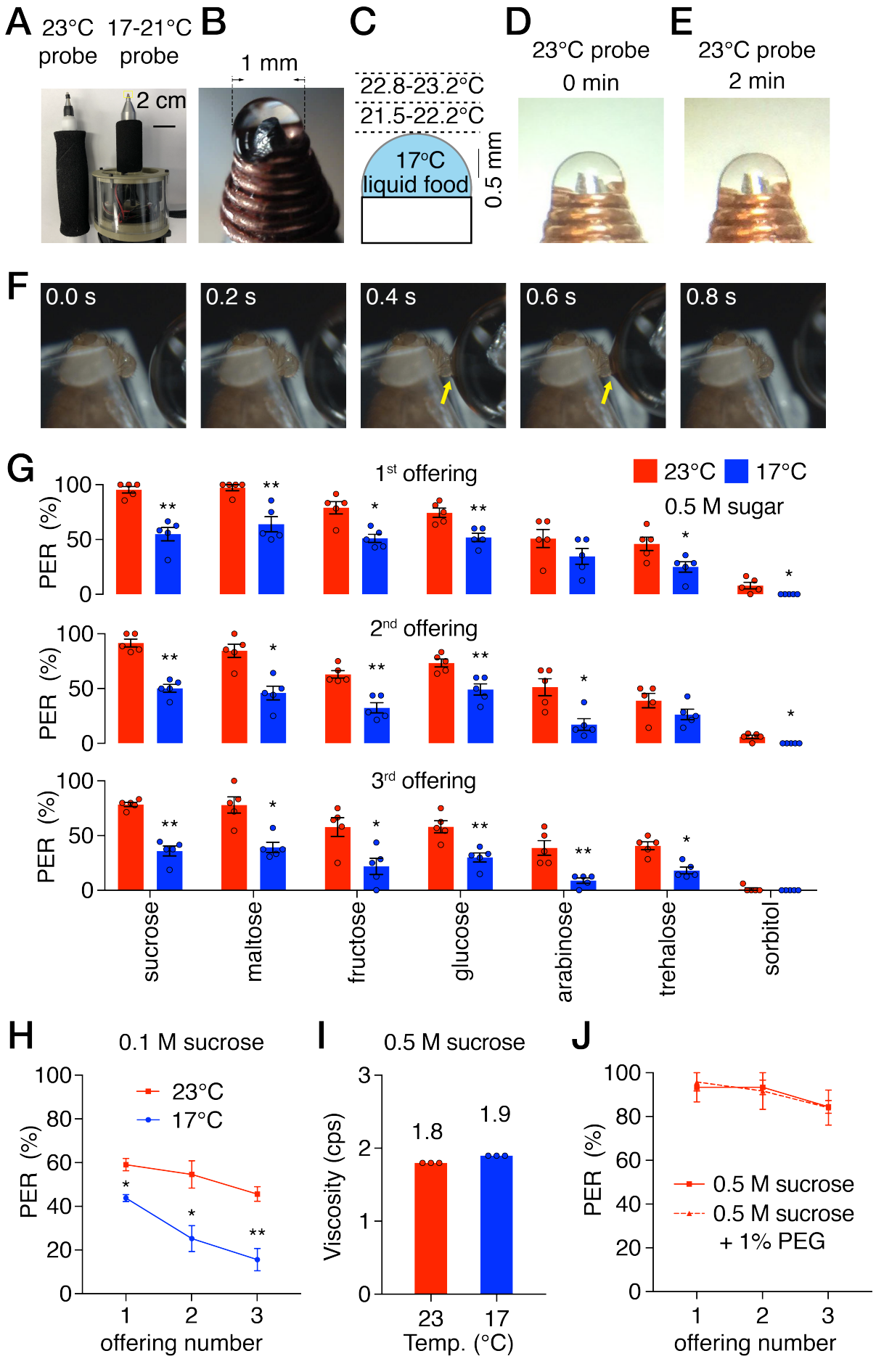
A
23°C probe
17-21°C probe
2 cm
B
1 mm
C
22.8-23.2°C
21.5-22.2°C
17°C liquid food
0.5 mm
D
23°C probe
0 min
E
23°C probe
2 min
F
0.0 s
0.2 s
0.4 s
0.6 s
0.8 s
G
1st offering
2nd offering
3rd offering
23°C
17°C
0.5 M sugar
PER (%)
100
50
0
sucrose
maltose
fructose
glucose
arabinose
trehalose
sorbitol
H
0.1 M sucrose
23°C
17°C
PER (%)
offering number
I
0.5 M sucrose
Viscosity (cps)
1.8
1.9
23
17
Temp. (°C)
J
PER (%)
0.5 M sucrose
0.5 M sucrose
+ 1% PEG
offering number

Figure S1. Temperature-dependent PER responses to different sugars. Related to Figure 1.

