

**MIR152, MIR200B, MIR338,
human positional and functional Neuroblastoma candidates, are involved in
neuroblast differentiation and apoptosis**

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Supplementary Table 1

Primers used to analyze MIR genomic region, methylation and expression of MIR targets

Name	Sequence	Notes
GADD45A Fw	5'-TGACGAATCCACATTCATCTCAATG-3'	Primer Express
GADD45A Rev	5'-TCACCGTTCAGGGAGATTAATCAC-3'	
CHUK Fw	5'-AAGTTGAACCATGCCAATGTTGT-3'	From PrimerBank*
CHUK Rev	5'-TCTCCTCCAGAACAGTATTCCAT-3'	
CUL5 Fw	5'-GATTTTATGCGCCCGATTGTTT-3'	From PrimerBank*
CUL5 Rev	5'-GCTGGGCCTTTATCATCCCAA-3'	
ZEB1 Fw	5'-AAGAAAGTGTACAGATGCAGCTG-3'	From RTPrimerDB **
ZEB1 Rev	5'-CCCTGGTAACACTGTCTGGTC-3'	
PTPRT Fw	5'-GAGATCCGAGTGCTCCTCAC-3'	Primer Express
PTPRT Rev	5'-CATGTACCGGATCTGCACAC-3'	
TFAP2A Fw	5'-ACTTTGGGTACGTGTGCGAAA-3'	From PrimerBank *
TFAP2A Rev	5'-CGGAATGTTGTGCGTTGAGAAAT-3'	
SP1 Fw	5'-GGTGCCTTTTCACAGGCTC-3'	From PrimerBank *
SP1 Rev	5'-CATTGGGTGACTCAATTCTGCT-3'	
CREB1 Fw	5'-TGCCACATTAGCCCAGGTATCTATG-3'	Primer Express
CREB1 Rev	5'-CATTGGGCAGCTGTACTAGAGTTACG-3'	
G-MIR152 Fw	5'-CCCCTGGGTGCAGAAAGGT-3'	Primer Express
G-MIR152 Rev	5'-CGGCCAGGTTCTGTGATACA-3'	
G-MIR338-Fw	5'-CCCTGCCATGCCCACTCA-3'	Primer Express
G-MIR338-Rev	5'-GGCGTAACCTGCATTCTTTCCA-3'	
G-MIR200B-Fw	5'-AGACACCGGGCCTTTGAGA-3'	Primer Express
G-MIR200B-Rev	5'-CACGTGCTGCCTTGCTGA-3'	
MIR200B Fw	5'-ATGGGAGTTTAGGGGATATATTG-3'	Assay Design Software
MIR200B Rev	5'-BIO-ACCCTAACACAAAAAATCAATTCA-3'	for Pyrosequencing
MIR200B S	5'-GGGAGTTTAGGGGATATATT-3'	Sequencing primer

GAPDH and HPRT primer sequences were from Vandesompele et al ***

***Wang X, Seed B (2003) A PCR primer bank for quantitative gene expression analysis. Nucleic Acids Res 31: e15415.**

****Lefever S, Vandesompele J, Speleman F, Pattyn F (2009) RTPrimerDB: the portal for real-time PCR primers and probes. Nucleic Acids Res 37: D942-945. DOI gkn777 [pii] 10.1093/nar/gkn777**

