Supplemental Data

MicroRNA Processing Pathway Regulates

Olfactory Neuron Morphogenesis

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Supplemental Experimental Procedures

Fly Stocks and Phenotypic Analysis

The insertions *LL03660* (in *pasha*), *LL06357* (in *Dicer-1*) and *LL06590* (in *aubergine*) originate from a *piggyBac* screen [1]. Information for all other mutant alleles used can be found in Flybase (http://flybase.bio.indiana.edu). Mosaic analyses with MARCM were performed as previously described using Gal4-GH146 and -Mz19 [2]. UAS-*Dicer-1* and UAS-*Dicer-2* transgene insertions on the 3rd chromosome and 2nd chromosome, respectively, were used for rescue and other experiments [3].

Transgene Construction

To generate UAS-*pasha-HA*, a full length cDNA (LD23072) was amplified using the following primers (5'-3'): CACCATGGCGGAGAAGCCGCTGGCC and AAGTTCCACGTTGTTCAAATTGGACCC. The PCR product was subcloned into pENTR-D/TOPO (Invitrogen) and recombined into pTWH (Gateway Collection, Drosophila Genomics Resource Center, Bloomington, IN) by using the Gateway LR clonase II enzyme mix (Invitrogen). A UAS-*pasha-HA* transgene insertion on the 2nd chromosome was used for all rescue experiments.

Generation of GH146-Gal4 Transgene

The p[GAL4, w+]^{GH146} enhancer trap was reproduced as a construct by inserting the 5P transposase promoter, GAL4 gene, and hsp70 terminator from pGaWB [4] into a cloned 12.6kb genomic fragment which flanks the p[GAL4, w+]^{GH146} insertion site. The GAL4 gene was inserted into the genomic fragment in the same position and orientation as the p[GAL4, w+]^{GH146} enhancer trap. The entire construct was cloned into a DsRed-marked *piggyBac* transformation vector [1]. A GH146-Gal4 transgene insertion on the X chromosome was used for MARCM experiments on chromosome 2R.

Clonal Analysis Using *bantam* Sensor

Mutant clones of *pasha*, *Dicer-1* and *AGO1* were generated in 3rd instar larval wing discs and assayed for *bantam* miRNA activity using the *bantam* sensor transgene on chromosome 2R (we determined to be at 60A11 by inverse PCR) as described in Brennecke et al. [5]. Discs were stained with anti-β-Galactosidase antibody to mark the clones, anti-GFP to visualize sensor expression and DAPI to mark nuclei. Clones were analyzed by confocal

microscopy and bantam activity was defined as ratios of mean fluorescence intensities of homozygous mutant divided by heterozygous tissue. Confocal images for clonal GFP level quantification in Figure S3 were taken under non-saturating conditions and black values were above 0.

Immunohistochemistry

Fly brains were dissected, fixed, stained and primary antibodies were diluted as described [1, 2, 6]. Rabbit anti-Pasha was used 1:500 [7], rabbit anti-Dicer-1 1:100 [8], chicken anti-GFP 1:500 (Aves Labs, cat.# GFP-1020) and rabbit anti-β-Galactosidase 1:1000 (Cappel, cat.# 55976).

