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Supplementary Information for
Requirement for an Otopetrin-Like protein for acid taste in *Drosophila*

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Figures S1 to S4

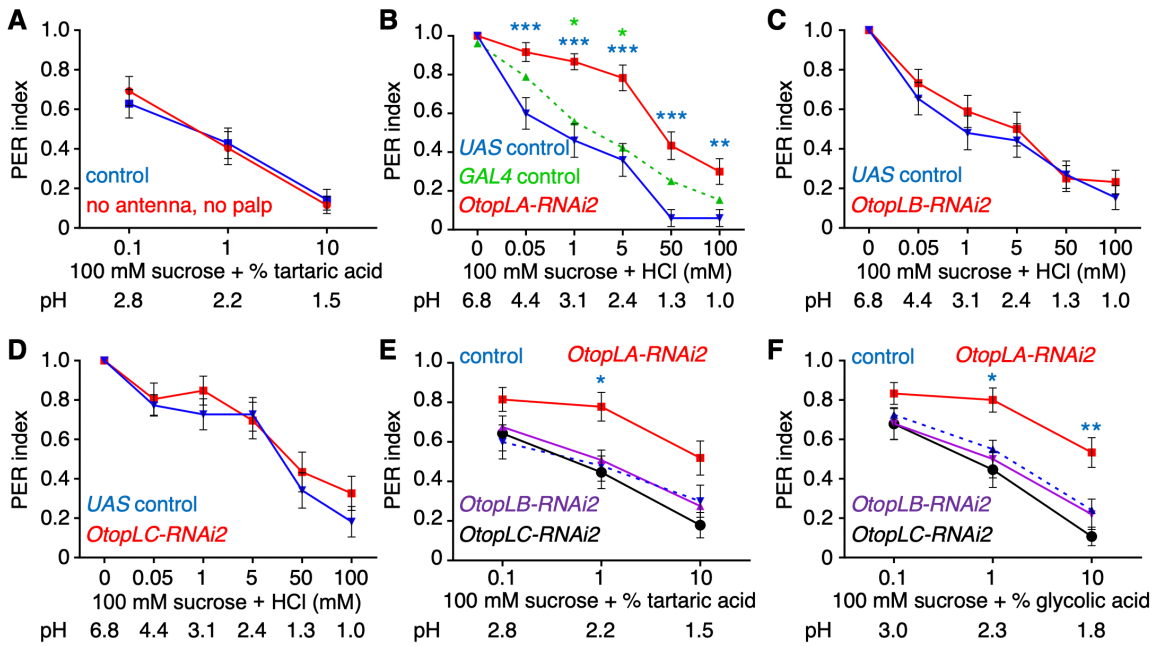


Fig. S1. Screen with second set of RNAi lines further supports that *OtopLA* is required for aversion of acid taste. PER assays using either 100 mM sucrose alone or sucrose plus the indicated concentrations of HCl (mM) or organic acids (%). All RNAi lines (B—F) were generated by crossing the indicated *UAS-RNAi* lines to *elav-GAL4;UAS-Dcr2* flies. (*A*) Effects of removing olfactory organs on the PER responses to the indicated concentrations of tartaric acid. The w^{1118} flies (intact flies) and the w^{1118} flies in which the antenna and maxillary palp were surgically removed (no antenna, no palp). Intact flies, n=35. No antenna and no maxillary palp, n=26. (*B—F*) Effects of RNAi knockdown of different *OtopL* genes on the PER responses to the indicated acids. (*B*) Effect of knockdown with *OtopLA*-RNA2 on the PER to sucrose plus HCl. *OtopLA-RNAi2* is *UAS-OtopLA-RNAi2* (v100847) crossed to *elav-GAL4;UAS-Dcr2* flies (n=32). The *UAS* control (n=26) and *GAL4* control (n=25) were generating by crossing w^{1118} to either *UAS-OtopLA-RNAi2* or *elav-GAL4;UAS-Dcr2*, respectively. The blue and green asterisks indicate statistically significance differences between *OtopLA* silenced flies and the *UAS* and *GAL4* controls, respectively. The results with the *GAL4* control (green dotted line) are the same as that used in Fig. 1*B*. (*C*) Effect of knockdown with *OtopLB-RNAi2* on the PER to sucrose plus HCl. *OtopLB-RNAi2* is *UAS-OtopLB-RNAi2* (v3452) crossed to *elav-GAL4;UAS-Dcr2* flies (n=27). *UAS* control (n=26). (*D*) Effect of knockdown of *OtopLC-RNAi2* on the PER to sucrose plus HCl. *OtopLC-RNAi1* is *UAS-OtopLC-RNAi* (v19613) crossed to *elav-GAL4;UAS-Dcr2* flies (n=26). *UAS* control (n=27). (*E*) Effect of knockdown with *OtopLA-RNAi2* (n=30), *OtopLB-RNAi2* (n=25) and *OtopLC-RNAi2* (n=28) on PERs using the indicated concentrations of tartaric acid. *OtopLA-RNAi1* (n=30), *OtopLB-RNAi1* (n=25), *OtopLC-RNAi1* (n=28). The *GAL4* "control" is *elav-GAL4;UAS-Dcr2* flies (n= 65) and is also presented in Fig. 1*E*. The blue asterisks indicate significant