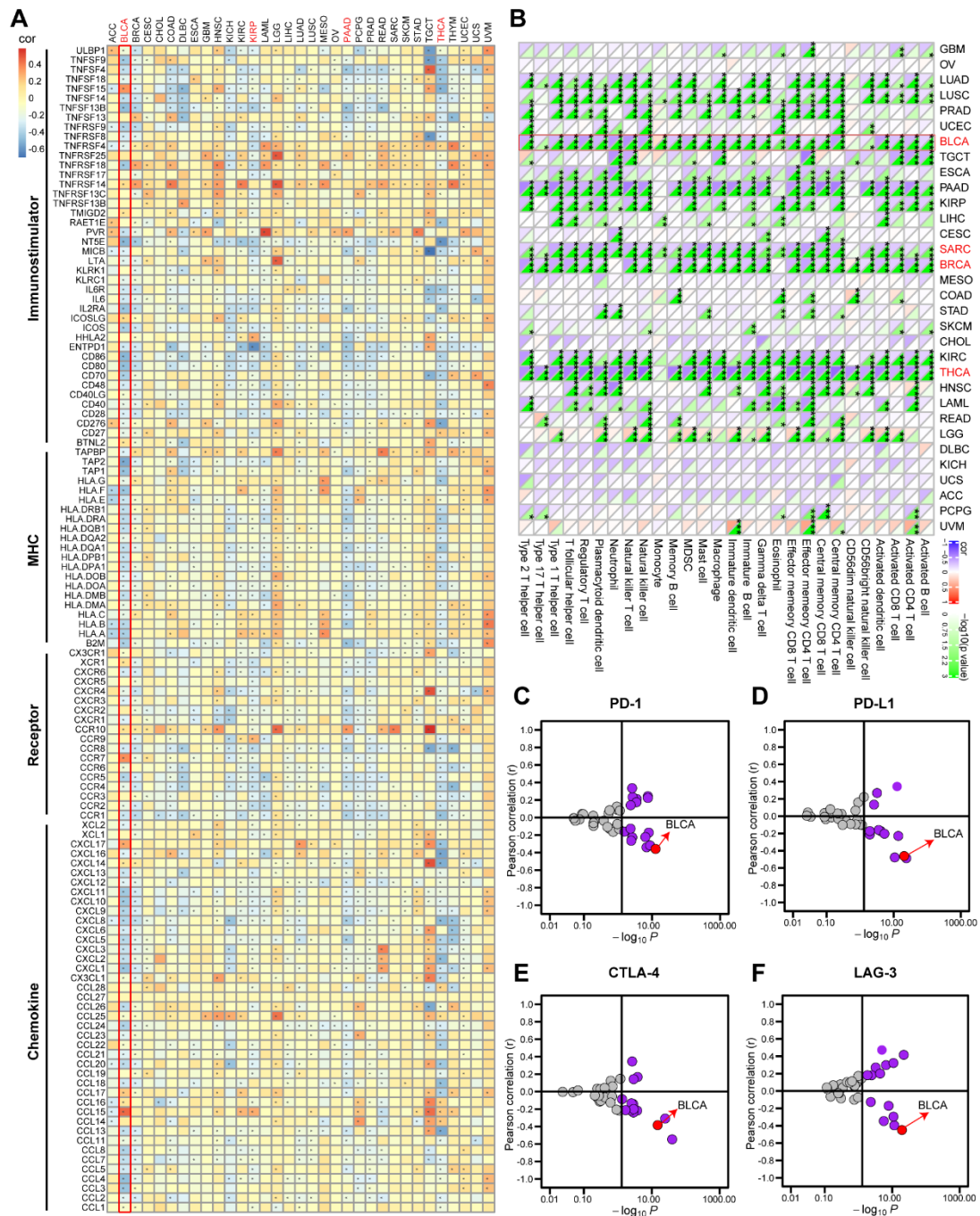


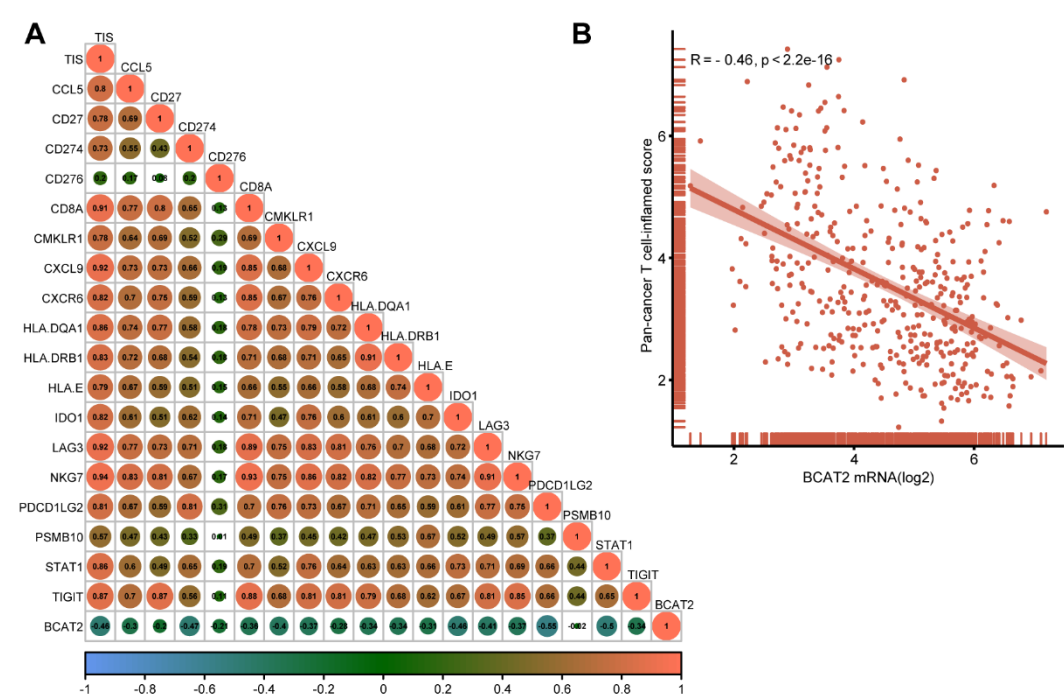
Supplementary Fig 1. Pan-cancer analysis of BCAT2 in tissues and cancer cell lines. (A) Expression patterns of BCAT2 in 33 types of cancer and adjacent normal tissues. (b) Expression pattern of BCAT2 in Xiangya BLCA cohort. (C) Expression patterns of BCAT2 in various cancer cell lines.



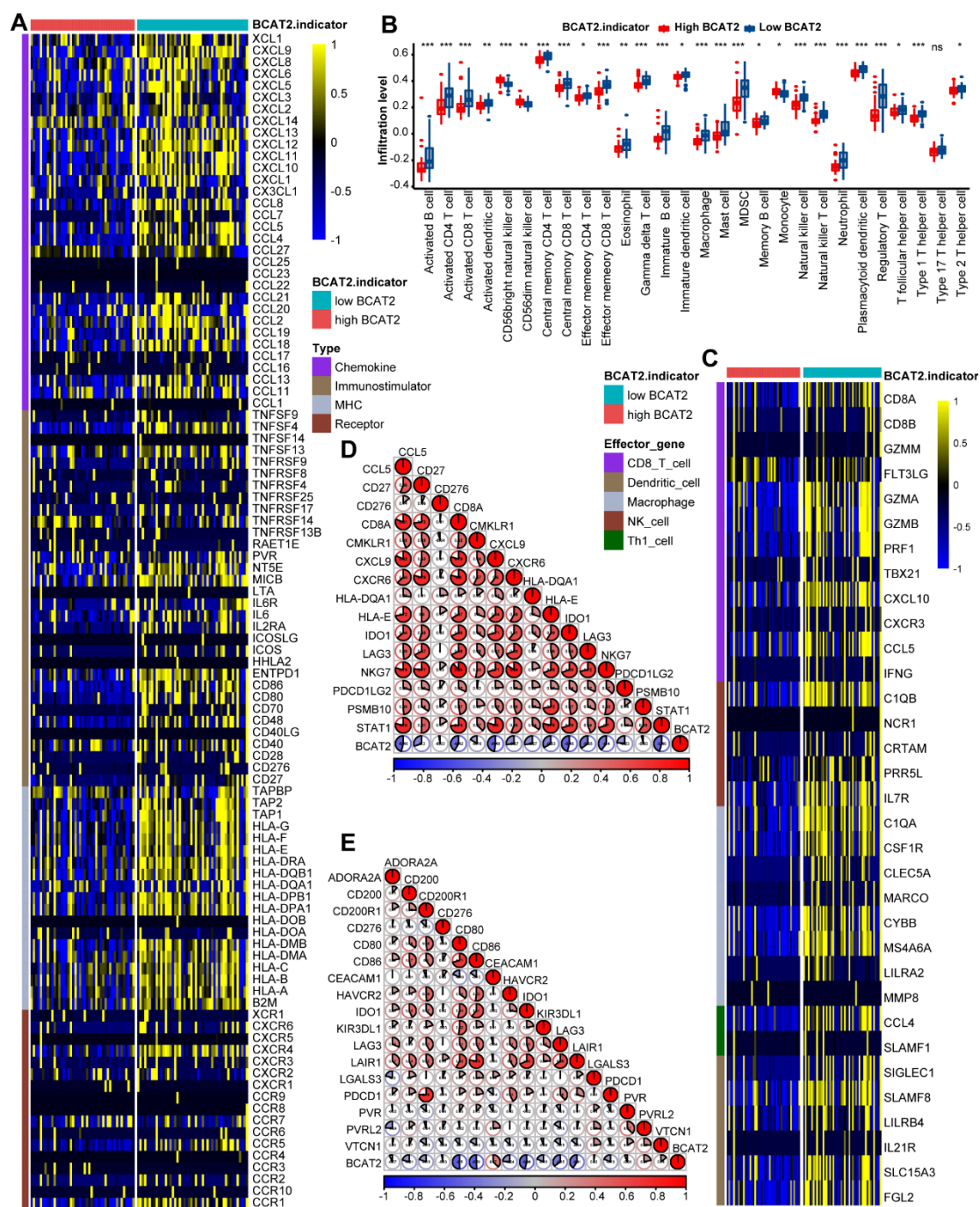
Supplementary Fig 2. Pan-cancer analysis of immunosuppressive role of BCAT2.

(A) Correlation analyses between BCAT2 and immunological related indicators in 33 types of cancer. Grid color represents positive or negative correlation. * $P < 0.05$. (B) Correlation analyses between BCAT2 and TIICs in 33 types of cancer. Grid color represents positive or negative correlation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Correlation analyses between BCAT2 and effector genes of ICB (C) PD-1 (D) PD-L1 (E) CTLA-4 (F) LAG-3 in 33 types of cancer. Purple circle means correlation with significance and grey circle means correlation with no significance. Circle in the upper

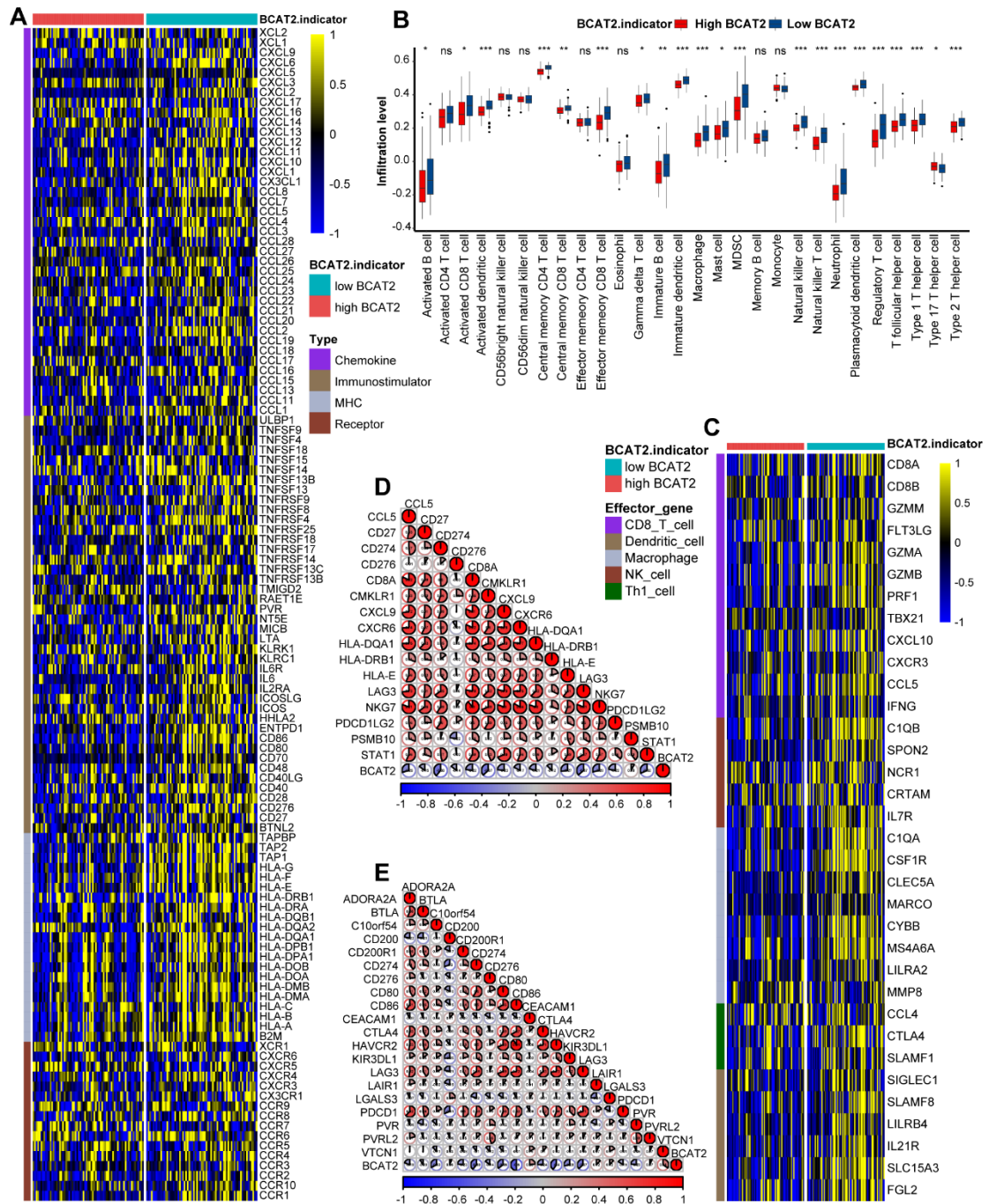
half of square represents positive relationship and circle in the bottom half square represents negative relationship. Red arrow points to BLCA.



