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Metformin kills and radiosensitizes cancer cells and preferentially kills cancer stem cells

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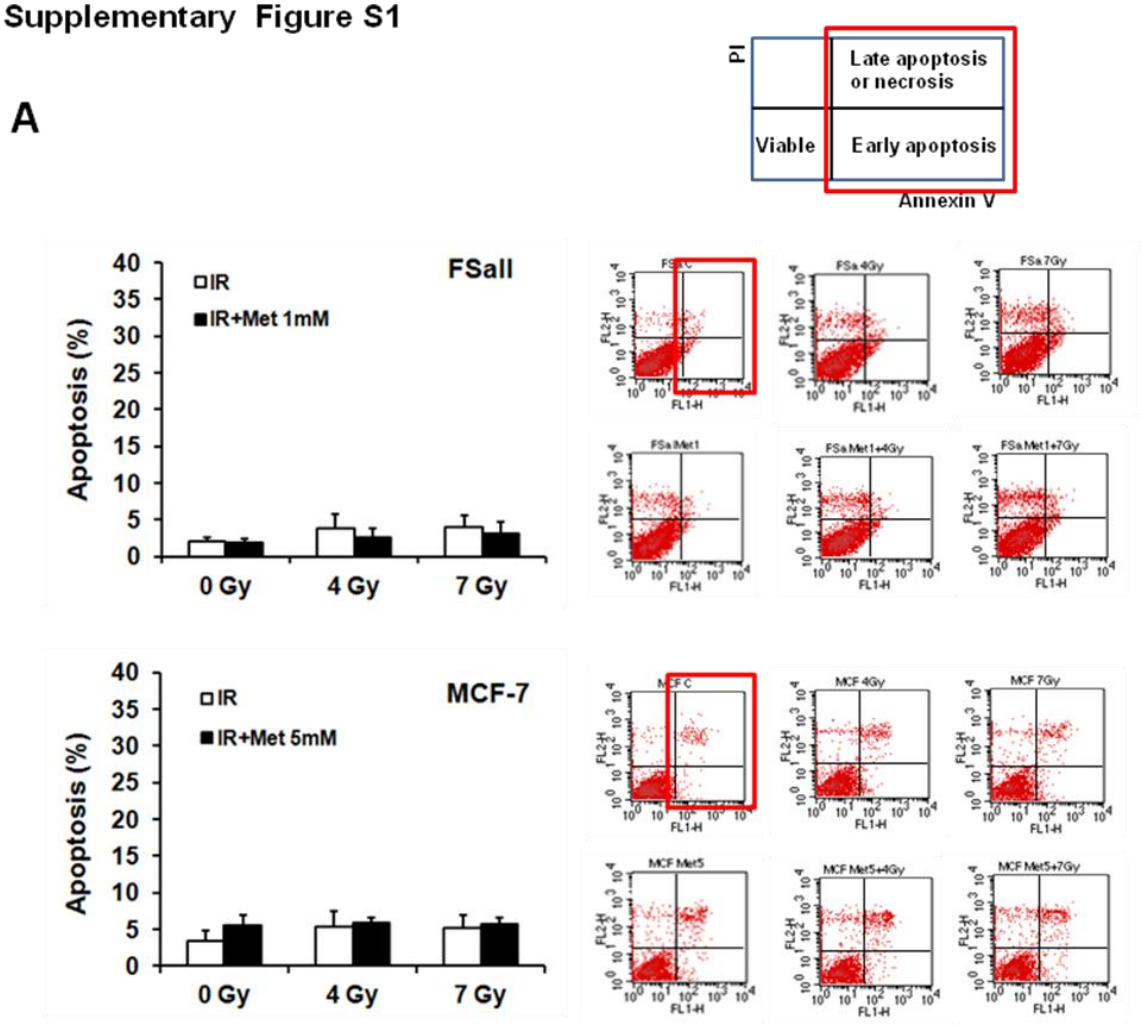
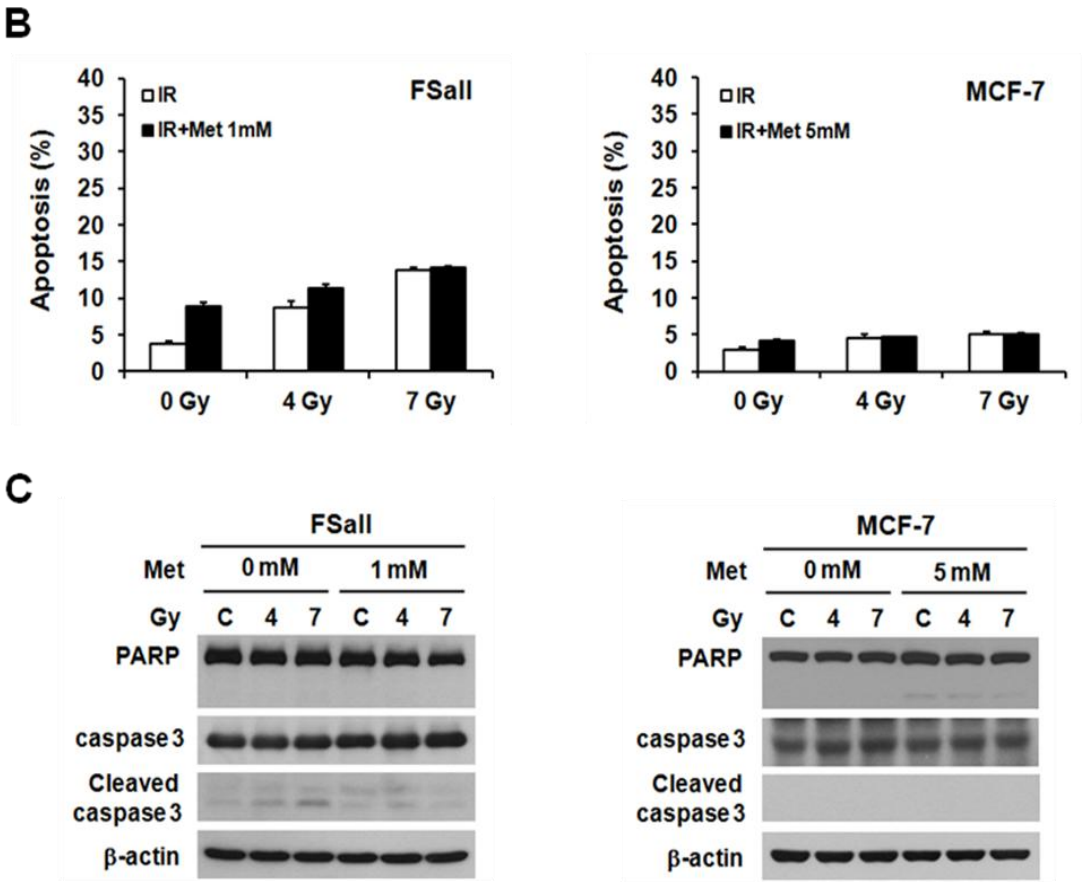
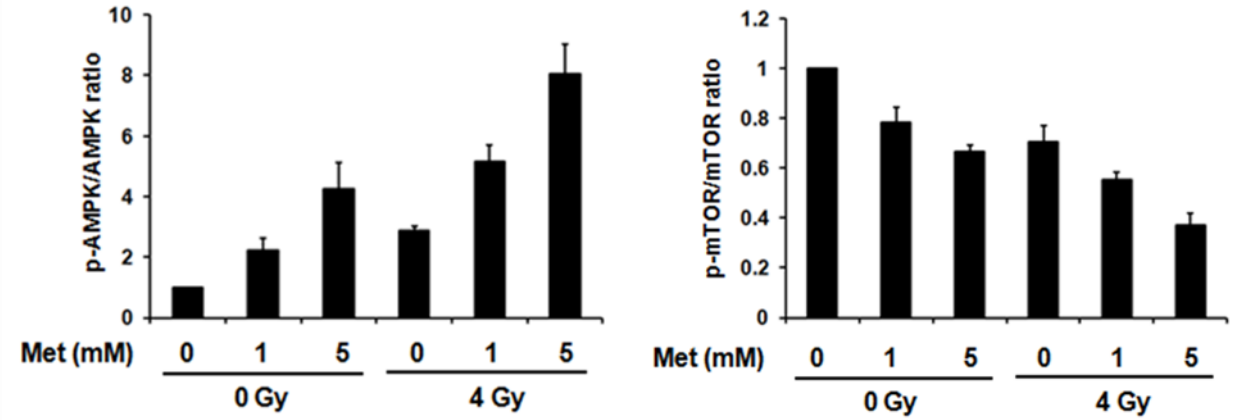
Supplementary Figure S1





