Jeong et al., http://www.jcb.org/cgi/content/full/jcb.201209002/DC1

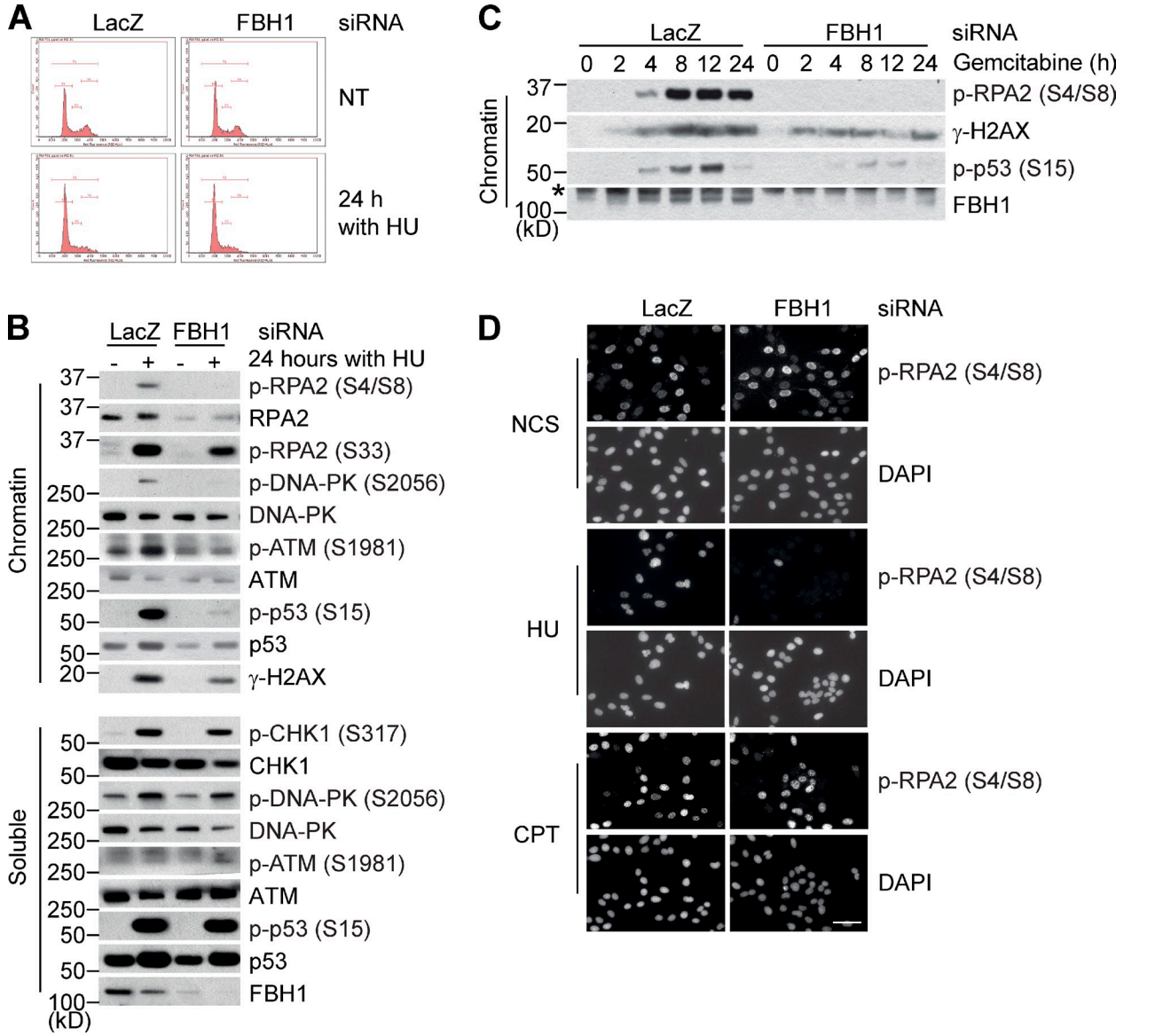

Figure S1. **FBH1 promotes the activation of ATM and DNA-PK and the phosphorylation of RPA2 on Ser4 and Ser8 after DNA replication stress induced by HU treatment.** (A) U2OS cells were transfected with siRNAs to either LacZ or *FBH1* mRNA. After 48 h, cells were left untreated or treated for 24 h with HU. Cells were then collected, and cell cycle populations were analyzed by flow cytometry. (B) IMR-90 cells (nontransformed, nonimmortalized, diploid human fibroblasts) were transfected with siRNAs to either LacZ or *FBH1* mRNA. After 48 h, cells were treated with either HU (+) or vehicle (−) for an additional 24 h. After harvesting, cells were fractionated into soluble and chromatin fractions, and lysates were immunoblotted for the indicated proteins. (C) U2OS cells were transfected with siRNAs against either LacZ or *FBH1* mRNA. After 48 h, cells were treated with gemcitabine for the indicated times. After harvesting, cells were fractionated into soluble and chromatin fractions, and lysates were immunoblotted as indicated. The asterisk denotes a nonspecific band present in the anti-FBH1 blot. (D) U2OS cells were transfected with siRNAs to either LacZ or *FBH1* mRNA. After 48 h, cells were treated with neocarzinostatin (NCS), hydroxyurea (HU), or camptothecin (CPT) and stained as indicated. Bar, 100 µm.

