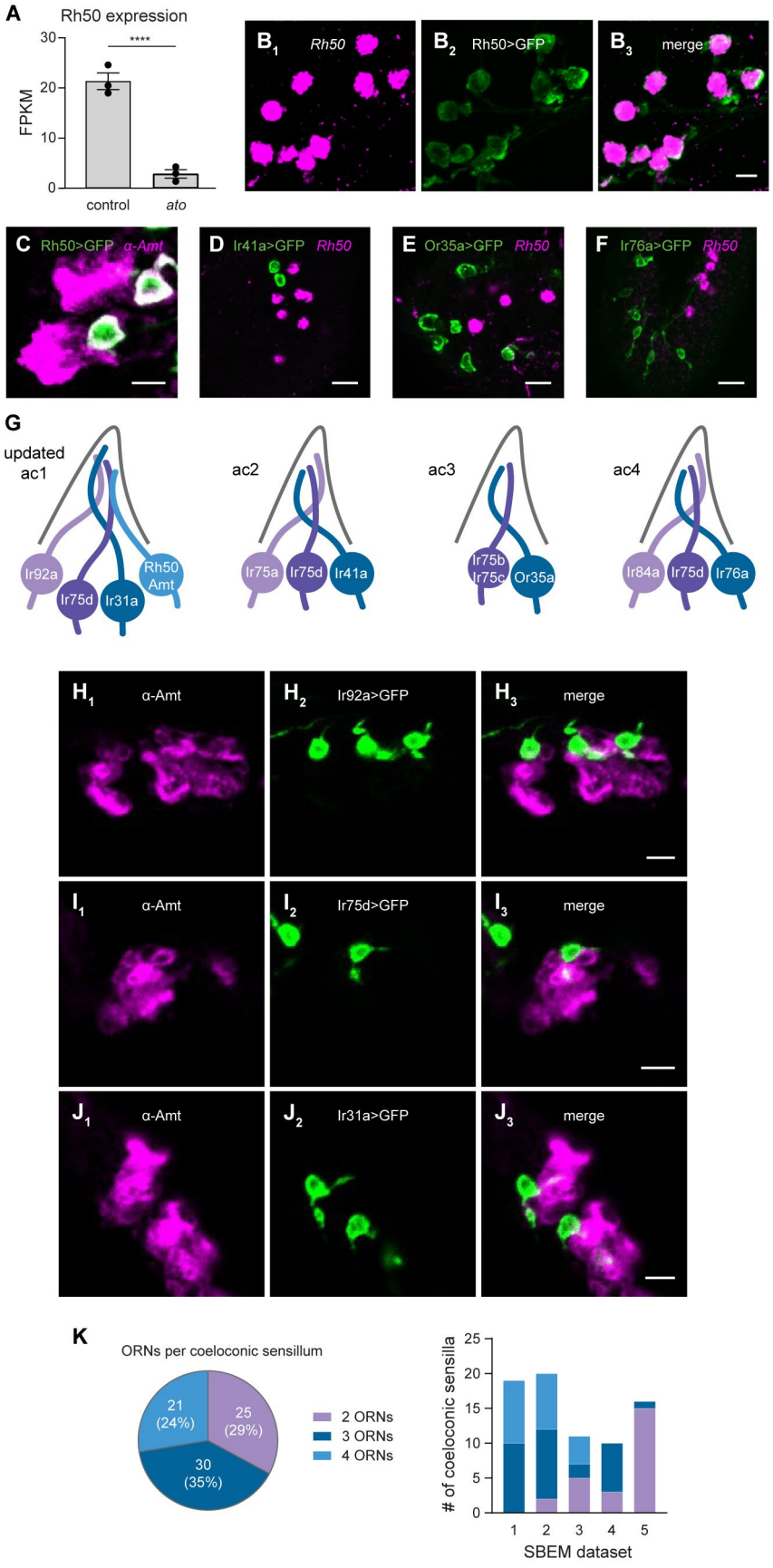
A
Rh50 expression
****
FPKM
30
20
10
0
control
ato
B1 Rh50
B2 Rh50>GFP
B3 merge
C Rh50>GFP α-Amt
D Ir41a>GFP Rh50
E Or35a>GFP Rh50
F Ir76a>GFP Rh50
G
updated ac1
Ir92a
Ir75d
Ir31a
Rh50 Amt
ac2
Ir75a
Ir75d
Ir41a
ac3
Ir75b Ir75c
Or35a
ac4
Ir84a
Ir75d
Ir76a
H1 α-Amt
H2 Ir92a>GFP
H3 merge
I1 α-Amt
I2 Ir75d>GFP
I3 merge
J1 α-Amt
J2 Ir31a>GFP
J3 merge
K
ORNs per coeloconic sensillum
21 (24%)
25 (29%)
30 (35%)
2 ORNs
3 ORNs
4 ORNs
# of coeloconic sensilla
25
20
15
10
5
0
1
2
3
4
5
SBEM dataset

Figure S1. Antennal ammonium transporter expression and coeloconic sensilla organization, related to Figure 1.

