Supporting Information

Feng et al. 10.1073/pnas.1203930109

SI Materials and Methods

Mouse Strains, Chronic Restraint, and IR and Corticosterone Administration. The $p53^{+/+}$, $p53^{+/-}$, and $p53^{-/-}$ C57BL6/J mice were purchased from The Jackson Laboratory. BALB/c nu/nu athymic nude mice were purchased from Taconic. Mouse restraint system (flat bottom restrainers; Kent Scientific) was used for chronic periodic restraint. The restrainer is well ventilated and has a hopper at the front allowing restrained mice to have access to food and water. Restrained mice were maintained horizontally in their home cages during the restraint sessions and released into the same cage during the free sessions. Mice were restrained for 6 h daily (10:00 AM to 4:00 PM). Age- and sex-matched C57BL6/J mice with or without restraint were subjected to 4 Gy of total-body IR with a 137 Cs γ-source irradiator. To determine p53 protein levels and function after IR, mice were killed at different time points after IR, and different tissues were collected for further experiments. At least five mice were used for each group. For IR-induced tumorigenesis assays, mice were examined three times per week until moribund. Tumor samples were processed for routine histological examination. For corticosterone administration, $p53^{+/+}$ C57BL6/J mice were administered (s.c.) with corticosterone (Sigma; 20 mg/kg, suspended in physiological saline containing 1% DMSO and 1% Tween 80) once a day for 1 wk. Mice administered with vehicle only served as controls.

Western Blot and ELISAs. Standard Western blot assays were used to analyze protein expression. Anti-p53 (FL393) (sc-6243) (Santa Cruz Biotechnology), antiphospho-p53 (Ser15) (9284) (Cell Signaling), anti-p21 (Ab-6) (Calbiochem), anti-SGK1 (SAB2104902) (Sigma), antiphospho-MDM2 (Ser166/186) (3521) (Cell Signaling), and anti–β-actin (A5441) (Sigma) antibodies were used to determine the levels of total p53, p-p53 Ser15, p21, SGK1, p-MDM2 Ser166/186, and β-actin, respectively. The mouse total p53 ELISA kit (R&D Systems) was used to detect the p53 protein levels in mouse tissues, and the corticosterone ELISA kit (Enzo Life Science) was used to detect mouse serum corticosterone levels.

Taqman Real-Time PCR Analysis. Total RNA was prepared from cells or mouse tissues with the RNeasy kit (Qiagen) and treated with DNase I to remove residual genomic DNA. Real-time PCR was performed in triplicate with Taqman PCR Mix (Applied Biosystems) in the ABI Step-One system. All primers were purchased from Applied Biosystems. The expression of genes was normalized to the actin gene.

Measurement of Cellular Apoptosis. To detect apoptotic cells, the splenocytes isolated from mice were stained with annexin V and analyzed in a flow cytometer as previously described (1). A TUNEL assay was used to detect apoptotic cells in the spleen tissues fixed in 10% (vol/vol) neutral buffered formalin solution for 24 h as previously described (1).

Xenograft Tumorigencity Analysis. Seven-week-old BALB/c nu/nu male athymic nude mice (Taconic) were used. Isogenic human colorectal HCT116 $p53^{+/+}$ and $p53^{-/-}$ cells (2×10^6 in 0.2 mL PBS) were injected s.c. into nude mice ($n = 10$ per group). After injection, mice were examined and tumor volumes were measured three times per week for 3 wk. Tumor volume = 1/2 (length × width^2). Tumor samples were processed for routine histology examination.

Cell Culture and Treatments. Human colorectal cancer HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cell lines were generous gifts from Dr. B. Vogelstein at The Johns Hopkins University, Baltimore, MD. Human breast cancer cell line MCF7 was purchased from ATCC. p53 $^{+/+}$ MEF cells were generated as previously described (2). For IR, a 137Cs γ-source irradiator was used to deliver 4 Gy to cells. Etoposide, cortisol, cortiosterone, and RU486 were purchased from Sigma. GSK650394 was purchased from Torcis Bioscience. Human pCMV-SGK1 expression plasmid was constructed and transfected into cells using Lipofectamine 2000 (Invitrogen). For siRNA knockdown, three different siRNA oligos against GR or SGK1 (IDT) were used. siRNAs were transfected into cells using Lipofectamine RNAiMAX (Invitrogen).

Statistical Analysis. Statistical differences in tumor latency induced by IR between mice with and without chronic restraint were analyzed by Kaplan-Meier analysis using GraphPad Prism software. Significance was determined by log-rank test. Statistical differences in tumor spectrum were determined by χ^2 test. Statistical differences in the growth of xenograft tumor were analyzed by two-way ANOVA.

1. Feng Z, et al. (2007) Declining p53 function in the aging process: A possible mechanism for the increased tumor incidence in older populations. *Proc Natl Acad Sci USA* 104: 16633–16638.

2. Harvey DM, Levine AJ (1991) p53 alteration is a common event in the spontaneous immortalization of primary BALB/c murine embryo fibroblasts. *Genes Dev* 5 (12B): 2375–2385.

