

Supplementary Information

TOX3 in hypothalamic POMC-lineage cells regulates energy balance via the PTEN-AKT signaling axis

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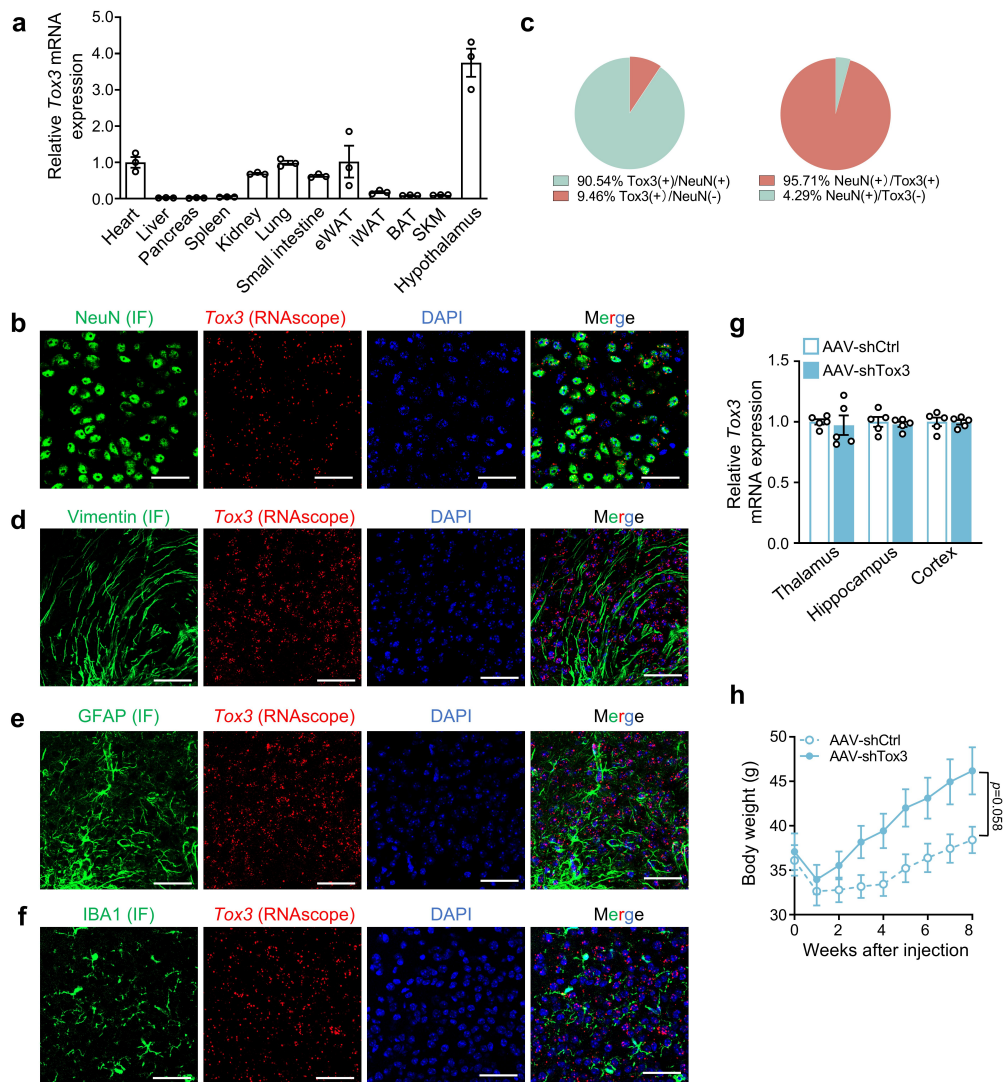


Figure S1. The expression patterns of TOX3 in hypothalamus, related to Figure 1. a *Tox3* mRNA expression across various tissues from WT C57BL/6J male mice (n=3). **b** Representative confocal images showing NeuN immunoreactivity (green) and *Tox3* RNAscope signals (red) in the ARC of WT C57BL/6J male mice. Scale bar, 25 μ m. **c** Quantification of hypothalamic *Tox3*-positive cells co-expressing NeuN (left) and NeuN-positive neurons co-expressing *Tox3* (right). **d-f** Representative confocal images of *Tox3* RNAscope signals (red) with immunostaining for: Vimentin (**d**, tanycyte marker, green), GFAP (**e**, astrocyte marker, green), and IBA1 (**f**, microglia marker, green) in the hypothalamus of WT male mice. Scale bar, 25 μ m. **g** Relative mRNA expression of *Tox3* in thalamus, hippocampus, and cortex of male mice injected with AAV-shCtrl or AAV-sh*Tox3* into the hypothalamus. **h** Body weight of male mice injected with AAV-shCtrl or AAV-sh*Tox3* into the hypothalamus. ARC, arcuate nucleus; WT, wild-type; GFAP, glial fibrillary acidic protein; IBA1, ionized calcium binding adapter molecule 1. Data are mean $\pm$ SEM. Numbers represents biological replicates. Significance was analyzed by two-way ANOVA

with Sidak's test (**h**) or unpaired two-tailed Student's t test (all others). Source data are provided as a Source Data file.

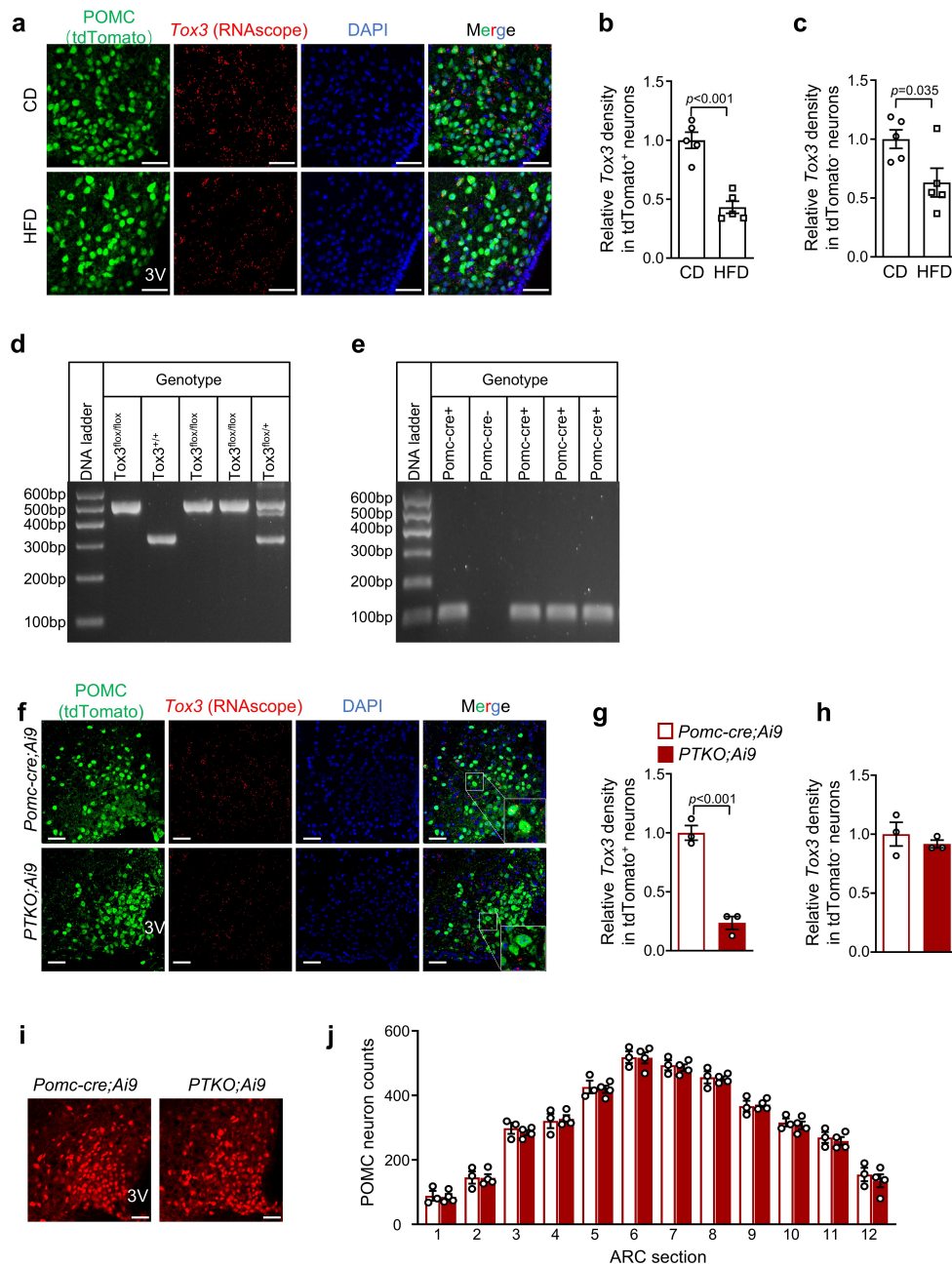


Figure S2. Expression pattern of TOX3 in hypothalamic POMC-lineage cells and characterization of mice with *Tox3* specifically deleted in POMC-lineage cells. **a** Representative confocal images of POMC (tdTomato⁺, green), *Tox3* mRNA (red), and DAPI (blue) in the ARC of *Pomc-cre;Ai9* male mice fed a standard CD or HFD for 16 weeks. Scale bar, 50 μ m. **b, c** Integrated density quantification of *Tox3* in tdTomato⁺ (**b**) and tdTomato⁻ (**c**) neurons from **a**. **d** Genotyping agarose-gel electrophoresis of *Tox3*^{+/+}, *Tox3*^{flx/+}, and *Tox3*^{flx/flx} mice. **e** Genotyping agarose-gel electrophoresis of *Pomc-Cre*⁺ and *Pomc-Cre*⁻ mice. **f** Representative confocal images of POMC (tdTomato⁺, green), *Tox3* mRNA (red),

and DAPI (blue) in the ARC of *Pomc-cre;Ai9* and *PTKO;Ai9* male mice. Scale bar, 50 μm . **g**, **h** Integrated density quantification of *Tox3* signal in tdTomato⁺ (**g**) and tdTomato⁻ (**h**) neurons from **f**. **i** Representative images of tdTomato⁺ cells in the ARC of *Pomc-cre;Ai9* and *PTKO;Ai9* male mice. Scale bar, 50 μm . **j** Distribution and number of POMC-lineage cells throughout the ARC of *Pomc-cre;Ai9* and *PTKO;Ai9* male mice (n=3 for *Pomc-cre;Ai9*, n=4 for *PTKO;Ai9*). ARC, arcuate nucleus; CD, chow diet; HFD, high fat diet; 3V, third ventricle; *Pomc-cre;Ai9*, *Pomc-Cre* mice with tdTomato-labeled POMC-lineage cells; *PTKO;Ai9*, POMC-lineage cell-specific *Tox3*-deficient mice with tdTomato-labeled POMC-lineage cells. Data are mean $\pm$ SEM. Numbers represents biological replicates. Significance was analyzed by unpaired two-tailed Student's t test. Source data are provided as a Source Data file.

