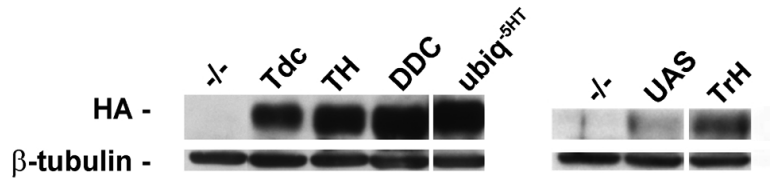

Figure S1 Protein expression and neurotransmitter content in genetically rescued *dVMAT* mutants. (A) Western blots show expression of the HA-tagged DVMAT transgene in *dVMAT* mutant (-/-) expressing *UAS-DVMAT* with the indicated drivers, *TH-Gal4* (TH), *Tdc2-Gal4* (Tdc), *TrH-Gal4* (TrH), *Ddc-Gal4* (DDC), and *da-Gal4* (ubiq^{-5HT}). Note that the *UAS-DVMAT* transgene (UAS) shows "leaky" expression in the absence of driver.

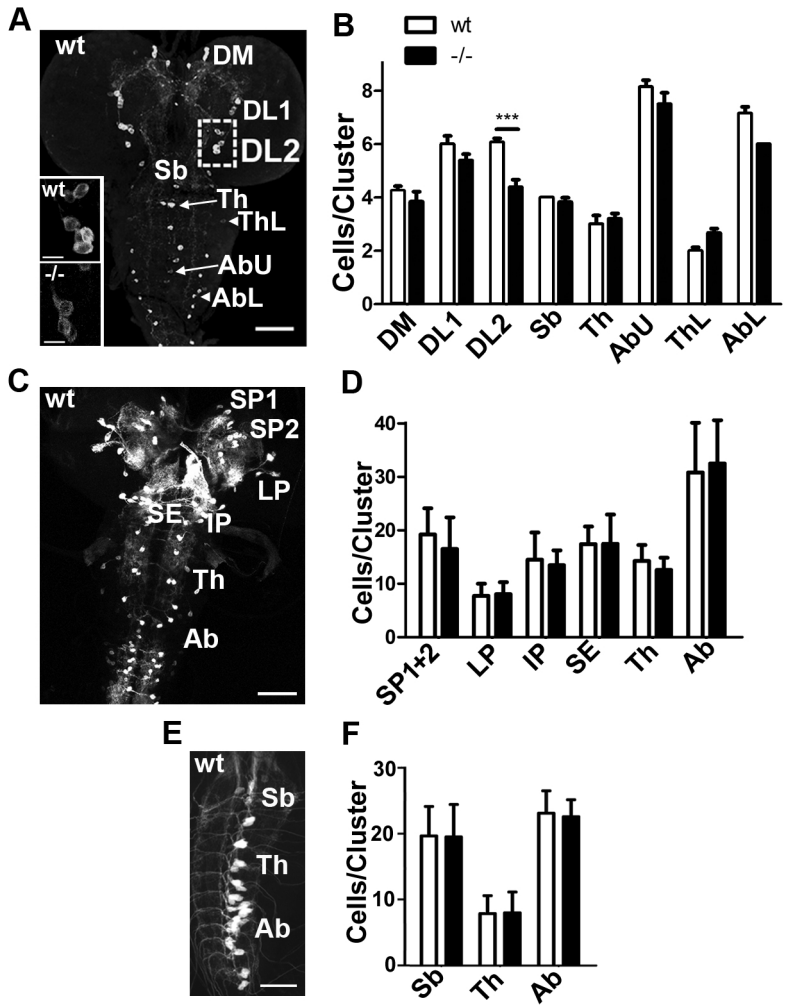

Figure S2 Aminergic cell counts in *dVMAT* mutants. (A, B) *dVMAT* mutants possess a reduced number of dopaminergic neurons in the DL2 cluster but are otherwise comparable to WT. (A) The arrangement of DA neuron clusters are pictured in wild-type larva with the broken rectangle indicating the DL2 cluster. Inset shows a representative image of DL2 cluster in the wild-type (WT) and *dVMAT* mutant (-/-). (B) Quantitation of each DA cluster (white bars: WT; black bars mutant, n=8 animals per genotype, mean +/- SEM) shows that there are significantly fewer neurons in DL2 in the mutant as compared WT (two way ANOVA, $p<0.0001$, Bonferroni post test, ***$p<0.001$ as indicated). Differences between the number of octopaminergic (C, D) or serotonergic (E, F) neurons in WT (n=9) versus mutant (n=12) are not detectable for each indicated cluster. Scale bars A, B, C: 50 um; A inset: 10 um.

