SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1: Endogenous interaction between RNF2 and H2AX: Whole cell extracts from 293T were subjected to immunoprecipitation with anti-RNF2 or anti-γ-H2AX antibodies and immunoblotting was performed with the indicated antibodies.

Supplemental Figure S2: IRIF formation was analyzed using antibodies against p-ATM, γ-H2AX, MDC1, FK2 and BMI1 antibodies after irradiation (4Gy, 15 min).

Supplemental Figure S3: RNF2 is required for *in vitro* ubiquitination of H2AX in an E3 ubiquitin ligase activity-dependent manner. H2AX proteins were incubated with adenosine triphosphate, E1, E2 along with GST, GST-RNF2, RNF2 H69Y or GST-BMI1 proteins as indicated for *in vitro* ubiquitination of H2AX.

Supplemental Figure S4: RNF2 is an E3 ubiquitin ligase for *in vivo* ubiquitination of H2AX. 293T cells were transfected with HA-ubiquitin (HA-Ubi) along with wild-type or mutant Myc-RNF2 and FLAG-H2AX. Then, cell lysate was subjected to immunoprecipitation with anti-FLAG antibodies and immunoblotting was performed with the indicated antibodies.

Supplemental Figure S5: Depletion of ubiquitin impairsγ-H2AX formation after exposure to IR. Time course analysis of H2AX phosphorylation was detected in U2OS cells transfected with control siRNA or ubiquitin siRNA and exposed to IR (4 Gy) at the indicated time points. In addition to reduced monoubiquitination of γ-H2AX**,** uH2A (ubiquitinated H2A) is also presented to indicate the overall reduced level of ubiquitination.

Supplemental Figure S6: Monoubiquitination of H2AX K120 is required for efficient H2AX phosphorylation after exposure to IR. A, $H2AX^{-/-}$ MEFs reconstituted with $H2AX^{WT}$ or H2AX with Arginine mutations at lysines 119 and 20 ($H2AX^{K119/120R}$) were measured by western blot analysis with indicated antibodies. B, U2OS cells were transfected with wild-type H2AX or mutant H2AX as indicated. Forty-eight hours later, cells were exposed to IR (4 Gy) harvested at indicated time points and analyzed for H2AX by immunoblottong with the indicated antibodies.

Supplemental Figure S7. A, H2AX K119/120R mutation does not impair ATM phosphrylation. Total cell lysates were harvested from H2AX-deficient MEF cells stably expression with wild-type H2AX or mutant H2AX K1119/120R after exposed to IR (4Gy) at the indicated time points. B, H2AX K120R mutant has reduced binding affinity for ATM. 293 T cells were transfected with wild-type H2AX or mutant H2AX. Forty-eight hours later, cells were exposed to IR (4 Gy) for 15 min and subjected to immunoprecipitation with anti-FLAG antibody.

Supplementary Figure S8. H2AX K119/120R impairs the recruitment of p-ATM (S1981), γ-H2AX and MDC1 to the DNA damaged sites. H2AX-deficient MEFs were transiently transfected with wild-type H2AX or mutant H2AX. Forty-eight hours later, cells were mock-irradiated or exposed to IR (4 Gy) for 15 min. Cells were analyzed for IR-induced foci with the indicated antibodies by immunofluorescent assay.

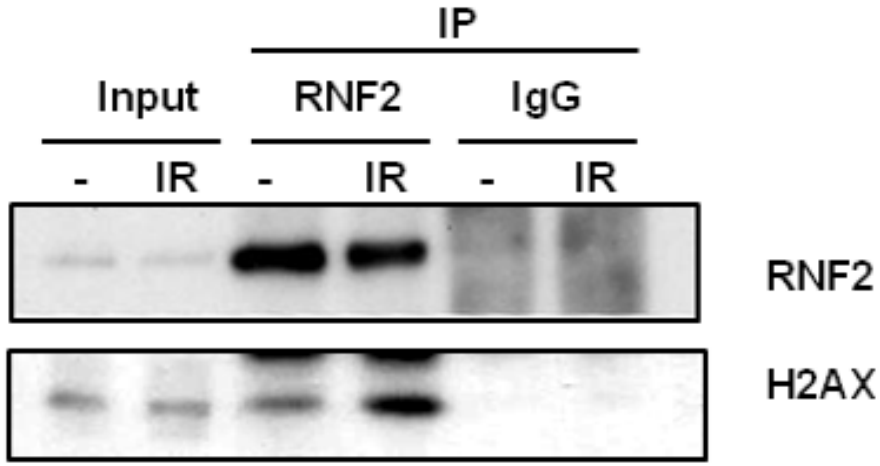
Supplemental Figure S9. A, Depletion of BMI1 or RNF2 or both leads to suppression of monoubiquitination of H2AX. H2AX-deficient MEFs were stably reconstituted with wild-type H2AX or H2AX K119/120R mutant. Chromatin fractionation was harvested after exposed IR (4 Gy) for 15 min to detect the monoubiquitination of H2AX in chromatin. B, RNF2 depletion does not affect ATM phosphorylation. ATM phosphorylation was performed by immunoblotting. U2OS cells were transfected with control siRNA or RNF2 siRNA. Seventy-two hours later, cells were exposed to IR (4 Gy) at the indicated time points and subjected to immunoblotting with the indicated antibodies.

