FIGURE S1

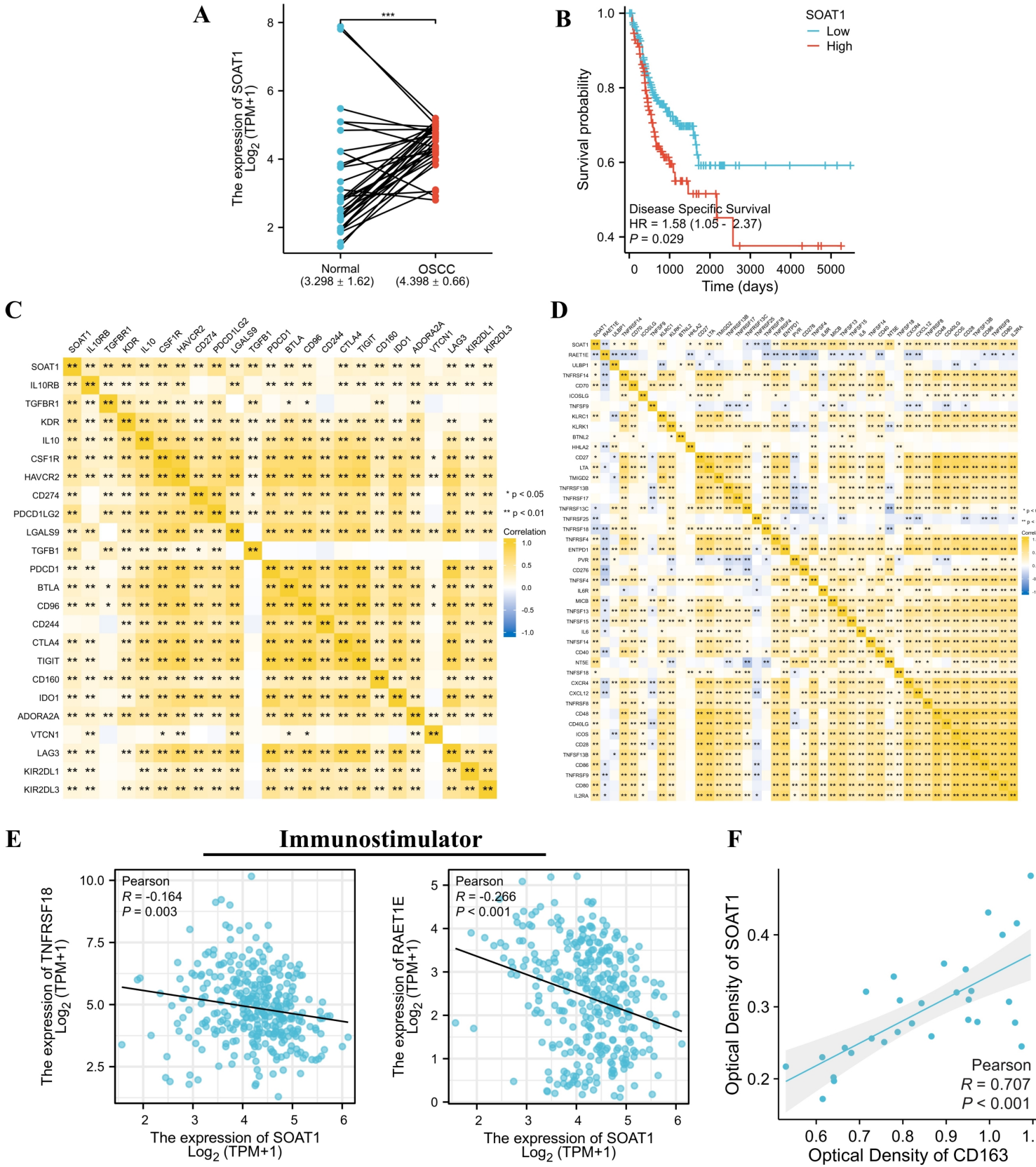

A
***
The expression of SOAT1
Log2 (TPM+1)
Normal
(3.298 ± 1.62)
OSCC
(4.398 ± 0.66)
B
SOAT1
Low
High
Survival probability
Disease Specific Survival
HR = 1.58 (1.05 - 2.37)
P = 0.029
Time (days)
C
SOAT1
IL10RB
TGFBR1
KDR
IL10
CSF1R
HAVCR2
CD274
PDCD1LG2
LGALS9
TGFB1
PDCD1
BTLA
CD96
CD244
CTLA4
TIGIT
CD160
IDO1
ADORA2A
VTCN1
LAG3
KIR2DL1
KIR2DL3
* p < 0.05
** p < 0.01
Correlation
D
* p < 0.05
** p < 0.01
Correlation
E
Immunostimulator
Pearson
R = -0.164
P = 0.003
The expression of TNFRSF18
Log2 (TPM+1)
The expression of SOAT1
Log2 (TPM+1)
Pearson
R = -0.266
P < 0.001
The expression of RAET1E
Log2 (TPM+1)
The expression of SOAT1
Log2 (TPM+1)
F
Optical Density of SOAT1
Pearson
R = 0.707
P < 0.001
Optical Density of CD163

