**Supplemental information**

Murine double minute 2 aggravates adipose tissue dysfunction through ubiquitin-mediated six-transmembrane epithelial antigen of prostate 4 degradation

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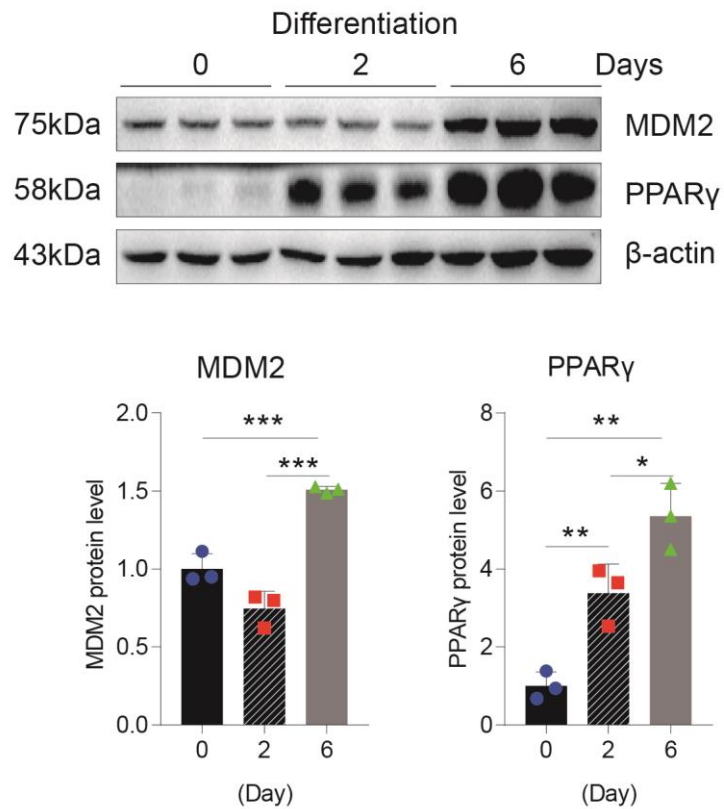
**Figure S1. The MDM2 expression is increased with the maturity of adipocyte differentiation. Related to Figure 1.**

Western blotting (up) and gray density (down) of MDM2 expression in SVF cells differentiation 0 day, 2 days and 6 days. n = 3. Data are represented as means ± SD. Statistical analysis was carried out by one-way ANOVA. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.
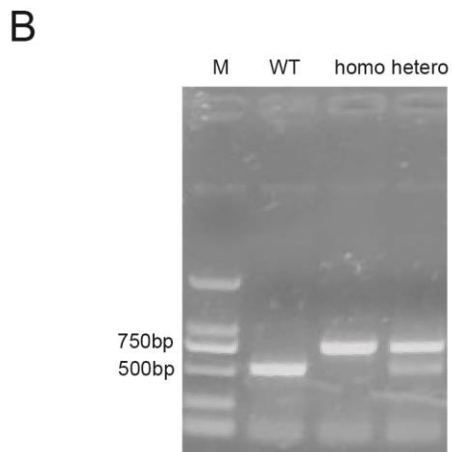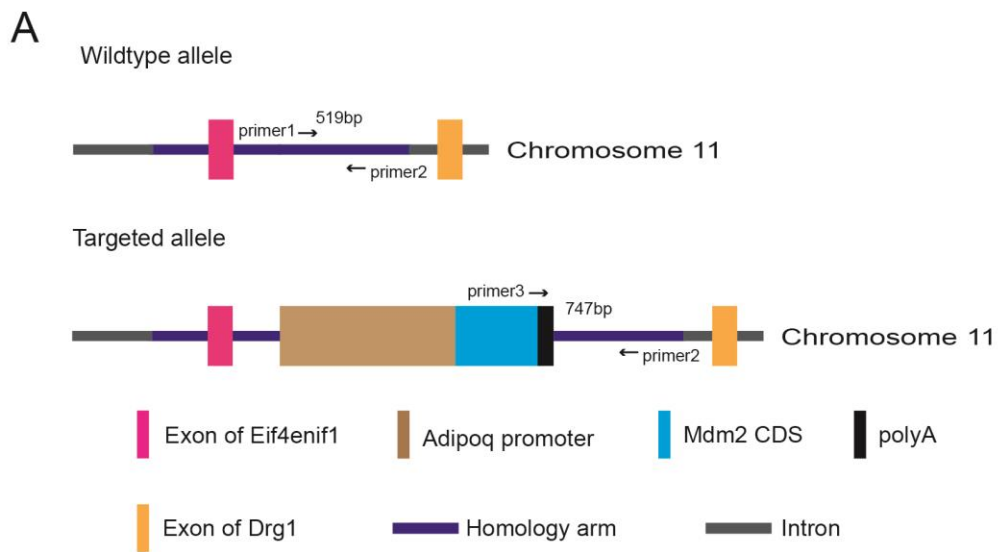
Figure S2. Creation of adipocyte-specific knock-in *Mdm2* mice. Related to Figure 2.

