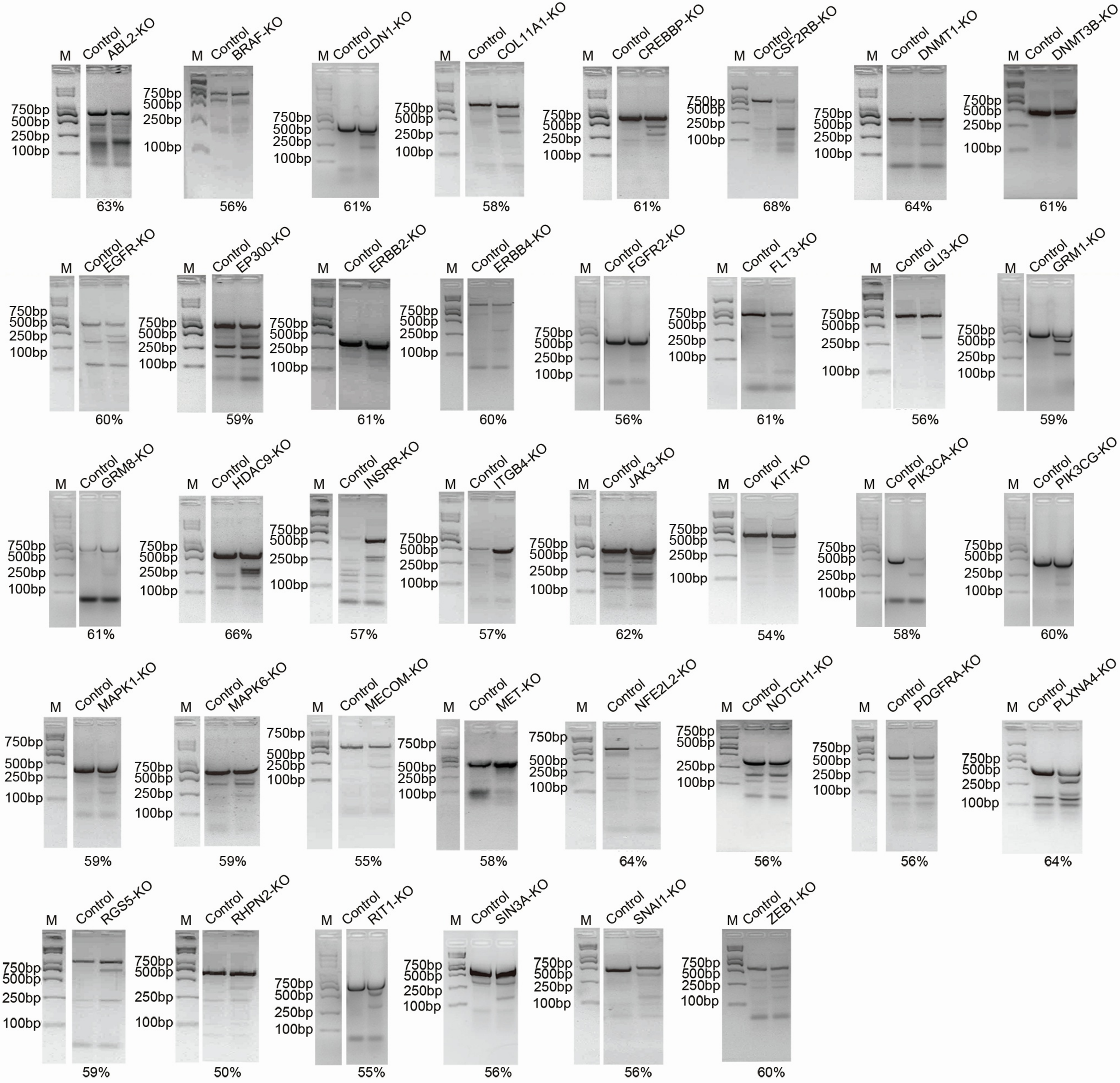
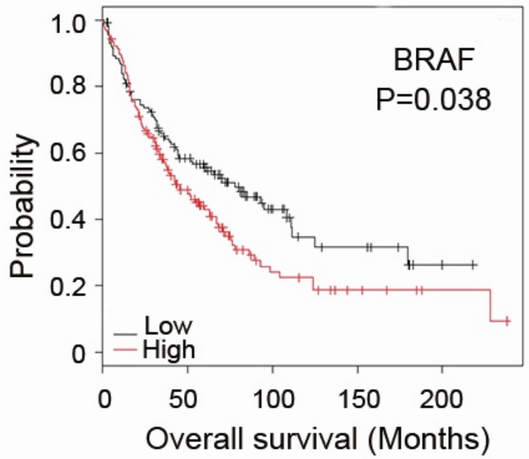
YMTHE, Volume *30*

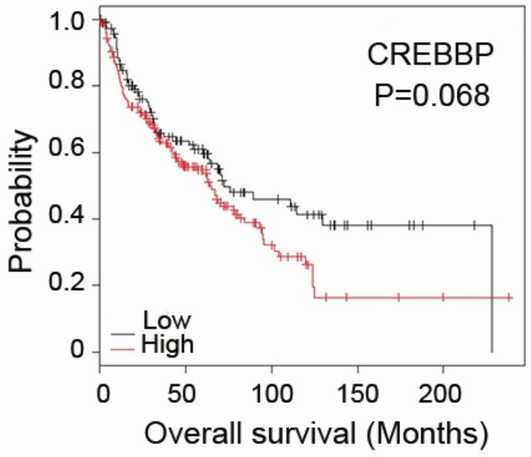
Supplemental Information

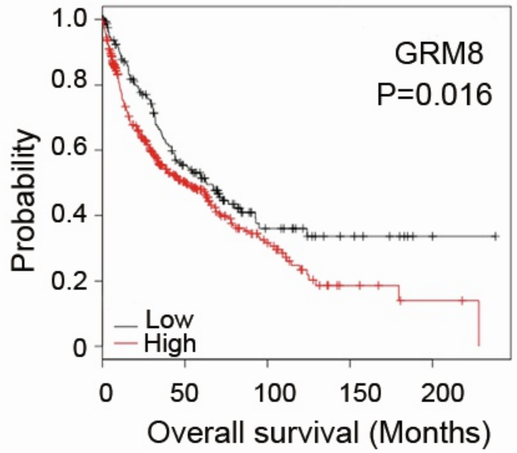
CRISPR-mediated *MECOM* depletion retards tumor growth by reducing cancer stem cell properties in lung squamous cell carcinoma

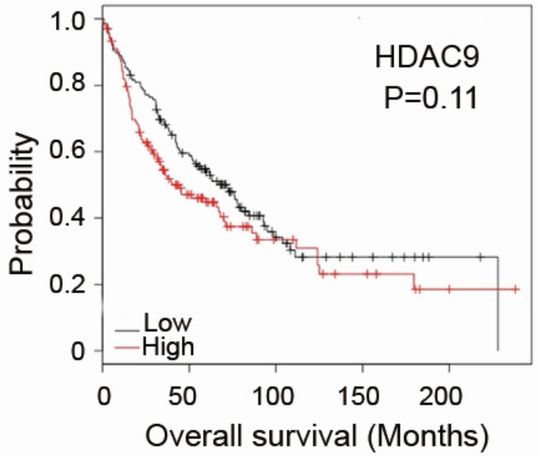
Yuanyuan Ma, Bin Kang, Shaolei Li, Guoyun Xie, Jiwang Bi, Fuqiang Li, Guo An, Bing Liu, Jing Li, Yue Shen, Xun Xu, Huanming Yang, Yue Yang, Ying Gu, and Nan Wu

