SUPPLEMENTARY METHODS

***Drosophila* stocks.** Fly stocks were maintained on conventional cornmeal-agar-molasses medium at 25°C. These following strains were used in this study: wild type-Berlin (M. Heisenberg); *w*^{1118}*; 70FLP,70I-SceI/Cyo* and *w*^{1118}*; 70FLP* (K. Golic); *UAS-CD8-GFP* (L. Luo); *Gr21a-GAL4* (K. Scott); *Or22a-GAL4*. *Gr63a*-sytRFP flies were constructed by fusing the coding region of n-synaptotagmin to RFP and placing this under the control of the *Gr63a* promoter.

RNA *In situ* hybridization. Double fluorescent RNA *in situ* hybridization was performed on fly antennae as described previously[1], and on mosquito maxillary palps without protocol modification. Adult mosquitoes (*Anopheles gambiae* strain G3; MRA-112) were obtained from MR4, CDC Atlanta, GA.

Immunofluorescence. Whole mount brain immunostaining of *Gr63a-syt-RFP; Gr21a-GAL4, UAS-CD8-GFP* flies was performed as previously described[2] using 1:1000 anti-GFP (Molecular Probes) and 1:10 nc82 (a gift of Reinhard Stocker) with 1:100 anti-rabbit-Alexa488 (Molecular Probes) and 1:100 anti-mouse-Cy3 (Jackson ImmunoResearch) secondary antibodies. Whole mount staining of *Gr21a*-Gal4;UAS-CD8-GFP and *Gr63a*-Gal4;UAS-CD8-GFP and *Gr21a*-Gal4/*Gr63a*-Gal4;UAS-CD8-GFP larvae was performed as described[3] using the antibodies above.

GR transgene generation. *Gr10a* was amplified from Oregon-R antennal cDNA using primers 5'- ATGACATCGCCGGATGAGCGT-3' and 5'- CTAGGACTTCTTGCGCAAATA-3'. *Gr63a* was amplified from *yw* genomic DNA using primers 5'- ATGCGTCCGTCTGGCGAAAAA -3' and 5'- CTAGCCTTTCCGGCCCTTTAG -3'. *GPRgr22* was amplified from *Anopheles gambiae* G3 antennal cDNA using primers 5'- ATGATTCACACACAGATGGAA-3' and 5'- TTAGGTGTTCACTTTGTCTGC-3'. *GPRgr24* was amplified from *Anopheles gambiae* G3 antennal cDNAs using primers 5'- ATGGTGTTTGAAAGCTCCAAA-3' and 5'- CTAAGAATGAGACGAATTACT-3'. PCR products produced using the Expand High Fidelity PCR kit (Roche) were subcloned into pGEM-T Easy (Promega). Fly GRs were subcloned into pUAST[4] and transgenic animals were produced (Genetic Services Inc., Cambridge, MA, USA) and balanced using standard methods.

Single sensillum electrophysiology. Extracellular recordings of ab1 and ab3 sensilla from individual flies (2-10 days old) were made as described[5,6] using a 10X AC probe connected to the Syntech IDAC-4 acquisition controller and analyzed offline using the software Autospike (Syntech, Hilversum, The Netherlands). Thirty µl of odorant, diluted 10^{-4} in paraffin oil, were added to filter paper strips and placed inside 1ml plastic tuberculin syringes. One-second odor stimuli were added to a constant air stream under the control of the Syntech CS-55 Stimulus controller. Prior to CO_2 recordings each sensillum was identified by its characteristic odorant response profile: ab3A, ethyl hexanoate; ab3B, 2-heptanone; ab1A, ethyl acetate; ab1B, acetoin; ab1D, methyl salicylate. All odorants were obtained from Sigma-Aldrich and were of the highest purity available. CO_2 stimuli were applied by filling 20 ml syringes from pre-mixed CO_2 in air tanks from Matheson Tri-Gas (Parsippany, NJ). Maximum stimulus concentrations (odours ~$3x10^{-5}$; CO_2 ~2-3%) at the exit point of the stimulus device were calculated by measuring system airflows. Response quantification was calculated by counting all spikes within a 500 ms window from the onset of the response. Air responses were then subtracted from CO_2 responses from the same sensillum and the resulting number of spikes was doubled to obtain a corrected CO_2 response in spikes/second. After checking the response distributions for normality, we proceeded with parametric means comparisons using the Tukey HSD test.

***Gr63a* targeting construct and mutant screen** Genomic DNA both 5' and 3' of the *Gr63a* coding sequence was amplified from *yw* flies using Expand High Fidelity PCR kit (Roche) and TA cloned into pGEM-T Easy (Promega, Madison, WI, USA). Inserts were sequenced from the ends and in any predicted internal coding regions, and then subcloned into CMC105[6]. 5' homologous arm: primers corresponding to nucleotides 3880204-3880228 and 3883041-3883062 of *Drosophila melanogaster* chromosome 3L (Genbank entry NT_037436) amplified a 2.859 kb fragment. 3' homologous arm: primers corresponding to nucleotides 3874643-3874664 and 3878509-3878530 of *Drosophila melanogaster* chromosome 3L (Genbank entry NT_037436) amplified a 3.888 kb fragment.

Four independent insertions of the targeting construct were screened as described[6]. The progeny of approximately 16,500 virgin mosaic or white-eyed females (~330,000 flies) were screened for re-insertion on the 3rd chromosome, and we recovered a single mutant allele, *Gr63a*[1]. PCR confirmation of *Gr63a*[1] was performed on genomic DNA preparations of the mutant line and its corresponding wild type parental targeting construct insertion with primers within *Gr63a* itself (nucleotides 3879912-3879930 and 3880180-3880204 of Genbank entry NT_037436) and within the neighbouring gene CG1079 (nucleotides 3877256-3877274 and 3877702-3877720 of Genbank entry NT_037436). A similar screen for a *Gr21a* mutant produced no mutants among ~350,000 progeny derived from five independent targeting construct insertions.

Behaviour. CO_2 T-maze avoidance experiments were performed essentially as described[7]. Mean avoidance index is calculated as # flies on CO_2 side - # flies on air side/ total # flies. Flies that failed to choose one of the two stimulus tubes were excluded from the index. Experiments were carried out in the dark at 25°C and 70% relative humidity with a 15W red-light positioned behind and perpendicular to the T-maze. Pure CO_2 (0.28 ml) was added to 14 ml tubes for a final concentration of ~2%. The stimulus was added to alternating sides to preclude any side bias. Each individual experiment included between 15 and 50 flies (mean = 30).

Phylogenetic tree. Multiple protein alignments were made using ClustalX version 1.83 with default parameters[8]. A neighbour-joining tree was generated with PHYLIP v. 3.6 [Felsenstein, J. 2005. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle] and viewed using the web-based Phylodendron tree viewer (http://iubio.bio.indiana.edu/treeapp/treeprint-form.html).

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