Supplementary Materials for

Notch signaling regulates adipose browning and energy metabolism

Pengpeng Bi, Tizhong Shan, Weiyi Liu, Feng Yue, Xin Yang, Xin-Rong Liang, Jinghua Wang, Jie Li, Nadia Carlesso, Xiaoqi Liu, Shihuan Kuang*

*Corresponding author. Email: skuang@purdue.edu

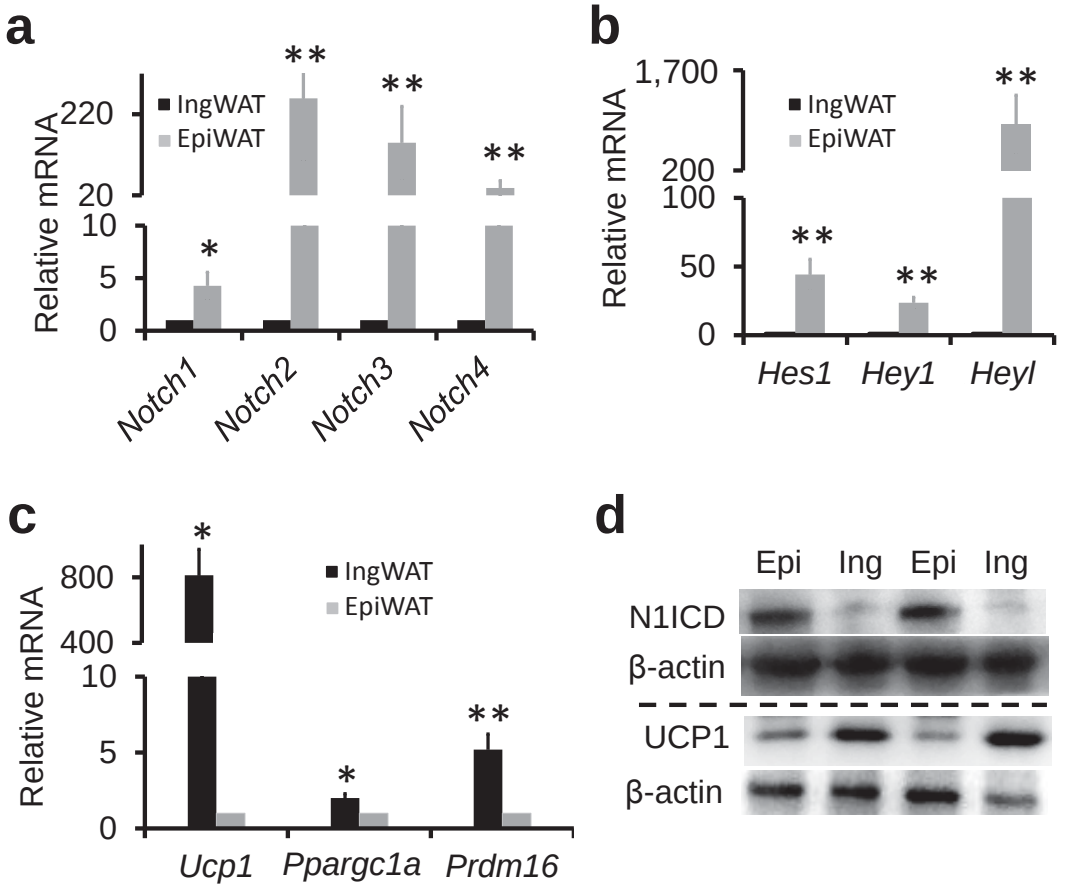
This PDF file includes:

