

Supplementary Information for

Trans-synaptic Fish-lips Signaling Prevents Misconnections between Non-synaptic Partner Olfactory Neurons

Qijing Xie, Bing Wu, Jiefu Li, Chuanyun Xu, Hongjie Li, David J Luginbuhl, Xin Wang, Alex Ward, Liqun Luo

Email: lluo@stanford.edu

This PDF file includes:

Figs. S1 to S8

Other supplementary materials for this manuscript include the following:

Dataset S1

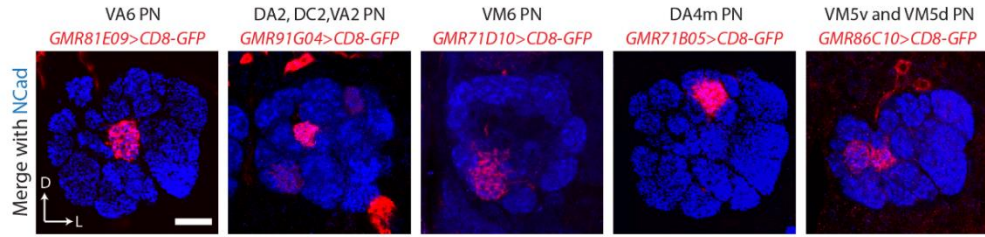


Fig. S1. *Enhancer-LexA* lines label specific PN neurons in adult.

Confocal sections of adult antennal lobe are shown. Neuronal processes are labeled by five different *enhancer-LexA*>*LexAop-mCD8GFP* (red) lines. Blue channel shows neuropil staining by the Ncad antibody.

Scale bars, 20 μ m.

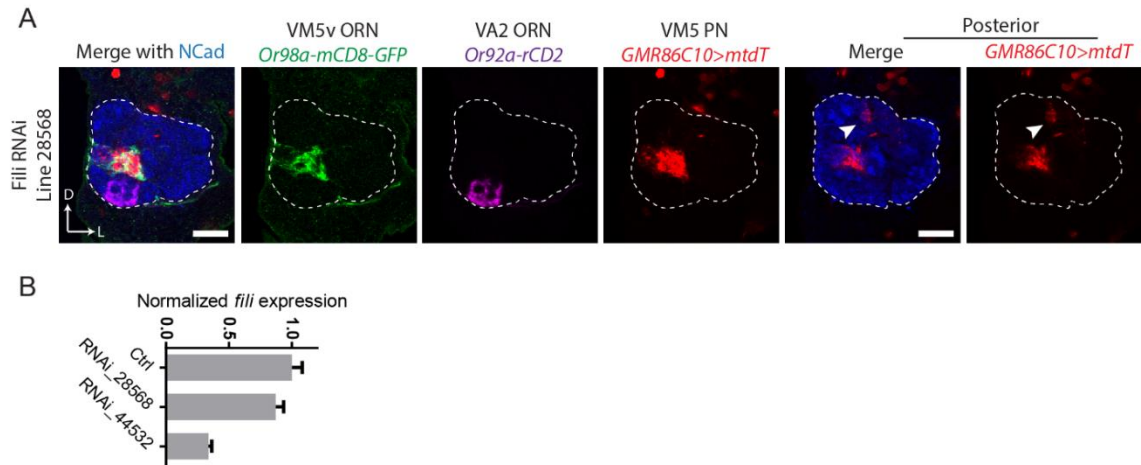


Figure S2. *fili* knockdown by two separate RNAi lines shows PN dendrite targeting defect.

(A) *C155-GAL4* drives *UAS-fili-RNAi* (Bloomington 28568) shows PN dendrite targeting defect. Ectopic PN target are indicated by arrowhead on the posterior section. (mistargeting in 7/20 antennal lobes). Scale bars, 20 μ m.

(B) Quantitative PCR (qPCR) measurement of the knockdown efficiency of *fili* using two *UAS-fili-RNAi* lines. *C155-GAL4* was crossed with either *w¹¹¹⁸* (control) or two Fili-RNAi lines. mRNA was extracted from 5-day-old adult fly heads (n=3 biological replicates of 10 heads pooled for each condition). Expression levels are normalized to *actin5C*. Error bars show SEM.

