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Supplemental Information

A Mouse Homolog of a Human *TP53* Germline Mutation Reveals a Lipolytic Activity of p53

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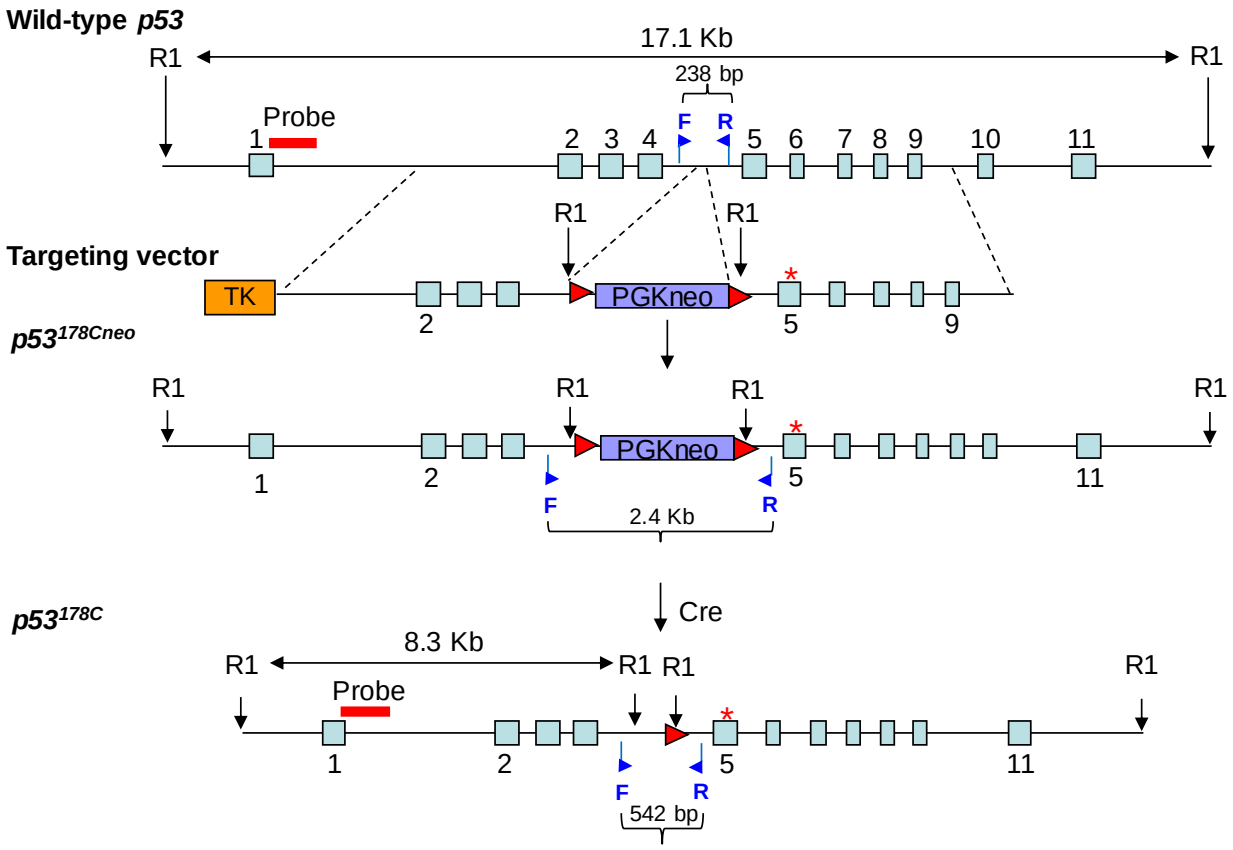

Figure S1. Strategy for generating *p53* R178C knockin mouse, Related to Figure 1. Conventional ES cell-mediated strategy was used to knockin the missense mutation into the *p53* gene ($p53^{R178C}$). Mice with germline transmission of the mutation were crossed with Cre-expressing mice to remove the neomycin (Neo) resistance gene flanked by *lox*P sites (triangles). Asterisk indicates the mutated nucleotide in exon 5 and horizontal arrowheads indicate forward and reverse primers for genotype screening. Genomic DNA was digested with *EcoR*I (R1) and analyzed with standard southern blot with the probe marked in red near Exon 1 region.

