



Figures and figure supplements

Transgenerational inheritance of ethanol preference is caused by maternal NPF repression

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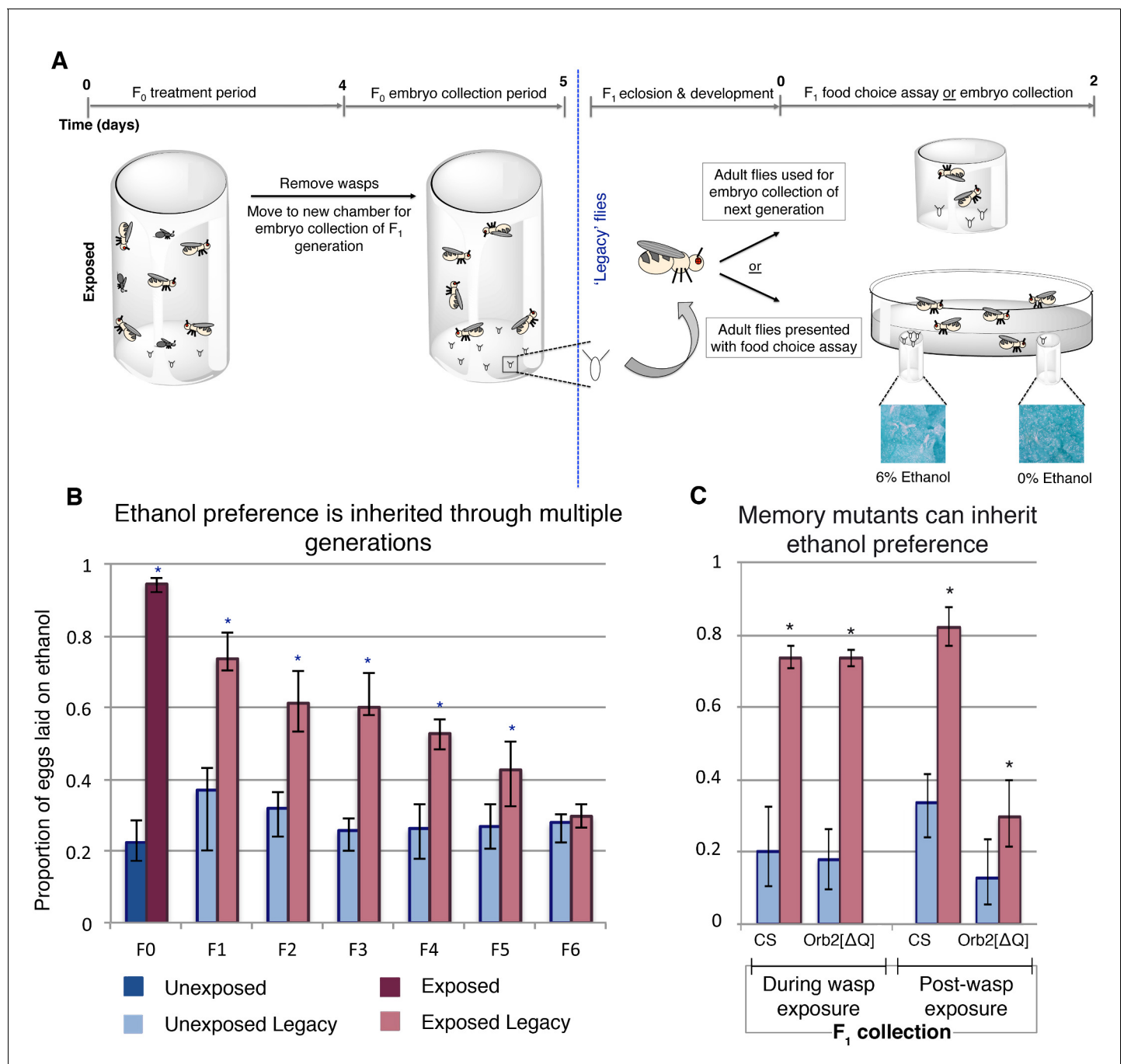


Figure 1. Maternally inherited ethanol preference persists for multiple generations. Schematic of experimental flow is shown (A). Flies are exposed to wasps for a period of four days prior to egg collection. The descendants from either wasp-exposed or unexposed treatment groups, termed 'legacy' flies, are separated from the previous generation and reared until maturity. Legacy flies are either used to propagate the next generation, or are assayed for ethanol preference. Flies from a particular generation are referred to as F_n , where n denotes the number of generations removed from the treatment. For example, the treatment group itself is F_0 , whereas their direct offspring are F_1 . Ethanol preference is quantified as proportion of eggs laid on ethanol food (B), illustrating that this behavior is heritable through the F_5 generation. Flies with deficient long-term memory ($Orb2[\Delta Q]$) were tested for transgenerational inheritance of ethanol along side the wild type control strain (CS) (C). Embryos (F_1 legacy flies) were collected during wasp exposure or in the 24 hr period following the wasp exposure; both CS and $Orb2[\Delta Q]$ exposed legacy flies are able to inherit the ethanol preference. Asterisk indicates p -value of <0.05 from a Mann-Whitney U test. Error bars are bootstrap 95% confidence intervals. Color-coding of bar charts indicates treatment and generation; dark blue (unexposed), light blue (unexposed legacy), dark magenta (exposed), light magenta (exposed legacy).

