

Title: The long noncoding RNA ASNR regulates degradation of Bcl-2 mRNA through its interaction with AUF1

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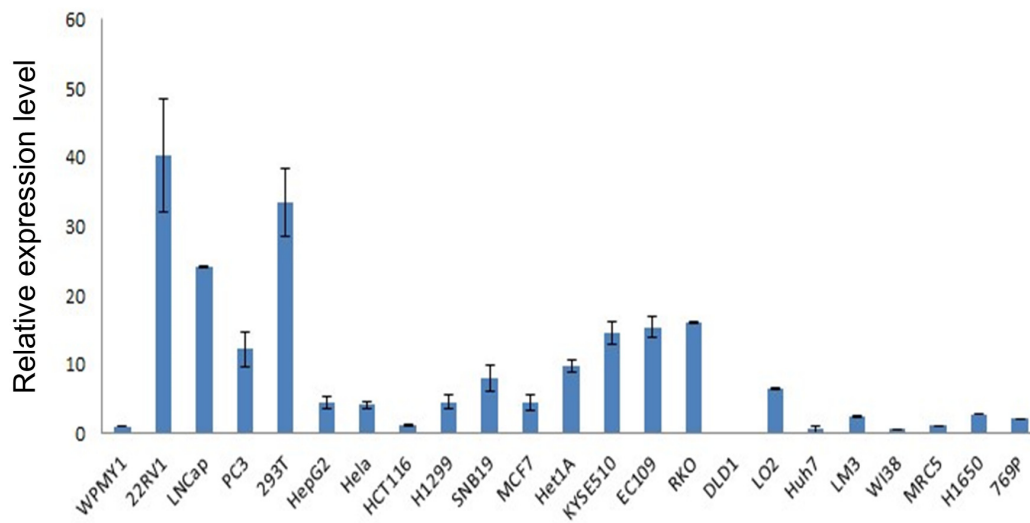
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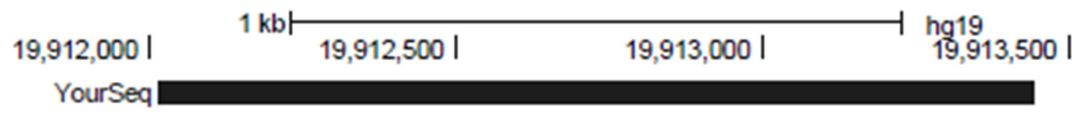
This PDF file includes:

Supplementary Figures 1-7 and Supplementary Table.



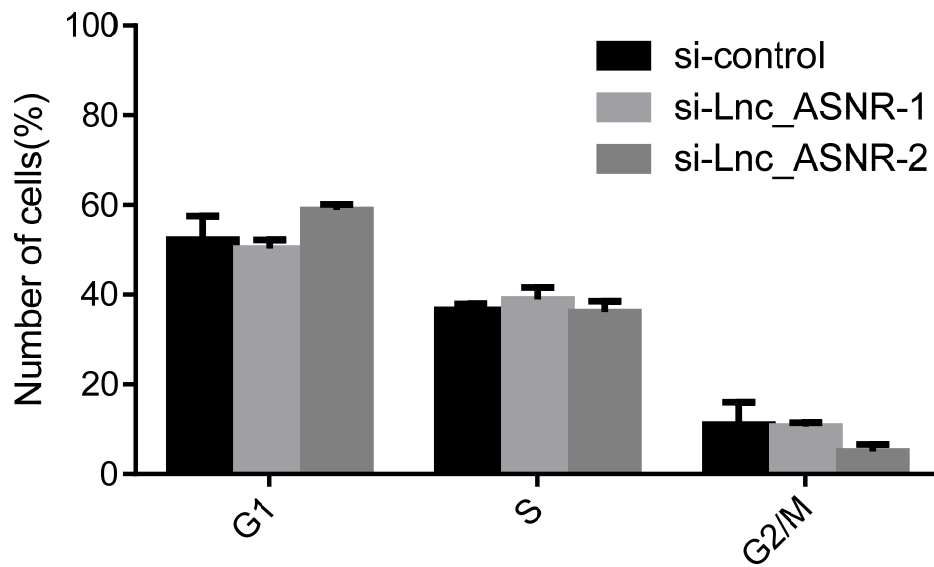
Supplementary Fig.1 Relative expression of Lnc_ASNR in different cells

Quantification of Lnc_ASNR expression in 23 kinds of cell lysates by qRT-PCR and GAPDH serve as controls for gene expression. Error bars represent SEM, n=3.