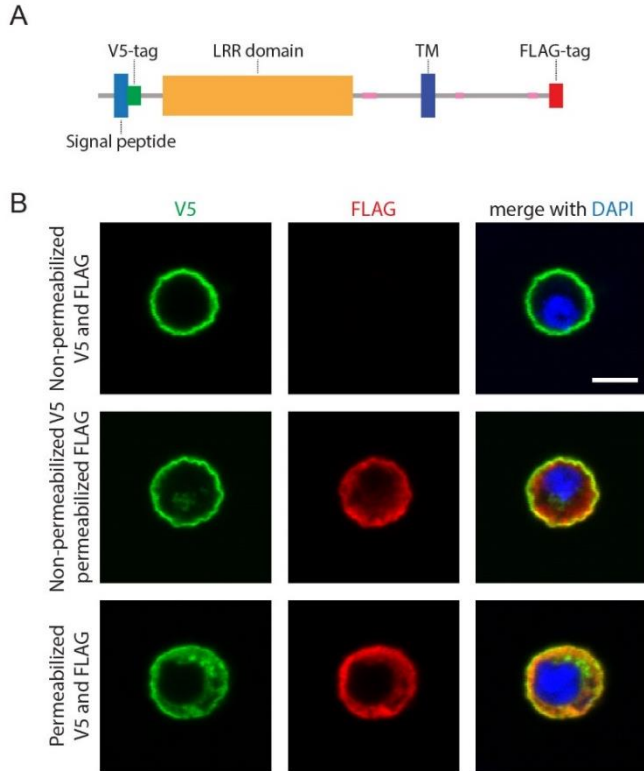


Figure S3. Fili is expressed on the plasma membrane.

(A) A schematic of the *SP-V5-Fili-Flag* construct used for membrane expression assay (see Figure S5A for a more detailed description of primary structure of Fili).

(B) Representative immunofluorescent images of S2 cells expressing *UAS-SP-V5-Fili-Flag*. Top panel: non-permeabilized staining of V5 and FLAG antibodies. Middle panel: non-permeabilized V5 antibody staining and permeabilized FLAG antibody staining. Bottom: permeabilized staining of V5 and FLAG antibodies.

Scale bar, 5 μm .

