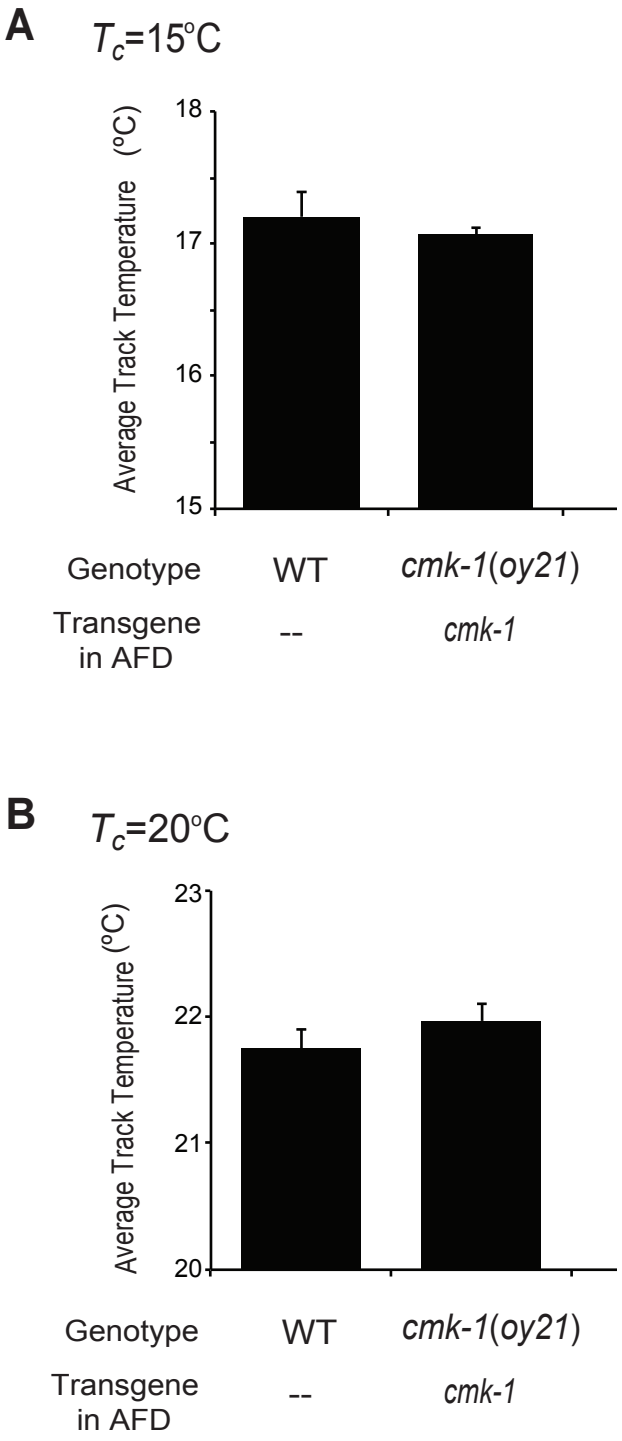