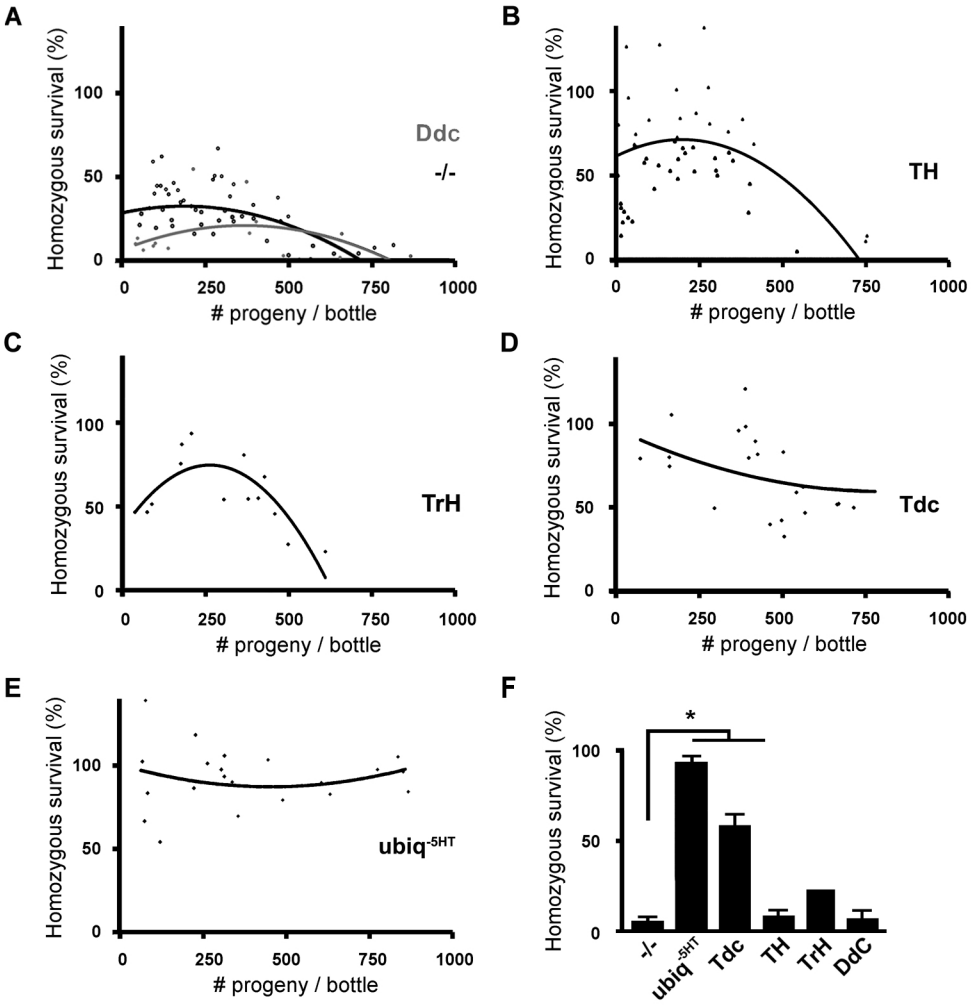

Figure S3 Homozygous survival plotted as a function of population density, and with *dVMAT* mutants expressing *UAS-DVMAT* using the indicated drivers. (A) Survival of homozygous *dVMAT* progeny (-/-) are plotted as a function of population density (black circles). Data for *dVMAT* mutants expressing *UAS-DVMAT* using the *Ddc-Gal4* are shown in gray. Rescue of *dVMAT* using *TH-Gal4* (B), *TrH-Gal4* (C), *Tdc-Gal4* (D), and *daughterless-Gal4* (E) is indicated. Second-order polynomial trendlines are displayed as solid lines. (F) Under standard culture situations (500-1000 progeny/bottle), approximately 6% of the homozygous *dVMAT* null mutant (-/-) progeny survive. Expression