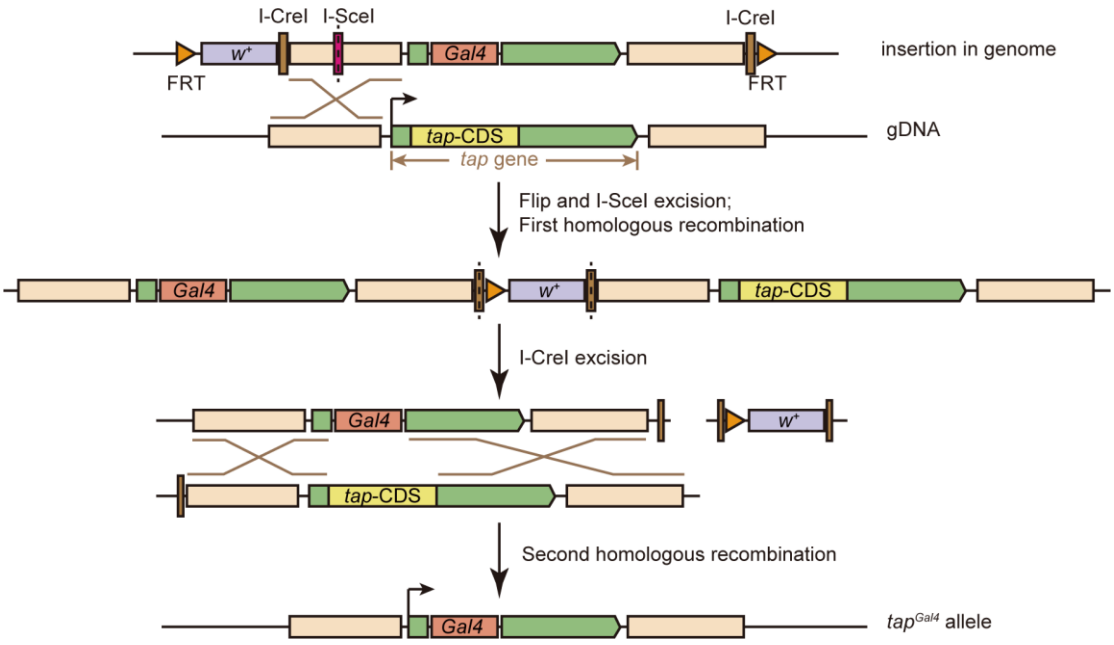


**Figure S1 Detailed schematic diagram of generation of *tap*$^{Gal4}$ allele.**

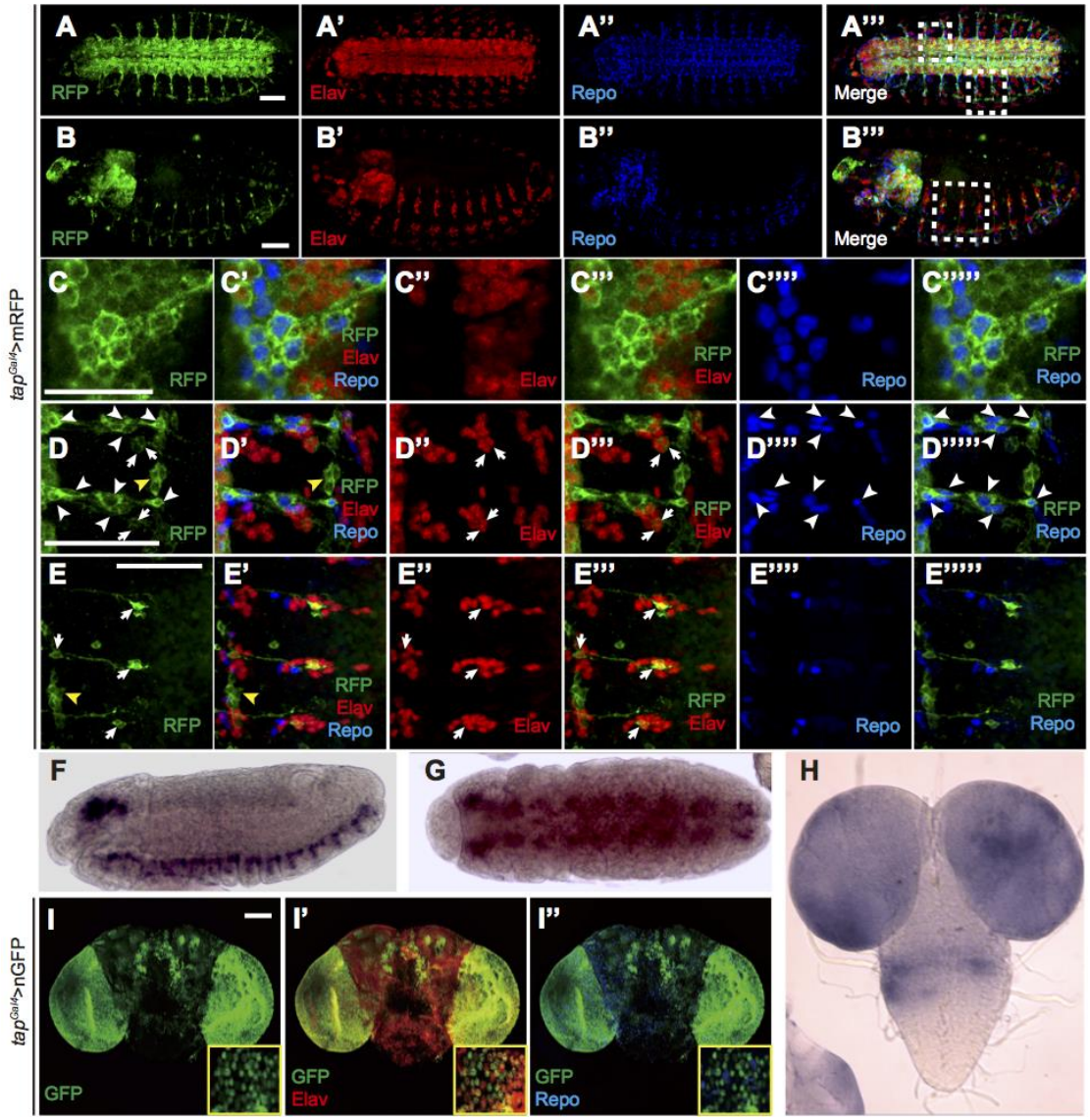


**Figure S2 Tap expression in the nervous system.**

**A-B**. Tap$^{+}$ cells co-stain with differentiated neuronal marker (red) or glial marker (blue). **A**. Dorsal view; **B**. Lateral view. **C**. A close look at VNC (the left framed region in **A'''**). Tap$^{+}$ cells overlap with both neuronal and glial regions. **D-E**. Different views of PNS. **D** is magnification of proximal PNS (right framed region in **A'''**). **E** is magnification of distal PNS (framed region in **B'''**). White arrows: Tap$^{+}$ cells overlap with Elav only; white arrowheads: Tap$^{+}$ cells overlap with Repo only; yellow arrowhead: Tap$^{+}$ cells which are neither neurons nor glias. **F-H**. *In situ* hybridization of *tap*. **F**. Lateral view of a stage 14 embryo. **G**. Dorsal view. **H**. L3 brain. **I**. Tap is expressed in neurons (**I'**) but not glia cells (**II''**) at adult stage. Scale bar=50 μm.

