Supporting Information

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SI Materials and Methods

Cell Culture and Reagents. HCT116 human colorectal carcinoma cells and their p53-null derivatives were a gift of Bert Vogelstein. Cells were grown in McCoy's 5A medium supplemented with 10% bovine serum, 1% glutamine, and antibiotics. Mouse embryonic fibroblasts (MEFs), and mouse embryonic lung fibroblasts (MRC5 and IMR90 cells) were grown in minimal Eagle's medium (MEM) supplemented with 10% bovine serum, 1% glutamine, 0.11 mg/mL sodium pyruvate, nonessential amino acids, and antibiotics. HepG2 cells stably expressing shp53 or shmNOXA were a gift of Varda Rotter. Human hepatocellular carcinoma HepG2 as well as U2OS human osteosarcoma cells were grown in DMEM supplemented with 10% bovine serum and antibiotics. Starvation was carried out in Earle's balanced salts solution (EBSS; Biological Industries or Invitrogen). EBSS contains 5.33 mM KCl, 26.19 mM $NaHCO_3$, 117.4 NaCl, 1.01 NaH_2PO_4-H_2O, and 5.56 mM D-glucose. Where indicated, 100 nM Bafilomycin A (Sigma), 50 μM zVAD-fmk (fluoro methyl ketone) (Sigma), or 50 μg/mL 5-fluorouracil (5-FU; ABIC) were added to the medium.

siRNA and Plasmid Transfections. siRNA experiments were carried out with Dharmafect 1 reagent (Dharmacon) for human cells and Dharmafect 2 reagent (Dharmacon) for MEFs, according to the manufacturer's protocol. siRNA oligos were ordered as smartpools from Dharmacon. Silencing of human MAP1-light chain 3 (LC3) was performed by transfection of a combination of siLC3A, siLC3B, and siLC3C smartpools (50 nM each; Dharmacon). As control, a combination of siLacZ smartpool (100 nM) and siControl 5 (50 nM; Dharmacon) was used. In the transfections described in Fig. 4*A* and Fig. S4, siLacZ was added to the siBeclin or siAtg5 transfections to reach equal total concentrations of siOligos in each transfection. Silencing of mouse LC3 was performed by transfection of a combination of 20 nM siLC3A (smartpool; Dharmacon) and 30 nM siLC3B (smartpool; Dharmacon) oligos. Plasmid transfections were carried out using Jet-PEI (Polyplus Transfection Inc.) according to the manufacturer's instructions.

Long-Term Cell Survival and Crystal Violet Staining. Cells were seeded in six-well plates at a density of 600,000 cells/well for complete starvation (100% EBSS) or 200,000 cells/well for mild starvation (90% EBSS + 10% standard full HCT116 culture medium). Microscopic images were taken using an Axiovert 135 microscope (Zeiss). For crystal violet staining, plates were washed one time with PBS and one time with cold methanol, and then, they were incubated in crystal violet solution (0.3% crystal violet in methanol) for 5 min at −20 °C. The plates were subsequently washed two times with double distilled water, air-dried, and scanned using a Canon scanner.

FACS Analysis. FACS-assisted cell cycle analysis for DNA content was performed as described. Samples were analyzed using an LSR-II (BD Biosciences) flow cytometer. Results presented in Fig. S4*B* were analyzed by the BD FACS-DIVA software (BD Biosciences) and are presented for the *x* axis in a logarithmic scale.

Bulk Protein Degradation. To measure the degradation of long-lived proteins under prolonged starvation conditions, cells were labeled for 16 h in medium containing [^{14}C]-valine (0.5 mCi/mL) and 5% FCS in valine-free αMEM. After three rinses with PBS, cells were incubated in valine-free αMEM supplemented with 10 mM nonradioactive valine. After 8 h of incubation, the medium was replaced with either McCoy's 5A or EBSS medium, also containing 0.1% BSA and 10 mM nonradioactive valine, and cultures were incubated for an additional 16 h. The medium was precipitated in 10% tri-chloric acid (TCA), and TCA-soluble radioactivity was measured. Total cell radioactivity was measured after lysis with 0.1 M NaOH. [^{14}C]-valine release was calculated as the percentage of the radioactivity in the TCA-soluble supernatant relative to total cell radioactivity.

