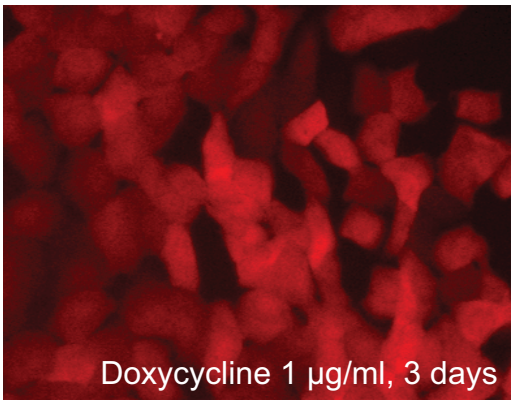
Supplementary Figure S1.

A



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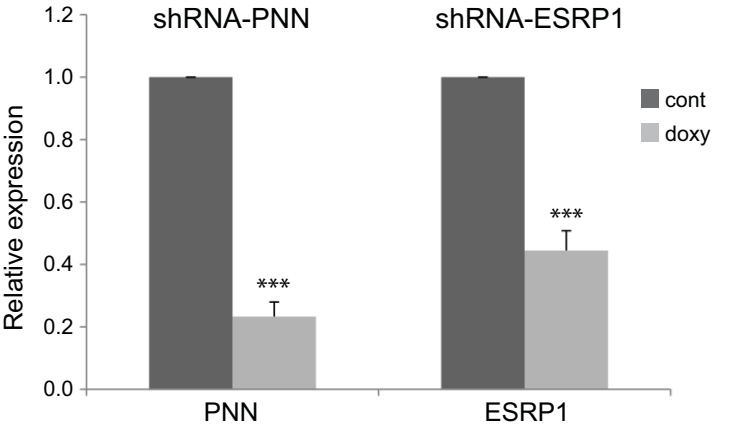

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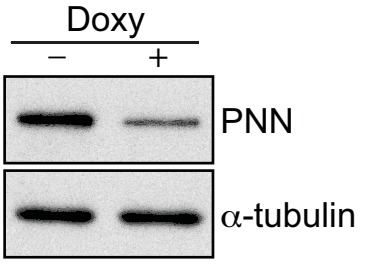

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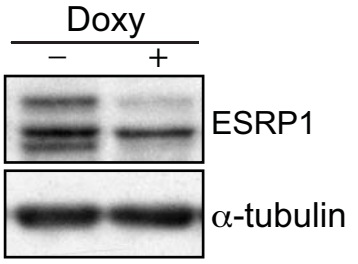

