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Supplemental information

FBF1 deficiency promotes beiging and healthy expansion of white adipose tissue

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Supplementary Figures

Figure S1

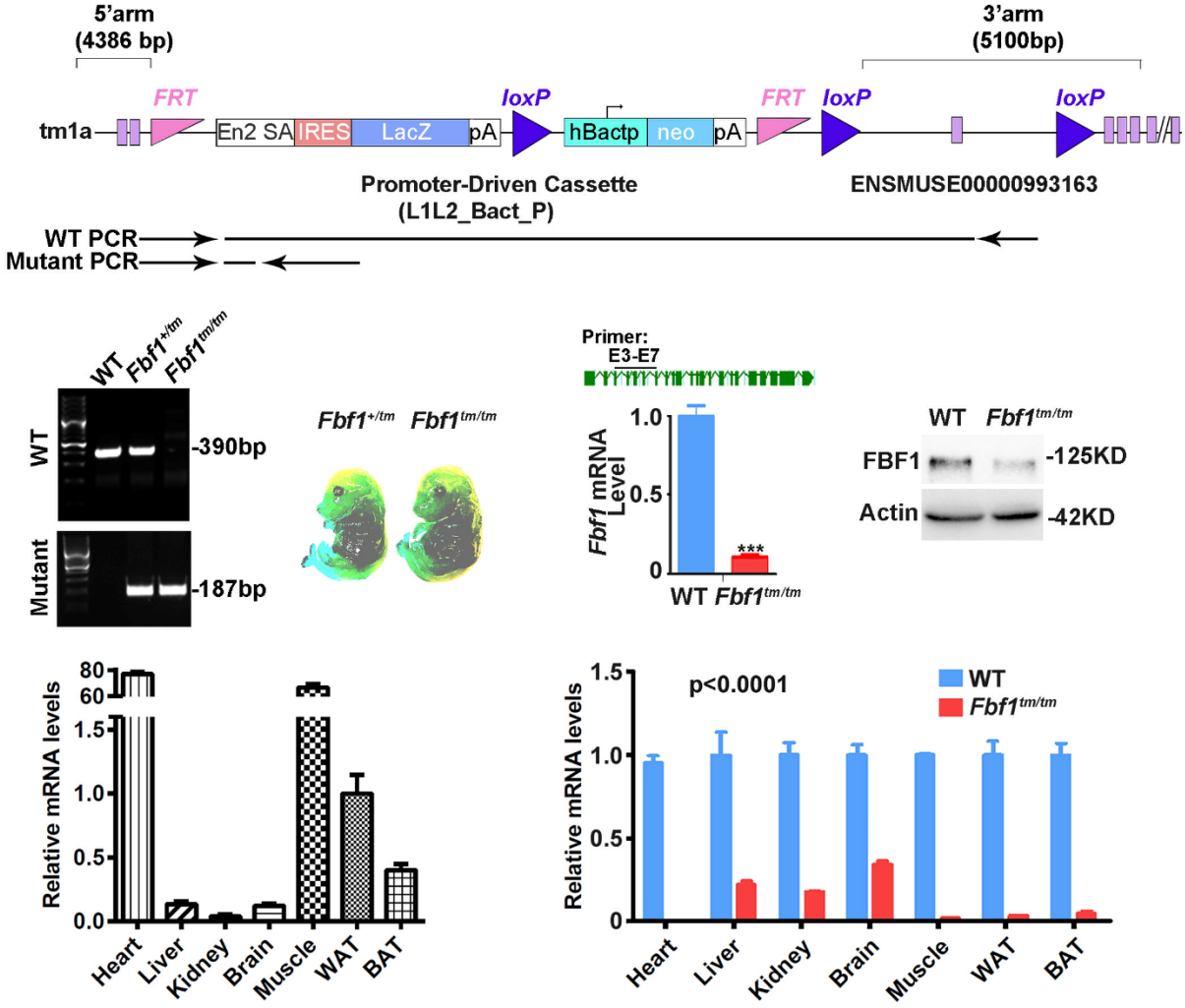

Figure S1. Generating *Fbf1*$^{tm/tm}$ homozygous mice and phenotyping. Related to Figure 1.

(A) Schematic of the 'knockout-first' conditional allele. The 'knockout-first' allele (*tm1a*) contains IRES: lacZ trapping cassette and a floxed promoter-driven neo cassette inserted into the intron of a gene, disrupting gene function.

(B) PCR genotyping results.

(C) LacZ reporter gene whole-mount analysis by X-gal staining of E15 embryos.

(D) Relative mRNA expression level of *Fbf1* in E13.5 embryos of *Fbf1*$^{tm/tm}$ and WT littermates. Values are expressed as mean ± SEM. n= 8, ***p < 0.001.

(E) Protein expression level of FBF1 in E13.5 embryos of *Fbf1*$^{tm/tm}$ and WT littermates.

(F) Relative mRNA expression level of *Fbf1* in WT adult tissues/organs.

(G) Relative mRNA expression level of *Fbf1* in adult tissues/organs of *Fbf1*$^{tm/tm}$ and WT littermates.

