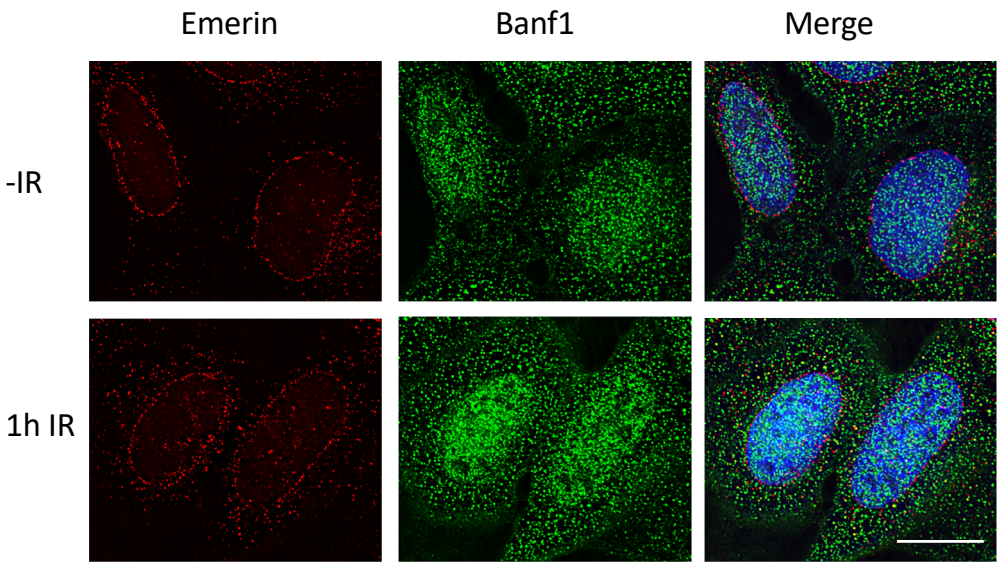
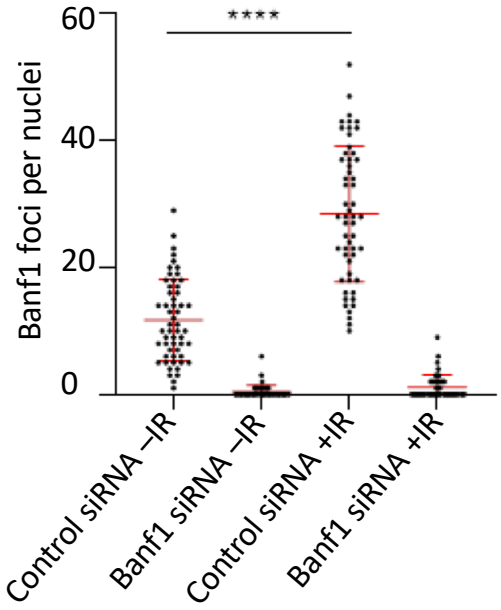
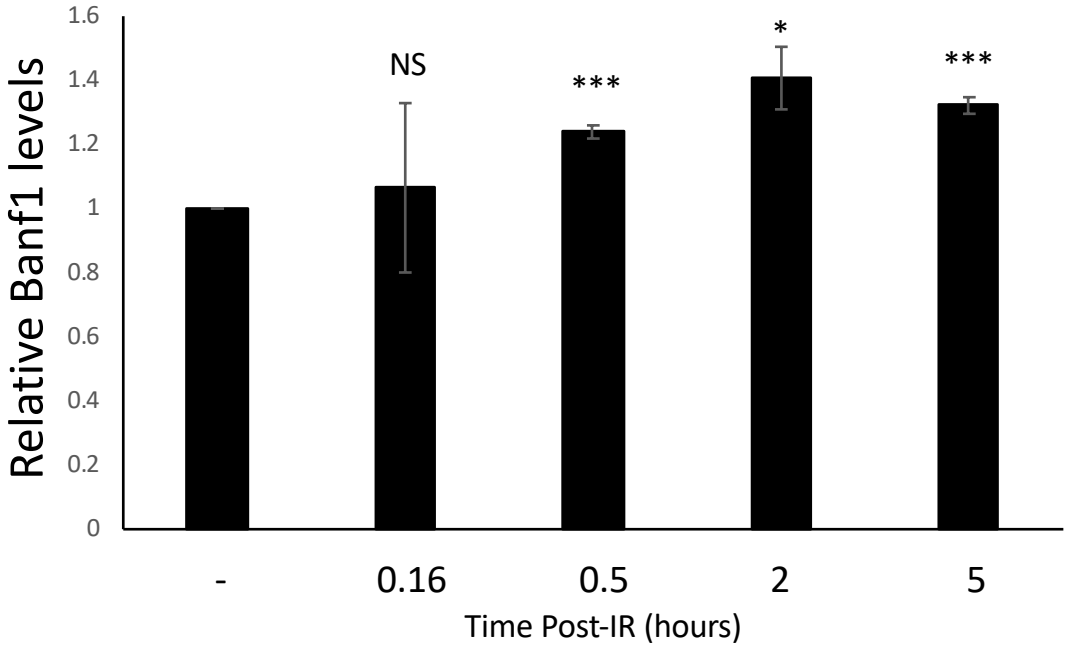