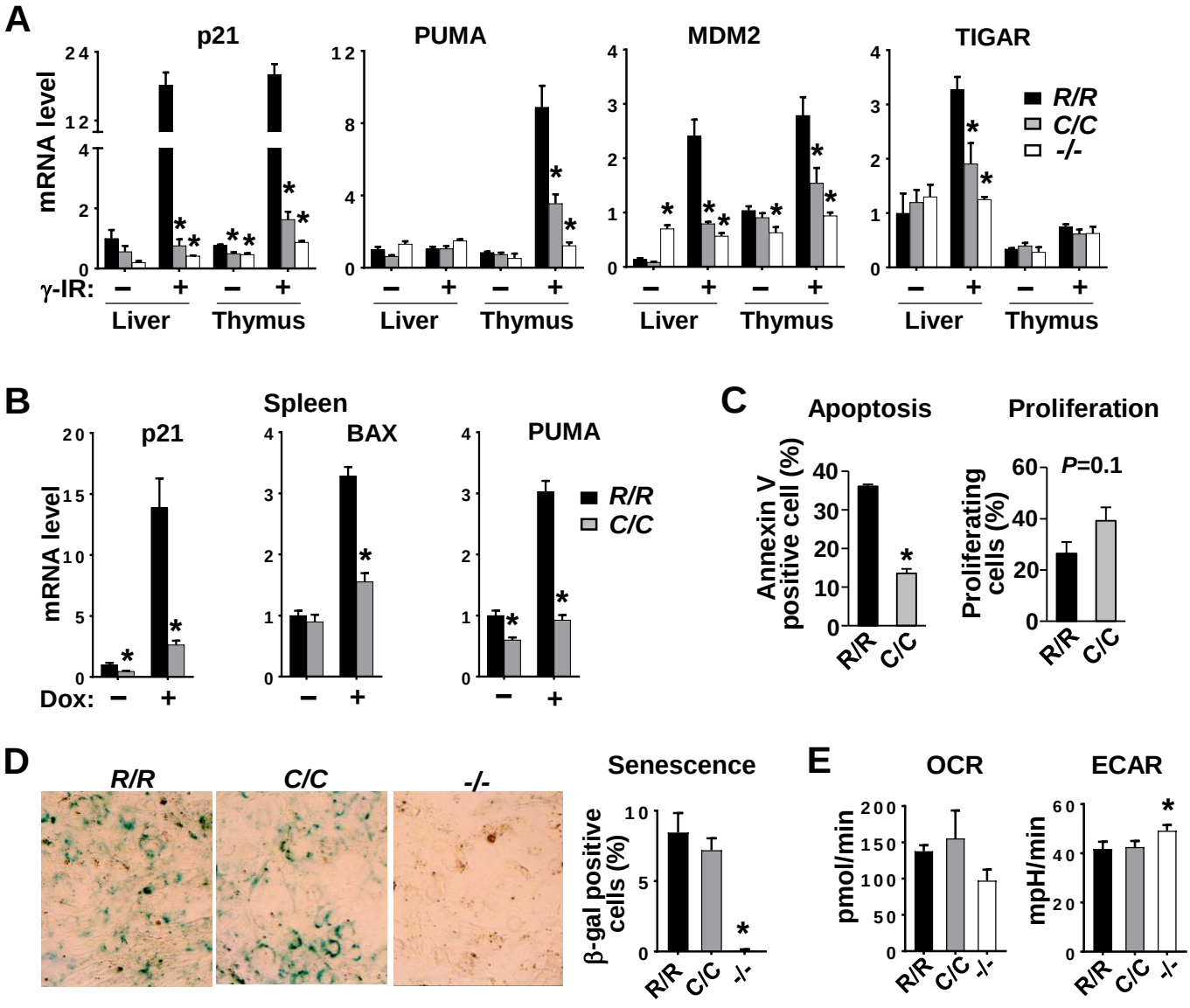

Figure S2. p53 R178C partially retains wild-type p53 activity, Related to Figure 1.
(A) mRNA levels of the indicated p53 target genes in γ-irradiated mouse tissues were quantified by RT-PCR (*R/R* and *C/C*, n = 4-5; -/-, n = 3).
(B) mRNA levels of the indicated p53 target genes in spleen of doxorubicin treated mice (n = 6).
(C) Apoptosis and proliferation of splenic T cells analyzed by flow cytometry (n = 4).
(D) Senescence in MEF cells imaged and quantified by β-galactosidase staining (n = 4).
(E) Oxygen consumption rate (OCR, mitochondrial respiration) and extracellular acidification rate (ECAR, glycolysis) in MEFs measured by Seahorse XF Analyzer (n = 6).
p53 R178 genotypes: wild-type (*R/R*); homozygous mutant (*C/C*); and null (-/-). Statistical difference by one-way ANOVA (A, D, E) or two-tailed unpaired *t* test (B, C) in comparison with wild-type (*R/R*). Values are mean ± SEM. **P* < 0.05