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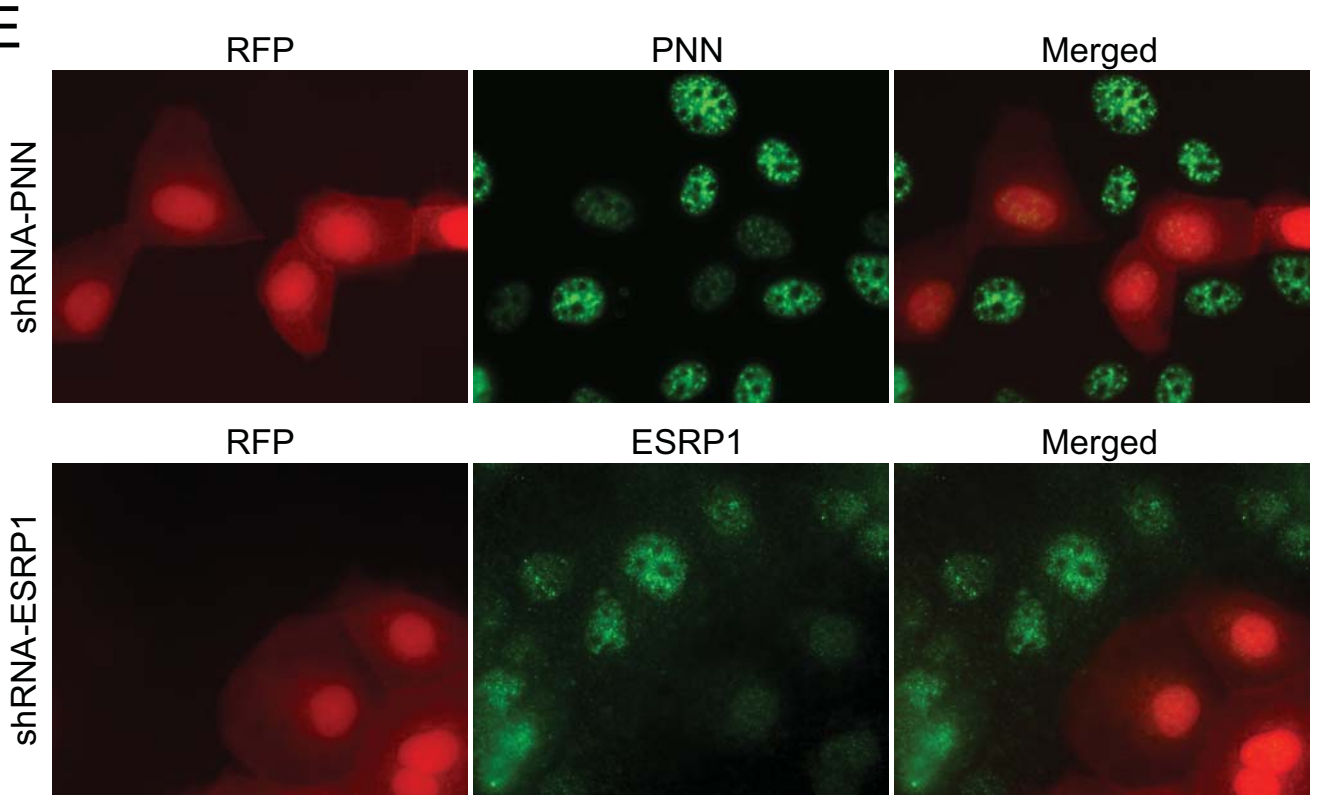
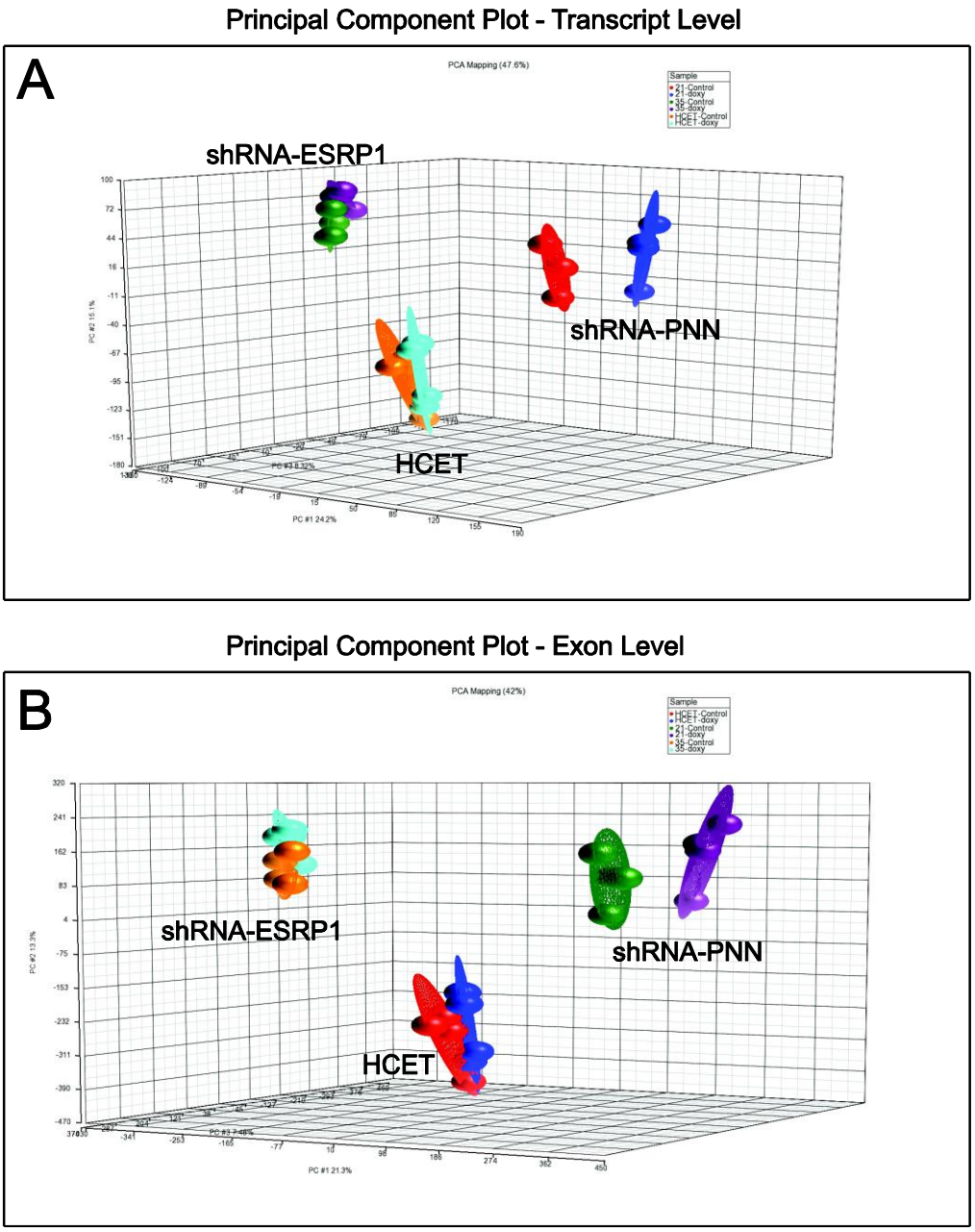


