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Supporting Online Material for

A Synthetic Maternal-Effect Selfish Genetic Element Drives Population Replacement in *Drosophila*

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This PDF file includes:

Supporting online material

Materials and methods

Construction of a modified *bicoid* promoter:

A *bicoid* promoter fragment was amplified from genomic DNA with primers Bic-5 5'- GGC CTC GAG TTA GAT CTC AAT TGT GCC ATC TCT ACA TCT CTT CGC TCA TCC CTA AAT AAA AGA ATG AAC ATC GAG GGA GG and Bic-3 5' GGC CAA TTG GGC GGC GGT TGC GCC GTT TTC C, cut with Xho1 and EcoRI [all restriction enzymes provided by New England Biolabs (NEB), Beverly MA], and ligated into pGMR (*1*) cut with the same enzymes. Two Serendipity (Sry) binding sites [CGC TCA TCC CTA AAT and GTG CCA TCT CTA CA (*2*)] were placed 5' to the bicoid promoter in order to enhance maternal expression levels. Primers 4bic-1 5'- GGC CTC GAG TTA GAT CTC AAT TGT GCC ATC TCT ACA TCT CTT CGC TCA TCC CTA AAT AAA AGA ATG AAC ATC GAG GGA GG and Bic-3 were annealed with the *bicoid* promoter fragment generated above and PCR carried out (Fig. S4A). This product was cloned into pGMR as above, generating pSry-Bic-GMR.

Construction of miRNAs targeting *myd88*.

The *Drosophila* miRNA mir6.1 is illustrated in Fig. S4B. The *Drosophila* miRNA mir6.1 stem-loop precursor and surrounding sequences is illustrated to the left, with the mature miRNA indicated in red. Processing sites for Drosha and Dicer are indicated (arrows). 22bp sequences corresponding to mature mir6.1 were replaced with sequences perfectly complementary to sequences from the *myd88* 5'UTR, generating two new miRNAs, mir6.1-myd88-1 and mir6.1-myd88-2. Two sites in the myd88 transcript were targeted to minimize the possibility that mRNA secondary structure would prevent the miRNA-RISC complex from being able to bind and cleave the mRNA. The use of multiple miRNAs targeting a common transcript, but at distinct positions, also provides a method for limiting the possibility that a single mutational event (in either the miRNA or the target sequence) will lead to a loss of toxin efficacy. *Myd88* mRNA sequences are indicated in black. The sites in *myd88* that are targeted are Myd88-1 CGA TCG GAA AAC TCG AAA AAA T and Myd88-2 TCA CGC GCT TCA TCG TTT TAT T (Fig. S4C). To generate a mir6.1 stem-loop backbone that generates a mature miRNA complementary to one or the other of these target sites we annealed pairs of primers. For example, to make a miRNA that targets myd88-1, primers Myd881-1 and Myd881- 2 (D-dMyd881-1: 5'-GGC AGC TTA CTT AAA CTT AAT CAC AGC CTT TAA TGT CGA TCG GAA AAC TCG AAA ACA TTA AGT TAA TAT ACC ATA TC and D-dMyd881- 2: 5'-AAT AAT GAT GTT AGG CAC TTT AGG TAC CGA TCG GAA AAC TCG AAA AAA TTA GAT ATG GTA TAT TAA CTT AAT GT) were annealed and filled in using PCR (Fig. S4C). This product was then amplified using primers Mir6 5' EcoRI/BglII (5'-GGC GAA TTC CGC CAG ATC TTT TAA AGT CCA CAA CTC ATC AAG GAA AAT GAA AGT CAA AGT TGG CAG CTT ACT TAA ACT TA) and Mir6 3' BamHI/NotI (5' GGC CGC GGC CGC ACG GAT CCA AAA CGG CAT GGT TAT TCG TGT GCC AAA AAA AAA AAA AAT TAA ATA ATG ATG TTA GGC AC). These primers add mir6.1 flanking sequences that are thought to promote miRNA processing, as well as several restriction sites (Fig. S4C). A miRNA that targets myd88-2 was generated similarly, beginning with primers Myd882-1 GGC AGC TTA CTT AAA CTT AAT CAC AGC CTT TAA TGT TCA CGC GCT TCA TCG TTT TCT TTA AGT

TAA TAT ACC ATA TC and Myd882-2 AAT AAT GAT GTT AGG CAC TTT AGG TAC TCA CGC GCT TCA TCG TTT TAT TTA GAT ATG GTA TAT TAA CTT AAA GA. PCR products were purified with Qiagen (Valencia, CA) PCR purification columns, and then digested with enzymes. For dMyd88-1 these were EcoRI and BamHI, for dMyd88-2 BglII and NotI. Digested products were then ligated into sry-bic-GMR cut with EcoRI and NotI, generating pBic-mir6.1-myd88. The structure of this construct is shown in Fig. S4D.

