Supplementary Information

Supersensitive detection and discrimination of enantiomers by dorsal olfactory receptors: evidence for hierarchical odour coding

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Other Supplementary Information for this manuscript includes the following:
(available at www.nature.com/scientificreports/)

Video S1. Odour setting and correct odour choice behaviour in the Y-maze.

Figure S1. A schematic diagram of the Y-maze. Two alternative forced choice assays, with mice choosing between odourant and solvent solutions, or between pairs of enantiomeric odourant solutions, were conducted in a Y-maze. Cotton balls moistened with diluted odourants were placed at the inlet ports of the maze arms. Odour vapour flows were guided into the Y-maze at the centres of the cavities by weak negative pressure. Flow rate at inlet port was adjusted to 0.5 L/min by regulating leakage through a 3-way valve. Runs to the target odour source (correct odour choice) against the (+)-enantiomers or the solvent were rewarded with a drop of water. Rewarded side was randomly switched by independently exchanging the terminal caps and funnels between the arms. A mouse was picked up in the start cup and set in the Y-maze to initiate a trial running to the end of one arm. When the mouse chose the wrong side, both terminal caps were removed to prevent the mouse from drinking water from the funnel. Each mouse ran and chose one of the two odours 18 to 24 times per day. The sums of the initial 18 trials for 6 mice of the same strain were statistically analysed.

Figure S2. Odour responses of the most sensitive OSNs for wine lactones in mice. Odour responses were measured using intracellular Ca^{2+}-imaging. A, OSN tuned to 10 nM (–)-wnl. B, OSN tuned to 10 nM (–)-wnl and (+)-wnl. C, OSN tuned to 1 μM (+)-wnl. D and E, OSNs tuned to 1 μM (–)-wnl. (–)-ro: (2*S*,4*R*)-(–)-cis-rose oxide; (+)-ro: (2*R*,4*S*)-(+)-cis-rose oxide. The arrows indicate the time at which odourant solutions of the indicated concentrations were applied to the OSNs for 4 s. The odourants that evoked characteristic odour responses are indicated by the asterisks. All OSNs, except for that shown in E, were obtained from the dorsal zone.

Figure S3. Dose-dependency of the peak areas of diluted odourant solutions in gas chromatography-mass spectrometry (GC-MS). The peak area per unit dose seemed too large for the 10^{-9}-w/w odourant solution compared with those at higher concentrations. This was likely due to background noise. This result suggests that the detection limit of this GC-MS measurement is approximately 10 ppb for (*R*)-(–)-carvone. The theoretical curve (dashed line) is the plot of the peak areas at the concentrations (C_x) with the correction by the term -(peak_area_at_10^{-9}) + (peak_area_at_$10^{-3} \times C_x/10^{-3}$).

Table ST1. COCRs of WT and ΔD mice in a Y-maze for serial 100-fold diluted odourants.

Table ST2. Dose-dependent subpopulations of wine lactone-responsive OSNs/ORs. Of 1,746 olfactory sensory neurons (OSNs) (1,207 dorsal zone: 539 ventral zone) assayed by

Ca^{2+} imaging, 2, 5, 17 and 46 OSNs/ORs responded to either or both wine lactone enantiomers at 10-100 nM, 1, 10 and 10 μM, respectively. They were classified by their tuning to target enantiomers of wine lactones. Target-best OSNs/ORs* are those more sensitive to the target enantiomer than to the non-target enantiomer. Percentage of each class responsive to the target at a given concentration is listed in the lower row. OR overlap indicates percentage (numbers) of overlapping OSN/OR in enantiomer OR codes at each concentration. (–)-wnl, (–)-wine lactone; (+)-wnl, (+)-wine lactone.

Table ST3. Dose-dependent subpopulations of carvone-responsive OSNs/ORs. Of 2,740 OSNs/ORs assayed by Ca^{2+} imaging, 13 and 89 OSNs/ORs (dorsal zone: ventral zone) responded to either or both carvone enantiomers at 1 and 10 μM, respectively, and were classified by their tuning to target odourants. Target-best OSNs/ORs* are those more sensitive to the target enantiomer than to the non-target enantiomer. Percentage of each class responsive to the target at a given concentration is listed in the lower row. The percentage† indicates OSNs/ORs with response amplitudes less than the half of the others. The percentages in parentheses indicate OSNs/ORs that were prepared from uncertain zones and counted in each zone by a factor of 1/2. OR overlap indicates percentage (numbers) of overlapping OSN/OR in enantiomer OR codes at each concentration. The largest class‡ is the sum of (+)-car and (+)-car/others OSNs/ORs. In the lower part, relative response amplitudes of 15 identified carvone ORs are shown with the lowest responsive concentrations (μM) for target (left column) and non-target carvone in parentheses (Fig. 3).

Table ST4. Significance of the COCR differences between WT and ΔD mice.

Table ST5. Peak areas of GC-MS and calculation of the dilution factors for the 100-fold dilution series. The dilution factors were obtained as the ratios of peak areas per unit dose between subsequent concentrations. (*R*)-(−)-carvone was used as the standard odourant. The dose-dependency of the peak areas was shown in Supplementary Information Fig. S3.

Table ST6. Estimated odourant concentrations. Averaged dilution factor, $D_{avg} = 1.0109 \pm 0.0515$, and the maximum dilution factor, $D_{max} = 1.21$, are shown in Supplementary Information Table ST5.

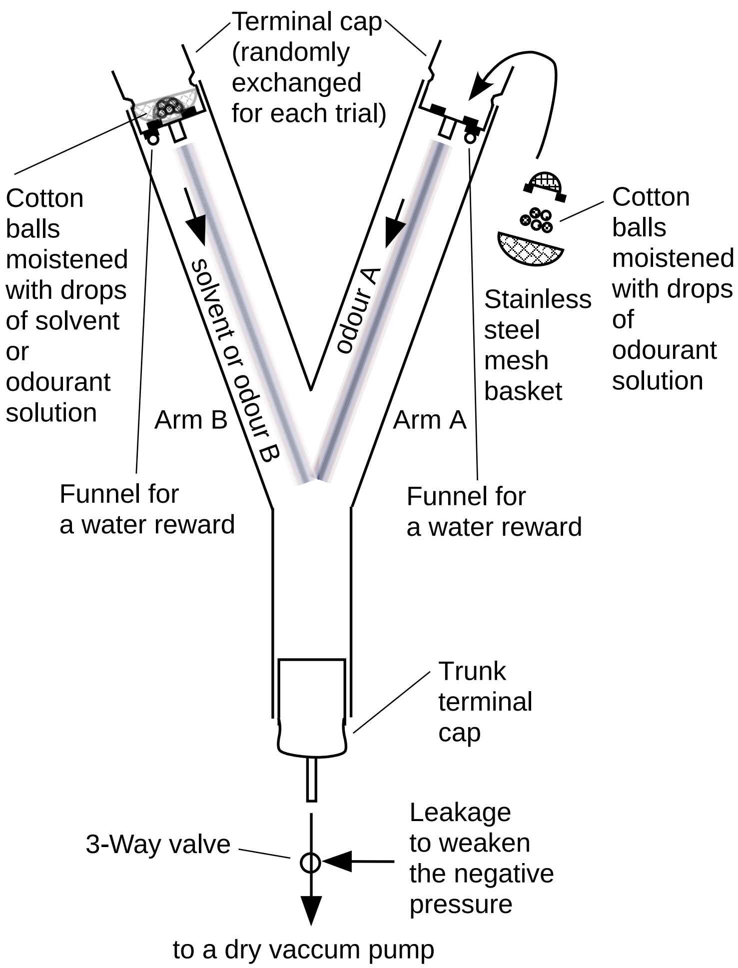

Figure S1. A schematic diagram of the Y-maze. Two alternative forced choice assays, with mice choosing between odourant and solvent solutions, or between pairs of enantiomeric odourant solutions, were conducted in a Y-maze. Cotton balls moistened with diluted odourants were placed at the inlet ports of the maze arms. Odour vapour flows were guided into the Y-maze at the centres of the cavities by weak negative pressure. Flow rate at inlet port was adjusted to 0.5 L/min by regulating leakage through a 3-way valve. Runs to the target odour source (correct odour choice) vs. the (+)-enantiomers or the solvent were rewarded with a drop of water. Rewarded side was randomly switched by independently exchanging the terminal caps and funnels between the arms. A mouse was picked up in the trunk terminal cap and set in the Y-maze to initiate a trial running to the end of one arm. When the mouse chose the wrong side, both terminal caps were removed to prevent the mouse from drinking water from the funnel. Each mouse ran and chose one of the two odours 18 to 24 times per day. Sums of initial 18 trials for 6 mice were statistically analysed.

