

# Supplementary Materials for

## **Notch signaling regulates adipose browning and energy metabolism**

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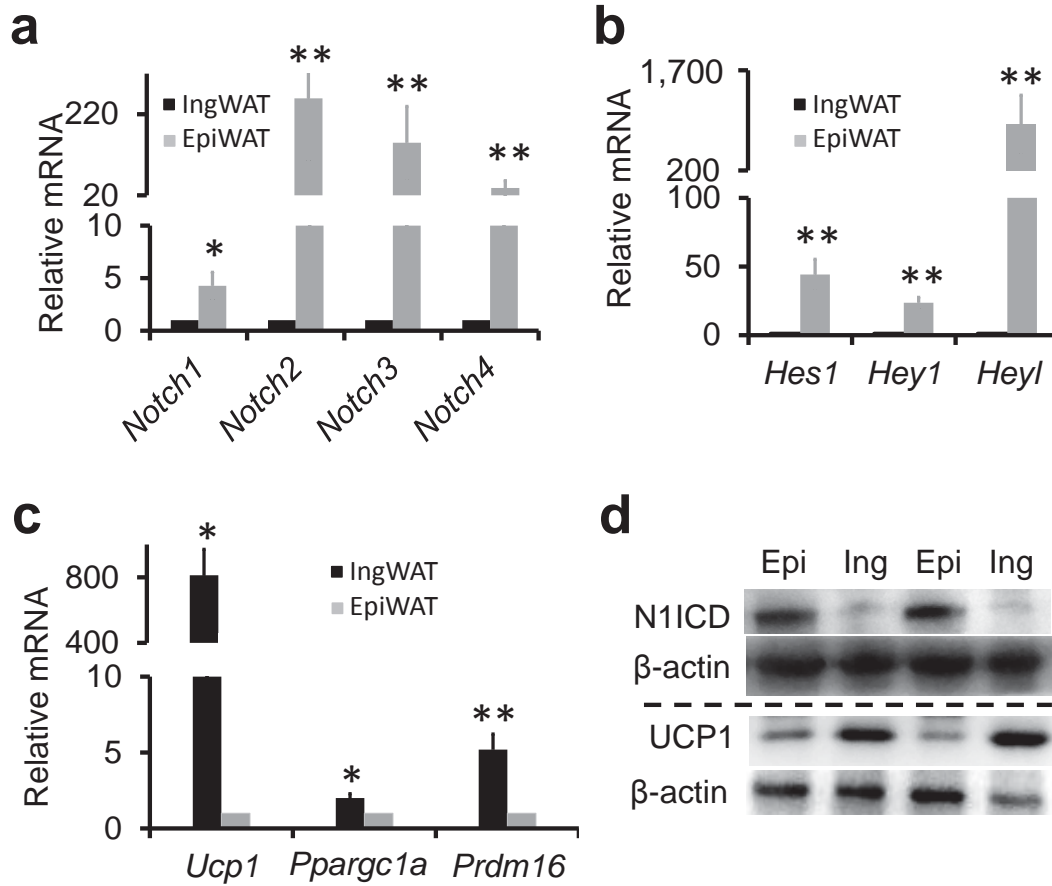
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Supplementary Figs. 1–7

