JAHA/2016/004629-T1R1

SUPPLEMENTARY DATA

NCKAP1 Modulated by miRNA-214 Determines Vascular Smooth Muscle Cell Migration, Proliferation and Neointima Hyperplasia

Tayyab Adeel Afzal[1#], Le Anh Luong[1#], Dan Chen[1,2], Cheng Zhang[1,2], Feng Yang[1,3], Qishan Chen[1,3], Weiwei An[1], Edmund Wilkes[5], Kenta Yashiro[4], Pedro R. Cutillas[5], Li Zhang[3*] and Qingzhong Xiao[1*]

[1]Centre for Clinical Pharmacology and [4]Translational Medicine & Therapeutics, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ, UK;
[2]Department of Cardiothoracic Surgery, The First Affiliated Hospital of Chongqing Medical University, 400016, Chongqing, China;
[3]Department of Cardiology, the First Affiliated Hospital, School of Medicine, Zhejiang University, 79 Qingchun Road, Hangzhou, 310003, Zhejiang, China;
[5]Centre for Haemato-Oncology, Barts Cancer Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ, UK.

[#]T Afzal and L Luong contributed equally to this study

*Correspondence to:
Dr Qingzhong Xiao (q.xiao@qmul.ac.uk***)***, Centre for Clinical Pharmacology, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Heart Centre, Charterhouse Square, London EC1M 6BQ, United Kingdom. Tel: +44(0)2078826584;
Or
Dr Li Zhang (li.zhang.uk@googlemail.com), Department of Cardiology, the First Affiliated Hospital, School of Medicine, Zhejiang University, 79 Qingchun Road, Hangzhou, 310003, Zhejiang, China; Tel: +86(0)571-87236500.

Supplementary Figures:

A

Mouse NCKAP1 (NM_016965.3), 3UTR: 3605~4403

Binding site-1 (BS1) - minimum loop-free energy (RNAhybrid):-51.803
3661 ggaactattt taccttaaag cctgaaaaca gtttgtgga tgaaattttc ttcatgctgt
miR-214: 3'-tgacggacagacac---------------ggacgaca-5'
BS1^{mut}: taccttaaag cctgaaaaca gtttgtgga tgaaattttc ttAaCgcGTG
Mlu I (ACGGCT)

Binding site-3 (BS3) - minimum loop-free energy (RNAhybrid): -51.80
4141 tccgaggttc tcagacagca gcacatgctg ccgccgcctg tagaaagcct actgtagaaa
miR-214: 3'-tgacggaca-gacacgga cgaca-5'
BS3^{mut}: tgctgccgccgcACgtagaaaCgcGtacACtagaaa
Mlu I (ACGCGT)

Binding site-2 (BS2) - minimum loop-free energy (RNAhybrid): -51.801
4201 catctcatcc cgtgctgccc ggctggcatc tgcactgcct ttac cctgctgtataataaa
miR-214: 3'-tgacg-gacagacac-ggacgaca-5'
BS2^{mut}: ggcatc tAcactgcct ttac AACgcGTGataataaa
Mlu I (ACGCGT)

B

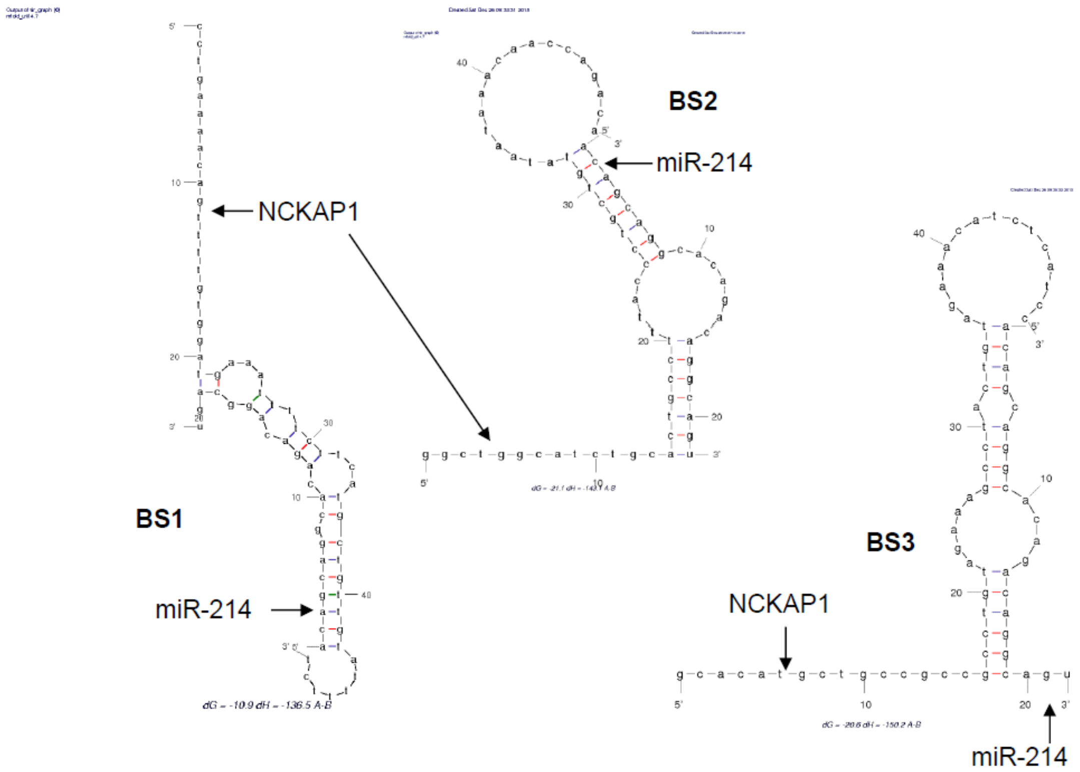

Figure S1. miR-214 binding sites within 3'UTR of NCKAP1 gene, and the miR-214:NCKAP1 3'UTR duplex stem-loop and respective minimum loop-free energy are depicted in this illustration.

(A) Three potential wild type binding sites (BS1-BS3) of miR-214 within NCKAP1 3'UTR as predicted by RNAhybrid and their mutants (BS1/2/3^{mut}) are depicted in this illustration. (B) The formation of the miR-214:NCKAP1 3'UTR (spanning through miR-214 BS1-3) duplex stem-loop and the minimum loop-free energy for individual loop (binding site) were calculated by and extracted from mFold software (http://mfold.rna.albany.edu/?q=DINAMelt/Two-state-melting).

