Supplementary Data

Gene[1]	Forward primer (5'-3')[2]	Reverse primer (5'-3')[2]	Length (bp)	Template	Vector	Integration site	Transgenic line code	Source[3]
Ir7a	AGATCTGGTGAAGAATAGAGTGTGGC	GAATTCTTTGAAACGAAACTGTTGCG	2318	OR genomic DNA	*pGAL4 attB*	attP2	BT58.1	(a)
Ir7b	AGATCTGGGATGAGAACGACATCGAT	GAATTCGGCTAAAGAGTTGCCAAAGG	578	OR genomic DNA	*pGAL4 attB*	attP2	BT47.1	(b)
Ir7c	CTCGAGGCCGGTTAGTGGTCCAAATA	AGATCTATCGTGTTTCATCGGTGGCT	1595	OR genomic DNA	*pGAL4 attB*	attP2	BT110.1	
Ir7d	AGATCTAACTTGTGTCAATGCGATCC	AGATCTGGCGAATGTGAAACATTTGG	1010	OR genomic DNA	*pGAL4 attB*	attP2	BT79.1	(b)
Ir7e	AGATCTTACTTCGGCAGAGGAACTAG	GAATTCTTGCTCCCCGGACAAATCGT	600	OR genomic DNA	*pGAL4 attB*	attP2	BT59.1	(b)
Ir7f	AGATCTGTCCGTCTATCGAAATCCGG	AGATCTATCGATCCTCGAATTCTCCA	766	OR genomic DNA	*pGAL4 attB*	attP2	BT98.1	
Ir7g	AGATCTATCGATCCTCGAATTCTCCA	AGATCTGTCCGTCTATCGAAATCCGG	766	OR genomic DNA	*pGAL4 attB*	attP2	BT99.1	(b)
Ir10a	GCGGCCGCGACACTATAGGTCCACTACC	GCGGCCGCTCGTATGGGATTTGTTAGCAC	2429	OR genomic DNA	*pGAL4 attB*	attP2	BT96.1	
Ir11a	AGATCTATGTATGTCATGCCACCAGC	GAATTCGACTGAATGGCCGTTGTGAA	2099	OR genomic DNA	*pGAL4 attB*	attP2	BT51.1	(a)
Ir20a	AGATCTACATTGTTCGGCACTCGCAG	AGATCTGTCGCCGGCATCGAAGGAAT	2488	OR genomic DNA	*pGAL4 attB*	attP2	BT61.1	
Ir47a	AGATCTGCTGAGTTGGGTGACGAATC	GAATTCTTTTTATGGCCTTTTGAAAC	2488	OR genomic DNA	*pGAL4 attB*	attP2	BT53.1	
Ir47bΨ								
Ir48aΨ								
Ir48b	AGATCTCCAGTCCAGTTCCAGATTGC	AGATCTCTGAAAGATATATAGAGCGT	2575	OR genomic DNA	*pGAL4 attB*	attP2	BT97.1	
Ir51aΨ								
Ir51b	AGATCTCAACCAATCAAGCTGGATAC	AGATCTGGTGGTTGATTCAATTGTGACA	2499	OR genomic DNA	*pGAL4 attB*	attP2	BT82.1	
Ir52a	AGATCTCCGACATTTCTTCGCGTAAC	AGATCTCACGAAACTGTTGACAATCC	2931	OR genomic DNA	*pGAL4 attB*	attP2	BT100.1	
Ir52b	AGATCTACTGGAGATATTGCGTTGCG	GAATTCTGTTTTCAAACAAACTGTTT	473	OR genomic DNA	*pGAL4 attB*	attP2	BT55.1	(a)
Ir52c	AGATCTAAACGCTGGATGAAATTCCG	AGATCTGGTGCTAAAGTGACTAATGG	644	OR genomic DNA	*pGAL4 attB*	attP2	BT80.1	
Ir52d	AGATCTTGAGATACTGGAGGAACTGC	GAATTCGGTGCAAGAGTTACTATTGC	664	OR genomic DNA	*pGAL4 attB*	attP2	BT56.1	
Ir52e[4]								
Ir54a	AGATCTGACGCAAGTCGACTCATTTG	AGATCTGTCCTTTCAATTATGTTGCC	2493	OR genomic DNA	*pGAL4 attB*	attP2	BT101.1	
Ir56a[5]	AGATCTATCATCACTGGCTGTCATGC	GAATTCGGCTGCCTTACCACTTTGAC	2400	OR genomic DNA	*pGAL4 attB*	attP2	BT52.1	(a)
Ir56b	GCGGCCGCATATCCTTCGGTCGAAGTGC	GCGGCCGCTGAAATAATTCTGCACTTGA	2461	OR genomic DNA	*pGAL4 attB*	attP2	BT62.1	
Ir56c	AGATCTGCAAAGACGTCCACAGTATG	GAATTCGACTTTCCCTTAGAAGCACC	319	OR genomic DNA	*pGAL4 attB*	attP2	BT57.1	(b)
