SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Detection of p53β mRNA. A. Analysis of enriched p53β mRNA expression on nuclear RPL26 in SY5Y cells 15 minutes post 10gy irradiation. Left: mRNA expression in immunoprecipitated nuclear fraction. Right: mRNA expression in input nuclear fraction. Shown is mean ± SEM. *TSp53*: total spliced p53. B. RT-PCR analysis of p53β and full-length p53 (FLp53) mRNA levels in MCF7 cells at different time points post 20gy irradiation. PCR primers locate at Exon 9 and Exon 10 separately. PCR products are separated in 3% agarose gel. Band intensity of p53β in each sample was quantified using ImageJ and normalized to untreated sample for fold change.

Figure S2: Regulation of p53β splicing by CP466722 (CP) and other protein factors. A. RT-qPCR (top) and immunoblot analysis (bottom) of p53β expression at different time points post 10μM CP treatment. The ratios of total spliced p53, p53β to p53 pre mRNA are calculated as described in Figure 1. The aliquots of the same samples for RT-qPCR analysis are subjected to immunoblot analysis for full-length (FL) p53, p53β protein expression. PARP and LDH are used as nuclear and cytoplasmic fraction marker respectively. B. Immunoblot (left panels) and RT-qPCR (right graph) analysis of p53β expression 3 days after p53β siRNA transfection and 8hr post CP treatment. Data are mean ± SEM of three independent experiments. C. Immunoblot analysis of p53β protein expression in ATM CRISPR knockout SY5Y cells treated with DMSO (D) or CP for 8hr. D. Immunoblot analysis of p53β expression post DNK-PK inhibitor Nu7026 treatment. E. Western blot analysis (Left) and RT-qPCR analysis (Middle) of p53β and/or hSMG1 expression 3 days post hSMG-1 siRNAs (SMG1si, SMG1siB) transfection. Data are mean ± SEM of three independent experiments. Right panel: Relative amount of p53β (p53β mRNA/p53 pre-mRNA) in MCF7 cells 3 days post SMG1siB transfection (This is a repeat of Figure 2D with a different SMG1 siRNA). The ratios of total spliced p53 and p53β to p53 pre mRNA are calculated as described in Figure 1. F. RT-qPCR analysis of nuclear and cytoplasmic p53β mRNA in the absence of SMG1. G. IP-western of Upf1 in MCF7 cells 3 days post hSMG-1 siRNA transfection. Immunoprecipitated Upf1 was immunoblotted with pan-phospho-TQ/SQ antibody and reblotted with Upf1 antibody. *P≤0.05, ** P≤0.01, ***P≤0.001, t-test.

Figure S3: Regulation of p53β expression by RPL26. A. RT-qPCR analysis (top) of p53β expression 3 days post RPL26 siRNA transfection and 1hr post 0 (-) or 10 (+) Gy irradiation. Data are mean ± SEM of three independent experiments. Immunoblot analysis (bottom) is performed to confirm the knocking down of RPL26 by siRNA treatment. Nucleolin (NCL) is the loading control. B. RT-PCR analysis of p53β and full-length p53 (FLp53) mRNA levels in MCF7 cells 2 days post RPL26 siRNA transfection and 8hr CP treatment. PCR primers locate at Exon 9 and Exon 10 separately. PCR products are separated in 3% agarose gel. Band intensity of p53β in each sample was quantified using ImageJ and normalized to ctrlsi/DMSO treated sample for fold change. C. Immunoprecipitation of nuclear RPL26 three days post hSMG-1 siRNA treatment. 1% of the input and 10% of the immuno-complex are subjected to Western blot analysis using appropriate antibodies. The rest of the immuno-complex was used for the IP-RT-qPCR described in Figure 3. D. Enrichment of p53 pre-mRNA on nuclear RPL26 in the absence of hSMG-1 (repeat of Figure 3B with a different hSMG1 siRNA (hSMG1siB). The bound mRNAs in nuclear RPL26 immunoprecipitated from MCF7 cells transfected with hSMG-1siB for 3 days were subjected to RT-qPCR analysis. Top: mRNA levels detected in RPL26-immunoprecipitate from nuclear fraction. Bottom: mRNA expression in input nuclear fraction. Shown is mean ± SEM of three independent experiments. *P≤0.05, t-test.

Figure S4: Regulation of p53β expression by SRSF7. A. Immunoblot (left panel) and RT-qPCR analysis (right graph) of p53β expression 3 days post SRSF3 siRNA treatment. Data are mean ± SEM of three independent experiments. B. RT-qPCR analysis of p53β mRNA expression 3 days post SRSF7 siRNAs (SRSF7si A, SRSF7siB) transfection. Data are mean ± SEM of three independent experiments. C. RT-qPCR analysis of the total spliced p53 in the absence of SRSF7. The ratio of the total spliced p53 mRNA level to p53 pre-mRNA level is calculated as described in Figure 4A. Data are mean ± SEM of three independent experiments. D. RT-qPCR analysis of nuclear p53β mRNA expression in MCF7 cells treated with 100μM desferoxamine (DFO) for 24 hr (left graph) or 100μM Hemin for 8hr (right graph). Data are fold changes of indicated mRNA expression in DFO or Hemin treated vs DMSO treated cells. E. RPL26 interacts with SRSF7 independent of RNA. Top panel: MCF7 cells are cotransfected with GFP-SRSF7 and Flag L26 for co-immunoprecipitation. Bottom panel: L26 is immunoprecipitated from MCF7