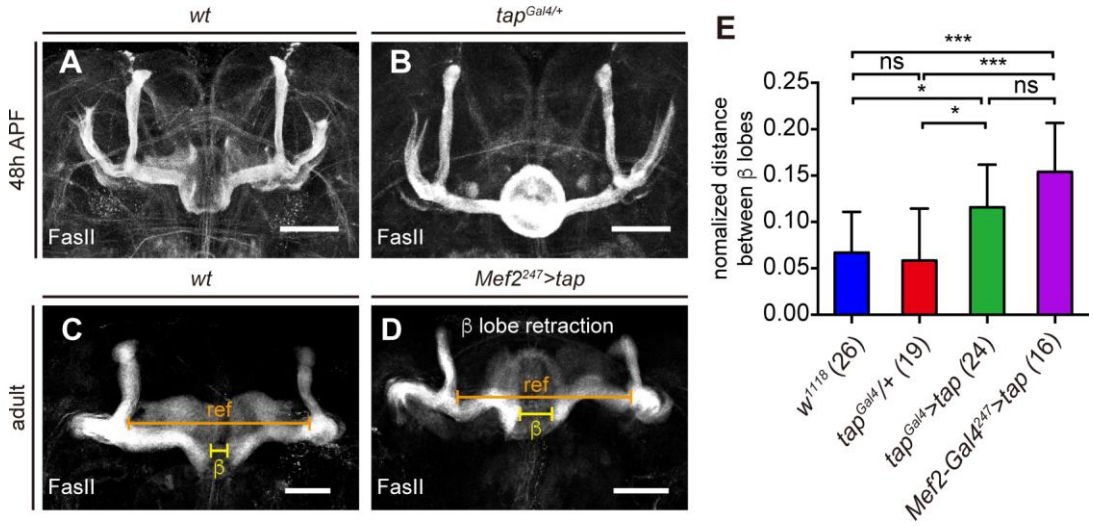


**Figure S3 Tap regulates axonal growth and guidance during development.**

**A**-**B**. Tap is required for the axonal growth at early stages during metamorphosis. Compared to the wild-type MB (**A**), the midline-crossing defect of β lobes in *tap* mutant was observed at early pupal stages (**B**). **C**-**E**. Ectopic expression of Tap increases the distance between contralateral β lobes. The normalized β distance in **E** is calculated by the gap between β lobes (yellow line in **C**, **D**) divided by the distance between two peduncles (orange line in **C**, **D**). Numbers of the brains being analysed were indicated in the bracket. Error bars represent the S.D.. ns: not significant; *: $p<0.05$; ***: $p<0.001$, One-way ANOVA test, Turkey correction. Scale bar=50 μm.

