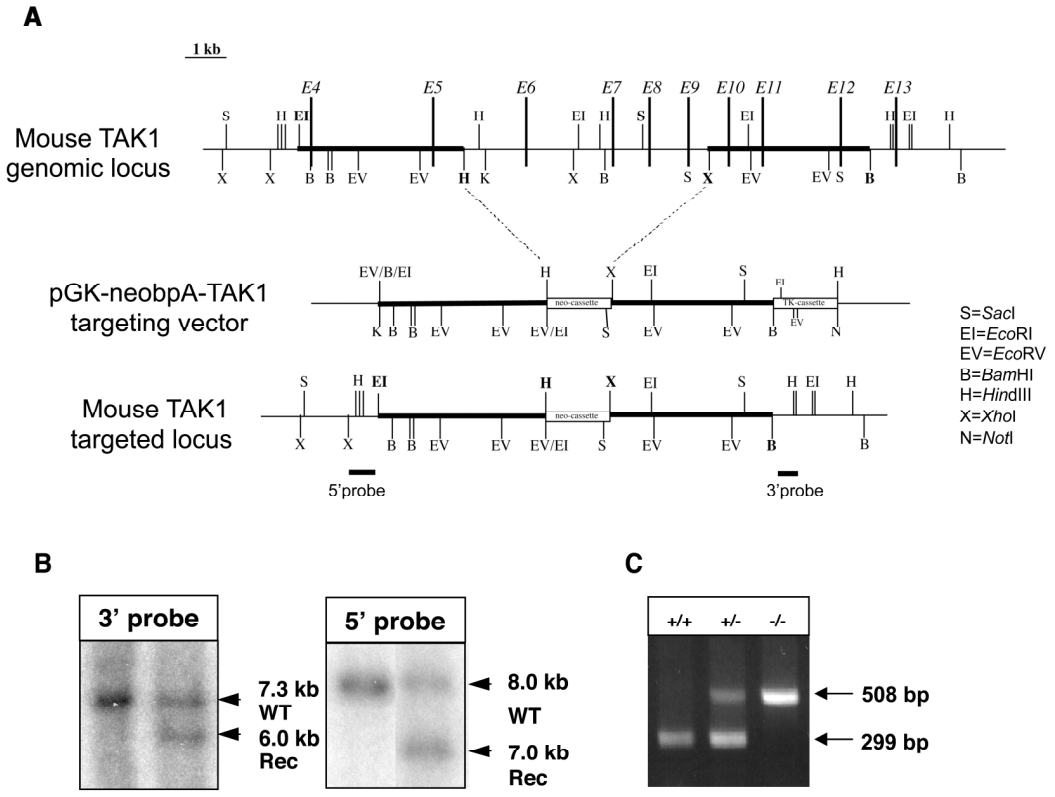
SUPPLEMENTARY METHODS

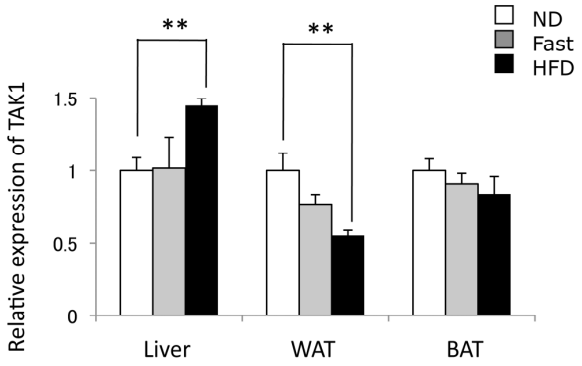
Microarray analysis. The quality and integrity of the RNA was assessed by bioanalyzer (Agilent, Santa Clara, CA) and agarose gel electrophoresis. RNA was amplified using the Agilent Low RNA Input Fluorescent Linear Amplification Kit. Starting with 0.5 mg of amplified total RNA, Cy3- or Cy5-labeled cRNA was produced according to manufacturer's protocol. For each two-color comparison, 750 ng of each Cy3- and Cy5-labeled cRNAs were mixed and fragmented using the Agilent *In Situ* Hybridization Kit. Hybridizations were performed for 17 h in a rotating hybridization oven using the Agilent 60-mer oligo microarray processing protocol. Data were retrieved using the Agilent Feature Extraction software (v8.1), using defaults for all parameters. Images and GEML files, including error and p-values, were exported from the Agilent Feature Extraction software and deposited into Rosetta Resolver (version 6.0, 6.0.0.0.304) (Rosetta Biosoftware, Kirkland, WA). The resultant ratio profiles were combined into ratio experiments as previously described (1). Intensity plots were generated for each ratio experiment and genes were considered "signature genes" if the p value was less than 0.001. The signature genes were sorted into different categories using GeneSpring software. The data discussed in this publication have been deposited in NCBIs Gene Expression Omnibus at (http://www.ncbi.nlm.nih.gov/geo; accession number GSE21903).

Glucose tolerance test (GTT) and insulin tolerance test (ITT). After an overnight fast, GTT or ITT were performed by intraperitoneal injection of glucose (2 g/kg) or insulin (0.75 U/kg) (Eli Lilly, Indianapolis, IN), respectively. Glucose levels were analyzed using glucose test strips (Nova Biomedical, Waltham, MA).

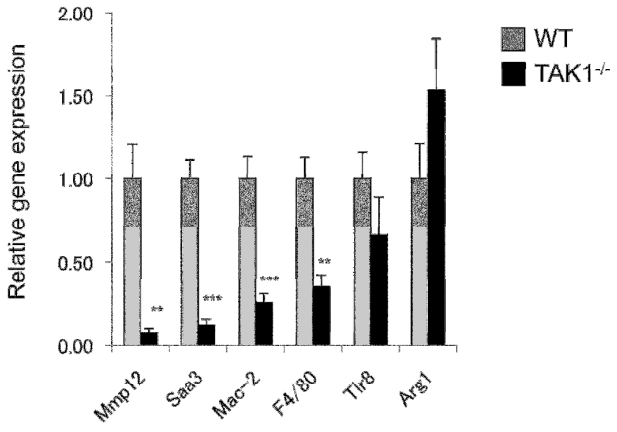