*****Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3: RESEARCH0034**

Supplementary Table 2

MIR positional candidates in NB

Name	Accession number miRBase	Chromosome	Start	End	Strand	Band
hsa-mir-197	MI0000239	1	109943038	109943112	-	1p13
hsa-mir-552	MI0003557	1	34907787	34907882	-	1p34
hsa-mir-30e	MI0000749	1	40992614	40992705	+	1p34
hsa-mir-30c-1	MI0000736	1	40995543	40995631	-	1p34
hsa-mir-101-1	MI0000103	1	65296705	65296779	+	1p31
hsa-mir-186	MI0000483	1	71305902	71305987	+	1p31
hsa-mir-200b	MI0000342	1	1092347	1092441	-	1p36
hsa-mir-200a	MI0000737	1	1093106	1093195	-	1p36
hsa-mir-429	MI0001641	1	1094248	1094330	-	1p36
hsa-mir-551a	MI0003556	1	3467119	3467214	-	1p36
hsa-mir-34a	MI0000268	1	9134314	9134423	-	1p36
hsa-mir-554	MI0003559	1	149784896	149784991	-	1q21
hsa-let-7g	MI0000433	3	52277334	52277417	-	3p21
hsa-mir-135a-1	MI0000452	3	52303275	52303364	-	3p21
hsa-mir-138-1	MI0000476	3	44130708	44130806	+	3p21
hsa-mir-191	MI0000465	3	49033055	49033146	-	3p21
hsa-mir-425	MI0001448	3	49032585	49032671	-	3p21
hsa-mir-564	MI0003570	3	44878384	44878477	+	3p21
hsa-mir-565	MI0003571	3	45705468	45705564	-	3p21
hsa-mir-566	MI0003572	3	50185763	50185856	+	3p21
hsa-mir-10a	MI0000266	17	44012199	44012308	-	17q21
hsa-mir-152	MI0000462	17	43469526	43469612	-	17q21
hsa-mir-196a-1	MI0000238	17	44064851	44064920	-	17q21
hsa-mir-338	MI0000814	17	76714278	76714344	-	17q25
hsa-mir-637	MI0003652	19	3912412	3912510	-	19p13
hsa-mir-7-3	MI0000265	19	4721682	4721791	+	19p13
hsa-mir-638	MI0003653	19	10690080	10690179	+	19p13
hsa-mir-199a-1	MI0000242	19	10789102	10789172	-	19p13
hsa-mir-24-2	MI0000081	19	13808101	13808173	-	19p13
hsa-mir-27a	MI0000085	19	13808254	13808331	-	19p13
hsa-mir-23a	MI0000079	19	13808401	13808473	-	19p13
hsa-mir-181c	MI0000271	19	13846513	13846622	+	19p13
hsa-mir-181d	MI0003139	19	13846689	13846825	+	19p13
hsa-mir-639	MI0003654	19	14501355	14501452	+	19p13
hsa-mir-640	MI0003655	19	19406872	19406967	+	19p13

Supplementary Table 3

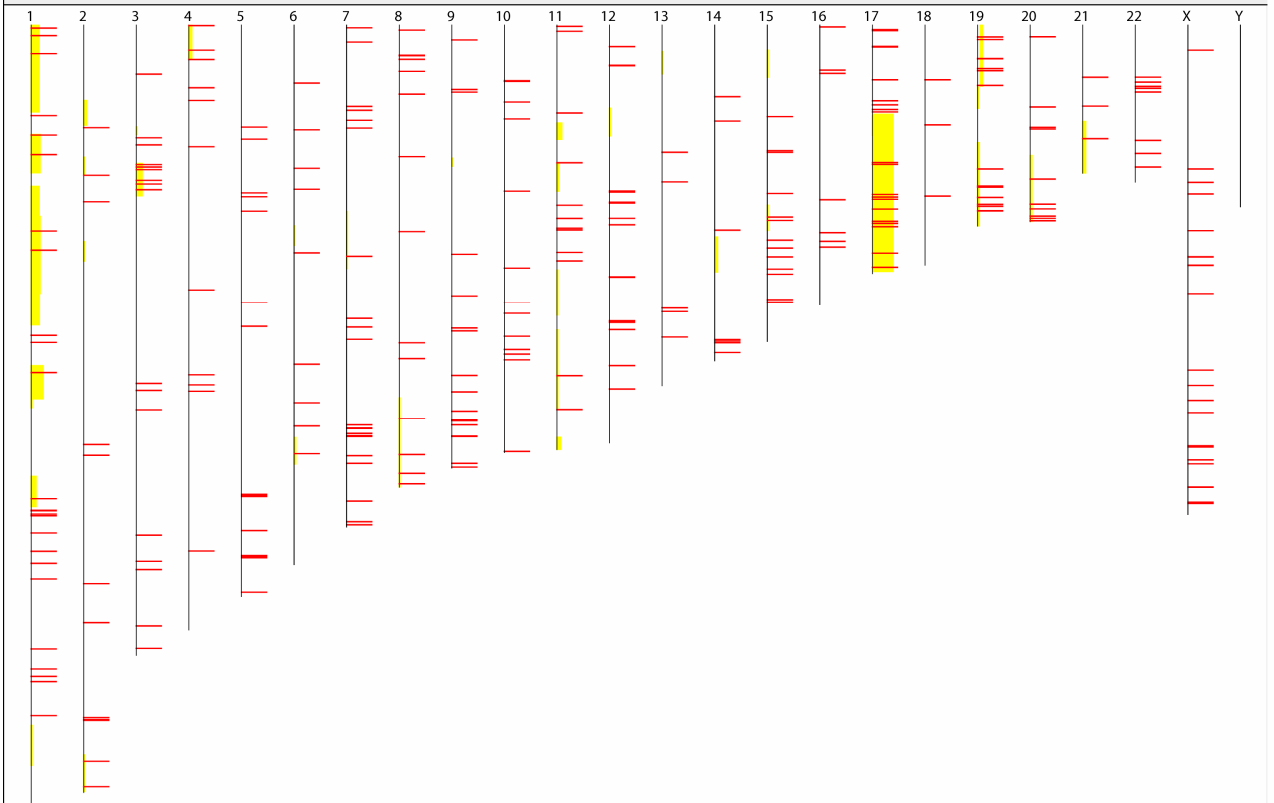
Most biologically relevant Gene Ontologies of MIR targets

