SUPPLEMENTARY METHODS

Animals

The experiments used transgenic Cx43$^{loxP/loxP}$, tdTomato^{POMC} and Cx43$^{loxP/loxP}$;tdTomato^{POMC} mice. tdTomato^{POMC} mice were generated by breeding Pomc-Cre mice with ROSA-tdTomato mice. These mice were bred with Cx43$^{loxP/loxP}$ mice, in which exon 2 of the Cx43 allele is flanked by two LoxP sites, to generate Cx43$^{loxP/loxP}$;tdTomato^{POMC} mice. Cx43$^{loxP/loxP}$, Pomc-cre and ROSA-tdTomato mice purchased from The Jackson Laboratories (Bar Harbor, ME; Pomc-cre line: 005965 – STOCK Tg(Pomc1-cre)16Lowl/J, ISMR Cat#JAX:005965, RRID:IMSR_JAX:005965; ROSA-tdTomato line: 007914 – B6.Cg-*Gt(ROSA)26Sor*$^{tm14(CAG-tdTomato)Hze}$/J, ISMR Cat#JAX:007914, RRID:IMSR_JAX007914; Cx43$^{loxP/loxP}$ line: 008039 – B6.129S7-*Gja1*^{tm1Dig}/J, IMSR Cat#JAX:008039, RRID_JAX008039). All three lines were maintained on a C57Bl6 background. *Cx43*$^{+/+}$;*tdTomato*$^{loxp\text{-}STOP\text{-}loxP}$ mice were generated by crossing *Cx43*$^{+/loxP}$;*tdTomato*$^{loxp\text{-}STOP\text{-}loxP}$ males with *Cx43*$^{+/loxP}$;*tdTomato*$^{loxp\text{-}STOP\text{-}loxP}$ females. In addition, wild-type C57Bl/6J (Stock No: 000664, B6, Jackson Laboratory) were also used for infection with viral vectors. All mice were bred and housed in a temperature- and humidity-controlled room on a 12h/12h light/dark cycle and with access to food and water ad libitum. Animal studies were performed with the approval of the Institutional Ethics Committees for the Care and Use of Experimental Animals of the University of Lille and the French Ministry of National Education, Higher Education and Research (APAFIS#2617-2015110517317420 v5), and under the guidelines defined by the European Union Council Directive of September 22, 2010 (2010/63/EU).

Lactate production and release assay in primary cultures of tanycytes

For lactate release/secretion assays, primary cultures of tanycytes were incubated in ACSF for 2h, then treated with either 2-desoxy-glucose (2-DG, 50 mM, Sigma Cat#D8375, CAS:154-17-6), 4-CIN (500 µM) or CBX (50 µM) for 30 minutes before cell lysis and medium sampling. Lactate concentrations in cell lysates and medium samples were analyzed using the Lactate-Glo™ assay (Promega) following kit instructions.

Isolation of tanycytes and POMC-expressing cells by fluorescence-activated cell sorting (FACS).

For the isolation of POMC-expressing cells, the mediobasal hypothalamus of *tdTomato*^{POMC} mice was microdissected under a stereomicroscope and enzymatically dissociated using a Papain Dissociation System (Worthington, Lakewood, NJ) to obtain a single-cell suspension.

For isolation of tanycytes, two weeks after the injection into the lateral ventricle of AAV1/2 vectors expressing EGFP (Dio2:EGFP or Cre-mediated expression of Mct1/4 shRNAs and EGFP), the median eminence was microdissected under a stereomicroscope and dissociated using a Papain Dissociation System (Worthington, Lakewood, NJ) to obtain a single-cell suspension. FACS was performed using a BD ARIA SORTER and FACSDiva software v8.0.3 (BD Biosciences, Inc). Sorting parameters were based on measurements of Tomato fluorescence (excitation 561nm; detection: bandpass 675+/-20nm) or GFP fluorescence (excitation: 488 nm; detection: GFP bandpass 530+/-30 nm), by comparing cell suspensions from Tomato-positive and wild-type animals. For each animal, 2000 Tomato-positive, 2000 GFP-positive and 2000 Tomato-negative cells were sorted directly into 10 µl of lysis buffer (0.1% Triton® X-100 and 0.4 unit/µl RNaseOUT™ (Life Technologies)) to perform qPCR.

Quantitative qPCR analyses

For gene expression analyses, total RNA obtained from FACS-sorted Tomato-positive, GFP-positive and negative cells was reverse transcribed using High-capacity cDNA Reverse transcription kit (Applied Biosystems™ ref 4368814) after a DNAse treatment (DNase I, Amplification Grade, Invitrogen™, ref 18068015). A linear preamplification step was performed using the TaqMan® PreAmp Master Mix Kit protocol (Applied Biosystems™ ref 4488593). Next, real-time PCR was carried out on an Applied Biosystems 7900HT Fast Real-Time PCR System using the TaqMan® probes listed in Table S2 and TaqMan™ Universal Master Mix II (Applied Biosystems™ ref 4440049). The control housekeeping gene r18S was used for normalization. Gene expression data were analyzed using the $2^{-\Delta\Delta Ct}$ method to compare between negative and positive FACS-sorted cells.

