Supporting Information

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SI Text

Zebrafish. Maintenance, larval collection, and developmental staging of zebrafish (*Danio rerio*) were performed essentially as described previously (1, 2). Collected larvae were maintained in 1/3 Ringer's solution (39 mM NaCl, 0.97 mM KCl, 1.8 mM $CaCl_2$, and 1.7 mM Hepes, pH 7.2) supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin. In some cases, 0.002% phenylthiourea was added at 12 h after fertilization to prevent pigmentation.

SAGFF, hspGFF, and hspGGFF fish were crossed with homozygous UAS:GFP reporter fish, and double-transgenic embryos were analyzed for GFP expression in cells along the olfactory neural pathways.

Inverse PCR. The chromosomal integration sites of SAGFF were analyzed by inverse PCR (3) and successfully identified for 2 lines: SAGFF27A insertion within the gene encoding retinoblastoma-binding protein 6 on chromosome 8 and SAGFF91B insertion at 7.8 kb upstream of the gene encoding retinal aldehyde reductase 12-like on chromosome 24. The expression and functional analyses of these genes are to be reported elsewhere.

Immunohistochemistry. Whole-mount immunohistochemistry was performed essentially as described previously (4) with the following modifications. Larvae were fixed in 10% (vol/vol) formalin in PBS for 30 min, further fixed in 2% (vol/vol) trichloroacetic acid in PBS for 3 h, and permeabilized in acetone for 7 min at −20 °C. Immunohistochemistry of OB sections was performed essentially as described previously (5).

Primary antibodies used in this study have been characterized previously in the literature and are summarized in Table S1. Cy3- and Cy5-conjugated secondary antibodies were purchased from Jackson ImmunoResearch. Alexa 488-conjugated secondary antibodies were purchased from Molecular Probes.

Data Analyses. To check whether fish prefer a specific side of the tank, each time bin was analyzed for a significant deviation from 0 using the Wilcoxon sign-rank test. Kruskal-Wallis ANOVA was applied to detect overall differences among several unpaired groups. When differences among groups occurred, the significantly different groups were filtered out by pair-wise comparisons using Mann-Whitney *U* tests. In all figures, 1, 2, and 3 asterisks indicate *P*-values less than 0.05, 0.01, and 0.001, respectively. Statistical analyses were performed with STATISTICA, version 8 (StatSoft).

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4. Miyasaka N, et al. (2005) Robo2 is required for establishment of a precise glomerular map in the zebrafish olfactory system. *Development* 132:1283–1293.
5. Sato Y, Miyasaka N, Yoshihara Y (2005) Mutually exclusive glomerular innervation by two distinct types of OSNs revealed in transgenic zebrafish. *J Neurosci* 25:4889–4897.

