SUPPLEMENTARY FIGURE LEGENDS

S1, related to Figure 1. **Somatotopic mapping of the trigeminal ganglia**
The trigeminal ganglia exhibits a 'coarse' but reproducible somatotopic organization. Gentle mechanical stimulation of the oral cavity by air puffs strongly activated neurons concentrated in a field located at the entrance of the mandibular nerve into the ganglia (green cells in overlay). Gentle mechanical stimulation of the facial hairy skin activated neurons in largely non-overlapping fields rostral and caudal to the oral field (red cells). Below, approximate extent of oral and facial somatotopic areas superimposed on a micrograph of the ganglia shown above. This somatotopic organization was consistent across three $Wnt1^{Cre}$; $Rosa^{GCaMP5}$ animals mapped. Scale bar is 100 micron, color scales indicate maximal percentage ΔF/F in response to stimulus.

S2, related to Figure 1. **Reliability and selectivity of thermosensory calcium responses.**
Repeated application of thermal stimuli produces highly reproducible spatial and temporal patterns of activity in trigeminal neurons. **A**) Overlay of population response to repeated stimulation in the noxious cold range demonstrates activation of nearly identical ensembles. **B**) Traces from 6 cold sensing neurons to 4 trials of a fixed cooling stimulus, illustrating reproducibility of calcium response kinetics. **C**) Responses are stimulus-dependent. Heat maps of maximal ΔF/F in the absence of thermal stimulus show little activity over a 40 second recording period (left panel), as compared to stimulation with a thermal stimulus (right panel). Data are from a single $TrpV1^{Cre}$ imaging field, level of basal activity is consistent across $TrpV1^{Cre}$ and $Wnt1^{Cre}$ lines. Similar reliability is observed with heat-sensing neurons (see text). **D, E**) Analysis of more than 800 responding neurons demonstrated that over 90% are selectively tuned for either heating or for cooling; red indicate ΔF/F responses to a 51° C, and blue are the ΔF/F responses to a 4° C stimulus. Note the high specificity of most neurons; a small subset of cold neurons show a 'paradoxical heat' response at extreme high temperatures as previously reported[46]. **F**) The relative proportions of cold-, hot- or bimodal-responding neurons seen in $TrpV1^{Cre}$ or $Wnt1^{Cre}$ animals are similar across genotypes.

S3, related to Figure 2. Representation of warmth and heat in trigeminal ganglia
A) Population responses to graded heating series in trigeminal imaging fields from three $TrpV1^{Cre}$;GCaMP mice, illustrating that population responses are not topographically organized, but that the progressive recruitment of warmth (yellow) and heat (red) neurons is consistent across animals. Intensity of labeling indicates normalized calcium response amplitude as percentage of maximal, scale bar is 100 micron. **B**) Response of an imaging field to repeated stimulation at 48° C, demonstrating repeatability of heat response. Image displays maximal ΔF/F as indicated by color scale. **C**) Calcium traces from two

warm neurons (top, magenta traces) and three heat sensing neurons (bottom, red traces) to a heating series from 37 to 52° C and to oral stimulation with 5mM capsaicin. **D)** Responses of a warmth sensing neuron to repeated stimulation with mild warming repeatedly tested over 100 minutes, illustrating consistency and reliability of warmth responses. **E**) Quantification of capsaicin responses for noxious heat neurons (N = 146, left) and warmth neurons (N = 17, right), red indicates number responding to stimulation with capsaicin and green unresponsive neurons. Data were recorded from N = 4 TrpV1Cre; RosaGCaMP5 mice. All low threshold warmth sensors express TRPV1 as demonstrated by capsaicin sensitivity, while over one quarter of noxious heat neurons are capsaicin insensitve.

S4, related to Figure 3. Effect of TRPV1 inhibition on noxious heat responses
A) Map of responses to noxious heat (~50° C) in an imaging field before and after pharmacological inhibition of TRPV1 by administration of JNJ-17203212. The two left panels show maximal ΔF/F, on the right neurons no longer responding to heating after TRPV1 inhibition are displayed in red, while the great majority of neurons still responding to heat are marked in red. **B**) Quantification of maximal response amplitude to 48° C before and after inhibition of TrpV1 for 53 noxious heat neurons. **C**) Sample traces taken from the set of 53 noxious heat-sensing neurons tested, showing that the majority of noxious heat sensors (39 neurons) continue to respond to heat after inhibition of TRPV1. We also observe a smaller subset (10 neurons), that no longer responds to 48° but still shows strong responses to 52° C, and a few neurons (~8%) that fail to respond to even extreme heat (52° C) upon TRPV1inhibition. Note that over 90% of the noxious-sensing neurons retain responses to a 52° C stimulus. Clearly additional thermosensors must mediate these responses. **D,E**) Inhibition of TRPV1 has no significant effect on innate avoidance of noxious heat. Panel **D** tracks the position of mice allowed to choose between room temperature and 48° C chambers and before and after inhibition of TRPV1, showing strong aversion to noxious heat even after treatment. As quantified in panel **E**, there is no detectable decrease in behavioral heat avoidance. Mean and SEM of preference index are plotted in **E**, p-values by Student's T-test. **F**) Inhibition of TRPV1 does not affect cold responses. The left panels show a maximal projection of fluorescence response (ΔF/F) for a trigeminal imaging field to a 25°C cooling stimulus before (upper) and after (lower) TRPV1 inhibition. On the right are examples of calcium traces from cold neurons pictured before and after pharmacological inhibition of TRPV1.

