Supplemental Figure Legends

Supplemental Figure 1. **A**) RNF8 deficient MEFs infected with viral particles carrying indicated expression constructs were mock treated or irradiated and immunostained with indicated antibodies. **B**) RNF8 deficient cells reconstituted with indicated expression constructs were mock treated or irradiated (10 Gy) and chromatin fractions were prepared as described previously (1). Levels of ubiquitylated H2AX were analyzed by immunoblotting using anti-pH2AX antibodies.

Supplemental Figure 2. **A**) RNF8 deficient MEFs infected with viral particles carrying indicated expression constructs were mock treated or irradiated and immunostained with indicated antibodies. **B**) Localization of endogenous UBC13 was determined by immunostaining using anti-UBC13 antibodies in RNF8 deficient cells expressing TRNF8-MMS2 or TRNF8-UEV1A. **C**) UBC13 deficient cells expressing UBC13-H2B or UBC13C87A-H2B were irradiated (10 Gy) and immunostained for 53BP1 and FK2 IRIFs.

Supplemental Figure 3. RNF8 deficient MEFs infected with viral particles carrying indicated expression constructs were irradiated and processed as that described in Methods and Materials.

Supplemental Figure 4. Ring domain mutants failed to restore RNF8 function in promoting 53BP1 and FK2 IRIFs. RNF8 deficient cells were infected with retrovirus

harboring TRNF8-RNF103C621S or TRNF8-ChfrC292S mutant. Cells were irradiated and processed to observe localization of the fusion proteins **(A)** or radiation-induced 53BP1 and FK2 foci formation (**B**).

Supplemental Figure 5. UBC13 E55Q and F57E mutations abolish interaction with its E2 variants MMS2 and UEV1A. 293T cells were transiently transfected with plasmids encoding SBP-Flag-tagged UBC13 together with plasmids encoding myc-MMS2 or myc-UEV1A. Cells were lysed in NETN buffer 24 hours post-transfection and co-immunoprecipitation experiments was performed as indicated.

Supplemental Figure 6. UBC13 is required for IRIF formation of 53BP1 and FK2, but not that of pH2AX. UBC13+/+ and UBC13-/- MEFs were irradiated (3 Gy) and 53BP1 (**A**), FK2 (**B**) and pH2AX (**C**) foci formation were monitored at different time intervals post treatment. Results represent mean ± S.E.M from 200 cells counted and experiments were repeated twice. White bars and shaded bars indicated respectively numbers of foci positive cells observed in UBC13+/+ and UBC13-/- cells.

Supplemental Figure 7. MMS2 depleted cells displayed elevated levels of DNA damage. HeLa cells treated with indicated siRNAs were irradiated and 53BP1 (**A**), FK2 (**B**) and pH2AX (**C**) foci formation were monitored at different time intervals post treatment. Results represent mean ± S.E.M from 200 cells counted and experiments were repeated twice.

Supplemental Figure 8. Rad18 is not required for 53BP1 and FK2 IRIF formation.

