Supporting Information

Controlled three-dimensional tumor microenvironments recapitulate phenotypic features and differential drug response in early vs. advanced stage breast cancer

Manjulata Singh,†,‡ Harini Venkata Krishnan,†,‡ Supraja Ranganathan,‡ Brian Kiesel,‡,§ Jan Hendrik Beumer,‡,§,⊥ Sreeja Sreekumar,∥ and Shilpa Sant*,‡,§,#,△

‡Department of Pharmaceutical Sciences, School of Pharmacy, #Department of Bioengineering, and △McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, United States
§University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania 15232, United States
⊥Division of Hematology-Oncology, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213, United States
∥Department of Pharmacology and Chemical Biology, Women’s Cancer Research Center, Magee-Women’s Research Institute, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania 15232, United States

†Contributed equally to the work.

* Corresponding author information:

Shilpa Sant, PhD
Assistant Professor
School of Pharmacy
Department of Pharmaceutical Sciences Department of Bioengineering
McGowan Institute for Regenerative Medicine University of Pittsburgh
3501 Terrace Street
808A Salk Hall
Pittsburgh, PA 15261
Phone: 614-648 9804; Email: shs149@pitt.edu

This supplementary information contains two figures S1 and S2 in a total of three pages.
Supplementary Figure S1. Controlling microtumor size regulates non-cellular components in the solid tumor microenvironment. (A) T47D cells seeded on the photo-crosslinked arrays of hydrogel microwells (150 and 600 µm in diameter) form uniform size 3D...
microtumors inside each well (top panel) and can be harvested on day 6 (bottom panel), scale bar: 500 µm. (B) Ruthenium-tris(4,7-diphenyl-1,10-phenanthroline) dichloride (Ru-dpp) staining revealed presence of hypoxic core (red) in large (600 µm) microtumors (right) compared to small (150 µm) ones on day 6 (Hoechst-blue, Ru-dpp-red), scale bar: 100 µm. (C) 2', 7'-dichlorofluorescin diacetate (DCHFDA) staining (green) used to estimate reactive oxygen species (ROS) showed negligible ROS signal in small microtumors in contrast to the strong green signal in core of large ones on day 6, scale bar: 100 µm; (D) Molecular marker of hypoxic microenvironment such as hypoxia inducible factor 1α (HIF-1α) was upregulated in large microtumors compared to 2D and small microtumors.
**Supplementary Figure S2:** Histograms showing population of cells in different cell cycle phases in 2D (day 6) and 150 and 600 µm microtumors harvested on days 1, 3 and 6. Colored arrows above each histogram represent different cell populations in sub G0/G1 (red); G0/G1 (green); S (blue) and G2/M (brown).