Supplemental Information for:

A Hypothalamic circuit that controls body temperature.

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Supplemental Materials and Methods

Surgeries

We delivered ~0.2 μl (unless specified) of AAV virus through a pulled-glass pipette and a pressure micro-injector (Nanoject II, #3-000-205A, Drummond). The injection coordinates were calculated according to Paxinos & Franklin mice brain coordinates (2nd edition) and the injection was performed using a small animal stereotaxic instrument (David Kopf Instruments, #PF-3983) under general anesthesia by isoflurane. A feedback heater was used to keep mice warm during surgeries. Mice were recovered in a warm bracket before there were transferred to housing cages for 2–4 weeks before performing behavioral evaluations. The fiber optic cables (200 μm in diameter, AniLab Co., China) were chronically implanted and secured with dental cement. For cholera toxin B subunit (CTb) injections, 150 nl of CTb conjugated with Alexa Fluor-488 dye (Invitrogen, #C22841) were sterotaxically injected to the DMD. Animals were sacrificed for histology 5 days after the injection.

Immunohistochemistry

Mice were transcardially perfused with PBS, followed by 4% PFA. Brains were then post-fixed overnight at 4 ^{o}C. Fixed brains were sectioned using a vibratome (Leica) to a thickness of 50 μm. Brain slices were incubated with primary antibodies for 12–24 h at 4 ^{o}C, followed by 3x washes in PBST (PBS with 0.1% Triton X-100, vol/vol) before incubation in secondary antibodies for 2 h at room temperature. Primary antibodies used were: rabbit anti-cFos (1:20, 000, Synaptic systems, #226003), chicken anti-GFP (1:1000, Abcam, #ab13970), rat anti-Tdt (1:1000, Chromotek, #5f8), rabbit anti- phospho-ribosomal protein S6 pSer244/pSer247 (1:1000, Invitrogen, #44-923). Antibodies were incubated in blocking buffer (PBS containing 2.5 % normal goat serum (vol/vol), 1.5 % Bovine serum albumin (weight/vol), 0.1% Triton™ X-100 (vol/vol) and 0.05% NaN_3 (weight/vol)). Secondary antibodies were Alexa Fluor-conjugated (Invitrogen). For pSTAT3 staining, slices were permeabilized for 10 min at –20°C in 100% methanol before immunostaining with rabbit anti-pSTAT3 (1:1000, Cell Signaling, #9145P). Images were captured on a Zeiss LSM710 confocal microscope or Olympus VS120 Virtual Microscopy Slide Scanning System.

Behavioral assays

Body temperature, oxygen consumption, and physical activity were monitored by Comprehensive Lab Animal Monitoring System with Temperature Telemetry Transmitter (Columbus, with G2 E-Mitter transponders). The counts of physical activity normalized to a 2-min interval per data point were shown in all figures. Temperature transponders were implanted into the peritoneal cavity 4–7 days before testing. Stimuli (light, drugs, or temperature) were delivered between 10 am and 3 pm in the dark phase. Mice were adapted in the metabolic chambers for 2 days before giving saline (volume (μl) = 10 x

body weight (grams)), CNO (ENZO, #BML-NS105-0025, i.p.,1.5 mg/kg body weight), or optogenetic stimulation (473 nm or 532 nm laser, AniLab). Stimulation protocol: for ChR2, bilateral or unilateral, light pulses for 2 s (10 mW, 20 Hz, 40%) followed by a 2-s break, with the sequence repeating for 30 min or 60 min as indicated; for hGtACR1, bilateral, light on for 30 s (473 nm, 10 mW except in Fig. 1*D* (6 mW)) followed by a 90-s break, with the sequence repeating for 30 min or 60 min as indicated.

Electrophysiological recordings

Adult mice were anesthetized with isoflurance, perfused transcardially with ice-cold oxygenated (95% O_2/5% CO_2) high-sucrose solution (2.5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM Na_2HPO_4, 2 mM $MgSO_4$, 213 mM sucrose, 26 mM $NaHCO_3$), and decapitated to remove the brain. After removal, the brain was immediately placed in ice-cold oxygenated high-sucrose solution and sectioned in coronal plane at 250 μm in this buffer using a vibratome (VT1200 S, Leica). After section, the slices was incubated in oxygenated aCSF (126 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 1.25 mM Na_2HPO_4, 2 mM $MgSO_4$, 10 mM Glucose, 26 mM $NaHCO_3$, 2 mM $CaCl_2$) at 34ºC for 1 h. The intracellular solution for action potential (spike) recordings contains 130 mM K-gluconate, 2 mM $MgSO_4$, 10 mM NaCl, 0.2 mM EGTA, 10 mM HEPES, 10 mM phosphocritine, 4 mM Tris-ATP and 0.5 mM Na_3-GTP (pH:7.3, 297 mOsm). The intracellular solution for IPSC recordings contains 125 mM CsCl, 5 mM NaCl, 0.6 mM EGTA, 10 mM HEPES, 4 mM Mg-ATP, 0.3 mM Na_3-GTP and 10 mM lidocaine hydrochloride (QX-314) (pH:7.3, 280 mOsm). For IPSC recordings, cells were clamped at 0 mV. 10 μM CNQX (Sigma) and 50 μM DL-AP5 (Sigma) were applied to the bath solution. To block IPSCs, 25 μM bicuculline (Tocris) was applied to the bath solution. All recordings were acquired using Multiclamp 700B amplifier and Digidata 1440A interface (Molecular Devices).

Fiber photometry

Following injection of a AAV5-Syn-Flex-GCaMP6f viral vector, an optical fiber (200 μm O.D., 0.37 numerical aperture, Thorlabs) was placed 200μm above the viral injection site. Post-surgery, mice were caged individually and allowed to recover for at least 2 weeks before any studies were performed. Fluorescence signals were acquired with a dual-channel fiber photometry system (Fscope, Biolinkoptics, China) equipped with a 488-nm excitation laser (OBIS, Coherent), 505-544 nm emission filter and a photomultiplier tube (R3896, Hamamatsu). The gain (voltage) on PMT was set to 600 V. The laser power at the tip of optical fiber was adjusted to as low as possible (~ 25 μW) to minimize bleaching. The analogue voltage signals fluorescent were low-pass (30 Hz) filtered and digitalized at 100 Hz, which were then recorded by Fscope software (Biolinkoptics). The temperature was monitored using T-type thermocouples and was recorded simultaneously with the fluorescent signals by Fscope software. An optical fiber (RJPSF2, Thorlabs) with integrated rotary joint preventing fiber damage from animal movement was used to guide the light between the fiber photometry system and the implanted optical fiber. The floor (15 x 15 cm) temperature for each individual mouse was controlled by a Peltier controller (#5R7-001, Oven Industry) with customized Labview code (National Instrument, ref. (1)).

We segmented the data based on behavioral events within individual trials. We derived the values of fluorescence change ($\Delta F/F$) by calculating $(F-F_0)/F_0$, where F_0 is the baseline fluorescence signal averaged over a 120-s-long control time window, which was typically set 0.5 s preceding the trigger events. Similar data processing has been described in (2).

Single cell Reverse Transcription PCR

Single cell RT-PCR was carried out using established procedures. First, a cDNA library of the transcriptome of individual cells after recording was generated using random hexamers and the SuperScript IV kit (Invitrogen, #18091050) according to the manufacturer's protocol, K-gluconate or CsCl inner solution was performed as negative control. Second, multiplex PCR was carried out with 2×Taq PCR MasterMix (Tiangen, #KT201-02) and primers for the 3 genes using the entire cDNA library from step 1 in a volume of 25 μl. Multiplex primers were designed to amplify exonic DNA sequences which spanned at least one exon-intron boundary. Multiplex PCR conditions were 60°C annealing temperature with 1 min elongation time using 40 cycles. Water control experiments were regularly performed to control for traces of contamination. Third, nested single gene PCR was carried out in a volume of 25 μl with a 1:50 dilution of the multiplex PCR reaction using the standard 2× Taq PCR MasterMix (Tiangen, #KT201-02). Nested primers were designed to amplify 100–400 bp DNA sequences within the multiplex PCR primer boundaries. Nested PCR conditions were 60°C annealing temperature with 30-s elongation time using 35 cycles. PCR products were visualized and documented using standard agarose gel electrophoresis with UV light. Primers were tested with dilutions of cDNA libraries from POA (3). The following primers are used.

genes	multiplex primer sequence	nested primer sequence
vgat (137 bp)	GTCACGACAAACCCAAGATCAC	GTCACGACAAACCCAAGATCAC
	GGCGAAGATGATGAGGAACAAC	GGCGAAGATGATGAGGAACAAC
vglut2 (891 bp,313 bp)	AGGGTTCGATGACGTTTCTGG	CTATTAGGAAACCCGTGGGCTG
	AGCGGATGCCGAAGGATATG	CTTCTTCTCCAGCACCCTGTA
gapdh (500 bp,138 bp)	ACCACAGTCCATGCCATCAC	GGTTGTCTCCTGCGACTTCA
	GGGTGGTCCAGGGTTTCTTA	TCCACCACCCTGTTGCTGTA

Note: The multiplex PCR and nested PCR of vgat shared the same primers with (4), while all the rest primers were self-designed via NCBI primer-blast.

