Supplemental Data

Table S1. Related to STAR Methods. Primer sequences for qPCR and gRNAs

Gene	Forward	Reverse
Adipoq	GCA CTG GCA AGT TCT ACT GCA A	GTA GGT GAA GAG AAC GGC CTT GT
Pparg2	TGG CAT CTC TGT GTC AAC CAT G	GCA TGG TGC CTT CGC TGA
Tbp	GAA GCT GCG GTA CAA TTC CAG	CCC CTT GTA CCC TTC ACC AAT
Ucp1	ACT GCC ACA CCT CCA GTC ATT	CTT TGC CTC ACT CAG GAT TGG
Cidea	TGC TCT TCT GTA TCG CCC AGT	GCC GTGT TAA GGA ATC TGC TG
Fabp4	ACA CCG AGA TTT CCT TCA AAC TG	CCA TCT AGG GTT ATG ATG CTC TTCA
Pgc1a	CCC TGC CAT TGT TAA GAC C	TGC TGC TGT TCC TGT TTT C
Prdm16	CCC TGC CAT TGT TAA GAC C	TGC TGC TGT TCC TGT TTT C
Cebpa	TGC GCA AGA GCC GAG ATA A	CGG TCA TTG TCA CTG GTC AAC T
Cpt1b	GCA CAC CAG GCA GTA GCT TT	CAG GAG TTG ATT CCA GAC AGG TA
Acadl	TCT TTT CCT CGG AGC ATG ACA	GAC CTC TCT ACT CAC TTC TCC AG
Acadm	AGG GTT TAG TTT TGA GTT GAC GG	CCC CGC TTT TGT CAT ATT CCG
Hmgcs2	GAA GAG AGC GAT GCA GGA AAC	GTC CAC ATA TTG GGC TGG AAA
Oxct1	CAT AAG GGG TGT GTC TGC TAC T	GCA AGG TTG CAC CAT TAG GAA T
Acaa2	CCT CAG TTC TTG TCT GTT CAG	AGG TGT GCG GTG ATT CTG
Slc27a2	TCC TCC AAG ATG TGC GGT ACT	TAG GTG AGC GTC TCG TCT CG
Mmp3-F	ACA TGG AGA CTT TGT CCC TTT TG	TTG GCT GAG TGG TAG AGT CCC
Acta2	GTC CCA GAC ATC AGG GAG TAA	TCG GAT ACT TCA GCG TCA GGA
Fbln2	CTG TGA AGA CCA AGA CGA GTG	CGT TGA GGA TAT AGC CCT CTG C
Lox	AGC ATG AAA GCA AGG CGC ATA	GTA CGC ATC TAC GCA GTT CTG
Mmp3	ACA TGG AGA CTT TGT CCC TTT TG	TTG GCT GAG TGG TAG AGT CCC
Col1a1	TGA CTG GAA GAG CGG AGA GT	GTT CGG GCT GAT GTA CCA GT
Col3a1	GTG CTC CTG GAC AGA ATG GT	CAC CCT TTA CAC CCT GAG GA
Col4a1	GCC AAG TGT GCA TGA GAA GA	AGC GGG GTG TGT TAG TTA CG

guide RNA	Forward	Reverse
R26R	CAC CGA AGA TGG GCG GGA GTC TTCT	AAA CAG AAG ACT CCC GCC CAT CTTC
Hif1a	CAC CGT TTC TTC TCG TTC TCG CCGC	AAA CGC GGC GAG AAC GAG AAG AAAC