Figure S2

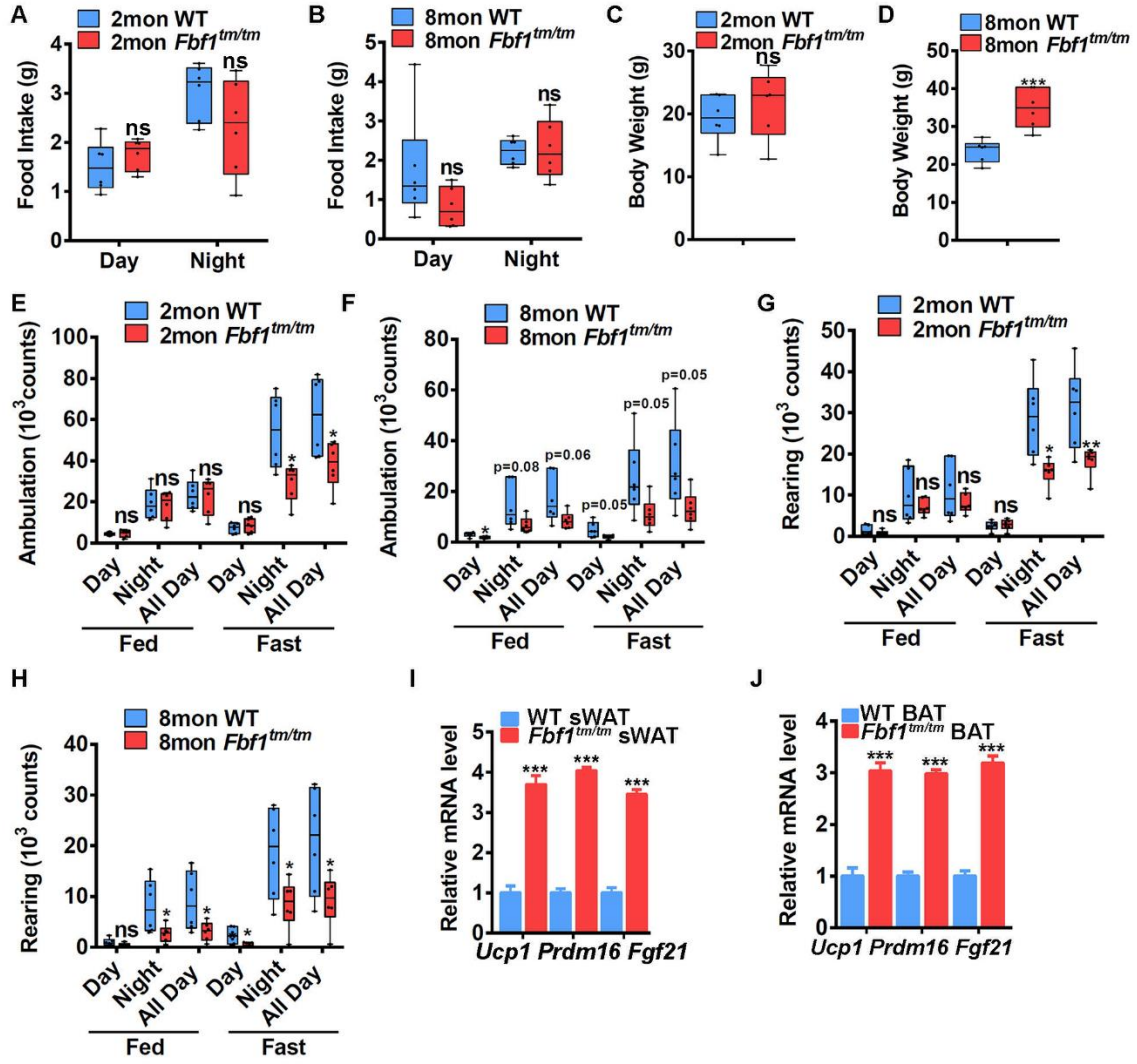

Figure S2. Comprehensive Laboratory Animal Monitoring System (CLAMS) data and relative mRNA levels of beiging genes in adipose tissues. Related to Figure 2.

(A-H) Multiple metabolic parameters were monitored in *Fbf1*$^{tm/tm}$ /WT littermates at 2 months and 8 months (n=6 in each group). (A and B) Food intake, (C and D) body weight, (E and F) ambulation and (G and H) rearing in 2-mon (A, C, E and G) and 8-mon mice (B, D, F and H).

(I) Relative mRNA levels of beiging program genes in sWAT of *Fbf1*$^{tm/tm}$ and WT control.

(J) Relative mRNA levels of beiging program genes in BAT of *Fbf1*$^{tm/tm}$ and WT control. Values are expressed as mean ± SEM, P-values were indicated in figures as follows: *p <0.05, **p < 0.01 and ***p < 0.001.

Figure S3

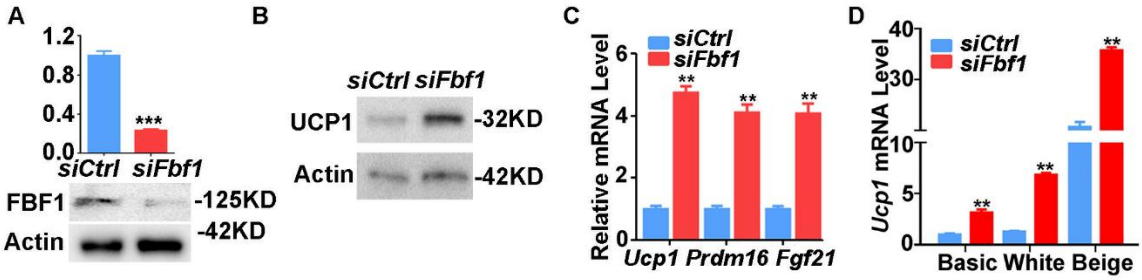

Figure S3. FBF1 Depletion Induces the Beiging of WAT. Related to Figure 3.

