SUPPLEMENTAL FIGURES AND FIGURE LEGENDS

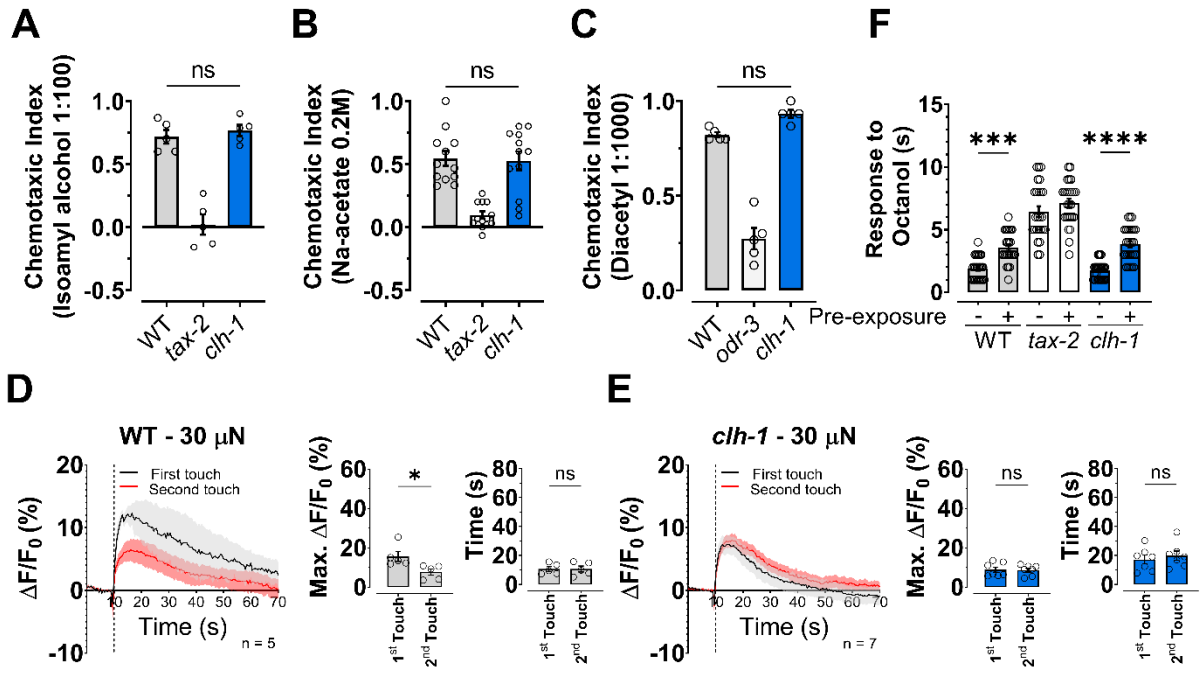

Figure S1. CLH-1 is not needed for chemotaxis; response of ASH neurons to lower force. Related to figure 1.

(**A-C**) Chemotaxis index of *clh-1* worms to 1:100 isoamyl alcohol (**A**), 0.2 M Na-acetate (**B**) and 1:1,000 diacetyl (**C**). Columns represent mean ± SEM. Each data point represents the chemotaxis index of independent experiments (n= 5, 12, and 5, respectively), in which at least 30 worms were tested each. Statistics were by one-way ANOVA followed by Tukey post-test (ns, not significant ($p>0.05$), ***$p<0.001$, and ****$p<0.0001$). (**D-E**) Left panels, calcium transients generated in ASH neurons by two 30 μN consecutive nose touch stimulations as measured by % increase of GCaMP-6s fluorescence above the baseline (ΔF/F) in wild type (**D**) and *clh-1(ok658)* (**E**). Data are shown as mean ± SEM (light gray and red). The first touch is in black, the second in red. The number of animals tested is in each panel. The vertical dashed line is when the touch stimulation was delivered. Middle panels, peak percentage (%) of GCaMP-6s ΔF/F. Right panels, time constants of fluorescence decay. Individual data points are shown as open circles, averages are shown as columns. Columns represent mean ± SEM. Statistics were calculated by two-tailed unpaired t-Test (ns, not significant ($p>0.05$), *$p<0.05$). (**F**) The response to 10% octanol of naïve and adapted individual worms (symbols) and the average (columns) are shown (n= 27, 25, 25, 26, 28 and 28, respectively).

