Supplemental data

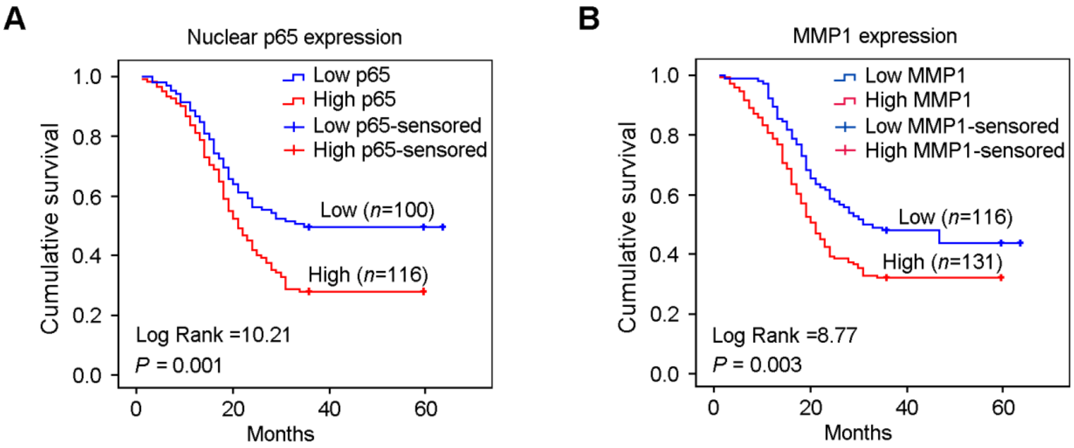

Fig. S1. Kaplan-Meier survival analysis comparing the overall survival time of ESCC patients with different nuclear p65 expression (A) or MMP1 expression (B) levels. Statistics: A and B, Kaplan-Meier analysis and log-rank test.

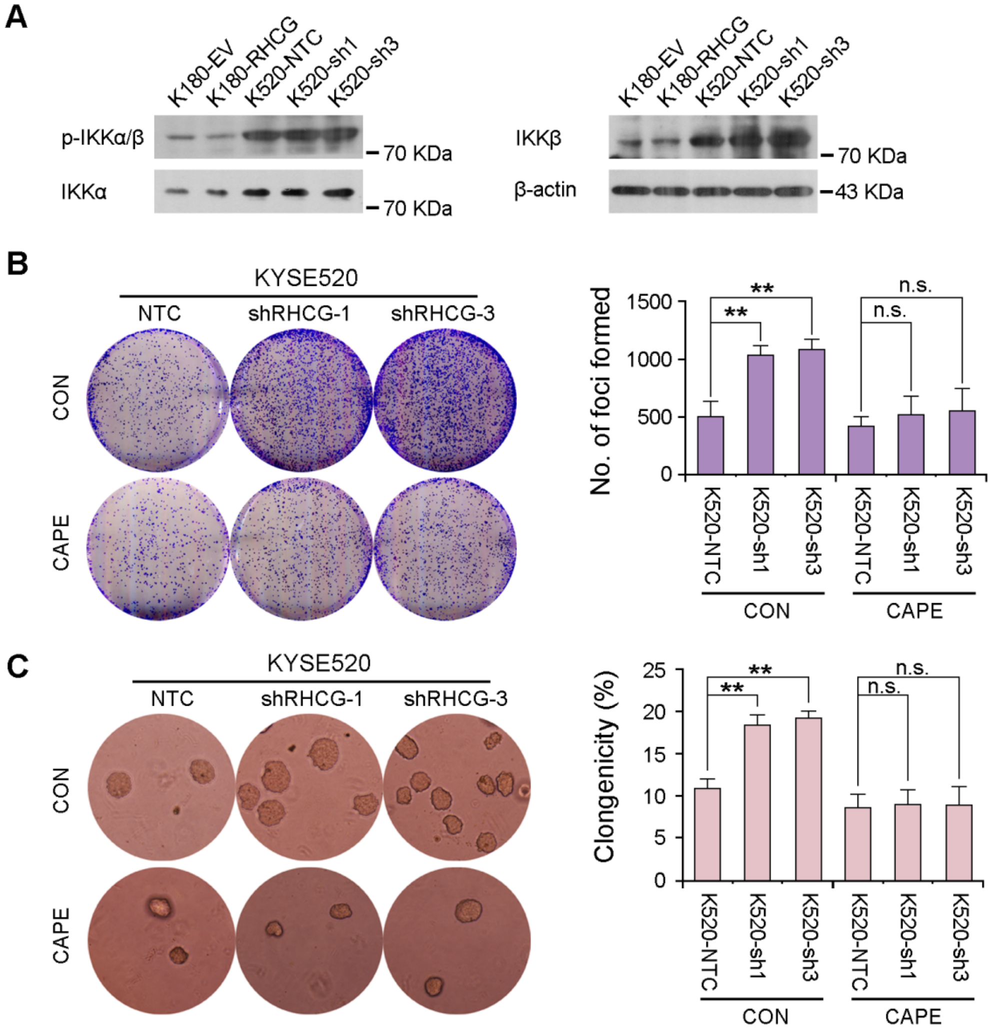

Fig. S2. NF-κB inhibitor CAPE suppresses ESCC tumor formation. (A) western blot analysis was used to detect total and phosphorylated IKKα/β in indicated cells. β-actin was used as a loading control. (B-C) Representative images and summaries of foci formation (B) and soft agar assays (C) performed in *RHCG*- repressed cells and control cells treated with or without CAPE. The values indicate the mean ± SD of three independent experiments. Statistics: B and C, Student *t* test. n.s., $P \geq 0.05$; **, $P < 0.001$.

Table S1. List of deregulated genes in primary ESCC vs. matched non-tumor tissue (cut off ＞2 folds).

