

Supplementary Information

Induction of Associative Olfactory Memory by Targeted Activation of Single Olfactory Neurons in *Drosophila* Larvae

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Legends to Supplementary Figures

Figure S1. The conditioning and test apparatus.

- a. **The conditioning set up.** We used a LED apparatus with dual flexible arms (3 Watt each). Blue LEDs (468 nm) with 490 nm short-path filter or red LEDs (625 nm) with 580 nm long-path filter were used. Larvae were placed on an agar plate set at the bottom of a cylinder-like covering made with aluminum foil (not shown), and illuminated from 12 cm above the agar surface with the dual LED apparatus. The agar plate was placed on a hot plate set to produce the indicated surface temperature measured with an infrared thermometer.
- b. **The blue/red LED quadrants.** Larval phototactic behaviors were tested on a 2.5 % agar plate with diametrically-opposed quadrants, each illuminated with 22 blue or red LEDs. For blue light, 468 nm LEDs (2,600 mcd) were used with 490 nm short-path filter of 50% transmission. For red light, 625 nm LEDs (2,600 mcd) were used with 580 nm long-path filter of 50% transmission.

Figure S2. Phototactic behaviors of naïve and trained Or-ChR2 larvae on blue/red quadrants.

- a. **Phototactic behaviors on the blue/red quadrants plate.** Larvae carrying only Tdc2-dTrpA1 or Or-ChR2 construct exhibited no alteration in their phototaxis toward blue light after training with blue light at 28°C (Mann-Whitney *U*-test). Note that naïve Or-ChR2 but not Tdc2-dTrpA1 larvae showed higher phototaxis toward blue light than the control *w* (*CS10*) larvae ($p < 0.001$ by Mann-Whitney *U*-test). Number of sample is indicated in the bar.
- b. **Phototactic response indices of Or-ChR2 larvae on the blue/red quadrants plate.** Larvae carrying only Tdc2-dTrpA1 or Or-ChR2 construct exhibited no alteration in their response indices after training with blue light at 28°C (Mann-Whitney *U*-test). Number of sample is indicated in the bar.

Figure S3. Naïve phototactic behaviors of Or-ChR2 larvae on light/dark quadrants.

- a. Light/dark preference test.** Naïve Or-ChR2 larvae were placed on the center of an agar plate (21°C) with diametrically opposed quadrants illuminated with white light. Typically, ~50 larvae were placed at the center and the numbers of animals moved in the light and dark areas were separately counted after 3 min. Response index (RI) was calculated as indicated.
- b. Phototactic behaviors on the light/dark quadrants plate.** No difference was detected among Or-ChR2 and *w* (*CS10*) control larvae (Kruskal-Wallis ANOVA), indicating normal visual acuity and locomotor activity of the transgenic animals. Number of sample is indicated in the bar.
- c. Phototactic response indices toward white light** No difference was detected among the response indices of Or-ChR2 and *w* (*CS10*) control larvae by Kruskal-Wallis ANOVA. Number of sample is indicated in the bar.

Figure S4. Thermotactic response indices of Or-ChR2 larvae.

As with the *w* (*CS10*) control larvae, transgenic larvae carrying Or-ChR2 construct alone preferred cooler temperatures exhibiting negative thermotactic RI. No difference was detected among the response indices of Or-ChR2 and *w* (*CS10*) control larvae by Kruskal-Wallis ANOVA. Number of sample is indicated in the bar.

Figure S5. Associative memory formation by different stimulation time.

- a. Phototactic response test with the blue/red quadrants plate.** Transgenic larvae carrying both Or24a-ChR2 and Tdc2-dTrpA1 were illuminated with blue light for the indicated time and then tested on the blue/red plate for their phototactic behaviors. *** $p < 0.001$ by Mann-Whitney *U*-test between 21°C and 28°C. Number of sample is indicated in the bar.
- b. Olfactory response test with acetophenone.** Transgenic larvae carrying both Or24a-ChR2 and Tdc2-dTrpA1 were illuminated with blue light for the indicated time and then tested for olfactory response behaviors using acetophenone. ** $p < 0.001$ by Mann-Whitney *U*-test between 21°C and 28°C. Number of sample is indicated in the bar.

