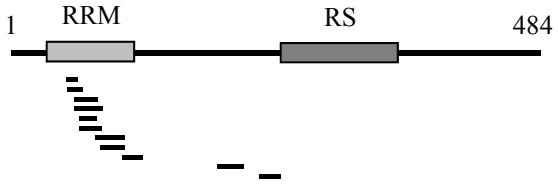
Supplementary figures

A



B

11 matched unique peptides to SRSF11

R.TLFGFLGK.I

R.TLFGFLGKIDELR.L

K.IDELRLFPPDDSPLPVSSR.V

K.IDELRLFPPDDSPLPVSSRVCFVK.F

R.LFPPDDSPLPVSSR.V

R.LFPPDDSPLPVSSRVCFVK.F

R.VCFVKFHDPDSAVVAQHLTNTVFVDR.A

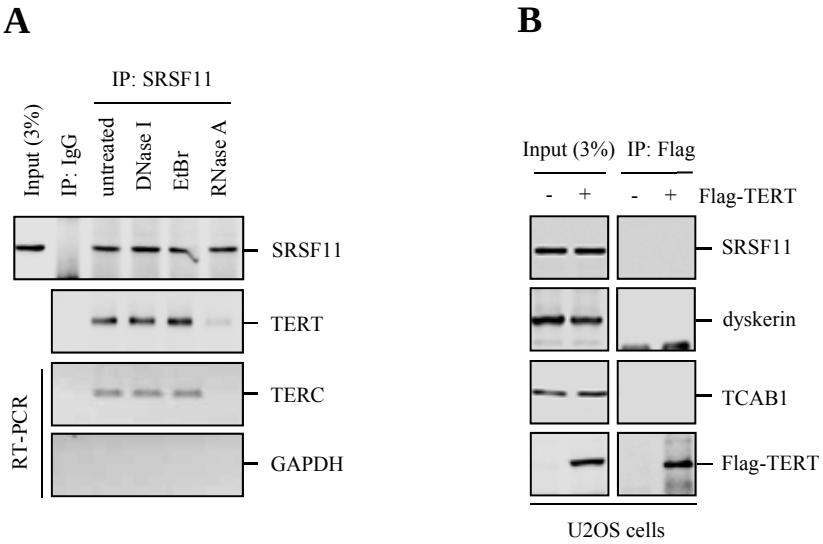
K.FHDPDSAVVAQHLTNTVFVDR.A

R.ALIVVPYAEGVIPDEAK.A

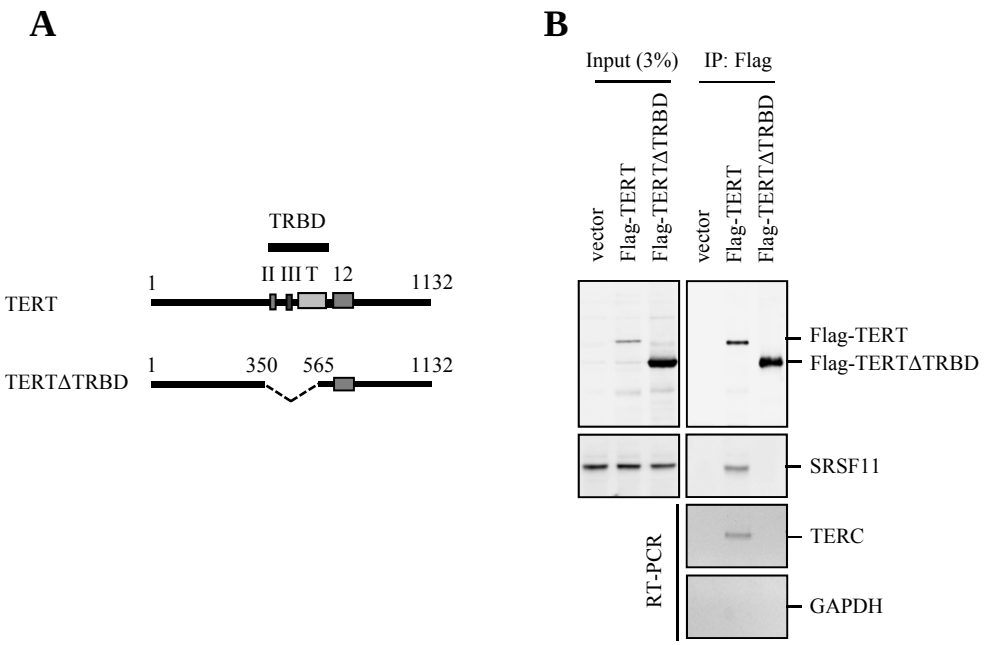
K.LMSTVDPKLNHVAAGLVSPSLK.S

R.EAQSLISAAIEPDKKEEK.R

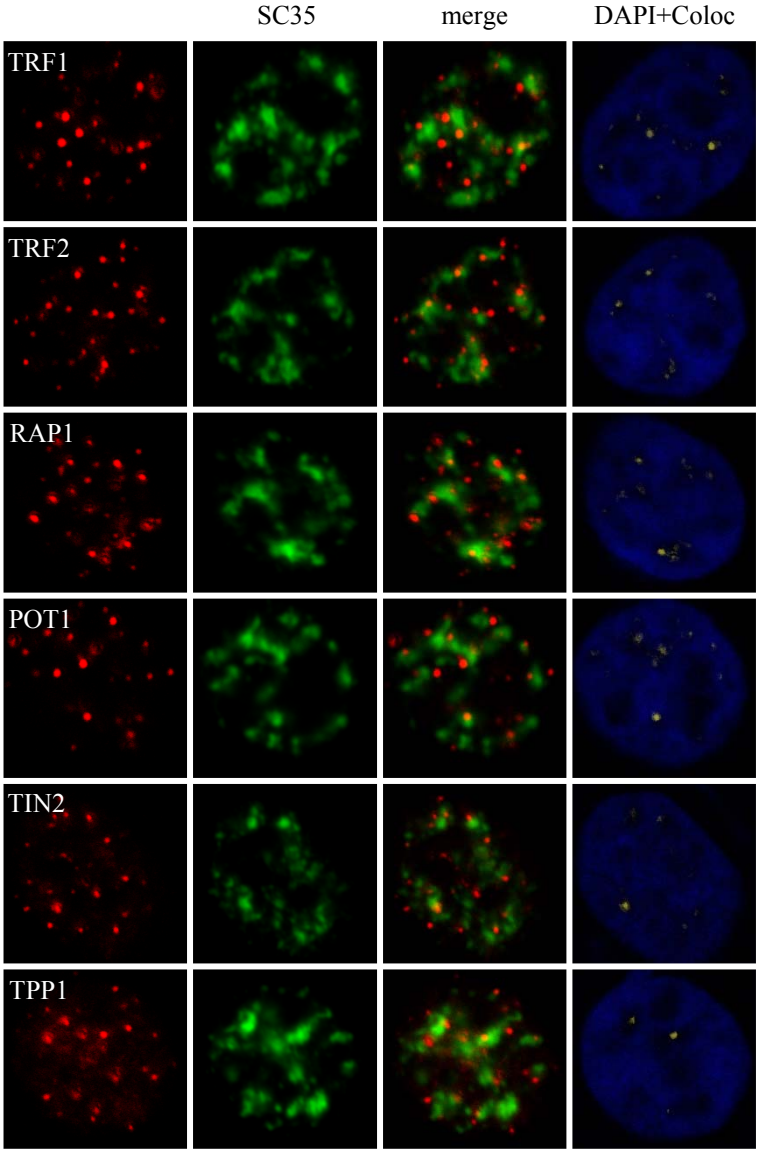
Supplementary Figure S1. Identification of SRSF11 as a TERC-interacting protein by nano-liquid chromatography-tandem mass spectrometry (nano LC-MS/MS). (**A**) RNA affinity purification of TERC complexes from HeLa S3 cells identified multiple unique peptides for SRSF11 by nano LC-MS/MS. Diagram of SRSF11 protein shows location of