Figure S3

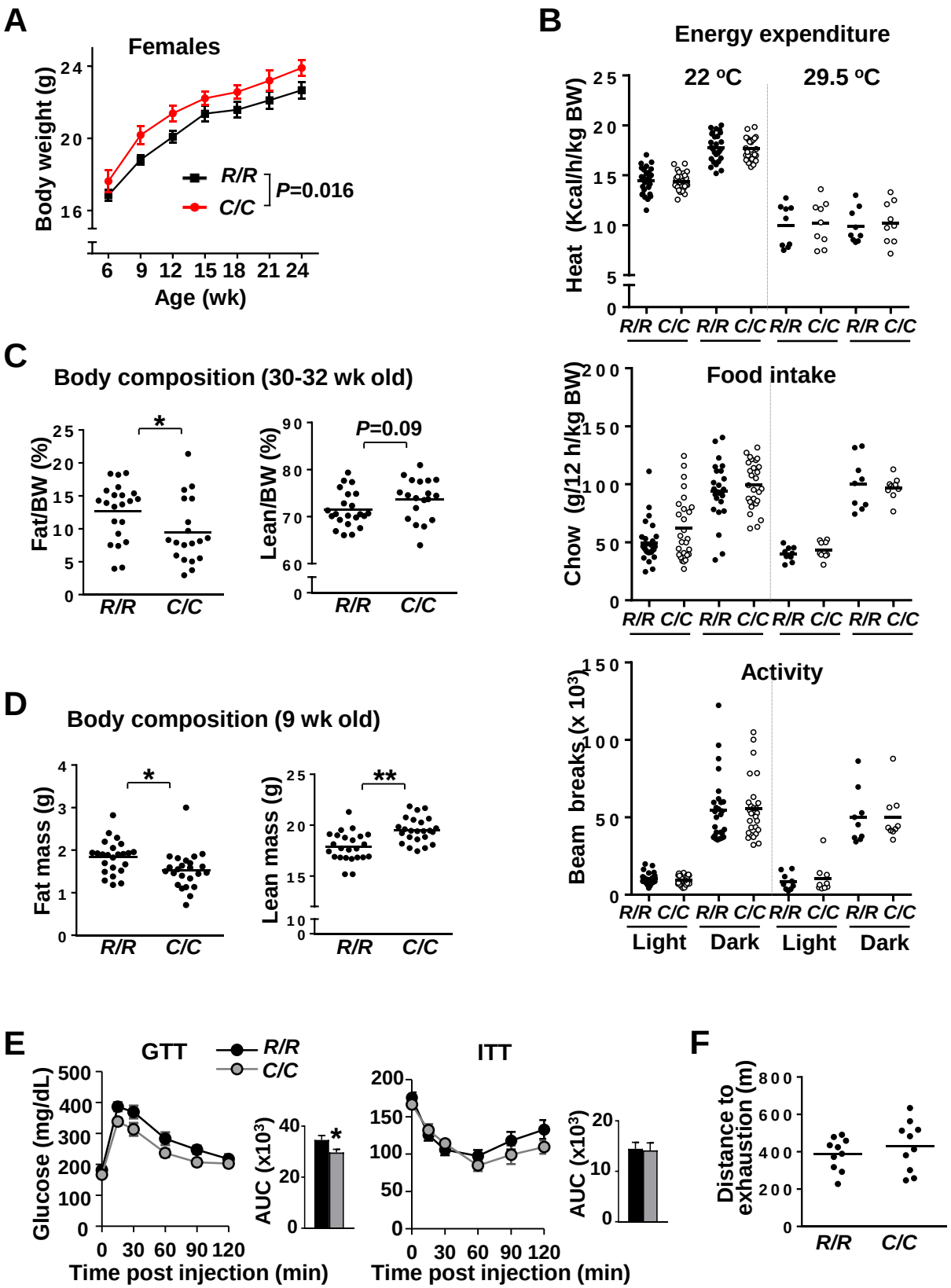
A
Females
Body weight (g)
24
20
16
R/R
C/C
P=0.016
6 9 12 15 18 21 24
Age (wk)
B
Energy expenditure
22 ºC
29.5 ºC
Heat (Kcal/h/kg BW)
25
20
15
10
5
0
R/R C/C R/R C/C R/R C/C R/R C/C
Food intake
Chow (g/12 h/kg BW)
200
150
100
50
0
R/R C/C R/R C/C R/R C/C R/R C/C
Activity
Beam breaks (x 10³)
150
100
50
0
R/R C/C R/R C/C R/R C/C R/R C/C
Light Dark Light Dark
C
Body composition (30-32 wk old)
Fat/BW (%)
25
20
15
10
5
0
*
R/R C/C
Lean/BW (%)
90
80
70
60
0
P=0.09
R/R C/C
D
Body composition (9 wk old)
Fat mass (g)
4
3
2
1
0
*
R/R C/C
Lean mass (g)
25
20
15
10
0
**
R/R C/C
E
GTT
R/R
C/C
Glucose (mg/dL)
500
400
300
200
100
0
0 30 60 90 120
Time post injection (min)
AUC (x10³)
40
20
0
*
ITT
200
150
100
50
0
0 30 60 90 120
Time post injection (min)
AUC (x10³)
20
10
0
F
Distance to exhaustion (m)
800
600
400
200
0
R/R C/C

