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Supplemental Information

Odor Information Processing by the Olfactory Bulb Analyzed in Gene-Targeted Mice

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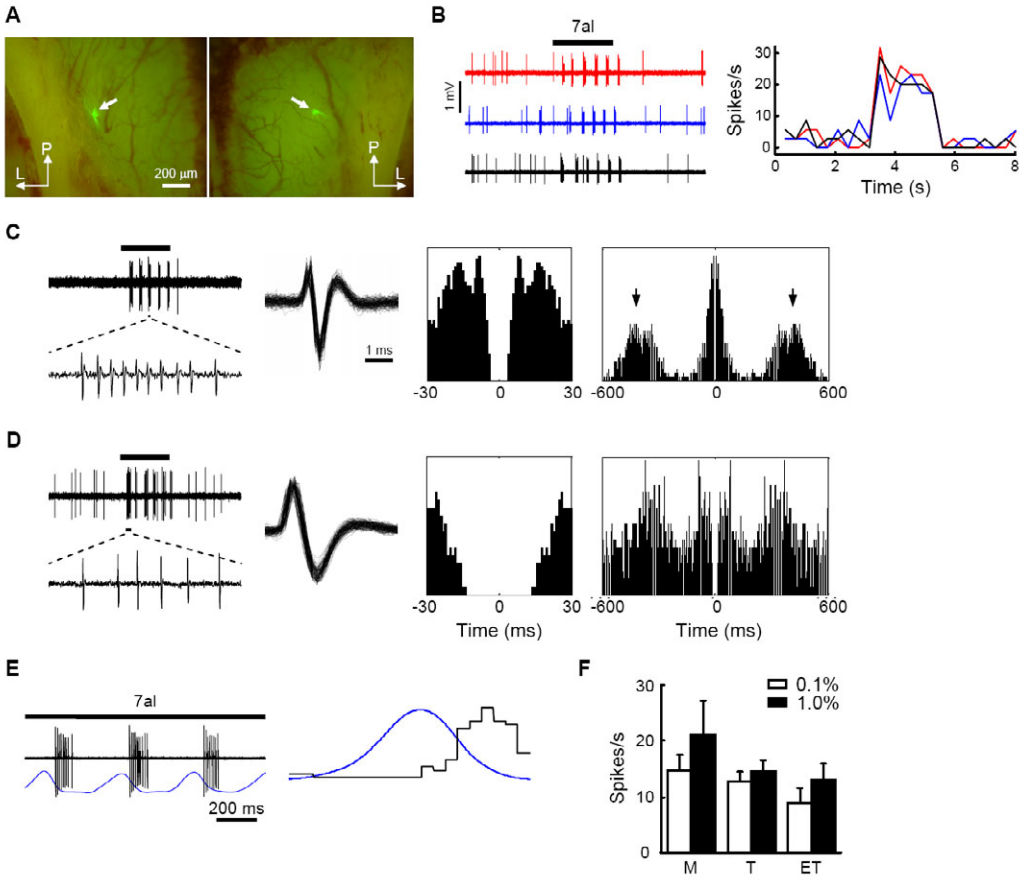

Figure S1, related to Figure 3. Methods of *in vivo* physiological recording from the MOB.
(A) An epifluorescent image shows the dorsal location of GFP-labeled I7 glomeruli (arrows) in bilateral olfactory bulbs from a mI7→M71-GFP mouse. P, posterior; L, lateral.
(B) Original physiological traces (left panel) and PSTH plots (right panel) of a mitral cell show the firing patterns evoked by 2-s heptanal application were reliable across three consecutive trials. Color codes indicate PSTHs corresponding to physiological traces of the same color.
(**C, D**) Confirmation of recordings from single-units by spike sorting and autocorrelograms of spike trains of two I7 M/T cells. Left panel, physiological traces showing the response to 2-s application of 1% (C) or 0.1% (D) heptanal. Middle panel, overlay of spike waveforms showing similar spike shape and amplitude. Right panel, autocorrelograms of spike trains at different time scales. Arrows point to the rhythm corresponding to breathing cycles.
(**E**) Heptanal-evoked firing of action potentials (black) was synchronized with respiratory rhythm (blue) in a mitral cell. The panel at right shows the average of breathing-triggered PSTH (black) and one respiratory cycle (blue), demonstrating that firing occurred during a specific phase of respiration.
(**F**) Similar response strengths of mitral, tufted and external tufted cells to heptanal at 0.1% and 1% dilutions. M, mitral cells; T, middle tufted cells; ET, external tufted cells. No significant difference was detected between the response strengths of different cell subtypes.

