Figure S1

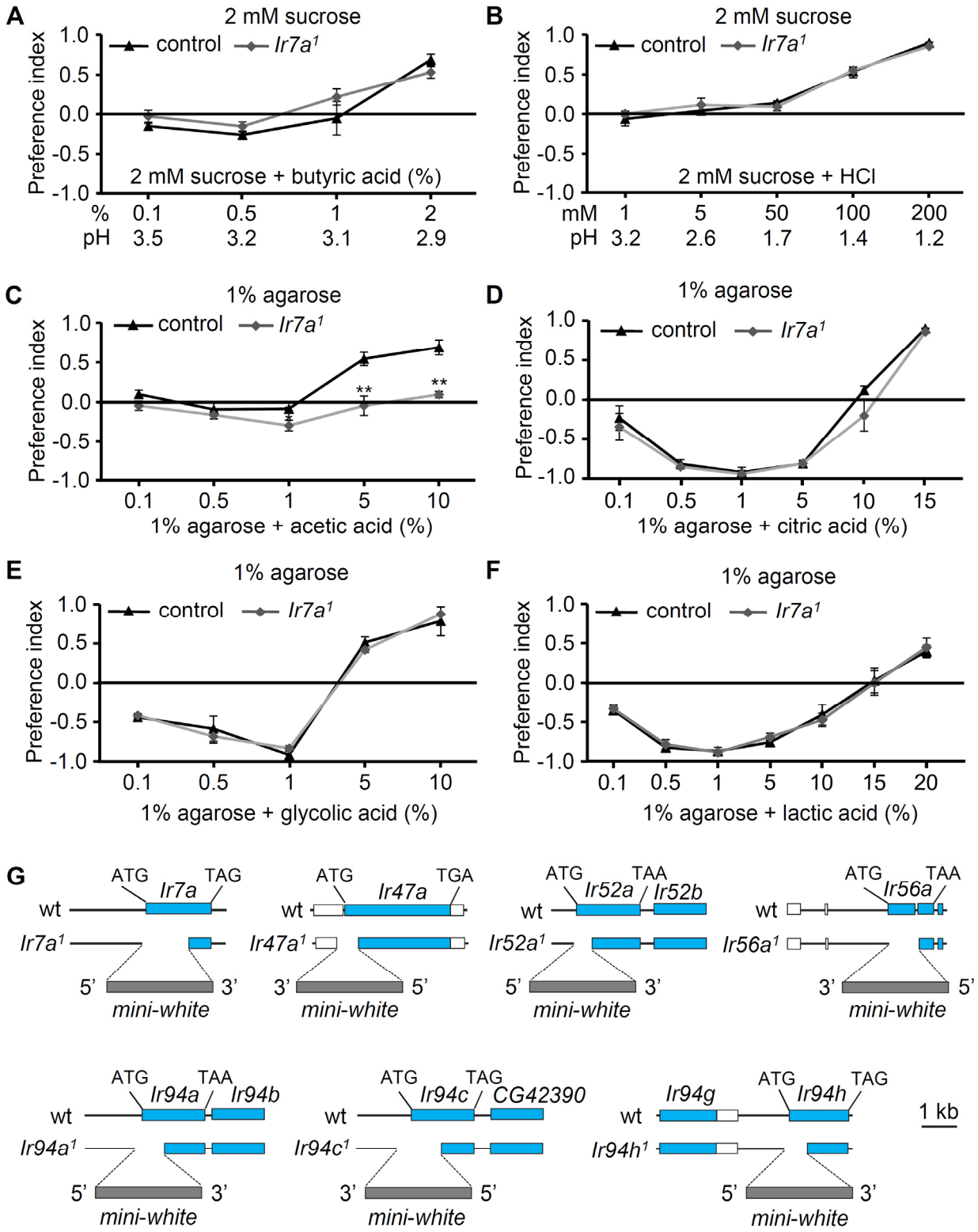
A
2 mM sucrose
control
Ir7a1
Preference index
2 mM sucrose + butyric acid (%)
% 0.1 0.5 1 2
pH 3.5 3.2 3.1 2.9
B
2 mM sucrose
2 mM sucrose + HCl
mM 1 5 50 100 200
pH 3.2 2.6 1.7 1.4 1.2
C
1% agarose
1% agarose + acetic acid (%)
**
D
1% agarose
1% agarose + citric acid (%)
E
1% agarose
1% agarose + glycolic acid (%)
F
1% agarose
1% agarose + lactic acid (%)
G
ATG Ir7a TAG
wt
Ir7a1
5' 3'
mini-white
ATG Ir47a TGA
Ir47a1
ATG Ir52a / Ir52b TAA
Ir52a1
ATG Ir56a TAA
Ir56a1
ATG Ir94a / Ir94b TAA
Ir94a1
ATG Ir94c / CG42390 TAG
Ir94c1
Ir94g ATG Ir94h TAG
Ir94h1
1 kb

