

Figure S1 The TMA of *ADCY2* and its relationship with prognosis. (A) Low-magnification images of bladder cancer TMA immunostained for *ADCY2*. (B) KM curve for DFI of *ADCY2* expression in TCGA-BLCA cohort; the groups were divided into high- and low-expression groups using the minimal P value approach. (C,D) Expression levels of *ADCY2* in bladder cancer tissues and normal tissues from the GEO data set GSE13507. GSE13507 comprises 165 primary bladder cancer samples, 23 recurrent non-muscle invasive tumour tissues, and 58 normal-looking bladder mucosae surrounding cancer, and 10 normal bladder mucosae. ***, $P < 0.001$; **, $P < 0.01$; ns, $P > 0.05$. T, tumour samples; N, matched normal samples; TCGA, The Cancer Genome Atlas; BLCA, bladder urothelial carcinoma; HR, hazard ratio; TMA, tissue microarray; *ADCY*, adenylyl cyclase; KM, Kaplan-Meier; DFI, disease-free interval; GEO, Gene Expression Omnibus.

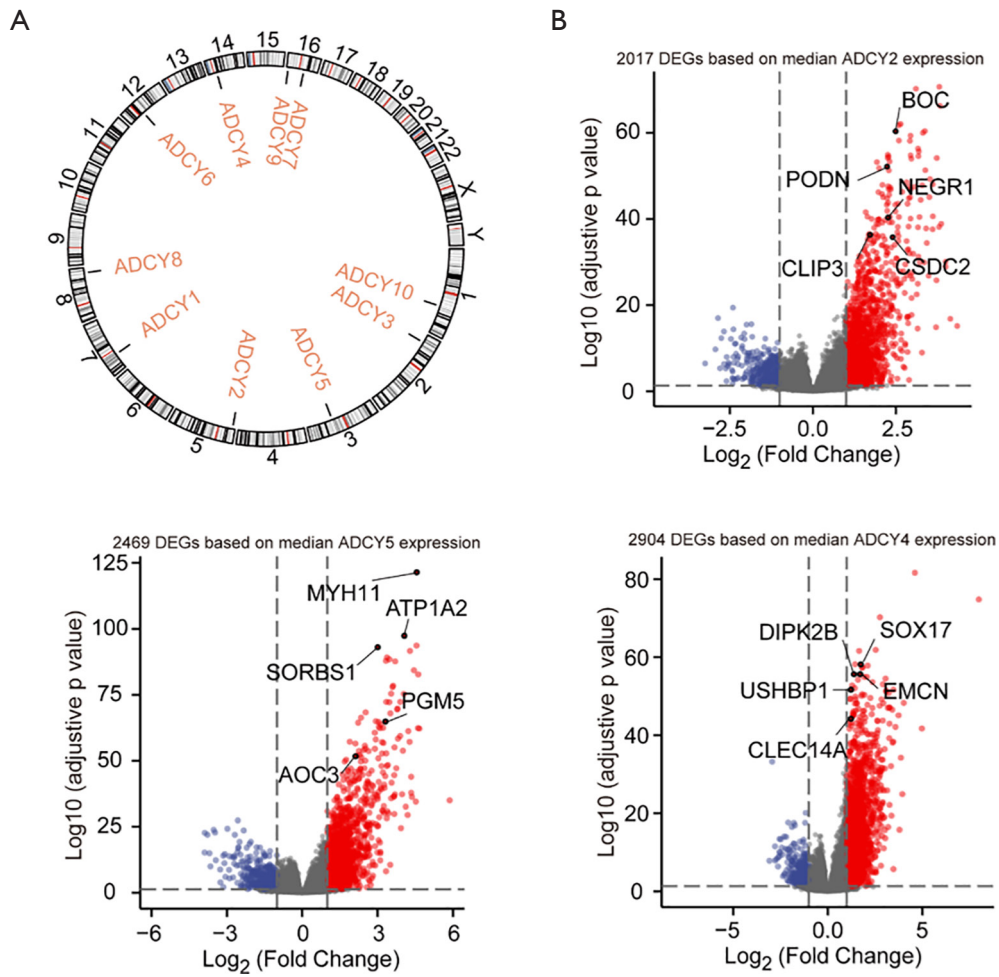


Figure S2 The position of *ADCYs* in chromosomes and the differential genes of *ADCY2*, *ADCY4*, and *ADCY5*. (A) Chromosomal localization of the 10 *ADCYs*. (B) Statistically significant DEGs were defined as those with $|\log_2\text{FC}| > 1$ and adjusted $P < 0.05$ as the cut-off criteria. The top 500 correlated genes for *ADCY2*, *ADCY4*, and *ADCY5* are marked in the volcano map. *ADCY*, adenylyl cyclase; DEG, differentially expressed gene; FC, fold change.

