Supplementary information

Matrix stiffness influences response to chemo and targeted therapy in brain metastatic breast cancer cells

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Supplementary Figures

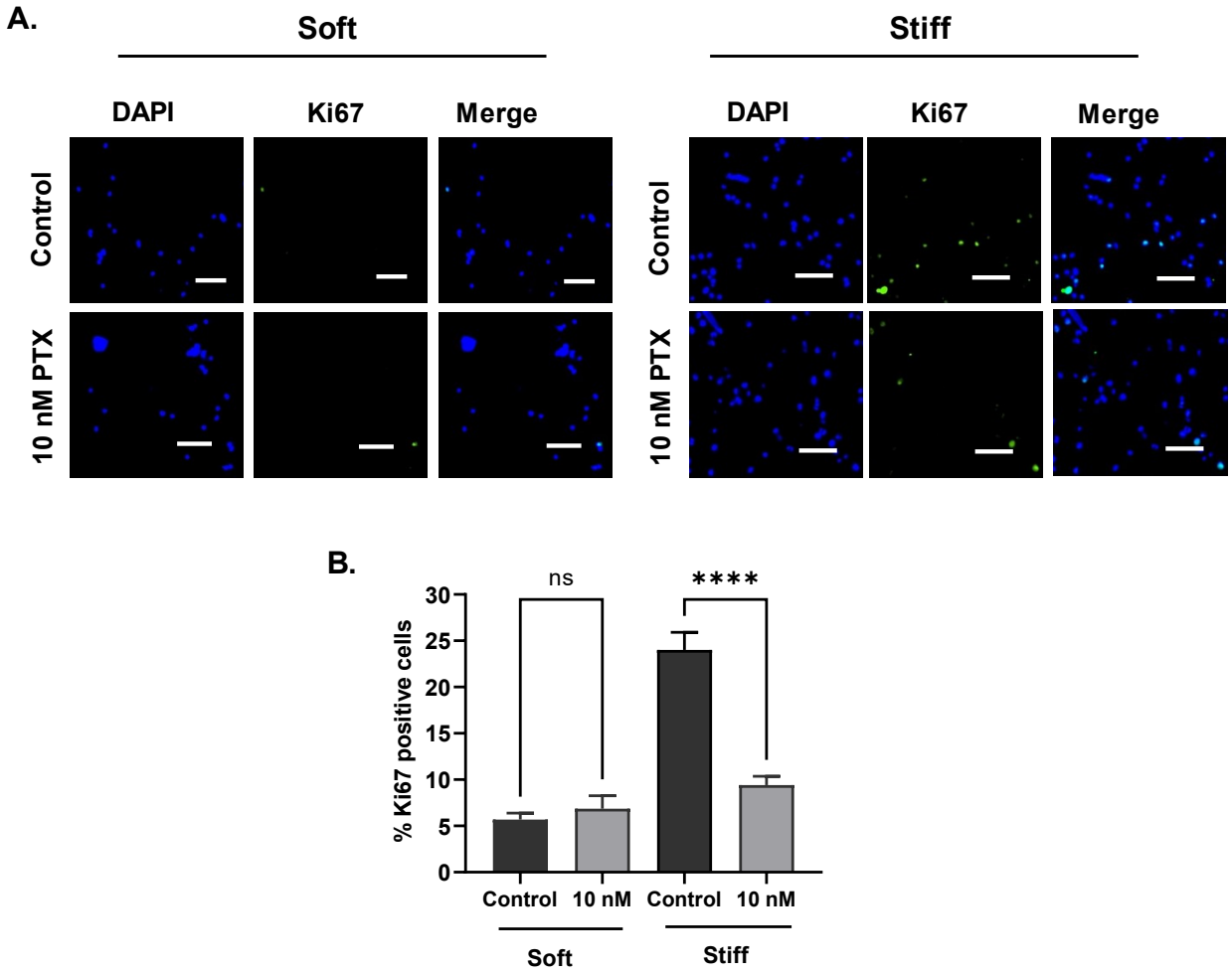

Figure S1. Percentage of Ki67 positive MDA-MB-231Br BMBC cells cultured on soft or stiff HA hydrogels treated with 10 nM PTX. (A) Fluorescence microscopic staining (blue: DAPI and green: Ki67) images of Ki67 positive MDA-MB-231Br cells cultured on soft or stiff HA hydrogels after 48 h without (control) or with 10 nM PTX treatment. (B) Quantification of percentage Ki67 positive MDA-MB-231Br cells. Scale bar = 100 μm. $N \geq 6$ hydrogels per condition. **** indicates $p < 0.0001$ when compared to control. Data presented as mean ± standard error.

