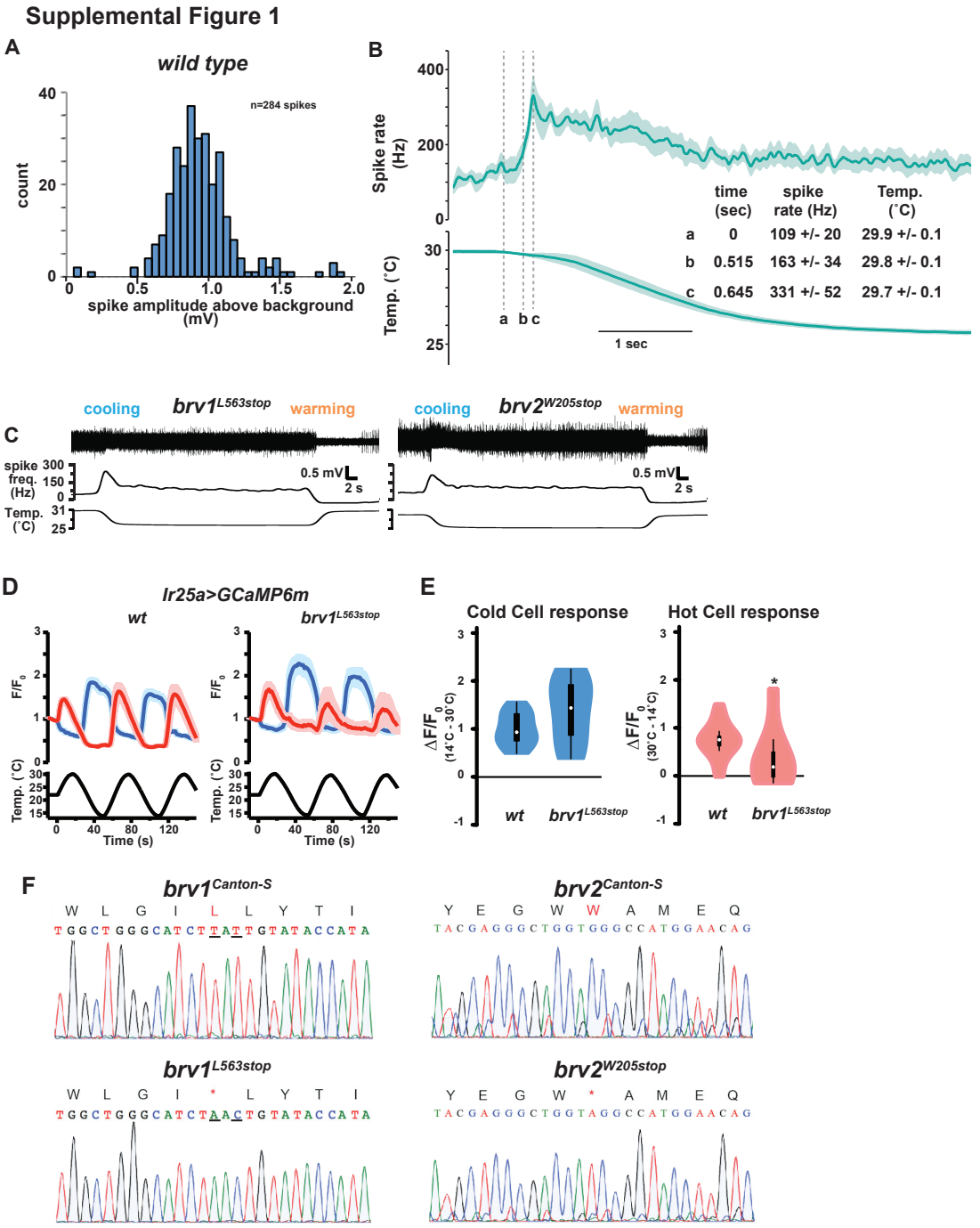
