Cell Reports, Volume *29*

Supplemental Information

Zc3h10 Acts as a Transcription Factor and Is Phosphorylated to Activate the Thermogenic Program

Danielle Yi, Jon M. Dempersmier, Hai P. Nguyen, Jose A. Viscarra, Jennie Dinh, Chihiro Tabuchi, Yuhui Wang, and Hei Sook Sul

Figure S1.

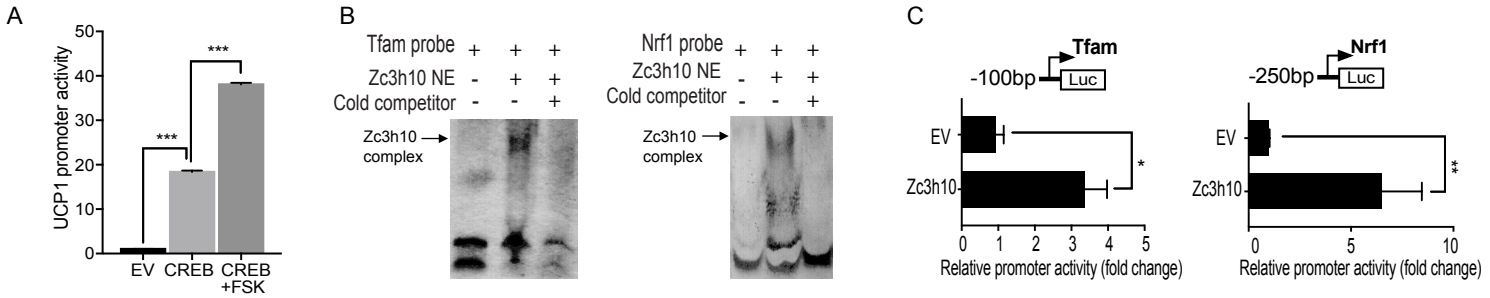

Figure S1. Zc3h10 is required for full activation of BAT gene program. Related to Figure 1.

(A) Luciferase activity in HEK293FT cells cotransfected with the -5.5 kb UCP1 promoter and indicated vector in the absence and presence of forskolin. (B) Gel shift assay performed with nuclear extracts HEK293FT cells transfected with Flag-Zc3h10 and oligos corresponding to the +50bp Tfam binding site and the -200bp Nrf1 binding site. (C) (Left) Luciferase activity in differentiated BAT cells cotransfected with the -100bp Tfam promoter and Zc3h10 (Right) Luciferase activity in differentiated BAT cells cotransfected with the -250bpNrf1 promoter and Zc3h10. (n=4).

Figure S2.

