Supplementary Figure 1.

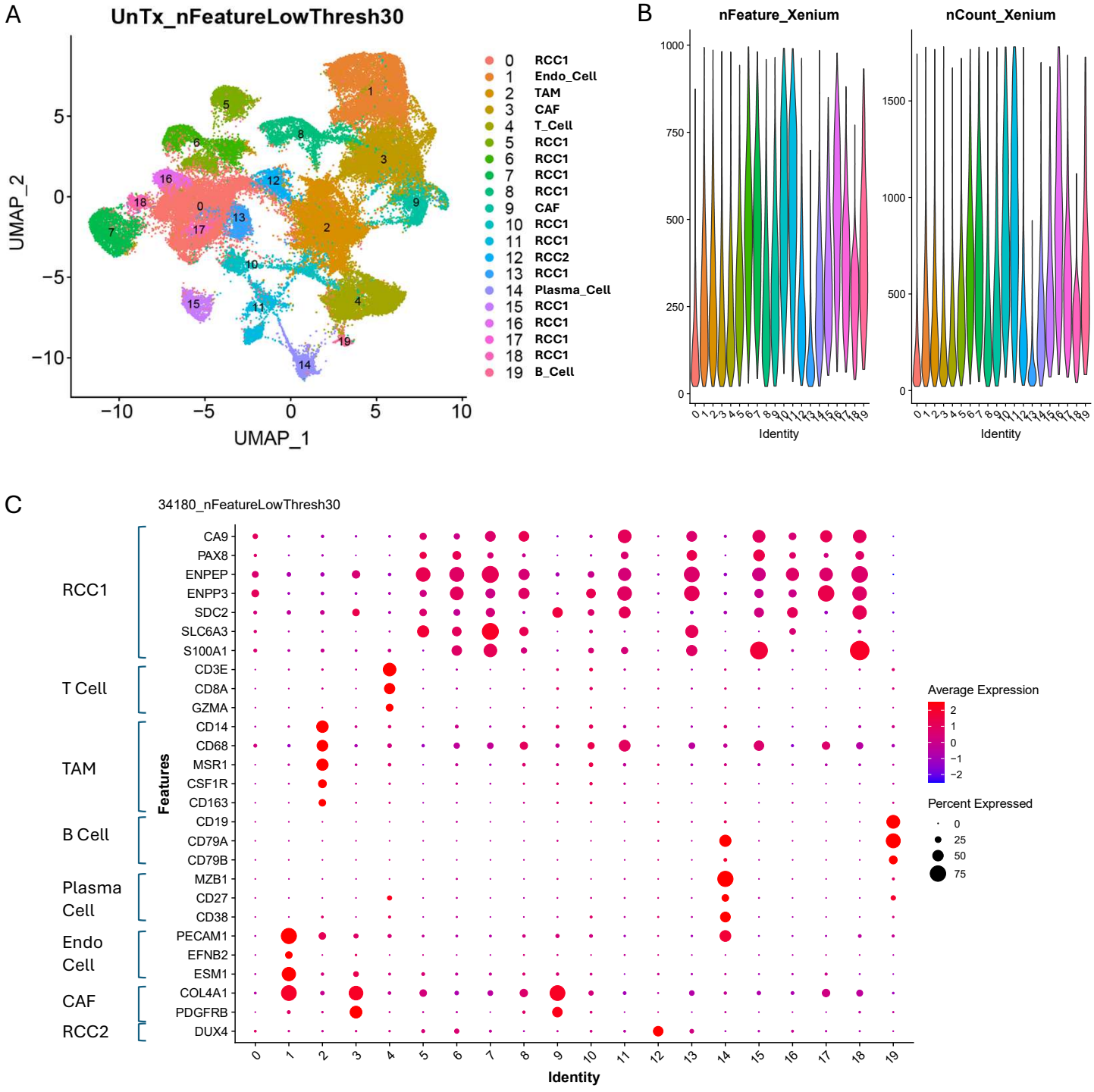


Fig. S1. Unsupervised clustering of cells from treatment naïve RCC tumors and marker-based annotation.

A. UMAP visualization and annotation of cell clusters within the tumor microenvironment of treatment-naïve tumors. B. Number of spatial features and total feature counts for each cluster. C. Dot plot displaying canonical marker genes used to annotate each cluster.

Supplementary Figure 2.

