Expanded View Figures

Figure EV1. ScRNA-seq profiling of the ecosystem in CC samples.

A UMAP plot showing the distribution of singlets (pink) and doublets (red) in CESC as evaluated using by R packages "DoubletFinder".
B Density plots showing the distribution of UMIs (left panel), genes (middle panel), and mitochondrial percents (right panel) of all single cells. The light gray dotted line denotes the cut-offs of quality control.
C Boxplots showing the T, Plasma and B cell three signature scores of B cell subtypes.
D UMAP plot showing the information of Batch from patients. Before (left panel) and after (right panel) batch effect removal.
E Boxplots showing the cell-type proportions of six patients for matched tumor and normal samples. $P < 0.05$ was considered statistical significance; The scCODA model and the ALDEx2 model were used to examine the differences in cell-type composition. Red bars indicate credible and significant results of scCODA. Stars indicate the significance calculated by ALDEx2 model (* $P < 0.05$).
F Scatter plots showing the quantification of T cells ($CD3^+$), B cells ($CD20^+$), macrophages ($CD68^+$) and NK cells ($CD56^+$) in tumor area (T) and adjacent normal tissue (N, $n = 6$). Represented Mean ± SEM. *P*-value was measured by paired Student's *t*-test.
G Violin plot showing the expression of marker genes in epithelial subclusters.
H Dot plot showing the selected signaling pathways (rows) with significant enrichment of GO, KEGG and Hallmark terms for four epithelial cell clusters.
I Stacked bar plot showing the epithelial cell clusters distribution among patients.
J Violin plot showing the expression of POSTN in EP2 and fibroblasts.
K OS rates for the high-correlation and low-correlation groups in TCGA, stratified using the EP2_POSTN signatures removed overlapped genes with fibroblasts. *P*-values are calculated using the log-rank test ($N = 255$).

Data information: Boxplots show the median and upper/lower quartiles (Related to Fig 1).

