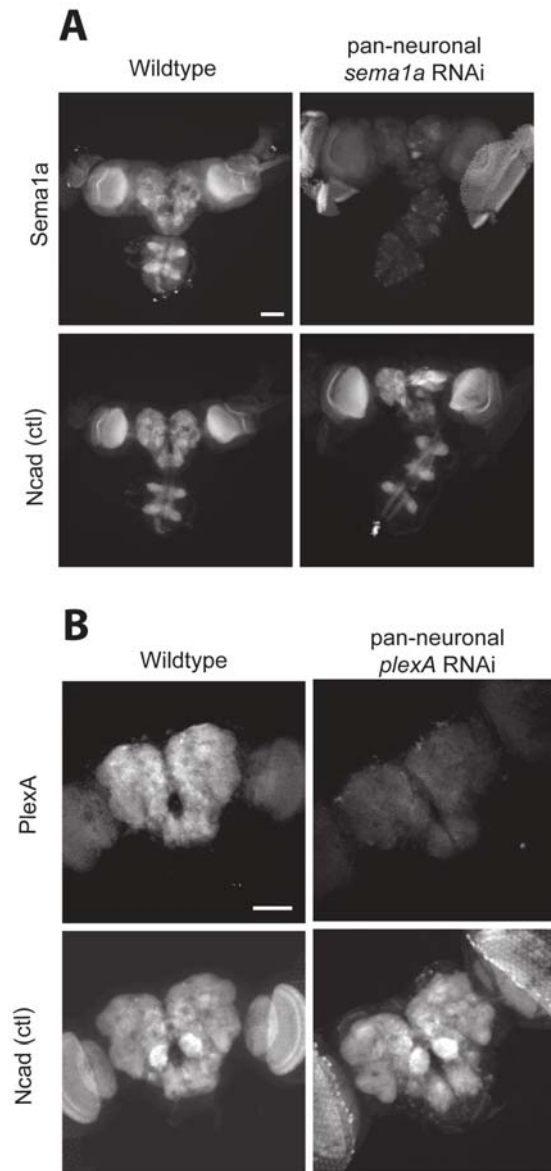# Supplemental Data

Temporal Target Restriction of Olfactory Receptor

Neurons by Semaphorin-1a/PlexinA-Mediated

Axon-Axon Interactions

Lora B. Sweeney, Africa Couto, Ya-Hui Chou, Daniela Berdnik, Barry J. Dickson, Liqun Luo, and Takaki Komiyama