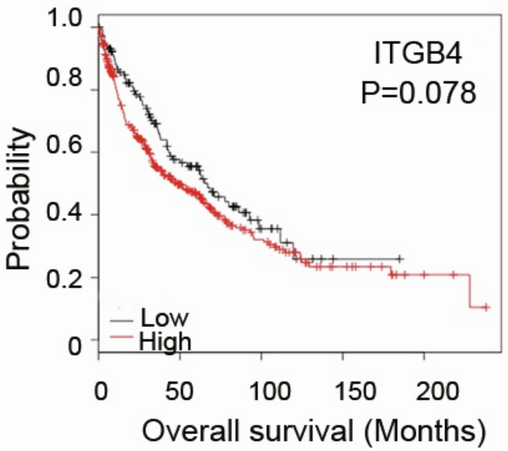

M
Control
ABL2-KO
750bp
500bp
250bp
100bp
63%
M
Control
BRAF-KO
750bp
500bp
250bp
100bp
56%
M
Control
CLDN1-KO
750bp
500bp
250bp
100bp
61%
M
Control
COL11A1-KO
750bp
500bp
250bp
100bp
58%
M
Control
CREBBP-KO
750bp
500bp
250bp
100bp
61%
M
Control
CSF2RB-KO
750bp
500bp
250bp
100bp
68%
M
Control
DNMT1-KO
750bp
500bp
250bp
100bp
64%
M
Control
DNMT3B-KO
750bp
500bp
250bp
100bp
61%
M
Control
EGFR-KO
750bp
500bp
250bp
100bp
60%
M
Control
EP300-KO
750bp
500bp
250bp
100bp
59%
M
Control
ERBB2-KO
750bp
500bp
250bp
100bp
61%
M
Control
ERBB4-KO
750bp
500bp
250bp
100bp
60%
M
Control
FGFR2-KO
750bp
500bp
250bp
100bp
56%
M
Control
FLT3-KO
750bp
500bp
250bp
100bp
61%
M
Control
GLI3-KO
750bp
500bp
250bp
100bp
56%
M
Control
GRM1-KO
750bp
500bp
250bp
100bp
59%
M
Control
GRM8-KO
750bp
500bp
250bp
100bp
61%
M
Control
HDAC9-KO
750bp
500bp
250bp
100bp
66%
M
Control
INSRR-KO
750bp
500bp
250bp
100bp
57%
M
Control
ITGB4-KO
750bp
500bp
250bp
100bp
57%
M
Control
JAK3-KO
750bp
500bp
250bp
100bp
62%
M
Control
KIT-KO
750bp
500bp
250bp
100bp
54%
M
Control
PIK3CA-KO
750bp
500bp
250bp
100bp
58%
M
Control
PIK3CG-KO
750bp
500bp
250bp
100bp
60%
M
Control
MAPK1-KO
750bp
500bp
250bp
100bp
59%
M
Control
MAPK6-KO
750bp
500bp
250bp
100bp
59%
M
Control
MECOM-KO
750bp
500bp
250bp
100bp
55%
M
Control
MET-KO
750bp
500bp
250bp
100bp
58%
M
Control
NFE2L2-KO
750bp
500bp
250bp
100bp
64%
M
Control
NOTCH1-KO
750bp
500bp
250bp
100bp
56%
M
Control
PDGFRA-KO
750bp
500bp
250bp
100bp
56%
M
Control
PLXNA4-KO
750bp
500bp
250bp
100bp
64%
M
Control
RGS5-KO
750bp
500bp
250bp
100bp
59%
M
Control
RHPN2-KO
750bp
500bp
250bp
100bp
50%
M
Control
RIT1-KO
750bp
500bp
250bp
100bp
55%
M
Control
SIN3A-KO
750bp
500bp
250bp
100bp
56%
M
Control
SNAI1-KO
750bp
500bp
250bp
100bp
56%
M
Control
ZEB1-KO
750bp
500bp
250bp
100bp
60%

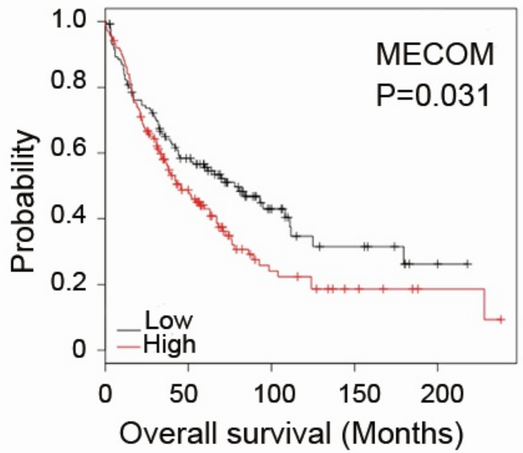

BRAF
P=0.038
Probability
1.0
0.8
0.6
0.4
0.2
0
Low
High
0
50
100
150
200
Overall survival (Months)

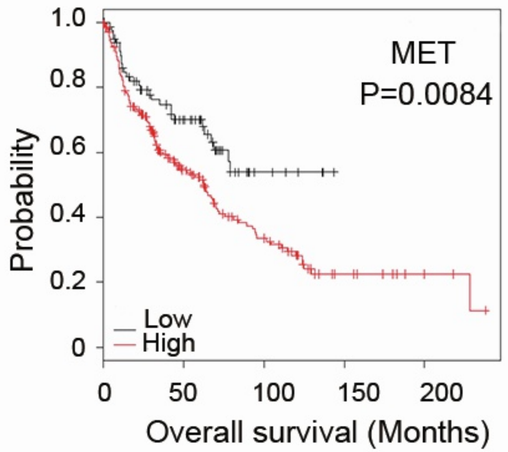

CREBBP
P=0.068
Probability
1.0
0.8
0.6
0.4
0.2
0
Low
High
0
50
100
150
200
Overall survival (Months)

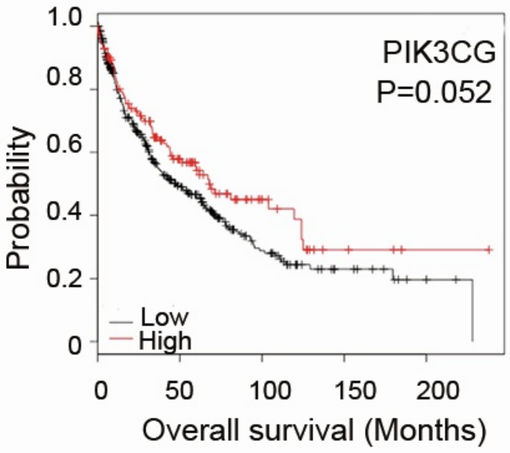

GRM8
P=0.016
Probability
1.0
0.8
0.6
0.4
0.2
0
Low
High
0
50
100
150
200
Overall survival (Months)

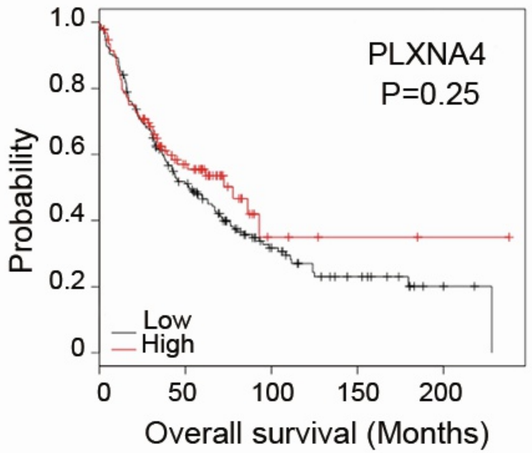

HDAC9
P=0.11
Probability
1.0
0.8
0.6
0.4
0.2
0
Low
High
0
50
100
150
200
Overall survival (Months)

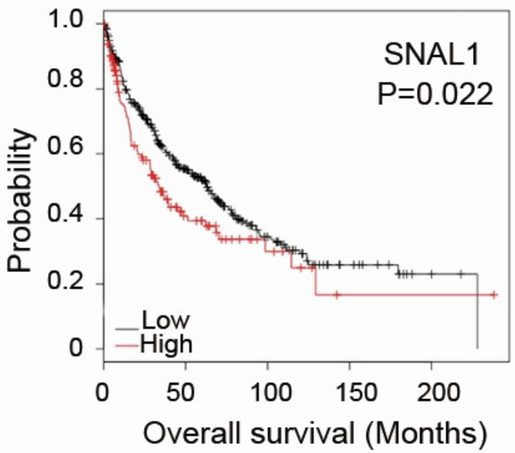

ITGB4
P=0.078
Probability
1.0
0.8
0.6
0.4
0.2
0
Low
High
0
50
100
150
200
Overall survival (Months)


MECOM
P=0.031
Probability
1.0
0.8
0.6
0.4
0.2
0
Low
High
0
50
100
150
200
Overall survival (Months)

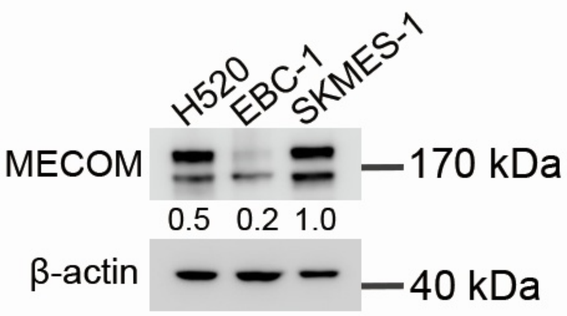

MET
P=0.0084
Probability
1.0
0.8
0.6
0.4
0.2
0
Low
High
0
50
100
150
200
Overall survival (Months)


PIK3CG
P=0.052
Probability
1.0
0.8
0.6
0.4
0.2
0
Low
High
0
50
100
150
200
Overall survival (Months)

