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Supplemental information

In situ observation of mitochondrial biogenesis as the early event of apoptosis

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Supplementary Information

Table S1. Oligonucleotides for amplify PCR product of COX8, related to STAR Methods.

Oligonucleotides	Sequence 5′ → 3′	Size (bp)
Oligo1	CTAGAGATCTGGTACCGTCGACTGCAGAGA TCCGCTAGGGGTACTCCGTGCCATCATGTCC GTCCTGACGCCGCTGCTGCTGCGGGGCTTGA CAGGCTCGGCCCGGCGGCTCCCAGTGCCGC GCGCCAAGATCCATTCGTTGCCGCCGGAGG GG	154
Oligo2	AGCTTCCCCTCCGGCGGCAACGAATGGATC TTGGCGCGCGGCACTGGGAGCCGCCGGGCC GAGCCTGTCAAGCCCCGCAGCAGCAGCGGC GTCAGGACGGACATGATGGCACGGAGTACC CCTAGCGGATCTCTGCAGTCGACGGTACCA GATCT	155

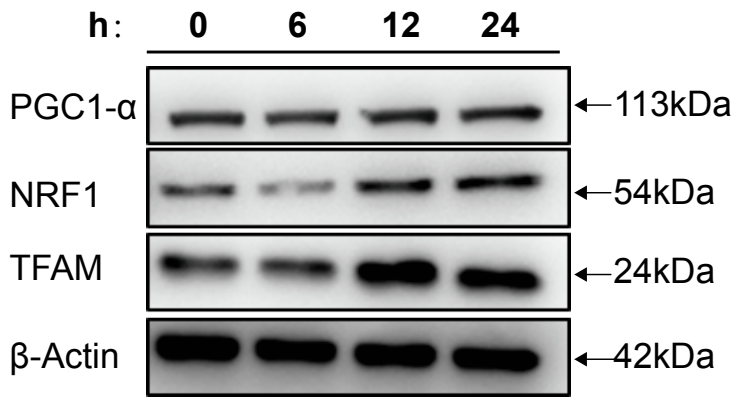

Figure S1. Western blot assay for verifying the effect of AICAR on mitochondrial biogenesis, related to Figure 2. HeLa cells were treated with mitochondrial biogenesis inducer drug (AICAR, 0.5 mM), and the whole cell extracts were collected at different time points. The mitochondrial biogenesis marker proteins (PGC-1α, NRF1 and TFAM) are indicated in the plot.