(A) 23°, and 17°—21°C temperature probes used to perform PER assays.
(B) Higher magnification view of the tip of the 17°—21°C temperature probe.
(C) Schematic of the temperature of the surrounding air when the liquid food at the end of the tip is set at 17°C.
(D) 23°C drop on a PER probe at 0 min.
(E) Same probe and drop shown in **D** after 2 min.
(F) Consecutive still images extracted from video S2. The frame rate is 5/sec. The times indicated are those at the beginning of each frame. The yellow arrows indicate two consecutive frames in which the probe containing 0.5 sucrose is in contact with the proboscis
(G) 1st, 2nd and 3rd PER assays in response to stimulation with the indicated sugars (0.5 M) at 23° or 17°C.
(H) PER assays in response to 0.1 M sucrose solution presented at 17° or 23°C.
(I) Viscosities (centipoise) of 0.5 M sucrose solutions at 23°C and 17°C.
(J) PER assays in response to 0.5 M sucrose (solid line) or 0.5 M sucrose plus 1% PEG (polyethylene glycol; dashed line). The food was presented at 23°C. 1% PEG causes the same viscosity increase (1.8 to 1.9 cps) as shifting the temperature from 23° to 17°C. Error bars represent mean ±SEMs. n=4—5 trials. Each trial includes ≥8 flies. *P<0.5, **P<0.01, nonparametric Mann-Whitney test.

