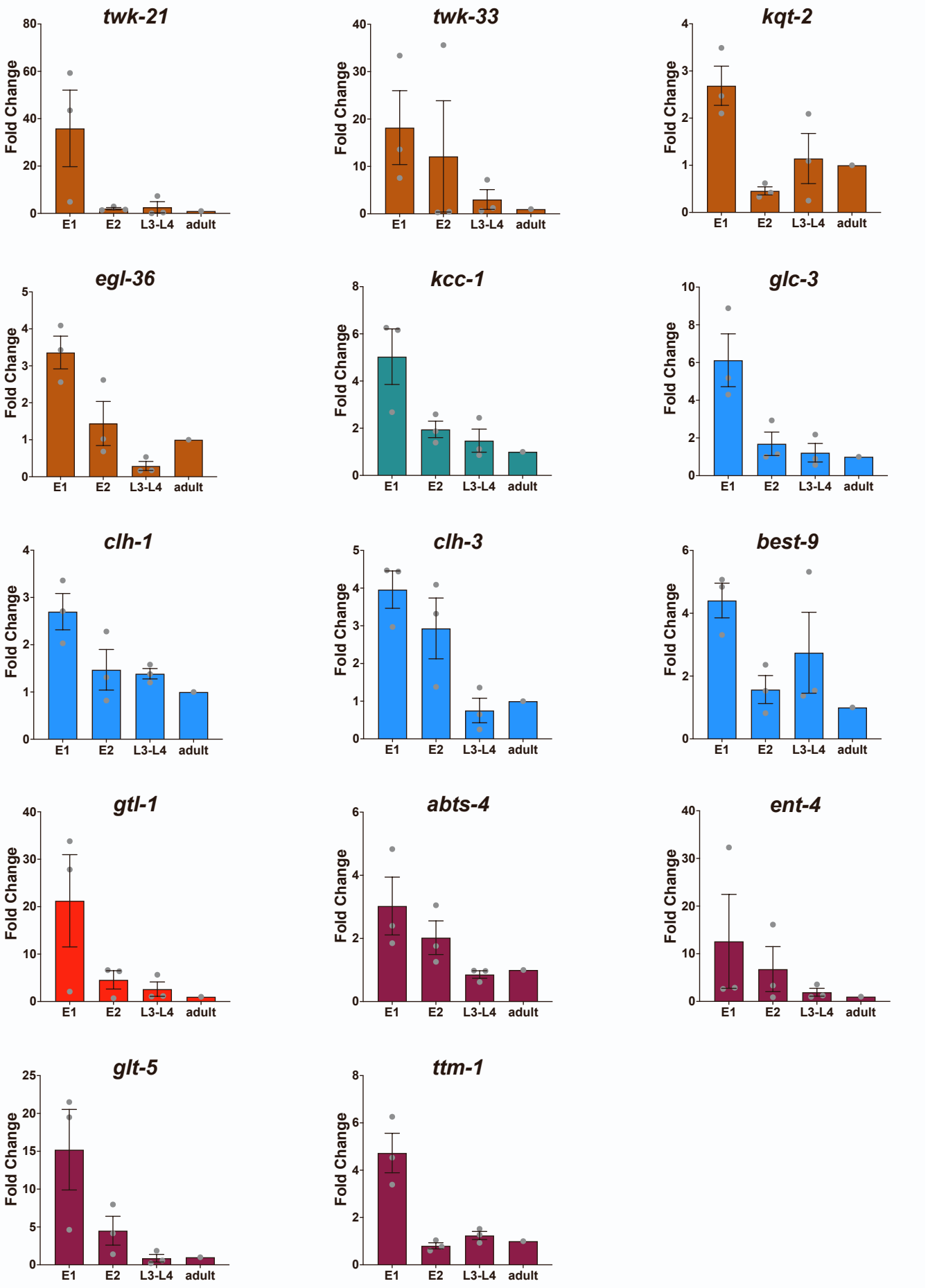
iScience, Volume *25*

Supplemental information

Glial regulators of ions and solutes required for specific chemosensory functions in *Caenorhabditis elegans*

Lei Wang, Bianca Graziano, Nicole Encalada, Jesus Fernandez-Abascal, Daryn H. Kaplan, and Laura Bianchi

