Supplementary Material

Inflammatory Signaling and DNA Damage Responses after Local Exposure to an Insoluble Radioactive Microparticle

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This supplementary material includes 6 figures, Figure S1: Geometry and dose profile of non-uniform exposure to 6MV-linac X-rays, Figure S2: Test for evaluating the effectiveness of the inhibitor, Figure S3 and S4: Spatial distribution of nuclear γ-H2AX foci for various inhibitor treatments for WI-38 cell line and HBEC3-KT cell line, respectively, Figure S5: NF-κB and COX-2 levels after non-uniform exposure, Figure S6: Dependency of irradiation area size on in- and out-of-field cell survival.

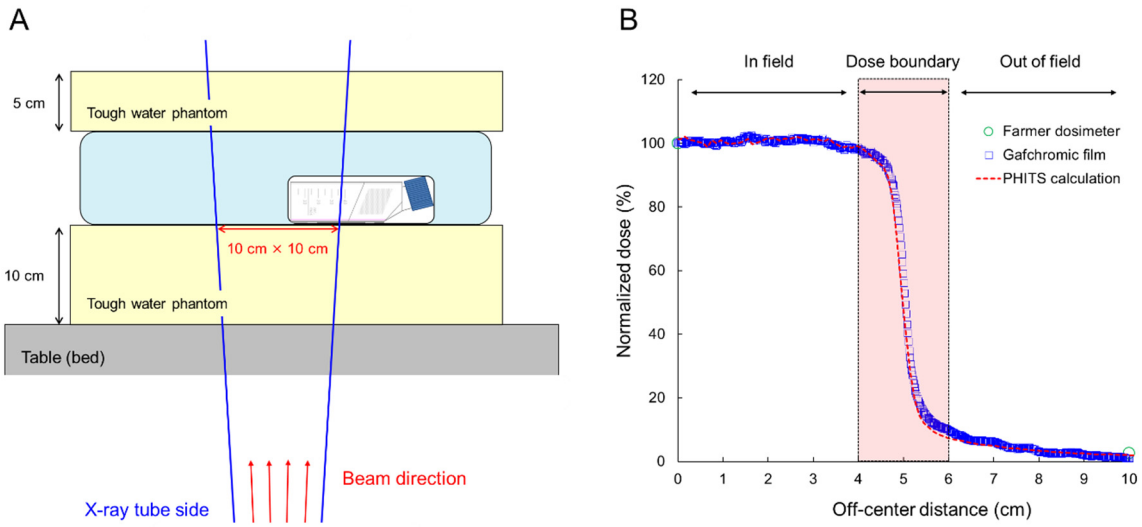

Figure S1. Geometry and dose profile of non-uniform exposure to 6 MV-linac X-rays: (A) Schematic illustration of geometry for irradiating 6 MV-linac X-rays. (B) Dose profile. As shown in Figure S1A, the 50% cells were exposed by the placement of cell culture container at the edge of the radiation field. The field size was 10 × 10 cm² and the depth was 10 cm. The dose profile was quantified by ionizing chamber, Gafchromic EBT3 film and the PHITS calculation using the phase-space file for Varian Clinac 600C 6MV photon (equivalent to Clinac 6EX). The out-of-field dose is 5%, on average, of in-field dose.