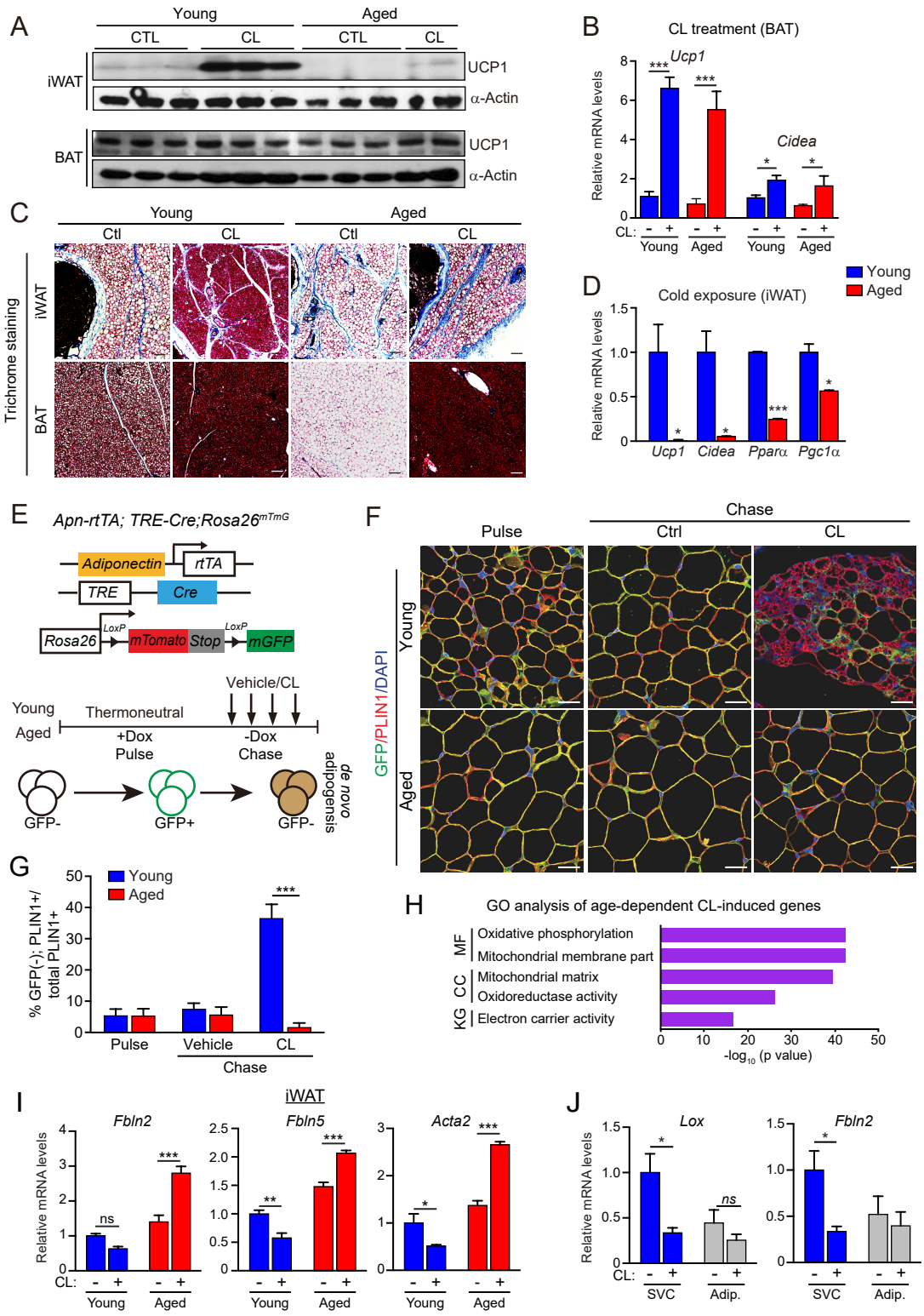

Fig. S1, Related to Fig. 1. Reduced beige adipocyte differentiation in aged mice

(A-C) Thermoneutral-housed young (2-month) and aged (12-month) mice were treated with vehicle or CL for 5 days. **(A)** Western blot analysis of UCP1 and α-Actin in iWAT and BAT. **(B)** mRNA levels of thermogenic genes in BAT. **(C)** Trichrome staining of collagen in iWAT and BAT. Scale bar, 100 μm. **(D)** mRNA levels of thermogenic genes in iWAT of young and aged mice following 1 wk of cold exposure. **(E)** Young and aged thermoneutral-housed *Adipochaser* mice were treated with doxycycline (Dox) to label adipocytes with mGFP (pulse). 2 days later, mice were treated with vehicle (Ctrl) or CL for 4 days (chase). **(F)** Immunofluorescence staining of Perlipin1 (PLIN1; red) and GFP (green) in iWAT. Nuclei (DAPI, blue); scale bar, 25 μm. **(G)** Quantification of adipogenesis (% of GFP-;PLIN1+/total PLIN1+ cells). **(H)** Gene ontology (GO) analysis of genes selectively induced by CL in young vs. aged mice (purple cluster from Fig.1C). MF, Molecular Function; CC, Cellular Component; KG, KEGG Pathway. **(I)** mRNA levels of fibrosis genes in young and aged mice treated with vehicle or CL. **(J)** mRNA levels of fibrosis genes in stromal vascular cells (SVC) and adipocytes (Adip) from young mice housed at thermoneutrality or exposed to 4°C for 1 wk. Data are mean ± sem; **p*< 0.05, ***p*<0.01, ****p*<0.001. n=3-5 per group.