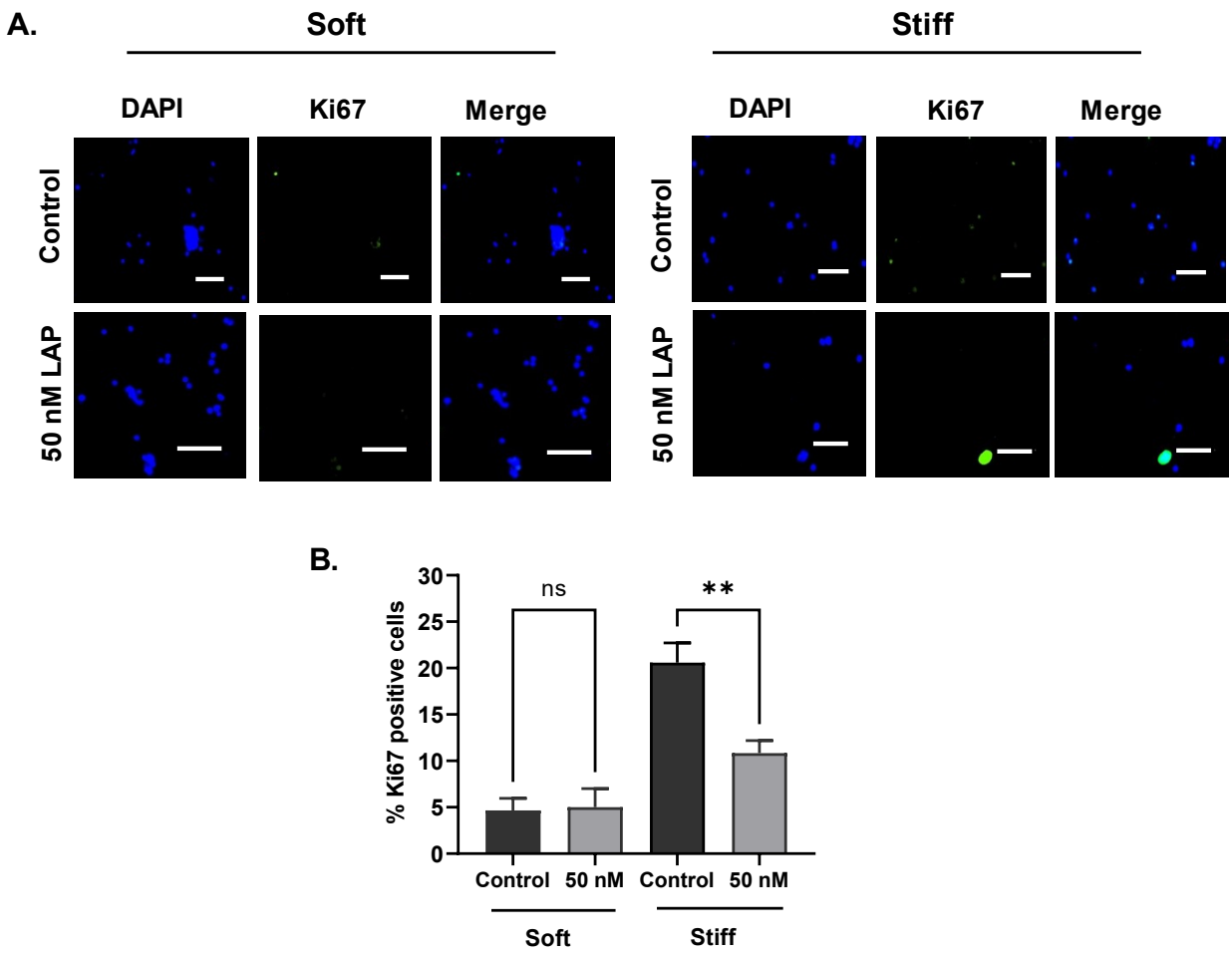

Figure S2. Percentage of Ki67 positive BT474Br3 BMBC cells cultured on soft or stiff HA hydrogels treated with 50 nM LAP. (A) Fluorescence microscopic staining (blue: DAPI and green: Ki67) images of Ki67 positive BT474Br3 cells cultured on soft or stiff HA hydrogels after 48 h without (control) or with 50 nM LAP treatment. (B) Quantification of percentage Ki67 positive BT474Br3 cells. Scale bar = 100 μm. N ≥ 6 hydrogels per condition. ** indicates $p < 0.01$ when compared to control. Data presented as mean ± standard error.

