Fig S1.

A

P60 Pulse P63

TM

Ucp1-Cre^{ERT2}-RFP (Pulse: P60-P63)

BAT
Heart
IGWup
Kidney
IGWlow
Liver
Epididymis
testis
PGW
Muscle
Ear
Spleen

BAT
Heart
IGWup
Kidney
IGWlow
Liver
PGW
Muscle
Ear
Spleen

Male

Female

B

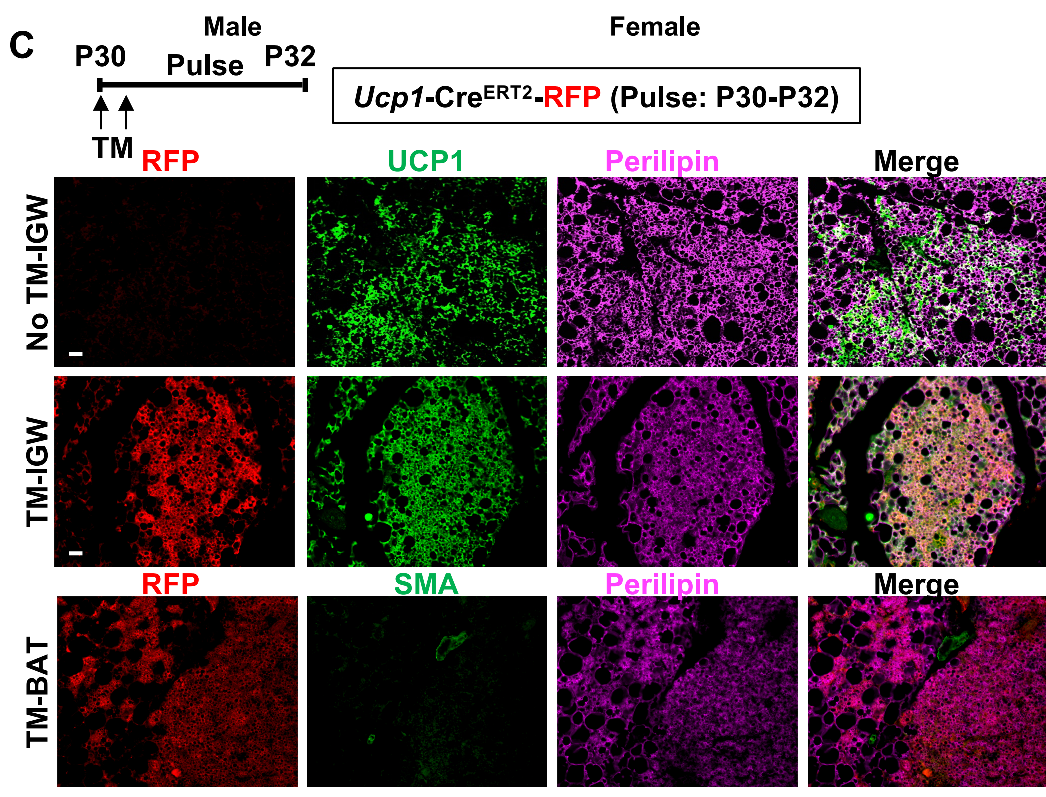
Ucp1-Cre^{ERT2}-mTmG

BAT
IGW
L
PGW
Kidney
Spleen
Heart
Liver
Muscle

Pulse: P60-P63

Male

C



D

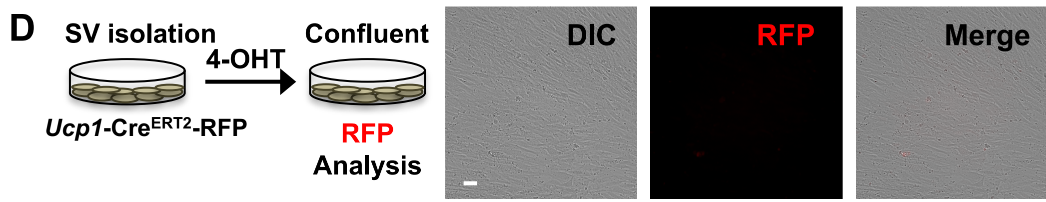


Figure S1. *Ucp1*-Cre^{ERT2}; *Rosa26R-RFP* faithfully labels UCP1+ cells at pulse, Related to Figure 1.

