Supplementary Information

Hedgehog-mediated gut-taste neuron axis controls sweet perception in *Drosophila*

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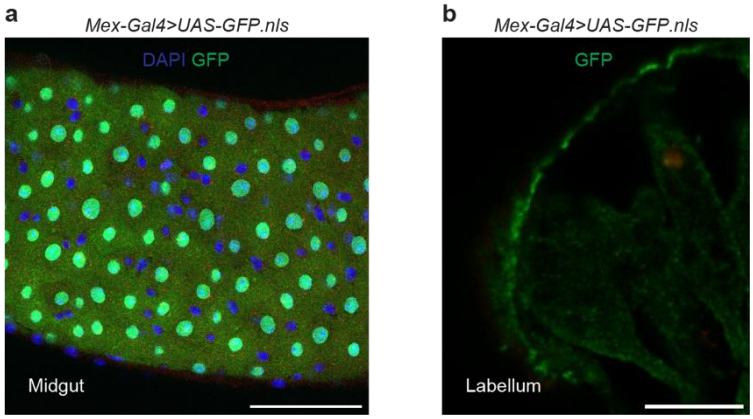
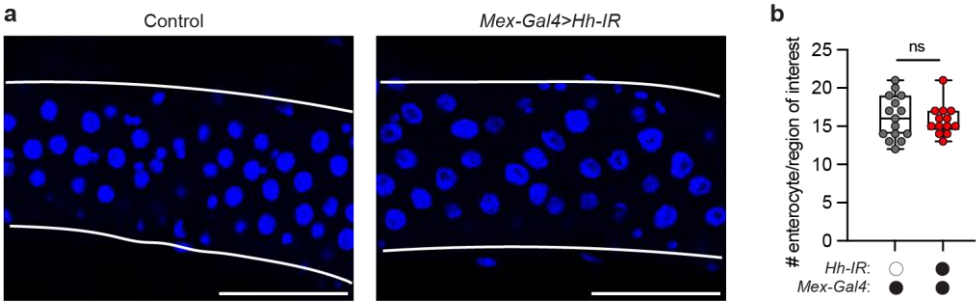
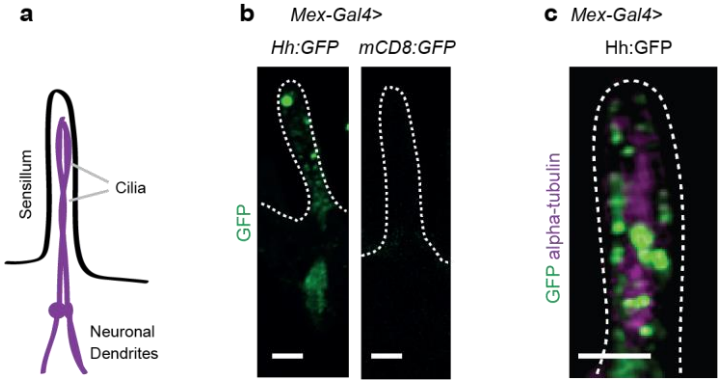
Supplementary Figures 1-5 and Supplementary Table 1-2



Supplementary Figure S1 *Mex-Gal4* expression is not detected in the labellum.
a Confocal image of an adult midgut (posterior part). *Mex-Gal4* drives nucleus localized GFP (GFP.nls) expression in the polyploid enterocytes. DAPI stains DNA blue. Scale bar, 50 μm. **b** Confocal image of *Mex-Gal4>UAS-GFP.nls* labellum. Scale bar, 20 μm.