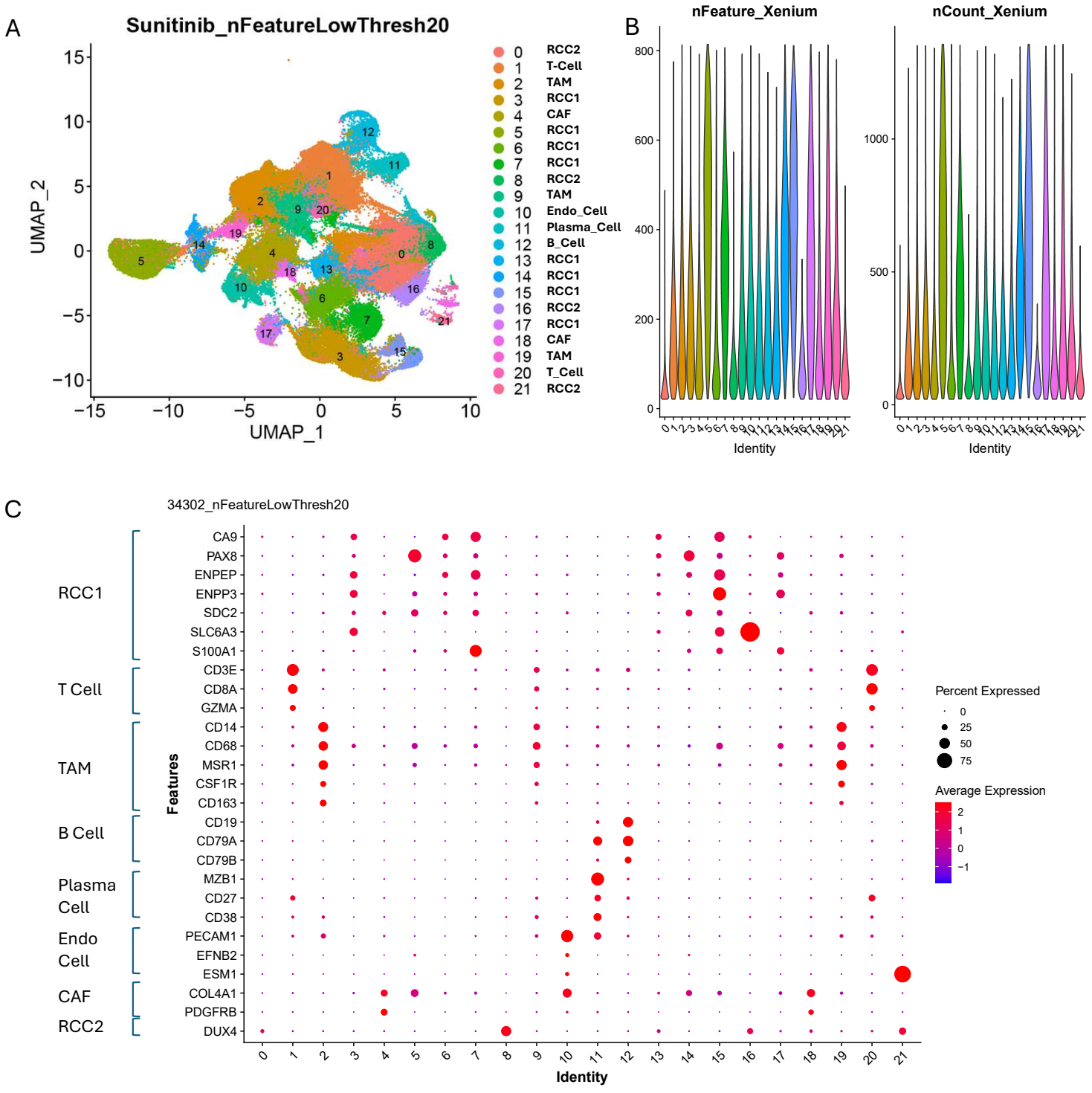


Fig. S2. Unsupervised clustering of cells from sunitinib-treated RCC tumors and marker-based annotation.

A. UMAP visualization and annotation of cell clusters within the tumor microenvironment of sunitinib-treated tumors. B. Number of spatial features and total feature counts for each cluster. C. Dot plot displaying canonical marker genes used to annotate each cluster.

Supplementary Figure 3.