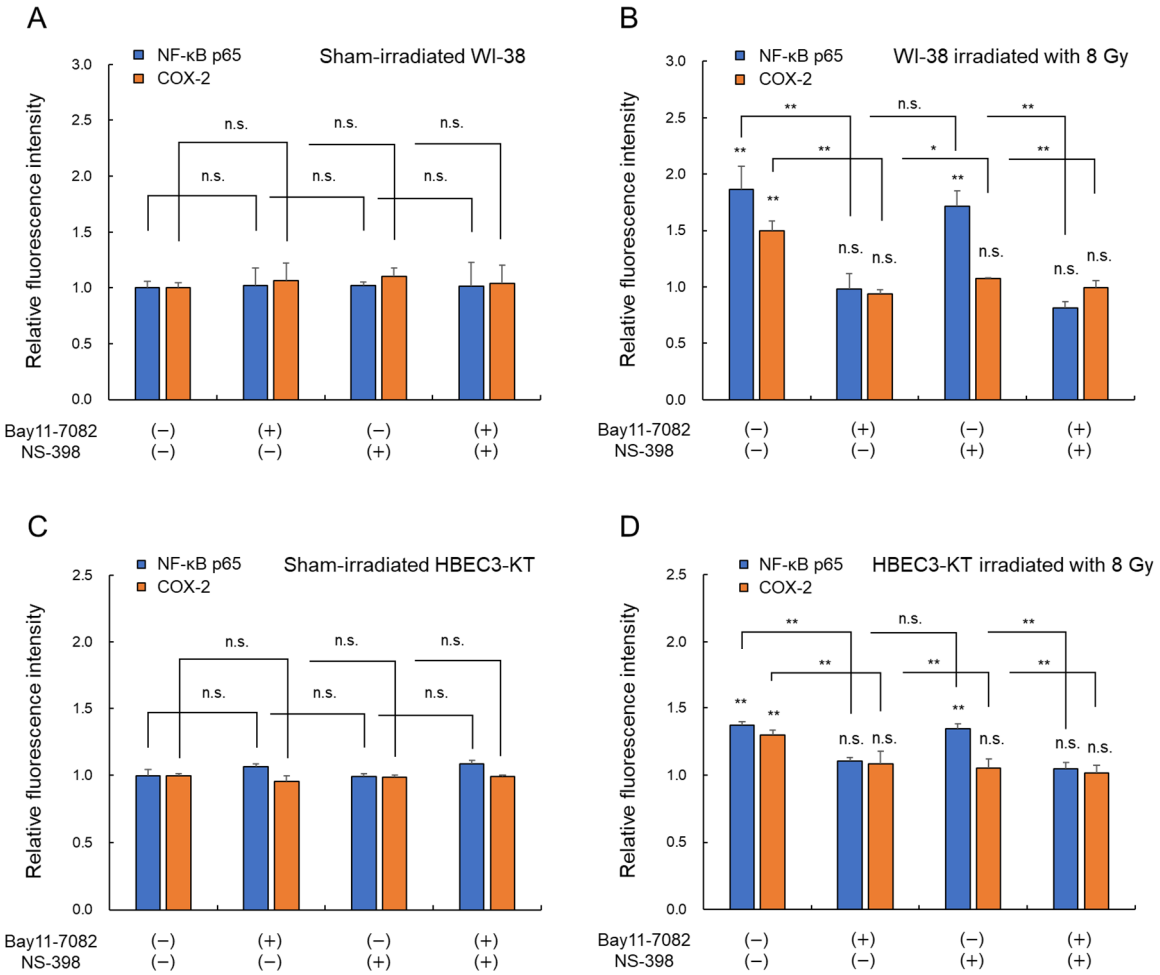

Figure S2. Test for evaluating the effectiveness of the inhibitor: (A) is for sham-irradiated WI-38 cells, (B) is for WI-38 cells irradiated with 8 Gy, (C) is for sham-irradiated HBEC3-KT, and (D) is for HBEC3-KT cells irradiated with 8 Gy. The inhibitors of NF-κB p65 and COX-2 used were 1 μM Bay-11-7082 (AG-CR1-0013-M010, Funakoshi) and 50 μM NS-398 (70590, Funakoshi), respectively. The irradiation was performed at room temperature with 150 kVp X-rays (1 mm Al filtration at 1.0 Gy/min) using an X-ray generator (MBR-1520R, Hitachi Medical Co., Tokyo, Japan). From Figure S2A and S2C, there was no significant difference among sham group and various inhibitor treatments. Bay-11-7082 inhibited both NF-κB p65 and COX-2 activations, and NS-398 reduced only COX-2 expression as shown in Figure S2B and S2D. Both treatments inhibited both NF-κB p65 and COX-2 activations in the same manner as NF-κB p65 treatment only. From these results, it was confirmed that NF-κB pathway upregulates COX-2 expression in inflammatory signaling pathways in WI-38 and HBEC3-KT cell lines. The symbols (*, **, n.s.) indicate the 5%, 1% significant difference and non-significant, respectively.