(A) Previous RNASeq analysis of Rh50 expression in antennal transcriptomes from control and *atonal* flies, which lack coeloconic sensilla (n = 3 sets of ~600 antennae) [14]. (B) Antennal section from an *Rh50>GFP* fly stained with an *in situ* hybridization probe to Rh50 (magenta, B_1) and an antibody against GFP (green, B_2). B_3, merged image. Scale bar, 5 μm. (C) Close-up view of antenna in Figure 1D showing co-expression (white) of Amt (magenta) in $Rh50^+$ ORNs (green). Scale bar, 10 μm. (D-F) Antennal sections from *Ir41a>GFP* (D), *Or35a>GFP* (E), and *Ir76a>GFP* (F) flies, which mark the location of ac2, ac3, and ac4 sensilla, respectively. The sections were labeled with an antisense probe for Rh50 (magenta) and an antibody against GFP (green). Scale bars, 10 μm. (G) An updated model of the ORNs and receptors found in each coeloconic sensillum. (H-J) Antennal sections from *Ir92a>GFP* (H), *Ir75d>GFP* (I), and *Ir31a>GFP* (J) flies labeled with an antibody against Amt (magenta) and an antibody against GFP (green). Scale bars, 5 μm. (K) Left, number and percentage of coeloconic sensilla with two, three, or four ORNs found in five serial block face electron microscopy (SBEM) datasets. Right, number of coeloconic sensilla with two, three or four ORNs found in each of the five SBEM datasets.

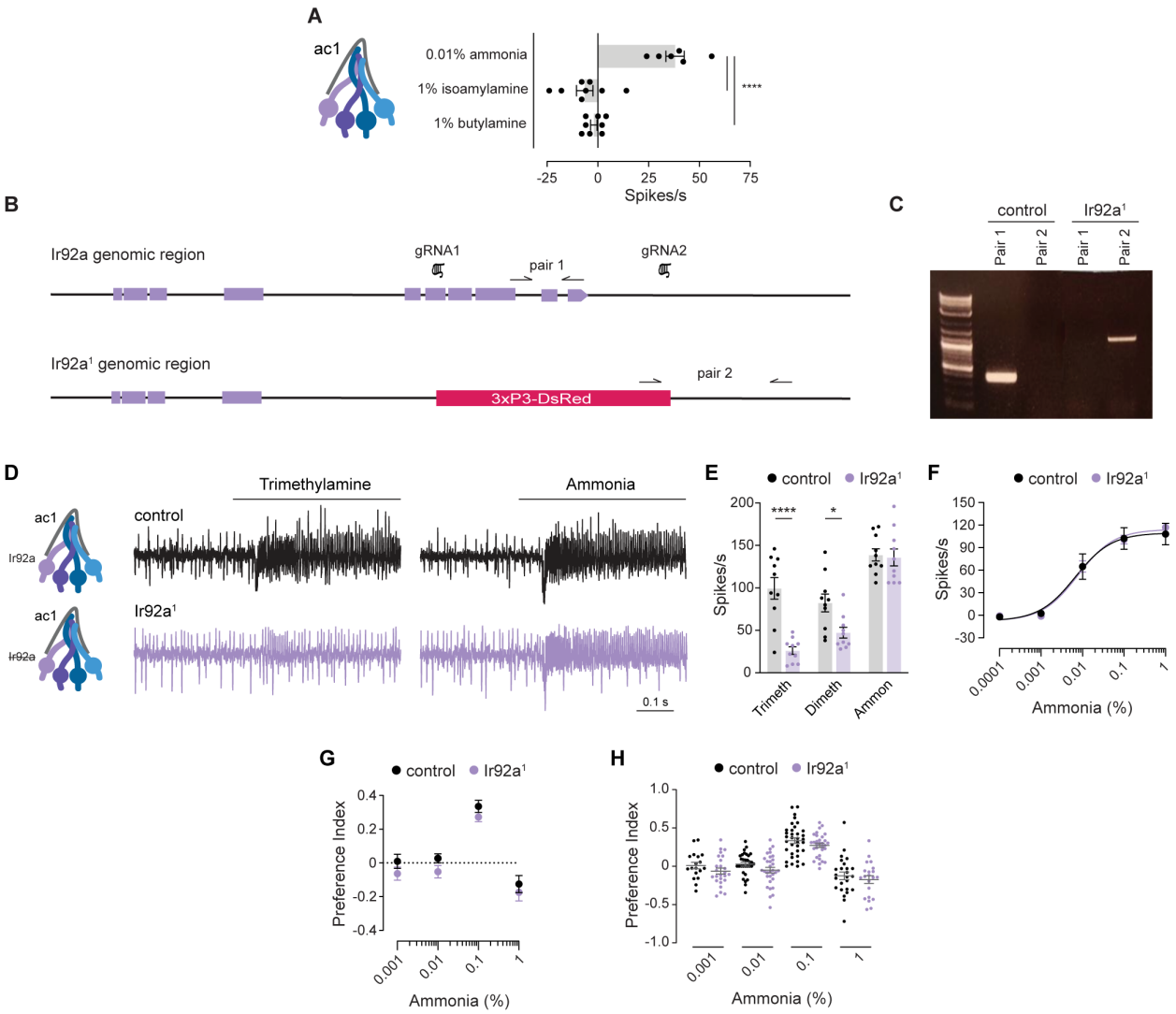
A
ac1
0.01% ammonia
1% isoamylamine
1% butylamine
****
-25
0
25
50
75
Spikes/s
B
Ir92a genomic region
gRNA1
pair 1
gRNA2
Ir92a¹ genomic region
pair 2
3xP3-DsRed
C
control
Ir92a¹
Pair 1
Pair 2
Pair 1
Pair 2
D
Trimethylamine
Ammonia
ac1
Ir92a
control
ac1
Ir92a
Ir92a¹
0.1 s
E
control
Ir92a¹
****
*
Spikes/s
Trimeth
Dimeth
Ammon
F
control
Ir92a¹
Spikes/s
0.0001
0.001
0.01
0.1
1
Ammonia (%)
G
control
Ir92a¹
Preference Index
0.001
0.01
0.1
1
Ammonia (%)
H
control
Ir92a¹
Preference Index
0.001
0.01
0.1
1
Ammonia (%)