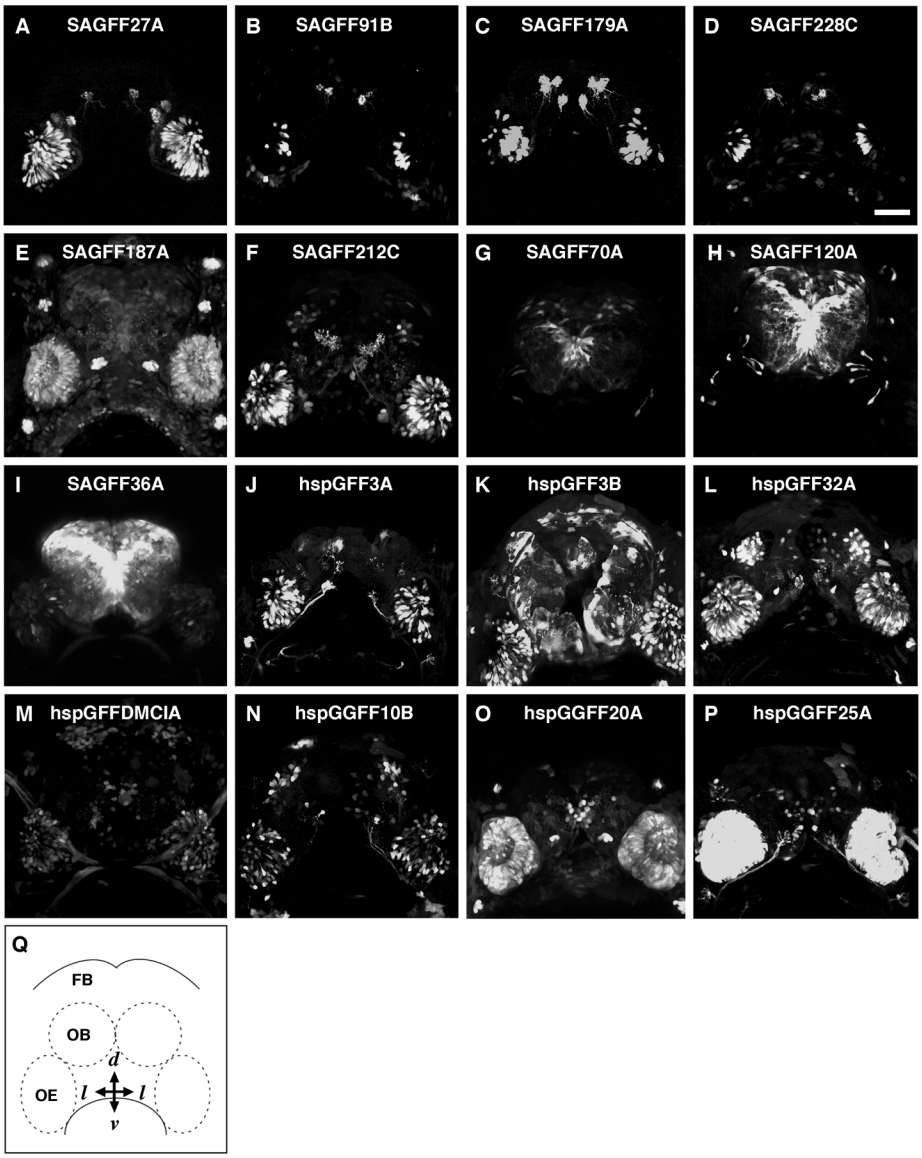


Fig. S1. Various patterns of GFP expression in the gene trap (*A–I*) and enhancer trap (*J–P*) zebrafish lines. Frontal views of 5-day-old larvae with ventral to the bottom. SAGFF27A (*A*), SAGFF91B (*B*), SAGFF179A (*C*), and SAGFF228C (*D*): distinct subsets of OSNs (see *Results* for details). (*E*) SAGFF187A: nonneuronal cells in the OE and neuromasts. (*F*) SAGFF212C: a subset of OSNs and cells in the forebrain. SAGFF70A (*G*), SAGFF120A (*H*), and SAGFF36A (*I*): cells in forebrain and small subsets of OSNs. (*J*) hspGFF3A: a subset of OSNs. (*K*) hspGFF3B: OSNs and epidermis. hspGFF32A (*L*), hspGFFDMCIA (*M*), and hspGGFF10B (*N*): OSNs and forebrain neurons. hspGGFF20A (*O*) and hspGGFF25A (*P*): nonneuronal cells in the OE and cells in the forebrain. (Q) A schematic diagram illustrating the frontal view of zebrafish larva. FB, forebrain; OB, olfactory bulb; OE, olfactory epithelium. (Scale bar: 50 μm.)

