**Single-cell RNA reveals a tumorigenic microenvironment in the interface zone of human breast tumors**

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## Supplementary Materials

## (Table S1 – S4 and Figure S1 – S3)

**Table S1.** Clinical parameters of the three breast cancer patients

| Patients | Histology | WHO Grade | Her2 status | ER status | PR status | ki67 | P53 |
|---|---|---|---|---|---|---|---|
| 1 | IBC-NST | 2 | 1+ | 80%+ | 70%+ | 15%+ | + |
| 2 | IBC-NST | 2 | 3+ | - | - | 40% | + |
| 3 | IBC-NST | 2 | 2+ | 90%+ | 90%+ | 30% | Pos |

WHO, World Health Organization; ER, estrogen receptor; Her2, human epidermal growth factor receptor 2; PR, progesterone receptor; IBC-NST, Invasive breast carcinoma of no special type.

**Table S2.** PCR primer sequence

| Gene | Forward Primer | Reverse Primer |
|---|---|---|
| GAPDH | GGAGTCCACTGGCGTCTTCA | GTCATGAGTCCTTCCACGATACC |
| BMPR1B | GAGGATGACTCTGGGTTGCC | AGGCAGTGTAGGGTGTAGGT |

**Table S3.** The specific information for each sample

| patient | zone | High quality cells | mean reads/cell | total genes |
|---|---|---|---|---|
| 1 | tumor | 11108 | 19727 | 35955 |
| 1 | interface | 12645 | 23938 | 36286 |
| 1 | normal | 13589 | 20801 | 35989 |
| 2 | tumor | 9597 | 38123 | 36618 |
| 2 | interface | 8539 | 32706 | 36680 |
| 2 | normal | 8960 | 36773 | 36821 |
| 3 | tumor | 8013 | 36323 | 36676 |
| 3 | interface | 8008 | 55136 | 37655 |
| 3 | normal | 8089 | 34588 | 36065 |

**Table S4.** Numbers of immune cells with immune hot and cold activities

| Patients | Zones | cluster 0 (CD8+ T) hot | cluster 1 (CD4+ T) hot | cluster 2 (CD4+ T) cold | cluster 3 (Tregs) cold | cluster 4 (CD8+ T) hot | cluster 5 (CD8+ T) hot | cluster 6 (Tregs) cold | cluster 7 (CD8+ T) hot | cluster 8 (CD4+ T) hot | Total cell Number (hot) | Total cell Number (cold) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Normal | 607 | 59 | 434 | 0 | 120 | 47 | 6 | 1 | 0 | 834 | 440 |
| 1 | Interface | 961 | 56 | 373 | 0 | 64 | 65 | 14 | 0 | 0 | 1146 | 387 |
| 1 | Tumor | 812 | 60 | 330 | 1 | 134 | 34 | 317 | 7 | 3 | 1050 | 648 |
| 2 | Normal | 73 | 35 | 75 | 1 | 60 | 60 | 20 | 4 | 3 | 235 | 96 |
| 2 | Interface | 20 | 985 | 33 | 174 | 381 | 94 | 507 | 73 | 127 | 1680 | 714 |
| 2 | Tumor | 15 | 63 | 46 | 1175 | 297 | 19 | 141 | 829 | 764 | 1987 | 1362 |
| 3 | Normal | 36 | 75 | 21 | 0 | 9 | 195 | 0 | 0 | 2 | 317 | 21 |
| 3 | Interface | 4 | 93 | 19 | 0 | 4 | 320 | 1 | 0 | 3 | 424 | 20 |
| 3 | Tumor | 37 | 207 | 138 | 2 | 138 | 312 | 88 | 7 | 5 | 706 | 228 |