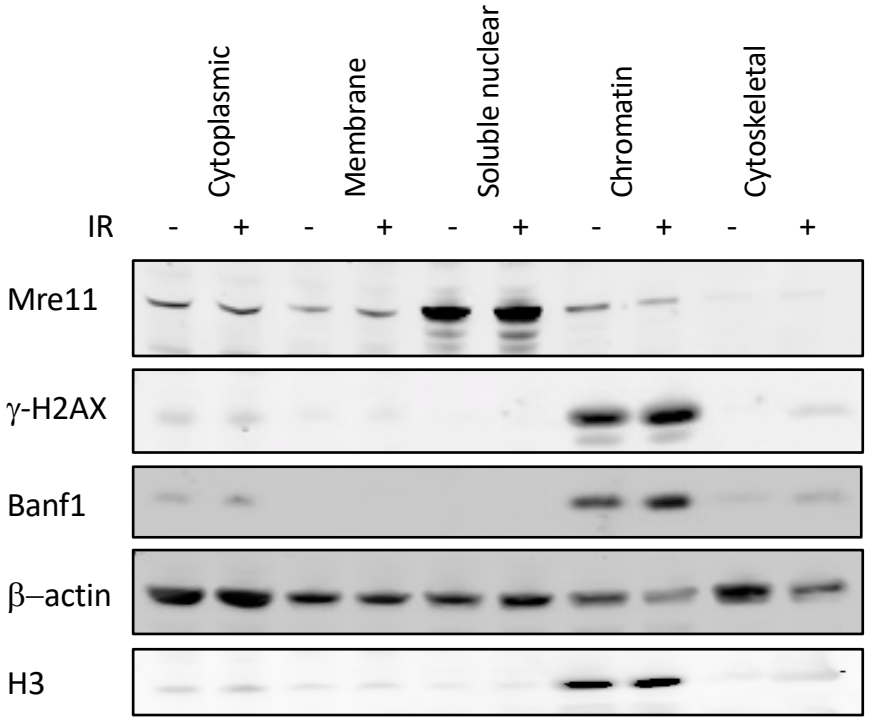
Supplementary Figure 1. Banf1 cellular localisation. U2OS cells were treated or mock treated with 6 Gy IR, cells were fixed (without pre-extraction) at the indicated timepoints and stained with the indicated antibodies. Immunofluorescence scale bars represent 10 μm.



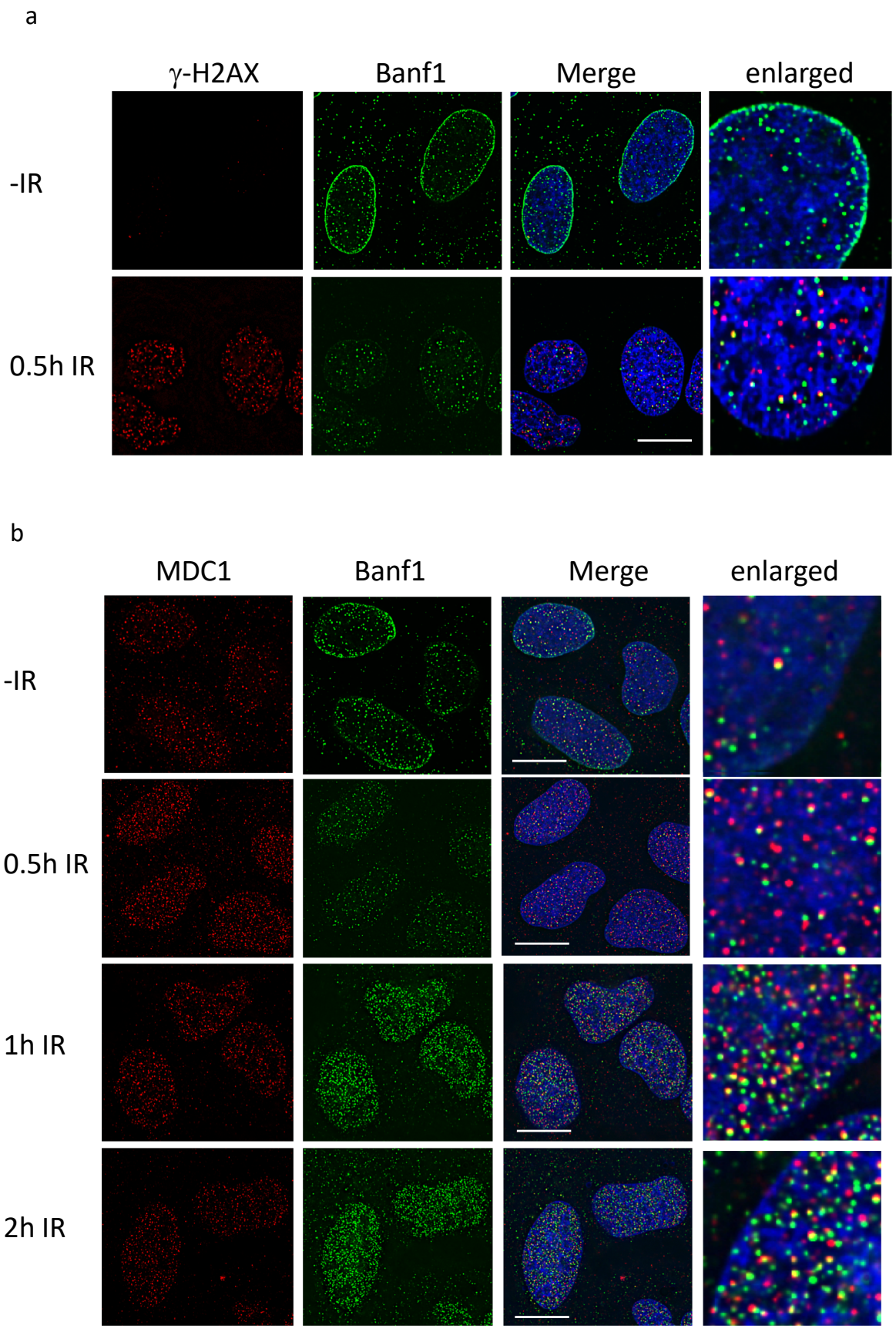
Supplementary Figure 2. Banf1 antibody specificity. U2OS cells were transfected with control or Banf1 siRNA. 72 hours after transfection, cells were treated or mock treated with 2 Gy IR, cells were fixed (following pre-extraction) at the indicated timepoints and stained with the indicated antibodies. The number of foci were counted in 50 cells per condition and t-test was used for statistical analysis ****P <0.0001.



