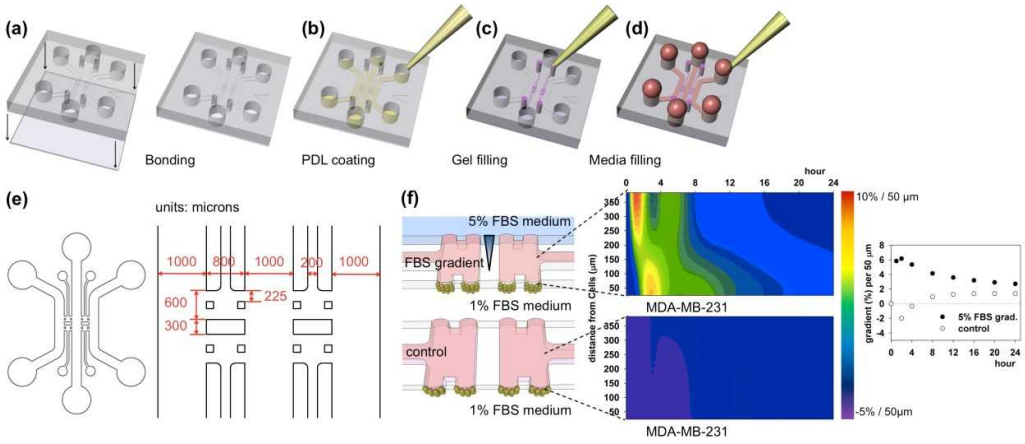
Supporting Information



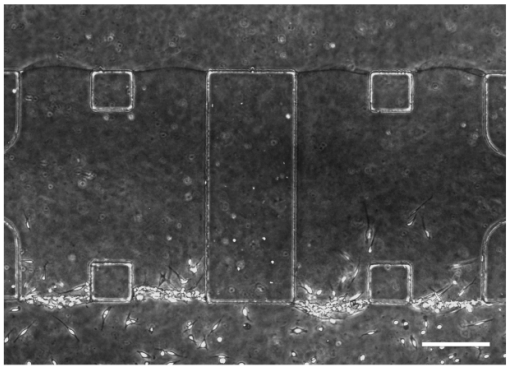
Supporting figure 1. Microfluidic assay preparation process used for Figure 1. PDMS (polydimethyl siloxane) microfluidic device fabricated using a soft lithography process was (a) bonded on the glass coverslip by oxygen plasma treatment. (b) Immediately after the bonding process, microfluidic channels were coated with PDL(poly-D-lysine hydrobromide (1 mg/ml) solution. (c) Collagen type I or matrigel was introduced into the hydrogel scaffold regions and allowed to gel in a 37°C incubator for 30 minutes. (d) After gelation, medium was filled and stored ready for cell seeding. (e) Dimension of the microfluidic channel (in microns). (f) Simulated gradient difference (% per 50 microns; average length of elongated cells) of FBS in hydrogel.

The color indicates the concentration gradient %,

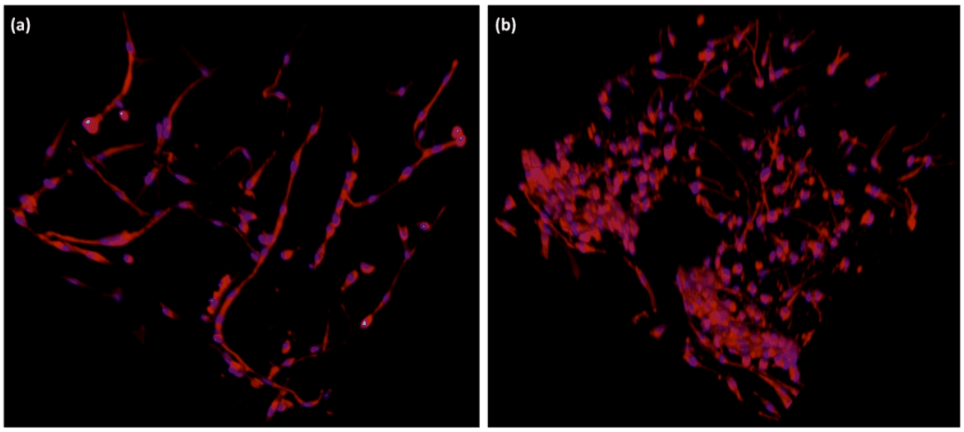
$\frac{C_x - C_{x+50\mu m}}{C_x} \times 100\%$: concentration at position x