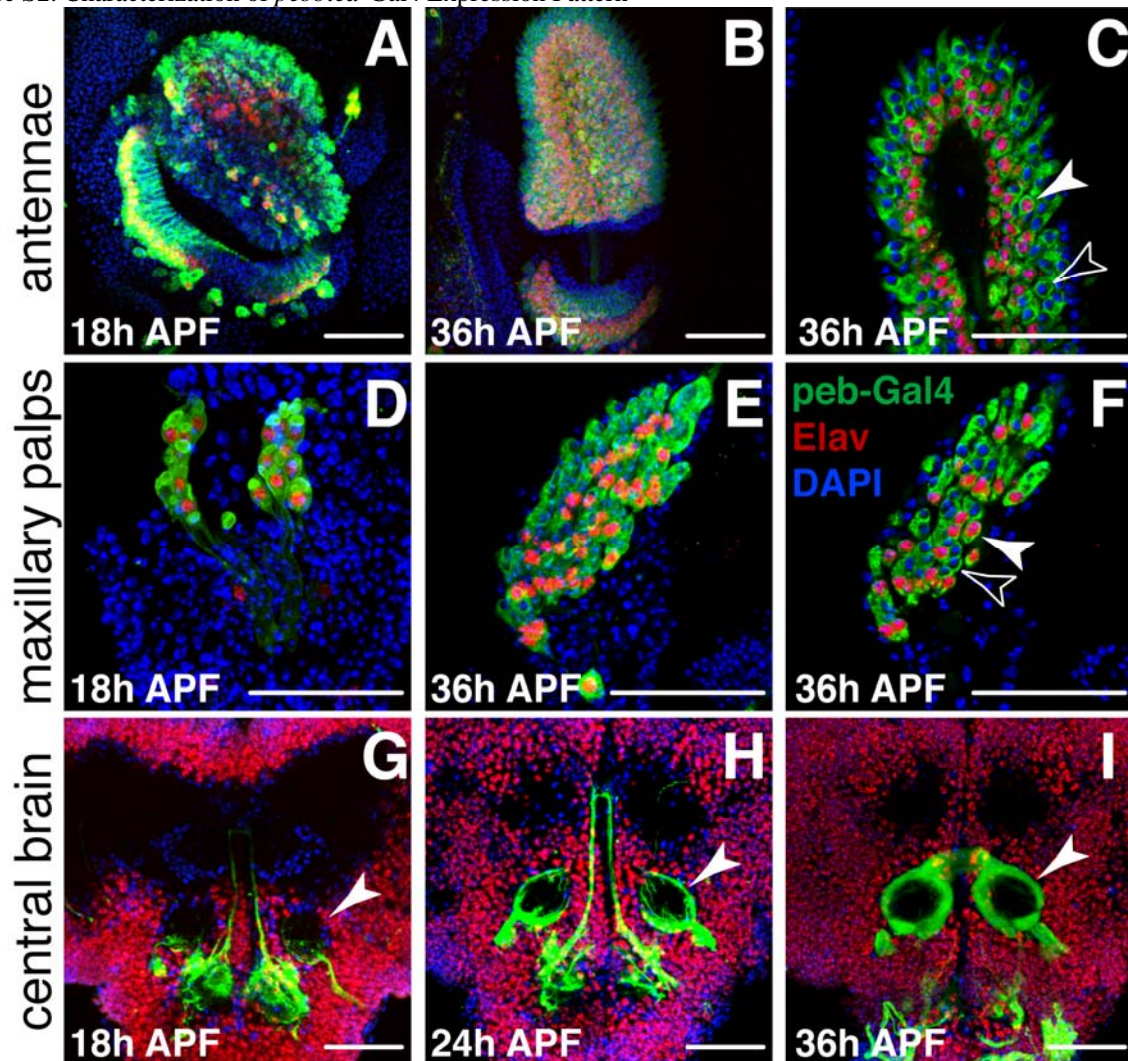Figure S1. RNAi Knockdown of Sema-1a and PlexinA Protein Expression

(A) Pan-neuronal expression of UAS-*sema-1a* RNAi using *elav*-Gal4 results in a marked reduction of the Sema-1a protein level in the brain (top), but does not affect levels of N-cadherin expression in the same sample (bottom). Pictures are mean intensity Z-projections. 18h APF at 29°C (roughly equivalent to 25h APF at 25°C).

