SUPPLEMENTARY INFORMATION

Figure S1

Figure S1. Generation of NRF2 knockout (KO) in A549 cells (A549-NRF2^{KO} cells) using CRISPR/Cas9 technology.

The DNA sequence in the image represents part of the NRF2 gene in a monoclonal cell. sgRNA: PCA03702 was designed with reference to the Human GeCKOv2 library. The yellow box represents the regions where the mutations occurred in clones (4 of the 105 monoclones with effective mutations). Clone 26# was used in experiments.

Figure S2

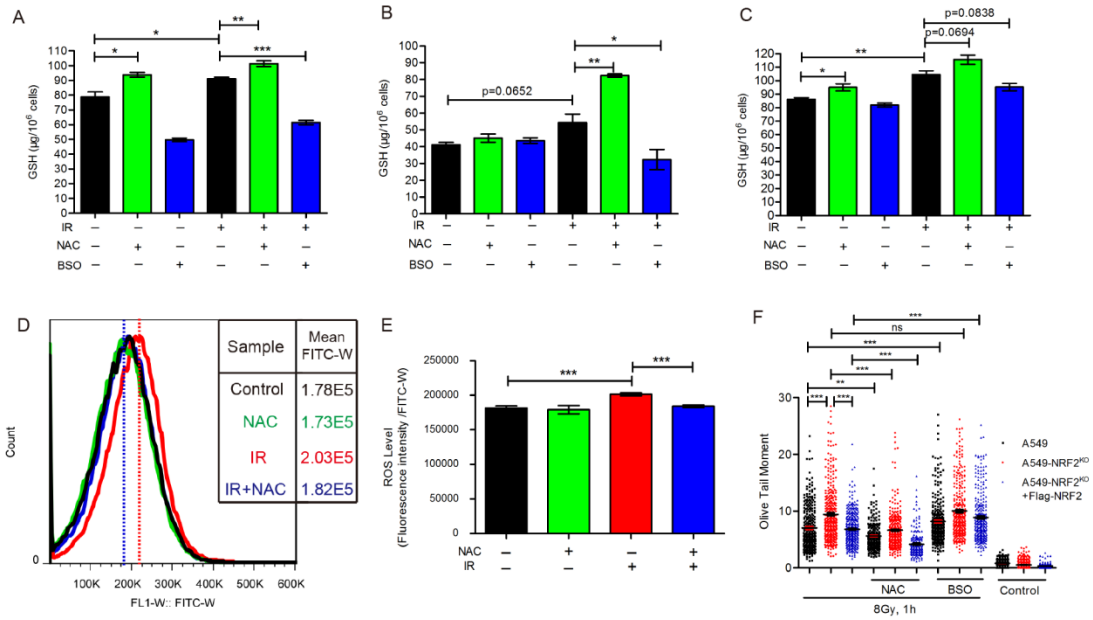

Figure S2. N-acetyl-L-cysteine (NAC) scavenges ROS.

(A-C) The glutathione (GSH) levels in A549 (A), A549-NRF2^{KO} (B) and A549-NRF2^{KO}+Flag-NRF2 (C) cells were measured using the Micro Reduced Glutathione (GSH) Assay Kit 2 h after IR (8 Gy). Cells were pretreated with 5 mM NAC for 1 h or 10 μM BSO for 2 h before IR. Data are means ± S.E.M. (n=3). *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

(D) The ROS levels in A549-NRF2^{KO} cells were measured using the Total Reactive Oxygen Species (ROS) Assay Kit 2 h after IR. Pretreatment with 5 mM NAC for 1 h before IR decreased ROS levels. Representative results from one of three independent experiments are shown.

(E) Statistical chart of changes in ROS levels of A549-NRF2^{KO} cells. The ROS levels in A549-NRF2^{KO} cells were measured by FACS 2 h after IR (8Gy). Cells were pretreated with 5 mM NAC for 1 h before IR. Data are means ± S.E.M. (n=3). ***p ≤ 0.001.

