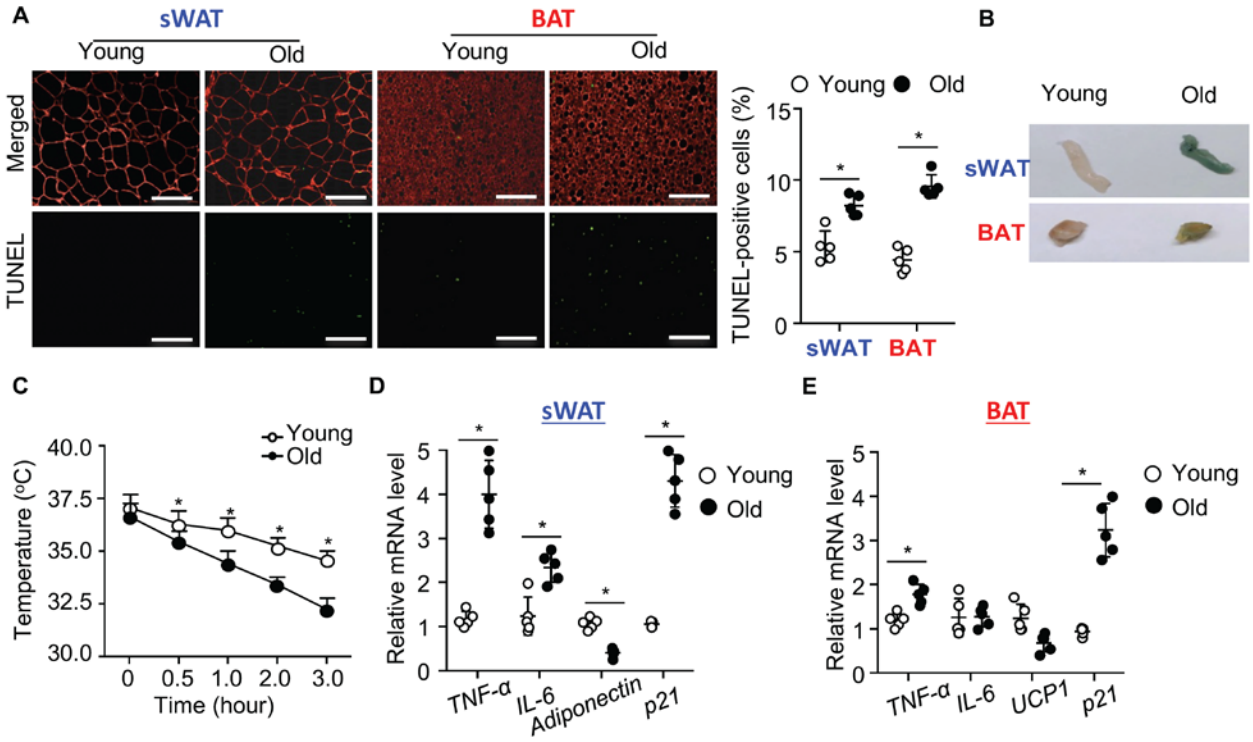
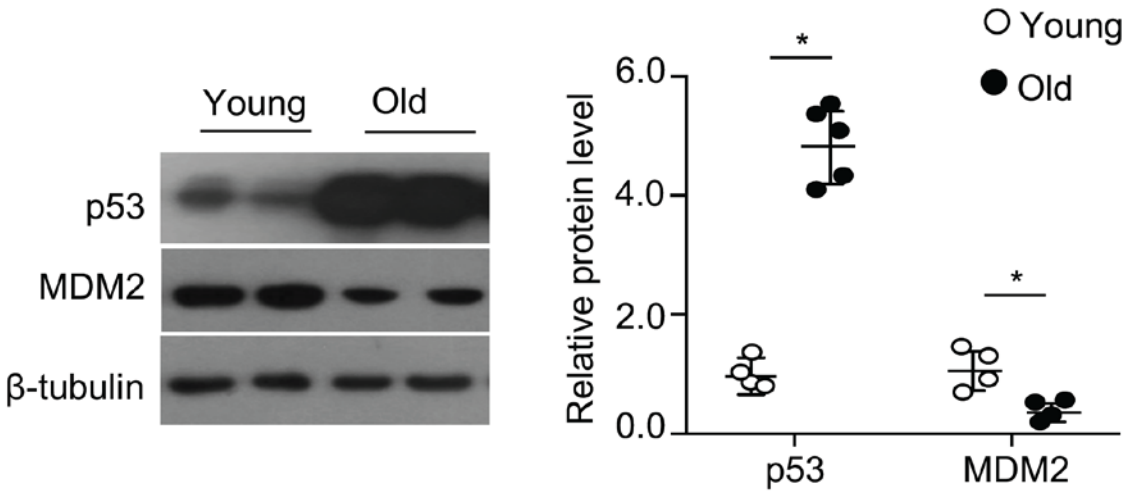
SUPPLEMENTARY DATA

Supplementary Figure 1. Increased adipocyte apoptosis and senescence and adipose tissue dysfunction in extreme old mice. Subcutaneous white adipose tissue (sWAT) and brown adipose tissue (BAT) were isolated from 12- week-old (young) and 2-year-old (old) male C57BL/6J mice. (A) Immunofluorescence staining of perilipin (red) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL; green) in the adipose tissue sections. Scale bar: 50 μm. The bar chart is quantification for the number of TUNEL-positive adipocytes. (B) Senescence β-galactosidase staining of the adipose tissues. (C) Core body temperature during cold exposure (4 oC). (D-E) QPCR analysis of genes related to inflammation (*TNF-α* and *IL-6*), senescence (*p21*) and adipocyte functions (*adiponectin* and *UCP1*) in BAT and sWAT. Expression level of target genes are normalized with *18S* and expressed as fold change over the young mice. *p<0.05 old group vs. young control group (Student's t-test) (n=5). All data are represented as mean ± SD.

