

## Phenotype and function of CXCR5<sup>+</sup>CD45RA<sup>-</sup>CD4<sup>+</sup> T cells were altered in HBV-related hepatocellular carcinoma and elevated serum CXCL13 predicted better prognosis

### Supplementary Materials

**Supplemental Table S1: Clinical features of the cohorts in this study**

|   | Cohort #1 (n = 21) | Cohort #2 (n = 111) |
|---|--------------------|---------------------|
| <b>Age</b>                              | 54 (29–72)         | 56 (23–78)          |
| <b>Gender (M/F)</b>                     | 13/8               | 91/20               |
| <b>HBV DNA (log<sub>10</sub> IU/ml)</b> | 4.09 (3.83–5.20)   | 4.16 (3.63–5.42)    |
| <b>Cirrhosis (Absent/Present)</b>       | 3/18               | 15/96               |
| <b>Liver Function index</b>             |                    |                     |
| ALT (IU/ml)                             | 39 (14–83)         | 35 (5–485)          |
| AST (IU/ml)                             | 37 (18–127)        | 37 (14–339)         |
| ALP (IU/ml)                             | 94 (1.5–261)       | 106 (33–371)        |
| γ-GT (IU/ml)                            | 80 (14–678)        | 74 (14–399)         |
| AFP (μg/L)                              | 316.9 (1.34–1211)  | 65.81 (0.3–1211)    |
| ALB (g/L)                               | 41.7 (24.9–49.7)   | 39 (21.4–50.2)      |
| TBI (μmol/L)                            | 15.2 (5.3–33.2)    | 14 (5.3–224.3)      |
| <b>Blood routine</b>                    |                    |                     |
| RBC (× 10 <sup>12</sup> /L)             | 4.13 (3.66–4.9)    | 4.2 (2.8–5.36)      |
| WBC (× 10 <sup>9</sup> /L)              | 6.5 (2.04–14.25)   | 5.4 (2–15.8)        |
| PLT (× 10 <sup>9</sup> /L)              | 141 (30–364)       | 151 (22–458)        |