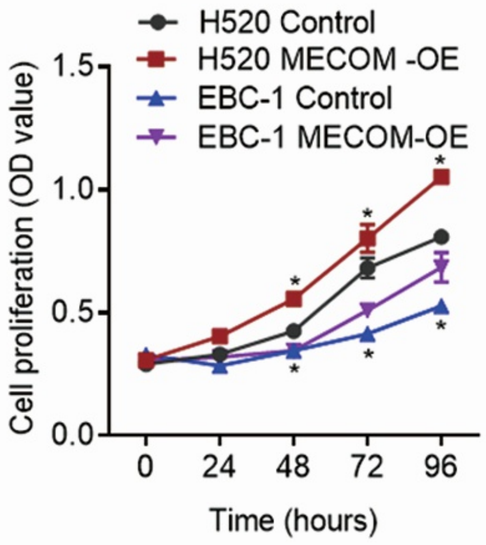

PLXNA4
P=0.25
Probability
1.0
0.8
0.6
0.4
0.2
0
Low
High
0
50
100
150
200
Overall survival (Months)


SNAL1
P=0.022
Probability
1.0
0.8
0.6
0.4
0.2
0
Low
High
0
50
100
150
200
Overall survival (Months)

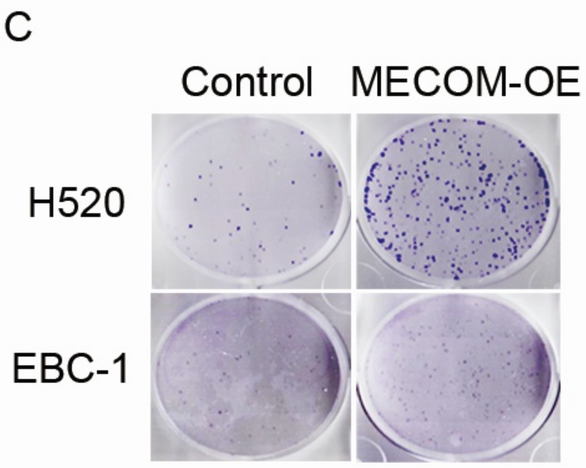
A

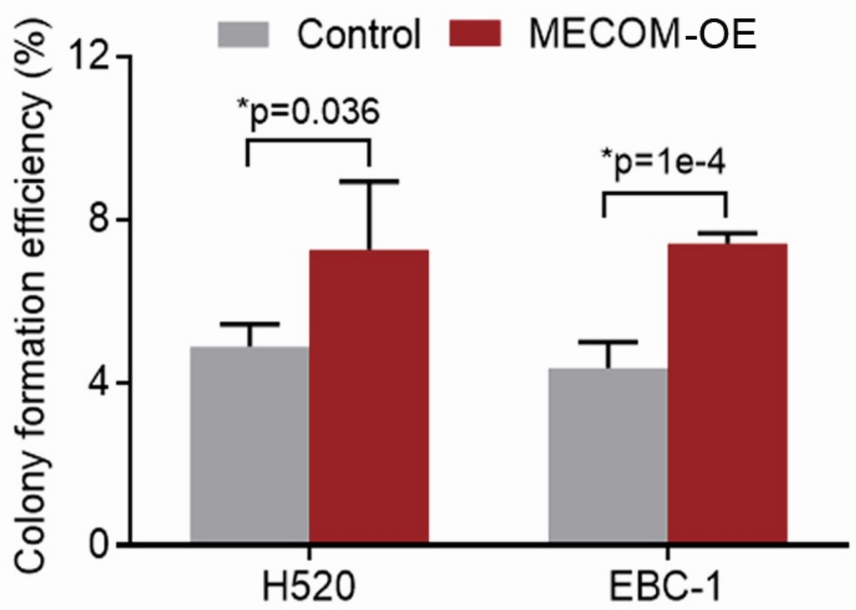

H520
EBC-1
SKMES-1
MECOM
170 kDa
0.5 0.2 1.0
β-actin
40 kDa

B

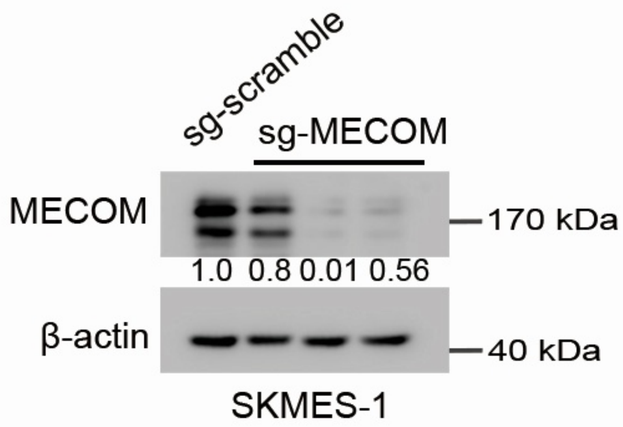

H520 Control
H520 MECOM -OE
EBC-1 Control
EBC-1 MECOM-OE
Cell proliferation (OD value)
1.5
1.0
0.5
0.0
0 24 48 72 96
Time (hours)

C

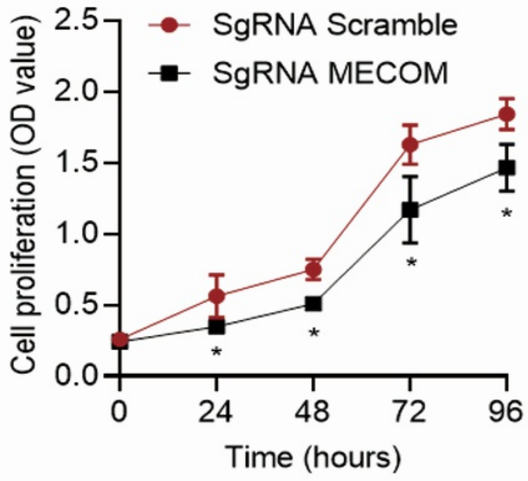

Control
MECOM-OE
H520
EBC-1


Control
MECOM-OE
Colony formation efficiency (%)
12
8
4
0
*p=0.036
*p=1e-4
H520
EBC-1