(A) Schematic diagram for the creation of adipocyte-specific knock-in *Mdm2* (*Mdm2*-AKI) mice.

(B) Identification of *Mdm2*-AKI mouse genotype.
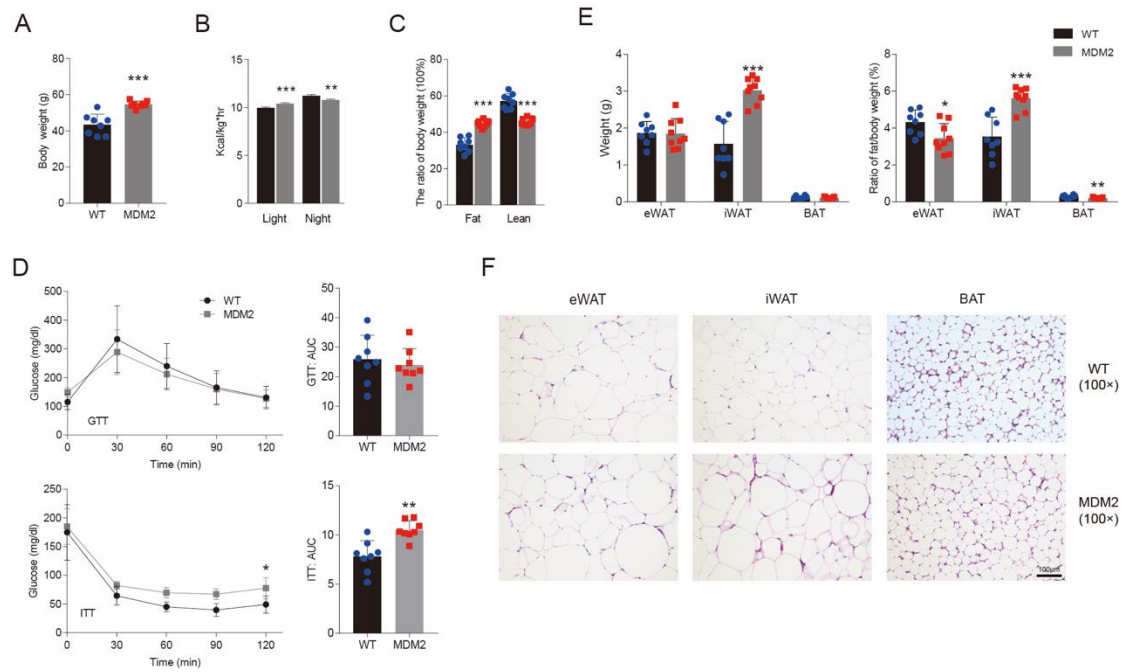
Figure S3. Adipose-specific MDM2 overexpression aggravates HFD-induced energy expenditure decrease and insulin resistance. Related to Figure 3.

