**Cancer Cell Invasion of Mammary Organoids with Basal-In Phenotype**

*Supplementary figures*

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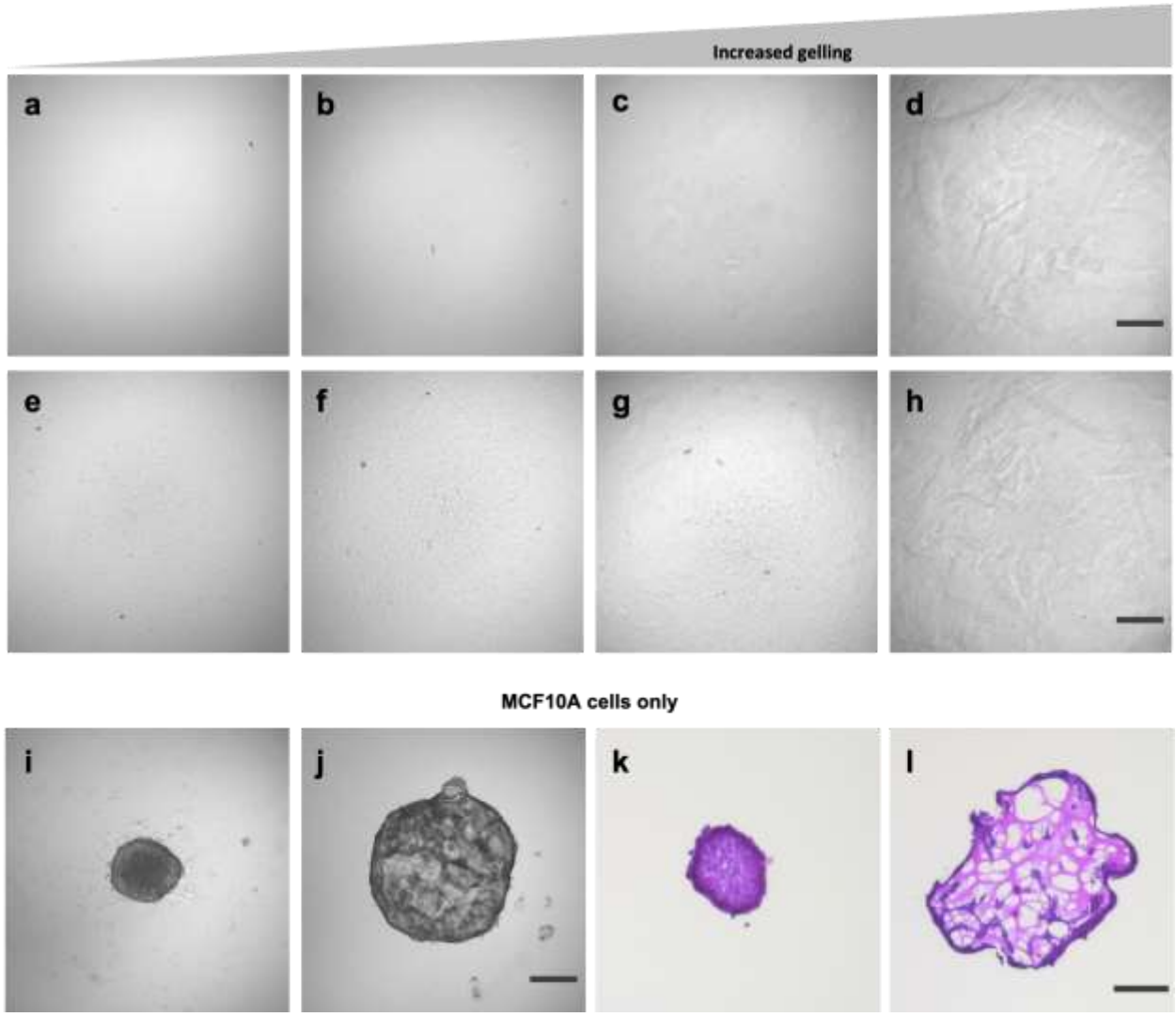
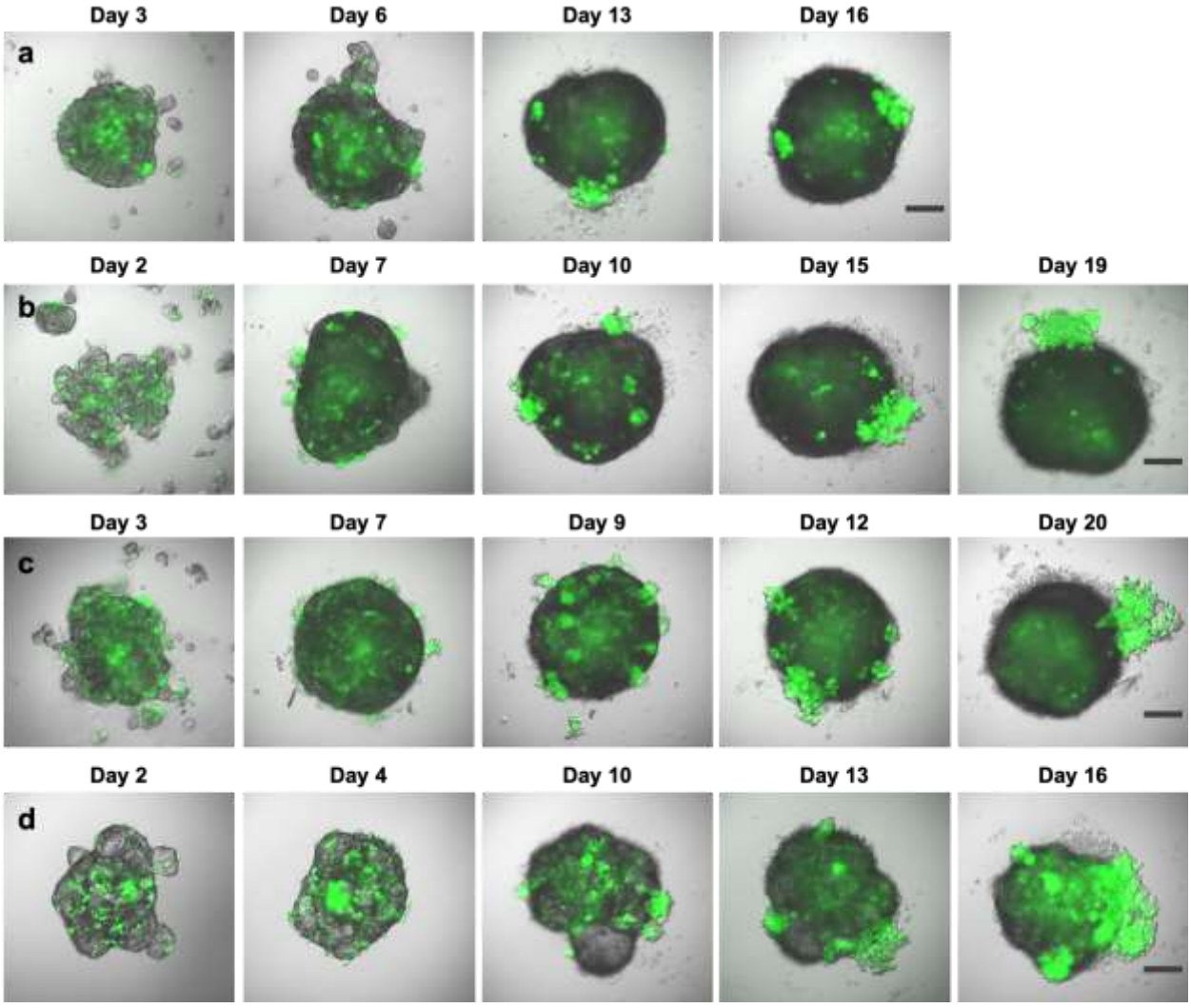
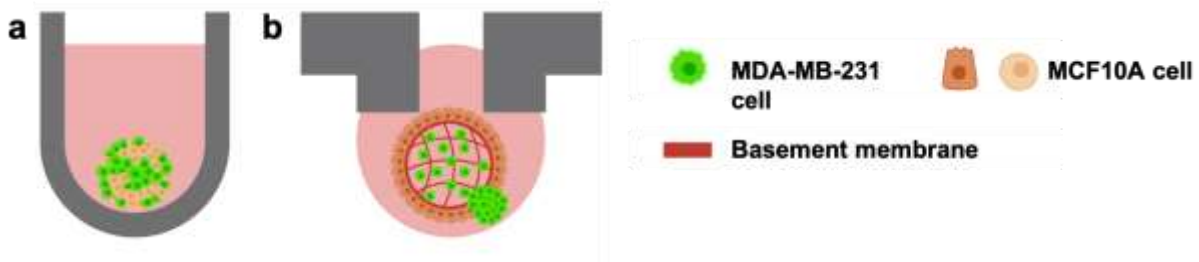