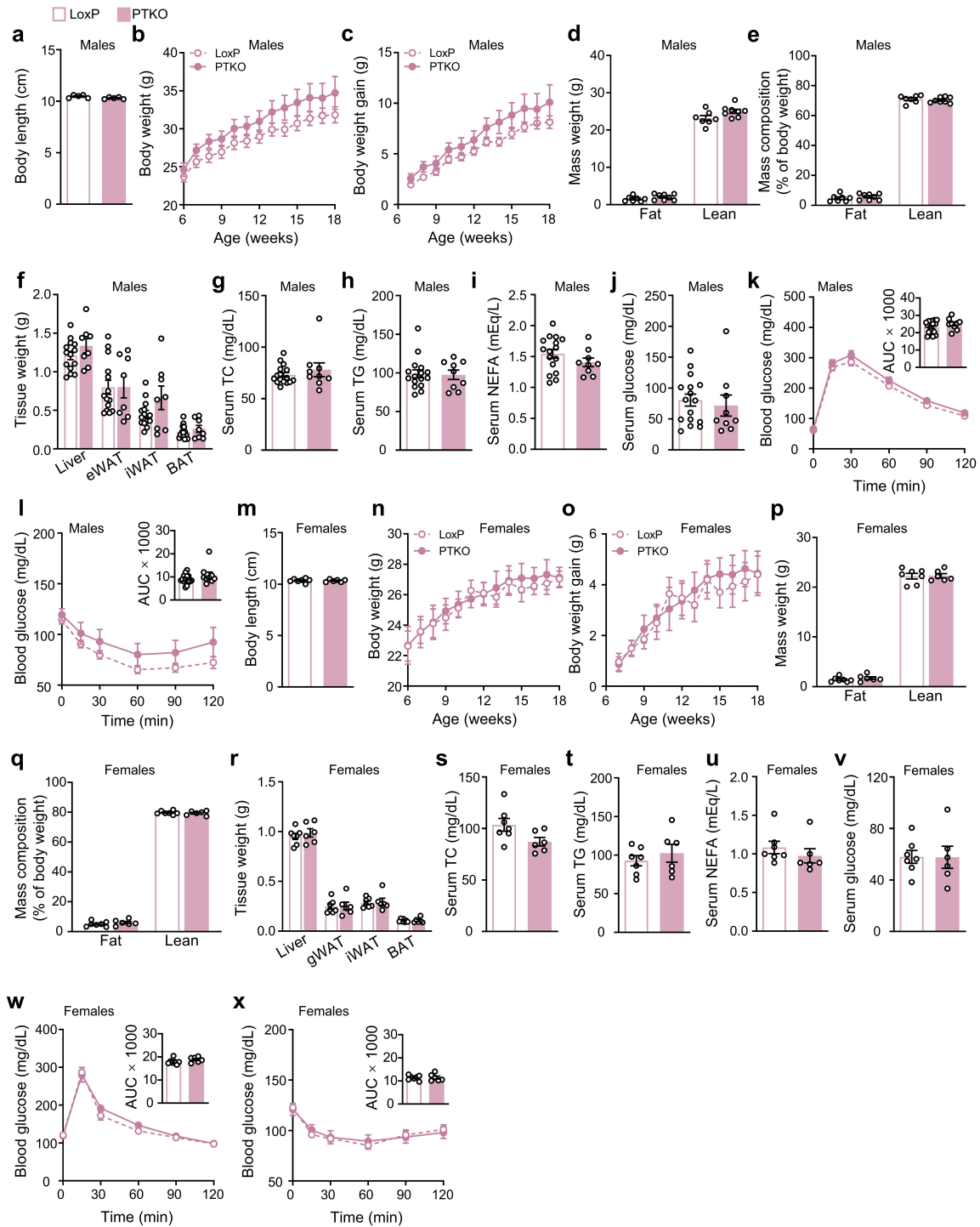


Figure S3. Deletion of TOX3 in POMC-lineage cells does not affect systemic metabolism in both male and female mice fed a CD. **a-l** Male LoxP and PTKO mice fed a CD. **a** Body length (n=5 per group). **b, c** Body weight (**b**) and body weight gain (**c**) (n=16 for LoxP, n=9 for PTKO). **d, e** Fat and lean mass weight (**d**) and body composition (**e**) (n=7 for LoxP, n=8 for PTKO). **f** Liver and adipose depots weight (n=14 for LoxP, n=8 for PTKO). **g-j** Serum levels of TC (**g**), TG (**h**), NEFA (**i**), and glucose (**j**) (n=16 for LoxP, n=9 for PTKO). **k, l** GTT (**k**) and ITT (**l**) (n=16 for LoxP, n=9 for PTKO). **m-x** Female LoxP and PTKO mice fed a CD.

m Body length (n=7 for LoxP, n=6 for PTKO). **n, o** Body weight (**n**) and body weight gain (**o**) (n=7 for LoxP, n=6 for PTKO). **p, q** Fat and lean mass (**p**) and body composition (**q**) (n=7 for LoxP, n=6 for PTKO). **r** Liver and adipose depots weight (n=7 for LoxP, n=6 for PTKO). **s-v** Serum levels of TC (**s**), TG (**t**), NEFA (**u**), and glucose (**v**) (n=7 for LoxP, n=6 for PTKO). **w, x** GTT (**w**) and ITT (**x**) (n=7 for LoxP, n=6 for PTKO). CD, chow diet; LoxP, *Tox3^{fllox/fllox}* mice; PTKO, *Pomc-cre;Tox3^{fllox/fllox}* mice; TC, total cholesterol; TG, triglycerides; NEFA, non-esterified fatty acid; GTT, glucose tolerance test; ITT, insulin tolerance test; AUC, area under the curve. Data are mean±SEM. Numbers represents biological replicates. Significance was analyzed by two-way ANOVA with Sidak's test (**b, c, k, l, n, o, w, x**) or unpaired two-tailed Student's t test (all others). Source data are provided as a Source Data file.

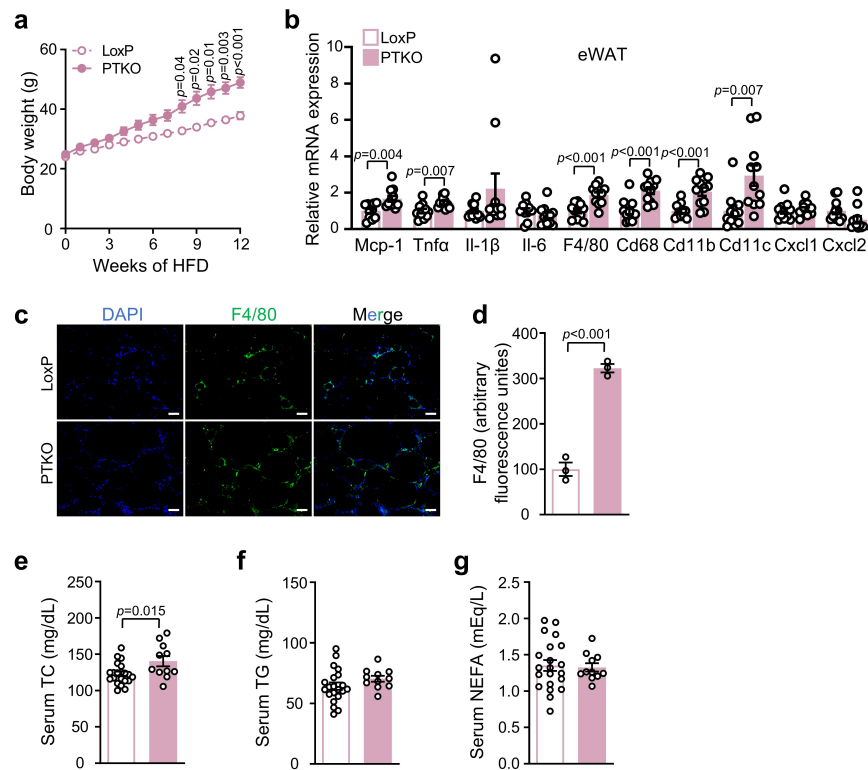


Figure S4. Effect of POMC-lineage cell-specific *Tox3* deletion on chronic inflammation infiltration in eWAT and serum lipids of HFD-fed male mice, related to Figure 2. **a** Body weight curve (n=18 for LoxP, n=12 for PTKO). **b** Relative mRNA expression of inflammation-related genes in eWAT (n=11 per group). **c, d** Representative images (**c**) and quantification (**d**, n=3 per group) of F4/80 immunofluorescence staining in eWAT. **e-g** Serum levels of TC (**e**), TG (**f**), and NEFA (**g**) (n=21 for LoxP, n=11 for PTKO). Scale bar, 50 μ m. HFD, high-fat diet; LoxP, *Tox3^{fllox/fllox}* mice; PTKO, *Pomc-cre;Tox3^{fllox/fllox}* mice; TC, total cholesterol; TG, triglycerides; NEFA, non-esterified fatty acid. Data are mean $\pm$ SEM. Numbers represents biological replicates. Significance was analyzed by two-way ANOVA with Sidak's test (**a**) or unpaired two-tailed Student's t test (all others). Source data are provided as a Source Data file.

