Figure S1. Histological and molecular characterization of adjacent normal ICC tissues. (A) Hematoxylin and eosin staining of (a) adjacent normal liver tissues and (b) ICC tumor tissues. (B) Immunohistochemistry staining of cytokeratin 19 of (a) adjacent normal liver tissues and (b) ICC tumor tissues (scale bars, 50 μm). (C) Visualization of cell-specific marker genes depicted in the UMAP plot. ICC, intrahepatic cholangiocarcinoma; UMAP, Uniform Manifold Approximation and Projection; ACTA2, actin α2; FABP1, fatty acid binding protein 1; EPCAM, epithelial cell adhesion molecule; NKG7, natural killer cell granule protein 7; MKI67, marker of proliferation Ki-67.

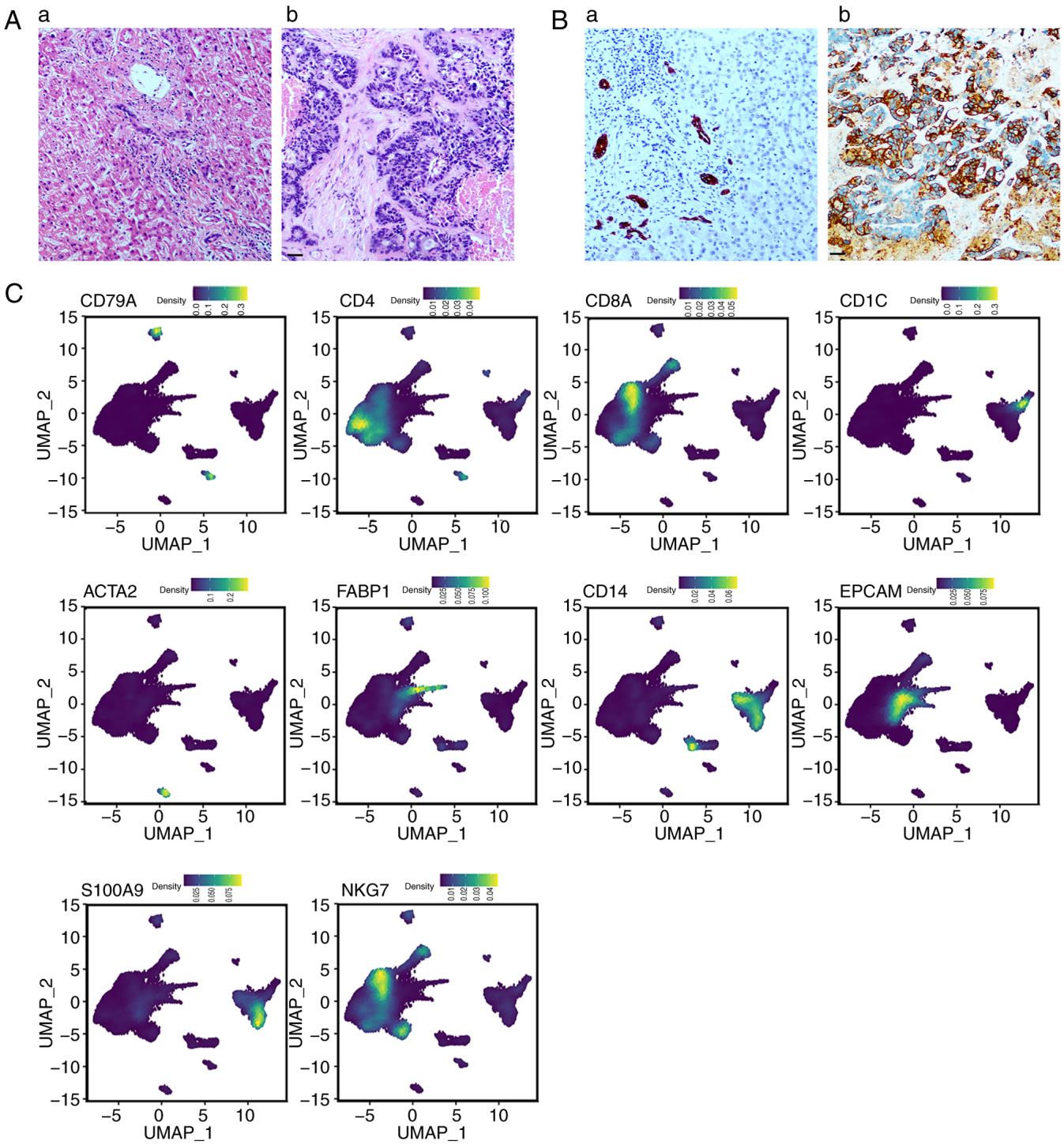

Figure S2. UMAP-based analysis of cell-specific marker genes, CD44 expression, BATF levels and diffusion pseudotime of T-cell sub-clusters in ICC. (A) Visualization of cell-specific marker gene depicted in the UMAP plot. (B) CD44 expression is upregulated in the T cells of tumor tissues compared with adjacent tissue. (C) BATF expression is upregulated in the T cells of tumor tissues compared with adjacent tissue. (D) BATF expression levels are correlated with poor prognosis in patients with ICC. (E) Diffusion pseudotime analysis of each sub-cluster of T cells in ICC. ***P<0.001. ICC, intrahepatic cholangiocarcinoma; BATF, basic leucine zipper ATF-like transcription factor; UMAP, Uniform Manifold Approximation and Projection; IL7R, interleukin 7 receptor; GZMK, granzyme K; FGFBP2, fibroblast growth factor binding protein 2; CXCL3, C-X-C motif chemokine ligand 13; FOXP3, forkhead box P3; MKI67, marker of proliferation Ki-67; KLRD1, killer cell lectin like receptor D1; HR, hazard ratio.