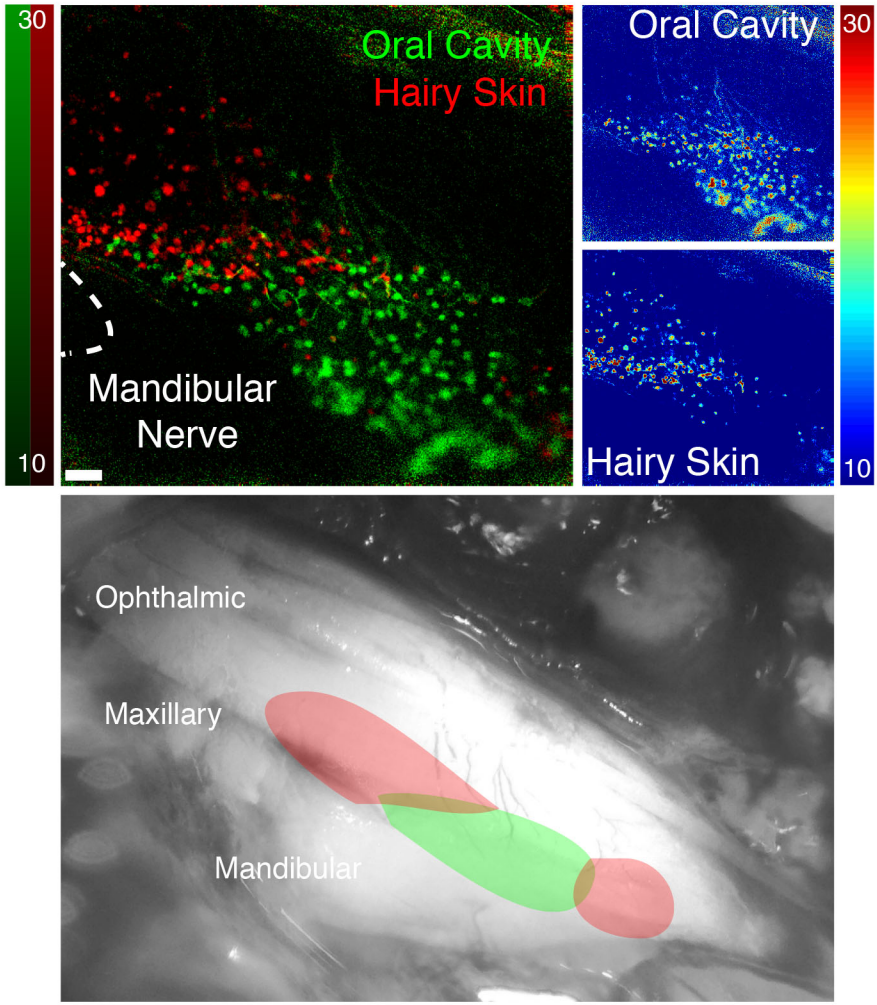
S5, related to Figure 4. Dynamic representation of cool and cold stimuli
Full response series for imaging field shown in **Fig. 4A**, showing temporal evolution of the population response to cold for 6 different stimulus temperatures. Colors indicate cold neuron type (blue represents Type I, green Type II, and magenta Type III), intensity of labeling indicates normalized calcium response amplitude as percentage of maximal,

scale bar is 100 micron.