FIGURE S2

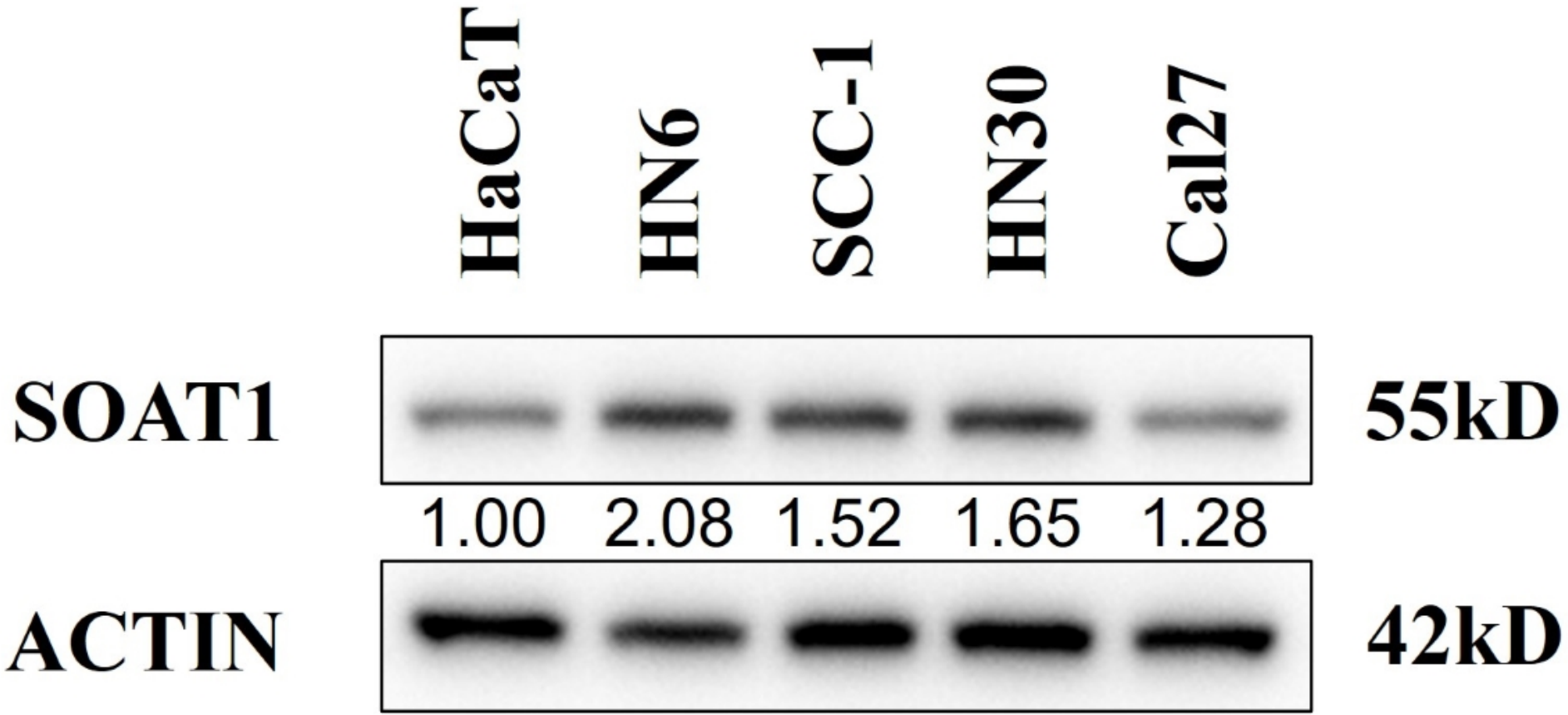

HaCaT
HN6
SCC-1
HN30
Cal27
SOAT1
55kD
1.00
2.08
1.52
1.65
1.28
ACTIN
42kD

FIGURE S3

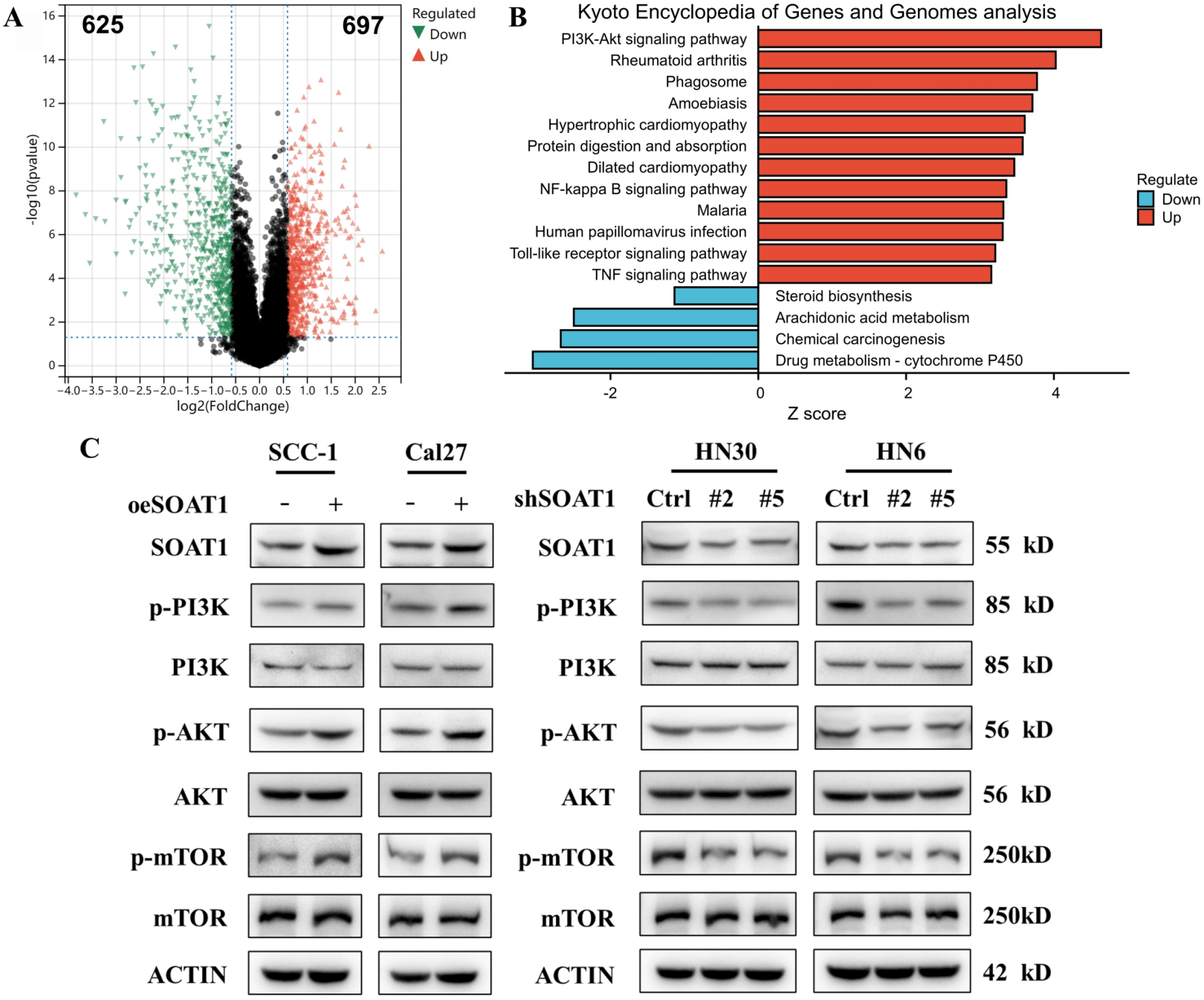

A
625
697
Regulated
Down
Up
-log10(pvalue)
log2(FoldChange)
B
Kyoto Encyclopedia of Genes and Genomes analysis
PI3K-Akt signaling pathway
Rheumatoid arthritis
Phagosome
Amoebiasis
Hypertrophic cardiomyopathy
Protein digestion and absorption
Dilated cardiomyopathy
NF-kappa B signaling pathway
Malaria
Human papillomavirus infection
Toll-like receptor signaling pathway
TNF signaling pathway
Steroid biosynthesis
Arachidonic acid metabolism
Chemical carcinogenesis
Drug metabolism - cytochrome P450
Regulate
Down
Up
Z score
C
SCC-1
Cal27
HN30
HN6
oeSOAT1
shSOAT1
Ctrl
#2
#5
SOAT1
p-PI3K
PI3K
p-AKT
AKT
p-mTOR
mTOR
ACTIN
55 kD
85 kD
85 kD
56 kD
56 kD
250kD
250kD
42 kD