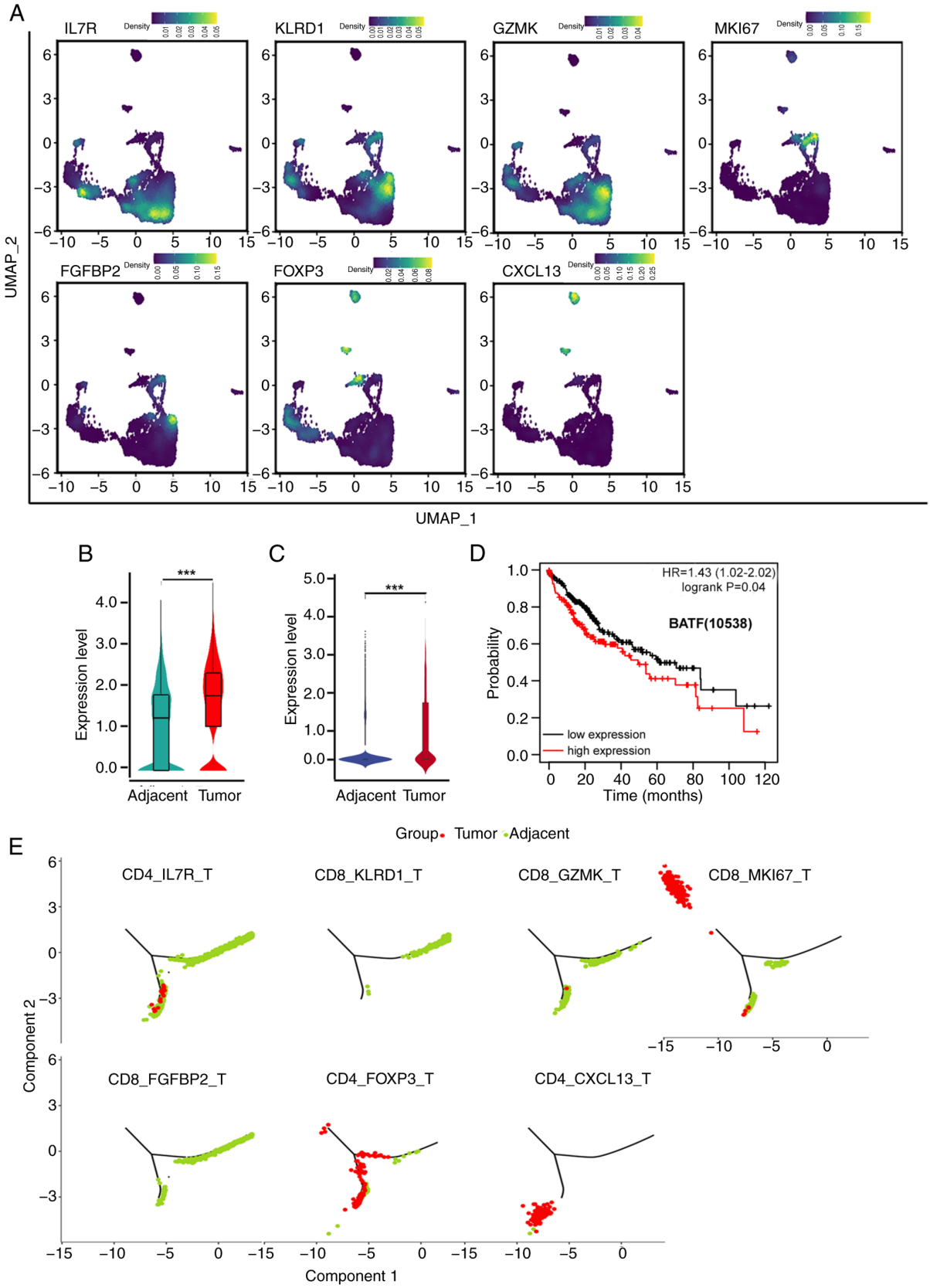

Figure S3. Expression of SPP1, S100A2 and SFN genes in ICC tumor cells and prognostic value of S100A2 and SFN. (A) SPP1 is highly expressed in ICC tumor tissue. (B) SPP1 is more highly expressed in clinical stage IV compared with stage I and II. (C) Reverse transcription-quantitative PCR analysis of SPP1 from 2 patients with ICC. (D) Representative immunohistochemistry staining for SPP1 from 2 patients with ICC (scale bars, 50 μm). (E) S100A2 and SFN genes are upregulated in ICC tumor cells compared with adjacent cells. (F) High expression levels of S100A2 gene are correlated with poor prognosis of patients with ICC. (G) High expression levels of SFN gene are associated with poor prognosis of patients with ICC. *P<0.05, **P<0.01, ***P<0.001. ICC, intrahepatic cholangiocarcinoma; SFN, stratifin; TPM, transcripts per million; HR, hazard ratio; SPP1, secreted phosphoprotein 1.

