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Supplemental Information

Chk1 promotes non-homologous end joining in G1 through direct phosphorylation of ASF1A

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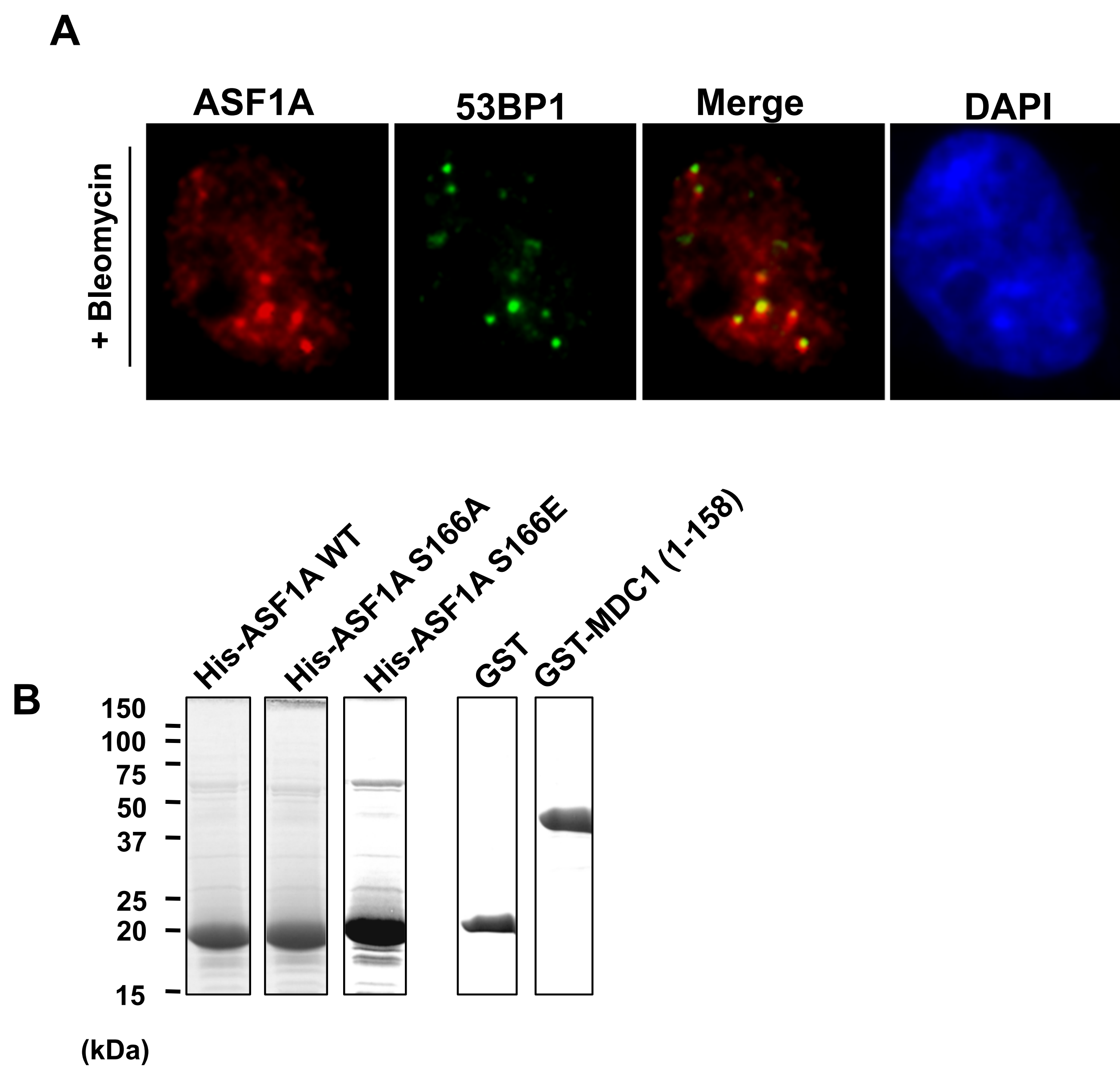

Figure S1. Related to Figure 1. (A) Representative images for overlapping ASF1A foci with 53BP1 foci upon bleomycin treatment. Cells were treated with 10 μg/ml bleomycin for 1 hr before fixation with 4% PFA. **(B)** Purification of recombinant his tagged-wild type, -S166A, and –S166E of ASF1A, GST, GST tagged-FHA domain of MDC1 (GST-N-MDC1), from a bacterial overexpression system.

