Supplementary Figures

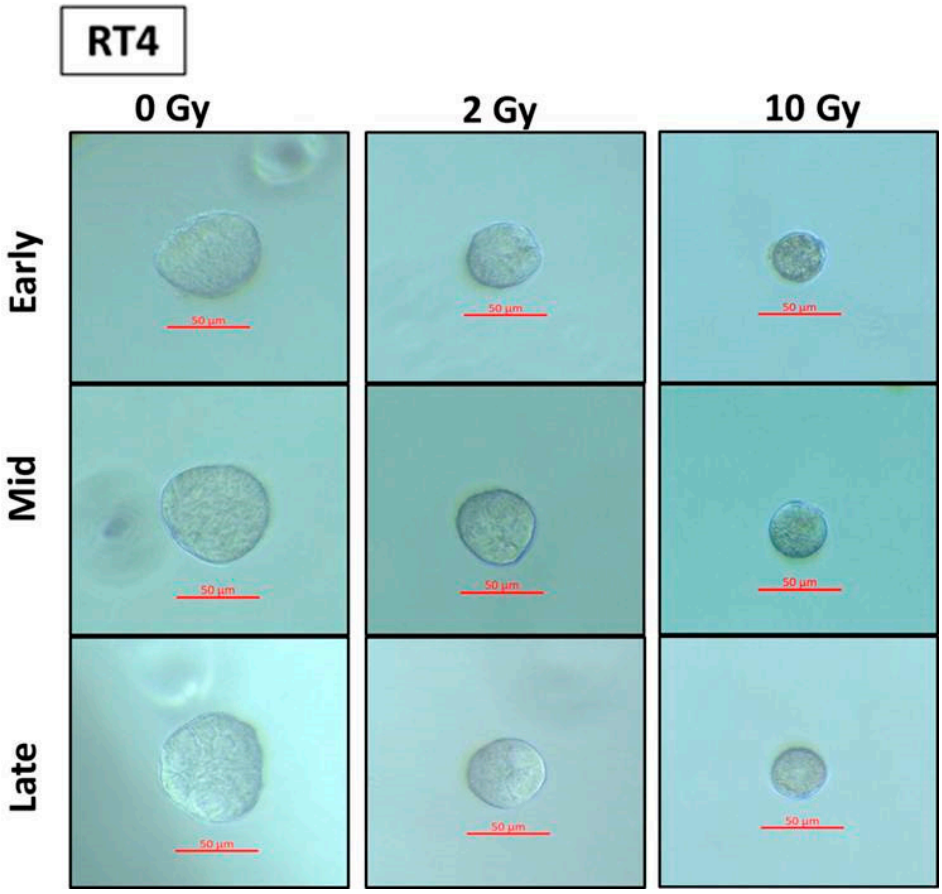

Figure S1. Representative images of RT4 spheres without IR and after single doses treatments with 2 Gy and 10 Gy.