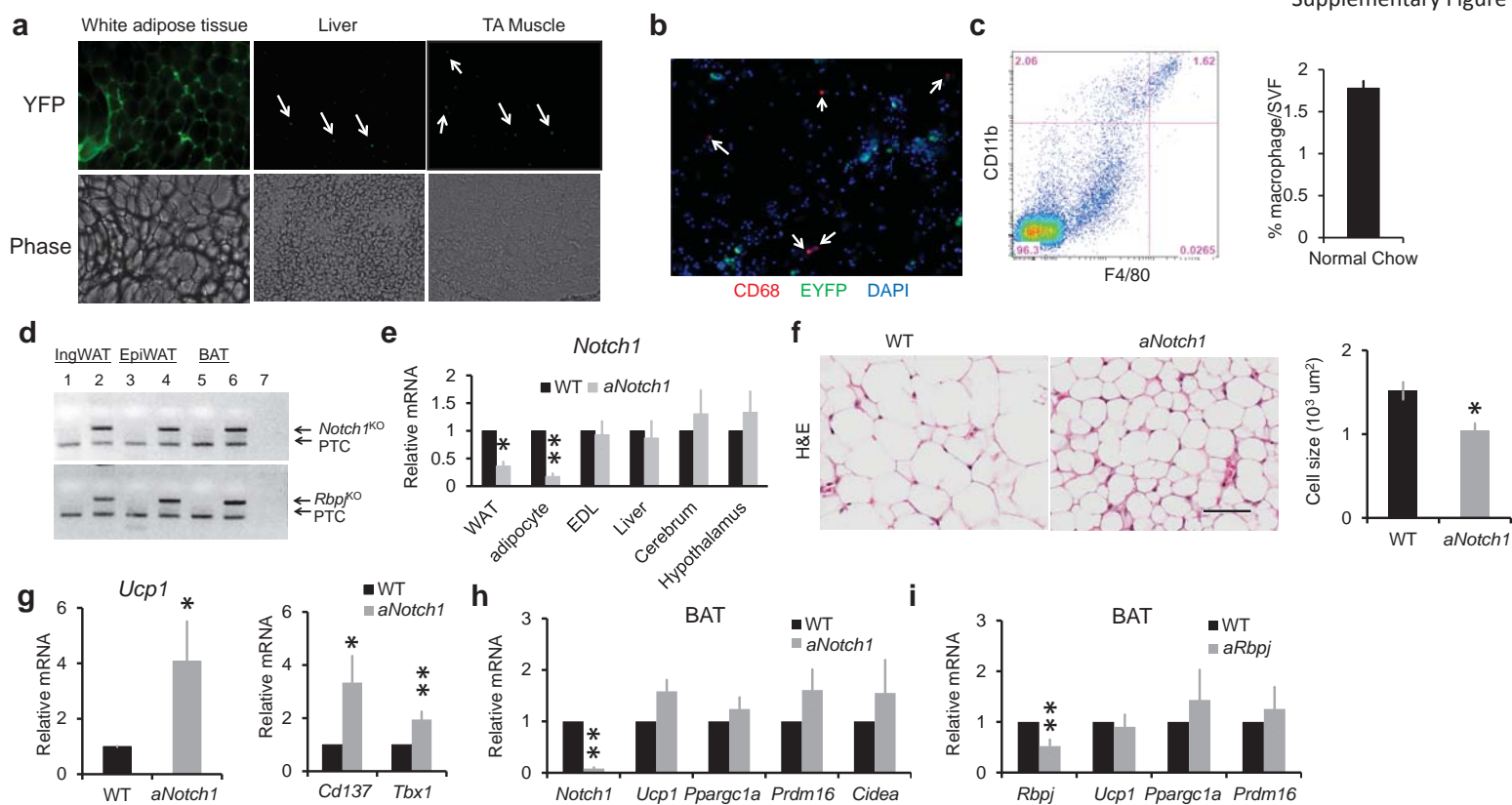
Supplementary Table 1

Supplementary Video 1

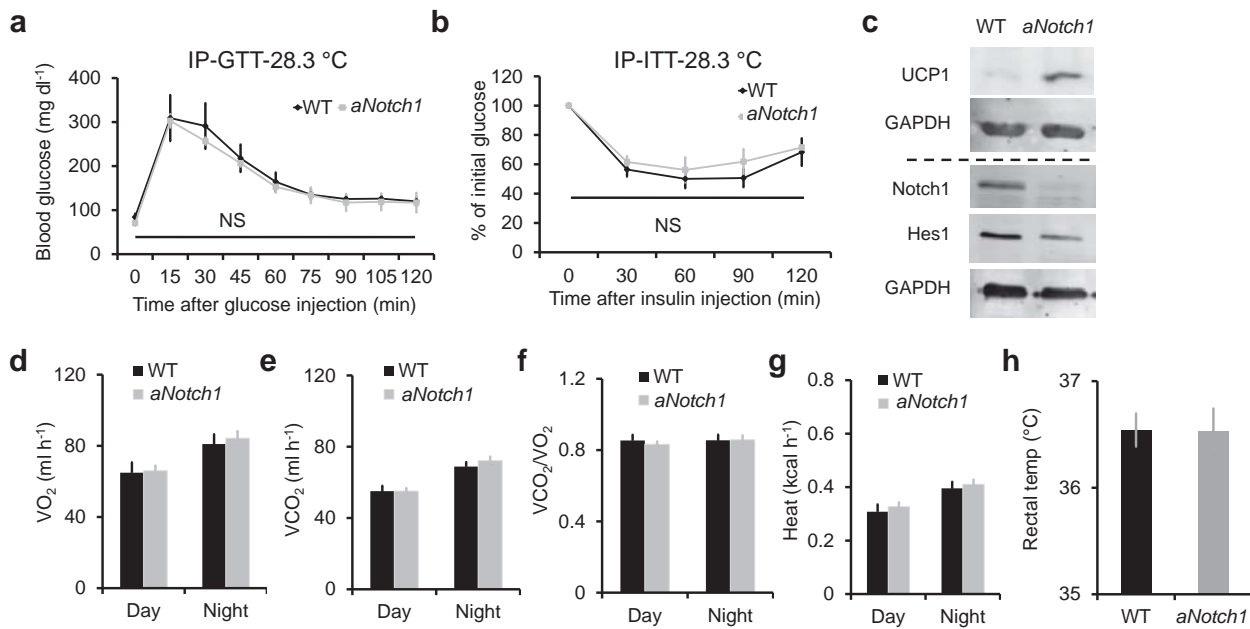
Supplementary Figure 1



**Supplementary Figure 1** Notch signaling is inversely correlated with expression of BAT-related genes. (**a–c**) Notch receptor (**a**), target (**b**), and brown fat-related gene (**c**) expression in EpiWAT and IngWAT,  $n = 5$ . (**d**) Representative western blots to show N1ICD and UCP1 expression in Epi-WAT and Ing-WAT. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Data are means  $\pm$  SEM.