Figure S6. Olfactory responses of transgenic larvae carrying Or-ChR2 or Tdc2-dTrpA1 alone.

a) Or24a-ChR2, b) Or42b-ChR2, c) Tdc2-dTrpA1. No difference was found between the larvae trained with blue light at 21 °C and 28°C. Mann-Whitney *U*-test. Number of sample is indicated in the bar.

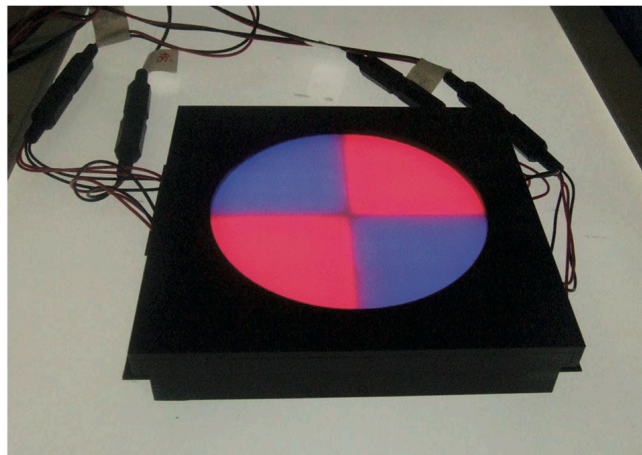
Movie 1. Thermotactic behavior of *w* (*CS10*) control larvae on a 21-28°C temperature gradient plate. Duration: 3min.

Movie 2. Thermotactic behavior of Or24a-ChR2;Tdc2-dTrpA1 larvae on a 21-28°C temperature gradient plate. Duration: 3 min.

