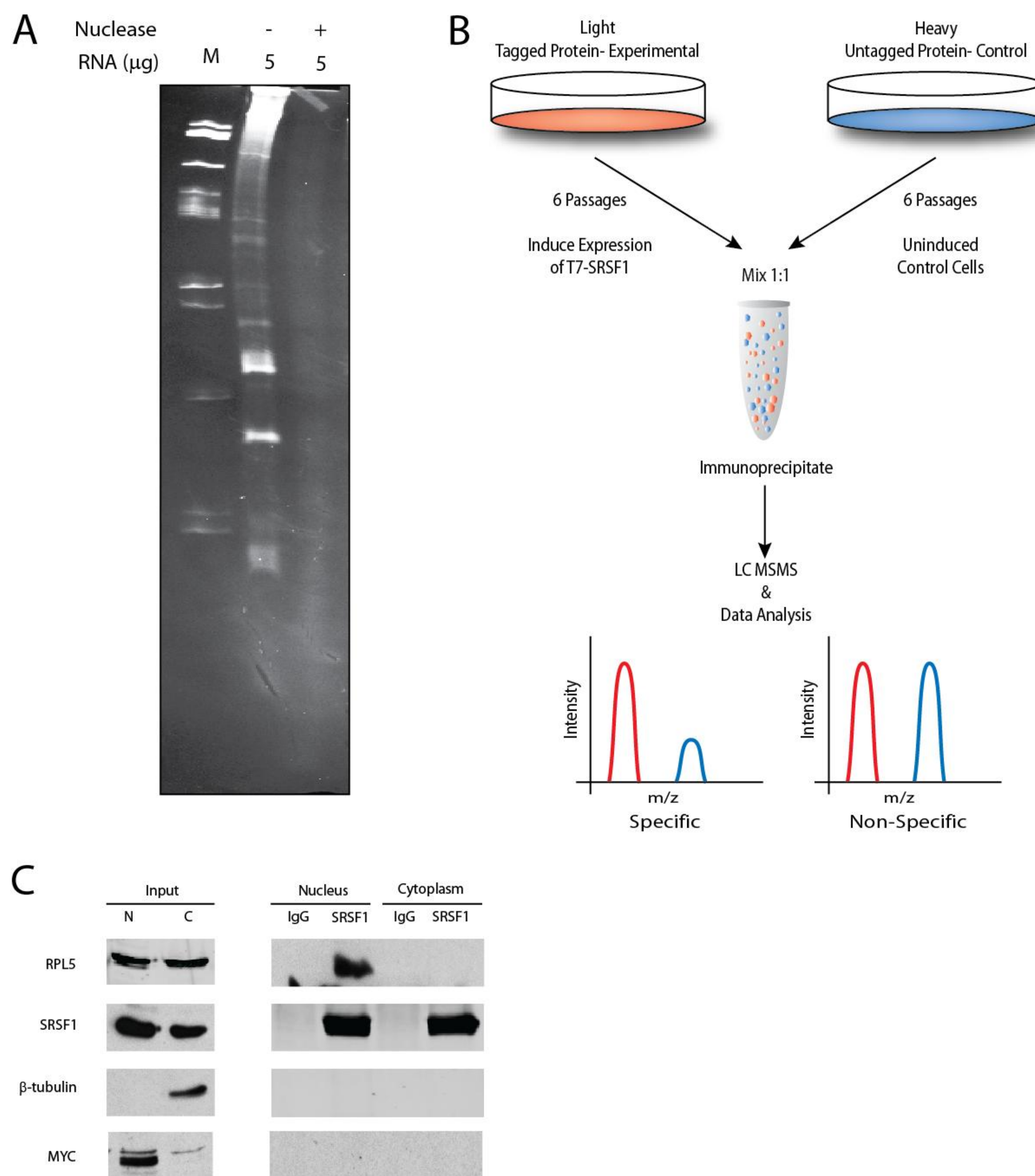

Figure S1. SRSF1 interacts with RPL5 independent of rRNA, mRNA or the ribosome; related to Figure 1. **(A)** Lysates from Hela TT7-SRSF1 were either treated or left untreated with RNase A + T1 Cocktail for 30 min. RNA was extracted from the lysate and analyzed on denaturing polyacrylamide gel following ethidium bromide staining. M refers to DNA marker. **(B)** I-DIRT