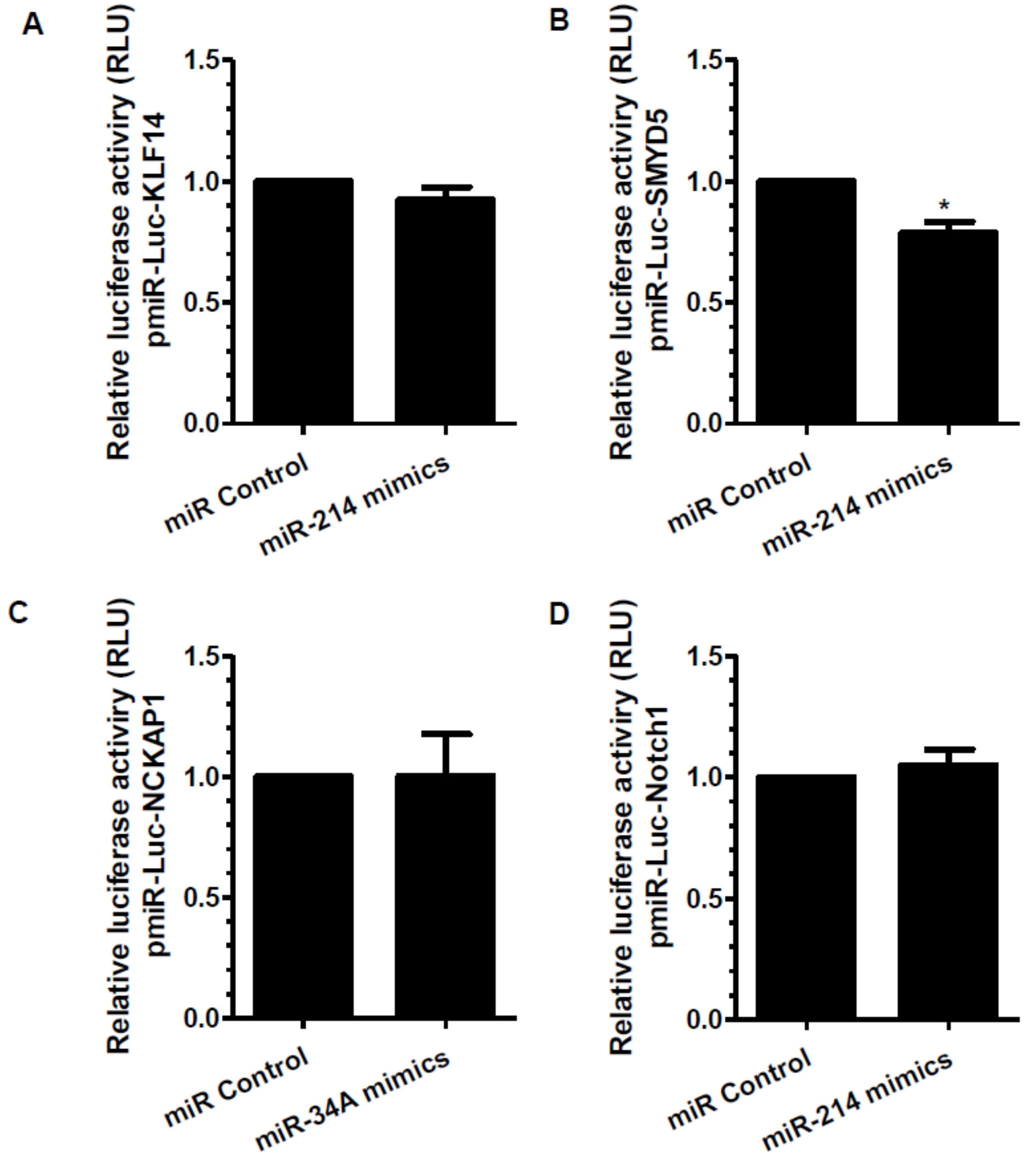

Figure S2. Gene 3’UTR reporter luciferase activity assays.
miR-214 mimics, miR-34A mimics or negative control was co-transfected into VSMCs with respective gene 3’UTR reporter (A, KLF14; B, SMYD5; C, NCKAP1; D, Notch1). Luciferase activity assay were measured at 48 hours post-transfection. The data presented here are representative or mean±S.E.M. of four to six independent experiments (n=4-6). *P<0.05 (versus miR control).

Supplementary table S1: Primer sets used in the present study

Gene names	Forward (5'-3')	Reverse (5'-3')	Application
U6 snoRNA (mu/hu)	GATGACACGCAAATTCGTG	miRNA universal reverse primer (Invitrogen, A11193-051)	Real-time RT-PCR
Mus/hu miR-214 (mature)	CAGGCACAGACAGGCAGT	miRNA universal reverse primer (Invitrogen, A11193-051)	Real-time RT-PCR
Mus Pri-miR-214	GCAAGGCTATGGCACTTACCTA	CCTGTTGTTACTGGCCCTCA	Real-time RT-PCR
Mus NCKAP1	GAGAAGCTCACCATCCTCAAC	CAAGAAGCAAGGACAAGTTTGG	Real-time RT-PCR
Mus PCNA	TTGCACGTATATGCCGAGACCT	ATTGCCAAGCTCTCCACTTGC	Real-time RT-PCR
Mus Twist1	GAGGTCTTGCCAATCAGCCA	CCAGTTTGATCCCAGCGTTT	Real-time RT-PCR
pmiR-Luc-NCKAP1-WT	GTGGTG GAGCTC AGAC AAGCACGAGTTTCTGTTG	CTGCTG AAGCTT CAAGGTGTGATACAGTCTAGTG	NCKAP1 3'UTR reporter clone (Sac I/Hind III)
pmiR-Luc-NCKAP1-$BS1^{mu}$	gtggatgaaattttcttAaCgcGTGtgtattttctgatc attggc	gccaatgatcagaaaataca CACgcGtT aagaaaatttcatccac	miR-214 binding site 1 mutation
pmiR-Luc-NCKAP1-$BS2^{mu}$	ggcatctAcactgcctttacAACgcGTGataataa acaaccagacac	gtgtctggttgtttattatCACgcGTTgtaaaggc agtgTagatgcc	miR-214 binding site 2 mutation
pmiR-Luc-NCKAP1-$BS3^{mu}$	tgctgccgccgcACgtagaaCgcGtacACtaga aacatctcatcc	ggatgagatgtttctaGTgtaCgcgttctacGTgc ggcggcagca	miR-214 binding site 3 mutation
pmiR-Luc-KLF14	GTCGTC GAGCTC GACTGTCTTGCTGTCTATCT	GTCGTC AAGCTT CTCAGCATTTAAAGATTTATAG	KLF14 3'UTR reporter clone (Sac I/Hind III)
pmiR-Luc-SMYD5	CACGAC GAGCTC TGTTATCTCACCTGGAAGGC	CACGAC AAGCTT TCACCACTCACATTTTATTGAGAC	SMYD5 3'UTR reporter clone (Sac I/Hind III)
pGL3-miR-214-FL	gaggag GAGCTC aggggggagccccaacttatctga	gaggag AAGCTT TTCCTGCACCAGGGGCTTGT	Mouse miR-214 gene promoter (-640:0) clone
pGL3-miR-214-short	gaggag GAGCTC aggggggagccccaacttatctga	ctggtc AAGCTT TGGGGCCCCAGTATGGAAAA	Mouse miR-214 gene promoter (-640:-357) clone

Supplementary table S2: **selected protein list regulated by miR-214 in VSMCs.** Note: Proteins/genes with yellow-highlighted have been implicated in regulation of cell migration, proliferation, adhesion, actin filament reorganization and actin polymerization, cell cycle, and gene expression, etc.