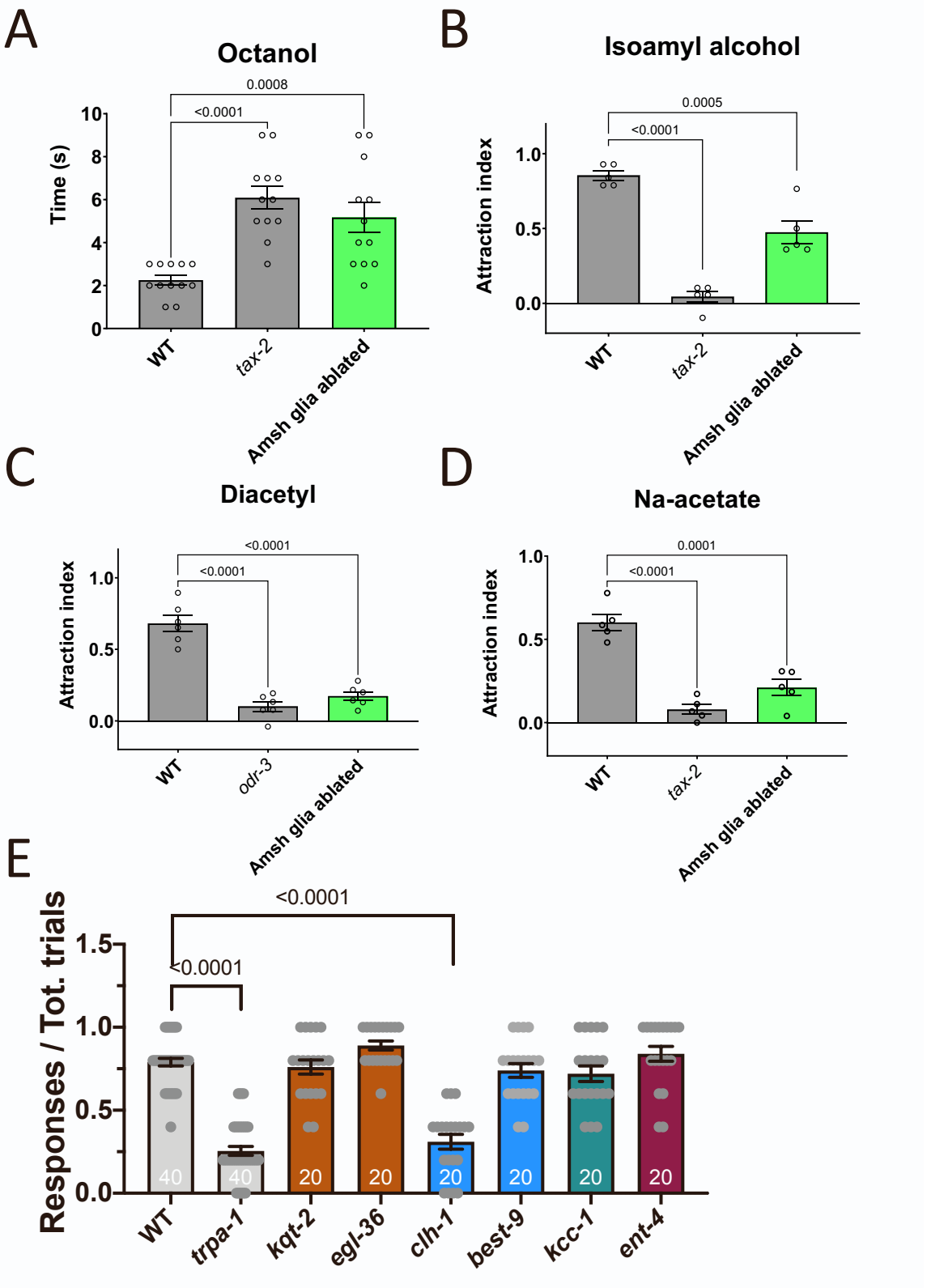


Wang et al., Fig. S1

Figure S1 (related to Figure 1A). The fold change in mRNA expression as compared to the rest of the worm cells in four independent datasets for the Amsh cells enriched ion channels and transporters. Fold change of 1 corresponds to the same expression level as compared to the rest of the cells. Embryo 1 (E1) corresponds to the dataset from Grant and colleagues (Grant *et al.*, 2015). This is an RNA sequencing dataset of embryonic Amsh cells labelled with *T02B11.3::PGM87* (a pH sensitive GFP). Embryo 2 (E2) corresponds to an oligo-nucleotide gene array data set obtained from embryonic Amsh cells labelled by *vap-1::GFP* (Bacaj *et al.*, 2008). The column labelled L3-L4 corresponds to an RNA sequencing dataset obtained from late-stage larvae (L3-L4) expressing *F16F9.3::dsRed* in the Amsh cells (Wallace *et al.*, 2016). The column labelled adult corresponds to single cell RNA sequencing from adult worms (Taylor *et al.*, 2021). Data are shown as individual data points and as mean +/- SE.

