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Supplemental Information

Modulation of Host Learning in *Aedes aegypti* Mosquitoes

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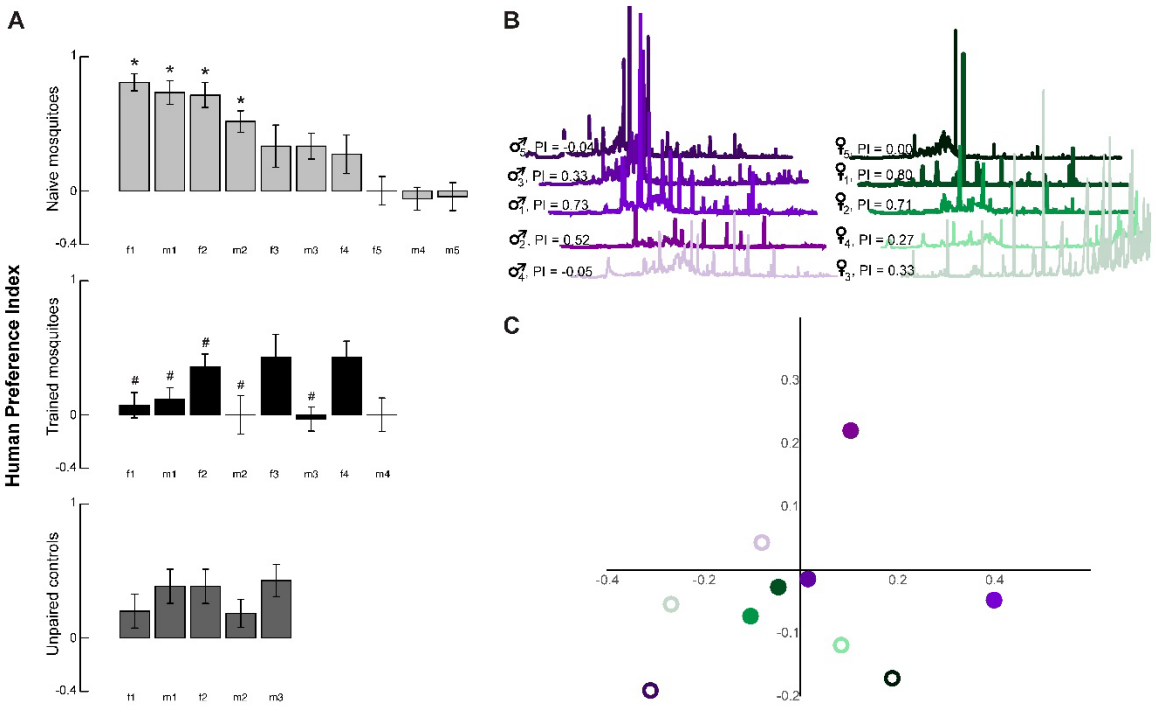

Figure S1. Human Host Scents are Distinct and do not Correlate with Behavioural Responses, Related to Figure 1. (**A**) Mosquito preference for each of the human host volunteers for the naive (light grey), trained (black) and unpaired (dark grey) groups. A total of 682 mosquitoes were tested. Each bar represents 7-34 responsive female mosquitoes (on average, approximately 25-30 mosquitoes were tested per treatment and human scent individual). Error bars represent the standard errors of the binary distribution. Asterisks indicate distributions that are significantly different from random ($p<0.05$, binomial test). Hash signs indicate trained groups that were significantly different from their respective naïve controls ($p<0.05$, binomial test). (**B**) Total ion chromatograms of the scent from the different human volunteers used in the learning assays. Scent from the human volunteers varied both in total abundance and composition. Mosquito innate and learned responses to scent from the different individuals did not significantly correlate with total scent abundance (Pearson's $r<0.58$; $p>0.21$). Chromatograms are colour-coded according to sex (purple: males; green: females) and behavioural preference by the mosquitoes: dark colour denotes the scent was significantly attractive to mosquitoes; light-coloured lines denotes no attraction. (**C**) NMDS plot of the individual scent profiles showed that individuals were significantly distinct in their body odours ($p = 0.007$, Anosim; $R = 0.46$; stress = 0.04). Filled symbols denote those individuals whose scents were significantly attractive to mosquitoes and whose scents mosquitoes could learn to avoid; unfilled symbols denote those individuals who are not innately attractive to mosquitoes. Symbol colours denote individual human volunteers and are the same as in (**A**).