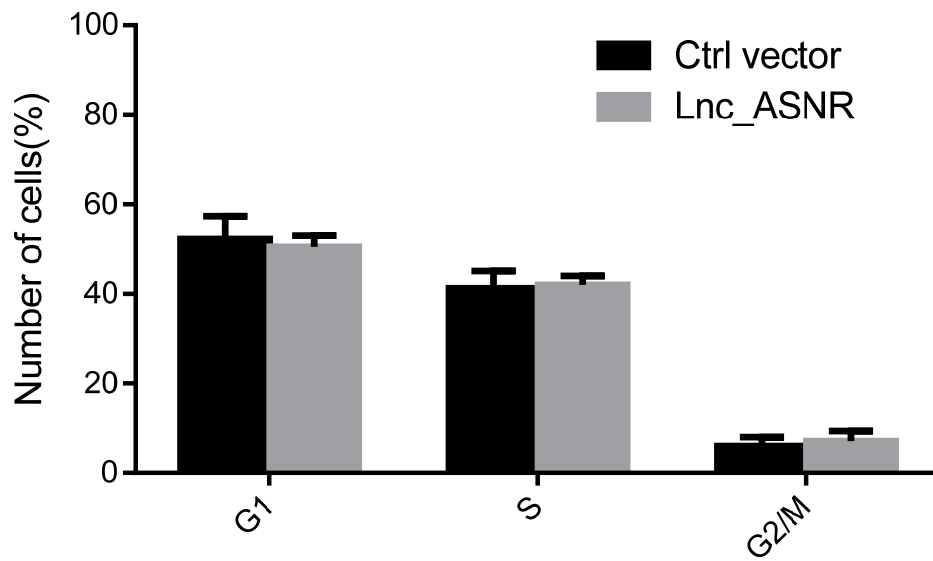
Chr14 >19913257-19912166

Supplementary Fig.2 3' RACE and 5' RACE of Lnc_ASNR.



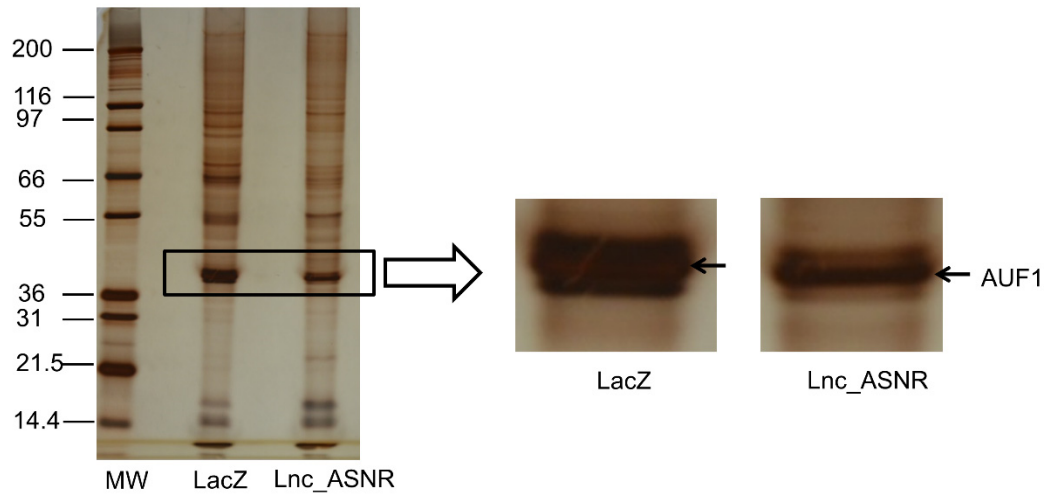
Supplementary Fig.3 Lnc_ASNR knockdown doesn't affect cell cycle regulation.

Cell cycle results of Lnc_ASNR knockdown were measured by flow cytometry. The X-axis represents cell cycle phases. The Y-axis gives the results from quantification (% cells) average of three independent replicate experiments. Bars indicate SEM.



