

Figure S1: Directional migration of stratified epithelium is not a result of localized cell death. (A-J) Cell death as detected by Lysotracker assay (green) during the time course of mammary epithelial migration toward beads soaked in BSA (A-D) or FGF10 (E-H). Cell death was quantified in one of the three evenly divided regions of an organoid, the front (f), middle (m), or rear (r) regions, depending on their distances from the bead (asterisk). Scale bars: 100μm. (I, J) Quantification of cell death in different regions of mammary organoids during epithelial migration. Only signals that overlap with cell bodies were counted as dying cells whereas background noise was discounted. Statistical data were analyzed using two-factorial Analysis of Variance (ANOVA), having time and section of organoid as the two factors.

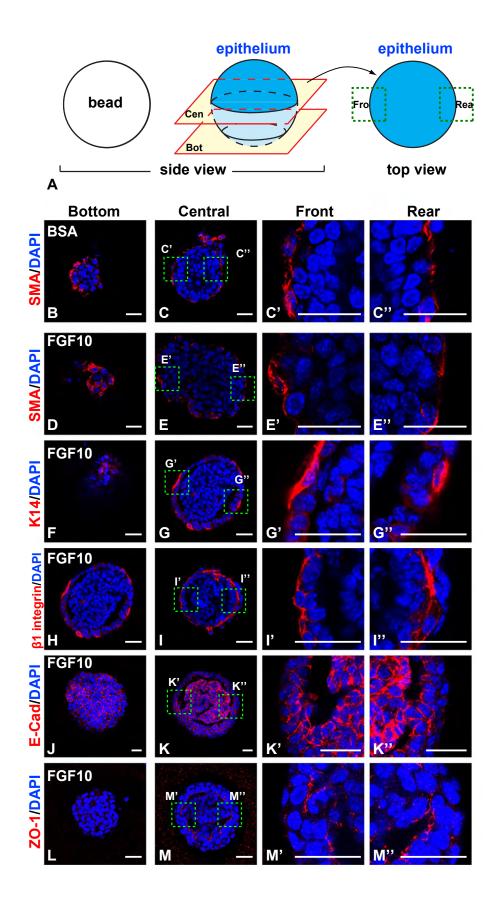


Figure S2: Directional migration of stratified epithelium lacks obvious front-rear polarity. (A) Schematic diagram depicting optical sectioning protocol of the mammary organoid in relation to bead location. Samples were sectioned optically along the Z-axis and images from the bottom (Bot) and central (Cen) are shown in (B-M). The front (Fro) and rear (Rea) areas of the central optical section were further shown in close-up views (C'-M''). (B-M) Immunofluorescence on mammary organoids to detect basal cells using antibodies against SMA (B-C'') and K14 (C-E'') and tissue polarity using antibodies against  $\beta$ 1 integrin (H-I''), E-Cadherin (J-K''), and Zo-1 (L-M''). Scale bars: 25  $\mu$ m.



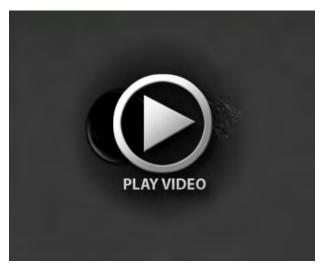
Movie 1: Time-lapse movie of mammary organoids stimulated by FGF2-soaked beads



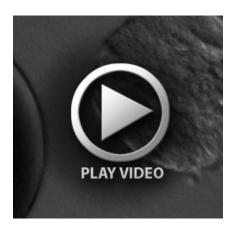
Movie 2: Time-lapse movie of mammary organoids stimulated by FGF7-soaked beads



Movie 3: Time-lapse movie of mammary organoids stimulated by BSA-soaked beads



Movie 4: Time-lapse movie of mammary organoids stimulated by FGF10-soaked beads



Movie 4': Time-lapse movie of close-up view of mammary collective epithelial migration when stimulated by FGF10-soaked beads



Movie 5: High-resolution time-lapse movie of mammary organoids stimulated by FGF10-soaked beads