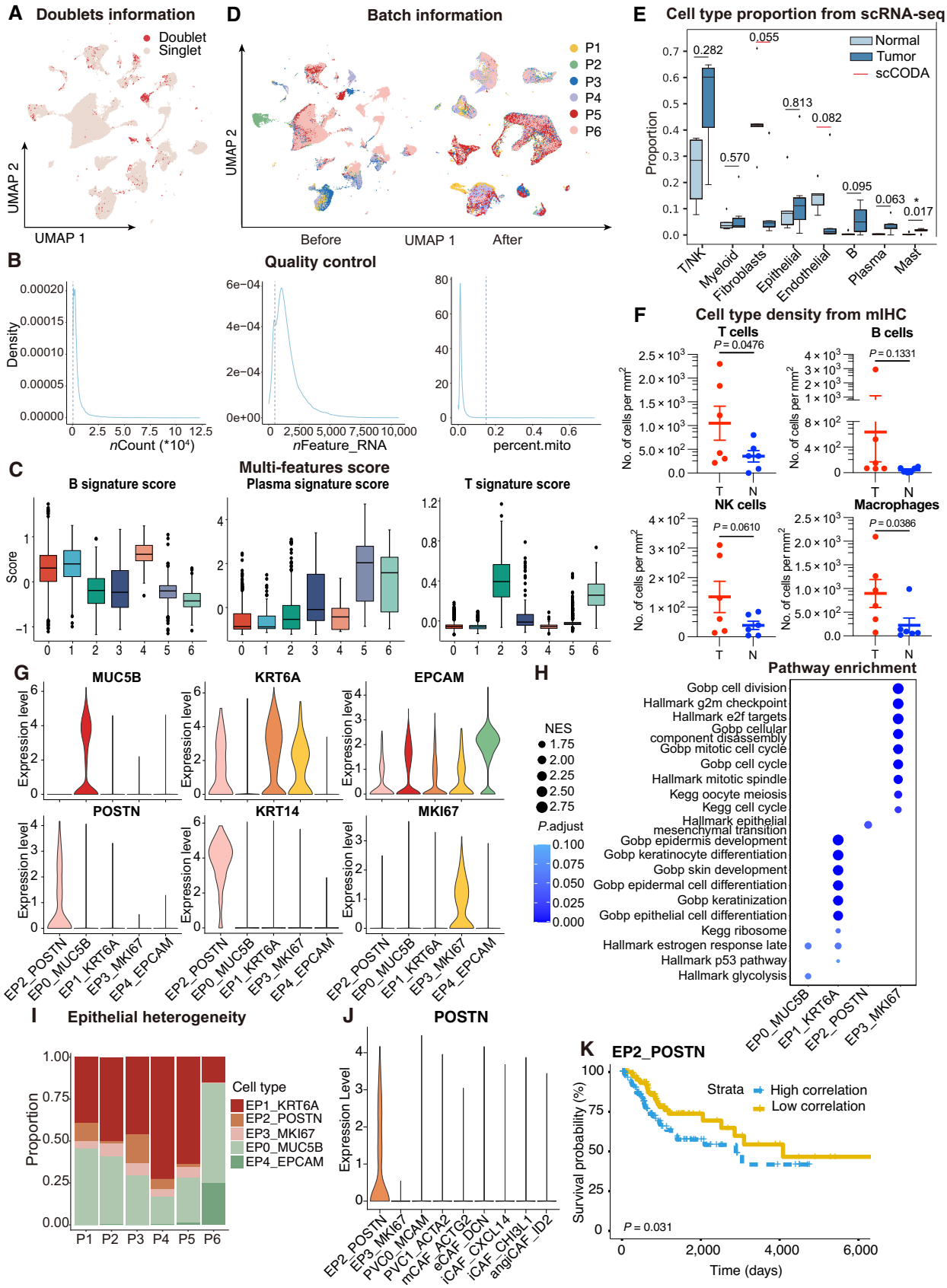

Figure EV1.

Figure EV2. The clinical function of NK cells and TCR repertoire profiling using TRUST predicted data.

A Heatmap showing the expression of marker genes in each subtype of T/NK cells.
B Comparison of Overall survival (OS) rates for the high-correlation and low-correlation groups, stratified using the NK_FCGR3A (left panel) and NK_KLRC1 (right panel) signatures in TCGA. *P*-values are calculated using the log-rank test ($N = 255$).
C Representative example of tumor versus normal clones from 10× TCR data, showing both chains, with filled points representing clones suggesting a significant change in frequency.
D Triangle heatmap showing the overlap of expanded TCR clonotypes across all possible combinations of T cell clusters. Data were aggregated for each of the indicated patient groups from TRUST predicted TCR data. Numbers indicate the normalized Jaccard index number of shared expanded TCR clonotypes for each cluster pair.
E Bubble plots showing the interactions between epithelial cells and T/NK cell populations using CellPhoneDB.
F UMAP as in Fig 2A, but clones from TRUST predicted TCR data.
G Dot plot showing the selected signaling pathways (rows) with significant enrichment of GO terms for large clones and small clones identified by 10× or predicted by TRUST (Related to Fig 2).