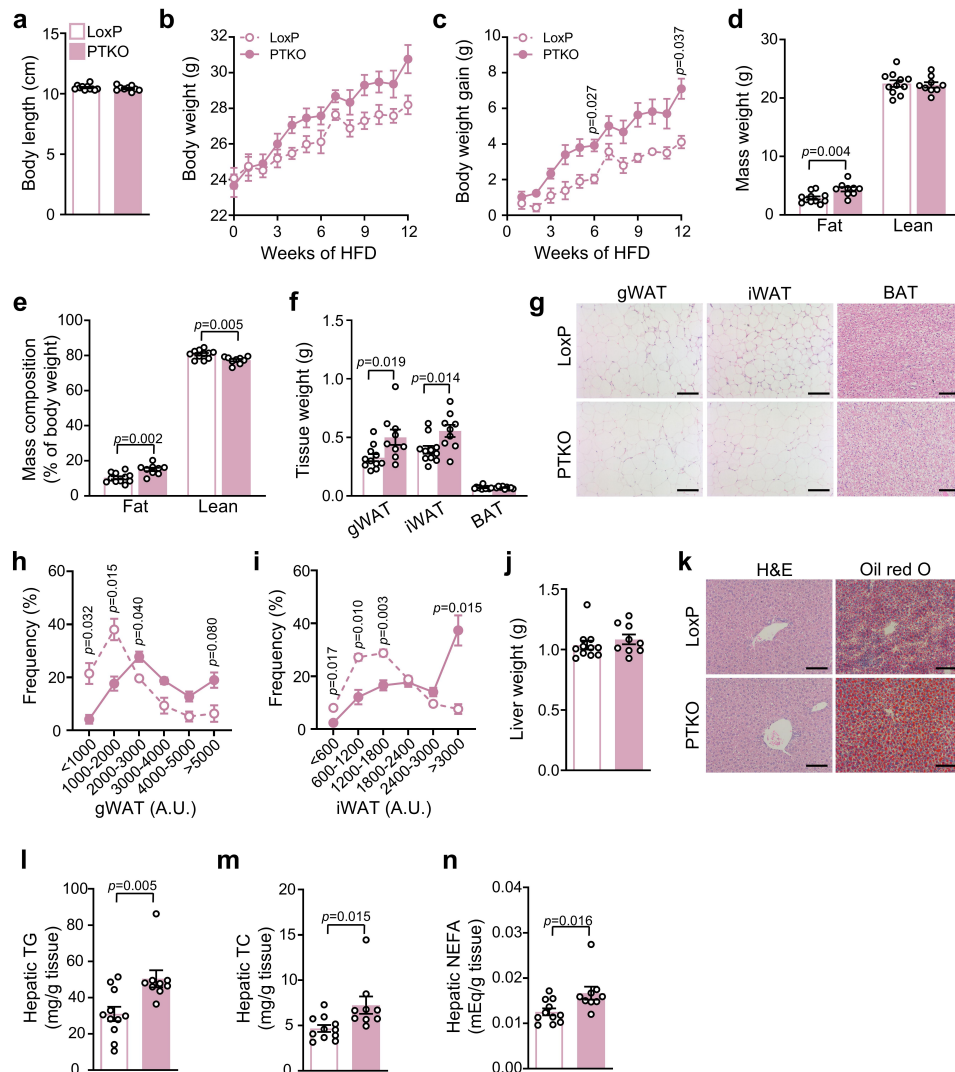


Figure S5. Deletion of TOX3 in POMC-lineage cells promotes fat accumulation and metabolic dysfunction in female mice upon HFD feeding. **a** Body length (n=11 for LoxP, n=9 for PTKO). **b, c** body weight (**b**) and body weight gain (**c**) (n=6 for LoxP, n=5 for PTKO). **d, e** Fat and lean mass (**d**) and body composition (**e**) (n=11 for LoxP, n=10 for PTKO). **f** Adipose tissue weights (n=11 for LoxP, n=9 for PTKO). **g** H&E staining of gWAT, iWAT, and BAT. Scale bar, 100 μ m. **h, i** Adipocyte size distribution of gWAT (**h**) and iWAT (**i**) (n=6 per group). **j** Liver weight (n=11 for LoxP, n=9 for PTKO). **k** Representative images of H&E- and Oil Red O- stained liver sections. Scale bar, 100 μ m. **l-n** Hepatic TG (**l**), TC (**m**), and NEFA (**n**) content (n=11 for LoxP, n=9 for PTKO). LoxP, *Tox3^{lox/lox}* mice; PTKO, *Pomc-cre;Tox3^{lox/lox}* mice; gWAT, gonadal white adipose tissue; iWAT, inguinal WAT; BAT, brown adipose tissue; TG, triglycerides; TC, total cholesterol; NEFA, non-esterified fatty acid. Data are mean $\pm$ SEM. Numbers represents biological replicates. Significance was analyzed by two-way ANOVA with Sidak's test (**b, c**) or unpaired two-tailed Student's t test (all others). Source data are provided as a Source Data file.

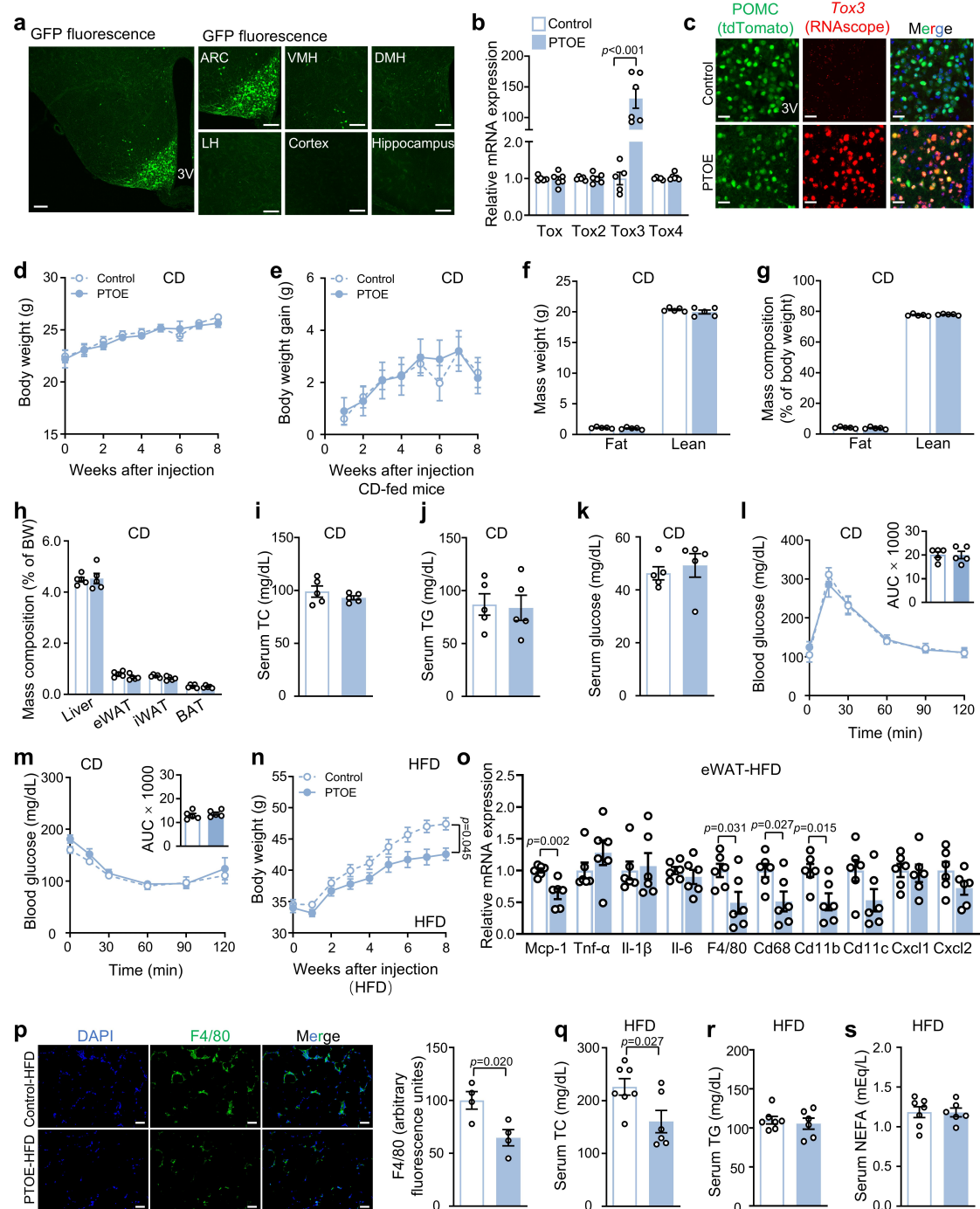


Figure S6. Effect of hypothalamic POMC-lineage cell-specific TOX3 overexpression on systemic metabolism in male mice fed a CD or HFD, related to Figure 3. **a** Representative images of GFP expression in the hypothalamus (left) and in specific brain regions (right) 8 weeks after the injecting AAV-GFP into the ARC of *Pomc*-Cre mice. Scale bars, 100 μ m (left) and 50 μ m (right). **b** Hypothalamic mRNA levels of *Tox* family genes of Control and PTOE mice ($n=5$ for Control, $n=6$ for PTOE). **c** Representative confocal images showing POMC (tdTomato⁺, green), *Tox3* mRNA (red), and DAPI (blue) fluorescence in the ARC of *Pomc*-Cre;*Ai9* mice injected with AAV-GFP or AAV-Tox3. Scale bar, 25 μ m. **d-m** Control and

PTOE mice maintained on a CD. **d, e** Body weight (**d**) and body weight gain (**e**) (n=5 per group). **f, g** Fat and lean mass (**f**) and body composition (**g**) (n=5 per group). **h** Liver and adipose depots weights (n=5 per group). **i-k** Serum levels of TC (**i**), TG (**j**), and glucose (**k**) (n=5 per group). **l, m** GTT (**l**) and ITT (**m**) (n=5 per group). **n** Body weight of HFD-fed Control and PTOE mice (n=5 for Control, n=6 for PTOE). **o** Relative mRNA expression of inflammation-related genes in eWAT of HFD-fed Control and PTOE mice (n=6 per group). **p** Representative images (left) and quantification analysis (right, n=4 per group) of F4/80 immunofluorescence staining in eWAT of HFD-fed Control and PTOE mice. Scale bar, 50 μ m. **q-s** Serum levels of TC (**q**), TG (**r**), and NEFA (**s**) in HFD-fed Control and PTOE mice (n=7 for Control, n=6 for PTOE). CD, chow diet; HFD, high-fat diet; GFP, green fluorescent protein; ARC, arcuate nucleus; VMH, ventromedial hypothalamus; DMH, dorsomedial hypothalamus; LH, lateral hypothalamus. Control, *Pomc*-Cre mice injected with AAV-GFP in the ARC; PTOE, *Pomc*-Cre mice injected with AAV-Tox3 in the ARC; TC, total cholesterol; TG, triglycerides; GTT, glucose tolerance test; ITT, insulin tolerance test; eWAT, epididymal white adipose tissue; NEFA, non-esterified fatty acid. Data are mean $\pm$ SEM. Numbers represents biological replicates. Significance was analyzed by two-way ANOVA with Sidak's test (**d, e, l, m, n**) or unpaired two-tailed Student's t test (all others). Source data are provided as a Source Data file.