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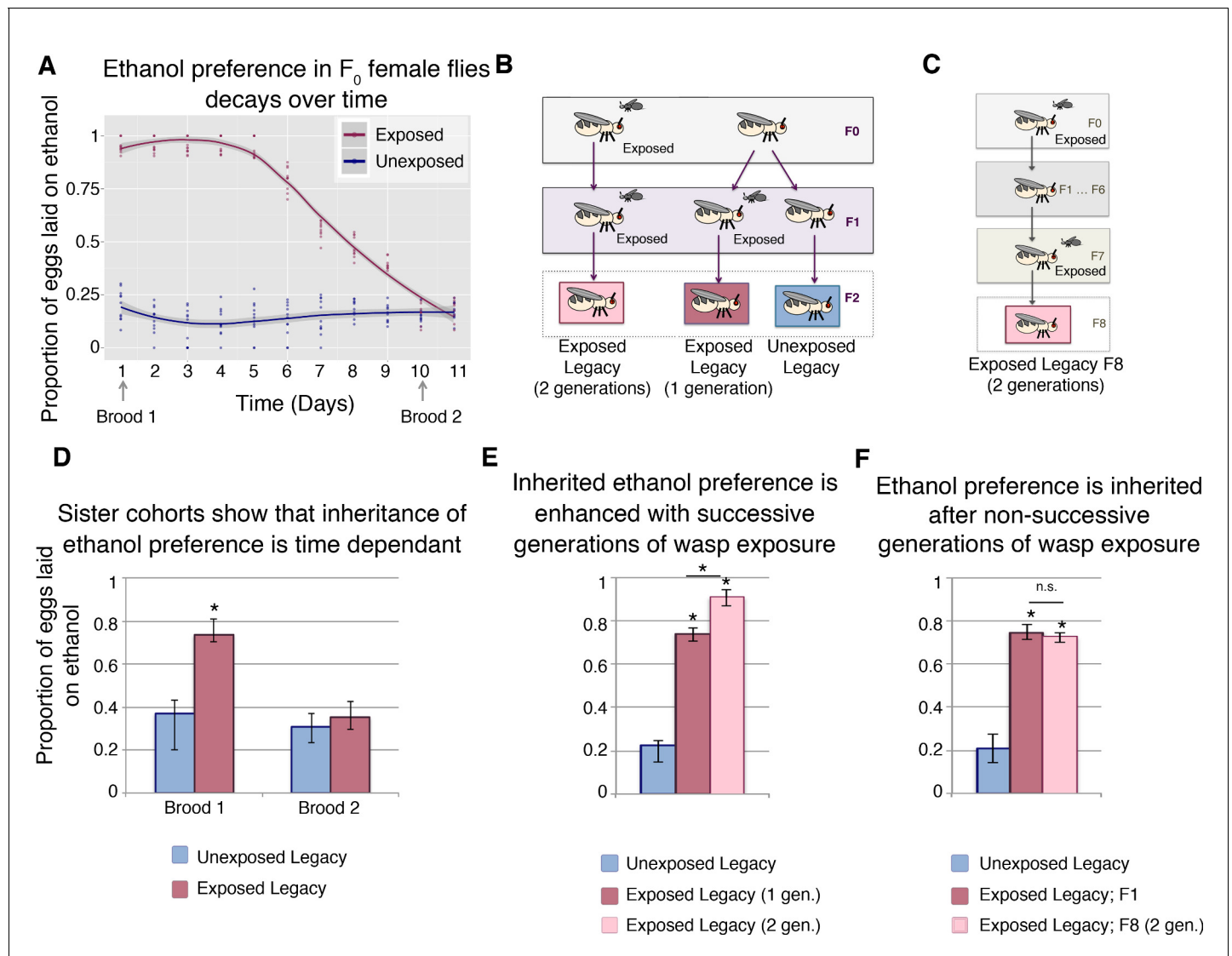


Figure 1—figure supplement 1. Temporal dynamics of wasp exposure effect inheritance of ethanol preference. Ethanol preference decays after wasp removal in F_0 flies over the course of 11 days: The decay curve is shown with loess regression, the shaded region indicates standard error (A). A diagram of the multigenerational exposure experiment is shown for successive generations (B), and non-consecutive generations (C). Sister cohorts of legacy flies were collected at different intervals post-wasp exposure, brood one immediately following wasp removal and brood two when the parental flies no longer exhibit an ethanol preference (A). These cohorts were assayed, and brood two did not demonstrate an ethanol preference (D). Flies with successive generations of wasp exposure exhibit an enhanced ethanol preference (B, E). Alternatively, flies from a second generation of non-consecutive wasp exposure (exposure of F_7 flies) exhibit an ethanol preference similar to that of one-generation wasp exposed flies (C, F). Asterisk indicates a p-value of <0.05 from a Mann-Whitney U test. Error bars are bootstrap 95% confidence intervals.

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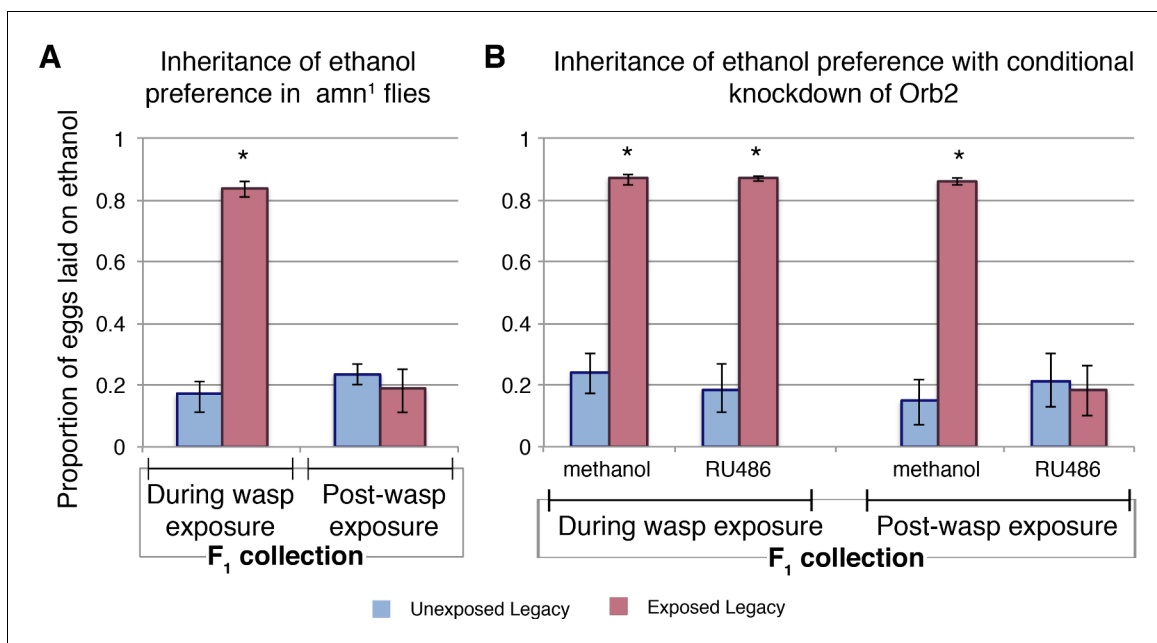