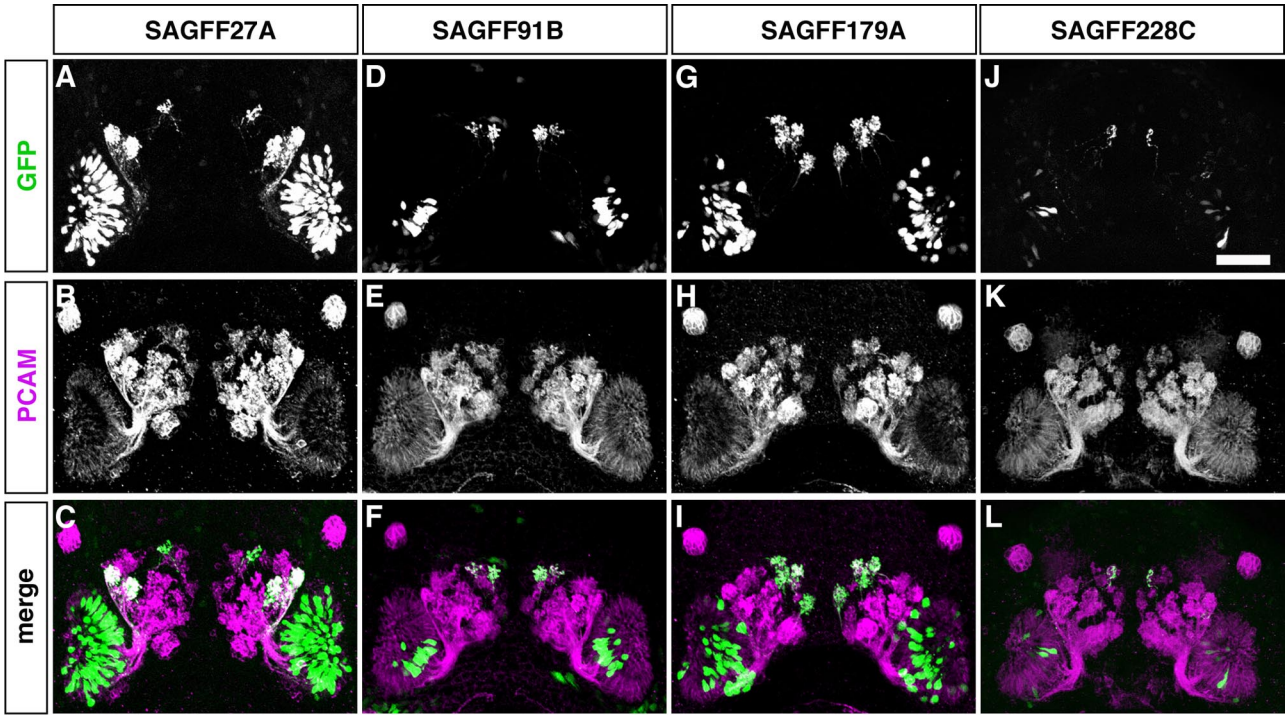


Fig. S2. PCAM expression in all the OSN axons. GFP expression patterns were examined in 5-day-old larvae of the 4 gene trap Gal4FF-expressing lines (*A–C*, SAGFF27A; *D–F*, SAGFF91B; *G–I*, SAGFF179A; and *J–L*, SAGFF228C) crossed with the UAS:GFP reporter strain. Whole-mount double-immunofluorescence labeling with antibodies against GFP (*A*, *D*, *G*, and *J*; green in *C*, *F*, *I*, and *L*) and PCAM (*B*, *E*, *H*, and *K*; magenta in *C*, *F*, *I*, and *L*). Single-label gray-scale images of PCAM demonstrate that PCAM is expressed in all the OSN axons, although its expression level is not equal among the axons. In particular, the axons projecting to the medial glomerular cluster show a relatively weak expression of PCAM compared with other axons. (Scale bar: 50 μm.)

