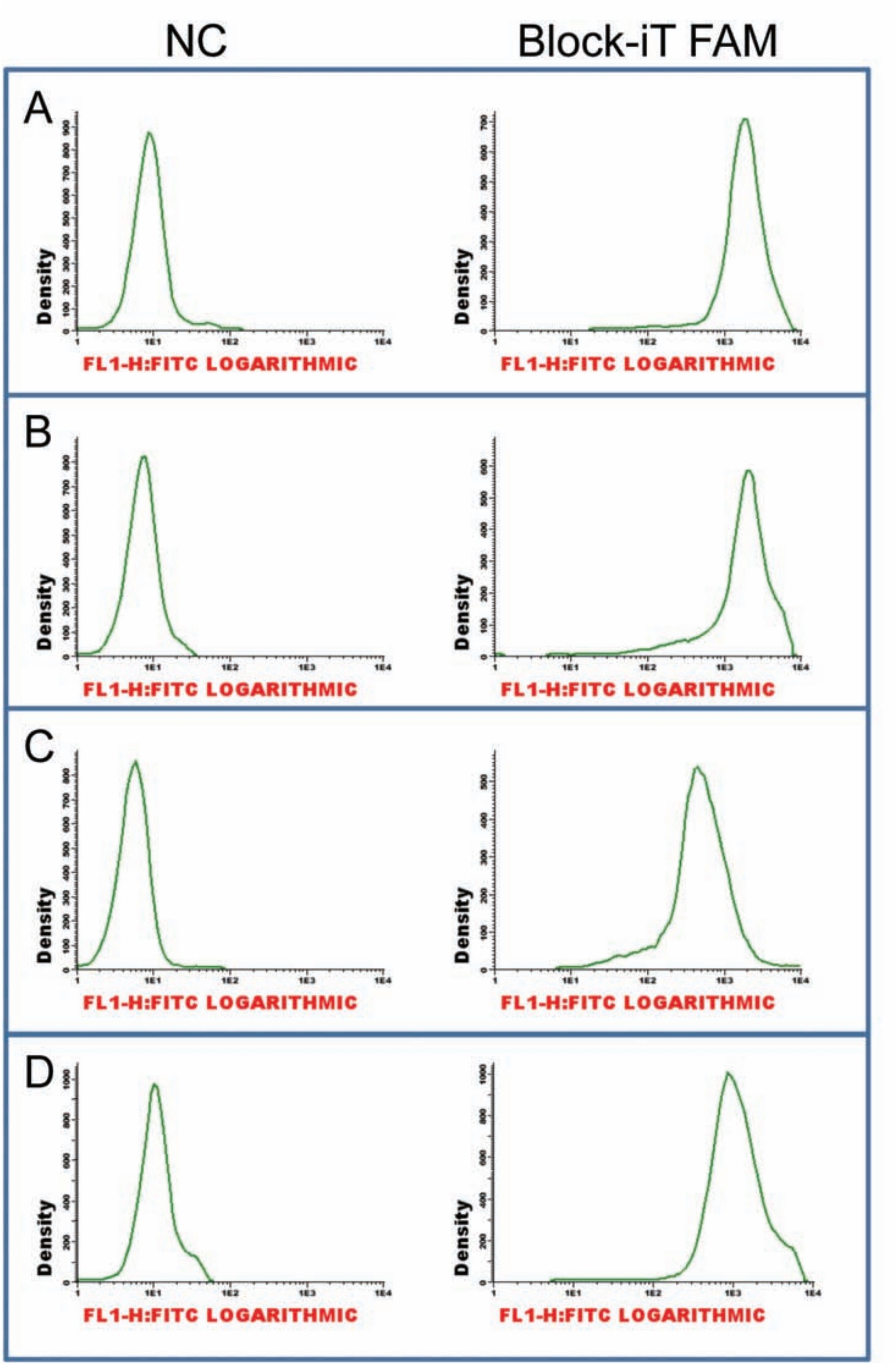
SUPPLEMENTARY APPENDIX

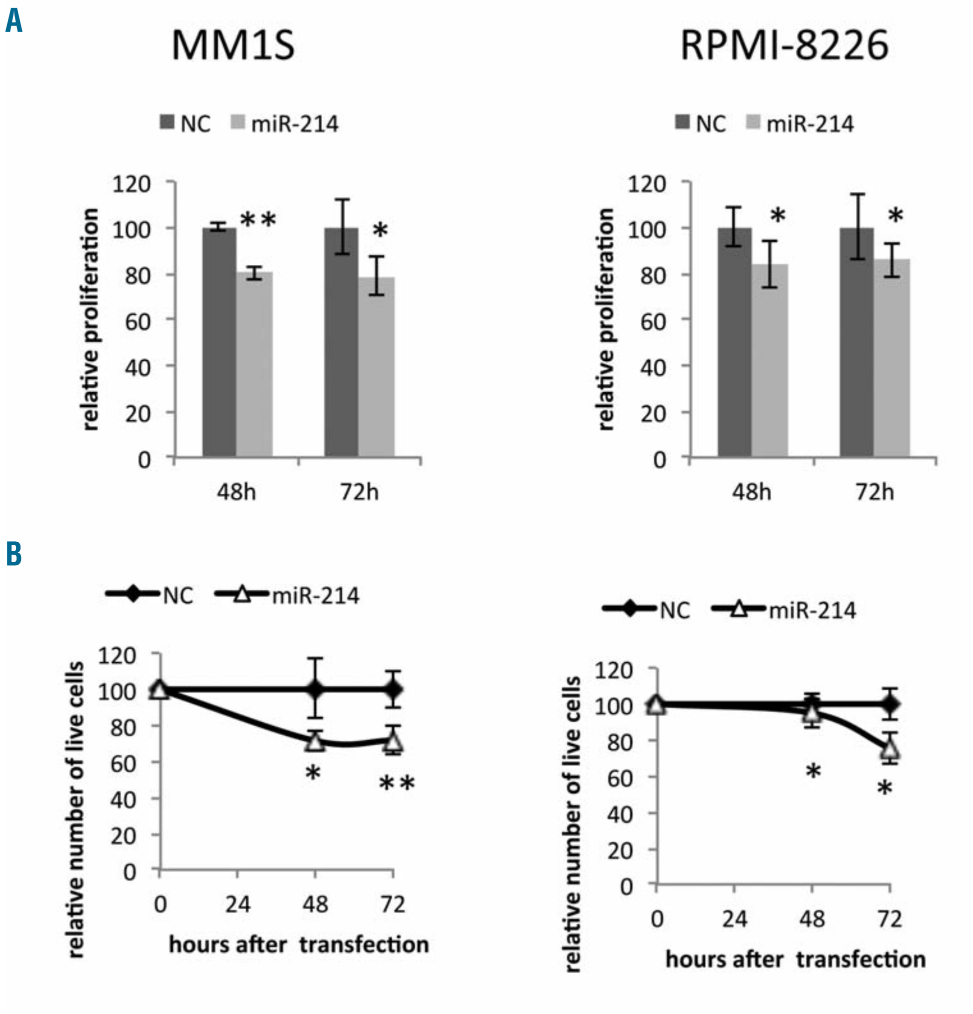
Restoration of microRNA-214 expression reduces growth of myeloma cells through positive regulation of P53 and inhibition of DNA replication

