SUPPLEMENTARY DATA

Supplemental Figure 1. 3E10 scFvs that do not bind DNA are still able to inhibit HDR when the 2xMBP-scFv expression constructs are simultaneously co-transfected with the HDR reporter constructs. A. The luciferase plasmid-based host cell reactivation assay was also performed in cells co-transfected with the pre-digested luciferase reporter construct and the 2xMBP-scFv expression constructs. B. Relative results, plotted as relative HDR (normalized to cells transfected with the luciferase constructs alone). Error bars represent the SEM; **P <0.01, and *P<0.05 by unpaired t-test.

Supplemental Figure 2. Neither full-length 3E10 nor 2XMBP-scFvs inhibit NHEJ. A. U2OS cells stably expressing the EJ5-GFP reporter construct were used to test the effect of 3E10 on NHEJ. The reporter construct is schematized. B. Cells were pre-treated with media control or purified full-length 3E10 for 24hours before nucleofection of an I-sceI construct. C. The percent of GFP positive cells was determined by flow cytometry, normalized to control and plotted as relative NHEJ. D. U2OS EJ5 cells were transfected with 2XMBP-scFv expression constructs 24hours before nucleofection of an I-sceI construct. E. The percent of GFP positive cells was determined by flow cytometry, normalized to control and plotted as relative NHEJ. F. A luciferase plasmid-based host cell reactivation assay for NHEJ was also performed in primary skin fibroblasts. The reporter construct is schematized. G. Cells were transfected with the 2XMBP-scFv expression constructs 24hours before transfection with the pre-digested luciferase reporter construct. H. Relative results, plotted as relative NHEJ (normalized to cells transfected with the luciferase constructs alone). Error bars represent the SEM.

Supplemental Figure 3. Full length 3E10 variants interact with endogenous RAD51. A. Hela cells were transfected with empty vectors or expression constructs for the WT full length 3E10 or the D31N full length 3E10. Cells were then lysed either in the presence or absence of benzonase, and the lysates were incubated with protein A/G beads (to which the full length 3E10 binds). The beads were washed and the immobilized proteins were eluted. B. The elution samples were run on a polyacrylamide gel and interrogated via western blots for a panel of DNA repair factors. C. Western blot results confirming the interaction between endogenous RAD51 and WT, D31N and D31N/R92N full length 3E10 expressed proteins. D. Results from a protein pull-down with purified D31N 2XMBP-scFv and purified RAD52. The samples were run on an SDS-PAGE gel and the gel was stained with coomassie.

Supplemental Figure 4. Purified WT 3E10 scFv has a higher binding affinity to purified RAD51 than the affinity between purified D31N scFv and RAD51. A. Protocol for an affinity binding assay to determine the Kd between purified WT or D31N scFv proteins and purified RAD51. B. SyproOrange

stained gels of standards of known concentrations of RAD51 and 2XMBP-scFv proteins (C) run in parallel with elution samples from the affinity binding experiment (D).

Supplemental Figure 5. Purified WT and D31N scFv proteins inhibit RAD51 nuclear localization and foci formation after irradiation. A. Primary skin fibroblasts were left untreated or pretreated with purified 2XMBP-scFv proteins and then irradiated with 10Gy IR and fixed for immunofluorescence. B. Representative images of treated primary skin fibroblasts for each fluorescent channel are shown as well as the three color merged images.

Supplemental Figure 6. Purified WT and D31N scFv proteins increase phospho-RPA nuclear foci formation after irradiation. A. Primary skin fibroblasts were left untreated or pretreated with purified 2XMBP-scFv proteins and then irradiated with 10Gy IR and fixed for immunofluorescence. B. Representative images of phospho-RPA foci from treated primary skin fibroblasts.

Supplemental Figure 7. Expressed scFv proteins inhibit RAD51 nuclear localization and foci formation after irradiation 24 hours after transfection with 2XMBP-scFv expression constructs. A. Primary skin fibroblasts were also transfected with 2XMBP-scFv expression constructs for a shorter time span and then irradiated with 10Gy IR and fixed for immunofluorescence. Cells with 10 or more RAD51 foci (B) and the cytoplasmic fraction of RAD51 (C) were scored for each condition. D. Transfected cells with 10 or more RPA foci were also scored. Error bars represent the SEM; ***P<0.001, **P <0.01, and *P<0.05 by unpaired t-test.

Supplemental Figure 8. Expressed scFv proteins inhibit RAD51 nuclear localization and foci formation after irradiation. A. Primary skin fibroblasts were left untransfected or transfected with 2XMBP-scFv expression constructs and then irradiated with 10Gy IR and fixed for immunofluorescence. B. Representative images of transfected primary skin fibroblasts for each fluorescent channel are shown as well as the three color merged images.

Supplemental Figure 9. Expressed scFv proteins increase phospho-RPA nuclear foci formation after irradiation. A. Primary skin fibroblasts were left untransfected or transfected with 2XMBP-scFv expression constructs and then irradiated with 10Gy IR and fixed for immunofluorescence. B. Representative images of phospho-RPA foci from transfected primary skin fibroblasts.

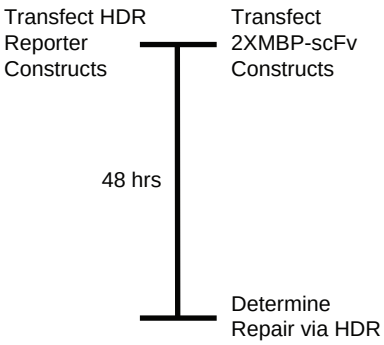
Supplemental Figure 10. Full length 3E10 also inhibits RAD51 foci formation after irradiation. A. Primary skin fibroblasts were left untreated or pretreated with full length 3E10, and then irradiated with 10Gy IR and fixed for immunofluorescence. B. Cells with 10 or more RAD51 foci were scored for each condition.