procedure to determine specific interactions of splicing factor SRSF1. Inducible cells are grown in light (experimental) or heavy (control) isotopic medium to allow for amino-acid incorporation. After six passages, experimental cells are induced, lysates from control and experimental cells are combined in equal amounts, and the epitope-tagged protein complex is immunoprecipitated. Immunoprecipitates are analyzed by MS, and specific interactions are identified as isotopically light, whereas non-specific, post-lysis interactions are identified as a mixture of isotopically light and heavy. Based on Tackett et al., 2005. **(C)** Nuclear and Cytoplasmic fractions obtained from sub-cellular fractionation of Hela cell lysate were subjected to immunoprecipitation with the AK96 antibody against SRSF1. Whole cell lysates and Immunoprecipitates were analysed by immunoblotting using the indicated antibodies. MYC protein was used as a nuclear marker while β-tubulin was used as a cytoplasmic marker to assess the quality of fractionation.

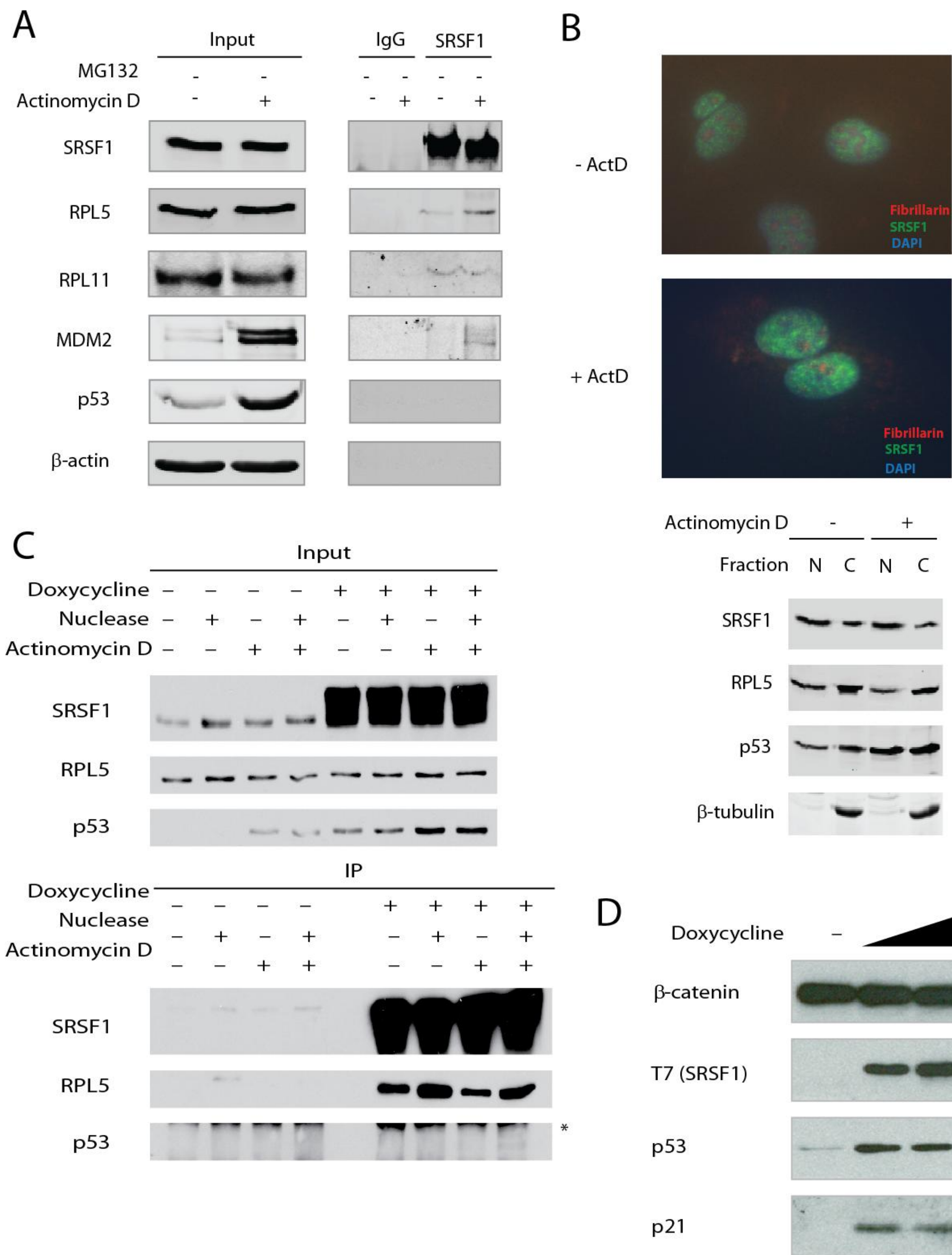

Figure S2. Endogenous SRSF1 physically interacts with MDM2 and RPL5 but not p53, yet induces expression of p53 and p53 target gene; related to Figure 2. **(A)** BJ fibroblast cells were