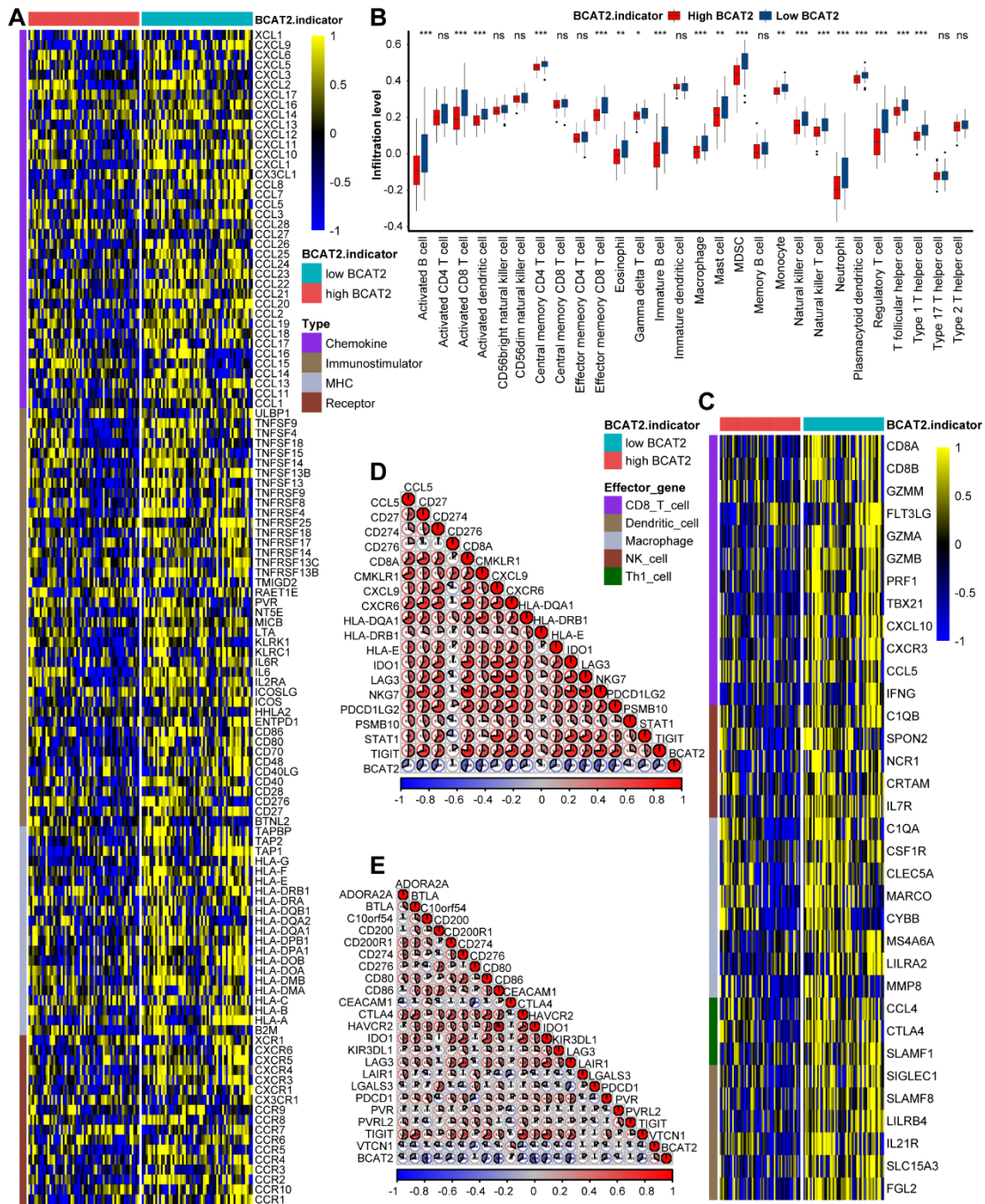
Supplementary Fig 3. Correlation between BCAT2 and TIS. (A) Correlation between BCAT2 and effector genes of TIS in BLCA. (B) Correlation between BCAT2 and TIS in pan-cancer analysis.



