

Supplementary Information for

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

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This PDF file includes:

Figs. S1 to S8

Other supplementary materials for this manuscript include the following:

Dataset S1

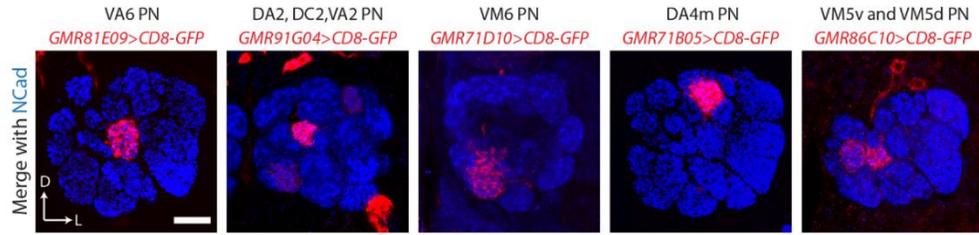


Fig. S1. *Enhancer-LexA* lines label specific PN neurons in adult.

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.

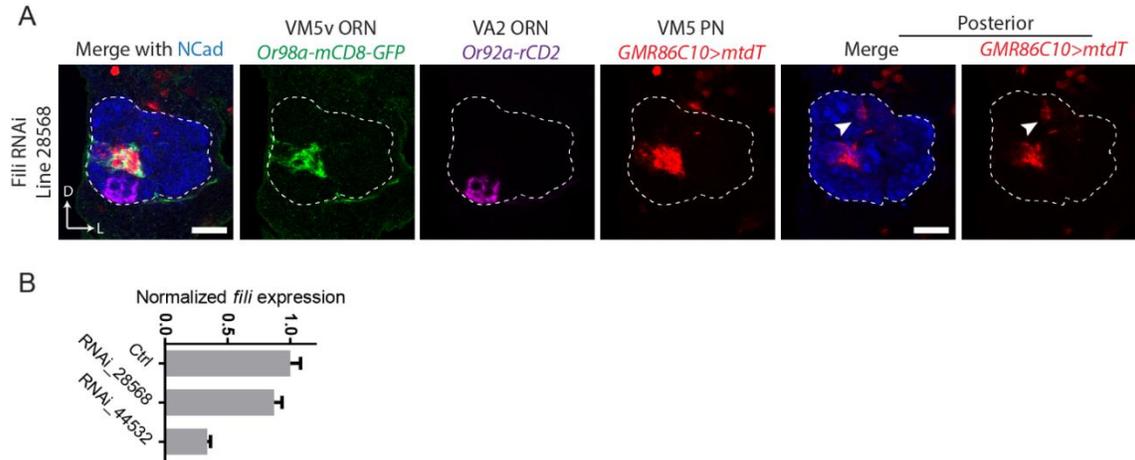


Figure S2. *fili* knockdown by two separate RNAi lines shows PN dendrite targeting defect.

(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.

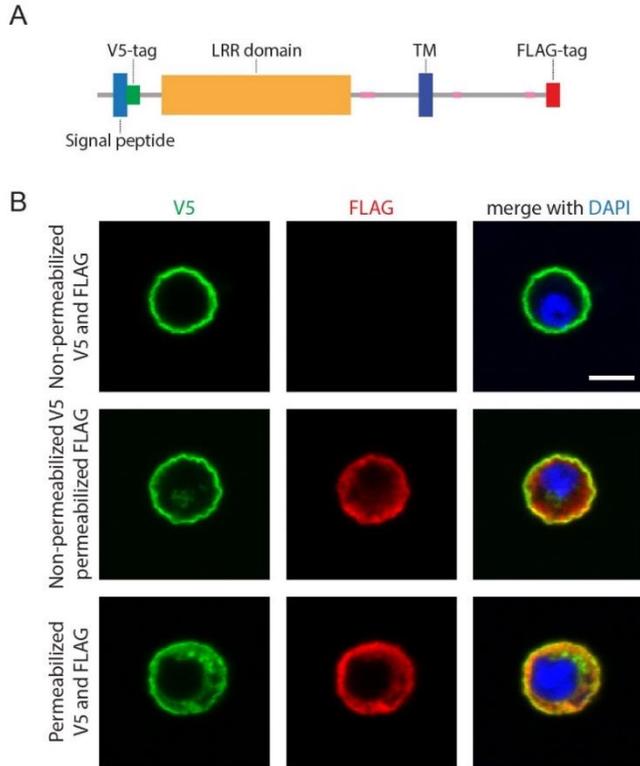


Figure S3. Fili is expressed on the plasma membrane.