Supplementary Figure S1. (**A**) A stable HCET cell line simultaneously expressing RFP reporter gene and shRNA-ESRP1 upon doxycycline treatment is shown. All established cell lines for PNN or ESRP1 knockdown are 100% RFP positive. (**B**) Quantitative real-time RT-PCR assays confirm reduction of PNN and ESRP1 transcripts upon doxycycline induction at day 3. Expression levels are normalized to Gapdh. Error bars represent standard deviation. All *p* values are compared to each control samples. ***: p <0.001 (two-tailed unpaired Student's t-tests, n=3). (**C, D**) Immunoblotting assays demonstrate successful knockdown of PNN and ESRP1 at protein level in doxycycline-treated cells (day 3). α-tubulin serves as an internal control for equal amount of protein between samples. (**E**) Immunofluorescence analyses also demonstrate specific knockdown of PNN and ESRP1 proteins after doxycycline induction. For the direct comparison of reduced protein levels in doxycycline-induced cells, shRNA-PNN and shRNA-ESRP1 HCET cells were mixed with normal HCET cells and treated with doxycycline for three days. Specific reduction of PNN or ESRP1 protein is evident in RFP-positive induced cells, while uninduced parental HCET cells exhibit normal protein levels.

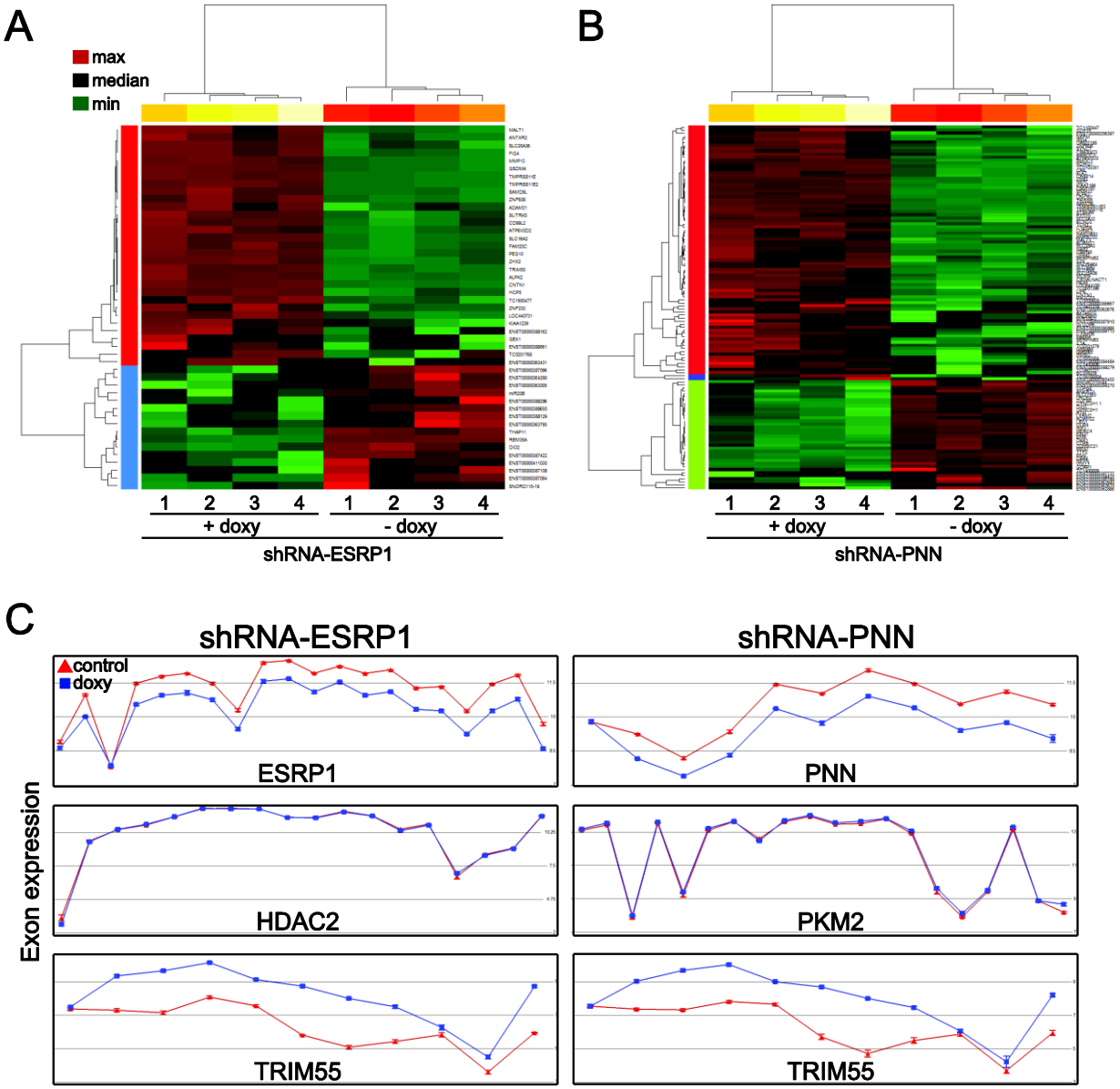
Supplementary Figure S2.



Supplementary Figure S2. (**A, B**) To visualize overall pattern of expression profile and to identify the potential outlier samples, principal component analyses (PCA) on normalized transcript levels and exon expression values from GG-H array were conducted. Four biological replicates of parental HCET, shRNA-PNN HCET, shRNA-ESRP1 HCET samples with/without doxycycline are plotted. Each cell group segregated apart from each other, however, doxycycline treatment did not seem to cause significant