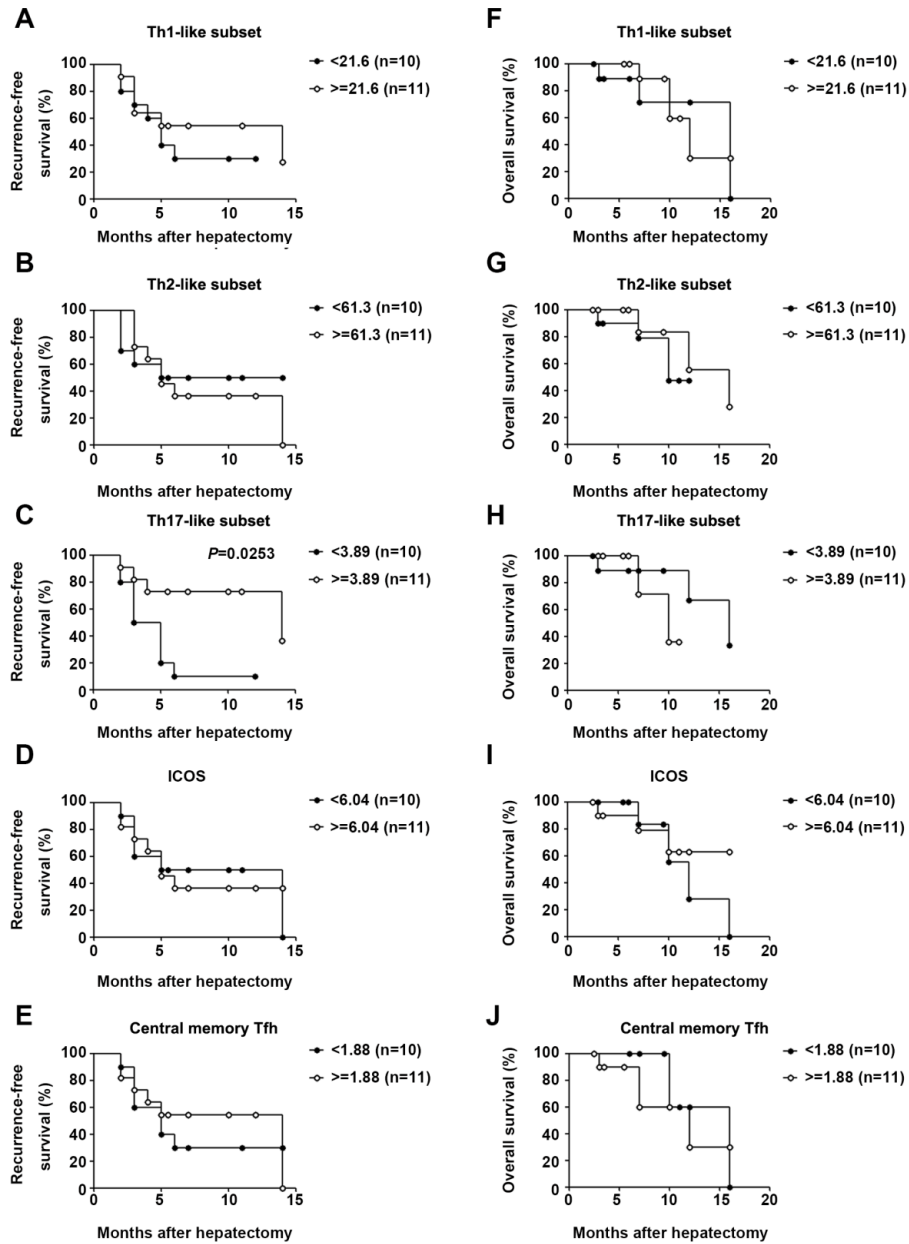
Data were displayed in the form of median (minimum-maximum) except gender and cirrhosis. ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline phosphatae; γ-GT: γ glutamyl transpeptidase; AFP: Alpha-fetoprotein; ALB: Albumin; TBI: Total Bilirubin; RBC: Red blood cell; WBC: White blood cell; PLT: Platelet.

**Supplemental Table S2: Association between the frequencies of Tfh sub-populations and clinical features of HCC subjects**