unique peptides. RRM, RNA recognition motif; RS, arginine and serine-rich domain. (**B**) Eleven SRSF11 peptides from mass spectrometry are shown.



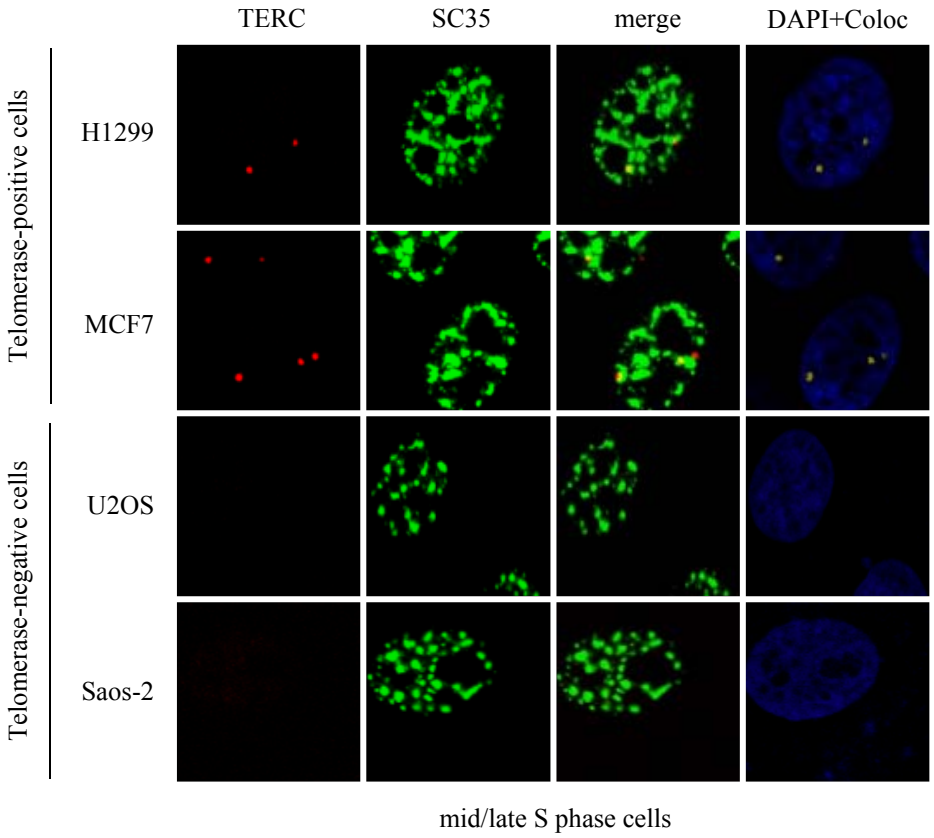
Supplementary Figure S2. SRSF11 association with TERT is RNase A-sensitive. (**A**) Lysates from HeLa S3 cells were immunoprecipitated with anti-SRSF11 antibody, followed by immunoblotting with anti-TERT antibody and quantitative RT-PCR to detect TERC. The extracts were treated with 0.1 mg/ml DNase I, 50 μg/ml ethidium bromide (EtBr), and 0.1 mg/ml RNase A during immunoprecipitation. IgG was used as a negative control. (**B**) Lysates from telomerase-negative U2OS cells expressing Flag-TERT were immunoprecipitated with anti-Flag antibody, followed by immunoblotting with anti-SRSF11, anti-dyskerin, anti-TCAB1, and anti-Flag antibodies.