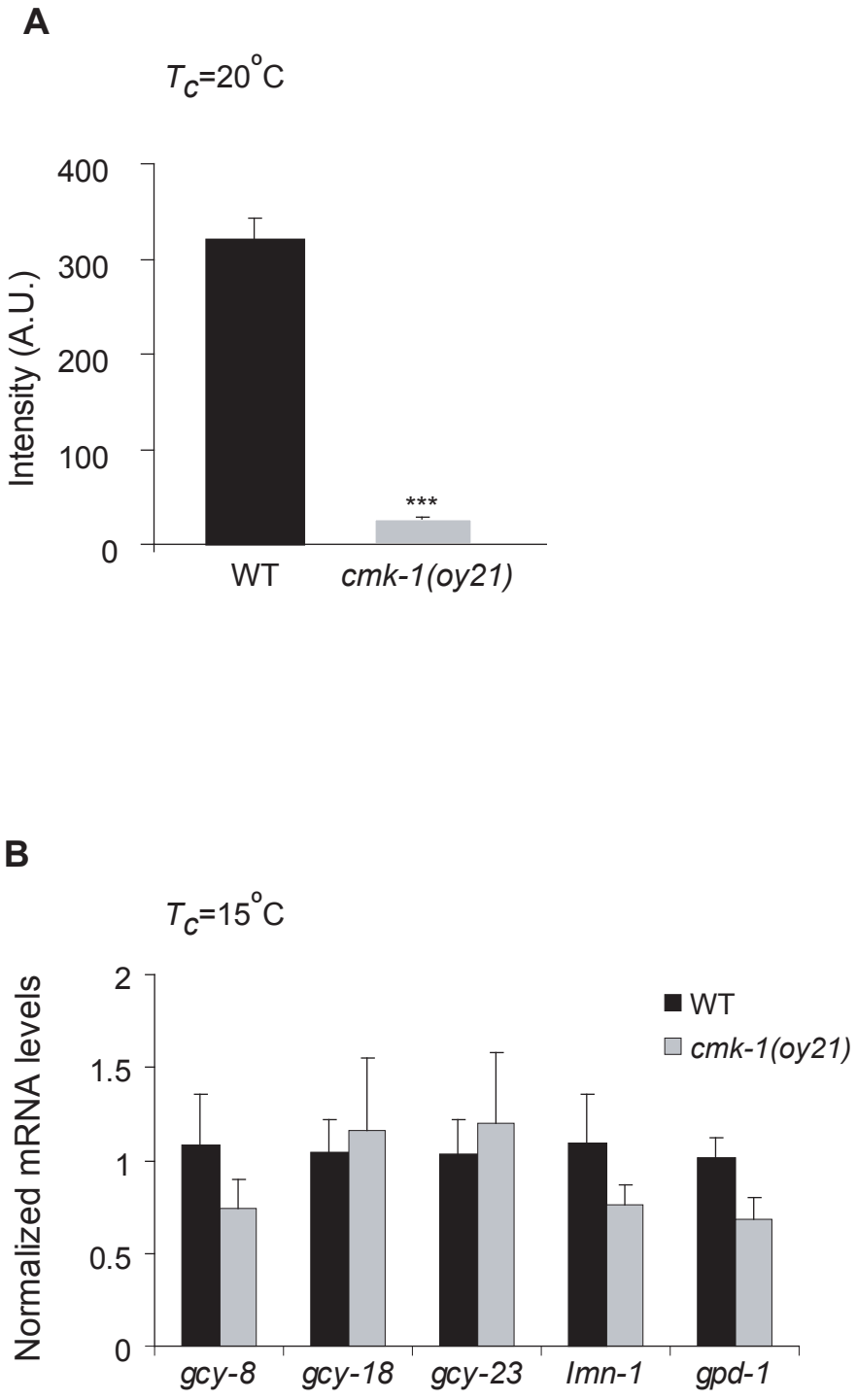
