

Translating mRNAs strongly correlate to proteins in a multivariate manner and their translation ratios are phenotype-specific

Tong Wang *, Yizhi Cui, Jingjie Jin, Jiahui Guo, Guibin Wang, Xingfeng Yin, Qing-Yu He *, Gong Zhang *

SUPPLEMENTARY MATERIALS

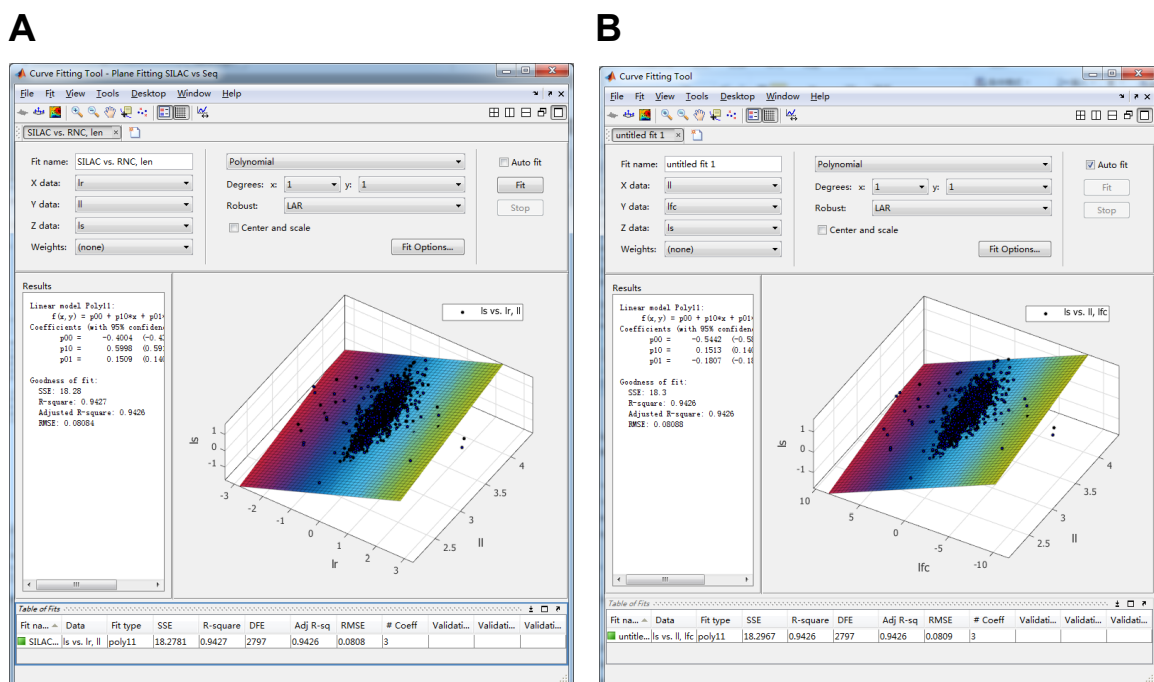


Figure S1. Raw screenshot of multivariate linear fitting of mRNA length, relative abundances of RNC-mRNAs and proteins, comparing A549 with HBE cells. The RNC-mRNA abundance ratio was calculated based on (A) rpkm method and (B) edgeR result. The fitting was performed using CurveFitting Toolbox in MATLAB R2012a.

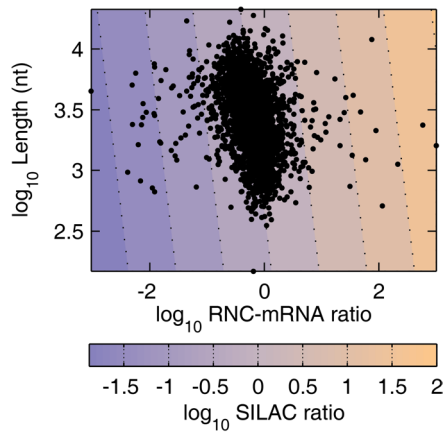


Figure S2 Top view of **Fig. 3C**, showing that relative abundances of RNC-mRNAs are not correlated with mRNA lengths.

