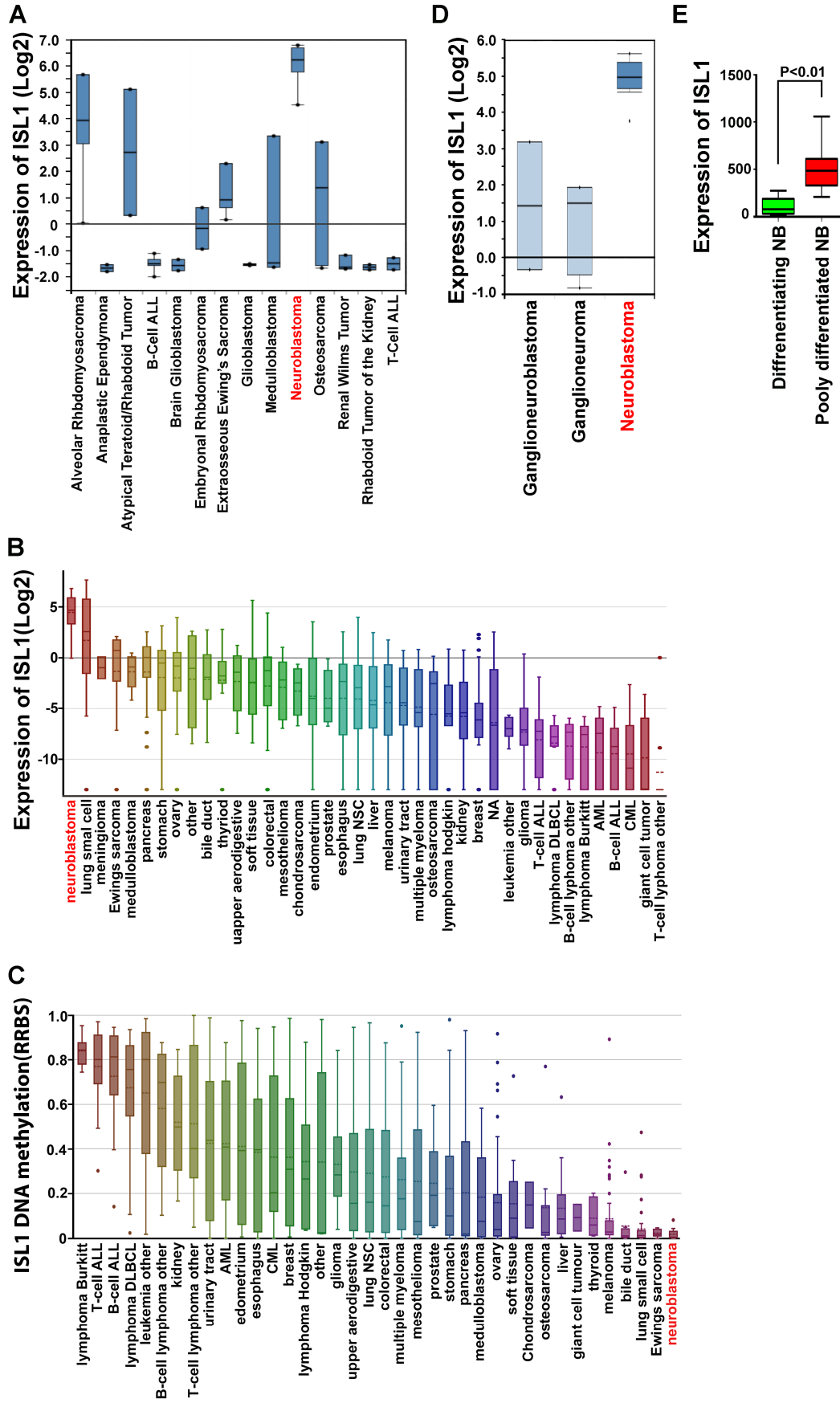
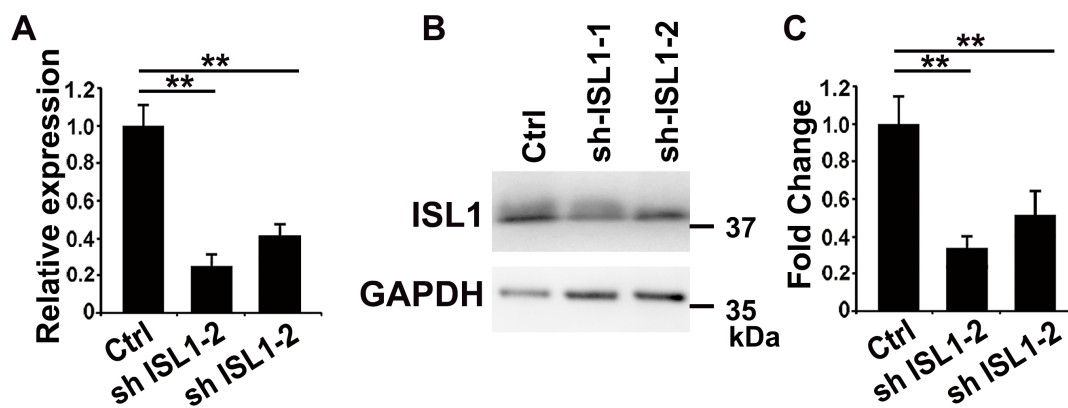