Ir56d[6]	GAATTCATGAGCAGTGCAACATGCTC	AGATCTATATTTGTACGGCGACTGCC	858	OR genomic DNA	*pGAL4 attB*	attP2	BT76.1	
Ir56eΨ								
Ir60a[7]	GAATTCTAGTCCGCGGACTGATTATC	GAATTCTATTGCTTCTGTCACGTCGG	2523	OR genomic DNA	*pGAL4 attB*	attP2	BT83.1	
Ir60b	AGATCTCATACGATTTCCCGAACAGC	AGATCTTTTCGAGTTGTCTGCTCTGG	2368	OR genomic DNA	*pGAL4 attB*	attP2	BT104.1	
Ir60cΨ	GAATTCGATTGGATACCACAGGTGGC	GAATTCGGCGACTATCCGAAACGAGC	560	OR genomic DNA	*pGAL4 attB*	attP2	BT81.1	(b)
Ir60d	AGATCTAGATTGGGTACCACAGATGG	GAATTCTTTTAAGGCGACTGCTCACA	339	OR genomic DNA	*pGAL4 attB*	attP2	BT63.1	
Ir60e	AGATCTAAATAATGAGCAGTCCCGAT	GAATTCAAGGCAGCGGGAAAATGCTT	2466	OR genomic DNA	*pGAL4 attB*	attP2	BT72.1	
Ir60fΨ								
Ir62a	GAATTCAAATCACCGAGTTCAATGGC	GAATTCATTTTCGCTCGTGAACCATG	2439	OR genomic DNA	*pGAL4 attB*	attP2	BT118.1	
Ir67a	AGATCTACAGACGTTTATCAGCAAAG	GAATTCATATCCTGGCTGAATGGCTG	2496	OR genomic DNA	*pGAL4 attB*	attP2	BT73.1	
Ir67b	AGATCTTGGTGTGTCAGCACTATAGC	GAATTCTGAAATGTCTCTGAAATCCT	515	OR genomic DNA	*pGAL4 attB*	attP2	BT74.1	
Ir67c	GCGGCCGCGGTGCTCCATCGTATCCTTC	GCGGCCGCGATGCAGTCCTGCCCGAAAA	2736	OR genomic DNA	*pGAL4 attB*	attP2	BT84.1	
Ir68b	AGATCTCCGGTTACTCGAAAGATATG	GAATTCGTTCTACGAGCAGACTAACC	637	OR genomic DNA	*pGAL4 attB*	attP2	BT48.1	
Ir85a	AGATCTAAGTCCTTCCTCAGTTGTCC	GAATTCTGCAATGCCAACTGTTTGAG	1369	OR genomic DNA	*pGAL4 attB*	attP2	BT49.1	
Ir87a	CTCGAGAGTTACCCATATGGACACCG	AGATCTGCCGCAAACGAATGACTGAT	2097	OR genomic DNA	*pGAL4 attB*	attP2	BT122.1	
Ir94a	AGATCTGCACACAGATAGATTCGCAG	AGATCTTTTCTACTTTAGCCAACAAT	2294	OR genomic DNA	*pGAL4 attB*	attP2	BT64.1	
Ir94b	AGATCTAAGATCAAGCGAAGATGACG	GAATTCATTTCGTAATTCACGTAGTG	482	OR genomic DNA	*pGAL4 attB*	attP2	BT77.1	
Ir94c	AGATCTTTCTGGCGAGCGTCTCTATC	AGATCTTTTTAGTTAGCCTTGGGTTA	2303	OR genomic DNA	*pGAL4 attB*	attP2	BT65.1	
Ir94d	CTCGAGACATTGTGTTCGGGTACGTG	AGATCTTTTTGAATGTGGGAATAGTTGGT	2420	OR genomic DNA	*pGAL4 attB*	attP2	BT111.1	
Ir94e	AGATCTTTGGCGACATAAGATGTGGC	GAATTCTTCCCAGGGGATTACACAAA	322	OR genomic DNA	*pGAL4 attB*	attP2	BT78.1	(b)
Ir94f	AGATCTGATTGTGGAGCGATCGATTG	GAATTCCTGTGCAGACGATGATGATG	2493	OR genomic DNA	*pGAL4 attB*	attP2	BT102.1	
Ir94g	GAATTCGAGCTCACTGTTCACTATGC	GAATTCCTTATAATACTGACTTCATT	388	OR genomic DNA	*pGAL4 attB*	attP2	BT75.1	
Ir94h	GAATTCTTGTTCACGCGGCAATTACG	GAATTCGACTTATACCGAAACCGACG	2000	OR genomic DNA	*pGAL4 attB*	attP2	BT60.1	(b)
Ir100a	AGATCTTTCATCGGAGTCGTAGCTAG	GAATTCGTCAGGAGTTACTGAACCGT	512	OR genomic DNA	*pGAL4 attB*	attP2	BT50.1	(a)