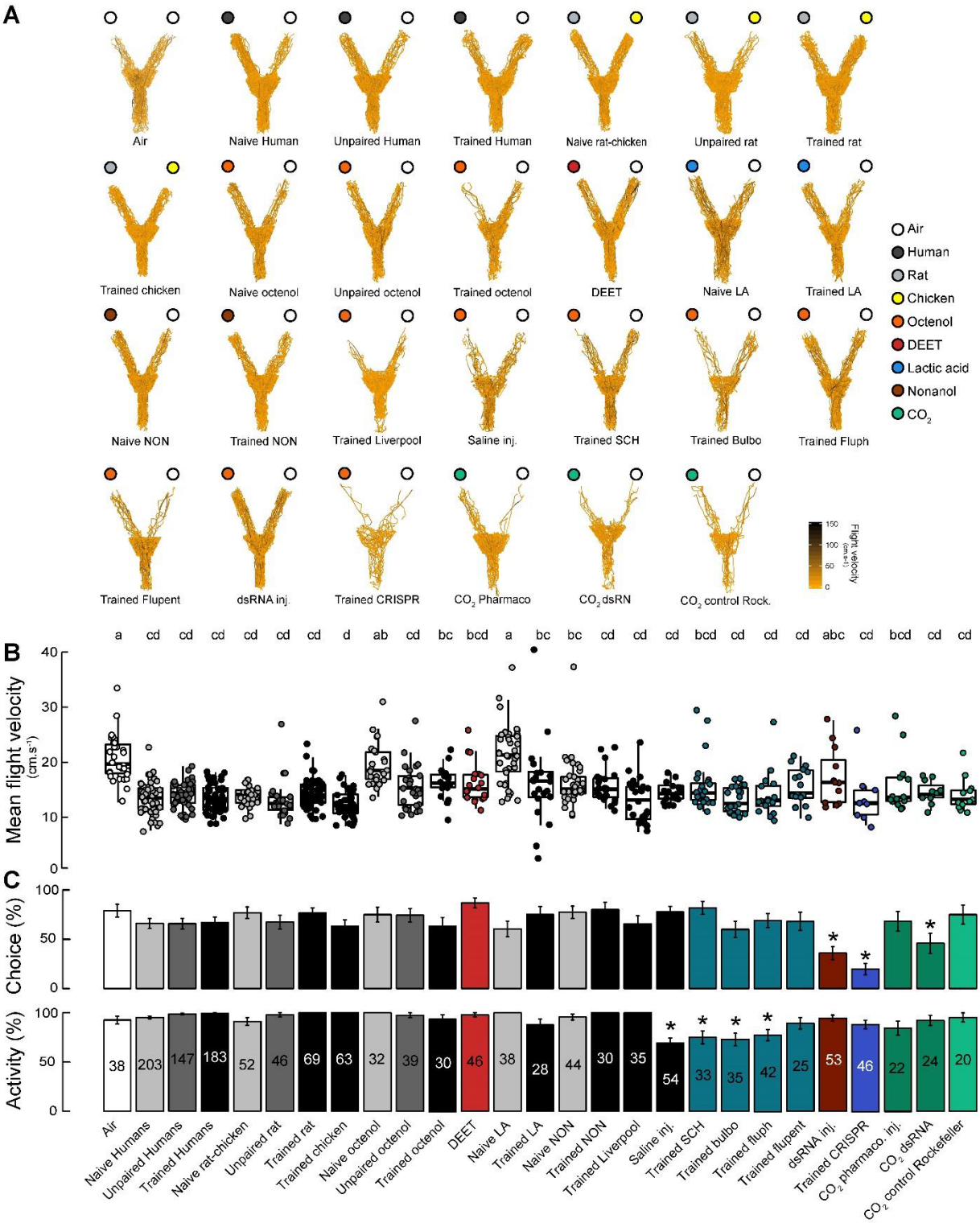

Figure S2. Learning-Evoked Responses do not Correlate with Activity Levels, Related to Figures 1 and 3. (**A**) Compiled flight tracks of individual female mosquitoes that were tested in the olfactometer (*n*=1740 mosquitoes). Trajectories are color-coded for each individual as a