of a DVMAT transgene in DA or 5-HT cells using *TH-Gal4* (TH), *TrH-Gal4* (TrH), or *Ddc-Gal4* (Ddc) respectively, do not rescue the survival deficit, whereas expression of DVMAT using *da-Gal4* (ubiq^{-5HT}) or *Tdc2-Gal4* (Tdc) significantly rescues lethality under standard, high density culture conditions (1-way ANOVA, * $p<0.05$, Bonferroni post test).

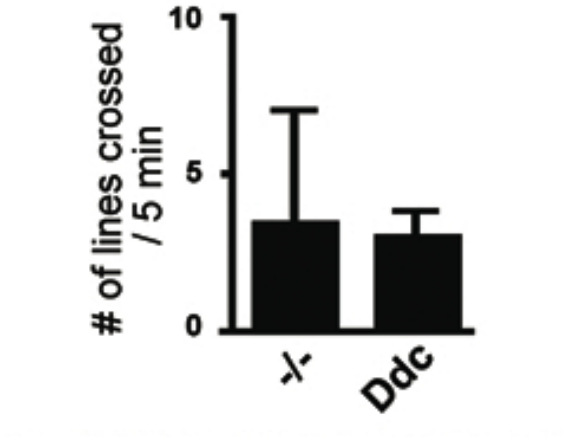

Figure S4 Expression of *UAS-DVMAT* using *Ddc-Gal4* does not rescue larval locomotion deficits of the *dVMAT* mutant. Expression of DVMAT using *Ddc2-Gal4* does not rescue the larval locomotion deficit of the *dVMAT* mutant (-/-). These data were obtained in a separate set of experiments from those shown in FIGURE 2A.

