# Supporting Information

## Canaan et al. 10.1073/pnas.1323426111

### SI Methods

**Mice.** HLA-F adjacent transcript 10 (FAT10) KO mice were backcrossed 12 times to their C57BL/6 WT background. Control mice in the lifespan studies consisted of $FAT10^{+/+}$ C57BL/6 mice that were derived from heterozygous crossings and C57BL/6 breeding. Overall, this control group showed mortality curves similar to those curves measured by the Jackson Laboratory for C57BL/6 lifespan. For the lifespan studies, mice were continuously bred in our animal facility and added to the evaluated groups. We have excluded from the studies mice that either died or had severe wounds because of fighting. Mice were housed under controlled temperature and lighting (12 h of light from 0700 to 1900 h; 12 h of dark from 1900 to 0700 h) with free access to water and food, and they were fed a normal chow diet (TD2018; Harlan Teklad). All of the studies involving mice were performed at our animal facility according to the guidelines and supervision of the Institutional Animal Care and Use Committee (IACUC) at Yale University School of Medicine.

**I.p. Insulin and Glucose Tolerance Tests.** Insulin tolerance tests were performed on food-deprived (6 h) nonanesthetized mice. Glucose measures were obtained from whole-tail vein blood using an automated glucometer at baseline and 30, 45, 60, and 90 min after i.p. injection of human insulin (either 0.75 or 1.0 mU/kg). Glucose tolerance tests were performed on food-deprived (12 h) nonanesthetized mice. Blood glucose was measured at baseline and 3, 7, 15, 30, 60, 90, and 120 min after i.p. injection of 2 mg/kg glucose bolus. Whole-tail vein blood (30 μL) was sampled at baseline and 3, 7, 15, and 30 min to measure insulin in a subset of mice from the glucose tolerance test.

**Insulin Signaling Experiments.** For assessment of insulin signaling in adipose, liver, and muscle tissues, mice were fasted at 0700 h for 6 h followed by i.p. injection of either saline or insulin (1.0 U/kg). Mice were euthanized by $CO_2$ narcosis followed by cervical dislocation 10 min postinjection, and tissues were harvested and immediately snap-frozen in liquid nitrogen. Tissues were homogenized, and 15–20 μg lysate protein was analyzed by SDS/PAGE analysis.

**Antibodies.** Rabbit polyclonal antibodies anti-AKT, phosphorylated anti-AKT, anti–acetyl-CoA carboxylase, phosphorylated anti-acetyl-CoA carboxylase, anti-Ubiquitin E1, and anti-Perilipin were purchased from Cell Signaling Technologies.

**Body Composition.** Body weights and composition were determined weekly using NMR technology (EchoMRI-100; Echo Medical Systems).

**Real-Time PCR.** All tissues were dissected, snap-frozen in liquid nitrogen, and stored at −80 °C. RNA was extracted from tissues using the RNeasy Lipid Mini or RNeasy Mini Kits (Qiagen).

**Statistics.** Evaluation of the mortality differences was done using a Kaplan–Meier estimator from the OriginPro software package (OriginLab). Calculations of averages, SDs and probability values were done using Excel software (Microsoft). Data are presented as means ± SEs. Data were determined to have a normal distribution with equal variance, and statistical differences were determined by PROC TTEST or PROC GLM using the Tukey least significant differences test with SAS v9.2. Significant differences were determined between groups at $P < 0.05$.