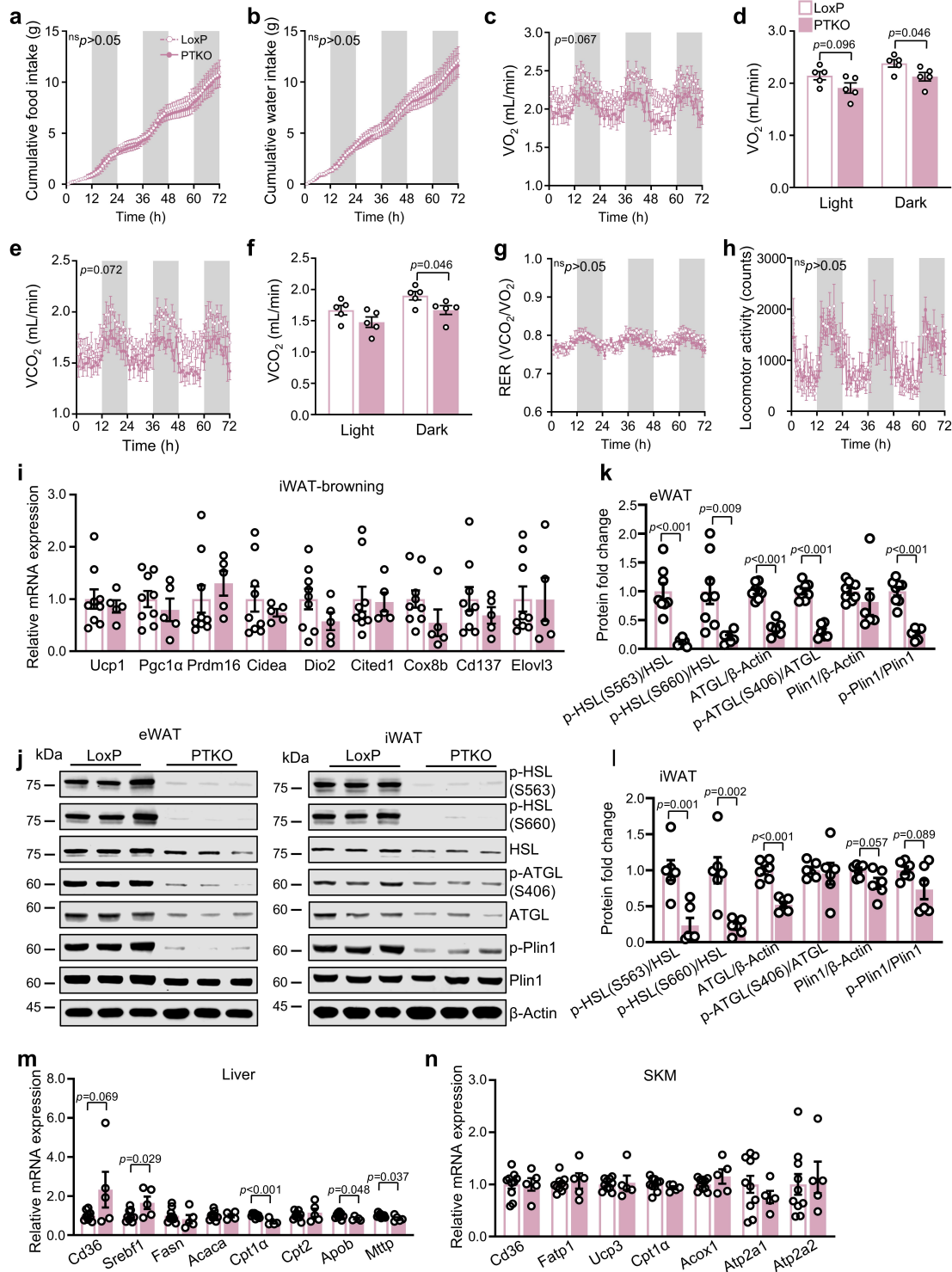


Figure S7. Effect of POMC-lineage cell-specific *Tox3* deletion on metabolic phenotypes in HFD-fed male mice, related to Figure 4. a-h 72-hour metabolic cage measurements. **a** Cumulative food intake (n=4 for LoxP, n=5 for PTKO). **b** Cumulative water intake (n=4 for LoxP, n=5 for PTKO). **c, d** Hourly O_2 consumption (**c**) and average O_2 consumption during dark and light phases (**d**) (n=5 per group). **e, f** Hourly CO_2 production (**e**) and average CO_2 production during dark and light phases (**f**) (n=5 per group). **g** RER (n=5 per group). **h**

Locomotor activity (n=5 per group). **i** Relative mRNA levels of browning-related genes in iWAT (n=9 for LoxP, n=5 for PTKO). **j-l** Western blot analysis (**j**) and quantification of key lipolytic enzymes in eWAT (**k**; n=8 for LoxP, n=6 for PTKO) and iWAT (**l**; n=6 per group). **m, n** Relative mRNA levels of metabolic genes in liver (**m**; n=9 for LoxP, n=5 for PTKO) and SKM (**n**; n=10 for LoxP, n=5 for PTKO). HFD, high-fat diet; LoxP, *Tox3^{flox/flox}* mice; PTKO, *Pomc-Cre;Tox3^{flox/flox}* mice; RER, Respiratory exchange ratio; iWAT, inguinal WAT; eWAT, epididymal WAT; SKM, skeletal muscle. Data are mean±SEM. Numbers represents biological replicates. Significance was analyzed by two-way ANOVA with Sidak's test (**c, e, g, h**) or unpaired two-tailed Student's t test (all others). Source data are provided as a Source Data file.

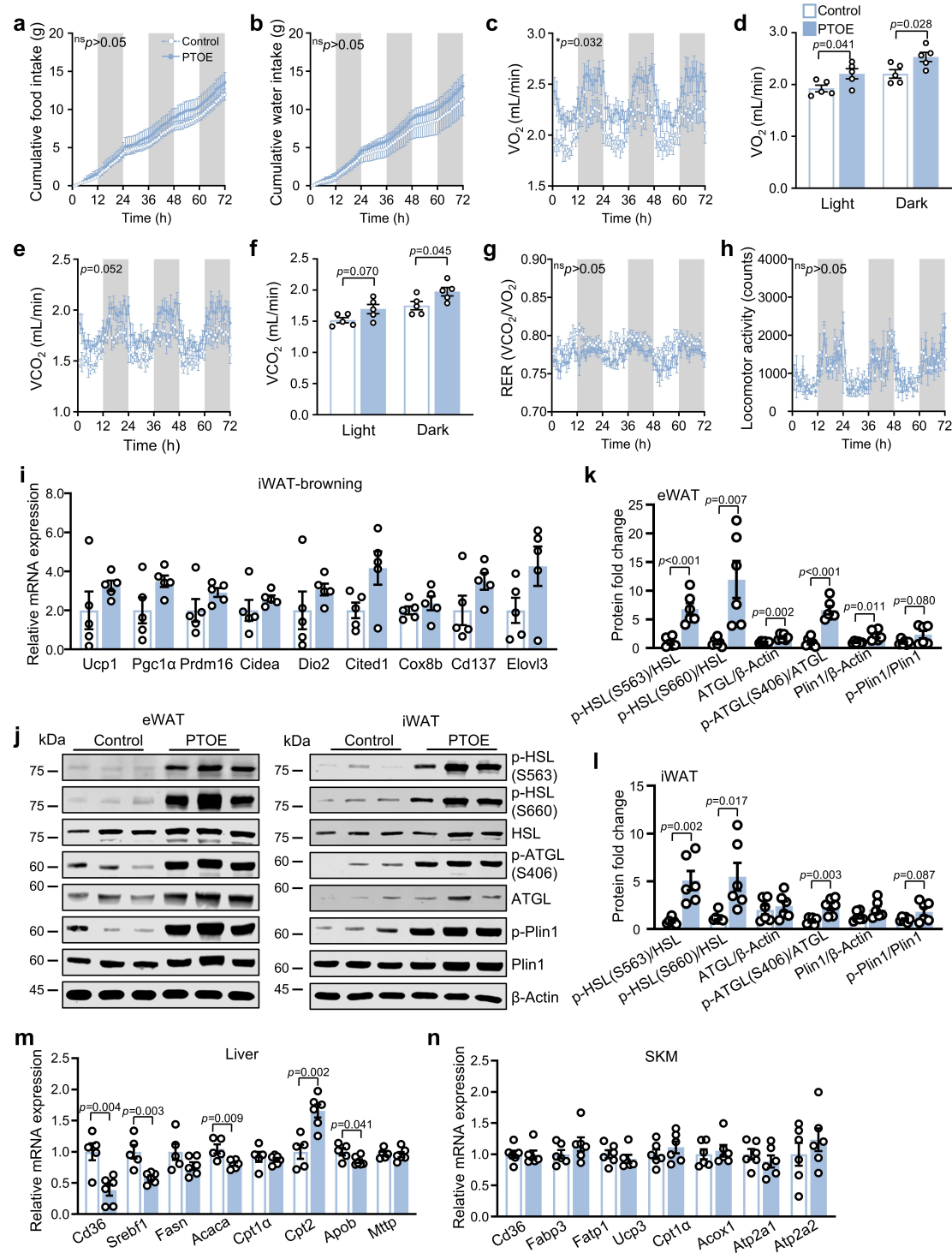


Figure S8. Effect of POMC-lineage cell-specific *Tox3* overexpression on metabolic phenotypes in HFD-fed male mice, related to Figure 4. **a-h** 72-hour metabolic cage measurements. **a** Cumulative food intake (n=5 for Control, n=4 for PTOE). **b** Cumulative water intake (n=5 for Control, n=4 for PTOE). **c, d** Hourly O_2 consumption (**c**) and average O_2 consumption during dark and light phases (**d**) (n=5 per group). **e, f** Hourly CO_2 production (**e**) and average CO_2 production during dark and light phases (**f**) (n=5 per group). **g** RER (n=5 per group). **h** Locomotor activity (n=5 per group). **i** Relative mRNA levels of browning-

related genes in iWAT (n=5 per group). **j-l** Western blot analysis (**j**) and quantification of key lipolytic enzymes in eWAT (**k**; n=6 per group) and iWAT (**l**; n=6 per group). **m, n** Relative mRNA levels of metabolic genes in liver (**m**; n=5 for Control, n=6 for PTOE) and SKM (**n**; n=6 per group). HFD, high-fat diet; Control, *Pomc*-Cre mice injected with AAV-GFP in the ARC; PTOE, *Pomc*-Cre mice injected with AAV-Tox3 in the ARC; RER, Respiratory exchange ratio; iWAT, inguinal WAT; eWAT, epididymal WAT; SKM, skeletal muscle. Data are mean±SEM. Numbers represents biological replicates. Significance was analyzed by two-way ANOVA with Sidak's test (**c, e, g, h**) or unpaired two-tailed Student's t test (all others). Source data are provided as a Source Data file.

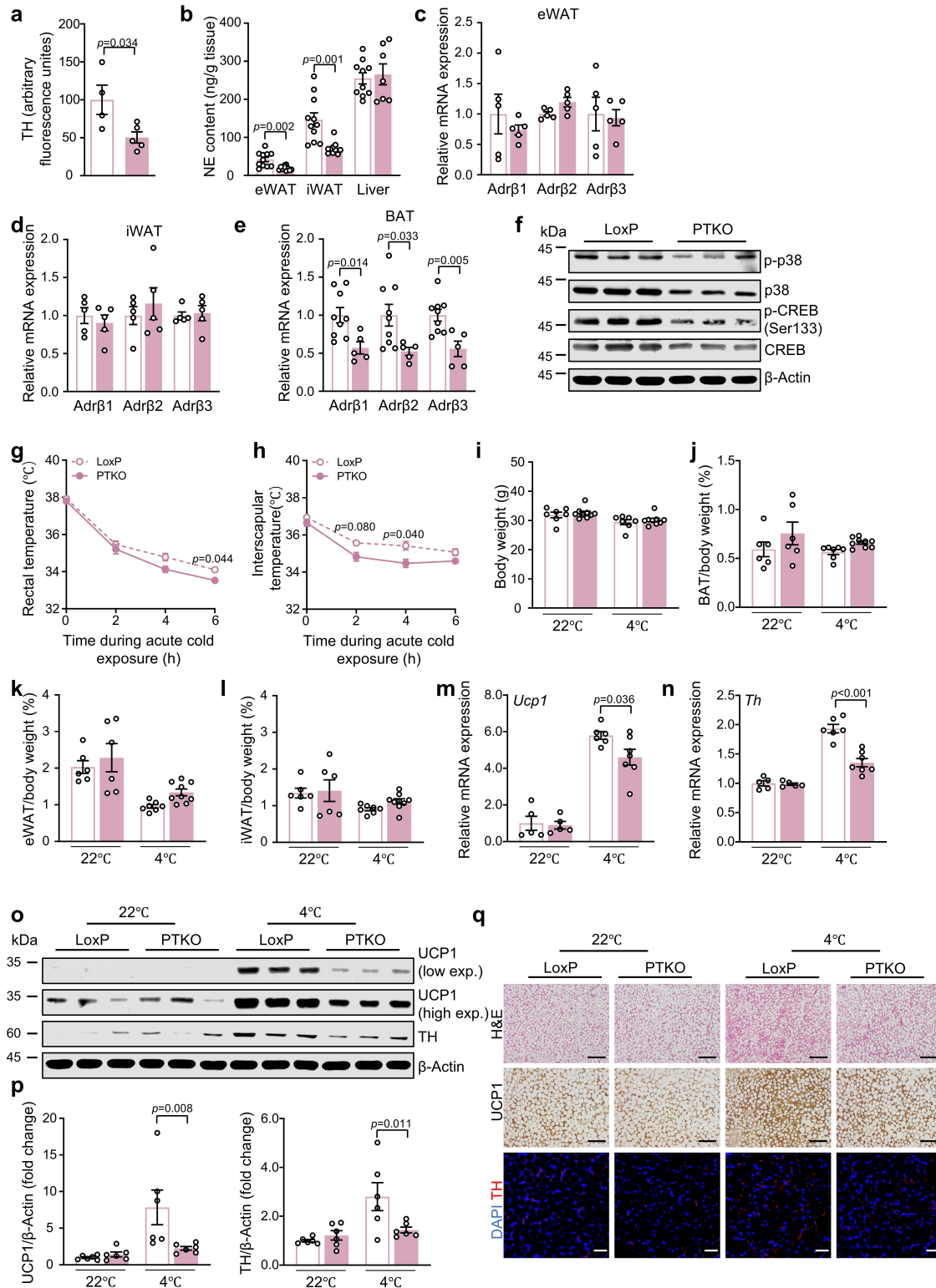


Figure S9. POMC-lineage cell-specific deletion of *Tox3* impairs sympathetic nerve activity in adipose tissues of male mice, related to Figure 5. **a** Quantification of TH immunofluorescence intensity in BAT (from Figure 5c). **b** NE contents in eWAT (n=11 for LoxP, n=10 for PTKO), iWAT (n=11 for LoxP, n=10 for PTKO), and liver (n=10 for LoxP, n=7 for PTKO). **c-e** Relative mRNA expression of β -adrenergic receptors in eWAT (**c**; n=5