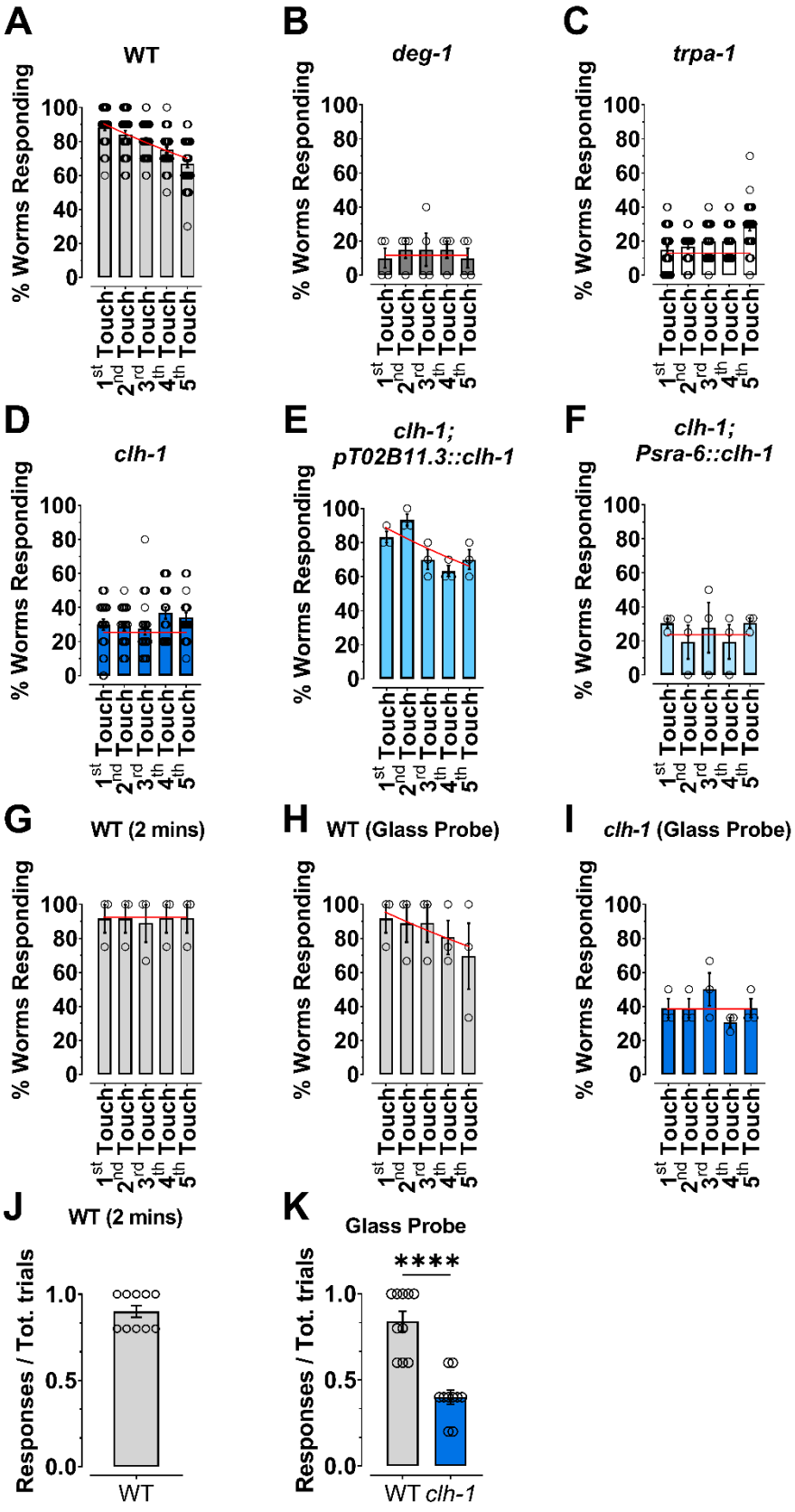
A
WT
B
deg-1
C
trpa-1
D
clh-1
E
clh-1;
pT02B11.3::clh-1
F
clh-1;
Psra-6::clh-1
G
WT (2 mins)
H
WT (Glass Probe)
I
clh-1 (Glass Probe)
J
WT (2 mins)
K
Glass Probe
% Worms Responding
Responses / Tot. trials
1st Touch
2nd Touch
3rd Touch
4th Touch
5th Touch
****
WT
WT clh-1

Figure S2. Behavioral responses to 5 touches. Related to figure 2.
Percentage of worms responding to 5 consecutive touches in wild type (**A**), *deg-1(u38u421)* (**B**), *trpa-1(ok999)* (**C**), *clh-1(ok658)* (**D**), CLH-1 AMsh glia rescue (**E**), and CLH-1 ASH rescue (**F**), wild type with a 2 minutes interval between touches (**G**) and in wild type (**H**) and *clh-1* (**I**) using a glass probe. **J** and **K** show the average response of individual worms to 5 touches for wild type with 2 minutes interval and wild type and *clh-1* stimulated with a glass probe, respectively. Data are shown as individual experiments (A-I, symbols) or individual worms (J and K) and as mean ± SEM (columns). In each experiment (A-I) 3 to 10 animals were tested. Fitting was by exponential decay with the following tau values: 15.39 (wild type, **A**), 13.29 (CLH-1 AMsh glia rescue, **E**) and 16.37 (WT with glass probe, 30 seconds intervals, **H**). The fitting for the rest of genotypes gave tau values > 10^6. Taus are expressed in number of touches. One-way ANOVA followed by Tukey post-test was used to compare touch 1 with touch 2. No statistical difference was found across the strains (not shown). Columns represent mean ± SEM and each point represent one worm. Statistics in **K** was by two-tailed unpaired t-Test (****$p<0.0001$).