Supplementary Figures



Supplementary Figure 1. The expression of *ISL1* in neuroblastoma

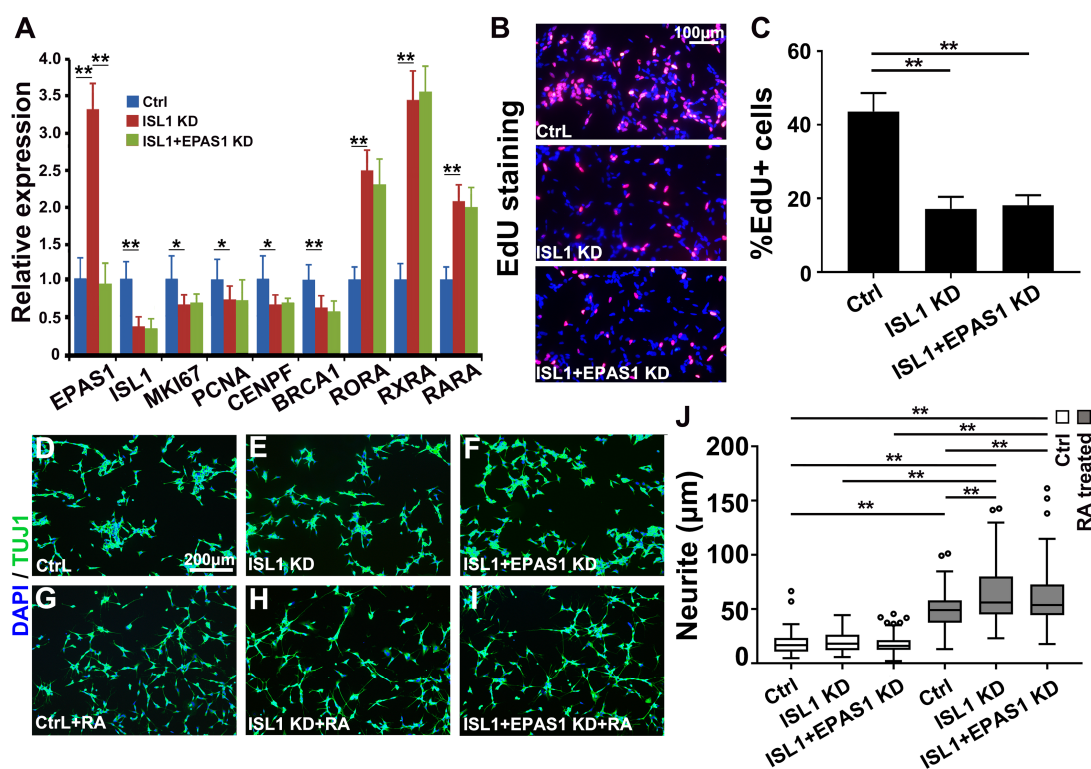
(A) Box-plots of *ISL1* expression in Neale Multi-cancer study. Data obtained through Oncomine.org showing that among these pediatric tumors analyzed, *ISL1* is most highly expressed in neuroblastoma. (B.C) Box-plots of expression of *ISL1*(B) and whole-genome DNA methylation profiling(C) showing the highest level of *ISL1* expression and the lowest methylation levels in the gene body of *ISL1* in neuroblastoma among 1457 neoplasm cell lines. Data were obtained from CCLE (<https://portals.broadinstitute.org/ccle>). (D) Box-plots of expression of *ISL1* among neuroblastic tumors in Albino Neuroblastic-Tumor study. Data were obtained from Oncomine.org. (E) Analysis of Bourdeut's published neuroblastomas gene expression microarray dataset showing that *ISL1* expression in poorly differentiated is markedly raised than more differentiated neuroblastomas.