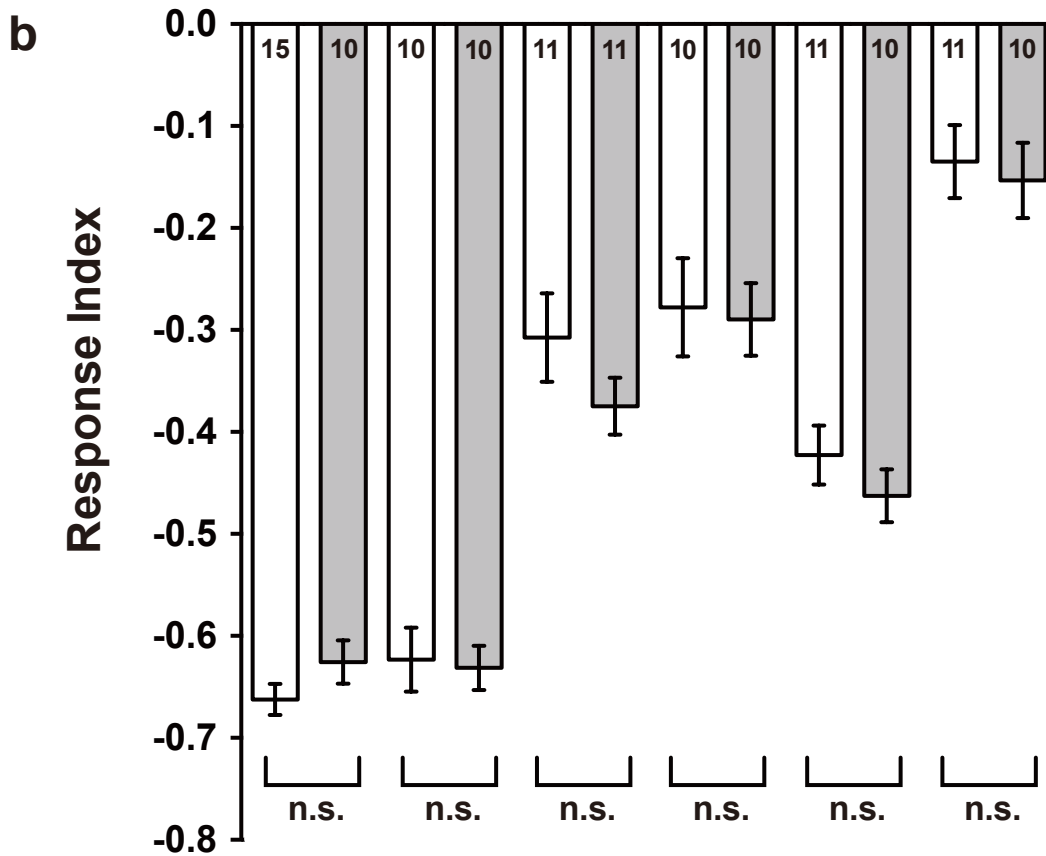
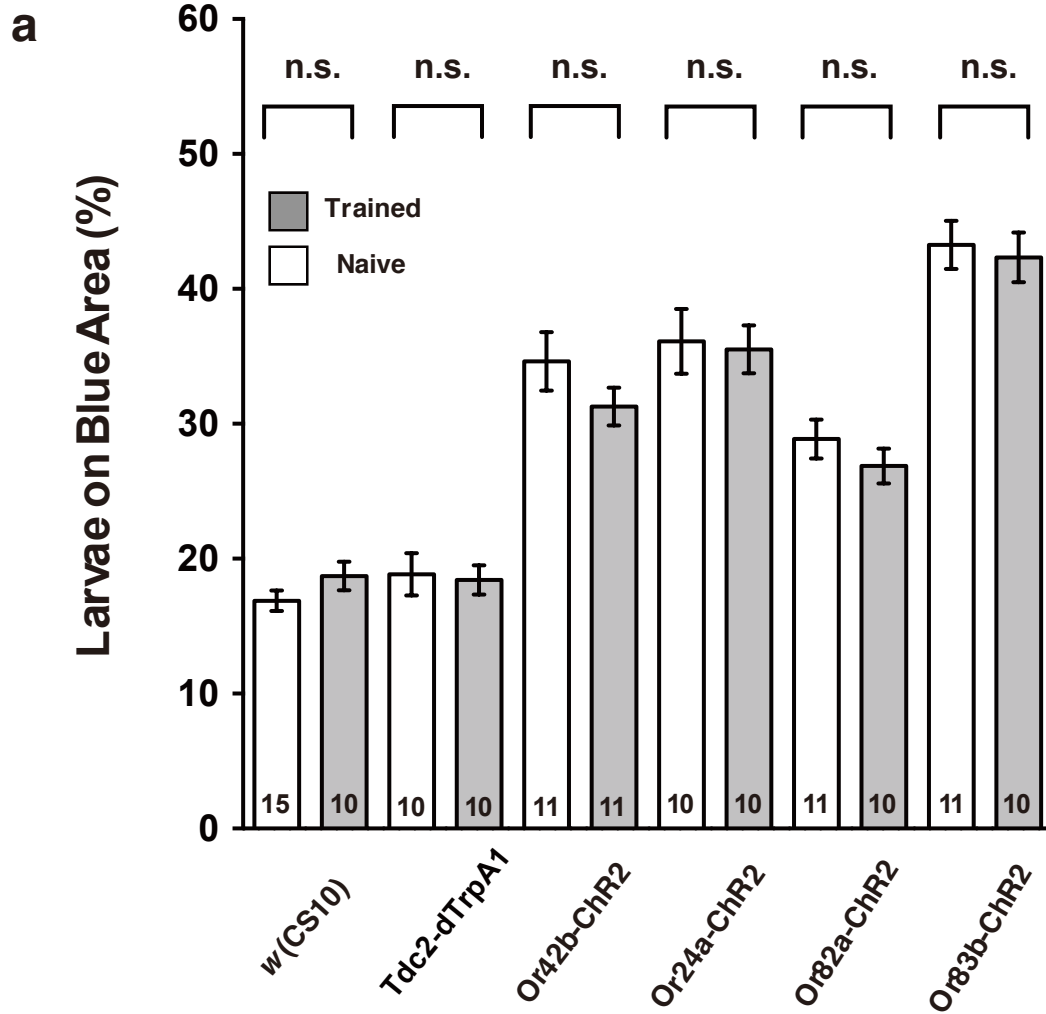
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