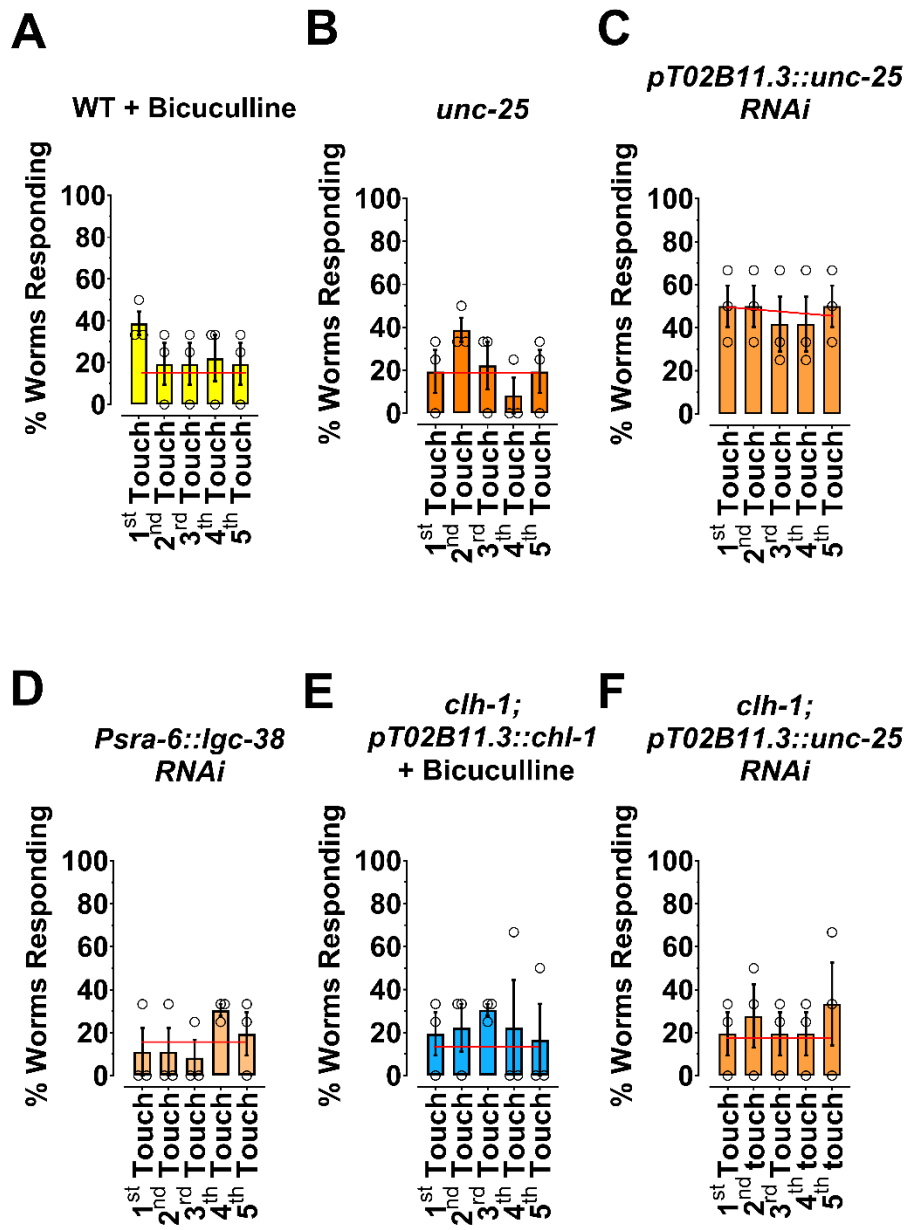

Figure S3. Nose touch behavior upon loss of GABA signaling (5 touches). Related to figure 4.

Percentage of worms responding to 5 touches in wild type treated with bicuculline (**A**), in *unc-25(e156)* mutants (**B**), in *unc-25* knockdown in AMsh glia (**C**), in *lgc-38* knockdown in ASH (**D**), in *clh-1* AMsh glia rescue treated with bicuculline (**E**), and in *unc-25* knockdown in AMsh glia in *clh-1* knockout (**F**). Data are shown as individual experiments (symbols) and as mean ± SEM (columns). In each experiment 3 to 10 animals were tested. Fitting was by exponential decay with the following tau values: 44.55 for AMsh glia specific *unc-25* RNAi (**C**), and $>10^6$ for all the other genotypes. One-way ANOVA followed by Tukey post-test was used to compare touch 1 with touch 2. No statistical difference was found across the strains (not shown).

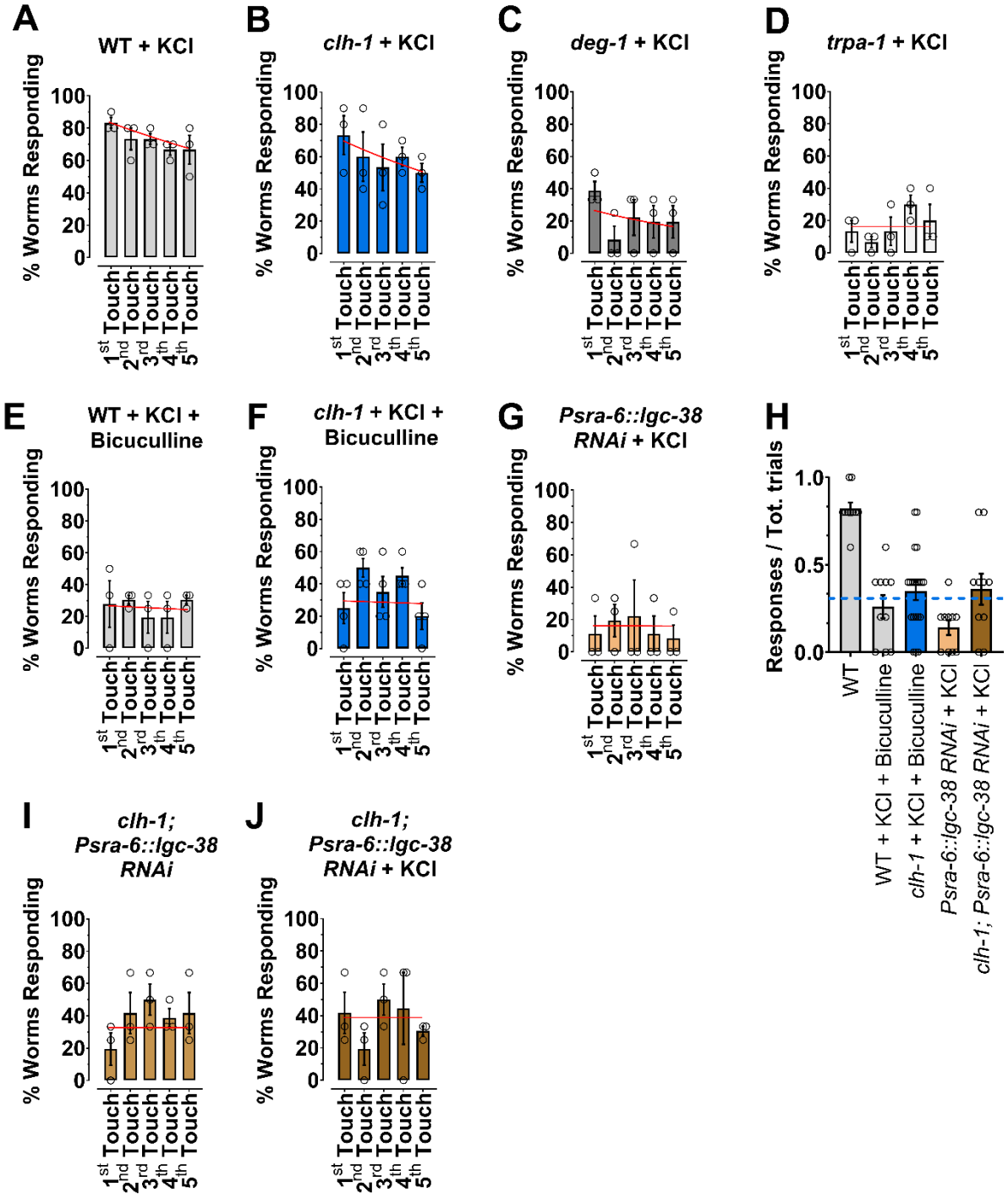

Figure S4. Nose touch behavior for experiments with KCl enriched plates (5 touches). Related to figure 5.