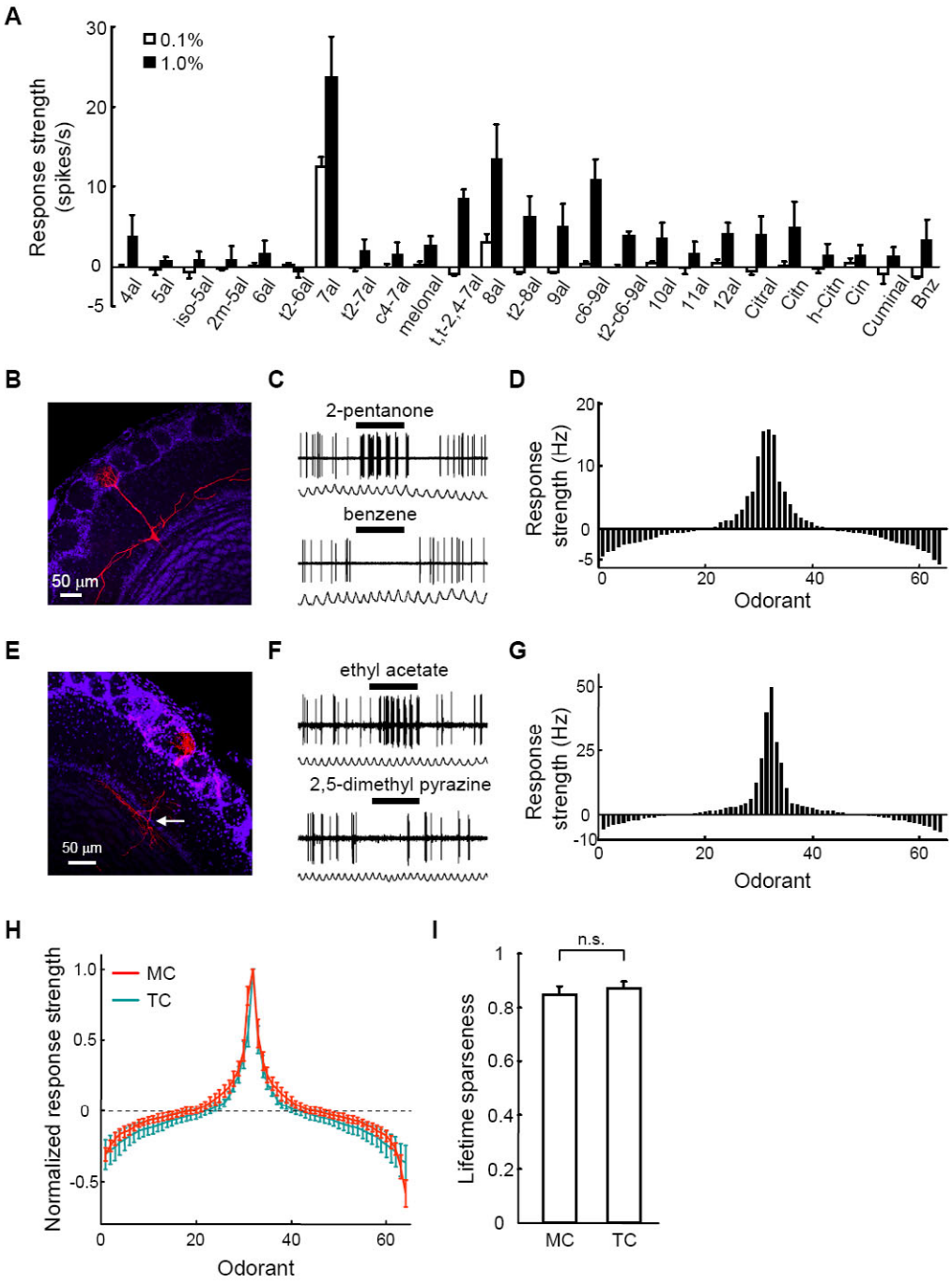

Figure S2, related to Figure 4. Both I7 and non-I7 mitral/tufted cells are narrowly tuned.
(A) Mean response strengths of I7 M/T cells to 25 aldehydes at concentrations of 0.1% and 1% dilution. Odorant names are abbreviated as: butanal, 4al; pentanal, 5al; isovaleraldehyde, iso-5al; 2-methyl pentanal; 2m-5al; hexanal, 6al; hexenal, t2-6al; trans-2-heptenal, t2-7al; trans,trans-2,4-heptandienal, t,t-2,4-7al; nonanal, 9al; trans-2,cis-6-nonadienal, t2-c6-9al; decanal, 10al; undecanal, 11al; dodecanal, 12al; (±)-citronellal, Citn; hydroxycitronellal, h-Citn; cinnamaldehyde, Cin; benzaldehyde, Bnz.
(B) The morphology of a mitral cell innervating a non-I7 glomerulus in the posterodorsal