Supplementary Figs. 1–7

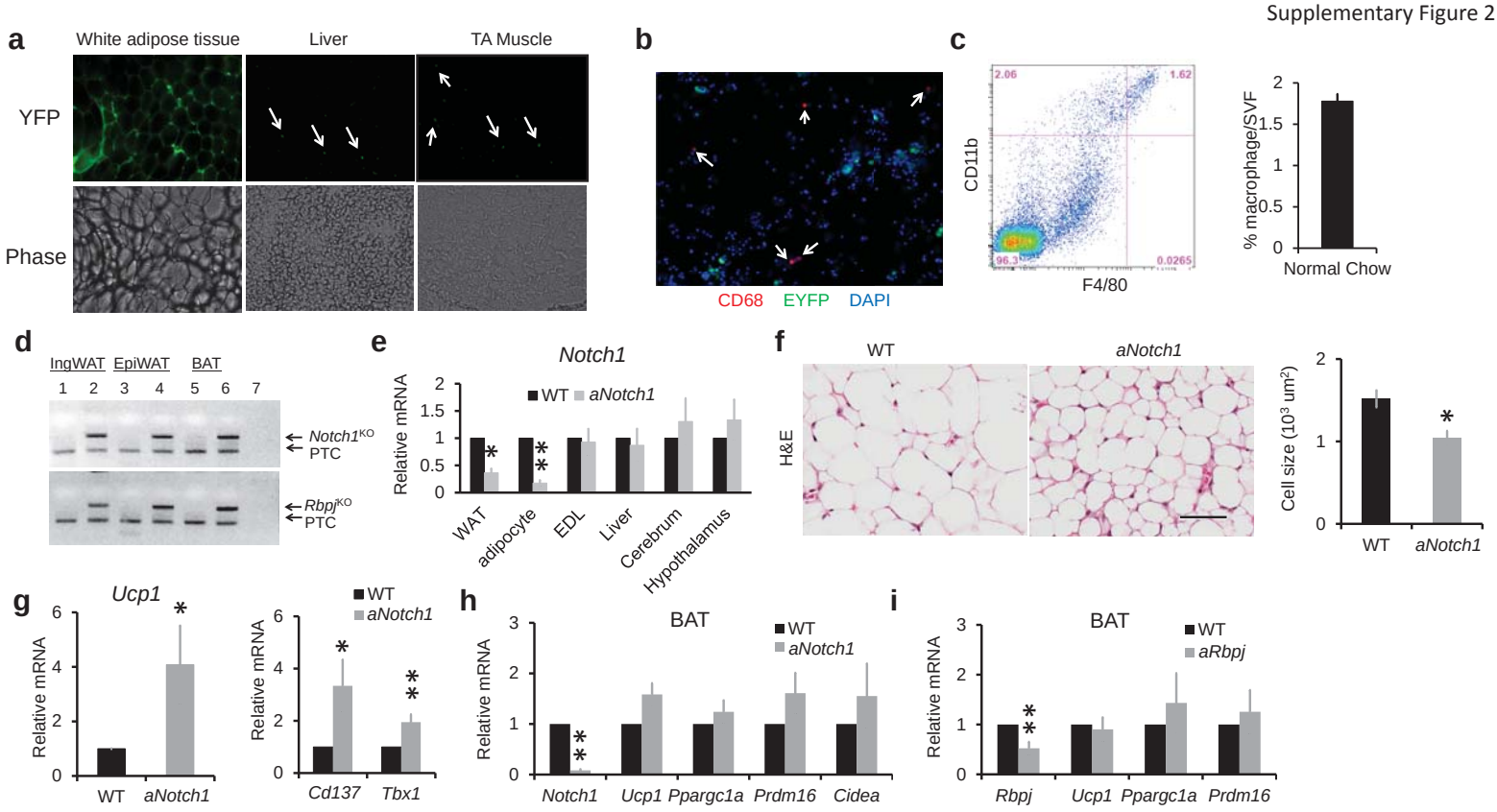
Supplementary Table 1

Supplementary Video 1

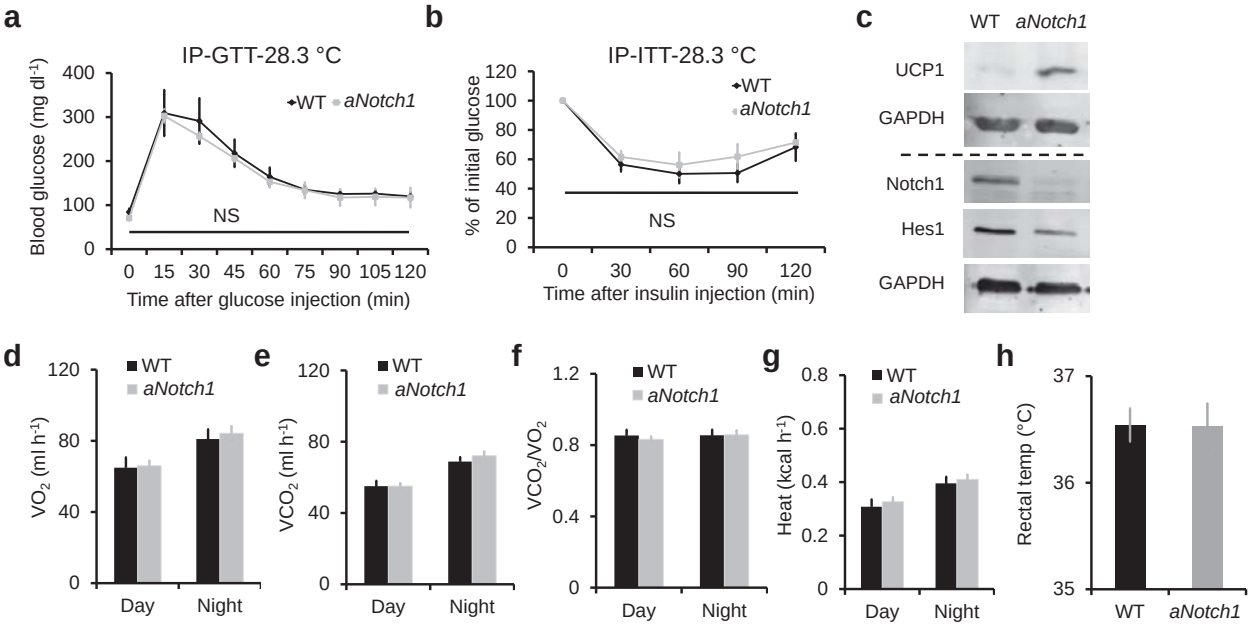
Nature Medicine: doi:10.1038/nm.3615



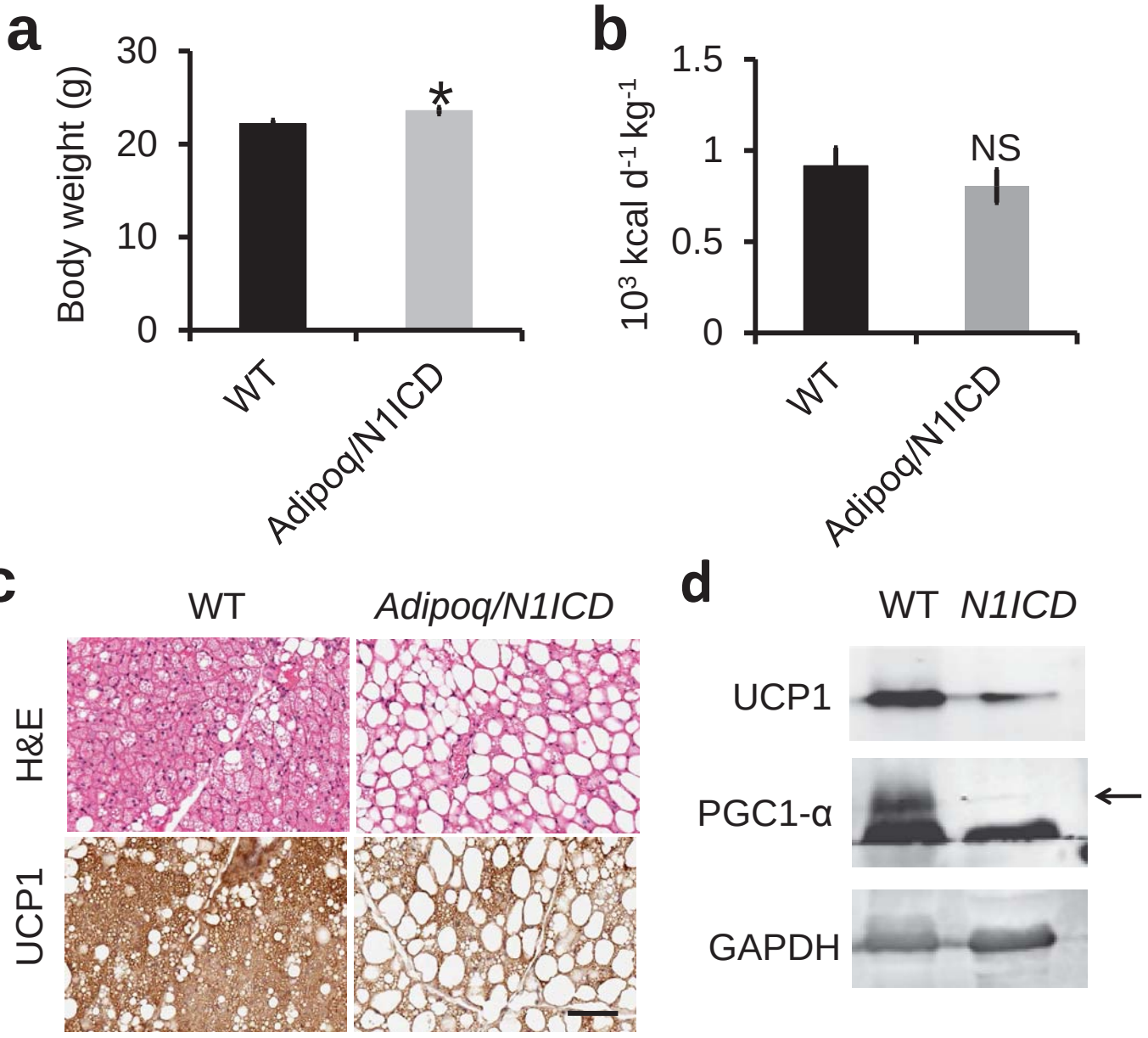
Supplementary Figure 1 Notch signaling is inversely correlated with expression of BAT-related genes. (**a**–**c**) Notch receptor (**a**), target (**b**), and brown fat-related gene (**c**) expression in EpiWAT and IngWAT, $n = 5$. (**d**) Representative western blots to show N1ICD and UCP1 expression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