Supplementary FIG. 1. Strategy of generating TAK1 null mice. (a) Schematic view of the mouse TAK1 locus, targeting vector pOSdupdel-TAK1, and recombination at the TAK1 locus. A 4 kb 5'-fragment, that included exons 4 and 5, was inserted into the *Kpn*I/*Hind*III sites of pGK-neobpA and a 4 kb 3'-fragment that included exons 10-12, was inserted into the *Xho*I/*Bam*HI sites of pGK-neobpA. pGK-neobpA-TAK1 was linearized by *Not*I and electroporated into 129/Sv ES cells. G418 resistant ES clones were isolated and screened by Southern blot analysis for homologous recombination events using 5' and 3' probes. The targeting vector was created such that, after homologous recombination, the 1.8 kb neomycin cassette would be inserted between intron 5 and 9, resulting in the deletion of exons 6-9. These exons encode the region ^{126}Gly and ^{311}Arg and include part of the first zinc finger, the entire second zinc finger, and the entire hinge domain of TAK1. In addition, this deletion places exon 10 out of frame with exon 5. This deletion removes the DNA binding domain and activation function and renders a transcriptionally inactive TAK1. Gene-targeted ES cells were microinjected into blastocysts from C57BL/6 mice and returned to pseudopregnant B6D2 mice. The disrupted TAK1 allele was transmitted through the germ line. (b) Diagnostic Southern blot analysis. Genomic DNA from WT and recombinant embryonic stem cells was cut with *Hind*III or *Xho*I, electrophoresed, and hybridized to the 3'- or 5'-flanking probe. The 3'-probe hybridized to a 7.3 and 6.0 kb mRNA while the 5'-probe hybridized to 8.0 and 7.0 kb mRNA in DNA from the WT and recombinant allele, respectively. (c) Diagnostic PCR genotyping. Genomic DNA from WT, heterozygous, and homozygous mice were analyzed by PCR. Genotypes were confirmed by PCR using 3 primer sets, WF (5'-CCCACATATCTGTTGTCTTCCA), WR (5'-AGGGAAGAAACACCCTGAGC), and Neo3'-2 (5'-TCTATCGCCTTCTTGACGAG). The 299 bp of WT allele was amplified with WF and WR primer set, and 508 bp of mutant allele was amplified with Neo3'-2 and WR primer set.



