Supplementary Figure Legends

Supplementary Figure 1. Strategies, strains and constructs related to Figure 1. A. Schematic of thermotaxis assays and behavior. (Left) Schematic of the behavioral choice assays used in most studies, in which animals are placed at the middle of the gradient, and allowed to migrate towards their preferred temperature region (shaded grey), where they perform isothermal tracking. (Right) Schematic of the assay using in this study, in which animal start sites (circles) were placed in an "H" configuration and trained to prefer 20C (shaded area in middle of assay), as to better capture behaviors of animals performing gradient migration up or down the gradient as they transition into isothermal tracking. **B.** Schematic and sequence information for the *inx-1(ola375)* allele isolated in this study from forward genetic screens. **C.** Schematic of the substractive labeling strategy to identify the INX-1 site of action. A promoter fragment of 2.5kb can drive expression of *inx-1* cDNA and rescue the observed thermotaxis defects for the *inx-1* mutants, while a promoter fragment of 1.5 kb is insufficient to do so. By creating transcriptional fusions of both promoter fragments, we identified candidate neurons which are uniquely labeled by the rescuing promoter fragment. **D.** Schematic of part of the thermotaxis circuit, highlighting the AIY interneuron position as the primary interneurons downstream of the thermosensory neuron AFD. **E.** Schematic of *inx-1(ola278)* a floxed allele engineered for conditional knockdowns of the *inx-1* gene.

Supplementary Figure 2. Examination of AIY coupling by INX-1, related to Figure 3. GCaMP6 signal strength over time in clamped AIY (AIY_c) and unclamped AIY (AIY_{uc}) of wild type (A and C) and the *inx-1* mutants (B and D). Shown here are results of individual animals normalized by the peak fluorescent signal of AIY_C. C and D are the same as Figure 3 C and D, and represent the cumulative results of the individual animals.

Supplementary Figure 3. Thermotaxis modeling parameterization and Pearson coefficient firing between AIY pairs. A. Data from a freely moving animal during a straight run, displaying the position of the nose tip (dots) and fit with a sinusoidal curve. **B.** Histogram of the speeds of runs of animals trained at 25C and placed at 20C, and moving up the gradient towards their preferred temperature, with a lognormal fit (in red). **C.** Pearson's coefficient in AIY pairs between wild-type animals (n = 8) and *inx-1(tm3524)* (n = 7). Values are shown as mean ± SE. the asterisks *** denote $p < 0.0005$ from two-tailed Mann-Whitney test.

Supplemental Movies.

Movie 1. Calcium responses of AIYs (ventral view) in wild type animals stimulated by simulated isotherm (+/- 0.01°C oscillations surrounding Tc, 20°C, related for Figure 4D)

Movie 2. As Movie 1, but in *inx-1(tm3524)* mutants.

Supplemental Strain Table.

Strain	Genotype	Source
N2	Bristol wild-type strain	CGC
CB4856	Hawaiian wild-type strain	CGC
DCR3056	*olaIs17 [Pmod-1::GCaMP6s (25ng/ul), Pttx-3::mCherry (25ng/ul), Punc-122::dsRed (40ng/ul)] I*	(57)
DCR3542	*pkc-1(nj1) V; inx-1(ola375) X*	This study
DCR3682	*inx-1(tm3524) X; olaEx2136[Pinx-1(2.5kb)::INX-1gene::SL2::GFP (10 ng/ul), Punc-122::RFP (35 ng/ul)]*	This study

DCR4116	*olaEx2390[Pinx-1(2.5kb)::GFP, Pinx-1(1.5kb)::mCherry, Punc-122::GFP]*	This study
DCR4466	*olaIs17 [Pmod-1::GCaMP6s (25ng/ul), Pttx-3::mCherry (25ng/ul), Punc-122::dsRed (40ng/ul)] I; inx-1(tm3524) X*	This study
DCR4708	*inx-1(ola278) X*	This study
DCR4984	*inx-1(ola278) X; olaEx2943[Pinx-1(2.5kb)::nCRE (25 ng/ul), Punc-122::RFP]*	This study
DCR4990	*inx-1(ola278) X; olaEx2949[Pinx-1(1kb)::nCRE (25 ng/ul), Punc-122::RFP]*	This study
DCR4995	*tmIs777[Prgef-1::cre, Punc-119::venus]; inx-1(ola278) X*	This study
DCR5027	*inx-1(ola278) X; olaEx2976[Pcex-1::nCRE (25 ng/ul), Punc-122::RFP]*	This study
DCR5030	*inx-1(ola278) X; olaEx2979[Podr-2b3a::nCRE (25 ng/ul), Punc-122::RFP]*	This study
DCR5035	*inx-1(ola278) X; olaex2984[Pttx-3::SL2::nCRE (25ng/ml), Punc122:RFP] #1*	This study
DCR5036	*inx-1(ola278) X; olaex2985[Pttx-3::SL2::nCRE (25ng/ml), Punc122:RFP] #2*	This study
DCR5043	*tmIs1091[Pttx-3::nCRE, Plin-44::GFP]; inx-1(ola278) X*	This study
DCR5080	*olaIs17 [Pmod-1::GCaMP6s (25ng/ul), Pttx-3::mCherry (25ng/ul), Punc-122::dsRed (40ng/ul)] I; inx-1(ola278) X*	This study
DCR5087	*olaIs17 [Pmod-1::GCaMP6s (25ng/ul), Pttx-3::mCherry (25ng/ul), Punc-122::dsRed (40ng/ul)] I; inx-1(ola278) X; olaEx3023[Pinx-1(2.5kb)::nCRE (25 ng/ul), Pmyo-3::RFP]*	This study
DCR5108	*inx-1(ola278) X; olaEx3041[Pceh-16::nCRE, Punc-122::RFP]*	This study
DCR5121	*olaIs17 [Pmod-1::GCaMP6s (25ng/ul), Pttx-3::mCherry (25ng/ul), Punc-122::dsRed (40ng/ul)] I; tmIs1091[Pttx-3::nCRE, Plin-44::GFP]; inx-1(ola278) X*	This study
DCR5122	*olaIs17 [Pmod-1::GCaMP6s (25ng/ul), Pttx-3::mCherry (25ng/ul), Punc-122::dsRed (40ng/ul)] I; inx-1(ola278) X; olaEx3053[Pinx-1(2.5kb)::nCRE (25 ng/ul), Pmyo-3::RFP]*	This study
DCR5281	*inx-1(tm3524) X* outcrossed 6 times	This study
DCR5282	*inx-1(ola278) X* outcrossed 5 times	This study
DCR5283	*inx-1(gk580946) X* outcrossed 4 times	This study
DCR5438	*olaIs17 [Pmod-1::GCaMP6s (25ng/ul), Pttx-3::mCherry (25ng/ul), Punc-122::dsRed (40ng/ul)] I; inx-1(gk580946) X*	This study