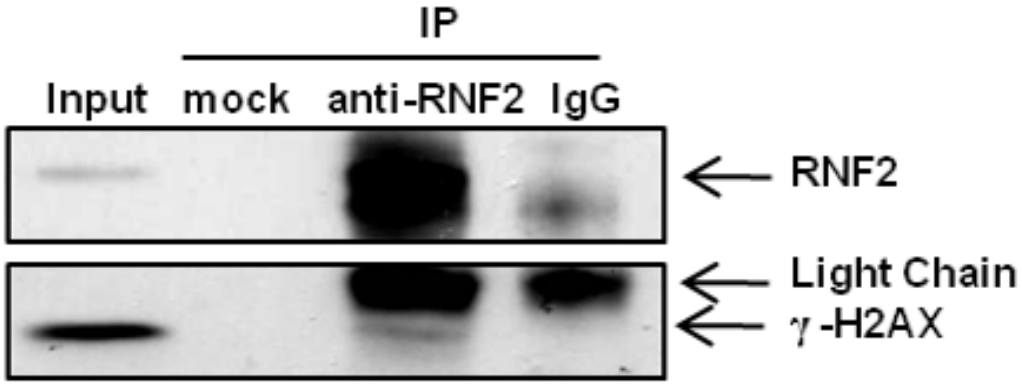
Supplemental Figure S10. RNF2-BMI1 complex is required for accumulation of IR-induced DNA-damage-associated proteins at the sites of DNA damage. U2OS cells were transfected with control siRNA and BMI1 siRNA. Seventy-two hours later, cells were exposed to IR (4 Gy) and analyzed by immunofluorescence assay with indicated antibodies fifteen minutes after IR.

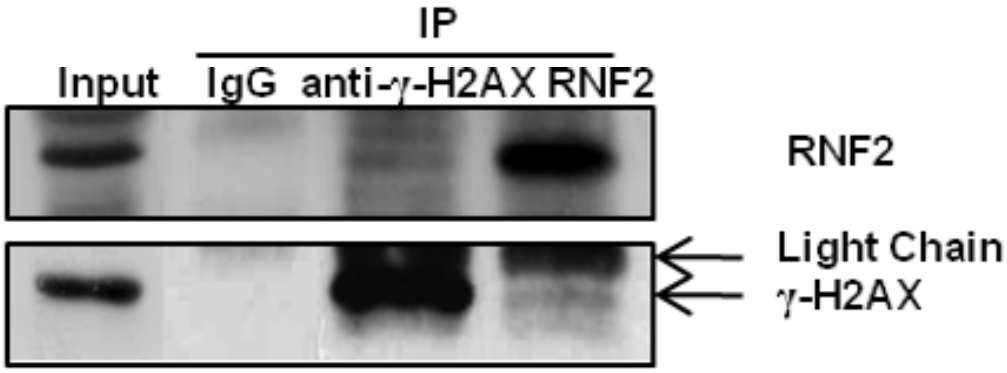
Supplemental Figure S11. Knockdown of RNF2 inhibits foci formation of γ-H2AX and BRIT1. 293T cells were transfected with control or GFP-tagged shRNA with specificity against RNF2 and exposed to IR (2Gy) for 15 min. Analysis of γ-H2AX and BRIT1 to the DNA damaged site were performed by immunofluorescent assay with specific antibodies. Western blot analyses to determine the efficiency of RNF2 knockdown were shown next to the representative images.

Supplemental Figure S12. RNF2 regulated the activation of H2AX formation in an ATM-dependent manner. U2OS cells were transfected with control siRNAs or RNF2 siRNA. Seventy-two hours later, cells were pre-treated with indicated inhibitor for 1 h. γ-H2AX was analyzed by Western blot analysis at indicated time points after IR (4Gy).

Supplemental Figure S1







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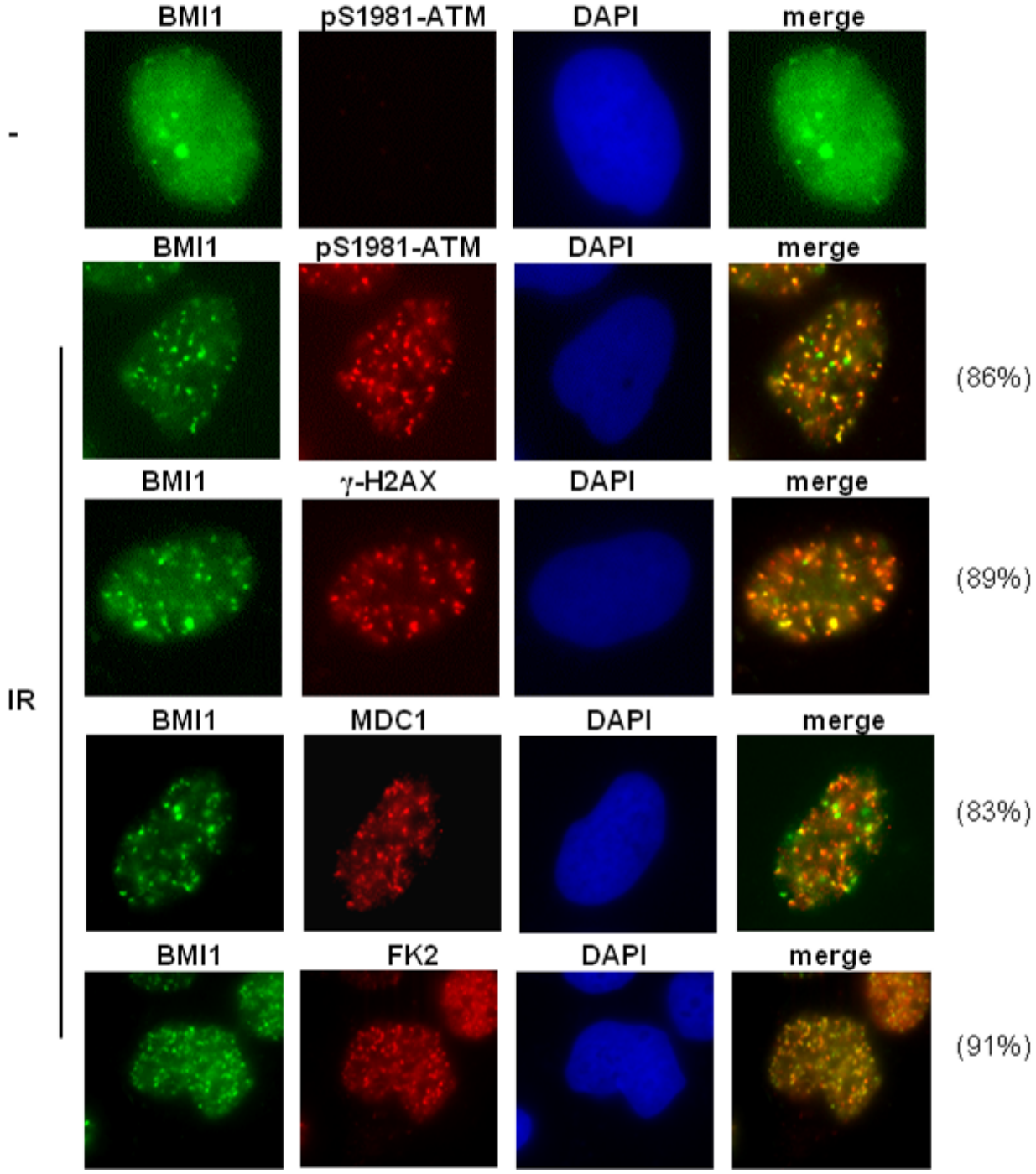