(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 μ m.

A Extracellular:

Fili	MPDHTRVTATTRSRLPWLCAIPVL---LLLLLTLVILPPETTAFCPCSKCQCLGGEANSRA	57
Caps	-----MSLAPHLGQAFSLCLCLCLVLAATLPVALGLANCPNGCECDDDT--LMV	48
Trn	-----MMIAFVGI--WCI-LASIGVEPAAGLANCPGCGCDDNT--LVV	39
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Fili	LCVDAALDVPPIQLNPE ²⁶ TKYINLTVNRIRTL ⁵⁴ LEFSLPFYMKLEILDLSQNI ²⁶ IETLGSKNFE	117
Caps	NCGEGTLDVLPALNPAL ²⁶ QRLV ⁵⁴ IKNNK ²⁶ LKTIDSSMQFYAQLTFLDLSFN ²⁶ DMLTI ⁵⁴ PERSFA	108
Trn	QCQEGQLDVLPTALNPS ²⁶ L ⁵⁴ QRLV ²⁶ IKSNK ⁵⁴ IKTIDSSI ²⁶ QFYAELTFLDLSN ²⁶ HMLTI ⁵⁴ PORTFA	99
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Fili	²⁶ YQSELR ⁵⁴ TLNLSRN ²⁶ LVSS ⁵⁴ LHKHAFKGL ²⁶ TNLLL ⁵⁴ DL ²⁶ SNRI ⁵⁴ ETV ²⁶ HPTAL ⁵⁴ SDLAS ²⁶ LV ⁵⁴ ELDL ²⁶ TN	177
Caps	²⁶ YHAKL ⁵⁴ QELH ²⁶ LDH ⁵⁴ NKIG ²⁶ QVSN ⁵⁴ KTF ²⁶ LGLST ⁵⁴ ISV ²⁶ LN ⁵⁴ RG ²⁶ NLIA ⁵⁴ ELEY ²⁶ RT ⁵⁴ FSP ²⁶ MV ⁵⁴ KLAE ²⁶ LN ⁵⁴ LG	168
Trn	²⁶ YQK ⁵⁴ KL ²⁶ QV ⁵⁴ HL ²⁶ NH ⁵⁴ NKIG ²⁶ Q ⁵⁴ LSN ²⁶ KTF ⁵⁴ IGLS ²⁶ AV ⁵⁴ TV ²⁶ LN ⁵⁴ RG ²⁶ NQ ⁵⁴ ISE ²⁶ LH ⁵⁴ QGT ²⁶ FT ⁵⁴ PL ²⁶ LK ⁵⁴ IE ²⁶ LN ⁵⁴ LG	159
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Fili	²⁶ NMIVS ⁵⁴ LED ²⁶ NC ⁵⁴ FK ²⁶ GM ⁵⁴ NT ²⁶ LEV ⁵⁴ LV ²⁶ FR ⁵⁴ NR ²⁶ LL ⁵⁴ DV ²⁶ PAS ⁵⁴ NL ²⁶ WH ⁵⁴ -L ²⁶ H ⁵⁴ AL ²⁶ KS ⁵⁴ LD ²⁶ MS ⁵⁴ LN ²⁶ LV ⁵⁴ F ²⁶ VR ⁵⁴ ND ²⁶ SF	236
