Supporting Information

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miRNA Design and Assembly

The *D. melanogaster* miRNA mir 6.1 stem loop was modified to target four sites in the *D. suzukii* myd88 5′UTR as previously described (34, 53). The *D. suzukii* myd88 gene ortholog was identified using the Augustus gene prediction tool (54), and sites were selected in the region spanning the ~300 bp upstream of the start codon, which was presumed to be the 5′UTR. To generate mir6.1 stem-loop backbones that create mature miRNAs complementary to each of these target sites, pairs of primers were annealed and products were utilized for two subsequent rounds of PCR and cloned into the pFusA backbone (from the Golden Gate TALEN and TAL Effector Kit 2.0, Addgene 1000000024) using Golden Gate assembly (55) to generate plasmid OA-961C. Assembled miRNAs were then subcloned into final plasmid OA-961B using PacI and FseI. The *D. suzukii* myd88 5′UTR region with target sequences, and sequences of primers used in the miRNA cloning, are listed in Tables S1 and S2, respectively.

Fly Culture and Strains

D. suzukii WT flies from Corvallis, OR, were maintained under 12L:12D conditions at 20 °C and 50% ambient humidity on a modified cornmeal medium, the recipe for which was obtained from the A. Ray Lab at University of California, Riverside. Rainbow Transgenics carried out the injections of construct OA-961B; the construct, along with a source of transposase, was injected into *D. suzukii* embryos using standard *D. melanogaster* injection procedures, and the surviving G_0 adults were individually outcrossed to WT individuals. G_1 progeny were screened for the presence of the *Medea* element (as evidenced by ubiquitous dsRed expression and eye-specific eGFP expression), and one G_1 transformant male was recovered and outcrossed to WT *D. suzukii* females.

Medea Genetic Behavior

To determine the genetic behavior of the *Medea* element in *D. suzukii* flies, male and female G_2 progeny from the single obtained G_1 transgenic male were individually outcrossed to WT (non-*Medea*-bearing, +/+) *D. suzukii* flies, and the resulting progeny were scored for the presence of the *Medea* element; this process was repeated until the G6 generation, and the resulting data are presented in Table 1. To assess whether the *Medea* system would function well in geographically distinct populations of *D. suzukii*, heterozygous *Medea*/+ males were individually introgressed with virgin females from eight geographically distinct *D. suzukii* populations, and resulting heterozygous *Medea*/+ virgins were crossed back to males from the eight populations to determine whether the *Medea* element functioned as expected (Fig. 2). A total of 1,319 progeny were counted, and 96.4% (50% expected given traditional Mendelian inheritance) were *Medea*-bearing.

Embryo Viability Determination

For embryo viability counts (Table 2), adult virgin females were mated with males of the relevant genotypes for 2–3 d in egg collection chambers with plates containing modified cornmeal medium, supplemented with dry yeast. Then, an overnight egg collection was carried out, after first having cleared old eggs from the females through a precollection period on a separate plate for several hours. All embryos (between 165–301) were counted and kept on an agar surface at 20 °C for 48 h. The percentage survival was then determined by counting the number of unhatched embryos. Each experiment was carried out in duplicate, and the results presented are averages from these two experiments. Embryo survival was normalized with respect to the percentage survival observed in parallel experiments carried out with WT flies, which was 91.63 ± 0.26% (percentage survival ± SD). For adult fly counts (Table 2), the adult flies used for each embryo count assay replicate were transferred from egg collection plates to 250-mL bottles containing modified cornmeal medium, allowed to lay eggs for 24 h, and then removed. One hundred percent of the resulting progeny (between 116–206 progeny) from these bottles were counted, and the results of the two replicates for each experiment were averaged together.

Population Cage Experiments

To determine whether the generated *D. suzukii Medea* is capable of spreading through populations, population cage experiments were set up as follows. Heterozygous *Medea*/+ males and virgin females were crossed to each other for multiple generations to generate homozygous stocks; homozygosity was confirmed by outcrossing. Then three types (low, medium, and high threshold, the first two in triplicate and the last in quadruplicate) of drive experiments were set up by crossing *Medea*-bearing males with WT flies of the strain from Corvallis, OR of a similar age in 250-mL bottles containing modified cornmeal medium. Low-frequency drives had 25 heterozygous *Medea*/+ and 25 WT +/+ males mated to 50 WT +/+ virgins, *Medea* allele frequency of ~12.5%; medium-frequency drives had 50 heterozygous *Medea*/+ males mated to 50 WT +/+ virgins, *Medea* allele frequency of ~25%; and high-frequency drives had 50 homozygous *Medea*/*Medea* males mated to 50 WT +/+ virgins, *Medea* allele frequency of ~50%. The total number of flies for each starting population was 100. After being placed together, adult flies were removed after 9 d. After another 7 d, half of the progeny (randomly selected) were counted, and the other half were placed in a new bottle to continue the simulation, and this process continued throughout the duration of the experiment. All fly experiments were carried out in the conditions described above.

Target Site Genotype Screening

To identify the genotypes of the four miRNA target sites of various flies from the *Medea* outcrosses and drive experiment and of different *D. suzukii* strains, genomic DNA was extracted from individual flies with the DNeasy Blood & Tissue kit (Qiagen) following the manufacturer's protocol. PCR was conducted using standard procedures to amplify the target loci using primers 807G and 807H (Table S2), which amplified a region of ~550 bp in the myd88 5′UTR region. The PCR program utilized was as follows: 98 °C for 30 s; 35 cycles of 98 °C for 10 s, 57 °C for 20 s, and 72 °C for 30 s; then 72 °C for 10 min. The PCR products were purified with the MinElute PCR Purification Kit (Qiagen) according to the manufacturer's protocol and sent for Sanger sequencing (SourceBioScience) using the same primers as utilized for the PCR. Target site sequences were analyzed and aligned with DNAStar software.

Model Fitting

We modeled *Medea* dynamics under laboratory cage conditions assuming random mating and discrete generations. To model resistance to the maternal toxin, we considered a *Medea* allele, “M,” and an unlinked toxin-resistant allele, “R,” that diminishes toxin efficacy in mothers having at least one copy of the *Medea* allele. We denote the absence of the *Medea* allele by “m” and the absence of the toxin-resistant allele by “r.” Consequently, there are nine possible genotypes: MMRR, MMRr, MMrr, MmRR, MmRr, Mmrr, mmRR, mmRr, and mmrr. We denote the proportion of organisms having each genotype at the *k*th generation by p_k^x, where x denotes one of the nine genotypes.

Given the large number of possible mating pairs (81), it is not feasible to show the complete equations for the next generation genotype frequencies here, so we instead describe them in brief. We considered the M and R loci as being independently inherited and following Mendelian inheritance rules, with the exception that mm offspring of mothers heterozygous for the *Medea* allele have reduced viability. If a *Medea*-heterozygous mother does not have any copies of the resistant allele (i.e., has the genotype Mmrr), the viability of their mm offspring is reduced by 100%; however, if the mother has two copies of the resistant allele (i.e., has the genotype MmRR), then the viability of their mm offspring is reduced by a fraction, e_R, denoting the maternal toxin efficiency in the presence of the resistant allele. We considered two models for maternal toxin efficiency in MmRr mothers; in model A the toxin efficiency is e_R, and in model B the toxin efficiency is $(1 + e_R)/2$ (i.e., midway between that of MmRR and Mmrr mothers).