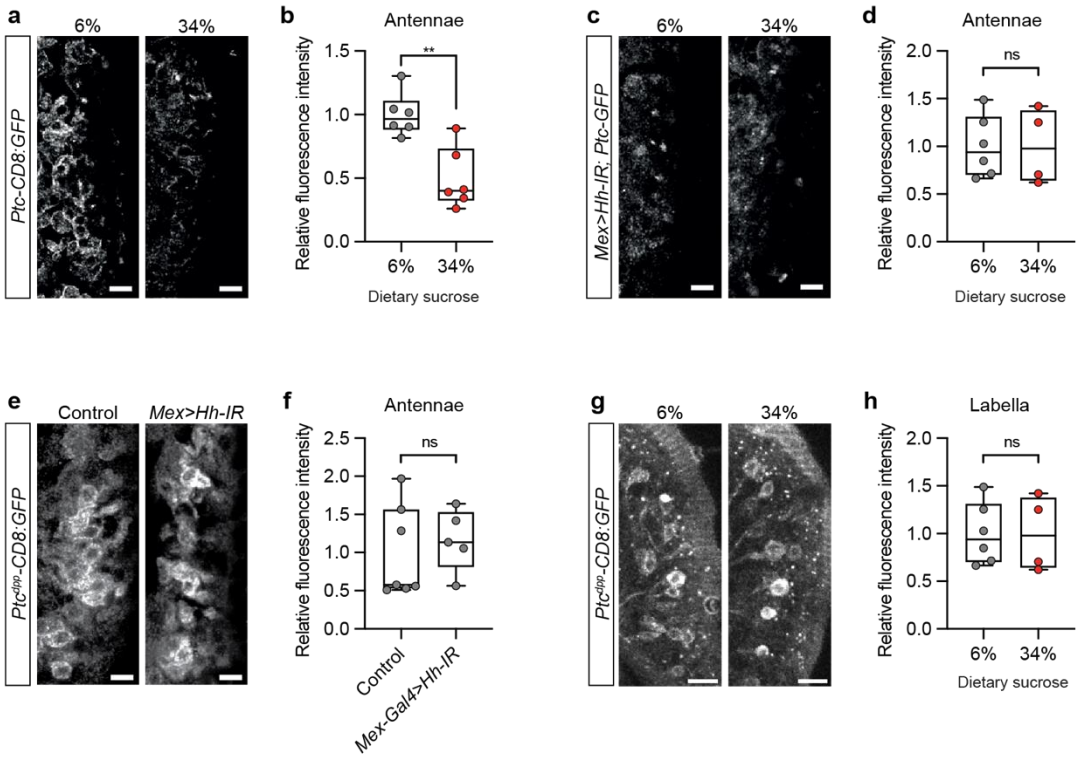


Supplementary Figure S2 *Mex-Gal4>Hh-IR* does not have a strong effect on enterocyte number.
a Representative confocal image of adult midguts from control (left) and *Mex-Gal4>Hh-IR* flies (right). DAPI staining appears in blue. Scale bars, 50 μm. **b** A box plot showing enterocyte number per region of interest in the midgut. No significant difference was observed between control and *Mex-Gal4>Hh-IR* flies. Median (middle line) is depicted, and whiskers indicate minimum to maximum. n = 15 and 13, respectively. Statistical analysis was performed via two-tailed t-test.



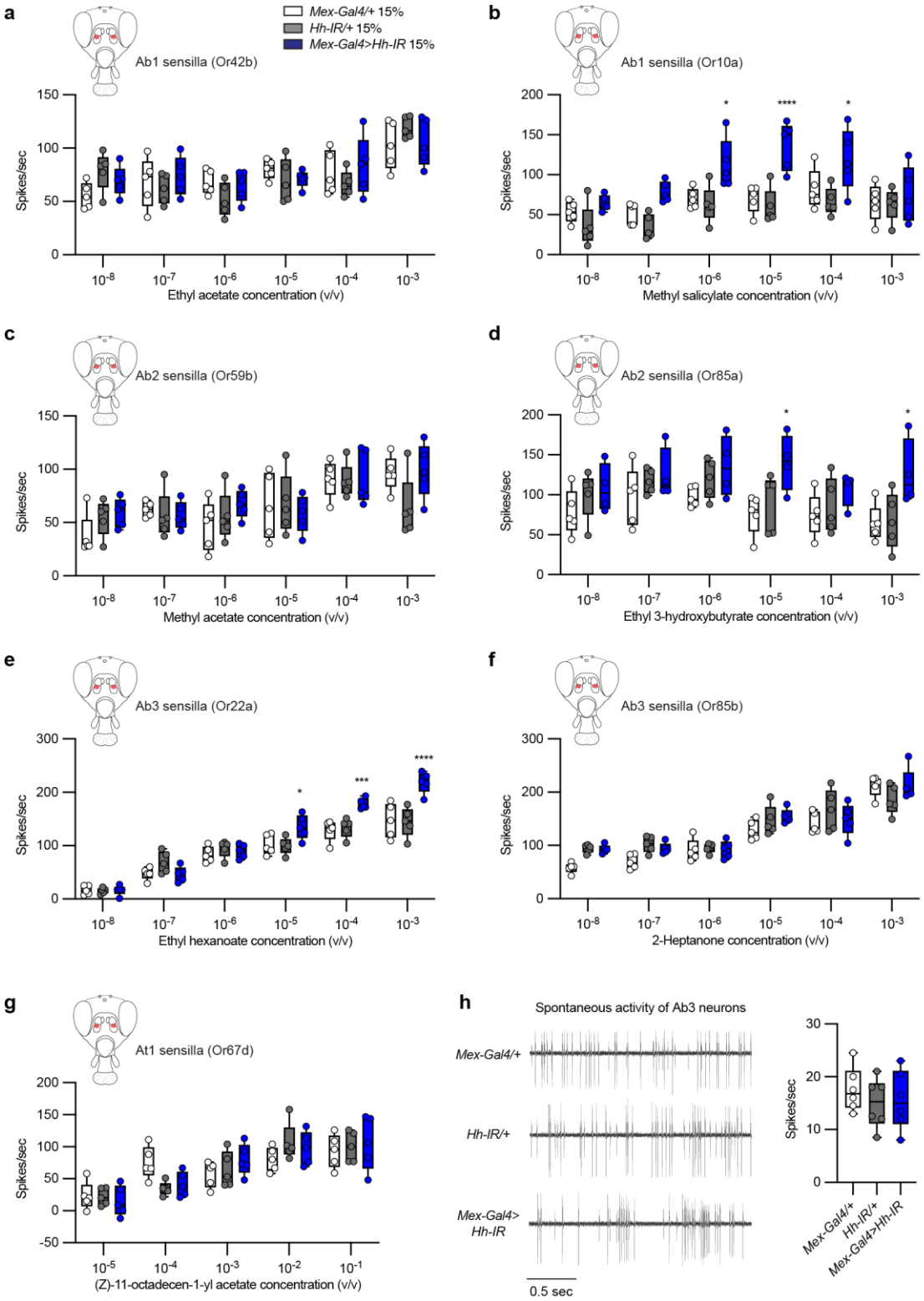
Supplementary Figure S3 Hh:GFP but not mCD8:GFP is transported from the gut to the olfactory sensillum.