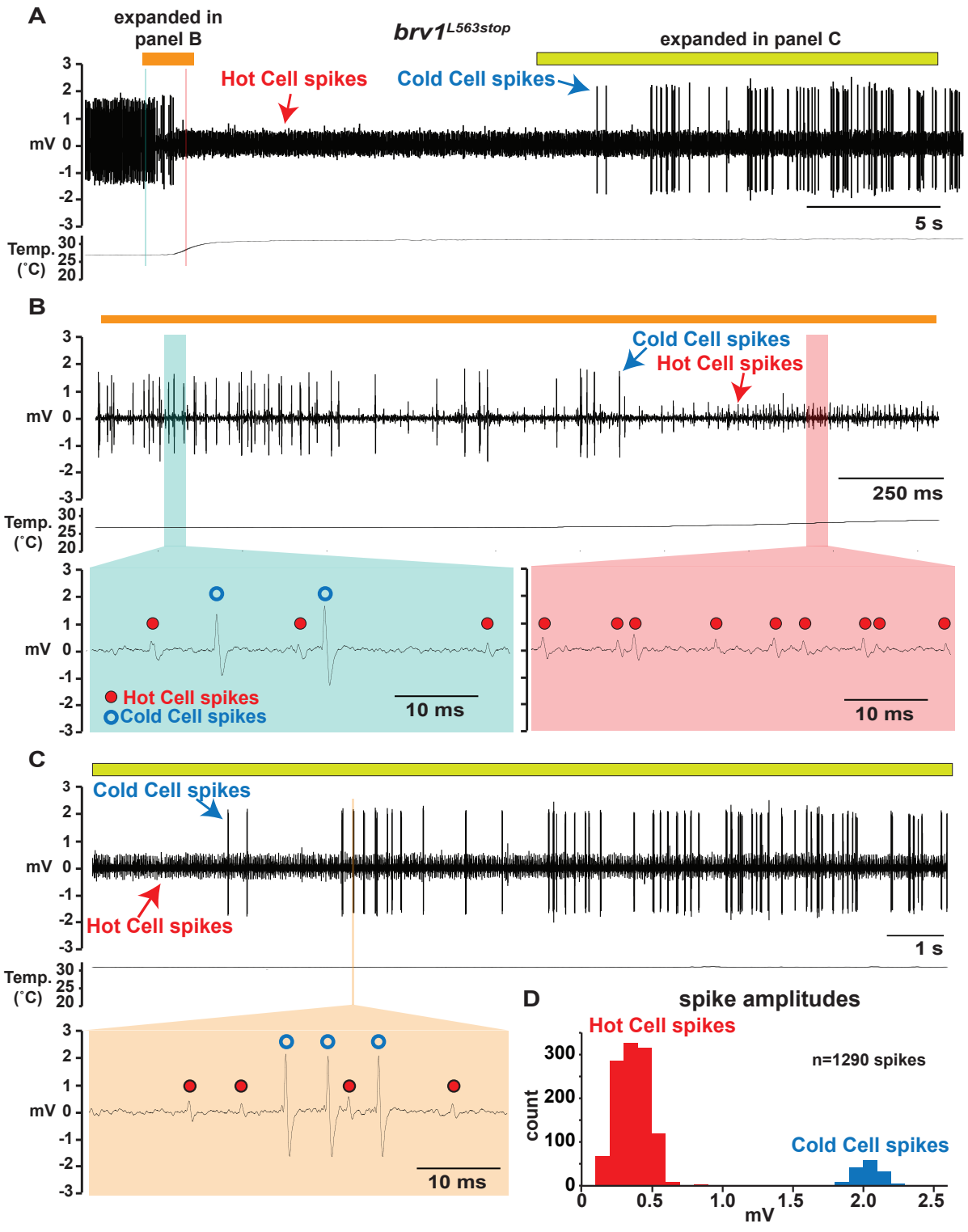