(**A-G**, **I-J**) Percentage of worms responding to 5 consecutive touches in wild type + KCl (**A**), *clh-1(ok658)* + KCl (**B**), *deg-1(u38u421)* + KCl (**C**), *trpa-1(ok999)* + KCl (**D**), wild type + KCl + bicuculline (**E**), *clh-1* + KCl + bicuculline (**F**), *Psra-6::lgc-38* RNAi + KCl (**G**), *clh-1; Psra-6::lgc-38* RNAi (**I**), and *clh-1;Psra-6::lgc-38* RNAi + KCl (**J**). (**H**) Average response of individual worms to 5 touches of panels **E-G** and **J**. The blue dotted line

represents the average nose touch response of *clh-1* knockout (Fig. 1B). Data are shown as individual experiments (**A-G**, **and I-J**) or individual worms (panel **H** only) (symbols) and as mean ± SEM (columns). In each experiment (**A-G**, **I-J**) 3 to 10 animals were tested. Fitting was by exponential decay with the following tau values: 17.84 (**A**), 41.07 (**E**), 12.28 (**B**), 65.52 (**F**), 8.33 (**C**), and $>10^6$ for the rest of genotypes. One-way ANOVA followed by Tukey post-test was used to compare touch 1 with touch 2. No statistical difference was found across the strains (not shown).

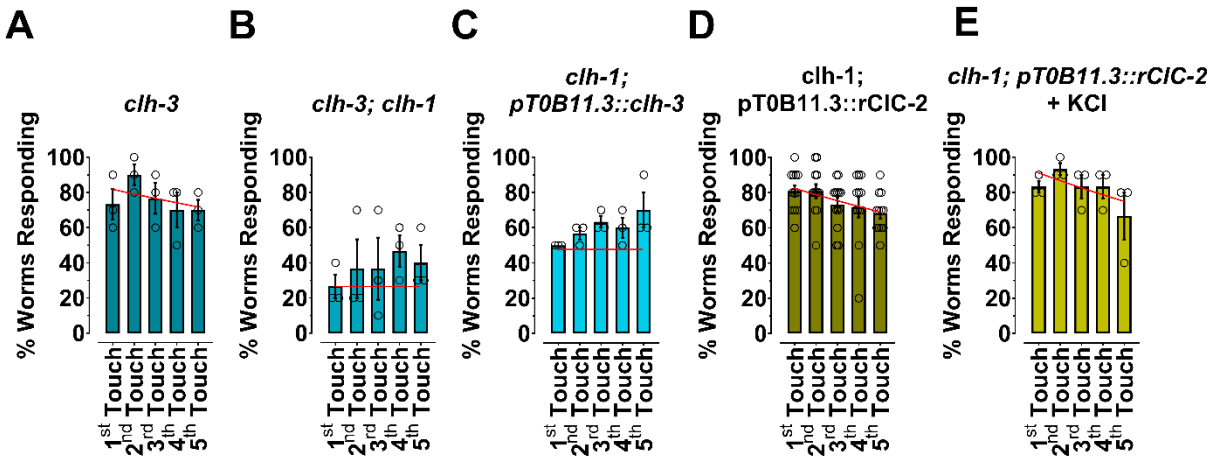

Figure S5. Nose touch behavior in ClC channel mutants and transgenics (5 touches). Related to figure 5.

Percentage of worms responding to 5 consecutive touches in *clh-3(ok763)* (**A**), *clh-3(ok763); clh-1(ok658)* (**B**), *clh-3* overexpression in AMsh glia (**C**), rat ClC-2 expression in AMsh glia (**D**) and rat ClC-2 expression in AMsh glia grown in KCl supplemented plates (**E**). Data are shown as individual experiments (symbols) and as mean ± SEM (columns). In each experiment 3 to 10 animals were tested. Fitting was by exponential decay with the following tau values: 29.54 (**A**), 21.99 (**D**), 19.94 (**E**) and $>10^6$ for the rest of genotypes. One-way ANOVA followed by Tukey post-test was used to compare touch 1 with touch 2. No statistical difference was found across the strains (not shown).

