Supporting Information

Abu et al. 10.1073/pnas.1719827115

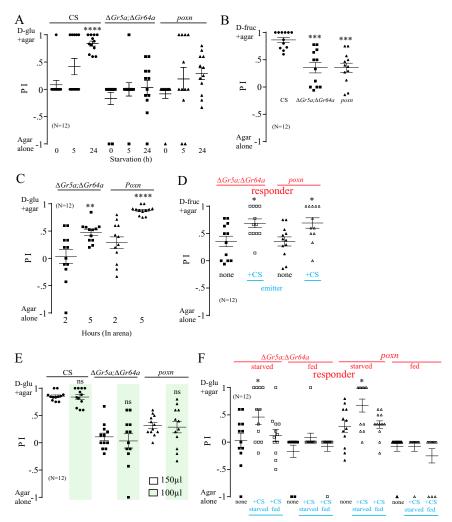


Fig. S1. (A) Preference of 24-h starved $\Delta Gr5a_i \Delta Gr64a$ mutants, $poxn^{\Delta M22-B5}$ (poxn) mutants, or WT flies in the two-choice assay after 0, 5, and 24 h of starvation. Asterisks indicate significant differences from 0 h of starvation (one-way ANOVA, followed by a Bonferroni test; n=12). (B) Preference of 24-h starved $\Delta Gr5a_i \Delta Gr64a$ and pox mutants or WT flies in a two-choice assay: p-fructose (p-fruc)+agar versus agar alone. Asterisks indicate significant differences from WT flies (one-way ANOVA, followed by a Bonferroni test; n=12). (C) Preference of 24-h starved $\Delta Gr5a_i \Delta Gr64a$ or poxn mutants in the two-choice assay after 2 h and 5 h. Asterisks indicate significant differences from those after 2 h (nonparametric Student's t test, followed by a Mann–Whitney U test; n=12). (D) Preference of 20 starved $\Delta Gr5a_i \Delta Gr64a$ or poxn responders when mixed with 10 starved WT emitters between p-fruc+agar and agar alone. Asterisks indicate significant differences from the control group, where no WT flies were mixed (nonparametric Student's t test, followed by a Mann–Whitney U test; n=12). (E) Preference of 24-h starved $\Delta Gr5a_i \Delta Gr64a$ mutants, poxn mutants, or WT flies in the two-choice assay in either 100-μL or 150-μL food drops. ns, nonsignificant differences from the control group (nonparametric Student's t test, followed by a Mann–Whitney U test; n=12). (F) Preference of $\Delta Gr5a_i \Delta Gr64a$ or $poxn^{\Delta M22-B5}$ responders, either fed or starved for 24 h, mixed with WT emitters (starved for either 0 h or 24 h) in the two-choice assay. Asterisks indicate significant differences from the control group, where no WT flies were mixed (one-way ANOVA, followed by a Bonferroni test; n=12). *P<0.05; **P<0.01; ****P<0.001; ****P<0.00

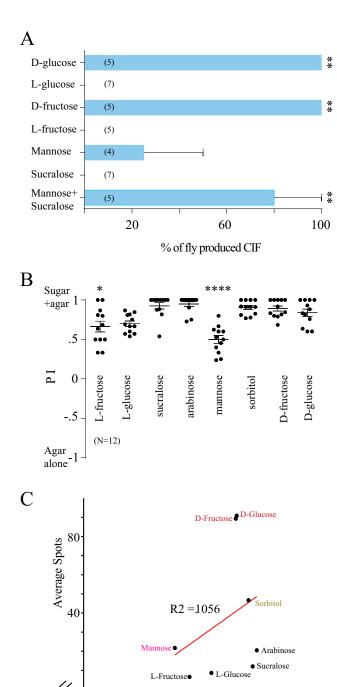


Fig. S2. (A) Percentage of starved male flies that produced CIF in the single-fly assay after they were fed with different sugars. The numbers of trials are shown in parentheses. Asterisks indicates significant differences from noncaloric sugars (one-way ANOVA, followed by a Kolmogorov–Smirnov test). (B) Preference of starved male WT flies in a two-choice arena: different sugar+agar and agar alone. ns, nonsignificant differences. Asterisks indicate significant differences from the preference for p-glucose+agar (one-way ANOVA, followed by a Bonferroni test; n = 12). (C) Linear correlation of spots produced to the preference of WT flies for different sugars used (n = 12). *P < 0.05; **P < 0.01; ****P < 0.0001. Error bars indicate SEM.