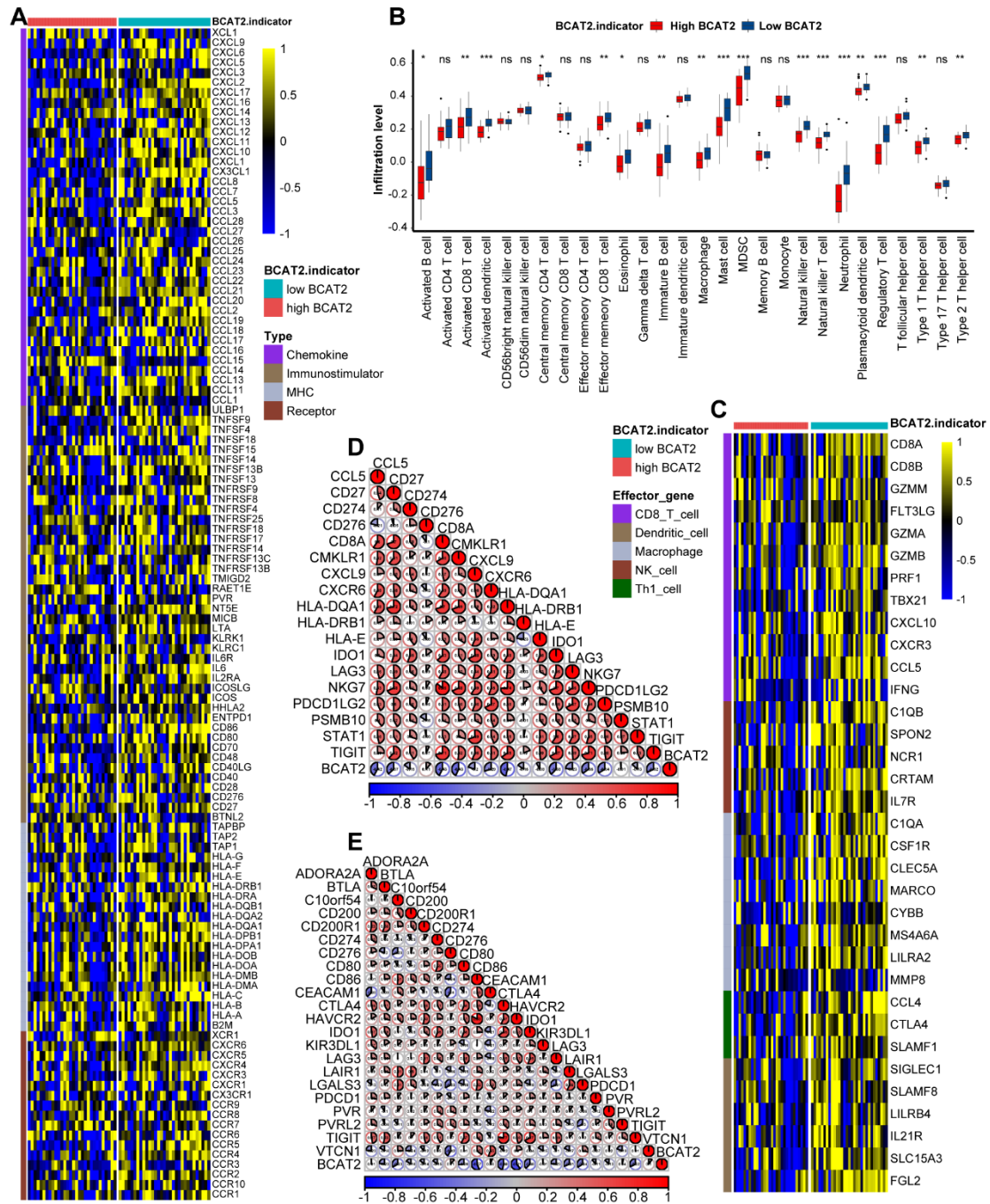
GSE31684



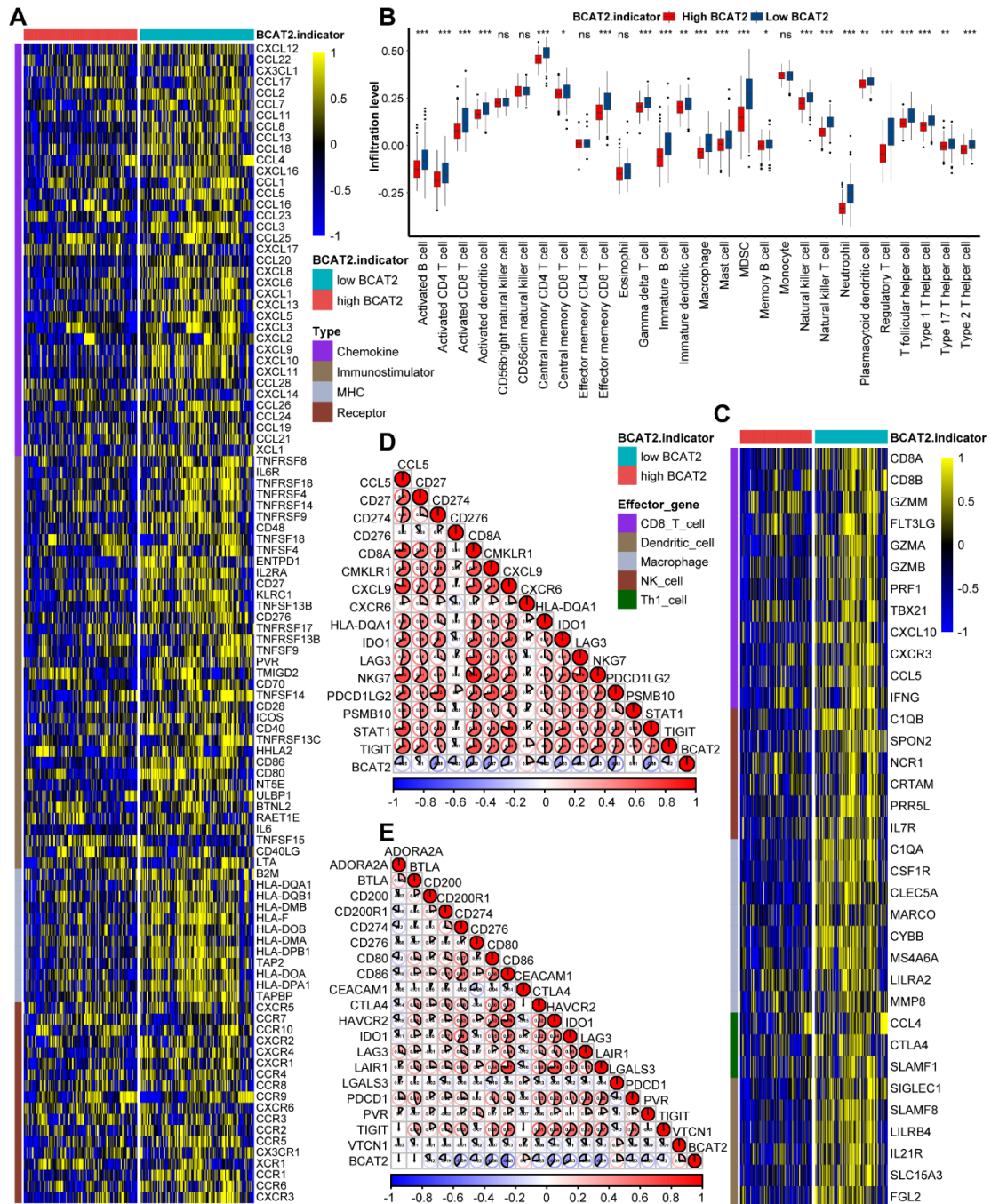
GSE48075



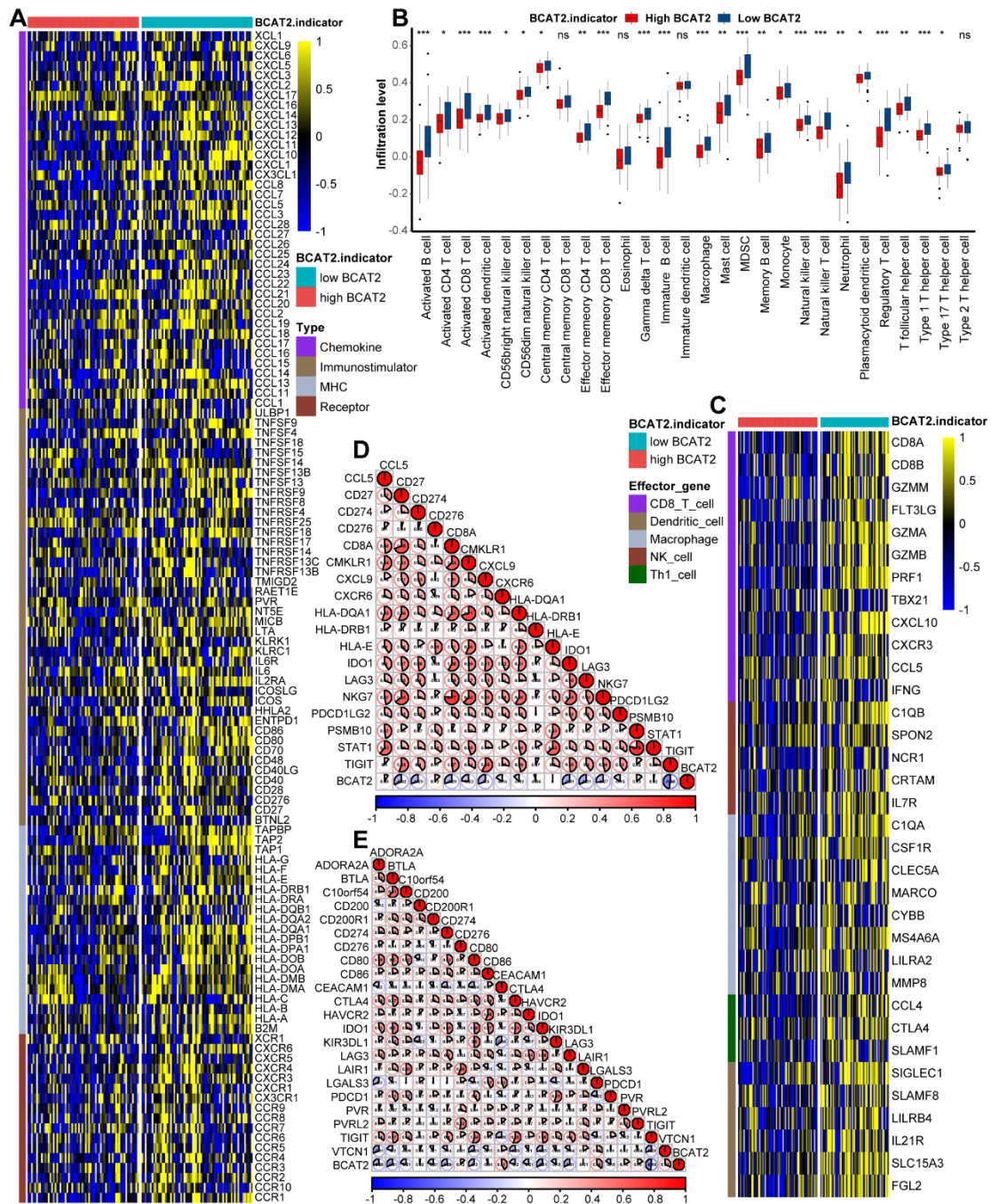
GSE48276



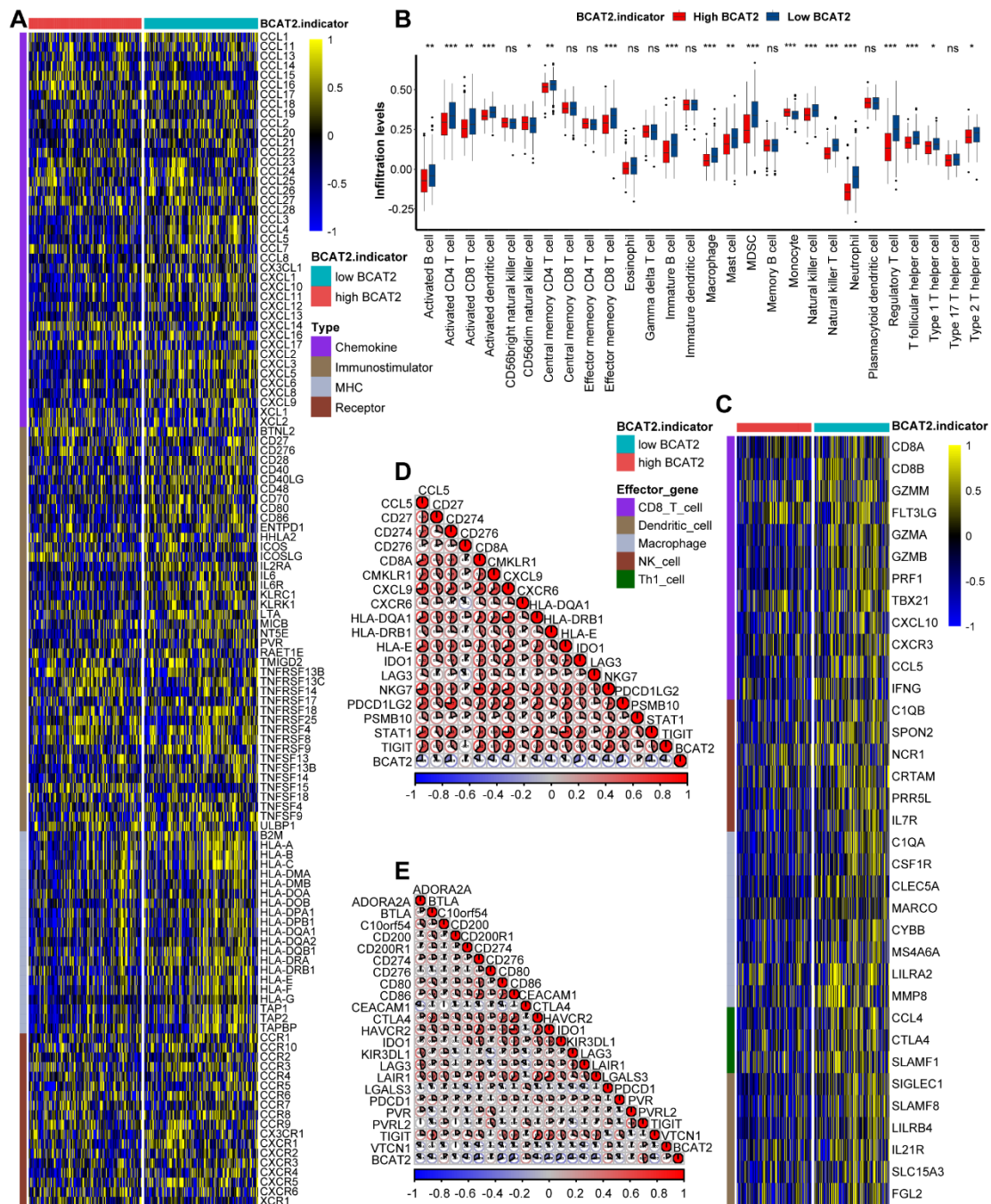
GSE69795



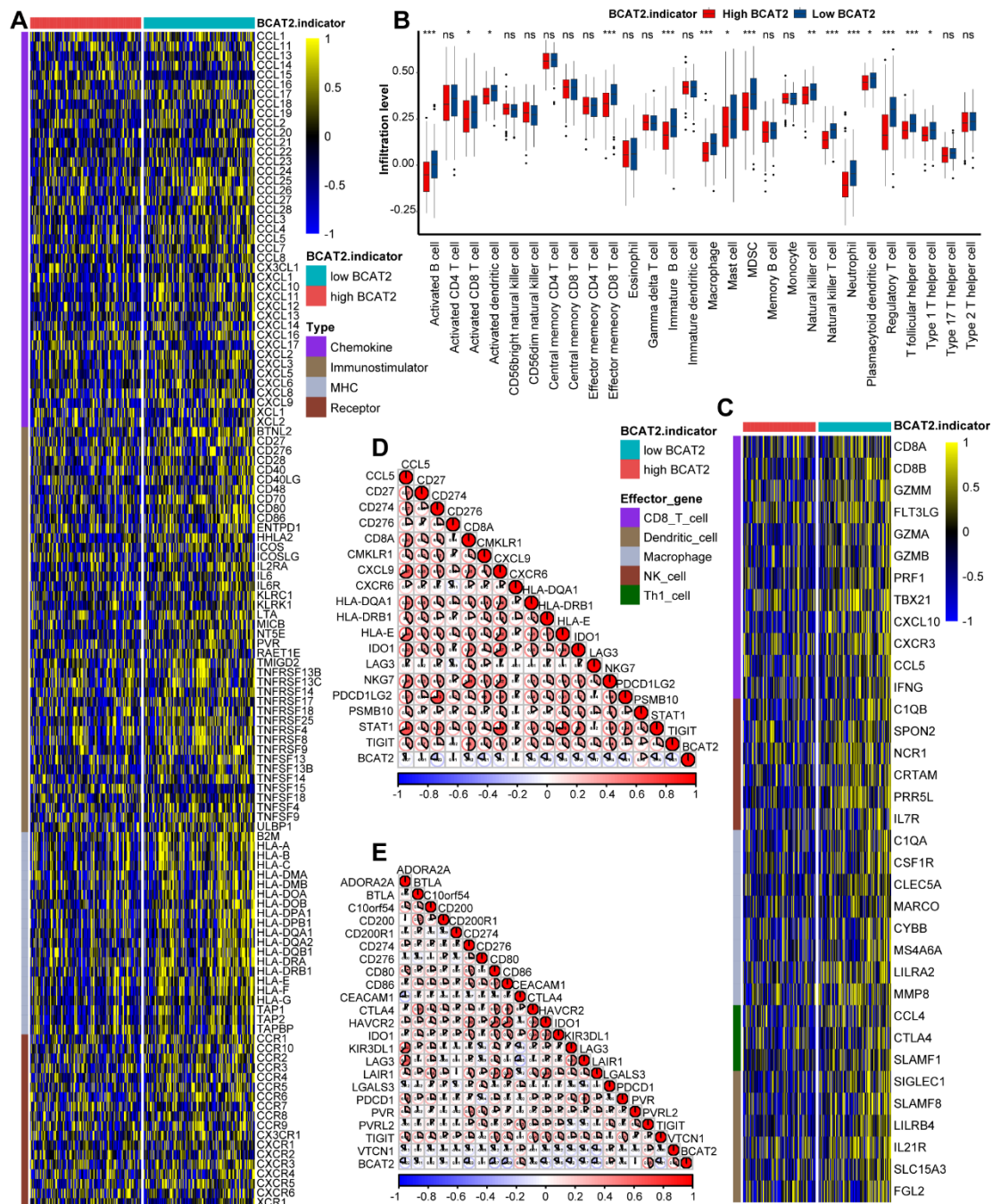
GSE83586



GSE86411



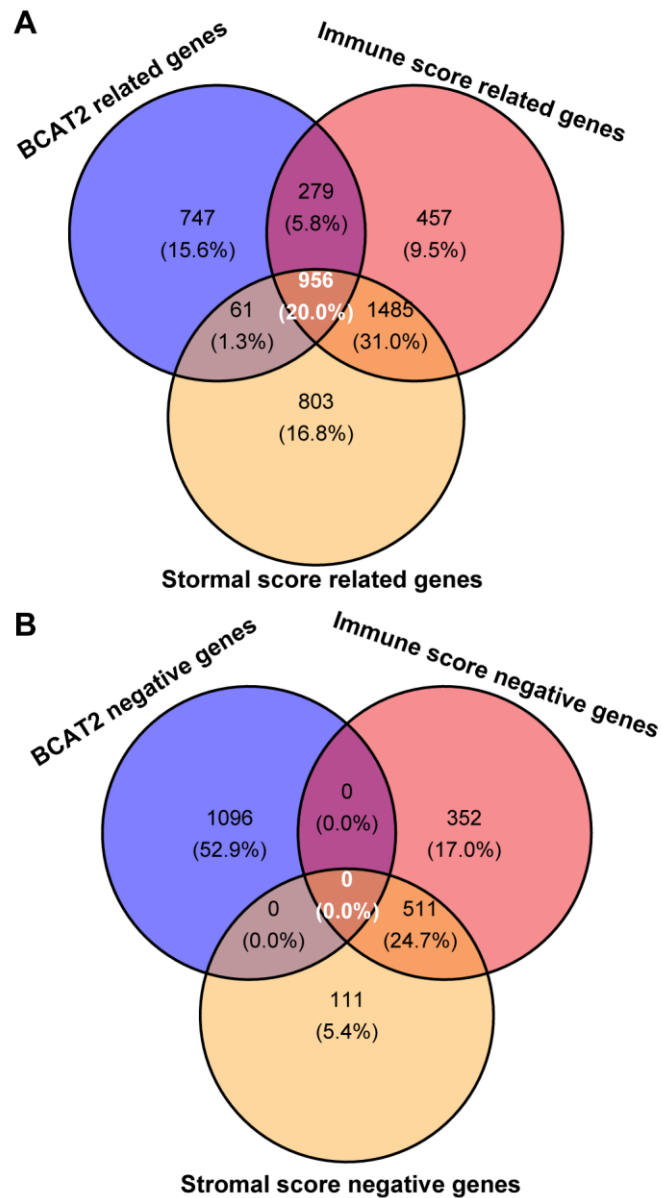
GSE87304



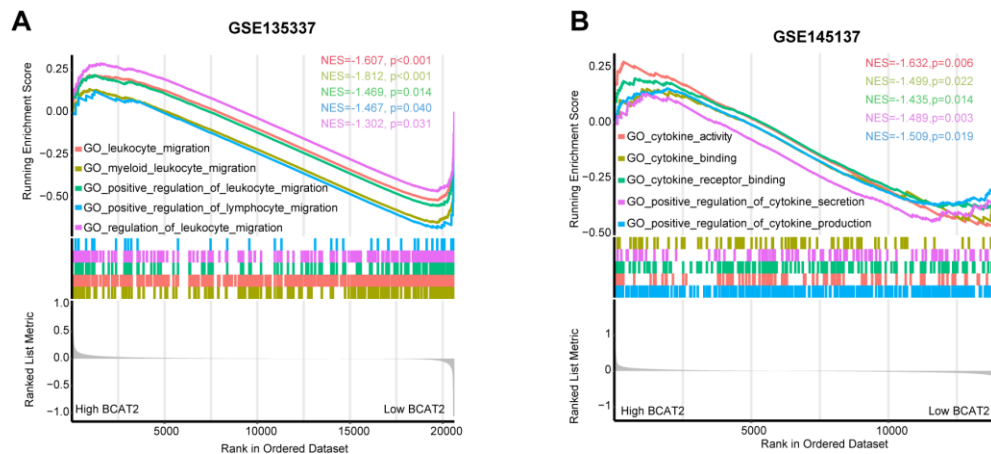
GSE128702

Supplementary Fig 4-12. Validation of immunosuppressive role of BCAT2 in nine BLCA cohorts (GSE31684, GSE32894, GSE48075, GSE48276, GSE69795, GSE83586, GSE86411, GSE87304 and GSE128702). (A) Expression patterns of chemokines, chemokine receptors, MHC effector genes and immunostimulators in high and low BCAT2 groups (B) Infiltration levels of TIICs in high and low BCAT2 groups (C) Effector genes of multiple subtypes of immune cell (CD8⁺ T cell, DC, Macrophage, NK and Th1) in high and low BCAT2 groups (D) Correlation between BCAT2 and TIS

