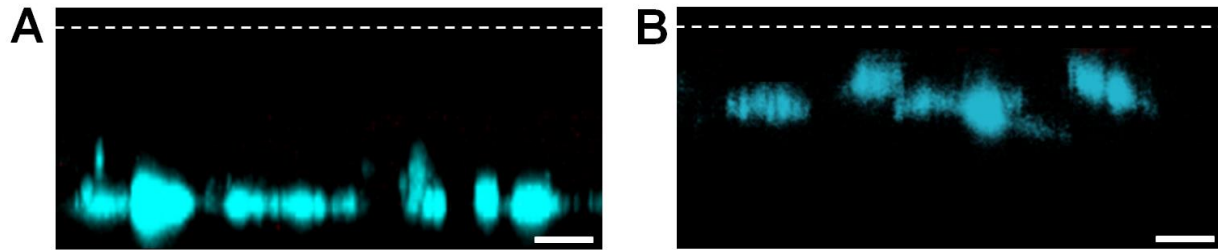
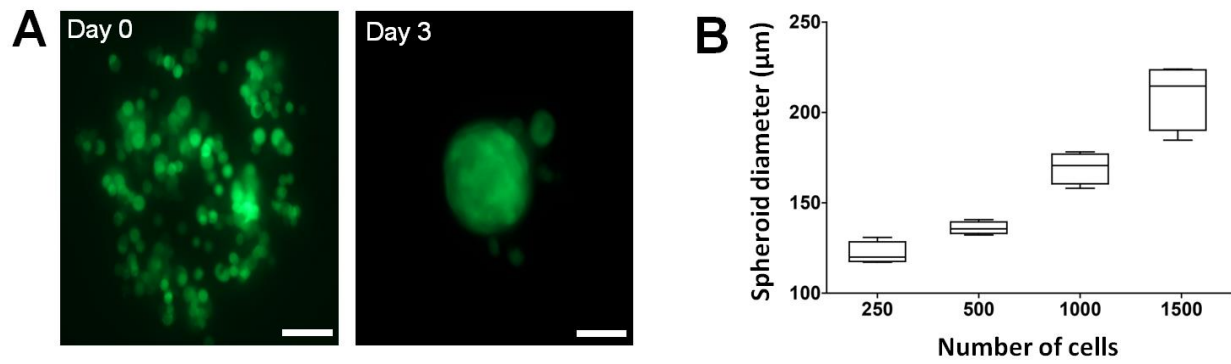


**Supplementary Figure 1. Design and fabrication of breast DCIS-on-a-chip.** **A.** The cross-sectional size of the cell culture chambers in the upper and lower layers is 1 mm (width: W) X 200  $\mu$ m (height: H), and the microchannels connecting the chambers to the access ports are 200  $\mu$ m (W) X 200  $\mu$ m (H). **B.** To prepare the ECM membrane, a drop of collagen solution is placed on the lower channel layer and incubated at 37°C overnight for 1 hour to induce gelation. Vitrification is achieved by drying the collagen gel at room temperature for 2 days, and this process leads to the formation of a thin vitrified collagen membrane attached to the lower cell culture chamber. Subsequently, the lower PDMS slab is briefly treated with plasma generated by a hand-held corona treater (Electro-Technic Products) and brought in conformal contact with the plasma-treated upper PDMS slab. Permanent bonding between the layers is accomplished by 3-hour incubation at 65°C.



**Supplementary Figure 2. Effect of BSA coating.** **A.** Without BSA coating, the contracting stromal layer detaches from the membrane and shrinks to the bottom of the lower chamber, as illustrated by the fibroblasts (cyan) detected at the channel floor. **B.** In BSA-coated channels, the fibroblast-laden collagen layer remains attached to the membrane surface. Scale bars: 100  $\mu\text{m}$ . The dotted lines show the location of the vitrified collagen membrane.



**Supplementary Figure 3. Optimization of the size of DCIS spheroids.** **A.** Hanging drop culture of GFP-DCIS.com cells. Scattered cells in the hanging drop aggregate over time and become a single spheroid after 3 days. Scale bar: 100  $\mu\text{m}$ . **B.** The effect of the number of seeded cells on the diameter of resulting DCIS spheroids at Day 3. Data represent mean  $\pm$  std.