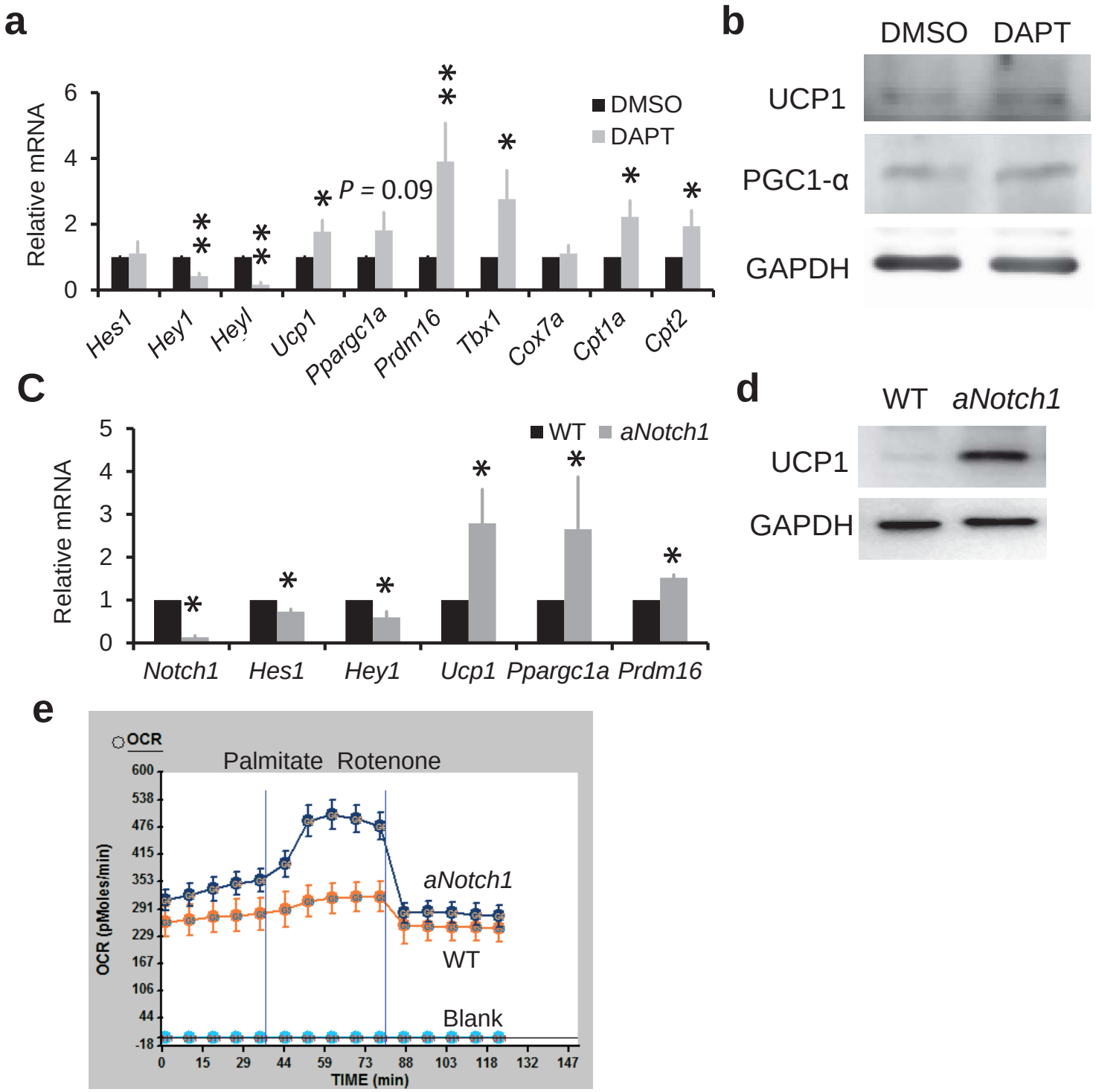
Supplementary Figure 2 *aP2* lineage tracing and gene expression assay. (**a**) YFP fluorescence in tissues of *aP2-Cre/Rosa*^{EYFP} mice, arrow points YFP^{+} cells. (**b**) CD68 (red color) immunostaining to show macrophage (arrow) in cultured inguinal SVF cells from *aP2-Cre/Rosa*^{EYFP} mice. (**c**) Fluorescence-activated cell sorting of CD11b^{+} and F4/80^{+} 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 μm (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 ± 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_2 consumption, CO_2 production, respiration exchange ratio (VCO_2/VO_2) and heat production, *n* = 5. (**h**) Rectal temperature measurement, *n* = 4. **P* < 0.05, ***P* < 0.01. Data are means ± SEM.