Figure S3. Metabolic phenotype characterization, Related to Figure 1.

(A) Body weight of female mice by age (n ≥ 12).

(B) Energy expenditure, food intake and activity of 10-13 wk old age-matched male mice with *p53*$^{178C/C}$ mutant (*C/C*) compared to wild-type (*R/R*). The parameters were measured using the Open Circuit Calorimetry system during the indicated cycle of the day; light cycle, 6 am to 6 pm; dark cycle, 6 pm to 6 am (n = 27, for room temperature at 22 ^{o}C, n = 9 for thermoneutral condition at 29.5 ^{o}C).

(C) Body composition measured by NMR expressed as percent of fat or lean of body weight (BW) in 30-32 wk old mice (*R/R,* n = 22*; C/C,* n = 19).

(D) Fat and lean mass of 9 wk old male mice (n = 24).

(E) Glucose tolerance test (GTT) and insulin tolerance test (ITT) (n = 11). AUC, area under the curve.

(F) The endurance capacity measured with treadmill exercise of male mice.

p53 R178 genotypes: wild-type (*R/R*); homozygous mutant (*C/C*); and null (-/-). Statistical difference by two-way ANOVA with repeated-measures (A) and two-tailed unpaired *t* test in comparison with wild-type (*R/R*) (B-H). Values are mean ± SEM. **P* < 0.05, ***P* < 0.01