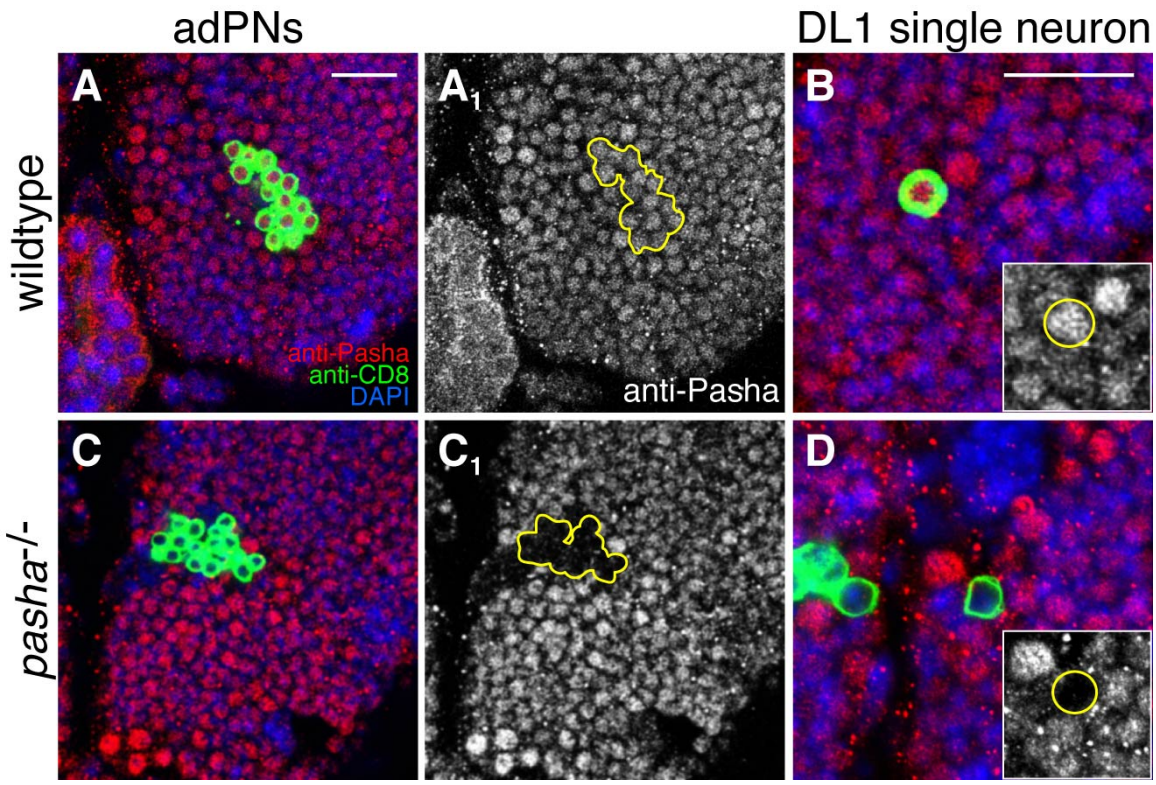

Figure S1. Pasha protein is absent in *pasha*$^{-/-}$ PNs at 18h APF.

(A and B) Pasha localizes to the nucleus of WT adPNs and DL1 single neurons and all surrounding cells in 18h APF pupal brains at equal levels. Pasha staining is shown separately in A_1 for the adPN clone and the inset in B for a single neuron, both outlined in yellow.
(C and D) Pasha protein is absent from *pasha*$^{-/-}$ adPNs (C and C_1) and DL1 single neurons (D and inset) at 18h APF while heterozygous neighboring cells express Pasha in the nucleus. The MARCM clones are outlined in yellow based on GFP-staining; the actual clone may also contain some Gal4-GH146 negative, and hence GFP-negative cells (e.g., in C_1).
Green is mCD8-GFP labeled MARCM clones, red labels anti-Pasha and blue is DAPI. Scale bars represent 20μm. All images are single confocal sections.