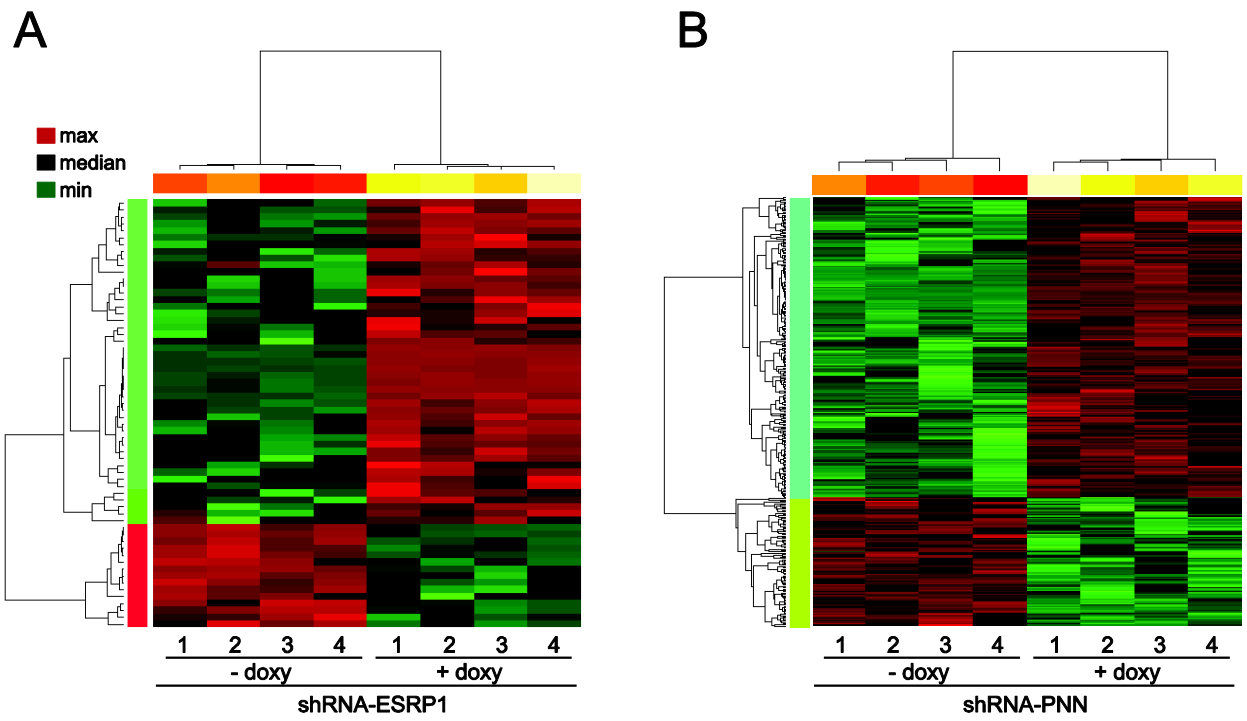
segregation within the same group. Principal component analysis (PCA) was conducted using Partek Genomics Suite (Partek Inc., St. Louis, MO).

Supplementary Figure S3.



Supplementary Figure S3. (**A, B**) Hierarchical clustering and heat map analysis of genes with differential transcript levels in ESRP1 or PNN knockdown HCET cells (>1.5-fold, p-value <0.05, $\log_2$-transformed). Four biological replicates for each condition are shown. Hierarchical clustering was performed based on Pearson's correlation and average linkage. (**C**) Exon-level profiles of representative genes are shown for ESRP1 or PNN knockdown HCET cells. All exons of ESRP1 and PNN show reduced level following their knockdown (top panel). While exon levels of HDAC2 and PKM2 are not affected by ESRP1 and PNN knockdown, respectively (middle panel), TRIM55 exhibited significant increase from both cell lines (bottom panel). Array data were RMA (robust multi-array analysis) background-corrected, quantile-normalized, and summarized into exon/gene expression using median-polish as implemented in Partek Genomics Suite software (v6.6).

Supplementary Figure S4.



Supplementary Figure S4. (**A, B**) Clustered heat maps of alternatively spliced exons from ESRP1 and PNN knockdown HCET cells demonstrate clear segregation of affected exons from control samples. 62 exons from 45 genes and 323 exons from 260 genes are analyzed from ESRP1 and PNN knockdown HCET cells, respectively. Due to the large number of genes, gene labels are not shown. Cut off criteria: |splicing index| >1.5 and MIDAS p-value <0.01, $\log_2$-transformed.

Supplementary Table S1. A full list of identified genes.

Supplementary Table S2. A list of transcripts commonly upregulated in both cell lines.