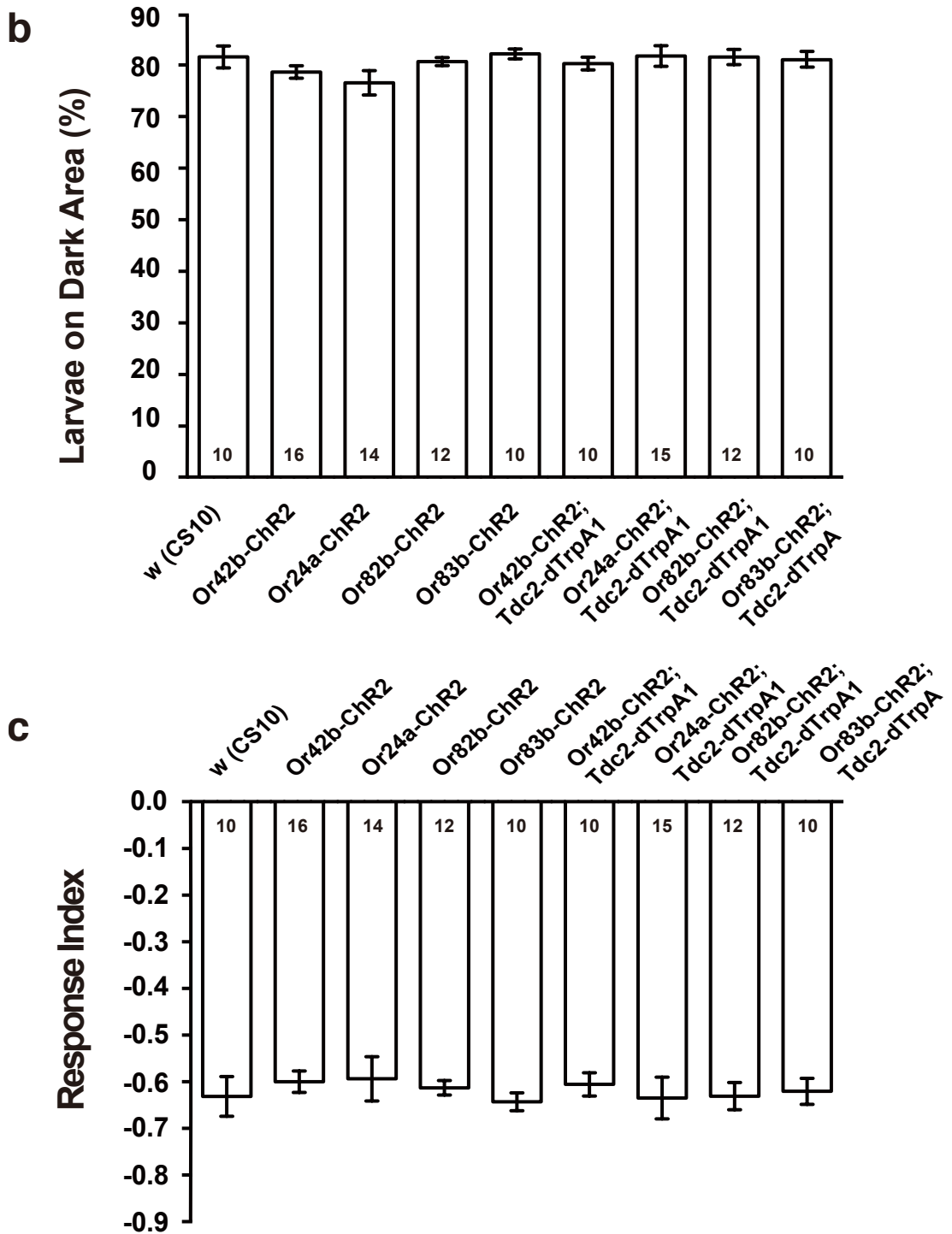
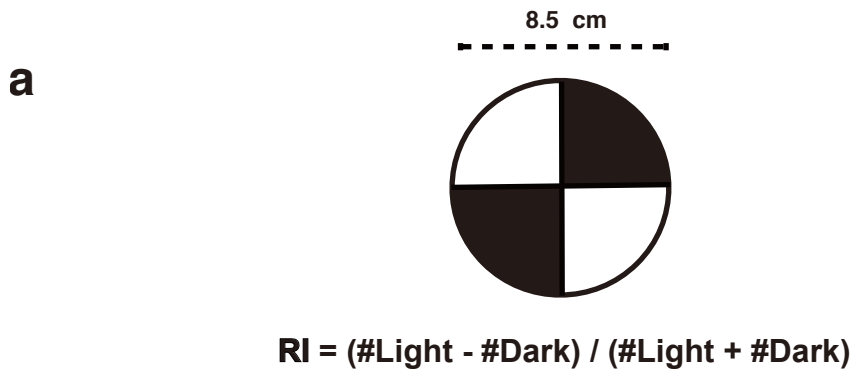