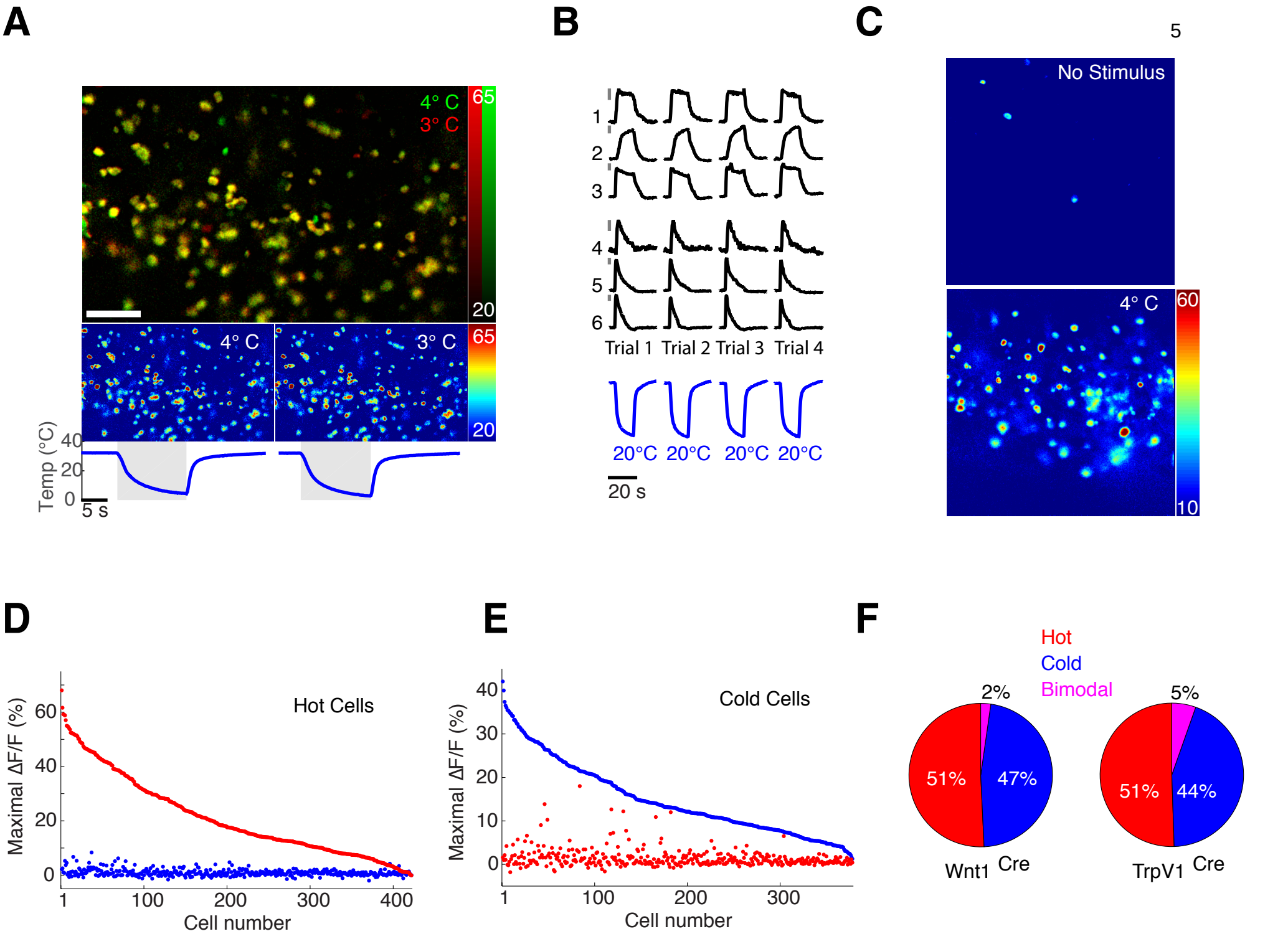
S6, related to Figure 4. Functional characterization of TrpA1 expressing neurons
A) Schematic of construct for the $TrpA1^{DIC}$ mouse line (Trankner and Zuker, unpublished); this line is also referred to as $TrpA1^{Cre}$ in the text. Diphtheria toxin receptor (DTR) and Cre are expressed from the endogenous TrpA1 locus as a single transcript separated by an internal ribosomal entry site (IRES). **B)** Neurons labeled by crossing $TrpA1^{Cre}$ to GCaMP reporter respond *in vivo* to heating (see Figure 5) and to oral application of the TrpA1 ligand allyl isothiocyanate (AITC). Intra-oral temperature traces and pharmacological stimuli are plotted above, bars to left of traces indicates 5% ΔF/F. **C**) Distribution of thermosensory response types for $TrpA1^{Cre}$ and $Trpm8^{Cre}$ labeled trigeminal neurons, showing TrpA1 neurons are highly selective for heating stimuli, while the great majority of Trpm8 neurons are cold sensing. **D)** Ablation of either TrpA1 or TrpV1 expressing neurons has no significant effect on avoidance of noxious cold (5° C) in a two-plate preference assay p=0.08 for TrpA1, p=0.24 for TrpV1, Student's T-test. Mice lacking $TrpA1^{DIC}$ neurons do not display significant avoidance to moderately noxious temperatures (45° C, p=0.76 , Student's T-test). As expected, an equivalent phenotype in seen in mice in which all TrpV1 expressing neurons are killed ($TrpV1^{DTR}$/DTX). Mean and SEM of preference ratios are plotted.

S7, related to Figure 8. Silent thermosensors are CGRP expressing $Trpm8^{Cre}$ neurons
Immunohistochemical staining for CGRP (following functional imaging of $Trpm8^{Cre}$; $Rosa^{GCaMP5}$ neurons) revealed that silent thermosensors are robustly labeled for CGRP (N = 5/5 neurons). The images show a field with a silent thermosensor marked with anti-CGRP antibodies (cell 1, top trace), while the neighboring TrpM8-expressing neurons are CGRP-negative (e.g. cell 2). Temperature ramps of cooling stimulation are shown above traces, gray bars indicate 5% ΔF/F. Scale bar is 20 micron.

Supplementary Table 1, related to Experimental Methods. Genotype, sex and age of experimental animals.
Table lists genotype, sex and for all transgenic animals used in imaging experiments.