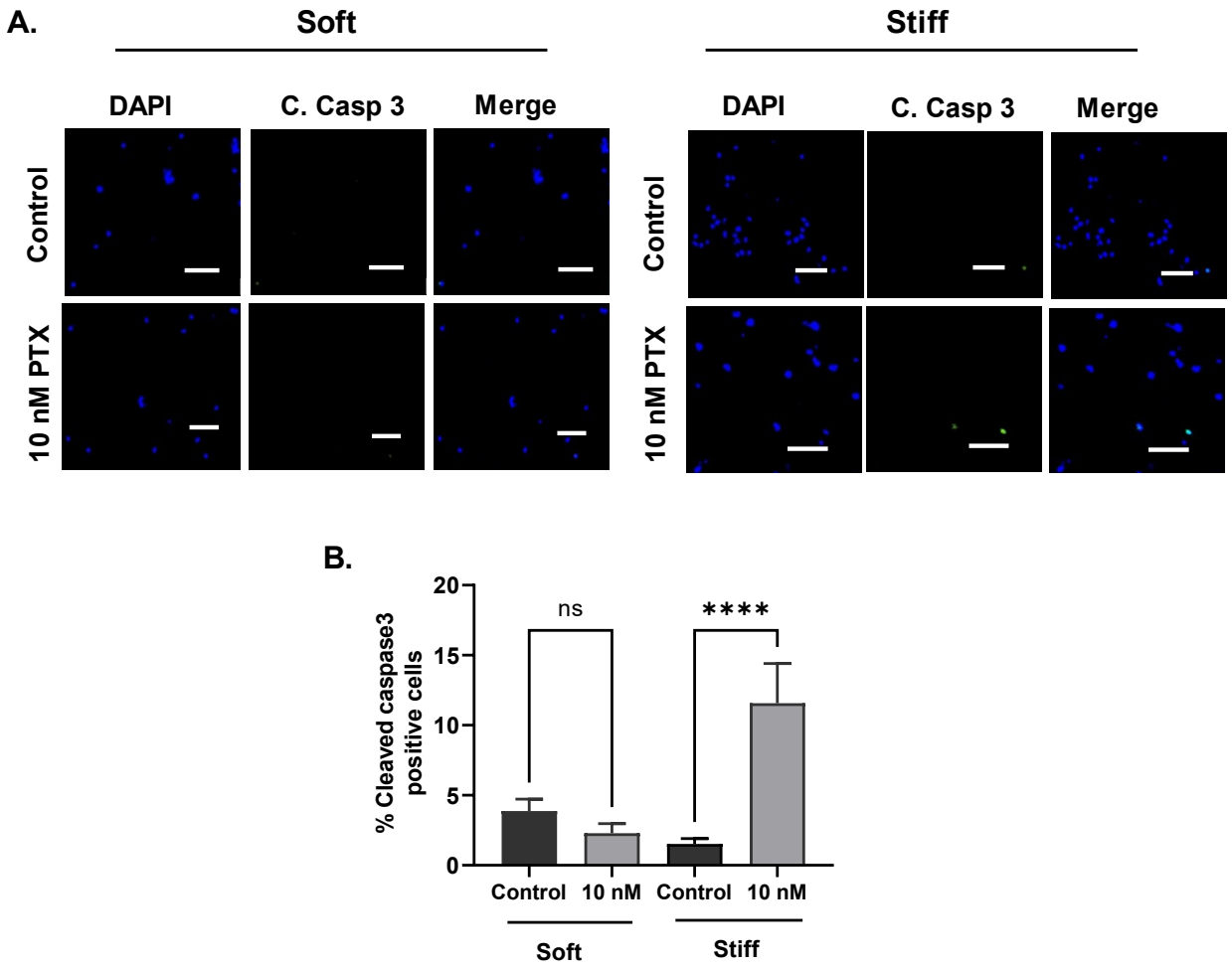

Figure S3. Percentage of Cleaved caspase 3 positive MDA-MB-231Br BMBC cells cultured on soft or stiff HA hydrogels treated with 10 nM PTX. (A) Fluorescence microscopic staining (blue: DAPI and green: Cleaved caspase 3) images of Cleaved caspase 3 positive MDA-MB-231Br cells cultured on soft or stiff HA hydrogels after 48 h without (control) or with 10 nM PTX treatment (B) Quantification of percentage Cleaved caspase 3 positive MDA-MB-231Br cells. Scale bar = 100 μm. N ≥ 6 hydrogels per condition. **** indicates $p < 0.0001$ when compared to control. Data presented as mean ± standard error.