(A) Relative mRNA and protein levels of FBF1 in 3T3-L1 cells treated with siRNAs.

(B) Immunodetection of UCP1 in control or *Fbf1*-knockdown 3T3-L1 cells with corresponding β-actin immunodetection as loading control.

(C) Relative mRNA levels of beiging program genes at day 4 of adipogenic differentiation of 3T3-L1 cells treated with control or *Fbf1* siRNA.

(D) Relative mRNA level of *Ucp1* in control or *Fbf1*-knockdown 3T3-L1 cells upon induced differentiation to beige adipocytes at day8. Values are expressed as mean ± SEM, **p < 0.01.

Figure S4

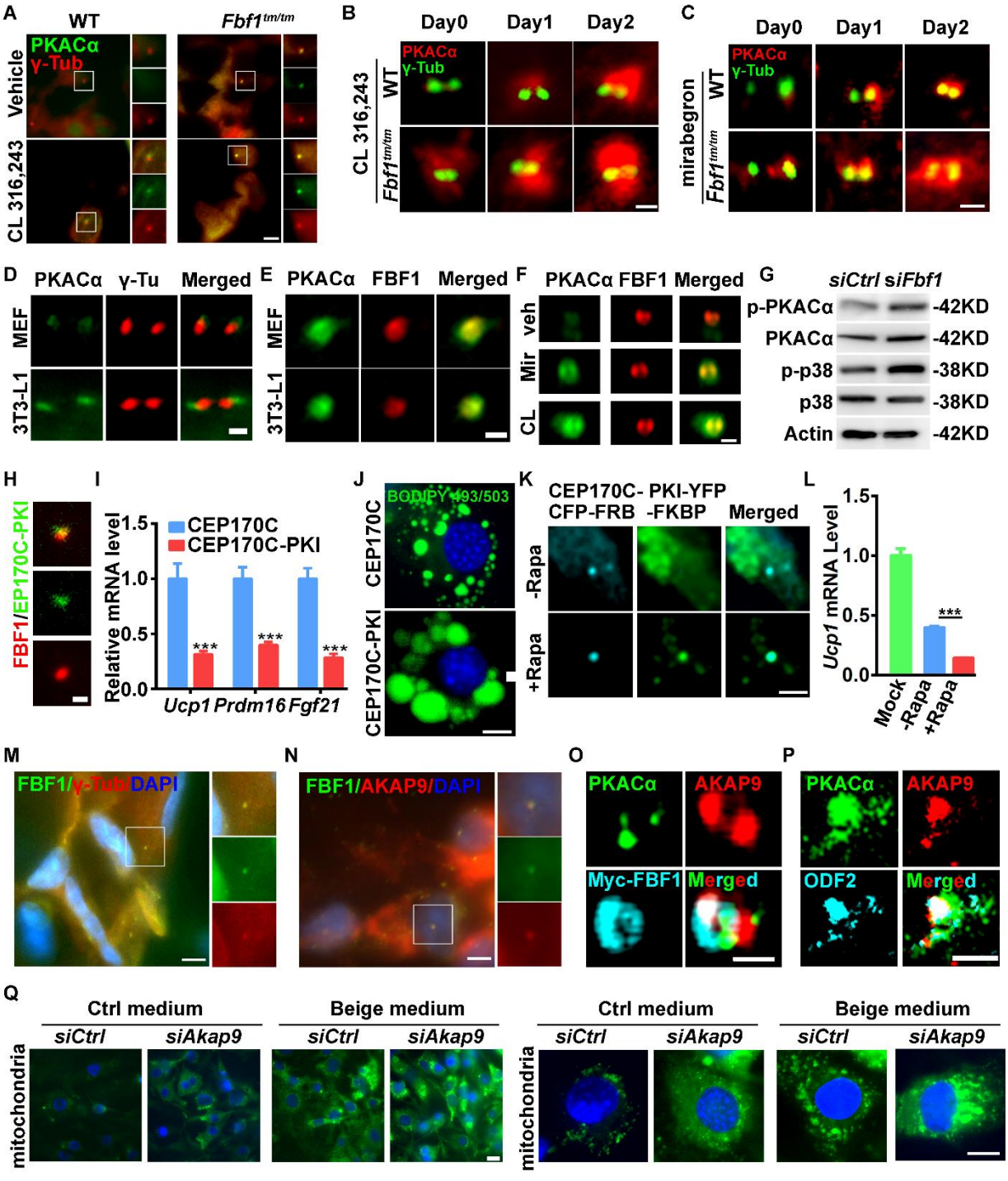
A
WT
Fbf1tm/tm
Vehicle
CL 316,243
PKACα
γ-Tub
B
Day0
Day1
Day2
CL 316,243
WT
Fbf1tm/tm
C
mirabegron
D
PKACα
γ-Tu
Merged
MEF
3T3-L1
E
FBF1
F
veh
Mir
CL
G
siCtrl siFbf1
p-PKACα
PKACα
p-p38
p38
Actin
-42KD
-38KD
H
FBF1/EP170C-PKI
I
CEP170C
CEP170C-PKI
Relative mRNA level
Ucp1 Prdm16 Fgf21
J
BODIPY 493/503
CEP170C
CEP170C-PKI
K
CEP170C- PKI-YFP
CFP-FRB -FKBP
-Rapa
+Rapa
L
Ucp1 mRNA Level
Mock
-Rapa
+Rapa
M
FBF1/γ-Tub/DAPI
N
FBF1/AKAP9/DAPI
O
AKAP9
Myc-FBF1
P
ODF2
Merged
Q
Ctrl medium
Beige medium
siCtrl
siAkap9
mitochondria

