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Supplemental information

Population encoding of cool and warm by thermoreceptors

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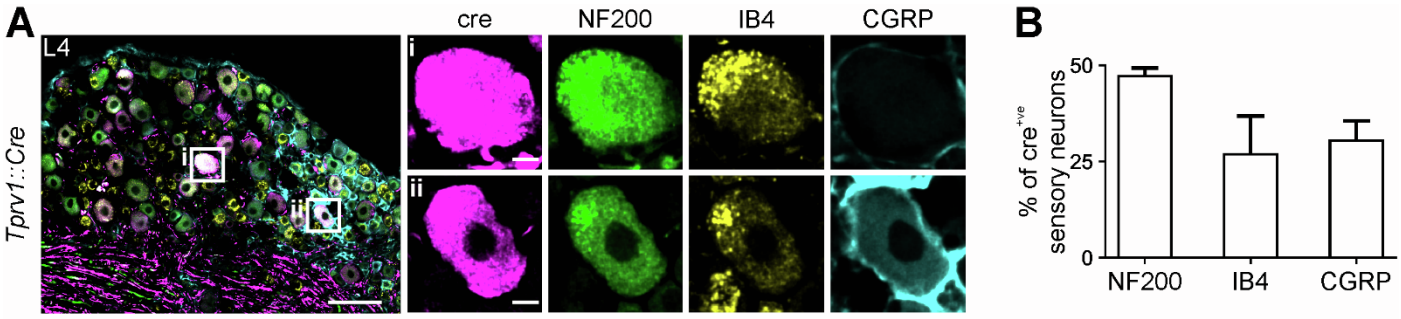

Figure S1. Characterization of cre labelling in molecularly distinct lumbar DRG neurons in the *Trpv1::Cre* mouse. Related to Figure 1.

(A) Left, representative image of a L4 DRG section showing expression of cre (TRPV1 neurons, magenta), CGRP (peptidergic neurons, cyan), IB4 (non-peptidergic neurons, yellow), and NF200 (myelinated neurons, green). Scale bar 100 µm. White staining highlights overlapping expression of different markers. Zoom of insets show example cre positive neurons (i and ii). Scale bar 10 µm.

(B) Quantification of the percent of cre expressing neurons expressing NF200, IB4 and CGRP in Trpv1::Cre mice (*n* = 4 thoraco-lumbar sections).

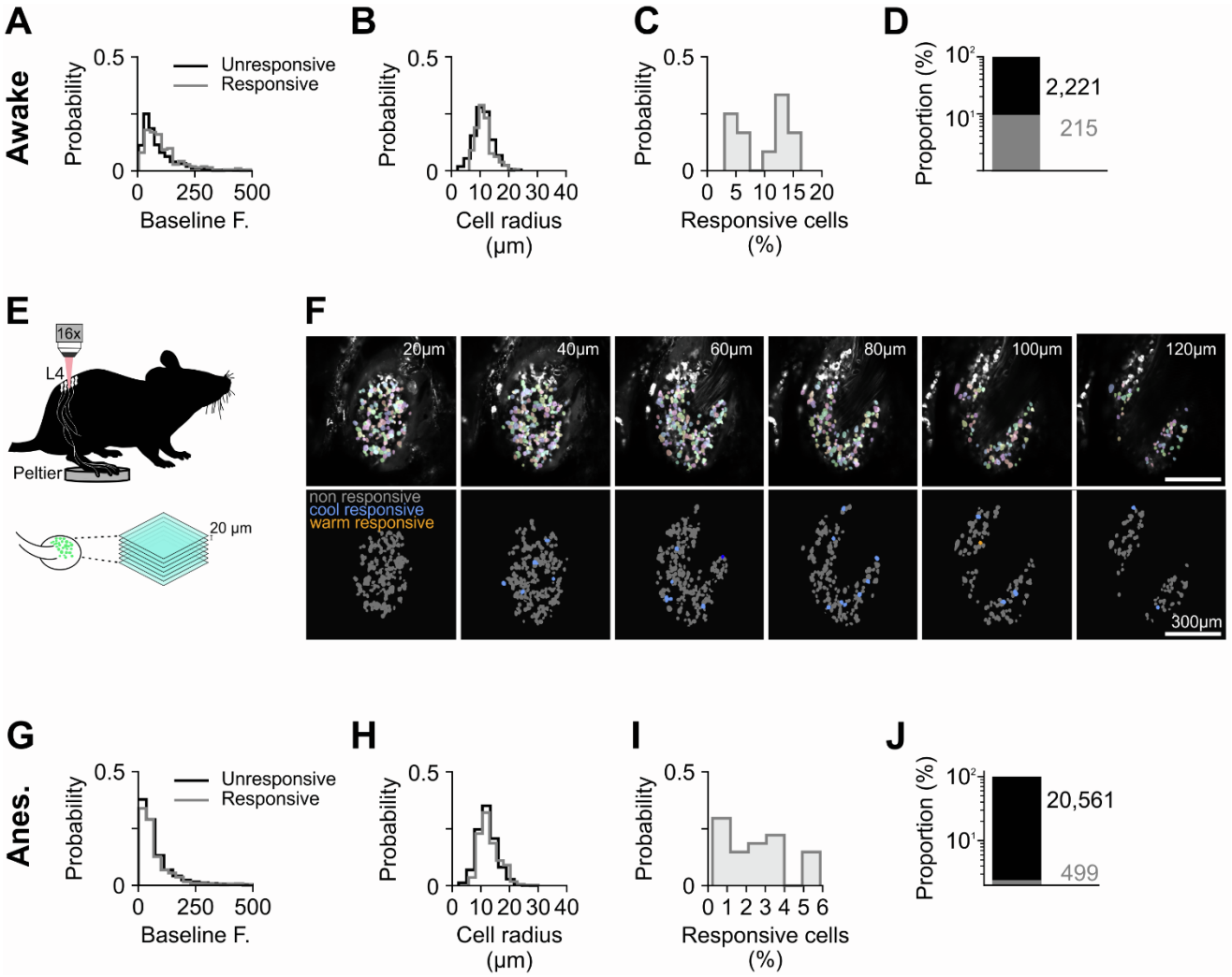

Figure S2. Sparse thermal sensitivity of DRG neurons *in vivo*. Related to Figure 1.
(A) Probability distribution of baseline fluorescence in awake mice for thermally sensitive (grey) or insensitive (black) neurons (*n* = 2,436 neurons, 12 mice).
(B) Probability distribution of cell radius for cells in awake mice for thermally sensitive (grey) or insensitive (black) (*n* = 2,436 neurons, 12 mice).
(C) Probability distribution of percent of recorded ROIs in awake mice classified as responsive (*n* = 215 neurons, 12 mice).
(D) Proportion of anatomically identified ROIs in awake mice with (grey, *n* = 215 neurons) or without (black, *n* = 2,221 neurons) thermal responses.
(E) Schematic depicting multi-plane imaging paradigm.
(F) Example *in vivo* multi-plane two-photon images showing DRG neurons (top) with functional classification as cool responsive (blue) or warm responsive (orange) overlaid on the image map (bottom). Scale bar, 300 μm.
(G to J) same as (A to D) except under anesthesia (*n* = 21,060 neurons total, 20,561 unresponsive and 499 responsive, 30 mice).

