SUPPLEMENTAL INFORMATION

Figure S1. Cell types in human and combined human/mouse vascular data. Related to Figure 1.

(A) UMAP plot of 2268 vascular cells extracted from human SVF of SAT.

(B) Violin plots for marker genes from clusters found in (A); *PECAM1* is shown as general marker for endothelial cells; *SPARCL1* is shown as a marker for non-lymphatic endothelial cells.

(C) UMAP plot of combined human/mouse vascular cells (see Fig. 1C and Fig. S1A).

(D) Breakdown of the origin of the cells comprising each cluster in (C); the y-axis shows the proportion of cells belonging to each depot (color) that belong to each cluster (x-axis).

(E) Violin plots for markers from Fig 1D; fenestrated capillary cells separate from the "Stalk" cell population (mouse).

Figure S2. Neurotensin(+) and *LYVE1*(+)/*Lyve1*(+) subpopulations of lymphatic endothelium. Related to Figure 2. Mouse gene nomenclature is used throughout the rest of this caption.

(A) UMAP-based feature plots of combined mouse/human lymphatic endothelial cells (see Fig. S1C, cluster "XsV6") for three lymphatic genes: *Mmrn1, Nts*, and *Lyve1*.

(B) Table showing the percent of Nts+ cells that co-express other lymphatic markers in our human and mouse scRNA-seq data.

(C) Co-expression plots for *Lyve1* (x-axis) and *Nts* (y-axis) in human and mouse cells; dot size indicates the number cells with the same expression levels; a single mouse outlier cell was excluded from the plot, due to visibility issues (expression level of *Nts* was ~10X the next nearest point).

(D) NTS protein measured by ELISA from isolated BAT of NTS-Cre mice, transduced ex vivo with AAV9-DIO-hM3Dq. $n = 2$; Mean ± SD. $^{**}P < 0.01$

(E) Representative image of isolated collecting lymphatic vessel from human mesenteric fat (n = 3 images from 3 isolated vessels). Scale bar, 75 μm.

(F) Western blot of lysates from collecting lymphatic vessel (LV) in human mesenteric fat, blank sample or human serum. One Western blot was performed.

(G) Data from Immgen showing *Nts* expression in thymic medullary epithelial cells and LECs.

(H) Representative image of intestinal lacteals of an NTS-Cre::Ai9 mouse, counterstained with anti-Lyve-1 (green) and anti-Prox1 (white). tdTomato (red) marks expression of NTS-Cre in lacteals. Blue staining is DAPI (n = 25 images from 5 mice). Scale bars, 50 μm and 5 μm.

(I) Ear skin collecting lymphatics of an NTS-Cre::Ai9 mouse counterstained with anti-Lyve-1 (green) and anti-Prox1 (white). tdTomato (red) marks expression of NTS-Cre. Yellow arrowheads indicate lymphatic valves. Scale bar, 100 μm.
(J) Diaphragmatic lymphatics of an NTS-Cre::Ai9 mouse counterstained with anti-Lyve-1 (green) and anti-Prox1 (white). tdTomato (red) marks expression of NTS-Cre. Scale bar, 100 μm.

Figure S3. The sympathetic nervous system controls NTS expression in LECs. Related to Figure 3.
(A) Expression of *Prox1* in the SVF of BAT, eWAT, and iWAT of mice housed at 30°C and 4°C. *n* = 5; Mean ± SD.
(B) Expression of *Prox1* in the Prox1+ cells isolated from BAT, eWAT, and iWAT of mice housed at 30°C and 4°C. *n* = 5; Mean ± SD.
(C, D, E) Prox1-CreERT2::Ai9 mice were treated with tamoxifen to activate tdTomato expression in Prox1+ cells (LECs). Tissue preparations of eWAT (C), iWAT (D), and interscapular BAT (E) were cleared using the Adipo-Clear method and stained with anti-tyrosine hydroxylase to visualize sympathetic nerves.
(F) Expression of *Nts* in the SVF of BAT SVF cells treated *ex vivo* with norepinephrine with and without phentolamine or propranolol. *n* = 2; Mean ± SD. ****P* <0.001
(G) Expression of *Nts* and *Prox1* in the SVF of BAT SVF cells treated *ex vivo* with phenylephrine or dexmedetomidine. *n* = 2; Mean ± SD. ***P* <0.01
(H) As in main Fig. 3F, AAV2/8-hSyn-DIO-hM3Dq-mCherry virus (or mCherry only) was injected into the RPa of Vglut3-IRES-Cre mice, resulting in specific expression of the DREADD hM3Dq in the glutamatergic neurons upstream of the SNS. Shown is the increase in c-Fos activation with CNO in the RPa.

Figure S4. NTS reduces BAT thermogenesis. Related to Figure 4.
(A) Oxygen consumption rate of WT mice after receiving AAV-NTS or AAV-GFP control in BAT as measured by indirect calorimetry at thermoneutrality (30°C), room temperature (23°C), and cold exposure (4°C). Data are normalized to lean body mass.
(B) CO_2 production rate of WT mice after receiving AAV-NTS or AAV-GFP control in BAT as measured by indirect calorimetry at thermoneutrality (30°C), room temperature (23°C), and cold exposure (4°C). Data are normalized to lean body mass.

(C) BAT from Prox1-CreERT2::DREADD and DREADD only (Cre-) mice treated with tamoxifen (2 mg/mouse daily for 11 days). LVs were identified by Lyve-1 staining (green), which co-localized with mCherry in Cre+ tissue.