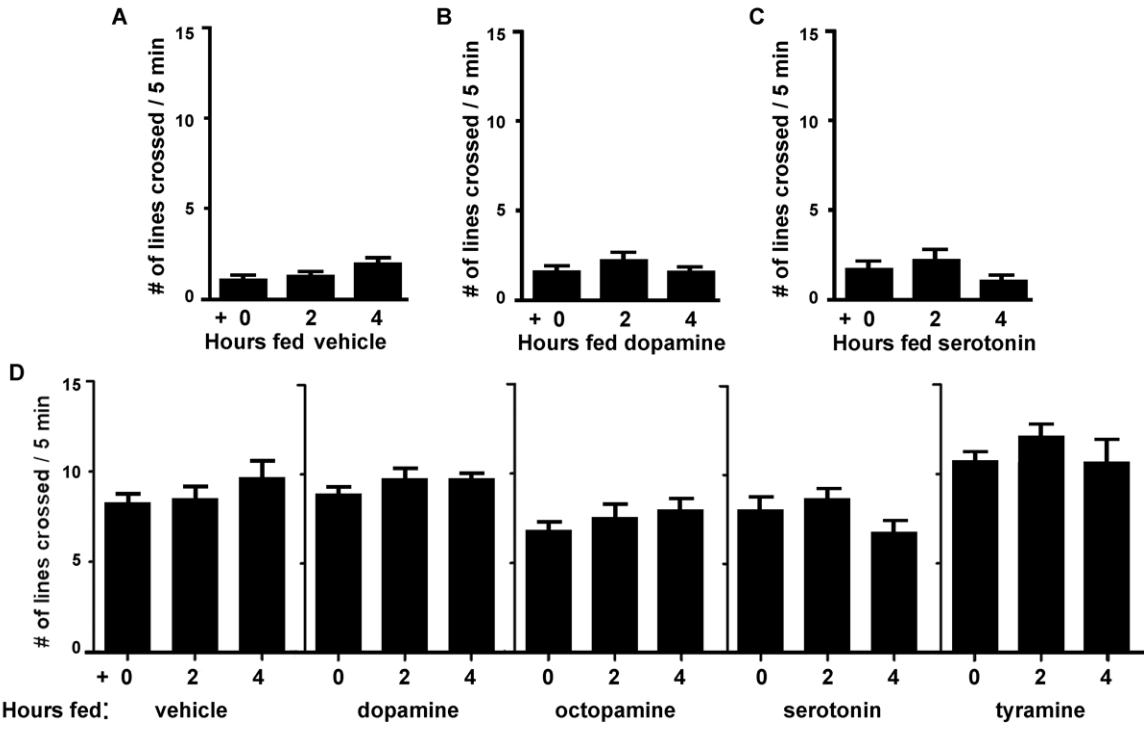

Figure S5 Controls for locomotion assays. (A-C) Feeding vehicle, dopamine (10 mg/mL) or serotonin (10mg/mL) does not alter *dVMAT* locomotion. (D) Locomotion was scored for WT larvae incubated on food containing the indicated amines (10 mg/ml) for 0, 2 and 4 hours. Locomotion of WT larvae is not detectably altered under these conditions.

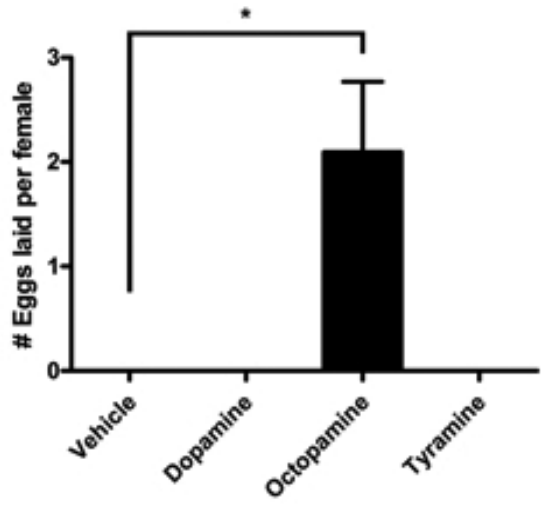

Figure S6 Octopamine partially rescues egg-laying. Three virgin (0-3 day old) *dVMAT* mutant females were collected and crossed to six virgin WT males (0-3 day old) and allowed to mate for three days on standard fly food. The males were then removed and discarded. The mated females were transferred into colored food containing 10mg/ml vehicle, dopamine, octopamine or tyramine and allowed to lay eggs for 2 days. The average number of eggs laid per female in a 24-hour period was calculated (2-way ANOVA with Bonferroni post-test, * $p<0.05$).