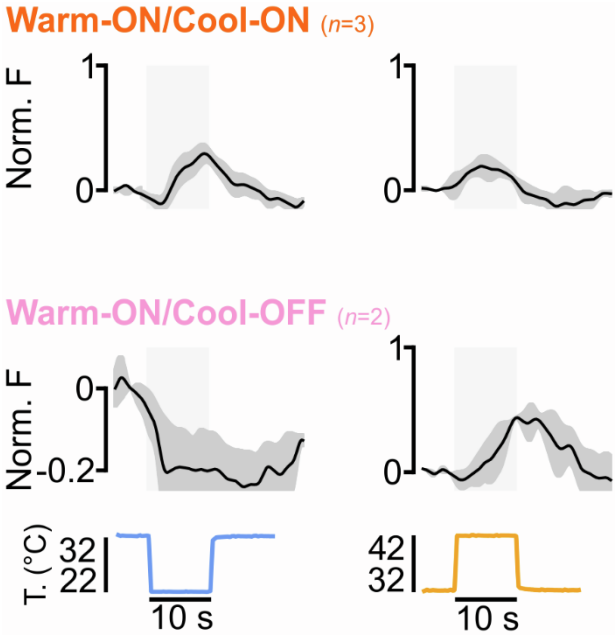

Figure S3. Rare thermosensory response profiles in the awake mouse. Related to Figure 1.
Grand average of rarely observed thermoreceptor responses types to cooling (left, 22°C) or warming (right, 42°C) stimuli showing, Top, an ON response to both warming and cooling (orange, *n* = 3 cells, 3 mice, *n* = 3 *TRPV1::cre;Ai162d*), or, Bottom, an ON response to warming and an OFF response to cooling (pink, *n* = 2 cells, 2 mice, *n* = 1 *TRPV1::cre;Ai148d*, *n* = 1 *TRPV1::cre;Ai162d*).

Data shown as mean ± SEM.

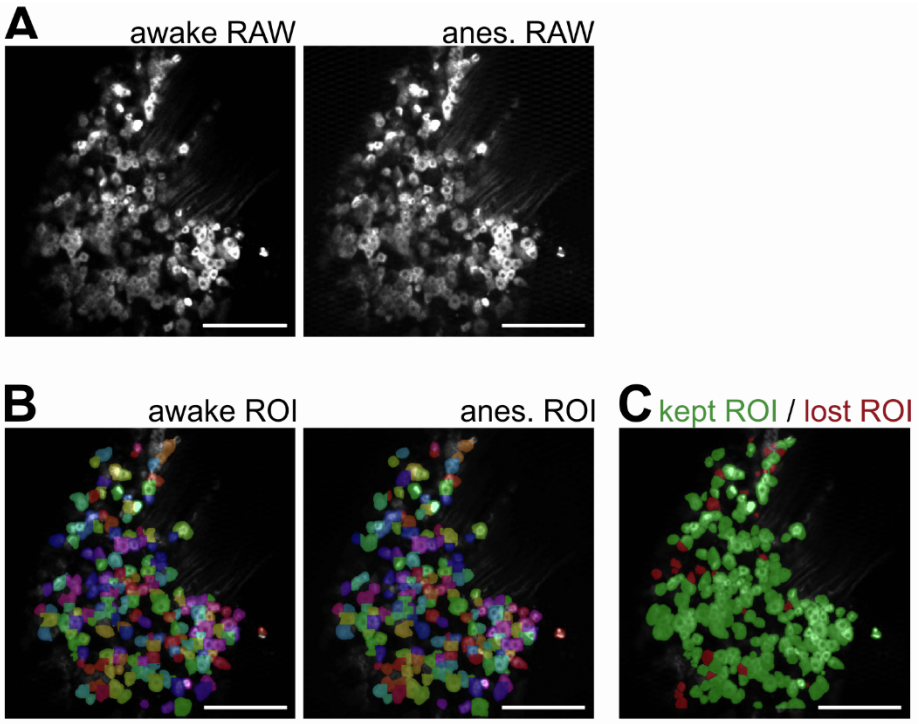

Figure S4: Methodological comparison of awake and anesthetized recordings. Related to Figure 2.
(A) Example *in vivo* image for an awake (left) and anesthetized (right) imaging session from the same mouse. Scale bar, 100 μm.
(B) Anatomical segmentation of the awake (left) and anesthetized (right) sessions using CellPose. Regions of interest (ROI) are pseudo colored and matched across each session. Scale bar, 100 μm.
(C) ROI segmentation of concatenated awake and anesthetized sessions using Suite2P. ROI are pseudo colored by those that are anatomically identified in both sessions and kept for further analysis (green) and those that were not anatomically identified in both sessions and excluded from further analysis (red). Scale bar, 100 μm.