either treated or untreated with Actinomycin D (5 nM) for 8 h. Lysates were immunoprecipitated with either control IgG or AK96 monoclonal antibody against SRSF1. Whole-cell lysates (Input) and Immunoprecipitations (IP) were analysed with the indicated antibodies. **(B)** BJ cells were treated with Actinomycin D (5 nM) for 8 h, as indicated. SRSF1 sub-cellular localization was analysed either by immunofluorescent staining of SRSF1 and nucleolar marker Fibrillarin (top panels) or sub cellular fractionation wherein lysates were fractionated to obtain nuclear and cytoplasmic fractions, and analyzed by immunoblotting with the indicated antibodies (bottom panels). **(C)** BJ TT7-SRSF1 cells were treated with doxycycline (0.1 μg/mL) for 36 h and actinomycin D (5 nM) for 8 h as indicated. Lysates, with or without nuclease treatment, were immunoprecipitated with T7 monoclonal antibody. Whole cell lysates (Input) and Immunoprecipitates (IP) were analyzed by immunoblotting with the indicated antibodies. The asterisk indicates non-specific binding. **(D)** BJ TT7-SRSF1 cells were induced with increasing concentrations of doxycycline, from 0.1 to 10 μg/mL. Cells were collected in RIPA buffer and analyzed by immunoblotting with the indicated antibodies.