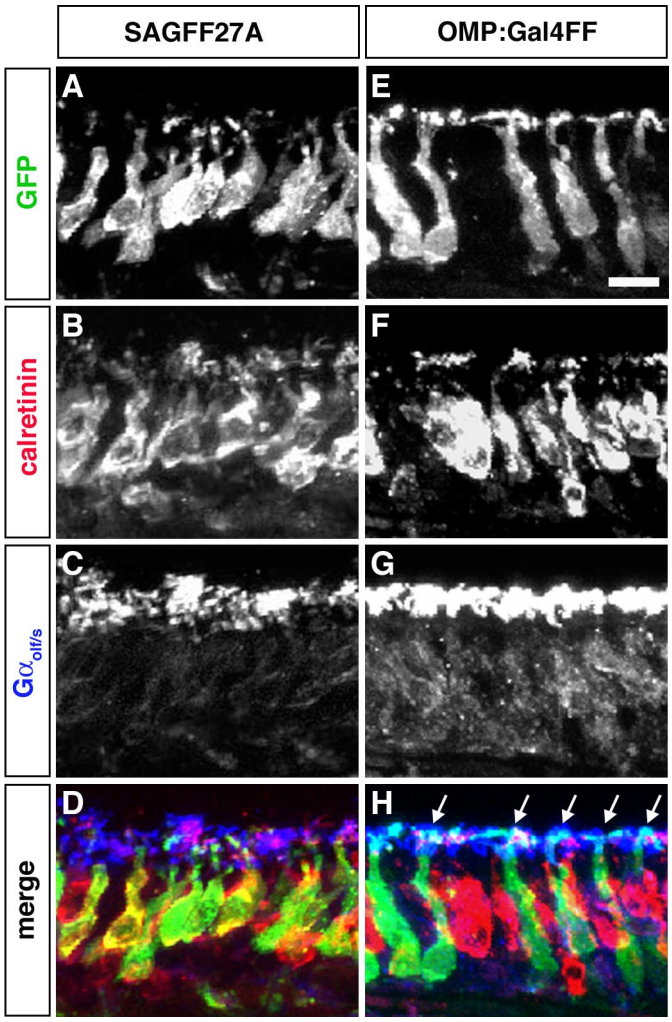


Fig. S3. Differential GFP expression in distinct types of OSNs in 2 transgenic fish lines, SAGFF27A (*A–D*) and OMP:Gal4FF (*E–H*). Adult OE sections were triple-labeled with antibodies against GFP (*A* and *E*; green in *D* and *H*), calretinin (*B* and *F*; red in *D* and *H*), and $G\alpha_{olf/s}$ (*C* and *G*; blue in *D* and *H*). In the SAGFF27A line, GFP is expressed in short dendrite-bearing and calretinin-positive OSNs in the apical layer of the OE (A, *B*, and *D*), suggesting that Gal4FF/GFP-expressing cells are the microvillous OSNs. In contrast, in the OMP:Gal4FF fish, GFP is observed in long dendrite-bearing OSNs, the cell bodies of which are located in the basal layer of the OE (*E*, *G*, and *H*). Arrows indicate the dendritic knobs that are positive for both GFP and $G\alpha_{olf/s}$ on the epithelial surface. These results indicate that Gal4FF/GFP-expressing cells in the OMP:Gal4FF fish are the ciliated OSNs. (Scale bar: 10 μm.)

