

Supplementary Information

Radiation-induced senescence in securin-deficient cancer cells promotes cell invasion involving the IL-6/STAT3 and PDGF-BB/PDGFR pathways

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Legends to suppl. Figures

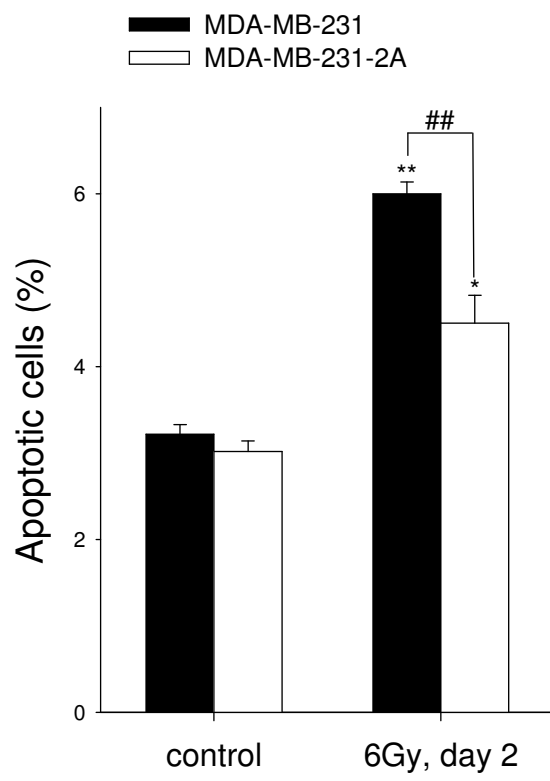
Suppl. Fig. S1. Radiation induced apoptosis in MDA-MB-231 but not in MDA-MB-231-2A cells. MDA-MB-231 and MDA-MB-231-2A cells exposed to 6 Gy radiation was collected 2 days after irradiation. Cell apoptosis was determined using Annexin V/PI double staining.

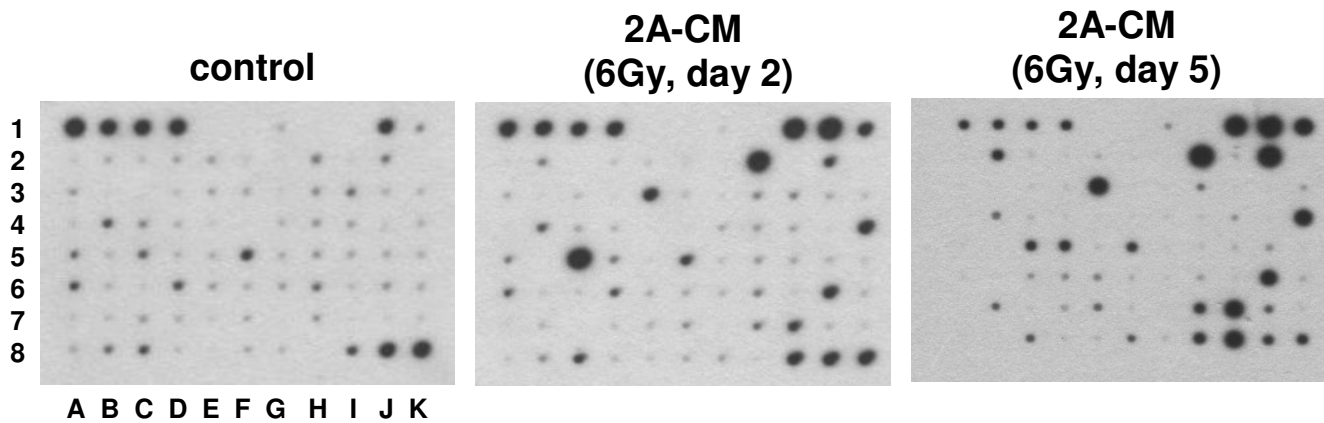
Suppl. Fig. S2. Cytokines produced by radiation-induced senescent MDA-MB-231-2A cells. The conditioned medium (CM) from MDA-MB-231-2A cells exposed to 6 Gy radiation was collected 2 and 5 days after irradiation. A Human Cytokine Antibody Array was used to analyse the presence of various cytokines and chemokines. The cytokines and chemokines listed in the table show stronger signal compared to control on the array. Quantization of the cytokines and chemokines in the CM from the irradiated cells with post-irradiation period of 2 days (left) and 5 days (right) are also listed.

Suppl. Fig. S3. Effect of STAT3 knockdown on radiation-induced STAT3 phosphorylation and IL-6 releases in MDA-MB-231-2A cells. MDA-MB-231-2A cells were transfected with vector or STAT3 dominant-negative (DN) plasmids followed by radiation. The levels of phospho-STAT3 and STAT3 were ascertained through western blot analyses **(A)**. IL-6 secreted from cells transfected with STAT3 siRNA followed by radiation was measured using an ELISA **(B)**. A p value of < 0.01 (**) indicates significant differences between irradiated and non-irradiated samples. A p value of < 0.01 (##) indicates a significant difference between vector- and STAT3-DN-transfected cells.