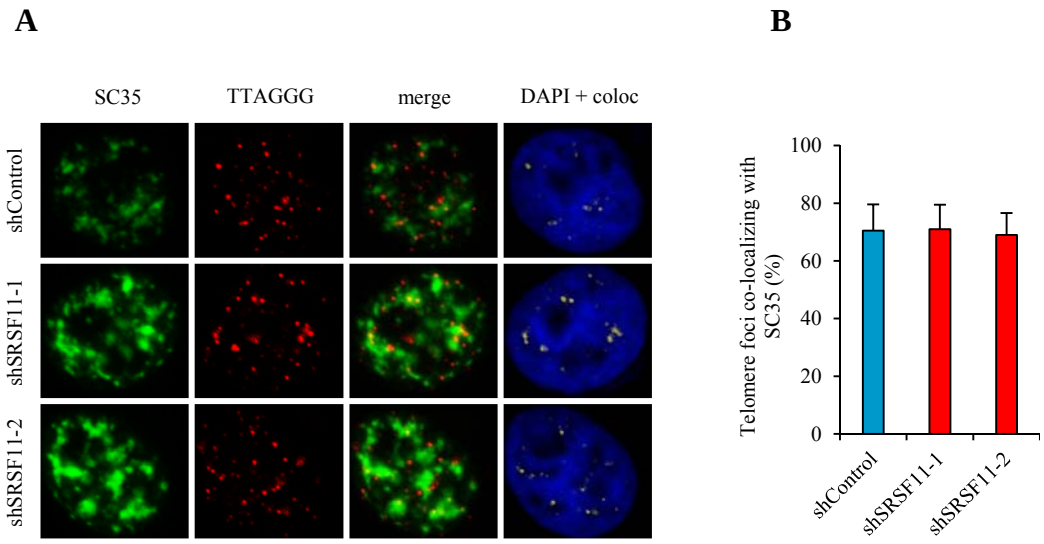
Supplementary Figure S3. Removing the TERC-binding domain (TRBD) on TERT abolishes SRSF11 association and TERC binding. (**A**) Schematic representation of mutant construct of hTERT in which the TERC-binding domain was deleted (TERTΔTRBD). (**B**) Lysates from HeLa S3 cells expressing Flag-TERT or TERTΔTRBD were immunoprecipitated with anti-Flag antibody, followed by immunoblotting with anti-SRSF11 antibody and quantitative RT-PCR to detect TERC.



