Supplementary data

Figure S1. Quality control of the single-cell NGS (A) The UMI number and percentage of the mitochondria. (B) The cell number in the normal lung (NL), primary lung cancer (PLC), pleural fluid of congestive heart failure (HP), and pleural metastasis (LCP) groups.

Figure S2. The cell profiles of primary and pleural metastasis tissue as determined by scRNA-seq. (A) Co-expressions of various cell markers on different cell clusters on the UMAP plot. (B) Proportion of various cell types. (C) Percentage of different cell types in different patients.

Figure S3. The MesoMT of pleural mesothelial cells (PMCs). (A) Visualization of PMCs by different patient groups. (B) Upregulated *ICAM1*, *SLC2A1,* and *TNC* in LCP. (C) The levels of MMP2 in pleural fluid obtained from the patients with congestive heart failure or pleural metastasis. (D) No significant difference of various factors in the pleural fluid of pleural metastasis lung cancer patients with wild type (WT) between mutated (MT) EGFR status. The impact of various secretory factors on the overall survival of lung cancer patients with WT (E) or MT (F) EGFR. Data are presented as mean ± SD. ns, not significant.

Figure S4. The evolution of cancer cells in the pleural niche. (A) InferCNV analysis of epithelial cells v.s. primary lung cancer and mesothelial cells v.s. pleural metastatic cancer. (B) The volcano plot of DEGs. Wiki pathway analysis (C) and IPA (D) of DEGs. (E) Heatmap of the ferroptosis driver gene set in pleural cancer. (F) Weight analysis of the ferroptosis driver gene set. (G) IHC showed upregulated GPX4 in pleural tumor than that in primary tumor in a paired tissue section. (H) The knockdown efficacy of *GPX4* siRNA. (I) Inhibition of *GPX4* by siRNA decreased cell viability of A549 in MPE. (J) A GPX4 inhibitor reduced cell viability of A549 in MPE. The cells were transfected with control or *GPX4* siRNA and the expression of GPX4 were assessed

after 24 h transfection by qRT-PCR. The data were presented as mean ± SD. ***$p < 0.001$.

Figure S5. LA-TAMs exhibited an immunosuppressive phenotype. (A) Cell markers of myeloid cells. Heatmaps of M1/M2 phenotype (B), complement (C), cholesterol metabolism (D), and IFN-related (E) pathways in IFN-TAMs and LA-TAMs. (F) The expressions of secretory factors across the monocytes and TAMs assessed by trajectory analysis.

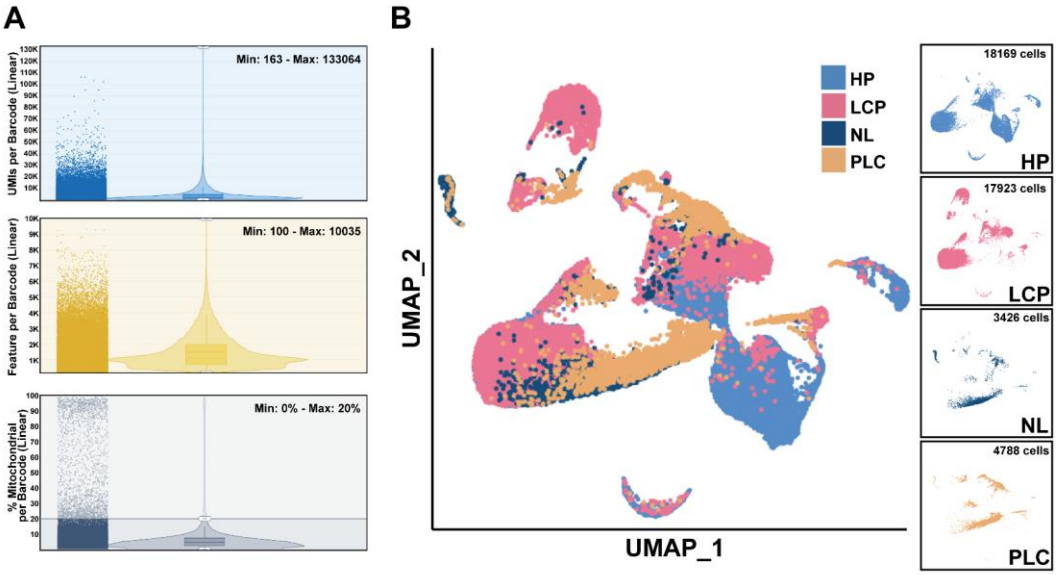
Figure S6. The levels of complement factors in MPE of lung cancer patients with wild-type (WT) or mutated (MT) EGFR. The concentrations of complement factors of (A) classical pathway, (B) lectin pathway, (C) alternative pathway, (D) C3 and (E) C5-related factors, and (F) complement factor H and complement factors I in the pleural fluid of pleural metastatic lung cancer patients with WT or MT EGFR. ns, not significant. *$p < 0.05$, **$p < 0.01$.

