US 20220127611A1

(19) **United States**

(12) **Patent Application Publication**

(10) **Pub. No.: US 2022/0127611 A1**

(43) **Pub. Date: Apr. 28, 2022**

Yeo et al.

(54) **METHODS OF MODULATING RNA TRANSLATION**

(71) Applicant: **The Regents of the University of California**, La Jolla, CA (US)

(72) Inventors: **Eugene Yeo**, La Jolla, CA (US); **En-Ching Luo**, La Jolla, CA (US)

(21) Appl. No.: **17/512,270**

(22) Filed: **Oct. 27, 2021**

Related U.S. Application Data

(60) Provisional application No. 63/106,631, filed on Oct. 28, 2020.

Publication Classification

(51) **Int. Cl.**

C12N 15/113 (2006.01)

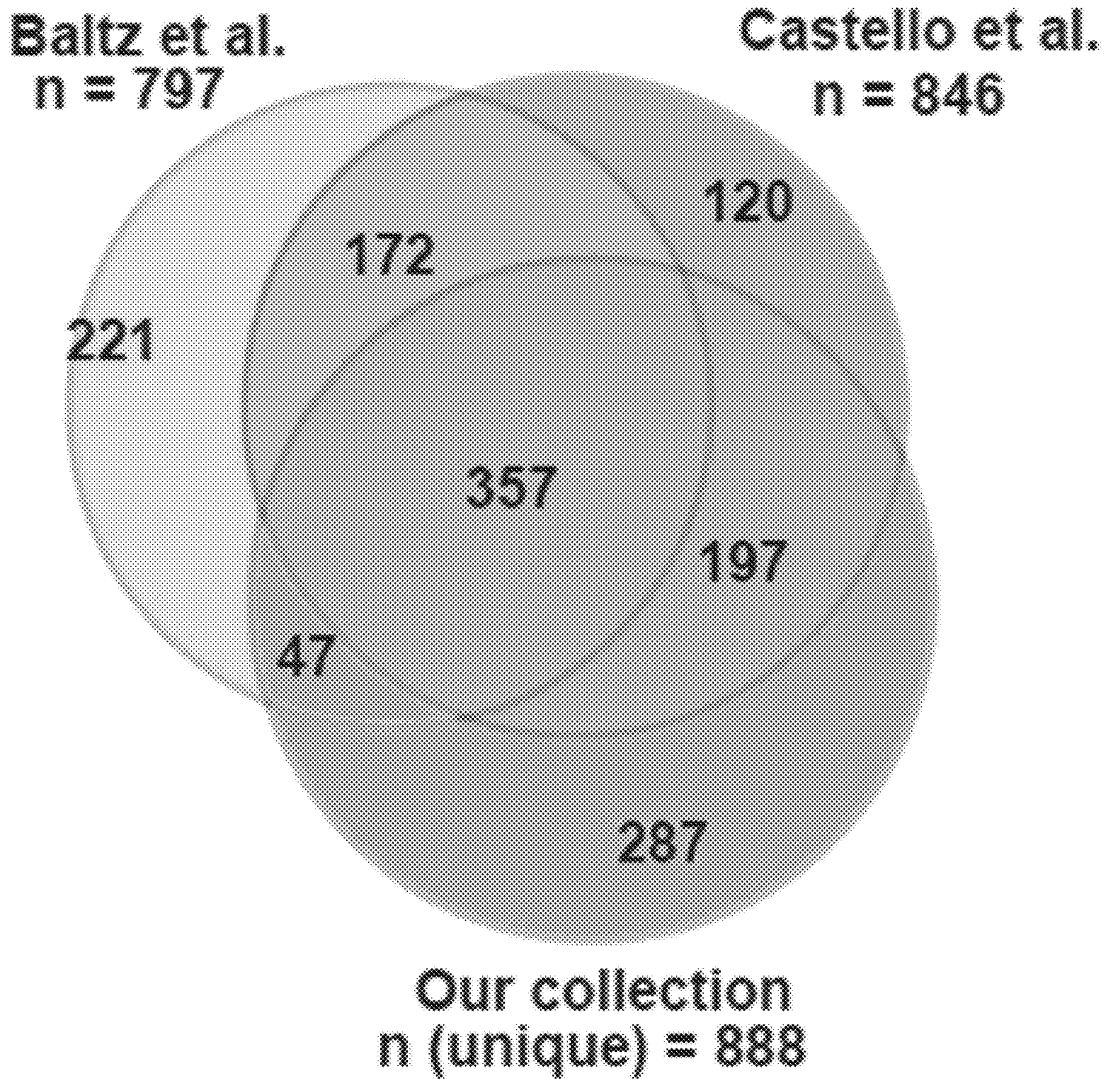
C12N 7/00 (2006.01)

(52) **U.S. Cl.**

CPC ***C12N 15/113*** (2013.01); ***C12N 7/00*** (2013.01); *C12N 2310/321* (2013.01); *C12N 2310/141* (2013.01); *C12N 2310/531* (2013.01); *C12N 2310/113* (2013.01)

(57) **ABSTRACT**

Provided are methods of modulating gene expression of a target RNA in a cell comprising (a) recruiting a modulation unit, wherein the modulation unit comprises an RNA binding protein (RBP), an exogenous RNA binding moiety, and a gene-editing agent; (b) delivering the modulation unit into the cell; and (c) detecting change in the target RNA translation, wherein the modulation unit modulates gene expression of the target RNA in the cell.



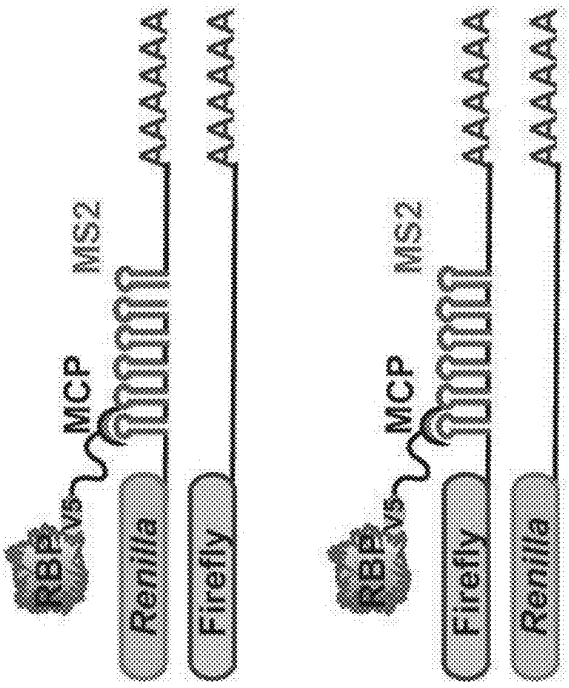

RBP
MCP
MS2
AAAAAA
Renilla
Firefly
RBP
MCP
MS2
AAAAAA
Firefly
Renilla

FIG. 1B

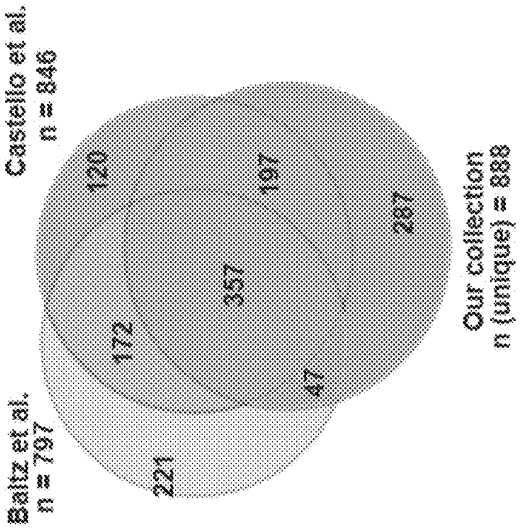

Castello et al.
n = 846
Baltz et al.
n = 797
Our collection
n (unique) = 888
120
172
357
197
47
287
221

FIG. 1A

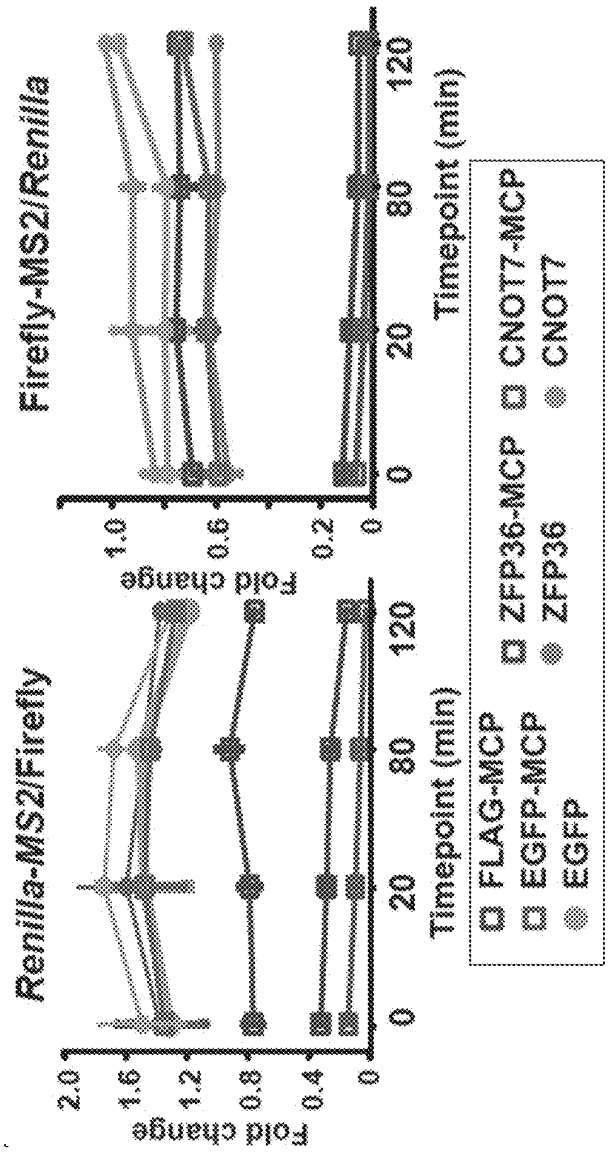

Renilla-MS2/Firefly
Firefly-MS2/Renilla
Fold change
Timepoint (min)
FLAG-MCP
EGFP-MCP
EGFP
ZFP36-MCP
ZFP36
CNOT7-MCP
CNOT7