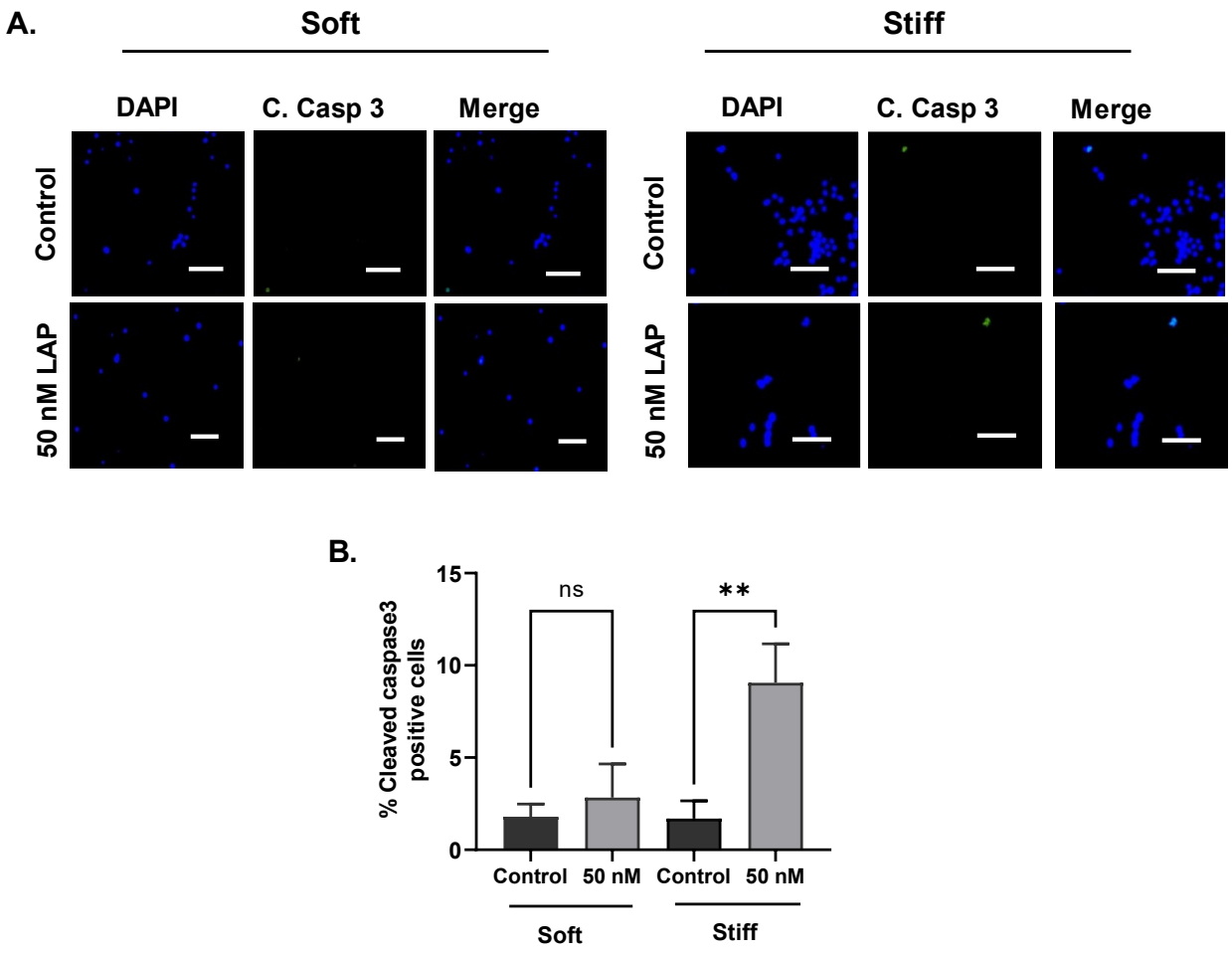

Figure S4. Percentage of Cleaved caspase 3 positive BT474Br3 BMBC cells cultured on soft or stiff HA hydrogels treated with 50 nM LAP. (A) Fluorescence microscopic staining (blue: DAPI and green: Cleaved caspase 3) images of Cleaved caspase 3 positive BT474Br3 cells cultured on soft or stiff HA hydrogels after 48 h without (control) or with 50 nM LAP treatment. (B) Quantification of percentage Cleaved caspase 3 positive BT474Br3 cells. Scale bar = 100 μm. N ≥ 6 hydrogels per condition. ** indicates $p < 0.01$ when compared to control. Data presented as mean ± standard error.