Figure S4. FBF1 Regulates Adipose Beiging through a cilia-specific PKA signaling. Related to Figure 4.
(A) The localization of PKACα (Green) in vWAT from 6-mon female WT mice or *Fbf1*$^{tm/tm}$ littermates. Centrosomes were stained with anti-γ-tubulin (Red). Scale bar, 5 μm.
(B and C) PKACα localization in WT and *Fbf1*$^{tm/tm}$ vWAT preadipocytes before and after treated with (B) CL316, 243 or (C) mirabegron. Centrosomes were stained with γ-tubulin. Scale bar, 1 μm.
(D) The localization of PKACα (Green) in 3T3-L1 cells and WT MEFs. Centrosomes were stained with anti-γ-tubulin (Red). Scale bar, 1 μm
(E) The localization of PKACα (Green) in 3T3-L1 cells and WT MEFs. FBF1 labels TFs (Red). Scale bar, 1 μm.
(F) The localization of PKACα (Green) in 3T3-L1 cells induced with β3-adrenergic agonists CL 316,243 or mirabegron. FBF1 labels basal bodies (Red). Scale bar, 1 μm.
(G) Immunoblotting in 3T3-L1 cells at day 2 after induced with β3-adrenergic agonists CL 316,243.
(H) 3T3-L1 cells labelled with GFP-tagged CEP170C-PKI. FBF1 labeled TFs. Scale bar, 1 μm.
(I) Relative mRNA levels of beiging genes in 3T3-L1 cells overexpressed CEP170C or CEP170C-PKI.
(J) 3T3-L1 cells overexpressed CEP170C or CEP170C-PKI were treated with CL 316, 243 and differentiated for 7 days, then fixed, stained and imaged. Green: lipids, Blue: nuclei. Scale bar, 50 μm.
(K) Transient treatment of 100 nM rapamycin for 6 hours induces strong recruitment of YFP–FKBP-PKI to the CFP-FRB–CEP170C at the ciliary base in WAT preadipocytes.
(L) Relative mRNA levels of *Ucp1* in WAT preadipocytes cells overexpressed YFP–FKBP-PKI and CFP-FRB–CEP170C with or without rapamycin treatment.
(M) The localization of FBF1 (Green) in vWAT from 6-mon female WT mice or *Fbf1*$^{tm/tm}$ littermates. Centrosomes were stained with anti-γ-tubulin (Red). Scale bar, 5 μm.
(N) The localization of AKAP9 (Red) in vWAT from 6-mon female WT mice or *Fbf1*$^{tm/tm}$ littermates. TFs were stained with FBF1 (Green). Scale bar, 5 μm.
(O) SIM image of the basal body of vWAT preadipocytes stained for PKACα, AKAP9, and FBF1. Scale bar, 1 μm
(P) SIM image of the basal body of *siFbf1*-treated 3T3-L1 cells stained for PKACα, AKAP9, and ODF2. Scale bar, 1 μm
(Q) Control or *Akap9*-knockdown 3T3-L1 cells induced with control or beige medium. At day 7, cells were labeled with Mitochondria-GFP. At day 8, cells were fixed, stained and imaged. Green: mitochondria, Blue: nuclei. Scale bar, 50 μm.

