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Supporting Information

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#These authors contributed equally
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Supplementary Figure 1

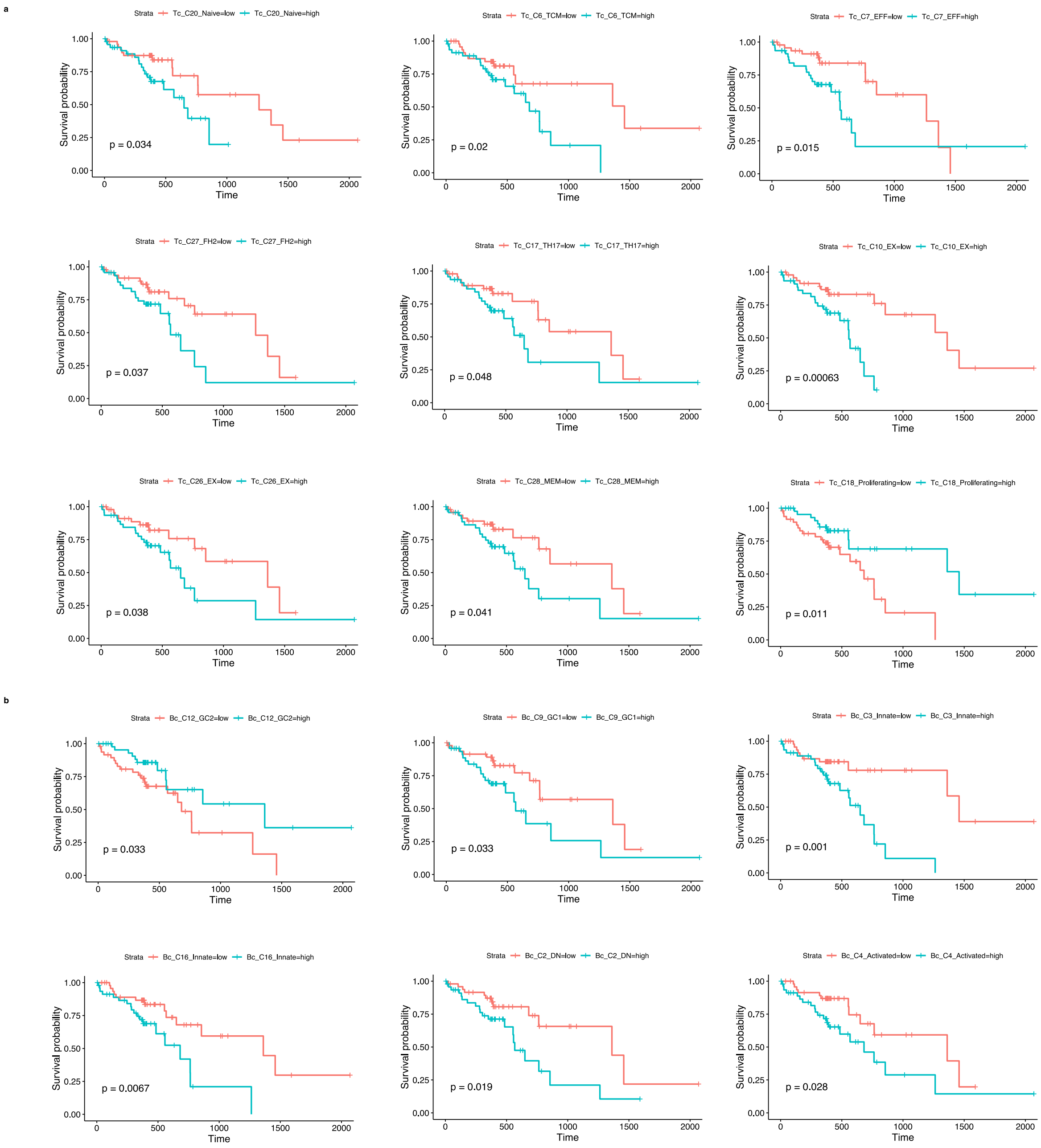


Figure S1. Kaplan-Meier curves for overall survival in the 95 patients in TCGA-ESCC cohort stratified according to high vs low score of the marker signature for each subclusters in T (a), B cells (b). (Only significant shown)

