Supporting Information

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SI Experimental Procedures

Cell Culture, Transfection, and Reagents. Mouse embryonic fibroblasts (MEFs) were isolated from embryos at embryonic day (E) 13.5 and were cultured in DMEM supplemented with 10% FBS, penicillin (100 IU/mL), and streptomycin (100 μg/mL) in a humidified 37 °C incubator in the presence of 5% CO_2 and 3% O_2. For all experiments, the MEF cells were used within the first five passages after isolation. To mimic starvation, MEF cells were seeded at a density of 2×10^4 cells per square centimeter. Twelve hours after plating, cells were washed twice with PBS solution, then cultured in DMEM with 0.1% FBS or in glucose-free DMEM containing 10% dialyzed FBS for the amount of time indicated. L-carnitine, palmitoyl CoA, malonyl CoA, coenzyme A, 4-hydroxytamoxifen, rotenone, carbonyl cyanide 4-(trifluoromethoxy)-phenylhydrazone, oligomycin, and antimycin A were purchased from Sigma.

Running Wheel Experiments. Mice were placed individually in a standard cage equipped with a running wheel (circumference, 1.12 m; model 80850; Lafayette Instrument). Running distance (in cumulative meters), average speed (in meters per minute), and maximum speed (in meters per minute) were recorded during 1-min intervals within a 24-h period.

Metabolomic Analysis. Mice were anesthetized with isoflurane, livers were removed and were immediately flash-frozen in liquid nitrogen, after which the livers were stored at −86 °C until processed for analysis. The livers were pulverized in liquid nitrogen, resuspended in 60% acetonitrile, vortexed, and incubated on ice for 15 min. Then, the samples were centrifuged at 4,500 × *g* for 5 min, after which the supernatants were collected and lyophilized. The powder was resuspended in D_2O containing 0.1 mM trimethylsilyl-2,2,3,3-tetradeuteropropionic acid and 1 mM formate as a reference for subsequent high-resolution NMR analysis. One-dimensional ^{1}H NMR spectra were acquired by using a 14.1-T INOVA apparatus (600 MHz ^{1}H frequency; Varian) equipped with a CapNMR Probe (Protasis/MRM). All NMR spectra were processed using ACD/1D NMR Manager software (version 12.0; Advanced Chemistry Development). Imported FIDs were zero-filled to 32,000 points, and an exponential line broadening of 0.5 Hz was applied before Fourier transformation. Phase and baseline correction were conducted for the spectra, which were referenced to the trimethylsilyl-2,2,3,3-tetradeuteropropionic acid peak at 0.00 ppm. Intelligent binning (into 0.04-ppm segments) was used to reduce the spectral data into a matrix of peak area vs. chemical shift and was exported as an Excel file (Microsoft), which was then imported into the multivariate statistical program SIMCA-P version 11.0 (Umetrics), autoscaled, and analyzed by unsupervised principle component analysis to assess differences in the spectra between WT and mutant mice bearing a single amino acid substitution at cysteine residue 305 of Mdm2 (*MDM2*^{C305F}). To create orthogonal partial least squares (OPLS) loading coefficient plots, ACD was used as described except the spectra were binned into segments of 0.01 ppm in size, and Excel files were created for four spectral groups for the serum and liver extracts. Only two spectral groups were compared in the OPLS loading coefficient plots using the SIMCA-P supervised OPLS feature. Loading coefficients and p-scaled correlation coefficients were exported to MatLab and were plotted as the Y-value (loading coefficient) or were color-coded (correlation coefficient) accordingly.

MEF Cell Differentiation and Neutral Lipid Staining. Cells were plated at 2.5×10^4 cells per square centimeter in 96-well plates in DMEM media containing 10% FBS (Gibco), 100 U/mL penicillin (Gibco), and 100 U/mL streptomycin (Gibco). Two days postconfluence, media was changed to adipocyte induction medium containing the following reagents: 1 μM rosiglitazone, 20 nM dexamethasone, 200 μM isobutylmethylxanthine, and 10 μg/mL human insulin. After 6 d of incubation, the media was changed to regular growth medium containing 10 μg/mL human insulin, and the cells were incubated for another 48 h. Neutral lipids were stained with AdipoRed (Lonza). Briefly, MEF cells were fixed in 10% formalin for 20 min at room temperature followed by one wash with PBS solution. Then, the cells were incubated in fresh PBS solution containing DAPI and AdipoRed (25 μL/mL) for 30 min at room temperature, after which the cells were washed with PBS solution. Neutral lipid staining was visualized by fluorescence microscopy or was quantified by scanning on a plate reader (AdipoRed, excitation, 485 nm; emission, 572 nm; DAPI, excitation, 350 nm; emission, 470 nm). The intensity of neutral lipid staining was normalized to DAPI signal.