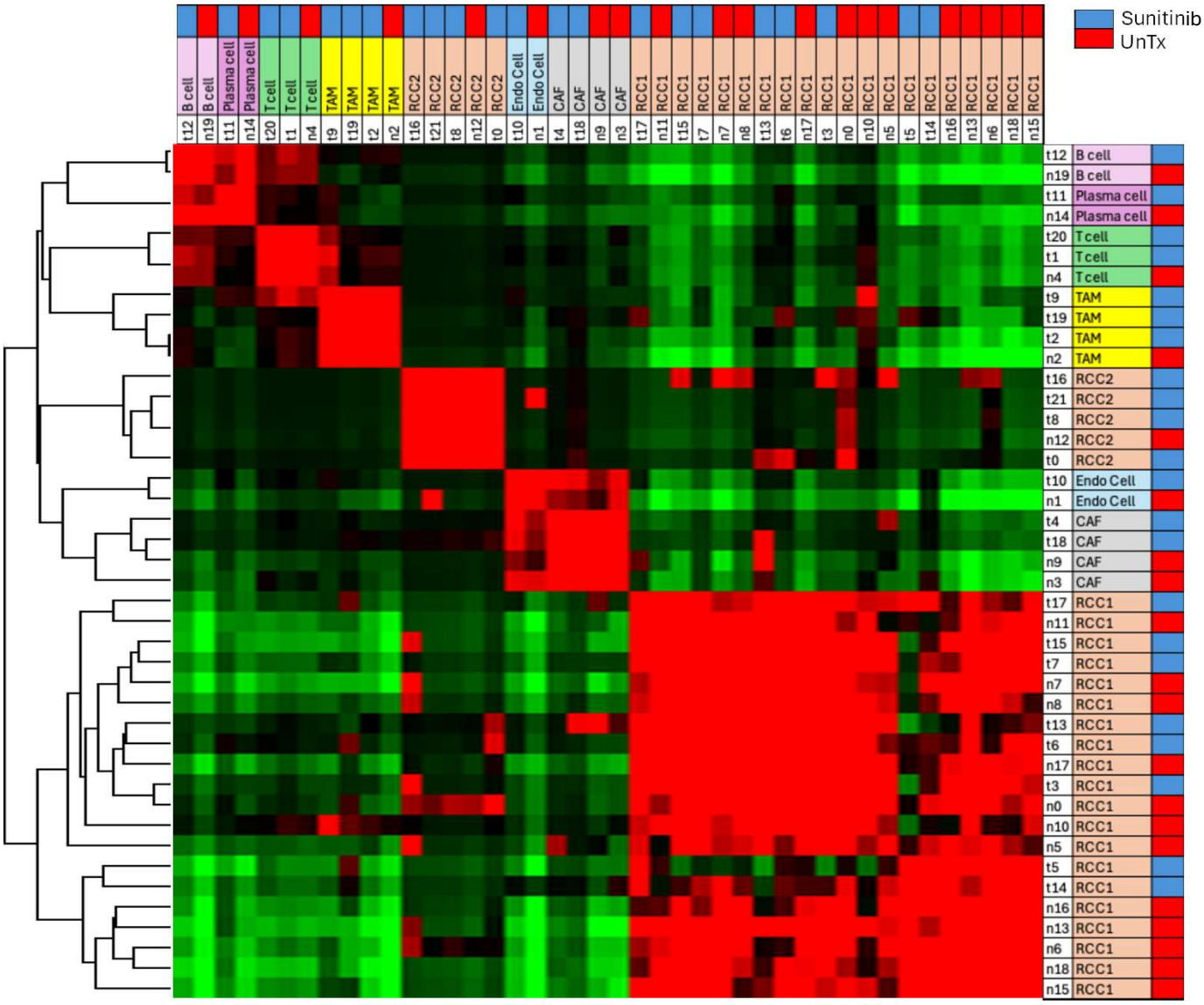


Fig. S3. Correlation analysis of clusters and cell types from treatment naïve and sunitinib treated tumors.

Hierarchical clustering of all clusters from both slides based on pairwise similarity of transcriptional profiles, defined by the relative activity of differentially upregulated genes on each slide.

Supplementary Figure 4.