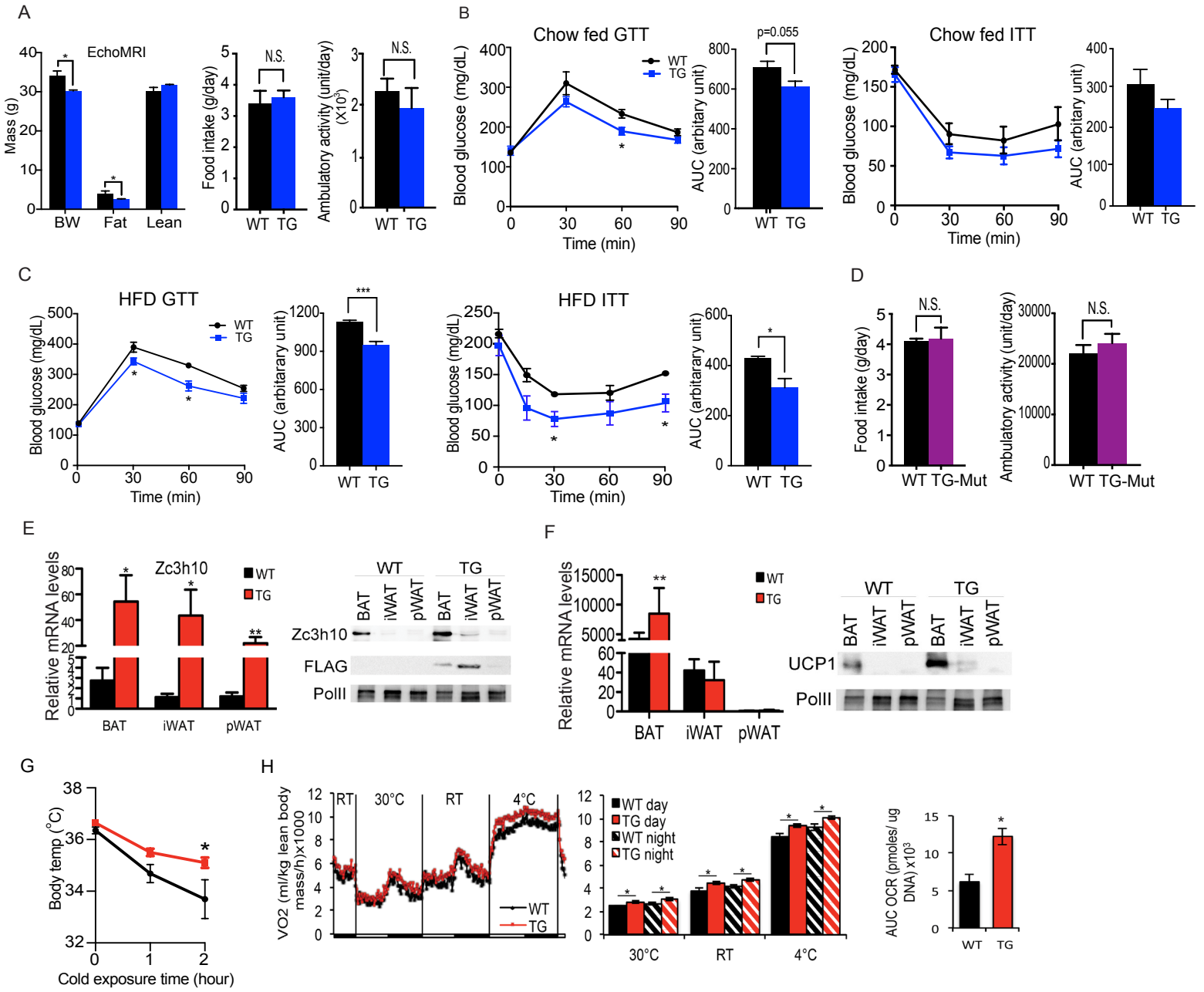

Figure S2. Overexpression of Zc3h10 promotes BAT gene program and enhances energy expenditure, preventing diet-induced obesity. Related to Figure 3.

(A) (Left) Body composition (Right) food intake and locomotor activity of AQ-Zc3h10 (TG) and their WT littermates. (B) (Left) Glucose tolerance test (GTT) of AQ-Zc3h10 after overnight fasting on chow diet.Glucose (1mg/g) was administered intraperitoneally. (Right) Insulin tolerance test (ITT) of AQ-Zc3h10 after 4 hr fasting on chow diet. Insulin (0.75U/kg) was adminstered intraperitoneally (n=5). (C) GTT and ITT of AQ-Zc3h10 mice at 16 wk of age that were on HFD for 3 weeks (n=5). (D) Food intake and locomotor activity of AQ-Zc3h10 (TG). (E) RT-qPCR and immunoblotting for Zc3h10 in various adipose depots of WT and aP2-Zc3h10 mice (TG). (F) (Left) RT-qPCR and immunoblotting for UCP1 in various adipose depots of WT and aP2-Zc3h10 mice (TG) (n=5-6 mice per group). (G) Rectal temperature measured in 15wk-old aP2-Zc3h10 mice at 4°C (n=6 mice per group) (H) (Left and Middle) VO2 assayed in aP2-Zc3h10 mice, housed at indicated ambient temperatures by indirect calorimetry using CLAMS (n=5 per group). (Right) OCR measured in BAT and iWAT of these mice using Seahorse XF24 Analyzer (n=5 per group). (I) Body weights of aP2-Zc3h10 mice (TG) on HFD and their body composition.