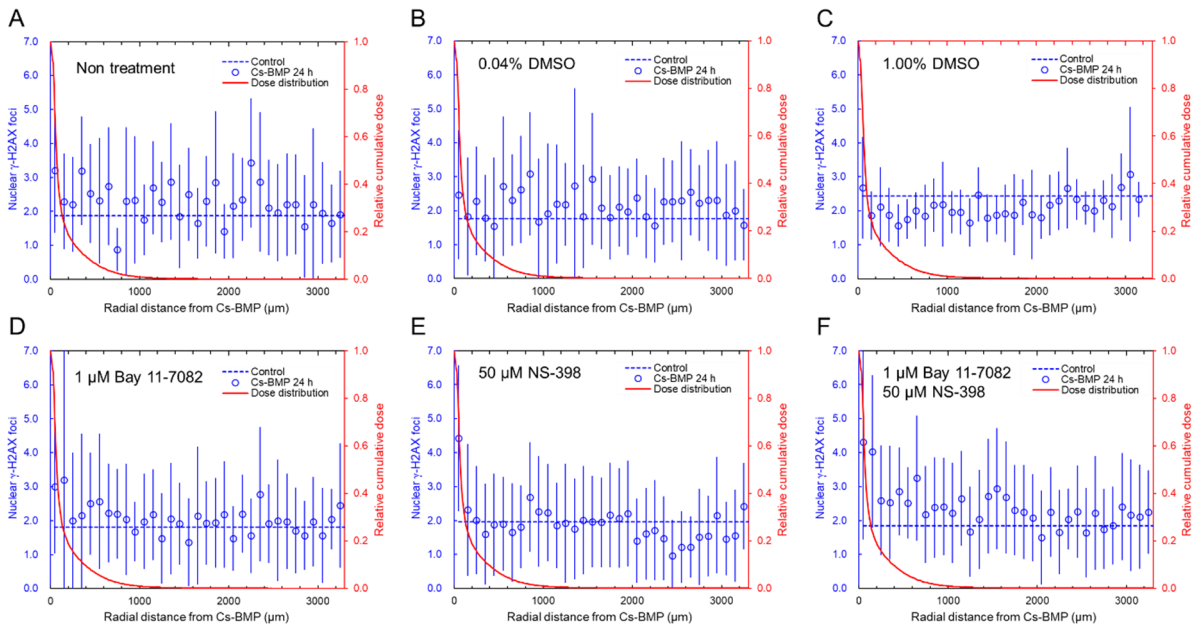

Figure S3. Spatial distribution of nuclear γ-H2AX foci for various inhibitor treatments for WI-38 cell line: (A) is for non-treatment, (B) is for 0.04% DMSO treatment, (C) is for 1.00% DMSO treatment, (D) is for 1 μM Bay 11-7082 treatment, (E) is for 50 μM NS-398 treatment, and (F) is for both treatments with 1 μM Bay 11-7082 and 50 μM NS-398. Blue dotted line is the non-irradiated level. Red solid line is the dose profile.

