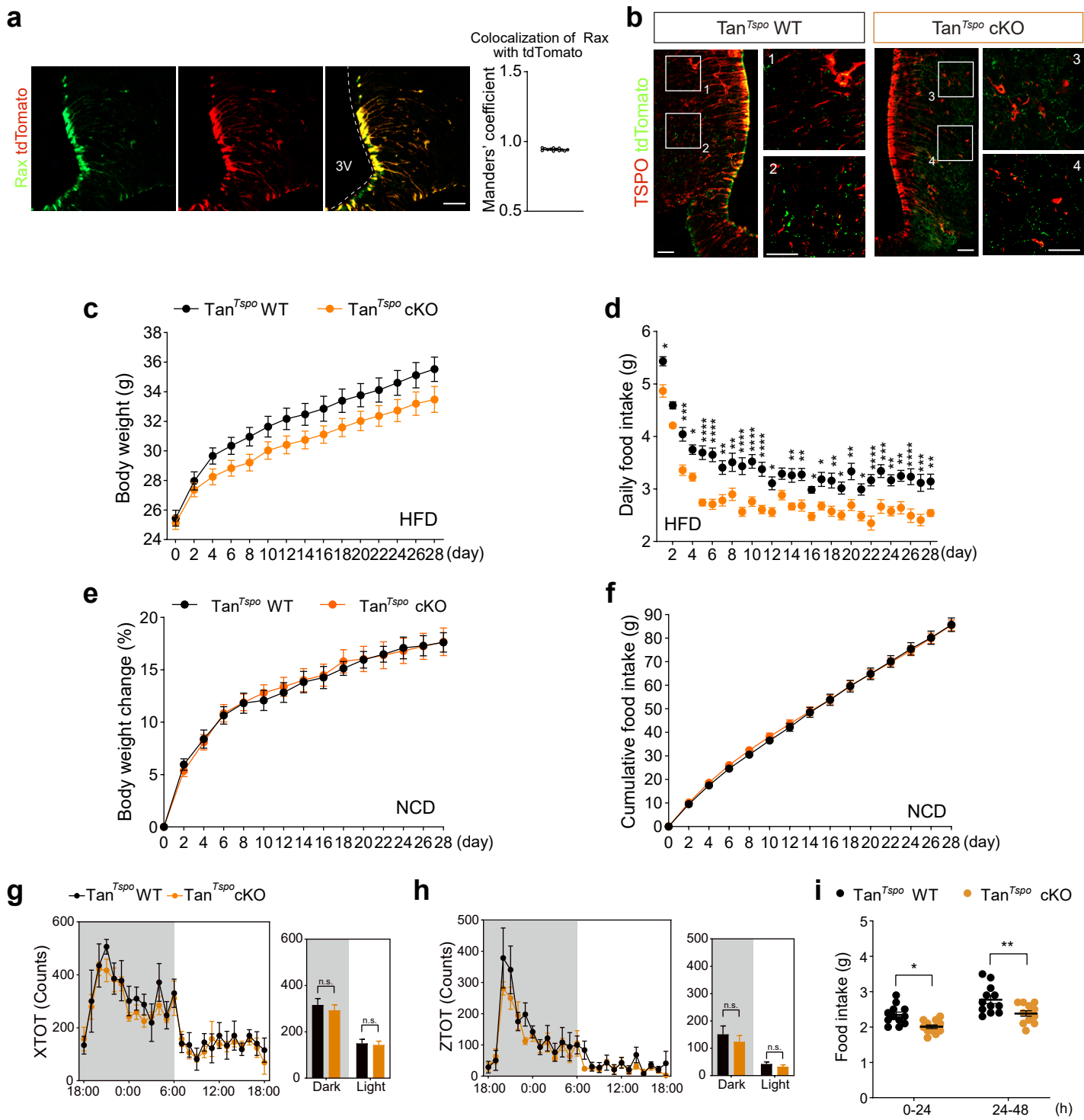