function of their instantaneous flight velocity. The white circle indicates the control side while a coloured circle indicates the tested odour side. (**B**) Average flight velocities obtained from video-tracking mosquitoes in the Y-maze olfactometer. Clean air (white dots), positive (green dots) and negative (red dots) controls, as well as naive (light grey dots), unpaired (dark grey dots), trained (black dots), trained drug-injected (blue dots), trained dsRNA-injected (brick dots), and CRISPR (mauve dots) groups are depicted as jitter dot plots. Boxplots represent median±95% confidence interval flight velocities. Different letters indicate statistical differences ($p<0.05$, *t*-test; $t>3.8$). (**C**) Arousal and activity levels depicted as the proportion of mosquitoes making a choice (either control arm or odour arm) over the total number of mosquitoes that flew during the experiments (top plots), as the proportion of mosquitoes that were active, i.e. that flew out of their individual container to enter the Y-maze. Asterisks indicate statistical differences from the respective control group ($p<0.05$, binomial test). Colour codes and groups correspond to those described in Figure S2A,B.

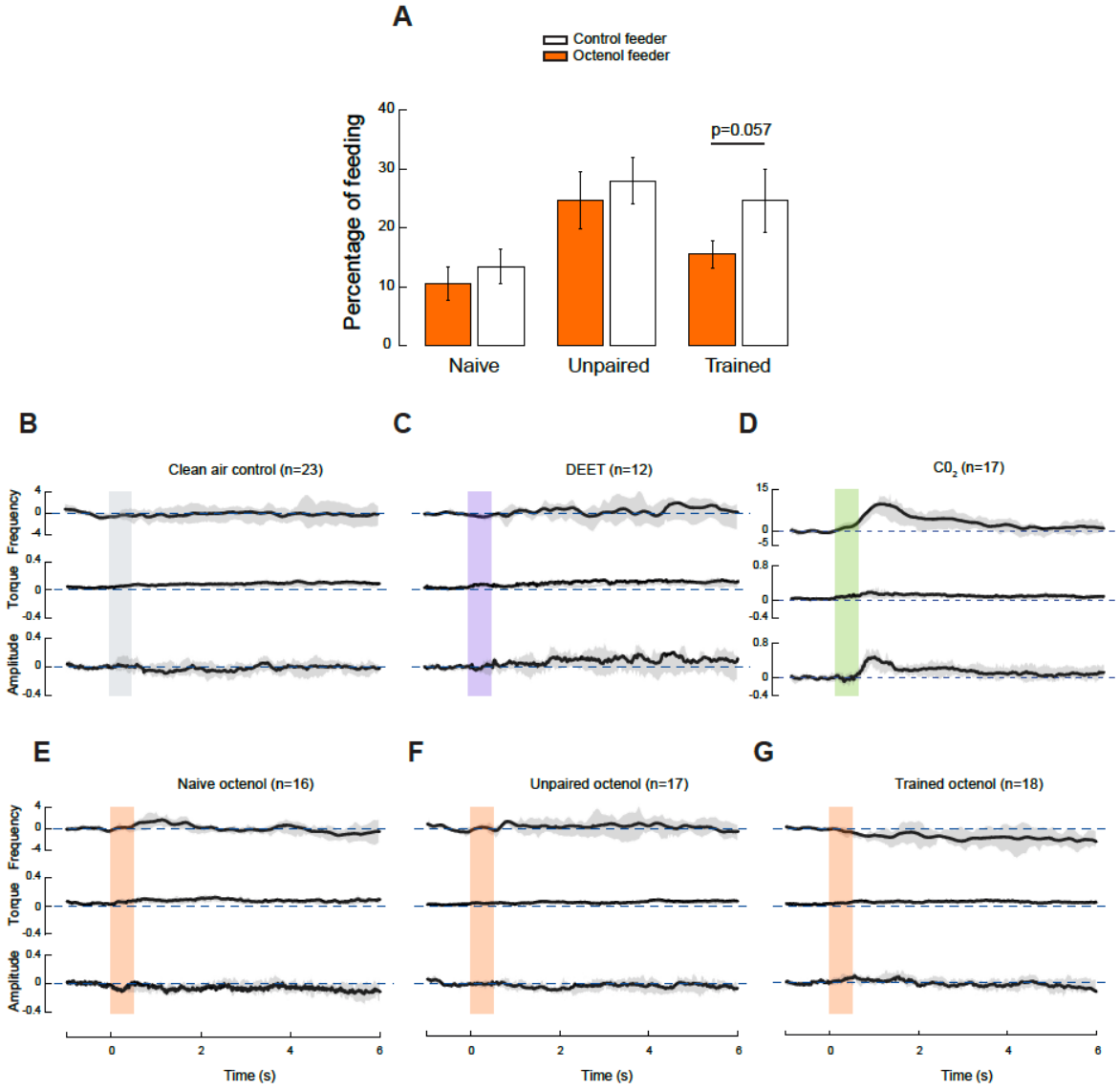

Figure S3. Mosquito Blood Feeding Responses and Tethered Mosquito Responses in the Flight Arena, Related to Figure 3. (**A**) Percentage of mosquito feeding on either one of the two artificial feeders (control feeder, white; 1-octen-3-ol feeder, orange), for the naive, unpaired and trained groups. Each bar represents 9-10 groups of 17 female mosquitoes. Error bars represent the standard errors of the binary distribution. (**B-G**) Wingbeat frequency, turning tendency (torque) and amplitude variations (black line) in response to a pulse of: (**B**) clean air (control), (**C**) DEET, (**D**) carbon dioxide, (**E-G**) 1-octen-3-ol for the naive, unpaired and trained groups. A total of 103 mosquitoes were tested; each line represents the average response of 12-23 individuals. The pulses are indicated as vertical bars and the shaded areas represent the mean ± the first quartiles.