$C_{x+50\mu m}$: concentration at position x+50μm in hydrogel

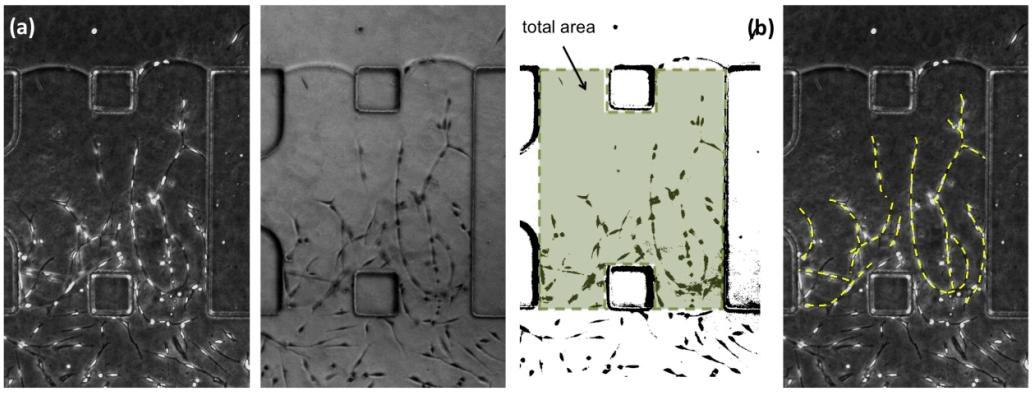
which was maintained at > 3% per 50 microns in the upper hydrogels in 24 hours. In the bottom hydrogels, the concentration difference was maintained at < 2% in 24 hours under control conditions.



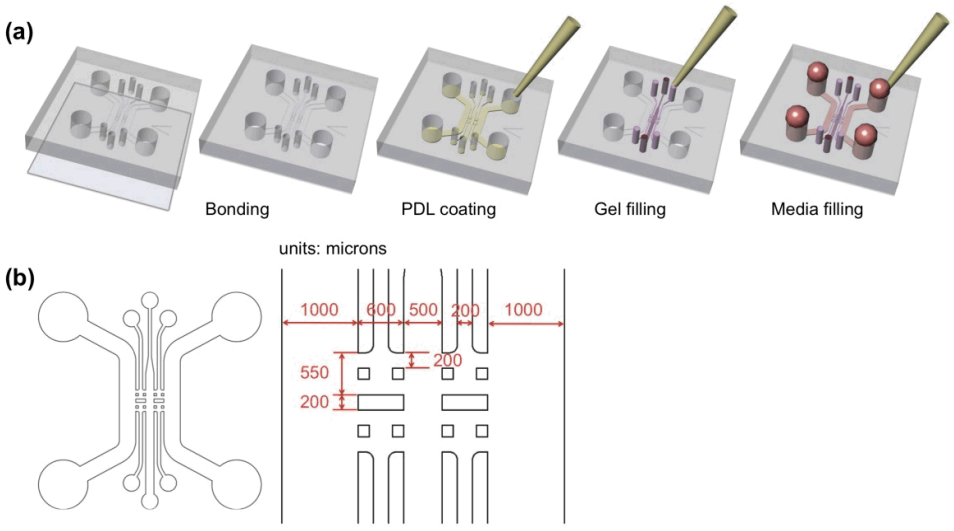
Supporting figure 2. Invasive nature of MDA-MB-231. Cells attached to collagen type 1 scaffold displayed invasion and migration in control medium without Fetal Bovine Serum (FBS). Scale bar: 250 μm.



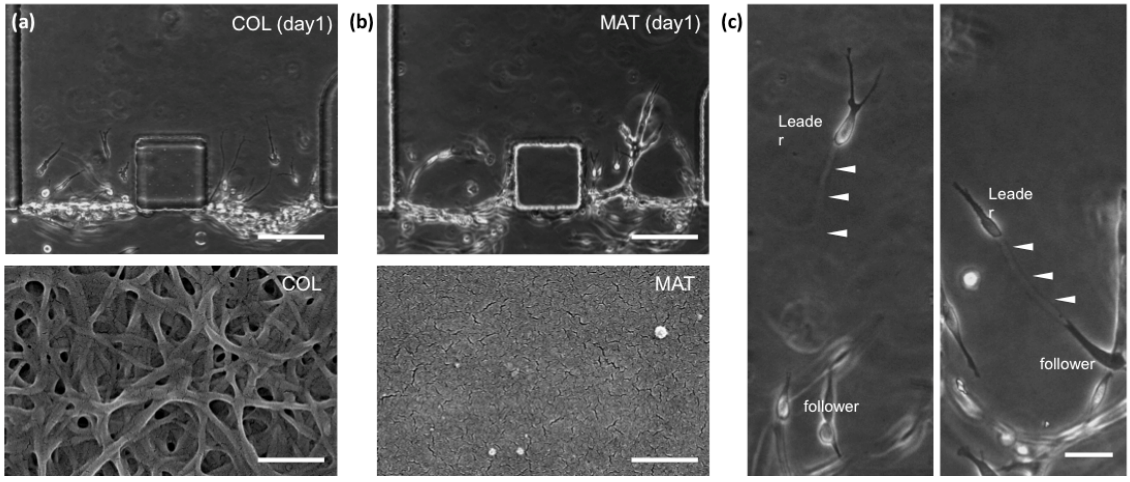
Supporting figure 3. 3D confocal images of MBA-MD-231 invading MAT (a) and COL (b). 3D images were obtained from raw image stacks used in Figure 2(a). Actin filaments and nuclei of cells were stained with rhodamine-phalloidin (red) and DAPI (blue), respectively.