Figure S1. Behavioral preferences of flies to acids and generation of *Ir* mutants. Related to Figure 1.

(A—B) Two-way choice feeding assays showing preferences of control (w^{1118}) and $Ir7a^{1}$ flies upon presentation of 2 mM sucrose alone, versus 2 mM sucrose with the indicated concentrations of (A) butyric acid, and (B) HCl. n=4-6.

(C—F) Preferences of control (w^{1118}) and $Ir7a^{1}$ flies to the indicated concentrations of (C) acetic acid, (D) citric acid, (E) glycolic acid, and (F) lactic acid without sucrose. n=4-6.

(G) *Ir* mutants generated by ends-out homologous recombination.

The error bars indicate SEMs. The asterisks indicate significant differences from the controls using ANOVA tests with Scheffe's *post hoc* analyses between wild-type and mutants. (**$p < 0.01$).

Figure S2

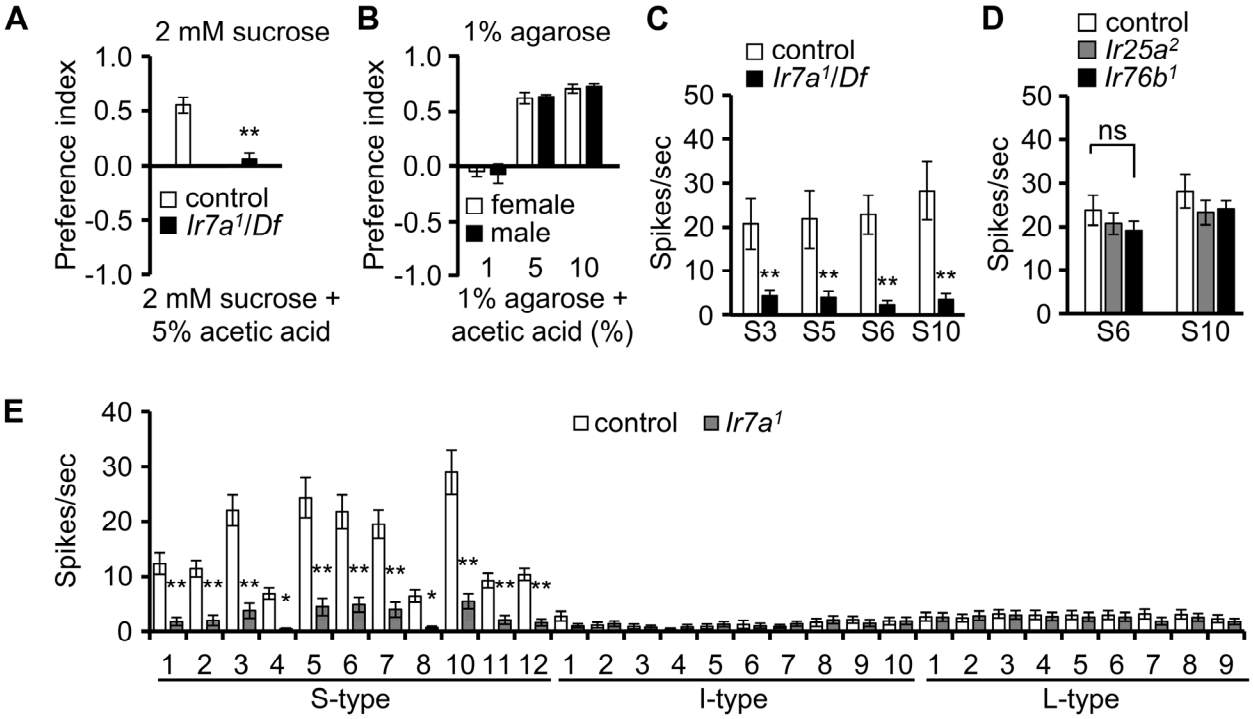

Figure S2. Analyses with control flies after starvation, *Ir7a*^{1}*/Df*, *Ir25a*^{2}, *Ir76b*^{1} flies, and behavioral response of bitter GRN ablated and rescue flies in the absence of sucrose. Related to Figures 1 and 2.

(A) Two-way choice feeding assay using flies harboring the *Ir7a*^{1} mutation *in trans* with a deficiency that removes *Ir7a* (*Ir7a*^{1}*/Df*). n=4-6.

(B) Two-way choice assay performed separately with males and females. n=4-6.

(C) Frequencies of action potentials elicited by controls, and *Ir7a*^{1}*/Df* flies. n=10-12.

(D) Frequencies of 1% acetic acid-induced action potentials elicited from S6 and S10 sensilla from control (*w*^{1118}), *Ir25a*^{2}, and *Ir76b*^{1} flies. n=20.

(E) Average frequencies of action potentials elicited from S, I, and L type sensilla in response to 1% acetic acid after the control and *Ir7a*^{1} flies were starved for 24 hrs. n=18-30.

The error bars indicate SEMs. The asterisks indicate significant differences from the controls using ANOVA tests with Scheffe's *post hoc* analyses. **p < 0.01.

