# Supplemental Information
# Calcium taste avoidance in *Drosophila*

Youngseok Lee, Seeta Poudel, Yunjung Kim, Dhananjay Thakur, and Craig Montell

## Figure S1 (Related to Figure 1)

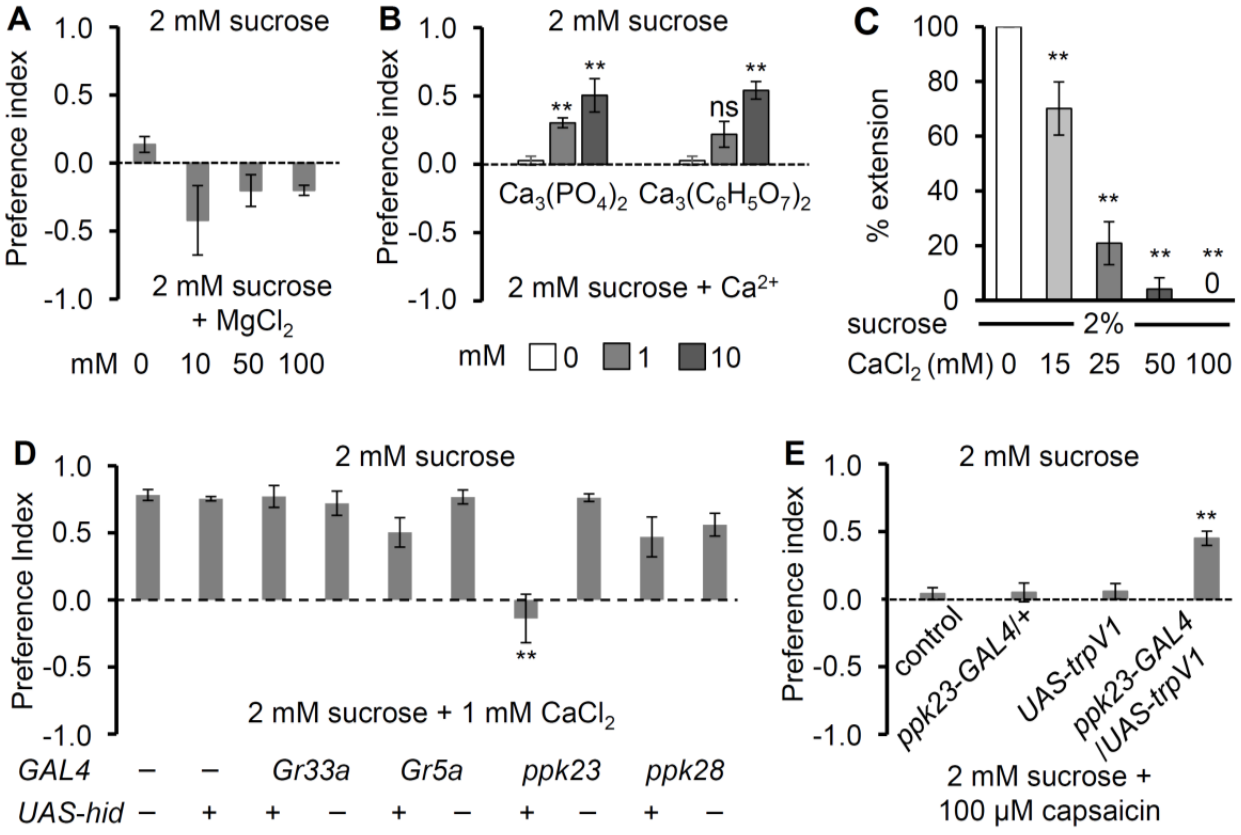



**Figure S1. Monitoring gustatory behavior using two-way choice and PER assays.**
(A) Two-way food choice assays performed with control flies. The animals were allowed to choice between 2 mM sucrose alone, and 2 mM sucrose mixed with the indicated $MgCl_2$ concentrations. n=4.
(B) Two-way food choice assays performed with control flies, using the indicated concentrations of calcium phosphite and calcium citrate. n=4.
(C) Proboscis extension response (PER) assays performed by applying 2% sucrose

