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Supporting Information

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TAOK3 Facilitates Esophageal Squamous Cell Carcinoma Progression and Cisplatin Resistance Through Augmenting Autophagy Mediated by IRGM

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Supporting information

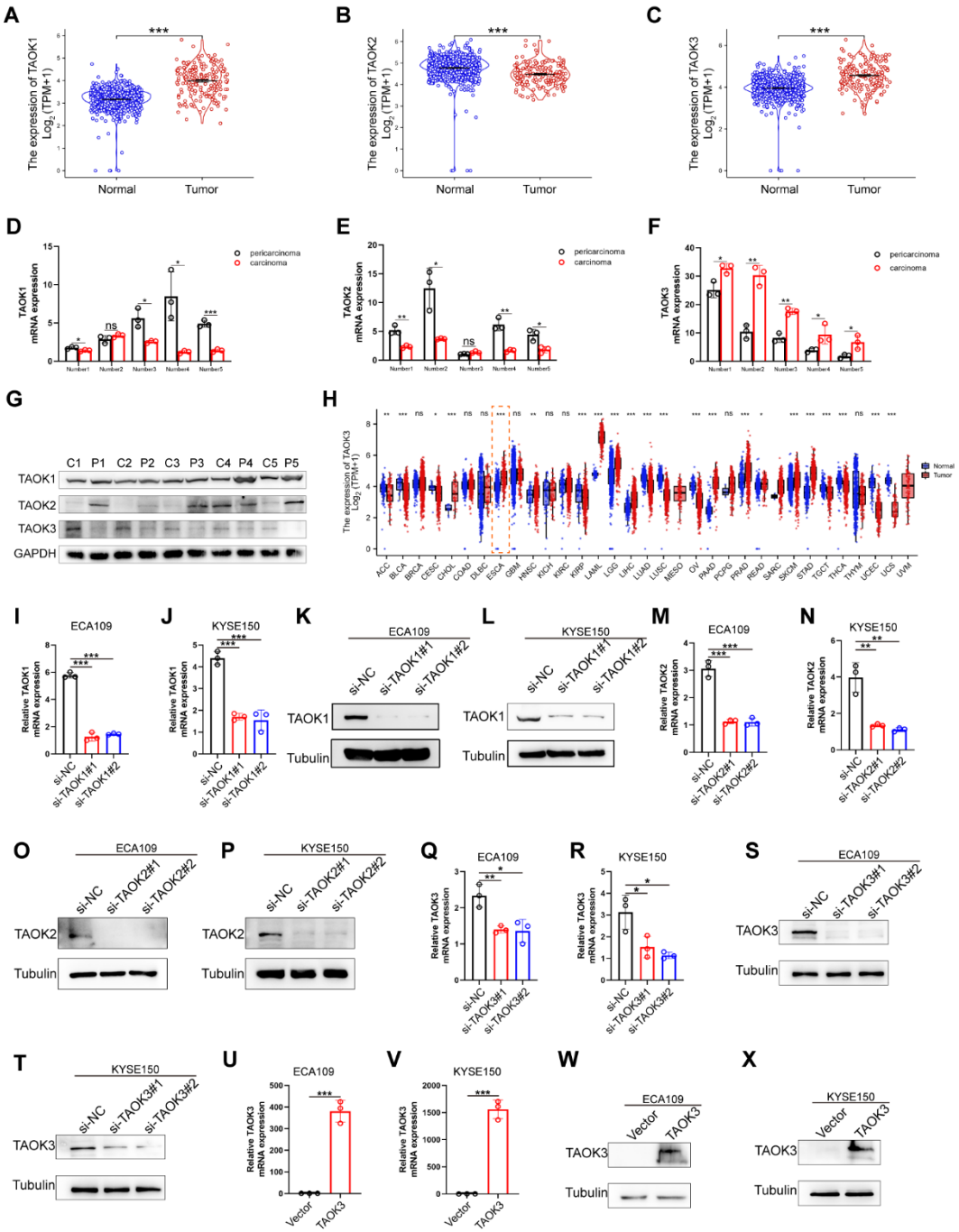


Fig. S1 TAOK3 is overexpressed in ESCC tissues from TCGA analysis and our samples, and the knockdown and overexpression efficiency of TAO kinases is high. A-C TCGA database analysis of the mRNA expression level of TAOK1, TAOK2, and TAOK3. D-F QPCR analysis of the

mRNA expression level of TAO kinases in 5 pairs of ESCC tissues. G Western blot analysis of the protein expression level of TAO kinases in 5 pairs of ESCC tissues. H TCGA analysis of pan-cancer mRNA expression of TAOK3. I-T QPCR and western blot analysis of the knockdown efficiency of siRNAs targeting TAOK1, TAOK2, and TAOK3. U-X QPCR and western blot analysis of the overexpression efficiency of pcDNA3.1-FLAG-TAOK3 transfection.