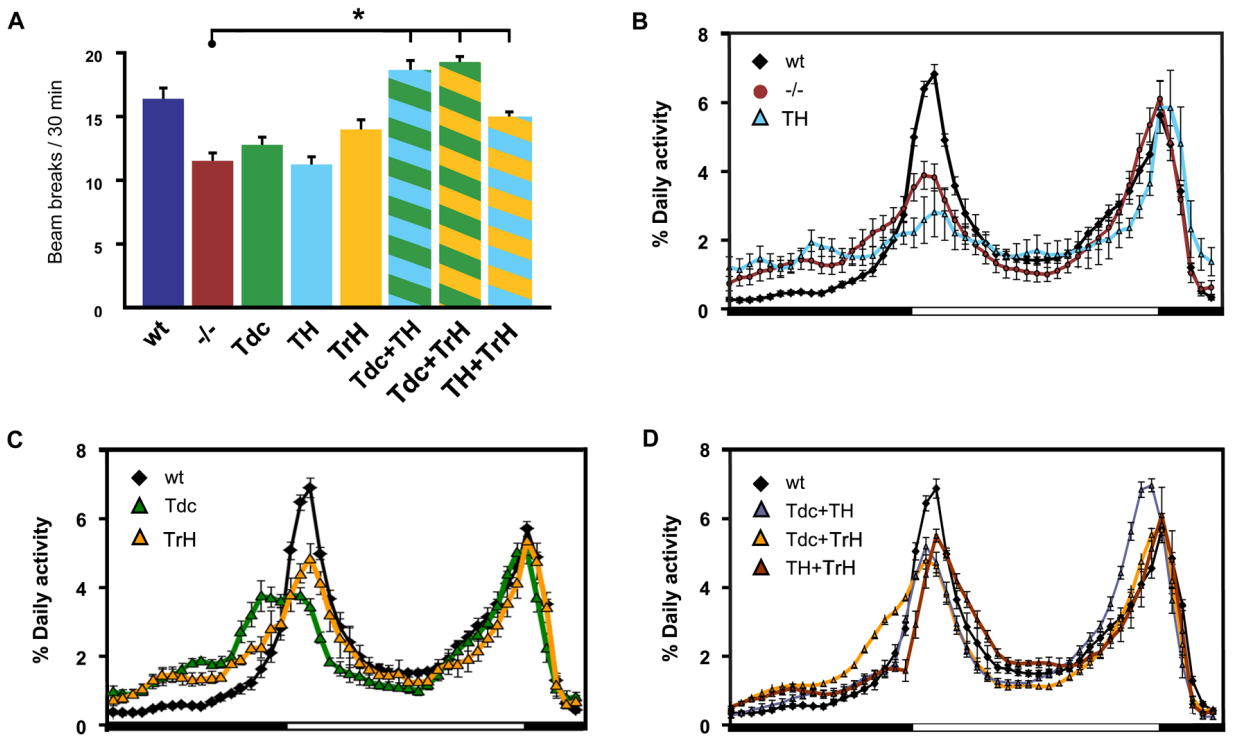

Figure S7 Average LD activity profiles for the *dVMAT* mutant and rescue lines. Average diurnal activity profiles were done in parallel but displayed in separate panels for clarity: (A) Histograms presenting the mean activity level as number of beam breaks per 30 min for the indicated genotypes. Genotypes showing improvement in the mean activity level compared to the *dVMAT* mutant are indicated (Kruskal-Wallis test with Dunn's Multiple comparisons test, $p<0.05$). (B) Activity of WT control flies (black diamonds), the *dVMAT* mutant (red circles) or the *dVMAT* mutant with DVMAT expression restored in dopaminergic neurons (*TH-Gal4* rescue, cyan triangles). The *dVMAT* mutant has a reduced morning activity peak that is exacerbated by rescue of DVMAT expression in DA neurons. (C) Activity of WT control flies (black diamonds), versus the *dVMAT* mutant rescued with *Tdc2-Gal4* (green triangles) or *Trh-Gal4* (yellow triangles). Expression of DVMAT with *Tdc2-Gal4* results in an abnormally broad and quasi-bimodal peak of morning activity. (D) Average activity plots for WT control (black diamonds), and the *dVMAT* mutant rescued using two drivers: Tdc + TH (gray triangles), Tdc + TrH (gold triangles) and TrH + TH (brown triangles). All three combinations rescue morning activity.