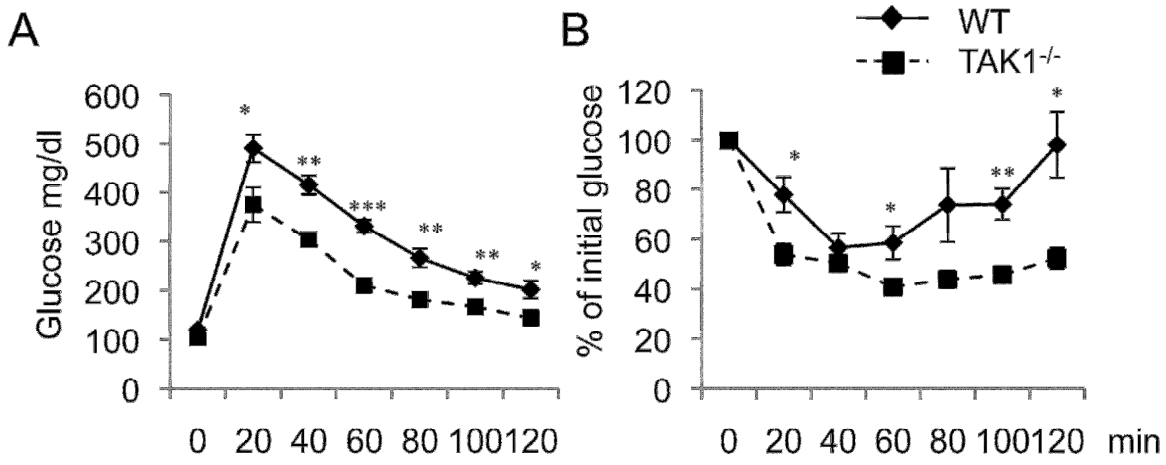
Supplementary FIG. 2. TAK1 is highly expressed in the liver and adipose tissues. TAK1 is highly expressed in several tissues that are critical in lipid and energy homeostasis, such as white adipose tissue (WAT), brown adipose tissue (BAT), and liver. TAK1 expression was analyzed in mice fed regular chow diet (RD), after fasting, or a HFD. No significant differences in TAK1 mRNA expression were observed in either liver or adipose tissues after 16 h fasting. In contrast, HFD feeding resulted in a modest, but significant, induction and repression of TAK1 mRNA in liver and WAT, respectively.