(A) Whole tissues were imaged for direct RFP. Two-month-old (P60) *Ucp1*-Cre^{ERT2}; *Rosa26R-RFP* (UCP1-RFP) male and female mice were administered TM for two doses and were analyzed at P63 (n=4 mice per group). White arrows indicate the Epididymis.
(B) Whole tissues were imaged for direct RFP and GFP. Two-month-old (P60) *Ucp1*-Cre^{ERT2}; *Rosa26R-mTmG* (UCP1-mTmG) male mice were administered TM for two doses and were analyzed at P63 (n=4 mice per group). Yellow arrows indicate brown/beige adipose tissue within WAT (IGW and PGW) and other organs, including kidney, heart, muscle.
(C) One-month-old (P30) *Ucp1*-Cre^{ERT2}; *Rosa26R-RFP* (UCP1-RFP) male mice were administered TM for two doses and were analyzed at P32 (n=4 mice per group). Representative immunostaining for UCP1-RFP (red), UCP1 (green), and Perilipin (magenta) on IGW and BAT sections of mice with or without TM (tamoxifen). Scale = 100 μm.
(D) SV cells isolated from UCP1-RFP mice (n=3 mice per group) were treated with 4-OH-Tamoxifen. Scale = 100 μm.

Fig S2.

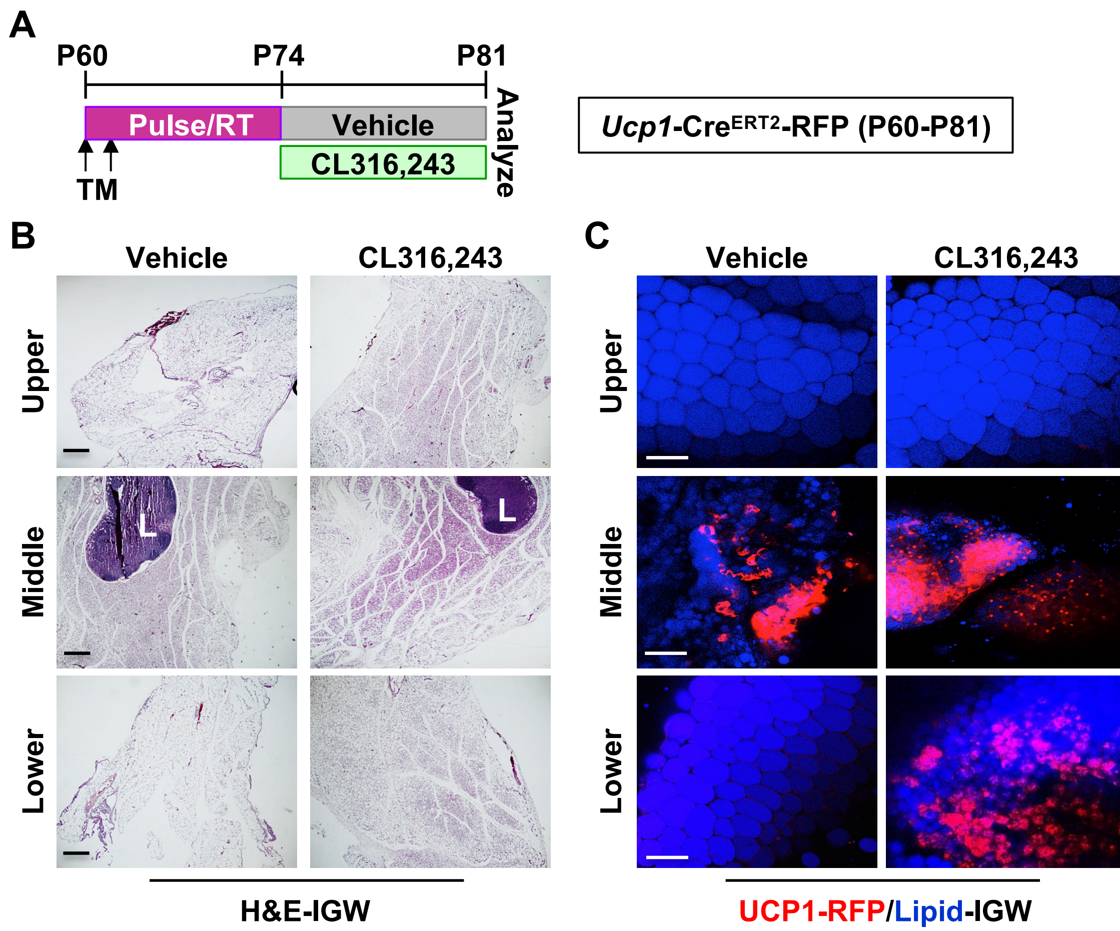
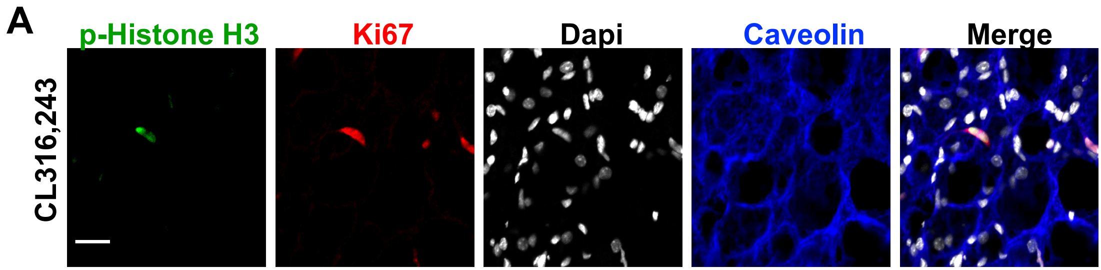
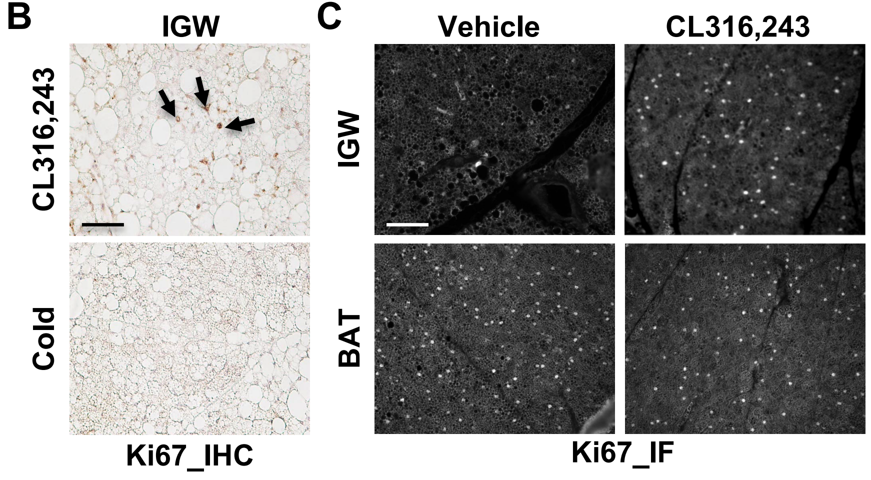