FIGURE S4

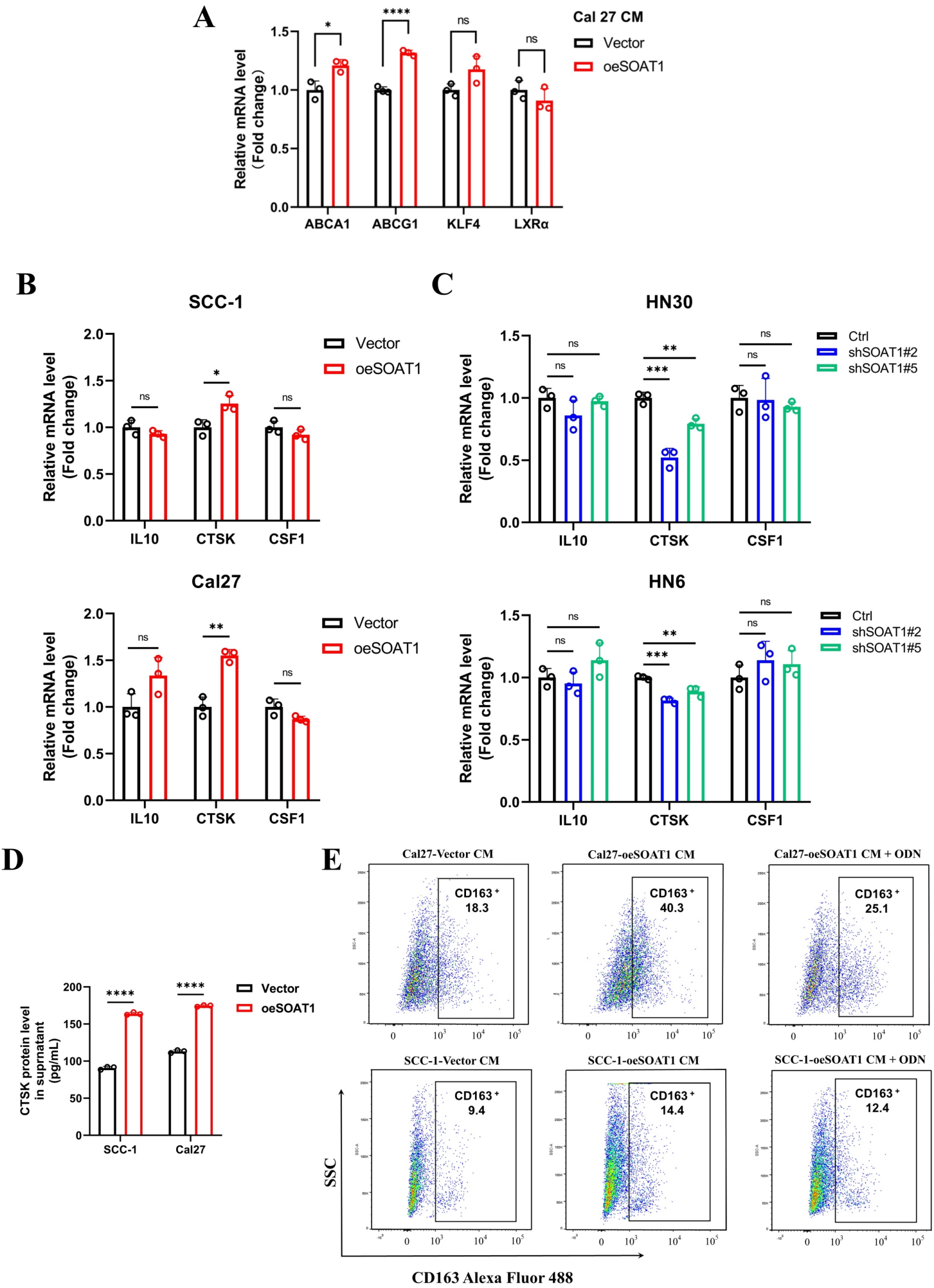

A
Cal 27 CM
Vector
oeSOAT1
Relative mRNA level (Fold change)
ABCA1
ABCG1
KLF4
LXRα
B
SCC-1
Cal27
IL10
CTSK
CSF1
C
HN30
HN6
Ctrl
shSOAT1#2
shSOAT1#5
D
CTSK protein level in supernatant (pg/mL)
SCC-1
Cal27
E
Cal27-Vector CM
Cal27-oeSOAT1 CM
Cal27-oeSOAT1 CM + ODN
SCC-1-Vector CM
SCC-1-oeSOAT1 CM
SCC-1-oeSOAT1 CM + ODN
CD163+ 18.3
CD163+ 40.3
CD163+ 25.1
CD163+ 9.4
CD163+ 14.4
CD163+ 12.4
SSC
CD163 Alexa Fluor 488