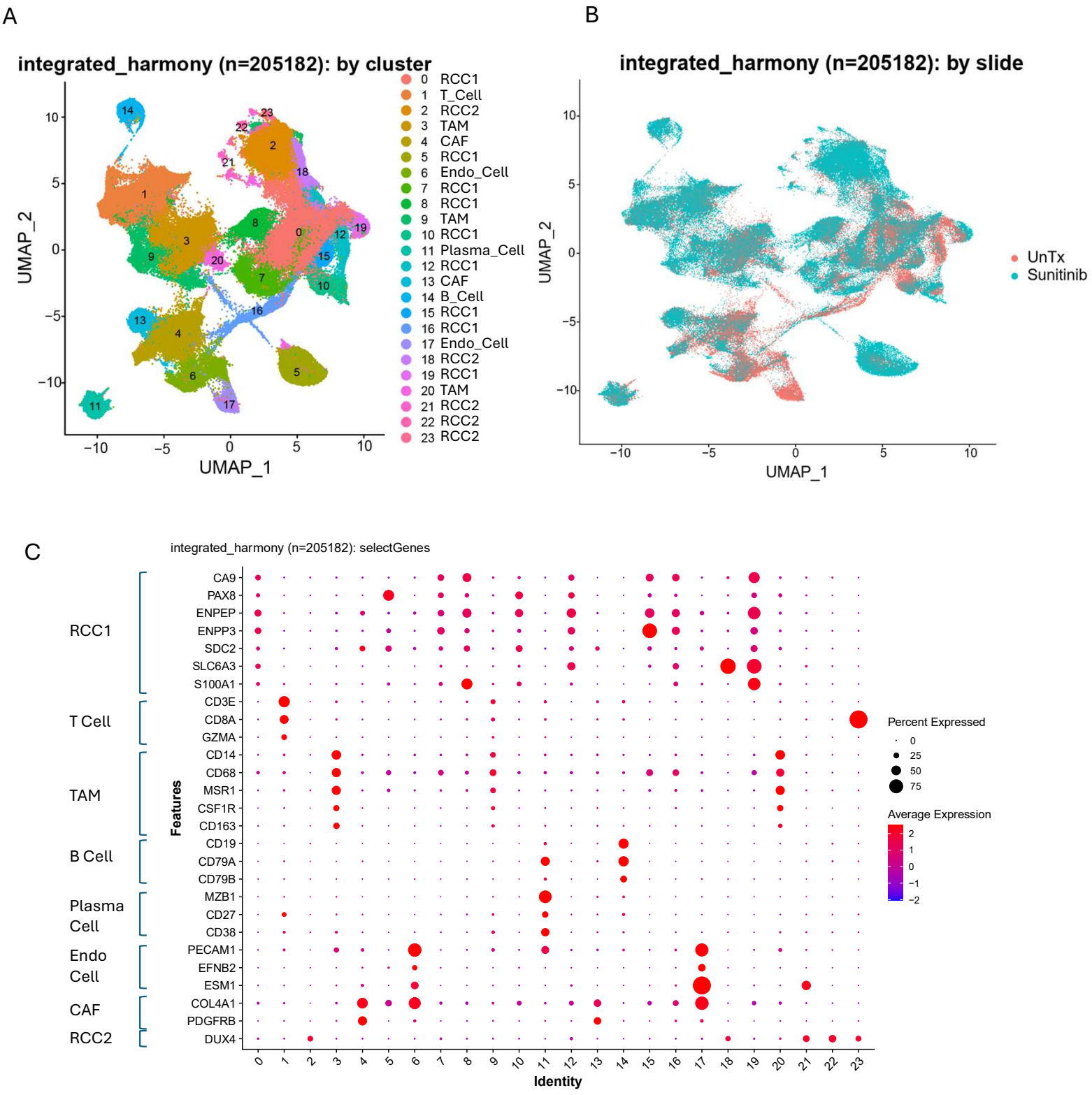


Fig. S4. Unsupervised clustering and annotation of cells integrated from treatment-naïve and sunitinib-treated RCC tumors.

A. UMAP visualization showing clustering and annotation of cell populations within the tumor microenvironment. B. Number of spatial features and total feature counts across each cluster. C. Dot plot illustrating canonical marker genes used for cluster annotation.

Supplementary Figure 5.