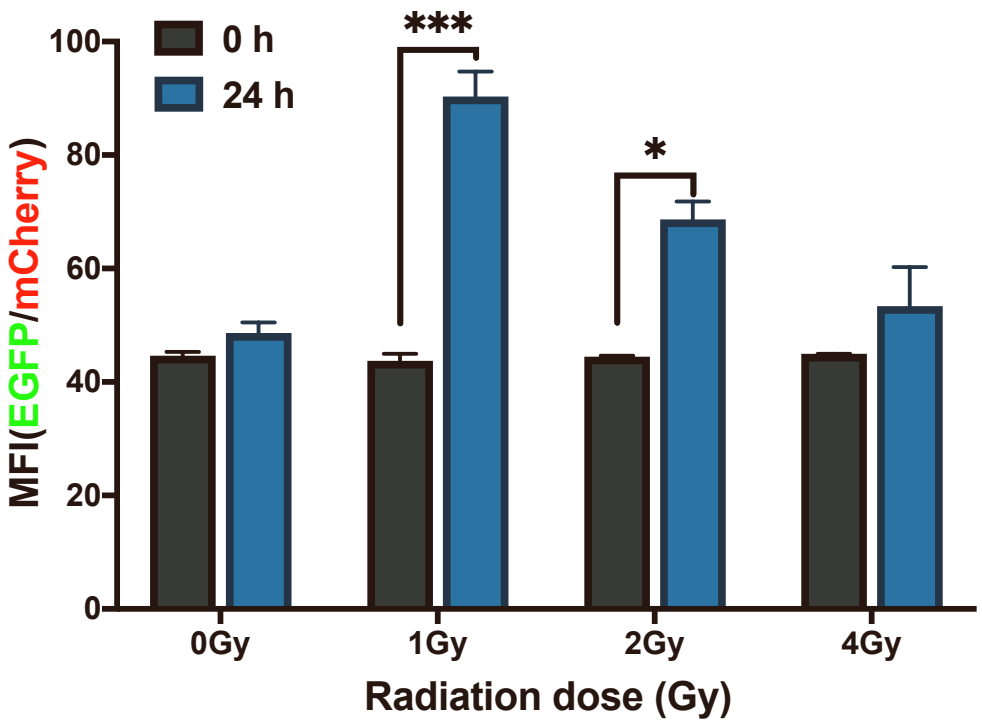

Figure S2. The mean fluorescence intensity (MFI) was determined by flow cytometry for the HeLa cells with or without irradiation, related to Figure 3. HeLa-cox8-EGFP-actin-mCherry cells were exposed to 1 Gy，2 Gy，4 Gy gamma-ray and incubated for the indicated periods. The analysis and comparison MFI obtained from the dual-fluorescent measurements by flow cytometry.

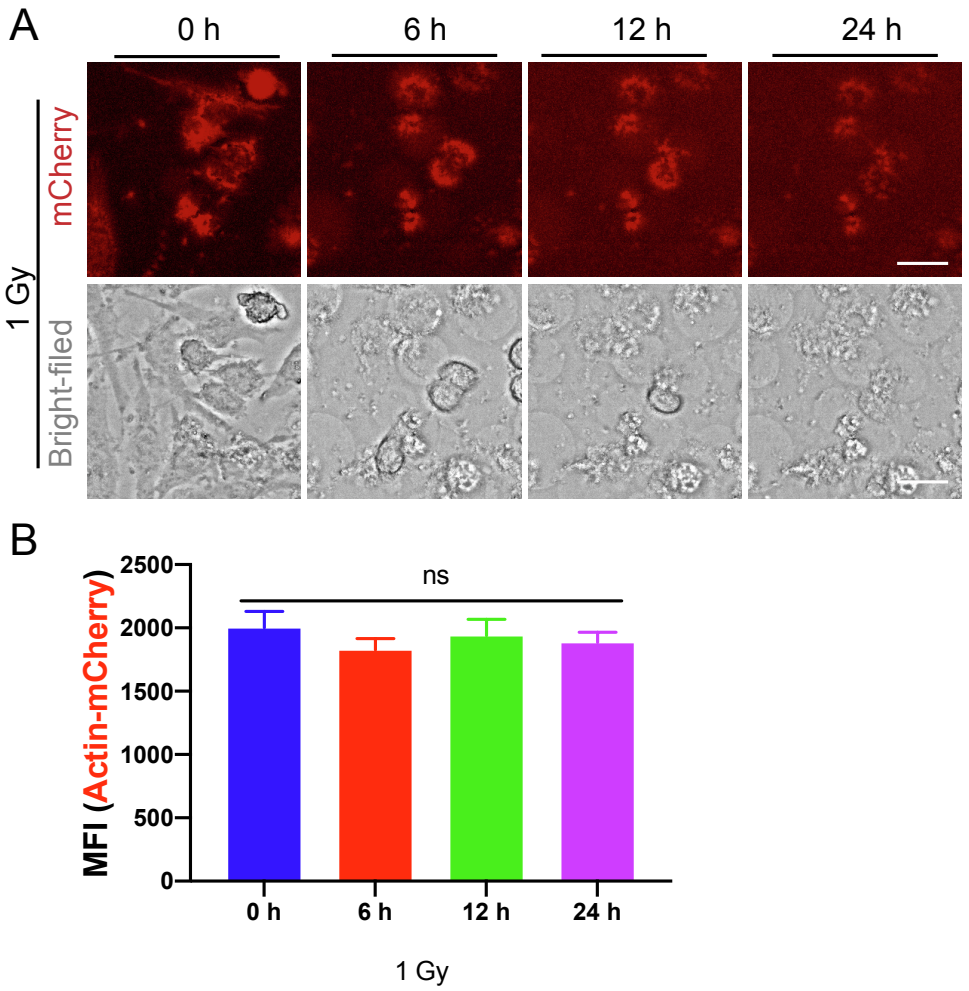

Figure S3. The mean fluorescence intensity (MFI) of Actin-mCherry was determined by living fluorescence microscopy for the HeLa cells with with or without irradiation (1 Gy), related to Figure 3. (**A**) Fluorescence images and bright field images were taken at 0, 6, 12 and 24 hours after irradiatied with 1 Gy dose. (**B**) The histogram is the statistical chart of the mean fluorescence intensity of Actin-mCherry.

