Supporting Information

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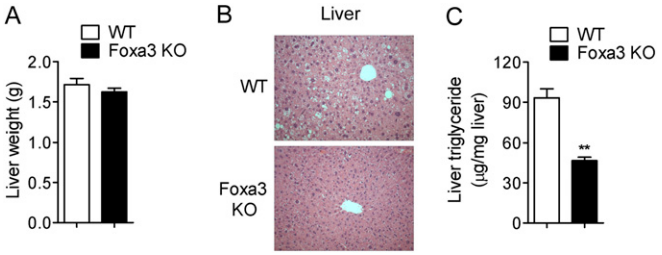

Fig. S1. Analysis of liver tissues obtained from 14-mo-old WT and forkhead box protein A3 (Foxa3)-null mice. Liver weights (*A*), representative images of H&E staining of liver tissues (*B*), and liver triglyceride levels (*C*) of 14-mo-old WT and Foxa3-null (Foxa3 KO) mice are shown. Data are presented as mean ± SEM. $^{**}P < 0.01$ compared with controls.

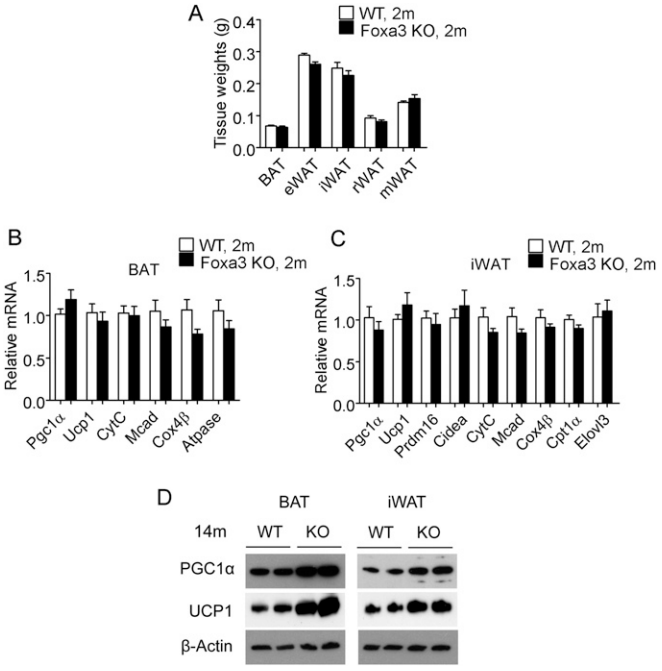

Fig. S2. Tissue weights and gene expression levels in 2-mo-old WT and Foxa3-null mice and peroxisome proliferator activated receptor γ coactivtor 1 α (PGC1α) and uncoupling protein 1 (UCP1) protein levels in 14-mo-old WT and Foxa3-null mice. (*A*) Fat tissue weight of 2-mo-old WT and Foxa3-null mice (Foxa3 KO). (*B* and *C*) Gene expression analysis of BAT (*B*) and iWAT (*C*) obtained from 2-mo-old mice ($n = 6$ per group). (*D*) Protein levels of PGC1α and UCP1 in BAT and iWAT obtained from 14-mo-old WT and Foxa3-null mice. β-actin was used for normalization. Data are presented as mean ± SEM.