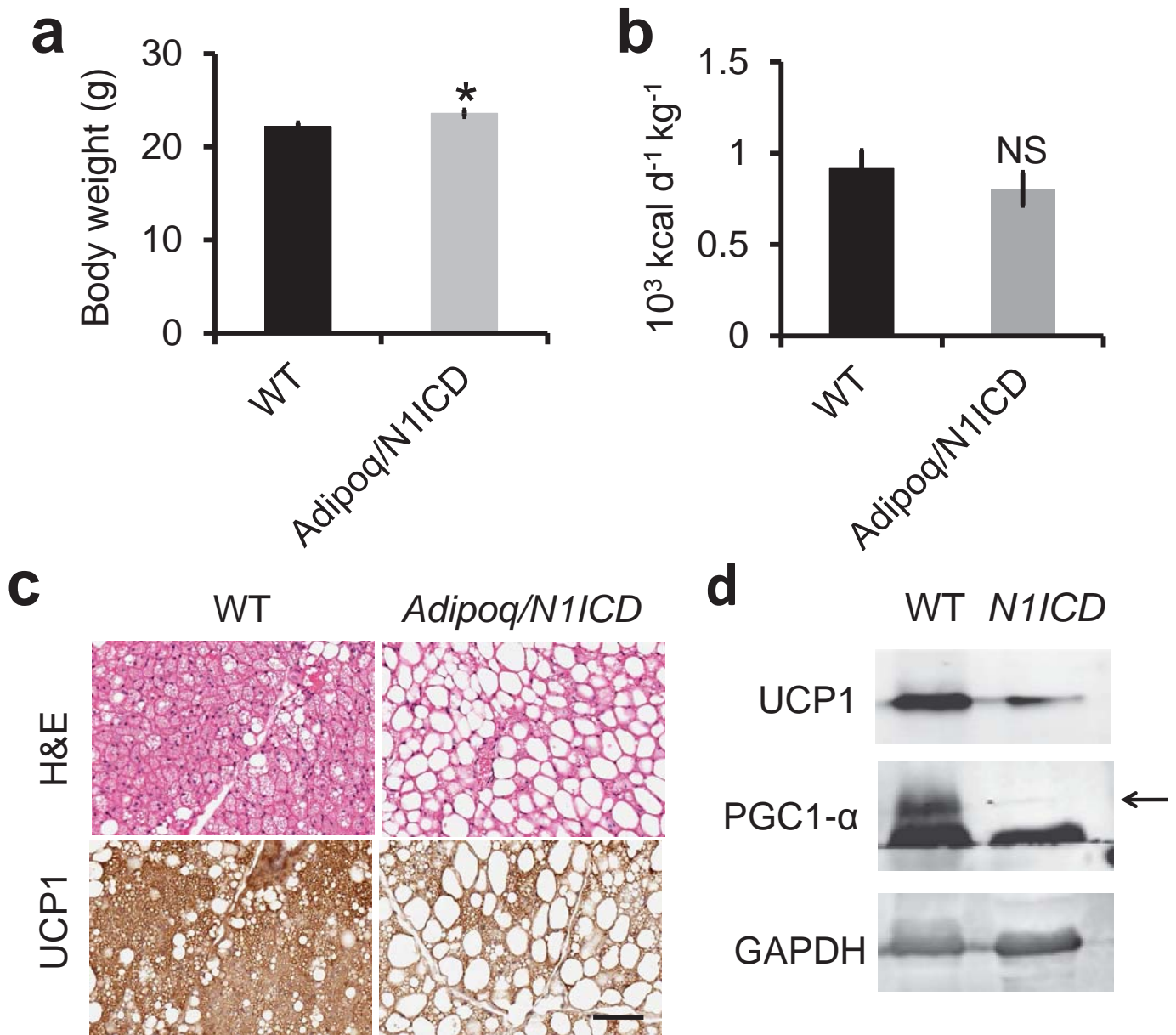


**Supplementary Figure 2** *aP2* lineage tracing and gene expression assay. (a) YFP fluorescence in tissues of *aP2-Cre/Rosa<sup>EYFP</sup>* mice, arrow points YFP<sup>+</sup> cells. (b) CD68 (red color) immunostaining to show macrophage (arrow) in cultured inguinal SVF cells from *aP2-Cre/Rosa<sup>EYFP</sup>* mice. (c) Fluorescence-activated cell sorting of CD11b<sup>+</sup> and F4/80<sup>+</sup> macrophages from inguinal SVF cells (left) and the ratio to SVF cells (right),  $n = 3$ . (d) Gel electrophoresis result of PCR product using primers to detect *Notch1* deletion and *Rbpj* deletion in *aNotch1* (top panel) and *aRbpj* (lower panel) mice tissue respectively, lanes 1, 3, 5 are WT tissues, lanes 2, 4, 6 are mutant tissues, lane 7 is PCR without template DNA, PTC, positive control of genomic DNA. (e) Expression of *Notch1* in various tissues or cells. (f) Representative H&E staining images of epididymal WAT, scale bar = 50  $\mu\text{m}$  (left), and adipocyte size (right). (g) Gene expression in epididymal WAT,  $n = 6$ . (h,i) Gene expression in brown adipose tissue (BAT) of *aNotch1* (h,  $n = 7$ ) and *aRbpj* (i,  $n = 4$ ) mice. \* $P < 0.05$ , \*\* $P < 0.01$ . Data are means  $\pm$  SEM.