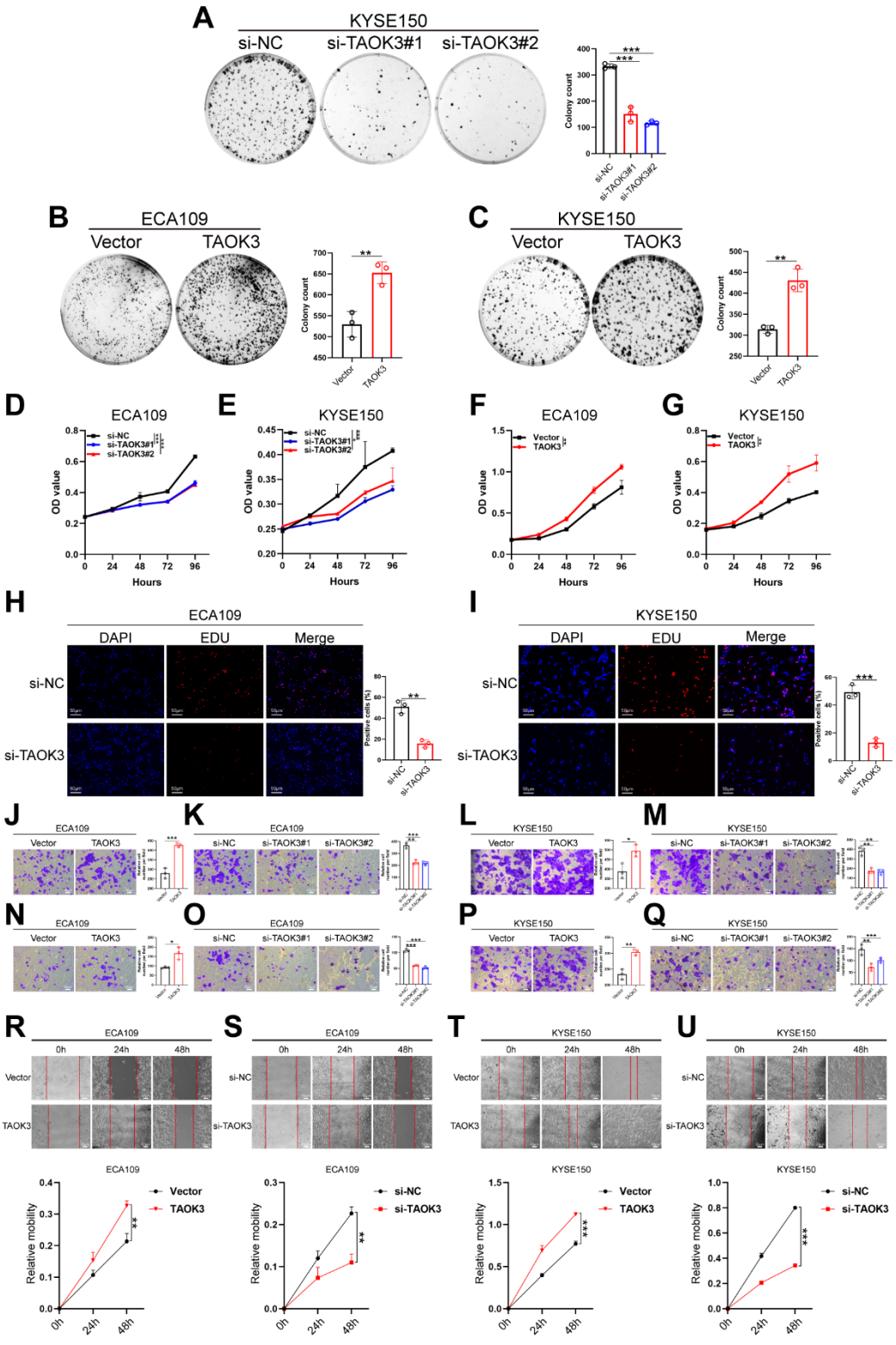
