

In Vitro Lung Cancer Local Invasion Modeling Using Patient-Derived Tumor Organoids and Hydrogel Pocket Chip

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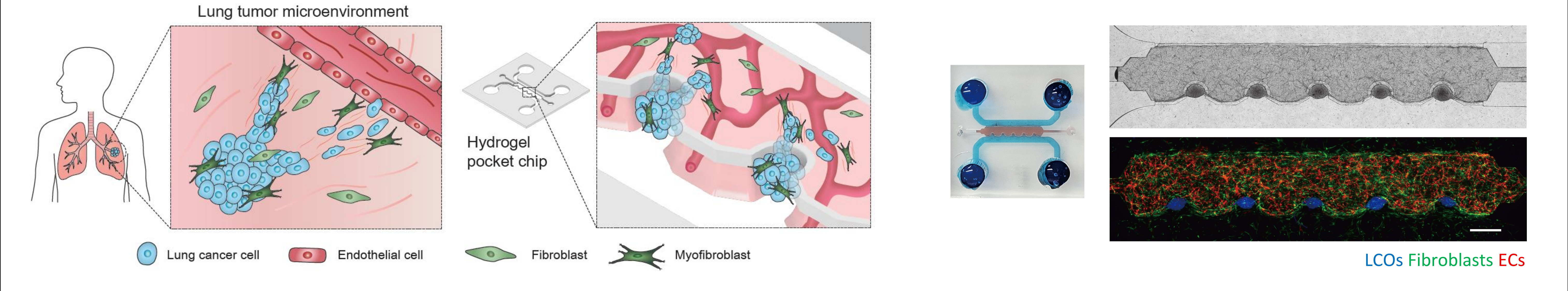
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Abstract

The tumor microenvironment (TME) plays a pivotal role in lung cancer invasion and metastasis by regulating complex physical and biochemical interactions. Local invasion of lung cancer, an early step in the multistep metastatic cascade, is under the dominant influence of TME components, but it remains incompletely understood. To investigate the specific impact of TME components on cancer cell invasion, we developed a 3D microfluidic system co-culturing cancer organoids with fibroblasts (HFL1) and endothelial cells (HUVEC) embedded within a hydrogel. We used a microfluidic chip comprising a single gel channel with a concave-shaped hydrogel pocket interface and two adjacent medium channels. Hydrogel mixture containing fibroblasts and HUVECs was injected into the gel channel, and crosslinked. Uniformly sized cancer organoids were pre-assembled using a multiwell device (Spherofilm), and five organoids were subsequently seeded into each chip. Co-culturing with endothelial cells prevented fibroblast-mediated extracellular matrix (ECM) collapse, thereby enabling long-term culture, while the inclusion of fibroblasts stabilized the vascular network. Lung cancer cells exhibited invasion when co-cultured with stromal cells, which was quantitatively assessed. By comparing mono-, co-, and tri-culture conditions, we discovered that fibroblasts act as the trigger for cancer cell invasion. Transcriptomic analysis via mRNA sequencing demonstrated that fibroblasts drive partial EMT in cancer cells. Concurrently, endothelial cells were found to promote fibroblast-to-myofibroblast transition through NOTCH signaling.