olfactory bulb.
(C) Firing patterns of the mitral cell showing representative excitatory (upper trace) and inhibitory (lower trace) responses to 2-s odorant pulses.
(D) Tuning curve of the cell shown in (B) in response to 64 different odorants at 1% concentration.
(E) The morphology of an external tufted cell in the posterodorsal olfactory bulb. For this cell, we observed intense axonal collaterals (arrow) in the underlying granule cell layer.
(F) Physiological traces showing excitatory (upper trace) and inhibitory (lower trace) responses of the external tufted cell to 2-s pulses of two different odorants (bars).
(G) Tuning curve for the cell shown in (E).
(H) Mean tuning curves of non-I7 mitral and tufted cells (n = 8 and 9 cells, respectively) at 1% odorant dilution. MC, mitral cells; TC, tufted cells.
(I) The lifetime sparseness values of non-I7 mitral and tufted cells were not significantly different (n.s.). These two values are higher than I7 M/T cells at the same concentration, likely because of the over-representation of aldehydes for testing the responses of I7 cells.

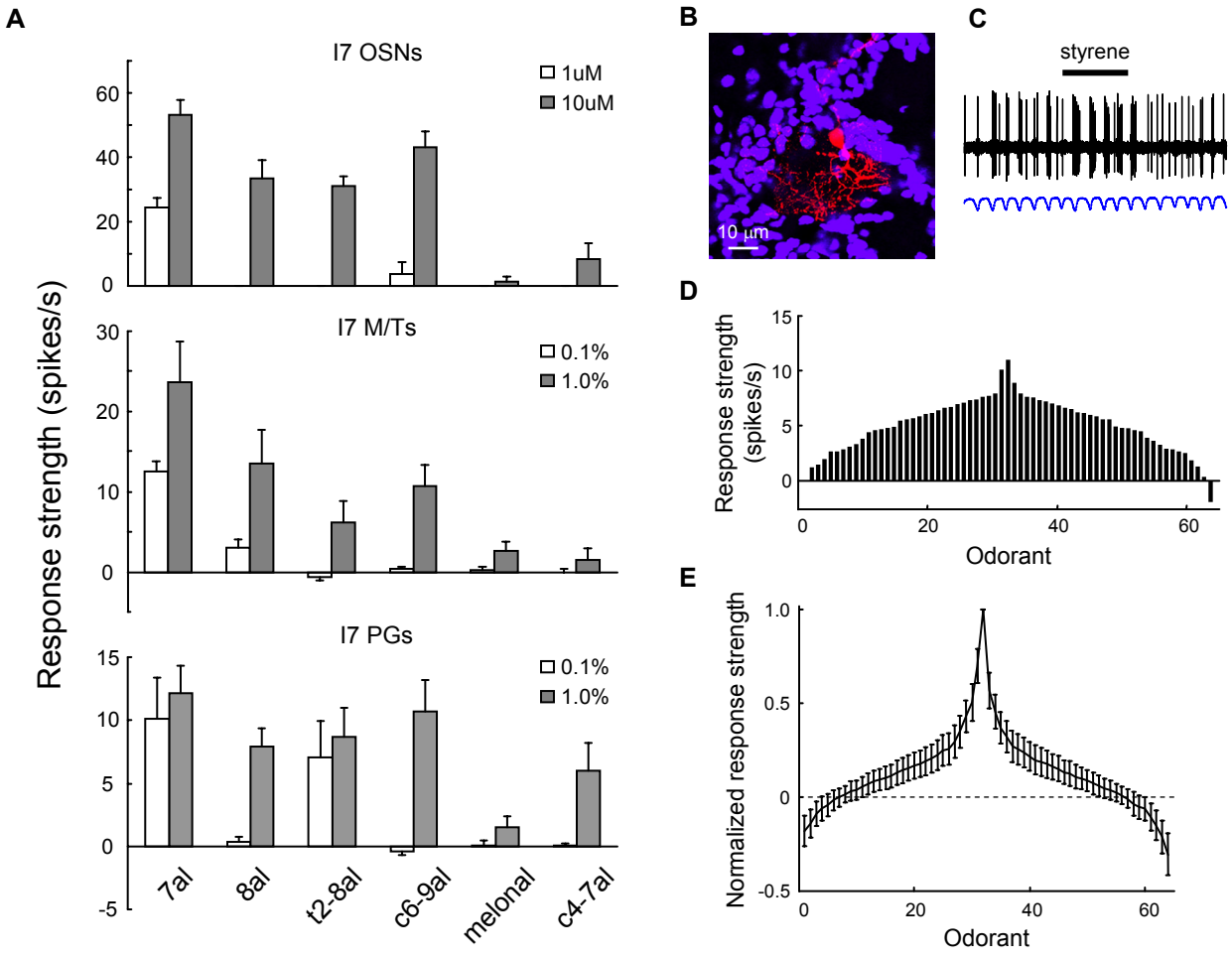

Figure S3, related to Figure 6. PG cells are broadly tuned.

(A) Comparison of the response profiles of I7 OSNs, I7 M/T cells and I7 PG cells to the same set of aldehydes at two concentrations.

(B) The morphology of a non-I7 PG cell. It extended its dendritic tuft into one glomerulus and axons to other glomeruli.

(C) Physiological trace showing an excitatory response of the cell to 2-s application of styrene.

(D) Tuning curve of the same PG cell as shown in (B). Note that most of the responses were excitatory.

(E) Mean tuning curve of all non-I7 PG cells (n = 8 cells).

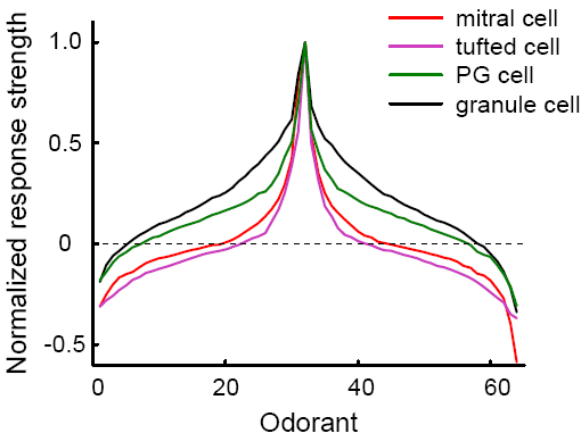

Figure S4, related to Figure 7. Comparison of the olfactory tuning of granule cells to those of non-I7 mitral cells, tufted cells, and PG cells. Odorant identities were aligned specifically for each experimental group so that each curve is smooth with maximal excitatory response in the middle and weak or inhibitory responses at the two sides.