Figure S5

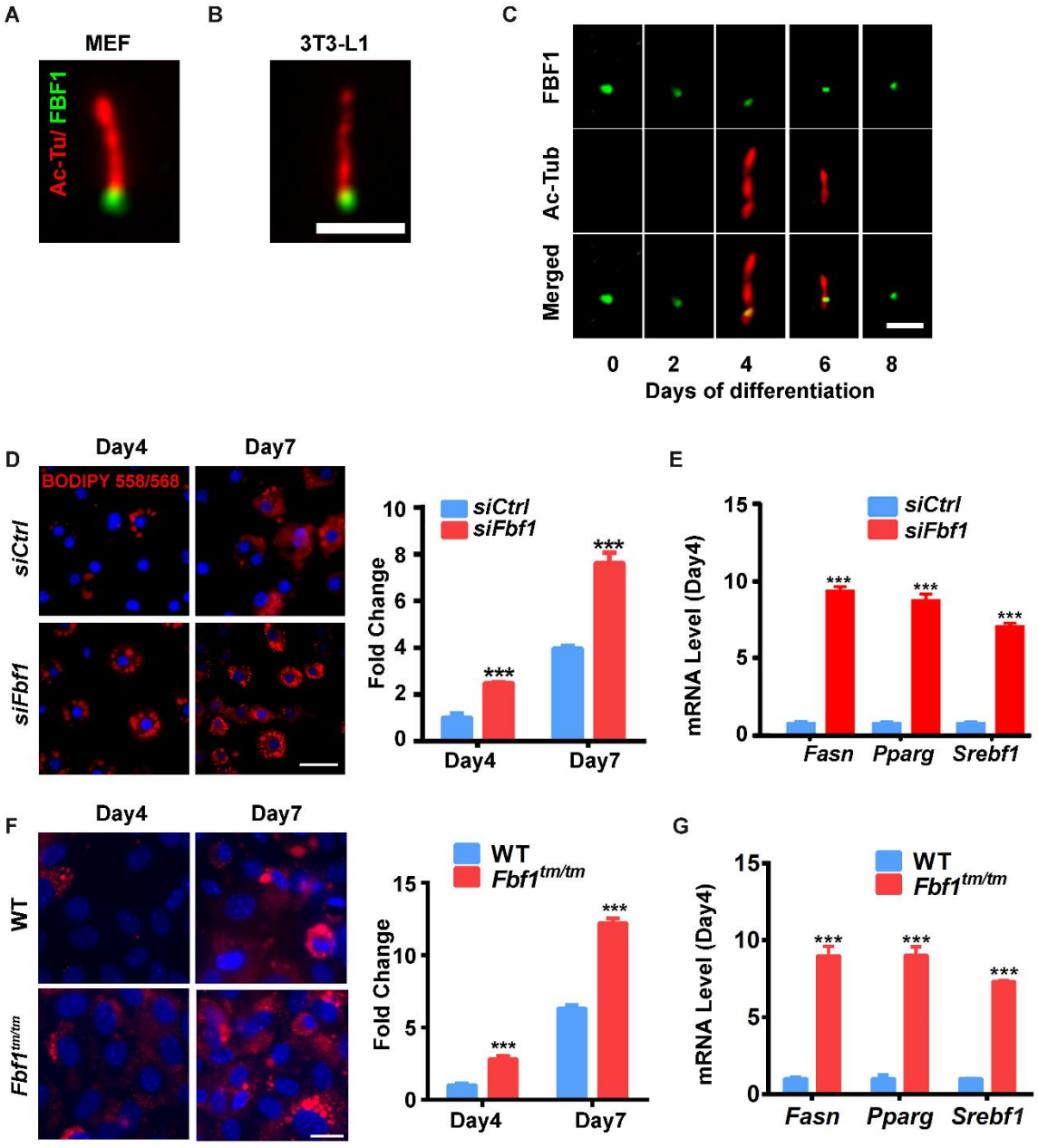

Figure S5. FBF1 Deficiency Enhances Adipogenic Program. Related to Figure 5.

(A and B) Primary cilia (red) present in WT MEFs (A) and 3T3-L1(B) cells after serum starvation. Scale bar, 5 μm.

(C) Immunofluorescence images of cilia (Ac-tubulin, Red) and (FBF1) staining during adipogenic differentiation of 3T3-L1 cells. Scale bar, 5 μm.

(D) 3T3-L1 cells were treated with siRNAs and then induced with adipogenic medium. At day 4/7, cells were fixed, stained and imaged by fluorescence microscopy. Red: lipids, Blue: nuclei. Scale bar, 60 μm.

(E) Relative mRNA levels of adipogenic regulatory genes at day 4 of differentiation of 3T3-L1 cells treated with siRNAs. Values are expressed as mean ± SEM, ***$p < 0.001$.
(F) vWAT preadipocytes were treated with siRNAs and then induced with adipogenic medium. At day 4/7, cells were fixed, stained, and imaged by fluorescence microscopy. Red: lipids, Blue: nuclei. Scale bar, 25 μm.
(G) Relative mRNA levels of adipogenic regulatory genes at day 4 of differentiation of vWAT preadipocytes cells treated with siRNAs. Values are expressed as mean ± SEM, ***$p < 0.001$.