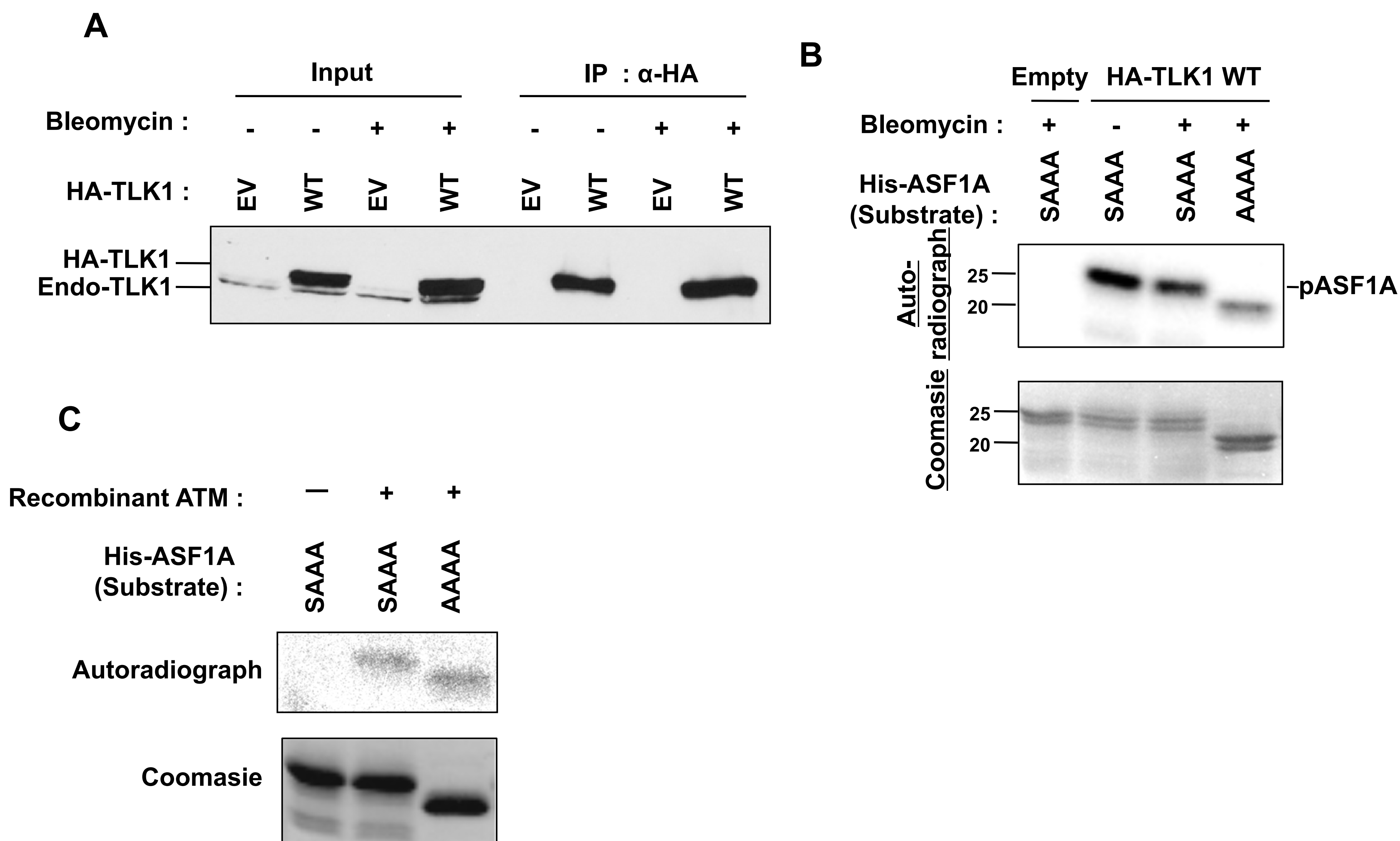

Figure S2. Related to Figure 4. S166 phosphorylation of ASF1A by TLK1 is suppressed upon DNA damage, and ATM is unable to phosphorylate S166 of ASF1A *in vitro*. (A and B) TLK is able to directly phosphorylate S166 of ASF1A, which is suppressed by DSBs. (A) An immunoblot to show immunoprecipitates of kinase, HA-TLK1, from unperturbed or bleomycin treated cells. (B) *In vitro* kinase reactions using immunopurified HA-TLK1 as in (A). TLK1 or a kinase associated with TLK1 can phosphorylate ASF1A at additional sites besides the four serines. **(C)** Recombinant ATM cannot directly phosphorylate S166 of ASF1A. *In vitro* kinase assay was performed using recombinant ATM.