and the indicated concentrations of $CaCl_2$ to the labellum of control flies. n=4.
(D) Binary food choice assays were performed to determine the type of GRN required for $CaCl_2$ avoidance. Different classes of GRNs were ablated by expressing a pro-apoptotic gene (*UAS-hid*) under the control of the *Gr33a-GAL4* (*Gr33a*$^{GAL4}$/+), *Gr5a-GAL4*, *ppk23-GAL4*, or *ppk28-GAL4*. n=4.
(E) Testing the gustatory response to activation of *ppk23* GRNs by feeding capsaicin to *trpV1*-expressing flies. Binary food choice assays were performed after feeding 100 μM capsaicin to the indicated flies. n=4. The error bars indicate SEMs. ANOVA tests with Scheffe's post hoc analyses. The comparisons were between: (A-C) control flies offered sucrose only, and (D-E) control flies without a *GAL4* or *UAS-hid*. $^{**}P < 0.01$.

# Figure S2 (Related to Figure 3)

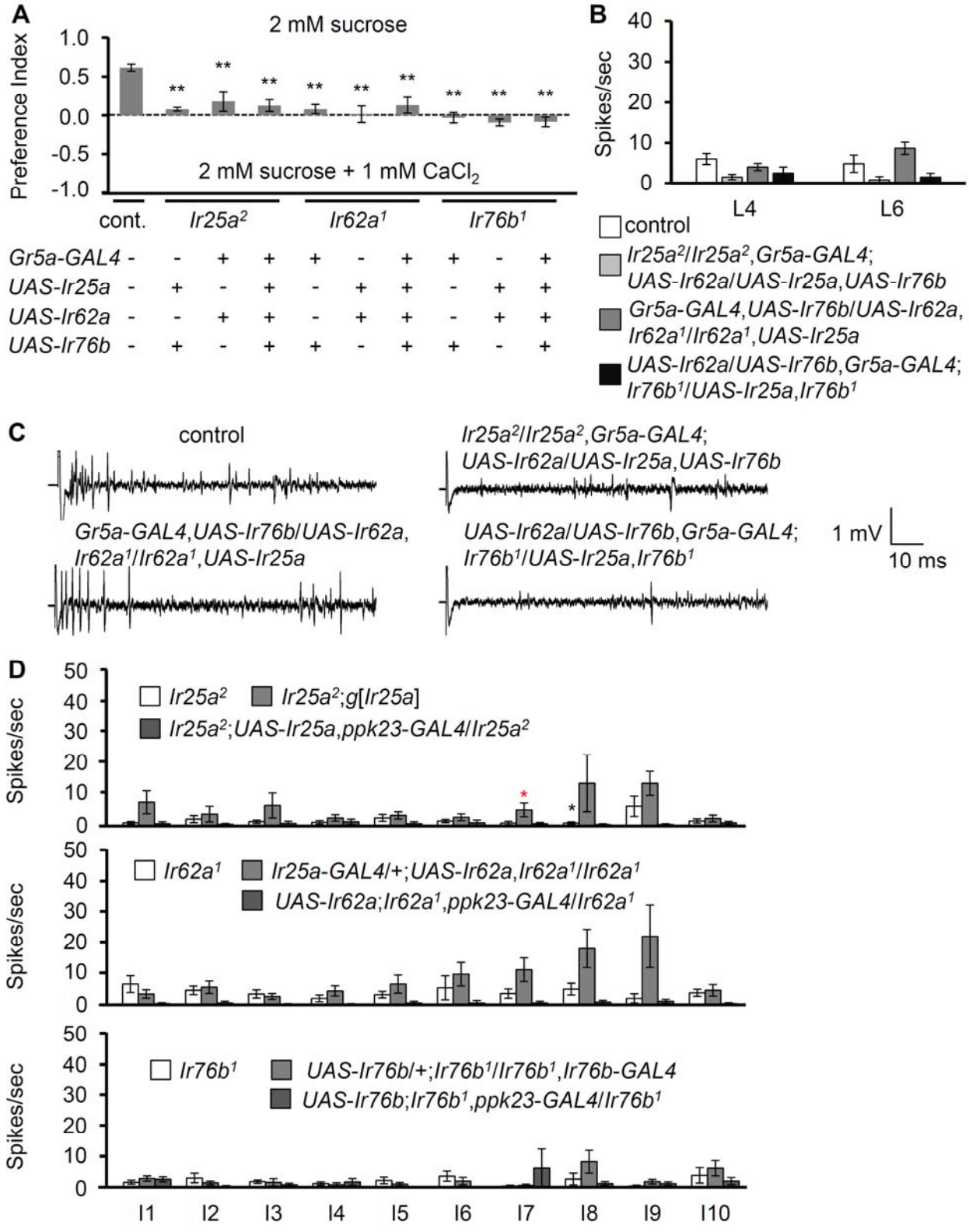


**Figure S2. Misexpression of *Ir25a*, *Ir62a* and *Ir76b* in sugar sensing GRNs and the $Ca^{2+}$ response from l-type sensilla**.
(A—C) *UAS-Ir25a*, *UAS-Ir62a* and *UAS-Ir76b* were expressed in sugar-sensing GRNs using the *Gr5a-Gal4* in $Ir25a^{2}$, *Ir62a* and $Ir76b^{1}$ mutant backgrounds. The flies were tested for acquisition of behavioral and elecrophysiological responses to 50 mM $CaCl_2$. (A) Binary food choice feeding assays. n=4. (B) Tip recordings on L4, and L6 sensilla using 50 mM $CaCl_2$ and the indicated flies. n=10. (C) Representative traces from L4 sensilla.
(D) Mean responses of the indicated l-type sensilla from the $Ir25a^{2}$, $Ir62a^{1}$ or $Ir76b^{1}$ mutants, or the mutants expressing either the genomic rescue transgene (*g*[*Ir25a*]) or a *UAS-cDNA* rescue transgene under control of the indicated *GAL4*s. n=10—11. The error bars indicate SEMs. ANOVA tests with Scheffe's post hoc were analyzed to compare two sets of data. $^{**}P < 0.01$.