Figure S3

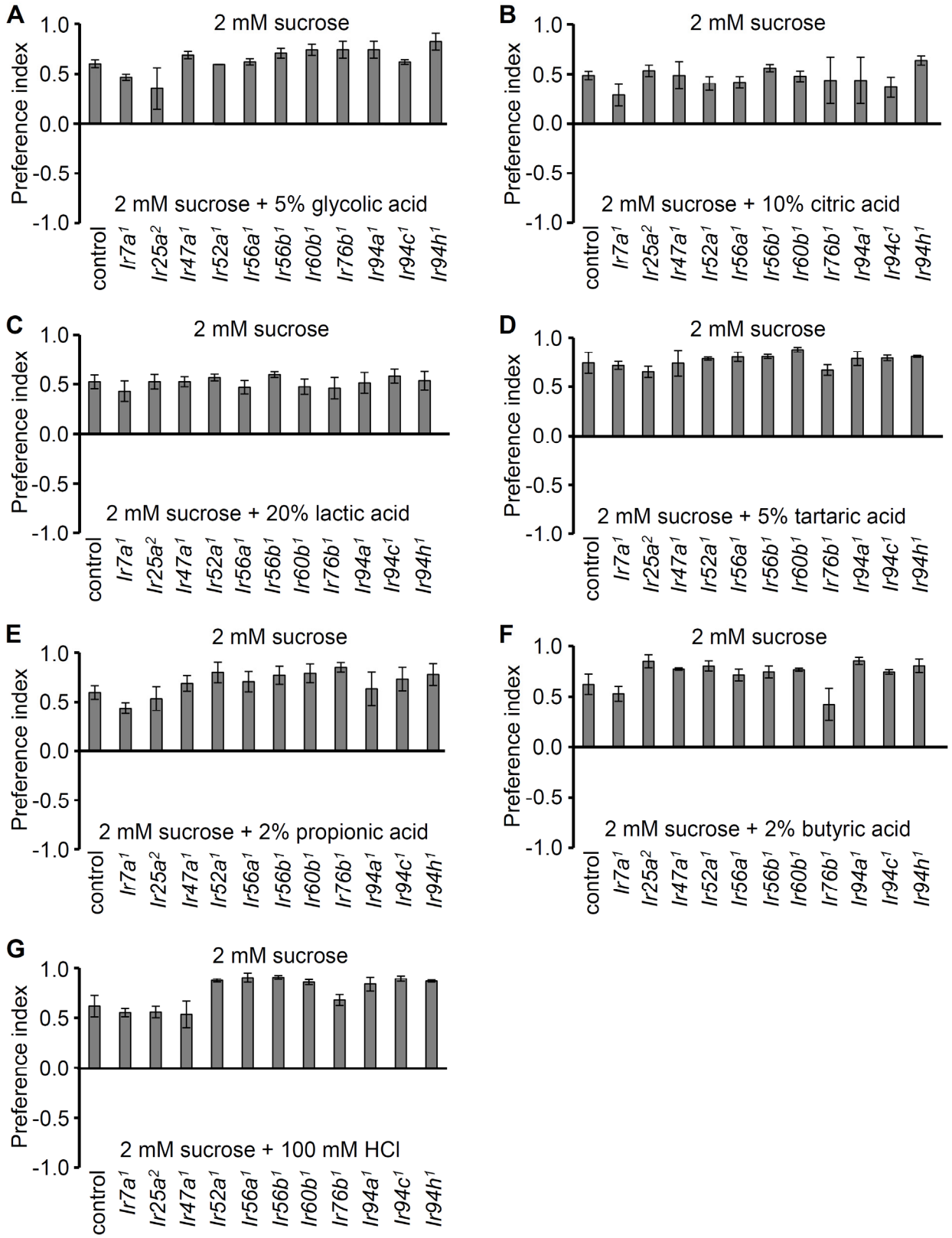
A
2 mM sucrose
Preference index
1.0
0.5
0.0
-0.5
-1.0
2 mM sucrose + 5% glycolic acid
control
Ir7a1
Ir25a2
Ir47a1
Ir52a1
Ir56a1
Ir56b1
Ir60b1
Ir76b1
Ir94a1
Ir94c1
Ir94h1
B
2 mM sucrose
2 mM sucrose + 10% citric acid
C
2 mM sucrose
2 mM sucrose + 20% lactic acid
D
2 mM sucrose
2 mM sucrose + 5% tartaric acid
E
2 mM sucrose
2 mM sucrose + 2% propionic acid
F
2 mM sucrose
2 mM sucrose + 2% butyric acid
G
2 mM sucrose
2 mM sucrose + 100 mM HCl

Figure S3. Screening of *Ir* mutants to test avoidances to the indicated acids using binary choice assays. Related to Figure 1.
The flies were given a choice between 2 mM sucrose versus 2 mM sucrose and the indicated acids. n=4-6.
Significant differences between the controls and the mutants were tested using ANOVA tests with Scheffe's *post hoc* analyses.