Figure 1—figure supplement 2. Intact long-term memory is dispensable for the transmission of ethanol preference. Long-term memory mutant flies *amn*¹ were tested for transgenerational inheritance of ethanol (A). Embryos (F₁ legacy flies) were collected during wasp exposure or in the 24 hr period following the wasp exposure; *amn*¹ flies collected during the wasp exposure were able to inherit the ethanol preference. Using a conditional knockdown of Orb2 specific to the mushroom body region of the brain, flies were manipulated to have impaired long-term memory when fed the drug RU486 (B). Offspring of these flies were able to inherit the ethanol preference if collected during the wasp exposure. However, legacy flies collected from memory impaired (RU486 fed) flies post-wasp exposure did not exhibit the ethanol preference. Asterisk indicates p-value of <0.05 from a Mann-Whitney U test. Error bars are bootstrap 95% confidence intervals.

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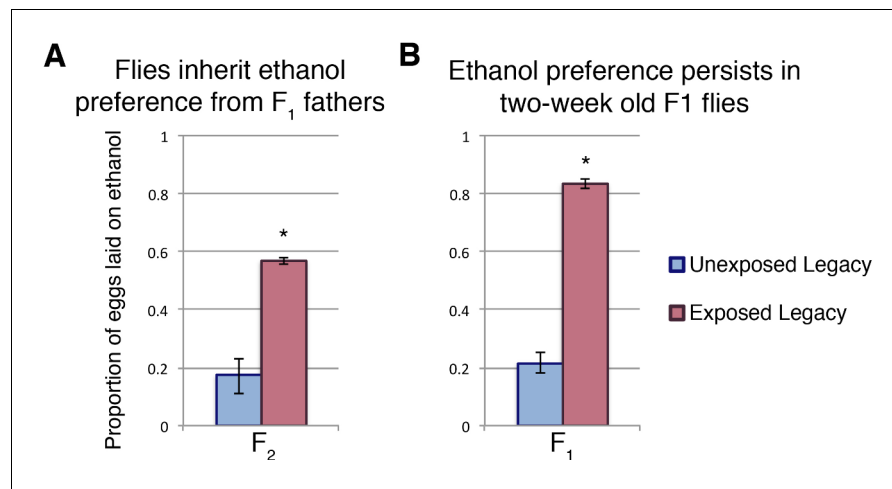


Figure 1—figure supplement 3. F_1 ethanol preference has distinct characteristics from those of the parental F_0 generation (pertaining to **Figure 1**). Male F_1 flies crossed to naïve females are able to pass on ethanol preference to their offspring; as demonstrated by the ethanol preference of F_2 legacy flies (**A**). The ethanol preference of F_1 flies does not decayed with age; ethanol preference was tested at two-weeks post eclosion (**B**). Asterisk indicates a p-value of <0.05 from a Mann-Whitney U test. Error bars are bootstrap 95% confidence intervals.

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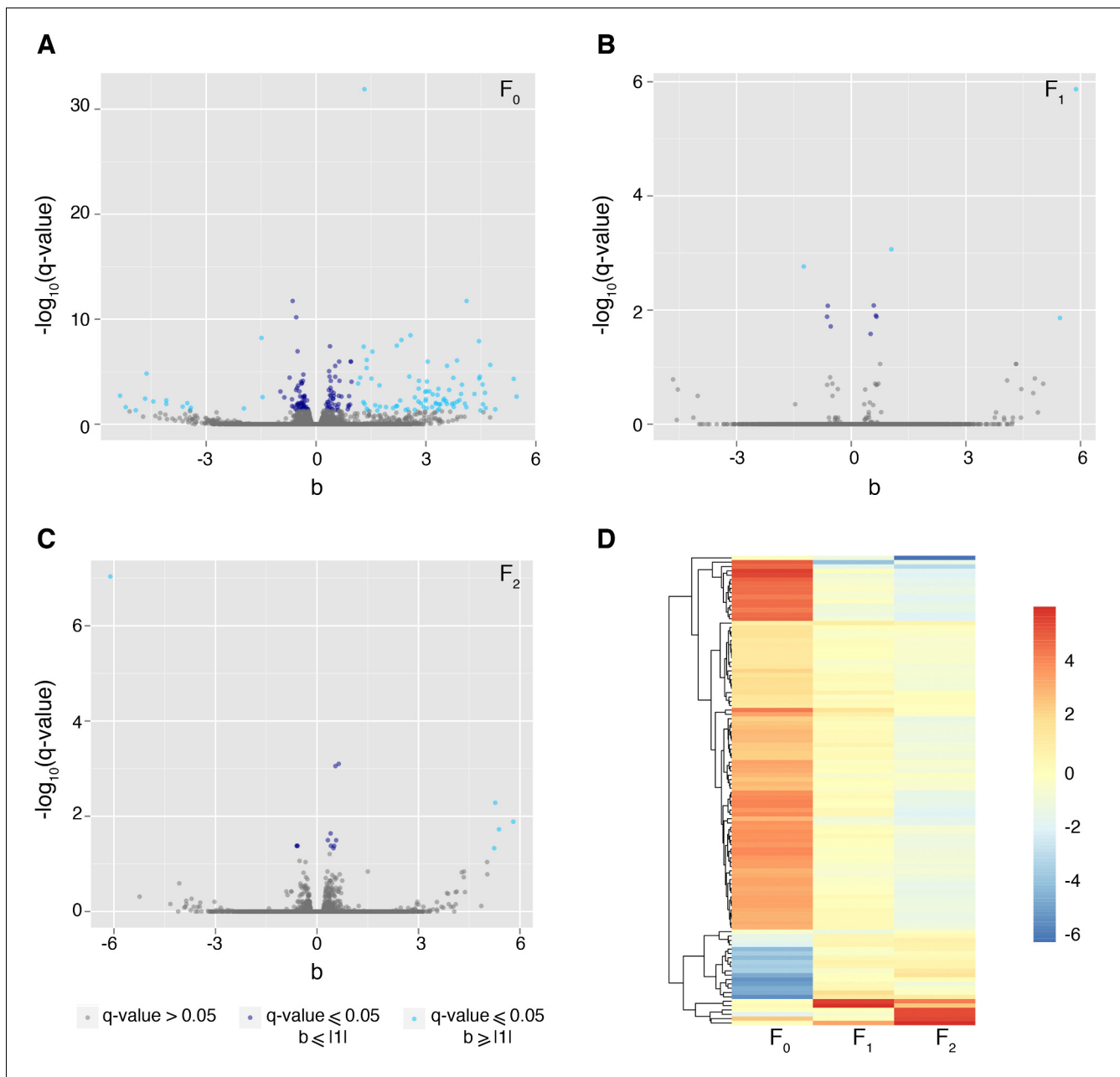