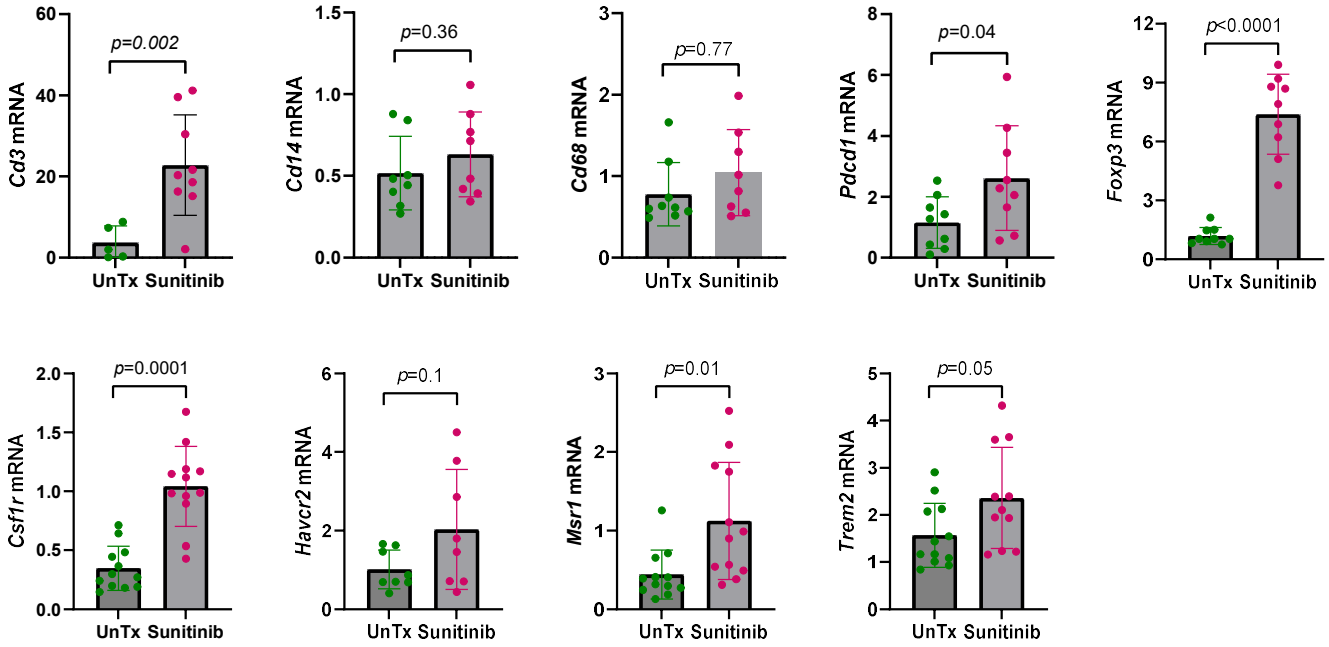


Fig. S5. Sunitinib treatment increases the expression of immunosuppressive genes in Renca-Balb/c tumors.

Real-time PCR analysis of immune-related gene expression in treatment-naïve and sunitinib-treated Renca tumors.

Supplementary Figure 6.

