Supplemental Material

FIG S1

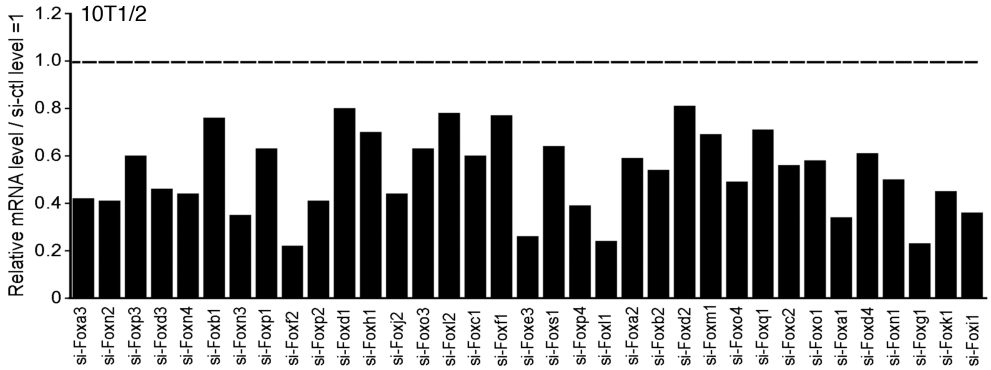


FIG S1 Relative mRNA levels of each individual Fox gene after siRNA knock-down in 10T1/2 cells.

FIG S2

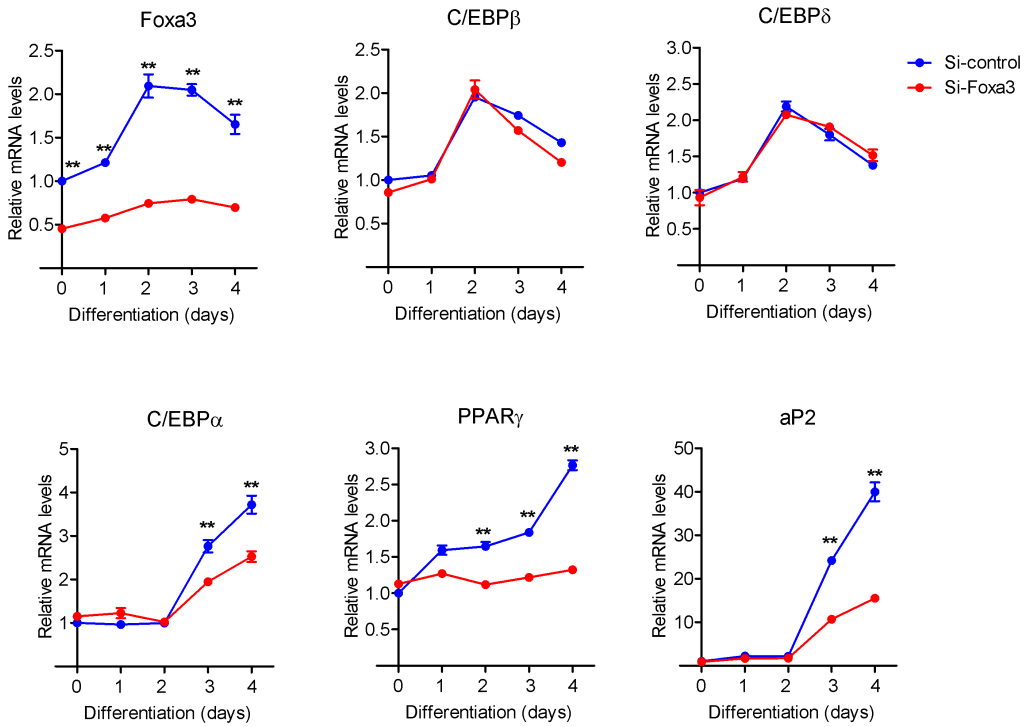
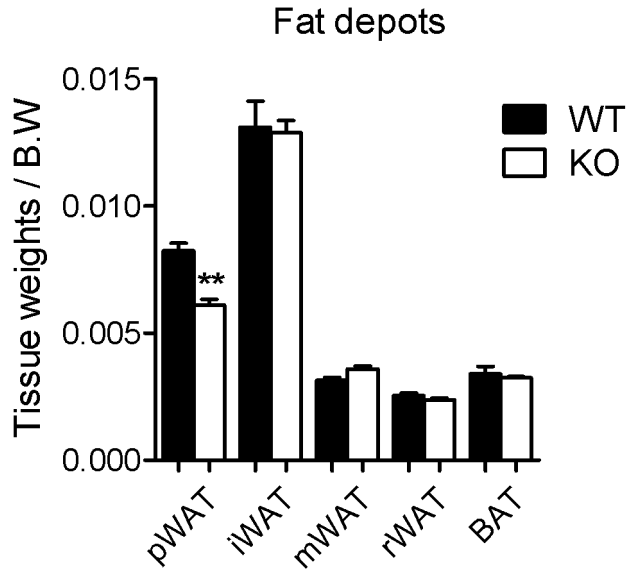