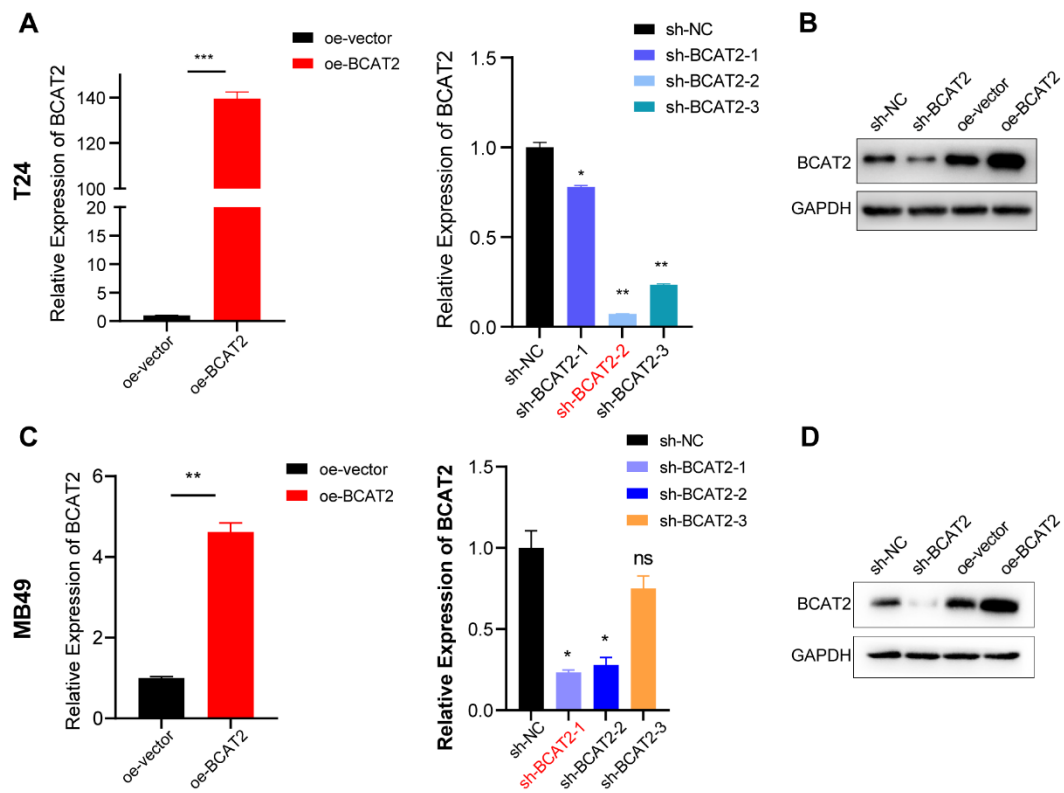
score (E) Correlation between BCAT2 and inhibitory immune checkpoints. Number in the circle means correlation coefficient. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns: no significance.



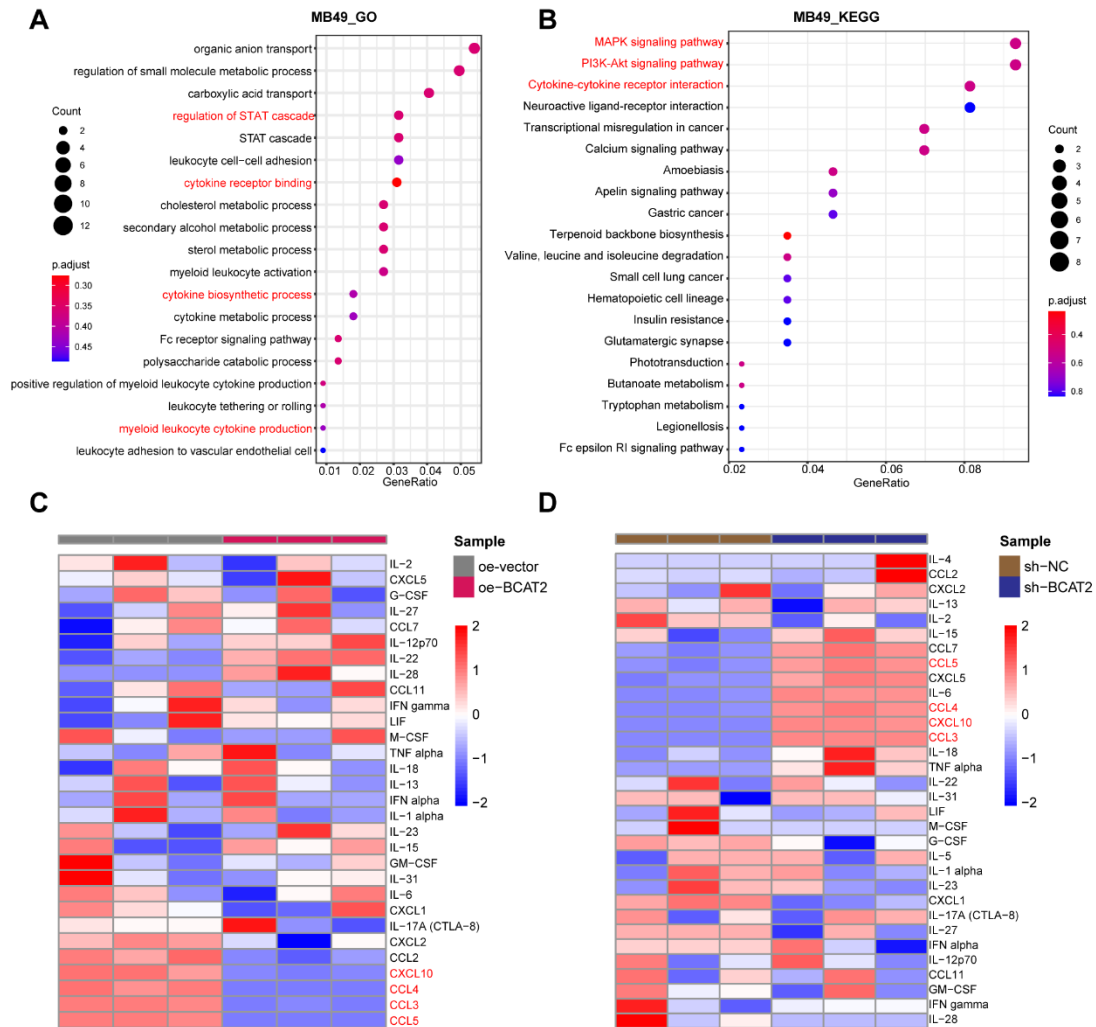
Supplementary Fig 13. Overlap of BCAT2, immune score and stromal score related genes. (A) Intersection of BCAT2, immune score and stromal score related genes. (B) Intersection of BCAT2, immune score and stromal score negative-related genes.



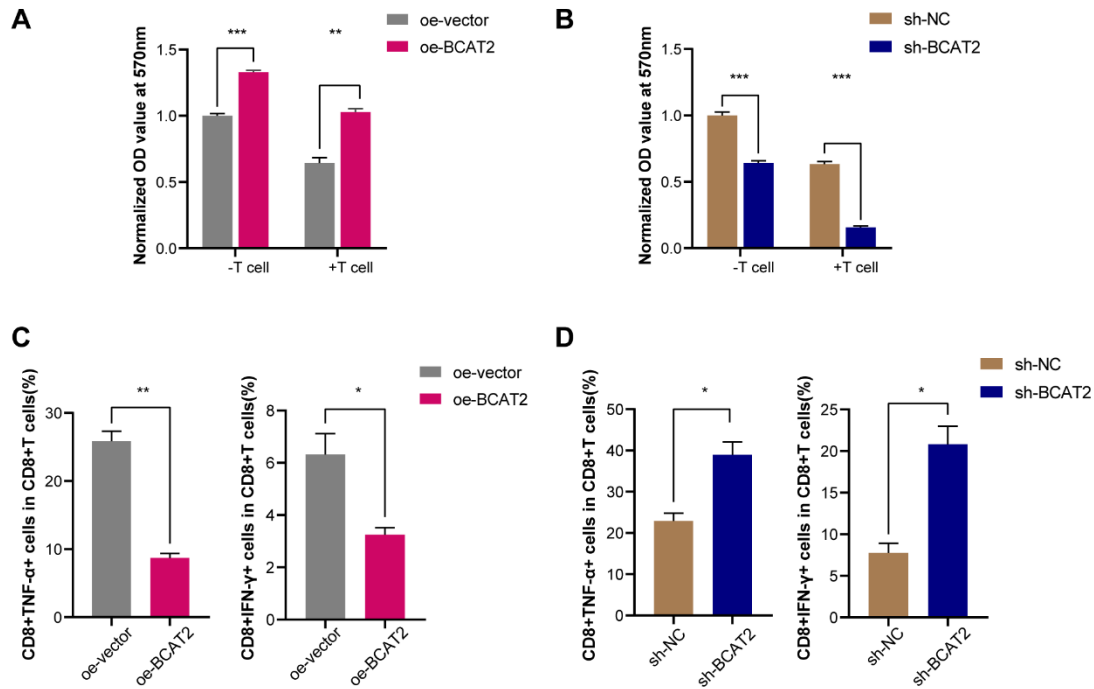
Supplementary Fig 14. GSEA analyses of differences on activity of TME related pathways between high and low BCAT2 groups by scRNA-seq. (A) GSEA analyses of GO terms indicate different activities of immune cell migration related pathways between high and low BCAT2 groups in GSE135337 scRNA cohort (B) GSEA analyses of GO terms indicate different activities of cytokine related pathways between high and low BCAT2 groups in GSE145137 scRNA cohort.



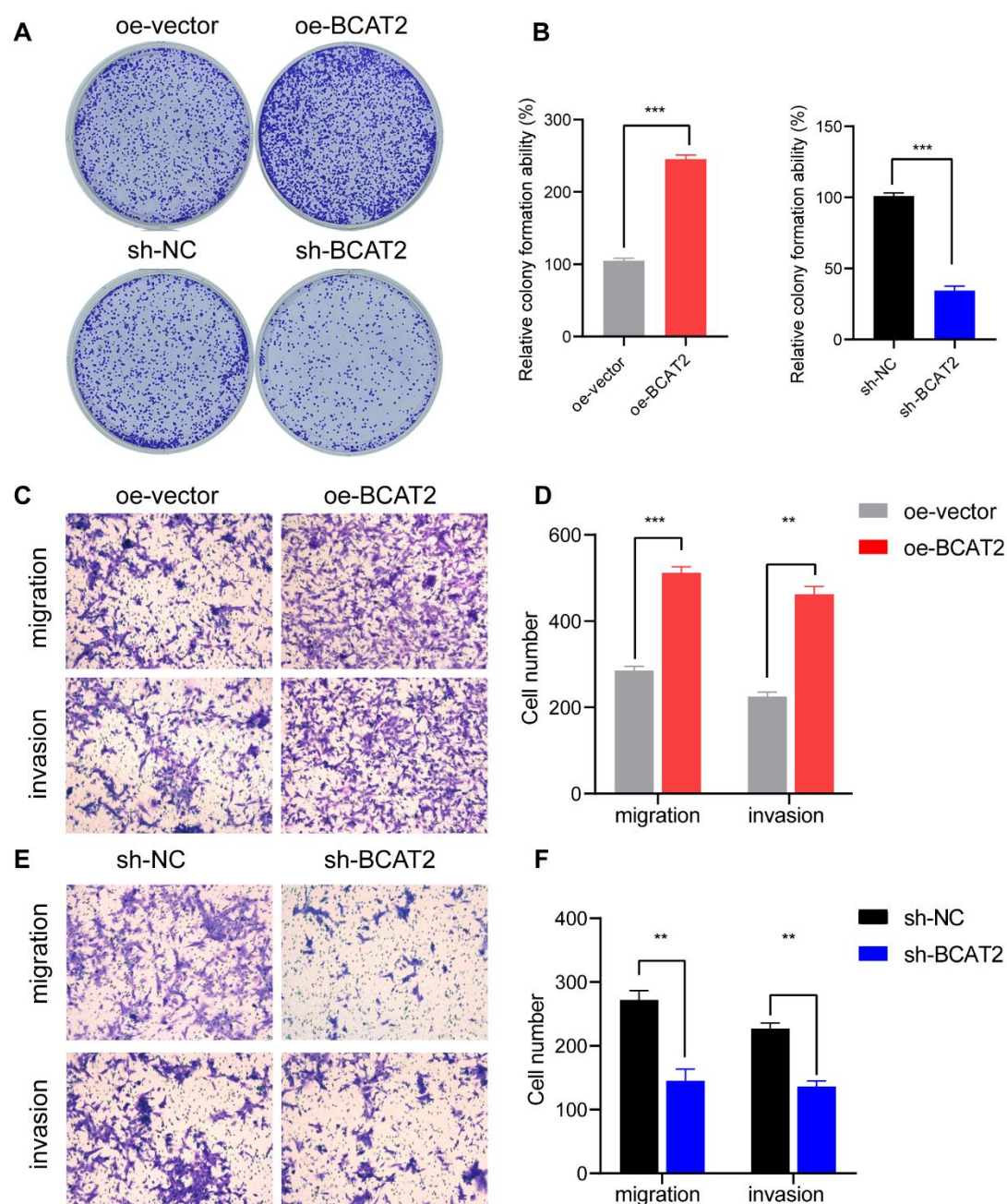
Supplementary Fig 15. Validation of BCAT2 OE and BCAT2 KD cell lines. (A) Validation of BCAT2 expression level in T24 cell line using qRT-PCR. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (B) Validation of BCAT2 expression level in T24 cell line using Western blot. (C) Validation of BCAT2 expression level in MB49 cell line using qRT-PCR. * $P < 0.05$, ** $P < 0.01$, ns: no significance. (D) Validation of BCAT2 expression level in MB49 cell line using Western blot.