* Predicted putative target of miR-214 (only the genes with binding sites within 3'UTR have been included in this table)

Proteins	Log FR (Treatment vs Control)	P-value	Fold changes (Treatment vs Control)	Binding sites*	Protein Name
Downregulated proteins by miR-214					
PEPD_MOUSE	-2.737973	0.4	0.149895	No	Xaa-Pro dipeptidase OS=Mus musculus GN=Pepd PE=2 SV=3
ABL2_MOUSE	-1.454600	0.5	0.364856	No	Abelson tyrosine-protein kinase 2 OS=Mus musculus GN=Abl2 PE=1 SV=1
LSM8_MOUSE	-1.454600	0.5	0.364856	No	U6 snRNA-associated Sm-like protein LSm8 OS=Mus musculus GN=Lsm8 PE=3 SV=3
NCKP1_MOUSE	-1.434939	0	0.369863	Yes (3)	Nck-associated protein 1 OS=Mus musculus GN=Nckap1 PE=1 SV=2
HYPK_MOUSE	-1.353127	0	0.391443	yes (1)	Huntingtin-interacting protein K OS=Mus musculus GN=Hypk PE=2 SV=2
COX2_MOUSE	-1.302748	0	0.405353	No	Cytochrome c oxidase subunit 2 OS=Mus musculus GN=Mtco2 PE=1 SV=1
EMAL1_MOUSE	-1.160103	0	0.447481	yes (1)	Echinoderm microtubule-associated protein-like 1 OS=Mus musculus GN=Eml1 PE=1 SV=1
SYAP1_MOUSE	-1.021893	0.5	0.49247	No	Synapse-associated protein 1 OS=Mus musculus GN=Syap1 PE=1 SV=1
SPTB1_MOUSE	-0.918821	0	0.528941	Yes (2)	Spectrin beta chain, erythrocytic OS=Mus musculus GN=Sptb PE=1 SV=4
ILEUC_MOUSE	-0.827446	0.5	0.563526	No	Leukocyte elastase inhibitor C OS=Mus musculus GN=Serpinb1c PE=2 SV=1
LYPA2_MOUSE	-0.806064	0	0.57194	Yes (1)	Acyl-protein thioesterase 2 OS=Mus musculus GN=Lypla2 PE=1 SV=1
KLF14_MOUSE	-0.793026	0	0.577132	Yes (2)	Krueppel-like factor 14 OS=Mus musculus GN=Klf14 PE=2 SV=1
ARPC2_MOUSE	-0.786859	0	0.579604	no	Actin-related protein 2/3 complex subunit 2 OS=Mus musculus GN=Arpc2 PE=1 SV=3
PGM1_MOUSE	-0.786171	0.1	0.579881	No	Phosphoglucomutase-1 OS=Mus musculus GN=Pgm1 PE=1 SV=4
PPM1F_MOUSE	-0.753456	0	0.593181	Yes (3)	Protein phosphatase 1F OS=Mus musculus GN=Ppm1f PE=2 SV=1
ABHEB_MOUSE	-0.704636	0.1	0.613597	No	Alpha/beta hydrolase domain-containing protein 14B OS=Mus musculus GN=Abhd14b PE=2 SV=1
TPP1_MOUSE	-0.689847	0	0.61992	Yes (5)	Tripeptidyl-peptidase 1 OS=Mus

					musculus GN=Tpp1 PE=1 SV=2
SNX12_MOUSE	-0.670723	0.1	0.628192		Sorting nexin-12 OS=Mus musculus GN=Snx12 PE=1 SV=1
CIRBP_MOUSE	-0.659948	0.1	0.632901	No	Cold-inducible RNA-binding protein OS=Mus musculus GN=Cirbp PE=1 SV=1
IDHC_MOUSE	-0.653167	0	0.635883	Yes (2)	Isocitrate dehydrogenase [NADP] cytoplasmic OS=Mus musculus GN=Idh1 PE=1 SV=2
INT3_MOUSE	-0.632013	0	0.645275	Yes (2)	Integrator complex subunit 3 OS=Mus musculus GN=Ints3 PE=2 SV=2
LIMS2_MOUSE	-0.599727	0	0.659879	No	LIM and senescent cell antigen-like-containing domain protein 2 OS=Mus musculus GN=Lims2 PE=1 SV=1
SNX5_MOUSE	-0.595149	0	0.661976	Yes (1)	Sorting nexin-5 OS=Mus musculus GN=Snx5 PE=1 SV=1
SMYD5_MOUSE	-0.572227	0	0.672578	Yes (4)	SET and MYND domain-containing protein 5 OS=Mus musculus GN=Smyd5 PE=2 SV=2
APC7_MOUSE	-0.553523	0	0.681354	Yes (2)	Anaphase-promoting complex subunit 7 OS=Mus musculus GN=Anapc7 PE=1 SV=3
ACOT9_MOUSE	-0.552868	0	0.681664	Yes (2)	Acyl-coenzyme A thioesterase 9, mitochondrial OS=Mus musculus GN=Acot9 PE=1 SV=1
LONM_MOUSE	-0.550077	0	0.682983	Yes (1)	Lon protease homolog, mitochondrial OS=Mus musculus GN=Lonp1 PE=1 SV=2
SNP29_MOUSE	-0.543593	0	0.68606	yes (3)	Synaptosomal-associated protein 29 OS=Mus musculus GN=Snap29 PE=2 SV=1
FKB10_MOUSE	-0.528053	0.4	0.69349	No	Peptidyl-prolyl cis-trans isomerase FKBP10 OS=Mus musculus GN=Fkbp10 PE=1 SV=2
GSTM1_MOUSE	-0.518952	0	0.697878	Yes (2)	Glutathione S-transferase Mu 1 OS=Mus musculus GN=Gstm1 PE=1 SV=2
KIME_MOUSE	-0.515848	0	0.699382	yes (1)	Mevalonate kinase OS=Mus musculus GN=Mvk PE=2 SV=1
ARL3_MOUSE	-0.512953	0	0.700787	Yes (1)	ADP-ribosylation factor-like protein 3 OS=Mus musculus GN=Arl3 PE=1 SV=1
SAMH1_MOUSE	-0.484792	0	0.7146	Yes (2)	Deoxynucleoside triphosphate triphosphohydrolase SAMHD1 OS=Mus musculus GN=Samhd1 PE=1 SV=2
SRRT_MOUSE	-0.483568	0	0.715207	Yes (1)	Serrate RNA effector molecule homolog OS=Mus musculus GN=Srrt PE=1 SV=1
AP2B1_MOUSE	-0.474446	0	0.719743	Yes (6)	AP-2 complex subunit beta OS=Mus musculus GN=Ap2b1 PE=1 SV=1
PROF1_MOUSE	-0.472765	0	0.720582	Yes (2)	Profilin-1 OS=Mus musculus GN=Pfn1 PE=1 SV=2
GSTM2_MOUSE	-0.470258	0	0.721836	Yes (1)	Glutathione S-transferase Mu 2 OS=Mus musculus GN=Gstm2 PE=1 SV=2