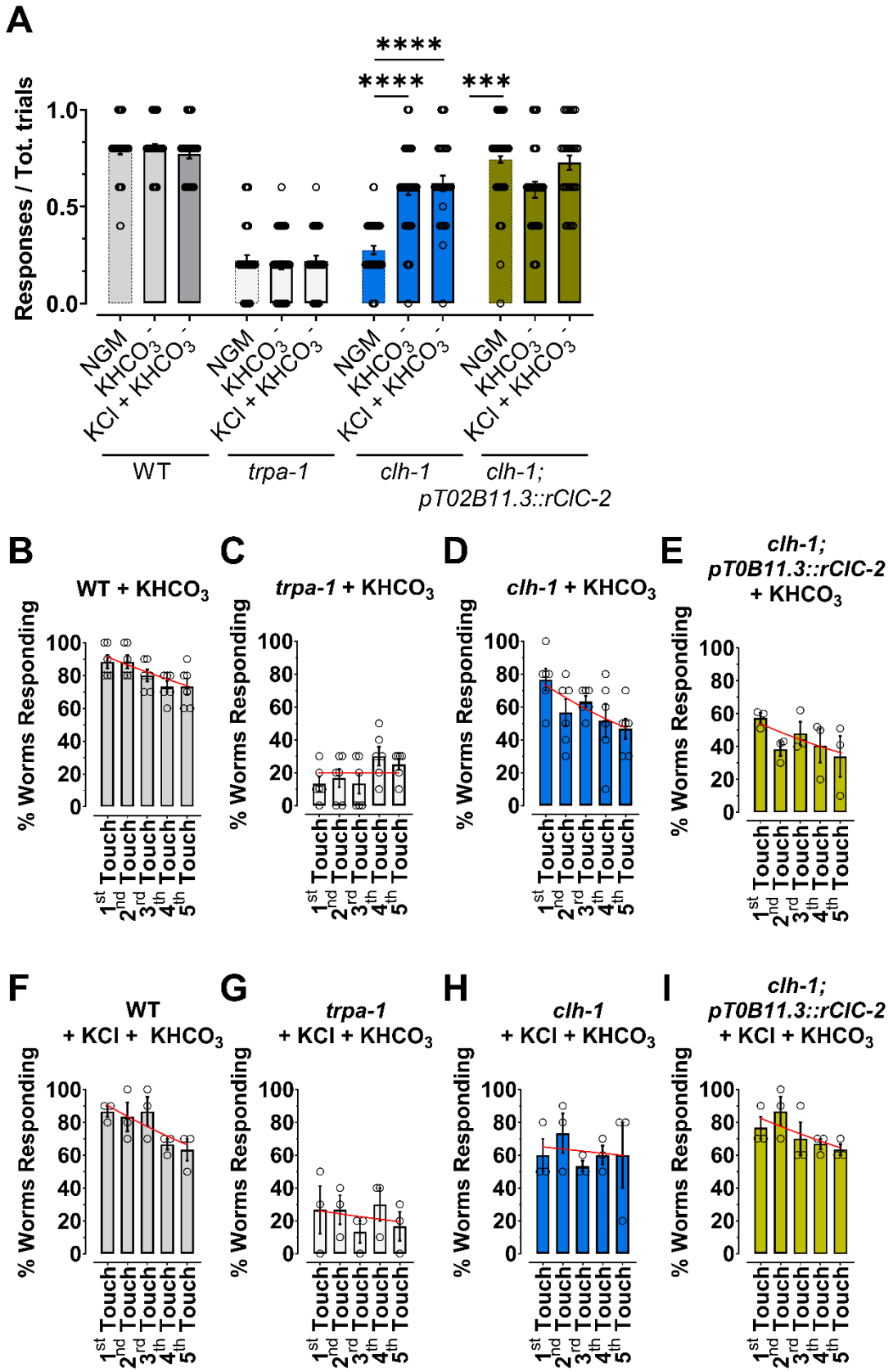
A
Responses / Tot. trials
1.0
0.5
0.0
****
****
***
NGM
KHCO3-
KCl + KHCO3-
WT
trpa-1
clh-1
clh-1;
pT02B11.3::rClC-2
B
WT + KHCO3
C
trpa-1 + KHCO3
D
clh-1 + KHCO3
E
clh-1;
pT0B11.3::rClC-2
+ KHCO3
% Worms Responding
1st Touch
2nd Touch
3rd Touch
4th Touch
5th Touch
F
WT
+ KCl + KHCO3
G
trpa-1
+ KCl + KHCO3
H
clh-1
+ KCl + KHCO3
I
clh-1;
pT0B11.3::rClC-2
+ KCl + KHCO3

Figure S6. Rescue of nose touch sensitivity in *clh-1* worms by bicarbonate. Related to figure 5.

(**A**) Effect of bicarbonate ($KHCO_3$) or KCl plus KHCO on nose touch in wild type, *trpa-1(ok999)*, *clh-1(ok658)*, and rat ClC-2 expression in AMsh glia. The dashed columns represent control data from Fig. 1B (WT, *trpa-1* and *clh-1*) and from Fig. 5H (AMsh glia rescue with rat *ClC-2*) and are shown here for comparison. Each data point represents one worm and columns represent mean ± SEM. n ≥ 30. (**B-E**) Percentage of worms responding to 5 touches in wild type (**B**), *trpa-1(ok999)* (**C**), *clh-1(ok658)* (**D**), and rat ClC-2 expression in AMsh glia (**E**) worms grown in plates supplemented with $KHCO_3$. (**F-I**) same as **B-E** for worms grown on plates supplemented with KCl + $KHCO_3$. Data are shown as individual experiments (**B-I**) (symbols) and as mean ± SEM (columns). In each experiment (**B-I**) 3 to 10 animals were tested. Fitting was by exponential decay with the following tau values: 17.92 (**B**), $>10^6$ (**C**), 8.91 (**D**), 9.66 (**E**), 12.61 (**F**), 13.38 (**G**), 46.03 (**H**) and 15.90 (**I**). One-way ANOVA followed by Tukey post-test was used to compare touch 1 with touch 2. No statistical difference was found across the strains (not shown).