GO TARGETS

MIR	NERVOUS SYSTEM DEVELOPMENT	POSITIVE REGULATION OF TRANSCRIPTION	TRANSCRIPTION FROM RNA POLYMERASE II PROMOTER	CELL PROLIFERATION	CELL DEATH
MIR152	CDK5R1, EDG1, EFNB2, EP300, NOG, NRP1, OTX1, POU4F2, ROBO1	ACVR1, DYRK1B, EDG1, EP300, NARG1, PPARGC1A, SMARCD1	EDG1, ELF5, EP300, MNT, OTX1, POU4F2, PPARGC1A, SMARCD1, TAF4, ZNF238	CDC25B, CDK5R1, CSF1, CUL5, EDG1, ELF5, KLF4, MNT, NRP1, SSR1	ACVR1, ATXN1, CDK5R1, CHUK, CUL5, EP300, GADD45A, MNT, PDIA3
MIR200B	ATRX, B3GNT1, CNTFR, CNTN4, DCX, EFNA1, EFNB2, EGR3, EIF2B5, EP300, FALZ, GATA2, GLI3, HDAC4, MBNL1, MYT1, NAB1, NDN, NFASC, NGEF, NOG, NR2C2, NTF3, PTPRZ1, SEMA6D, SIAH1, SLITRK1, TCF8, TUBB3, VLDLR, YWHAG, ZFH1B	ADRB2, ASF1A, CITED2, CREBBP, EP300, ESRRG, FALZ, GATA4, KLF13, NCOA2, NFYA, NOT7, NRIP1, NTF3, PCAF, SMARCD1, SOX2, SRF, THRAP1, ZFPM2, ZIC3	ADRB2, CITED2, CNOT7, E2F3, ELF2, EP300, FALZ, GABPA, GATA2, GATA4, GF11, GLI3, GTF2E1, HDAC4, HLF, ID2, KLF10, KLF12, KLF13, MXD4, MYCN, NCOA2, NFYA, NR3C1, NR5A2, NRIP1, NTF3, PRDM1, SOX2, SRF, TAF4, TARDBP, TBP, TCEB1, TCF8, THRAP1, TRPS1, TSC22D1, ZFPM2, ZIC3	ADRB2, APRIN, BAP1, BHLHB3, CNOT8, CNTFR, COL4A3, CTBP2, EDNRA, EPS8, ETS1, EVI5, GLI3, HDAC4, KHDRBS1, KLF10, KLF4, LAMC1, MXD4, MYCN, NDN, NPM1, PCAF, RAP1B, SESN1, SHC1, TCF8, TOB1	ADRB2, ARHGDI1A, ATXN1, CNTN4, COL4A3, DYRK2, EP300, HSPA9B, MMD, NPM1, NTF3, PDCD10, PPM1F, PPP2CA, PRKCA, SIAH1, SLK, SULF1, TBP, UBQLN1
MIR338	CNP, EN1, FOS, KIF2, MTPN, NRP1, SEMA4F, SEMA6A, TITF1, YWHAH	CNP, EN1, FOS, KIF2, MTPN, NRP1, SEMA4F, SEMA6A, TITF1, YWHAH	CBFB, CSDA, FOS, FOSB, GATA6, IRF2, MAFB, MYCN, NFE2L1, NRL, TITF1, ZNF238	ETS1, FGF9, IRF2, MYCN, NRP1, PTPRT, SKP2, TBC1D8	ACVR1, DYRK2, FAF1, PRKCG, PRKCZ, SEMA6A, SLK, SON, YWHAH