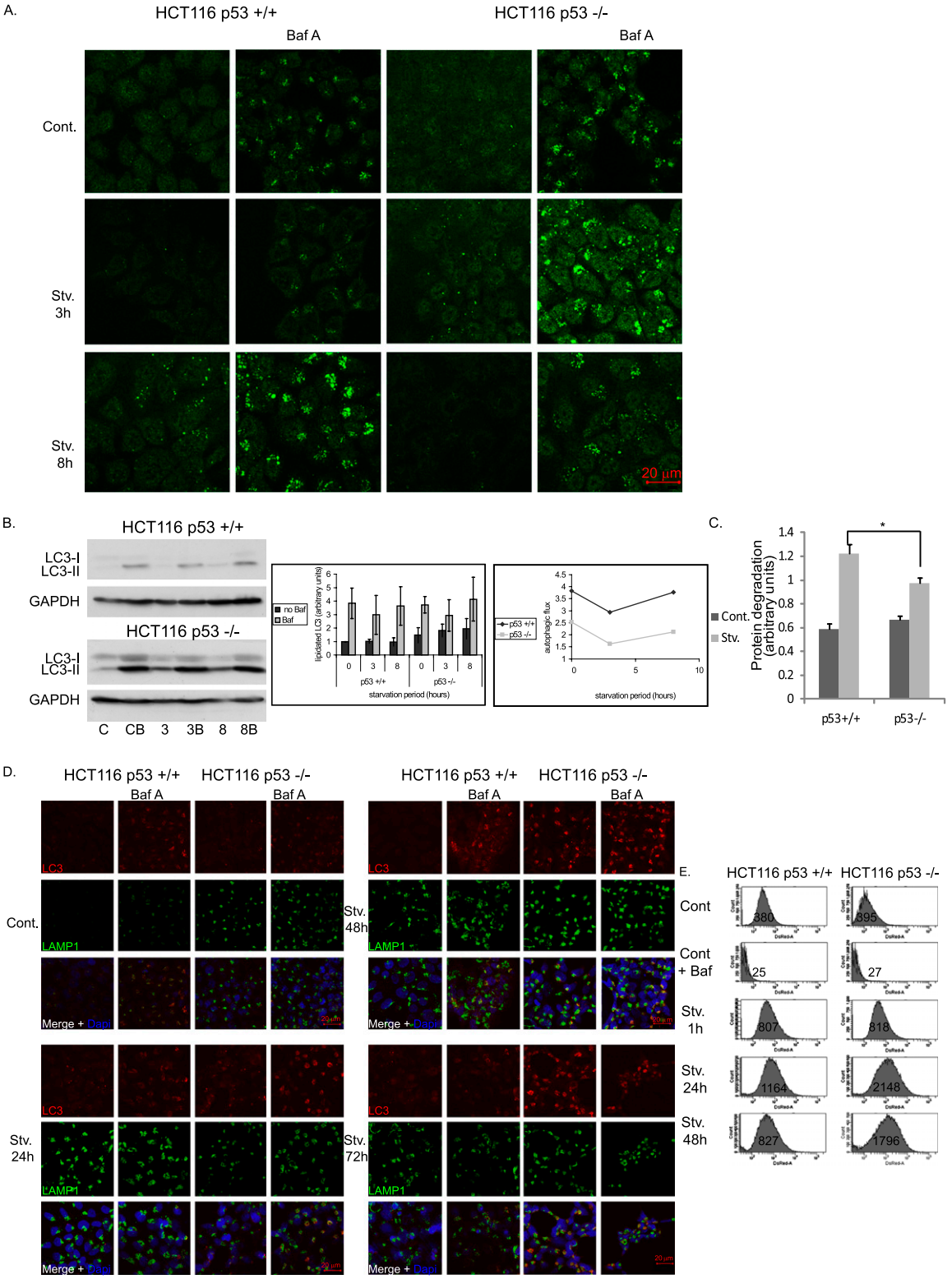

Fig. S1. Autophagic flux is not affected by p53 during short starvation. (*A*) HCT116 p53$^{+/+}$ or p53$^{-/-}$ were incubated in control medium or EBSS for the indicated time points; 1 mM Bafilomycin A (BafA) was added, where indicated, for the last 3 h of incubation, and the cells were subsequently (*A*) fixed for immunostaining with anti-LC3 antibodies (the experiment was repeated three times, and representative images are shown) or (*B*) lysed, resolved by 15% SDS/PAGE, Western blotted, and quantified using Image-J software. For each sample, lipidation was calculated as the ratio between LC3II and GAPDH. The lipidation of nonstarved p53$^{+/+}$ cells was set to 1, and the rest of the samples were normalized accordingly. Average results of three separate experiments are presented in *Middle*. Autophagic flux was calculated by dividing the value of lipidated LC3 in the presence of BafA by that without BafA (*Right*). (*C*) Bulk protein degradation was measured in HCT116 p53 $^{+/+}$ or p53$^{-/-}$ cells incubated in either control medium or EBSS for 24 h. Representative results of four separate experiments, preformed in triplicates, are shown. *$P < 0.05$. (*D*) Low magnification images of the cells enlarged in Fig. 1 *A* and *C*. HCT116 p53$^{+/+}$ or

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$p53^{-/-}$ cells kept in control medium or starved in EBSS for the indicated time periods in the presence or absence of 1 mM BafA for the last 3 h of incubation, where indicated, were fixed for immunostaining with anti-LC3 antibodies (red), anti-lysosomal-associated membrane protein (LAMP)1 antibodies (green), and DAPI (blue). (*E*) HCT116 $p53^{+/+}$ or $p53^{-/-}$ cells were incubated in control medium or EBSS for the indicated time points; 1 mM BafA was added, where indicated, for 16 h. LysoTracker Red (50 nM) was added to the medium for the last 30 min of incubation, after which the cells were trypsinized and analyzed by FACS.

