Supplementary Materials for

Notch signaling regulates adipose browning and energy metabolism

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Supplementary Figs. 1–7
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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  

(a) YFP fluorescence in tissues of aP2-Cre/RosaEYFP mice, arrow points YFP* cells.  
(b) CD68 (red color) immunostaining to show macrophage (arrow) in cultured inguinal SVF cells from aP2-Cre/RosaEYFP 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 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.
**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.

<table>
<thead>
<tr>
<th>Site</th>
<th>Sequence 5'-3'</th>
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| PTC        | 5'-TAAGCCTGCCCAGAAGACTC-3'  
|            | 5'-AAAGTCGCTCTGAGTTGTTAT-3'                                                   |
| Notch1KO   | 5'-TGGCCTGCCTGTCTGGAACACAGTTTCAGG-3'                                          
|            | 5'-ACCCCTTGCTCAGTTCAAACACAAGATACG-3'                                          |
| RbpjKO     | 5'-CTTGATAATTCTGTAAGAGA-3'                                                    
|            | 5'-CCACAGGCAACAATTGAG-3'                                                      |
| Primer1    | 5'-GCCGTGTTAGCAGGGATTTA-3'                                                    
|            | 5'-AGGTCTCTTGGGGGACAGT-3'                                                     |
| Primer2    | 5'-TGAGGTGAAGACCGAGAAGG-3'                                                    
|            | 5'-CGCACAGAGCACTCAATCTG-3'                                                    |
| Primer3    | 5'-CACAAGAGCTGCTGTCTGGA-3'                                                    
|            | 5'-GCACCTTCTGCACTTTTTTC-3'                                                    |
| Primer4    | 5'-TTTCAGTGTGGTCCTTCATCT-3'                                                   
|            | 5'-CCCAGAAAAACAAATGCTAGA-3'                                                   |

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