

Supplementary figures

Fig. S1. Calcium from intracellular stores contributes to the odor mediated calcium response in HEK293T cells. (A) Ionomycin response is independent of extracellular calcium. Violin plot of ionomycin response ($\log(\text{ionomycin response}) = \log(R_i/B_i)$) of transfected cells with ($n = 536$ cells from 10 independent experiments) or without ($n = 1,407$ cells from 20 independent experiments) the presence of calcium in extracellular buffer tested to 10^{-2} M EtBE. Median with same letters are not significant (Mann-Whitney U test, $p = 0.38$, $536 \leq n \leq 1,407$ cells). (B) Odor responses reduce available intracellular calcium. Violin plot of ionomycin response ($\log(\text{ionomycin response}) = \log(R_i/B_i)$) of control ($n = 1,161$ cells from 20 independent experiments) and transfected cells, plotted for responders ($n = 2,099$ cells from 50 independent experiments) and non-responders ($n = 4,574$ cells from 50 independent experiments) separately, tested to 10^{-2} M EtBE in the presence of extracellular calcium. Median with different letters differ significantly (Kruskal Wallis test, $p < 2.2e-16$, $1,161 \leq n \leq 4,574$ cells).

Fig. S2. Classification of responders and non-responders. Plot of odor response (R_o/B_o , Y-axis) against basal fluorescence (B_o , X-axis). Red circles and green triangles indicates transfected (dORs, $n = 6,663$ cells from 50 independent experiments) and control group ($n = 1,161$ cells from 12 independent experiments) of cells respectively. R_o/B_o value higher (responder) or lower (non-responder) than 1.5 (black horizontal line) was used as a threshold to distinguish responding and non responding cells.

Fig. S3. Expression levels of dORs were unaltered by over-expression of $G\alpha_{o/i}$. (A) Western blots of transfected HEK293T cell membranes showing the levels of dORs (dOr22a+Orco; 1st

blot), $G\alpha_{i1/2}$ (2nd blot), $G\alpha_o$ (3rd blot) and β -tubulin (4th blot) levels. Representative blot from four individual transfections. (B) Quantification of levels of dORs (Or22a+Orco) shown on Western blots after normalization to total protein applied to the gel (tubulin controls) from four different experiments (independent transfections). Data is shown as mean \pm SEM, ANOVA, $F_{1,14} = 0.09$, $p = 0.76$, $n = 4$ independent experiments.

Fig. S4. Adapted and tonic responses are less variable than phasic responses. (A) Plot of response max of adapted response (Y-axis) against response max of phasic response of all odorants and concentrations tested (X-axis) in female flies. Red circles, green triangles and blue pluses indicate control, $G\alpha_i$ RNAi (downregulation of $G\alpha_i$) and PTX (reduction of $G\alpha_o$) groups of flies, respectively. Red, green and blue colored lines indicate linear regression fit for the corresponding genotypes and the color coding is maintained throughout the figure. (B) Plot of response max of tonic response (Y-axis) against response max of phasic response of best ligands (statistically significant data alone included) at all concentrations tested (X-axis) in female flies. Different odor and concentrations from 5 to 7 flies for each genotype are shown.

Figure S1

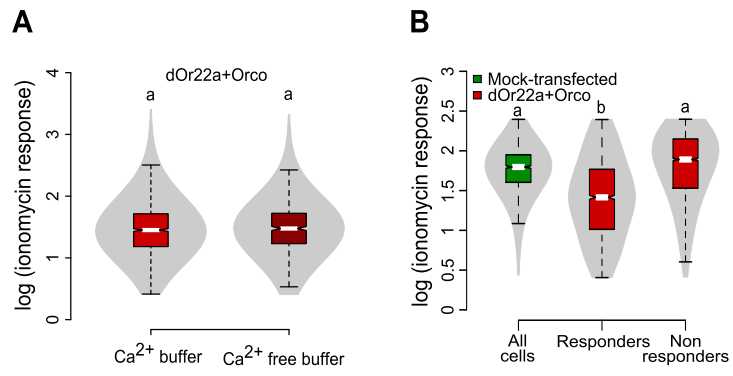


Figure S2

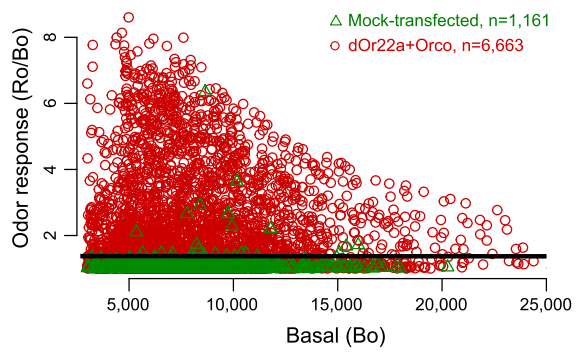


Figure S3

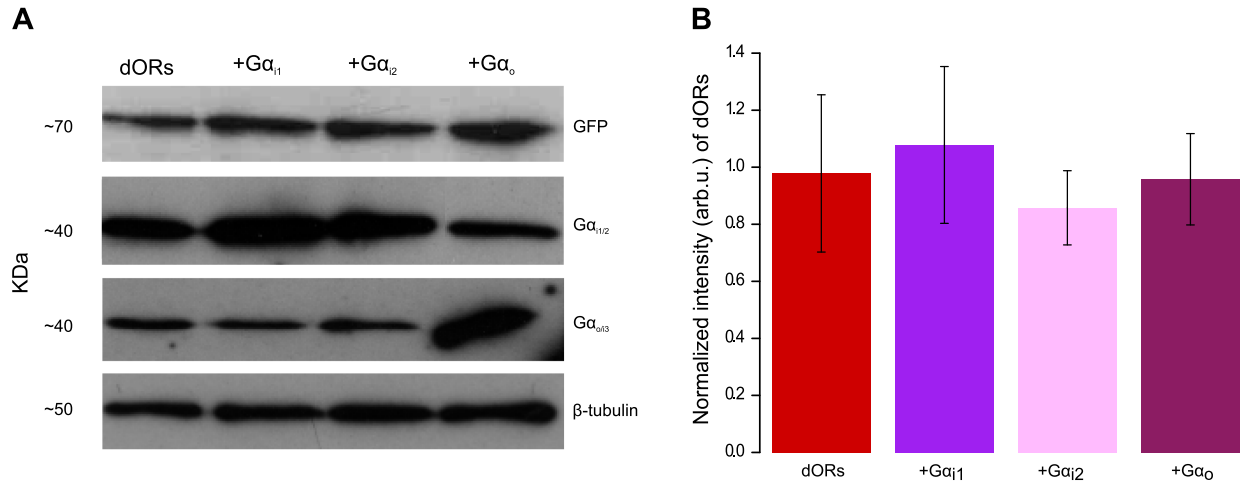


Figure S4