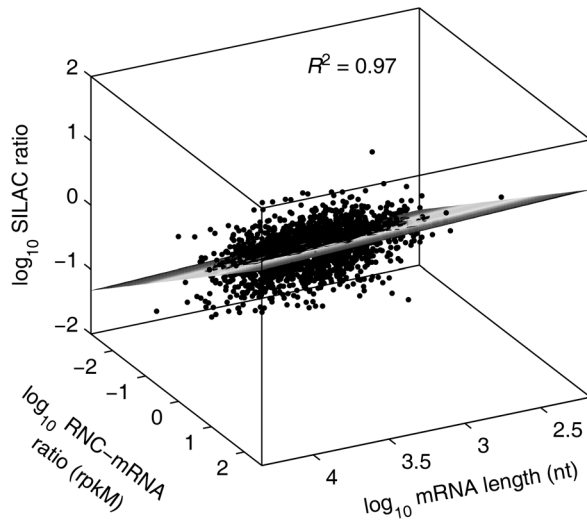


Figure S3 Multivariate linear model fitting SILAC ratio (H1299/HBE), mRNA length and RNC-mRNA ratio (H1299/HBE), calculated based on rpkm normalization.

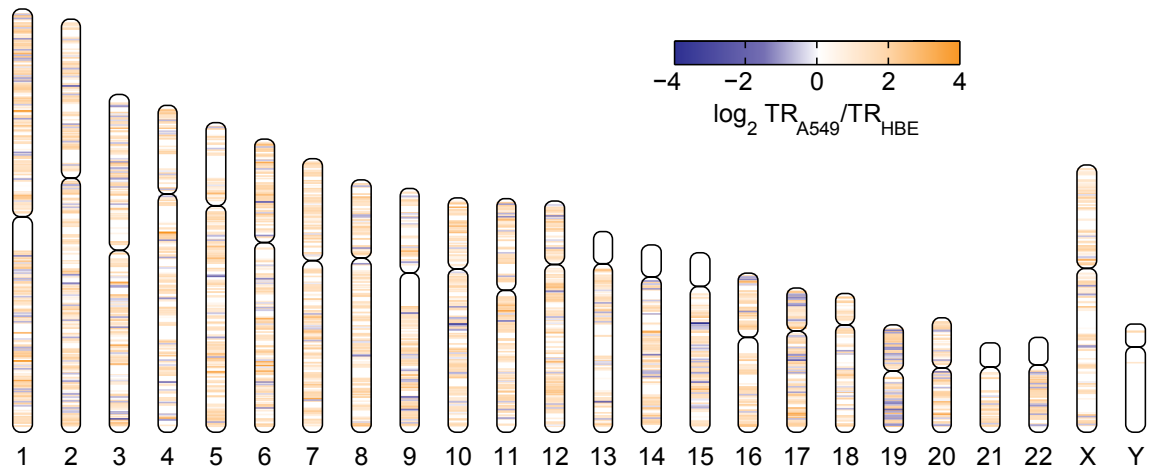


Figure S4 Chromosomal locations of up-regulated (orange) and down-regulated (blue) genes in terms of TR ratios (A549/HBE). The color intensity represents the grade of TR regulation as indicated in the color scale.

A

Top Networks		
ID	Associated Network Functions	Score
1	View RNA Post-Transcriptional Modification, RNA Damage and Repair, Cell Morphology	45
2	View Cellular Assembly and Organization, Nucleic Acid Metabolism, Small Molecule Biochemistry	42
3	View Cell Cycle, Cell Morphology, Cellular Assembly and Organization	37
4	View Connective Tissue Disorders, Dermatological Diseases and Conditions, Developmental Disorder	37
5	View Carbohydrate Metabolism, Lipid Metabolism, Small Molecule Biochemistry	37

Top Bio Functions		
Diseases and Disorders		
Name	p-value	# Molecules
Cancer	1.82E-12 - 5.13E-03	580
Neurological Disease	1.99E-12 - 2.15E-03	194
Skeletal and Muscular Disorders	5.30E-11 - 2.15E-03	160
Gastrointestinal Disease	8.18E-09 - 4.43E-03	220
Hematological Disease	1.74E-08 - 4.92E-03	143

Molecular and Cellular Functions		
Name	p-value	# Molecules
Cell Death	4.33E-13 - 4.52E-03	448
DNA Replication, Recombination, and Repair	6.11E-13 - 3.15E-03	160
Cellular Growth and Proliferation	2.88E-11 - 5.04E-03	439
Free Radical Scavenging	4.80E-10 - 2.15E-03	89
Cellular Assembly and Organization	1.44E-09 - 5.04E-03	289

Top Canonical Pathways		
Name	p-value	Ratio
NRF2-mediated Oxidative Stress Response	3.53E-11	44/191 (0.23)
Mitochondrial Dysfunction	1.18E-10	36/174 (0.207)
Glycolysis/Gluconeogenesis	1.71E-10	28/130 (0.215)
Butanoate Metabolism	6.39E-10	22/128 (0.172)
Integrin Signaling	4.39E-09	43/210 (0.205)