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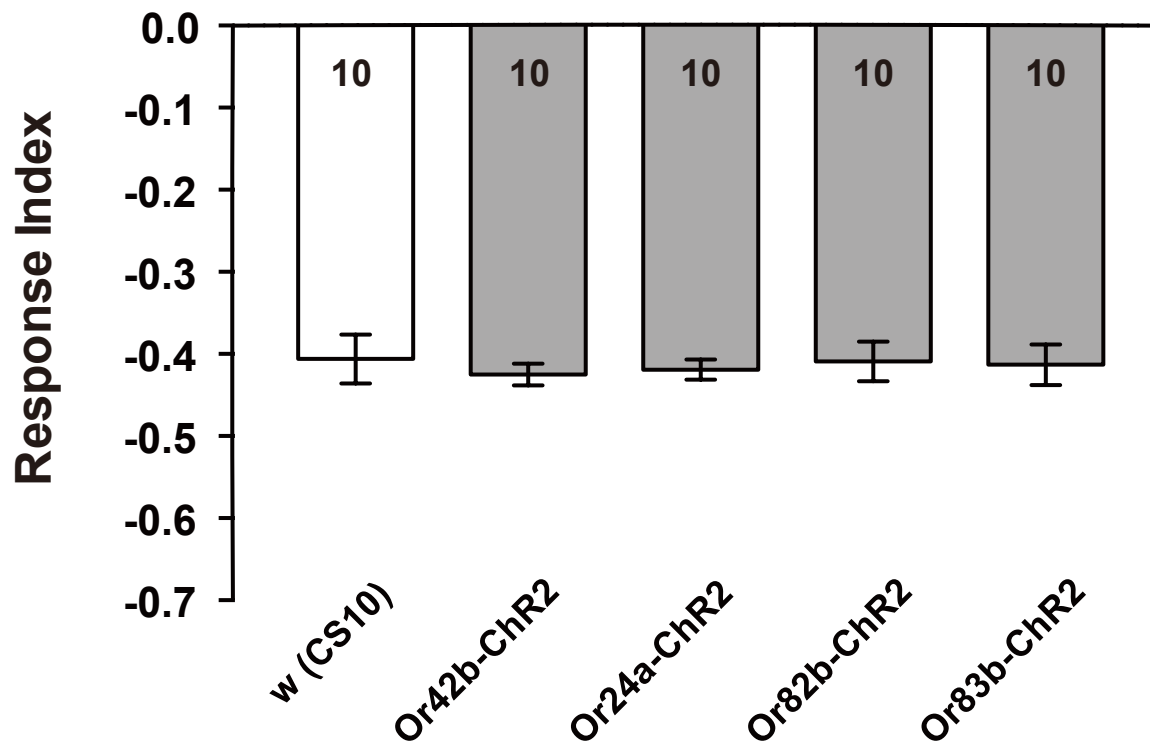
Supplemental Figure 1