FIG. 1C

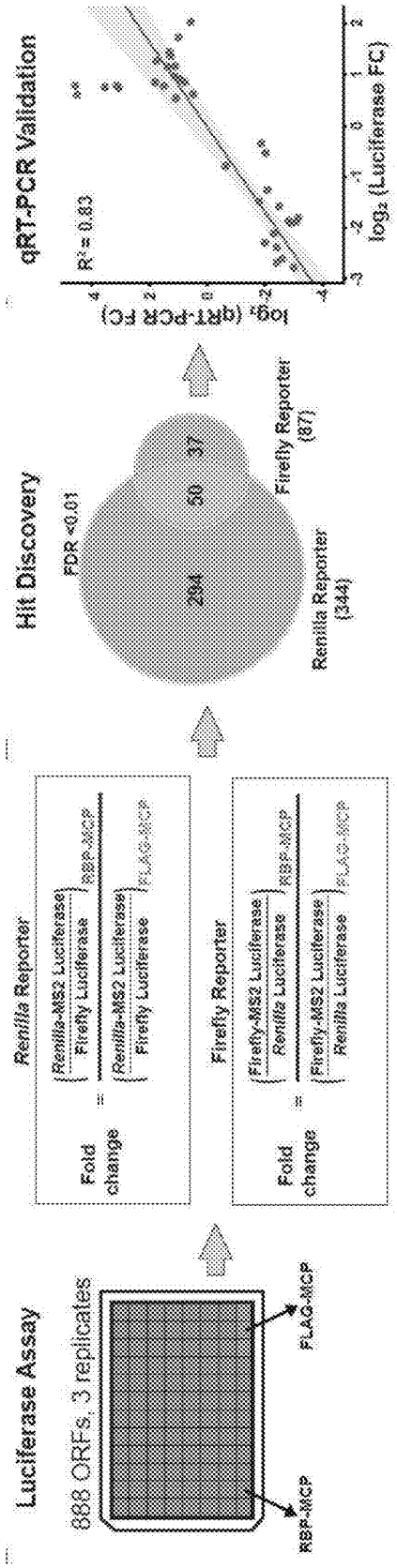


FIG. 1D

FIG. 1E

FIG. 1F

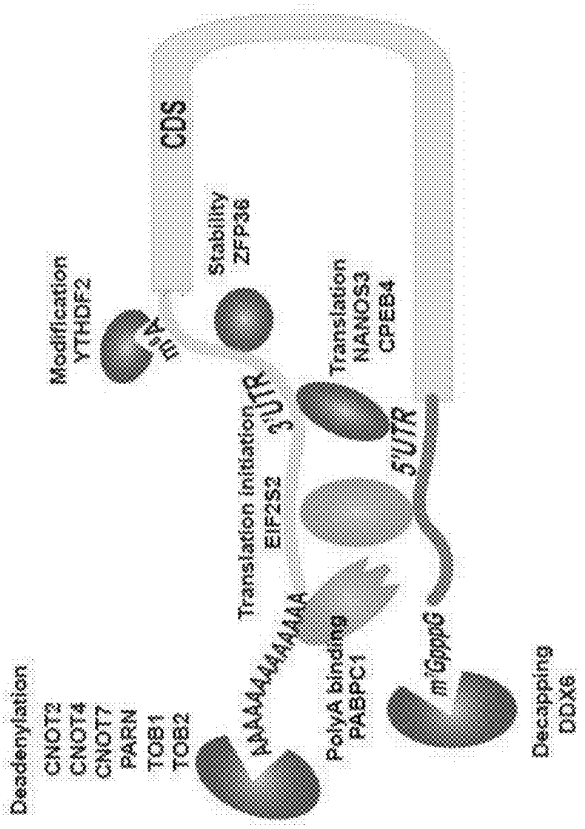

CDS
Modification
YTHDF2
m⁶A
Stability
ZFP36
Translation
NANOS3
CPEB4
Translation initiation
EIF2S2
3'UTR
5'UTR
AAAAAAAAAA
PolyA binding
PABPC1
m⁷GpppG
Deadenylation
CNOT2
CNOT4
CNOT7
PARN
TOB1
TOB2
Decapping
DDX6

FIG. 1G

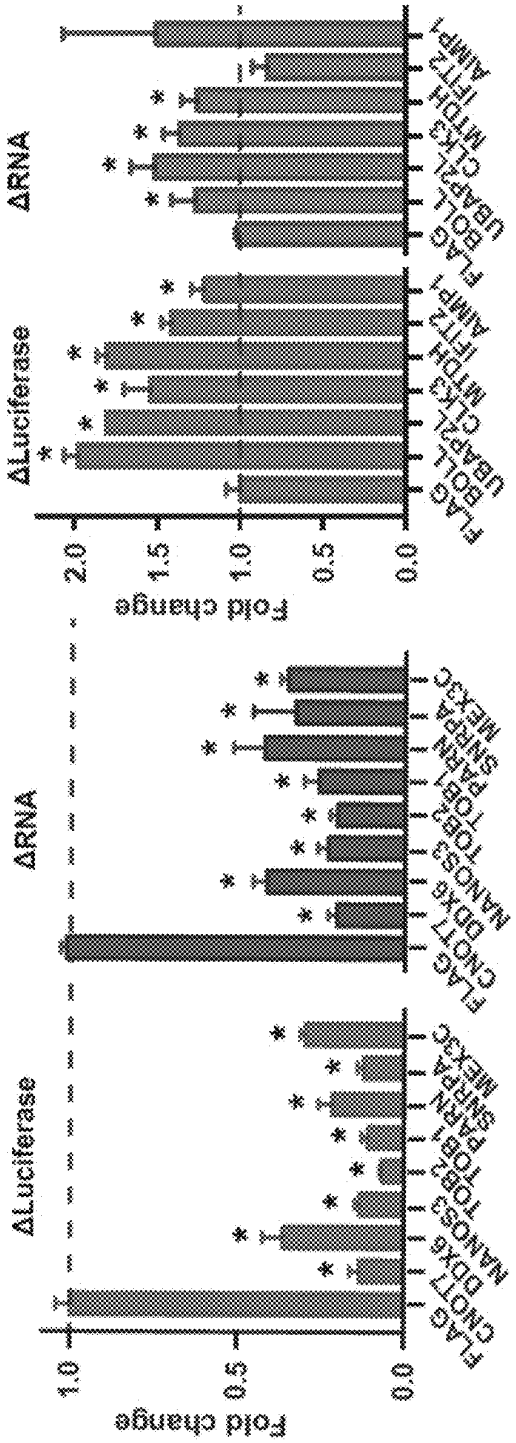

ΔLuciferase
ΔRNA
Fold change
2.0
1.5
1.0
0.5
0.0
FLAG
FBOLL
UBAP2L
CLK3
MTOR
FTL2
AIMP1
FLAG
CNOT7
DDX6
NANOS3
TOB2
TOB1
PABPN1
SNRPA
MEX3C

FIG. 1I

FIG. 1H

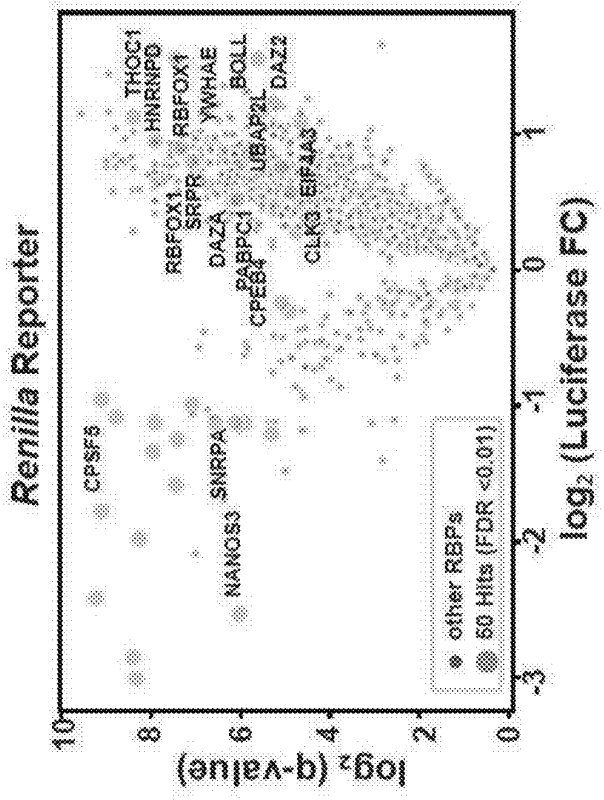

Renilla Reporter
log2 (q-value)
log2 (Luciferase FC)
other RBPs
60 Hits (FDR <0.01)
CPSF6
NANOS3
SNRPA
CPEB4
RBFOX1
SRPR
DAZA
PABPC1
CLK3
EIF4A3
UBAP2L
YWHAE
BOLL
DAZ3
RBFOX1
HNRNPD
THOC1

FIG. 1K

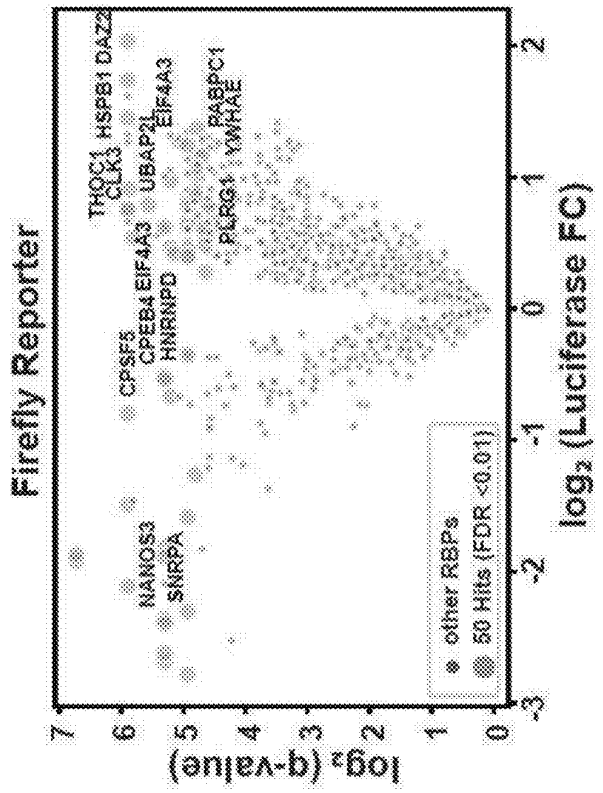

Firefly Reporter
log2 (q-value)
log2 (Luciferase FC)
other RBPs
60 Hits (FDR <0.01)
NANOS3
SNRPA
CPSF5
CPEB4
EIF4A3
HNRNPD
THOC1
CLK3
UBAP2L
PLRG1
PABPC1
YWHAE
HSPB1
DAZ2

FIG. 1J

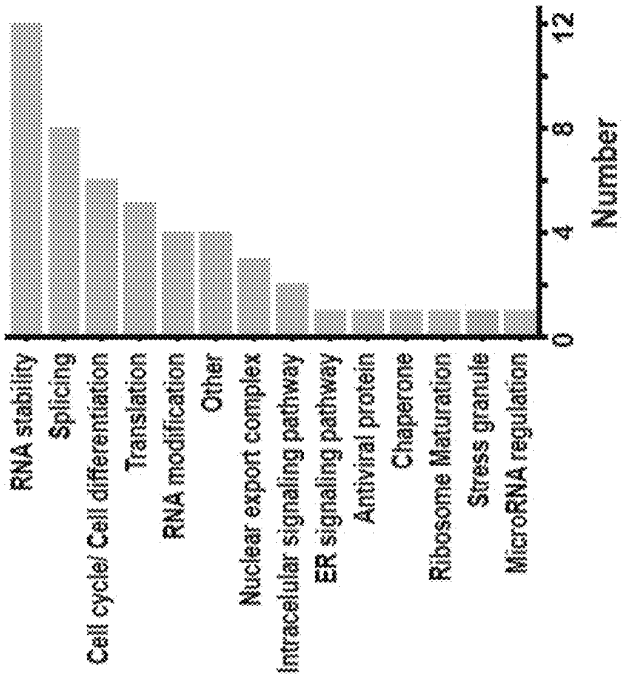

RNA stability
Splicing
Cell cycle/ Cell differentiation
Translation
RNA modification
Other
Nuclear export complex
Intracelular signaling pathway
ER signaling pathway
Antiviral protein
Chaperone
Ribosome Maturation
Stress granule
MicroRNA regulation
0
4
8
12
Number