**Quantitative PCR Primers.**

TNF-α: 5′-ATGGGCTTTCCGAATTCAC-3′, 5′-GAGGCAACCTGACCACTC-3′

FAT10: 5′-CAGACATGGCTGTCCGCACCTGT-3′, 5′- GAGACCTTGGTTTGGGACCT-3′

Cpt1b: 5′-ATCTGGGCTATCTGTGTCCG-3′, 5′-TCCTTGGCCAATGTCTCCAT-3′

Acc2: 5′-CGGGTGAAGTACATCAAGCG-3′, 5′-ACTTGGTGTAGCTTCTCCCC-3′

Mcd: 5′-ATCCAGGGCATTGTGAAGGA-3′, 5′-TTCCTTCTGCAGCTCCTTGA-3′

Acox: 5′-GCTGAGGAACCTGTGTCTCT-3′, 5′-TCAAAGGCATCCACCAAAGC-3′

Acot1: 5′-GCTATGGCCTCCTTCCTGAA-3′, 5′-TCCACGGGAATGAAGCTCTT-3′

Acot3: 5′-AAGAGCTTGATTCCCGTGGA-3′, 5′-ACAGTGGGAAGTAAGGAGGC-3′

Ppard: 5′-AGTGGCTTGAAACTTGCTGG-3′, 5′-CACCCCAAGACCTGATCAGT-3′

Ppara: 5′-ACCTTGTGTATGGCCGAGAA-3′, 5′-AAGGAGGACAGCATCGTGAA-3′

Pgc1a: 5′-AGCCTCTTTGCCCAGATCTT-3′, 5′-GGCAATCCGTCTTCATCCAC-3′

Pgc1b: 5′-TCTGCCAACGGAAACAAAGG-3′, 5′-GCTGCTGTCCTCAAATACGG-3′

Ucp2: 5′-CGGCCTAAAGTGATGGCTTC-3′, 5′-CTCATTTGCTGTGCCCTTGT-3′

Ucp3: 5′-CAGCTTCCTCCCTGAACTGA-3′, 5′-CAGGGGAAAAGTGAGGAGGT-3′

Tnfa: 5′-GACCCCTTTACTCTGACCCC-3′, 5′-AGGCTCCAGTGAATTCGGAA-3′

Il1b: 5′-ACTCATTGTGGCTGTGGAGA-3′, 5′-TTGTTCATCTCGGAGCCTGT-3′

Il6: 5′-GCCAGAGTCCTTCAGAGAGA-3′, 5′-GGTCTTGGTCCTTAGCCACT-3′