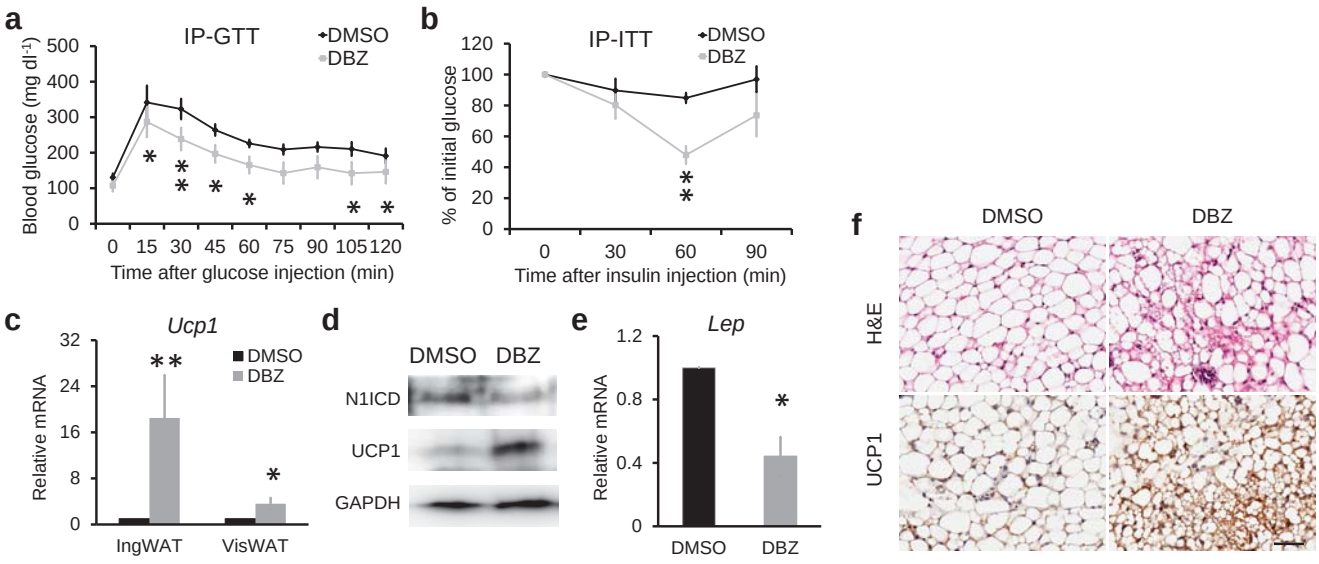


Supplementary Figure 4 Characterization of *Adipoq/N1ICD* mice**.** (**a**) Body weight measurement at 7–11 weeks old, *n* = 8 pairs of mice. (**b**) Food intake normalized to body weight, *n* = 6 pairs of mice. (**c**) Representative H&E and UCP1 staining images of IngWAT from mice acclimated at 4 °C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