PhosphoTRAP and mRNA sequencing

The procedure of ribosome immunoprecipitation using anti-pS6 antibody was similar as described in (5), except we used the same antibody (rabbit anti-S6(pSpS244/247), Invitrogen, #44-923) for both staining and immunoprecipitation. We did not use any blocking peptides during immunoprecipitation since this pS6 antibody recognized the last two phosphorylation sites at the C terminal, which has less background than the one used in (5). Briefly, we loaded Protein A Dynabeads (150 μL, Invitrogen, #10002D) with 4 μg of pS6 antibody (Invitrogen, #44-923) in Buffer A (10 mM HEPES [pH 7.4], 150 mM KCl, 5 mM $MgCl_2$, 1% NP40, 0.05% IgG-free BSA) at 4 ^oC. Beads were washed three times with Buffer A immediately before use.

Control (29 ^oC) or temperature challenged (38 ^oC) mice were sacrificed by cervical dislocation. The anterior hypothalamus in between Bregma = 0.5 and 0.0 was rapidly dissected in Buffer B on ice (1x HBSS, 4 mM $NaHCO_3$, 2.5 mM HEPES [pH 7.4], 35 mM Glucose, 100 μg/ml cycloheximide). Hypothalami were pooled (typically 6-8 per IP), transferred to a glass homogenizer, and resuspended in 1 ml of buffer C (10 mM HEPES [pH 7.4], 150 mM KCl, 5 mM $MgCl_2$,100 nM calyculin A, 2 mM DTT, 100 U/ml RNasin, 100 μg/ml cycloheximide, protease and phosphatase inhibitor cocktails). Samples were homogenized manually. Homogenates were transferred to a microcentrifuge tube and clarified at 2,000x g for 10 min at 4^oC. To 1.0 mL supernatant, add 100 μL of 10% NP40 and 100 μL of 1,2-diheptanoyl-sn-glycero-3-phosphocholine (DHPC, Avanti Polar Lipids: 100 mg/0.69 ml). Mix by inversion and incubate on ice for 5 min. This solution was mixed and then clarified at 17000x g for 20 min at 4 ^oC. The resulting high-speed supernatant

was transferred to a new tube. A 20 μl aliquot of this solution was removed, transferred to a new tube containing 350 μl buffer RLT (QIAGEN), and stored at -80°C for purification as input RNA. The remainder was used for immunoprecipitation. Immunoprecipitations were allowed to proceed 10 min at 4°C. The beads were then washed four times with buffer D (10 mM HEPES [pH 7.4], 350 mM KCl, 5 mM $MgCl_2$, 0.5 mM DTT, 1% NP40, 100 U/ml RNasin, and 100 μg/ml cycloheximide). After the final wash the RNA was eluted by addition of buffer RLT (350 μL) to the beads on ice, the beads removed by magnet, and the RNA purified using the RNeasy Micro Kit (QIAGEN). The RNA integrity number (RIN) was assessed using an Agilent 2100 bioanalyzer. Only samples with RIN > 7.5 were processed further.

For RNA-seq analysis, cDNA was prepared using the SMARTer Ultralow Input RNA for Illumina Sequencing Kit (Clontech, #634935). Paired-end libraries were synthesized by using the TruSeq™ RNA Sample Preparation Kit (Illumina, USA) following TruSeq™ RNA Sample Preparation Guide. Briefly, the poly-A containing mRNA molecules were purified using poly-T oligo-attached magnetic beads. Following purification, the mRNA was fragmented into small pieces using divalent cations under 94°C for 8 min. The cleaved RNA fragments were copied into first strand cDNA using reverse transcriptase and random primers. This was followed by second strand cDNA synthesis using DNA polymerase I and RNase H. These cDNA fragments then went through an end repair process, the addition of a single ‘A’ base, and then ligation of the adapters. The products were then purified and enriched with PCR to create the final cDNA library. Purified libraries were quantified by Qubit® 2.0 Fluorometer (Life Technologies, USA) and validated by Agilent 2100 bioanalyzer (Agilent Technologies, USA) to confirm the insert size and calculate the mole concentration. Cluster was generated by cBot with the library diluted to 10 pM and then was sequenced on the Illumina HiSeq 2500 (Illumina, USA). The library

construction and sequencing was performed by Shanghai Biotechnology Corporation (China).

Data analysis

The RNA quantification data after sequencing was analyzed in two steps. First the RNA in the IP for each gene was divided by the input, to determine the fold-enrichment (IP/Input). This analysis was performed separately for 38 ^{o}C and 29 ^{o}C groups. To calculate the differential enrichment, the fold-enrichment values for 38 ^{o}C group were then divided by the fold-enrichment values for the 29 ^{o}C group from the same day. The top candidate genes were then selected for further quantitative PCR analysis and validated by RNA in situ with heat-induced cFos staining.

RNA Fluorescent in situ hybridization (FISH)

RNA probes against Vgat, Vglut2, GAD67 and BDNF were constructed according to the description on Allen Brain Atlas (http://www.brain-map.org). The primers used for probe cloning were as follows:

Vgat (Forward, GCCATTCAGGGCATGTTC; reverse, AGCAGCGTGAAGACCACC),

Vglut2 (Forward, CCAAATCTTACGGTGCTACCTC, reverse, AGCCATCTTTCCTGTTCCACT), GAD67 (Forward, TGTGCCCAAACTGGTCCT; reverse, TGGCCGATGATTCTGGTT), BDNF (Forward, CGACGACATCACTGGCTG; reverse, CGGCAACAAACCACAACA).

All probes were cloned from mouse brain cDNA and ligated into pGM-T vector (TianGen, #VT202). The RNA probes labeled by digoxygenin-UTP (Roche, #112770739) or Fluorescein-UTP (Roche, #11685619910) were generated by in vitro transcription (T7 RNA Polymerase, Promega, #P2077). For FISH, mice were transcardially perfused with

DEPC-PBS, followed by 4% PFA. Brains were then post-fixed overnight at 4 °C and vibratome sectioned. 40 μm free-floating sections were washed sequentially with PBS and PBS containing 0.1% Tween 20 (0.1% PBSTw) for 10 min and quenched with 0.5% H_2O_2 (vol/vol) in PBS for 30min followed by wash with 0.1% PBSTw for 10 min. Then they were premeabilized with 0.5% Triton X-100 in 2 × SSC for 20 min, followed by another round of wash with 0.1% PBSTw for 10 min. Slices were acetylated by 0.25% acetic anhydride (vol/vol) in 0.1 M triethanolamine (pH 8.0) for 10 min, followed by two rounds of washing with PBS for 10min. Afterward, sections were incubated in pre-hybridization solution (50% formamide (vol/vol), 5× SSC, 0.1% Tween 20 (vol/vol), 0.1% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS, wt/vol) and 5 mM EDTA) for 2 hours in 60 °C water bath, and then incubated in the hybridization solution (pre-hybridization solution plus 0.3 mg/ml yeast tRNA, 100 mg/ml heparin, 1× Denhardt's) containing 0.5 μg ml^{-1} RNA probes for 16~20 h at 60 °C water bath. After hybridization, the sections were sequentially washed with pre-hybridization solution for 30 min and 1:1 pre-hybridization solution / 1% TBST (500 mM NaCl, 20 mM Tris (pH 7.5) and 1% Tween 20 (vol/vol)) for 30 min at 60 °C. After that, the sections were rinsed sequentially with 1% TBST, 1:1 TBST / TAE (40 mM Tris-Acetate, 1 mM EDTA) and TAE at room temperature. Unbind RNAs were removed using electrophoresis (I = 80 mA) for 2 hours in TAE buffer followed by twice washes in 1% TBST. When immunostaining was combined with FISH, the sections were incubated with Anti-digoxigenin, AP-conjungate (1:500, Roche, #11093274910) and cFos primary antibody (1:5,000, Synaptic System, #226003) in Blocking buffer (0.5% Blocking reagent (Roche, #11096176001), 0.1 M Tris, 0.15 M NaCl) for 12h~24h at 4°C. Afterward, the sections were washed thrice with AGT buffer (1× PBS, 2 mM $MgCl_2$, 0.1% Triton X-100, 0.5% Goat serum), and incubated with AGT buffer containing Alexa Fluor® 488 (1:500, Invitrogen, #A-11008) for detection of cFos protein signal. Finally, the RNA signal was detected by alkaline phosphatase activity using SIGMAFAST™ Fast Red

Tablets (Sigma, #F4648). When carrying out double RNA fluorescent in situ hybridization, BDNF probe was detected by AP system as described above, and Vgat or Vglut2 probes were detected as follows: after electrophoresis, sections were washed by TBST twice, then incubated with Anti-Fluorescein-HRP (1:2000, PerkinElmer, # NEF710) for 2 h at room temperature. Afterward, they were washed three times with TBST for 10 min. The fluorescein signal was amplified by TSA Plus DNP (HRP) System (PerkinElmer). Finally, the deposited fluorophores were visualized by anti-DNP, Alexa 488 conjugated (Invitrogen, #A-11097).