Supplemental Figure 11. Purified WT and D31N scFv proteins inhibits RAD51 nuclear localization and foci formation after treatment with etoposide. A. U2OS cells were left untreated or pretreated with purified 2XMBP-scFv proteins and then treated with etoposide and fixed for immunofluorescence. B. Representative images of each experimental condition of primary skin fibroblasts for each fluorescent channel are shown, as well as the three color merged images.

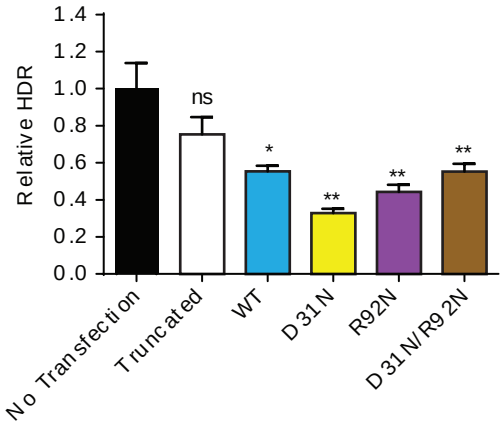
Supplemental Figure 12. The D31N mutation in 3E10scFv decreases chemosensitization of cells to Etoposide. BRCA2 deficient PEO-1 cells and PTEN deficient U251 cells were left untransfected or transfected with 2XMBP-scFv expression constructs and then reseeded for a clonogenic survival assay. A. Western blot confirming expression of 2XMBP-scFv variants in PEO-1 cells. B. PEO-1 clonogenic survival assay results. C. Western blot confirming expression of 2XMBP-scFv variants in U251 cells. D. U251 clonogenic survival assay results after transfection with 2XMBP-scFv expression constructs. PTEN deficient U251 cells were also treated with purified WT or D31N 2XMBP-scFv proteins and then reseeded for a clonogenic survival assay. E. U251 clonogenic survival assay results after treatment with 2XMBP-scFv proteins.

Supplemental Figure 1

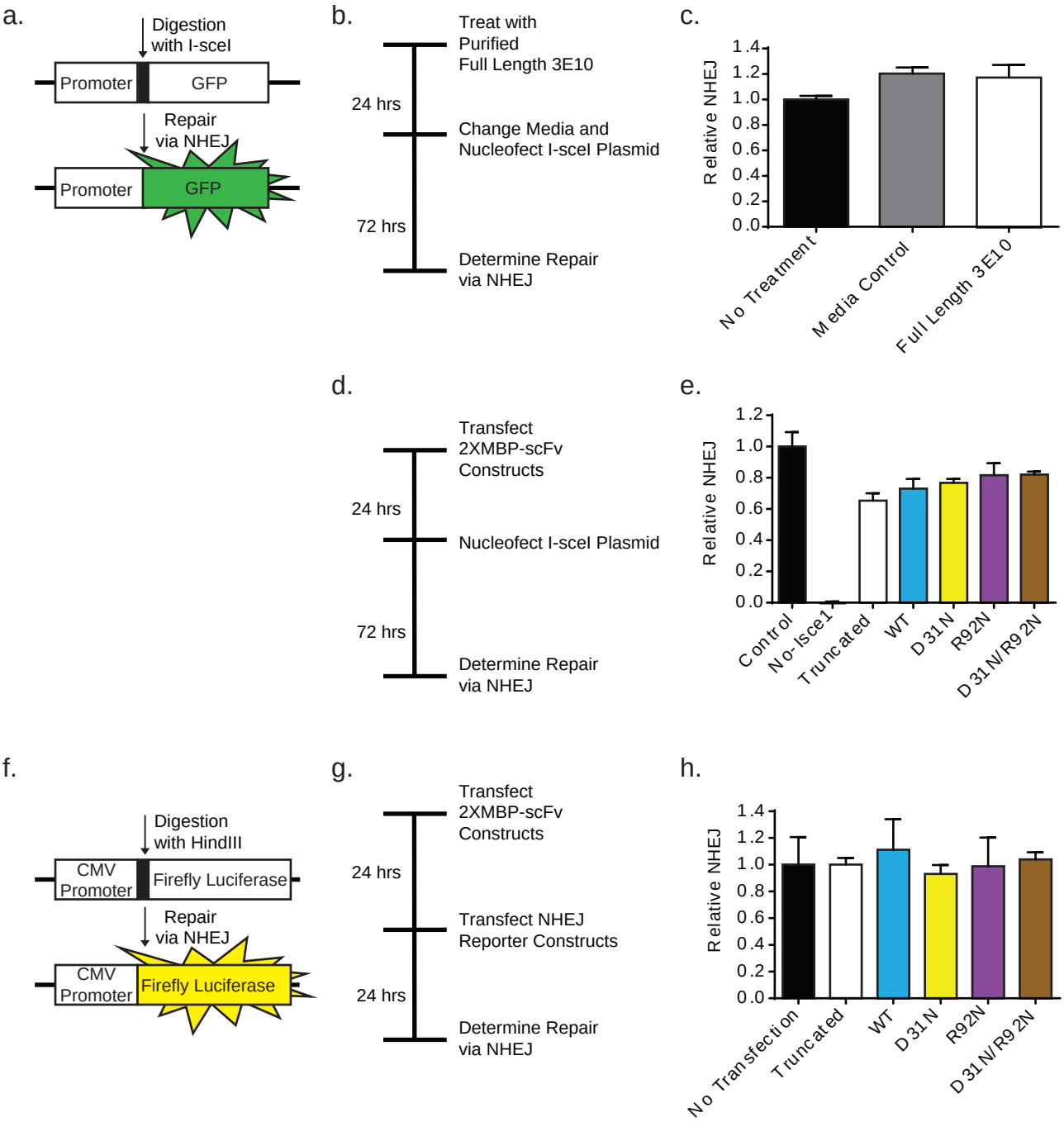
a.



b.

