Supplementary Materials

Human Plasma-Derived 3D Cultures Model Breast Cancer Treatment Responses and Predict Clinically Effective Drug Treatment Concentrations



**Figure S1.** (**a**) Cell proliferation data of each of the independent BCa cell lines. (**b**) Quantification of confocal microscopy images. Quantified number of MDA-MB-231 cells detected growing within HuP3D cultures after 3 and 7 days and representative confocal images reflecting the selection pane used via binary editor (DiO tagged green cancer cells are highlighted red when selected for counting considerations). (**c**) Quantified number of MDA-MB-231 cell clusters only in scanned z-stack within HuP3D cultures after 3 and 7 days and representative confocal images reflecting the selection pane used via binary editor for counting MDA-MB-231 cell clusters (clusters of DiO tagged green cancer cells are highlighted red when selected for counting considerations), (\*) *p* <0.05.



**Figure S2.** Micrographs of the 5 BCa cell lines and primary cells (single cells and organoids) growing in HuP3D cultures at day 3. Scale Bar = 100 µm.



**Figure S3.** HuP3D high-throughput drug screening. (**a**) BCa cell lines were previously labeled with DiD and incorporated in HuP3D cultures. After matrix stabilization, cells were treated with a DMSO control (γCtrl) and increasing concentrations 0.1 nM–300 µM of seven standard-of-care chemotherapeutic drugs. Media was refreshed at day 4, and HuP3D cultures were enzymatically digested to be analyzed using flow cytometry at day 0 (γ0) and day 7. Data was analyzed utilizing the GR calculator to determine 10 drug response metrics. (**b**) Cytotoxic and cytostatic effects of HuP3D drug treatments depicting the HuP3D culture drug metrics from the relative cell count curve including the half maximum inhibitory concentration (IC50), maximal measured efficacy (Emax), area under the curve (AUC), half maximal response concentration (EC50), and the effect of the drug concentration at infinite concentration (Einf), as well as from the GR curve including the half maximum growth rate inhibition concentration (GR50), the maximum effect of the drug at the highest tested concentration (GRmax), area over the curve (GRAOC), the half maximal growth rate inhibition response concentration (GEC50), and the effect of the drug at infinite concentration (Einf).

Western Blot