Irena Misiewicz-Krzeminska,[1,2] María E. Sarasquete,[1] Dalia Quwaider,[1] Patryk Krzeminski,[1] Fany V. Ticona,[1] Teresa Paíno,[1] Manuel Delgado,[1] Andreia Aires,[1] Enrique M. Ocio,[1] Ramón García-Sanz,[1] Jesús F. San Miguel,[1] and Norma C. Gutiérrez[1]

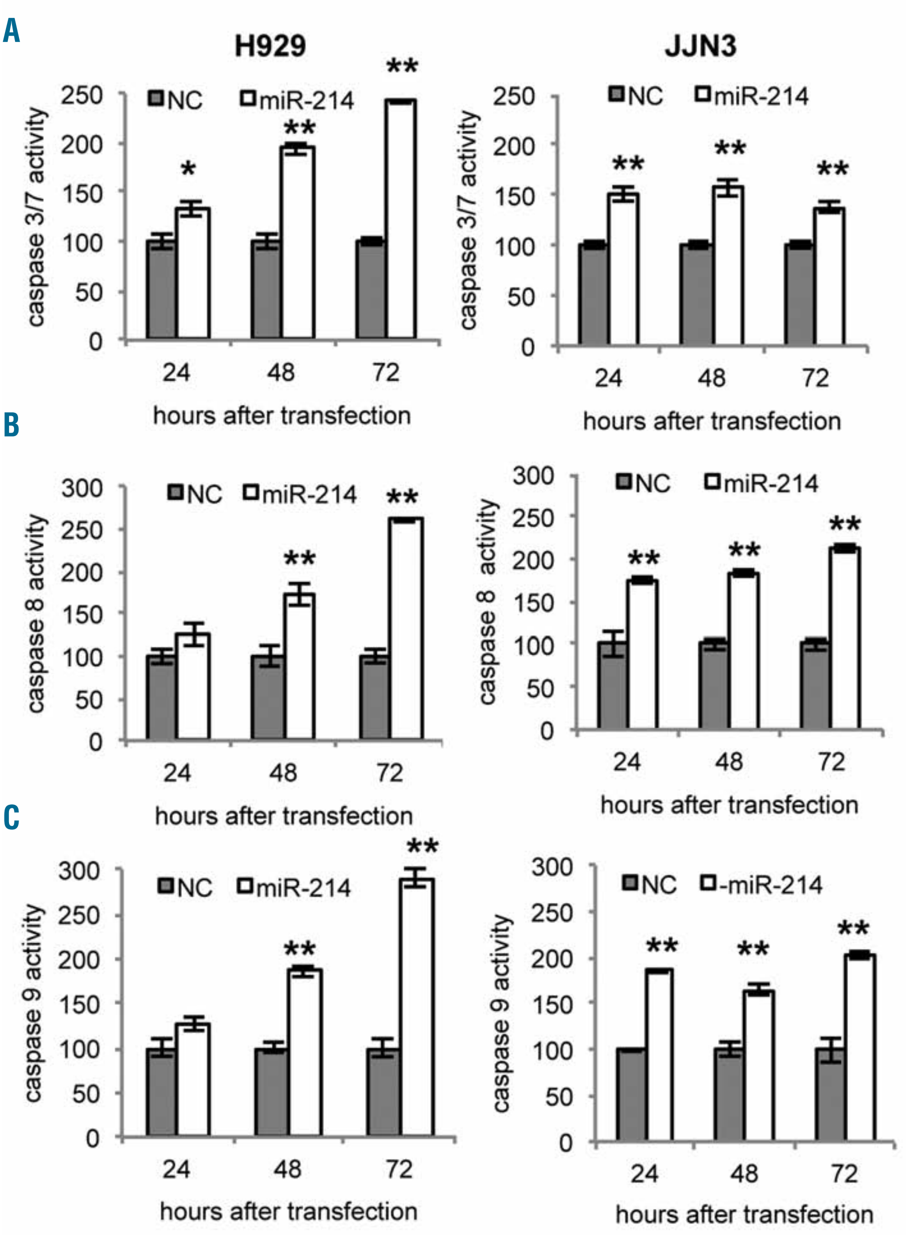
[1]Servicio de Hematología, Hospital Universitario. IBSAL, IBMCC (USAL-CSIC), Salamanca, Spain, and [2]National Medicines Insitute, Warsaw, Poland



