Moriel-Carretero et al., https://doi.org/10.1083/jcb.201702136

A

	Count in gene set (219)	false discovery rate
Cellular Component (GO)		
Ribonucleoprotein complex (0030529)	96	1.43e-84
Nuclear lumen (0031981)	150	1.33e-67
Molecular function (GO)		
RNA binding (0003723)	152	8.38e-119
Nucleic acid binding (0003676)	173	2.54e-87
Biological process (GO)		
RNA processing (0006396)	92	1.1e-74
RNA splicing (0008380)	72	1.79e-72
KEGG pathway		
Splicesosome (03040)	44	2.35e-53
Ribosome (03010)	38	1.48e-42
PFAM Protein Domains		
RNA recognition motif (PF00076)	31	1.38e-31

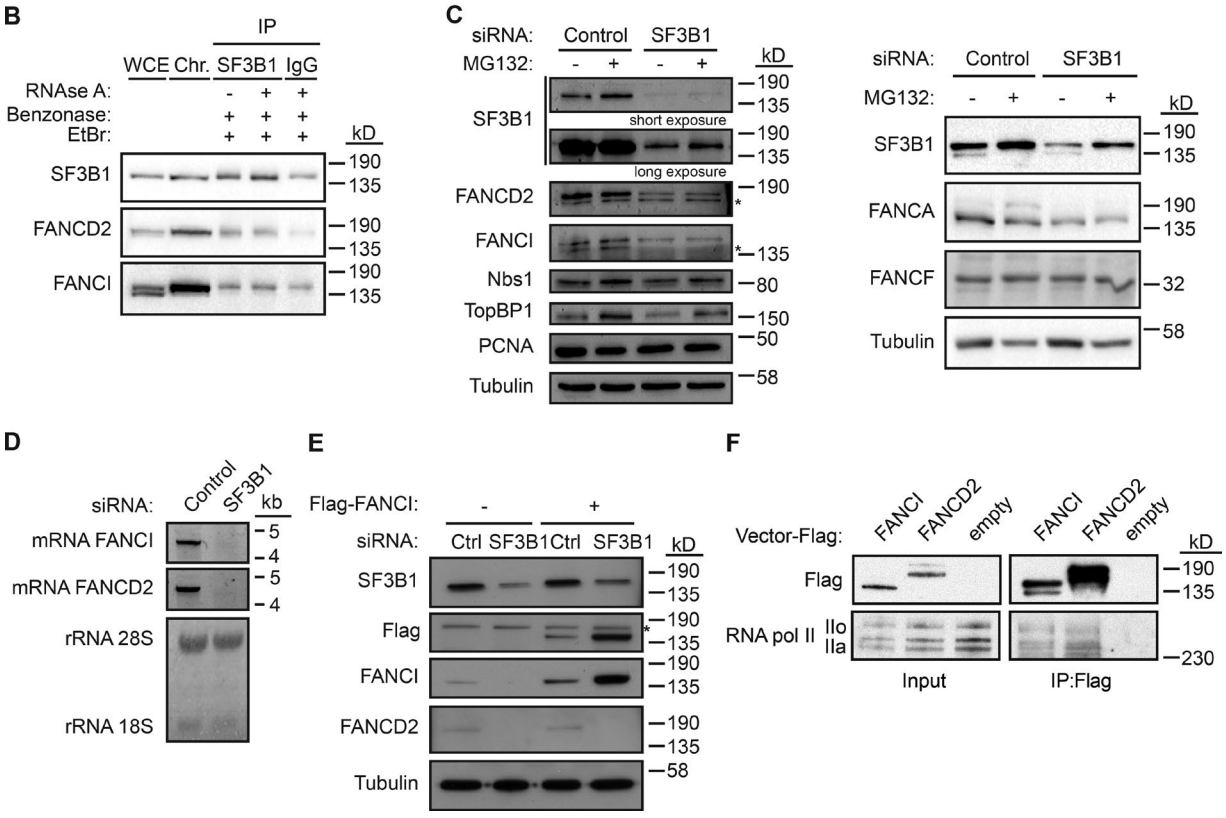

Figure S1. **Characterization of the relationship between SF3B1 and FANCI/FANCD2.** (A) Functional enrichments of proteins detected by at least three peptides in Flag-FANCI–eluted fractions and absent in the control purification. 219 proteins fulfilled this criterion. Count in gene set is the number of hits in this set of 219 genes. Geneset analysis was performed using STRING (http://string-db.org/). GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PFAM, database of protein domain families. (B) SF3B1 IP from chromatin extract of HEK293 cells in the presence of benzonase and ethidium bromide (EtBr) with or without RNase A. (C) U2OS cells were transfected with the indicated siRNAs, and total protein extracts were prepared 48 h after transfection. Where indicated, cells were further treated with 10 µM MG132 for 4 h. WB of the indicated proteins was performed to score for their relative amount. (D) Total RNA was prepared from U2OS cells 48 h after their transfection with the indicated siRNAs. After quantification, an equal amount of RNA was loaded on a gel (see rRNAs as loading control) or retrotranscribed and subjected to nonsaturating, semiquantitative PCR to check for the presence of FANCI and FANCD2 mRNAs. (E) U2OS cells were simultaneously transfected with the indicated siRNAs (Ctrl, siControl) and a Flag-empty vector (–) or a Flag-FANCI–expressing vector (+). IB of the indicated proteins was performed to score for their relative amount. *, nonspecific band. (F) HEK293T cells were transfected with Flag-FANCI, Flag-FANCD2, or a Flag-empty vector. Chromatin extracts were prepared and subjected to IP with Flag antibodies to detect interaction with RNA-PolII. IIa and IIo indicate the hypo- and hyperphosphorylated forms of RNA-PolII, respectively.

