

## Supplementary Material

**Table S1.** The *Irx.447* target site conserved in mosquitoes was not found in non-mosquito genomes.

Species/Taxon Group	Gene/Contig
<i>Aedes aegypti</i> *	AAEL007505
<i>Aedes albopictus</i>	AALF026228
<i>Anopheles albimanus</i>	AALB016292
<i>Anopheles arabiensis</i>	AARA016724
<i>Anopheles atroparvus</i>	AatrE3_2L
<i>Anopheles coluzzii</i>	ACON005010
<i>Anopheles culicifacies</i>	KI423683
<i>Anopheles darlingi</i>	ADMH02001638
<i>Anopheles dirus</i>	ADIR015668
<i>Anopheles epiroticus</i>	KB672242
<i>Anopheles farauti</i>	KI915044
<i>Anopheles funestus</i>	AFUN016038
<i>Anopheles gambiae</i>	AGAP005010
<i>Anopheles maculatus</i>	AMAM012126
<i>Anopheles melas</i>	AXCO02001637
<i>Anopheles merus</i>	AMEM019330
<i>Anopheles minimus</i>	AMIN011495
<i>Anopheles quadriannulatus</i>	AQUA016748
<i>Anopheles sinensis</i>	KE525340
<i>Anopheles stephensi</i>	ASTEI09300
<i>Culex pipiens pallens</i>	LOC120426920
<i>Culex quinquefasciatus</i>	CPIJ000125
Amphibians	None
Arthropods (non-mosquito)	None
Birds	None
Fish	None
Fungi	None
Human	None
Mammals	None
Plants	None
Reptiles	None

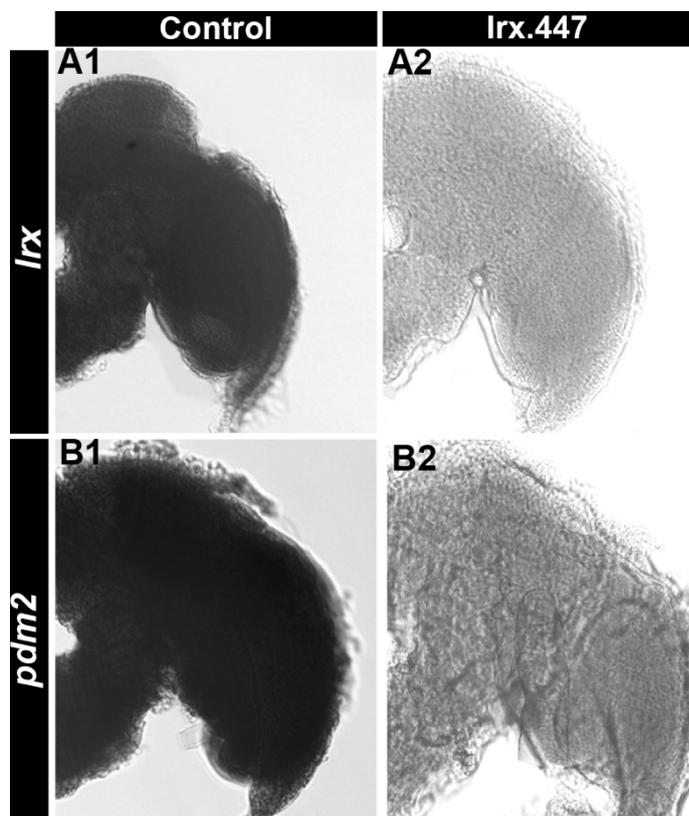
\* The *Irx.447* target site is identically conserved in multiple disease vector mosquito *Irx* genes, but this identical 21 bp target site was not detected in the genomes of non-target species in the National Center Biotechnology Information (NCBI) database [47].