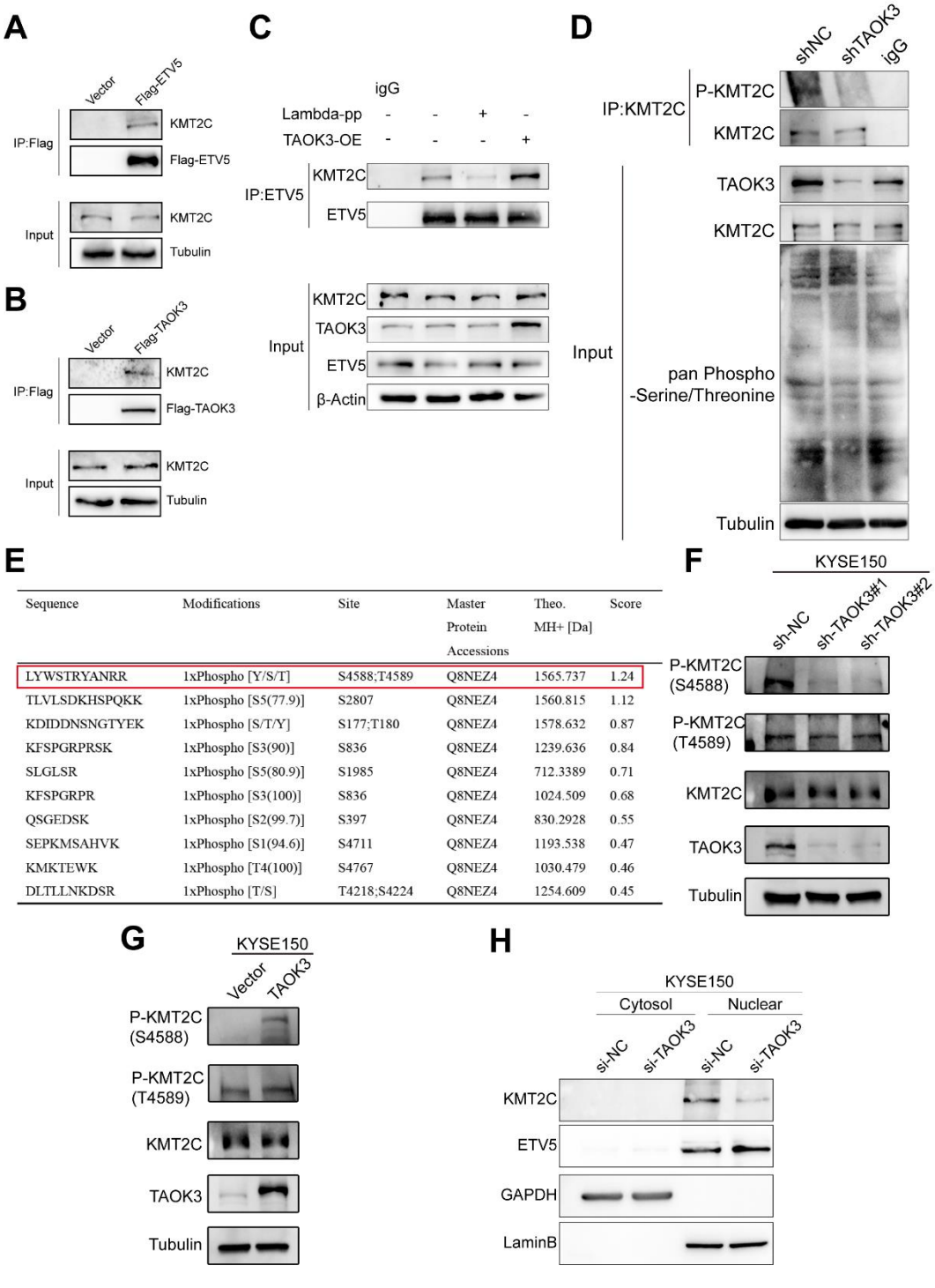