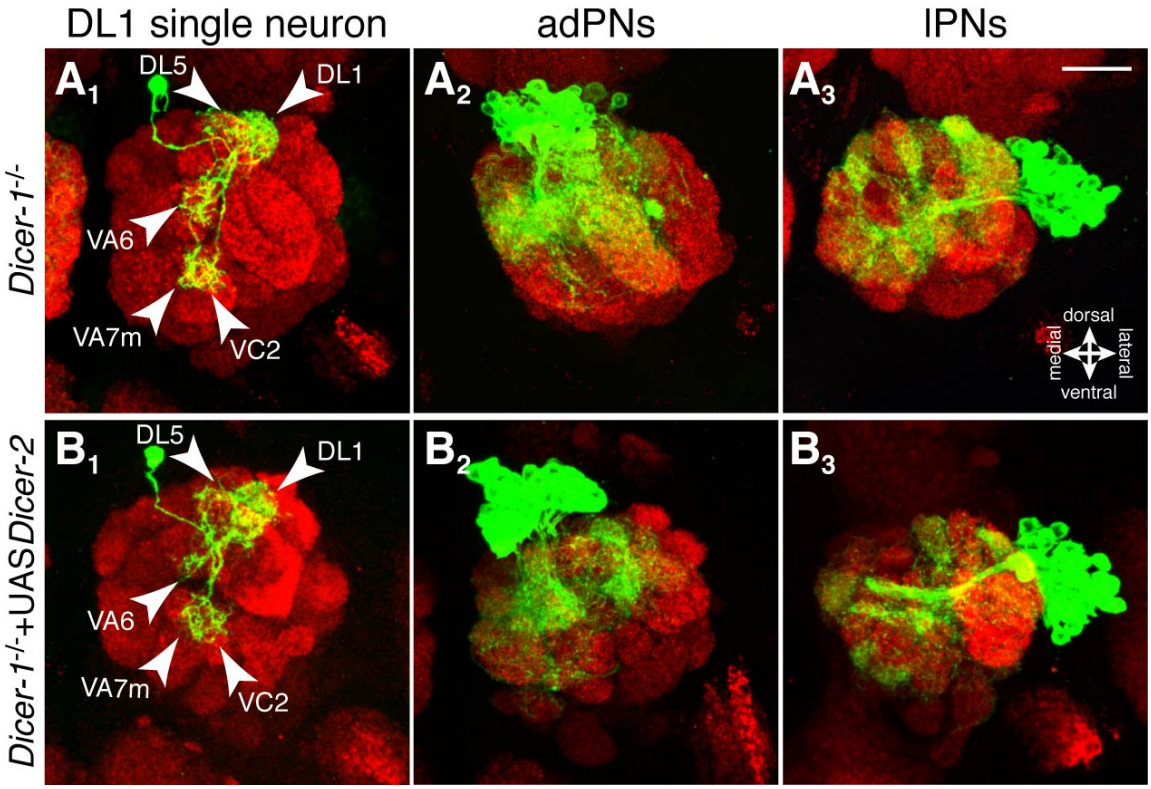

Figure S2. Dicer-2 cannot compensate for Dicer-1 function during PN targeting.

(A_1) In *Dicer-1*$^{-/-}$ DL1 single neurons dendrites mistarget to other glomueruli besides DL1 (arrowheads). In *Dicer-1*$^{-/-}$ adPNs (A_2) and lPNs (A_3) the dendritic mass is significantly reduced and dendrites spill non-specifically into inappropriate glomeruli. All these dendritic phenotypes cannot be rescued or altered by overexpressing Dicer-2 in *Dicer-1*$^{-/-}$ DL1 single neurons (arrowheads in B_1, compare to A_1), adPNs (B_2, compare to A_2), and lPNs (B_3, compare to A_3). Green is mCD8-GFP labeled MARCM clones, red labels the presynaptic marker nc82. Scale bar represents 20μm. All images are *z*-projections of confocal stacks.