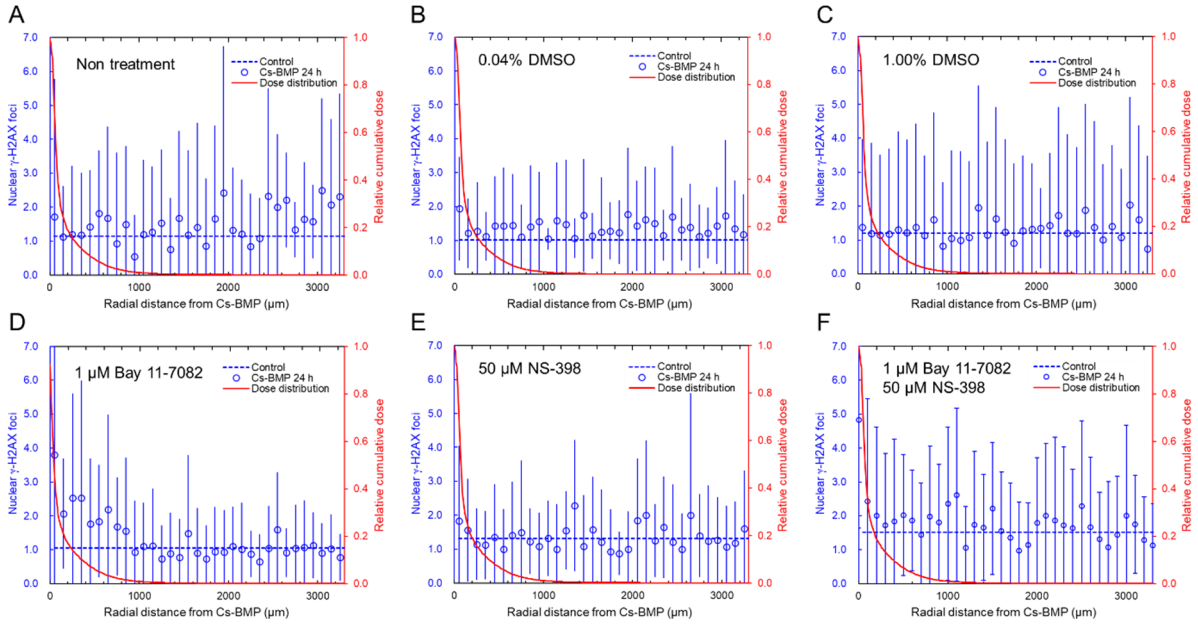

Figure S4. Spatial distribution of nuclear γ-H2AX foci for various inhibitor treatments for HBEC3-KT cell line: (A) is for non-treatment, (B) is for 0.04% DMSO treatment, (C) is for 1.00% DMSO treatment, (D) is for 1 μM Bay 11-7082 treatment, (E) is for 50 μM NS-398 treatment, and (F) is for both treatments with 1 μM Bay 11-7082 and 50 μM NS-398. Blue dotted line is the non-irradiated level. Red solid line is the dose profile.