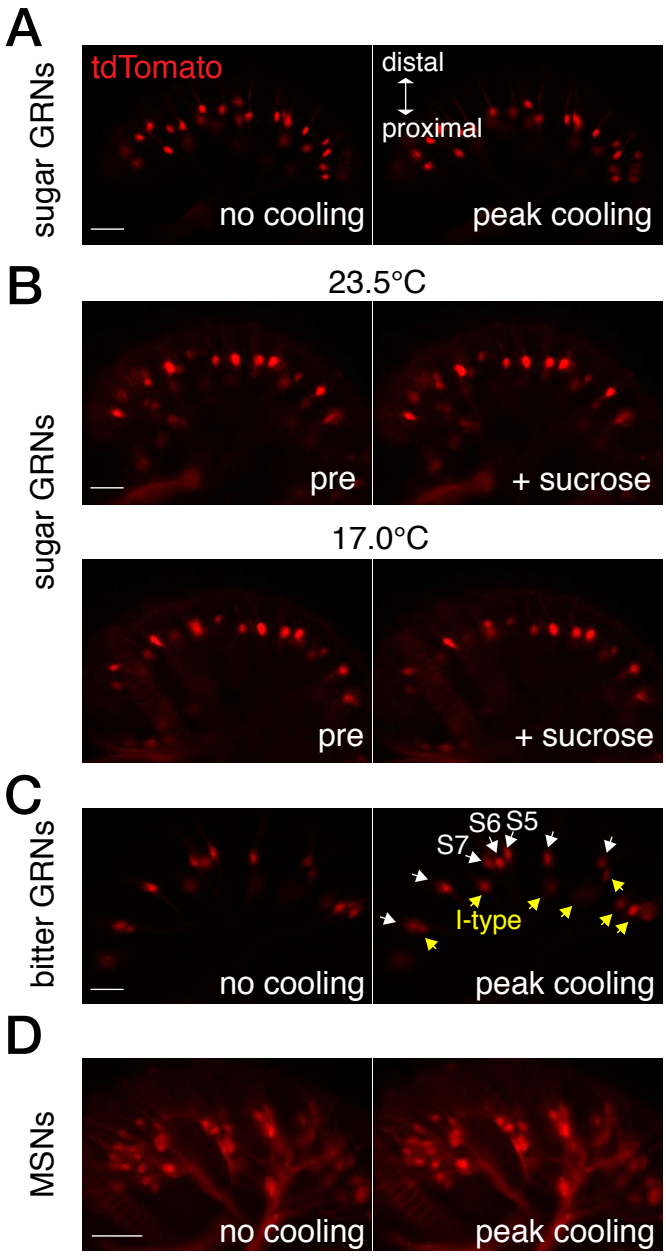

Figure S2. tdTomato fluorescence provides a baseline control for GCaMP6f fluorescence. Related to Figure 2.
tdTomato fluorescence exhibited by neurons in the same labella shown in Figure 2. *UAS-tdTomato*/+ was co-expressed in the same neurons as *UAS-GCaMP6f*/+ using the *Gal4s* indicated below. Scale bars represent 20 μm.
(A) Sugar GRNs; *Gr64f-Gal4*/+ (corresponding to Figure 2A).
(B) Sugar GRNs; *Gr64f-Gal4*/+ (corresponding to Figure 2C).
(C) Bitter GRNs; *Gr66a-Gal4*/+ (corresponding to Figure 2E).
(D) MSNs; *R41E11-Gal4*/+ (corresponding to Figure 2G).

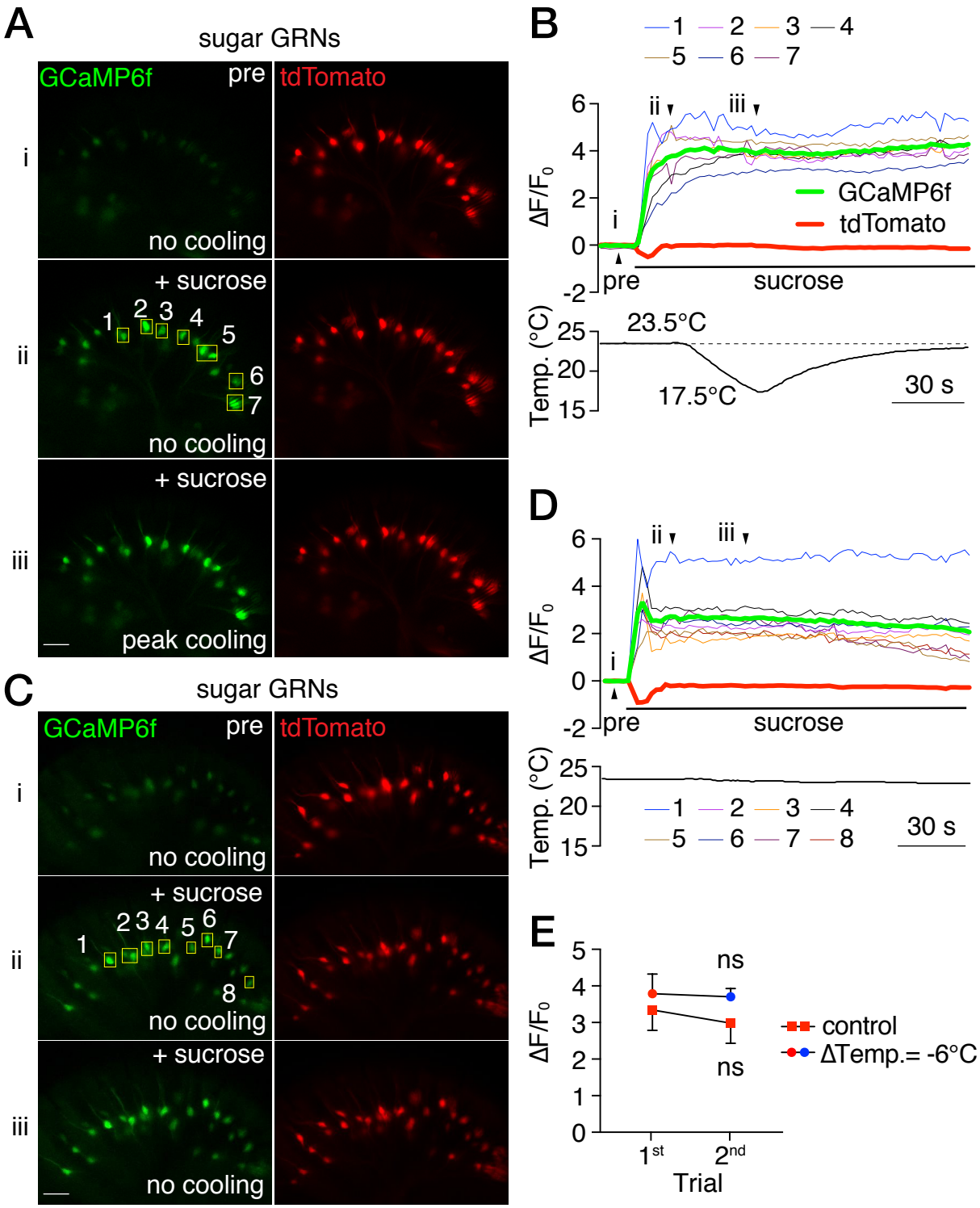

Figure S3. Cooling does not alter GCaMP6f responses of sugar GRNs. Related to Figure 2.