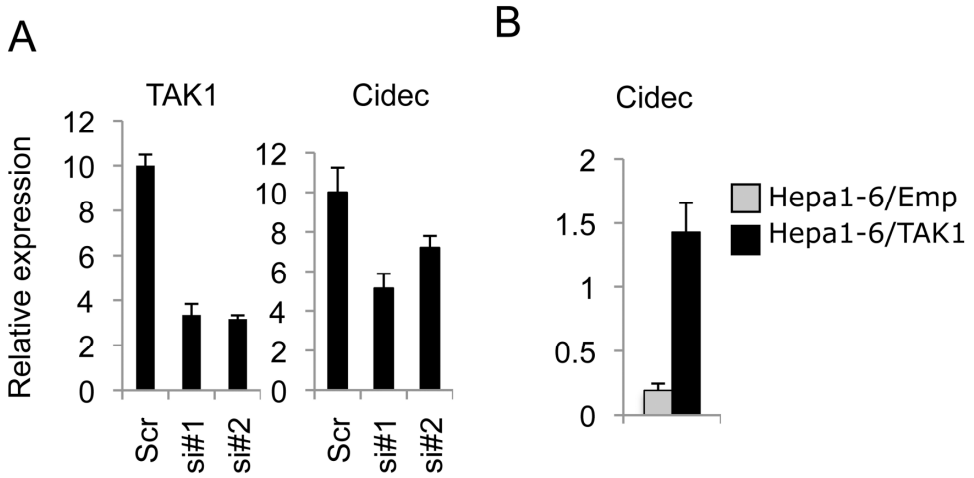
Supplementary FIG. 3. Male TAK1$^{-/-}$ mice are protected from age-induced adipose tissue-associated inflammation. Induction of several inflammatory genes was greatly decreased in WAT of one-year old TAK1$^{-/-}$ mice compared to WT littermates (n=5). Gene expression was analyzed by QRT-PCR. Data represent mean ± SEM. ** p<0.01, *** p<0.001.



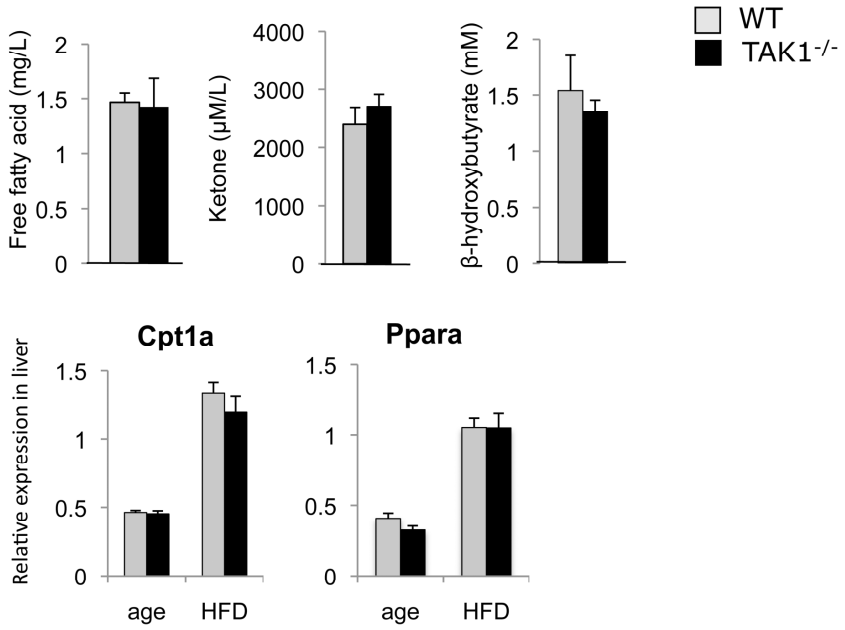
Supplementary FIG. 4. 4-5 month-old TAK1$^{-/-}$ mice fed with a regular chow diet exhibited a significant greater glucose tolerance and insulin sensitivity than WT littermates. A, B: GTT and ITT analyses in 4-5 month-old WT and TAK1$^{-/-}$ mice (WT, n=5; TAK1$^{-/-}$, n=4).