per group), iWAT (**d**; n=5 per group), and BAT (**e**; n=9 for LoxP, n=5 for PTKO). **f** Western blot analysis of protein levels of p-p38, p38, p-CREB, and CREB in BAT. **g-q** CD-fed male LoxP and PTKO mice kept at room temperature or exposed to acute cold (6 h). **g** Rectal temperature (n=7 for LoxP, n=9 for PTKO). **h** Interscapular BAT temperature (n=7 for LoxP, n=9 for PTKO). **i** Body weight (n=7 for LoxP, n=9 for PTKO). **j-l** Weight of BAT (**j**), eWAT (**k**), and iWAT (**l**) after room-temperature (n=6 per group) or cold exposure (n=7 for LoxP, n=9 for PTKO). **m, n** Relative mRNA levels of *Ucp1* and *Th* in BAT of mice subjected to room temperature (n=5 per group) or cold exposure (n=6 for LoxP, n=7 for PTKO). **o, p** Western blot analysis (**o**) and quantification (**p**) of UCP1 and TH in BAT of mice subjected to room temperature (n=6 per group) or acute cold exposure (n=6 per group). **q** Representative images of H&E-, UCP1-, and TH- stained BAT sections of LoxP and PTKO mice under room temperature and cold exposure. Scale bars, 100 μ m (images for H&E and UCP1) and 50 μ m (images for TH). eWAT, epididymal WAT; iWAT, inguinal WAT; BAT, brown adipose tissue; LoxP, *Tox3^{fllox/fllox}* mice; PTKO, *Pomc-cre;Tox3^{fllox/fllox}* mice; Data are mean $\pm$ SEM. Numbers represents biological replicates. Significance was analyzed by two-way ANOVA with Sidak's test (**g, h, i, j, k, l, m, n, p**) or unpaired two-tailed Student's t test (all others). Source data are provided as a Source Data file.

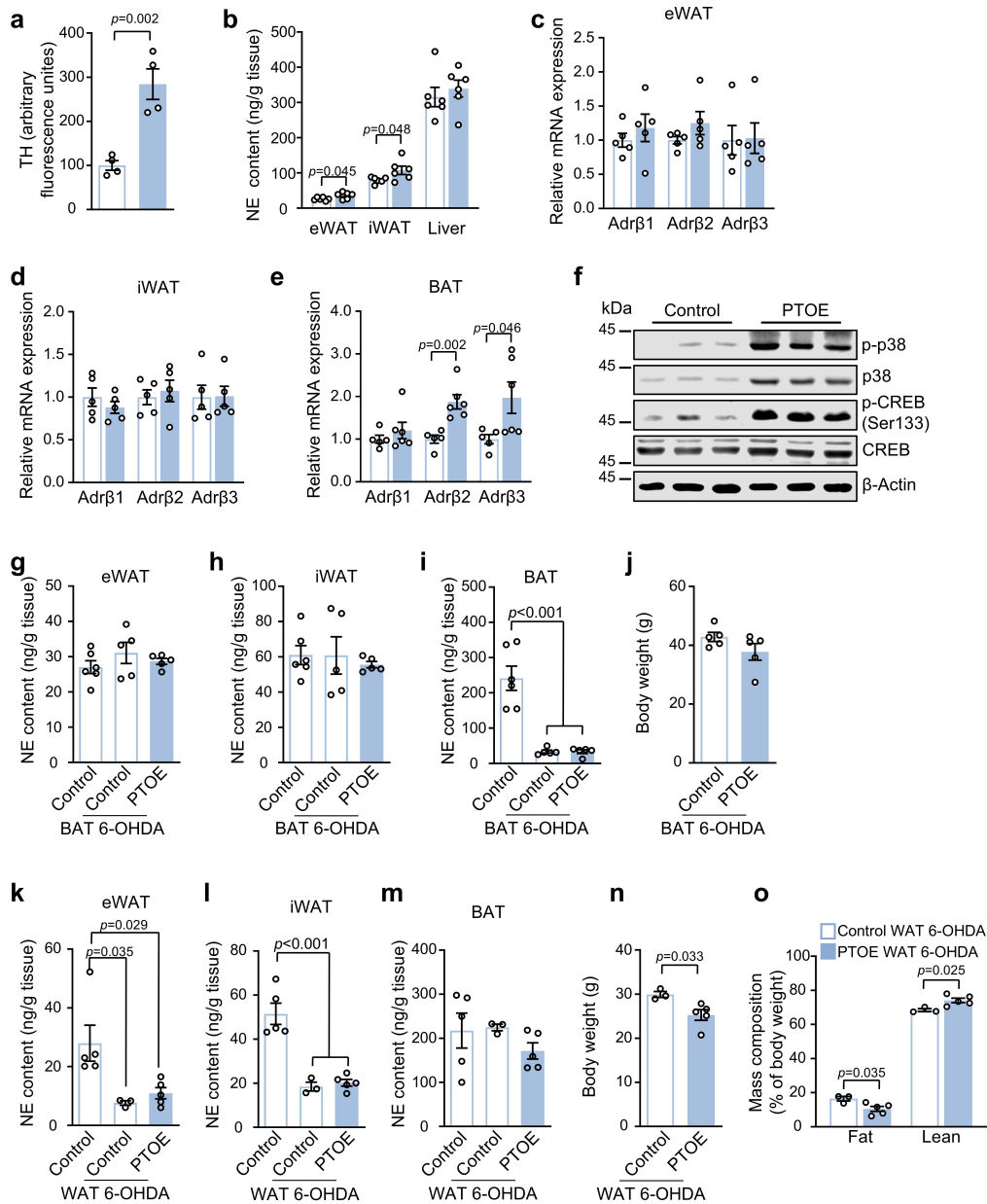


Figure S10. Overexpression of TOX3 in POMC-lineage cells enhances sympathetic nerve activity in adipose tissues of HFD-fed male mice, related to Figure 5. a Quantification of TH immunofluorescence intensity in BAT (from Figure 5n). **b** NE contents in eWAT, iWAT, and liver (n=6 per group). **c-e** Relative mRNA expression of β -adrenergic receptors in eWAT (**c**; n=5 per group), iWAT (**d**; n=5 per group), and BAT (**e**; n=5 for Control, n=6 for PTOE). **f** Western blot analysis of p-p38, p38, p-CREB, and CREB protein levels in BAT of HFD-fed Control and PTOE mice. **g-j** HFD-fed Control and PTOE mice bilaterally injected with saline or 6-OHDA into BAT. **g-i** NE contents in eWAT (**g**), iWAT (**h**), and BAT (**i**) (n=6 for Control-saline, n=5 for Control-6-OHDA, n=5 for PTOE-6-OHDA). **j** Body weight of mice after BAT injection of 6-OHDA (n=5 per group). **k-o** HFD-fed Control and PTOE mice bilaterally injected with either saline or 6-OHDA into both eWAT and iWAT. **k-m**

NE contents in eWAT (**k**), iWAT (**l**), and BAT (**m**) (n=5 for Control-saline, n=3 for Control-6-OHDA, n=5 for PTOE-6-OHDA). **n** Body weight of mice after WAT injection of 6-OHDA (n=5 per group). **o** Body composition of mice after WAT injection of 6-OHDA (n=3 for Control-6-OHDA, n=5 for PTOE-6-OHDA). HFD, high-fat diet; eWAT, epididymal WAT; iWAT, inguinal WAT; BAT, brown adipose tissue; Control, *Pomc*-Cre mice injected with AAV-GFP in the ARC; PTOE, *Pomc*-Cre mice injected with AAV-Tox3 in the ARC; NE, Norepinephrine. Data are mean±SEM. Numbers represents biological replicates. Significance was analyzed by one-way ANOVA (**g**, **h**, **i**, **k**, **l**, **m**) unpaired two-tailed Student's t test. Source data are provided as a Source Data file.