Supplemental References:

1. Luo J, Shen WL, & Montell C (2017) TRPA1 mediates sensation of the rate of temperature change in Drosophila larvae. *Nat Neurosci* 20(1):34-41.
2. Li Y*, et al.* (2016) Serotonin neurons in the dorsal raphe nucleus encode reward signals. *Nature communications* 7:10503-10517.
3. Pfeffer CK, Xue M, He M, Huang ZJ, & Scanziani M (2013) Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. *Nature neuroscience* 16(8):1068-1076.
4. Padilla SL*, et al.* (2016) Agouti-related peptide neural circuits mediate adaptive behaviors in the starved state. *Nat Neurosci* 19(5):734-741.
5. Knight ZA*, et al.* (2012) Molecular profiling of activated neurons by phosphorylated ribosome capture. *Cell* 151(5):1126-1137.

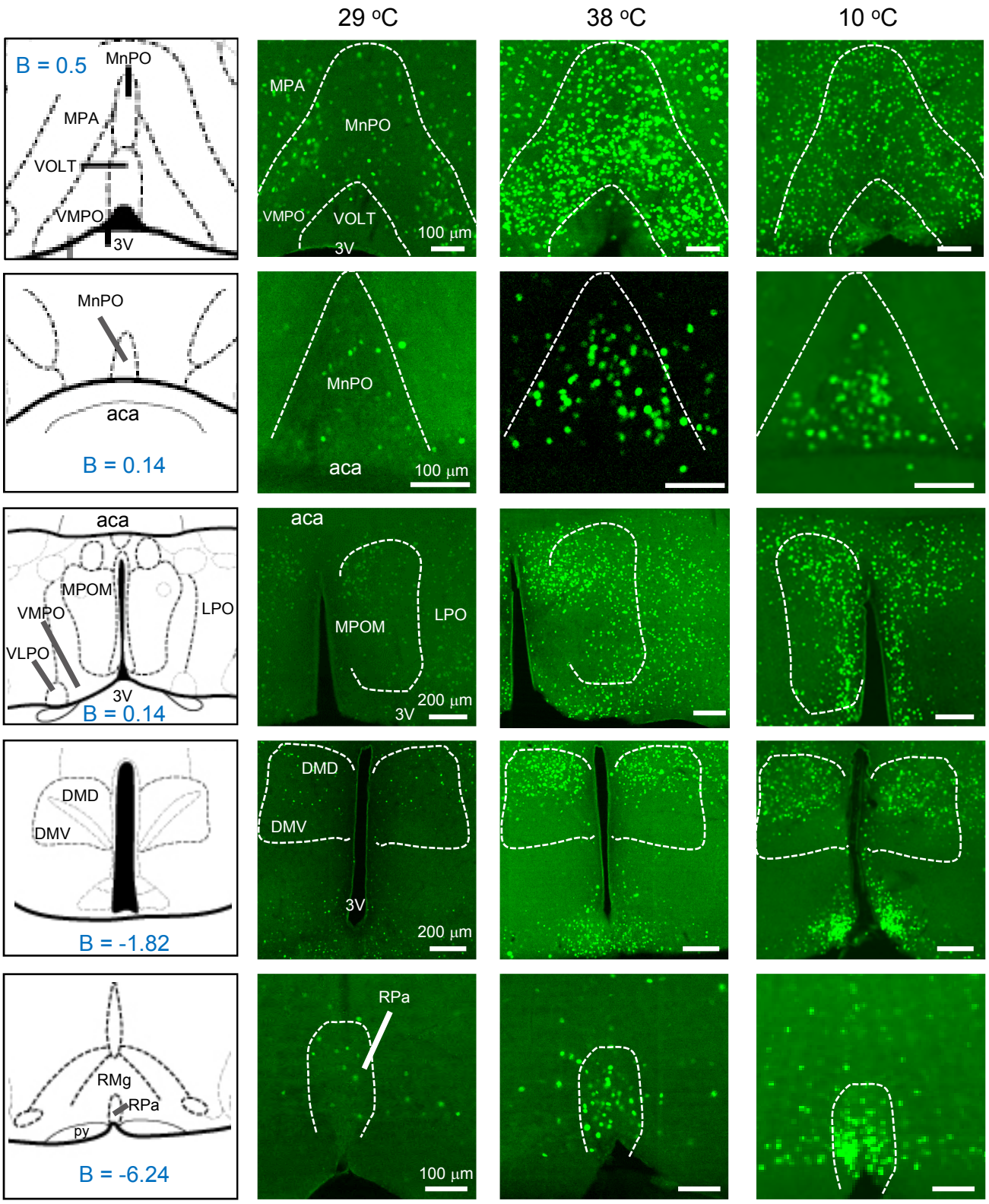

Figure S1. Brain areas activated by thermal stimuli.

Thermal neutral temperature (29 ºC) was used as the control. cFos (in green) staining were seen in multiple areas after 2 h at indicated temperature. Brain coordinates and nomenclatures were according to *Mouse Brain in Stereotaxic Coordinates* (by Paxinos & Franklin, 2nd edition, 2001). Scale bars are indicated in the figure. B, Bregma; MPA, medial preoptic area; MnPO, median preoptic nucleus; VOLT, vascular organ of the lamina terminalis; VMPO, ventromedial preoptic nucleus; 3V, third ventricle; aca, anterior commissure, anterior part; MPOM, medial preoptic nucleus, medial part; LPO, lateral preoptic area; VLPO, ventrolateral preoptic nucleus; DMD, dorsomedial hypothalamic nucleus, dorsal Part; DMV, dorsomedial hypothalamic nucleus, ventral part; Rmg, raphe magnus nucleus; Rpa, raphe pallidus nucleus; py, pyramidal tract.