differences from the control. (*F*) Effect of knockdown with *OtopLA-RNAi2* (n=30), *OtopLB-RNAi2* (n=25) and *OtopLC-RNAi2* (n=28) on PERs using the indicated concentrations of glycolic acid. The *GAL4* "control" is *elav-GAL4;UAS-Dcr2* flies (n= 65) and is also presented in Fig. 1*F*. Mann-Whitney U tests. Error bars, s.e.m.s. *p < 0.05, **p < 0.01, and ***p < 0.001.

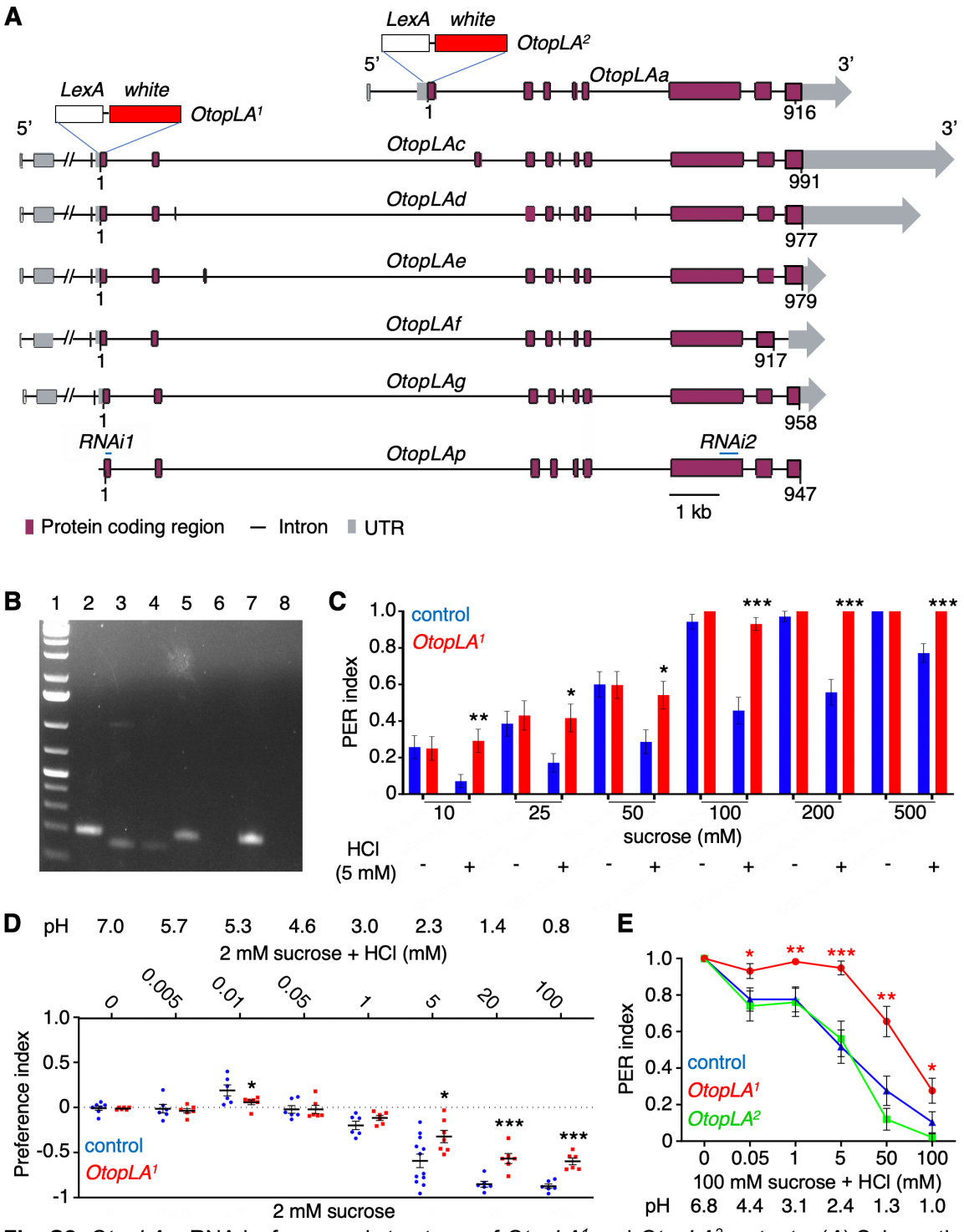


Fig. S2. *OtopLA* mRNA isoforms and structures of *OtopLA¹* and *OtopLA²* mutants. (*A*) Schematic of the *OtopLA* isoforms and the *OtopLA¹* and *OtopLA²* mutants. The protein coding exons are indicated in purple and the horizontal lines indicate introns. To create *OtopLA¹* the first 40 base pairs of the coding sequence common to *OtopLAc—OtopLAg* and *OtopLAp* were replaced with *lexA* and *mini-white*. To generate *OtopLA²*, the first 52 base pairs of the coding region of *OtopLAa* were replaced with *lexA* and *mini-white*. The regions targeted by *RNAi1* and *RNAi2* are indicated.