Supplementary Figure 2

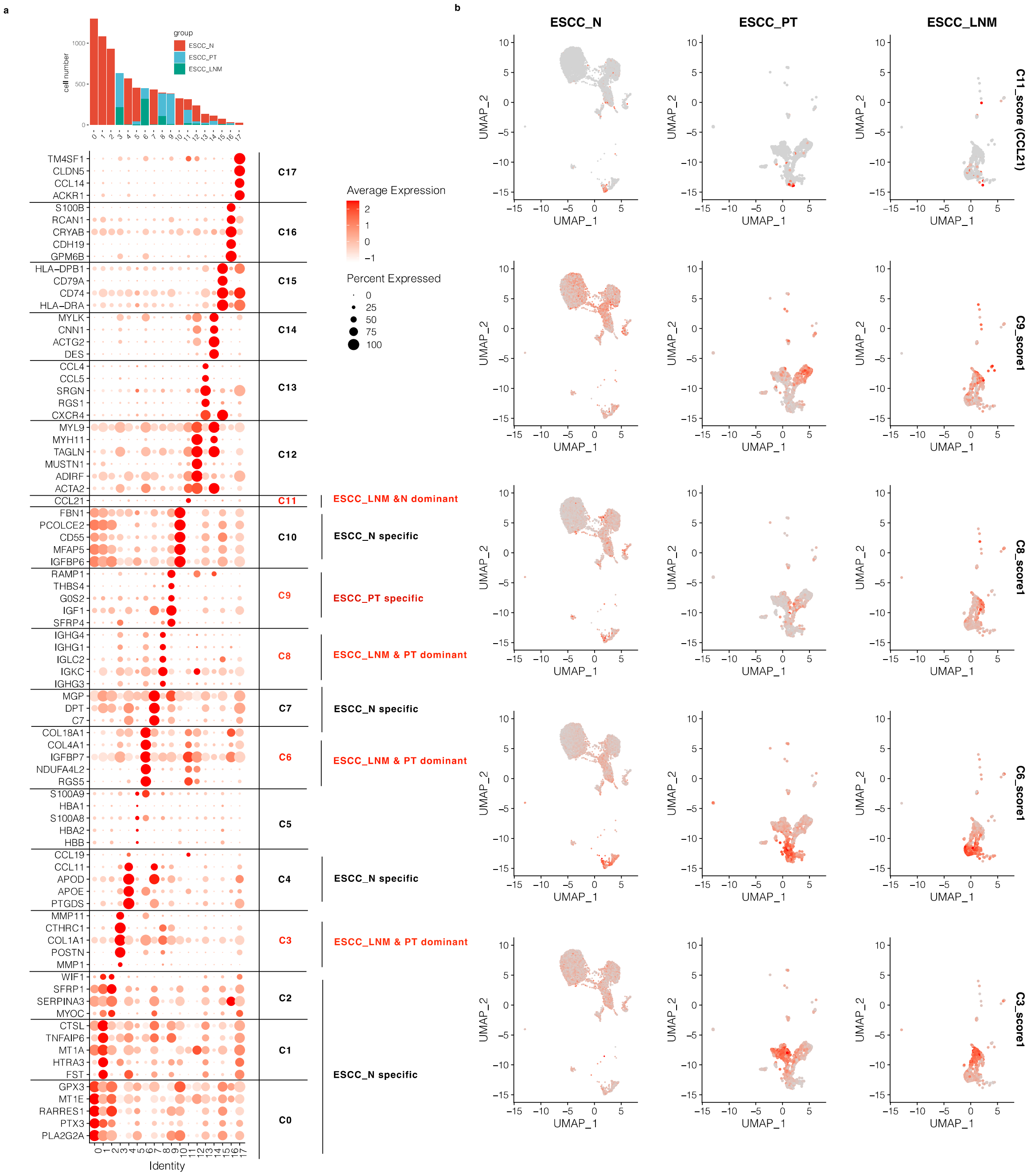


Figure S2. Fibroblast subclusters.

(a) Dot plot showing the differentially expressed genes (DEGs) among clusters of Fibroblast cells.

(b) The expression of the differentially expressed genes (DEGs) defined in (a) for fibroblast cells.