Figure S2. Pre-existing UCP1+ cells generate new beige adipocytes in response to β3-adrenergic stimulation, Related to Figure 1.

(A) Experimental procedure. TM-induced 2-month-old UCP1-RFP male mice were administered vehicle or CL316,243 (β3 agonist) for 7 consecutive days. (n=5 mice per group)
(B) H&E staining on IGW sections of mice described in (A). IGW depots were separated into 3 portions: upper, middle, and lower. L: lymph node. Scale = 20 μm.
(C) Whole IGW depots of mice described in (A) were imaged for direct UCP1-RFP (red) and LipidTox staining (blue). Scale = 200 μm.

Fig S3.





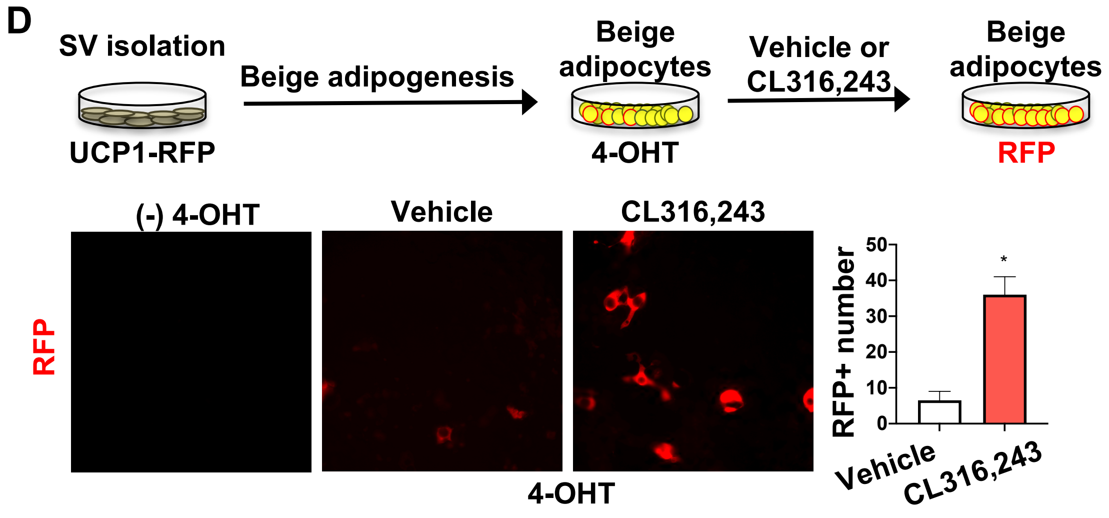


Figure S3. A subset of UCP1+ beige adipocytes mimic progenitor-like characteristics in response to β3-agonist, Related to Figure 2.