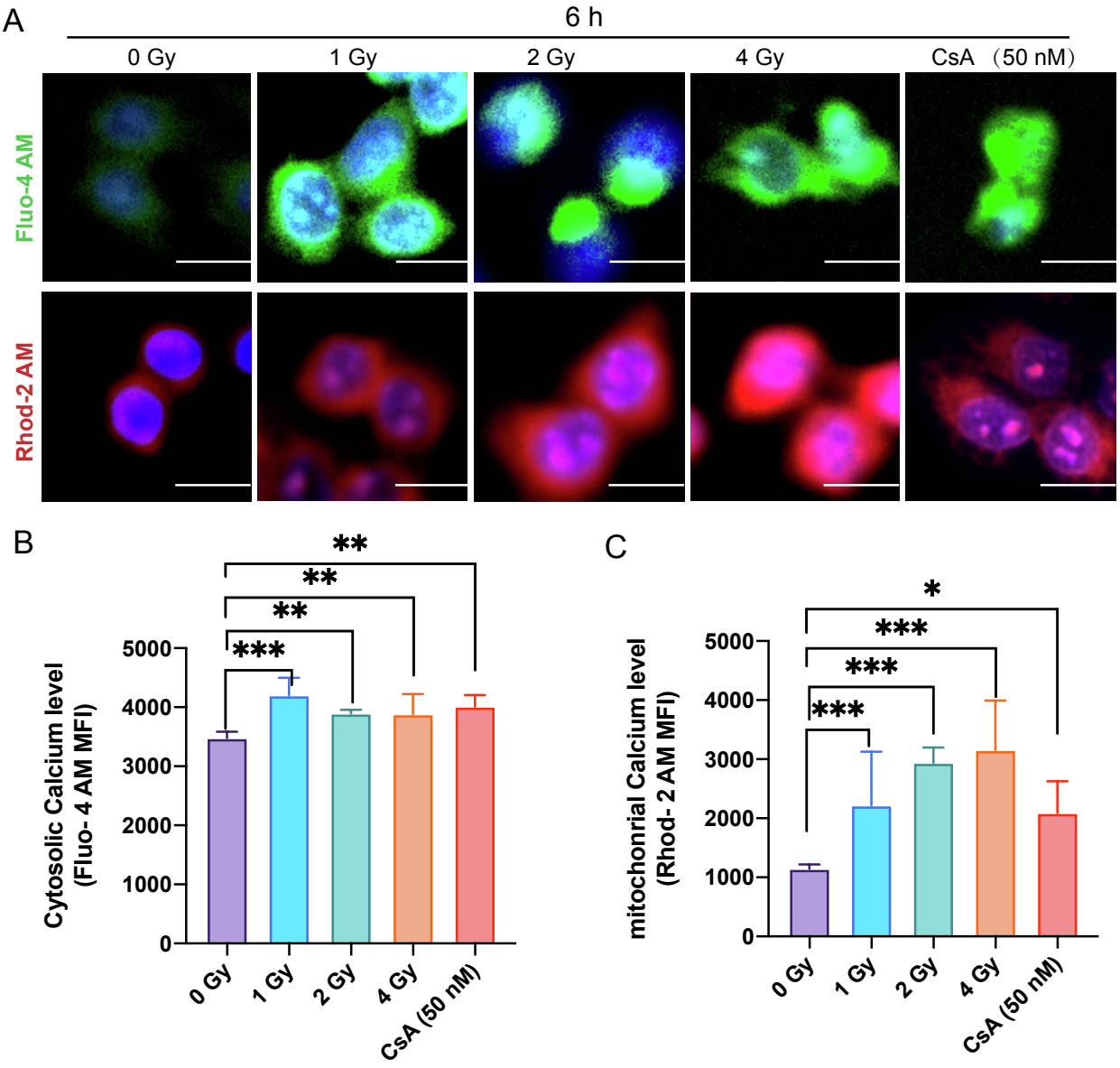

Figure S4. Radiation induces mitochondrial Ca^{2+} accumulation, related to Figure 5. (**A**) Photomicrograph shows the radiation induced change and cellular distribution of Ca^{2+} stained with Fluo-4 AM and mitochondrial calcium using Rhod-2 AM. (**B-C**) Bar diagram shows alteration in Ca^{2+} concentration in HeLa cells stained with Fluo-4 AM (5 µM) and Rhod-2 AM (1 µM), respectively, analyzed using fluorescence microscope and imaging by imageJ software. Images were captured