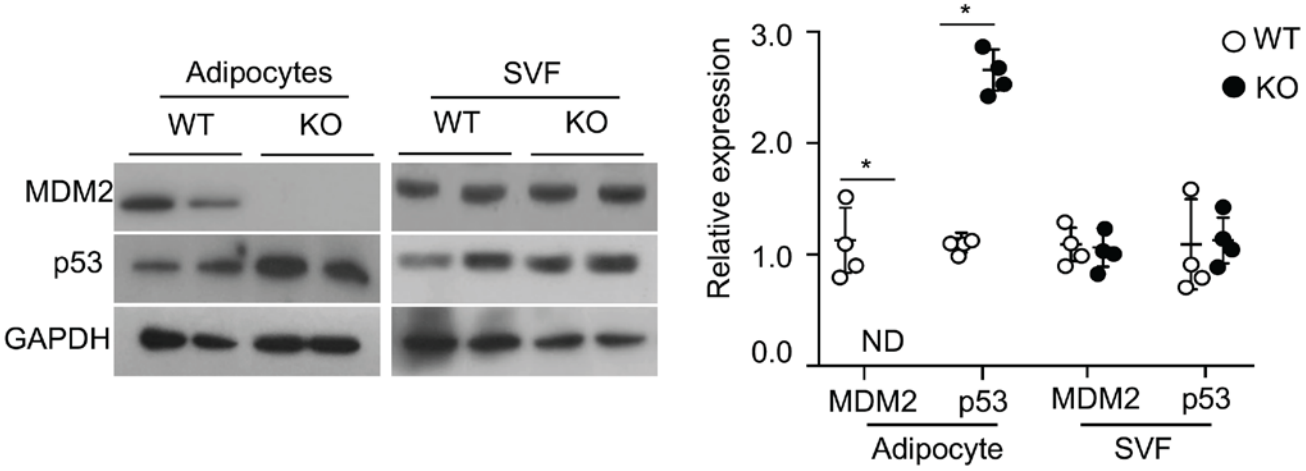


SUPPLEMENTARY DATA

Supplementary Figure 2. Dysfunctional MDM2-p53 axis in epididymal white adipose tissue of aged mice. eWAT were isolated from 12-week-old (young) and 2-year-old (old) male C57BL/6J mice. Immunoblotting analysis of p53, MDM2 and β-tubulin in eWAT. The right panel is the densitometric analysis for the relative abundance of MDM2 and p53 normalized with β-tubulin. Representative immunoblots were shown. *p<0.05 old group vs. young control (Student's t-test) (n=4-5). All data are represented as mean ± SD.

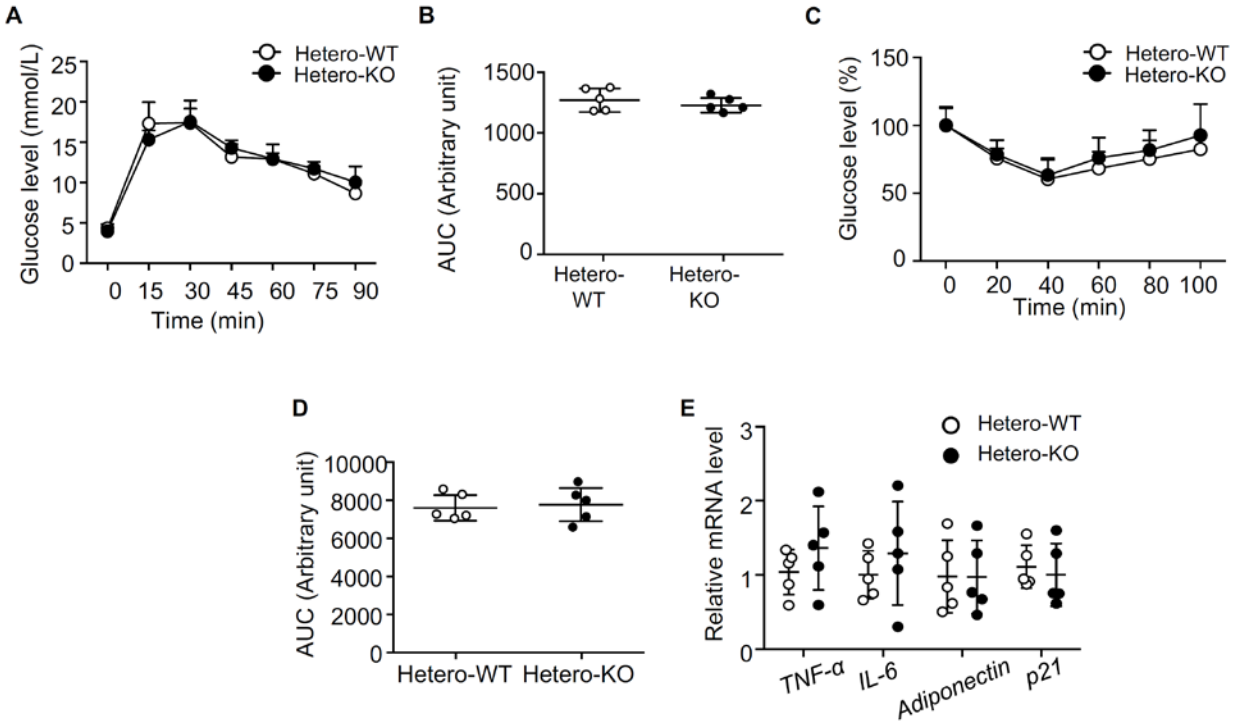


Supplementary Figure 3. Generation of adipocyte-specific MDM2 knockout (KO) mice. Immunoblotting analysis of MDM2 and p53 in adipocytes and stromal vascular fraction (SVF) isolated from sWAT of 3-week-old Adipo-MDM2- KO mice and their WT littermates. Representative immunoblot images were shown from four independent biological samples. The bar chart in the right panel is densitometric analysis for the relative abundance of MDM2 and p53 normalized with GAPDH. *p<0.05 KO vs. WT group (Student's t-test) (n=4). ND (undetectable). All data are represented as mean ± SD.