Odourant pairs	COCRs of individual WT mice (%)						avg. of 3 mice (wt1-3)	S.E.M.	avg. of 3 mice (wt4-6)	S.E.M.	avg. of 6 WT mice	S.E.M.	COCR for P = 0.05
	wt1	wt2	wt3	wt4	wt5	wt6							
(-)-wnl vs. solvent													
10^{-3} (-)-wnl vs. solvent	100.0	72.2	88.9	77.8	83.3	100.0	87.0	8.1	87.0	6.7	87.0	4.7	59.4
10^{-5} (-)-wnl vs. solvent	77.8	77.8	83.3	77.8	77.8	72.2	79.6	1.9	75.9	1.9	77.8	1.4	59.4
10^{-7} (-)-wnl vs. solvent	88.9	66.7	66.7	83.3	66.7	61.1	74.1	7.4	70.4	6.7	72.2	4.5	59.4
10^{-9} (-)-wnl vs. solvent	77.8	72.2	61.1	83.3	72.2	77.8	70.4	4.9	77.8	3.2	74.1	3.1	59.4
10^{-11} (-)-wnl vs. solvent	72.2	66.7	72.2	88.9	88.9	88.9	70.4	1.9	88.9	0.0	79.6	4.2	59.4
10^{-13} (-)-wnl vs. solvent	72.2	66.7	72.2	94.4	83.3	88.9	70.4	1.9	88.9	3.2	79.6	4.5	59.4
10^{-15} (-)-wnl vs. solvent	83.3	77.8	72.2	77.8	83.3	83.3	77.8	3.2	81.5	1.9	79.6	1.9	59.4
10^{-17} (-)-wnl vs. solvent	66.7	72.2	72.2	83.3	83.3	77.8	70.4	1.9	81.5	1.9	75.9	2.7	59.4
10^{-19} (-)-wnl vs. solvent	77.8	61.1	61.1	77.8	72.2	77.8	66.7	5.6	75.9	1.9	71.3	3.3	59.4
10^{-21} (-)-wnl vs. solvent	66.7	77.8	61.1	72.2	61.1	66.7	68.5	4.9	66.7	3.2	67.6	2.7	59.4
10^{-23} (-)-wnl vs. solvent	61.1	61.1	44.4	66.7	61.1	61.1	55.6	5.6	63.0	1.9	59.3	3.1	59.4
10^{-25} (-)-wnl vs. solvent	55.6	44.4	55.6	50.0	44.4	66.7	51.9	3.7	53.7	6.7	52.8	3.4	59.4
PO assays													
10^{-13} (-)-wnl vs. solvent	66.7	55.6	83.3	72.2	72.2	83.3	68.5	8.1	75.9	3.7	72.2	4.3	59.4
10^{-13} (-)-wnl vs. (-)-wnl	55.6	50.0	61.1	44.4	50.0	50.0	55.6	3.2	48.1	1.9	51.9	2.3	59.4
(+)-wnl vs. solvent													
10^{-3} (+)-wnl vs. solvent	94.4	77.8	66.7	75.0	75.0	70.8	79.6	8.1	73.6	1.4	76.6	3.9	59.4
10^{-5} (+)-wnl vs. solvent	66.7	66.7	77.8	83.3	83.3	83.3	72.2	5.6	83.3	0.0	77.8	3.5	59.4
10^{-7} (+)-wnl vs. solvent	83.3	77.8	66.7	75.0	83.3	79.2	72.2	8.5	79.2	2.4	75.7	4.2	59.4
10^{-9} (+)-wnl vs. solvent	77.8	88.9	88.9	79.2	79.2	95.8	81.5	3.7	84.7	5.6	83.1	3.1	59.4
10^{-11} (+)-wnl vs. solvent	72.2	77.8	55.6	79.2	79.2	83.3	68.5	6.7	80.6	1.4	74.5	4.1	59.4
10^{-13} (+)-wnl vs. solvent	88.9	88.9	77.8	75.0	83.3	83.3	85.2	3.7	80.6	2.8	82.9	2.3	59.4
10^{-15} (+)-wnl vs. solvent	83.3	77.8	77.8	75.0	79.2	75.0	74.1	6.7	76.4	1.4	75.2	3.1	59.4
10^{-17} (+)-wnl vs. solvent	83.3	77.8	77.8	75.0	62.5	75.0	79.6	1.9	70.8	4.2	75.2	2.8	59.4
10^{-19} (+)-wnl vs. solvent	77.8	83.3	88.9	66.7	75.0	66.7	79.6	1.9	69.4	2.8	74.5	2.7	59.4
10^{-21} (+)-wnl vs. solvent	50.0	50.0	33.3	62.5	58.3	58.3	44.4	5.6	59.7	1.4	52.1	4.3	59.4
10^{-23} (+)-wnl vs. solvent				58.3	50.0	58.3			55.6	2.8			63.3
PO assays													
10^{-19} (+)-wnl vs. solvent	72.2	72.2	77.8	77.8	77.8	66.7	70.4	1.9	74.1	3.7	72.2	2.0	59.4
10^{-19} (+)-wnl vs. (+)-wnl	50.0	38.9	55.6	33.3	33.3	66.7	46.3	3.7	44.4	11.1	45.4	5.3	59.4
(-)-wnl vs. (+)-wnl													
10^{-3} (-)-wnl vs. (+)-wnl	55.6	77.8	66.7	72.2	66.7	77.8	66.7	6.4	72.2	3.2	69.4	3.4	59.4
10^{-5} (-)-wnl vs. (+)-wnl	55.6	55.6	66.7	46.7	40.0	61.1	59.3	3.7	49.3	6.2	54.3	3.9	59.4
10^{-7} (-)-wnl vs. (+)-wnl	83.3	88.9	77.8	55.6	55.6	77.8	83.3	3.2	63.0	7.4	73.1	5.8	59.4
10^{-9} (-)-wnl vs. (+)-wnl	72.2	66.7	61.1	61.1	72.2	77.8	66.7	3.2	70.4	4.9	68.5	2.7	59.4
10^{-11} (-)-wnl vs. (+)-wnl	77.8	61.1	55.6	50.0	61.1	66.7	64.8	6.7	59.3	4.9	62.0	3.9	59.4
10^{-13} (-)-wnl vs. (+)-wnl	88.9	88.9	66.7	61.1	61.1	66.7	81.5	7.4	63.0	1.9	72.2	5.4	59.4
10^{-15} (-)-wnl vs. (+)-wnl	72.2	66.7	55.6	66.7	83.3	61.1	64.8	4.9	70.4	6.7	67.6	3.9	59.4
10^{-17} (-)-wnl vs. (+)-wnl	44.4	55.6	50.0	77.8	83.3	55.6	50.0	3.2	72.2	8.5	61.1	6.4	59.4
10^{-19} (-)-wnl vs. (+)-wnl	50.0	61.1	55.6	66.7	61.1	50.0	55.6	3.2	59.3	4.9	57.4	2.7	59.4
10^{-21} (-)-wnl vs. (+)-wnl	50.0	55.6	72.2	66.7	61.1	44.4	59.3	6.7	57.4	6.7	58.3	4.2	59.4
10^{-23} (-)-wnl vs. (+)-wnl	66.7	55.6	44.4	55.6	55.6	44.4	55.6	6.4	51.9	3.7	53.7	3.4	59.4
PO assays													
10^{-13} (-)-wnl vs. (+)-wnl	72.2	77.8	94.4	66.7	61.1	72.2	81.5	6.7	66.7	3.2	74.1	4.7	59.4
10^{-13} (-)-wnl vs. solvent	83.3	83.3	66.7	88.9	94.4	88.9	79.2	4.2	90.7	1.9	84.3	3.9	59.4
(-)-car vs. solvent													
10^{-3} (-)-car vs. solvent	83.3	77.8	83.3	72.2	100.0	72.2	81.5	1.9	81.5	9.3	81.5	4.2	59.4
10^{-5} (-)-car vs. solvent	77.8	83.3	83.3	72.2	94.4	83.3	81.5	1.9	83.3	6.4	82.4	3.0	59.4
10^{-7} (-)-car vs. solvent	75.0	75.0	79.2	66.7	61.1	77.8	76.4	1.4	68.5	4.9	72.5	2.9	59.4
10^{-9} (-)-car vs. solvent	55.6	66.7	61.1	83.3	66.7	72.2	61.1	3.2	74.1	4.9	67.6	3.9	59.4
10^{-11} (-)-car vs. solvent	50.0	72.2	72.2	83.3	94.4	83.3	64.8	7.4	87.0	3.7	75.9	6.2	59.4
10^{-13} (-)-car vs. solvent	61.1	61.1	72.2	83.3	88.9	88.9	64.8	3.7	87.0	1.9	75.9	5.3	59.4
10^{-15} (-)-car vs. solvent	77.8	66.7	72.2	77.8	61.1	77.8	72.2	3.2	72.2	5.6	72.2	2.9	59.4
10^{-17} (-)-car vs. solvent	66.7	72.2	61.1	88.9	77.8	77.8	66.7	3.2	81.5	3.7	74.1	4.0	59.4
10^{-19} (-)-car vs. solvent	72.2	72.2	72.2	66.7	61.1	61.1	72.2	0.0	63.0	1.9	67.6	2.2	59.4
10^{-21} (-)-car vs. solvent	66.7	61.1	55.6	55.6	44.4	55.6	61.1	3.2	51.9	3.7	56.5	3.0	59.4
10^{-23} (-)-car vs. solvent	61.1	61.1	61.1	50.0	55.6	55.6	61.1	0.0	53.7	1.9	57.4	1.9	59.4
10^{-25} (-)-car vs. solvent	55.6	61.1	55.6	61.1	38.9	44.4	57.4	1.9	48.1	6.7	52.8	3.7	59.4
PO assays													
10^{-11} (-)-car vs. solvent	72.2	77.8	72.2	70.8	79.2	70.8	74.1	1.9	73.6	2.8	74.1	1.9	59.4
10^{-11} (-)-car vs. (-)-car	44.4	61.1	44.4	38.9	61.1	44.4	50.0	5.6	48.1	6.7	50.0	5.6	59.4