Caps	²⁶ NRISH ⁵⁴ ID ²⁶ PH ⁵⁴ AL ²⁶ DG ⁵⁴ LN ²⁶ RV ⁵⁴ LY ²⁶ LD ⁵⁴ DN ²⁶ LT ⁵⁴ TV ²⁶ PG ⁵⁴ EL ²⁶ TF ⁵⁴ QAL ²⁶ HS ⁵⁴ LA ²⁶ EY ⁵⁴ LT ²⁶ NS ⁵⁴ FMT ²⁶ IP ⁵⁴ GG ²⁶ AF	228
Trn	²⁶ NRIGY ⁵⁴ LD ²⁶ PK ⁵⁴ AF ²⁶ DG ⁵⁴ LS ²⁶ QL ⁵⁴ RI ²⁶ LY ⁵⁴ LD ²⁶ DN ⁵⁴ AL ²⁶ TV ⁵⁴ PD ²⁶ PV ⁵⁴ I ²⁶ Q ⁵⁴ AMP ²⁶ SL ⁵⁴ AE ²⁶ FL ⁵⁴ GM ²⁶ NT ⁵⁴ L ²⁶ QS ⁵⁴ I ²⁶ Q ⁵⁴ AG ²⁶ AF	219
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Fili	²⁶ EGLKEL ⁵⁴ LALS ²⁶ VQ ⁵⁴ GN ²⁶ VS ⁵⁴ E ²⁶ LD ⁵⁴ LS ²⁶ AFE ⁵⁴ GL ²⁶ IS ⁵⁴ LK ²⁶ HL ⁵⁴ DL ²⁶ SD ⁵⁴ NN ²⁶ LT ⁵⁴ MV ²⁶ PT ⁵⁴ Q ²⁶ OLS ⁵⁴ K ²⁶ LS ⁵⁴ N ²⁶ LY ⁵⁴ LN ²⁶ LG	296
Caps	²⁶ QDLK ⁵⁴ GL ²⁶ TR ⁵⁴ LD ²⁶ LR ⁵⁴ G ²⁶ AG ⁵⁴ LHN ²⁶ IS ⁵⁴ GD ²⁶ AL ⁵⁴ K ²⁶ GL ⁵⁴ V ²⁶ SL ⁵⁴ RF ²⁶ LD ⁵⁴ SD ²⁶ NR ⁵⁴ LP ²⁶ AI ⁵⁴ PT ²⁶ AA ⁵⁴ F ²⁶ Q ⁵⁴ RL ²⁶ GR ⁵⁴ LE ²⁶ QL ⁵⁴ NI ²⁶ G	288
Trn	²⁶ QDLK ⁵⁴ GL ²⁶ TR ⁵⁴ LE ²⁶ LK ⁵⁴ GA ²⁶ SL ⁵⁴ RNI ²⁶ SH ⁵⁴ DS ²⁶ FL ⁵⁴ GL ²⁶ Q ⁵⁴ EL ²⁶ RI ⁵⁴ LD ²⁶ SD ⁵⁴ NR ²⁶ LD ⁵⁴ RI ²⁶ PS ⁵⁴ V ²⁶ GL ⁵⁴ SK ²⁶ LV ⁵⁴ RL ²⁶ QL ⁵⁴ SL ²⁶ G	279
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Fili	²⁶ GNRFS ⁵⁴ QL ²⁶ PA ⁵⁴ VAF ²⁶ LN ⁵⁴ FL ²⁶ HL ⁵⁴ RL ²⁶ HL ⁵⁴ SR ²⁶ LD ⁵⁴ FL ²⁶ QR ⁵⁴ IDS ²⁶ RA ⁵⁴ FV ²⁶ DN ⁵⁴ TH ²⁶ L ⁵⁴ Q ²⁶ TL ⁵⁴ HL ²⁶ NN ⁵⁴ P ²⁶ QL ⁵⁴ SD ²⁶ IP ⁵⁴ MR	356