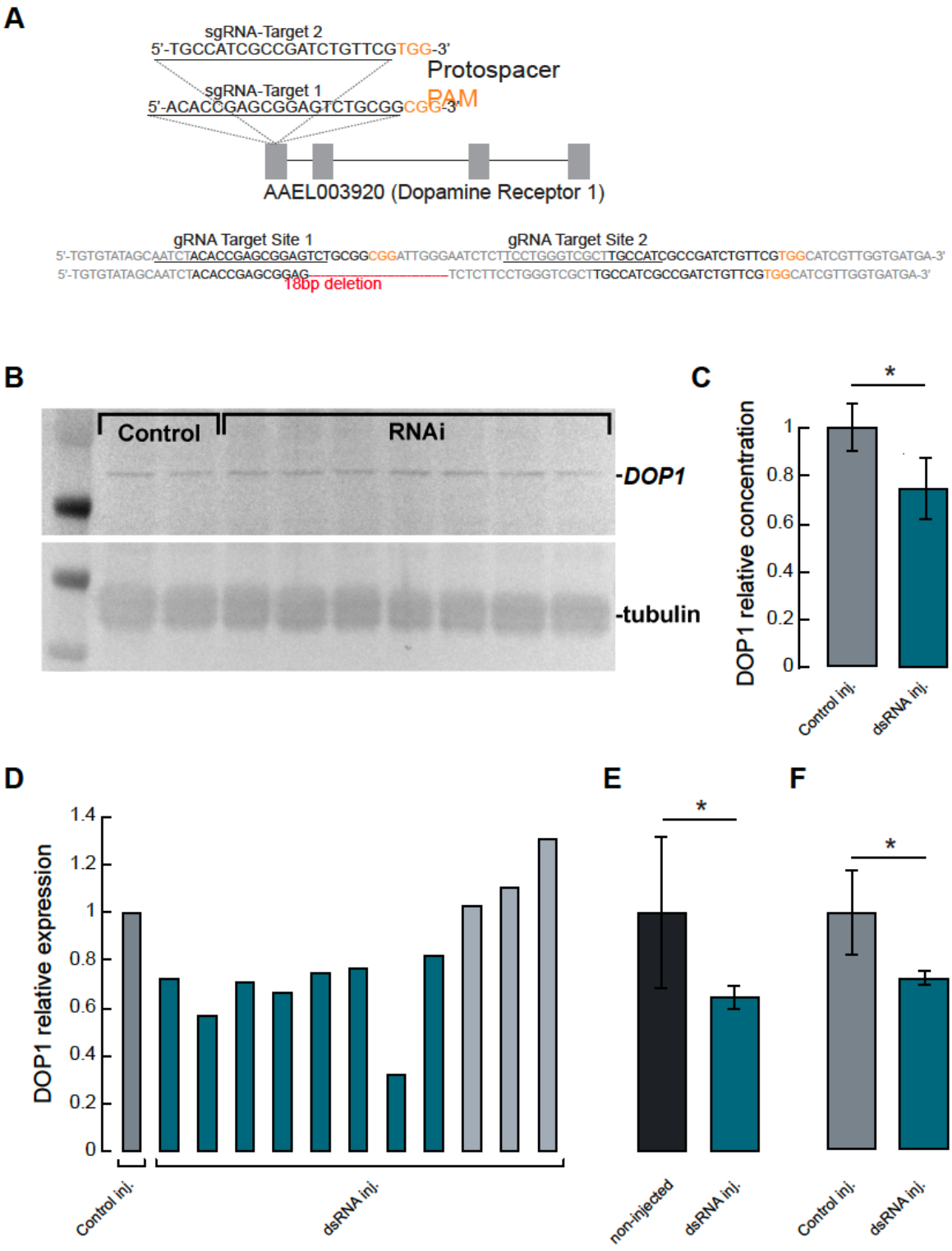

Figure S4. Dopamine Receptor 1 CRISPR Target Sites and Sequencing, and Knockdown of DOP1 in *Ae. Aegypti*, Related to Figure 3. (**A**) Two sgRNAs were designed to target the first exon of Dopamine receptor 1 (AAEL03920). Sequencing results illustrating the 18bp nucleotide deletion generated using CRISPR/Cas9 are shown in red. PAM is indicated with orange and

Protospacer is indicated with black. (**B**) Western blot assay of protein from whole *Ae. aegypti* heads from females performed 8 days after injection with either 100 ng of dsRNA (RNAi) or non-target dsRNA (Control). The blot was probed with antibodies against *DOP1* and tubulin. (**C**) Quantification of relative concentration of *DOP1* from the western blot assay in dsRNA injected mosquitoes compared to non-target dsRNA injected controls. (**D**) mRNA quantification by qPCR of *DOP1* in mosquitoes injected with 100 ng of dsRNA. Each bar represents the relative expression of *DOP1* of a single mosquito head 8 days post-injection compared to a non-target dsRNA injected control mosquito. Blue bars indicate individuals showing an efficient knock-down, light-grey bars denote individuals that were not affected by the injections. (**E,F**) Relative expression of *DOP1* in mosquitoes injected with *DOP1* dsRNA showing a knock-down compared to a non-injected control (**E**) or a non-target dsRNA injected control (**F**). Each bar corresponds to mRNA quantified by qPCR with RNA extracted from 6-18 mosquito heads 8 days post-injection.