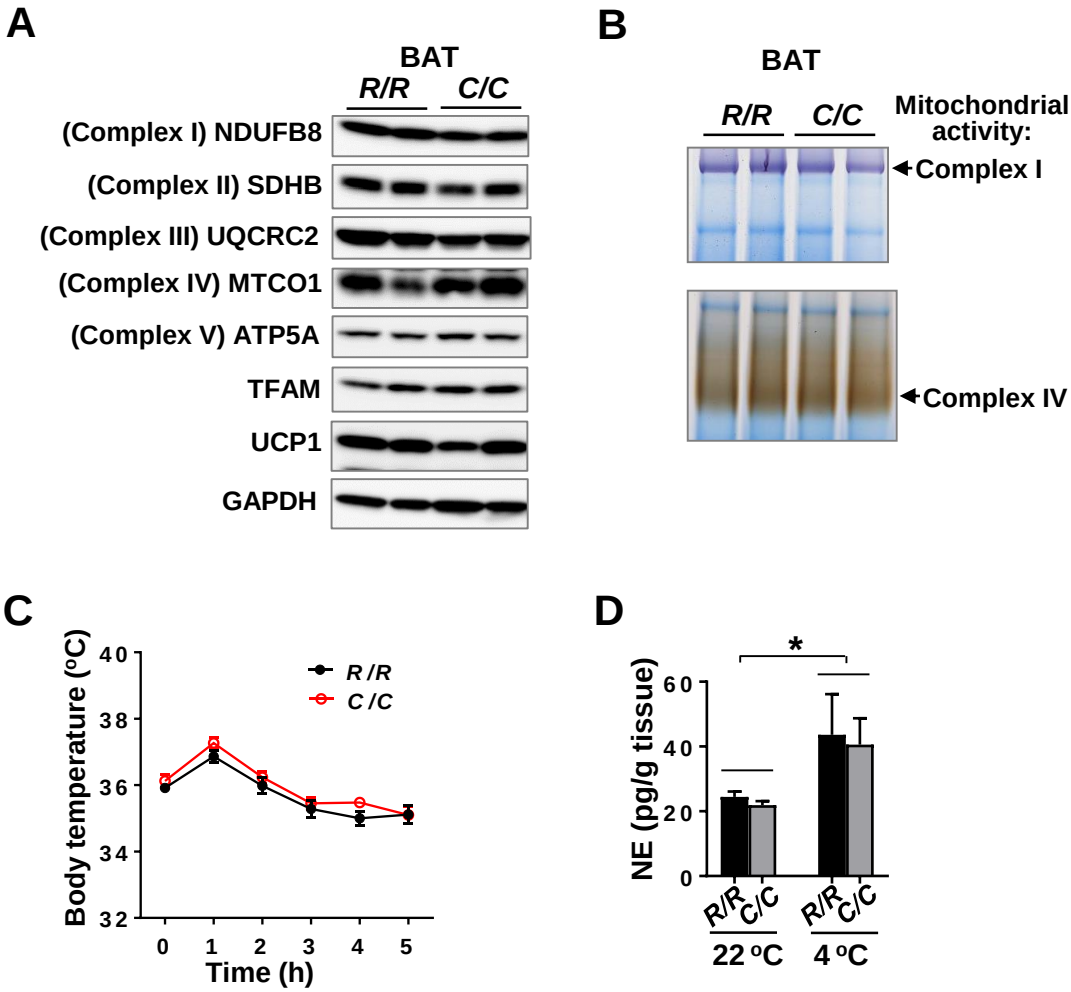

Figure S4. Brown adipose tissue (BAT) is not significantly affected by *p53* R178C mutation, Related to Figure 2.

(A) Western blot analysis of BAT tissue lysates.

(B) Mitochondrial activity staining. Mitochondria were purified from BAT tissues and their respiratory complex activities were visualized by blue-native in-gel staining.

(C) Time course of core body temperature measured over 5 h of cold exposure (4 °C) (*R/R,* n = 15*; C/C,* n = 13). .

(D) Norepinephrine (NE) level in iWAT tissue of mice at room temperature (22 °C, n = 6) or after cold (4°C) exposure for 5 h (n = 3). The significant increase in NE level after cold exposure serves as positive control in both wild-type and mutant p53 mice.

p53 R178 genotypes: wild-type (*R/R*); and homozygous mutant (*C/C*). Statistical difference by two-tailed unpaired *t* test (C) or two-way ANOVA (D). Values are mean ± SEM. **P* < 0.05