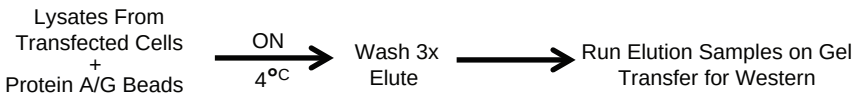


Supplemental Figure 2



Supplemental Figure 3

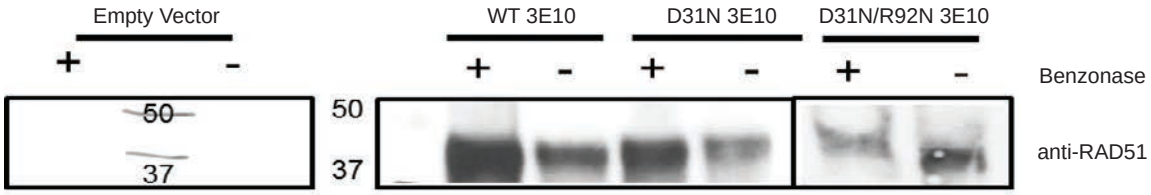
a.



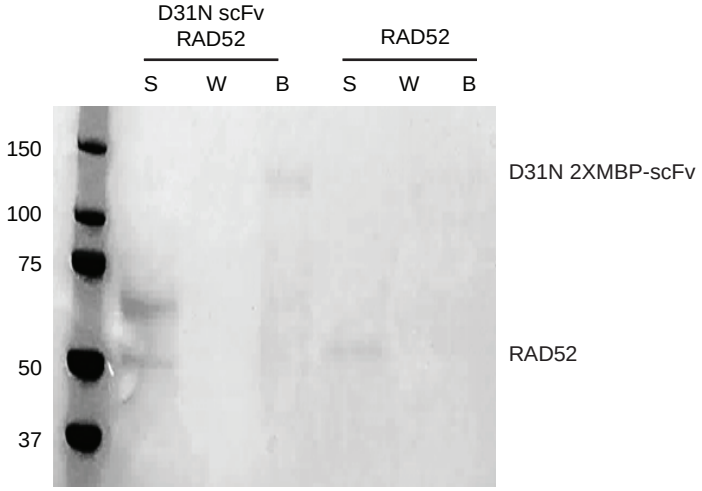
b.

	Empty Vector	WT Full Length 3E10	D31N Full Length 3E10
RAD51	No	Yes	Yes
RAD52	No	Yes	Yes
BRCA1	Yes	Yes	Yes
KU80	Yes	Yes	Yes
yH2Ax	No	No	No
MRE11	No	No	No
CHK1	No	No	No
RAD50	No	No	No
BRCA2	No	No	No
PTEN	No	No	No
AKT	No	No	No
NBS1	No	No	No
ATRIP	No	No	No
53BP1	No	No	No
XRCC3	No	No	No
RPA	No	No	No

c.

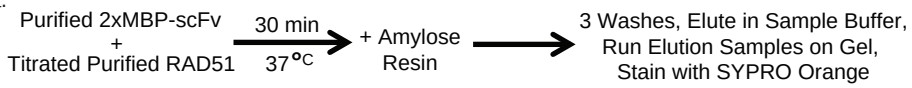


d.

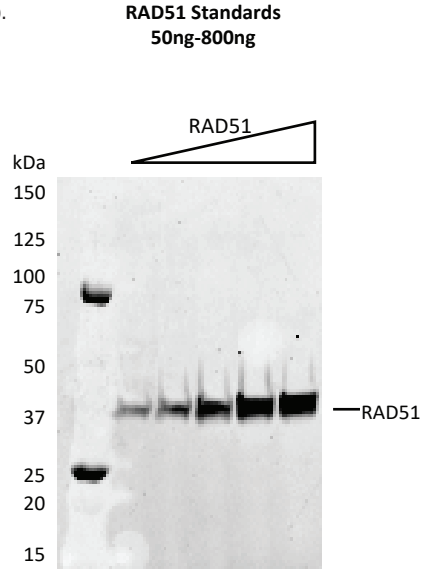


Supplemental Figure 4

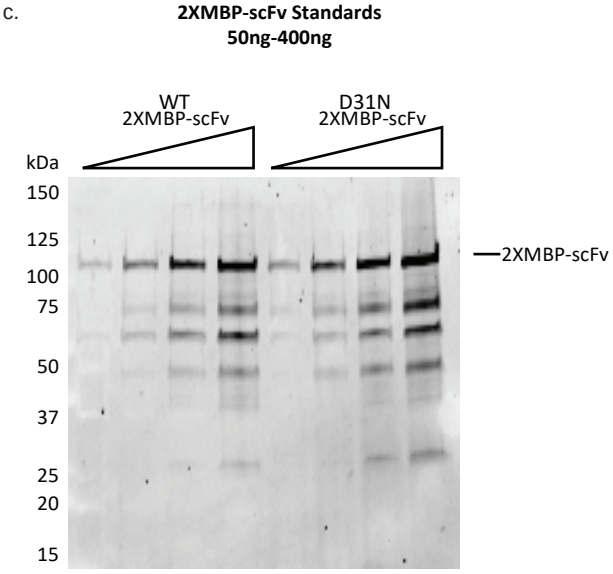
a.