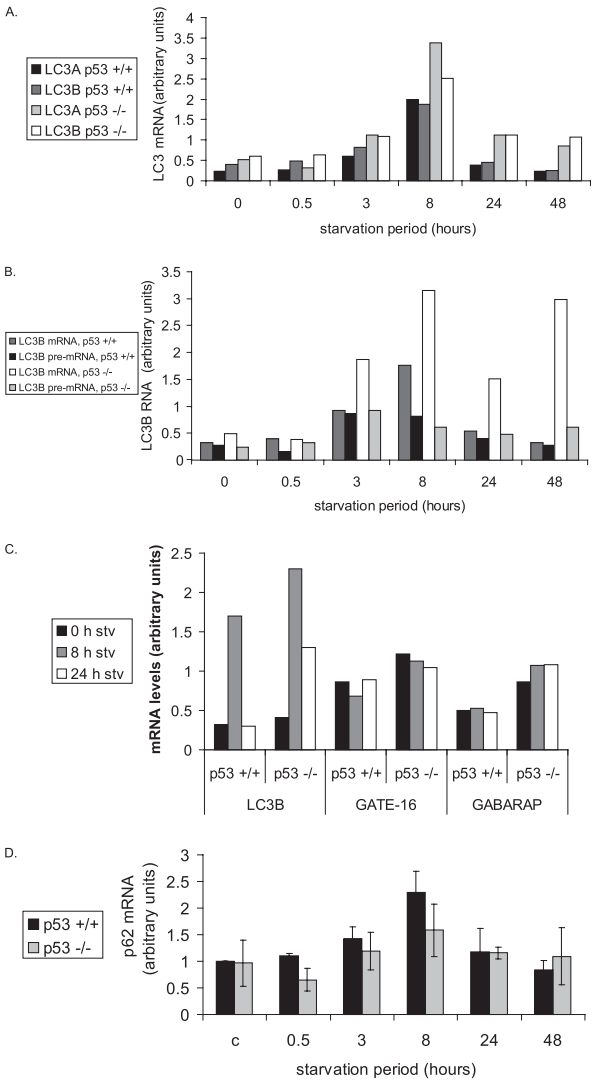

Fig. S2. p53 down-regulates LC3 mRNA but not Golgi associated ATPase enhancer (GATE)-16, GABA-receptor associated protein (GABARAP), or p62 mRNA. HCT116 $p53^{+/+}$ or $p53^{-/-}$ cells kept in control medium or starved in EBSS for the indicated time periods were harvested, and RNA was extracted and subjected to quantitative RT-PCR analysis. (*A*) Quantification of LC3A and LC3B mRNA. The experiment was repeated two times; a representative result is shown. (*B*) qRT-PCR analysis of LC3B mRNA (using exon–exon junction primers) and pre-mRNA (using intronic primers). For the pre-mRNA, RT-PCR results are shown after subtraction of the values obtained for the corresponding no reverse transcriptase control (details in *Materials and Methods*). A representative result of three experiments is shown. (*C*) Quantification of LC3B, GATE-16, and GABARAP mRNA. The experiment was repeated two times; a representative result is shown. (*D*) Quantification of p62 mRNA. Values obtained for nonstarved $p53^{+/+}$ cells were set to 1, and the rest of the samples were normalized accordingly. Averaged results of three experiments are presented.