Supporting figure 4. Quantification method. (a) Cell migration was monitored from images obtained daily using phase-contrast microscopy. Area fraction was measured as the percentage of the projected area of invading MDA-MB-231 to the total area (green). (b) Length of connected cancer stalks, estimated as the total pixel of each stalk marked by yellow lines.

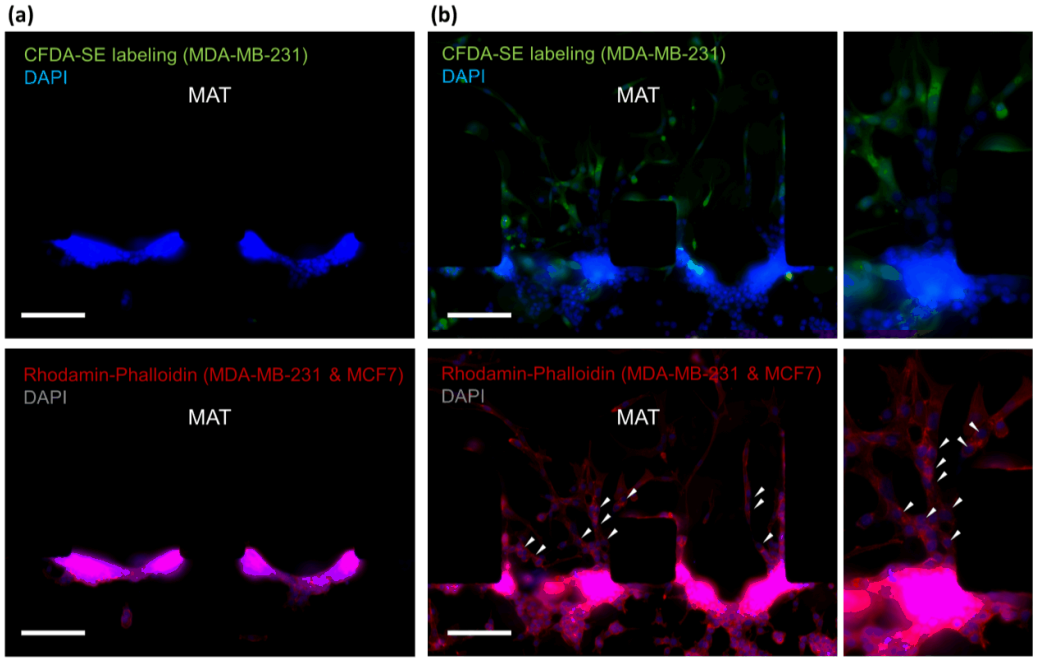


Supporting figure 5. Fabrication and preparation of the microfluidic assay used in Figure 3, composed of connected hydrogel scaffolds. (a) Basic assay preparation processes (similar to those described in supporting information (1). (b) Dimension of the microfluidic assay (unit, microns).