(*B*) RT-PCR using cDNAs synthesized from heads of control, *OtopLA*^{1} and *OtopLA*^{2}. Lane 1: GeneRuler 1 kb Plus DNA Ladder (Thermo Fisher, SM1331). Lanes 2, 5 and 7 show *syt1* RT-PCR products (192 bp) from control, *OtopLA*^{1} and *OtopLA*^{2} cDNAs (primers: forward, tctggtcgtgcttcgagaag; reverse, cggatccctatgtcaaggtg). Lanes 3 and 6 are *OtopLAc-p* RT-PCR products using control (143 bp band) and *OtopLA*^{1} (no band) cDNAs, respectively (primers: forward, cagcggtgtccctacatt; reverse, ccatcgccctgcagctggttg). Lanes 4 and 8 are *OtopLAa* RT-PCR products from control (141 bp) and *OtopLA*^{2} (no band), respectively (primers: forward, atgggcggcggtgaagtgaaggt; reverse, ttccatctccttgttggcgg). *(C)* PERs elicited by control (*w*^{1118}) and *OtopLA*^{1} flies in response to stimulation with the indicated concentrations of sucrose with or without 5 mM HCl as indicated. Control (*w*^{1118}), n=35. *OtopLA*^{1}, n=36. (*D*) Binary choice assays showing preferences of control (*w*^{1118}) and *OtopLA*^{1} flies to 2 mM sucrose versus 2 mM sucrose mixed with the indicated concentrations of HCl. n=6—12. *(E)* PER assays showing that *OtopLA*^{2}, which specifically disrupts *OtopLAa*, does not impact on the aversion to HCl. Control (*w*^{1118}), n=29. *OtopLA*^{1}, n=29. *OtopLA*^{2}, n=25. Mann-Whitney U tests. Error bars, s.e.m.s. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