FIGURE S5

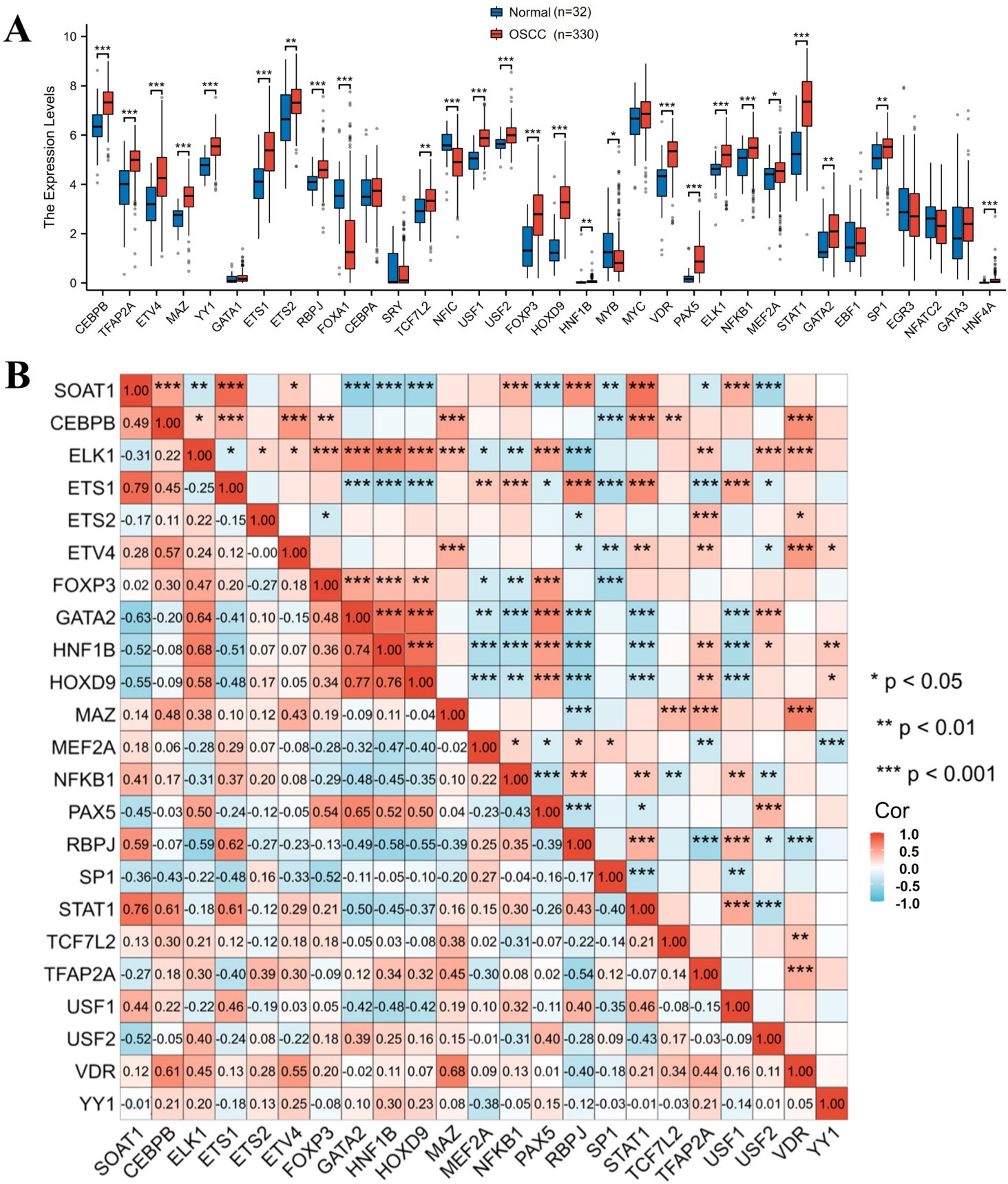
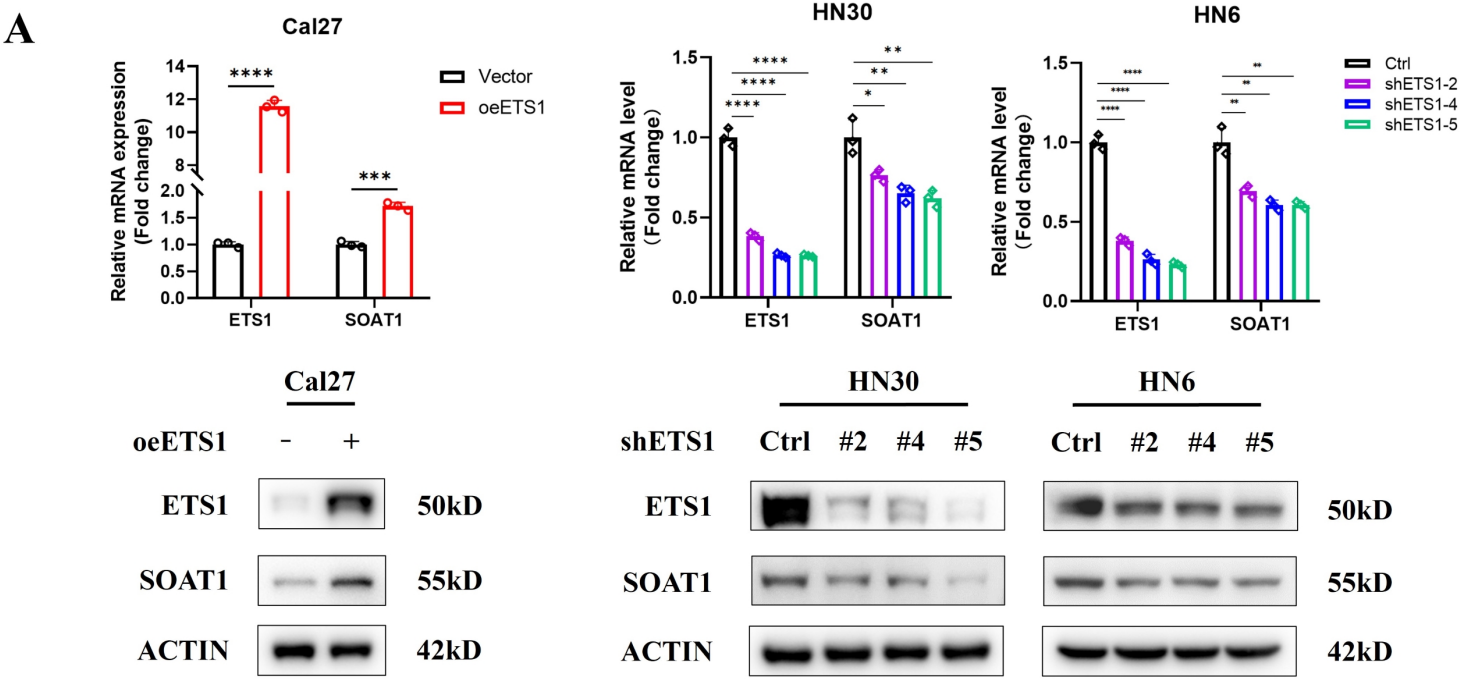

A
Normal (n=32)
OSCC (n=330)
The Expression Levels
CEBPB
TFAP2A
ETV4
MAZ
YY1
GATA1
ETS1
ETS2
RBPJ
FOXA1
CEBPA
SRY
TCF7L2
NFIC
USF1
USF2
FOXP3
HOXD9
HNF1B
MYB
MYC
VDR
PAX5
ELK1
NFKB1
MEF2A
STAT1
GATA2
EBF1
SP1
EGR3
NFATC2
GATA3
HNF4A
B
SOAT1
CEBPB
ELK1
ETS1
ETS2
ETV4
FOXP3
GATA2
HNF1B
HOXD9
MAZ
MEF2A
NFKB1
PAX5
RBPJ
SP1
STAT1
TCF7L2
TFAP2A
USF1
USF2
VDR
YY1
* p < 0.05
** p < 0.01
*** p < 0.001
Cor
1.0
0.5
0.0
-0.5
-1.0

FIGURE S6


A
Cal27
HN30
HN6
Relative mRNA expression (Fold change)
Relative mRNA level (Fold change)
Vector
oeETS1
Ctrl
shETS1-2
shETS1-4
shETS1-5
ETS1
SOAT1
oeETS1
shETS1
Ctrl
#2
#4
#5
ETS1
SOAT1
ACTIN
50kD
55kD
42kD