Footnotes

1: Ψ = predicted pseudogene in the reference *D. melanogaster* genome (Adams et al., Science 2000); for most of these we therefore did not construct a driver line. For *Ir48a-Gal4* we observed very variable expression in the central and peripheral nervous system (data not shown). For *Ir60c*, this locus is predicted to be intact in a w^{1118} strain

2: Restriction enzyme sites in cloning primers are highlighted in blue

3: Lines previously published: (a) Croset *et al*., PLOS Genetics (2010); (b) Croset *et al*., Scientific Reports (2016)

4: *Ir52e* is not present in the reference *D. melanogaster* genome, so no driver line was constructed for the locus in this study (see also Koh *et al.*, Neuron 2014)

5: *Ir56a-Gal4* displays expression in several olfactory sensory neuron populations; this is likely to be non-specific as there is no evidence for antennal expression of *Ir56a* (Menuz *et al*., PLOS Genetics 2014); as *Ir56a* is located within the intron of another gene (*5-HT1A*) this may reflect overlapping regulatory elements of these genes

6: *Ir56d-Gal4* was also detected in some larval head chemosensory neurons, but this expression weak and was not confirmed in the $Ir56d^{Gal4}$ reporter allele.

7: *Ir60a-Gal4* displays extensive and variable non-neuronal expression (data not shown), which is likely to be non-specific; as *Ir60a* is located within the intron of another gene (*nord*), this may reflect overlapping regulatory elements of these genes

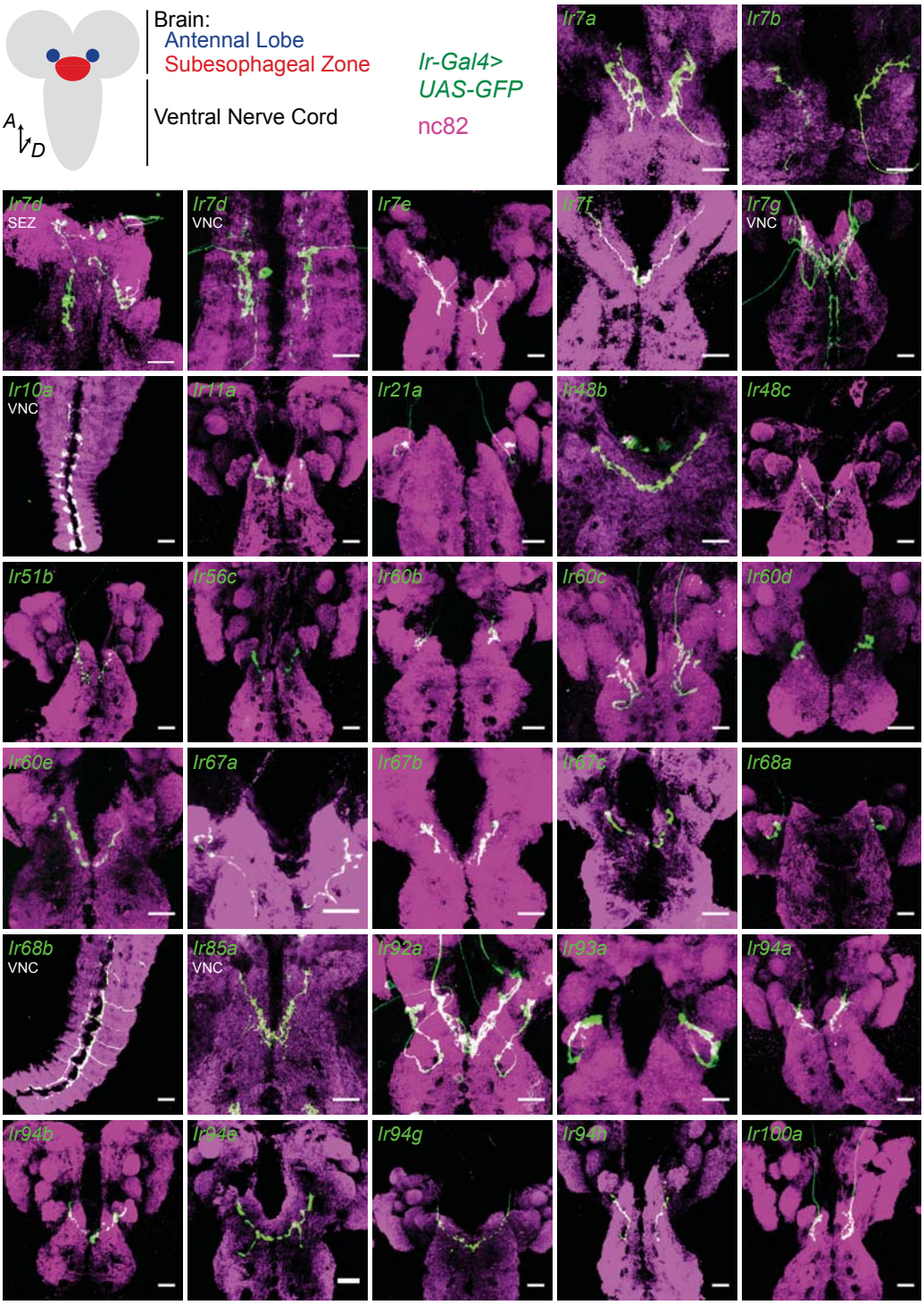
Supplementary Table 1. Oligonucleotide sequences and construction of *Ir-Gal4* lines