(D) Addition of CNO to the medium induces release of NTS protein specifically from Prox1-CreERT2+ BAT, as measured by ELISA, $n = 2$; Mean ± SD. $^{**}P < 0.01$

(E) *Ucp1* mRNA levels are reduced in BAT by CNO treatment of Prox1-CreERT2+ BAT, $n = 4$; Mean ± SD. $^{**}P < 0.01$

(F) UCP-1 protein levels are reduced in BAT by CNO treatment of Prox1-CreERT2+::DREADD mice.

Figure S5. NTS signals through MAP kinase to repress UCP-1 expression. Related to Figure 7.

(A) Expression of thermogenic genes in BAT after direct delivery of AAV-shNtsr2 or a control shRNA for three weeks. $n = 5$; Mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

(B) BAT histology three weeks after injection of AAV-shNtsr2 (vs. AAV-shControl) at 30^{o}C.

(C) BAT explants were treated with the indicated amount of NTS for 60 minutes and lysates were collected for Western blotting.

(D) BAT explants were treated with 25 uM NTS for the times indicated prior to lysate collection and Western blotting.

(E) *Ucp1* mRNA expression was detected in BAT explants after treatment with 50 uM NTS for 24 hours with or without simultaneous treatment with the MEK inhibitor PD0325901. $n = 2$; Mean ± SD. $^{*}P < 0.05$

(F) UCP-1 protein expression was detected by Western blotting in BAT explants after treatment with 50 uM NTS for 24 hours with or without simultaneous treatment with the MEK inhibitor PD0325901 at 5uM and 10uM.

Figure S6. Inhibition of NTSR2 increases thermogenesis in BAT. Related to Figure 7.

(A) Schematic of the proneurotensin protein with the internal Neuromedin N (NN; green) and Neurotensin (NTS; orange) peptide sequences indicated.

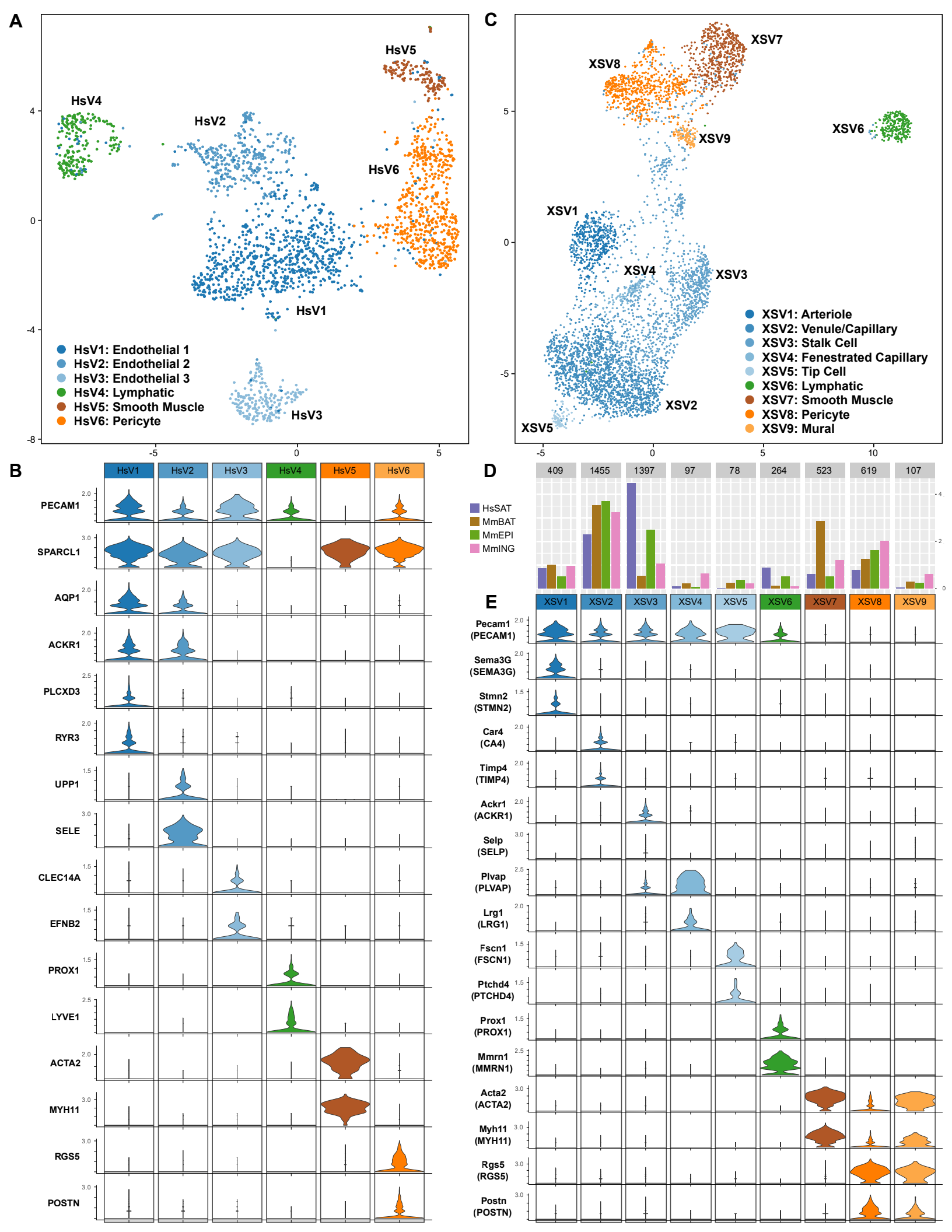
(B) BAT explants were treated with the indicated concentrations of NN for 0.5 hrs prior to lysate production and Western blotting. $n = 2$; Mean ± SD.