Zygotic promoter and *myd88* antidote rescue construct:

A 500 bp fragment of DNA containing the transient, early zygotic *bnk* promoter (*3*) was amplified from genomic DNA using primers Bnk 5' XhoI (5'-GGC CTC GAG TAT TTC ACA AAT TCA ATT TTA ATA TTT AAG) and Bnk3' EcoRI (5'-GGC GAA TTC GTT GAC GGT TGA AGT ACG AAT GTG CTG T), cut with XhoI and EcoRI and inserted into similarly cut pGMR, generating P-BNK. The myd88 coding region was amplified from a cDNA library using primers myd88-5 (5'- GGC GAA TTC ATG CGC CCT CGA TTT GTA TGC CAT C and myd88-3 (5'- GGC GCG GCC GCT CAG CCC GGC GTC TGC AGC TTC), cut with EcoRI and NotI, and ligated into similarly cut P-BNK, generating P-BNK-dMYD88 (Fig. S5A). Note that because this *myd88* transcript lacks a 5' UTR present in the endogenous *myd88* transcript, it is not silenced by mir6.1-myd88-1 or mir6.1-myd88-2, which target the *myd88* 5' UTR.

Generation of *Medea*Myd88:

P-BNK-dMYD88 (Fig. S5A) was cut with XhoI and 5' ends were dephospholated with Calf Intestinal Alkaline Phosphatase (NEB, Beverly MA). A gypsy insulator-containing DNA fragment was amplified from genomic DNA using a 5' primer (gypsy 5') that contained a number of restriction enzyme target sites (SalI, HpaI, AvrII, NheI, SpeI, KpnI, BglII) (5'- GGC GTC GAC GTT AAC CTA GGC TAG CAC TAG TGG TAA CCC CGA GAT CTT CAC GTA ATA AGT GTG CGT TGA ATT TAT TCG C) and a second primer (gypsy 3'), which contains an XhoI site, (5'- GGC CTC GAG AAT TGA TCG GCT AAA TGG TAT GGC AAG AAA AG). This PCR fragment was cut with SalI and XhoI and ligated into XhoI cut P-BNK-dMYD88. This created Pgypsy-BNK-dMYD88 (Fig. S5B), which was cut with AvrII and dephosphorylated with Calf Intestinal Alkaline Phosphatase. The modified *bicoid* promoter and myd88-targeting miRNAs in pBic-mir6.1-myd88 (Fig. S4D) were amplified using primers 4 BIC Myd 1+2 5' AvrII- 5' GGC CCT AGG GTC GAG TTA GAT CTC AAT T and 4 BIC Myd 1+2 3' HpaI /SpeI/ Nhe- 5' GGC GTT AAC ACT AGT GCT AGG GCC TTC TAG ACC CCG GCC GC. The PCR product was cut with AvrII and NheI and ligated into AvrII cut P-gypsy-BNK-dMYD88. One plasmid product, designated P-bicoid-myd88RNAi-gypsy-BNK-dMYD88, was selected in which the *bicoid* and *myd88* promoters were oriented so as to transcribe in opposite directions (Fig. S5C).

Generation of *Medea*myd88-int:

The plasmid P-Bnk-dMyd88 was cut was cut with EcoRI and NotI, the vector purified and then ligated with PCR fragments dMyd88 exon 1/intron 1(EcoRI, SpeI) and intron 1/ exon2 (SpeI, NotI), generating P-Bnk-dMyd88-intron. PCR fragment dMyd88 exon 1/intron contains dMyd88 exon 1and a 5' splice site. This fragment was amplified

by PCR with primer Myd88 EcoRI 5' (5'-GGC GAA TTC ATG CGC CCT CGA TTT GTA TGC C) and Myd 88 exon 1 /intron SpeI /NheI 3' (5'- GGC ACT AGT GGC CGC TAG CAG CGA CTA CCA TAA GTA AAA AAT AGT TAA TGC CTA CCC AGA TTC TCC TGG ATA TCG TCG CAG). The second PCR fragment, dMyd88 intron 1/ exon2, contains intron 1, a pyrimidine-rich trait and 3' splice site as well as the 3' exons of dMyd88 derived from the cDNA. This fragment was amplified by PCR with primers myd88-EXON2–intron-SpeI-5 (5'- GGC ACT AGT TAG TAA AAC TGT TTT AAT TTT GCT CTC CTC AAA AGC CAA GGA CAC CCA GCG CTT CAT CAT G) and myd88-NotI-3 (5'- GGC GCG GCC GCT CAG CCC GGC GTC TGC AGC TTG C). P-Bnk-dMyd88-intron was cut with SpeI, which cuts within the intron. The product was dephosphorylated with Calf Intestinal Alkaline Phosphatase according to the manufacturers instructions (NEB, Beverley MA) and ligated with a PCR product containing the modified *bic* promoter and the Myd88- targeting miRNAs. Primers used were 4 BIC Myd 1+2 5' SpeI, which also contains a consensuses branch point site GAT TAG ATG, (5' GGC ACT AGT TAT TGA TTA GAT GTC GAG TTA GAT CTC AAT T) and 4 BIC Myd 1+2 3' NheI (5'- GGC GCT AGC GGC CTT CTA GAC CCC GGC CGC). The final product was designated as Pw+*Medea*$^{myd88\text{-}int}$.

