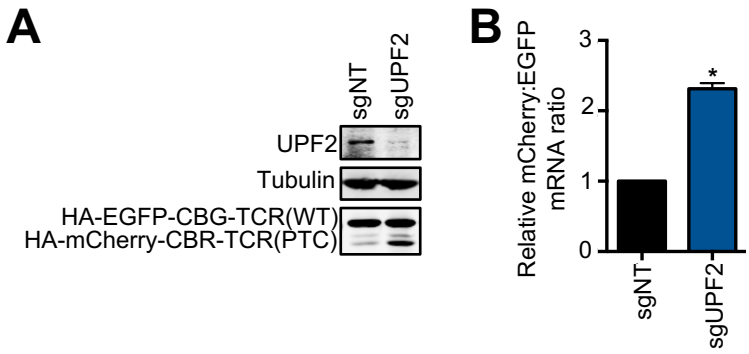
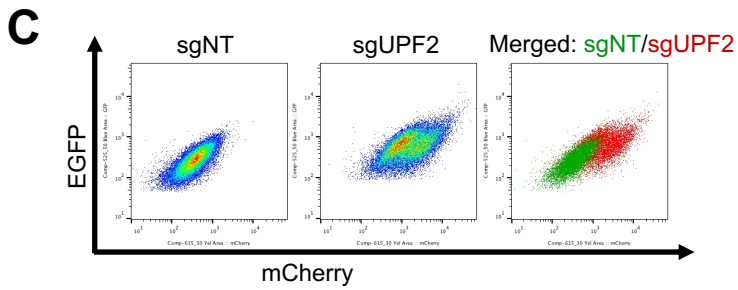
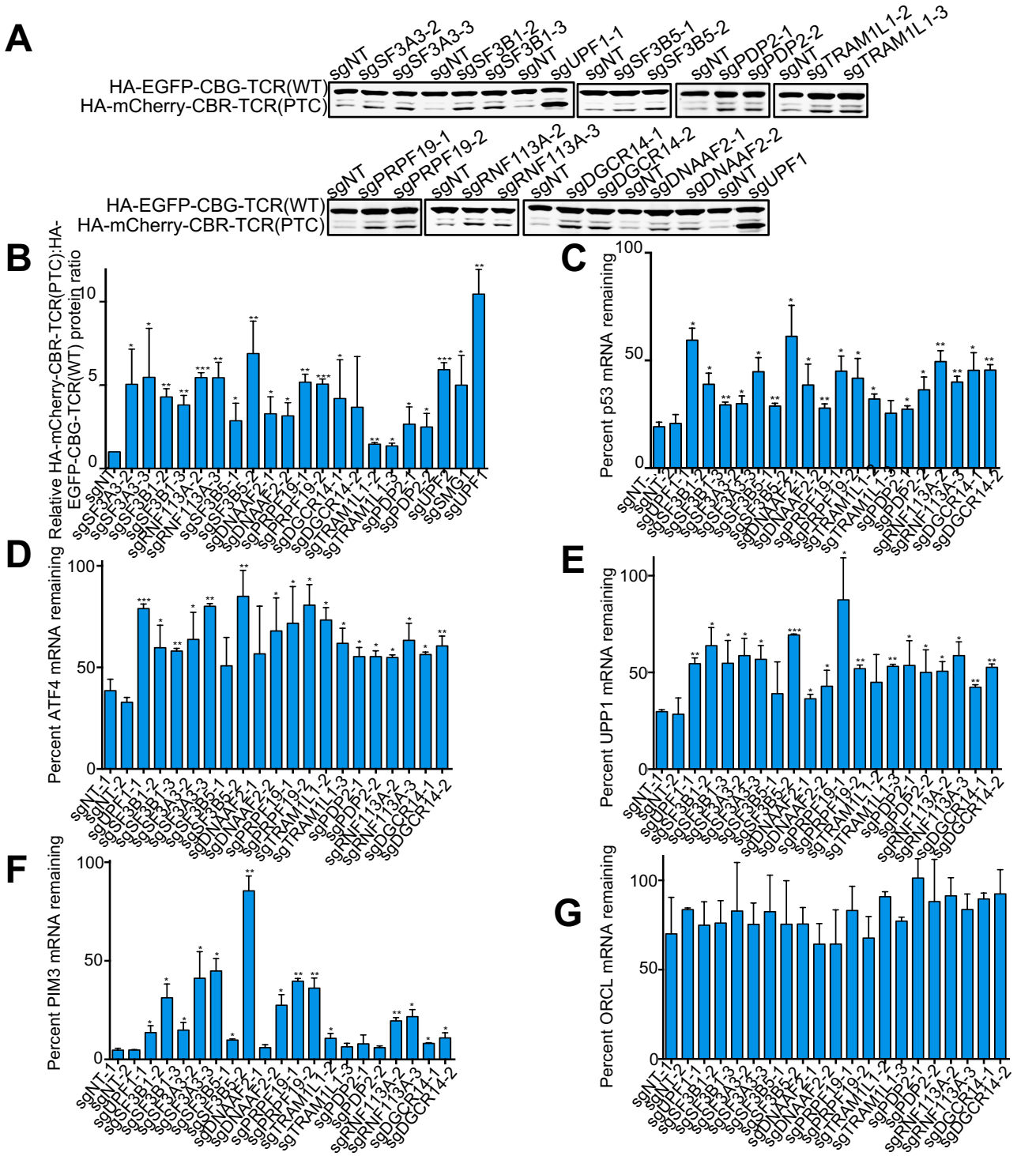