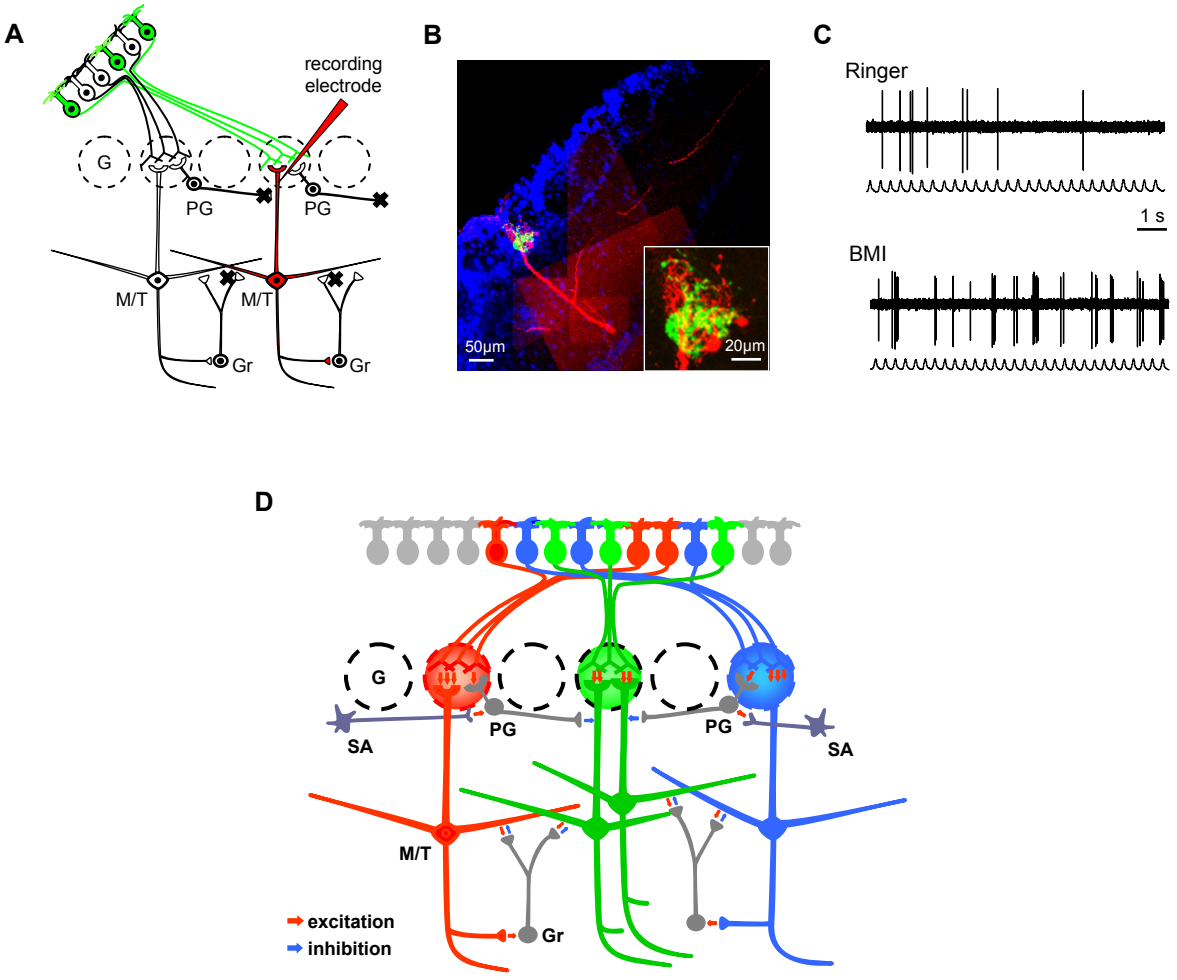

Figure S5, related to Figure 8. Method of *in vivo* BMI applications and our hypothesis on the function of intrabulbar circuitry.

(A) Schematic diagram showing the recording site and BMI targets.

(B) The morphology of an I7 mitral cell. Inset shows the enlarged dendritic tufts within the GFP glomerulus.

(C) Spontaneous firing patterns of the cell in (B) before (upper panel) and after (lower panel) BMI application.

(D) A schematic diagram illustrating our hypothesis on the function of intrabulbar circuitry. In addition to convergent excitatory input from OSNs, M/T cells interact laterally via their connections with PG cells in the glomerular layer and granule cells in the granule cell layer. Individual M/T cells innervating the same glomerulus (green) may interact with different subsets of PG cells and granule cells. In addition, individual PG cells may receive input from distinct short-axon cells. These differences in lateral connections may result in the response heterogeneity of iso-glomerular M/T cells to odorants at high concentrations. For simplicity, PG cells associated with the green glomerulus are not shown.

heptanal 1.89	octanal 0.80	trans-2-heptenal 0.59	pentanal 13.98	cis-6-nonenal 0.11	melonal 0.29	cis-4-heptenal 1.02	