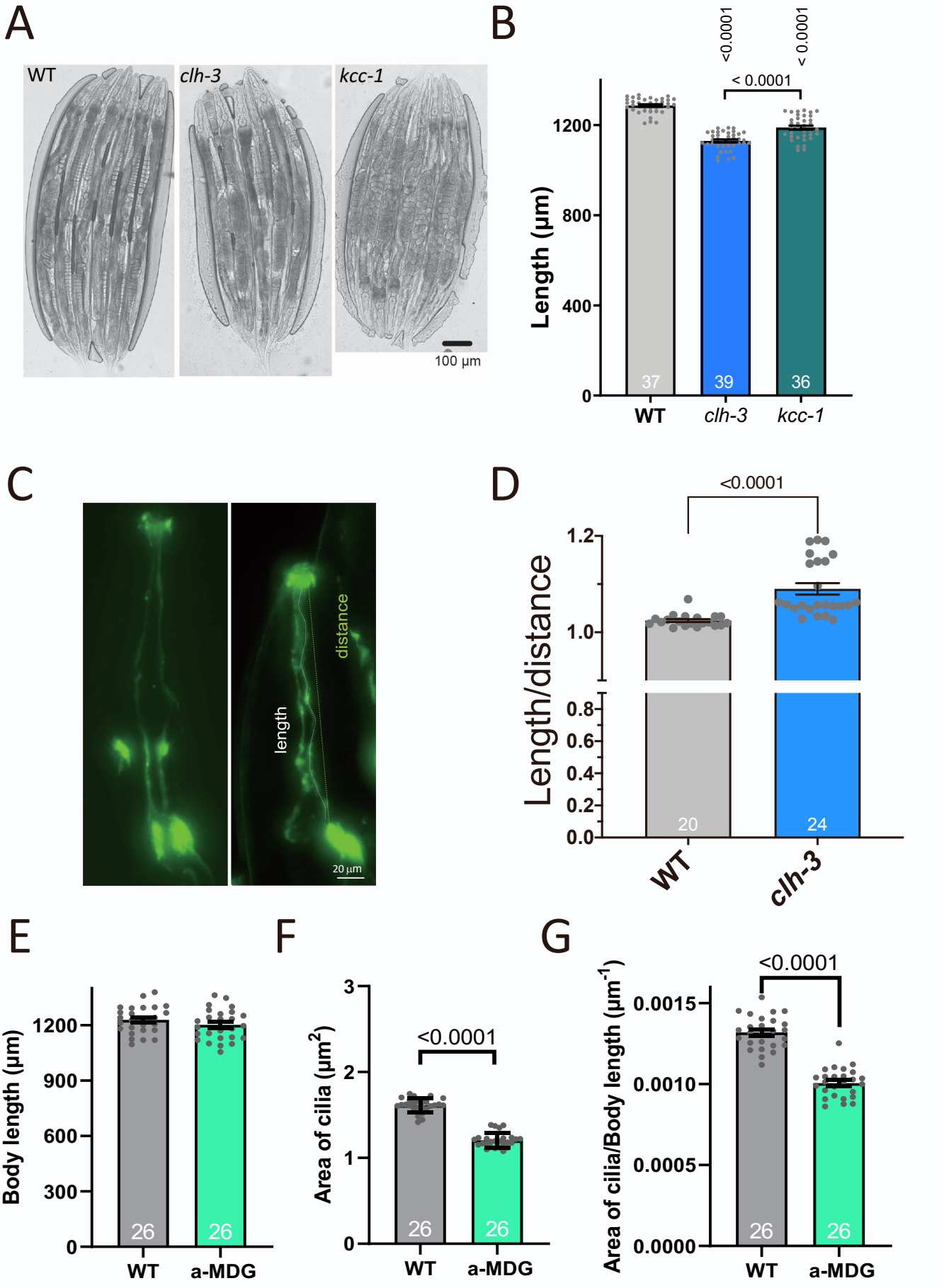
Wang et al., Fig. S2

Figure S2 (related to Figure 1A). The expression level in Amsh glia versus the rest of *C. elegans* of ion channels and transporters. The Fragments Per Kilobase of transcript per Million mapped reads (FPKM) in ion channels and transporters mRNA expression in Amsh glia as compared to the rest of the worm cells in Grant *et al.*, 2015. The log2 FPKM values of each ion channel and transporter gene were used to generate the heatmap. ND stands for not detected.