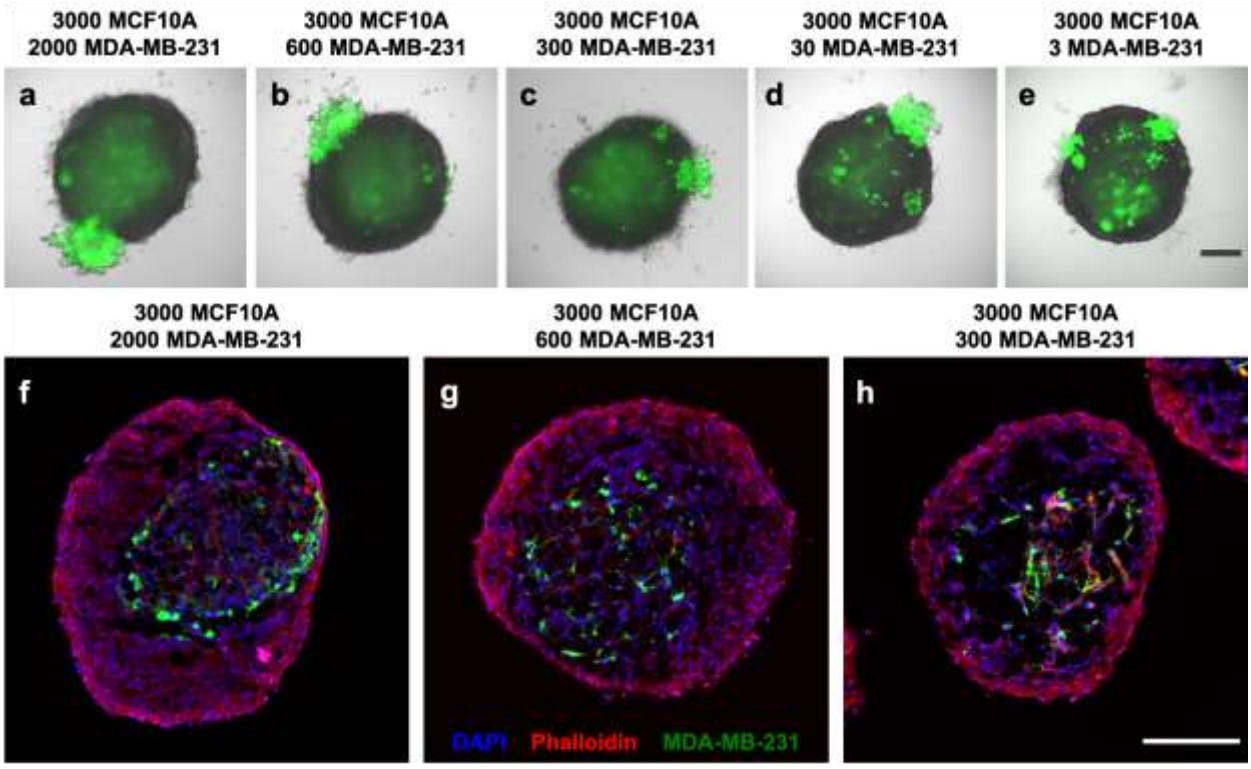

**Supplementary Figure 1. Sensitivity of Matrigel gelling to temperature and presence of fetal bovine serum.** Images of acellular hanging drop media shortly after seeding that is cold without FBS (a), cold with 10% FBS (b), warm without FBS (c), and warm with 10% FBS (d). Cold without FBS (e), cold with 10% FBS (f), warm without FBS (g), and warm with 10% FBS (h) after 72 hours at 37 °C and 5% $CO_2$. Brightfield images of 3 day organoid formation with FBS and without (i) and with (j) Matrigel. H&E images without (k) and with (l) Matrigel. All scale bars represent 200 μm.