A. Western blot analysis of the protein products of the NMD reporter in Cas9-expressing U2OS reporter cells after sgRNA-mediated depletion of UPF2.

B. Ratios of mCherry-containing reporter mRNA to EGFP-containing reporter mRNA in Cas9-expressing U2OS reporter cells after UPF2 depletion. The mCherry/EGFP mRNA ratio of the sgNT (nontargeting) control was normalized to 1. Data represent the mean ± SD of three independent experiments. *p ≤ 0.05 (paired t-test).

C. FACS analysis of Cas9-expressing U2OS reporter cells after UPF2 depletion.

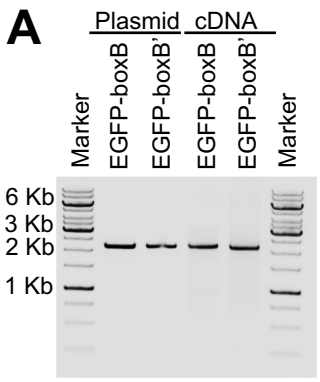


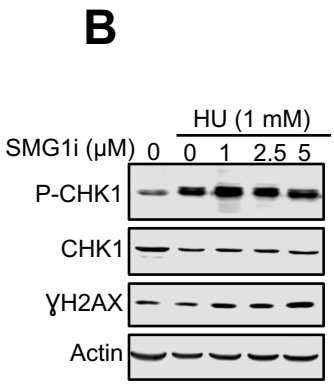
A. Western blot analysis of the protein products of the NMD reporter in Cas9-expressing U2OS reporter cells after sgRNA-mediated depletion of nine top-ranked genes individually. Two sgRNAs that are distinct from that in the original GeCKOv2 library were used for knockdown.

B. Quantified results of the samples depicted in A. The ratios of the sgNT control was normalized to 1. Data represent the mean ± SD of three independent experiments. ***p ≤ 0.001; **p ≤ 0.01; *p ≤ 0.05 (paired t-test).

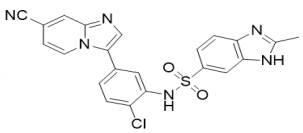
C. Effects of depletion of the 9 top-ranked genes individually on the stability of endogenous PTC-containing p53 mRNA in Calu-6 cells. Total mRNA was collected before and after actinomycin D treatment. p53 mRNA levels were measured using RT-qPCR. Data represent the mean ± SD of three independent experiments. *p ≤ 0.05; **p ≤ 0.01; (paired t-test).