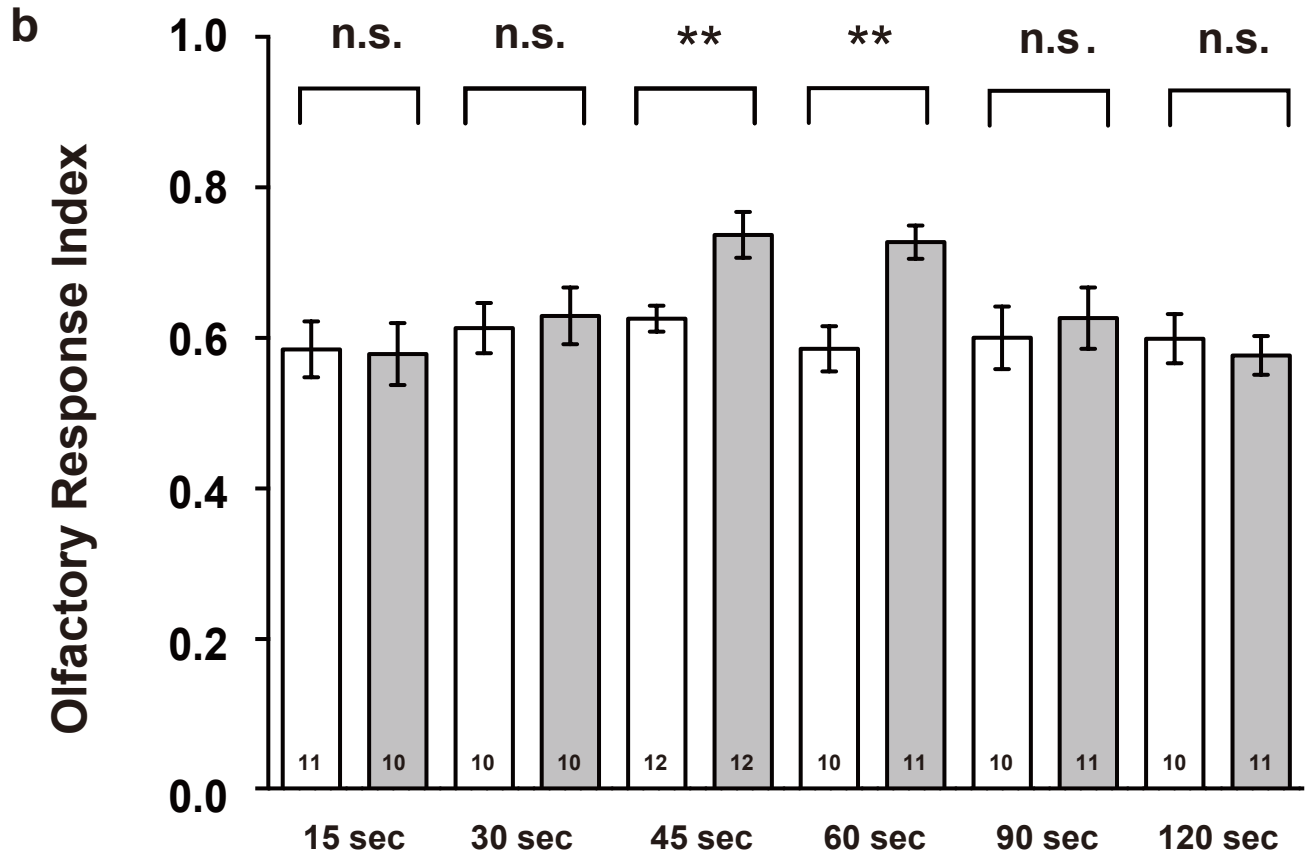