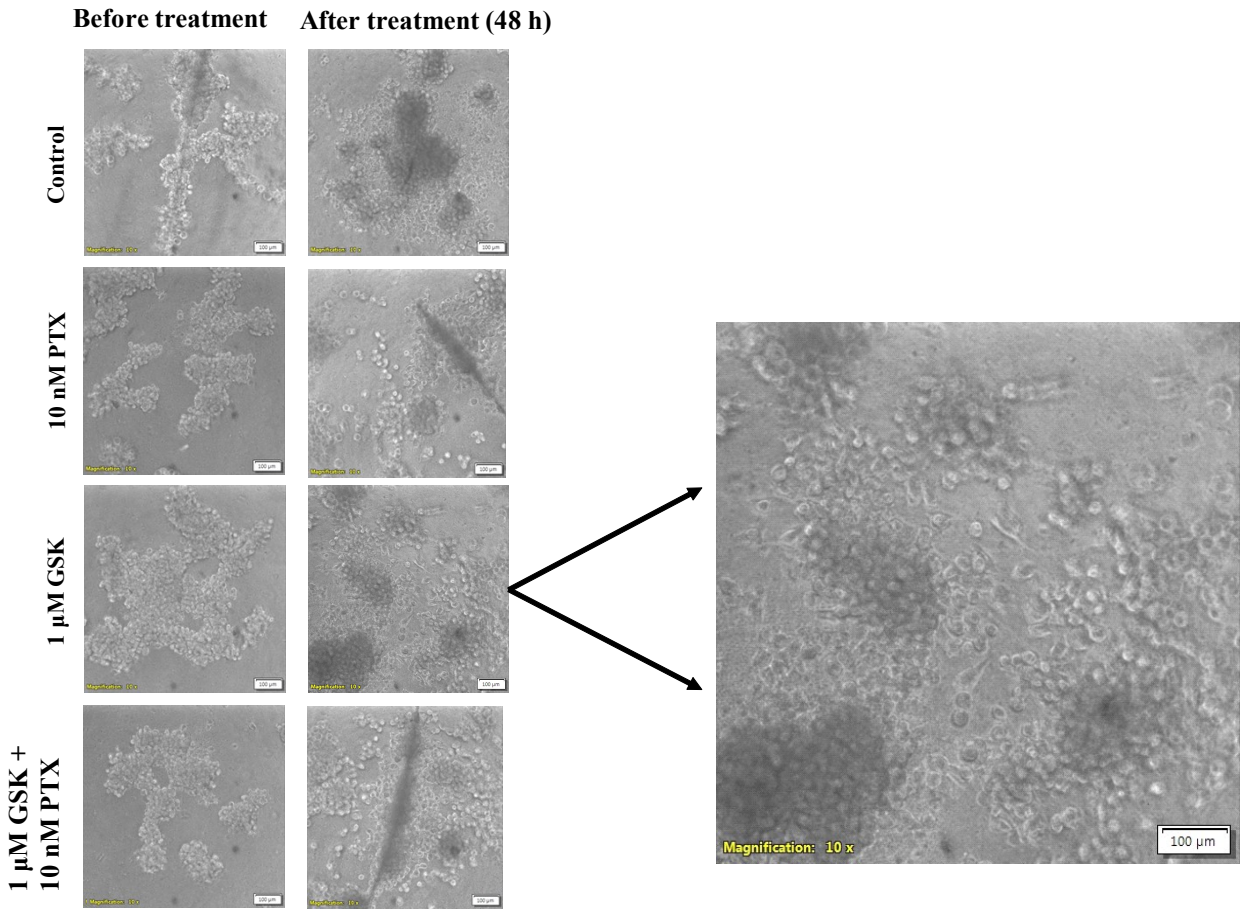

Figure S5. Morphology of MDA-MB-231Br BMBC cells cultured on soft HA hydrogel before (on day 2) and after treatment (day 4) in control (untreated), 10 nM PTX, 1 µM GSK, and 10 nM PTX + 1 µM GSK conditions.

