

## **Supplemental Material to:**

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and Shu-Jun Chiu**

**Autophagy promotes radiation-induced senescence but  
inhibits bystander effects in human breast cancer cells**

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**[www.landesbioscience.com/journals/autophagy/article/28772](http://www.landesbioscience.com/journals/autophagy/article/28772)**

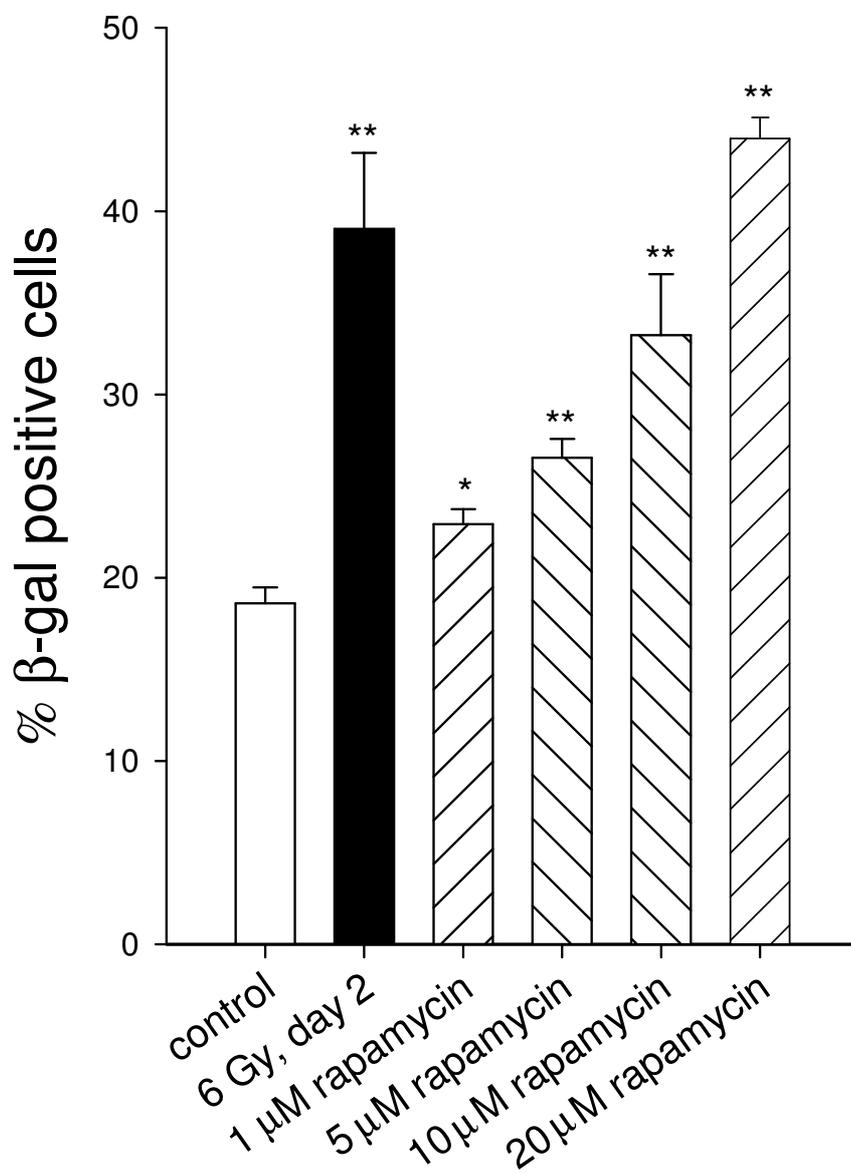
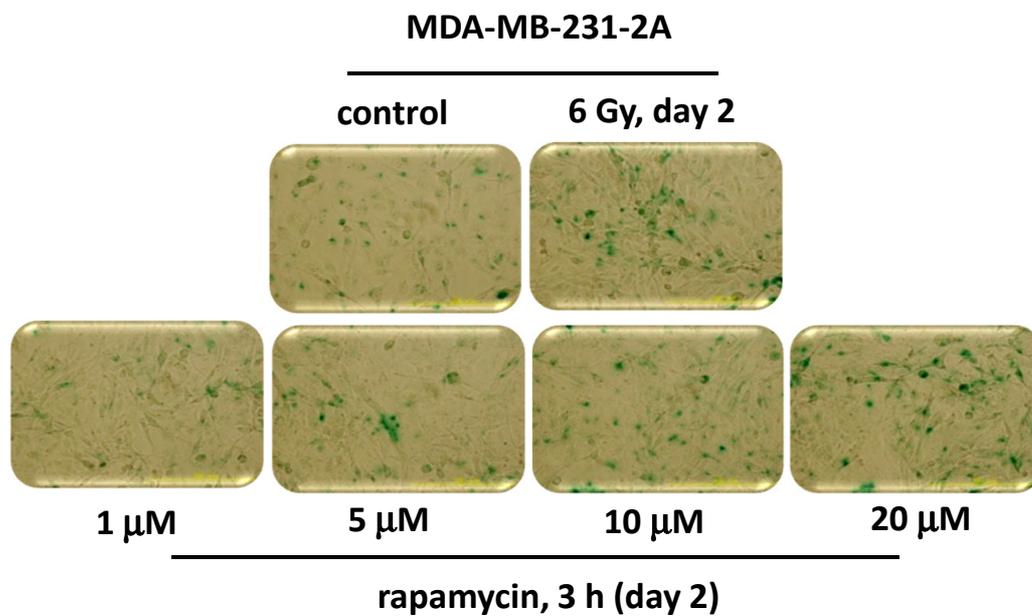
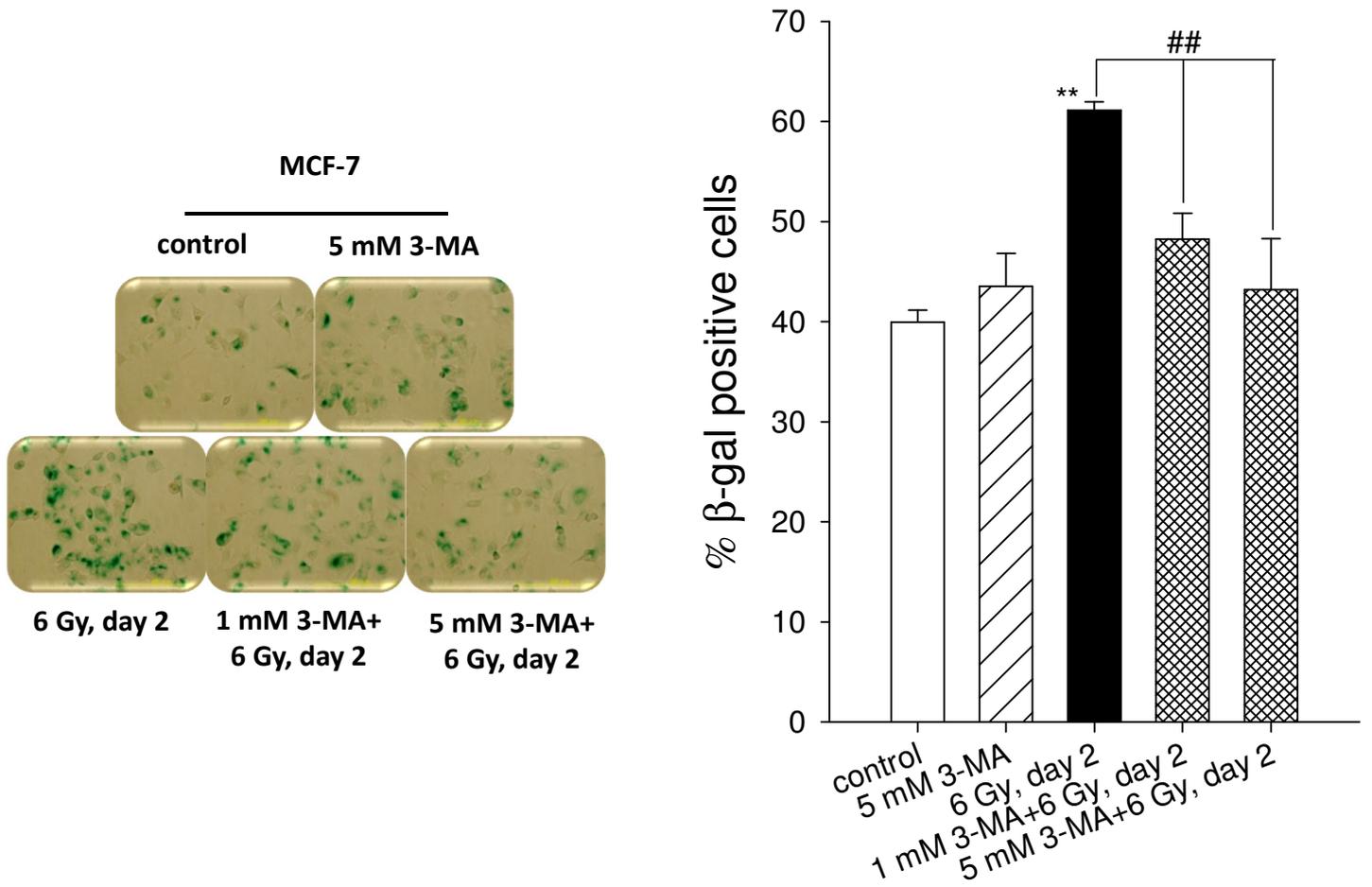