Supplementary Figure 2. Validation of shRNA-mediated knockdown *ISL1* in SK-N-BE(2) cells

(A) qPCR analysis of *ISL1* expression in *ISL1* KD SK-N-BE(2) cells mediated by *sh ISL1-1* and *sh ISL1-2*. Error bars represent \pm SD, n=5, *p<0.05; **p<0.01. (B, C)

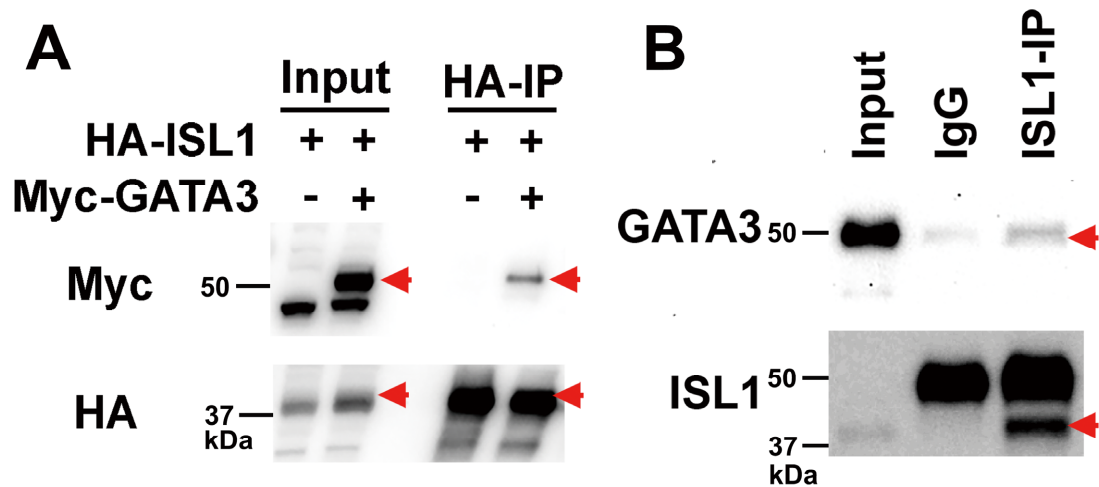
Validation by Western blot analysis of shRNA-mediated knockdown *ISL1* in SK-N-BE(2) cells. (C) Quantification of Western blots. Error bars represent \pm SD, n=5, *p<0.05; **p<0.01.



Supplementary Figure 3. Prevention of *EPAS1* elevation in *ISL1* KD SH-SY5Y cells is not able to restore neuroblastoma proliferation and RA-induced neurite outgrowth.