Supplemental Figure 1



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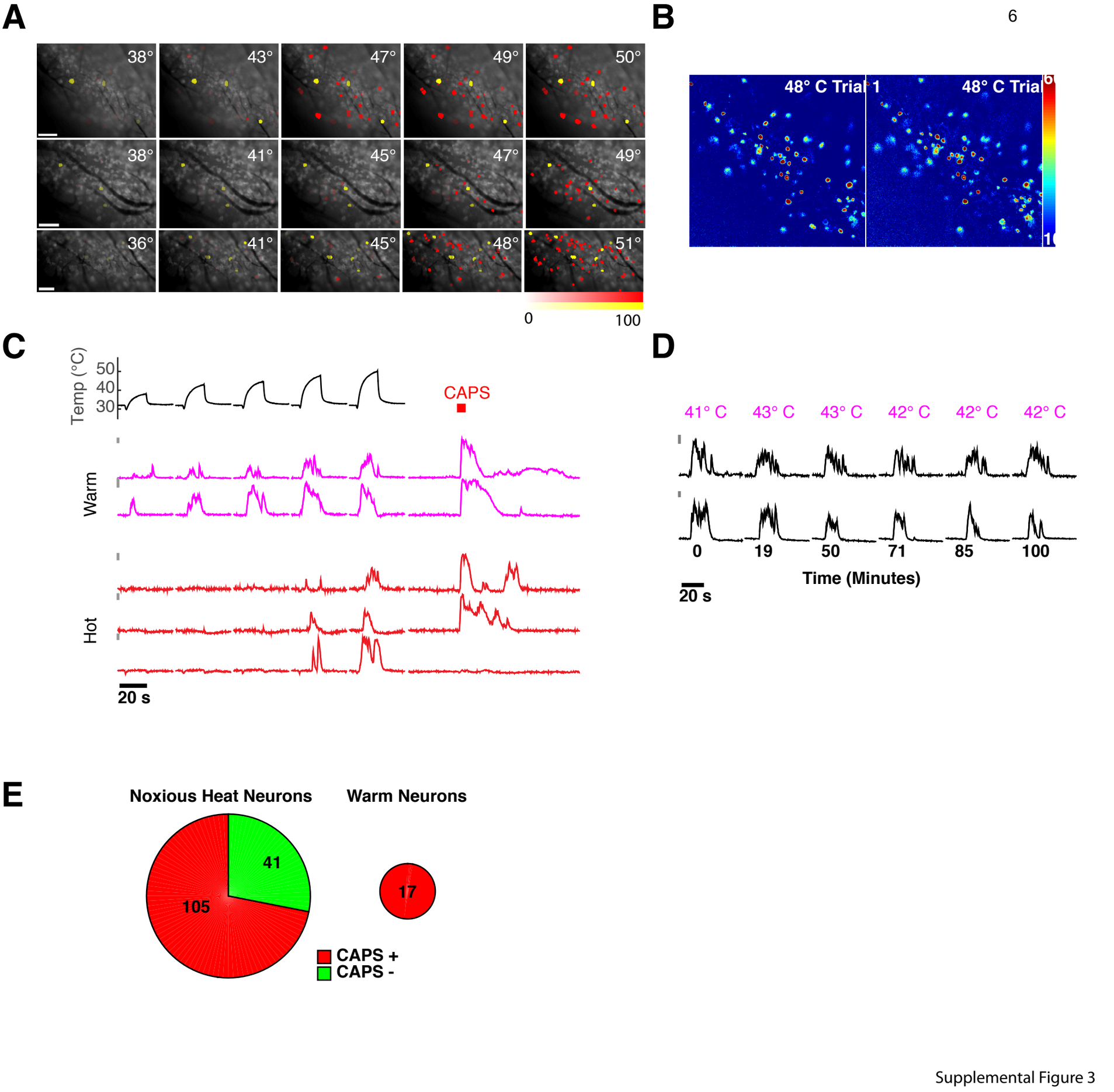

