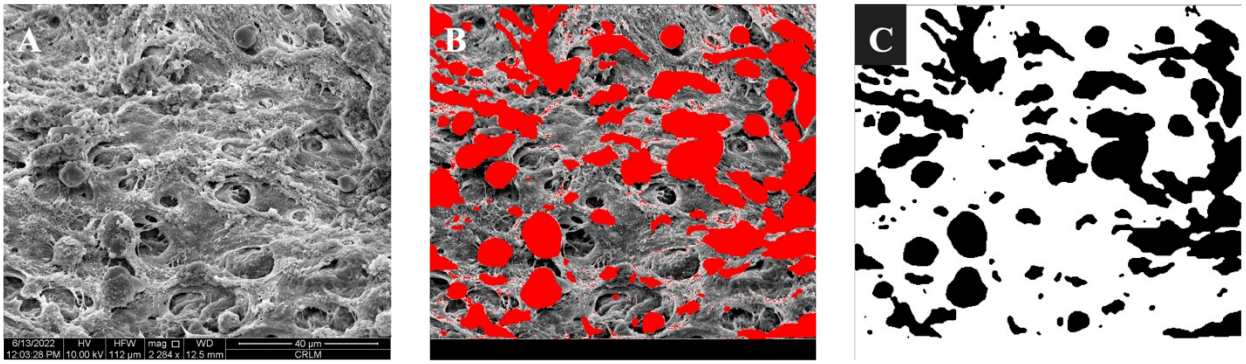
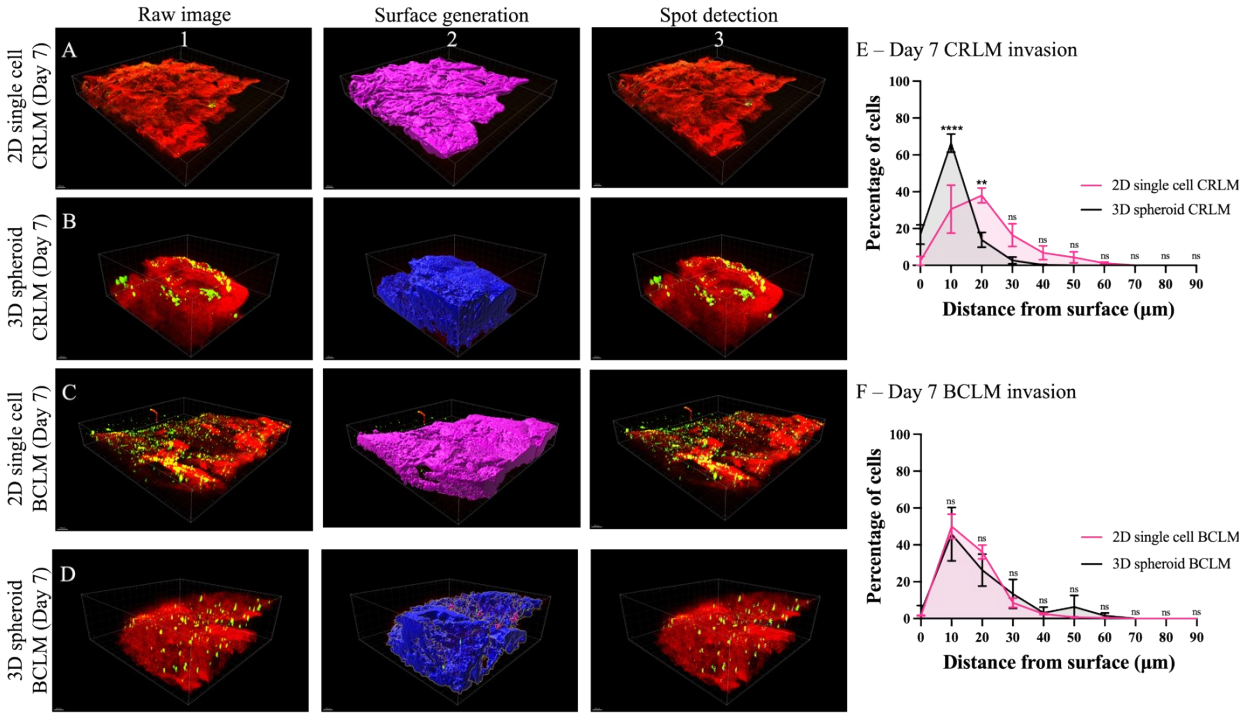
Supplementary Figure 1. Masking technique for quantifying cell area occupancy in scanning electron micrographs. Scanning electron micrographs were analyzed with ImageJ software in order to quantify the cell area occupancy of colonized biomatrices under various metastatic nest seeding conditions. **A)** Shown is a representative image of CRLM established by HCT116 spheroids cultured in the liver biomatrix for 3 days. **B)** Cell area occupancy was quantified by manually tracing areas of the image containing cellular material using the "Freehand" tool.and thresholding to display these occupied areas in red. **C)** After thresholding, the images were converted to binary masks with reduced noise and area fraction was measured with the software.



Supplementary Figure 2. Stepwise quantification of biomatrix invasion. Multiphoton z-stack images were stitched together to form 3D renderings of the recellularized liver and lung biomatrix scaffolds. SHG signal from ECM collagen is shown in red and GFP-tagged cancer cells in green. The quantification process is shown for a representative image of each of 4 conditions: **A)** 2D single cell CRLM on day 7; **B)** 3D spheroid CRLM on day 7; **C)** 2D single cell BCLM on day 7; and **D)** 3D spheroid BCLM on day 7. In step 1, the multiphoton z-stack is stitched to form the 3D structure. Step 2 involves creating a surface (shown in pink or blue) from the collagen matrix with the smoothness parameter set to 4 µm. In step 3, cells are identified with the "Spot" feature set to an estimated 6 µm radius and their invaded distance relative to the surface calculated. Quantification of invasion of HCT116 and MDA-MB-231 single cells and spheroids into the liver and lung biomatrices is shown here to compare seeding conditions after 7 days of culture. **E)** CRLM established with single cells invaded further into the liver biomatrix than spheroid-seeded cells (compare pink trace to black trace). Single cells were significantly more invaded (**$p<0.001$, one-way ANOVA) at Day 7 between 15-25µm. **F)** Single cell-seeded BCLM was similarly invasive following 7 days of culture to spheroids in the lung biomatrix, but no statistical significance was noted.



Supplementary Figure 3. Effect of MMP-9 Inhibitor on CRC proliferation. To test whether reduced invasion initiated by a small molecule inhibitor of MMP-9 presented in Figure 8A could be attributed to MMP activity rather than changes in proliferation (colonization), proliferation was measured with MTS assay across a range of doses. **A)** HCT116 spheroids treated with 0-1000 nM MMP-9 inhibitor showed no significant change in proliferation over 7 days of culture across all doses. B) HCT116 spheroids were treated with 1000 nM MMP-9 inhibitor one day prior to seeding on the liver biomatrix to establish CRLM. Upon seeding, they were submerged in media containing the inhibitor, and received supplemented media with the inhibitor on day 3 of culture, as well. There was no significant difference in the proliferation of the treated cells compared to control samples. This indicates that any reduced invasion can be attributed to lower MMP-9 activity initiated by the inhibitor rather than the inhibitor causing changes in cell proliferation.