Figure S3.

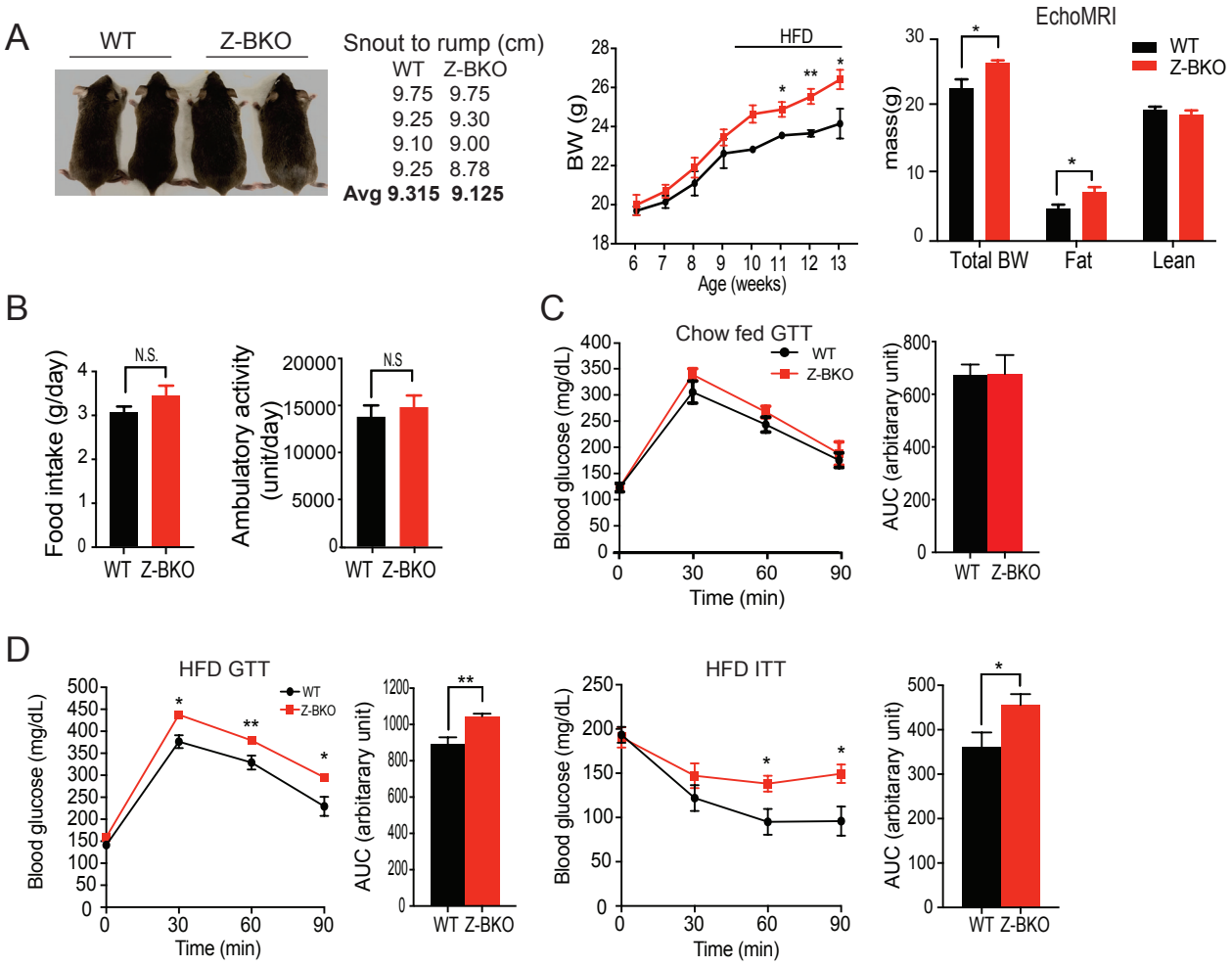

Figure S3. Zc3h10 is required for full activation of BAT gene program. Related to Figure 4.