B

Top Networks		
ID	Associated Network Functions	Score
1	View Cellular Assembly and Organization, Cellular Function and Maintenance, Cardiovascular System Development and Function	25
2	View Amino Acid Metabolism, Developmental Disorder, Hematological Disease	25
3	View Endocrine System Development and Function, Small Molecule Biochemistry, Nervous System Development and Function	23
4	View Cellular Assembly and Organization, Cellular Compromise, Organ Morphology	22
5	View Cell Signaling, Nucleic Acid Metabolism, Small Molecule Biochemistry	22

Top Bio Functions		
Diseases and Disorders		
Name	p-value	# Molecules
Immunological Disease	4.84E-07 - 5.52E-03	24
Dermatological Diseases and Conditions	8.33E-07 - 5.52E-03	25
Inflammatory Disease	8.33E-07 - 5.52E-03	30
Cancer	2.10E-06 - 5.52E-03	48
Developmental Disorder	2.91E-06 - 5.52E-03	21

Molecular and Cellular Functions		
Name	p-value	# Molecules
Cell Death and Survival	2.48E-07 - 5.52E-03	41
Cell Cycle	3.88E-07 - 5.52E-03	23
Cell Signaling	4.08E-07 - 5.52E-03	19
Molecular Transport	4.08E-07 - 3.99E-03	17
Nucleic Acid Metabolism	4.08E-07 - 2.97E-04	11

Top Canonical Pathways		
Name	p-value	Ratio
Role of Tissue Factor in Cancer	2.96E-04	5/114 (0.044)
Airway Pathology in Chronic Obstructive Pulmonary Disease	8.24E-04	2/9 (0.222)
Hepatic Fibrosis / Hepatic Stellate Cell Activation	1.02E-03	5/146 (0.034)
SAPK/JNK Signaling	1.8E-03	4/102 (0.039)
IGF-1 Signaling	1.94E-03	4/105 (0.038)

Figure S5. General reports of Ingenuity pathway analysis (IPA) of (a) differentially expressed proteins (DEPs, fold change ≥ 1.5) and (b) genes with TR fold change ≥ 4.0 .