Figure S4

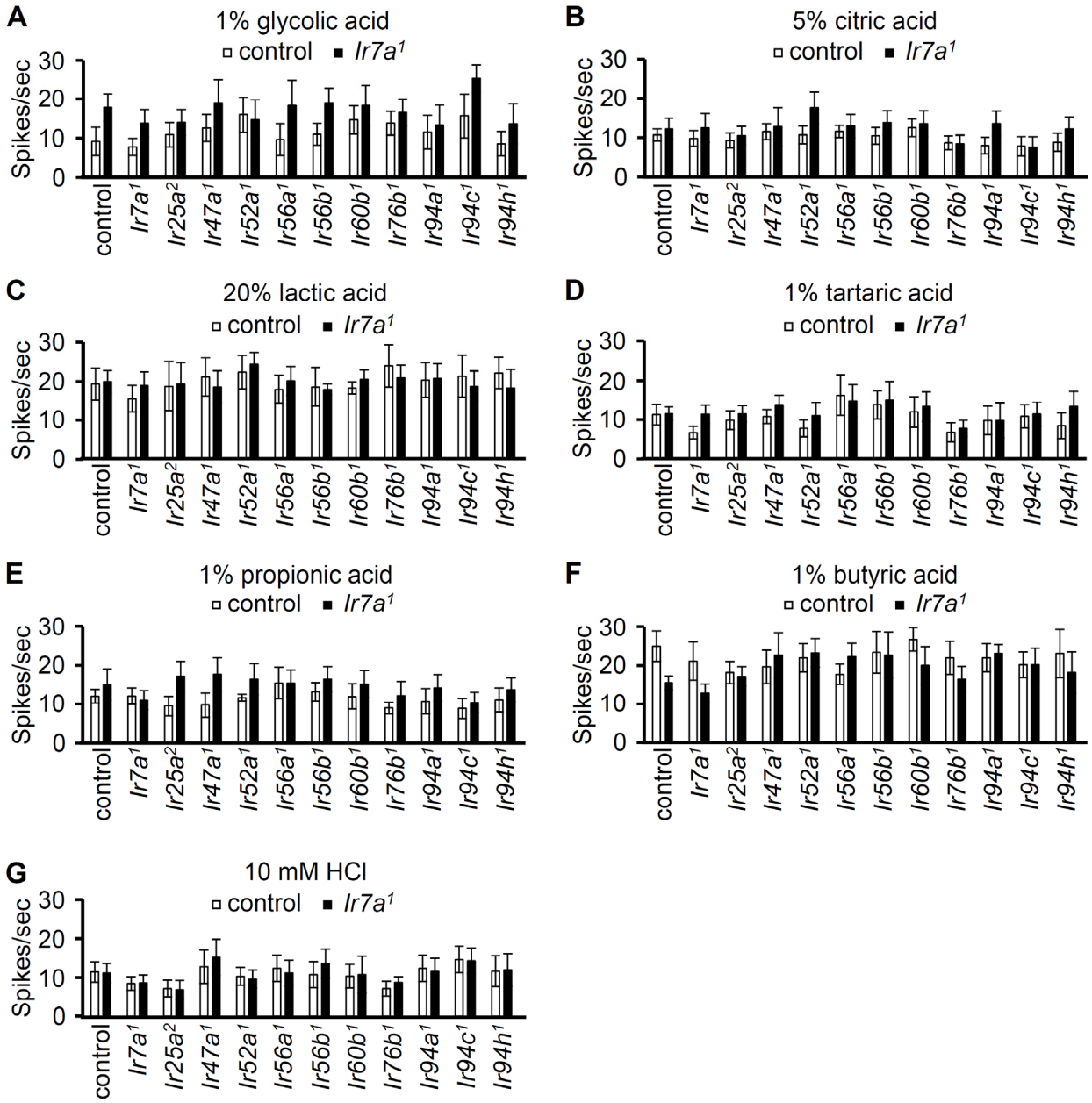

Figure S4. Acid-induced action potentials displayed by *Ir* mutants. Related to Figure 2.

Frequencies of action potentials obtained by performing tip recording on S6 and S10 sensilla from control (w^{1118}) and *Ir* mutant flies in response to the indicated concentrations of organic acids and HCl. n=12-20.

Significant differences between the controls and the mutants were tested using ANOVA tests with Scheffe's *post hoc* analyses.