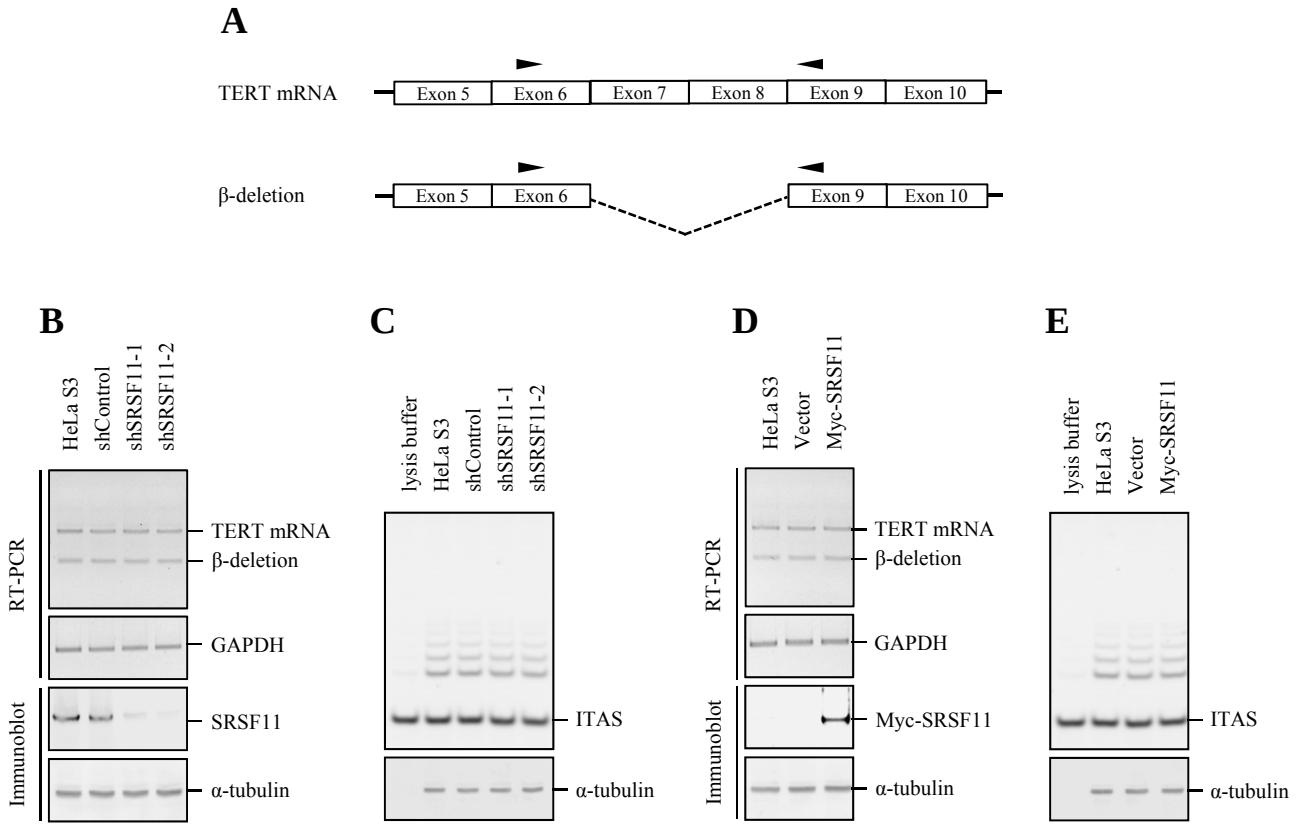
Supplementary Figure S4. The shelterin components co-localize to nuclear speckles. HeLa S3 cells were analyzed by indirect immunofluorescence for co-localization of the various shelterin components with SC35 (nuclear speckle marker). Co-localization channels were calculated using Meta Imaging Series^{R} MetaMorph software and appear in white in all figures. DNA was stained with DAPI. Coloc, co-localization.



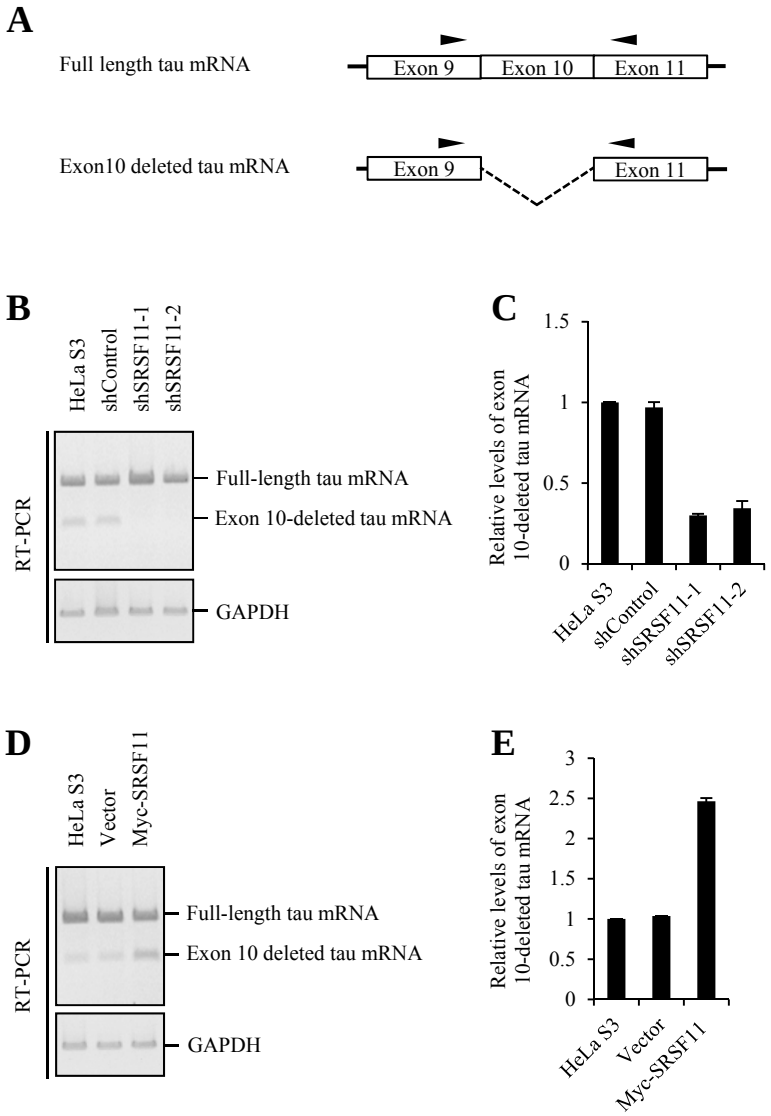
Supplementary Figure S5. TERC co-localizes to nuclear speckles during S phase in telomerase-positive cells. Telomerase-positive H1299 and MCF7 cells and telomerase-negative U2OS and Saos-2 cells were synchronized in mid/late S phase and analyzed by indirect immunofluorescence for co-localization of TERC with SC35. Co-localization channels were calculated using Meta Imaging Series^{R} MetaMorph software and appear in white in all figures. DNA was stained with DAPI. Coloc, co-localization.



