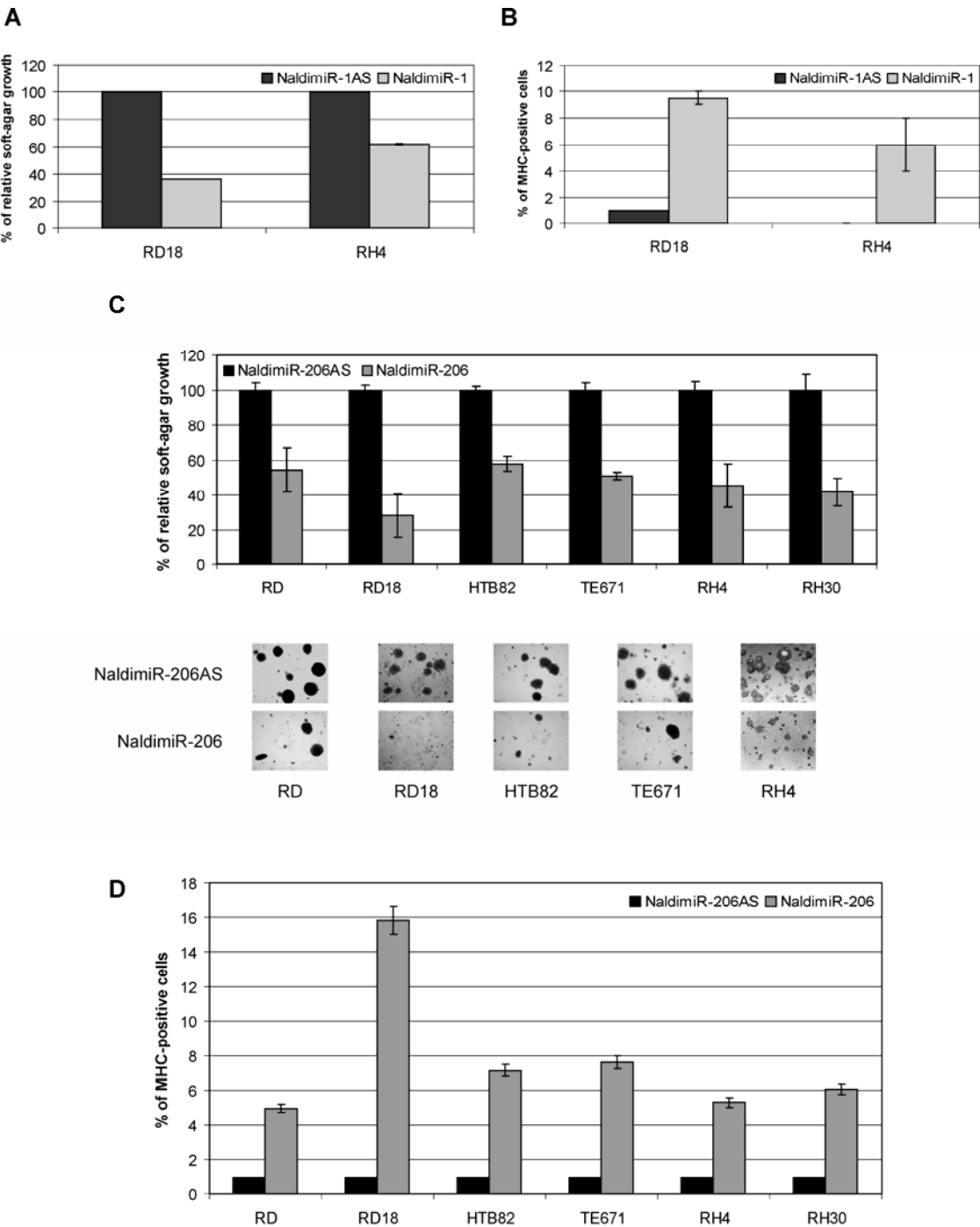
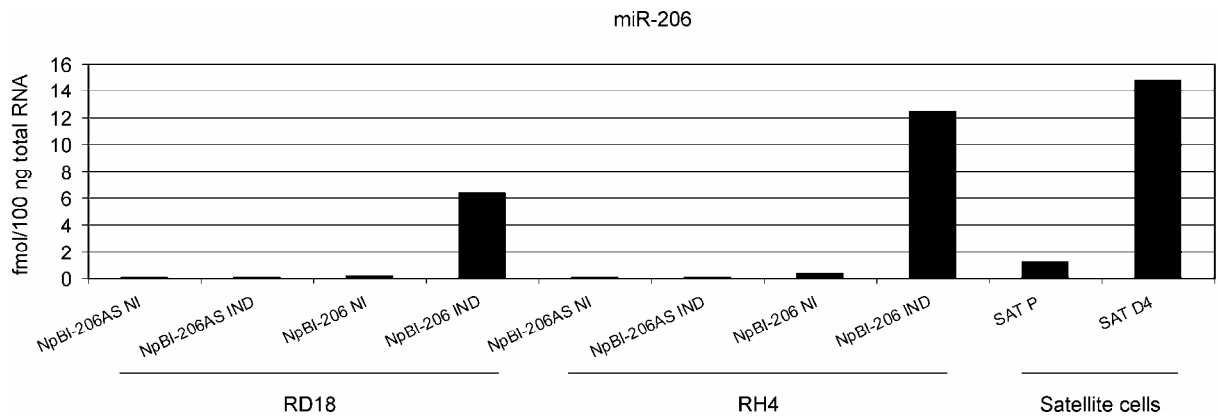