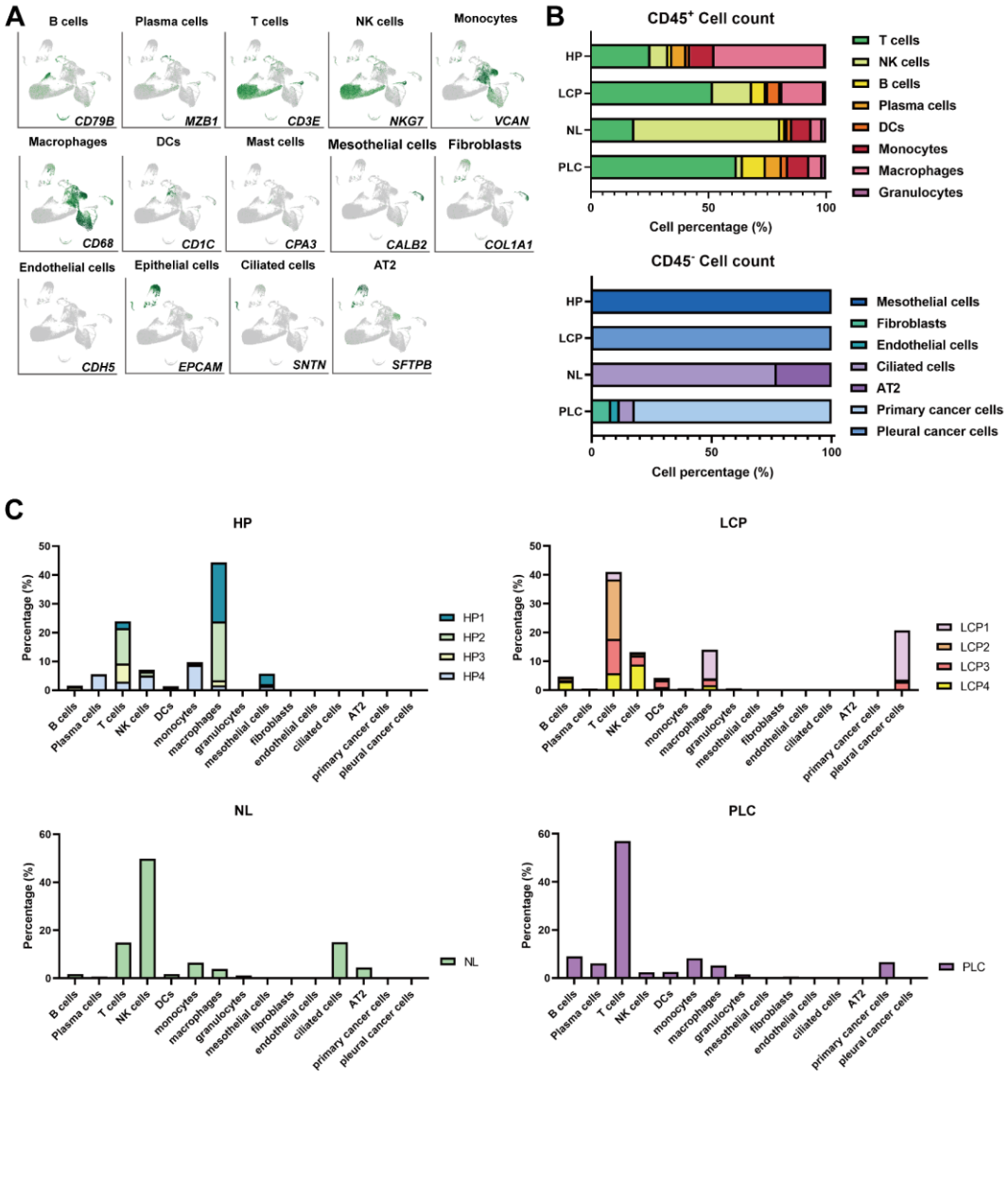
Figure S7. Kaplan-Meier curves representing overall survival (OS) in the cohort stratified by complement factor expressions. The impact of complement factors of (A) C5-related factors, (B) classical pathway, (C) lectin pathway, (D) alternative pathway, (E) C3-related, and (F) complement factors H and I on the OS of pleural metastatic lung cancer patients with WT EGFR. The impact of complement factors of (G) C5a, (H) classical pathway, (I) lectin pathway, (J) alternative pathway, (K) C3-related, and (L) complement factors H and I in the OS of pleural metastatic lung cancer patients with MT EGFR. The OS of patients were calculated using the Kaplan-Meier method. CFB, complement factor B. CFD, complement factor D. CFH, complement factor H. CFI, complement factor I.