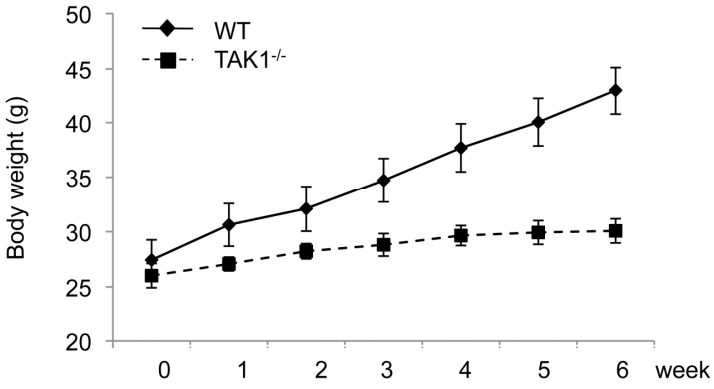
Supplementary FIG. 5. Regulation of Cidec expression by TAK1 in Hepa1-6 cells. A: TAK1 expression was down-regulated by TAK1-specific siRNAs #1 and #2, and 48 hrs later the expression of TAK1 and Cidec analyzed by QRT-PCR. B: Induction of Cidec in Hepa1-6 cells by exogenous expression of TAK1. Hepa1-6 cells/Emp and Hepa1-6/TAK1 stably expressing pLXIN or pLXIN-TAK1 were analyzed for the expression of Cidec. The results plotted represents the analysis of 4 or 5 different pLXIN or pLXIN-TAK1 expressing clones, respectively.



Supplementary FIG. 6. No apparent difference was observed between the level of β-oxydation in WT and TAK1$^{-/-}$ mice. Free fatty acids, ketone, and β-hydroxybutyrate levels were analyzed in sera from WT(HFD) and TAK1$^{-/-}$(HFD) mice. The expression of Cpt1a and Ppara was analyzed in liver of aged WT and TAK1$^{-/-}$ mice or mice fed with a HFD.



Supplementary FIG. 7. Comparison of the absolute body weights of WT and TAK1$^{-/-}$ mice fed with a HFD.



Supplementary Table 1. QRT-PCR probes and primers. SYBRG and TaqMan QRT-PCR analyses were performed to validate genes identified by microarray analyses as described previously (2). Primers and probes were designed using the ABI PrimerExpress 2.0 software. Predesigned Assays-on-Demand probe sets were purchased from Applied Biosystem (Foster City, CA). All results were normalized relatively to the 18S or GAPDH transcripts.