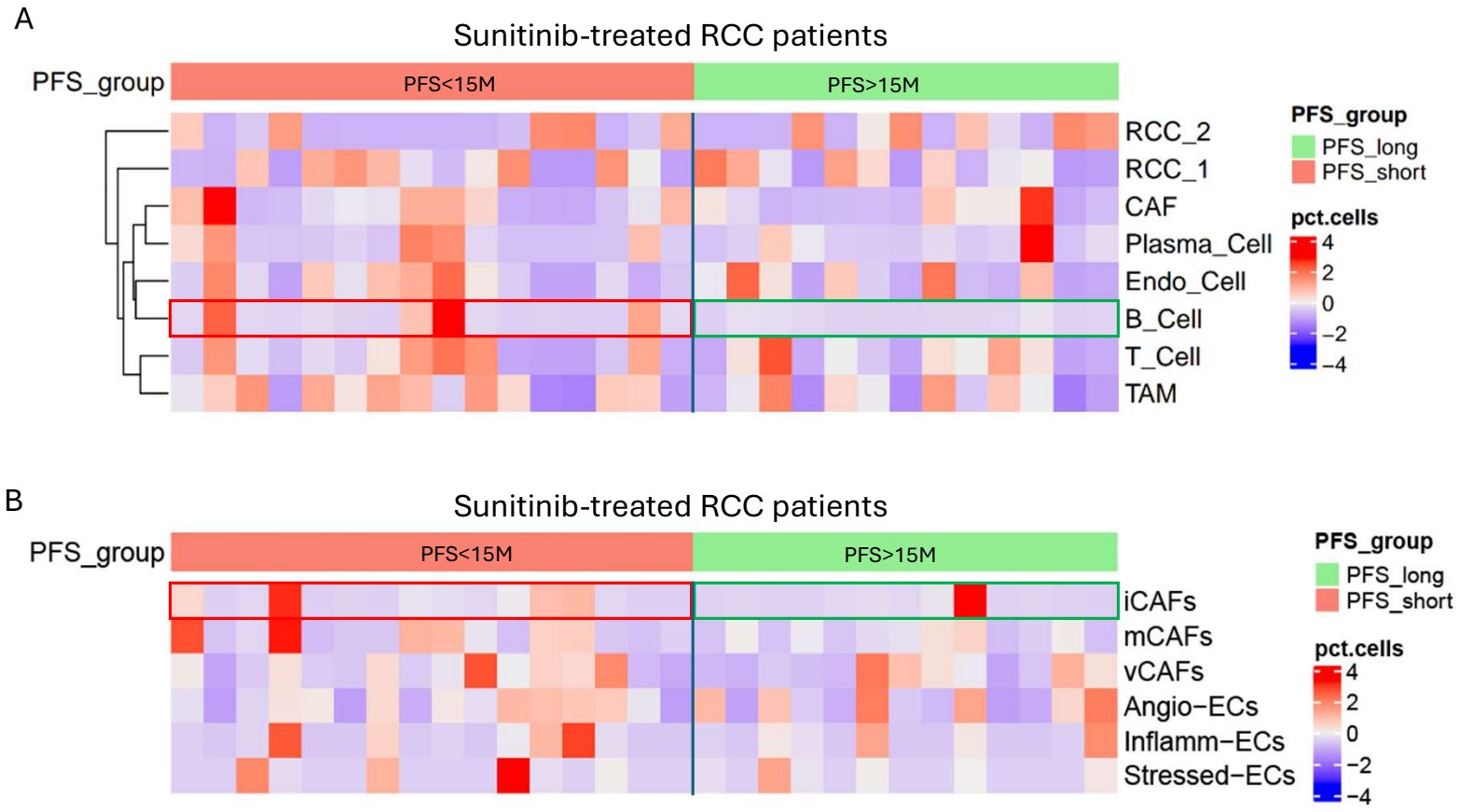


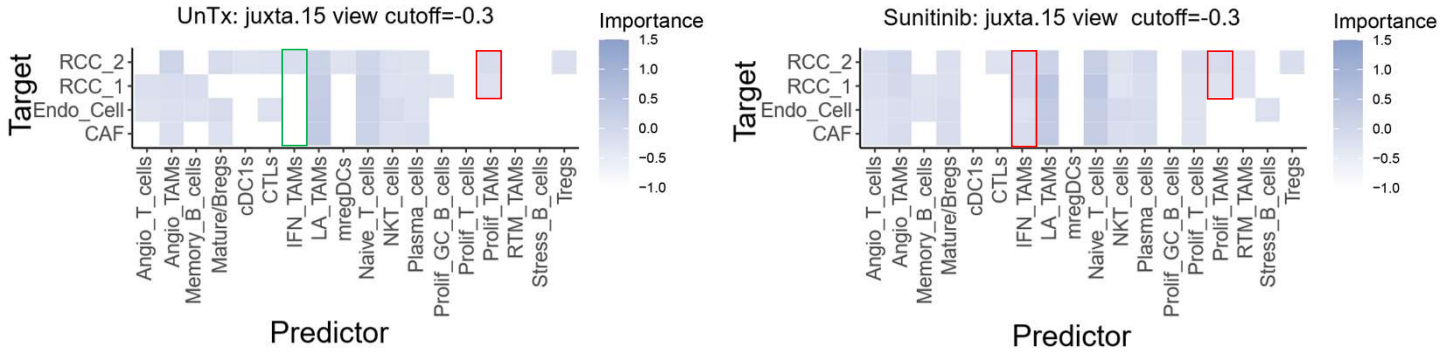
Fig. S6. Association between PFS and major cell types, CAF subtypes, and endothelial cell subtypes in sunitinib-treated cohorts.