CAPZB_MOUSE	-0.463590	0.3	0.725179	No	F-actin-capping protein subunit beta OS=Mus musculus GN=Capzb PE=1 SV=3
TGM2_MOUSE	-0.461355	0	0.726304	Yes (4)	Protein-glutamine gamma-glutamyltransferase 2 OS=Mus musculus GN=Tgm2 PE=1 SV=4
RCN3_MOUSE	-0.457328	0	0.728334	No	Reticulocalbin-3 OS=Mus musculus GN=Rcn3 PE=2 SV=1
SMC3_MOUSE	-0.451019	0	0.731526	Yes (5)	Structural maintenance of chromosomes protein 3 OS=Mus musculus GN=Smc3 PE=1 SV=2
RHOA_MOUSE	-0.447904	0	0.733107	Yes (1)	Transforming protein RhoA OS=Mus musculus GN=Rhoa PE=1 SV=1
G3BP1_MOUSE	-0.444125	0	0.73503	Yes (7)	Ras GTPase-activating protein-binding protein 1 OS=Mus musculus GN=G3bp1 PE=1 SV=1
LAMP1_MOUSE	-0.443885	0	0.735152	Yes (1)	Lysosome-associated membrane glycoprotein 1 OS=Mus musculus GN=Lamp1 PE=1 SV=2
SMC1A_MOUSE	-0.443237	0	0.735483	Yes (1)	Structural maintenance of chromosomes protein 1A OS=Mus musculus GN=Smc1a PE=1 SV=4
SPI2_MOUSE	-0.441805	0.5	0.736213	No	Serpin I2 OS=Mus musculus GN=Serpini2 PE=2 SV=1
PLSI_MOUSE	-0.440962	0	0.736643	Yes (2)	Plastin-1 OS=Mus musculus GN=Pls1 PE=2 SV=1
LTOR3_MOUSE	-0.439528	0	0.737376	Yes (3)	Ragulator complex protein LAMTOR3 OS=Mus musculus GN=Lamtor3 PE=1 SV=1
PSB4_MOUSE	-0.438911	0	0.737691	No	Proteasome subunit beta type-4 OS=Mus musculus GN=Psmb4 PE=1 SV=1
THIM_MOUSE	-0.437870	0	0.738224	Yes (2)	3-ketoacyl-CoA thiolase, mitochondrial OS=Mus musculus GN=Acaa2 PE=1 SV=3
STK25_MOUSE	-0.434782	0	0.739805	Yes (1)	Serine/threonine-protein kinase 25 OS=Mus musculus GN=Stk25 PE=1 SV=2
CATD_MOUSE	-0.434733	0	0.739831	Yes (4)	Cathepsin D OS=Mus musculus GN=Ctsd PE=1 SV=1
ULA1_MOUSE	-0.433753	0	0.740333	No	NEDD8-activating enzyme E1 regulatory subunit OS=Mus musculus GN=Nae1 PE=1 SV=1
TBAL3_MOUSE	-0.433621	0	0.740401	Yes (1)	Tubulin alpha chain-like 3 OS=Mus musculus GN=Tubal3 PE=2 SV=2
CSN5_MOUSE	-0.431786	0.1	0.741343	No	COP9 signalosome complex subunit 5 OS=Mus musculus GN=Cops5 PE=1 SV=3
PPP6_MOUSE	-0.421643	0	0.746574	Yes (2)	Serine/threonine-protein phosphatase 6 catalytic subunit OS=Mus musculus GN=Ppp6c PE=2 SV=1
RS27A_MOUSE	-0.421124	0	0.746842	No	Ubiquitin-40S ribosomal protein S27a OS=Mus musculus GN=Rps27a PE=1 SV=2
CBX1_MOUSE	-0.419343	0	0.747765	Yes (2)	Chromobox protein homolog 1 OS=Mus musculus GN=Cbx1 PE=1 SV=1

MK10_MOUSE	-0.418101	0	0.748409	Yes (3)	Mitogen-activated protein kinase 10 OS=Mus musculus GN=Mapk10 PE=1 SV=2
Upregulated proteins by miR-214					
PSB7_MOUSE	0.323204	0.2	1.251106		Proteasome subunit beta type-7 OS=Mus musculus GN=Psmb7 PE=1 SV=1
DPOE1_MOUSE	0.323513	0.1	1.251374		DNA polymerase epsilon catalytic subunit A OS=Mus musculus GN=Pole PE=2 SV=3
CALX_MOUSE	0.325062	0.3	1.252718		Calnexin OS=Mus musculus GN=Canx PE=1 SV=1
COPZ1_MOUSE	0.326198	0.3	1.253705		Coatomer subunit zeta-1 OS=Mus musculus GN=Copz1 PE=2 SV=1
VPS29_MOUSE	0.327551	0.1	1.254881		Vacuolar protein sorting-associated protein 29 OS=Mus musculus GN=Vps29 PE=1 SV=1
NASP_MOUSE	0.328329	0.1	1.255558		Nuclear autoantigenic sperm protein OS=Mus musculus GN=Nasp PE=1 SV=2
HNRPK_MOUSE	0.328623	0.2	1.255814		Heterogeneous nuclear ribonucleoprotein K OS=Mus musculus GN=Hnrnpk PE=1 SV=1
RTCB_MOUSE	0.328922	0.5	1.256075		tRNA-splicing ligase RtcB homolog OS=Mus musculus GN=Rtcb PE=2 SV=1
UBL4A_MOUSE	0.329965	0.2	1.256983		Ubiquitin-like protein 4A OS=Mus musculus GN=Ubl4a PE=2 SV=1
H12_MOUSE	0.330383	0.3	1.257347		Histone H1.2 OS=Mus musculus GN=Hist1h1c PE=1 SV=2
PHB2_MOUSE	0.332705	0	1.259372		Prohibitin-2 OS=Mus musculus GN=Phb2 PE=1 SV=1
SC61B_MOUSE	0.335326	0.2	1.261662		Protein transport protein Sec61 subunit beta OS=Mus musculus GN=Sec61b PE=1 SV=3
CNPY2_MOUSE	0.339157	0.3	1.265017		Protein canopy homolog 2 OS=Mus musculus GN=Cnpy2 PE=1 SV=1
TRXR1_MOUSE	0.340636	0.1	1.266315		Thioredoxin reductase 1, cytoplasmic OS=Mus musculus GN=Txnrd1 PE=1 SV=3
QCR1_MOUSE	0.341453	0.2	1.267032		Cytochrome b-c1 complex subunit 1, mitochondrial OS=Mus musculus GN=Uqcrc1 PE=1 SV=2
RFA2_MOUSE	0.341865	0	1.267394		Replication protein A 32 kDa subunit OS=Mus musculus GN=Rpa2 PE=1 SV=1
UBE2K_MOUSE	0.343689	0.2	1.268997		Ubiquitin-conjugating enzyme E2 K OS=Mus musculus GN=Ube2k PE=1 SV=3
NUCB2_MOUSE	0.348476	0.3	1.273215		Nucleobindin-2 OS=Mus musculus GN=Nucb2 PE=1 SV=2
ALR_MOUSE	0.349409	0.5	1.274038		FAD-linked sulfhydryl oxidase ALR OS=Mus musculus GN=Gfer PE=2 SV=2
TIA1_MOUSE	0.352377	0.1	1.276662		Nucleolysin TIA-1 OS=Mus musculus GN=Tia1 PE=1 SV=1
TBC15_MOUS	0.353875	0.4	1.277988		TBC1 domain family member 15