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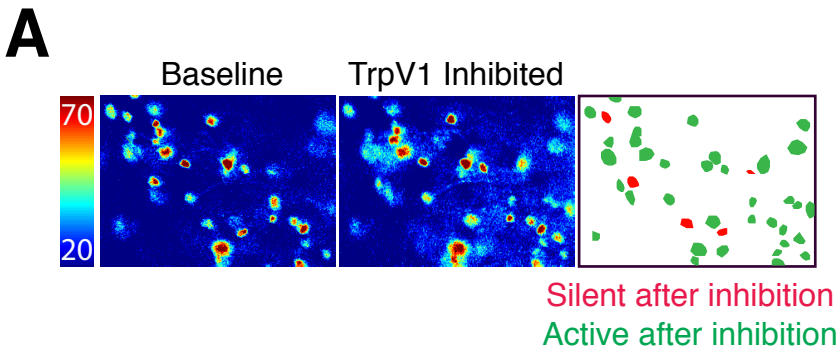
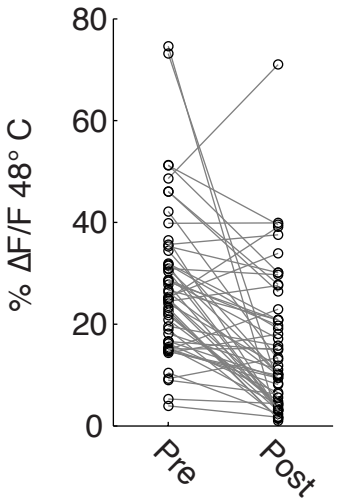
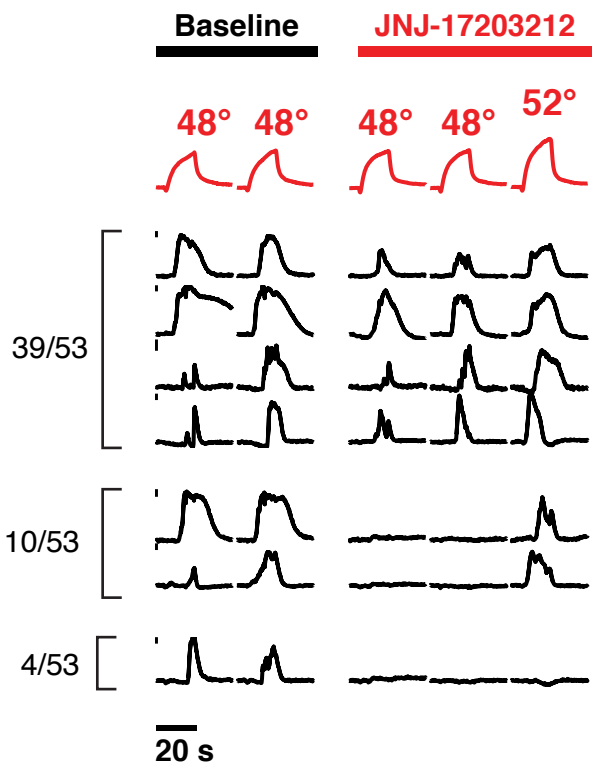
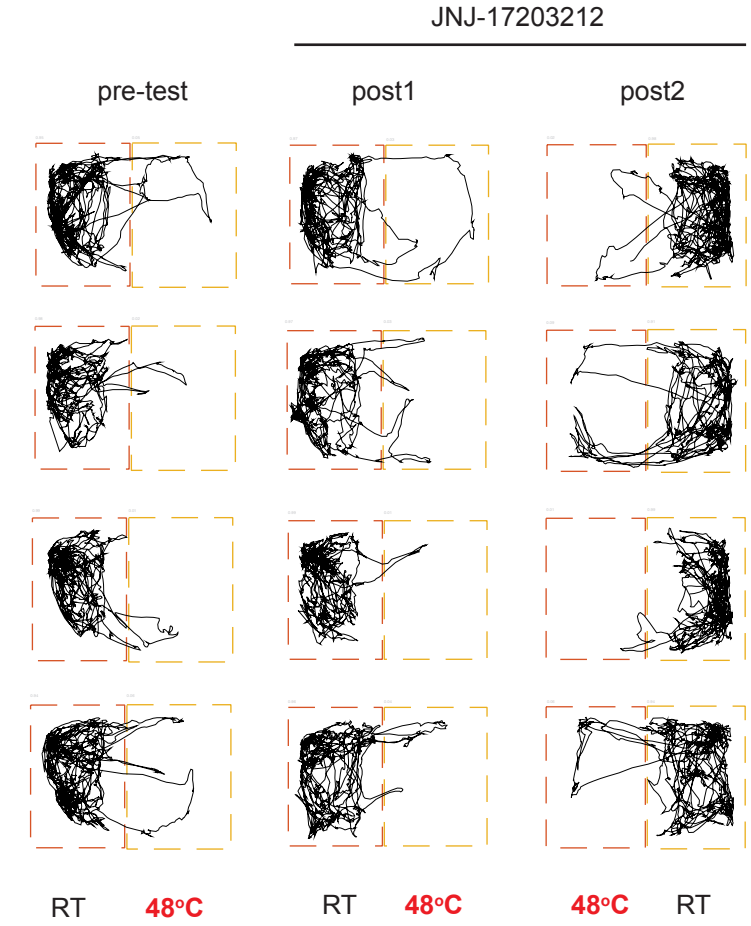
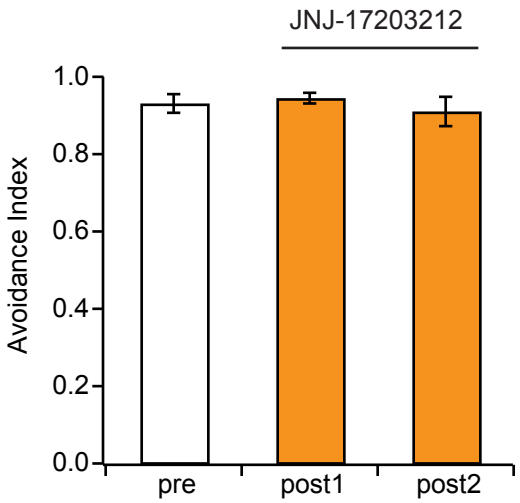
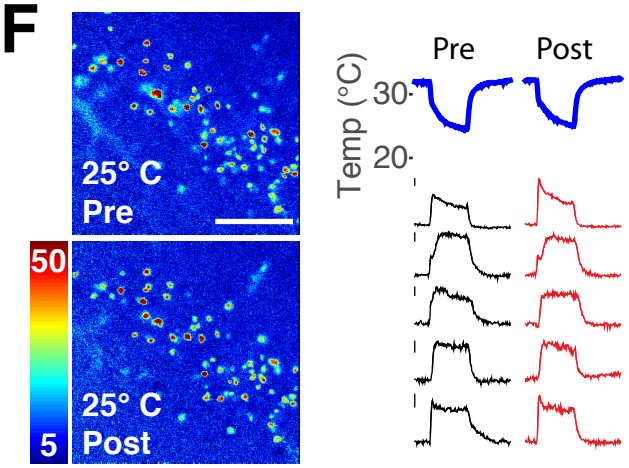




