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Supplementary Material

Preclinical Studies with Glioblastoma Brain Organoid Co-Cultures Show Efficient 5-ALA Photodynamic Therapy

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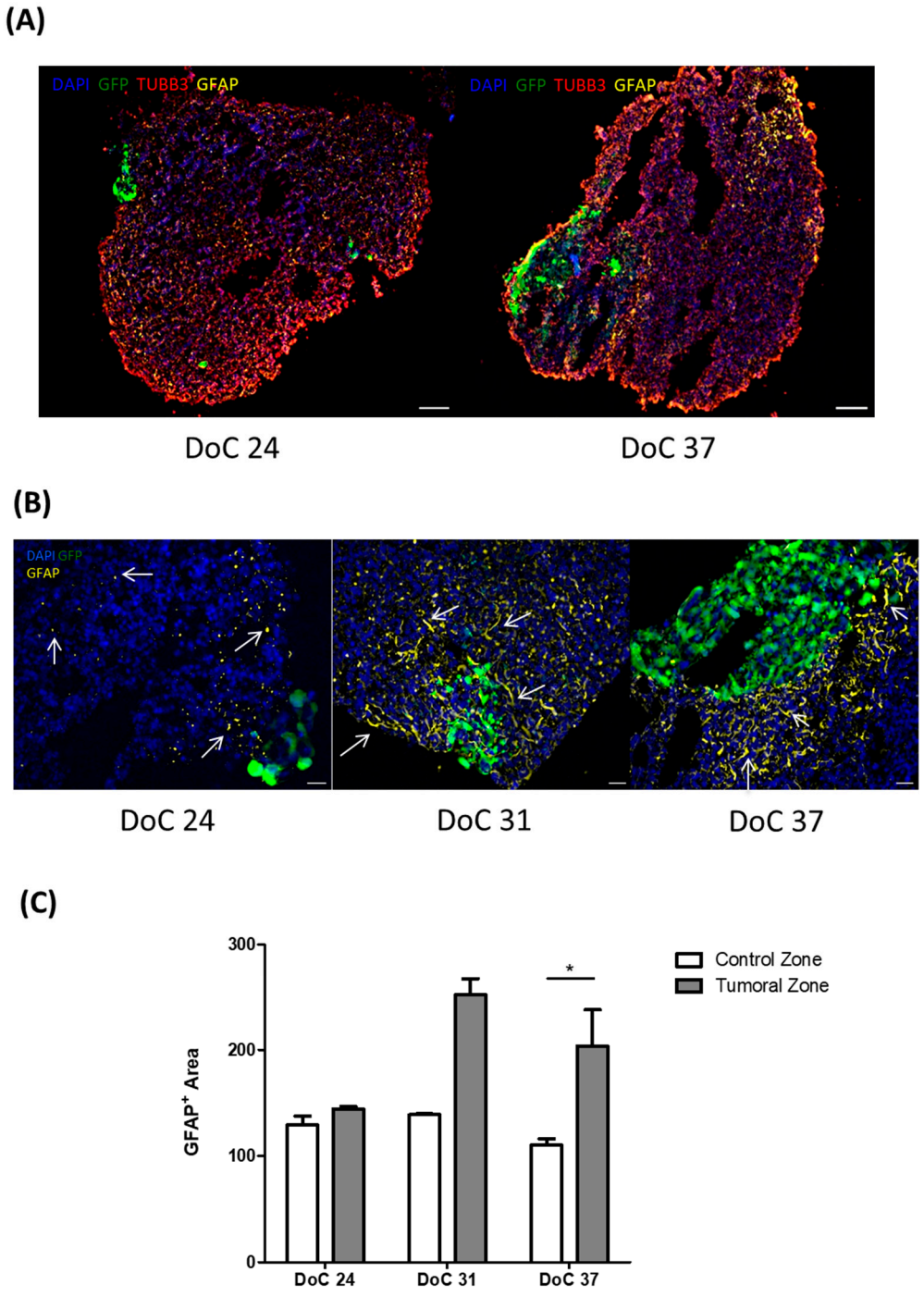