Figure S1. Huang *et al.*

A



B

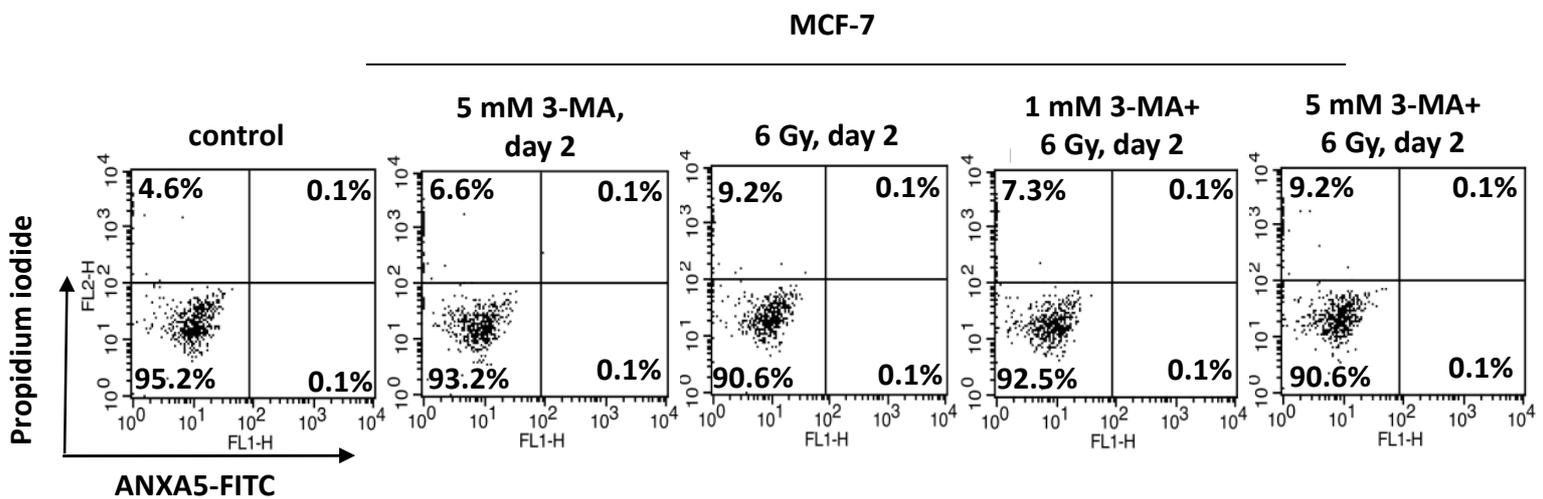