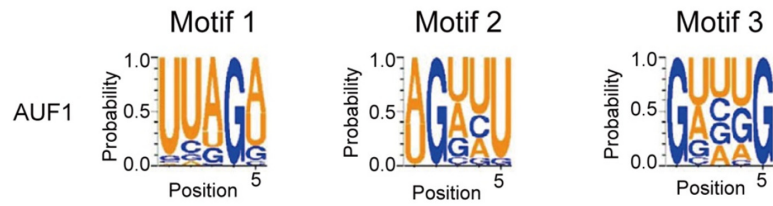
Supplementary Fig.4 Overexpression of Lnc_ASNR doesn't affect cell cycle regulation.

Cell cycle results of Lnc_ASNR overexpression were measured by flow cytometry. The X-axis represents cell cycle phases. The Y-axis gives the results from quantification (% cells) average of three independent replicate experiments. Bars indicate SEM.



Supplementary Fig.5 Silver stains gel of RNA-pulldown.

SDS-PAGE gel of proteins pulled down by the Lnc_ASNR and LacZ probes. Band indicated by arrows were submitted for mass spectrometry.



Motif 1 :

No	start	end
1	19912323	19912327
2	19912585	19912589

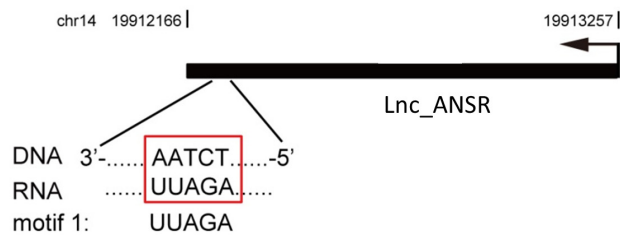
Motif 3 :

No	start	end
1	19912399	19912403
2	19912579	19912583
3	19913579	19913583

Motif 2 :

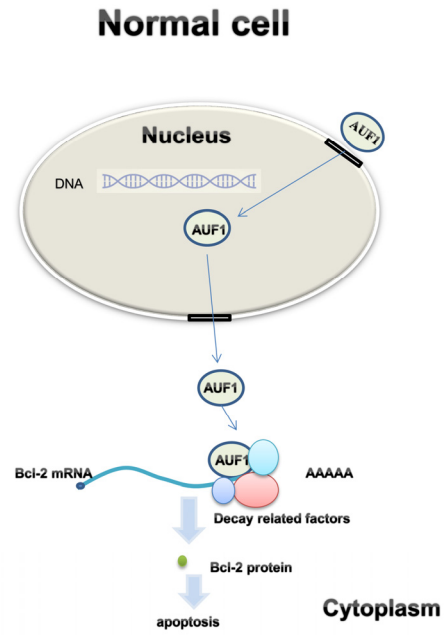
No	start	end
1	19912290	19912294
2	19912398	19912402
3	19912578	19912582
4	19912695	19912699
5	19912855	19912859
6	19913578	19913582
7	19913585	19913589

Example : Site 2 of motif 1



Supplementary Fig.6 Computational search the binding motifs of AUF1 across the full-length of Lnc_ASNR revealed the potential binding sites.

The binding motifs of AUF1 information from published reference. The binding sites across the full length of Lnc_ASNR were shown on the chromatin location. Example of binding position (UUAGA) of AUF1 showed that AUF1 binds to sequence UUAGA of Lnc_ASNR.



Supplementary Fig.7 The reported model of AUF1 mediated Bcl-2 degradation in normal cell.

In normal cells, AUF1 binds to the Bcl-2 mRNA ARE region in the cytoplasm, which resulting in Bcl-2 mRNA decay.

Supplementary Table

RACE primers

Lnc_ASNR-5'RACE-R1 5' GAAGAACAACAAATAAGACTTAAGAGGAAAAGGAAT 3'
Lnc_ASNR-5'RACE-R2 5' TGCAGCCTACTAAATCAGAATGAAAATAGA 3'
Lnc_ASNR-3'RACE-F1 5' AGTTCCTGCTTTCTTGTCAAAAATTGAAG 3'
Lnc_ASNR-3'RACE-F2 5' CTTCTAGGAACCCATCATCCAAGAC 3'