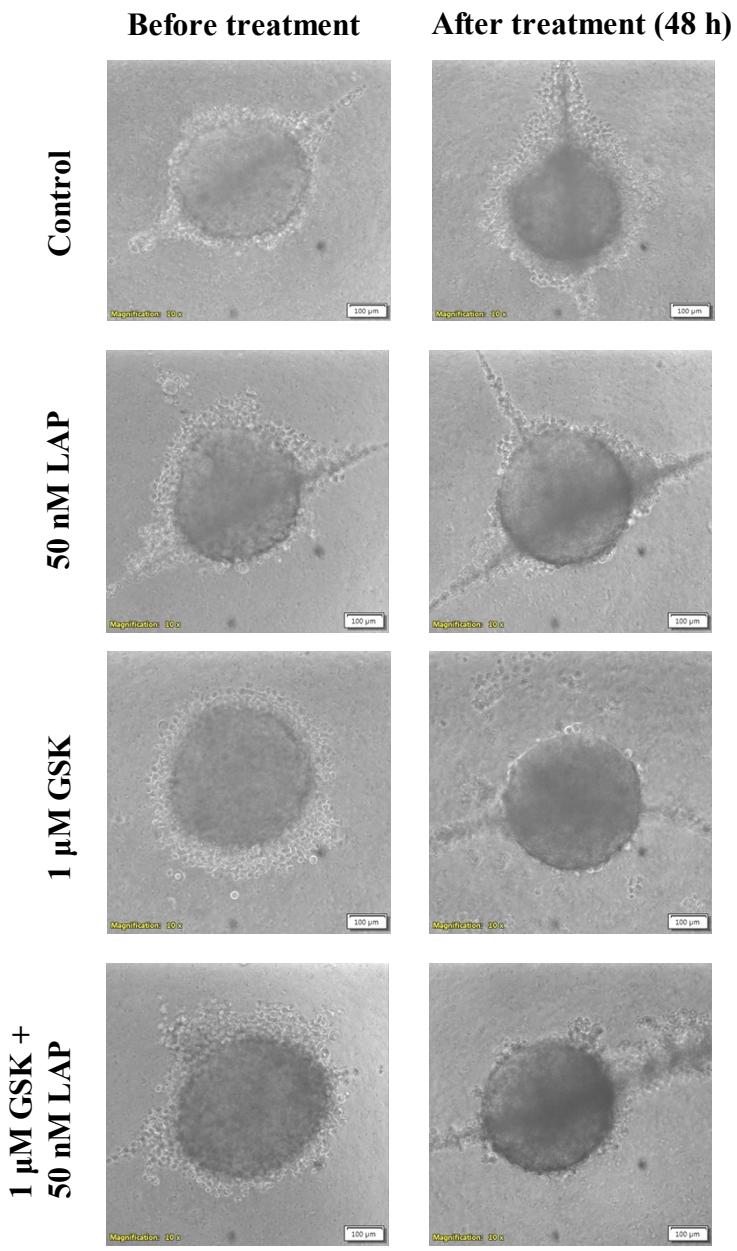

Figure S6. Morphology of BT474Br3 BMBC cells cultured on soft HA hydrogel before (on day 2) and after treatment (day 4) in control (untreated), 50 nM LAP, 1 µM GSK and 50 nM LAP + 1 µM GSK conditions.

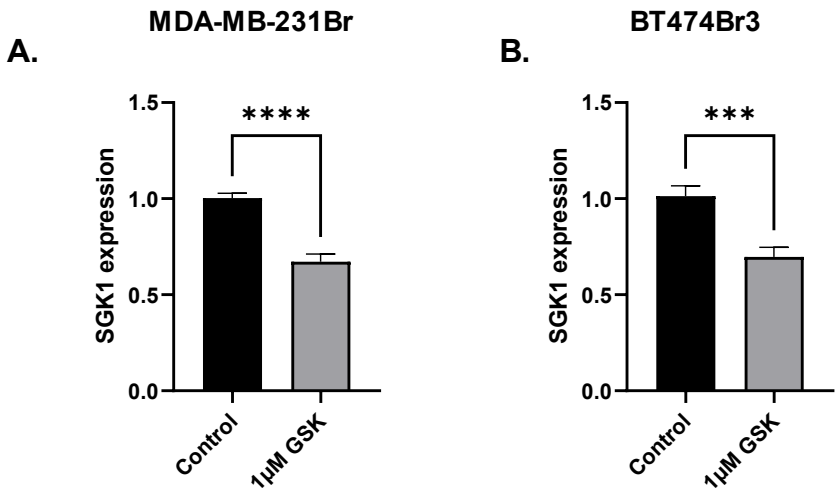

Figure S7. SGK1 gene expression in BMBC cells cultured on soft HA hydrogels with or without treatment. (A) SGK1 gene expression in MDA-MB-231Br cells cultured on soft HA hydrogels after 48 h without (control) or with 1 μM GSK treatment. (B) SGK1 gene expression in BT474Br3 cells cultured on soft HA hydrogels after 48 h without (control) or with 1 μM GSK treatment. The relative expression was normalized with control (of soft hydrogel). $N \geq 6$ hydrogels per condition. ***indicates $p < 0.001$ and **** indicates $p < 0.0001$. Data presented as mean ± std error.