Table ST1. COCRs of WT and ΔD mice in a Y-maze for serial 100-fold diluted odourants.

Odourant pairs	COCRs of individual WT mice (%)						avg. of 3 mice (wt1-3)	S.E.M.	avg. of 3 mice (wt4-6)	S.E.M.	avg. of 6 WT mice	S.E.M.	COCR for *P* = 0.05
	wt1	wt2	wt3	wt4	wt5	wt6							
(+)-car vs. solvent													
10^{-3} (+)-car vs. solvent	72.2	72.2	72.2	94.4	83.3	83.3	72.2	0.0	87.0	3.7	79.6	3.7	59.4
10^{-5} (+)-car vs. solvent	83.3	66.7	72.2	88.9	77.8	88.9	74.1	4.9	85.2	3.7	79.6	3.7	59.4
10^{-7} (+)-car vs. solvent	77.8	77.8	88.9	77.8	72.2	83.3	81.5	3.7	77.8	3.2	79.6	2.3	59.4
10^{-9} (+)-car vs. solvent	72.2	61.1	66.7	66.7	61.1	61.1	66.7	3.2	63.0	1.9	64.8	1.9	59.4
10^{-11} (+)-car vs. solvent	66.7	77.8	77.8	66.7	61.1	77.8	74.1	3.7	68.5	4.9	71.3	3.0	59.4
10^{-13} (+)-car vs. solvent	83.3	77.8	66.7	66.7	72.2	66.7	75.9	4.9	68.5	1.9	72.2	2.9	59.4
10^{-15} (+)-car vs. solvent	72.2	77.8	88.9	61.1	66.7	66.7	79.6	4.9	64.8	1.9	72.2	4.1	59.4
10^{-17} (+)-car vs. solvent	61.1	72.2	77.8	61.1	61.1	55.6	70.4	4.9	59.3	1.9	64.8	3.4	59.4
10^{-19} (+)-car vs. solvent	55.6	55.6	61.1	55.6	55.6	61.1	57.4	1.9	57.4	1.9	57.4	1.2	59.4
10^{-21} (+)-car vs. solvent	61.1	61.1	50.0	61.1	66.7	61.1	57.4	3.7	63.0	1.9	60.2	2.2	59.4
10^{-23} (+)-car vs. solvent	50.0	50.0	55.6	55.6	50.0	55.6	51.9	1.9	53.7	1.9	52.8	1.2	59.4
PO assays													
10^{-5} (-)-car vs. solvent	66.7	66.7	77.8	83.3	83.3	77.8	70.4	3.7	81.5	1.9	75.9	3.1	59.4
(-)-car vs. (+)-car													
10^{-3} (-)-car vs. (+)-car	72.2	94.4	72.2	77.8	83.3	66.7	79.6	7.4	75.9	4.9	77.8	4.1	59.4
10^{-5} (-)-car vs. (+)-car	72.2	66.7	83.3	72.2	66.7	77.8	74.1	4.9	72.2	3.2	73.1	2.7	59.4
10^{-7} (-)-car vs. (+)-car	66.7	72.2	66.7	83.3	72.2	88.9	68.5	1.9	81.5	4.9	75.0	3.7	59.4
10^{-9} (-)-car vs. (+)-car	61.1	61.1	72.2	66.7	61.1	72.2	64.8	3.7	66.7	3.2	65.7	2.2	59.4
10^{-11} (-)-car vs. (+)-car	66.7	77.8	83.3	61.1	66.7	61.1	75.9	4.9	63.0	1.9	69.4	3.7	59.4
10^{-13} (-)-car vs. (+)-car	55.6	61.1	55.6	72.2	50.0	77.8	57.4	1.9	66.7	8.5	62.0	4.4	59.4
10^{-15} (-)-car vs. (+)-car	66.7	66.7	66.7	66.7	55.6	88.9	66.7	0.0	70.4	9.8	68.5	4.5	59.4
10^{-17} (-)-car vs. (+)-car	72.2	61.1	72.2	61.1	55.6	77.8	68.5	3.7	64.8	6.7	66.7	3.5	59.4
10^{-19} (-)-car vs. (+)-car	61.1	61.1	66.7	61.1	55.6	61.1	63.0	1.9	59.3	1.9	61.1	1.4	59.4
10^{-21} (-)-car vs. (+)-car	44.4	50.0	61.1	55.6	50.0	61.1	51.9	4.9	55.6	3.2	53.7	2.7	59.4
10^{-23} (-)-car vs. (+)-car	55.6	44.4	61.1	44.4	55.6	55.6	53.7	4.9	51.9	3.7	52.8	2.8	59.4
PO assays													
10^{-5} (-)-car vs. solvent	94.4	66.7	83.3	88.9	66.7	83.3	81.5	8.1	79.6	6.7	80.6	4.7	59.4
nTMT vs dpg													
10^{-9} nTMT vs. solvent	94.4	61.1	50.0	66.7	66.7	72.2	68.5	13.4	68.5	1.9	68.5	6.0	59.4
10^{-11} nTMT vs. solvent	66.7	66.7	55.6	66.7	72.2	77.8	63.0	3.7	72.2	3.2	67.6	3.0	59.4
10^{-13} nTMT vs. solvent	83.3	72.2	61.1	83.3	83.3	88.9	72.2	6.4	85.2	1.9	78.7	4.2	59.4
10^{-15} nTMT vs. solvent	83.3	72.2	61.1	77.8	66.7	94.4	72.2	6.4	79.6	8.1	75.9	4.9	59.4
10^{-17} nTMT vs. solvent	77.8	77.8	66.7	61.1	55.6	72.2	74.1	3.7	63.0	4.9	68.5	3.7	59.4
10^{-19} nTMT vs. solvent	66.7	66.7	61.1	66.7	66.7	72.2	64.8	1.9	68.5	1.9	66.7	1.4	59.4
10^{-21} nTMT vs. solvent	88.9	55.6	55.6	72.2	50.0	61.1	66.7	11.1	61.1	6.4	63.9	5.9	59.4
10^{-23} nTMT vs. solvent	66.7	61.1	55.6	55.6	44.4	50.0	61.1	3.2	50.0	3.2	55.6	3.2	59.4
PO assays													
10^{-21} nTMT vs. solvent	72.2	66.7	61.1	61.1	72.2	61.1	66.7	3.2	64.8	3.7	65.7	2.2	59.4