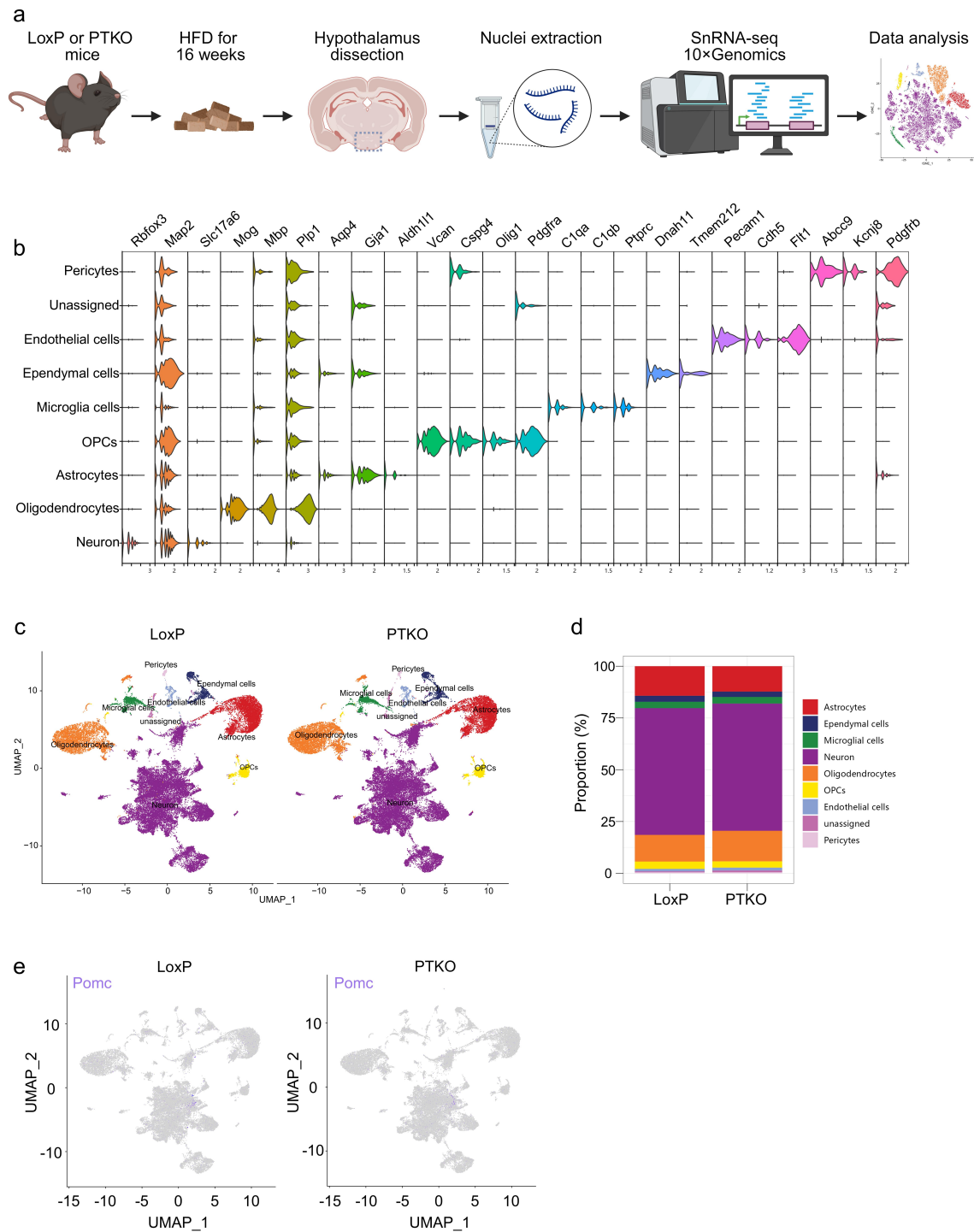


Figure S11. Single-nucleus RNA sequencing (snRNA-seq) of the hypothalamus in HFD-fed LoxP and PTKO mice, related to Figure 7. a Schematic diagram of the experimental procedure. Created in BioRender. Tang, Q. (2026) <https://BioRender.com/o1kmjdo>. **b** Violin plots showing expression of known cell type markers for different clusters. **c** UMAP plot of cells. **d** Proportions of hypothalamic cell populations. **e** Feature plots of *Pomc* expression levels in neurons from LoxP and PTKO mice.

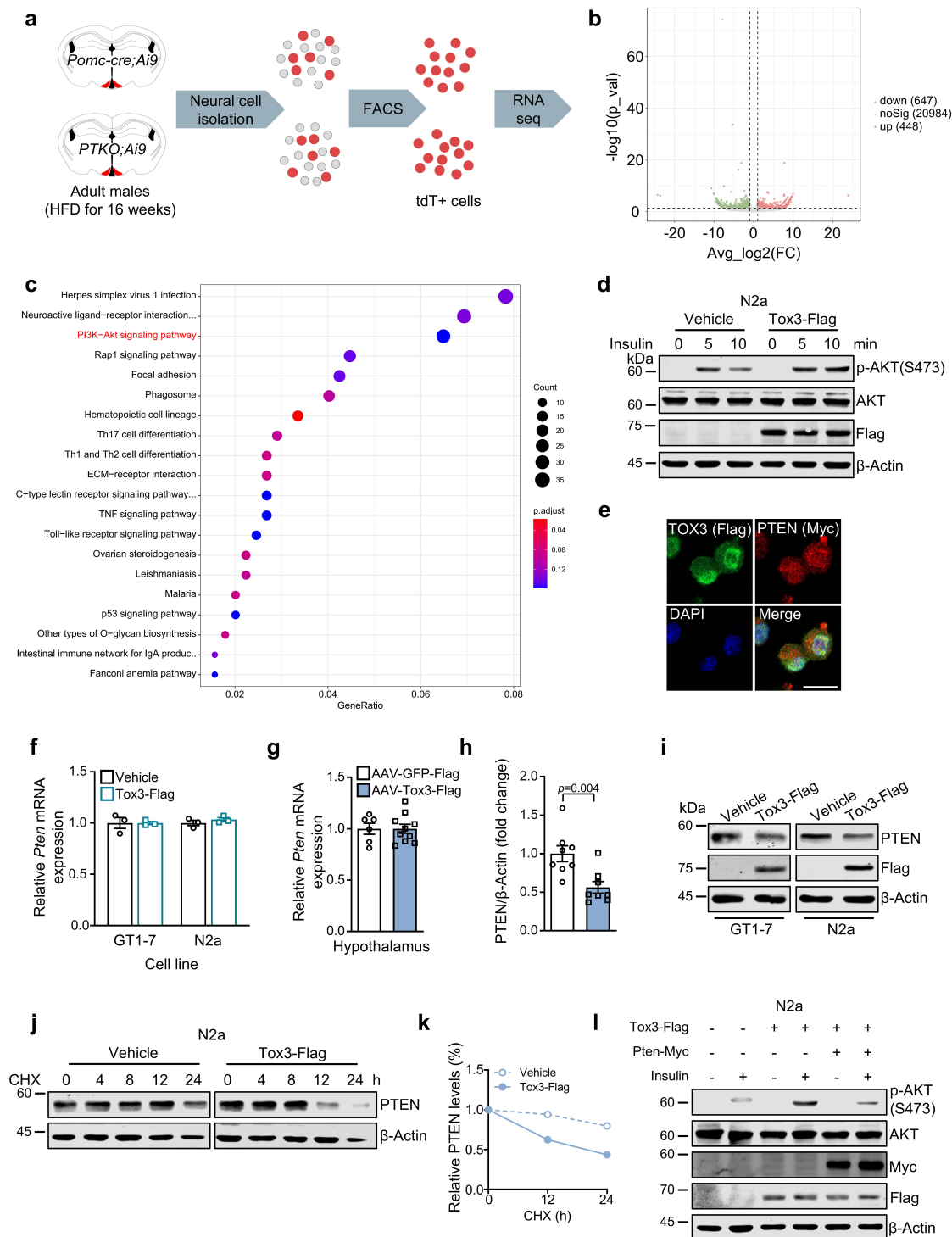


Figure S12. TOX3 modulates AKT signaling by regulating PTEN degradation, related to Figure 7. **a** Schematic of sample preparation for RNA sequencing of POMC-lineage cells from HFD-fed *Pomc-cre;Ai9* and *PTKO;Ai9* male mice. **b** Volcano plot of differentially expressed genes in POMC-lineage cells. **c** Top 20 enriched KEGG pathways. **d** Western blot analysis of insulin-treated (0, 5, 10 min) N2a cells transduced with control or Tox3-Flag constructs. **e** Immunofluorescence staining showing colocalization of PTEN and TOX3 in N2a cells. Scale bar, 20 μ m. **f** *Pten* mRNA expression in GT1-7 and N2a cells transfected

with control or Tox3-Flag constructs. **g** *Pten* mRNA expression in the hypothalamic tissues of mice injected with AAV-GFP-Flag or AAV-Tox3-Flag. **h** Quantification of PTEN protein levels in hypothalamus (from Figure 7j). **i** Western blot analysis of PTEN protein levels in GT1-7 and N2a cells transduced with control or Tox3-Flag. **j, k** Cycloheximide (CHX) chase assay (50 μ g/mL) in control- or Tox3-Flag-transduced N2a cells, assessing PTEN protein stability by Western blot analysis at indicated timepoints in three independent experiments. **l** Insulin response (10 min) in N2a cells co-transfected with vehicle/ Tox3-Flag and *Pten*-Myc plasmid. *Pomc-cre;Ai9*, *Pomc*-Cre mice with tdTomato-labeled POMC-lineage cells; *PTKO;Ai9*, POMC-lineage cell-specific *Tox3*-deficient mice with tdTomato-labeled POMC-lineage cells. Data are mean $\pm$ SEM. Numbers represents biological replicates. Significance was analyzed by unpaired two-tailed Student's t test. Source data are provided as a Source Data file.