A. Relationship between PFS and the abundance of major cell types. B. Relationship between PFS and CAF and endothelial cell subtypes. RCC patients were grouped into PFS-long (>15 months) and PFS-short cohorts. For each patient, the percentage of each cell type or subtype across different tumor regions was averaged, and cell abundance was aligned with patient PFS.

Supplementary Figure 7.

Mixture: cutoff = -0.3, all immune subtype

A



B

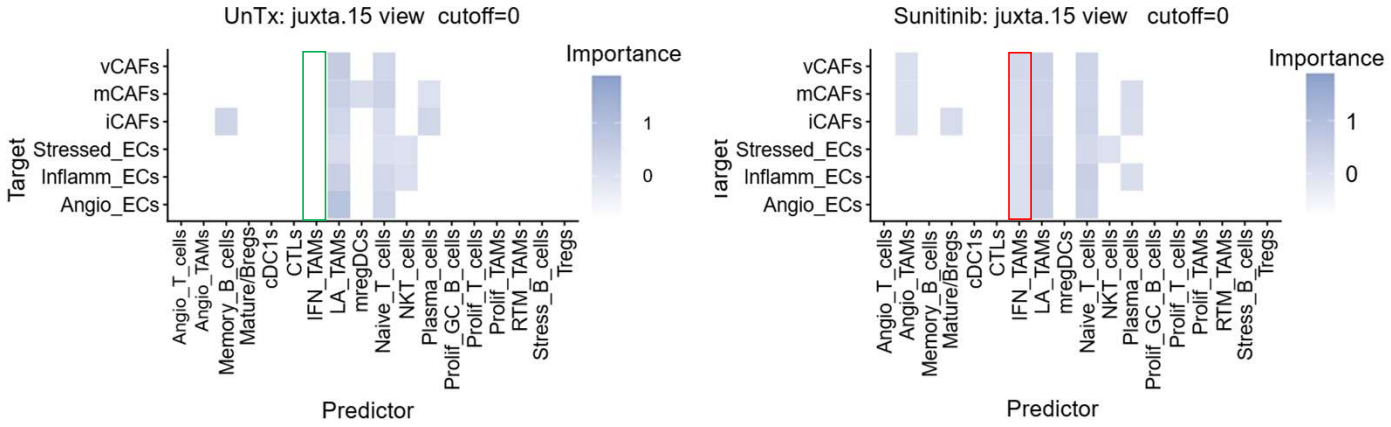


Fig. S7. MISTy analysis of intercellular interactions between immune subsets and RCC cells, endothelial cells, CAFs or their subtypes.

A. Interactions between immune subsets and RCC cells, endothelial cells, and CAFs. B. Interactions between immune subsets and endothelial cell and CAF subtypes.

Cell type	Marker genes
RCC1	*RCC1; CA9, PAX8, ENPEP, ENPP3, SDC2, SLC6A3, S100A1*
RCC2	*DUX4*
CAF	*COL4A1, PDGFRB*
T Cell	*CD3, CD8A, GZMA*
TAM	*CD68, MSR1, CSF1R, CD163*
B cell	*CD19, CD79A, CD79B*
Plasma	*MZB1, CD27, CD38*

Table S1. Marker genes of cell types.

	B Cell	CAF	Endo Cell	Plasma	RCC1	RCC2	T Cell	TAM
B Cell	3654	53	12	12	15	32	67	27
CAF	21	17987	643	88	570	219	258	255
Endo Cell	5	512	10881	17	333	53	62	225
Plasma	30	29	9	4528	59	15	24	6
RCC1	16	650	586	9	73513	3882	399	815
RCC2	244	1101	297	271	1290	23870	478	564
T Cell	262	1001	300	147	435	373	22127	855
TAM	36	616	134	161	2325	456	722	26545

Table S2. Agreement of cell annotations before and after integration.
Comparison of cell type annotations assigned before and after integration. Cells with agreement were highlighted in purple. Cells with discordant annotations between rows and columns are labeled as “uncertain.” Rows represent annotations after integration, and columns represent annotations before integration.