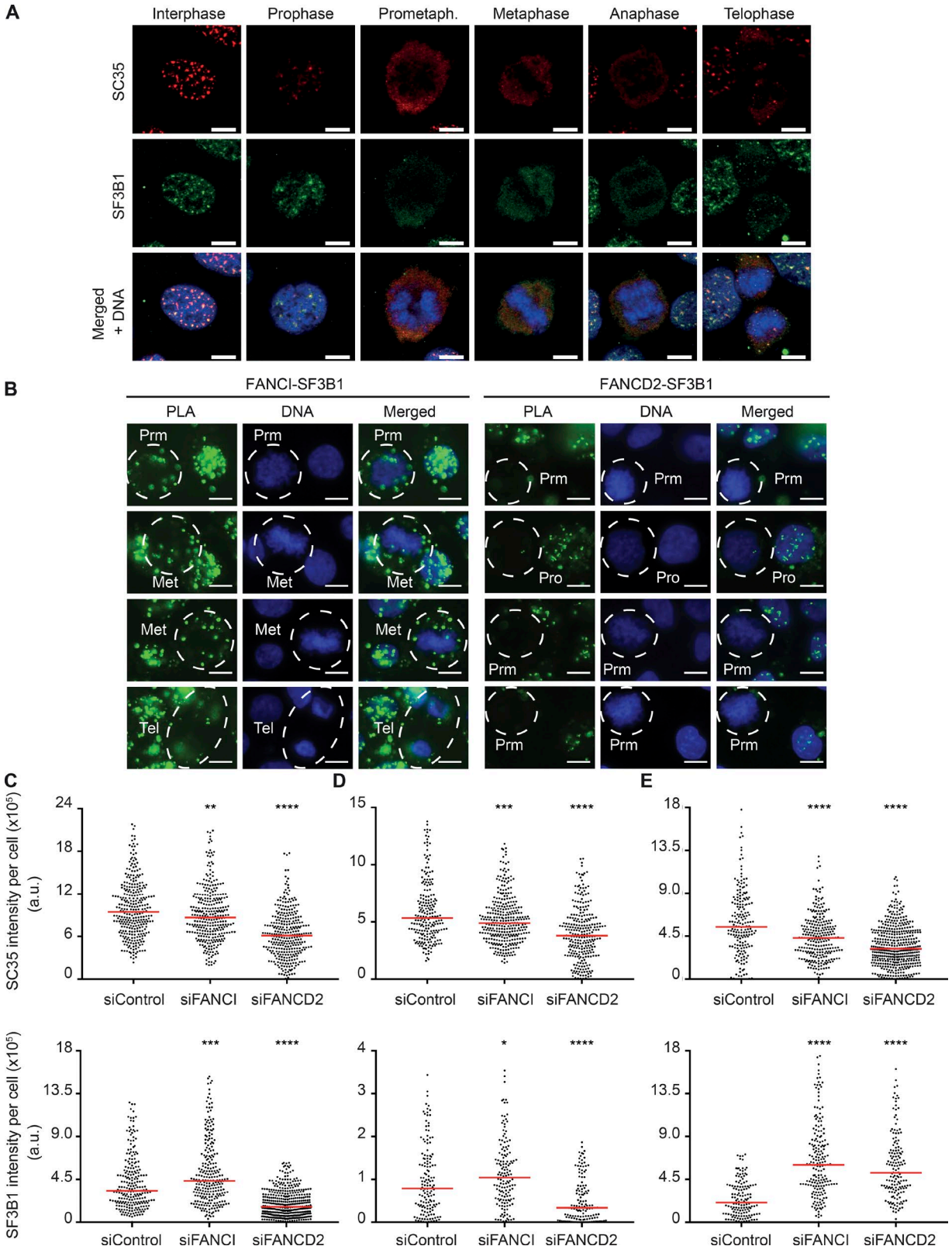

Figure S2. **FANCI and FANCD2 localize differently with SF3B1 during the cell cycle and control SF dynamics.** (A) U2OS cells were fixed with methanol, and SC35 (red) and SF3B1 (green) were detected by IF. DNA (blue) dyed with Hoechst. Representative images of each mitosis phase are shown. (B) Additional images for PLA signals for FANCI-SF3B1 and FANCD2-SF3B1 in U2OS cells in mitosis. Discontinuous circles highlight mitoses. Pro, prophase; Prm, prometaphase; Met, metaphase; Tel, telophase. (C–E) The experiments displayed in Fig. 3 were repeated three additional times, presented here. C and D are two experiments performed with the same set of siRNA, whereas E corresponds to a repetition performed with an siRNA targeting different FANCI and FANCD2 sequences. The red bar marks the median value in each condition. The significance by Student's *t* test is indicated by asterisks and p-value (****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.1$). a.u., arbitrary units. Bars, 10 µm.