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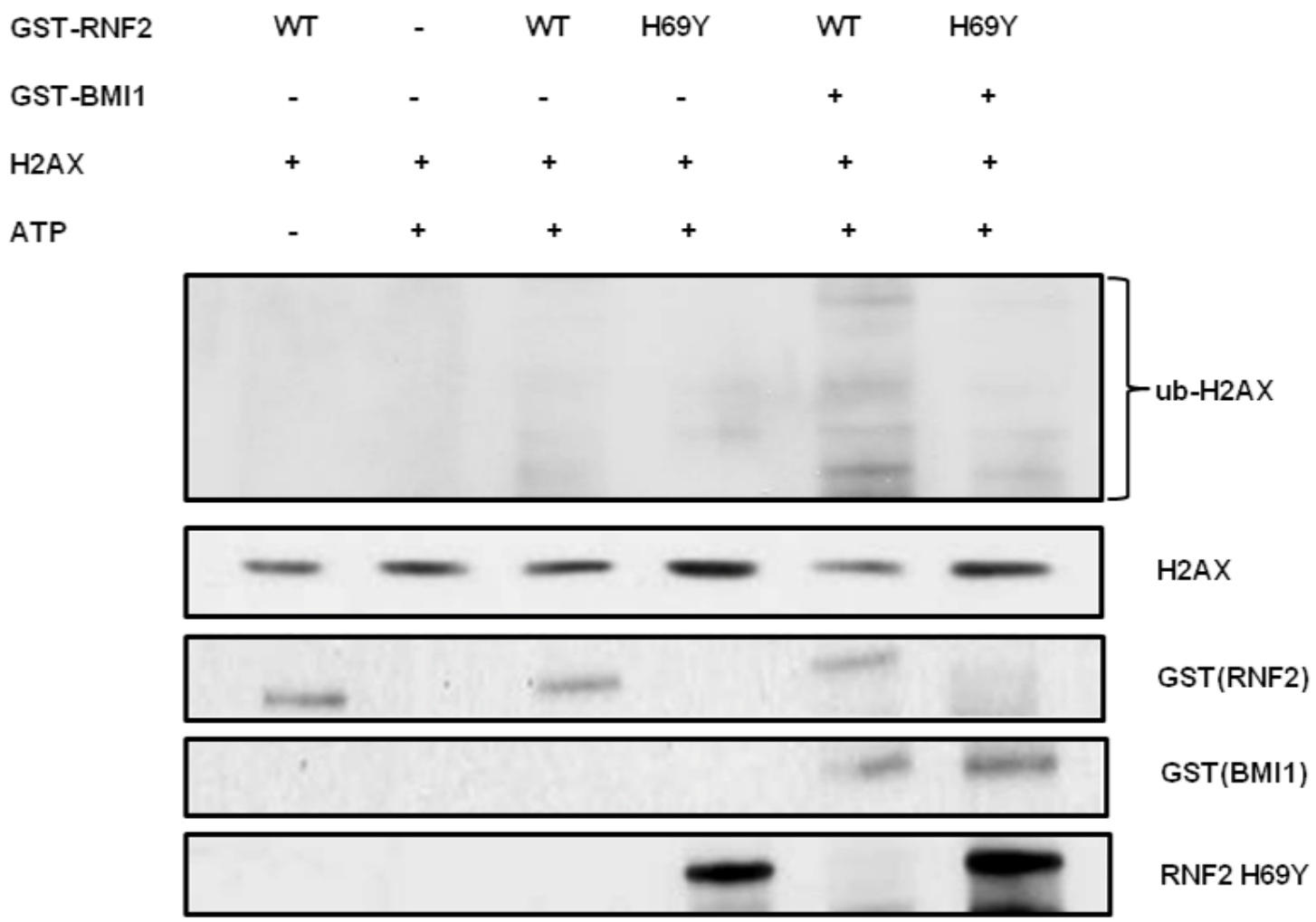