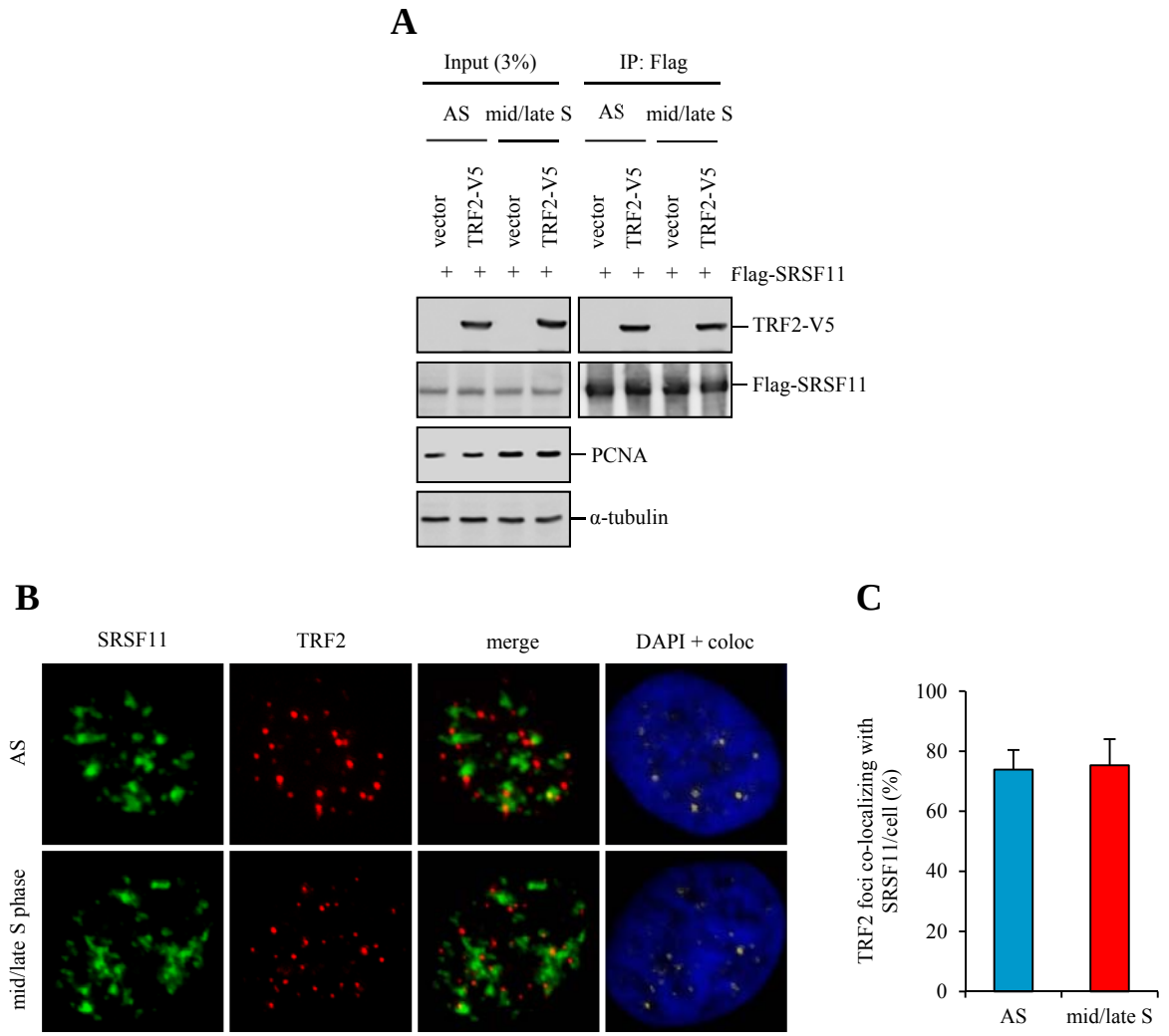
Supplementary Figure S6. Telomeres localize to nuclear speckles after depletion of SRSF11. (**A**) HeLa S3 cells expressing control shRNA (shControl) or SRSF11 shRNAs (shSRSF11-1 and shSRSF11-2) were analyzed by indirect immunofluorescence for co-localization of SC35 with telomeric sites marked by TTAGGG-specific FISH probe. Co-localization channels were calculated using Meta Imaging Series^{R} MetaMorph software and appear in white in all figures. DNA was stained with DAPI. Coloc, co-localization. (**B**) The average percentage of telomere foci co-localizing with SC35 per cell and standard deviation were determined by analyzing >100 nuclei.