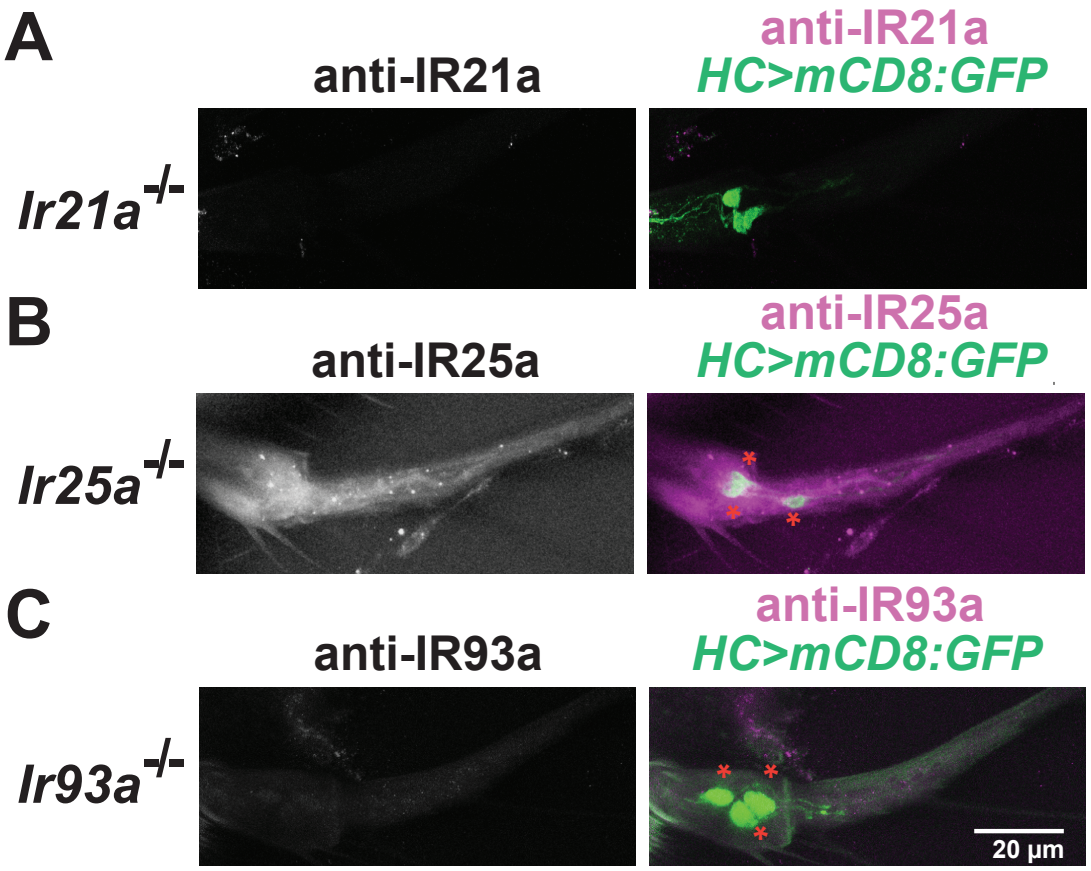
Supplemental Figure 1, related to Figure 1: Thermoreceptor responses in *wild type* and *brivido* (*brv*) mutant animals. A, Spike amplitude analysis of spikes from a *wild type* arista held at 25˚C. **B**, PSTH of wild type arista response generated using the same primary data as in Fig. 1B, but shown on an expanded time scale and using a narrower triangular window (100 msec) for improved temporal resolution. Time, temperature, and spike rates provided for indicated time points. n=7 animals, average +/- SEM. **C,** Representative arista thermoreceptor recordings from indicated genotypes. **D,** Thermoreceptor responses in *wild type* (*Ir25a-Gal4/UAS-GCaMP6m*) and $brv1^{L563stop}$ (*Ir25a-Gal4*, $brv1^{L563stop}$ */UAS-GCaMP6m*, $brv1^{L563stop}$). Traces, F/F_0, average +/- SEM. Blue line, Cold Cells; red line, Hot Cells. *wild type*, n=19 Cold Cells and 18 Hot Cells from 10 animals, one trial per cell. $brv1^{L563stop}$, n=12 Cold Cells and 7 Hot Cells from 6 animals. **E,** Responses ($F/F_{0\,(14°C)} - F/F_{0\,(30°C)}$) for Cold Cells, converse for Hot Cells. Cold Cell responses were not statistically distinct between samples. Hot Cell responses were distinct between *wild type* and $brv1^{L563stop}$ (*$p<0.05$, Wilcoxon). **F**, Sequence traces (with virtual translations) of *brv1* and *brv2* genes confirm the presence of mutations in the indicated genotypes. Nucleotides affected in the mutant alleles are underlined.