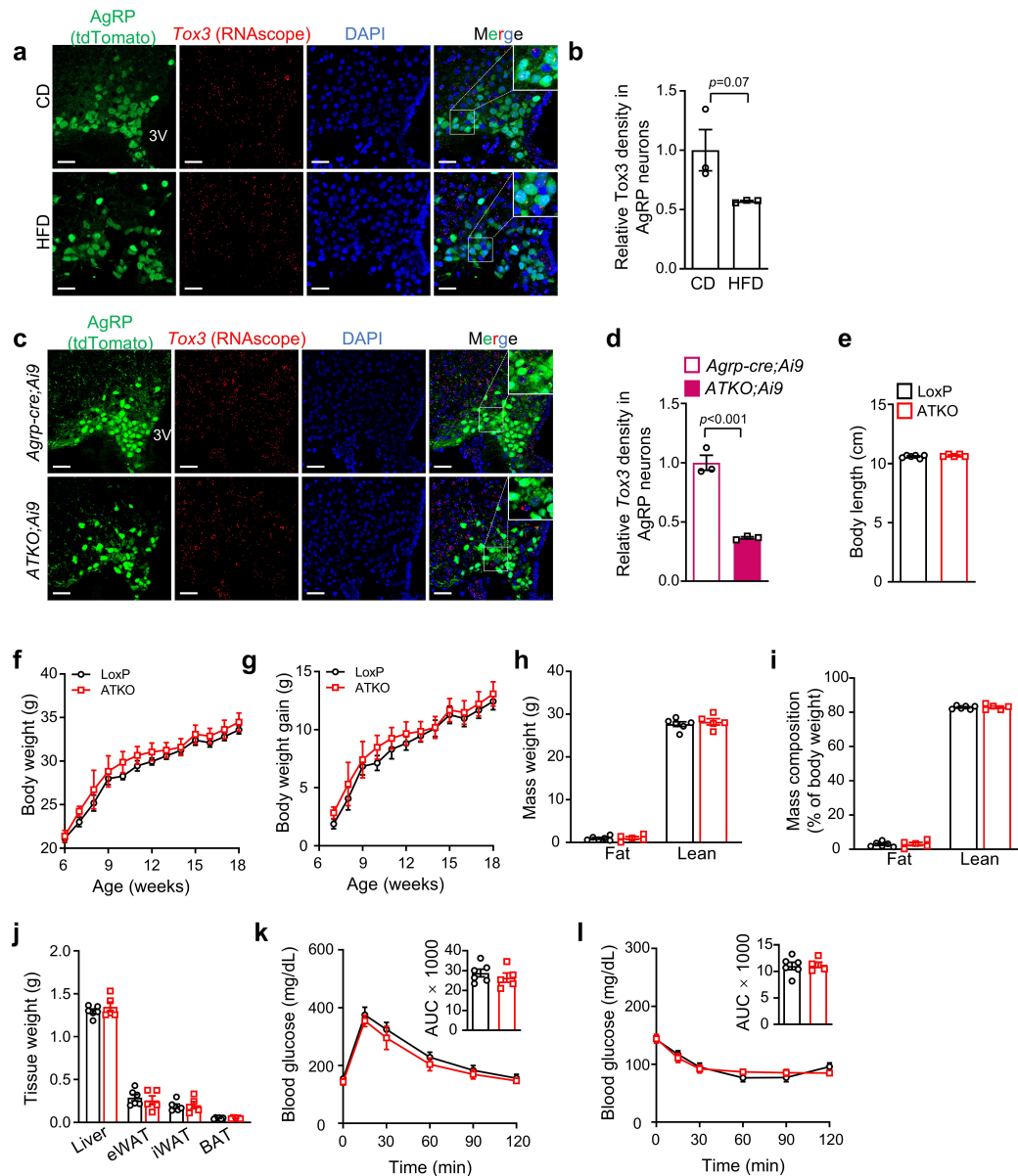


Figure S13. Deletion of TOX3 in AgRP neurons does not affect metabolic parameters in CD-fed male mice. **a** Representative confocal images showing AgRP (tdTomato⁺, green), *Tox3* mRNA (red), and DAPI (blue) in the ARC of CD- or HFD-fed *Agrp-cre;Ai9* mice. Scale bar, 50 μ m. **b** Integrated density quantification of *Tox3* signal in **a**. **c** Representative confocal images showing AgRP (tdTomato⁺, green), *Tox3* mRNA (red), and DAPI (blue) in the ARC of *Agrp-cre;Ai9* mice and *Agrp-cre;Tox3^{fl/fl};Ai9* (ATKO;Ai9) mice. Scale bar, 50 μ m. **d** Quantification of **c**. **e-l** Metabolic parameters in CD-fed LoxP and ATKO male mice. **e** Body length (n=6 for LoxP, n=5 for ATKO). **f, g** Body weight (**f**) and body weight gain (**g**) (n=6 for LoxP, n=5 for ATKO). **h, i** Fat and lean mass (**h**) and body composition (**i**) (n=6 for LoxP, n=5 for ATKO). **j** Liver and adipose depot weights (n=6 for LoxP, n=5 for ATKO). **k, l** GTT (**k**) and ITT (**l**) (n=6 for LoxP, n=5 for ATKO). CD, chow diet; HFD, high-fat diet; LoxP,

Tox3^{flox/flox} mice; ATKO, *Agrp-cre;Tox3^{flox/flox}* mice; GTT, glucose tolerance test; ITT, insulin tolerance test. Data are mean±SEM. Numbers represents biological replicates. Significance was analyzed by two-way ANOVA with Sidak's test (**f**, **g**, **k**, **l**) or unpaired two-tailed Student's t test (all others). Source data are provided as a Source Data file.

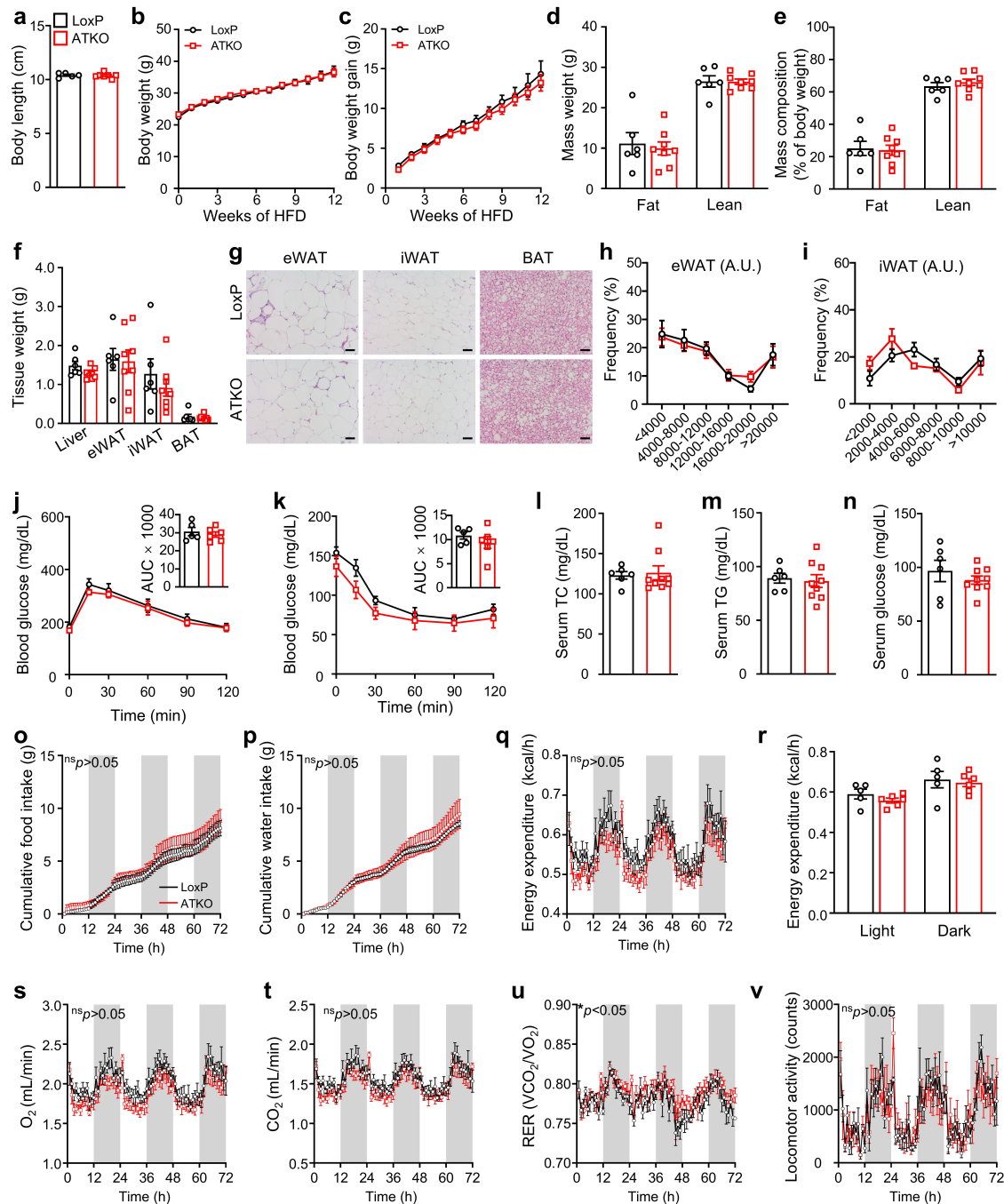


Figure S14. Deletion of TOX3 in AgRP neurons does not alter metabolic parameters in HFD-fed male mice. **a** Body length (n=5 for LoxP, n=7 for ATKO). **b, c** Body weight (b) and body weight gain (c) (n=9 for LoxP, n=10 for ATKO). **d, e** Mass weight (d) and body composition (e) (n=6 for LoxP, n=8 for ATKO). **f** Liver and adipose depot weights (n=6 for LoxP, n=8 for ATKO). **g** H&E staining of eWAT, iWAT, and BAT. Scale bar, 100 μ m. **h, i** Adipocyte size distribution in eWAT (h) and iWAT (i) (n=5 per group). **j, k** GTT (j) and ITT (k) (n=5 for LoxP, n=6 for ATKO). **l-n** Serum TC (l), TG (m), and glucose (n) levels (n=6 for LoxP, n=9 for ATKO). **o-v** 72-hour metabolic cage measurements. **o** Cumulative food intake (n=4 for LoxP, n=5 for ATKO). **p** Cumulative water intake (n=4 for LoxP, n=5 for ATKO). **q, r** Energy expenditure (q, r) (n=4 for LoxP, n=5 for ATKO). **s, t** O_2 (s) and CO_2 (t) (n=4 for LoxP, n=5 for ATKO). **u** RER (u) (n=4 for LoxP, n=5 for ATKO). **v** Locomotor activity (v) (n=4 for LoxP, n=5 for ATKO).

r Hourly energy expenditure (**q**) and average energy expenditure during dark and light phases (**r**) (n=5 for LoxP, n=6 for ATKO). **s-v** Hourly O₂ consumption (**s**), CO₂ production (**t**), RER (**u**), and Locomotor activity (**v**) (n=5 for LoxP, n=6 for ATKO). LoxP, *Tox3^{lox/lox}* mice; ATKO, *Agrp-cre;Tox3^{lox/lox}* mice; eWAT, epididymal WAT; iWAT, inguinal WAT; BAT, brown adipose tissue; GTT, glucose tolerance test; ITT, insulin tolerance test; TC, total cholesterol; TG, triglycerides; RER, respiratory exchange ratio. Data are mean±SEM. Numbers represents biological replicates. Significance was analyzed by two-way ANOVA with Sidak's test (**b**, **c**, **j**, **k**, **q**, **s**, **t**, **u**, **v**) or unpaired two-tailed Student's t test (all others). Source data are provided as a Source Data file.