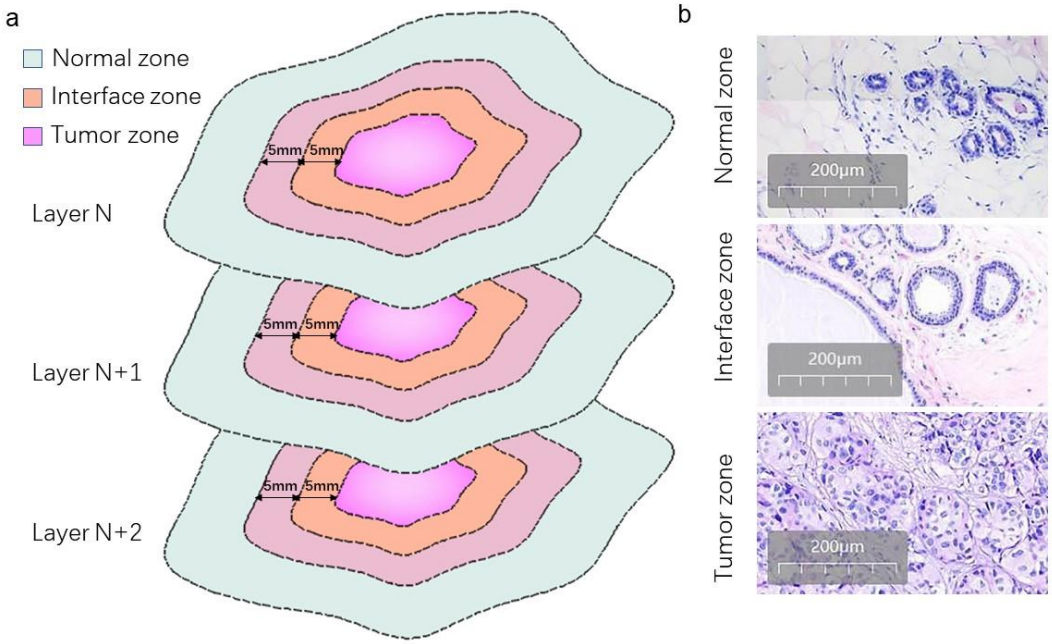


**Figure S1. Identification and collection of representative samples. a** Breast cancer tissues from the normal zone, interface zone and tumor zone were acquired by macroscopic dissection based on geographical mapping**.** Layer N, N+1, N+2 represented three continuous sections that have similar tumor boundary in the cutting surface. **b** Histological appearances of the normal, interface and tumor zone confirmed by hematoxylin and eosin (H&E) staining of representative tissues.