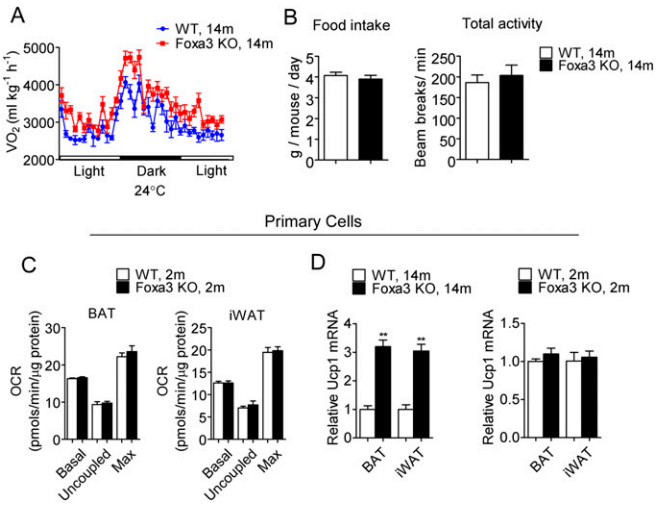

Fig. S3. Metabolic parameters in 14-mo-old WT and Foxa3-null mice and oxygen consumption and UCP1 levels in BAT and iWAT primary cells obtained from of WT and Foxa3-null mice. (*A*) Oxygen consumption, (*B*) food intake and total locomotor activity of 14-mo-old WT (WT, 14m) and Foxa3-null (Foxa3 KO, 14m) mice, $n = 4$ per group. (*C*) Oxygen consumption rate (OCR) in differentiated primary cells obtained from BAT and iWAT of 2-mo-old WT and Foxa3-null mice. (*D*) *Ucp1* mRNA levels in differentiated primary cells obtained from BAT and iWAT of 2- and 14-mo-old WT and Foxa3-null mice. Data are presented as mean ± SEM. $^{**}P < 0.01$ compared with controls.