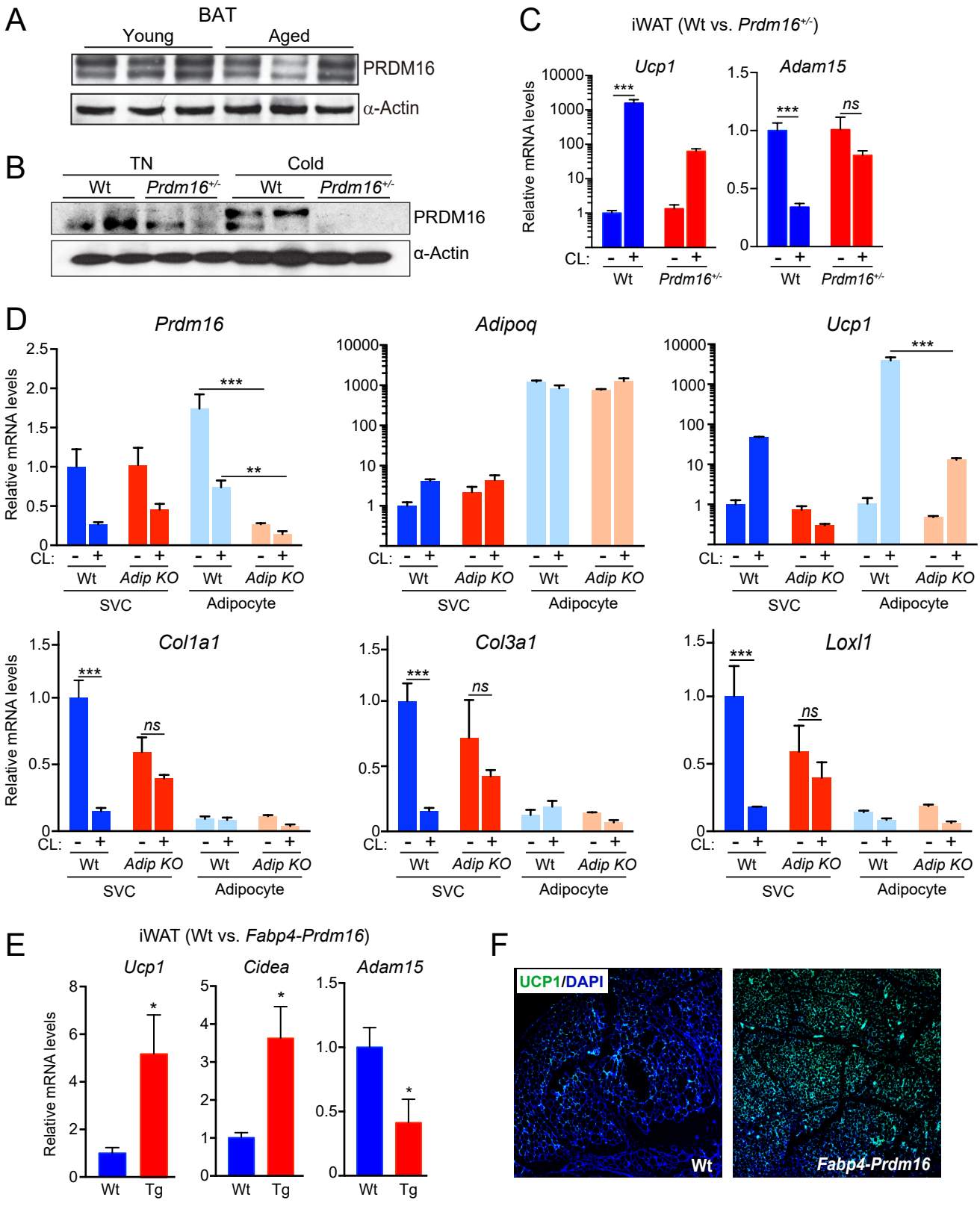

Fig. S2, Related to Fig. 2. PRDM16 regulates beige fat remodeling

(A) Western blot analysis of PRDM16 and α-Actin in BAT from young and aged mice housed at thermoneutrality. **(B)** Western blot analysis of PRDM16 and α-Actin in iWAT from wildtype (Wt) and *Prdm16*$^{+/-}$ mice treated with vehicle (Ctrl) or CL. **(C)** mRNA levels of *Ucp1* and fibrosis marker *Adam15* in iWAT from 3-month-old wildtype (Wt) and *Prdm16*$^{+/-}$ mice treated with vehicle or CL for 4 days. **(D)** mRNA levels of adipocyte and fibrosis genes in stromal vascular cells (SVC) and adipocytes isolated from wildtype (Wt) or adipocyte-selective *Prdm16*-knockout (*Adip. KO*) mice, treated with vehicle or CL. **(E,F)** 1-year-old wildtype and *Fabp4-Prdm16* transgenic mice were housed at thermoneutrality and treated with CL for 4 days. **(E)** mRNA levels of thermogenic genes and *Adam15*. **(F)** Immunofluorescence staining for UCP1 (green) in iWAT. Nuclei (DAPI, blue). Data are mean ± sem; *p< 0.05, **p<0.01, ***p<0.001. n=3-5 per group.

