## SUPPLEMENTAL FIGURE LEGENDS

**Supplemental Figure 1.** The initial 53BP1 foci formation observed in H2AX-/- cells occurs in a dose-dependent manner. Cells were irradiated with indicated doses of ionizing radiation and fixed 30 minutes later. Immunostaining was carried out using 53BP1 antibodies. Means and standard deviation (error bars) shown are results obtained from 10 cells counted. Bar, 10 μm.

**Supplemental Figure 2.** The initial NBS1 focus formation observed in H2AX-deficient cells does not require BRCA1. Single or double depletion of H2AX and BRCA1 was performed in U2OS cells. Following siRNA transfection, cells were irradiated (5 Gy) and fixed 30 minutes later. Immunostaining was carried out using the indicated antibodies. Bar, 10 μm.

**Supplemental Figure 3.** G2/M checkpoint assays (**A**) and cell cycle analysis (**B**) were performed in cells depleted of H2AX, NBS1, or both.

**Supplemental Figure 4.** IR-induced 53BP1 phosphorylation is greatly impaired in cells depleted of NBS1 or NBS1/H2AX. HeLa cells transfected with indicated siRNAs were treated with IR (10 Gy). One hour later, cells were collected and lysed. Immunoblotting was carried out using anti-53BP1, anti-NBS1, anti-H2AX and anti-phospho-53BP1 (S25/29P) antibodies as previously described (1).

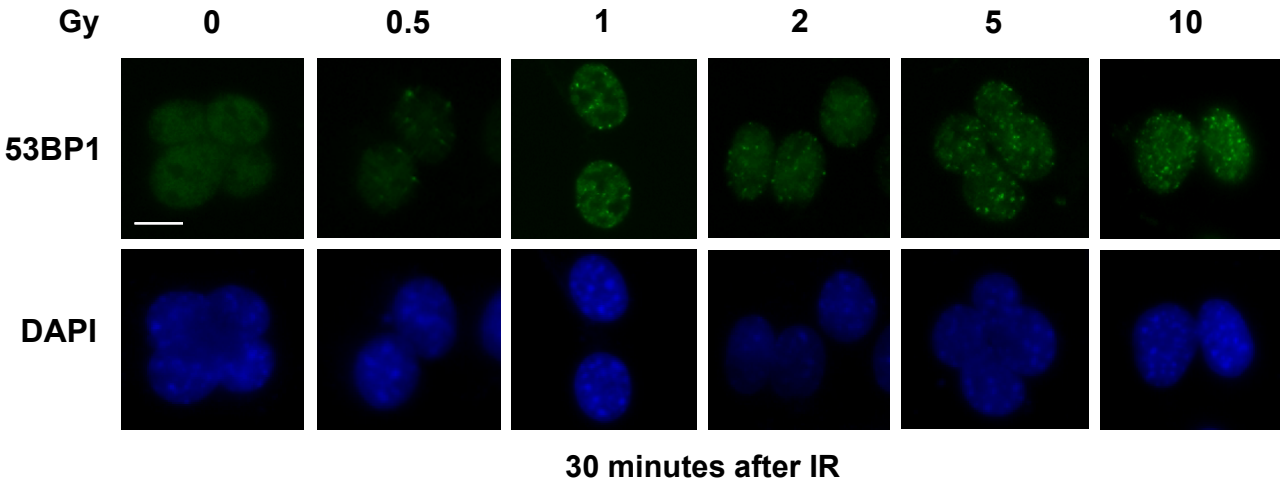
**Supplemental Figure 5.** (**A**) Depletion of H2AX in U2OS cells does not impair RPA foci formation. U2OS cells were transfected with indicated siRNAs. 48 hours later, cells were irradiated (10 Gy) and allowed to recover for 6 hours before fixation and immunostaining with antibodies as indicated. (**B**) RPA foci formation in wild type and ATM deficient cells was determined at different time points following IR treatment. For each sample in (**A**) or (**B**), at least two hundred cells were counted and the percentage of cells with indicated foci was determined. The results represent the average of three experiments. Bars, 10 μm.

**Supplemental Figure 6.** (**A**) 53BP1 interacts with NBS1. (**B**) Both of FHA and BRCT1 domains of NBS1 may be involved in 53BP1 binding. 293T cells were transfected with indicated expression constructs. Cells were lysed 24 hours after transfection. Immunoprecipitation was carried out using S-protein beads and immunoblotting was performed using antibodies as indicated.