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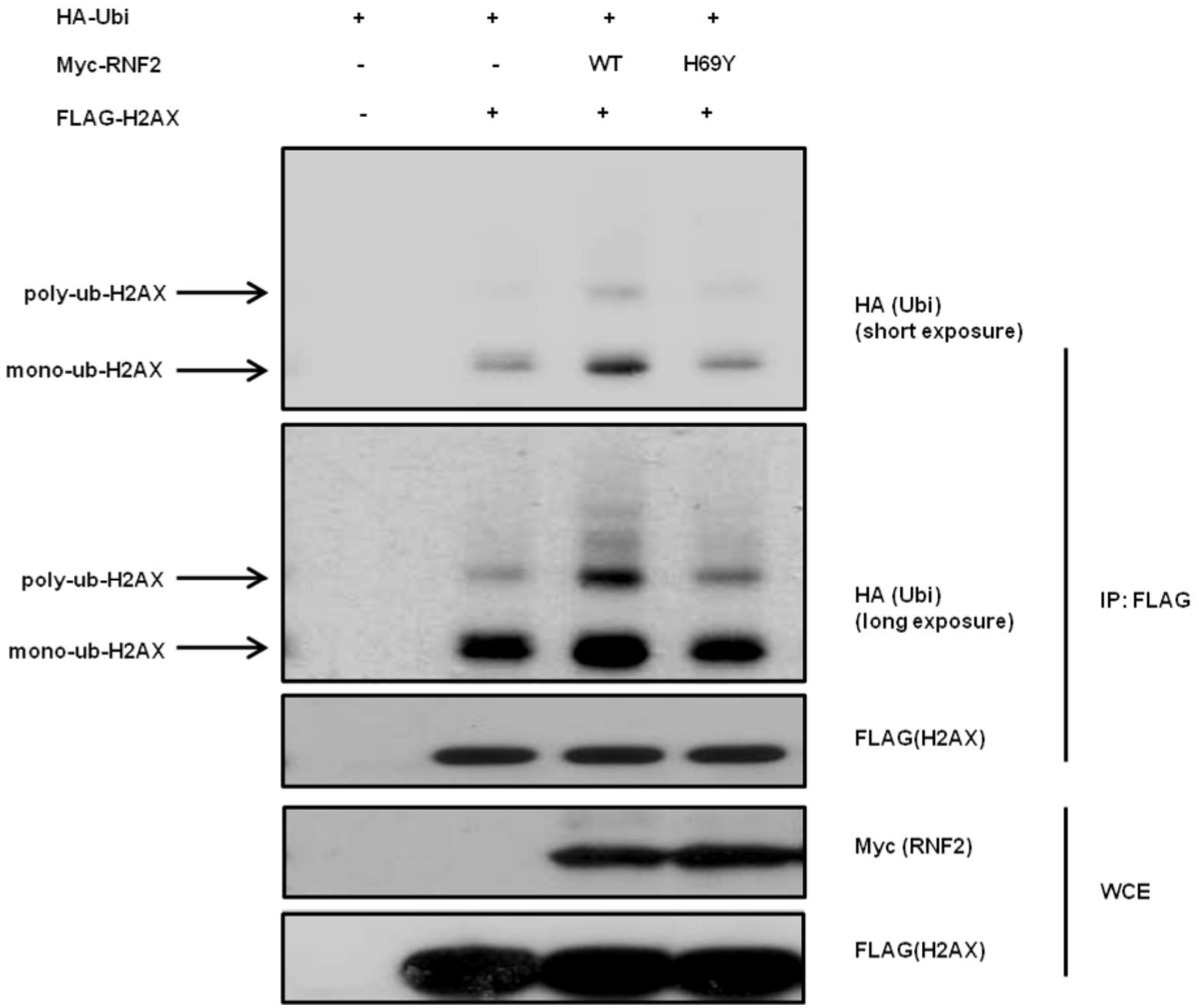

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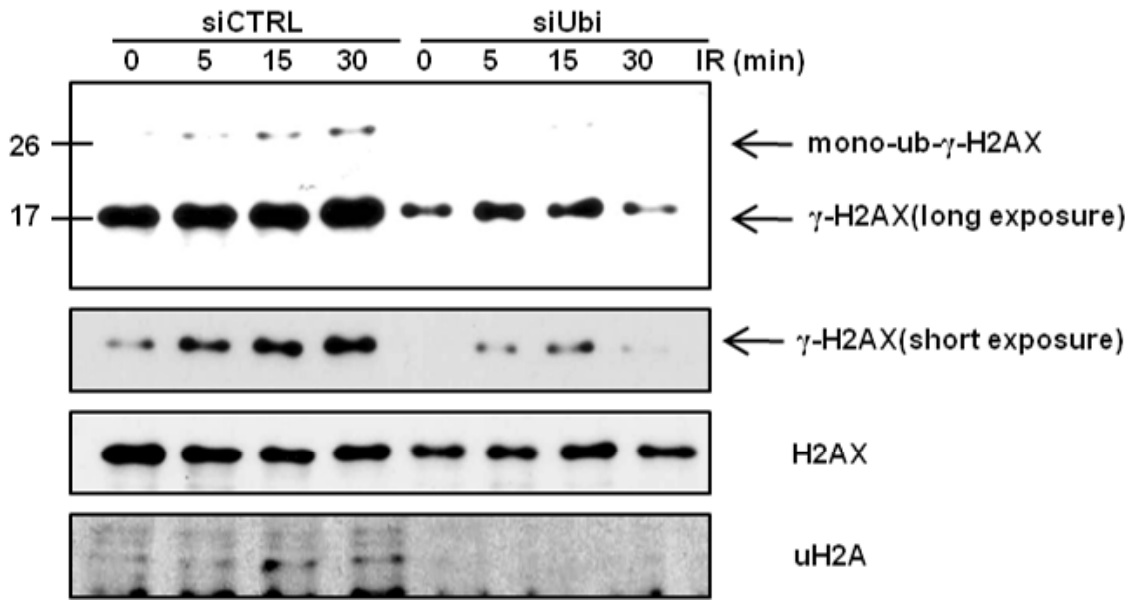