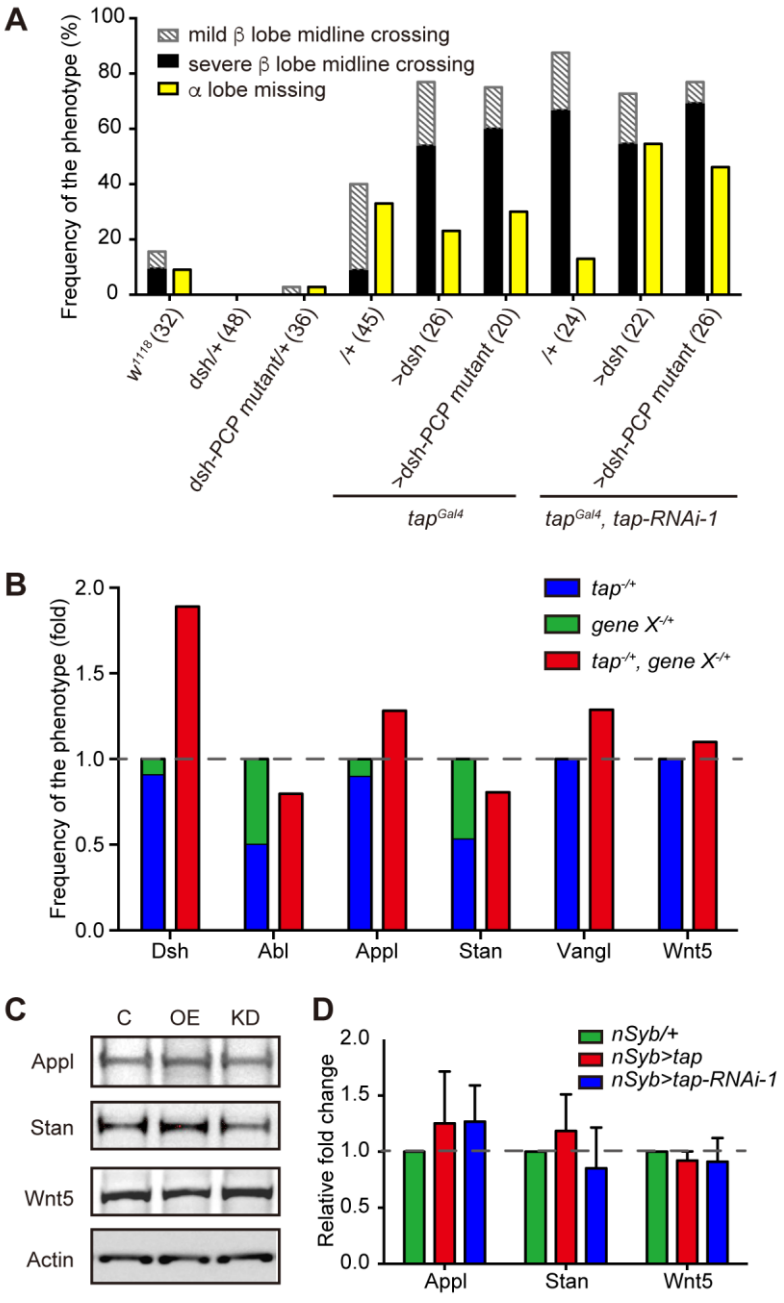


**Figure S4 Tap does not interact with the ligands and receptors in PCP pathway.**

**A.** Ectopic expression of Dsh in *tap* loss of function conditions enhances the β lobe overgrowth defect. Numbers of the brains analysed are indicated in the bracket. **B**. Double heterozygous mutant screen of the protein in PCP signalling for the defects in MB. The penetrance of total β lobe overgrowth defect was normalized to the sum of the penetrance of *tap* mutant plus the mutant of PCP genes. Dashed line indicates 1, which represents the normalized defect severity aggregated by the two single mutants. **C**. Western blot analysis showing the protein levels of some PCP components in

driver alone (C), Tap overexpression (OE) or knockdown (KD) cases. Actin is used as a loading control. **D**. Quantification of relative changes in protein levels of PCP components when OE or KD Tap, normalized by control. Error bars represent the S.D., n=3.