(B) Pan-neuronal expression of UAS-*PlexinA* RNAi using *elav*-Gal4 results in a marked reduction of the PlexinA protein level in the brain (top), but does not affect levels of N-Cadherin expression in the same sample (bottom). 36h APF.
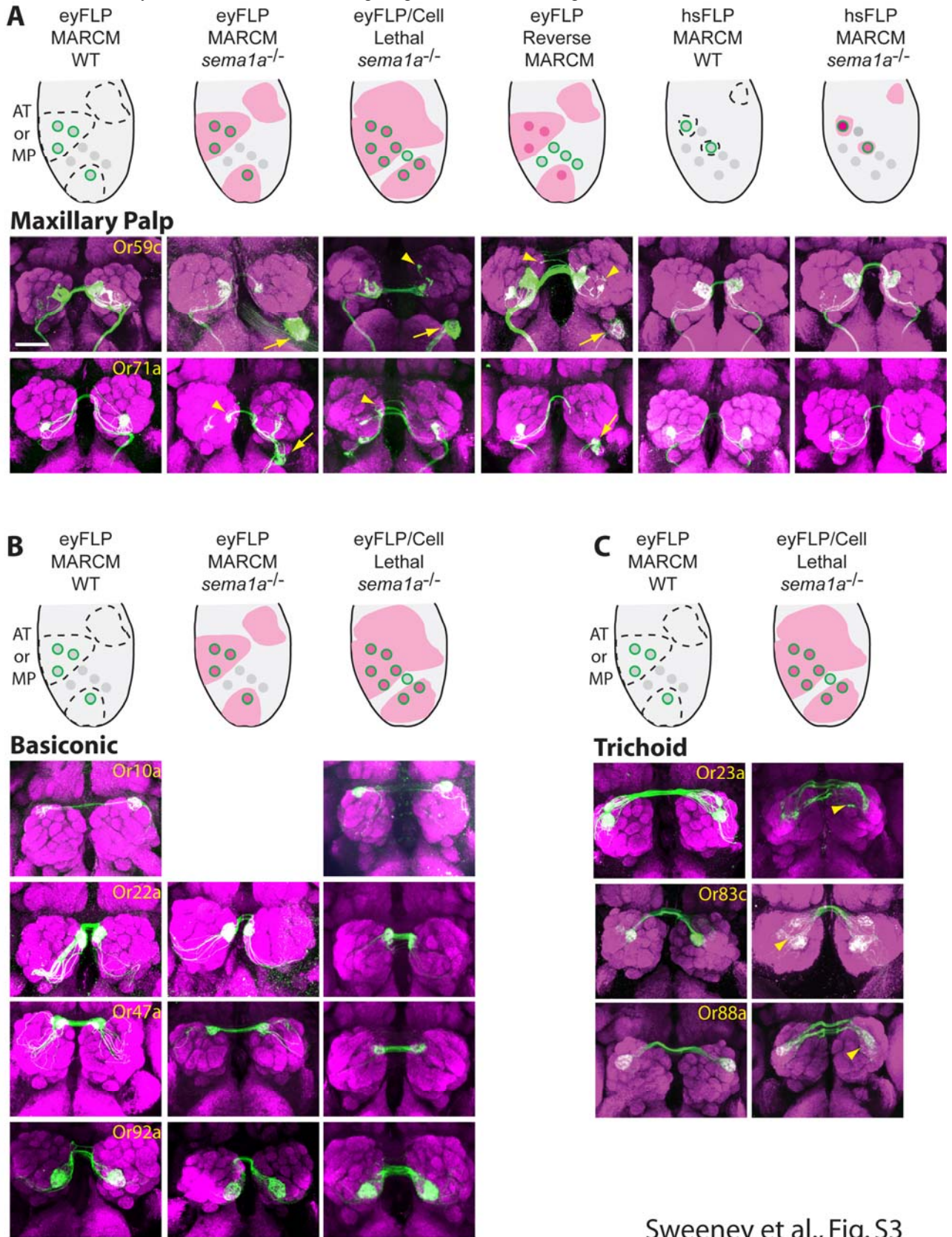
Scale bars, 100 μm. Genotype: (A) WT: *UAS-sema-1a RNAi*. RNAi: *elav-Gal4, UAS-mCD8GFP/UAS-sema-1a RNAi*. (B) WT: *UAS-plexA RNAi/+*. RNAi: *elav-Gal4, UAS-mCD8GFP/+; UAS-plexA RNAi/+*.