Transgenesis and Population cage experiments:

Germline transformants were generated in a w^{1118} background using standard techniques, by Rainbow Transgenic Flies, Inc (www.rainbowgene.com, Newbury Park, CA). The exact origin of the chromosomes present in the w^{1118} strain used for transgenesis and population replacement experiments, and the relationship of these chromosomes to those present in the OR strain, is unknown. The second and third chromosomes were isogenized in a w^{1118} background in the early 1990s as a prelude to a large mutagenesis screen for regulators of Ras pathway signaling (*6*). The stock has been maintained continuously in the laboratory since then. All fly experiments were carried out at 25^{o}C, ambient humidity in 250 ml bottles containing Lewis medium (*4*) supplemented with live dry yeast. Fly rearing was carried out in a light tight chamber placed in an incubator or in a darkened incubator. In a first set of experiments (three green lines in Fig. 1E,F), three populations of 50 males heterozygous for *Medea*^{myd88}-1 (w^{1118}/Y; P^{w+}*Medea*^{myd88}-1/+) were each crossed with 50 w^{1118} females in separate bottles. In a second set of experiments (four blue lines in Fig. 1E, F) 25 wildtype (+/+) males and 25 homozygous *Medea*^{myd88}-1/ *Medea*^{myd88}-1 males were crossed with 50 w^{1118} females in separate bottles. For both sets of experiments flies were allowed to lay eggs for four days, after which adults were removed. Progeny were allowed to develop, eclose and mate for another 10 days. All adult progeny were collected at this single timepoint and their genotypes determined using eye color as a marker (no *Medea*, w^{1118} = white eyed; *Medea*^{myd88}-1/+ = yellow/orange eyed; *Medea*^{myd88}-1/ *Medea*^{myd88}-1 = darker red eyed). Note that adult progeny continued to eclose after the time of collection. These were not counted or transferred into the subsequent generation population. Numbers of adults scored per population per generation ranged between 102 and 601 (mean 333; s.d.105). Following counting, progeny were transferred to fresh bottles and allowed to lay eggs for four days, and the cycle repeated.

In a second set of population cage experiments, *Medea*^{myd88}-1 was first introduced into a w^{+} background in order to decrease the possibility that P^{w+} expression from the

vector carrying *Medea*^{myd88} was providing these animals with an unknown (vision-independent) fitness advantage. Females homozygous for *Medea*^{myd88}-1 were mated with Oregon R (OR) males, which carry a wildtype copy of the *w* gene at the endogenous locus on the X chromosome. Progeny males, which are w^+/Y; *Medea*^{myd88}-1 were then mated as above to OR virgin females to initiate the population cage experiments. The presence of the endogenous w^+ gene completely prevents direct identification of *Medea*^{myd88}-1-bearing individuals based on eye color, since the endogenous w^+ gene is expressed at very high levels compared with the Pw^+ in our transformed strain. We determined genotypes at the end of the 12th generation in the following way. 200 males from each population were mated singly in vials with w^{1118} females. All male progeny inherit the w^{1118} chromosome from their mothers (making them w^{1118} with respect to the endogenous locus on the X chromosome). However, those males that carry one copy of *Medea*^{myd88}-1 give rise to 50% red-eyed progeny, while those homozygous for *Medea*^{myd88}-1 give rise to all red-eyed progeny. In each of the three populations the % of non-element-bearing males was less than 1%: population #1 = 0/200 +/+, 30/200 *Medea*^{myd88}-1/+, 170/200 *Medea*^{myd88}-1/ *Medea*^{myd88}-1; Population #2 = 1/200 +/+, 67/200 *Medea*^{myd88}-1/+, 132/200 *Medea*^{myd88}-1/ *Medea*^{myd88}-1; Population #3 = 0/200 +/+, 58/200 *Medea*^{myd88}-1/+, 142/200 *Medea*^{myd88}-1/ *Medea*^{myd88}-1. Note that the OR genetic background into which Medea$^{myd88-1}$ was introduced should be assumed to be distinct from that of the w^{1118} strain used for transgenesis and the population replacement experiments described above. Thus, while evidence for population replacement was obtained in both sets of experiments they are not strictly comparable in the sense that more variables have been changed than just the status of the *w* gene (w^{1118} versus w^+).