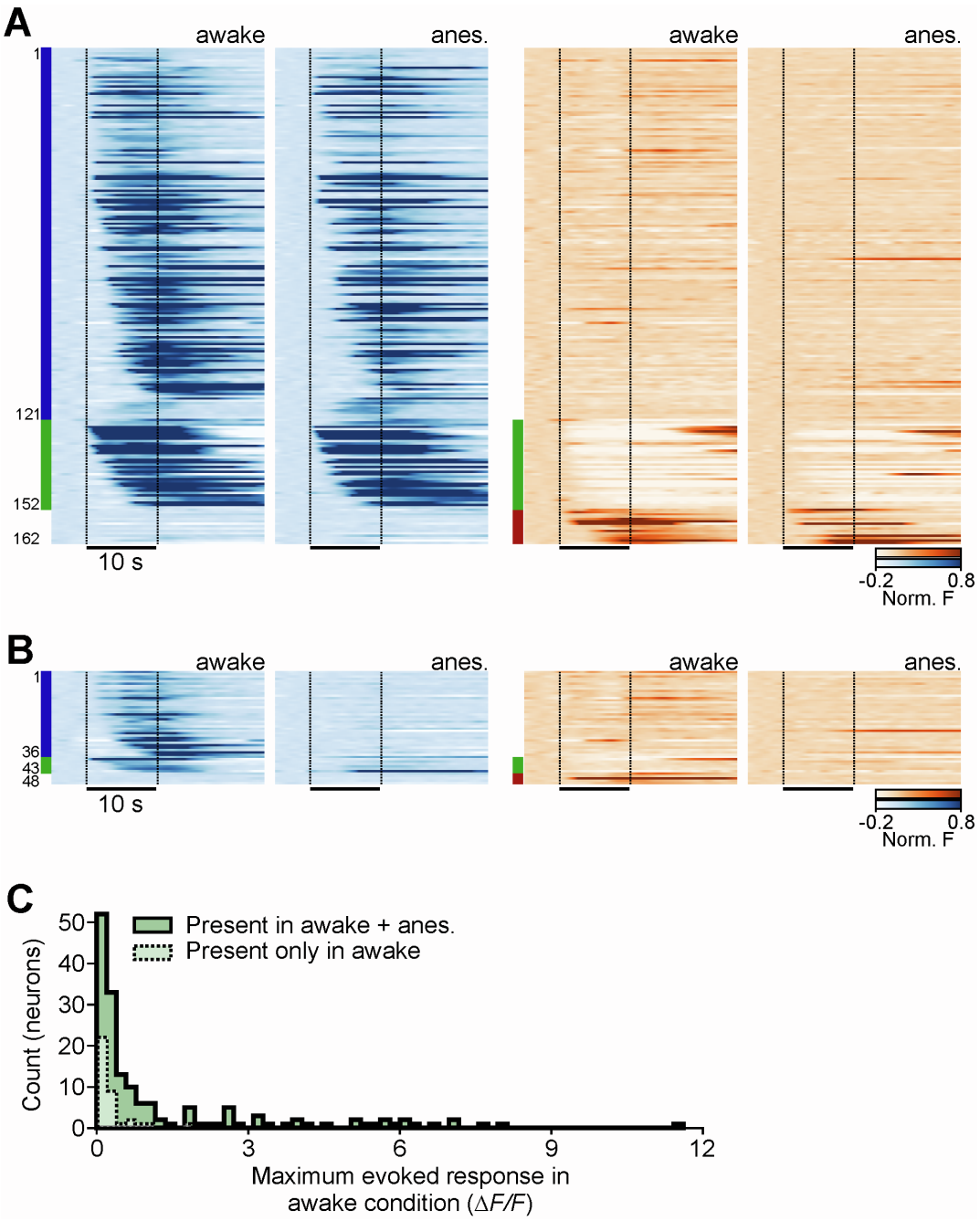

Figure S5. Thermoreceptors unresponsive under anesthesia have low amplitude responses in the awake mouse. Related to Figure 2.

(A) Single thermoreceptor responses to cooling (left, 22ºC) and warming (right, 42ºC) stimuli in awake and anesthetized mice. Each row represents a single neuron, sorted based on response latency and response type. Vertical dashed lines indicate the onset and end of the plateau phase of a thermal stimulus (*n* = 162 thermally responsive neurons, 12 mice, *n* = 5 *TRPV1::cre;Ai148d*, *n* = 7 *TRPV1::cre;Ai162d*).

(B) as in (A), except neurons that are only present in awake mice and are lost when mice are anesthetized (*n* = 48 thermally response neurons, 12 mice, *n* = 5 *TRPV1::cre;Ai148d*, *n* = 7 *TRPV1::cre;Ai162d*).

(C) Neurons anatomically identified in both sessions, but functionally present only in the awake session (dotted outline) showed a lower stimulus evoked response in awake mice than neurons that were functionally responsive in both sessions (solid outline).

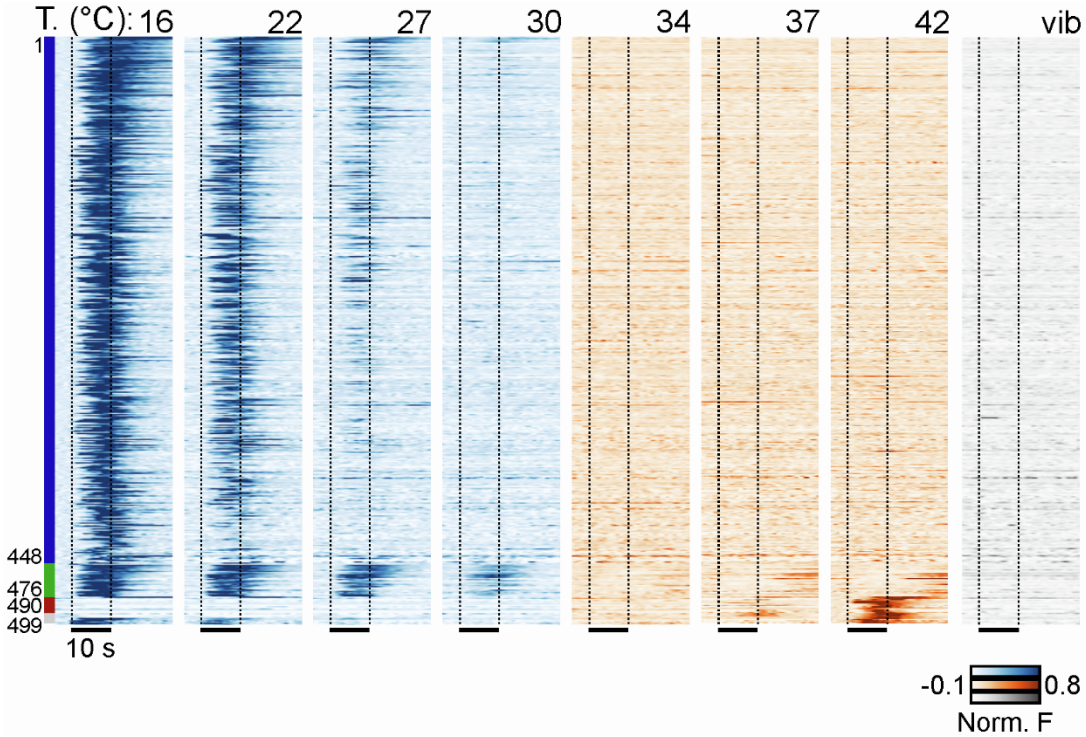

Figure S6. Thermoreceptors do not show tactile responses. Related to Figure 3.
Thermoreceptor calcium responses to cooling (blue), warming (orange) and 40 Hz vibration (grey) stimuli applied to the right hindpaw. Each line represents a single neuron; sorted based on both the response duration and the response type. Vertical dashed lines indicate the onset and end of the plateau phase of a thermal or tactile stimulus (*n* = 499 thermally responsive neurons, 27 mice, *n* = 15 *TRPV1::cre;Ai148d*, *n* = 12 *TRPV1::cre;Ai162d*). No significant response to vibration was observed.