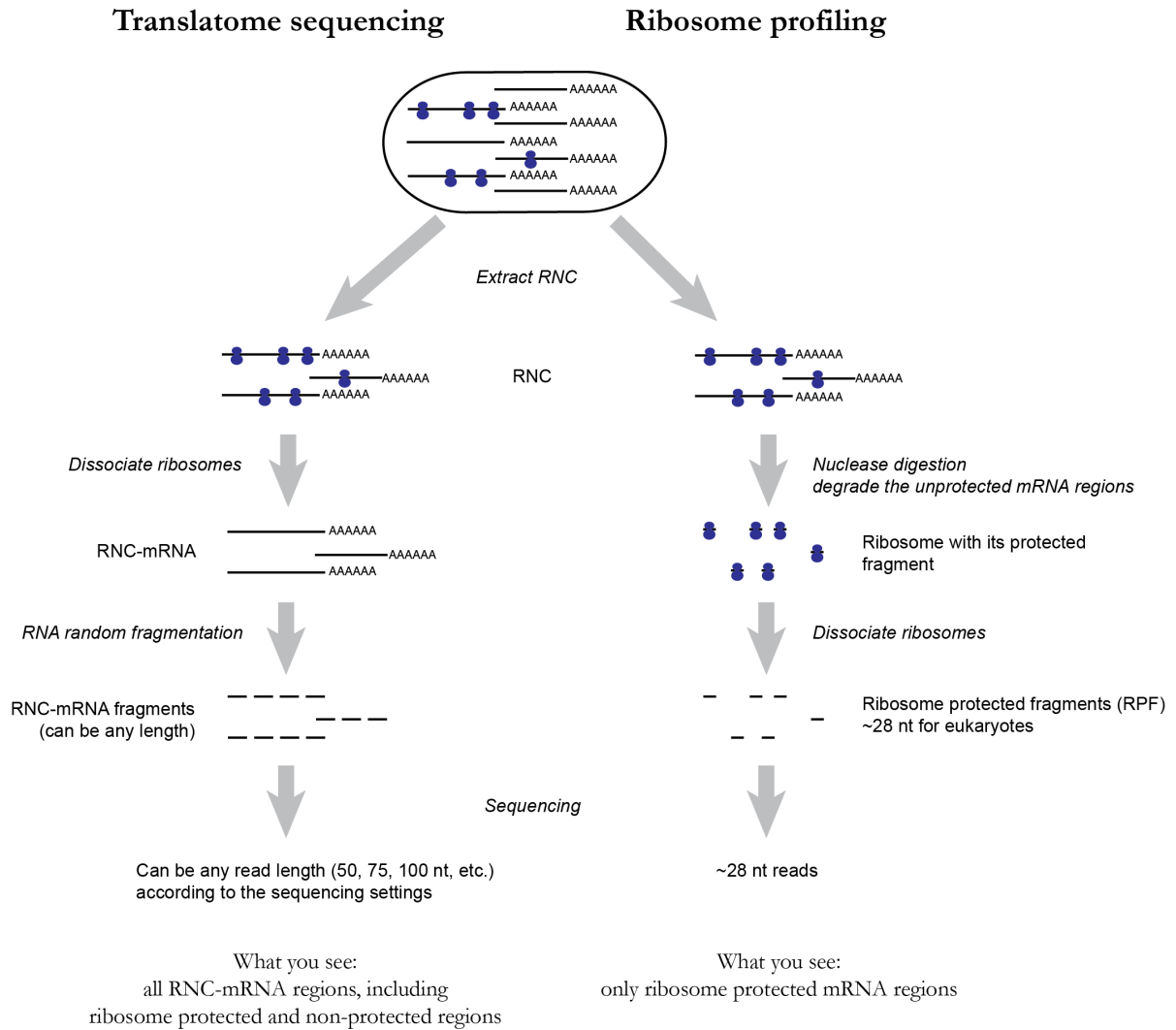


Figure S6: Comparison of translatome sequencing (applied in this study) and ribosome profiling. Both techniques require the extraction of RNC fraction. Next, ribosome profiling degrades unprotected mRNA regions using nuclease and then dissociates ribosomes, leaving the ribosome protected mRNA fragments (RPF), usually ~28nt for eukaryotes, for sequencing (1). In contrast, translatome profiling dissociates ribosomes from translating mRNAs immediately after RNC extraction. The entire translating mRNA molecules are then random fragmented and sequenced. These fragments contain both ribosome protected and unprotected regions. The fragments can be of any length, and the sequencing read lengths are totally dependent on the sequencing settings.

1. Ingolia, N.T., Ghaemmaghami, S., Newman, J.R. and Weissman, J.S. (2009) Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. *Science*, **324**, 218-223.