Figure S1. Immunofluorescence (IF) analyses of GFP-PG88-co-cultured with the cerebral organoid. (**A**) IF merged TUJ1 (red) and GFAP (yellow) at 24 (left) and 37 (right) days of co-culture (DoC). TUJ1 is homogenously expressed in all organoids on both days. *N* = 3. (**B**) Sequential images of samples on DoC 24 (left panel), on DoC 31 (middle panel), and on DoC 37 (right panel) after engraftment with GFP-PG88. Anti-GFAP antibody stain visualizing astrocytes in the human organoids are merged with GFP$^+$ cells signal and nuclei staining. Images are representative images of 3 different organoids analyzed, 6 fields with tumor and 9 fields without tumor, are obtained with the objective 10× and 40× with Microscopy Olympus BX41. Scale bar 100 µm (10×) and 20 µm (40×). White arrows highlight the GFAP$^+$ cells. **(C)** GFAP$^+$-cells area was studied in the tumor and control area far from the tumor-induced with PG88 cells at different days of organoid and GFP-GIC co-culture. The histogram represents the area of the GFAP$^+$ cells of organoid and GFP-GIC co-culture: day 24 (*n* = 3, 6 fields with tumor and 9 fields without tumor), day 31 (*n* = 2, 4 fields with tumor and 4 fields without tumor) and day 37 (*n* = 3, 8 fields with tumor and 6 fields without tumor). The GFAP

area was analyzed, by ImageJ, in GFP-GICs tumor area, and in areas without infiltrating GFP-GICs (control zone). ANOVA test was used to compare the means of different groups (* $p < 0.05$).