Gene Symbol	**Probe set ID**	**Entrez Gene**	**Fold change** (ESCC vs. non-tumor tissues)			
			#301	**#327**	**#351**	**#363**
Up-regulated genes in primary ESCC						
COL1A1	202310_s_at	1277	2.44	18.13	2.72	11.50
COL1A1	202311_s_at	1277	6.81	47.81	3.19	26.98
COL1A2	202404_s_at	1278	3.57	22.58	2.25	10.82
FOXM1	202580_x_at	2305	5.47	2.71	2.10	3.93
CCNA2	203418_at	890	8.14	4.41	2.12	3.67
DLGAP5	203764_at	9787	33.10	6.60	2.34	6.57
IGF2BP3	203819_s_at	10643	20.67	28.37	4.74	4.21
IGF2BP3	203820_s_at	10643	31.73	31.97	5.13	3.91
IFI6	204415_at	2537	2.68	4.85	2.28	4.29
MMP1	204475_at	4312	101.99	296.87	2.01	729.27
NEK2	204641_at	4751	27.81	10.00	2.17	3.65
KIF23	204709_s_at	9493	11.73	7.48	2.38	3.24
CENPA/SLC35F6	204962_s_at	1058/54978	16.12	4.42	2.13	3.86
MMP3	205828_at	4314	53.92	39.43	4.00	33.52
CST1	206224_at	1469	81.01	17.62	4.12	42.72
MDK	209035_at	4192	2.84	7.23	2.06	4.63
SPP1	209875_s_at	6696	3.14	24.51	4.04	81.32
HIST1H2BD	209911_x_at	3017	2.01	9.13	2.15	3.33
MAGEA3	209942_x_at	4102	3.23	178.10	2.19	2.68
FAP	209955_s_at	2191	2.06	6.66	2.37	4.90
KIF2C	211519_s_at	11004	11.53	4.07	2.11	5.38
KIAA1199	212942_s_at	57214	5.05	17.22	2.65	3.63
OIP5	213599_at	11339	11.19	2.09	2.16	3.13
IGF2BP3	216493_s_at	10643	2.36	2.02	3.89	4.30
NCAPG	218663_at	64151	10.70	4.46	2.17	2.93
NETO2	218888_s_at	81831	3.82	10.75	2.11	3.03
RPP25	219143_s_at	54913	3.76	2.56	2.36	2.24
SHCBP1	219493_at	79801	3.25	7.51	2.22	3.06
ASPM	219918_s_at	259266	21.89	2.02	2.15	3.42
EPB41L4B	220161_s_at	54566	11.06	2.75	2.15	5.71
DEPDC1	220295_x_at	55635	13.72	6.42	2.30	6.29
MCM10	220651_s_at	55388	8.90	2.56	2.09	7.29
COL5A2	221729_at	1290	2.91	13.20	2.85	6.21
COL5A2	221730_at	1290	2.42	9.63	2.79	7.46
NETO2	222774_s_at	81831	3.17	8.17	2.72	5.26
DEPDC1	222958_s_at	55635	19.90	8.78	2.04	6.45
CDCA3	223307_at	83461	6.40	5.76	2.09	2.60
CDCA7	224428_s_at	83879	10.56	5.48	2.14	3.32
CTHRC1	225681_at	115908	6.90	89.21	2.55	20.25
CCDC34	226287_at	91057	5.24	2.14	2.66	2.01
FNDC1	226930_at	84624	3.73	9.23	5.10	5.86
RHPN2	227196_at	85415	28.78	9.88	2.53	9.05
ERP27	227450_at	121506	2.60	2.20	4.83	4.40
SIX1	228347_at	6495	7.30	2.37	2.86	5.16
COL1A2	229218_at	1278	2.61	24.84	2.39	5.58
WISP1	229802_at	8840	4.71	20.69	2.48	10.74
SHISA2	230493_at	387914	3.38	2.30	2.21	5.23
DEPDC1	232278_s_at	55635	19.67	5.09	2.42	8.04
COL3A1	232458_at	1281	2.34	23.40	2.20	6.05

KIF14	236641_at	9928	13.86	2.74	2.02	2.54
C11orf80	238593_at	79703	3.27	4.05	2.05	2.23
RASEF	1553185_at	158158	8.68	7.79	2.29	8.06
GPNMB	1554018_at	10457	2.46	4.78	2.05	2.19
Down-regulated genes in primary ESCC						
MYL9	201058_s_at	10398	-3.71	-11.45	-2.26	-2.57
EMP1	201325_s_at	2012	-2.77	-13.83	-2.21	-7.33
GPX3	201348_at	2878	-5.87	-3.30	-3.92	-7.15
MYH11	201495_x_at	4629	-8.18	-142.47	-5.07	-4.96
MYH11	201496_x_at	4629	-6.97	-244.28	-3.53	-11.85
MYH11	201497_x_at	4629	-2.47	-49.81	-2.43	-7.76
FHL1	201539_s_at	2273	-5.66	-88.56	-3.56	-13.61
FHL1	201540_at	2273	-2.88	-18.82	-2.51	-11.84
SFRP1	202036_s_at	6422	-6.80	-10.76	-2.64	-9.96
SFRP1	202037_s_at	6422	-10.36	-12.39	-2.53	-29.23
DES	202222_s_at	1674	-3.15	-89.67	-2.52	-6.04
ACTG2	202274_at	72	-2.54	-33.58	-3.42	-4.69
MYLK	202555_s_at	4638	-2.19	-6.15	-2.35	-2.35
CRABP2	202575_at	1382	-3.36	-2.71	-2.77	-3.34
C7	202992_at	730	-4.65	-86.74	-8.29	-35.96
PTPRN2	203029_s_at	5799	-2.78	-7.58	-2.13	-4.55
ATP1A2	203296_s_at	477	-8.35	-39.40	-2.93	-11.21
F13A1	203305_at	2162	-2.66	-5.85	-2.60	-4.17
PPL	203407_at	5493	-2.77	-5.87	-2.29	-8.15
PLA2G2A	203649_s_at	5320	-4.10	-11.04	-4.27	-51.57
TMOD1	203661_s_at	7111	-3.44	-4.49	-2.18	-2.43
LMOD1	203766_s_at	25802	-4.33	-34.70	-2.07	-6.80
SLIT3	203813_s_at	6586	-3.17	-4.23	-2.29	-3.86
TSPAN8	203824_at	7103	-2.09	-79.61	-3.59	-15.51
IGFBP6	203851_at	3489	-5.29	-2.83	-4.30	-5.42
CNN1	203951_at	1264	-4.36	-41.00	-3.07	-5.17
TPM2	204083_s_at	7169	-3.45	-9.03	-2.16	-2.01
PPP1R3C	204284_at	5507	-2.79	-8.16	-4.07	-11.39
REEP1	204364_s_at	65055	-5.61	-17.70	-2.43	-6.41
LMO3	204424_s_at	55885	-4.45	-22.54	-2.83	-3.76
SNCA	204467_s_at	6622	-3.08	-2.91	-3.13	-2.02
SERPINB2	204614_at	5055	-2.63	-173.61	-2.01	-18.65
TFF3	204623_at	7033	-2.39	-86.21	-5.70	-9.01
TGFBR3	204731_at	7049	-2.07	-3.51	-2.04	-12.33
IL11RA	204773_at	3590	-3.94	-2.58	-2.03	-3.88
MAL	204777_s_at	4118	-3.55	-321.54	-9.78	-318.87
AOC3	204894_s_at	8639	-4.41	-5.84	-2.09	-2.83
PLN	204938_s_at	5350	-4.24	-59.91	-4.16	-4.02
PLN	204939_s_at	5350	-3.28	-36.76	-2.57	-4.62
PLN	204940_at	5350	-3.91	-34.06	-2.30	-4.70
SPINK5	205185_at	11005	-3.99	-81.19	-4.70	-92.74
CLEC3B/EXOSC7	205200_at	7123/23016	-14.19	-26.37	-2.03	-41.82
EPM2A	205231_s_at	7957	-3.24	-5.44	-2.31	-2.40
CLDN10	205328_at	9071	-6.18	-19.67	-4.51	-11.06
AGTR1	205357_s_at	185	-10.48	-2.53	-2.94	-2.79
CFD	205382_s_at	1675	-12.40	-20.38	-4.02	-20.28
FXYD1	205384_at	5348	-7.89	-10.14	-2.12	-6.57
DCLK1	205399_at	9201	-6.18	-4.74	-2.38	-8.75
IL1R2	205403_at	7850	-2.15	-17.16	-2.40	-25.78
BCHE	205433_at	590	-5.46	-86.77	-3.76	-34.11
PCP4	205549_at	5121	-4.31	-95.54	-3.09	-19.93