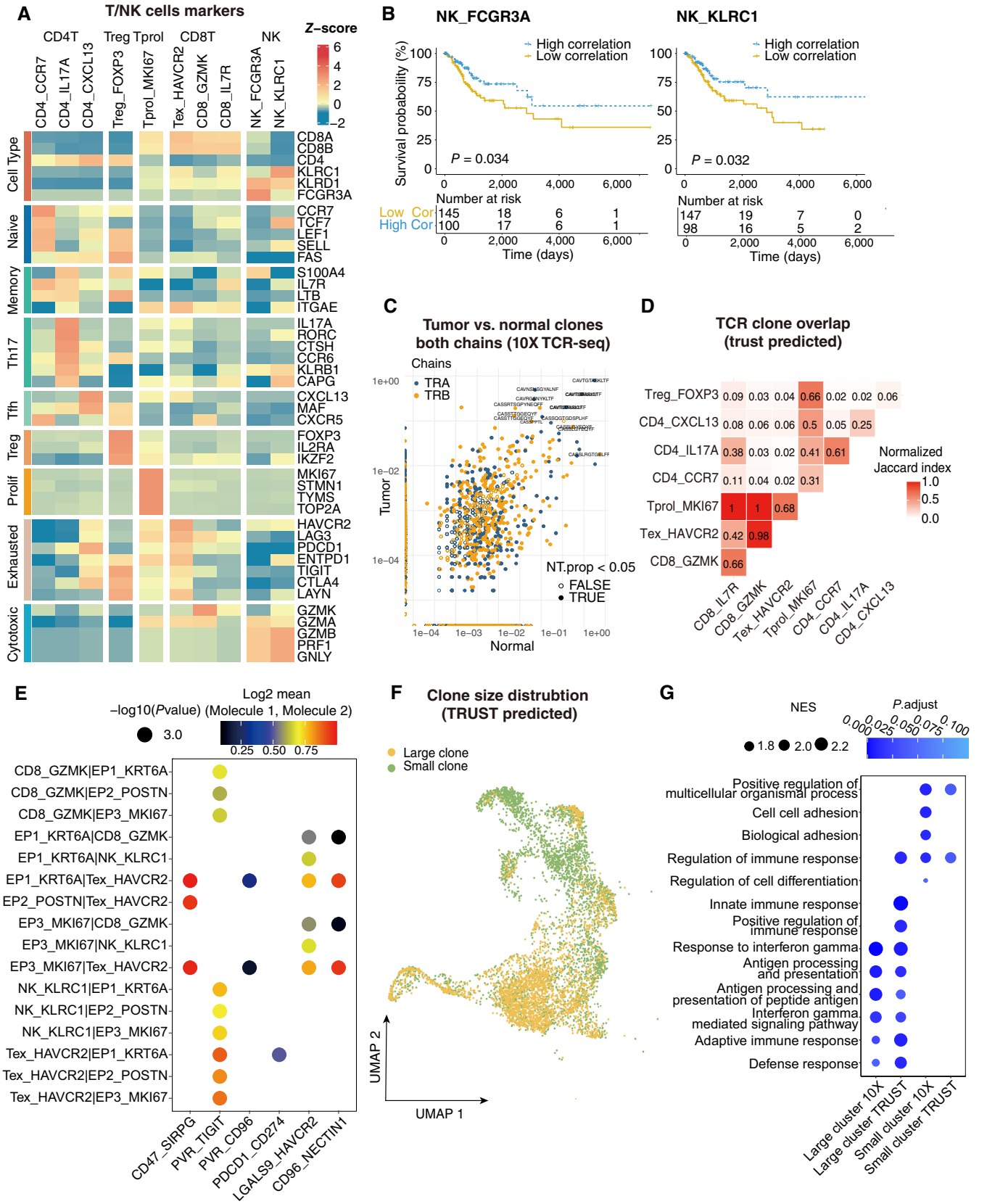

Figure EV2.