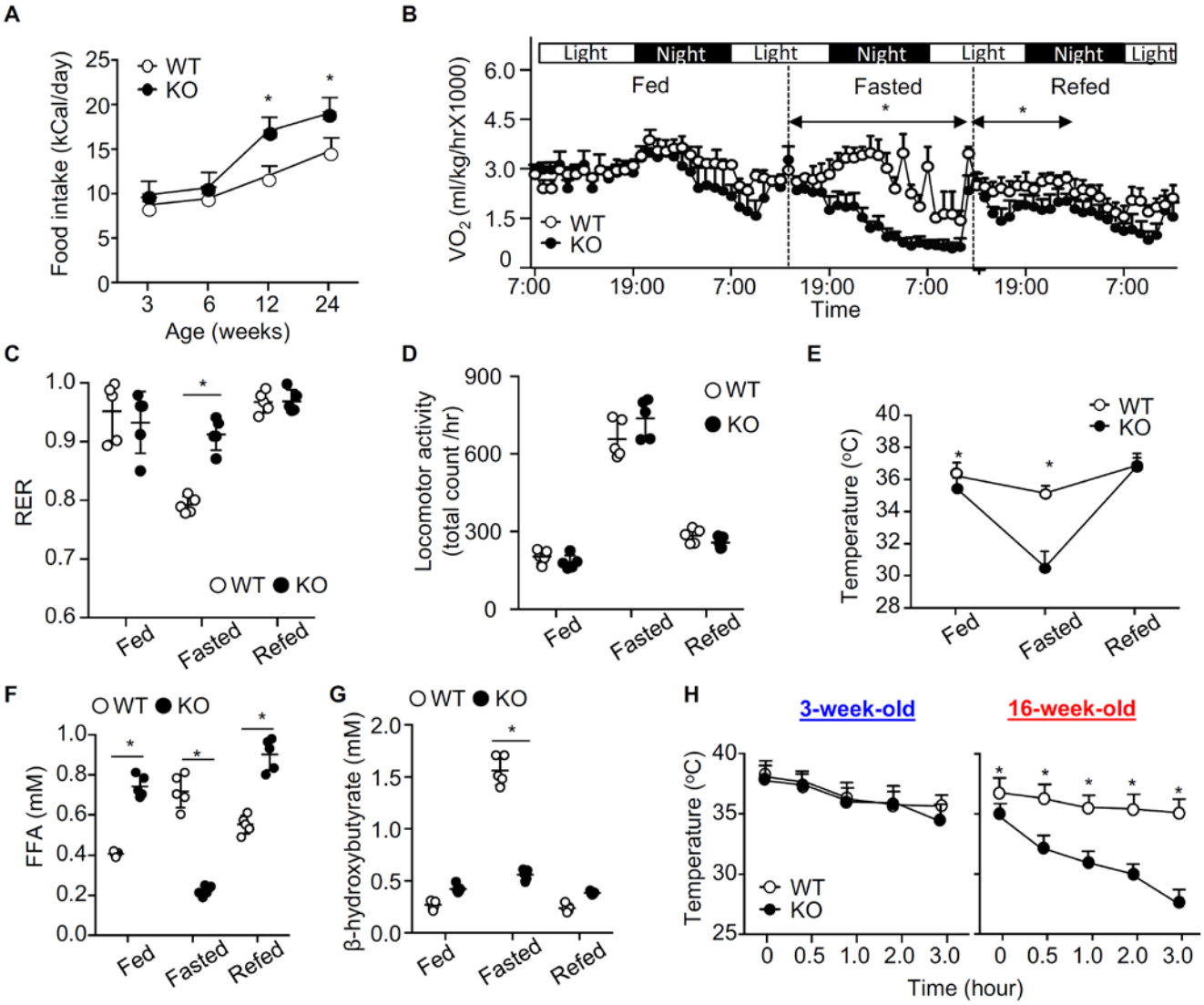
SUPPLEMENTARY DATA

Supplementary Figure 4. Heterozygous adipocyte specific MDM2 KO (Hetero-Adipo-MDM2-KO) mice exhibit normal glucose metabolism. Hetero-Adipo-MDM2-KO mice (MDM2floxed/--Adiponectin-Cre) and their WT littermates (MDM2floxed/-) were used. (A) GTT in 12-week-old mice. (B) AUC for the GTT in panel A. (C) ITT in 13-week-old mice. (D) AUC for ITT in panel C. (E) QPCR analysis of *TNF-α, IL-6, p21* and *adiponectin* the in sWAT. *p<0.05 Hetero-WTvs. Hetero-KO group (Student's t-test) (n=5). All data are represented as mean ± SD.



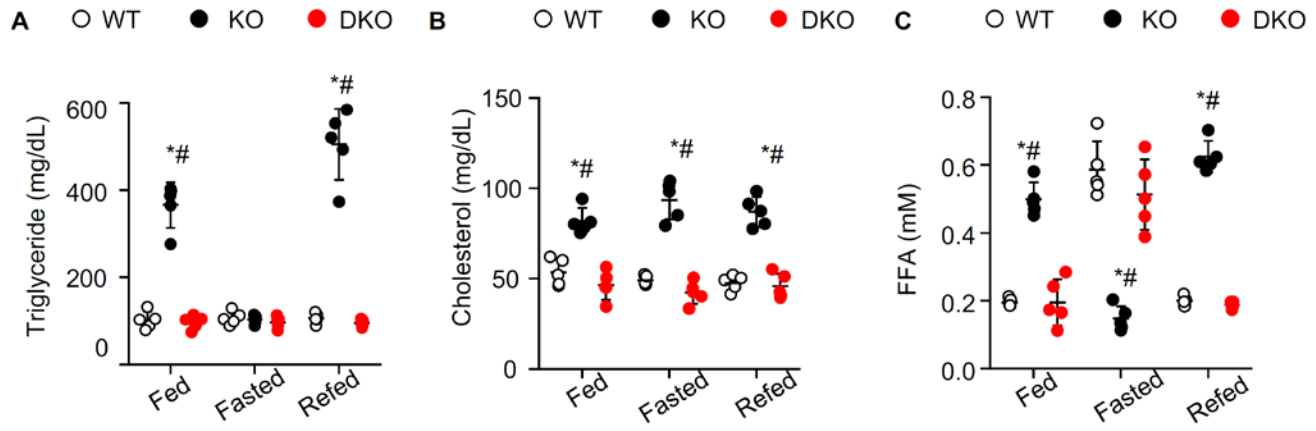
SUPPLEMENTARY DATA

Supplementary Figure 5. Adipo-MDM2 KO mice are unable to maintain energy balance under starvation and cold environment. Male Adipo-MDM2-KO mice and their WT littermates were used. (A) Daily food intake. (B-D) 12- week-old mice were subjected to measurement of (B) oxygen consumption (VO2), (C) respiratory exchange ratio (RER) and (D) locomotor activity in the metabolic cage under *ad libitum* (fed), fasted (24 hours) and refed (6 hours) conditions. (E) Core body temperature, serum levels of (F) free fatty acid (FFA) and (G) β-hydroxybutyrate in different nutritional status as in Panel B-D. (H) Core body temperature in the 3- week-old and 16-week-old mice upon 4oC cold exposure at indicated time points. *p<0.05 KO vs. WT group (Student's t-test) (n=5). All data are represented as mean ± SD.