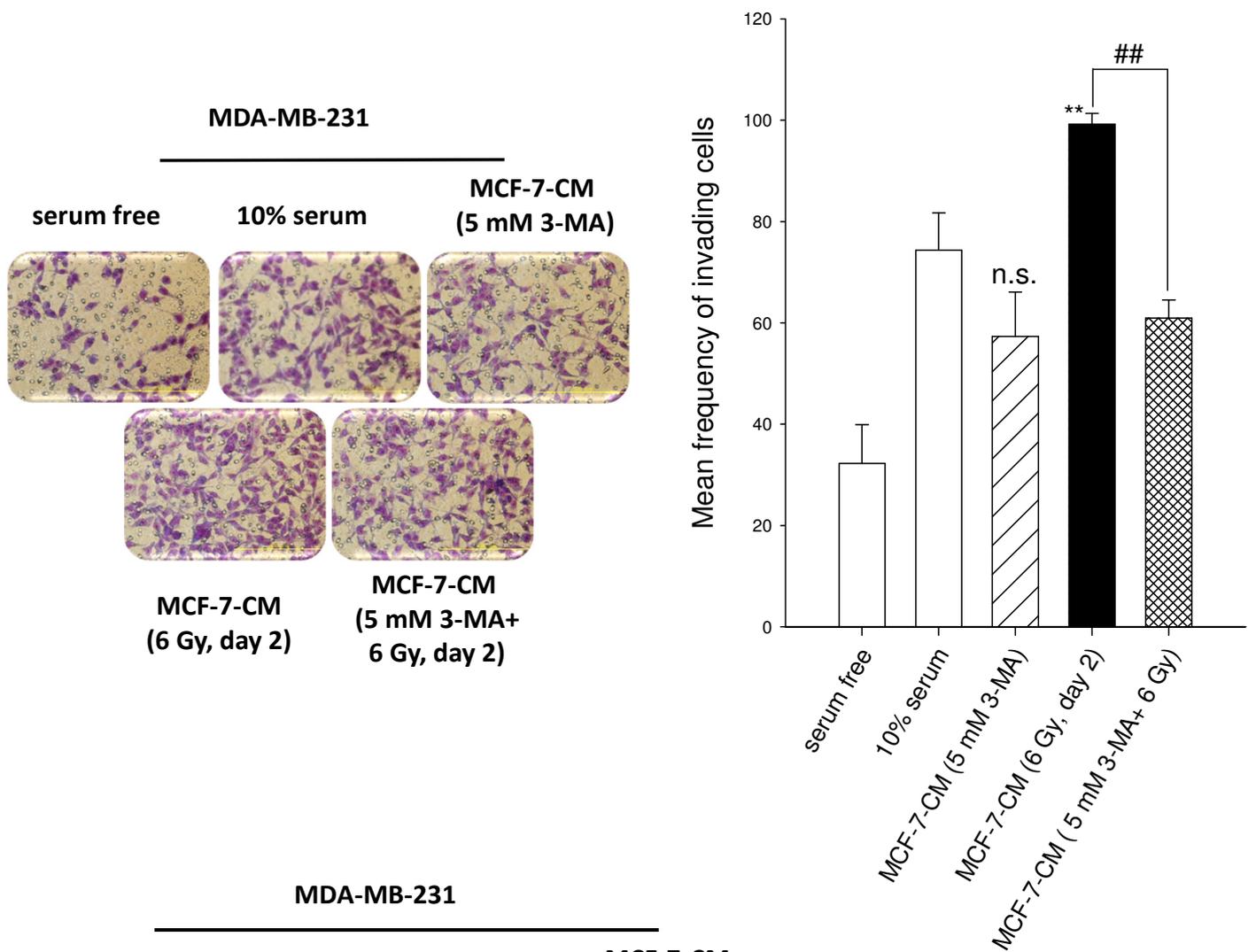
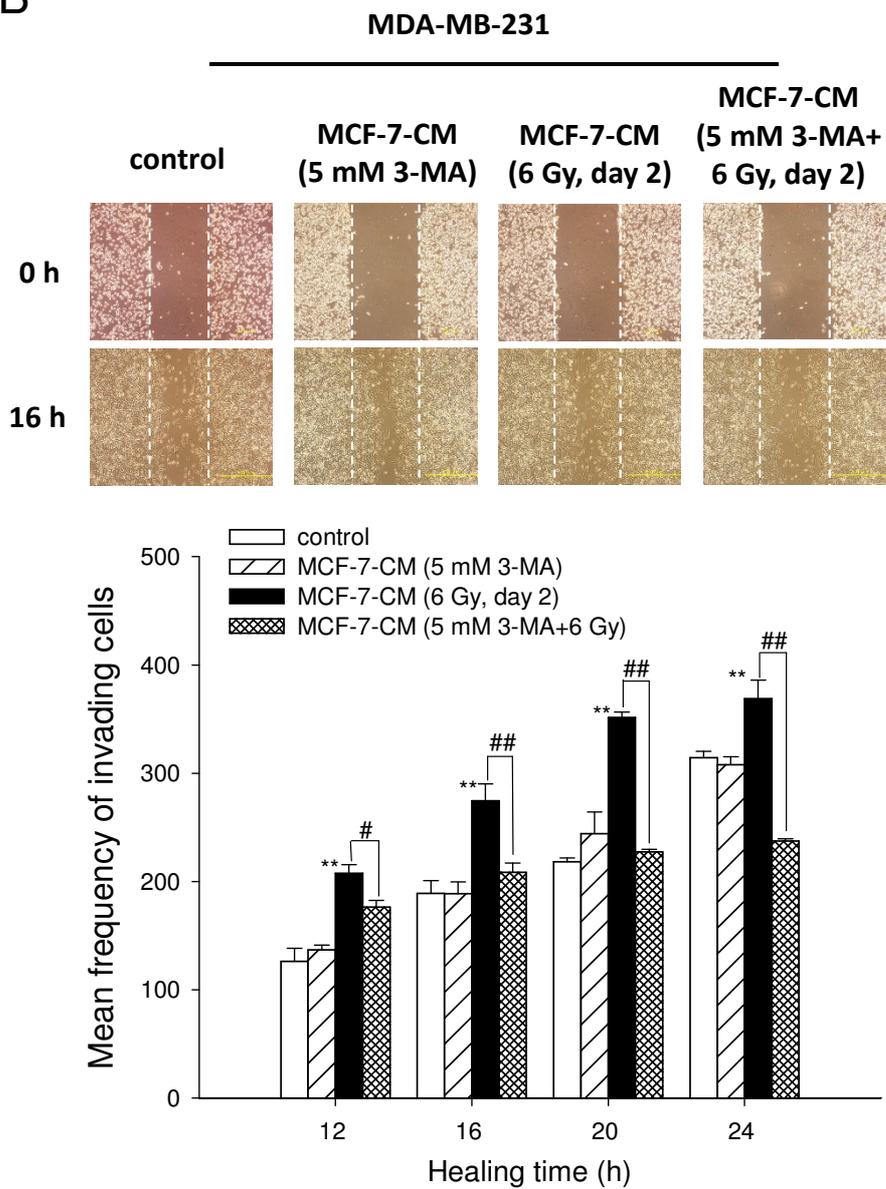