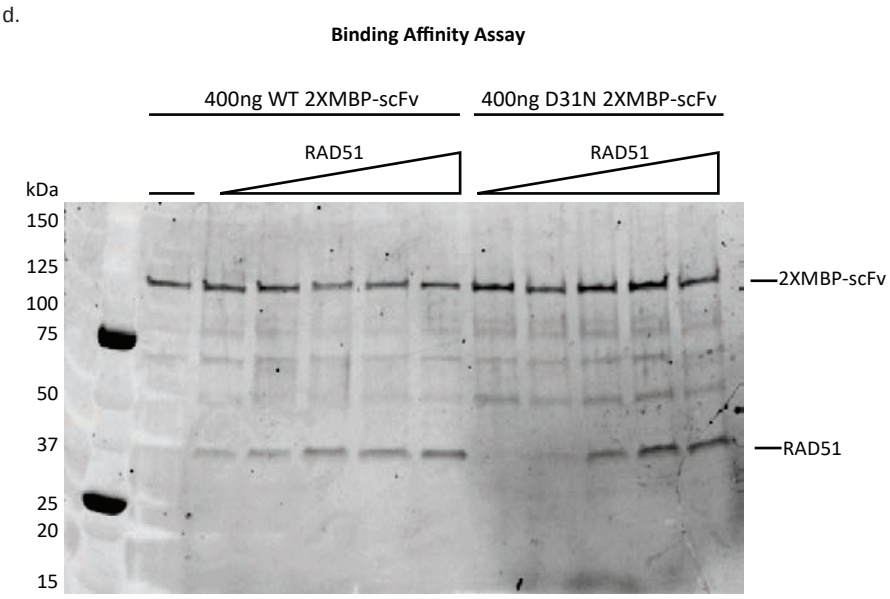
b.



c.

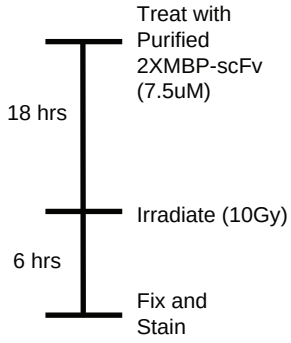


d.

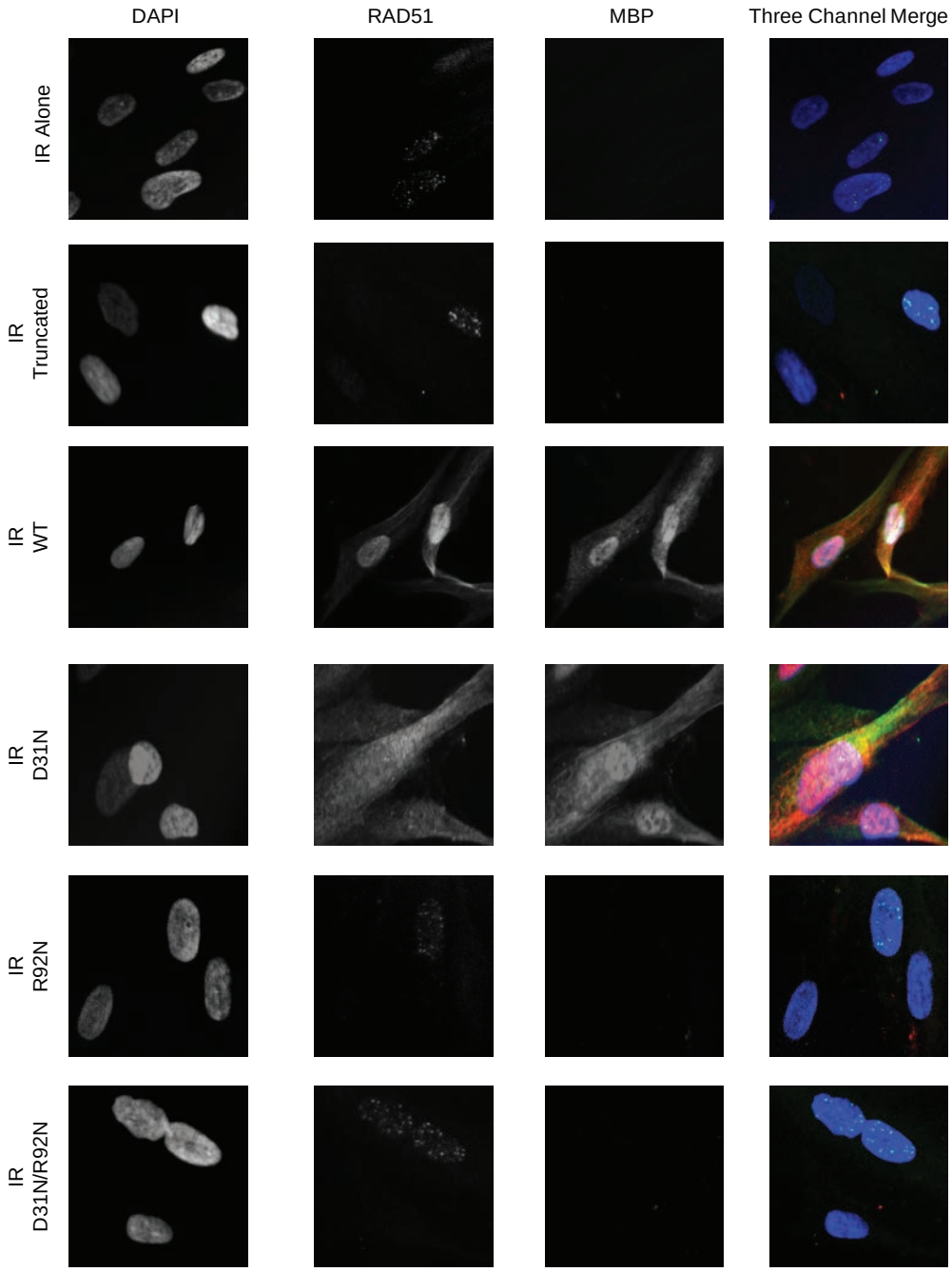


Supplemental Figure 5

a.