Embryo and adult viability determination:

Adult viability for the crosses presented in Table 1 and Table 2 was determined as follows. 50 adult males of the indicated genotype were allowed to mate with 50 virgin females in bottles supplemented with dry yeast for three days. 10 bottles were established for each cross. Adults were then removed. Adult progeny from each bottle were collected, genotyped and counted (either directly or by weighing and comparing with a standard) for 10 days following eclosion of the first progeny. For embryo viability counts, 2-4 day old adult virgin females were allowed to mate with males of the relevant genotypes for 2-3 days in egg collection chambers supplemented with wet yeast paste. On the following day, a 3 hr egg collection was carried out, after first having cleared old eggs from the females through a pre-collection period on a separate plate for three hrs. Embryos were isolated into groups of 100 and kept on an agar surface at 25^{o}C for 48-72 hrs. The % survival was then determined by counting the number of unhatched embryos. Four groups of 100 embryos per cross were scored in each experiment, and each experiment was carried out three times. The results presented are averages from these three experiments. Embryo survival was normalized with respect to the % survival observed in parallel experiments carried out with the w^{1118} strain used for transgenesis.

Modeling *Medea* population spread

In order to model the spread of *Medea* and compare this with our experimental observations, a deterministic model was created. In this model, we used as our initial conditions 25% non-element-bearing (wildtype; WT) males, 25% homozygous *Medea*-

bearing males, and 50% WT females for *Medea*. These initial conditions, which mimic those of the first set of population cage experiments (green lines in Fig. 1E, F) are mathematically equivalent to the second set of crosses carried out, involving heterozygous *Medea*^{myd88}/+ males crossed to wildtype (+/+) females) (blue lines in Fig. 1E, F),. Genotypes for each generation were calculated beginning with genotype frequencies from the previous generation. We assumed random mating, nonoverlapping generations, and interfamily competition. We assume that maternal effect lethality for progeny of *Medea* that fail to inherit the element is 100%, as observed for *Medea*^{myd88} (Table 1).

We kept track of the frequency of homozygous, heterozygous, and WT male and female flies in each generation. For this model, aside from the first generation, the frequency of WT males and females are equal, the frequency of heterozygous males and females are equal, and the frequency of homozygous males and females are equal. From the frequency of each type of fly, we calculated the frequency of each allele type. For example, in order to calculate the frequency of WT females in generation n+1 we begin with the frequency of WT females in generation n. We calculate the percent of the next generation that will be wild type by finding the frequency of viable WT female embryos normalized (divided by) the total viable population (WT, heterozygotes and homozygotes). WT female flies can arise from (½ WT males x WT females) + (1/4 of WT female x heterozygous males). WT embryos derived from crosses between heterozygous females x WT males will die as embryos and not contribute to the population. We calculate the total viable population by summing the WT females, WT males, heterozygous females, heterozygous males, homozygous females and homozygous males.

When we assessed a fitness cost (as in Fig. 1C, D and Fig. S3), this cost was assumed to be an additive fitness cost. The fitness cost was expressed as a fraction of the embryos that die. That is, for a 5 percent fitness cost, 95 percent of the heterozygous embryos are viable, while 90 percent of the homozygous embryos were viable. For the model presented in Fig. S3, a specific additional fitness cost of 80% was incurred by progeny of homozygous *Medea*$^{myd88\text{-}int}$ mothers that inherited only one copy of the element (the fathers being either *Medea*$^{myd88\text{-}int}$/+ or +/+).

The formulas used are shown below. WTf, Hetf, Homof, WTm, Hetm, and Homom refer to the fraction of the adult population that is WT female, heterozygous female, homozygous female, WT male, heterozygous male, homozygous male, respectively. FitCost is the fitness cost, TotalViablePopulation=the total viable population, WTembryo, Hetembryo, HomoembryoWT embryo, and Hetembryo refer to the number of WT, heterozygous, and homozygous embryos produced before the effects of the medea element are included and without normalization. All subscripts refer to the generation.

$$WTf_{n+1} = WTm_{n+1} = \frac{0.5 * WTembryo_{n+1}}{TotalViablePopulation_{n+1}}$$

$$Hetf_{n+1} = Hetm_{n+1} = \frac{0.5 * Hetembryo_{n+1} * (1 - FitCost)}{TotalViablePopulation_{n+1}}$$

$$Homof_{n+1} = Homom_{n+1} = \frac{0.5 * Homoembryo_{n+1} * (1 - 2 * FitCost)}{TotalViablePopulation_{n+1}}$$

where,

$$TotalViablePopulation_{n+1} = WTembryo_{n+1} + Hetembryo_{n+1} * (1 - FitCost) + Homoembryo_{n+1} * (1 - 2 * FitCost)$$

$$WTembryo_{n+1} = WTf_n * WTm_n + 0.5 * WTf_n * Hetm_n$$

$$Hetembryo_{n+1} = WTm_n * Homof_n + 0.5 * Wtm_n * Hetf_n + 0.5 Hetm_n * WTf_n + 0.5 * Hetm_n * Hetf_n + 0.5 * Hetm_n * Homof_n + Homom_n * Wtf_n + 0.5 * Homom_n * Hetf_n$$

$$Homoembryo_{n+1} = 0.5 * Homom_n * Hetf_n + 0.5 * Hetm_n * Homof_n + 0.25 * Hetm_n * Hetf_n + Homom_n * Homof_n$$

All model calculations were carried out in Excel for Windows XP. Matrix manipulation calculations were carried out in Maple 9.01 (Maplesoft, a division of Waterloo Maple Inc, 2003).