These considerations allow us to calculate the expected genotype frequencies in the next generation before accounting for fitness costs. Let us denote these frequencies by $\hat{p}_{k+1}^x$, where x denotes the genotype and $k + 1$ denotes the next generation. Normalizing these ratios to account for a fitness cost, s_{Het}, associated with being heterozygous for the *Medea* allele, and s_{Hom}, associated with being homozygous for the *Medea* allele, the genotype frequencies in the next generation are given by

$$\left(p_{k+1}^{MmRR}, p_{k+1}^{MmRr}, p_{k+1}^{Mmrr}\right) = \left(\hat{p}_{k+1}^{MmRR}, \hat{p}_{k+1}^{MmRr}, \hat{p}_{k+1}^{Mmrr}\right)(1 - s_{Het})/W_{k+1}, \tag{S1}$$

$$\left(p_{k+1}^{MMRR}, p_{k+1}^{MMRr}, p_{k+1}^{MMrr}\right) = \left(\hat{p}_{k+1}^{MMRR}, \hat{p}_{k+1}^{MMRr}, \hat{p}_{k+1}^{MMrr}\right)(1 - s_{Hom})/W_{k+1}, \tag{S2}$$

$$\left(p_{k+1}^{mmRR}, p_{k+1}^{mmRr}, p_{k+1}^{mmrr}\right) = \left(\hat{p}_{k+1}^{mmRR}, \hat{p}_{k+1}^{mmRr}, \hat{p}_{k+1}^{mmrr}\right)/W_{k+1}. \tag{S3}$$

Here, W_{k+1} is a normalizing term given by

$$W_{k+1} = \left(\hat{p}_{k+1}^{MmRR} + \hat{p}_{k+1}^{MmRr} + \hat{p}_{k+1}^{Mmrr}\right)(1 - s_{Het}) + \left(\hat{p}_{k+1}^{MMRR} + \hat{p}_{k+1}^{MMRr} + \hat{p}_{k+1}^{MMrr}\right)(1 - s_{Hom}). + \left(\hat{p}_{k+1}^{mmRR} + \hat{p}_{k+1}^{mmRr} + \hat{p}_{k+1}^{mmrr}\right). \tag{S4}$$

Note that here we have assumed equal fitness costs due to the *Medea* element in males and females and have assumed no fitness cost due to the toxin-resistant allele. The fitness costs due to the *Medea* element represent average fitness costs in males and females and may originate from the maternal toxin, zygotic antidote (the promoter for which is expressed in both males and females), marker gene (also expressed in both males and females) and/or insertion site. While these fitness costs may differ between the sexes, we do not expect to be able to resolve this difference from the population cage data, which do not keep track of sex, and so present them as averages here. We assumed that the toxin-resistant allele does not have a significant fitness cost as it is located in the 5′UTR of the target gene and not its coding sequence.

The likelihood of the population cage data was calculated by assuming a binomial distribution of WT and *Medea*-bearing individuals and by using the model predictions to generate expected proportions for each set of parameter values (i.e., by calculating the log likelihood):

$$\begin{aligned} \log L(\theta) = \sum_{i=1}^{10} \sum_{k=1}^{n_i} \log \binom{M_{i,k} + \mathrm{WT}_{i,k}}{M_{i,k}} &+ \mathrm{WT}_{i,k} \log\left(p_k^{mmRR}(\theta) + p_k^{mmRr}(\theta) + p_k^{mmrr}(\theta)\right) \\ &+ M_{i,k} \log\left(\hat{p}_k^{MMRR}(\theta) + \hat{p}_k^{MMRr}(\theta) + \hat{p}_k^{MMrr}(\theta) + p_k^{MmRR}(\theta) + p_k^{MmRr}(\theta) + p_k^{Mmrr}(\theta)\right). \end{aligned} \tag{S5}$$

Here, $M_{i,k}$ and $\mathrm{WT}_{i,k}$ are the number of *Medea*-bearing and WT individuals at generation k in experiment i, the ith experiment is run for n_i generations, and the expected genotype frequencies are dependent on the model parameters $\theta = \{e_R, p_R, s_{Het}, s_{Hom}\}$, where e_R, s_{Het}, and s_{Hom} are as defined earlier and p_R is the resistant allele frequency at the beginning of the experiment. The initial condition for each experiment was such that, for the *Medea* allele, heterozygote frequency was determined according to the first generation data with the remaining individuals being WT, and for the resistant allele Hardy–Weinberg equilibrium was assumed with a resistant allele frequency of p_R and the resistant allele being independently distributed from the *Medea* allele.

Prior information on *Medea* toxin efficiency in *Medea*-resistant mothers was inferred from G_5 and G_6 outcrosses in which heterozygous *Medea* females were mated with WT males and the proportion of WT offspring, $p(\mathrm{WT})$, was nonzero. In *Medea*-resistant mothers, for a toxin efficiency of e_R, the ratio of WT to *Medea*-bearing offspring should be $(1 - e_R) : 1$, and hence the proportion of WT offspring should be $(1 - e_R)/(1 + 1 - e_R)$. Rearranging this equation, then toxin efficiency in terms of $p(\mathrm{WT})$ is given by $e_R = (1 - 2p(\mathrm{WT}))/(1 - p(\mathrm{WT}))$. Using this equation, we calculated the mean and variance of toxin efficiency in *Medea*-resistant mothers from the results of 17 G_5 and G_6 outcrosses displaying resistance, from which we parameterized a normally distributed prior on e_R.

We used a Bayesian MCMC sampling procedure to estimate our model parameters, including 95% credible intervals, and used the deviance information criterion (DIC) as a criterion to select between the two models for toxin efficiency in MmRr mothers. Following ref. 1, we calculated the DIC as

$$\mathrm{DIC} = -2 \log L(\overline{\theta}) + 2p_D. \tag{S6}$$

Here, $\overline{\theta}$ is the posterior mean of the model parameters and p_D is the effective number of parameters as inferred from the MCMC chain, which may be calculated as

$$p_D = 2\mathrm{Var}(\log L(\theta)). \tag{S7}$$

This favored model B, in which the toxin efficiency in MmRr mothers is midway between that of MmRR and Mmrr mothers (the model with smallest DIC value is favored and model A had a DIC value of 1,146.5, while model B had a DIC value of 1,145.3).

Model Predictions

We modeled the expected dynamics of the generated *Medea* element and one with its fitness costs halved in both a fully *Medea*-susceptible population and a fully *Medea*-resistant population. Under these scenarios, a simpler model could be used since the resistant allele frequency is not expected to change. Assuming random mating and discrete generations, the proportions of the kth generation that are WT, heterozygous, and homozygous for *Medea* are denoted by p_k^{mm}, p_k^{Mm}, and p_k^{MM}, respectively. Considering all possible mating pairs, and taking into account that most WT offspring of heterozygous mothers are unviable—the viable fraction is denoted by $(1-e)$, where e represents the maternal toxin efficiency—the genotypes of embryos in the next generation are described by the ratio $\hat{p}_{k+1}^{mm}:\hat{p}_{k+1}^{Mm}:\hat{p}_{k+1}^{MM}$, where

$$\hat{p}_{k+1}^{mm} = \left(p_k^{mm}\right)^2 + 0.5p_k^{mm}p_k^{Mm}(2-e) + 0.25\left(p_k^{Mm}\right)^2(1-e), \tag{S8}$$

$$\hat{p}_{k+1}^{Mm} = 2p_k^{mm}p_k^{MM} + 0.5\left(p_k^{Mm}\right)^2 + p_k^{mm}p_k^{Mm} + p_k^{MM}p_k^{Mm}, \tag{S9}$$

$$\hat{p}_{k+1}^{MM} = \left(p_k^{MM}\right)^2 + p_k^{MM}p_k^{Mm} + 0.25\left(p_k^{Mm}\right)^2. \tag{S10}$$