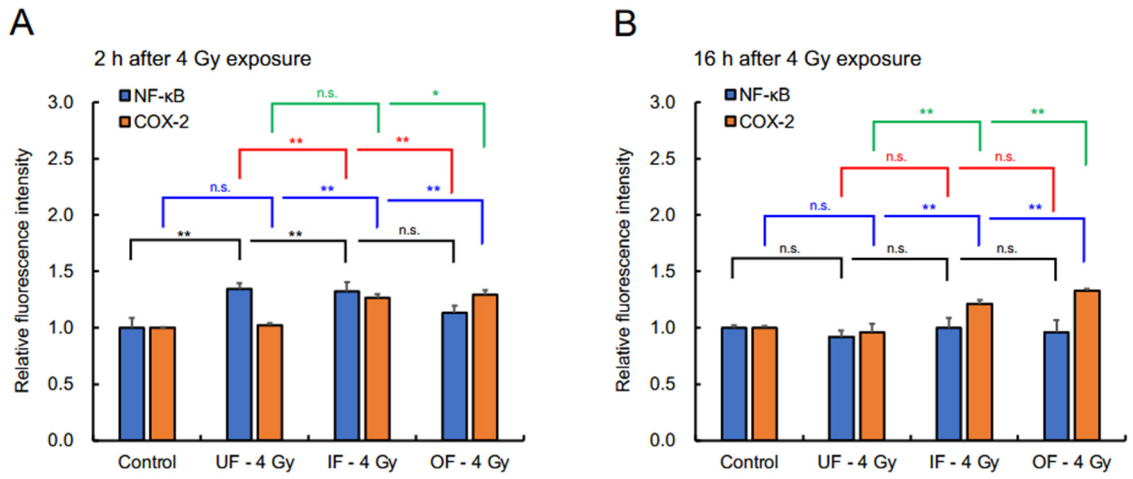

Figure S5. NF-κB and COX-2 levels after non-uniform exposure: (A) is the relative fluorescence to sham-irradiated WI-38 cells at 2 h post-irradiation, (B) is the relative value to sham-irradiated WI-38 cells at 16 h post-irradiation. In this non-uniform exposure, the dose was delivered to 50% of the area of culture dish containing the cells, and the in-field dose was 4 Gy. The irradiation was performed at room temperature with 150 kVp X-rays (1 mm Al filtration at 1.0 Gy/min) using an x-ray generator (MBR-1520R, Hitachi Medical Co., Tokyo, Japan). The significant activations of NF-κB and COX-2 in out-of-field cells were detected at 2 h after non-uniformexposure to 4 Gy (figure S5A) as bystander responses. Meanwhile, the persistent COX-2 expression was also detected at 16 h after the exposure (figure S5B). The symbols (*, **, n.s.) indicate the 5%, 1% significant difference and non-significant, respectively.

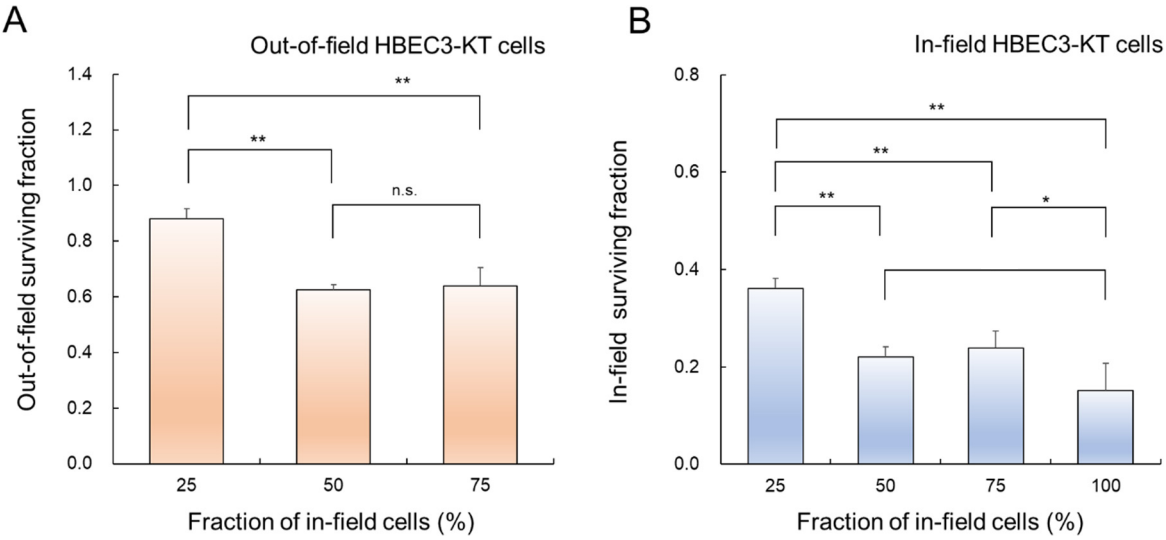

Figure S6. Dependency of irradiation area size on in- and out-oh-field cell survival: (A) is out-of-field survival of HBEC3-KT cells, (B) is in-field survival of HBEC3-KT cells. The partial irradiation was performed and the in-field dose was 4 Gy. The irradiation was performed at room temperature with 6-MV linac X-rays (Clinac 6EX, Varian). The geometry and dose profile are described in Figure S1. As shown in Figure S6A, the bystander effects (reduced clonogenicity) from in-field cells to out-of-field seems to be saturated for larger fraction of in-field cells than 50%. Meanwhile, the protective effects (reduced cell killing) are maximized in case of the smallest fraction of in-field cells of 25%. The symbols (*, **, n.s.) indicate the 5%, 1% significant difference and non-significant, respectively.