(F) Comet assay was used to detect DNA damage. The Olive Tail Moments (OTM) were analyzed statistically. A549, A549-NRF2^{KO}, and A549-NRF2^{KO}+Flag-NRF2 cells were pretreated with 5 mM NAC for 1 h before IR (8 Gy) or 10 μM BSO for 2 h before IR (8 Gy). Comet assay was performed 1 h after IR. Data are means ± S.E.M. More than 150 cells were counted in three independent experiments. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ns: no significance.

Figure S3

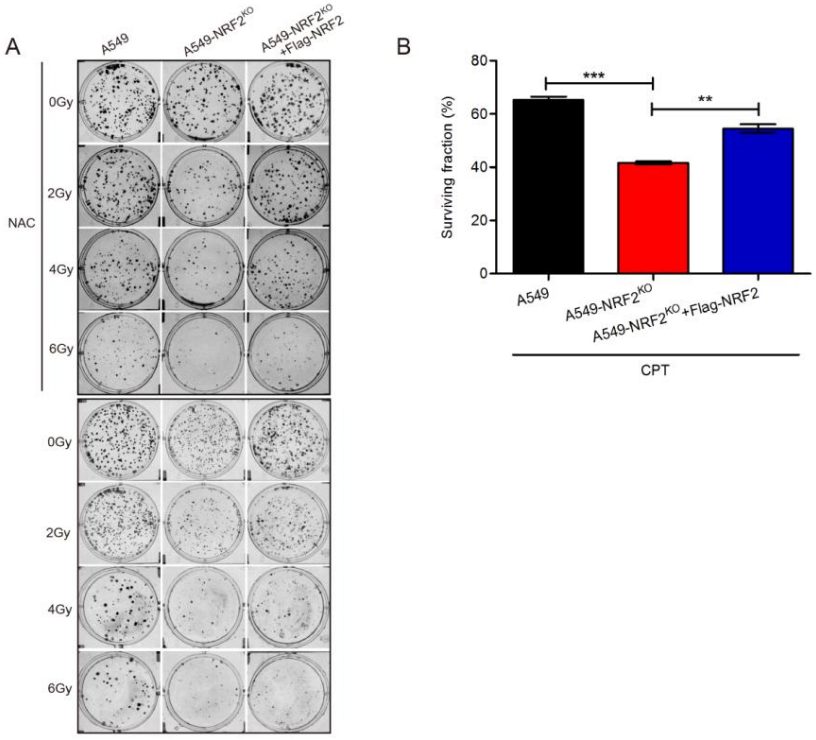

Figure S3. Clonogenic survival of A549, A549-NRF2^{KO}, and A549-NRF2^{KO}+Flag-NRF2 cells.

A549-NRF2^{KO} cells were generated using CRISPR-Cas9 and transfected with a Flag-NRF2 expression vector to generate A549-NRF2^{KO}+Flag-NRF2 cells. (A) Cells were pretreated with 5 mM NAC for 1 h or not and then exposed to 0, 2, 4, or 6 Gy of IR. (B) Cells were treated with 30 nM CPT for 6 h, and the percentages of surviving cells were calculated after 1–2 weeks. Data are means ± S.E.M. (n=3). **$p \leq 0.01$, ***$p \leq 0.001$.

Figure S4

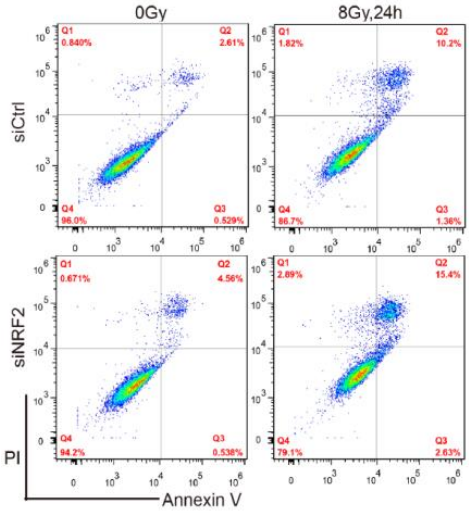

Figure S4. Depletion of NRF2 increases apoptosis after exposure to IR.