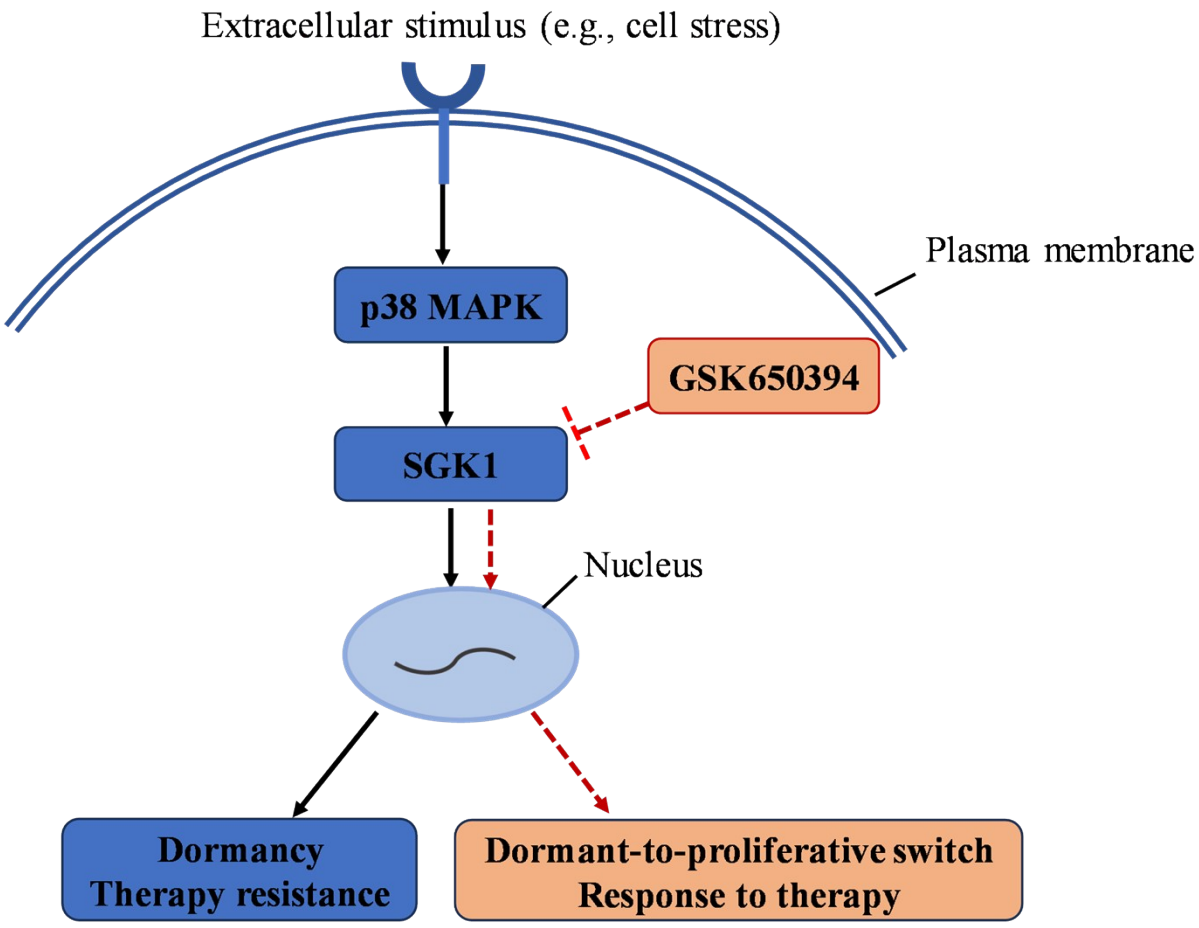

Figure S8. Schematic representation of p38 MAPK mediated SGK1 signaling pathway in dormancy associated chemoresistance. Inhibition of SGK1 using SGK inhibitor (GSK650394) resulted in a dormant-to-proliferative switch and response to therapy.