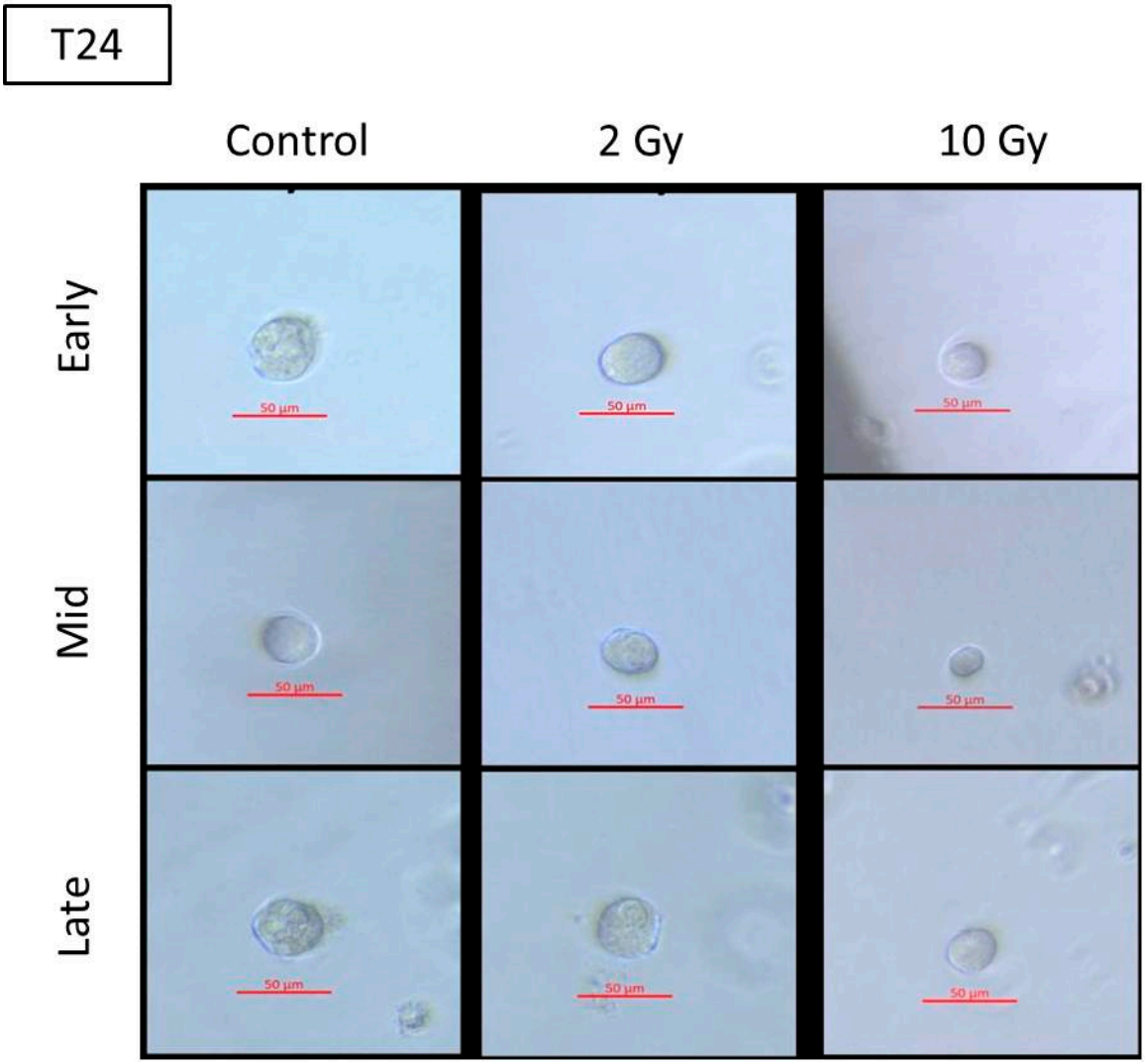

Figure S2. Representative images of T24 spheres without IR and after single doses treatments with 2 Gy and 10 Gy.