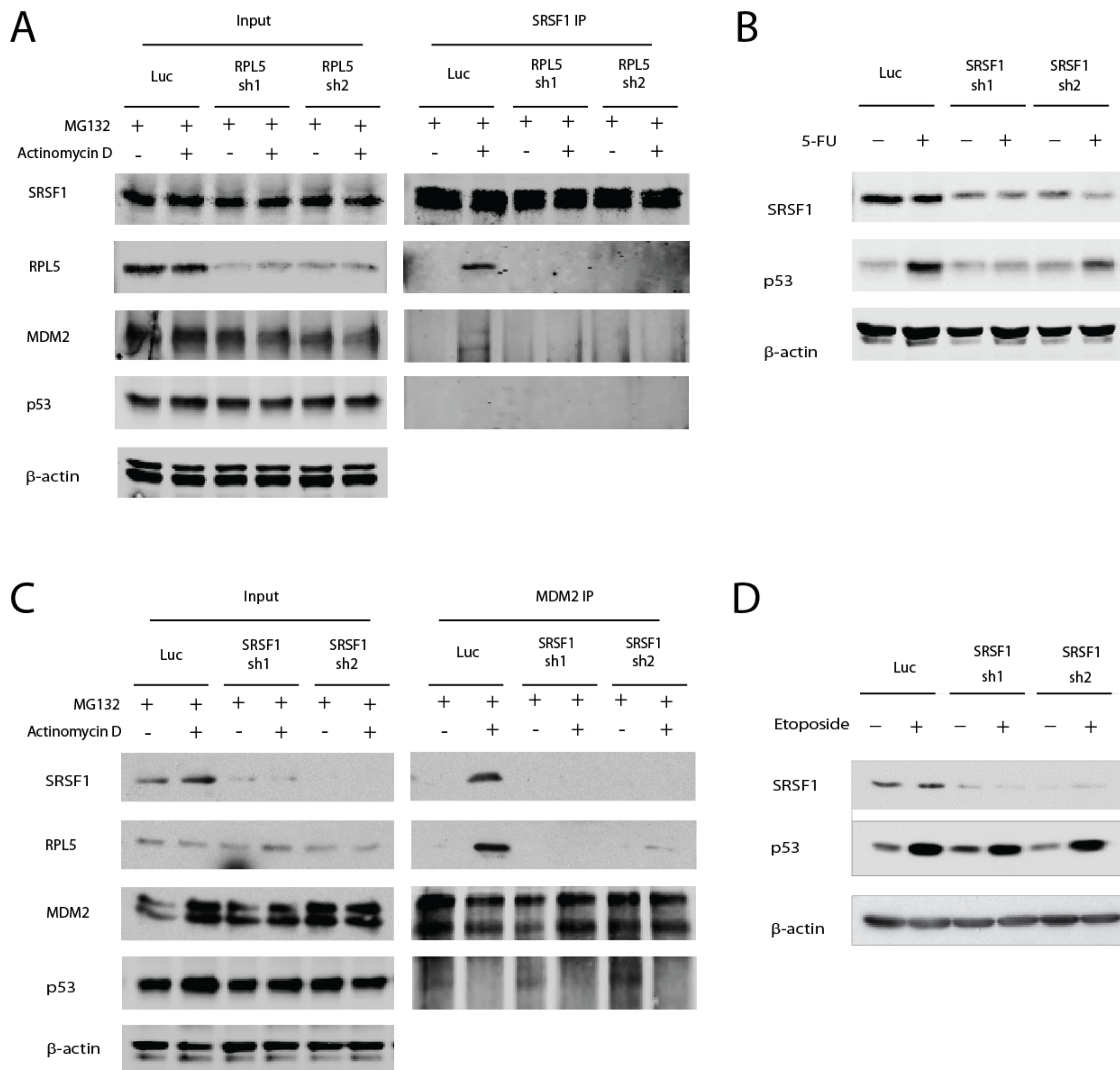

Figure S3. SRSF1 functions specifically through the ribosomal stress pathway to stabilize p53 protein; related to Figure 3. **(A)** U2OS cells transduced with luciferase or RPL5 shRNA were treated with 5 nM actinomycin D for 8 h, followed by 50 µM MG132 for 8h as indicated. Lysates were immunoprecipitated with AK96 monoclonal antibody against SRSF1. Whole cell lysates (Input) and Immunoprecipitates (IP) were analysed by immunoblotting with the indicated

antibodies. **(B)** U2OS cells were transduced with luciferase or SRSF1 shRNA, selected with puromycin, followed by 5-fluorouracil (10 ug/mL) treatment for 8 h, as indicated, and analyzed by immunoblotting. **(C)** U2OS cells transduced with luciferase or SRSF1 shRNA were treated with 5 nM actinomycin D for 8 h, followed by 50 µM MG132 for 8h as indicated. Lysates were immunoprecipitated with anti-MDM2 antibody (2A10, Abcam). Whole cell lysates (Input) and Immunoprecipitates (IP) were analysed by immunoblotting with the indicated antibodies. **(D)** U2OS cells were transduced with luciferase or SRSF1 shRNA, followed by etoposide treatment for 12 h, as indicated, and analyzed by immunoblotting.