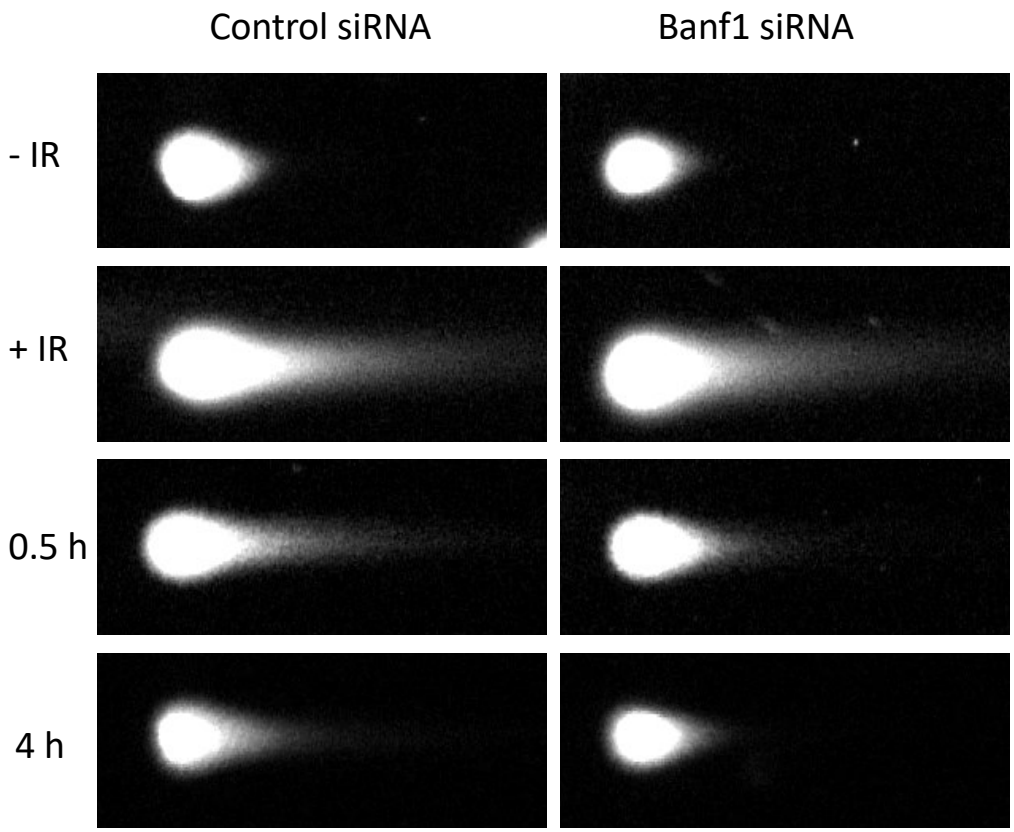
Supplementary Figure 3. Banf1 is stabilised following IR treatment. U2OS cells were treated or mock treated with 6 Gy IR and cells were lysed at the indicated timepoints (as in Figure 1d). Lysates were immunoblotted with Banf1 and β-Actin. The densitometry of the Banf1 bands were analysed and normalised to β-Actin. The histogram data represents the mean and S.D. of 2 independent experiments, t-test was used for statistical analysis, comparing each point back to the untreated sample *P <0.05, ***P <0.001.