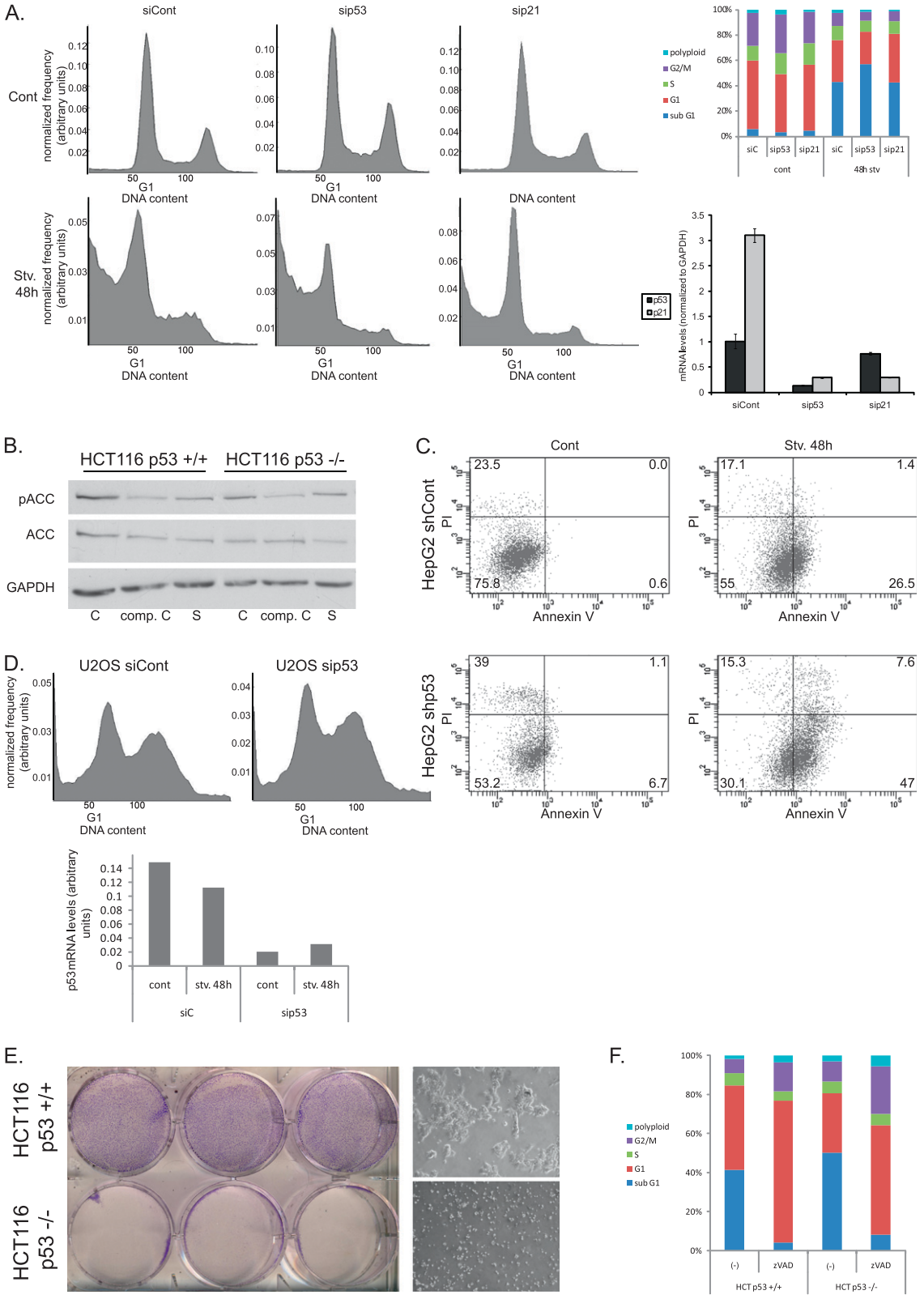

Fig. S3. (*A*) HCT116 $p53^{+/+}$ cells were transfected with 50 nM control (siLacZ), p53-specific (sip53), or p21-specific (sip21) siRNA oligonucleotides; 24 h later, the cells were placed in EBSS for 48 h and subsequently, trypsinized, fixed, and analyzed in an LSR-II flow cytometer. The experiment was repeated three times; a representative result is shown. Data analysis was performed by Matlab using FCS data reader script (*Left*). Quantification is shown in *Upper Right*. (*Lower Right*) RNA was extracted and subjected to qRT-PCR analysis to confirm efficient siRNA-mediated knockdown. (*B*) HCT116 $p53^{+/+}$ or $p53^{-/-}$ cells were kept in control medium, starved in EBSS for 48 h, or treated with 20 μM Compound C (comp C, an AMP kinase inhibitor serving as a positive control for regulation of phospho acetyl-CoA-carboxylase (pACC) by AMPK) for 48 h before lysis and Western blotting with the indicated antibodies. Whereas compound C reduced pACC levels as expected, no effect of starvation on pACC levels was observed. (*C*) HepG2 cells stably expressing control shRNA (pRetroSuper-shmNOXA-Blast) or

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p53-specific shRNA (pRetroSuper-p53 shRNA-Blast) were starved in EBSS or maintained in control medium for 48 h before trypsinization and staining with Annexin V and propidium iodide. The percentage of cells in each quadrant is indicated. (*D*) U2OS human osteosarcoma cells were transfected with 20 nM control (siLacZ) or p53-specific (sip53) siRNA oligonucleotides; 24 h later, the cells were starved in EBSS for 72 h and subsequently, trypsinized, fixed, and analyzed as in *A*. Data analysis was performed by Matlab using FCS data reader script (*Upper*). (*Lower*) RNA was extracted and subjected to qRT-PCR analysis to confirm efficient p53 knockdown. (*E*) HCT116 $p53^{+/+}$ or $p53^{-/-}$ cells were exposed to mild starvation in 90% EBSS supplemented with 10% full-standard culture medium for 4 wk, during which the medium was replaced every 4 d. At the end of this period, live cell imaging was preformed (*Right*), and then, cells were fixed and stained with crystal violet (*Left*). (*F*) Quantification of cell cycle analysis of HCT116 cells transfected and treated as explained in Fig. 3*D*.