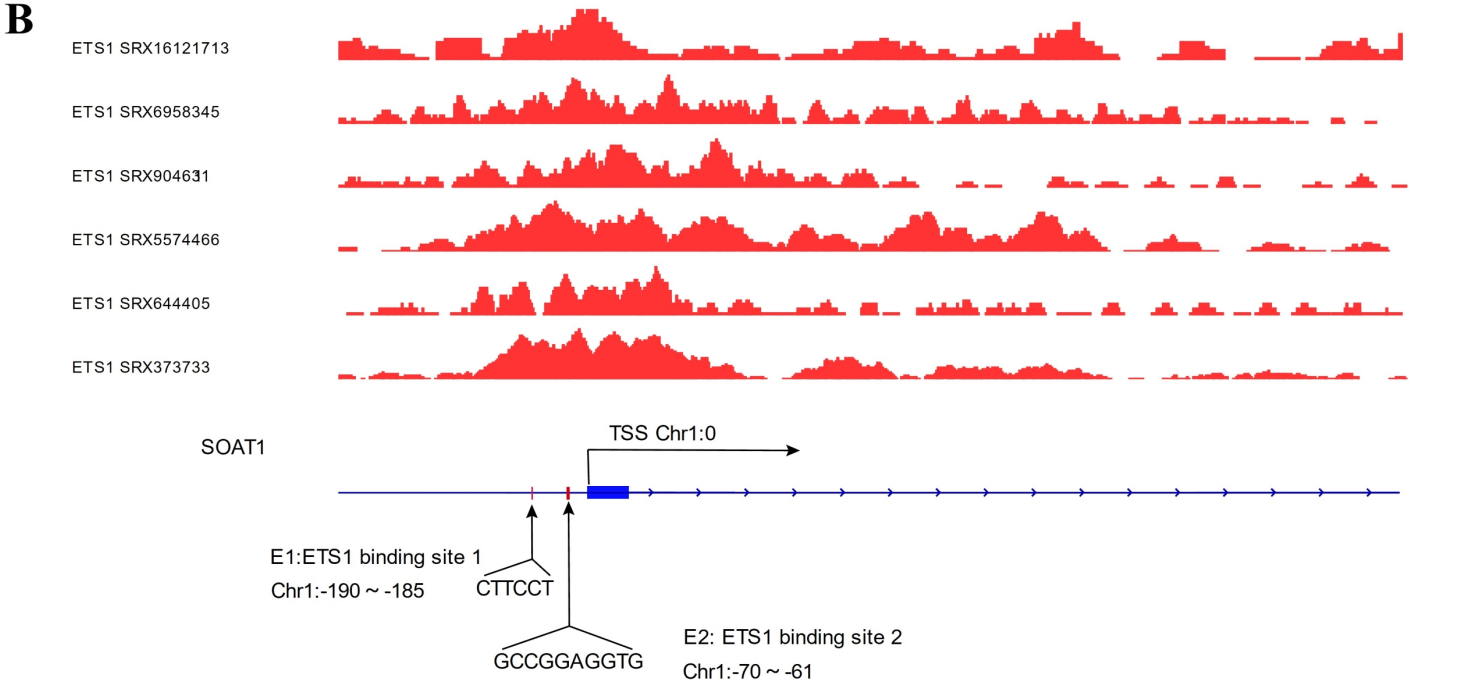

B
ETS1 SRX16121713
ETS1 SRX6958345
ETS1 SRX904631
ETS1 SRX5574466
ETS1 SRX644405
ETS1 SRX373733
SOAT1
TSS Chr1:0
E1:ETS1 binding site 1
Chr1:-190 ~ -185
CTTCCT
GCCGGAGGTG
E2: ETS1 binding site 2
Chr1:-70 ~ -61