Supplementary Figure 1

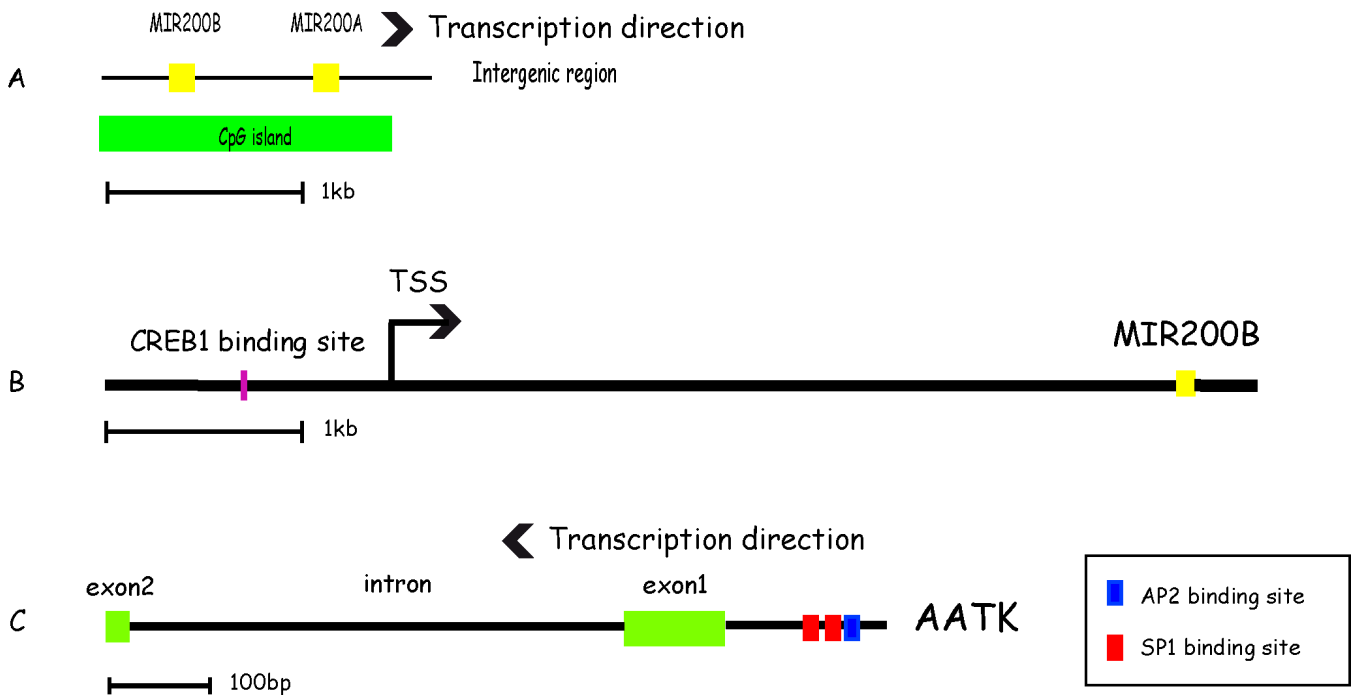
Overlapping between the Human Genome Map of MIRs and the Map of Chromosomal Structural Mutations in Neuroblastoma