**Table S2.** Mosquito ATSB feeding rates observed in laboratory simulated field trials.

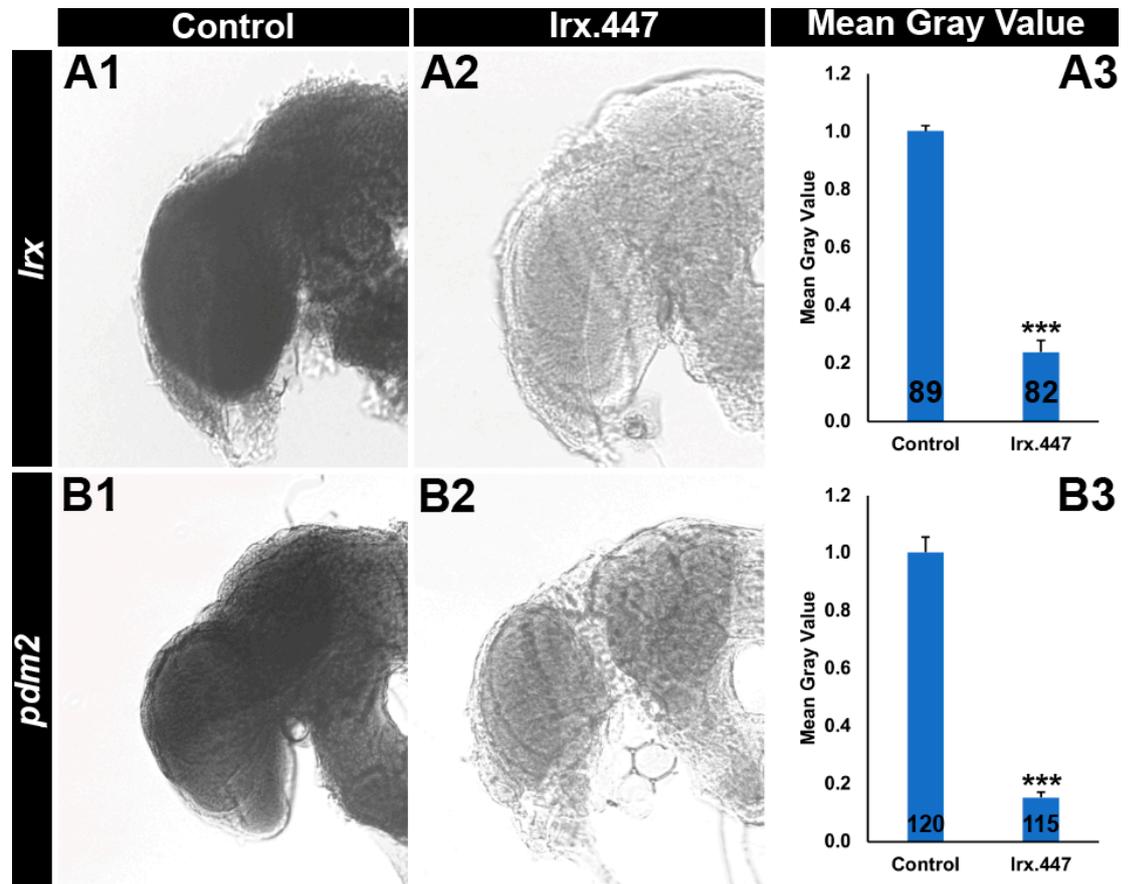
	Species	Feeding rate (%)		n/treatment
		Control	<i>Irx.447</i>	
siRNA/ATSB	<i>Aedes aegypti</i> *	57 ± 7	63 ± 12	68
	<i>Aedes aegypti</i>	100 ± 0	100 ± 0	150
yeast/ATSB	<i>Anopheles gambiae</i>	100 ± 0	100 ± 0	75
	<i>Culex quinquefasciatus</i>	100 ± 0	100 ± 0	225
	<i>Aedes albopictus</i>	87 ± 0.5	87 ± 2	225

\* The percentages of total insects that took sugar meals including each of the indicated treatments are shown. The species of mosquito, feeding rates with SEMs, as well as the n number of individuals receiving each treatment are shown. No significant differences

between feeding rates for the insecticidal or control treatments were identified (Student's t-test).



**Figure S1.** *Irx.447* yeast ATSB induces target gene silencing and decreased *pdm2* expression in *A. aegypti*. Consumption of *Irx.447* yeast ATSB (**A2,B2**) resulted in a significant loss of *Irx* (**A2**) and *pdm2* (**B2**) transcripts in the adult female *A. aegypti* brain (compare to control-treated female brains in **A1** and **B1**).



**Figure S2.** *Irx.447* yeast ATSB results in target gene silencing and significantly impacts *pdm2* expression in *A. gambiae*. Consumption of *Irx.447* yeast ATSB resulted in a significant reduction in *Irx* (A2,A3) and *pdm2* (B2,B3) transcripts in the adult female *A. gambiae* brain (compare to control-treated female brains in A1 and B1), as evidenced by mean gray value analyses (A3,B3). The numbers of brains analyzed (*n*) are specified below each bar in the graphs; \*\*\* =  $p < 0.001$  vs. control (Student's *t*-test).

## References

- Sayers, E.W.; Beck, J.; Bolton, E.E.; Bourexis, D.; Brister, J.R.; Canese, K.; Comeau, D.C.; Funk, K.; Kim, S.; Klimke, W.; et al. Database resources of the national center for biotechnology information. *Nucleic Acids Res.* **2021**, *49*, D10–D17. <https://doi.org/10.1093/nar/gkaa892>.