Toxin efficiency, e, equals 1 in a fully *Medea*-susceptible population, e_R in a fully *Medea*-resistant population, and 0 for a non-*Medea* allele. Normalizing these ratios and taking into account fitness costs, the genotype frequencies in the next generation are given by

$$p_{k+1}^{mm} = \hat{p}_{k+1}^{mm}/W_{k+1}, \tag{S11}$$

$$p_{k+1}^{Mm} = \hat{p}_{k+1}^{Mm}(1-s_{Het})/W_{k+1}, \tag{S12}$$

$$p_{k+1}^{MM} = \hat{p}_{k+1}^{MM}(1-s_{Hom})/W_{k+1}. \tag{S13}$$

Here, s_{Het} and s_{Hom} represent the fitness costs associated with being heterozygous or homozygous for the *Medea* element, and W_{k+1} is a normalizing term given by

$$W_{k+1} = \hat{p}_{k+1}^{mm} + \hat{p}_{k+1}^{Mm}(1-s_{Het}) + \hat{p}_{k+1}^{MM}(1-s_{Hom}). \tag{S14}$$

The only further change to the model used for projections is that release proportion refers to a release of homozygous *Medea* males, with the remainder of the first generation being half WT males and half WT females. Denoting the release proportion by r_M, then the *Medea* genotype frequencies in the second generation are given by

$$p_2^{mm} = 0.5(1-r_M)/(0.5(1-r_M) + r_M(1-s_{Het})), \tag{S15}$$

$$p_2^{Mm} = r_M(1-s_{Het})/(0.5(1-r_M) + r_M(1-s_{Het})), \tag{S16}$$

$$p_2^{MM} = 0. \tag{S17}$$

The model described in Eqs. **S8–S14** applies from generation 3 onward.

Use of *Medea* to Introduce a Target Site for a Population-Suppressing Homing Construct

One potential strategy by which the *Medea* system could be used to suppress a population is to use the *Medea* element to introduce a target site for a population-suppressing homing construct that would be introduced subsequently. To model this strategy, we use the same framework as above with the addition of a homing allele, H, that targets an allele required in at least one copy for female fertility, the target site for which is introduced by the *Medea* element. This expands the number of possible genotypes from three to six (i.e., mm, Mm, MM, Hm, HM, and HH). We ignore the emergence of homing-resistant alleles in this analysis. Homing-resistant alleles have been observed as a result of imperfect cleavage and repair for all recently engineered homing constructs (e.g., refs. 2–4); however, in previous work (5) we showed that the rate of resistant allele generation must be extremely low in order for population suppression-based homing strategies to have a high chance of eliminating a population of small-to-moderate size (e.g., the rate must be less than ~10^{-5} imperfect copies per drive-mediated cleavage event to have a >90% chance of suppressing a population of ~10,000 insects). Furthermore, it is theoretically possible that resistant allele generation rates this low may be achievable through multiplexing guide RNAs within the homing construct (5), essentially making the resistant allele generation rate negligible. This assumption allows us to ignore model complications due to homing-resistant alleles and instead to focus on complications due to incomplete *Medea* spread before release of the homing system in the insect population.

Of note for the proposed strategy, homing only occurs in HM individuals due to the presence of both the homing construct (H) and its target site (M)—an important biosecurity feature given the potential invasiveness of homing systems. HM males and females therefore produce H gametes in the germline at a frequency equal to $(1 + h)/2$, where h denotes the "homing efficiency," which represents the proportion of M gametes that are converted into H gametes in the germline of HM heterozygotes through the act of homing. Ignoring homing-resistant alleles, HM individuals also produce M gametes at a frequency equal to $(1 - h)/2$, although this frequency is expected to be very low in the presence of multiplexed guide RNAs. All other individuals produce gametes at standard Mendelian frequencies, with the exception that HH females produce no viable offspring, and due to the action of the *Medea* construct most non-*Medea*-bearing (mm, Hm, and HH) offspring of *Medea*-heterozygous (Mm and HM) mothers are unviable, with the fraction unviable determined by the *Medea* toxin efficiency, e, as described above. To favor the spread of the homing allele, we also consider the case in which the homing construct includes a copy of the zygotic antidote present in the *Medea* allele (but not the maternal toxin). Under this scenario, Hm offspring of Mm mothers and Hm and HH offspring of HM mothers are fully viable, while mm offspring of Mm mothers continue to have reduced viability.

Assuming random mating and discrete generations, the proportions of the kth generation that have the genotypes mm, Mm, MM, Hm, HM, and HH are denoted by p_k^{mm}, p_k^{Mm}, p_k^{MM}, p_k^{Hm}, p_k^{HM}, and p_k^{HH}, respectively. Considering all possible mating pairs, and taking into account homing in the germline of HM individuals, the infertility of HH females, and the offspring having reduced viability due to the action of the *Medea* construct, the genotypes of embryos in the next generation are described by the ratio $\hat{p}_{k+1}^{mm}:\hat{p}_{k+1}^{Mm}:\hat{p}_{k+1}^{MM}:\hat{p}_{k+1}^{Hm}:\hat{p}_{k+1}^{HM}:\hat{p}_{k+1}^{HH}$, where

$$\hat{p}_{k+1}^{mm} = \left(p_k^{mm}\right)^2 + 0.5p_k^{mm}p_k^{Mm}(2-e) + 0.25\left(p_k^{Mm}\right)^2(1-e) + p_k^{mm}p_k^{Hm} + 0.25\left(p_k^{Hm}\right)^2 + 0.25p_k^{Hm}p_k^{Mm}(2-e_{NZ}), \tag{S18}$$

$$\hat{p}_{k+1}^{Mm} = 2p_k^{mm}p_k^{MM} + 0.5\left(p_k^{Mm}\right)^2 + p_k^{mm}p_k^{Mm} + p_k^{MM}p_k^{Mm} + 0.5p_k^{Mm}p_k^{Hm} + p_k^{MM}p_k^{Hm} + (1-h)p_k^{mm}p_k^{HM} + 0.5(1-h)p_k^{Mm}p_k^{HM} + 0.5(1-h)p_k^{Hm}p_k^{HM}, \tag{S19}$$

$$\hat{p}_{k+1}^{MM} = \left(p_k^{MM}\right)^2 + p_k^{MM}p_k^{Mm} + 0.25\left(p_k^{Mm}\right)^2 + 0.5(1-h)p_k^{Mm}p_k^{HM} + (1-h)p_k^{MM}p_k^{HM} + 0.25(1-h)^2\left(p_k^{HM}\right)^2. \tag{S20}$$

$$\begin{aligned}\hat{p}_{k+1}^{Hm} = {} & p_k^{mm}p_k^{HH} + 0.5\left(p_k^{Hm}\right)^2 + p_k^{mm}p_k^{Hm} + 0.5p_k^{HH}p_k^{Hm} + 0.25p_k^{Mm}p_k^{Hm}(2-e_{NZ}) + 0.5p_k^{HH}p_k^{Mm}(1-e_{NZ}) + 0.5(1+h)p_k^{mm}p_k^{HM}(2-e_{NZ}) \\ & + 0.5(1+h)p_k^{Mm}p_k^{HM}(1-e_{NZ}) + 0.25(1+h)p_k^{Hm}p_k^{HM}(2-e_{NZ}),\end{aligned} \tag{S21}$$

$$\begin{aligned}\hat{p}_{k+1}^{HM} = {} & p_k^{MM}p_k^{HH} + 0.5(1+h)(1-h)\left(p_k^{HM}\right)^2 + (1+h)p_k^{MM}p_k^{HM} + 0.5(1-h)p_k^{HH}p_k^{HM} + p_k^{MM}p_k^{Hm} + 0.5p_k^{HH}p_k^{Mm} + 0.5(1+h)p_k^{Mm}p_k^{HM} \\ & + 0.5p_k^{Mm}p_k^{Hm} + 0.5(1-h)p_k^{Hm}p_k^{HM},\end{aligned} \tag{S22}$$

$$\hat{p}_{k+1}^{HH} = 0.5(1+h)p_k^{HH}p_k^{HM}(1-e_{NZ}) + 0.5p_k^{HH}p_k^{Hm} + 0.25\left(p_k^{Hm}\right)^2(1-e_{NZ}) + 0.25(1+h)p_k^{Hm}p_k^{HM}(2-e_{NZ}) + 0.25(1+h)^2\left(p_k^{HM}\right)^2. \tag{S23}$$