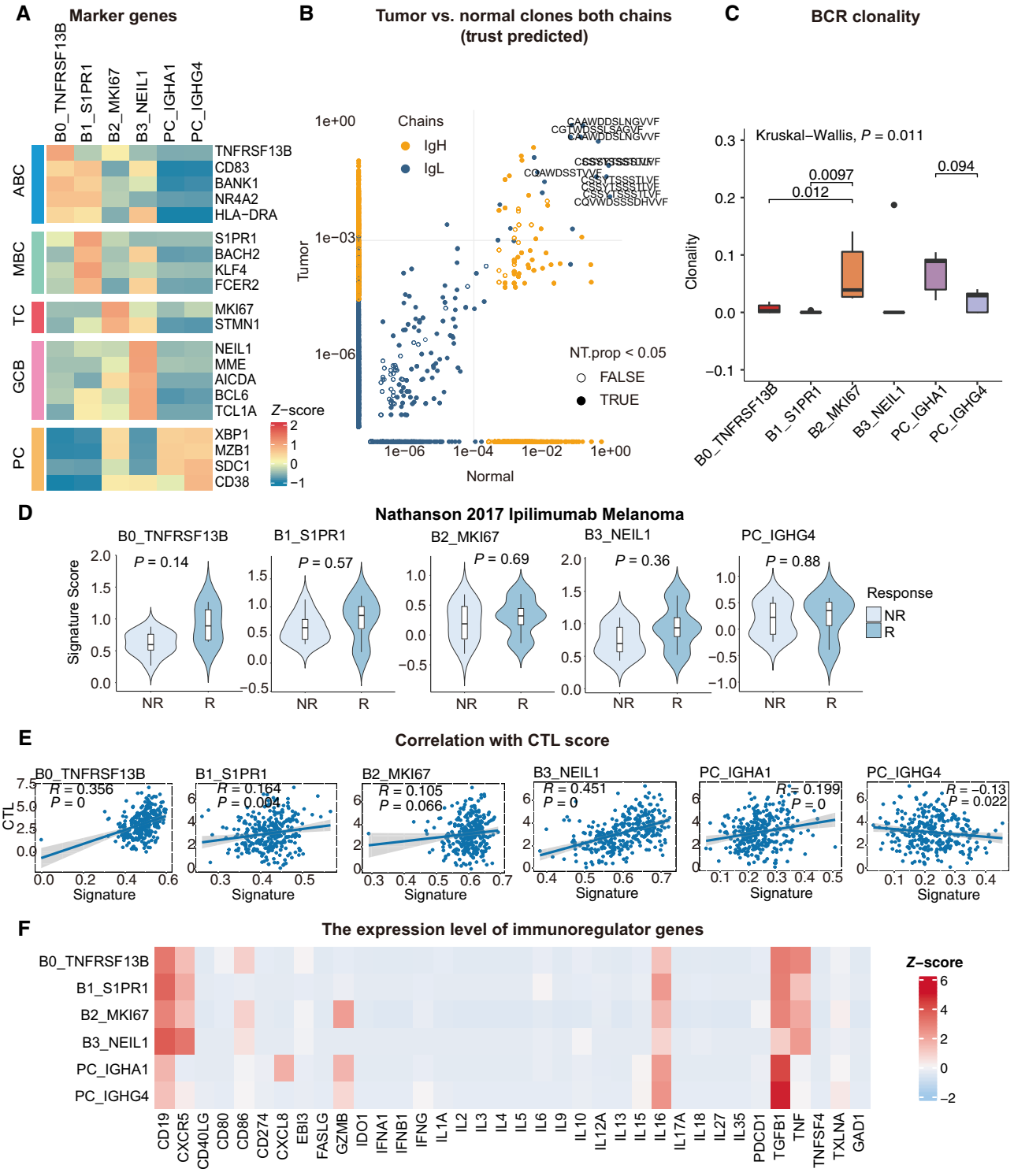
A
Marker genes
B0_TNFRSF13B
B1_S1PR1
B2_MKI67
B3_NEIL1
PC_IGHA1
PC_IGHG4
ABC
MBC
TC
GCB
PC
TNFRSF13B
CD83
BANK1
NR4A2
HLA−DRA
S1PR1
BACH2
KLF4
FCER2
MKI67
STMN1
NEIL1
MME
AICDA
BCL6
TCL1A
XBP1
MZB1
SDC1
CD38
Z−score
B
Tumor vs. normal clones both chains (trust predicted)
Chains
IgH
IgL
Tumor
Normal
NT.prop < 0.05
FALSE
TRUE
C
BCR clonality
Kruskal–Wallis, P = 0.011
0.0097
0.012
0.094
Clonality
D
Nathanson 2017 Ipilimumab Melanoma
Signature Score
P = 0.14
P = 0.57
P = 0.69
P = 0.36
P = 0.88
Response
NR
R
E
Correlation with CTL score
CTL
R = 0.356
P = 0
R = 0.164
P = 0.004
R = 0.105
P = 0.066
R = 0.451
P = 0
R = 0.199
P = 0
R = −0.13
P = 0.022
Signature
F
The expression level of immunoregulator genes
Z−score
CD19
CXCR5
CD40LG
CD80
CD86
CD274
CXCL8
EBI3
FASLG
GZMB
IDO1
IFNA1
IFNB1
IFNG
IL1A
IL2
IL3
IL4
IL5
IL6
IL9
IL10
IL12A
IL13
IL15
IL16
IL17A
IL18
IL27
IL35
PDCD1
TGFB1
TNF
TNFSF4
TXLNA
GAD1

Figure EV3.

