Supplementary materials

miR-486 expression is regulated by DNA methylation in osteosarcoma

Short title: Epigenetic regulation of miR-486 in osteosarcoma

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Supplementary Table 1

Overview of TaqMan assays for miRNA qRT-PCR experiments

miRNA	AssayID	Mature sequence
miR-486-5p	1278	UCCUGUACUGAGCUGCCCCGAG

Endogenous control

Assay	AssayID	Control sequence
RNU44	1094	CCTGGATGATGATAGCAAATGCTGACTGAACATGAAGGTCTTAATTAGCTCTAACTGACT

Overview of TaqMan assays for mRNA qRT-PCR experiments

mRNA	AssayID	Transcript variant
ANK1	Hs00986630_m1	Variant 1-4
ANK1	Hs00986660_m1	Variant 9
ANK1	Hs00252830_m1	Variant 5,7,10 (sANK1).
GAPDH	Hs99999905_m1	

Supplementary Table 2

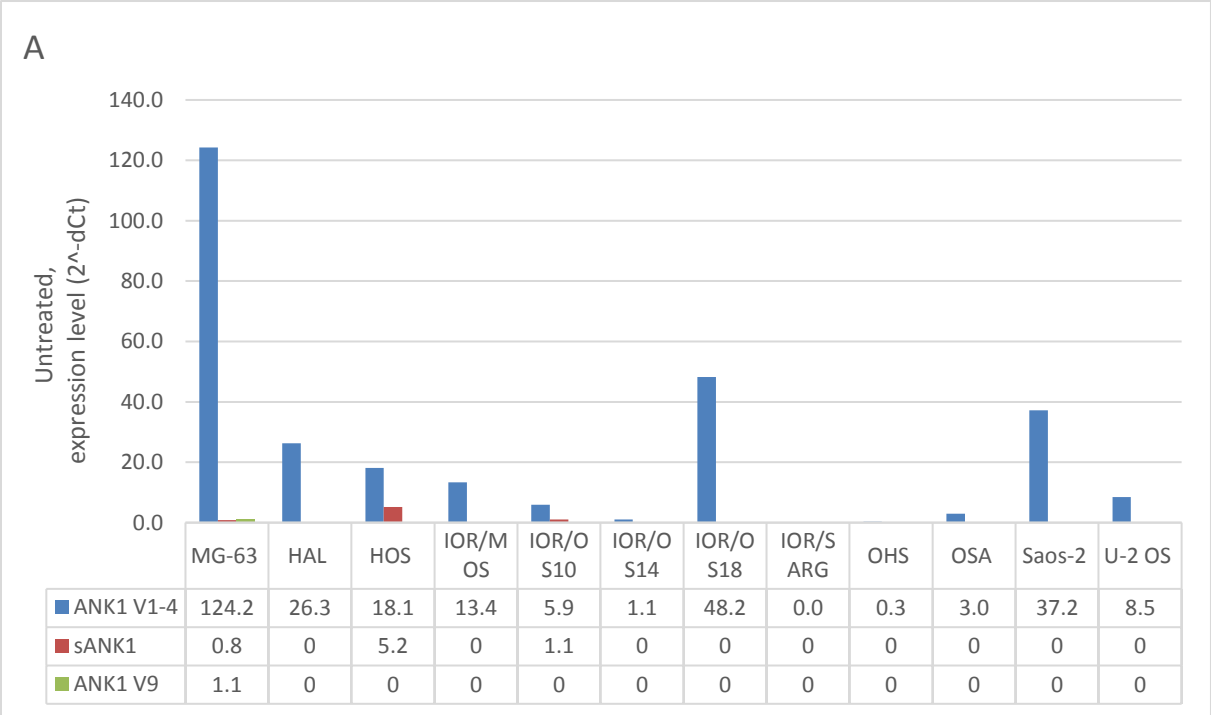
Primers used for qualitative Methylation-Specific Polymerase Chain Reaction (MSP) and bisulfite sequencing (BS).