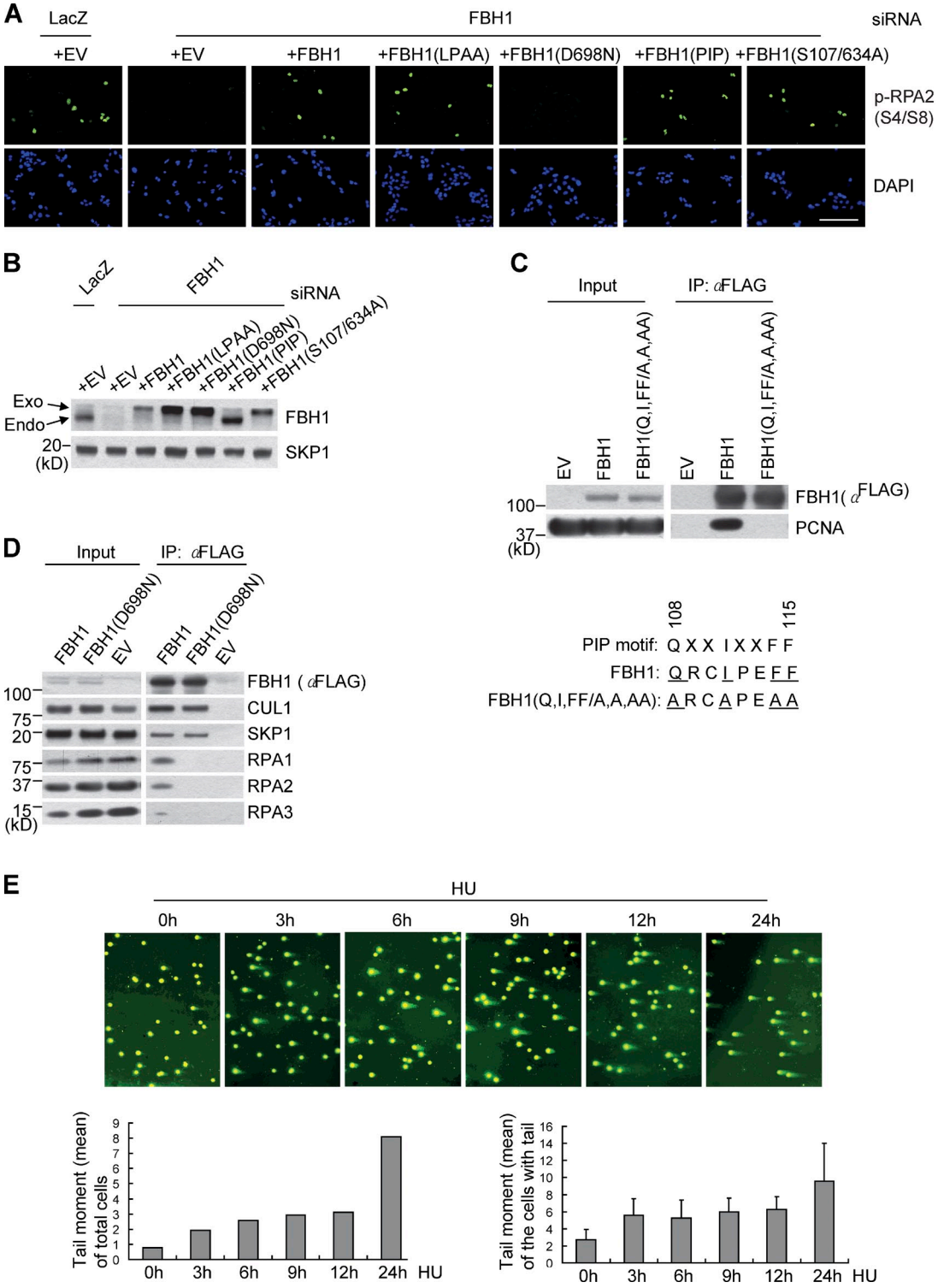

Figure S2. **The helicase domain of FBH1 is required for phosphorylation of RPA2 on Ser4 and Ser8 after HU treatment.** (A) U2OS cells stably infected with either an empty vector (EV), wild-type FBH1, or the indicated FBH1 mutants were transfected with siRNAs to either LacZ or *FBH1* mRNA. After 48 h, cells were treated with hydroxyurea (HU) for an additional 24 h and stained as indicated. Bar, 100 µm. (B) U2OS cell lysates from the experiment shown in A were immunoblotted for SKP1 (loading normalization) and both endogenous (Endo) and exogenous (Exo) FBH1. (C) HEK-293T cells were transfected with FLAG-tagged wild-type FBH1, FLAG-tagged FBH1(Q,I,FF/A,A,AA), or an empty vector (EV). 24 h after transfection, cells were harvested and lysed. Whole-cell extracts (WCE) were subjected to immunoprecipitation (IP) with α-FLAG resin and immunoblotted as indicated. The schematic representation below the blots illustrates that FBH1(Q,I,FF/A,A,AA) is a mutant in which Gln108, Ile111, Phe114, and Phe115 were mutated to Ala. (D) HEK-293T cells were transfected with the indicated FLAG-tagged wild-type FBH1, FLAG-tagged FBH1(D698N), or an empty vector (EV). 24 h after transfection, cells were harvested and lysed. Whole-cell extracts (WCE) were subjected to immunoprecipitation (IP) with α-FLAG resin and immunoblotted as indicated. (E) U2OS cells were treated with HU for the indicated hours and analyzed for the presence of DSBs using a neutral comet assay. Images are representative of multiple experiments quantified using CometScore software from TriTek Corp. (Bottom left) tail moment calculated for the entire cell population. This experiment was completed once (n = ~300 cells). (Bottom right) tail moment (±SD) calculated only for cells displaying a comet tail.