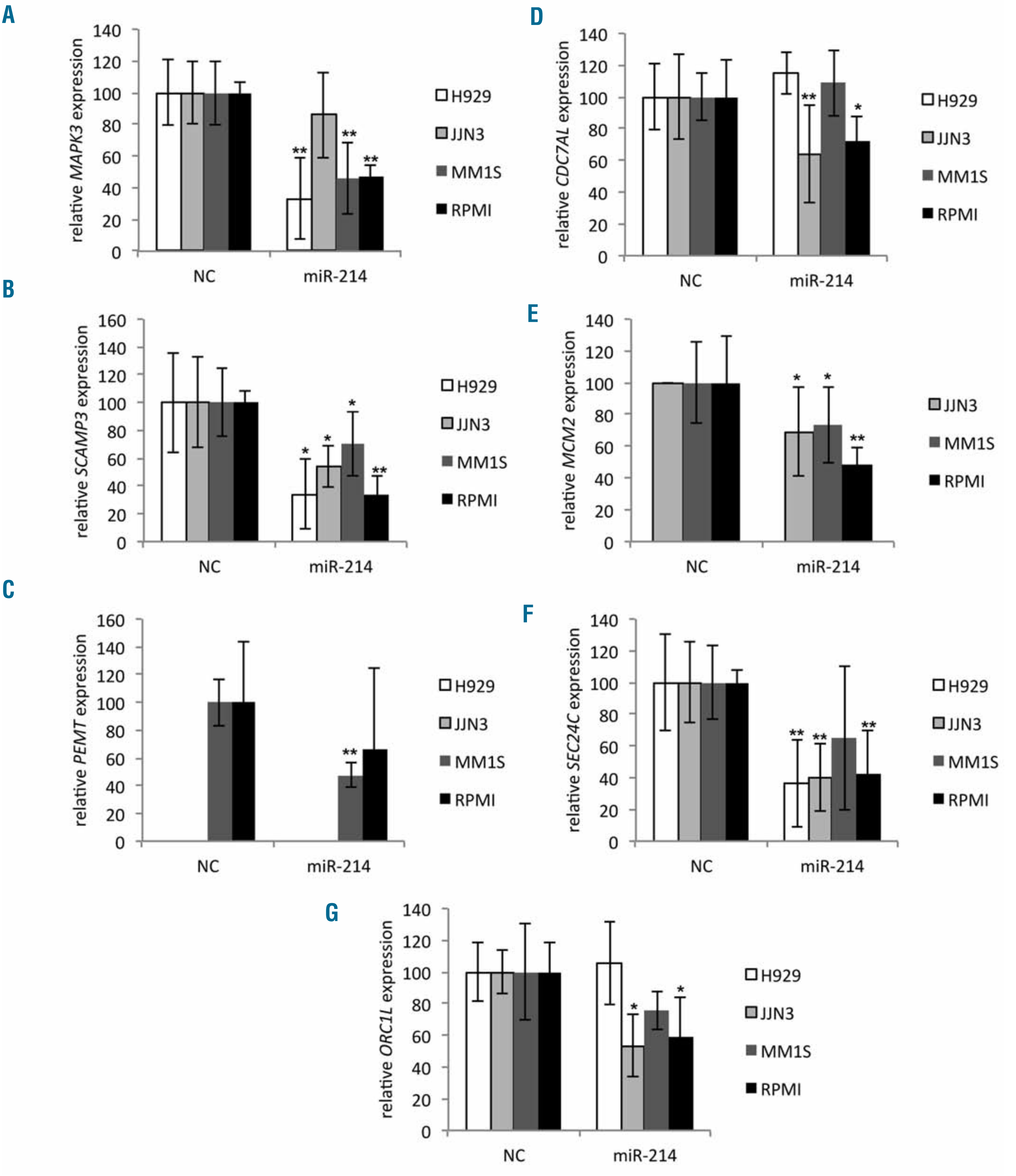
Online Supplementary Figure S1. Transfection efficiency measured by flow cytometric assessment of Block-iT FAM fluorescence in myeloma cell lines; H929 (A), JJN3 (B), MM1S (C) and RPMI-8226 (D).



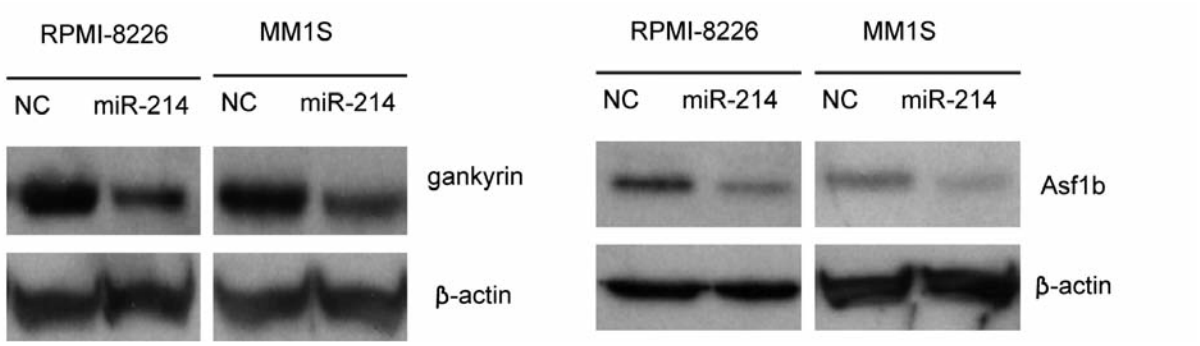
Online Supplementary Figure S2. MM1S left panel, RPMI-8226 right panel. (A) Proliferation evaluated by ATP quantitation (CellTiter-Glo®) of myeloma cells transfected with miR-214. The results are presented as the mean ± SD of three different experiments and considering the result after non-targeting control (NC) transfection as 100%. (B) Relative number of live cells in a time course evaluated by trypan blue counting after miR-214 or non-targeting control (NC) transfection. *$P<0.05$; **$P<0.01$.