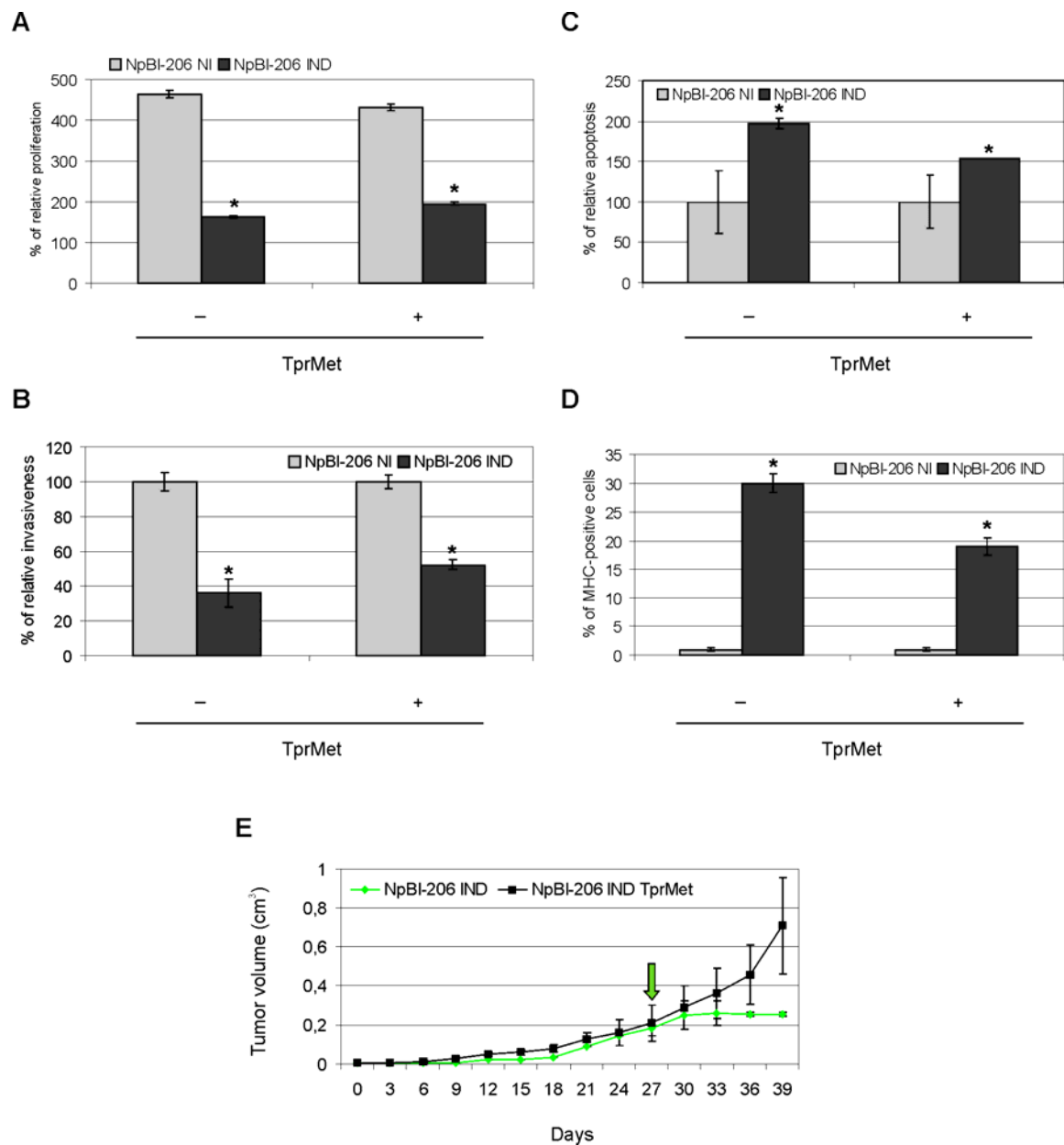
SUPPLEMENTAL DATA



Supplementary Figure 1 Expression of miR-1 and miR-206 in RMS cells impairs anchorage-independent growth and promotes myogenic differentiation. Cells were infected with the following lentiviral vectors: NaldimiR-1/206 and NaldimiR-1/206 antisense (AS) as a control. (A and C) soft agar growth assay. The number of colonies obtained from cells infected with the control vectors was set at 100%. Mean values ($\pm$ SEM) are from three independent experiments performed in triplicate. C, Bottom panel: representative images of soft agar colonies formation in cells expressing miR-206AS or miR-206. (B and D) MHC expression in RMS cells upon infection with NaldimiR-1 and-206 or with the control vectors. Values represent counts of 6 fields for each sample normalized against the number of DAPI-positive cells in the same fields (20X). Mean values ($\pm$ SEM) are from three independent experiments.



Supplementary Figure 2 Quantitative real-time PCR for mature miR-206 on RMS cells inducibly expressing miR-206. Cells infected with either the control (NpBI-206AS) or the miR-206-expressing (NpBI-206) vector (Tet-off) were first sorted for high GFP expression (after a brief induction in absence of doxycycline) and then treated (Non-Induced, NI) or not (Induced, IND) with doxycycline for 3 days. Proliferating and differentiated murine satellite cells were included in the analysis.