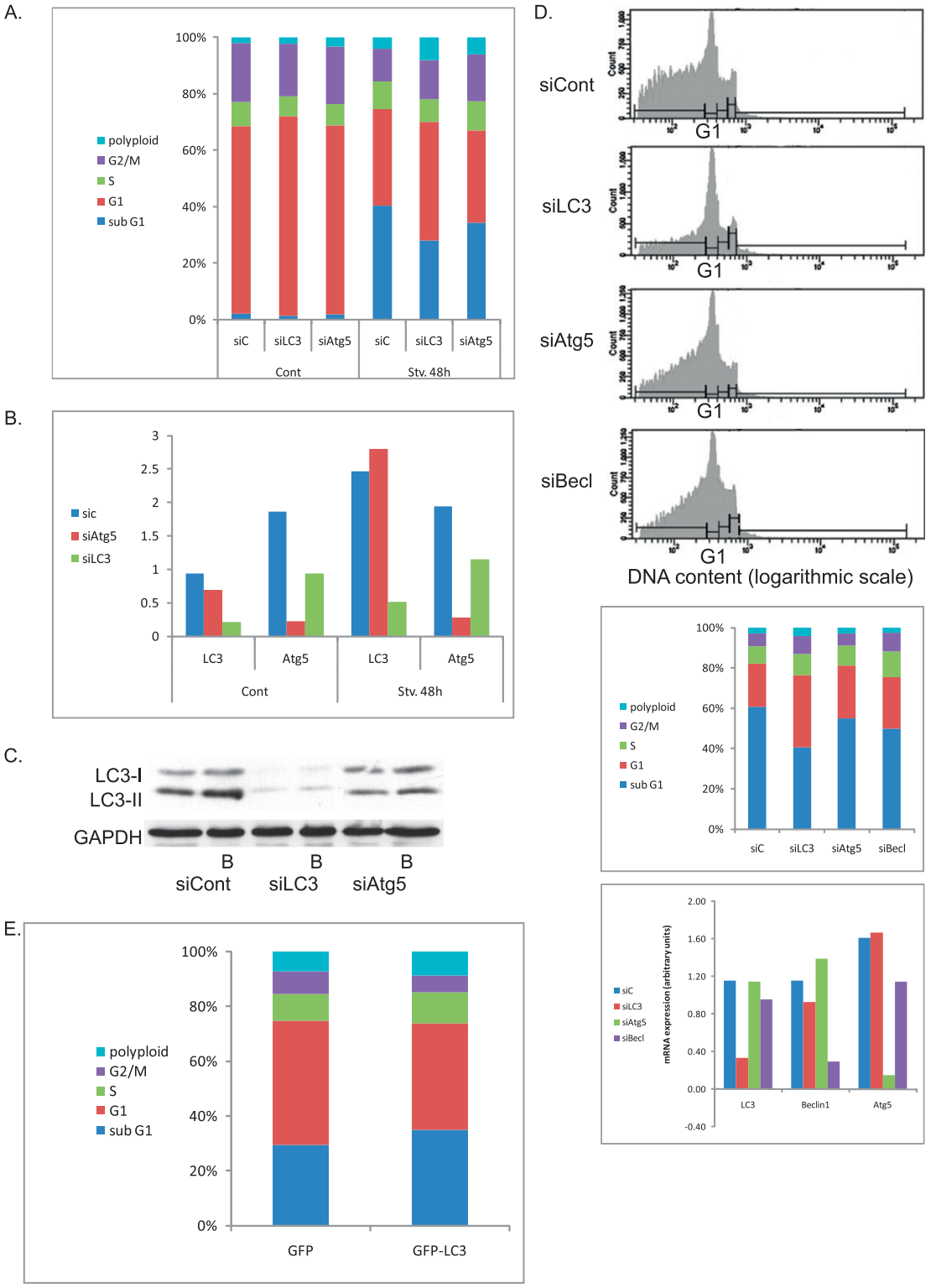

Fig. S4. (*A*) Quantification of cell cycle analysis after siRNA-mediated knockdown of LC3 or Atg5. HCT116 $p53^{-/-}$ cells were transfected with 150 nM control (100 nM siLacZ plus 50 nM nontargeting siControl 2), LC3-specific (50 nM siLC3A, 50 nM siLC3B, and 50 nM siLC3C), or Atg5-specific (50 nM siAtg5 plus 100 nM