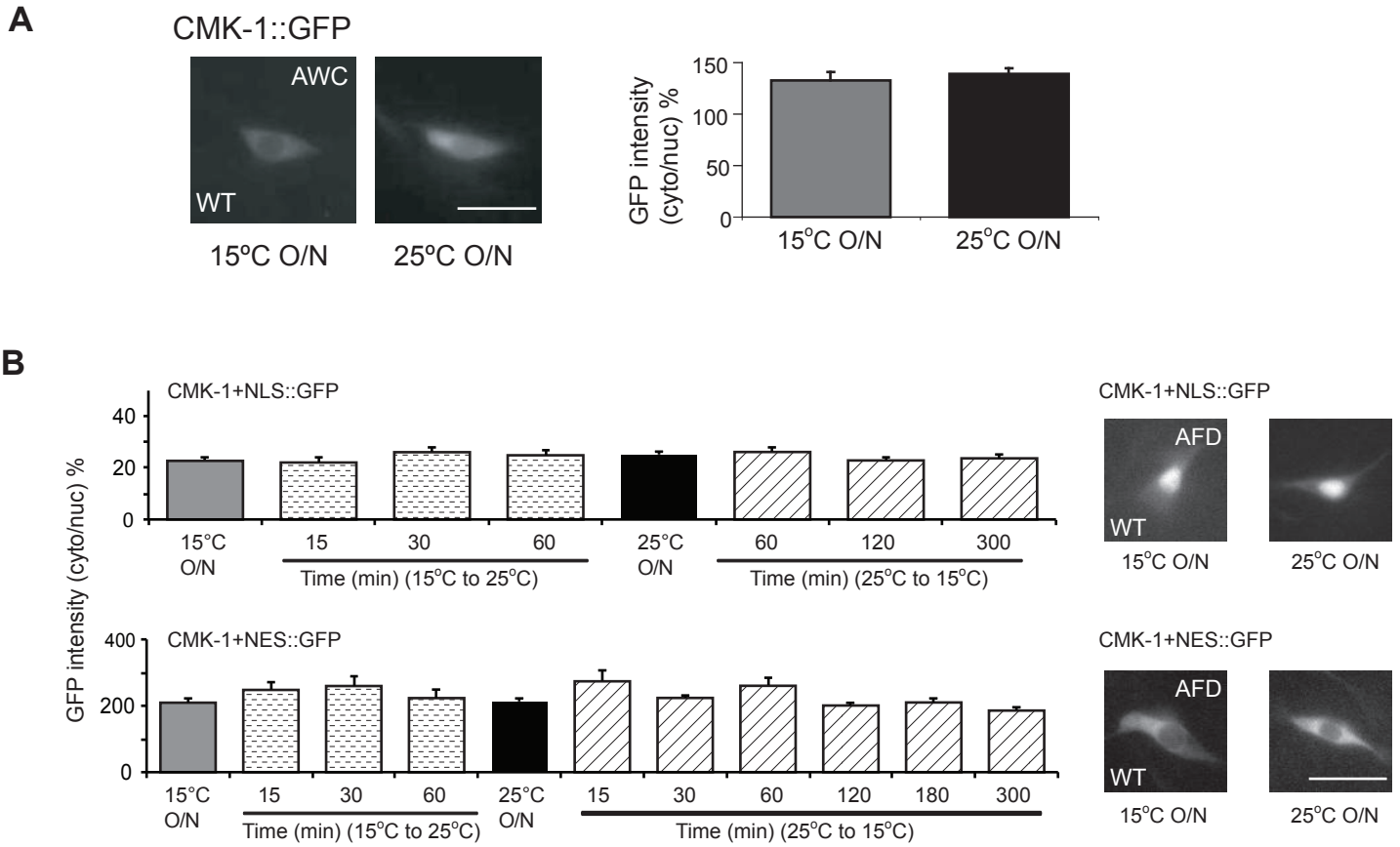