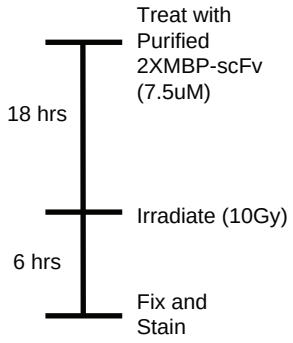


b.

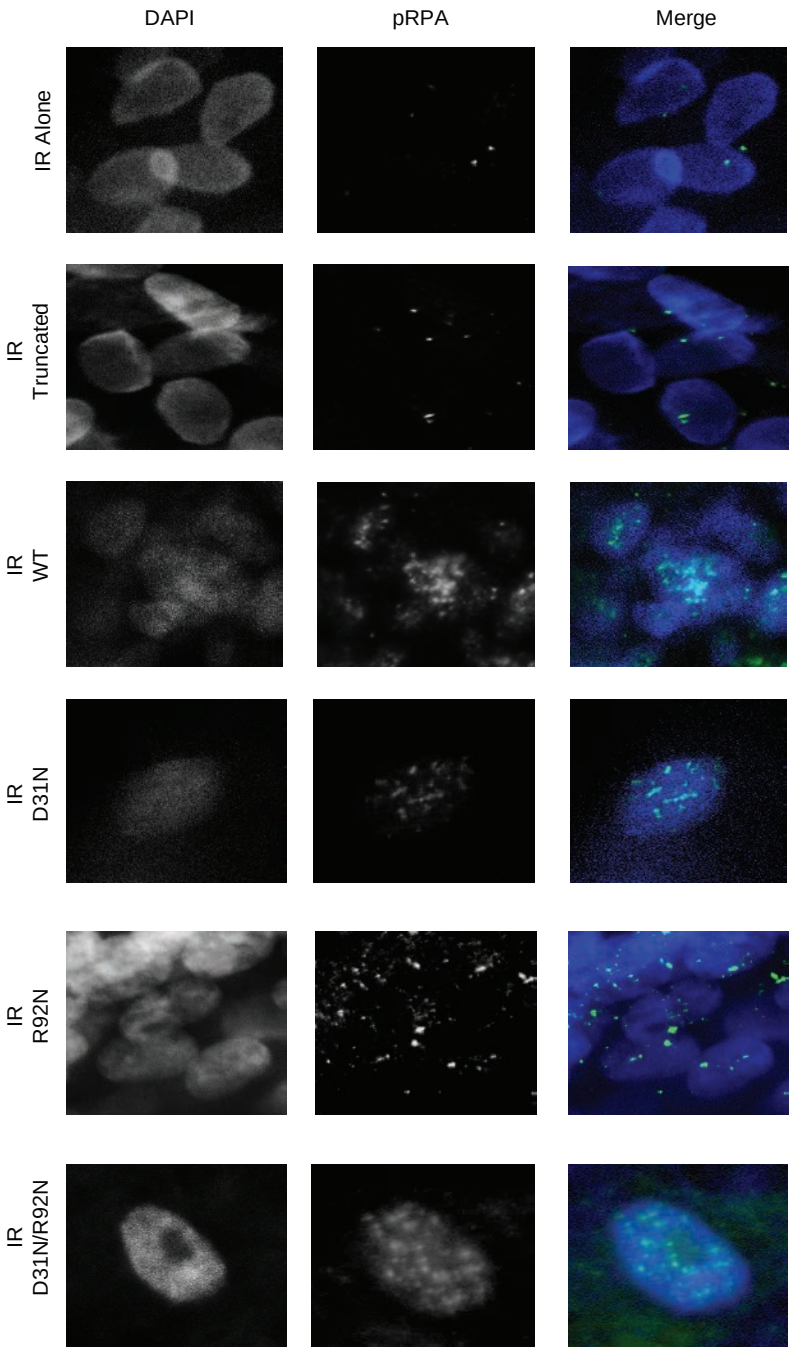


Supplemental Figure 6

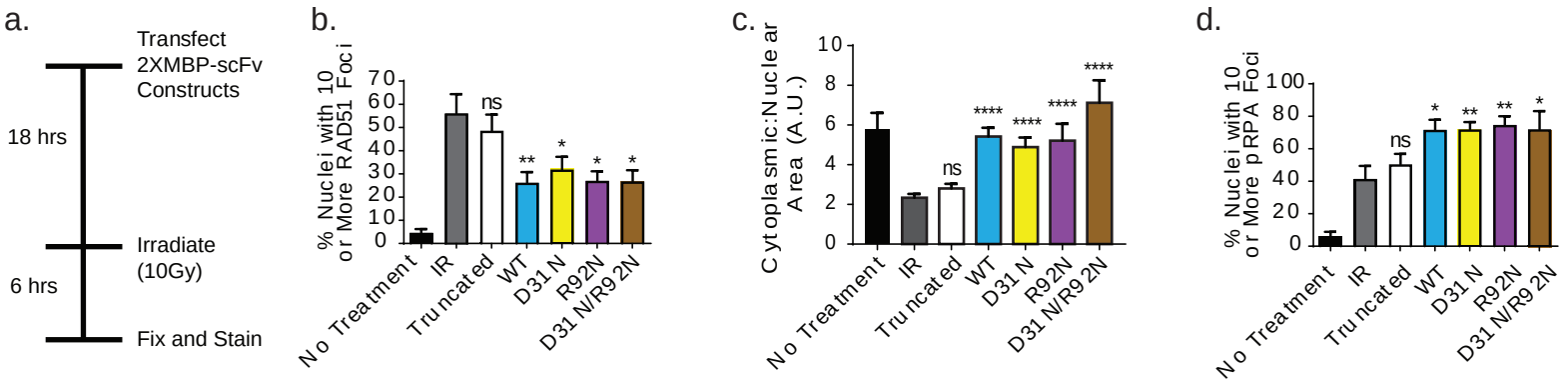
a.



b.