A549 cells transfected with siCtrl or siNRF2 were pretreated with NAC for 1 h and exposed to 8 Gy Cs137 γ-rays. At 24 h post-irradiation, the percentage of apoptotic cells was determined by co-staining with FITC-Annexin V and PI. Representative results from one of three independent experiments are shown.

Figure S5

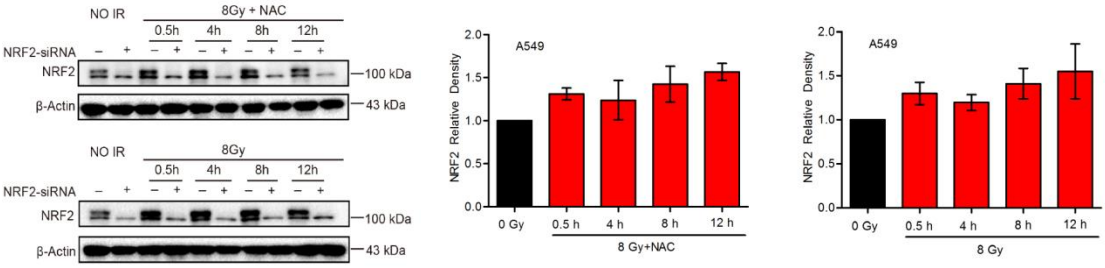

Figure S5. IR induces upregulation of NRF2 protein levels with or without ROS.

Western blotting of NRF2 in A549 cells transfected with siCtrl or siNRF2, pretreated with 5 mM NAC for 1 h or not, and then exposed to 8 Gy Cs137 γ-rays.

Figure S6

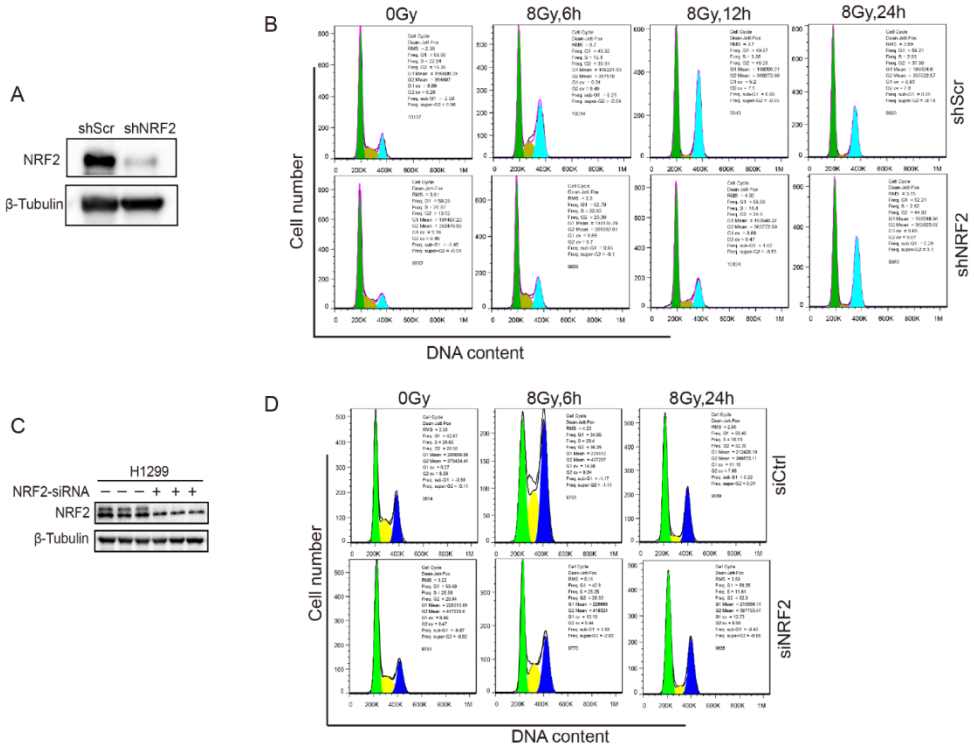

Figure S6. NRF2 facilitates G2 cell cycle arrest in H460 and H1299 cells

(A) Western blotting of NRF2 in H460 cells treated with shScr#1 and shNRF2#1.