To determine if the experimental population data presented in Fig. 1 conformed to the model, the methods of Wilson (5) were used. Briefly, these methods are based on a Chi-square analysis of allele frequency corrected for the non-independence of generations (the frequency of allele p at generation 3 is dependent on the frequency of allele p (the *Medea* allele) in generation 2). Because of the non-independence of generations, a covariation matrix is used. The covariation matrix for a particular category (experimental trial), c, is denoted W_c and is calculated as

$$(W_c)_{tt} = \frac{1}{n_{c,t}} + \left(1 - \frac{1}{n_{c,t}}\right)\left[1 + (w_c - 1)\prod_{j=1}^{t-1}\left(1 - \frac{1}{N_{c,j}}\right)\right]$$

and

$$(W_c)_{t,t+r} = (W_c)_{t+r,t} = 1 + (w_c - 1)\prod_{j=1}^{t-1}\left(1 - \frac{1}{N_{c,j}}\right)$$

$N_{c,j}$ is the number of genes (twice the number of individuals) taken at generation j to give be parents of the next generation. $n_{c,j}$ is the number of genes (twice the number of individuals) used for genetic analysis. To be conservative (that is, to make the assumption that our population has the largest possible variation), w_c was calculated as $(V_c)_{O,O}/\pi_{C,O}(1-\pi_{c,o})$, where V_c is the sample variance of the initial population and π_C is the theoretical variance of the initial population.

The difference vector, γ_c^m, for any particular model, m, is calculated as the difference between the normalized gene frequencies observed experimentally and the normalized gene frequencies expected from our model using a particular fitness cost. Each of these frequencies are normalized using the arcsin transformation: $2 * \sin^{-1}\left(\sqrt{p}\right)$, modified such that if p=0, the transform equals $2 * \sin^{-1}\left(\sqrt{\frac{1}{2} * n_t}\right)$, where n_t is the number of individuals in a particular generation, t. We calculate the Chi-square test statistic for a particular model, m, as $(\chi^m)^2 = \sum_c (\gamma_c^m)^T W_c^{-1} \gamma_c^m$. The degrees of freedom in each category are calculated as the number of generations observed less the number of fitted parameters in the model. To find the total degrees of freedom, the degrees of freedom in each category are summed.

The values of p range from 0 (all individuals are homozygous for the construct) to 1 (all individuals are homozygous wildtype). When all 7 experimental trials were considered as a group, the Chi-square test statistic for no fitness cost was 88.3.

There are 122 degrees of freedom, leading to a critical $\chi 2$=148.8, with a probability error threshold (P value) of 0.05. Our Chi-square value is less than the critical value, indicating the data from our experiments is not significantly different from the theoretical model. The minimal Chi-square value to the nearest percent occurs at 0 fitness cost. Due to the conservative nature of this test, the confidence interval is large. To the nearest 0.01, the fitness cost can range from -0.23 to 0.10 and be consistent with our observations at a P value of .05. This range is calculated by finding the first value resulting in a Chisquare value falling outside the critical value, 147.7 (d.f=121).

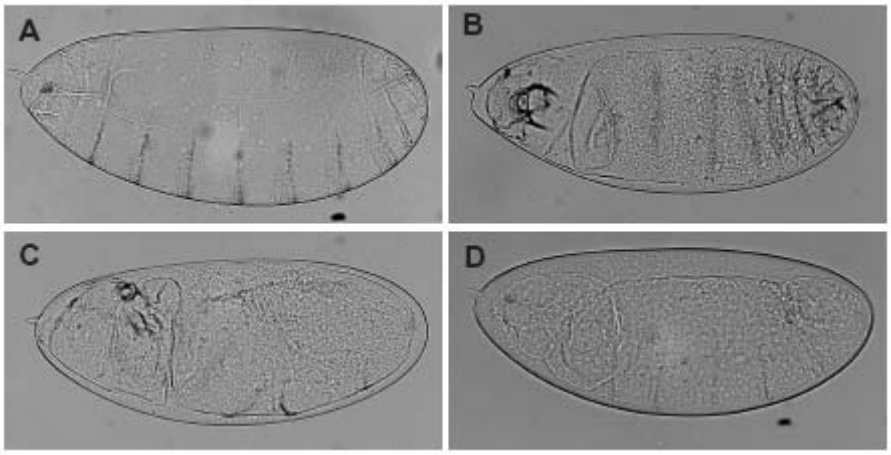

Fig. S1. Embryos of *Medea*^{myd88} mothers that fail to inherit *Medea*^{myd88} have dorsal-ventral patterning defects. (A) Cuticle preparation of an embryo from wildtype parents. Anterior is to the left, and dorsal is up. Rows of denticle belts are visible on the ventral side of the embryo. (B-D) Embryos from *Medea*^{myd88}/+ mothers. Ventral denticle belts are decreased in size (B) or largely absent (C,D), consistent with the dorsalization expected on loss of maternal Toll signaling.