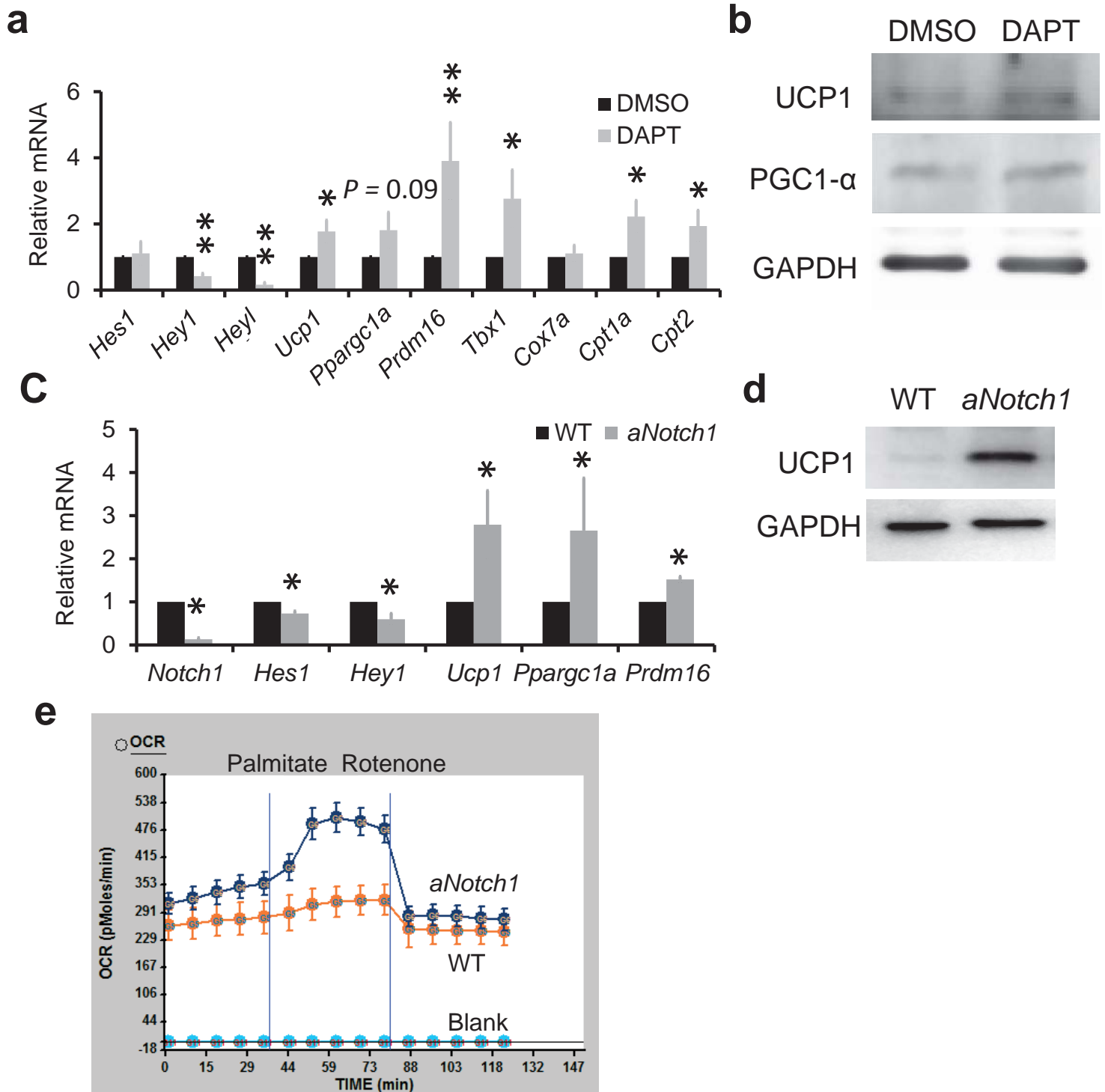


**Supplementary Figure 3** Characterization of *aNotch1* mice acclimated at thermoneutral condition (28.3 °C). (a,b) Blood glucose concentrations during IP-GTT (a,  $n = 4$ ) or IP-ITT (b,  $n = 3$ ). (c) Western blot result of UCP1, Notch1 and Hes1 in inguinal WAT. (d–g) Averaged day and night O<sub>2</sub> consumption, CO<sub>2</sub> production, respiration exchange ratio (VCO<sub>2</sub>/VO<sub>2</sub>) and heat production,  $n = 5$ . (h) Rectal temperature measurement,  $n = 4$ . \* $P < 0.05$ , \*\* $P < 0.01$ . Data are means  $\pm$  SEM.