D-G. Effects of depletion of the 9 top-ranked genes individually on the stability of physiological NMD targets *ATF4* (D), *UPP1* (E), and *PIM3* (F) in Calu-6 cells. *ORCL* (G) is non-NMD target control. Samples were generated as depicted in C. Data represent the mean ± SD of three independent experiments. ****p ≤ 0.0001; ***p ≤ 0.001; **p ≤ 0.01; *p ≤ 0.05 (paired t-test).

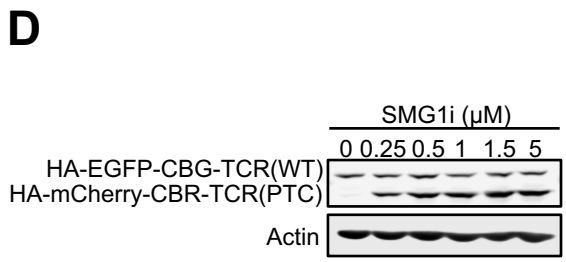


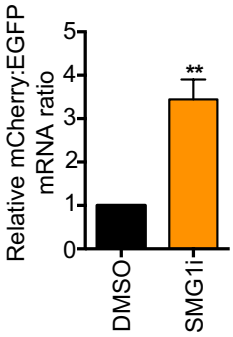


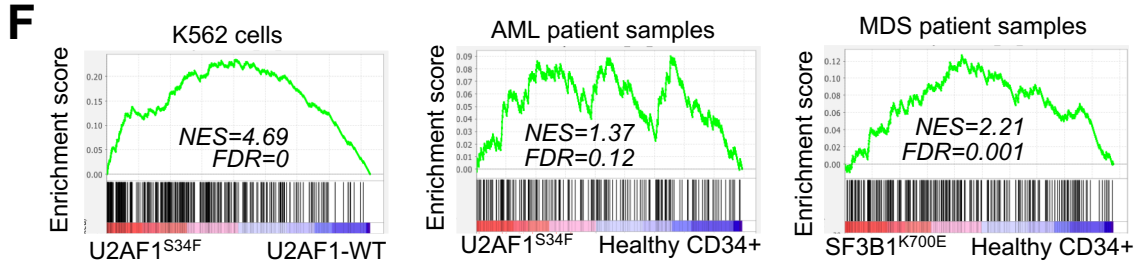
C



SMG1i						
kinase	SMG1	PI3Kα	PI3Kβ	PI3Kδ	PI3Kγ	mTOR
IC_{50}(μM)	0.0004	1.6	2.7	1.8	0.4	0.6







A. No cryptic splicing was detected in the intronless EGFP-boxB or EGFP-boxB' tethering reporter RNA. Total RNA was extracted from U2OS cells expressing boxB or boxB' reporter mRNAs, followed by RT using an oligo-dT primer. PCR was then used to generate cDNAs with primers that anneal to 5'UTR and 3'UTR of the reporter transcripts.