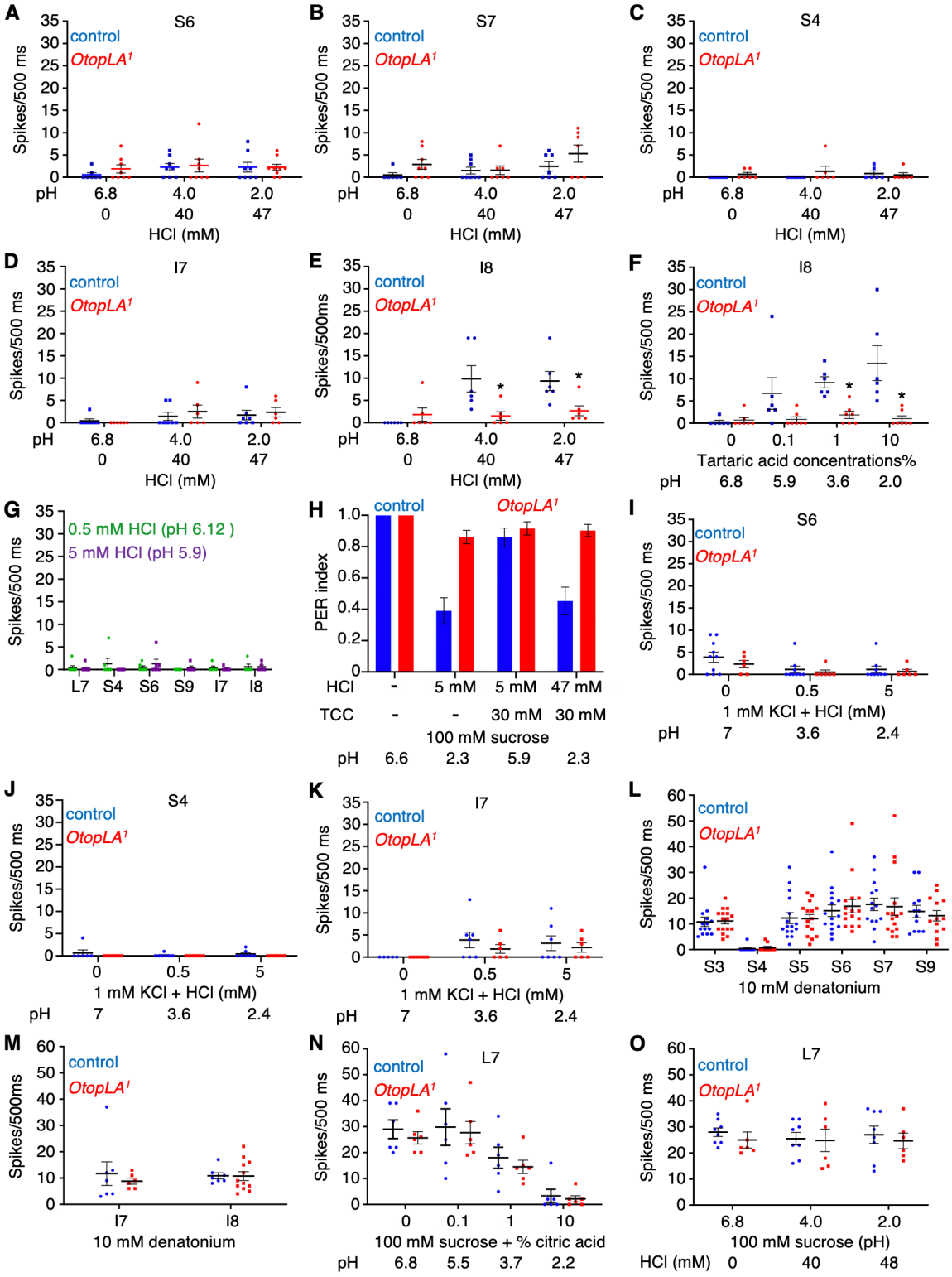
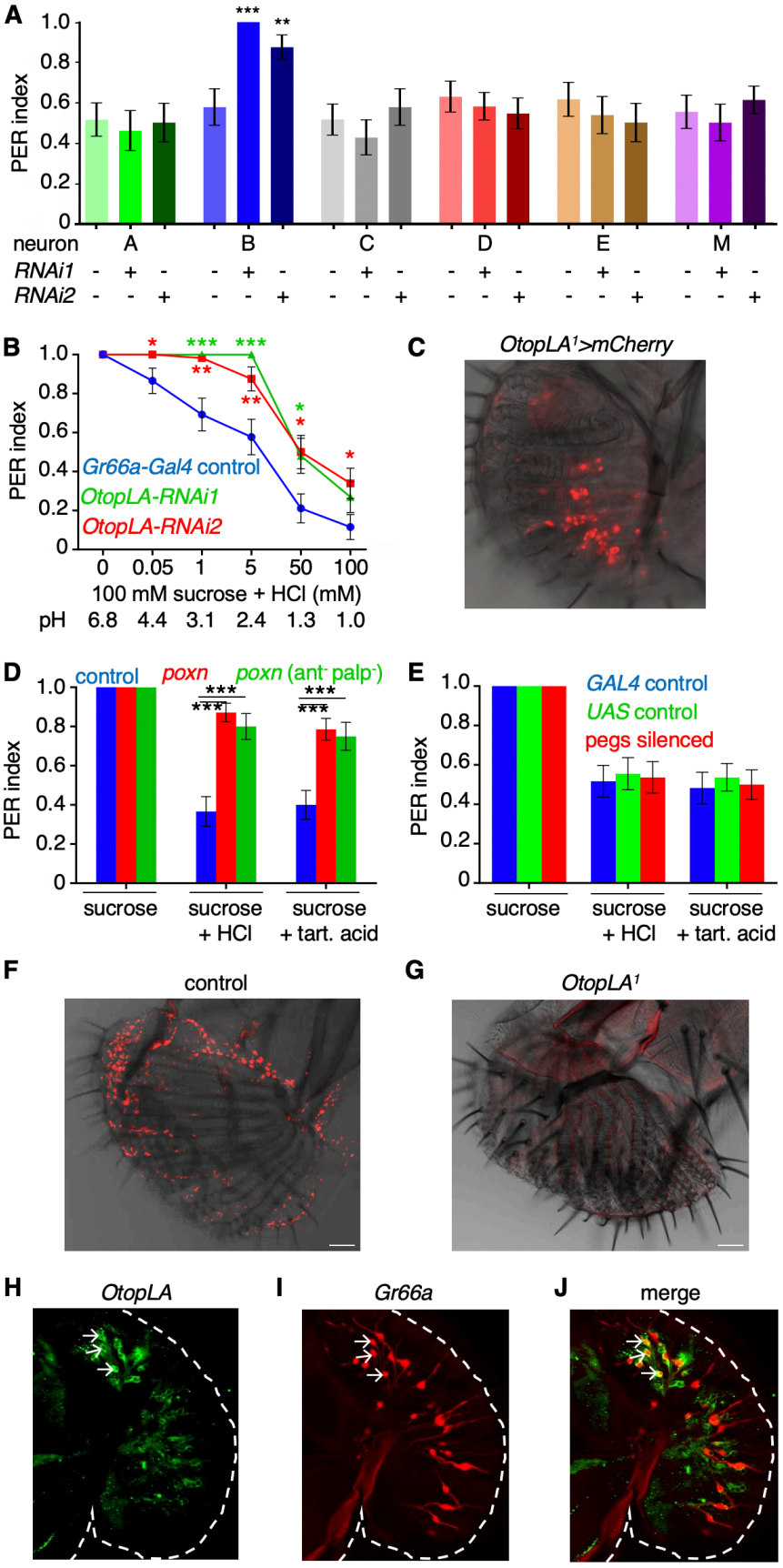


Fig. S3. Tip recordings to test responses of control and *OtopLA¹* sensilla to acids, denatonium and sucrose. Mean action potentials elicited by the control (w^{1118}) and the *OtopLA¹* mutant during the first 500 msec upon stimulating the indicated sensilla with the indicated chemical. (*A*—*E*) Stimulation of the indicated sensilla with HCl solutions at the indicated pHs. The pH 6.8 solution

contained only the electrolyte (30 mM 3 tricholine citrate). (*A*) Responses of S6 sensilla to HCl. Control, n=7—8. *OtopLA*^{1}, n=8—9. (*B*) Responses of S7 sensilla to HCl. Control, n=6—8. *OtopLA*^{1}, n=6—8. (*C*) Responses of S4 sensilla to HCl. Control, n=6. *OtopLA*^{1}, n=6. (*D*) Responses of I7 sensilla to HCl. Control, n=7. *OtopLA*^{1}, n=6. (*E*) Responses of I8 sensilla to HCl. Control, n=6. *OtopLA*^{1}, n=6. (*F*) Responses of I8 sensilla to the indicated concentrations of tartaric acid. Control, n=6. *OtopLA*^{1}, n=6. *(G)* Responses of the indicated class of sensilla from control (w^{1118}) flies to 5 mM HCl and 0.5 mM