Gene Symbol	lgFC*			p-value			FDR†		
	control HCET	shRNA -ESRP1	shRNA -PNN	Control HCET	shRNA -ESRP1	shRNA -PNN	Control HCET	shRNA -ESRP1	shRNA -PNN
TMPRSS11E	-0.150	**3.874**	**2.744**	1.1E-01	0.0E+00	0.0E+00	6.6E-01	0.0E+00	0.0E+00
TMPRSS11E2	-0.144	**3.632**	**2.499**	6.9E-02	0.0E+00	0.0E+00	5.8E-01	0.0E+00	0.0E+00
TRIM55	-0.112	**1.971**	**1.782**	4.8E-02	0.0E+00	0.0E+00	5.3E-01	0.0E+00	0.0E+00
ADAM21	-0.240	**1.492**	**1.623**	7.1E-02	0.0E+00	0.0E+00	5.9E-01	0.0E+00	0.0E+00
ALPK2	-0.037	**1.432**	**1.568**	5.2E-01	0.0E+00	0.0E+00	9.1E-01	0.0E+00	0.0E+00
MMP13	-0.085	**1.350**	**2.333**	1.4E-01	0.0E+00	0.0E+00	7.0E-01	0.0E+00	0.0E+00
ATP6V0D2	0.046	**1.256**	**0.778**	5.6E-01	0.0E+00	0.0E+00	9.3E-01	0.0E+00	1.0E-06
SAMD9L	0.150	**1.249**	**1.175**	2.9E-02	0.0E+00	0.0E+00	4.6E-01	0.0E+00	0.0E+00
ZHX2	0.053	**0.824**	**1.605**	1.8E-01	0.0E+00	0.0E+00	7.3E-01	0.0E+00	0.0E+00
ZNF608	0.069	**0.806**	**0.884**	2.1E-01	0.0E+00	0.0E+00	7.6E-01	0.0E+00	0.0E+00
SLC16A2	0.039	**0.786**	**0.581**	3.4E-01	0.0E+00	0.0E+00	8.4E-01	0.0E+00	0.0E+00
CNTN1	-0.011	**0.692**	**0.596**	8.3E-01	0.0E+00	0.0E+00	9.8E-01	0.0E+00	0.0E+00
SLC25A36	-0.082	**0.672**	**0.769**	1.5E-01	0.0E+00	0.0E+00	7.1E-01	0.0E+00	0.0E+00
MALT1	-0.132	**0.596**	**0.659**	1.2E-02	0.0E+00	0.0E+00	3.7E-01	0.0E+00	0.0E+00

* $\log_2$-transformed fold-change

† False discovery rate, Benjamini and Hochberg approach

Supplementary Table S3. A list of alternatively spliced genes identified in both cell lines with analogous splicing pattern.

Gene Symbol	Probe Set Id	Probe Set Type	Splicing Index		DABG* p-value		MADS† p-value		MIDAS‡ p-value	
			shRNA -ESRP1	shRNA -PNN	shRNA -ESRP1	shRNA -PNN	shRNA -ESRP1	shRNA -PNN	shRNA -ESRP1	shRNA -PNN
CENPT	893032	Exon 4	**1.9302**	**1.6500**	2.3E-06	1.3E-03	8.0E-15	6.7E-07	1.9E-04	1.5E-04
CENPT	787992	Exon 4	**5.5598**	**4.8870**	1.0E-14	5.4E-09	0.0E+00	0.0E+00	5.2E-07	2.8E-04
CENPT	761865	Exon 3	**5.4875**	**5.5347**	0.0E+00	0.0E+00	0.0E+00	0.0E+00	2.2E-06	1.3E-03
HMGCL	1120573	exon 2	**15.2577**	**5.3569**	0.0E+00	1.9E-08	0.0E+00	4.0E-09	2.3E-04	1.0E-03
CNGB1	337389	exon 31	**1.8118**	**3.2908**	9.3E-05	3.6E-05	3.5E-06	7.3E-08	4.4E-05	4.8E-05
CNGB1	976241	exon 29	**1.8664**	**1.8823**	4.5E-06	1.3E-07	7.3E-08	2.5E-09	5.3E-03	1.6E-04
CNGB1	457224	exon 26	**1.8656**	**2.5835**	4.8E-11	4.9E-13	1.5E-06	2.2E-13	3.7E-03	8.3E-05
CNGB1	346222	exon 20	**1.6389**	**1.8234**	3.0E-10	1.9E-11	3.9E-11	5.7E-12	3.2E-04	8.9E-04
HDGF	1183381	exon 3	**1.5503**	**2.2286**	1.8E-04	7.4E-05	2.5E-04	3.2E-06	4.1E-03	3.9E-03
SIRT2	544944	exon 1	**1.6806**	**1.9379**	3.5E-05	2.9E-08	1.2E-08	5.7E-09	1.1E-03	1.4E-03
GMPR2	1178334	exon 1	**1.5088**	**1.6554**	3.4E-09	2.8E-11	5.0E-08	1.2E-13	5.2E-05	2.6E-04
UBC	915477	intron 1	**10.4632**	**4.1772**	0.0E+00	1.5E-14	0.0E+00	0.0E+00	2.0E-05	5.1E-07
UBC	346556	intron 1	**8.0629**	**5.5113**	0.0E+00	0.0E+00	0.0E+00	0.0E+00	5.4E-06	1.4E-05
UBC	23958	intron 1	**7.1765**	**5.6587**	1.4E-14	1.4E-13	0.0E+00	0.0E+00	5.9E-08	5.1E-05