| Variables              | Th1 subset/Tfh (%)  |          | Th2 subset/Tfh (%)  |          | Th17 subset/Tfh (%) |          | ICOS + /Tfh (%)     |          | Central Memory Tfh  |          |
|------------------------|---------------------|----------|---------------------|----------|---------------------|----------|---------------------|----------|---------------------|----------|
|                        | Values              | <i>P</i> | Values              | <i>P</i> | Values              | <i>P</i> | Values              | <i>P</i> | Values              | <i>P</i> |
| <b>Age (years)</b>     |                     | 0.550    |                     | 0.130    |                     | 0.113    |                     | 0.418    |                     | 0.860    |
| ≤ 50                   | 24.2<br>(3.41–34.3) |          | 66.1<br>(44.1–81.9) |          | 3.12<br>(0.29–9.7)  |          | 5.79<br>(1.21–11.3) |          | 1.55<br>(0.54–4.32) |          |
| > 50                   | 20.7<br>(12.8–33.3) |          | 58.4<br>(33.7–74)   |          | 5.16<br>(1.81–13.3) |          | 6.42<br>(1.81–25.4) |          | 1.96<br>(0.47–3.59) |          |
| <b>Gender</b>          |                     | 0.856    |                     | 0.328    |                     | 0.690    |                     | 0.328    |                     | 0.180    |
| Female                 | 22.5<br>(16–34.3)   |          | 58.9<br>(47.1–68.1) |          | 4.52<br>(1.91–8.62) |          | 8.46<br>(3.44–18.7) |          | 1.29<br>(0.47–3.23) |          |
| Male                   | 21.5<br>(3.41–33.3) |          | 65.5<br>(33.7–81.9) |          | 3.52<br>(0.29–13.3) |          | 5.55<br>(1.21–25.4) |          | 1.94<br>(0.55–4.32) |          |
| <b>Liver cirrhosis</b> |                     | 0.063    |                     | 0.079    |                     | 0.078    |                     | 0.039    |                     | 0.651    |
| Absent                 | 31.6<br>(23.8–33.3) |          | 57.1<br>(51.2–57.5) |          | 6.45<br>(5.16–13.3) |          | 3.62<br>(1.81–4.58) |          | 1.03<br>(0.54–3.59) |          |
| Present                | 21.1<br>(3.41–34.3) |          | 64.6<br>(33.7–81.9) |          | 3.44<br>(0.29–10.3) |          | 6.57<br>(1.21–25.4) |          | 1.91<br>(0.47–4.31) |          |
| <b>Tumor number</b>    |                     | 0.967    |                     | 0.029    |                     | 0.200    |                     | 0.592    |                     | 0.091    |
| 1                      | 21.6<br>(3.41–34.3) |          | 64.6<br>(44.1–81.9) |          | 3.25<br>(0.29–13.3) |          | 5.79<br>(1.21–17)   |          | 2.05<br>(0.47–4.32) |          |
| > 1                    | 23.8<br>(12.8–31.6) |          | 57.1<br>(33.7–65.5) |          | 5.16<br>(3.52–7.26) |          | 6.73<br>(1.81–25.4) |          | 1.03<br>(0.49–1.96) |          |
| <b>Encapsulation</b>   |                     | 0.303    |                     | 0.964    |                     | 0.560    |                     | 0.893    |                     | 0.165    |
| Complete               | 23.4<br>(14.3–34.3) |          | 61.3<br>(44.1–81.9) |          | 3.95<br>(0.29–13.3) |          | 6.04<br>(1.21–18.7) |          | 1.94<br>(0.47–4.32) |          |
| Incomplete             | 17.6<br>(3.41–31.6) |          | 63.5<br>(33.7–73.2) |          | 1.83<br>(2.35–5.16) |          | 8.13<br>(1.81–25.4) |          | 1.07<br>(0.55–1.96) |          |
| <b>AFP (µg/L)</b>      |                     | 0.879    |                     | 1.000    |                     | 0.849    |                     | 0.820    |                     | 0.649    |
| ≤ 200                  | 21.5<br>(3.41–33.3) |          | 61.3<br>(33.7–73.2) |          | 3.15<br>(1.81–13.3) |          | 5.55<br>(1.81–25.4) |          | 1.40<br>(0.47–3.59) |          |
| > 200                  | 23.8<br>(14.3–34.3) |          | 59.4<br>(44.1–81.9) |          | 3.95<br>(0.29–9.7)  |          | 6.73<br>(1.21–18.7) |          | 1.88<br>(0.49–4.32) |          |
| <b>TBI (µmol/L)</b>    |                     | 0.294    |                     | 0.405    |                     | 0.971    |                     | 0.971    |                     | 0.492    |
| ≤ 17.1                 | 23.4<br>(12.8–33.3) |          | 61.3<br>(33.7–69.4) |          | 3.52<br>(0.54–13.3) |          | 6.04<br>(1.81–25.4) |          | 1.94<br>(0.47–4.32) |          |
| > 17.1                 | 19.3<br>(3.41–34.3) |          | 62.5<br>(47.1–81.9) |          | 3.92<br>(0.29–7.26) |          | 7.76<br>(1.21–18.7) |          | 1.50<br>(0.49–3.23) |          |
| <b>ALB (g/L)</b>       |                     | 0.651    |                     | 0.802    |                     | 0.880    |                     | 0.175    |                     | 0.175    |
| ≤ 35                   | 19.5<br>(16.9–24.6) |          | 58.1<br>(47.1–74.0) |          | 3.95<br>(2.46–7.26) |          | 17.0<br>(5.03–18.7) |          | 0.49<br>(0.47–2.62) |          |
| > 35                   | 22.5<br>(3.41–34.3) |          | 62.5<br>(33.7–81.9) |          | 3.71<br>(0.29–13.3) |          | 5.80<br>(1.21–25.4) |          | 1.91<br>(0.54–4.32) |          |
| <b>ALT (U/L)</b>       |                     | 0.538    |                     | 0.856    |                     | 0.971    |                     | 0.800    |                     | 0.538    |