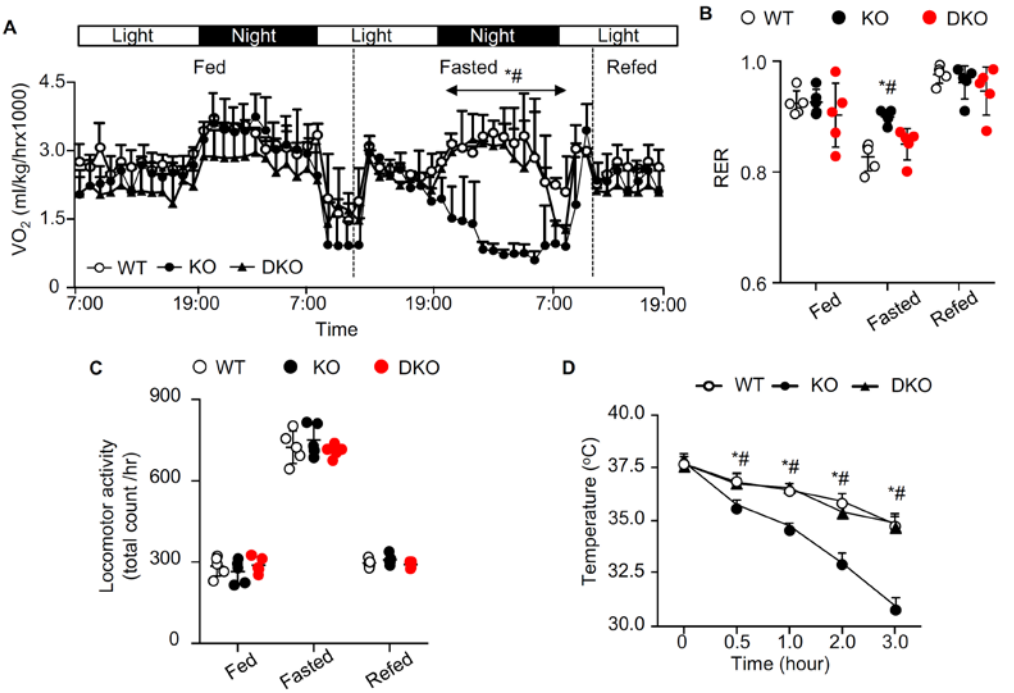


SUPPLEMENTARY DATA

Supplementary Figure 6. Genetic deletion of adipocyte p53 restores lipid homeostasis in Adipo-MDM2-KO mice. Serum levels of (A) triglyceride, (B) cholesterol and (C) FFA in 12-week-old male Adipo-MDM2-KO mice, DKO mice and their WT littermates under *ad libitum* (fed), fasted (24 hours) and refed (6 hours) conditions. *p<0.05 WT vs. KO, #p<0.05 KO vs. DKO (One-way ANOVA with Bonferroni correction for multiple comparisons) (n=5). All data are represented as mean ± SD.

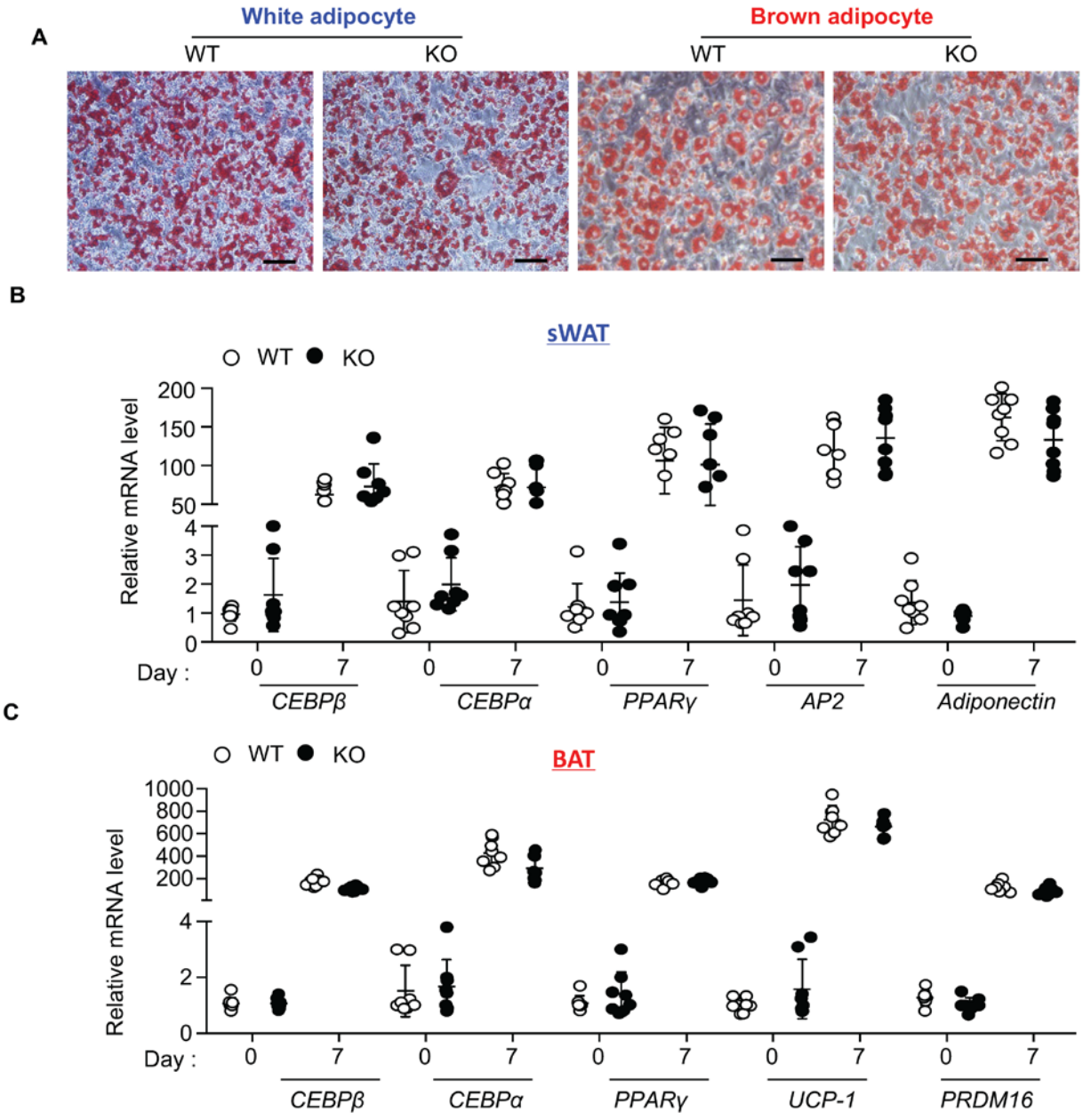


Supplementary Figure 7. Concomitant deletion of adipocyte p53 rescues the disrupted energy metabolism in Adipo-MDM2-KO mice. (A-C) 12-week-old male Adipo-MDM2-KO mice, DKO mice and their WT littermates were fed *ad libitum* (fed), fasted for 24 hours (fasted) and re-fed for 6 hours. (A) Oxygen consumption (volume of oxygen consumed [VO2] is normalized with body weight), (B) RER and (C) locomotor activity. (D) Core temperature of 15- week-old male mice upon cold exposure (4 oC) at indicated time points (n=5). *p<0.05 WT vs. KO, #p<0.05 KO vs. DKO (One-way ANOVA with Bonferroni correction for multiple comparisons) (n=5). All data are represented as mean ± SD.