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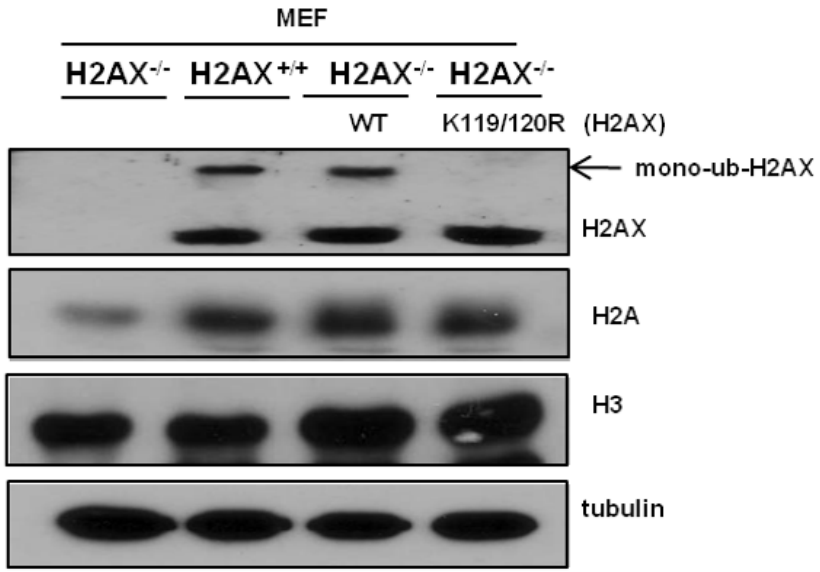
A



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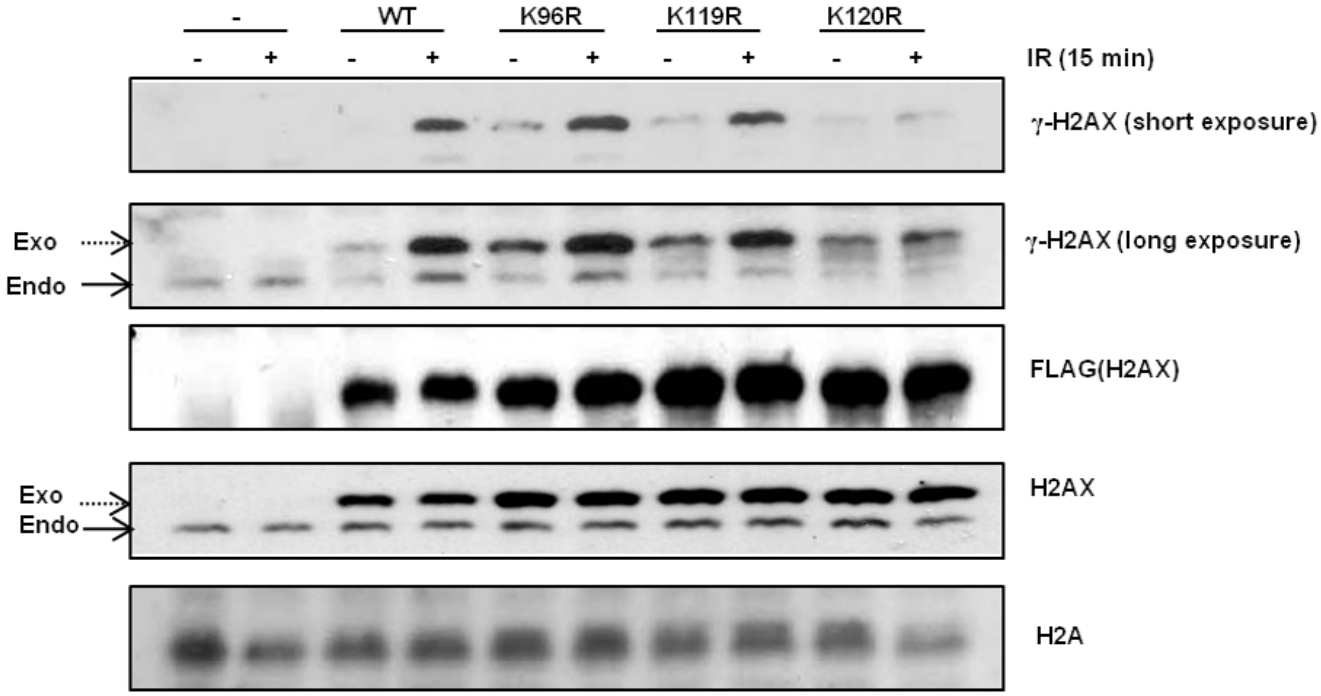

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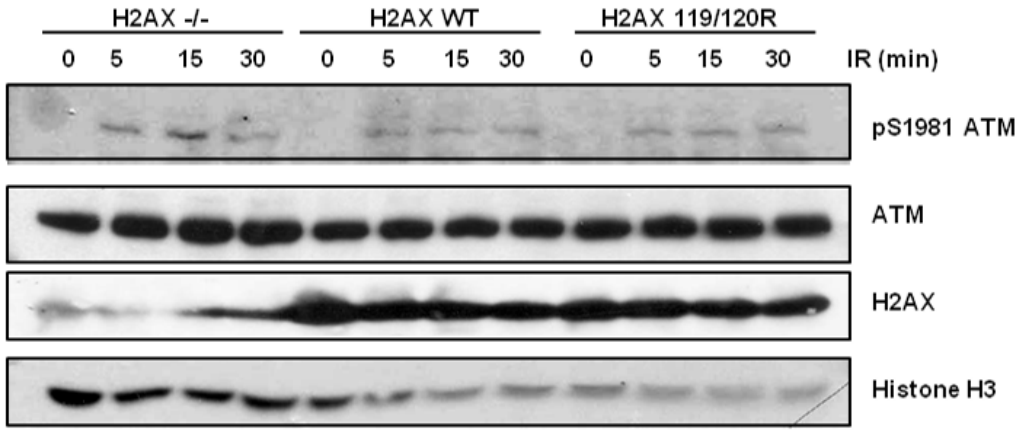