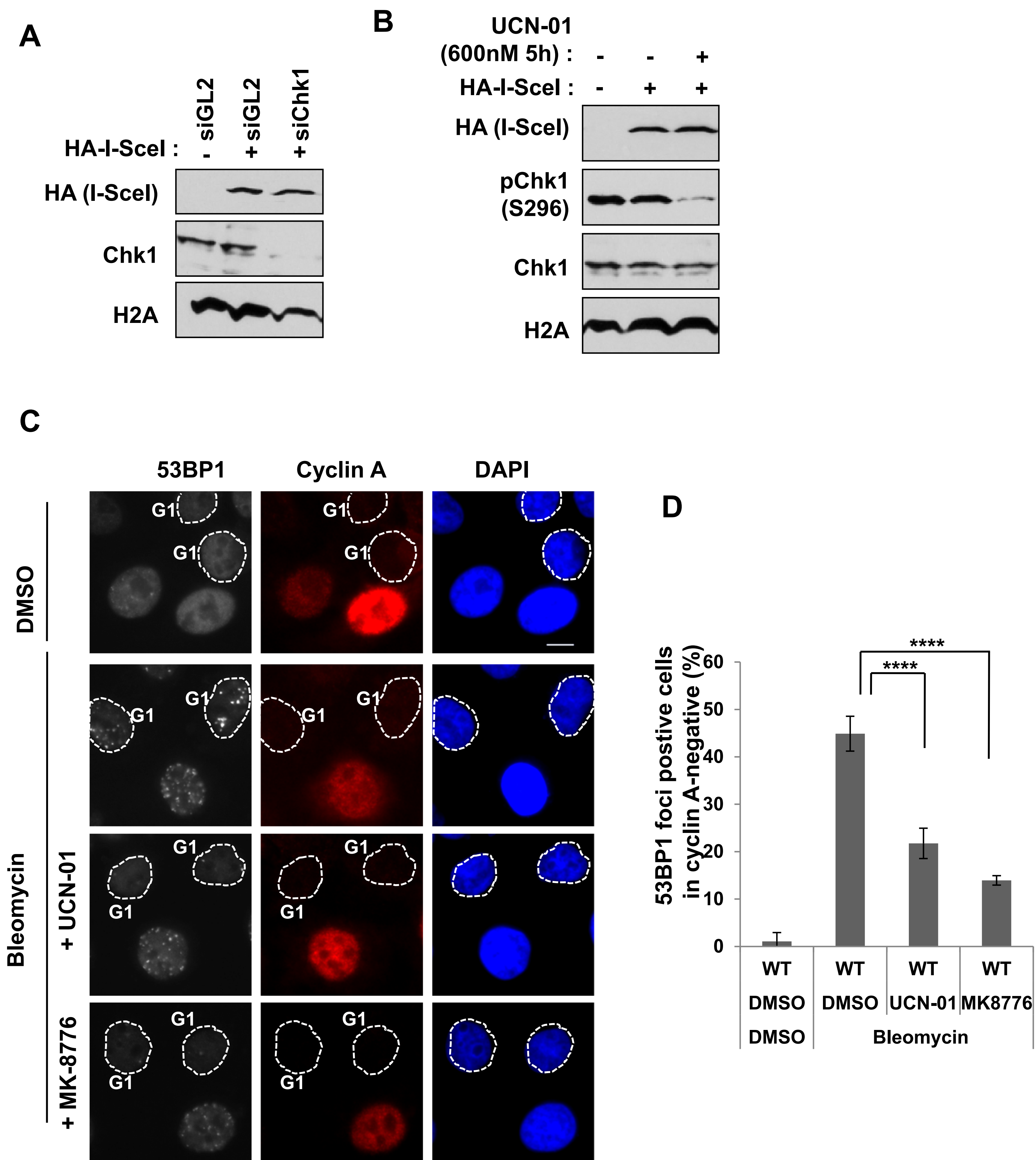

Figure S3. Related to Figure 5. (A and B) Immunoblots of the 293B lysates to show that HA-I-SceI expression is not altered by siChk1 or Chk1 inhibition by UCN01 as used in **Figure 5A**. **(C)** Chk1 inhibition decreases 53BP1 foci in G1 cells in a second cell line. HeLa DR13-9 cells were treated with 300 nM UCN-01 or 20 μM MK8776 for 1 hr followed by 20 μg/ml bleomycin for additional 1 hr. Representative images. **(D)** Quantitation of 53BP1 foci positive cells in cyclin A-negative cells from the experiment in (C). One way ANOVA (Tukey's post-hoc test); ****, P<0.0001.