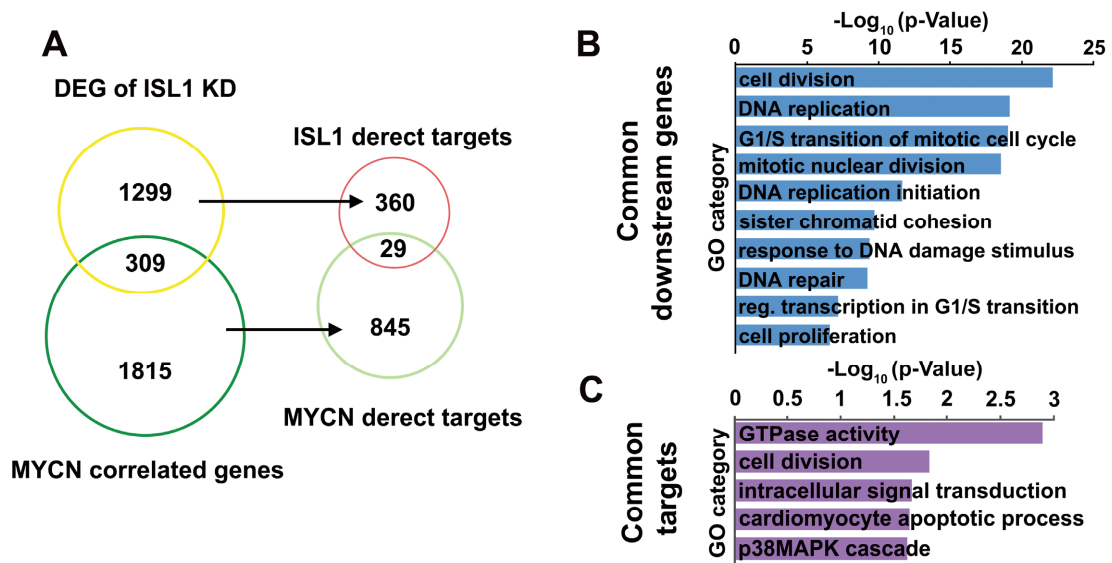
(A) qPCR analysis of *ISL1* KD and *ISL1+EPAS1* KD SH-SY5Y cells, showing downregulation of *EPAS1* in *ISL1* KD cells cannot restore expression of cell cycle-related genes and RA receptors. Error bars represent \pm SD, n=3, *p<0.05; **p<0.01, 2-tailed t-test. (B) EdU staining showing Knockdown of *EPAS1* is not able to restore the proliferation of *ISL1* KD neuroblastoma cells. (C) Quantification of EdU-positive cells. Error bars represent \pm SD, n=5, *p<0.05; **p<0.01. (D, E) neurite outgrowth of *ISL1*

KD, *ISL1/EPAS1*-double KD and control SH-SY5Y cells in the presence or absence of RA. Cells were fixed at 72 hours after RA or DMSO treatment and stained with *TUJ1* antibody. The images from three biological replicates were pooled, and the length of longest neurite of individual cells was measured. The number of measured cells (n) per culture is 100. (*p<0.05; **p<0.01; 2-tailed t-test).



Supplementary Figure 4. Coimmunoprecipitation of ISL1 and GATA3

(A) Coimmunoprecipitation (IP) of ISL1 and GATA3 in HEK293T cells co-transfected with HA-ISL1 and Myc-GATA3. (IP with anti-HA antibody). Myc-GATA3 and HA-ISL1 (red arrowhead) were shown by Western blot using anti-Myc antibody and anti-HA antibody, respectively. (B) Coimmunoprecipitation of endogenous ISL1 and GATA3. Anti-ISL1 antibody was used for IP in SH-SY5Y cells. GATA3 and ISL1 (red arrowhead) were shown by Western blot.



Supplementary Figure 5. Common downstream genes and common targets of *ISL1* and *MYCN*.

(A) Overlay of the 1608 DEGs (differentially expressed genes) found in *ISL1* KD SH-SY5Y cells with 2124 *MYCN*-correlated genes identified in Hsu's study by calculating the Spearman correlation coefficient between *MYCN* and other genes in neuroblastoma gene expression data. 309 genes are common downstream genes of *ISL1* and *MYCN* (left panel), only 29 common targets of *ISL1* and *MYCN* found by intersection of 389 *ISL1* direct targets and 874 *MYCN* direct targets identified in Hsu's study (right panel).

(B) GO analysis of common downstream genes of *ISL1* and *MYCN*. (C) GO analysis of common targets of *ISL1* and *MYCN*.