UAS-GCaMP6f and *UAS-tdTomato* were co-expressed in sugar GRNs under the control of the *Gr64f-Gal4*, and GCaMP6f and tdTomato fluorescence was monitored before (pre) and during stimulation with sucrose. Scale bars represent 20 μm.

(A) GCaMP6f imaging in labella initially held at 23.5°C before (i) and during the initiation of 0.5 M sucrose stimulation (ii). The temperature was gradually reduced to 17.5°C (iii) as indicated in **B**. Video S5.

(B) Traces showing the GCaMP6f ($\Delta F/F_0$) and tdTomato fluorescence exhibited by sugar GRNs held at 23.5°C before and during initiation of the stimulation with 0.5 M sucrose. The temperature was then gradually decreased to 17.5°C. The lower trace displays the temperature changes during the recordings. The colored thin traces represent each single sugar GRN (ROI) indicated in **(A)**. The bold red and green traces indicate the average tdTomato and GCaMP6f fluorescence.
(C) GCaMP6f imaging in labella maintained at 23.5°C before (i) and during [+5 s (ii) and +35 s (iii)] stimulation with 0.5 M sucrose as indicated in **D**.
(D) Traces showing the GCaMP6f ($\Delta F/F_0$) and tdTomato fluorescence exhibited by sugar GRNs held at 23.5°C before and during stimulation with 0.5 M sucrose. The temperature trace is shown below. The colored thin traces represent each single sugar GRN (ROI) indicated in **(C)**. The bold red and green traces indicate the average tdTomato and GCaMP6f fluorescence. The slight changes in fluorescence during the initial 3 sec after addition of sucrose was due to an adjustment in focus. Cells that remained in focus before and after sucrose application were analyzed.
(E) Statistics showing that there are no significant changes in GCaMP6f fluorescence ($\Delta F/F_0$) in sugar neurons due to exposure to 0.5 M sucrose at 23.5°C (ii, +5 sec) and 17°C (iii, +35 sec). n= 5 animals. Nonparametric Mann-Whitney test for Figure S3E. ns, not significant. Error bars represent means ±SEMs.