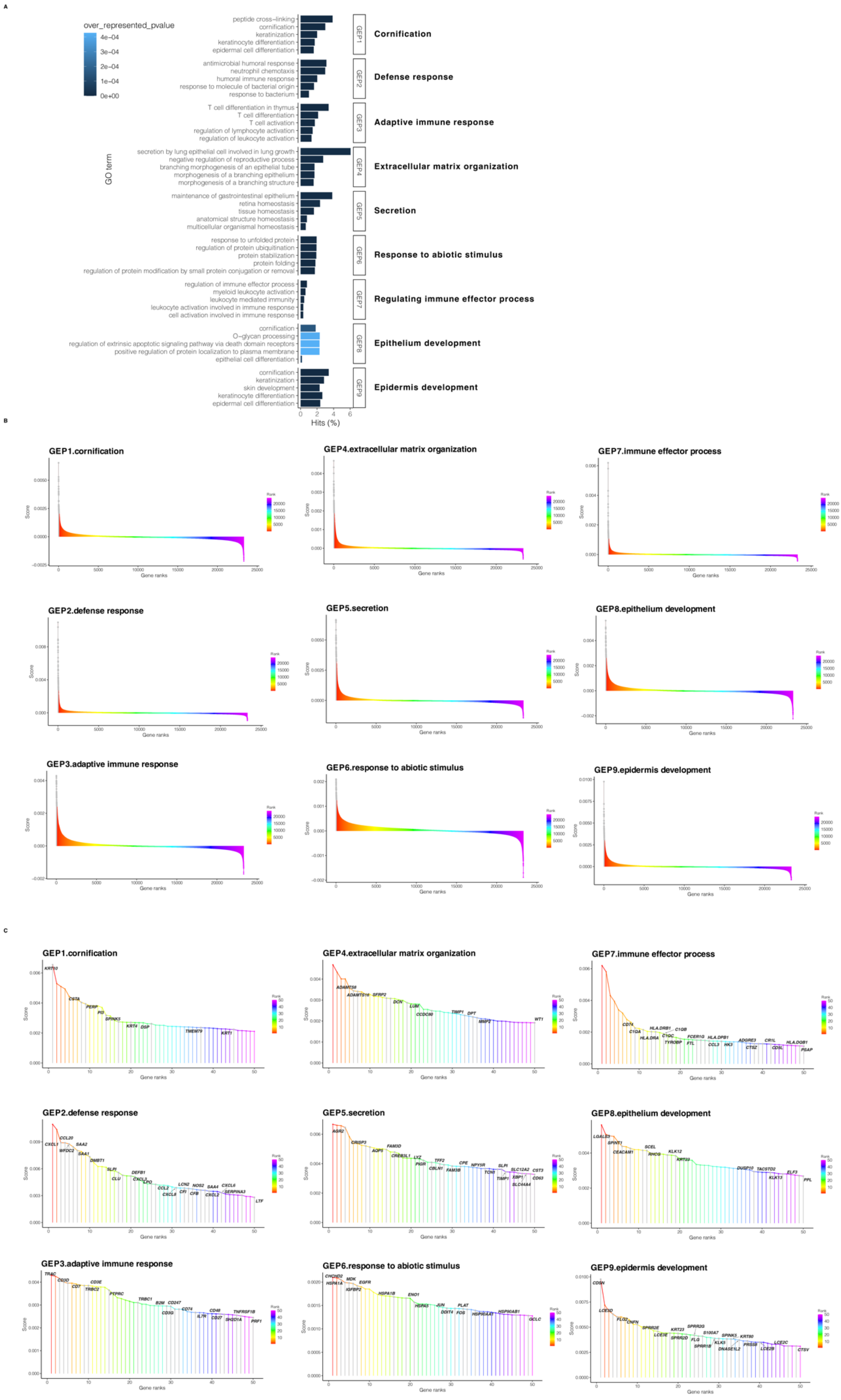