CDA	205627_at	978	-4.87	-2.10	-2.21	-7.45
CTSG	205653_at	1511	-4.17	-9.15	-2.00	-6.63
EREG	205767_at	2069	-2.64	-11.25	-4.86	-23.79
FGF7	205782_at	2252	-5.84	-10.20	-2.10	-4.75
ZBTB16	205883_at	7704	-3.62	-16.52	-2.52	-5.25
TGM3	206004_at	7053	-4.93	-204.50	-3.96	-136.01
TGM1	206008_at	7051	-2.91	-7.97	-2.07	-4.93
ASPA	206030_at	443	-7.45	-14.60	-2.16	-10.25
CILP	206227_at	8483	-8.08	-5.20	-3.76	-7.70
USP9Y	206624_at	8287	-3.51	-2.37	-3.21	-3.20
PDE5A	206757_at	8654	-3.47	-12.84	-2.26	-2.41
SCEL	206884_s_at	8796	-2.81	-186.31	-4.52	-20.56
CDH19	206898_at	28513	-4.54	-12.08	-2.86	-3.43
NCRNA00185	207063_at	55410	-2.75	-2.68	-2.07	-3.56
CASQ2	207317_s_at	845	-4.50	-20.72	-2.85	-10.54
SMTN	207390_s_at	6525	-5.36	-7.71	-2.27	-4.22
TPSAB1	207741_x_at	7177	-3.67	-7.70	-2.06	-6.76
CRISP3	207802_at	10321	-8.67	-677.90	-3.92	-453.99
KRT13	207935_s_at	3860	-2.20	-1825.09	-2.85	-4.88
MYH11	207961_x_at	4629	-2.88	-46.38	-2.42	-5.17
DPT	207977_s_at	1805	-12.02	-14.72	-2.68	-9.19
FAM107A	209074_s_at	11170	-16.92	-30.31	-2.44	-18.87
ECM1	209365_s_at	1893	-2.71	-10.80	-2.62	-16.35
SMTN	209427_at	6525	-5.12	-2.11	-2.10	-2.60
ADH1B	209612_s_at	125	-23.77	-136.03	-3.98	-261.97
ADH1B	209613_s_at	125	-29.30	-175.36	-4.20	-254.59
ADH1B	209614_at	125	-11.03	-2.25	-3.25	-8.68
NKX3-1	209706_at	4824	-3.67	-34.79	-7.35	-6.56
CHRDL1	209763_at	91851	-4.97	-47.77	-3.05	-23.77
KCNMB1	209948_at	3779	-4.42	-41.94	-2.67	-3.50
PLP1	210198_s_at	5354	-15.57	-26.25	-3.51	-7.93
FHL1	210298_x_at	2273	-5.00	-74.56	-3.03	-14.34
FHL1	210299_s_at	2273	-4.50	-53.04	-2.86	-14.71
TCEAL2	211276_at	140597	-4.54	-15.65	-4.62	-6.19
IL1R2	211372_s_at	7850	-2.73	-13.84	-2.24	-12.11
FCER1A	211734_s_at	2205	-9.71	-34.70	-4.03	-7.10
PTN	211737_x_at	5764	-4.26	-7.20	-2.18	-23.56
MFAP4	212713_at	4239	-7.66	-12.60	-2.21	-10.59
SYNM	212730_at	23336	-3.00	-37.03	-2.34	-10.64
PRUNE2	212805_at	158471	-2.79	-36.65	-2.01	-2.69
PRUNE2	212806_at	158471	-3.56	-12.87	-2.11	-2.89
DPT	213068_at	1805	-10.23	-14.20	-2.67	-21.21
DPT	213071_at	1805	-10.60	-10.99	-3.45	-13.36
TNXA	213451_x_at	7148	-4.68	-10.44	-2.04	-11.07
MFAP5	213764_s_at	8076	-4.67	-2.11	-2.17	-2.06
MFAP5	213765_at	8076	-4.95	-3.12	-2.53	-2.78
DES	214027_x_at	1674	-3.16	-30.58	-2.07	-3.71
GPX3	214091_s_at	2878	-6.93	-3.36	-3.29	-7.86
FHL1	214505_s_at	2273	-4.79	-50.55	-3.17	-11.01
SLURP1	214536_at	57152	-3.59	-9.79	-2.02	-26.58
HSPB6	214767_s_at	126393	-5.13	-7.22	-2.26	-2.42
IL1RN	216244_at	3557	-4.19	-9.01	-2.01	-24.33
MT1M	217546_at	4499	-3.69	-60.45	-2.66	-20.65
C3	217767_at	718	-2.18	-2.30	-2.21	-7.25
PHLDA1	217996_at	22822	-2.31	-2.19	-2.04	-3.18
SORBS1	218087_s_at	10580	-4.45	-33.33	-2.79	-9.23