Table ST1. COCRs of WT and ΔD mice in a Y-maze for serial 100-fold diluted odourants (continued).

Odourant pairs	COCRs of individual ΔD mice (%)						avg. of 3 mice (ΔD1-3)	S.E.M.	avg. of 3 mice (ΔD4-6)	S.E.M.	avg. of 6 ΔD mice	S.E.M.	COCR for *P* = 0.05
	ΔD1	ΔD2	ΔD3	ΔD4	ΔD5	ΔD6							
(-)-wnl vs. solvent													
10^{-3} (-)-wnl vs. solvent	66.7	87.5	91.7	61.1	61.1	61.1	81.9	7.7	61.1	0.0	71.5	5.8	59.4
10^{-5} (-)-wnl vs. solvent	61.1	83.3	77.8	61.1	61.1	61.1	74.1	6.7	61.1	0.0	67.6	4.2	59.4
10^{-7} (-)-wnl vs. solvent	77.8	66.7	66.7	75.0	91.7	83.3	70.4	3.7	83.3	4.8	76.9	4.0	59.4
10^{-9} (-)-wnl vs. solvent	77.8	66.7	77.8	72.2	72.2	83.3	74.1	3.7	75.9	3.7	75.0	2.4	59.4
10^{-11} (-)-wnl vs. solvent	83.3	77.8	77.8	77.8	72.2	66.7	79.6	1.9	72.2	3.2	75.9	2.3	59.4
10^{-13} (-)-wnl vs. solvent	66.7	72.2	72.2	72.2	66.7	61.1	70.4	1.9	66.7	3.2	68.5	1.9	59.4
10^{-15} (-)-wnl vs. solvent	61.1	66.7	61.1	66.7	61.1	66.7	63.0	1.9	64.8	1.9	63.9	1.2	59.4
10^{-17} (-)-wnl vs. solvent	66.7	61.1	50.0	66.7	61.1	66.7	59.3	4.9	64.8	1.9	62.0	2.7	59.4
10^{-19} (-)-wnl vs. solvent	55.6	55.6	72.2	55.6	61.1	66.7	61.1	5.6	61.1	3.2	61.1	2.9	59.4
10^{-21} (-)-wnl vs. solvent	50.0	50.0	33.3	38.9	50.0	61.1	44.4	5.6	50.0	6.4	47.2	4.0	59.4
10^{-23} (-)-wnl vs. solvent	38.9	55.6	44.4	38.9	55.6	50.0	46.3	4.9	48.1	4.9	47.2	3.1	59.4
PO assays													
10^{-13} (-)-wnl vs. solvent	72.2	66.7	77.8	77.8	66.7	61.1	72.2	3.2	68.5	4.9	71.3	3.3	59.4
10^{-13} (-)-wnl vs. (-)-wnl	50.0	50.0	38.9	61.1	50.0	50.0	46.3	3.7	53.7	3.7	50.0	2.5	59.4
(+)-wnl vs. solvent													
10^{-3} (+)-wnl vs. solvent	72.2	61.1	50.0	55.6	61.1	66.7	61.1	6.4	61.1	3.2	61.1	3.2	59.4
10^{-5} (+)-wnl vs. solvent	72.2	72.2	72.2	66.7	72.2	72.2	72.2	0.0	70.4	1.9	71.3	0.9	59.4
10^{-7} (+)-wnl vs. solvent	66.7	66.7	83.3	66.7	66.7	55.6	72.2	5.6	63.0	3.7	67.6	3.6	59.4
10^{-9} (+)-wnl vs. solvent	72.2	72.2	72.2	66.7	66.7	72.2	72.2	0.0	68.5	1.9	70.4	1.2	59.4
10^{-11} (+)-wnl vs. solvent	77.8	66.7	77.8	72.2	72.2	72.2	74.1	3.7	72.2	0.0	73.1	1.7	59.4
10^{-13} (+)-wnl vs. solvent	55.6	55.6	55.6	61.1	44.4	38.9	55.6	0.0	48.1	6.7	51.9	3.4	59.4
10^{-15} (+)-wnl vs. solvent	61.1	61.1	61.1	77.8	44.4	50.0	61.1	0.0	57.4	10.3	59.3	4.7	59.4
10^{-17} (+)-wnl vs. solvent	44.4	61.1	44.4	61.1	44.4	50.0	50.0	5.6	51.9	4.9	50.9	3.3	59.4
PO assays													
10^{-11} (+)-wnl vs. solvent	77.8	66.7	77.8	72.2	72.2	72.2	74.1	3.7	72.2	0.0	73.1	1.7	59.4
10^{-9} (+)-wnl vs. (+)-wnl	61.1	55.6	33.3	55.6	50.0	44.4	50.0	8.5	50.0	3.2	50.0	4.1	59.4
(-)-wnl vs. (+)-wnl													
10^{-3} (-)-wnl vs. (+)-wnl	27.8	50.0	55.6	61.1	38.9	44.4	44.4	8.5	48.1	6.7	46.3	4.9	59.4
10^{-5} (-)-wnl vs. (+)-wnl	38.9	33.3	38.9	38.9	55.6	44.4	37.0	1.9	46.3	4.9	41.7	3.1	59.4
10^{-7} (-)-wnl vs. (+)-wnl	55.6	44.4	66.7	44.4	33.3	44.4	55.6	6.4	40.7	3.7	48.1	4.7	59.4
10^{-9} (-)-wnl vs. (+)-wnl	38.9	66.7	55.6	38.9	44.4	50.0	53.7	8.1	41.7	2.8	48.9	5.4	59.4
10^{-11} (-)-wnl vs. (+)-wnl	55.6	61.1	50.0	33.3	55.6	38.9	55.6	3.2	42.6	6.7	49.1	4.4	59.4
10^{-13} (-)-wnl vs. (+)-wnl	66.7	50.0	61.1	61.1	55.6	50.0	59.3	4.9	55.6	3.2	57.4	2.7	59.4
PO assays													
10^{-13} (-)-wnl vs. (+)-wnl	54.2	54.2	54.2	62.5	54.2	54.2	54.2	0.0	56.9	2.8	55.6	1.4	59.4
10^{-13} (-)-wnl vs. solvent	70.8	70.8	66.7	66.7	66.7	70.8	69.4	1.4	68.1	1.4	68.8	0.9	59.4
(-)-car vs. solvent													
10^{-3} (-)-car vs. solvent	66.7	77.8	77.8	66.7	72.2	66.7	74.1	3.7	68.5	1.9	71.3	2.2	59.4
10^{-5} (-)-car vs. solvent	55.6	77.8	83.3	83.3	83.3	72.2	72.2	8.5	79.6	3.7	75.9	4.5	59.4
10^{-7} (-)-car vs. solvent	72.2	83.3	77.8	72.2	66.7	66.7	77.8	3.2	68.5	1.9	73.1	2.7	59.4
10^{-9} (-)-car vs. solvent	77.8	77.8	83.3	77.8	61.1	72.2	79.6	1.9	70.4	4.9	75.0	3.1	59.4
10^{-11} (-)-car vs. solvent	83.3	72.2	88.9	94.4	72.2	72.2	81.5	4.9	79.6	7.4	80.6	4.0	59.4
10^{-13} (-)-car vs. solvent	83.3	77.8	66.7	83.3	72.2	72.2	75.9	4.9	75.9	3.7	75.9	2.7	59.4
10^{-15} (-)-car vs. solvent	61.1	66.7	66.7	66.7	72.2	61.1	64.8	1.9	66.7	3.2	65.7	1.7	59.4
10^{-17} (-)-car vs. solvent	77.8	61.1	72.2	72.2	55.6	66.7	70.4	4.9	64.8	4.9	67.6	3.3	59.4
10^{-19} (-)-car vs. solvent	61.1	55.6	50.0	61.1	72.2	50.0	55.6	3.2	61.1	6.4	58.3	3.4	59.4
PO assays													
10^{-9} (-)-car vs. solvent	83.3	66.7	72.2	66.7	72.2	66.7	74.1	4.9	68.5	1.9	71.3	2.7	59.4
10^{-9} (-)-car vs. (-)-car	55.6	55.6	55.6	55.6	55.6	38.9	55.6	0.0	50.0	5.6	52.8	2.8	59.4