Dye-coupling analysis

After electrophysiological recordings, slices were immediately fixed with 4% paraformaldehyde (PFA) overnight at 4°C. After several washes in phosphate buffered saline (PBS) solution, slices were treated with Alexa Fluor 488 streptavidin (1:250; Invitrogen) with 0.5% Triton X-100 in PBS overnight at 4°C. After rinsing several times in PBS solution, slices were incubated with Hoechst 33342 (1:10,000) for 1 minute. In some experiments, biocytin detection was combined with immunolabeling for the neuronal nuclear marker (NeuN). In this case, slices were incubated with a primary mouse anti-NeuN antibody (1:1000; Millipore, Cat#MAB377, RRID:AB_2298772) in Triton/normal goat serum (NGS)/PBS for 3 days at 4°C, washed several times, then treated with a secondary goat anti-mouse Alexa Fluor 546

antibody (1:500; Invitrogen, Invitrogen Cat#A-11030; RRID: AB_2534089) and Alexa Fluor 488 streptavidin in Triton/NGS/PBS overnight at 4°C. After several rinses with PBS solution, slices were mounted on slides with Mowiol. Images were captured using an Axio Imager Z2 Apotome microscope.

Immunochemistry

For immunocytofluorescence in tanycyte primary cultures, tanycytes were seeded onto poly-L-lysine coated glass coverslips. Cells were fixed for 15 minutes in 4% PFA in PBS, washed three times with PBS and stored at 4°C in PBS-Na azide 0.02%. After three washes with PBS, cells were permeabilized in PBS-Triton 0.25% (PBS-T) for 15 minutes, washed three times with PBS and incubated in a blocking solution of PBS-T BSA 1% for 30 minutes. After blocking, tanycytes were incubated with primary antibodies (Cx43, Millipore Cat#AB1727, RRID:AB_2294609, 1:200; MCT-1, Millipore Cat#AB1286, RRID:AB_11212410, 1:100; MCT-4, Millipore, 1:200; Vimentin, Millipore Cat#AB3314P, RRID:AB_2286063, 1:1000; Vimentin, DAKO Cat#M0725, RRID:AB_10013485, 1:100) in blocking solution overnight at 4°C. Cells were then washed 3 times with PBS and incubated with secondary antibodies in PBS-T for 1 hour at room temperature. After three PBS washes, cells were incubated with DAPI (1:5000, Sigma Cat#D9542, CAS:28718-90-3) for 5 minutes at room temperature and washed three times with PBS. Images were captured using an Axio Imager Z2 Apotome microscope.

For immunohistochemical detection, mice were deeply anesthetized with 50-100 mg/kg of ketamine hydrochloride and xylazine hydrochloride and perfused via the left ventricle with 20 ml PBS solution followed sequentially by 100 ml of 4% PFA in 0.1 M PBS, pH 7.4. Brains were rapidly removed and postfixed in 4% PFA for 24 hours at 4°C. After fixation, they were cryoprotected by immersing for 24 hours in a solution of 30% sucrose in PBS. Coronal 40 μm-thick hypothalamic sections containing the arcuate nucleus were cut serially at 200 μm intervals on a freezing microtome (SM2010R; Leica) and then stored in an antifreeze solution at -20°C until immunolabeling. To determine tanycytic Cx43 expression, double immunofluorescence labeling for Cx43 and vimentin was performed on free-floating sections. Sections were incubated with 5% normal goat serum (NGS), 0.3% triton X-100 in 0.1 M PBS blocking solution for 1 hour at room temperature, then with primary mouse anti-Cx43 (1:500; Millipore) and chicken anti-vimentin antibodies (1:2000; Millipore Cat#AB5733, RRID:AB_11212377), or rabbit anti-POMC propeptide (1:200; Phoenix pharmaceuticals, Cat#H02930, RRID:AB_2307442), and a chicken anti-GFP (1:1000; Invitrogen, Cat#A10262, RRID:AB_2534023) overnight at 4°C on a shaking

platform. After several washes in PBS, sections were treated with secondary goat anti-mouse Alexa Fluor 546 (1:1000; Invitrogen, Cat#A-11030, RRID:AB_2534089) and goat anti-chicken Alexa Fluor 633 (1:500; Invitrogen Cat##A-21103, RRID:AB_2535756). All sections were counterstained with Hoechst 33342 (1:10000; Invitrogen Cat#H3570, CAS:23491-52-3), and mounted on slides with Mowiol. Immunofluorescence images were captured using an LSM 710 Zeiss confocal microscope.

SUPPLEMENTARY TABLE

Taqman probe	Gene	
Mm00475829_g1	Agrp	Agouti-related peptide
Mm04210469_m1	Cartpt	Cocaine- and amphetamine-regulated transcript protein
Mm00515664_m1	Dio2	Iodothyronine deiodinase 2
Mm01151962_m1	Elavl3	ELAV-like protein 3 (Hu-antigen C, HuC)
Mm00439129_m1	Gck	Glucokinase
Mm01179639_s1	Gja1	Cx43
Mm00433661_s1	Gjb6	Cx30
Mm00439147_m1	Gpr50	Melatonin-related receptor (G protein-coupled receptor 50) (H9)
Mm01612132_g1	Ldh1	Lactate dehydrogenase a
Mm05874166_g1	Ldh5	Lactate dehydrogenase b
Mm01410146_m1	NPY	Neuropeptide Y
Mm00435874_m1	POMC	Pro-opiomelanocortin
Mm00454892_m1	Ppp1r1b	Darpp32
Mm03928990_g1	Rn18s	Ribosomal 18S
Mm01306379_m1	Slc16a1	Mct1, solute carrier family 16 (monocarboxylic acid transporters), member 1
Mm00446102_m1	Slc16a3	Mct4, solute carrier family 16 (monocarboxylic acid transporters), member 3
Mm00441442_m1	Slc16a7	Mct2, solute carrier family 16 (monocarboxylic acid transporters), member 7
Mm00441480_m1	Slc2a1	Glut1
Mm00446229_m1	Slc2a2	Glut2
Mm00436615_m1	Slc2a4	Glut4
Mm01333430_m1	Vim	Vimentin

Table S2. Taqman probes used for qPCR.