Supplementary Table 3 - Homor Known Motif Enrichment Results (ISL1 CHIP-seq peak)

Rank	Motif	Name	P-value	log P-value	q-value (Benjamini)	# Target Sequences with Motif	% of Targets Sequences with Motif	# Background Sequences with Motif	% of Background Sequences with Motif
1		Phox2a(Homeobox)/Neuron-Phox2a-ChIP-Seq(GSE31456)/Homer	1e-474	-1.092e+03	0.0000	1699.0	22.33%	2464.9	6.08%
2		GATA3(Zf)/Treg-Gata3-ChIP-Seq(GSE20898)/Homer	1e-449	-1.036e+03	0.0000	3021.0	39.71%	7110.6	17.55%
3		Isl1(Homeobox)/Neuron-Isl1-ChIP-Seq(GSE31456)/Homer	1e-434	-9.996e+02	0.0000	3438.0	45.20%	8949.5	22.08%
4		Gata4(Zf)/Heart-Gata4-ChIP-Seq(GSE35151)/Homer	1e-420	-9.686e+02	0.0000	2263.0	29.75%	4522.6	11.16%
5		Nkx6.1(Homeobox)/Isl1-Nkx6.1-ChIP-Seq(GSE40975)/Homer	1e-384	-8.854e+02	0.0000	4519.0	59.41%	14466.9	35.70%
6		Lhx3(Homeobox)/Neuron-Lhx3-ChIP-Seq(GSE31456)/Homer	1e-375	-8.636e+02	0.0000	3388.0	44.54%	9300.9	22.95%
7		Gata2(Zf)/K562-GATA2-ChIP-Seq(GSE18829)/Homer	1e-356	-8.204e+02	0.0000	1628.0	21.40%	2830.5	6.98%
8		Gata1(Zf)/K562-GATA1-ChIP-Seq(GSE18829)/Homer	1e-338	-7.794e+02	0.0000	1481.0	19.47%	2478.7	6.12%
9		Lhx1(Homeobox)/EmbryoCarcinoma-Lhx1-ChIP-Seq(GSE70957)/Homer	1e-295	-6.805e+02	0.0000	2545.0	33.46%	6574.5	16.22%
10		Lhx2(Homeobox)/HFSC-Lhx2-ChIP-Seq(GSE48068)/Homer	1e-236	-5.438e+02	0.0000	2241.0	29.46%	5951.6	14.69%
11		Olig2(bHLH)/Neuron-Olig2-ChIP-Seq(GSE30882)/Homer	1e-234	-5.399e+02	0.0000	2476.0	32.55%	6928.3	17.10%
12		Atoh1(bHLH)/Cerebellum-Atoh1-ChIP-Seq(GSE22111)/Homer	1e-233	-5.386e+02	0.0000	1606.0	21.11%	3568.1	8.80%
13		Nanog(Homeobox)/mES-Nanog-ChIP-Seq(GSE11724)/Homer	1e-200	-4.627e+02	0.0000	4730.0	62.18%	18185.8	44.88%
14		NeuroD1(bHLH)/Isl1-NeuroD1-ChIP-Seq(GSE30298)/Homer	1e-190	-4.377e+02	0.0000	1192.0	15.67%	2476.5	6.11%