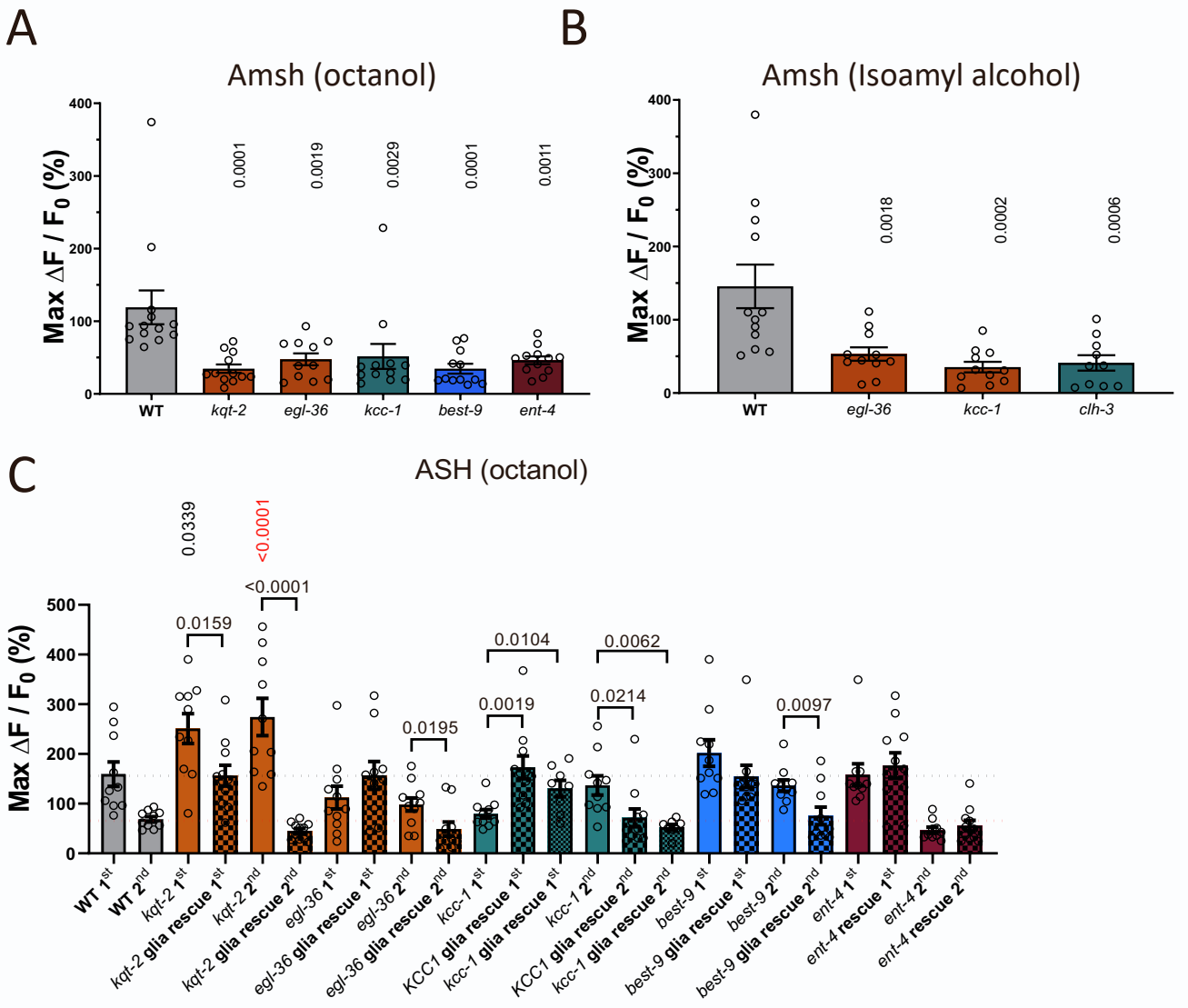
Wang et al., Fig. S3

Figure S3 (related to Figure 1C). Amsh glia are needed for response to odorants and tastants in *C. elegans*. Chemotaxis assays of wild type, *tax-2*, *odr-3*, and Amsh glia ablated strain. **(A)** Time to response to the odorant octanol (10%) in seconds (n=12 each), **(B)** attraction index to isoamyl alcohol (1:100) (n=5 each), **(C)** attraction index to diacetyl (1:1000) (n=6 each), and **(D)** attraction index to Na-acetate (0.2 M) (n=5 each). **(E)** The nose touch response of wild type and mutants that have reduced octanol avoidance: *kqt-2*, *egl-36*, *best-9*, *kcc-1*, and *ent-4. clh-1* responds normally to octanol, but it is nose touch insensitive, and it is shown here for comparison (Fernandez-Abascal *et al.*, 2022). Positive and negative controls were wild type and *tax-2(p691)*, respectively for octanol avoidance and chemotaxis to isoamyl alcohol, and Na-acetate. Negative controls for chemotaxis to diacetyl and for nose touch were *odr-3,* and *trpa-1* and *clh-1*, respectively. Data are shown as individual data points and as mean +/- SE. p values are shown in the panels and were obtained by ANOVA with Tukey correction.



