CACTATACACGAACGATCAG	91 bp
<i>RpL19</i>	CCAACTCGCGACAAAACATTC	ACCGGCTTCTTGATGATCAGA	61 bp

Supplementary Table 3: Model statistics. Related to Figures 2–7, Figure S2, Figure S4 and STAR Methods. JMP statistical software was used to construct models for data analysis when multiple variables needed to be accounted for in an experiment. Depending on the data set, a Generalized Linear Model, Standard Least Squares Model, or Nominal Logistic Regression Model was used and non-significant interaction terms ($p > 0.05$) were removed. Effect test outputs are reported here. See main text for abbreviation definitions.

Figure	Comparison	Test/Model	Effect Test Outputs
2C	8 d pIBM oocysts	Generalized Linear Model, Poisson distribution	treatment $p = 0.0011$ replicate $p < 0.0001$
3B	7 d pIBM oocysts	Generalized Linear Model, Poisson distribution	treatment $p = 0.0006$ replicate $p < 0.0001$ replicate x treatment $p = 0.0182$
4A	24 h pIBM ookinetes	Generalized Linear Model, Poisson distribution	treatment $p > 0.05$ replicate $p = 0.0003$
4B	2 d pIBM oocysts	Log(y) transformed, Generalized Linear Model, normal distribution	treatment $p < 0.0001$ replicate $p < 0.0001$
5B	5 d pIBM mean oocyst size	Standard Least Squares Model	treatment $p = 0.001$ replicate $p < 0.0001$
	7 d pIBM mean oocyst size	Standard Least Squares Model	treatment $p < 0.0001$ replicate $p > 0.05$
	8 d pIBM mean oocyst size	Standard Least Squares Model	treatment $p = 0.0004$ replicate $p = 0.0004$
	10 d pIBM mean oocyst size	Standard Least Squares Model	treatment $p < 0.0001$ replicate $p = 0.0093$
	12 d pIBM mean oocyst size	Standard Least Squares Model	treatment $p > 0.05$ replicate $p = 0.0024$
6B, S4A	Total lipids	Standard Least Squares Model	treatment $p > 0.05$ replicate $p = 0.0296$ time pBM $p > 0.05$ treatment x time pBM $p = 0.0121$
	TAGs	Standard Least Squares Model	treatment $p > 0.05$ replicate $p < 0.0001$ time pBM $p > 0.05$ treatment x time pBM $p = 0.0192$
	DAGs	Standard Least Squares Model	treatment $p = 0.0202$ replicate $p < 0.0001$ time pBM $p < 0.0001$
	PCs	Standard Least Squares Model	treatment $p > 0.05$ replicate $p > 0.05$ time pBM $p > 0.05$ treatment x time pBM $p = 0.0404$

	LPEs	Standard Least Squares Model	treatment $p > 0.05$ replicate $p < 0.0001$ time pBM $p > 0.05$ treatment x time pBM $p = 0.0108$
7A, S4B	Total lipids	Standard Least Squares Model	treatment $p < 0.0001$ replicate $p = 0.0051$ time pBM $p = 0.0191$
	TAGs	Standard Least Squares Model	treatment $p < 0.0001$ replicate $p > 0.05$ time pBM $p > 0.05$ treatment x time pBM $p = 0.0411$
	DAGs	Standard Least Squares Model	treatment $p = 0.0002$ replicate $p > 0.05$ time pBM $p = 0.0313$
	PCs	Standard Least Squares Model	treatment $p = 0.0074$ replicate $p = 0.0056$ time pBM $p = 0.0205$
	PSs	Standard Least Squares Model	treatment $p = 0.0265$ replicate $p = 0.0226$ time pBM $p = 0.0235$
	PIs	Standard Least Squares Model	treatment $p = 0.0002$ replicate $p > 0.05$ time pBM $p > 0.05$
	PEs	Standard Least Squares Model	treatment $p = 0.0269$ replicate $p = 0.0052$ time pBM $p = 0.0065$
7C	<i>Lp</i> expression	Standard Least Squares Model	treatment $p = 0.0226$ replicate $p = 0.0309$
S2C	Sporozoite prevalence	Nominal Logistic Regression Model	treatment $p = 0.0367$ replicate $p = 0.0027$

Supplementary Table 4: Additional primer sequences. Related to STAR Methods and Key Resources Table.

	Forward primer	Reverse primer
<i>vasa2 promoter for Cas9</i>	CAGGTCTCAATCCCG ATGTAGAACGCGAG	CGGTCTCACATATTG TTTCCTTTCTTTATTACACCGG
<i>SpCas9</i>	CAGGTCTCATATGGA CTATAAGGACCACGACGGAG	CAGGTCTCAAAGCTT ACTTTTTCTTTTTTGCCTGGCC
<i>vasa2 promoter for EYFP</i>	CGGTCTCACGCGCGA TGTAGAACGCGAGCAA	CAGGTCTCACCATAT TGTTTCCTTTCTTTATTACACCGG
<i>EYFP-SV40</i>	CAGGTCTCAATGGTG AGCAAGGGCG	CAGGTCTCACGCGGC TTTAAGATACATTGATGAGTTTGGAC
<i>USP dsRNA region</i>	GGAAGCAATGGAGGTGGAG	CATAGAATTCTGGCCAACGC
<i>T7 promoter</i>	TAATACGACTCACTATAGGG	
<i>TEP1 dsRNA region-T7</i>	taatacgactcactataggg TTTGTGGGGCTGAAAGCGCTG	taatacgactcactataggg ACCACGTAACCGCTCGGTAAG
<i>TL2 dsRNA region-T7</i>	taatacgactcactataggg CTTTAGTCGGGTGGAGGACA	taatacgactcactataggg GATGATCGGTGTTTCGGCTT
<i>pCR2.1-T7</i>	taatacgactcactataggg CCGCCAGTGTGCTGGAA	taatacgactcactataggg GGATATCTGCAGAATTCGCCC