Primer Set	Forward Primer	Reverse Primer	Frgagment size	Fragment Location
mir-486 MSP-M	GTAATTTGAGGTTTTTAGGC	ACTAACGAACTCACCGCA	152	-128 to +24
mir-486 MSP-U	TTTGTAATTTGTGGTTTTTAGGT	AACTAACAAACTCACCACAAC	156	-131 to +25
mir-486 BS	GTGGTTTGATGTTTAATTTTGG	TAACCCCCTCCTAACATCTC	424	-261 to +163

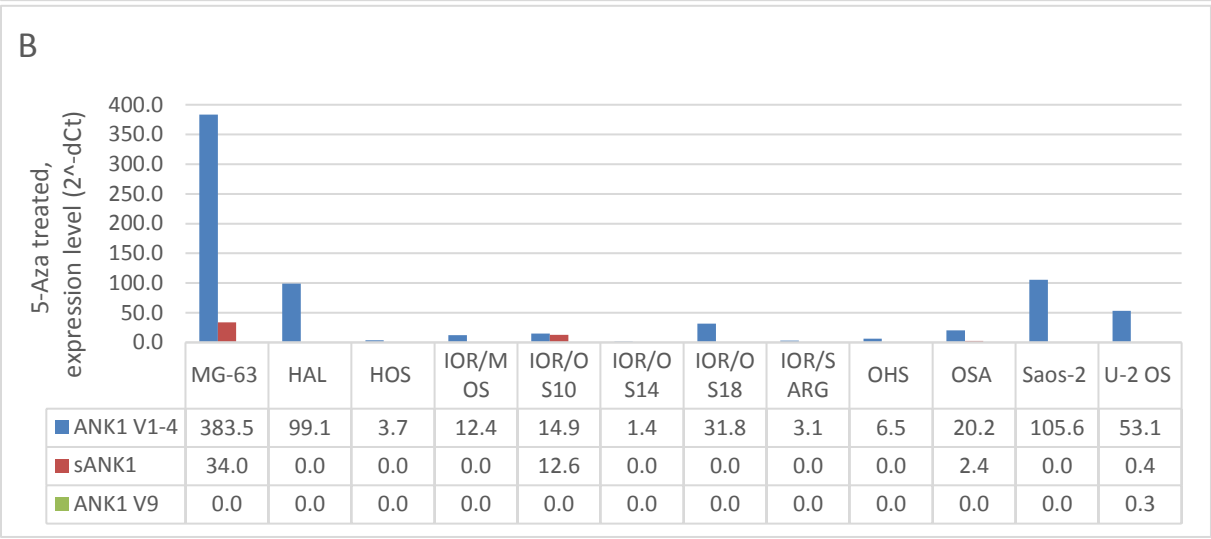
Abbreviations: MSP, methylation-specific polymerase chain reaction; BS, bisulfite sequencing; M, methylated-specific primers; U, unmethylated-specific primers; Frg. Size, fragment size (in bases); Fragment location indicates the start and end point (in bases, provided by the USCS Genome Browser) of each fragment relative to the transcription start site of *ANK1* variant 1-4 for *mir-486*.

Primers and probe used for quantitative Methylation-Specific Polymerase Chain Reaction (qMSP).

Assay	Forward Primer	Reverse Primer	Probe	Fragment size
mir-486	TTAGGCGGGCGTTATTTTTTC	GTACGAACCAAACCCCCGA	TTCGGTTCGATAGTAAGC	100



	MG-63	HAL	HOS	IOR/MOS	IOR/OS10	IOR/OS14	IOR/OS18	IOR/SARG	OHS	OSA	Saos-2	U-2 OS
ANK1 V1-4	124.2	26.3	18.1	13.4	5.9	1.1	48.2	0.0	0.3	3.0	37.2	8.5
sANK1	0.8	0	5.2	0	1.1	0	0	0	0	0	0	0
ANK1 V9	1.1	0	0	0	0	0	0	0	0	0	0	0



	MG-63	HAL	HOS	IOR/MOS	IOR/OS10	IOR/OS14	IOR/OS18	IOR/SARG	OHS	OSA	Saos-2	U-2 OS
ANK1 V1-4	383.5	99.1	3.7	12.4	14.9	1.4	31.8	3.1	6.5	20.2	105.6	53.1
sANK1	34.0	0.0	0.0	0.0	12.6	0.0	0.0	0.0	0.0	2.4	0.0	0.4
ANK1 V9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3

Supplementary Figure 1. Expression of *ANK1* transcript variants in untreated and 5-Aza treated osteosarcoma cell lines. Quantification of *ANK1* using qRT-PCR in cell lines. **A.** Expression of untreated cells. **B.** Expression of 5-Aza treated cells. Expression normalized against GAPDH. Cell lines with undetected expression is set to 0.