Table ST1. COCRs of WT and ΔD mice in a Y-maze for serial 100-fold diluted odourants (continued).

Odourant pairs	COCRs of individual ΔD mice (%)						avg. of 3 mice (ΔD1-3)	S.E.M.	avg. of 3 mice (ΔD4-6)	S.E.M.	avg. of 6 ΔD mice	S.E.M.	COCR for *P* = 0.05
	ΔD1	ΔD2	ΔD3	ΔD4	ΔD5	ΔD6							
(+)-car vs. solvent													
10^{-3} (+)-car vs. solvent	72.2	72.2	72.2	61.1	72.2	61.1	72.2	0.0	64.8	3.7	68.5	2.3	59.4
10^{-5} (+)-car vs. solvent	61.1	72.2	72.2	55.6	55.6	61.1	68.5	3.7	57.4	1.9	63.0	3.1	59.4
10^{-7} (+)-car vs. solvent	77.8	77.8	77.8	77.8	72.2	66.7	77.8	0.0	72.2	3.2	75.0	1.9	59.4
10^{-9} (+)-car vs. solvent	72.2	77.8	61.1	72.2	66.7	61.1	70.4	4.9	66.7	3.2	68.5	2.7	59.4
10^{-11} (+)-car vs. solvent	72.2	83.3	72.2	66.7	66.7	61.1	75.9	3.7	64.8	1.9	70.4	3.1	59.4
10^{-13} (+)-car vs. solvent	72.2	72.2	61.1	61.1	61.1	61.1	68.5	3.7	61.1	0.0	64.8	2.3	59.4
10^{-15} (+)-car vs. solvent	66.7	50.0	55.6	66.7	55.6	55.6	57.4	4.9	59.3	3.7	58.3	2.8	59.4
PO assays													
10^{-11} (+)-car vs. solvent	61.1	66.7	66.7	66.7	66.7	66.7	64.8	1.9	66.7	0.0	65.7	0.9	59.4
(-)-car vs. (+)-car													
10^{-3} (-)-car vs. (+)-car	61.1	61.1	55.6	66.7	55.6	61.1	59.3	1.9	61.1	3.2	60.2	1.7	59.4
10^{-5} (-)-car vs. (+)-car	61.1	72.2	66.7	72.2	55.6	61.1	66.7	3.2	63.0	4.9	64.8	2.7	59.4
10^{-7} (-)-car vs. (+)-car	72.2	61.1	61.1	66.7	55.6	55.6	64.8	3.7	59.3	3.7	62.0	2.7	59.4
10^{-9} (-)-car vs. (+)-car	55.6	72.2	61.1	72.2	55.6	55.6	63.0	4.9	61.1	5.6	62.0	3.3	59.4
10^{-11} (-)-car vs. (+)-car	50.0	55.6	66.7	50.0	44.4	44.4	57.4	4.9	46.3	1.9	51.9	3.4	59.4
10^{-13} (-)-car vs. (+)-car	61.1	55.6	55.6	61.1	66.7	50.0	57.4	1.9	59.3	4.9	58.3	2.4	59.4
10^{-15} (-)-car vs. (+)-car	55.6	50.0	61.1	61.1	55.6	50.0	55.6	3.2	55.6	3.2	55.6	2.0	59.4
10^{-17} (-)-car vs. (+)-car	50.0	55.6	61.1	61.1	61.1	50.0	55.6	3.2	57.4	3.7	56.5	2.2	59.4
10^{-19} (-)-car vs. (+)-car	50.0	55.6	66.7	55.6	61.1	50.0	53.7	1.9	55.6	3.2	54.6	1.7	59.4
PO assays													
10^{-11} (-)-car vs. solvent	77.8	72.2	77.8	72.2	83.3	66.7	75.9	1.9	74.1	4.9	75.0	2.4	59.4
nTMT vs dpg													
10^{-7} nTMT vs. solvent	61.1	61.1	66.7	66.7	61.1	66.7	63.0	1.9	64.8	1.9	63.9	1.2	59.4
10^{-9} nTMT vs. solvent	83.3	72.2	66.7	61.1	66.7	88.9	74.1	4.9	72.2	8.5	73.1	4.4	59.4
10^{-11} nTMT vs. solvent	77.8	72.2	77.8	72.2	72.2	88.9	75.9	1.9	77.8	5.6	76.9	2.7	59.4
10^{-13} nTMT vs. solvent	77.8	94.4	83.3	77.8	83.3	72.2	85.2	4.9	77.8	3.2	81.5	3.1	59.4
10^{-15} nTMT vs. solvent	77.8	66.7	83.3	77.8	72.2	66.7	75.9	4.9	72.2	3.2	74.1	2.7	59.4
10^{-17} nTMT vs. solvent	77.8	88.9	83.3	55.6	50.0	61.1	83.3	3.2	55.6	3.2	69.4	6.5	59.4
10^{-19} nTMT vs. solvent	77.8	55.6	72.2	50.0	55.6	50.0	68.5	6.7	51.9	1.9	60.2	4.8	59.4
10^{-21} nTMT vs. solvent	44.4	61.1	50.0	50.0	55.6	50.0	51.9	4.9	51.9	1.9	51.9	2.3	59.4
PO assays													
10^{-17} nTMT vs. solvent	61.1	83.3	77.8	61.1	66.7	83.3	74.1	6.7	70.4	6.7	72.2	4.3	59.4

Table ST1. COCRs of WT and ΔD mice in a Y-maze for serial 100-fold diluted odourants (continued).