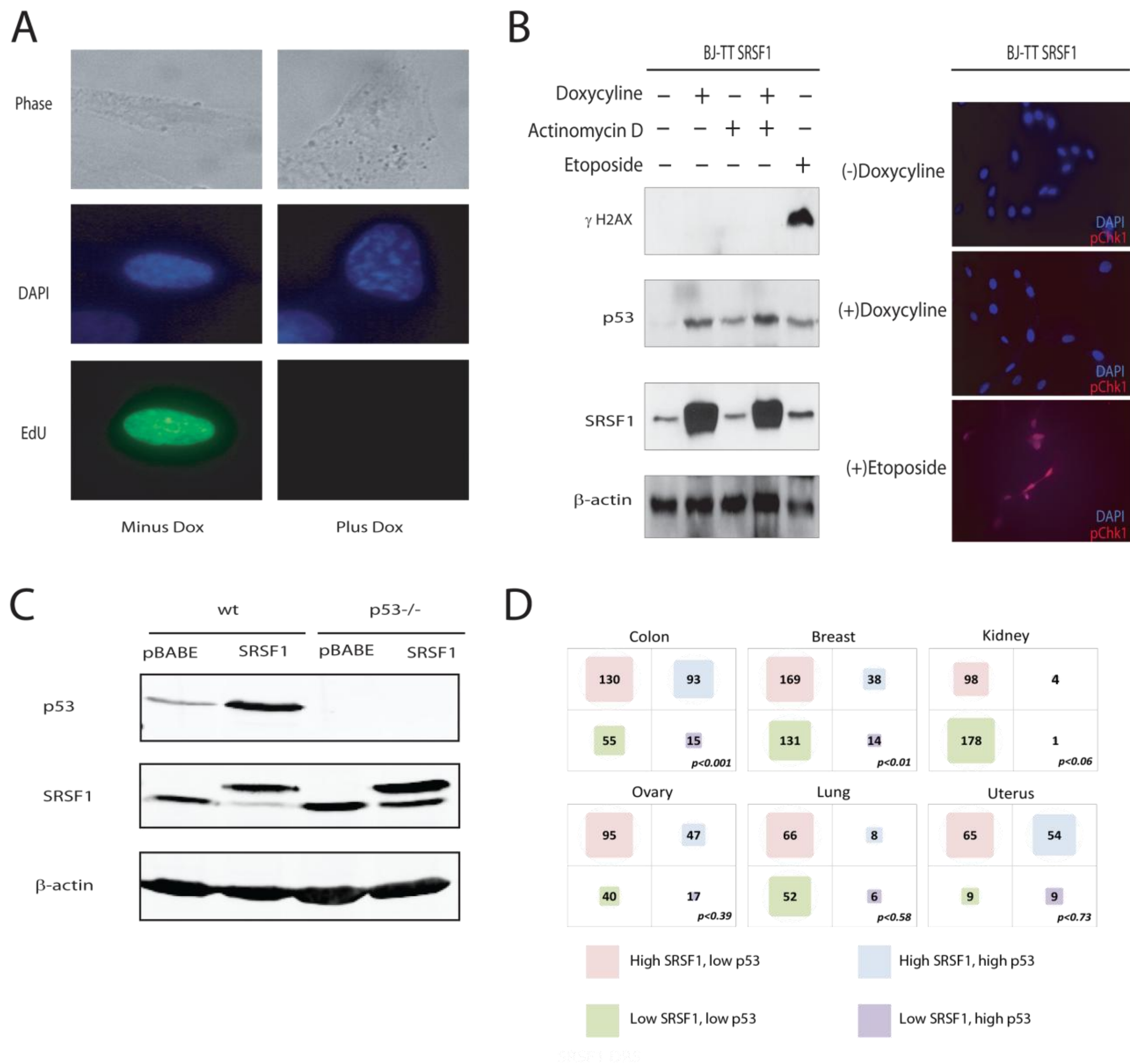

Figure S4. SRSF1-induced p53 stability and senescence are not associated with DNA damage; related to Figure 4. **(A)** Representative day-7 induced and control BJ TT7-SRSF1 cells imaged at 63X for morphology (phase, top), DNA (DAPI, middle), and proliferation (EdU, bottom). **(B)** BJ TT7-SRSF1 cells were treated with or without doxycycline, actinomycin D, or etoposide, and analyzed by immunoblotting (left) or immunofluorescence (right) for the DNA-damage markers γ-H2AX or phospho-CHK1, respectively. 50 nM etoposide was used as a positive control. **(C)**

Wild-type and p53-null MEFs were transduced to overexpress SRSF1. Cells were lysed under denaturing conditions and immunoblotted with the indicated antibodies. **(D)** Expression of *SRSF1* and *TP53* were profiled from microarray data from a collection of human tumors (GSE2109). The data were normalized to Z-score (see Supplemental Experimental Procedures) and divided into categories corresponding to tumors expressing high or low *SRSF1* or *TP53* levels. The plot shows the distribution of the tumors for each condition. The size of the squares is proportional to the number of observations. Fisher test P-values are shown at the bottom.