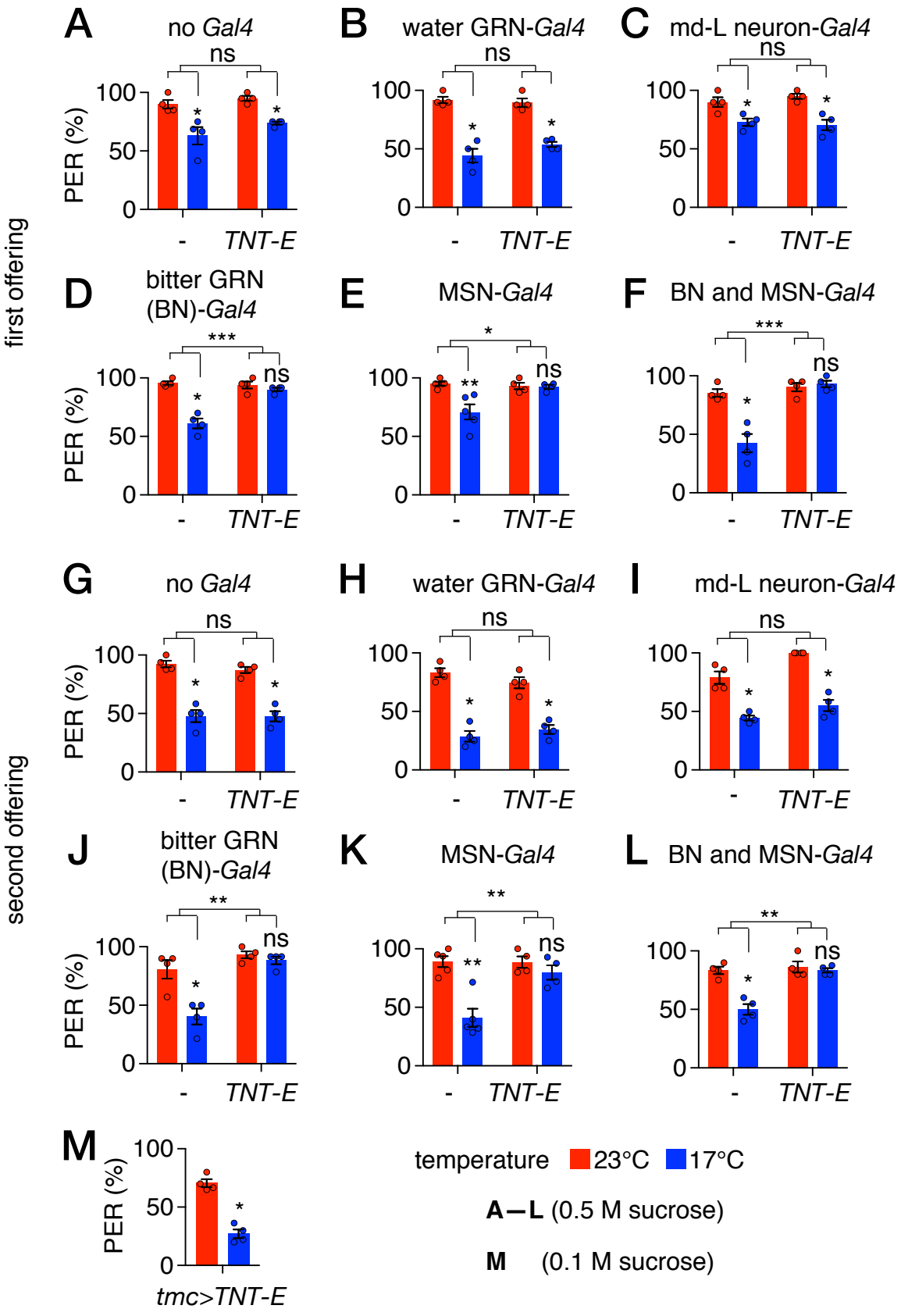

Figure S4. 1st and 2nd PER offerings showing distaste for cooler food depends on bitter GRNs and MSNs. Related to Figure 3.

PER assays in response to 0.5 M sucrose (**A—L**) or 0.1 M sucrose (**M**) offered at either 23°C (red bars) or 17°C (blue bars) after inhibiting different classes of neurons by expressing *UAS-TNT-E* under control of the indicated Gal4 lines. Shown are the results of the 1st and 2nd offerings (see Figure 3 for the results

of the 3rd offering). Two-way ANOVA was used to analyze statistically significant differences between flies of the same genotype when offered sucrose at 23° versus 17°C. We used two-way ANOVA to determine whether the temperature-dependent differences between groups (e.g. *UAS-TNT-E* alone versus *UAS-TNT-E* plus the *Gal4*) were significant. n=4—5 trials. Each trial includes ≥8 flies. *P≤0.05, **P≤0.01, ***P≤0.001. Error bars represent means ±SEMs.
(A) 1st offering, no *Gal4*.
(B) 1st offering, water GRNs (*ppk28-Gal4*).
(C) 1st offering, md-L neurons (*tmc-Gal4*).
(D) 1st offering, bitter GRNs (*Gr66a-Gal4*).
(E) 1st offering, MSNs (*R41E11-Gal4*).
(F) 1st offering, bitter GRNs and MSNs (*Gr66a-Gal4* and *R41E11-Gal4*).
(G) 2nd offering, no *Gal4*.
(H) 2st offering, water GRNs (*ppk28-Gal4*).
(I) 2st offering, md-L neurons (*tmc-Gal4*).
(J) 2st offering, bitter GRNs (*Gr66a-Gal4*).
(K) 2st offering, MSNs (*R41E11-Gal4*).
(L) 2st offering, bitter GRNs and MSNs (*Gr66a-Gal4* and *R41E11-Gal4*).
(M) 3rd PERs using flies expressing *UAS-TNT-E* under control of the *tmc-Gal4*. The flies were presented 0.1 M sucrose at 23° or 17°C. Error bars represent mean ±SEMs. n=4 trials. Each trial includes ≥8 flies. *P≤0.05. Nonparametric Mann-Whitney test.