Odourant concentration	Odourants										
	(–)-wnl					Total of target-best ORs	(+)-wnl				
	Tuning classes of OSNs/ORs			OR overlap	The largest class		The largest class	OR overlap	Tuning classes of OSNs/ORs		
	(–)-wnl*	(–)-/(+)-wnl	(+)-wnl						(–)-wnl	(–)-/(+)-wnl	(+)-wnl*
10 nM	1 (1:0) 50%	1 (1:0) 50%	0 (0:0) 0%	50% (1/2)	(–), (–)/(+)	>	(–)/(+)	100% (1/1)	0 (0:0) 0%	1 (1:0) 100%	0 (0:0) 0%
100 nM	1 (1:0) 50%	1 (1:0) 50%	0 (0:0) 0%	50% (1/2)	(–), (–)/(+)	>	(–)/(+)	100% (1/1)	0 (0:0) 0%	1 (1:0) 100%	0 (0:0) 0%
1 μM	3 (2:1) 75%	1 (1:0) 25%	0 (0:0) 0%	25% (1/4)	(–)	>	(–)/(+), (+)	50% (1/2)	0 (0:0) 0%	1 (1:0) 50%	1 (1:0) 50%
10 μM	14 (7:7) 87.5%	2 (2:0) 12.5%	0 (0:0) 0%	25% (4/16)	(–)	>	(–)/(+), (–)	80% (4/5)	2 (2:0) 40%	2 (2:0) 40%	1 (1:0) 20%
100 μM	38 (21:17) 84%	3 (3:0) 7%	4 (2:2) 9%	42% (19/45)	(–)	>	(–)	95% (19/20)	12 (6:6) 60%	3 (3:0) 15%	5 (3:2) 25%

Table ST2. Dose-dependent subpopulations of wine lactone-responsive OSNs/ORs. Of 1,746 olfactory sensory neurons (OSNs) (1,207 dorsal zone: 539 ventral zone) assayed by Ca^{2+} imaging, 2, 5, 17 and 46 OSNs/ORs responded to either or both wine lactone enantiomers at 10-100 nM, 1, 10, 100 μM, respectively. They were classified by their tuning to target enantiomers. Target-best OSNs/ORs* are those more sensitive to the target enantiomer than to non-target enantiomer. Percentage of each class responsive to the target at a given concentration is listed in the lower row. OR overlap indicates percentage (Numbers) of overlapping OSN/OR in enantiomer OR codes at each concentration. (–)-wnl, (–)-wine lactone; (+)-wnl, (+)-wine lactone.

Odourant conc.	Odourants												
	(–)-car						Total of target-best ORs	(+)-car					
	Tuning classes of OSNs/ORs				OR overlap	The largest class		The largest class	OR overlap	Tuning classes of OSNs/ORs			
	(–)-car*	(–)-car /others	(–)-/(+)-car	(+)-car						(–)-car	(–)-/(+)-car	(+)-car /others	(+)-car*
1 μM	4 (2:2) 45%	2 (1:1) 22%	3 (2:1) 33%	0 (0:0) 0%	55% (5/9)	(–)	>	(–)/(+), (+)‡	55% (5/9)	2 (0:2) 22%†	3 (2:1) 33%	2 (1:1) 22%	2(1.5:0.5) 22%(11)
10 μM	18(7.5:10.5) 25.5%(7)	6 (2:4) 8.5%(3)	44 (20:24) 62%(5.5)	3 (2.5:0.5) 4%(1.5)	79% (56/71)	(–)/(+)	=	(–)/(+)	62% (51/82)	9 (4:5) 11%(5)	44 (20:24) 53.5%(5)	8 (2.5:5.5) 10%(3.5)	21(5.5:15.5) 25.5%(3.5)
	Identified carvone-responsive ORs and their sensitivities to carvone enantiomers												
1 μM	*car-c5* (+)car(10)												
			car-n266 less								*car-n266* greater		
			car-b85 greater								*car-b85* less		
			car-n272 greater								*car-n272* less		
10 μM	*car-c10* (+)car(100)												
													car-b158 (–)car(100)
			car-b130 greater								*car-b130* less		
			car-n270 greater								*car-n270* less		
	car-257 (+)car(100)												
			car-n271 equal								*car-n271* equal		
100 μM	*car-c260*												
													car-c258
			car-c6 equal								*car-c6* equal		
			car-c255 less								*car-c255* greater		
	car-c15												

Table ST3. Dose-dependent subpopulations of carvone-responsive OSNs/ORs. Of 2,740 OSNs/ORs assayed by Ca^{2+} imaging, 13 and 89 OSNs/ORs (dorsal zone: ventral zone) responded to either or both of carvone enantiomers at 1 and 10 μM, respectively, and were classified by their tuning to target odourants. Target-best OSNs/ORs* are those more sensitive to the target enantiomer than to the non-target enantiomer. Percentage of each class responsive to target at a given concentration is listed in the lower rows. Percentage† indicates OSNs/ORs with response amplitudes less than the half of the others. The percentages in parentheses indicate OSNs/ORs that were prepared from uncertain zones and counted in each zone by a factor of 1/2. OR overlap indicates percentage (numbers) of overlapping OSN/OR in enantiomer OR codes at each concentration. The largest class‡ is the sum of (+)-car and (+)-car/others OSNs/ORs. In the lower part, relative response amplitudes of 15 identified carvone ORs are shown with the lowest responsive concentrations (μM) of target (left column) and non-target carvone in parentheses (see Fig. 3).

