

Electronic Supplementary Information

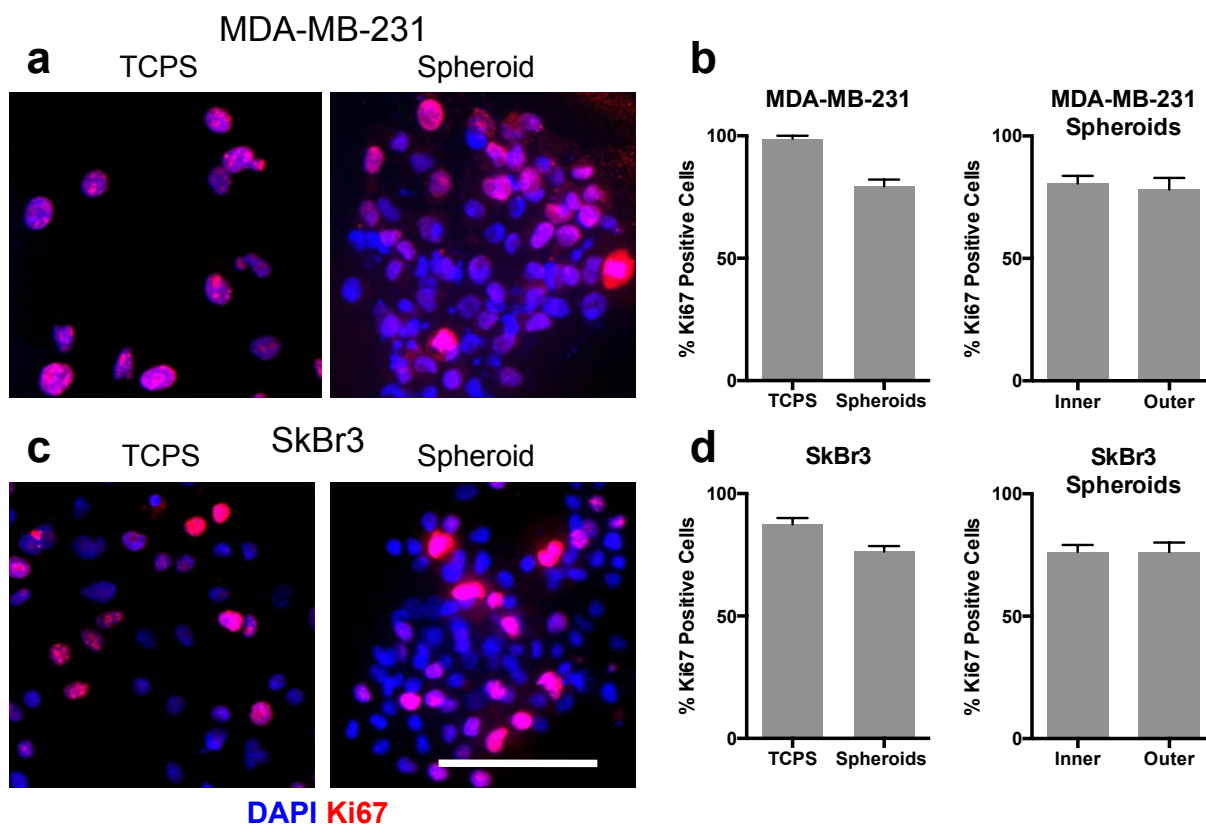


Figure S1. Cells are proliferating throughout spheroids. a. MDA-MB-231 Ki67 staining of cells on TCPS or spheroids encapsulated in 3 kPa 3D gels for one day. b. Quantification of percentage of cells Ki67-positive on TCPS and as spheroids (left), and based upon location in spheroid (right). c. SkBr3 Ki67 staining and d. quantification. Scale bar: 100 μ m.