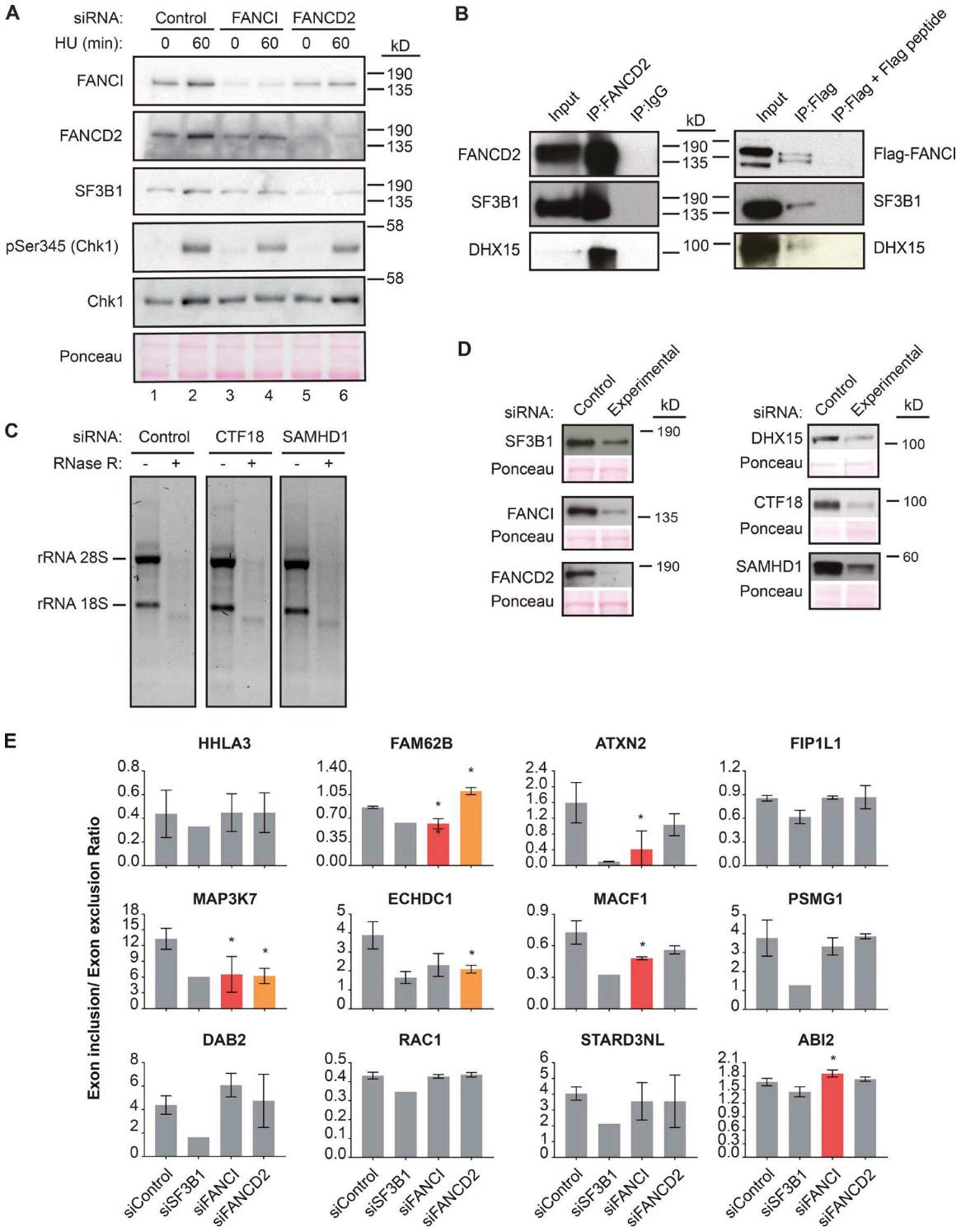

Figure S3. **FANCI and FANCD2 down-regulation impacts SF3B1-sensitive alternative splicing events.** (A) Cell lysates from U2OS cells transfected for 48 h with the indicated siRNAs obtained from Qiagen for FANCI and FANCD2 were analyzed by IB. Depletion levels are representative of the experiments showed in Fig. S2 E and Fig. S5 (D and E). Where indicated, for Fig. S5 (D and E), cells were treated for 60 min with 5 mM HU to induce replication stress, and the effect of this treatment was verified by phosphorylation of Ser345-Chk1. (B) Immunoprecipitates of FANCD2 and Flag-FANCI from Flag-FANCI stable HEK293 chromatin extracts were analyzed for FANCD2, FANCI, SF3B1, and DHX15 by IB. (C) As an additional control, the same experiment as in Fig. 4 A was performed in CTF18- and SAMHD1-depleted cells. (D) Representative images of siRNA-mediated depletions performed to accomplish the experiments of Figs. 4 A and S3 C. (E) Quantifications of the impact on alternative splicing in HEK293T cells of the depletion of SF3B1, FANCI, or FANCD2 from three biological replicates (n = 3), ≥120 cells each, as described in the legend to Fig. 4 C.