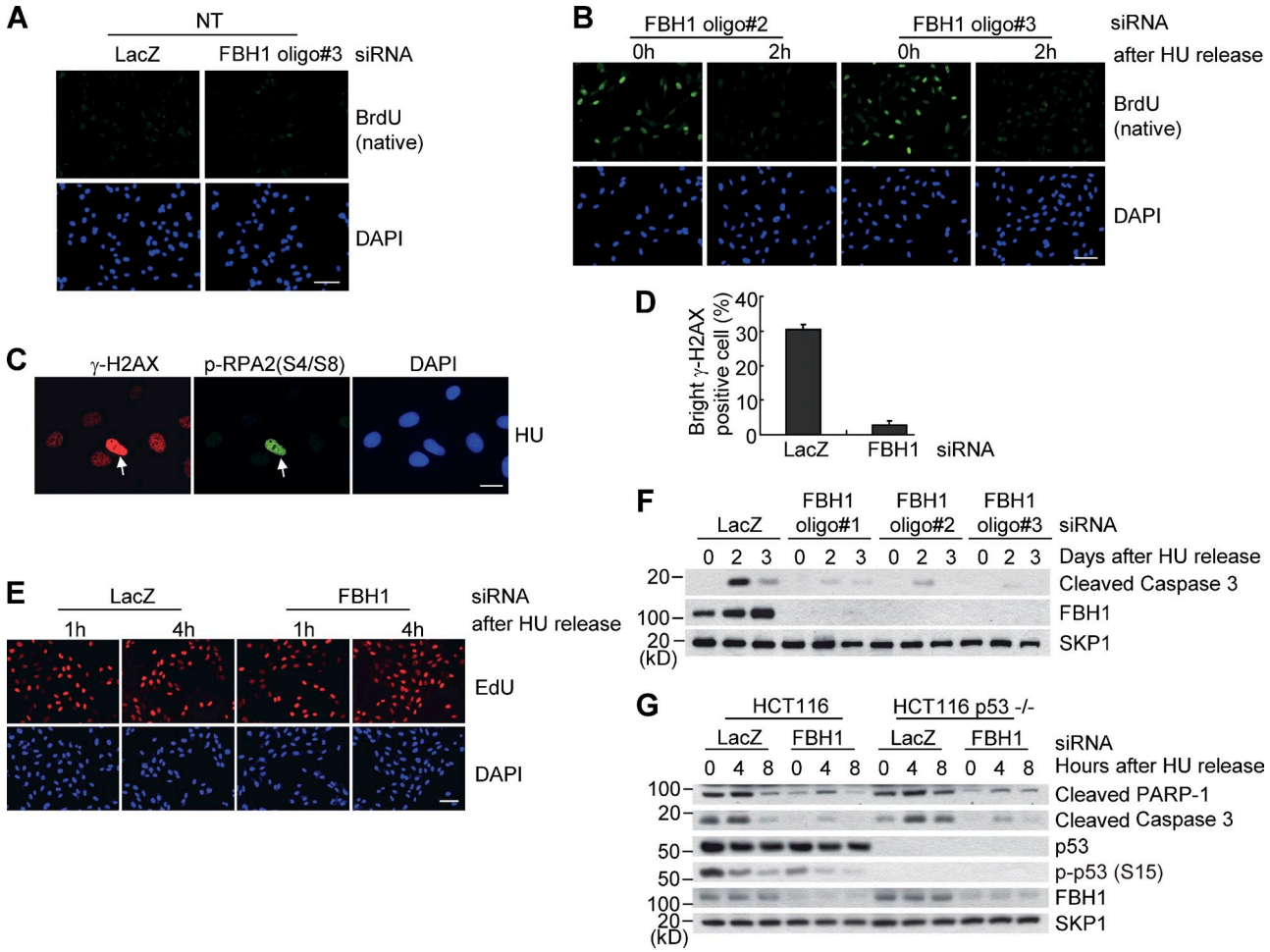

Figure S3. **FBH1 confers sensitivity to HU.** (A) In the absence of HU treatment, BrdU cannot be detected under native conditions. U2OS cells were transfected with siRNAs to either LacZ or *FBH1* mRNA, labeled with BrdU, and stained under native conditions as indicated. (B) U2OS cells were transfected with siRNAs to either LacZ or *FBH1* mRNA. After 48 h, cells were treated with HU for 24 h, immediately fixed (0 h), or released for 2 h into fresh medium, fixed, and stained under native conditions as indicated. BrdU was added 24 h before HU incubation. Bar, 100 μm. (C) U2OS cells were treated with HU for 24 h and stained as indicated. All cells displaying strong positivity for γ-H2AX (indicated by arrows) are positive for p-RPA2(S4/S8). However, ~15% of the cells that are positive for p-RPA2(S4/S8) display only weak γ-H2AX staining (not depicted). (D) Percentage of bright γ-H2AX foci in control and FBH1-depleted cells. The means (±SD) of at least three independent experiments are shown. (E) U2OS cells were transfected with siRNAs to either LacZ or *FBH1* mRNA. After 48 h cells were treated with HU for 24 h, released for 1 or 4 h into fresh medium, fixed, and stained as indicated. EdU was added 10 min before fixing the cells. Bar, 100 μm. (F) FBH1 silencing protects from apoptosis. U2OS cells were transfected with siRNAs to either LacZ or *FBH1* mRNA. After 48 h, cells were treated with HU for an additional 24 h and released for the indicated days. Cells were harvested, and their lysates were immunoblotted as indicated. (G) HCT116 and HCT116 $p53^{-/-}$ cells were transfected with siRNAs to either LacZ or *FBH1* mRNA, treated with HU for an additional 36 h, and released for the indicated times. Cells were harvested, and their lysates were immunoblotted as indicated.