HCl. n≥5. (*H*) PERs elicited by control (w^{1118}) and *OtopLA*^{1} flies in response to stimulation with 100 mM sucrose with or without 30 mM TCC (tricholine citrate) mixed with HCl at the indicated concentrations. Control (w^{1118}), n=32. *OtopLA*^{1}, n=35. (*I*—*K*) Stimulation of the indicated sensilla with HCl at the indicated concentrations and pHs. 1 mM KCl was used as the electrolyte in all cases. (*I*) Responses of S6 sensilla to HCl. Control, n=9—10. *OtopLA*^{1}, n=6. (*J*) Responses of S4 sensilla to HCl. Control, n=7. *OtopLA*^{1}, n=6. (*K*) Responses of I7 sensilla to HCl. Control, n=5—7. *OtopLA*^{1}, n=6. *(L)* Responses of the indicated S-type sensilla to 10 mM denatonium. Control, n≥12. *OtopLA*^{1}, n≥12. (*M*) Responses of the indicated I-type sensilla to 10 mM denatonium. Control, n≥6. *OtopLA*^{1}, n≥6. (*N*) Responses of L7 sensilla to 100 mM sucrose mixed with citric acid at the indicated concentrations and pHs. Control, n=6. *OtopLA*^{1}, n=6. (*O*) Responses of L7 sensilla to 100 mM sucrose plus HCl at the indicated pHs. Control, n=8. *OtopLA*^{1}, n=6. Student's unpaired *t*-tests. Error bars, s.e.m.s. *p < 0.05, **p < 0.01, and ***p < 0.001.