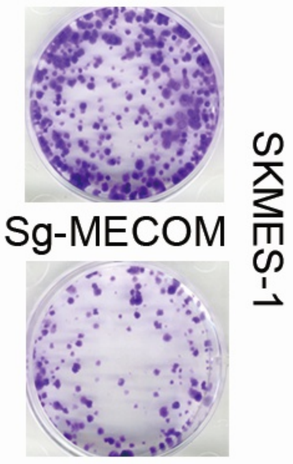
D

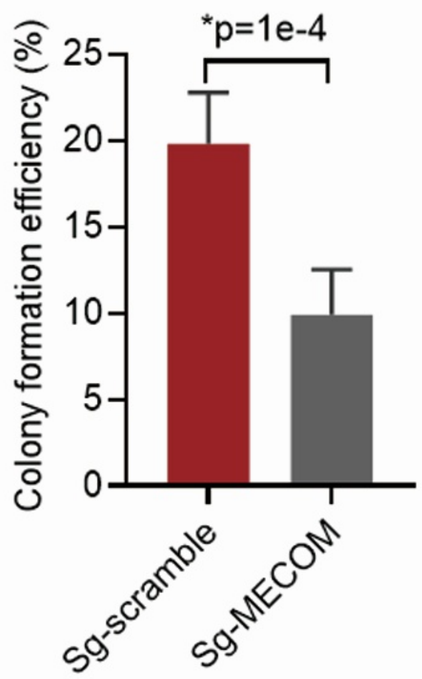

sg-scramble
sg-MECOM
MECOM
170 kDa
1.0 0.8 0.01 0.56
β-actin
40 kDa
SKMES-1

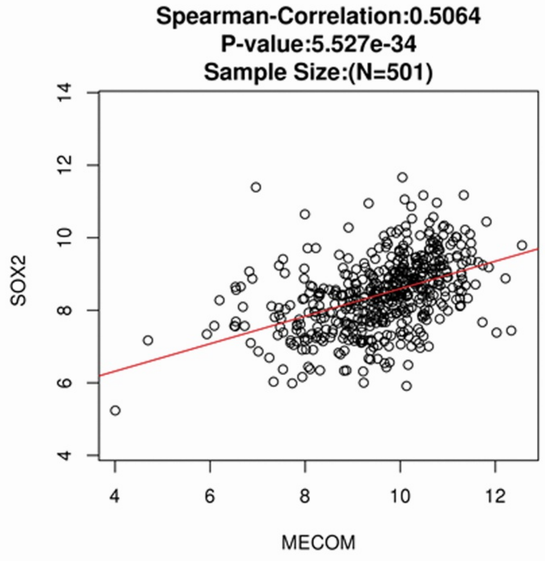
E

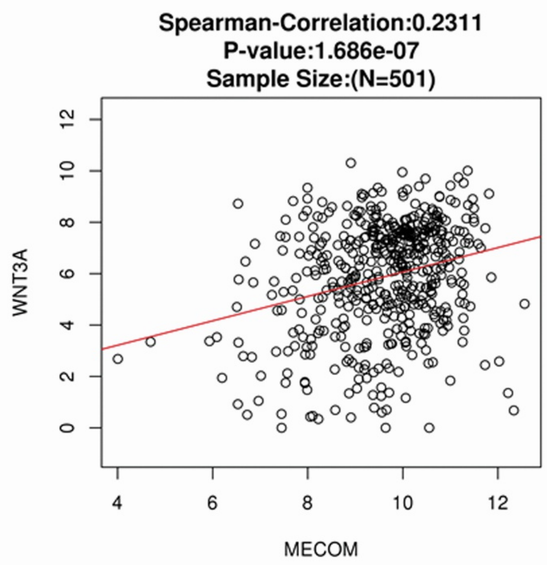

SgRNA Scramble
SgRNA MECOM
Cell proliferation (OD value)
2.5
2.0
1.5
1.0
0.5
0.0
0 24 48 72 96
Time (hours)

