

# Supplementary Materials: Ewing's Sarcoma: An Analysis of miRNA Expression Profiles and Target Genes in Paraffin-Embedded Primary Tumor Tissue

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**Table S1.** Analysis of the change in expression of 954 miRNAs in 20 ES biopsies compared to MSCs from 4 normal donors. (Miltenyi Biotec).

**Table S2.** Analysis of the change in expression of 954 miRNAs in 20 ES biopsies compared to MSCs from 4 normal donors. 58 miRNAs which are suitable for further evaluation ( $p$ -value  $\leq 0.05$  was considered statistically-significant) are highlighted in yellow.

**Table S3.** Functional analysis [KEGG Pathway Enrichment] [Gene Ontology Enrichment].

Target Gene	Description	Target Gene	Description
<i>CDC34</i>	cell division cycle 34 homolog	<i>JAK1</i>	Janus kinase 1
<i>CDC25A</i>	cell division cycle 25 homolog A	<i>MAP2K1</i>	mitogen-activated protein kinase kinase 1
<i>CCND1</i>	Cyclin D1	<i>COL1A1</i>	Collagen, type I, alpha 1
<i>EWSR1</i>	Ewing sarcoma breakpoint region 1	<i>SP1</i>	Sp1 transcription factor
<i>NF2</i>	neurofibromin 2 (merlin)	<i>MCL1</i>	myeloid cell leukemia sequence 1 (BCL2-related)
<i>KRAS</i>	Kirsten rat sarcoma viral oncogene homolog	<i>TCL1A</i>	T-cell leukemia/lymphoma 1A
<i>E2F2</i>	E2F transcription factor 2	<i>ARHGD1A</i>	Rho GDP dissociation inhibitor (GDI) alpha
<i>IL6</i>	Interleukin 6 (Interferon, Beta 2)	<i>NKRF</i>	NFkB repressing factor
<i>CCR7</i>	chemokine (C-C motif) receptor 7	<i>MEOX2</i>	mesenchyme homeobox 2
<i>BCL2</i>	B-cell CLL/lymphoma 2	<i>RUNX3</i>	runt-related transcription factor 3
<i>HMGA2</i>	High mobility group AT-hook 2	<i>PTEN</i>	phosphatase and tensin homolog
<i>HMGA1</i>	High mobility group AT-hook 1.	<i>BMI1</i>	BMI1 polycomb ring finger oncogene
<i>MYC</i>	V-myc myelocytomatosis viral oncogene homolog (avian)	<i>TGFBR1</i>	transforming growth factor, beta receptor 1
<i>CDKN2A</i>	Cyclin-dependent kinase inhibitor 2A	<i>FBXW7</i>	F-box and WD repeat domain containing 7, E3 ubiquitin protein ligase
<i>BCL2L1</i>	B-cell CLL/lymphoma 2-LIKE PROTEIN 1	<i>TCL1A</i>	T-cell leukemia/lymphoma 1A
<i>COL1A2</i>	collagen, type I, alpha 2	<i>HOXC8</i>	homeobox C8
<i>HOXA5</i>	homeobox A5	<i>IKBK8</i>	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta
<i>RUNX3</i>	Runt related transcription factor 3	<i>HIF1A</i>	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
<i>PPARG</i>	peroxisome proliferator-activated receptor gamma	<i>CAV1</i>	caveolin 1, caveolae protein, 22 kDa
<i>CDKN1B</i>	cyclin-dependent kinase inhibitor 1B (p27, Kip1)	<i>ERBB2</i>	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog
<i>RNF2</i>	ring finger protein 2	<i>GSK3B</i>	glycogen synthase kinase 3 beta
<i>RALA</i>	v-ral simian leukemia viral oncogene homolog A (ras related)	<i>JAG1</i>	jagged 1
<i>WEE1</i>	WEE1 homolog (S. pombe)	<i>MET</i>	met proto-oncogene (hepatocyte growth factor receptor)
<i>E2F3</i>	E2F transcription factor 3	<i>CD44</i>	CD44 molecule (Indian blood group)
<i>CDK6</i>	cyclin-dependent kinase 6	<i>MTOR</i>	mechanistic target of rapamycin (serine/threonine kinase)
<i>RUNX2</i>	Runt-Related Transcription Factor 2	<i>SOD2</i>	superoxide dismutase 2, mitochondrial
<i>RAF1</i>	v-raf-1 murine leukemia viral oncogene homolog 1	<i>MMP1</i>	matrix metalloproteinase 1 (interstitial collagenase)
<i>SOX5</i>	SRY (sex determining region Y)-box 5	<i>CDKN1C</i>	cyclin-dependent kinase inhibitor 1C (p57, Kip2)
<i>E2F1</i>	E2F transcription factor 1	<i>TMED7</i>	transmembrane emp24 protein transport domain containing 7
<i>PTEN</i>	phosphatase and tensin homolog	<i>DNMT3A</i>	DNA (cytosine-5-)-methyltransferase 3 alpha
<i>TGFBR2</i>	transforming growth factor, beta receptor II (70/80 kDa)	<i>DNMT3B</i>	DNA (cytosine-5-)-methyltransferase 3 beta
<i>TIMP3</i>	TIMP metalloproteinase inhibitor 3	<i>COL4A2</i>	collagen, type IV, alpha 2
<i>PDCD4</i>	programmed cell death 4 (neoplastic transformation inhibitor)	<i>COL4A1</i>	collagen, type IV, alpha 1
<i>FGFRL1</i>	fibroblast growth factor receptor-like 1	<i>PPM1D</i>	protein phosphatase, Mg2+/Mn2+ dependent, 1D
<i>BDNF</i>	brain-derived neurotrophic factor	<i>PIK3R1</i>	phosphoinositide-3-kinase, regulatory subunit 1 (alpha)
<i>PTPN1</i>	protein tyrosine phosphatase, non-receptor type 1	<i>SPARC</i>	secreted protein, acidic, cysteine-rich (osteonectin)
<i>ISCU</i>	iron-sulfur cluster scaffold homolog ( <i>E. coli</i> )	<i>SERPINB9</i>	serpin peptidase inhibitor, clade B (ovalbumin), member 9