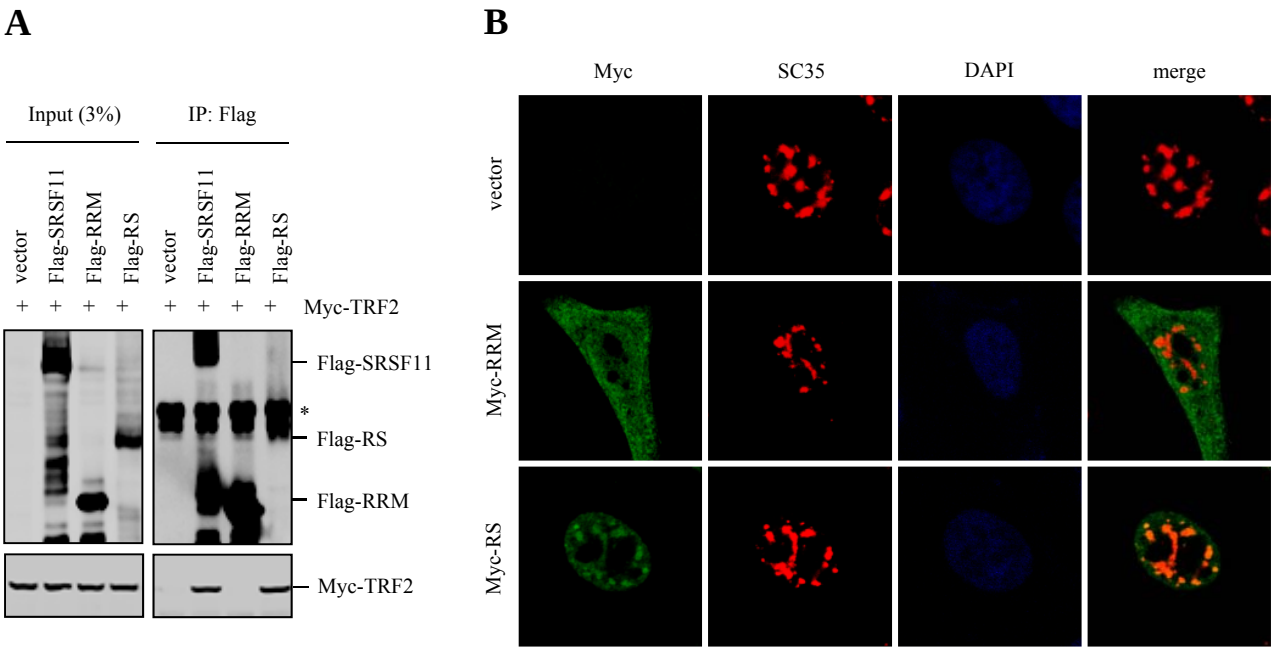
Supplementary Figure S7. The levels of TERT mRNA and β-deletion transcript were not significantly changed upon depletion or overexpression of SRSF11. (**A**) Schematic representation of full-length TERT mRNA and β-deletion transcript. Primers for quantitative RT-PCR analysis are indicated by a pair of arrowheads and reveal a 333-bp fragment from full-length TERT mRNA and a 150-bp fragment from β-deletion transcript. (**B**) HeLa S3 cells expressing control shRNA (shControl) or SRSF11 shRNA (shSRSF11-1 and shSRSF11-2) were subjected to quantitative RT-PCR to measure the levels of TERT variant mRNAs and immunoblotting with anti-SRSF11 antibody. (**C**) HeLa S3 cells expressing shControl or shSRSF11 were analyzed for telomerase activity by the TRAP assay. ITAS, internal telomerase assay standard. (**D**) HeLa S3 cells expressing the empty vector or Myc-SRSF11 were subjected to quantitative RT-PCR to measure the levels of TERT variant mRNAs and immunoblotting with anti-SRSF11 antibody. (**E**) HeLa S3 cells expressing the empty vector or Myc-SRSF11 were analyzed for telomerase activity by the TRAP assay. ITAS, internal telomerase assay standard.