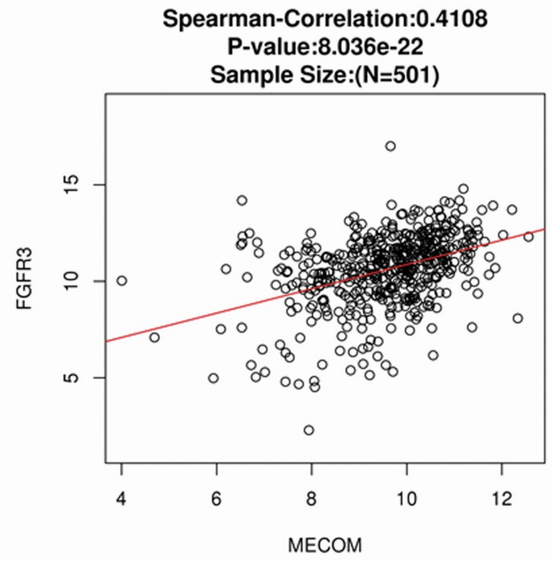
F

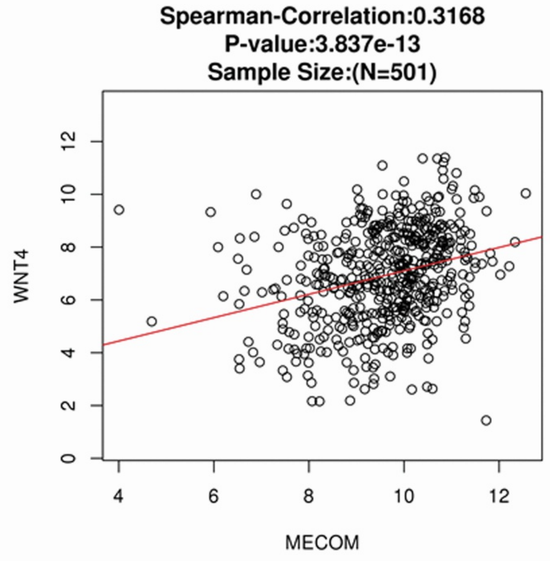

Sg-scramble
Sg-MECOM
SKMES-1

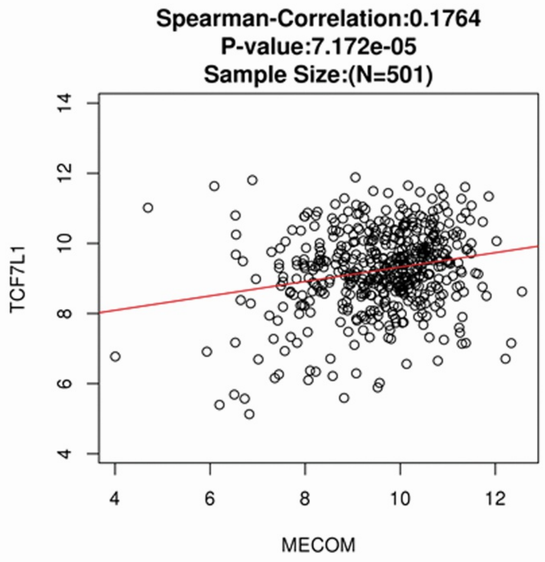

*p=1e-4
Colony formation efficiency (%)
25
20
15
10
5
0
Sg-scramble
Sg-MECOM

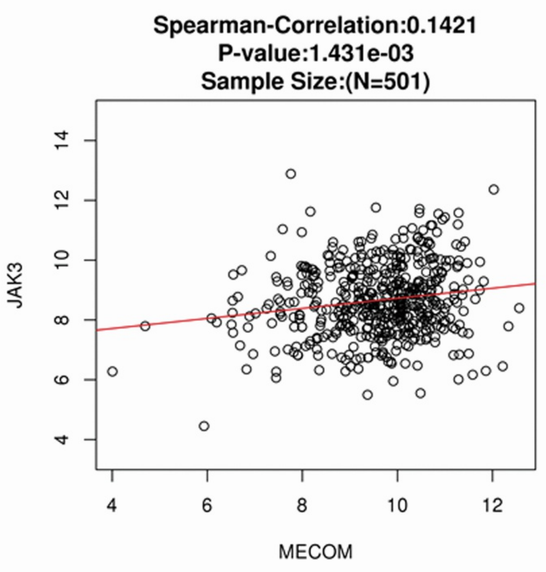

Spearman-Correlation:0.5064
P-value:5.527e-34
Sample Size:(N=501)
SOX2
MECOM

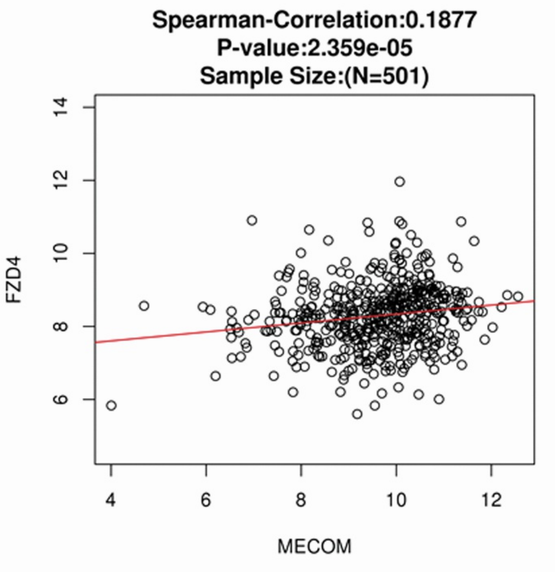

Spearman-Correlation:0.2311
P-value:1.686e-07
Sample Size:(N=501)
WNT3A
MECOM

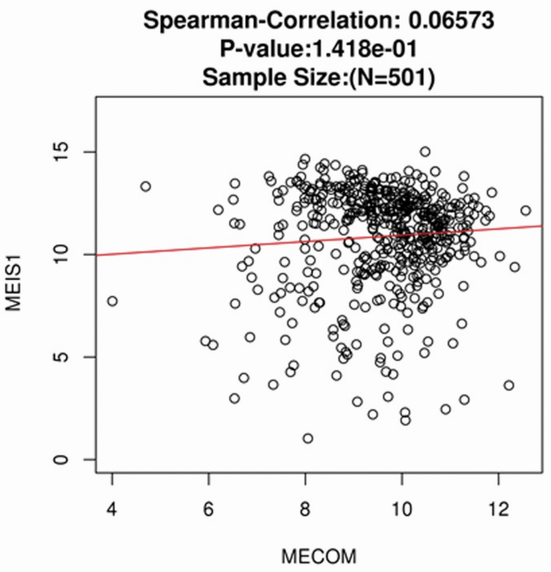

Spearman-Correlation:0.4108
P-value:8.036e-22
Sample Size:(N=501)
FGFR3
MECOM

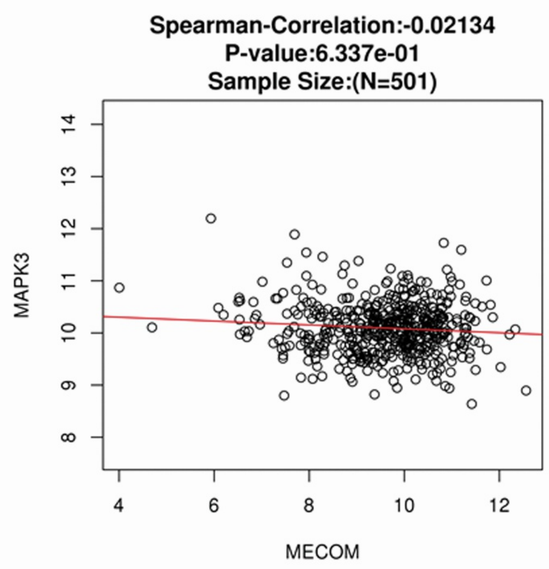

Spearman-Correlation:0.3168
P-value:3.837e-13
Sample Size:(N=501)
WNT4
MECOM

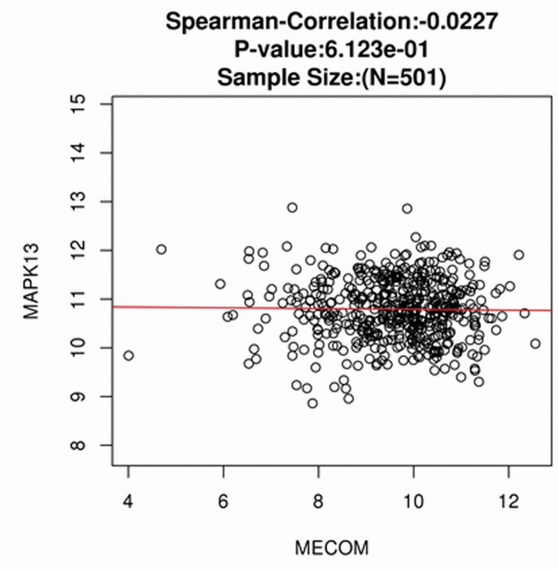

Spearman-Correlation:0.1764
P-value:7.172e-05
Sample Size:(N=501)
TCF7L1
MECOM

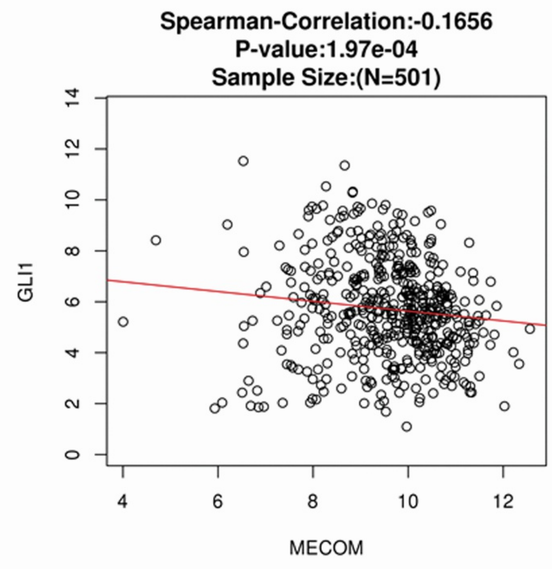

Spearman-Correlation:0.1421
P-value:1.431e-03
Sample Size:(N=501)
JAK3
MECOM

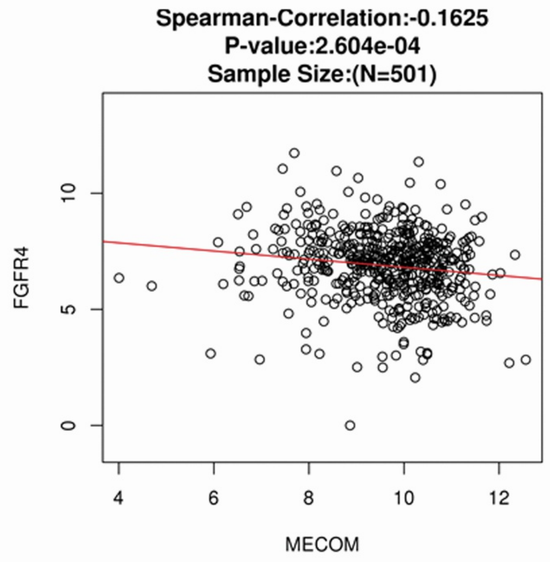

Spearman-Correlation:0.1877
P-value:2.359e-05
Sample Size:(N=501)
FZD4
MECOM

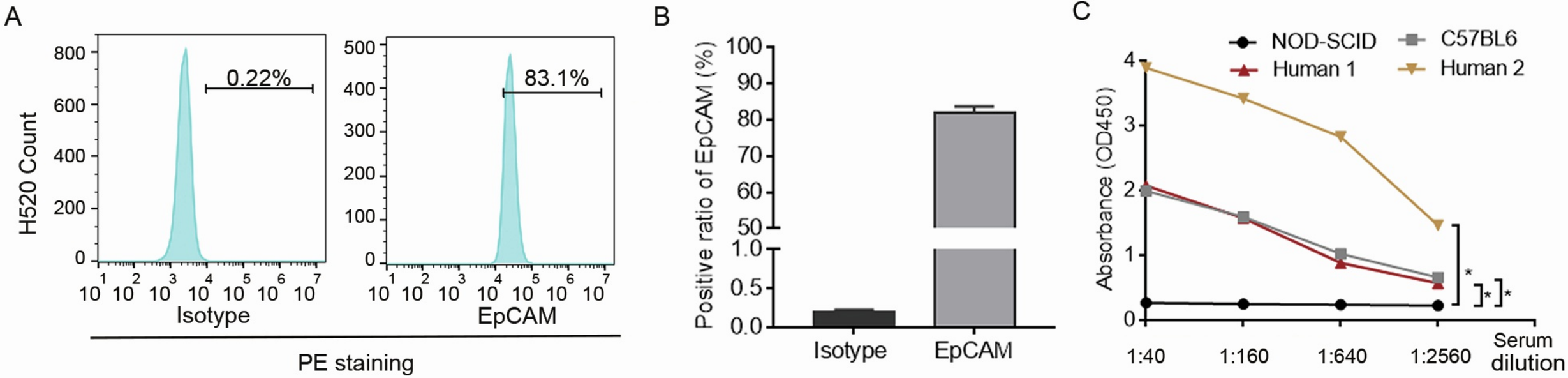

Spearman-Correlation: 0.06573
P-value:1.418e-01
Sample Size:(N=501)
MEIS1
MECOM

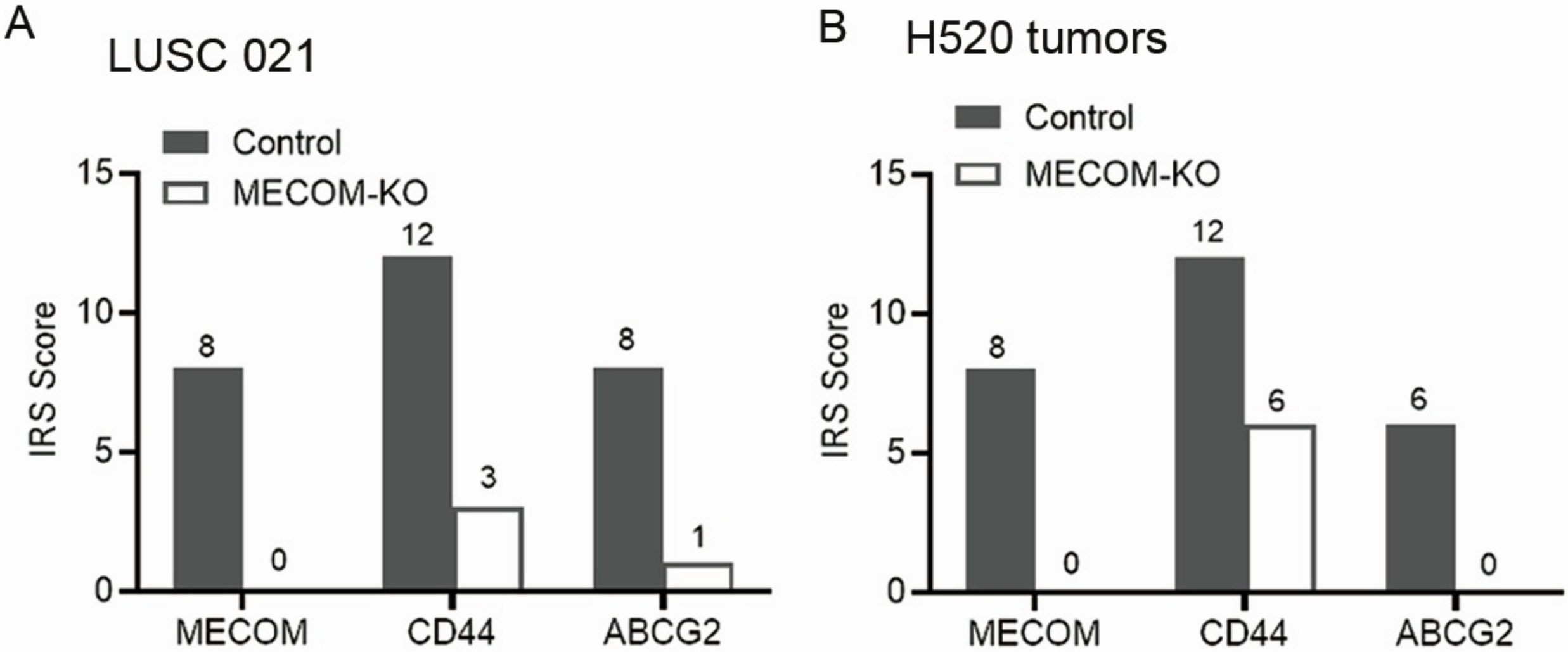

Spearman-Correlation:-0.02134
P-value:6.337e-01
Sample Size:(N=501)
MAPK3
MECOM


Spearman-Correlation:-0.0227
P-value:6.123e-01
Sample Size:(N=501)
MAPK13
MECOM


Spearman-Correlation:-0.1656
P-value:1.97e-04
Sample Size:(N=501)
GLI1
MECOM


Spearman-Correlation:-0.1625
P-value:2.604e-04
Sample Size:(N=501)
FGFR4
MECOM


A
H520 Count
0.22%
83.1%
Isotype
EpCAM
PE staining
B
Positive ratio of EpCAM (%)
Isotype
EpCAM
C
NOD-SCID
C57BL6
Human 1
Human 2
Absorbance (OD450)
1:40
1:160
1:640
1:2560
Serum dilution


A
LUSC 021
Control
MECOM-KO
IRS Score
15
10
5
0
8
0
12
3
8
1
MECOM
CD44
ABCG2
B
H520 tumors
Control
MECOM-KO
IRS Score
15
10
5
0
8
0
12
6
6
0
MECOM
CD44
ABCG2

SUPPLEMENTAL FIGURE LEGEND

Figure S1. T7E1 analyses. A specific sgRNA for 38 drive genes was inserted in the vector of pLEX_305-CMV::SaCas9-2A-GFP;U6::BsaI-sgRNA which was further performed for lentivirus packaging. The lentivirus was transfected into the HEK293FT cells for 48 h. Then, the images of T7E1 assay in these HEK293FT cells were shown. The editing efficiencies were listed below the images.

Figure S2. Kaplan-Meier survival curves of 10 candidate genes in LUSC. The survival curves were analyzed in 524 LUSC cases using Kaplan-Meier plotter (https://kmplot.com).

Figure S3. MECOM promotes proliferation and colony formation.

(A) Western blots show MECOM and β-actin expression in H520, EBC-1 and SKMES-1 cell lines. The numerical value under the band shows densitometric analyses of MECOM, compared to this protein expressed in SKMES-1, which was normalized as "1.0". (B) Proliferation curves of H520 and EBC-1 cells with MECOM overexpression (MECOM-OE). (C) Representative images (left panel) and efficiencies (right panel) of colony formation in H520 and EBC-1 cells with MECOM overexpression (MECOM-OE). (D) Western blots show MECOM and β-actin expression in several clones of SKMES-1 cells transfected with sg-MECOM. The numerical value under the band shows densitometric analyses of MECOM, compared to this protein expressed in the cells with sg-scramble, which was normalized as "1.0". (E) Proliferation curves of SKMES-1 cells with MECOM depletion (sg-MECOM). (F) Representative images (left

panel) and efficiencies (right panel) of colony formation in SKMES-1 cells with MECOM depletion (sg-MECOM). *p＜0.05, unpaired two-tailed t-test.

Figure S4. Correlation analyses in the tumor tissues of 501 cases of LUSC patients derived from public database of LinkedOmics.

(http://www.linkedomics.org/login.php**).**

Figure S5. EpCAM level and anti-ADV antibodies analysis. (**A-B**).