* Detection Above Background, † Microarray Analysis of Differential Splicing, ‡ Microarray Detection of Alternative Splicing

Supplementary Table S4. Validated genes exhibiting specific changes in alternative splicing pattern.

	Gene Symbol	Probe Set ID	Probe Set Type	TC Exp FC*	PS Exp FC†	Splicing Index	DABG‡ p-value	MADS§ p-value	MIDAS‖ p-value
shRNA -ESRP1	**ARHGEF11**	793026	exon 38	1.0356	2.2565	**2.1797**	2.3E-05	2.8E-11	1.4E-04
	SLC37A2	922657	exon 18	0.9350	0.5297	**0.5667**	0.0E+00	3.8E-15	2.0E-04
	CYBASC3	1101610	exon 7	1.0073	1.5947	**1.5810**	1.4E-05	9.4E-09	1.3E-05
	EPB41	1163951	exon 16a	1.0192	0.5546	**0.5446**	9.5E-12	2.6E-14	1.1E-05
	EPB41	479236	exon 16a	1.0192	0.6691	**0.6561**	2.5E-13	1.6E-11	3.7E-04
	LAS1L	399614	Exon 9	0.9634	1.6425	**1.7068**	3.2E-06	8.3E-13	9.6E-04
	MLPH	655367	Exon 9	1.0361	1.7513	**1.6853**	1.8E-10	6.5E-13	6.0E-04
	RALGPS2	920195	Exon 15	1.1278	0.7328	**0.6490**	0.0E+00	0.0E+00	6.5E-04
shRNA -PNN	**FOXJ3**	63542	int 11	1.0167	2.3105	**2.2728**	9.1E-16	0.0E+00	4.2E-06
	PAX6	981701	exon 5a	1.1617	2.2506	**1.9243**	5.2E-04	1.9E-04	5.7E-03
	NCSTN	681696	int 15	0.9851	0.9851	**2.3708**	6.9E-06	9.2E-11	2.8E-03
	PSENEN	1110849	int 3	1.2727	1.2727	**2.2115**	1.3E-10	0.0E+00	2.9E-05
	ECT2	968469	exon 24a	0.9808	0.9808	**1.5548**	1.4E-14	1.3E-09	4.9E-04
	FAM50A	770545	int 9	0.9568	0.9568	**2.0498**	5.1E-05	1.3E-15	2.1E-04
	GLT8D1	578917	Int 9	1.1082	1.1082	**0.7049**	2.4E-06	1.4E-02	1.9E-02

* Average transcript expression level fold change, † Average probeset expression level fold change
‡ Detection Above Background, § Microarray Analysis of Differential Splicing, ‖ Microarray Detection of Alternative Splicing