Supplemental Data

Research Article

Adipose is a conserved, dosage-sensitive anti-obesity gene

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Supplemental Experimental Procedures

Negative geotaxis assays. Ten sex and age matched flies were put into empty vials with a line drawn one inch above the bottom. Vials were shaken several times and flies were tapped down to the bottom of the vial. The time required for 50% of flies to migrate one inch above the bottom of the vial was measured. The experiment was performed twice and with triplicate groups of flies per genotype.

Fractionation of adipose tissue. Explanted fat depots from 6-week old mice were minced with a razor blade, treated with collagenase (1 mg/ml) for 1.5 hr at 37°C in 100 mM HEPES, pH 7.4, 120 mM NaCl, 50 mM KCl, 5 mM glucose, 1 mM $CaCl_2$, 1.5% bovine serum albumin (w/v), then debris removed with a 200 μm mesh. The filtrate was centrifuged at 100 x g for 5 min producing a floating adipocyte fraction and a pelleted stromal-vascular fraction.

Plasmids. mAdipose (NM_199306**,** WDTC1**)** was PCR amplified from cDNA derived from 3T3-L1s and placed into the pMX retroviral vector (gift of Dr. Nolan) generating pMX-Adp. pMX-AdpC1 was generated by PCR amplification and cloning into pMX. For shRNA knockdown experiments, three hairpins targeting different regions of Adp were cloned into the mU6-neo shRNA plasmid and tested for knock-down efficiency by qPCR and the two most efficient were used for further experiments. pMX-GFP-Adp and pMX-GFP-AdpC1 were constructed by inserting GFP N-terminal to full-length mouse Adp or AdpC1. The pMX-NLS-Adp was constructed by cloning the large T antigen NLS (DPKKKRKV) N-terminal to full-length mouse Adp. pMX-NES-Adp was constructed by cloning the human protein kinase inhibitor α nuclear export signal

(NSNELALKLAGLDINKTE) N-terminal to full-length mouse Adp. FLAG- and myc-tagged versions of mouse Adp, H2B and H4 were generated by subcloning appropriate fragments into pCMV-3x FLAG 7.1 (Sigma) and pCS2+-6x myc-N-terminal vectors. FLAG-HDAC3 expression plasmid was a generous gift of Dr. Eric Olson. All clones were sequenced verified. Primer sequences are available upon request.

Plasma analyses. Overnight fasted mice were euthanized and blood was drawn by cardiac puncture into EDTA-coated syringes. After a brief centrifugation plasma samples were collected and aliquots were kept at -80°C until analysis was performed. Plasma insulin and leptin levels were measured by ELISA (Linco Research Inc., MENDO-75K-02).

Confocal microsopy. pMX-GFP-Adp or pMX-GFP-AdpC1 were transfected into C3H10T1/2 cells plated on poly-L-lysine (Sigma) coated coverslips. 48 hrs after transfection, samples were collected, washed with PBS, fixed with 4% paraformaldehyde, and coverslips were mounted on a glass slide. Images were acquired using a Zeiss LSM510 confocal microscope.

Co-immunoprecipitations. HEK293 cells, maintained in DMEM with 10% fetal calf serum, 10 units/ml penicillin, 10 μg/ml streptomycin, were transfected with the indicated plasmids using FuGene6 (Roche). 5 μM MG132 (Sigma) was added to the culture 16 hours prior to harvest. Cells were harvested and lysed in RIPA buffer containing Protease Inhibitor Cocktail (Sigma) and 1 mM PMSF. Cell lysates were then incubated with anti-myc or anti-FLAG monoclonal antibody for 2 hours at 4ºC, and immunoprecipitated with standard protocols. Eluted proteins were resolved by SDS-PAGE, blotted onto nitrocellulose membranes, and then detected with anti-FLAG or anti-myc antibodies and mouse TrueBlot (eBiosciences) HRP-conjugated secondary antibodies.

Luciferase assays. tk-PPRE3X-luc plasmid (generous gift of Dr. Ron Evans) was used as a reporter for PPARγ transcriptional activation. A pRL-cytomegalovirus (CMV) vector (Promega Corp.) expressing the *Renilla* luciferase gene served as an internal control, and all results were normalized against this control. Transfections were performed in 24-well plates using Fugene 6 (Roche) and transfections typically consisted of 20 ng tk-PPRE3X, 5 ng pRL-CMV, 50 ng pCS2+-PPARγ2, and 200 ng effector or control expression vector. pMX-GFP or pMX-Adp was used for Adp

overexpression experiments and mU6neo shRNA or mU6neo-Adp shRNA plasmid was used for RNAi experiments. The mU6neo-Adp shRNA plasmid used in luciferase assays targets a region of Adp mRNA that is common to both murine and human Adp mRNA and knockdown efficiency was validated (see Figure 4A). Other luciferase reporter systems were comprised of pCS2+-C/EBPβ/-85cMGF-luc, CMV-N-SREBP1a or –SREBP2/LDL-SRE3X-luc, (generous gifts of Drs. Achim Leutz, Michael Brown and Joseph Goldstein). 36-48 hrs. post-transfection, cell lysates were harvested and luciferase assays were performed with the Dual Luciferase Reporter Assay System (Promega Corp.) following the manufacturer's guidelines. Luciferase activity was measured using the POLARstar OPTIMA plate reader (BMG labtech).

Statistical analyses. Differences between groups were assessed using the paired Student's t-test.

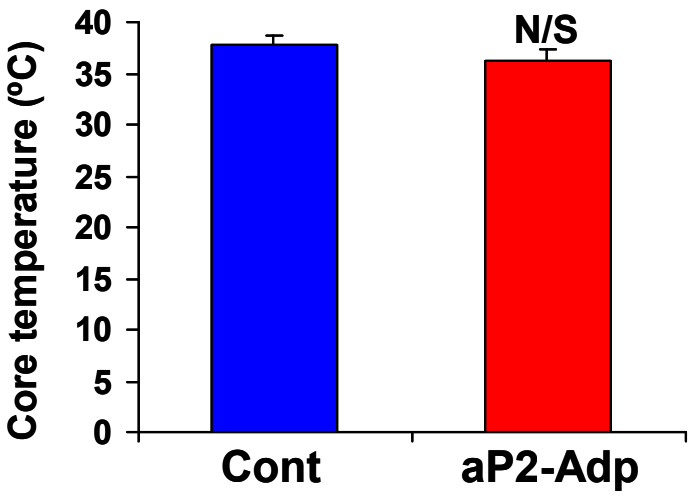

Figure S1 Core Body Temperature of aP2-Adp Transgenic Mice.

Core body temperature of control and aP2-Adp littermates was measured and the average temperature was plotted (n = 8). Error bars represent SEM. N/S not significant by t-test.

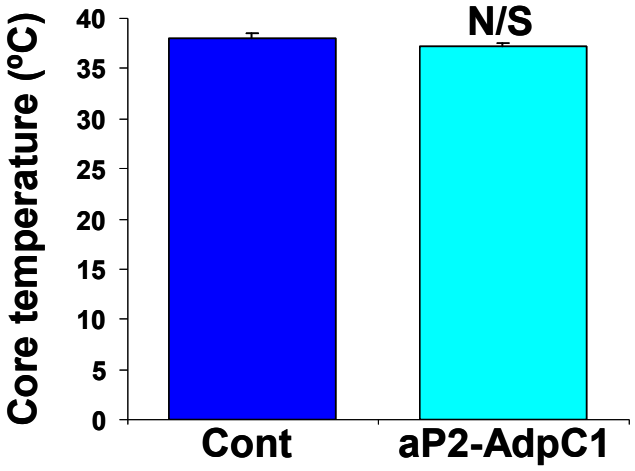

Figure S2 Core Body Temperature of aP2-AdpC1 Transgenic Mice.

Core body temperature of control and aP2-AdpC1 founders was measured and the average temperature was plotted (n = 10). Error bars represent SEM. N/S not significant by t-test.

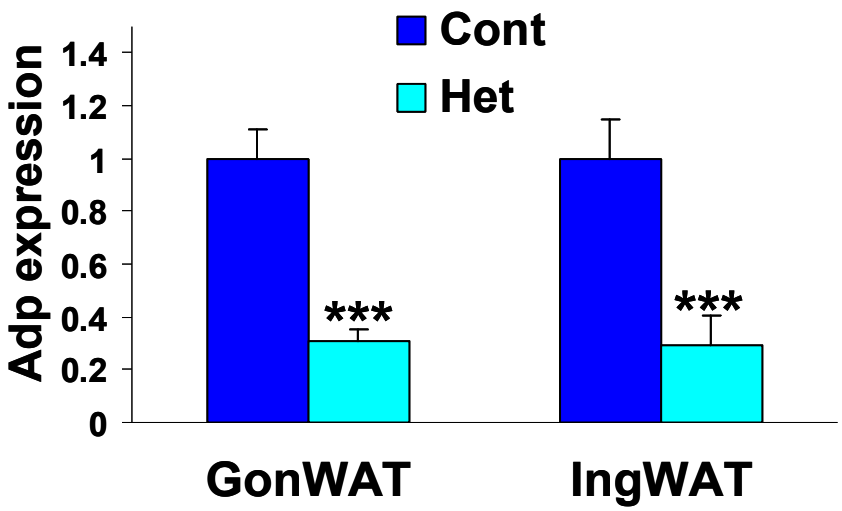

Figure S3 Adp Expression Levels in *Adp* Heterozygous Mice.

RNA was extracted from perigonadal (Gon) and inguinal (Ing) white adipose tissues (WAT) of mice harboring a heterozygous (Het) insertion and wild-type control littermates (Cont). Real-time PCR was then used to quantify Adp expression. ***$p<0.005$ by t-test. Error bars indicate SEM.

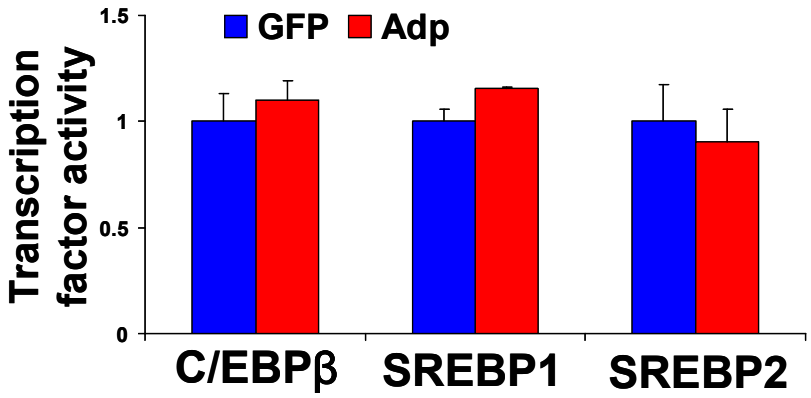

Figure S4 C/EBP and SREBP Transcription Factor Activity Are Not Affected by Adp. GFP or Adp and the indicated transcription factors along with their cognate luciferase reporter and the renilla luciferase control reporter were transfected into HEK293 cells. Luciferase reporter activity was normalized with the renilla control and the normalized data was plotted as transcription factor activity. Error bars indicate SEM.