FIG S2 Foxa3 knock-down in 3T3-L1 reduces the levels of late adipocyte markers. si-Foxa3 expression in 3T3-L1 cells decreased Foxa3 levels and reduced the mRNAs of PPARγ, C/EBPα, and aP2, during the course of differentiation but did not affect the levels of the early regulators C/EBPβ and C/EBPδ. Mean ± SEM, **p<0.01.

FIG S3

A



B

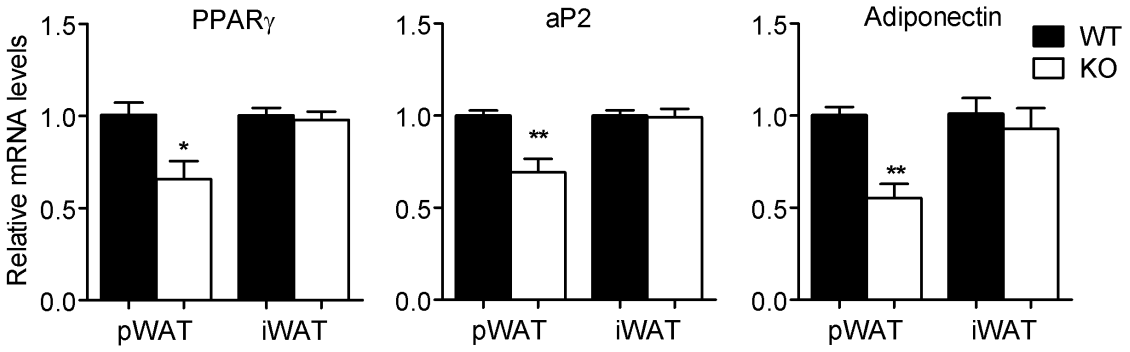


FIG S3 Analysis of fat depot weights (A) and of mRNA levels of PPARγ and its targets genes aP2 and adiponectin (B) in perigonadal fat (pWAT) and in inguinal fat (iWAT) of WT (n=4) and Foxa3-null (n=5) two weeks-old female mice. Mean ± SEM, *p<0.05 and **p<0.01.

FIG S4

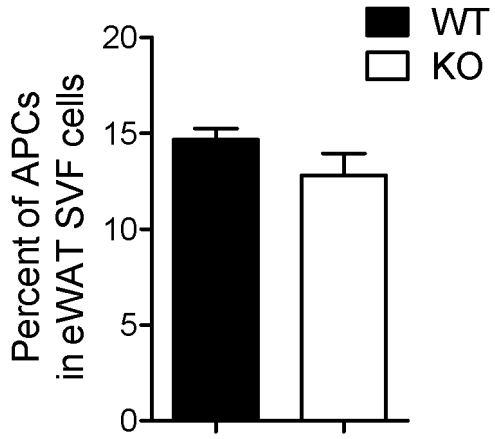


FIG S4 Percentage of adipose progenitor cells (APC) in SVF cells obtained from the epididymal fat depot of WT (n=3) and Foxa3-null (KO) (n=3) two week-old male mice. Mean ± SEM.

FIG S5

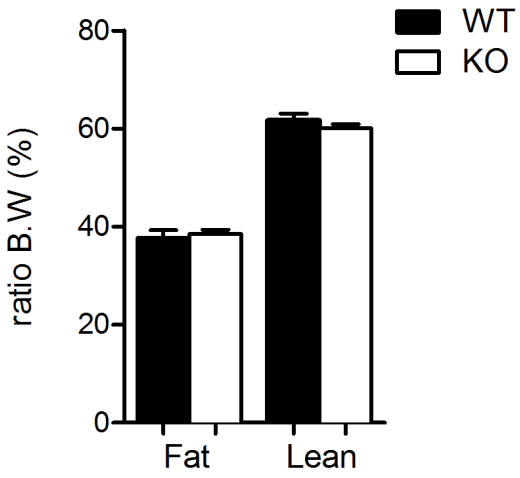


FIG S5 Fat and lean mass in WT and Foxa3-null (KO) mice after HFD (mean ±SEM).