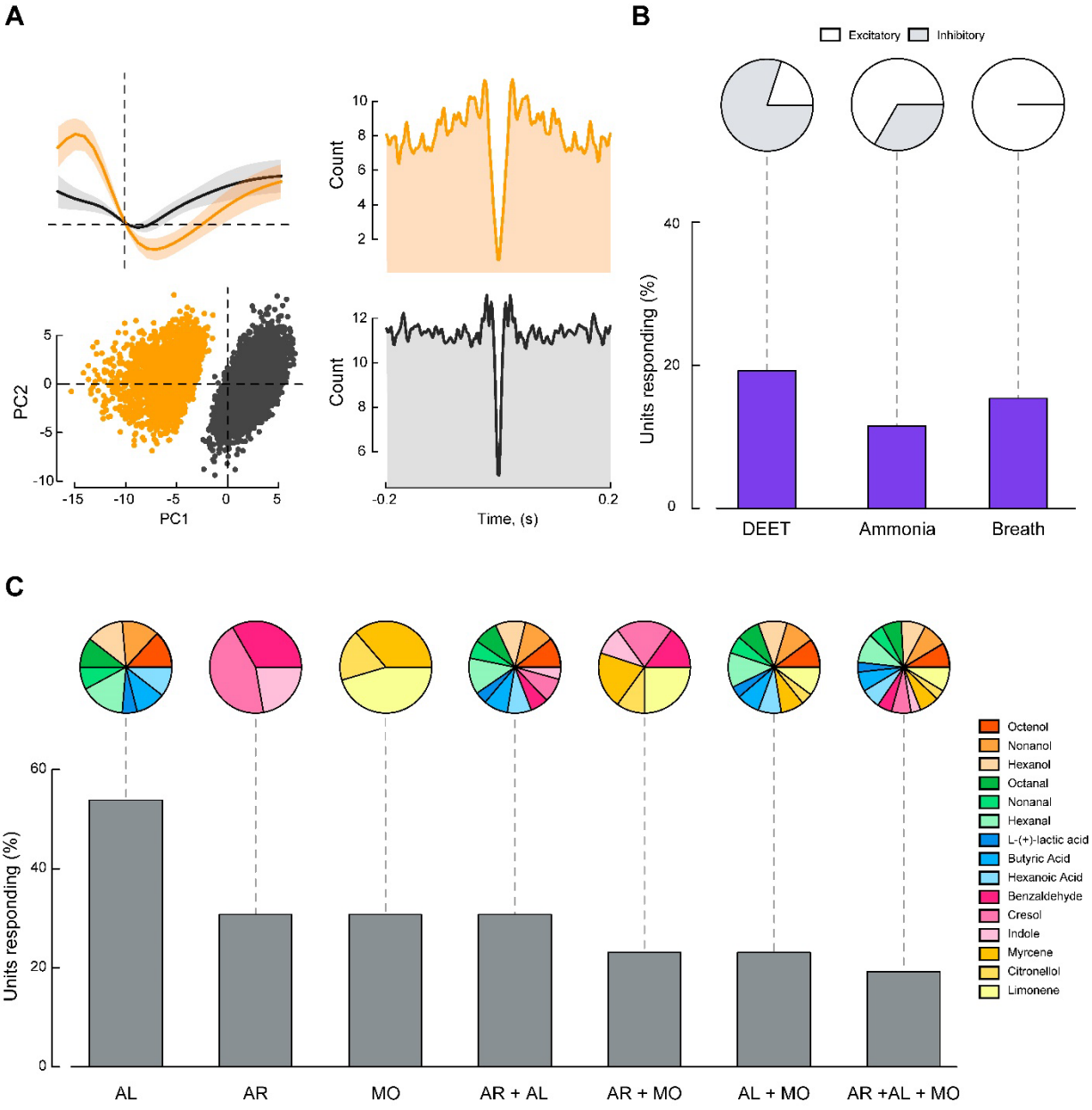

Figure S5. Sorting of Recorded Units and Tuning of AL Neural Units to Odour Stimuli, Related to Figures 4 and 6. (**A**) Neural activity was recorded using a suction electrode, plotted in 2-dimensional space, and sorted according to waveform characteristics. (*Top, left*) Mean waveshape (± SD) of simultaneously recorded units. (*Bottom, left*) First two principal components and autocorrelograms (*Bottom and Top*, *right*) based on the waveform characteristics of the two units. Units are colour-coded (orange, dark-grey) throughout panels. (**B**) Percentage of excitatory and inhibitory units that show significant response to DEET, ammonia and human breath. Pie charts denote the percentage of responsive units that were inhibitory (dark grey) and excitatory (light grey). (**C**) Percentage of units showing significant

responses to aliphatic odorants (AL), aromatics (AR), and monoterpenes (MO). Some units were also broadly responsive to odorants from different chemical classes (AR+AL; AR+MO; AL+MO; AR+AL+MO). Pie charts at the top are the percentage of units responding to the individual odorants; colours denote odorant identity.