A
PER index
1.0
0.8
0.6
0.4
0.2
0
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neuron A B C D E M
RNAi1 - + - - + - - + - - + - - + - - + - - + -
RNAi2 - - + - - + - - + - - + - - + - - + - - +
B
PER index
Gr66a-Gal4 control
OtopLA-RNAi1
OtopLA-RNAi2
0 0.05 1 5 50 100
100 mM sucrose + HCl (mM)
pH 6.8 4.4 3.1 2.4 1.3 1.0
C
OtopLA1>mCherry
D
control
poxn
poxn (ant- palp-)
PER index
sucrose
sucrose + HCl
sucrose + tart. acid
E
GAL4 control
UAS control
pegs silenced
PER index
sucrose
sucrose + HCl
sucrose + tart. acid
F
control
G
OtopLA1
H
OtopLA
I
Gr66a
J
merge

Fig. S4. (*A*) PER assays to test effects on acid aversion after RNAi knockdown of *OtopLA* in different classes of GRNs (A—E) and the mechanosensory (M) neuron associated with taste hairs. PERs were in response to 100 mM sucrose mixed with 5 mM HCl. *OtopLA* was knocked down in A—E GRNs and the M neuron using two independent RNAi lines. *GAL4* lines used to specifically express the RNAi lines in different neurons: *Gr5a-GAL4* for A GRNs, *Gr66a-GAL4* for B GRNs, *ppk28-GAL4* for C GRNs, *ppk23-GAL4* for D GRNs, *Ir94e-GAL4* for E GRNs and *nompC-GAL4* for M neurons. The controls without RNAi lines were the *GAL4* lines crossed to w^{1118}. n≥25. (*B*) PER assays to test the effects of RNAi knockdown of *OtopLA* upon stimulation with 100 mM sucrose plus different concentrations of HCl. *Gr66a-GAL4* control, n= 26. *OtopLA-RNAi1*, n= 26. *OtopLA-RNAi2*, n= 28. (*C*) Confocal image of $OtopLA^{1}$ (*lexA*) neurons (red; anti-DsRed) in the labellum. The image focuses on neurons in taste pegs. Genotype: $OtopLA^{1}$;*UAS-mCherry*. (*D*) PERs elicited by control (w^{1118}), intact $poxn^{70}$ flies, and $poxn^{70}$ flies with the antennae and maxillary palps surgically ablated in response to stimulation with 100 mM sucrose, 100 mM sucrose mixed with either with 5 mM HCl or with 1% tartaric acid. Control, n=30. $poxn^{70}$ (intact), n=35. $poxn^{70}$ (antenna-less, maxillary palp-less), n=30. (*E*) PER elicited from flies in which the peg neurons are silenced show normal aversion to acids. Labella were stimulated with 1) 100 mM sucrose, 2) 100 mM sucrose mixed with 5 mM HCl, or 3) 100 mM sucrose plus 1% tartaric acid. *GAL4* control: *57F03-GAL4/+*, n=29. *UAS* control: *UAS-Kir2.1/+*, n=27. Pegs silenced: *UAS-kir2.1/+*;*57F03-GAL4/+*, n=27. (*F*) *In situ* hybridization of *OtopLA* transcripts in a control (w^{1118}) labellum. Scale bar indicates 10 μm. (*G*) *In situ* hybridization of *OtopLA* transcripts in an $OtopLA^{1}$ labellum. Scale bar indicates 10 μm. (*H*—*J*) Confocal images testing for overlap between the *OtopLA-LexA* and *Gr66a* reporters. The images focused on neurons in taste hairs. The arrows indicate examples of overlapping expression. (*H*) $OtopLA^{1}$ (*lexA*) was visualized using anti-GFP (green). Genotype: $OtopLA^{1}$(*lexA*);*Gr66a-GAL4;UAS-tdTomato*/*lexAop-GFP*. (*I*) *Gr66a-GAL4* visualized using anti-DsRed (red). Genotype: $OtopLA^{1}$(*lexA*);*Gr66a-GAL4;UAS-tdTomato*/*lexAop-GFP*. (*J*) Merge of *G* and *H*. Mann-Whitney U tests. Error bars, s.e.m.s. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.