SUPPLEMENTARY FIGURES

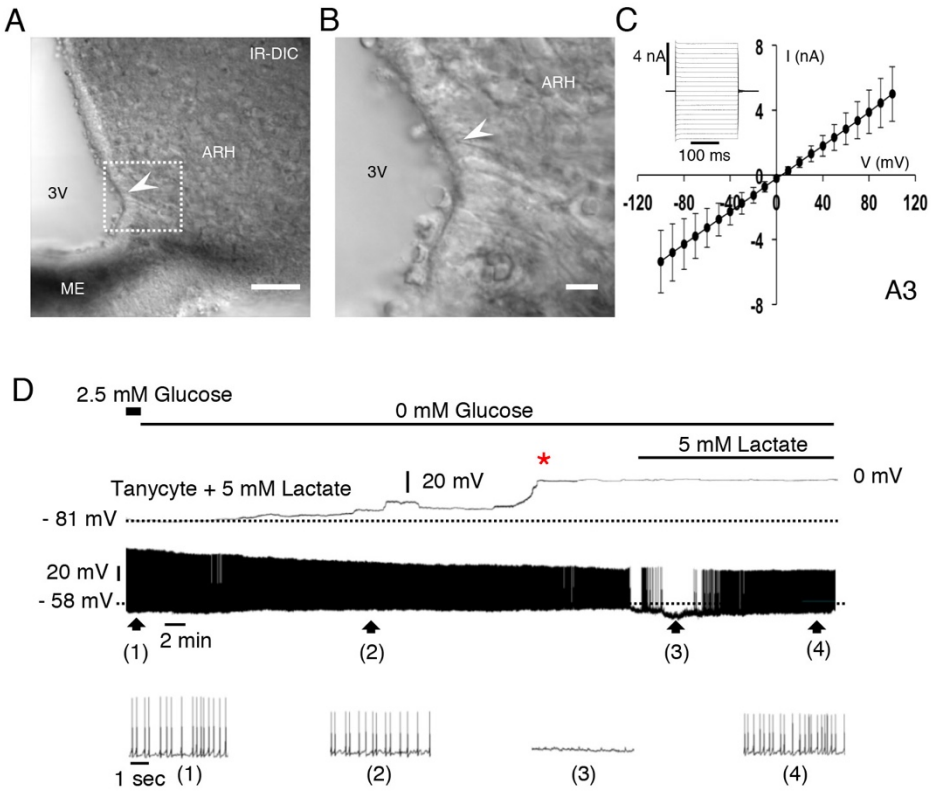

Figure S1. Intracellular lactate delivery to tanycytes maintains the electrical activity of POMC neurons during exogenous glucose deprivation.

(A) Infrared differential interference contrast (IR-DIC) image of an arcuate nucleus (ARH) slice (250 µm-thick) at the level of the median eminence (ME). 3V: third ventricle. Scale bar, 50 µm.

(B) Higher magnification of the area delineated by the white square in (A1) showing the strip of ARH tanycytic cell bodies (arrowhead) lining the wall of the third ventricle (3V) and a single process (arrowhead) extending into the ARH. Scale bar, 20 µm.

(C) Representative membrane current of an ARH tanycyte in response to voltage pulses and average I(V) curve for ARH tanycytes (n=8 cells).

(D) Recording of a paired ARH tanycyte and POMC neuron (dyad) in whole-cell patch-clamp mode showing that filling the tanycyte with 5 mM lactate allows the electrical activity of a POMC neuron to be maintained during exogenous glucose deprivation (0 mM), and that the loss of the patched tanycyte, represented by the red asterisk, leads to a decrease in the firing rate of the neuron that can be

compensated by the bath application of 5 mM lactate. Bottom traces show expansions of the recording at the indicated time points 1, 2, 3 and 4.