Table S3. Cont.

Target Gene	Description	Target Gene	Description
<i>POU4F2</i>	POU class 4 homeobox 2	<i>MYBL2</i>	v-myb myeloblastosis viral oncogene homolog (avian)-like 2
<i>IL6R</i>	interleukin 6 receptor	<i>NOTCH1</i>	Neurogenic locus notch homolog protein 1
<i>MYH1</i>	myosin, heavy chain 1, skeletal muscle, adult	<i>CDK4</i>	Cyclin-dependent kinase 4
<i>MYH2</i>	myosin, heavy chain 2, skeletal muscle, adult	<i>NOTCH2</i>	Neurogenic locus notch homolog protein 2
<i>MYH4</i>	myosin, heavy chain 4, skeletal muscle	<i>PDGFRA</i>	platelet-derived growth factor receptor, alpha polypeptide
<i>FOXO1</i>	Forkhead box O1	<i>MAP3K9</i>	mitogen-activated protein kinase kinase kinase 9
<i>PHB</i>	prohibitin	<i>IGF1R</i>	insulin-like growth factor 1 receptor
<i>SPRY2</i>	sprouty homolog 2	<i>ACVR1C</i>	activin A receptor, type IC
<i>IGF1</i>	insulin-like growth factor 1 (somatomedin C)	<i>GRB2</i>	growth factor receptor-bound protein 2
<i>ST14</i>	suppression of tumorigenicity 14 (colon carcinoma)	<i>CDC42</i>	cell division cycle 42 (GTP binding protein, 25 kDa)
<i>CCNT1</i>	cyclin T1	<i>OSCP1</i>	organic solute carrier partner 1
<i>MMP13</i>	matrix metalloproteinase 13 (collagenase 3)	<i>SOX2</i>	SRY (sex determining region Y)-box 2
<i>CYP1B1</i>	Cytochrome P450 1B1	<i>SP2</i>	Sp2 transcription factor
<i>BCL6</i>	B-cell CLL/lymphoma 6	<i>JUNB</i>	jun B proto-oncogene
<i>SOCS1</i>	suppressor of cytokine signaling 1	<i>JUND</i>	jun D proto-oncogene
<i>SNAI1</i>	snail homolog 1	<i>TGFB1</i>	transforming growth factor, beta 1
<i>UBE2I</i>	ubiquitin-conjugating enzyme E2I	<i>MECP2</i>	methyl CpG binding protein 2 (Rett syndrome)
<i>KIT</i>	V-Kit Hardy-Zuckerman 4 Feline Sarcoma Viral Oncogene Homolog	<i>TJP1</i>	tight junction protein 1
<i>PDGFB</i>	Platelet-derived growth factor beta polypeptide	<i>PTCH1</i>	patched 1
<i>ATXN1</i>	ataxin 1	<i>MEF2C</i>	myocyte enhancer factor 2C
<i>BMPR2</i>	bone morphogenetic protein receptor, type II (serine/threonine kinase)	<i>NFIA</i>	nuclear factor I/A
<i>TLR2</i>	toll-like receptor 2	<i>FOXO1</i>	forkhead box O1
<i>CDKN1A</i>	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	<i>PARP1</i>	poly (ADP-ribose) polymerase 1
<i>RB1</i>	Retinoblastoma 1	<i>RUNX1</i>	runt-related transcription factor 1
<i>TCEAL1</i>	transcription elongation factor A (SII)-like 1	<i>CALN1</i>	calneuron 1
<i>BCL2L11</i>	BCL2-like 11 (apoptosis facilitator)	<i>TGFB1</i>	transforming growth factor, beta-induced, 68 kDa