Supplemental Figure 2



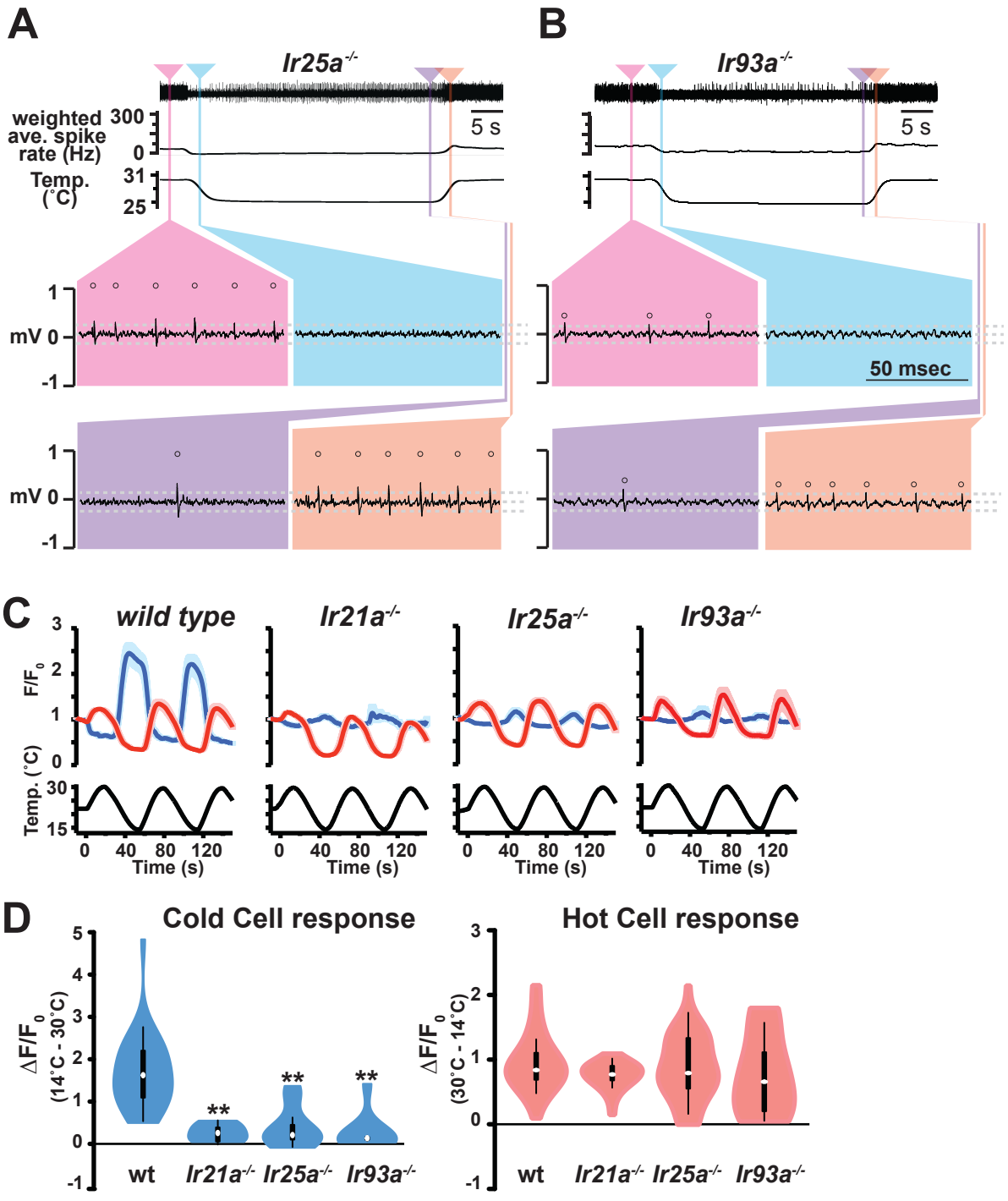
Supplemental Figure 2, related to Figure 1: Arista recording with unusually high signal to noise ratio. A, Recording of *brv1*^{L563stop} animal in which both Hot Cell and Cold Cell spikes were readily detected above noise. **B,** Indicated section of trace from panel A, with two regions shown on an expanded timescale below to highlight individual Hot Cell and Cold Cell spikes. Note the warming-induced decrease in Cold Cell spiking and increase in Hot Cell spiking. **C,** Indicated section of trace from panel A, with one region shown on an expanded timescale below to highlight individual Hot Cell and Cold Cell spikes. **D.** Amplitude histogram of spikes in panel C.