All terms in these equations are as described earlier, with the addition of *Medea* toxin efficiency with no zygotic antidote, e_{NZ}, which is equal to e for a homing construct having no zygotic antidote and 0 for a homing construct having the antidote. Normalizing these ratios and taking into account fitness costs due to the *Medea* allele, the genotype frequencies in the next generation are given by

$$\left(p_{k+1}^{mm}, p_{k+1}^{Hm}, p_{k+1}^{HH}\right) = \left(\hat{p}_{k+1}^{mm}, \hat{p}_{k+1}^{Hm}, \hat{p}_{k+1}^{HH}\right)/W_{k+1}, \tag{S24}$$

$$\left(p_{k+1}^{Mm}, p_{k+1}^{HM}\right) = \left(\hat{p}_{k+1}^{Mm}, \hat{p}_{k+1}^{HM}\right)(1-s_{Het})/W_{k+1}, \tag{S25}$$

$$p_{k+1}^{MM} = \hat{p}_{k+1}^{MM}(1-s_{Hom})/W_{k+1}. \tag{S26}$$

Here, s_{Het} and s_{Hom} represent the fitness costs associated with being heterozygous or homozygous for the *Medea* element, and W_{k+1} is a normalizing term given by

$$W_{k+1} = \hat{p}_{k+1}^{mm} + \hat{p}_{k+1}^{Hm} + \hat{p}_{k+1}^{HH} + \left(\hat{p}_{k+1}^{Mm} + \hat{p}_{k+1}^{HM}\right)(1-s_{Het}) + \hat{p}_{k+1}^{MM}(1-s_{Hom}). \tag{S27}$$

For simplicity, we have assumed no additional fitness costs due to the homing allele, although these could easily be accommodated.

The release of MM males in the first generation at a proportion equal to r_M is as described in Eqs. **S15**–**S17**. The only further change to the model is to accommodate a release of males homozygous for the homing construct once the *Medea* system has driven the homing system target site into the population. Denoting the release generation for the homing construct by x and the release proportion by r_H, we must adjust Eqs. **S18**–**S27** to account for the increased proportion of fertile HH males that contribute to the $(x + 1)th$ generation. To accommodate this, Eqs. **S21**–**S23** become

$$\begin{aligned}\hat{p}_{x+1}^{Hm} = {} & p_x^{mm}\left(p_x^{HH}+2r_H/(1-r_H)\right)+0.5\left(p_x^{Hm}\right)^2+p_x^{mm}p_x^{Hm}+0.5\left(p_x^{HH}+2r_H/(1-r_H)\right)p_x^{Hm}+0.25p_x^{Mm}p_x^{Hm}(2-e_{NZ})+0.5\left(p_x^{HH}\right. \\ & \left.+2r_H/(1-r_H)\right)p_x^{Mm}(1-e_{NZ})+0.5(1+h)p_x^{mm}p_x^{HM}(2-e_{NZ})+0.5(1+h)p_x^{Mm}p_x^{HM}(1-e_{NZ})+0.25(1+h)p_x^{Hm}p_x^{HM}(2-e_{NZ}),\end{aligned} \tag{S28}$$

$$\begin{aligned}\hat{p}_{x+1}^{HM} = {} & p_x^{MM}\left(p_x^{HH}+2r_H/(1-r_H)\right)+0.5(1+h)(1-h)\left(p_x^{HM}\right)^2+(1+h)p_x^{MM}p_x^{HM}+0.5(1-h)p_x^{HM}\left(p_x^{HH}+2r_H/(1-r_H)\right)+p_x^{MM}p_x^{Hm} \\ & +0.5p_x^{Mm}\left(p_x^{HH}+2r_H/(1-r_H)\right)+0.5(1+h)p_x^{Mm}p_x^{HM}+0.5p_x^{Mm}p_x^{Hm}+0.5(1-h)p_x^{Hm}p_x^{HM},\end{aligned} \tag{S29}$$

$$\begin{aligned}\hat{p}_{x+1}^{HH} = {} & 0.5(1+h)p_x^{HM}\left(p_x^{HH}+2r_H/(1-r_H)\right)(1-e_{NZ})+0.5p_x^{Hm}\left(p_x^{HH}+2r_H/(1-r_H)\right)+0.25\left(p_x^{Hm}\right)^2(1-e_{NZ})+0.25(1+h)p_x^{Hm}p_x^{HM}(2-e_{NZ}) \\ & +0.25(1+h)^2\left(p_x^{HM}\right)^2.\end{aligned} \tag{S30}$$

Eqs. **S18**–**S20** and **S24**–**S27** apply to generation ($x + 1$) as they do to previous generations, and Eqs. **S18**–**S27** apply for generation ($x + 2$) onward. Due to the release of HH males at generation x, the genotype frequencies at the xth generation should also be adjusted, following the calculation of genotype frequencies for the ($x + 1$)th generation, to be

$$p_x^{HH} \rightarrow p_x^{HH}(1-r_H)+r_H, \tag{S31}$$

$$\left(p_x^{mm}, p_x^{Mm}, p_x^{MM}, p_x^{Hm}, p_x^{HM}\right) \rightarrow \left(p_x^{mm}, p_x^{Mm}, p_x^{MM}, p_x^{Hm}, p_x^{HM}\right)(1-r_H). \tag{S32}$$

The results of this modeling are shown in Fig. S3. Results of another *Medea*-based population suppression strategy, in which *Medea* is used to introduce a conditional lethal gene into the population, are described in ref. 6.