**Table S4.** Target Gene prediction of deregulated miRNAs.

miRNA	Target Gene
miR-937	CPOX. DCAF8. GLUL
miR-1303	SOAT. FHOD3. CDK6
miR-1908	COX2. COQ4. ATP6
miR-1915 *	PTCDI. WDR33
miR-762	AMD1
miR-379	IL11
miR-1286	U/I
miR-1275	U/I
miR-665	U/I
miR-602	U/I
miR-1248	U/I

\* From miRTarBase prediction tool (less or no evidence).

**Table S5.** Summary of chromosomal loci of deregulated miRNAs in ES tissue.

Chromosomal Locus	miRNA	up/down
11q23.1	miR-34c-3p	down
11p15.5	miR-210	up
11q12.1	miR-130a	up
11p15.5	miR-675	down
11	miR-1908	down
22q13.31	LET-7B	up
22q13.1	miR-659	down
22	miR-1286	down
19p13.13	miR-27a	up
19p13.13	miR-23a	up
19p13.2	miR-199b-3p	up
19p13.2	miR-199a-5p	up
19p13.2	miR-638	down
19q13.32	miR-330-3p	down
19q13.41	LET-7E	up
17p13.1	miR-195	up
17p13.1	miR-497	up
17p13.3	miR-212	down
17q21.32	miR-196a	up
17q22	miR-301a	up
17q23.1	miR-21	up
9q22.32	LET-7F	up
9q22.32	miR-27b	up
9q22.32	LET-7A	up
9q34.3	miR-602	down
8q24.3	miR-937	down
8q24.22	miR-30b	up
8	miR-151-5p	up
1p36.22	miR-34a	up
1p34.2	miR-30e	up
1q32.1	miR-181a	up
1q32.1	miR-181b	up

**Table S6.** miRXplore™ Microarrays: 954 microRNAs probe-sequence.

**Table S7.** TaqMan® MicroRNA Human Assays form Applied biosystem used in this study.

miRNA	AB Assay ID	Target Sequence (5'–3')
hsa-miR-181b	1098	AACAUUCAUUGCUGUCGGUGGG
hsa-miR-1915	121111_mat	CCCCAGGGCGACGCGGCGGG
hsa-miR-1275	2840	GUGGGGGAGAGGCUGUC
RNU6B	1093	CGCAAGGAUGACACGCAAUUCGUGAAGCGUCCAUUUUUU