Supplementary Figure 3 Tpr-Met expression increases proliferation, invasiveness and tumorigenicity of miR-206-expressing RD18 cells, while it reduces apoptosis and the number of terminally differentiated cells. RD18 cells stably transduced with miR-206-expressing vector (NpBI-206, Tet-On) were infected with a Tpr-Met retrovirus and then treated (IND) or not (NI) with

doxycycline (1 μ g/ml). (A) proliferation was evaluated 5 days after doxycycline administration. The number of cells at day 0 was set at 100%. (B) invasiveness was evaluated after 72 hrs from seeding RMS cells in Matrigel-coated transwell chambers. Invasiveness of NI cells was set at 100% (not shown). (C) apoptosis was measured by Annexin V-APC staining and FACS analysis. The number of apoptotic cells in NI RD18 was set at 100%. (D) MHC expression in RMS cells upon miR-206 induction in high serum, compared to NI controls. Values represent counts of 6 fields for each group normalized against the number of DAPI-positive cells in the same fields (20X). All mean values ($\pm$ SD) are from three independent experiments. *, *P* value < 0.05 (Student's T-test). (E) tumor growth was analyzed using the Tet-On system: mice bearing NpBI-206 Tpr-Met xenografts (*n*=5) and NpBI-206 xenografts (*n*=3) were given drinking water containing 1 mg/ml of doxycycline starting on the day indicated by the arrow. Tumor growth was measured every three days starting when the tumors became palpable. Bars indicate SEM.