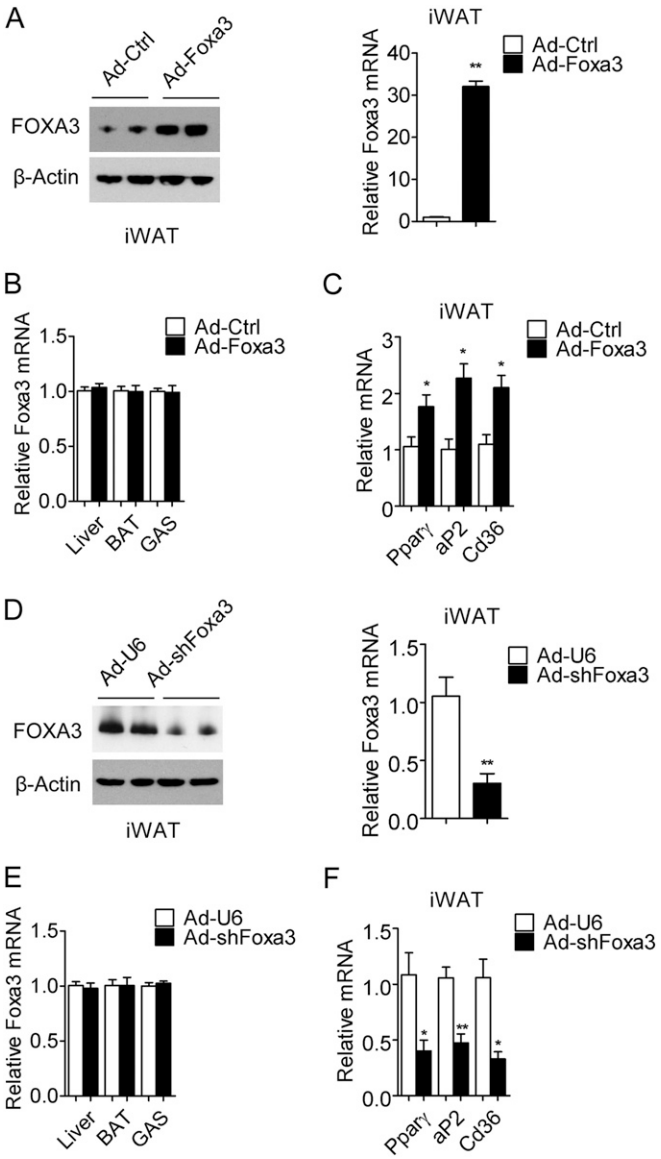

Fig. S4. Analysis of Foxa3 levels and differentiation markers in iWAT of mice injected with control or Foxa3 adenoviruses. (*A* and *B*) Foxa3 protein and mRNA levels in iWAT (*A*) and Foxa3 gene expression levels (*B*) in tissues of mice injected with adenoviruses expressing control (Ad-Ctrl) or Foxa3 (Ad-Foxa3). (*C*) mRNA levels of white fat differentiation markers in iWAT of mice injected with adenoviruses expressing control (Ad-Ctrl) or Foxa3 (Ad-Foxa3). (*D* and *E*) Foxa3 protein and mRNA levels in iWAT (*D*) and Foxa3 gene expression levels (*E*) in tissues of mice injected with adenoviruses expressing control (Ad-U6) or shFoxa3 (Ad-shFoxa3). (*F*) mRNA levels of differentiation markers in iWAT of mice injected with adenoviruses expressing control (Ad-U6) or shFoxa3 (Ad-shFoxa3). Data are presented as mean ± SEM. $*P < 0.05$, $**P < 0.01$ compared with controls.