Mcp1: 5′-AGGTGTCCCAAAGAAGCTGT-3′, 5′-ACAGAAGTGCTTGAGGTGGT-3′

iNos: 5′-CCCCGCTACTACTCCATCAG-3′, 5′-CCACTGACACTTCGCACAAA-3′

Il10: 5′-GGTGAGAAGCTGAAGACCCT-3′, 5′-TGTCTAGGTCCTGGAGTCCA-3′

Lipe: 5′-TGAGATTGAGGTGCTGTCGT-3′, 5′-GTACCTTGCTGTCCTGTCCT-3′

Pnpla2: 5′-CAACGCCACTCACATCTACG-3′, 5′-ACCAGGTTGAAGGAGGGATG-3′

Ptprf: 5′-CTGGAGATCACCGAGGAGTC-3′, 5′-TTGAAAAGGGAAAGGCGGTG-3′

Dgat1: 5′-GTGGCCAGGACAGGAGTATT-3′, 5′-CATACATGAGCACAGCCACC-3′

Dgat2: 5′-GCGCTACTTCCGAGACTACT-3′, 5′-ATCCGGAAGTTACCAGCCAA-3′

Scd1: 5′-GCTTCCAGATCCTCCCTACC-3′, 5′-ACCCTCG-CATTCAGTGGTTA-3′

Gpat1: 5′-CGAAGGTCACTACAATGGCG-3′, 5′-TTGAA-GGAAGGATCGCTGGT-3′

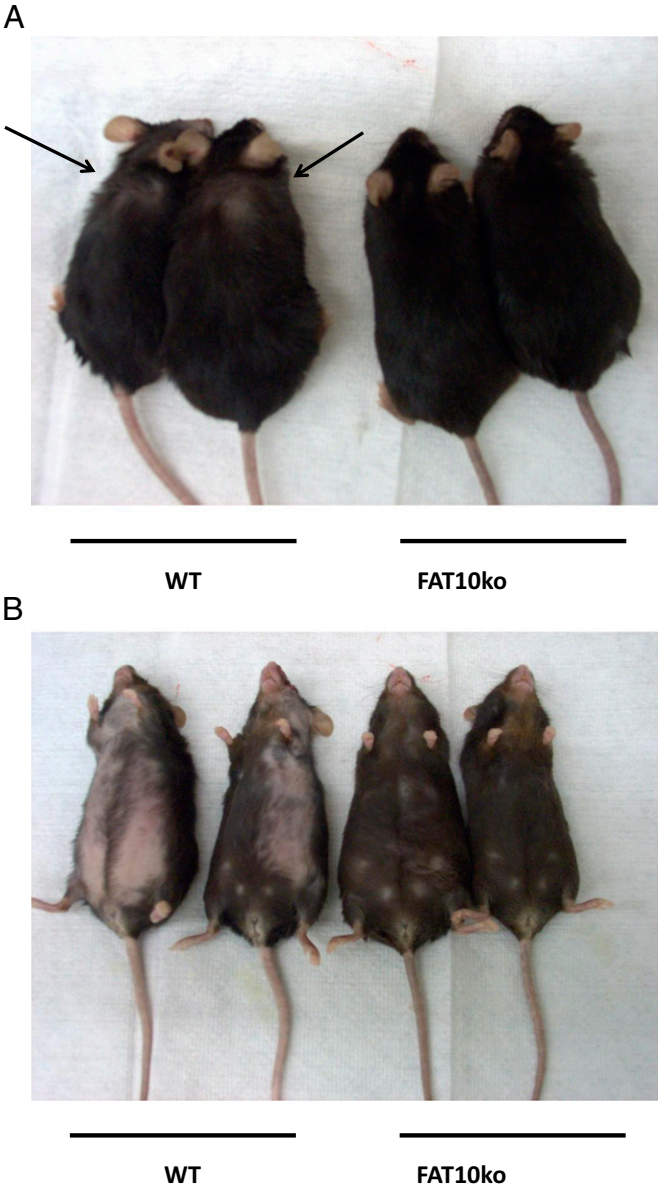


**Fig. S1.** FAT10ko mice show reduced phenotypic characteristics of aging and prolonged lifespan. Observation of 2.5-y-old female mice reveals the slower aging process of the KO mice as manifested in (*A*) the smoother and denser fur seen in the dorsal view and (*B*) the striking decrease of white hair patches in the KO mice. Mortality of KO mice and WT controls was recorded over the course of 1,220 d from birth.