Figure S2. Analysis of ac1 pH sensitivity and *Ir92a*^{1} mutants, related to Figures 2 and 3.

(A) Single sensillum recordings of action potentials in ac1 sensilla from a wild-type fly in response to ammonia (pKa 9.4) and higher concentrations of two more basic odorants: isoamylamine (pKa 10.6) and butylamine (pKa 10.8) (n = 6-8 sensilla). (B) Upper, a depiction of the Ir92a genomic region, with Ir92a exons in purple. The location of gRNAs 1 and 2 is shown. Lower, the Ir92a genomic region in *Ir92a*^{1} mutant flies after homology directed repair. The region between gRNAs 1 and 2 was replaced with the 3xP3-DsRed marker. Genotyping primer pairs 1 and 2 are shown. (C) Agarose gel showing genotyping PCR bands from control and *Ir92a*^{1} mutant flies with primer pairs 1 and 2. (D) Representative traces of extracellular recordings of action potentials elicited by 1% trimethylamine and 0.1% ammonia in ac1 sensilla from control flies (black) and *Ir92a*^{1} receptor mutants (purple). (E) Quantification of solvent corrected odor responses in control (black) and *Ir92a*^{1} (purple) flies (n = 10 sensilla). (F) Dose-dependent responses to ammonia in control (black) and *Ir92a*^{1} flies (purple) (n = 8-10 sensilla). (G) Preference indices of control (black) and *Ir92a*^{1} flies (purple) when given the choice between water and ammonia at various concentrations. (H) Individual trials of the behavioral assays in (G). Each dot represents one assay of ~20-30 flies (n = 18-35 assays each).

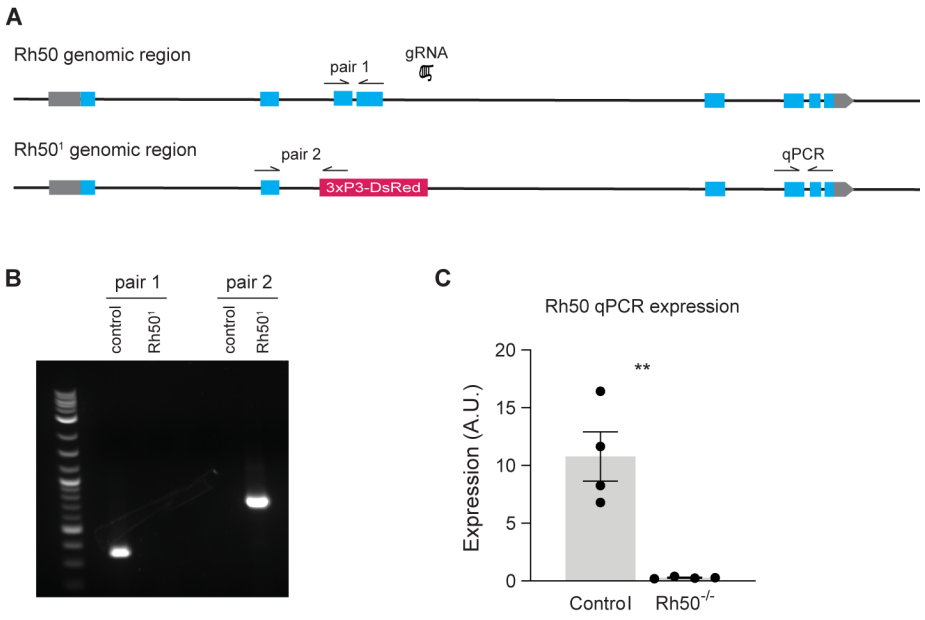

Figure S3. Generation and validation of *Rh50¹* mutants, related to Figure 4.