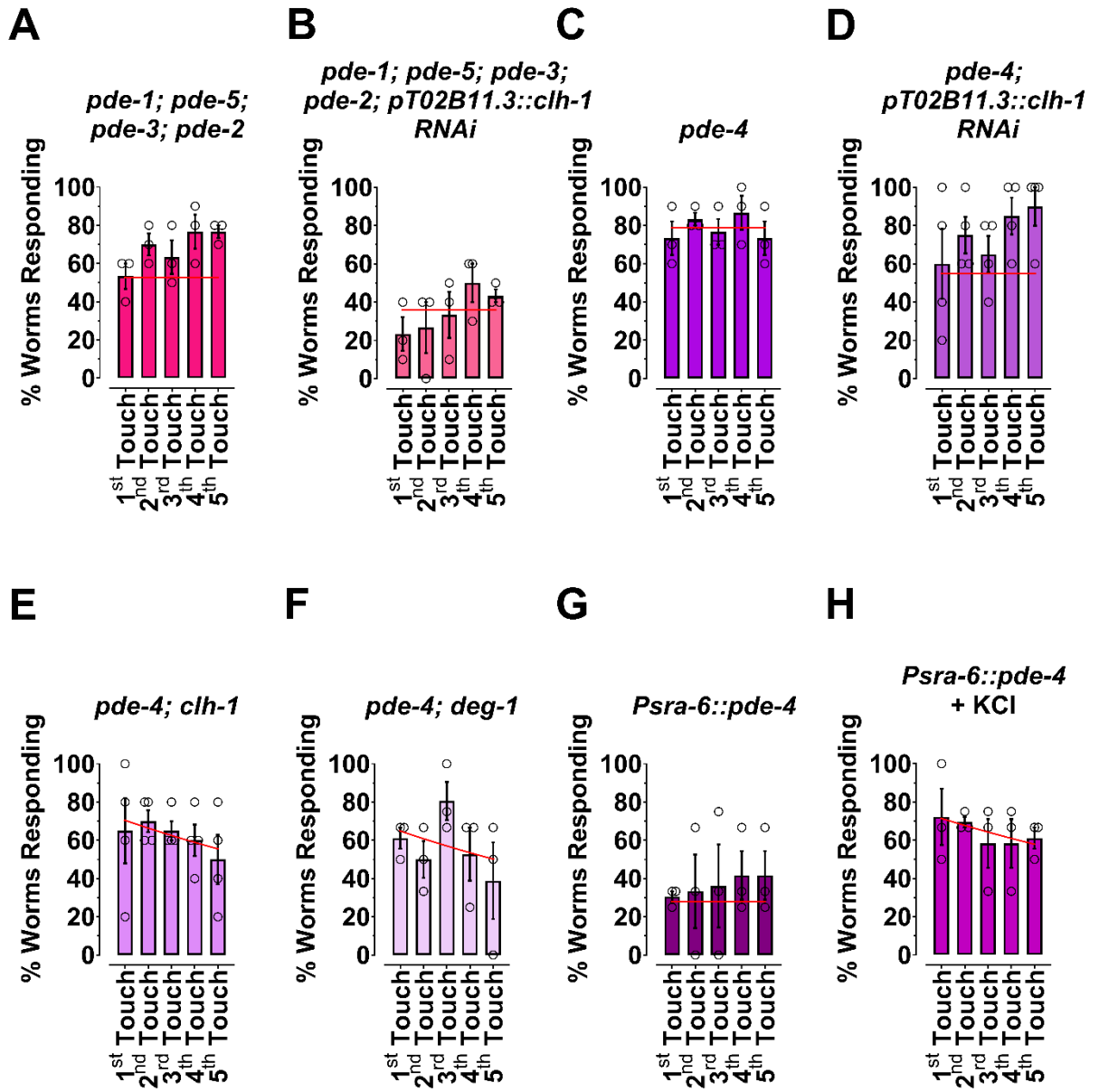

Figure S7. Nose touch behavior of *pde* mutants (5 touches). Related to figure 6. Percentage of worms responding to 5 touches in *pde-1; pde-5; pde-3; pde-2* (**A**), AMsh glia specific *clh-1* knockdown in *pde-1; pde-5; pde-3; pde-2* (**B**), *pde-4* (**C**), AMsh glia specific *clh-1* knockdown in *pde-4* (**D**), *pde-4; clh-1* (**E**), *pde-4; deg-1* (**F**), overexpression of *pde-4* in ASH (**G**), and overexpression of *pde-4* in ASH + KCl (**H**). Data are individual experiments (symbols) and mean ± SEM (columns). In each experiment 3 to 10 animals were tested. Fitting by exponential decay had the following tau values: $>10^6$ (**A-D, G**), 16.27 (**E**), 15.13 (**F**) and 18.52 (**H**). One-way ANOVA followed by Tukey post-test was used to compare touch 1 with touch 2. No statistical difference was found across the strains (not shown).

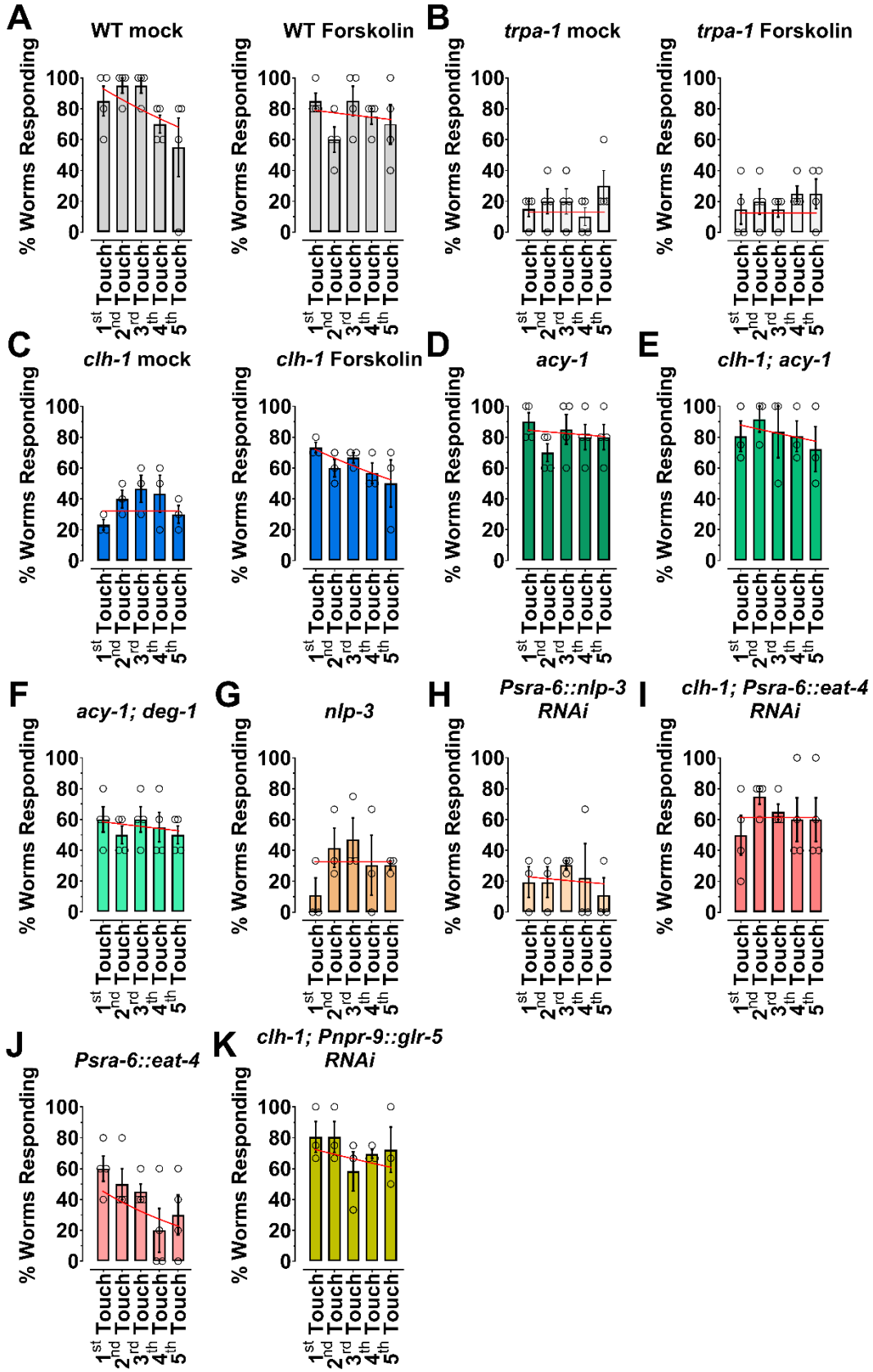
A
WT mock
WT Forskolin
B
trpa-1 mock
trpa-1 Forskolin
C
clh-1 mock
clh-1 Forskolin
D
acy-1
E
clh-1; acy-1
F
acy-1; deg-1
G
nlp-3
H
Psra-6::nlp-3 RNAi
I
clh-1; Psra-6::eat-4 RNAi
J
Psra-6::eat-4
K
clh-1; Pnpr-9::glr-5 RNAi
% Worms Responding
0
20
40
60
80
100
1st Touch
2nd Touch
3rd Touch
4th Touch
5th Touch