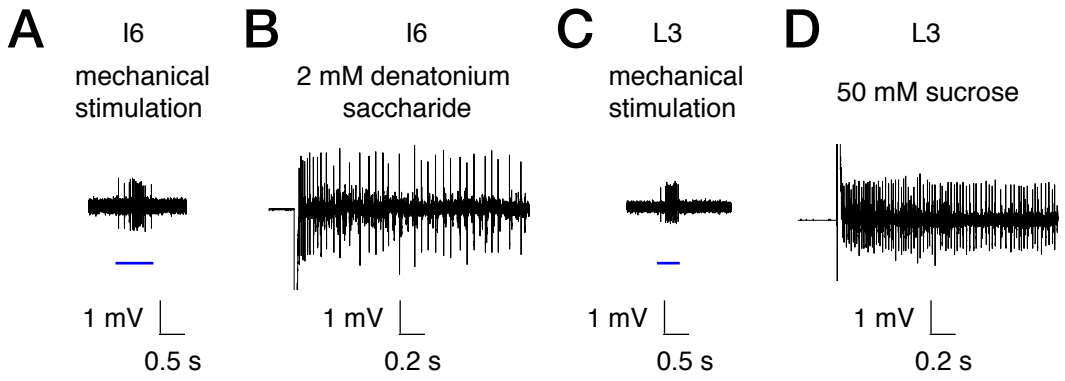

Figure S5. Action potentials of I6 and L3 sensilla in response to mechanical and chemical stimulation. Related to Figure 4.

Tip recordings to assess action potentials in of the indicated sensilla from control flies in response to the indicated stimuli. Time and mV scales are shown.

(A) I6 sensillum exposed to a 20 μm deflection (blue line).

(B) I6 sensillum stimulated with 2 mM denatonium saccharide.

(C) L3 sensillum exposed to a 20 μm deflection (blue line).

(D) L3 sensillum stimulated with 50 mM sucrose.