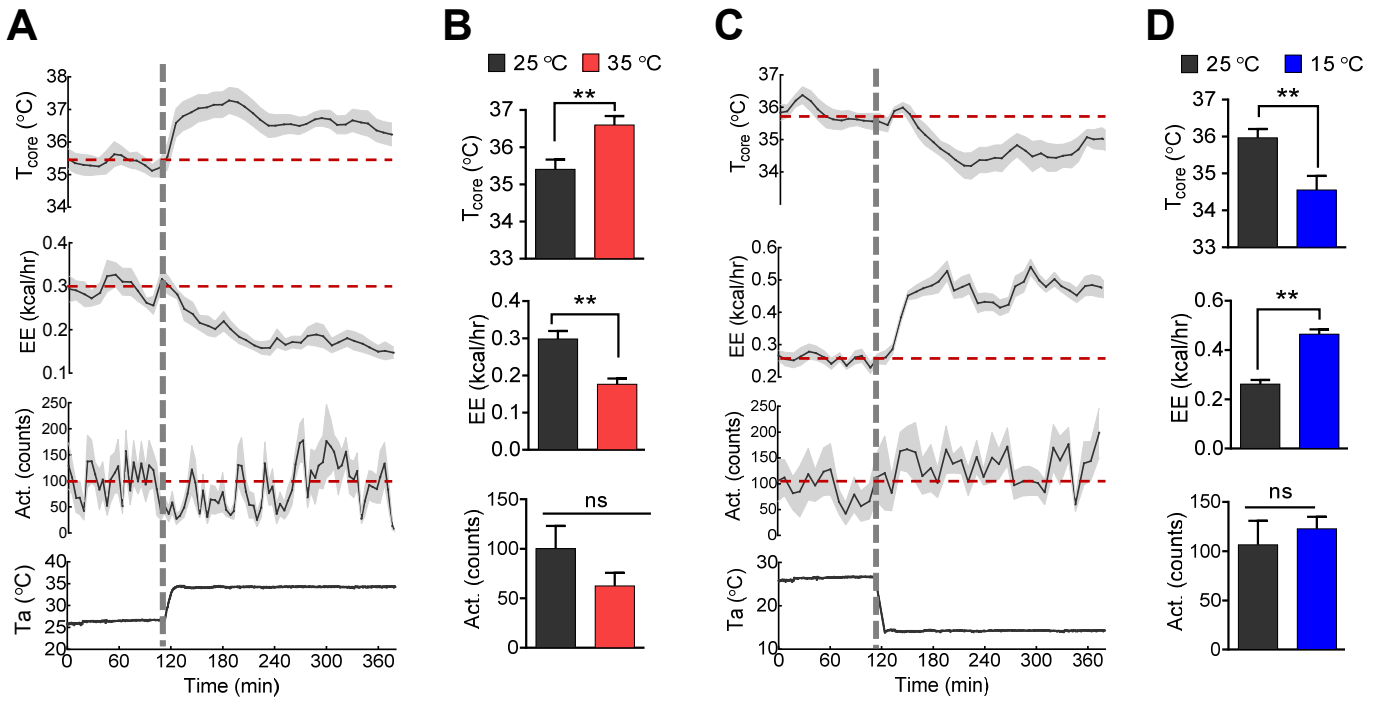

Figure S2. T_{core}, EE and activity changes under different environmental temperature. **A,** Changes of T_{core}, EE and activity over time under high ambient temperature (Ta, 25 ➝ 35 °C). (n = 8 mice). **B,** The average of T_{core}, EE and activity at the indicated Ta during 1h interval. **C,** Changes of T_{core}, EE and activity over time under cold ambient temperature (Ta, 25 ➝ 15 °C). (n = 8 mice). **D,** The average of T_{core}, EE and activity at the indicated Ta during 1 h interval. The time intervals used to calculate data shown in (**B**) and (**D**) are: 25 °C, 0 – 60 min; 35 °C, 240 – 300 min; 15 °C, 240 – 300 min). All data are plotted as mean ± SEM. The p-values compared to control group (25 °C) are calculated using two-side unpaired t tests. *p ≤ 0.05; **, p ≤ 0.01; ns, not significant.

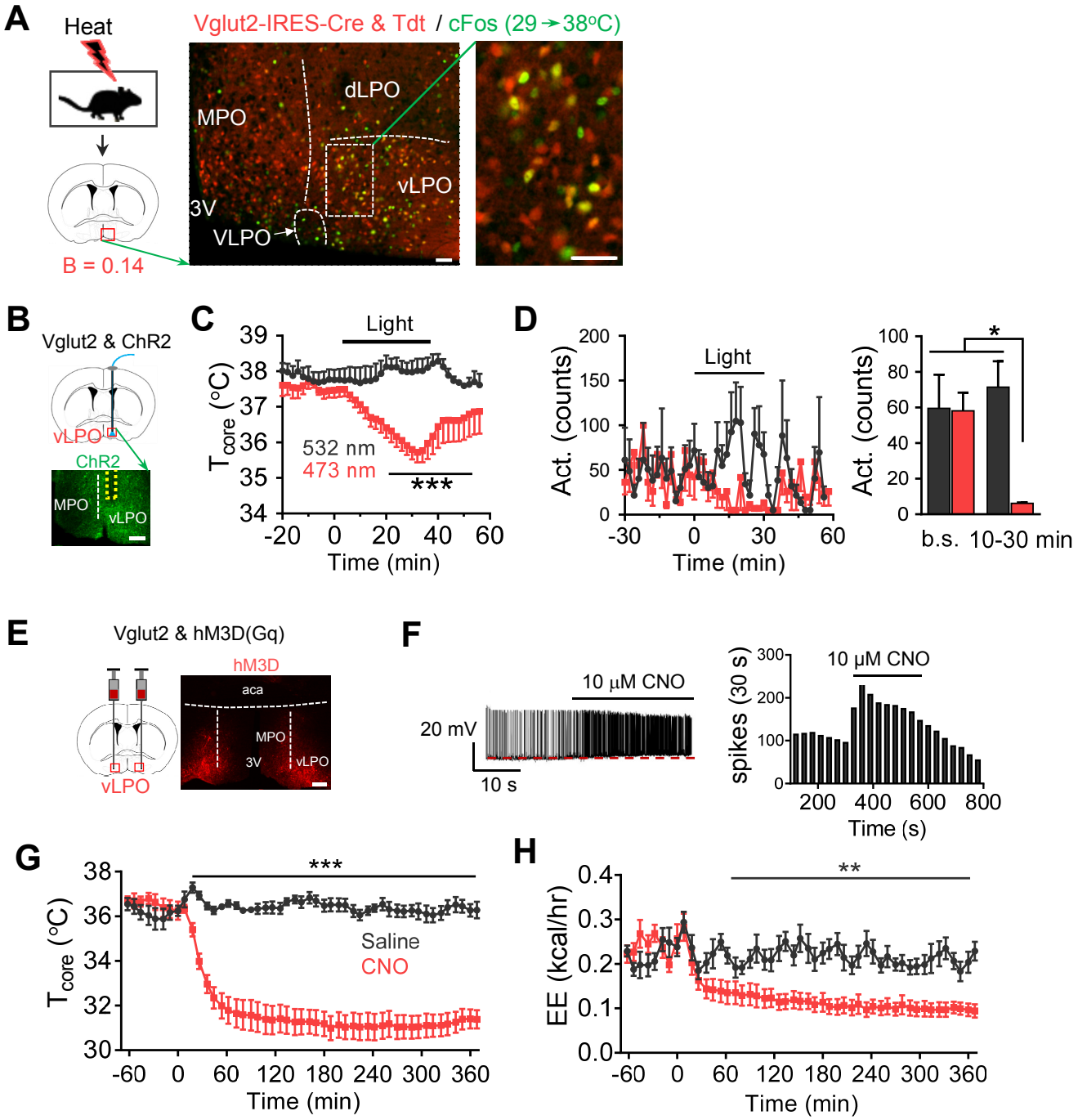

Figure S3. Activation of vLPO glutamatergic neurons reduced body temperature.
A, Heat (38 °C, 2h) induced cFos co-localized with glutamatergic markers (Vglut2-IRES-Cre, lox-*tdTomato*) in the vLPO. No. of cFos+ & Vglut2+ / No. of cFos+ = 37.3 ± 2.7 %. (n = 3). **B,** Scheme of optogenetic stimulation and viral expression of ChR2 in vLPO^{Vglut2} neurons. **C-D,** Optogenetic activation of vLPO glutamatergic (Vglut2-IRES-Cre+) neurons by blue lights reduced T_{core} (**C**) and activity (**D**) (n = 4). Unilateral illumination, light pulses for 2 s (488 nm or 532 nm laser, 10 mW, 20 Hz, 40%) followed by a 2-s break, with the sequence repeating for 30 min. For activity (Act.), baselines (b.s.) represents the average of counts in between t = (-30 – -10 min). (10 – 30 min) represents the average of counts in between t = (10 – 30 min). **E,** Scheme of viral injection and expression of hM3D(Gq) in vLPO^{Vglut2} neurons. **F,** Representative trace in the right shows bath application of CNO induced an increase in spontaneous firing rates in vLPO$^{Vglut2\ \&\ hM3D}$ neurons. The quantification of spike numbers during 30 s period is shown in the right. **G-H,** Stimulation of vLPO^{Vglut2} neurons by CNO (1.5 mg/kg body weight, i.p. at t = 0) strongly reduced T_{core} (**F**) and EE (**G**) (n = 7). Scale bars are 100 μm in (**A**) and 200 μm in (**B**) and (**E**). All data are plotted as mean ± s.e.m.. The p-values compared to *Saline* group unless specified are calculated based on two-way ANOVA. *, p-value ≤ 0.05; **, p-value ≤ 0.01; ***, p-value ≤ 0.001. B, bregma; 3V, third ventricle; aca, anterior commissure, anterior part; MPO, medial preoptic nucleus; dLPO and vLPO, dorsal and ventral part of lateral preoptic nucleus respectively; VLPO, ventrolateral preoptic nucleus.

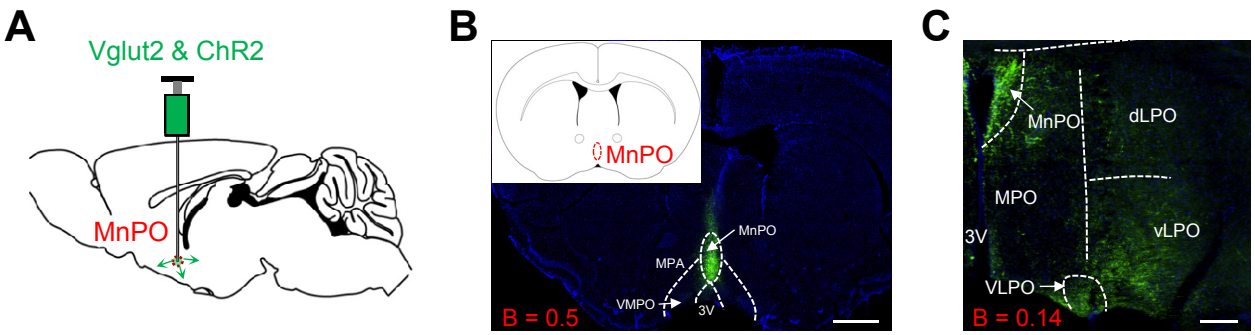

Figure S4. The vLPO receives glutamatergic input from the MnPO.
A, Scheme for anterograde tracing using ChR2. We injected 50 nl of Cre-dependent AAV5 viruses expressing ChR2-eYFP into the MnPO of Vglut2-IRES-Cre driver mice. **B,** Expression of ChR2-eYFP in MnPO^{Vglut2} neurons. Cell bodies were seen in the MnPO (B = 0.5). **C,** Representative image showing nerve terminals in the vLPO derived from MnPO^{Vglut2} neurons. The cell bodies were also seen in the MnPO (B = 0.14). Scale bars are 1 mm (**B**) and 200 µm (**C**). B, bregma; 3V, third ventricle; MnPO, median preoptic nucleus; MPO, medial preoptic nucleus; dLPO and vLPO, dorsal and ventral part of lateral preoptic nucleus respectively; VLPO, ventrolateral preoptic nucleus.