UPREGULATED GENES

Category	Term	Corrected <i>P</i> value
GOTERM_BP_ALL	muscle system process	5,80E-13
GOTERM_BP_ALL	muscle contraction	5,80E-13
GOTERM_BP_ALL	muscle development	1,00E-05
GOTERM_BP_ALL	regulation of muscle contraction	3,00E-03
GOTERM_BP_ALL	system process	2,40E-02
GOTERM_BP_ALL	system development	2,80E-02
GOTERM_BP_ALL	striated muscle contraction	3,10E-02
GOTERM_BP_ALL	anatomical structure development	3,10E-02
GOTERM_BP_ALL	cellular component organization and biogenesis	4,30E-02
GOTERM_BP_ALL	organ development	3,90E-02
GOTERM_BP_ALL	multicellular organismal process	3,60E-02

DOWNREGULATED GENES

Category	Term	Corrected <i>P</i> value
GOTERM_BP_ALL	cell cycle	1,30E-65
GOTERM_BP_ALL	cell cycle process	7,10E-58
GOTERM_BP_ALL	M phase	8,80E-58
GOTERM_BP_ALL	mitosis	2,40E-57
GOTERM_BP_ALL	M phase of mitotic cell cycle	3,70E-57
GOTERM_BP_ALL	cell cycle phase	3,60E-57
GOTERM_BP_ALL	mitotic cell cycle	5,40E-56
GOTERM_BP_ALL	cell division	8,10E-54
GOTERM_BP_ALL	DNA metabolic process	1,20E-45
GOTERM_BP_ALL	DNA replication	8,50E-32
GOTERM_BP_ALL	organelle organization and biogenesis	2,40E-24
GOTERM_BP_ALL	regulation of progression through cell cycle	8,30E-24
GOTERM_BP_ALL	regulation of cell cycle	1,00E-23
GOTERM_BP_ALL	response to DNA damage stimulus	1,50E-23
GOTERM_BP_ALL	chromosome organization and biogenesis	8,50E-22
GOTERM_BP_ALL	DNA repair	5,20E-21
GOTERM_BP_ALL	response to endogenous stimulus	1,20E-19
GOTERM_BP_ALL	chromosome segregation	1,40E-18
GOTERM_BP_ALL	DNA-dependent DNA replication	1,30E-16
GOTERM_BP_ALL	cell cycle checkpoint	6,20E-16
GOTERM_BP_ALL	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	5,80E-15
GOTERM_BP_ALL	regulation of mitosis	7,20E-15
GOTERM_BP_ALL	nucleosome assembly	5,10E-14
GOTERM_BP_ALL	chromatin assembly	7,30E-14
GOTERM_BP_ALL	biopolymer metabolic process	1,90E-13
GOTERM_BP_ALL	mitotic sister chromatid segregation	1,00E-12
GOTERM_BP_ALL	sister chromatid segregation	1,70E-12
GOTERM_BP_ALL	microtubule-based process	2,40E-12
GOTERM_BP_ALL	spindle organization and biogenesis	2,60E-12
GOTERM_BP_ALL	chromatin assembly or disassembly	8,50E-12
GOTERM_BP_ALL	cellular component organization and biogenesis	3,00E-11
GOTERM_BP_ALL	DNA packaging	1,00E-10