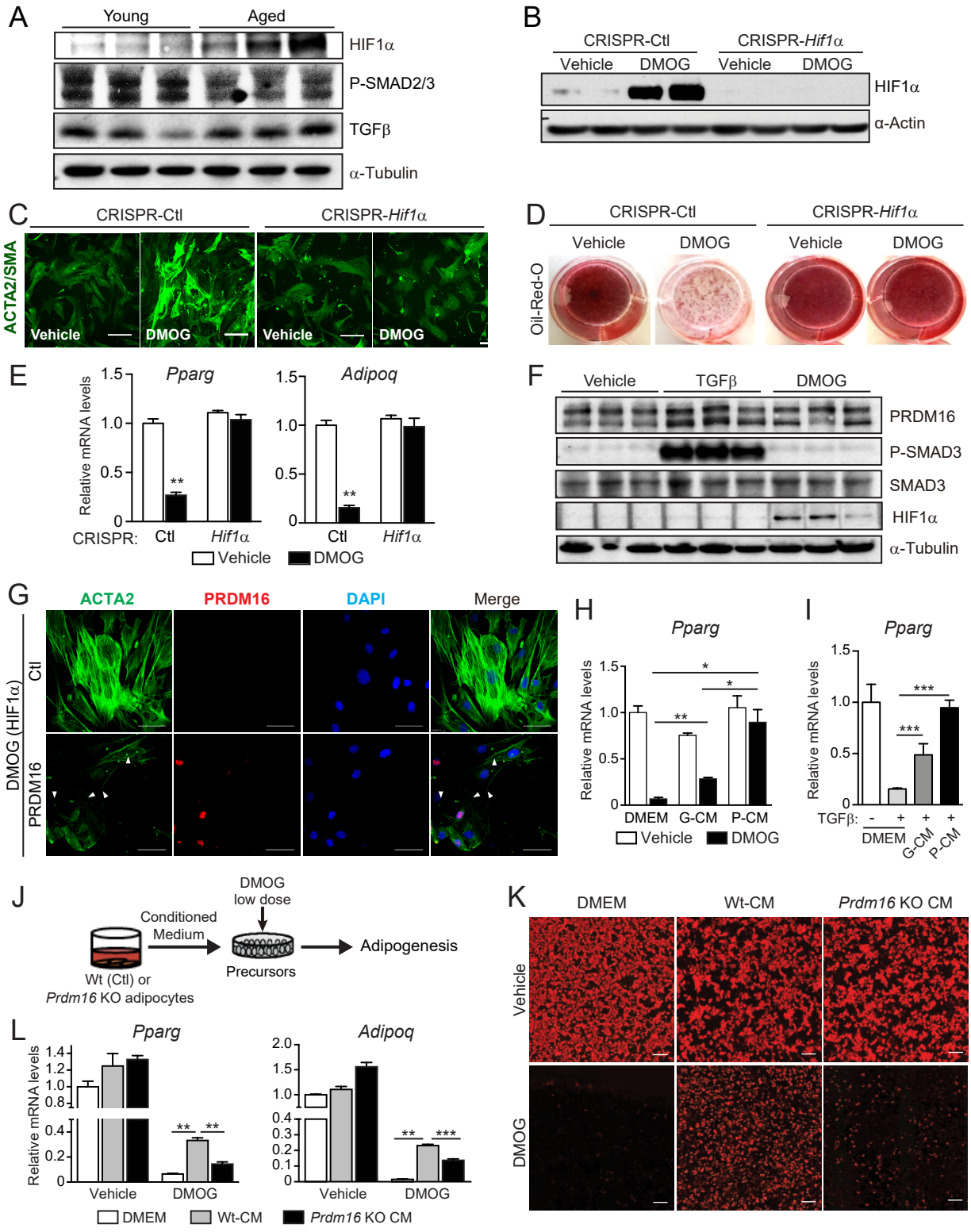

Fig. S3, Related to Fig. 3. PRDM16 suppresses precursor fibrogenesis

(A) Western blot analysis of indicated proteins in iWAT of young and aged mice. **(B-E)** iWAT precursor cells were transduced with retrovirus expressing CRISPR/Cas9 and *Rosa26* (Ctl) or *Hif1α* guide RNAs (gRNAs) and then treated with vehicle or DMOG. **(B)** Western blot analysis of HIF1α and α-Actin. **(C)** Immunofluorescence staining for ACTA2. Scale bar, 100 μM. **(D)** Oil-Red-O staining of adipocytes. **(E)** mRNA levels of adipocyte genes. **(F)** Western blot analysis of indicated proteins in day 8 differentiated adipocytes treated with vehicle, TGFβ, or DMOG for 2 days. **(G)** Immunofluorescence staining of ACTA2 (green) and PRDM16 (red) in iWAT precursors expressing control (Ctl) or PRDM16 retrovirus. Nuclei (DAPI, blue); scale bar, 100 μm. **(H-I)** iWAT precursor cells were treated with: (1) control medium (DMEM), GFP-CM or P-CM; and (2) vehicle, DMOG or TGFβ. After 24 h, cultures were induced to differentiate into adipocytes for 5 days. **(H,I)** mRNA levels of *Pparg*. **(J-L)** DMOG-treated iWAT precursor cells were incubated with CM from wildtype (Wt) or *Prdm16*-knockout (KO) adipocytes during adipogenic differentiation. **(K)** LipidTOX staining of adipocytes. Scale bar, 200 μm. **(L)** mRNA levels of adipocyte genes. Data are mean ± s.e.m; **p*<0.05, ***p*<0.01, ****p*<0.001. n=3-5.