Figure 1—figure supplement 4. Global transcriptional changes in the female head. RNA sequencing was performed on heads of exposed and unexposed F₀, F₁, and F₂ females (four replicates each). Volcano plots show the distribution of transcript expression and significance. F₀ flies have a considerable number of differentially expressed transcripts (A). Whereas F₁ and F₂ heads have very few changes in transcripts (B) and (C). The beta value is approximately analogous to the natural log fold change of the transcript, and the q-value is the measure of significance. Gray points indicate a transcript with non-significant q-value, dark blue points indicate transcripts with significant q-value but that do not meet the beta value threshold. Light blue dots have significant q-value and an absolute value of beta greater or equal to one. Heat map shows the trend of transcript expression over the three generations (D). Transcripts meeting the threshold criteria (q-value and beta) for any one generation was included in the map.

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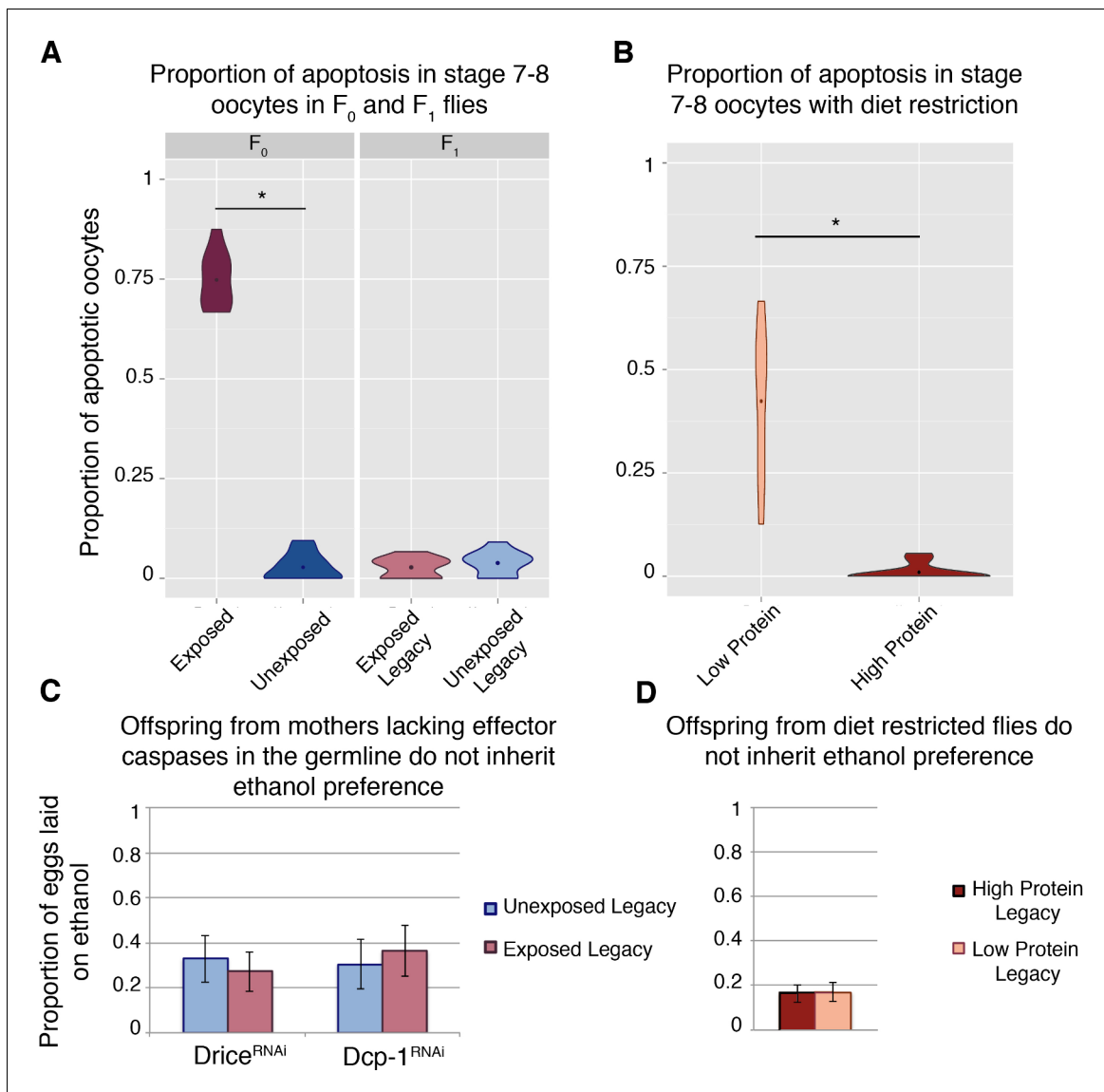


Figure 2. Germline apoptosis and activated caspases play a role in the inheritance of ethanol preference. Apoptosis in stage 7–8 egg chambers was quantified in F_0 and F_1 (legacy) flies (A); wasp exposure leads to elevated levels of apoptosis but is not persistent across the next (F_1) generation. Similarly, apoptosis was quantified in stage 7–8 egg chambers in flies fed different diets: Flies fed a protein-restricted diet have elevated levels of stage 7–8 oocyte apoptosis (B). Genetic knockdown of the germline effector caspases, Drice or Dcp-1, was achieved by expressing a RNA hairpin in the female germline (driven by the maternal- α tubulin-Gal4). Offspring from these flies were collected and tested for ethanol preference: Ethanol preference is not inherited from mothers with Dcp-1 or Drice knockdown (C). Offspring from the diet treatments were similarly tested; progeny from protein-restricted parents don't inherit an ethanol preference (D). Points within violin plots denote the group mean. Sample size was 10 for each experimental group. Asterisk indicates a p-value of <0.05 from a Mann-Whitney U test. Error bars are bootstrap 95% confidence intervals.