15		NF1(CTF)/LNCaP-NF1-ChIP-Seq(Unpublished)/Homer	1e-154	-3.568e+02	0.0000	661.0	8.69%	1045.9	2.58%
16		GATA(Zf),IR3/Treg-Gata3-ChIP-Seq(GSE20898)/Homer	1e-147	-3.401e+02	0.0000	514.0	6.76%	686.2	1.69%
17		SCL(bHLH)/HPC7-Sc1-ChIP-Seq(GSE13511)/Homer	1e-143	-3.312e+02	0.0000	4259.0	55.99%	16776.4	41.40%
18		GATA(Zf),IR4/Treg-Gata3-ChIP-Seq(GSE20898)/Homer	1e-124	-2.875e+02	0.0000	344.0	4.52%	366.3	0.90%
19		Bapx1(Homeobox)/VertebralCol-Bapx1-ChIP-Seq(GSE36672)/Homer	1e-112	-2.599e+02	0.0000	2124.0	27.92%	7061.6	17.43%
20		Ascl1(bHLH)/NeuralTubes-Ascl1-ChIP-Seq(GSE55840)/Homer	1e-108	-2.499e+02	0.0000	1603.0	21.07%	4883.7	12.05%
21		Tcf21(bHLH)/ArterySmoothMuscle-Tcf21-ChIP-Seq(GSE61369)/Homer	1e-105	-2.426e+02	0.0000	1158.0	15.22%	3131.5	7.73%
22		NF1-halfsite(CTF)/LNCaP-NF1-ChIP-Seq(Unpublished)/Homer	1e-94	-2.165e+02	0.0000	1839.0	24.18%	6124.6	15.11%
23		Ptf1a(bHLH)/Panc1-Ptf1a-ChIP-Seq(GSE47459)/Homer	1e-89	-2.067e+02	0.0000	2362.0	31.05%	8573.6	21.16%
24		Pdx1(Homeobox)/Isl1-Pdx1-ChIP-Seq(SRA008281)/Homer	1e-87	-2.008e+02	0.0000	1557.0	20.47%	5015.2	12.38%
25		Nkx2.5(Homeobox)/HL1-Nkx2.5.biotin-ChIP-Seq(GSE21529)/Homer	1e-85	-1.969e+02	0.0000	2093.0	27.51%	7420.7	18.31%
26		Pax7(Paired,Homeobox),long/Myoblast-Pax7-ChIP-Seq(GSE25064)/Homer	1e-81	-1.877e+02	0.0000	171.0	2.25%	133.0	0.33%
27		Tlx?(NR)/NPC-H3K4me1-ChIP-Seq(GSE16256)/Homer	1e-80	-1.856e+02	0.0000	541.0	7.11%	1140.6	2.81%
28		Tgif2(Homeobox)/mES-Tgif2-ChIP-Seq(GSE55404)/Homer	1e-78	-1.805e+02	0.0000	3166.0	41.62%	12711.5	31.37%
29		Nkx3.1(Homeobox)/LNCaP-Nkx3.1-ChIP-Seq(GSE28264)/Homer	1e-75	-1.730e+02	0.0000	2126.0	27.95%	7788.4	19.22%
30		Smad2(MAD)/ES-SMAD2-ChIP-Seq(GSE29422)/Homer	1e-71	-1.643e+02	0.0000	1414.0	18.59%	4670.0	11.52%