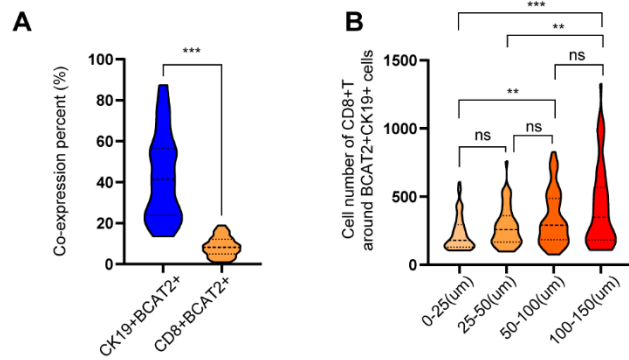
Supplementary Fig 16. Exploring the role of BCAT2 in TME by murine cell line. (A-B) GO and KEGG analyses of DEGs between murine BCAT2 OE and BCAT2 KD cell lines. (C-D) ProcartaPlex multiple immunoassays detected secretion levels of cytokines and chemokines in BCAT2 OE, BCAT2 KD and negative control cell lines.



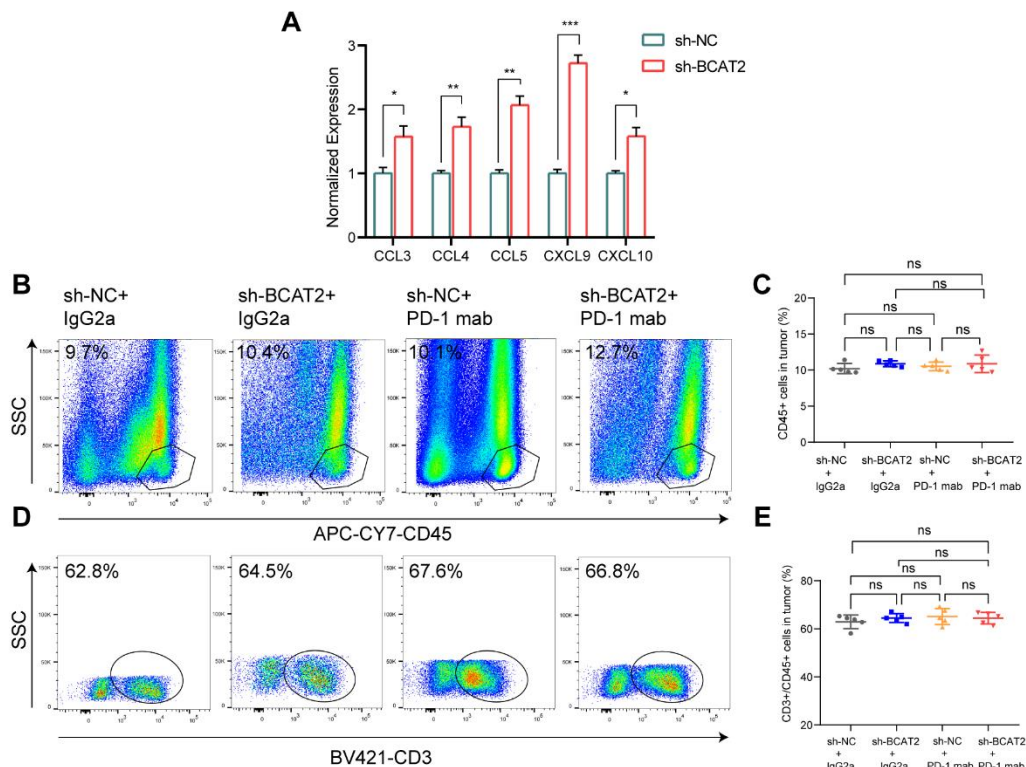
Supplementary Fig 17. The variation of cytotoxicity of T cells and activity of CD8+T cells in different coculture system. (A) Normalized OD value at 570 nm of remained cancer cells (BCAT2-OE and negative control) with and without coculturing with T cells (n=3 per group). **P<0.01, ***P<0.001. (B) Normalized OD value at 570 nm of remained cancer cells (BCAT2-KD and negative control) with and without coculturing with T cells (n=3 per group). ***P<0.001. (C-D) Flow cytometry analysis of proportions of CD8⁺TNF-α⁺ T cells and CD8⁺IFN-γ⁺ T cells in different coculture systems (n=3 per group). *P<0.05, **P<0.01.



Supplementary Fig 18. The oncogenic role of BCAT2 in vitro. (A-B) Overexpression of BCAT2 significantly enhanced proliferation ability of T24 and knock down of BCAT2 significantly inhibited proliferation ability of T24. ***P < 0.001. (C-F) Overexpression of BCAT2 significantly stimulated migration and invasion abilities of T24 and knock down of BCAT2 significantly impaired migration and invasion abilities of T24. **P < 0.01, ***P < 0.001.

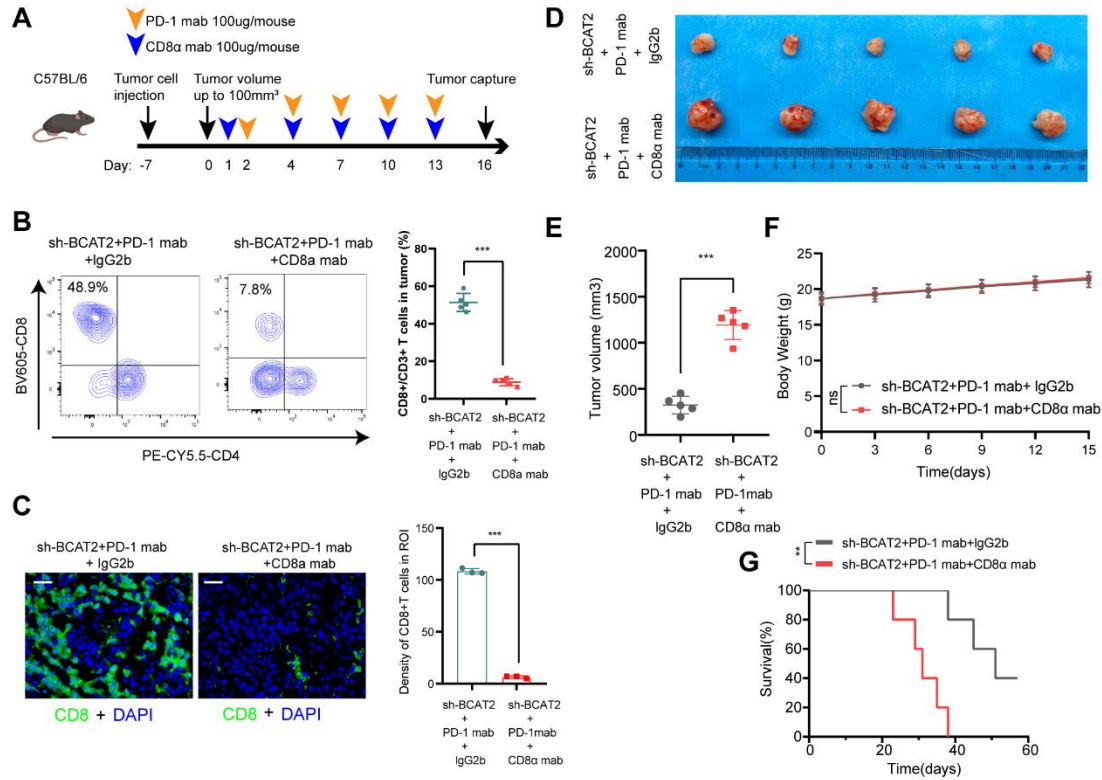


Supplementary Fig 19. Exploring exclusive spatial relationship between BCAT2⁺ tumor cells and CD8⁺T cells on human tissue level. (A) Co-expression proportions of BCAT2⁺CK19⁺ cells and BCAT2⁺CD8⁺ cells in TMA of Xiangya BLCA cohort (n=56). ***P<0.001. (B) Multi-dimensional distance gradient analyses of CD8⁺T cells around BCAT2⁺ tumor cells in TMA of Xiangya BLCA cohort (n=56). **P<0.01, ***P<0.001, ns: no significance.

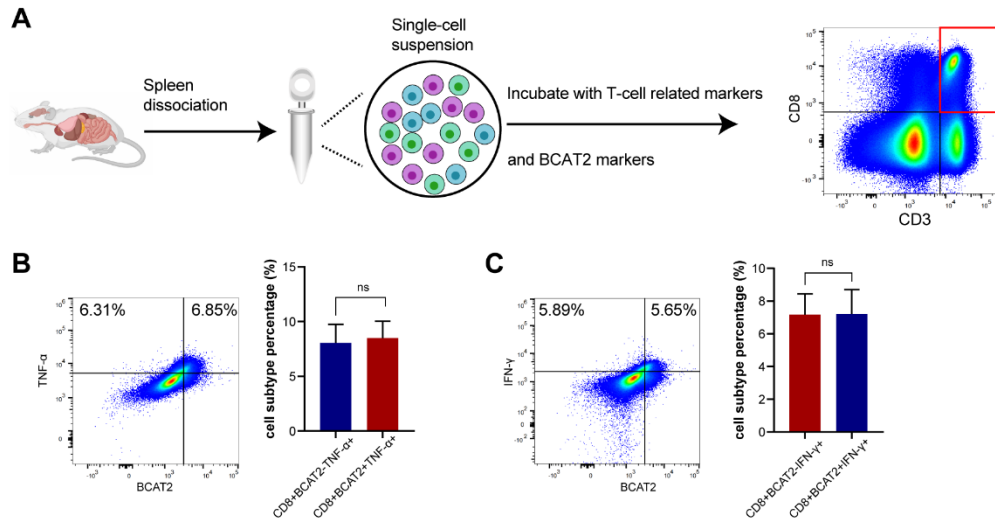


Supplementary Fig 20. The role of BCAT2 on CD8⁺T cell related chemokines, leukocytes and T cells in vivo. (A) Exploring the role of BCAT2 on CD8⁺T cell related chemokines in vivo by qRT-PCR (n=3 per group). *p<0.05, **p<0.01, ***p<0.001. (B-