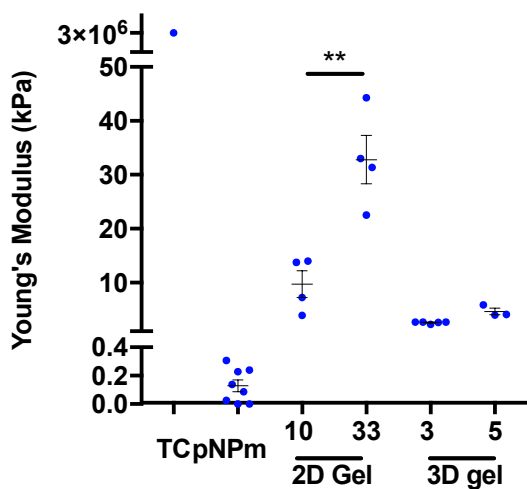


Figure S2. Mechanical properties of biomaterials used. Young's modulus was measured using indentation on pNIPAAm-PEG (pNPm) at 37°C and rheology on 3D and 2D gels (the number indicates the average stiffness of each biomaterial, and N is between 3-5 samples per

condition). These are compared to the Young's Modulus of tissue culture polystyrene (TCPS) (Yang et al., 2014).

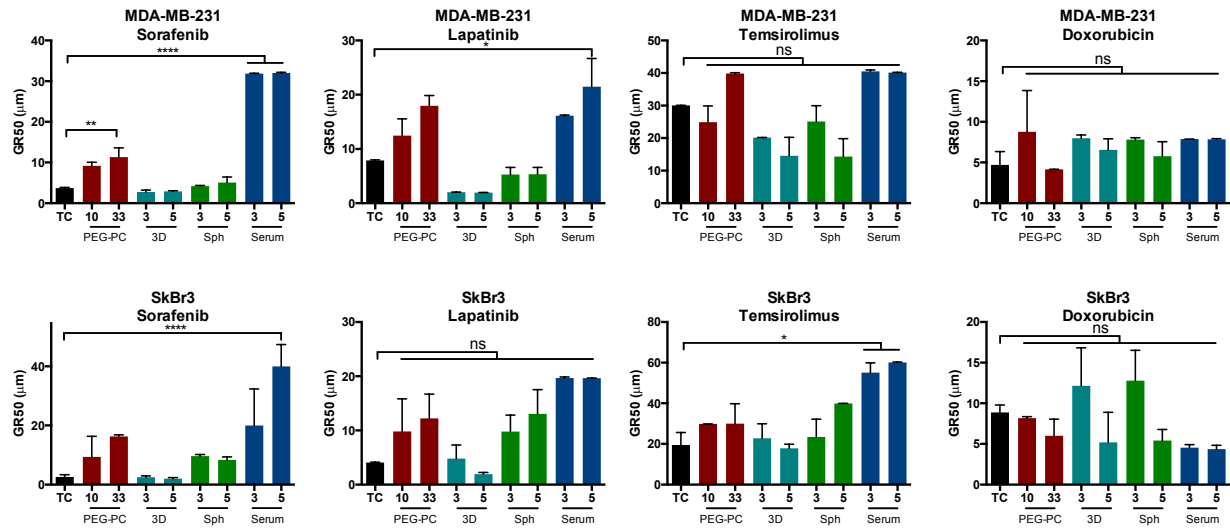


Figure S3. GR₅₀ for MDA-MB-231 and SkBr3 across all microenvironment conditions. For clarity, only statistical comparisons to TCPS are shown.

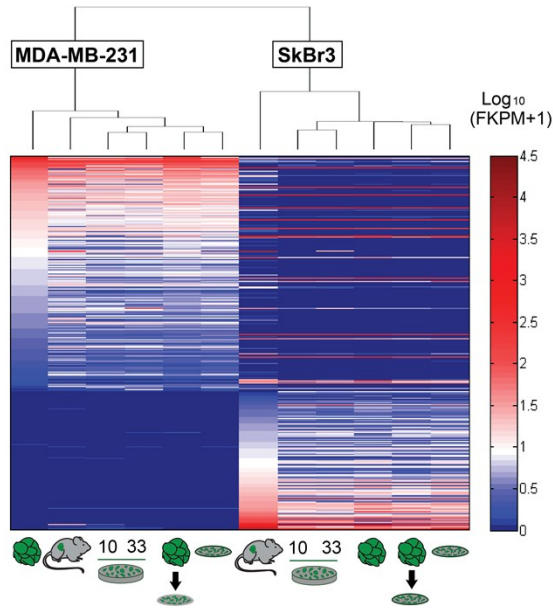


Figure S4. Gene expression clustering separates cell lines and microenvironment conditions. Significantly differentially expressed genes first distinguish between MDA-MB-231 and SkBr3 cells, then by biomaterial platform.

