

Supplemental Data

The 20-hydroxyecdysone agonist, halofenozide, promotes anti-*Plasmodium* immunity in *Anopheles gambiae* via the ecdysone receptor

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Supplemental Information

Supplemental Figure Legends

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Figure S2. Halofenozide does not influence bacteria.

Figure S3. Increasing concentrations of halofenozide stimulate higher levels of ecdysone signaling.

Figure S4. Targeting the heterodimeric ecdysone receptor (EcR/USP) by RNAi.

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Table S1. Primers for qRT-PCR analysis.

Table S2. Primers for dsRNA synthesis.=

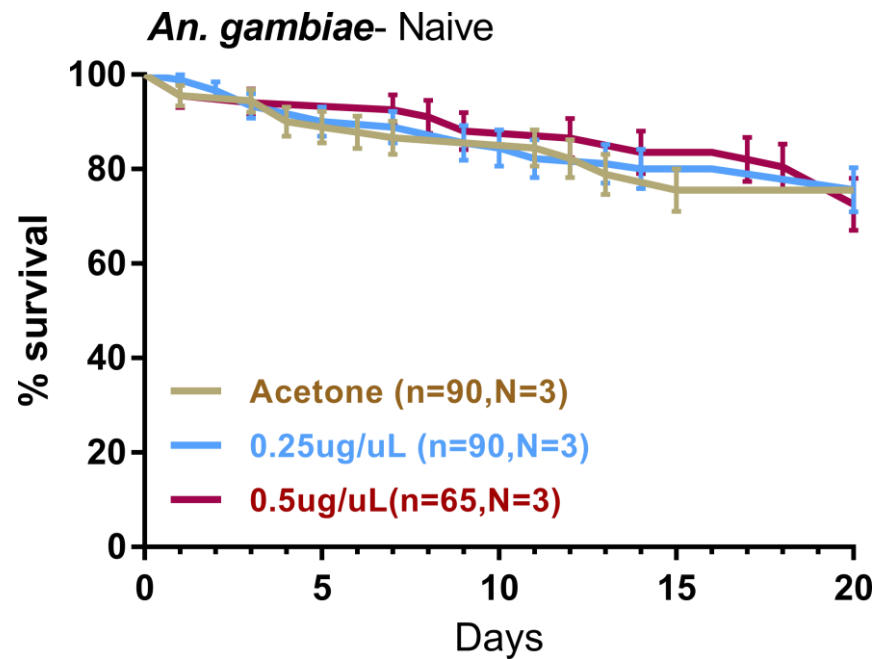


Figure S1. Mosquito survival following halofenozide application. Naive female *An. gambiae* mosquitoes were topically applied with either acetone (control) or halofenozide (0.25 $\mu\text{g}/\mu\text{L}$ and 0.5 $\mu\text{g}/\mu\text{L}$). Mosquito survival was monitored for 20 days post-application. No significant differences between treatments were detected when a Log-rank (Mantel-Cox) test was performed.

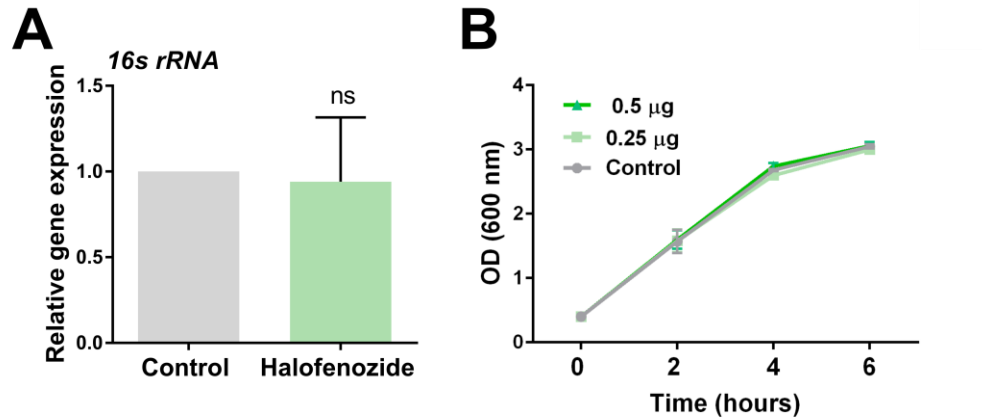


Figure S2. Halofenozide does not influence bacteria. The influence of halofenozide on the mosquito microbiota was examined in control- or halofenozide-treated (0.5 µg) mosquitoes using 16s rRNA primers by qRT-PCR (**A**). Results display previously results from four independent experiments. Data were examined using Mann-Whitney analysis. ns, not significant. Bacteria growth was also examined *in vitro*, in which halofenozide (0.25 and 0.5 µg/ml) or acetone (control) were added to liquid cultures of *E. coli* and their growth was examined for 6 hours using measurements of optical density (OD) at 600 nm (**B**). Experiments were performed in two independent experiments and analyzed using a two-way ANOVA and Bonferroni's multiple comparison test. No significant differences were detected between treatments at any experimental timepoint.