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siLacZ) siRNA oligonucleotides; 48 h posttransfection, cells were starved in EBSS or kept in control medium for 48 h before trypsinization, fixation, and FACS analysis as explained in Fig. S3. (*B*) Validation of LC3 and Atg5 knockdown efficiency. Cells from *A* were used, after trypsinization, for mRNA extraction and qRT-PCR analysis. (*C*) Autophagic flux is attenuated after knockdown of LC3 or Atg5. HCT116 p53$^{-/-}$ cells transfected with 150 nM control (100 nM siLacZ plus 50 nM nontargeting siControl 2), LC3-specific (50 nM siLC3A, 50 nM siLC3B, and 50 nM siLC3C), or Atg5-specific (50 nM siAtg5 plus 100 nM siLacZ) siRNA oligonucleotides and retransfected with the same amounts of siRNA 24 h later were starved 48 h posttransfection for 3 h in the presence or absence of 1 mM BafA. The cells were then lysed and Western blotted with the indicated antibodies. (*D*) HCT116 p53$^{-/-}$ cells were transfected with 150 nM control (100 nM siLacZ plus 50 nM nontargeting siControl 2), siLC3 (50 nM siLC3A, 50 nM siLC3B, and 50 nM siLC3C), siAtg5 (50 nM siAtg5 and 100 nM siLacZ), or siBeclin1 (50 nM siBeclin1 and 100 nM siLacZ); 48 h posttransfection, the cells were starved in EBSS for 24 h before trypsinization, fixation, and FACS analysis (*Top*; quantified in *Middle*) or subjected to mRNA extraction and qRT-PCR analysis (*Bottom*). (*E*) Quantification of cell cycle analysis of HCT116 p53$^{+/+}$ cells transfected with enhanced GFP vector or with eGFP-LC3 and starved in EBSS for 24 h as explained in Fig. 4*B*.