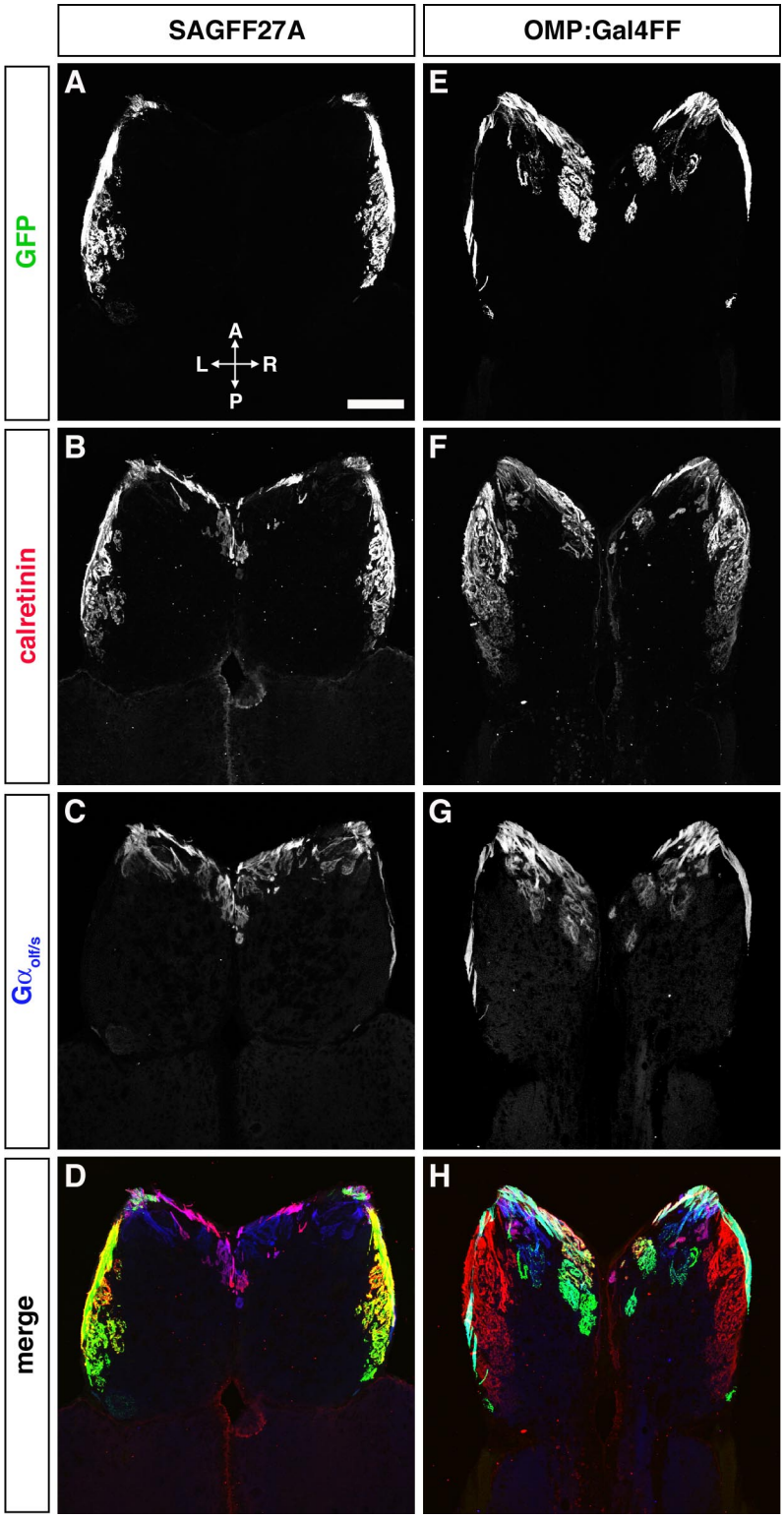


Fig. S4. Differential glomerular innervation by distinct OSN axons in SAGFF27A (*A–D*) and OMP:Gal4FF (*E–H*). Horizontal sections through the OB were triple-labeled with anti-GFP (*A* and *E*; green in *D* and *H*), anti-calretinin (*B* and *F*; red in *D* and *H*), and anti-G$\alpha_{olf/s}$ (*C* and *G*; blue in *D* and *H*) antibodies. In the SAGFF27A line, GFP-expressing microvillous OSNs project their axons exclusively to calretinin-positive glomeruli in the lateral region of the OB. In contrast, GFP-expressing ciliated OSNs in the OMP:Gal4FF fish project their axons mainly to G$\alpha_{olf/s}$-positive glomeruli in the anterior and medial regions of the OB. (Scale bar: 100 μm.)