Figure S5

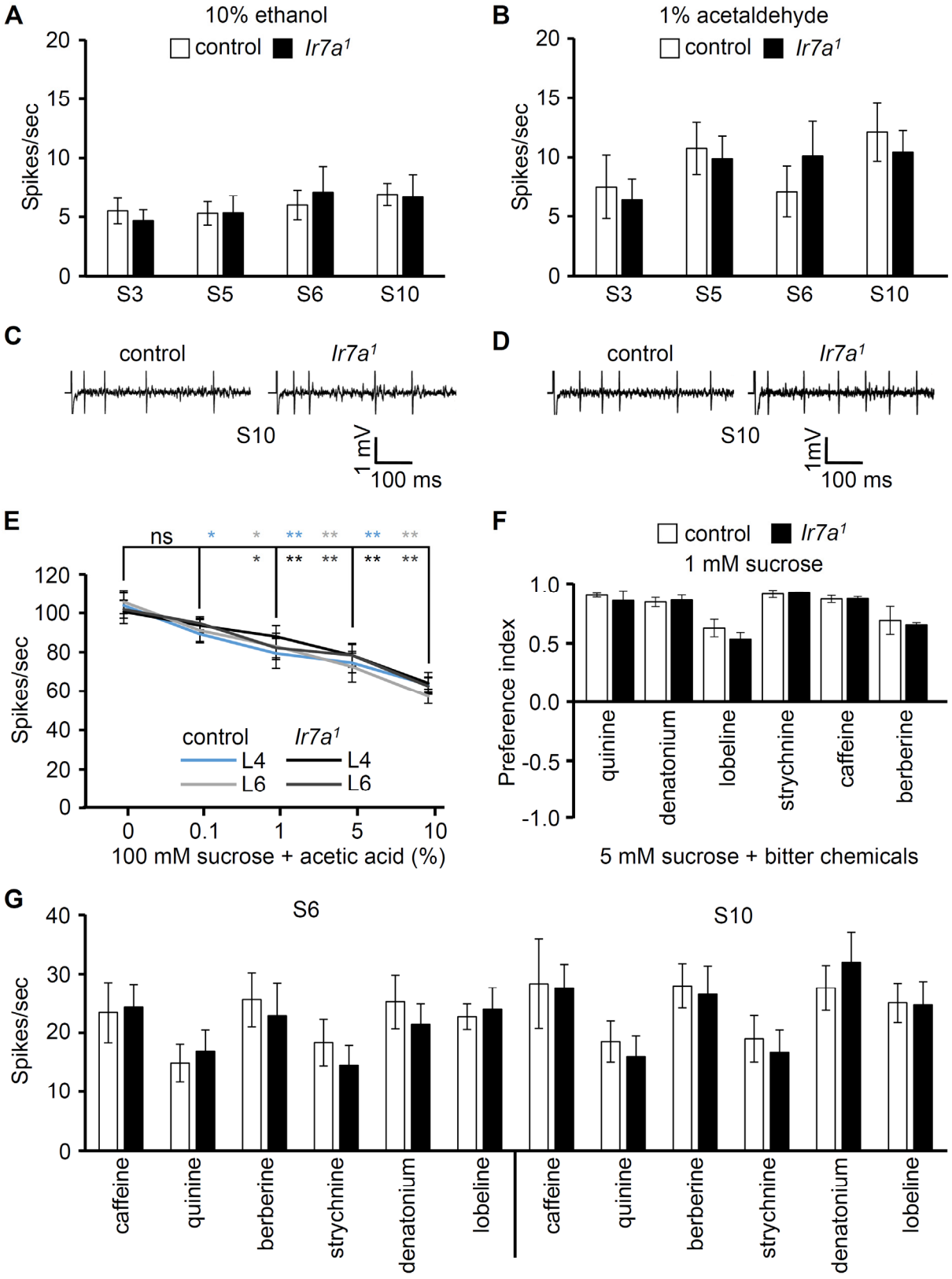
A
10% ethanol
control
Ir7a1
Spikes/sec
S3
S5
S6
S10
B
1% acetaldehyde
C
S10
1 mV
100 ms
D
E
ns
100 mM sucrose + acetic acid (%)
L4
L6
F
1 mM sucrose
Preference index
quinine
denatonium
lobeline
strychnine
caffeine
berberine
5 mM sucrose + bitter chemicals
G
S6
S10

Figure S5. Testing roles for *Ir7a* for acetic acid inhibition of sugar GRNs, and for sensing molecules with similar carbon backbones as acetic acid. Related to Figure 3.

(A—B) Average frequencies of action potentials elicited by several S-type sensilla from control and *Ir7a*^{1} flies in response to ethanol and acetaldehyde, which have similar carbon backbones as acetic acid.

(A) 10% ethanol. n=12-14.

(B) 1% acetaldehyde. n=15-19.

(C—D) Representative tip recording traces obtained from S10 sensilla from control and *Ir7a*^{1} flies.

(C) 10% ethanol.

(D) 1% acetaldehyde.