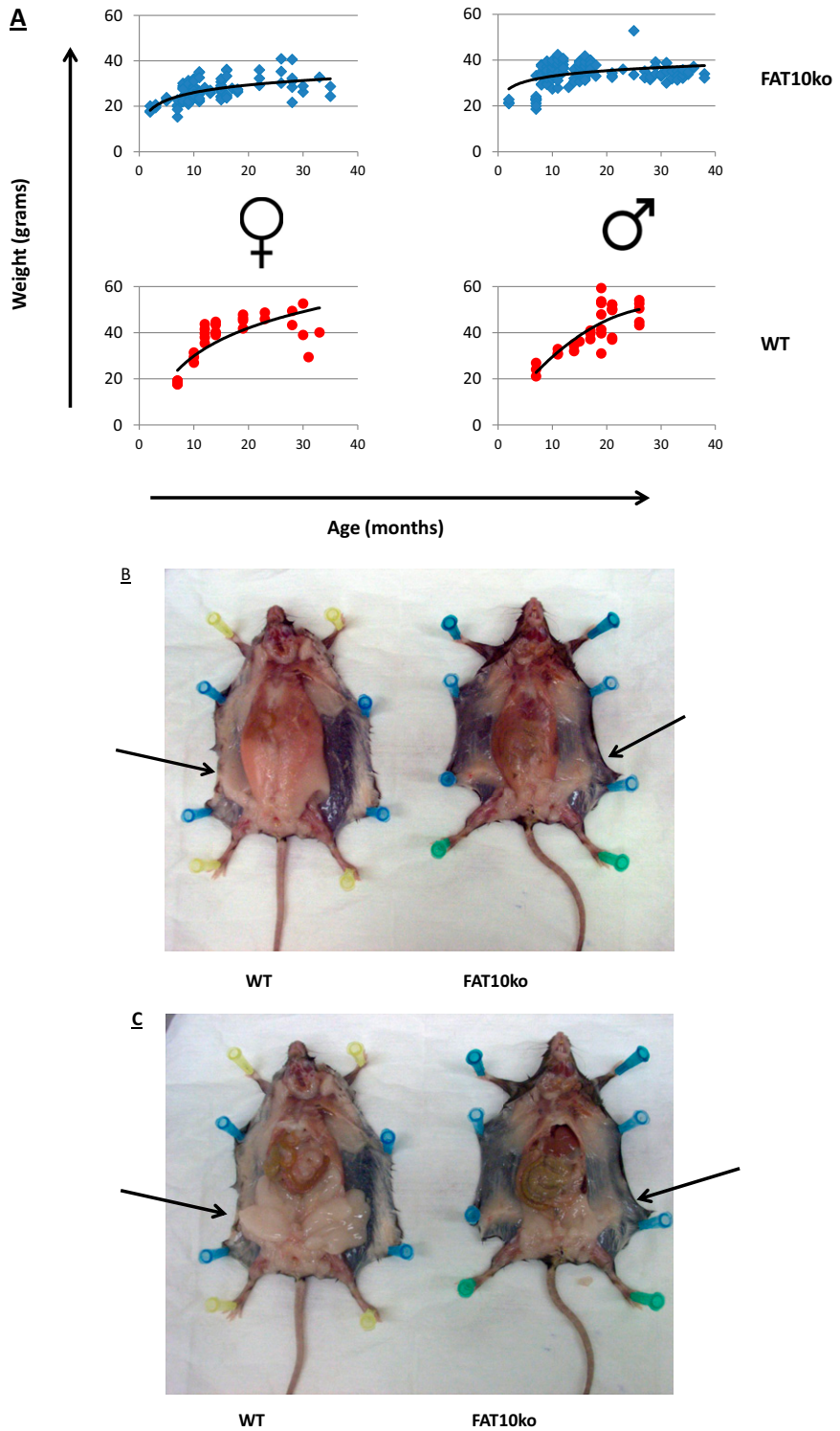


**Fig. S2.** FAT10ko mice have reduced fat tissues and a lower body weight throughout their lifespan. (*A*) FAT10ko mice maintain a lower weight while aging compared with WT controls. Data weight was recorded during the longevity studies (Fig. 1). The mice were periodically measured as shown in the graphs. A regression line was generated for a scatter plot using EXCEL. Comparison of the white adipose depots in 2.5-y-old female mice reveals a major decrease in (*B*) s.c. and (*C*) periovarian fat (arrows) white adipose depots in the KO mouse (*Right*).