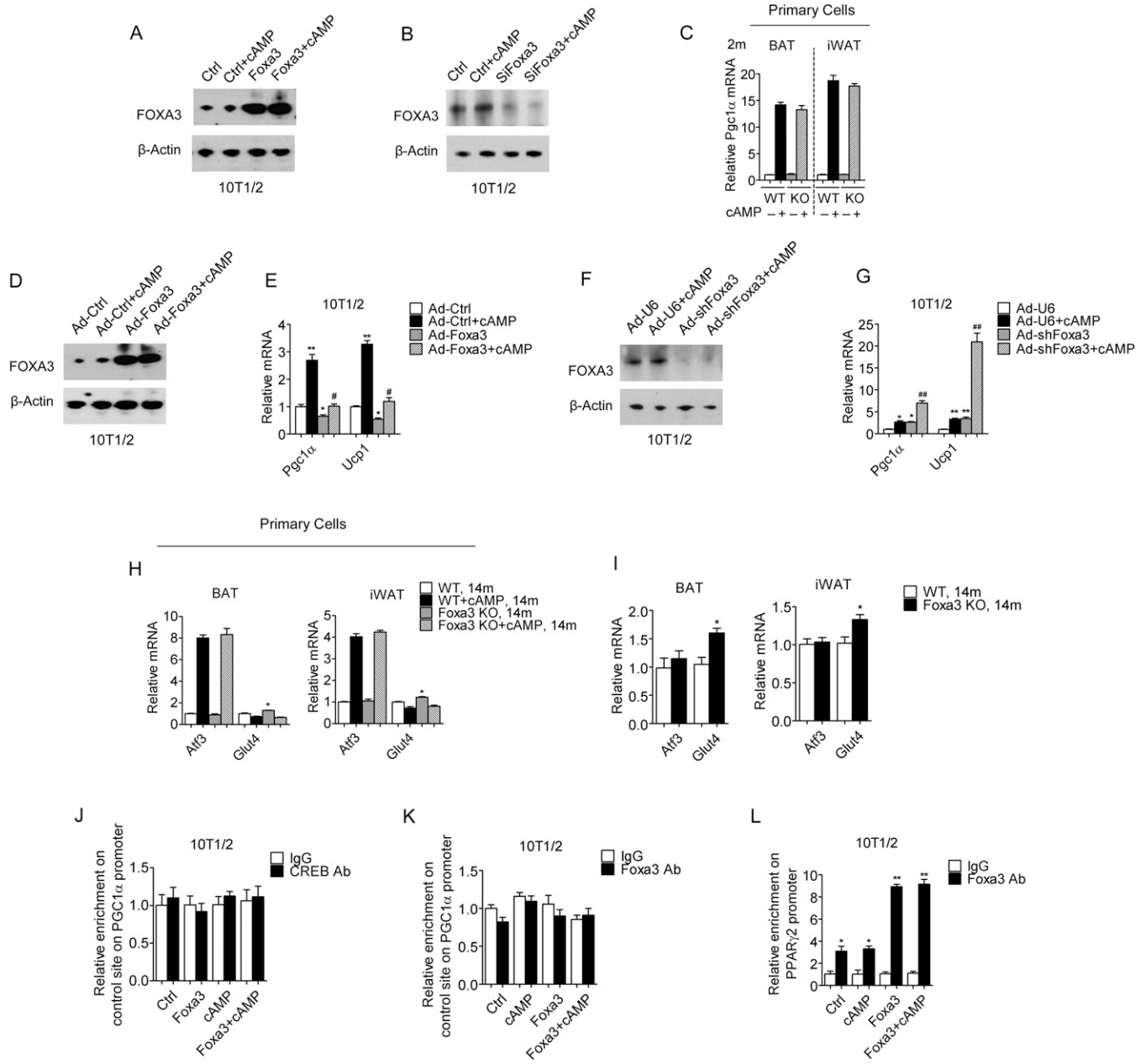

Fig. S5. Foxa3 interference with cAMP responsive element binding protein 1 (CREB) downstream pathways is PGC1α promoter-selective. (*A* and *B*) Foxa3 protein levels in 10T1/2 cells expressing vector (Ctrl), Foxa3 (*A*), or siFoxa3 (*B*) in the presence of vehicle or cAMP. (*C*) Analysis of *PGC1α* mRNA levels in differentiated primary cells obtained from BAT and iWAT of 2-mo-old WT and Foxa3-null (Foxa3 KO) mice treated with vehicle or cAMP. (*D* and *E*) Foxa3 protein levels (*D*) and *PGC1α* and *UCP1* mRNA levels (*E*) in differentiated 10T1/2 cells infected with control or Foxa3 (Ad-Foxa3) adenoviruses treated with cAMP or vehicle. (*F* and *G*) Foxa3 protein levels (*F*) and *PGC1α* and *UCP1* mRNAs (*G*) in differentiated 10T1/2 cells infected with control (Ad-U6) or shFoxa3 (Ad-shFoxa3) adenoviruses treated with cAMP or vehicle. (*H*) mRNA levels of the CREB target gene *ATF3* and of *Glut4* in differentiated primary cells obtained from BAT and iWAT of 14-mo-old WT and Foxa3-null mice treated with vehicle or cAMP. (*I*) mRNA levels of *ATF3* and *Glut4* in BAT and iWAT of 14-mo-old WT and Foxa3-null mice. (*J* and *K*) ChIP analysis of CREB and Foxa3 binding at a control site, adjacent to the Foxa-responsive (*J*) and CRE-responsive (*K*) elements, in the PGC1α promoter, in untreated or cAMP-treated 10T1/2 cells expressing either vector (Ctrl) or Foxa3. (*L*) ChIP analysis of Foxa3 binding at the Foxa-responsive element present in the PPARγ2 promoter in the presence or absence of cAMP in 10T1/2 cells expressing vector (Ctrl) or Foxa3. Data are presented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with controls. $^{\#\#}P < 0.01$ compared with controls + cAMP.

Table S1. Hyperinsulinemic-euglycemic clamp analysis of 14-mo-old WT and Foxa3-null mice

Variable	WT	Foxa3 KO
Basal plasma glucose, mg/dL	149.1 ± 12.4	141.1 ± 8.4
Basal insulin, ng/mL	3.2 ± 1.1	0.4 ± 0.2
Clamp plasma glucose, mg/dL	218.5 ± 4.0	201.7 ± 2.7
Clamp insulin, mg/dL	7.0 ± 3.0	1.6 ± 0.3
Basal EGP, μmol/kg/min	90.9 ± 8.1	86.9 ± 4.9
Clamp EGP, μmol/kg/min	47.6 ± 9.6	13.6 ± 11.2*
Supression of EGP, %	38.2 ± 5.0	83.2 ± 13.5†
GIR, μmol/kg/min	270.8 ± 9.7	326.7 ± 12.6‡
Rd, μmol/kg/mL	318.4 ± 11.2	340.3 ± 11.3
WAT 2-DG uptake, μmol/kg/min	7.8 ± 0.9	14.0 ± 1.7‡
Muscle 2-DG uptake, μmol/kg/min	108.9 ± 20.9	66.6 ± 9.1
BAT 2-DG uptake, μmol/kg/min	717.5 ± 138.8	908.3 ± 234.8