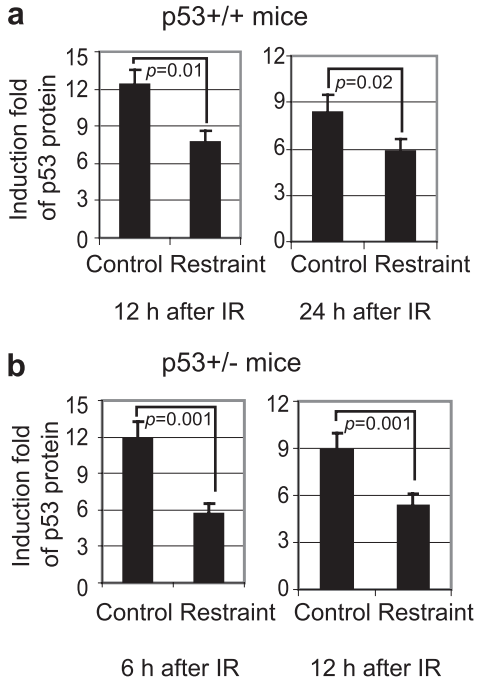

Fig. S1. Chronic restraint decreases p53 protein accumulation in response to IR in the spleen from $p53^{+/+}$ and $p53^{+/-}$ mice. Seven-week-old $p53^{+/+}$ (*A*) or $p53^{+/-}$ (*B*) C57BL6/J mice with or without periodic restraint were treated with 4 Gy IR ($n = 5$ per group). Induction fold of p53 protein induced by IR in the spleen tissues was determined at 12 and 24 h after IR in $p53^{+/+}$ mice (*A*) and at 6 and 12 h after IR in $p53^{+/-}$ mice (*B*), respectively, by ELISAs. Data are presented as mean ± SD.

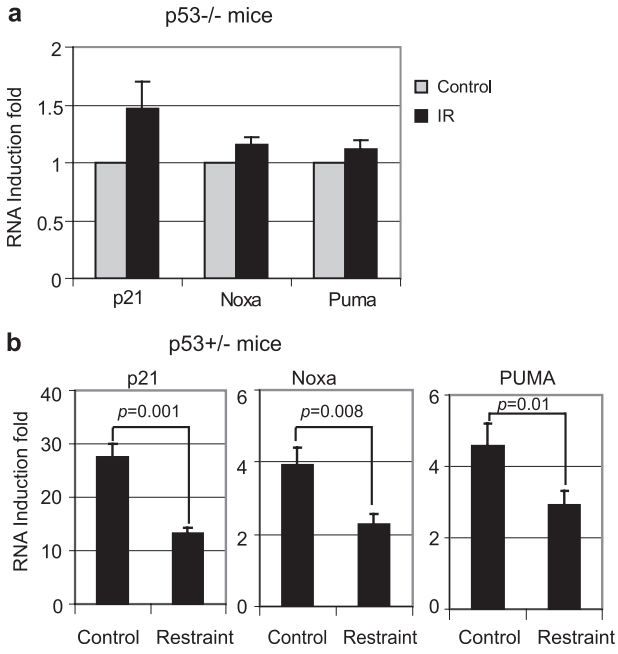

Fig. S2. mRNA levels of p53 target genes (*p21*, *Noxa*, and *Puma*) in response to IR in $p53^{-/-}$ and $p53^{+/-}$ mice. Seven-week-old $p53^{-/-}$ C57BL6/J mice without restraint (*A*) or $p53^{+/-}$ C57BL6/J mice with or without periodic restraint (*B*) were treated with 4 Gy IR, and spleen tissues were collected at 6 h after IR. mRNA levels of *p21*, *Noxa*, and *Puma* were determined by Taqman real-time PCR and normalized with actin.

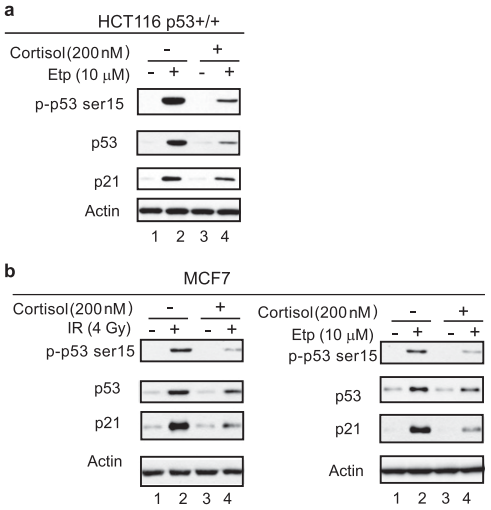

Fig. S3. Cortisol decreases p53 protein levels and function in HCT116 $p53^{+/+}$ and MCF7 cells. (*A*) Cortisol decreases p-p53 Ser15 levels, accumulation of p53 protein, and induction of p21 protein in response to etoposide treatments in HCT116 $p53^{+/+}$ cells. HCT116 $p53^{+/+}$ cells were treated with cortisol for 12 h, followed by etoposide (Etp; 10 μM for 6 h) treatment. (*B*) Cortisol decreases p-p53 Ser15 levels, accumulation of p53 protein, and induction of p21 protein in response to IR or etoposide treatments in MCF7 cells. p53 wild-type MCF7 cells treated with cortisol for 12 h were subjected to IR (*Left*) or etoposide (*Right*) treatments. p-p53 Ser15, p53, and p21 protein levels were determined at 6 h after IR or etoposide treatments by Western blot assays.