DCR5790	*olaIs17 [Pmod-1::GCaMP6s (25ng/ul), Pttx-3::mCherry (25ng/ul), Punc-122::dsRed (40ng/ul)] I; olaIs72 [Pelt-7::mCherry (25ng/ul), Pttx-3::Cx36::mCherry (25ng/ul)]*	(57)
DCR7342	*inx-1(ola375) X* outcrossed 1 time	This study
DCR8285	*olaIs72 [Pelt-7::mCherry (25ng/ul), Pttx-3::Cx36::mCherry (25ng/ul)]; inx-1(tm3524) X*	This study
DCR8945	*olaIs17 [Pmod-1::GCaMP6s (25ng/ul), Pttx-3::mCherry (25ng/ul), Punc-122::dsRed (40ng/ul)] I; inx-1(tm3524) X; olaEx5354[Pttx-3::SL2::Cx36::mCherry (25ng/ul), Pelt-7::mCherry (25ng/ul)]*	This study
DCR8946	*olaIs17 [Pmod-1::GCaMP6s (25ng/ul), Pttx-3::mCherry (25ng/ul), Punc-122::dsRed (40ng/ul)] I; inx-1(tm3524) X; olaEx5355[Pttx-3::SL2::Cx36::mCherry (25ng/ul), Pelt-7::mCherry (25ng/ul)]*	This study
DCR8947	*inx-1(tm3524) X; olaEx5356[Pttx-3::SL2::Cx36::mCherry (25ng/ul), Pelt-7::mCherry (25ng/ul)]*	This study
FX03524	*inx-1(tm3524) X*	Shohei Mitani/ NBRP
FX14215	*tmIs777[Prgef-1::cre, Punc-119::venus]*	Shohei Mitani/ NBRP
FX16643	*tmIs1091[Pttx-3::nCRE, Plin-44::GFP]*	Shohei Mitani/ NBRP
IK105	*pkc-1(nj1) V*	CGC
VC40335	*inx-1(gk580946) X*	CGC

inx-1(ola278) **sequence (insertions in bold)**

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atcctagtcagtgtacggtactagaaggcatgaa

<u>Genotyping protocols</u>

<u>Genotyping the *inx-1(tm3524) X* allele:</u>

inx1_tm_F: GCCTGTCAGTTGCCAAATCT
inx1_tm_R1: GCAGTGTCCATGTTCCATTG
inx1_tm_R2: ATGTGTGTCCAGAAGCGATG

Anneal at 55ºC, elongate for 1 min at 72ºC. Run amplified products on a 2% agarose gel. Homozygous wild-type will produce 542bp & 159bp bands, homozygous *inx-1(tm3524) X* will produce a single 304bp band, and a heterozygous *inx-1(+/tm3524) X* will produce three bands at 542bp, 304bp & 159bp.

<u>Primer set for *inx-1* exome sequencing:</u>

ex1_inx1_F: CGGCACGGATGAAGTAGTTT
ex1_inx1_R: TTGGTTTCATCTCCCGTTTC
ex1_inx1 product: 576bp

ex2_inx1_F: AGACGGGAGACCTACGTTGT
ex2_inx1_R: CCGCAACTTAGCCGATCTTA
ex2_inx1 product: 982bp

ex3_inx1_F: AAACACGCAATTTTCTGCAA
ex3_inx1_R: CACACACGCACATCCATACA
ex3_inx1 product: 994bp

ex4_inx1_F: CTGCTGTCTGCTCGCTAATG
ex4_inx1_R: ACGTGGTCGGGTTAGATGAG
ex4_inx1 product: 250bp

Anneal at 55ºC, elongate for 1 min 15 seconds at 72ºC. Send resulting products for Sanger Sequencing performed by GENEWIZ (Azenta Life Sciences).