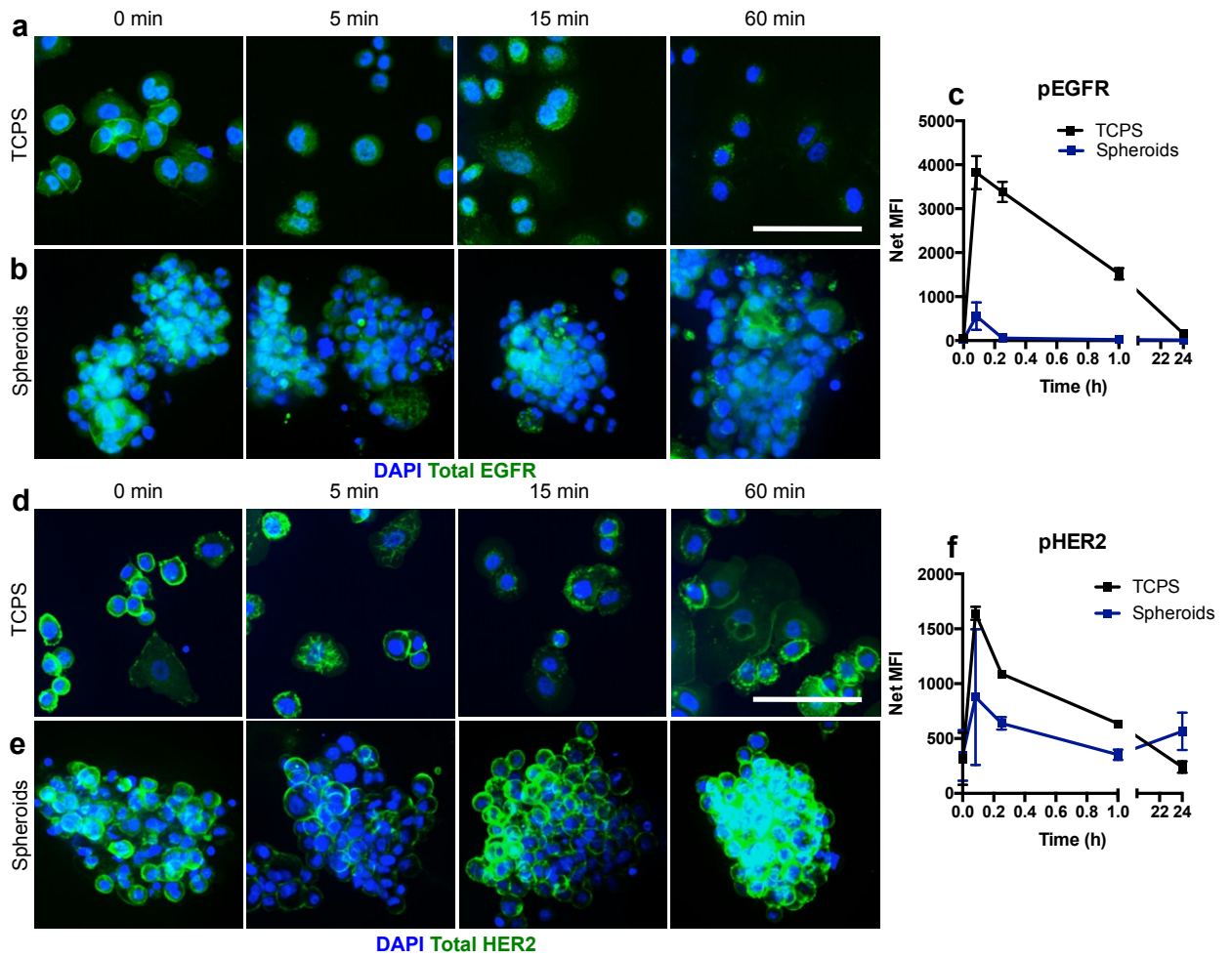


Figure S5. SkBr3 spheroids have suppressed response to EGF stimulation. a-b. EGFR localization, and c. pEGFR in response to 100 ng/ml EGF stimulation at 0, 5, 15, and 60 minutes post-stimulation. d-e. HER2 localization, and f. pHER2 in response to EGF stimulation at 0, 5, 15, and 60 minutes post-stimulation. Scale bars: 100 μ m.

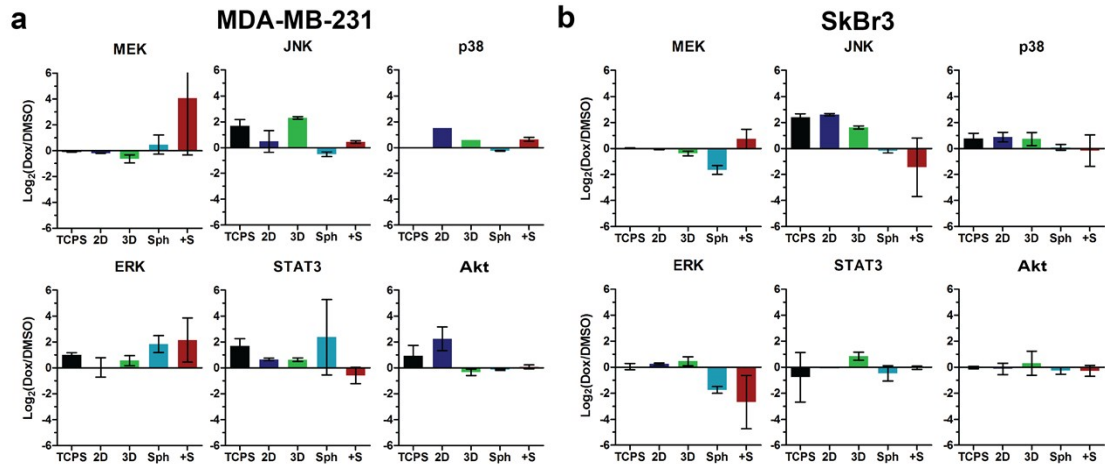


Figure S6. Doxorubicin treated cells show limited change in phosphorylation of key signaling analytes. a-b. The \log_2 fold change of cells treated with doxorubicin (Dox) compared to the vehicle control (DMSO) across 5 screening environments; TCPS (black), 2D gel (dark blue), single cells in 3D (green), spheroids in a 3D gel (light blue) and 3D gel spheroids with serum (red) in MDA-MB-231 cells (a) and SkBr3 cells (b).