## Supplementary Figure 4

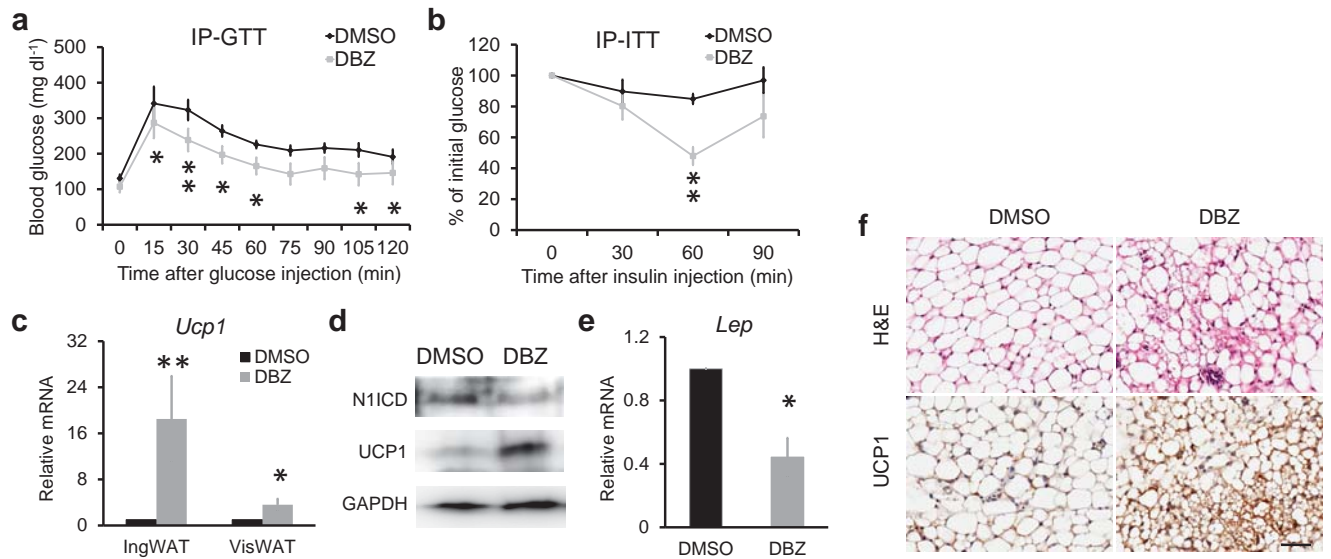


**Supplementary Figure 4** Characterization of *Adipoq/N1ICD* mice. (a) Body weight measurement at 7–11 weeks old,  $n = 8$  pairs of mice. (b) Food intake normalized to body weight,  $n = 6$  pairs of mice. (c) Representative H&E and UCP1 staining images of IngWAT from mice acclimated at 4 °C for 2 weeks, scale bar = 100  $\mu\text{m}$ . (d) Western blot of IngWAT sample as in panel c. \* $P < 0.05$ . Data are means  $\pm$  SEM. \* $P < 0.05$ . Data are means  $\pm$  SEM.