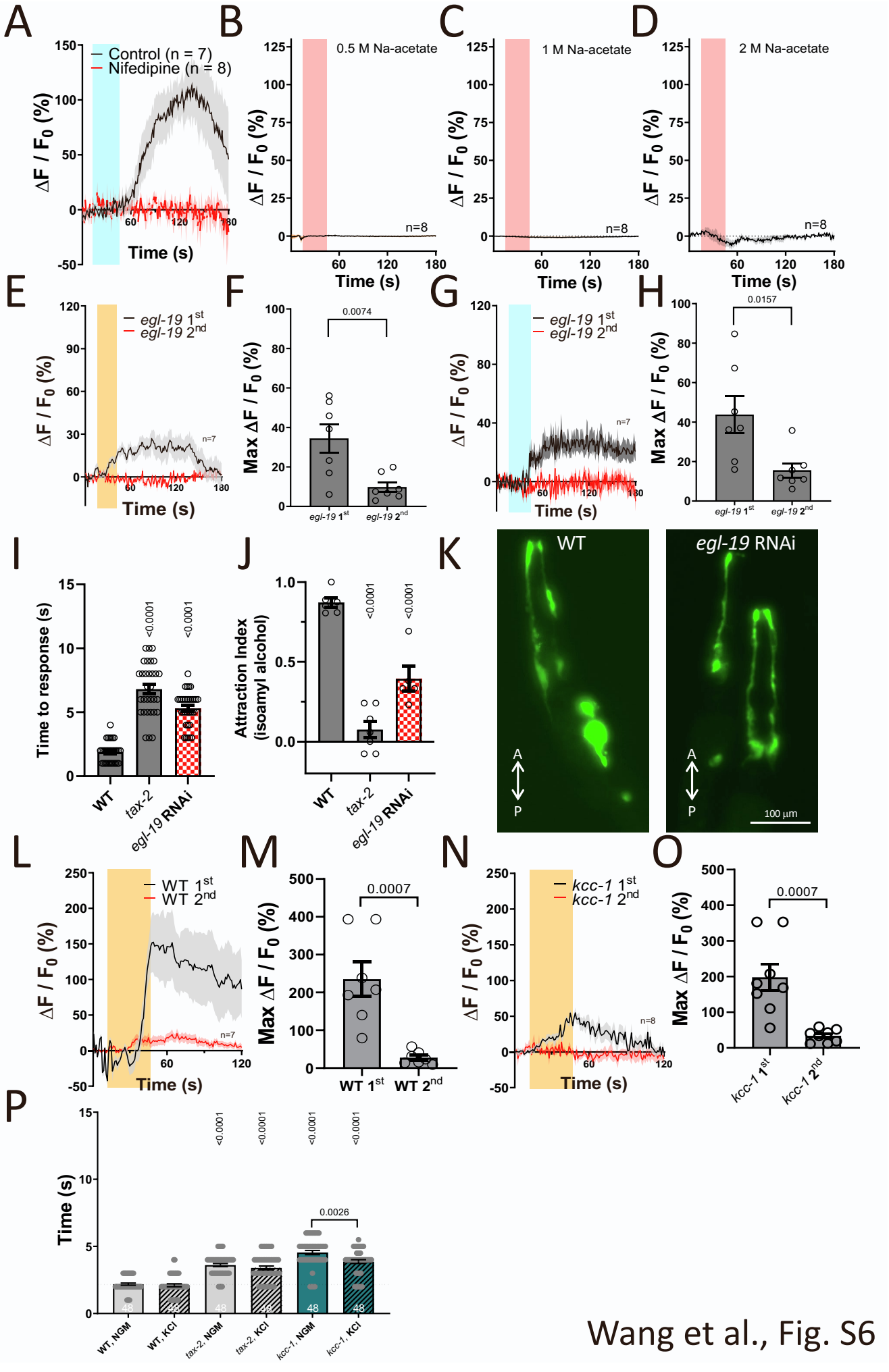
Wang et al., Fig. S4

Figure S4 (related to Figure 2). *clh-3* and *kcc-1* worms are smaller. (A) Bright field images of wild type (WT), *clh-3*, and *kcc-1* mutant worms. Scale bar is 100 μm. **(B)** Quantification of the length of the body of wild type, *clh-3*, and *kcc-1* mutant worms. **(C)** Fluorescence images of the head of wild type and *clh-3* mutants expressing GCaMP-6s in Amsh glia. Scale bar is 50 μm. The length of the Amsh glia processes and the distance from the cell body to the tip of the nose are shown as white and yellow dotted lines. **(D)** Quantification of the length/distance ratio of the Amsh glia processes of the wild type and *clh-3* mutant worms. **(E)** Quantification of the length of the body and **(F)** the area of ASH cilia of worms reared on control and α-MDG supplemented plates. **(G)** Quantification of the area of cilia/length ratio. The n is shown in the columns. Data are expressed as individual data points and as mean +/- SE. p values are shown in the panels and were obtained by unpaired student t-Test.