FIG. 1L

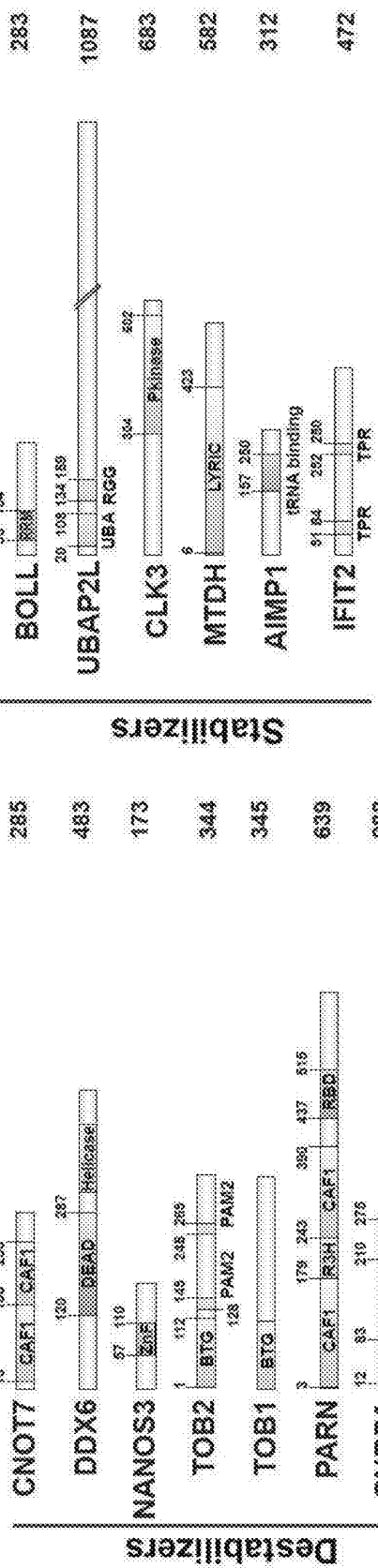

Destabilizers
Stabilizers
Amino acids
CNOT7
DDX6
NANOS3
TOB2
TOB1
PARN
SNRPA
MEX3C
BOLL
UBAP2L
CLK3
MTDH
AIMP1
IFIT2
CAF1
DEAD
Helicase
ZnF
BTG
PAM2
RGH
RRM
KH
RBD
PPIase
LYRIC
tRNA binding
TPR

FIG. 2A

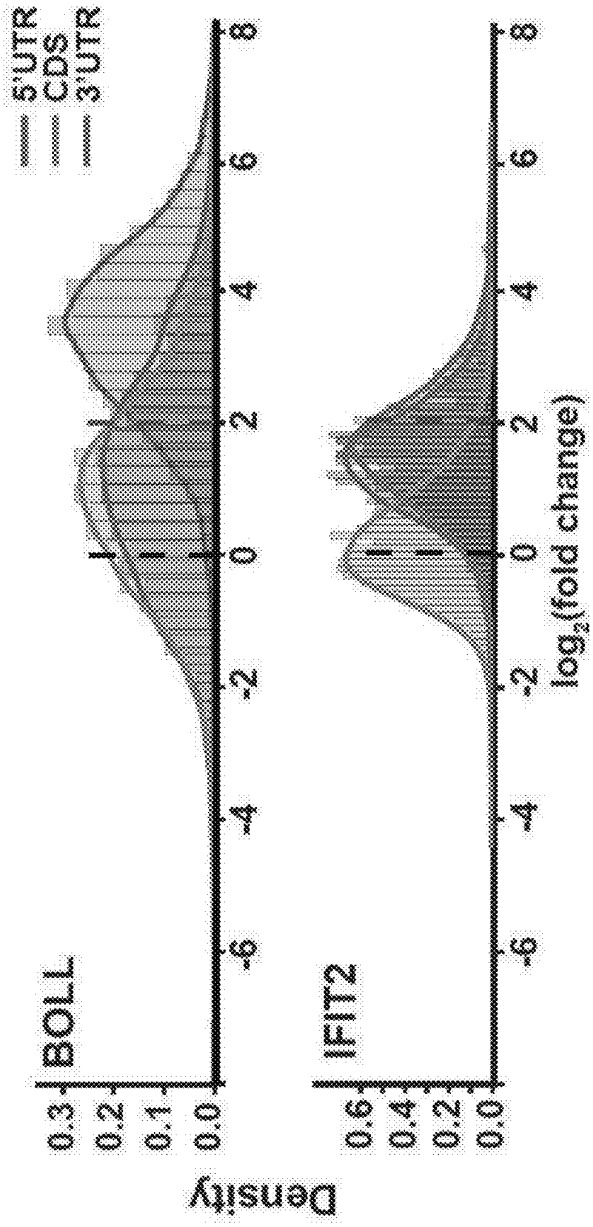

5'UTR
CDS
3'UTR
BOLL
IFIT2
Density
0.3
0.2
0.1
0.0
0.6
0.4
0.2
0.0
-6
-4
-2
0
2
4
6
8
log2(fold change)

FIG. 2B

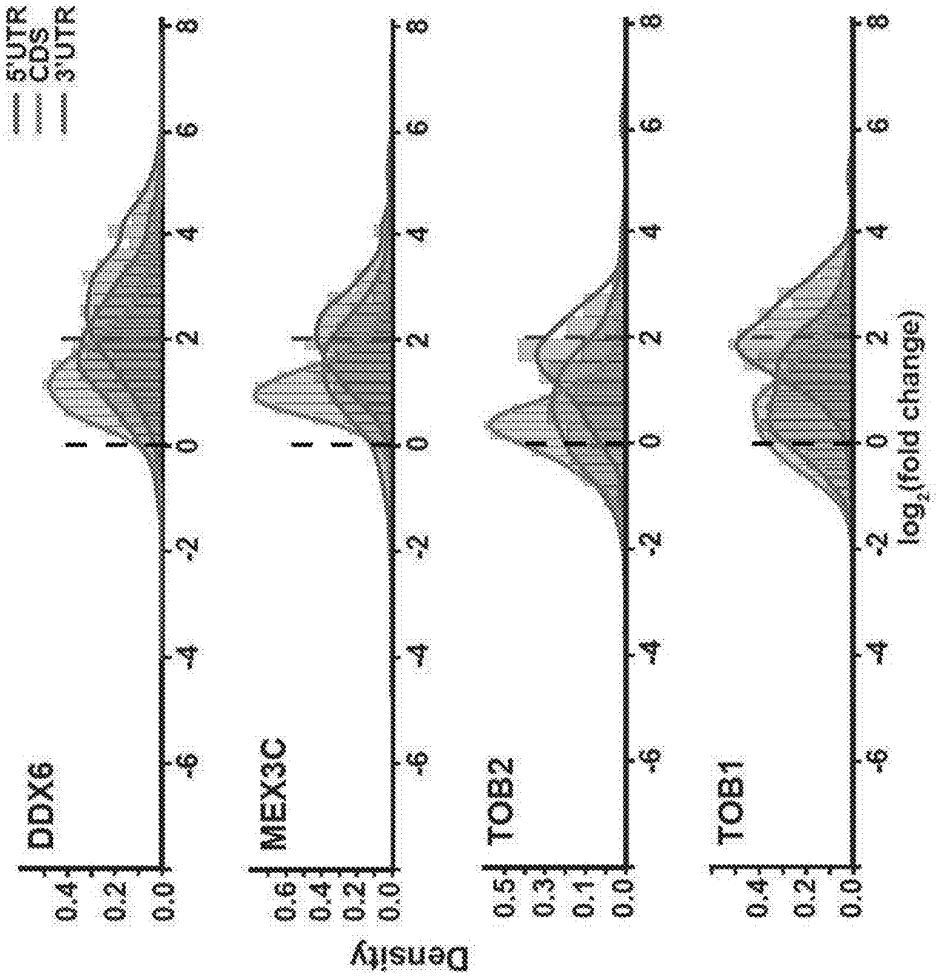


FIG. 2C

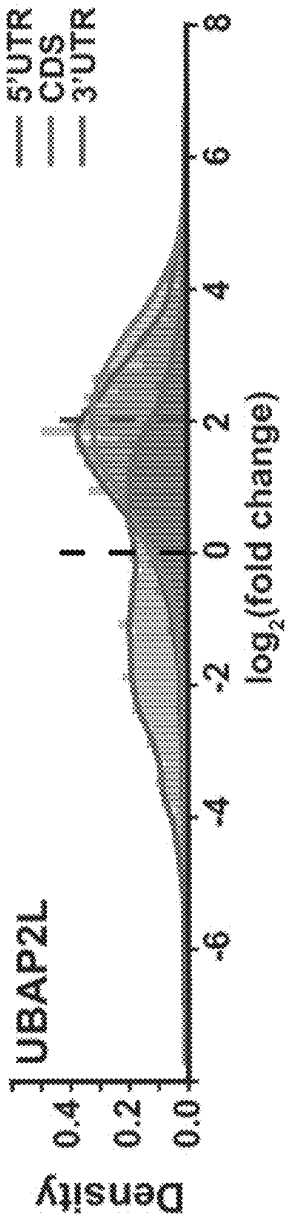

5'UTR
CDS
3'UTR
UBAP2L
Density
0.4
0.2
0.0
-6
-4
-2
0
2
4
6
8
log2(fold change)

FIG. 2D

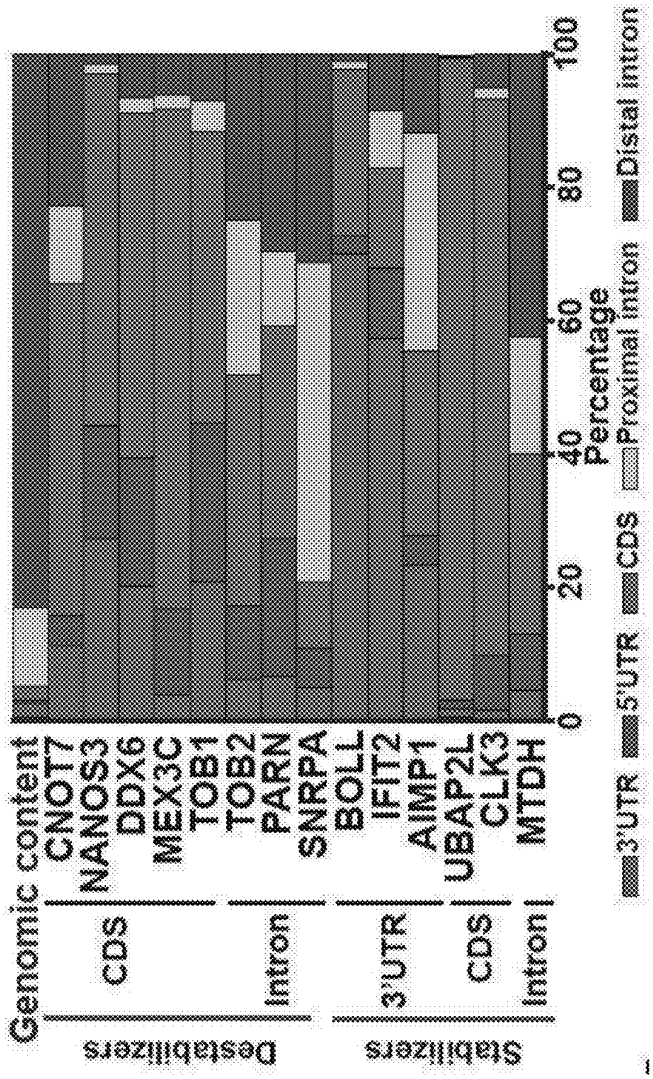

Genomic content
Destabilizers
Stabilizers
CDS
Intron
3'UTR
CDS
Intron
CNOT7
NANOS3
DDX6
MEX3C
TOB1
TOB2
PARN
SNRPA
BOLL
IFIT2
AIMP1
UBAP2L
CLK3
MTDH
0
20
40
60
80
100
Percentage
3'UTR
5'UTR
CDS
Proximal intron
Distal intron