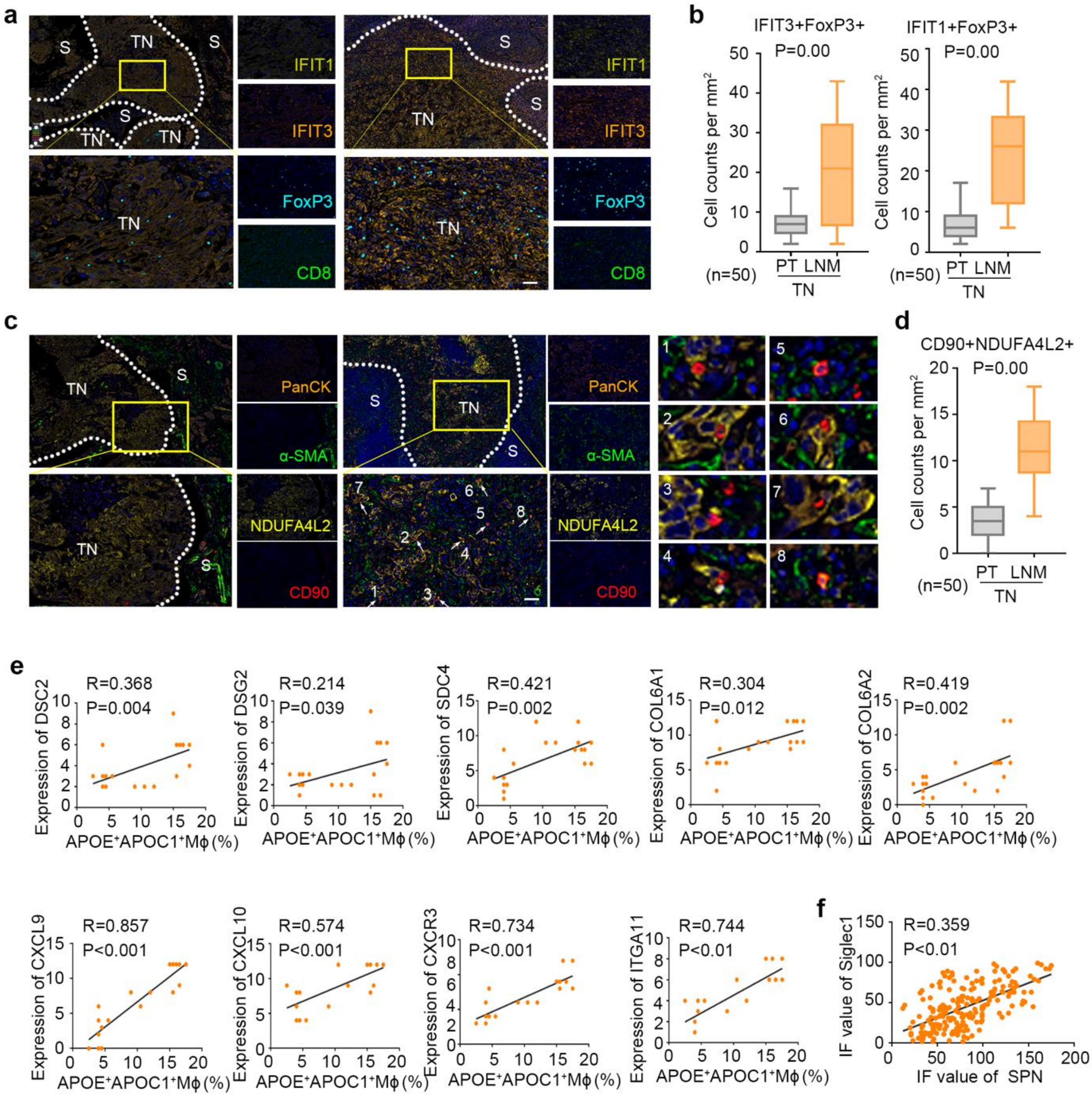