- MiR genes
- █ Frequencies of Chromosomal Structural Mutations

Supplementary Figure 2

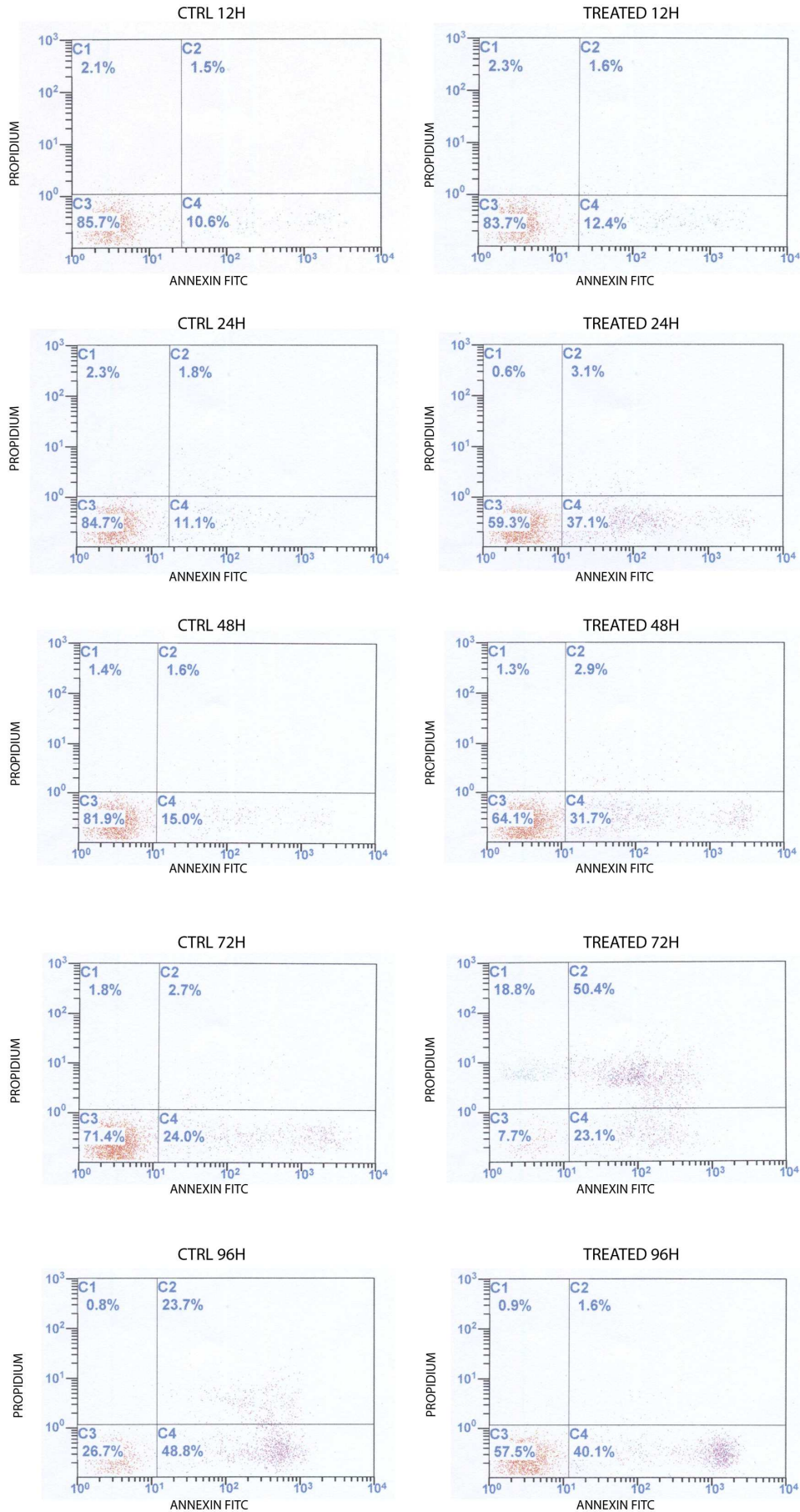
CpG island near the gene encoding MIR200B and predicted conserved TFBSs upstream of MIR200B and MIR338



(A) CpG island (5.5 Kb long) extending over the genomic region comprising the genes encoding MIR200B and MIR200A. (B) A conserved TFBS for CREB1 was found at position -4382 bp from the MIR200B gene TSS (Transcription Start Site). (C) Conserved TFBSs regulating MIR338 expression, located within the promoter of the host gene AATK. TFBSs for AP2 α and Sp1 were found about 100 bp upstream of the transcription start site of the AATK gene.