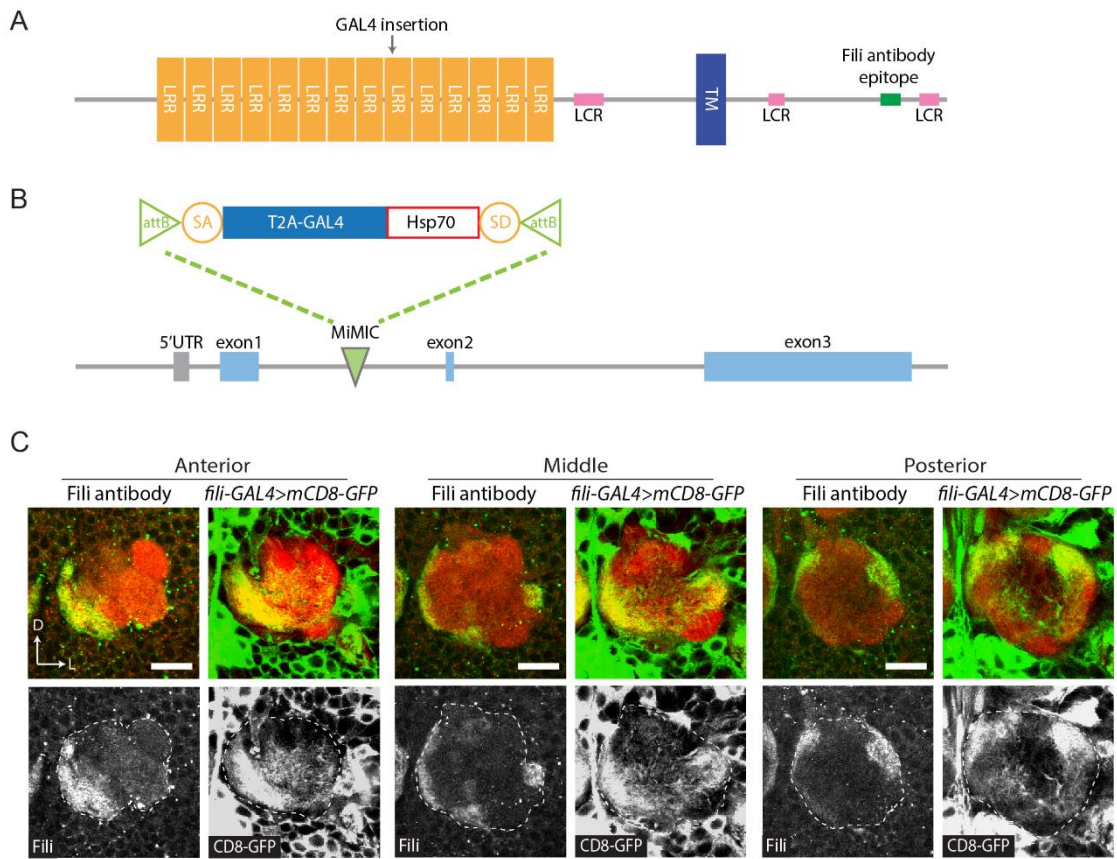


Figure S5. Fili expression revealed by antibody and transcriptional reporter GAL4 showed similar pattern.

(A) Fili protein domain prediction. The blue bar (TM) represents transmembrane domain. Orange bars (LRR) indicates leucine-rich repeat domains. Pink bars (LCR) indicate low-complexity regions. The epitope for Fili antibody is represented by the green bar.

(B) Design of *fili-GAL4*. A *T2A-GAL4* cassette was inserted into a MiMIC locus in the first intron of *fili*. SA, splicing acceptor. Hsp70, terminator sequence of *Hsp70*. SD, splicing donor. Blue bars: coding exons of *fili*.

(C) Fili antibody staining and *fili-GAL4* shows consistent pattern at 48h APF. Three optical sections (anterior, middle, and posterior) are shown for both Fili antibody staining and CD8-GFP (green). Neuropil is visualized by antibody against either NC82 or NCad (red). Dashed lines outline the antennal lobe.

Scale bars, 20 μ m.

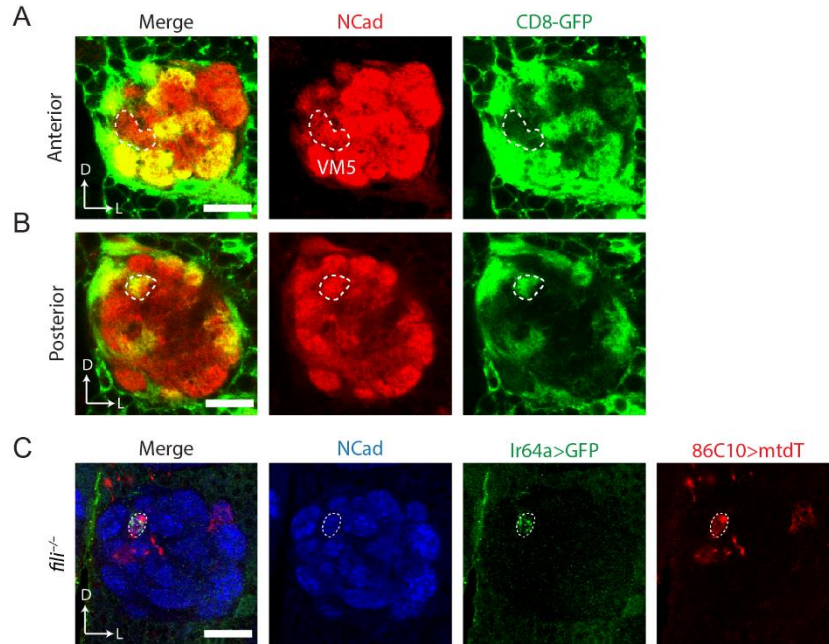


Figure S6. The ectopic target site of VM5 PN dendrites is innervated by *fili-GAL4+* ORN axons.

ey-FLP intersecting with *fili-GAL4* together with *UAS-FRT-stop-FRT-mCD8GFP* shows *fili* expression pattern in ORNs in the 48 hours APF antennal lobe.