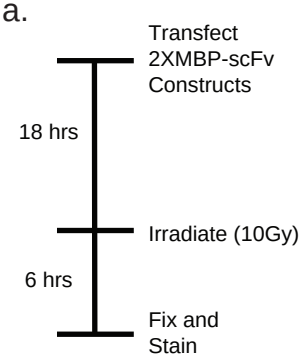


Supplemental Figure 7

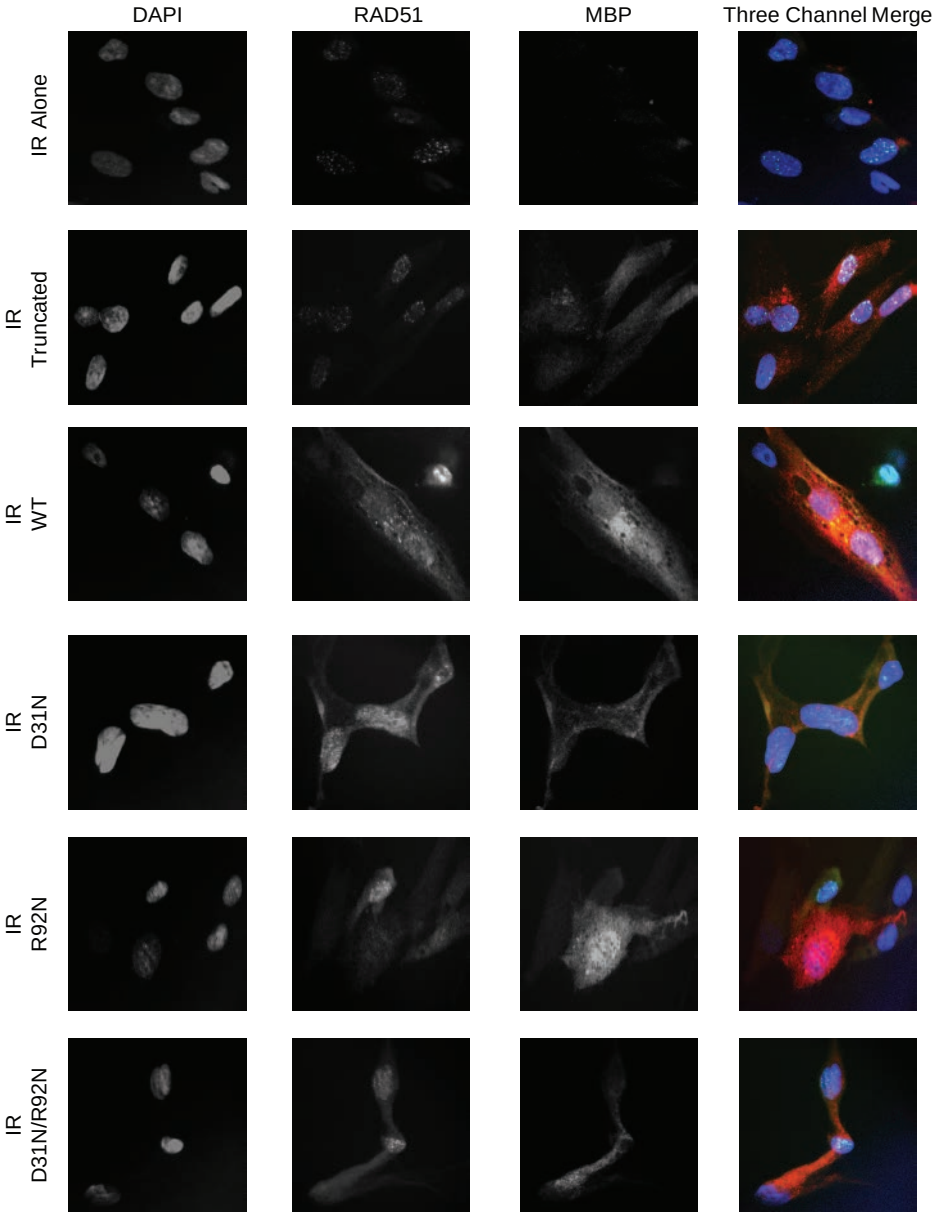


Supplemental Figure 8

a.

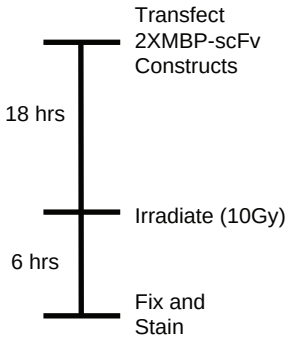


b.