(A) Body weight of WAT in WT and *Mdm2*-AKI mice on a HFD for 8 months (B). n = 8/group. Data are represented as mean ± SD. Statistical analysis was carried out by Student's t test. *** p < 0.001.
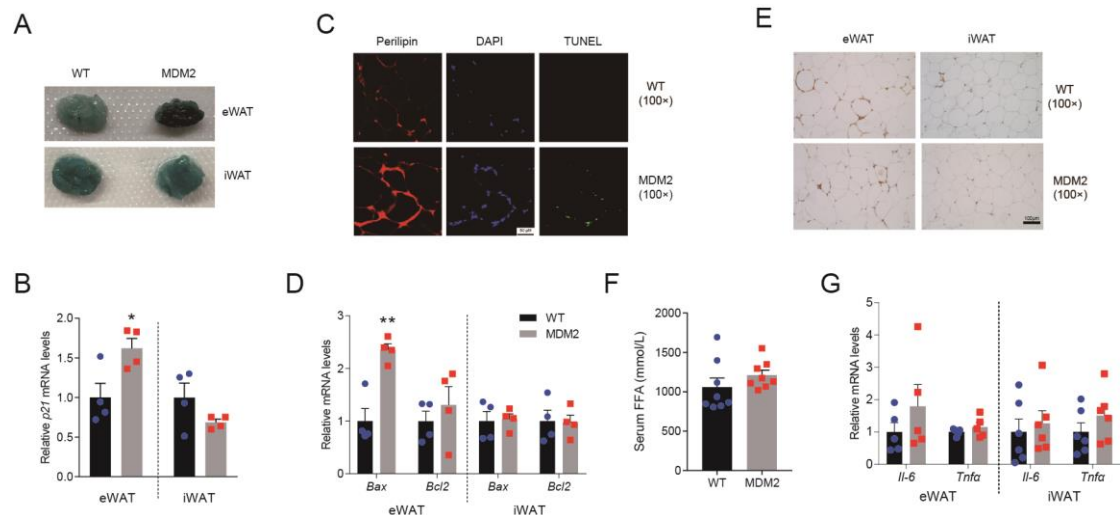
(B) Heat production of WT and *Mdm2*-AKI mice on a HFD for 8 months was measured by CLAMS. n = 8/group. Data are represented as mean ± SEM. Statistical analysis was carried out by Student's t test. ** p < 0.01 and *** p < 0.001.

(C) Fat and lean mass of WT and *Mdm2*-AKI mice on a HFD for 8 months were determined by noninvasive EchoMRI. n = 8/group. Data are represented as mean ± SD. Statistical analysis was carried out by Student's t test. *** p < 0.001.

(D) GTT (up), ITT (down) and area under the curve (AUC) of WT and *Mdm2*-AKI mice on a HFD for 8 months were analyzed. n = 8/group. Data are represented as mean ± SD. Statistical analysis was carried out by two-way ANOVA for GTT and ITT, and Student's t test for AUC. * p < 0.05 and ** p < 0.01.

(E) Weight (left) and ratio (right) of fat weight/body weight of WAT in WT and *Mdm2*-AKI mice on a HFD for 8 months. n = 8/group. Data are represented as mean ± SD. Statistical analysis was carried out by one-way ANOVA. * p < 0.05, ** p < 0.01 and *** p < 0.001.

(F) H&E staining of WAT and BAT of WT and *Mdm2*-AKI mice on a HFD for 8 months. Scare bars, 100 μm.

Figure S4. MDM2 overexpression in adipose tissues increases HFD-induced eWAT dysfunction. Related to Figure 4.

(A) Senescence β-galactosidase staining in WAT of WT and *Mdm2*-AKI mice on a HFD for 8 months.