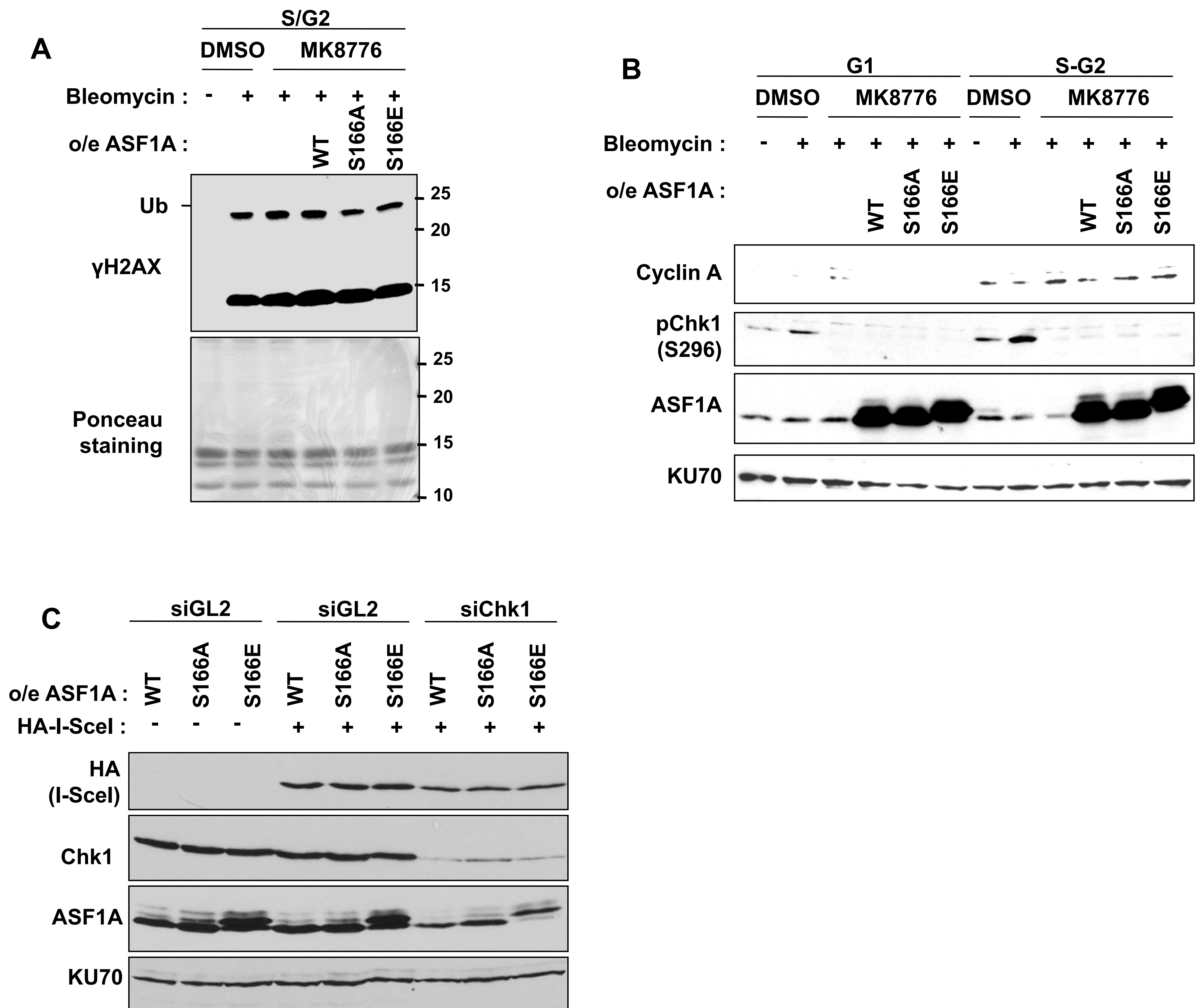

Figure S4. Related to Figure 6. (A) Histone ubiquitination on γH2AX upon DSBs, not altered by Chk1 inhibition or S166 mutants of ASF1A in S/G2. **(B)** Immunoblots of whole cell extracts used in acidic histone extraction in **Figure 6C** and **Figure S4A**. **(C)** Immunoblots of the 293B lysates for NHEJ assay in **Figure 6F** to show equal expression of HA-I-SceI, knockdown of Chk1 and equal expression of the three forms of ASF1A in each triplet comparison.

Table S1. Oligonucleotides used in this study. Related to STAR Methods.

PCR primers for ASF1A 1-160	Forward	GGGGGGAATTCATGGCAAAGGTTCAGGTGAAC
	Reverse	GGGGGGGGGCGGCCGCTCACAGTTTTTCTGTGTTATCTTCCCAAT
PCR primers for ASF1A 1-170	Forward	GGGGGGAATTCATGGCAAAGGTTCAGGTGAAC