Figure S2. Characterization of *pebbled*-Gal4 Expression Pattern

pebbled-Gal4 drives mCD8-GFP expression (green) in most cells residing in the antenna and maxillary palp during development. All ORNs in the 3rd antennal segment (A-C) and maxillary palp (D-F) identified via the pan-neuronal marker Elav (red) are positive for *pebbled*-Gal4 driven GFP expression (closed arrowheads in C and F), as are many non-neuronal cells shown by DAPI staining in blue (open arrowheads in C and F). At 18h APF, ORN axons reach the antennal lobe in the central brain (G) and start to innervate it at later stages (H and I). During this developmental time frame, areas surrounding the antennal lobe (arrowheads) are devoid of labeled cell bodies (G-I). C and F are single confocal sections; the rest are partial maximum-intensity confocal stack *z*-projections. Scale bars, 50 μm. Genotype: *pebbled-Gal4 UAS-mCD8GFP; Pin/CyO*.

Figure S3. Mosaic Analysis of *sema-1a* in ORN Targeting (Continuation of Figure 4)
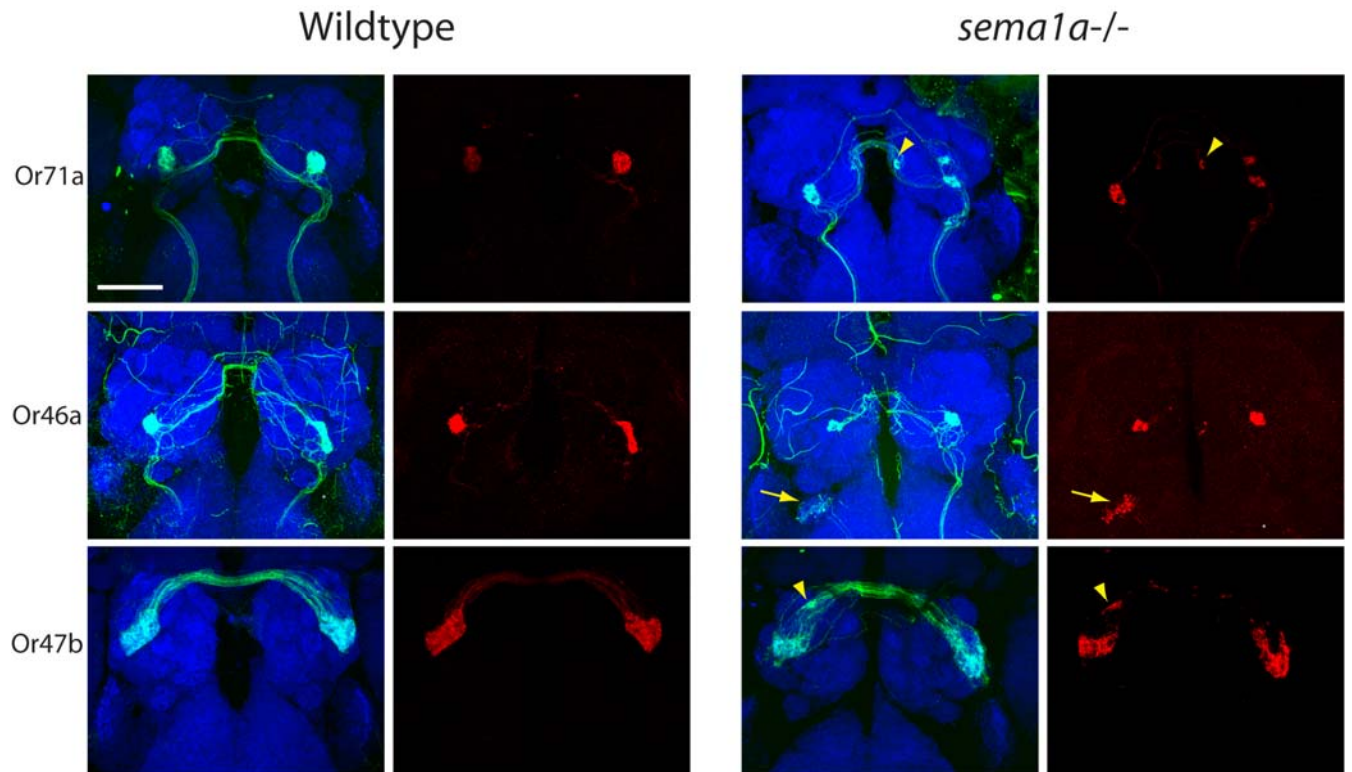

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(A) Axon targeting defects in the remaining two MP ORN classes not shown in Figure 4. Top row, schematic as in Figure 4A.

