Supporting Information for

Okamoto KW, Robert MA, Gould F, Lloyd AL (2014) Feasible Introgression of an Anti-pathogen Transgene into an Urban Mosquito Population without Using Gene-Drive. PLoS Negl Trop Dis 8(7): e2827. doi:10.1371/journal.pntd.0002827

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Figure S1.

The average reduction in vector-competent females from 30 simulation runs two years after releases end when both male and female transgenic mosquitoes are released (A) at all sites for a single year, (B) at all sites for three years, (C) at 10% of the sites across a regular grid for a single year, and (D) at 10% of the sites across a regular grid for a single year, and (D) at 10% of the sites across a regular grid for three years. In panels (C) and (D), males and females are released in equal numbers. In all panels, the black lines represent results in the presence of a fitness cost associated with the anti-pathogen transgene, and grey lines represent the results assuming no fitness cost. The end points on the error bars represent the 2.5th and 97.5th percentile abundances across simulation runs.



Figure S2.

A comparison of the reduction in competent female vectors across three transgenic control strategies: direct population reduction (an FK strategy aiming exclusively at population reduction), a "reduce and replace" (an RR strategy) strategy, and the "replacement-alone" strategy (an AP strategy), when adult male transgenic mosquitoes are released in all sites, which is the scenario that provided the highest extinction rates for transgenic control measures based on vector population reduction alone (e.g., Legros et al. 2012we note that female releases are assumed to be undesirable when released females carry only a conditionally-lethal gene). In both panels, the lines represent the number of vector competent females averaged over all 30 model runs, and thus incorporate simulations resulting in population extinction. Panel (A) describes the results of modeling the release of 4 males per site for a single and three years, while panel (B) describes the results of modeling the release of 8 males per site for a single year. In both panels, grey lines represent an AP strategy, black lines represent an RR strategy, and blue lines represent a strategy based on population reduction alone. For the RR and AP strategies (black and grey lines), solid lines represent scenarios with a fitness cost associated with the anti-pathogen transgene, while dashed lines represent scenarios where no fitness cost is associated with the anti-pathogen transgene. For the strategy based on population reduction alone, the solid line represents a single year release while the dashed line represents a three year release. Here, and in Supporting Information Figure S3, we assume that the conditionally-lethal construct carries no additional fitness costs beyond dominant female adult lethality.

Reference: Legros, M., Xu, C., Okamoto, K., Scott, T.W., Morrison, A.C., Lloyd, A.L. & Gould, F. (2012). Assessing the feasibility of controlling *Aedes aegypti* with transgenic methods: A model-based evaluation. PLoS ONE **7**(12): e52235



Figure S3.

The average number of vector competent mosquitoes for each day across 30 simulation runs modeling "reduce and replace" (RR) transgenic control strategies and a strategy releasing mosquitoes carrying the anti-pathogen construct alone (an AP strategy). The same release regimes are analyzed as in Figure 4 in the main text, but unlike Figure 4 in the main text, simulations resulting in population elimination are not omitted in calculating the daily average number of vector competent mosquitoes. (A) adult males are released at all sites, (B) adult male and adult female releases at all sites, (C) eggs are released in 10% of the sites along a regular grid and (D) adult males and females are released in 10% of the sites along a regular grid. In all panels, solid lines represent releases for a single year and dashed lines represent 3 years releases. RR releases are illustrated in black and AP only releases are in grey. The numbers of mosquitoes released per site for the illustrated time series are: (A) 2 adult males, (B) 1 adult male and 1 adult female, (C) 100 eggs, (D) 10 adult males and 10 adult females.



Figure S4.

The average number of vector competent mosquitoes for each day across 30 simulation runs modeling "reduce and replace" (RR) transgenic control strategies and a strategy releasing mosquitoes carrying the anti-pathogen construct alone (an AP strategy) under differing fitness costs associated with the anti-pathogen gene. The same release regimes are analyzed as in Figure 5 in the main text, but unlike Figure 5 in the main text, simulations resulting in population elimination are not omitted in calculating the daily average number of vector competent mosquitoes. The results are for (A) adult male releases at all sites, (B) adult male and adult female releases at all sites, (C) adult male releases at 10% of the sites along a regular grid, and (D) adult male and adult female releases at 10% of the sites along a regular grid, and (D) adult male and adult female releases cost associated with the anti-pathogen gene, solid lines represent the absence of a fitness cost per copy). RR releases are illustrated in black and AP-only releases are in grey. We illustrate the time series from a single year of releases with per-site release numbers of (A) 7 adult males, (B) 1 adult male and 1 adult female, and (C) 80 adult males, and (D) 15 adult males.



Figure S5.

An illustration of minimal spatial variation in the dynamics of transgenic and wild-type adult females through space and time for a single run. Results are from a simulated weekly release of 6 males at all sites for one year. The anti-pathogen transgene is assumed to carry a fitness cost of 5% per copy. Colors represent the frequency of female adults carrying the anti-pathogen gene at each site, from blue (wild-type only) through red (anti-pathogen gene is at fixation). The number at the top of each panel represents the frequency of adult females carrying the anti-pathogen gene on the corresponding date.



Figure S6.

A comparison of the average reduction across 10 simulation runs in competent female vectors with and without pre-release traditional control interventions (e.g., chemical insecticide spraying). The dashed line represents the reduction in the absence of a pre-release traditional control intervention, the dotted line represents the reduction with a pre-release traditional control intervention lasting two weeks, and the solid line represents the reduction assuming a pre-release traditional control intervention lasting two months before releases begin. The simulations model the release of 100 eggs in 10% of the sites randomly determined for a single year. In all control situations, the anti-pathogen transgene is assumed to carry a fitness cost of 5% per copy.

Supplementary Information S1 Measuring the release area for 1 the Wolbachia-based field trials $\mathbf{2}$

3 Because [1] do not specifically report the size of the areas where releases occurred, we estimated the size of the release area based on the regions illustrated in Supplementary Fig. S2 of [1]. We manually traced 45the perimeter of the release regions specified in [1] and used an automated area calculator ([2]) based on 6 Google Maps ([3]) to estimate the size of the release area enclosed within the perimeter. Based on this 7analysis, we found the total release area to encompass a region of approximately 200 hectares.

References 8

1. Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, et al. (2011) Successful 9 10establishment of Wolbachia in Aedes populations to suppress dengue transmission. Nature 476: 454-7.

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