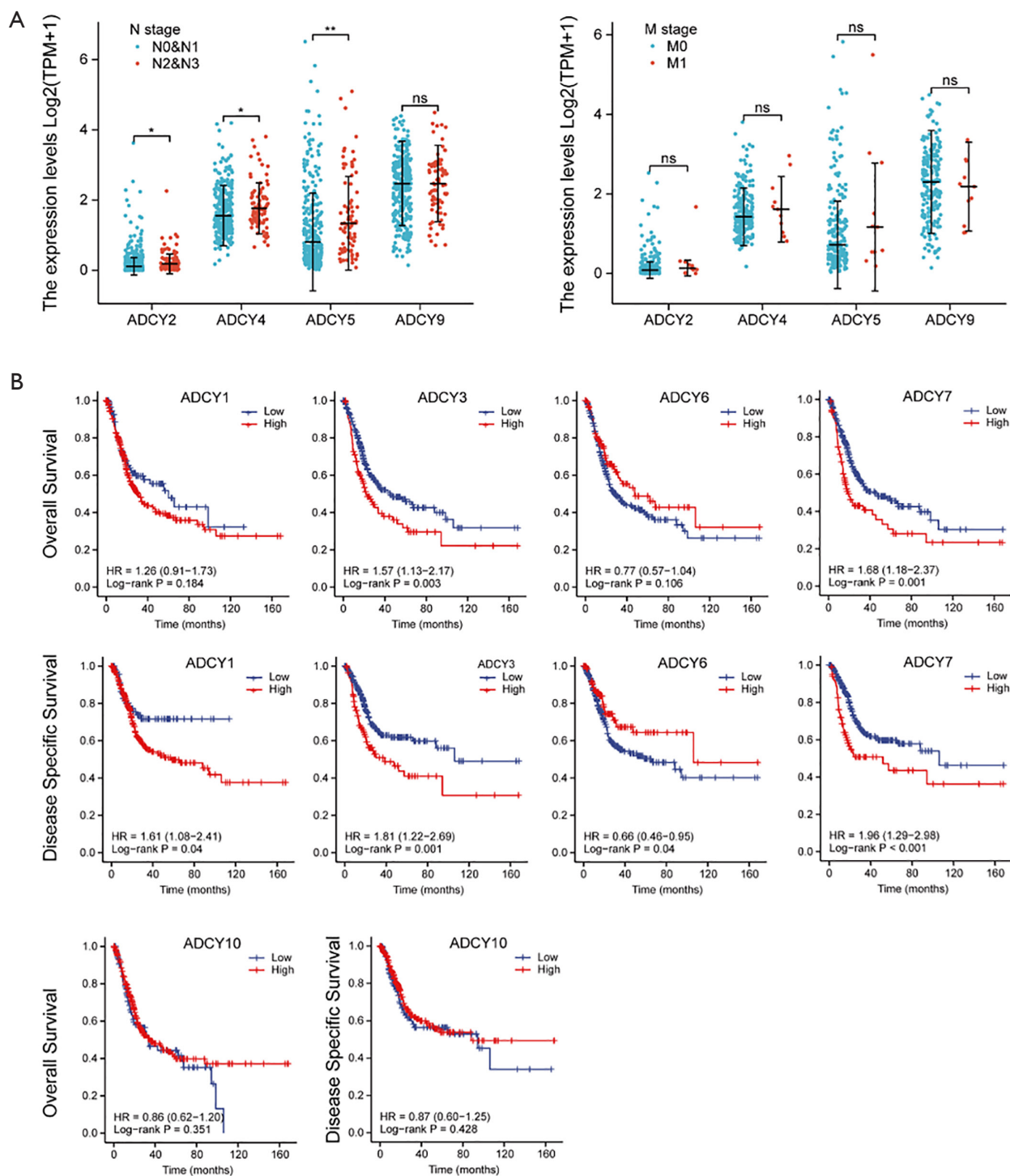


Figure S3 The role of *ADCY* family in the prognosis of bladder cancer. (A) Relationship of *ADCY2*, *ADCY4*, *ADCY5*, and *ADCY9* gene expression with clinical N stage and M stage of patients with bladder cancer in TCGA cohort. (B) KM curve for OS and DSS of *ADCY* expression in BLCA patients of TCGA cohort. There was no prognostic information for *ADCY8* because its expression in more than half of the samples was the minimum value (0), and patients could not be divided into high- and low-expression groups. **, $P < 0.01$; *, $P < 0.05$; ns, $P > 0.05$. *ADCY*, adenylyl cyclase; TPM, transcript per million; HR, hazard ratio; TCGA, The Cancer Genome Atlas; KM, Kaplan-Meier; OS, overall survival; DSS, disease-specific survival; BLCA, bladder urothelial carcinoma.