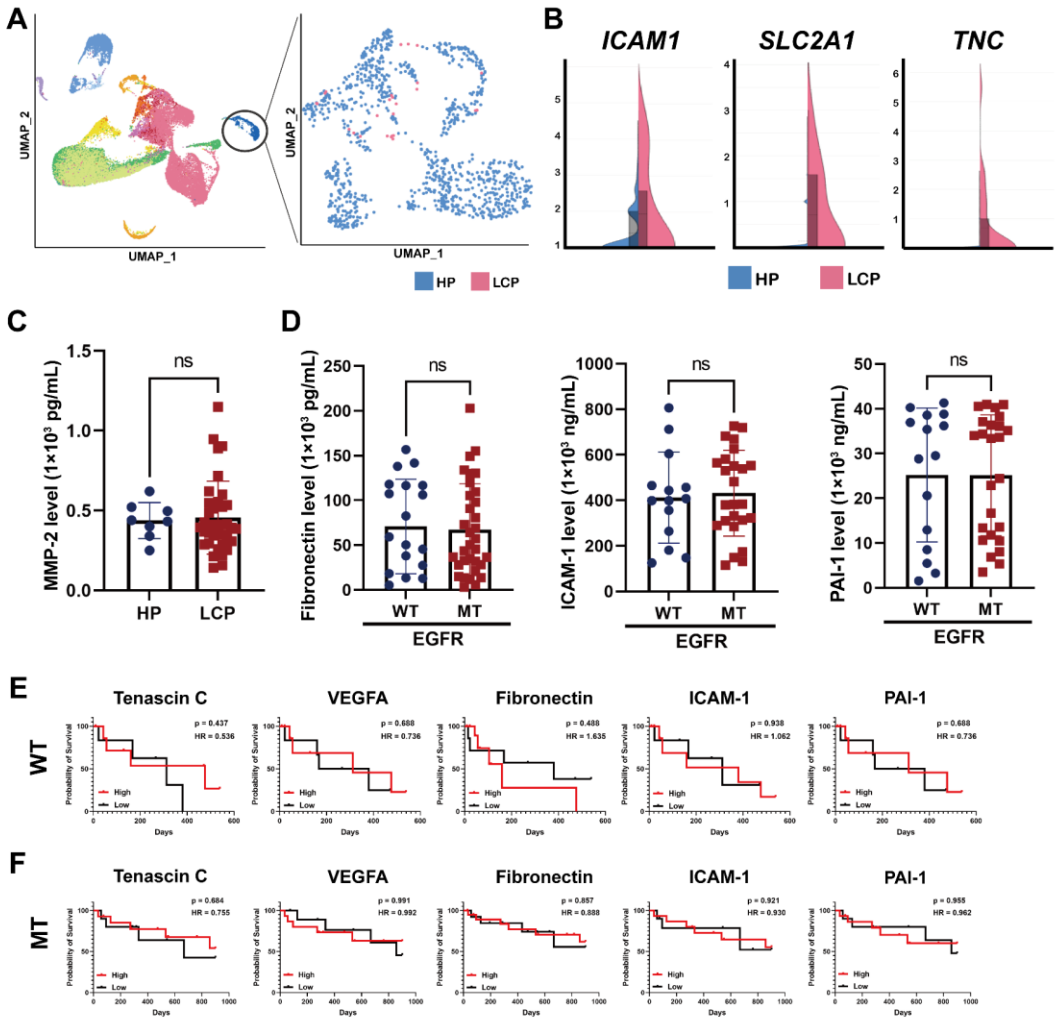
F**igure S8. The levels of DC-derived factors in the pleural fluid of MPE.** (A) The levels of various factors in the pleural fluid of MPE obtained from lung cancer patients with WT or MT EGFR. The impact of various DC-derived factors on the OS of WT

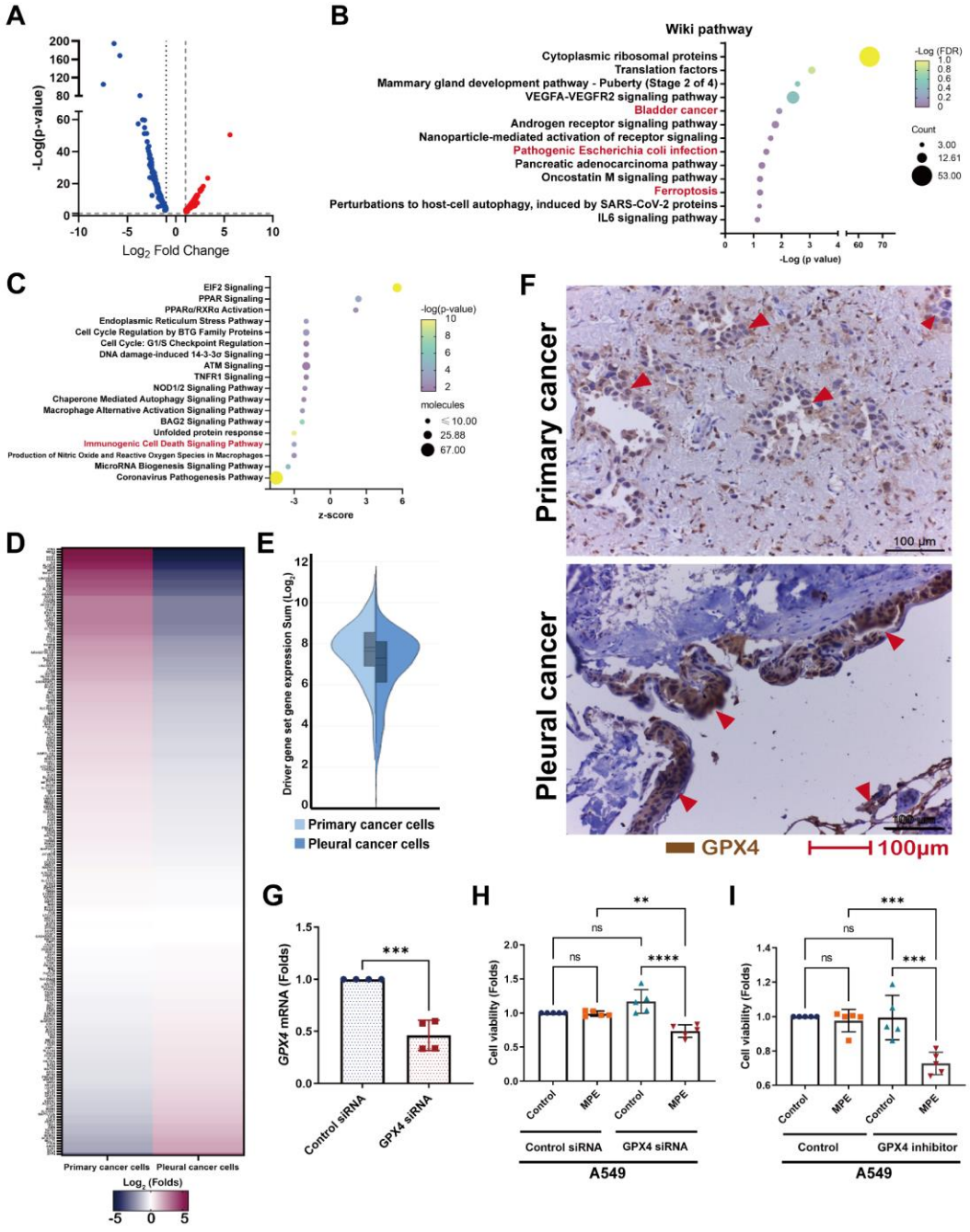
EGFR (B) and MT (C) lung cancer patients. ns, not significant.