Figure EV3. Clinical performance of B cell populations and BCR repertoire profiling using TRUST predicted data.

A Heatmap showing the expression of marker genes in each subtype of B cells.
B Representative example of tumor versus normal clones from TRUST predicted BCR data, showing both chains, with filled points representing clones suggesting a significant change in frequency.
C Boxplots showing each cell type BCR Clonality level for matched tumor and normal samples by using TRUST predicted BCR data. Student's *t*-test, $P < 0.05$ was considered statistical significance.
D Violin plots showing the association of different B-cell signatures with the response (R, $N = 4$) and no response (NR, $N = 11$) to Ipilimumab in the Nathanson 15 cohort. *P*-values calculated using the student's *t*-test.
E Scatter plot showing the correlation between the bulk sample with CTL score and the single-cell B cell clusters.
F Heatmap showing the expression level of immunoregulator genes in B-cell populations.

Data information: Boxplots show the median and upper/lower quartiles (Related to Fig 3).

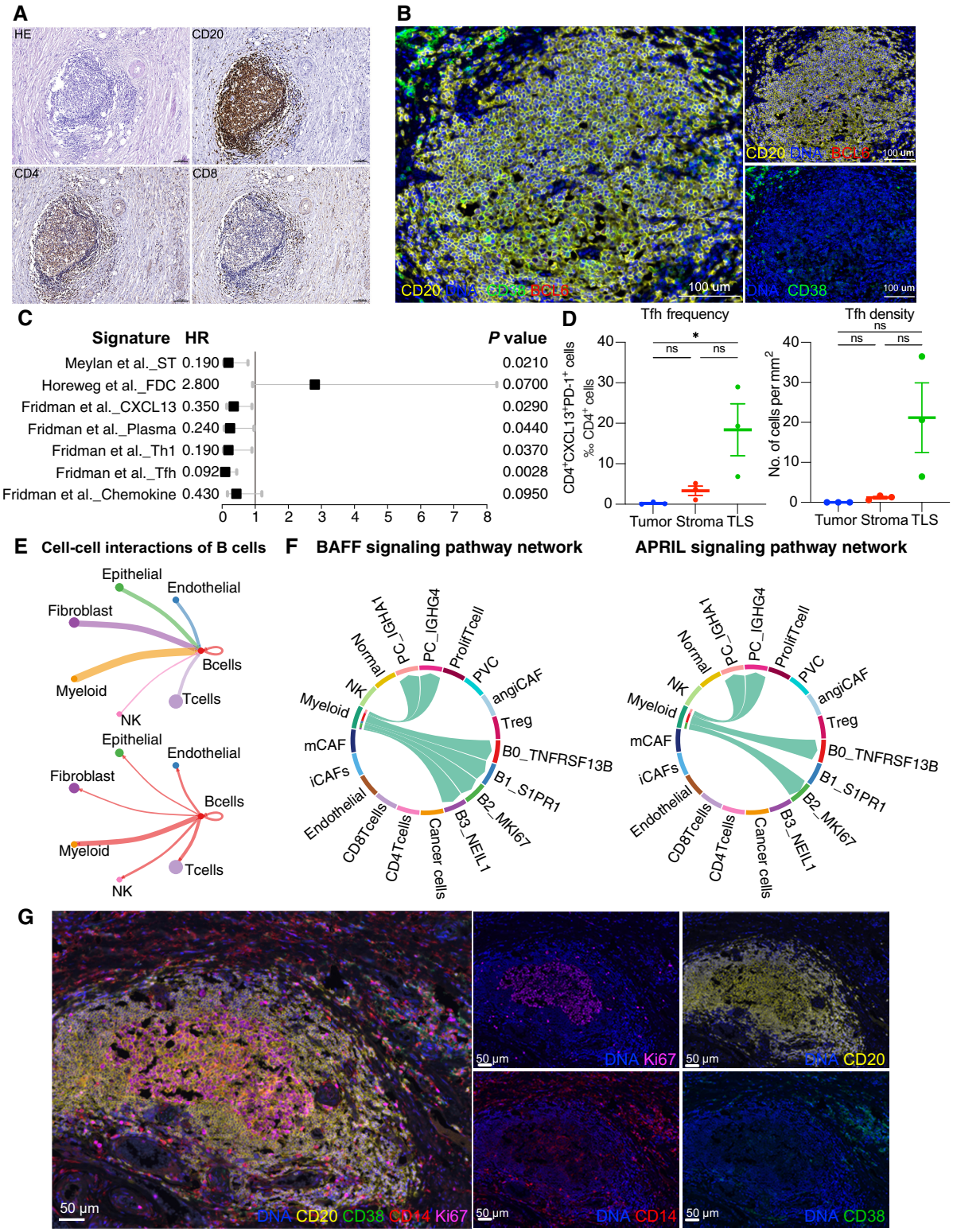
A
HE
CD20
CD4
CD8
B
CD20 DNA CD38 BCL6
100 um
CD20 DNA BCL6
100 um
DNA CD38
100 um
C
Signature HR
P value
Meylan et al._ST 0.190 0.0210
Horeweg et al._FDC 2.800 0.0700
Fridman et al._CXCL13 0.350 0.0290
Fridman et al._Plasma 0.240 0.0440
Fridman et al._Th1 0.190 0.0370
Fridman et al._Tfh 0.092 0.0028
Fridman et al._Chemokine 0.430 0.0950
0 1 2 3 4 5 6 7 8
D
Tfh frequency
CD4+CXCL13+PD-1+ cells
‰ CD4+ cells
ns ns *
Tumor Stroma TLS
Tfh density
No. of cells per mm2
ns ns ns
Tumor Stroma TLS
E Cell-cell interactions of B cells
Epithelial
Endothelial
Fibroblast
Bcells
Myeloid
Tcells
NK
F BAFF signaling pathway network
APRIL signaling pathway network
PC_IGHA1
PC_IGHG4
ProlifTcell
PVC
angiCAF
Treg
B0_TNFRSF13B
B1_S1PR1
B2_MKI67
B3_NEIL1
Cancer cells
CD4Tcells
CD8Tcells
Endothelial
iCAFs
mCAF
Myeloid
NK
Normal
G
50 μm
DNA CD20 CD38 CD14 Ki67
DNA Ki67
DNA CD20
DNA CD14
DNA CD38