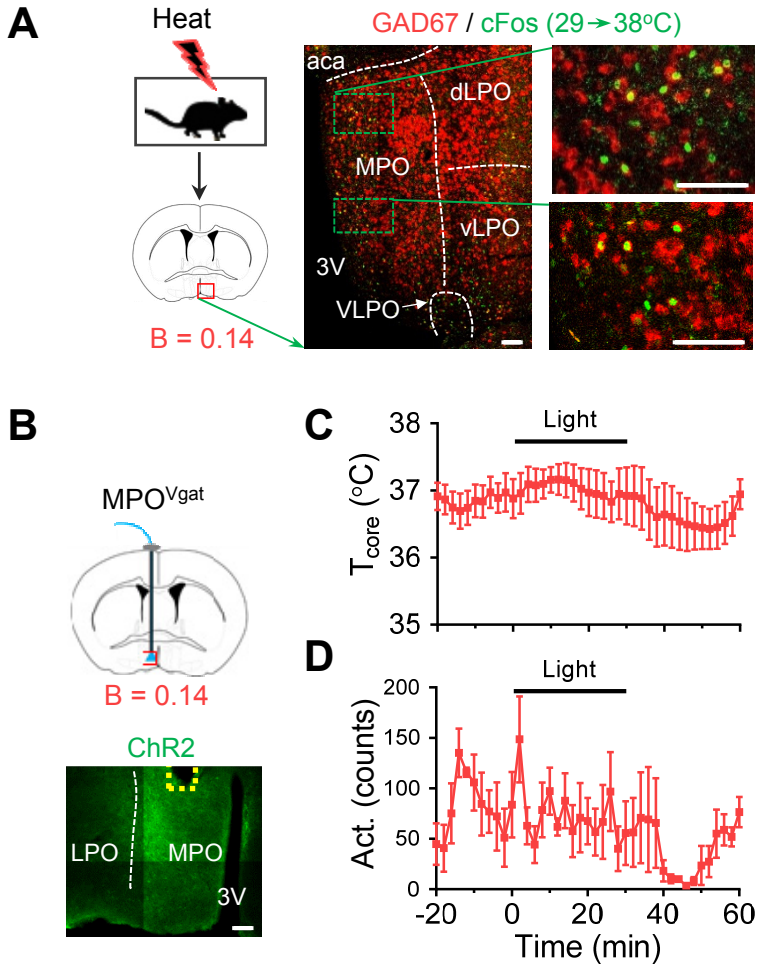

Figure S5. Activation of MPO GABAergic neurons did not change body temperature.
A, Heat-induced (38 °C, 2h) cFos co-localized with the GABAergic marker GAD67 in the MPO (No. of cFos+ & GAD67+ / No. of cFos+ = 30 ± 3.6%, n = 3). **B,** Scheme of optogenetic stimulation and viral expression of ChR2 in MPO^{Vgat} neurons. **C-D,** Optogenetic activation of MPO GABAergic neurons (Vgat-IRES-Cre+) by blue light did not significantly change T_{core} (**C**) and activity (**D**) (n = 3). Unilateral illumination, light pulses for 2 s (488 nm laser, 10 mW, 20 Hz, 40%) followed by a 2-s break, with the sequence repeating for 30 min. Scale bars are 100 μm in (**A**) and (**B**). All data are plotted as mean ± s.e.m. B, bregma; 3V, third ventricle; aca, anterior commissure, anterior part; MPO, medial preoptic nucleus; dLPO and vLPO, dorsal and ventral part of lateral preoptic nucleus respectively; VLPO, ventrolateral preoptic nucleus.

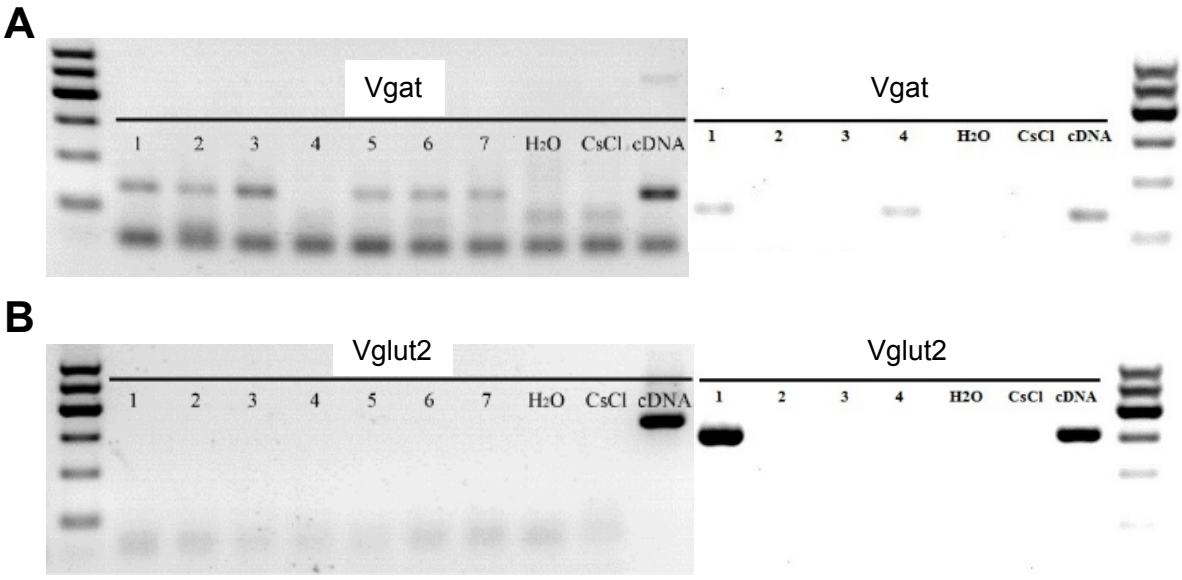

Figure S6. **Single-cell RT-PCR analysis (related to Fig. 2*H*).**
The intracellular fluid containing mRNAs was sucked directly using the recording pipette, revered transcribed and amplified by two around of PCR reactions (from n = 3 mice). **A,** The final PCR products for Vgat primers (band: 137 bp). **B,** The final PCR products for Vglut2 primers (band: 313 bp). DNA markers (upper to lower: 600, 500, 400, 300, 200,100 bp). The cDNA library prepared from brain lysates were used as a positive control. H_2O and CsCl buffer were negative controls.