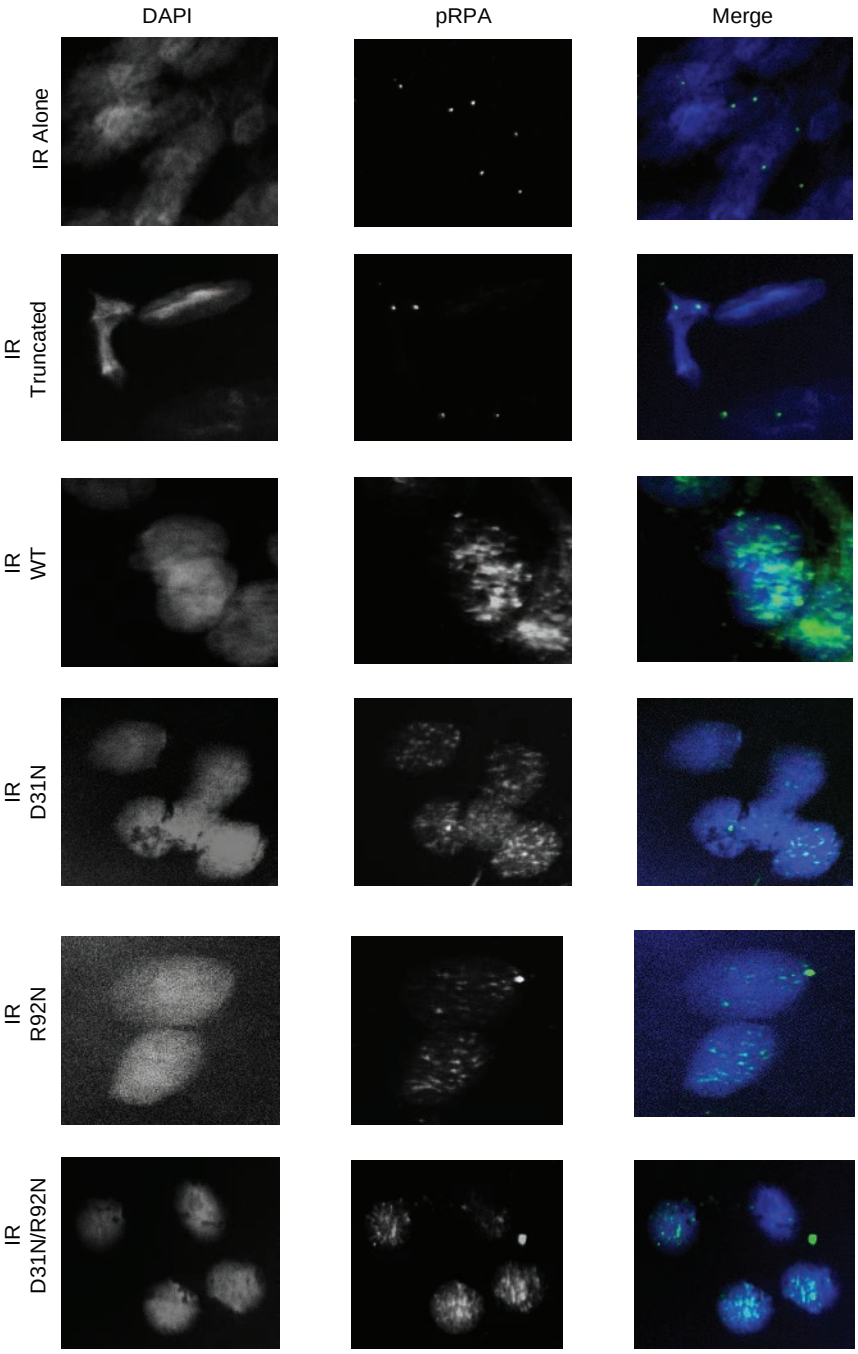


Supplemental Figure 9

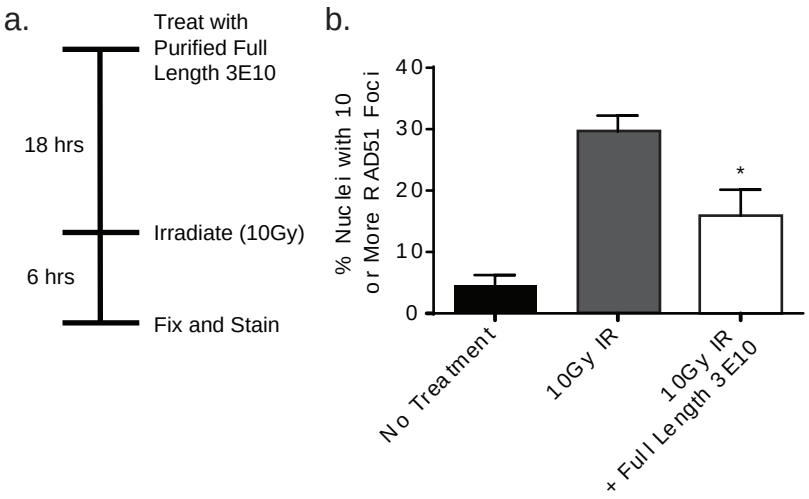
a.



b.