Supplementary Table 6 - sequence sets used for RNAi

Gene symbols	sequences
sh ISL1-1	GCAAGTGAAGTAGCATCAATGT
sh ISL1-2	GGCATGAGGAACATTTCATTCT
sh GATA3	GGGCTCTACTACAAGCTTCAC
sh Ctrl	CCTAAGGTTAAGTCGCCCTCG

Gene symbols	sequences
si Ctrl sense	UUCUCCGAACGUGUCACGUTT
si Ctrl anti-sense	ACGUGACACGUUCGGAGAATT
si MYCN sense	GCCACUGAGUAUGUCCACUTT
si MYCN anti-sense	AGUGGACAUACUCAGUGGCTT
si EPAS1 sense	GCCACAGCAUGGACAUGAATT
si EPAS1 anti-sense	UUCAUGUCCAUGCUGUGGCTT

Supplementary Table 7 - primer list

Gene	odut long	Primer Sequence
qISL1_for	114	GTGTATCACATCGAGTGTTC
qISL1_rev	114	CACCACATCGTGGTCTGC
qISL1_for	85	AAGGACAAGAAGCGAAGCAT
qISL1_rev	85	TTCCTGTCATCCCCTGGATA
qGATA3_for	170	AATGAACGGACAGAACCGG
qGATA3_rev	170	TGTGAAGCTTGTAGTAGAGCCC
qMYCN_for	176	ACCCGGACGAAGATGACTTCT
qMYCN_rev	176	CAGCTCGTTCTCAAGCAGCAT
qMYCN_for	113	TGATCCTCAAACGATGCCTTC
qMYCN_rev	113	GGACGCCTCGCTCTTTATCT
qMKI67_for	152	AAGAAGAGGTCCTACCAGTCG
qMKI67_rev	152	CATCCCAGTTCATAGTTTGC
qCENPF_for	97	ATAAACTCACATCAGTAAAGCAACA
qCENPF_rev	97	ATCTGACTCGCCTGGAACG
qBRCA1_for	173	GTTACTGAGCCACAGATAATACAAG
qBRCA1_rev	173	TGACCATTCTGCTCCGTTT
qBARD1_for	94	CTGCTCGCGTTGTAACAT
qBARD1_rev	94	TCCAATGCAGTCACTTACACAAT
qCCNE1_for	104	GCCAGCCTTGGACAATAATG
qCCNE1_rev	104	CTTGACGTTGAGTTTGGGT
qCDCA4_for	161	TCAGCACAGGCACCAAGGCA
qCDCA4_rev	161	GGGCTGTCCACGTCTGAGAACA
qAURKA_for	108	GGAATATGCACCACTTGAACA
qAURKA_rev	108	TAAGACAGGGCATTGCCAAT
qLMO1_for	121	TCTGCTGAAGGCATTGGA
qLMO1_rev	121	TCGGCACAGGATGAGGTT
qLIN28B_for	199	ATATCGGTGTGCTGTGATGC
qLIN28B_rev	199	TGAGCAACGCTTATCATGTTTT
qCTBP2_for	108	ATCCACGAGAAGGTTCTAAACGA
qCTBP2_rev	108	CCGCACGATCACTCTCAGG
qMYB_for	127	GAGGTGGCATAACCACTTGAA
qMYB_rev	127	AGGCAGTAGCTTTGCGATTTT
qLOX_for	128	CGGCGGAGGAAAACGTCT
qLOX_rev	128	TCGGCTGGGTAAGAAATCTGA
qLUM_for	120	TGAGCTGGGCTGTCCTTTCTCC
qLUM_rev	120	CCCAGTCACGCCAGTCTCTGAA
qMMP2_for	112	GATACCCCTTTGACGGTAAGGA
qMMP2_rev	112	CCTTCTCCAAGGTCCATAGC

qRXRA_for	89	GACGGAGCTTGTGTCCAAGAT
qRXRA_rev	89	AGTCAGGGTTAAAGAGGACGAT
qRORA_for	102	ACTGGTGTGCATAGCGGAGGTT
qRORA_rev	102	CCTGCGGACTGGCAATAATCGG
qRARA_for	109	GGCAAATACACTACGAACAACA
qRARA_rev	109	CTCCACAGTCTTAATGATGCACT
qRET_for	100	AAAGTGGCATTGGGCCTCTAC
qRET_rev	100	GCAGGGCATGGACGTACAG
qVIP_for	114	GACACCAGAAATAAGGCCAG
qVIP_rev	114	GTCACCCAACCTGAGAGCAG
qEPAS1_for	115	CGGAGGTGTTCTATGAGCTGG
qEPAS1_rev	115	AGCTTGTGTGTTCCGAGGAA
qSYT4_for	113	ATGGGATACCCTACACCCAAAT
qSYT4_rev	113	TCCCAGAGAGAGGAATTAGAACTT
qSPRY1_for	102	GAGAGAGATTCAGCCTACTGCT
qSPRY1_rev	102	GCAGGTCTTTTCACCACCGAA
qTP53_for	110	GTGCGTGTTTGTGCCTGTCCT
qTP53_rev	110	AGTGCTCGCTTAGTGCTCCCT
qHAND2_for	118	GATTCCGGGTTAGCTTCGGTGC
qHAND2_rev	118	GGAGGCAGAATCCTCTCGTGCT
qCDKN1A_for	139	TGTCCGTCAGAACCCATGC
qCDKN1A_rev	139	AAAGTCGAAGTTCCATCGCTC
qPCNA_for	85	GTTCGCCCGCTCGCTCTGA
qPCNA_rev	85	TGGCAACAACGCCGCTACA
LMO1 luc_fov	553	GTAGGGGTTGGAGTTCAGC
LMO1 luc_rev	553	AGGGGCTCTGTAGTCTCCT