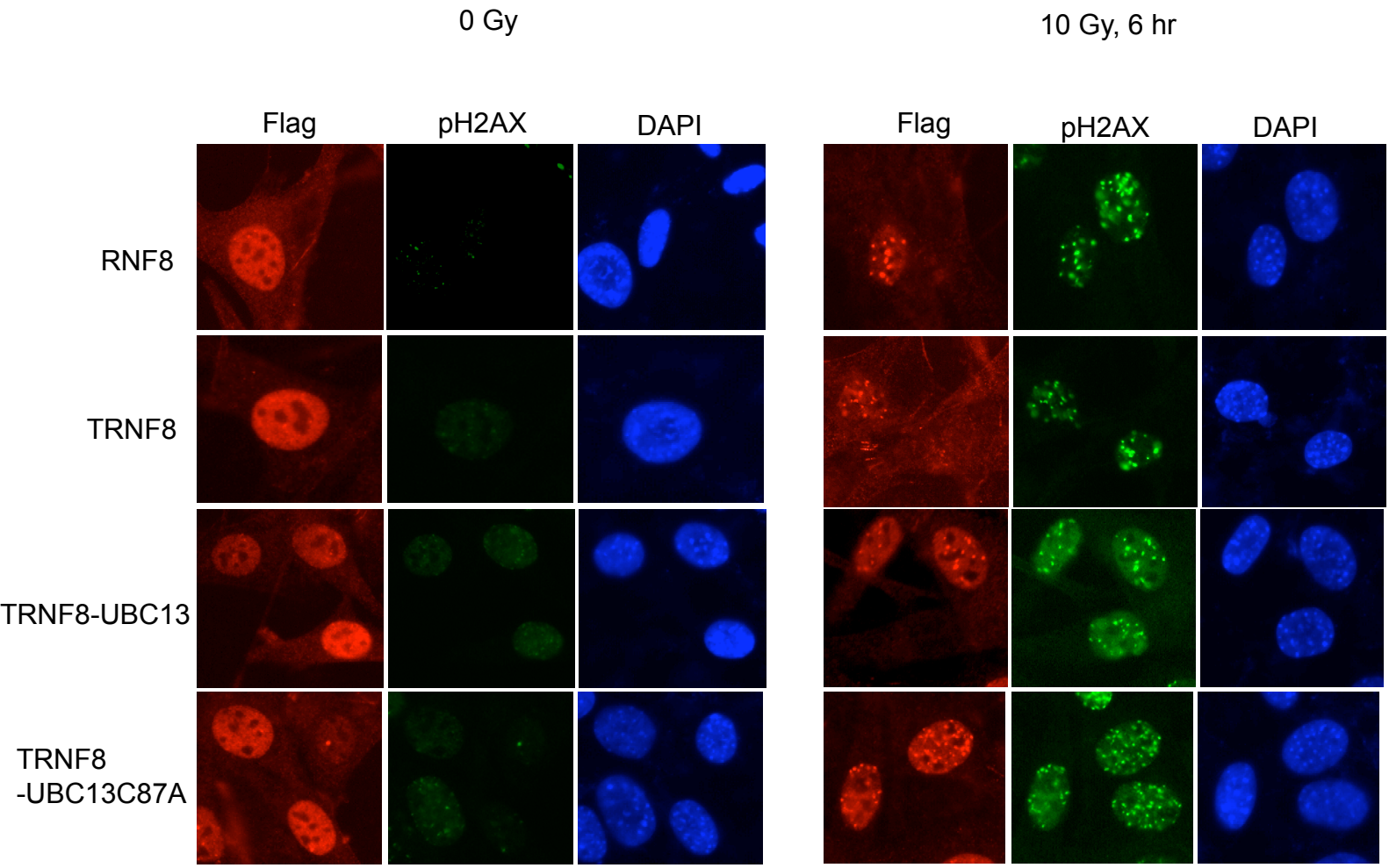
HeLa cells depleted of RNF8 or Rad18 were irradiated (10Gy) and fixed 6 hours post IR. Cells were processed as described in Methods and Materials and localization of ubiquitin conjugates (FK2; **A**) and 53BP1 (**B**) were observed using indicated antibodies. siRNA sequence for Rad18 is ACUCAGUGUCCAACUUGCUdTdT.

Reference

1. **Huen, M. S., R. Grant, I. Manke, K. Minn, X. Yu, M. B. Yaffe, and J. Chen. 2007. RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell 131:901-14.**