(B) H460 cells treated with shScr or shNRF2 were exposed to 8 Gy Cs137 γ-rays. The distribution of cells in G1/S/G2 phases was determined by PI staining and flow cytometry at 6, 12, and 24 h post-irradiation. Representative results from one of three independent experiments are shown.

(C) Western blotting of NRF2 in H1299 cells transfected with siCtrl or siNRF2 for 24 h.

(D) H1299 cells transfected with siCtrl or siNRF2 were exposed to 8 Gy Cs137 γ-rays. The distribution of cells in G1/S/G2 phases was determined by PI staining and flow cytometry at 6 and 24 h post-irradiation. Representative results from one of three independent experiments are shown.

Figure S7

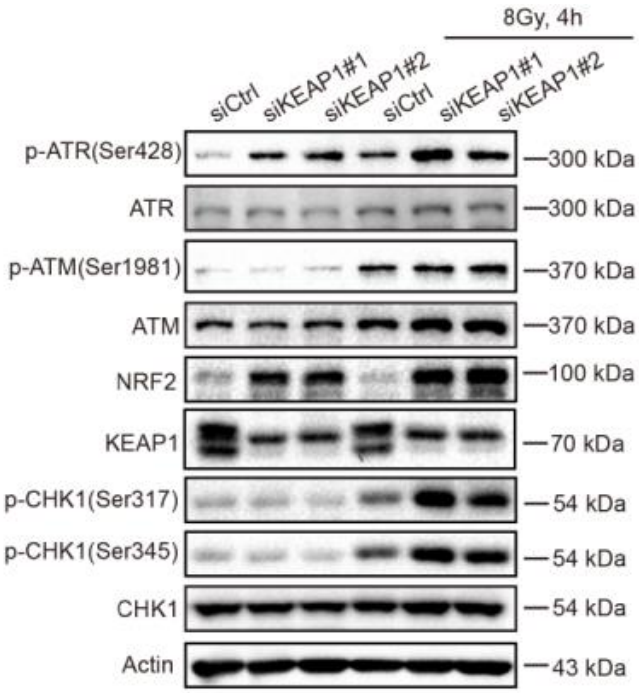

Figure S7. NRF2 activates the ATR-CHK1 pathway in U2OS cells

Western blotting of NRF2, KEAP1, p-ATM, ATM, p-ATR, ATR, CHK1 and p-CHK1 in U2OS siCtrl and U2OS siKEAP1 cells, which were pretreated with NAC for 1 h and exposed to 0 or 8 Gy γ-rays.