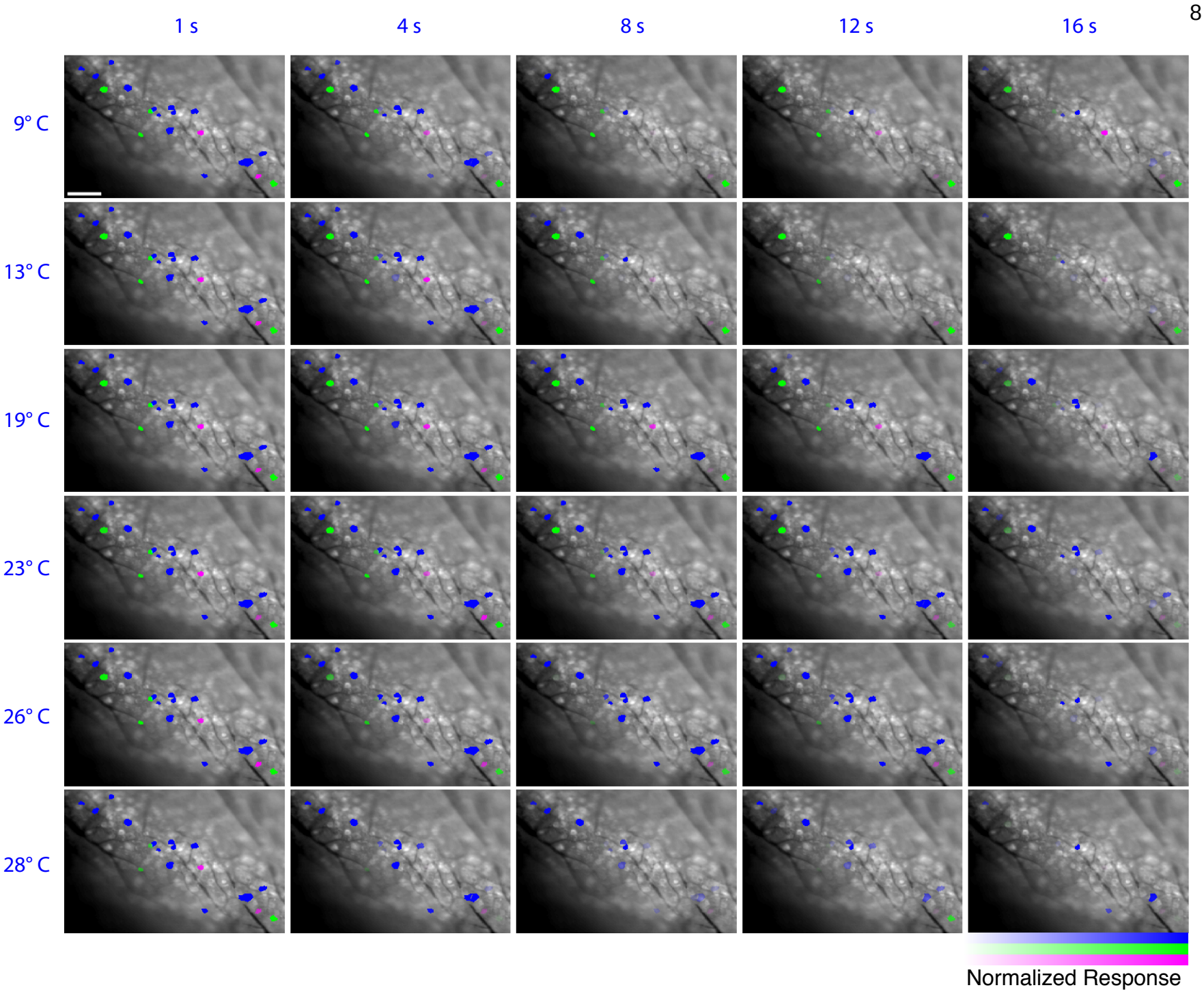








Supplemental Figure 4



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