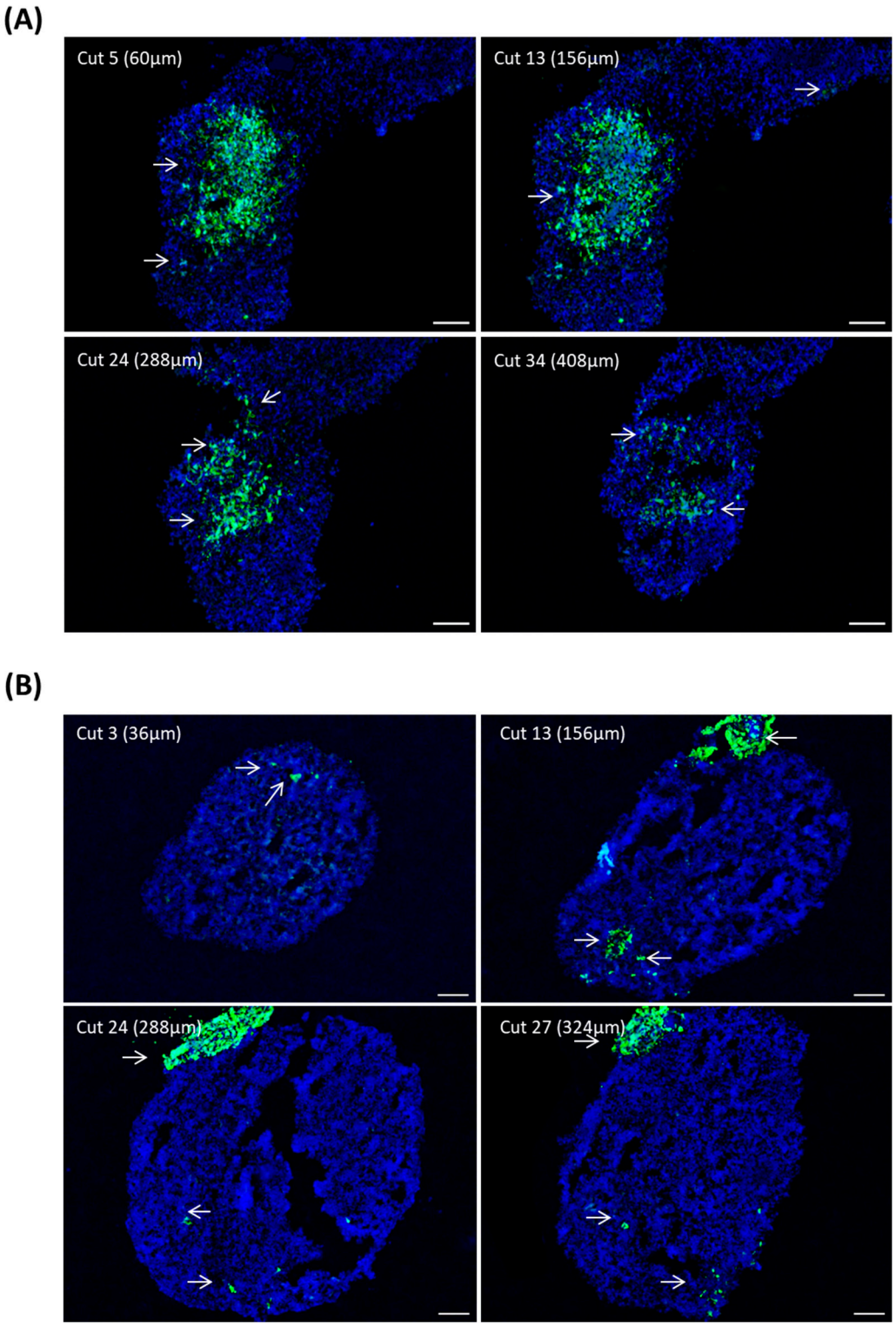

Figure S2. Invasive ability of GFP-GICs into organoids. Consecutive cuts in the same sample show infiltration of GFP-GIC7 (**A**) and GFP-PG88 (**B**) into the organoid at DoC 37. (**A**) For GFP-GIC7 the cut numbers 5, 13, 24, and 34 are shown, corresponding to 60, 156, 288, and 408 µm of depth into the organoid, respectively. (**B**) For GFP-PG88, the cut numbers 3, 13, 24, and 27 are shown, corresponding to 36, 156, 288, and 324 µm of depth into the organoid, respectively. The GFP-GICs are found in all cuts (white arrows)—nuclei labelled by Dapi. Scale bar 100 µm (10×).