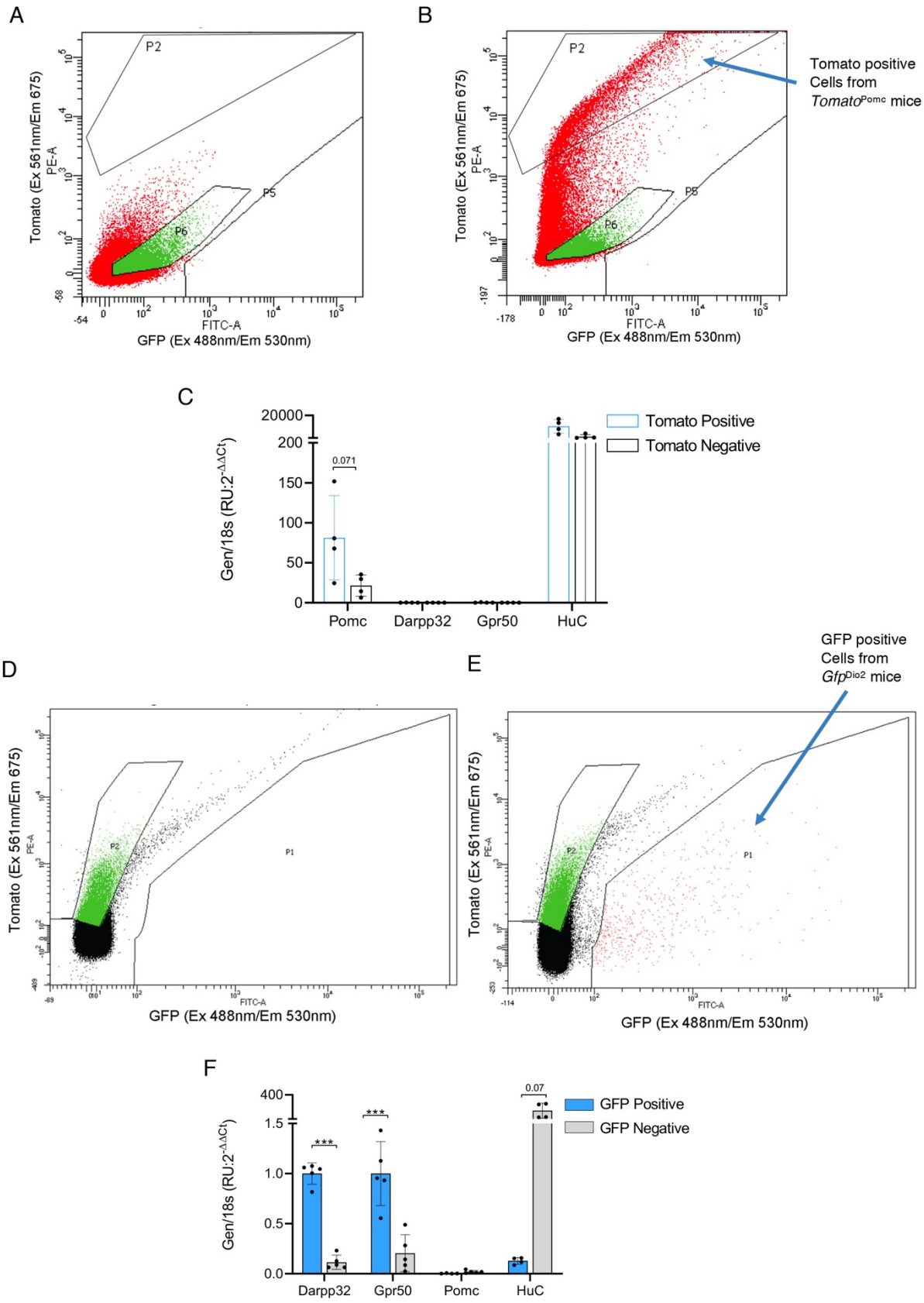
A
B
P2
P5
P6
Tomato (Ex 561nm/Em 675)
PE-A
FITC-A
GFP (Ex 488nm/Em 530nm)
Tomato positive
Cells from
Tomato^Pomc mice
C
Gen/18s (RU:2^-ΔΔCt)
0.071
Tomato Positive
Tomato Negative
Pomc
Darpp32
Gpr50
HuC
D
E
P1
GFP positive
Cells from
Gfp^Dio2 mice
F
***
0.07
GFP Positive
GFP Negative

Figure S2. FACS and qPCR analyses in mice in wild-type mice selectively expressing tdTomato and green fluorescent protein (GFP) in POMC neurons and tanycytes, respectively.

(A and **B)** Gating strategy for sorting Tomato-positive putative POMC neurons from *Pomc::cre*; *tdTomato*$^{loxP\text{-}STOP\text{-}loxP}$ mice.

(C) mRNA expression levels of control markers in Tomato-positive and -negative cells (unpaired student's t-tests; *Pomc*, P=0.071; *Darpp32*, P=0.158; *Gpr50*, P=0.319; *Huc*, P=0.027; n=4 mice).

(D and **E)** Gating strategy for sorting GFP expressing cells.

(F) mRNA expression levels of tanycytic markers in GFP-positive and -negative cells (unpaired Student's t-tests; *Darpp32*, P<0.001; *Gpr50*, P=0.001; *Pomc*, P=0.06; *Huc*, P=0.07; n=5 mice).