FIGURE S7

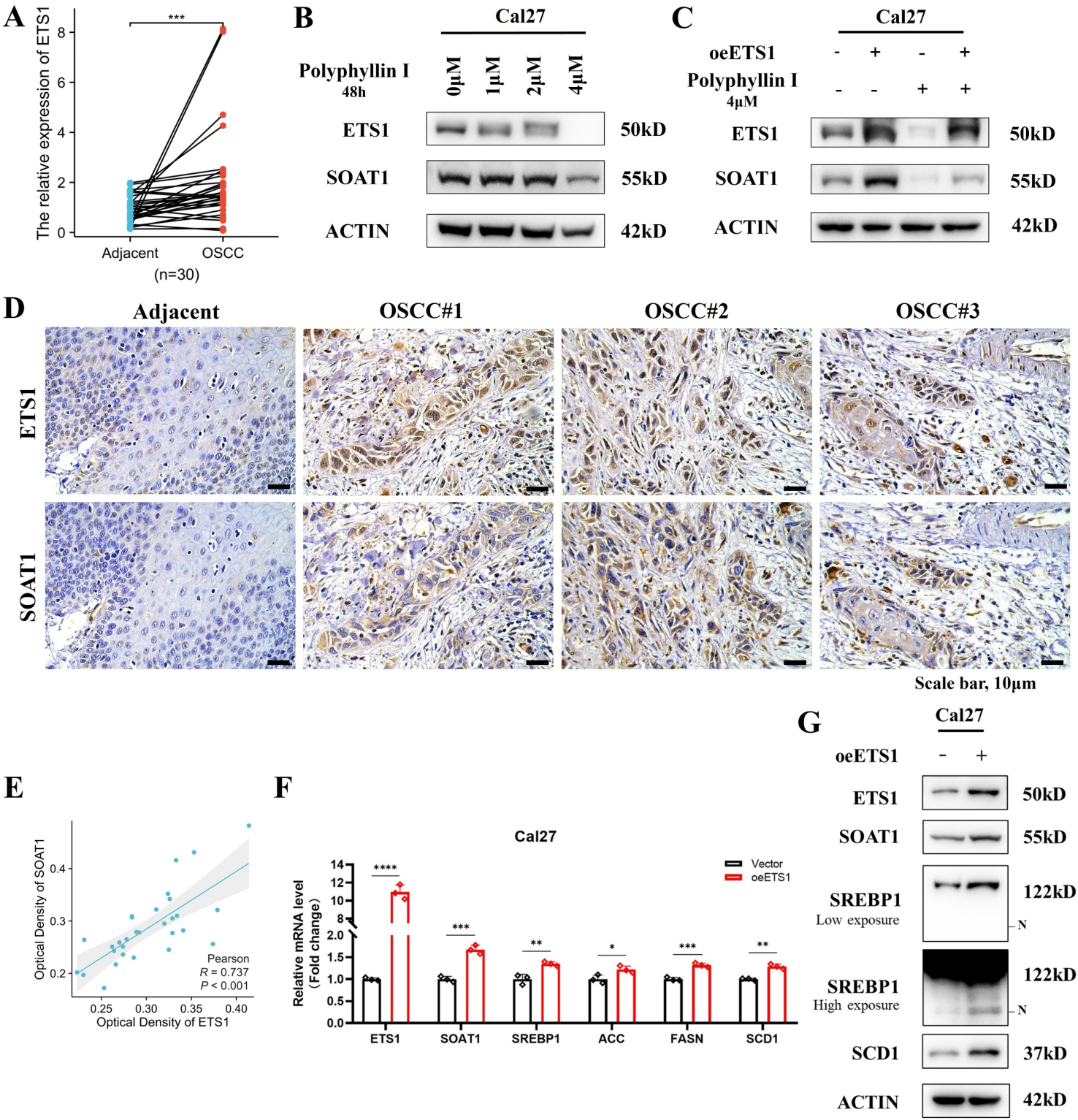
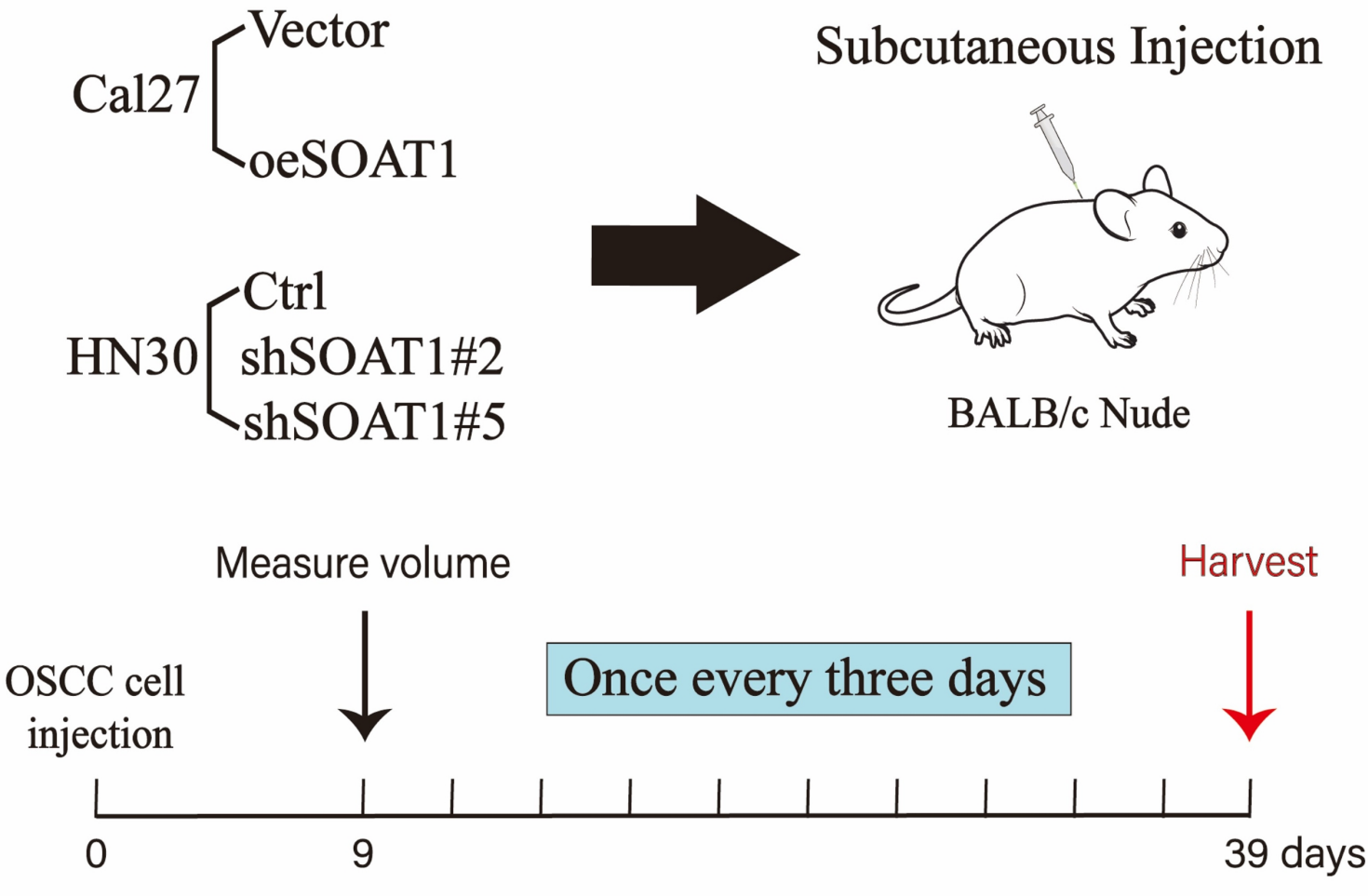