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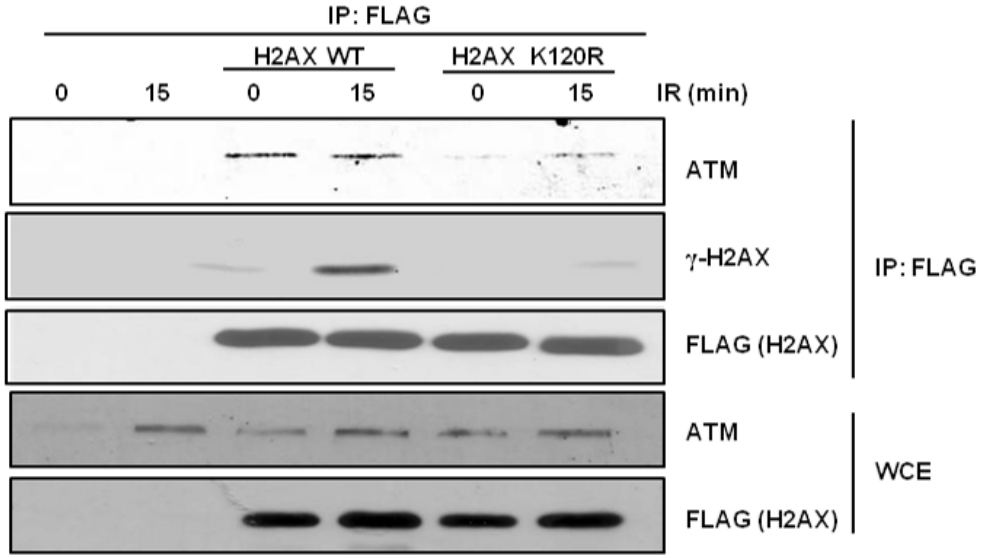

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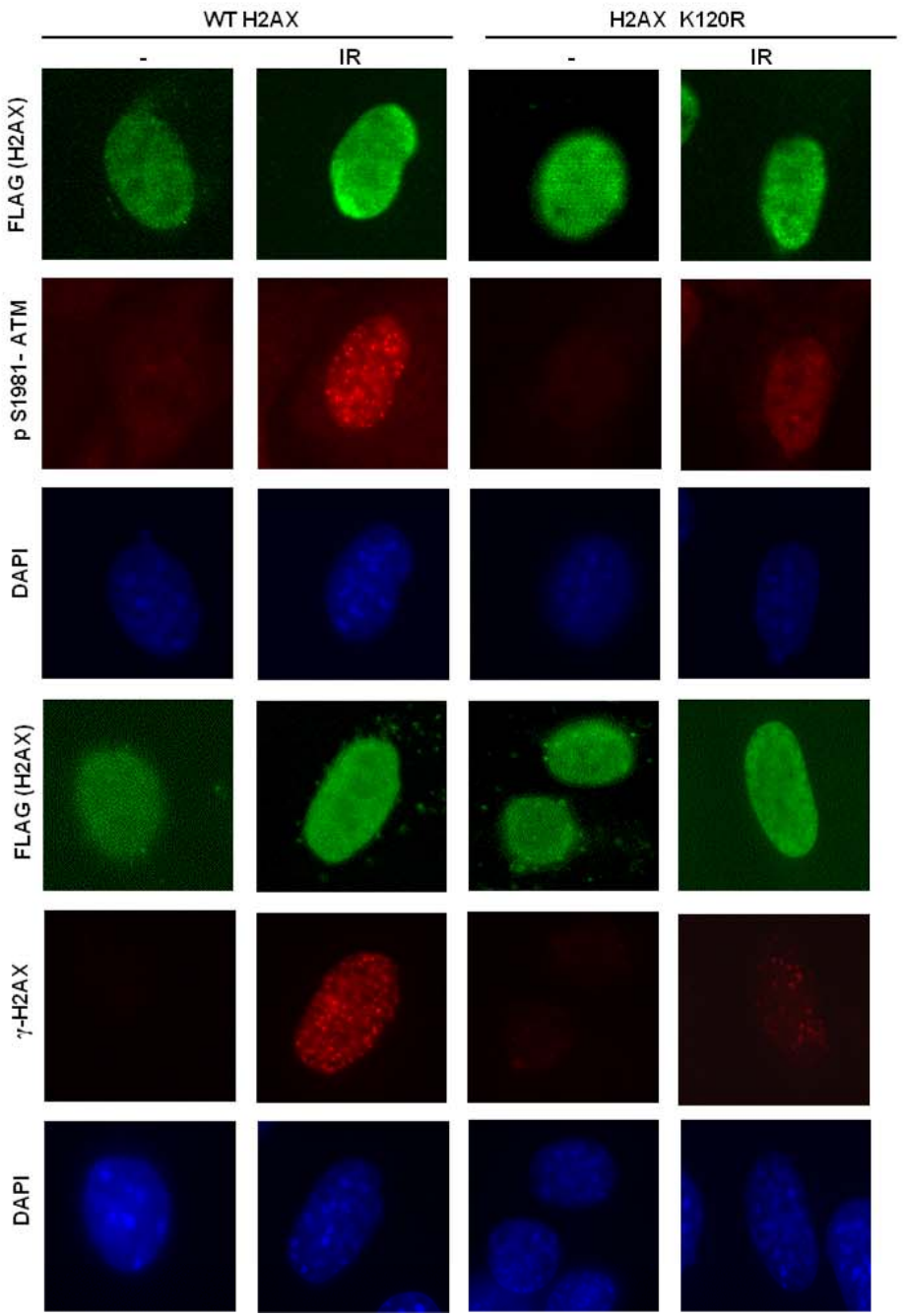