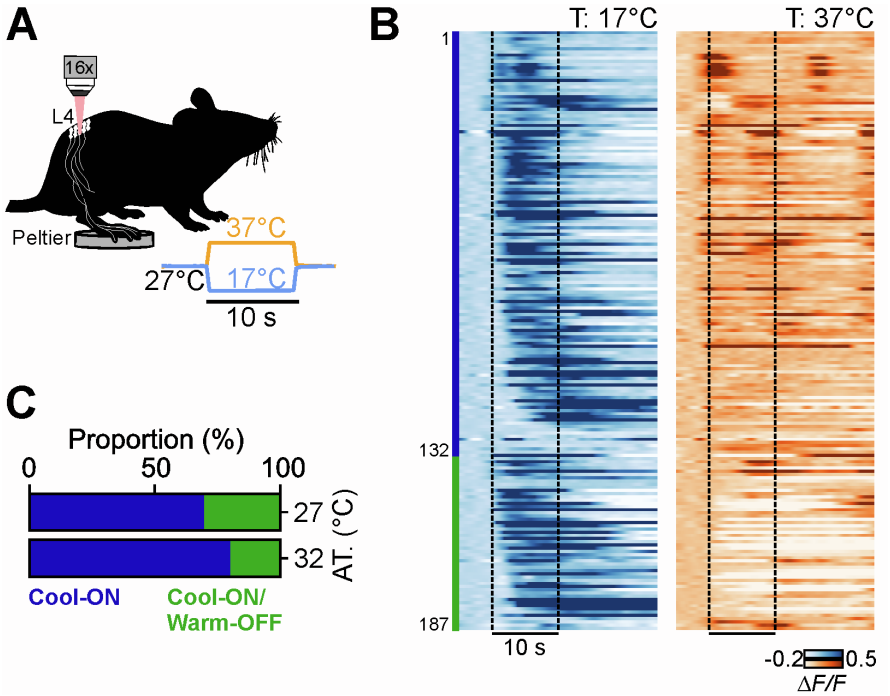

Figure S7. Thermoreceptor encoding of cool and warm from an adapted temperature of 27°C in the awake mouse. Related to Figure 4.

(A) Schematic of an awake mouse with the right hindpaw tethered to a Peltier element for cutaneous thermal stimulation during two-photon calcium imaging of the right L4 DRG; insert shows temporal dynamics of cooling and warming stimuli from an adapted temperature of 27°C.

(B) Single thermoreceptor calcium responses to cooling (left) and warming (right) stimuli. Vertical dashed lines indicate the onset and end of the plateau phase of a thermal stimulus (n = 187 thermally responsive neurons, 12 mice, n = 5 *TRPV1::cre;Ai148d*, n = 7 *TRPV1::cre;Ai162d*). Index bar on the left indicates the functional response types Cool-ON (blue) and Cool-ON/Warm-OFF (green).

(C) Proportion of Cool-ON and Cool-ON/Warm-OFF thermoreceptors from an adapted temperature of 27°C (top) or 32°C (bottom) in the awake mouse.