E					OS=Mus musculus GN=Tbc1d15 PE=1 SV=1
LSM6_MOUSE	0.356710	0	1.280502		U6 snRNA-associated Sm-like protein LSm6 OS=Mus musculus GN=Lsm6 PE=3 SV=1
IMPA2_MOUSE	0.357044	0.6	1.280799		Inositol monophosphatase 2 OS=Mus musculus GN=Impa2 PE=1 SV=1
VATA_MOUSE	0.360771	0.2	1.284112		V-type proton ATPase catalytic subunit A OS=Mus musculus GN=Atp6v1a PE=1 SV=2
CTBP1_MOUSE	0.364133	0	1.287108		C-terminal-binding protein 1 OS=Mus musculus GN=Ctbp1 PE=1 SV=2
SZT2_MOUSE	0.365471	0.2	1.288303		Protein SZT2 OS=Mus musculus GN=Szt2 PE=1 SV=1
RBM25_MOUSE	0.365489	0	1.288318		RNA-binding protein 25 OS=Mus musculus GN=Rbm25 PE=1 SV=2
PTMS_MOUSE	0.366225	0.7	1.288976		Parathymosin OS=Mus musculus GN=Ptms PE=1 SV=3
INF2_MOUSE	0.367510	0.1	1.290124		Inverted formin-2 OS=Mus musculus GN=Inf2 PE=1 SV=1
ILF3_MOUSE	0.368909	0	1.291376		Interleukin enhancer-binding factor 3 OS=Mus musculus GN=Ilf3 PE=1 SV=2
PABP2_MOUSE	0.371446	0.1	1.293649		Polyadenylate-binding protein 2 OS=Mus musculus GN=Pabpn1 PE=2 SV=3
ALD2_MOUSE	0.372046	0.2	1.294187		Aldose reductase-related protein 2 OS=Mus musculus GN=Akr1b8 PE=1 SV=2
TCOF_MOUSE	0.373927	0	1.295875		Treacle protein OS=Mus musculus GN=Tcof1 PE=1 SV=1
I2BP2_MOUSE	0.374380	0.3	1.296283		Interferon regulatory factor 2-binding protein 2 OS=Mus musculus GN=Irf2bp2 PE=1 SV=1
H32_MOUSE	0.375301	0.2	1.29711		Histone H3.2 OS=Mus musculus GN=Hist1h3b PE=1 SV=2
XRN2_MOUSE	0.376271	0.3	1.297983		5'-3' exoribonuclease 2 OS=Mus musculus GN=Xrn2 PE=1 SV=1
CK5P2_MOUSE	0.376365	0.2	1.298067		CDK5 regulatory subunit-associated protein 2 OS=Mus musculus GN=Cdk5rap2 PE=1 SV=3
RPN1_MOUSE	0.377760	0.1	1.299323		Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 OS=Mus musculus GN=Rpn1 PE=1 SV=1
RS28_MOUSE	0.378317	0.3	1.299825		40S ribosomal protein S28 OS=Mus musculus GN=Rps28 PE=2 SV=1
API5_MOUSE	0.378529	0.3	1.300016		Apoptosis inhibitor 5 OS=Mus musculus GN=Api5 PE=1 SV=2
IF2B3_MOUSE	0.379407	0.2	1.300807		Insulin-like growth factor 2 mRNA-binding protein 3 OS=Mus musculus GN=Igf2bp3 PE=1 SV=1
CUL4B_MOUSE	0.379845	0.2	1.301202		Cullin-4B OS=Mus musculus GN=Cul4b PE=1 SV=1
NXT2_MOUSE	0.380032	0.1	1.30137		NTF2-related export protein 2 OS=Mus musculus GN=Nxt2 PE=2