FIG. 2E

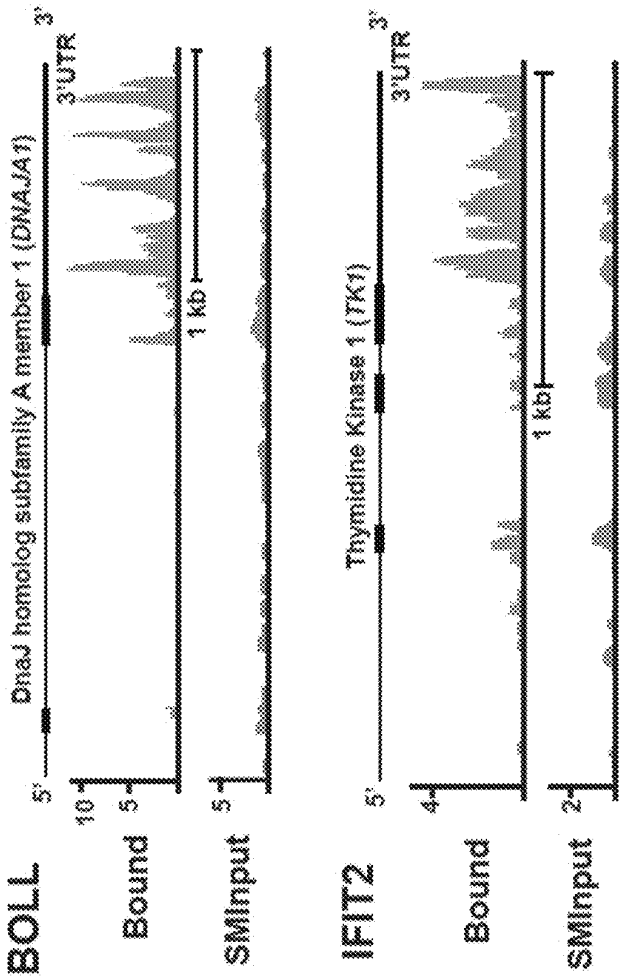

BOLL
DnaJ homolog subfamily A member 1 (DNAJA1)
5'
3'
3'UTR
10
5
1 kb
Bound
5
SMInput
IFIT2
Thymidine Kinase 1 (TK1)
5'
3'
3'UTR
4
1 kb
Bound
2
SMInput

FIG. 2F

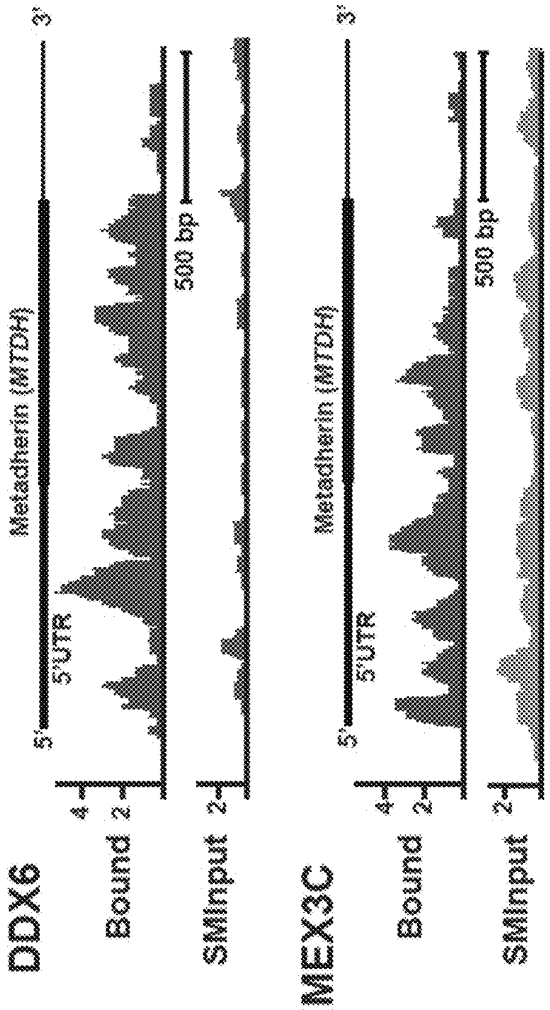

DDX6
Bound
SMInput
MEX3C
Bound
SMInput
Metadherin (MTDH)
5'UTR
5'
3'
500 bp
4
2
2
Metadherin (MTDH)
5'UTR
5'
3'
500 bp
4
2
2

FIG. 2G

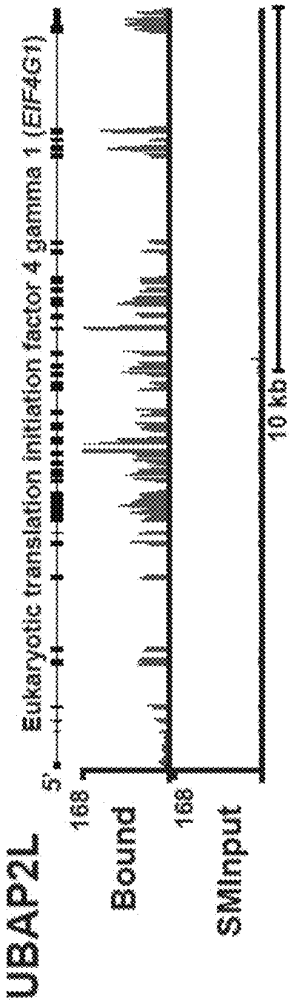

UBAP2L
Eukaryotic translation initiation factor 4 gamma 1 (EIF4G1)
5'
168
Bound
168
SMInput
10 kb

FIG. 2H

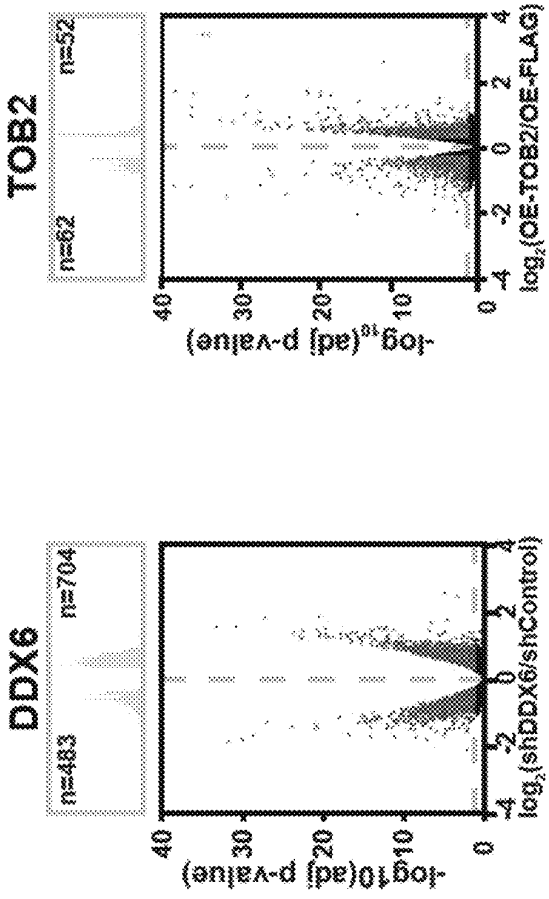

DDX6
n=704
n=483
-log10(adj p-value)
log2(shDDX6/shControl)
TOB2
n=52
n=62
-log10(adj p-value)
log2(OE-TOB2/OE-FLAG)

FIG. 3A

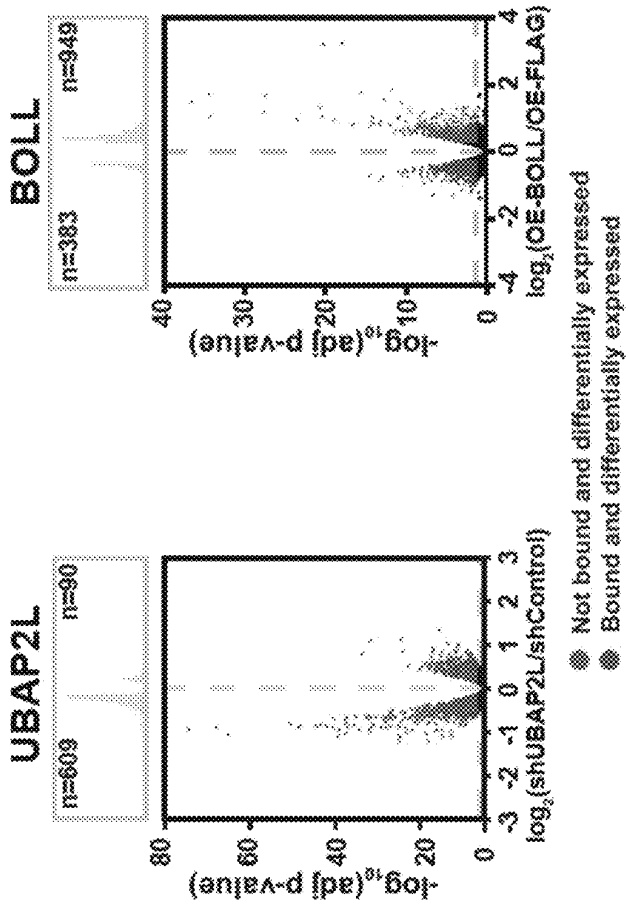

UBAP2L
n=609
n=90
-log10(adj p-value)
log2(shUBAP2L/shControl)
BOLL
n=383
n=649
-log10(adj p-value)
log2(OE-BOLL/OE-FLAG)
Not bound and differentially expressed
Bound and differentially expressed

FIG. 3B

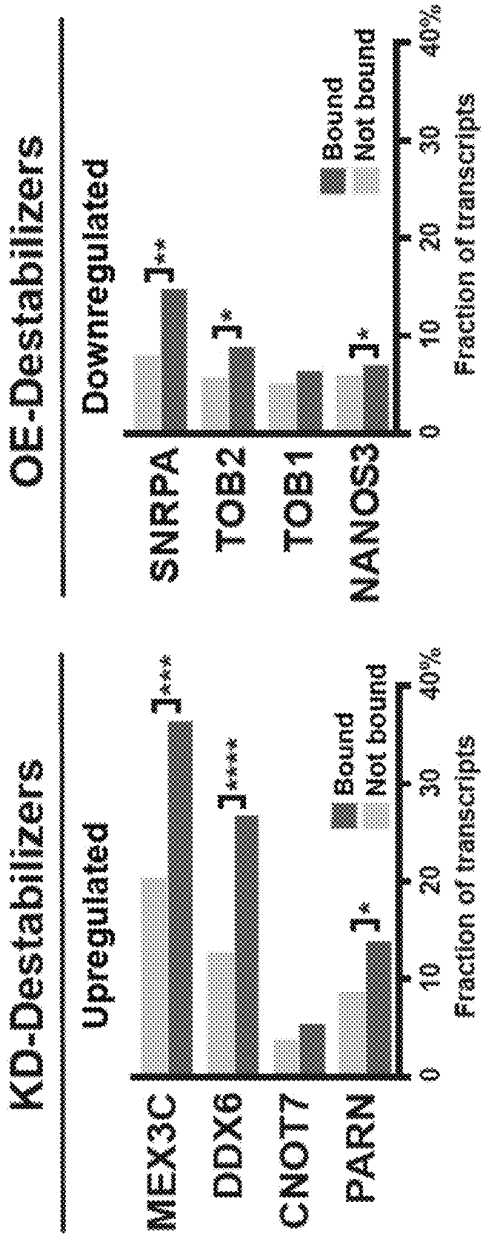

KD-Destabilizers
Upregulated
MEX3C
DDX6
CNOT7
PARN
*
****
***
Bound
Not bound
0
10
20
30
40%
Fraction of transcripts
OE-Destabilizers
Downregulated
SNRPA
TOB2
TOB1
NANOS3
**
*
*
Bound
Not bound
0
10
20
30
40%
Fraction of transcripts