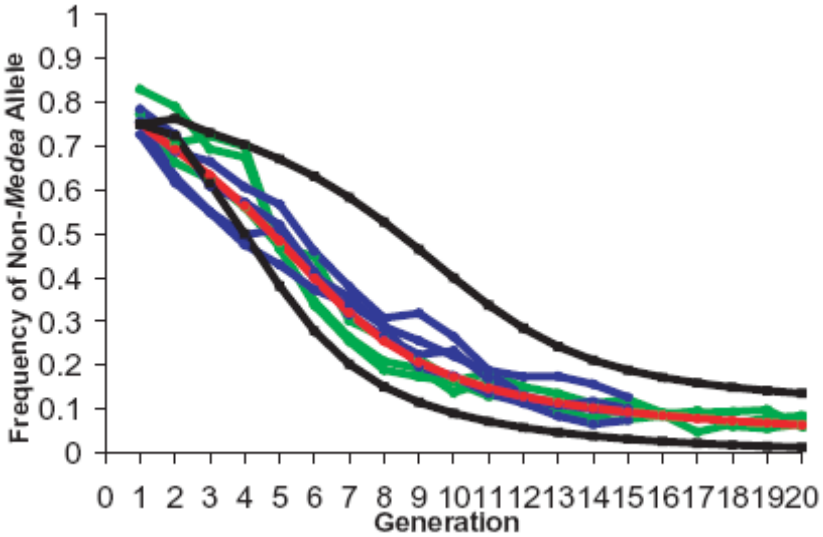

Figure S2. The population replacement behavior of *Medea*^{myd88}-1 is consistent with that of a *Medea* carrying little or no fitness cost. The frequency of the non-*Medea*-bearing chromosome (the + allele) is plotted over generations, from Fig. 1F. The black lines identify boundary conditions for a *Medea* with a fitness gain of 23% or a fitness cost of 10%, values at which we would reject the hypothesis that *Medea*^{myd88}-1 conforms to the model. See SOM methods for details.