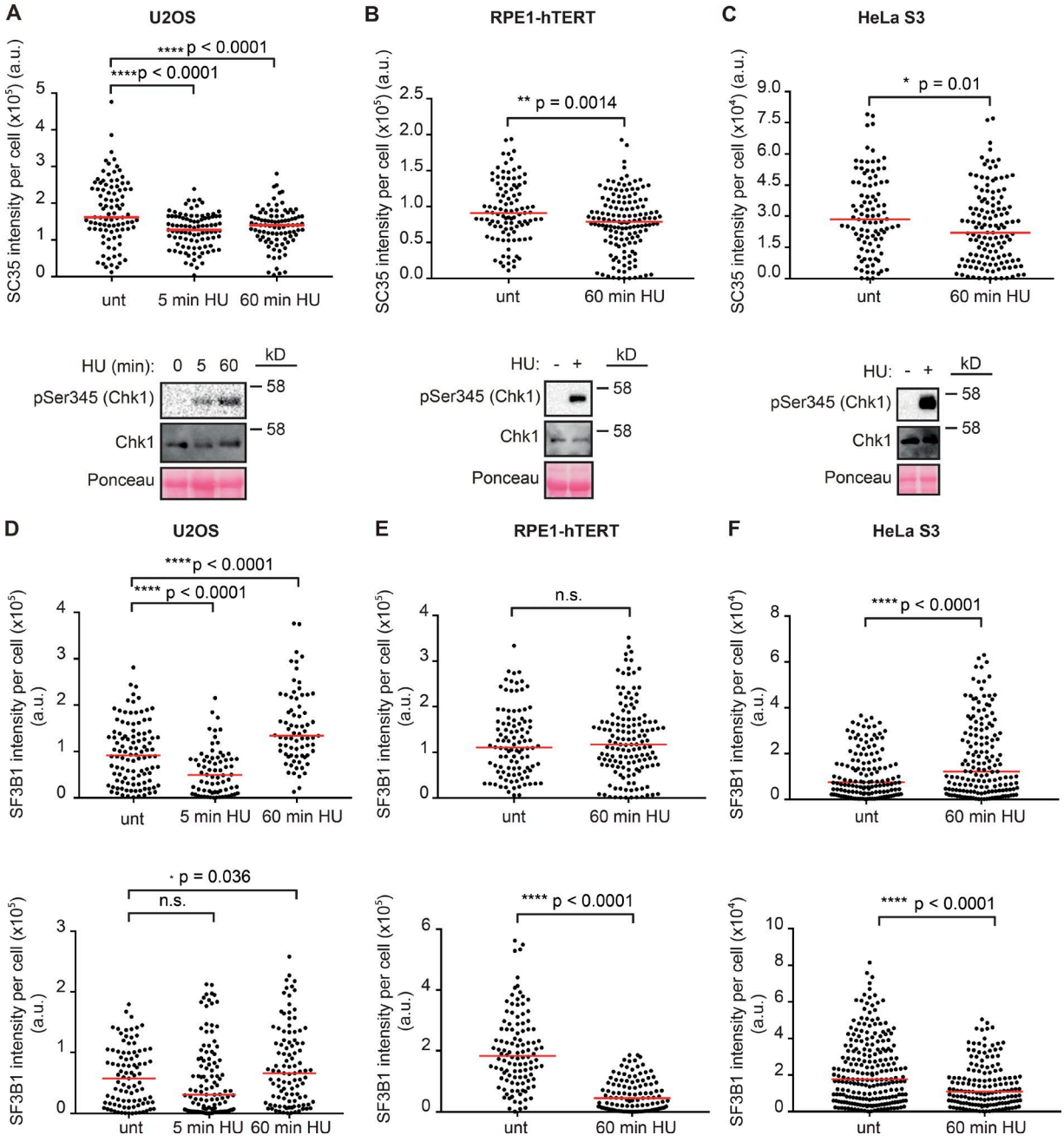

Figure S4. **HU-induced replication stress promotes SF release from speckles in tumoral and non-tumoral cell lines.** (A–C) Replicates of experiments shown in Fig. 6 A performed in U2OS (A), RPE1-hTERT (B), and HeLa S3 (C) cells. Cells were exposed to 5 mM HU for the indicated times, fixed, and processed to detect SC35. Speckles intensity per cell from a minimum of 100 cells is plotted; the red bar marks the median value in each condition. The significance after by Student's *t* test is indicated by asterisks and *p*-values. Immunoblots show Chk1 phosphorylation on Ser345 as a marker to evaluate the efficiency of HU-induced checkpoint activation in each cell line. (D–F) Graphs show two biological replicates performed as in Figs. 6 A and S4 A in U2OS (D), RPE1-hTERT (E), and HeLa S3 (F) cells. Quantification of SF3B1-labeled speckle intensity performed as described in Fig. 3.