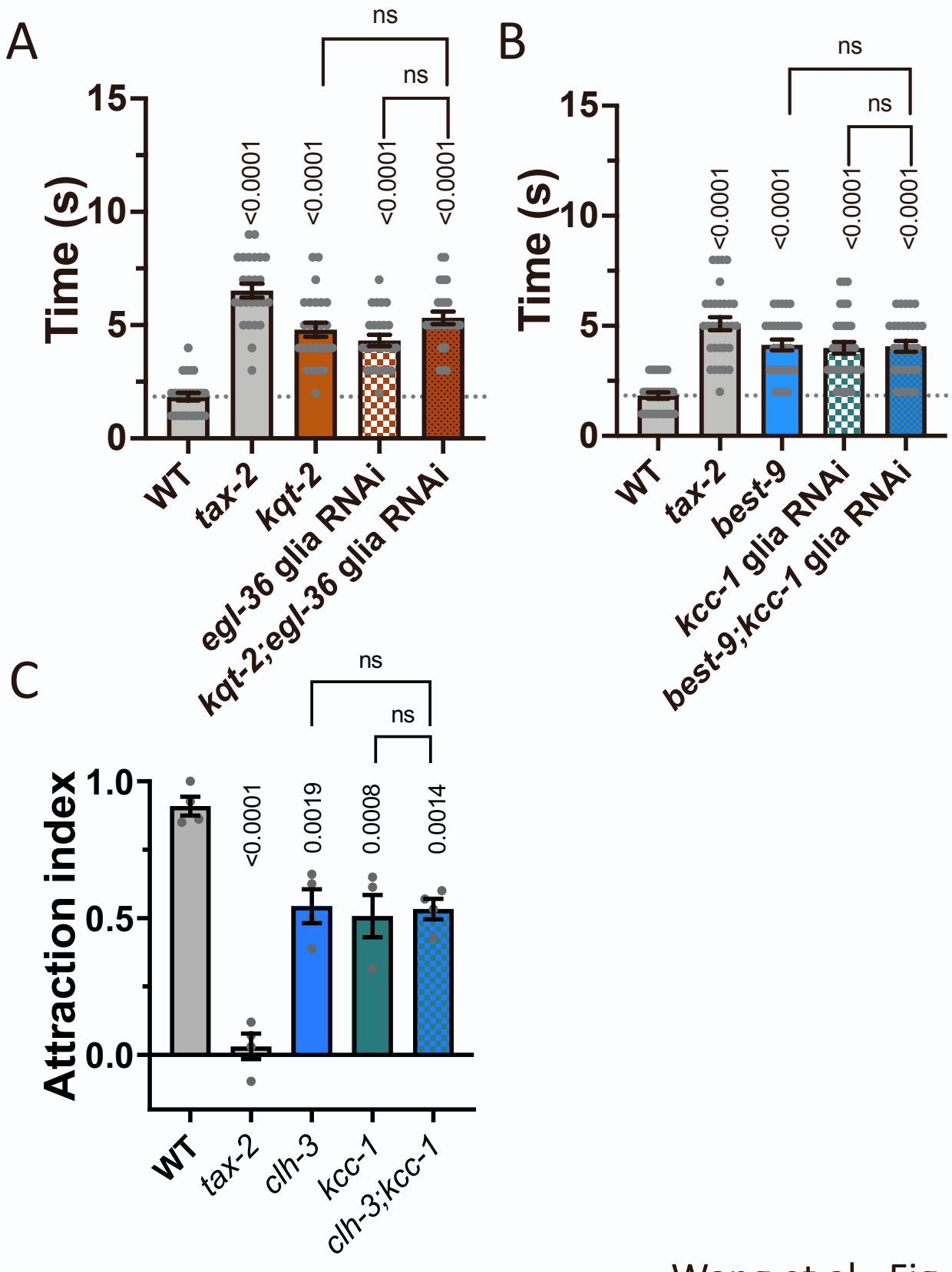
Wang et al., Fig. S5

Figure S5 (related to Figures 3 and 4). The amplitude of the calcium transients in response to odorants in Amsh glia and ASH neurons. (A) Peak percentage of GCaMP-6s fluorescence ($\Delta F/F_0$) in Amsh glia upon perfusion with octanol (first exposure, 1:1,000). **(B)** Same as in (A) for Amsh glia upon perfusion with isoamyl alcohol (1:100). **(C)** Same as in (A) and (B) for ASH neurons upon perfusion with octanol (1:1,000). Data are from figures 3 and 4, they correspond to the first perfusion with the odorant, and they are shown here together for easy comparison. N was 11-13 for (A), 11,12 for (B), and 10,11 for (C). In C the dashed black line indicates the average amplitude of the first calcium transients of wild type worms. The dashed red line indicates the average amplitude of the second calcium transients of wild type worms. Data are expressed as individual data points and as mean +/- SE. p values are shown in the panels and were obtained by ANOVA with Tukey correction. The p value shown in red is a comparison between the second calcium transient in wild type and *kqt-2*.