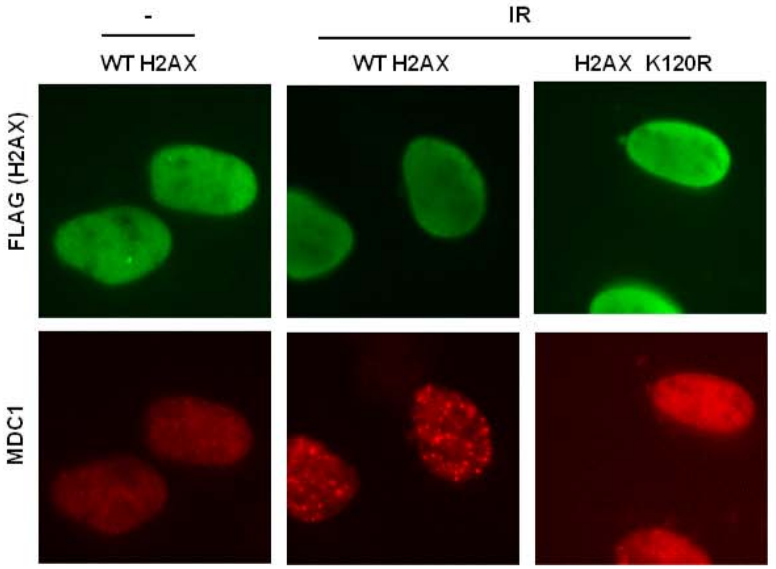

Supplemental Figure S9

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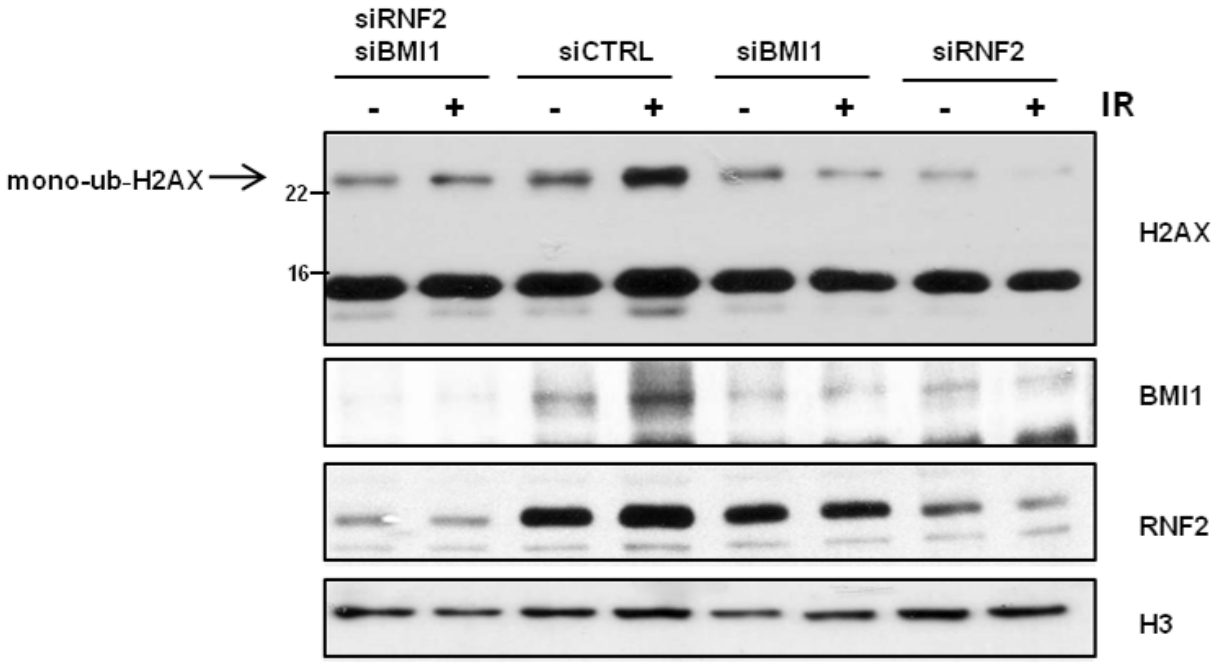

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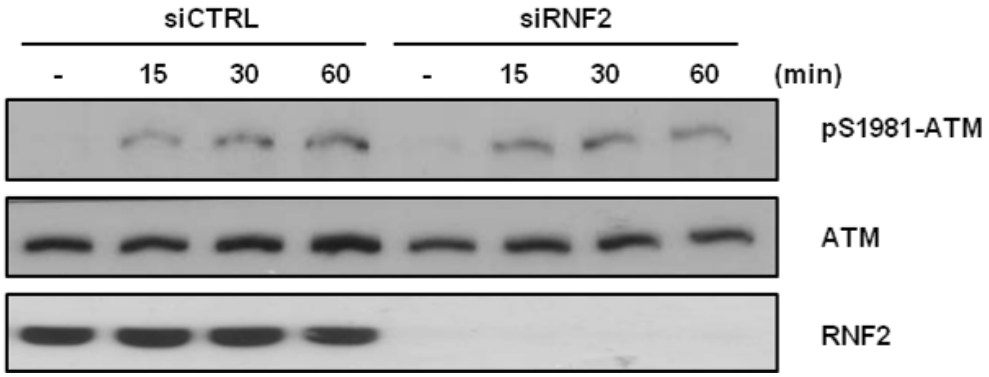