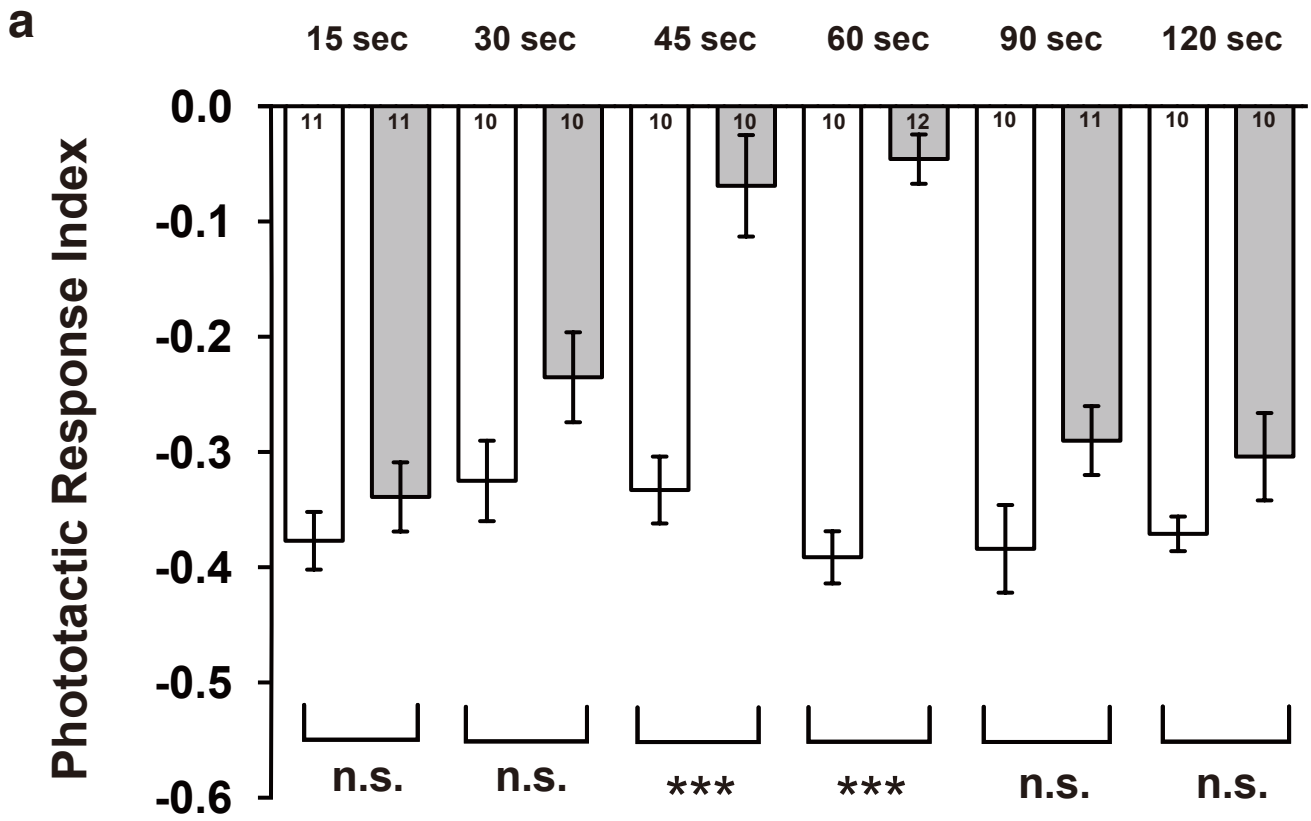
Supplemental Figure 2