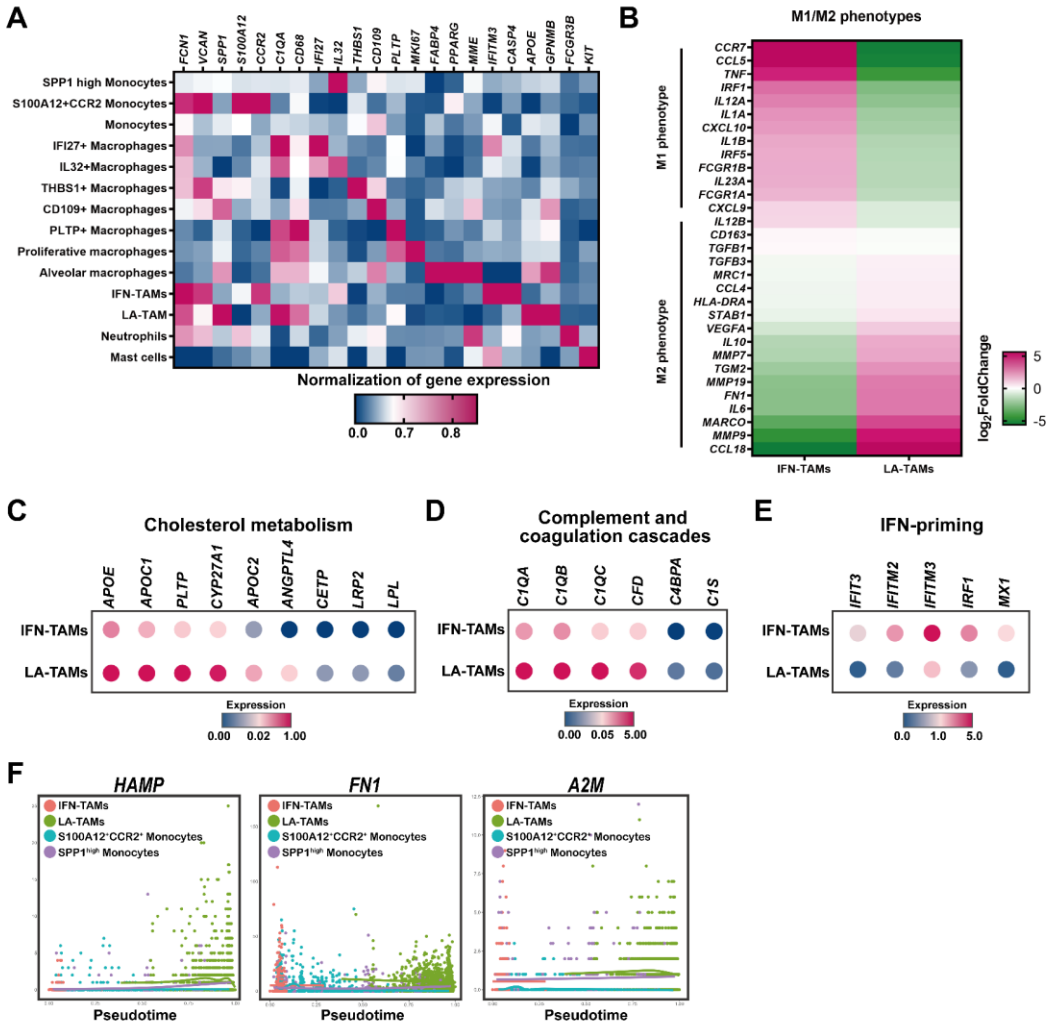
Figure S9. The levels of various ligands involved in the cell-cell interaction of pleural microenvironment. (A) The interaction of pleural cancer cells by autocrine pattern. (B) The interaction of pleural cancer cells with LA-TAMs. (C) The interaction of pleural cancer cells with the subsets of DCs. (D) The interaction of IFN-TAMs with pleural cancer cells or mesothelial cell. (E) The levels of various factors in the MPE obtained from lung cancer patients with WT or MT EGFR. The impact of various ligands on the OS of EGFR wild type (F) and mutated (G) lung cancer patients. ns, not significant.