		Calcium imaging Figure 5c	
Tastant	**CAS**	**Concentration**	**Notes**
water	-	-	
glycerol	56-81-5	10% (v/v)	
fructose	57-48-7	1 M	
trehalose	6138-23-4	1 M	
sucrose	57-50-1	1 M	
caffeine	58-08-2	15 mg/ml	
denatonium	3734-33-6	10 mM	
arginine	74-79-3	100 mM	
histidine	71-00-1	100 mM	
lysine	56-87-1	100 mM	
aspartic acid	56-84-8	100 mM	
glutamic acid	56-86-0	100 mM	
acetic acid	64-19-7	1% (v/v)	
NaCl (high)	7647-14-5	1 M	
NaCl (low)	7647-14-5	10 mM	
$NaHCO_3$ (pH 5)	144-55-8	200 mM	0.5 ml of 200 mM $NaHCO_3$ (pH 6.5) + 50 µl of 5 M H_2PO_4
$CsHCO_3$ (pH 7)	29703-01-3	200 mM	pH set with HCl immediately before use
NaH_2PO_4	13472-35-0	500 mM	
PBS pH 4	-	100%	7.8 mM NaH_2PO_4 + 12.2 mM Na_2HPO_4 + 153.8 mM NaCl (pH set with HCl or NaOH)
PBS pH 7	-	100%	
PBS pH 10	-	100%	
carbonated water	-	-	Aproz ® (in mg/ml: Ca^{2+} 360; Mg^{2+} 70; Na^+ 6; K^+ 2.5; HCO_3^- 250; NO_3^- 1.5; SO_4^{2-} 930; $Si0_2$ 7) + gaseous CO_2
non-carbonated water	-	-	Aproz ® (in mg/ml: Ca^{2+} 360; Mg^{2+} 70; Na^+ 6; K^+ 2.5; HCO_3^- 250; NO_3^- 1.5; SO_4^{2-} 930; $Si0_2$ 7)
hexanoic acid	142-62-1	-	