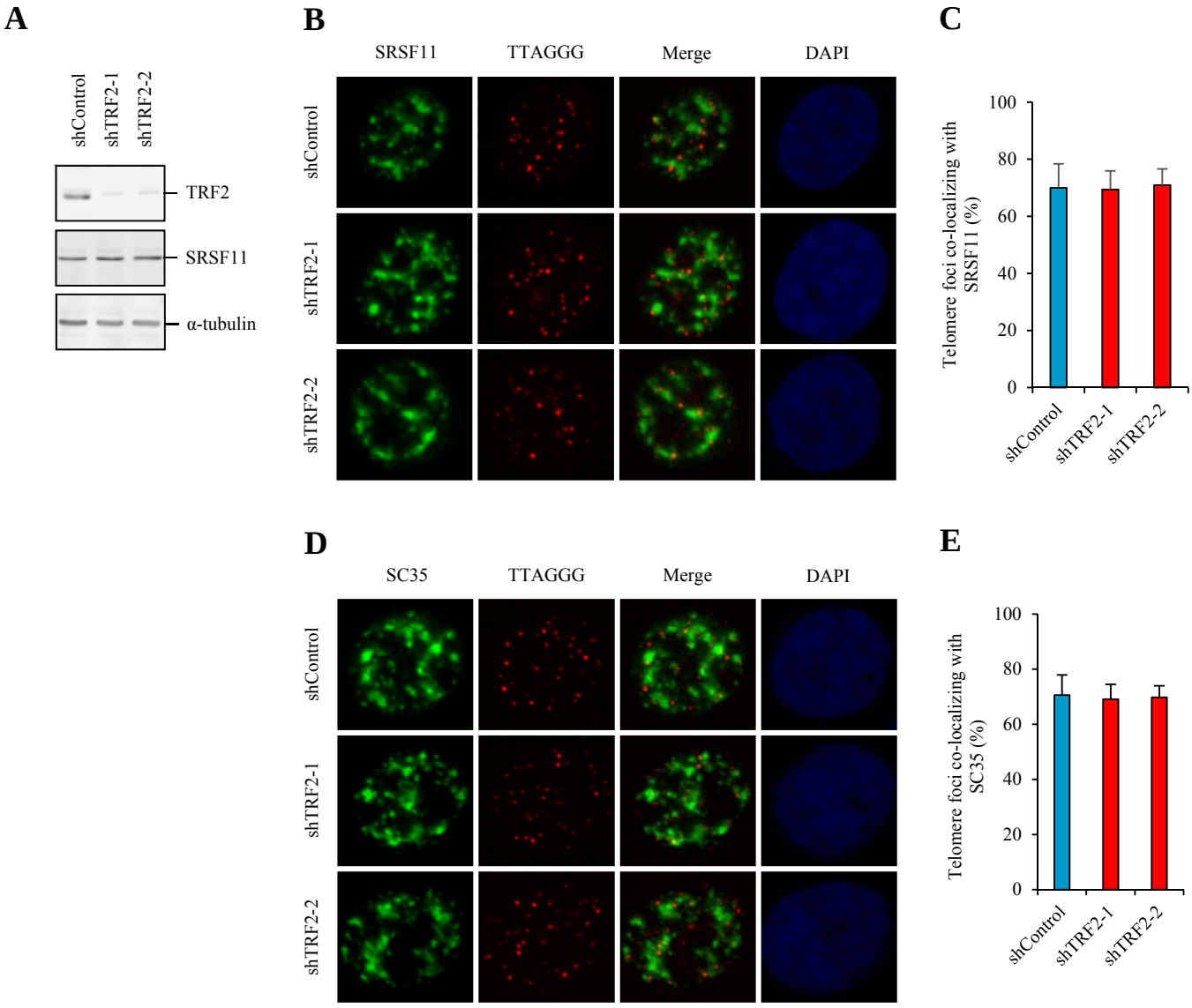
Supplementary Figure S8. The levels of full-length tau mRNA and exon 10-deleted tau mRNA were measured upon depletion or overexpression of SRSF11. (**A**) Schematic representation of full-length tau mRNA and exon 10-deleted tau mRNA. Primers for quantitative RT-PCR analysis are indicated by a pair of arrowheads and reveal a 280-bp fragment from full-length tau mRNA and a 187-bp fragment from exon 10-deleted tau mRNA. (**B**) HeLa S3 cells expressing control shRNA (shControl) or SRSF11 shRNA (shSRSF11-1 and shSRSF11-2) were subjected to quantitative RT-PCR to measure the levels of tau variant mRNAs. (**C**) Graphical representation of the relative levels of exon 10-deleted tau mRNA normalized against full-length tau mRNA. The mRNA levels were quantified with the average and standard deviation from three independent experiments. (**D**) HeLa S3 cells expressing the empty vector or Myc-SRSF11 were subjected to quantitative RT-PCR to measure the levels of tau variant mRNAs. (**E**) Graphical representation of the relative levels of exon 10-deleted tau mRNA normalized against full-length tau mRNA.