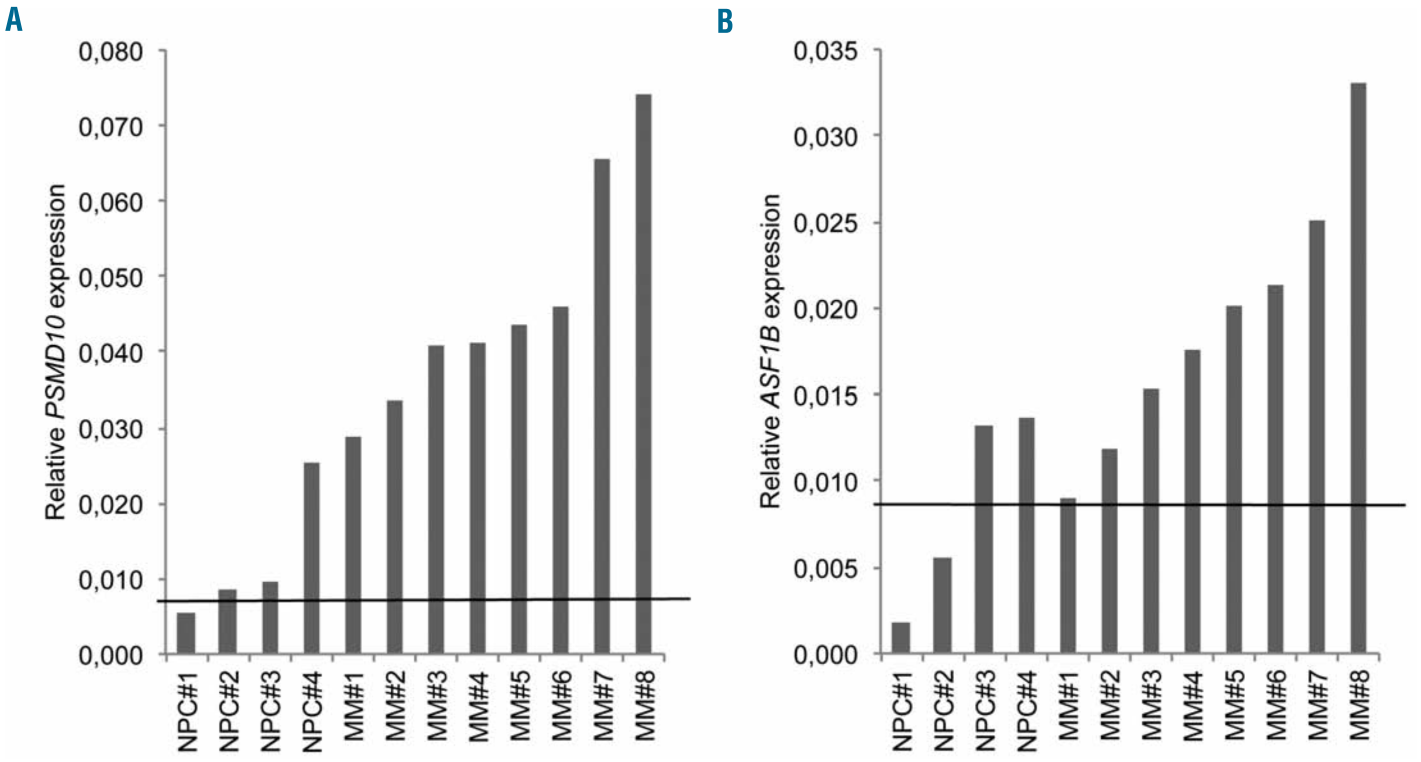
Online Supplementary Figure S3. Effect of miR-214 over-expression on caspase activity. H929 cell line (left panel) and JJN3 cell line (right panel). (A) Luminescent assays in a time course of caspases 3/7, (B) caspase 8 and (C) caspase 9. The results are presented as the mean ± SD of three different experiments and considering the result after non-targeting control (NC) transfection as 100%. *$P<0.05$; **$P<0.01$.