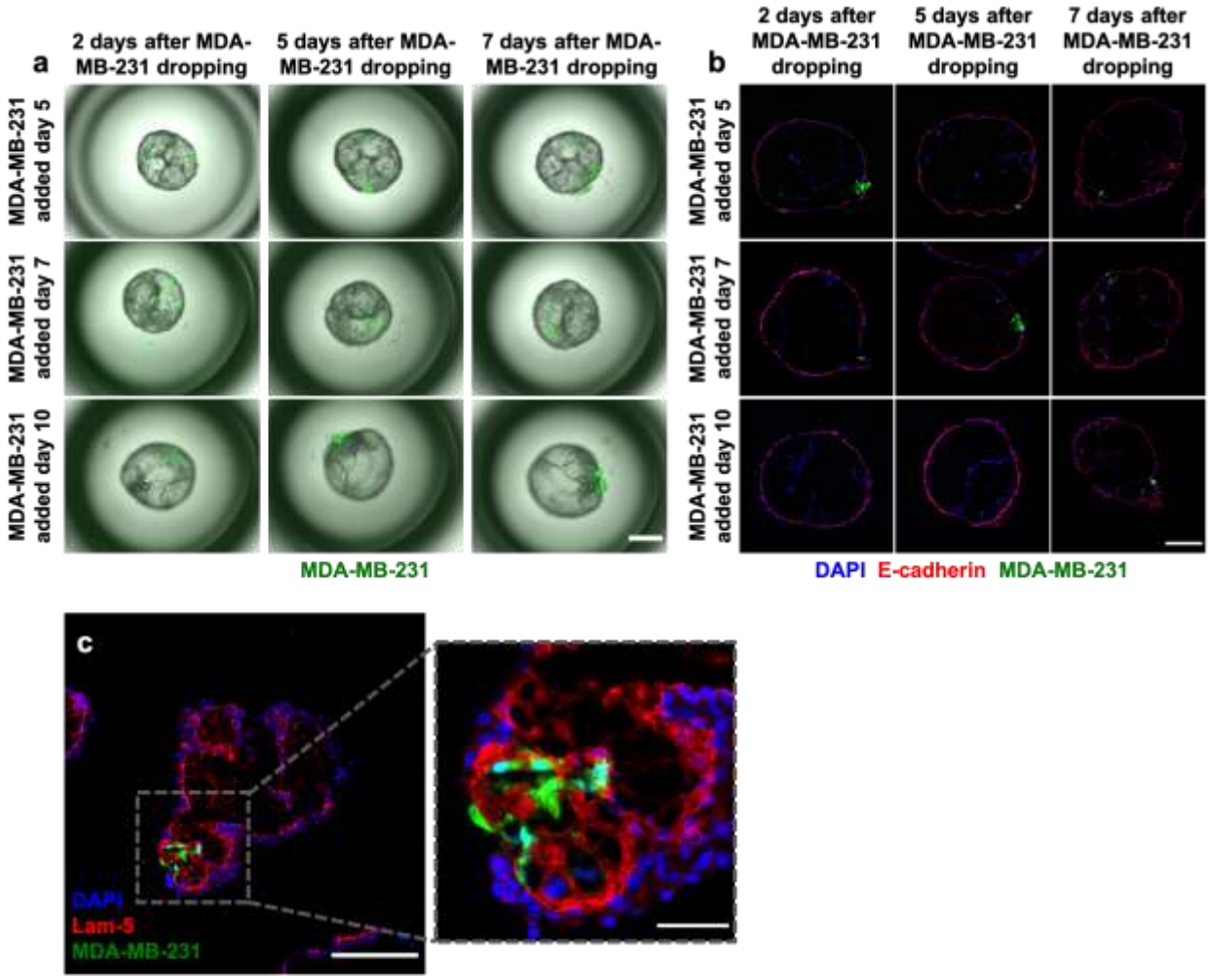

**Supplementary Figure 2. MCF10A and MDA-MB-231 co-culture over time.** (a-d) The tracking of four co-culture organoids (3000 MCF10A cells and 300 MDA-MB-231 cells) from four independent experiments shows a robust and reproducible self-organizing pattern that can be maintained for 20+ days. MDA-MB-231 cells are shown in green. Scale bars represent 200 μm.