(A) Representative fluorescence imaging of p-Histone H3 (green), Ki67 (red) and Caveolin (blue) immunostaining on IGW sections with CL316,243 for 7 days. DAPI stained the nuclei. Scale = 100 μm.
(B) Representative IHC of Ki67 staining on IGW sections from mice treated with CL316,243 or cold for 7 days. Arrows showed Ki67+ cells Scale = 200 μm.
(C) Representative fluorescence imaging of Ki67 (white) on IGW and BAT sections. Scale = 200 μm.
(D) SV cells isolated from IGW of UCP1-RFP mice were differentiated into beige adipocytes *in vitro* and treated with vehicle or CL316,243. Scale = 100 μm. *$P<0.01$. ($n=4$ mice per group).

Fig S4.

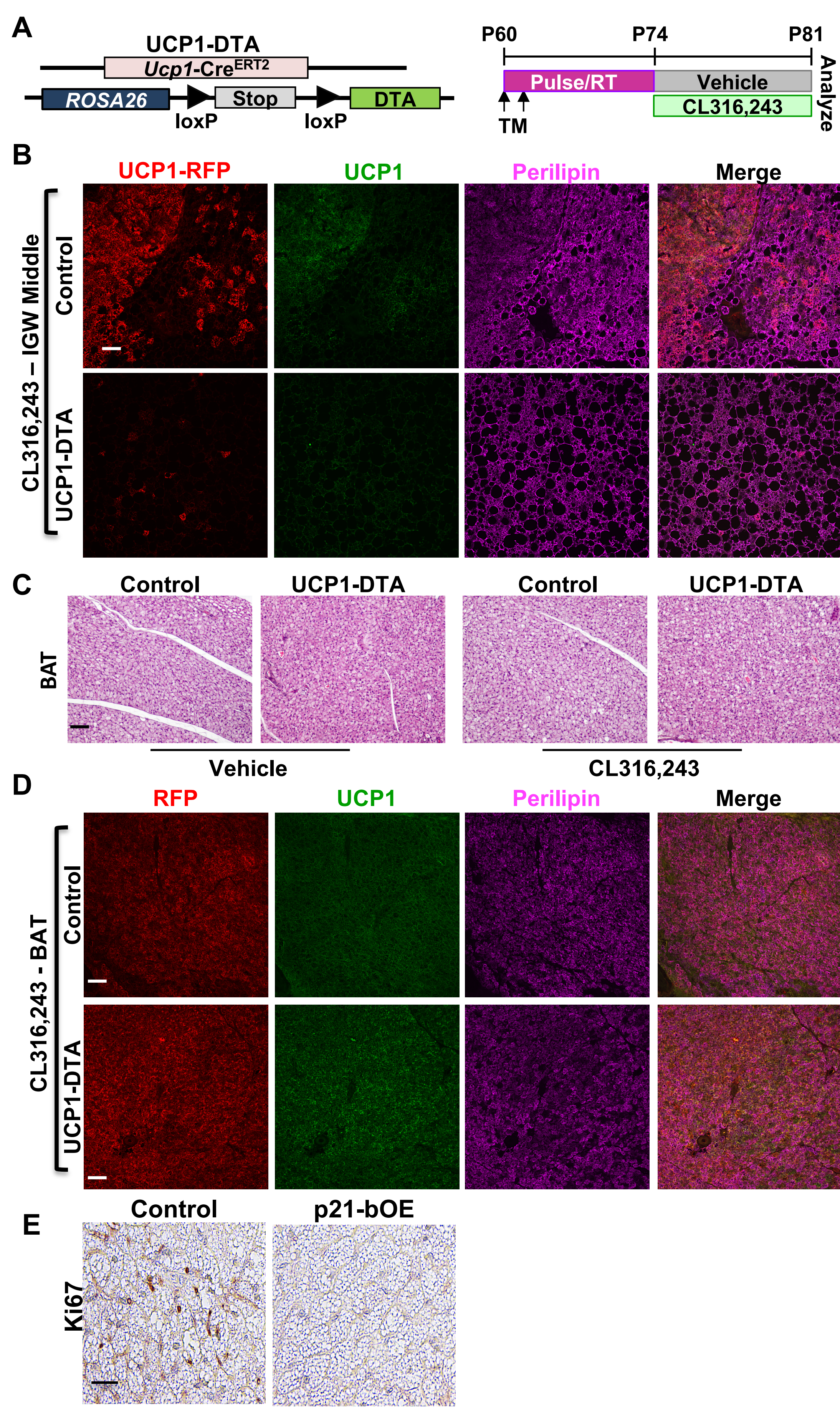


Figure S4. Ablation of UCP1+ cells reduces beige formation, Related to Figure 3.

(A) Experimental scheme. TM-induced 2-month-old male UCP1-DTA mice were treated with vehicle or CL316,243 (β3 agonist) (n=5 mice per group).
(B) Representative fluorescence imaging of UCP1-RFP (red), UCP1 (green), and Perilipin (Magenta) immunostaining on IGW sections. Scale = 100 μm.
(C) Representative H&E staining on BAT sections. Scale = 100 μm.
(D) Representative immunostaining images for UCP1-RFP (red), UCP1 (green), and Perilipin (magenta) and on BAT sections. Scale = 100 μm.
(E) Representative images for Ki67 staining on control or p21-bOE depots . Scale = 200 μm.

Fig S5.