Supplemental Figure 1

A



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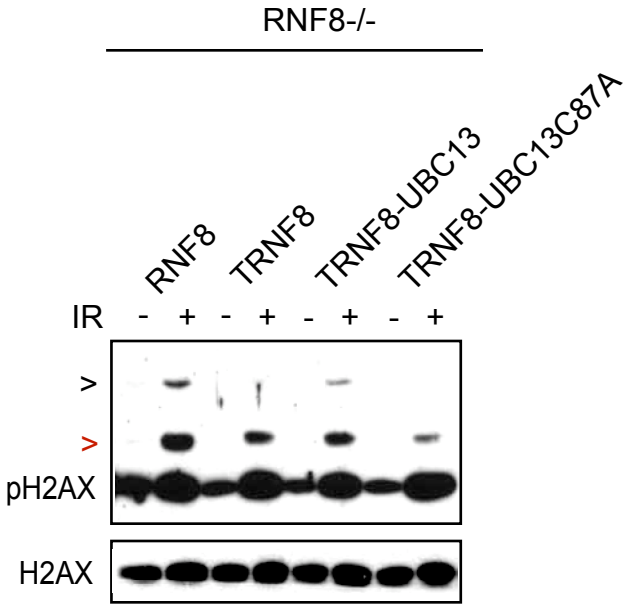