cells in the presence of Benzonase. The bound proteins are subjected to Western blot analysis to detect RPL26 and SRSF7 by appropriate antibodies. IgG was used as a negative control for IP. 10% lysate before IP was loaded as input. F. Model of "SMG1-SRSF7-RPL26" pathway regulation on p53β splicing in response to IR. ***P≤0.001, t-test. G. Immunoblot (top) and RT-qPCR analysis (bottom) of p53 splice variants expression in MMS treated cells. Representative immunoblots shown in top panel were performed in nuclear (Nuc) or cytoplasmic (Cyto) fractions of MCF7 cells 16hr post 118μM MMS or 1mM H_2O_2 exposure. Bottom graph is a representative RT-qPCR analysis of basal p53β mRNA expression 3 days after RPL26si RNA (left graph) or SRSF7si RNA (right graph) transfection and 16hr post 118μM MMS treatment. Data are mean ± SEM. P values were calculated for p53β mRNA level in MMS treated cells vs untreated cells. UT: untreated. H. Immunoblot (top) and RT-qPCR analysis (bottom) of p53 splice variants expression in 10J/m^2 irradiated cells. Representative immunoblots shown in top panel were performed in whole cell extract (WCE) and nuclear (Nuc) or cytoplasmic (Cyto) fraction of MCF7 cells at different time points post UV irradiation. Bottom graph is a representative RT-qPCR analysis of nuclear Δp53 mRNA expression after UV treatment. Data are mean ± SEM. I. Representative immunoblot analysis of p53 splice variants 3 days after ctrl (ctrlsi), SRSF7 (SRSF7si) or RPL26 (L26si) siRNA transfection and 16 hr post 10 J/m^2 irradiation. *P≤0.05, ** P≤0.01, ***P≤0.001, t-test.

Figure S5: Characterization of stable cell lines overexpressing p53β and p53β CRISPR knockout cells. A. Overexpression of p53β induces senescence phenotype in p53-wild-type cells. A549 stable lines overexpressing GFP or GFP-p53β were observed under light microscope (left images) or fluorescent microscope (middle images). SA-β-galactosidase staining of A549 stable lines is presented in right images. B. p53β has no effect on p53 induced apoptosis. H1299 cells were transfected with GFP-p53β alone or together with GFP-FLp53. Left panel: Western blot analysis of the transfected proteins using anti-GFP antibody. Total 4μg of DNA (2μg for each construct) was used in each reaction. Right panel: Apoptotic cells were assessed by PI staining. The percentage of subG1 population in each sample was presented. Data are mean ± SEM of three independent experiments. ** P≤0.01, t-test. C. p53β induced senescence phenotype requires wild type p53. Top: Growth rate of HCT116 p53-wild-type (Left) or p53-null cell line (Right) constitutively expressing GFP or GFP-p53β (GFP-beta). Data are mean ± SEM of three independent experiments. Bottom: SA-β-gal staining of stable cell lines described in the top panel. D. p53β does not interact with full-length p53 (FLp53). GFP-FLp53 cotransfected with flag tagged p53β or FLp53 for coIP in MCF7 cells. 5μg of each construct was used in the transfection. E. Differential expression of p53 target genes in p53β stable cell lines analyzed by RT-qPCR. The list presents the fold changes (GFP-p53β/GFP stable line) of p53 target gene mRNA levels in HCT116 wild type (WT) and p53 knockout (p53-/-) stable lines. F. Sequence around Cas9 targeting site at p53 gene locus. The sequences of p53β CRISPR clones were aligned against published p53 gene sequence (wild type). Donor sites (red rectangle) and acceptor sites (green rectangle) were highlighted in the sequences. G. Characterization of p53β CRISPR knockout cells. Left graph: Growth curves of two p53β CRISPR knockout clones. Right panel: Western blot analysis of full-length p53 and p21 protein expression at different time points post 5gy treatment in p53β CRISPR knockout clones.

Figure S6: Modulators of DNA damage induced cellular senescence. A. Modulation of p53β level affects IR induced cellular senescence. Left, Middle: SA-β-gal staining of p53β CRISPR knockout clones 6 days after 5gy irradiation. The average counts of 5 fields under light microscope (Left images) in irradiated samples were graphed (Middle graph). Since no staining was observed in untreated samples, data were not shown. Right: flow β-gal assay to assess senescent cells in MCF7 transfected with p53βsi for 3 days and 6 days post 20gy irradiation. *P≤0.05, ** P≤0.01, ***P≤0.001, t-test. B. RT-qPCR analysis of SASP cytokines in p53β CRISPR knockout cells post 20gy irradiation. Data are mean ± SEM of three independent experiments. *P≤0.05, t-test. C. Modulation of hSMG1 and SRSF7 levels affects IR induced cellular senescence. Flow β-gal assay to assess senescent cells in p53β CRISPR knockout clones 5 days post transfection with SMG1siB (Left, repeat of Figure 5D with a different siRNA to hSMG1) or in MCF7 cells transfected with SRSF7siB for 3 days and 6 days post 20gy irradiation (Right, repeat of Figure 5E with a different siRNA to SRSF7). Data are mean ± SEM of three independent experiments. D. Left: Venn diagram of genes that overlap among three groups of comparison: p53β CRISPR no IR vs ctrl no IR (β-CRISPR IR-/ctrl IR-), ctrl IR vs no IR (ctrl IR+/-), and p53β CRISPR IR vs no IR (β-CRISPR IR+/-). Right: Venn diagram of genes with more than 2 fold increase (Top) or 2 fold decrease (Bottom) between β-CRISPR IR+/- vs ctrl IR+/-.