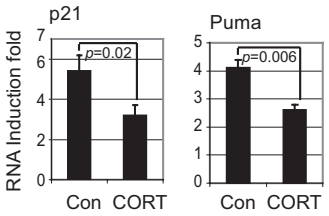

Fig. S4. Corticosterone decreases p53 transcriptional induction of *p21* and *Puma* in MEFs. MEFs were treated with corticosterone (CORT; 200 nM) for 12 h, followed by IR (4 Gy). mRNA levels of *p21* and *Puma* were determined at 6 h after IR by Taqman real-time PCR and normalized with actin.

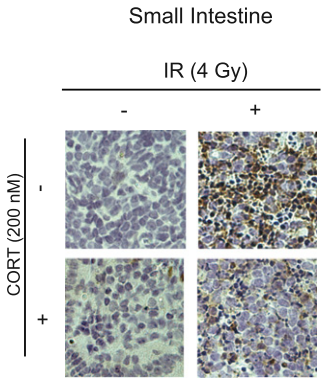

Fig. S5. Corticosterone injection greatly decreased p53-mediated apoptosis in small intestine in response to IR in mice. $p53^{+/+}$ C57BL6/J male mice were s.c. injected with corticosterone (CORT; 20 mg/kg) daily for 1 wk. Mice with or without corticosterone injection were exposed to IR (4 Gy), and small intestine was collected at 12 h after IR. TUNEL assay was used to detect apoptotic cells.

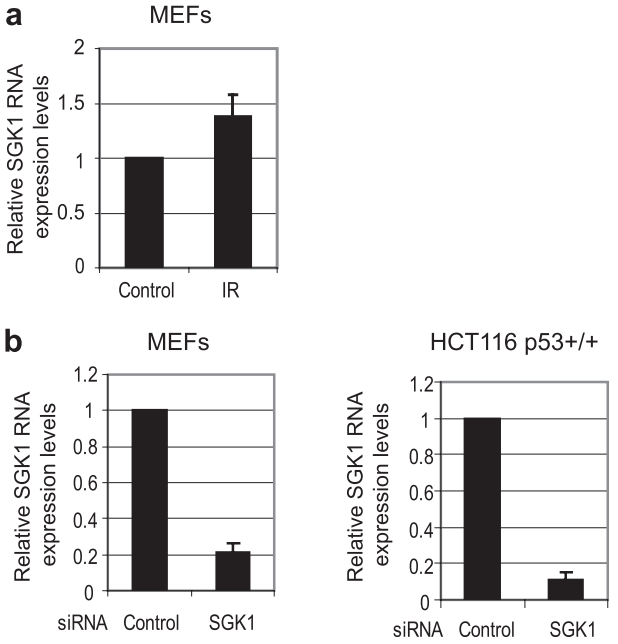

Fig. S6. mRNA levels of SGK1 in cells treated with IR or treated with siRNA against SGK1. (*A*) p53$^{+/+}$ MEFs were treated with IR (4 Gy). mRNA levels of SGK1 were determined at 15 h after IR by Taqman real-time PCR and normalized with actin. (*B*) siRNAs against mouse and human SGK1 were transfected into MEFs and HCT116 p53$^{+/+}$ cells, respectively. mRNA levels of SGK1 were determined in MEFs (*Left*) and HCT116 p53$^{+/+}$ cells (*Right*) by Taqman real-time PCR and normalized with actin.

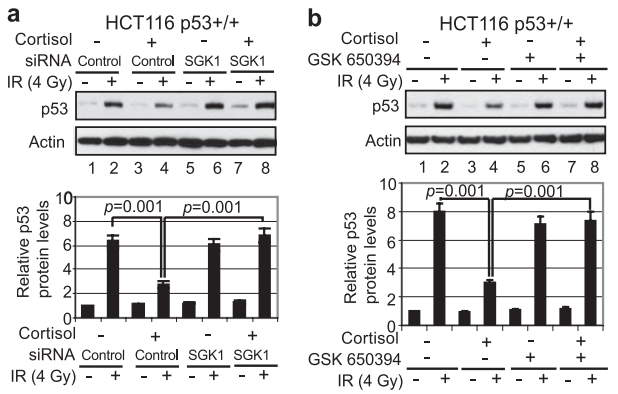

Fig. S7. SGK1 mediates the inhibitory effect of glucocorticoids on p53 in HCT116 p53$^{+/+}$ cells. Blocking SGK1 function by SGK1 siRNA (*A*) or GSK650394 (200 nM) (*B*) abolished the effect of cortisol (200 nM) on IR-induced p53 accumulation in HCT116 p53$^{+/+}$ cells. Bar graphs represent quantification of p53 levels in Western blot assays from three experiments.