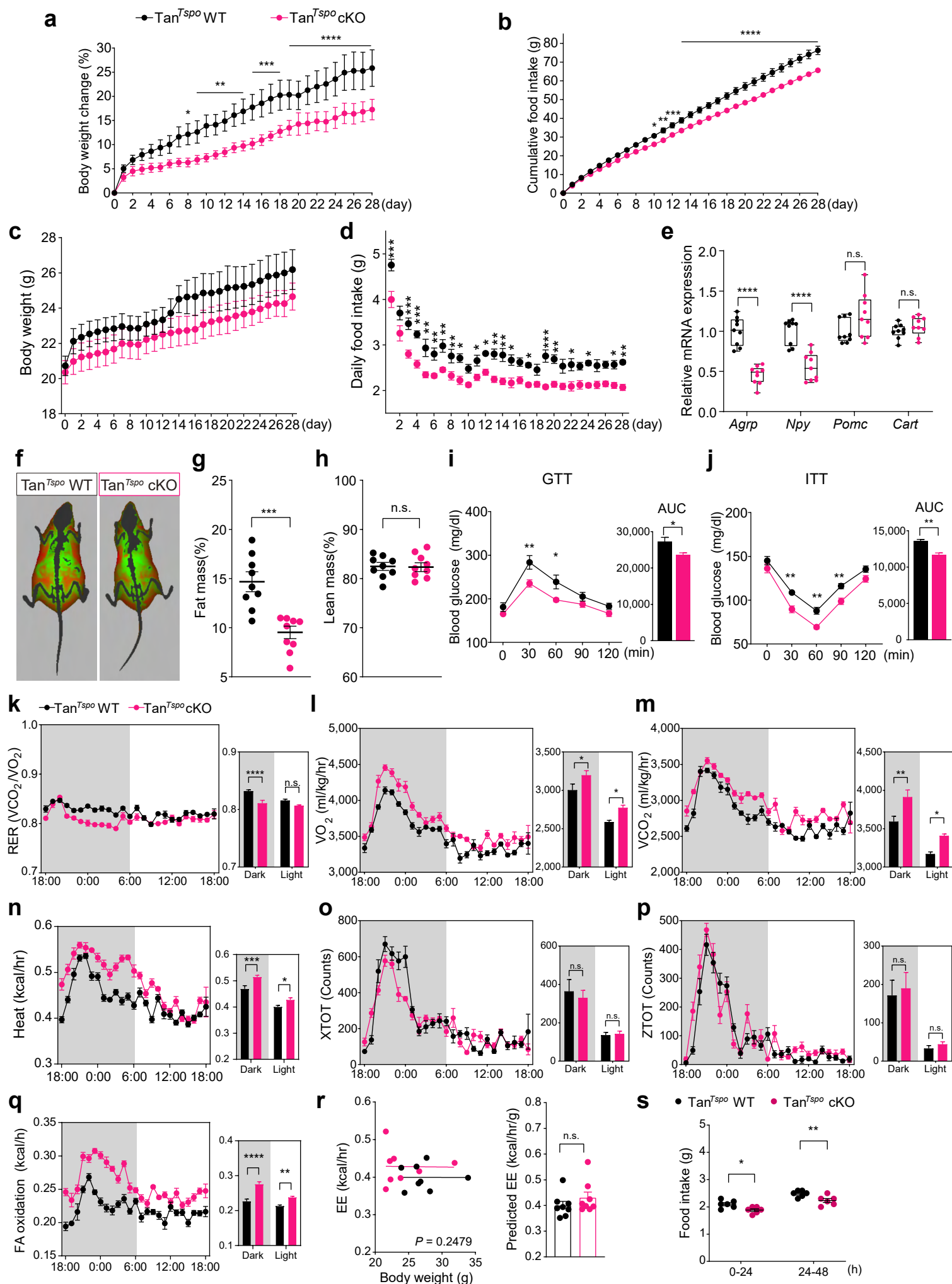