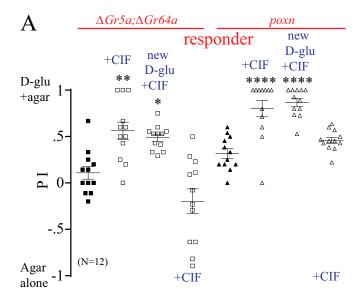
Preference Index

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Sugar +agar

.4

alone



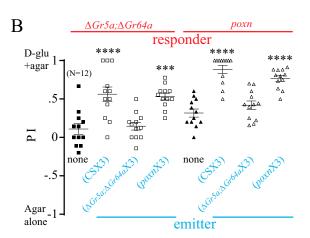


Fig. S3. (A) Preference of starved $\Delta Gr5a_i \Delta Gr64a$ or $poxn^{\Delta M22-85}$ (poxn) responders in a preexposed (CSX3) arena with starved WT flies, with a choice between p-glucose (p-glu)+agar with CIF and p-glu+agar without CIF. As a control, the mutants were given a choice between p-glu+agar without CIF and p-glu+agar without CIF. Asterisks indicate significant differences from the control group without CIF (one-way ANOVA, followed by a Bonferroni test; n=12). (B) Preference of starved $\Delta Gr5a_i \Delta Gr64a$ or poxn responders in a preexposed (CSX3) arena with starved WT, $\Delta Gr5a_i \Delta Gr64a$, or pox emitters for 2 h. Asterisks indicate significant differences from the control group when not preexposed to emitters (one-way ANOVA, followed by a Bonferroni test; n=12). *P < 0.05; **P < 0.01; ****P < 0.001; ****P < 0.001; ****P < 0.0001. Error bars indicate SEM.

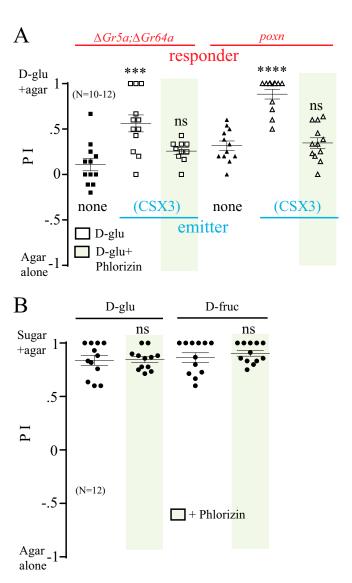


Fig. S4. (A) Preference of 30 starved $\triangle Gr5a$; $\triangle Gr64a$ or $poxn^{\triangle M22-B5}$ (poxn) responders in a two-choice assay: p-glucose (p-glu) with or without phlorizin +1% agar versus 1% agar alone. The two-choice arena was previously preexposed to WT emitters for 2 h for three rounds (CSX3) or was not preexposed. Asterisks indicate significant differences from the control group when not preexposed to WT (one-way ANOVA, followed by a Bonferroni test; n=10-12). (B) Preference of 30 starved WT flies in a two-choice assay: p-glu+agar with or without phlorizin versus agar alone and p-fructose (p-fruc)+agar with or without phlorizin versus agar alone. ns, insignificant differences from the control group without phlorizin (nonparametric Student's t test, followed by a Mann–Whitney t test; t 12). ****t2 0.001; ****t3 0.001. Error bars indicate SEM.