Quantitative Real-Time PCR. RNA was extracted by using the RNeasy Mini kit (Qiagen), and cDNA was synthesized by using SuperScript III reverse transcriptase (Invitrogen). Quantitative RT-PCR was performed by using SYBR Green probes with an Applied Biosystems 7900HT Fast Real-Time PCR system. Thermocycling conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The primers used for the PCR reactions were as follows: *mcd* forward primer, 5′- GGAATTTCCTCAGCTGGGGGCC-3′; *mcd* reverse primer, 5′-CCAGGTAGTAGCGGTAGTT-GACC -3′; *g6pase* forward primer, 5′-GAAAGCTAAGAGATG-GTGTGAGCGG-3′; and *g6pase* revere primer, 5′-ATGCAGG-CGAAGCGGAATGG-3′. All experiments were performed in triplicate. Data are expressed as the ΔCt values [ΔCt = Ct of the target gene − Ct of GAPDH]. To calculate the fold change compared with untreated MEFs or liver tissue from fed mice, the $2^{-\Delta\Delta Ct}$ equation was used. All statistical analyses were performed based on ΔCt values.

Protein Analysis. Procedures and conditions for immunoprecipitation and immunoblotting have been previously described (1). Briefly, cells were lysed in 0.1% Nonidet P-40 lysis buffer for the immunoprecipitation experiments, and cells were lysed in 0.5% Nonidet P-40 lysis buffer for straight Western blot experiments. Mouse tissue was homogenized and lysed in tissue lysis buffer (50 mM Tris·HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 0.2% Triton X-100, 0.3% Nonidet P-40, and protease inhibitor mixture). Mouse monoclonal anti-Mdm2 (2A10; Calbiochem), anti-actin (MAB1501; Chemicon), rabbit polyclonal anti–phospho-p53 (S15; equivalent to mouse phospho-p53 S18; Cell Signaling), rabbit polyclonal anti-Sco2 (H-45; Santa Cruz), and rabbit polyclonal anti- malonyl-CoA decarboxylase (MCD; ProteinTech) antibodies were purchased commercially. Mouse monoclonal anti-p53 (pAb122) was purified in-house. Rabbit polyclonal antibody recognizing L11 was purified as previously described (2).

1. Itahana K, et al. (2003) Tumor suppressor ARF degrades B23, a nucleolar protein involved in ribosome biogenesis and cell proliferation. *Mol Cell* 12(5):1151–1164.

2. Lindström MS, Jin A, Deisenroth C, White Wolf G, Zhang Y (2007) Cancer-associated mutations in the MDM2 zinc finger domain disrupt ribosomal protein interaction and attenuate MDM2-induced p53 degradation. *Mol Cell Biol* 27(3):1056–1068.