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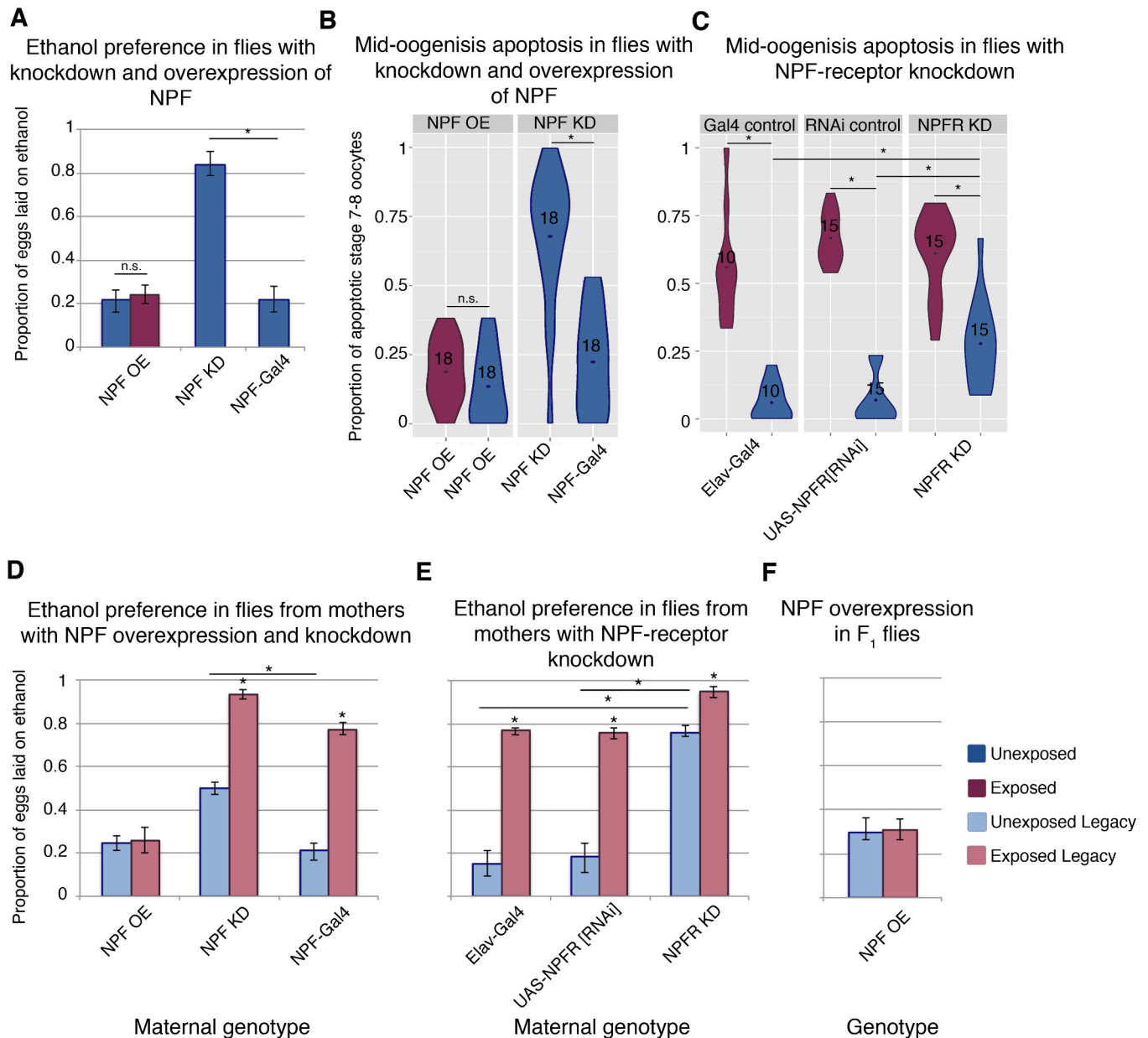


Figure 3. NPF affects ethanol preference and germline apoptosis. Genetic manipulation of NPF levels can alter ethanol preference (A). NPF overexpression (OE) or knockdown (KD) was achieved in the NPF-expression pattern (NPF-Gal4). This genetic manipulation of NPF can alter levels of germline apoptosis as well (B). Knockdown of the NPF-receptor in neurons (using the pan neuronal driver Elav-Gal4) leads to increased germline apoptosis (C). F_1 legacy flies have altered ethanol preference depending on the maternal NPF genotype (D). Similarly, maternal knockdown of the NPF-receptor in neurons can drive inheritance of ethanol preference in legacy flies (E). Genetic manipulation of NPF in the F_1 legacy flies (overexpression in the NPF-expression pattern) can alter the inheritance of ethanol preference (F). Points within violin plots denote the group mean; the number within the violin plot indicates sample size for the group. Asterisk indicates a p-value of <0.05 from a Mann-Whitney U test. Error bars are bootstrap 95% confidence intervals. Color-coding of charts indicates treatment and generation; dark blue (unexposed), light blue (unexposed legacy), dark magenta (exposed), light magenta (exposed legacy).

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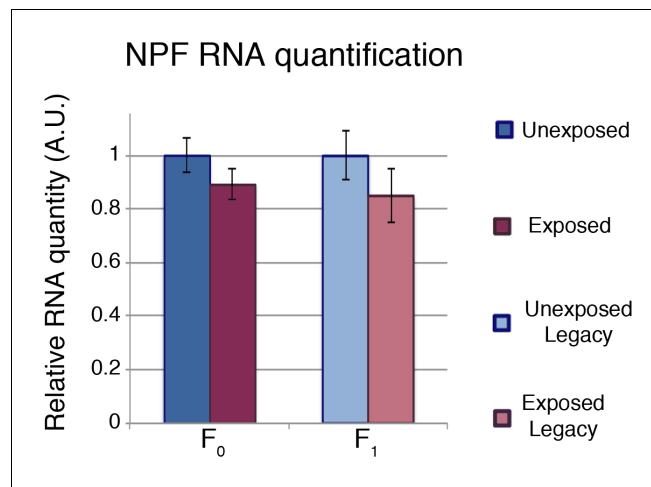


Figure 3—figure supplement 1. Ethanol preference in F₁ legacy flies for transgene control lines. The fly lines carrying either the transgene UAS-NPF or UAS-NPF[RNAi] are able to transmit the ethanol preference behavior following wasp exposure. Asterisk indicates a p-value of <0.05 from a Mann-Whitney U test. Error bars are bootstrap 95% confidence intervals.