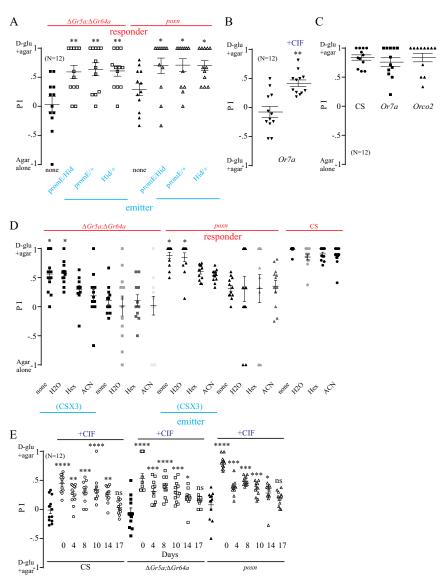


Fig. S5. (A) Preference of starved $\Delta Gr5a;\Delta Gr64a$ or $poxn^{\Delta M22-B5}$ (poxn) responders (20 flies) when mixed with starved promE-Gal4 > UAS-Hid flies as emitters (10 flies) and UAS-Hid/ $^+$, promE-Gal4/ $^+$ flies as a control in the two-choice assay. Asterisks indicate significant differences from the control group when no promE-Gal4 > UAS-Hid flies were mixed (one-way ANOVA, followed by a Bonferroni test; n = 12). (B) Preference of starved Or7a knockout flies in a two-choice arena with p-glucose (p-glu)+agar with CIF and p-glu+agar without CIF. Asterisks indicate significant differences from the control group without CIF (non-parametric Student's t test, followed by a Mann-Whitney U test; n = 12). (C) Preference of 30 starved male Or7a knockout or $Orco^2$ mutants in the two-choice assay. ns, insignificant differences from the control group (one-way ANOVA, followed by a Bonferroni test; n = 12). (D) Preference of starved $\Delta Gr5a;\Delta Gr64a$, poxn, or WT responders in the preexposed arena (p-glu+agar and agar alone) washed with water, hexane, or acetonitrile. As a control, the preference of starved $\Delta Gr5a;\Delta Gr64a$, poxn, or WT responders in the arena not exposed to emitters washed with water (H₂O), hexane (Hex), or acetonitrile (ACN) was measured. Asterisks indicate significant differences from the control group without being preexposed or washed (one-way ANOVA, followed by a Bonferroni test; n = 12). (E) Preference of starved male $\Delta Gr5a;\Delta Gr64a$, poxn, or WT responders in the arena with a choice of p-glu+agar with CIF versus p-glu+agar without CIF after differences from the control group without CIF (one-way ANOVA, followed by a Bonferroni test; n = 12). P0.05; **P0.05; **P0.001; ****P0.001; ****P0.0001. Error bars indicate SEM.

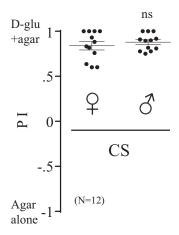


Fig. S6. Preference of male or female WT flies in the two-choice assay. D-glu, D-glucose. ns, nonsignificant difference (nonparametric Student's t test, followed by a Mann–Whitney U test; n = 12). Error bars indicate SEM.

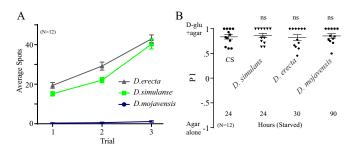


Fig. 57. (A) Average number of spots produced by 30 starved male *Drosophila simulans*, *Drosophila erecta*, and *Drosophila mojavensis* flies in a two-choice arena containing p-glucose (p-glu)+agar or agar alone for 2 h [trial 1 (T1)]. Trials 2 and 3 refer to second and third additional 2-h exposures to new sets of 30 starved WT flies. (B) Preference of *Drosophila melanogaster* WT CS or other species (D. simulans, D. erecta, and D. mojavensis) in the two-choice assay with respect to corresponding starvation periods. ns, nonsignificant differences from WT (one-way ANOVA, followed by a Bonferroni test; n = 12). Error bars indicate SEM.



Movie S1. Starved female fly releasing CIF after ingesting p-glucose.



Movie S2. Starved male fly releasing CIF after ingesting D-fructose.



Movie S3. Starved male fly not releasing CIF after ingesting sucralose.

Movie S3



 $\textbf{Movie S4.} \quad \text{Starved male fly not releasing CIF after ingesting ι-glucose}.$

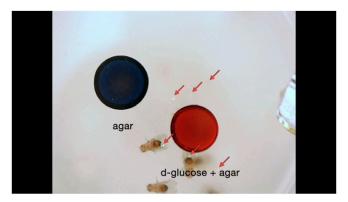


Movie S5. Starved male fly not releasing CIF after ingesting L-fructose.



Movie S6. Starved male fly releasing CIF after ingesting mannose.

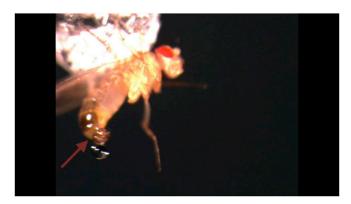
Movie S6



Movie 57. Starved male flies releasing CIF in two-choice arena with agar containing p-glucose versus plain agar, recorded from the bottom of the arena.



Movie S8. Starved male fly releasing CIF after ingesting mannose+sucralose.



Movie S9. Starved male fly releasing CIF after ingesting D-glucose.