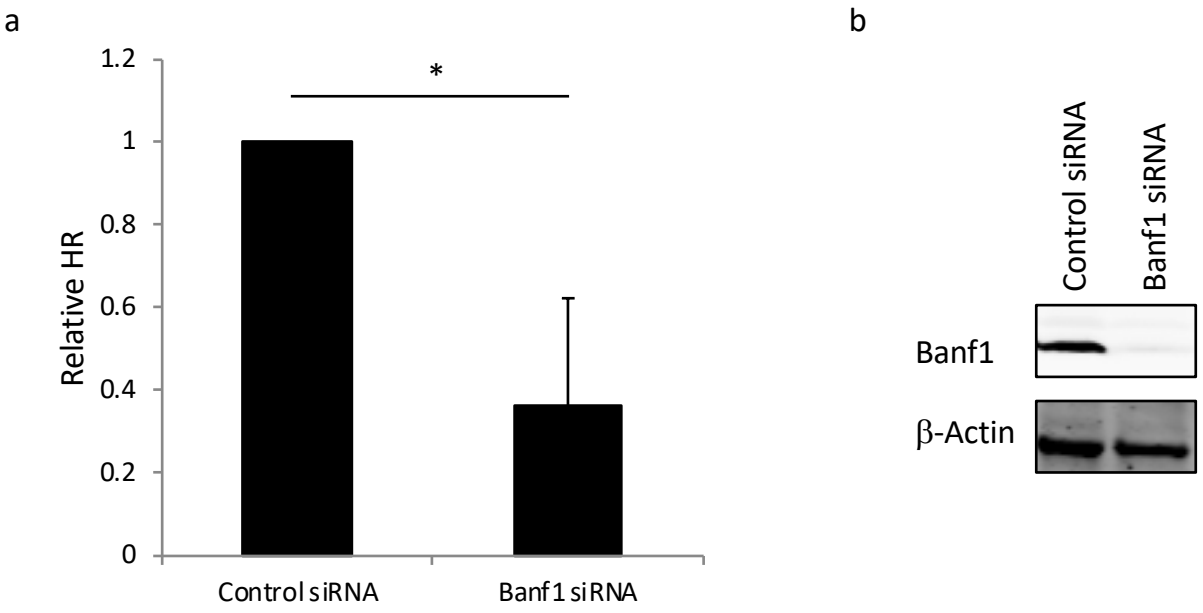
Supplementary Figure 4. Banf1 cellular localisation. U2OS cells were treated or mock treated with 6 Gy IR and cells lysed 1 hour post-IR. Lysates were fractionated using a fractionation kit and immunoblotted with the indicated antibodies. Images are representative of 3 independent experiments.



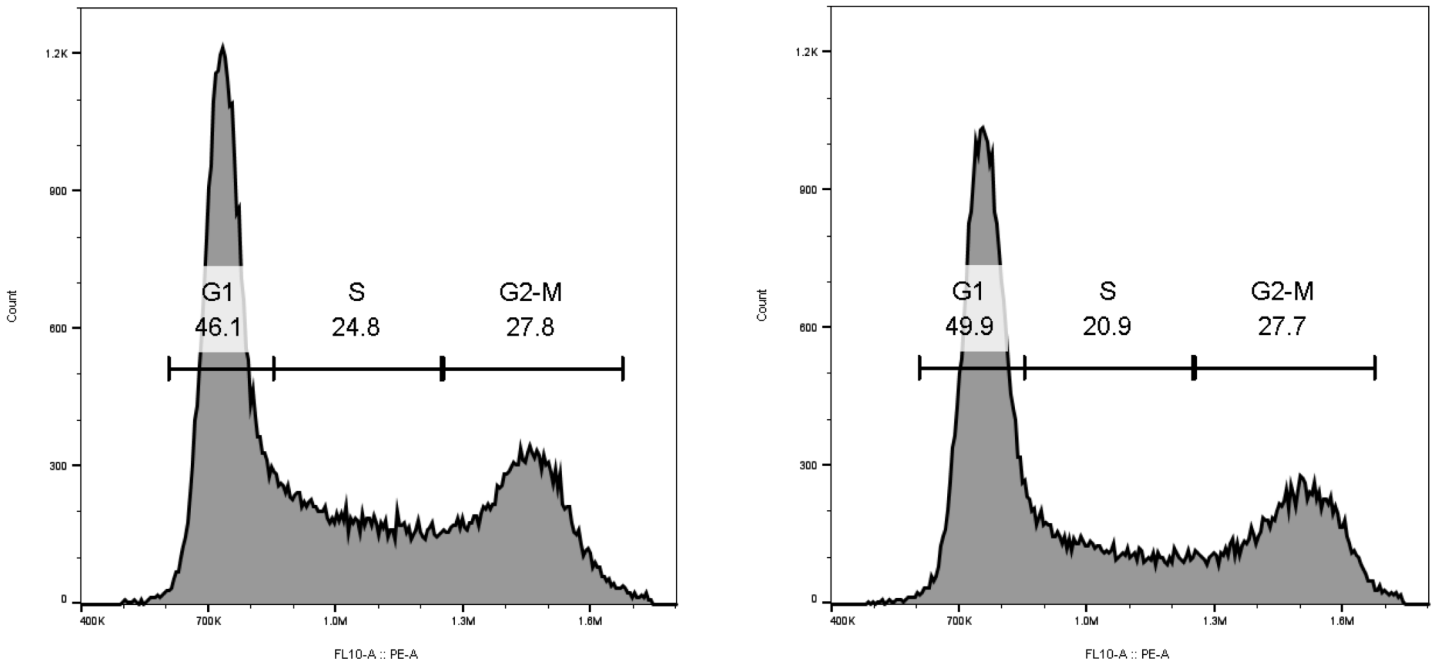
Supplementary Figure 5. Banf1 colocalises with DNA repair proteins after IR. **a, b** Cells were pre-extracted to remove soluble proteins, fixed at the indicated time after 6 Gy IR and stained with the indicated antibodies. Images are representative of 3 independent experiments. Immunofluorescence scale bars represent 10 μm.