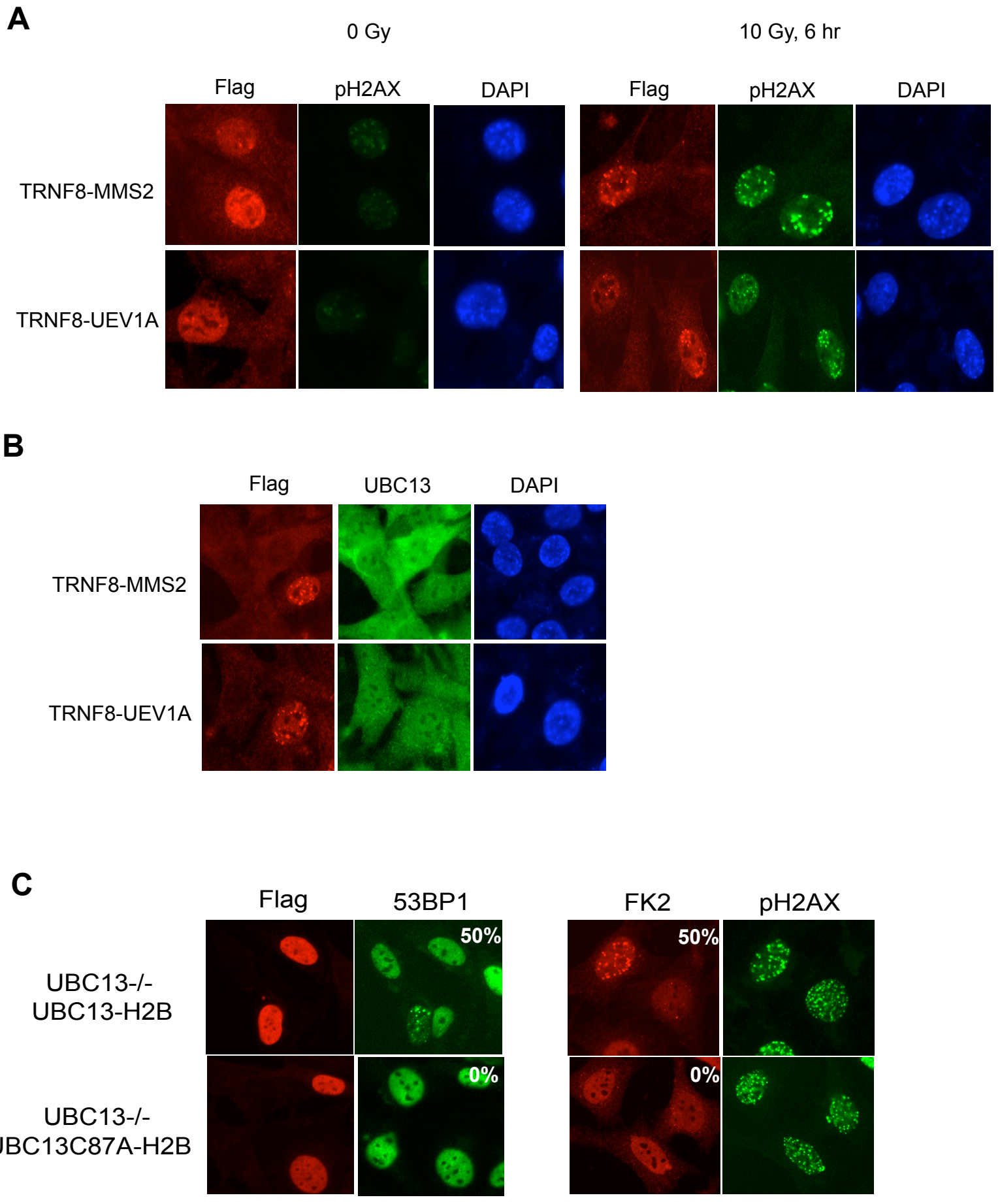
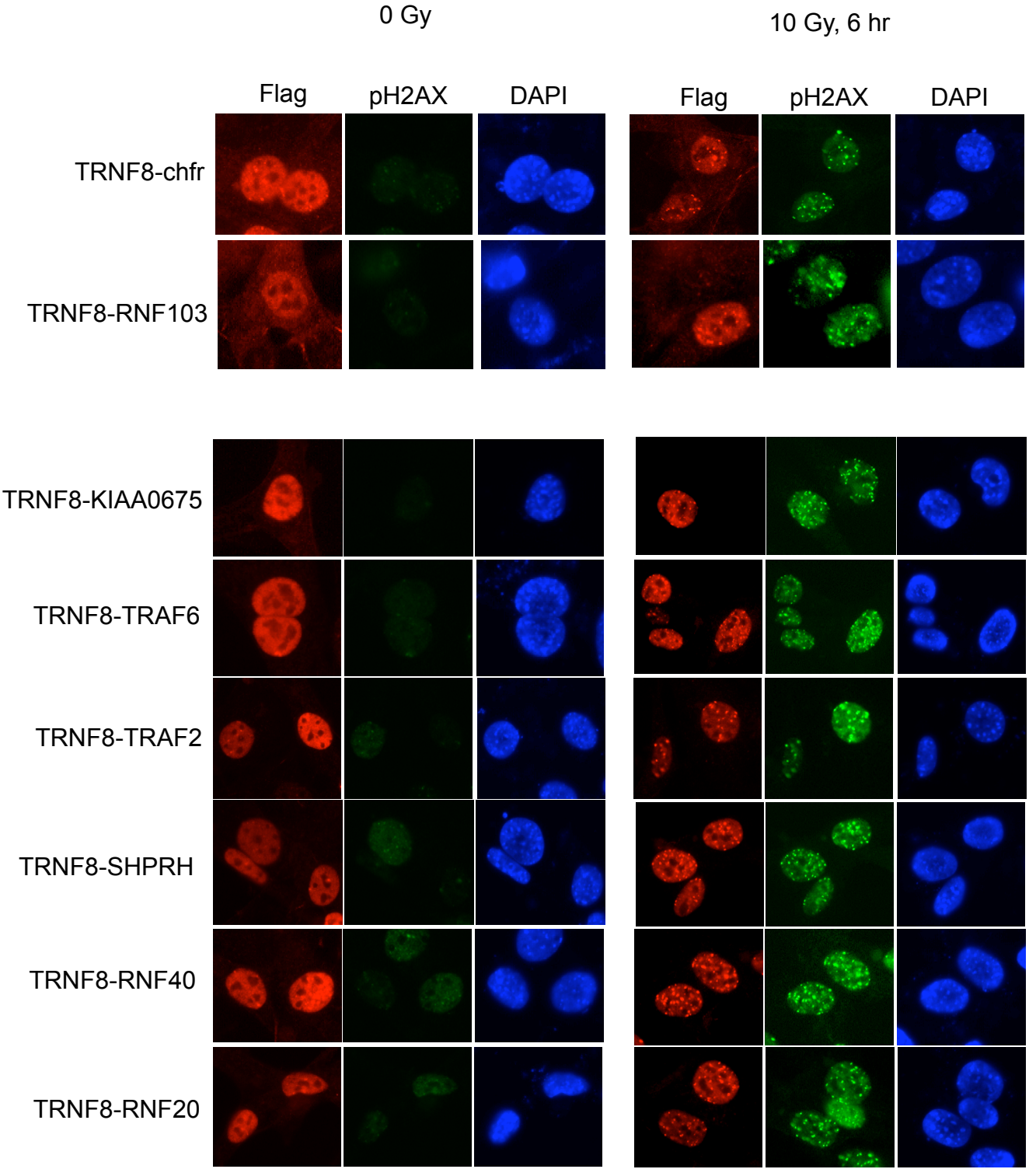