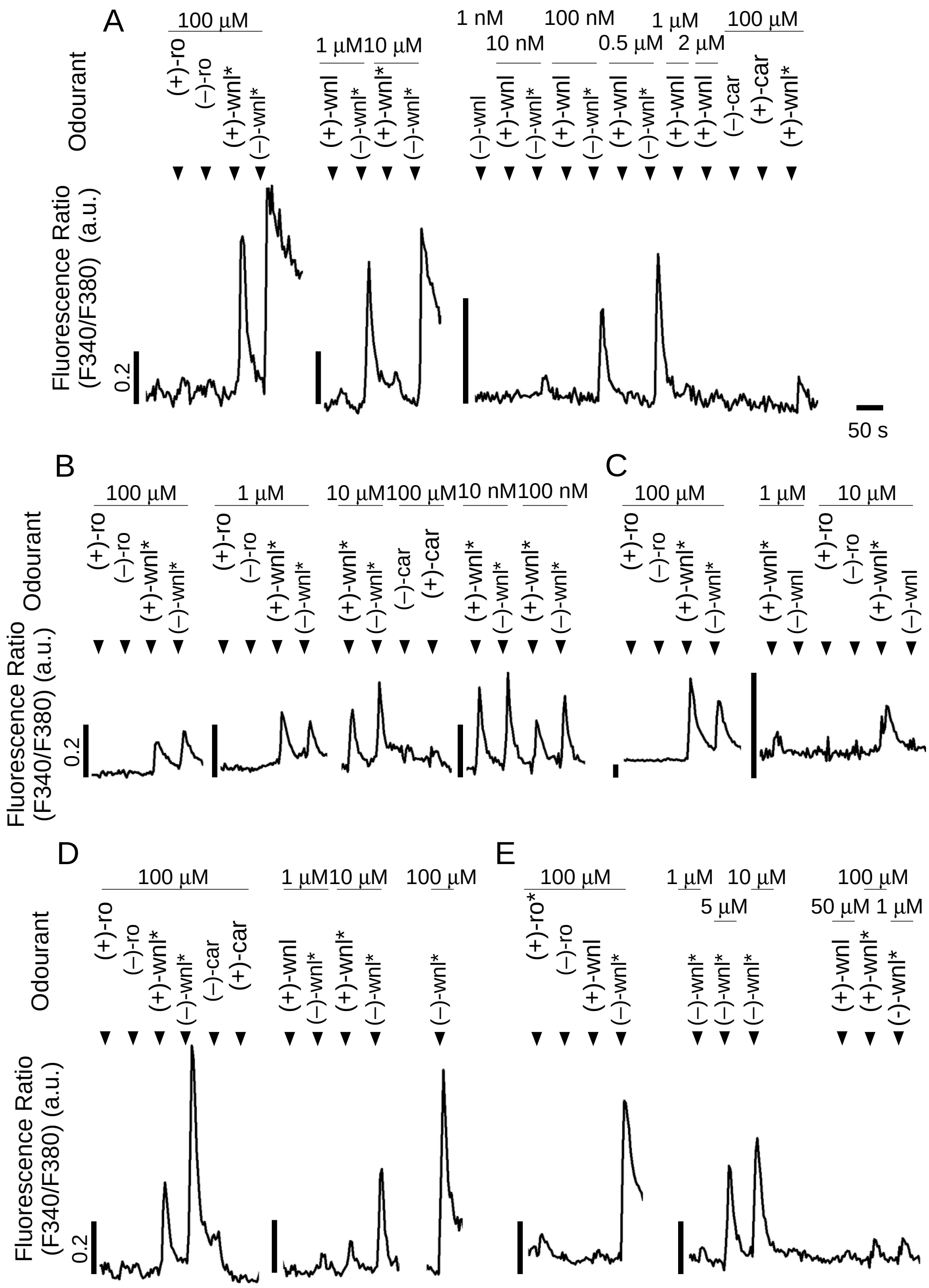

Figure S2. Odour responses of the most sensitive OSNs for wine lactones in mice. Odour responses were measured using intracellular Ca^{2+}-imaging. A, OSN tuned to 10 nM (–)-wnl . B, OSN tuned to 10 nM (–)-wnl and (+)-wnl. C, OSN tuned to 1 μM (+)-wnl. D and E, OSNs tuned to 1 μM (–)-wnl. (–)-ro: (2*S*,4*R*)- (–)-cis-rose oxide; (+)-ro:(2*R*,4*S*)-(+)-cis-rose oxide. The arrows indicate the time at which odourant solutions of the indicated concentrations were applied to the OSNs for 4 s. The odourants that evoked characteristic odour responses are indicated by the asterisks. All OSNs, except for that shown in E, were obtained from the dorsal zone.

Odourant pairs	significance of difference between 2 strains		WT mice		ΔD mice	
	F value	*P* value	COCR (%) (n = 6)	S.E.M.	COCR (%) (n = 6)	S.E.M.
(-)-wnl vs. solvent						
10^{-3} (-)-wnl vs. solvent	4.13	7.0×10^{-2}	87.0	4.7	71.5	5.8
10^{-5} (-)-wnl vs. solvent	5.52	4.1×10^{-2}	77.8	1.4	67.6	4.2
10^{-7} (-)-wnl vs. solvent	0.61	0.45	72.2	4.5	76.9	4.0
10^{-9} (-)-wnl vs. solvent	0.07	0.80	74.1	3.1	75.0	2.4
10^{-11} (-)-wnl vs. solvent	0.58	0.46	79.6	4.2	75.9	2.3
10^{-13} (-)-wnl vs. solvent	5.35	4.3×10^{-2}	79.6	4.5	68.5	1.9
10^{-15} (-)-wnl vs. solvent	47.57	4.2×10^{-5}	79.6	1.9	63.9	1.2
10^{-17} (-)-wnl vs. solvent	12.86	5.0×10^{-3}	75.9	2.7	62.0	2.7
10^{-19} (-)-wnl vs. solvent	5.18	4.6×10^{-2}	71.3	3.3	61.1	2.9
10^{-21} (-)-wnl vs. solvent	18.06	1.7×10^{-3}	67.6	2.7	47.2	4.0
10^{-23} (-)-wnl vs. solvent	6.86	2.6×10^{-2}	59.3	3.1	47.2	3.1
(+)-wnl vs. solvent						
10^{-3} (+)-wnl vs. solvent	9.79	1.1×10^{-2}	76.6	3.9	61.1	3.2
10^{-5} (+)-wnl vs. solvent	2.92	0.12	77.8	3.5	71.3	0.9
10^{-7} (+)-wnl vs. solvent	5.08	4.8×10^{-2}	75.7	4.2	67.6	3.6
10^{-9} (+)-wnl vs. solvent	21.00	1.0×10^{-3}	83.1	3.1	70.4	1.2
10^{-11} (+)-wnl vs. solvent	0.09	0.76	74.5	4.1	73.1	1.7
10^{-13} (+)-wnl vs. solvent	54.55	2.4×10^{-5}	82.9	2.3	51.9	3.4
10^{-15} (+)-wnl vs. solvent	14.72	3.3×10^{-3}	75.2	3.1	59.3	4.7
10^{-17} (+)-wnl vs. solvent	31.87	2.1×10^{-4}	75.2	2.8	50.9	3.3
(-)-wnl vs. (+)-wnl						
10^{-3} (-)-wnl vs. (+)-wnl	15.37	2.9×10^{-3}	69.4	3.4	46.3	4.9
10^{-5} (-)-wnl vs. (+)-wnl	6.36	3.0×10^{-2}	54.3	3.9	41.7	3.1
10^{-7} (-)-wnl vs. (+)-wnl	11.45	7.0×10^{-3}	73.1	5.8	48.1	4.7
10^{-9} (-)-wnl vs. (+)-wnl	13.54	4.3×10^{-3}	68.5	2.7	48.9	5.4
10^{-11} (-)-wnl vs. (+)-wnl	4.78	5.4×10^{-2}	62.0	3.9	49.1	4.4
10^{-13} (-)-wnl vs. (+)-wnl	6.01	3.4×10^{-2}	72.2	5.4	57.4	2.7

Table ST4. Significance of the COCR differences between WT and ΔD mice.