Figure S2. Huang *et al.*

**A**

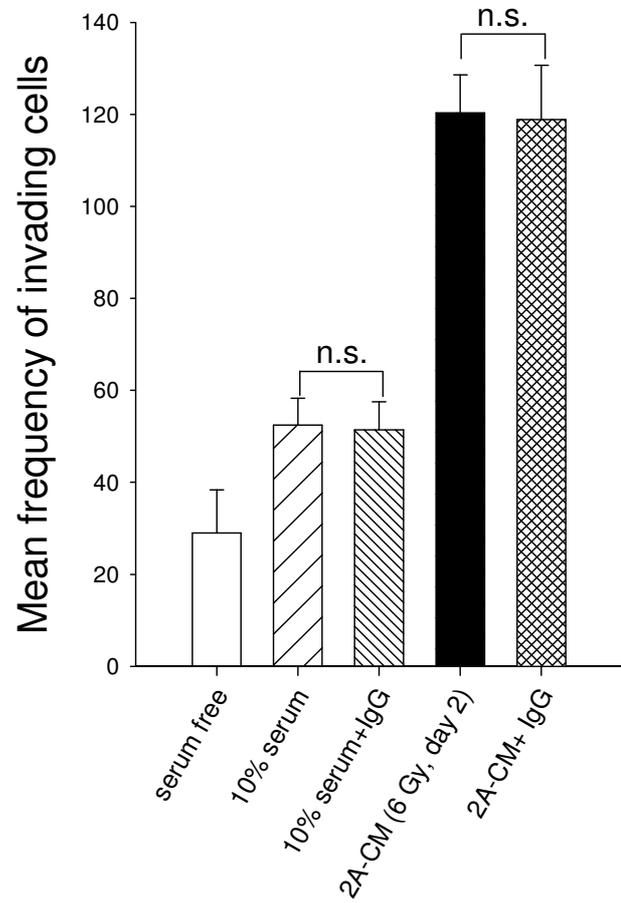
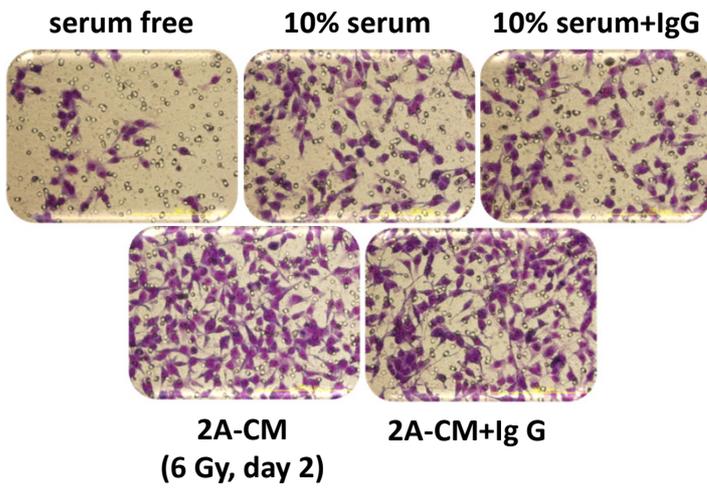