Supplementary Figure 6. Representative comet assay images showing the relative Olive tail moment in control or Banf1 siRNA transfected cells, at the indicated times post-IR. +IR represents immediately following IR treatment.



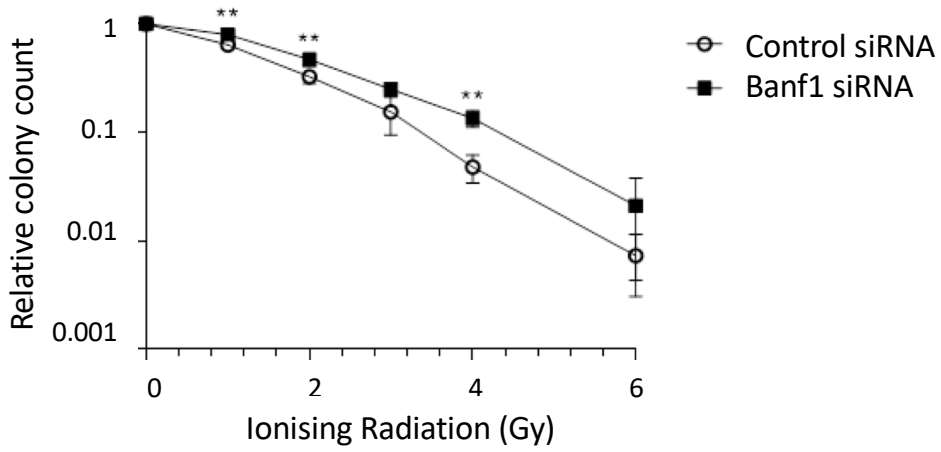
Supplementary Figure 7. Banf1 depletion inhibits homologous recombination. **a,** MCF7 cells stably expressing a HR DRGFP reporter were transfected with control or Banf1 esiRNA. Cells were then transfected with an ISCE1-expressing plasmid and GFP positive cells were detected via FACs. A separate GFP transfection was used as a control for each condition to correct for transfection efficiency. **b,** lysates were extracted from cells from a, and immunoblotted with the indicated antibodies. Histogram data represent the mean and S.D. from 4 independent experiments. T Test *P <0.05.