Figure S6

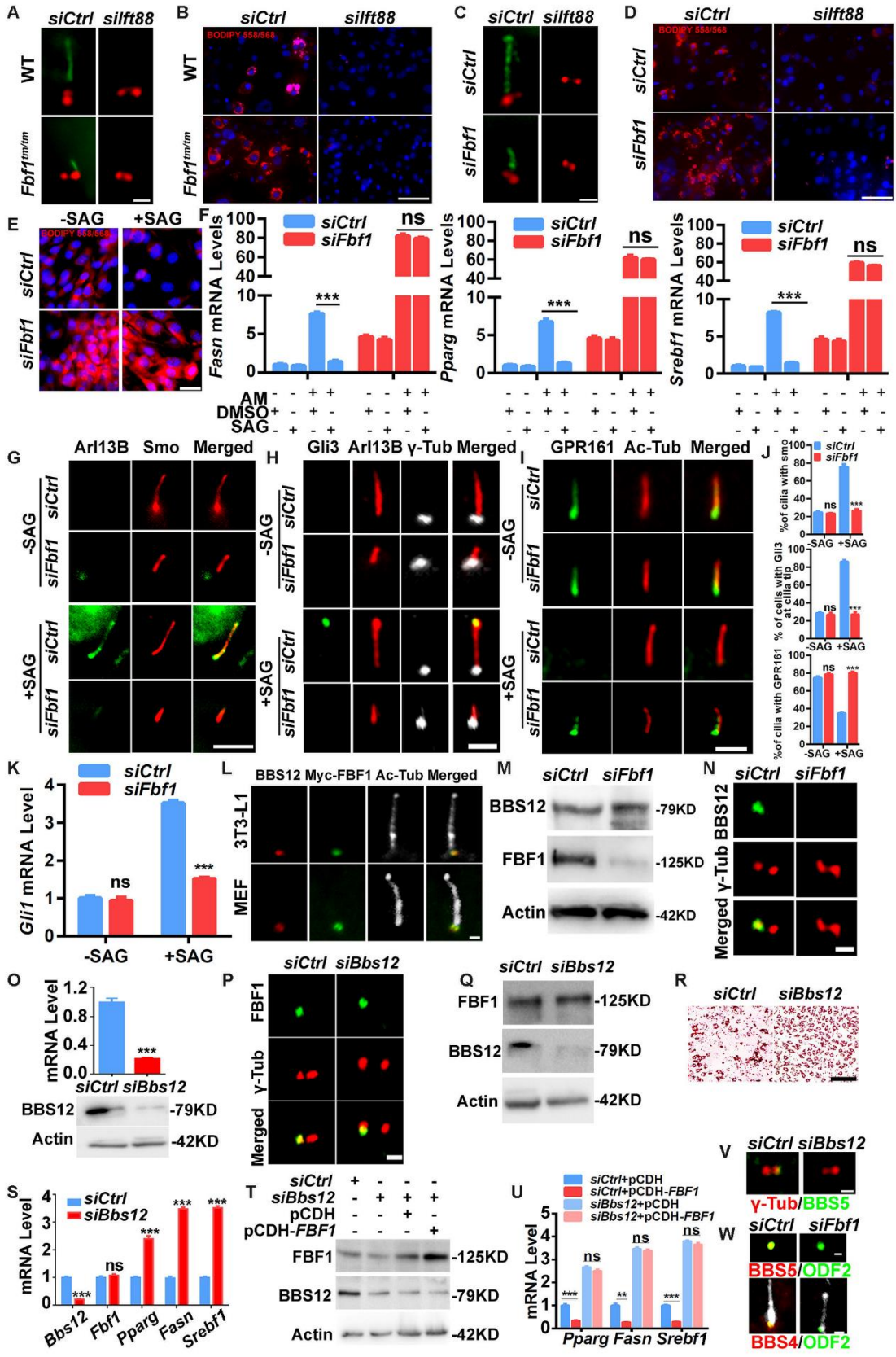
A
siCtrl
silft88
WT
Fbf1tm/tm
B
BODIPY 558/568
C
siFbf1
D
E
-SAG
+SAG
F
Fasn mRNA Levels
Pparg mRNA Levels
Srebf1 mRNA Levels
ns
***
AM
DMSO
SAG
G
Arl13B
Smo
Merged
H
Gli3
γ-Tub
I
GPR161
Ac-Tub
J
%of cilia with smo
% of cells with Gli3 at cilia tip
%of cilia with GPR161
K
Gli1 mRNA Level
L
BBS12 Myc-FBF1 Ac-Tub Merged
3T3-L1
MEF
M
BBS12
FBF1
Actin
-79KD
-125KD
-42KD
N
Merged γ-Tub BBS12
O
mRNA Level
P
Q
R
S
Bbs12
Fbf1
Pparg
Fasn
Srebf1
T
pCDH
pCDH-FBF1
U
siCtrl+pCDH
siCtrl+pCDH-FBF1
siBbs12+pCDH
siBbs12+pCDH-FBF1
V
γ-Tub/BBS5
W
BBS5/ODF2
BBS4/ODF2

Figure S6. FBF1 Controls Hh Signaling by Recruiting BBS Chaperonin and BBSome to TFs. Related to Figure 6.

(A and C) Primary cilia were stained with Arl13B (Green) and the centrosomes/basal bodies were stained with γ-tubulin (Red) in (A) WT/*Fbf1*$^{tm/tm}$ MEFs or (C) control/*Fbf1*-knockdown 3T3-L1 cells treated with control or *Ift88* siRNA. Scale bar, 2 μm.

(B) WT/*Fbf1*$^{tm/tm}$ MEFs and (D) control/*Fbf1*-knockdown 3T3-L1 cells were treated with control/ or *Ift88* siRNAs and differentiated for 7 days, then stained with BIDIPY. Red: lipids, Blue: nuclei. Scale bar, 150 μm.

(E) Control or *Fbf1*-knockdown 3T3-L1 cells were treated with DMSO or SAG and differentiated. At day 7, cells were fixed, stained, and imaged. Red: lipids, Blue: nuclei. Scale bar, 150 μm.

(F) Relative mRNA level of adipogenic regulatory genes at day 4 of adipogenic differentiation in control or *Fbf1*-knockdown 3T3-L1 cells that were treated with DMSO or SAG.

(G-I) The localization of (G) SMO, (H) Gli3 and (I) GPR161 in primary cilia of control or *Fbf1*-knockdown 3T3-L1 cells treated with DMSO or SAG. Basal bodies and cilia were labeled with anti-γ-tubulin and anti-acetylated tubulin. Scale bar, 5 μm.

(J) Statistical analysis of SMO-positive cilia, Gli3-positive cilia tip and GPR161-positive cilia. Data were calculated from 300 cilia in each group. Values are expressed as mean ± SEM, ***$p < 0.001$.

(K) *Gli1* mRNA levels in control or *Fbf1*-knockdown 3T3-L1 cells before and after Hh activation. (L) BBS12 Localization in WT MEFs or 3T3-L1 cells. Ac-tubulin labeled primary cilia. FBF1 labeled Basal bodies. Scale bar, 1 μm.

(M) Cell lysates from 3T3-L1 cells treated with control or *Fbf1* siRNAs were immunoblotted.

(N) The localization of BBS12 (Green) in control or *Fbf1*-knockdown 3T3-L1 cells. Centrosomes were stained with anti-γ-tubulin (Red). Scale bar, 1 μm.

(O) Relative mRNA and protein levels of BBS12 in 3T3-L1 cells treated with control or *Bbs12* siRNA.

(P) The localization of FBF1 (Green) in control or BBS12-knockdown 3T3-L1 cells. Centrosomes were stained with anti-γ-tubulin (Red). Scale bar, 1 μm.

(Q) Protein level of FBF1 in 3T3-L1 cells treated with control or *Bbs12* siRNA.