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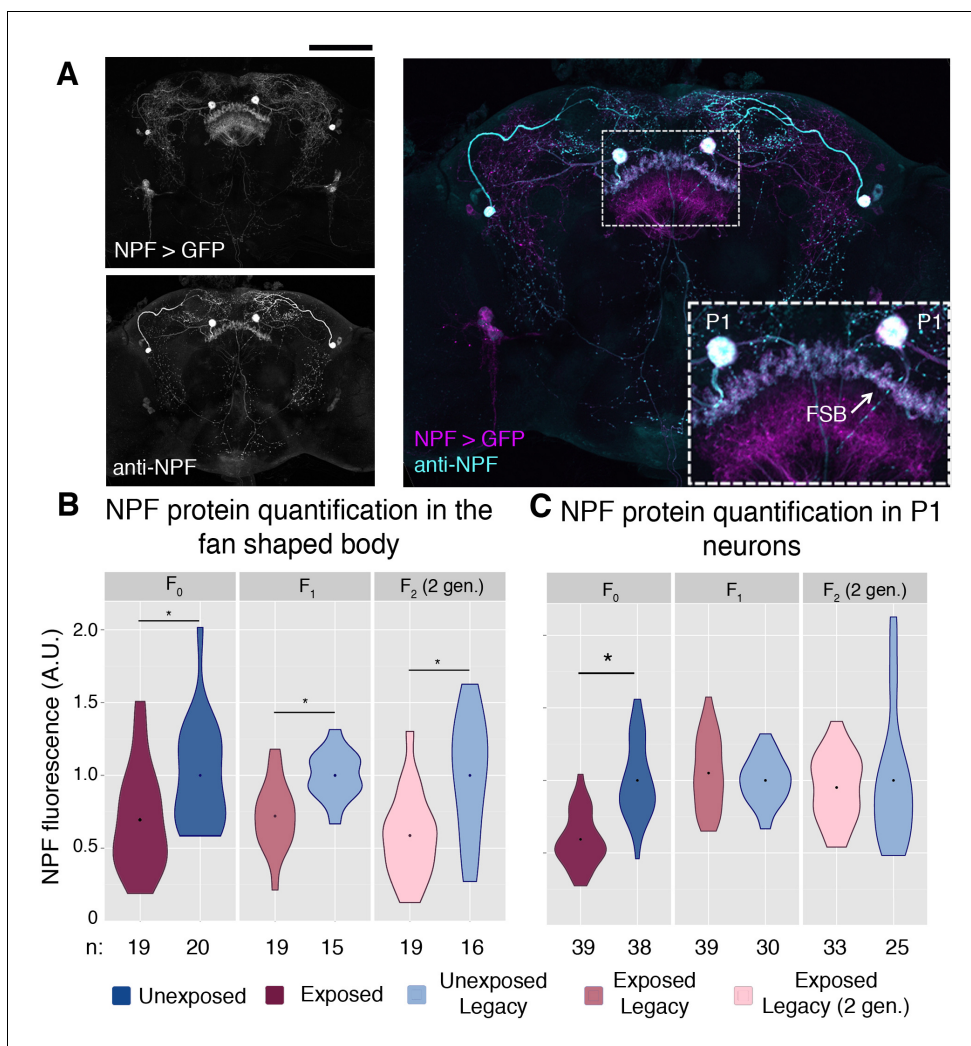


Figure 4. NPF protein is reduced in the fan shaped body following wasp exposure. NPF antibody staining has a similar pattern to that of NPF-Gal4 expression in an adult female brain, inset shows a magnification of the two large P1 neurons and the fan shaped body (FSB) (A). NPF protein levels are reduced in the fan shaped body across generations (B). NPF depression in P1 neurons is observed only in the F₀ generation (C). Points within violin plots denote the group mean. Sample size (n) is indicated at the bottom of the graph for each group. Asterisk indicates a p-value of <0.05 from a Mann-Whitney U test. Scale bar is 100 microns.

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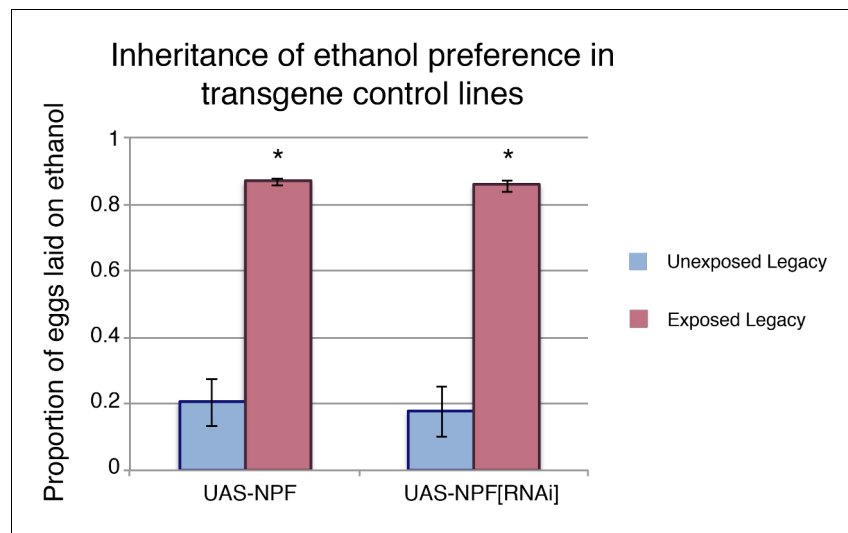


Figure 4—figure supplement 1. mRNA quantification of NPF in female fly heads. Asterisk indicates a p-value of <0.05 from a students t-test. Error bars represent standard error of the mean. Sample size is three per experimental group.