Figure S8

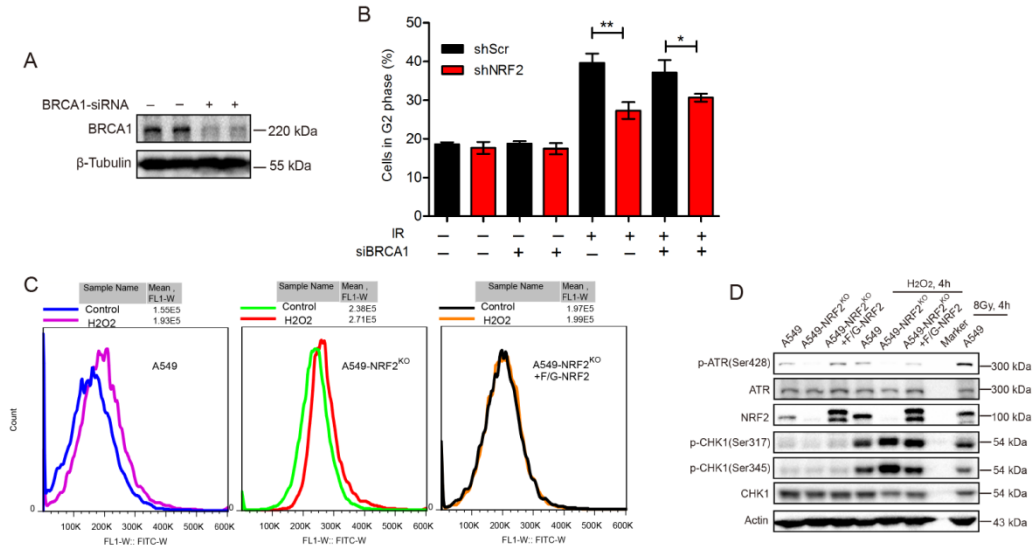

Figure S8. Activation of ATR-CHK1 pathway by NRF2 may not be affected by BRCA1 and ROS.

(A) Western blotting of BRCA1 in H1299 cells treated with shScr and transfected with siBRCA1 for 24 h.

(B) Flow cytometric analysis of G2 cell cycle arrest in H1299 cells treated with shScr or shNRF2, transfected with siBRCA1 for 24 h, and exposed or not to 8 Gy Cs137 γ-rays. Data are means ± S.E.M. (n=3). *$p \leq 0.05$, **$p \leq 0.01$.

(C) The ROS levels in A549, A549-NRF2^{KO}, and A549-NRF2^{KO}+F/G-NRF2 cells were measured using the Total Reactive Oxygen Species (ROS) Assay Kit 4 h after treatment with 200 μM H_2O_2. Representative results from one of three independent experiments are shown.

(D) Western blotting of p-ATR, NRF2 and p-CHK1 in A549, A549-NRF2^{KO}, and A549-NRF2^{KO}+F/G-NRF2 cells. A549, A549-NRF2^{KO}, and A549-NRF2^{KO}+F/G-NRF2 cells were treated with 200 μM H_2O_2 for 4 h. A549 cells were exposed to 8 Gy Cs137 γ-rays.