a Schematic illustration of cilia. **b** Representative confocal images of adult olfactory sensilla. GFP fluorescence is shown in green. Left, *Mex-Gal4>Hh:GFP*; Right, *Mex-Gal4>mCD8:GFP*. **c** Confocal image of an adult olfactory sensillum (*Mex-Gal4>Hh:GFP*). GFP fluorescence is shown in green. Anti-alpha-Tubulin is shown in magenta. Scale bars 1 μm.



Supplementary Figure S4 Sugar-induced Hh supress Ptc expression in olfactory sensory neurons.

a Representative confocal image of adult olfactory sensilla in *ptc-CD8:GFP* flies maintained on the 6% and 34% sugar diets, respectively. GFP is shown in grey. **b** Quantification of relative ptc-CD8:GFP expression in a. *n* = 6. **c** Representative confocal images of adult olfactory sensilla of *Mex-Gal4>Hh-IR* flies maintained on the 6% and 34% sugar diets. **d** Quantification of relative ptc-CD8:GFP expression in c. *n* = 6 and 4, respectively. **e** Confocal image of adult olfactory sensilla of *ptc^{dpp}-mCD8:GFP* flies. This reporter has a mutation in its Ci binding motifs, rendering it insensitive to Hh signalling. **f** Quantification of the relative fluorescence intensity of ptc^{dpp}-mCD8:GFP expression in e. Left, control (*Mex-Gal4*); right, *Mex-Gal4>Hh-IR*. *n* = 7 and 5, respectively. **g** Confocal image of adult labella from *ptc^{dpp}-mCD8:GFP* flies maintained on the 6% and 34% sugar diets. **h** Quantification of the relative fluorescence intensity of ptc^{dpp}-mCD8:GFP expression in g. *n* = 6 and 4, respectively. Scale bars, 5 µm (a, c, and e) or 10 µm (g). Median (middle line) is depicted, whiskers represent minimum to maximum (b, d, f, and h). Statistical analyses were performed via two-tailed *t*-test with the Welch correction. **, $p < 0.01$.



Supplementary Figure S5 Midgut Hh regulates odour responses.

a Electrophysiological responses of ab1A neurons (Or42B) to ethyl acetate. *n* = 5. **b** Electrophysiological responses of ab1D neurons (Or10a) to methyl salicylate. *n* = 5. **c** Electrophysiological responses of ab2A neurons (Or85a) to methyl acetate. *n* = 5. **d** Electrophysiological responses of ab2B neurons (Or85a) to ethyl 3-hydroxybutyrate. *n* = 5. **e** Electrophysiological responses of ab3A neurons (Or22a) to ethyl hexanoate. *n* = 5, 5, and 4, respectively. **f** Electrophysiological responses of ab3B neurons (Or85b) to 2-heptanone. *n* = 5. **g**

Electrophysiological responses of at1 neurons (Or67d) to (Z)-11-octadecen-1-yl acetate. *n* = 5. **h** Left: Electrophysiological traces of the spontaneous activity of ab3 in the resting state. Right: spikes within 2 seconds. Median (middle line) is depicted, whiskers represent minimum to maximum. Statistical significance was assessed via two-way ANOVA with the Sidak correction (a-g) or one way ANOVA with the Tukey correction (h). *, $p < 0.05$; ***, $p < 0.001$; ****, $p < 0.0001$.

Supplementary Table 1: Fly lines used in this work

Flyline	Source	Identifyer
w^{1118}	Bloomington Drosophila Stock Center (BDSC)	#3605
UAS-Hh-IR	BDSC	#32489
UAS-ptc^{1130X}	BDSC	#52215
Hh-Gal4	BDSC	#67493
Gr64f-Gal4	BDSC	#57669
Mex-Gal4	BDSC	#91368
tub-Gal80ts	BDSC	#7017
UAS-disp-IR	BDSC	#44633
UAS-GFP.nls	BDSC	#4776
UAS-Hh:GFP	Isabel Guerrero, Madrid university, Madrid, Spain	
ptc-CD8:GFP	This work	
ptc^{dpp}-CD8:GFP	This work	

Supplementary Table 2: Oligo sequences used for qPCR

Primer name	Sequence (5' to 3')
Actin F	CACACCAAATCTTACAAAATGTGT
Actin R	AATCCGGCCTTGCACATG
Hedgehog F	CGCCAGTGTCACCTGTCTC
Hedgehog R	TTCTTGCGGGATTGCGGAG