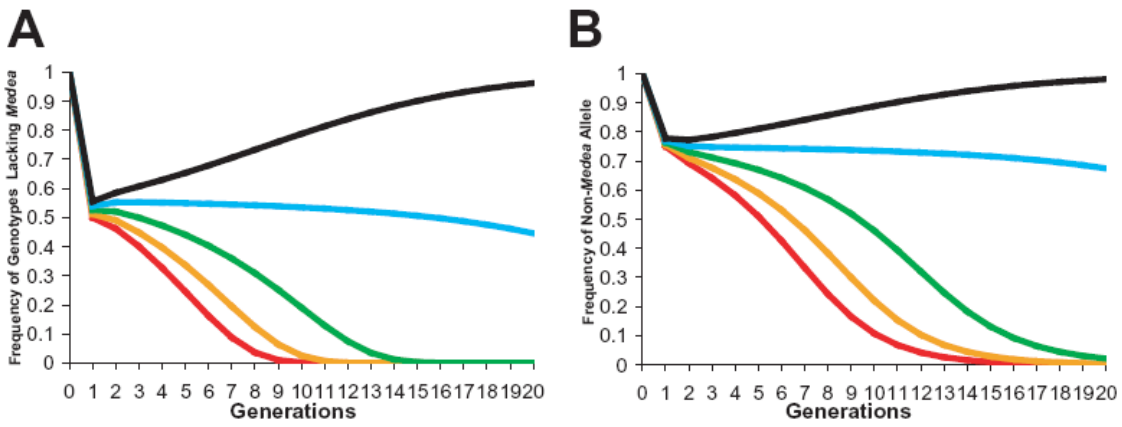

Figure S3. Drive characteristics of a *Medea* elements with the fitness characteristics of *Medea*$^{myd88\text{-}int}$. *Medea* is modeled as being introduced into the population at an allele frequency of 25%, representing a scenario in which equal numbers of wildtype and homozygous *Medea*-bearing males are mated with wild, non-element- bearing females, as in Fig. 1C, D. (A) Frequency of individuals lacking *Medea* for an element in which progeny of homozygous *Medea* females that inherit only one copy of the *Medea* suffer an 80% fitness cost (embryo mortality), in addition to either no fitness cost (red line), a five percent additive fitness cost (yellow line), a 10 percent additive fitness cost (green line), a 15% additive fitness cost (blue line), or a 20% additive fitness cost (black line) over generations. (B) Frequency of the + allele (non-element bearing chromosome) for the populations described in (A). For a *Medea* that has a fixed, additive fitness cost (such as described in Fig. 1C, D), the frequency of non-*Medea* bearing chromosomes initially decreases rapidly, but slows and eventually reaches a stable equilibrium value. This is due to *Medea*-dependent selection against the non element-bearing chromosome, which is balanced by its increased fitness relative to that of *Medea*-bearing counterparts, as detailed by Wade and Beeman (*13*). In contrast, when the progeny of homozygous *Medea*-bearing females cannot be rescued by a single *Medea*, then the non-element bearing homolog comes under selective pressure that increases with the frequency of homozygous *Medea*-bearing females in the population. For situations in which the fixed additive fitness costs are modest (0-10%) this results in a more dramatic reduction of the non-Medea-bearing chromosome from the population. *Medea* elements with this characteristic may be useful for population replacement since the presence of two effector copies in each individual should also delay the re-appearance of disease carriers when the effector mutates to inactivity.

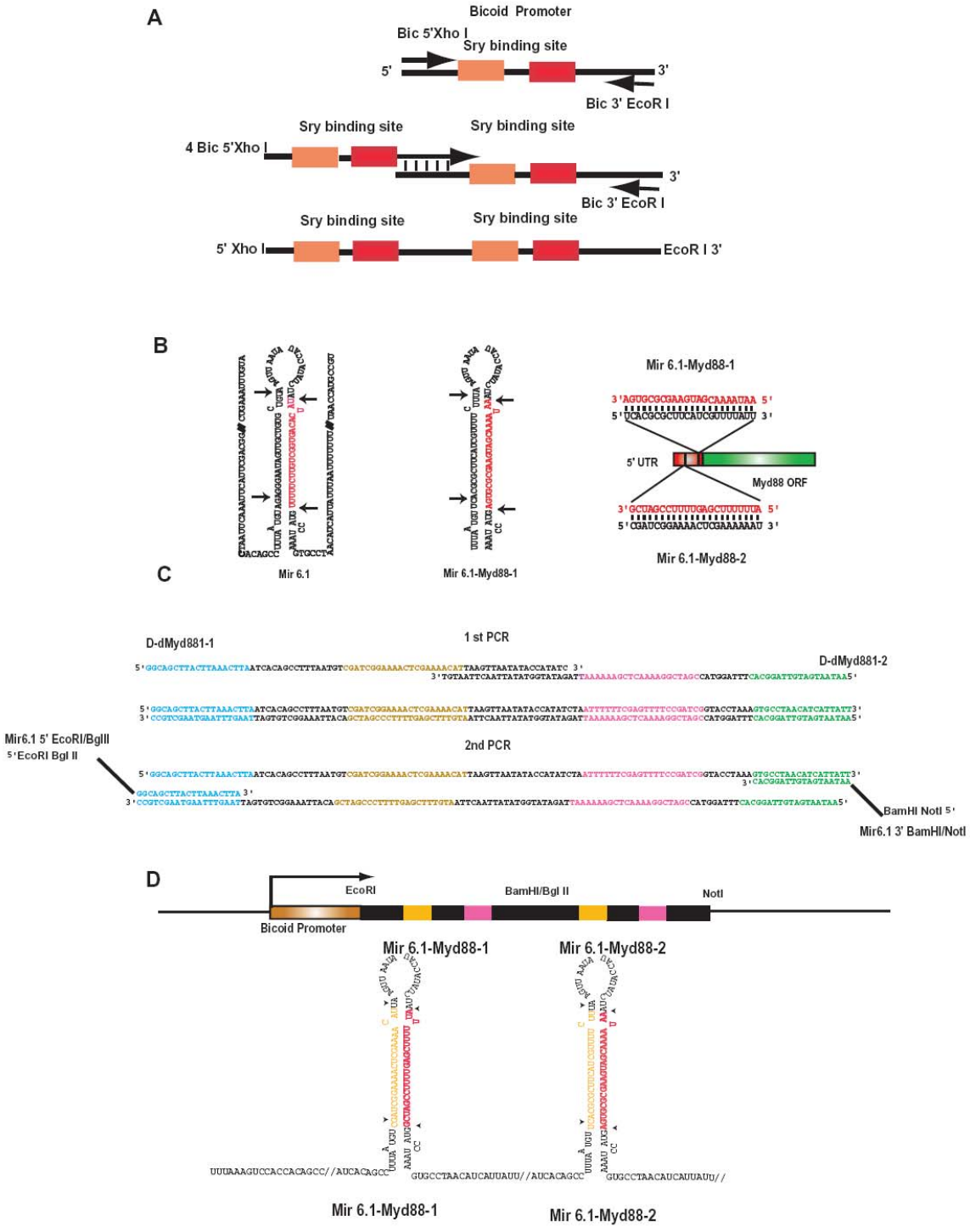

Figure S4. Scheme for the generation of a stronger version of the *bic* promoter, and for the generation of a transcript carrying two miRNAs designed to silence maternal *myd88*. See SOM methods for details. (A) Generation of a stronger bic promoter. (A, top) PrimersBic 5'Xho1 and Bic 3' EcoR1 were used to amplify the bicoid promoter. (A, middle) This fragment was re-amplified with Bic 3' EcoR1 and a second 5' primer (4 Bic