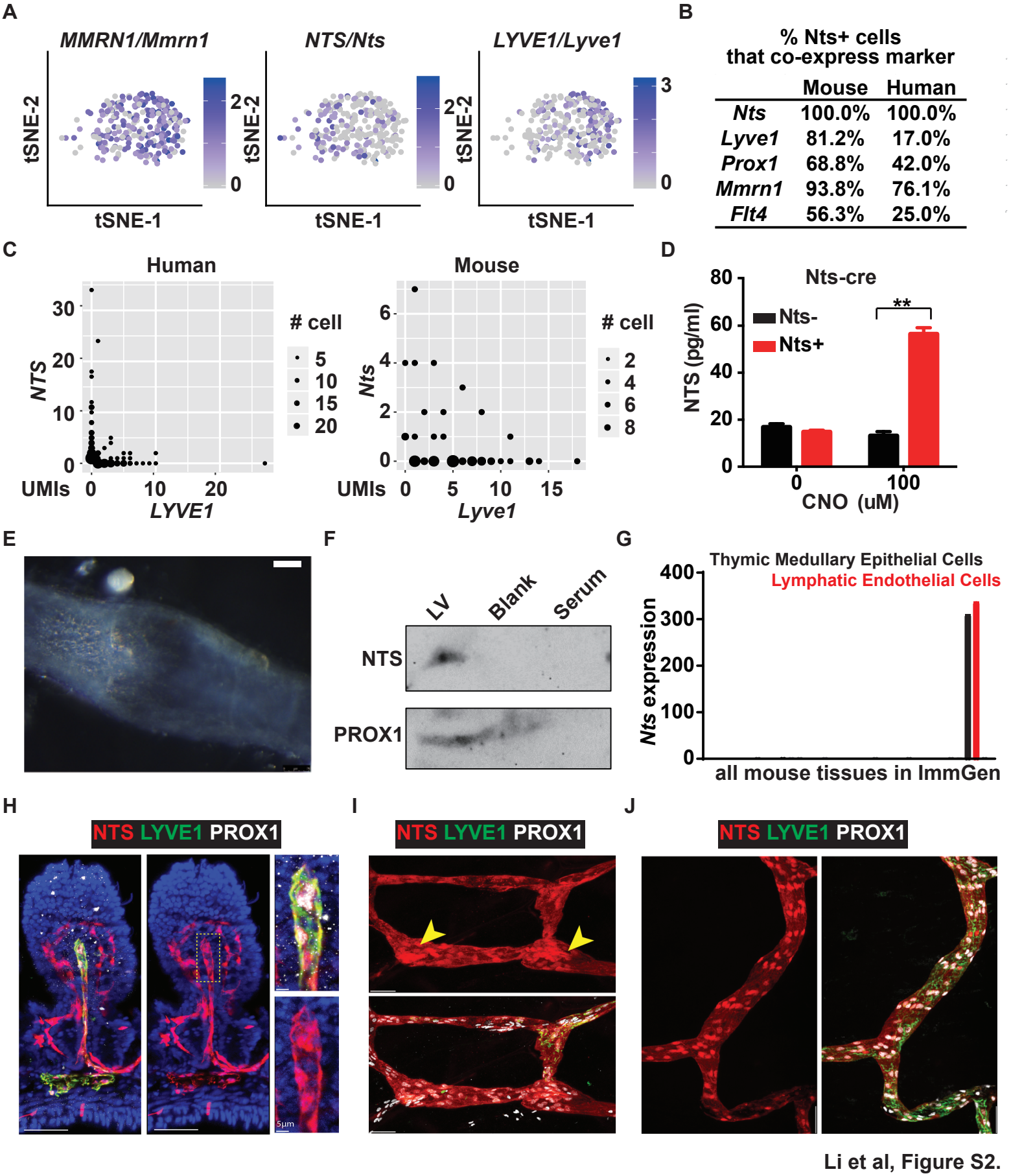
(C) BAT explants were treated with the indicated concentrations of NN for 24 hrs prior to harvest and qRT-PCR. Gene expression was normalized to *36b4*. $n = 2$; Mean ± SD.

(D) BAT explants were treated with 40 uM NN for 24 hrs prior to lysate production and Western blotting.
(E, F) BAT explants were treated 25 uM NTS for 0.5 hrs with or without 10 uM NTRC-824 prior to harvest for Western blotting. *n* = 3; Mean ± SD. **P* <0.05, ***P* <0.01.
(G) BAT explants were treated with vehicle, 50 uM NTS, or 50 uM NTS plus 10 uM NTRC-824 for 24 hours prior to harvest for qRT-PCR. *n* = 2; Mean ± SD. **P* <0.05, ***P* <0.01, ****P* <0.001
(H) 8-week-old lean male mice received intraperitoneal (IP) injections of NTRC-824 (5 mg/kg) or DMSO while housed at 30°C. The mice were treated for two weeks prior to harvest of BAT for qRT-PCR. *n* = 5; Mean ± SD. ****P* <0.001
(I) Mice were treated as in (H) before harvesting BAT for histology.
(J) Mice were treated as in (H) before placement in CLAMS unit. *n* = 6-7; Mean ± SD. **P* <0.05

Figure S7. Pharmacological blockade of NTSR2 improves the metabolic state of obese mice. Related to Figure 7.
(A) Body weights of 20-week-old obese male mice receiving IP injections of NTRC-824 (5 mg/kg) or DMSO every day for four weeks while housed at 30°C. *n* = 10; Mean ± SD. **P* <0.05
(B, C) % fat and lean mass of mice from (A).
(D) Daily food intake of mice from (A).
(E) Glucose tolerance testing was performed on mice from (A) after 15 days of treatment. The inset shows the AUC. *n* = 10; Mean ± SD. **P* <0.05
(F) Insulin tolerance testing was performed on mice from (A) after 24 days of treatment. The inset shows the AAC. *n* = 10; Mean ± SD. **P* <0.05
(G) Thermogenic gene expression in BAT from mice in (A) after 4 weeks of treatment. *n* = 5; Mean ± SD. **P* <0.05, ***P* <0.01, ****P* <0.001
(H) Histology of BAT from mice in (A).