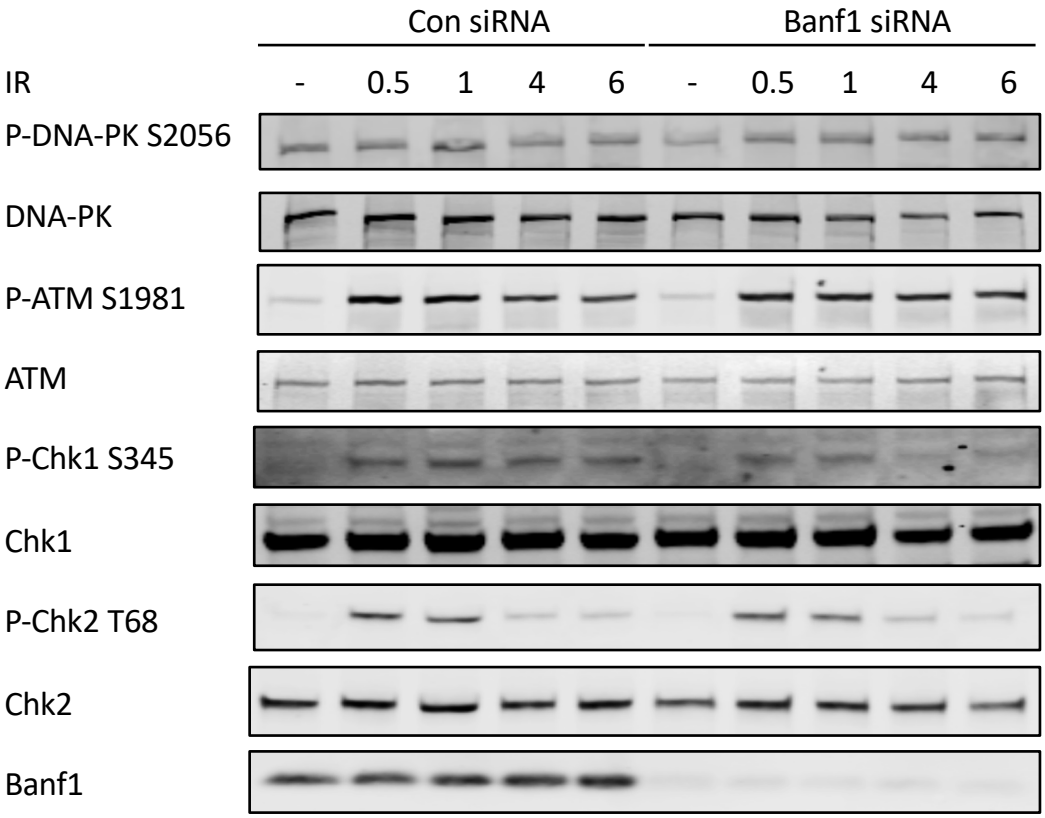
Control siRNA

Banf1 siRNA

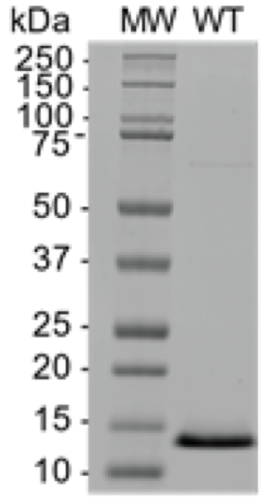
Supplementary Figure 8. Depletion of Banf1 in U2OS cells does not significantly change the cell cycle distribution. Representative cell cycle profiles of control or Banf1 siRNA transfected cells. Histogram data are representative of 3 independent experiments.



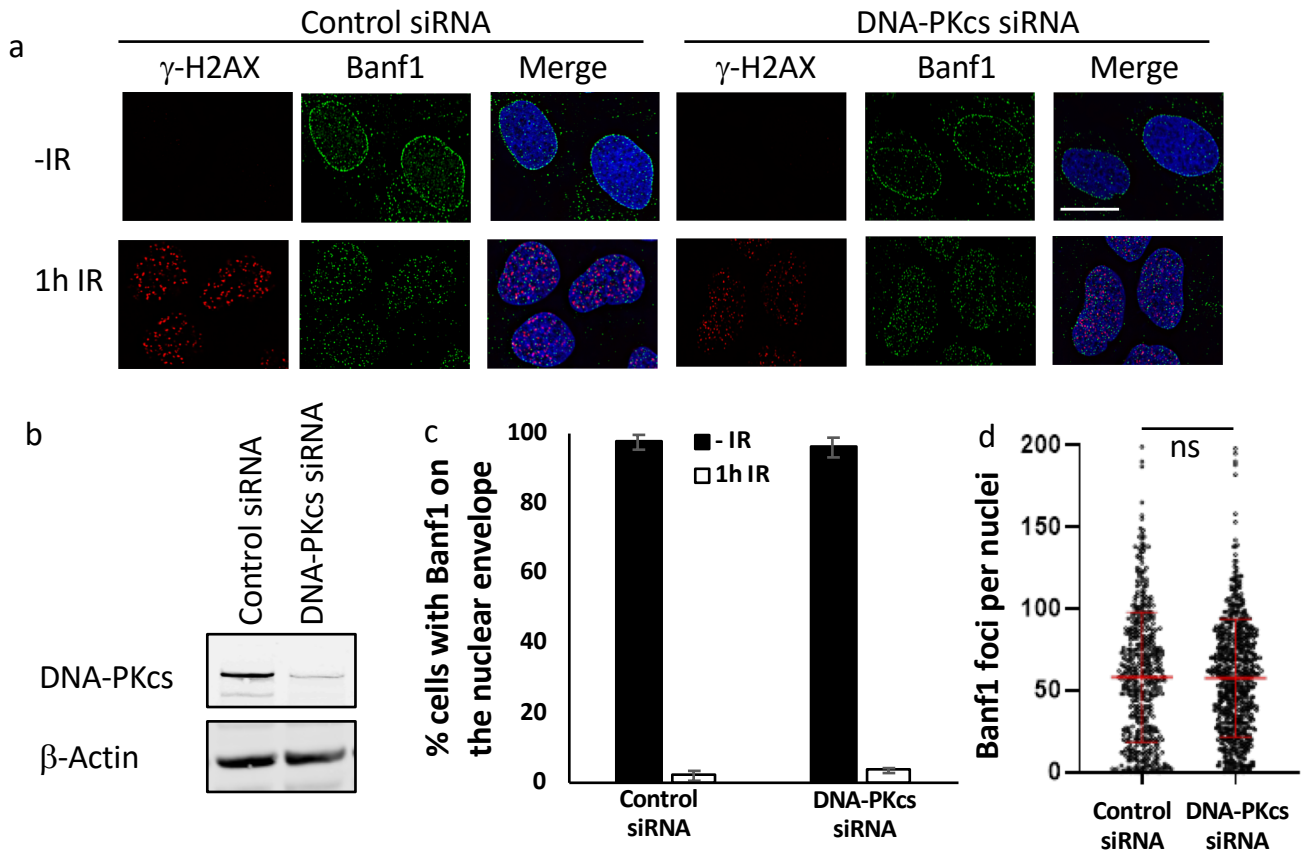
Supplementary Figure 9. Banf1-depletion induces radioresistance in U2OS cells. U2OS cells were transfected with control or Banf1 siRNA. 48 hours after transfection 500 cells were seeded into 6-well plates. Cells were treated or mock-treated with the indicated doses of IR and colonies were counted 10 days after exposure to IR. Histogram data represent the mean and S.D. from 3 independent experiments and t Test was used for statistical analysis **P <0.01.