Experiment



Results & Discussions

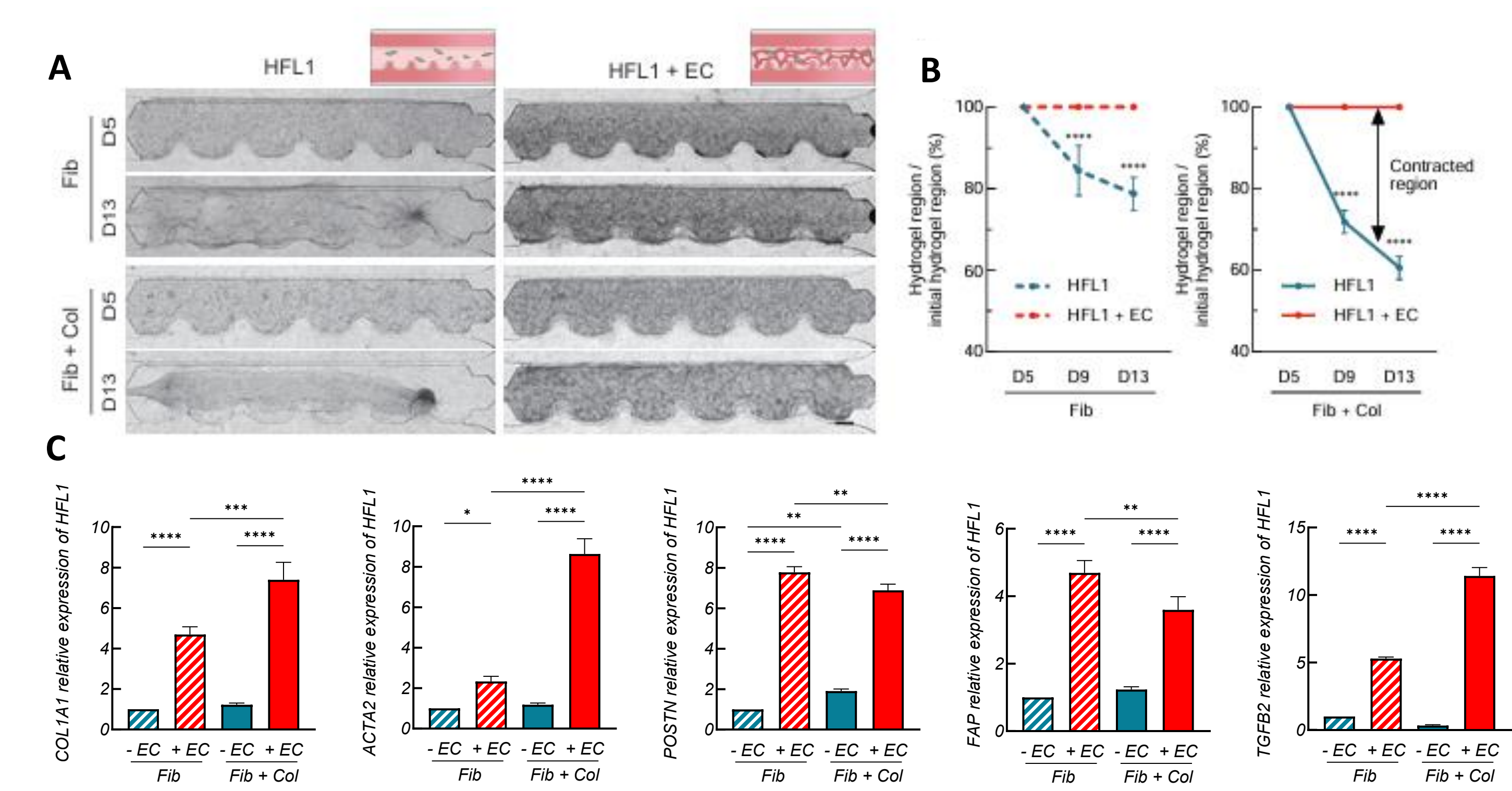


Fig. 1. Blood vessels prevented hydrogel contraction caused by HFL1 and activated HFL1. (Scale bar: 200 μ m).
(A), (B) B.F. Images and quantification graph of hydrogel contraction
(C) mRNA expression of HFL1 (Fib: fibrin 5 mg/mL, Fib + Col: fibrin 5 mg/mL + collagen type 1 3 mg/mL)