# Supplemental Materials and Methods

### Fly Husbandry and Transgenic Lines

Flies were kept at 25°C or 29°C on standard medium. Experiments involving RNAi technique, or genetic interaction were performed at 29°C, and the others were performed at 25°C. The following lines were obtained from the Bloomington Drosophila Stock Center (BL): *dpp-Gal4/TM6B* (1553), *UAS-mCD8::GFP* (L. Luo), *UAS-mCD8::mRFP* (27398), *nSyb-Gal4* (51914), *UAS-Stinger2* (J. Posakony), *Mef2-Gal4*$^{247}$ (50742), *UAS-dsh.myc* (9453), *UAS-dsh.myc-DEP domain mutant* (9525). The following lines were obtained from the Vienna Drosophila RNAi Center (VDRC): *UAS-tap-RNAi-1* (110705), *UAS-tap-RNAi-2* (12638). For gain-of-function analyses, the following strains were generated: *UAS-mNgn1*, *UAS-ato*, *UAS-tap-GFP*. For loss-of-function analyses in embryo stages, the following strain was generated: *UAS-mCD8-RFP; tap*$^{Gal4}$*/TM6B, Dfd-EYFP*. For MARCM experiments, flies of *hsFlp, UAS-mCD8::GFP/FM7; FRT2A, tub-Gal80/TM6C; ey*$^{OK107}$ were crossed with (1) FRT2A; (2) *FRT2A, tap*$^{Gal4}$*/TM6C*; (3) *UAS-tap/CyO; FRT2A, tap*$^{Gal4}$*/TM6C* flies. The progenies were heat-shocked at 37°C for 1 hour during 0-24h APF, and shifted back to 25°C. The mutants for genetic interaction screen were the following: *Dsh*$^{6}$*/FM7* (BL5297), *Dsh*$^{1}$ (BL5298), *Drl*$^{Red2}$, *Slit*$^{2}$*/CyO* (BL3266), *Dscam1*$^{21}$ (D. Schmucker), *Abl*$^{4}$*/TM6B, Appl*$^{d}$, *Stan*$^{E59}$*/CyO* (BL41776), *Vang*$^{stbm-6}$ (BL6918) *and Wnt5*$^{400}$ (J. Noordermeer).

### Cloning and gene targeting

The fragments of 5' homologous recombination arm of *tap* ORF, Gal4 and 3' recombination arm of *tap* ORF were amplified using the primers below: F1: GTACGGTACCGCTAGCAATGGAGCAAATGGAACGAC, R1: GTACTCTAGACCTAGGATTGTAGCAGGCGGCCATGA, F2: GTACCCTAGGATGAAGCTACTGTCTTCTATCGAACAAG; R2: GTACCCTAGGTTACTCTTTTTTTGGGTTTGGTGG, F3: ACGTACCCTAGGCACAGTTTTGTGGGCCAAACTAC, R3: GTACGCGGCCGCCATGAAACCGTTGTTTTGACG, and cloned into pED13(M) vector (gifted from B. Dickson lab). *tap* targeting was achieved by ends-in homologous recombination described in Fig. S1 and (Rong and Golic, 2000).

### Immuohistochemistry

Embryos and dissected tissues were stained using the protocol described in (Hassan et al., 2000; Langen et al., 2013). Flat preparation of embryonic fillets at stage 17 were generated and stained on polylysine-coated glass following the protocol described in (Featherstone et al., 2009). Primary and secondary antibodies were diluted as follows: rabbit anti-GFP 1:500 (Invitrogen A-11122), guinea pig anti-Sens 1:1000 (gifted from H. Bellen lab (Nolo et al., 2000)), rabbit anti-Ngn1 1:250 (gifted from H. Bellen lab), rabbit anti-Ato 1:1000 (generated in house (Quan et al., 2004)), mouse anti-Futsch 1:100 (Hybridoma Bank 22C10), rat anti-CadN 1:20 (Hybridoma Bank DN-Ex #8), rabbit anti-DsRed 1:500 (Clontech 632496), rat-Elav 1:100 (Hybridoma Bank 7E8A10), mouse anti-Repo 1:10 (Hybridoma Bank 8D12), mouse anti-FasII 1:50 (Hybridoma Bank 1D4), Alexa 488, 555, and 647 coupled secondary antibodies 1:500 (Invitrogen). Pictures were acquired using either LEICA TCS SP8 or LEICA TCS SP5 and processed with ImageJ.

### Western blot

Proteins from 20 adult fly brains in each genotype were resolved and probed using the protocol described in (Okray et al., 2015). Primary antibodies were used as: rat anti-Dsh 1:1000 (gifted from T. Uemura lab (Srahna et al., 2006)), mouse anti-Actin 1:5000 (Abcam ab8227), rabbit anti-App-C-term 1:100 (a gift from B. De Strooper (Soldano et al., 2000)), mouse anti-Stan 1:100 (Hybridoma Bank Flamingo #74) and rabbit anti-Wnt5 1:250 (a gift from L. Fradkin).

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