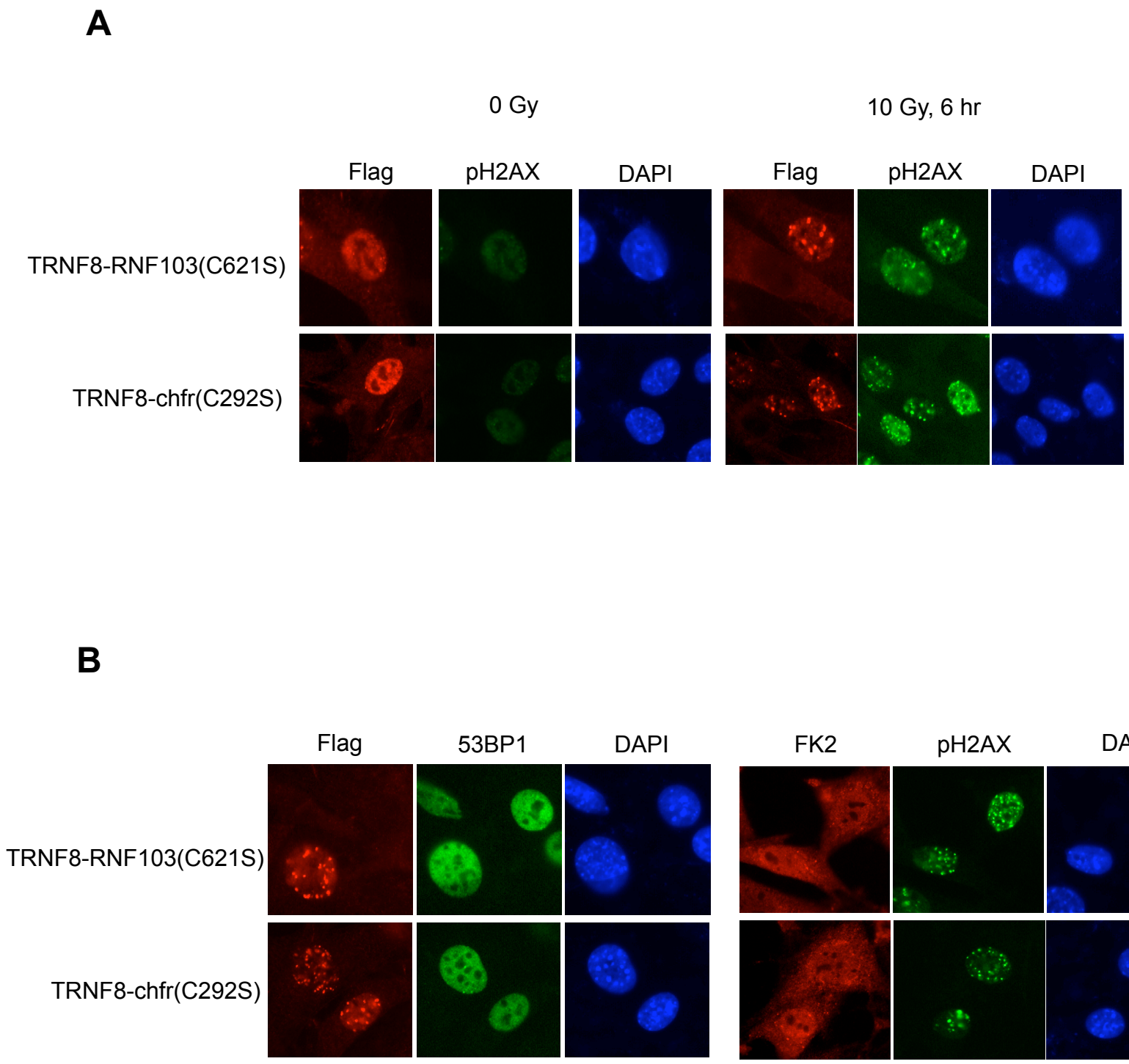
Supplemental Figure 2



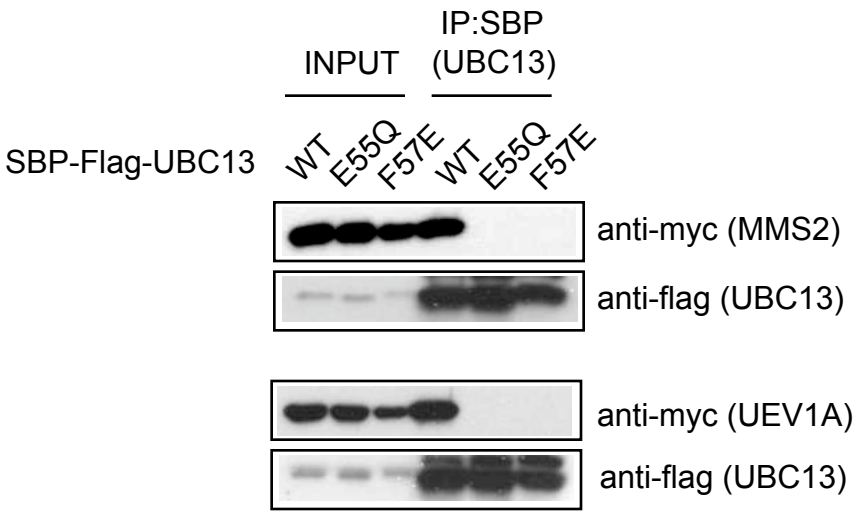
