Supplementary Methods

Cell culture and transfection
HEK293 cells were maintained under standard conditions in MEM supplemented with 10% FBS, 100 units/ml penicillin and streptomycin, and 2 mM L-glutamine. Transfections were performed using a standard calcium phosphate precipitation technique. Cells were harvested and used 48 hours post-transfection.

DNA constructs and plasmids
Drosophila odorant receptors DOR83b, DOR43a and DOR22a were amplified from RNA obtained from D. melanogaster antennae by RT-PCR using specific primers which amplify the complete open reading frame and contain restriction sites for further subcloning. The receptor constructs for DOR83b and DOR43a, which contain ClaI restriction sites 3’upstream of the termination codon, were cloned into pcDNA3 (Invitrogen). The pcDNA3-DOR83b-Luc, pcDNA3-DOR83b-GFP, pcDNA3-DOR43a-Luc and pcDNA3-DOR43a-GFP plasmids were generated by subcloning the coding sequences of Renilla luciferase (Luc) and GFP² from the plasmid pGFP²-MCS-Rluc(h) (Packard BioScience) into the ClaI-linearized, odorant-receptor-containing plasmids. PCR product of DOR22a was cloned into a linearised plasmid containing GFP². This plasmid was generated by amplification of GFP² from pGFP²-MCS-Rluc(h) (Packard BioScience) by PCR using primers that contain restriction sites for the generation of N-terminal fusion constructs. Drosophila CNG channel (acc no. X89601) was cloned from D. melanogaster cDNA from complete heads, rat β2-adrenergic receptor (β2-AR) was cloned directly from rat genomic DNA; both were tagged with GFP² and luciferase in the same way as the Drosophila odorant receptors. All PCR-amplified stretches of DNA were verified by sequencing.
**BRET assay**

Bioluminescence resonance energy transfer assays used to detect GPCR dimerization were performed as previously described\(^1\). Briefly, transfected HEK293 cells were resuspended in Ringer’s solution (140 mM NaCl, 5 mM KCl, 2 mM CaCl\(_2\), 2 mM MgCl\(_2\), 10 mM Hepes, 10 mM glucose) and 3\(\times\)10\(^5\) cells were distributed in each well of a 96-well microplate (white Optiplate from Packard Bioscience). The luciferase substrate DeepBlueC (PackardBioScience) was added at a final concentration of 5 M and readings were performed using a Fusion™ universal microplate analyser (Packard BioScience), which allows the sequential integration of signals detected at 370-450 nm (luciferase) and 500-530 nm (GFP\(^\alpha\)). BRET signals were calculated as the ratio of the light emitted at 500-530 nm by BRET acceptors (receptors fused to GFP\(^\alpha\)), over the light emitted at 370-450 nm by BRET donors (receptors fused to luciferase). In each experiment, values were corrected by subtracting the background obtained by measuring before the application of DeepBlueC.

**Western blot**

HEK293 cells were transfected with pCDNA3-DOR43a-GFP alone or together with pCDNA3-DOR83b. Two days after transfection the cells were harvested, resuspended in homogenisation buffer (320mM sucrose, 4mM HEPES, pH 7.0) with protease inhibitors (Roche Complete® protease inhibitor cocktail) and homogenized using a dounce homogenizer. Cell debris and nuclei were removed by centrifugation (1000g; 10 min), the pellet was discarded and the supernatant was ultracentrifuged (20,000g; 1 hr) in a Beckmann TLA100.1 rotor. The resultant membrane pellet was solubilised using 1% (w/v) L-α-lysophosphatidylcholine (Sigma); protein concentration was determined by the Bradford microassay (Bio-Rad).

An aliquot of solubilised HEK293 cell membrane was denatured in Lämmli-Buffer containing DTT at 40°C for 5 min. Samples were loaded on 8 % SDS-PAGE gels, transferred to nitrocellulose membrane (Protran, Schleicher & Schuell) and stained with Ponceau S (Sigma).
The nitrocellulose-membranes were blocked with TBS (150 mM NaCl, 50 mM TrisCl pH 7.4) containing 5 % non-fat dry milk (Biorad) and incubated with rabbit α-GFP antibody (Abcam) 1:1000 in 3 % milk for 120 min. After washing in TBS, membranes were incubated with secondary goat-α-rabbit antibodies coupled to HRP (Bio-Rad) diluted in 3 % milk. Detection was performed with ECL plus (Amersham) on Hyperfilm ECL (Amersham).

**Calcium Imaging**

Transfected HEK293 cells were incubated for 45 min at 37°C in loading buffer (pH 7.4) containing standard Ringer’s solution and 7.5 M fura-2-AM (Molecular Probes). After 45min the solution was exchanged for fura-free buffer. Ca-Imaging experiments were performed on the stage of a Zeiss inverted microscope equipped for ratiometric imaging to collect and quantify spatiotemporal Ca^{2+}-dependent fluorescence signals (f_{340}/f_{380} ratio). Cells were viewed with 630X magnification. Images were acquired in a randomly selected field of view, and integrated fluorescence ratios were measured. Exposure to odorants was accomplished using a specialized microcapillary application system.

**RNAi**

*Drosophila* is amenable to genetic manipulation including gene silencing through a process known as RNA interference (RNAi)\(^2\). Typically, RNAi is triggered by double-stranded RNA (dsRNA), which is first processed by the Dicer RNase into 21- to 23-nt fragments. These fragments form a silencing complex that binds specifically to the dsRNA-complementary endogenous mRNA and leads to the destruction of the mRNA. For the RNAi experiments, double-stranded RNA was synthesized by *in-vitro* transcription of a PCR-generated fragment containing the DOR83a coding sequence flanked by T7-promoter sequences, essentially by the method described by Dzitoyeva et al.\(^3\). dsRNA (300 ng/μl) was injected into early-stage
embryos (preformed by the EMBL Drosophila injection service, Heidelberg, Germany).

**Electroantennograms**

EAG recordings were performed as described⁶. Briefly, 1-day-old flies were mounted in truncated micropipette tips with the anterior portion of the head protruding from the end of the tip. The indifferent electrode was inserted into the haemolymph of the head capsule. The recording electrode was placed on the frontal surface of the anterior aspect of the antenna. After obtaining a stable baseline, EAG recordings were initiated by a short pulse of odor (~1s), applied through a syringe into an air stream (0.5 liter/min) that was directed toward the antenna. All odorants were dissolved in paraffin oil at the given concentrations. Odorants used were ethanol, cyclohexanone, cyclohexanol, 1-butanol, cineole and ethyl acetate.

**In-situ hybridization**

Antisense RNA probes were labeled with digoxigenin-UTP (Roche) by in-vitro transcription from the linearized plasmid pcDNA3-OR83b using SP6 RNA-polymerase (Roche). The RNA probes were hydrolyzed under alkaline conditions to yield probes with a length under 500 nt. ISH on cryosections was essentially performed as described⁶. Briefly, Drosophila antennae were frozen in liquid nitrogen and 10-μm sections were collected on Super Frost Plus slides (Menzel-Gläser). After fixation with 4% paraformaldehyde for 10 min sections were acetylated for 10 min and washed several times in phosphate buffer saline. Slides were incubated thereafter for 1h at 58°C in prehybridization buffer and then overnight in hybridization buffer containing the digoxigenin-labeled probe. After blocking sections in normal goat serum, hybridization was visualized by incubating slides overnight with alkaline phosphatase-coupled α-digoxigenin antibody (Roche), and subsequent dye development with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.
References