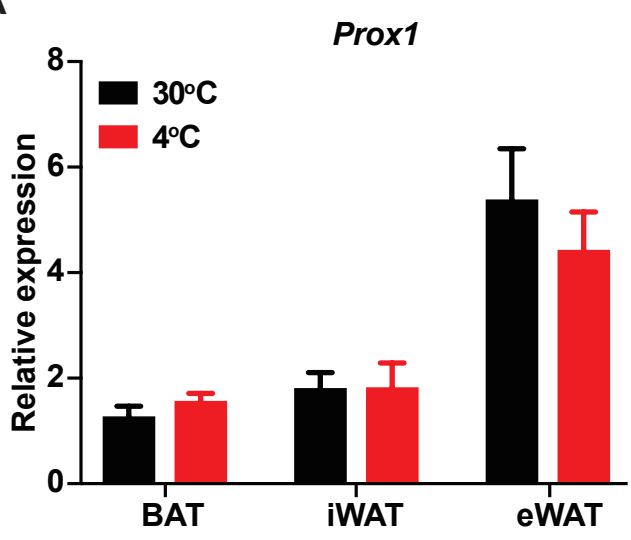
Table S1. Primers used in this study. Related to Figs.2-7.


A
HsV1
HsV2
HsV3
HsV4
HsV5
HsV6
HsV1: Endothelial 1
HsV2: Endothelial 2
HsV3: Endothelial 3
HsV4: Lymphatic
HsV5: Smooth Muscle
HsV6: Pericyte
C
XSV1
XSV2
XSV3
XSV4
XSV5
XSV6
XSV7
XSV8
XSV9
XSV1: Arteriole
XSV2: Venule/Capillary
XSV3: Stalk Cell
XSV4: Fenestrated Capillary
XSV5: Tip Cell
XSV6: Lymphatic
XSV7: Smooth Muscle
XSV8: Pericyte
XSV9: Mural
B
PECAM1
SPARCL1
AQP1
ACKR1
PLCXD3
RYR3
UPP1
SELE
CLEC14A
EFNB2
PROX1
LYVE1
ACTA2
MYH11
RGS5
POSTN
D
HsSAT
MmBAT
MmEPI
MmING
409
1455
1397
97
78
264
523
619
107
E
Pecam1 (PECAM1)
Sema3G (SEMA3G)
Stmn2 (STMN2)
Car4 (CA4)
Timp4 (TIMP4)
Ackr1 (ACKR1)
Selp (SELP)
Plvap (PLVAP)
Lrg1 (LRG1)
Fscn1 (FSCN1)
Ptchd4 (PTCHD4)
Prox1 (PROX1)
Mmrn1 (MMRN1)
Acta2 (ACTA2)
Myh11 (MYH11)
Rgs5 (RGS5)
Postn (POSTN)



Li et al, Figure S2.

A



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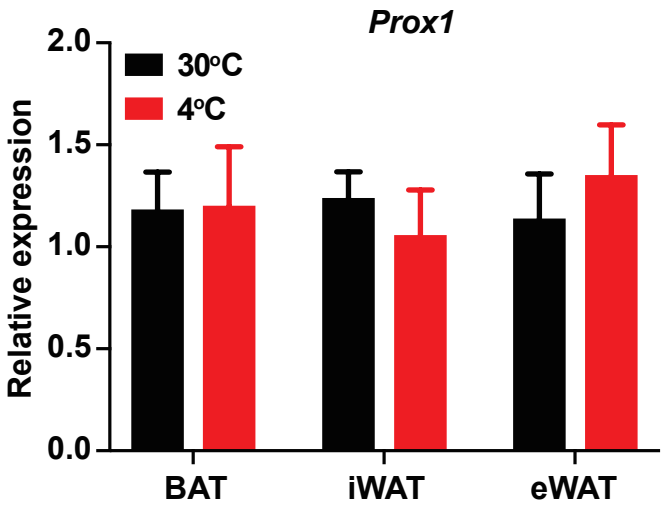
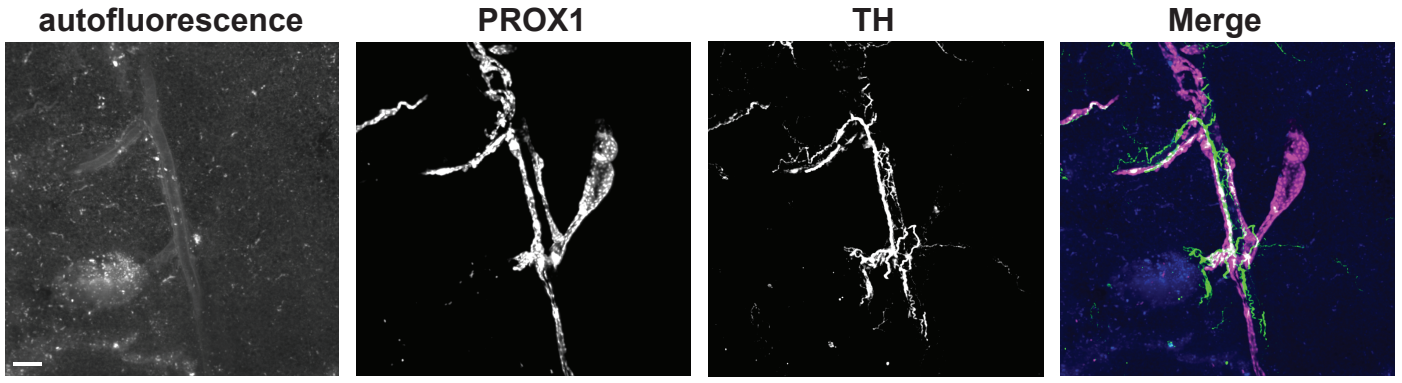