Supplemental Figure 3



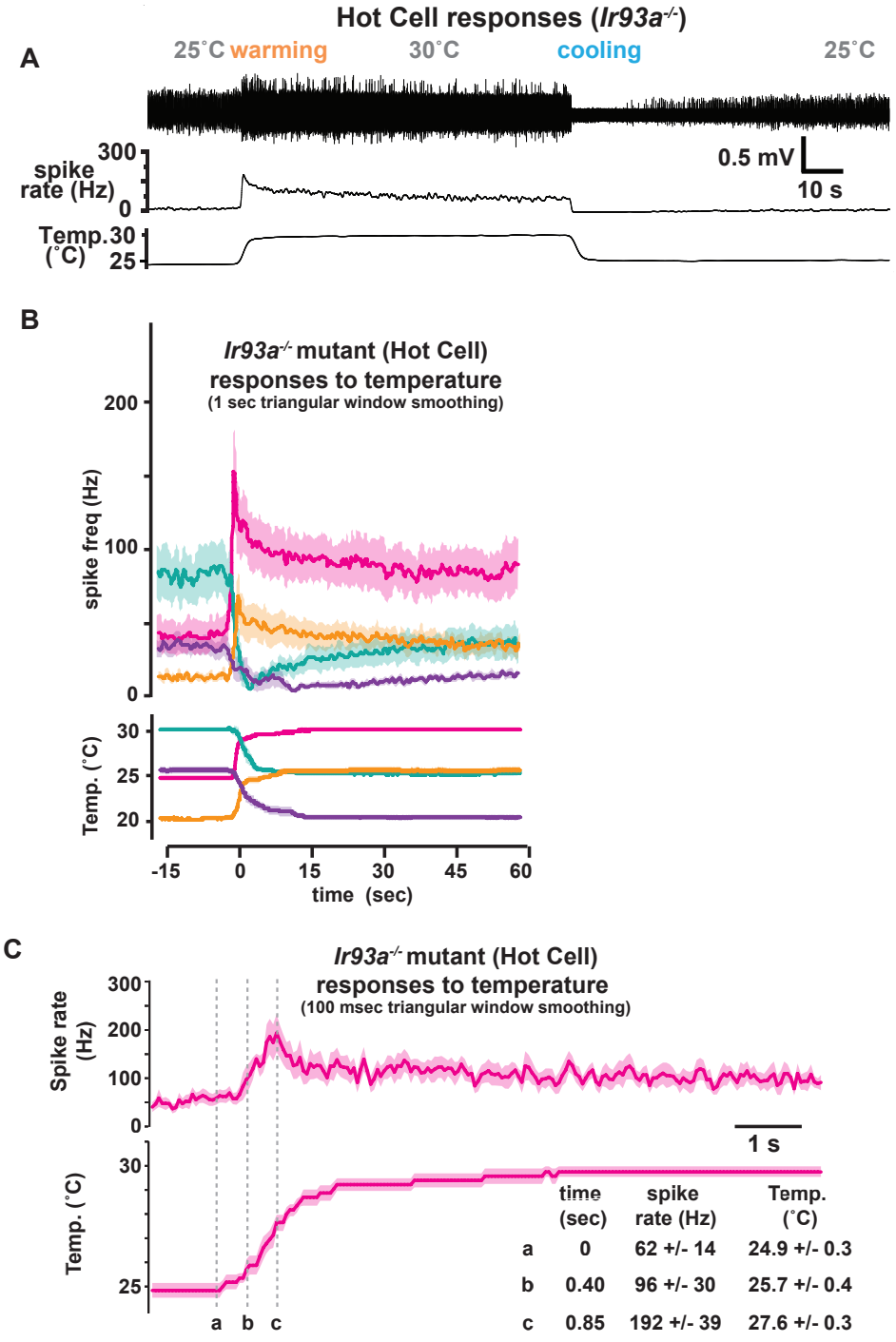
Supplemental Figure 3, related to Figure 2: IR immunostaining is absent in corresponding mutant animals. A-C, anti-IR21a (**A**), anti-IR25a (**B**), and anti-IR93a (**C**) immunostaining in indicated mutant strains. Right, merge with Hot Cell-specific *HC-Gal4;UAS-GFP*. Red asterisks, Hot Cells.