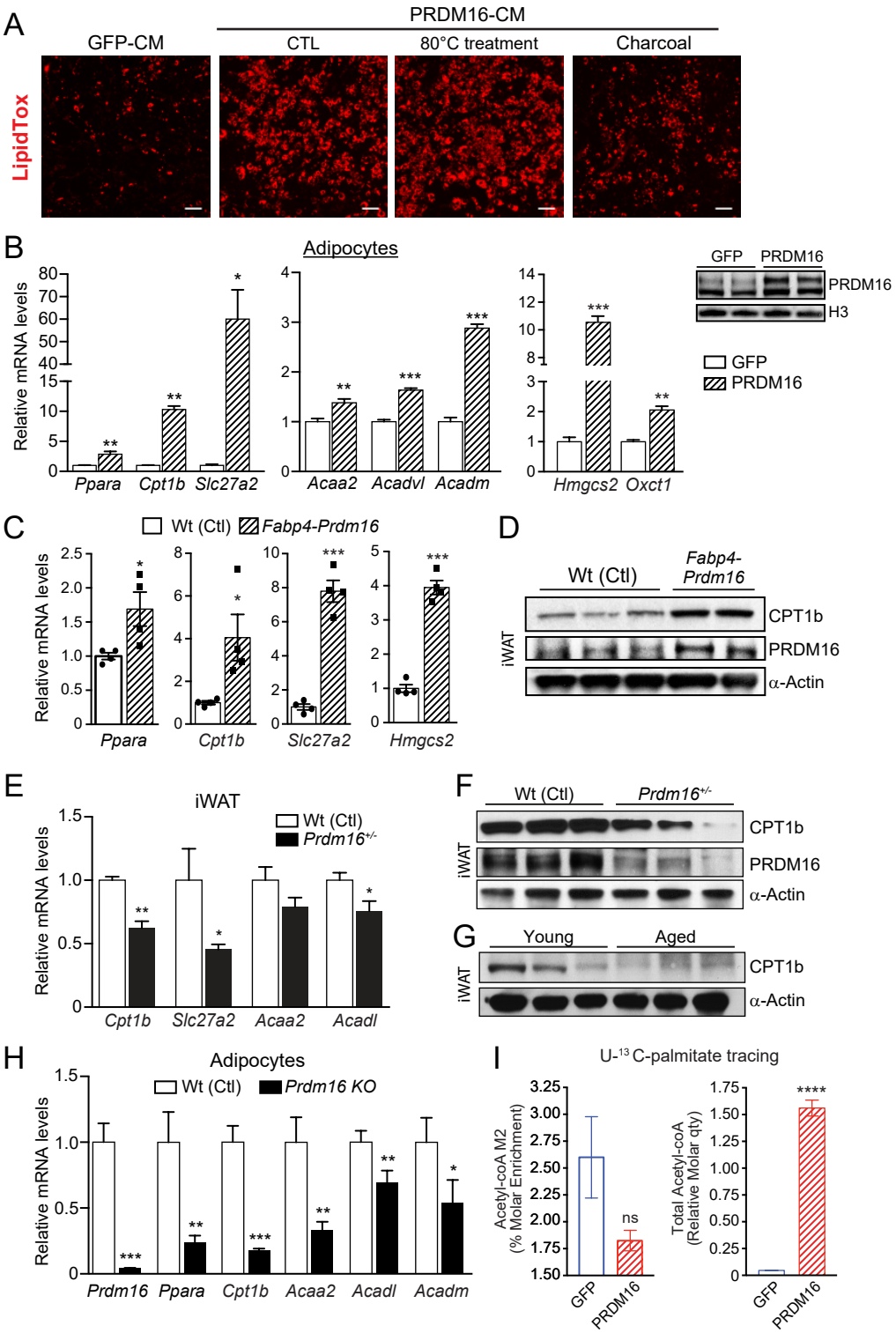

Fig. S4, Related to Fig. 4. PRDM16 regulates the fatty acid oxidation (FAO) program in adipocytes