Supplementary Table 2. Gustatory stimuli

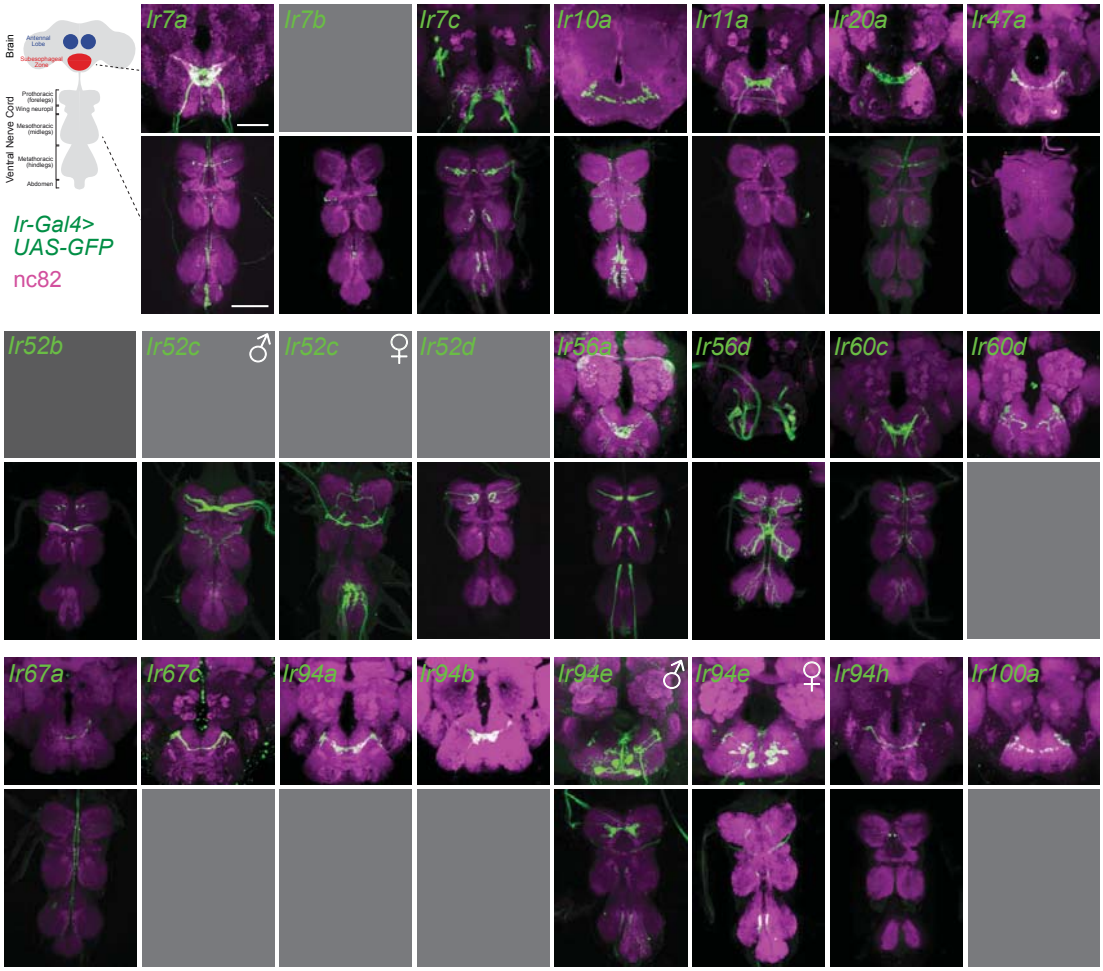
Oligonucleotide	Sequence (5'-3')	Notes
CRISPRsgR	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC	
CRISPRsgF-*Ir56d*[1]	GAAAT*TAATACGACTCACTATA* GGTCATCACGGAGCGCATGTGTTTTAGAGCTAGAAATAGC	T7 promoter sequence is italicised; *Ir56d* target sequence is underlined
CRISPRsgF-*Ir56d*[2]	GAAAT*TAATACGACTCACTATA* GGTCAGCTATAGCTATCCCATGTTTTAGAGCTAGAAATAGC	T7 promoter sequence is italicised; *Ir56d* target sequence is underlined
Ir56d sgRNAs-fwd	GCGGCCCGGGTTCGATTCCCGGCCGATGCAAAATATGGATAATCGTGCTGGTTTTAGAGCTAGAAATAGCAAG	sgRNA cloning into *pCFD5*
Ir56d sgRNAs-rev	ATTTTAACTTGCTATTTCTAGCTCTAAAACGCAAGCCCAGATCGTTTCTCTGCACCAGCCGGGAATCGAACCC	sgRNA cloning into *pCFD5*
Ir56d Gal4-HA1-fwd	GATCCACCTGCGATCTCGCCCCACGCACTGTGCATCCTTGAAGTGC	Homology Arm 1-*Gal4* ORF fusion
Ir56d Gal4-HA1-rev	GATCCACCTGCGATCCTACGGATCCAGATCCACTAGTCAAGGCAC	Homology Arm 1-*Gal4* ORF fusion
Ir56d Gal4-HA1-internal-	ACTGGCAGTCGCCGTACAAATATGAAGCTACTGTCTTCTATCGAACAAGC	Homology Arm 1-*Gal4* ORF fusion
Ir56d Gal4-HA1-internal-	CGATAGAAGACAGTAGCTTCATATTTGTACGGCCGACTGCCACGTGGGTAAC	Homology Arm 1-*Gal4* ORF fusion
Ir56d -HA2-fwd	GATCGCTCTTCGTATAGCCCAGATCGTTTCTCAGGCGCTTCATG	Homology Arm 2
Ir56d -HA2-rev	GATCGCTCTTCGGACGATGCCTTGCAATTGATACGTGAACG	Homology Arm 2