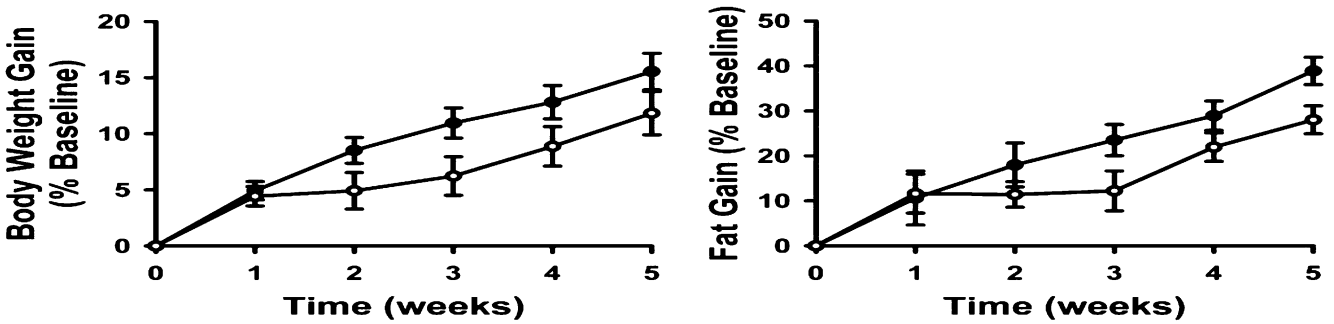


**Fig. S3.** Body weight and fat gain of FAT10ko and WT mice housed at thermoneutrality. Increase in (*Left*) body weight and (*Right*) adiposity are expressed as percent change relative to baseline values. Adiposity was measured weekly by MRI. Data are presented as means ± SEMs ($n$ = 8 per group).

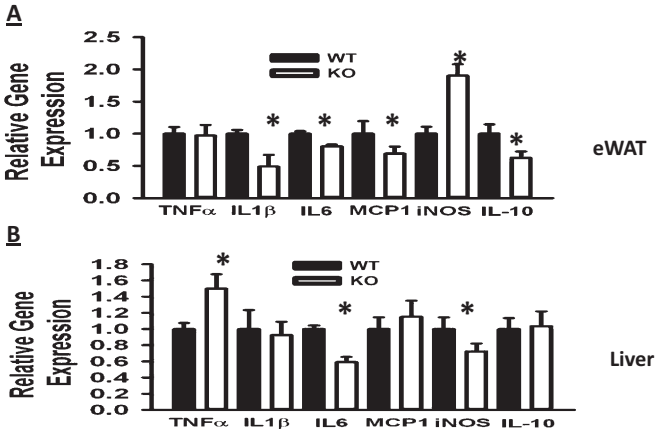


**Fig. S4.** Inflammatory gene expression in adipose tissue and liver of WT and FAT10ko mice. Gene expression in (*A*) epididymal white adipose tissue (eWAT) and (*B*) liver of KO mice is expressed relative to levels in WT mice. Data are expressed as means ± SEMs. *$P$ < 0.05 ($n$ = 6 per group). iNOS, induced nitric oxide synthase; MCP1, monocyte chemotactic protein 1.

**Table S1. Metabolic readouts in serum, liver, and quadriceps muscle of WT and FAT10ko mice**

| | WT | KO |
|---|---|---|
| Serum | | |
| Glucose (mg/dL) | 99 ± 1.83[a] | 71 ± 1.99[b] |
| Insulin (ng/mL) | 1.03 ± 0.14[a] | 0.53 ± 0.11[b] |
| Nonesterified fatty acid (mM) | 0.58 ± 0.093[a] | 0.72 ± 0.07[b] |
| Adiponectin (μg/mL) | | |
| Total | 31.46 ± 0.11[a] | 26.12 ± 0.35[b] |
| High molecular weight | 2.17 ± 0.11[a] | 1.5 ± 0.07[b] |
| Liver | | |
| Triglycerides (μg/mg protein) | 52.16 ± 6.56[a] | 52.16 ± 5.54[b] |
| Glycogen (pg/μg protein) | 8.78 ± 0.93[a] | 3.86 ± 0.67[b] |
| Muscle | | |
| Triglycerides (μg/mg protein) | 91.88 ± 0.88[a] | 84.24 ± 0.51[b] |
| Glycogen (pg/μg protein) | 0.62 ± 0.16 | 0.65 ± 0.11 |

Measurements were obtained from mice fasted for 12 h. Data identified by different letters are significantly different ($P$ < 0.05; $n$ = 6–8 per group).

**Dataset S1. Kaplan–Meier estimator with three independent statistical evaluations**

Dataset S1

Mortality differences for data in Fig. 1 were identified using the Kaplan–Meier estimator with the statistical tests set to a level of 99% confidence. Analysis examined mortality differences among (*A*) females and (*B*) males separately. Mice numbers are shown in Fig. 1. (*C*) Median lifespan sex differences for FAT10ko mice were also examined (the number of mice in each group is shown in parentheses).