(A) (Left) Representative image, body length measurements (Middle) body weight of 14wk old mice that were on HFD for 4 weeks (Right) EchoMRI of the mice. (B) Food intake and locomotor activity of Z-BKO and control littermates. (C) (Left) Glucose tolerance test of Z-BKO that were fasted overnight. Glucose (1mg/g) was administered intraperitoneally. (Right) Insulin tolerance test of Z-BKO on chow diet after 4 hr fasting. Insulin (0.75U/kg) was adminstered intraperitoneally (n=4). (D) (Left) Glucose tolerance test and (Right) insulin tolerance test of Z-BKO and control littermates on HFD (n=4).

Figure S4.

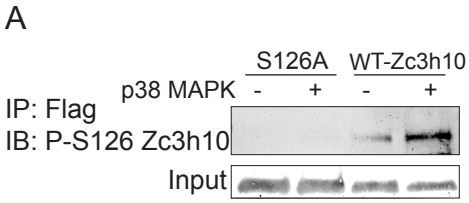

Figure S4. Phosphorylation of Zc3h10-S126 by p38 MAPK for UCP1 transcription during cold exposure. Related to Figure 5.

(A) Specificity of P-126 Zc3h10 antibody tested in lysates from HEK293 cells, transfected with eith S126A or WT-Zc3h10 plasmids with or without p38 MAPK plasmid as indicated. IP with flag antibody followed by immunoblotting with P-126 Zc3h10.

Table S1. Primer sets for Rt-qPCR. Related to STAR Methods.

	Forward primer	Reverse Primer
Zc3h10	CGA CTA ATG CTG CAC CTC CT	AGG AGT AGT GGT GTG GCT CA
UCP1	ACT GCC ACA CCT CCA GTC ATT	CTT TGC CTC ACT CAG GAT TGG
Tfam	GTC CAT AGG CAC CGT ATT GC	CCC ATG CTG GAA AAA CAC TT
Nrf1	GAC AAG ATC ATC AAC CTG CCT GTA G	GCT CAC TTC CTC CGG TCC TTT G
PGC1a	CCC TGC CAT TGT TAA GAC C	TGC TGC TGT TCC TGT TTT C
PPARg	GTG CCA GTT TCG ATC CGT AGA	GGC CAG CAT CGT GTA GAT GA
18s	AGT CCC TGC CCT TTG TAC ACA	CGA TCC GAG GGC CTC ACT A
-4.6kb UCP1 ChIP	CTC AGA GTG CAA CCC CTC AC	GAG GTC GCA GAT CTG TTC CA
-2.5kb UCP1 ChIP	AGC GGTC ACA GAG GGT CAG T	GTG AGG CTG GAT CCC CAG A
Intron 2 UCP1 ChIP	TGA CAT TCA GGG CAG ACA GAG	TCG AGT CGC TGT ATG GGT AG
-200 Nrf1 ChIP	CTC GGG CCG ACG AAT GAT G	CGG GCT TCC CAC AGT ACC C
+50 Tfam ChIP	GGT CCA ATC AGA TAG CCG GG	CCT CAC TCA TTG GTG GGT CC

Table S2. Oligos for gel shift assays. Related to STAR Methods.

Tfam	5'-GGC CCG GCG CCG TCC GGT TCG TCT CAC GCA ATA CGG TGC CTA TGG ACT GCC ACA CTA CCA GCG TGG GAA CTC CGG GGG CT-3'
Nrf1	5'-CTC AGC CGG TTG CCC TCC TGG TCC GGG CCC CGG CCA GCG CTG CCG AGC CGC ACC AGA GGC CGA CGA GGC GGC CGG GGG TC-3'