FIG. 3C

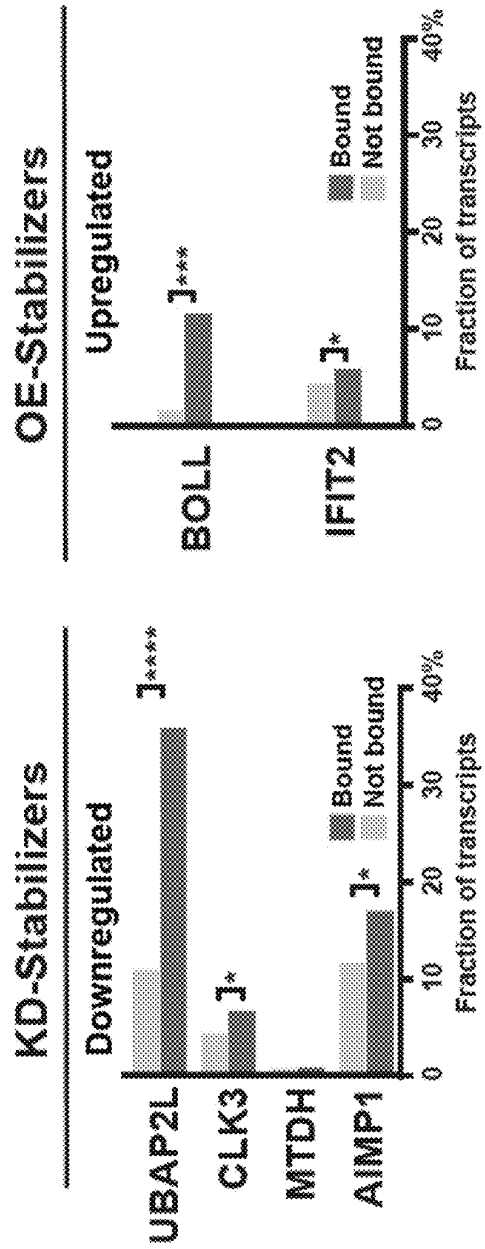

KD-Stabilizers
Downregulated
UBAP2L
CLK3
MTDH
AIMP1
*
****
Bound
Not bound
0
10
20
30
40%
Fraction of transcripts
OE-Stabilizers
Upregulated
BOLL
IFIT2
*
***
Bound
Not bound
0
10
20
30
40%
Fraction of transcripts

FIG. 3D

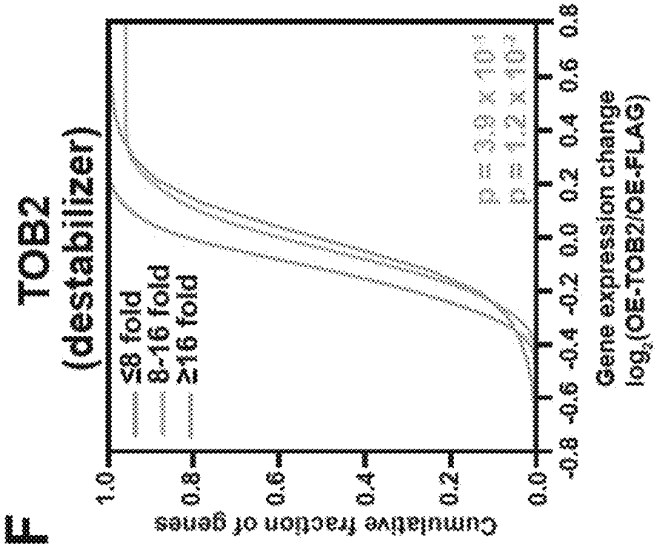

F
TOB2
(destabilizer)
≤8 fold
8-16 fold
≥16 fold
p = 3.9 x 10⁻¹
p = 1.2 x 10⁻²
Cumulative fraction of genes
1.0
0.8
0.6
0.4
0.2
0.0
-0.8 -0.6 -0.4 -0.2 0.0 0.2 0.4 0.6 0.8
Gene expression change
log2(OE-TOB2/OE-FLAG)

FIG. 3F

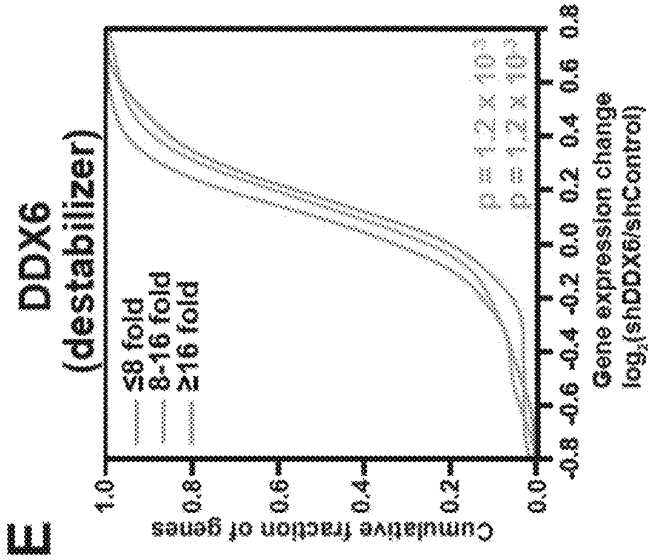

E
DDX6
(destabilizer)
≤8 fold
8-16 fold
≥16 fold
p = 1.2 x 10⁻³
p = 1.2 x 10⁻²
Cumulative fraction of genes
1.0
0.8
0.6
0.4
0.2
0.0
-0.8 -0.6 -0.4 -0.2 0.0 0.2 0.4 0.6 0.8
Gene expression change
log2(shDDX6/shControl)

FIG. 3E

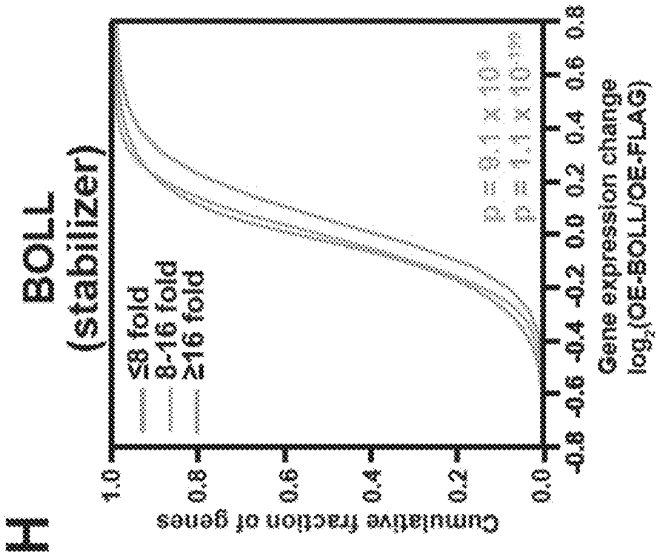

H
BOLL
(stabilizer)
≤8 fold
8-16 fold
≥16 fold
p = 8.1 x 10^-6
p = 1.1 x 10^-10
Cumulative fraction of genes
Gene expression change
log2(OE-BOLL/OE-FLAG)

FIG. 3H

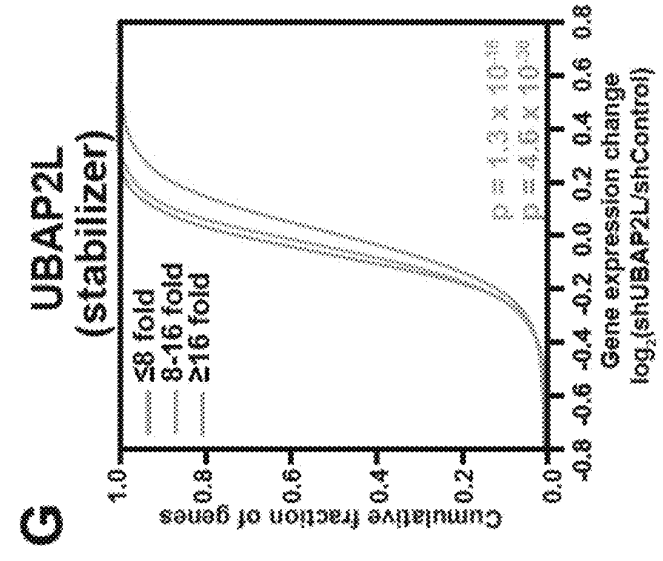

G
UBAP2L
(stabilizer)
≤8 fold
8-16 fold
≥16 fold
p = 1.3 x 10^-15
p = 4.6 x 10^-4
Cumulative fraction of genes
Gene expression change
log2(shUBAP2L/shControl)

FIG. 3G

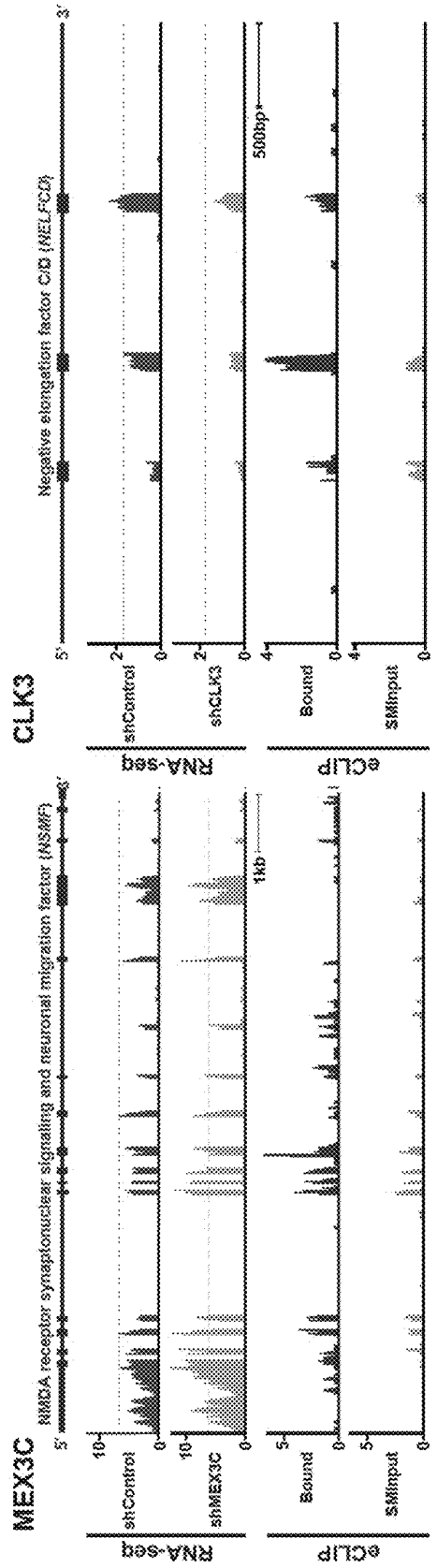

CLK3
Negative elongation factor C/D (NELFCD)
5'
3'
RNA-seq
shControl
shCLK3
eCLIP
Bound
SMInput
500bp
MEX3C
NMDA receptor synaptonuclear signaling and neuronal migration factor (NSMF)
shMEX3C
1kb