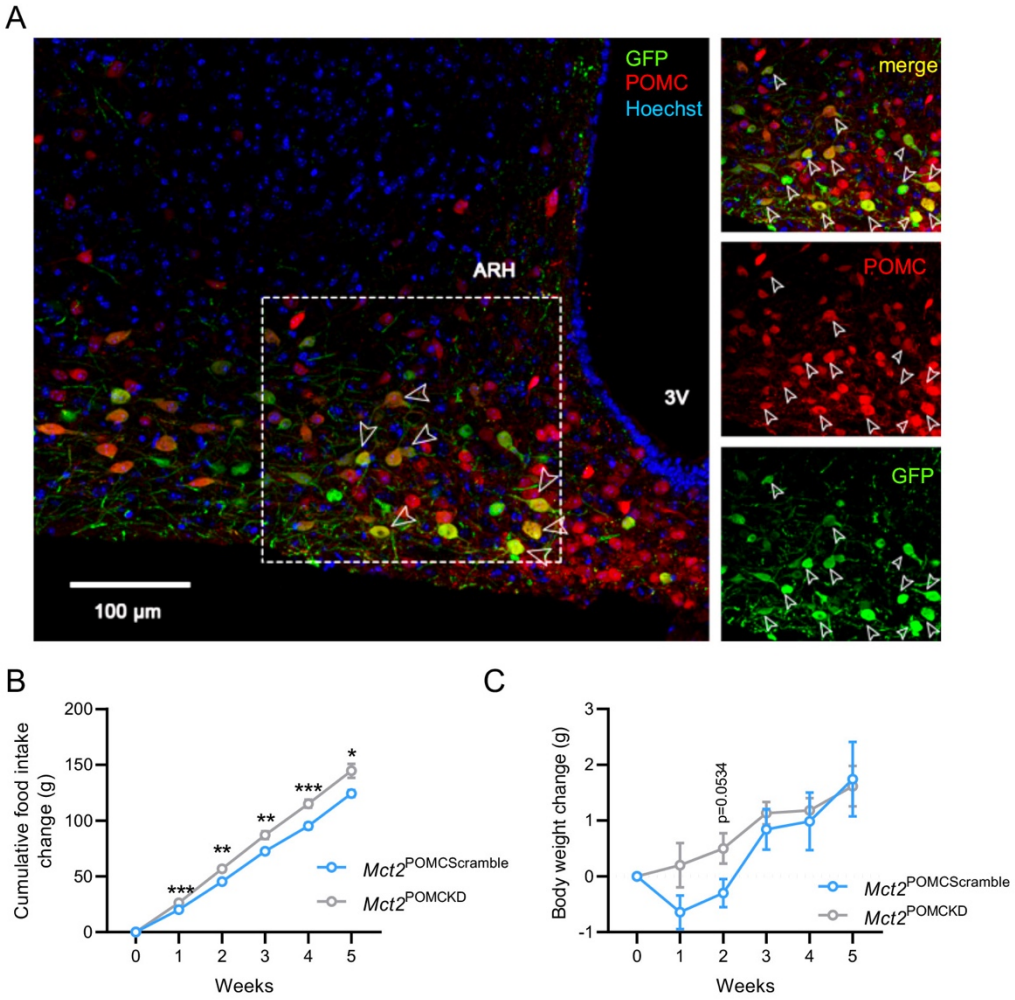

Figure S3. Effect of selectively knocking down Mct2 expression in POMC neurons on cumulative food intake and body weight.

(A) Immunofluorescence image showing that GFP transgene expression (green) was restricted to POMC neurons (red) in *Pomc::cre* mice injected into the arcuate nucleus of the hypothalamus (ARH) with AAV8 expressing shRNA:GFP in a Cre-dependent manner.

(B and C) Effect of the viral-vector-induced knockdown of *Mct2* expression selectively in POMC neurons (*Mct2*^{POMCKD}) on cumulative food intake (B) and the body weight (C) in male mice compared to mice expressing a scrambled shRNA in POMC neurons (*Mct2*^{POMCScramble}) (unpaired Student's t-tests; (B), Week 1, *P*=0.0003; Week 2, *P*=0.0018; Week 3, *P*=0.0045; Week 4, *P*=0.0008; Week 5, *P*=0.0114; (C), Week 1, *P*=0.1135; Week 2, *P*=0.0534; Week 3, *P*=0.5188; Week 4, *P*=0.7470; Week 5, *P*=0.8773; n=7 Mct2^{POMCScramble} and 6 Mct2^{POMCKO} mice). * P<0.05, **P<0,01, ***P<0,001.