(B) Relative mRNA levels of *p21* in WAT of WT and *Mdm2*-AKI mice on a HFD for 8 months, relative to β-actin. Data are represented as mean ± SEM. Statistical analysis was carried out by Student's t test. ** $p < 0.01$.

(C) Representative immunofluorescence staining of TUNEL (green) in eWAT of WT and *Mdm2*-AKI mice on a HFD for 8 months. Scare bars, 50 μm.

(D) Relative mRNA levels of *Bax* and *Bcl2* in WAT of WT and *Mdm2*-AKI mice on a HFD for 8 months, relative to β-actin. Data are represented as mean ± SEM. Statistical analysis was carried out by Student's t test. ** $p < 0.01$.

(E) F4/80 antigen positivity in WAT of WT and *Mdm2*-AKI mice on a HFD for 8 months. Scare bars, 100 μm.

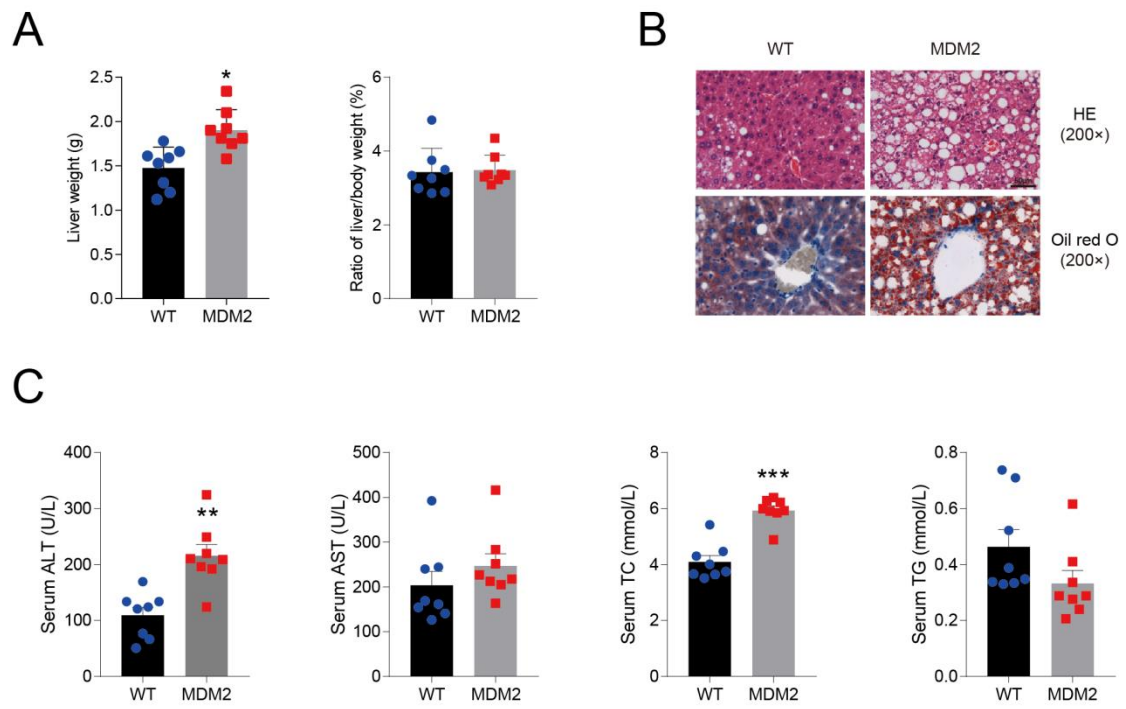(F) Biochemical analysis of serum FFA in WT and *Mdm2*-AKI mice on a HFD for 8 months. Data are represented as mean ± SD. Statistical analysis was carried out by Student's t test.