(B) Axon targeting of remaining basiconic ORN classes not shown in Figure 4. Top row, schematic as in Figure 4A for mosaic manipulations shown. These four basiconic classes are not affected by *sema-1a* loss-of-function.

(C) Mild targeting imprecision in the remaining trichoid ORN classes not shown in Figure 4, as exemplified by a spill-over of axons from the normal targets (arrowheads). Top row, schematic as in Figure 4A of mosaic manipulations shown. Green: mCD8-GFP marking axons of a given ORN class; magenta: synaptic marker nc82. Scale bar, 50 μm. Genotype: see Figure 4.
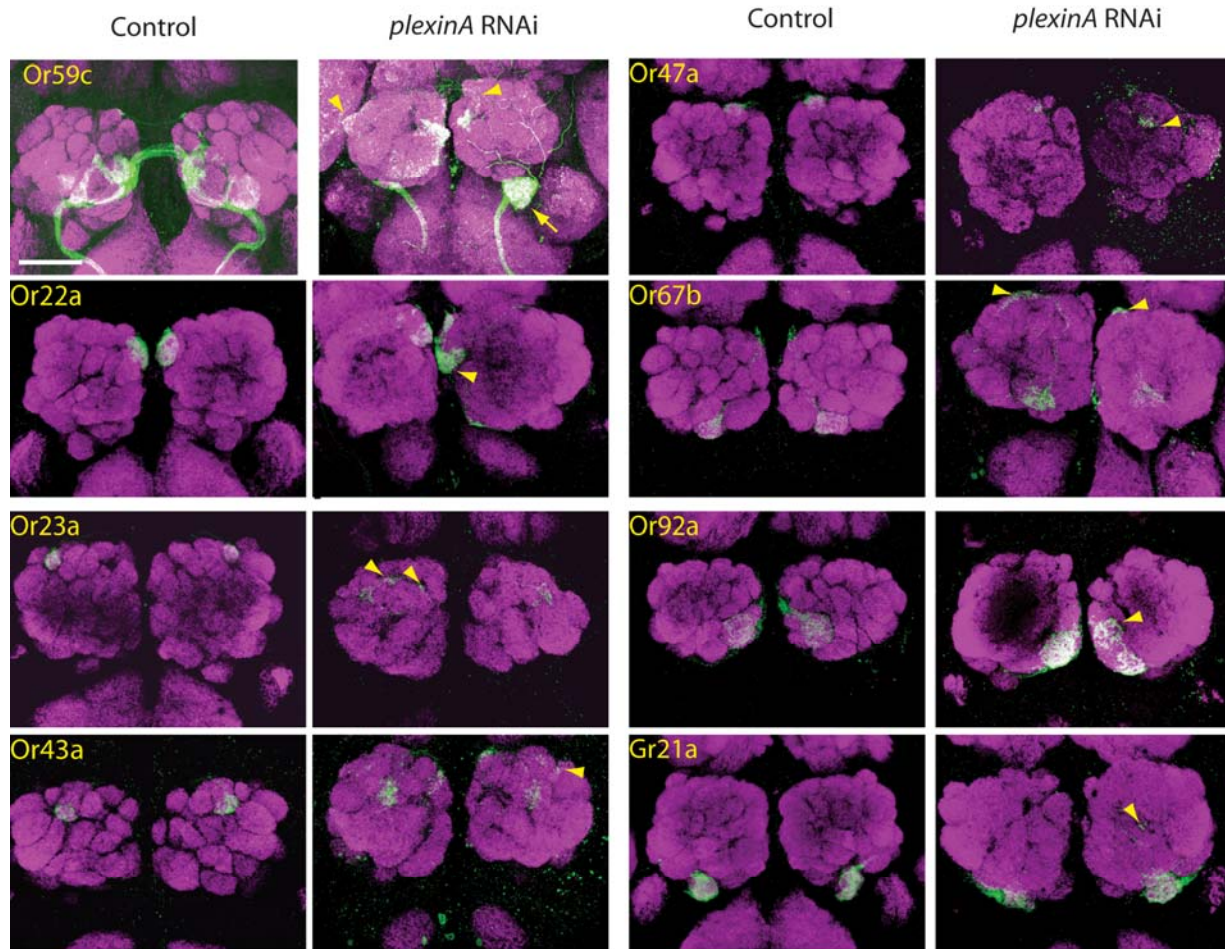
Figure S4. Mistargeted MP ORN Axon Terminals in *sema-1a*^{-/-} Mutants Are Enriched for a Presynaptic Terminal Marker
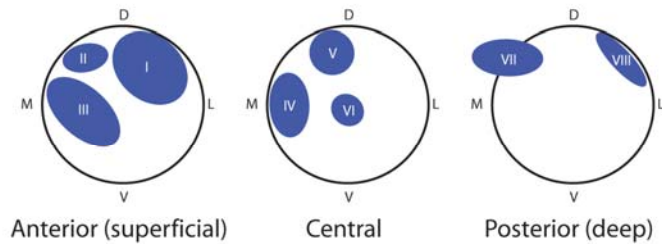