C



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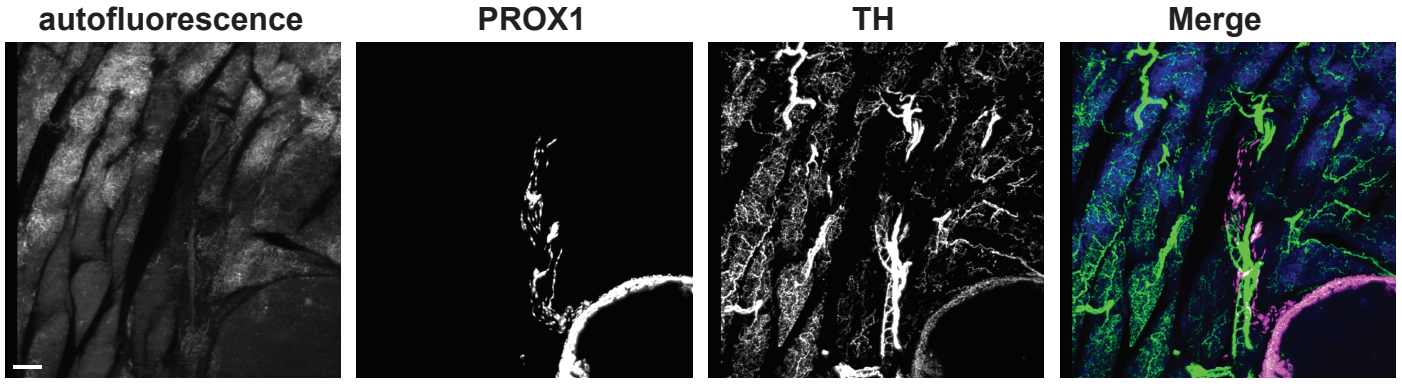

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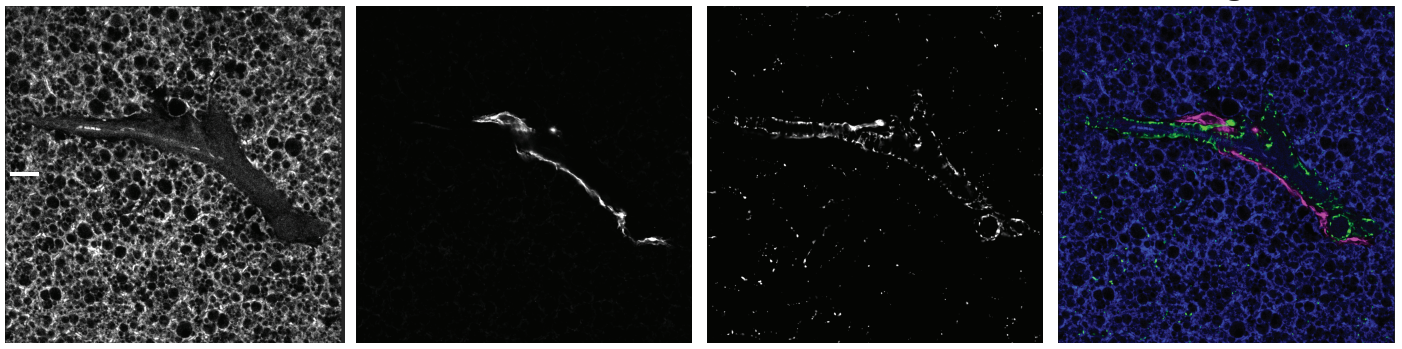
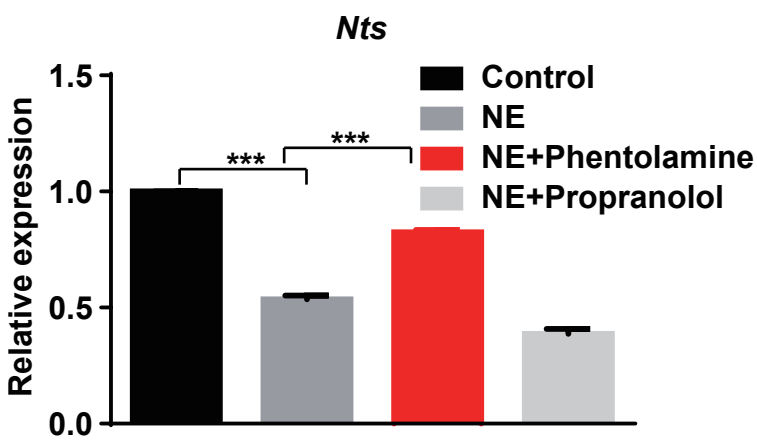
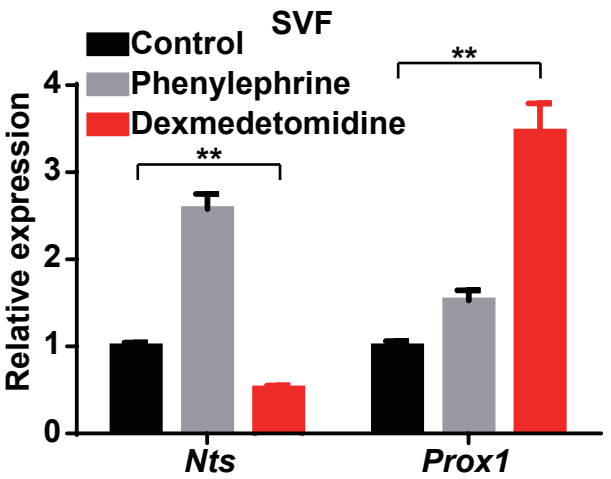