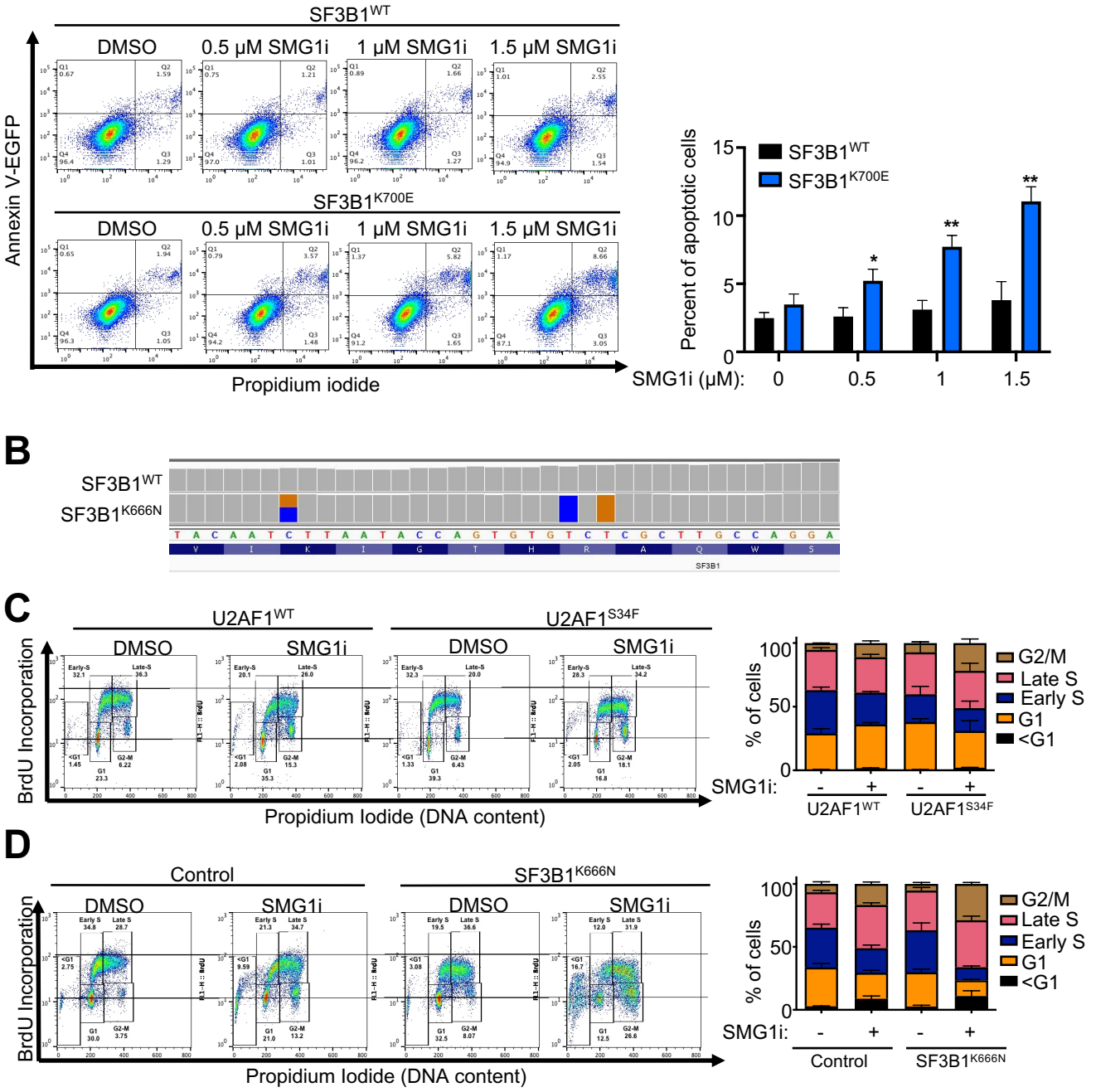
B. SMG1i did not inhibit ATR activity towards CHK1 after replication stress. Western blot analysis of phospho-CHK1 (pS345), total CHK1, and γH2AX in U2OS cells pre-treated with the indicated concentrations of SMG1i for 24 hours, and then treated with 1 mM hydroxyurea (HU) for 6 hours.

C. SMG1i structure and its kinase inhibition activity against SMG1, and other PI3 kinase family members (PI3Kα, PI3Kβ, PI3Kγ, PI3Kδ, mTOR).

D. Western blot analysis of the protein products of our new NMD reporter after treatment with SMG1i at indicated concentrations for 24 hours.

E. Ratios of mCherry-containing reporter mRNA to EGFP-containing reporter mRNAs in U2OS reporter cells treated with 1 μM SMG1i for 24 hours. DMSO-treated cells were normalized to 1. Data represent the mean ± SD of three independent experiments. **p ≤ 0.01 (paired t-test).