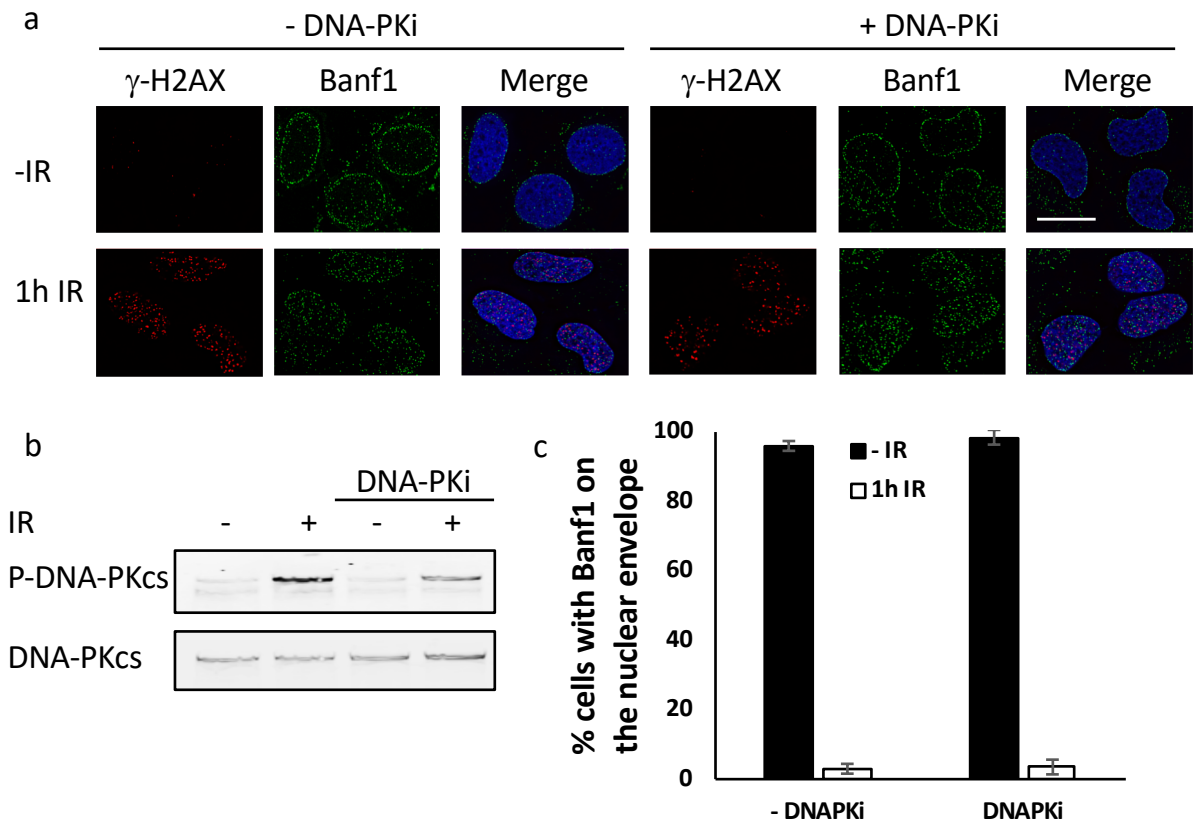
Supplementary Figure 10. IR-induced DNA damage signalling in Banf1 depleted cells is comparable to control cells U2OS cells were transfected with control or Banf1 siRNA and incubated for 72 hours before exposure to 6 Gy IR. Cells were lysed at the indicated times post-IR and lysates were immunoblotted with the indicated antibodies.



Supplementary Figure 11. Purification of recombinant Banf1. Recombinant Banf1 was expressed and purified from E.Coli. Recombinant Banf1 was run on a SDS PAGE gel and stained with Coomassie.