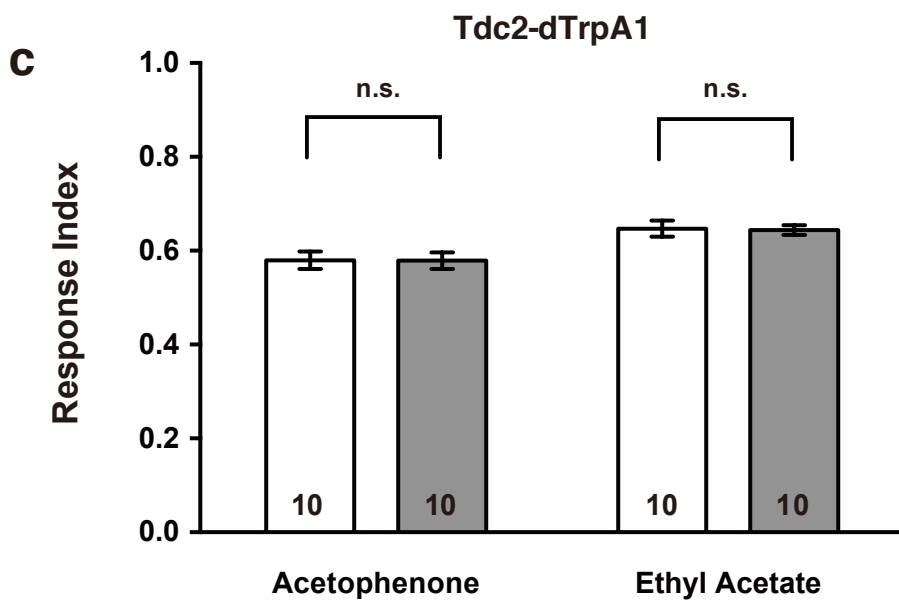
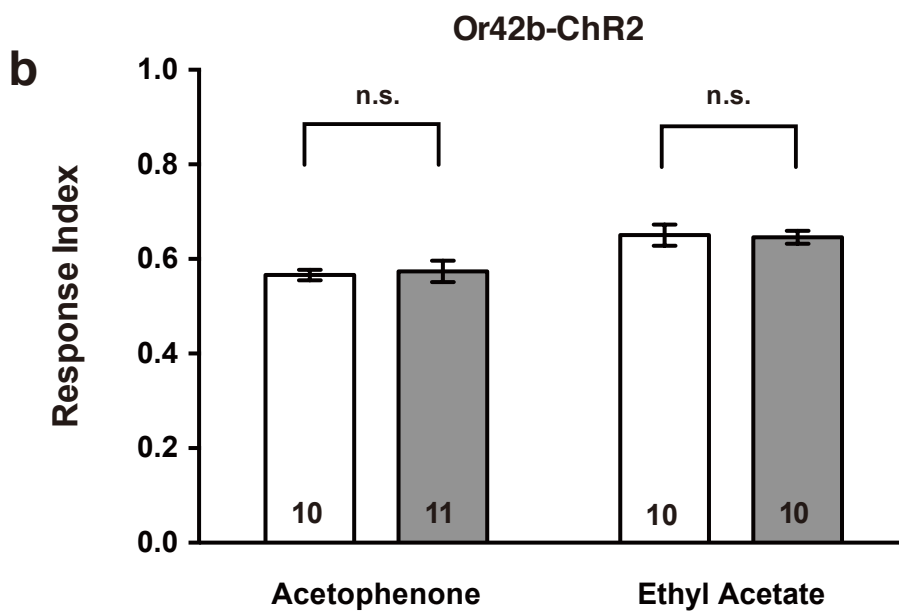
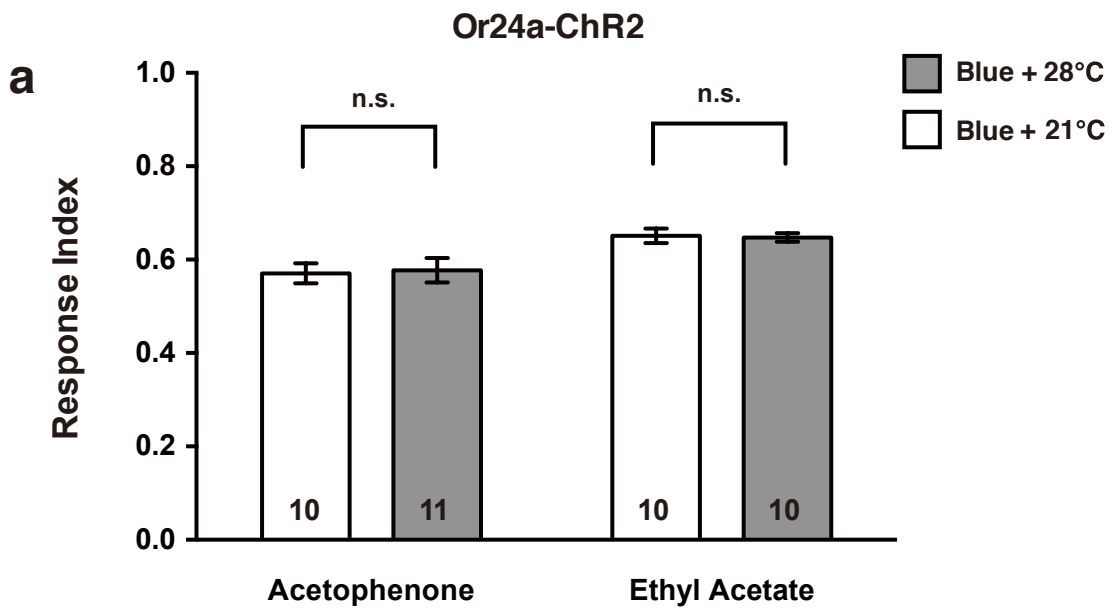
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6