Supplementary Table 3. Oligonucleotide sequences used for CRISPR/Cas9-mediated editing of the *Ir56d* locus



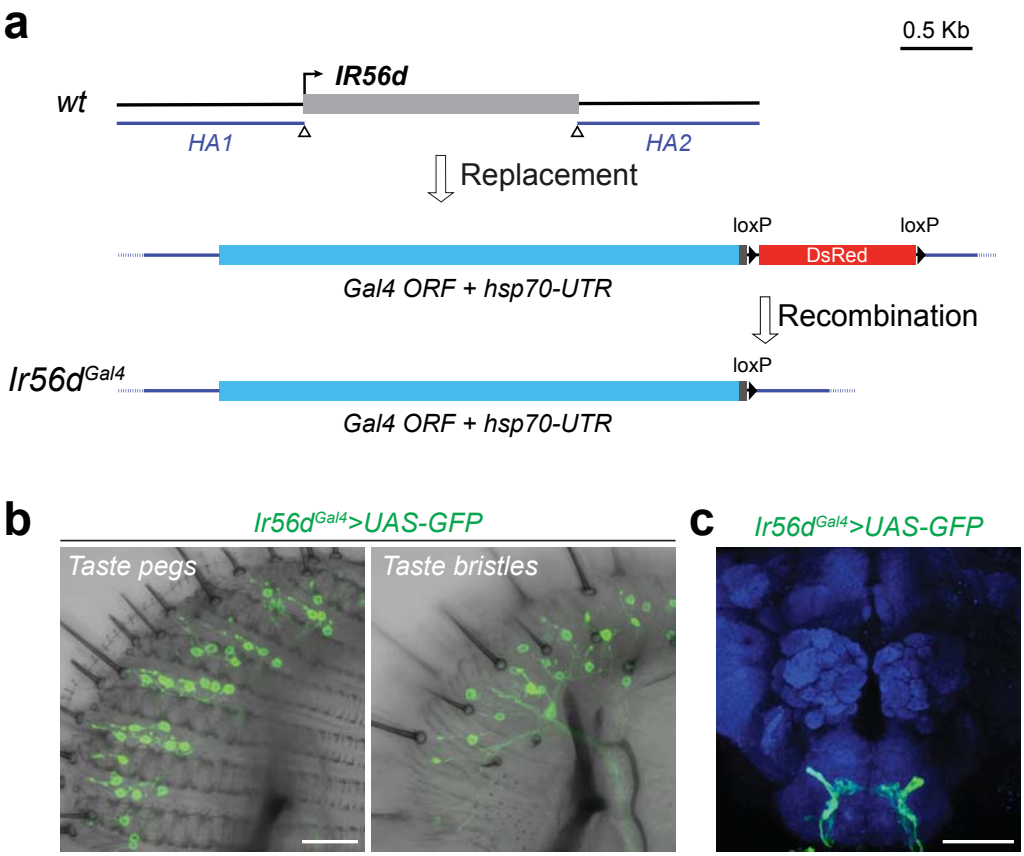
Supplementary Figure 1. Projections of *Ir-Gal4* expressing sensory neurons in the larval central nervous system

Immunofluorescence with anti-GFP (green) and nc82 (magenta) on whole-mount brains of third instar larvae, revealing the projection patterns of *Ir-Gal4*-expressing neuron populations in the brain and ventral nerve cord (as schematised in the cartoon at the top left). Images for *Ir7a*, *Ir7b*, *Ir7g*, *Ir94e* and *Ir94h* drivers have been adapted from Croset et al., 2016[28]. Genotypes are of the form: *w;UAS-mCD8:GFP;IrX-Gal4*. SEZ: Subesophageal Zone; VNC: Ventral Nerve Cord. Scale bars: 20 μm.