(A) Upper, a depiction of the Rh50 genomic region, with Rh50 exons in blue. The location of the gRNA is shown. Lower, the location of the 3xP3-DsRed insertion in the Rh50 genomic region in *Rh50¹* mutant flies as determined with PCR and sequencing. Exons three and four are eliminated. Genotyping primer pairs 1 and 2 and qPCR primers are also shown. (B) Agarose gel showing genotyping PCR bands from control and *Rh50¹* mutant flies with primer pairs 1 and 2. (C) qPCR analysis of Rh50 expression in cDNA from heads of control and *Rh50¹* flies (n = 4 biological replicates).

***Drosophila* stock name**	**Figure**
w; Rh50-GAL4; UAS-mCD8::GFP	Figures 1A, 1B, 1D and 3B Figures S1B and S1C
w; Amt-GAL4; UAS-mCD8::GFP	Figures 1C, 1L, 1M and 3C
w; UAS-mCD8::GFP; Ir92a-GAL4	Figures 1E and S1H
w; Ir31a-GAL4; UAS-mCD8::GFP	Figures 1F and S1J
w; UAS-mCD8::GFP; Ir75d-GAL4	Figures 1G and S1I
w; 10X UAS-myc-APEX2-Orco; Or56a-GAL4 *w; 10X UAS-myc-APEX2-Orco; Or47b-GAL4* *w; 10X UAS-myc-APEX2-Orco, Or88a-GAL4; +* *w; 10X UAS-myc-APEX2-Orco; Ir75c-GAL4* *w; 10X UAS-myc-APEX2-Orco; Or47a-GAL4*	Figure 1I Figure S1K
w ; Rh50-Gal4/repo-GAL80; Rh50-Gal4/UAS-mCD8::GFP	Figures 1J and 1K
w; UAS-GCaMP7s/Bl^{1}*; Rh50-GAL4/+*	Figure 2A
w; UAS-GCaMP7s/Sp; Ir92a-GAL4/+	Figure 2B
w; UAS-DTA/Cyo; Ir92a-GAL4-1	Figures 2C and 2D
w; UAS-DTA/Cyo; + *w; UAS-DTA/Cyo; Rh50-GAL4*	Figures 2C, 2D, 3L and 3M
w; Rh50-GAL4/+; UAS-GCaMP7s/+	Figures 3A, 3E-3J
w; Ir64a-GAL4; UAS-mCD8::GFP	Figure 3D
w; + ; Rh50-GAL4	Figures 3L and 3M
w; Ir25a-GAL4; UAS-mCD8::GFP	Figure 4A
w; UAS-mCD8::GFP; Ir76b-GAL4	Figure 4B
w; UAS-mCD8::GFP; Ir8a-GAL4	Figure 4C
w; UAS-mCD8::GFP; Orco-GAL4	Figure 4D
w; Ir25a^{2}*; +*	Figure 4E
+; +; + (Canton-S, CS)	Figure 4E Figures S2A, S2C and S2F-H
w; +; Rh50^{1}	Figure 4F Figures S3B and S3C
w; +; + (wCS)	Figure 4F Figures S2D, S3E, S3B and S3C
w^{1118}*; +; Amt*^{1} *w*^{1118}*; +; + (isogenic background)*	Figure 4G
w; UAS-GCamp6s; Ir75d-GAL4 *w; UAS-GCamp6s/UAS-Amt; Ir75d-GAL4/+*	Figure 4H
w; UAS-Amt; + *w; +; Ir75a-GAL4* *w; UAS-Amt; Ir75a-GAL4*	Figure 4I
w; +; Or22a-GAL4 *w; UAS-Amt; Or22a-GAL4*	Figure 4J
w; UAS-AgAmt; Ir75a-GAL4 *w; UAS-AgAmt; +*	Figure 4K
w; Ir41a-GAL4; UAS-mCD8::GFP	Figure S1D
w; UAS-mCD8::GFP; Or35a-GAL4	Figure S1E
w; Ir76a-GAL4; UAS-mCD8::GFP	Figure S1F
w; +; Ir92a^{1}	Figures S2D and S2E
+; +; Ir92a^{1}	Figures S2C and S2F-H

Table S1. *Drosophila* genotypes used in this study. Related to STAR Methods.