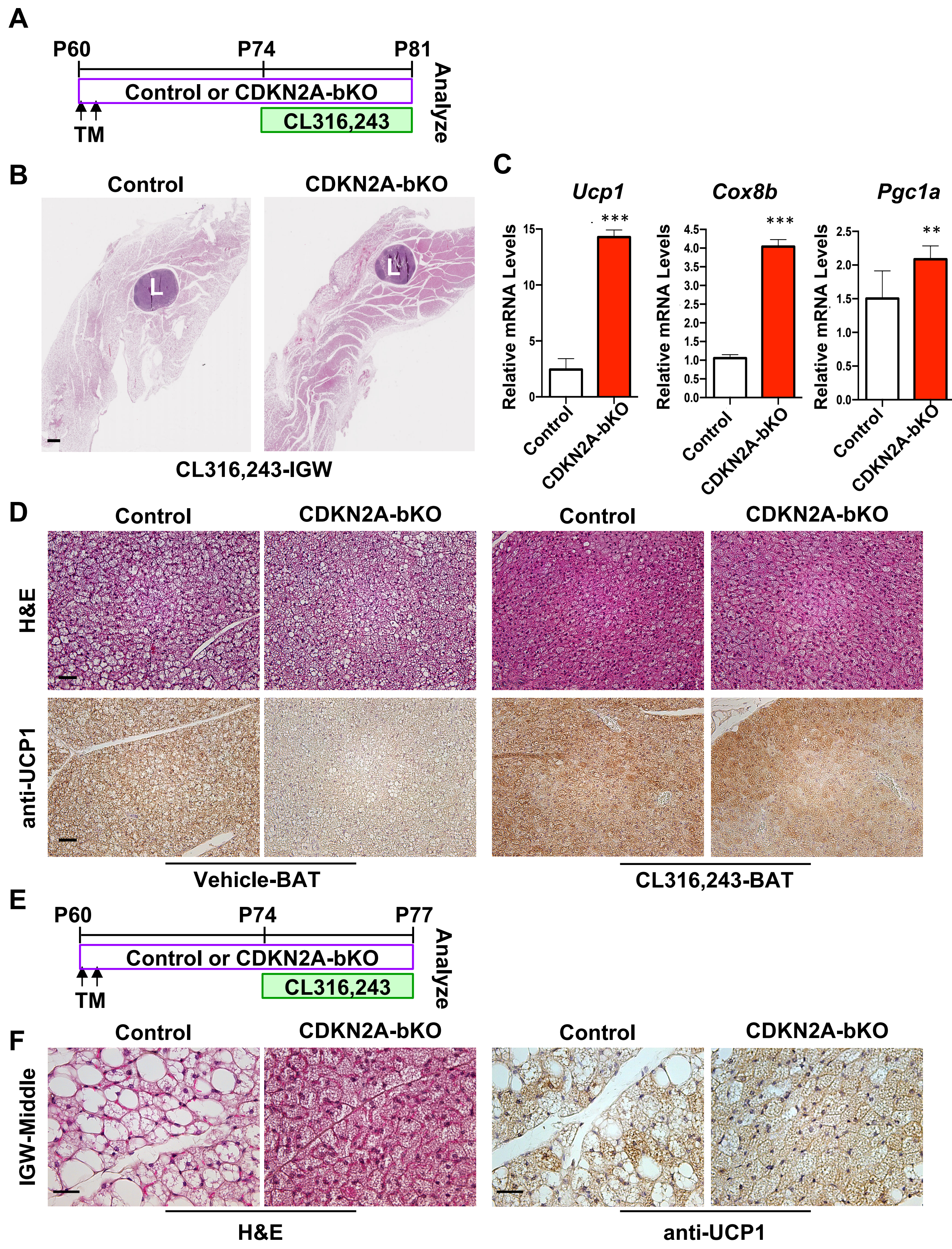


Figure S5. Loss of CDKN2A in UCP1+ cells promotes beige adipocyte formation but does not affect brown adipocytes, Related to Figure 5.

(A) Experimental scheme. TM-induced 2-month-old control or Cdkn2a-bKO mice were administered CL316,243 for 7 consecutive days (n=6 mice per group)
(B) Representative H&E staining on IGW sections. Scale = 0.5mm
(C) Thermogenic gene expression in IGW. Data are expressed as means ± SEM. **$P<0.001$, ***$P<0.0001$
(D) Representative H&E staining and IHC for UCP1 on BAT sections. Scale = 200 µm.
(E) Experimental scheme. TM-induced 2-month-old control or Cdkn2a-bKO mice were administered CL316,243 for 3 consecutive days. (n=3 mice per group)
(F) Representative H&E staining and IHC for UCP1 on IGW sections. Scale = 200 µm.

Fig S6.

