SUPPLEMENTARY METHODS

Cell culture

Mouse NIH3T3 fibroblasts and 3T3-L1 preadipocytes were maintained in growth media (DMEM with 10% calf serum, 10 units/ml penicillin, 10 μg/ml streptomycin) at 37 °C in 5% CO_2. Cells were passed before confluence and discarded after 10 passages. Media was changed every other day during cell maintenance and adipogenic induction. For the necessity studies, 3T3-L1 cells were transfected with the shRNAi plasmids using Lipofectamine (Invitrogen) and selected in 10% calf serum in DMEM + 400 μg/ml G418 for 2 weeks as described (McKay *et al.* 2003). Hundreds of resistant clones were then pooled and seeded for further studies. (Some cells were harvested at Day 0 and tested for knockdown of TPPII message.) 3T3-L1s were grown to confluency in growth media and induced to form adipocytes as described (MacDougald and Lane 1995). For the sufficiency studies, GFP, TPPII, TPPII-D44A, TPPII-ΔN, TPPII-Cterm, and TPPII-PD were cloned into the retroviral vector pMX and viral supernatants were used to infect subconfluent NIH3T3 or 3T3-L1 cells. Cells were then induced to form adipocytes as described (Suh *et al.* 2006). Oil Red O staining and lipid quantification were as described (Suh *et al.* 2006).

RNAi

For worm TPPII-RNAi, the dsRNA encompassed all of the TPPII cDNA (F21H12.6). CKR-1 dsRNA included the first 8 exons of the gene (T23B3.4). CKR-2 dsRNA included the 5th exon through the first half of the 9th exon (Y39A3B.5).

For the mouse 3T3-L1 cell line RNAi experiments, targeting oligos were annealed and cloned into the mU6 vector as described (McKay *et al.* 2003). RNAi sequences for hairpin RNAi were as follows:

mU6-TPPII #1 target sequence: AACAGGAAGTGGTGATGTAAA

mU6-TPPII #2 target sequence: AAGCAACTCATTGGCCAAATT

RT-PCR

Total RNA was extracted with Trizol (Invitrogen), DNAse I-treated, and cDNA synthesized using random hexamers (LeSueur and Graff 1999; Suh *et al.* 2006). For real-time PCR, PCR reactions were performed with Applied Biosystems *Power* SYBR Green PCR Master Mix on an Applied Biosystems 7500 Real Time PCR system. For mouse samples, gene expression was normalized to β-actin (Suh *et al.* 2006). For worm samples, gene expression of CKR-1 and CKR-2 was normalized against two different genes, *ard-1* and *nhr-23* (Van Gilst *et al.* 2005). Primer sequences are available upon request.

Generation of *Tpp2* null mice

ES cells containing an insertion in the 2nd intron of the *Tpp2* gene were purchased from Bay Genomics (ES cell line XB429) and then analyzed with a series of molecular studies, including PCR analyses and direct sequencing by inverse PCR of the insertion junctions, which showed that the *Tpp2* locus was appropriately disrupted. After molecular confirmation, the *Tpp2* mutant ES cells were injected into C57BL/6 donor blastocysts and implanted into pseudopregnant ICR females. Chimeric male pups were then mated to C57BL/6J mice and transmission of the insertion was monitored by PCR genotyping.

Mice used in the metabolic studies were ingressed at least six generations to C57BL/6J mice.

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Suh, J., X. Gao, J. P. McKay, R. M. McKay, Z. Salo, et al. 2006 Hedgehog signaling plays a conserved role in inhibiting fat formation. *Cell Metabolism* **3:** 25-34.

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