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Strain	Target Sequence	# of Flies Sequenced (n)
Corvalis, OR (reference strain)	Target 1 Target 2 Target 3 Target 4 ATCTGAAAAAAATTAAAAAAAAATAGTAATA.....TCCCGCGCTTCATCGTTTTATT.....ACTGAAAAACGTCCCGTTGATAAATACATATATCATCG	**4**
Medea+ from drive (2 alleles)	ATCTGAAAAAAATTAAAAAAAAATAGTAATA.....TCACGCGCTTCATCGTTTTATT.....ACTGATAAACGTCCCGTTGATAAATACATATATCATCG ATCTGAAAAAAATTTAAAAAAAATAGTAATA.....TCACGCGCTTCATCGTTTTATT.....ACTGATAAACGTCCCGTTGATAAATACATATATCATCG	**28**
Medea- from drive (2 alleles)	ATCTGAAAAAAATTTAAAAAAAATAGTAATA.....TCACGCGCTTCATCGTTTTATT.....ACTGATAAACGTCCCGTTGATAAATACATATATCATCG ATCTGAAAAAAATTAAAAAAAAATAGTAATA.....TCACGCGCTTCATCGTTTTATT.....ACTGATAAACGTCCCGTTGATAAATACATATATCATCG A	**26**
Clayton, WA	ATCTGAAAAAAATTAAAAAAAA_TAGTAATA.....TCACGCGCTTCATCGTTTTATT.....ACTGATAAACGTCCCGTTGATAAATACATATATCATCG	**16**
Brentwood, CA	ATCTGAAAAAAATTAAAAAAAA_TAGTAATA.....TCACGCGCTTCATCGTTTTATT.....ACTGATAAACGTCCCGTTGATAAATACATATATCATCG	**10**
Oahu, HI	ATCTGAAAAAAATTAAAAAAAA_TAGTAATA.....TCACGCGCTTCATCGTTTTATT.....ACTGATAAACGTCCCGTTGATAAATACATATATCATCG	**9**
Enime, Japan	ATCTGAAAAAAATTTAAAAAAAATAGTAATA.....TCACGCGCTTCATCGTTTTATT.....ACTGATAAACGTCCCGTTGATAAATACATATATCATCG	**10**
Beltsville, MD	ATCTGAAAAAAATTAAAAAAAA_TAGTAATA.....TCACGCGCTTCATCGTTTTATT.....ACTGATAAACGTCCCGTTGATAAATACATATATCATCG	**6**
Mt. Hood, OR	ATCTGAAAAAAATTTAAAAAAAATAGTAATA.....TCACGCGCTTCATCGTTTTATT.....ACTGATAAACGTCCCGTTGATAAATACATATATCATCG	**14**
Watsonville, CA	ATCTGAAAAAAATTTAAAAAAAATAGTAATA.....TCACGCGCTTCATCGTTTTATT.....ACTGATAAACGTCCCGTTGATAAATACATATATCATCG	**21**
Tracy, CA	ATCTGAAAAAAATTAAAAAAAA_TAGTAATA.....TCACGCGCTTCATCGTTTTATT.....ACTGATAAACGTCCCGTTGATAAATACATATATCATCG	**9**

Fig. S1. Genomic DNA sequences of the myd88 5′UTR region of various strains/fly types targeted by the *Medea* toxin miRNAs. Green and black nucleotides represent sequence perfectly complementary to the miRNAs; red and other colored nucleotides represent specific mutations and target sites that are not perfectly complementary to the miRNAs, respectively. Target site four is not highlighted/underlined as it is perfectly conserved among all sequenced flies.

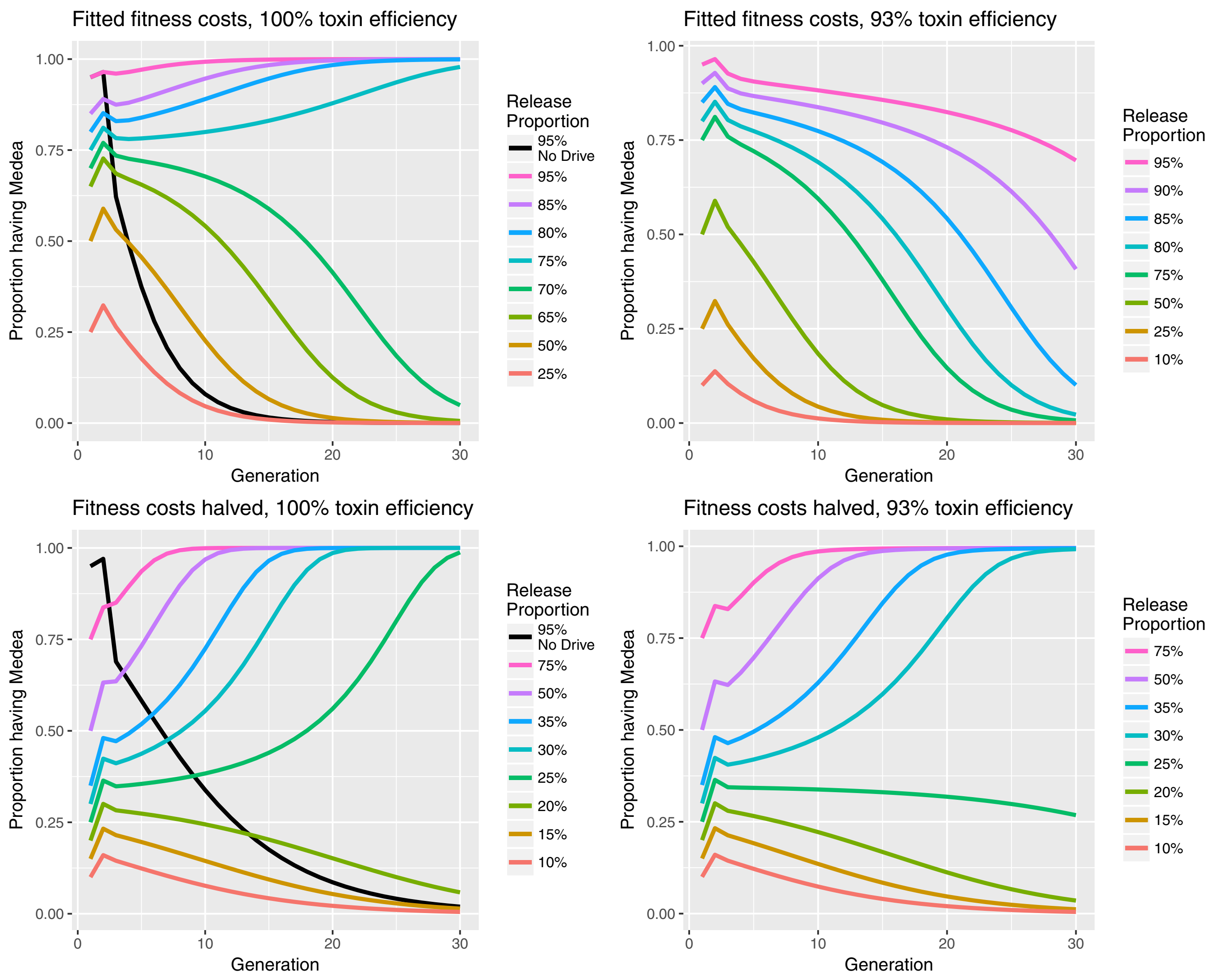

Fig. S2. Predicted dynamics of *D. suzukii Medea* element. In all cases, releases of homozygous *Medea* males are assumed, except for black lines, which describe the dynamics of an equivalent release of non-*Medea* males. (*A*) In a *Medea*-susceptible population, the generated element (toxin efficiency of 100%, heterozygote fitness cost of 28%, and homozygote fitness cost of 65%) displays threshold dynamics, spreading to fixation for release proportions of 73% or higher. (*B*) In a *Medea*-resistant population in which toxin efficiency is 93%, as inferred from the laboratory studies, the *Medea* element can be maintained at high frequencies following a high release proportion; however, its eventual elimination is inevitable unless supplemental releases are carried out. (*C*) In a *Medea*-susceptible population, if fitness costs are halved, the element displays threshold dynamics, spreading to fixation for release proportions of 23% or higher. (*D*) In a *Medea*-resistant population in which toxin efficiency is 93%, if fitness costs are halved, the critical release threshold is raised slightly to 25%.