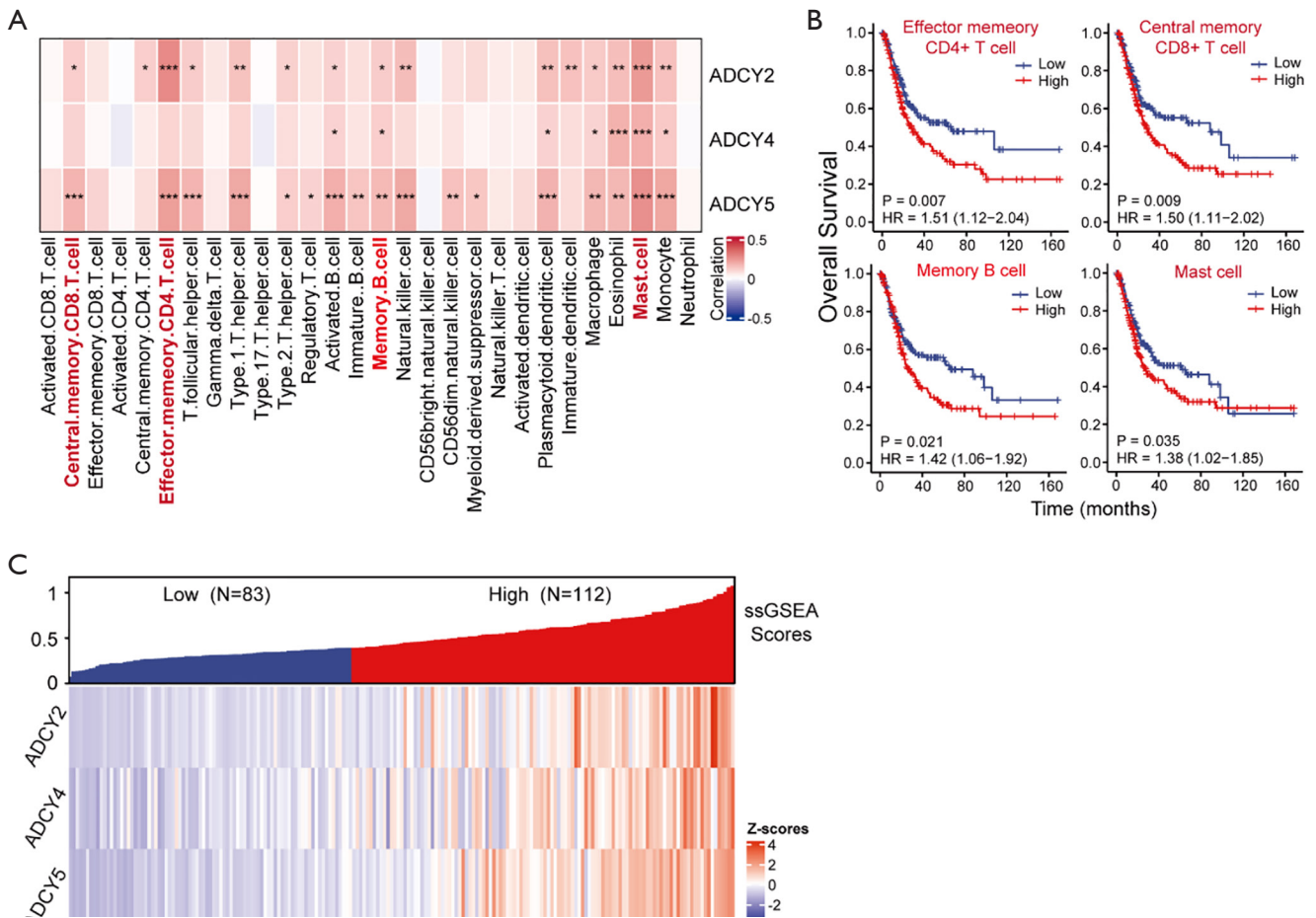


Figure S4 The correlation between *ADCY2*, *ADCY4*, *ADCY5* and immune cells and the impact of immune microenvironment on prognosis. (A) Heat map showing the correlation between the expression of genes (*ADCY2*, *ADCY4*, and *ADCY5*) and enrichment of 28 common immune cells. The expression of *ADCY2*, *ADCY4*, and *ADCY5* was positively correlated to the infiltration of CD8⁺ T cells, CD4⁺ T cells, B cells, and mast cells as determined using Spearman relation analysis. (B) KM curve of OS in four immune cell types. The groups were divided using the minimal P value approach. (C) Heat map of 195 bladder cancer samples in the IMvigor210 cohort based on *ADCY2*, *ADCY4*, and *ADCY5* expressions. The ssGSEA was used to divide the samples into groups according to the minimal P value approach. ***, P < 0.001; **, P < 0.01; *, P < 0.05. *ADCY*, adenylyl cyclase; HR, hazard ratio; ssGSEA, single sample gene set enrichment analysis; KM, Kaplan-Meier; OS, overall survival.

