

Supplementary Information for:

Receptive range analysis of a mouse odorant receptor subfamily

Jingyi Li, Rafi Haddad, Vanessa Santos, Selvan Bavan, Charles W. Luetje

Supplementary Table 1.

Composition of odorant mixtures (with CAS numbers) used in Figure 1.

Mixture 1	Mixture 2	Mixture 3	Mixture 4
(-)-carvone (6485-40-1) (-)-isopulegol (89-79-2) (+)-citronellal (2385-77-5) benzyl cinnamate (103-41-3) 1,4-cineole (470-67-7) D-Camphor (464-49-3) trans,trans-2,4-octadienal (5577-44-6)	(-)-carvyl acetate (97-42-7) (-)-citronellal (5949-05-3) (-)-dihydrocarveol (20549-47-7) (-)-limonene (5989-54-8) (+)-carvone (2244-16-8) 2,5-dimethylpyrazine (123-32-0) 2-acetylfuran (1192-62-7) 2-acetylpyridine (1122-62-9)	(-)-menthol (2216-51-5) (-)-carveol (99-48-9) (+)-dihydrocarvone (7764-50-3) (+)-isopulegol (104870-56-6) 2-heptanone (110-43-0) 2-isobutylthiazole (18640-74-9) 2-methylanisole (578-58-5) benzyl salicylate (118-58-1)	(-)-menthone (14073-97-3) (+)-limonene (5989-27-5) 2-nonanone (821-55-6) 2-pentanol (6032-29-7) 4-pentenoic acid (591-80-0) 5-methylfurfural (620-02-0) amyl acetate (628-63-7) α -ionone (127-41-3)
Mixture 5	Mixture 6	Mixture 7	
anisole (100-66-3) α -pinene (80-56-8) cinnamaldehyde (104-55-2) cinnamic acid (140-10-3) citral (5392-40-5) cuminaldehyde (122-03-2) diethyl succinate (123-25-1) ethyl 2-furoate (614-99-3)	ethyl butyrate (105-54-4) ethyl octanoate (106-32-1) geraniol (106-24-1) geranyl acetate (105-87-3) guaiacol (90-05-1) hydrocinnamaldehyde (104-53-0) isoamyl acetate (123-92-2) isopentylamine (107-85-7)	linalool (78-70-6) phenylacetic acid (103-82-2) piperidine (110-89-4) pyrrolidine (123-75-1) trans-2-hexenal (6728-26-3) trans-2-hexen-1-ol (928-95-0) veratrole (91-16-7)	

Supplemental Table 2.

Composition of odorant mixtures (with CAS numbers) used in Figure 4. These mixtures contain all of the odorants from Figure 1, plus 101 additional odorants.