Figure S8. Adenylyl cyclase and glutamate in nose touch behavior. Related to figure 6.

(**A-C**) Percentage of worms responding to 5 touches in wild type, *trpa-1* and *clh-1* worms mock treated (**A-C**, left panels) or treated with forskolin (**A-C**, right panels). (**D-K**) Same as in A-C for the following strains *acy-1* (**D**), *clh-1; acy-1* (**E**), *acy-1; deg-1* (**F**), *nlp-3* (**G**), *nlp-3* knock-down in ASH (**H**), *eat-4* knock-down in ASH in *clh-1* knockout (**I**), *eat-4* overexpression in ASH (**J**), and *glr-5* knock-down in AIB (**K**). Data are individual experiments (symbols) and mean ± SEM (columns). In each experiment 3 to 10 animals were tested. Fitting by exponential decay had the following tau values: 12.53 (**A**, left panel), 48.64 (**A**, right panel), 12.34 (**C**, right panel), 78.98 (**D**), 30.62 (**E**), 36.99 (**F**), 17.79 (**H**), 8.84 (**J**), 24.62 (**K**) and $>10^6$ (**B**, **C** left panel, and **I**). One-way ANOVA followed by Tukey post-test was used to compare touch 1 with touch 2. No statistical difference was found across the strains (not shown).

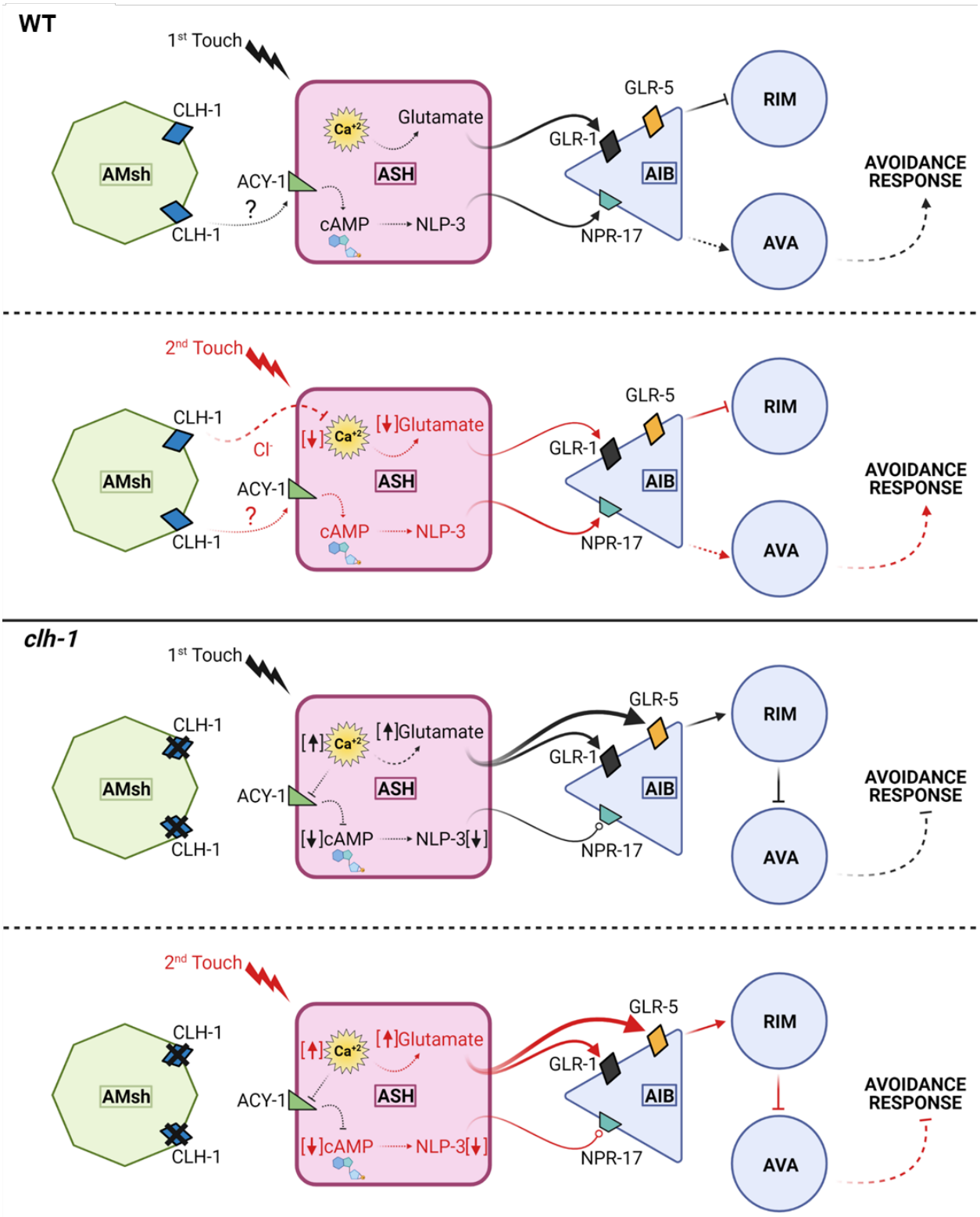

Figure S9. Glial CLH-1's regulation of glutamate and NLP-3 release from ASH neurons and avoidance response. Related to figure 8.