OGN	218730_s_at	4969	-7.05	-63.48	-4.57	-72.97
LYVE1	219059_s_at	10894	-12.85	-24.79	-2.56	-17.47
TMEM100	219230_at	55273	-6.11	-12.59	-4.20	-26.38
PCOLCE2	219295_s_at	26577	-5.03	-9.60	-4.64	-22.71
CLIC3	219529_at	9022	-3.81	-4.28	-2.34	-13.56
RHCG	219554_at	51458	-2.52	-17.93	-3.49	-327.98
TMEM35	219685_at	59353	-6.05	-10.12	-3.95	-3.11
CLCA4	220026_at	22802	-2.86	-912.27	-2.62	-123.97
LYVE1	220037_s_at	10894	-22.97	-61.28	-2.51	-16.57
CRNN	220090_at	49860	-3.61	-489.28	-11.91	-261.59
FHL5	220170_at	9457	-15.74	-6.32	-4.01	-7.29
RERGL	220276_at	79785	-35.41	-6.72	-2.40	-19.27
ADAMTS9	220287_at	56999	-2.75	-3.63	-2.22	-4.57
TMPRSS11E	220431_at	28983	-2.44	-64.86	-3.94	-47.06
CRCT1	220620_at	54544	-4.24	-21.33	-5.39	-73.20
GREM2	220794_at	64388	-8.42	-17.98	-2.77	-10.33
HSPB8	221667_s_at	26353	-4.59	-3.69	-2.28	-7.83
ADAMTS1	222162_s_at	9510	-9.03	-17.95	-2.05	-7.99
ADAMTS1	222486_s_at	9510	-12.33	-19.59	-2.14	-5.94
SORBS1	222513_s_at	10580	-3.75	-12.99	-2.13	-3.66
OGN	222722_at	4969	-6.13	-50.41	-3.98	-80.10
TMEM40	222892_s_at	55287	-2.36	-3.36	-2.55	-4.51
RAB9B	222918_at	51209	-8.11	-10.18	-3.33	-9.09
SLMAP	222924_at	7871	-5.42	-9.26	-2.34	-5.47
C1orf21	223126_s_at	81563	-2.60	-6.34	-2.03	-5.78
C2orf40	223623_at	84417	-13.64	-63.70	-2.06	-34.56
SPINK7	223720_at	84651	-4.01	-326.75	-7.21	-256.81
CNFN	224329_s_at	84518	-2.83	-2.99	-4.34	-135.09
ANGPTL1	224339_s_at	9068	-9.19	-14.13	-3.05	-12.87
PDK4	225207_at	5166	-10.20	-248.03	-2.18	-40.60
SYNPO2	225720_at	171024	-5.74	-143.24	-2.73	-11.38
SYNPO2	225721_at	171024	-4.68	-41.90	-2.48	-8.06
MSRB3	225782_at	253827	-3.28	-6.92	-2.05	-2.12
CGNL1	225817_at	84952	-3.47	-3.22	-2.09	-10.08
SYNPO2	225894_at	171024	-8.11	-49.88	-3.14	-8.59
SYNPO2	225895_at	171024	-4.88	-127.87	-2.58	-12.88
CAB39L	225915_at	81617	-6.34	-20.14	-2.08	-13.43
STEAP4	225987_at	79689	-6.10	-16.48	-3.09	-24.38
NEXN	226103_at	91624	-3.13	-10.64	-2.06	-2.07
PGM5	226303_at	5239	-6.65	-76.02	-2.48	-16.10
HSPB6	226304_at	126393	-4.83	-10.15	-2.33	-4.60
MUSTN1	226856_at	389125	-19.54	-7.18	-2.77	-13.85
NOSTRIN	226992_at	115677	-3.77	-7.19	-2.03	-3.00
C11orf96	227099_s_at	387763	-4.03	-2.20	-2.15	-5.90
CELF2	227178_at	10659	-2.95	-5.69	-2.11	-4.64
PLAC9	227419_x_at	219348	-5.46	-13.95	-2.48	-8.66
GFRA1	227550_at	2674	-3.64	-5.34	-4.21	-9.07
SYNPO2	227662_at	171024	-2.79	-57.14	-2.89	-9.46
NAP1L5	228062_at	266812	-4.22	-7.17	-2.36	-6.29
NAP1L5	228063_s_at	266812	-3.41	-4.87	-2.15	-9.03
MYH11	228133_s_at	4629	-8.79	-308.03	-3.22	-4.41
SCN7A	228504_at	6332	-4.12	-39.57	-3.98	-28.62
GCOM1	228568_at	145781	-3.99	-15.43	-2.10	-11.01
CD36	228766_at	948	-16.02	-31.27	-2.75	-6.55
MAMDC2	228885_at	256691	-4.17	-24.87	-3.72	-17.89
MYOCD	229339_at	93649	-4.87	-38.66	-2.78	-7.15

NEGR1	229461_x_at	257194	-7.20	-30.40	-3.00	-8.46
JPH2	229578_at	57158	-2.99	-7.75	-2.21	-2.16
DCLK1	229800_at	9201	-8.32	-4.59	-2.85	-5.07
SCARA5	229839_at	286133	-19.04	-11.43	-3.44	-26.07
NFIA	229994_at	4774	-7.77	-10.48	-2.31	-2.79
PGM5-AS1	230595_at	572558	-9.28	-23.50	-4.60	-26.41
IGSF10	230670_at	285313	-2.92	-2.72	-2.38	-13.41
SGCD	230730_at	6444	-2.25	-2.81	-2.09	-2.33
DSTN	230933_at	11034	-6.89	-9.02	-2.02	-2.49
KGFLP2	231031_at	654466	-4.02	-2.38	-2.11	-2.11
ANGPTL1	231773_at	9068	-8.38	-28.72	-3.17	-9.67
SCEL	232056_at	8796	-2.89	-186.90	-4.54	-20.85
PRSS27	232074_at	83886	-3.47	-9.07	-4.30	-15.84
SYNPO2	232119_at	171024	-7.86	-39.09	-3.57	-2.74
MBNL1-AS1	232298_at	401093	-2.99	-25.22	-3.26	-2.15
ANXA1	233011_at	301	-12.74	-39.80	-2.64	-38.12
CDC37L1	235787_at	55664	-2.74	-3.75	-2.60	-2.24
SCARA5	235849_at	286133	-13.45	-11.21	-3.67	-24.47
KIAA1377	235956_at	57562	-5.15	-3.50	-2.30	-3.75
SPG20	236600_at	23111	-2.99	-2.25	-2.04	-3.12
MYOCD	237206_at	93649	-5.24	-45.73	-3.33	-6.66
SSBP2	238484_s_at	23635	-4.79	-2.26	-2.77	-2.82
VSIG10L	238654_at	147645	-3.77	-16.04	-2.86	-30.08
ANGPTL1	239183_at	9068	-12.51	-5.65	-3.77	-6.35
NEGR1	243357_at	257194	-4.37	-15.61	-2.73	-3.09
SYNPO2	244108_at	171024	-6.88	-15.67	-2.70	-2.34
KCNT2	244455_at	343450	-2.01	-2.45	-2.00	-2.91
KRT78	1553212_at	196374	-4.91	-13.84	-3.97	-36.39
KRT78	1553213_a_at	196374	-5.17	-52.68	-3.72	-105.98
SCEL	1554921_a_at	8796	-2.89	-62.02	-3.83	-21.40
FHL5	1555191_a_at	9457	-16.86	-3.42	-3.07	-4.61
RHOJ	1555234_a_at	57381	-2.23	-2.23	-2.17	-2.32
CAPN14	1557321_a_at	440854	-9.23	-103.34	-4.04	-160.42
A2ML1	1564307_a_at	144568	-3.45	-15.17	-2.70	-45.82
TMPRSS11B	1560712_at	132724	-4.46	-586.57	-4.24	-679.72
C9orf169	1569144_a_at	375791	-3.03	-20.75	-2.42	-18.93

Table S2. Cox proportional hazard regression analysis for overall survival

Clinicopathologic features	Univariable analysis		Multivariable analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Gender (male *vs.* female)	1.130 (0.855-1.495)	0.390	1.199 (0.878-1.636)	0.469
Age (<60 *vs.* ≥60)	1.009 (0.994-1.025)	0.227	1.119 (0.825-1.519)	0.253
Differentiation (well/moderate *vs.* poor)	1.633 (1.210-2.225)	**0.001**	1.310 (0.936-1.833)	0.115
TNM stage (I/II *vs.* III/IV)	2.365 (1.788-3.129)	**<0.001**	1.971 (1.439-2.7)	**<0.001**
RHCG negative expression	0.497 (0.366-0.676)	**<0.001**	0.541 (0.395-0.743)	**<0.001**

Table S3. List of deregulated genes in KYSE180-EV vs. KYSE180-RHCG cells (cut off ＞1.5 folds).

Gene	Gene Name	Transcripts	Entrez	Fold
Up-regulated genes in KYSE180-RHCG				
ALDH1L2	aldehyde dehydrogenase 1 family member L2	16769481	160428	1.94
ANKRD1	ankyrin repeat domain 1	16716478	27063	1.98
APOC1	apolipoprotein C1	16863124	341	1.65
ATP12A	ATPase H+/K+ transporting non-gastric alpha 2 subunit	16773260	479	1.57
BIRC3	baculoviral IAP repeat containing 3	16730522	330	1.63
CA8	carbonic anhydrase 8	17077525	767	1.58
CAPNS2	calpain, small subunit 2	16819099	84290	1.56
CARD17	caspase recruitment domain family member 17	16743922	440068	1.68
CASP14	caspase 14	16859090	23581	1.88
CCDC80	coiled-coil domain containing 80	16957396	151887	1.62
CCL2	chemokine (C-C motif) ligand 2	16833204	6347	1.64
CD24	CD24 molecule	17117110	100133941	1.54
CXCL10	chemokine (C-X-C motif) ligand 10	16977052	3627	1.54
CXCL14	chemokine (C-X-C motif) ligand 14	17000168	9547	1.60
CYR61	cysteine-rich angiogenic inducer 61	16666738	3491	1.69
DAPL1	death associated protein-like 1	16886806	92196	1.86
DLX5	distal-less homeobox 5	17060049	1749	1.52
EGR1	early growth response 1	16989736	1958	1.74
EPB41L4A	erythrocyte membrane protein band 4.1 like 4A	16998850	64097	1.82
FAM171B	family with sequence similarity 171 member B	16888554	165215	1.57
FGFR2	fibroblast growth factor receptor 2	16719025	2263	1.51
FIGN	fidgetin, microtubule severing factor	16904410	55137	1.60
FLT1	fms related tyrosine kinase 1	16780478	2321	1.56
FOS	Fos proto-oncogene, AP-1 transcription factor subunit	16786587	2353	1.63
FREM2	FRAS1 related extracellular matrix protein 2	16774130	341640	1.64
GPR135	G protein-coupled receptor 135	16793475	64582	1.51
HERC5	HECT and RLD domain containing E3 ubiquitin protein ligase 5	16968765	51191	1.56
HMGCS1	3-hydroxy-3-methylglutaryl-CoA synthase 1	16995890	3157	1.64
HS3ST3B1	heparan sulfate-glucosamine 3-O-sulfotransferase 3B1	16831306	9953	1.90
ICAM1	intercellular adhesion molecule 1	16858137	3383	1.57
IFI44L	interferon-induced protein 44 like	16666485	10964	1.50
ITGA2	integrin alpha 2	16984689	3672	1.51
ITGB8	integrin beta 8	17043982	3696	1.68
IVNS1ABP	influenza virus NS1A binding protein	16697245	10625	1.66
KIT	KIT proto-oncogene receptor tyrosine kinase	16966855	3815	1.71
KRT1	Keratin 1	16765029	3848	2.77
KRT10	keratin 10	16844477	3858	1.66
LUZP2	leucine zipper protein 2	16722987	338645	1.89
MAP3K5	mitogen-activated protein kinase kinase kinase 5	17024079	4217	1.51
MPPED2	metallophosphoesterase domain containing 2	16736942	744	1.61

MSMO1	methylsterol monooxygenase 1	16972155	6307	1.58
MT-TD	mitochondrially encoded tRNA aspartic acid	17100659	4555	1.96
MT-TT	mitochondrially encoded tRNA threonine	17100685	4576	1.81
MT-TW	mitochondrially encoded tRNA tryptophan	17100655	4578	1.52
MX1	MX dynamin like GTPase 1	16923031	4599	1.68
NEDD9	neural precursor cell expressed, developmentally down-	17118190	4739	1.76
NPIPB3	nuclear pore complex interacting protein family member B3	17117684	23117	1.52
OLR1	oxidized low density lipoprotein (lectin-like) receptor 1	16761293	4973	1.90
OR8G2	olfactory receptor, family 8, subfamily G, member 2	16732840	26492	2.32
PBX1	PBX homeobox 1	16673191	5087	1.56
PLXDC2	Plexin domain containing 2	16703036	84898	2.71
PTPRZ1	protein tyrosine phosphatase, receptor-type Z1	17050847	5803	1.60
RBMS3	RNA binding motif, single stranded interacting protein 3	16938527	27303	1.93
RHCG	Rh family, C glycoprotein	16813112	51458	8.37
SAMD5	sterile alpha motif domain containing 5	17013507	389432	1.69
SEMA3C	semadomain 3C	17059119	10512	1.53
SEMA3D	semadomain 3D	17059355	223117	2.11
SLC16A12	solute carrier family 16 member 12	16716403	387700	1.53
SLC1A3	solute carrier family 1 member 3	16984056	6507	1.71
SLC25A27	solute carrier family 25 member 27	17009333	9481	1.59
SLITRK5	SLIT and NTRK like family member 5	16775732	26050	1.61
SMAD6	SMAD family member 6	16802232	4091	2.11
SNTB1	syntrophin beta 1	17080630	6641	1.58
SQLE	squalene epoxidase	17072552	6713	1.60
TAS2R19	taste 2 receptor member 19	16761514	259294	1.61
TAS2R30	taste 2 receptor member 30	16761526	259293	2.28
TGFBR2	transforming growth factor beta receptor 2	16938562	7048	1.63
Down-regulated genes in KYSE180-RHCG				
ABCA1	ATP binding cassette subfamily A member 1	17096728	19	1.71
ADH7	alcohol dehydrogenase 7 (classIV), mu or sigma polypeptide	16978261	131	1.54
AKR7L	aldo-keto reductase family 7 like	16682671	246181	1.74
ANKRD22	ankyrin repeat domain 22	16716341	118932	1.53
ANKRD29	ankyrin repeat domain 29	16854301	147463	1.57
ANXA10	annexin A10	16972229	11199	2.90
AREG	amphiregulin	16967863	374	2.55
ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta	16761843	397	1.68
CALB1	calbindin 1	17078943	793	1.71
CD68	CD68 molecule	16830577	968	2.91
CKMT1A	creatine kinase, mitochondrial 1A	16800242	548596	1.67
DKK1	dickkopf WNT signaling pathway inhibitor 1	16705011	22943	1.51
DSG3	desmoglein 3	16851749	1830	1.63
FAM72C	family with sequence similarity 72 member C	16691883	554282	1.90
FBN2	fibrillin 2	16999475	2201	1.72
FST	follistatin	16984730	10468	1.65
GAL	galanin	16728141	14419	2.57

GBP6	guanylate binding protein family member 6	16666943	163351	1.60
GLIPR1	GLI pathogenesis related 1	16754373	11010	1.62
GTF3C6	general transcription factor IIIC subunit 6	17011683	112495	1.51
HAS3	hyaluronan synthase 3	16820528	3038	1.53
HIST1H2BG	histonecluster 1 H2B family member g	17016390	8339	2.17
HIST1H2BH	histone cluster1 H2B family member h	17005600	8345	1.56
HIST1H2BJ	histonecluster 1 H2B family member j	17016451	8970	2.01
HIST1H3C	histone cluster 1 H3 family member c	17005539	8352	1.61
HIST1H3H	histone cluster 1 H3 family member h	17005862	8357	1.63
HIST2H3D	histone cluster 2 H3 family member d	16692614	653604	1.70
IFI16	interferon gamma inducible protein 16	16672390	3428	1.67
IL1A	interleukin 1 alpha	16901974	3552	2.24
KRT14	keratin 14	16844804	3861	1.53
KRT32	keratin 32	16844702	3882	1.69
KYNU	kynureninase	16886174	8942	1.75
LAMC2	laminin subunit gamma 2	16674845	3819	1.82
LCP1	lymphocyte cytosolic protein 1	16778849	3936	1.72
MFAP5	microfibrillar associated protein 5	16760953	8076	1.71
MMP1	matrix metallopeptidase 1	16743721	4312	1.58
MRPS12	mitochondrial ribosomal protein S12	16861907	6183	1.52
NPR3	natriuretic peptide receptor 3	16983765	4883	1.71
ODC1	ornithine decarboxylase 1	16894402	4953	1.52
OR56A3	olfactory receptor family 56 subfamily A member 3	16721320	390083	1.58
PBDC1	polysaccharide biosynthesis domain containing 1	17104983	51260	1.56
PGAM4	phosphoglycerate mutase family member 4	17112334	441531	1.52
PLA2G4A	phospholipase A2 group IVA	16675197	5321	1.65
PLIN3	perilipin 3	16867414	10226	1.54
PRPS1	phosphoribosyl pyrophosphate synthetase 1	17106031	5631	1.54
SERPINE1	serpin family E member 1	17049676	5054	1.76
SERPINI1	serpin family I member 1	16947715	5274	1.63
SFN	stratifin	16661314	2810	1.85
SGK1	serum/glucocorticoid regulated kinase 1	17023810	6446	1.86
SPRR3	small proline-rich protein 3	16671108	6707	1.52
TAGLN	transgelin	16731654	6876	1.89
TMPRSS11E	transmembrane protease, serine 11E	16967327	28983	3.27
TMPRSS15	transmembrane protease, serine 15	16924349	5651	1.59
TNFRSF9	TNF receptor superfamily member9	16681288	3604	1.53
UBASH3B	ubiquitin associated and SH3 domain containing B	16732676	84959	1.51
ZNF165	zinc finger protein 165	17005888	7718	1.53
ZNF326	zinc finger protein 326	16667011	284695	1.59
ZNF429	zinc finger protein 429	16860203	353088	1.52

Table S4. Association of nuclear p65 expression with clinicopathologic features in 216 primary ESCCs

Features	Total	Nuclear p65 expression		*P*-value[b]
		Low	High	
Sex				
Female	92	48 (52.2%)	44 (47.8%)	
Male	124	52 (41.9%)	72 (58.1%)	0.136
Age, years				
<60	116	47 (40.5%)	69 (59.5%)	
≥60	100	39 (39%)	61 (61%)	0.820
Differentiation				
Well/moderate	161	74 (46%)	87 (54%)	
Poor	55	26 (47.3%)	29 (52.7%)	0.866
Invasion				
Absent	84	50 (59.5%)	34 (40.5%)	
Present	132	50 (37.9%)	82 (62.1%)	**0.002**
Clinical Stage[a]				
Early stage I-II	155	90 (58.1%)	65 (41.9%)	
Advanced stage III-IV	61	10 (16.4%)	51 (83.6%)	**<0.001**
Lymph node metastasis				
Absent	135	80 (59.3%)	55 (40.7%)	
Present	81	20 (24.7%)	61 (75.3%)	**<0.001**

[a] AJCC/UICC TNM staging system.

[b] Pearson χ^2 test.

Table S5. Association of MMP1 expression with clinicopathologic features in 247 primary ESCCs

Features	Total	MMP1 expression		*P*-value[b]
		Low	High	
Sex				
Female	108	53 (49.1%)	55 (50.9%)	
Male	139	63 (45.3%)	76 (54.7%)	0.558
Age, years				
<60	130	66 (50.8%)	64 (49.2%)	
≥60	117	50 (42.7%)	67 (57.3%)	0.206
Differentiation				
Well/moderate	186	81 (43.5%)	105 (56.5%)	
Poor	61	22 (36.1%)	39 (63.9%)	0.304
Invasion				
Absent	90	52 (57.8%)	38 (42.2%)	
Present	157	64 (40.8%)	93 (59.2%)	**0.010**
Clinical Stage[a]				
Early stage I-II	165	92 (55.8%)	73 (44.2%)	
Advanced stage III-IV	82	24 (29.3%)	58 (70.7%)	**<0.001**
Lymph node metastasis				
Absent	137	75 (54.7%)	62 (45.3%)	
Present	110	41 (37.3%)	69 (62.7%)	**0.006**

[a] AJCC/UICC TNM staging system.

[b] Pearson χ^2 test.

Table S6. List of PCR primers for expression and DNA methylation

Gene Name	**Primer Sequence (5'-3')**
For Real-time PCR	
RHCG	Forward: TTCCAAGTGACCCTCTTCGC
NM_016321.2	Reverse: TAGGTTGCGTCGGTAGAGGA
AREG	Forward: GAGCCGACTATGACTACTCAGA
NM_001657	Reverse: TCACTTTCCGTCTTGTTTTGGG
IL1A	Forward: TGGTAGTAGCAACCAACGGGA
NM_000575	Reverse: ACTTTGATTGAGGGCGTCATTC
LAMC2	Forward: TGGAGAACGCTGTGATAGGTG
NM_018891	Reverse: CAGGAGACCCATTTCGTTGGA
ABCA1	Forward: ACATCCTGAAGCCAATCCTGA
NM_005502	Reverse: CTCCTGTCGCATGTCACTCC
MMP1	Forward: GGGGCTTTGATGTACCCTAGC
NM_002421	Reverse: TGTCACACGCTTTTGGGGTTT
CXCL10	Forward: GTGGCATTCAAGGAGTACCTC
NM_001565	Reverse: TGATGGCCTTCGATTCTGGATT
APOC1	Forward: TCCAGTGCCTTGGATAAGCTG
NM_001645	Reverse: GGCTGATGAGTTCCCGAGC
RBMS3	Forward: GGGGAACAGTTGAGTAAAACCA
NM_001003793	Reverse: ACAATTTTTCCATACGGTTGGCA
SMAD6	Forward: GCTACCAACTCCCTCATCACT
NM_001142861	Reverse: CGTACACCGCATAGAGGCG
EGR1	Forward: GGTCAGTGGCCTAGTGAGC
NM_001964	Reverse: GTGCCGCTGAGTAAATGGGA
GAPDH	Forward: GGAGCGAGATCCCTCCAAAAT
NM_001256799	Reverse: GGCTGTTGTCATACTTCTCATGG
For full length cDNA	
RHCG	Forward: ccgctcgagATGGCCTGGAACACCAACCT
NM_016321.2	Reverse: tccccgcggCTAGGGTACCAAGGGTACCGAG
For DNA methylation	
RHCG-BGS	Forward: AGAATTTGGGGTATTTTGATTTTTT
-193 - +77	Reverse: CAAACCATACTACAAAAATACCTAACC
RHCG-MSP-M	Forward: TTGTTTTTTAGTTCGACGCG
-138 - +15	Reverse: GACGAAACTTTTAAAACCCGAA
RHCG-MSP-U	Forward: TGTTTTTTAGTTTGATGTGG
-137 - +16	Reverse: CAACAAAACTTTTAAAACCCAAA

Table S7. List of antibodies used in this project

Antibody	Vendor	Cat No.	Application
Rabbit anti-human RHCG	Sigma-Aldrich	HPA043317	WB 1:1000, IHC 1:100
Mouse anti-human β-actin	Abcam	ab6276	WB 1:5000
Rabbit anti-human Histone H3	Abcam	Ab8580	WB 1:2000
Mouse anti-human MMP1	Santa Cruz	sc-58377	WB 1:500, IHC 1:50
Mouse anti-human NF-κB p65	Santa Cruz	sc-8008	IF 1:100
Rabbit anti-human IKKα	Cell Signaling	#2682	WB 1:1000
Rabbit anti-human IKKβ	Cell Signaling	#2678	WB 1:1000
Rabbit anti-human IKKα/β (Ser176/180)	Cell Signaling	#2697	WB 1:1000
Rabbit anti-human NF-κB p65	Cell Signaling	#4764	WB 1:1000
Rabbit anti- human NF-κB p65 (Ser536)	Cell Signaling	#3033	WB 1:1000
Mouse anti-human IκB-α	Cell Signaling	#4814	WB 1:1000
Rabbit anti-human IκB-α (Ser32)	Cell Signaling	#2859	WB 1:1000
Goat anti-mouse IgG HRP antibody	Sigma-Aldrich	A4416	WB 1:5000
Goat anti-rabbit IgG HRP antibody	Sigma-Aldrich	A9169	WB 1:5000
CF^{TM} goat anti-mouse IgG antibody	Biotium	20030-1	IF 1:5000

Table S8. The shRNA sequences for lentiviral transduction

Gene	shRNA Sequence
RHCG (NM_016321.2)	TRCN0000059853: CCGGCCAGCCTTGAAGTCTATGGAACTCGAGTTCCATAGACTTCAAGGCTGGTTTTTG
	TRCN0000059856: CCGGCCACTTCTTACAAGACCGCTACTCGAGTAGCGGTCTTGTAAGAAGTGGTTTTTG
Non-Target Control	SHC002: CCGGCAACAAGATGAAGAGCACAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTT

Supplemental Methods

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIZOL Reagent (Invitrogen), and cDNA was synthesized using a reverse transcription (RT)-PCR Kit (Roche) according to the manufacturer's instructions. qRT-PCR was performed using the SYBR Green PCR Kit (Applied Biosystems) and an ABI PRISM 7900 Sequence Detector (Applied Biosystems). Specificity of primers was verified by dissociation curve analysis. Data were analyzed using ABI SDS v2.4 software (Applied Biosystems). All qRT-PCR reactions were performed in duplicates. Housekeeping gene GAPDH was used as an internal control. Using primers listed in Table S6.

5-aza-2'-deoxycytidine (5-aza-dC) and trichostatin A (TSA) treatment

ESCC cell lines with absent/low RHCG expression (EC109, KYSE30, KYSE140 and KYSE180) were treated with 1-100 μmol/L 5-aza-dC, or 100-400 nmol/L TSA (Sigma-Aldrich, St. Louis, MO) for 72 hours with a daily medium change.

Wound healing assay

Cell mobility was studied by a scratch wound-healing assay. Briefly, cells were cultured in a 6-well plate until confluence. The culture medium was replaced by serum-free medium 24 h before wound creation. The cell layer was wounded using a sterile tip. After wounding, the medium was changed to fresh serum-free medium to remove cellular debris. Serial photographs were obtained at different time points.

Immunofluorescence

Immunofluorescence (IF) was performed on ESCC cell lines. First, cells on the coverslips were fixed with 4% paraformaldehyde, and incubated with primary antibody (mouse anti-p65, 1:100 diluted; Santa Cruz) overnight at 4°C. After washing, cells underwent incubation with PEconjugated secondary antibodies for 1 hour, and subsequently counterstained with DAPI for 5 minutes at room temperature (Roche Diagnostics). All images were visualized under Carl Zeiss LSM700 fluorescence microscope (ZEISS, Germany) and analyzed using ZEN 2010 software.

Western blotting

Quantified protein lysates were resolved on SDS-PAGE, transferred onto a polyvinylidenedifluoride (PVDF) membrane (Millipore), and then blocked with 5% non-fat milk in Tris-buffered saline-Tween 20 (TBS-T) for 1 hour at room temperature. The blocked membrane was then incubated with primary antibody at 4°C overnight. After washing with TBS-T, the membrane was incubated for 1 hour with horseradish peroxidase (HRP)-conjugated secondary antibody. A complex of primary and secondary antibodies labeled proteins were detected by enhanced chemiluminescence (ECL) system (GE Healthcare) and X-ray film (GE Healthcare). β-actin was used as a housekeeping control. Using antibodies listed in Table S7.

RHCG overexpression and knockdown

The full-length wild-type *RHCG* cDNA (NM_016321.2) was cloned into the pLenti6 expression vector (Invitrogen) and transfected into EC109 and KYSE180 cells with Lipofectamine 2000 Reagent (Invitrogen). Stable *RHCG*-expressing clones were selected for 2 weeks with Blasticidin (Sigma-Aldrich). Empty vector (EV)-transfected cells were used as controls. For *RHCG* knockdown, the scrambled shRNA plasmid (pLKO.1-NTC) and the *RHCG*-specific shRNA expression vectors (pLKO.1-shRHCG) were purchased from Sigma-Aldrich. Two constructs against *RHCG* were used: sh*RHCG*-1 (TRCN0000059853) and sh*RHCG*-3 (TRCN0000059856). The shRNA sequences listed in Table S8. The pLKO.1-sh*RHCG* or the scrambled shRNA plasmid was transfected into KYSE520, and stable clones were selected using puromycin (Sigma-Aldrich).

Cell fractionation

Cell fractionation was performed using the ReadyPrep™ Protein Extraction Kit (Cytoplasmic/Nuclear; Bio-Rad, #163-2089) according to manufacturer’s instruction. The concentration of protein samples was determined by Plus One 2-D Quant Kit (GE Healthcare, Hatfield, Hertfordshire, UK). β-Actin and Histone H3 served as loading controls for cytoplasmic extract and nuclear extract, respectively.