The expression of EpCAM in H520 cells was analyzed by flow cytometry. Anti-human EpCAM labelled with phycoerythrin (Mitenyi Biotec, 130-113-826) was used. The positive ratio of this protein was respectively indicated by presentative images (A) and graph (B). (C) Abundance of anti-ADV antibodies in serum of NOD-SCID mice, C57BL/6N mice and two lung cancer patients by ELISA analyses. * p＜0.05, unpaired two-tailed t-test.

Figure S6. IRS score for immunohistochemistry.

(A) Histogram shows IRS scores of MECOM, ABCG2 and CD44 expression in the ADV/protein treated tumors (LUSC 021) with MECOM depletion (MECOM-KO). (B) Histogram demonstrates IRS scores of MECOM, ABCG2 and CD44 expression in the ADV/protein treated tumors derived from H520 orthotopic xenograft with MECOM depletion (MECOM-KO).

Table S1 SNVs and CNVs of 38 genes in the primary and corresponding PDX tumors of LUSC

Sample		Primary tumor	PDX tumor	Common SNV/CNV in primary and PDX tumors	Ratio of consistency (%)*
LUSC012	SNV	18	27	18	100
LUSC019	SNV	11	105	11	100
LUSC021	SNV	5	14	5	100
LUSC012	CNV	22	38	14	63.6
LUSC019	CNV	36	38	36	100
LUSC021	CNV	31	38	31	100

* The ratio of consistency was calculated through dividing the common SNV/CNV number in both the primary and PDX tumors by the SNV/CNV number in the primary tumors.

Table S2 SNVs in both primary and corresponding PDX tumors of LUSC

Sample ID	Entrez Gene ID	Gene Name	Gene description	Nucleotide	Amino acid	Exonic Funct
LUSC-012	27	ABL2	ABL proto-oncogene 2, non-receptor tyrosine kinase	c.889G>T	p.G297C	Missense
LUSC-012	1301	COL11A1	collagen, type XI, alpha 1	c.4715G>T	p.G1572V	Missense
LUSC-012	1301	COL11A1	collagen, type XI, alpha 1	c.1479G>T	p.G493G	Silent
LUSC-012	1387	CREBBP	CREB binding protein	c.6903G>T	p.M2301I	Missense
LUSC-012	1786	DNMT1	DNA (cytosine-5-)-methyltransferase 1	c.3152A>G	p.Y1051C	Missense
LUSC-012	1786	DNMT1	DNA (cytosine-5-)-methyltransferase 1	c.1770C>T	p.P590P	Silent
LUSC-012	2033	EP300	E1A binding protein p300	c.221C>T	p.A74V	Missense
LUSC-012	2033	EP300	E1A binding protein p300	c.754C>T	p.P252S	Missense
LUSC-012	2911	GRM1	glutamate receptor, metabotropic 1	c.1091G>T	p.R364M	Missense
LUSC-012	9734	HDAC9	histone deacetylase 9	c.2048G>T	p.R683L	Missense
LUSC-012	3645	INSRR	insulin receptor-related receptor	c.2111C>A	p.A704E	Missense
LUSC-012	3718	JAK3	Janus kinase 3	c.1639G>C	p.E547Q	Missense
LUSC-012	5594	MAPK1	mitogen-activated protein kinase 1	c.404G>A	p.R135K	Missense
LUSC-012	5597	MAPK6	mitogen-activated protein kinase 6	c.1426C>G	p.L476V	Missense
LUSC-012	4851	NOTCH1	notch 1	c.6142dupC	p.L2048fs	Frame shift
LUSC-012	5294	PIK3CG	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma	c.1328G>T	p.S443I	Missense
LUSC-012	25942	SIN3A	SIN3 transcription regulator family member A	c.3215A>T	p.Q1072L	Missense

LUSC-012	6935	ZEB1	zinc finger E-box binding homeobox 1	c.601C>T	p.R201C	Missense
LUSC-019	9076	CLDN1	claudin 1	c.372G>T	p.A124A	Silent
LUSC-019	1387	CREBBP	CREB binding protein	c.4336C>T	p.R1446C	Missense
LUSC-019	2033	EP300	E1A binding protein p300	c.7086G>C	p.G2362G	Silent
LUSC-019	2263	FGFR2	fibroblast growth factor receptor 2	c.1153G>T	p.G385W	Missense
LUSC-019	2911	GRM1	glutamate receptor, metabotropic 1	c.442C>A	p.P148T	Missense
LUSC-019	2918	GRM8	glutamate receptor, metabotropic 8	c.335C>G	p.A112G	Missense
LUSC-019	4780	NFE2L2	nuclear factor, erythroid 2-like 2	c.235G>C	p.E79Q	Missense
LUSC-019	4851	NOTCH 1	notch 1	c.1290C>G	p.I430M	Missense
LUSC-019	91584	PLXNA4	plexin A4	c.3900C>A	p.I1300I	Silent
LUSC-019	91584	PLXNA4	plexin A4	c.2636G>A	p.R879Q	Missense
LUSC-019	6615	SNAI1	snail family zinc finger 1	c.521G>A	p.R174Q	Missense
LUSC-021	2263	FGFR2	fibroblast growth factor receptor 2	c.1995G>T	p.R665R	Silent
LUSC-021	3815	KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	c.2055A>T	p.K685N	Missense
LUSC-021	5156	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	c.3070G>T	p.D1024Y	Missense
LUSC-021	5294	PIK3CG	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma	c.159C>A	p.S53R	Missense
LUSC-021	6935	ZEB1	zinc finger E-box binding homeobox 1	c.1079C>T	p.T360I	Missense