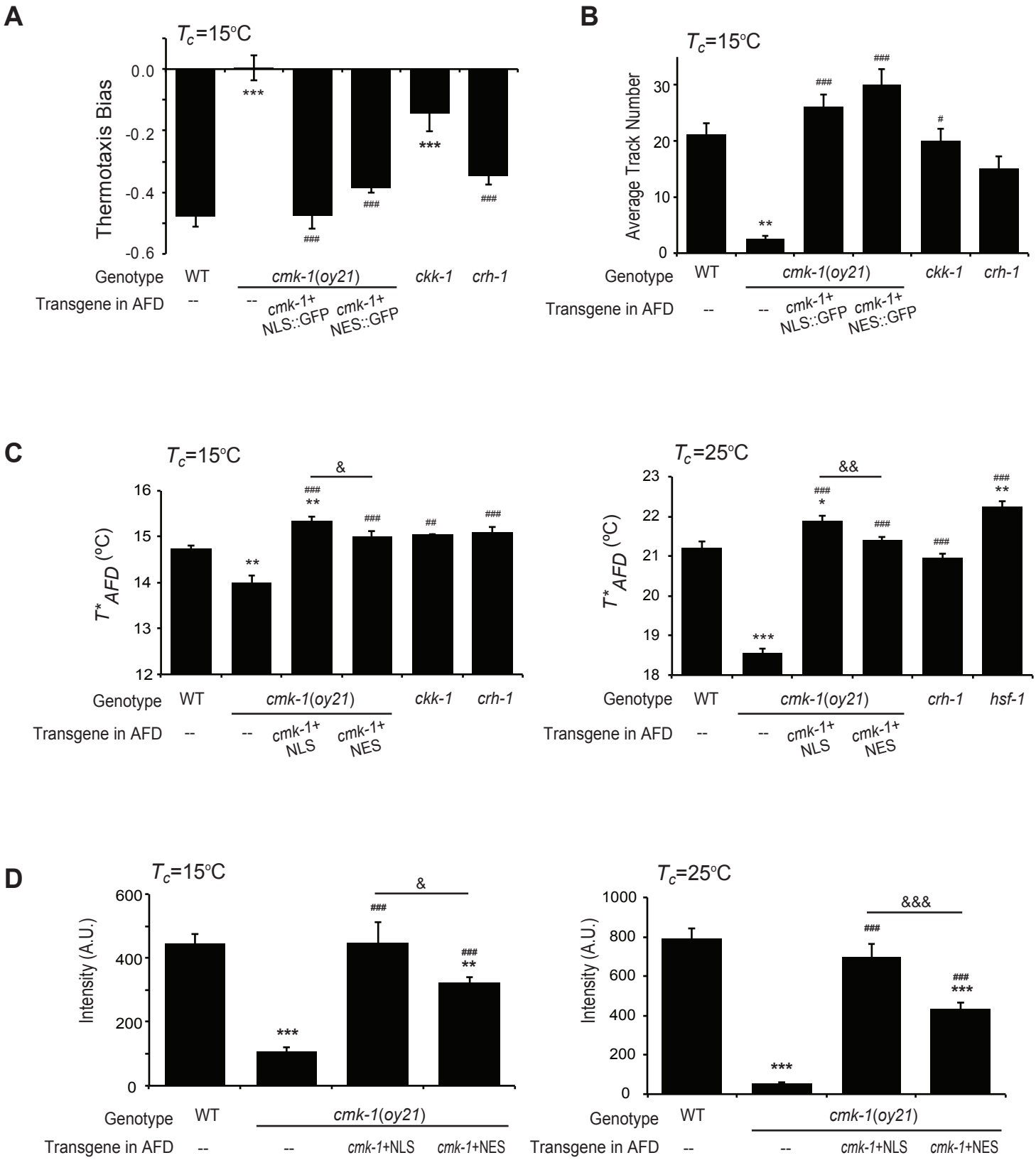
Yu, Bell et al, Fig. S1, related to Fig. 1