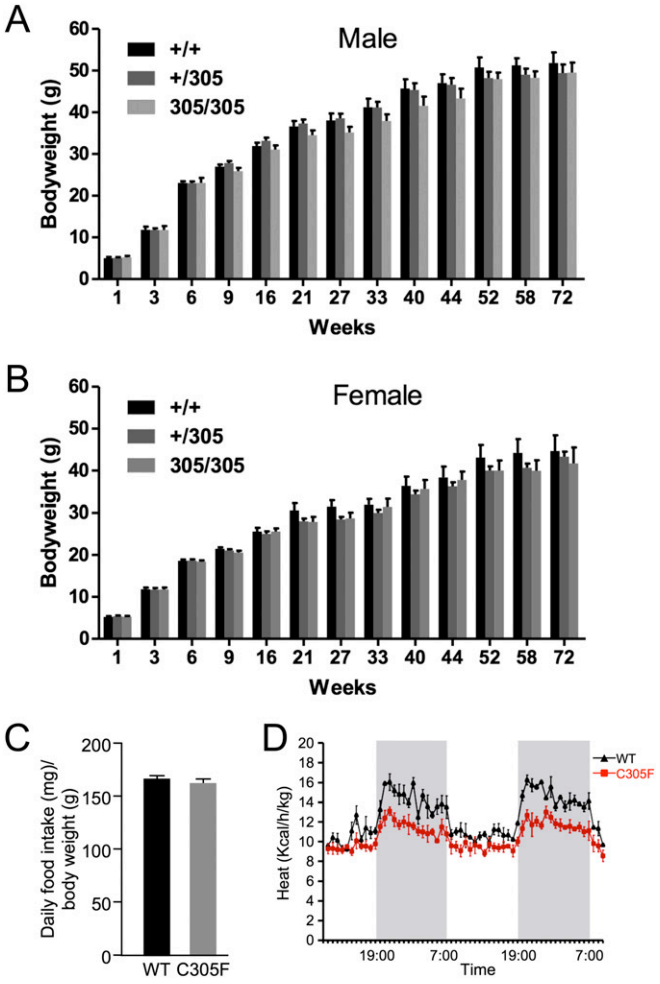

Fig. S1. Body weight of male (*A*; WT, $n = 10$; $Mdm2^{+/C305F}$, $n = 19$; $Mdm2^{C305F/C305F}$, $n = 9$) and female (*B*; WT, $n = 12$; $Mdm2^{+/C305F}$, $n = 19$; $Mdm2^{C305F/C305F}$, $n = 10$) mice over time (in weeks) fed a normal chow diet ad libitum. (*C*) Food intake of WT and $Mdm2^{C305F}$ mice normalized by body weight (in milligrams per gram; $n = 15$ per genotype). (*D*) Energy expenditure (heat) of WT (black line) and $Mdm2^{C305F}$ (red line) mice fed a normal chow diet ad libitum. The white background indicates light hours, and the shaded background indicates dark hours.

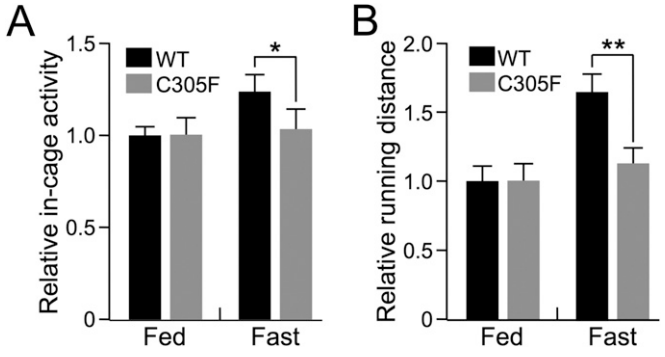

Fig. S2. (*A*) In-cage activity of mice housed in a metabolic chamber and (*B*) total distance traveled on a running wheel by WT and $Mdm2^{C305F}$ mice over a 24-h period before (Fed) or after (Fast) fasting ($n = 3$).