Figure EV4.

Figure EV4. Additional analysis of TLS.

A Cellular compositions of TLS are exhibited by immunohistochemistry. Scale = 100 μm.

B B-cell subsets are exhibited by mIHC via differential markers as follows: total B cell: $CD20^+$, GCB: $CD20^+BCL6^-$, PC: $CD20^-CD38^+$. Scale = 100 μm.

C A forest plot showing the association of TLSs-associated signatures with survival across the TCGA SCC cohort ($n = 255$). Squares and lines indicate hazard ratios (HRs) and 95% confidence intervals (CIs), respectively. HRs calculated using univariable Cox regression; *P*-values calculated using log-rank test; ST, spatial transcriptomics.

D Scatter plots showing the frequency and density of Tfh cells ($CD4^+PD\text{-}1^+CXCL13^+$) in TLSs, tumor and stroma ($n = 3$). Represented Mean ± SEM. *P*-value was measured by the Holm–Sidak's multiple comparisons test, $^*P < 0.05$.

E Circle plots showing the number of interaction weights between B cell groups and other cell groups. Edge colors are consistent with the sources as the sender, and edge weights are proportional to the interaction strength. A thicker edge line indicates a stronger signal.

F Chord diagram showing the communication networks of APRIL (left panel) and BAFF (right panel) signaling pathways. Arrows indicate the interactions (signaling pathways) from some cell groups to other cell groups.

G Representative mIHC of $CD14^+$ cell localization in TLS. Scale bars are 50 μm (Related to Fig 4).