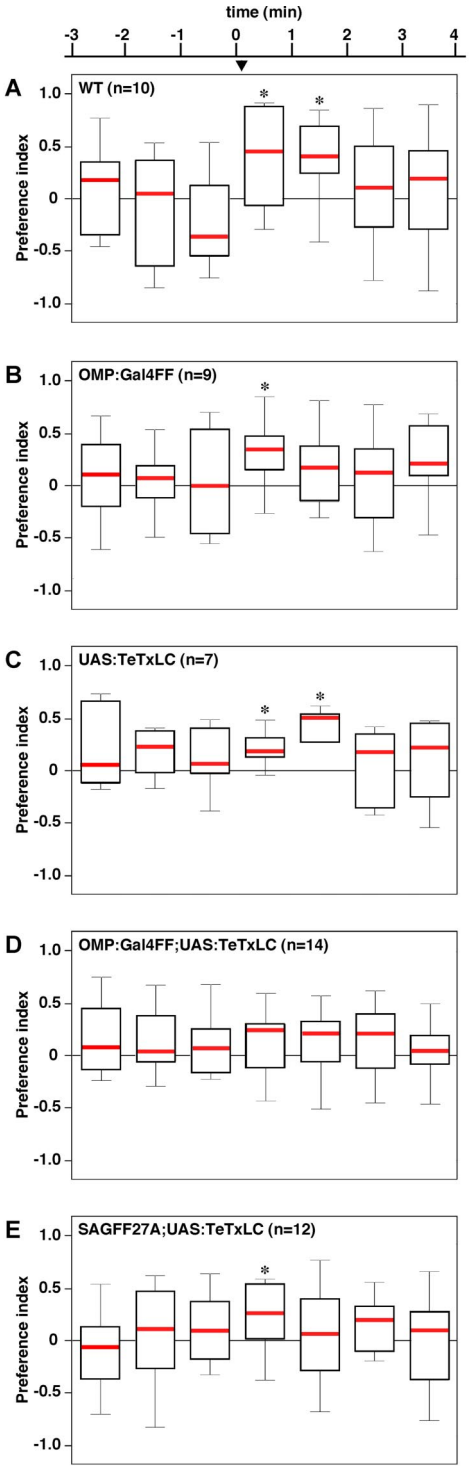
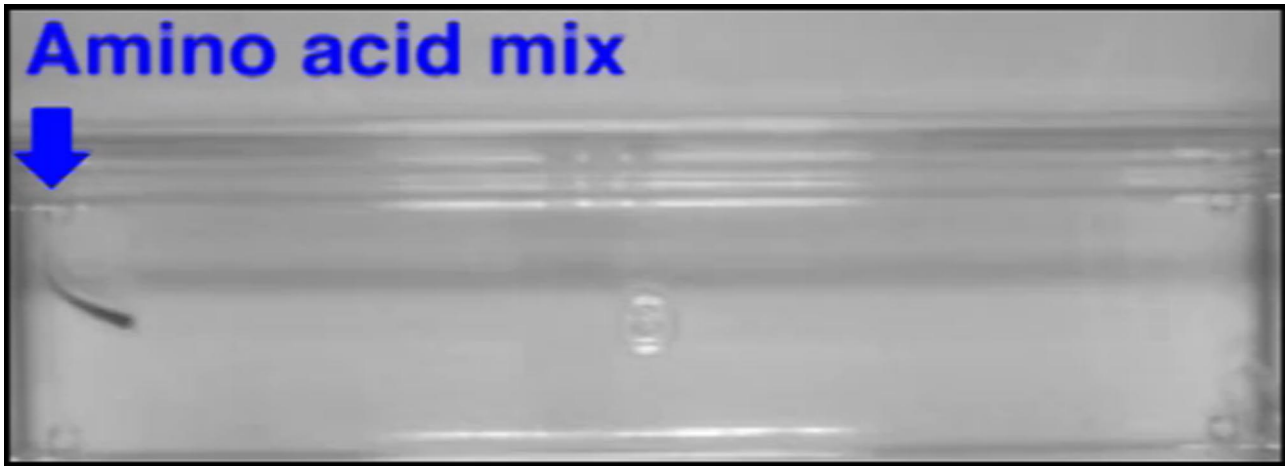
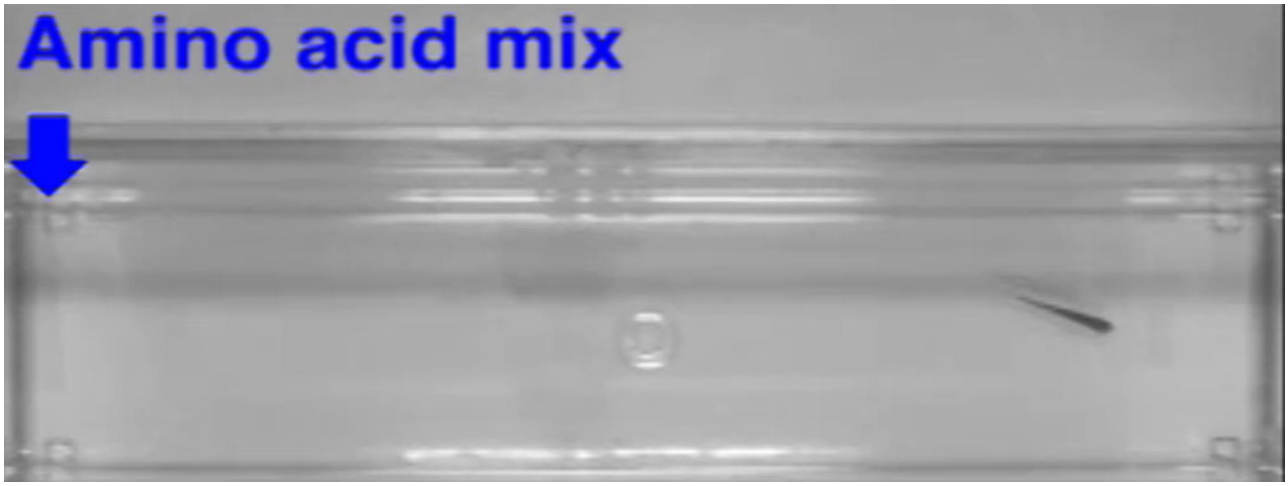