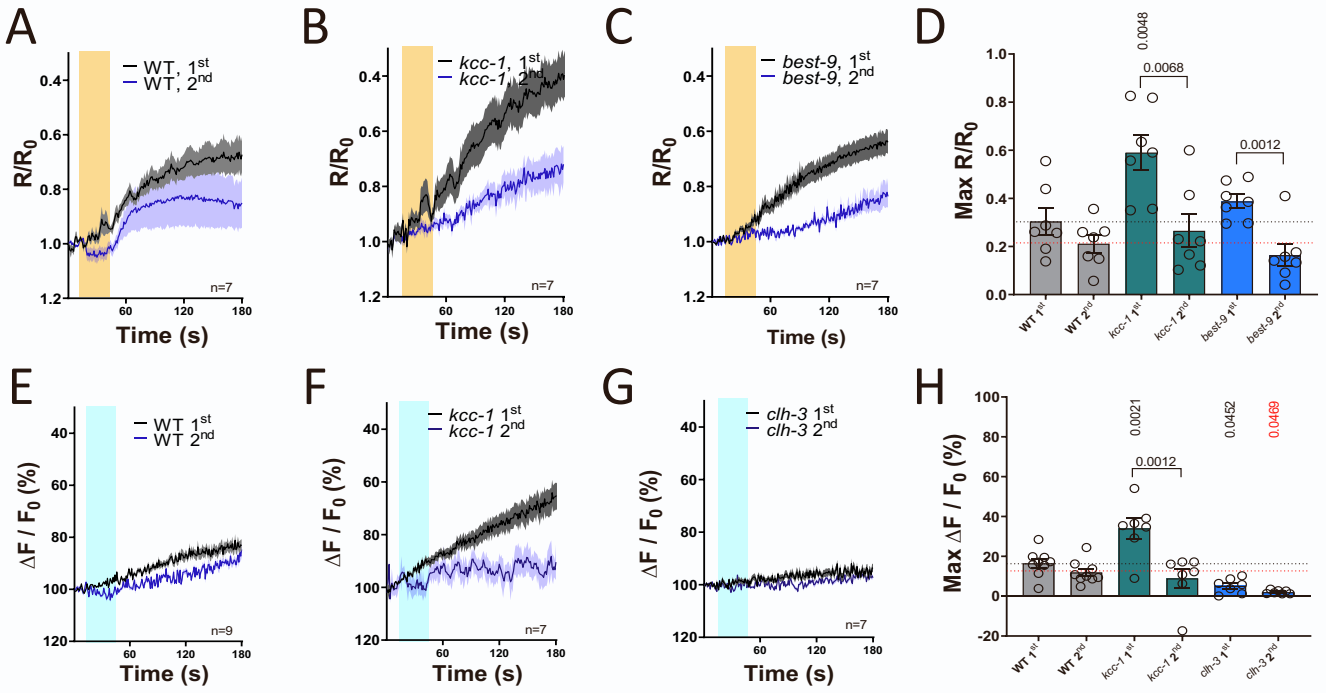
Wang et al., Fig. S6

Figure S6 (related to Figures 2 and 3). Calcium transients in glia and animal behavior. (A) The effect of the calcium-channel blocker nifedipine on intracellular calcium in Amsh glia. Intracellular calcium transients measured as % increase of GCaMP-6s fluorescence above the baseline ($\Delta F/F_0$) generated by perfusion with octanol (1:1,000) in Amsh glia from wild type worms in control and following preincubation with nifedipine (purple line). **(B)** to **(D)** Intracellular calcium in Amsh glia following perfusion with 0.5, 1, and 2 M Na-acetate. **(E-H)** Amsh glia RNAi of *egl-19*. The blue shaded area indicates the time of perfusion with isoamyl alcohol. The yellow shaded area indicates the time of perfusion with octanol. Calcium transients generated in ASH neurons were measured by % increase of GCaMP-6s fluorescence above the baseline ($\Delta F/F0$) in *egl-19* glial specific RNAi worms for the 1st and 2nd stimulation. **(I)** Time to response to octanol (10%), n=33, 31, and 31, respectively, **(J)** attraction index to isoamyl alcohol of *egl-19* Amsh glial specific RNAi worms, n=6, 7, and 5, respectively. **(K)** The morphology of Amsh glial cells in control and *egl-19* glial specific RNAi worms. A represents anterior, P represents posterior. **(L-O)** Calcium transients recorded in Amsh glia of wildtype and *kcc-1* mutant worms reared on plates containing 150 mM KCl. **(P)** Time to response to octanol for WT, *tax-2*, and *kcc-1* mutant worms reared on control (NGM) plates and plates containing 150 mM KCl. The dashed black line indicates the average response time of wild type worms in NGM plates. Data are expressed as mean +/- SE. The number of cells tested in calcium imaging is shown in each panel, the number of worms tested is shown in the columns.