|                   |                     |       |                     |       |                     |       |                     |       |                     |       |
|-------------------|---------------------|-------|---------------------|-------|---------------------|-------|---------------------|-------|---------------------|-------|
| ≤ 40              | 21.5<br>(3.41–34.3) |       | 63.7<br>(33.7–73.2) |       | 3.52<br>(1.81–13.3) |       | 6.42<br>(3.44–25.4) |       | 1.94<br>(0.54–3.59) |       |
| > 40              | 24.0<br>(14.3–31.6) |       | 59.7<br>(44.1–81.9) |       | 4.55<br>(0.29–9.7)  |       | 5.77<br>(1.21–18.7) |       | 1.98<br>(0.47–4.32) |       |
| <b>AST (U/L)</b>  |                     | 0.915 |                     | 0.972 |                     | 0.915 |                     | 0.145 |                     | 0.859 |
| ≤ 40              | 21.6<br>(12.8–33.3) |       | 62.5<br>(33.7–81.9) |       | 4.34<br>(0.29–13.3) |       | 5.23<br>(1.21–25.4) |       | 1.79<br>(0.47–3.59) |       |
| > 40              | 23.8<br>(3.41–34.3) |       | 59.4<br>(44.1–74)   |       | 3.89<br>(0.54–9.7)  |       | 7.25<br>(4.58–18.7) |       | 2.62<br>(0.54–0.49) |       |
| <b>ALP (U/L)</b>  |                     | 0.190 |                     | 0.190 |                     | 0.256 |                     | 0.163 |                     | 0.930 |
| ≤ 150             | 23.6<br>(12.8–33.3) |       | 59.9<br>(33.7–81.9) |       | 5.16<br>(0.29–13.3) |       | 5.53<br>(1.21–25.4) |       | 1.79<br>(0.47–4.32) |       |
| > 150             | 16.9<br>(3.41–34.3) |       | 68.1<br>(58.4–74)   |       | 3.35<br>(1.81–3.95) |       | 10.5<br>(5.03–15.2) |       | 2.16<br>(1.12–2.62) |       |
| <b>γ-GT (U/L)</b> |                     | 0.447 |                     | 0.365 |                     | 0.137 |                     | 0.294 |                     | 0.971 |
| ≤ 50              | 22.5<br>(12.8–33.3) |       | 59.9<br>(33.7–69.4) |       | 5.16<br>(2.35–13.3) |       | 5.23<br>(1.81–25.4) |       | 1.17<br>(0.55–3.59) |       |
| > 50              | 19.5<br>(3.41–34.3) |       | 65.5<br>(44.1–81.9) |       | 3.35<br>(0.29–9.7)  |       | 6.73<br>(1.21–18.7) |       | 1.88<br>(0.47–4.32) |       |

Data listed were displayed as median (minimum-maximum)



**Supplementary Figure S1: Association of Tfh sub-populations with HCC prognosis.** (A)–(E) Association of the frequencies of Th1-like subset, Th2-like subset, Th17-like subset, ICOS<sup>+</sup> subset and central memory Tfh cells with recurrence-free survival; (F)–(J) Association of the frequencies of Th1-like subset, Th2-like subset, Th17-like subset, ICOS<sup>+</sup> subset and central memory Tfh cells with overall survival. (Patients were divided into two groups according to median frequencies of each subset).