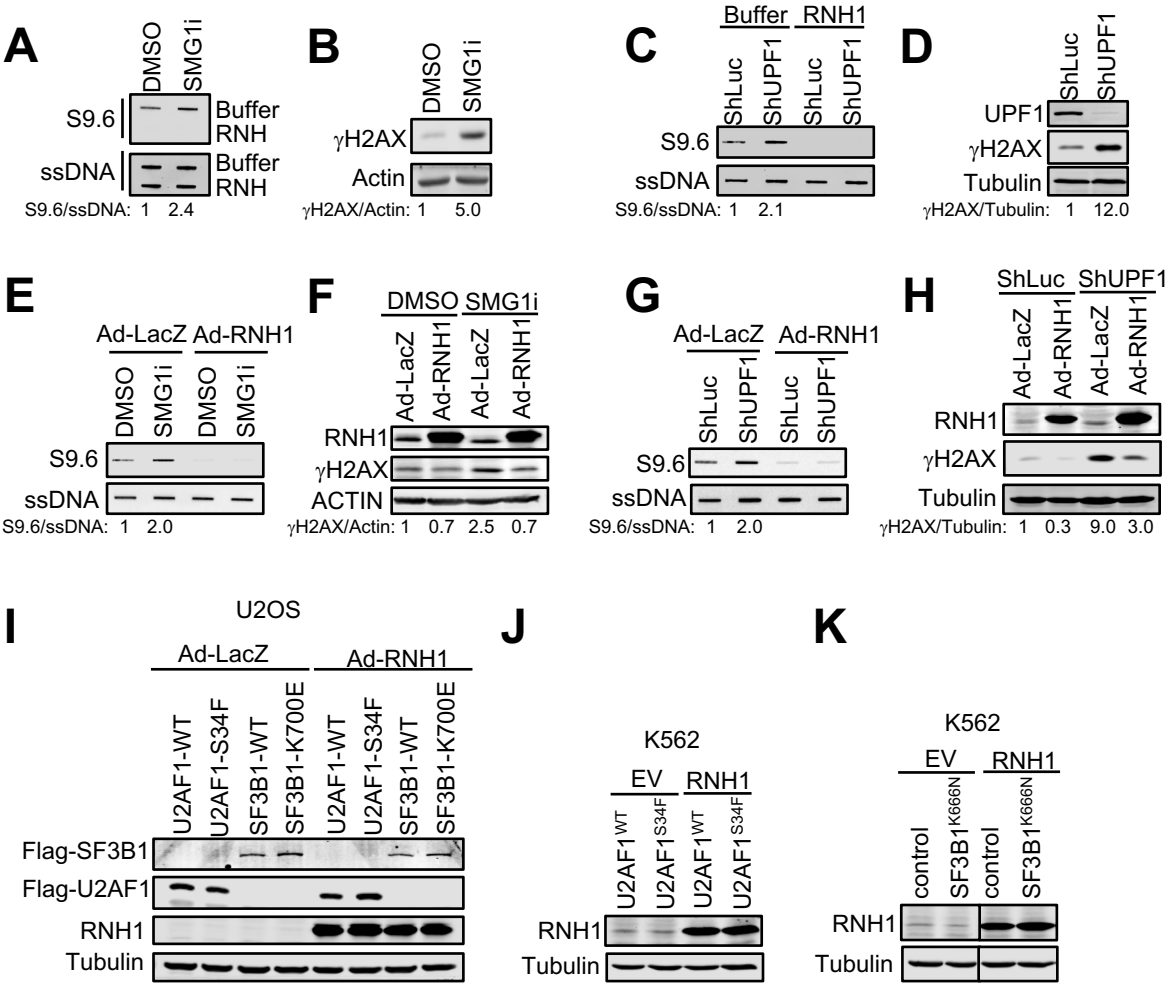
F. Gene Set Enrichment Analysis (GSEA) enrichment score plots for NMD target genes that are upregulated by SMG1i treatment. We curated a list of NMD target genes that were upregulated following treatment with SMG1i, an inhibitor of the SMG1 kinase required for NMD. These NMD target genes were upregulated in K562 cells expressing mutant U2AF1(S34F) (top), in AML patient samples expressing mutant U2AF1 (S34F) (middle), and in MDS patient samples expressing mutant SF3B1 (K700E) (bottom, analysis of the data in Pellagatti et al.(39)). Individual genes in the NMD target gene set are represented by a black vertical bar at the bottom of the plot.