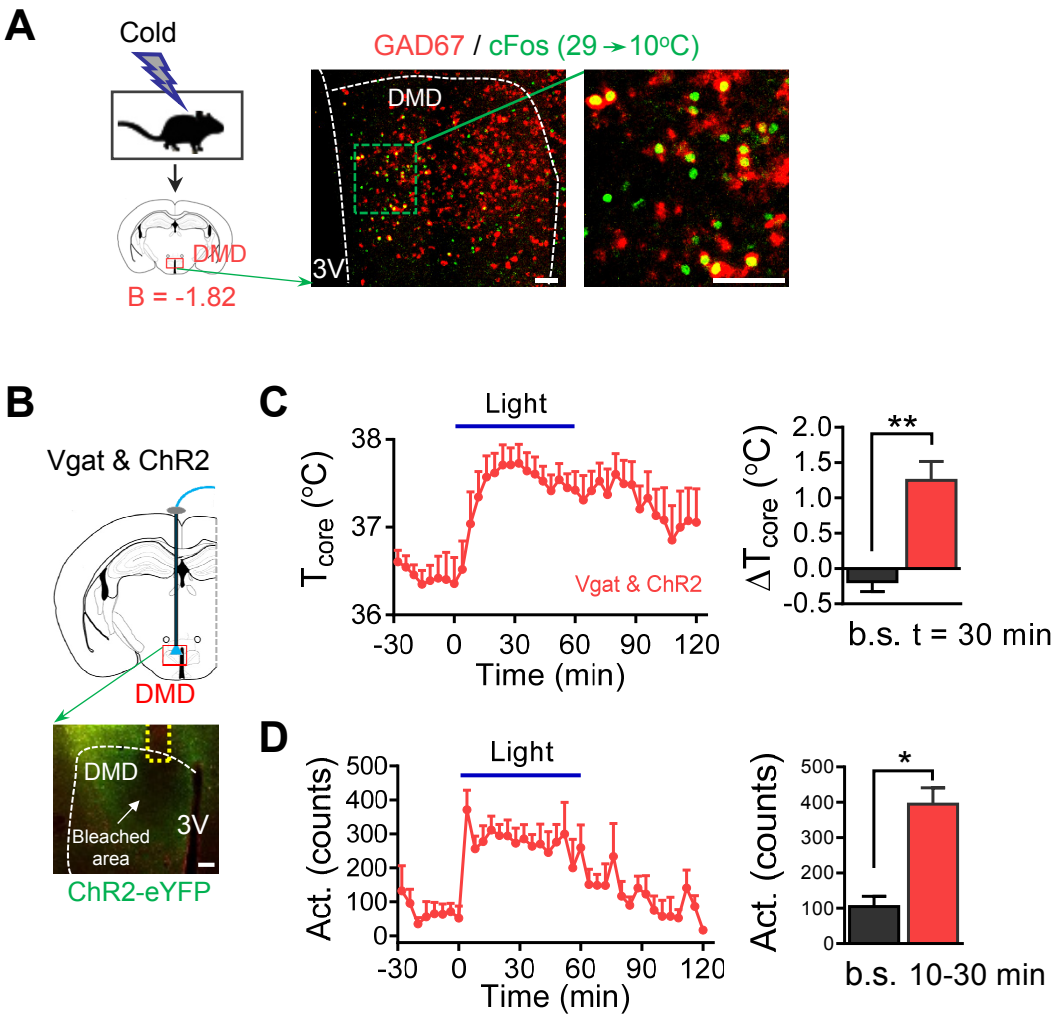

Figure S7. Activation of DMD GABAergic neurons increases T_{core}.
A, Cold-induced (10°C, 2h) cFos co-localized with GAD67 in the DMD. No. of cFos+ & GAD67+ / No. of cFos+ = 57 ± 3.2 %. (n = 3). **B,** Schematic of optogenetic stimulation and representative image of viral expression of ChR2-eYFP in DMD GABAergic neurons (Vgat-IRES-Cre+). Mice were sacrificed right after optogenetic stimulation. The GFP florescence (without immunostaining) was bleached by laser illumination. The dashed yellow lines indicate the positions of optical inserts. The dashed white lines indicate boundaries between subregions. **C-D,** Optogenetic activation of DMD^{Vgat} neurons (n = 4 mice) resulted in significant increases in T_{core} (**C**) and activity (**D**). ΔT_{core} represents the T_{core} changes from the mean level before light delivery (t = –30 to –10 min). The time point t = –10 min (baseline, b.s) and t = 30 min are in the bar graph. The average of activity in 20 min interval in between t = (–30 to –10 min) (baseline, b.s.) and in between t = (10–30 min) are shown in the bar graph. Stimulation protocol for ChR2: unilateral illumination, light pulses for 2 s (488 nm laser, 10 mW, 20 Hz, 40%) followed by a 2-s break, with the sequence repeating for 60 min. Scale bars in (**A**) and (**B**) are 100 μm. The p-values compared to baseline are calculated based on two-tailed unpaired t tests. *p-value ≤ 0.05; **p-value ≤ 0.01. B, bregma; 3V, third ventricle; DMD, dorsal part of dorsomedial hypothalamic nucleus.

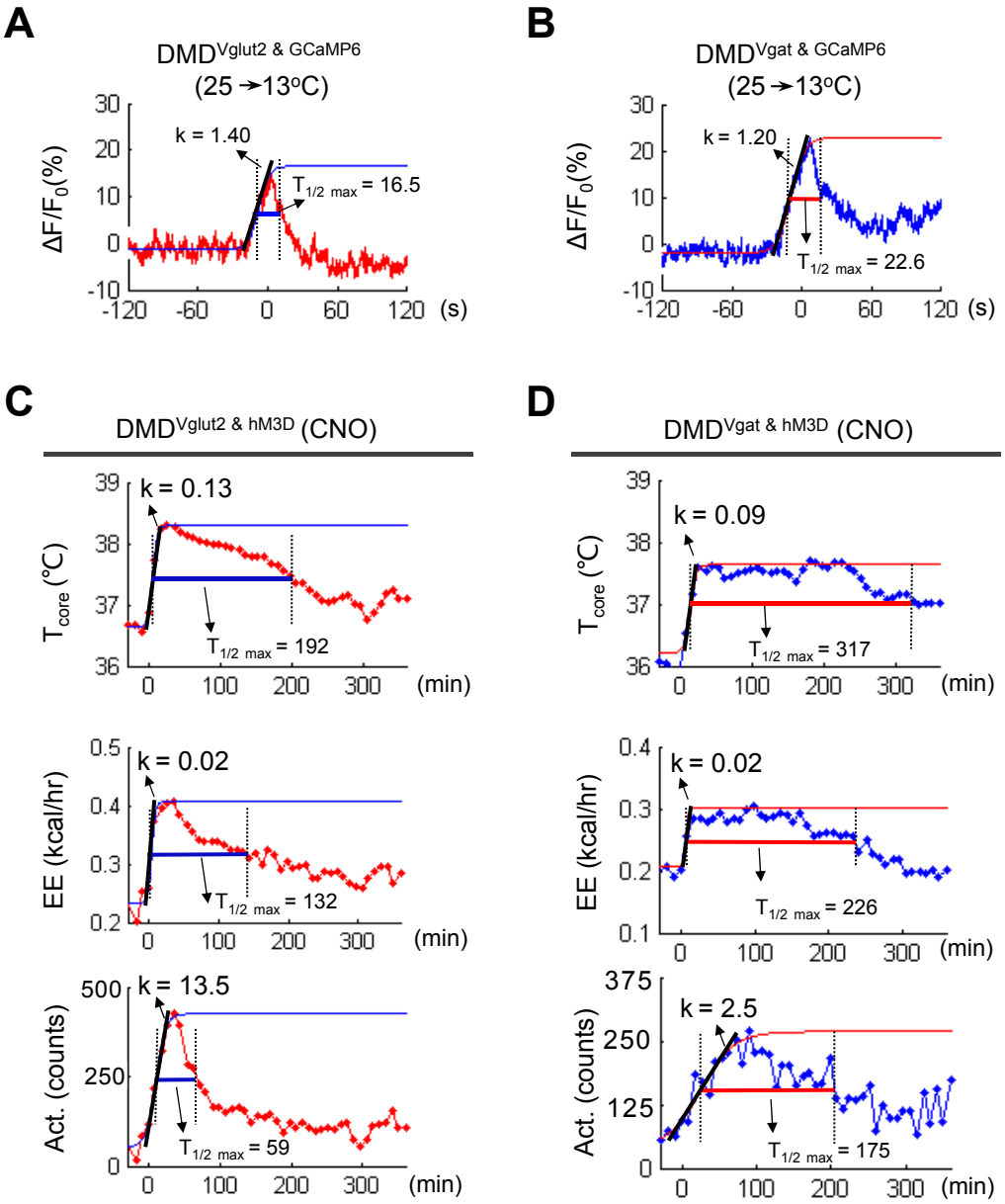

Figure S8. Fitting of the response curve of by a sigmoidal function.
A-B, Fitting of calcium transients from DMD^{Vglut2} and DMD^{Vgat} neurons in response to cooling as shown in Fig. 3*D-E*. k is the maximum change rate from the sigmoidal function. $T_{1/2\ max}$ is the full width at half maximum (FWFM). **C-D,** Fitting of T_{core}, EE and activity curve from mice with DREADD activation of DMD^{Vglut2} and DMD^{Vgat} neurons (related to Fig. 4*B-D*,*F-H*). CNO injected at t = 0.