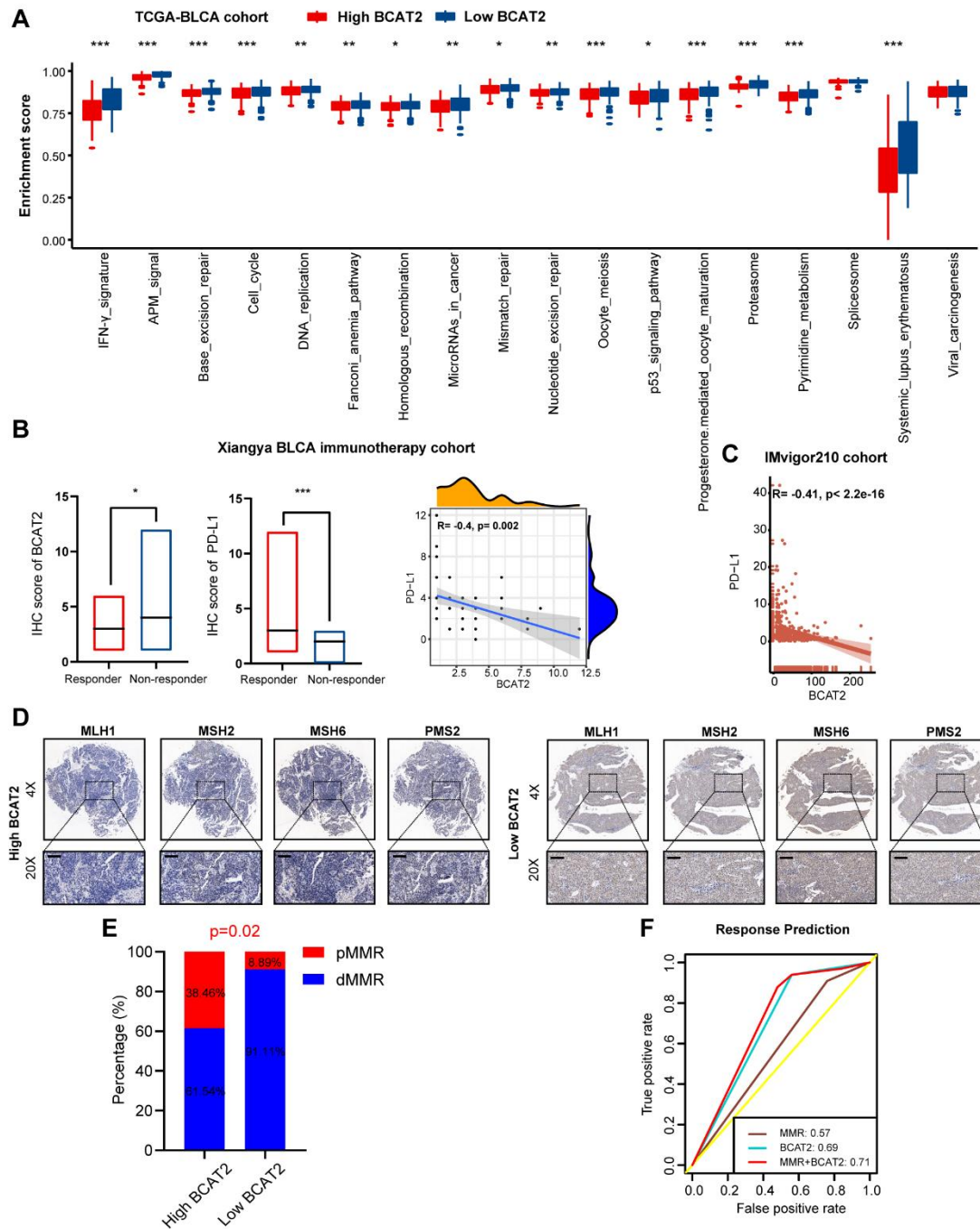
C) Flow cytometry analysis of proportion of leukocytes (CD45⁺) (n=5 per group) and (D-E) T cells (CD45⁺CD3⁺) in different therapy regimens (n=5 per group). ns: no significance.



Supplementary Fig 21. CD8⁺T cells act an indispensable part in synergistic effect of cotreatment. (A) Flow diagram of treatment plan. (B) Validation of successful depletion of CD8⁺T cells (n=5 per group) by flow cytometry analysis. ***P<0.001. (C) Validation of successful depletion of CD8⁺T cells by IF (n=3 per group). Scale bar: 20um. ***P<0.001. (D) Subcutaneous tumor model of different therapy regimens. (E) Tumor volume (F) body weight and (G) survival time of tumor-bearing mice (n=5 per group) in different therapy regimens. **P<0.01, ***P<0.001, ns: no significance.

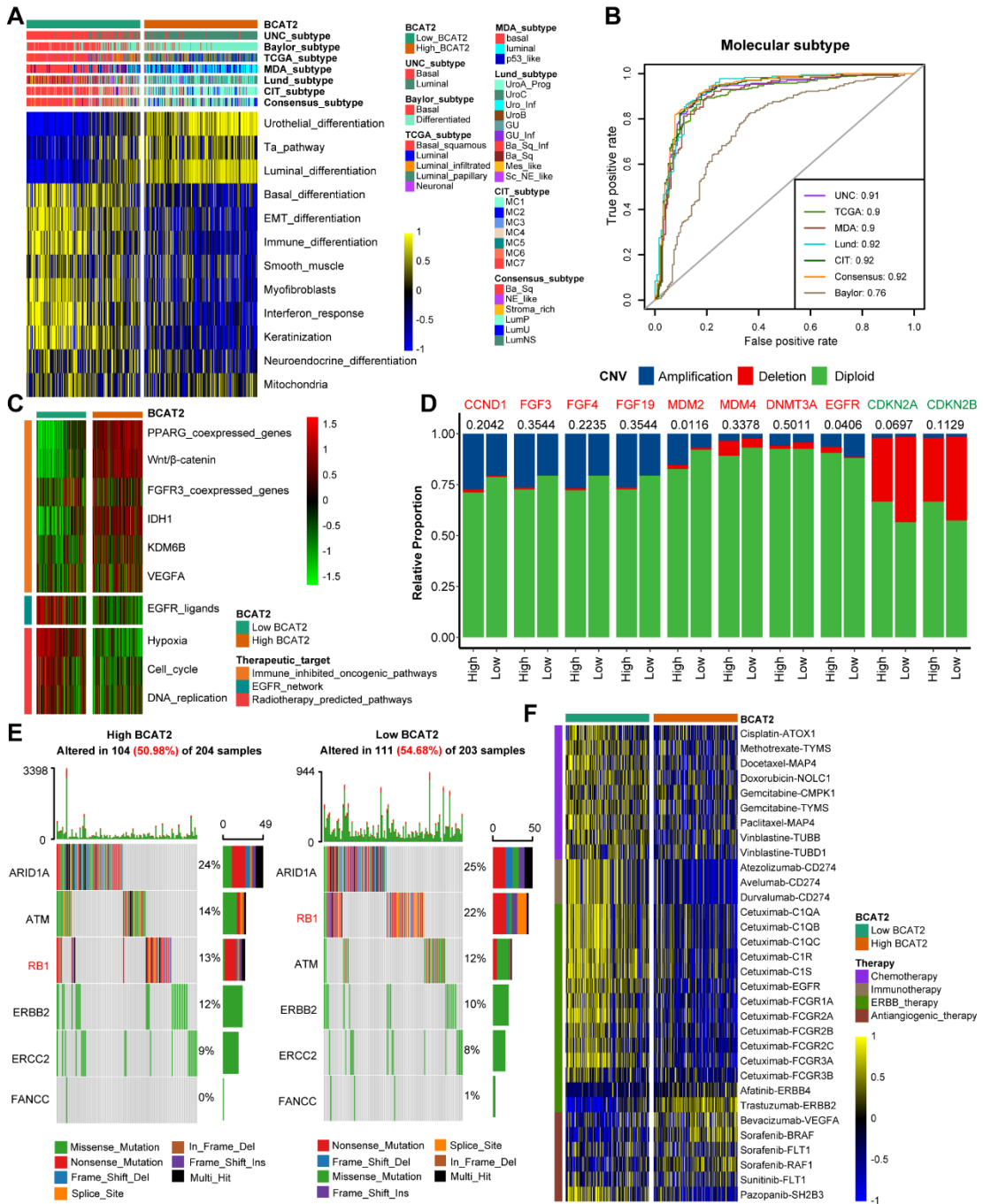


Supplementary Fig 22. The interaction between expression level of BCAT2 on CD8⁺T cell and activity of CD8⁺T cell. (A) Flow diagram of interaction exploration. (B) Proportions of CD8⁺TNF- α ⁺ T cells and (C) CD8⁺IFN- γ ⁺ T cells between BCAT2⁺CD8⁺ and BCAT2⁻CD8⁺ groups (n=3 per group). ns: no significance.



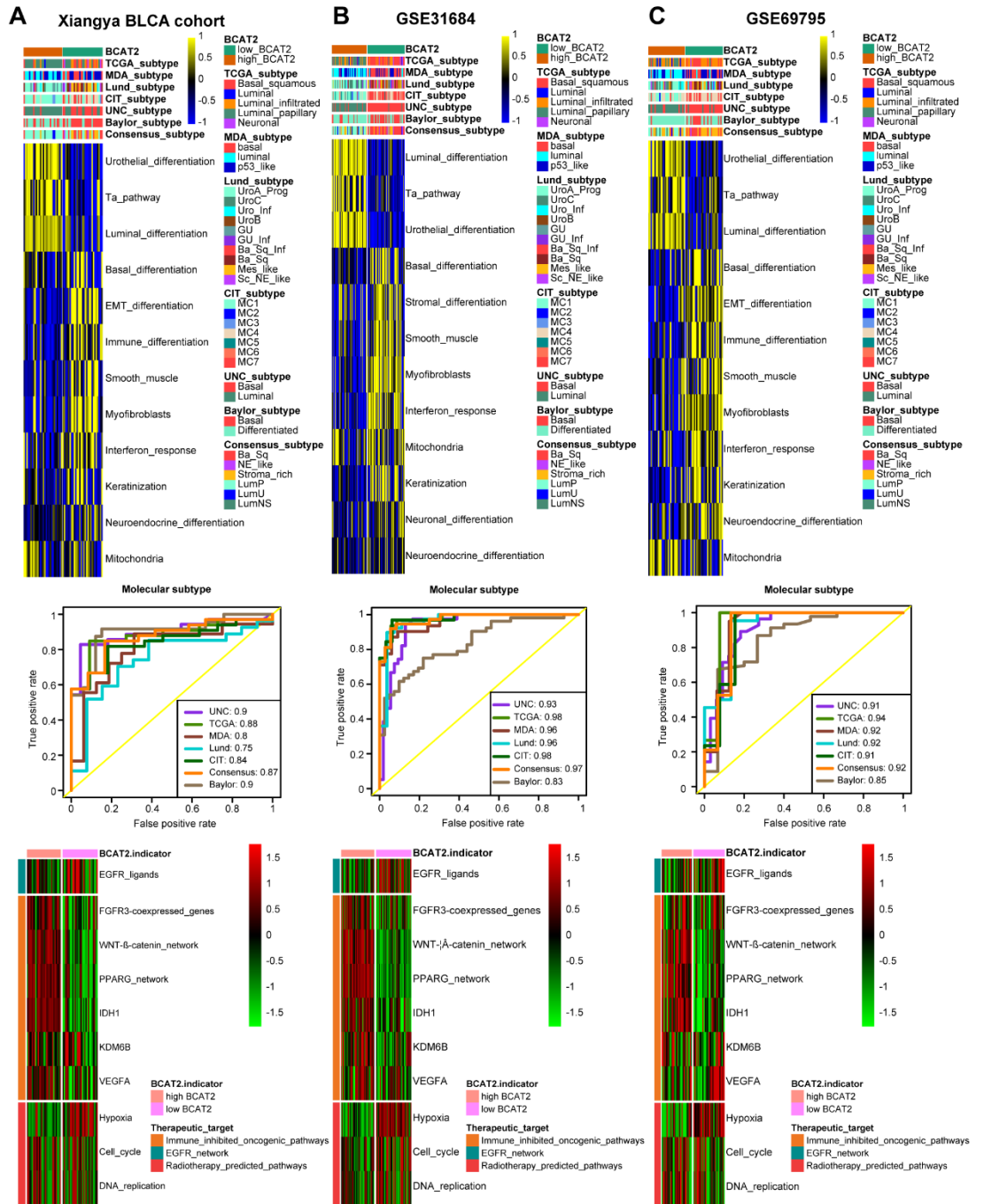
Supplementary Fig 23. The potential of BCAT2 in predicting efficacy of immunotherapy. (A) Enrichment scores of immunotherapy-related pathways between high and low BCAT2 groups in TCGA-BLCA cohort. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (B) IHC score and correlation of BCAT2 and PD-L1 in Xiangya BLCA immunotherapy cohort. * $P < 0.05$, *** $P < 0.001$. (C) Correlation between BCAT2 and PD-L1 in IMvigor210 cohort. (D) IHC image (scale bar: 50um) of marker genes of MMR and (E) difference of MMR's status (pMMR and dMMR) between high and low BCAT2 groups in Xiangya BLCA immunotherapy cohort. (F) Prediction accuracy of MMR, BCAT2