## Figure S3 (Related to Figure 4)

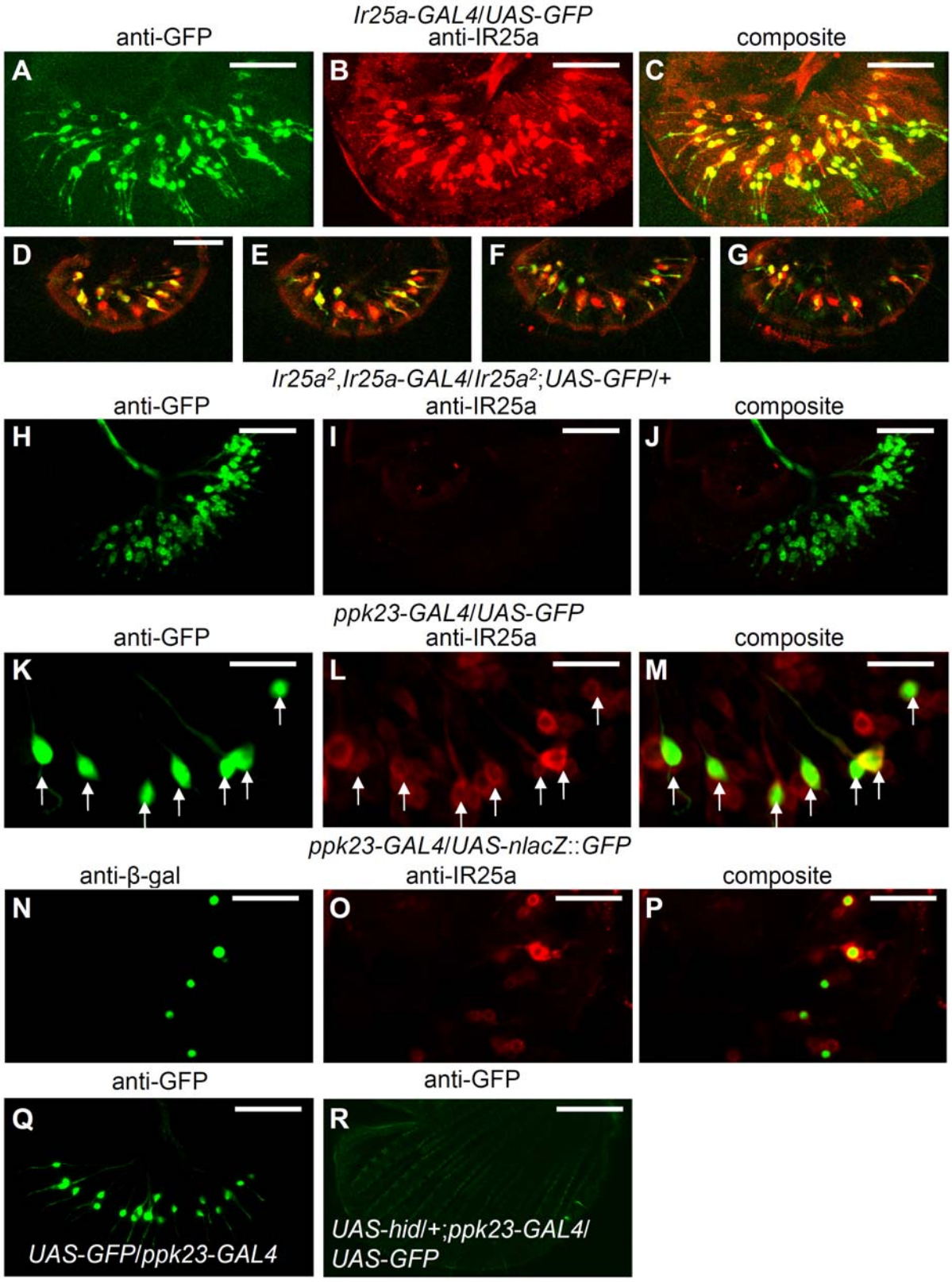