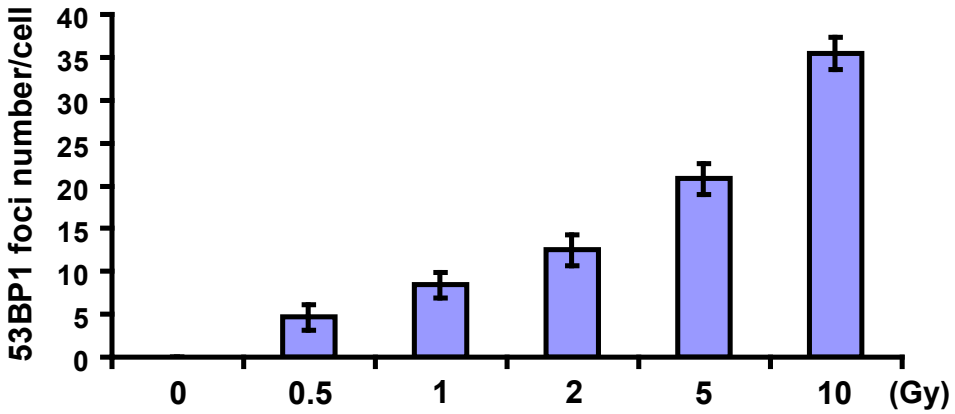
## REFERENCES

1. Ward, I. M., Minn, K., Jorda, K. G., and Chen, J. (2003) *J Biol Chem* **278**(22), 19579-19582

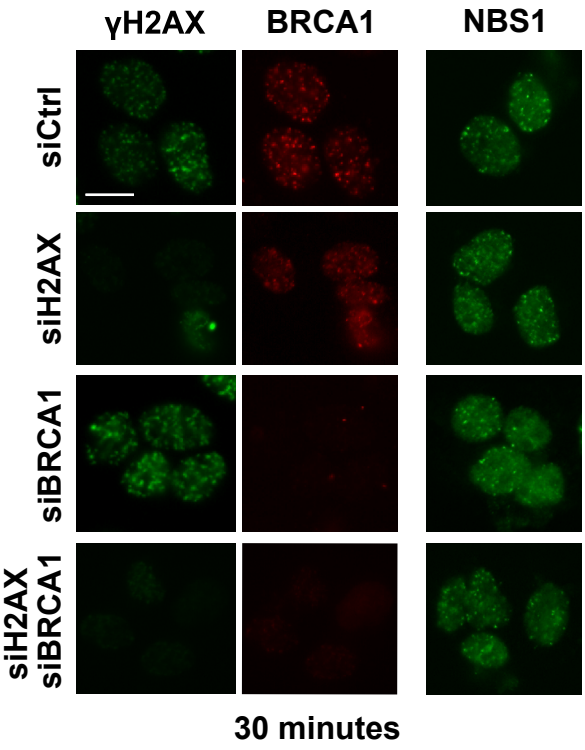
**Supplemental Figure 1**



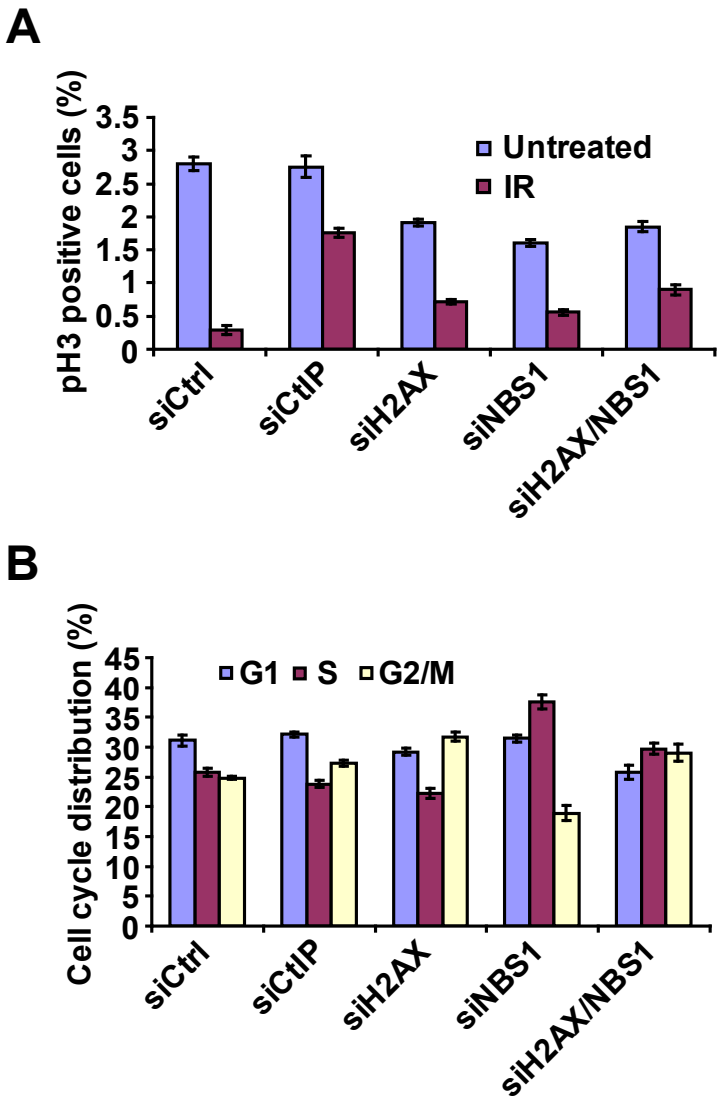

