# Supplementary material

# Characteristic T cell receptor repertoire of infiltrated T lymphocytes in breast tumor and adjacent tissues

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Running title: TCR repertoire of breast tumor and adjacent tissues

**Supplementary Figure legends:**

Supplementary Figure 1. Amplification bias assessment across multiplex PCR primer mix. We amplified the equimolar mixture of 40 synthesized standard templates with the primer mix. The amplification bias of each primer was calculated according to the following formula: Amplification bias = (observed value – mean) / mean

Supplementary Figure 2. *TRB* repertoire diversity of lymph node, non-tumor and tumor tissue. (A) Cumulative frequency of total clones with cumulative unique clones. The X axis showed unique clones ranked with increasing frequency. Each curve represents a sample, and the steeper of ascending curve indicates the higher clonality and lower diversity of the repertoire. (B) Abundant clones (frequency > 0.01%) percentage of *TRB* clone in Lymph node, non-tumor and tumor tissue.

(\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 according to Mann-Whitney U test)

Supplementary Figure 3. Comparison of *TRBV* or *TRBJ* segment usage between tumor tissue and other two tissues. (A-D) The biased *TRBJ* usage between lymph node and tumor tissue (no *TRBV* bias identified). (E) The biased TRBV segment usage between non-tumor and tumor tissue.

All p-values were calculated according to Mann-Whitney U test.

Supplementary Figure 4. Shared clone frequency between lymph node and tumor tissue in lymph node and tumor tissue. All p-values were calculated according to paired two-tailed t test.

LN: lymph node tissue; T: tumor tissue

Supplementary Figure 5. Expanded clones (>0.1%) percentage in tumor tissue and lymph node. (A) Expanded *TRB* clones percentage in total tumor clones and in tumor clones shared with lymph node tissue. (B) Expanded clones percentage in total LN clones and in LN clones shared with tumor tissue. All p-values were calculated according to Mann-Whitney U test.

Supplementary Figure 6. The similarity between paired lymph node, non-tumor and tumor tissue.

(A) Pearson coefficient index between tumor and paired lymph node tissue of patients with high LNR (>30%) and LN-negative patients. (B) Pearson coefficient index among lymph node, non-tumor and tumor tissue. All p-values were calculated according to Mann-Whitney U test.

**Supplementary Tables:**

|  |  |  |  |  |  |  |
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| **Table S1. The reproducibility of IHC scoring of infiltrated T cells.** | | | | | | |
| Patient ID | tumor infiltration T cells(%) | | | non-tumor infiltration T cells (%) | | |
| result | by the same pathologist | by another pathologist | result | by the same pathologist | by another pathologist |
| BC0001A | NA | NA | NA | NA | NA | NA |
| BC0002A | 10 | 10 | 10 | 2 | 3 | 2 |
| BC0003A | 20 | 20 | 25 | 0.1 | 0 | 0 |
| BC0004A | 8 | 10 | 10 | 1 | 2 | 1 |
| BC0005A | 0.3 | 0.5 | 1 | 0.1 | 0 | 0 |
| BC0006A | 25 | 25 | 23 | 0.5 | 0.5 | 0.5 |
| BC0007A | 2 | 5 | 2 | 1 | 2 | 2 |
| BC0008A | NA | NA | NA | NA | NA | NA |
| BC0009A | 30 | 30 | 25 | 0.1 | 0 | 0 |
| BC0010A | 40 | 40 | 38 | 0.2 | 0.2 | 0.2 |
| BC0011A | 8 | 10 | 7 | 0.1 | 0 | 0 |
| BC0012A | 3 | 3 | 3 | 1 | 0.5 | 0.5 |
| BC0013A | 2 | 2 | 2 | 0.2 | 0.2 | 0.3 |
| BC0014A | 0.2 | 0.2 | 0.5 | 2 | 2 | 1 |
| BC0015A | 15 | 15 | 18 | 3 | 3 | 2 |
| BC0016A | 12 | 10 | 10 | 0.1 | 0 | 0 |
|  |  |  |  |  |  |  |
|  | NA indicates Not Analyzed. | | |  |  |  |