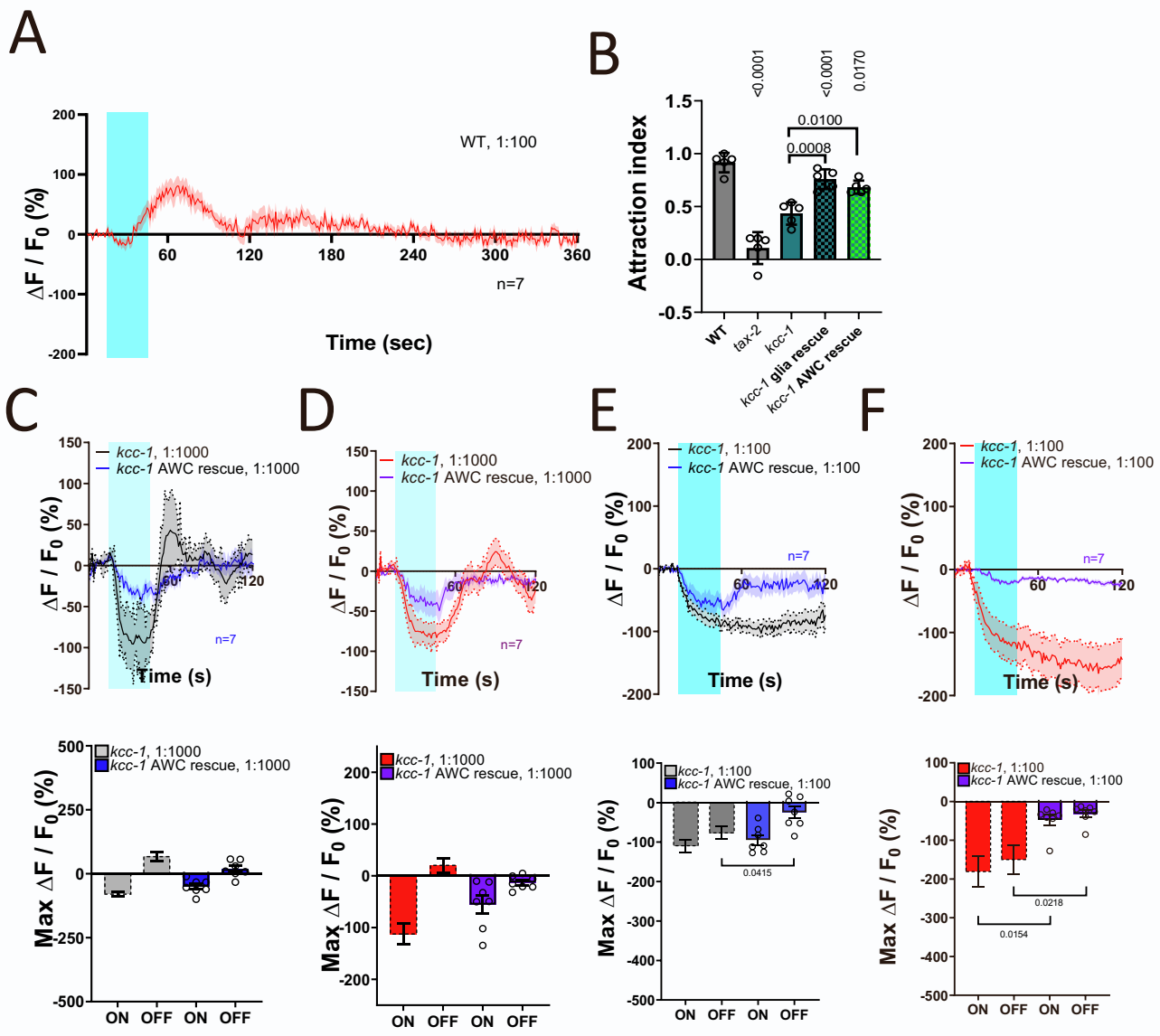
Wang et al., Fig. S7

Figure S7 (related to Figure 1C). Response to octanol and isoamyl alcohol in double mutants. (A) Time to response to octanol (10%) for *kqt-2*, *egl-36* Amsh RNAi, and *kqt-2*;*egl-36* Amsh RNAi. n=25 each. **(B)** Same as in a for *best-9*, *kcc-1*, and *best-9*;*kcc-1* Amsh RNAi. n = 30-33. **(C)** Attraction index to isoamyl alcohol (1:100) for *clh-3* and *kcc-1* single and double mutants, n=4 each. Data are expressed as individual data points and as mean +/- SE. p values are shown in the panels and were obtained by ANOVA with Tukey correction.



Wang et al., Fig. S8

Figure S8 (related to Figure 3). Chloride concentration in glia of channels and transporters' mutants. (A-C) Intracellular changes in chloride concentration generated in Amsh by perfusion with octanol (1:1,000, yellow shaded area) as measured by SuperClomeleon fluorescence. Data represent the YFP/CFP ratio (R) change with respect to the baseline (R_0, 10 seconds before touch). The 1st stimulation is in black and 2nd stimulation is in blue. **(D)** Average R/R_0 change of the last 10 seconds of recording with respect to the baseline for wild type, *kcc-1*, and *best-9* mutants. **(E-G)** Same as in a-d but for perfusion with isoamyl alcoholand mutants *kcc-1* and *clh-3* (1:100, blue shaded area). The dashed black line indicates the average amplitude of the first chloride transients of wild type worms. The dashed red line indicates the average amplitude of the second chloride transients of wild type worms. Data are expressed as individual data points and as mean +/- SE. p values are shown in the panels and were obtained by ANOVA with Tukey correction.



Wang et al., Fig. S9

Figure S9 (related to Figures 6 and 7). AWC calcium transients in response to isoamyl alcohol in *kcc-1* mutants with AWC specific *kcc-1* rescue. (A) The second calcium transients generated in AWC neurons by perfusion with Isoamyl alcohol (1:100, cyan shaded area) as measured by % increase of GCaMP5 fluorescence above the baseline ($\Delta F/F_0$) in wild type worms recorded for 6 minutes. **(B)** The attraction index to isoamyl alcohol of *kcc-1, kcc-1* AWC rescue, and *kcc-1* Amsh glia rescue worms, n was 5 each. p values are shown in the panels and were obtained by ANOVA with Tukey correction. **(C-F)** Calcium transients in AWC neurons by perfusion with isoamyl alcohol (1:100 and 1:1,000, cyan shaded area) as measured by % increase of GCaMP5 fluorescence above the baseline ($\Delta F/F_0$) in *kcc-1* and *kcc-1* AWC rescue worms (up panel). The peak percentage of calcium concentration change ($\Delta F/F_0$) in *kcc-1* and *kcc-1* AWC rescue worms (down panel). Data shown by dotted lines are from figures 6 and 7 and they are shown here for easy comparison. Data are expressed as individual data points and as mean +/- SE. p values are shown in the panels and were obtained by unpaired student's test.