Yu, Bell et al, Fig. S2, related to Fig. 3



Yu, Bell et al, Fig. S3, related to Fig. 4



Yu, Bell et al, Fig. S4, related to Fig. 4

SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIGURES

Figure S1, related to Figure 1. Temperature range of IT behavior.

Average temperature at which IT behavior is exhibited by animals of the indicated genetic backgrounds grown at 15ºC (**A**) or 20ºC (**B**). Data are from Figure 1. *cmk-1(oy21)* mutants exhibit few tracks and thus are not included in this analysis. Error bars are SEM.

Figure S2, related to Figure 3. Expression levels of *gcy-8*, *gcy-18* and *gcy-23* in wild-type and *cmk-1(oy21)* mutants.

A. GFP fluorescence levels in AFD of a stably integrated *gcy-8*p*::gfp* reporter gene in animals grown overnight at 20ºC in wild-type and *cmk-1(oy21)* mutants. Fluorescence levels in AFD were normalized as described in the legend to Figure 3. Bars (± SEM) are the average of n>30 neurons for each condition. *** indicates different from corresponding wild-type values at $P<0.001$.

B. Fold-change in mRNA levels of the indicated genes in animals grown at 15ºC in the indicated genetic backgrounds. Gene expression was normalized as indicated in the legend to Figure 3. Shown are the averages of data from 3-4 independent biological replicates each analyzed in triplicate.

Figure S3, related to Figure 4. Nucleocytoplasmic shuttling of CMK-1::GFP in AFD is required for CMK-1 functions.

A. Representative images (left) and quantification (right) of fluorescence intensity of CMK-1::GFP localization in the AWC sensory neurons in wild-type animals grown at the indicated T_c. n>10 neurons at each temperature. Expression was driven under the *ceh-36Δ* promoter (Kim et al., 2010). Scale bar – 10 μm.

B. Quantification of fluorescence levels of the indicated proteins in the cytoplasm or nucleus of AFD upon temperature shift. Bars (± SEM) are the average of 2 independent assays of n>9 neurons. Representative images of localization of the indicated proteins in AFD at different grown temperatures are shown at right. Scale bar – 10 μm.

C. GFP fluorescence levels in AFD of a stably integrated *gcy-8*p*::gfp* reporter gene in animals grown overnight in wild-type and *ckk-1* mutants at the indicated temperatures. Data for T_c=25ºC are from Figure 4D. Fluorescence levels in AFD were normalized as indicated in the legend to Figure 3. Bars (± SEM) are the average of >14 neurons for each condition. * and *** indicates different from corresponding wild-type values at P<0.05 and 0.001, respectively (ANOVA and Bonferroni post-hoc corrections).

D. Representative images of CMK-1+NLS::GFP localization in AFD in *ckk-1(ok1033)* mutants at the indicated T_c. Scale bar – 10 μm.

E. Representative images of CMK-1(T179A)::DsRed localization in wild-type AFD at the indicated T_c. Scale bar – 10 μm.

F. T^*_{AFD} of the indicated strains upon overnight growth at 25ºC. Bars (± SEM) are the average of 8-10 neurons each. *** indicates different from wild-type at P<0.001 (ANOVA and Bonferroni post-hoc corrections).

Figure S4, related to Figure 4. CMK-1+NLS::GFP and CMK-1+NES::GFP rescue thermosensory phenotypes of *cmk-1* mutants.

A-C. CMK-1+NLS::GFP and CMK-1+NES:GFP can rescue the negative thermotaxis and IT behavioral defects (**A, B**) and T^*_{AFD} adaptation phenotypes (**C**) of *cmk-1(oy21)* mutants. Wild-type and *cmk-1(oy21)* values are repeated from Figures 1-3 for clarity; control and mutant strains were interleaved and examined together over independent assays. Also shown are behavioral and T^*_{AFD} phenotypes of of *ckk-1(ok1033), crh-1(tz2)* and *hsf-1(sy441)* mutants (**A-C**). *hsf-1(sy441)* results in temperature-sensitive phenotypes (Hajdu-Cronin et al., 2004); thus, responses were only examined at the restrictive temperature of 25ºC. Bars (± SEM) are the average of 10 independent assays of 15 animals each **(A, B)**, 8-10 neurons each **(C)**. ** and *** indicate different from corresponding wild-type values at P<0.01, and <0.001, respectively; $^{\#}$, $^{\#\#}$ and $^{\#\#\#}$ indicates different from corresponding *cmk-1(oy21)* values at P<0.05, <0.01 and <0.001, respectively (ANOVA and Bonferroni post-hoc corrections except for **B** which was analyzed by a Kruskal-Wallis nonparametric test followed by Bonferroni post-hoc corrections). $^{\&}$, $^{\&\&}$ and $^{\&\&\&}$ indicate different between the indicated values at P<0.05, 0.01 and 0.001, respectively (t-test).

D. CMK-1+NLS and CMK-1+NES also rescue the *gcy-8*p*::gfp* gene expression defects of *cmk-1(oy21)* mutants. Statistical values as indicated above. n=15-22 neurons each.