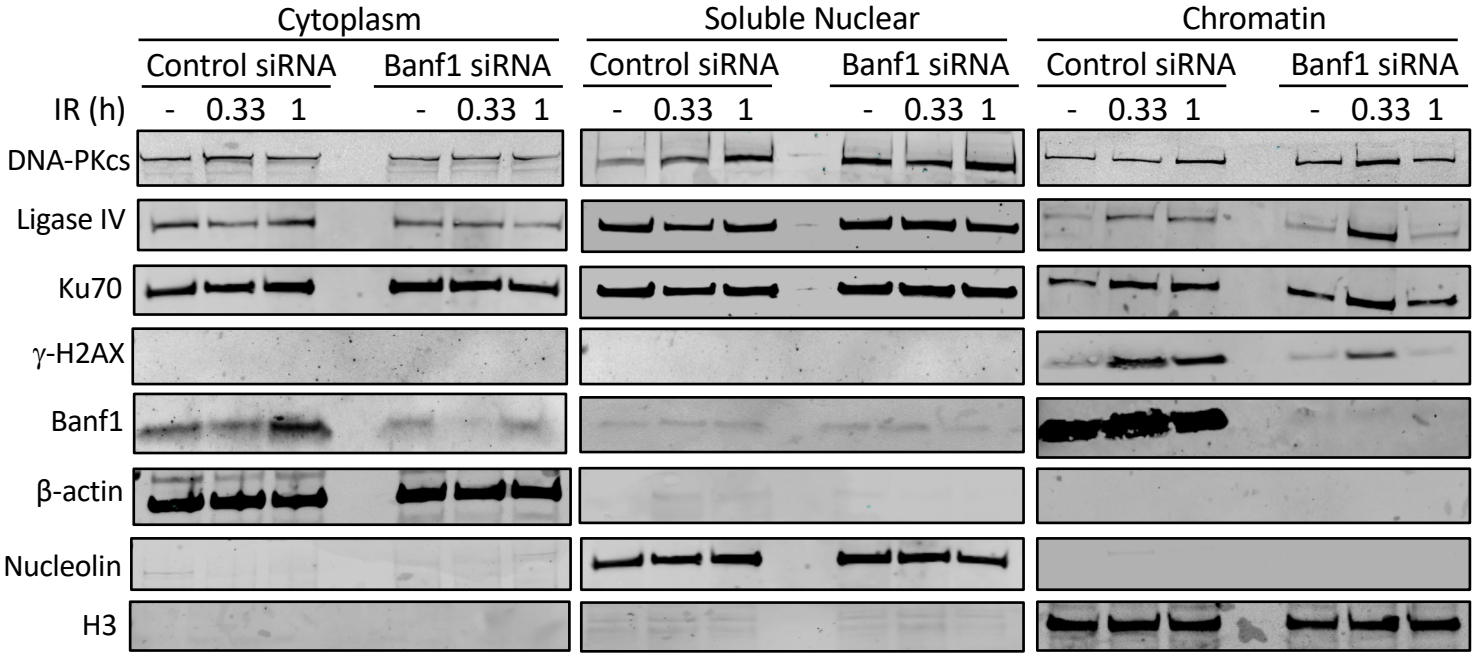


Supplementary Figure 12. Banf1 relocalisation from the nuclear envelope following ionising radiation is not dependent on DNA-PK in U2OS cells. **a, b** U2OS cells were transfected with control or DNA-PKcs siRNA and treated or mock treated with 6 Gy IR. Cells were fixed and stained with the indicated antibodies for immunofluorescence, a, or lysed for immunoblotting to show DNA-PKcs depletion, b. Representative cells stained with the indicated antibodies are shown in a. **c,** Nuclear envelope localisation (from a) was manually quantified using a Delta Vision PDV microscope. **d**, The Banf1 foci per nuclei from a, were imaged using an Incell 6500 and analysed with Incarta to calculate the Banf1 foci per nuclei. Statistical analysis was performed using a t-test. Histogram data represent the mean and S.D. from 3 independent experiments. 100 cells were scored for each condition in c. Immunofluorescence scale bars represent 10 μm.

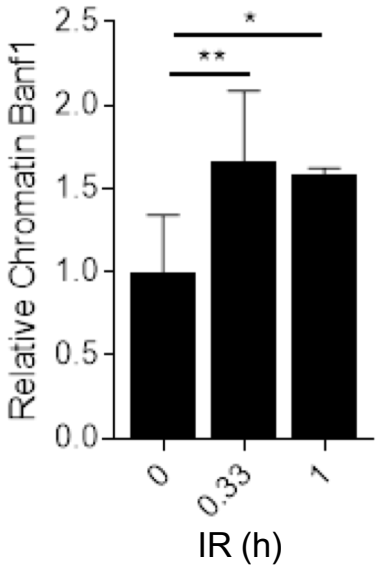


Supplementary Figure 13. Banf1 relocalisation from the nuclear envelope following ionising radiation is not dependent on DNA-PK in U2OS cells. **a, b,** U2OS cells were treated or mock-treated with DNA-PKcs inhibitor (DNA-Pki) for an hour before treatment or mock treatment with 6 Gy IR. Cells were fixed and stained with the indicated antibodies for immunofluorescence a, or lysed for immunoblotting to show DNA-PKcs **c**, Nuclear envelope localisation (from a) was manually quantified using a Delta Vision PDV microscope. Histogram data represent the mean and S.D. from 3 independent experiments. 100 cells were scored for each condition in c. Immunofluorescence scale bars represent 10 μm.

a



b



Supplementary Figure 14. Depletion of Banf1 using siRNA leads to increased association of NHEJ proteins with the chromatin post-IR. a, U2OS cells were transfected with control or Banf1 siRNA. Cells were treated or mocked treated with 6 Gy IR and cellular fractionations processed at the indicated time post-treatment. Cellular fractions were immunoblotted with the indicated antibodies. Data shown are representative of 4 independent experiments. **b,** The bands of Banf1 in the chromatin fraction were analysed via densitometry and normalised to Histone H3 bands. The histogram data shown, represent the mean and S.D. of 4 independent experiments. Paired t-test was used for statistical analysis *P <0.05 **P <0.01.