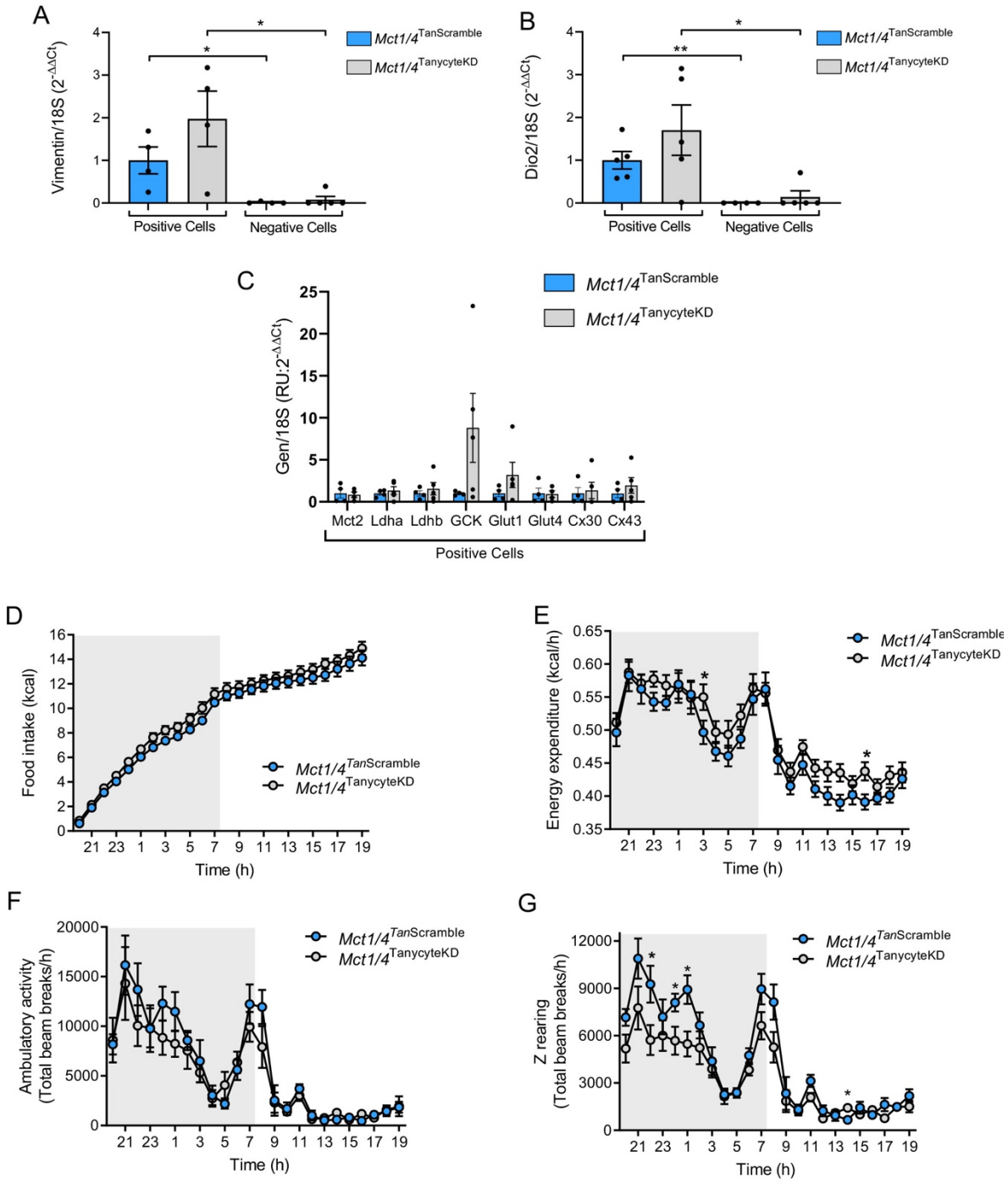

Figure S4. Gene expression and metabolic profiling in mice in which *Mct1* and *Mct4* have been selectively knocked down in *Dio2*-expressing tanycytes.

(A) mRNA expression levels of tanycytic markers, *Vimentin* (A1) and *Dio2* (A2) in GFP-positive and -negative cells (unpaired Student's t-tests; (A1) *Mct1/4*$^{\text{TanScramble}}$/positive cells vs. *Mct1/4*$^{\text{TanScramble}}$/negative cells P=0.0200, *Mct1/4*$^{\text{TanycyteKD}}$/positive cells vs. *Mct1/4*$^{\text{TanycyteKD}}$/negative

cells, *P*=0.0134; (A2) *Mct1/4*$^{\text{TanScramble}}$/positive cells vs. *Mct1/4*$^{\text{TanScramble}}$/negative cells *P*=0.0037, *Mct1/4*$^{\text{TanycyteKD}}$/positive cells vs. *Mct1/4*$^{\text{TanycyteKD}}$/negative cells, *P*=0.0324; n=4 mice). **P*<0.05.

(B) Real-time PCR analysis for various gene transcripts (*Mct2*, *Ldha*, *Ldhb*, Glucokinase (*Gck*), *Glut1*, *Glut4*, *Cx30* and *Cx43*) in GFP-positive cells, between *Mct1/4*$^{\text{TanScramble}}$ and *Mct1/4*$^{\text{TanycyteKD}}$ mice (unpaired Student's tests; *Mct2*, *P*=0.8204; *Ldha*, *P*=0.5561; *Ldhb*, *P*=0.5457, *Gck, P*=0.1381; *Glut1*, *P*=0.2479; *Glut4*, *P*=0.9174; *Cx30*, *P*=0.7825; *Cx43*, *P*=0.4315; n=6 mice).

(D-G) Cumulative food intake (**D**), energy expenditure (**E**), ambulatory activity (**F**) and Z rearing (**G**) in *Mct1/4*$^{\text{TanScramble}}$ and *Mct1/4*$^{\text{TanycyteKD}}$ mice (two-way ANOVA followed by an uncorrected Fisher's LSD test; **D**, *P*=0.2310, n=6 and 8 mice; **E**, *P*=0.04480, n=7 and 8 mice; **F**, *P*=0.5215, n=7 and 8 mice; **G**, *P*=0.0305, n=7 and 8 mice). **P*<0.05.