					SV=1
MYL6B_MOUSE	0.382474	0	1.303576		Myosin light chain 6B OS=Mus musculus GN=Myl6b PE=2 SV=1
PYR1_MOUSE	0.384037	0.2	1.304988		CAD protein OS=Mus musculus GN=Cad PE=2 SV=1
RTN4_MOUSE	0.384912	0.1	1.30578		Reticulon-4 OS=Mus musculus GN=Rtn4 PE=1 SV=2
KTN1_MOUSE	0.385735	0.1	1.306525		Kinectin OS=Mus musculus GN=Ktn1 PE=2 SV=1
E2F8_MOUSE	0.386205	0	1.306951		
MALT1_MOUSE	0.386487	0.4	1.307206		Mucosa-associated lymphoid tissue lymphoma translocation protein 1 homolog OS=Mus musculus GN=Malt1 PE=1 SV=2
KLC2_MOUSE	0.388877	0.4	1.309374		Kinesin light chain 2 OS=Mus musculus GN=Klc2 PE=1 SV=1
GFPT2_MOUSE	0.393627	0	1.313692		Glutamine--fructose-6-phosphate aminotransferase [isomerizing] 2 OS=Mus musculus GN=Gfpt2 PE=2 SV=3
BAP31_MOUSE	0.396808	0.1	1.316592		B-cell receptor-associated protein 31 OS=Mus musculus GN=Bcap31 PE=1 SV=4
RAB10_MOUSE	0.405410	0.4	1.324465		Ras-related protein Rab-10 OS=Mus musculus GN=Rab10 PE=1 SV=1
H1T_MOUSE	0.406704	0.2	1.325653		Histone H1t OS=Mus musculus GN=Hist1h1t PE=1 SV=4
NDUA4_MOUSE	0.411036	0.6	1.32964		NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4 OS=Mus musculus GN=Ndufa4 PE=1 SV=2
RS27L_MOUSE	0.411764	0.2	1.330311		40S ribosomal protein S27-like OS=Mus musculus GN=Rps27l PE=2 SV=3
RRBP1_MOUSE	0.414189	0.2	1.33255		Ribosome-binding protein 1 OS=Mus musculus GN=Rrbp1 PE=1 SV=2
D19L1_MOUSE	0.414387	0.2	1.332733		Probable C-mannosyltransferase DPY19L1 OS=Mus musculus GN=Dpy19l1 PE=2 SV=1
PRUNE_MOUSE	0.414520	0.5	1.332855		Protein prune homolog OS=Mus musculus GN=Prune PE=2 SV=1
UCHL5_MOUSE	0.415921	0.2	1.33415		Ubiquitin carboxyl-terminal hydrolase isozyme L5 OS=Mus musculus GN=Uchl5 PE=1 SV=2
SH3L3_MOUSE	0.420241	0.2	1.338151		SH3 domain-binding glutamic acid-rich-like protein 3 OS=Mus musculus GN=Sh3bgrl3 PE=1 SV=1
AN32B_MOUSE	0.422776	0.4	1.340504		Acidic leucine-rich nuclear phosphoprotein 32 family member B OS=Mus musculus GN=Anp32b PE=1 SV=1
TYB10_MOUSE	0.423131	0.4	1.340834		Thymosin beta-10 OS=Mus musculus GN=Tmsb10 PE=2 SV=3
VASP_MOUSE	0.425311	0.3	1.342862		Vasodilator-stimulated phosphoprotein OS=Mus musculus GN=Vasp PE=1 SV=4
IDHP_MOUSE	0.427113	0.2	1.344541		Isocitrate dehydrogenase [NADP], mitochondrial OS=Mus musculus

					GN=Idh2 PE=1 SV=3
SFR1_MOUSE	0.428830	0.3	1.346141		Swi5-dependent recombination DNA repair protein 1 homolog OS=Mus musculus GN=Sfr1 PE=1 SV=2
CO6A1_MOUSE	0.435420	0.2	1.352304		Collagen alpha-1(VI) chain OS=Mus musculus GN=Col6a1 PE=2 SV=1
CCD38_MOUSE	0.438081	0.4	1.354801		
PPP5_MOUSE	0.439429	0.5	1.356067		Serine/threonine-protein phosphatase 5 OS=Mus musculus GN=Ppp5c PE=1 SV=3
ILF2_MOUSE	0.445389	0.3	1.361681		Interleukin enhancer-binding factor 2 OS=Mus musculus GN=Ilf2 PE=1 SV=1
ERLN2_MOUSE	0.449599	0.1	1.36566		Erlin-2 OS=Mus musculus GN=Erlin2 PE=1 SV=1
TTC9C_MOUSE	0.456300	0	1.372019		Tetratricopeptide repeat protein 9C OS=Mus musculus GN=Ttc9c PE=2 SV=1
SRA1_MOUSE	0.458491	0.1	1.374104		Steroid receptor RNA activator 1 OS=Mus musculus GN=Sra1 PE=1 SV=3
RAB1A_MOUSE	0.461907	0.5	1.377361		Ras-related protein Rab-1A OS=Mus musculus GN=Rab1A PE=1 SV=3
QCR6_MOUSE	0.462570	0.4	1.377995		Cytochrome b-c1 complex subunit 6, mitochondrial OS=Mus musculus GN=Uqcrh PE=1 SV=2
SAE2_MOUSE	0.463759	0	1.379131		SUMO-activating enzyme subunit 2 OS=Mus musculus GN=Uba2 PE=1 SV=1
H2AZ_MOUSE	0.467525	0.2	1.382735		Histone H2A.Z OS=Mus musculus GN=H2afz PE=1 SV=2
SMAD3_MOUSE	0.476613	0.2	1.391473		Mothers against decapentaplegic homolog 3 OS=Mus musculus GN=Smad3 PE=1 SV=2
IKIP_MOUSE	0.477947	0	1.39276		Inhibitor of nuclear factor kappa-B kinase-interacting protein OS=Mus musculus GN=Ikbip PE=2 SV=2
HBA_MOUSE	0.478404	0.4	1.393201		Hemoglobin subunit alpha OS=Mus musculus GN=Hba PE=1 SV=2
CALL3_MOUSE	0.479895	0.5	1.394642		Calmodulin-like protein 3 OS=Mus musculus GN=Calml3 PE=2 SV=1
FBRL_MOUSE	0.481143	0.1	1.395849		rRNA 2'-O-methyltransferase fibrillarin OS=Mus musculus GN=Fbl PE=2 SV=2
ARF4_MOUSE	0.486244	0.2	1.400793		ADP-ribosylation factor 4 OS=Mus musculus GN=Arf4 PE=1 SV=2
GCR_MOUSE	0.488620	0.1	1.403102		Glucocorticoid receptor OS=Mus musculus GN=Nr3c1 PE=1 SV=1
TBCA_MOUSE	0.491108	0.4	1.405524		Tubulin-specific chaperone A OS=Mus musculus GN=Tbca PE=2 SV=3
OFUT2_MOUSE	0.495153	0.4	1.40947		GDP-fucose protein O-fucosyltransferase 2 OS=Mus musculus GN=Pofut2 PE=1 SV=1
LTOR2_MOUSE	0.499423	0.2	1.413648		Ragulator complex protein LAMTOR2 OS=Mus musculus GN=Lamtor2 PE=1 SV=1