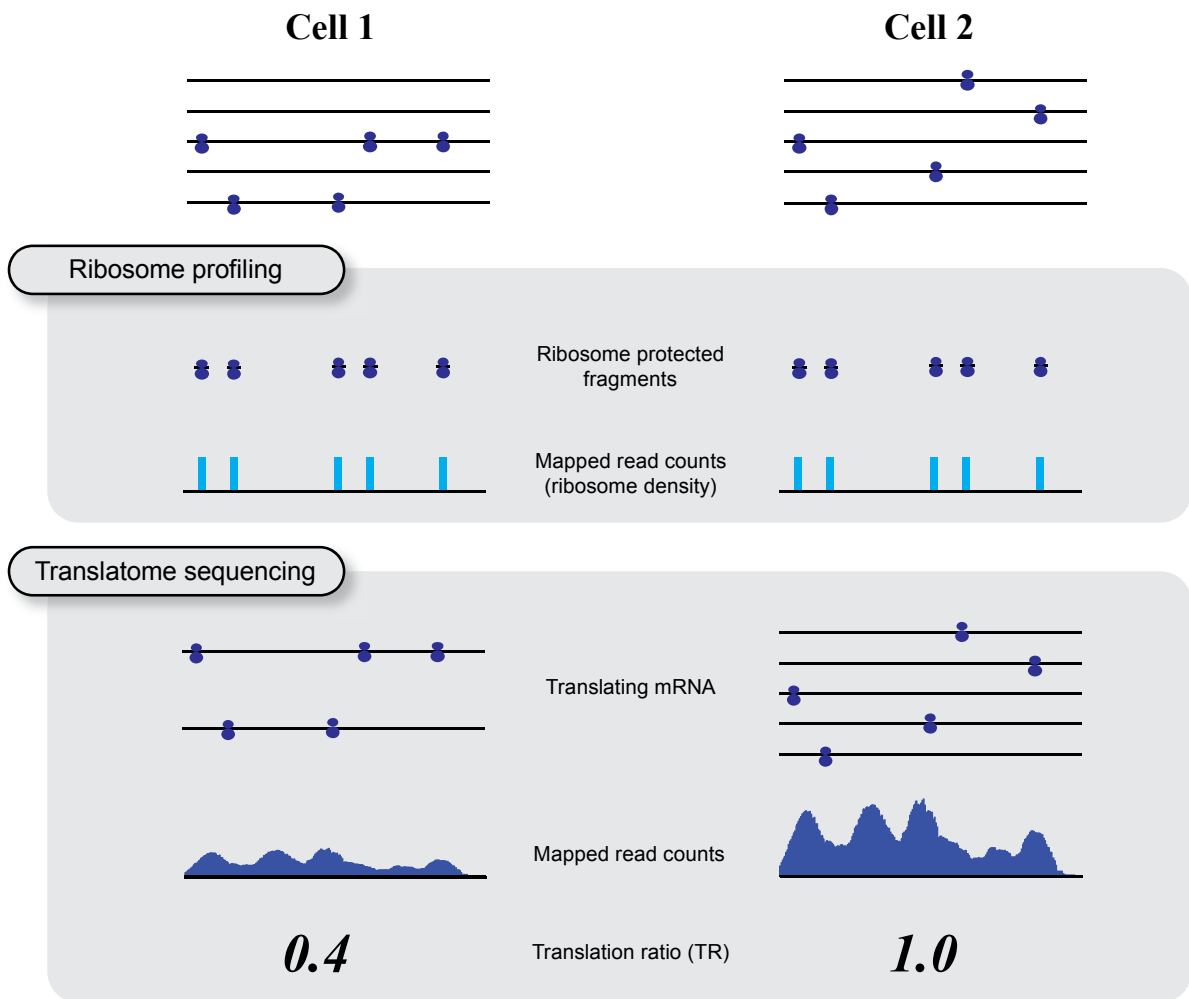


Figure S7: An example of differential outcomes comparing ribosome profiling and translatoem sequencing. Assume a gene X in two cells with obvious translation scenarios: in cell 1, only a small fraction of mRNA is subjected to translation and multiple ribosomes are attached to each translating mRNA molecule; in cell 2, almost all the mRNA is subjected to translation and only one ribosome is attached to each translating mRNA molecule. Using ribosome profiling, the ribosome protected fragments in the two cells may be identical, resulting in the same ribosome density. In contrast, translatoem sequencing is able to distinguish the difference of the amount of the translating mRNA molecules, thus can accurately determine the TR of this gene in the two cells. However, the sequencing reads from translatoem sequencing include both ribosome protected and unprotected regions, while ribosome profiling can accurately locate the positions of ribosomes.

Table S1. Number of sequenced and mapped reads with high quality of mRNA and RNC-mRNA of A549 and HBE cells.

Sample	Sequenced reads	Mapped reads
A549 mRNA	19370847	15700378
A549 RNC-mRNA	13538864	11851482
HBE mRNA	12326472	9622423
HBE RNC-mRNA	16782103	14437109
H1299 mRNA	14800743	11248989
H1299 RNC-mRNA	14801206	12139690

Table S2. RT-PCR primers for validation of the six randomly selected genes.

Gene	Database accessions			RT-PCR primers (5'-3')		Abundance (rpKM)			
	RNA RefSeq	Protein RefSeq	UniProt accession	Forward primer	Reverse primer	A549	A549	HBE	HBE
						mRNA	RNC-mRNA	mRNA	RNC-mRNA
TGIF2	NM_001199513	NP_068581	Q9GZN2	CTCCGCACAAAGTTTAC	CTTCGTCTCACCTAG	2.92	4.488	27.887	28.351
BLCAP	NM_001167823	NP_006689	P62952	GTGTAGAAAGAGCCCTGAC	CCTGCCATCACGTCAG	9.975	16.705	108.438	101.268
DIDO1	NM_001193369	NP_149072	Q9BTC0	CATTGTACGTACTACAG	AACCTATGGACACTTGG	19.442	11.825	123.585	54.446
HMGB3P1	NR_002165	n/a	n/a	GAAGTGACCCAGGAAAC	CACAAAGAAGGCAAAAGCAG	23.468	65.714	48.852	290.81
ACSS2	NM_001242393	NP_001229322	n/a	GGGCTAGATGGGGTC	CCAGAATTCTGCACTCAC	6.511	8.263	39.924	34.794
FAM217B	NM_001190826	NP_071389	n/a	CAGTGCTCTGGCTATTG	GGAAAGGTCTCCTACCAC	8.197	11.362	51.46	46.07

n/a = not available

Table S3 List of gene identifications of RNA-seq of total mRNA and RNC-mRNA in A549 and HBE cells. The splice variants were merged.
(Separate Excel file)

Table S4 List of proteins identified by SILAC-based MS (A549/HBE).
(Separate Excel file)

Table S5 List of gene identifications of RNA-seq of total mRNA and RNC-mRNA in H1299 cells. The splice variants were merged.
(Separate Excel file)

Table S6 List of proteins identified by SILAC-based MS (H1299/HBE).
(Separate Excel file)

Table S7. Genes with TR fold change ≥ 4.0 (\log_2 scale 2.0), comparing A549 with HBE cells.