FIG. 3J

FIG. 3I

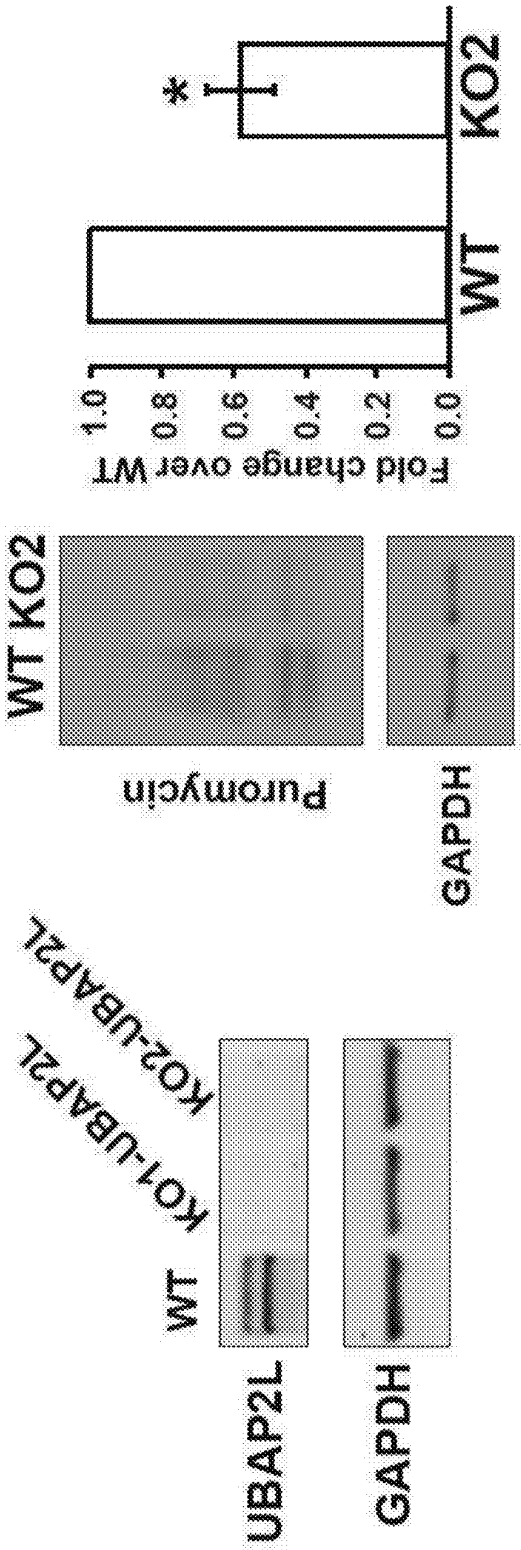

UBAP2L
GAPDH
WT
KO1-UBAP2L
KO2-UBAP2L

FIG. 4A

WT KO2
Puromycin
GAPDH
Fold change over WT
1.0
0.8
0.6
0.4
0.2
0.0
WT
KO2
*

FIG. 4B

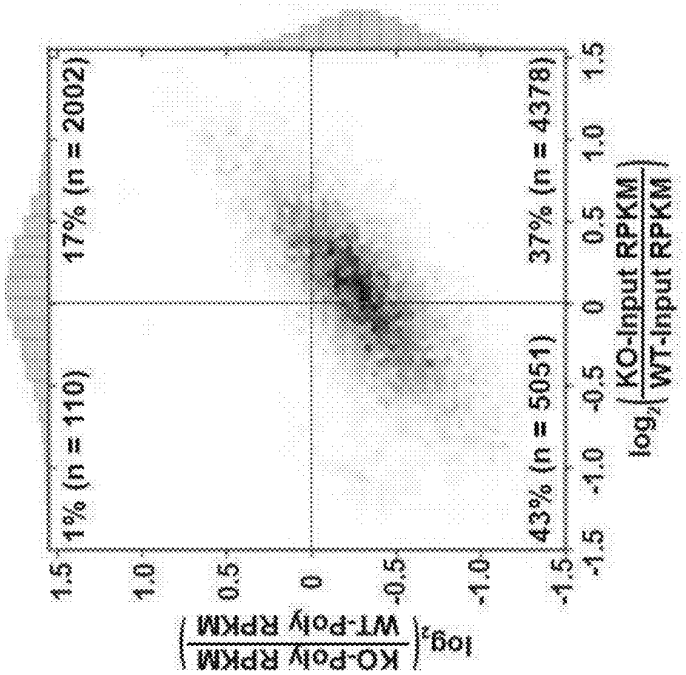

1% (n = 110)
17% (n = 2002)
43% (n = 5051)
37% (n = 4378)
1.5
1.0
0.5
0
-0.5
-1.0
-1.5
log2(KO-Poly RPKM / WT-Poly RPKM)
log2(KO-Input RPKM / WT-Input RPKM)

FIG. 4D

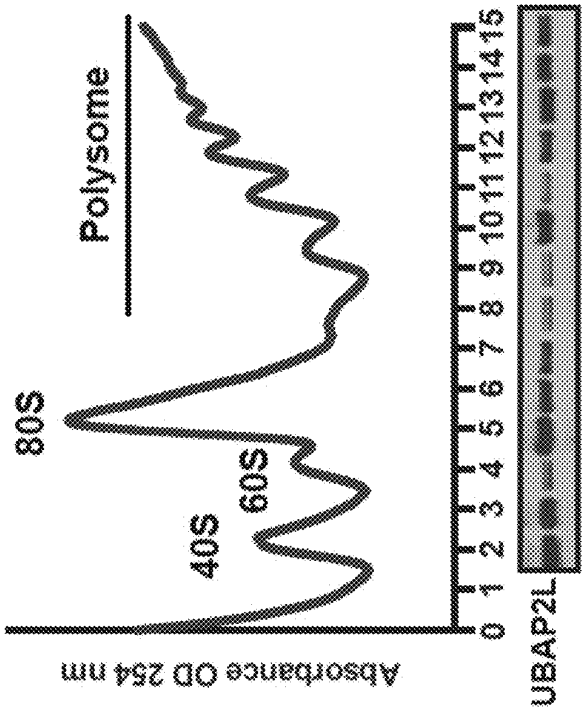

Absorbance OD 254 nm
80S
60S
40S
Polysome
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
UBAP2L

FIG. 4C

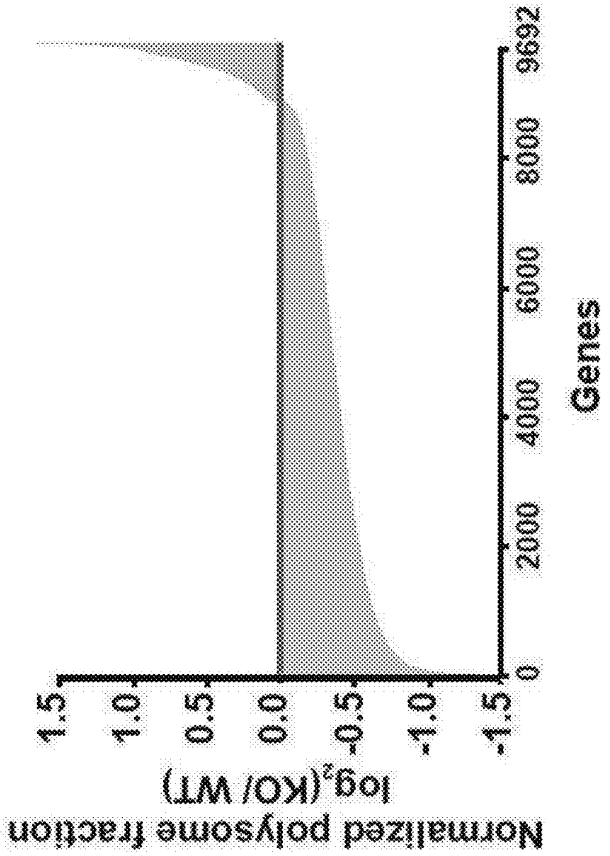

Normalized polysome fraction
log2(KO/ WT)
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
0
2000
4000
6000
8000
9692
Genes

FIG. 4F

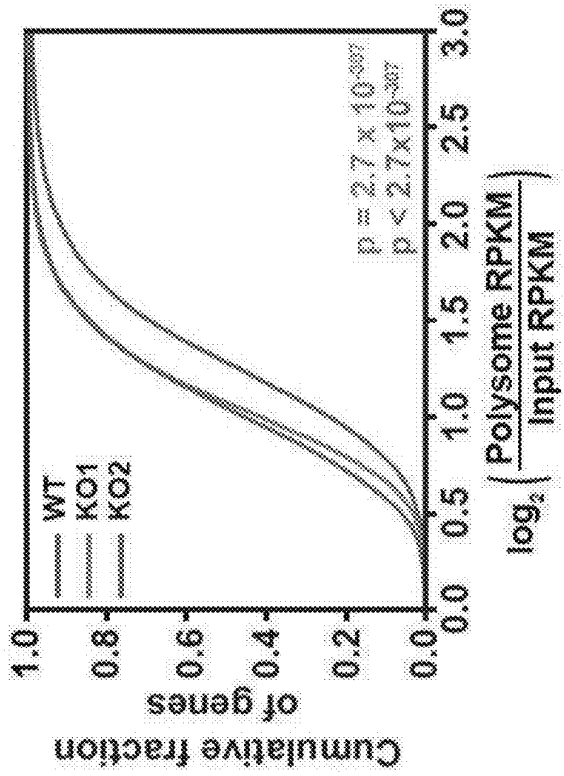

Cumulative fraction
of genes
1.0
0.8
0.6
0.4
0.2
0.0
WT
KO1
KO2
p = 2.7 x 10^-307
p < 2.7x10^-307
0.0
0.5
1.0
1.5
2.0
2.5
3.0
log2(Polysome RPKM / Input RPKM)