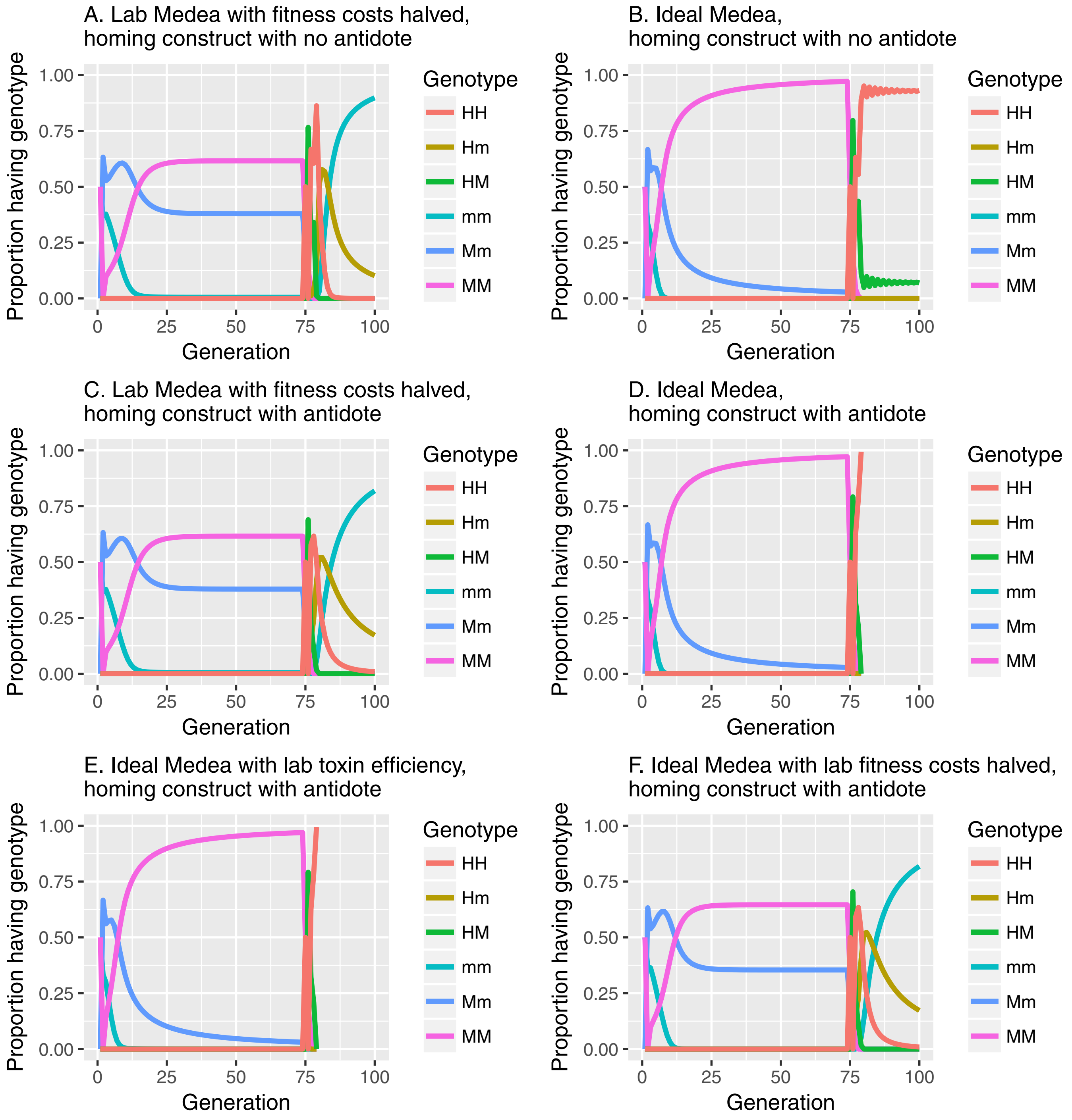

Fig. S3. Predicted dynamics of the *D. suzukii Medea* element followed by release of a population-suppressing homing construct. In all cases, releases of homozygous *Medea* males (MM) representing 50% of the population upon release (generation 1) are assumed, followed by releases of males homozygous for the homing construct (HH) representing 50% of the population upon release (generation 75). The WT genotype is represented by mm, and heterozygotes are represented by the genotypes Mm, Hm, and HM. The *Medea* element targets a gene required for female fertility and includes a recoded copy of this gene. It also includes a target site for the homing construct within the recoded copy of the female fertility gene. The laboratory *Medea* construct is assumed to have a toxin efficiency of 93% (i.e., its efficiency in a population having the *Medea*-resistant allele), a heterozygote fitness cost of 28%, and homozygote fitness cost of 65%. We modeled a homing construct with a homing efficiency of 99% and no associated fitness costs. We considered the case in which the homing construct includes a copy of the zygotic antidote for the maternal toxin within the *Medea* construct, in addition to the case that it does not. (*A*) For the laboratory *Medea* construct with fitness costs halved, the M allele does not reach fixation and the m allele prevents a population crash by acting as a homing-resistant allele following release of the homing construct. (*B*) For an ideal *Medea* construct (100% toxin efficiency and no fitness costs), the M allele spreads sufficiently for the m allele to be eliminated upon release of the homing construct; however, the H allele does not spread sufficiently to cause a population crash because HM offspring are selected for in matings between HH males and HM females as they have the antidote to the maternal toxin, while HH offspring do not. (*C*) Including a zygotic antidote in the homing construct does not help if the *Medea* construct has a significant fitness cost because the M allele still does not reach fixation and so the m still allele serves as a homing-resistant allele. (*D*) For an ideal *Medea* construct, however, the m allele is eliminated upon release

Legend continued on following page

of the homing construct and a population crash is achieved within a small number of generations for a homing construct having the zygotic antidote. (*E*) A population crash can still be achieved if the toxin efficiency is relaxed (here, it is 93%). (*F*) For this strategy to work, however, it is important that the fitness costs of the *Medea* construct are negligible, as otherwise the m allele remains in the population and serves as a homing-resistant allele (here, *Medea* fitness costs are half those of the laboratory construct).

Table S1. *D. suzukii* genomic DNA sequences used in constructing synthetic *Medea*