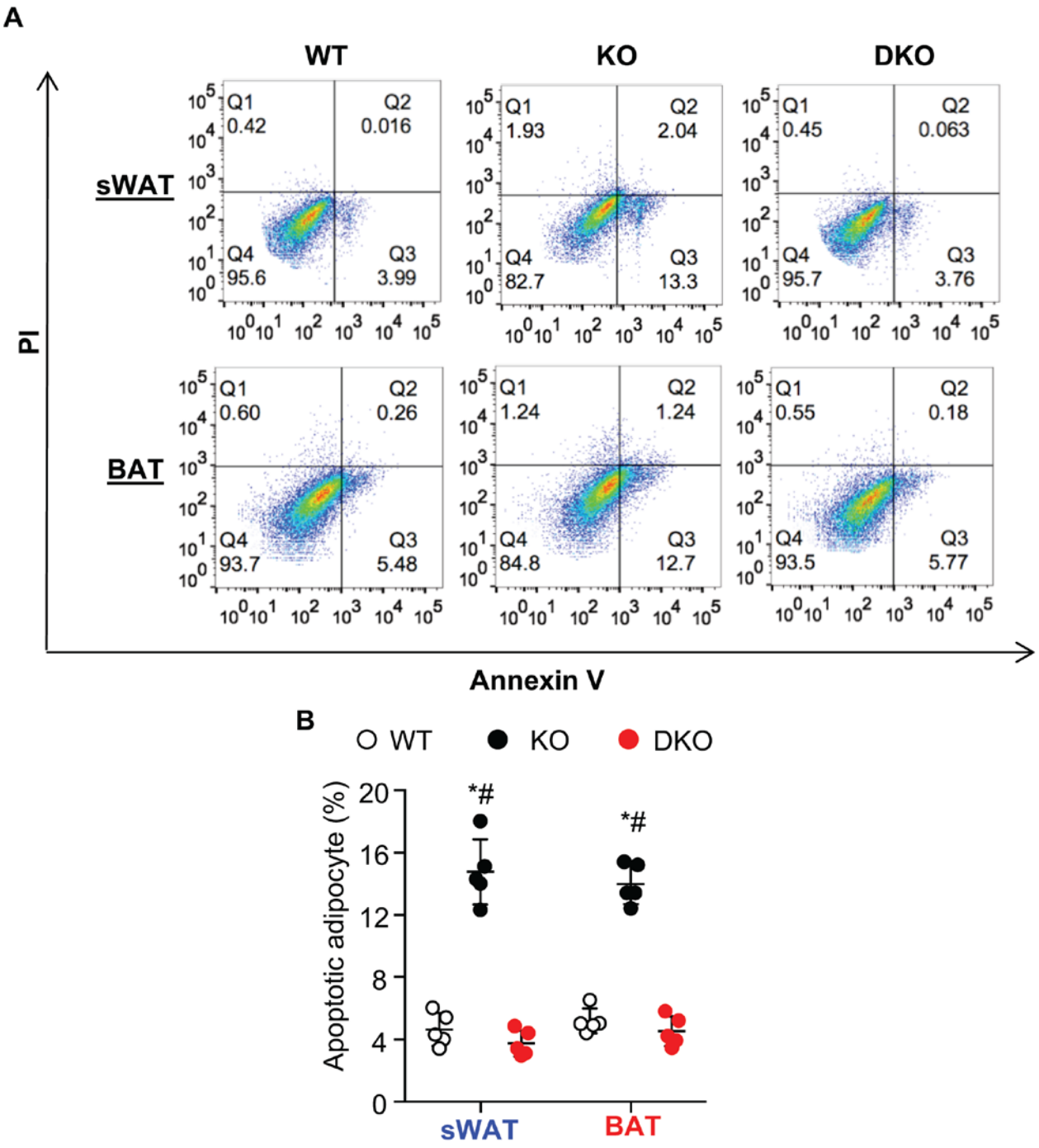
SUPPLEMENTARY DATA

Supplementary Figure 8. Effect of adiponectin-Cre mediated deletion of MDM2 on adipogenesis. SVF were isolated from sWAT and BAT of 4-week-old male Adipo-MDM2-KO mice and their WT littermates, followed by induction of adipocyte differentiation. The first day of differentiation is defined as day 1. (A) Oil-Red-O staining in the SVF at day- 7. Scale bar: 100 µm. (B-C) Relative expression of adipogenic markers in the SVF of (B) sWAT and (C) BAT after 0- or 7-day differentiation. All the target genes were normalized with *18S* and are expressed as fold change over WT controls. *p<0.05 KO vs. WT group (Student's t-test) (n=8). All data are represented as mean ± SD