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| **Table S2. The infiltration level of CD4+ and CD8+ T cells in tumors and non-tumors.** | | | | | | |
| Patient ID | tumor infiltration score | | non-tumor infiltration score | |  |  |
| CD4+ | CD8+ | CD4+ | CD8+ |  |  |
| BC0001A | 1 | 2 | 0 | 0 |  |  |
| BC0002A | 2 | 2 | 0 | 1 |  |  |
| BC0003A | 1 | 3 | 0 | 1 |  |  |
| BC0004A | 1 | 1 | 0 | 1 |  |  |
| BC0005A | 1 | 1 | 0 | 1 |  |  |
| BC0006A | 1 | 1 | 0 | 0 |  |  |
| BC0007A | 0 | 4 | 1 | 1 |  |  |
| BC0008A | NA | NA | NA | NA |  |  |
| BC0009A | 1 | 1 | 0 | 0 |  |  |
| BC0010A | 1 | 3 | 0 | 1 |  |  |
| BC0011A | 0 | 1 | 0 | 0 |  |  |
| BC0012A | 1 | 1 | 3 | 4 |  |  |
| BC0013A | 1 | 1 | 0 | 0 |  |  |
| BC0014A | 1 | 1 | 0 | 2 |  |  |
| BC0015A | 1 | 1 | 0 | 2 |  |  |
| BC0016A | 3 | 4 | 0 | 0 |  |  |
|  | : |  |  |  |  |  |
| NA indicates this sample is not analyzed. | | |  |  |  |  |
| The score corresponds to the percentage of CD4 or CD8 positive cells in the total lymphocytes. | | | | | |  |
| 0-25%: score 1; 26-50%: score 2; 51-75%: score 3;76-100%: score 4 | | | | |  |  |

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| --- | --- | --- | --- | --- |
| **Table S3. The clinical characteristics of 49 patients.** | | |  |  |
| **Patient ID** | **Tumor Subtype** | **Age** | **Ki-67** | **Lymph Node Ratio** |
| BC01 | Luminal A | 39 | 13% | 0/18 |
| BC02 | Basal-like | 39 | 50% | 0/14 |
| BC03 | Luminal B | 46 | 30% | NA |
| BC04 | Luminal B | 45 | 20% | 2/20 |
| BC05 | Luminal B | 60 | 70% | 1/15 |
| BC06 | Luminal A | 42 | 10% | 0/18 |
| BC07 | Luminal B | 47 | 20% | 1/4 |
| BC08 | Luminal B | 38 | 25% | 0/18 |
| BC09 | Her-2 | 62 | 35% | 0/11 |
| BC10 | NA | 43 | NA | 0/22 |
| BC11 | Luminal A | 49 | NA | 0/11 |
| BC12 | Luminal B | 41 | 25% | 1/3 |
| BC13 | Luminal A | 46 | 10% | 1/21 |
| BC14 | Her-2 | 71 | 50% | 2/19 |
| BC15 | Luminal A | 50 | 12% | 0/13 |
| BC16 | Her-2 | 61 | 25% | 11/17 |
| BC17 | Luminal B | 40 | 25% | 0/16 |
| BC18 | Luminal A | 58 | 10% | 0/16 |
| BC19 | Luminal A | 41 | 9% | 0/8 |
| BC20 | Her-2 | NA | NA | NA |
| BC21 | Luminal B | 60 | 20% | 0/5 |
| BC22 | Her-2 | 59 | 25% | 0/16 |
| BC23 | Luminal B | 67 | 25% | 1/19 |
| BC24 | Luminal A | 78 | 8% | 14/14 |
| BC25 | Luminal A | 62 | 7% | 0/16 |
| BC26 | Her-2 | 35 | 15% | 0/21 |
| BC27 | Her-2 | 57 | 18% | 0/14 |
| BC28 | Luminal B | 57 | 20% | 5/19 |
| BC29 | Luminal A | 43 | 8% | 0/17 |
| BC30 | Basal-like | 57 | 16% | 6/19 |
| BC31 | Basal-like | 60 | 75% | 7/19 |
| BC32 | Her-2 | 51 | 85% | 3/15 |
| BC33 | Luminal B | 58 | 40% | 0/14 |
| BC34 | Basal-like | 62 | 25% | 0/12 |
| BC35 | Luminal B | 45 | 25% | 0/11 |
| BC36 | Luminal A | 55 | 15% | 0/9 |
| BC37 | Luminal B | 47 | 40% | 2/25 |
| BC38 | Luminal B | 56 | 25% | 1/13 |
| BC39 | Luminal A | NA | NA | NA |
| BC40 | Basal-like | 41 | 30% | 2/17 |
| BC41 | Luminal B | 46 | 20% | 2/21 |
| BC42 | Luminal A | 59 | 8% | 0/12 |
| BC43 | Luminal B | 49 | 20% | 1/17 |
| BC44 | Luminal B | 66 | 25% | 10/22 |
| BC45 | Luminal A | 53 | 15% | 7/18 |
| BC46 | Luminal B | 36 | 35% | 0/5 |
| BC47 | Her-2 | 67 | 10% | 0/18 |
| BC48 | Luminal B | 39 | 30% | 0/2 |
| BC49 | Her-2 | 62 | 15% | 1/14 |
|  |  |  |  |  |
| NA refers to Not Analyzed. |  |  |  |  |