(R) Control or *Bbs12*-knockdown 3T3-L1 cells were induced to differentiate 14 days, and cells were then stained with Oil Red O. Scale bar, 150 μm.

(S) Relative mRNA levels of adipogenic regulatory genes at day 4 of adipogenic differentiation in control or *Bbs12*-knockdown 3T3-L1 cells.

(T) Immunodetection of BBS12 and FBF1 in control or *Bbs12*-knockdown 3T3-L1 cells overexpressed with vehicle or FBF1.

(U) Relative mRNA levels of adipogenic regulatory genes at day 4 of adipogenic differentiation in *Bbs12*-knockdown 3T3-L1 cells overexpressed with vehicle or FBF1.

(V) BBS5 localization in control or *BBS12*-knockdown 3T3-L1 cells. Centrosomes were stained with anti-γ-tubulin. Scale bar, 1 μm.

(W) BBS4/5 localization in control or *Fbf1*-knockdown 3T3-L1 cells. Primary cilia were stained with anti-Ac-tubulin. ODF2 labels basal bodies. Scale bar, 1 μm. Values are expressed as mean ± SEM, n=3 in each group. P-values were indicated in figures as follows: **$p < 0.01$ and ***$p < 0.001$.

Figure S7

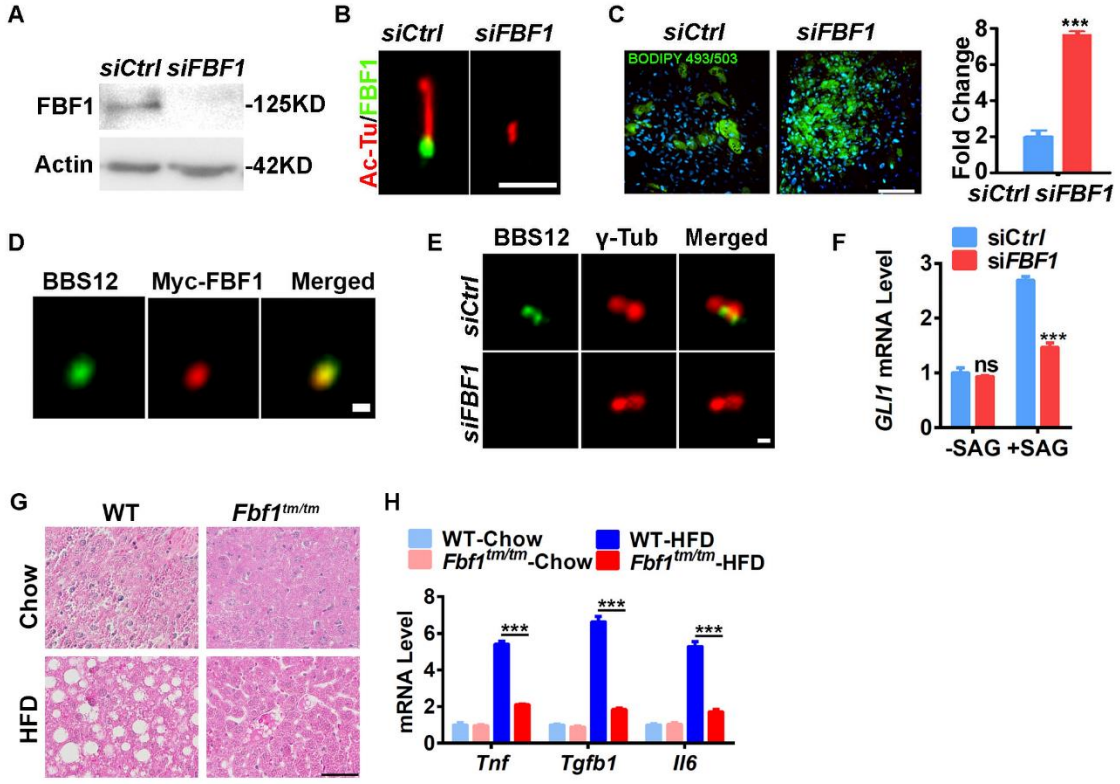

Figure S7. FBF1 Depletion Promotes Adipogenic Program during Human Preadipocyte differentiation and Protects animal organs. Related to Figure 7.

(A) Immunodetection of FBF1 in human adipocyte progenitors treated with control or *FBF1* siRNA.

(B) The localization of FBF1 (Green) in human adipocyte progenitors treated with control or *FBF1* siRNA. Cilia were stained with anti-Ac-tubulin (Red). Scale bar, 5 μm.

(C) Human adipocyte progenitors treated with control or *FBF1* siRNA were induced to differentiate for 14 days, and cells were then fixed, stained, and imaged. Green: lipids, Blue: nuclei. Scale bar, 200 μm. Images are representative of n=6 replicates.

(D) The localization BBS12 (Green) in human adipocyte progenitors. FBF1 labeled basal bodies (Red). Scale bar, 1 μm.

(E) BBS12 localization in human adipocyte progenitors treated with control or *FBF1* siRNA. Centrosomes were stained with γ-tubulin. Scale bar, 5 μm.

(F) *GLI1* mRNA level in human adipocyte progenitors treated with control or *FBF1* siRNA before and after Hh activation. P-values were indicated in figures as follows: ***p < 0.001.

(G) H&E staining of the liver sections of 5-mon-old *Fbf1*$^{tm/tm}$ mice and WT littermates treated with HFD for 3 months. n=6 in each group. Scale bar, 50 μm.