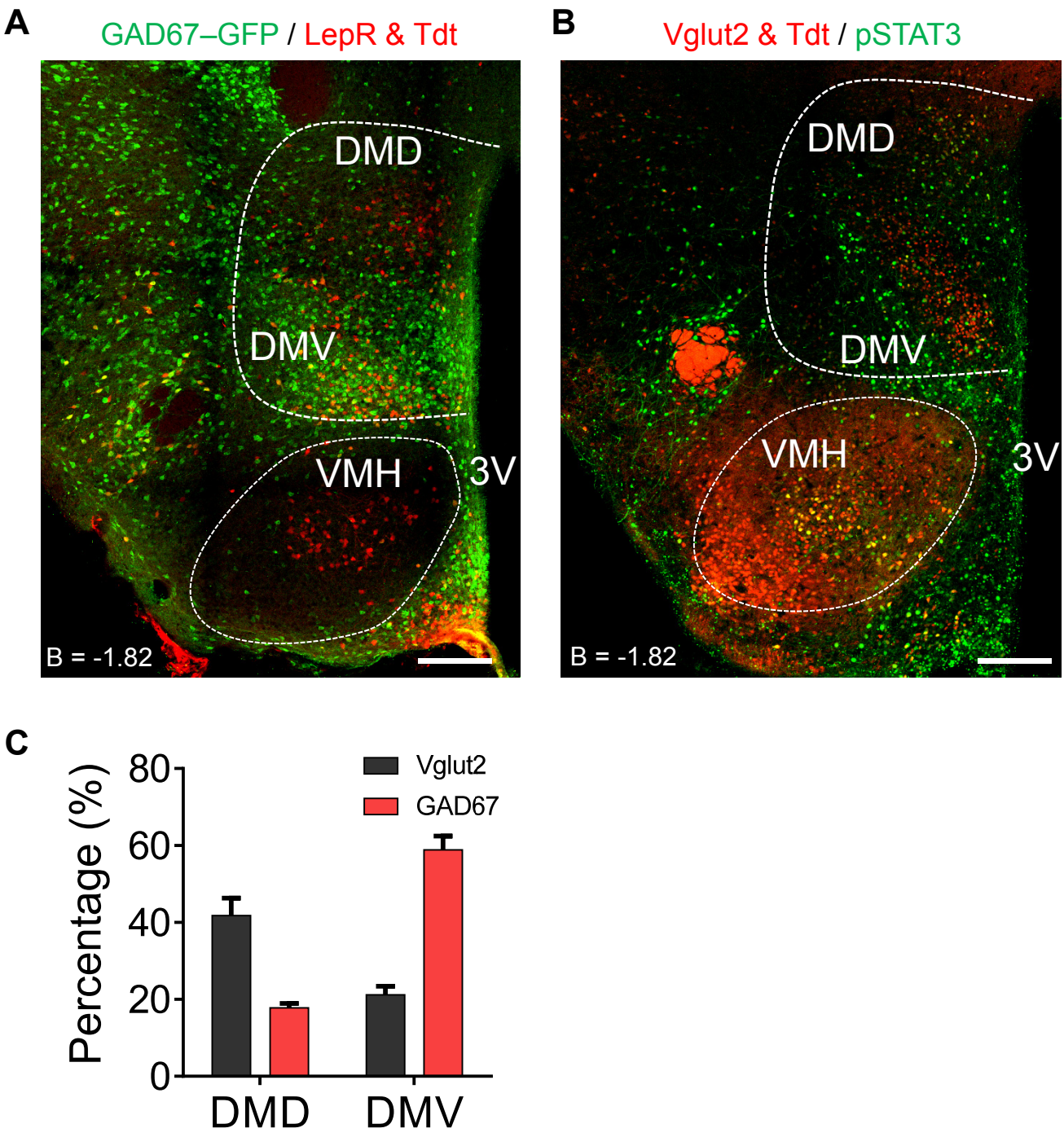

Figure S9. LepR neurons in the DMH.
A, Representative image of double immunostaining of GAD67-GFP and LepR (LepR-Cre, lox-*tdTomato*). The overlapping ratio is shown in (**C**). **B,** Representative image of double immunostaining of Vglut2 (Vglut2-IRES-Cre, lox-*tdTomato*) and pSTAT3 after leptin injection (I.P. leptin, 4 mg/kg body weight). pSTAT3 is a positive indicator for leptin signaling. The overlapping ratio is shown in (**C**). **C,** Quantification of overlapping ratio for (**A**) and (**B**). No. of GAD67-GFP+ & LepR+ / No. of LepR+ cells in (**A**) is in red. No. of Vglut2+ & pSTAT3+ / No. of pSTAT3+ cells in (**B**) is in black. Scale bars in (**A**) and (**B**) are 200 μm. B, bregma; DMD and DMV, dorsal and ventral part of dorsomedial hypothalamic nucleus respectively; 3V, third ventricle; VMH, ventromedial nucleus of the hypothalamus.

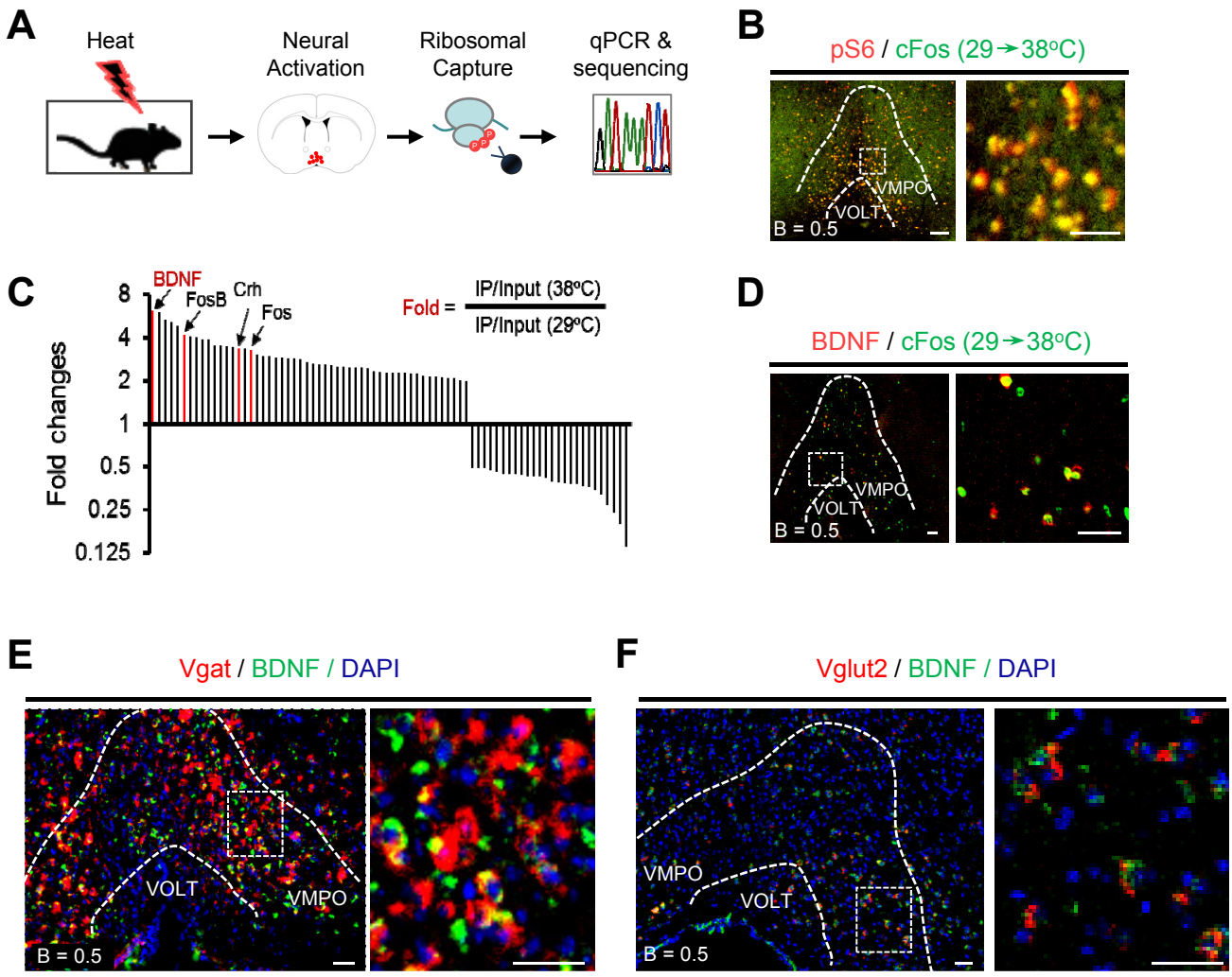

Figure S10. Identification of BDNF as a novel marker for heat activated neurons.
A, Scheme for phosphor-TRAP followed by mRNA sequencing. **B,** pS6 immune activity were colocalized with heat induced cFos in the POA. **C,** BDNF was highly enriched after heat induction (2h, 38 ºC) based mRNA sequencing reads number. The genes are listed in Table S2. **D,** BDNF mRNAs co-localized with heat induced cFos in the POA. The overlapping ratio (No. of BDNF+ & cFos+ / No. of cFos+) in the VMPO is 67.2 ± 2.8% (n = 3). **E,** Double FISH analysis of Vgat and BDNF. The overlapping ratio (No. of BDNF+ & Vgat+ / No. of BDNF+) in the VMPO is 32.9 % (n = 2). **F,** Double FISH analysis of Vglut2 and BDNF. The overlapping ratio (No. of BDNF+ & Vglut2+ / No. of BDNF+) in the VMPO is 59.8 % (n = 2). Scale bars are 50 μm. B, bregma; VMPO, ventromedial preoptic nucleus; VOLT, vascular organ of the lamina terminalis.

