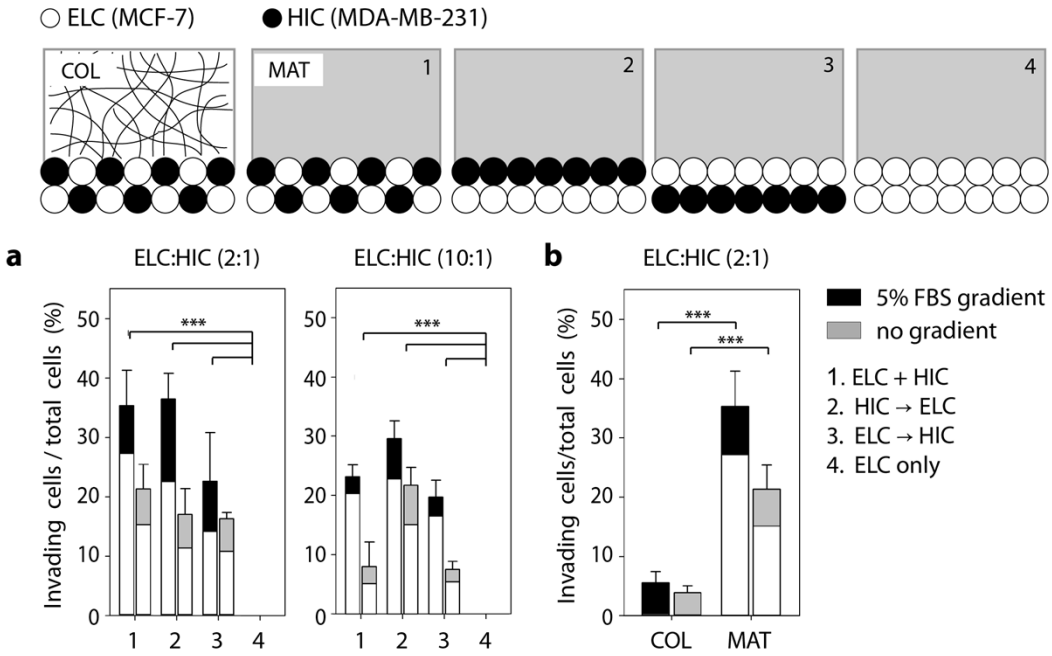
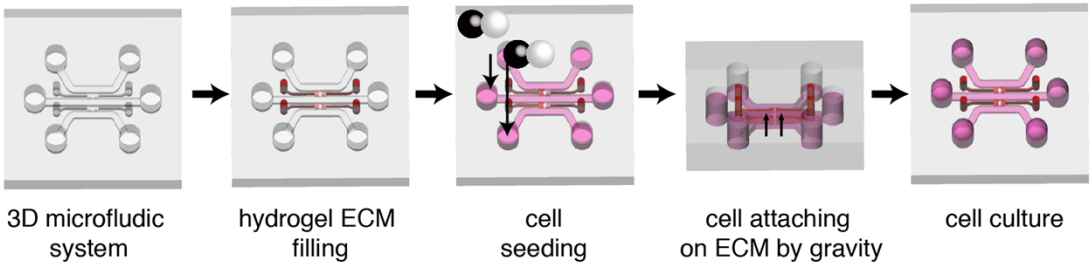
Supplementary information



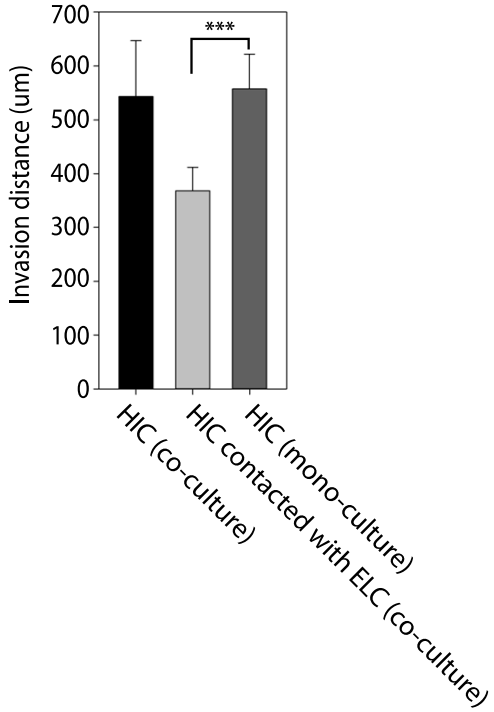
Supplementary Figure 1

(a) Quantification of total cell invasion into MAT in a different seeding order (#1~#4) at a seeding ratio of 2:1 and 10:1 (ELC:HIC) in the absence or presence of FBS gradient. (b) Quantification of total cell invasion into COL and MAT in the presence or absence of HICs under 5% FBS gradient or no gradient. Black, gray and white represent the proportion of HIC (black under 5% FBS gradient, gray under no gradient) and ELC (white) in total invading cells, respectively. All data are expressed as mean ± SD. (***$p < 0.001$)



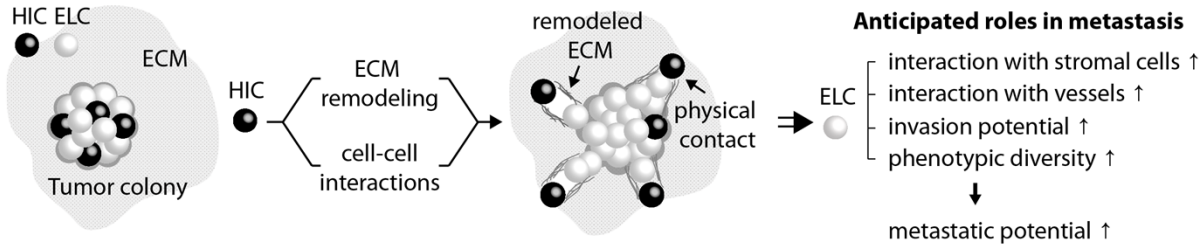
Supplementary Figure 2.

Preparation process for the *in vitro* intratumor heterogeneity model. Collagen type 1 and Matrigel were filled in hydrogel regions as ECM in the PDMS (polydimethylsiloxane)-based 3D microfluidic system. After gelling, cancer cells were seeded into cell culture channels and attached on hydrogel ECM via tiling the device.



Supplementary Figure 3.

Quantification of invasion distance of the five fastest HICs; 1) among all HICs (co-culture), 2) contacting with ELC (co-culture), and HICs only (mono-culture).



Supplementary Figure 4.

An anticipated role of intratumor heterogeneity as an encourager of cancer invasion. HICs would promote the metastatic potential of ELCs by improving the opportunity to interact with stromal cells and vessels, inducing invasiveness and generating phenotypic diversity via combined activities of ECM remodeling and cell-cell interactions.