under fluorescence microscope with 20X objective. n = 500 cells calculated per group. Scale bar: 20 µm.

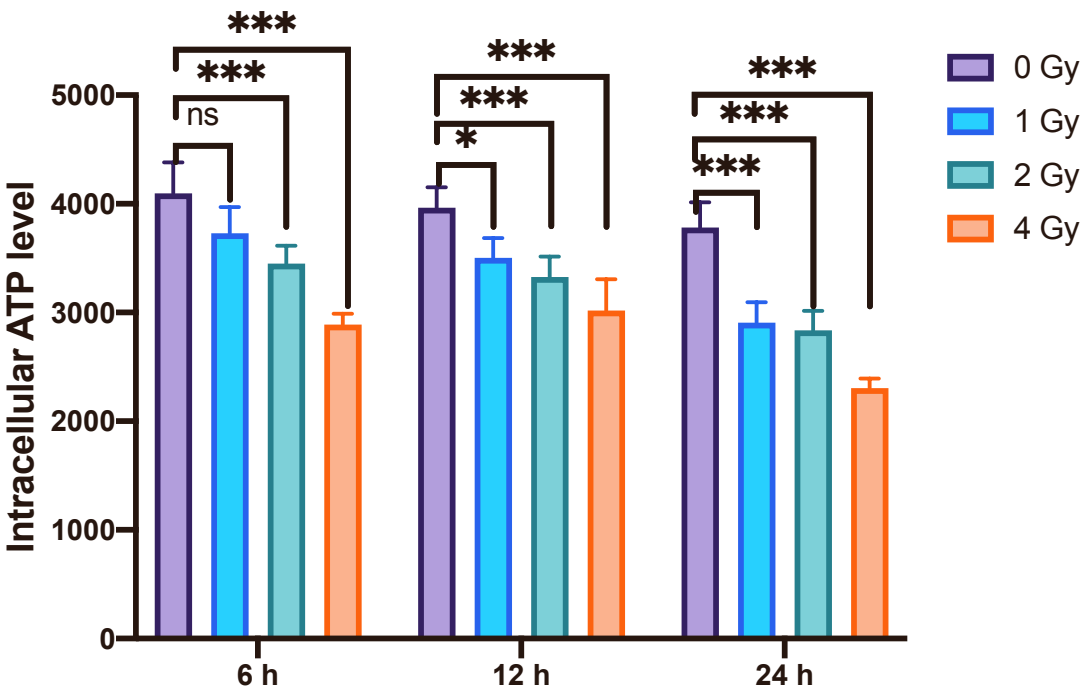

Figure S5. Effects of intracellular ATP depletion by radiation, related to Figure 6. HeLa cells treated with different doses of radiation (0, 1, 2 and 4Gy). Quantitative analysis of intercellular ATP level in percentage. *P<0.05, **P<0.01 and ***P < 0.001 indicated significant differences compared to non-treated cells group. All the results are presented as mean ± SD; n=3.