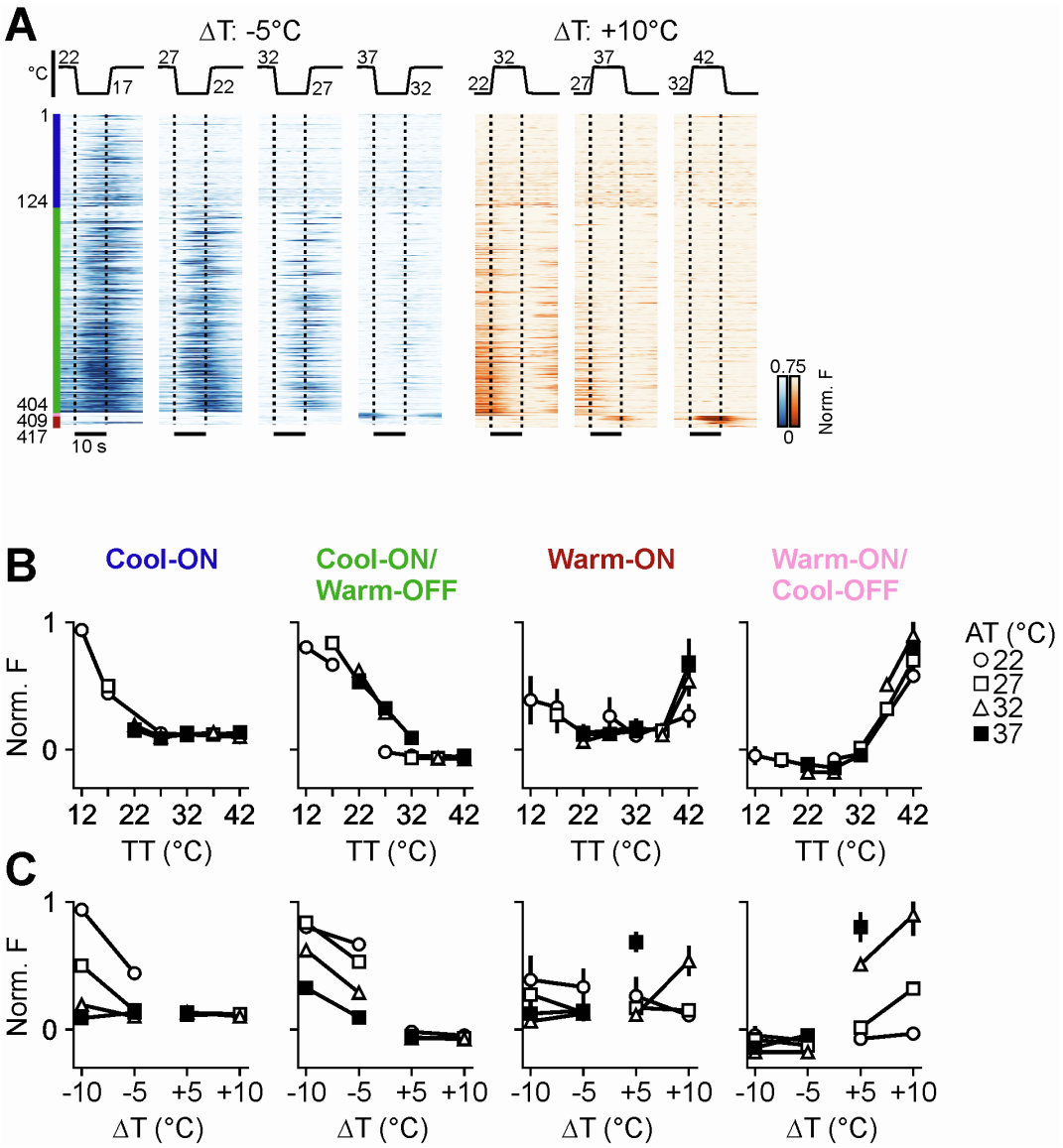

Figure S8. Adaptation temperature affects evoked thermosensory responses. Related to Figure 5.
(A) Single-cell thermoreceptor calcium responses to cooling (blue, ΔT = -5°C) or warming (orange, ΔT = +10°C) at different adapted temperatures. Each line represents a single neuron; sorted based on response latency and response type. Vertical dashed lines indicate the onset and end of the plateau phase of a thermal stimulus (*n* = 417 thermally responsive neurons, 16 mice, *n* = 6 *TRPV1::cre;Ai148d*, *n* = 10 *TRPV1::cre;Ai162d*).
(B) Normalized peak fluorescence values by target temperature (TT) of each thermoreceptor type. Marker indicates the adapted temperature (AT = 22°C: open circle, AT = 27°C: open square, AT = 32°C: open triangle, AT = 37°C: closed square).
(C) Same as (B), but shown as a change in temperature (ΔT).

Data are shown as mean ± SEM.

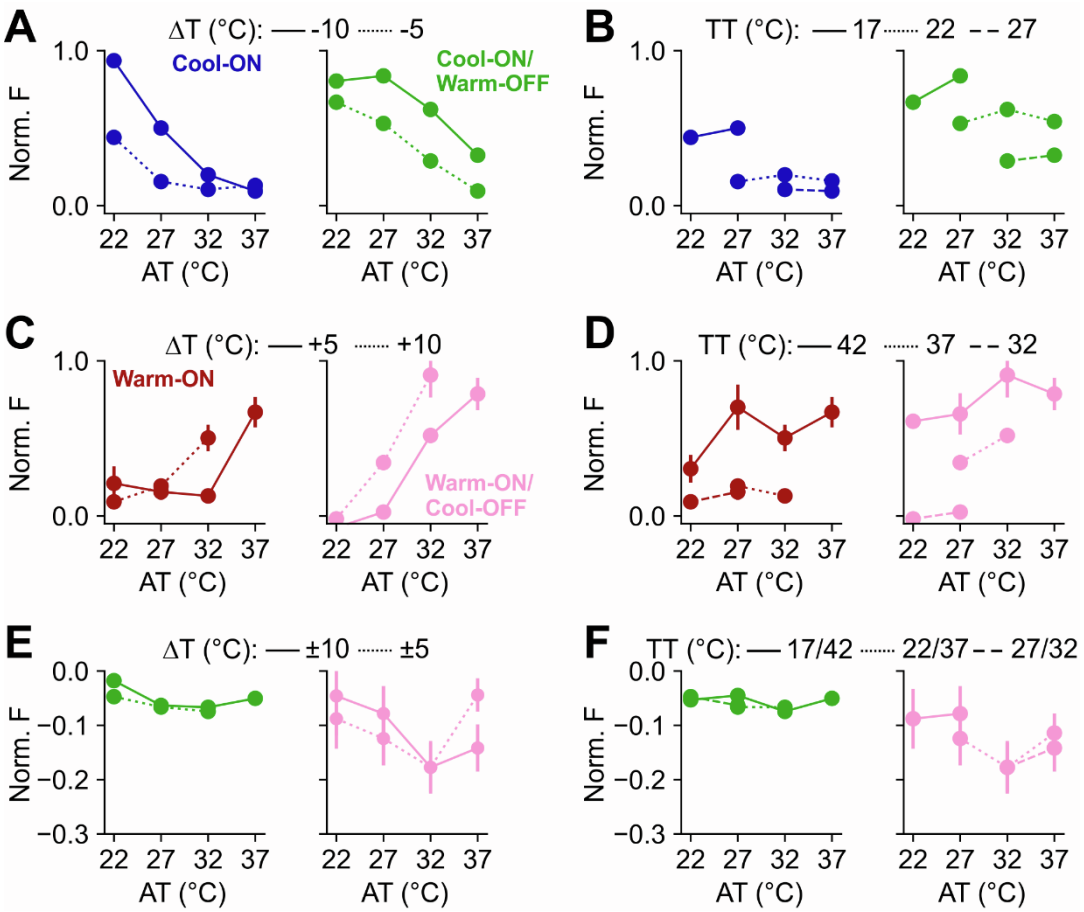

Figure S9. ON/OFF cells show greater sensitivity than ON-only cells. Related to Figure 5.
(A) Normalized peak fluorescence response values across adapted temperatures in response to a ΔT of -10°C (solid) or -5°C (dotted) for Cool-ON (left: *n* = 184 cells, 16 mice, *n* = 6 *TRPV1::cre;Ai148d*, *n* = 10 *TRPV1::cre;Ai162d*, blue) and Cool-ON/Warm-OFF (right: *n* = 280 cells, 16 mice, *n* = 6 *TRPV1::cre;Ai148d*, *n* = 10 *TRPV1::cre;Ai162d*, green).
(B) Same as (A) except for TT = 17°C (solid), 22°C (dotted), or 27°C (dashed).
(C) Same as (A) except for ΔT = +5°C (solid) or +10°C (dotted) for Warm-ON (left: *n* = 8 cells, 16 mice, *n* = 6 *TRPV1::cre;Ai148d*, *n* = 10 *TRPV1::cre;Ai162d*, maroon) and Warm-ON/Cool-OFF (right: *n* = 5 cells, 16 mice, *n* = 6 *TRPV1::cre;Ai148d*, *n* = 10 *TRPV1::cre;Ai162d*, pink).
(D) Same as (C) except for TT = 42°C (solid), 37°C (dotted), or 32°C (dashed).
(E) Same as (A), but for the OFF responses for warming (left: Cool-ON/Warm-OFF, green, ΔT = +5°C (dotted) or +10°C (solid)) and cooling (right: Warm-ON/Cool-OFF, pink, ΔT = -5°C (dotted) or -10°C (solid)).
(F) Same as (E), but for TT = 17/42°C (solid), 22/37°C (dotted), or 27/32°C (dashed).