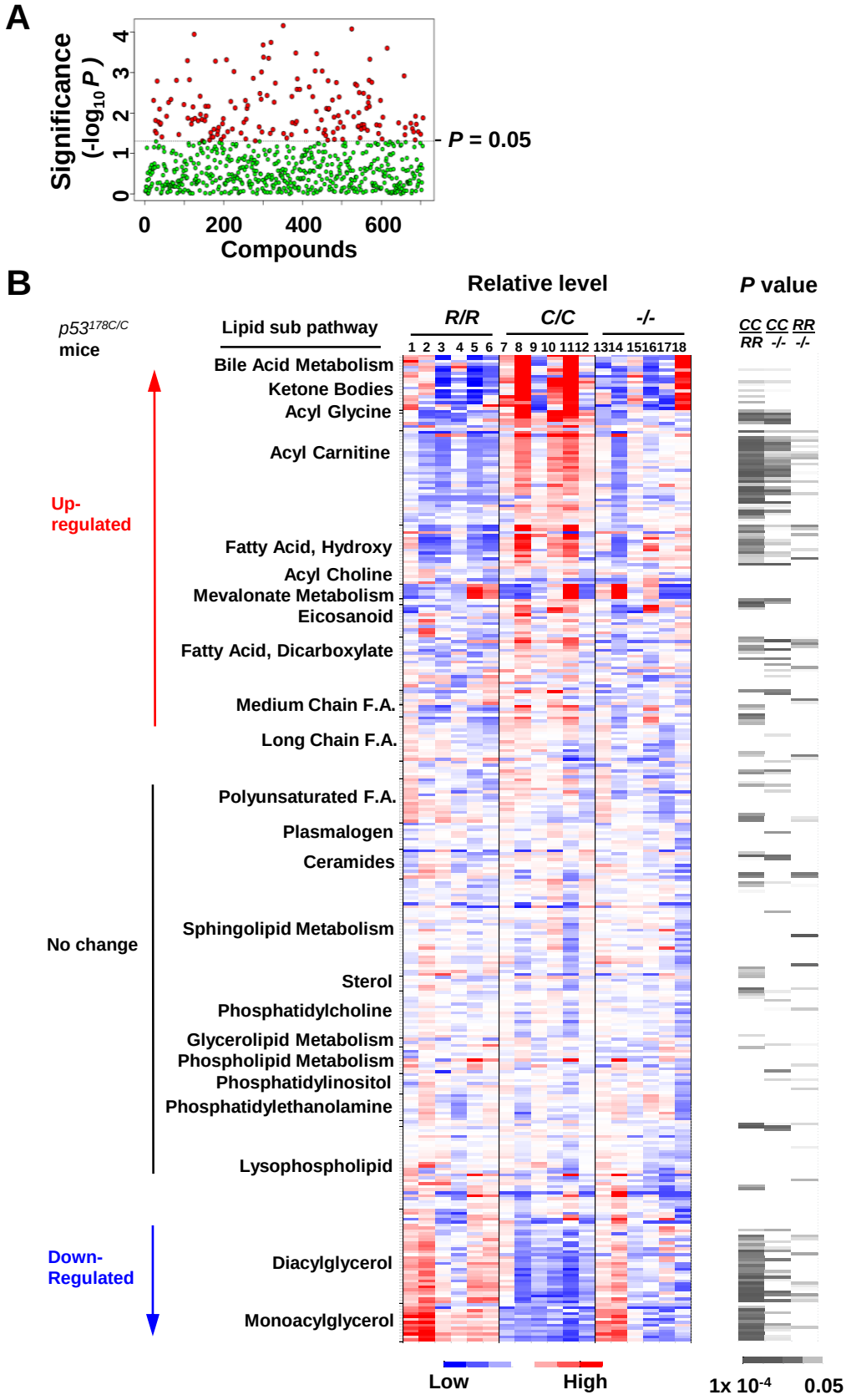

Figure S5. Plasma metabolomics, Related to Figure 3.
(A) One-way ANOVA analysis of all metabolites. Out of 706 total metabolites, 140 were significantly changed by p53 status (red, $P < 0.05$).
(B) Heat map comparison of all lipid metabolites identified in plasma from *R/R*, *C/C* and *-/-* mice (n = 6). Relative levels of the metabolites are color coded (left) and $P < 0.05$ between each group is presented as a gradient (right).