(G) Relative mRNA levels of *Tnfα* and *Il-6* in WAT of WT and *Mdm2*-AKI mice on a HFD for 8 months, relative to β-actin. Data are represented as mean ± SEM. Statistical analysis was carried out by Student's t test.
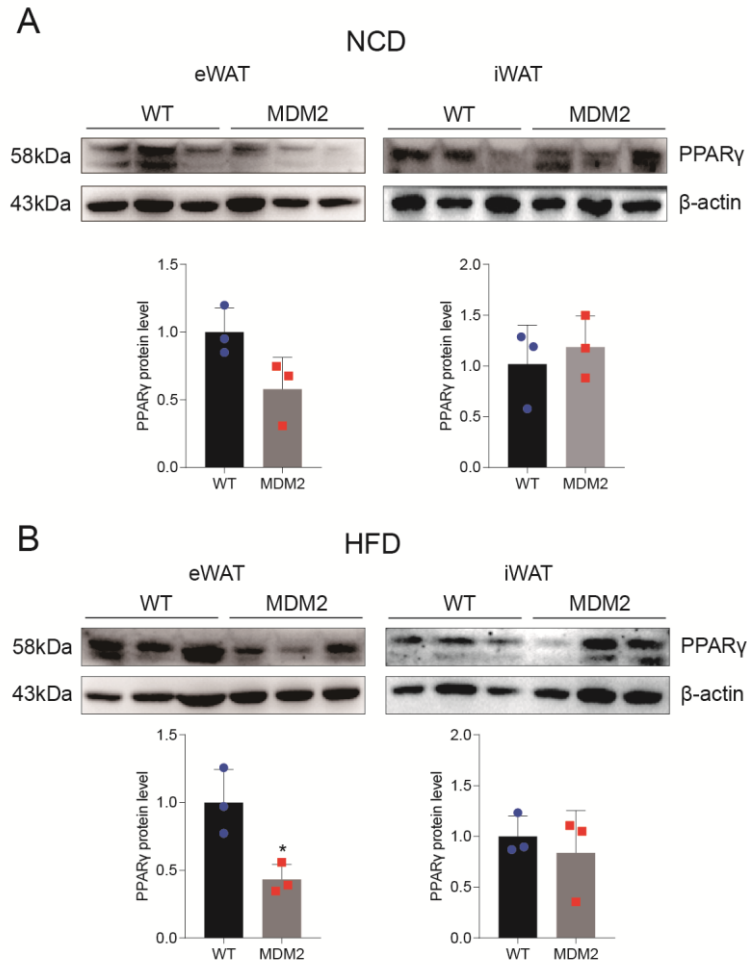
Figure S5. Western blotting (up) and protein gray value (down) of PPARγ in WAT of WT and *Mdm2*-AKI mice on a NCD for 8 weeks (A) and on a HFD for 12 weeks(B). Related to Figure 4.

n = 3/group. Data are represented as mean ± SD. Statistical analysis was carried out by Student's t test. * $p < 0.05$.

Figure S6. MDM2 overexpression in adipose tissues aggravates HFD-induced hepatic steatosis. Related to Figure 5.

(A) Liver weight (left) and ratio of liver weight/body weight (right) in WT and *Mdm2*-AKI mice on HFD for 8 months. n = 8/group. Data are represented as mean ± SD. Statistical analysis was carried out by Student's t test. * p < 0.05.

(B) H&E (up) and oil red O staining (down) of livers of WT and *Mdm2*-AKI mice on a HFD for 8 months. Scare bars, 50 μm.