Data shown as mean ± SEM.

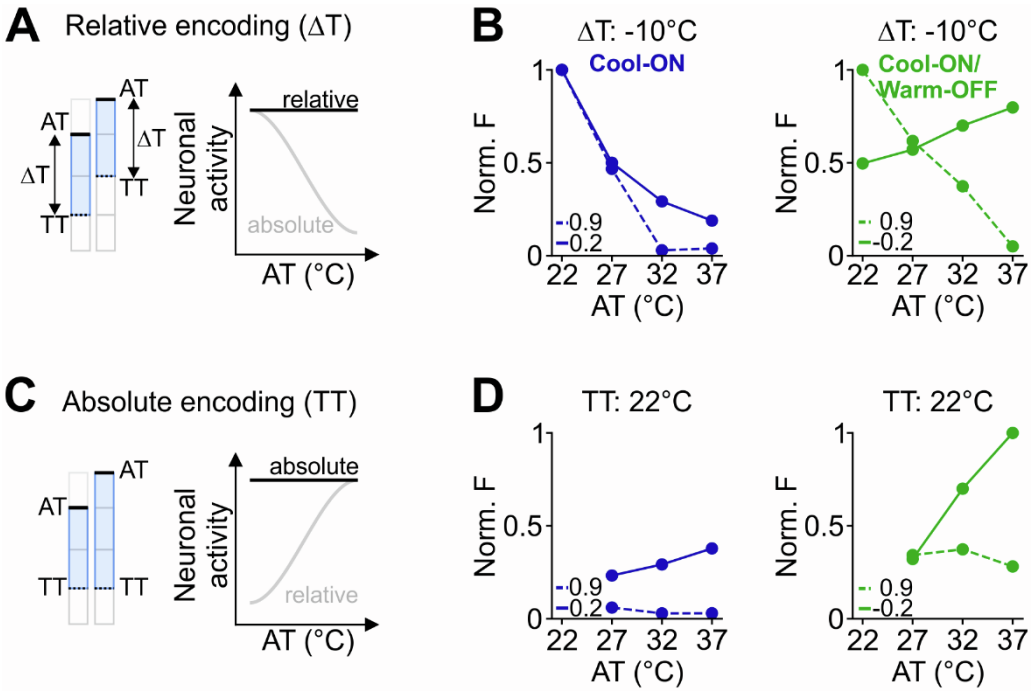

Figure S10. Examples of Cool-ON and Cool-ON/Warm-OFF thermoreceptors with different relative/absolute encoding index values. Related to Figure 5.

(A) Schematic of a relative encoding scheme in which the amplitude of neural responses to a fixed amplitude thermal stimulus (ΔT) is independent of the adapted temperature (AT). Neurons following a purely relative encoding scheme would have a relative/absolute encoding index of -1.

(B) Four example neural responses to a ΔT of -10°C from different adapted temperatures (AT). Left, the dashed blue line shows responses amplitudes of an example Cool-ON thermoreceptor with a high relative/absolute encoding index (+0.9) consistent with absolute encoding, while the solid blue line shows a thermoreceptor with a lower relative/absolute encoding index (+0.2) suggesting an intermediate coding scheme that is neither perfectly absolute or relative. Right, green dashed line shows an example Cool-ON/Warm-OFF thermoreceptor with a high relative/absolute encoding index (+0.9) consistent with absolute encoding; green, solid line shows an example Cool-ON/Warm-OFF thermoreceptor with a lower relative/absolute encoding index (-0.2) indicated a more relative encoding scheme.

(C) Schematic of an absolute encoding scheme in which the amplitude of the neural responses to a thermal stimulus with a fixed target temperature (TT*)* is independent of the adapted temperature (AT). Neurons following a purely absolute encoding scheme would have a relative/absolute encoding index of +1.

(D) Same responses as (B) except for a TT of 22°C.