Odourant pairs	significance of difference between 2 strains		WT mice		ΔD mice	
	F value	*P* value	COCR (%) (n = 6)	S.E.M.	COCR (%) (n = 6)	S.E.M.
(-)-car vs. solvent						
10^{-3} (-)-car vs. solvent	4.24	6.7×10^{-2}	81.5	4.2	71.3	2.2
10^{-5} (-)-car vs. solvent	1.45	0.26	82.4	3.0	75.9	4.5
10^{-7} (-)-car vs. solvent	0.03	0.87	72.5	2.9	73.1	2.7
10^{-9} (-)-car vs. solvent	2.21	0.17	67.6	3.9	75.0	3.1
10^{-11} (-)-car vs. solvent	0.41	0.54	75.9	6.2	80.6	4.0
10^{-13} (-)-car vs. solvent	0.00	1.00	75.9	5.3	75.9	2.7
10^{-15} (-)-car vs. solvent	3.70	0.08	72.2	2.9	65.7	1.7
10^{-17} (-)-car vs. solvent	1.58	0.24	74.1	4.0	67.6	3.3
10^{-19} (-)-car vs. solvent	5.14	4.7×10^{-2}	67.6	2.2	58.3	3.4
(+)-car vs. solvent						
10^{-3} (+)-car vs. solvent	6.43	3.0×10^{-2}	79.6	3.7	68.5	2.3
10^{-5} (+)-car vs. solvent	12.34	5.6×10^{-3}	79.6	3.7	63.0	3.1
10^{-7} (+)-car vs. solvent	2.22	0.17	79.6	2.3	75.0	1.9
10^{-9} (+)-car vs. solvent	1.21	0.30	64.8	1.9	68.5	2.7
10^{-11} (+)-car vs. solvent	0.07	0.79	71.3	3.0	70.4	3.1
10^{-13} (+)-car vs. solvent	4.49	6.0×10^{-2}	72.2	2.9	64.8	2.3
10^{-15} (+)-car vs. solvent	7.67	2.0×10^{-2}	72.2	4.1	58.3	2.8
(-)-car vs. (+)-car						
10^{-3} (-)-car vs. (+)-car	28.13	3.5×10^{-4}	84.3	4.2	60.2	1.7
10^{-5} (-)-car vs. (+)-car	8.55	1.5×10^{-2}	77.8	3.5	64.8	2.7
10^{-7} (-)-car vs. (+)-car	9.13	1.3×10^{-2}	73.1	2.7	62.0	2.7
10^{-9} (-)-car vs. (+)-car	1.60	0.23	68.5	3.7	62.0	3.3
10^{-11} (-)-car vs. (+)-car	27.17	3.9×10^{-4}	75.9	3.1	51.9	3.4
10^{-13} (-)-car vs. (+)-car	2.43	0.15	64.8	3.4	58.3	2.4
10^{-15} (-)-car vs. (+)-car	10.61	8.6×10^{-3}	66.7	2.9	55.6	2.0
10^{-17} (-)-car vs. (+)-car	17.90	1.7×10^{-3}	75.0	3.7	56.5	2.2
10^{-19} (-)-car vs. (+)-car	8.43	1.6×10^{-2}	67.6	2.7	54.6	1.7
nTMT vs. solvent						
10^{-9} nTMT vs. solvent	0.40	0.54	68.5	6.0	73.1	4.4
10^{-11} nTMT vs. solvent	5.02	4.9×10^{-2}	67.6	3.0	76.9	2.7
10^{-13} nTMT vs. solvent	0.30	0.59	78.7	4.2	81.5	3.1
10^{-15} nTMT vs. solvent	0.09	0.77	75.9	4.9	74.1	2.7
10^{-17} nTMT vs. solvent	0.01	0.91	68.5	3.7	69.4	6.5
10^{-19} nTMT vs. solvent	1.67	0.23	66.7	1.4	60.2	4.8
10^{-21} nTMT vs. solvent	3.72	8.3×10^{-2}	63.9	5.9	51.9	2.3

Table ST4. Significance of the COCR differences between WT and ΔD mice (continued).

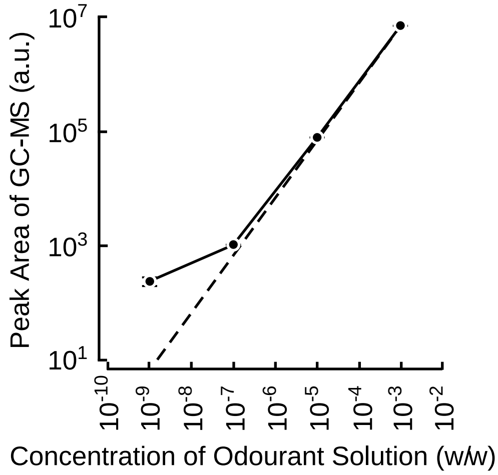

Figure S3. Dose-dependency of the peak areas of diluted odourant solutions in gas chromatography-mass spectrometry (GC-MS). The peak area per unit dose seemed too large for the 10^{-9}-w/w odourant solution compared with those at higher concentrations. This was likely due to background noise. This result suggests that the detection limit of this GC-MS measurement is approximately 10 ppb for (*R*)-(-)-carvone. The theoretical curve (dashed line) is the plot of the peak areas at the concentration (C_x) with correction by the term -(peak_area_at_10^{-9}) + (peak_area_at_ $10^{-3} \times C_x/10^{-3}$)

target conc. (w/w)	measured peak area (a.u.)					dilution factor (D, ratio of peak area per unit dose)		
	dilution 1 (D1)	dilution 2 (D2)	dilution 3 (D3)	average ± S.E.M.		D1	D2	D3
					average	1.01	1.00	1.02
10^{-3}	7,029,438.5	6,806,454.0	6,703,118.0	$6.85 \times 10^6 \pm 9.63 \times 10^4$		1.18	1.11	1.07
10^{-5}	83,192.0	75,972.5	71,889.0	$7.70 \times 10^4 \pm 3.30 \times 10^3$		1.04	0.90	1.21
10^{-7}	1,033.5	893.5	1,181.0	$1.04 \times 10^3 \pm 8.30 \times 10^1$		0.82	1.00	0.77
10^{-9}	178.5	219.0	321.0	$2.40 \times 10^2 \pm 4.24 \times 10^1$	D_{avg}	1.0109 ± 0.0515		

Table ST5. Peak areas in GC-MS and calculation of the dilution factors for the 100-fold dilution series. The dilution factors were obtained as the ratios of peak areas per unit dose between subsequent concentrations. (*R*)-(−)-carvone was used as the standard odourant. The dose-dependency of the peak areas was shown in Supplementary Information Fig. S3.

Target concentration (C) (w/w)	Number of dilution steps (N)	Accumulated average dilution factor ($A_{avg} = D_{avg}$^N)	Estimated conc. = C × A_{avg} (w/w)	Accumulated maximum dilution factor ($A_{max} = D_{max}$^N)	Estimated maximum conc. = C × A_{max} (w/w)
10^{-3}	1	1.01	1.0×10^{-3}	1.21	1.2×10^{-3}
10^{-5}	2	1.02	1.0×10^{-5}	1.47	1.5×10^{-5}
10^{-7}	3	1.03	1.0×10^{-7}	1.78	1.8×10^{-7}
10^{-9}	4	1.04	1.0×10^{-9}	2.15	2.2×10^{-9}
10^{-11}	5	1.06	1.1×10^{-11}	2.60	2.6×10^{-11}
10^{-13}	6	1.07	1.1×10^{-13}	3.15	3.2×10^{-13}
10^{-15}	7	1.08	1.1×10^{-15}	3.82	3.8×10^{-15}
10^{-17}	8	1.09	1.1×10^{-17}	4.62	4.6×10^{-17}
10^{-19}	9	1.10	1.1×10^{-19}	5.60	5.6×10^{-19}
10^{-21}	10	1.11	1.1×10^{-21}	6.78	6.8×10^{-21}
10^{-23}	11	1.13	1.1×10^{-23}	8.21	8.2×10^{-23}
10^{-25}	12	1.14	1.1×10^{-25}	9.94	9.9×10^{-25}

Table ST6. Estimated odourant concentrations for the 100-fold dilution series. Averaged dilution factor, Davg = 1.0109 ± 0.0515, and the maximum dilution factor, D_{max} = 1.21, are shown in Supplementary Information Table ST5.