Supplemental Figure 4



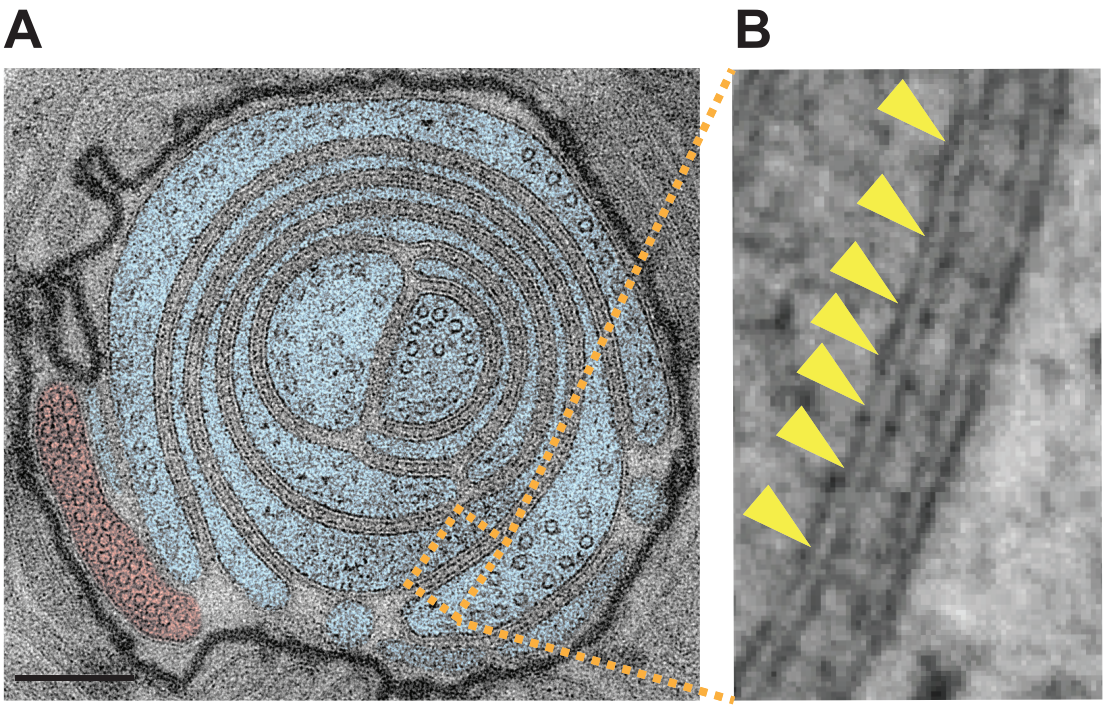
Supplemental Figure 4, related to Figure 3: *Ir21a*, *Ir25a* and *Ir93a* are specifically required for Cold Cell thermosensing. A, B, Representative recordings from indicated genotypes, with portions displayed below using an expanded time scale, as in **Fig. 3. C, D,** Temperature responses of arista thermoreceptors monitored using *GMR11F02>GCaMP6m*. Traces, average +/- SEM. Blue line denotes Cold Cell responses, Red line denotes Hot Cell responses. *wild type* (*GMR11F02-Gal4; UAS-GCaMP6m*), n=27 Cold Cells, 28 Hot Cells, 10 flies. *Ir21a*$^{-/-}$ (*Ir21a*$^{\Delta 1}$/ *Ir21a*$^{\Delta 1}$; *GMR11F02-Gal4/UAS-GCaMP6m*), n=5 Cold Cells, 24 Hot Cells, 10 flies. *Ir25a*$^{-/-}$ (*Ir25a*^{2}/ *Ir25a*^{2}; *GMR11F02-Gal4/UAS-GCaMP6m*), n=14 cold cells, 36 Hot Cells, 14 flies. *Ir93a*$^{-/-}$ (*Ir93a*^{MI05555},*GMR11F02-Gal4/ Ir93a*^{MI05555},*UAS-GCaMP6m*): n=8 Cold Cells, 18 Hot Cells, 8 flies. **P<0.01 compared to wild type, Steel-Dwass.

Supplemental Figure 5



Supplemental Figure 5, related to Figure 3: Hot Cell responses. A, Representative arista thermoreceptor recording from an *Ir93a* mutant. **B,** PSTHs of responses to indicated temperature stimuli (pink and green traces, n=9 animals each; purple and orange traces, n=10). Instantaneous spike frequencies were smoothed using a 1 sec triangular window. **C**, Time expanded view of initial response of pink trace in panel B. Instantaneous spike frequencies were smoothed using a 100 msec window to maximize temporal resolution.

Supplemental Figure 6



Supplemental Figure 6, related to Figure 4: BOSS elements in wild type Cold Cells. Transmission electron micrographs. **A,** EM section through *wild type* arista sensilla near sensillum's distal tip. (The same micrograph as presented in Figure 4, panel B). Hot Cell, red shading. Cold Cell, blue shading. Scale bar: 10 µm, **B.** Expanded view of the indicated region in panel A. Yellow arrowheads denote positions of BOSS elements.