ATPD_MOUSE	0.500446	0.3	1.41465		ATP synthase subunit delta, mitochondrial OS=Mus musculus GN=Atp5d PE=1 SV=1
MKLN1_MOUSE	0.500446	0.3	1.41465		
MP2K1_MOUSE	0.501973	0.1	1.416149		Dual specificity mitogen-activated protein kinase kinase 1 OS=Mus musculus GN=Map2k1 PE=1 SV=2
REPS1_MOUSE	0.508337	0.1	1.42241		RalBP1-associated Eps domain-containing protein 1 OS=Mus musculus GN=Reps1 PE=1 SV=2
DHRS4_MOUSE	0.509393	0.2	1.423451		Dehydrogenase/reductase SDR family member 4 OS=Mus musculus GN=Dhrs4 PE=1 SV=3
PTMA_MOUSE	0.525322	0.4	1.439254		Prothymosin alpha OS=Mus musculus GN=Ptma PE=1 SV=2
NC2B_MOUSE	0.527111	0.1	1.441041		Protein Dr1 OS=Mus musculus GN=Dr1 PE=2 SV=1
FETUA_MOUSE	0.530992	0.3	1.444922		Alpha-2-HS-glycoprotein OS=Mus musculus GN=Ahsg PE=1 SV=1
LAP2B_MOUSE	0.531212	0.1	1.445143		Lamina-associated polypeptide 2, isoforms beta/delta/epsilon/gamma OS=Mus musculus GN=Tmpo PE=1 SV=4
PRS10_MOUSE	0.532698	0.4	1.446632		26S protease regulatory subunit 10B OS=Mus musculus GN=Psmc6 PE=1 SV=1
NEST_MOUSE	0.533669	0.2	1.447606		Nestin OS=Mus musculus GN=Nes PE=1 SV=1
NPTN_MOUSE	0.533795	0.1	1.447732		Neuroplastin OS=Mus musculus GN=Nptn PE=1 SV=3
E41L3_MOUSE	0.537226	0.5	1.451179		Band 4.1-like protein 3 OS=Mus musculus GN=Epb41l3 PE=1 SV=1
MEPCE_MOUSE	0.537434	0.2	1.451389		7SK snRNA methylphosphate capping enzyme OS=Mus musculus GN=Mepce PE=1 SV=2
LAP2A_MOUSE	0.537485	0	1.45144		Lamina-associated polypeptide 2, isoforms alpha/zeta OS=Mus musculus GN=Tmpo PE=1 SV=4
SRSF4_MOUSE	0.550978	0	1.465078		Serine/arginine-rich splicing factor 4 OS=Mus musculus GN=Srsf4 PE=2 SV=1
PR40A_MOUSE	0.552884	0	1.467015		Pre-mRNA-processing factor 40 homolog A OS=Mus musculus GN=Prpf40a PE=1 SV=1
H33_MOUSE	0.562486	0.2	1.476811		Histone H3.3 OS=Mus musculus GN=H3f3a PE=1 SV=2
DHRS1_MOUSE	0.567058	0.1	1.481499		Dehydrogenase/reductase SDR family member 1 OS=Mus musculus GN=Dhrs1 PE=2 SV=1
IF1A_MOUSE	0.571096	0	1.485652		Eukaryotic translation initiation factor 1A OS=Mus musculus GN=Eif1a PE=2 SV=3
PP14B_MOUSE	0.595869	0.2	1.511382		Protein phosphatase 1 regulatory subunit 14B OS=Mus musculus GN=Ppp1r14b PE=1 SV=2
IMMT_MOUSE	0.599209	0.1	1.514886		Mitochondrial inner membrane protein OS=Mus musculus GN=Immt

					PE=1 SV=1
MBB1A_MOUSE	0.601030	0	1.5168		Myb-binding protein 1A OS=Mus musculus GN=Mybbp1a PE=1 SV=2
RIR1_MOUSE	0.603171	0	1.519051		Ribonucleoside-diphosphate reductase large subunit OS=Mus musculus GN=Rrm1 PE=1 SV=2
IF2B2_MOUSE	0.607204	0.2	1.523304		Insulin-like growth factor 2 mRNA-binding protein 2 OS=Mus musculus GN=Igf2bp2 PE=1 SV=1
H13_MOUSE	0.627204	0	1.544569		Histone H1.3 OS=Mus musculus GN=Hist1h1d PE=1 SV=2
RANB3_MOUSE	0.630777	0.3	1.548399		Ran-binding protein 3 OS=Mus musculus GN=Ranbp3 PE=1 SV=2
SPF27_MOUSE	0.635218	0.3	1.553173		Pre-mRNA-splicing factor SPF27 OS=Mus musculus GN=Bcas2 PE=2 SV=1
P20L1_MOUSE	0.637010	0	1.555103		PHD finger protein 20-like protein 1 OS=Mus musculus GN=Phf20l1 PE=2 SV=2
HMGA2_MOUSE	0.643604	0.1	1.562227		High mobility group protein HMGI-C OS=Mus musculus GN=Hmga2 PE=1 SV=1
EDF1_MOUSE	0.643840	0.3	1.562482		Endothelial differentiation-related factor 1 OS=Mus musculus GN=Edf1 PE=1 SV=1
TECR_MOUSE	0.644426	0.2	1.563117		Very-long-chain enoyl-CoA reductase OS=Mus musculus GN=Tecr PE=1 SV=1
ITAL_MOUSE	0.649337	0.1	1.568448		Integrin alpha-L OS=Mus musculus GN=Itgal PE=1 SV=2
ABCE1_MOUSE	0.650618	0.1	1.56984		ATP-binding cassette sub-family E member 1 OS=Mus musculus GN=Abce1 PE=2 SV=1
C1QBP_MOUSE	0.665730	0.2	1.586371		Complement component 1 Q subcomponent-binding protein, mitochondrial OS=Mus musculus GN=C1qbp PE=1 SV=1
AT2B2_MOUSE	0.671810	0.2	1.59307		Plasma membrane calcium-transporting ATPase 2 OS=Mus musculus GN=Atp2b2 PE=1 SV=2
DVL3_MOUSE	0.674132	0.3	1.595637		Segment polarity protein dishevelled homolog DVL-3 OS=Mus musculus GN=Dvl3 PE=1 SV=2
RFPLA_MOUSE	0.691521	0.2	1.614985		Ret finger protein-like 4A OS=Mus musculus GN=Rfpl4a PE=2 SV=1
WNK1_MOUSE	0.703097	0.6	1.627996		Serine/threonine-protein kinase WNK1 OS=Mus musculus GN=Wnk1 PE=1 SV=2
NDUS5_MOUSE	0.703673	0.1	1.628646		NADH dehydrogenase [ubiquinone] iron-sulfur protein 5 OS=Mus musculus GN=Ndufs5 PE=1 SV=3
FKB11_MOUSE	0.704716	0.6	1.629824		Peptidyl-prolyl cis-trans isomerase FKBP11 OS=Mus musculus GN=Fkbp11 PE=2 SV=1
SC22B_MOUSE	0.707687	0.1	1.633183		Vesicle-trafficking protein SEC22b OS=Mus musculus GN=Sec22b PE=1 SV=3
PUR8_MOUSE	0.718084	0.1	1.644996		Adenylosuccinate lyase OS=Mus