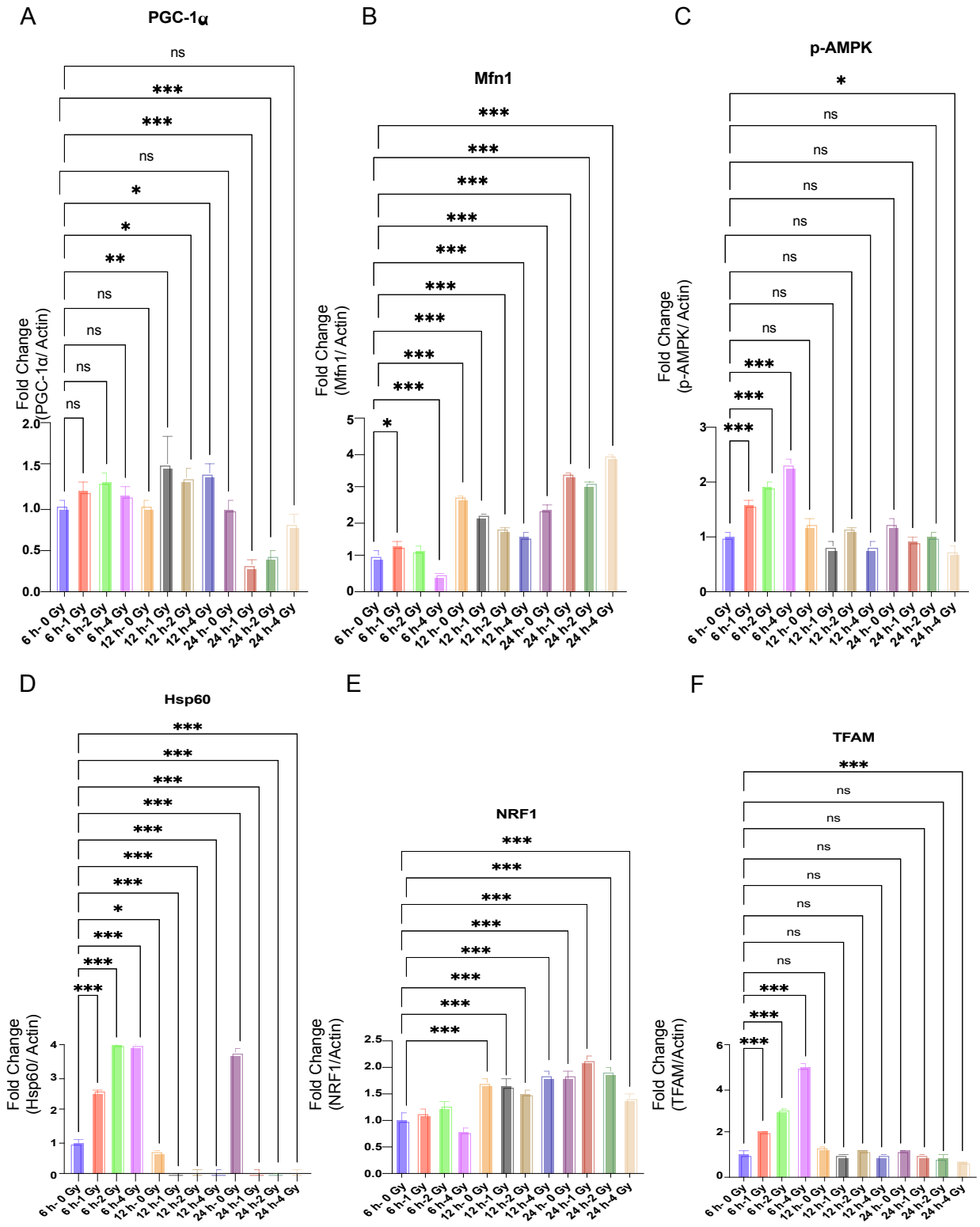

Figure S6. Densitometric analysis of immunoblotting of mitochondrial biogenesis-related proteins, related to Figure 8E. (A-F) Western blot bands of PGC-1α, Mfn1, p-AMPK, Hsp60,

NRF1 and TFAM were analyzed by densitometry with ImageJ software. Intensity of each band was normalized to the respective actin. Results are representative of three independent experiments.