A
over_represented_pvalue
4e−04
3e−04
2e−04
1e−04
0e+00
GO term
peptide cross−linking
cornification
keratinization
keratinocyte differentiation
epidermal cell differentiation
antimicrobial humoral response
neutrophil chemotaxis
humoral immune response
response to molecule of bacterial origin
response to bacterium
T cell differentiation in thymus
T cell differentiation
T cell activation
regulation of lymphocyte activation
regulation of leukocyte activation
secretion by lung epithelial cell involved in lung growth
negative regulation of reproductive process
branching morphogenesis of an epithelial tube
morphogenesis of a branching epithelium
morphogenesis of a branching structure
maintenance of gastrointestinal epithelium
retina homeostasis
tissue homeostasis
anatomical structure homeostasis
multicellular organismal homeostasis
response to unfolded protein
regulation of protein ubiquitination
protein stabilization
protein folding
regulation of protein modification by small protein conjugation or removal
regulation of immune effector process
myeloid leukocyte activation
leukocyte mediated immunity
leukocyte activation involved in immune response
cell activation involved in immune response
cornification
O−glycan processing
regulation of extrinsic apoptotic signaling pathway via death domain receptors
positive regulation of protein localization to plasma membrane
epithelial cell differentiation
cornification
keratinization
skin development
keratinocyte differentiation
epidermal cell differentiation
0
2
4
6
Hits (%)
GEP1
GEP2
GEP3
GEP4
GEP5
GEP6
GEP7
GEP8
GEP9
Cornification
Defense response
Adaptive immune response
Extracellular matrix organization
Secretion
Response to abiotic stimulus
Regulating immune effector process
Epithelium development
Epidermis development
B
GEP1.cornification
GEP4.extracellular matrix organization
GEP7.immune effector process
GEP2.defense response
GEP5.secretion
GEP8.epithelium development
GEP3.adaptive immune response
GEP6.response to abiotic stimulus
GEP9.epidermis development
Score
Gene ranks
Rank
20000
15000
10000
5000
C
GEP1.cornification
KRT10
CSTA
PERP
PI3
SPINK5
KRT4
DSP
TMEM79
KRT1
GEP4.extracellular matrix organization
ADAMTS8
ADAMTS16
SFRP2
DCN
LUM
CCDC80
TIMP1
DPT
MMP2
WT1
GEP7.immune effector process
CD74
C1QA
HLA.DRB1
C1QB
HLA.DRA
C1QC
TYROBP
FCER1G
FTL
HLA.DPB1
CCL3
HK3
ADGRE3
CTSZ
CR1L
CD5L
HLA.DQB1
PSAP
GEP2.defense response
CXCL1
CCL20
WFDC2
SAA2
SAA1
DMBT1
SLPI
CLU
DEFB1
CXCL3
LPO
CCL2
CXCL8
LCN2
CFI
NOS2
CFB
SAA4
CXCL2
CXCL6
SERPINA3
LTF
GEP5.secretion
AGR2
CRISP3
AQP5
FAM3D
CREB3L1
LYZ
PIGR
TFF2
CBLN1
FAM3B
CPE
NPY5R
TCN1
SLPI
TIMP1
SLC12A2
XBP1
SLC44A4
CST3
CD63
GEP8.epithelium development
LGALS3
SPINT1
CEACAM1
SCEL
RHCG
KLK12
KRT23
DUSP10
TACSTD2
KLK13
ELF3
PPL
GEP3.adaptive immune response
TRAC
CD3D
CD7
CD3E
TRBC2
PTPRC
TRBC1
B2M
CD247
CD3G
CD74
IL7R
CD48
CD27
SH2D1A
TNFRSF1B
PRF1
GEP6.response to abiotic stimulus
CHCHD2
HSPA1A
MDK
IGFBP2
EGFR
HSPA1B
ENO1
HSPA5
JUN
DDIT4
FOS
PLAT
HSP90AA1
HSP90AB1
GCLC
GEP9.epidermis development
CDSN
LCE3D
FLG2
CNFN
SPRR2E
LCE3E
KRT23
SPRR2D
SPRR2G
FLG
SPRR1B
S100A7
KLK5
SPINK5
DNASE1L2
KRT80
PRSS8
LCE2B
LCE2C
CTSV
Score
Gene ranks
Rank
50
40
30
20
10