In wild type, the first touch triggers the increase of intracellular calcium concentrations ($[Ca^{2+}]_I$) and thus the release of glutamate. NLP-3 is also released by ASH and its

release is dependent on the cAMP pathway. CLH-1 may regulate the function of ACY-1 directly via Cl^- binding or indirectly via membrane hyperpolarization and reduced Ca^{2+} levels. Glutamate activates glutamate receptor GLR-1 in AIB interneurons, which in turn leads to the inhibition of inhibitory neuron RIM (Zou et al., 2018). Additionally, NLP-3 activates NPR-17 receptors in AIB which activates interneuron AVA (Harris et al., 2010). Finally, AVA initiates the avoidance response. Upon stimulation with a second touch, GABA signaling hyperpolarizes ASH neurons thus decreasing the $[Ca^{2+}]_i$. Glial channel CLH-1 mediates the flux of Cl^- ions needed for GABA signaling. Upon reduction of $[Ca^{2+}]_i$ the amount of glutamate released onto the AIB neurons is decreased, preventing the saturation of its GLR-1 receptors and thus resulting in an outcome similar to the one seen with the first touch. In the *clh-1* mutants, the absence of Cl^- flux from glia contributes to an increased basal $[Ca^{2+}]_I$ and to a decrease in the activity of adenylyl cyclase ACY-1. As a consequence, there is higher glutamate and lower NLP-3 release. Elevated levels of glutamate are expected to saturate GLR-1 and activate a second glutamate receptor, GLR-5, which in turn activates inhibitory RIM neurons (Zou et al., 2018). Together all these effects lead to reduced avoidance response. Upon stimulation with the second touch, reduced avoidance response persists due to added lack of GABA inhibition. Black and red lines indicate signaling during the first touch and second touch, respectively. Arrows indicate activation or positive interactions, blunted lines inhibition or negative interactions, and lines with an open circle indicate lower activation.

Genotype	Condition	ASH adaptation	Nose touch response
Wild type	-	Yes	Yes
clh-1	-	No	No
clh-1; pSra-6::clh-1	-	No	No
clh-1; pT02B11.3::clh-1	-	Yes	Yes
deg-1	-	No	No
trpa-1	-	No	No
Wild type	Bicuculline	No	No
unc-25	-	No	No
pT02B11.3::unc-25 RNAi	-	No	No
pSra-6::lgc-38 RNAi	-	No	No
Wild type	KCl	Yes	Yes
clh-1	KCl	Yes	Yes
Wild type	KCl + Bicuculline	No	No
clh-1	KCl + Bicuculline	No	No
pSra-6::lgc-38 RNAi	KCl	No	No
clh-1; pSra-6::lgc-38 RNAi	KCl	No	No
pde-4	-	Yes	Yes
pde-4; clh-1	-	Yes	Yes
acy-1	-	Yes	Yes
clh-1; acy-1	-	Yes	Yes

Table S1. Related to figures 2, and 4-6.
List of strains and conditions, in which we performed both Ca^{2+} imaging and behavioral assays, with the corresponding cellular and behavioral phenotypes. *ASH adaptation* is the adaptation in Ca^{2+} transients seen upon two consecutive touches in ASH neurons. *Nose touch response* indicates a positive behavioral nose touch avoidance response. Yes and No indicate whether the phenotype was observed or not.

Oligonucleotides		
pPD95_75 (pSra-6::RFP)	This study	N/A
pPD95_75 (pSra-6::delm-1)	(Han et al., 2013)	N/A
pPD95_75 (P_{delm-1}::RFP)	(Han et al., 2013)	N/A
pPD95_75 (pSra-6::clh-1 cDNA)	This study	N/A
pT02B11.3::clh-1 RNAi	This study	N/A
pGEM+clh-1 cDNA	(Grant et al., 2015)	N/A
pPD95_75 (pSra-6::GCaMP-6s)	This study	N/A
pPD95_75 (pT02B11.3::GCaMP-6s)	This study	N/A
pPD95_75 (pT02B11.3::clh-1 cDNA)	This study	N/A
pENTR221+clh-3b cDNA	(Branicky et al., 2014)	N/A
pTLN+rClC-2 cDNA	A gift from Michael Push	N/A
pPD95_75 (pT02B11.3)	(Bacaj et al., 2008)	N/A
pPD95_75 (pT02B11.3::GFP1-10)	This study	N/A
pPD95_75 (pT02B11.3::pGM87)	(Grant et al., 2015)	N/A
pPD95_75 (Punc-122::GFP)	(Grant et al., 2015)	N/A
pPD95_75 (Pvap-1::RFP)	(Johnson et al., 2020)	N/A
pPD95_75 (Pvap-1::clh-1 cDNA)	This study	N/A
Psra-6::clh-1 RNAi	This study	N/A
pPD95.75 (pT02B11.3::twk-33 RNAi sense)	This study	N/A
pPD95.75 (pT02B11.3::twk-33 RNAi antisense)	This study	N/A
pG-gcy-5p::SuperClomeleon	(Park et al., 2021)	N/A
pPD95_75 (pT02B11.3::SuperClomeleon)	This study	N/A
Psm [aptf-1p::unc25::sl2gfp]	(Liu et al., 2018)	N/A
pT02B11.3::unc-25 RNAi	This study	N/A
Psra-6::lgc-38 RNAi	This study	N/A
KG #203 (Prab-3::pde-4d (+) cDNA)	(Charlie et al., 2006)	N/A
pPD95_75 (Psra-6::pde-4d (+) cDNA)	This study	N/A
Psra-6::nlp-3 RNAi	This study	N/A
Psra-6::eat-4 RNAi	This study	N/A
pCR2.1-TOPO (eat-4 cDNA)	This study	N/A
pPD95_75 (Psra-6::eat-4 cDNA)	This study	N/A
Pnpr-9::glr-5 RNAi	This study	N/A

Table S2. Related to Key Resource Table and molecular biology section in STAR methods.

Oligonucleotides used in this study to generate transgenic strains or other constructs.