					musculus GN=Adsl PE=2 SV=2
BIEA_MOUSE	0.734828	0	1.664199		Biliverdin reductase A OS=Mus musculus GN=Blvra PE=2 SV=1
ADT4_MOUSE	0.752110	0	1.684254		ADP/ATP translocase 4 OS=Mus musculus GN=Slc25a31 PE=2 SV=1
H2AY_MOUSE	0.761745	0.3	1.69554		Core histone macro-H2A.1 OS=Mus musculus GN=H2afy PE=1 SV=3
GSLG1_MOUSE	0.771952	0.3	1.707579		Golgi apparatus protein 1 OS=Mus musculus GN=Glg1 PE=1 SV=1
EVL_MOUSE	0.778213	0	1.715005		Ena/VASP-like protein OS=Mus musculus GN=Evl PE=1 SV=2
RPC2_MOUSE	0.780418	0	1.717628		DNA-directed RNA polymerase III subunit RPC2 OS=Mus musculus GN=Polr3b PE=2 SV=2
SLK_MOUSE	0.789592	0.1	1.728586		STE20-like serine/threonine-protein kinase OS=Mus musculus GN=Slk PE=1 SV=2
LARP7_MOUSE	0.790086	0	1.729178		La-related protein 7 OS=Mus musculus GN=Larp7 PE=1 SV=2
ATPG_MOUSE	0.849791	0	1.80224		ATP synthase subunit gamma, mitochondrial OS=Mus musculus GN=Atp5c1 PE=1 SV=1
H2A2B_MOUSE	0.867560	0	1.824574		Histone H2A type 2-B OS=Mus musculus GN=Hist2h2ab PE=1 SV=3
ZBT43_MOUSE	0.883939	0.1	1.845407		Zinc finger and BTB domain-containing protein 43 OS=Mus musculus GN=Zbtb43 PE=2 SV=2
HECD1_MOUSE	0.898640	0.3	1.864307		E3 ubiquitin-protein ligase HECTD1 OS=Mus musculus GN=Hectd1 PE=1 SV=2
ALBU_MOUSE	0.907684	0.3	1.876031		Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3
MEP50_MOUSE	0.921289	0.4	1.893807		Methylosome protein 50 OS=Mus musculus GN=Wdr77 PE=1 SV=1
MAP6_MOUSE	0.960701	0	1.946255		Microtubule-associated protein 6 OS=Mus musculus GN=Map6 PE=1 SV=2
PLP2_MOUSE	0.968741	0.4	1.957132		Proteolipid protein 2 OS=Mus musculus GN=Plp2 PE=2 SV=1
M4K4_MOUSE	1.004881	0.1	2.006777		Mitogen-activated protein kinase kinase kinase kinase 4 OS=Mus musculus GN=Map4k4 PE=1 SV=1
EHD3_MOUSE	1.008355	0	2.011615		EH domain-containing protein 3 OS=Mus musculus GN=Ehd3 PE=1 SV=2
FBLN3_MOUSE	1.010373	0.1	2.014431		EGF-containing fibulin-like extracellular matrix protein 1 OS=Mus musculus GN=Efemp1 PE=2 SV=1
PI3R4_MOUSE	1.022615	0.2	2.031597		Phosphoinositide 3-kinase regulatory subunit 4 OS=Mus musculus GN=Pik3r4 PE=1 SV=3
HAP28_MOUSE	1.123091	0.7	2.178132		28 kDa heat- and acid-stable phosphoprotein OS=Mus musculus GN=Pdap1 PE=1 SV=1
ATPO_MOUSE	1.164437	0.6	2.241457		ATP synthase subunit O, mitochondrial OS=Mus musculus

					GN=Atp5o PE=1 SV=1
NUFP2_MOUSE	1.298795	0.1	2.460232		Nuclear fragile X mental retardation-interacting protein 2 OS=Mus musculus GN=Nufip2 PE=1 SV=1
MRCKB_MOUSE	1.353744	0.3	2.555745		Serine/threonine-protein kinase MRCK beta OS=Mus musculus GN=Cdc42bpb PE=1 SV=2
CPPED_MOUSE	1.474910	0	2.779663		Serine/threonine-protein phosphatase CPPED1 OS=Mus musculus GN=Cpped1 PE=2 SV=1
ABRA_MOUSE	1.561250	0.3	2.951095		
H2AX_MOUSE	1.567391	0.1	2.963682		Histone H2AX OS=Mus musculus GN=H2afx PE=1 SV=2
BRCC3_MOUSE	1.613716	0.4	3.060391		Lys-63-specific deubiquitinase BRCC36 OS=Mus musculus GN=Brcc3 PE=2 SV=1
ARP3B_MOUSE	1.795556	0.6	3.471492		Actin-related protein 3B OS=Mus musculus GN=Actr3b PE=2 SV=1
FRIL2_MOUSE	2.195888	0.4	4.581715		Ferritin light chain 2 OS=Mus musculus GN=Ftl2 PE=2 SV=2
Other reported targets of miR-214					
CTNB1_MOUSE	-0.181716	0.6	0.881654		Catenin beta-1 OS=Mus musculus GN=Ctnnb1 PE=1 SV=1
PCBP2_MOUSE	-0.042262	0.8	0.971131		Poly(rC)-binding protein 2 OS=Mus musculus GN=Pcbp2 PE=1 SV=1

Supplementary table S3: **GO term enrichment analysis of down-regulated proteins by miR-214 in VSMCs.**

Analysis Type:	PANTHER Overrepresentation Test (release 20160321)
Annotation Version and Release Date:	GO Ontology database Released 2016-04-23
Analyzed List:	Down-regulated (Mus musculus)
Reference List:	Mus musculus (all genes in database)
Bonferroni correction:	TRUE

GO biological process complete	**Mus musculus - REFLIST (22320)**	**Proteins (56)**	**Proteins (expected)**	**Proteins (over/under)**	**Proteins (fold Enrichment)**	**Proteins (P-value)***	**Protein name**
regulation of actin filament polymerization (GO:0030833)	137	6	0.34	+	17.46	1.05E-02	NCKPA1, RHOA, ARPC2, SPTB1, PROF1, CAPZB
regulation of protein polymerization (GO:0032271)	174	7	0.44	+	16.03	2.29E-03	NCKPA1, RHOA, ARPC2, SPTB1, PROF1, CAPZB, EMAL1
regulation of actin polymerization or depolymerization (GO:0008064)	156	6	0.39	+	15.33	2.21E-02	NCKPA1, RHOA, ARPC2, SPTB1, PROF1, CAPZB
regulation of actin filament length (GO:0030832)	157	6	0.39	+	15.23	2.30E-02	NCKPA1, RHOA, ARPC2, SPTB1, PROF1, CAPZB
regulation of actin cytoskeleton organization (GO:0032956)	278	7	0.7	+	10.04	4.99E-02	NCKPA1, RHOA, ARPC2, SPTB1, PROF1, CAPZB, PPM1F
organelle organization (GO:0006996)	2779	21	6.97	+	3.01	1.30E-02	NCKAP1, RHOA, STK25, SNX12, PLSI, THIM, TPP1, CATD, EMAL1, ARPC2, PPM1F, PROF1, ABL2, SMC3, ARL3, SNX5, SNP29, LONM, SMC1A, CAPZB, APC7