GOTERM_BP_ALL	protein-DNA complex assembly	2,50E-10
GOTERM_BP_ALL	cellular process	3,80E-10
GOTERM_BP_ALL	interphase	6,30E-10
GOTERM_BP_ALL	DNA replication initiation	8,90E-10
GOTERM_BP_ALL	establishment and/or maintenance of chromatin architecture	1,80E-09
GOTERM_BP_ALL	interphase of mitotic cell cycle	2,40E-09
GOTERM_BP_ALL	mitotic cell cycle checkpoint	4,60E-08
GOTERM_BP_ALL	cytoskeleton organization and biogenesis	6,30E-08
GOTERM_BP_ALL	microtubule cytoskeleton organization and biogenesis	6,50E-08
GOTERM_BP_ALL	macromolecular complex assembly	3,00E-07
GOTERM_BP_ALL	macromolecule metabolic process	4,30E-07
GOTERM_BP_ALL	cellular component assembly	5,10E-07
GOTERM_BP_ALL	primary metabolic process	2,90E-06
GOTERM_BP_ALL	response to stress	3,60E-06
GOTERM_BP_ALL	cellular metabolic process	4,20E-06
GOTERM_BP_ALL	cell proliferation	6,30E-06
GOTERM_BP_ALL	chromosome condensation	8,50E-06
GOTERM_BP_ALL	phosphoinositide-mediated signaling	1,20E-05
GOTERM_BP_ALL	regulation of progression through mitotic cell cycle	1,20E-05
GOTERM_BP_ALL	microtubule-based movement	2,20E-05
GOTERM_BP_ALL	mitotic chromosome condensation	3,10E-05
GOTERM_BP_ALL	regulation of cyclin-dependent protein kinase activity	4,30E-05
GOTERM_BP_ALL	cytoskeleton-dependent intracellular transport	1,30E-04
GOTERM_BP_ALL	establishment of chromosome localization	1,70E-04
GOTERM_BP_ALL	chromosome localization	1,70E-04
GOTERM_BP_ALL	DNA integrity checkpoint	1,70E-04
GOTERM_BP_ALL	traversing start control point of mitotic cell cycle	3,80E-04
GOTERM_BP_ALL	centrosome duplication	3,80E-04
GOTERM_BP_ALL	centrosome cycle	5,40E-04
GOTERM_BP_ALL	regulation of DNA metabolic process	5,70E-04
GOTERM_BP_ALL	spindle checkpoint	7,10E-04
GOTERM_BP_ALL	metabolic process	1,80E-03
GOTERM_BP_ALL	microtubule organizing center organization and biogenesis	1,80E-03
GOTERM_BP_ALL	centrosome organization and biogenesis	1,80E-03
GOTERM_BP_ALL	cytokinesis	2,60E-03
GOTERM_BP_ALL	meiosis	2,70E-03
GOTERM_BP_ALL	M phase of meiotic cell cycle	2,70E-03
GOTERM_BP_ALL	meiotic cell cycle	3,30E-03
GOTERM_BP_ALL	double-strand break repair	3,40E-03
GOTERM_BP_ALL	mitotic spindle organization and biogenesis	4,10E-03
GOTERM_BP_ALL	establishment of organelle localization	1,00E-02
GOTERM_BP_ALL	DNA unwinding during replication	1,10E-02
GOTERM_BP_ALL	DNA damage checkpoint	1,40E-02
GOTERM_BP_ALL	DNA synthesis during DNA repair	1,60E-02
GOTERM_BP_ALL	mitotic cell cycle spindle assembly checkpoint	1,60E-02
GOTERM_BP_ALL	DNA geometric change	1,70E-02
GOTERM_BP_ALL	DNA duplex unwinding	1,70E-02
GOTERM_BP_ALL	regulation of DNA replication	1,80E-02
GOTERM_BP_ALL	DNA damage response, signal transduction	1,80E-02
GOTERM_BP_ALL	second-messenger-mediated signaling	1,90E-02
GOTERM_BP_ALL	S phase	3,00E-02

GOTERM_BP_ALL	G1 phase of mitotic cell cycle	3,00E-02
GOTERM_BP_ALL	negative regulation of DNA metabolic process	3,00E-02
GOTERM_BP_ALL	organelle localization	3,40E-02
GOTERM_BP_ALL	regulation of kinase activity	3,70E-02
GOTERM_BP_ALL	regulation of transferase activity	4,40E-02

Supplementary Table 1. Comprehensive list of the enriched functional categories of genes modulated by miR-206 induction in RD18 cells with a corrected *P* value < 0.05.