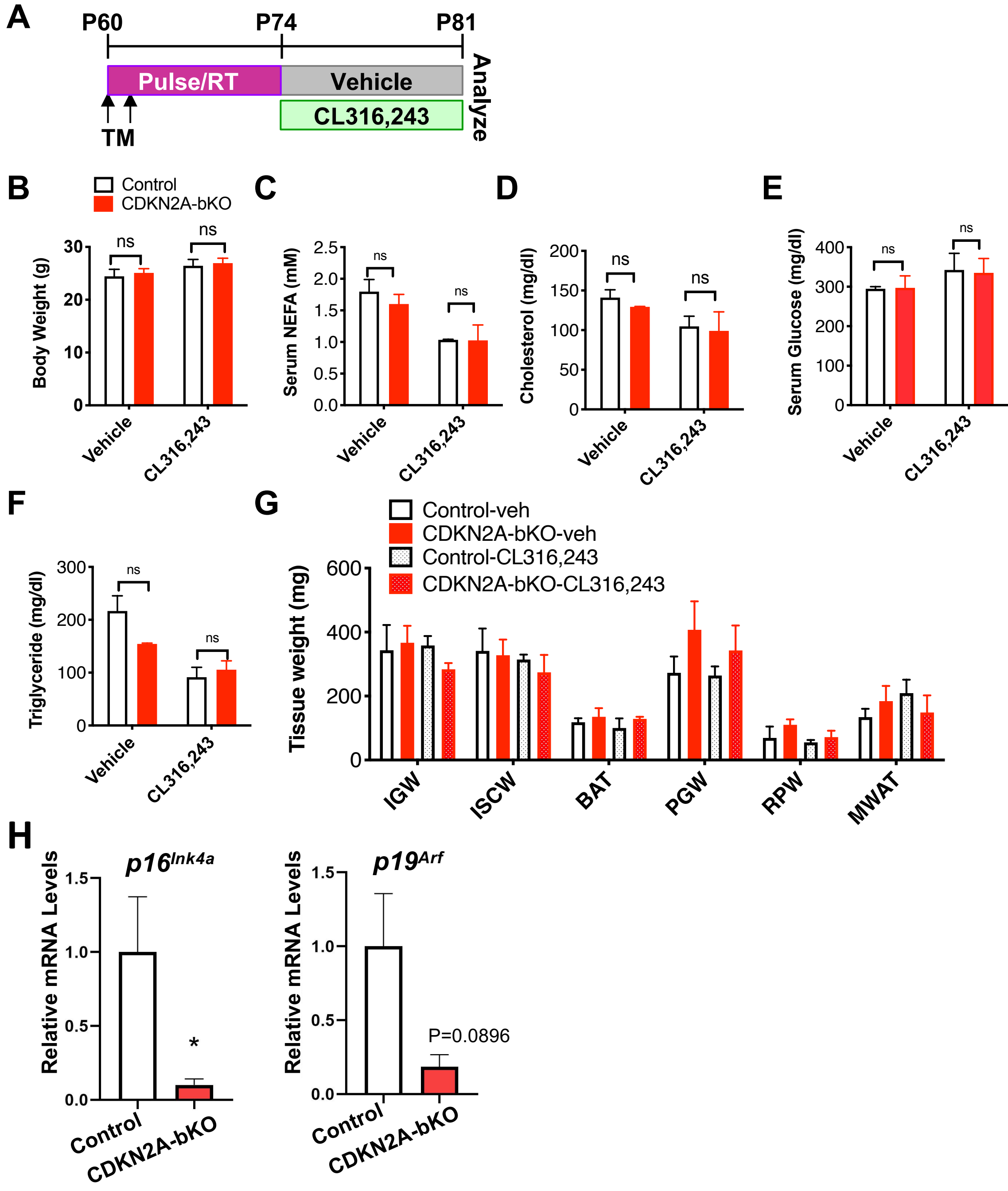


Figure S6. Short-term loss of CDKN2A in UCP1+ cells does not display metabolic phenotypes, Related to Figure 5.

(A) Experimental scheme. TM-induced 2-month-old control or Cdkn2a-bKO mice were administered vehicle or CL316,243 for 7 consecutive days (n=6 mice per group).
(B) Body weights of control and CDKN2A-bKO mice after vehicle or CL316,243 treatment. Data are expressed as means ± SEM (n=4 mice per group).
(C-F) Serum NEFA (C), Cholesterol (D), Glucose (E), and Triglyceride (F) levels of vehicle or CL316,243-treated control and CDKN2A-bKO mice. Data are expressed as means ± SEM (n=4 mice per group).
(G) Tissue weights of vehicle or CL316,243-treated control and CDKN2A-bKO mice (n=4 mice per group). Data are expressed as means ± SEM. PGW, Perigonadal WAT; RPW, Retroperitoneal WAT; MWAT, Mesenteric WAT.
(H) *Cdkn2a* gene expression in control and CDKN2A-bKO IGW depots (n=4 mice per group). Data are expressed as means ± SEM. *P<0.01.

Fig S7.