Table S3 CNVs in both primary and corresponding PDX tumors of LUSC

Sample	LUSC 012	LUSC 019	LUSC 021
ABL2	1.32192809488736	1.321928095	0.584962501
BRAF	0.584962501	1	0.584962501
CLDN1	1	2.169925001	1.807354922
CREBBP		1	0.584962501
CSF2RB		1.321928095	
DNMT1		1	0.584962501
DNMT3B		1	0.584962501
EGFR		1	0.584962501
EP300		1.321928095	
ERBB2		1	1
ERBB4		0.584962501	
FGFR2		0.584962501	0.584962501
FLT3		0.584962501	
GLI3		1	0.584962501
GRM1			0.584962501
GRM8	0.584962501	1	0.584962501
HDAC9		1	0.584962501
INSRR	1.321928095	1.321928095	0.584962501
ITGB4	0.584962501	1	1
JAK3		1	0.584962501
KIT		1	0.584962501
MAPK1		1.321928095	
MAPK6		1	1
MECOM	1	2.169925001	1.807354922
MET	0.584962501	1	0.584962501
NFE2L2		0.584962501	
NOTCH1		0.584962501	0.584962501
PDGFRA		1	0.584962501
PIK3CA	1	2.169925001	1.807354922
PIK3CG	0.584962501	0.584962501	0.584962501
PLXNA4	0.584962501	1	0.584962501
RGS5	1.321928095	1.321928095	1.807354922
RHPN2		1	1.807354922
RIT1	1.321928095	1.321928095	0.584962501
SIN3A			1
SNAI1	0.584962501	1	0.584962501
ZEB1		0.584962501	0.584962501

The copy number log-ratio of total sequence read count in the tumor to that in the normal, which is provided by the software facets.

Table S4 CNVs and SNVs frequencies of 10 genes in TCGA database

Gene name	501 cases of LUSC in TCGA database*	
	CNV (Amplification, %)	SNV (%)
PLXNA4	41.72	10.67
PIK3CG	46.91	8.99
CREBBP	16.17	8.43
HDAC9	51.50	7.87
GRM8	42.51	7.30
MECOM	89.82	5.06
ITGB4	47.50	4.49
BRAF	42.71	4.49
MET	44.51	2.25
SNAI1	52.89	2.25

*LUSC indicates the CNV/SNV of 501 cases of squamous cell lung cancer in TCGA database.

Table S5 MECOM amplification in the primary and corresponding PDX tumors of LUSC

Samples	MECOM CNV (amplification)
LUSC 006	0.584962501
LUSC 012	1
LUSC 018	1.321928095
LUSC 019	2.169925001
LUSC 021	1.807354922

The copy number log-ratio of total sequence read count in the tumor to that in the normal, which is provided by the software facets.

Table S6 Univariate and multivariate cox regression analyses for disease-free survival in LUSC with MECOM staining (n=150)

Characteristic	Frequency (%)	Univariable analysis		Multivariable analysis	
		HR (95% CI)	P value	HR (95% CI)	P value
Age group, y					
<60	17/63 (27.0)	1	0.077	1	0.022
⩾60	34/87 (39.1)	1.729 (0.941-3.176)		2.060 (1.111-3.819)	
Sex					
Female	4/11 (36.4)	1	0.812	-	-
Male	47/139 (33.8)	0.883 (0.318-2.457)		-	
Smoking history					
No	5/11 (45.5)	1	0.874	-	-
Yes	46/139 (33.1)	0.920 (0.331-2.560)		-	
Lymphovascular invasion					
No	42/132 (31.8)	1	0.126	-	-
Yes	9/18 (50.0)	1.760 (0.854-3.629)		-	
Differentiation					
Well	23/80 (28.8)	1	0.112	-	-
Poorly	28/70 (40.0)	1.599 (0.896-2.852)		-	
TNM Stage					
I	18/83 (21.7)	1	0.001	1	<0.001
II and III	33/67 (49.3)	2.696 (1.507-4.823)		3.259 (1.803-5.890)	
MECOM					
Low expression	9/43 (20.9)	1	0.022	1	0.031
High expression	42/107 (39.3)	2.466 (1.108-5.489)		2.632 (1.179-5.873)	

Table S7 Univariate and multivariate cox regression analyses for overall survival in LUSC with MECOM staining (n=150)

Characteristic	Frequency (%)	Univariable analysis		Multivariable analysis	
		HR (95% CI)	P value	HR (95% CI)	P value
Age group, y					
<60	9/63 (14.3)	1	0.036	1	0.010
≥60	25/87 (28.7)	2.260 (1.055-4.843)		2.829 (1.289-6.208)	
Sex					
Female	1/11 (9.1)	1	0.302	-	-
Male	33/139 (23.7)	2.851 (0.390-20.845)		-	
Smoking history					
No	3/11 (27.3)	1	0.776	-	-
Yes	31/139 (22.3)	0.842 (0.257-2.755)		-	
Lymphovascular invasion					
No	25/132 (18.9)	1	0.003	1	0.051
Yes	9/18 (50.0)	3.135 (1.462-6.724)		2.288 (0.995-5.264)	
Differentiation					
Well	12/80 (15.0)	1	0.009	1	0.010
Poorly	22/70 (31.4)	2.698 (1.277-5.703)		2.807 (1.282-6.146)	
TNM Stage					
I	13/83 (15.7)	1	0.027	1	0.018
II, III	21/67 (31.3)	2.183 (1.093-4.361)		2.483 (1.171-5.268)	
MECOM					
Low expression	4/43 (9.3)	1	0.024	1	0.046
High expression	30/107 (28.0)	3.113 (1.096-8.836)		2.931 (1.021-8.412)	

Table S8. Primers of T7E1-PCR, qRT-PCR, and ChIP

	Name	Primer (5'→3')
T7E1-PCR	ABL2 Forward	ACTTAACTCTGCCTATAACACA
	ABL2 Reverse	GAGTCTCGCTCTATTGCC
	BRAF Forward	CTCCCCATTTAATTTACAGA
	BRAF Reverse	AGTTTGCCTTATCTAACCC
	CLDN1 Forward	CTGTATATTGGGTTACCAGC
	CLDN1 Reverse	ACTTCCTCCTTAGCGTTT
	COL11A1 Forward	CTGCCTTTCACTTTAACTCA
	COL11A1 Reverse	CAAAGGGACTATAATGCGAT
	CREBBP Forward	CCCACATTGATGCCGTTT
	CREBBP Reverse	TTTAAGACATGCCTATGAGT
	CSF2RB Forward	ACCCCGGCAGACATGAACACA
	CSF2RB Reverse	CCCCTCCCAGACACGTCCACA
	DNMT1 Forward	TACATTCTCTCATTGCCTC
	DNMT1 Reverse	CATCACAATGACTTGGCCTA
	DNMT3B Forward	TCTTGCTTCTAGGTCCGAAC
	DNMT3B Reverse	GAAATGTTGCCATACCCGCTA
	EGFR Forward	CTCATTATCACAGGGGTCA
	EGFR Reverse	TTATTCACTGCCTACACAC
	EP300 Forward	ACAAAATTTAGCTCGGTGT
	EP300 Reverse	TAGGCATTATCCCTTGTCC
	ERBB2 Forward	GCTACGTGCTCATCGCTCA
	ERBB2 Reverse	GACGCAAGCTACAACTTCC
	ERBB4 Forward	ATAAAATTCCTTCACGCACA
	ERBB4 Reverse	ATCTTGAAACTCTAAAGGCA
	FGFR2 Forward	GAAAACCACCCCTAAATGCAA
	FGFR2 Reverse	CAAGGCAGTTTTCTTATCCCT
	FLT3 Forward	AATCCGCAATTTTCTAGGGAG
	FLT3 Reverse	TTTTGTGCATCTTTGTTGCT
	GLI3 Forward	TGCCTTGAATCAGACGTT
	GLI3 Reverse	TCGCTAACTCAAAATAGTGT
	GRM1 Forward	TATCTGGCTACTTCTATGGGA
	GRM1 Reverse	CTTTGGATCTCTAGCCCTG
	GRM8 Forward	AGCCAGGTGTTCAGAATCACA
	GRM8 Reverse	CATTAGCACACTTCACATCCG
	HDAC9 Forward	GCTGAATGAAAATTAGCCTA
	HDAC9 Reverse	CACAATTCCCTTCAAGCCAT
	INSRR Forward	CGGCCACAGTCCTTGTCCTC
	INSRR Reverse	CCCCACCCTCCCTACACTCAC
	ITGB4 Forward	AGAGCAGCTTCCAAATCACA
	ITGB4 Reverse	ACCGAGATTCTTCCCTTGAGA
	JAK3 Forward	AGATAGTGTGTTGCATCCCCT
	JAK3 Reverse	CGAAGCCCCACTTGTCAGC