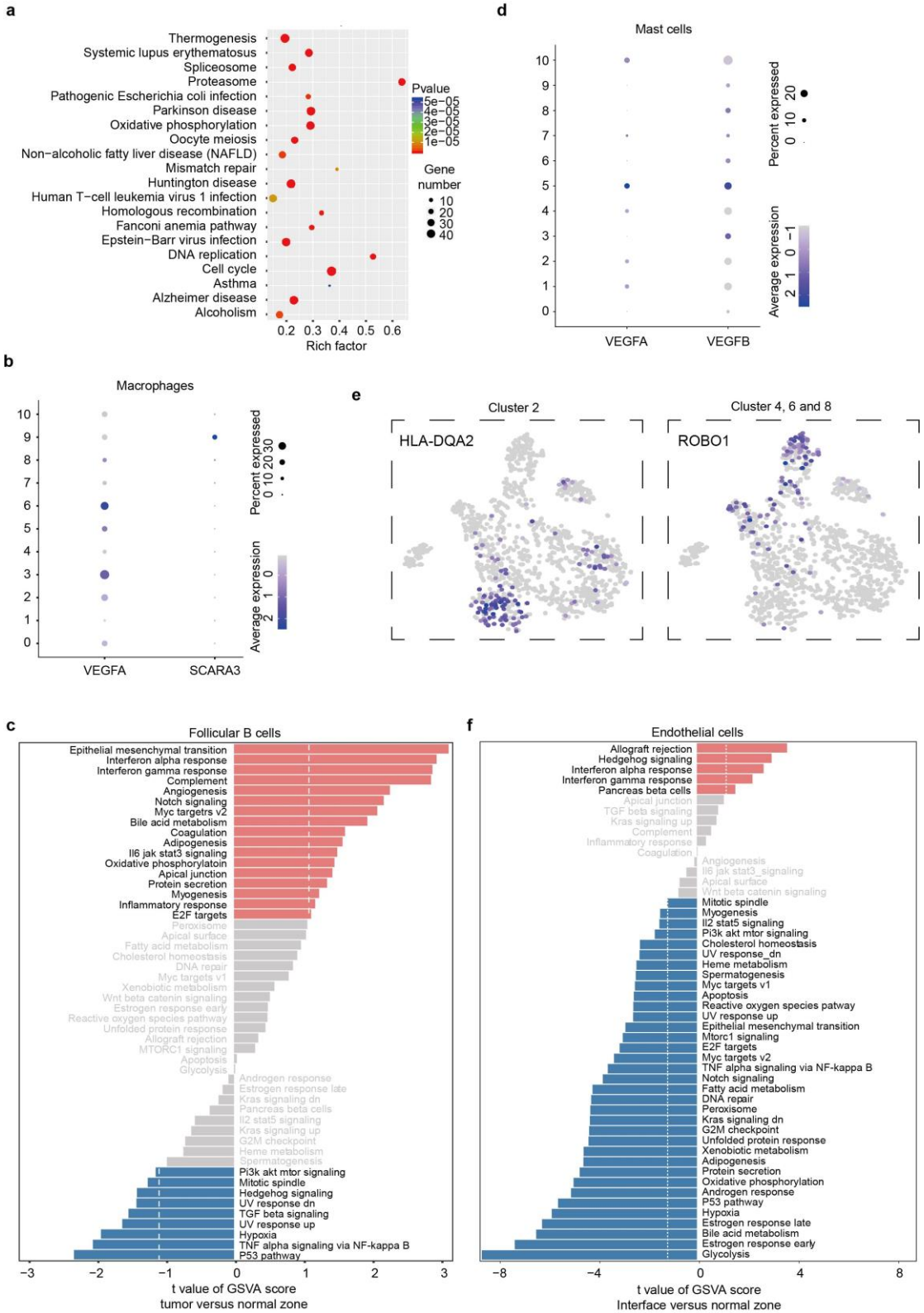


**Figure S2. Gene expression and pathway analysis in stromal cells. a** Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of marker genes in TOP2A+ T cells. **b** Bubble plots displaying expressions of selected genes in macrophage clusters. **c** Differences in pathway activity of each cell scored by GSVA between follicular B cells isolated from normal and tumor zones. **d** Bubble plots displaying the expression of selected genes in mast cell clusters. **e** tSNE

plots of the specific gene expression of endothelial cell clusters. **f** Differences in pathway activity of each cell scored by GSVA between endothelial cells isolated from normal and interface zones.