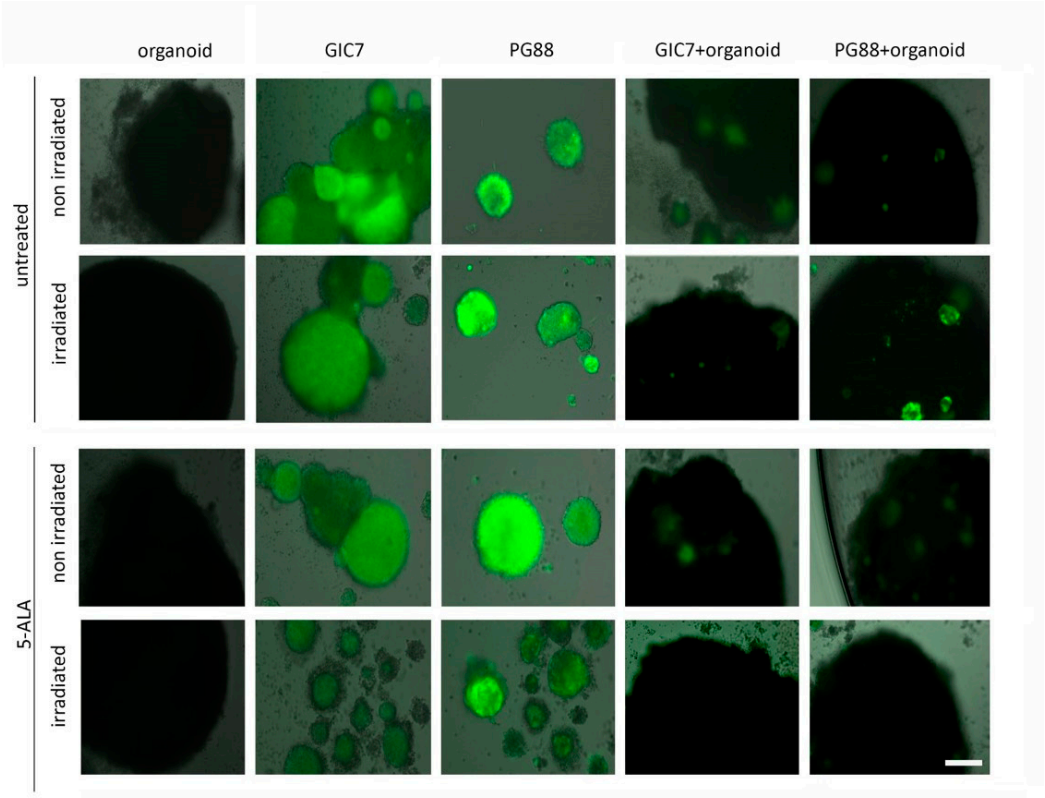

Figure S3. Comparison between co-cultures of brain organoids engrafted with GIC7 and PG88 GFP-GICs cells. Green fluorescence pictures of brain organoids and GICs taken 72 h after cells were exposed to the photodynamic treatment consisted on 50 µg/mL of 5-ALA incubated for 24 h. Pictures are representative of many pictures made in each of the plate wells. Scale bar: 250 µm.

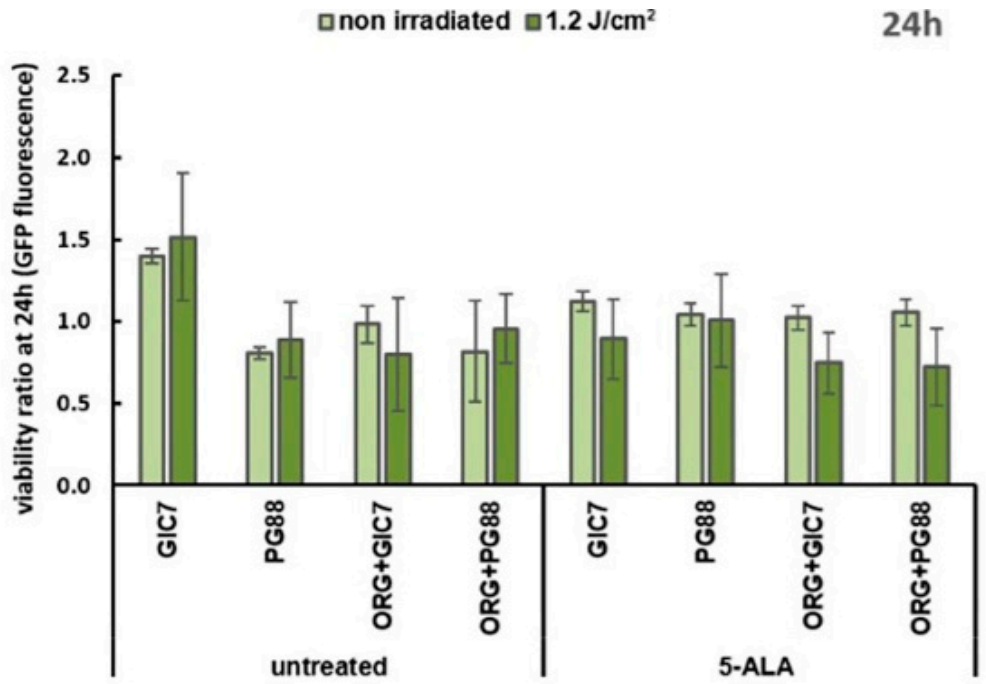

Figure S4. Cell viability after PDT of tumorspheres alone and co-cultured with brain organoids. GICs viability after 24 h post-irradiation using the GFP fluorescence ratio between each endpoint and time 0. Results are the mean ± SE of two independent experiments (n = 2) performed using six replicates.