(E) Average frequencies of action potentials elicited from L4 and L6 sensilla using 100 mM sucrose and the indicated concentrations of acetic acid. n=10-12. There are no significant differences between the control and the *Ir7a*^{1} mutant.

(F) Two-way choice assays in response to 1 mM sucrose alone versus 5 mM sucrose plus bitter compounds at the following concentrations: 1 mM quinine, 0.3 mM denatonium, 0.3 mM lobeline, 0.5 mM strychnine, 10 mM caffeine, and 0.1 mM berberine. n=4-6.

(G) Average frequencies of action potentials elicited from S6 and S10 sensilla in response to 1 mM quinine, 1 mM denatonium, 1 mM lobeline, 1 mM strychnine, 10 mM caffeine, and 0.1 mM berberine. n=10-12.

The error bars represent SEMs. The asterisks indicate significant differences (*$p < 0.05$, **$p < 0.01$) using unpaired Student *t*-tests for comparing two sets of data or ANOVA with Scheffe's analysis as a *post hoc* test to compare two sets of data.

Figure S6

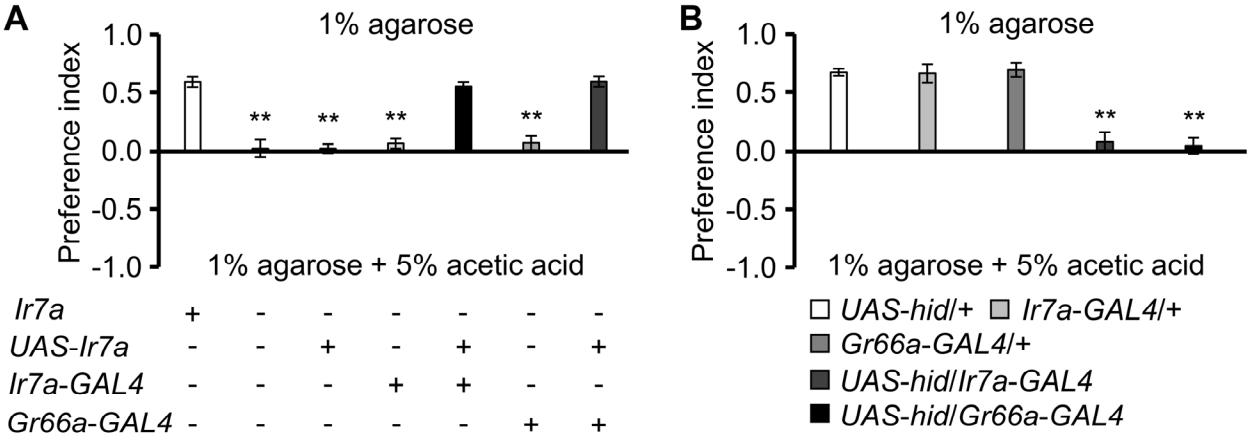

Figure S6. Testing for rescue of *Ir7a*-dependent acetic acid repulsion and for the effect of killing *Ir7a* GRNs with *hid* using two-way choice assays without sugar. Related to Figure 4.

(A) Two-way choice feeding assays without sucrose showing rescue of the avoidance defect in $Ir7a^{1}$ in response to 5% acetic acid. n=6.

(B) Two-way food choice assays without sucrose after expressing the cell death gene, *hid* (*UAS-hid*), under control of either the *Ir7a-GAL4* or the *Gr66a-GAL4*. n=6.

The error bars indicate SEMs. The asterisks indicate significant differences from the controls using ANOVA tests with Scheffe's *post hoc* analyses. **p < 0.01.