	Reverse	GGGGGGGGGCGGCCGCTCATAGATTTGGATTACTGCTCTCTGC
PCR primers for MDC1 1-158	Forward	GGGGGGGATCCCCATGGAGGACACCCAGGCTATTG
	Reverse	GGGGGGGGGCGGCCGCTTGAGTTTCTCCCTGTACTCTGGGTG
PCR primers for ASF1A S166A	Forward	CTGGAAGATGCAGAGAGCGCTAATCCAAATCTACAGTC
	Reverse	GACTGTAGATTTGGATTAGCGCTCTCTGCATCTTCCAG
PCR primers for ASF1A S166E	Forward	CTGGAAGATGCAGAGAGCGAGAATCCAAATCTACAGTC
	Reverse	GACTGTAGATTTGGATTCTCGCTCTCTGCATCTTCCAG
PCR primers for ASF1A S175A	Forward	CTACAGTCACTTCTTGCAACAGATGCATTACC
	Reverse	GGTAATGCATCTGTTGCAAGAAGTGACTGTAG
PCR primers for ASF1A S192A	Forward	GGTCCACATCAGAAAACGCACTAAATGTCATGTTAG
	Reverse	CTAACATGACATTTAGTGCGTTTTCTGATGTGGACC
PCR primers for ASF1A S199A	Forward	CTAAATGTCATGTTAGAAGCCCACATGGACTGCATG
	Reverse	CATGCAGTCCATGTGGGCTTCTAACATGACATTTAG