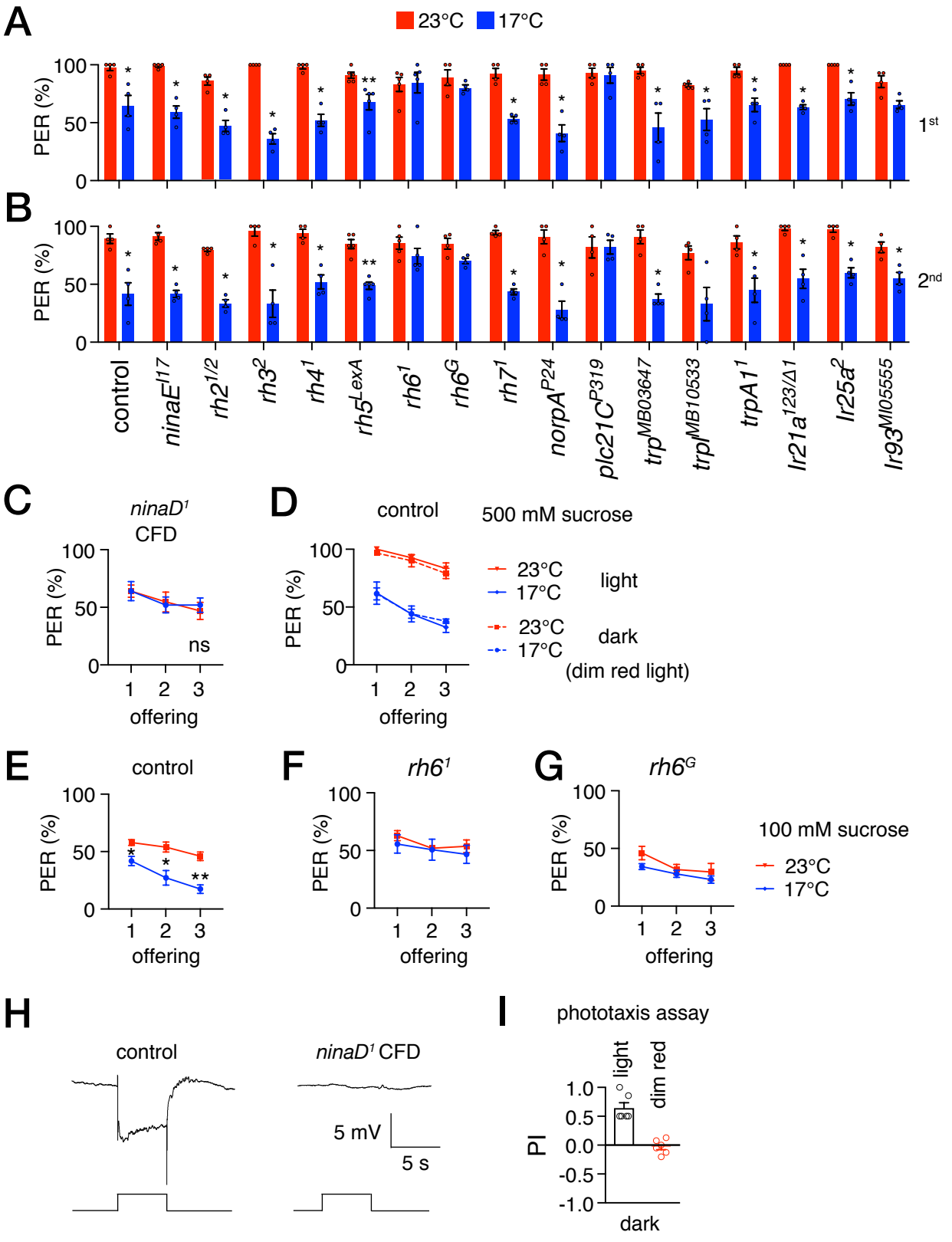

Figure S6. Testing for roles for opsins and other signaling proteins in suppression of sugar appeal by cool temperatures. Related to Figure 5.
(A—E) PERs using 0.5 M sucrose presented at 23° or 17°C.
(A, B) Screening mutants for defects in inhibition of sugar attraction by cool temperatures.
(A) 1st offerings.
(B) 2nd offerings.

(C—E) PERs using 100 mM sucrose presented at 23° or 17°C.
(C) Control.
(D) *rh6*^{1}.
(E) *rh6*^{G}.
(F) ERG responses of control and *ninaD*^{1} flies raised for five generations on a carotenoid-free diet (CFD).
(G) PER assays using *ninaD*^{1} flies raised for five generations on a CFD.
(H) Y tube phototaxis assays using control animals. n=6 groups. 100 ±10 flies per group. The preference index (PI)=

$$\frac{(\#\text{ flies in upper transparent tube}) - (\#\text{ flies in upper dark tube})}{(\#\text{ flies in upper transparent tube}) + (\#\text{ flies in upper dark tube})}.$$

(I) PER assays using 0.5 M sucrose offered at 23° and 17°C under normal light or under a dim, red photographic safety light.
Error bars represent means ±SEMs. n=4—5 trials (**A—E, G** and **I**). Each trial includes ≥8 flies.*P≤0.05. Nonparametric Mann-Whitney tests.