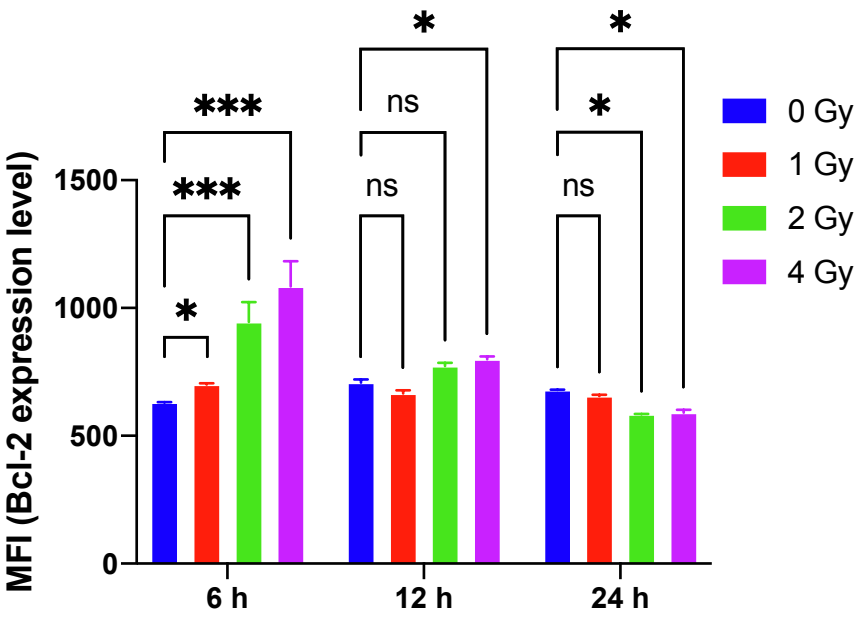

Figure S7. Quantitative analysis of the expression level of anti-apoptotic protein Bcl-2 at different time points, related to Figure 11A. Cells were subjected to different doses (0, 1, 2, 4 Gy) of radiation and then harvested at different time points to detect MFI values by flow cytometry.