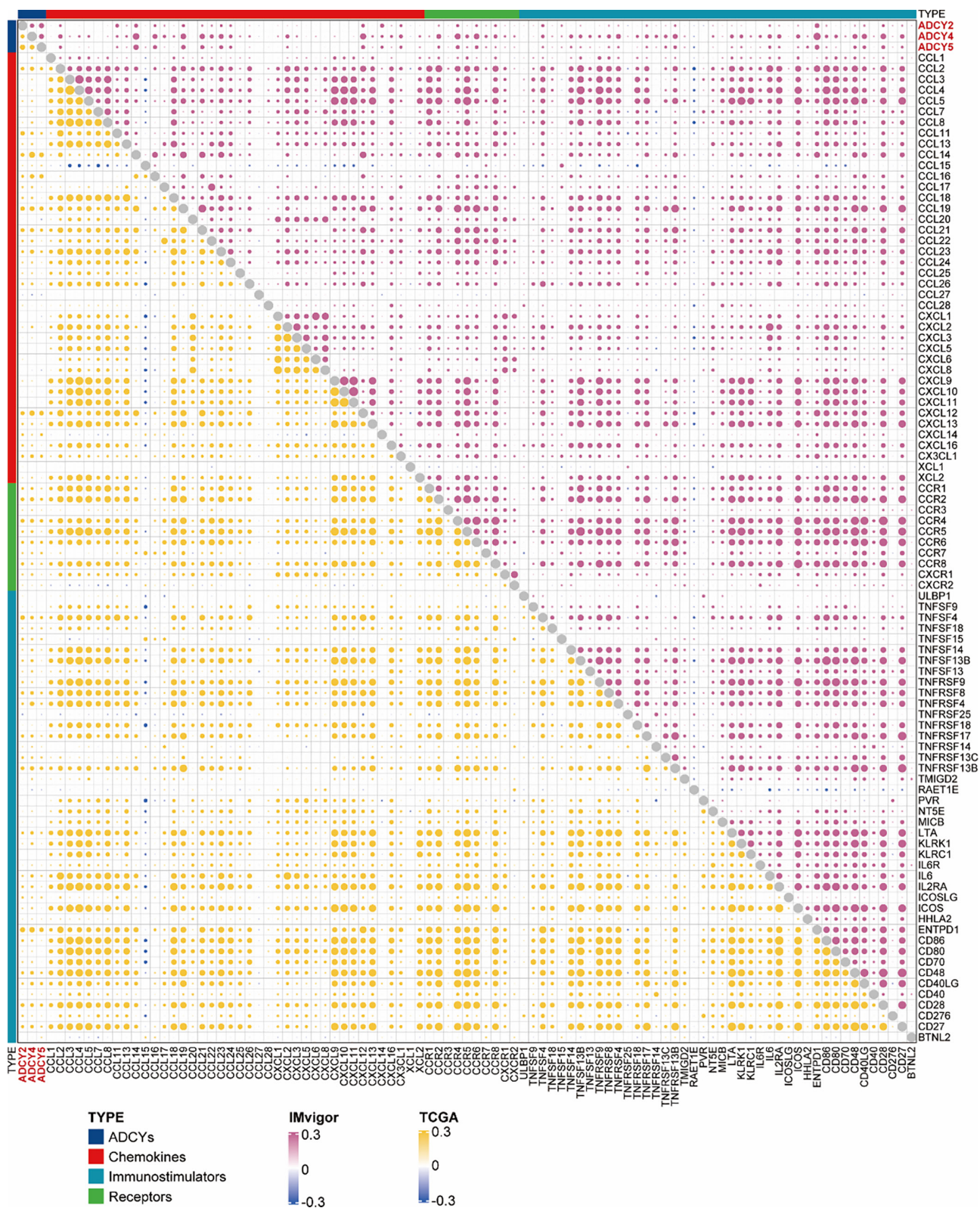


Figure S5 In TCGA and IMvigor210 cohorts, *ADCY2*, *ADCY4*, and *ADCY5* expression was positively associated with the expression of immunomodulators, chemokines, and chemokine receptors. *ADCY*, adenylyl cyclase; TCGA, The Cancer Genome Atlas.