(A) Precursor cells were exposed to DMOG and either: G-CM, P-CM, P-CM heated to 80°C, or P-CM treated with charcoal. Cultures were induced to differentiate into adipocytes for 5 days and stained with LipidTOX. Scale bar, 200 μm. **(B)** mRNA levels of FAO genes in GFP- and PRDM16-expressing adipocytes. (Inset) Western blot analysis of PRDM16 and Histone H3. **(C)** mRNA levels of FAO genes in iWAT from wildtype (Ctl) and *Fabp4-Prdm16* mice. **(D)** Western blot analysis of CPT1b, PRDM16, and α-Actin in iWAT from samples in (C). **(E)** mRNA levels of FAO genes in iWAT from 2-month-old wildtype (Ctl) and *Prdm16*$^{+/-}$ mice. **(F)** Western blot analysis of CPT1b, PRDM16, and α-Actin in samples from (E). **(G)** Western blot analysis of CPT1b and α-Actin in iWAT from young and aged mice. **(H)** mRNA levels of FAO genes in wildtype (Ctl) and *Prdm16*-knockout (KO) adipocytes. **(I)** Quantification of FAO. Adipocytes were cultured in medium containing U-^{13}C-palmitate for 6 h prior to metabolite extraction and analysis by LC-MS. Percentage of molar enrichment of acetyl-CoA M2 was determined by correcting the detected AUCs for the acetyl-CoA isotopologues M0, M1, M2 M3 to matched control samples that had not been treated with $^{13}C_6$-palmitate (left). Acetyl-CoA relative molar quantity was determined by summing the AUCs for acetyl-CoA isotopolgues M0, M1, M2, M3 and normalizing to the AUC for the $^{13}C_3{}^{15}N_1$-acetyl-CoA internal standard (right).. All data are mean ± sem. **p*<0.05, ***p*<0.01, ****p*<0.001. n=3-5 per group.