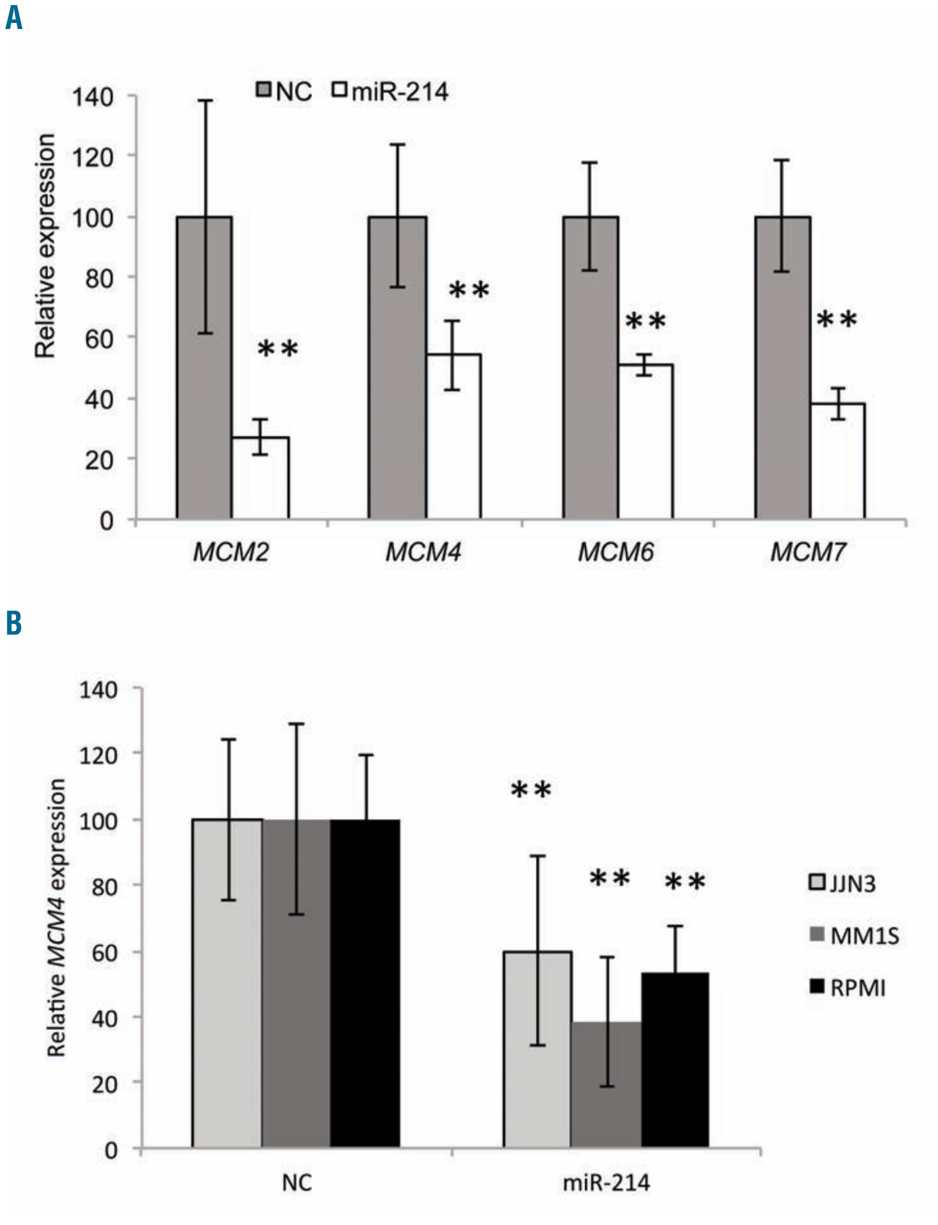
Online Supplementary Figure S4. mRNA level of (A) *MAPK3*, (B) *SCAMP*, (C) *PEMT*, (D) *CDC7AL*, (E) *MCM2* (F) *SEC24C*, and (G) *ORC1L*, assessed by qRT-PCR after transfecting H929, JJN3, MM1S and RPMI-8226 cells with miR-214 precursor or NC. The results are shown as an average of three experiments after normalization with *GAPDH*. **P*<0.05, ***P*<0.01. The H-929 cell line is not included in Figure 4E, because it is shown in *Online Supplementary Figure S7*. In H929 and JJN3 the level of expression of *PEMT* was below the detection limit.



Online Supplementary Figure S5. Levels of gankyrin and Asf1b proteins in RPMI-8226 and MM1S cells at 48 h after transfection with miR-214 or NC, determined by western blotting.

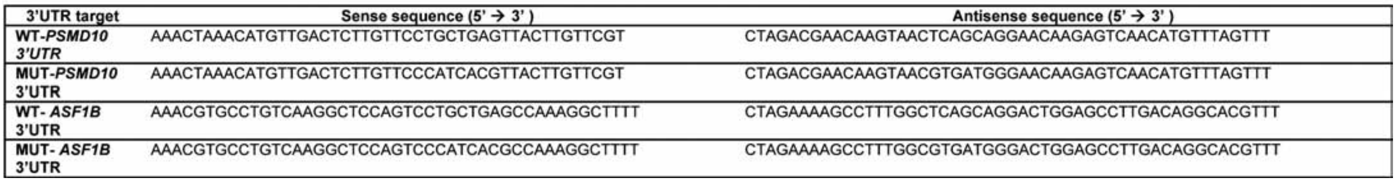


Online Supplementary Figure S6. Expression of *PSMD10* and *ASFB1* determined by qRT-PCR, in normal plasma cells and MM cells from patients. Relative values were calculated by the $2^{-\Delta Ct}$ method. The dark line indicates the median of *PSMD10* and *ASFB1* expression in the four samples of normal plasma cells.