(C) Biochemical analysis of serum in WT and *Mdm2*-AKI mice on a HFD for 8 months. n = 8/group. Data are represented as mean ± SD. Statistical analysis was carried out by Student's t test. ** p < 0.01 and *** p < 0.001.
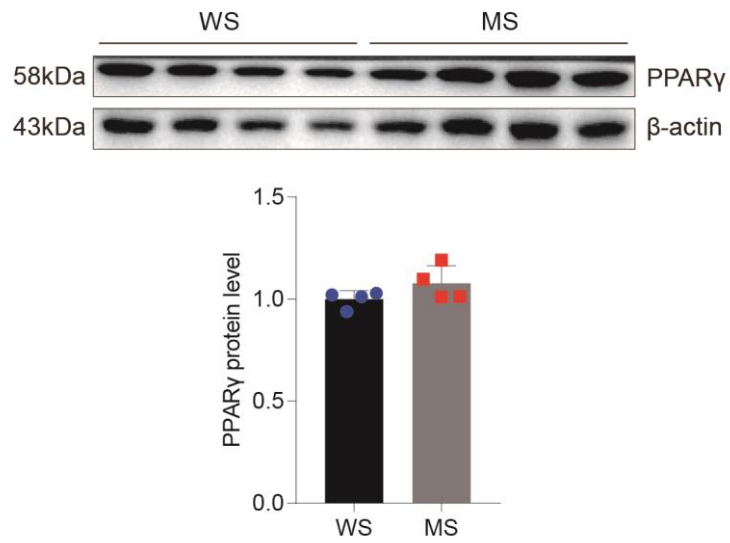
Figure S7. Western blotting (up) and protein gray value (down) of PPARγ in eWAT of WS and MS mice on a HFD for 12 weeks. Related to Figure 8.

n = 4/group. Data are represented as mean ± SD. Statistical analysis was carried out by Student's t test.
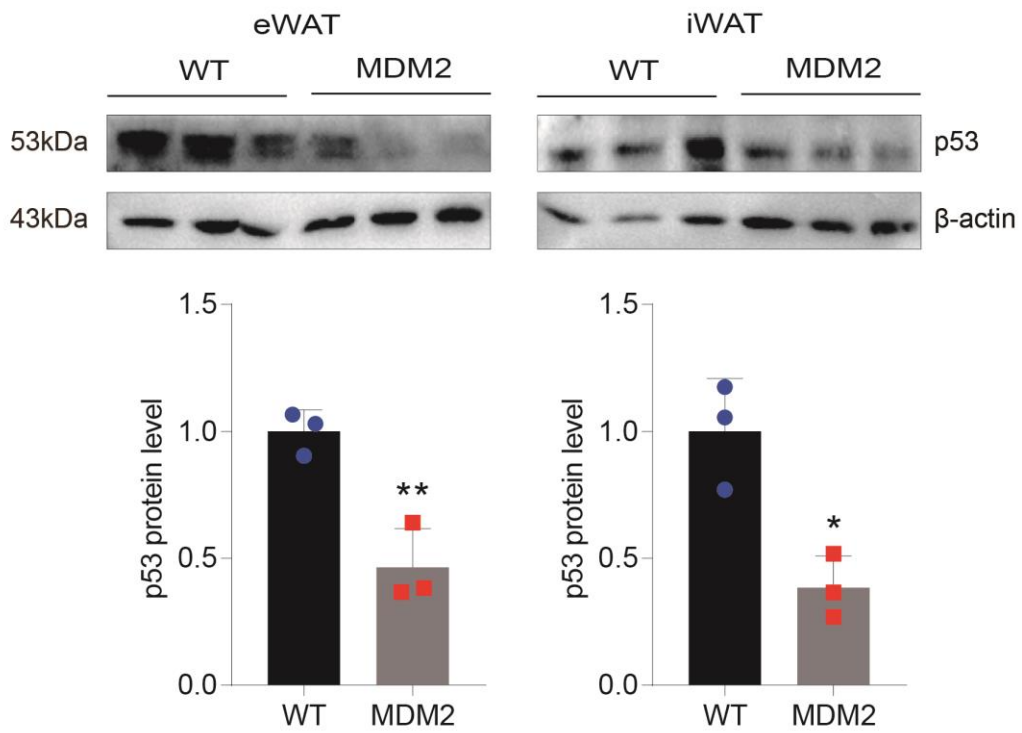
Figure S8. Western blotting (up) and protein gray value (down) of p53 in eWAT (left) and iWAT (right) of WT and *Mdm2*-AKI mice on a HFD for 12 weeks. Related to Figure 7.

n = 3/group. Data are represented as mean ± SD. Statistical analysis was carried out by Student's t test. * p < 0.05 and ** p < 0.01.