Fig. S5. Attractive response of zebrafish to a bile acid, taurocholic acid (TCA). Results are presented for every 1 min from 3 min before (*Left*) to 4 min after (*Right*) the application of 0.6 mL of 1 mM TCA into 600 mL of water. The arrowhead indicates the time point of TCA application. WT (*A*), OMP:Gal4FF (*B*), UAS:TeTxLC (*C*), OMP:Gal4FF;UAS:TeTxLC (*D*), and SAGFF27A;UAS:TeTxLC (*E*). The PI is shown by the box plot representing the median (red horizontal line), 25 to 75% quantiles (boxes), and ranges (whiskers) of data. Each time bin was analyzed for a significant deviation from 0 with the Wilcoxon sign-rank test (*$P < 0.05$).



Movie S1. The movie shows the attractive response to amino acids in WT zebrafish. The corner of amino acid application is highlighted with a blue arrow.

Movie S1



Movie S2. Impairment of the attractive response in SAGFF27A;UAS:TeTxLC double-transgenic fish.

Movie S2

Table S1. Primary antibodies used in the present study

Antibody	Species	Antigen	Dilution or concentration	Source catalog no.	References
GFP	Rat	His-GFP (full-length) fusion protein	1:1000	Nacalai Tesque 0440426	(1–4)
$G\alpha_{olf/s}$	Rabbit	C-terminus of rat $G\alpha_s$ (aa 377–394)	1:1000	Santa Cruz sc-383	(5–7)
Calretinin	Mouse	Recombinant human calretinin	1:1000	Swant 6B3	(1, 8–10)
PCAM	Rabbit	C-terminus of zebrafish PCAM (aa 1012–1031)	0.4 μg/ml	Yoshihara Yoshihiro, RIKEN Brain Science Institute (Japan)	(1, 11)
SV2	Mouse	Synaptic vesicles purified from Ommata electrical organ	1:50	Developmental Studies Hybridoma Bank	(1, 3, 4, 12)

1. Miyasaka N, et al. (2005) Robo2 is required for establishment of a precise glomerular map in the zebrafish olfactory system. *Development* 132:1283–1293.
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3. Sato Y, Miyasaka N, Yoshihara Y (2005) Mutually exclusive glomerular innervation by two distinct types of olfactory sensory neurons revealed in transgenic zebrafish. *J Neurosci* 25:4889–4897.
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