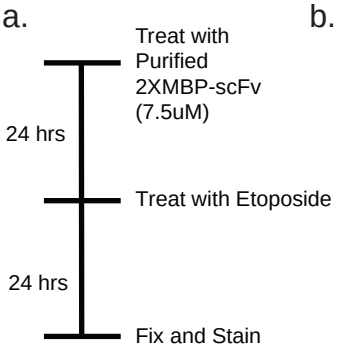


Supplemental Figure 10

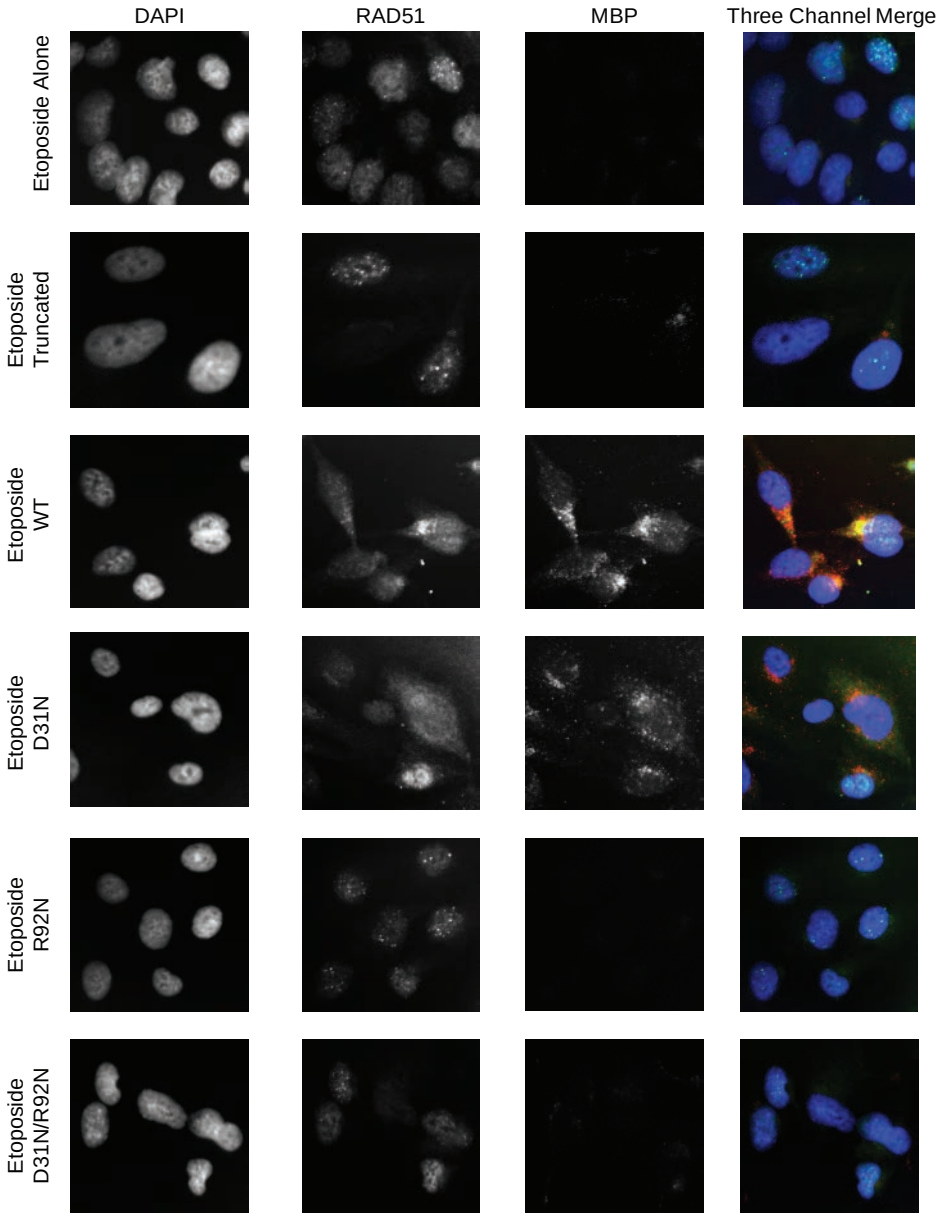


Supplemental Figure 11

a.

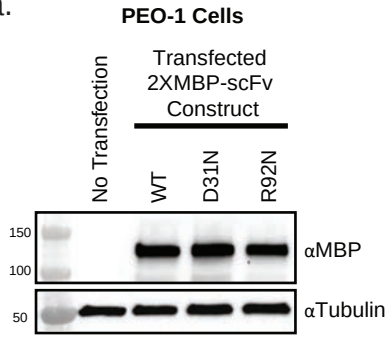


b.

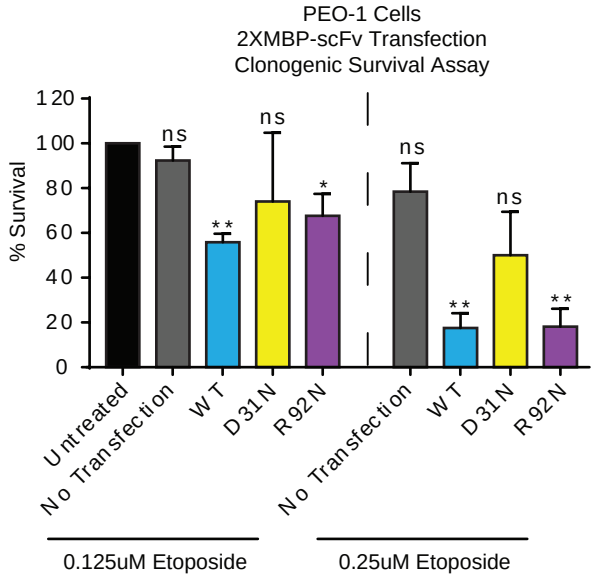


Supplemental Figure 12

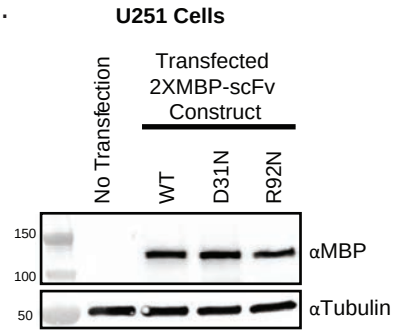
a.



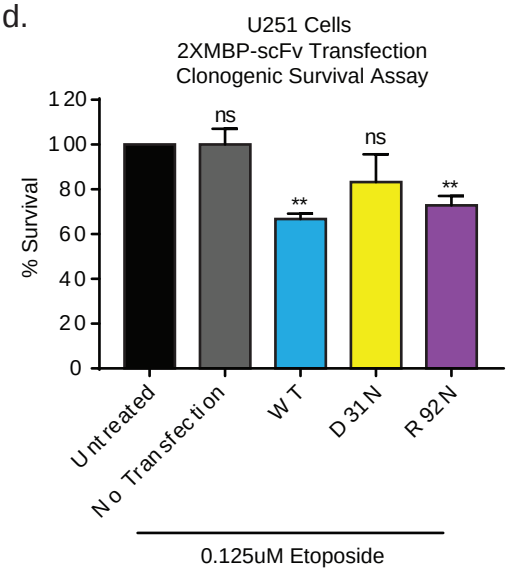
b.



c.



d.



e.