Caps	²⁶ QND ⁵⁴ FE ²⁶ V ⁵⁴ ISS ²⁶ GA ⁵⁴ FS ²⁶ GL ⁵⁴ REL ²⁶ R ⁵⁴ HL ²⁶ EL ⁵⁴ TGA ²⁶ QR ⁵⁴ LR ²⁶ RV ⁵⁴ ES ²⁶ GA ⁵⁴ FS ²⁶ GN ⁵⁴ T ²⁶ LE ⁵⁴ HL ²⁶ N ⁵⁴ LS ²⁶ SN ⁵⁴ QL ²⁶ N ⁵⁴ EL ²⁶ SSI	348
Trn	²⁶ QND ⁵⁴ FE ²⁶ V ⁵⁴ IS ²⁶ GA ⁵⁴ F ²⁶ MG ⁵⁴ L ²⁶ K ⁵⁴ Q ²⁶ LR ⁵⁴ LV ²⁶ NG ⁵⁴ AL ²⁶ RI ⁵⁴ K ²⁶ RV ⁵⁴ MT ²⁶ GA ⁵⁴ FS ²⁶ D ⁵⁴ NG ²⁶ N ⁵⁴ LE ²⁶ YL ⁵⁴ N ²⁶ LS ⁵⁴ SN ²⁶ K ⁵⁴ ML ²⁶ EV ⁵⁴ Q ²⁶ EG	339
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Fili	²⁶ LFQGN ⁵⁴ PN ²⁶ LI ⁵⁴ EV ²⁶ MQ ⁵⁴ S ²⁶ NS ⁵⁴ L ²⁶ Q ⁵⁴ TL ²⁶ YS ⁵⁴ AO ²⁶ FP ⁵⁴ VD ²⁶ Q ⁵⁴ L ²⁶ K ⁵⁴ LY ²⁶ LD ⁵⁴ GN ²⁶ PL ⁵⁴ QC ²⁶ NS ⁵⁴ L ²⁶ WL ⁵⁴ WR ²⁶ LV ⁵⁴ T ²⁶ GN ⁵⁴ FEG	416
Caps	²⁶ ALGG ⁵⁴ L ²⁶ PH ⁵⁴ LS ²⁶ TV ⁵⁴ V ²⁶ L ⁵⁴ KAN ²⁶ QL ⁵⁴ SS ²⁶ LD ⁵⁴ E ²⁶ GL ⁵⁴ V ²⁶ W ⁵⁴ AD ²⁶ L ⁵⁴ Q ²⁶ TL ⁵⁴ DL ²⁶ SEN ⁵⁴ PF ²⁶ EC ⁵⁴ DR ²⁶ LL ⁵⁴ WL ²⁶ RL ⁵⁴ HL ²⁶ LS ⁵⁴ VR ²⁶ NAS	408
Trn	²⁶ ALS ⁵⁴ GL ²⁶ S ⁵⁴ QL ²⁶ K ⁵⁴ H ²⁶ V ⁵⁴ L ²⁶ KAN ⁵⁴ AL ²⁶ TS ⁵⁴ LA ²⁶ E ⁵⁴ GL ²⁶ FP ⁵⁴ WK ²⁶ DL ⁵⁴ Q ²⁶ TL ⁵⁴ DL ²⁶ SEN ⁵⁴ PL ²⁶ SC ⁵⁴ DR ²⁶ V ⁵⁴ M ²⁶ HL ⁵⁴ LN ²⁶ LV ⁵⁴ AK ²⁶ NAS	399
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Fili	VDPGMEHAAGGAVAALAKEADDEELADEATAVASTDDGVAALAAAYIAEQHIVNALHTTEP	476
Caps	G-QYAP----VICAYPTALRDLPLAHLAEPLLGCAHGAASKQA-----	446
Trn	QDDVSE----LLCEPPERLRGESLRHLNPAMMGCTHADPRKQA-----	438
	: . . . * . . : . . . *	
Fili	SAYELATSSSNRNSGILRMDRQQIGCDIWRDKVTRRKLTMSEGEITCPAHI	529
Caps	-----	446
Trn	-----	438