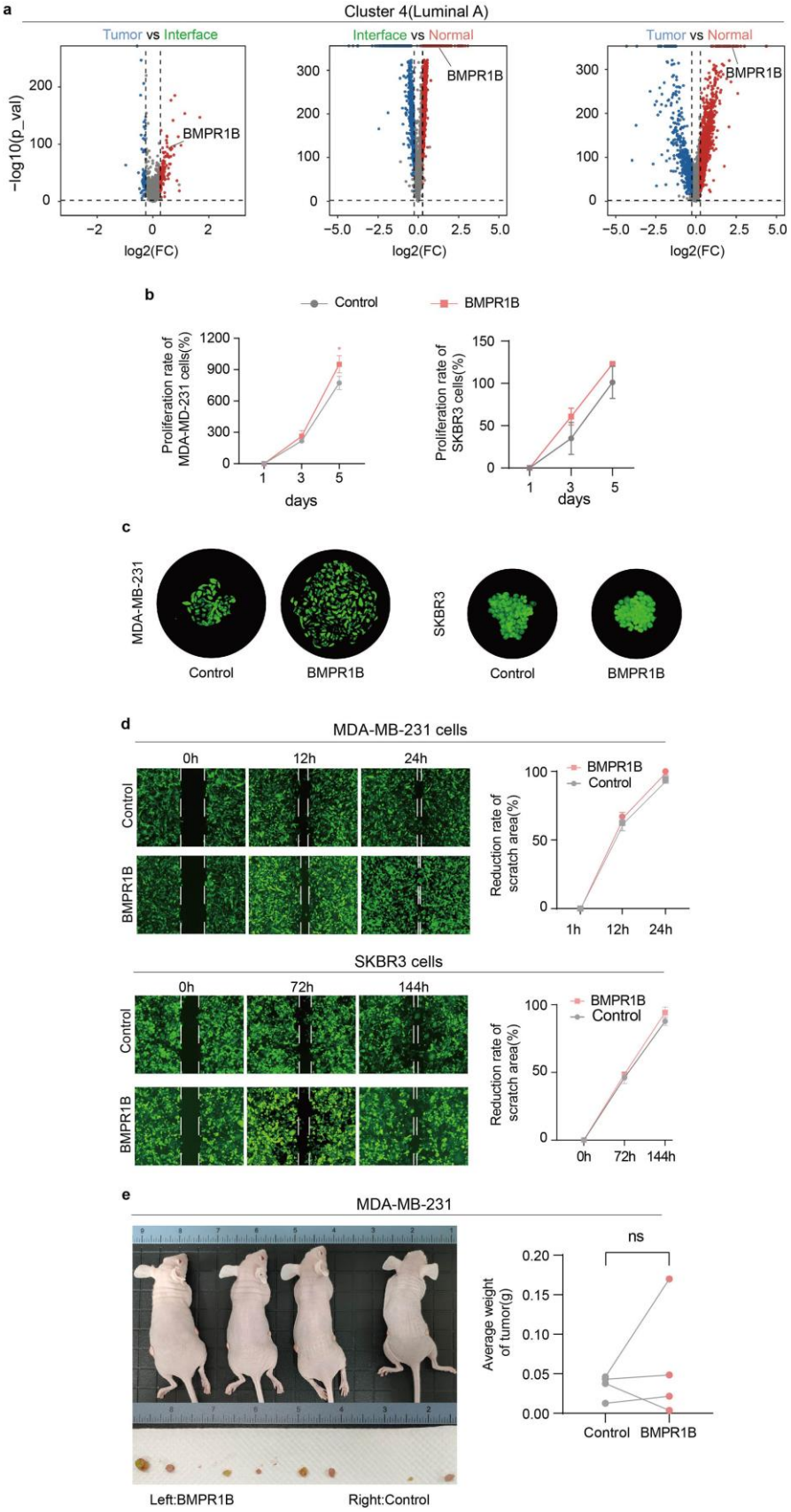

**Figure S3. The expression and function of BMPR1B gene in epithelial cells. a** Volcano plots of BMPR1B gene expression in cluster 4 from different zones here (left to right): Tumor zone versus interface zone, interface zone versus normal zone and tumor zone versus normal zone. **b** Line chart of cell proliferation measured at 450 nm. Cells with raw vector served as control group. **c** Photos of cell clones observed under a microscope. Cloning formation of BMPR1B-expressing MDA-MB-231 and SKBR3 cells versus matched cells with raw vectors. **d** Wound-healing assay of MDA-MB-231 and SKBR3 cells with or without BMPR1B overexpression. Left: representative images of BMPR1B-overexpressed cells and control cells at three time point after wounding. Right: quantification of the reduction rate of scratch area (mean±SD). **e** Photographs of BALB/cNj-Foxn1$^{nu}$/Gpt mice. (left) BMPR1B-overexpressed MDA-MB-231 cells and control cells with raw vector were respectively injected into subcutaneous location of left and right groin in nude mice. 8th week post-transplantation, mice were photographed, and tumors were removed surgically and weighed. (right) Statistical analysis of mice tumor weights, (n = 3, t test).