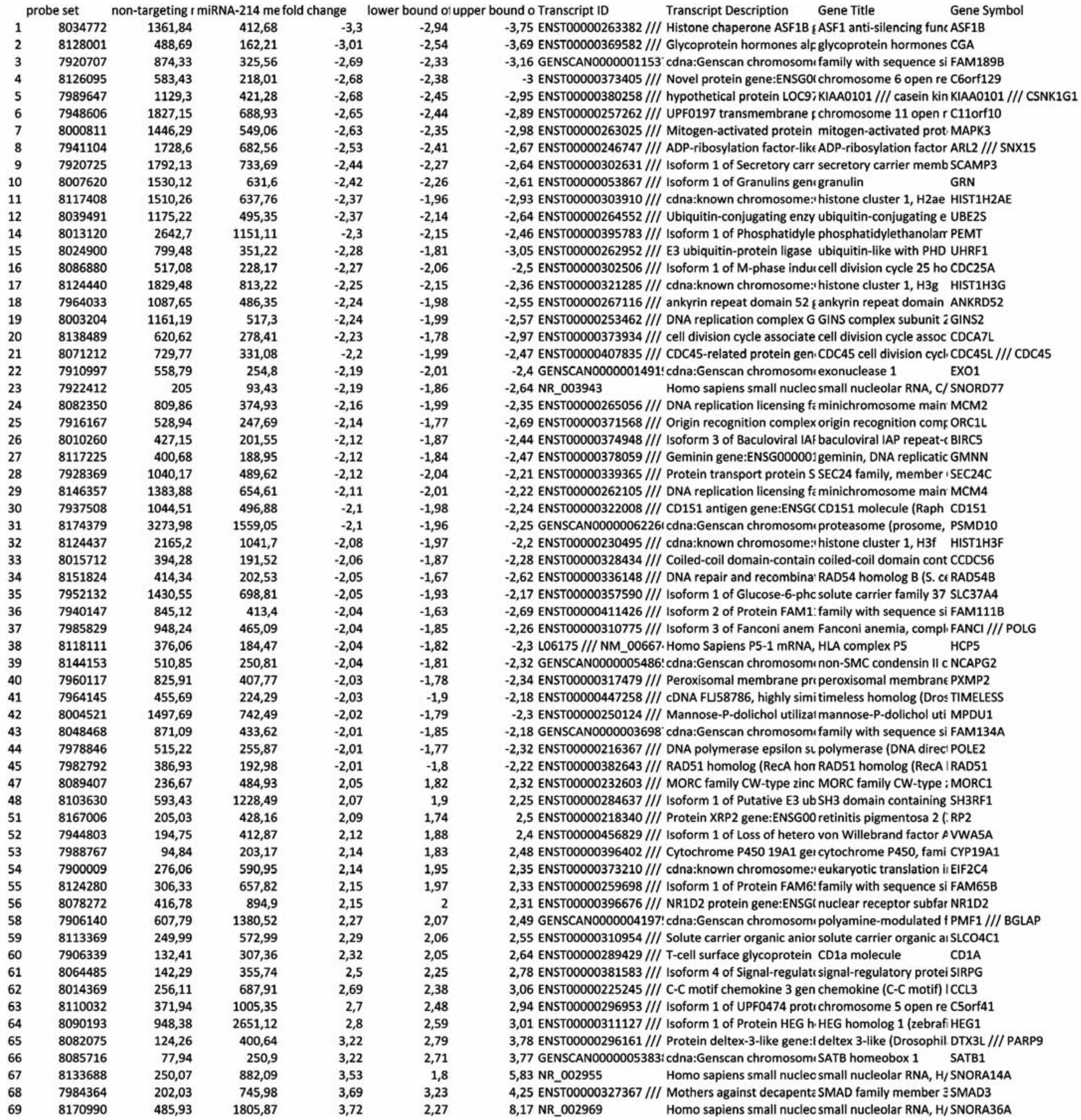
Online Supplementary Figure S7. Effect of miR-214 transfection on the MCM complex. (A) *MCM2, 4, 6* and *7* mRNA expression in H929 cells, determined by qRT-PCR after H929 transfection with miR-214 precursor or NC. (B) *MCM4* expression in JJN3, MM1S and RPMI-8226 cells, determined by qRT-PCR after transfection with miR-214 precursor or NC.

Online Supplementary Table S1. Sequences of oligonucleotides for the wild-type-3’UTR and the mutated-3’UTR cloned into pmirGLO reporter vector.



3’UTR target	Sense sequence (5’ → 3’)	Antisense sequence (5’ → 3’)
WT-*PSMD10* *3’UTR*	AAACTAAACATGTTGACTCTTGTTCCTGCTGAGTTACTTGTTCGT	CTAGACGAACAAGTAACTCAGCAGGAACAAGAGTCAACATGTTTAGTTT
MUT-*PSMD10* 3’UTR	AAACTAAACATGTTGACTCTTGTTCCCATCACGTTACTTGTTCGT	CTAGACGAACAAGTAACGTGATGGGAACAAGAGTCAACATGTTTAGTTT
WT- *ASF1B* 3’UTR	AAACGTGCCTGTCAAGGCTCCAGTCCTGCTGAGCCAAAGGCTTTT	CTAGAAAAGCCTTTGGCTCAGCAGGACTGGAGCCTTGACAGGCACGTTT
MUT- *ASF1B* 3’UTR	AAACGTGCCTGTCAAGGCTCCAGTCCCATCACGCCAAAGGCTTTT	CTAGAAAAGCCTTTGGCGTGATGGGACTGGAGCCTTGACAGGCACGTTT

Online Supplementary Table S2. Genes differentially expressed by >2-fold after ectopic transfection of the H-929 cell line with miR-214, compared to non-targeting control using dChip software.