## SUPPLEMENTARY TABLE S1: Primers used in qPCR.

| Gene name    | Forward sequence $(5' \rightarrow 3')$ | Reverse sequence $(5' \rightarrow 3')$ |  |
|--------------|--|--|--|
| Fgfl         | ggacaccgaagggcttttat                   | gcatgcttcttggaggtgtaa                  |  |
| Fgf2         | cggctctactgcaagaacg tgcttggagttgtag    |  |  |
| Fgf3         | tgagaacagcgcctatagca                   | gtaccgcccagaaaagagc                    |  |
| Fgf4         | gcaagetetteggtgtge                     | cgtaggattcgtaggcgttg                   |  |
| Fgf5         | cgaggagttttcagcaacaaa                  | tccgtaaatttggcacttgc                   |  |
| Fgf6         | tcagtggaacacacgaggag                   | cccgttctaccgtggagat                    |  |
| Fgf7         | aagggacccaggagatgaag                   | actgccacggtcctgattt                    |  |
| Fgf8         | caggtcctggccaacaag                     | ggtctccacaatgagcttcg                   |  |
| Fgf9         | actgcaggactggatttcatttag               | ccaggcccactgctatactg                   |  |
| Fgf10        | cgggaccaagaatgaagact                   | aacaactccgatttccactga                  |  |
| Fgf11        | ctttgccagaaacagetcet                   | gcctttgagctgaggctct                    |  |
| Fgf12        | gacgaaaacagcgactacacc                  | tetecatteatggecacata                   |  |
| Fgf13        | caggcagatggaaccattg                    | cccacagggatgaggttaaa                   |  |
| Fgf14        | tgctgtacaggcaacaggag                   | ttctcggtacatggcaacttc                  |  |
| Fgf15        | ggcaagatatacgggctgatt                  | tccatttcctccctgaaggt                   |  |
| Fgf16        | agtggactctggcctgtaccta                 | cattcacgtgtgagtttcttcg                 |  |
| Fgf17        | tatgaacaagaggggcaagc                   | ctcggtgaacacgcagtct                    |  |
| Fgf18        | aggacggggacaagtatgc                    | ggacttgactcccgaaggtat                  |  |
| Fgf20        | cggcaggatcacagtctctt                   | aaggtacaggccactgtcca                   |  |
| Fgf21        | agatggagctctctatggatcg                 | gggcttcagactggtacacatt                 |  |
| Fgf22        | ctatgtggccatgaatcgc                    | cggaacctacagtccacagag                  |  |
| Fgf23        | atctccacggcaacattttt                   | gtccactggcggaacttg                     |  |
| Etv5         | aggaccccaggctgtacttt                   | tggccgattcttctggatac                   |  |
| K8           | ategagateaceacetaceg                   | tgaagccagggctagtgagt                   |  |
| K18          | agatgacaccaacatcacaagg                 | tccagaccttggacttcctc                   |  |
| Notch1       | acaacaacgagtgtgagtcc                   | acacgtggctcctgtatatg                   |  |
| Hes6         | getgeteetegtttgtaacaca                 | cgatgggatggcaaccaa                     |  |
| Gata3        | agccacatctctcccttcag                   | agggctctgcctctctaacc                   |  |
| K14          | atcgaggacctgaagagcaa                   | ggctctcaatctgcatctcc                   |  |
| p63          | ggatgatttggcaagtctga                   | acttggggtcctcaggagat                   |  |
| Mmp2         | taacctggatgccgtcgt                     | ttcaggtaataagcacccttgaa                |  |
| Mmp3         | ttgttctttgatgcagtcagc                  | gatttgcgccaaaagtgc                     |  |
| <i>Mmp14</i> | gagaacttcgtgttgcctga                   | ctttgtgggtgaccctgact                   |  |

| Actb         | ggetgtatteecetecateg   | ccagttggtaacaatgccatgt |
|--------------|------------------------|------------------------|
| 18S          | gtaacccgttgaaccccatt   | gtaacccgttgaaccccatt   |
| <i>EEF1g</i> | ggattctgtgtgtttgagagca | cagcaaagctgacccactg    |
| GAPDH        | ttcaccaccatggagaaggc   | cccttttggctccaccct     |

SUPPLEMENTARY TABLE S2. Effects of MEK inhibitor (U0126) and PI3K inhibitor (LY294002), alone or in combination, on migration of mammary epithelium when stimulated by beads soaked in FGF10.

| U0126 [μM] | LY294002 [μM] | Complete migration | Partial migration | No<br>migration | Numbers tested |
|------------|---------------|--------------------|-------------------|-----------------|----------------|
| 2.0        | 0             | 0                  | 2                 | 3               | 5              |
| 1.3        | 0             | 4                  | 0                 | 0               | 4              |
| 1.0        | 0             | 3                  | 0                 | 1               | 4              |
| 0          | 200.0         | 0                  | 0                 | 9               | 9              |
| 0          | 20.0          | 0                  | 0                 | 6               | 6              |
| 0          | 13.3          | 0                  | 0                 | 4               | 4              |
| 0          | 10.0          | 1                  | 1                 | 2               | 4              |
| 0          | 5.0           | 2                  | 0                 | 2               | 4              |
| 0          | 3.3           | 4                  | 0                 | 0               | 4              |
| 2.0        | 3.0           | 0                  | 3                 | 0               | 3              |
| 2.0        | 5.0           | 0                  | 3                 | 1               | 4              |
| 1.3        | 13.3          | 0                  | 0                 | 3               | 3              |
| 1.3        | 10.0          | 0                  | 0                 | 7               | 7              |
| 1.3        | 5.0           | 0                  | 2                 | 2               | 4              |
| 1.3        | 3.3           | 0                  | 3                 | 1               | 4              |