FIG S6

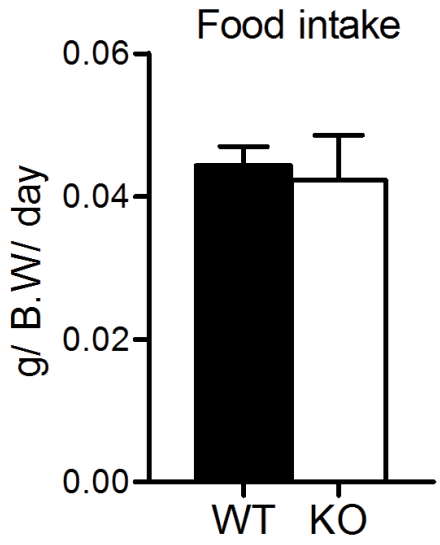


FIG S6 Food intake in WT and Foxa3-null (KO) mice after HFD (60%) (mean ± SEM).

FIG S7

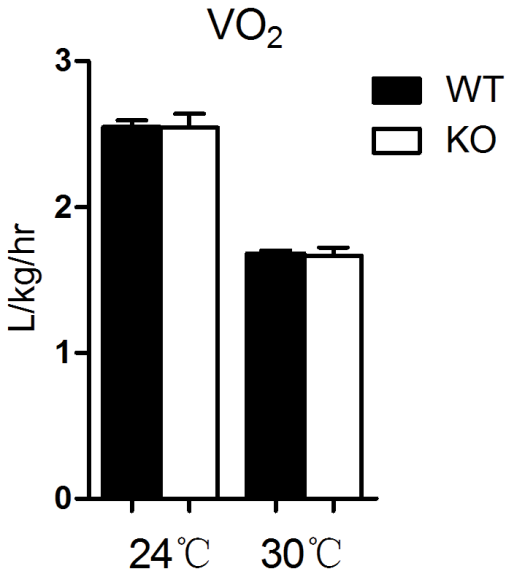


FIG S7 Total energy expenditure in WT and Foxa3-null (KO) mice after HFD (60%) (mean ± SEM).

TABLE S1 Sequence of primers used for Foxa3 DNA binding motif mutagenesis.

	Forward (F) primers 5'-3'	Reverse (R) primers 5'-3'
Foxa3-R162P/N165I	aaccagcaac**c**ttggcaga**t**ctccatccgg	ccggatggag**a**tctgccaa**g**gttgctggtt
Foxa3-M202R	ccagctctgggaaca**a**gtttgagaacggctg	cagccgttctcaaac**t**tgttcccagagctgg
Foxa3-R210P	acggctgctatctcc**c**ccggcagaagcgctt	aagcgcttctgccgg**g**ggagatagcagccgt

Supplementary Methods

Body composition, food intake and energy expenditure measurements. Body composition was measured by NMR (Echo Medical). Energy expenditure was measured by indirect calorimetry at 24ºC or at thermoneutrality, for 24 hours after a 48 hours adaptation period, as described previously (1). Food intake was measured for 48 hours during indirect calorimetry testing, as previously described.

Isolation of Adipocyte Precursors Cells (APC) and flow cytometry. Epididymal fat pads obtained from WT and Foxa3-null mice were minced and incubated with 1 mg/mL collagenase I for 30 min. The cell suspension was filtered through a nylon mesh, and the SVF was isolated by centrifugation at 800×g. Pelleted cells were resuspended in 1% BSA-PBS and incubated with anti-FcγR antibody (BD). SVF cells were subsequently labeled with antibodies against CD45, CD31, SCA1 and CD34 for 30 min on ice and fixed in 1% paraformaldehyde solution in PBS and subjected to flow cytometry on a FACSCalibur and LSR II (BD). Data were analyzed using FlowJo software (Tree Star, Inc.). Cells negative for CD45 and CD31 and positive for both SCA1 and CD34 were considered adipocyte precursors cells (APCs). CD45-APC, CD31-PE-Cy5, SCA1-PE and CD34-FITC antibodies were purchased from BD Biosciences.

Supplementary Reference

1. **Chatterjee R, Bhattacharya P, Gavrilova O, Glass K, Moitra J, Myakishev M, Pack S, Jou W, Feigenbaum L, Eckhaus M, Vinson C.** 2011. Suppression of the C/EBP family of transcription factors in adipose tissue causes lipodystrophy. J. Mol. Endocrinol. **46:**175-192.