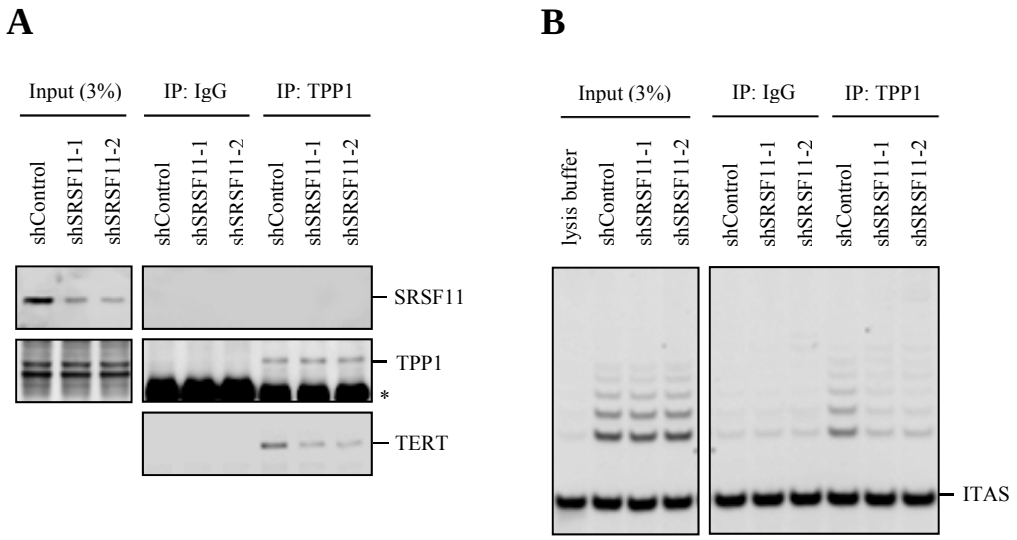
Supplementary Figure S9. SRSF11 interacts with TRF2 irrespective of cell cycle phase. (**A**) HeLa S3 cells expressing Flag-SRSF11 and TRF2-V5 were synchronized in mid/late S phase and subjected to immunoprecipitation with Flag-antibody, followed by immunoblotting with anti-V5 antibody. Immunoblot for proliferation cell nuclear antigen (PCNA) was used for an independent marker of S phase. AS, asynchronous cells. (**B**) HeLa S3 cells were synchronized in mid/late S phase and analyzed by indirect immunofluorescence for co-localization of SRSF11 with TRF2. Co-localization channels were calculated using Meta Imaging Series^{R} MetaMorph software and appear in white in all figures. DNA was stained with DAPI. Coloc, co-localization. (**C**) The average percentage of TRF2 foci co-localizing with SRSF11 per cell and standard deviation were determined by analyzing >100 nuclei for each cell cycle phase.



Supplementary Figure S10. Identification of the domain in SRSF11 that is required for TRF2 binding. (**A**) HeLa S3 cells were co-transfected with Myc-TRF2 and Flag-SRSF11, Flag-RRM, or Flag-RS and subjected to immunoprecipitation with anti-Flag antibody, followed by immunoblotting with anti-Myc antibody. The asterisk marks the position of nonspecific immunoglobulin chains. (**B**) Subcellular localization of the SRSF11 deletion mutants, RRM and RS. HeLa S3 cells were transfected with Myc-RRM or Myc-RS and analyzed by indirect immunofluorescence for co-localization of RRM or RS with SC35. DNA was stained with DAPI.



Supplementary Figure S11. Depletion of TRF2 has no effect on co-localization of telomeres with nuclear speckles. (**A**) HeLa S3 cells expressing control shRNA (shControl) or TRF2 shRNAs (shTRF2-1 and shTRF2-2) were subjected to immunoblotting to measure the levels of endogenous TRF2 and SRSF11. (**B**) HeLa S3 cells expressing shControl or shTRF2 were analyzed by indirect immunofluorescence for co-localization of SRSF11 with telomeric sites marked by TTAGGG-specific FISH probe. DNA was stained with DAPI. (**C**) The average percentage of telomere foci co-localizing with SRSF11 per cell and standard deviation were determined by analyzing >100 nuclei. (**D**) HeLa S3 cells expressing shControl or shTRF2 were analyzed by indirect immunofluorescence for co-localization of SC35 with telomeric sites marked by TTAGGG-specific FISH probe. DNA was stained with DAPI. (**E**) The average percentage of telomere foci co-localizing with SC35 per cell and standard deviation were determined by analyzing >100 nuclei.



Supplementary Figure S12. Telomerase recruitment to telomeres by SRSF11 precedes TPP1. (**A**) HeLa S3 cells expressing control shRNA (shControl) or SRSF11 shRNAs (shSRSF11-1 and shSRSF11-2) were subjected to immunoprecipitation with anti-TPP1 antibody, followed by immunoblotting with anti-TERT antibody. IgG was used as a negative control. The asterisk marks the position of nonspecific immunoglobulin chains. (**B**) HeLa S3 cells expressing shControl or shSRSF11 were subjected to immunoprecipitation with anti-TPP1 antibody. Immunoprecipitates of endogenous TPP1 were analyzed for telomerase activity by the TRAP assay. ITAS, internal telomerase assay standard.