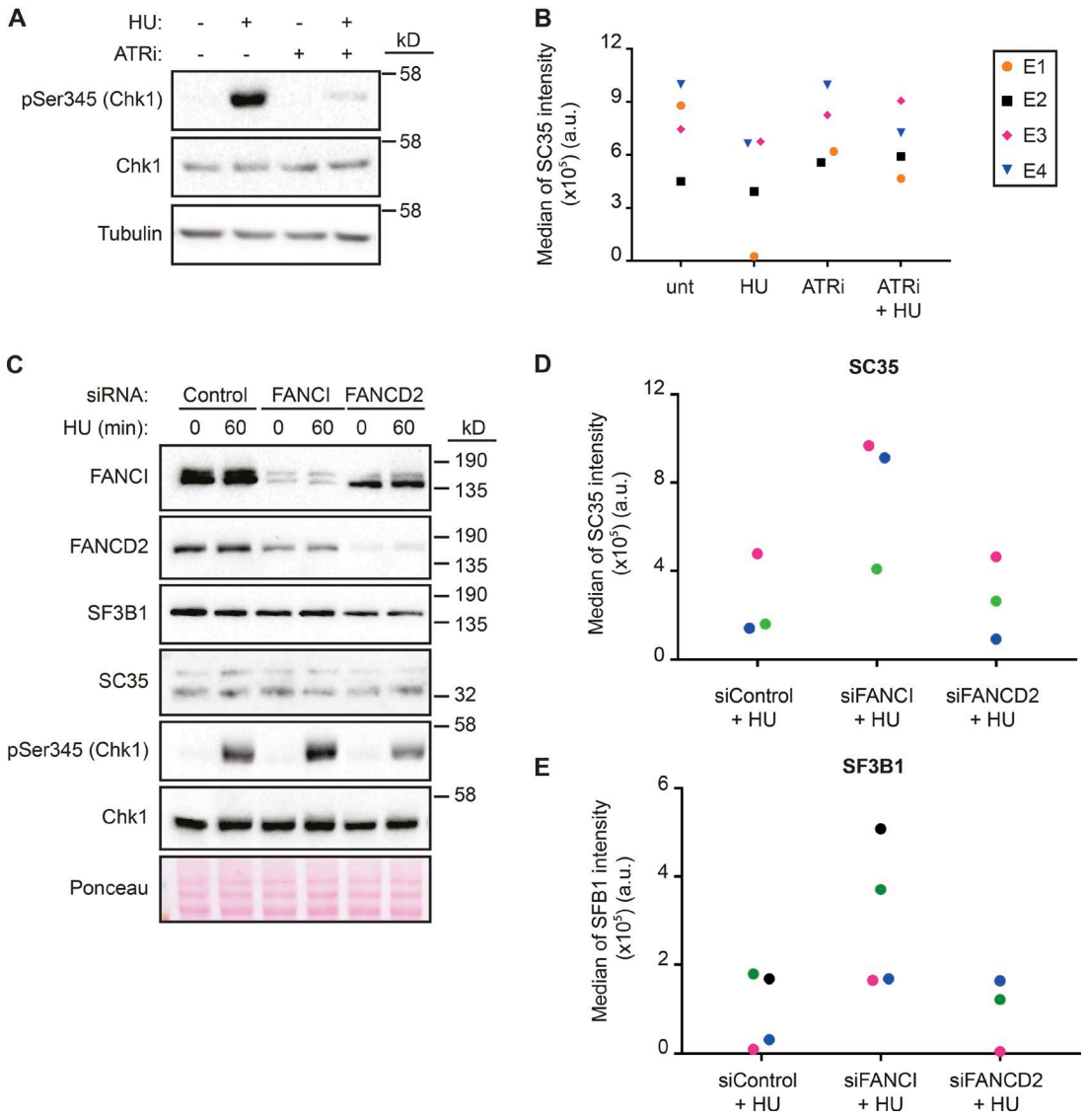

Figure S5. **Release of SFs from speckles induced by replication stress is dependent on ATR activity and FANCI.** (A) Representative immunoblot showing ATR inhibition and HU treatment efficiencies in U2OS cells analyzed in Figs. 6 D and S5 B. (B) The graph shows the median of each of the four biological replicates of the representative experiment presented in Fig. 6 D. (C) Immunoblots indicate FANCI and FANCD2 depletion levels and Chk1 phosphorylation on Ser345 in cells analyzed in Fig. 7 B. SF3B1 and SC35 levels are also shown. (D and E) Graphs showing the median of three (D) and four (E) independent biological replicates ($n = 3$) in support of Fig. 7 B. Each median of SF3B1 and SC35 intensity derives from quantification of ≥120 cells. Green dots correspond to biological replicates performed with different siRNAs targeting distinct sequences in FANCI and FANCD2.

Provided online is a supplemental ZIP file containing all the individual signals of a nucleus that were recorded for each nucleus using the enclosed ImageJ macro (signals in nuclei.ijm). Two custom algorithms (nuclei.ijm and Galaxy-Workflow-Intensity-per-nucleus_IFs-quantification.ga [Texts S1 and S2]) are included to quantify IF signals as described in Materials and methods.