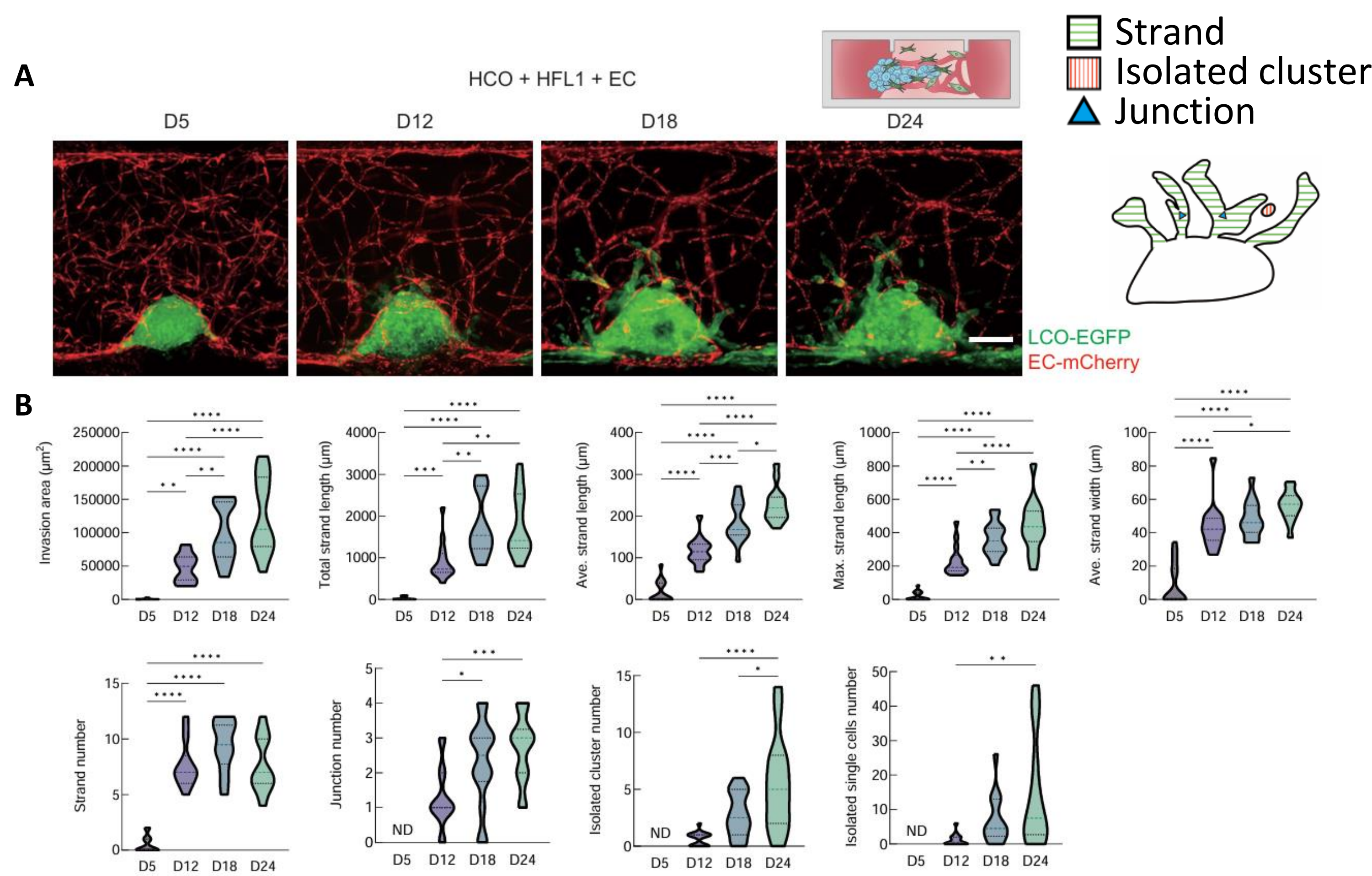


Fig. 2. LCOs initially exhibit collective migration and subsequently shift to single-cell dissemination through EMT. (Scale bar: 200 μ m).
(A) Fluorescent images of the migration of LCOs.
(B) Quantification graphs of the migration of LCOs.