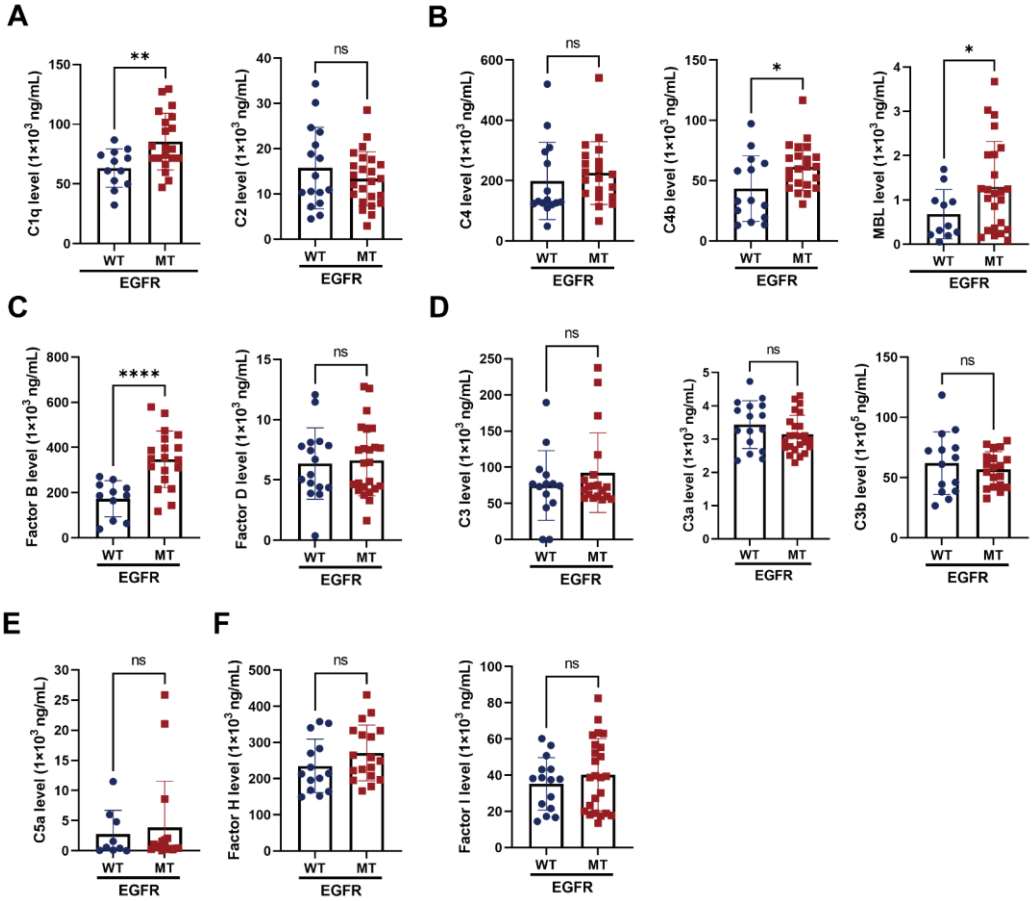

A
UMIs per Barcode (Linear)
Min: 163 - Max: 133064
Feature per Barcode (Linear)
Min: 100 - Max: 10035
% Mitochondrial per Barcode (Linear)
Min: 0% - Max: 20%
B
HP
LCP
NL
PLC
UMAP_2
UMAP_1
18169 cells
HP
17923 cells
LCP
3426 cells
NL
4788 cells
PLC