(A) VM5v and VM5d glomeruli (outlined by dashed line) do not have detectable ORN *Fili* signal.

(B) The ectopic targeting site (outlined by dashed line) of VM5 PNs have high ORN *Fili* signal.

(C) VM5 PN dendrites, labeled by *86C10-LexA>LexAop-mtdT*, mistarget to DC4 glomerulus (outlined by dashed line) which is labeled by *Ir64a-GAL4>UAS-mCD8-GFP*.

Scale bars, 20 μm .

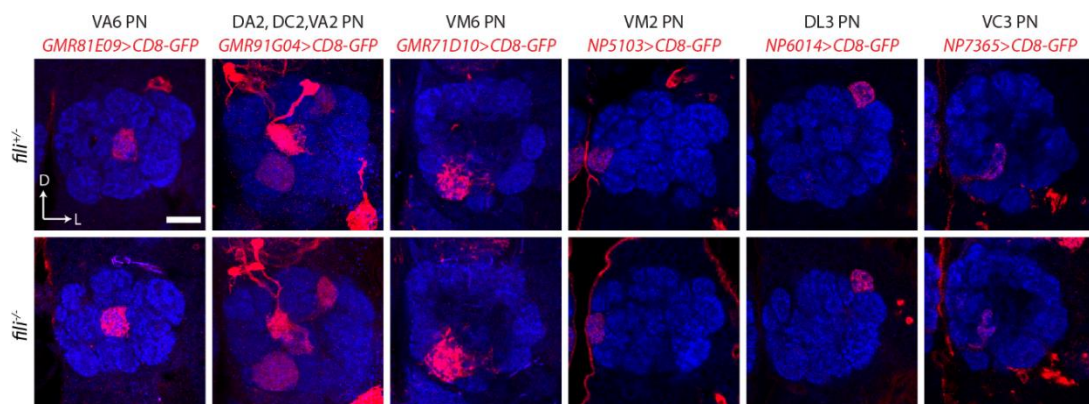


Figure S7. Fili is not required for the targeting of the 8 tested PN classes.

PN dendrites (red) are visualized in *fil^{+/-}* control animal and *fil^{-/-}* for VA6 PN (*GMR81E09-GAL4*); DA2, DC2, and VA2 PN (*GMR91G04-GAL4*); VM6 PN (*GMR71D10-GAL4*); VM2 PN (*NP5103-GAL4*); DL3 PN (*NP6014-GAL4*); VC3 PN (*NP7365-GAL4*). Maximum z-projection is used for *GMR91E04-GAL4* images to show dendrite targeting of all 3 PN classes, all other images are single sections.

Scale bar, 20 μ m.

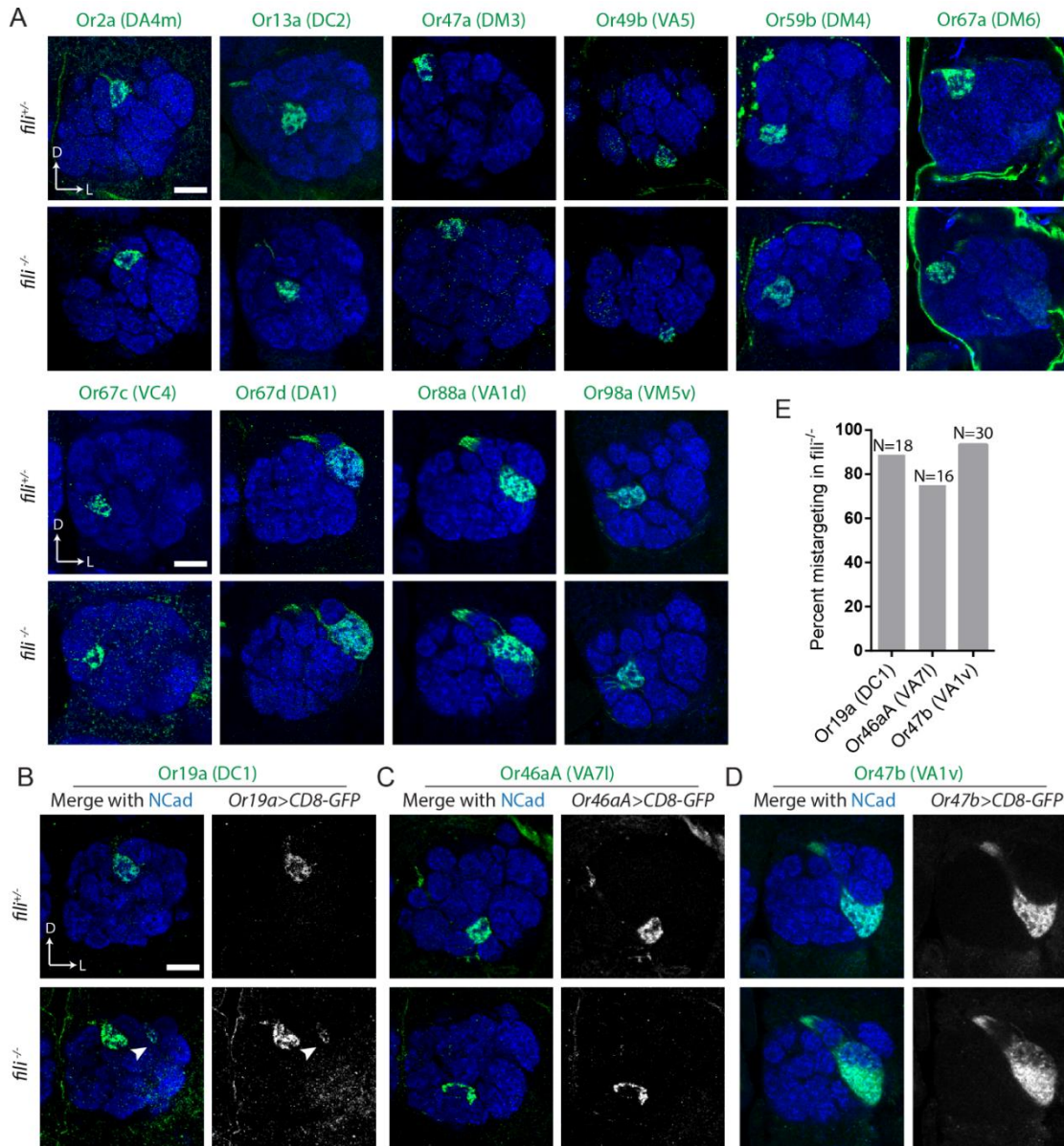


Figure S8 Fili is required for the axon targeting of some ORN classes.

ORN axons (green) in *fili*^{+/-} and *fili*^{-/-} animals visualized using either Or-promoter fused upstream of GAL4 to drive *UAS-mCD8-GFP* expression, or direct fusion of Or-promoter and *mCD8-GFP*, *mtdTomato*, or *rCD2*.

(A) ORN classes whose axon targeting does not require Fili.

(B) *Or19a-mCD8-GFP* labeled DC1 ORN axons shows an ectopic target on the lateral side of DC1 in *fili*^{-/-}. Arrowhead points to the ectopic target. (mistargeting in 16/18 antennal lobes).

(C) *Or46aA-mCD8-GFP* labeled VA7I ORN axons are misshapen in *fili*^{-/-} animal but no ectopic target site is observed (mistargeting in 12/16 antennal lobes).

(D) *Or47b-rCD2* labeled VA1v ORN axons invade the VA1d glomerulus in *fili*^{-/-} animals (mistargeting in 28/30 antennal lobes).

(E) Quantification of mistargeting of DC1, VA7I, and VA1v ORN axons shown in (B–D).

Scale bars, 20 μm .