B Intracellular:

Fili	FVKRRRKL ⁵⁴ LHERG ²⁶ PM ⁵⁴ RT ²⁶ SKS ⁵⁴ II-----NVH ⁵⁴ DR ²⁶ IL ⁵⁴ QGH ²⁶ NP ⁵⁴ GG ²⁶ LGL ⁵⁴ MS ²⁶ MT ⁵⁴ LG ²⁶ GG ⁵⁴ NH	50
Caps	----RHR----IREMLK ⁵⁴ GHS ²⁶ AL ⁵⁴ GR ²⁶ KERE ⁵⁴ YQ ²⁶ TF ⁵⁴ S ²⁶ DEE ⁵⁴ YMS ²⁶ R ⁵⁴ PP ²⁶ PGG ⁵⁴ --GGV ⁵⁴ HPAA----	45
Trn	--RCR ⁵⁴ HK ²⁶ IRE ⁵⁴ TIK ²⁶ GGL ⁵⁴ W ²⁶ GN ⁵⁴ SAL ²⁶ GR ⁵⁴ KERE ²⁶ YQ ⁵⁴ KTF ²⁶ CE ⁵⁴ DE ²⁶ YMS ⁵⁴ RR ²⁶ H ⁵⁴ HP ²⁶ CS ⁵⁴ LGI ²⁶ HST ⁵⁴ FP----	54
	* : : . . * : : . . * : : . . * : : . .	
Fili	VNGLG ⁵⁴ MT ²⁶ LN ⁵⁴ YP ²⁶ PHA ⁵⁴ Q ²⁶ TL ⁵⁴ Q ²⁶ AHH ⁵⁴ HYH ²⁶ -Q ⁵⁴ AM ²⁶ PL ⁵⁴ Q ²⁶ SH ⁵⁴ GG ²⁶ NG ⁵⁴ -N ²⁶ HEY ⁵⁴ Q ²⁶ TT ⁵⁴ LP ²⁶ QLD-----	99
Caps	-----GGYPY ⁵⁴ IAG---NS---R---M ⁵⁴ IP ²⁶ VT ⁵⁴ EL-----	63
Trn	-----NTYT ⁵⁴ AP ²⁶ HH ⁵⁴ PG---ATH ⁵⁴ HY ²⁶ GM ⁵⁴ CP ²⁶ ME ⁵⁴ VND ²⁶ LG ⁵⁴ AI ²⁶ DP ⁵⁴ Q ²⁶ K ⁵⁴ F ²⁶ Q ⁵⁴ LV ²⁶ PT ⁵⁴ AT ²⁶ MI ⁵⁴ SE ²⁶ K ⁵⁴ LN	106
	* : : * : : . .	
Fili	-----KLE ⁵⁴ LERY ²⁶ LAA ⁵⁴ Q ²⁶ TIAN---EYRA	118
Caps	-----	63
Trn	NKALV ⁵⁴ S ²⁶ QGA ⁵⁴ ID ²⁶ DS ⁵⁴ AS ²⁶ FV ⁵⁴ L ²⁶ HK ⁵⁴ SAT ²⁶ MGR ⁵⁴ DV ²⁶ H ⁵⁴ QQ ²⁶ N ⁵⁴ P ²⁶ QL ⁵⁴ N ²⁶ HY ⁵⁴ TK ²⁶ P ⁵⁴ QL ²⁶ SAT ⁵⁴ AT ²⁶ V ⁵⁴ GD ²⁶ SC ⁵⁴ YS ²⁶ YAD	166
Fili	L-----KPWE-----LPV ⁵⁴ KEAD ²⁶ PE ⁵⁴ PHY ²⁶ LER-----FD ⁵⁴ H ²⁶ YEY ⁵⁴ PD ²⁶ HT ⁵⁴ MS ²⁶ KL ⁵⁴ QAASL	160
Caps	-----	63
Trn	VPMVH ⁵⁴ GAP ²⁶ LG ⁵⁴ PN ²⁶ Q ⁵⁴ QL ²⁶ RL ⁵⁴ T ²⁶ Q ⁵⁴ EH ²⁶ FK ⁵⁴ Q ²⁶ RE ⁵⁴ LY ²⁶ D ⁵⁴ Q ²⁶ EM ⁵⁴ G ²⁶ SE ⁵⁴ IL ²⁶ D ⁵⁴ HN ²⁶ Y ⁵⁴ IS ²⁶ N ⁵⁴ TH ²⁶ YS ⁵⁴ MP ²⁶ LE ⁵⁴ QL ²⁶ GR-	225
Fili	NHSN ⁵⁴ SS ²⁶ AG ⁵⁴ SP ²⁶ VP ⁵⁴ PP ²⁶ SS ⁵⁴ GK ²⁶ PH ⁵⁴ VYV-----	184
Caps	-----	63
Trn	---SK ⁵⁴ TP ²⁶ PP ⁵⁴ MP ²⁶ PP ⁵⁴ AL ²⁶ PL ⁵⁴ R ²⁶ NG ⁵⁴ LC ²⁶ ATT ⁵⁴ GR ²⁶ RS ⁵⁴ FQ ²⁶ QS ⁵⁴ AS ²⁶ Q ⁵⁴ Q ²⁶ Q ⁵⁴ NN ²⁶ NT ⁵⁴ L ²⁶ RQ ⁵⁴ F ²⁶ TH	274

Figure S4. Sequence alignment of Fili, Trn, and Caps.

Alignment of Fili, Trn, and Caps extracellular (A) and intracellular (B) regions using Cluster Omega (53). The LRR motifs of Fili, Caps, and Trn are highlighted in yellow (26, 54). The transmembrane region is highlighted in blue. Asterisk (*) indicates identical residue, colon (:) indicates strongly similar residues, and period (.) indicates weakly similar residues.

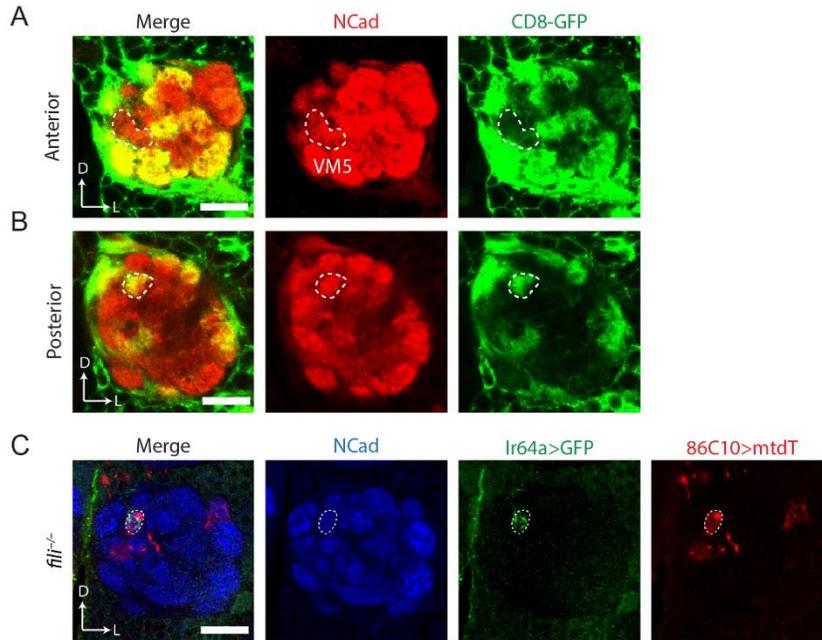


Figure S6. The ectopic target site of VM5 PN dendrites is innervated by *fili-GAL4+* ORN axons.

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 .

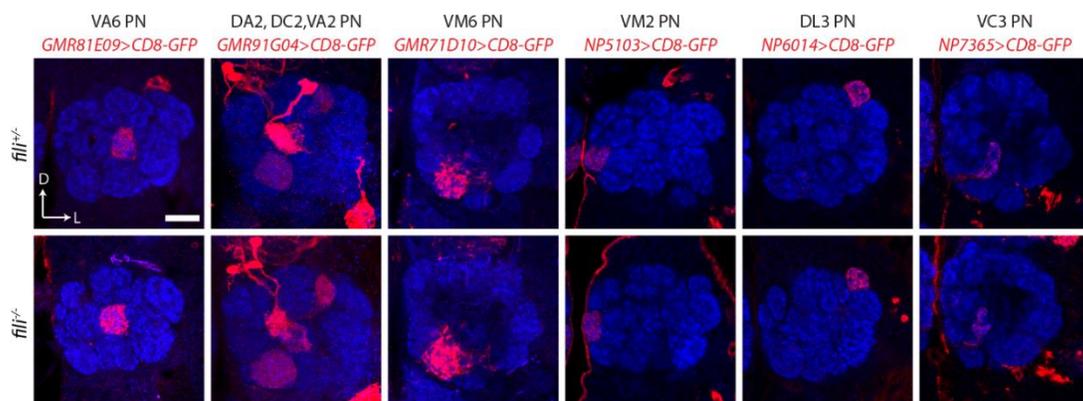


Figure S7. Fili is not required for the targeting of the 8 tested PN classes.