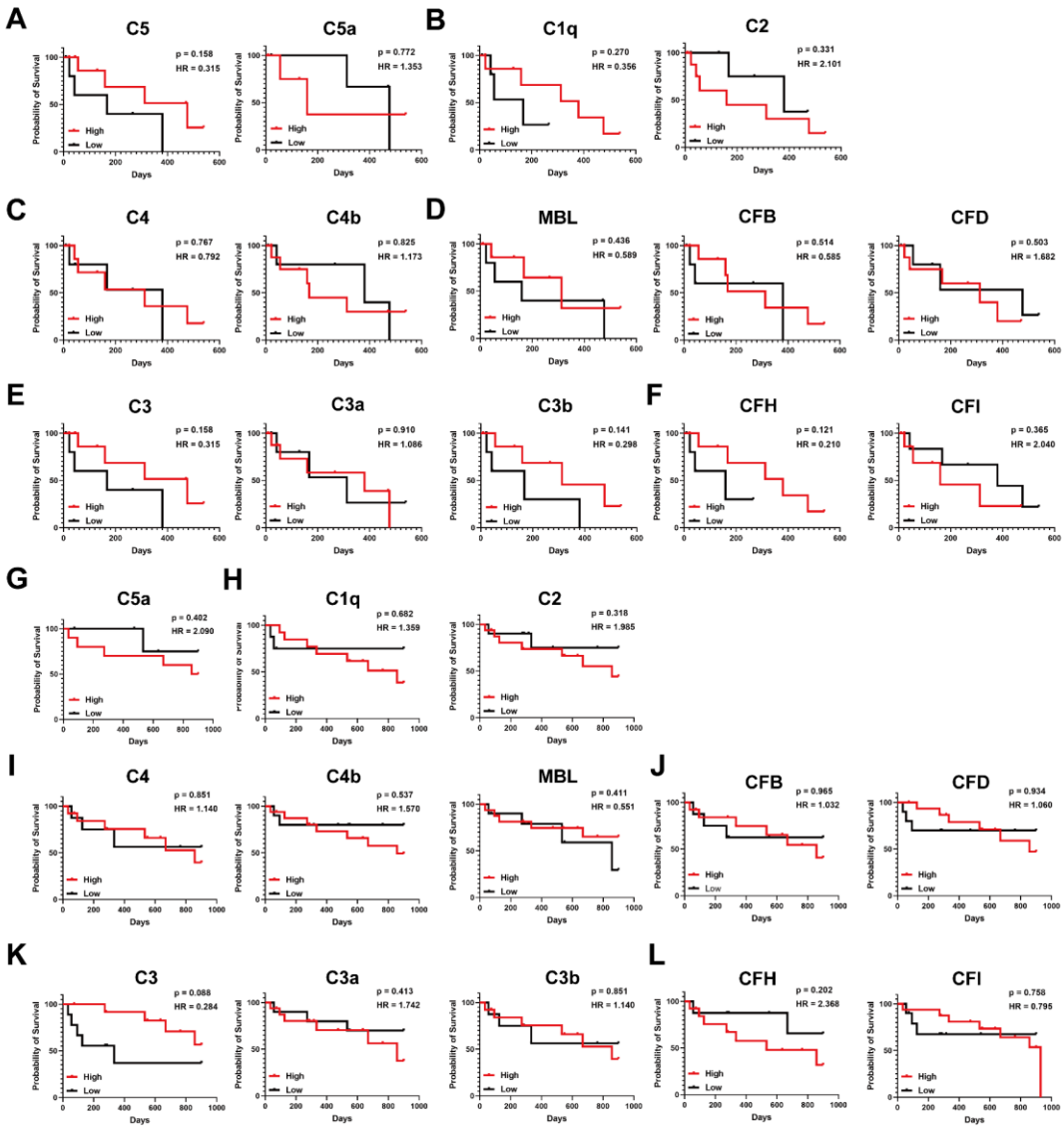

A
B cells
Plasma cells
T cells
NK cells
Monocytes
CD79B
MZB1
CD3E
NKG7
VCAN
Macrophages
DCs
Mast cells
Mesothelial cells
Fibroblasts
CD68
CD1C
CPA3
CALB2
COL1A1
Endothelial cells
Epithelial cells
Ciliated cells
AT2
CDH5
EPCAM
SNTN
SFTPB
B
CD45+ Cell count
HP
LCP
NL
PLC
0
50
100
Cell percentage (%)
T cells
NK cells
B cells
Plasma cells
DCs
Monocytes
Macrophages
Granulocytes
CD45- Cell count
Mesothelial cells
Fibroblasts
Endothelial cells
Ciliated cells
AT2
Primary cancer cells
Pleural cancer cells
C
HP
LCP
NL
PLC
Percentage (%)
HP1
HP2
HP3
HP4
LCP1
LCP2
LCP3
LCP4
NL
PLC
B cells
Plasma cells
T cells
NK cells
DCs
monocytes
macrophages
granulocytes
mesothelial cells
fibroblasts
endothelial cells
ciliated cells
AT2
primary cancer cells
pleural cancer cells

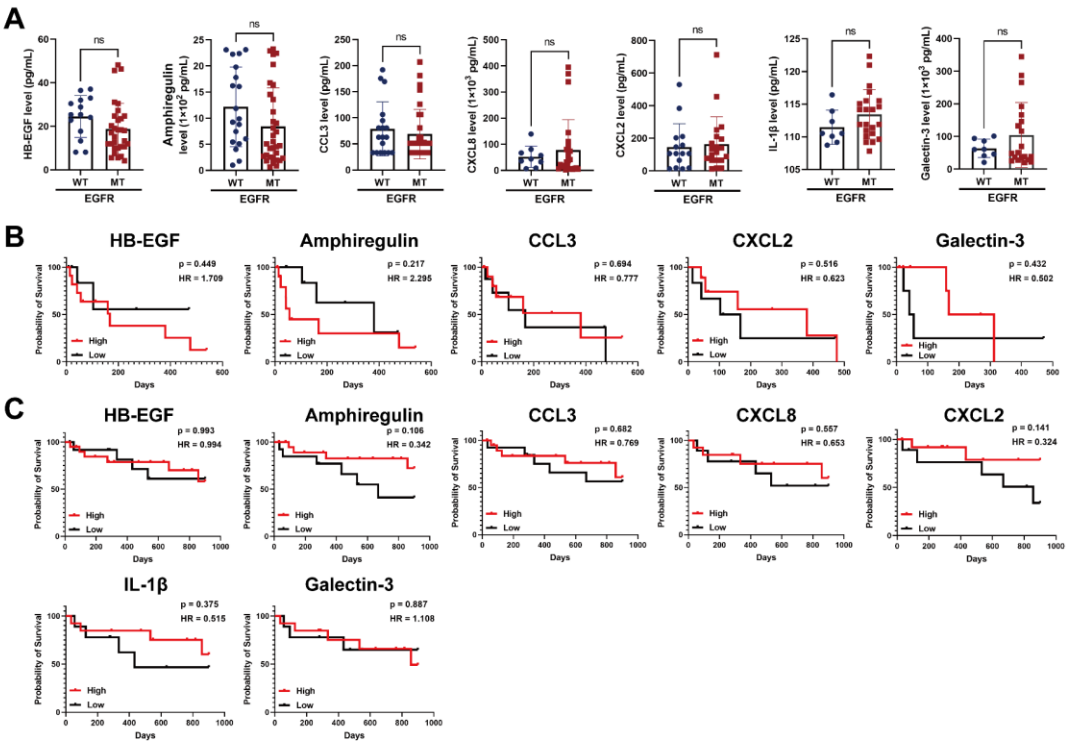

A
UMAP_2
UMAP_1
UMAP_2
UMAP_1
HP
LCP
B
ICAM1
SLC2A1
TNC
HP
LCP
C
MMP-2 level (1×10³ pg/mL)
ns
HP
LCP
D
Fibronectin level (1×10³ pg/mL)
ns
WT
MT
EGFR
ICAM-1 level (1×10³ ng/mL)
ns
WT
MT
EGFR
PAI-1 level (1×10³ ng/mL)
ns
WT
MT
EGFR
E
WT
Tenascin C
p = 0.437
HR = 0.536
VEGFA
p = 0.688
HR = 0.736
Fibronectin
p = 0.488
HR = 1.635
ICAM-1
p = 0.938
HR = 1.062
PAI-1
p = 0.688
HR = 0.736
Probability of Survival
High
Low
Days
F
MT
Tenascin C
p = 0.684
HR = 0.755
VEGFA
p = 0.991
HR = 0.992
Fibronectin
p = 0.857
HR = 0.888
ICAM-1
p = 0.921
HR = 0.930
PAI-1
p = 0.955
HR = 0.962
Probability of Survival
High
Low
Days

