cancers

MDPI

Table S1. Primers for conventional RT-PCR, QPCR and ribozyme synthesis.

PRIMER NAME	PRIMER SEQUENCE (5′-3′)
GAPDHF8	GGCTGCTTTTAACTCTGGTA
GAPDHR8	GACTGTGGTCATGAGTCCTT
GAPDHF1	AAGGTCATCCATGACAACTT
GAPDHZR1	ACTGAACCTGACCGTACAGCCATCCACAGTCTTCTG
SIPA1F1	AGAACTCGCTGTCACCAC
SIPA1ZR	ACTGAACCTGACCGTACATACTGATGGCTTGGCTGT
CLDN1F	GAAGTGTATGAAGTGCTTGG
CLDN1ZR	ACTGAACCTGACCGTACAGACCTGCAAGAAGAAATA
CLDN2F1	AAGTCTTTGACTCCTTGCTG
CLDN2ZR	ACTGAACCTGACCGTACAGCCACAAAGATTGCTATCAC
CLDN3F1	ACTGCCACAGGACCTTCA
CLDN3ZR	ACTGAACCTGACCGTACAATGGTGATCTTGGCCTTG
CLDN4F1	GCAGCAACATTGTCACCT
CLDN4ZR	ACTGAACCTGACCGTACAGTACACCTTGCACTGCATC
CLDN 5F1	TTCCTGGACCACAACATC
CLDN5ZR1	ACTGAACCTGACCGTACACACCGAGTCGTACACTTTGC
CLDN5F2	TTCCTGGACCACAACATC
CLDN5ZR2	ACTGAACCTGACCGTACACACCGAGTCGTACACTTTGC
CLDN6F1	ACGTGCCCTCTGTGTCAT
CLDN6ZR	ACTGAACCTGACCGTACACTCCACACAGGTGGTACACT
CLDN7F1	ATAACCCTTTGATCCCTACC
CLDN7ZR	ACTGAACCTGACCGTACAACAGGAACAGGAGAGCAGT
CLDN8F1	GCTTGGAGAAGCTCTCTACTT
CLDN8ZR	ACTGAACCTGACCGTACAAGCTACTGCTCTTTTCGTTG
CLDN9F1	GTGCCCTCTGTGTCATTG
CLDN9ZR	ACTGAACCTGACCGTACATCCACACACGTGGTACACT
CLDN10F1	GTCTCCAACTGCAAGGACT
CLDN10ZR	ACTGAACCTGACCGTACAGCAAATATGGAACCAAAGAA
CLDN11F1	CCGGTGTGGCTAAGTACA
CLDN11ZR	ACTGAACCTGACCGTACACACACAGGGAACCAGATG
CLDN12F1	CTCTGCCTGATTGGAATG
CLDN12ZR	ACTGAACCTGACCGTACAACCTGCACTATTGACCAGAC
CLDN14F1	CACCCTACAGGCCCTACC
CLDN14ZR	ACTGAACCTGACCGTACAGTCTTTGTAGGCAGCTGGT
CLDN15F1	CCTGGGCGTCTACAACTG
CLDN15ZR	ACTGAACCTGACCGTACAATAGACATCAGCCGGACA
CLDN17F1	CTGCTTATTGGCATCTGTG
CLDN17ZR	ACTGAACCTGACCGTACATGAAGTTCCCAGAAGGTATG
CLDN18F1	GGATCATGTTCATTGTCTCA
CLDN18ZR	ACTGAACCTGACCGTACACATCCAGAAGTTAGTCACCAG
CLDN19F1	GGAGCCCTCTTCATCCTG
CLDN19ZR	ACTGAACCTGACCGTACAGTGCTTGGGTTGAAGAACT
CLDN20F1	AGCAAACTTTCTGGATCTGA
CLDN20ZR	ACTGAACCTGACCGTACACAGAAAATCATGCCAGAGAT
CLDN22F1	CAGGCTCTTGTAACCTCAAC
CLDN22ZR	ACTGAACCTGACCGTACATTGGATGACACAGGTTTG
CLDN23F1	CCGCCATCAAGTACTACAG
CLDN23ZR1	ACTGAACCTGACCGTACACCACCGAGTTGGTGTAGG
CLDN24F1	GTGGACGCACATGACTGT
CLDN24ZR	ACTGAACCTGACCGTACACAGGAGGAAGGTGAGCTG
JAM1F2	AACAAGATCACAGCTTCCTA
JAM1ZR	ACTGAACCTGACCGTACAACAAGTGTATGTCCCAGTGT

Cancers **2021**, *13*, 1747. https://doi.org/10.3390/cancers13071747

JAM2F1	CTGATAGGGGCTGTAAATCT
JAM2ZR	ACTGAACCTGACCGTACATAATGATGCAAGACAGTTCC
JAM3F1	TTGATGAGATTGTGATCGAG
JAM3ZR	ACTGAACCTGACCGTACAATCTTGCCTACTGGTACAGC
ZO1ZF2	TGGGTATGACACACATGGTA
ZO1ZR2	ACTGAACCTGACCGTACAGGTGGTACTTGCTCGTAA
ZO2F1	CAAAAGAGGATTTGGAATTG
ZO2ZR1	ACTGAACCTGACCGTACAGAGCACATCAGAAATGACAA
ZO3F1	CTGACATGGAGGAGCTGA
ZO3ZR1	ACTGAACCTGACCGTACAGCTTAGCTTCCCTTCTGACT
AF6F	ATCATCCACCACTGGAAC
AF6ZR	ACTGAACCTGACCGTACATCATCACTCCATGGAACTC
OCLNZF	G AATTCAAACCGAATCATTG
OCLNZR	ACTGAACCTGACCGTACATGAAGAATTTCATCTTCTGG

Z sequence 'ACTGAACCTGACCGTACA' highlighted in blue. Z-sequence presents on the specific primers containing a 5′ hairpin structure labelled with a fluorophore (FAM).

Table S2. Primary and secondary antibodies used in this study.

Antibodies	**Molecular weight (kDa)**	**Supplier**	**Product code**
Mouse anti SIPA1	112	Abcam	ab219085
Mouse anti GAPDH	37	Insight Biotechnology	sc-32233
Mouse anti AF6	200	Insight Biotechnology	sc-74433
Rabbit anti ZO1	220	Thermo Fisher Scientific	61-7300
Goat anti ZO2	160	Insight Biotechnology	sc-12386
Goat anti ZO3	80-160	Insight Biotechnology	sc-11475
Goat anti Claudin1	22	Insight Biotechnology	sc-17658
Rabbit anti Claudin5	23	Insight Biotechnology	sc-28670
Goat anti Claudin7	24	Insight Biotechnology	sc-17670
Goat anti Claudin9	23	Insight Biotechnology	sc-17672
Rabbit anti Claudin10	23/19	Insight Biotechnology	sc-25710
Rabbit anti Claudin11	20	Insight Biotechnology	sc-25711
Goat anti Claudin15	23	Insight Biotechnology	sc-17683
Rabbit anti Claudin19	22	Insight Biotechnology	sc-134618
Rabbit anti Claudin20	23.5	Novusbio	H00049861-D01P
Goat anti Claudin23	32	Insight Biotechnology	sc-87047
Mouse anti JAM1	36	Insight Biotechnology	sc-53623
Goat anti JAM2	54	Insight Biotechnology	sc-23002
Goat anti JAM3	38	Insight Biotechnology	sc-23006
Goat anti Occludin	60-82	Insight Biotechnology	sc-8145
Rabbit anti-mouse (whole molecule) IgG peroxidise conjugate	Dependent on primary	Sigma-Aldrich	A5278
Goat anti-rabbit (whole molecule) IgG peroxidise conjugate	Dependent on primary	Sigma-Aldrich	A0545
Rabbit anti-goat (whole molecule) IgG peroxidise conjugate	Dependent on primary	Sigma-Aldrich	A5420

Table S3. Top signalling pathways which were downregulated by SIPA1 knockdown on A549 cells. (Generated from: www.reactome.org.).

Pathway name	Entities				Reactions	
	found	ratio	p-value	FDR*	found	ratio
Signaling by SCF-KIT	6 / 51	0.004	4.86e-08	2.76e-05	21 / 36	0.003
CD28 co-stimulation	5 / 39	0.003	4.48e-07	1.27e-04	10 / 19	0.002
Interleukin-3, Interleukin-5 and GM-CSF signaling	5 / 50	0.003	1.50e-06	2.84e-04	10 / 38	0.003
Regulation of signaling by CBL	4 / 24	0.002	2.44e-06	3.44e-04	4 / 7	5.61e-04
ESR-mediated signaling	8 / 256	0.018	5.67e-06	6.26e-04	37 / 110	0.009
Signaling by Receptor Tyrosine Kinases	11 / 554	0.038	6.66e-06	6.26e-04	92 / 657	0.053
PECAM1 interactions	3 / 14	9.70e-04	2.31e-05	0.002	1 / 7	5.61e-04
Signaling by Interleukins	11 / 639	0.044	2.51e-05	0.002	52 / 490	0.039
Fc epsilon receptor (FCERI) signaling	7 / 235	0.016	3.10e-05	0.002	27 / 63	0.005
Costimulation by the CD28 family	5 / 97	0.007	3.61e-05	0.002	12 / 34	0.003
Generic Transcription Pathway	17 / 1,553	0.108	4.17e-05	0.002	61 / 824	0.066
EPHB-mediated forward signaling	4 / 51	0.004	4.60e-05	0.002	9 / 26	0.002
Regulation of KIT signaling	3 / 18	0.001	4.85e-05	0.002	3 / 8	6.41e-04
Signal Transduction	26 / 3,305	0.229	6.20e-05	0.002	240 / 2,303	0.184
Oxidative Stress Induced Senescence	5 / 114	0.008	7.70e-05	0.003	17 / 40	0.003
Fcgamma receptor (FCGR) dependent phagocytosis	6 / 193	0.013	9.45e-05	0.003	7 / 42	0.003
Signaling by Nuclear Receptors	8 / 385	0.027	1.02e-04	0.003	37 / 191	0.015
Cellular Senescence	6 / 199	0.014	1.12e-04	0.003	26 / 90	0.007
RNA Polymerase II Transcription	17 / 1,692	0.117	1.23e-04	0.004	61 / 885	0.071
CTLA4 inhibitory signaling	3 / 25	0.002	1.27e-04	0.004	2 / 5	4.00e-04
Developmental Biology	14 / 1,207	0.084	1.31e-04	0.004	109 / 511	0.041
PIP3 activates AKT signaling	7 / 316	0.022	1.95e-04	0.005	9 / 86	0.007
Cellular responses to stress	10 / 690	0.048	2.52e-04	0.006	45 / 227	0.018
EPHA-mediated growth cone collapse	3 / 33	0.002	2.87e-04	0.006	3 / 4	3.20e-04
Estrogen-dependent gene expression	5 / 154	0.011	3.09e-04	0.006	27 / 66	0.005

Table S4. Top signalling pathways which were up regulated by SIPA1 knockdown on A549 cells. (Generated from: www.reactome.org.).

Pathway name	Entities				Reactions	
	found	ratio	p-value	FDR*	found	ratio
MyD88:MAL(TIRAP) cascade initiated on plasma membrane	13 / 111	0.008	7.25e-11	5.41e-09	45 / 64	0.005
Toll Like Receptor TLR6:TLR2 Cascade	13 / 111	0.008	7.25e-11	5.41e-09	45 / 66	0.005
MAP kinase activation	11 / 69	0.005	9.09e-11	5.41e-09	24 / 32	0.003
Toll Like Receptor TLR1:TLR2 Cascade	13 / 114	0.008	1.00e-10	5.41e-09	45 / 66	0.005
Toll Like Receptor 2 (TLR2) Cascade	13 / 114	0.008	1.00e-10	5.41e-09	45 / 68	0.005
MyD88 cascade initiated on plasma membrane	12 / 94	0.007	1.53e-10	6.56e-09	30 / 58	0.005
Toll Like Receptor 5 (TLR5) Cascade	12 / 94	0.007	1.53e-10	6.56e-09	30 / 59	0.005
Toll Like Receptor 10 (TLR10) Cascade	12 / 94	0.007	1.53e-10	6.56e-09	30 / 59	0.005
Interleukin-17 signaling	11 / 77	0.005	2.85e-10	1.17e-08	24 / 35	0.003
Signaling by Interleukins	26 / 639	0.044	3.60e-10	1.30e-08	125 / 490	0.039
TRAF6 mediated induction of NFkB and MAP kinases upon TLR7/8 or 9 activation	12 / 102	0.007	3.81e-10	1.30e-08	30 / 48	0.004
Toll Like Receptor 3 (TLR3) Cascade	12 / 102	0.007	3.81e-10	1.30e-08	31 / 61	0.005
MyD88 dependent cascade initiated on endosome	12 / 103	0.007	4.25e-10	1.32e-08	30 / 63	0.005
Toll Like Receptor 7/8 (TLR7/8) Cascade	12 / 103	0.007	4.25e-10	1.32e-08	30 / 64	0.005
Signaling by Receptor Tyrosine Kinases	24 / 554	0.038	5.54e-10	1.61e-08	174 / 657	0.053
TRIF(TICAM1)-mediated TLR4 signaling	12 / 107	0.007	6.51e-10	1.76e-08	31 / 58	0.005
MyD88-independent TLR4 cascade	12 / 107	0.007	6.51e-10	1.76e-08	31 / 60	0.005
Toll Like Receptor 9 (TLR9) Cascade	12 / 107	0.007	6.51e-10	1.76e-08	30 / 67	0.005
Generic Transcription Pathway	41 / 1,553	0.108	7.51e-10	1.95e-08	279 / 824	0.066
Toll Like Receptor 4 (TLR4) Cascade	13 / 144	0.01	1.64e-09	4.10e-08	48 / 95	0.008
RNA Polymerase II Transcription	42 / 1,692	0.117	2.77e-09	6.64e-08	281 / 885	0.071
Gene expression (Transcription)	43 / 1,850	0.128	1.20e-08	2.88e-07	293 / 996	0.08
Nuclear Receptor transcription pathway	10 / 86	0.006	1.30e-08	2.99e-07	2 / 2	1.60e-04
Toll-like Receptor Cascades	13 / 184	0.013	2.89e-08	6.35e-07	53 / 182	0.015
VEGFA-VEGFR2 Pathway	11 / 126	0.009	4.33e-08	9.54e-07	37 / 77	0.006