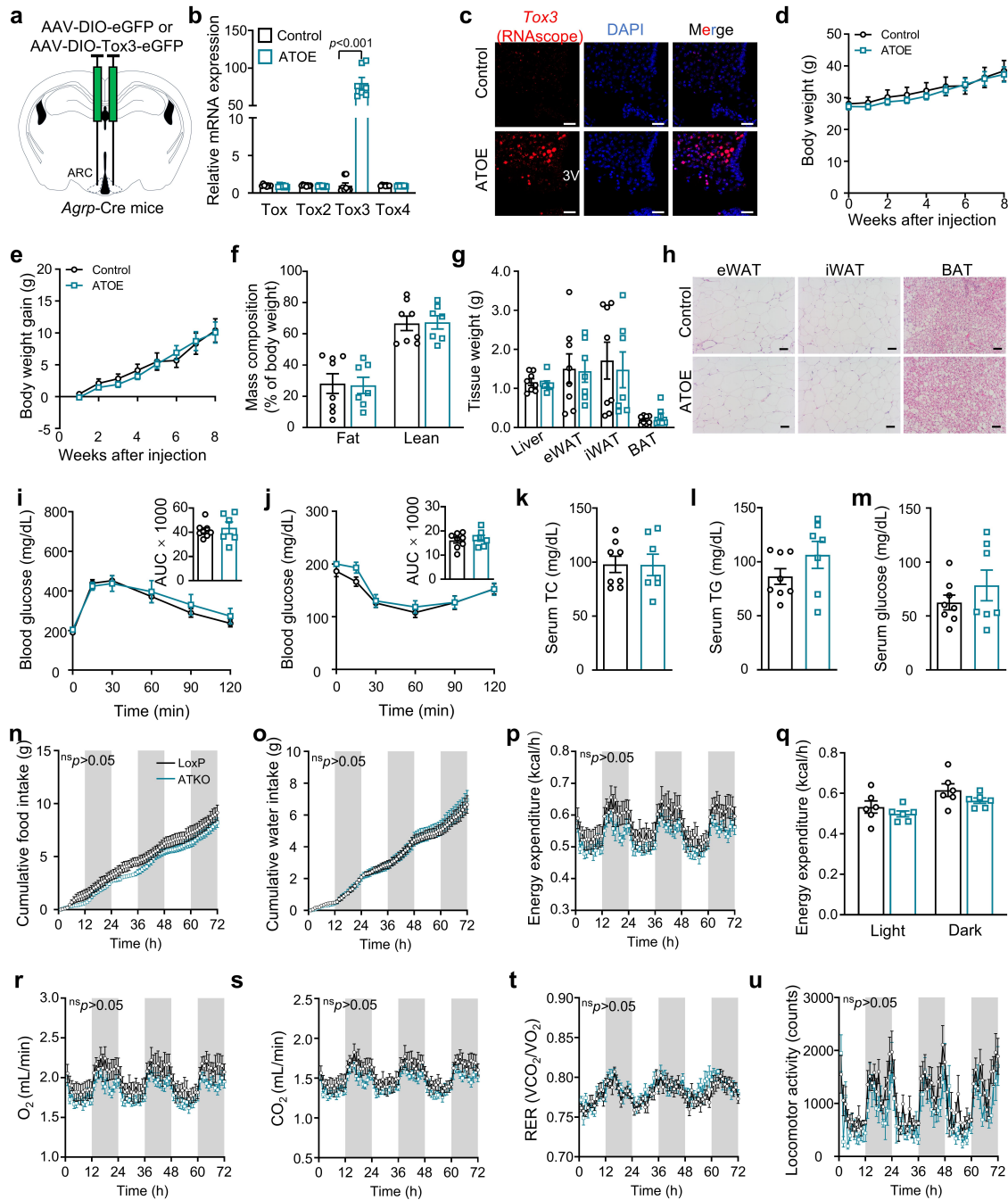


Figure S15. Overexpression of TOX3 in AgRP neurons does not alter metabolic parameters in male mice fed an HFD. **a** Schematic of bilateral ARC injections of AAV-GFP or AAV-Tox3 in *AgRP-Cre* mice, named Control and ATOE mice, respectively. **b** Relative mRNA expression of *Tox* family genes in the hypothalamus of Control and ATOE mice (n=8 for Control, n=7 for ATOE). **c** Representative confocal images showing *Tox3* mRNA (red) and DAPI (blue) fluorescence in the ARC of Control and ATOE mice. Scale bar, 50 μ m. **d, e** Body weight (**d**) and body weight gain (**e**) (n=8 for Control, n=7 for ATOE). **f** Body composition (n=8 for Control, n=7 for ATOE). **g** Liver and adipose depot weights (n=8 for Control, n=7 for ATOE). **h** H&E staining of eWAT, iWAT, and BAT. Scale bar, 50 μ m. **i, j**

GTT (**i**; n=8 for Control, n=6 for ATOE) and ITT (**j**; n=8 for Control, n=7 for ATOE). **k-m** Serum TC (**k**), TG (**l**), and glucose (**m**) levels (n=8 for Control, n=7 for ATOE). **n-u** 72-hour metabolic cage measurements. **n** Cumulative food intake (n=5 for Control, n=6 for ATOE). **o** Cumulative water intake (n=5 for Control, n=6 for ATOE). **p, q** Hourly energy expenditure (**p**) and average energy expenditure during dark and light phases (**q**) (n=6 per group). **r-u** Hourly O₂ consumption (**r**), CO₂ production (**s**), RER (**t**), and Locomotor activity (**u**) (n=6 per group). eWAT, epididymal WAT; iWAT, inguinal WAT; BAT, brown adipose tissue; GTT, glucose tolerance test; ITT, insulin tolerance test; TC, total cholesterol; TG, triglycerides; RER, respiratory exchange ratio. Data are mean±SEM. Numbers represents biological replicates. Significance was analyzed by two-way ANOVA with Sidak's test (**d, e, i, j, p, r, s, t, u**) or unpaired two-tailed Student's t test (all others). Source data are provided as a Source Data file.

Table S1. Mouse-specific primer sequences for qPCR analysis.

Gene	Forward (5'-3')	Reverse (5'-3')
<i>l8s</i>	TTGACTCAACACGGGAAACC	AGACAAATCGCTCCACCAAC
<i>Acaca</i>	TGTACAAGCAGTGTGGGCTGGCT	CCACATGGCCTGGCTTGGAGGG
<i>Acox1</i>	CCACATATTGACCCCAAGACC	AGGCATGTAACCCGTAGCAGC
<i>Adrb1</i>	CTCATCGTGGTGGGTAACGTG	ACACACAGCACATCTACCGAA
<i>Adrb2</i>	ATGTCGGTTATCGTCTGGC	GGTTTGTAGTCGCTCGAACTTG
<i>Adrb3</i>	GGCCCTCTCTAGTTCCCAG	TAGCCATCAAACCTGTTGAGC
<i>Agrp</i>	GCGGAGGTGCTAGATCCA	AGGACTCGTGCAGCCTTA
<i>Apob</i>	AGGTGGCCACAGCCAATAAT	TTATGCCAGCTTG GTTGCAG
<i>Atp2a1</i>	CAATTCGGTGATCCGCCAAC	GGGGCAGATCGTCAAACCTCA
<i>Atp2a2</i>	GCAACACATCTACCAGCCCT	TGAACTGACGCTGAGCACTT
<i>Cart</i>	AAACGCATTCCGATCTACG	GGAAAGAGGGAATATGGGAACC
<i>Cd11b</i>	TTTTAGGAGCACCTCGGTAT	TGAGGATCAAGTTGGTATTG
<i>Cd137</i>	GGTGGACAGCCGAACGTAA	TCTTAAATGCTGGTCTCCCTC
<i>Cd36</i>	TACCTGGGAGTTGGCGAGAA	GTTCCGATCACAGCCCATTCT
<i>Cd68</i>	TGTCTGATCTTGCTAGGACCG	GAGAGTAACGGCCTTTTTGTGA
<i>Cidea</i>	TCCTATGCTGCACAGATGACG	TGCTCTTCTGTATCGCCCAGT
<i>Cited1</i>	CTCCCACAACCTATCGGCG	ATGCAGGTTGAAGGATGGGG
<i>Cox8b</i>	TGCTGGAACCATGAAGCCAAC	AGCCAGCCAAAACCTCCCACTT
<i>Cpt1a</i>	TTGCACGAGGGAAAAATAAGC	CCCTGCATGCGGTGGAAAAGGC
<i>Cpt2</i>	GAGGCATTTGTCAGG	GGACAGGATGTTGTGG
<i>Cxcl1</i>	CCAACCACCAGGCTACAGG	GCGTCACACTCAAGCTCTG
<i>Cxcl2</i>	CCAACCACCAGGCTACAGG	GCGTCACACTCAAGCTCTG
<i>Dio2</i>	CATTGATGAGGCTCACCTTTC	GGTTCCGGTGCTTCTTAACCT
<i>Elovl3</i>	GTGTGCTTTGCCATCTACACG	CTCCCAGTTCAACAACCTTGC-
<i>F4/80</i>	TGACTCACCTTGTGGTCCTAA	CTTCCCAGAATCCAGTCTTTCC
<i>Fasn</i>	GAGGACACTCAAGTGGCTGA	GTGAGGTTGCTGTCGTCTGT
<i>Fatp1</i>	AGCCTGGTCAAGTTCTGTTCTGGA	AGCCTGGTCAAGTTCTGTTCTGGA
<i>Il-1β</i>	CCGTGGACCTTCCAGGATGA	GGGAACGTACACACCAGCA
<i>Il-6</i>	TTCTTGGGACTGATGCTG	CTCATTTCCACGATTTCCC
<i>Mcp-1</i>	GCTGGAGAGCTACAAGAGGATC	GTCAACTTCACATTCAAAGGTGC
<i>Mtp</i>	TGAGAGGCCAGTTGTGTGAC	ATGATCCTCTTGGCAGTGCTT
<i>Npy</i>	CTCCGCTCTGCGACACTAC	AATCAGTGTCTCAGGGCT
<i>Pgc1a</i>	GCACCAGAAAACAGCTCCAAG	CGTCAAACACAGCTTGACAGC
<i>Pomc</i>	GAGGCCACTGAACATCTTTGTC	GCAGAGGCAAACAAGATTGG
<i>Prdm16</i>	ACACGCCAGTTCTCCAACCTGT	TGCTTGTTGAGGGAGGAGGTA
<i>Pten</i>	AGCCTCTTGATGTGTGCATT	CCATTGGTAGCCAAACGGAAC

Table S1. Continued.

Gene	Forward (5'-3')	Reverse (5'-3')
<i>Srebf1</i>	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
<i>Th</i>	CCAAGGTTTCATTGGACGGC	CTCTCCTCGAATACCACAGCC
<i>Tnf-α</i>	GGCGGTGCCTATGTCTCA	AGGGTCTGGGCCATAGAA
<i>Tox</i>	AGATCAACTGGGGTCCCTTG	GCGTGATGGGTGGGATGTTA
<i>Tox2</i>	ATGCAGCAGCATCTCACAGA	TGAGGGTCTCTTCTCTCCCG
<i>Tox3</i>	GATGTGAGGTTCTACCCCGC	TGGAATGTCTCACTGGCAGC
<i>Tox4</i>	GGGTAGGGGACACTATTGCG	TTGCCTCTTCTTTCCCCCTT
<i>Ucp1</i>	TCTCAGCCGGCTTAATGACTG	GGCTTGCATTCTGACCTTCAC
<i>Ucp3</i>	ACCCGATACATGAACGCTCC	TCATCACGTTCCAAGCTCCC

Table S2. Primary antibodies used for immunohistochemistry analysis and western blot analysis.

Antibody	Catalog no.	Company	Application
β -Actin	#AC026	Abclonal, Wuhan, China	WB
ATGL	#2138	Cell Signaling Technology, MA, USA	WB
AKT	#ET1609-51-50	Huabio, Hangzhou, China	WB
c-FOS	#2250	Cell Signaling Technology, MA, USA	IF
c-FOS	#OB-PGP080	Oasis, Zhejiang, China	IF
CREB	#ET1601-15	Huabio, Hangzhou, China	WB
F4/80	#70076	Cell Signaling Technology, MA, USA	IF
Flag	#F1804	Sigma-Aldrich, Missouri, USA	IP
Flag	#M1403-2	Huabio, Hangzhou, China	WB
GFAP	#ab7260	Abcam, MA, USA	IF
GFP	#ab6556	Abcam, MA, USA	IF
HSL	#A15686	Abclonal, Wuhan, China	WB
IBA1	#ET1705-78	Proteintech, Wuhan, China	IF
Myc	#AE009	Abclonal, Wuhan, China	WB
NeuN	#ET1602-12	Proteintech, Wuhan, China	IF
p-AKT (S473)	#4060	Cell Signaling Technology, MA, USA	WB, IF
p-ATGL (S406)	#ab135093	Abcam, MA, USA	WB
p-CREB (Ser133)	#9198	Cell Signaling Technology, MA, USA	WB
p-HSL (Ser563)	#4139	Cell Signaling Technology, MA, USA	WB
p-HSL (Ser660)	#45804	Cell Signaling Technology, MA, USA	WB
p-p38	#BS-2210R	Bioss, Beijing, China	WB
p-PKA substrate	#9624	Cell Signaling Technology, MA, USA	WB
P38	#ET1602-26	Proteintech, Wuhan, China	WB
Plin1	#9349	Cell Signaling Technology, MA, USA	WB
POMC	#H-029-30	Phoenix Pharmaceuticals, CA, USA	IF
PTEN	#9188	Cell Signaling Technology, MA, USA	WB
tdTomato	#orb182397	Biorbyt, Cambridge, UK	IF
TH	#AB152	Sigma-Aldrich, Missouri, USA	WB, IF, IHC
UCP1	#ab10983	Abcam, MA, USA	WB, IF
Vimentin	#ET1610	Huabio, Hangzhou, China	IF

Abbreviations: WB, western blot; IF, immunofluorescence; IHC, immunohistochemistry.