**B**



**Figure S3. Huang et al.**

**A**



**B**

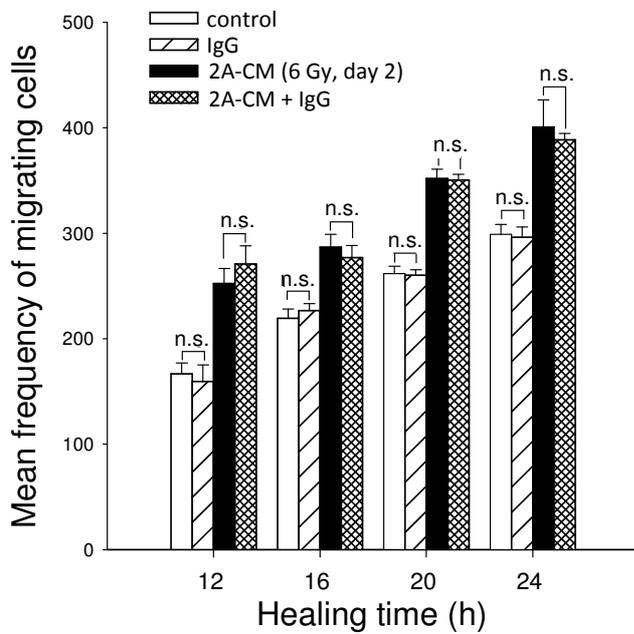
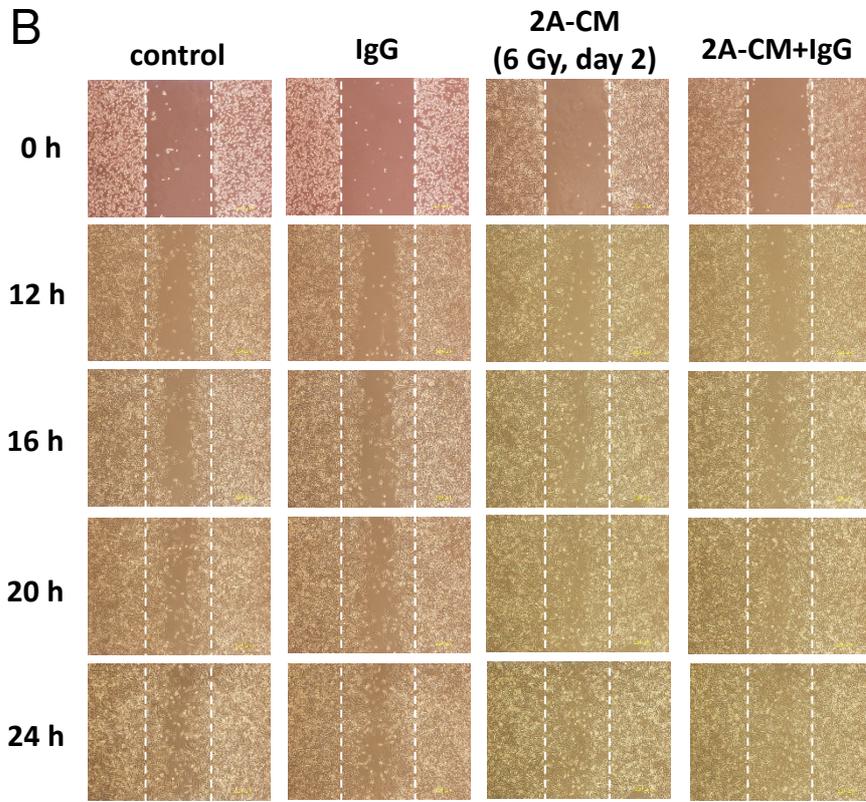