	probe set	non-targeting r	miRNA-214 me	fold change	lower bound o	upper bound o	Transcript ID	Transcript Description	Gene Title	Gene Symbol
1	8034772	1361,84	412,68	-3,3	-2,94	-3,75	ENST00000263382 ///	Histone chaperone ASF1B	ASF1 anti-silencing func	ASF1B
2	8128001	488,69	162,21	-3,01	-2,54	-3,69	ENST00000369582 ///	Glycoprotein hormones al	glycoprotein hormones	CGA
3	7920707	874,33	325,56	-2,69	-2,33	-3,16	GENSCAN0000001153	cdna:Genscan chromosom	family with sequence si	FAM189B
4	8126095	583,43	218,01	-2,68	-2,38	-3	ENST00000373405 ///	Novel protein gene:ENSG0	chromosome 6 open re	C6orf129
5	7989647	1129,3	421,28	-2,68	-2,45	-2,95	ENST00000380258 ///	hypothetical protein LOC9	KIAA0101 /// casein kin	KIAA0101 /// CSNK1G1
6	7948606	1827,15	688,93	-2,65	-2,44	-2,89	ENST00000257262 ///	UPF0197 transmembrane	chromosome 11 open r	C11orf10
7	8000811	1446,29	549,06	-2,63	-2,35	-2,98	ENST00000263025 ///	Mitogen-activated protein	mitogen-activated prot	MAPK3
8	7941104	1728,6	682,56	-2,53	-2,41	-2,67	ENST00000246747 ///	ADP-ribosylation factor-lik	ADP-ribosylation factor	ARL2 /// SNX15
9	7920725	1792,13	733,69	-2,44	-2,27	-2,64	ENST00000302631 ///	Isoform 1 of Secretory carr	secretory carrier memb	SCAMP3
10	8007620	1530,12	631,6	-2,42	-2,26	-2,61	ENST00000053867 ///	Isoform 1 of Granulins gen	granulin	GRN
11	8117408	1510,26	637,76	-2,37	-1,96	-2,93	ENST00000303910 ///	cdna:known chromosome:	histone cluster 1, H2ae	HIST1H2AE
12	8039491	1175,22	495,35	-2,37	-2,14	-2,64	ENST00000264552 ///	Ubiquitin-conjugating enzy	ubiquitin-conjugating e	UBE2S
14	8013120	2642,7	1151,11	-2,3	-2,15	-2,46	ENST00000395783 ///	Isoform 1 of Phosphatidyle	phosphatidylethanolan	PEMT
15	8024900	799,48	351,22	-2,28	-1,81	-3,05	ENST00000262952 ///	E3 ubiquitin-protein ligase	ubiquitin-like with PHD	UHRF1
16	8086880	517,08	228,17	-2,27	-2,06	-2,5	ENST00000302506 ///	Isoform 1 of M-phase indu	cell division cycle 25 ho	CDC25A
17	8124440	1829,48	813,22	-2,25	-2,15	-2,36	ENST00000321285 ///	cdna:known chromosome:	histone cluster 1, H3g	HIST1H3G
18	7964033	1087,65	486,35	-2,24	-1,98	-2,55	ENST00000267116 ///	ankyrin repeat domain 52	ankyrin repeat domain	ANKRD52
19	8003204	1161,19	517,3	-2,24	-1,99	-2,57	ENST00000253462 ///	DNA replication complex G	GINS complex subunit	GINS2
20	8138489	620,62	278,41	-2,23	-1,78	-2,97	ENST00000373934 ///	cell division cycle associate	cell division cycle assoc	CDCA7L
21	8071212	729,77	331,08	-2,2	-1,99	-2,47	ENST00000407835 ///	CDC45-related protein gen	CDC45 cell division cycl	CDC45L /// CDC45
22	7910997	558,79	254,8	-2,19	-2,01	-2,4	GENSCAN0000001491	cdna:Genscan chromosom	exonuclease 1	EXO1
23	7922412	205	93,43	-2,19	-1,86	-2,64	NR_003943	Homo sapiens small nucleo	small nucleolar RNA, C/	SNORD77
24	8082350	809,86	374,93	-2,16	-1,99	-2,35	ENST00000265056 ///	DNA replication licensing f	minichromosome main	MCM2
25	7916167	528,94	247,69	-2,14	-1,77	-2,69	ENST00000371568 ///	Origin recognition complex	origin recognition comp	ORC1L
26	8010260	427,15	201,55	-2,12	-1,87	-2,44	ENST00000374948 ///	Isoform 3 of Baculoviral IA	baculoviral IAP repeat-	BIRC5
27	8117225	400,68	188,95	-2,12	-1,84	-2,47	ENST00000378059 ///	Geminin gene:ENSG00000	geminin, DNA replicatic	GMNN
28	7928369	1040,17	489,62	-2,12	-2,04	-2,21	ENST00000339365 ///	Protein transport protein S	SEC24 family, member	SEC24C
29	8146357	1383,88	654,61	-2,11	-2,01	-2,22	ENST00000262105 ///	DNA replication licensing f	minichromosome main	MCM4
30	7937508	1044,51	496,88	-2,1	-1,98	-2,24	ENST00000322008 ///	CD151 antigen gene:ENSG	CD151 molecule (Raph	CD151
31	8174379	3273,98	1559,05	-2,1	-1,96	-2,25	GENSCAN0000006226	cdna:Genscan chromosom	proteasome (prosome,	PSMD10
32	8124437	2165,2	1041,7	-2,08	-1,97	-2,2	ENST00000230495 ///	cdna:known chromosome:	histone cluster 1, H3f	HIST1H3F
33	8015712	394,28	191,52	-2,06	-1,87	-2,28	ENST00000328434 ///	Coiled-coil domain-contain	coiled-coil domain cont	CCDC56
34	8151824	414,34	202,53	-2,05	-1,67	-2,62	ENST00000336148 ///	DNA repair and recombina	RAD54 homolog B (S. c	RAD54B
35	7952132	1430,55	698,81	-2,05	-1,93	-2,17	ENST00000357590 ///	Isoform 1 of Glucose-6-ph	solute carrier family 37	SLC37A4
36	7940147	845,12	413,4	-2,04	-1,63	-2,69	ENST00000411426 ///	Isoform 2 of Protein FAM1	family with sequence si	FAM111B
37	7985829	948,24	465,09	-2,04	-1,85	-2,26	ENST00000310775 ///	Isoform 3 of Fanconi anem	Fanconi anemia, compl	FANCI /// POLG
38	8118111	376,06	184,47	-2,04	-1,82	-2,3	L06175 /// NM_00667	Homo Sapiens P5-1 mRNA,	HLA complex P5	HCP5
39	8144153	510,85	250,81	-2,04	-1,81	-2,32	GENSCAN0000005486	cdna:Genscan chromosom	non-SMC condensin II c	NCAPG2
40	7960117	825,91	407,77	-2,03	-1,78	-2,34	ENST00000317479 ///	Peroxisomal membrane pr	peroxisomal membran	PXMP2
41	7964145	455,69	224,29	-2,03	-1,9	-2,18	ENST00000447258 ///	cDNA FLJ58786, highly simi	timeless homolog (Dro	TIMELESS
42	8004521	1497,69	742,49	-2,02	-1,79	-2,3	ENST00000250124 ///	Mannose-P-dolichol utiliza	mannose-P-dolichol uti	MPDU1
43	8048468	871,09	433,62	-2,01	-1,85	-2,18	GENSCAN0000003698	cdna:Genscan chromosom	family with sequence si	FAM134A
44	7978846	515,22	255,87	-2,01	-1,77	-2,32	ENST00000216367 ///	DNA polymerase epsilon s	polymerase (DNA direc	POLE2
45	7982792	386,93	192,98	-2,01	-1,8	-2,22	ENST00000382643 ///	RAD51 homolog (RecA hon	RAD51 homolog (RecA	RAD51
47	8089407	236,67	484,93	2,05	1,82	2,32	ENST00000232603 ///	MORC family CW-type zinc	MORC family CW-type	MORC1
48	8103630	593,43	1228,49	2,07	1,9	2,25	ENST00000284637 ///	Isoform 1 of Putative E3 ub	SH3 domain containing	SH3RF1
51	8167006	205,03	428,16	2,09	1,74	2,5	ENST00000218340 ///	Protein XRP2 gene:ENSG00	retinitis pigmentosa 2 (	RP2
52	7944803	194,75	412,87	2,12	1,88	2,4	ENST00000456829 ///	Isoform 1 of Loss of hetero	von Willebrand factor A	VWA5A
53	7988767	94,84	203,17	2,14	1,83	2,48	ENST00000396402 ///	Cytochrome P450 19A1 ge	cytochrome P450, fami	CYP19A1
54	7900009	276,06	590,95	2,14	1,95	2,35	ENST00000373210 ///	cdna:known chromosome:	eukaryotic translation i	EIF2C4
55	8124280	306,33	657,82	2,15	1,97	2,33	ENST00000259698 ///	Isoform 1 of Protein FAM6	family with sequence si	FAM65B
56	8078272	416,78	894,9	2,15	2	2,31	ENST00000396676 ///	NR1D2 protein gene:ENSG	nuclear receptor subfar	NR1D2
58	7906140	607,79	1380,52	2,27	2,07	2,49	GENSCAN0000004197	cdna:Genscan chromosom	polyamine-modulated f	PMF1 /// BGLAP
59	8113369	249,99	572,99	2,29	2,06	2,55	ENST00000310954 ///	Solute carrier organic anior	solute carrier organic a	SLCO4C1
60	7906339	132,41	307,36	2,32	2,05	2,64	ENST00000289429 ///	T-cell surface glycoprotein	CD1a molecule	CD1A
61	8064485	142,29	355,74	2,5	2,25	2,78	ENST00000381583 ///	Isoform 4 of Signal-regulat	signal-regulatory protei	SIRPG
62	8014369	256,11	687,91	2,69	2,38	3,06	ENST00000225245 ///	C-C motif chemokine 3 gen	chemokine (C-C motif)	CCL3
63	8110032	371,94	1005,35	2,7	2,48	2,94	ENST00000296953 ///	Isoform 1 of UPF0474 prot	chromosome 5 open re	C5orf41
64	8090193	948,38	2651,12	2,8	2,59	3,01	ENST00000311127 ///	Isoform 1 of Protein HEG h	HEG homolog 1 (zebrafi	HEG1
65	8082075	124,26	400,64	3,22	2,79	3,78	ENST00000296161 ///	Protein deltex-3-like gene:	deltex 3-like (Drosophil	DTX3L /// PARP9
66	8085716	77,94	250,9	3,22	2,71	3,77	GENSCAN0000005383	cdna:Genscan chromosom	SATB homeobox 1	SATB1
67	8133688	250,07	882,09	3,53	1,8	5,83	NR_002955	Homo sapiens small nucleo	small nucleolar RNA, H/	SNORA14A
68	7984364	202,03	745,98	3,69	3,23	4,25	ENST00000327367 ///	Mothers against decapent	SMAD family member	SMAD3
69	8170990	485,93	1805,87	3,72	2,27	8,17	NR_002969	Homo sapiens small nucleo	small nucleolar RNA, H/	SNORA36A