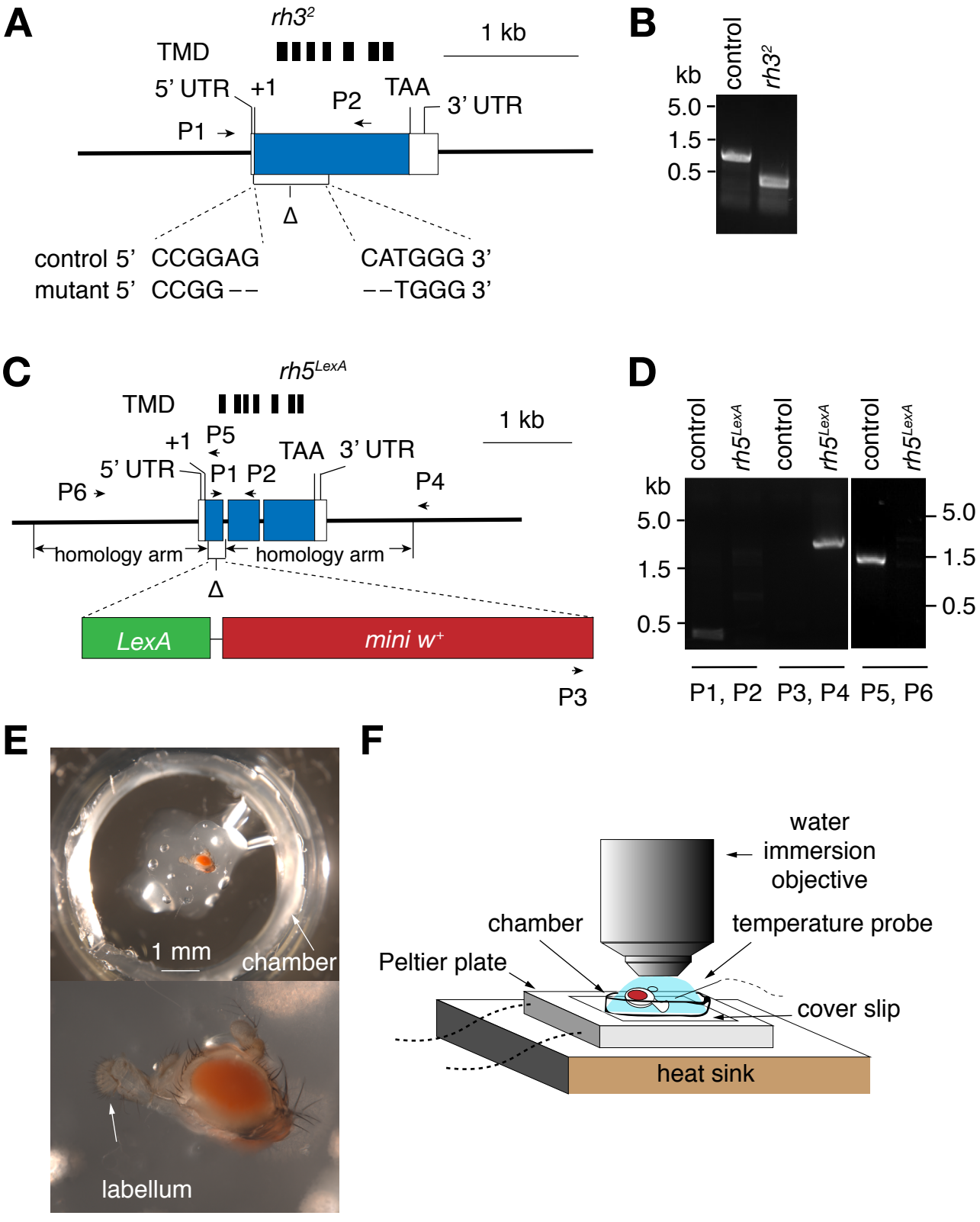

Figure S7. Generation of *rh3²* and *rh5LexA* mutants. Related to Figure 5. and setup for performing GCaMP6 imaging on neurons in the labellum. Related to Figure 2.

(A) Schematic illustrating the strategy for generating the *rh3²* mutant allele. The region encompassing the ATG start site and the transmembrane segments 1 to 4 (nucleotides -3—+555) were deleted using CRISPR/Cas9 induced nonhomologous end joining. The white boxes indicate the 5' and 3' untranslated regions (UTR) and the blue

box represents the protein coding region. +1 indicates the first nucleotide in the coding region.
(B) Identification of the *rh3*^{2} mutant by PCR using the P1 and P2 primers indicated in **A**. The PCR products for the control and *rh3*^{2} were 1021 and 463 base pairs, respectively.
(C) Schematic illustrating the strategy for generating the *rh5*^{LexA} mutant allele. The region encoding the N-terminus and transmembrane segments 1 were deleted (+51—+236 spanning exon 1) and replaced with *LexA* and the *mini white* (*w*$^{+}$) gene by CRISPR/Cas9-induced homologous dependent repair.
(D) Identification of the rh5LexA mutant by PCR using the primers (P1—P6) indicated in C. The PCR products are: 1) primers P1 and P2: 365 base pairs (control) and no band (*rh5*^{LexA}), 2) primers P3 and P4: no band (control) and 2602 base pairs (*rh5*^{LexA}), and 3) primers P5 and P6: 1390 base pairs (control) and no band (*rh5*^{LexA}).
(E) Fly head immobilized in grease in a circular chamber (~6 mm in diameter) containing 1xPBS. Note that the upper side of the labellum is not submerged in the grease.
(F) The setup for cooling the labellum includes a Peltier plate and a heat sink. The temperature changes were monitored by inserting a temperature probe in the chamber. The GCaMP6f responses were monitored using a 20x water immersion objective.