Table S1, related to Figure 2. Average T^*_{AFD} and amplitude in animals grown at different temperatures.

Strain	T_c	Average T^*_{AFD} (°C)	*P*-value[a]	Average amplitude[b] (% ΔR/R)	*P*-value[a]
Wildtype	15°C	14.7 ± 0.1	-	15.0 ± 1.8	-
	20°C	18.3 ± 0.1	-	31.5 ± 4.7	-
	25°C	21.2 ± 0.2	-	26.8 ± 4.1	-
cmk-1(oy21)	15°C	14.0 ± 0.4	<0.001	5.9 ± 0.8	<0.001
	20°C	17.2 ± 0.1	<0.001	12.9 ± 0.8	<0.01
	25°C	18.5 ± 0.1	<0.001	7.4 ± 0.7	<0.001

[a]*P*-values indicate different from wild-type values at the corresponding T_c.
[b]See Supplemental Experimental Procedures.
Errors are SEM. n > 10 neurons each.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

C. elegans strains

Worms were maintained using standard methods with *E.coli* OP50 as the food source. Strains used were *C. elegans* variety Bristol, strain N2 (wild-type), PY8980 *cmk-1(oy20)*, PY1589 *cmk-1(oy21)*, PY1157 [*oyIs17* (*gcy-8*p::*gfp*)], PS3551 *hsf-1(sy441)*, VC691 *ckk-1(ok1033)* and YT17 *crh-1(tz2)*. Transgenic animals were generated by injecting test plasmids and the *unc-122*p::*gfp* coinjection marker at 30-50 ng/µl each. Constructs used to assess rescue or subcellular localization were injected at 1 ng/µl.

Molecular biology

cmk-1 cDNAs with or without C-terminally tagged GFP sequences were expressed specifically in AFD and AWC under the *ttx-1* (Satterlee et al., 2001) and *ceh-36*Δ (Kim et al., 2010) regulatory sequences, respectively. CMK-1+NLS and CMK-1+NES constructs were generated with or without C-terminal tagged GFP sequences by inserting one copy each of the NES and NLS sequences from HIV Rev (Solinger et al., 2010) or EGL-13 (Lyssenko et al., 2007), respectively. To generate *srtx-1*p::YC3.60, 1.2 kb of *srtx-1* upstream regulatory sequences were inserted upstream of YC3.60 coding sequences in a *C. elegans* expression vector. Although the *srtx-1* promoter was reported to drive expression in AFD and AWC (Colosimo et al., 2004), we observed YC3.60 expression almost exclusively in AFD.

Quantitative real-time PCR

To collect RNA, a ~50 µl pellet containing growth synchronized young adult worms was collected and washed in 1.5 ml tubes. Total RNA was extracted from each sample using 0.8 ml of TRIzol reagent (Invitrogen) and treated with DNaseI. Two µg of total RNA was reverse transcribed into cDNA using Superscript III First Strand Synthesis System (Invitrogen) according to the manufacturer's manual. Quantitative PCR amplification was performed using the Qiagen Rotor-Gene Sybr Green PCR master-mix and the Rotor-Gene 6000 real-time PCR detection system. The expression level of each gene was calculated using the comparative C_T method and normalized to *act-1* actin RNA levels at each temperature. Each data point is the average of ≥3 biologically independent samples, each performed in triplicate. Primer sequences are available on request.

Quantification of GFP fluorescence

To quantify *gcy-8*p*::gfp* intensity in the AFD neurons, transgenic animals were transferred to a 2% agarose pad on a glass slide and immobilized with 5 µl of 10mM levamisole for 1-2 min. Images of AFD neurons were captured with a 40X oil objective (NA 1.30) using MetaMorph software (Molecular Devices) and a digital camera (Hamamatsu). Exposure time was adjusted to ensure that image intensity was in the linear range, and kept constant for all examined worms in an experiment. Fluorescence levels in AFD were normalized to fluorescence levels of GFP expressed in the PVQ non-thermosensory neuron type under the *sra-6* promoter in animals grown under identical conditions (Troemel et al., 1995). Captured images were analyzed using custom written scripts in MATLAB (The Mathworks). GFP intensity was corrected by subtracting background intensity in all cases.