Figure S3. Annotation and characterization of 9 gene expression programs in Epithelial cells.

(a) Gene oncology analysis of top50 genes ranked by contribution score in each gene expression programs.

(b) The distribution of contributing score for all genes in each gene expression programs.

(c) The distribution of contributing score for top50 genes in each gene expression programs.



Supplementary Figure 4

Figure S4

(a) Representative images of multiplexed IF staining of IFIT1, IFIT3 and Foxp3 in primary tumor and metastatic lymph node (n=50). Scale bar, 50 µm.

(b) Percentage of IFIT1+FoxP3+, IFIT3+FoxP3+ T cells in tumor nest between primary tumor and metastatic lymph node.

(c) Representative images of multiplexed IF staining of Pan-CK, α-SMA, CD90 and NDUFA4L2 in primary tumor and metastatic lymph node (n=50). Scale bar, 50 µm. The white arrows indicate the location of CD90 and NDUFA4L2 positive cells.

(d) Density of CD90+NDUFA4L2+ CAFs in tumor nest between primary tumor and metastatic lymph node.

(e) Correlation between the expression of DSG2, DSG2, SDC4, COL6A1, COL6A2, CXCL9, CXCL10, CXCR3, ITGA11 and APOE+APOC1+ macrophages in metastatic lymph-node (n=20).

(f) The intensity of IF staining for Siglec1 and SPN in tumor (cell number n = 200) was analyzed with ImageJ software, and a linear regression analysis was performed to test their correlations. Data are presented as means ± SD. PT: primary tumor, LNM: metastatic lymph node.

Table S1. Clinical characteristic of patients recruited in this study.

Name	Gender	Age	Pathology	Grade	Vessel Invasion	Nerve Invasion	TNM	Stage
P1	Male	53	ESCC	G2	Yes	Yes	T3N2M0	III B
P2	Male	53	ESCC	G1	Yes	No	T3N1M0	III B
P3	Female	66	ESCC	G1	Yes	No	T3N1M0	III B
P4	Female	57	ESCC	G2	No	No	T1bN0M0	I B

Table S2. List of antibodies used in this project.

Antibody	Cat No.	Vendor	Application
For IHC			
Rabbit anti-human APOE	**ab279714**	**Abcam**	**IHC, 1:100**
Rabbit anti-human	ab1898	Abca	IHC: 1:100

APOC1	66	m	
Rabbit anti-human COL6A1	ab151422	Abcam	IHC, 1:200
Rabbit anti-human COL6A2	PA5-65085	Invitrogen	IHC, 1:200
Mouse anti-human DSC2	ab218377	Abcam	IHC, 1:200
Rabbit anti-human DSG2	**ab96761**	**Abcam**	**IHC, 1:200**
Rabbit anti-human CXCL9	ab118543	Abcam	IHC, 1:200
Rabbit anti-human CXCL10	MA5-23774	Invitrogen	IHC, 1:200
Rabbit anti-human CXCR3	ab288437	Abcam	IHC, 1:200
Rabbit anti-human SDC4	GTX02062	GeneTex	IHC, 1:200
For IF			
Rabbit anti-human SIGLEC1	GTX131703	GeneTex	IF: 1:100
Mouse anti-human SPN	ab257315	Abcam	IF: 1:100
For Multiplexed staining			
Rabbit anti-human CD163	ab182422	Abcam	IF: 1:500
Rabbit anti-human CD68	ab213363	Abcam	IF: 1:1000
Rabbit anti-human PD-1	D4W2J, 86163S	CST	IF: 1:200
Rabbit anti-human PD-L1	E1L3N, 13684S	CST	IF: 1:400
Rabbit anti-human CD3	A0452	Dako	IF: 1:1
Rabbit anti-human CD4	ab133616	Abcam	IF: 1:100
Rabbit anti-human CD8	ab178089,	Abcam	IF: 1:200
Rabbit anti-human CD56	ab75813	Abcam,	IF: 1:1000
Rabbit anti-human CD20	L26, IR604	Dako	IF: 1:1
Rabbit anti-human FOXP3	ab20034	Abcam	IF: 1:100
Rabbit anti-human APOE	**ab183597**	**Abcam**	**IF: 1:100**
Rabbit anti-human APOC1	ab198288	Abcam	IF: 1:100
Rabbit anti-human IFIT1	**23247-1-AP**	**proteintech**	**IF: 1:100**
Rabbit anti-human IFIT3	15201-1-AP	proteintech	IF: 1:100
Rabbit anti-human α-SMA	**67735-1-**	**proteintech**	**IF: 1:100**

	Ig		
Rabbit anti-human CD90	ab133350	Abcam	IF: 1:100
Rabbit anti-human NDUFA4L2	**16480-1-AP**	**proteintech**	**IF: 1:100**
Rabbit anti-human RGS5	11590-1-AP	proteintech	IF: 1:100
Rabbit anti-human pan-CK	ab7753	Abcam	IF: 1:100

Data S1. Metadata information of 85, 263 cells.

Data S2. Cluster signature genes (Top Rank 50).

Data S3. The expression level of cluster signature genes in the 95 patients from TCGA-ESCC cohort, inferred by the GSVA method based on bulk RNA-seq data.

Data S4. Top50 signature genes and their contribution score of 9 gene expression program in epithelial cells.

Data S5. The expression level of 9 epithelial gene expression program in the 95 patients from TCGA-ESCC cohort, inferred by the GSVA method based on bulk RNA-seq data.