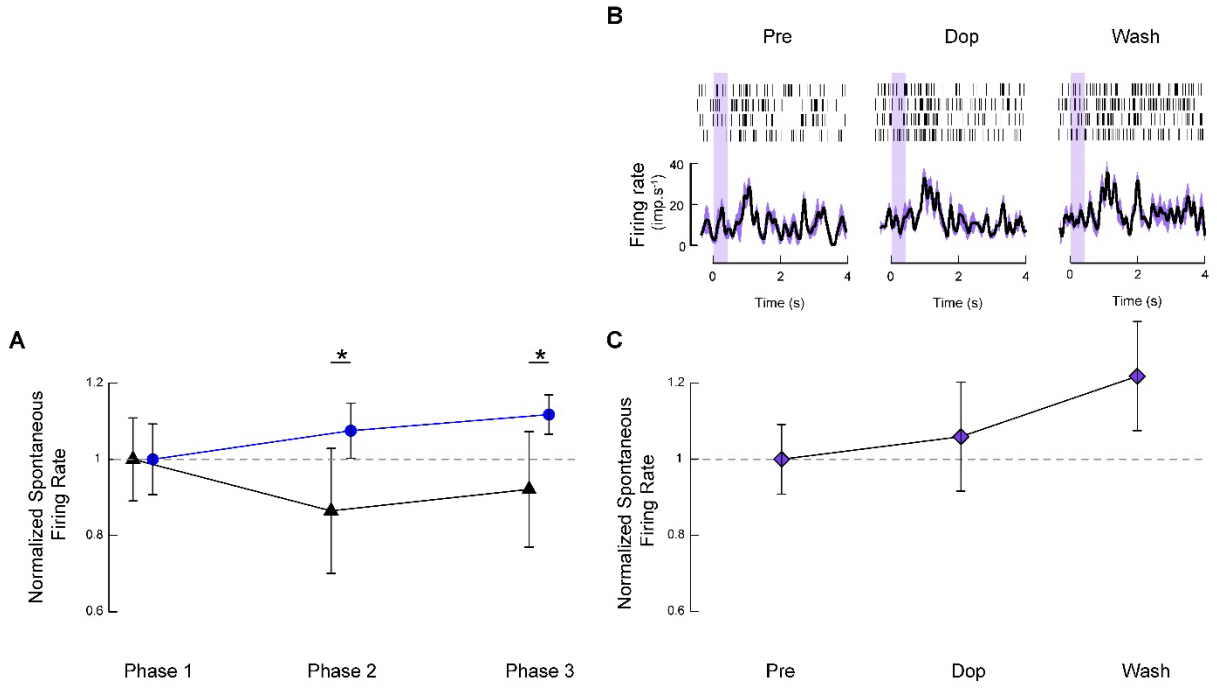

Figure S6. Spontaneous Firing Rate is Affected by Dopamine, but not by the Recording Time, and Odour-Evoked Responses and Spontaneous Activity of CRISPR Mutants are not Affected by Dopamine Application, Related to Figures 3 and 6. (**A**) Normalised mean spontaneous firing rate (± SEM) of 8 preparations before (Phase 1), during (Phase 2) and after (Phase 3) dopamine application (black triangles), and 5 "control" preparations where saline was applied (blue circles). Although there was a slight increase in spontaneous firing rate of the saline control (blue line), there is no significant difference between the three phases of saline application ($p>0.05$, pairwise comparisons using paired *t*-tests with Holm p value adjustment; $n=45$; $t=0.91$ [Phase1-Phase2], 1.42 [Phase1-Phase3] and 1.52 [Phase2-Phase3]). Similarly, there is no significant difference between the three phases of the dopamine application group (black line) ($p>0.05$, pairwise comparisons using paired *t*-tests with Holm p value adjustment; $n=72$; $t=1.96$ [Phase1-Phase2], 0.80 [Phase1-Phase3] and -1.29 [Phase2-Phase3]). By contrast, dopamine application elicited a significant reduction in spontaneous activity compared to the saline control ($p < 0.05$, pairwise comparisons using *t*-tests with pooled SD, and Holm p value adjustment; $n=39$; $t=-3.33$ for [Phase2 saline - Phase2 dopamine], and $t=-3.67$ for [Phase3 saline - Phase3 dopamine];). Asterisks denote significant differences ($p<0.05$). (**B**) Peri-event histograms of the mean (± variance) responses of an isolated unit from the extracellular recording. Vertical shaded bars represent the odour stimulus, ammonia (purple). Each column corresponds to the responses before (Pre), during (Dop) and after (Wash) dopamine application. (**C**) Mean spontaneous firing rate (± SEM) before (Pre), during (Dop) and after (Wash) dopamine application. There is no significant difference between the three phases of the dopamine application ($p>0.05$, pairwise comparisons using paired *t*-tests with Holm p value adjustment; $n=45$; $t=0.78$ [Pre-Dop], 1.67 [Pre-Wash] and 1.11 [Dop-Wash]).

Table S1. Primers for CRISPR *DOP1*, Related to Figure 3 and STAR Methods.

ID	Sequence 5'-3'
Primer 1	TGCAGGTGTTTTTCTATCGATTGTGAT
Primer 2	ACATGACATCGAACGCCACCC
Primer 3	GAAATTAATACGACTCACTATAGGACACCGAGCGGAGTCTGCGGGTTTTAGAGCTAGAAATAGC
Primer 4	GAAATTAATACGACTCACTATAGGTGCCATCGCCGATCTGTTCGGTTTTAGAGCTAGAAATAGC
Primer 5	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC