

A ENG		Primary			Post-CT			Metastasis			Relapse		
Intensity	Score	# scored	% total per group		# scored	% total per group		# scored	% total per group		# scored	% total per group	
Negative	0	1	2.86	22.86	6	26.09	60.87	0	0.00	31.25	1	12.50	37.50
Low	(0,1]	7	20.00		8	34.78		5	31.25		2	25.00	
Intermediate	(1,2]	17	48.57	71.14	6	26.09	39.13	9	56.25	68.75	2	25.00	62.50
High	(2,3]	10	28.57		3	13.04		2	12.50		3	37.50	
Total		35	100	100	23	100	100	16	100	100	8	100	100

B ENG		Primary			Post-CT			Metastasis			Relapse		
IxA	Score	# scored	% total per group		# scored	% total per group		# scored	% total per group		# scored	% total per group	
Negative	0	1	2.86	28.57	6	26.09	65.22	1	6.25	31.25	1	12.50	37.50
Low	(0,3]	9	25.71		9	39.13		4	25.00		2	25.00	
Intermediate	(3,6]	17	48.57	71.43	5	21.74	34.78	9	56.25	68.75	2	25.00	62.50
High	(3,9]	8	22.86		3	13.04		2	12.50		3	37.50	
Total		35	100	100	23	100	100	16	100	100	8	100	100

C ENG		# scored					
Intensity	Score	Primary	% All total	Post-CT	% All total	Metastasis	% All total
Negative	0	1	9.8	6	17.1	0	6.1
Low	(0,1]	7		8		5	
Intermediate	(1,2]	17	32.9	6	11.0	9	13.4
High	(2,3]	10		3		2	
Total		35	42.7	23	28.0	16	19.5

Table S1. Distribution of ENG intensity in ES patients. (A) Frequency of ENG intensity in IHC samples from ES patients. (B) Frequency of ENG intensity considering area of staining in IHC samples from ES patients. (C) Frequency of ENG intensity considering the frequency of each stage as part of a whole group.

A MMP14		Primary			Post-CT			Metastasis			Relapse		
Intensity	Score	# scored	% total	% total per group	# scored	% total	% total per group	# scored	% total	% total per group	# scored	% total	% total per group
Negative	0	1	2,86	20.00	4	17.39	60.87	2	12.50	37.50	1	12.50	62.50
Low	(0,1]	6	17.14		10	43.48		4	25.00		4	50.00	
Intermediate	(1,2]	22	62.86	80.00	6	26.09	39.13	5	31.25	62.50	2	25.00	37.50
High	(2,3]	6	17.14		3	13.04		5	31.25		1	12.50	
Total		35	100	100	23	100	100	16	100	100	8	100	100

B MMP14		Primary			Post-CT			Metastasis			Relapse		
IxA	Score	# scored	% total	% total per group	# scored	% total	% total per group	# scored	% total	% total per group	# scored	% total	% total per group
Negative	0	1	2.86	22.86	5	21.74	65.22	2	12.50	43.75	1	12.50	62.50
Low	(0,3]	7	20.00		10	43.48		5	31.25		4	50.00	
Intermediate	(3,6]	21	60.00	77.14	5	21.74	34.78	4	25.00	56.25	2	25.00	37.50
High	(3,9]	6	17.14		3	13.04		5	31.25		1	12.50	
Total		35	100	100	23	100	100	16	100	100	8	100	100

C MMP14		# scored					
Intensity	Score	Primary	% All total	Post-CT	% All total	Metastasis	% All total
Negative	0	1	8.5	4	17.1	2	7.3
Low	(0,1]	6		10		4	
Intermediate	(1,2]	22	34.1	6	11.0	5	12.2
High	(2,3]	6		3		5	
Total		35	42.7	23	28.0	16	19.5

Table S2. Distribution of MMP14 intensity in ES patients. (A) Frequency of MMP14 intensity in IHC samples from ES patients. (B) Frequency of MMP14 intensity considering area of staining in IHC samples from ES patients. (C) Frequency of MMP14 intensity considering the frequency of each stage as part of a whole group.

shENG	Database Molecular Signature	NES	pvalue	p.adjust	qvalues
RM82	GO_EXTRACELLULAR_MATRI X	-1,56	1,49E-03	1,32E-01	1,23E-01
SKNMC		-2,10	2,04E-03	6,76E-02	6,08E-02
RM82	GO_REGULATION_OF_TRAN SMEMBRANE_TRANSPORT	-1,45	1,49E-03	1,32E-01	1,23E-01
SKNMC		1,34	1,58E-02	2,24E-01	2,01E-01
RM82	GO_SIDE_OF_MEMBRANE	-1,46	1,51E-03	1,32E-01	1,23E-01
SKNMC		-1,35	6,00E-03	1,40E-01	1,26E-01
RM82	GO_REGULATION_OF_TRAN S_SYNAPTIC_SIGNALING	-1,38	1,51E-03	1,32E-01	1,23E-01
SKNMC		-1,28	1,61E-02	2,24E-01	2,02E-01
RM82	GO_SYNAPTIC_MEMBRANE	-1,53	1,51E-03	1,32E-01	1,23E-01
SKNMC		-1,43	4,07E-03	1,10E-01	9,93E-02
RM82	BOQUEST_STEM_CELL_CUL TURED_VS_FRESH_UP	-1,46	1,51E-03	1,32E-01	1,23E-01
SKNMC		-1,33	1,20E-02	1,97E-01	1,77E-01
RM82	GO_EXTRACELLULAR_STRU CTURE_ORGANIZATION	-1,60	1,52E-03	1,32E-01	1,23E-01
SKNMC		-2,24	2,00E-03	6,76E-02	6,08E-02
RM82	GO_SYNAPSE_ORGANIZATIO N	-1,43	1,54E-03	1,32E-01	1,23E-01
SKNMC		-1,78	2,04E-03	6,76E-02	6,08E-02
RM82	GO_CELL_SUBSTRATE_JUNC TION	-1,57	1,54E-03	1,32E-01	1,23E-01
SKNMC		-1,52	2,06E-03	6,76E-02	6,08E-02
RM82	BOQUEST_STEM_CELL_UP	-1,88	1,54E-03	1,32E-01	1,23E-01
SKNMC		-2,06	2,05E-03	6,76E-02	6,08E-02

Table S3. GSEA from RM82- and SKNMC- shENG models.

Cell line	Database	NES	p value	p adjust	q value
shENG/pENG	Molecular Signature				
RM82	RIGGI_EWING_SARCOMA _PROGENITOR_DN	-1,43	1,03E-02	2,52E-01	2,33E-01
TC71		1,35	2,00E-02	1,43E-01	1,19E-01
RM82	GO_ACTOMYOSIN	-1,72	1,80E-03	1,88E-01	1,75E-01
TC71		1,45	1,82E-02	1,33E-01	1,11E-01
RM82	GO_COLLAGEN_BINDING	-1,54	9,06E-03	2,45E-01	2,26E-01
TC71		1,53	2,07E-02	1,45E-01	1,21E-01
RM82	GO_CELL_SUBSTRATE_A DHESION	-1,30	3,04E-02	3,53E-01	3,27E-01
TC71		1,42	1,95E-03	3,15E-02	2,63E-02
RM82	GO_CELL_SUBSTRATE_J UNCTION	-1,66	1,58E-03	1,88E-01	1,75E-01
TC71		1,72	1,93E-03	3,15E-02	2,63E-02
RM82	GO_CELL_LEADING_EDG E	-1,45	1,58E-03	1,88E-01	1,75E-01
TC71		1,56	1,92E-03	3,15E-02	2,63E-02

Table S4. GSEA from RM82-shENG and TC71- pENG models.

Subpathway_name	log2(FC)	Pvalue	FDR	UniprotKB_functions
Leukocyte transendothelial migration: PXN	-2,03E-01	9,70E-07	2,42E-04	Cell adhesion
Ras signaling pathway: MLLT4*	1,50E-01	1,10E-06	2,42E-04	Cell adhesion
Ras signaling pathway: MLLT4	1,40E-01	1,71E-06	2,69E-04	Cell adhesion
Sphingolipid signaling pathway: PRKCA	-1,79E-01	1,70E-05	1,06E-03	Apoptosis, Angiogenesis, Cell adhesion, Transcription regulation, Transcription, Adaptive immunity, Immunity, Biological rhythms
Tight junction: TJP1 CTNNB1 CTNNA1	-4,60E-02	1,73E-05	1,06E-03	Cell adhesion, Neurogenesis, Wnt signaling pathway, Transcription regulation, Transcription, Differentiation
Wnt signaling pathway: PRKCA	-1,66E-01	2,22E-05	1,29E-03	Apoptosis, Angiogenesis, Cell adhesion, Transcription regulation, Transcription, Adaptive immunity, Immunity, Biological rhythms
Melanogenesis: PRKACA	-1,84E-01	3,11E-05	1,63E-03	Angiogenesis, Cell adhesion, Apoptosis
Natural killer cell mediated cytotoxicity: PRKCA	-1,59E-01	7,74E-05	2,75E-03	Apoptosis, Cell adhesion, Angiogenesis, Adaptive immunity, Immunity, Transcription regulation, Transcription, Biological rhythms
Retrograde endocannabinoid signaling: PRKCA	-1,62E-01	7,78E-05	2,75E-03	Apoptosis, Angiogenesis, Cell adhesion, Adaptive immunity, Transcription regulation, Transcription, Immunity, Biological rhythms
Cholinergic synapse: PRKCA	-1,62E-01	7,78E-05	2,75E-03	Apoptosis, Angiogenesis, Cell adhesion, Transcription regulation, Transcription, Adaptive

Subpathway_name	log2(FC)	Pvalue	FDR	UniprotKB_functions
				immunity, Immunity, Biological rhythms
Serotonergic synapse: PRKCA	-1,62E-01	7,78E-05	2,75E-03	Apoptosis, Angiogenesis, Cell adhesion, Adaptive immunity, Transcription regulation, Transcription, Immunity, Biological rhythms
Insulin secretion: PRKCA	-1,95E-01	8,64E-05	2,79E-03	Angiogenesis, Apoptosis, Cell adhesion, Adaptive immunity, Immunity, Transcription regulation, Transcription, Biological rhythms
Circadian entrainment: PRKCA	-1,71E-01	9,62E-05	2,86E-03	Angiogenesis, Apoptosis, Cell adhesion, Adaptive immunity, Transcription regulation, Transcription, Immunity, Biological rhythms
Ras signaling pathway: PRKCA	-2,20E-01	3,59E-04	6,69E-03	Apoptosis, Angiogenesis, Cell adhesion, Adaptive immunity, Transcription regulation, Transcription, Immunity, Biological rhythms
Signaling pathways regulating pluripotency of stem cells: CTNNB1*	4,53E-02	4,08E-04	6,69E-03	Transcription regulation, Transcription, Neurogenesis, Wnt signaling pathway, Cell adhesion
ErbB signaling pathway: PRKCA	-2,36E-01	4,91E-04	7,82E-03	Apoptosis, Angiogenesis, Cell adhesion, Adaptive immunity, Transcription regulation, Transcription, Immunity, Biological rhythms
cAMP signaling pathway: AFDN	3,36E-01	1,55E-03	2,03E-02	Cell adhesion

Subpathway_name	log2(FC)	Pvalue	FDR	UniprotKB_functions
Thyroid hormone signaling pathway: ATP1B4	-8,51E-02	2,08E-03	2,51E-02	Ion transport, Transcription regulation, Transcription, Transport, Sodium/potassium transport, Sodium transport, Potassium transport, Cell adhesion
Leukocyte transendothelial migration: BCAR1	-1,34E-01	3,54E-03	4,01E-02	Cell adhesion
Ras signaling pathway: PIK3R5*	-9,98E-02	4,52E-03	4,86E-02	Angiogenesis, Endocytosis, Autophagy, Cell adhesion, Chemotaxis, Adaptive immunity, Inflammatory response, Innate immunity, Differentiation, Immunity, Stress response, Host-virus interaction, Protein transport, Transport
Progesterone-mediated oocyte maturation: PIK3R5	-9,28E-02	4,63E-03	4,87E-02	Angiogenesis, Endocytosis, Autophagy, Cell adhesion, Chemotaxis, Inflammatory response, Adaptive immunity, Innate immunity, Differentiation, Immunity, Stress response, Protein transport, Host-virus interaction, Transport

Table S5. HiPathia analysis on RM82-shENG with shared cell adhesion UniprotKB function.

Antibody	Manufacturer	Reference
FAK1	CST	3285
FAK1 P Y397	CST	3283
CamKII P Thr286	CST	3361
MEK1/2	CST	9122
MEK1/2 P Ser217/221	CST	9154
PI3 Kinase p110-alpha	CST	4249
PKC (pan) P Ser660 (beta-2)	CST	9371
PKC-zeta	CST	9372
PKC-zeta/lambda P Thr410/403	CST	9378
ErbB-1/EGFR	CST	2232
EGFR P Tyr1173	CST	4407
Akt P Thr308	CST	2965
Src	CST	2109
Akt	CST	9272
Akt P Ser473	CST	4060
beta-actin	CST	4970
E-Cadherin	CST	3195
beta-Catenin	CST	9562
beta-Catenin P Ser33,Ser37,Thr41	CST	9561
beta-Catenin P Thr41,Ser45	CST	9565
VEGFR P Tyr1175	CST	2478
PTEN	CST	9552
PTEN P Ser380,Thr382,Thr383	CST	9554
GSK-3-alpha/beta P Ser21/Ser9	CST	9331
mTOR P Ser2448	CST	2971
PLC-gamma1 P Tyr783	CST	2821
Src (family) P Tyr416	CST	2101
Smad2/3 P Ser465/Ser423,Ser467/Ser425	CST	8828
Smad1/5 P Ser463/Ser465	CST	9516
EGFR P Tyr1086	Thermo Fisher Scientific	36-9700
GSK-3-beta P Ser9	CST	9336
GSK-3-beta	CST	9315
PKC-alpha	Beckton Dickinson	610108
PLC-gamma1	CST	2822
VEGFR P Tyr951	CST	4991
VEGFR P Tyr1059	CST	3817
Calmodulin	Calbiochem	NB12
PDGFR P Tyr1021	CST	2227
PDGFR P Tyr751	CST	4549
Zyxin	CST	3553

Antibody	Manufacturer	Reference
ILK1 (4G9)	CST	3856
Akt P Ser473	CST	9271
Akt P Thr308	CST	9275
EGFR P Y992	CST	2235
EGFR P Y1086	CST	2234
mTOR (7C10)	CST	2983
Integrin Beta 1 [EP1041Y]	Abcam	ab52971
mTOR P S2448 [EP2426(2)Y]	Abcam	ab109268
Smad2 (C86F7)	CST	3122
Caveolin-1 (D46G3) XP R	CST	3267
Integrin alpha 4	CST	4600
Integrin beta3	CST	4702
Integrin beta4	CST	4707
Smad1 (D59D7)	CST	6944
EphA2 (D4A2)	CST	6997
beta-Tubulin	Abcam	ab6046
PKC-alpha P Thr638	Abcam	ab32502
GAPDH	Abcam	ab9484
PKC-gamma P Thr514	GeneTex	GTX25778
mTOR P Ser2481	Millipore (Upstate)	09-343SP

Table S6. Antibodies used in RPPA.

ID	Description	sgMMP14	NES	pvalue	p.adjust	qvalues
GO:0000226	microtubule cytoskeleton organization	RM82	-1,53	3,39E-03	1,33E-01	1,19E-01
		SKNMC	-1,42	1,31E-03	1,78E-01	1,65E-01
GO:0034333	adherens junction assembly	RM82	-1,43	2,70E-02	2,69E-01	2,41E-01
		SKNMC	-1,83	1,64E-03	1,78E-01	1,65E-01
GO:0051893	regulation of focal adhesion assembly	RM82	-1,50	2,21E-02	2,46E-01	2,20E-01
		SKNMC	-1,78	6,57E-03	2,46E-01	2,29E-01
GO:0033627	cell adhesion mediated by integrin	RM82	-1,49	2,43E-02	2,58E-01	2,32E-01
		SKNMC	-1,66	8,20E-03	2,46E-01	2,29E-01
GO:0031012	extracellular matrix	RM82	1,61	1,40E-03	7,16E-02	6,27E-02
		SKNMC	-2,11	1,29E-03	1,00E-01	8,92E-02
GO:0030545	receptor regulator activity	RM82	1,80	1,42E-03	1,26E-01	1,12E-01
		SKNMC	-1,54	2,63E-03	2,73E-01	2,61E-01
GO:0050840	extracellular matrix binding	RM82	1,83	1,69E-03	1,26E-01	1,12E-01
		SKNMC	-1,70	8,67E-03	2,88E-01	2,75E-01
GO:0070006	metalloamino peptidase activity	RM82	-1,87	6,96E-03	1,62E-01	1,45E-01
		SKNMC	-1,70	1,84E-02	3,66E-01	3,49E-01
GO:0005201	extracellular matrix structural constituent	RM82	1,49	9,58E-03	1,75E-01	1,56E-01
		SKNMC	-2,13	1,52E-03	2,73E-01	2,61E-01
WP3888	VEGFA-VEGFR2 Signaling	RM82	-1,35	1,45E-02	1,83E-01	1,64E-01
		SKNMC	-1,41	5,19E-03	1,80E-01	1,68E-01

Table S7. GSEA from RM82- and SKNMC-sgMMP14 models

Gene name	Protein name	ENG / IgG	
		LFQ Ratio	p-value
ACTR1A	Alpha-centractin	1,00E+08	3,66E-03
ILKAP	Integrin-linked kinase-associated	3,70E+07	7,66E-06
	serine/threonine phosphatase 2C		
APOF	Apolipoprotein F	1,50E+07	2,44E-02
DCTN3	Dynactin subunit 3	1,20E+07	1,98E-02
DCTN4	Dynactin subunit 4	8,50E+06	2,21E-03
PGAM5	Serine/threonine-protein phosphatase PGAM5,	7,90E+06	4,93E-03
	mitochondrial		
PPIL1	Peptidyl-prolyl cis-trans isomerase-like 1	7,50E+06	3,45E-02
ADORA3	Adenosine receptor A3	3,00E+06	2,67E-02
sep-09	Septin-9	2,70E+06	2,20E-04
VRK1	Serine/threonine-protein kinase VRK1	9,60E+05	3,27E-02
CEP290	Centrosomal protein of 290 kDa	9,30E+03	2,28E-02
ENG	Endoglin	3,20E+02	6,13E-03
IQCB1	IQ calmodulin-binding motif-containing protein 1	8,80E+01	2,20E-02
SORBS2	Sorbin and SH3 domain-containing protein 2	4,20E+01	2,64E-05
CALM3	Calmodulin-3	3,00E+01	7,23E-03
COL1A1	Collagen alpha-1(I) chain	2,10E+01	2,78E-02
CRABP1	Cellular retinoic acid-binding protein 1	1,90E+01	1,22E-04
DCTN2	Dynactin subunit 2	1,70E+01	1,57E-03
IGKV3OR2-268	Ig kappa chain V-III region POM	8,60E+00	7,41E-05
IARS	Isoleucine--tRNA ligase, cytoplasmic	5,30E+00	1,40E-02
PLAA	Phospholipase A-2-activating protein	5,20E+00	1,98E-02

Table S8. Table depicting the most enriched ENG -interactors identified by IP/MS. LFQ ratios ENG/IgG and associated *p*-values are shown. Herein, three biological replicates were used for each individual experiment. Statistical analysis was performed by paired two-tailed Student's *t*- test.