Gene name	log2 TR fold change	Function
<i>C1orf74</i>	4.185	Homo sapiens chromosome 1 open reading frame 74 (C1orf74), mRNA
<i>IL8</i>	4.123	Homo sapiens interleukin 8 (IL8), mRNA
<i>JUN</i>	4.059	Homo sapiens jun proto-oncogene (JUN), mRNA
<i>GEM</i>	3.737	Homo sapiens GTP binding protein overexpressed in skeletal muscle (GEM), mRNA
<i>SGK1</i>	3.596	Homo sapiens serum/glucocorticoid regulated kinase 1 (SGK1), mRNA
<i>SNORD46</i>	3.449	Homo sapiens adrenoceptor beta 2, surface (ADRB2), mRNA
<i>PLCXD2</i>	3.359	Homo sapiens phosphatidylinositol-specific phospholipase C, X domain containing 2 (PLCXD2), mRNA
<i>C14orf182</i>	3.349	Homo sapiens chromosome 14 open reading frame 182 (C14orf182), mRNA
<i>DUSP10</i>	3.292	Homo sapiens dual specificity phosphatase 10 (DUSP10), mRNA
<i>FOSL1</i>	3.282	Homo sapiens FOS-like antigen 1 (FOSL1), mRNA
<i>MTRNR2L6</i>	3.276	Homo sapiens MT-RNR2-like 6 (MTRNR2L6), mRNA
<i>EDN1</i>	3.193	Homo sapiens endothelin 1 (EDN1), mRNA
<i>C17orf110</i>	3.165	Homo sapiens chromosome 17 open reading frame 110 (C17orf110), mRNA
<i>MTRNR2L3</i>	3.111	Homo sapiens MT-RNR2-like 3 (MTRNR2L3), mRNA
<i>PRDM1</i>	3.074	Homo sapiens PR domain containing 1, with ZNF domain (PRDM1), mRNA
<i>MTRNR2L10</i>	3.036	Homo sapiens MT-RNR2-like 10 (MTRNR2L10), mRNA
<i>CSRNP1</i>	3.013	Homo sapiens cysteine-serine-rich nuclear protein 1 (CSRNP1), mRNA
<i>ANKRD37</i>	3.001	Homo sapiens ankyrin repeat domain 37 (ANKRD37), mRNA
<i>MTRNR2L1</i>	2.998	Homo sapiens MT-RNR2-like 1 (MTRNR2L1), mRNA
<i>TNFAIP3</i>	2.983	Homo sapiens tumor necrosis factor, alpha-induced protein 3 (TNFAIP3), mRNA
<i>CTGF</i>	2.976	Homo sapiens connective tissue growth factor (CTGF), mRNA
<i>TM4SF1</i>	2.968	Homo sapiens transmembrane 4 L six family member 1 (TM4SF1), mRNA