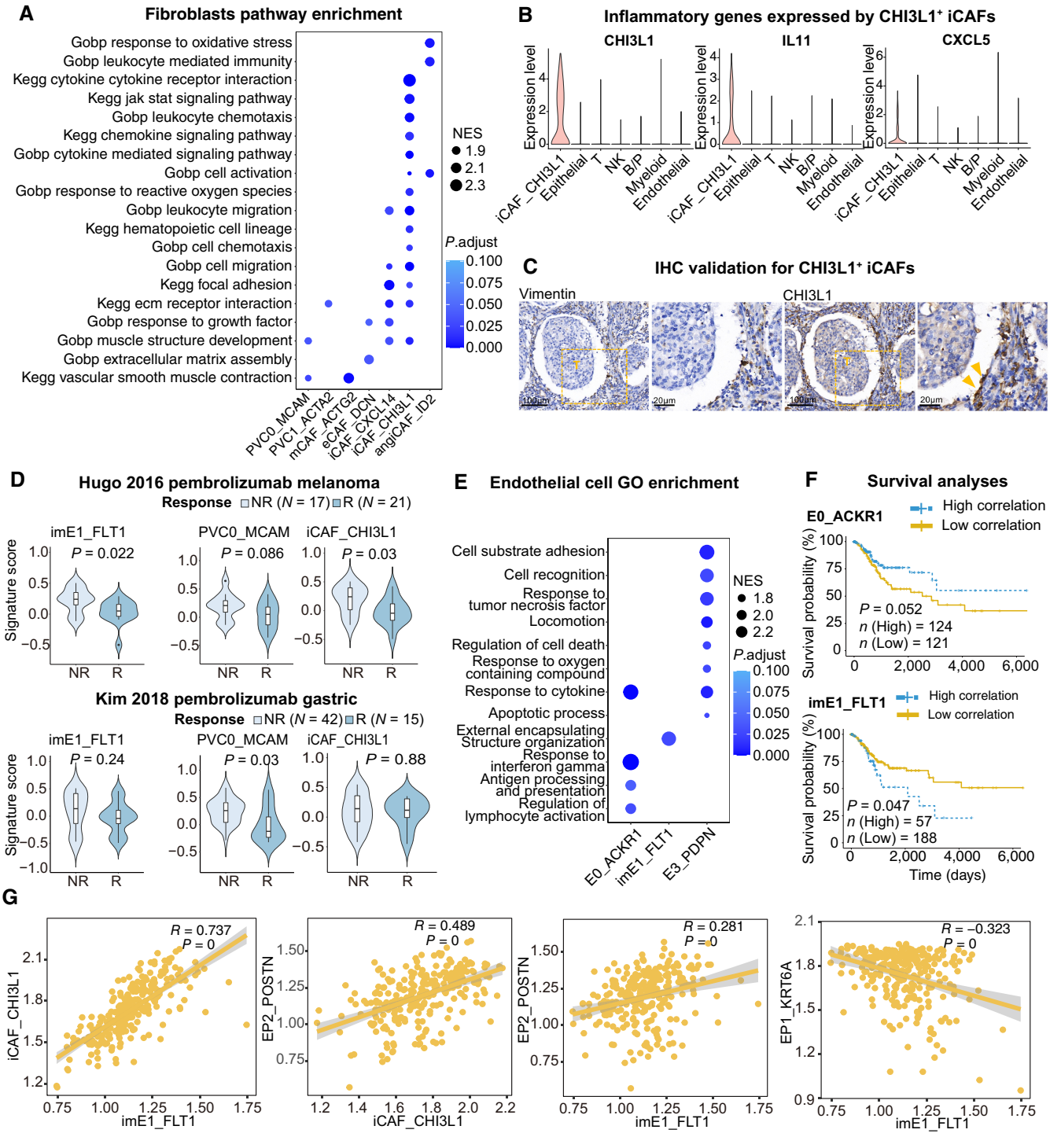
A
Fibroblasts pathway enrichment
Gobp response to oxidative stress
Gobp leukocyte mediated immunity
Kegg cytokine cytokine receptor interaction
Kegg jak stat signaling pathway
Gobp leukocyte chemotaxis
Kegg chemokine signaling pathway
Gobp cytokine mediated signaling pathway
Gobp cell activation
Gobp response to reactive oxygen species
Gobp leukocyte migration
Kegg hematopoietic cell lineage
Gobp cell chemotaxis
Gobp cell migration
Kegg focal adhesion
Kegg ecm receptor interaction
Gobp response to growth factor
Gobp muscle structure development
Gobp extracellular matrix assembly
Kegg vascular smooth muscle contraction
PVC0_MCAM
PVC1_ACTA2
mCAF_ACTG2
eCAF_DCN
iCAF_CXCL14
iCAF_CHI3L1
angiCAF_ID2
NES
1.9
2.1
2.3
P.adjust
0.100
0.075
0.050
0.025
0.000
B
Inflammatory genes expressed by CHI3L1+ iCAFs
CHI3L1
IL11
CXCL5
Expression level
iCAF_CHI3L1
Epithelial
T
NK
B/P
Myeloid
Endothelial
C
IHC validation for CHI3L1+ iCAFs
Vimentin
CHI3L1
100μm
20μm
D
Hugo 2016 pembrolizumab melanoma
Response NR (N = 17) R (N = 21)
imE1_FLT1
P = 0.022
PVC0_MCAM
P = 0.086
iCAF_CHI3L1
P = 0.03
Signature score
NR
R
Kim 2018 pembrolizumab gastric
Response NR (N = 42) R (N = 15)
imE1_FLT1
P = 0.24
PVC0_MCAM
P = 0.03
iCAF_CHI3L1
P = 0.88
E
Endothelial cell GO enrichment
Cell substrate adhesion
Cell recognition
Response to tumor necrosis factor
Locomotion
Regulation of cell death
Response to oxygen containing compound
Response to cytokine
Apoptotic process
External encapsulating Structure organization
Response to interferon gamma
Antigen processing and presentation
Regulation of lymphocyte activation
E0_ACKR1
imE1_FLT1
E3_PDPN
NES
1.8
2.0
2.2
P.adjust
0.100
0.075
0.050
0.025
0.000
F
Survival analyses
E0_ACKR1
High correlation
Low correlation
P = 0.052
n (High) = 124
n (Low) = 121
imE1_FLT1
P = 0.047
n (High) = 57
n (Low) = 188
Survival probability (%)
Time (days)
G
R = 0.737
P = 0
iCAF_CHI3L1
imE1_FLT1
R = 0.489
P = 0
EP2_POSTN
iCAF_CHI3L1
R = 0.281
P = 0
EP2_POSTN
imE1_FLT1
R = −0.323
P = 0
EP1_KRT6A
imE1_FLT1

Figure EV5.

◀ **Figure EV5. Functional and clinical analysis of stromal cells.**

A Dot plot showing the selected signaling pathways (rows) with significant enrichment of GO, and KEGG terms for fibroblasts.
B Violin plot showing the expression of immune regulator genes in iCAF_CHI3L1 and other cell lineages.
C IHC staining of representative tumor section showing the expression of CHI3L1 in CAFs (yellow arrows) close to cancer cells; The scale bars for the left slide of each marker are 100 μm, and the scale bars for the right slide are 20 μm.
D Violin plots showing the association of different stromal cell signatures with the response (R) and no response (NR) to Pembrolizumab across on Hugo 26 cohort (upper panel) and Kim 45 cohort (lower panel). *P*-values calculated using student's *t*-test. Sample sizes are indicated in figure.
E Dot plot showing the selected signaling pathways (rows) with significant enrichment of GO, and endothelial cell clusters.
F Comparison of Overall survival (OS) rates for the high-correlation and low-correlation groups, stratified using the E0_ACKR1 (left panel) and imE1_FLT1 (right panel) signatures in TCGA ($N = 255$). *P*-values are calculated using the log-rank test.
G Scatter plot showing the correlation among imE1, EP1, EP2 and iCAF_CHI3L1 subsets.

Data information: Violin plots show the median and upper/lower quartiles (Related to Fig 6).