A
KYSE150
si-NC
si-TAOK3#1
si-TAOK3#2
Colony count
B
ECA109
Vector
TAOK3
C
KYSE150
D
ECA109
E
KYSE150
F
ECA109
G
KYSE150
OD value
Hours
H
ECA109
DAPI
EDU
Merge
I
KYSE150
Positive cells (%)
J
K
L
M
N
O
P
Q
R
S
T
U
Relative mobility
0h
24h
48h

Fig. S2 TAOK3 promotes proliferation, migration and invasion in vitro. A-C Colony formation analysis of the proliferation of ESCC cells when TAOK3 was overexpressed or knocked down. D-G CCK8 analysis of the viability of ESCC cells when TAOK3 was overexpressed or knocked down. H-I Edu analysis of the proliferation of ESCC cells when TAOK3 was overexpressed. J-M Migration assay showed the alteration when TAOK3 was overexpressed or knocked down. N-Q Invasion assay showed the alteration when TAOK3 was overexpressed or knocked down. R-U Wound healing analysis of the migration and invasion of ESCC cells when TAOK3 was overexpressed or knocked down.



Sequence	Modifications	Site	Master Protein Accessions	Theo. MH+ [Da]	Score
LYWSTRYANRR	1xPhospho [Y/S/T]	S4588;T4589	Q8NEZ4	1565.737	1.24
TLVLSDKHSPQKK	1xPhospho [S5(77.9)]	S2807	Q8NEZ4	1560.815	1.12
KDIDDNSNGTYEK	1xPhospho [S/T/Y]	S177;T180	Q8NEZ4	1578.632	0.87
KFSPGRPRSK	1xPhospho [S3(90)]	S836	Q8NEZ4	1239.636	0.84
SLGLSR	1xPhospho [S5(80.9)]	S1985	Q8NEZ4	712.3389	0.71
KFSPGRPR	1xPhospho [S3(100)]	S836	Q8NEZ4	1024.509	0.68
QSGEDSK	1xPhospho [S2(99.7)]	S397	Q8NEZ4	830.2928	0.55
SEPKMSAHVK	1xPhospho [S1(94.6)]	S4711	Q8NEZ4	1193.538	0.47
KMKTEWK	1xPhospho [T4(100)]	S4767	Q8NEZ4	1030.479	0.46
DLTLLNKDSR	1xPhospho [T/S]	T4218;S4224	Q8NEZ4	1254.609	0.45

Fig. S3 TAOK3 phosphorylates KMT2C at S4588. A-B Exogenous CO-IP analysis of the interaction between TAOK3, KMT2C, and ETV5 in ECA109 cells. C CO-IP analysis of the interaction between KMT2C and ETV5 in ECA109 cells after L-PP was used, or TAOK3 was overexpressed. D CO-IP analysis of the expression of the pan-serine/threonine phosphorylated KMT2C in ECA109 cells when TAOK3 was knocked down. E MS analysis of the potential phospho-sites of TAOK3 to KMT2C. F Western blot analysis of the expression of P-KMT2C (S4588) and P-KMT2C (T4589) when TAOK3 was knocked down in KYSE150 cells. G Western blot analysis of the expression of P-KMT2C (S4588) and P-KMT2C (T4589) when TAOK3 was overexpressed in KYSE150 cells. H Western blot analysis of nuclear and cytosol fractionated proteins in KYSE150 cells.