FIG. 4E

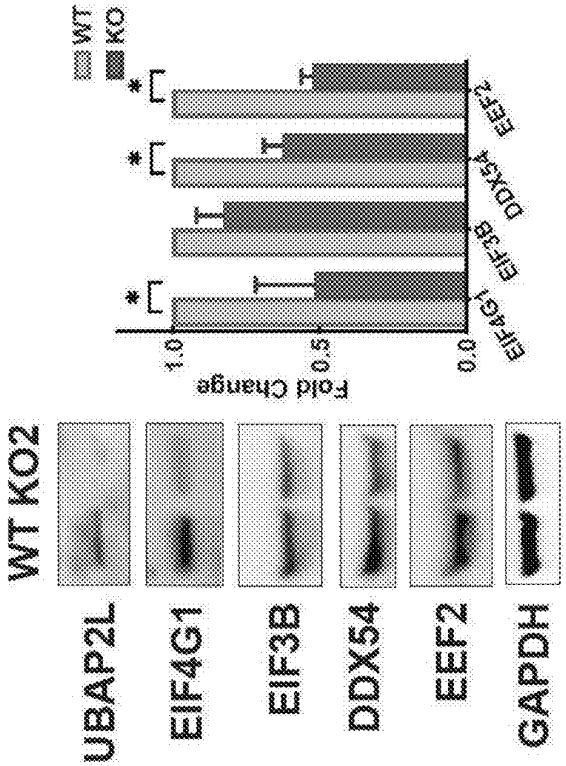


FIG. 4I

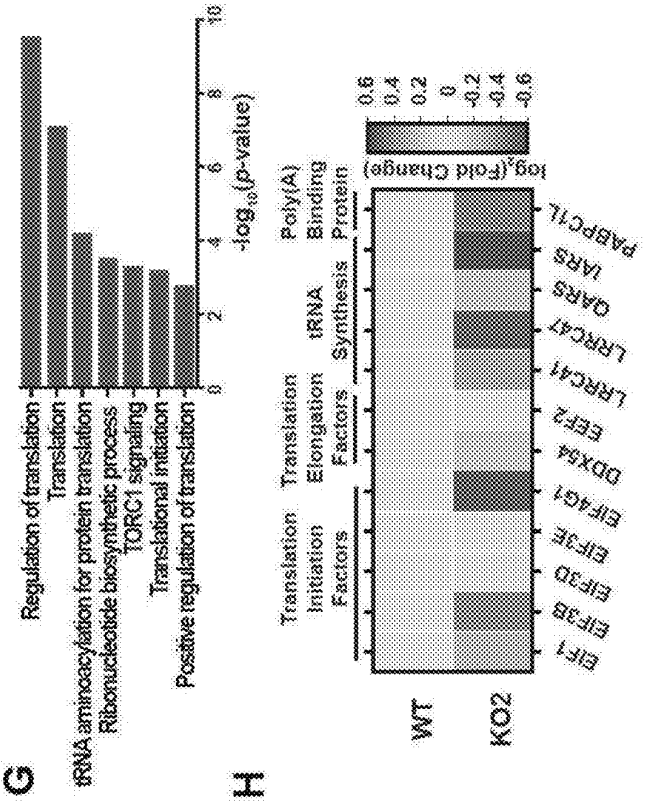


FIG. 4G

FIG. 4H

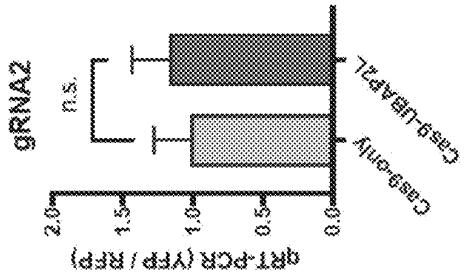


FIG. 4L

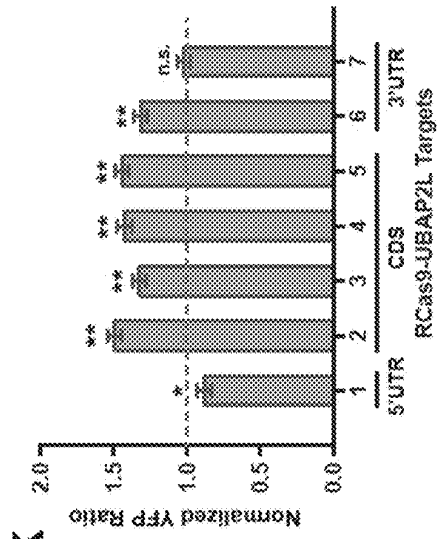


FIG. 4K

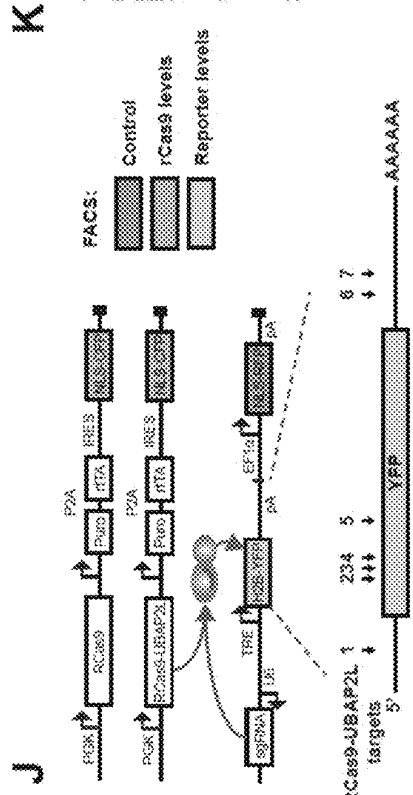


FIG. 4J

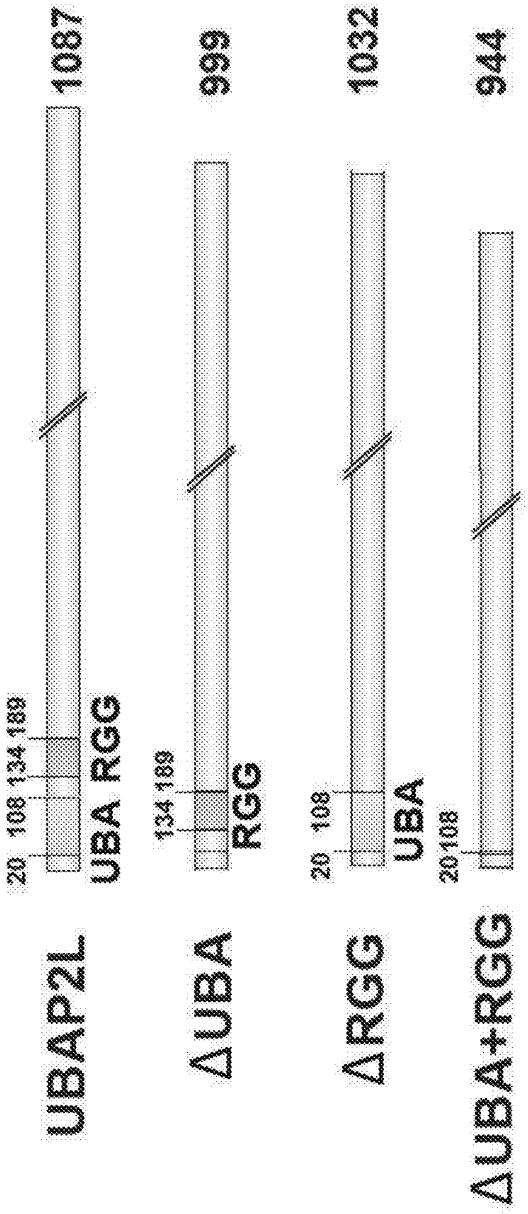

UBAP2L
1087
20
108
134
189
UBA
RGG
ΔUBA
999
134
189
RGG
ΔRGG
1032
20
108
UBA
ΔUBA+RGG
944
20
108

FIG. 5A

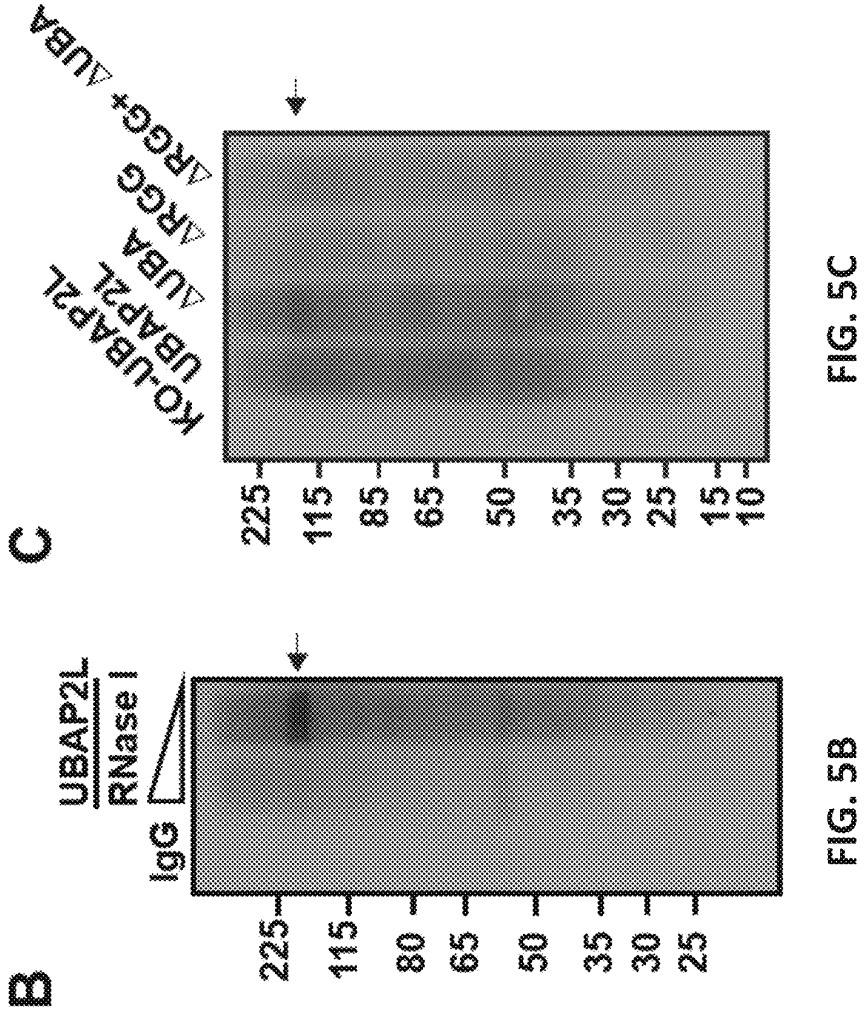

B
UBAP2L
RNase I
IgG
225
115
80
65
50
35
30
25
C
KO-UBAP2L
UBAP2L
ΔUBA
ΔRGG
ΔRGG+ ΔUBA
225
115
85
65
50
35
30
25
15
10

FIG. 5B

FIG. 5C

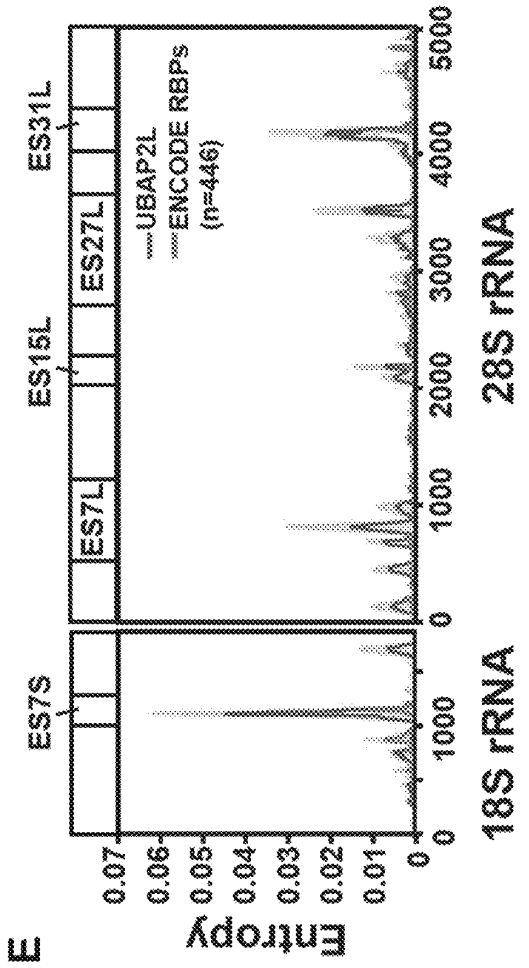

E
Entropy
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
ES7S
ES7L
ES15L
ES27L
ES31L
UBAP2L
ENCODE RBPs (n=446)
0
1000
0
1000
2000
3000
4000
5000
18S rRNA
28S rRNA

FIG. 5E

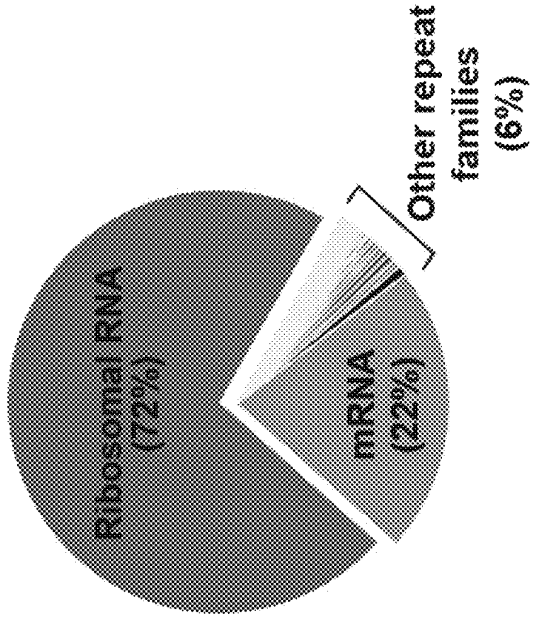

Ribosomal RNA (72%)
mRNA (22%)
Other repeat families (6%)