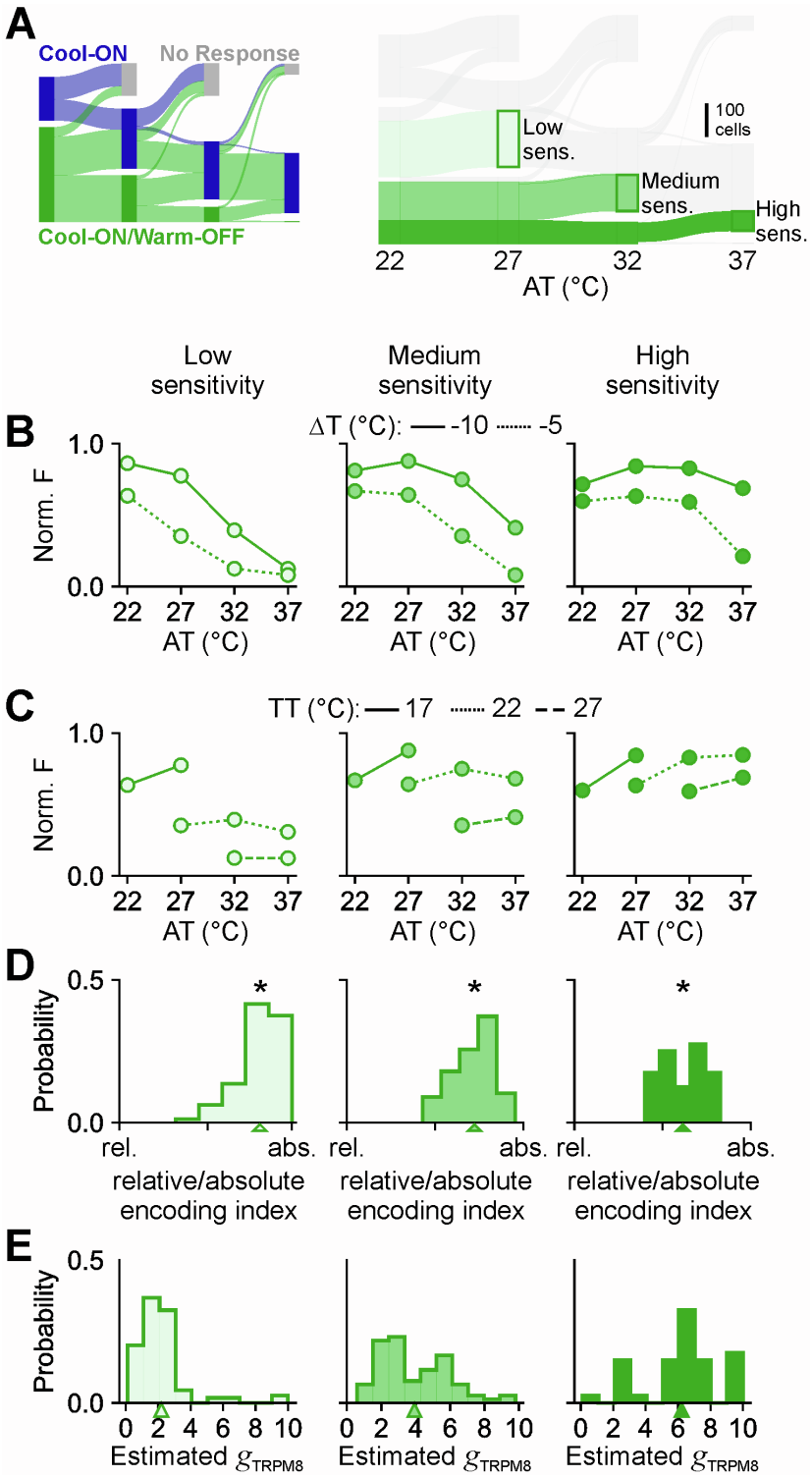

Figure S11. Thermoreceptors classified as Cool-ON/Warm-OFF at higher adapted temperatures indicates higher thermal sensitivity. Related to Figure 5.

(A) Left. Sankey plot depicting the transition of functional type classification for thermoreceptors with an ON response to cool at different adapted temperatures. Bar color indicates thermoreceptor classification at each adapted temperature (AT) while ribbon color indicates classification at the lowest AT (22°C). Right. Sankey plot of the numbers of Cool-ON/Warm-OFF responses at different adapted temperatures. Low sensitivity Cool-ON/Warm-OFF cells no longer display an OFF response at 27°C AT. Medium sensitivity maintained an OFF response at 27°C AT but did not at 32°C AT. High sensitivity maintained the warm OFF response at 32°C AT but not at 37°C AT.

(B) Normalized peak fluorescence response values across ATs in response to a ΔT of -10°C (solid) or -5°C (dotted) for Low to High sensitivities (left to right).

(C) Same as (B), except for TT = 17°C (solid), 22°C (dotted), or 27°C (dashed).

(D) Encoding bias distribution, defined as the similarity of an individual unit tuning to absolute (+1) or relative (-1) encoding in response to cooling for Low to High sensitivities (left to right).

(E) Model-fit TRPM8 conductance values to each experimental neuron in Low (left: *n* = 114 neurons), Medium (middle: *n* = 78 neurons), and High sensitivity (right: *n* = 40 neurons) groups.

Data shown as mean ± SEM. Data from 16 mice, *n* = 6 *TRPV1::cre;Ai148d*, *n* = 10 *TRPV1::cre;Ai162d.*

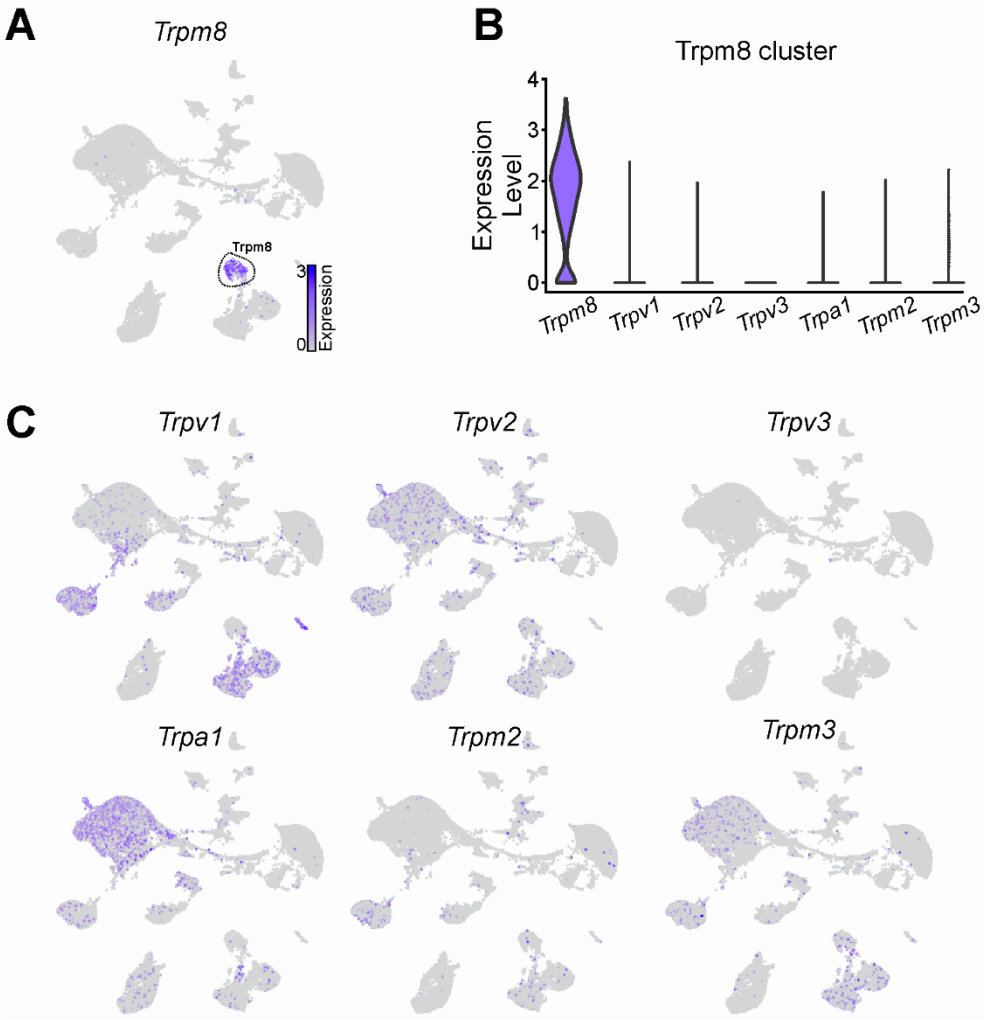

Figure S12. Genes encoding different thermosensory TRP ion channels are not enriched within the TRPM8 cluster of sensory afferent neurons. Related to Figure 6.
(A) UMAP visualization of DRG scRNA-seq data showing enrichment for Trpm8.
(B) Violin plots displaying expression profiles of different thermosensory TRP genes within the Trpm8 cluster.
(C) UMAP visualization of DRG scRNA-seq data showing broad and distributed expression of different thermosensory TRP genes.