5' Xho1) which included a dimer of Sry binding sites. (A, bottom) The final product, which carries an Xho1 site at the 5' end, and an EcoR1 site at the 3' end, constitutes the modified bicoid promoter. (B) The *Drosophila* miRNA mir6.1 stem-loop precursor and surrounding sequences is illustrated to the left, with the mature miRNA indicated in red. Processing sites for Drosha and Dicer are indicated (arrows). 22bp sequences corresponding to mature mir6.1 were replaced with sequences perfectly complementary to sequences from the *myd88* 5'UTR, generating two new miRNAs, mir6.1-myd88-1 (shown) and mir6.1-myd88-2. Mature mir6.1-myd88-1 and mir6.1-myd88-2 are indicated in red, and complementary *Myd88* mRNA sequences targeted by these miRNAs are indicated in black. (C) Strategy for the synthesis of mir6.1-myd88-1 using two rounds of PCR. The first round of PCR amplifies the miRNA stem loop (miRNA and miRNA* strand indicated in pink and yellow, respectively). This product was amplified in a second round of PCR using oligonucleotides that provide mir6.1 flanking sequences (not shown) and restriction sites for cloning. (D, upper) Schematic of the sequences that make up the bic-driven miRNAs that target myd88. Cloning sites are indicated. (D, lower) Stem loop regions and surrounding sequences of bic-mir6.1-myd88-1 + myd88-2. The *bic* promoter is located to the left.

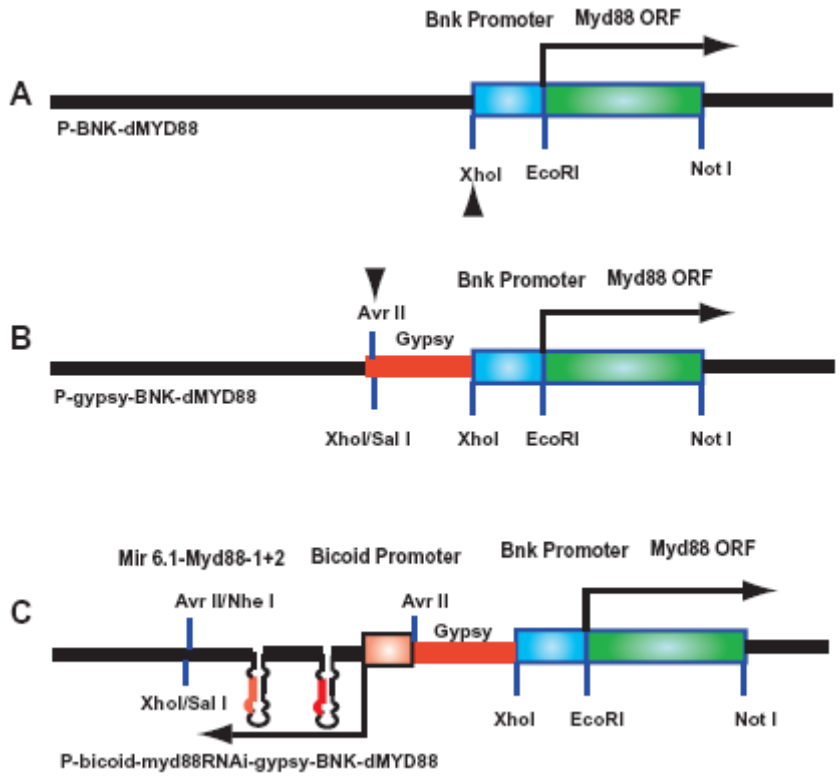

Figure S5. Schematic depicting key intermediates in the generation of a P element expressing *myd88*-silencing miRNAs under the control of the maternal *bic* promoter, and a miRNA-insensitive version of *myd88* under the control of the transient, early *bnk* promoter. See SOM methods for details.

Supplementary References

S1. B. A. Hay, T. Wolff, G. M. Rubin, *Development* **120**, 2121 (1994).
S2. C. Ruez, F. Payre, A. Vincent, *Mech. Dev.* **78**, 125 (1998).
S3. E. D. Schejter, E. Wieschaus, *Cell* **75**, 373 (1993).
S4. E.B. Lewis, *Dros. Inf. Serv*. **34**, 117 (1960).
S5. S. R. Wilson, *Genetics* **95**, 489 (1980).
S6. F. D. Karim, H. C. Chang, M. Therrien, D. A. Wassarman, T. Laverty, and G. M. Rubin, *Genetics* **143**, 315 (1996).