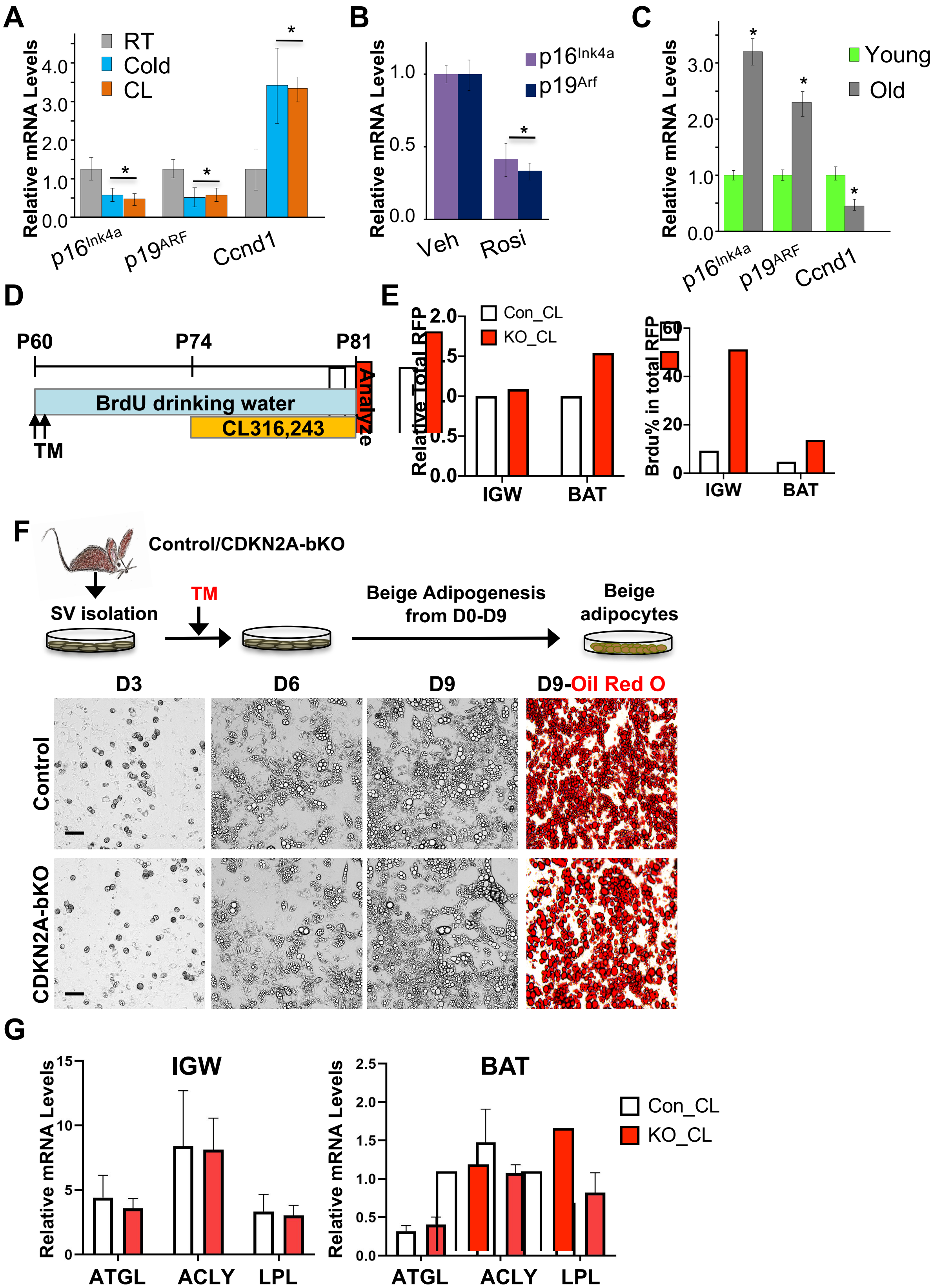


Figure S7. An inverse correlation between p16^{Ink4a} and p14^{ARF} expressions in iWAT and beiging activity, Related to Figure 6.

(A-C) qPCR analysis of cell cycle gene expression in IGW depots with 7 days of cold or CL treatment (**A**), with rosiglitazone (Rosi) treatment (**B**), and of young and old mice (**C**). Data are expressed as means ± SEM. *P<0.01. (n=4-5 mice per group). Young: 2-month-old; Old: 6-month-old.
(D) Experimental scheme. TM-induced 2-month-old control or CDKN2A-bKO mice were given BrdU containing drinking water for 3 weeks and treated with CL for 7 days.
(E) Quantification BrdU and RFP flow analysis of IGW and BAT from control or CDKN2A-bKO mice (mixed samples from n=3 male mice per group).
(F) Experimental scheme to treat SV cells with 4-OHT and then induce beige adipogenesis. DIC imaging at day 3 (D3), D6, and D9; and Oil Red O staining on D9 from control and mutants; Scale = 100 μm. n=4 mice per group.
(G) Lipolysis- and lipogenesis-related gene expression in IGW and BAT. ATGL, adipose triglyceride lipase; ACLY: ATP citrate lyase; LPL: lipoprotein lipase. Data are expressed as means ± SEM.

Table S1, Related to STAR Methods.
Primer sequences used for qRT-PCR.

Gene	Forward	Reverse
Ucp1	CACCTTCCCGCTGGACACT	CCCTAGGACACCTTTATACCTAATGG
Pgc1a	CCGATCACCATATTCCAGGT	GTGTGCGGTGTCTGTAGTGG
Cox8b	GAACCATGAAGCCAACGACT	GCGAAGTTCACAGTGGTTCC
Irf4	CAGGACTACAATCGTGAGGAGG	GCACATCGTAATCTTGTCTTCCA
Cidea	TGACATTCATGGGATTGCAGAC	GGCCAGTTGTGATGACTAAGAC
Tbx1	CTGTGGGACGAGTTCAATCAG	TTGTCATCTACGGGCACAAAG
ATGL	AACACCAGCATCCAGTTCAA	GGTTCAGTAGGCCATTCCTC
Acly	ACCCTTTCACTGGGGATCACA	GACAGGGATCAGGTATTCCTTG
LPL	GGCCAGATTCATCAACTGGAT	GCTCCAAGGCTGTACCCTAAG
P21	GTACTTCCTCTGCCCTGCTG	TCTGCGCTTGGAGTGATAGA
Ccnd1	ACTGACAACTCTATCCGGCC	GTCTGCTTGTTCTCATCCGC
p19^{ARF}	GCAGGTTCTTGGTCACTGT	TCGCACGAACTTCACC
p16^{Ink4a}	CGAACTCTTTCGGTCGTAC	ATCATCATCACCTGAATCGGGGT
Rn18S	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG