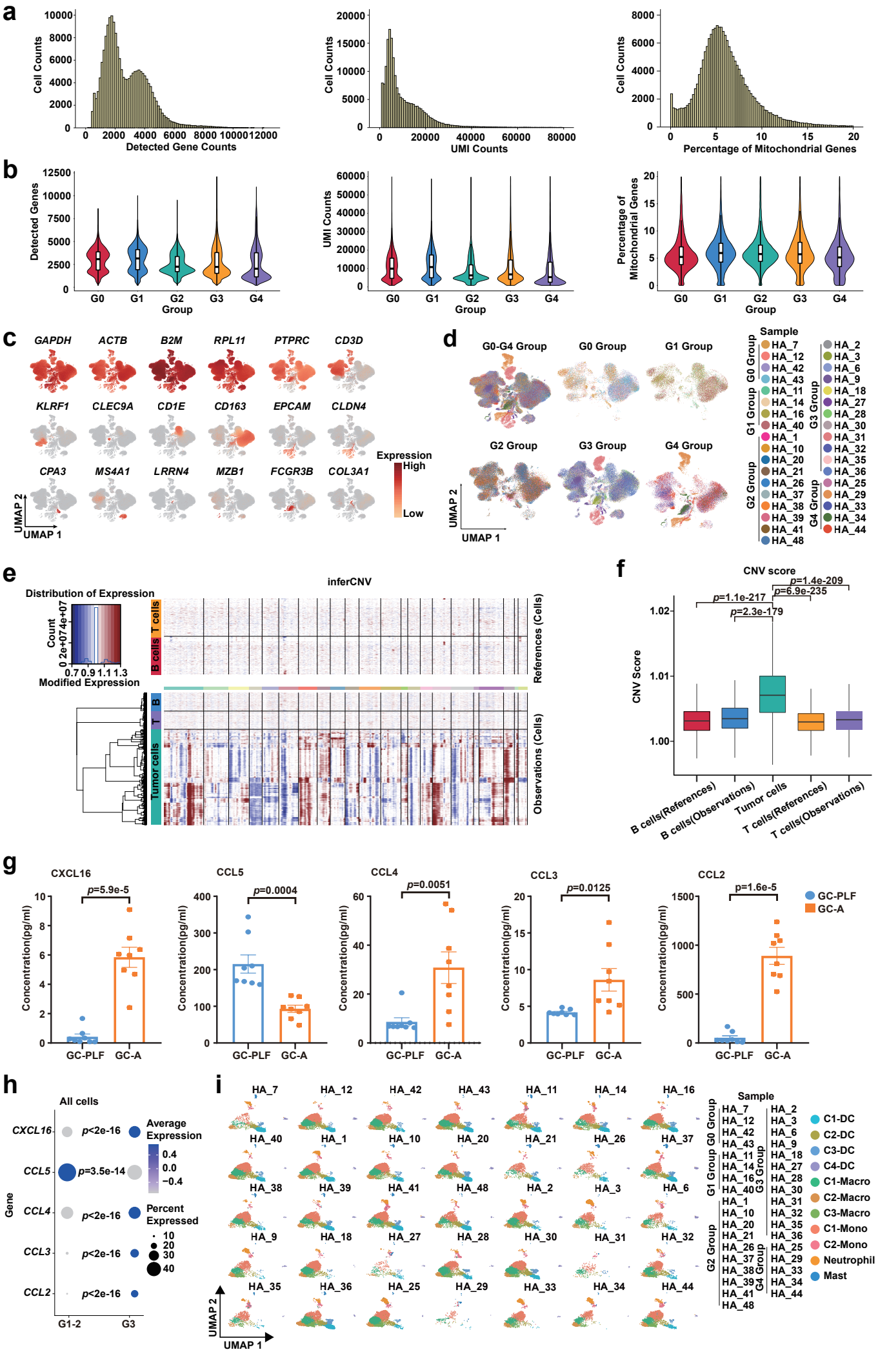
**Single-cell sequencing of ascites fluid illustrates heterogeneity and therapy-induced evolution during gastric cancer peritoneal metastasis**

**Supplementary Figure and Supplementary Tables:**

**Supplementary Figure and Supplementary Figure Legends:**

**Supplementary Fig. 1-7**


a
Cell Counts
Detected Gene Counts
UMI Counts
Percentage of Mitochondrial Genes
b
Detected Genes
UMI Counts
Percentage of Mitochondrial Genes
Group
G0
G1
G2
G3
G4
c
GAPDH
ACTB
B2M
RPL11
PTPRC
CD3D
KLRF1
CLEC9A
CD1E
CD163
EPCAM
CLDN4
CPA3
MS4A1
LRRN4
MZB1
FCGR3B
COL3A1
Expression
High
Low
UMAP 2
UMAP 1
d
G0-G4 Group
G0 Group
G1 Group
G2 Group
G3 Group
G4 Group
Sample
G0 Group
G1 Group
G2 Group
G3 Group
G4 Group
HA_7
HA_12
HA_42
HA_43
HA_11
HA_14
HA_16
HA_40
HA_1
HA_10
HA_20
HA_21
HA_26
HA_37
HA_38
HA_39
HA_41
HA_48
HA_2
HA_3
HA_6
HA_9
HA_18
HA_27
HA_28
HA_30
HA_31
HA_32
HA_35
HA_36
HA_25
HA_29
HA_33
HA_34
HA_44
e
Distribution of Expression
Count
Modified Expression
inferCNV
T cells
B cells
References (Cells)
B
T
Tumor cells
Observations (Cells)
f
CNV score
p=1.4e-209
p=6.9e-235
p=1.1e-217
p=2.3e-179
CNV Score
B cells(References)
B cells(Observations)
Tumor cells
T cells(References)
T cells(Observations)
g
CXCL16
CCL5
CCL4
CCL3
CCL2
p=5.9e-5
p=0.0004
p=0.0051
p=0.0125
p=1.6e-5
Concentration(pg/ml)
GC-PLF
GC-A
h
All cells
Gene
CXCL16
CCL5
CCL4
CCL3
CCL2
p<2e-16
p=3.5e-14
Average Expression
Percent Expressed
G1-2
G3
i
Sample
C1-DC
C2-DC
C3-DC
C4-DC
C1-Macro
C2-Macro
C3-Macro
C1-Mono
C2-Mono
Neutrophil
Mast

**Supplementary Fig. 1. scRNA-seq profiles of cells identified in the peritoneal ecosystem**

(a) Histogram showing the distributions of detected gene counts, unique molecular identifier (UMI) counts, and percentage of mitochondrial genes per cell.

(b) Violin plots showing the distributions of detected genes, UMI counts and percentage of mitochondrial genes for cells in G0-G4 Groups (G0: n = 19581 cells; G1: n = 16768 cells; G2: n = 56153 cells; G3: n = 69648 cells; G4: n = 29837 cells). Box represents median ± interquartile range; whiskers represent 1.5x interquartile range.

(c) Uniform Manifold Approximation and Projection (UMAP) plots indicating the expression of marker genes to annotate cell types.

(d) Uniform Manifold Approximation and Projection (UMAP) plot showing the cell type distribution in G0-G4 Groups. Each color represents a sample.

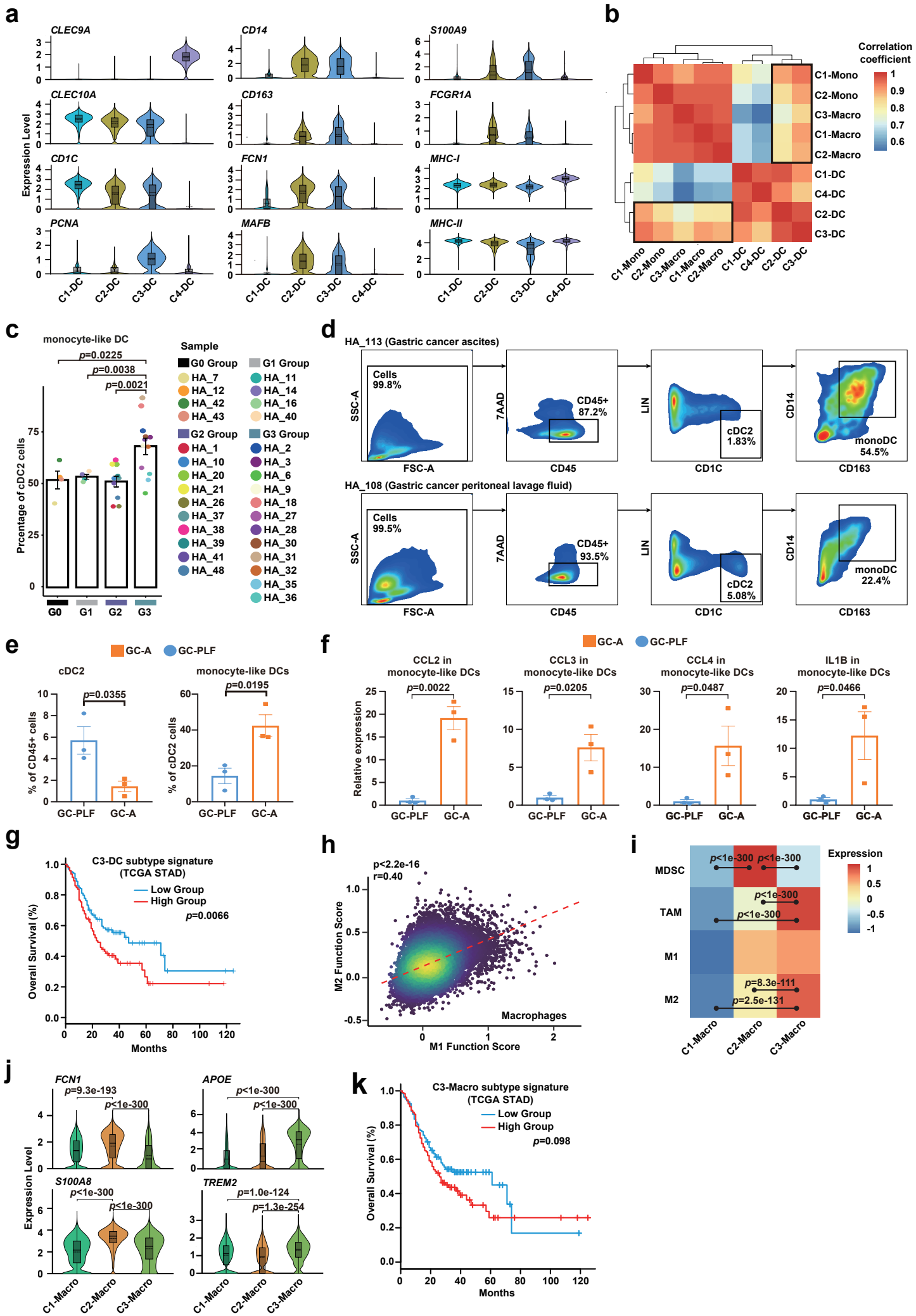
(e) Heatmap indicating chromosomal copy number variation (CNV) of T cells, B cells, and tumor cells. Upper panel shows the CNVs of 1000 random-selected T and B cells as reference cells. Lower panel shows the CNVs of another 1000 random-selected T and B cells for validation and all tumor cells for observation.

(f) Box plots showing CNV scores of T cells (n = 1000 cells) and B cells (n = 1000 cells) in references and observations, respectively, and tumor cells (n = 5235 cells). Box represents median ± interquartile range; whiskers represent 1.5x interquartile range; the *p* values are calculated by two-sided unpaired Wilcoxon test.

(g) ELISA of chemokines in the gastric cancer ascites (GC-A, n = 8 samples) and gastric cancer peritoneal lavage fluids (GC-PLF, n = 8 samples). Data are presented as mean ± SEM; the *p* values are calculated by two-sided unpaired Student's t test.

(h) Dotplots showing the expression levels of chemokine genes in all cells. Dot size represents percent of expressing cells in each group, color represents expression level of selected genes. G1-2: gastric cancer without peritoneal metastasis, as GC-PLF in (g); G3, gastric cancer with peritoneal metastasis, as GC-A in (g). *p* values are calculated by Wilcoxon test.

(i) UMAP plot showing the distribution of myeloid cells in G0-G4 Groups. Each color represents a cell type.


a
CLEC9A
CD14
S100A9
CLEC10A
CD163
FCGR1A
CD1C
FCN1
MHC-I
PCNA
MAFB
MHC-II
Expression Level
C1-DC
C2-DC
C3-DC
C4-DC
b
Correlation coefficient
C1-Mono
C2-Mono
C3-Macro
C1-Macro
C2-Macro
C1-DC
C4-DC
C2-DC
C3-DC
c
monocyte-like DC
p=0.0225
p=0.0038
p=0.0021
Prcentage of cDC2 cells
Sample
G0 Group
G1 Group
G2 Group
G3 Group
HA_7
HA_11
HA_12
HA_14
HA_42
HA_16
HA_43
HA_40
HA_1
HA_2
HA_10
HA_3
HA_20
HA_6
HA_21
HA_9
HA_26
HA_18
HA_37
HA_27
HA_38
HA_28
HA_39
HA_30
HA_41
HA_31
HA_48
HA_32
HA_35
HA_36
G0
G1
G2
G3
d
HA_113 (Gastric cancer ascites)
Cells 99.8%
SSC-A
FSC-A
7AAD
CD45+ 87.2%
CD45
LIN
cDC2 1.83%
CD1C
CD14
monoDC 54.5%
CD163
HA_108 (Gastric cancer peritoneal lavage fluid)
Cells 99.5%
CD45+ 93.5%
cDC2 5.08%
monoDC 22.4%
e
GC-A
GC-PLF
cDC2
monocyte-like DCs
p=0.0355
p=0.0195
% of CD45+ cells
% of cDC2 cells
f
CCL2 in monocyte-like DCs
CCL3 in monocyte-like DCs
CCL4 in monocyte-like DCs
IL1B in monocyte-like DCs
p=0.0022
p=0.0205
p=0.0487
p=0.0466
Relative expression
g
C3-DC subtype signature (TCGA STAD)
Low Group
High Group
p=0.0066
Overall Survival (%)
Months
h
p<2.2e-16
r=0.40
M2 Function Score
M1 Function Score
Macrophages
i
MDSC
TAM
M1
M2
p<1e-300
p=8.3e-111
p=2.5e-131
Expression
j
FCN1
p=9.3e-193
APOE
S100A8
TREM2
p=1.0e-124
p=1.3e-254
k
C3-Macro subtype signature (TCGA STAD)
p=0.098

**Supplementary Fig. 2. Characteristics of myeloid cells in the peritoneal microenvironment during gastric cancer peritoneal metastasis**

(a) Violin plots showing the expression levels of selected marker genes in dendritic cell (DC) (C1-DC: n = 8753 cells; C2-DC: n = 8430 cells; C3-DC: n = 2173 cells; C4-DC: n = 921 cells), color-coded by cell type. Box represents median ± interquartile range; whiskers represent 1.5x interquartile range; horizontal dotted line represents mean value.

(b) Heatmap showing the correlation between myeloid subtypes. Black boxes highlight the C2/C3-DC sharing with monocyte-lineage features.

(c) The proportion of monocyte-like DCs in different groups from G0 (n = 4 samples), G1 (n = 4 samples), G2 (n = 10 samples) and G3 (n = 12 samples) Group. Each color represents a sample. Data are presented as mean values ± SEM (error bars); the *p* values are calculated by two-sided unpaired Student's t test.

(d) FACS strategy of monocyte-like DCs from gastric cancer ascites and gastric cancer peritoneal lavage fluids.

(e) Bar plots showing the proportions of cDC2 in CD45+ cells (Left) and monocyte-like DCs in cDC2 (Right) between gastric cancer ascites (GC-A, n= 3 independent experiments) and gastric cancer peritoneal lavage fluids (GC-PLF, n= 3 independent experiments). Data are presented as mean values ± SEM (error bars); the *p* values are calculated by two-sided unpaired Student's t test.

(f) Bar plots showing qPCR results on chemokines for monocyte-like DCs between gastric cancer ascites (GC-A, n= 3 independent experiments) and gastric cancer peritoneal lavage fluids (GC-PLF, n= 3 independent experiments). Data are presented as mean values ± SEM (error bars); the *p* values are calculated by two-sided unpaired Student's t test.

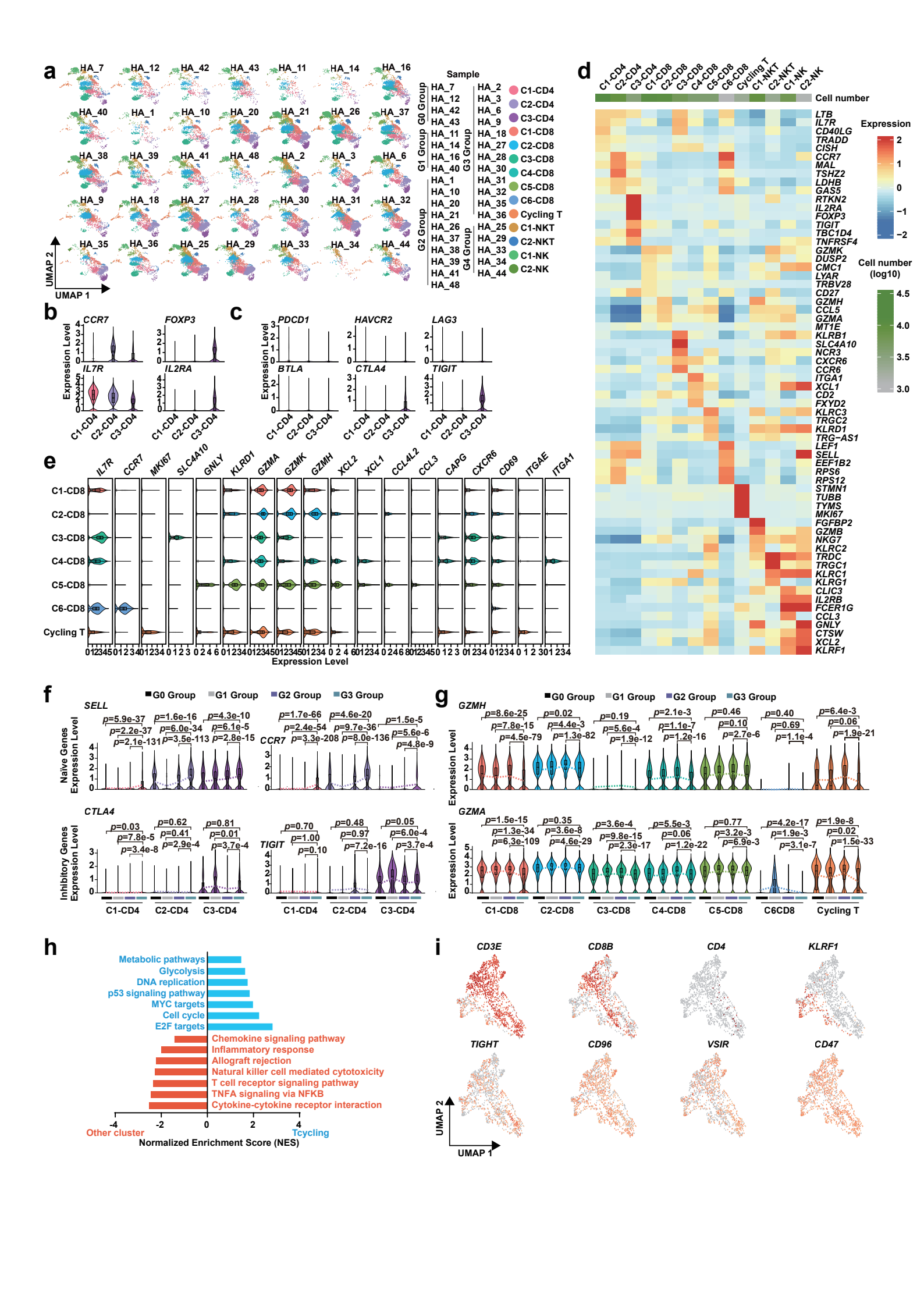
(g) Kaplan-Meier curve showing the overall survival of patients from The Cancer Genome Atlas Stomach Adenocarcinoma (TCGA STAD) database based on the expression level of the C3-DC signature. *p* value is calculated by log-rank test.

(h) Scatter plots showing the correlation between M1 and M2 function score in macrophages. *p* value is calculated by Pearson correlation test.

(i) Heatmap indicating the expression levels of specific gene signatures in macrophage subtypes. Macro, macrophage. *p* values are calculated by Wilcoxon test.

(j) Violin plots indicating the expression levels of *FCN1, APOE, S100A8* and *TREM2* genes in macrophage types (C1-Macro: n = 16571 cells; C2-Macro: n = 9995 cells; C3-Macro: n = 6323 cells). Box represents median ± interquartile range; whiskers represent 1.5x interquartile range; horizontal dotted line represents mean value. The *p* values are calculated by Wilcoxon test.

(k) The overall survival of TCGA STAD patients with high or low expression group of the C3-Macro subtype signature. *p* value is calculated by log-rank test.


a
HA_7 HA_12 HA_42 HA_43 HA_11 HA_14 HA_16
HA_40 HA_1 HA_10 HA_20 HA_21 HA_26 HA_37
HA_38 HA_39 HA_41 HA_48 HA_2 HA_3 HA_6
HA_9 HA_18 HA_27 HA_28 HA_30 HA_31 HA_32
HA_35 HA_36 HA_25 HA_29 HA_33 HA_34 HA_44
UMAP 2
UMAP 1
Sample
G0 Group
G1 Group
G2 Group
G3 Group
G4 Group
HA_7
HA_12
HA_42
HA_43
HA_11
HA_14
HA_16
HA_40
HA_1
HA_10
HA_20
HA_21
HA_26
HA_37
HA_38
HA_39
HA_41
HA_48
HA_2
HA_3
HA_6
HA_9
HA_18
HA_27
HA_28
HA_30
HA_31
HA_32
HA_35
HA_36
HA_25
HA_29
HA_33
HA_34
HA_44
C1-CD4
C2-CD4
C3-CD4
C1-CD8
C2-CD8
C3-CD8
C4-CD8
C5-CD8
C6-CD8
Cycling T
C1-NKT
C2-NKT
C1-NK
C2-NK
b
CCR7
FOXP3
IL7R
IL2RA
Expression Level
C1-CD4
C2-CD4
C3-CD4
c
PDCD1
HAVCR2
LAG3
BTLA
CTLA4
TIGIT
d
Cell number
Expression
Cell number (log10)
LTB
IL7R
CD40LG
TRADD
CISH
CCR7
MAL
TSHZ2
LDHB
GAS5
RTKN2
IL2RA
FOXP3
TIGIT
TBC1D4
TNFRSF4
GZMK
DUSP2
CMC1
LYAR
TRBV28
CD27
GZMH
CCL5
GZMA
MT1E
KLRB1
SLC4A10
NCR3
CXCR6
CCR6
ITGA1
XCL1
CD2
FXYD2
KLRC3
TRGC2
KLRD1
TRG-AS1
LEF1
SELL
EEF1B2
RPS6
RPS12
STMN1
TUBB
TYMS
MKI67
FGFBP2
GZMB
NKG7
KLRC2
TRDC
TRGC1
KLRC1
KLRG1
CLIC3
IL2RB
FCER1G
CCL3
GNLY
CTSW
XCL2
KLRF1
e
IL7R
CCR7
MKI67
SLC4A10
GNLY
KLRD1
GZMA
GZMK
GZMH
XCL2
XCL1
CCL4L2
CCL3
CAPG
CXCR6
CD69
ITGAE
ITGA1
C1-CD8
C2-CD8
C3-CD8
C4-CD8
C5-CD8
C6-CD8
Cycling T
Expression Level
f
G0 Group
G1 Group
G2 Group
G3 Group
SELL
CCR7
CTLA4
TIGIT
Naïve Genes Expression Level
Inhibitory Genes Expression Level
C1-CD4
C2-CD4
C3-CD4
g
GZMH
GZMA
Expression Level
C1-CD8
C2-CD8
C3-CD8
C4-CD8
C5-CD8
C6CD8
Cycling T
h
Metabolic pathways
Glycolysis
DNA replication
p53 signaling pathway
MYC targets
Cell cycle
E2F targets
Chemokine signaling pathway
Inflammatory response
Allograft rejection
Natural killer cell mediated cytotoxicity
T cell receptor signaling pathway
TNFA signaling via NFKB
Cytokine-cytokine receptor interaction
Other cluster
Tcycling
Normalized Enrichment Score (NES)
i
CD3E
CD8B
CD4
KLRF1
TIGHT
CD96
VSIR
CD47
UMAP 2
UMAP 1

**Supplementary Fig. 3. Characteristics of T cells in the peritoneal microenvironment during gastric cancer peritoneal metastasis**

(a) Uniform Manifold Approximation and Projection (UMAP) plot showing the distribution of T/NK cells in different groups. Each color represents a cell type.

(b, c) Violin plots showing expression levels of marker genes (b) and inhibitory genes (c) in CD4 subtypes (C1-CD4: n = 12855 cells; C2-CD4: n = 8528 cells; C3-CD4: n = 3485 cells). Box represents median ± interquartile range; whiskers represent 1.5x interquartile range; horizontal dotted line represents mean value.

(d) Heatmap plot indicating expression levels of selected genes among T/NK cell types. Expression level is projected by color: red represents high expression and blue represents low expression.

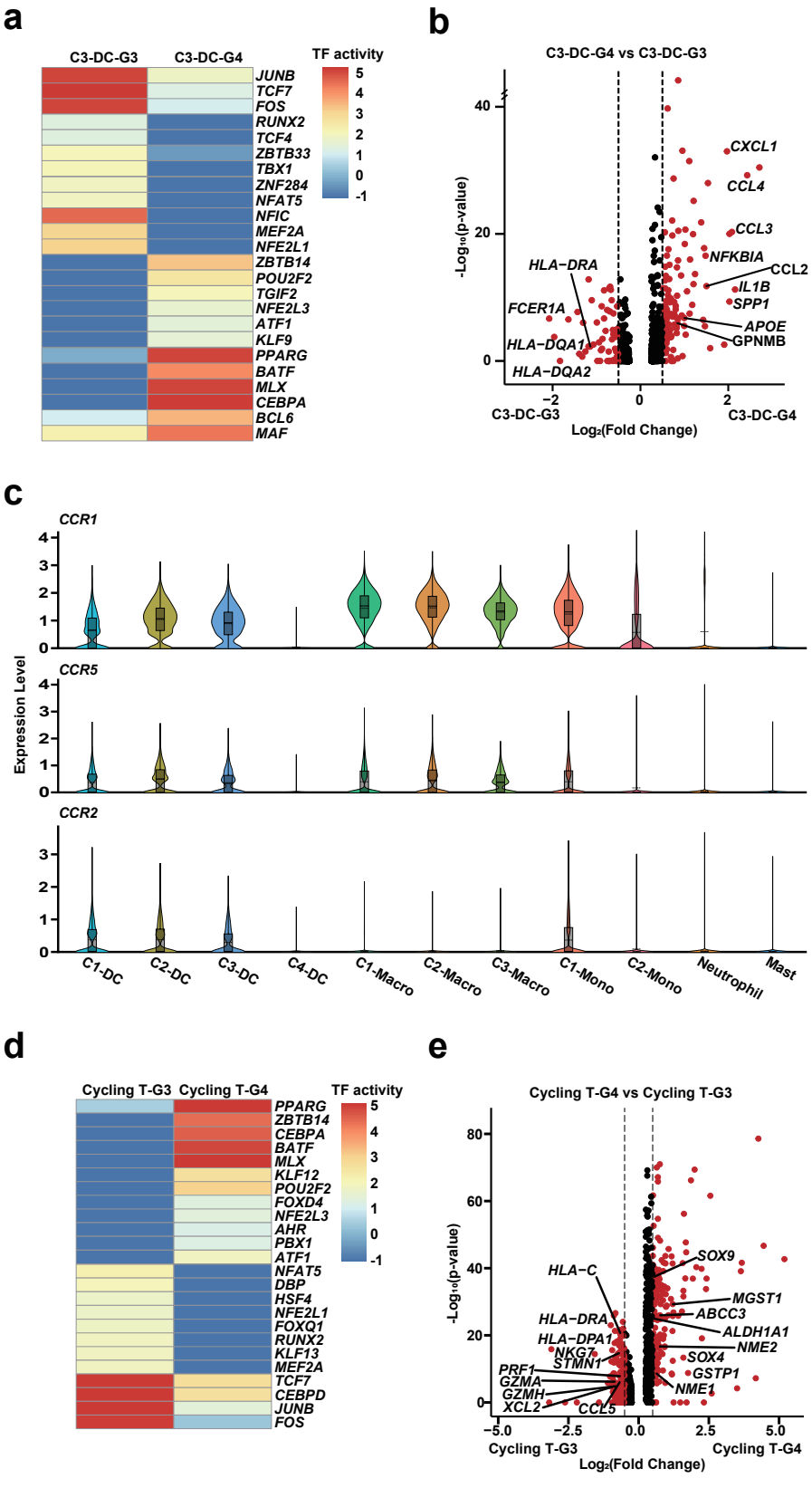
(e) Violin plots of selected marker genes (columns) for CD8 T cell subsets and cycling T cells (rows) (C1-CD8: n = 11021 cells; C2-CD8: n = 10285 cells; C3-CD8: n = 5623 cells; C4-CD8: n = 4025 cells; C5-CD8: n = 4007 cells; Cycling T: n = 1404 cells).

(f) Violin plot showing expression levels of selected naïve and inhibitory genes in CD4 T cells in G0 (n = 4 samples), G1 (n = 4 samples), G2 (n = 10 samples) and G3 (n = 12 samples) Group. Horizontal dotted line represents mean value. Box represents median ± interquartile range; whiskers represent 1.5x interquartile range; the *p* values are calculated by two-sided unpaired Wilcoxon test.

(g) Violin plot showing expression levels of selected cytotoxic genes in CD8 T cell types and cycling T cells in G0 (n = 4 samples), G1 (n = 4 samples), G2 (n = 10 samples) and G3 (n = 12 samples) Group. Horizontal dotted line represents mean value. Box represents median ± interquartile range; whiskers represent 1.5x interquartile range; the *p* values are calculated by two-sided unpaired Wilcoxon test.

(h) Bar chart showing the normalized enrichment score (NES) of specific pathways enriched in cycling T (blue lines) and other CD8 T cells (red lines) based on Gene Set Enrichment Analyses (GSEA) analysis.

(i) Uniform Manifold Approximation and Projection (UMAP) plots indicating the expression of selected marker genes to determine cycling T subtypes.


a
C3-DC-G3
C3-DC-G4
TF activity
JUNB
TCF7
FOS
RUNX2
TCF4
ZBTB33
TBX1
ZNF284
NFAT5
NFIC
MEF2A
NFE2L1
ZBTB14
POU2F2
TGIF2
NFE2L3
ATF1
KLF9
PPARG
BATF
MLX
CEBPA
BCL6
MAF
b
C3-DC-G4 vs C3-DC-G3
-Log10(p-value)
CXCL1
CCL4
CCL3
NFKBIA
CCL2
IL1B
SPP1
APOE
GPNMB
HLA−DRA
FCER1A
HLA−DQA1
HLA−DQA2
C3-DC-G3
C3-DC-G4
Log2(Fold Change)
c
CCR1
CCR5
CCR2
Expression Level
C1-DC
C2-DC
C3-DC
C4-DC
C1-Macro
C2-Macro
C3-Macro
C1-Mono
C2-Mono
Neutrophil
Mast
d
Cycling T-G3
Cycling T-G4
TF activity
PPARG
ZBTB14
CEBPA
BATF
MLX
KLF12
POU2F2
FOXD4
NFE2L3
AHR
PBX1
ATF1
NFAT5
DBP
HSF4
NFE2L1
FOXQ1
RUNX2
KLF13
MEF2A
TCF7
CEBPD
JUNB
FOS
e
Cycling T-G4 vs Cycling T-G3
-Log10(p-value)
SOX9
HLA−C
MGST1
HLA−DRA
ABCC3
ALDH1A1
HLA−DPA1
NME2
NKG7
SOX4
STMN1
GSTP1
PRF1
GZMA
NME1
GZMH
XCL2
CCL5
Cycling T-G3
Cycling T-G4
Log2(Fold Change)

**Supplementary Fig. 4. Distinct states of monocyte-like DC and cycling T cells in therapy-naïve and therapy groups**

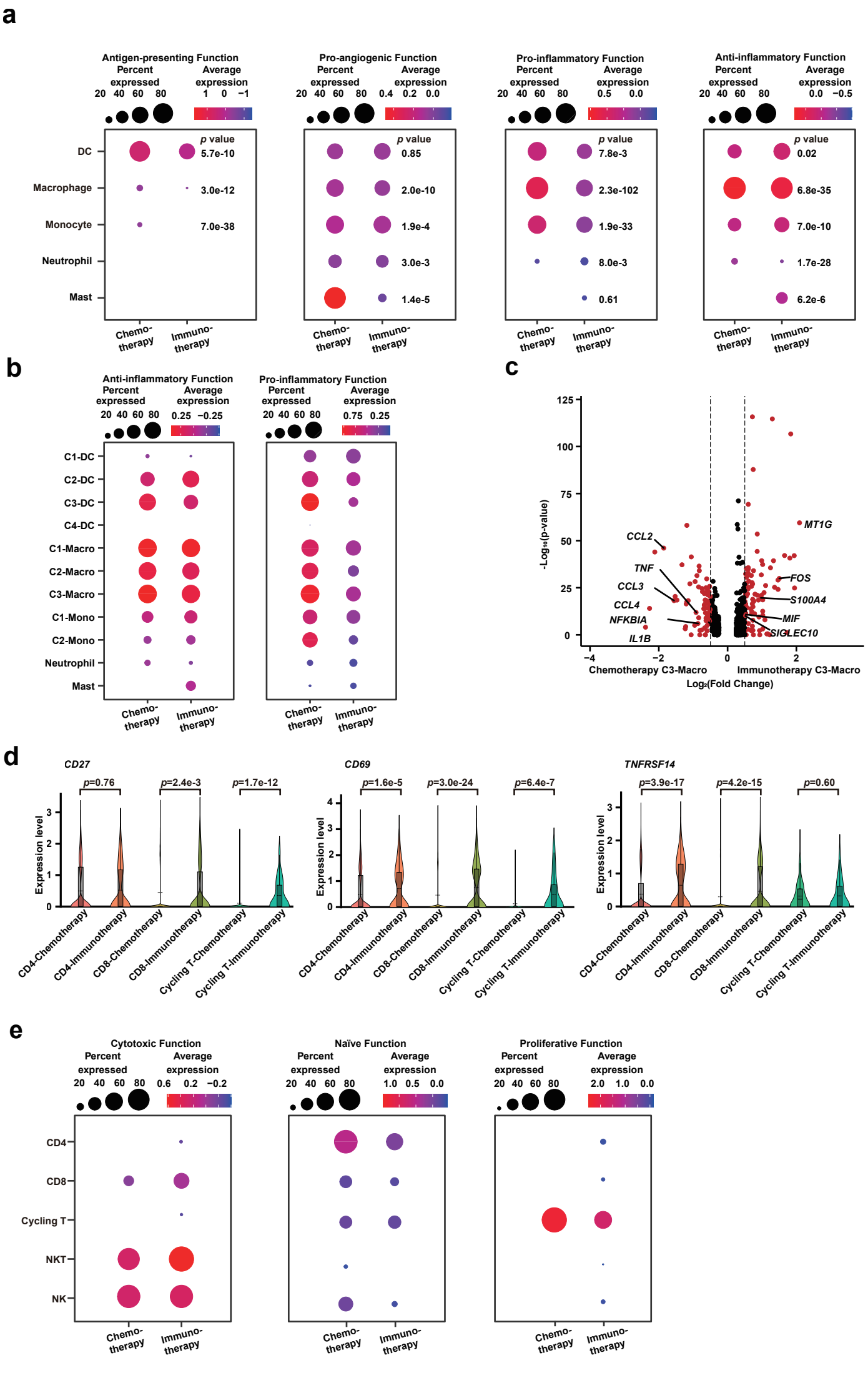
(a) Heatmap showing transcription factor (TF) activity of C3-DC in the G3 and G4 Groups using SCENIC analysis.

(b) Volcano plot showing differentially expressed genes between C3-DC in the G3 and G4 Groups.

(c) Violin plots indicating expression levels of chemokine receptors in myeloid cells (C1-DC: n = 8753 cells; C2-DC: n = 8430 cells; C3-DC: n = 2173 cells; C4-DC: n = 921 cells; C1-Macro: n = 16571 cells; C2-Macro: n = 9995 cells; C3-Macro: n = 6323 cells; C1-Mono: n = 35729 cells; C2-Mono: n = 3682 cells; Neutrophil: n = 6602 cells; Mast: n = 1504 cells). Box represents median ± interquartile range; whiskers represent 1.5x interquartile range; horizontal dotted line represents mean value. DC, dendritic cell; Macro, macrophage; Mono, monocyte.

(d) Heatmap showing TF activity of cycling T cells in the G3 (n = 531 cells) and G4 (n = 137 cells) Groups using SCENIC analysis.

(e) Volcano plot showing differentially expressed genes between cycling T cells in the G3 and G4 Groups.


a
Antigen-presenting Function
Pro-angiogenic Function
Pro-inflammatory Function
Anti-inflammatory Function
Percent expressed
Average expression
p value
DC
Macrophage
Monocyte
Neutrophil
Mast
5.7e-10
3.0e-12
7.0e-38
0.85
2.0e-10
1.9e-4
3.0e-3
1.4e-5
7.8e-3
2.3e-102
1.9e-33
8.0e-3
0.61
0.02
6.8e-35
7.0e-10
1.7e-28
6.2e-6
Chemo-therapy
Immuno-therapy
b
Anti-inflammatory Function
Pro-inflammatory Function
C1-DC
C2-DC
C3-DC
C4-DC
C1-Macro
C2-Macro
C3-Macro
C1-Mono
C2-Mono
Neutrophil
Mast
c
-Log10(p-value)
CCL2
TNF
CCL3
CCL4
NFKBIA
IL1B
MT1G
FOS
S100A4
MIF
SIGLEC10
Chemotherapy C3-Macro
Immunotherapy C3-Macro
Log2(Fold Change)
d
CD27
CD69
TNFRSF14
p=0.76
p=2.4e-3
p=1.7e-12
p=1.6e-5
p=3.0e-24
p=6.4e-7
p=3.9e-17
p=4.2e-15
p=0.60
Expression level
CD4-Chemotherapy
CD4-Immunotherapy
CD8-Chemotherapy
CD8-Immunotherapy
Cycling T-Chemotherapy
Cycling T-Immunotherapy
e
Cytotoxic Function
Naïve Function
Proliferative Function
CD4
CD8
Cycling T
NKT
NK

**Supplementary Fig. 5. Immune phenotype evolution of monocyte-like DC and cycling T cells after immunotherapy**

(a) Dotplots showing the cell expression percentage (dot size) and related function scores (dot color) of antigen-presenting, pro-angiogenic, pro-inflammatory, and anti-inflammatory function scores in main myeloid subtypes in the Chemotherapy (three patients) and Immunotherapy (two patients) groups. DC, dendritic cell. *p* values are calculated by two-sided unpaired Wilcoxon test.

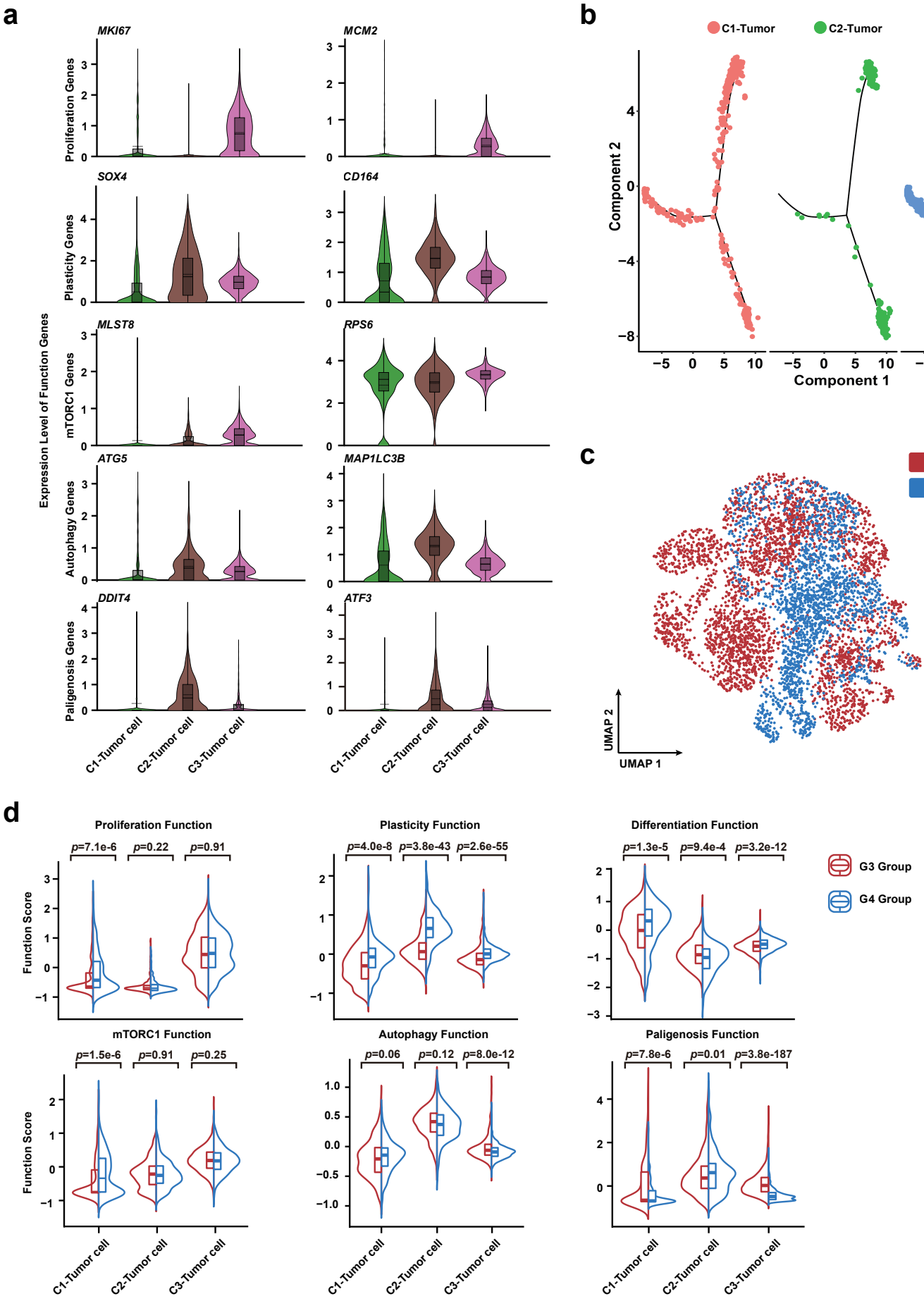
(b) Similar to (a), dotplots showing anti-inflammatory and pro-inflammatory function scores of myeloid subtypes in Chemotherapy (three patients) and Immunotherapy (two patients) groups. DC, dendritic cell; Macro, macrophage; Mono, monocyte.

(c) Volcano plot showing differentially expressed genes of C3-Macro cells between the Chemotherapy (three patients) and Immunotherapy (two patients) groups.

(d) Violin plot showing expression levels of co-stimulatory markers of T cells in the Chemotherapy (three patients) and Immunotherapy (two patients) groups. Box represents median ± interquartile range; whiskers represent 1.5x interquartile range; horizontal dotted line represents mean value. *p* values are calculated by two-sided unpaired Wilcoxon test.

(e) Similar to (a), dotplots showing the cytotoxic, naïve, and proliferative function scores of main T/NK cell clusters in the Chemotherapy (three patients) and Immunotherapy (two patients) groups.

Source data are provided as a Source Data file.


a
MKI67
MCM2
SOX4
CD164
MLST8
RPS6
ATG5
MAP1LC3B
DDIT4
ATF3
Proliferation Genes
Plasticity Genes
mTORC1 Genes
Autophagy Genes
Paligenosis Genes
Expression Level of Function Genes
C1-Tumor cell
C2-Tumor cell
C3-Tumor cell
b
C1-Tumor
C2-Tumor
C3-Tumor
Component 1
Component 2
c
G3 Group
G4 Group
UMAP 1
UMAP 2
d
Proliferation Function
p=7.1e-6
p=0.22
p=0.91
Plasticity Function
p=4.0e-8
p=3.8e-43
p=2.6e-55
Differentiation Function
p=1.3e-5
p=9.4e-4
p=3.2e-12
mTORC1 Function
p=1.5e-6
p=0.91
p=0.25
Autophagy Function
p=0.06
p=0.12
p=8.0e-12
Paligenosis Function
p=7.8e-6
p=0.01
p=3.8e-187
Function Score
G3 Group
G4 Group
C1-Tumor cell
C2-Tumor cell
C3-Tumor cell

**Supplementary Fig. 6. Characteristics of tumor cells in the peritoneal microenvironment during gastric cancer peritoneal metastasis**

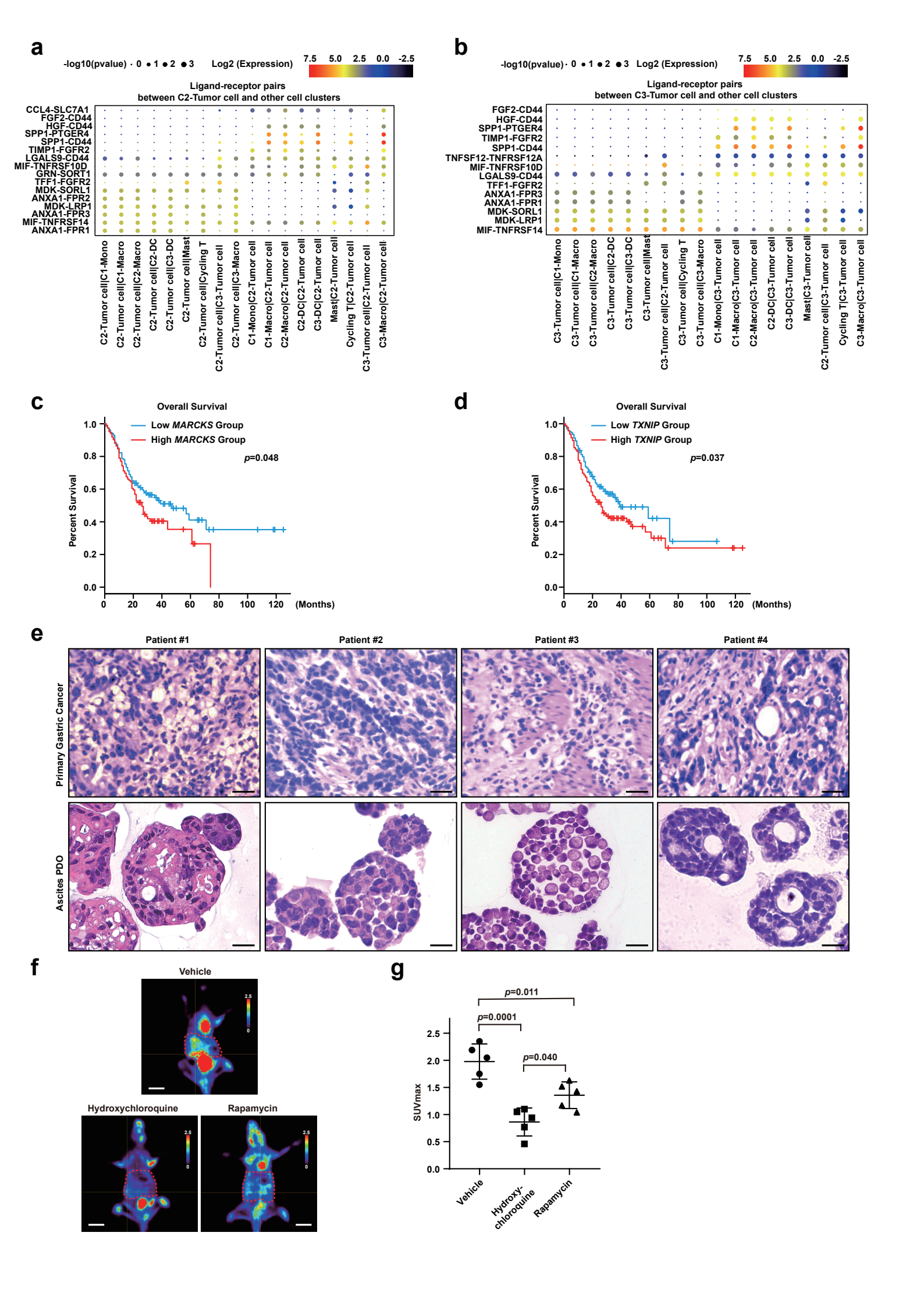
(a) Violin plots showing expression levels of function-related genes, including proliferation, plasticity, mTORC1, autophagy, and paligenosis genes, in tumor subtypes (C1-Tumor cell: n = 713 cells; C2-Tumor cell: n = 549 cells; C3-Tumor cell: n = 2545 cells). Box represents median ± interquartile range; whiskers represent 1.5x interquartile range; horizontal dotted line represents mean value.

(b) Transition trajectories of tumor cell subtypes along pseudotime. Each dot corresponds to a single cell.

(c) Uniform Manifold Approximation and Projection (UMAP) plot showing the distribution of tumor cells in G3 (Red) and G4 (blue) Groups.

(d) Split violin plots representing function scores of proliferation, plasticity, differentiation, mTORC1, autophagy, and paligenosis for tumor cell subtypes in the G3 (red) and G4 (blue) Groups. Box represents median ± interquartile range; *p* values are calculated by Wilcoxon test.

Source data are provided as a Source Data file.


a
-log10(pvalue) 0 1 2 3
Log2 (Expression)
7.5 5.0 2.5 0.0 -2.5
Ligand-receptor pairs
between C2-Tumor cell and other cell clusters
CCL4-SLC7A1
FGF2-CD44
HGF-CD44
SPP1-PTGER4
SPP1-CD44
TIMP1-FGFR2
LGALS9-CD44
MIF-TNFRSF10D
GRN-SORT1
TFF1-FGFR2
MDK-SORL1
ANXA1-FPR2
MDK-LRP1
ANXA1-FPR3
MIF-TNFRSF14
ANXA1-FPR1
C2-Tumor cell|C1-Mono
C2-Tumor cell|C1-Macro
C2-Tumor cell|C2-Macro
C2-Tumor cell|C2-DC
C2-Tumor cell|C3-DC
C2-Tumor cell|Mast
C2-Tumor cell|Cycling T
C2-Tumor cell|C3-Tumor cell
C2-Tumor cell|C3-Macro
C1-Mono|C2-Tumor cell
C1-Macro|C2-Tumor cell
C2-Macro|C2-Tumor cell
C2-DC|C2-Tumor cell
C3-DC|C2-Tumor cell
Mast|C2-Tumor cell
Cycling T|C2-Tumor cell
C3-Tumor cell|C2-Tumor cell
C3-Macro|C2-Tumor cell
b
-log10(pvalue) 0 1 2 3
Log2 (Expression)
7.5 5.0 2.5 0.0 -2.5
Ligand-receptor pairs
between C3-Tumor cell and other cell clusters
FGF2-CD44
HGF-CD44
SPP1-PTGER4
TIMP1-FGFR2
SPP1-CD44
TNFSF12-TNFRSF12A
MIF-TNFRSF10D
LGALS9-CD44
TFF1-FGFR2
ANXA1-FPR3
ANXA1-FPR1
MDK-SORL1
MDK-LRP1
MIF-TNFRSF14
C3-Tumor cell|C1-Mono
C3-Tumor cell|C1-Macro
C3-Tumor cell|C2-Macro
C3-Tumor cell|C2-DC
C3-Tumor cell|C3-DC
C3-Tumor cell|Mast
C3-Tumor cell|C2-Tumor cell
C3-Tumor cell|Cycling T
C3-Tumor cell|C3-Macro
C1-Mono|C3-Tumor cell
C1-Macro|C3-Tumor cell
C2-Macro|C3-Tumor cell
C2-DC|C3-Tumor cell
C3-DC|C3-Tumor cell
Mast|C3-Tumor cell
C2-Tumor cell|C3-Tumor cell
Cycling T|C3-Tumor cell
C3-Macro|C3-Tumor cell
c
Overall Survival
Low MARCKS Group
High MARCKS Group
p=0.048
Percent Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
(Months)
d
Overall Survival
Low TXNIP Group
High TXNIP Group
p=0.037
Percent Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
(Months)
e
Patient #1
Patient #2
Patient #3
Patient #4
Primary Gastric Cancer
Ascites PDO
f
Vehicle
Hydroxychloroquine
Rapamycin
2.5
0
g
p=0.011
p=0.0001
p=0.040
SUVmax
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
Hydroxy-chloroquine
Rapamycin

**Supplementary Fig. 7. Characteristics of tumor cell involved in paligenosis and gastric cancer patient-derived organoids**

(a,b) Dotplot showing intercellular interaction between C2-Tumor cell (a) and C3-Tumor cell (b) and immune cell subtypes, based on ligand-receptor pairs by CellPhoneDB analysis. Dot size represents the significance of the interactions and dot color represents expression levels.

(c,d) Kaplan-Meier curves showing the overall survival of patients from The Cancer Genome Atlas Stomach Adenocarcinoma (TCGA-STAD) database with high (red line) or low (blue line) expression group of *MARCKS* (c) and *TXNIP* (d), respectively. *p* value is calculated by log-rank test.

(e) Hematoxylin-eosin staining for matched patient biopsies (top) and patient-derived organoid from ascites (bottom), showing that the organoids maintain the growth patterns and differentiation of the primary gastric cancer. Scale bars, 100 μm.

(f) 18F-FDG PET images of mice at the five weeks after intraperitoneal injection with PDOs. Scale bar, 2cm.

(g) Quantification of SUVmax in patient-derived organoid xenograft (PDOX) as in (f) (n = 5 mice in each group). Data are presented as mean values ± SEM (error bars); *p* values are calculated by one-way ANOVA with Tukey post hoc test.

## Supplementary Table and Supplementary Table Legends

**Supplementary Table 1. Summary of scRNA-seq patient sample information.**

| Patient ID | Age(years) | Sex | Group | Sample type | Pathologic AJCC stage | Therapeutic history |
|---|---|---|---|---|---|---|
| HA_7 | 42-66 | Female | G0 | Normal peritoneal lavage fluid | N/A* | Treatment-naïve |
| HA_12 | 42-66 | Female | G0 | Normal peritoneal lavage fluid | N/A* | Treatment-naïve |
| HA_42 | 42-66 | Female | G0 | Normal peritoneal lavage fluid | N/A* | Treatment-naïve |
| HA_43 | 42-66 | Female | G0 | Normal peritoneal lavage fluid | N/A* | Treatment-naïve |
| HA_11 | 38-63 | Male | G1 | Peritoneal lavage fluid | pT1bN0M0, IA | Treatment-naïve |
| HA_14 | 38-63 | Male | G1 | Peritoneal lavage fluid | pT1aN0M0, IA | Treatment-naïve |
| HA_16 | 38-63 | Male | G1 | Peritoneal lavage fluid | pT1aN0M0, IA | Treatment-naïve |
| HA_40 | 38-63 | Female | G1 | Peritoneal lavage fluid | pT1aN0M0, IA | Treatment-naïve |
| HA_1 | 52-84 | Male | G2 | Peritoneal lavage fluid | pT2N2M0, IIB | Treatment-naïve |
| HA_10 | 52-84 | Male | G2 | Peritoneal lavage fluid | pT3N1M0, IIB | Treatment-naïve |
| HA_20 | 52-84 | Male | G2 | Peritoneal lavage fluid | pT3N2M0, IIIA | Treatment-naïve |
| HA_21 | 52-84 | Male | G2 | Peritoneal lavage fluid | pT4aN1M0, IIIA | Treatment-naïve |
| HA_26 | 52-84 | Male | G2 | Peritoneal lavage fluid | pT3N0M0, IIA | Treatment-naïve |
| HA_37 | 52-84 | Male | G2 | Peritoneal lavage fluid | pT4aN2M0, IIIA | Treatment-naïve |
| HA_38 | 52-84 | Female | G2 | Peritoneal lavage fluid | pT3N3aM0, IIIB | Treatment-naïve |
| HA_39 | 52-84 | Male | G2 | Peritoneal lavage fluid | pT4aN2M0, IIIA | Treatment-naïve |
| HA_41 | 52-84 | Male | G2 | Peritoneal lavage fluid | pT2N0M0, IB | Treatment-naïve |
| HA_48 | 52-84 | Male | G2 | Peritoneal lavage fluid | pT3N1M0, IIB | Treatment-naïve |
| HA_2 | 36-81 | Male | G3 | Ascites | N/A* | Treatment-naïve |
| HA_3 | 36-81 | Male | G3 | Ascites | N/A* | Treatment-naïve |
| HA_6 | 36-81 | Male | G3 | Ascites | N/A* | Treatment-naïve |
| HA_9 | 36-81 | Male | G3 | Ascites | N/A* | Treatment-naïve |
| HA_18 | 36-81 | Female | G3 | Ascites | N/A* | Treatment-naïve |
| HA_27 | 36-81 | Male | G3 | Ascites | N/A* | Treatment-naïve |
| HA_28 | 36-81 | Female | G3 | Ascites | N/A* | Treatment-naïve |
| HA_30 | 36-81 | Female | G3 | Ascites | N/A* | Treatment-naïve |
| HA_31 | 36-81 | Female | G3 | Ascites | N/A* | Treatment-naïve |
| HA_32 | 36-81 | Male | G3 | Ascites | N/A* | Treatment-naïve |
| HA_35 | 36-81 | Male | G3 | Ascites | N/A* | Treatment-naïve |
| HA_36 | 36-81 | Mele | G3 | Ascites | N/A* | Treatment-naïve |
| HA_25 | 46-83 | Female | G4 | Ascites | N/A* | S-1+Paclitaxel |
| HA_29 | 46-83 | Male | G4 | Ascites | N/A* | S-1+Paclitaxel+Apatinib |
| HA_33 | 46-83 | Female | G4 | Ascites | N/A* | S-1+Atezolizumab |
| HA_34 | 46-83 | Male | G4 | Ascites | N/A* | S-1+Oxaliplatin+Toripalimab |
| HA_44 | 46-83 | Female | G4 | Ascites | N/A* | S-1+Oxaliplatin |

N/A*: Not applicable due to benign hysteromyoma or gastric cancer peritoneal metastasis without surgery, age as a range for each patient within each group.

**Supplementary Table 2. Summary of scRNA-seq transcriptomic profiles.**

| Sample | Group | Cell counts per sample | Median genes per cell | Median UMI counts per cell | Mitochondrial genes per cell (%) |
|---|---|---|---|---|---|
| HA_7 | G0 | 4689 | 2010 | 5006 | 5.58 |
| HA_12 | G0 | 4746 | 3177 | 10412 | 4.25 |
| HA_42 | G0 | 4111 | 3913 | 14720 | 6.47 |
| HA_43 | G0 | 6035 | 3205 | 10927 | 6.50 |
| HA_11 | G1 | 2707 | 3028 | 9961 | 4.34 |
| HA_14 | G1 | 2689 | 4057 | 18129 | 6.41 |
| HA_16 | G1 | 5483 | 2390 | 6962 | 6.16 |
| HA_40 | G1 | 5889 | 3675 | 13036 | 6.92 |
| HA_1 | G2 | 5393 | 2321 | 6389 | 7.33 |
| HA_10 | G2 | 6099 | 2954 | 8847 | 7.02 |
| HA_20 | G2 | 5680 | 2030 | 6703.5 | 5.56 |
| HA_21 | G2 | 6423 | 1867 | 5096 | 6.08 |
| HA_26 | G2 | 5926 | 1996.5 | 5126.5 | 4.99 |
| HA_37 | G2 | 5699 | 2217 | 6060 | 5.63 |
| HA_38 | G2 | 5957 | 2368 | 6313 | 5.51 |
| HA_39 | G2 | 4456 | 2969 | 9866.5 | 7.08 |
| HA_41 | G2 | 6341 | 2832 | 8706 | 5.82 |
| HA_48 | G2 | 4179 | 3352 | 13019 | 6.18 |
| HA_2 | G3 | 8844 | 1731.5 | 5823.5 | 8.62 |
| HA_3 | G3 | 5018 | 2056 | 6740 | 5.04 |
| HA_6 | G3 | 5130 | 1931 | 6295 | 5.29 |
| HA_9 | G3 | 4796 | 2008 | 6045.5 | 4.51 |
| HA_18 | G3 | 5038 | 2430.5 | 7579.5 | 5.81 |
| HA_27 | G3 | 5320 | 2172 | 6054.5 | 5.08 |
| HA_28 | G3 | 6066 | 3890 | 15862.5 | 5.54 |
| HA_30 | G3 | 6470 | 2674 | 7387 | 6.26 |
| HA_31 | G3 | 6480 | 1603 | 4834.5 | 6.69 |
| HA_32 | G3 | 5933 | 2062 | 6945 | 4.88 |
| HA_35 | G3 | 3406 | 1989 | 5888 | 5.03 |
| HA_36 | G3 | 7147 | 3655 | 14717 | 8.78 |
| HA_25 | G4 | 12655 | 1790 | 5030 | 4.36 |
| HA_29 | G4 | 5786 | 1442.5 | 4004 | 6.60 |
| HA_33 | G4 | 3658 | 1972 | 5222 | 5.43 |
| HA_34 | G4 | 2701 | 1702 | 4173 | 4.23 |
| HA_44 | G4 | 5037 | 3141 | 10494 | 7.72 |

UMI: Unique Molecular Identifier

**Supplementary Table 3. Summary of PDOs sample information.**

| Patient ID | Age | Gender | Sample type | AJCC stage |
|---|---|---|---|---|
| PDO#1 | 57-83 | Male | Ascites | N/A* |
| PDO#2 | 57-83 | Male | Ascites | T4N3bM1 |
| PDO#3 | 57-83 | Male | Ascites | N/A* |
| PDO#4 | 57-83 | Female | Ascites | T3N3aM1 |

N/A*: Not applicable due to gastric cancer peritoneal metastasis without surgery; PDOs: patient-derived organoids.

**Supplementary Table 4. Antibodies application and dilution**

| Antibodies | Company | Application | Dilution |
|---|---|---|---|
| Mouse Anti-TXNIP (JY2) | Novus (NBP1-54578) | IF | 1:50 |
| Mouse Anti-β-Tubulin | Cell Signaling Technology (2146S) | IF | 1:200 |
| Rabbit Anti-MARCKS (D88D11) | Cell Signaling Technology (5607S) | IF | 1:100 |
| Rabbit Anti-LC3B | Cell Signaling Technology (2775S) | IF | 1:50 |
| Rabbit Anti-SOX9 | Merck (AB5535) | IF | 1:100 |
| Mouse Anti-SOX9 | Abcam (ab76997) | IF | 1:100 |
| Rabbit Anti-REDD1 | Proteintech (10638-1-AP) | IF | 1:100 |
| Rabbit Anti-ATF3 | Sigma (HPA001562) | IF | 1:200 |
| Rabbit Anti-PS6 Ribosomal Protein (Ser235/236) | Cell Signaling Technology (4858S) | IF | 1:250 |
| Rabbit Anti-Ki67 (SP6) | Thermo Fisher Scientific (MA5-14520) | IF | 1:100 |
| Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 594 | Invitrogen (A-21203) | IF | 1:500 |
| Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 | Invitrogen (A-21202) | IF | 1:200 |
| Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 594 | Invitrogen (A-21207) | IF | 1:500 |
| Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 | Invitrogen (A-21206) | IF | 1:200 |
| Anti-human CD45-eFluor | eBioscience (69-0459-42) | FACS | 5 μL/test |
| Anti-human CD3-FITC | eBioscience (11-0037-42) | FACS | 5 μL/test |
| Anti-human CD56-FITC | eBioscience (11-0566-42) | FACS | 5 μL/test |
| Anti-human CD19-FITC | eBioscience (11-0199-42) | FACS | 5 μL/test |
| Anti-human CD1c-APC | BioLegend (331524) | FACS | 5 μL/test |
| Anti-human CD163-PE | eBioscience (12-1639-42) | FACS | 5 μL/test |
| Anti-human CD14-BV421 | BD Biosciences (563743) | FACS | 5 μL/test |
| 7-AAD Viability Staining Solution | eBioscience (00-6993-50) | FACS | 5 μL/test |

Abbreviation, IF: Immunofluorescence; FACS: Fluorescence-activated cell sorting.