Supplementary Tables

Table S1 Combining analysis proteomics and ubiquitinomics. Related to Figure 6.

Protein accession	Gene name	Downregulation Ratio	Ubiquitin Modified Upregulation Position
Q9D2R0	*Aacs*	0.242	24
Q61285	*Abcd2*	0.619	615
Q9WTQ5	*Akap12*	0.486	164
O70423	*Aoc3*	0.604	596
O54754	*Aox1*	0.641	1103
Q63918	*Cavin2*	0.582	156
Q9DB34	*Chmp2a*	0.639	9
P56198	*Cidec*	0.577	6, 112, 115, 180, 236
P56395	*Cyb5a*	0.57	39
P56387	*Dynlt3*	0.572	63
Q3TGW2	*Eepd1*	0.631	156
P54310	*Lipe*	0.664	323
P26645	*Marcks*	0.472	11, 30, 40
Q61753	*Phgdh*	0.465	21, 384
Q8BJ56	*Pnpla2*	0.594	92
P31324	*Prkar2b*	0.626	174, 202, 285, 370
Q99K85	*Psat1*	0.633	323
Q9ET01	*Pygl*	0.665	618
P07758	*Serpina1a*	0.443	186
P22599	*Serpina1b*	0.517	292
P82347	*Sgcd*	0.554	23
P51912	*Slc1a5*	0.53	7, 384, 502, 534
Q9Z0F7	*Sncg*	0.463	23, 110, 119
Q923B6	*Steap4*	0.658	18, 97, 161

Table S2 Primers of plasmids construction for Co-Immunoprecipitation and In vitro ubiquitination assay. Related to Plasmid construction in STAR Methods.

Plasmids	Forward primer(5'---3')	Reverse primer(5'---3')
pEGFP-C1 -STEAP4	ATTCTGCAGTCGACGGTACC GAGAAAGCACATGCA	TAGATCCGGTGGATCCTTAA ATATCCGATT
pCMV3-myc -mSTEAP4	GCCGCCACCAAGCTTGGTA	GAATTCGGCGGCCGCTCTAG
pCMV3-myc -mSTEAP4(K18R)	CTGATTCCTCAGAAAGGCAA GGGGTTGTC	GACAACCCCTTGCCTTTCTG AGGAATCAG
pCMV3-myc -mSTEAP4(K97R)	CTAGTTGATTATCTCAGAGG AAAAGTATTG	CAATACTTTTCCTCTGAGATA ATCAACTAG
pCMV3-myc -mSTEAP4(K161R)	GTGGAAATGACAGCAGGG CCAAACAAAGAG	CTCTTTGTTTGGCCCTGCTG TCATTTCCAC

Table S3 The qPCR primers. Related to Quantitative RT-PCR in STAR Methods.

Gene name	Forward primer (5'---3')	Reverse primer(5'---3')
actin	CCAGCCTTCCTTCTTGGGTAT	TGCTGGAAGGTGGACAGTGAG
Mdm2	CATGTCTGTGTCTACCGAGGG	TAAGTGTCGTTTTGCGCTCC
p21	TAAGGACGTCCCACTTTGCC	GACAACGGCACACTTTGCTC
Bax	GCGTGGTTGCCCTCTTCTACTTTG	AGTCCAGTGTCCAGCCCATGATG
Bcl2	TGAGTACCTGAACCGGCATCT	GCATCCCAGCCTCCGTTAT
Tnfα	CGTCAGCCGATTTGCTATCT	CGGACTCCGCAAAGTCTAAG
Il-6	AGTTGCCTTCTTGGGACTGA	TCCACGATTTCCCAGAGAAC