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.

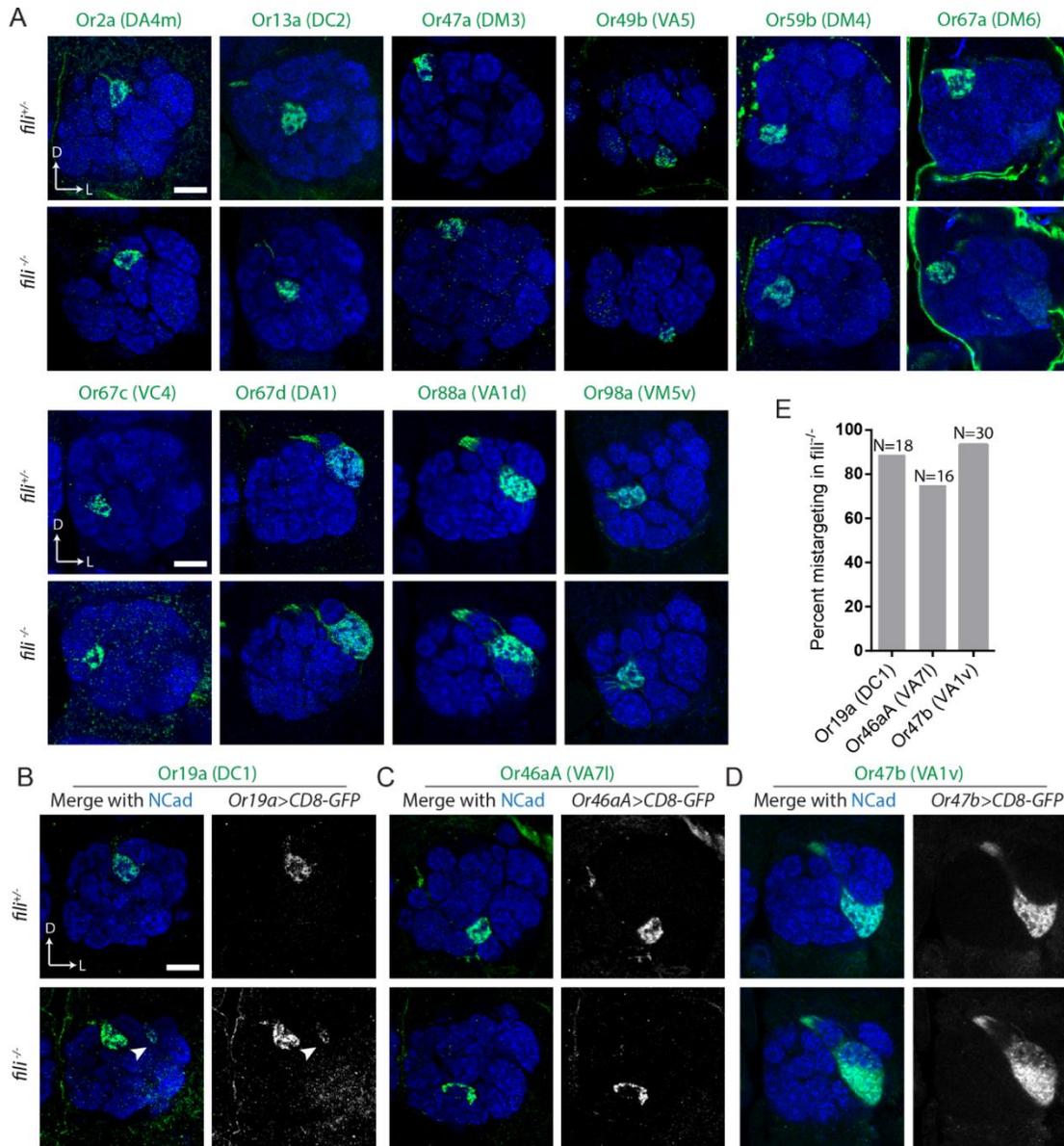


Figure S8 Fili is required for the axon targeting of some ORN classes.

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) *Or46a-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 .