Suppl. Fig. S4. Effects of STAT3 knockdown on CM-induced STAT3 phosphorylation and invasion in MDA-MB-231 cells. MDA-MB-231 cells were transfected with vector or STAT3 dominant-negative (DN) plasmids followed by 2A-CM treatment. The levels of phospho-STAT3 and total STAT3 in MDA-MB-231 cells treated with 2A-CM were examined through western blot analyses **(A)**. The effects of cell invasion were analysed using a Boyden chamber assay **(B)**. The population of invasion cells was quantified. A p value of < 0.01

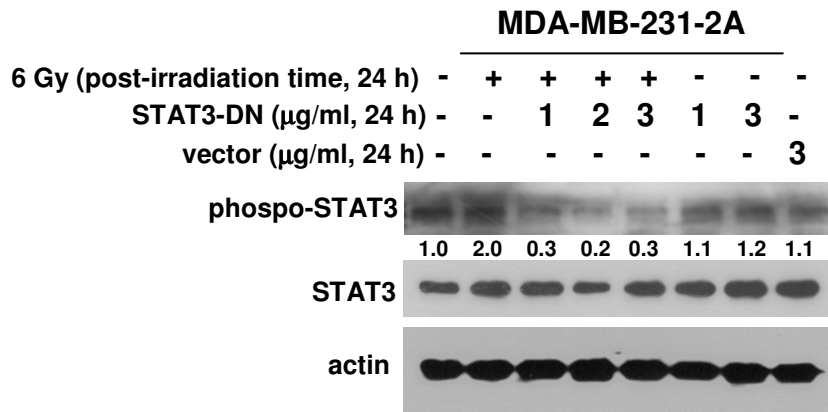
(**) indicates significant differences between CM-treated and untreated samples. A p value of < 0.01 (##) indicates a significant difference between vector- and STAT3-DN-transfected cells.



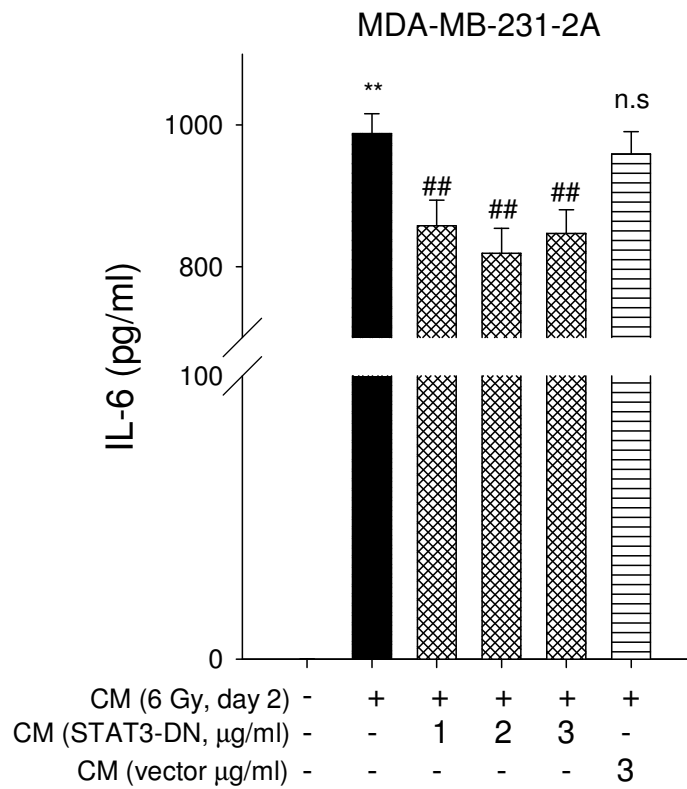


| Position | Cytokines/ Chemokines | MDA-MB-231-2A | |
|----------|--------------------------|---------------|---------------|
| | | CM (day 2) | CM (day 5) |
| 7-I | MIP-3 α | 5.69 | 68.73 |
| 1-I | GM-CSF | 18.22 | 59.85 |
| 3-E | MCP-1 | 3.59 | 36.00 |
| 2-J | IL-8 | 1.34 | 25.34 |
| 2-H | IL-6 | 3.86 | 25.14 |
| 1-K | GRO- α | 2.71 | 24.16 |
| 4-K | Angiogenin | 3.71 | 23.74 |
| 6-J | IGFBP-1 | 2.61 | 18.99 |
| 5-D | PDGF-BB | 0.87 | 18.54 |
| 8-I | TIMP-2 | 1.49 | 14.35 |
| 1-J | GRO | 1.41 | 9.62 |
| 5-C | VEGF | 2.91 | 5.55 |

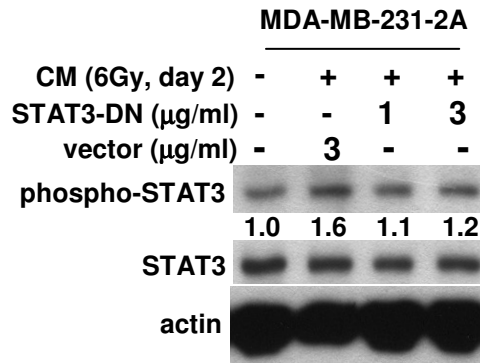
A



B



A



B