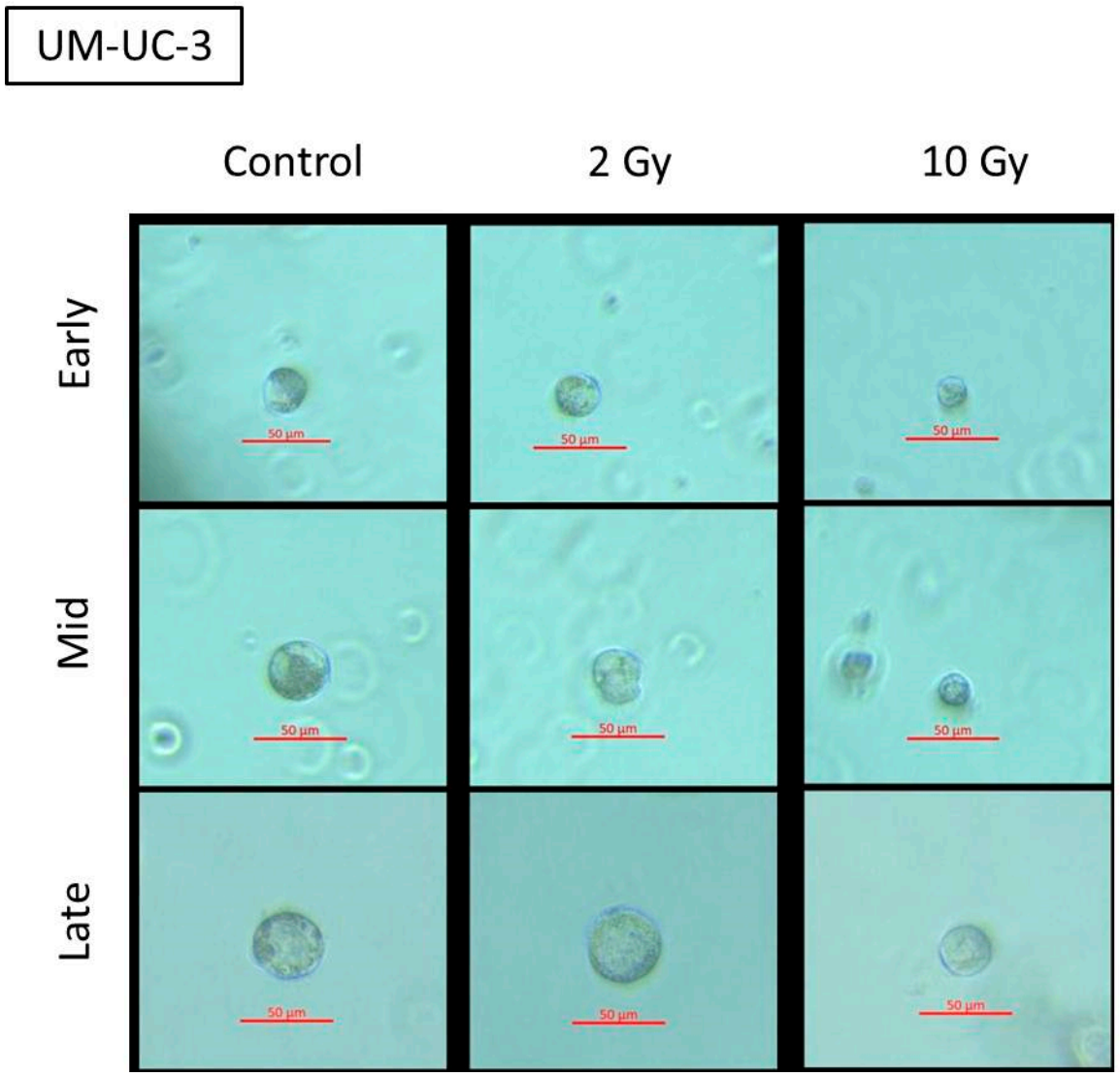

Figure S3. Representative images of UM-UC-3 spheres without IR and after single doses treatments with 2 Gy and 10 Gy.

Supplementary Materials and Methods

Sphere Formation Assay:

Sphere formation assay was performed as previously described [1]. In brief, single cells were suspended, in duplicates, in a 50 µL volume of 1:1 cold growth factor-reduced Matrigel™ (BD Biosciences)/ DMEM Ham's F-12 (serum-free) at a density of 2,000 cells/well. This cell suspension was plated gently around the rim of each well of a 24-well plate and left for 1 hour at 37°C in a 5% CO2 humidified incubator to solidify. Afterwards, 500 µL of DMEM Ham's F-12 cell growth medium supplemented with 3% FBS was gently added to the center of each well and replenished.

Every 2–3 days. Spheres' count was assessed using the sphere formation efficiency or sphere formation unit (SFU) formula:

$$SFU = \frac{number\ of\ spheres\ counted}{number\ of\ cells\ seeded} \times 100$$

Each experiment was performed 3 times. Results are shown as mean ± SEM.

Clonogenic Assay:

To assess the radiosensitivity of the three bladder cell lines, clonogenic assay was performed as before [1]. In brief, cells were seeded in 12-well plates and upon reaching 70% confluency, cells were treated with a dose of 2Gy or left untreated (control group). After 24hrs, cells were trypsinized, counted and plated in 60mm petri dishes at two

different cell densities based on their best plating efficiency (PE). After 12-14 days of incubation, colonies were fixed with 95% ethanol, washed with PBS and stained with cresyl violet. Colonies were then counted (each colony >50 cells) and consequently, the surviving fraction was calculated according to the following formula:

References

1. Bodgi, L.; Bahmad, H.F.; Araji, T.; Al Choboq, J.; Bou-Gharios, J.; Cheaito, K.; Zeidan, Y.H.; Eid, T.; Geara, F.; Abou-Kheir, W. Assessing Radiosensitivity of Bladder Cancer in vitro: A 2D vs. 3D Approach. *Front Oncol* **2019**, *9*, 153, doi:10.3389/fonc.2019.00153.