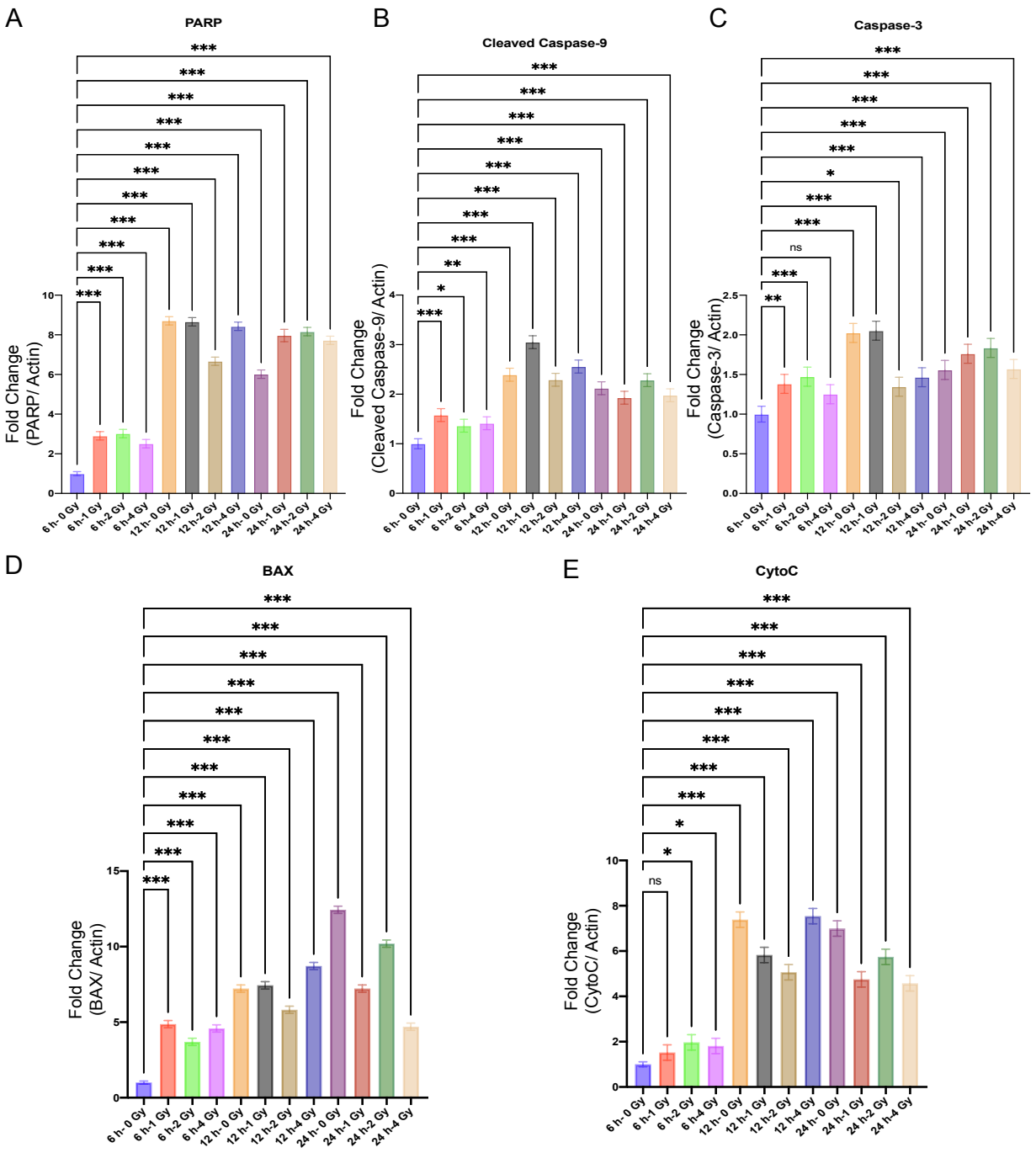

Figure S8. Densitometric analysis of immunoblotting of apoptosis-related proteins, related to Figure 11I. (A-E) Western blot bands of PARP, Cleaved Caspase- 9, Caspase-3, BAX and CytoC were analyzed by densitometry with ImageJ software. Intensity of each band was normalized to the respective beta-actin. Results are representative of three independent experiments.

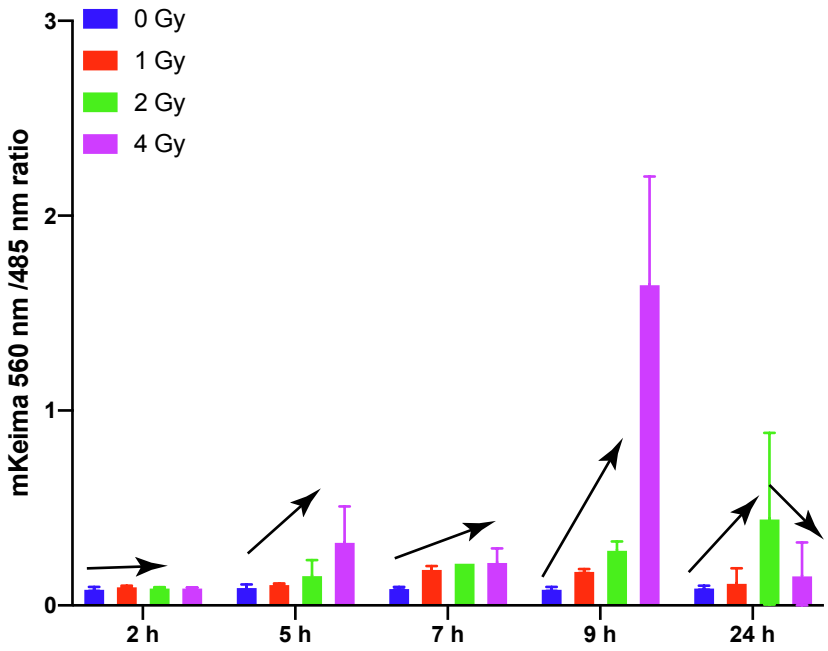

Figure S9. Mitophagy activity assessed using mt-Keima in HeLa cells, related to Figure 12. Elevated levels of mitophagy were observed following irradiation with 1 Gy, 2 Gy, 4 Gy. The fluorescence ratio of red fluorescence to green fluorescence (560 nm/ 485 nm) at different time points (2, 5, 7, 9 and 24 hours) was calculated by high content fluorescence analysis system. n = 1000 cells calculated per group.