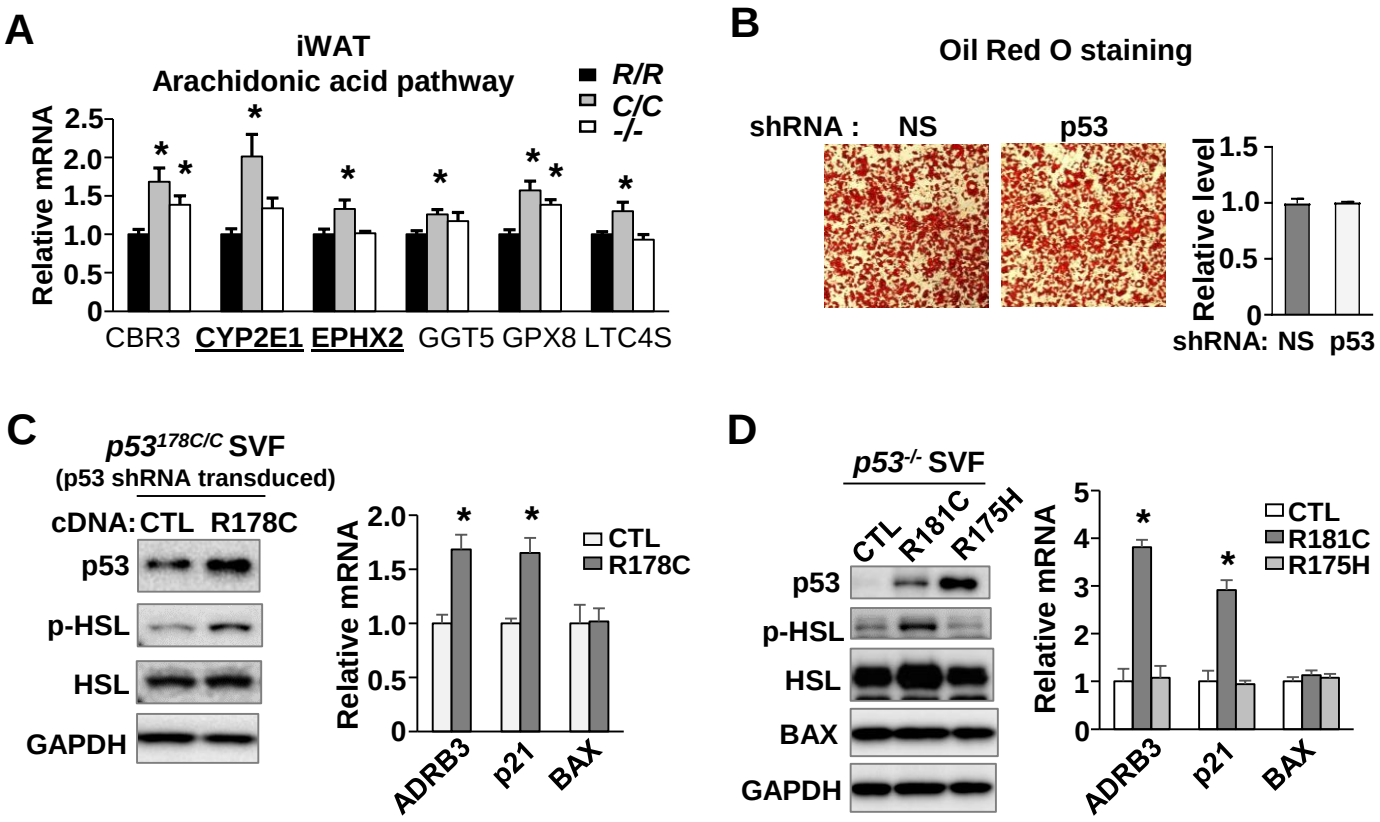

Figure S6. p53 R178C is associated with regulation of lipid metabolism, Related to Figure 4 and 6.
(A) Arachidonic acid pathway genes enriched in *C/C* iWAT. Genes related to 12,13-diHOME biosynthesis are shown in bold and underlined; CYP2E1 (Cytochrome P450, family 2, subfamily e, polypeptide 1) and EPHX2 (Epoxide hydrolase 2, cytoplasmic).
(B) SVF cells isolated from *C/C* iWAT were transduced with p53 or non-specific control (NS) shRNA lentivirus, differentiated into adipocytes for 6 d, and stained with Oil-Red O. The stain was eluted with 100% isopropanol and quantified by measuring A490 nm (n = 3).
(C) Control experiment to assess for off-target effect of shRNA. Control (CTL) or p53 R178C cDNA containing lentivirus was transduced for 2 d in $p53^{178C/C}$ SVF adipocytes stably expressing p53 shRNA (described in B) to ensure that p53 R178C re-expression can rescue HSL phosphorylation and *ADRB3* mRNA levels. Representative immunoblot and mRNA levels of the respective genes are shown (n = 4).
(D) Effect of human p53 R181C, in contrast to a loss of DNA binding mutant p53 R175H, on HSL phosphorylation and *ADRB3* expression. Control (CTL) or p53 cDNA lentivirus was transduced into $p53^{-/-}$ SVF cells and differentiated into adipocytes. Representative immunoblot and mRNA levels are shown (n = 3).
Statistical difference by one-way ANOVA or two-tailed unpaired t test in comparison with wild-type (*R/R*). Values are mean ± SEM. *P < 0.05

A

Wild-type p53	
Motif	***P* value**
TP53	9E-240
PPARG::RXRA	2E-87
HIC1	3E-85
RXRB	2E-74
PRDM4	9E-70
ZFP187	2E-69
EN1	5E-61
E2F1	1E-58
NFIC	4E-53
NFIX	1E-52

Mutant p53 R178C	
Motif	***P* value**
TP53	2E-304
RXRB	1E-107
PRDM4	1E-106
HIC1	3E-94
E2F3	4E-89
PPARG::RXRA	7E-83
NR4A2	6E-75
FOXJ3	9E-75
GATA2	3E-74
LMO2	6E-73