Sequence name	Sequence, 5′ to 3′
Myd88 5′UTR portion (miRNA target sites in bold)	CGCGACTGTGACGCGGCAAGGTGCTGGAGGTCACTCGGCCCATTTCGCATAGCAGCGCCACCCCCAACCCCCTTCCAGCCATTTCCAGTTCCAGTTCCAGACGCCCGCAAACAGCACGCGCGATCTGAAA**AAAATTAAAAAAAATAGTAATA**ATAGAAAAAAAAACACACCAGCAAAC**TCACGCGCTTCATCGTTTTATT**GCCCGCCCTCTCG**ACTGATAAACGTCCCGTTGATAAATACATA**TATCATCGCATCACACCAAAAACAAACACAGCAGCGCAGCAAACACCAATTATTCCAATAAAGCCAGCGCCAAGTGCGA
Myd88 CDs	ATGCGCCCTCGATTTGTATGCCATCAGCAGCACTCGGTGGCCCATTCCCACTTCCATCCCCTGTCTCACTTCCAGACCCATTCCCATACCCATACCCACTTCCAGCGCCATCCCAATCCCCATCCCCATCCCCATCACTTTTACGACGCCACTGACGTCGGCTATCGGCGTTATCGCACCGCCAACATGGTGGTGGCCGAGGGAGTTATGGACTCCGGATCGGGGATCGGTACGGGAATGGGAATGGGGCACTTCAACGAGACCCCCTTATCCGAGCTGGGCGTGGAGACCCGCTCGCAGCTGTCCCGCATGCTGAACCGCAAGAAGGTGCTGCGCTCCGAGGAGGGCTACCAGCGGGACTGGCGGGGCATCTCGGAGCTGGCCAAGCAGAAGGGATTCGTGGACGAAAACGCCAACAATCCCATGGATCTGGTGCTGATCAGCTGGAGCCAGCGGAGCCCACAGACCGCCAAGGTGGGCCATCTCGAGAACTTCCTGGGCATCATCGATCGGTGGGATGTCTGTGACGATATCCAGGAGAATCTGGCCAAGGACACCCGCCGCTTTATTATAAAGCAGGAGCAGCGGCAGACCTCTCTGGTGGAGGCGTGTCCCCCGCCCCCCAGCGACTGTTTCGAGACCAACAACAACTACAGCAGCAACAACAACATCACAGTGGGCCAAAGTGTCCAGATCCTGAGCGACGAGGACCAGAGATGTGTGCAGATGGGCCAACCGCTGCCCAGATACAATGCCTGTGTTCTGTACGCCGAGGCAGACATCGACCATGCCACCGAGATCATGAATAACCTAGAGTCTGAGCGATACAATCTCAGGCTTTTCCTGCGCCATCGCGACATGCTAATGGGCGTACCCTTCGAGCATGTCCAACTCTCCCACTTCATGGCCACCCGCTGTAATCACCTGATCGTCGTGCTCACCGAGGAGTTTCTTCGGAGTCCGGAGAACACGTACCTCGTGAACTTCACCCAGAAGATACAGATCGAGAACCACACTCGCAAGATCATACCGATTCTGTACAAGCCAGACATGCACATACCCCAGACCCTGGGCATCTATACGCACATCAAGTACGCCGGGGACTCCAAGCTGTTCAACTTCTGGGATAAGTTGGCTCGATCGCTGCACGATCTGGATGCCTGTTCCATCTACTCCACGCGCCAGGTGCAAACACCCTCTCCAGTGGAGGAATCGACTCCCCAGCGGGTAACCACGCCCAGCATTCGGATACAGATCAACGACAAGGATGTGACCGACATGCCCAACTTCAAGGTGCCAGAGGCGGAGACCACCATCGTTTCGGTTTCCGGAGATACCGGTTCCCCCCTGCCGGAACACAAGCCGAAGAAAAAGGATCGCTTTCTGCGCAGAATCACGCACAGTTTCGCCAAGACGCCGAAGAATGAGGGAGGCAGTGCAAAGACCCTGCGGCACGCGCACTCCGTCAGCACCATAAACGTCACGGAACGGGAGAGGACACTCAGTGCCAGCAGCTCCAATATCTCCACCACGTCGGAGAGCAAGAAGAGCTTCATCAAGTGGCAGCCGAATATCCTGAAGAAGGCCCTATTCTCCCGATCCAGCAACAAGCTGCAGACGCCGGGTTGA
Bnk promoter region	ATGAACCCTATTAAGATCCCGACACCTCTTTTCGCCTTTCTGCATAATAATTTCGCCATTCCACCGCTGTATAATTGCGTATTCGCCTTCAGTAAATGAAAATTGCAGTTGTATTTTATAATTTCGTTTTTCATTTTCCCTCTCTCATTTTCTACGTCTGTTTGGCCAGCTGTCAATTGCCGAGTGTCGACTGAATTATTGGCCATTAATTGCATTGATTTTGATTGTAATGAACCAGCTAATGAAACGTCAACAGCTAAGAGTGGGCCATAAAACCCGGAGGAATTATCTTCTAGAAACCTTGAACATCTGTCGCGAATTTAAGTTTTAAGCTGTTTATGAATTAGTACCCTCGCTAATCCTTTTCGAAAGGACCTTATAAAAGTGCGCGGATTAGCAAAAAGATTTGTGTAAAATTGGTGCGCGGAAAACACGCAAAATTCGCGTGCTCGGGTTGCAATTACCGGGAAATGTGGGCCTTTTTTACTTGCAGGGCTTAGGTAGTTTCCCGGAATGGAGTCAAGCTAAGCTAGCCAGGTACCTGAGTTCCCCGGACCAGCTGTCCGGTGCTGAAACTAGAGCAGGTAGTCCCCAGAATCCCAGCTATATAAGGCCTGCCTTCCTGGCAACAGCATCACATTCGTTCATCAGCCTTCAACA
BicC promoter region	CTGCTGAAACCATCGGCGTAAACCTCTAATTAAGGCTAGTAACCTTTGTAGAAAATTATTTAGTTTATATTTTTAAACATAAATTATTTTTGAAATTGTAACTAAAAATGTATGCCTATTTTAAAAATTCCTTCNTAAGAAAATTAGTTTAAGTAGTACACTTTTGACGCTCACTGTATCAAAATTTTTCTGGAGCGCCATCTGGGGAGCTTACTCAATTTCAAAAGCTTTACTTTACTTAGGTAAAGGGCCGAAGATAAAAGCTGACGTAGGATTTTAACTGAATGGGAGCTTTCTAAGGTTGTTTATGCACTGGAGAGATAGTAGATAAATGCACTTCCCACAGAACCCAGAGCTTTCGGATCTGAAGGTCAGTTGGGACTTGGACCCACAAGTCGAGCTTAGTTTAATAGTCAGCGCGCTTAAACGACGACAACTGCACGGCGCGCCCCCATTAATAATATATATTTTTCCAAAAATAAACTTAAAAAAAATAATTAAATAAAAAATAATATTAACATTGAACGCGCGGTGCACGATTTTTTGACAACAATTCAGTTTCGCTTTTCCTTAGATTTCCATATAATTTTTTTCCTTTGTGTTTTCCACACACGTGGTGTTCTTCTGTTACTCGCACTTCGGTAGTGGTTATTTTTTTTGGGTTACATTGAAAAAAGTACATCGACTGCCAGCCGAATTCCCATTCGAACCTTAATTTGACCAATTGACGGAAAATTAATTGCGCACAAAATTAATTAGCAAGCGAATATATATTTTTTTTTTTTACGTGAAACGAGCGTGTTATTTGTCAAAAAGATTTTACAACTGATTGTGTTTAAGTAAATTAACCTGAGATATATCTATGTGTTTGTGCTACGATCCGAAATTCCAATATTGCGGTAAGTGGTGTGATTTTTGGCGGGAATAAAATCGGTTTCCCTTTGCGTCGTATTTCTCTCCCGATTTTTCTGGCGCCAAAATGAATGAAAACGGTTGGCAACATTTTCAACTTAACATGGCAACATGCGAATCTAACCGCTGTTAGGGGAACTTGTAAGGGAACATGTTAGAAATTCTGGAACACCTCTCCAACTTTGTTTTGTTTGGCGCTCTCTCTTTGAAATGTAGAATCTCTTATTTTGGAACGGGAGCGGTTCATTGACCCAGCAACAACAATATTCGCCATATCTTCTCCTTCCCAGCGATTTGCAGAATTCATCCGTACTACAGTTAGAAATAATTCTAGTCCAACAGTAAGCAAAGTCGATCGGCGAAATCGGGAGCCGCGTTCAGTTGCCTTCGAGCCGTACTTAGAAGTGGTAGAGCCCCCAGGAATCGGAGAGATTATCCCGTAACTTGATACTCTGACGAAATCCCCCAGACAGACCCCTCGCAATCACACACAGCTATATACTGATATATAGATCTATATATATTGCGCAGATCACCCTTTAAATGCCTTTCGTCCAGAGATTCCAACAAATACGTAATTTTGTTATGAACGTCTGCCTCGTCGAGGCCATCCAATTCGTGCAGGACCTCACGCATGCCCTCTGCCTGGACCTCCAGGGCCTGTTCAACCTCAAGTAGAGATTCCCGAGCGATCTCCCAGCGGTAAGTCACGCCATTTGGCCGCGGGCCGCGAAAAGTGAAAGTGCGGGAGAAATTACGGTTATCGGGTTTTCGGGAGAAAGGGGAAAGTGGAAACCTCATGGCAGCAGCCCTCCATTTTGAGAGGTTATGTCAAAGGAAAGGAAGAGAAAGGAAGTTAAAAAATGTTAGCAAGGAAAATATTAGCCTTCGTTTTTCATAAGTACACCAATAAACCACATTTTACTGGGTGTCAAAAGTTAGAGAATTAAACAGCAAGTTACCAAAATTAGTTTGAAGGCAAAACTTTAGCTAGTTACGTTTTTAGAGGGTTCTGGAATTACAAATGCCCACAGCAGGAGAAATATAAATTTGTGTTTTCATTTTAAACAGATTATGTTTTAAAAAGTAATACATCAAAATTGACTTTAGCCGTTTTTTAAATAGGTAATTTGGTATATAAACATACATTTTTTAATAAAAATATGAACACTTTGTTTTTATTTTTGTACTGGTAGGCAAACTACAAGATATAACTGTTAAATAAAAAAAGATTTTAAGTGTAGGCAATAAAAAATCAAATCAAAAATATATTTTGAATAAAAGTGAAGGTCAAAAATGAAAGACTTCAAAGTACCACTCTACTCATGAAGCTATAATAATAATGGGTAAATAAACAGTATATTACATGTTCGTTTTAAATATTTTCAATGCAAAAATGCCCTGCTTATATTAAGCTTTACTTTCACCACAACTGTTCCCATCATCTATTTTTAATATTATTGACCTCGGACTTCTTACATTTCATCGCCAGCTCATCCCATCAATCACCCAGATAAACTACCACCTTCATTAACACACCGCAAGCATCTCACAACGCTGACCAATAAACGCCTCGACATCCAGAATCCCCAACTGAGACGGACGTTTTGCTTGGTTTTTAAAACTAAACGACCCTTTGGTAGCCGCACCTATTGACCTTTCACACATTCATATCCCTCGGTCCACAAGCCGACCCACTGATCCATTCATTCACCTCCCACG