Supplemental Figure 3



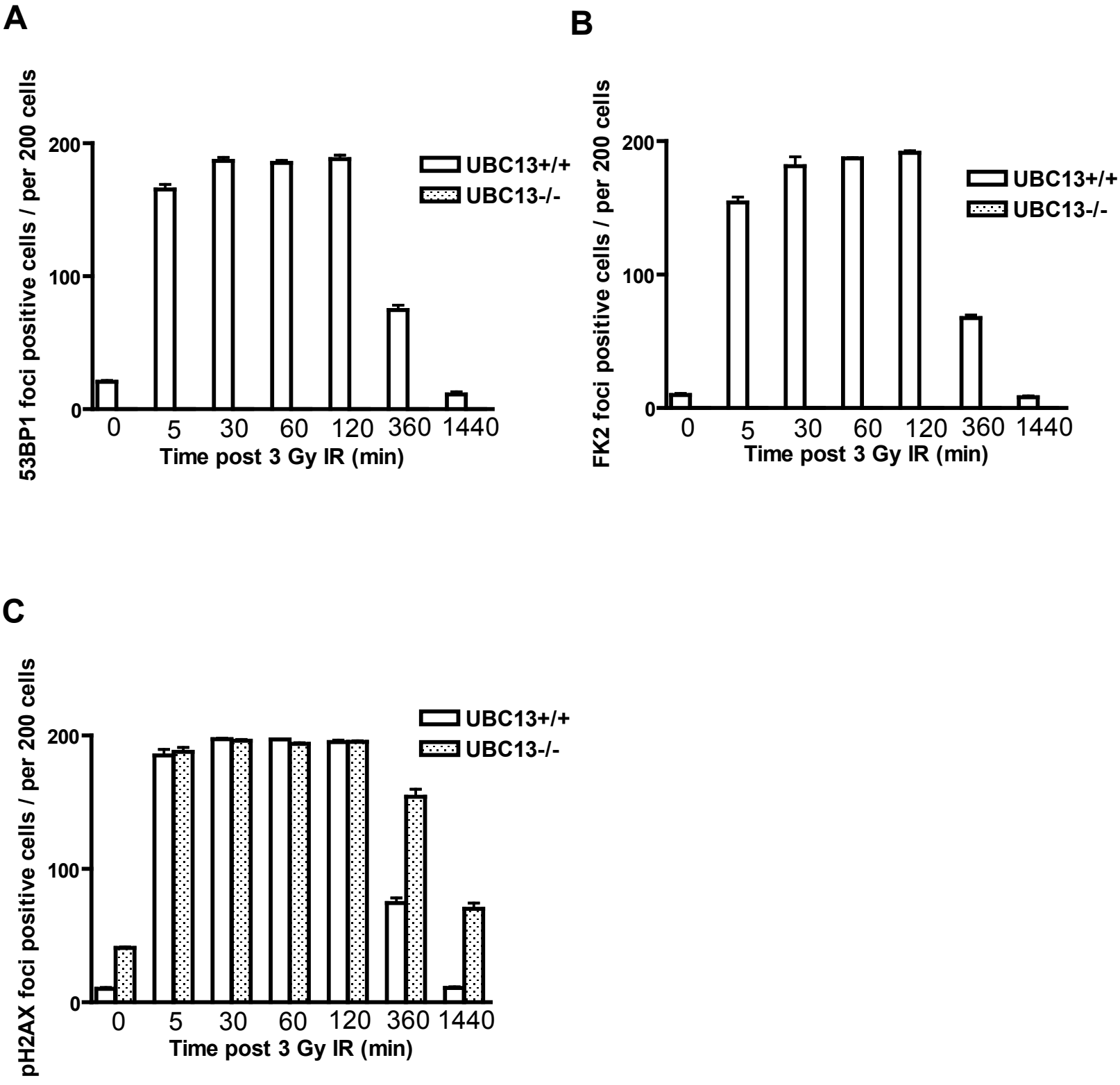
Supplemental Figure 4



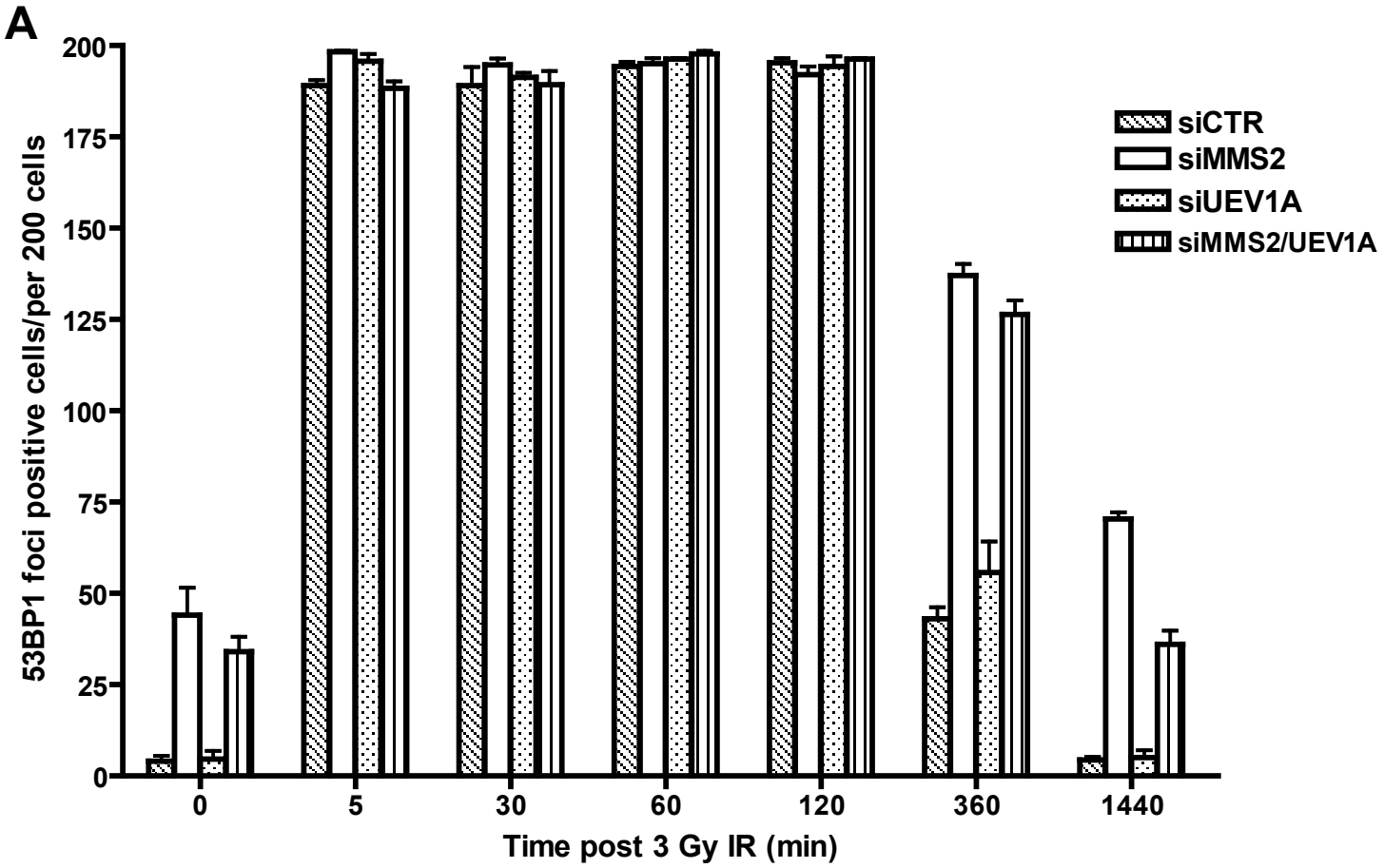