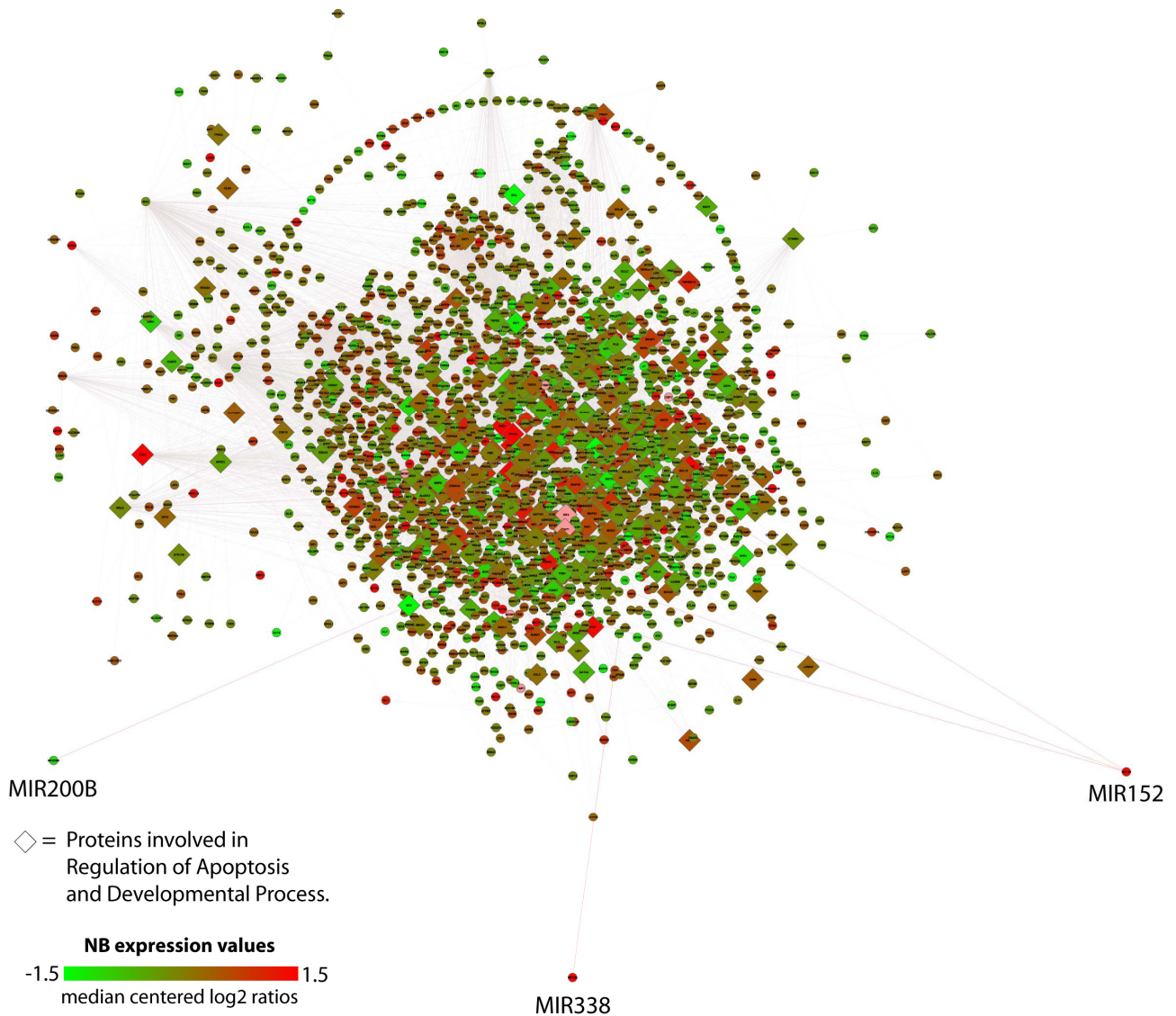
Supplementary Figure 3

Annexin-Propidium cytofluorimeter raw data



Supplementary Figure 4

Cellular Network based on molecular interactions among targets of MIR152, MIR200B, MIR338



Network was built up by using interactions from cytoscape APID2NET and MIMI plugins for MIR targets and their second level neighbours nodes. The colours of the nodes indicate their expression levels on NB, according to the bar shown above the network. The over-sized nodes with rhombus shape are proteins involved in Regulation of Apoptosis and Developmental Process.