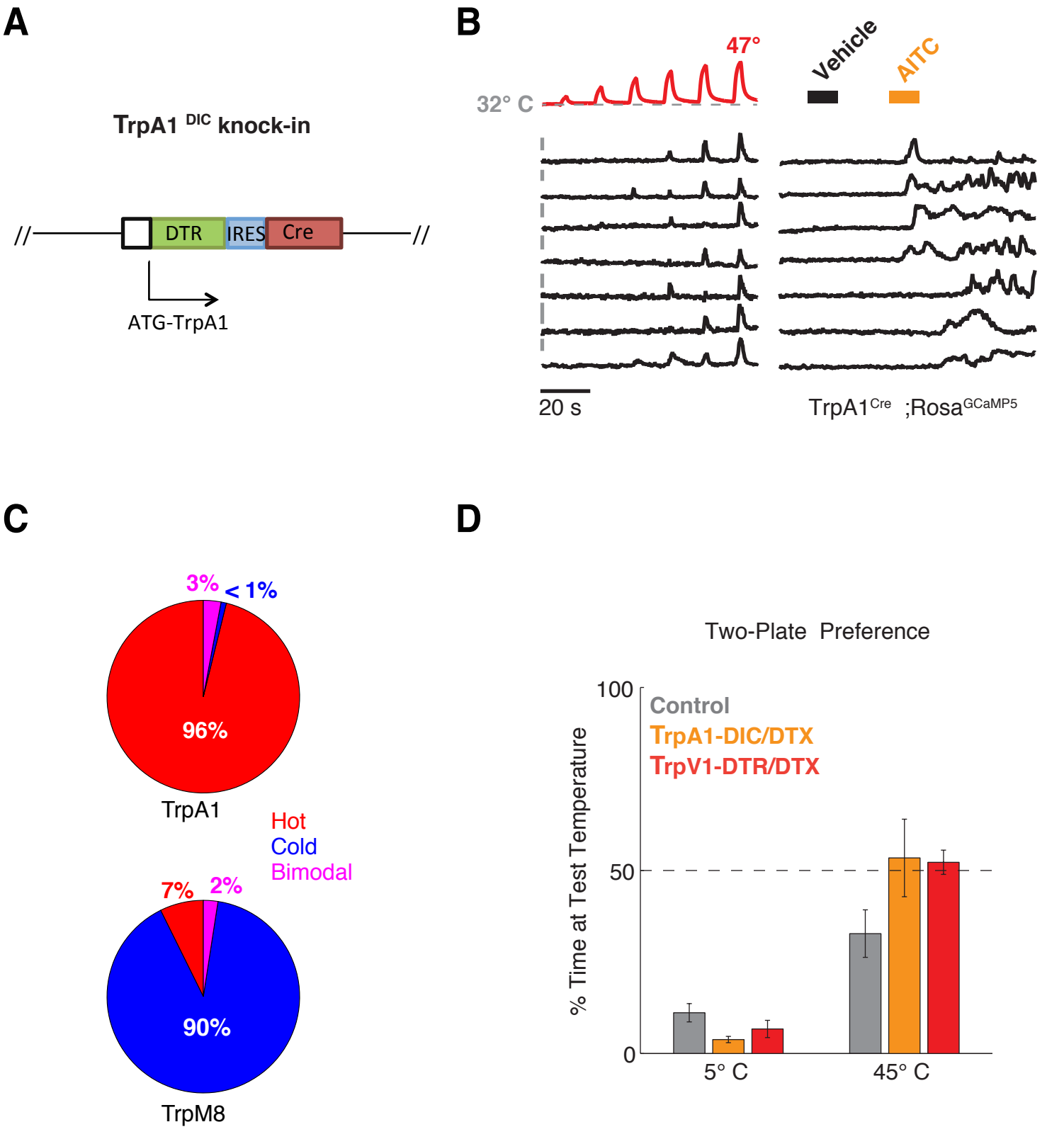
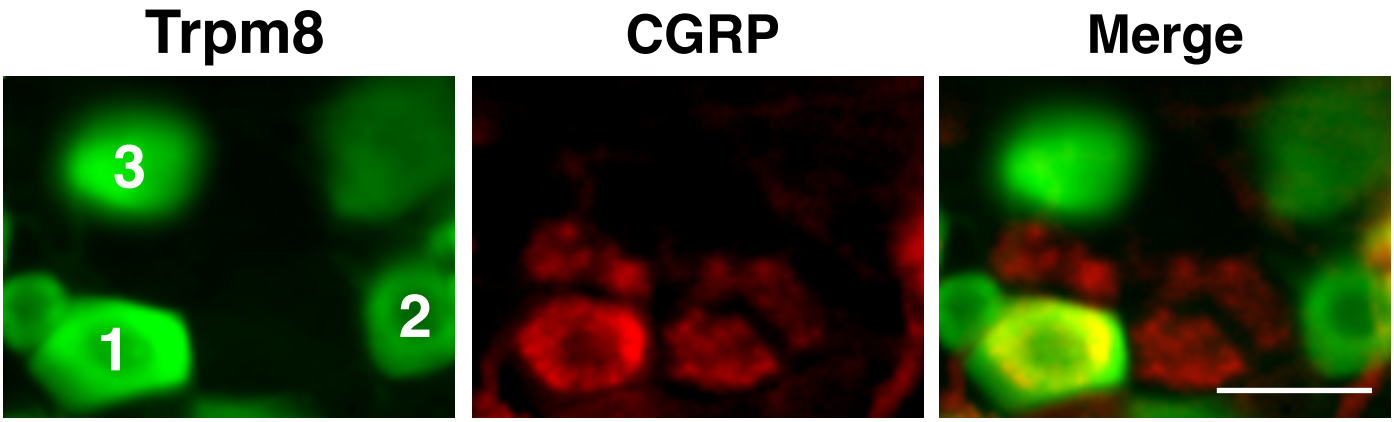
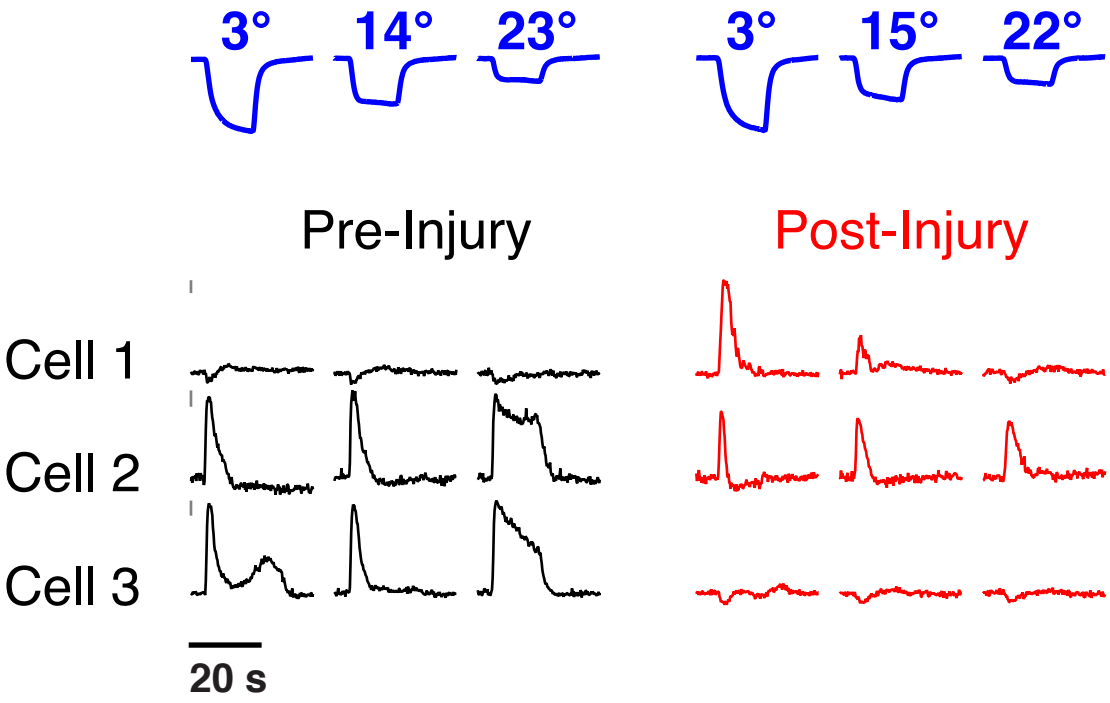