Cell subsets	Marker genes
Angiogenic EC	*EDNRB*, *PTP4A3*, *HEY1*
Inflammatory EC	*CADM3*, *POSTN*, *JAM2*, *VCAM1*, *ADGRG6*
Stressed EC	*ENPP2* and *ESM1*
Vessel-associated CAF	*MCAM*, *NOTCH3*, *COL18A1*
Inflammatory CAF	*CCL19*, *HGF*, *C4B*, *MMP14*, *PDGFRA*
Matrix-producing CAF	*POSTN*, *SPON2*, *COL5A1*
Naïve T cell	*CD8B, CCR7, CXCR4*
Angiogenic T cell	*CD8B, VEGFA* and *ANGPTL4*
Proliferating T cell	*CD8B*, *MKI67*, *TOP2A*, *TYMS*, *CXCL13*
Cytotoxic T lymphocyte	*CD8B*, *IFNG*, *GZMA*, *GZMB*, *GZMK*, *PRF1*, *XCL2*
Regulatory T cell	*CD4*, *FOXP3*, *CTLA4*, *CCR4*
Natural killer T cell	*PFFBP2*, *KLRC1*, *KLRD1*
Angiogenic TAMs	*PLVAP*, *ADAM8*, *KDR*, *MCAM*, *ESM1*, *NR4A1*, *PLAUR*
Lipid-associated TAMs	*ACP5*, *PLA2G7*, *F13A1*
Interferon-primed TAMs	*CXCL9*, *CXCL10*, *CXCL11*, *IRF1*
Proliferating TAMs	*MKI67*, *TOP2A*, *RRM2*, *CDK1*
Resident tissue macrophage–like TAMs	*CD5L*, *VCAM1*, *CETP*, *FOLR2*
mregDC	*LAMP3*, *CD83*, *FSCN1*, *CCR7*, *CCL19*, *CCL22*
cDC1	*CLEC9A*, *XCR1*, *CLNK*, *DNASE1L3*, *IRF8*, *BATF3*
Plasma cell	*MZB1*, *CD27*, *CD38*, *XBP1*, *PIM2*
Marure B cell	*CD19*, *CD79A*, *CD79B*, *MS4A1*, *FCRL1*, *IL4R*, *CCR6*, *CXCL13*, *TLR10*, *SELL*, *CIITA*
Proliferating or germinal center like B cell	*MYBL2*, *TYMS*, *STMN1*, *TUBB*, *PCNA*, *SMC4*, *NUSAP1*, *CENPF*, *MKI67*, *TOP2A*, *CDK1*
Atypical memory B cell	*LDHA*, *PKM*
Stress B cell	*IRF7*, *HSPG2*

Table S3. Marker genes of cell subtypes.

Murine Genes	Forward primer	Reverse Primer
Actb	5’-TGTCCACCTTCCAGCAGATGT-3’	5’-AGCTCAGTAACAGTCCGCCTAG-3’
Cd68	5’-TGTCTGATCTTGCTAGGACCG-3’	5’-GAGAGTAACGGCCTTTTTGTGA-3’
Cd86	5’-CTGGACTCTACGACTTCACAATG-3’	5’-AGTTGGCGATCACTGACAGTT-3’
Cd163	5’-TGTGCAGTAACGGCTGGAG-3’	5’-ATCATGTTTGCAGTCCCAAAGA-3’
Trem2	5’-CTGGAACCGTCACCATCACTC-3’	5’-GACCCACAGGATGAAACCTGC-3’
Havcr2	5’-ACTGGTGACCCTCCATAATAACA-3’	5’-GCAGTTCTGATCGTTTCTCCA-3’
Pim2	5’-CGGGTGTGATACGCCTTCTTG-3’	5’-CGGGTGTGATACGCCTTCTTG-3’
Csf1r	5’-TGTCATCGAGCCTAGTGGC-3’	5’-CGGGAGATTCAGGGTCCAAG-3’
Cd8	5’-CCGTTGACCCGCTTTCTGT-3’	5’-TTCGGCGTCCATTTTCTTTGG-3’
Gzmb	5’-CCACTCTCGACCCTACATGG-3’	5’-GGCCCCCAAAGTGACATTTATT-3’
Foxp3	5’-CACCTATGCCACCCTTATCCG-3’	5’-CATGCGAGTAAACCAATGGTAGA-3’

Table. S4 Real-Time PCR Primers for Gene Expression.
Primers used for real-time PCR. All the sequences were from PrimerBank (https://pga.mgh.harvard.edu/primerbank/).