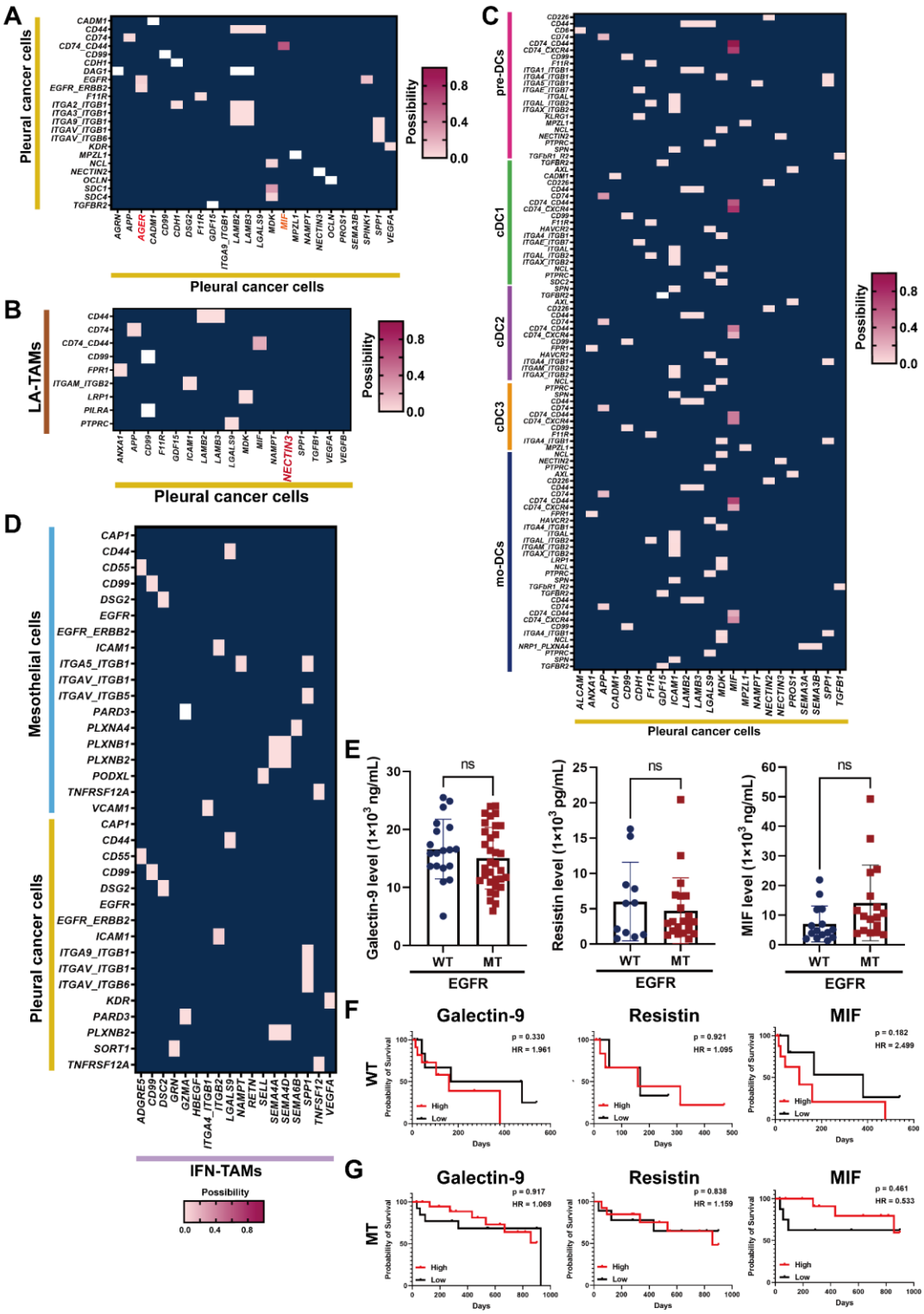

A
-Log(p-value)
Log2 Fold Change
B
Wiki pathway
Cytoplasmic ribosomal proteins
Translation factors
Mammary gland development pathway - Puberty (Stage 2 of 4)
VEGFA-VEGFR2 signaling pathway
Bladder cancer
Androgen receptor signaling pathway
Nanoparticle-mediated activation of receptor signaling
Pathogenic Escherichia coli infection
Pancreatic adenocarcinoma pathway
Oncostatin M signaling pathway
Ferroptosis
Perturbations to host-cell autophagy, induced by SARS-CoV-2 proteins
IL6 signaling pathway
-Log (FDR)
Count
-Log (p value)
C
EIF2 Signaling
PPAR Signaling
PPARα/RXRα Activation
Endoplasmic Reticulum Stress Pathway
Cell Cycle Regulation by BTG Family Proteins
Cell Cycle: G1/S Checkpoint Regulation
DNA damage-induced 14-3-3σ Signaling
ATM Signaling
TNFR1 Signaling
NOD1/2 Signaling Pathway
Chaperone Mediated Autophagy Signaling Pathway
Macrophage Alternative Activation Signaling Pathway
BAG2 Signaling Pathway
Unfolded protein response
Immunogenic Cell Death Signaling Pathway
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages
MicroRNA Biogenesis Signaling Pathway
Coronavirus Pathogenesis Pathway
-log(p-value)
molecules
z-score
D
Primary cancer cells
Pleural cancer cells
Log2 (Folds)
E
Driver gene set gene expression Sum (Log2)
Primary cancer cells
Pleural cancer cells
F
Primary cancer
Pleural cancer
100 μm
GPX4
100μm
G
GPX4 mRNA (Folds)
Control siRNA
GPX4 siRNA
H
Cell viability (Folds)
Control
MPE
Control siRNA
GPX4 siRNA
A549
I
Cell viability (Folds)
Control
MPE
Control
GPX4 inhibitor
A549