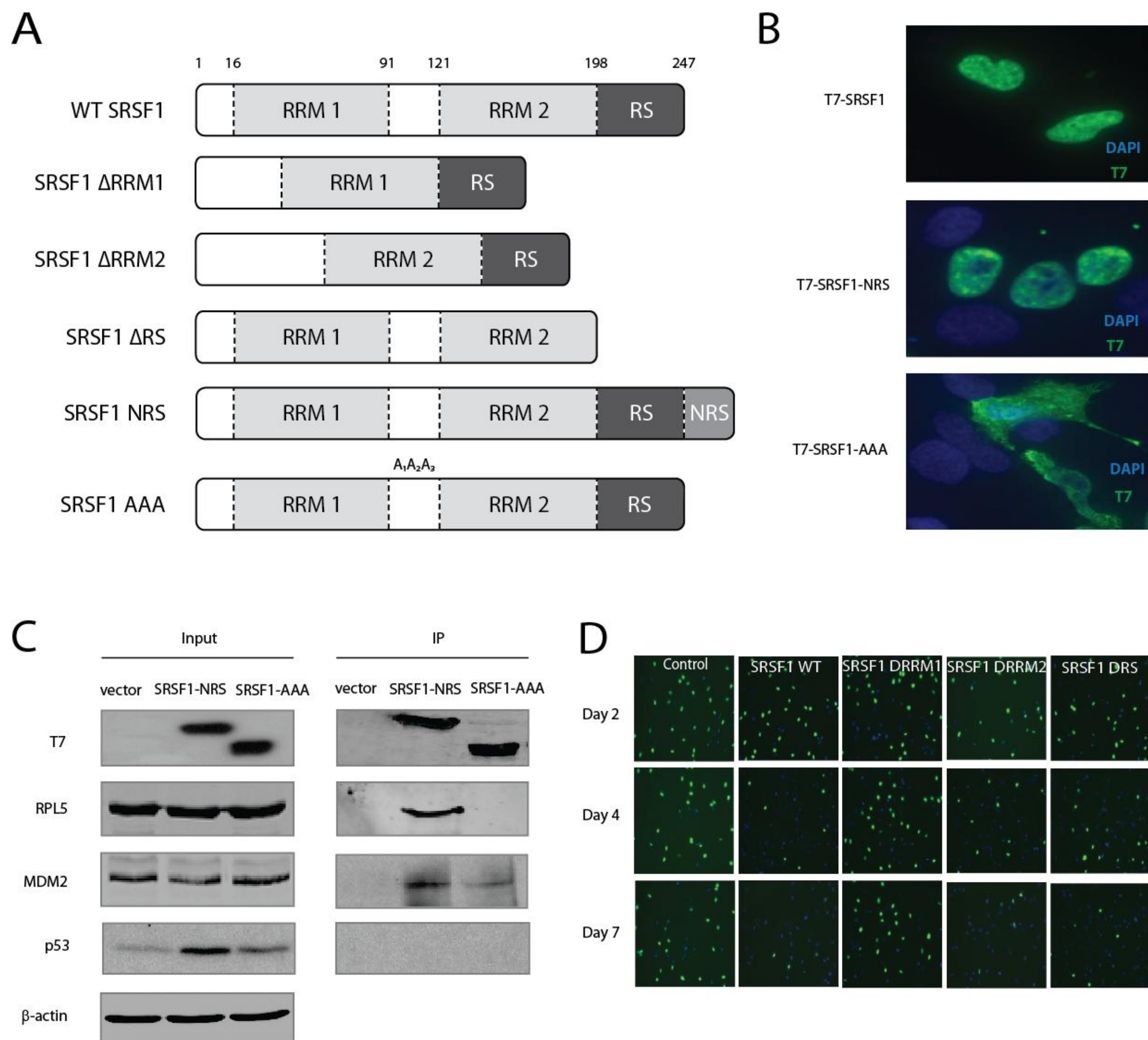

Figure S5. The RRM1 domain of SRSF1 is required for the formation of the ternary SRSF1-RPL5-MDM2 complex in the nucleus and for induction of p53 and cellular senescence; related to Figure 5. **(A)** Schematic representation of wild-type SRSF1, deletion mutants lacking either RRM1, RRM2, or the RS domain, NRS1 construct consisting of a C-terminal fusion to a nuclear retention signal from SRSF2, and the AAA mutant. **(B)** Immunofluorescence staining using anti-T7

monoclonal antibody to show the localization of T7-tagged SRSF1-NRS or SRSF1-AAA mutant proteins, transfected into HeLa cells. DAPI was used to stain the nucleus. **(C)** Lysates from HeLa cells transfected with T7-SRSF1-NRS or T7-SRSF1-AAA mutant construct were immunoprecipitated with T7 monoclonal antibody. Whole-cell lysates (Input) and Immunoprecipitate (IP) were analysed by immunoblotting with the indicated antibodies. **(D)** BJ TT7-SRSF1, BJ-TT7-SRSF1-ΔRRM1, BJ-TT7-SRSF1-ΔRRM2 and BJ-TT7-SRSF1-ΔRS cells were induced with doxycycline for 2, 4, or 7 d, treated with 10 μM EdU, and observed at 20x magnification.