Gene	Forward primer	Reverse primer	Probe (5'-3-FAM 3'-TAMRA)
18S	CGGCTACCACATCCAAGGAA	GCTGGAATTACCGCGGCT	TGCTGGCACCAGACTTGCCCTC
Acaa	GACTGTACCTTTGTCTACGGT	TGCCAATGTCATAAGACCCATT	
Acox1	GGGAGTGCTACGGGTTACATG	CCGATATCCCCAACAGTGATG	
Agpat6	TGGGACAGGTTCACGGGGGC	ACCAGACATGGGGGCAGGCT	
Arg1	CATTGGCTTGCGAGACGTAGA	TTGCCAATCCCCAGCTTGT	
Cd36	TCATGCCAGTCGGAGACATG	TGTCTGTACACAGTGGTGCCTGT	
Cidea	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT	
Cideb	GACCCTTCCGTGTCTGTGAT	GTAGCAGCAAGGTCTCCAGG	
Cidec	CCTATGACCTGCACTGCTACAAG	CATGTAGCTGGAGGTGCCAAG	
CoxIV	AAGTTCAGTTGTACCGCATCCA	TCCCAAATCAGAACGAGCG	
CyclophilinB	GGTGGAGAGCACCAAGACAGA	GCCGGAGTCGACAATGATG	
Dgat1	GTGCACAAGTGGTGCATCAG	CAGTGGGATCTGAGCCATCA	
Dyx1c1	CCAACAGCACTTCGGGAATC	TCTCCGTGCTTCTGCTTGTTT	CAGCCACTCCTCCTCTTCTGCGACTTG
Errα	GCAGGGCAGTGGGAAGCTA	CCTCTTGAAGAAGGCTTTGCA	
F4/80	ACCCTCCAGCACATCCAGCCA	TCACAGCCCGAGGGTGTCCA	
Fabp2	ACGGCACGTGGAAAGTAGAC	GTCATGAGCTCCAAGCTTCC	
G6Pase	ATGAACATTCTCCATACTTTGGG	GACAGGGAACTGCTTTATTATAGG	
Gpam	ACGCACACAAGGCACAGAG	TGCTGCTCAGTACATTCTCAGTA	
Gprc5b	ATCTCACACGGGAAGACACC	GCCCTCAAGAAAGACACAGC	
IL6	GAGAAAAGAGTTGTGCAATGGC	CCAGTTTGGTAGCATCCATCAT	
Lxrα	GAGTTGTGGAAGACAGAACCTCAA	GGGCATCCTGGCTTCCTC	
Mac-2	ATGAAGAACCTCCGGGAAAT	TTAGATCATGGCGTGGTTAGC	
Mmp-12	CCCACTTCGCCAAAAGGTTT	CATGAGCTCCTGCCTCACATC	
Mogat1	CCAGCGCAAAGGGTTTGTT	CACCAAAAGAAAATACTGGAACCA	
Pgc-1a	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC	
Pparγ	GGAAGACCACTCGCATTCCTT	TCGCACTTTGGTATTCTTGGAG	
Pxr	ATCTGGAACTACCAACCCCC	AGTTGATGACGCCCTTGAAC	
Retn	AAGCCATCGACAAGAAGATCAAA	TCCAGCAATTTAAGCCAATGTTC	
Rip140	CTGGCCACAAAGAGGAAGAC	GACAGTGGGACCATTGCTTT	
Srebp1c	ACGGAGCCATGGATTGCAC	TGTCTCACCCCCAGCATAG	

Saa3	Assays-on-demand	Mm00441203_m1	
Tak1	ATCAGTCAACACCCATCATTGAAG	GGGCATTGTAAGCTCTTGAATGTG	AGGCCCTCTCCTTTCAGACACACA
Tnfα	CAAAATTCGAGTGACAAGCCTG	CACTCCAGCTGCTCCTCCAC	
Trl8	GGCACAACTCCCTTGTGATT	TGTTGTTTGGCATTGTGGTT	
Ucp-1	TCCTAGGGACCATCACCACC	GCAGGCAGACCGCTGTACA	TGGCAAAAACAGAAGGATTGCCGAAA

Supplementary Table 2. Heterozygous TAK1 mating pairs generated offspring with abnormal Mendelian ratios. The genotype ratios generated by heterozygous mating pairs did not correspond to the expected Mendelian ratios. The surviving $TAK1^{-/-}$ pups comprised about 13% of the total offspring suggesting increased mortality of $TAK1^{-/-}$ embryos during development in agreement with a previous report (3). Normal implantation of $TAK1^{-/-}$ embryos was observed at E6.5, but by E12.5 a significant number of $TAK1^{-/-}$ embryos were resorbed (data not shown). Expected Mendelian percentage is indicated in parenthesis

	WT	$TAK1^{+/-}$	$TAK1^{-/-}$
$TAK1^{+/-}$ x $TAK1^{+/-}$	51/167	94/167	22/167
Percentage (%)	30.5 (25)	56.5 (50)	13 (25)

REFERENCES

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2. Kang HS, Angers M, Beak JY, Wu X, Gimble JM, Wada T, Xie W, Collins JB, Grissom SF, Jetten AM: Gene expression profiling reveals a regulatory role for ROR alpha and ROR gamma in phase I and phase II metabolism. *Physiol Genomics* 31:281-294, 2007
3. Collins LL, Lee YF, Heinlein CA, Liu NC, Chen YT, et al. Growth retardation and abnormal maternal behavior in mice lacking testicular orphan nuclear receptor 4. *Proc Natl Acad Sci USA* 101:15058-15063, 2004