Supplementary Figure 1



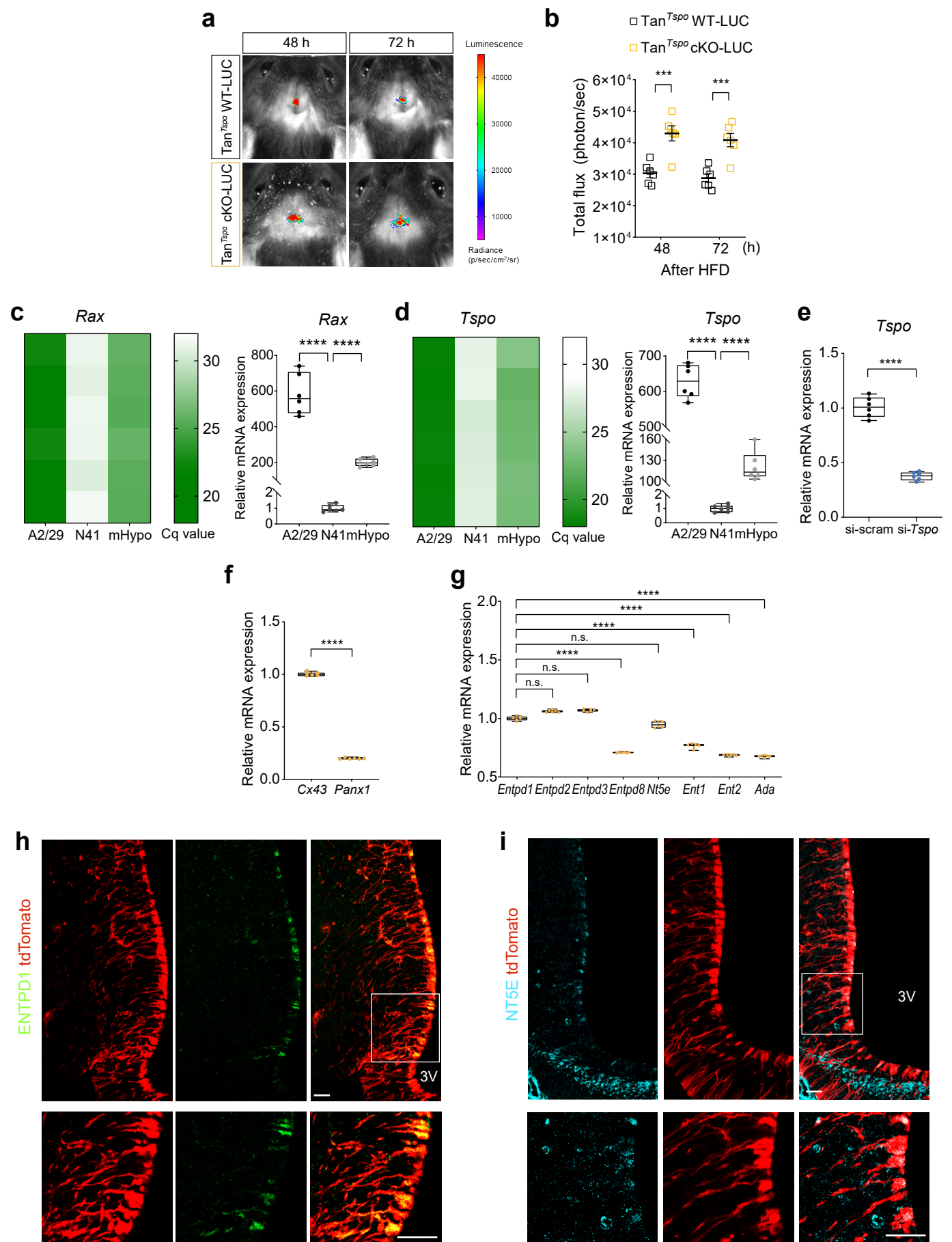
Supplementary Figure 1. Additional phenotypes of Tan^{Tspo} cKO versus Tan^{Tspo} WT male mice. Related to Figure 1. **a** Representative image and quantification of Rax (green) colocalized with tdTomato (red) in Tan^{Tspo} WT mice. **b** Representative images of TSPO (green) colocalized with tdTomato (red) in Tan^{Tspo} WT and Tan^{Tspo} cKO mice. White boxes indicated the mediobasal hypothalamus. **c** Body weight (g) and **d** daily food intake in Tan^{Tspo} WT and Tan^{Tspo} cKO male mice during 4 weeks of high-fat diet (HFD) (n=12 per group). **e** Body weight change (%) and **f** cumulative food intake during 4 weeks of normal chow diet (NCD) in Tan^{Tspo} WT and Tan^{Tspo} cKO male mice (n=5 per group). Locomotor activities of **g** X-axis and **h** Z-axis in Tan^{Tspo} WT and Tan^{Tspo} cKO male mice after 4 weeks of HFD. **i** Daily food intake during indirect calorimetry measurements in Tan^{Tspo} WT and Tan^{Tspo} cKO male mice fed HFD. Scale bar in **a** and **b**, 20 μ m. Data represent mean $\pm$ s.e.m. Significance was determined by two-way ANOVA with Sidak's multiple comparisons test (* p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001; n.s., not significant).