air	2-methoxy-4-methylphenol 0.01	(-)-fenchone 0.005	2-pentanone 14.52	2-acetylphenol 0.11	(-)-carvone 0.22	(-)-menthone 0.27	geranyl acetone 0.008
butyl propionate 2.38	phenyl acetate 0.19	triethyl citrate 0.38	2-phenethyl acetate 0.02	butyro phenone 0.05	benzyl alcohol 0.08	isobutyl Isobutyrate 2.33	butyric acid 0.23
toluene 15.27	3-heptanone 1.40	2-hexanol 1.34	2'-amino acetophenone 0.005	2',4'-dimethyl acetophenone 0.04	n-amyl acetate 2.15	linalool 0.09	styrene 2.69
benzyl ether 0.001	2-hexanone 6.45	benzene 40.11	mineral oil	propio phenone 0.09	propyl acetate 13.44	acetophenone 0.22	2-butanone 38.17
ethyl vanillin 0.005	2-heptanone 1.15	2,5-dimethyl pyrazine 0.81	ethyl propionate 19.30	3-octanol 0.54	ethyl acetate 50.11	geraniol 0.11	(-)-isopulegol 0.003
anisole 1.90	3-decanone 0.24	octanoic acid 0.002	l-carvcol 0.007	trans-anethole 0.03	ethanol 23.98	hydroxy citronellal 0.003	butanal 48.39
cinnam aldehyde 0.02	citral 0.11	(±)-citronellal 0.15	trans-2-octenal 0.46	nonanal 0.14	decanal 0.08	hexanal 5.38	trans-2, cis-6-nonadienal 0.12
dodecanal 0.02	isovaler aldehyde 16.13	2-methyl pentanal 9.62	trans-2-hexenal 5.38	benzaldehyde 0.54	undecanal 0.03	trans,trans-2,4-heptandienal 0.32	cuminal 0.03