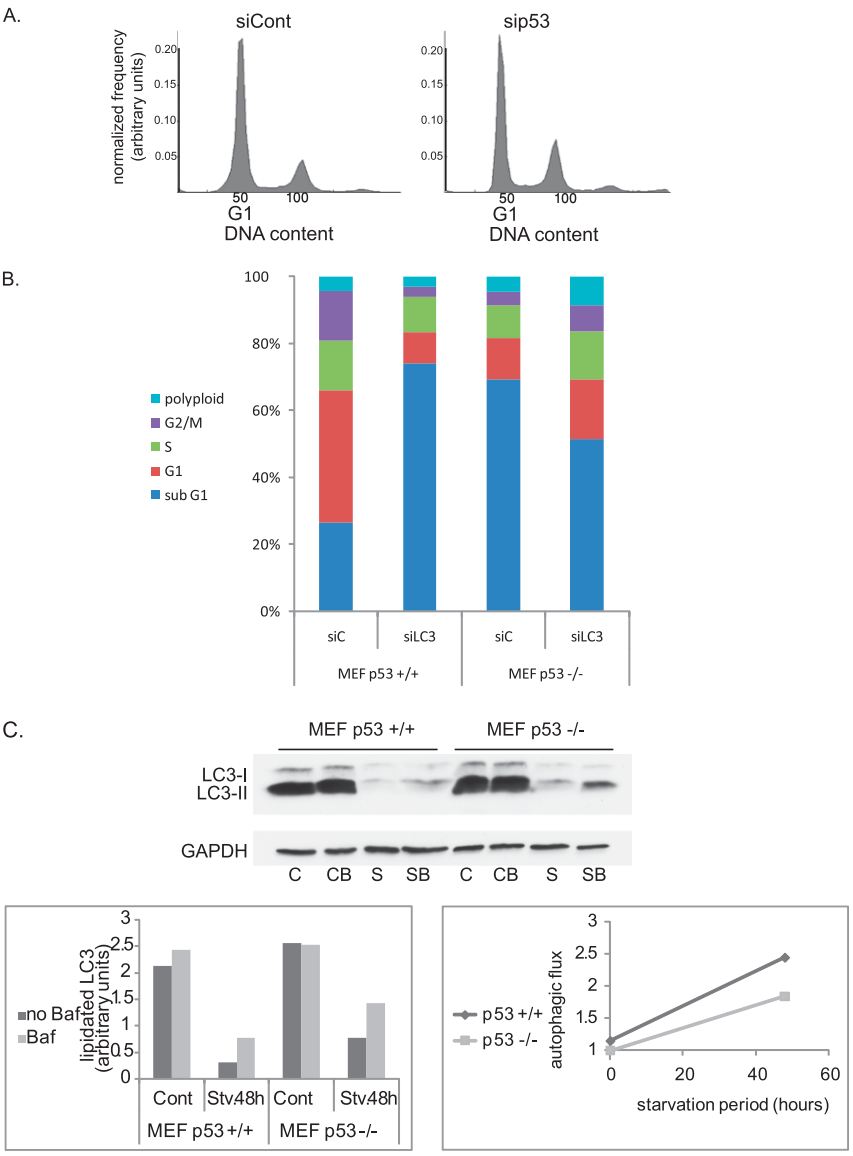

Fig. S5. (*A*) Knockdown of p53 does not induce cell death in nonstarved MRC5 cells. MRC5 cells transfected as explained in Fig. 5*A* were fixed, stained with propidium iodide, and analyzed by FACS. (*B*) Quantification of cell cycle analysis of MEFs transfected and treated as explained in Fig. 5*B*. (*C*) Analysis of wild-type or p53 $^{-/-}$ MEFs kept in control medium or starved in EBSS for 48 h; 1 mM BafA was added, where indicated, for the last 3 h of incubation, and the cells were subsequently lysed in RIPA buffer. Proteins were resolved by 15% SDS/PAGE, Western blotted, and quantified using Image-J software. For each sample, LC3-II readings were normalized for the corresponding GAPDH values. The experiment was repeated two times; a representative result is shown in *Lower Left*. Autophagic flux was calculated by dividing the value of LC3-II in the presence of BafA by that without BafA (*Lower Right*).

Table S1. Primers for qRT-PCR

Primer name	Sequence
hLC3A F	TCCCGGACCATGTCAACAT
hLC3A R	ACCATGCTGTGCTGGTTCAC
hLC3B F	ACCATGCCGTCGGAGAAG
hLC3B R	ATCGTTCTATTATCACCGGGATTTT
LC3B premRNA F	AGTTCCAGACACCGGTTTGG
LC3B premRNA R	AGTCCACCGCGATGAACAC
mLC3A F	TTGGTCAAGATCATCCGGC
mLC3A R	GCTCACCATGCTGTGCTGG
mLC3B F	CCCACCAAGATCCCAGTGAT
mLC3B R	CCAGGAACTTGGTCTTGTCCA
GATE-16 F	ccgctgtaggccacatataagaa
GATE-16 R	CAGTCCCACAGTGCTGGTTCAC
GABARAP F	CGGGTGCCGGTGATAGTAGA
GABARAP R	TGAGATCAGAAGGCACCAGGTA
p62 F	aggcgcactaccgcgat
p62 R	CGTCACTGGAAAAGGCAACC
p53 F	CCCAAGCAATGGATGATTTGA
p53 R	GGCATTCTGGGAGCTTCATCT
Atg5 F	GCAGATGGACAGTTGCACACA
Atg5 R	TTTCCCCATCTTCAGGATCAA
Beclin1 F	CTGGACACGAGTTTCAAGATCCT
Beclin1 R	TGTGGTAAGTAATGGAGCTGTGAGTT
p21 F	ggcagaccagcatgACAGATT
p21 R	GCGGATTAGGGCTTCCTCTT
mHPRT F	GCAGTACAGCCCCAAAATGG
mHPRT R	GGTCCTTTTCACCAGCAAGCT