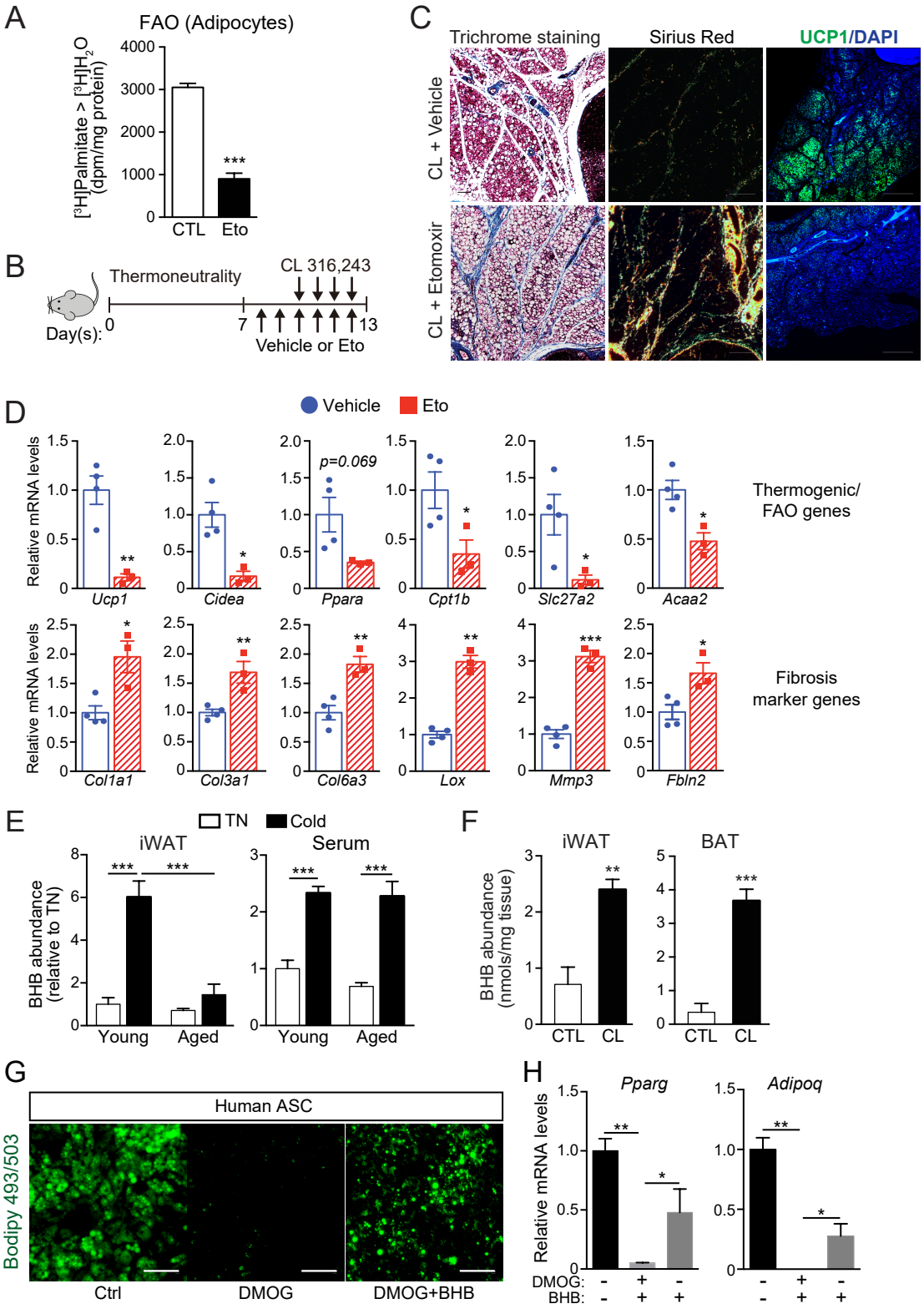

Fig. S5, Related to Figs. 4 & 5. Acute inhibition of FAO blocks beige fat development; and BHB promotes adipogenesis in human adipose-derived stem cells (ASCs)

(A) FAO rate in IWAT adipocyte cultures treated with vehicle (CTL) or 50 μM Etomoxir (Eto). CTL or Eto-treated adipocytes were incubated with [^{3}H]palmitate for 2 h, followed by measurement of [^{3}H]H_2O in the medium. **(B-D)** 2-month-old mice were housed at thermoneutrality and treated with CL and either vehicle or Eto. **(C)** Trichrome (left), Sirius Red (middle), and immunofluorescence staining for UCP1 (right) in iWAT. Nuclei (DAPI, blue); scale bar, 100 μm. **(D)** mRNA levels of indicated genes. **(E)** BHB levels in iWAT and serum from young and aged mice housed at thermoneutrality or exposed to 5°C cold for 7 days. **(F)** BHB levels in iWAT (left) or BAT (right) of mice treated with vehicle (Ctrl) or CL. **(G-H)** Human ASCs were treated with vehicle, DMOG or DMOG plus 3 mM BHB, and induced to differentiate into adipocytes for 16 d. **(G)** Bodipy 493/503 (green) staining of adipocytes. Scale bar, 100μM. **(H)** mRNA levels of *Pparg*, and *Adipoq*. Data are mean ± sem. **p*<0.05, ***p*<0.01, ****p*<0.001. n=4-5 per group.