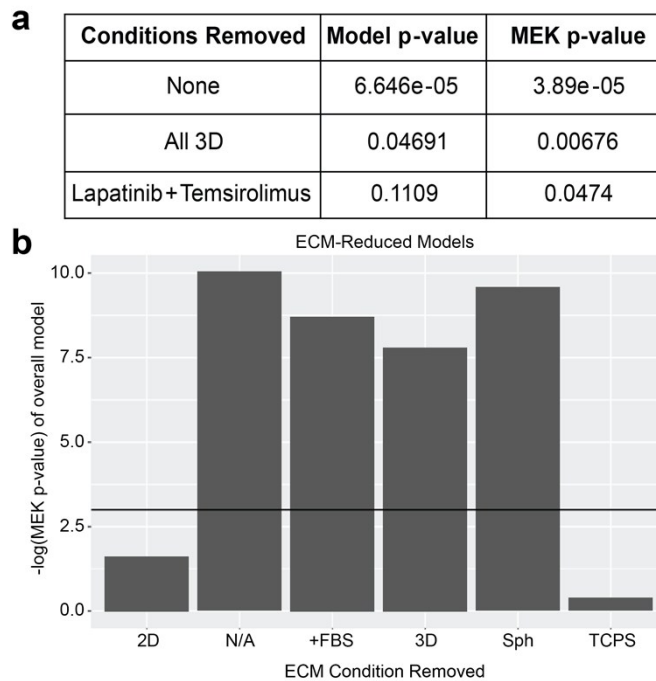


Figure S7. Multiple biomaterials are required for success of the multiple linear regression. a. Exclusion of either all 3D conditions or lapatinib and temsirolimus increase the p-value of the overall model and MEK in MDA-MB-231 cells. b. The impact of exclusion of each individual biomaterial (x-axis) on the p-value of the overall model (y-axis: 2D or 3D indicated the geometry of the hydrogel, N/A is the complete model with no conditions removed, Sph is spheroids, +FBS is spheroids in serum, and TCPS is tissue culture polystyrene).

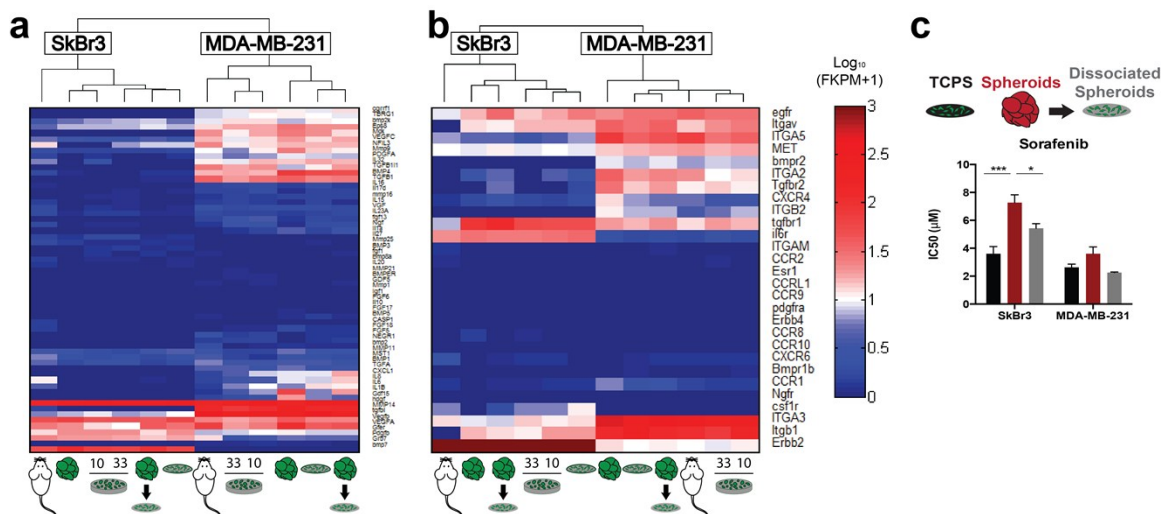


Figure S8. Gene expression of growth factors and receptors reveals possible reversible autocrine signaling in drug resistance across biomaterials. a. Differentially expressed soluble factors across in vitro and in vivo culture conditions. b. Expression of receptors and resulting sample clustering. c. Sorafenib IC₅₀ for cells treated on TCPS, treated as spheroids, or grown as spheroids, dissociated, and re-seeded onto TCPS.

Table S1, Related to Figure 1. Two-way ANOVA analysis of drug resistance across biomaterial microenvironments. Percent of total variation (%). * denotes significance by two-way ANOVA.

	SkBr3 (%)		MDA-MB-231 (%)					
	Culture Format		Only 3D Spheroids		Culture Format		Only 3D Spheroids	
	Geometry	Modulus	Medium	Modulus	Geometry	Modulus	Medium	Modulus
Proliferation	35 *	10 *	57 *	3	49 *	0.8	59 *	28 *
Doxorubicin	8	2	49	0.01	1	11	27	37
Temsirolimus	80 *	3	87 *	0.3	11	22	22	10
Sorafenib	51 *	3	81	2	91 *	2	96 *	2 *
Lapatinib	35	10	28	0.08	76 *	14 *	86 *	9 *