Arrows and arrowheads indicate respectively extra-lobe terminations and intra-lobe mistargeting events. Green: mCD8-GFP marking ORN axons of a given class; blue: synaptic marker nc82; red: HA-synaptotagmin expressed in the same ORN class. Scale bar, 50 μm. Genotype (eyFLP/cell lethal): *UAS-mCD8GFP eyFLP/UAS-HA-synaptotagmin; cycE FRT40A/sema-1a*[P1] *FRT40A; Or-Gal4/+.*

Figure S5. ORN Axon Targeting Defects in ORN Knockdown of *plexinA* (Continuation of Figure 5A)

A

B

	I	II	III	IV	V	VI	VII	VIII
Or22a(20)	0%	0%	0%	45%	40%	0%	40%	0%
Or23a(18)	94%	0%	0%	0%	0%	0%	11%	0%
Or43a(17)	94%	0%	0%	0%	0%	0%	0%	0%
Or47a(19)	5%	84%	0%	5%	0%	0%	0%	0%
Or67b(20)	0%	0%	80%	0%	0%	0%	15%	60%
Or92a(18)	0%	0%	50%	0%	0%	0%	6%	22%
Gr21a(19)	0%	0%	0%	0%	0%	37%	0%	0%

(A) Axon targeting of 8 ORN classes as indicated in controls (left panels) and in *plexinA* RNAi conditions (right panels). Arrows and arrowheads indicate respectively extra-lobe terminations and intra-lobe ectopic terminations.

Scale bar, 50 μm. Genotype: Control: *pebbled-Gal4; Or-mCD8GFP*. *plexinA* RNAi: *pebbled-Gal4; UAS-plexinA RNAi/+; Or-mCD8GFP/+*.

(B) Quantification of the penetrance of antennal ORN axon mistargeting to regions defined as follows: I: D, DA2, DA3, DA4l, DA4m; II: VM5d, DM6; III: VA2, VM2, VM5v, VA7l, VA7m; IV: DM5, VM7; V: DM3, DC4; VI: VC1, VC2; VII: DM4 and commissure; VIII: DL1. Data are represented as % of total brains examined that have mistargeting to defined regions. The number of brains examined is indicated in parentheses.