Figure S7

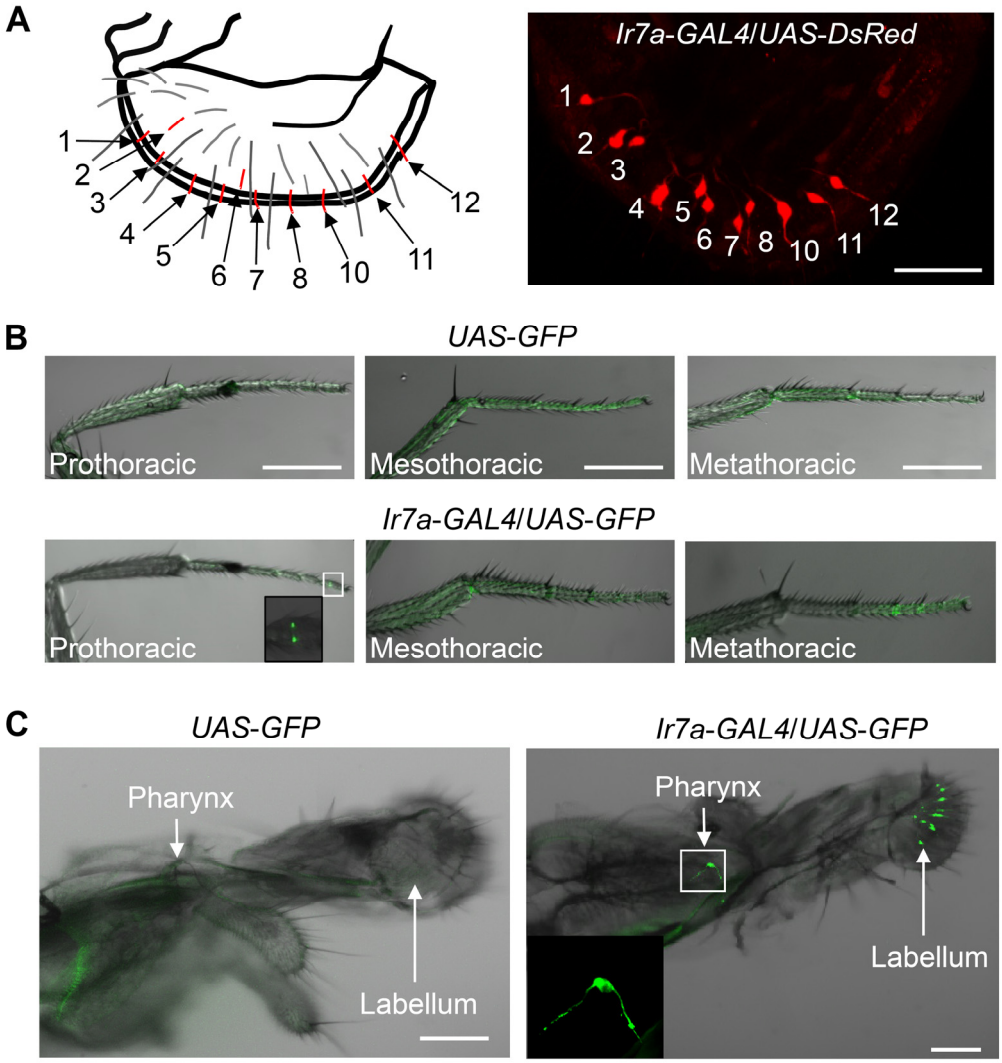

Figure S7. Expression pattern of the *Ir7a-GAL4* reporter in the labellum, legs and pharynx. Related to Figure 5.

UAS-DsRed was expressed under the control of the *Ir7a-GAL4*. The signals were detected by staining with anti-DsRed and viewed by confocal microscopy.

(A) Schematic representation of the 11 S-type sensilla in the labellum that were labeled by the *Ir7a-GAL4* reporter. This is the same image presented in Figure 5A. The scale bars represent 25 μm.

(B) Expression of the *Ir7a* reporter in the legs of control (w^{1118}) flies. The upper and lower panels show flies harboring the *UAS-GFP* transgene only, and *UAS-GFP* plus the *Ir7a-GAL4* transgenes, respectively. The inset in the image of the prothoracic leg is a magnified region indicated by the box. The scale bars represent 100 μm.

(C) *Ir7a* reporter expression in the pharynx of control (w^{1118}) flies. The panel on the left is a proboscis from a fly containing *UAS-GFP* transgene only. The panel on the right shows expression of *UAS-GFP* under control of the *Ir7a-GAL4*. The inset is a magnified region indicated by the box. The scale bars represent 100 μm.