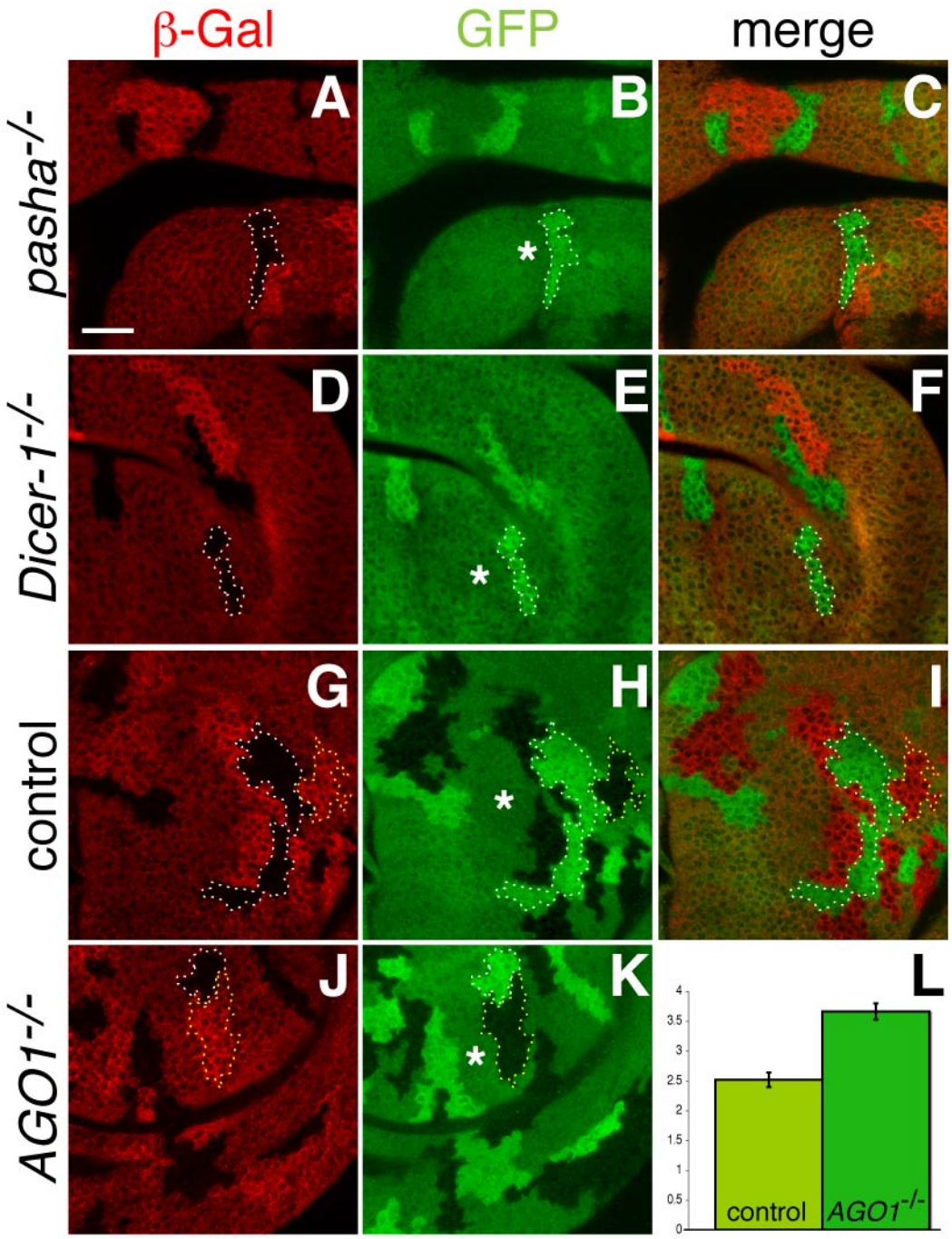

Figure S3. *bantam* sensor is de-repressed in *AGO1*^{k08121} mutant clones of larval wing discs.

(A-C) *pasha*$^{-/-}$ clones are marked by the absence of a *LacZ* reporter gene (red, A). An example is outlined by dashed line. GFP levels are elevated compared to

heterozygous tissue (asterisk), presumably due to the absence of mature miRNA within the clone (B). n=11.
(D-F) In *Dicer-1*$^{-/-}$ clones the effect of *bantam* sensor de-repression is similar. n=12.
(G-K) FRT42D control (G) or FRT42D *AGO1*^{k08121} mutant clones (J), respectively, are marked by the lack of β-Gal staining (examples outlined by white dashed line) and contain two copies of the same *bantam* sensor transgene as in B and E, which is located also on chromosome 2R (H, K). Twin spot clones contain two copies of *LacZ* (examples marked by yellow dashed line) but lack any *bantam* sensor transgene (H, K). Heterozygous tissue bears one copy of *LacZ* and *bantam* sensor transgene and is marked by an asterisk (H, K).
(L) The extent of *bantam* sensor de-repression in *AGO1*^{k08121} mutant clones was determined as follows: we subtracted the mean fluorescence intensity of twin spot clones (no *bantam* sensor) from the homozygous (two copies of *bantam* sensor) and heterozygous clones (one copy of *bantam* sensor) separately, and then calculated the ratio of the homozygous and the heterozygous values. In control clones, *bantam* sensor expression is increased by ~2.5 fold compared to heterozygous tissue. In *AGO1*^{k08121} mutant clones *bantam* sensor expression is up-regulated ~3.7 fold, showing a significant increase in GFP levels compared to the control ($p=1.7x10^{-6}$; n= 13 or 16 independent clones for control or *AGO1*^{k08121} mutants, respectively). Error bars indicate SEM.
Larval genotypes: (A-C) Hs-FLP1; *bantam* sensor/ +; *arm-LacZ* FRT82B/ FRT2A FRT82B *pasha*^{LL03660} y+; (D-F) Hs-FLP1; *bantam* sensor/ +; *arm-LacZ* FRT82B/ FRT2A FRT82B *Dicer-1*^{LL06357} y+; (G-I) Hs-FLP1; FRT42D *bantam* sensor/ FRT42D *arm-LacZ*; (J-K) Hs-FLP1; FRT42D *AGO1*^{k08121} *bantam* sensor/ FRT42D *arm-LacZ*
Green represents *bantam* sensor GFP expression, red anti-ß-Galactosidase. Scale bar represents 20*μ*m. All images are single confocal sections of 3rd instar wing imaginal discs.

Table S1. Seven miRNA mutants exhibit WT DL1 targeting.

miRNA	allele generated by	reference	WT DL1 targeting*
miR1	homologous recombination	[9]	7/7
miR7	imprecise excision	[10]	10/10
miR8	imprecise excision	[11]	19/19
miR9a	homologous recombination	[12]	14/14
miR14	imprecise excision	[13]	6/6
miR279	P-element insertion	[14]	13/13
bantam	imprecise excision	[5]	6/6

* numbers represent antennal lobes scored for DL1 targeting phenotypes

Supplemental References

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