Table S1. Summary of statistical analyses

Figure	Sample size (n)	Statistical test	P values
1D-Chr2	4 mice	RM-two-way ANOVA. Factor one: time Factor two: treatment (control, ChR2) Bonferroni's multiple comparisons test.	Time: F (40, 120) = 5.19, P < 0.0001 Treatment: F (1, 3) = 5.94, P = 0.09 Interaction: F (40, 120) = 0.71, P < 0.0001
1D-hGtACR	3 mice	RM-two-way ANOVA. Factor one: time Factor two: treatment (control, hGtACR1) Bonferroni's multiple comparisons test.	Time: F (40, 80) = 2.96, P < 0.0001 Treatment: F (1, 2) = 51.66, P = 0.02 Interaction: F (40, 80) = 9.81, P < 0.0001
1E-Chr2-bar graph	4 mice	RM-two-way ANOVA. Factor one: time bin (baseline, 20 – 30 min) Factor two: treatment (control, ChR2) Bonferroni's multiple comparisons test.	Time bin: F (1, 3) = 28.88, P = 0.01 Treatment: F (1, 3) = 0.36, P = 0.59 Interaction, F (1, 3) = 9.56, P = 0.05
1E-hGtACR-bar graph	3 mice	RM-two-way ANOVA. Factor one: time bin (baseline, 20 – 30 min) Factor two: treatment (control, hGtACR1) Bonferroni's multiple comparisons test.	Time bin: F (1, 2) = 52.33, P = 0.02 Treatment: F (1, 2) = 0.53, P = 0.54 Interaction: F (1, 2) = 27.29, P = 0.03
2E	5 mice	RM-two-way ANOVA. Factor one: time Factor two: treatment (473 nm, 532 nm) Bonferroni's multiple comparisons test.	Time: F (49, 196) = 8.07, P < 0.0001 Treatment: F (1, 4) = 38.14, P = 0.004 Interaction: F (49, 196) = 7.99, P < 0.0001
2F	5 mice	Paired t test (two side)	P = 0.04
3D	3 mice	Paired t test (two side)	25-13: 0-10 vs b.s.: P < 0.001 110 -120 vs b.s.: P = 0.09 25-38: 0-10 vs b.s.: P = 0.27 110 -120 vs b.s.: P = 0.09
3E	4 mice	Paired t test (two side)	25-13: 0-10 vs b.s.: P < 0.001 110 -120 vs b.s.: P = 0.003 25-38: 0-10 vs b.s.: P = 0.26 110 -120 vs b.s.: P = 0.04
4B	5 mice	RM-two-way ANOVA. Factor one: time Factor two: treatment (Saline , CNO) Bonferroni's multiple comparisons test.	Time: F (27, 108) = 6.58, P < 0.0001 Treatment: F (1, 4) = 27.19, P = 0.006 Interaction: F (27, 108) = 8.01, P < 0.0001
4C	5 mice	RM-two-way ANOVA. Factor one: time Factor two: treatment (Saline , CNO) Bonferroni's multiple comparisons test.	Time: F (27, 108) = 6.24, P < 0.0001 Treatment: F (1, 4) = 75.66, P = 0.001 Interaction: F (27, 108) = 8.58, P < 0.0001
4D	5 mice	RM-two-way ANOVA. Factor one: time Factor two: treatment (Saline , CNO) Bonferroni's multiple comparisons test.	Time: F (27, 108) = 6.16, P < 0.0001 Treatment: F (1, 4) = 76.60, P = 0.0009 Interaction: F (27, 108) = 7.23, P < 0.0001
4F	8 mice	RM-two-way ANOVA. Factor one: time Factor two: treatment (Saline , CNO) Bonferroni's multiple comparisons test.	Time: F (34, 238) = 12.69, P < 0.0001 Treatment: F (1, 7) = 61.97, P = 0.0001 Interaction: F (34, 238) = 7.15, P < 0.0001
4G	8 mice	RM-two-way ANOVA. Factor one: time Factor two: treatment (Saline , CNO) Bonferroni's multiple comparisons test.	Time: F (34, 238) = 4.63, P < 0.0001 Treatment: F (1, 7) = 27.57, P = 0.001 Interaction: F (34, 238) = 2.85, P < 0.0001
4H	8 mice	RM-two-way ANOVA. Factor one: time Factor two: treatment (Saline , CNO) Bonferroni's multiple comparisons test.	Time: F (34, 238) = 4.31, P < 0.0001 Treatment: F (1, 7) = 47.29, P = 0.0002 Interaction: F (34, 238) = 5.49, P < 0.0001

5A	4 mice	Paired t test (two side)	P = 0.009
5B	4 mice	Paired t test (one side)	P = 0.04
5C	4 mice	Paired t test (two side)	P = 0.003
5D	4 mice	Paired t test (one side)	P = 0.04

Table S2. Enriched or depleted genes after heat challenge (from pS6-TRAP).

Symbol	Definition	Fold change
Bdnf	brain derived neurotrophic factor	6.09
Rrad	Ras-related associated with diabetes	6.06
Npas4	neuronal PAS domain protein 4	5.25
Apold1	apolipoprotein L domain containing 1	5.12
Fosl1	fos-like antigen 1	4.87
Fosb	FBJ osteosarcoma oncogene B	4.14
Calml4	calmodulin-like 4	4.07
Serinc2	serine incorporator 2	4.02
Inhba	inhibin beta-A	3.87
Junb	jun B proto-oncogene	3.87
Csrnp1	cysteine-serine-rich nuclear protein 1	3.56
Gem	GTP binding protein	3.51
Crem	cAMP responsive element modulator	3.44
Nr4a3	nuclear receptor subfamily 4, group A, member 3	3.39
Crh	corticotropin releasing hormone	3.37
Fos	FBJ osteosarcoma oncogene	3.27
Nr4a1	nuclear receptor subfamily 4, group A, member 1	2.95
Fam90a1b	family with sequence similarity 90, member A1B	2.95
Igfbpl1	insulin-like growth factor binding protein-like 1	2.92
Sertad1	SERTA domain containing 1	2.92
Adcyap1	adenylate cyclase activating polypeptide 1	2.84
Slc37a2	solute carrier family 37 (glycerol-3-phosphate transporter), member 2	2.84
Nms	neuromedin S	2.79
Gal	galanin	2.64
Dusp14	dual specificity phosphatase 14	2.58
Yap1	yes-associated protein 1	2.54
Nts	neurotensin	2.52
Galr1	galanin receptor 1	2.52
Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)	2.50
Gabre	gamma-aminobutyric acid (GABA) A receptor, subunit epsilon	2.48
Diap3	diaphanous homolog 3 (Drosophila)	2.47
Btg2	B cell translocation gene 2, anti-proliferative	2.46
Skap1	src family associated phosphoprotein 1	2.33
Cebpb	CCAAT/enhancer binding protein (C/EBP), beta	2.28
Egr1	early growth response 1	2.26
Dnajc25	DnaJ (Hsp40) homolog, subfamily C, member 25	2.26
Dusp1	dual specificity phosphatase 1	2.26
Fosl2	fos-like antigen 2	2.26
Magoh	mago homolog, exon junction complex core component	2.23
Tnfrsf12a	tumor necrosis factor receptor superfamily, member 12a	2.23
Tmc3	transmembrane channel-like gene family 3	2.15
Col8a1	collagen, type VIII, alpha 1	2.14
Gadd45b	growth arrest and DNA-damage-inducible 45 beta	2.11
Rps2-ps6	ribosomal protein S2, pseudogene 6	2.08
Cd36	CD36 antigen	2.07
F2rl2	coagulation factor II (thrombin) receptor-like 2	2.03
Ier3	immediate early response 3	2.00
Adgre5	adhesion G protein-coupled receptor E5	0.50
Slc6a13	solute carrier family 6 (neurotransmitter transporter, GABA), member 13	0.50
Fpgs	folylpolyglutamyl synthetase	0.50
Fam72a	family with sequence similarity 72, member A	0.48
Fbn2	fibrillin 2	0.46
Plcb3	phospholipase C, beta 3	0.45
Brsk1	BR serine/threonine kinase 1	0.45
Serpina9	serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 9	0.44
Akap1	A kinase (PRKA) anchor protein 1	0.44
Tmem173	transmembrane protein 173	0.43
Pmch	pro-melanin-concentrating hormone	0.43
Kif26a	kinesin family member 26A	0.43
Ddx58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	0.42
Alas2	aminolevulinic acid synthase 2, erythroid	0.40
Fam83d	family with sequence similarity 83, member D	0.39
Spg11	spastic paraplegia 11	0.38
Hck	hemopoietic cell kinase	0.38
Svil	supervillin	0.38
Dapp1	dual adaptor for phosphotyrosine and 3-phosphoinositides 1	0.37
Sdc1	syndecan 1	0.33
Cfh	complement component factor h	0.27
Chst3	carbohydrate (chondroitin 6/keratan) sulfotransferase 3	0.24
Foxc1	forkhead box C1	0.20
Col1a1	collagen, type I, alpha 1	0.14