Table S1 Provided as Excel Spreadsheet

Table S1. Ribosomal proteins identified by I-DIRT; related to Figure 1. All ribosomal proteins identified in IP-MS and quantified by stable isotope labeling in culture without nuclease and with nuclease. Locus refers to IPI protein ID; Average Ratio is quantified as light/heavy peptides for a given protein; Standard Deviation based on peptide score; Peptide Number is the total number of peptides identified for a given protein that contain labeled lysine or arginine amino acids; Spectral Number is the total number of spectra identified for a given protein; Description refers to the protein name.

Supplemental Experimental Procedures

Heavy Amino Acid Incorporation

To analyze heavy amino acid incorporation for I-DIRT, nanoflow high-performance liquid chromatography (HPLC) was carried out with an Agilent microfluidic HPLC-ChipCube and analyzed by a quadrupole time-of-flight mass spectrometer (Q-TOF, Agilent 6520) controlled by MassHunter software (Agilent Technologies). Q-TOF data were extracted, searched (EBI-IPI human non-redundant database, Version 3.35), and quantified using the Spectrum Mill MS Proteomics Workbench (Agilent Technologies).

Protein Identification

For protein identification of IPs, peptide mixtures were separated by 13-step MudPIT chromatography carried out with an Eksigent Nano2D HPLC. Peptides were electrosprayed directly into an LTQ Orbitrap XL ion-trap mass spectrometer (Thermo Finnigan). Mass spectrometer scan functions and HPLC solvent gradients were controlled by the Xcalibur data system (Thermo Finnigan). Tandem mass spectra were extracted by RawXtract (fields.scripps.edu) and searched against the EBI-IPI human protein database (version 3.61) as well as a decoy database (Elias et al., 2007). SEQUEST (Thermo Finnigan) and DTASelect (Tabb et al., 2002) were used to identify proteins, and peptide ion intensity ratios were calculated using Census (Park et al., 2008).

Microarray Data Analysis

The GEO GES2109 dataset from the Expression Project for Oncology (http://www.ncbi.nlm.nih.gov/projects/geo/query/acc.cgi?acc=GSE2109) comprises 2158 human cancer samples from clinically annotated tumors. Each sample was standardized by calculating Z-scores based on the sample average and s.d. through the whole set of genes. Expression profiles of *SRSF1* and *TP53* were extracted for all the samples. A contingency table was built, showing for every tissue, the number of samples with high expression (Z score ≥ 1) of both *SRSF1* and *TP53*, only *SRSF1*, only *TP53*, or neither. Fisher exact P-values were then calculated.

RNA Interference

The RPL5 shRNA were purchased from Open Biosystems (Thermo Scientific).

List of Primers

Cloning:

Xho-1 Fwd:

(5'-GGAACTCGAGGCCACCATGGCATCGATGACAGGTGGCCAACAGATGGGTATGTC GGGAGGTGGTGTGAT-3')

EcoR1 Rev:

(5'- GGGGAAGAATTCTTATGTACGAGAGCGAGATC-3')

RT-PCR:

*SRSF1*F: 5'-ATGTCGGGAGGTGGTGTGATTC-3'

*SRSF1*R: 5'-TGTTCCACGGCCGCTTCGAG-3'

p53F: 5’-TACAGTCAGAGCCAACCTCAG-3

p53R: 5’-AGATGAAGCTCCCAGAATGCC-3’

p21F: 5’-ATGTCAGAACCGGCTGGGGATG-3’

p21R: 5’-TTAGGGCTTCCTCTTGGAGAAG-3’

ARF/p14F: 5’-CCCTCGTGCTGATGCTACTGA-3’

ARF/p14R: 5’-ACCACCAGCGTGTCCAGGAA-3’

β-actinF: 5’-GTGCCCATTTATGAGGGCTA-3’

β-actinR: 5’-CTGGCAGCTCGTAGCTCTTT-3’

Supplemental References

Elias, J.E., and Gygi, S.P. (2007). Target decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry. Nature Methods *4*, 207-214.

Park, S. K., Venable, J. D., Xu, T., and Yates, J. R., 3rd. (2008). A quantitative analysis software tool for mass spectrometry-based proteomics. Nature Methods *5*, 319-322.

Tabb, D.L., McDonald, W.H., and Yates, J.R., 3rd. (2002). DTASelect and contrast: tools for selecting and comparing protein identifications from shotgun proteomics. J. Proteome Res. *1*, 21-26.