A. Effects of SMG1i treatment (3 days) on apoptosis of U2OS cells expressing SF3B1^{WT} or SF3B1^{K700E}. Treated cells were harvested and stained with Annexin V-EGFP and PI for 5 minutes and analyzed using flow cytometry. Data represent the mean ± SD of three independent experiments. **p ≤ 0.01; *p ≤ 0.05 (unpaired t-test)

B. Genetically modified SF3B1^{K666N} K562 cells express 50% SF3B1^{WT} mRNA and 50% SF3B1^{K666N} mRNA. Sequence fragment density reads from RNA sequencing of K562 cells with or without SF3B1^{K666N} knock-in mutation aligned in IGV. The control cells express 100% SF3B1^{WT} mRNA. The knock-in cells express 50% mRNA containing the C to G mutation at position 1998 (which changes K at codon 666 to N, colored bars on the left) and 100% mRNA containing the blocking modifications (which do not change the amino acid) to prevent additional editing by Cas9 (colored bars on the right).

C-D. Left, effects of SMG1i treatment (1 μM, 3 days) on the cell cycle and DNA replication of K562 cells expressing U2AF1^{WT} or U2AF1^{S34F} (C), or of K562 cells with or without SF3B1^{K666N} knock-in mutation (D). Treated cells were pulsed labeled with BrdU for 30 min before being harvested for propidium iodide staining and flow cytometry. Right, percentages of cells in different cell cycle phases after SMG1i treatment. Data represent the mean ± S.E.M. of three independent experiments.



A. Effects of SMG1i treatment on R-loop levels. U2OS cells were treated with SMG1i (5 μM, 24 hours) followed by genomic DNA extraction for R-loop analysis. Total genomic DNA with or without RNase H (RNH) digestion were slotted on a membrane and S9.6 antibody was used to detect R-loops via slot blotting. ssDNA signal from denatured total genomic DNA was used as an input control.

B. Effects of SMG1i treatment (5 μM, 24 hours) on γH2AX in U2OS cells. Samples were generated as described in A.

C. Effects of shRNA-mediated knockdown of UPF1 on R-loop levels in U2OS cells. R-loops were detected via slot blotting in isolated total genomic DNA with or without RNase H (RNH) digestion

D. Effects of shRNA-mediated knockdown of UPF1 on γH2AX in U2OS cells. Samples were generated as described in C.

E. Effects of RNH1 expression on R-loops in SMG1i-treated U2OS cells. U2OS infected with adenovirus expressing lacZ control or RNH1 for 48 hours were treated with SMG1i (5 μM) for 24 hours. R-loops were detected by slot blotting.

F. Effects of RNH1 expression on γH2AX in SMG1i-treated U2OS cells. Samples were generated as described in E.

G. Effects of RNH1 expression on R-loop levels in UPF1-depleted U2OS cells. U2OS cells infected with lentivirus expressing shLuc or shUPF1 were infected with adenovirus expressing lacZ control or RNH1.

H. Effects of RNH1 expression on γH2AX in UPF1-depleted U2OS cells. Samples were generated as described in G.

I. Western blot analysis of RNH1, Flag-U2AF1 and Flag-SF3B1 in U2OS cells expressing $U2AF1^{WT}$, $U2AF1^{S34F}$, $SF3B1^{WT}$, or $SF3B1^{K700E}$ that were infected with adenovirus expressing lacZ or RNH1.

J. Western blot analysis of RNH1 in K562 cells expressing inducible $U2AF1^{WT}$ or $U2AF1^{S34F}$ that were infected with lentivirus expressing empty vector (EV) or RNH1.

K. Western blot analysis of RNH1 in wild type or $SF3B1^{K666N}$ knock-in K562 cells that were infected with lentivirus expressing empty vector (EV) or RNH1.