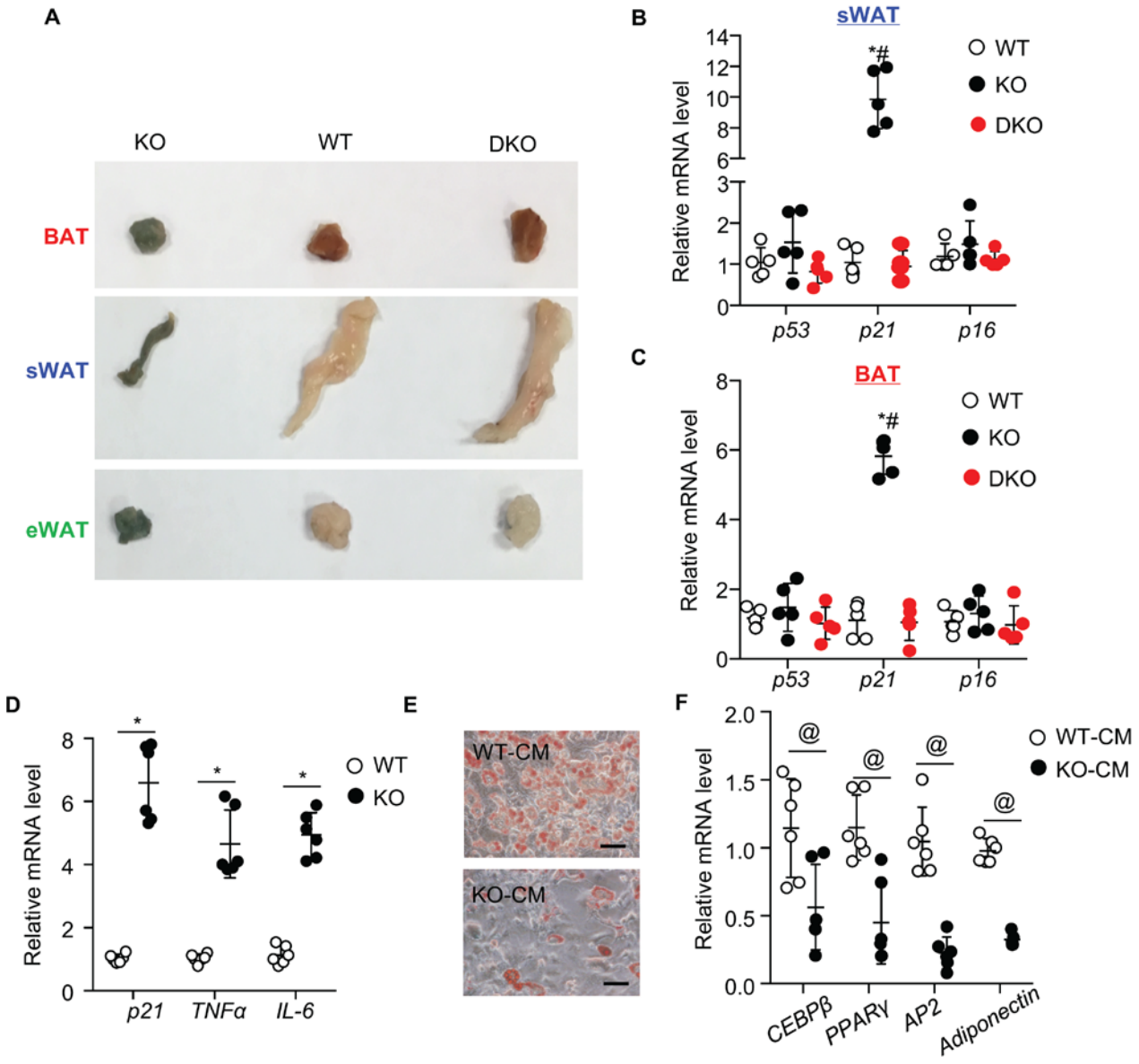
SUPPLEMENTARY DATA

Supplementary Figure 9. Additional deletion of p53 rescues the augmented adipocyte apoptosis in Adipo- MDM2-KO mice. (A) Adipocytes were isolated from sWAT and BAT of 3-week-old male Adipo-MDM2-KO mice, DKO mice and their WT littermates, followed by staining with Annexin V (FITC) and propidium iodide (PI). PI and Annexin V positive cells were identified by cytometry analysis. Representative contour plots were shown from five individual biological samples. (B) Percentage of apoptotic adipocytes (Annexin V positive [Q3] plus Annexin V and PI double positive [Q2]). *p<0.05 WT vs. KO, #p<0.05 KO vs. DKO (One-way ANOVA with Bonferroni correction for multiple comparisons) (n=5). All data are represented as mean ± SD