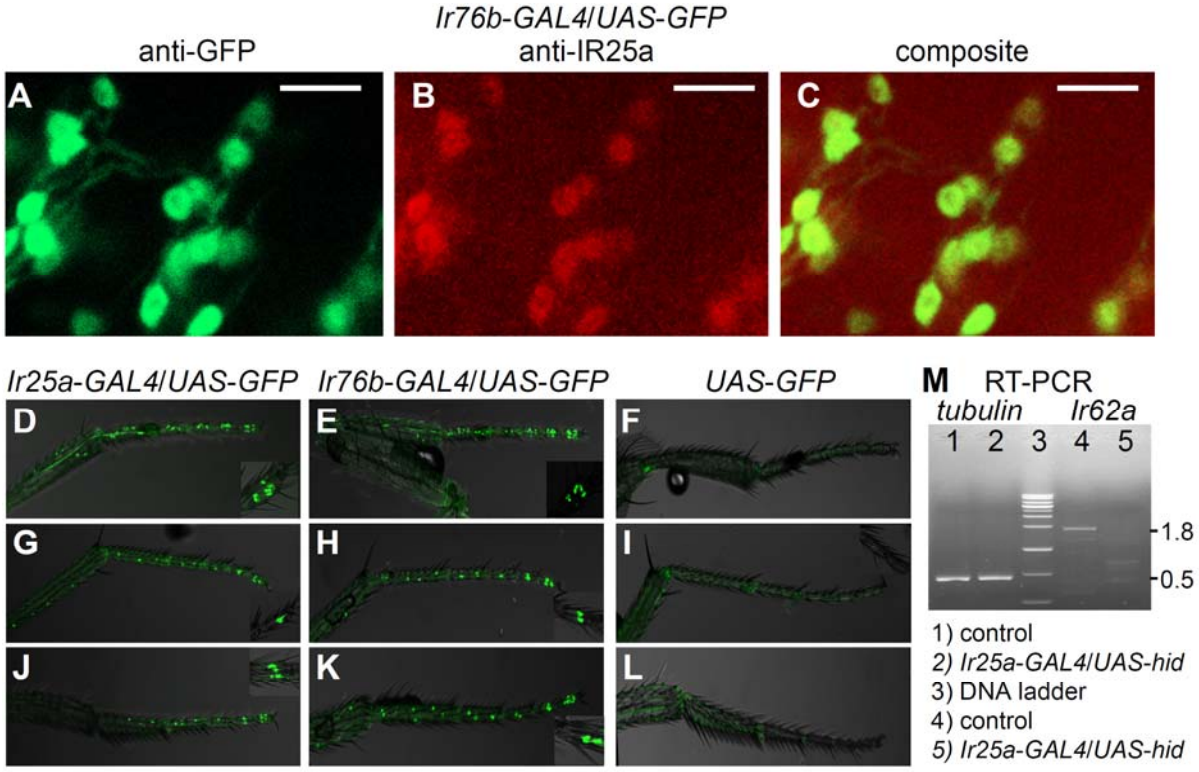
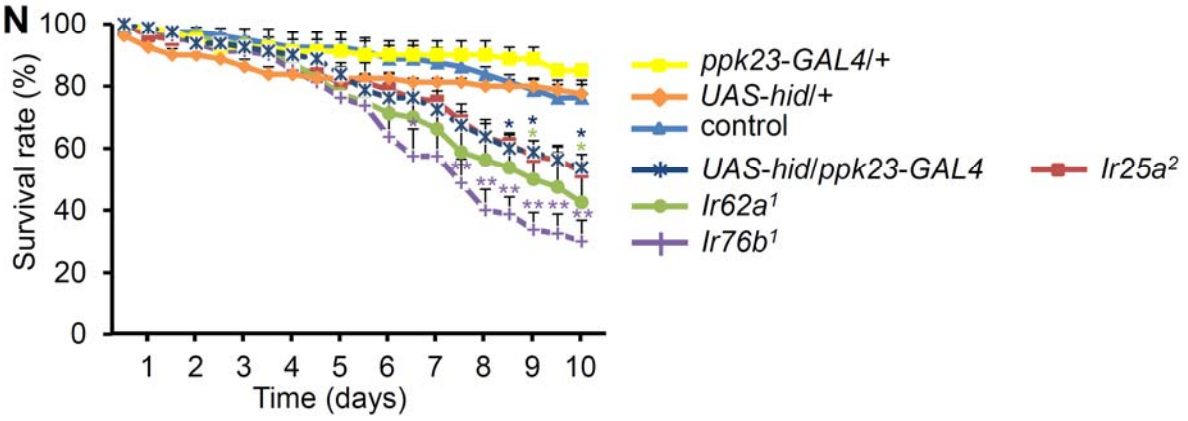