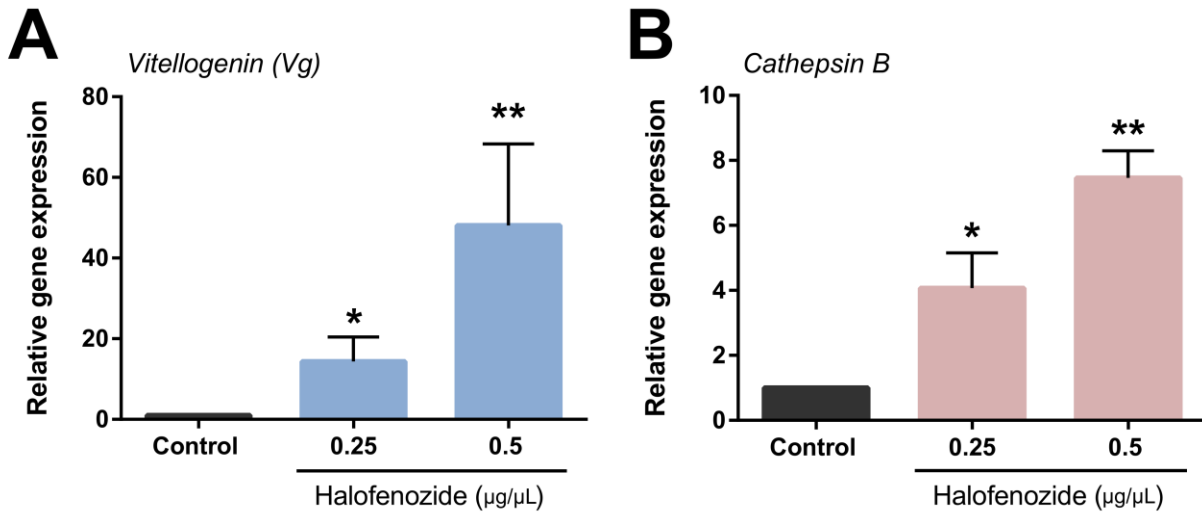


Figure S3. Increasing concentrations of halofenozide stimulate higher levels of ecdysone signaling. Analysis of vitellogenin (A) and cathepsin B (B) gene expression by qRT-PCR in response to increasing concentrations of halofenozide in whole naive female *An. gambiae*. Data from three or more experiments were compared to control (acetone) and analyzed using a Mann-Whitney test in GraphPad Prism 6.0 to determine significance (*, $P < 0.05$; **, $P < 0.01$).

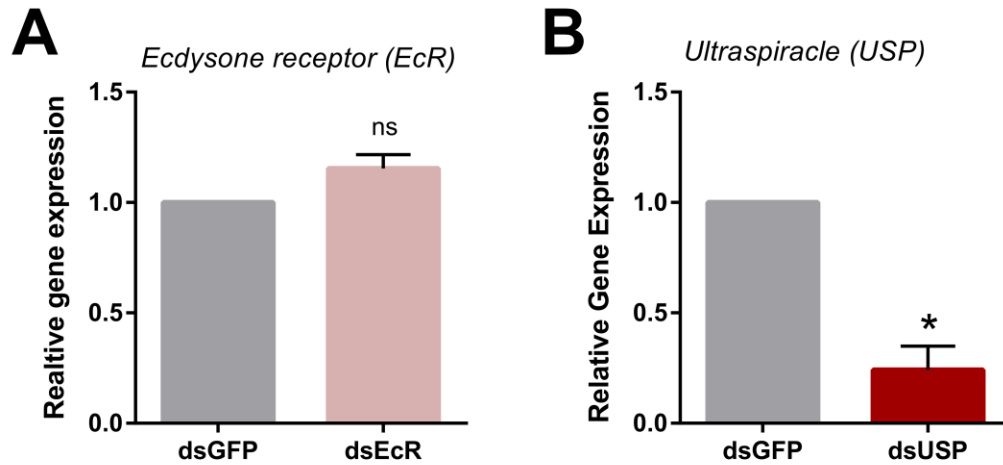


Figure S4. Targeting the heterodimeric ecdysone receptor (EcR/USP) by RNAi. To disrupt the heterodimeric ecdysone receptor (EcR/USP), EcR and USP were individually targeted by the injection of specific dsRNA. To validate the presence or absence of a knockdown, the expression of *EcR* **(A)** and *USP* **(B)** in whole mosquitoes 2 days post-dsRNA injection by qRT-PCR. Data from three or more experiments were analyzed using a Mann-Whitney test in GraphPad Prism 6.0 to determine significance (*, $P < 0.05$).

Table S1. Primers for qRT-PCR analysis

Primer	Gene ID	Sequence (5'- 3')
Cathepsin B-F Cathepsin B-R	AGAP004534	GCCAACGGTCTAGTGTCTCGAGTGTCTG ACTCGTACCGTCTGATCGGCACCTT
Cecropin 1-F Cecropin 1-R	AGAP000693	TTCATCTTTGTCGTGCTGGC GCACTGCCAGCACGACAAAG
Cecropin 3-F Cecropin 3-R	AGAP000694	ACGTACTGAACCACCTGCGCGTT GCGCTGTGTGCGCCGATGAA
rpS7-F rpS7-R	AGAP010592	ACCCCATCGAACACAAAGTTGACACT CTCCGATCTTTCACATTCCAGTAGCAC
USP-F USP-R	AGAP002095	TGAAGTCCGAAGAAATCAACTCGAC GGGCAAACCTCGATTAGCTGGTAGAT
Vg-F Vg-R	AGAP004203	TGCAGTACATCGAGCAGGGTGACAA CTTGACGGTCTTGGTGACCGACTTG
Universal bacteria 16S-F Universal bacteria 16S-R	N/A	TCCTACGGGAGGCAGCAGT GGACTACCAGGGTATCTAATCCTGTT

Table S2. Primers for dsRNA synthesis

Primer	Gene ID	Sequence (5'- 3')
GFP T7-F	AGAP002095	TAATACGACTCACTATAGGGAGAATGGTGAGCAAGGGCGAGGAGCTGT
GFP T7-R		TAATACGACTCACTATAGGGAGATTACTTGTACAGCTCGTCCATGCC
USP T7-F		TAATACGACTCACTATAGGGCCTAAAATGTCG
USP T7-R		TAATACGACTCACTATAGGGATCGGGAGACACACGCAGT