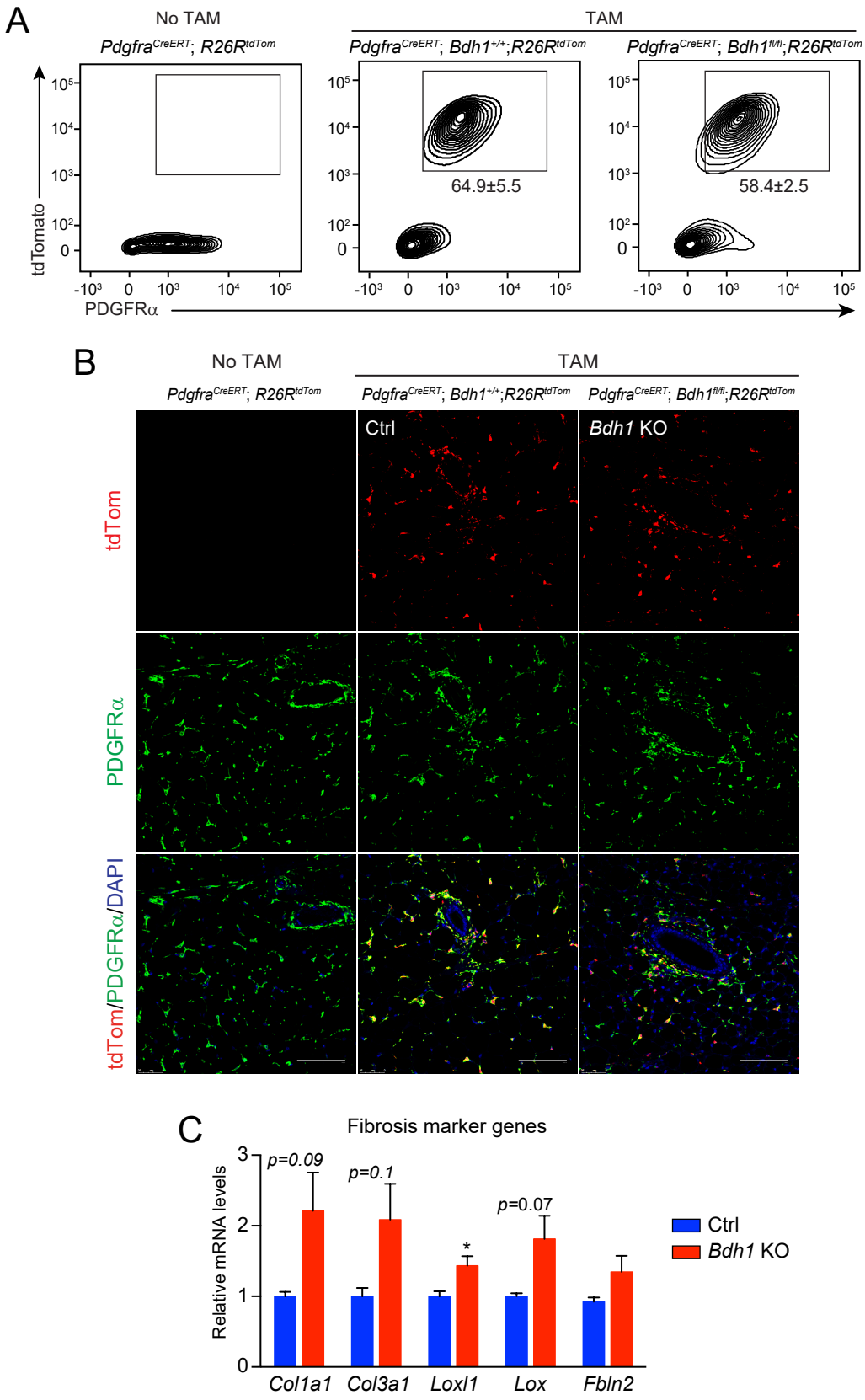

Fig. S6, Related to Fig. 6. Precursor *Bdh1* is required for beige fat development

Bdh1 KO (*Pdgfra*^{CreERT2}*; Bdh1*$^{flox/flox}$*; R26R*^{tdTomato}) and corresponding control (ctrl; *Pdgfra*^{CreERT2}*; Bdh1*$^{+/+}$*; R26R*^{tdTomato}) mice were injected with tamoxifen for 5 days. Mice were then: **(A,B)** immediately analyzed (Pulse); or **(C)** subjected to cold exposure. Mice injected with corn oil served as controls. **(A)** Flow cytometry plots of tdTomato and PDGFRα expression in CD31(-);CD45(-) cells from iWAT of indicated animals. **(B)** Immunofluorescence analysis of tdTomato (red) and PDGFRα (green) in iWAT of indicated animals. Scale bar, 200 μM. DAPI was used to stain nuclei (blue). **(C)** mRNA levels of fibrosis genes in iWAT after cold exposure. Data are mean ± sem. **p*<0.05. n=3-4 mice per group.

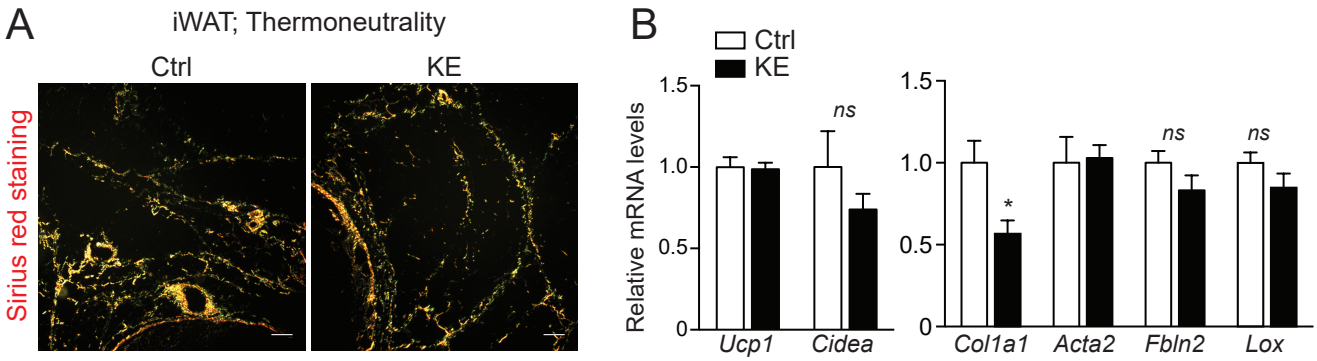

Fig.S7, Related to Fig. 7. KE diet is not sufficient to induce beiging in thermoneutral-housed mice

(A-B) Thermoneutral-acclimated aged mice were pair-fed a control (Ctrl) or Ketone ester diet (KE) for 4 weeks. **(A)** Sirius red staining of collagen fibers in iWAT. Scale bar, 100 μM. **(B)** mRNA levels of thermogenic and fibrosis genes in iWAT of indicated animals. n=10 mice per group. Data are mean ± sem. *p<0.05.