A
FCN1
VCAN
SPP1
S100A12
CCR2
C1QA
CD68
IFI27
IL32
THBS1
CD109
PLTP
MKI67
FABP4
PPARG
MME
IFITM3
CASP4
APOE
GPNMB
FCGR3B
KIT
SPP1 high Monocytes
S100A12+CCR2 Monocytes
Monocytes
IFI27+ Macrophages
IL32+Macrophages
THBS1+ Macrophages
CD109+ Macrophages
PLTP+ Macrophages
Proliferative macrophages
Alveolar macrophages
IFN-TAMs
LA-TAM
Neutrophils
Mast cells
Normalization of gene expression
0.0
0.7
0.8
B
M1/M2 phenotypes
M1 phenotype
M2 phenotype
CCR7
CCL5
TNF
IRF1
IL12A
IL1A
CXCL10
IL1B
IRF5
FCGR1B
IL23A
FCGR1A
CXCL9
IL12B
CD163
TGFB1
TGFB3
MRC1
CCL4
HLA-DRA
STAB1
VEGFA
IL10
MMP7
TGM2
MMP19
FN1
IL6
MARCO
MMP9
CCL18
IFN-TAMs
LA-TAMs
log2FoldChange
5
0
-5
C
Cholesterol metabolism
APOE
APOC1
PLTP
CYP27A1
APOC2
ANGPTL4
CETP
LRP2
LPL
IFN-TAMs
LA-TAMs
Expression
0.00
0.02
1.00
D
Complement and coagulation cascades
C1QA
C1QB
C1QC
CFD
C4BPA
C1S
IFN-TAMs
LA-TAMs
Expression
0.00
0.05
5.00
E
IFN-priming
IFIT3
IFITM2
IFITM3
IRF1
MX1
IFN-TAMs
LA-TAMs
Expression
0.0
1.0
5.0
F
HAMP
FN1
A2M
IFN-TAMs
LA-TAMs
S100A12+CCR2+ Monocytes
SPP1high Monocytes
Pseudotime
Pseudotime
Pseudotime


A
B
C
D
E
F
C1q level (1×10³ ng/mL)
C2 level (1×10³ ng/mL)
C4 level (1×10³ ng/mL)
C4b level (1×10³ ng/mL)
MBL level (1×10³ ng/mL)
Factor B level (1×10³ ng/mL)
Factor D level (1×10³ ng/mL)
C3 level (1×10³ ng/mL)
C3a level (1×10³ ng/mL)
C3b level (1×10⁵ ng/mL)
C5a level (1×10³ ng/mL)
Factor H level (1×10³ ng/mL)
Factor I level (1×10³ ng/mL)
**
ns
*
****
WT
MT
EGFR


A
C5
p = 0.158
HR = 0.315
C5a
p = 0.772
HR = 1.353
B
C1q
p = 0.270
HR = 0.356
C2
p = 0.331
HR = 2.101
C
C4
p = 0.767
HR = 0.792
C4b
p = 0.825
HR = 1.173
D
MBL
p = 0.436
HR = 0.589
CFB
p = 0.514
HR = 0.585
CFD
p = 0.503
HR = 1.682
E
C3
p = 0.158
HR = 0.315
C3a
p = 0.910
HR = 1.086
C3b
p = 0.141
HR = 0.298
F
CFH
p = 0.121
HR = 0.210
CFI
p = 0.365
HR = 2.040
G
C5a
p = 0.402
HR = 2.090
H
C1q
p = 0.682
HR = 1.359
C2
p = 0.318
HR = 1.985
I
C4
p = 0.851
HR = 1.140
C4b
p = 0.537
HR = 1.570
MBL
p = 0.411
HR = 0.551
J
CFB
p = 0.965
HR = 1.032
CFD
p = 0.934
HR = 1.060
K
C3
p = 0.088
HR = 0.284
C3a
p = 0.413
HR = 1.742
C3b
p = 0.851
HR = 1.140
L
CFH
p = 0.202
HR = 2.368
CFI
p = 0.758
HR = 0.795
Probability of Survival
Days
High
Low


A
HB-EGF level (pg/mL)
Amphiregulin level (1×10² pg/mL)
CCL3 level (pg/mL)
CXCL8 level (1×10³ pg/mL)
CXCL2 level (pg/mL)
IL-1β level (pg/mL)
Galectin-3 level (1×10³ pg/mL)
ns
WT
MT
EGFR
B
HB-EGF
p = 0.449
HR = 1.709
Amphiregulin
p = 0.217
HR = 2.295
CCL3
p = 0.694
HR = 0.777
CXCL2
p = 0.516
HR = 0.623
Galectin-3
p = 0.432
HR = 0.502
C
HB-EGF
p = 0.993
HR = 0.994
Amphiregulin
p = 0.106
HR = 0.342
CCL3
p = 0.682
HR = 0.769
CXCL8
p = 0.557
HR = 0.653
CXCL2
p = 0.141
HR = 0.324
IL-1β
p = 0.375
HR = 0.515
Galectin-3
p = 0.887
HR = 1.108
Probability of Survival
Days
High
Low


A
Pleural cancer cells
Possibility
Pleural cancer cells
B
LA-TAMs
Possibility
Pleural cancer cells
C
pre-DCs
cDC1
cDC2
cDC3
mo-DCs
Possibility
Pleural cancer cells
D
Mesothelial cells
Pleural cancer cells
IFN-TAMs
Possibility
E
Galectin-9 level (1×10³ ng/mL)
Resistin level (1×10³ pg/mL)
MIF level (1×10³ ng/mL)
ns
WT
MT
EGFR
F
Galectin-9
Resistin
MIF
WT
p = 0.330
HR = 1.961
p = 0.921
HR = 1.095
p = 0.182
HR = 2.499
Probability of Survival
High
Low
Days
G
Galectin-9
Resistin
MIF
MT
p = 0.917
HR = 1.069
p = 0.838
HR = 1.159
p = 0.461
HR = 0.533