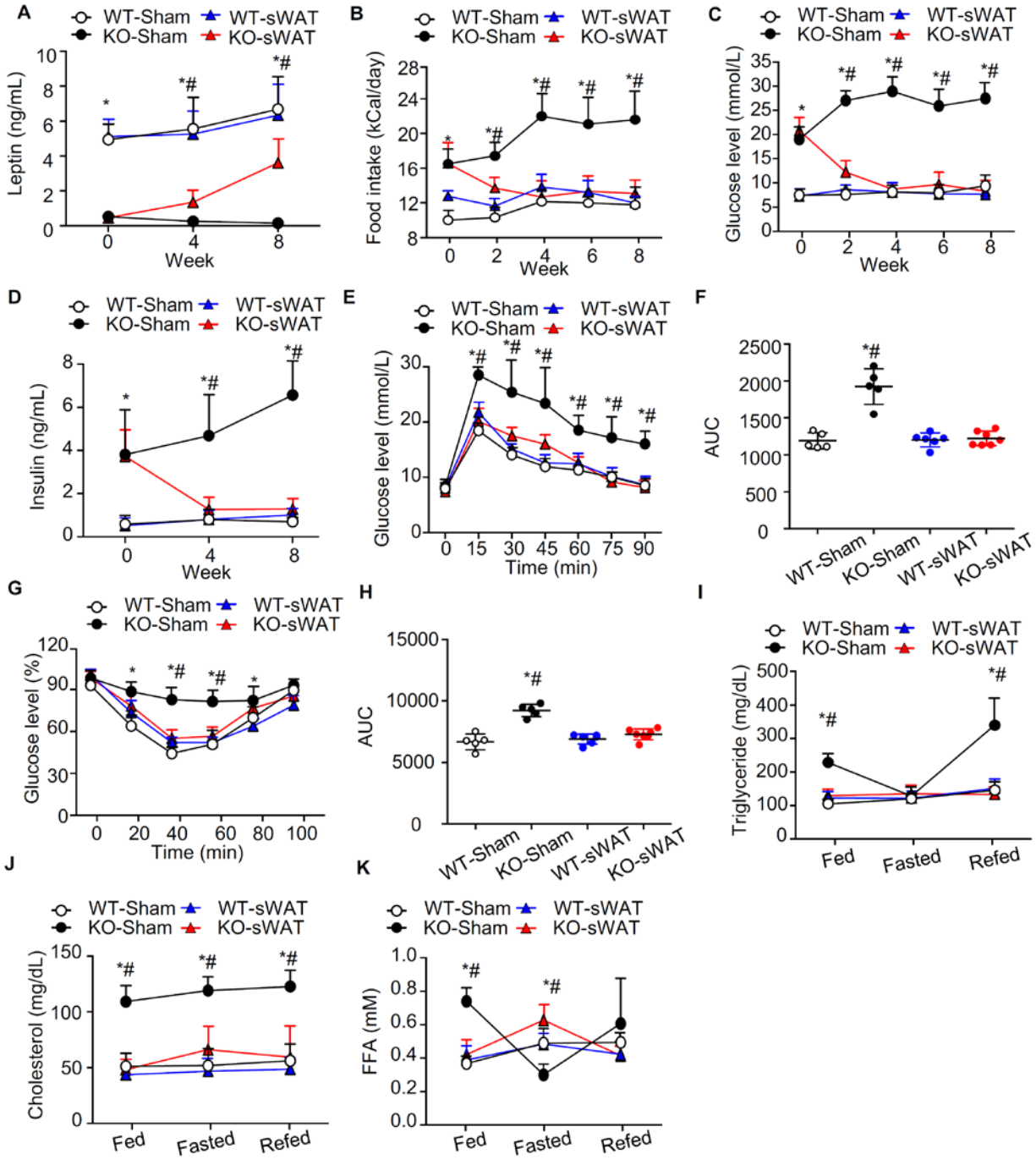
SUPPLEMENTARY DATA

Supplementary Figure 10. Dysfunctional MDM2-p53 axis triggers adipocyte senescence, leading to impairment of adipogenesis in progenitors. (A-C) 4-week-old male Adipo-MDM2-KO mice, DKO mice and their WT littermates were used (n=5). (A) Representative images of senescence associated-β-Gal staining in the fat pads. (B-C) Relative mRNA abundance of *p53, p21* and p*16* in (B) sWAT and (C) BAT of the 4-week-old mice. All target genes are normalized with *18S* and expressed as fold change over WT group. (D-F) SVF isolated from sWAT were differentiated into mature adipocytes. Conditional medium was collected and mixed with the adipocyte differentiation medium in a ratio of 50:50 (n=6). (D) QPCR analysis of *p21*, *TNF-α* and *IL-6* in mature adipocytes differentiated from SVF of KO and WT mice. (E-F) SVF isolated from sWAT of C57/BL6J mice were treated with the conditional mixture during differentiation for 7 days. (E) Oil-red-O staining and (F) mRNA abundance of adipogenic genes (*CEBPβ*, *PPARγ*, *AP2* and *adiponectin*) in SVF after differentiation for 7 days. The target genes were normalized with *18S* and expressed as fold change over WT. Scale bar: 100 μm. Representative images were shown. *p<0.05 WT vs. KO, #p<0.05 KO vs. DKO (One-way ANOVA with Bonferroni correction for multiple comparisons); @WT-CM vs KO-CM (Student's t-test). All data are represented as mean ± SD.



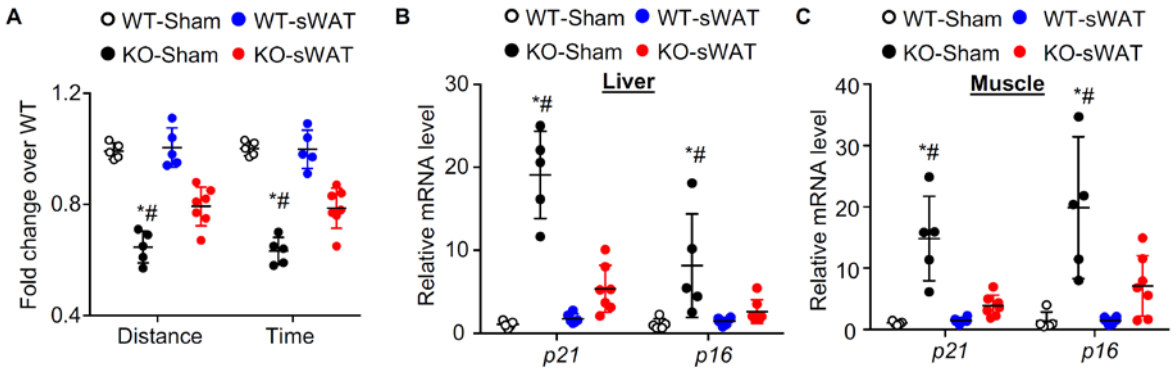
SUPPLEMENTARY DATA

Supplementary Figure 11. Fat transplantation alleviates glucose and lipid disturbances in Adipo-MDM2-KO mice. 12-week-old Adipo-MDM2-KO mice and their WT littermates were subcutaneously implanted with sWAT (WTsWATand KO-sWAT) or subjected to sham operation (WT-Sham and KO-Sham). The week of transplantation is defined as week-0. (A) Serum level of leptin. (B) Daily food intake after fat transplantation. (C-D) Serum levels of (C) glucose and (D) insulin under *ad libitum* feeding condition after transplantation. (E) GTT at week-6. (F) The value of AUC of GTT in panel E. (G) ITT at week-7. (H) The value of AUC of ITT in panel G. (I-K) Serum levels of (I) triglyceride, (J) cholesterol and (K) free-fatty acid (FFA) in the mice under *ad libitum* (fed), fasted (24 hours) and refed (6 hours) conditions at week-8. *p<0.05 WT-Sham vs. KO-Sham; #p<0.05 KO-Sham vs. KO-sWAT (Student's t-test). WT-Sham group and KO-Sham group (n=5). WT-sWAT group and KO-sWAT group (n=7). All data are represented as mean ± SD.