Table S1, related to Figure 3. The identity and molar concentrations of 69 odorants used in this study. The odorants contain acetate, aldehyde, acid, ketone, alcohol, ether, and aromatic functional groups. Some belong to presumptive mouse pheromones. Color codes for at least one of the functional groups of individual odorants, but some odorants can have multiple functional groups. The molar concentration of each odorant at 1% dilution (in μM) is shown below the odorant name.

odor #	M/T cells in Fig 4A-C	PG cells in Fig 6D, E
1	mineral oil	melonal
2	cuminal	air
3	cinnamaldehyde	pentanal
4	toluene	cuminal
5	octanoic acid	toluene
6	ethanol	octanoic acid
7	undecanal	n-amyl acetate
8	ethyl acetate	2-methoxy-4-methylphenol
9	2-methylpentanal	2-butanone
10	hexanal	2'-aminoacetophenone
11	ethyl propionate	benzyl alcohol
12	(-)-isopulegol	ethyl acetate
13	n-amyl acetate	anisole
14	benzaldehyde	l-carveol
15	trans-anethole	benzaldehyde
16	decanal	2-acetylphenol
17	linalool	ethanol
18	trans-2,cis-6-nonadienal	3-heptanone
19	2-butanone	trans-anethole
20	propyl acetate	cis-4-heptenal
21	styrene	hydroxycitronellal
22	nonanal	2-pentanone
23	butyric acid	2-heptanone
24	trans-2-heptenal	isobutyl isobutyrate
25	acetophenone	geranyl acetone
26	butyrophenone	2-phenethyl acetate
27	3-decanone	3-octanol
28	triethyl citrate	linalool
29	propiophenone	phenyl acetate
30	trans,trans-2,4-heptandienal	(-)-carvone
31	2-phenethyl acetate	(-)-isopulegol
32	phenyl acetate	butanal
33	3-heptanone	butyrophenone
34	2-pentanone	trans-2-octenal
35	octanal	cis-6-nonenal
36	heptanal	heptanal
37	cis-6-nonenal	citral
38	(-)- menthone	2',4'-dimethylacetophenone

39	2-acetylphenol	propiophenone
40	isobutyl isobutyrate	octanal
41	2-hexanone	(±)-citronellal
42	geranyl acetone	acetophenone
43	trans-2-octenal	nonanal
44	butyl propionate	2-hexanol
45	citral	(-)-menthone
46	(-)-carvone	triethyl citrate
47	2,5-dimethyl pyrazine	2,5-dimethyl pyrazine
48	2-heptanone	ethyl vanillin
49	2,4-dimethylacetophenone	hexanal
50	2-hexanol	3-decanone
51	(-)-fenchone	2-hexanone
52	dodecanal	benzyl ether
53	3-octanol	undecanal
54	(±)- citronellal	geraniol
55	melonal	butyric acid
56	ethyl vanillin	trans,trans-2,4-heptandienal
57	benzyl alcohol	cinnamaldehyde
58	anisole	styrene
59	butanal	(-)-fenchone
60	geraniol	decanal
61	air	butyl propionate
62	pentanal	trans-2,cis-6-nonadienal
63	2-aminoacetophenone	dodecanal
64	benzyl ether	2-methylpentanal
65	cis-4-heptenal	ethyl propionate
66	isovaleraldehyde	hexenal
67	l-carveol	propyl acetate
68	2-methoxy-4-methylphenol	trans-2-heptenal
69	hydroxycitronellal	isovaleraldehyde
70	benzene	benzene
71	trans-2-hexenal	mineral oil

Table S2, related to Figures 4 & 6. Alignment of odorant identity for plotting the tuning curves of I7 M/T cells and PG cells. Two controls (air and mineral oil at the same flow rate) were included. These two different alignments were used for plots in Fig 4A-C and Fig 6D, E.