To characterize the subcellular localization of CMK-1::GFP, transgenic animals grown or shifted to specific temperatures were transferred to a 10% agarose pad on a glass slide and immobilized with 6 μl of 0.1M sodium azide. Images of AFD or AWC neurons were captured on a spinning disk confocal microscope using a 63X oil objective and an EMCCD camera (Photometrics QuantEM 512SC). For intensity quantification, images were acquired on an epifluorescent microscope as described above, and background intensity was subtracted from fluorescence levels measured in a box of defined dimensions drawn in the putative nuclear and cytoplasmic regions of the neuron. Fluorescence quantification was performed using ImageJ (NIH).

Thermotaxis behavioral assays

Worms were cultivated at the desired temperature with food overnight. Prior to the start of the assay, 15 young adult animals were transferred to a 6 cm unseeded NGM plate to clear bacterial food. Animals were then transferred to a 10 cm unseeded NGM plate which was in turn placed on an aluminum plate. The gradient on the aluminum plate was established and maintained by Peltier thermoelectric temperature controllers (Oven Industries). The temperature of the NGM plate was confirmed by measuring with a two-probe digital thermometer (Fluke Electronics). For negative thermotaxis assays performed with animals grown at T_c=15ºC, the gradient steepness and range was 0.5ºC/cm and 18-23ºC; animals were placed on the gradient at 20ºC at the start of the assay. For IT behavioral assays, gradient steepness and range was 1.0ºC/cm and 12-22ºC (T_c=15ºC) or 15-25ºC (T_c=20ºC).

Animals moved freely for 5 minutes, and were then recorded for the following 30 minutes at a rate of 1 Hz using a CCD camera (Panasonic WV-CP484) and LabView software (National Instruments). Animals were illuminated by a ring of red LED lights to ensure visible contrast between animals and the agar plate. Videos were analyzed using custom written scripts in LabView and MATLAB. Worms were treated as particles and centers of mass were determined by applying a threshold. Isothermal tracks were defined as persistent movements perpendicular to the temperature gradient (within ±6° of the perpendicular axis) that were longer than the average track lengths on isothermal plates (3.6 mm). For negative thermotaxis, runs longer than 6 sec and within 45–315° or 135–225° of the perpendicular axis were used to calculate bias.

***in vivo* calcium imaging**

To measure temperature-evoked calcium responses in AFD, animals expressing *YC3.60* in AFD under the *srtx-1* promoter were cultivated overnight at a specific temperature. Prior to initiation of imaging, individual animals were placed on a 10% agarose pad on a coverslip, and immobilized with 1.5 µl of a 1% (v/v) solution of 0.1 µm polystyrene beads (Polysciences) (Kim et al., 2013) and covered with a second glass coverslip. The coverslips containing the worm were transferred to a glass slide mounted onto a Peltier on the microscope stage pre-warmed to the desired starting temperature, with 5 µl of glycerol between the coverslip and the mounted slide to ensure thermal conductivity. Temperature was measured using a 15K thermistor (McShane Inc). The Peltier was controlled via temperature-regulated feedback using LabView to achieve the desired target temperature. Imaging was initiated within 3 min of removing animals from

the growth incubator. We measured responses to a rising linear temperature ramp with a superimposed sinusoidal oscillation of 0.04 Hz and 0.2ºC amplitude (Figure 2A-D); this stimulus allows accurate quantification of T^*_{AFD} over background noise (Clark et al., 2006). Temperature ramps were started at 13°C and 17°C for animals cultivated at 15°C and 25°C, respectively.

For temperature upshift and downshift experiments, ramps were started at different temperatures to ensure that all neurons were imaged for similar time periods. We ensured that animals were exposed to the new temperature for the correct period of time when shifting for periods <10 minutes by placing single animals on slides pre-warmed to the new temperature for the required time period prior to imaging intracellular calcium dynamics in response to a rising temperature ramp.