A
The relative expression of ETS1
***
Adjacent
OSCC
(n=30)
B
Cal27
Polyphyllin I
48h
0μM
1μM
2μM
4μM
ETS1
SOAT1
ACTIN
50kD
55kD
42kD
C
Cal27
oeETS1
Polyphyllin I
4μM
ETS1
SOAT1
ACTIN
50kD
55kD
42kD
D
Adjacent
OSCC#1
OSCC#2
OSCC#3
ETS1
SOAT1
Scale bar, 10μm
E
Optical Density of SOAT1
Optical Density of ETS1
Pearson
R = 0.737
P < 0.001
F
Cal27
Relative mRNA level
(Fold change)
Vector
oeETS1
ETS1
SOAT1
SREBP1
ACC
FASN
SCD1
G
Cal27
oeETS1
ETS1
SOAT1
SREBP1
Low exposure
SREBP1
High exposure
N
SCD1
ACTIN
50kD
55kD
122kD
122kD
37kD
42kD

FIGURE S8


Cal27
Vector
oeSOAT1
HN30
Ctrl
shSOAT1#2
shSOAT1#5
Subcutaneous Injection
BALB/c Nude
Measure volume
Harvest
OSCC cell injection
Once every three days
0
9
39 days

Supplementary figure legends

Figure S1 (A) The expression of *SOAT1* in OSCC and normal paired samples. (B) Kaplan-Meier curves of disease-specific survival for high and low groups of *SOAT1* expression. (C) Heat map of *SOAT1* correlation with suppressive immunomodulatory genes. (D) Heat map of *SOAT1* correlation with stimulatory immunomodulatory genes. (E) Scatter plots of *SOAT1* correlation with stimulatory immunomodulatory genes. (F) IHC staining intensity correlation analysis of *SOAT1* and *CD163* in 30 pairs of clinical samples of OSCC paired with adjacent tissue. The asterisks represented the statistical *p*-value (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Figure S2 Western Blot assay was performed to detect the protein expression level of SOAT1 in four OSCC cell lines and HaCaT cell.

Figure S3 (A) Volcano plot of differentially expressed genes in high and low groups of *SOAT1* expression in TCGA-OSCC. (B) KEGG analysis of DEGs between high and low expression groups. (C) The effects of overexpression and knockdown of *SOAT1* on the expression of p-PI3K, PI3K, p-AKT, AKT, p-mTOR and mTOR in OSCC cells were analyzed by Western Blot.

Figure S4 (A) RT-qPCR analysis of macrophages after cultured with CM of *SOAT1*-overexpressing Cal27 cell. (B) RT-qPCR analysis of pro-M2 polarization genes in *SOAT1* overexpressing OSCC cells. (C) RT-qPCR analysis of pro-M2 polarization genes in *SOAT1* knockdown OSCC cells. (D) ELISA assay to detect CTSK levels in the supernatants of SCC-1 and Cal27 cells overexpressing *SOAT1*. (E) Flow cytometry was used to assess the expression level of CD163 in macrophages after culture in conditioned medium and treatment with ODN. (ODN: Odanacatib, a CTSK-specific inhibitor) The asterisks represented the statistical *p*-value (ns = no significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

Figure S5 (A) Box plot display the expression levels of 34 potential upstream

transcription factors in OSCC (n=330) and normal tissues (n=32) analyzed using the TCGA-OSCC data. (B) Heatmap of the correlation of 22 potential upstream transcription factors with *SOAT1*. The asterisks represented the statistical *p*-value (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Figure S6 (A) RT-qPCR and Western Blot were used to verify the efficiency of *ETS1* overexpression and knockdown, simultaneously inspected the expression of *SOAT1* after altering *ETS1* expression. (B) Prediction of *ETS1* binding sites in the promoter region of *SOAT1*. The asterisks represented the statistical *p*-value (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

Figure S7 (A) RT-qPCR was used to determine the expression levels of *ETS1* in OSCC and paracancerous tissues (n=30). (B) Western Blot explores the effects of different concentrations of Polyphyllin I on the expression of *ETS1* and *SOAT1* in Cal27 cells after 48 h of treatment. (C) Western Blot was designed to test for changes in the expression of *ETS1* and *SOAT1* in PPI-treated Vector/oeETS1 Cal27 cells for 48 h. (D-E) IHC analysis of *ETS1* and *SOAT1* in 30 pairs of clinical samples of OSCC paired with adjacent tissue. (F-G) The expression levels of downstream lipid metabolism genes in OSCC cells with *ETS1*-overexpressing were checked using RT-qPCR and Western Blot. The asterisks represented the statistical *p*-value (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

Figure S8 Schematic diagram of nude mice treatment.