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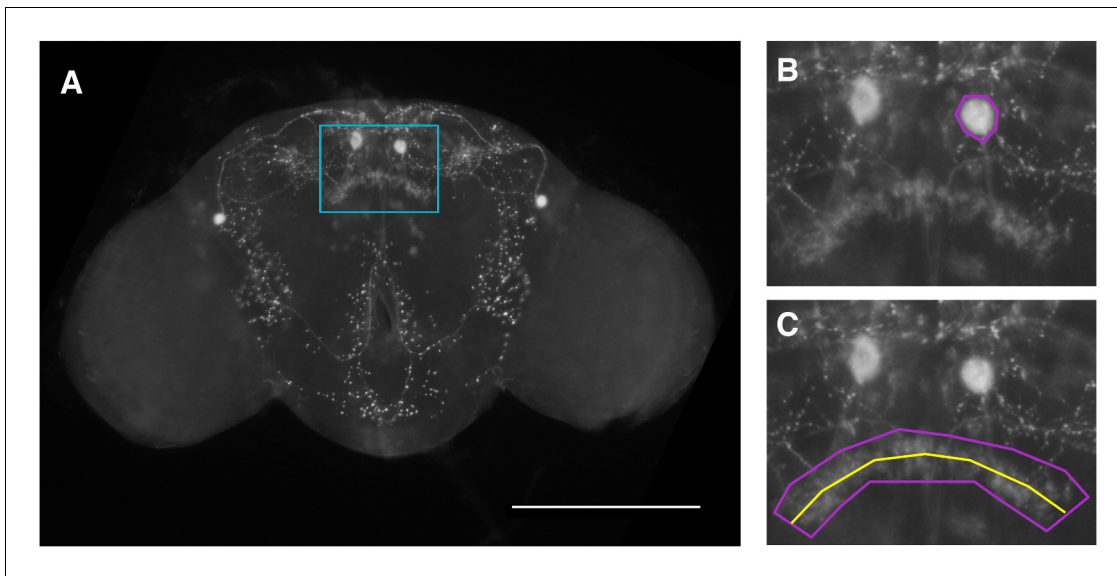


Figure 4—figure supplement 2. Region of interest for NPF protein quantification. NPF antibody staining of a female fly brain is shown (A). Blue box indicates region of magnification for subsequent panels. The cell body of the P1 neuron is outlined (magenta) as a demonstration of the region of interest for NPF protein quantification (B). The fan shaped body is outlined (magenta) as a demonstration of the region of interest for the NPF fan shaped body quantification (C). The yellow line was drawn to approximate the middle of the fan shaped body structure and would be used as the 'length' measure for quantification. Scale bar is 200 microns.

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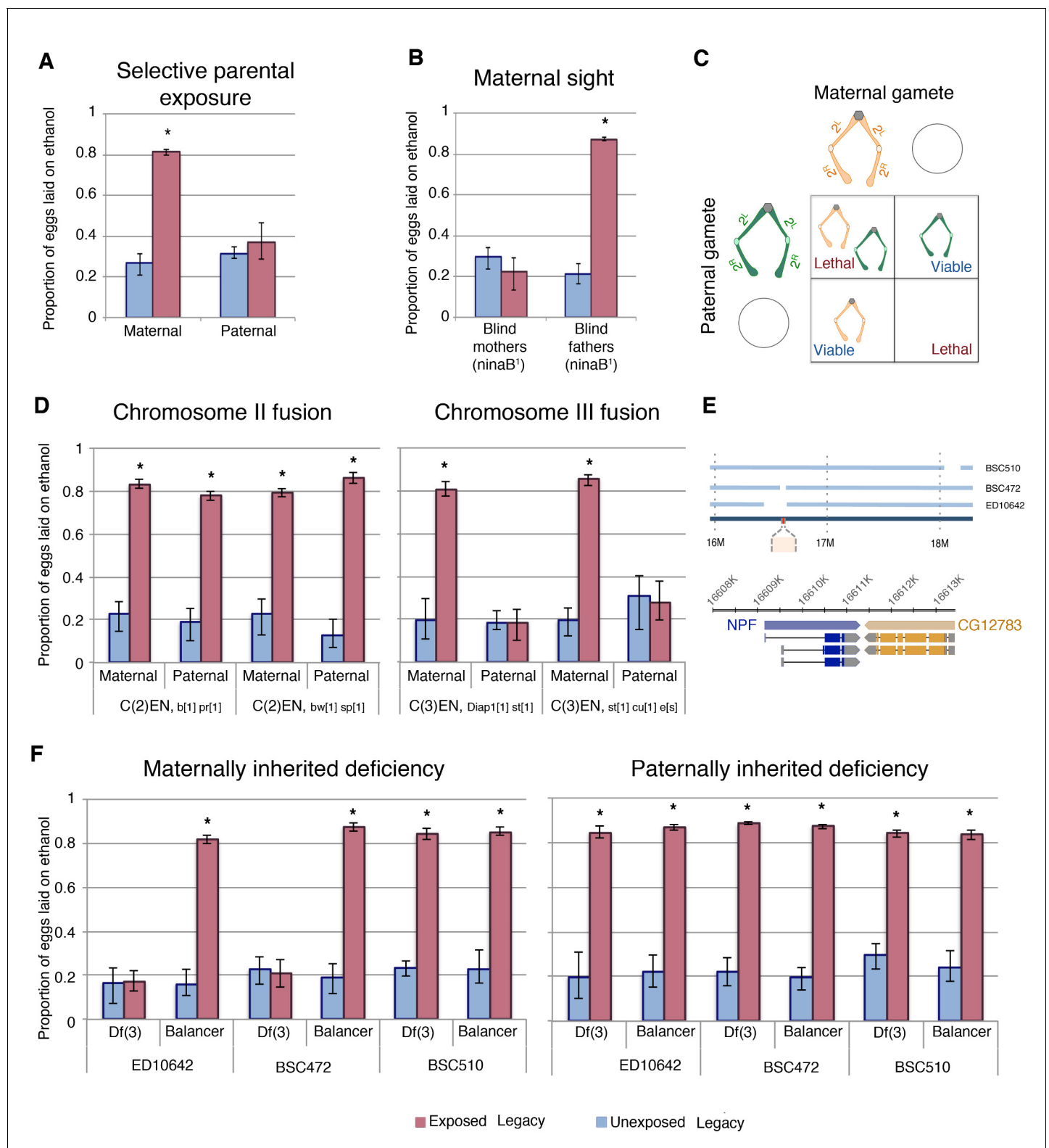