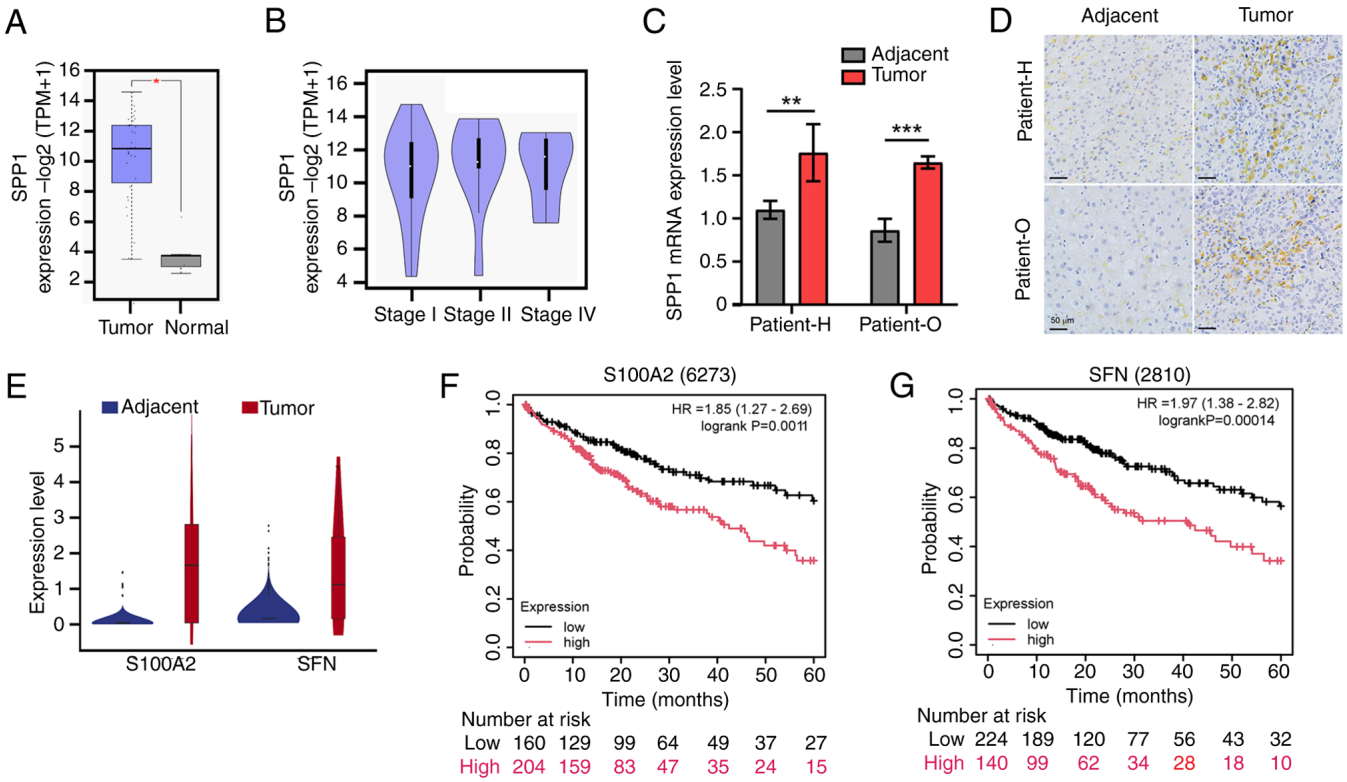

Figure S4. Comprehensive analysis of differential gene expression, prognosis, pathway alterations and T-cell sub-cluster dynamics in early- and late-stage ICC. (A) Volcano plot showing the differential gene expression in late-stage ICC tumor cells compared with early-stage ICC tumor cells. (B) Expression levels of ARK1C2, IL32, VTN and S100P in early and late stages. (C) High expression levels of ARK1C2 gene are correlated with poor prognosis of patients with ICC. (D) Low expression levels of IL32 gene are correlated with poor prognosis of patients with ICC. (E) High expression levels of S100P gene are correlated with poor prognosis of patients with ICC. (F) Low expression levels of VTN gene are correlated with poor prognosis of patients with ICC. (G) Kyoto Encyclopedia of Genes and Genomes pathway analysis of the differentially expressed genes in late-stage ICC tumor cells compared with early-stage ICC tumor cells. The circle size represents the level of statistical significance and the circle color represents the intensity of the interaction. (H) Diffusion pseudotime analysis of each sub-cluster of T cells in early-stage and late-stage ICC. ***P<0.001. VTN, vitronectin; ICC, intrahepatic cholangiocarcinoma; ARK1C2, aldo-keto reductase family 1 member C2; HR, hazard ratio.