Northern Blotting Probe Primers

Lnc_ASNR LNA probe: /5DigN/TAATGGTGTAAGCAATGTGGAT the probe designed from exiqon webset

hGAPDH_F GGAAGGTGAAGGTCGGAGT capital nucleotides indicated T7 promoter sequences
hGAPDH_T7_R agtcatTAATACGACTCACTATAGGAAGGGGTGCTAAGCAGTTGGT

qPCR primers

Lnc_ASNR_q_F GAGAATTCCTCTGGTGGCTAAA
Lnc_ASNR_q_R GGAAGTGAATCTCACTGGACAA
AUF1-q-F ATGTCGGAGGAGCAGTTCGGCGGGGAC
AUF1-q-R TTAGTATGGTTTGTAGCTATTTTGATG
Bcl-2-q-F GGAGGATTGTGGCCTTCTTT
Bcl-2-q-R GTTCAGGTACTCAGTCATCCAC
linc01296-Qper-F TAATGATCACCTGCGCCTTC
linc01296-Qper-R AGCAACCATCGCCCTTATC
linc01296.1-Qper-F CATAGGTCACCAGCTTCACTTC
linc01296.1-Qper-R CACTCCATACCTTGCTCAACTAC
BMSF18/17-Qper-F GAAACCAGTGGGTGAGAAAGA
BMSF18/17-Qper-R CAGGCTGCATCTCCACTAATAC
DUXAP10-Qper-F GCAAGGTTTCTGAGGGACTT
DUXAP10-Qper-R TCACCATGTTCTGGCTCTTG
GAPDH_q_F TGCCATCAATGACCCCTTC
GAPDH_q_R CATCGCCCCACTTGATTTTG
U1_q_F GGGAGATACCATGATCACGAAGGT
U1_q_R CCACAAATTATGCAGTCGAGTTTCCC
β-actin_q_F ATTTAAAACTGGAACGGTGAAGGT
β-actin_q_R CTGTAACAACGCATCTCATATTTGG

Construct primers

Lnc_ASNR-F 5' GG **GGTACC** CAGGAAACTCCAAACTGTCCAAGGA 3'
 Lnc_ASNR-R 5' CG **GGATCC** GTAACAACTTTATTTTTTGGATGAGTGAAGACT 3'

siRNAs

	sense(5'-3')	antisense (5'-3')	r represents ribonucleic acid.
si-Lnc_ASNR-1	rGrGrArGrArArUrGrCrArGrArArUrGrGrUrCrArGrArCrUAC	rGrGrArGrArArUrGrCrArGrArArUrGrGrUrCrArGrArCrUAC	
si-Lnc_ASNR-2	rGrCrArGrGrArArCrUrUrGrArUrUrArCrUrUrUrGrArGrUGC	rGrCrArCrUrCrArArArGrUrArArUrCrArArGrUrUrCrCrUrGrCrUrU	
si-Lnc_ASNR-3	rArCrArCrCrArUrUrArGrGrCrArUrArGrArUrUrCrArGrUGT	rArCrArCrUrGrArArUrCrUrArUrGrCrCrUrArArUrGrGrUrGrUrArA	
si-AUF1-1	rCrCrArCrArArUrGrUrUrGrGrUrCrUrUrArGrUrArArArUGT	rArCrArUrUrUrArCrUrArArGrArCrCrArArCrArUrUrGrUrGrGrUrA	
si-AUF1-2	rArCrArArUrArUrCrArGrCrArArCrArGrCrArArCrArGrUGG	rCrCrArCrUrGrUrUrGrCrUrGrUrUrGrCrUrGrArUrArUrUrGrUrUrC	

Pulldown 3' biotinylated probes

Target	Probe Name	Sequence of Oligo (5'- to -3')
Lnc_ASNR	ASNR_p1	caacagaactatctccttgg
	ASNR_p2	cacctgagatttgcctac
	ASNR_p3	ccaagactgaggcactaaaa
	ASNR_p4	ctgtgacagtggcagaagaa
	ASNR_p5	ctcaagatgaaagactccct
	ASNR_p6	gcagaatggcagactacca
	ASNR_p7	ctatgcctaagtgtgaagc
	ASNR_p8	atctggatcaagcagtttgg
	ASNR_p9	gtgtgcagcctactaaatcag
Non-Targetting Control (LacZ)	lacZ_p1	GCCAGTGAATCCGTAATCAT
	lacZ_p2	AATGTGAGCGAGTAACAACC
	lacZ_p3	GATCTTCCAGATAACTGCCG
	lacZ_p4	GTCATCGATAATTTACCG
	lacZ_p5	TTAACGCCTCGAATCAGCAA
	lacZ_p6	ATTTGATCCAGCGATACAGC
	lacZ_p7	GGGTTGCCGTTTTCATCATA
	lacZ_p8	CACTTACGCCAATGTCGTTA