Prox1-CreERT2::Ai9

F



G



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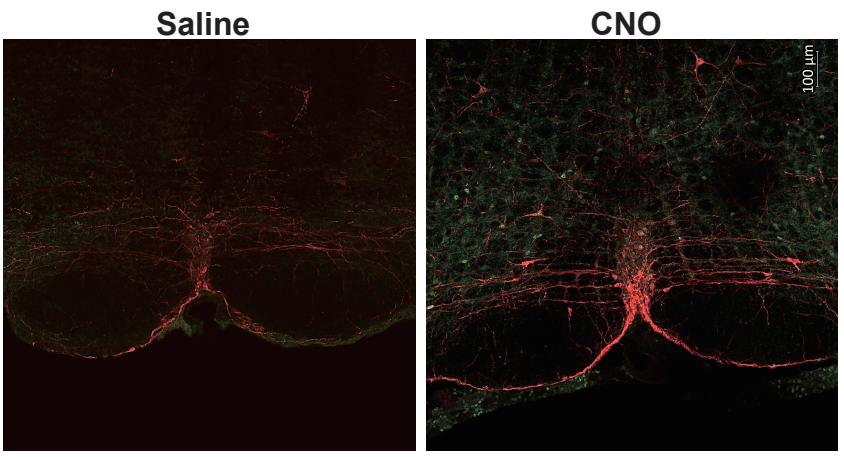
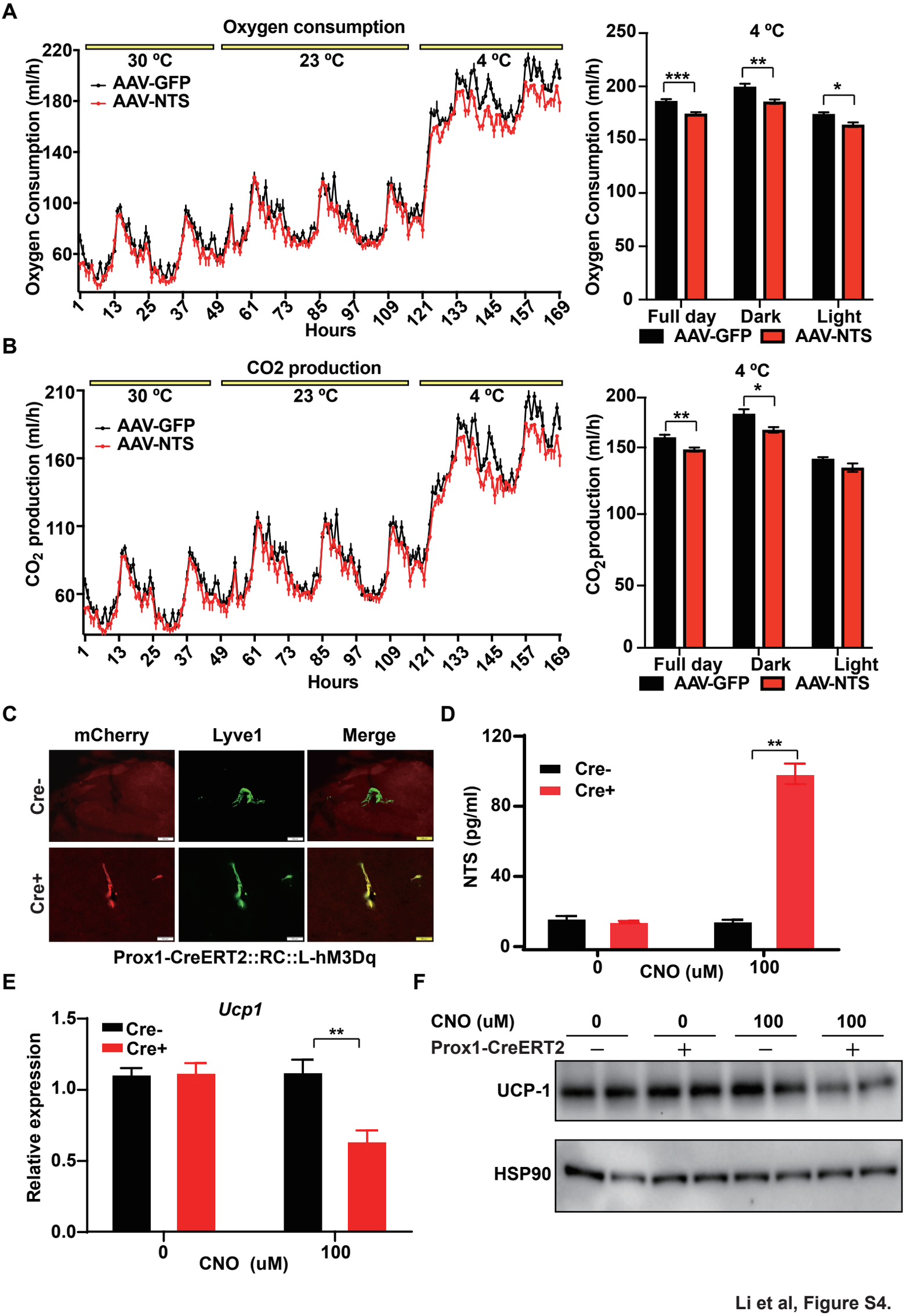
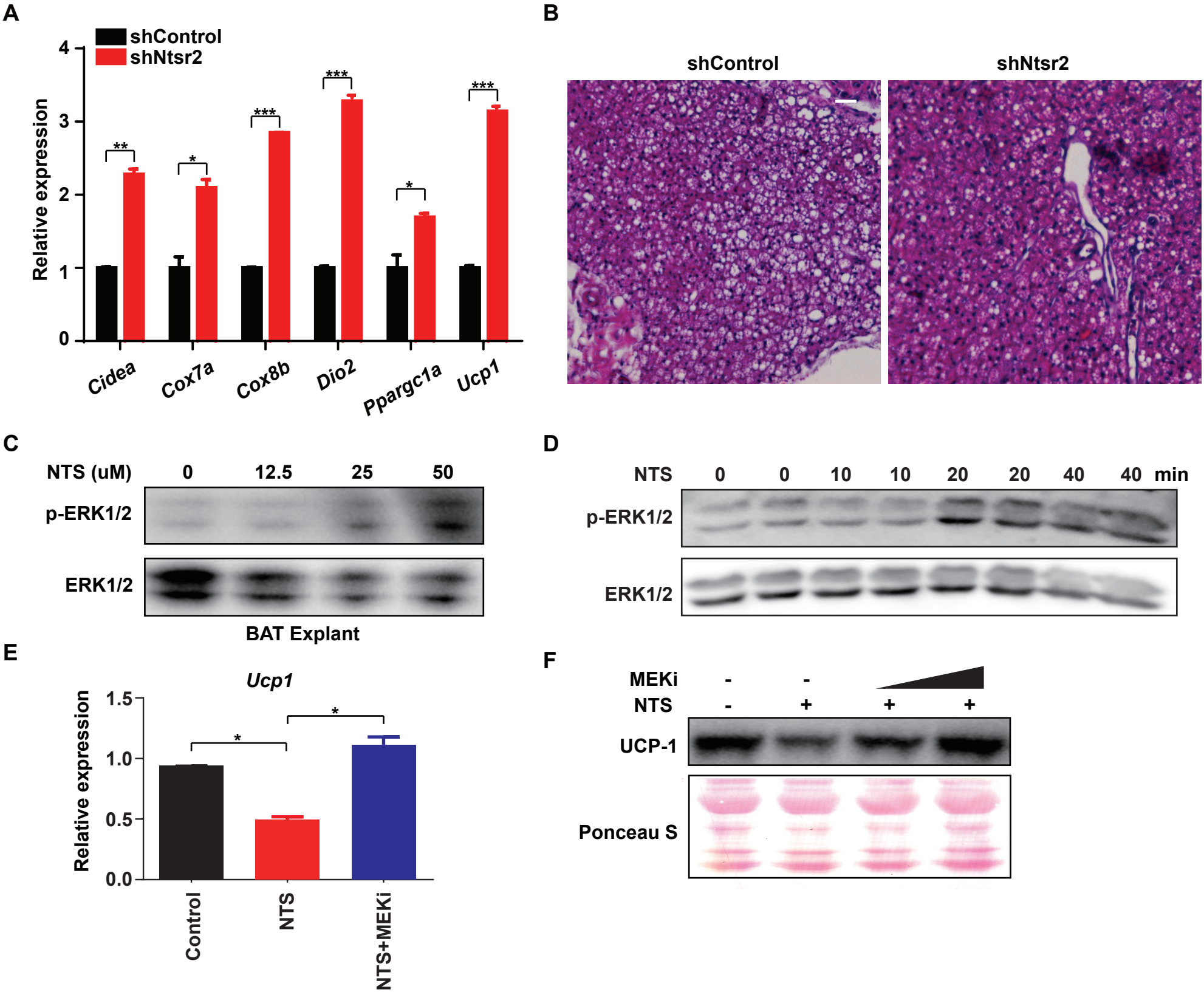