	KIT Forward	CAGATAGGTTAGCACCAT
	KIT Reverse	ACGGTATCAACAATAGCTT
	MAPK1 Forward	ATGCTTCTTAAAGTGTGCTC
	MAPK1 Reverse	CAAACTCTCAACGCAGAGG
	MAPK6 Forward	CAAACATGCTCTACGTGA
	MAPK6 Reverse	AAAGTTGAAATAGCATCCCC
	MECOM Forward	TTGAAAATGGAACCCCAA
	MECOM Reverse	CATTTAAGTACCCACGCAT
	MET Forward	ATCCTTGCCATTATCCTC
	MET Reverse	CATTGTTTGGCTTTCAGTC
	NFE2L2 Forward	GTCCAGAAGCCAAACTGA
	NFE2L2 Reverse	TATATCCCGAATTAATGCAA
	NOTCH1 Forward	TTTCCCCAGCCTCCATGCCTT
	NOTCH1 Reverse	GGGCACTCGCACTGGAACTCA
	PDGFRA Forward	ATGTAGCCTTTGTACCTC
	PDGFRA Reverse	TTAATCTAGGCATCTAACCC
	PIK3CA Forward	GAATTATTACTACTTAGCCTA
	PIK3CA Reverse	ATCTTTTCTTCACGGTTG
	PIK3CG Forward	CTGCTGATAGACCACCGTTT
	PIK3CG Reverse	ATCATCGTCCTCCAAGCTCT
	PLXNA4 Forward	CAATTTCCTTGACGCTCCC
	PLXNA4 Reverse	CACAAGCCCAAATTGAACACA
	RGS5 Forward	CCAGCTCATCAAACCCAA
	RGS5 Reverse	TGATGCACTGGTATTAGCTT
	RHPN2 Forward	CCAGAGTTTACGATGCCAGT
	RHPN2 Reverse	TCCCCAGTGATACAACGAG
	RIT1 Forward	CTTGTCCCTACTGTGCCGAGA
	RIT1 Reverse	CTCACAGTTACAGAGCGAGT
	SIN3A Forward	GTTTTGTAGTGCATCCCT
	SIN3A Reverse	GAAACAGCCCAATAGTCCA
	SNAI1 Forward	CCATCACTGCCAGCCGTTG
	SNAI1 Reverse	TCAGCCTTTGTCCTGTAGCTC
	ZEB1 Forward	TAATGTAATAAGGCAAGTGT
	ZEB1 Reverse	CTCTTCTGCACTTGGTTG
qRT-PCR	MECOM Forward	AAGAGAAGCCATTTAAGTGTC
	MECOM Reverse	ATCCAGAATCGCACCTGT
	FGFR3 Forward	CCCACTCCCTCCATCTCCT
	FGFR3 Reverse	GCTGCCAAACTTGTTCTCCAC
	FGFR4 Forward	GCATTGGAGGCATTCGG
	FGFR4 Reverse	CACGGCTGTGGTGTTGG
	FZD4 Forward	TCCCACCACAGAACGAC
	FZD4 Reverse	AAGCCAGCATCATAGCC
	GLI1Forward	TTCCTACCAGAGTCCCAAGT
	GLI1 Reverse	CCCTATGTGAAGCCCTATTT

	JAK3Forward	TCCTTCCGAGCCGTCAT
	JAK3 Reverse	TCGCCTAGCGGGTCATAG
	MAPK3Forward	GGGGAGGTGGAGATGGTGA
	MAPK3 Reverse	CTGGCAGTAGGTCTGATGTTCG
	MAPK13Forward	CTCACCCATCCCTTCTTTG
	MAPK13 Reverse	TGTGCTGCTTCCATTCATC
	MEIS1Forward	ACACCCTTACCCTTCTGA
	MEIS1 Reverse	CTTACTGCTCGGTTGGA
	SOX2 Forward	CCCCTGTGGTTACCTCTTCCT
	SOX2 Reverse	CCGTTAATGGCCGTGCC
	TCF7L1Forward	GTCAACGAGTCGGAGAACCA
	TCF7L1 Reverse	TCTCACTTCGGCGAAATAGTC
	WNT3AForward	TCCACGCCATTGCCTCAG
	WNT3A Reverse	CACCATCCCACCAAACTCG
	WNT4 Forward	AGCGGAACCTGGAAGTCAT
	WNT4 Reverse	GAGTCCCTTGCGTCACCA
	SaCas9 Forward	GCAACAAACTGAACGCCCAT
	SaCas9 Reverse	TCCAGATTCTTCACGGTCAC
	hActin Forward	TTAGTTGCGTTACACCCTTT
	hActin Reverse	ACCTTCACCGTTCCAGTTT
	mActin Forward	CCATCCAGGCTGTGCTGTCCCTGTA
	mActin Reverse	ACGCACGATTTCCCTCTCAGCTGTG
ChIP	SOX2-promoter Forward	GTCTGGGAACATAAACA
	SOX2-promoter Reverse	AAGATAACTGGGAGGAT

Table S9 Amino acid sequences for adaptor (CFS) and protector (HF)

Protein name	Amino acid sequences
CFS	MYRMQLLSCIALSLALVTNSEFEGSHHHHHHLSITTPEEMIEKAKGETAYLPCKFTL SPEDQGPLDIEWLISPADNQKVDQVIILYSGDKIYDDYYPDLKGRVHFTSNDLKSG DASINVTNLQLSDIGTYQCKVKKAPGVANKKIHLVVLVKPSGARCYVDGSEEIGSD FKIKCEPKEGSLPLQYEWQKLSDSQKMPTSWLAEMTSSVISVKNASSEYSGTYSCT VRNRVGSDQCLLRLNVVPPSNKAGGSRGLTNSIKANETNIASVTQEVNTAKGNISS LQGDVQALQEAGYIPEAPRDGQAYVRKDGEWVFLSTFLSPATRGGGGSGGGGSG GGGSGGGGSELQMTQSPSSLSASVGDRVTITCRTSQSISSYLNWYQQKPGQPPKLLI YWASTRESGVPDRFSGSGSGTDFTLTISSLQPEDSATYYCQQSYDIPYTFGQGTKLE IKGGGGSGGGGSGGGGSEVQLLESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVR QAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCAKDMGWGSGWRPYYYYGMDVWGQGTTVTVSSG
HF	MYRMQLLSCIALSLALVTNSEFEGSMGSSHHHHHHSSGLVPRGSHQVQLVQSGAE DKKPGASVKVSCKVSGFSLGRYGVHWVRQAPGQGLEWMGVIWRGGTTDYNAKF QGRVTITKDDSKSTVYMELSSLRSEDTAVYYCARQGSNFPLAYWGQGTLVTVSSG GGGSGGGGSGGGGSDIVMTQSPSSLSASVGDRVTITCKASQSVTNDAAWYQKKPG KAPKLLIYQASTRYTGVPSRFSGSGYGTDFTLTISSLQPEDFATYFCHQDYSSPLTFG QGTKVEIKRGGGGSGGGGSGGGGSTRRGLTNSIKANETNIASVTQEVNTAKGNISS LQGDVQALQEAGYIPEAPRDGQAYVRKDGEWVFLSTFLSPALEVLFQ

SUPPLEMENTAL MATERIALS AND METHODS

Proliferation and colony formation

CCK-8 Kit (Dojindo Laboratories) was used to validate cell proliferation. The cells were planted in the 96-well plates, and the cells were tested by CCK-8 for 24 h, 48 h, 72 h and 96 h. The colony formation assay was performed by seeding 1000 cells in one well of a 6-well plate. After 7 to 10 days later, the colonies were stained with crystal violet solution, and the colony numbers were counted.