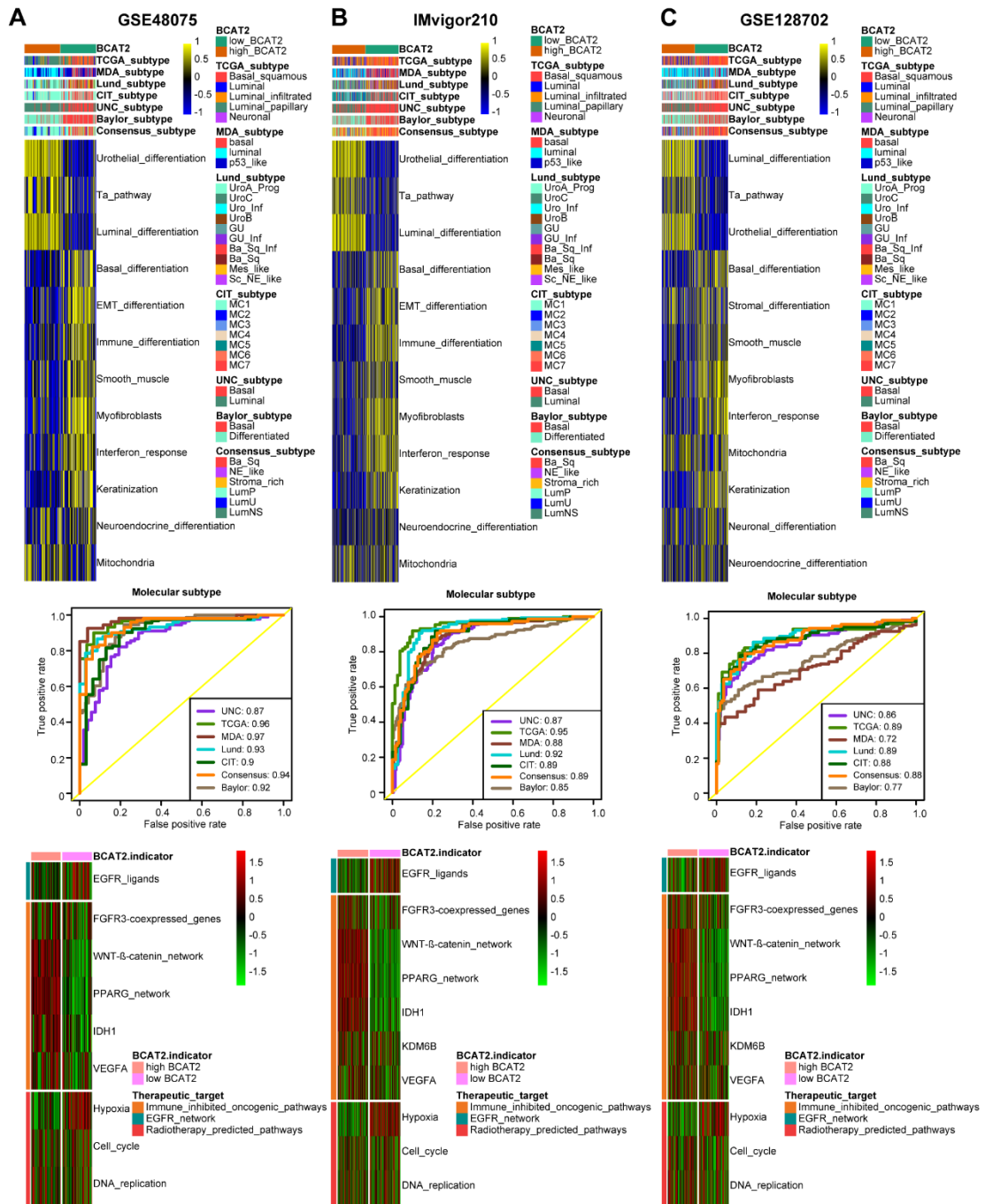
and combination index (MMR+BCAT2) in Xiangya BLCA immunotherapy cohort.

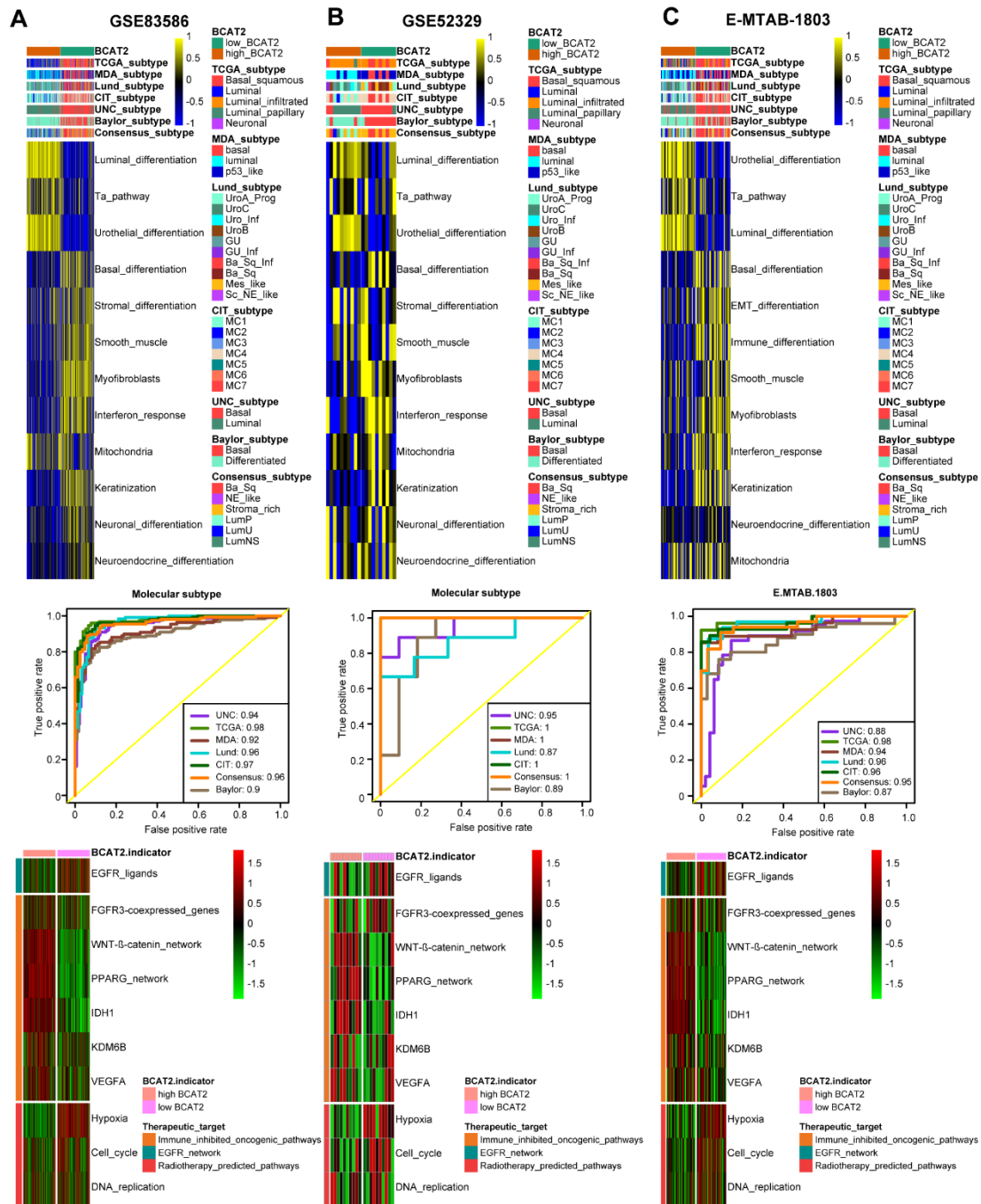


Supplementary Fig 24. The value of BCAT2 in forecasting molecular subtype and guiding precision therapy. (A) The effect of different expression level of BCAT2 on molecular classification systems and activities of basal and luminal related pathways. (B) Prediction accuracy of BCAT2 on molecular subtypes. (C) Activities of EGFR target therapy and radiotherapy related pathways in high and low BCAT2 groups (D) CNV rates of HPD associated biomarkers in different expression of BCAT2. Positive

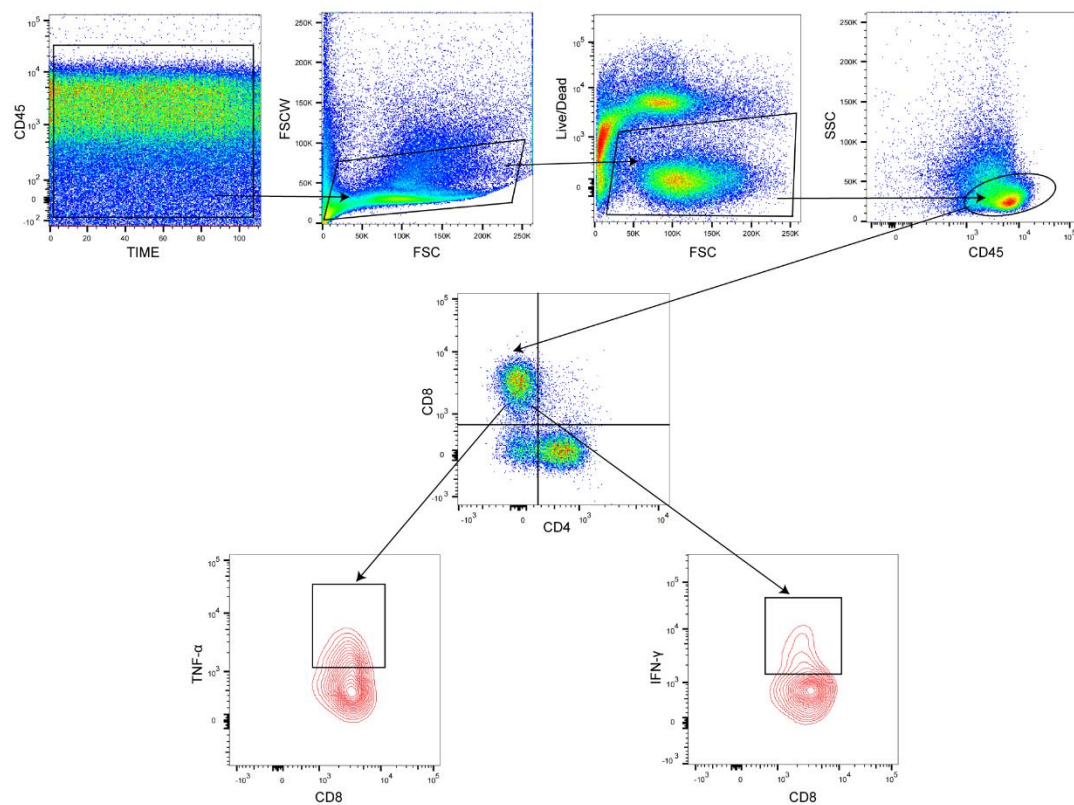
effector genes of HPD were marked in red and negative effector genes of HPD were marked in green. (E) Mutation rates of NAC related genes in high and low BCAT2 groups. (F) The effect of different expression level of BCAT2 on effector genes of chemotherapy, immunology, ERBB therapy and antiangiogenic therapy.