Images were captured using a 40X air objective (NA 0.9) using MetaMorph software (Molecular Devices), and a digital camera (Orca, Hamamatsu) at a rate of 1 Hz. The yellow and cyan emissions of cameleon were split onto a single image using a DualView DV2 emission splitter (Photometrics). Data were analyzed using custom written scripts in MATLAB. Raw data were smoothed with a local regression filter. T^*_{AFD} was identified by manual inspection and defined as the temperature at which the variation in the YFP/CFP ratio initially phase-locked to the sinusoidal variation in the stimulus. Response amplitude was defined as the average amplitude of the second, third and fourth peaks following the first peak after T^*_{AFD}. We confirmed that thermotaxis behaviors of the *srtx-1*p::YC3.60-expressing strain used in all imaging experiments were similar to those of wild-type animals.

To calculate dynamics of T^*_{AFD} adaptation to temperature upshift, data were fit with a double exponential equation using MATLAB:

$T_{adp}(t) = T_{initial} + A\ ((1-\exp(-t/\tau_1) + B\ (1-\exp(-t/\tau_2)))$, where $T_{initial}$, A, B, τ_1, and τ_2 are free parameters. A nested F test indicated that the use of a second exponential term in this equation provided a better fit than a single exponential ($P<0.001$) for temperature upshifts. A single exponential (nested F test, $P<0.5$) was sufficient to fit the data for corresponding to temperature downshifts.

REFERENCES

Clark, D.A., Biron, D., Sengupta, P., and Samuel, A.D.T. (2006). The AFD sensory neurons encode multiple functions underlying thermotactic behavior in *C. elegans*. J. Neurosci. *26*, 7444-7451.

Colosimo, M.E., Brown, A., Mukhopadhyay, S., Gabel, C., Lanjuin, A.E., Samuel, A.D., and Sengupta, P. (2004). Identification of thermosensory and olfactory neuron-specific genes via expression profiling of single neuron types. Curr. Biol. *14*, 2245-2251.

Hajdu-Cronin, Y.M., Chen, W.J., and Sternberg, P.W. (2004). The L-type cyclin CYL-1 and the heat-shock-factor HSF-1 are required for heat-shock-induced protein expression in *Caenorhabditis elegans*. Genetics *168*, 1937-1949.

Kim, K., Kim, R., and Sengupta, P. (2010). The HMX/NKX homeodomain protein MLS-2 specifies the identity of the AWC sensory neuron type via regulation of the *ceh-36 Otx* gene in *C. elegans*. Development *137*, 963-974.

Kim, E., Sun, L., Gabel. C.V., and Fang-Yen, C. (2013). Long-term imaging of *Caenorhabditis elegans* using nanoparticle-mediated immobilization. PLoS ONE *8*, e53419,

Lyssenko, N.N., Hanna-Rose, W., and Schlegel, R.A. (2007). Cognate putative nuclear localization signal effects strong nuclear localization of a GFP reporter and facilitates gene expression studies in *Caenorhabditis elegans*. Biotechniques *43*, 596, 598, 560.

Satterlee, J.S., Sasakura, H., Kuhara, A., Berkeley, M., Mori, I., and Sengupta, P. (2001). Specification of thermosensory neuron fate in *C. elegans* requires *ttx-1*, a homolog of *otd/Otx*. Neuron *31*, 943-956.

Solinger, J.A., Paolinelli, R., Kloss, H., Scorza, F.B., Marchesi, S., Sauder, U., Mitsushima, D., Capuani, F., Sturzenbaum, S.R., and Cassata, G. (2010). The *Caenorhabditis elegans* Elongator complex regulates neuronal alpha-tubulin acetylation. PLoS Genet. *6*, e1000820.

Troemel, E.R., Chou, J.H., Dwyer, N.D., Colbert, H.A., and Bargmann, C.I. (1995). Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. Cell *83*, 207-218.