Supplementary Figure 2. Projections of *Ir-Gal4* expressing sensory neurons in the adult central nervous system

Immunofluorescence with anti-GFP (green) and nc82 (magenta) on whole-mount adult brains and ventral nerve cords (schematised top left), revealing the projection patterns of *Ir-Gal4*-expressing neuron populations. Grey panels indicate no expression was detected for that Gal4 driver. Genotypes are of the form: *UAS-mCD8:GFP;IrX-Gal4*. Scale bars: 50 μm (brain), 100 μm (ventral nerve cord).

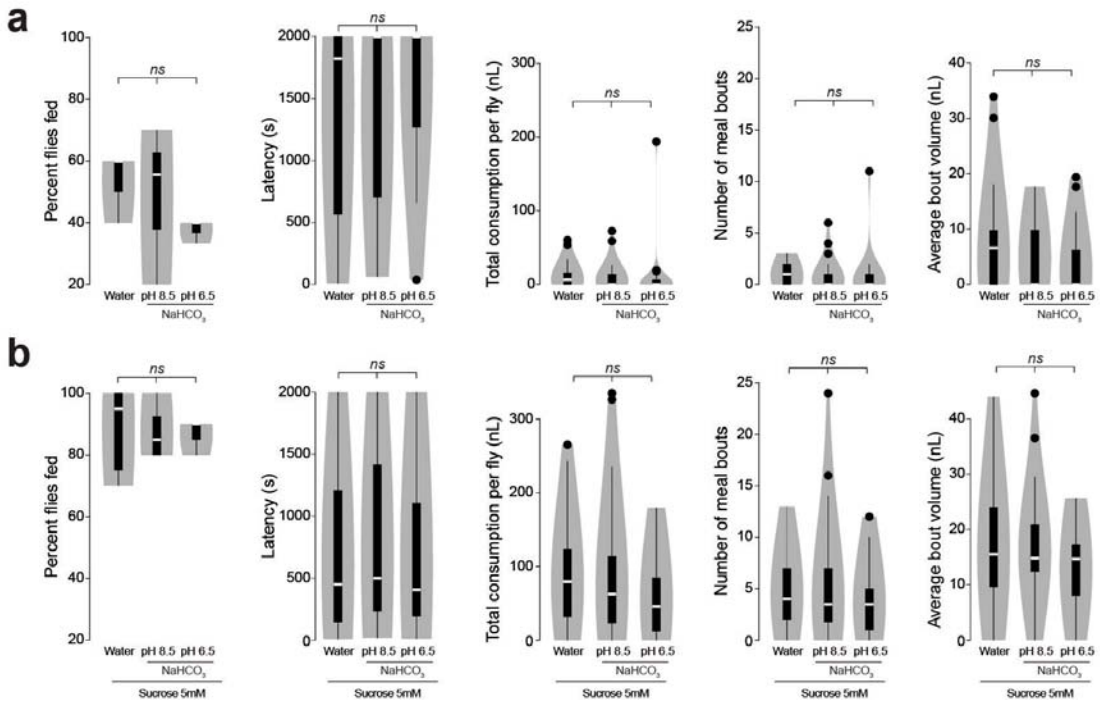


Supplementary Figure 3. An *Ir56d*^{Gal4} allele shows a similar expression pattern to *Ir56d-Gal4*

(a) Schematic representing the generation of the *Ir56d*^{Gal4} allele. The entire *Ir56d* exon was substituted with the *Gal4* sequence. In an additional step, the *DsRed* marker used for screening of positive recombination events was removed with Cre recombinase. HA: Homology Arm. Unfilled arrowheads point to the sgRNA targets.

(b) Immunofluorescence with anti-GFP (green), overlaid on a bright-field image, of a whole mount proboscis of a *w;Ir56d*^{Gal4}*;UAS-mCD8:GFP* animal. Scale bar: 25 μm.