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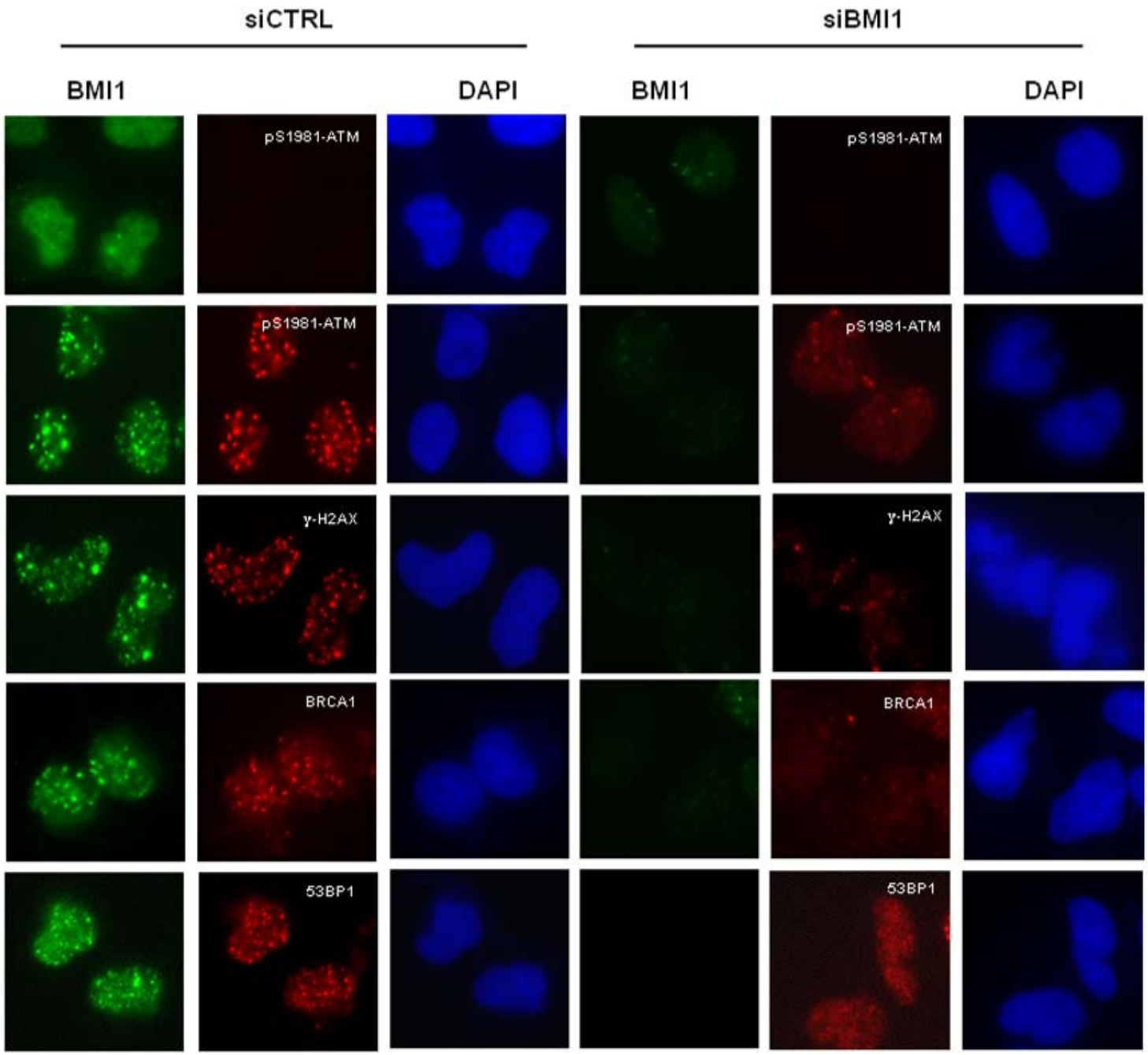

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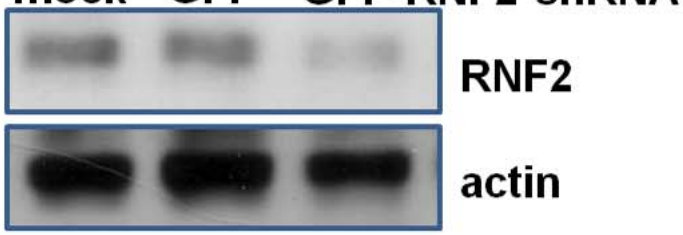

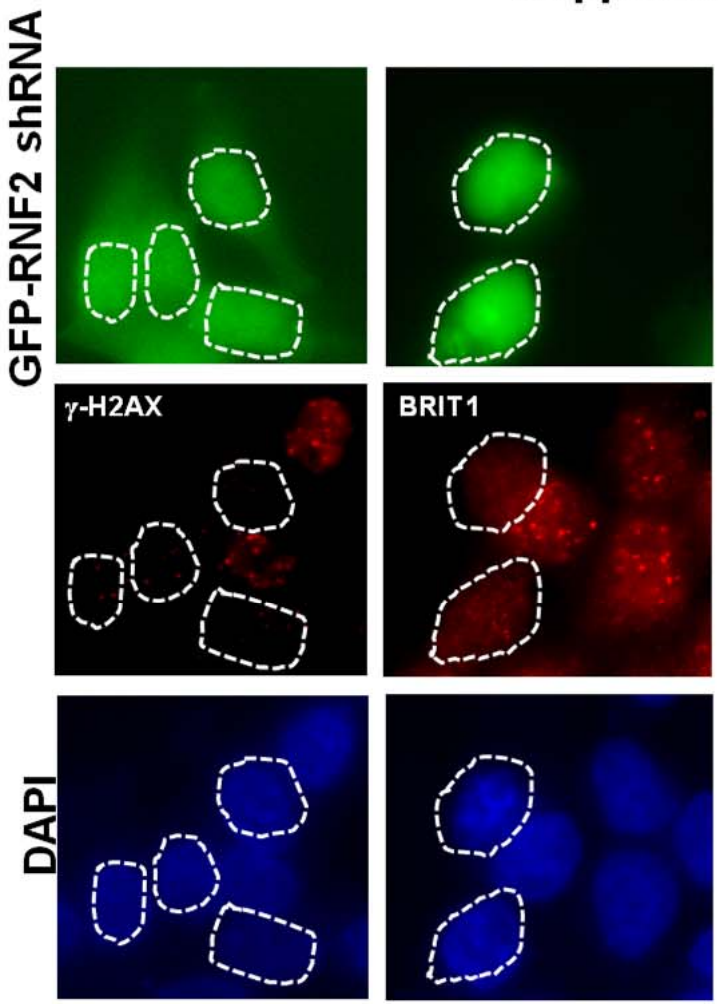

Supplemental Figure S12