Figure 5. Maternal chromosome three is required for inherited ethanol preference. Experiments with exclusively maternal or paternal wasp exposure demonstrate that maternal wasp exposure is necessary for ethanol preference inheritance (A). 'Blind' flies, with a mutation in *ninaB* were used to test the requirement of sight: maternal or paternal *ninaB¹* flies (mated with wild type counterparts) were wasp exposed and offspring tested for ethanol preference (B). Schematic of compound chromosome 2; progeny inherit both copies of the chromosome from either maternal or paternal source and Figure 5 continued on next page

Figure 5 continued

are identified based on chromosomal markers (C). Flies receiving either maternal or paternal copies of the compound chromosome two are able to inherit the ethanol preference, but compound chromosome three must be maternally derived to facilitate inheritance of ethanol preference (D). The relative location of NPF (red) on chromosome three and the deleted region of the deficiency stock is shown in a diagram (E). The inheritance of ethanol preference was observed in flies receiving an intact maternal NPF locus on a balancer chromosome and not in flies from receiving a maternal NPF deficiency (Df3) chromosome: Paternal inheritance of the NPF deficiency had no effect on transmission of ethanol preference (F). Asterisk indicates a p-value of <0.05 from a Mann-Whitney U test. Error bars are bootstrap 95% confidence intervals.

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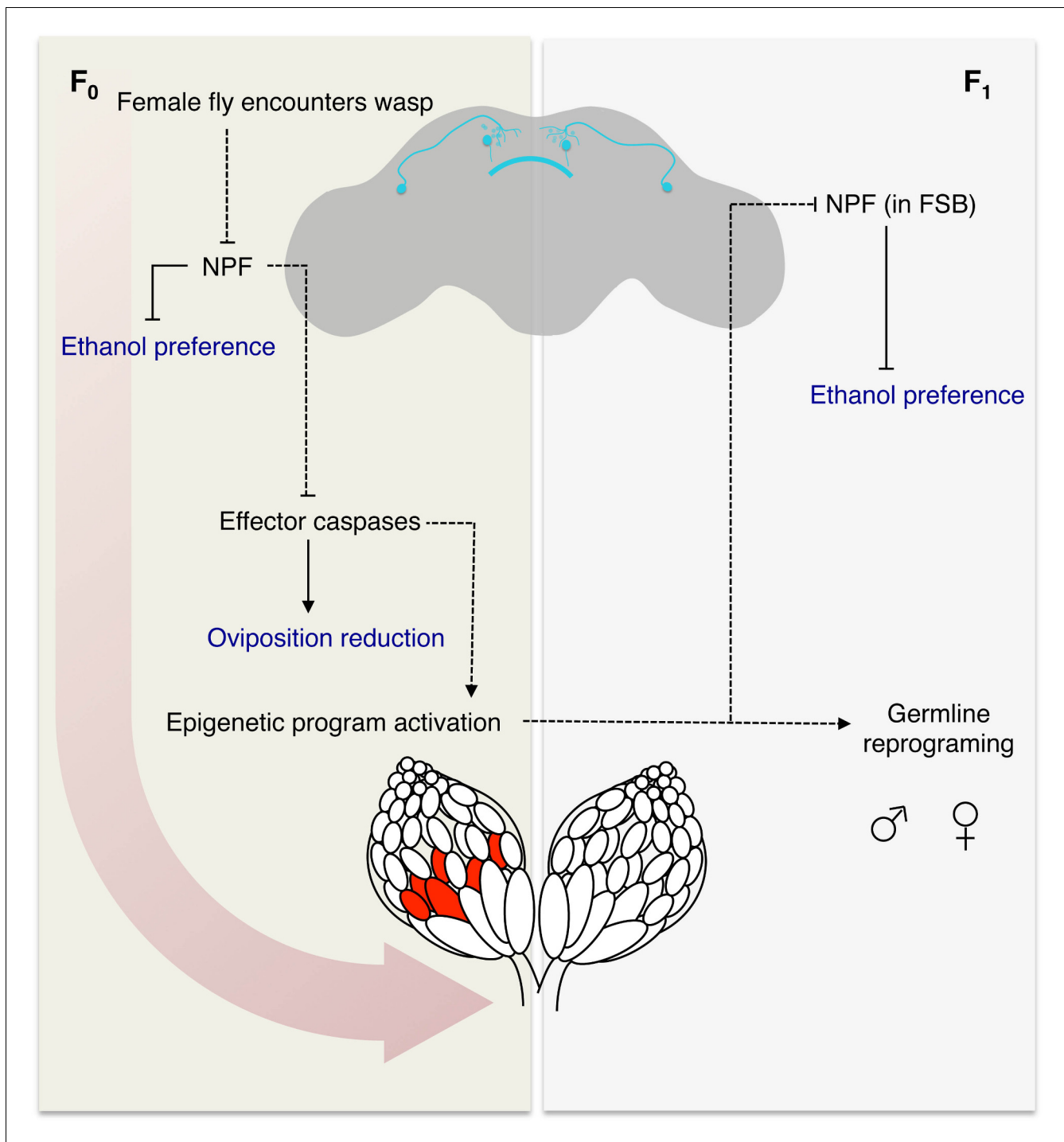


Figure 6. Model for fly-wasp mediated ethanol preference. A female fly encounters a wasp, based in part on visual signals, leading to a cascade of physiological and behavioral changes. One of the initiating factors following wasp exposure is the depression of NPF in the female fly brain. Under normal conditions, NPF inhibits the ethanol preference behavior and caspase mediated germline apoptosis. Therefore, the reduction of NPF triggers ethanol preference and germline caspases. Activation of the germline effector caspases Dcp-1 and Drice in turn reduced egg laying and participates in the epigenetic reprogramming of the female germline and chromosome 3. The epigenetic program is passed to both sexes of the F₁ generation, in that both male and female progeny can pass on the ethanol preference. Further, legacy F₁ female flies inherit depressed NPF in the fan shaped body (FSB), which drives the ethanol preference behavior. Model legend: Measured behavioral outputs are in blue, the dashed lines indicate a speculative or unknown mechanism of action.

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