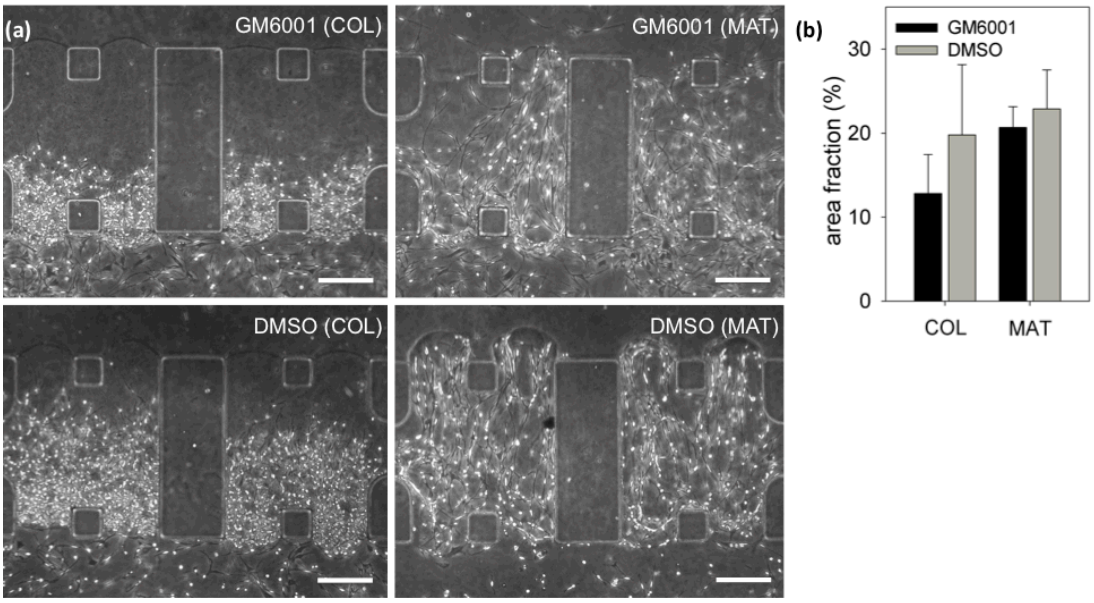


Supporting figure 6. Images of MDA-MB-231 cells invading into (a) COL and (b) MAT and their SEM images. When MDA-MB-231 cells invade into fibrous COL, each cell has a chance to contact with collagen fibers and pores. Then, each cancer cell individually migrates into the COL by adopting mesenchymal movement with cell-matrix focal adhesion [S1, S2]. On the other hands, MDA-MB-231 cells need proteolytic activity to invade into MAT that has no fibers and gaps. Among cancer cells, cell expressing higher progeolytic activity than others migrates into Matrigel as a leader by generating microtrack [S1, S2]. Remiaining cancer cells prefer to migrate into Matrigel through the pre-existing gaps (microtracks) rather than to migration proteolytically, resulting in multicellular migration mode [S3]. (c) Degradation of MAT by MDA-MB-231 leaders and consequent microtracks (white arrowheads) for followers to migrate through. Scale bar: 50 μm.

[S1] P. Friedl, Current Opinion in Cell Biology 2004, 16, 14.
[S2] P. Friedl, S. Alexander, Cell 2011, 147, 992.
[S3] Pavlo G. Gritsenko, Journal of Pathology 2012, 255, 185.



Supporting figure 7. Multicellular invasion of non-invasive MCF-7 following highly invasive MDA-MB-231 cells (green). In the single culture model (a), MCF-7 cells (1x106 cells/ml) were seeded and cultured on MAT. In the co-culture model (b), MCF-7 cells (1x106 cells/ml) were seeded on MAT, and after 3 hours, MDA-MB-231 cells (1x106 cells/ml) were added. Images were obtained following 3 days of culture. (a) Single culture model with MCF-7 cells. Non-invasive MCF-7 did not invade MAT, even under the FBS gradient. (b) Co-culture model with MCF-7 and MDA-MB-231. MDA-MB-231 cells overtook MCF-7 and actively invaded MAT. Non-invasive MCF-7 also invaded MAT (white arrowheads) by following the traction generated by invading MDA-MB-231 cells. MDA-MB-231 (green) and MCF-7 cells were stained with rhodamine-phalloidin (red) for actin filament, DAPI (blue) for nuclei, and CFDA-SE for MDA-MB-231 (green), respectively. MDA-MB-2131 cells were specifically treated with CFDA-SE before seeding into the device. Scale bar: 150 μm.



Supporting figure 8. Effects of GM6001, a MMP inhibitor, on cancer cell invasion into MAT and COL. MDA-MB-231 cells were treated with GM6001 (25 μM in DMSO) or DMSO as a control. (a) Invasion of GM6001-treated cells was suppressed in COL but not in MAT. (b) Quantification graph of the average area fraction of invading GM6001-treaded MDA-MB-231 cells, compared to control (average area fraction of DMSO-treated MDA-MB-231) in COL & MAT; n=4 for all experimental cases. Scale bar: 250 μm.

The limited suppression in COL was due to the morphological change (from mesenchymal to amoeboid) of MDA-MB-231 by GM6001 treatment[S4].GM6001-treated cells could invade into pores of COL by amoeboid migration showing reduced amount of invasion. Interestingly, GM6001-treated MDA-MB-231 showed no reduction in invasion potential in MAT. It can be explained by the experimental results in Supporting figure 7; GM6001-treated cells (non invasive) invaded following the tracks made by small amount of survivors (less treated cells or proliferated cells), but still requires further investigations.

[S4] P. Friedl, K.Wolf, Nature Reviews Cancer 2003, 3, 362.