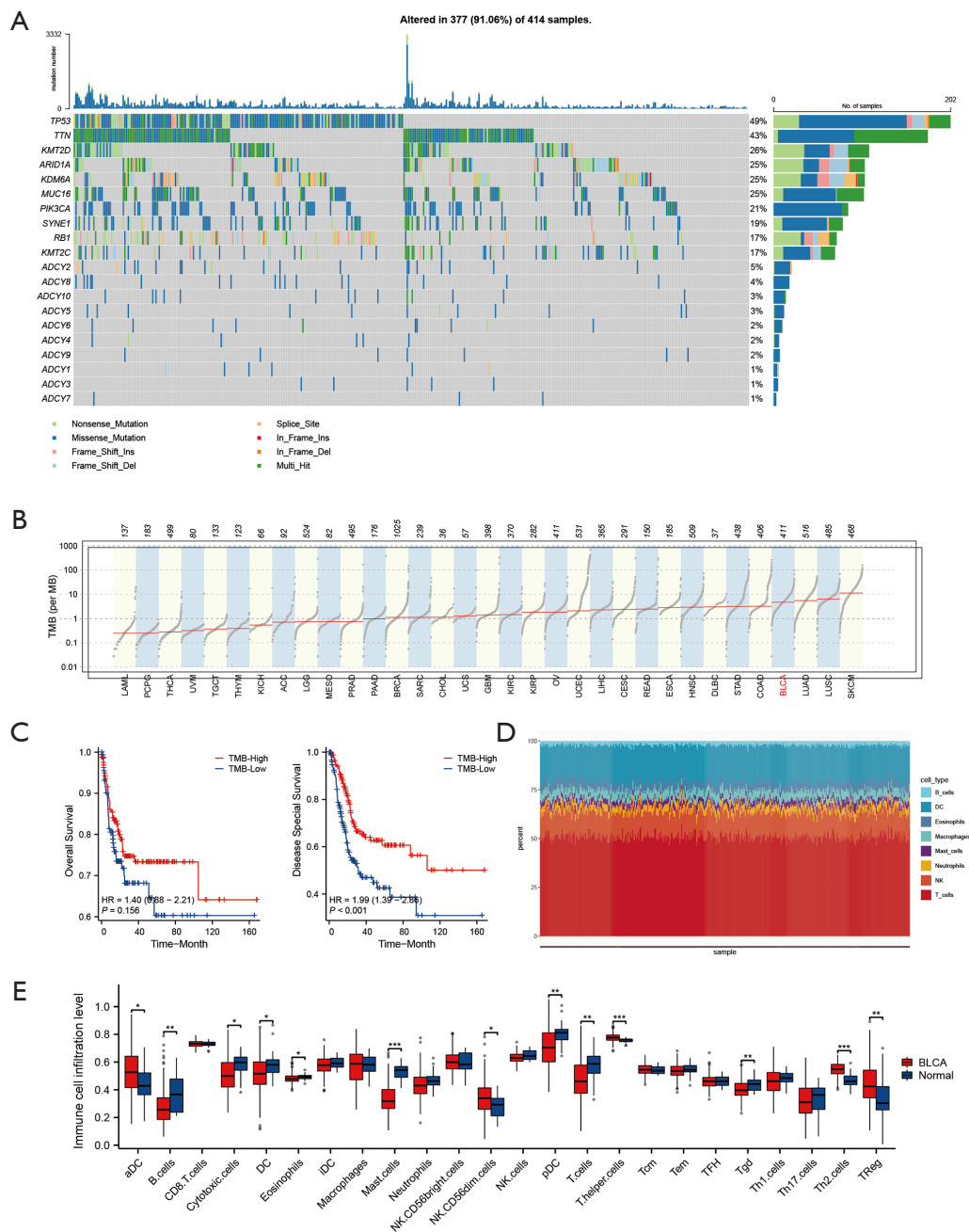


Figure S6 The role of *ADCYs* in TMB and immune microenvironment. (A) Mutations are displayed as an oncoplot, including the 10 genes with the highest mutation frequency and the *ADCY* family genes in bladder cancer. (B) The level of TMB in BLCA among 33 types of tumours in TCGA. The red line represents the median level of TMB for various tumours. The full name of the TCGA abbreviations sees the website: <https://gdc.cancer.gov/resources-tcga-users/tcga-code-tables/tcga-study-abbreviations>. (C) OS and DSS of bladder cancer under the median grouping of TMB. (D) The infiltration level of immune cells was calculated by ssGSEA method, and the proportion of immune cells in bladder cancer samples was displayed. (E) The infiltration levels of 24 types of immune cells between BLCA and normal tissues. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. *ADCY*, adenylyl cyclase; TMB, tumour mutational burden; HR, hazard ratio; DC, dendritic cell, NK, natural killer; aDC, activated DC; iDC, immature DC; NK, natural killer; pDC, plasmacytoid DC; Tcm, central memory T cell; Tem, effector memory T cell; TFH, T follicular helper; Tgd, gamma-delta T cell; Th, T helper; TReg, regulatory T cell; BLCA, bladder urothelial carcinoma; TCGA, The Cancer Genome Atlas; OS, overall survival; DSS, disease-specific survival; ssGSEA, single sample gene set enrichment analysis.