All data has been reanalyzed from Qi et al. 2024[1] using code built from Ghitani et al. 2025[2] (https://doi.org/10.5281/zenodo.14907827).

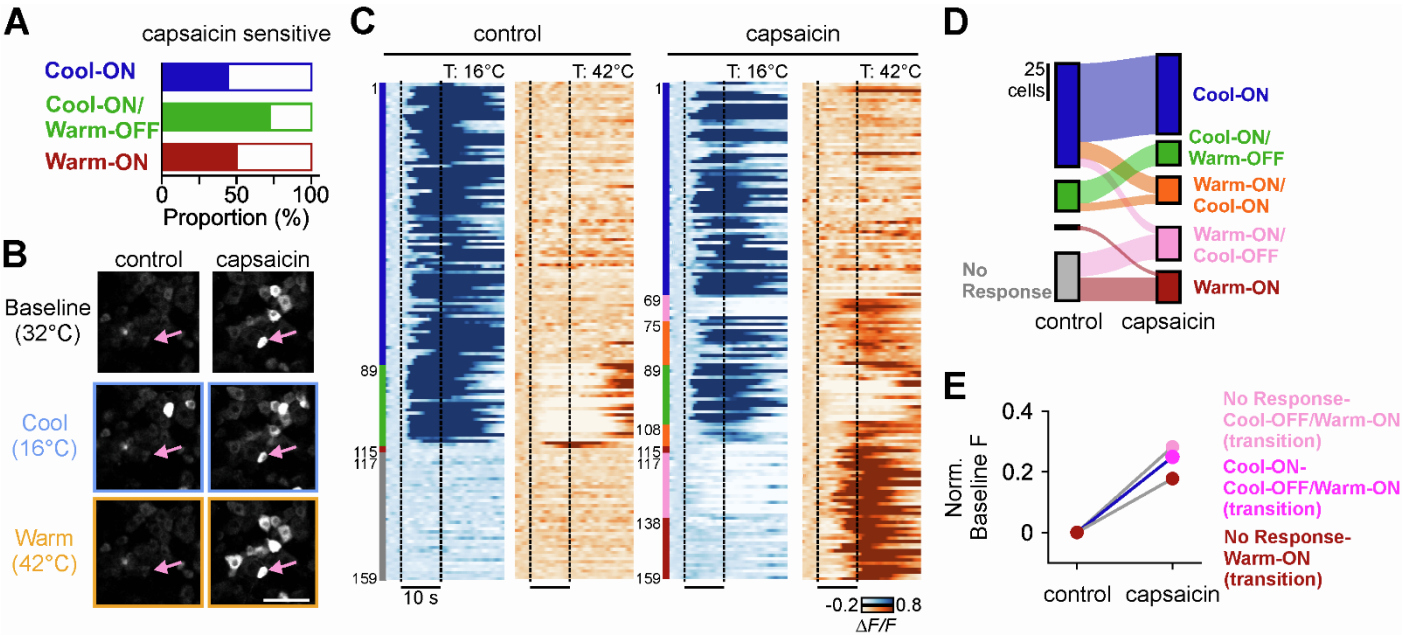

Figure S13. TRPV1 sensitization unmasks Warm ON responses within thermoreceptors. Related to Figure 8.

(A) Proportion of thermoreceptor types that display a response to the application of TRPV1 agonist capsaicin to the skin (blue: Cool-ON, *n* = 89 neurons; green: Cool-ON/Warm-OFF, *n* = 26 neurons, maroon: Warm-ON, *n* = 2 neurons).

(B) Example *in vivo* two-photon image of thermoreceptors before (left) and after (right) application of capsaicin during the pre-stimulus phase (top, 32ºC), during a cooling (middle, 16ºC) or warming stimulus (bottom, 42ºC). Example neurons during capsaicin application that unmask a Warm-ON response are indicated by a pink arrow. Scale bar, 100 µm.

(C) Single thermoreceptor calcium responses to cooling (AT: 32ºC, TT: 16ºC, blue) and warming (AT: 32ºC, TT: 42ºC, orange) stimuli before (left) and after (right) application of capsaicin. Each row represents a single neuron; sorted based on the response width and response type. Vertical dashed lines indicate the onset and end of the plateau phase of a thermal stimulus (*n* = 159 thermally responsive neurons, 7 mice, *n* = 3 *TRPV1::cre;Ai148d*, *n* = 4 *TRPV1::cre;Ai162d*).

(D) Sankey plot depicting transitions of thermoreceptor functional type following capsaicin application. Bar color indicates classification as denoted in A while ribbon color indicates thermoreceptor classification after application of capsaicin.

(E) Baseline fluorescence before and after application of capsaicin for three functional thermoreceptor groups: (i) thermoreceptors with No Response to warm or cool stimuli that transition to Cool-OFF/Warm-ON (light pink, *n* = 21 neurons) (ii) Cool-ON that transition to Cool-OFF/Warm-ON (dark pink, *n* = 6 neurons), (iii) No Response that transition to Warm-ON (maroon, *n* = 21 neurons).

Data in (E) show mean ± SEM.

References

1. Qi, L., Iskols, M., Shi, D., Reddy, P., Walker, C., Lezgiyeva, K., Voisin, T., Pawlak, M., Kuchroo, V.K., Chiu, I.M., et al. (2024). A mouse DRG genetic toolkit reveals morphological and physiological diversity of somatosensory neuron subtypes. Cell *187*, 1508-1526.e16. https://doi.org/10.1016/j.cell.2024.02.006.

2. Ghitani, N., Von Buchholtz, L.J., MacDonald, D.I., Falgairolle, M., Nguyen, M.Q., Licholai, J.A., Ryba, N.J.P., and Chesler, A.T. (2025). A distributed coding logic for thermosensation and inflammatory pain. Nature *642*, 1016–1023. https://doi.org/10.1038/s41586-025-08875-6.