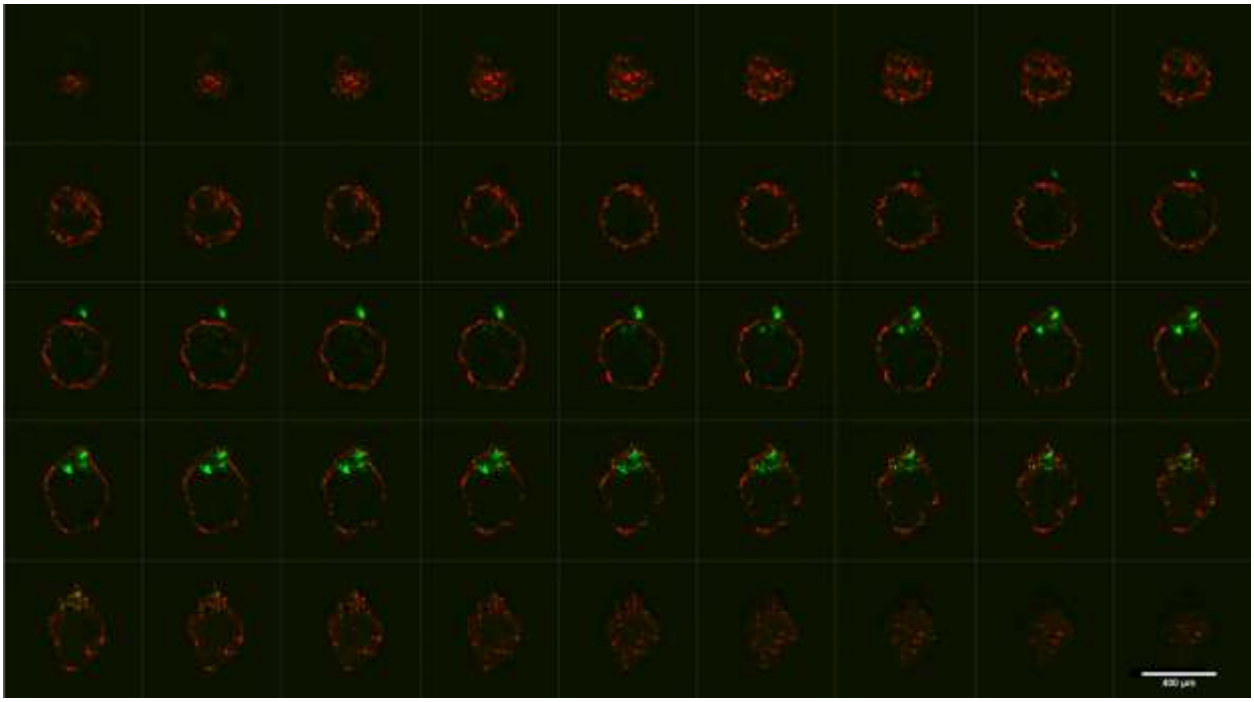

**Supplementary Figure 3. Comparison of inverted mammary organoids to existing co-culture models.** Schematic of co-culture comparison between the multicellular spheroid (MCS) model presented by Carey *et al.* in 2013 (a) and the work presented here (b).




**Supplementary Figure 4. MDA-MB-231 cell number does not significantly alter organoid 3D organization.** Adding 2000 (a), 600 (b), 300 (c), 30 (d), or 3 (e) MDA-MB-231 cells to 3000 MCF10A cells resulted in a very similar organization after 16 days in 3D co-culture. Top scale bar represents 200 μm. Cryosectioning and phalloidin staining of 10 μm sections confirmed a similar presence of MDA-MB-231 cells in the organoid core for the conditions of 2000 (f), 600 (g), and 300 (h) MDA-MB-231 cells. Bottom scale bar represents 200 μm.




**Supplementary Figure 5. Invasion of MDA-MB-231 cells into MCF10A organoids.** Brightfield images of whole organoids (a) and E-cadherin immunostaining of 10 μm sections (b) when MDA-MB-231 cells were introduced at days 5, 7, and 10 of MCF10A organoid culture and imaged/fixed 2, 5, and 7 days after cancer cell introduction. Scale bar in (a) represents 500 μm and scale bar in (b) represents 200 μm. (c) Laminin-5 staining after MDA-MB-231 invasion. Scale bar represents 200 μm. Inset shows region where cancer cells invaded. Scale bar represents 50 μm.



**Supplementary Figure 6.** Confocal stack of representative MCF10A organoid that experienced MDA-MB-231 cancer cell invasion inside of the organoid core. MDA-MB-231 cells were added on day 7 of the MCF10A organoid cultures and they were then maintained for an addition 9 days. The z-planes have 4.4 micron spacing. MCF10A cells express RFP and MDA-MB-231 cells express GFP.