30 minutes after IR



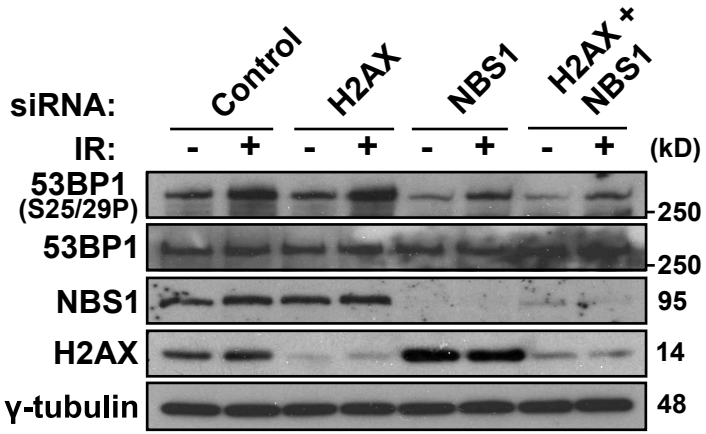
**Supplemental Figure 2**



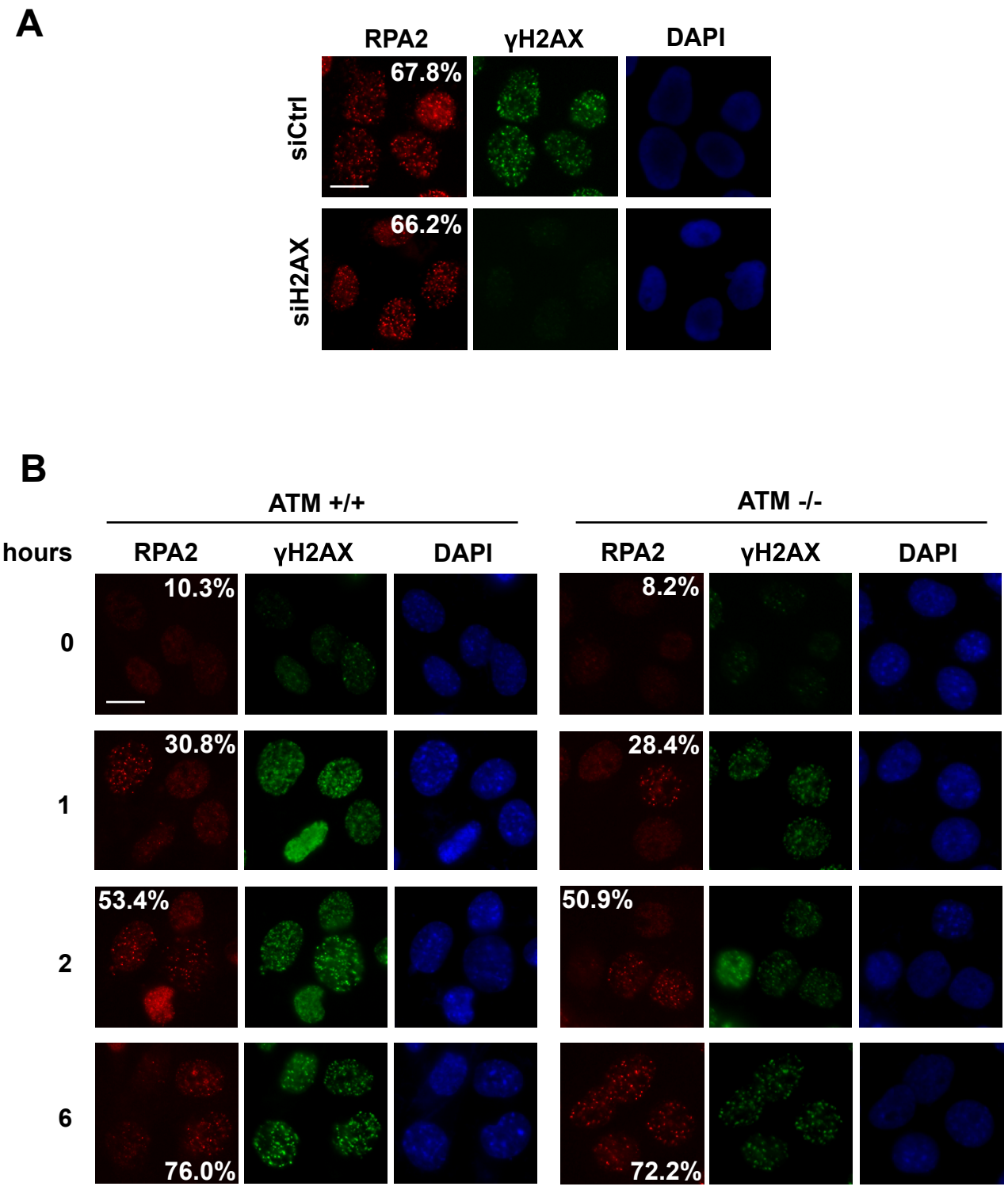
**Supplemental Figure 3**



**Supplemental Figure 4**



**Supplemental Figure 5**



**Supplemental Figure 6**