Supplemental Figure 5

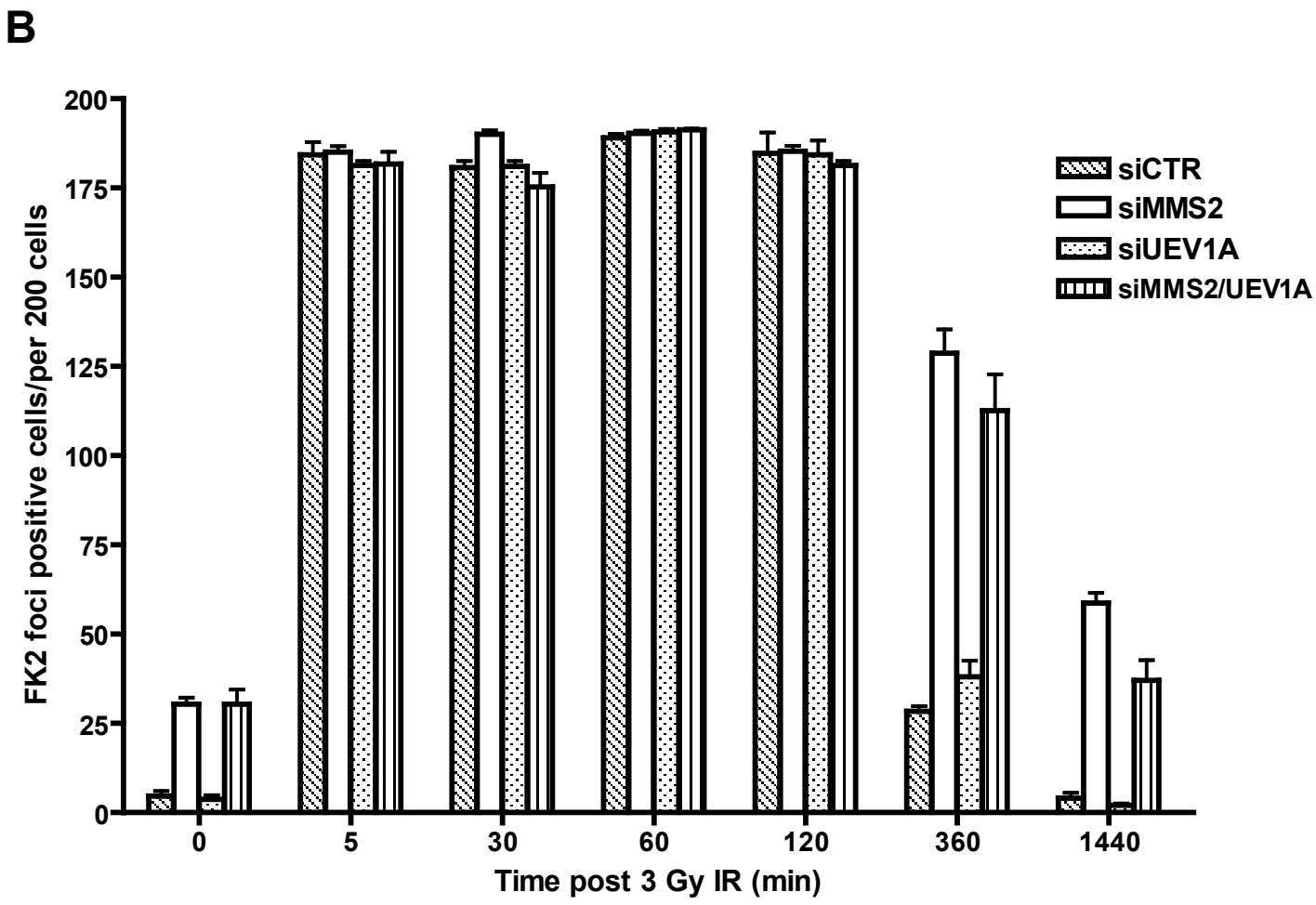


Supplemental Figure 6

