



**Figure S1. Diagram of the female tTA overexpression system.**

(A) Diagram of the FL3 gene construct. tTA expression is driven by a core promoter and upstream enhancer that contains 21 copies of the tTA binding site from the tet operator (tetO). The tTA gene contains the *Cochliomyia hominivorax tra* intron, which is sex-specifically spliced. The ZsGreen marker gene is driven by the constitutive *Lucilia cuprina hsp83* promoter. The gene construct contains the 5' and 3' ends of the *piggyBac* transposon for germline transformation.

(B) Schematic illustration of the female lethal system. In females, the *C. hominivorax tra* intron is correctly spliced out and functional tTA protein is produced. In the absence of tetracycline, tTA binds to the tetOx21 enhancer site to promote production of more tTA. tTA bound to the tetOx21 enhancer also increases expression of the linked ZsGreen marker gene. In males, the *tra* intron is alternatively spliced incorporating an additional exon that contains multiple in frame translation stop codons.