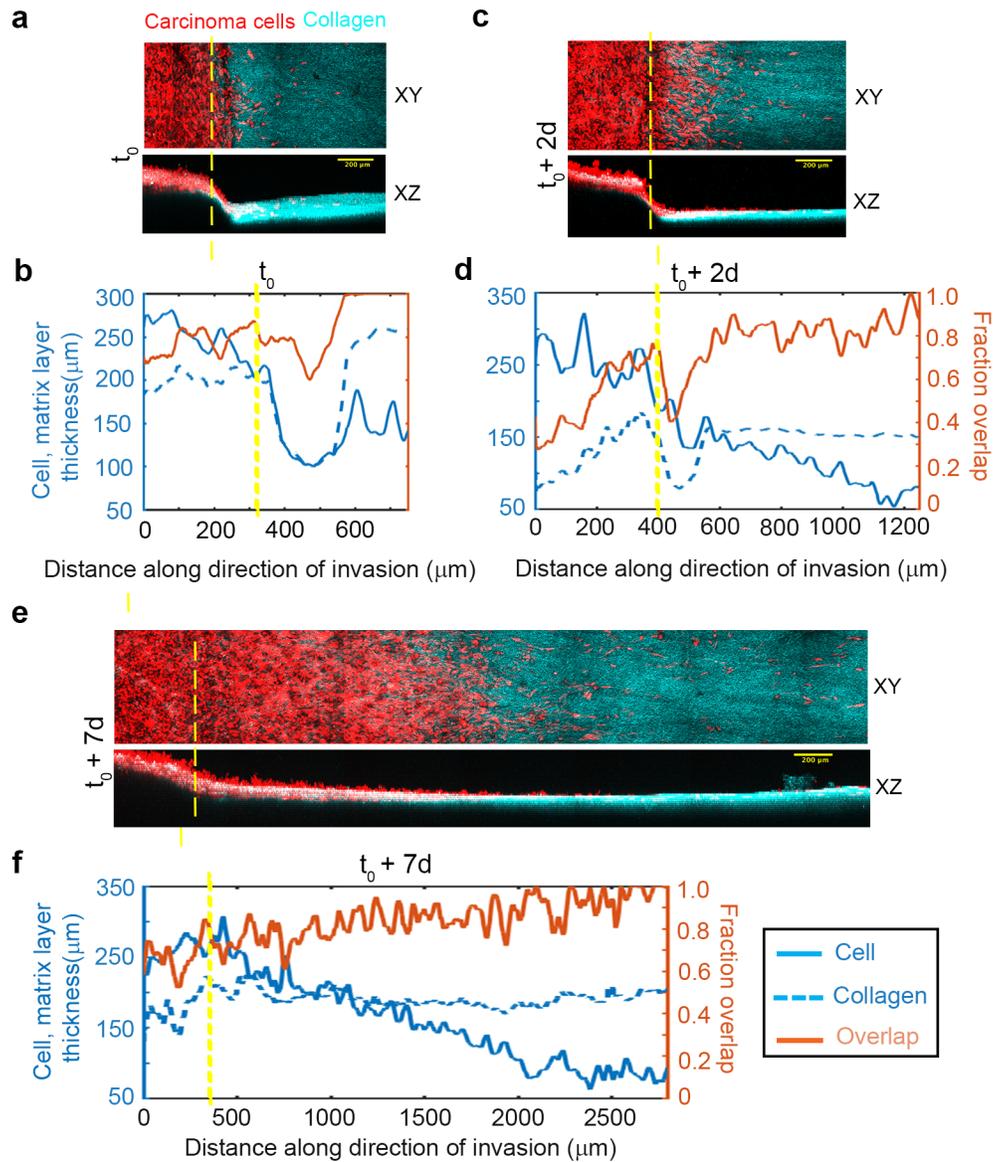
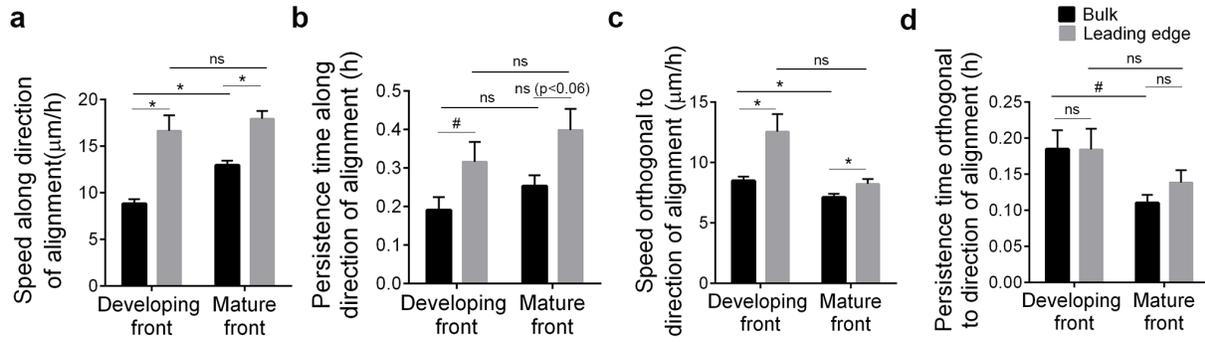


Supplementary Fig.1: Schematic figure showing possible scenarios leading to an optimal strategy to interface a cell-loaded ECM plug to a pre-aligned collagen matrix in 3D.



Supplementary Fig.2: (a, c, e) XY and XZ views of invading carcinoma cells and SHG showing collagen associated with the invasion fronts at various time points analyzed in Figure 2, yellow dotted lines represent the interface between the plug and the pre-aligned matrix. Note that the XY projection images in Fig.2 only represent the invaded region, i.e., the portion to the right of the dotted yellow line. **(b, d, f)** Plots showing image analysis-based estimation of the thickness of the cell and collagen layers along with their fractional overlap as a function of distance along the axis of invasion for the three time points analyzed. Scale bars=200 μm .



Supplementary Fig.3: Comparison of (a) speed and (b) persistence times along the direction of alignment and comparison of (c) speed and (d) persistence time orthogonal to the direction of alignment for MDA-MB-231 cells at the bulk (black bars) and leading edge (grey bars) of developing and mature invasive fronts (n=40-70 cells/group, ns=no significance, *p<0.001, #p<0.05, unpaired Mann Whitney test).