Figure S4. Huang *et al.*

**Figure S1.** Rapamycin induced senescence in MDA-MB-231-2A cells. MDA-MB-231-2A cells were exposed to 6 Gy radiation or various doses of rapamycin for 3 h. After 2 days of recovery time, the cells were observed by bright-field microscopy and stained with SA- $\beta$ -gal. \* and \*\* indicate significant differences (\* $P$ <0.05, \*\* $P$ <0.01) between the control and treated cells.

**Figure S2.** Effect of 3-MA on radiation-induced senescence in MCF-7 cells. (A) MCF-7 cells were pretreated with or without 3-MA before exposure to 6 Gy radiation. Bright-field microscopy observation and SA- $\beta$ -gal staining were performed 2 days after irradiation. \*\* indicates significant differences ( $P$ <0.01) between the control and irradiated cells. ## indicates significant differences ( $P$ <0.01) between the inhibitor-treated and untreated cells. (B) MCF-7 cells were pretreated with or without 3-MA before exposure to 6 Gy radiation. Apoptotic cell death was measured by ANXA5-PI double staining 2 days after irradiation.

**Figure S3.** Effect of 3-MA on MCF-7-CM-induced invasion and migration of unirradiated MDA-MB-231 cells. MDA-MB-231 cells were treated with serum free, 10% serum, MCF-7-CM from MCF-7 cells treated with 3-MA alone or MCF-7-CM from MCF-7 cells pretreated with or without 3-MA before exposure to irradiation. The invasion (A) and migration (B) of MDA-MB-231 cancer cells were measured using a Boyden chamber and a wound-healing assay, respectively. The numbers of the invaded and migrated cells were quantified. \*\* indicates significant differences ( $P$ <0.01) between the control and CM-treated cells. ## indicates significant differences ( $P$ <0.01) between the CM-treated and CM (3-MA)-treated cells. n.s. indicates no significant differences between the control and CM-treated cells.

**Figure S4.** Effect of isotype IgG on 2A-CM-induced invasion and migration of unirradiated MDA-MB-231 cells. Isotype IgG was added to the 2A-CM for 1 h and then incubated with MDA-MB-231 cells. MDA-MB-231 cells were treated with serum free, 10% serum, 10% serum and IgG, 2A-CM or 2A-CM from MDA-MB-231-2A cells neutralized with or without IgG after exposure to irradiation. The invasion (A) and migration (B) of MDA-MB-231 cancer cells were measured using a Boyden chamber and a wound-healing assay, respectively. The numbers of the invaded and migrated cells were quantified. n.s. indicates no significant differences between the IgG-treated and untreated cells.