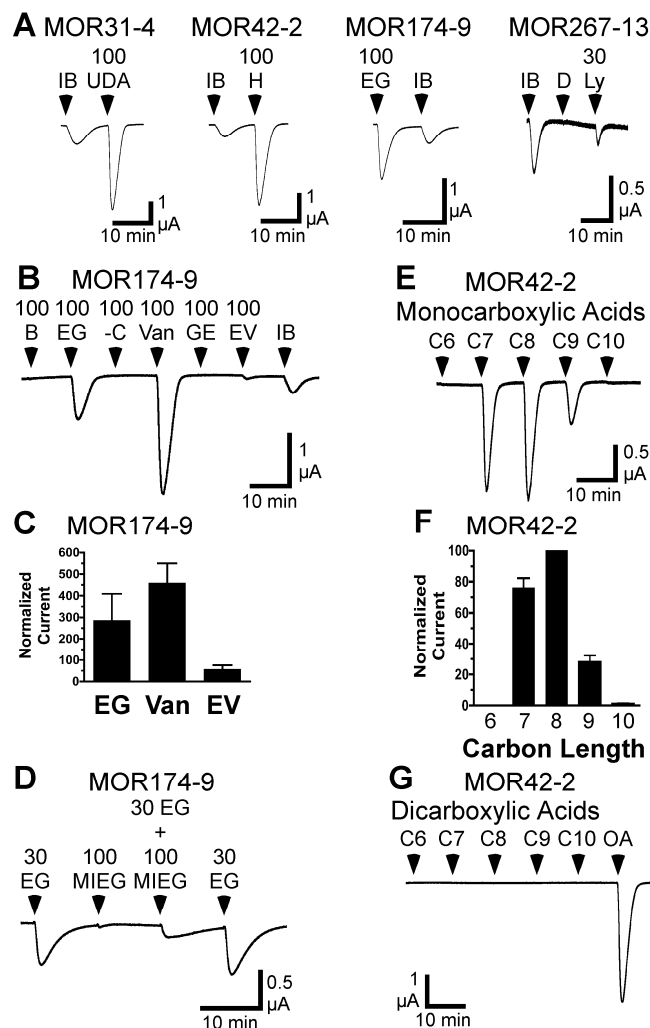
Mixture 1	Mixture 2	Mixture 3	Mixture 4
(-)-ambroxide (6790-58-5) (-)-carvyl acetate (97-42-7) (-)-limonene (5989-54-8) 1-hexanethiol (111-31-9) 2-acetylfuran (1192-62-7) 2-methylpentanoic acid (97-61-0) 2-pentanol (6032-29-7) 2,3-dimethylpyrazine (5910-89-4) benzyl salicylate (118-58-1) butylamine (109-73-9) ethyl octanoate (106-32-1) furfuryl isopropyl sulfide (1883-78-9) linalool (78-70-6) methyl phenylacetate (101-41-7) p-cresol (106-44-5) phenethylamine (64-04-0) piperidine (110-89-4)	(+)-limonene (5989-27-5) 2-acetylpyrazine (22047-25-2) 2-acetylthiazole (24295-03-2) 2-heptanol (543-49-7) 2-methylanisole (578-58-5) 2-pentanone (107-87-9) 2,3,5,6-tetramethylpyrazine (1124-11-4) 4-pentenoic acid (591-80-0) allyl butyrate (2051-78-7) anisyl alcohol (105-13-5) cinnamyl formate (104-65-4) isopentylamine (107-85-7) linalyl formate (115-99-1) nerolidol (7212-44-4) propyl disulfide (629-19-6) propyl formate (110-74-7) pyrrolidine (123-75-1) α -pinene (80-56-8)	(-)-menthone (14073-97-3) (+)-citronellal (2385-77-5) 2-acetylpyridine (1122-62-9) 2-ethoxythiazole (15679-19-3) 2-methyl butanal (96-17-3) 2-nonanol (628-99-9) 2-nonanone (821-55-6) 2-phenylpropionaldehyde (93-53-8) 2,5-dimethylpyrazine (123-32-0) 3-heptanone (106-35-4) 4-allylanisole (140-67-0) allyl heptanoate (142-19-8) cinnamic acid (140-10-3) cinnamyl alcohol (104-54-1) citral (5392-40-5) furfuryl heptanoate (39481-28-2) geranyl acetate (105-87-3) guaiacol (90-05-1) propyl mercaptan (107-03-9) trans-cinnamaldehyde (14371-10-9)	(-)-fenchone (7787-20-4) (-)- β -pinene (18172-67-3) (+)-carvone (2244-16-8) (+)-isopulegol (104870-56-6) 2-ethylpyrazine (13925-00-3) 2-isobutylthiazole (18640-74-9) 2-thiophenethiol (7774-74-5) 2,3-pentanedione (600-14-6) 3-heptanol (589-82-2) 3-nonanone (925-78-0) 3-phenyl-1-propanol (122-97-4) allyl tiglate (7493-71-2) anisole (100-66-3) cuminaldehyde (122-03-2) heptyl butyrate (5870-93-9) isobutyl phenylacetate (102-13-6) phenylacetic acid (103-82-2) γ -Octalactone (104-50-7) trans,trans-2,4-heptandienal (4313-03-5) trans,trans-2,4-octadienal (5577-44-6)
Mixture 5	Mixture 6	Mixture 7	Mixture 8
(-)-carvone (6485-40-1) (-)-isopulegol (89-79-2) (+)-dihydrocarvone (7764-50-3) 1-nonanol (143-08-8) 1,9-nonanedithiol (3489-28-9) 2-mercaptopropionic acid (79-42-5) 2-pentylfuran (3777-69-3) 3-octen-2-one (18402-82-9) 5-methyl-2-phenyl-2-hexenal (21834-92-4) 5-phenyl-1-pentanol (10521-91-2) amyl acetate (628-63-7) diphenyl ether (101-84-8) farnesal (19317-11-4) isobornyl propionate (2756-56-1) myrcene (123-35-3) nonyl acetate (143-13-5) octyl isobutyrate (109-15-9) trans-2-hexenal (6728-26-3) trans-2-hexenoic acid (13419-69-7) α -ionone (127-41-3)	(-)-carveol (99-48-9) (-)-citronellal (5949-05-3) (+)-menthone (3391-87-5) 2,4-dimethylthiazole (541-58-2) 3-methylthio butanal (16630-52-7) 3-penten-2-one (625-33-2) allyl-2-furoate (4208-49-5) anisyl acetate (104-21-2) cis-4-decenal (21662-09-9) citronellol (106-22-9) citronellyl valerate (7540-53-6) eucalyptol (470-82-6) heptyl acetate (112-06-1) hydrocinnamaldehyde (104-53-0) phenylacetaldehyde (122-78-1) pinacol (76-09-5) prenyl acetate (1191-16-8) skatole (83-34-1) theaspirane (36431-72-8) trans-2-heptenal (18829-55-5)	(-)-dihydrocarveol (20549-47-7) (-)-menthol (2216-51-5) 1-octene-3-one (4312-99-6) 4-ethylguaiacol (2785-89-9) 4-oxoisophorone (1125-21-9) 5-methylfurfural (620-02-0) allyl mercaptan (870-23-5) benzyl butyrate (103-37-7) D-Camphor (464-49-3) diethyl succinate (123-25-1) ethyl 2-furoate (614-99-3) farnesol (4602-84-0) hydroxycitronellal (107-75-5) isoamyl acetate (123-92-2) methyl-2-nonenoate (111-79-5) p-cymene (99-87-6) p-tolyl phenylacetate (101-94-0) safranal (116-26-7) trans-2-methyl-2-pentenoic acid (16957-70-3) veratrole (91-16-7)	(S)-(-)-perillyl alcohol (18457-55-1) 1,4-cineole (470-67-7) 2-heptanone (110-43-0) 2-methyl pentanal (123-15-9) 6-methyl-5-hepten-2-one (110-93-0) allyl sulfide (592-88-1) benzyl cinnamate (103-41-3) carvacrol (499-75-2) dimethyl anthranilate (85-91-6) ethyl butyrate (105-54-4) geraniol (106-24-1) hexyl isobutyrate (2349-07-7) indole (120-72-9) isopropyl tiglate (1733-25-1) o-methoxycinnamaldehyde (1504-74-1) p-tolyl acetate (140-39-6) terpinyl formate (2153-26-6) thymol (89-83-8) trans-3-hexenoic acid (1577-18-0) γ -terpinene (99-85-4)

Supplemental Table 3.

Sequence comparisons for MORs in this study. Amino acid sequences were aligned using ClustalW. Amino acid identity (or identity + similarity) is presented as a percentage for each pair of receptor sequences.

	263-3	263-9	263-10	104-2	165-2	171-17
263-2	48 (62)	48 (62)	48 (62)	42 (62)	41 (61)	43 (61)
263-3		90 (93)	91 (94)	40 (57)	38 (55)	39 (54)
263-9			90 (93)	39 (57)	38 (54)	39 (55)
263-10				40 (58)	38 (55)	40 (55)
104-2					38 (58)	40 (61)
165-2						60 (72)

Supplementary Figure 1. Functional expression of MORs without an N-terminal extension. A, Oocytes expressing MOR31-4, MOR42-2, MOR174-9, or MOR267-13, as well as $G\alpha_{\text{off}}$ and CFTR, were challenged with 15 sec applications of 1 mM IBMX (IB) and a cognate ligand: 100 μM undecanedioic acid (UDA) for MOR31-4, 100 μM heptanoic acid (H) for MOR42-2, 100 μM eugenol (EG) for MOR174-9, 30 μM lyral (Ly) for MOR267-13. The oocyte expressing MOR267-13 was also exposed to 0.06% DMSO (D). **B)** An oocyte expressing MOR174-9, $G\alpha_{\text{off}}$ and CFTR was challenged with 100 μM butanol (B), eugenol (EG), (-)-carvone (-C), vanillin (Van), Geraniol (GE), ethyl vanillin (EV) and 1mM IBMX. **C)** Responses of MOR174-9 expressing oocytes to eugenol, vanillin and ethyl vanillin (each at 100 μM) are normalized to the response of the same oocyte to 1 mM IBMX (mean \pm SEM, $n=6-10$). **D)** An oocyte expressing MOR174-9, $G\alpha_{\text{off}}$ and CFTR was challenged with 30 μM eugenol (EG), 100 μM methylisoeugenol (MIEG), 30 μM eugenol plus 100 μM methylisoeugenol, followed by 30 μM eugenol again. Responses to eugenol in the presence of 100 μM methylisoeugenol were $36 \pm 3\%$ of the response to eugenol alone (mean \pm SEM, $n=5$). **E)** An oocyte expressing MOR42-2, $G\alpha_{\text{off}}$ and CFTR was challenged with 100 μM of monocarboxylic acids of varying carbon length (C6-C10). **F)** Responses of MOR42-2 expressing oocytes to 100 μM of monocarboxylic acids (C6-C10) are normalized to the response of the same oocyte to 100 μM octanoic acid (mean \pm SEM, $n=4$). **G)** An oocyte expressing MOR42-2, $G\alpha_{\text{off}}$ and CFTR was challenged with 100 μM of dicarboxylic acids of varying carbon length (C6-C10) and octanoic acid (OA).



Supplementary Figure 2. Concentration–response analysis of MOR263-3 activation by *trans,trans*-2,4-octadienal (ODL).

Current responses of oocytes expressing MOR263-3, $G\alpha_{\text{off}}$ and CFTR to a range of ODL concentrations were normalized to the response of the same oocyte to application of 1 μM ODL. Normalized data were then fit as described in Experimental Procedures ($EC_{50} = 21 \pm 6 \mu\text{M}$, Hill coefficient = 0.98 ± 0.17 , $n = 4$).