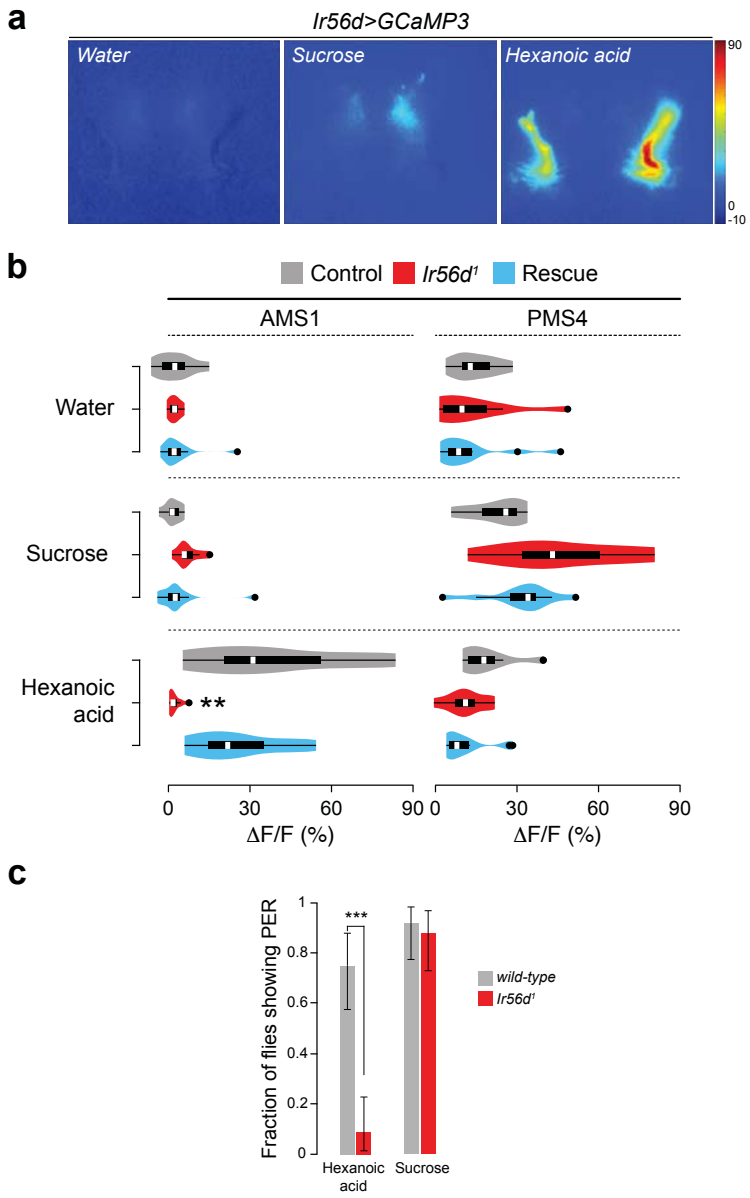
(c) Immunofluorescence with anti-GFP (green) and anti-nc82 (blue) on a whole mount brain of a *w;Ir56d*^{Gal4}*;UAS-mCD8:GFP* animal. Scale bar: 50 μm (right).



Supplementary Figure 4. Analysis of carbonation-evoked feeding by Expresso

(a) Feeding success, latency to first bout, total consumption per fly, number of meal bouts and average bout volume for male w^{1118} flies (n=30 per tastant) feeding from water, 100 mM $NaHCO_3$ pH 8.5 or 100 mM $NaHCO_3$ pH 6.5 solutions.

(b) Feeding success, latency to first bout, total consumption per fly, number of meal bouts and average bout volume for male w^{1118} flies (n=30 per tastant) feeding from solutions containing 5 mM sucrose, 5 mM sucrose + 100 mM $NaHCO_3$ pH 8.5 or 5 mM sucrose + 100 mM $NaHCO_3$ pH 6.5 solutions. *ns*: non-significant (pairwise comparisons using Tukey and Kramer (Nemenyi) test with Tukey-Dist approximation for independent samples).



Supplementary Figure 5. IR56d is required for physiological and behavioural responses to hexanoic acid

(a) Colour-coded images (reflecting the maximal GCaMP3 fluorescence intensity changes) in *w;Bl/+;UAS-GCaMP3,Ir56d-Gal4/+* animals stimulated with distilled water, 1 M sucrose and 1% (v/v) hexanoic acid.

(b) Quantification of changes in GCaMP3 fluorescence (ΔF/F) upon stimulation with the indicated chemicals (as in (a)) to the proboscis of the flies. Genotypes: Control: *w;Bl/+;UAS-GCaMP3,Ir56d-Gal4/+* (n=7); Mutant: *w;Ir56d^{1}/Ir56d^{1};UAS-GCaMP3,Ir56d-Gal4/+* (n=8); Rescue: *w;Ir56d^{1},UAS-Ir56d/Ir56d^{1};UAS-GCaMP3,Ir56d-Gal4/+* (n=11). *P<0.05, **P<0.01, ***P<0.001 (Wilcoxon rank sum test with Bonferroni correction for multiple comparisons).

(c) Fraction of *w^{1118}* (n=36) or *Ir56d^{1}* mutant (n=33) flies showing proboscis extension reflex (PER) to 1% (v/v) hexanoic acid and 100 mM sucrose. Error bars represent the ±95% binomial confidence intervals ***P<0.001 (Fisher exact test).