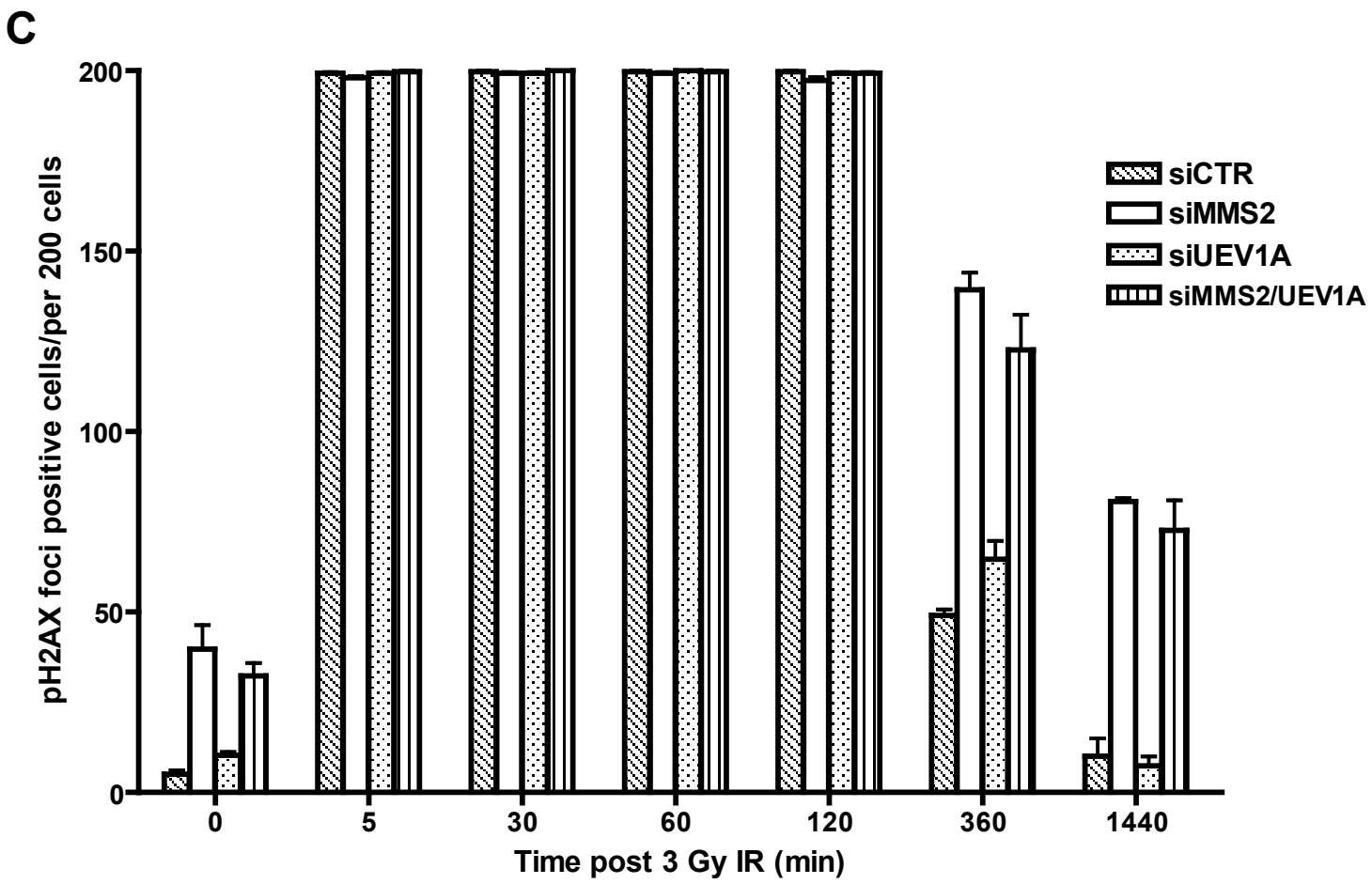


Supplemental Figure 7





Supplemental Figure 7



Supplemental Figure 8