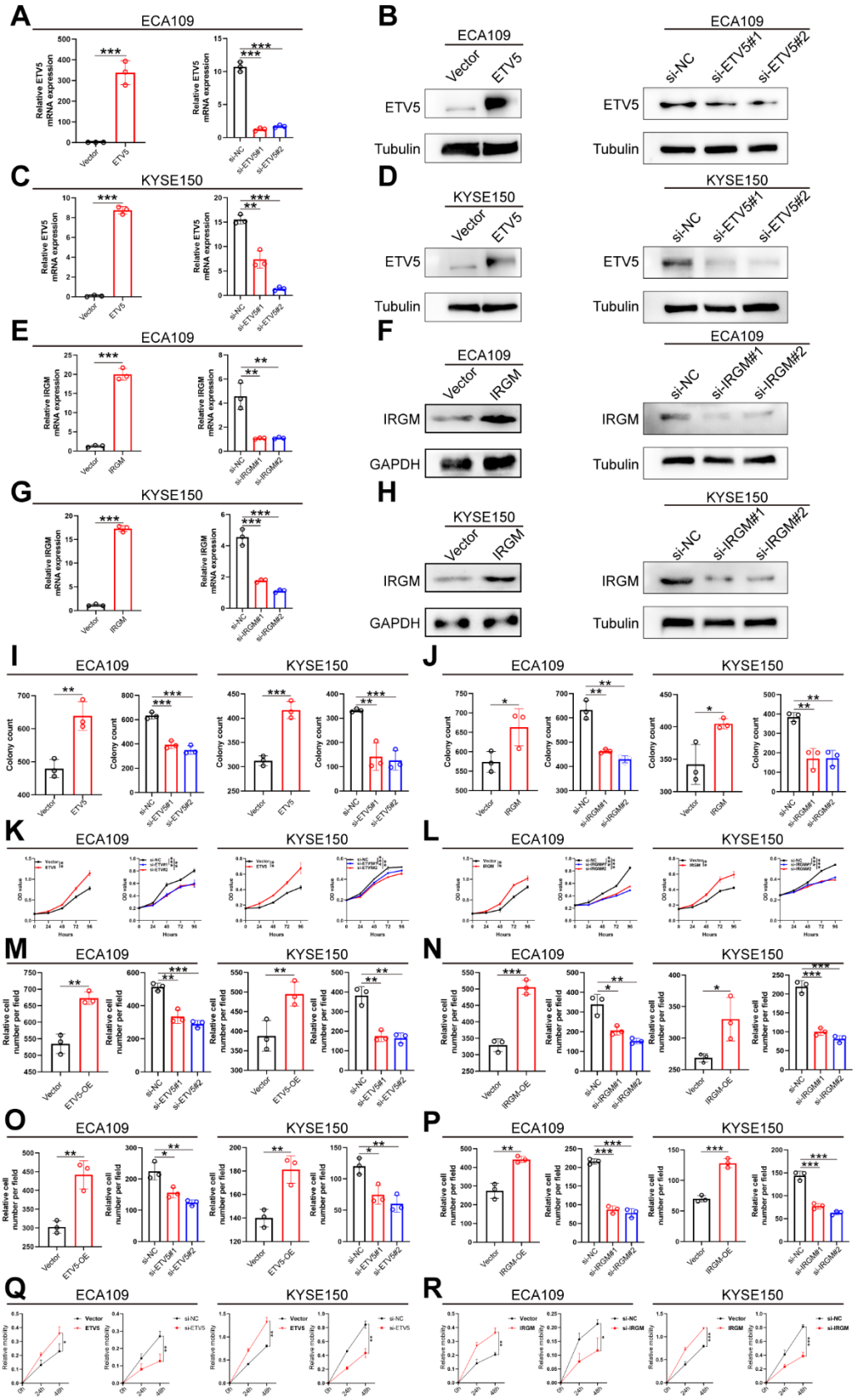

A
ECA109
Relative ETV5 mRNA expression
Vector
ETV5
si-NC
si-ETV5#1
si-ETV5#2
B
ECA109
Vector
ETV5
ETV5
Tubulin
si-NC
si-ETV5#1
si-ETV5#2
C
KYSE150
D
KYSE150
E
ECA109
Relative IRGM mRNA expression
IRGM
si-IRGM#1
si-IRGM#2
F
ECA109
IRGM
GAPDH
Tubulin
G
KYSE150
H
KYSE150
I
ECA109
KYSE150
Colony count
J
ECA109
KYSE150
K
ECA109
KYSE150
OD value
Hours
L
ECA109
KYSE150
M
ECA109
KYSE150
Relative cell number per field
ETV5-OE
N
ECA109
KYSE150
IRGM-OE
O
ECA109
KYSE150
P
ECA109
KYSE150
Q
ECA109
KYSE150
Relative mobility
R
ECA109
KYSE150

Fig. S4 ETV5 and IRGM promote proliferation, migration and invasion in vitro. A-H QPCR and western blot analysis of the knockdown and overexpression efficiency of ETV5 and IRGM. I-J Colony formation analysis of the proliferation of ESCC cells when ETV5 or IRGM was overexpressed or knocked down. K-L CCK8 analysis of the viability of ESCC cells when ETV5 or IRGM was overexpressed or knocked down. M-N Migration assay showed the alteration when ETV5 or IRGM was overexpressed or knocked down. O-P Invasion assay showed the alteration when ETV5 or IRGM was overexpressed or knocked down. Q-R Wound healing analysis of the migration and invasion of ESCC cells when ETV5 or IRGM was overexpressed or knocked down.

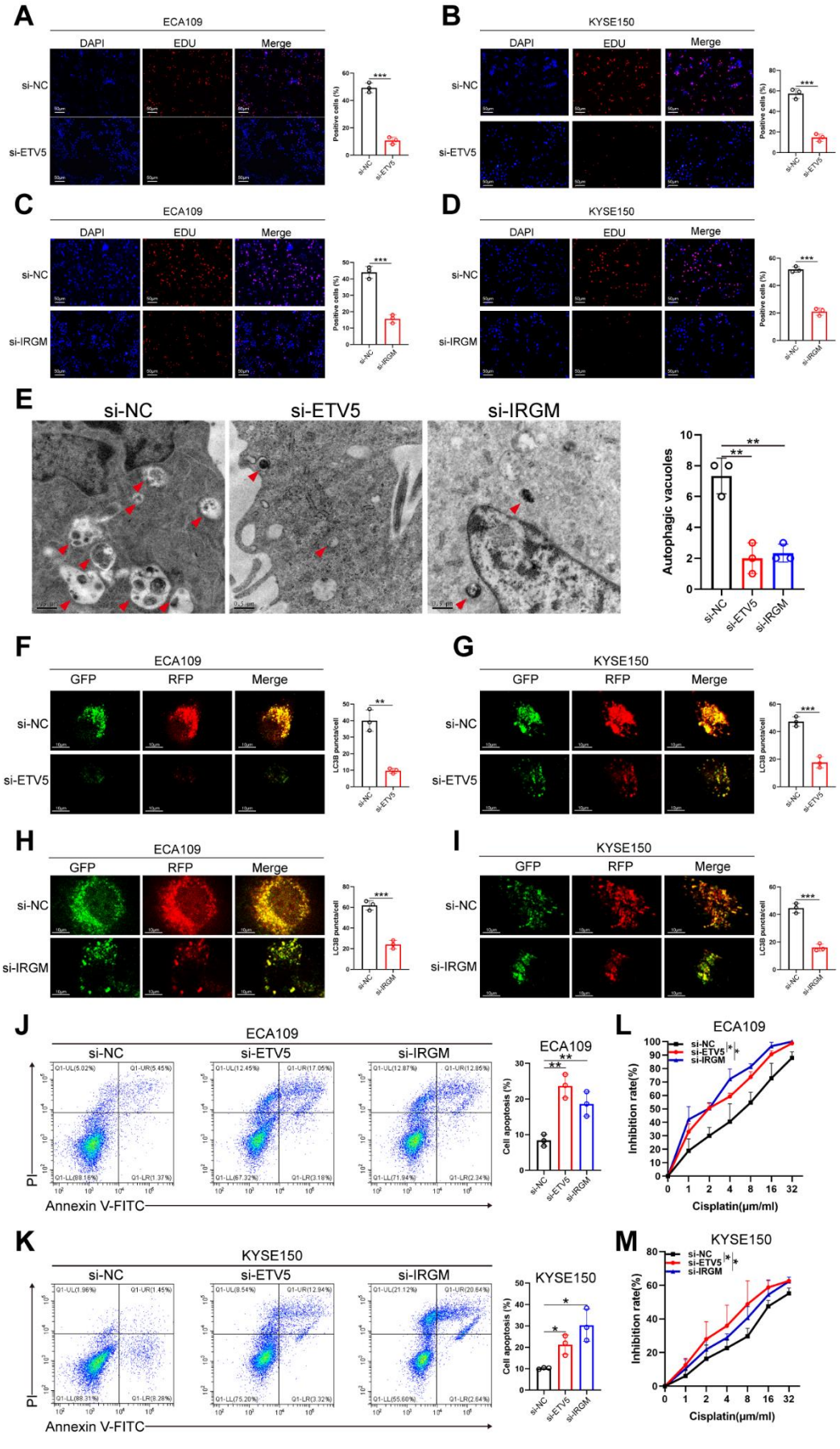

A
ECA109
DAPI
EDU
Merge
si-NC
si-ETV5
Positive cells (%)
B
KYSE150
C
si-IRGM
D
E
si-NC
si-ETV5
si-IRGM
Autophagic vacuoles
F
GFP
RFP
LC3B puncta/cell
G
H
I
J
Q1-UL(5.02%)
Q1-UR(5.45%)
Q1-LL(89.16%)
Q1-LR(1.37%)
Q1-UL(12.45%)
Q1-UR(17.05%)
Q1-LL(67.32%)
Q1-LR(3.18%)
Q1-UL(12.87%)
Q1-UR(12.86%)
Q1-LL(71.94%)
Q1-LR(2.34%)
PI
Annexin V-FITC
Cell apoptosis (%)
K
Q1-UL(1.96%)
Q1-UR(1.45%)
Q1-LL(96.31%)
Q1-LR(0.28%)
Q1-UL(8.54%)
Q1-UR(12.94%)
Q1-LL(75.20%)
Q1-LR(3.32%)
Q1-UL(21.12%)
Q1-UR(20.64%)
Q1-LL(55.60%)
Q1-LR(2.64%)
L
Inhibition rate(%)
Cisplatin(μm/ml)
M

Fig. S5 ETV5 and IRGM promote autophagy and cisplatin resistance but inhibit cell apoptosis in vitro. A-D Edu analysis of the proliferation of ESCC cells when ETV5 or IRGM was knocked down. E TEM analysis of Autophagic vesicle formation when ETV5 or IRGM was knocked down in ECA109 cells. F-I Confocal microscopy analysis of autophagy-lysosome formation when ETV5 or IRGM was knocked down after lentivirus vector carrying GFP-RFP-LC3 was transfected. J-K Annexin V-FITC/PI staining analysis of cell apoptosis level when ETV5 or IRGM was knocked down in ESCC cells. L-M CCK8 analysis of the inhibition rate of ESCC cells growth when ETV5 or IRGM was knocked down.

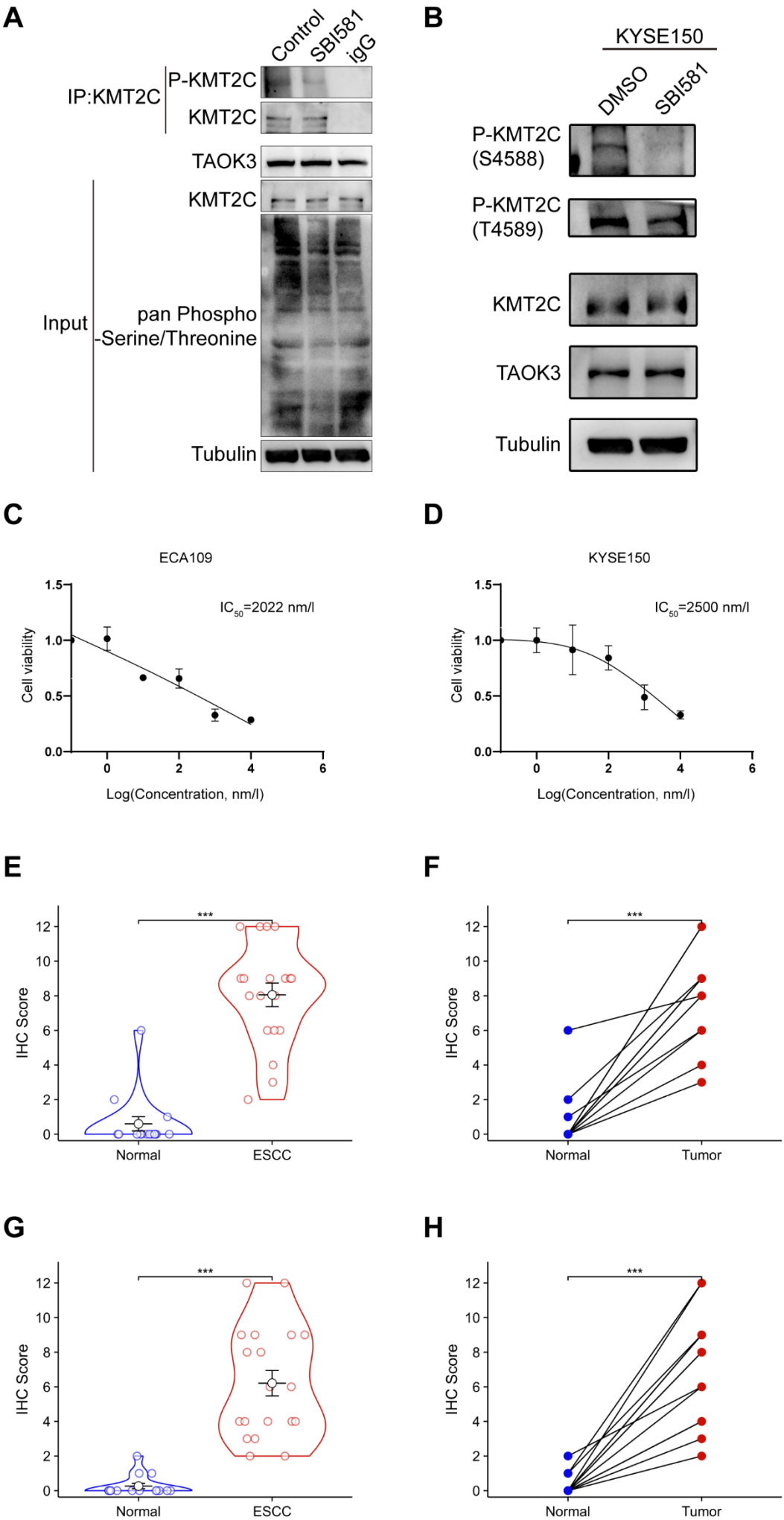

A
Control
SBI581
igG
IP:KMT2C
P-KMT2C
KMT2C
TAOK3
Input
KMT2C
pan Phospho
-Serine/Threonine
Tubulin
B
KYSE150
DMSO
SBI581
P-KMT2C
(S4588)
P-KMT2C
(T4589)
KMT2C
TAOK3
Tubulin
C
ECA109
IC50=2022 nm/l
Cell viability
Log(Concentration, nm/l)
D
KYSE150
IC50=2500 nm/l
Cell viability
Log(Concentration, nm/l)
E
IHC Score
***
Normal
ESCC
F
IHC Score
***
Normal
Tumor
G
IHC Score
***
Normal
ESCC
H
IHC Score
***
Normal
Tumor

Fig. S6 The inhibitory effect of SBI-581 to TAOK3 and the IC_{50} of SBI-581 affecting ESCC cells survival at 48h are confirmed, and both TAOK3 and IRGM are overexpressed in paired or non-paired ESCC tissues of the metastasis subgroup. A CO-IP analysis of the expression level of the pan-serine/threonine phosphorylated KMT2C when SBI-581 (100nm/L) was used. B Western blot analysis of the expression of P-KMT2C (S4588) and P-KMT2C (T4589) when SBI-581 was applied in KYSE150 cells. C-D CCK8 analysis of the IC_{50} of SBI-581in terms of affecting ECA109 and KYSE150 survival at 48h. E-F IHC analysis of the expression of protein TAOK3 in paired or non-paired ESCC tissues of metastasis subgroup. G-H IHC analysis of the expression of protein IRGM in paired or non-paired ESCC tissues of metastasis subgroup.

Tables

Supplementary table 1 Primers for qPCR assays

TAOK1	forward	5’-ATGTTGGCTGTCCTGGTGTATTGC-3’
	reverse	5’-AATGCTGTTAGGAAGAAGGGTGGTG-3’
TAOK2	forward	5’-CTGCCGTAACCGAGACCACTTTG-3’
	reverse	5’-GCTGTCGTCGCATCCGCTTATAG-3’
TAOK3	forward	5’- GCTTCTCCTGCCAACTCCTTCG -3’
	reverse	5’- CCGTTCCGCCAATTCAATACAAGTG -3’
ETV5	forward	5’-CAGCACACGGGTTCCAGTCAC-3’
	reverse	5’-TGGCAGTTAGGCACTTCTGAATCG-3’
IRGM	forward	5’- AATCTTGCTGCTGCTCATTCTTTGG -3’
	reverse	5’- GCGGCGAGTCTGGAGTTGTTC -3’
IRGM-site1(for CHIP)	forward	5’-TTTTCTGATAATGATCAAACAGA-3’
	reverse	5’-CATGTGAGGTTATATATAGTTAA-3’
IRGM-site2(for CHIP)	forward	5’- ATATGCTCACTTAGTAAATTTA -3’
	reverse	5’-AGATATATTGCCAGATACC-3’
IRGM-site3(for CHIP)	forward	5’- GAACCCACCGGAAGGAAGAAACTC-3’
	reverse	5’- TGCTTATCATTGCATTCGCCAAACC -3’

Supplementary table 2 The relationship between clinical factors and TAOK3 expression

Clinicopathological factors	TAOK3 expression		
	High	Low	P-value
Gender			
Male	44	21	0.806
Female	9	5	
Age(y)			
≤60	19	7	0.428
> 60	34	19	
Tumor size			
≤3cm	16	14	0.042
> 3cm	37	12	
Metastasis*			
Negtive	35	25	0.002
Positive	18	1	
TNM stage			
0-II	34	26	< 0.001
III-IV	19	0	

* Metastasis here includes lymph node metastasis and other organs metastasis.

Supplementary table 3 The relationship between clinical factors and IRGM expression

Clinicopathological factors	IRGM expression		
	High	Low	P-value
Gender			
Male	38	27	0.286
Female	6	8	
Age(y)			
≤60	18	8	0.090
> 60	26	27	
Tumor size			
≤3cm	14	16	0.206
> 3cm	30	19	
Metastasis*			
Negtive	27	33	0.001
Positive	17	2	
TNM stage			
0-II	25	35	< 0.001
III-IV	19	0	

* Metastasis here includes lymph node metastasis and other organs metastasis.