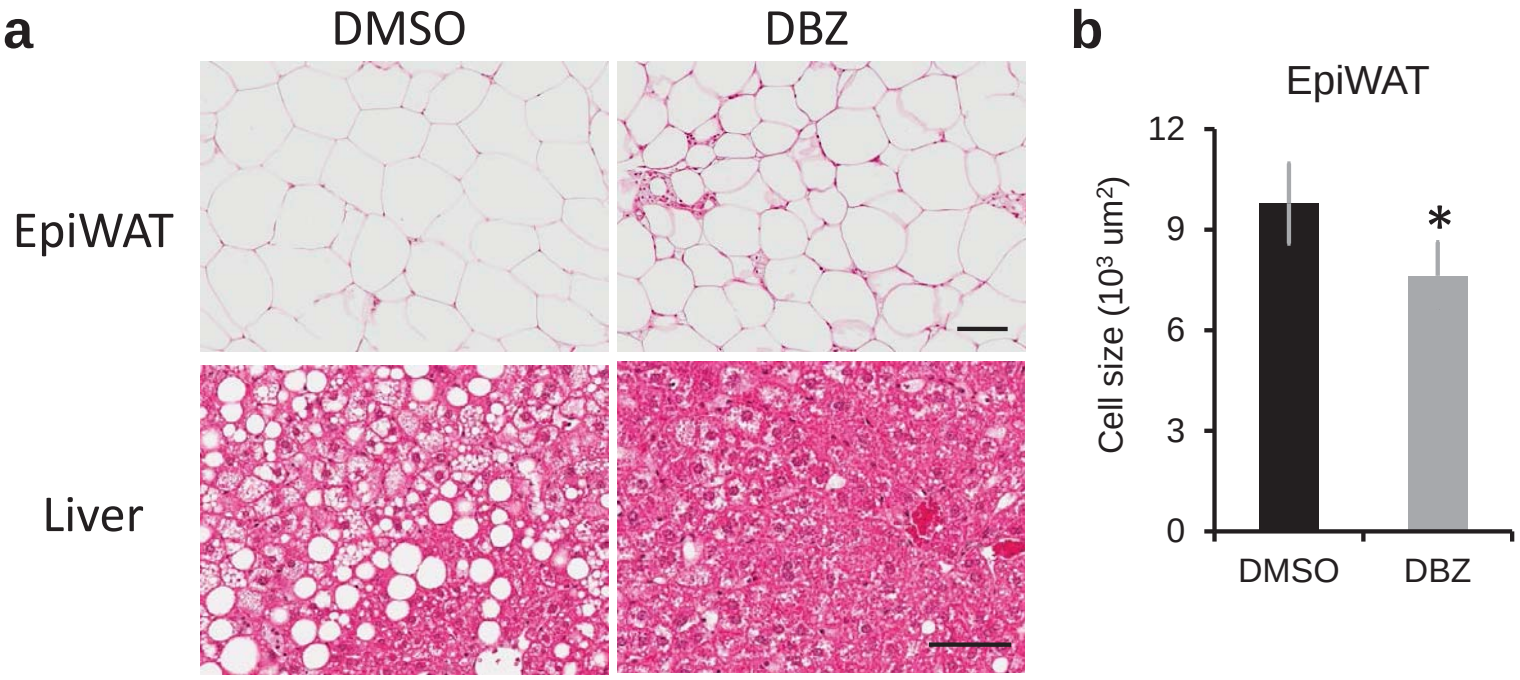
Supplementary Figure 5 Browning of cultured white adipocyte with inhibition of Notch signaling. (**a**,**b**) Gene expression in cultured EpiWAT adipocytes treated with DAPT. (**c**,**d**) Relative expression of Notch targets and BAT-related gene in cultured WT and *aNotch1* inguinal adipocytes. (**e**)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

Nature Medicine: doi:10.1038/nm.3615



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

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

Site	**Sequence** 5'-3'
PTC	5'-TAAGCCTGCCCAGAAGACTC-3' 5'-AAAGTCGCTCTGAGTTGTTAT-3'
Notch1^{KO}	5'-TGGCCTGCCTGTCTGGAACAACAGTTCAGG-3' 5'-ACCCTTGCCTCAGTTCAAACACAAGATACG-3'
Rbpj^{KO}	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.

Nature Medicine: doi:10.1038/nm.3615