$n = 6$ for WT and $n = 4$ for Foxa3 KO mice. Data are presented as mean ± SEM. 2-DG, 2-deoxyglucose; EGP, endogenous glucose production; GIR, glucose infusion rate; Rd, glucose disposal rate.
*$P = 0.053$.
†$P < 0.05$.
‡$P < 0.01$.

Table S2. Primers used for gene expression analysis

Primer	Forward primer, 5′-3′	Reverse primer, 5′-3′
m36B4	GCTTCATTGTGGGAGCAGAC	ATGGTGTTCTTGCCCATCAG
mPGC1α	ACCATGACTACTGTCAGTCACTC	GTCACAGGAGGCATCTTTGAAG
mUCP1	GGCCCTTGTAAACAACAAAATAC	GGCAACAAGAGCTGACAGTAAAT
mPRDM16	CCACCAGCGAGGACTTCAC	GGAGGACTCTCGTAGCTCGAA
Mcidea	TGACATTCATGGGATTGCAGAC	CGAGCTGGATGTATGAGGGG
McytC	AAATCTCCACGGTCTGTTCGG	GGGTATCCTCTCCCCAGGTG
MMCAD	ATGACGGAGCAGCCAATGAT	TCGTCACCCTTCTTCTCTGCTT
mCPT1α	TTGCCCTACAGCTCTGGCATTTCC	GCACCCAGATGATTGGGATACTGT
mCOX4β	CTGCCCGGAGTCTGGTAATG	CAGTCAACGTAGGGGGTCATC
mElovl3	TTCTCACGCGGGTTAAAAATGG	TCTCGAAGTCATAGGGTTGCAT
mKlhl13	AGAATTGGTTGCTGCAATACTCC	AAGGCACAGTTTCAAGTGCTG
mCD40	TTGTTGACAGCGGTCCATCTA	CCATCGTGGAGGTACTGTTTG
mEar2	CCTGTAACCCCAGAACTCCA	CAGATGAGCAAAGGTGCAAA
mTmem26	ACCCTGTCATCCCACAGAG	TGTTTGGTGGAGTCCTAAGGTC
mCD137	CGTGCAGAACTCCTGTGATAAC	GTCCACCTATGCTGGAGAAGG
mSp100	TGATGGAGGGAACCCAAACTC	CTTCCTTGAGAATAGCTGGCAC
mTbx1	GGCAGGCAGACGAATGTTC	TTGTCATCTACGGGCACAAAG
mSlc27a1	CTGGGACTTCCGTGGACCT	TCTTGCAGACGATACGCAGAA
mAtf3	AAGACAGAGTGCCTGCAGAA	GTGCCACCTCTGCTTAGCTC
mGlut4	AAAAGTGCCTGAAACCAGAG	TCACCTCCTGCTCTAAAAGG

Table S3. Primers used for ChIP assays

Primer	Forward primer, 5′-3′	Reverse primer, 5′-3′
Foxa3-binding site on PGC1α promoter	CAAAGGCCAAGTGTTTCCTT	TCCTGTGCAAGCTTGCTGC
Control site on PGC1α promoter	AAGACAGGTGCCTTCAGTTCA	TGCACATGTCCCAAGCCAT
Foxa3-binding site on PPARγ2 promoter	TCACTTAAACATCAACCATTGGA	GGTCCAAAATGTTACTGCTATCC