**Figure S3. Testing for co-localization of the *Ir25a-Gal4*, and the *ppk23-GAL4* reporters with anti-IR25a in the labellum.**
(A—C) Labella from *Ir25a-GAL4*/*UAS-GFP* flies were stained with: (A) anti-GFP and (B) anti-IR25a. (C) Merged images from A and B.
(D—G) A series of confocal sections from *Ir25a-GAL4*/*UAS-GFP* flies showing extensive overlap between anti-GFP (green) and anti-IR25a (red) staining.
(H—J) Loss of anti-IR25a staining in $Ir25a^{2}$,*Ir25a-GAL4*/$Ir25a^{2}$;*UAS-GFP*/+ flies.
(K—M) *ppk23-GAL4*/*UAS-GFP* flies stained with: (K) anti-GFP and (L) anti-IR25a. (M) Merge of K and L.
(N—P) Labella from *ppk23-GAL4*/*UAS-nlacZ*::*GFP* flies stained with: (N) anti-β-galactosidase (anti-β-gal) and (O) anti-IR25a. (P) Merge of N and O.
(Q and R) Effect of expression of *UAS-hid* under the control of the *ppk23-GAL4* on cell survival in the labella. The indicated flies were stained with anti-GFP. The scale bars represent 25 μm.

## Figure S4 (Related to Figure 4)



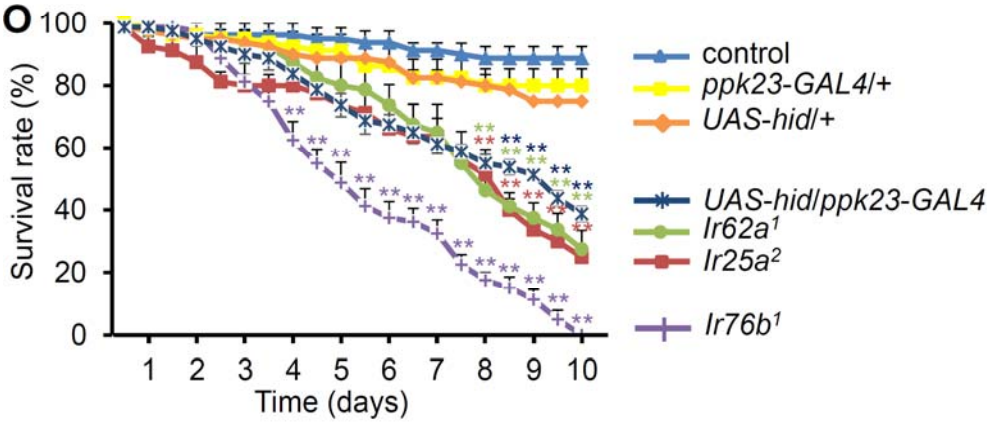



**Figure S4. *Ir* expression in the labellum and legs, and increased lethality of *Ir* mutants in a binary $Ca^{2+}$ feeding assay.**
(A—C) Co-localization of the *Ir76b-GAL4* reporters with anti-IR25a. (A) anti-GFP. (B) anti-IR25a. (C) Merged image of A and B.
(D—L) Expression of *UAS-GFP* in the legs under control of the *Ir25a-Gal4*, and *Ir76b-GAL4*. (D—F) Forelegs. (G—I) Mid legs. (J—L) Hind legs.
(M) RT-PCR analyses using RNA isolated from control ($w^{1118}$) and *Ir25a-GAL4*/*UAS-hid* labella. The flies used for the indicated RT-PCR analyses (lanes 1, 2, 4 and 5) are indicated below. *tubulin* served as the control. The DNA ladder (lane 3) was from Enzynomics (DM002). The sizes of the RT-PCR products are indicated to the right in kb.
(N and O) Survival of flies in a food environment in which they were allowed to choose between 100 mM fructose versus: (N) 200 mM fructose and 50 mM $CaCl_2$. n=4. (O) 200 mM fructose and 100 mM $CaCl_2$. n=4. The error bars represent SEMs. The asterisks indicate significant differences from the fructose only feeding (***P*<0.01, **P*<0.05.) using single factor ANOVA with Scheffe's analysis as a *post hoc* test to compare two sets of data.