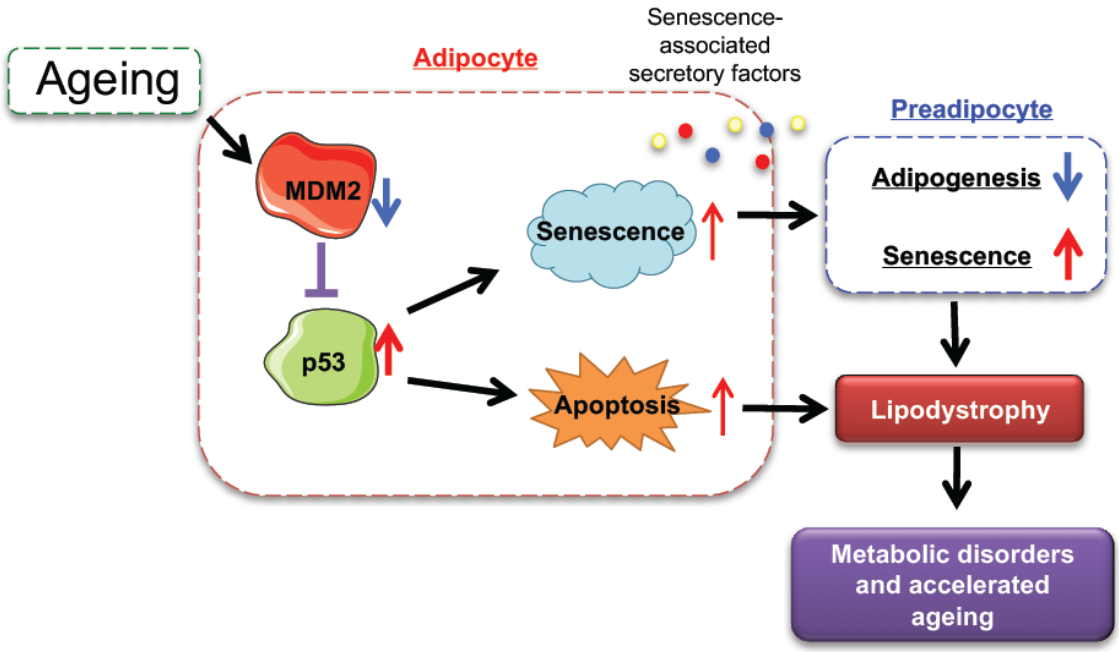


SUPPLEMENTARY DATA

Supplementary Figure 12. Fat transplantation improves exercise ability and global ageing in Adipo-MDM2-KO mice. The mice with fat transplantation and sham operation were used (Please refer the detail in Supplementary Fig. 11). (A) Exercise ability of mice at week-6. "Time" is running time to exhaustion; "Distance" is distance travelled at time of exhaustion; Data are expressed as fold change over time of WT-Sham group. (B-C) Relative mRNA levels of *p21 and p16* in (B) liver and (C) skeletal muscle of mice. All target genes are normalized with *18S* and expressed as fold change over WT-Sham group *p<0.05 KO-Sham vs WT-Sham; #p<0.05 KO-Sham vs KO-sWAT (Student's t-test). WT-Sham group and KO-Sham group (n=5). WT-sWAT group and KO-sWAT group (n=7). All data are represented as mean ± SD.



Supplementary Figure 13. The MDM2-p53 axis maintains adipose dynamics and metabolic health. Diminished expression of MDM2 results in augmented p53 activation, leading to adipocyte senescence and apoptosis. Senescentadipocytes release senescence-associated secretory factors that impede differentiation of pre-adipocyte into mature adipocytes and trigger senescence in the neighboring cells. The interaction between adipocyte apoptosis, senescenceand defective adipogenesis synergistically leads to severe lipodystrophy and subsequent multiple metabolic disorders



SUPPLEMENTARY DATA

Supplementary Table 1. Primer sequences used for this study.

Gene	Forward (5'-3')	Reverse (5'-3')
MDM2	GGGAGTGATCTGAAGGATCC	CTCATCTGTGTTCTCTTCTGTC
MDM4	GGAAAAGCCCAGGTTTGACC	GCCAAATCCAAAAATCCCACT
WWP1	CCTTGGAGTTCCGAGTTTGGA	AGTTCCCCAGTTTGCACTATTC
E4F1	CTCAAGGCCCACATGGTAA	CACACTTGGCACATTTGTAGG
PIRH2	CAGACTTGTGAAGACTGTAGCAC	CGAAGATTCGTGGTTAGGCAT
TNFα	ACGGCATGGATCTCAAAGAC	AGATAGCAAATCGGCTGACG
IL-6	CTCTGGGAAATCGTGGAAAT	CCAGTTTGGTAGCATCCATC
MCP-1	CCACTCACCTGCTGCTACTCA	TGGTGATCCTCTTGTAGCTCTCC
p16	CGGTCGTACCCCGATTCAG	GCACCGTAGTTGAGCAGAAGAG
p21	GCAGATCCACAGCGATATCC	CAACTGCTCACTGTCCACGG
PUMA	ATGGCGGACGACCTCAAC	AGTCCCATGAAGAGATTGTACATGAC
Bax	GCGTGGTTGCCCTCTTCTACTTTG	AGTCCAGTGTCCAGCCCATGATG
Bcl-2	TGAGTACCTGAACCGGCATCT	GCATCCCAGCCTCCGTTAT
p53	ACTGCATGGACGATCTGTTG	GTGACAGGGTCCTGTGCTG
AP2	ACACCGAGATTTCCTTCAAACTG	CCATCTAGGGTTATGATGCTCTTCA
Adiponectin	GGAGAGAAAGGAGATGCAGGT	CTTTCCTGCCAGGGGTTC
CEBPβ	GGGTTTCGGGACTTGATGC	ACATCAACAACCCCGCAGG
CEBPα	GGGCAAAGCCAAGAAGTCG	AGTACCCGGTACTCGTTGCT
PPARγ	TCCAGCATTTCTGCTCCACA	ACAGACTCGGCACTCAATGG
UCP1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
PRDM16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
GAPDH	CTCATGACCACAGTCCATGC	CACATTGGGGGTAGGAACAC
18S	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACTA

SUPPLEMENTARY DATA

Supplementary Table 2. Tissue weight, body weight and lean mass of Adipo-MDM2-KO mice and their WT littermates at different ages. *p<0.05 KO vs. WT group (Student's t-test) (n=9). All data are represented as the mean ± SD.

	Age			
	3-week	***6-week***	***12-week***	***24-week***
sWAT (g)				
WT	0.074±0.036	0.103±0.042	0.247±0.060	0.490±0.090
KO	0.068±0.030	0.067±0.022*	0*	0*
eWAT (g)				
WT	0.070±0.030	0.091±0.048	0.455±0.107	0.629±0.252
KO	0.066±0.024	0.063±0.027*	0*	0*
BAT (g)				
WT	0.063±0.045	0.071±0.033	0.156±0.034	0.144±0.075
KO	0.047±0.021	0.051±0.019*	0*	0*
Liver (g)				
WT	0.796±0.106	0.970±0.147	1.103±0.477	1.511±0.774
KO	0.905±0.483	1.215±0.254*	3.786±1.233*	6.844±3.435*
Body weight (g)				
WT	14.136±2.110	19.991±1.882	23.817±1.881	28.753±1.672
KO	13.106±2.140	19.481±1.989	26.856±1.904*	31.97±2.442*
Lean mass (g)				
WT	1.639±0.116	3.103±0.173	3.880±0.216	5.168±0.414
KO	1.542±0.129	2.973±0.224	4.511±0.554*	6.189±0.534*