Supplementary Figure 2



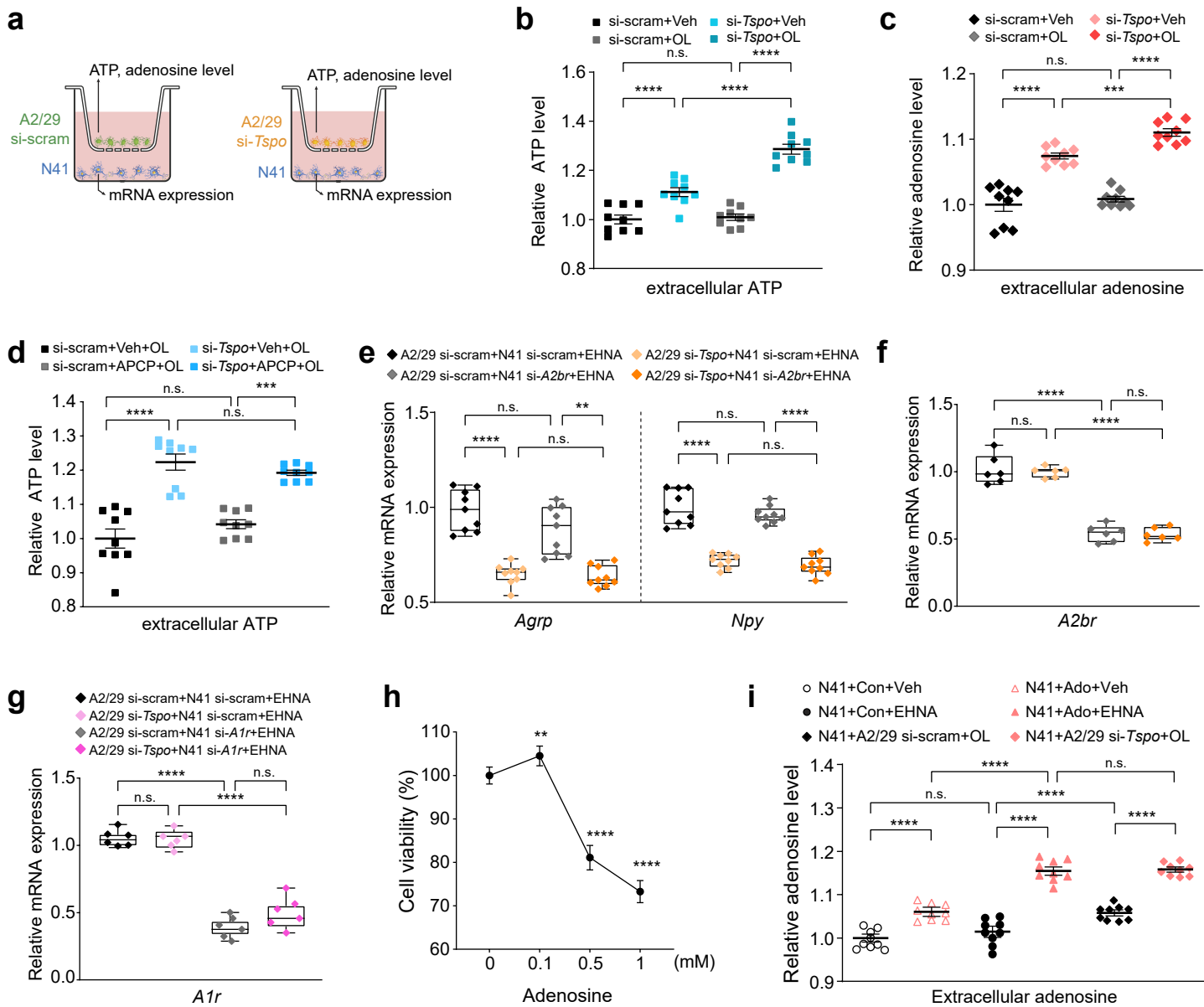
Supplementary Figure 2. Tan^{Tspo} cKO female DIO mice show a reduction of appetite and improvement of glucose homeostasis. **a** Body weight change (%), **b** cumulative food intake, **c** body weight (g), and **d** daily food intake during 4 weeks of HFD in Tan^{Tspo} WT and Tan^{Tspo} cKO female mice (n=9 per group). **e** Relative mRNA levels of neuropeptides in Tan^{Tspo} WT and Tan^{Tspo} cKO female mice after 4 weeks of HFD (n=9 per group). **f** Representative images of body composition. Red, fat mass; green, lean mass. **g** Fat mass and **h** lean mass after 4 weeks of HFD (n=9 per group). **i** GTT and **j** ITT in Tan^{Tspo} WT and Tan^{Tspo} cKO female mice (n=9 per group). **k–s** Indirect calorimetry parameters in Tan^{Tspo} WT and Tan^{Tspo} cKO female mice after 4 weeks of HFD (n=6 per group); **k** Respiratory exchange ratio (RER), **l** O_2 consumption, **m** CO_2 production, **n** heat generation, **o** and **p** locomotor activities X-axis and Z-axis, respectively. **q** Fatty acid (FA) oxidation, **r** regression plots of energy expenditure (EE) against body weight and predicted EE at the mean of body weight of each group, and **s** daily food intake during indirect calorimetry measurements. Data represent mean $\pm$ s.e.m. or boxes indicating the interquartile range with whiskers. Significance was determined by two-tailed unpaired Student's *t*-test (* p <0.05, ** p <0.01, *** p <0.001) in **g** and **h**, AUC in **i** and **j**, and using ANCOVA in **r** or otherwise by two-way ANOVA with Sidak's multiple comparisons test (* p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001). n.s., not significant; AUC, area under the curve.

Supplementary Figure 3



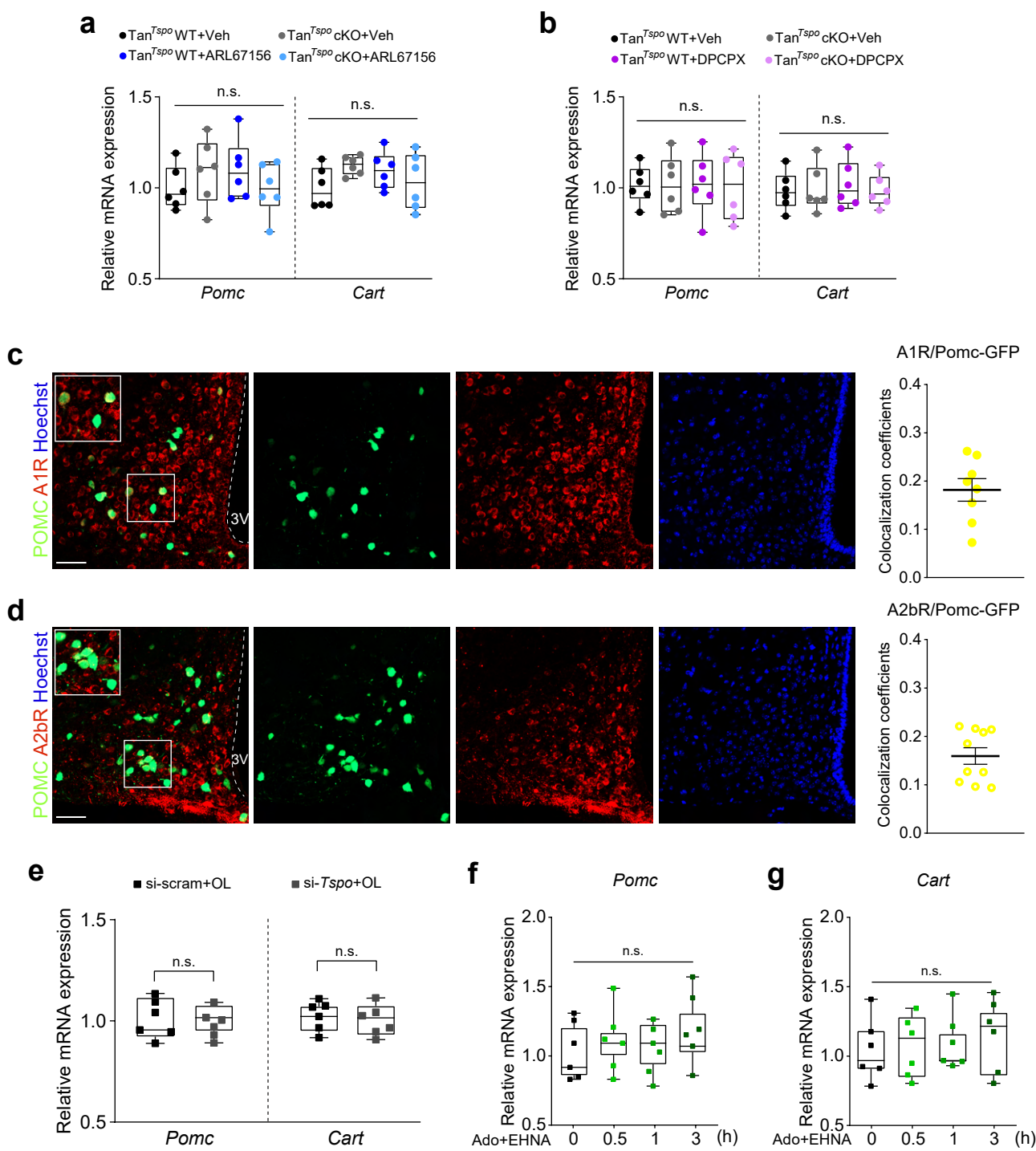
Supplementary Figure 3. Bioluminescence imaging of Tan^{Tspo} WT and Tan^{Tspo} cKO mice and expression of hemichannels, ectonucleotidases, and nucleoside transporters in the hypothalamus. a–f Related to Figure 2; **g–i** related to Figure 3. **a** Representative bioluminescence images and **b** quantification of the total flux in Tan^{Tspo} WT and Tan^{Tspo} cKO mice after 48 h and 72 h of HFD feeding (n=6 per group). **c** Heatmap of *Rax* quantification in A2/29 cells, N41 cells, and mouse hypothalamus (left) and relative mRNA expression levels of *Rax* in A2/29 cells, N41 cells, and mouse hypothalamus (right). **d** Heatmap of *Tspo* quantification in A2/29 cells, N41 cells, and mouse hypothalamus (left) and relative mRNA expression levels of *Tspo* in A2/29 cells, N41 cells, and mouse hypothalamus (right). **e** Efficiency of knockdown using si-*Tspo* in A2/29 cells (n=6 per group). Relative mRNA expression of **f** *Cx43* and *Panx1* and **g** ectonucleotidases and nucleoside transporters in the mouse hypothalamus (n=6). Representative immunostaining images of **h** ENTPD1 (green) and **i** NT5E (turquoise) in tanycytes (red). Lower images are enlargements of the white boxes in the upper images. Scale bars in **h** and **i**, 20 μ m. Data are mean $\pm$ s.e.m. or boxes indicating the interquartile range with whiskers. Significance was determined by two-way ANOVA with Sidak's multiple comparisons test (** p <0.001, **** p <0.0001) in **b**, two-way ANOVA with Tukey's multiple comparisons test (**** p <0.0001) in **c** and **d**, two-tailed unpaired Student's *t*-test (**** p <0.0001) in **e** and **f**, and one-way ANOVA with Dunnett's multiple comparisons test (**** p <0.0001) in **g**. mHypo, mouse hypothalamus; n.s., not significant.

Supplementary Figure 4



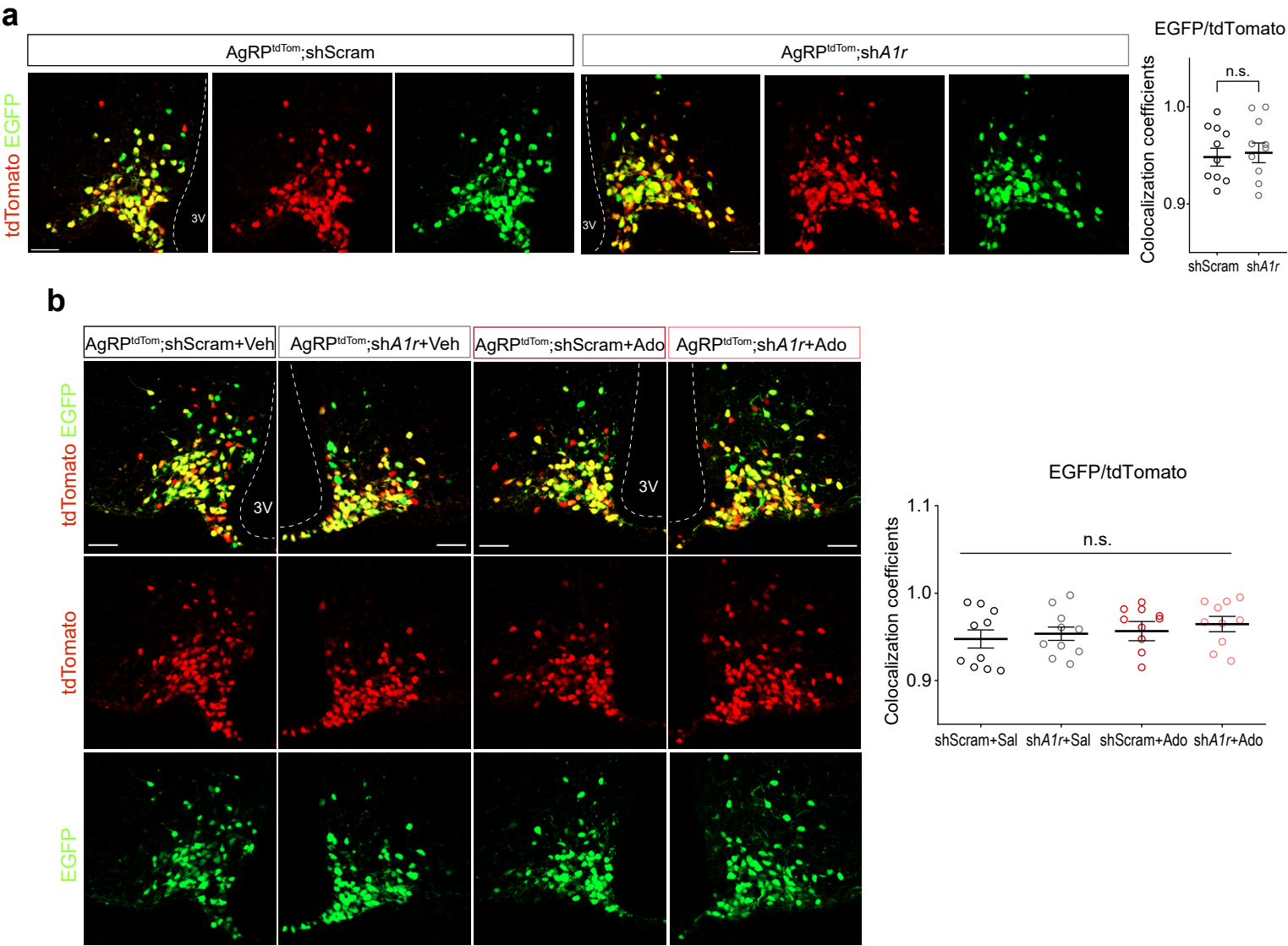
Supplementary Figure 4. Expression of orexigenic neuropeptides in co-culture systems with A2/29 and N41 cells and optimization of the concentration of adenosine. a–d Related to Figure 4; **e–i** related to Figure 5. **a** Schematic illustration of indirect co-culture using a transwell to investigate intercellular crosstalk between A2/29 cells and hypothalamic AGRP/NPY-expressing cells (mHypoE-N41, N41). A2/29 cells were grown on permeable membranes, transfected with si-RNAs, and then transferred over the wells containing N41 cells, which were separately cultured at the bottom of the wells. A2/29 cells were collected to examine the ATP and adenosine levels and N41 cells were used for analysis of mRNA expression. **b** Relative extracellular ATP levels and **c** relative extracellular adenosine levels in si-scram or si-*Tspo* A2/29 cells with or without OL treatment for 3 h (n=9 per group). **d** Relative extracellular ATP levels in si-scram or si-*Tspo* A2/29 cells indirectly co-cultured with N41 cells in the presence or absence of APCP under OL treatment for 3 h (n=9 per group). **e** Relative *Agrp* and *Npy* expression levels in si-scram or si-*A2br* N41 cells indirectly co-cultured with si-scram or si-*Tspo* A2/29 cells for 3 h under EHNA treatment (n=9 per group). Efficiency of knockdown using **f** si-*A2br* or **g** si-*A1r* in N41 cells (n=6 per group). **h** Concentration-dependent viability of N41 cells for 24 h (n=6). **i** Extracellular adenosine levels in N41 cells in the presence or absence of EHNA with or without adenosine (Ado) treatment and in N41 cells co-cultured with *Tspo*-knockdown A2/29 cells under OL treatment for 3 h (n=9 per group). Data are mean±s.e.m. or boxes indicating the interquartile range with whiskers and dot lines separate individual significance. Significance was determined by one-way ANOVA with Dunnett's multiple comparisons test (** $p<0.01$, **** $p<0.0001$) in **h** or otherwise by two-way ANOVA with Sidak's multiple comparisons test (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$). n.s., not significant; Con, untreated control.

Supplementary Figure 5



Supplementary Figure 5. Adenosine does not affect *Pomc* and *Cart* expression via adenosine A1 receptors. Related to Figure 6. **a** Relative mRNA levels of *Pomc* and *Cart* in Tan^{Tspo} WT and Tan^{Tspo} cKO mice injected with Veh or ARL67156 4 h after icv injection and HFD feeding (n=6 per group). **b** Relative mRNA levels of *Pomc* and *Cart* in Tan^{Tspo} WT and Tan^{Tspo} cKO mice injected with Veh or DPCPX 4 h after icv injection and HFD feeding (n=6 per group). **c** Representative image of A1R (red) expressed in POMC neurons (green) in the hypothalamus and colocalization coefficients of A1R with POMC neurons (n=8). **d** Representative image of A2bR (red) expressed at POMC neurons (green) in the hypothalamus and colocalization coefficients of A2bR with POMC neurons (n=10). **e** Relative mRNA levels of *Pomc* and *Cart* in hypothalamic POMC/CART-expressing cells (mHypoE-N43/5, N43/5) indirectly co-cultured with si-scram or si-*Tspo* A2/29 cells under 0.06 mM OL treatment for 3 h (n=6 per group). Time course of relative **f** *Pomc* and **g** *Cart* expression levels in N43/5 cells treated with Ado+EHNA (n=6 per group). Scale bars in **c** and **d**, 20 μ m. Data are mean $\pm$ s.e.m. or boxes indicating the interquartile range with whiskers and dot lines separate individual significance. Significance was determined by two-way ANOVA with Sidak's multiple comparisons test in **a** and **b**, two-tailed unpaired Student's *t*-test in **e**, and by one-way ANOVA with Dunnett's multiple comparisons test in **f** and **g**. n.s., not significant.

Supplementary Figure 6



Supplementary Figure 6. The efficiency of AAV transduction in $\text{AgRP}^{\text{tdTom}};\text{shScram}$ and $\text{AgRP}^{\text{tdTom}};\text{ShA1r}$. Related to Figure 8. **a** Representative images and analysis of the functional efficiency of AAV in $\text{AgRP}^{\text{tdTom}};\text{shScram}$ and $\text{AgRP}^{\text{tdTom}};\text{shA1r}$ mice (n=10 per group). **b** Representative images and colocalization coefficients of tdTomato (red) and EGFP (green) in $\text{AgRP}^{\text{tdTom}};\text{shScram}$ and $\text{AgRP}^{\text{tdTom}};\text{shA1r}$ mice with or without Ado icv injection (n=10 per group). Scale bar, 20 μm . Data are mean $\pm$ s.e.m. Significance was determined by two-tailed unpaired Student's *t*-test in **a** and by two-way ANOVA with Sidak's multiple comparisons test in **b**. n.s., not significant.

Supplementary Table 1. The sequence of primers for genotyping PCR

Primer	Sequence (5' to 3')
Tspo ^{fl/fl} forward	GGA TTA CCA CAC CCA ACC AG
Tspo ^{fl/fl} reverse	TAG GAG TGC AAA GCC AGT CA
Rax-Cre ^{ERT2} forward-common	CCC TGA GGC TAA ACT TGC AG
Rax-Cre ^{ERT2} reverse-wild	AGG TGT CTA GGA TGC CGT CT
Rax-Cre ^{ERT2} reverse-mutant	AGG CAA ATT TTG GTG TAC GG
Ai14-tdTomato forward-wild	AAG GGA GCT GCA GTG GAG TA
Ai14-tdTomato reverse-wild	CCG AAA ATC TGT GGG AAG TC
Ai14-tdTomato forward-mutant	GGC ATT AAA GCA GCG TAT CC
Ai14-tdTomato reverse-mutant	CTG TTC CTG TAC GGC ATG G
AgRP-ires-Cre	GGG CCC TAA GTT GAG TTT TCC T
AgRP-ires-Cre	GAT TAC CCA ACC TGG GCA GAA C
AgRP-ires-Cre	GGG TCG CTA CAG ACG TTG TTT G

