Supplementary information

Methods

Clonal analysis. All of the wild-type and mutant clones were generated using MARCM technique as previously described\(^1\). To label ORNs, one of the following Gal4 drivers was used; Gal4-AM29, OR-Gal4s (Or47a, Or23a, Or43a, Or46a\(^2\); Or92a, Or71a\(^3\)), or Gal4-C155. MARCM clones were induced either by a 30-min (for large clones) or by a 10-min (for small clones) heat shock at 37 °C. The heat shock was given at various developmental stages depending on the experiments; 72 h to 6 h BPF for mam\(^{\kappa\ell\kappa\ell^4}\) clones, 0 h to 6 h APF for nb\(^2\) clones, and 72 h BPF to 6 h APF for wild-type small clones.

The genotypes of flies used for various experiments are as follows:

1. wild-type clones labeled with AM29: y w hsFLP/w or Y; Gal4-AM29 FRTG13 UAS-mCD8::GFP/FRTG13 tubP-Gal80; +/UAS-mCD8::GFP
2. wild-type clones labeled with C155: w Gal4-C155 hsFLP/w or Y; FRTG13 UAS-mCD8::GFP/FRT13 tubP-Gal80; +/UAS-mCD8::GFP
3. mam\(^{\kappa\ell\kappa\ell^4}\) clones labeled with AM29: y w hsFLP/w or Y; Gal4-AM29 FRTG13 mam\(^{\kappa\ell\kappa\ell^4}\) UAS-mCD8::GFP/FRTG13 tubP-Gal80; +/UAS-mCD8::GFP
4. mam\(^{\kappa\ell\kappa\ell^4}\) clones labeled with C155: w Gal4-C155 hsFLP/w or Y; FRTG13 mam\(^{\kappa\ell\kappa\ell^4}\) UAS-mCD8::GFP/FRTG13 tubP-Gal80; +/UAS-mCD8::GFP
5. mam\(^{\kappa\ell\kappa\ell^4}\) clones labeled with OR-Gal4s: y w hsFLP/w or Y; FRTG13 mam\(^{\kappa\ell\kappa\ell^4}\) UAS-mCD8::GFP/FRTG13 tubP-Gal80; OR-Gal4 UAS-mCD8::GFP/+  
6. nb\(^2\) clones labeled with AM29: y w hsFLP/w or Y; Gal4-AM29 nb\(^2\) FRT40A/tubP-Gal80 FRT40A; UAS-mCD8::GFP /UAS-mCD8::GFP  
7. nb\(^2\) clones labeled with C155: w Gal4-C155 hsFLP UAS-mCD8::GFP/w or Y; nb\(^2\) FRT40A/tubP-Gal80 FRT40A; +/UAS-mCD8::GFP  
8. nb\(^2\) clones labeled with OR-Gal4s: y w hsFLP/w or Y; nb\(^2\) FRT40A/tubP-Gal80 FRT40A; OR-Gal4 UAS-mCD8::GFP/UAS-mCD8::GFP.

Immunostaining. For all clonal analyses, adult brains were dissected out, fixed and immunostained by anti-mouse CD8α (1:100; Caltag Laboratories) and mAb nc82\(^4\)
(1:10), except for experiments with a single-pair clone of AM29-labeled ORNs, where brains and antennae were simultaneously dissected out at a mid-pupal stage. To analyze OR-Gal4 expression in ORNs, antennae and maxillary palps were dissected from adult flies and their GFP fluorescence was directly observed. Mouse or rat anti-Elav (1:20, DSHB) was used to label ORN clones in the pupal antenna. To characterize the cell differentiation in the olfactory organ lineage, the developing antennae carrying MARCM clones were dissected out at 20–24 h APF and were immunolabeled with anti-Sens5 (1:1000), anti-Svp6 (1:100), anti-Cut7 (1:50; 2B10 from DSHB) and anti-Pon8 (1:1000) using a standard method. Optical sections of the ALs and antennae were collected using a Leica TCS SP2 or Zeiss LSM510 confocal microscope.

**Glomerular analysis.** Identities of glomeruli were determined by carefully comparing adjacent optical sections of the antennal lobes while using the previous descriptions as references3,8,10. During the analysis, we named one previously unreported glomerulus, VC5, located posterior to VC3l, and renamed two glomeruli “1” and VM7 as VM7v and VM7d, respectively.

In the analysis of small Gal4-C155 clones, many samples showed projections from more than one cluster of ORNs. To identify each cluster of ORN projections in such samples, we reasoned that projections always labeled together should be from a single cluster of ORNs.