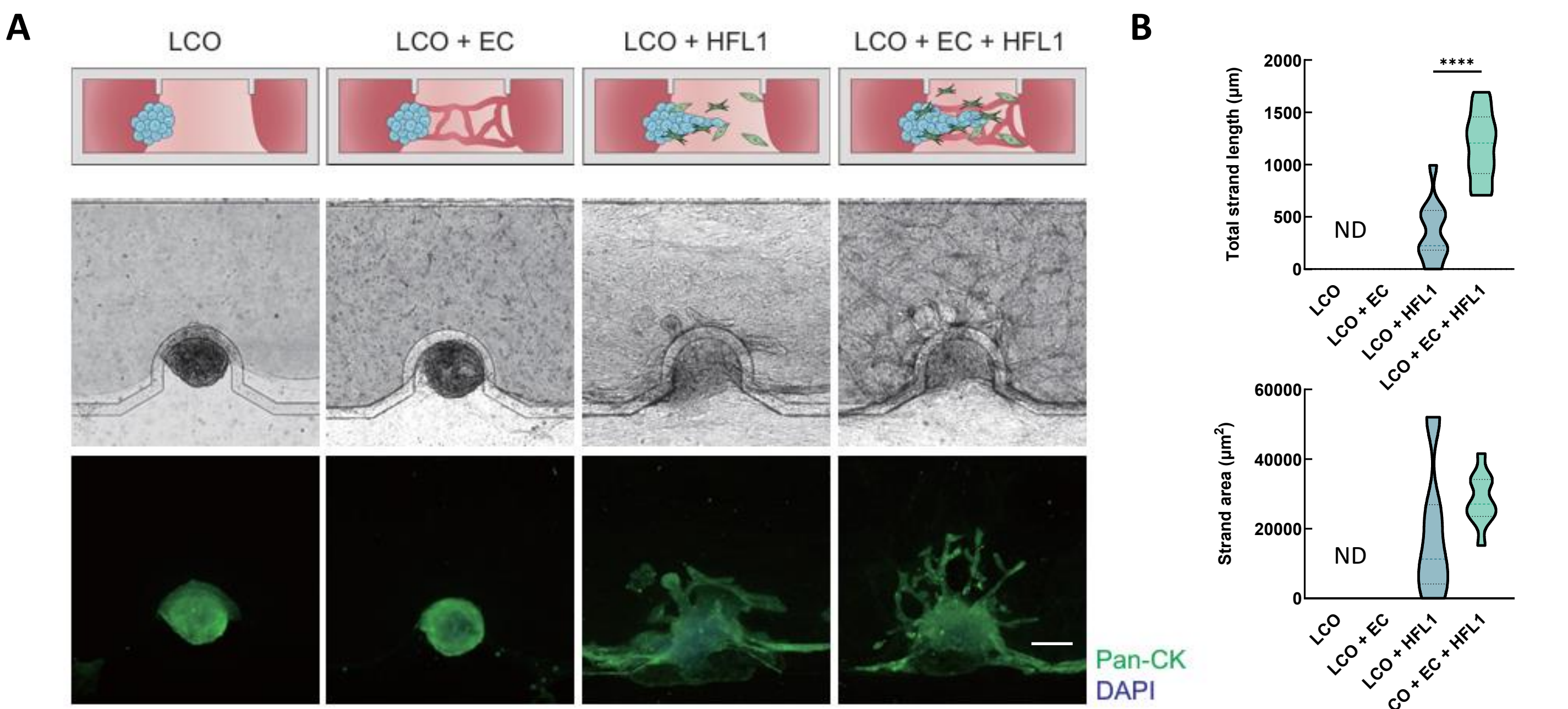


Fig. 3. Fibroblasts triggered the invasion of LCOs in the hydrogel pocket chip.
(A) Bright-field and immunofluorescent images of LCOs (scale bar: 200 μ m).
(e) Quantification graphs of LCOs invasion (ND: not detected).

Fig. 4. Fibroblasts induce EMT in lung cancer cells, while endothelial cells enhance fibroblast activation, thereby amplifying lung cancer EMT

(A-B) mRNA sequencing analysis of HFL1.
(C-D) mRNA sequencing analysis of LCO.
(E) ssGSEA scores of HFL1 and LCO.
(F) Ligand-receptor analysis between HFL1 and LCO

(CO: LCO + HFL1 co-culture
TRI: LCO + EC + HFL1 tri-culture)

* All tools were used for translation assistance.

Acknowledgement

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References

[1] Peter Friedl, Stephanie Alexander, "Cancer invasion and the microenvironment: plasticity and reciprocity", cell. 147 (2011).