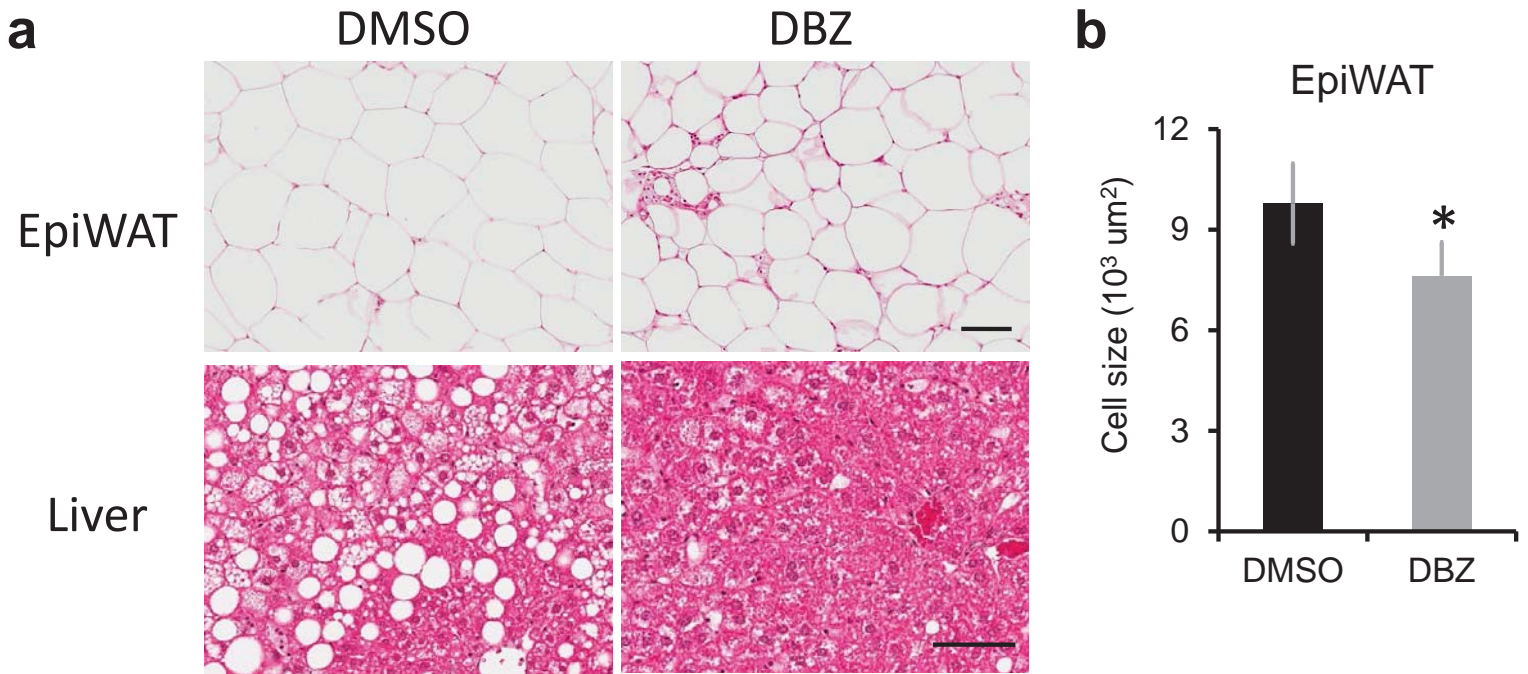


**Supplementary Figure 5** Browning of cultured white adipocyte with inhibition of Notch signaling. (a,b) Gene expression in cultured EpiWAT adipocytes treated with DAPT. (c,d) Relative expression of Notch targets and BAT-related gene in cultured WT and *aNotch1* inguinal adipocytes. (e) OCR of cultured WT and *aNotch1* inguinal adipocytes.  $n = 3$ . \* $P < 0.05$ , \*\* $P < 0.01$ . Data are means  $\pm$  SEM.

Supplementary Figure 6



**Supplementary Figure 6** Pharmacological inhibition of Notch using dibenzazepine (DBZ) induces browning and ameliorates glucose metabolism. **(a,b)** Blood glucose concentrations during IP-GTT **(a)** or IP-ITT **(b)** in mice treated with vehicle control (DMSO) or DBZ for 5 days,  $n = 4$ . **(c)** Relative expression of *Ucp1* in IngWAT and VisWAT after treatment with DMSO or DBZ,  $n = 5$ . **(d)** Representative western blot of N1ICD and UCP1 in EpiWAT. **(e)** Relative expression of *Lep* in EpiWAT,  $n = 6$ . **(f)** H&E and UCP1 staining of IngWAT from mice after 5 days DMSO or DBZ treatment, scale bar = 50  $\mu$ m. \* $P < 0.05$ , \*\* $P < 0.01$ . Data are means  $\pm$  SEM.



**Supplementary Figure 7** Histological analysis of mice after DBZ treatment. **(a)** H&E staining of EpiWAT and liver, scale bars represent  $100 \mu\text{m}$ . **(b)** Averaged epididymal adipocyte size.  $n = 4$ .  $*P < 0.05$ . Data are means  $\pm$  SEM.



**Supplementary Table 1.** Primers for genomic DNA recombination detection and ChIP-qPCR.

Site	Sequence 5'-3'
PTC	5'-TAAGCCTGCCCAGAAGACTC-3' 5'-AAAGTCGCTCTGAGTTGTTAT-3'
<i>Notch1</i> <sup>KO</sup>	5'-TGGCCTGCCTGTCTGGAACAACAGTTCAGG-3' 5'-ACCCTTGCCTCAGTTCAAACACAAGATACG-3'
<i>Rbpj</i> <sup>KO</sup>	5'-CTTGATAATTCTGTAAAGAGA-3' 5'-CCACAGGCAACAATTGAG-3'
Primer1	5'-GCCGTGTTAGCAGGGATTTA-3' 5'-AGGTCCTCTTGGGGAACAGT-3'
Primer2	5'-TGAGGTGAAGACCGAGAAGG-3' 5'-CGCACAGAGCACTCAATCTG-3'
Primer3	5'-CACAAAAGCTGTCGTCTGGA-3' 5'-GCACCTTCTGCACCTTTTTC-3'
Primer4	5'-TTTCAGTGTTTTTCCTTCATT-3' 5'-CCCAGAAAACAAATGCTAGA-3'

**Supplementary Video 1.** Movement of WT (left) and *aNotch1* (right) mice in the new cages.