(H) Relative mRNA levels of inflammation genes in the liver of 5-mon-old *Fbf1*$^{tm/tm}$ mice and WT littermates treated with HFD for 3 months. n=6 in each group.

Table S1 Specific primer pairs used in this study. Related to STAR Methods.

Oligo Type	Oligo name	Oligo sequence (5' to 3')
Specific primer pairs used in *Fbf1*$^{tm1a/tm1a}$ mice genotyping	CAS_R1_Term	TCGTGGTATCGTTATGCGCC
	Fbf1_33719_F	GGCCTATATCTTGAGCCTCTGA
	Fbf1_33719_R	CAAAAGCCTCCCAGACAGAC
	LacZ_2_small_F	ATCACGACGCGCTGTATC
	LacZ_2_small_R	ACATCGGGCAAATAATATCG
Specific primer pairs used in quantitative Real-time PCR experiments (Mouse)	*Fbf1*_F	ATGACTGGACAGTGCTGTGAGG
	*Fbf1*_R	CTCAGCATCAGCTCCTGCCAG
	*Akap9*_F	CCATGAACAACAGACAGATGG
	*Akap9*_R	GCTATTTCTTCTTCCAGTCG
	*Fasn*_F	AGCGGCCATTTCCATTGCCC
	*Fasn*_R	CCATGCCCAGAGGGTGGTTG
	*Srebf1*_F	GAACAGACACTGGCCGAGAT
	*Srebf1*_R	GAGGCCAGAGAAGCAGAAGAG
	*Pparg*_F	AAGATTTGAAAGAAGCGGTGAAC
	*Pparg*_R	CAATGGCCATGAGGGAGTTAG
	*Gli1*_F	ATGAAGCTAGGGGTCCAGGT
	*Gli1*_R	AGAAGGGAACTCACCCCAGT
	*Ucp1*_F	GTGAACCCGACAACTTCCGAA
	*Ucp1*_R	TGAAACTCCGGCTGAGAAGAT
	*Prdm16*_F	CCACCAGCGAGGACTTCAC
	*Prdm16*_R	GGAGGACTCTCGTAGCTCGAA
	*Fgf21*_F	GGGATGGGTCAGGTTCAGA
	*Fgf21*_R	CAGCCTTAGTGTCTTCTCAGC
	*Bbs12*_F	TTCCTCTTGGATGGCCTCAT
	*Bbs12*_R	GCCAGGGACTTCAGCACAGT
	*Tnf*_F	GCCTCTTCTCATTCCTGCTTG
	*Tnf*_R	CTGATGAGAGGGAGGCCATT
	*Mcp1*_F	CCACTCACCTGCTGCTACTCAT
	*Mcp1*_R	TGGTGATCCTCTTGTAGCTCTCC
	*Tgfb1*_F	TACCATGCC AACTTCTGTCTGGG A
	*Tgfb1*_R	ATGTTGGACAAC TGCTCCACCTTG
	*Il6*_F	CTGCAAGAGACTTCCATCCAG
	*Il6*_R	AGTGGTATAGACAGGTCTGTTGG
	*Actin*_F	GTGGGCCGCCCTAGGCACCAG
	*Actin*_R	TTGGCCTTAGGGTTCAGGGGGG
Specific primer pairs used in quantitative Real-time PCR experiment (Human)	*UCP1*_F	TCTCTCAGGATCGGCCTCTA
	*UCP1*_R	GTGGGTTGCCCAATGAATAC
	*FGF21*_F	ACCTGGAGATCAGGGAGGAT
	*FGF21*_R	GCACAGGAACCTGGATGTCT
	*PRDM16*_F	AAATACTGACGGACGTGGAAGT
	*PRDM16*_R	GACACTGGTCGCATTTGTACTC
	*COX1*_F	AAGGAGATGGCAGCAGAGTT
	*COX1*_R	GTGGCCGTCTTGACAATGTT
	*HK2*_F	CAAAGTGACAGTGGGTGTGG
	*HK2*_R	GCCAGGTCCTTCACTGTCTC
	*COX7A*_F	TGACATCCCGTTGTACCTGAA

	*COX7A*_R	AAGGAGGCCCAGCCAAG
	*SREBF1*_F	TCAGCGAGGCGGCTTTGGAGCAG
	*SREBF1*_R	CATGTCTTCGATGTCGGTCAG
	*FASN*_F	CTTCCGAGATTCCATCCTACGC
	*FASN*_R	TGGCAGTCAGGCTCACAAACG
	*PPARG*_F	CGGTTTCAGAAGTGCCTTG
	*PPARG*_R	GGTTCAGCTGGTCGATATCAC
	*GLI1*_F	TAAAGCTCCAGTGAACACA
	*GLI1*_R	TCCCACTTTGAGAGGCCCAT
	*Actin*_F	AGCTACGAGCTGCCTGACGG
	*Actin*_R	CCAGACAGCACTGTGTTGG

Table S2 Sequences of siRNA. Related to STAR Methods.

Oligo name	Oligo sequence (5' to 3')
Fbf1 siRNA	TGAACAGTTCTTCCTGGAG
	CAGCTCAGCATGCGTCATATT
	ACAAGCCTGCTGGCACATTAAC
Ift88 siRNA	(Deren et al., 2016)
Bbs12 siRNA	CCTGGGATTTAATAAGTCTGTAAAT
	CCTTTCCGAGTGATTCTCATT
Akap9 siRNA	GAATATTGATGGAATTATT
	GCAGCAGCACATGAAGACAAA
FBF1 siRNA	(Wei et al., 2013)