Supplementary Table 2. List of antibodies

Antibodies	Manufacturer
Phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204)	Cell Signaling Technology (#4370, #9101)
P44/42 MAPK (ERK1/2)	Cell Signaling Technology (#9107)
Phospho-AKT (Ser473)	Cell Signaling Technology (#4060)
AKT	Cell Signaling Technology (#9272)
Phospho-CREB (Ser133)	Cell Signaling Technology (#9198)
CREB	Cell Signaling Technology (#9197)
GAPDH	Cell Signaling Technology (#2118)
TSPO	Abcam (ab109497)
A1R	Invitrogen (MA5-38140)
A2bR	Abcam (ab229671)
Rax	Takara (M229)
Cx43	Sigma-Aldrich (C6219)
ENTPD1	Cell Signaling Technology (#14481)
NT5E	Cell Signaling Technology (#13160)
Alexa flour® 488 Donkey anti-chicken IgG (H+L)	Jackson ImmunoResearch (703-545-155)
Alexa flour® 647 Donkey anti-chicken IgY(IgG) (H+L)	Jackson ImmunoResearch (703-605-155)
Alexa flour® 488 Donkey anti-mouse IgG (H+L)	Jackson ImmunoResearch (715-545-150)
Alexa flour® 647 Donkey anti-mouse IgG (H+L)	Jackson ImmunoResearch (715-605-151)
Alexa flour® 488 Donkey anti-sheep IgG (H+L)	Jackson ImmunoResearch (713-545-003)
Alexa flour® 647 Donkey anti-sheep IgG (H+L)	Jackson ImmunoResearch (713-605-147)
Alexa flour® 488 Donkey anti-rabbit IgG (H+L)	Jackson ImmunoResearch (711-545-152)
Alexa flour® 647 Donkey anti-rabbit IgG (H+L)	Jackson ImmunoResearch (711-605-152)
Alexa flour® 488 Donkey anti-guinea pig IgG (H+L)	Jackson ImmunoResearch (706-545-148)

Supplementary Table 3. The sequence of primers for qRT-PCR

Gene	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
<i>Cx43</i>	TCA TCT TCA TGC TGG TGG TGT CCT	TGG TGA GGA GCA GCC ATT GAA GTA
<i>Panx1</i>	GCT CCC TGC AGA GCG AGT CTG G	CTC TTG GCA GCC TTG ATG GCG C
<i>Entpd1</i>	AGC TGC CCC TTA TGG AAG AT	TCA GTC CCA CAG CAA TCA AA
<i>Entpd2</i>	TTC CTG GGA TGT CAG GTC TC	GTC TCT GGT GCT TGC CTT TC
<i>Entpd3</i>	ACC TGT CCC GTG CTT AAA TG	AGA CAG AGT GAA GCC CCT GA
<i>Entpd8</i>	CAC ACA GGA CCT TCT GAG CA	AGC CTT CTG AGG TGG CAC TA
<i>Nt5e</i>	CAG GAA ATC CAC CTT CCA AA	AAC CTT CAG GTA GCC CAG GT
<i>Ent1</i>	CTT GGG ATT CAG GGT CAG AA	ATC AGG TCA CAC GAC ACC AA
<i>Ent2</i>	CAT GGA AAC TGA GGG GAA GA	GTT CCA AAG GCC TCA CAG AG
<i>Ada</i>	ACA CCC GCA TTC AAC AAA CC	TGC CTC TCT TCT TGC CAA AGT
<i>Agrp</i>	CTG CAG ACC GAG CAG AAG A	TGC GAC TAC AGA GGT TCG TG
<i>Npy</i>	CAG AAA ACG CCC CCA GAA	AAA AGT CGG GAG AAC AAG TTT CAT T
<i>Gapdh</i>	ATC ACT GCC ACC CAG AAG AC	ACA CAT TGG GGG TAG GAA CA
<i>A1r</i>	GCA AGA GGC GGA CAT CAC A	TCA CTC ACC CTA GAA GCC ATA CTC
<i>A2ar</i>	CTT TGT CCT GGT CCT CAC GC	TCG CAA TGA TGC CCT TCG
<i>A2br</i>	TCT TCC TCG CCT GCT TCG T	CTC GTG TCC CAG TGA CCA AAC
<i>A3r</i>	CGG GAG TTC AAG ACA GCT AAG T	CAC ATT GCG ACA TCT GGT ATC T
<i>Tspo</i>	TGC AGA AAC CCT CTT GGC ATC	GAA ACC TCC CAG CTC TTT CC
<i>Pomc</i>	GAA CAG CCC CTG ACT GAA AA	ACG TTG GGG TAC ACC TTC AC
<i>Cart</i>	CGA GAA GAA GTA CGG CCA AGT CC	GGA ATA TGG GAA CCG AAG GTG G