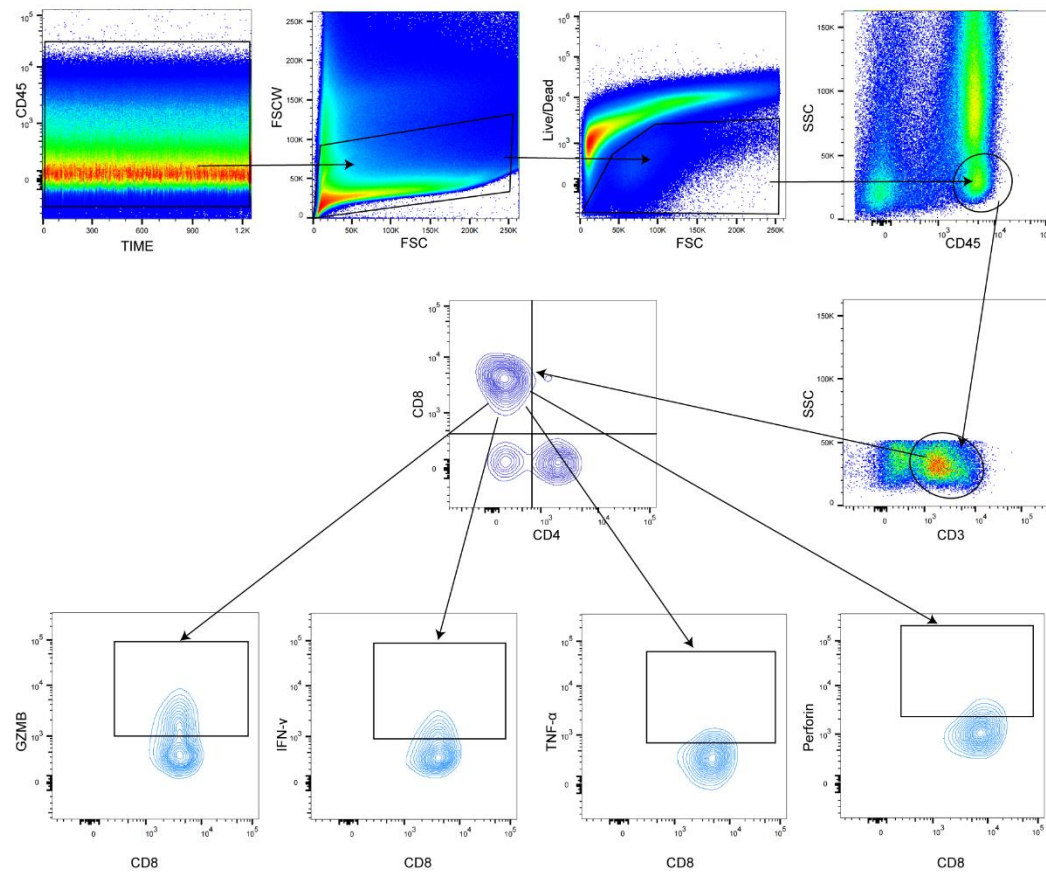




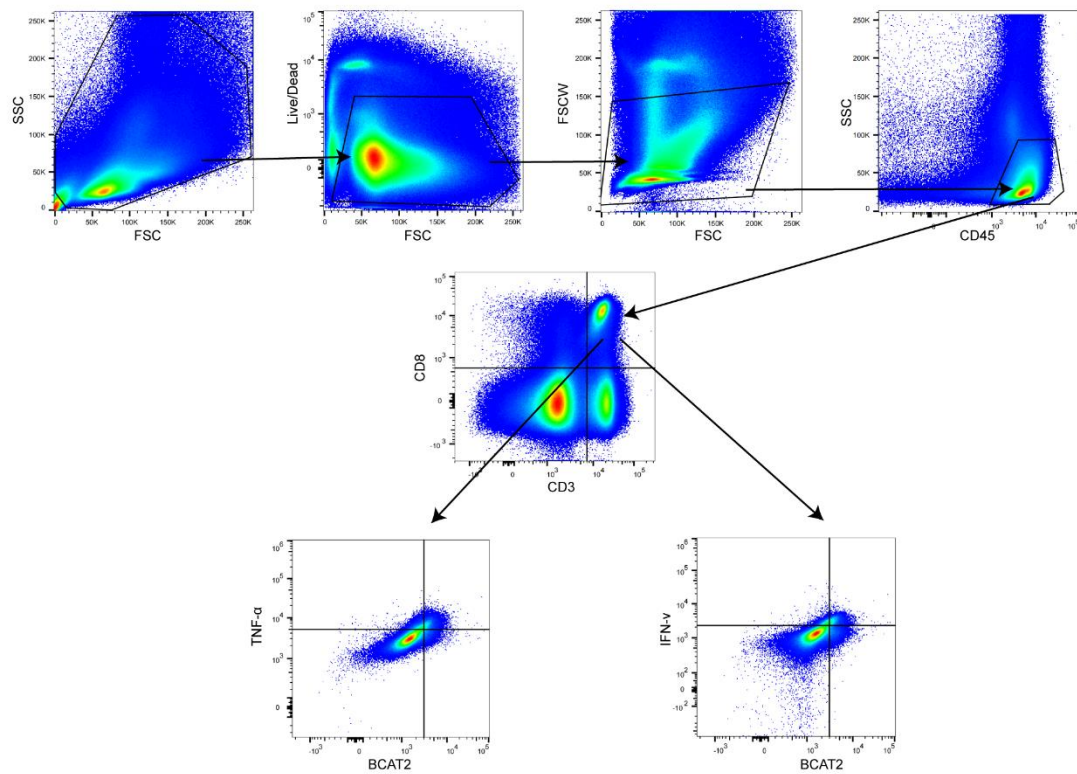
Supplementary Fig 25-27. Validation of prediction accuracy of BCAT2 on molecular classification and treatment sensitivity in nine BLCA cohorts (Xiangya BLCA cohort, GSE31684, GSE69795, GSE48075, IMvigor210, GSE128702, GSE83586, GSE52329 and E-MTAB-1803). (A) The effect of different expression level of BCAT2 on molecular classification systems and activities of basal and luminal related pathways. (B) Prediction accuracy of BCAT2 on molecular subtypes. (C) Activities of EGFR target therapy and radiotherapy related pathways in high and low BCAT2 groups.



Supplementary Fig 28. Gate strategy of flow cytometry analysis on coculture assay.



Supplementary Fig 29. Gate strategy of flow cytometry analysis on subcutaneous tumorigenesis model



Supplementary Fig 30. Gate strategy of flow cytometry analysis on the interaction between expression level of BCAT2 on CD8⁺T cell and activity of CD8⁺T cell.

Supplementary Table 1. The clinicopathological data of Xiangya BLCA cohort.

| ID | Age | Gender | Overall survival (day) | Pathological grade | Pathological stage |
|--------|-----|--------|---------------------------|--------------------|--------------------|
| XYBC-1 | 58 | Female | 377 | High | T4N0M0 |
| XYBC-2 | 50 | male | 1518 | High | T2N1M0 |
| XYBC-3 | 63 | male | 946 | High | T1N0M0 |
| XYBC-4 | 53 | male | 1258 | High | T1N0M0 |
| XYBC-5 | 46 | male | 1213 | High | T3bN1M0 |
| XYBC-6 | 72 | Female | 1192 | Low | T1N0M0 |
| XYBC-7 | 69 | Female | 1047 | High | T3N0M0 |
| XYBC-8 | 60 | male | 970 | High | T1N0M0 |
| XYBC-9 | 57 | male | 443 | High | T3N0M0 |

| | | | | | |
|---------|----|--------|-----|------|---------|
| XYBC-10 | 70 | male | 975 | Low | T1N0M0 |
| XYBC-11 | 54 | male | 877 | High | T1N0M0 |
| XYBC-12 | 61 | male | 911 | High | T1N0M0 |
| XYBC-13 | 67 | male | 914 | High | T2N0M0 |
| XYBC-14 | 62 | male | 662 | High | T3N0M0 |
| XYBC-15 | 55 | male | 905 | High | T1N0M0 |
| XYBC-16 | 66 | male | 423 | High | T2aN0M0 |
| XYBC-17 | 66 | male | 851 | High | T1N0M0 |
| XYBC-18 | 45 | male | 876 | High | T4N0M0 |
| XYBC-19 | 71 | male | 857 | High | T2N0M0 |
| XYBC-20 | 74 | male | 807 | High | T3bN1M0 |
| XYBC-21 | 63 | male | 797 | High | T4N0M0 |
| XYBC-22 | 62 | Female | 766 | High | T2N0M0 |
| XYBC-23 | 55 | male | 781 | Low | T1N0M0 |
| XYBC-24 | 59 | male | 881 | High | T4N0M0 |
| XYBC-25 | 70 | male | 797 | High | T4N1M0 |
| XYBC-26 | 54 | male | 736 | High | T1N0M0 |
| XYBC-27 | 69 | male | 736 | High | T2N0M0 |
| XYBC-28 | 73 | male | 859 | High | T2bN0M0 |
| XYBC-29 | 75 | male | 659 | High | T1N0M0 |
| XYBC-30 | 77 | male | 671 | High | T3N1M0 |
| XYBC-31 | 50 | Female | 689 | High | T3N0M0 |
| XYBC-32 | 66 | male | 645 | Low | T1N0M0 |
| XYBC-33 | 77 | Female | 653 | High | T3N0M0 |
| XYBC-34 | 60 | male | 646 | High | T1N0M0 |
| XYBC-35 | 70 | male | 622 | High | T2N0M0 |
| XYBC-36 | 60 | male | 147 | High | T3N0M0 |
| XYBC-37 | 80 | male | 972 | High | T3N0M0 |
| XYBC-38 | 66 | male | 606 | High | T2bN0M0 |

| | | | | | |
|---------|----|--------|-----|------|---------|
| XYBC-39 | 77 | male | 592 | Low | T1N0M0 |
| XYBC-40 | 83 | male | 565 | Low | T1N0M0 |
| XYBC-41 | 73 | male | 592 | Low | T1N0M0 |
| XYBC-42 | 70 | male | 549 | High | T2N0M0 |
| XYBC-43 | 33 | male | 556 | Low | T1N0M0 |
| XYBC-44 | 45 | male | 526 | High | T4N0M0 |
| XYBC-45 | 56 | male | 523 | High | T4N0M1 |
| XYBC-46 | 64 | male | 534 | High | T2N0M1 |
| XYBC-47 | 65 | Female | 239 | High | T3aN1M1 |
| XYBC-48 | 78 | male | 299 | High | T2bN0M0 |
| XYBC-49 | 67 | Female | 725 | Low | T1N0M0 |
| XYBC-50 | 71 | male | 159 | High | T4N0M1 |
| XYBC-51 | 62 | male | 235 | Low | T1N0M0 |
| XYBC-52 | 65 | male | 240 | High | T1N0M0 |
| XYBC-53 | 51 | male | 230 | High | T1N0M0 |
| XYBC-54 | 71 | male | 204 | Low | T0N0M0 |
| XYBC-55 | 66 | Female | 242 | High | T4N0M1 |
| XYBC-56 | 64 | Female | 226 | Low | T2N0M0 |

Supplementary Table 2. The clinicopathological data of Xiangya BLCA immunotherapy cohort.

| ID | Response | Age | Gender | Surgical Treatment |
|---------|----------|-----|--------|--------------------|
| XYBIC-1 | CR | 49 | male | TURBT |
| XYBIC-2 | CR | 71 | male | Radical cystectomy |
| XYBIC-3 | CR | 49 | male | TURBT |
| XYBIC-4 | CR | 66 | male | Radical cystectomy |
| XYBIC-5 | CR | 71 | male | TURBT |
| XYBIC-6 | CR | 46 | male | TURBT |

| | | | | |
|----------|----|----|--------|--------------------|
| XYBIC-7 | CR | 56 | male | TURBT |
| XYBIC-8 | CR | 57 | male | TURBT |
| XYBIC-9 | CR | 63 | male | Radical cystectomy |
| XYBIC-10 | CR | 69 | male | TURBT |
| XYBIC-11 | CR | 56 | male | Radical cystectomy |
| XYBIC-12 | CR | 73 | male | Radical cystectomy |
| XYBIC-13 | CR | 62 | male | Radical cystectomy |
| XYBIC-14 | CR | 63 | male | Radical cystectomy |
| XYBIC-15 | CR | 47 | female | TURBT |
| XYBIC-16 | CR | 50 | male | TURBT |
| XYBIC-17 | CR | 59 | male | Radical cystectomy |
| XYBIC-18 | PR | 63 | male | Radical cystectomy |
| XYBIC-19 | PR | 69 | male | Radical cystectomy |
| XYBIC-20 | PR | 57 | male | Radical cystectomy |
| XYBIC-21 | PR | 71 | male | Radical cystectomy |
| XYBIC-22 | PR | 73 | male | Radical cystectomy |
| XYBIC-23 | PR | 52 | male | Radical cystectomy |
| XYBIC-24 | PR | 57 | female | Radical cystectomy |
| XYBIC-25 | PR | 57 | female | Radical cystectomy |
| XYBIC-26 | PR | 63 | male | Radical cystectomy |
| XYBIC-27 | PR | 58 | male | Radical cystectomy |
| XYBIC-28 | PR | 68 | male | Radical cystectomy |
| XYBIC-29 | PR | 66 | male | Radical cystectomy |
| XYBIC-30 | PR | 54 | male | Radical cystectomy |
| XYBIC-31 | PR | 68 | female | Radical cystectomy |
| XYBIC-32 | PR | 72 | female | Radical cystectomy |
| XYBIC-33 | PR | 79 | male | Radical cystectomy |
| XYBIC-34 | SD | 67 | male | Radical cystectomy |
| XYBIC-35 | SD | 72 | male | Radical cystectomy |

| | | | | |
|----------|----|----|--------|--------------------|
| XYBIC-36 | SD | 81 | male | Radical cystectomy |
| XYBIC-37 | SD | 71 | male | Radical cystectomy |
| XYBIC-38 | SD | 56 | male | Radical cystectomy |
| XYBIC-39 | SD | 67 | female | Radical cystectomy |
| XYBIC-40 | SD | 74 | female | Radical cystectomy |
| XYBIC-41 | SD | 32 | male | Radical cystectomy |
| XYBIC-42 | SD | 73 | male | Radical cystectomy |
| XYBIC-43 | SD | 62 | male | Radical cystectomy |
| XYBIC-44 | SD | 60 | male | Radical cystectomy |
| XYBIC-45 | SD | 80 | male | Radical cystectomy |
| XYBIC-46 | SD | 63 | male | Radical cystectomy |
| XYBIC-47 | SD | 58 | male | Radical cystectomy |
| XYBIC-48 | SD | 49 | male | Radical cystectomy |
| XYBIC-49 | SD | 61 | female | Radical cystectomy |
| XYBIC-50 | SD | 74 | male | Radical cystectomy |
| XYBIC-51 | PD | 69 | male | Radical cystectomy |
| XYBIC-52 | PD | 57 | male | Radical cystectomy |
| XYBIC-53 | PD | 46 | male | Radical cystectomy |
| XYBIC-54 | PD | 57 | female | Radical cystectomy |
| XYBIC-55 | PD | 63 | female | Radical cystectomy |
| XYBIC-56 | PD | 68 | male | Radical cystectomy |
| XYBIC-57 | PD | 68 | male | Radical cystectomy |
| XYBIC-58 | PD | 71 | male | Radical cystectomy |

Supplementary Table 3. Target sequences of shRNA

| Target sequences of shRNA for human species | |
|---|-----------------------|
| sh-BCAT2-1 | gtGCACCGAATCCTGTACAAA |
| sh-BCAT2-2 | acTACAAGTTAGGTGGGAATT |
| sh-BCAT2-3 | tgAAGTGCAATACGAAATAAA |

| Target sequences of shRNA for murine species | |
|--|------------------------|
| sh-BCAT2-1 | ccACATACCTACCATGGAGAA |
| sh-BCAT2-2 | caAGGTCACCTATGAAGGAATT |
| sh-BCAT2-3 | ccCTTCCAGAACCTCACGCTA |

Supplementary Table 4. Primer sequences of qRT-PCR

Species: human

| | Forward | Reverse |
|--------|--------------------------|--------------------------|
| CCL3 | CATGGCTCTCTGCAACCAGTTCTC | CTGGCTGCTCGTCTCAAAGTAGTC |
| CCL4 | TCTGCGTGACTGTCCTGTCTCTC | TCTACCACAAAGTTGCGAGGAAGC |
| CCL5 | CAGCAGTCGTCCACAGGTCAAG | TTTCTTCTCTGGGTTGGCACACAC |
| CXCL9 | AAGACCTTAAACAATTTGCCCC | TGCTGAATCTGGGTTTAGACAT |
| CXCL10 | CTCTCTCTAGAACTGTACGCTG | ATTCAGACATCTCTTCTCACCC |

Species: mouse

| | Forward | Reverse |
|--------|------------------------|-------------------------|
| CCL3 | CCCAGCCAGGTGTCATTT | AGTTCCAGGTCAGTGATGTATTC |
| CCL4 | CCACTTCCTGCTGTTTCTCTTA | GCAAAGACTGCTGGTCTCATA |
| CCL5 | GCCCACGTCAAGGAGTATTT | CCCACTTCTTCTCTGGGTTG |
| CXCL9 | AGGCACGATCCACTACAAATC | GCAGGTTTGATCTCCGTTCT |
| CXCL10 | GCTGCAACTGCATCCATATC | CGTGGCAATGATCTCAACAC |