SPHK1	2.875	Homo sapiens sphingosine kinase 1 (SPHK1), mRNA
GRHL3	2.839	Homo sapiens grainyhead-like 3 (Drosophila) (GRHL3), mRNA
KLF6	2.818	Homo sapiens Kruppel-like factor 6 (KLF6), mRNA
BCL2A1	2.815	Homo sapiens BCL2-related protein A1 (BCL2A1), mRNA
EMBP1	2.810	Homo sapiens suppressor of cytokine signaling 3 (SOCS3), mRNA
SRR	2.807	Homo sapiens serine racemase (SRR), mRNA
MTRNR2L4	2.767	Homo sapiens MT-RNR2-like 4 (MTRNR2L4), mRNA
C1orf56	2.660	Homo sapiens chromosome 1 open reading frame 56 (C1orf56), mRNA
SOX9	2.655	Homo sapiens SRY (sex determining region Y)-box 9 (SOX9), mRNA
PPM1L	2.651	Homo sapiens protein phosphatase, Mg ²⁺ /Mn ²⁺ dependent, 1L (PPM1L), mRNA
TPM2	2.626	Homo sapiens tropomyosin 2 (beta) (TPM2), mRNA
KLF4	2.619	Homo sapiens Kruppel-like factor 4 (gut) (KLF4), mRNA
C6orf226	2.610	Homo sapiens chromosome 6 open reading frame 226 (C6orf226), mRNA
UFSP1	2.610	Homo sapiens UFM1-specific peptidase 1 (non-functional) (UFSP1), mRNA
PTGER4	2.599	Homo sapiens prostaglandin E receptor 4 (subtype EP4) (PTGER4), mRNA
SATB1	2.593	Homo sapiens SATB homeobox 1 (SATB1), mRNA
CXCL3	2.593	Homo sapiens chemokine (C-X-C motif) ligand 3 (CXCL3), mRNA
BCORP1	2.579	Homo sapiens regulator of G-protein signaling 2, 24kDa (RGS2), mRNA
PPP1R15A	2.576	Homo sapiens protein phosphatase 1, regulatory subunit 15A (PPP1R15A), mRNA
TMCC3	2.575	Homo sapiens transmembrane and coiled-coil domain family 3 (TMCC3), mRNA
CXCL2	2.570	Homo sapiens chemokine (C-X-C motif) ligand 2 (CXCL2), mRNA
PMAIP1	2.562	Homo sapiens phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1), mRNA
MTRNR2L8	2.558	Homo sapiens MT-RNR2-like 8 (MTRNR2L8), mRNA
FAM43A	2.557	Homo sapiens family with sequence similarity 43, member A (FAM43A), mRNA
KRT16	2.539	Homo sapiens keratin 16 (KRT16), mRNA
FOXQ1	2.502	Homo sapiens forkhead box Q1 (FOXQ1), mRNA
ATAD3C	2.479	Homo sapiens ATPase family, AAA domain containing 3C (ATAD3C), mRNA
HBEGF	2.475	Homo sapiens heparin-binding EGF-like growth factor (HBEGF), mRNA
CLYBL	2.470	Homo sapiens citrate lyase beta like (CLYBL), mRNA
GNG11	2.457	Homo sapiens guanine nucleotide binding protein (G protein), gamma 11 (GNG11), mRNA
GSTM2	2.444	Homo sapiens glutathione S-transferase mu 2 (muscle) (GSTM2), mRNA
CTU1	2.436	Homo sapiens cytosolic thiouridylase subunit 1 homolog (S. pombe) (CTU1), mRNA
MTRNR2L2	2.435	Homo sapiens MT-RNR2-like 2 (MTRNR2L2), mRNA
SRRM5	2.430	Homo sapiens serine/arginine repetitive matrix 5 (SRRM5), mRNA
POLL	2.428	Homo sapiens polymerase (DNA directed), lambda (POLL), mRNA
FAM224B	2.412	Homo sapiens tenascin C (TNC), mRNA
HSPA1L	2.406	Homo sapiens heat shock 70kDa protein 1-like (HSPA1L), mRNA
FAM27A	2.389	Homo sapiens AHNAK nucleoprotein (AHNAK), mRNA
KCNH3	2.381	Homo sapiens potassium voltage-gated channel, subfamily H (eag-related), member 3

		(KCNH3), mRNA
CSPG4P1Y	2.381	Homo sapiens cysteine-rich, angiogenic inducer, 61 (CYR61), mRNA
PTGIS	2.374	Homo sapiens prostaglandin I2 (prostacyclin) synthase (PTGIS), mRNA
ADAT3	2.361	Homo sapiens adenosine deaminase, tRNA-specific 3 (ADAT3), mRNA
CDNF	2.356	Homo sapiens cerebral dopamine neurotrophic factor (CDNF), mRNA
PKD2L2	2.347	Homo sapiens polycystic kidney disease 2-like 2 (PKD2L2), mRNA
ARL5B	2.323	Homo sapiens ADP-ribosylation factor-like 5B (ARL5B), mRNA
MIR298	2.321	Homo sapiens zinc finger protein 34 (ZNF34), mRNA
TPPP	2.301	Homo sapiens tubulin polymerization promoting protein (TPPP), mRNA
RPL31P11	2.290	Homo sapiens cyclin-dependent kinase 17 (CDK17), mRNA
C14orf132	2.285	Homo sapiens chromosome 14 open reading frame 132 (C14orf132), mRNA
IL17D	2.270	Homo sapiens interleukin 17D (IL17D), mRNA
STAG3	2.269	Homo sapiens stromal antigen 3 (STAG3), mRNA
FAM131B	2.238	Homo sapiens family with sequence similarity 131, member B (FAM131B), mRNA
FOXD1	2.227	Homo sapiens forkhead box D1 (FOXD1), mRNA
MC1R	2.223	Homo sapiens melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor) (MC1R), mRNA
TM4SF19	2.214	Homo sapiens transmembrane 4 L six family member 19 (TM4SF19), mRNA
ARHGAP30	2.206	Homo sapiens Rho GTPase activating protein 30 (ARHGAP30), mRNA
SH2B2	2.188	Homo sapiens SH2B adaptor protein 2 (SH2B2), mRNA
MYL5	2.180	Homo sapiens myosin, light chain 5, regulatory (MYL5), mRNA
RPS6KA6	2.170	Homo sapiens ribosomal protein S6 kinase, 90kDa, polypeptide 6 (RPS6KA6), mRNA
RTL1	2.169	Homo sapiens retrotransposon-like 1 (RTL1), mRNA
ZNF789	2.161	Homo sapiens zinc finger protein 789 (ZNF789), mRNA
C15orf41	2.161	Homo sapiens chromosome 15 open reading frame 41 (C15orf41), mRNA
NIPAL1	2.156	Homo sapiens NIPA-like domain containing 1 (NIPAL1), mRNA
WDR63	2.144	Homo sapiens WD repeat domain 63 (WDR63), mRNA
ZNF331	2.144	Homo sapiens zinc finger protein 331 (ZNF331), mRNA
POLR2J3	2.138	Homo sapiens polymerase (RNA) II (DNA directed) polypeptide J3 (POLR2J3), mRNA
LYPD6	2.138	Homo sapiens LY6/PLAUR domain containing 6 (LYPD6), mRNA
MYO5B	2.132	Homo sapiens myosin VB (MYO5B), mRNA
ZNF593	2.125	Homo sapiens zinc finger protein 593 (ZNF593), mRNA
YOD1	2.124	Homo sapiens YOD1 OTU deubiquinating enzyme 1 homolog (S. cerevisiae) (YOD1), mRNA
MIR379	2.123	Homo sapiens kelch repeat and BTB (POZ) domain containing 8 (KBTBD8), mRNA
FAM101B	2.116	Homo sapiens family with sequence similarity 101, member B (FAM101B), mRNA
CLDN23	2.110	Homo sapiens claudin 23 (CLDN23), mRNA
E2F7	2.110	Homo sapiens E2F transcription factor 7 (E2F7), mRNA
CCDC96	2.110	Homo sapiens coiled-coil domain containing 96 (CCDC96), mRNA
IQCD	2.099	Homo sapiens IQ motif containing D (IQCD), mRNA