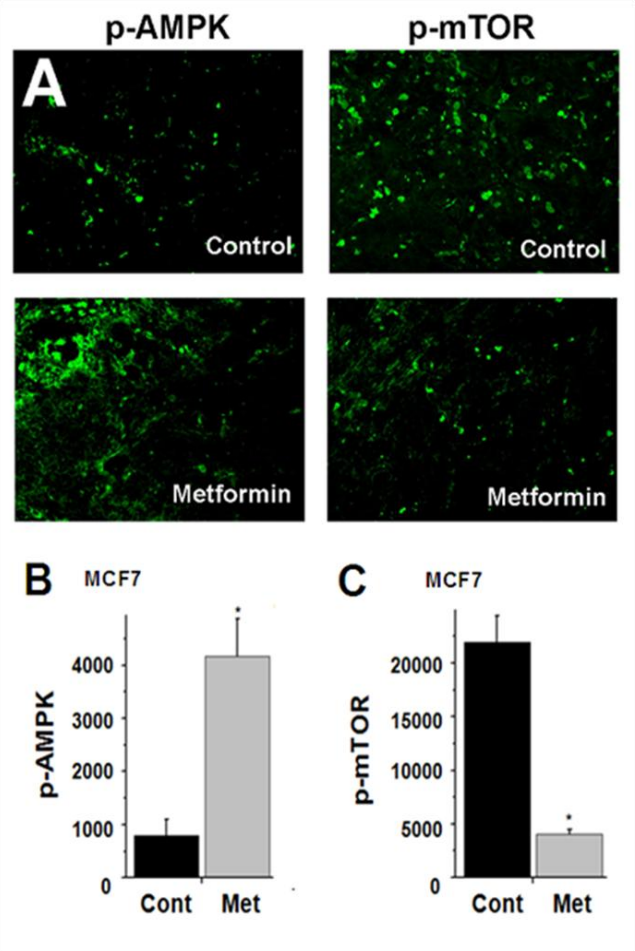
Supplement Fig 1. Apoptotic cell death of FSaII and MCF-7 cells treated with metformin alone or with radiation. (**A**): Cells were incubated with metformin for 24 h, irradiated with various doses of X-rays, and further incubated with 1 or 5 mM metformin for 24 h. Cells were stained with Annexin V/PI and apoptotic changes were measured by flow cytometry. (**B**): Cells were incubated with metformin for 24 h, irradiated with various doses of X-rays, and further incubated with 1 or 5 mM metformin for 24 h. Cells were stained with PI and the percentage of cells with a sub-G1 DNA content was measured by flow cytometry. (**C**): Cells were incubated with metformin for 24 h, irradiated with various doses of X-rays, further incubated with 1 or 5 mM metformin for 24 h. PARP, caspase-3 and β-actin were then analyzed by western blotting.

Supplementary Figure S2



Supplement Fig 2. Effects of metformin or irradiation and combined on the expression of p-AMPK and p-mTOR. This is supplemental data for the figures 2. The band intensities of p-AMPK, AMPK, p-mTOR, mTOR were measured using ImageJ 1.41 software (NIH, Bethesda, MD). Averages of 4-5 experiments are shown.

Supplementary Figure S3



Supplement Fig 3. Immunohistological study for the expression of p-AMPK and p-mTOR in MCF-7 tumors. The cross-sections were stained and quantified for p-AMPK (A left panels and B) and p-mTOR (A right panels and C). All results are expressed as mean pixel counts per image ± standard error from 20 images derived from 3 tumors per group (magnification 200x). Representative images illustrating the average amount of staining are depicted in panel A for each treatment modality as indicated. *$P < 0.001$ Metformin group vs. vehicle