Online Supplementary Table S3. Genes that were down-regulated in H929 cells transfected with miR-214 compared to cells transfected with non-targeting control (NC), and which were predicted as potential direct targets for miR 214.

Gene symbol	Transcript ID	Chromosome location	Description	Protein function*	Fold change**
ASF1B	NM_018154	19p13.12	Anti-silencing function 1 homolog B	Histone H3/H4 chaperone Promotes replication-dependent chromatin assembly	-3.3
MAPK3	NM_002746 /// NM_001040056 /// NM_001109891	16p11.2	Mitogen-activated protein kinase 3	Initiation and regulation of meiosis, mitosis, and postmitotic functions in differentiated cells	-2.63
SCAMP3	NM_005698 /// NM_052837	1q21	Secretory carrier-associated membrane protein 3	Functions in post-Golgi recycling pathways. Acts as a recycling carrier to the cell surface	-2.44
PEMT	NM_148172 /// NM_148173 /// NM_007169	17p11.2	Phosphatidylethanolamine N-methyltransferase	Catalyzes three sequential methylation of phosphatidylethanolamine	-2.3
CDCA7L	NM_018719 /// NM_001127370/// NM_001127371	7p15.3	Cell division cycle-associated 7-like protein	Transcriptional regulation as a repressor that inhibits monoamine oxidase A activity	-2.23
MCM2	NM_004526	3q21	Minichromosome maintenance complex component 2	Component of the MCM2-7 complex which is the putative replicative helicase essential for DNA replication initiation and elongation	-2.16
ORC1L	NM_004153	1p32	Origin recognition complex, subunit 1	Component of the origin recognition complex (ORC) that binds origins of replication	-2.14
SEC24C	NM_004922 /// NM_198597	10q22.2	Protein transport protein Sec24C	Transport from the endoplasmic reticulum to the Golgi apparatus	-2.12
PSMD10	NM_170750 /// NM_002814	Xq22.3	26S proteasome non-ATPase regulatory subunit 10/gankyrin	Proteasome subunit. Acts as an proto-oncoprotein by being involved in negative regulation of tumor suppressors RB1 and p53/TP53	-2.1

**According to UniProtKB/Swiss-Prot database. **Fold change expressed using the H929 cells transfected with NC as the baseline.*