Gene description	(*) Expected number of seed matches under evolutionary selective pressure	Location of the seed matches within the 3'UTR
	EIMMo score (*)	
Homo sapiens stress-associated endoplasmic reticulum protein 1 (SERP1), mRNA.	2,0213	3'UTR -X----- X----
Homo sapiens protein tyrosine phosphatase-like A domain containing 1 (PTPLAD1), mRNA.	1,8954	3'UTR -X----- X--
Homo sapiens solute carrier family 35, member B4 (SLC35B4), mRNA.	1,8726	3'UTR ----- X-----X- XX--X-
Homo sapiens gap junction protein, alpha 1, 43kDa (GJA1), mRNA.	1,7662	3'UTR --X----- X-
Homo sapiens glucose-6-phosphate dehydrogenase (G6PD), transcript variant 1, mRNA.	1,7147	3'UTR XXX-
Homo sapiens pogo transposable element with KRAB domain (POGK), mRNA.	1,7019	3'UTR ---- XXX--
Homo sapiens exportin 6 (XPO6), mRNA.	1,4451	3'UTR --X-
Homo sapiens met proto-oncogene (hepatocyte growth factor receptor) (MET), transcript variant 2, mRNA.	1,3449	3'UTR --X-X-- ----
Homo sapiens glycerol-3-phosphate dehydrogenase 2 (mitochondrial) (GPD2), nuclear gene encoding mitochondrial protein, transcript variant 2, mRNA.	1,1701	3'UTR -X----- --X-----
Homo sapiens polypyrimidine tract binding protein 1 (PTBP1), transcript variant 3, mRNA.	1,1417	3'UTR X----- X-
Homo sapiens polypyrimidine tract binding protein 1 (PTBP1), transcript variant 1, mRNA.	1,1417	3'UTR X----- X-
Homo sapiens polypyrimidine tract binding protein 1 (PTBP1), transcript variant 2, mRNA.	1,1417	3'UTR X----- X-
Homo sapiens polypyrimidine tract binding protein 1 (PTBP1), transcript variant 4, mRNA.	1,1417	3'UTR X----- X-
PREDICTED: Homo sapiens hypothetical protein MGC5139, transcript variant 3 (MGC5139), mRNA.	1,1341	3'UTR ----- --X---XX
Homo sapiens LIM and SH3 protein 1 (LASP1), mRNA.	1,1037	3'UTR X-X----- -----
Homo sapiens prothymosin, alpha (gene sequence 28) (PTMA), transcript variant 2, mRNA.	0,8649	3'UTR --X--
Homo sapiens ring finger protein 138 (RNF138), transcript variant 1, mRNA.	0,8436	3'UTR -----X- ----
Homo sapiens ring finger protein 138 (RNF138), transcript variant 2, mRNA.	0,8436	3'UTR -----X- ----
Homo sapiens splicing factor, arginine/serine-rich 9 (SFRS9), mRNA.	0,8436	3'UTR X--