Genomic DNA sequences of the targeted portion of the myd88 5′UTR, with miRNA target sites indicated in bold (sites three and four overlap), the myd88 CDs used as the rescue gene, the bnk predicted promoter region utilized to drive expression of the rescue, and the BicC predicted promoter region utilized to drive expression of the rescue.

Table S2. Sequences of primers utilized in cloning myd88-targeting miRNA and verifying miRNA target site regions

Primer name	Primer sequence, 5′ to 3′	Source
miRNA 1		Self-annealing primers
807A-1	TAATCACAGCCTTTAATGTAAAATTAAAAAAAATAGTACTATAAGTTAATATACCATATCTATATT	
807B-1	ATGTTAGGCACTTTAGGTACAAAATTAAAAAAAATAGTAATATAGATATGGTATATTAACTTATAG	
miRNA 2		Self-annealing primers
807A-2	AAACTTAATCACAGCCTTTAATGTTCACGCGCTTCATCGTTTTCTTTAAGTTAATATACCATATCTAAATA	
807B-2	TGATGTTAGGCACTTTAGGTACTCACGCGCTTCATCGTTTTATTTAGATATGGTATATTAACTTAAAGA	
miRNA 3		Self-annealing primers
807A-3	CTTAATCACAGCCTTTAATGTACTGATAAACGTCCCGTTGCTATAAGTTAATATACCATATCTATATC	
807B-3	ATGTTAGGCACTTTAGGTACAACTGATAAACGTCCCGTTGATATAGATATGGTATATTAACTTATAGC	
miRNA 4		Self-annealing primers
807A-4	ACTTAATCACAGCCTTTAATGTACGTCCCGTTGATAAATACCTATAAGTTAATATACCATATCTATAT	
807B-4	TGATGTTAGGCACTTTAGGTACACGTCCCGTTGATAAATACATATAGATATGGTATATTAACTTATAGG	
720C	TTTAAAGTCCACAACTCATCAAGGAAAATGAAAGTCAAAGTTGGCAGCTTACTTAAACTTAATCACAGCCTTTAATGT	Self-annealed products of 807A/807B
720D	AAAACGGCATGGTTATTCGTGTGCCAAAAAAAAAAAAAATTAAATAATGATGTTAGGCACTTTAGGTAC	
miRNA 1		Self-annealed products of 720C/720D-1
807E-1	TCTAGAGGTCTCGCTATCGGCGGCCGCGCTTAATTAACCGGATCCTTTAAAGTCCACAACTCATCAAGGAA	
720F-1	CTCGAGGGTCTCCCATGGTCAAAACGGCATGGTTATTCGTG	
miRNA 2		Self-annealed products of 720C/720D-2
720E-2	TCTAGAGGTCTCCCATGGCTTTAAAGTCCACAACTCATCAAGGA	
720-F2	CTCGAGGGTCTCGGTCCTGAAAACGGCATGGTTATTCGTGTGC	
miRNA 3		Self-annealed products of 720C/720D-3
720-E3	TCTAGAGGTCTCAGGACCATTTAAAGTCCACAACTCATCAAGGAA	
720-F3	CTCGAGGGTCTCCCTGGCAAAAACGGCATGGTTATTCGTGTGC	
miRNA 4		Self-annealed products of 720C/720D-4
720-E4	TCTAGAGGTCTCGCCAGGATTTAAAGTCCACAACTCATCAAGGA	
807F-4	CTCGAGGGTCTCCCGCCACTGCGGCCGCGGCGCGCCTGGCCGGCCAAAGATCTAAAACGGCATGGTTATTCGTGTGC	
Sequencing primers		Genomic DNA
807G	GTGTGCGTGCCTTGTGTATATTTAAAC	
807H	GGAATGGGTCTGGAAGTGAGACA	

Table S3. Sequences of primers used to assemble plasmid OA-961B

Primer name	Primer sequence, 5′ to 3′	Source
961B.1	TATCTTAAAGCTTATCGATACGCGTACGGAGCGCTTGGAACCAGACATGATAAGATACATTG	Addgene plasmid 78897
961B.2	GCAACAAGCTGCAGACGCCGGGTTGACATATGGATCTTTGTGAAGGAACCTTACTTCTGTG	
961B.3	TCACACCACAGAAGTAAGGTTCCTTCACAAAGATCCATATGTCAACCCGGCGTCTGCAG	*D. suzukii* genomic DNA
961B.4	AACAGCATCACATTCGTTCATCAGCCTTCAACCCTAGGATGCGCCCTCGATTTGTATG	
961B.5	CTGATGGCATACAAATCGAGGGCGCATCCTAGGGTTGAAGGCTGATGAACGAATGT	*D. suzukii* genomic DNA
961B.6	TACCTCAGGTCGACGTCCCATGGCCATTCGAATGTTTAAACATGAACCCTATTAAGATCC	
961B.7	CGAAAAGAGGTGTCGGGATCTTAATAGGGTTCATGTTTAAACCTGCTGAAACCATCGGCG	*D. suzukii* genomic DNA
961B.8	AGAGTCGCGGGGCCGGCCAAAGATCTGGATCCGGTTAATTAACGTGGGAGGTGAATGAAT	
961B.9	ACCTCCCACGTTAATTAACCGGATCCAGATCTTTGGCCGGCCCCGCGACTCTAGATCATA	Addgene plasmid 78897
961B.10	AGCTTATGACTCATACTTGATTGTGTTTTACGCGGCGATCGCAAGCTTTAAGATACATTG	
961B.11	AACTCATCAATGTATCTTAAAGCTTGCGATCGCCGCGTAAAACACAATCAAGTATGAGTCA	Novagen plasmid 71235-3
961B.12	CTTGATGACGTTCTTGGAGGAGCGCACCATCTCGAGGTCACTTGGTTGTTCACGATCTTG	
961B.13	TGGCGGCGACAAGATCGTGAACAACCAAGTGACCTCGAGATGGTGCGCTCCTCCAAGAAC	Addgene plasmid 64703
961B.14	CTTCGGTGTGTCCGTCAGTACAGCGATCGCATTTAAATAAGCTTTAAGATACATTGATGAGTTTGGA	