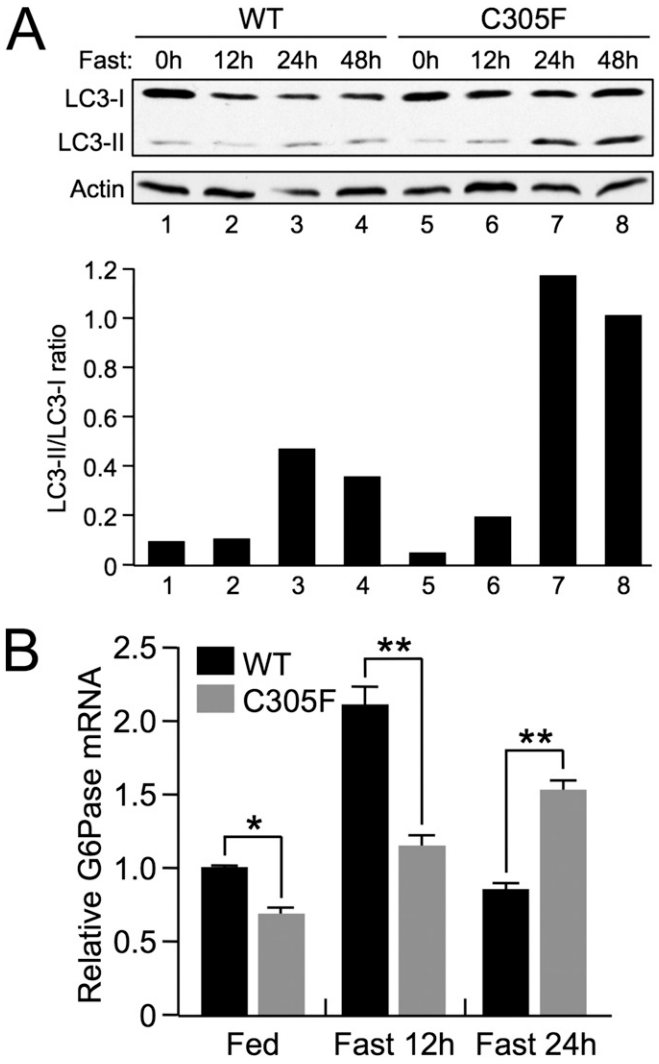

Fig. S3. (*A*) WT and *Mdm2*^{C305F} mice were fasted for the indicated period, and muscle homogenates were immunoblotted for LC3B (*Upper*). The LC3B-II/ LC3B-I ratio is shown (*Lower*) as an indicator of autophagy. (*B*) Quantitative RT-PCR analysis of glucose-6-phosphatase in the liver of WT and *Mdm2*^{C305F} mice fasted for the indicated amount of time.

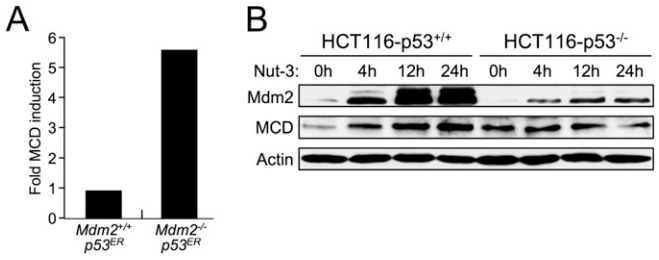

Fig. S4. (*A*) MEFs harboring a single p53ER fusion allele (*p53*$^{ER/-}$) with the indicated *Mdm2* genotypes were treated for 24 h with 4-hydroxytamoxifen. Cells were harvested, and total RNA was extracted and subjected to microarray analysis. The fold induction of MCD is shown. (*B*) HCT116 *p53*-WT and HCT116 *p53*-null cells were treated with nutlin-3 (10 μM) for the indicated amount of time, and MCD protein expression was assessed by immunoblot. Mdm2 is shown as an indicator of p53 activity.

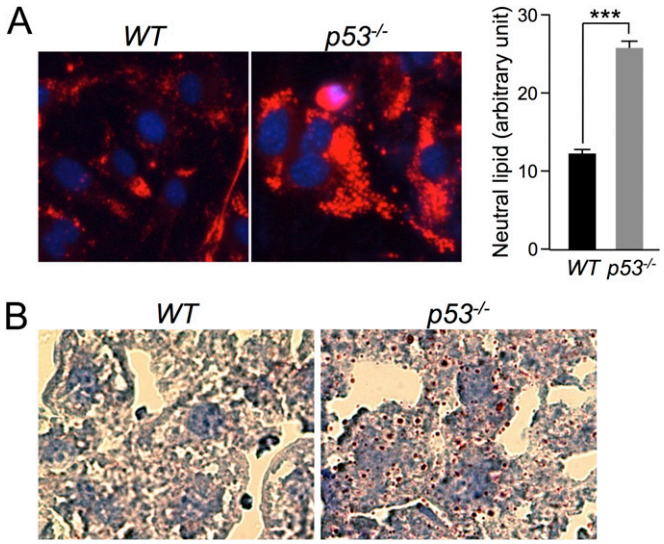

Fig. S5. (*A*) Representative pictures show neutral lipid staining in MEF cells. WT and $p53^{-/-}$ MEF cells were differentiated to adipocytes followed by staining with AdipoRed. The bar graph shows the statistical analysis of AdipoRed signal normalized by DAPI staining for nuclei. (*B*) Representative images of oil red O-stained liver sections from WT and $p53^{-/-}$ mice fed a normal chow diet ad libitum.