TSC22D1	2.098	Homo sapiens TSC22 domain family, member 1 (TSC22D1), mRNA
ZBTB43	2.098	Homo sapiens zinc finger and BTB domain containing 43 (ZBTB43), mRNA
CCDC71L	2.088	Homo sapiens coiled-coil domain containing 71-like (CCDC71L), mRNA
SLC52A1	2.087	Homo sapiens solute carrier family 52, riboflavin transporter, member 1 (SLC52A1), mRNA
RAPGEF3	2.075	Homo sapiens Rap guanine nucleotide exchange factor (GEF) 3 (RAPGEF3), mRNA
HIST2H2AC	2.068	Homo sapiens histone cluster 2, H2ac (HIST2H2AC), mRNA
GADD45A	2.051	Homo sapiens growth arrest and DNA-damage-inducible, alpha (GADD45A), mRNA
MED26	2.051	Homo sapiens mediator complex subunit 26 (MED26), mRNA
DENND2D	2.050	Homo sapiens DENN/MADD domain containing 2D (DENND2D), mRNA
NAPRT1	2.050	Homo sapiens nicotinate phosphoribosyltransferase domain containing 1 (NAPRT1), mRNA
HS3ST1	2.046	Homo sapiens heparan sulfate (glucosamine) 3-O-sulfotransferase 1 (HS3ST1), mRNA
PLEKHF1	2.046	Homo sapiens pleckstrin homology domain containing, family F (with FYVE domain) member 1 (PLEKHF1), mRNA
ZNF771	2.045	Homo sapiens zinc finger protein 771 (ZNF771), mRNA
PRDM7	2.036	Homo sapiens PR domain containing 7 (PRDM7), mRNA
ADM	2.032	Homo sapiens adrenomedullin (ADM), mRNA
FBXO6	2.026	Homo sapiens F-box protein 6 (FBXO6), mRNA
KRT17	2.024	Homo sapiens keratin 17 (KRT17), mRNA
BMP7	2.016	Homo sapiens bone morphogenetic protein 7 (BMP7), mRNA
XBP1	2.016	Homo sapiens X-box binding protein 1 (XBP1), mRNA
TMEM116	2.014	Homo sapiens transmembrane protein 116 (TMEM116), mRNA
XAGE1C	2.002	Homo sapiens ribosomal protein L13 (RPL13), mRNA
CBWD6	2.002	Homo sapiens COBW domain containing 6 (CBWD6), mRNA
ZNF846	2.001	Homo sapiens zinc finger protein 846 (ZNF846), mRNA
DDIT4	-2.652	Homo sapiens DNA-damage-inducible transcript 4 (DDIT4), mRNA
BMF	-4.034	Homo sapiens Bcl2 modifying factor (BMF), mRNA