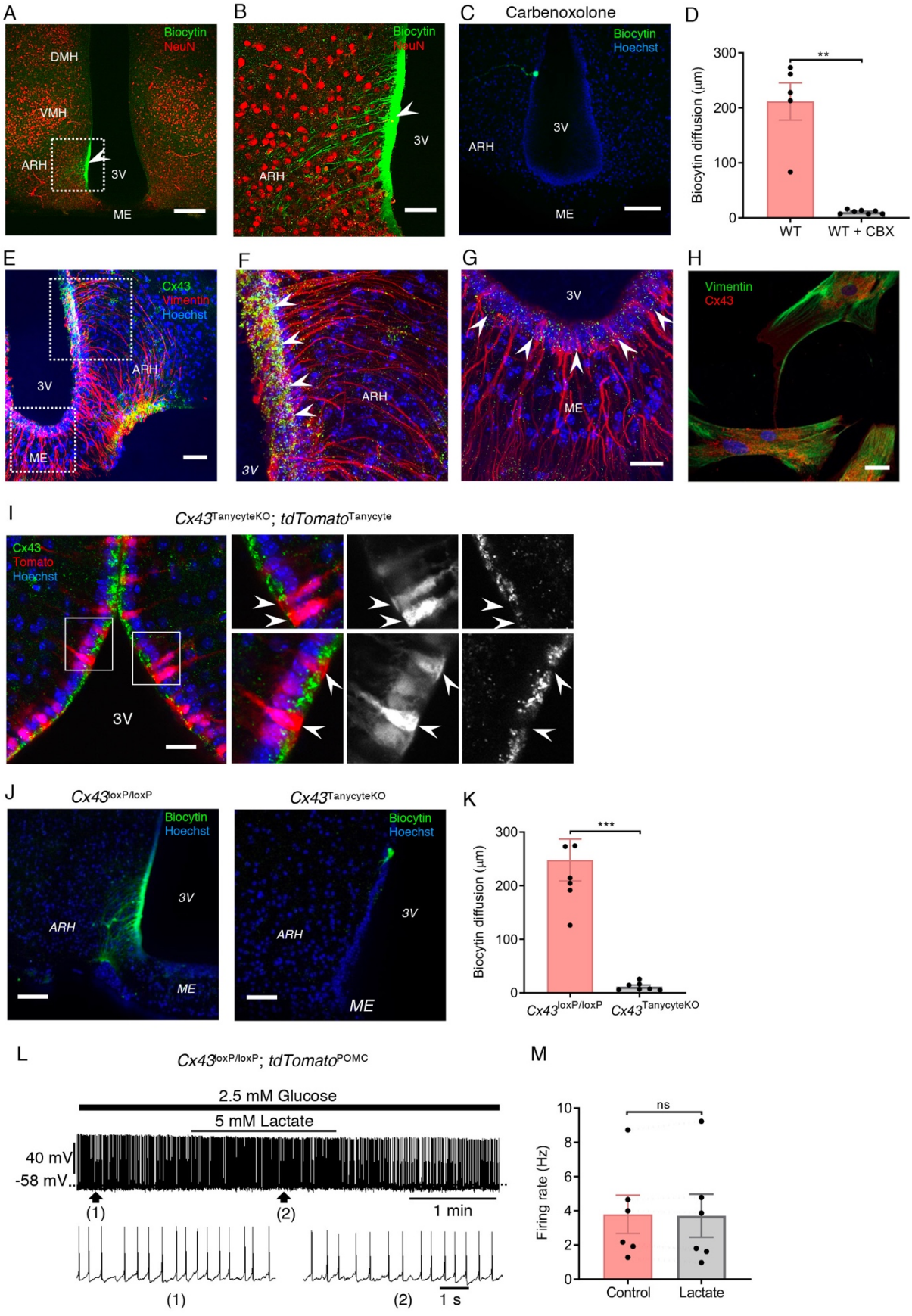

Figure S5. ARH tanycytes form a network of cells interconnected by connexin 43 gap junctions.

(A) Extensive intercellular dye-coupling between ARH tanycytes visible after fixation of the WT hypothalamic slice and revelation of biocytin by fluorochrome-conjugated streptavidin (green), combined with immunofluorescence labeling for NeuN (red). The arrow indicates the single tanycyte that was recorded for 20 minutes with a whole-cell patch-clamp electrode containing biocytin. DMH: dorsomedial hypothalamus. VMH: ventromedial hypothalamus. Scale bar, 200 μm.

(B) Higher magnification of the area delineated by the white square in (B1). Scale bar, 100 μm.

(C) Treatment of a WT slice with the gap-junction blocker carbenoxolone (CBX, 50 μM) abolished the intercellular diffusion of biocytin (green) between ARH tanycytes.

(D) Extent of biocytin diffusion in WT slices and slices treated with CBX. Statistical differences were tested using a Mann-Whitney U test (WT vs. WT+ CBX, U=0, P=0.0025; n=5 and 7 cells, 4 and 5 mice). ** P<0.01.

(E) Confocal image of a hypothalamic section showing Hoechst nuclear staining (blue), Cx43 immunolabeling (green) and vimentin immunolabeling (red) in the arcuate nucleus of the hypothalamus (ARH) and the median eminence (ME). 3V: third ventricle. Scale bar, 50 μm.

(F and **G)** Higher magnification of the ARH (**F**) and ME (**G**) regions delineated by white squares in (**E**), showing the dense punctiform expression of Cx43 in the cell bodies of ARH tanycytes lining the 3V (**F**, arrowheads) compared to almost non-existent labeling in the cell bodies of ME tanycytes (B3, arrowheads). Scale bar, 25 μm.

(H) Primary cultures of tanycytes immunolabeled with anti-connexin 43 (Cx43) antibody and anti-vimentin antibodies and counterstained with DAPI to reveal cell nuclei. Scale bar, 10 μm.

(I) Confocal image of a hypothalamic section showing Cx43 immunolabeling (green), tdTomato expression (red) and Hoechst staining (blue) in *Cx43*$^{\text{TanycyteKO}}$;tdTomato mice. Scale bar, 25 μm.

(J) Representative diffusion of biocytin through ARH tanycytes in *Cx43*$^{\text{loxP/loxP}}$ and *Cx43*$^{\text{TanycyteKO}}$ slices. Scale bar, 50 μm.

(K) Extent of biocytin diffusion in *Cx43*$^{\text{loxP/loxP}}$ and *Cx43*$^{\text{TanycyteKO}}$ slices. Statistical differences were tested using a Mann-Whitney U test (*Cx43*$^{\text{loxP/loxP}}$ vs. *Cx43*$^{\text{TanycyteKO}}$, P=0.0006; n=7 and 7 cells, 6 and 6 mice). *** P<0.001.