Figure S9

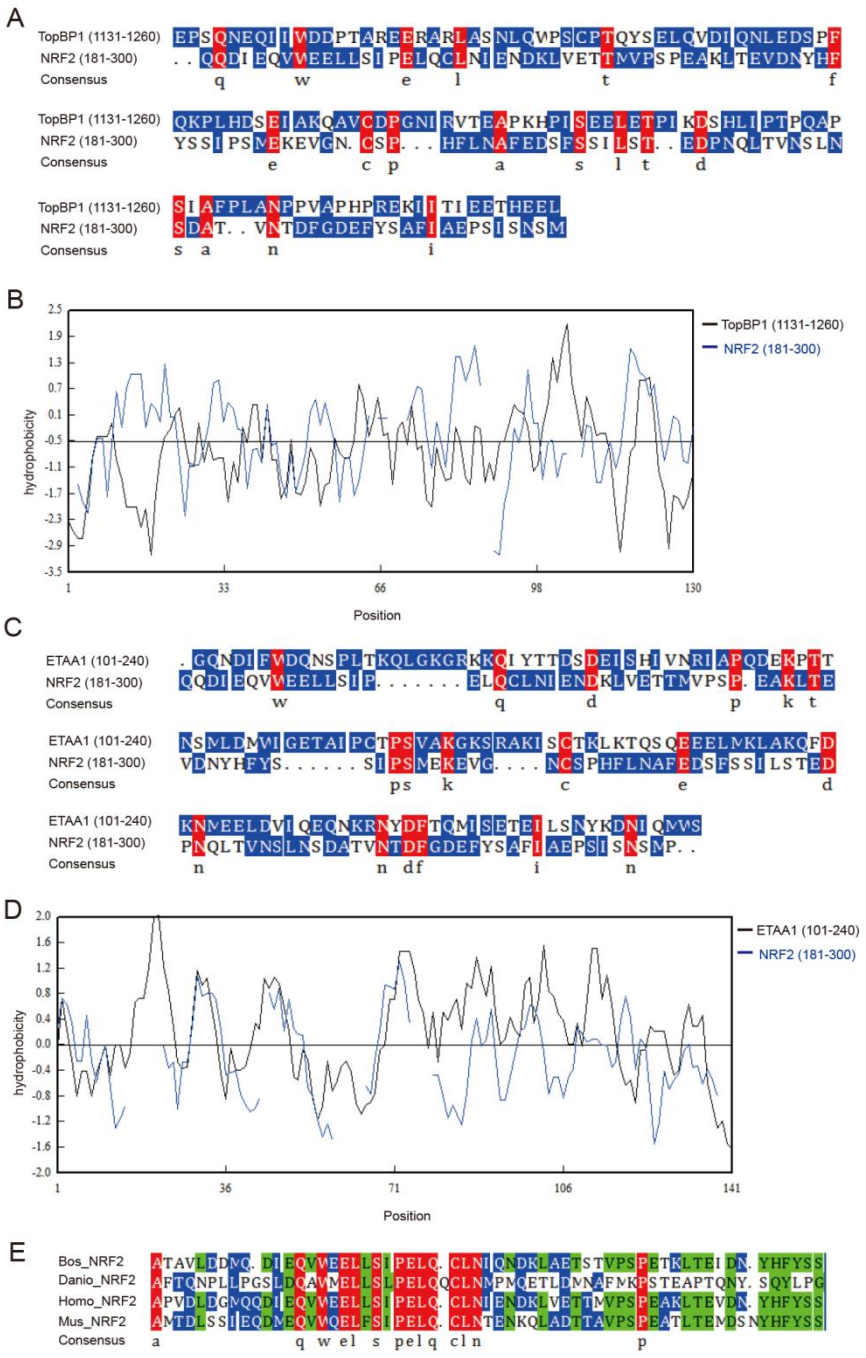

Figure S9. NRF2 protein sequence analyses.

(A) Protein sequence alignment of TopBP1 (1131–1260) and NRF2 (181–300).

(B) Hydrophobicity analysis of TopBP1 (1131–1260) and NRF2 (181–300).

(C) Protein sequence alignment of ETAA1 (101–240) and NRF2 (181–300).

(D) Hydrophobicity analysis of ETAA1 (101–240) and NRF2 (181–300).

(E) Sequence characteristics of NRF2 from different species.

Figure S10

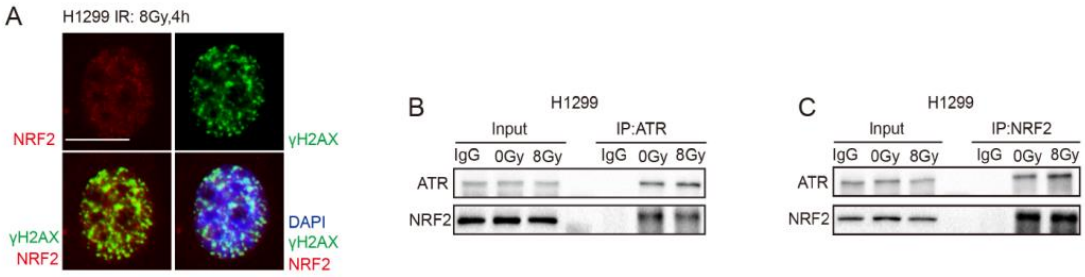

Figure S10. NRF2 interacts with ATR in H1299 cells

(A) Immunofluorescence analysis of NRF2 (red), γH2AX (green), and DAPI (blue) in H1299 cells exposed to 8 Gy Cs137 γ-rays. Scale bars, 20 μm.

(B) Extracts of H1299 cells exposed to 0 or 8 Gy Cs137 γ-rays were subjected to immunoprecipitation with an anti-ATR antibody or control IgG. Samples were immunoblotted with the indicated antibodies.

(C) Extracts of H1299 cells exposed to 0 or 8 Gy Cs137 γ-rays were subjected to immunoprecipitation with an anti-NRF2 antibody or control IgG. Samples were immunoblotted with the indicated antibodies.