Supplemental Figure 6





Supplemental Figure 7

Genotype	Sex	Age (days)
Rosa26^{GCaMP5};Trpa1$^{Cre\ +/-}$	♂	39
Rosa26^{GCaMP5};Trpa1$^{Cre\ +/-}$	♂	43
Rosa26^{GCaMP5};Trpa1$^{Cre\ +/-}$	♀	64
Rosa26^{GCaMP5};Trpa1$^{Cre\ +/-}$	♂	48
Rosa26^{GCaMP5};Trpa1$^{Cre\ +/-}$	♂	65
Rosa26^{GCaMP5};Trpa1$^{Cre\ +/-}$	♂	79
Rosa26^{GCaMP5};Trpa1$^{Cre\ +/-}$	♀	122
Rosa26^{GCaMP5};Trpa1$^{Cre\ +/-}$	♀	57
Rosa26^{GCaMP5};Trpa1$^{Cre\ +/-}$	♀	65
Rosa26^{GCaMP5};Trpm8$^{Cre\ +/-}$	♂	87
Rosa26^{GCaMP5};Trpm8$^{Cre\ +/-}$	♂	46
Rosa26^{GCaMP5};Trpm8$^{Cre\ +/-}$	♀	48
Rosa26^{GCaMP5};Trpm8$^{Cre\ +/-}$	♂	87
Rosa26^{GCaMP5};Trpm8$^{Cre\ +/-}$	♂	75
Rosa26^{GCaMP5};Trpm8$^{Cre\ +/-}$	♂	75
Rosa26^{GCaMP5};Trpm8$^{Cre\ +/-}$	♀	86
Rosa26^{GCaMP5};Trpm8$^{Cre\ +/-}$	♂	74
Rosa26^{GCaMP5};Trpv1$^{Cre\ +/-}$	♂	52
Rosa26^{GCaMP5};Trpv1$^{Cre\ +/-}$	♀	51
Rosa26^{GCaMP5};Trpv1$^{Cre\ +/-}$	♀	125
Rosa26^{GCaMP5};Trpv1$^{Cre\ +/-}$	♂	63
Rosa26^{GCaMP5};Trpv1$^{Cre\ +/-}$	♂	134
Rosa26^{GCaMP5};Trpv1$^{Cre\ +/-}$	♀	75
Rosa26^{GCaMP5};Wnt1$^{Cre\ +/-}$	♂	42
Rosa26^{GCaMP5};Wnt1$^{Cre\ +/-}$	♂	42
Rosa26^{GCaMP5};Wnt1$^{Cre\ +/-}$	♀	45
Rosa26^{GCaMP5};Wnt1$^{Cre\ +/-}$	♀	45
Rosa26$^{\Delta GCaMP5}$;Trpv1$^{-/-}$	♂	59
Rosa26$^{\Delta GCaMP5}$;Trpv1$^{-/-}$	♀	63
Rosa26$^{\Delta GCaMP5}$;Trpv1$^{-/-}$	♂	63
Rosa26$^{\Delta GCaMP5}$;Trpv1$^{+/+}$	♂	54
Rosa26$^{\Delta GCaMP5}$;Trpv1$^{+/+}$	♂	55
Rosa26$^{\Delta GCaMP5}$;Trpv1$^{+/+}$	♀	69