(L) Whole-cell current-clamp recording performed in ACSF containing 2,5 mM glucose showing that the bath application of 5 mM lactate has no effect on the spontaneous activity of a POMC neuron from a *Cx43*$^{\text{loxP/loxP}}$; *tdTomato*$^{\text{POMC}}$ mouse.

(M) Firing rate of POMC neurons in *Cx43*$^{loxP/loxP}$; *tdTomato*^{POMC} mice following the bath application of lactate. Statistical differences were tested using a paired Student's t-test (Control vs. Lactate, *P*=0.6042; n=6 cells, 6 mice). ns: non-significant.

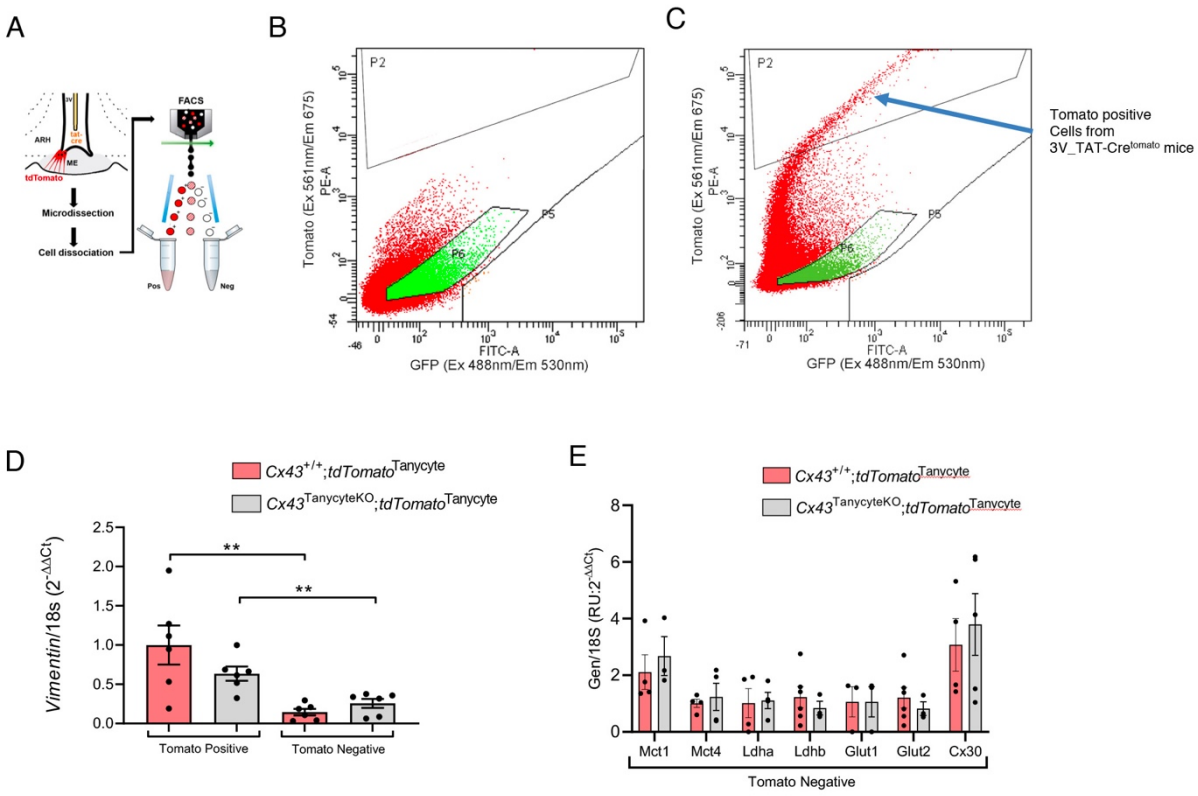

Figure S6. FACS and qPCR analyses in wild-type and mutant mice selectively expressing tdTomato in tanycytes

(A) Schematic model for fluorescence-activated cell sorting (FACS) used to isolate putative Tomato-positive tanycytes in *Cx43*$^{loxP/loxP}$;*tdTomato*$^{loxP\text{-}STOP\text{-}loxP}$ mice injected with TAT-Cre into the third ventricle (3V).

(B-C) Gating strategy for sorting Tomato-positive cells in control (**B**) and Tomato-expressing tissue (**C**).

(D) mRNA expression levels for *Vimentin*, a tanycytic marker, in tdTomato-positive and -negative cells (unpaired Student's t-test; cells Positive vs. Negative for Tomato in *Cx43*$^{+/+}$;*tdTomato* mice, *P*=0.0070; cells Positive vs. Negative for Tomato in *Cx43*^{TanycyteKO};*tdTomato mice*, *P*=0.0056; n=6 mice). ***P*<0.01.

(E) Real-time PCR analysis for various gene transcripts (*Mct1*, *Mct4*, *Ldha*, *Ldhb*, *Glut1*, *Glut4* and *Cx30*) in tdTomato-negative cells from *Cx43*$^{+/+}$;*tdTomato*^{Tanycyte} and *Cx43*^{TanycyteKO};*tdTomato*^{tanycyte} mice (unpaired Student's t-tests; *Mct1*, *P*=0.5679; *Mct4*, *P*=0.6651; *Ldha*, *P*=0.8780; *Ldhb*, *P*=0.5127, *Glut1*, *P*>0.9999; *Glut2*, *P*=0.5127; *Cx30*, *P*=0.6421; n=4 mice).