Homo sapiens adenosine deaminase, RNA-specific (ADAR), transcript variant 2, mRNA.	0,8206	3'UTR ----- ---X
Homo sapiens adenosine deaminase, RNA-specific (ADAR), transcript variant 3, mRNA.	0,8206	3'UTR ----- ---X
Homo sapiens adenosine deaminase, RNA-specific (ADAR), transcript variant 4, mRNA.	0,8206	3'UTR ----- ---X
Homo sapiens adenosine deaminase, RNA-specific (ADAR), transcript variant 1, mRNA.	0,8206	3'UTR ----- ---X
Homo sapiens neuropilin 2 (NRP2), transcript variant 3, mRNA.	0,8206	3'UTR X----- -----
Homo sapiens neuropilin 2 (NRP2), transcript variant 2, mRNA.	0,8206	3'UTR X----- -----
Homo sapiens neuropilin 2 (NRP2), transcript variant 1, mRNA.	0,8206	3'UTR X----- -----
Homo sapiens phosphoglucomutase 2 (PGM2), mRNA.	0,8204	3'UTR -X-----
Homo sapiens CAP, adenylate cyclase-associated protein 1 (yeast) (CAP1), transcript variant 1, mRNA.	0,8151	3'UTR ---X---
Homo sapiens acidic (leucine-rich) nuclear phosphoprotein 32 family, member E (ANP32E), transcript variant 1, mRNA.	0,7940	3'UTR -X----- ---
Homo sapiens tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide (YWHAQ), mRNA.	0,7685	3'UTR --X----
Homo sapiens high-mobility group nucleosome binding domain 1 (HMGN1), mRNA.	0,7484	3'UTR -X---
Homo sapiens neuropilin (NRP) and tolloid (TLL)-like 2 (NETO2), mRNA.	0,7270	3'UTR --X-X--- -
Homo sapiens abhydrolase domain containing 3 (ABHD3), mRNA.	0,7195	3'UTR --X--
Homo sapiens transketolase (Wernicke-Korsakoff syndrome) (TKT), mRNA.	0,6725	3'UTR X-
Homo sapiens acidic (leucine-rich) nuclear phosphoprotein 32 family, member B (ANP32B), mRNA.	0,6725	3'UTR -X--
Homo sapiens chromosome 15 open reading frame 42 (C15orf42), mRNA.	0,6724	3'UTR -X----
Homo sapiens ornithine aminotransferase (gyrate atrophy) (OAT), nuclear gene encoding mitochondrial protein, mRNA.	0,6591	3'UTR X--X
Homo sapiens DnaJ (Hsp40) homolog, subfamily B, member 1 (DNAJB1), mRNA.	0,6384	3'UTR -X----
Homo sapiens solute carrier family 39 (zinc transporter), member 9 (SLC39A9), mRNA.	0,6093	3'UTR ---XX--- ---
Homo sapiens polymerase (DNA directed), alpha 1 (POLA1), mRNA.	0,5803	3'UTR ---X--
Homo sapiens gap junction protein, alpha 7, 45kDa (GJA7), transcript variant 1, mRNA.	0,5569	3'UTR -----X--- X----- -----
Homo sapiens tyrosyl-DNA phosphodiesterase 1 (TDP1), transcript variant 2, mRNA.	0,5569	3'UTR -X--X--- -
Homo sapiens tyrosyl-DNA phosphodiesterase 1 (TDP1), transcript variant 1, mRNA.	0,5569	3'UTR -X--X--- -
Homo sapiens nuclear transport factor 2-like export factor 2 (NXT2), mRNA.	0,5286	3'UTR -X----- --
Homo sapiens nucleoside phosphorylase (NP), mRNA.	0,4619	3'UTR --X-
Homo sapiens PC4 and SFRS1 interacting protein 1 (PSIP1), transcript variant 1, mRNA.	0,4619	3'UTR --X-
Homo sapiens actin-like 6A (ACTL6A), transcript variant 1, mRNA.	0,4618	3'UTR --X
Homo sapiens actin-like 6A (ACTL6A), transcript variant 3, mRNA.	0,4618	3'UTR --X
Homo sapiens actin-like 6A (ACTL6A), transcript variant 2, mRNA.	0,4618	3'UTR --X
Homo sapiens CP110 protein (CP110), mRNA.	0,4542	3'UTR ---X----- ---