B

GO: Mutant p53 R178C associated genes

Term	***P* value**
Positive regulation of reactive oxygen species metabolic process	6E-04
Intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator	6E-04
Response to drug	1E-03
Oxidation-reduction process	7E-03
Response to mechanical stimulus	9E-03
Negative regulation of apoptotic process	1E-02
Response to endoplasmic reticulum stress	2E-02
Response to glucose	2E-02
Aging	2E-02
Response to toxic substance	2E-02
Apoptotic process	3E-02
Transcription, DNA-templated	4E-02

Table S1. Motif and pathway analysis of p53 binding regions, Related to Figure 5.
(A)Top 10 transcription factor binding motifs enriched in p53 binding regions from wild type (*R/R*) and *p53*$^{178C/C}$(*C/C*) ChIP-Seq. High confidence (FDR < 1E-10) peaks were subjected to motif analyses by SeqPos motif tool in Galaxy Cistrome.
(B) Gene Ontology (GO) biological process analysis of genes proximal to mutant p53 R178C binding regions.

Table S2. Primer sequences, Related to Star Methods.

	Forward (5′ → 3′)	Reverse (5′ → 3′)
RT-PCR		
ADRB3	GGTAGTGGGACTCCTCGTAATG	GGGTTGGTGACAGCTAGGTA
BAX	CCGGCGAATTGGAGATGAACT	CCAGCCCATGATGGTTCTGAT
CDKN1A (p21)	CTGTGGGTCTCTGCCAGCTGC	GAGGCCTGTCTCACCACCAAG
MDM2	GCCATTGAACCTTGTGTGATTT	CATACTGGGCAGGGCTTATT
LIPE (HSL)	GTCCTCTGCTTCTCCCTCTC	CGGAGGTCTCTGAGGAACAG
BBC3 (PUMA)	AGCAGCACTTAGAGTCGCC	CCTGGGTAAGGGGAGGAG
TIGAR	GTGGCCATCTTCCGAGAAACC	GAAGCCAGCCCACCAAACT
EIF3F (TIF)	CTGAGGATGTGCTGTCTGGGAA	CCTTTGCCTCCACTTCGGTC
Genotyping		
p53 R178C geno	CGAGGCCATCTCTGACTACACAG	GTCCAAGCACCATTGGACGC
Southern probe		
p53 Exon1	GTAGCTTCAGTTCATTGGGACC	GTGTGTACAACGCGTAGCGGAG
Cloning		
p53 R178C	CCCACCATGAGTGCTGCTCCGATGGTG ATGGCCTGGCTC	GAGCCAGGCCATCACCATCGGAGCAGC ACTCATGGTGGG
pLEX-*p53*	CCGACTCTACTAGAGGATCCTGGATGA CTGCCATGGAGG	GGCCCTCTAGACTCGAGTCAGTCTGAG TCAGGCC
pGL4.10-*Adrb3*RE	CTGAGCTCGCTAGCCTCGAGACATCCC CTGTGCTAAAACAG	AGTACCGGATTGCCAAGCTTGGAGGAA GTGCGTCACTTTG
pGL4.10-p*21*RE	GAGCTCGCTAGCCTCGAGTGTGTGAAT GTGTGTGCATGTTTG	GTACCGGATTGCCAAGCTTTGTGACCTC CTGTGCCTTTAC
ADRB3-AT(Mut1)	GTCTGTGTTTCCAAGAACTGAACAAGTC TAGACAAGTTCAACCCTCAGAAGCTGCC CTG	CAGGGCAGCTTCTGAGGGTTGAACTTG TCTAGACTTGTTCAGTTCTTGGAAACAC AGA
ADRB3-T(Mut2)	CTAAGTCTGTGTTTCCAAGAACTGGGTA ATTCCAGGTAATTCCAACCCTCAGAAGC TG	CAGCTTCTGAGGGTTGGAATTACCTGGA ATTACCCAGTTCTTGGAAACACAGACTT AG
p21-GC(Mut1)	CTAGCTTTCTGGCCTTCAGGGGCATGTC CTGGCATGTC	CCTCTTCAATTCCAGGGCTGGACATGCC AGGACATGCC
p21-T(Mut2)	GGGACTAGCTTTCTGGCCTTCAGGAATA TTTCTTGATATTTTCAGCCC	CCACCTCTTCAATTCCAGGGCTGAAAAT ATCAAGAAATATTCCTGAA
Sequencing		
p53 cDNA	CAGTTCATTGGGACCATCCT	CAGCAGAGACCTGACAACTATC
pGL4.10	CTAACTGGCCGGTACCTGAG	GGCTTTACCAACAGTACCGGATTG