mCherry/c-Fos

Li et al, Figure S3.

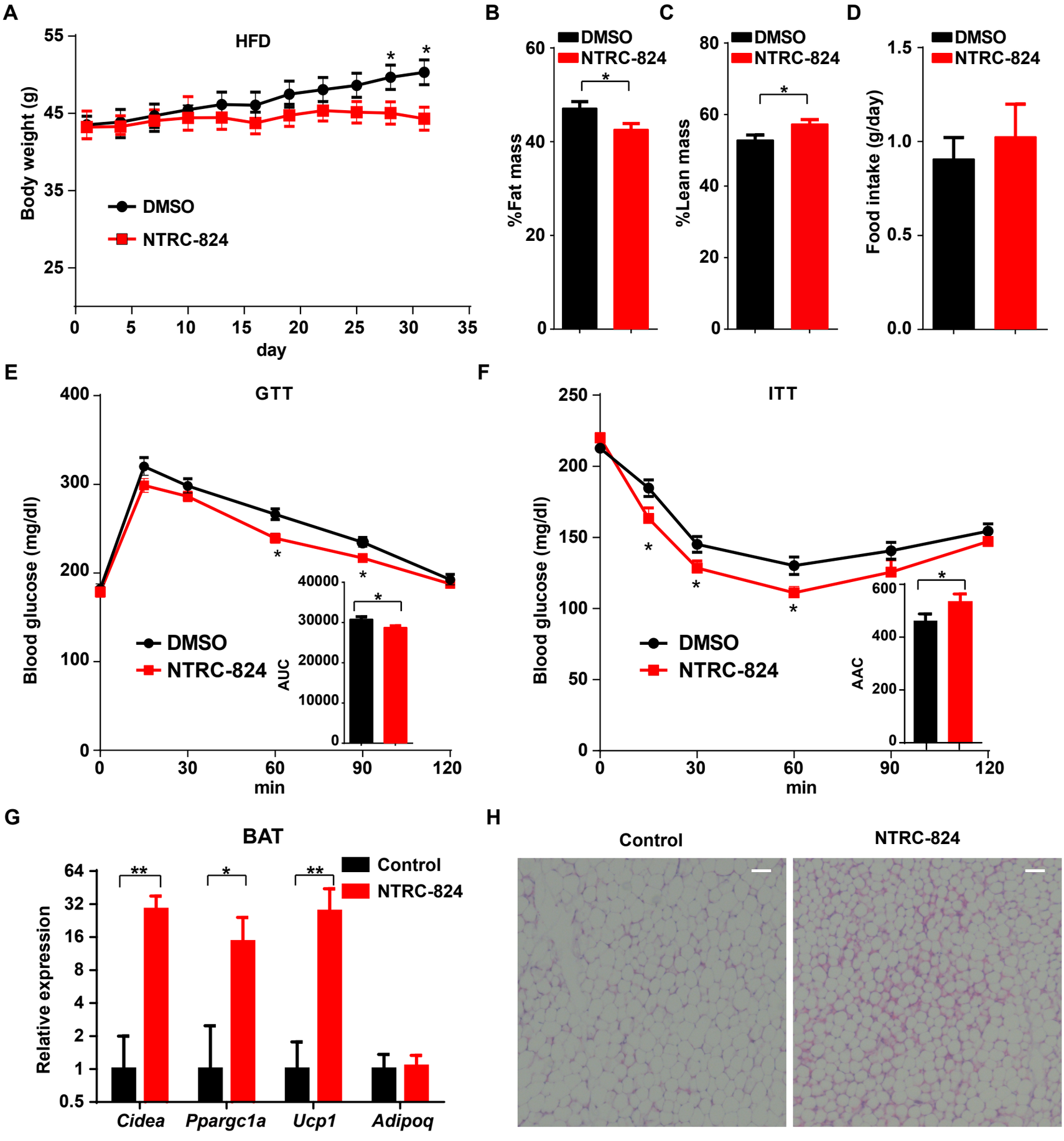


Li et al, Figure S4.



Li et al. Figure S5.

A
Neuromedin N
KIPYIL
Proneurotensin
QLYENKPRRPYIL
Neurotensin
B
NN (uM) 0 0 20 20 40 40
p-ERK
ERK
Relative abundance
1.5
1.0
0.5
0.0
Control
NN-20uM
NN-40uM
p-ERK1/ERK1
p-ERK2/ERK2
C
Relative expression
2.0
1.5
1.0
0.5
0.0
Control
NN-5uM
NN-10uM
NN-20uM
NN-40uM
D
Control
NN
UCP1
GAPDH
E
Control
NTS
NTS+NTRC-824
p-ERK
ERK
F
Relative abundance
2.0
1.0
0.0
* *
** **
Control
NTS
NTS+NTRC-824
p-ERK1/ERK1
p-ERK2/ERK2
G
BAT Explant
Relative expression
2.0
1.5
1.0
0.5
0.0
*
** **
******
** **
* **
Control
NTS
NTS+NTRC-824
Adipoq
Cidea
Dio2
Ppargc1a
Ucp1
H
BAT
Relative expression
8
6
4
2
0
***
***
Control
NTRC-824
Ucp1
Cidea
Ppargc1a
I
Control
NTRC-824
J
Hourly Plot
Relative Body Temperature (°C)
3
2
1
0
*
*
*
Control
NTRC-824
0 1 2 3 4 5 6 7 8 9 10 11 12
Hours
Li et al, Figure S6



Li et al, Figure S7.

Supplemental Table 1. qRT-PCR primers used in this study, Related to Figures 2-7

Gene	Left	Right
Nts	GTGTGGACCTGCTTGTCAGA	TGCTTTGCTGATCTTGGATG
Nts (OE)	CATCCAAGATCAGCAAAGCA	TTCTGGAGCTGGAAGATGGT
Ntsr2	TGCACGGTGCTAGTAAGTCG	GAGTTGACTTGGGCAGAAGC
Prox1	GGAGATGGCTGAGAACAAGC	AGACTTTGACCACCGTGTCC
Flt4	CCCAGCCATGTACAGAAGGT	GGCTGGAGTCAGAGGAGTTG
Pard6g	GATTACAACGCCCTGCATCT	AGGAAACACGGATGGAACAG
Reln	CCATACTGTGGCCATGACTG	CACCTGGTTGTCCATGTGAG
Ucp1	AGGCTTCCAGTACCATTAGGT	CTGAGTGAGGCAAAGCTGATTT
36b4	GAGGAATCAGATGAGGATATGGGA	AAGCAGGCTGACTTGGTTGC
Ppargc1a	GGACATGTGCAGCCAAGACTCT	CACTTCAATCCACCCAGAAAGCT
Cidea	TACTACCCGGTGTCCATTTCT	ATCACAACTGGCCTGGTTACG
Cox7a	ATGAGGGCCCTACGGGTCTC	CATTGTCGGCCTGGAAGAG
Cox8b	GAACCATGAAGCCAACGACT	GCGAAGTTCACAGTGGTTCC
Dio2	CAGTGTGGTGCACGTCTCCAATC	TGAACCAAAGTTGACCACCAG
Adipoq	TGTTCCTCTTAATCCTGCCCA	CCAACCTGCACAAGTTCCCTT
Cox1	GCCTTTCAGGAATACCACGA	AGGTTGGTTCCTCGAATGTG
Cox2	ACGAAATCAACAACCCCGTA	GGCAGAACGACTCGGTTATC