Homo sapiens hydroxysteroid (17-beta) dehydrogenase 11 (HSD17B11), mRNA.	0,3918	3'UTR ---X-
Homo sapiens cytoskeleton associated protein 2 (CKAP2), transcript variant 1, mRNA.	0,3790	3'UTR -----X-
Homo sapiens Mov10, Moloney leukemia virus 10, homolog (mouse) (MOV10), transcript variant 1, mRNA.	0,3790	3'UTR -X
Homo sapiens leucine zipper-EF-hand containing transmembrane protein 2 (LETM2), mRNA.	0,3790	3'UTR -X
Homo sapiens cell division cycle 25 homolog C (S. pombe) (CDC25C), transcript variant 1, mRNA.	0,3325	3'UTR X--
Homo sapiens ring finger and WD repeat domain 3 (RFWD3), mRNA.	0,3325	3'UTR -----X--- -----
Homo sapiens cell division cycle 25 homolog C (S. pombe) (CDC25C), transcript variant 2, mRNA.	0,3325	3'UTR X--
Homo sapiens PAS domain containing serine/threonine kinase (PASK), mRNA.	0,3325	3'UTR -X-
Homo sapiens TATA box binding protein (TBP)-associated factor, RNA polymerase I, B, 63kDa (TAF1B), mRNA.	0,3325	3'UTR X--
Homo sapiens intraflagellar transport 52 homolog (Chlamydomonas) (IFT52), mRNA.	0,3325	3'UTR -X
Homo sapiens tripartite motif-containing 59 (TRIM59), mRNA.	0,3266	3'UTR -----X-- ----
Homo sapiens antigen identified by monoclonal antibody Ki-67 (MKI67), transcript variant 1, mRNA.	0,3266	3'UTR ----- X---
Homo sapiens BRI3 binding protein (BRI3BP), mRNA.	0,3266	3'UTR -X-
Homo sapiens pirin (iron-binding nuclear protein) (PIR), transcript variant 2, mRNA.	0,3266	3'UTR X-
Homo sapiens DBF4 homolog B (S. cerevisiae) (DBF4B), transcript variant 1, mRNA.	0,3266	3'UTR ---X--
Homo sapiens cyclin-dependent kinase 2 (CDK2), transcript variant 2, mRNA.	0,3266	3'UTR ---X---
Homo sapiens chromosome 18 open reading frame 54 (C18orf54), mRNA.	0,3266	3'UTR ----- X-----
Homo sapiens elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 2 (ELOVL2), mRNA.	0,3266	3'UTR --X----- -----
Homo sapiens structural maintenance of chromosomes 4 (SMC4), transcript variant 2, mRNA.	0,3266	3'UTR --X---
Homo sapiens kinesin family member 4A (KIF4A), mRNA.	0,3266	3'UTR --X-
Homo sapiens methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase (MTHFD2), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA.	0,3266	3'UTR -X---
Homo sapiens structural maintenance of chromosomes 4 (SMC4), transcript variant 1, mRNA.	0,3266	3'UTR --X---
Homo sapiens transmembrane protein 97 (TMEM97), mRNA.	0,3266	3'UTR -----X-- --
Homo sapiens apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C (APOBEC3C), mRNA.	0,3266	3'UTR -X-
Homo sapiens pirin (iron-binding nuclear protein) (PIR), transcript variant 1, mRNA.	0,3266	3'UTR X-
Homo sapiens cyclin-dependent kinase 2 (CDK2), transcript variant 1, mRNA.	0,3266	3'UTR ---X---
Homo sapiens cell division cycle associated 8 (CDCA8), mRNA.	0,3266	3'UTR -----X--
Homo sapiens family with sequence similarity 57, member A (FAM57A), mRNA.	0,2949	3'UTR X-----
Homo sapiens killer cell lectin-like receptor subfamily A, member 1 (KLRA1), mRNA.	0,2617	3'UTR ---X---
Homo sapiens gastric cancer up-regulated-2 (GCUD2), mRNA.	0,2492	3'UTR --X---
Homo sapiens histone cluster 1, H2bc (HIST1H2BC), mRNA.	0,2322	3'UTR X
Homo sapiens origin recognition complex, subunit 6 like (yeast) (ORC6L), mRNA.	0,2322	3'UTR X---
Homo sapiens hydroxyacyl-Coenzyme A dehydrogenase (HADH), nuclear gene	0,2303	3'UTR X---

encoding mitochondrial protein, mRNA.		
Homo sapiens regulator of chromosome condensation 1 (RCC1), transcript variant 3, mRNA.	0,2303	3'UTR ----X 3'UTR -----
Homo sapiens non-SMC condensin I complex, subunit H (NCAPH), mRNA.	0,2303	X--
Homo sapiens RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae) (RAD51), transcript variant 2, mRNA.	0,2303	3'UTR ----X-
Homo sapiens RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae) (RAD51), transcript variant 1, mRNA.	0,2303	3'UTR ----X-
Homo sapiens SHC SH2-domain binding protein 1 (SHCBP1), mRNA.	0,2303	3'UTR ----X-
Homo sapiens WEE1 homolog (S. pombe) (WEE1), mRNA.	0,0751	3'UTR ----X-

Supplementary Table 2. Comprehensive list of the predicted miR-206 targets

found among the downregulated transcripts in miR-206-induced versus non-induced RD18 cells.