Table S1. **The RPA complex present in 293T cell extract co-purify with FBH1, but not FBXW5**

		293T cells transient FBH1 double IP			293T cells transient FBXW5 double IP		
Locus	**Gene**	**Peptides**	**Unique peptides**	**dNSAF**	**Peptides**	**Unique peptides**	**dNSAF**
NP_116196.3	FBH1	57	0	0.07398089	0	0	0
NP_002936.1	RPA1	3	3	0.00061772	0	0	0
NP_002937.1	RPA2	2	2	0.00930143	0	0	0
NP_002938.1	RPA3	1	1	0.00220131	0	0	0
NP_733779.1	SKP1	15	11	0.23834582	19	13	0.14537007
NP_003583.2	CUL1	21	21	0.02182065	34	34	0.03707393
NP_061871.1	FBXW5	0	0	0	35	35	0.31376635

MudPIT analysis of FBH1 and FBXW5 double immunopurifications (IP), listing derived normalized spectral abundance factors (dNSAFs) for the indicated proteins.

Table S2. **The RPA complex present in HeLa cell extract co-purify with FBH1, but not FBXW5**

		HeLa cells stable FBH1 single IP			HeLa cells stable FBXW5 single IP		
Locus	**Gene**	**Peptides**	**Unique peptides**	**dNSAF**	**Peptides**	**Unique peptides**	**dNSAF**
NP_116196.3	FBH1	44	0	0.03109872	0	0	0
NP_002936.1	RPA1	9	9	0.00312626	0	0	0
NP_002937.1	RPA2	2	2	0.00906421	0	0	0
NP_002938.1	RPA3	6	6	0.01061032	0	0	0
NP_733779.1	SKP1	10	8	0.13463679	11	6	0.29463134
NP_003583.2	CUL1	24	24	0.02155948	18	18	0.02156502
NP_061871.1	FBXW5	4	4	0.00070884	26	26	0.12998618

MudPIT analysis of FBH1 and FBXW5 single immunopurifications (IP), listing derived normalized spectral abundance factors (dNSAFs) for the indicated proteins.