FIG. 5D

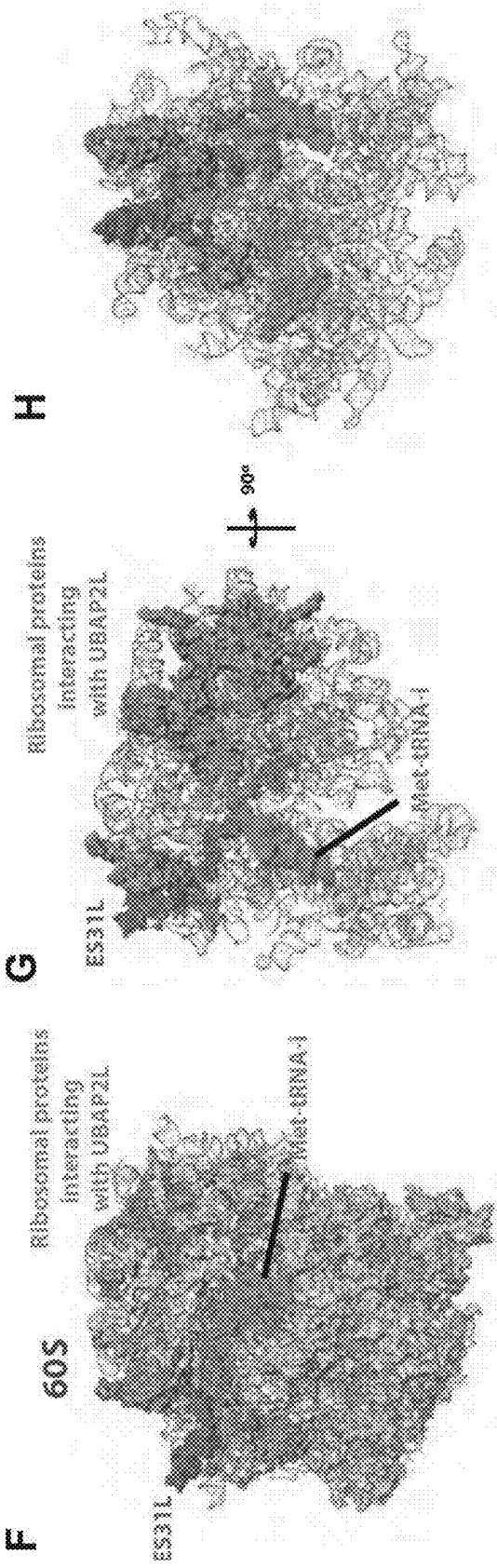


FIG. 5F

FIG. 5G

FIG. 5H

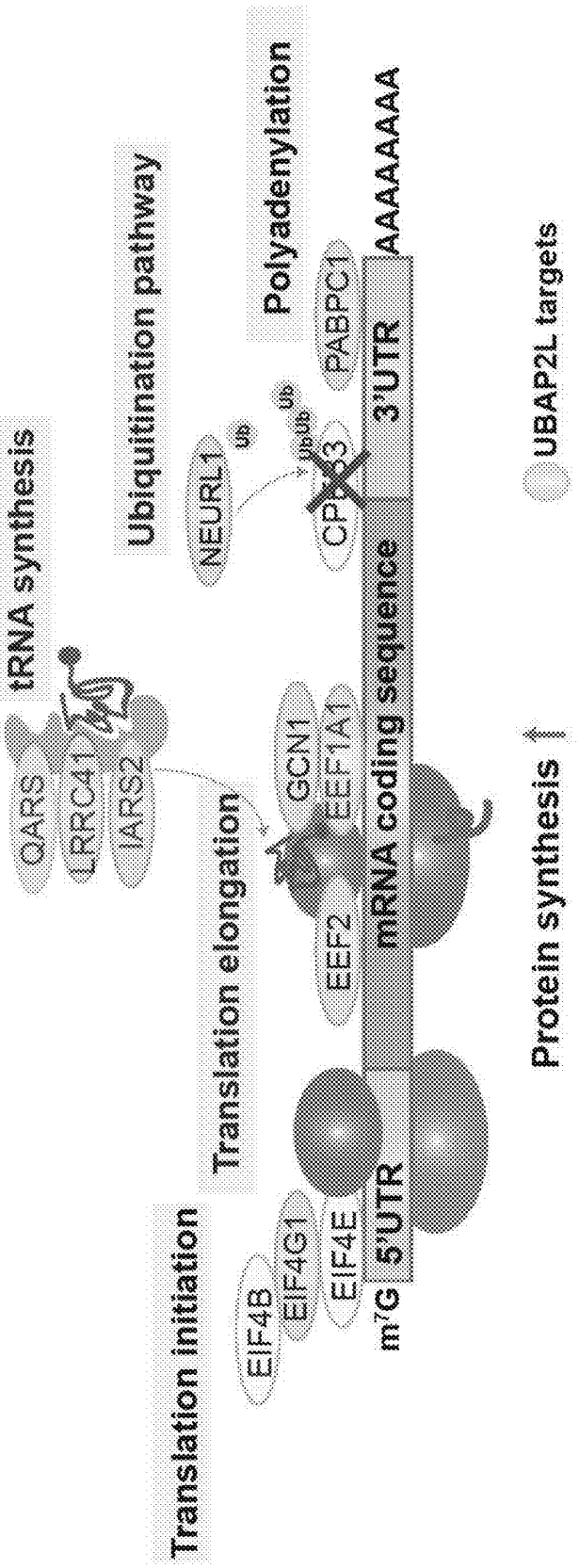

Translation initiation
Translation elongation
Ubiquitination pathway
Polyadenylation
tRNA synthesis
QARS
LRRC41
IARS2
NEURL1
Ub
Ub
Ub
Ub
CP
53
PABPC1
GCN1
EEF1A1
EEF2
EIF4B
EIF4G1
EIF4E
m7G
5'UTR
mRNA coding sequence
3'UTR
AAAAAAA
Protein synthesis
UBAP2L targets

FIG. 6A

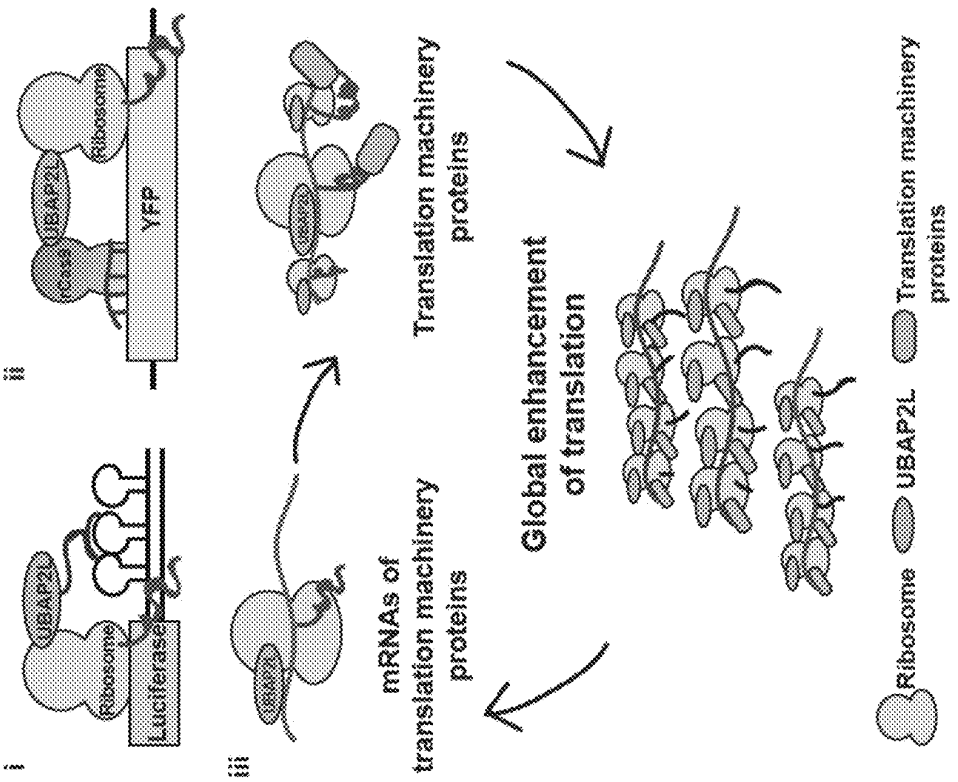

i
Ribosome
UBAP2L
Luciferase
ii
Ribosome
UBAP2L
YFP
iii
mRNAs of translation machinery proteins
Translation machinery proteins
Global enhancement of translation
Ribosome
UBAP2L
Translation machinery proteins

FIG. 6B

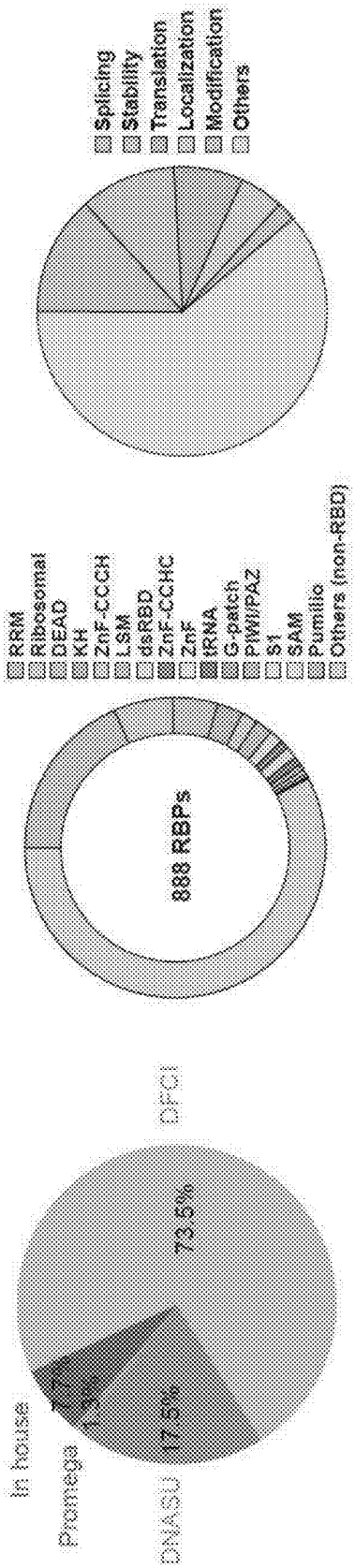


FIG. 7A

FIG. 7B

FIG. 7C

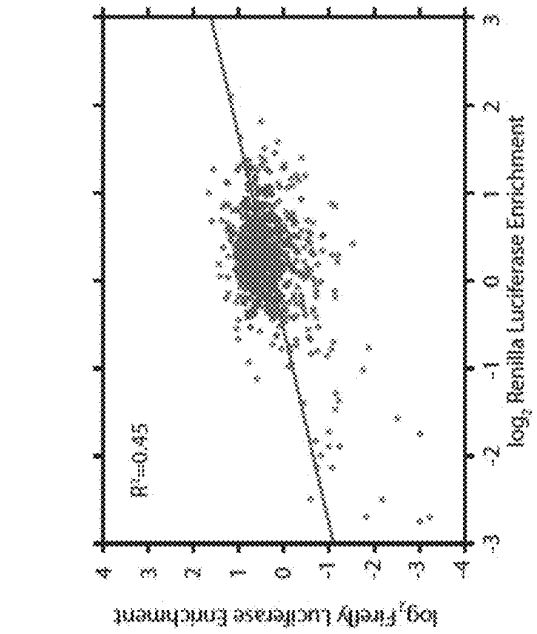


FIG. 7F

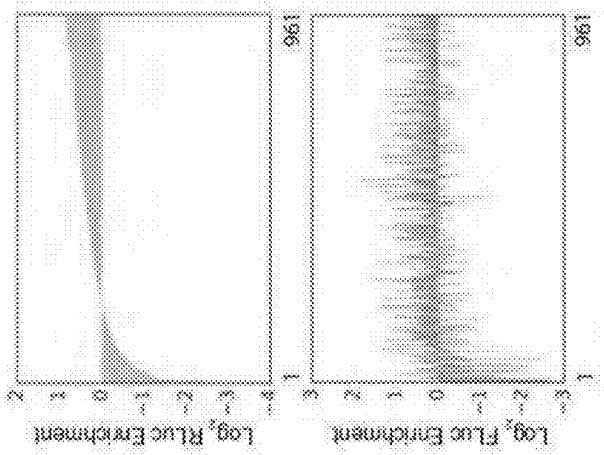


FIG. 7E

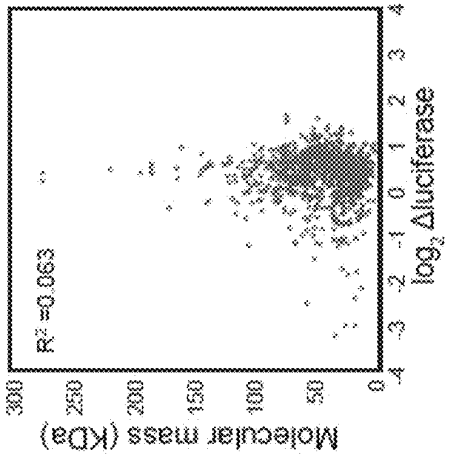


FIG. 7D

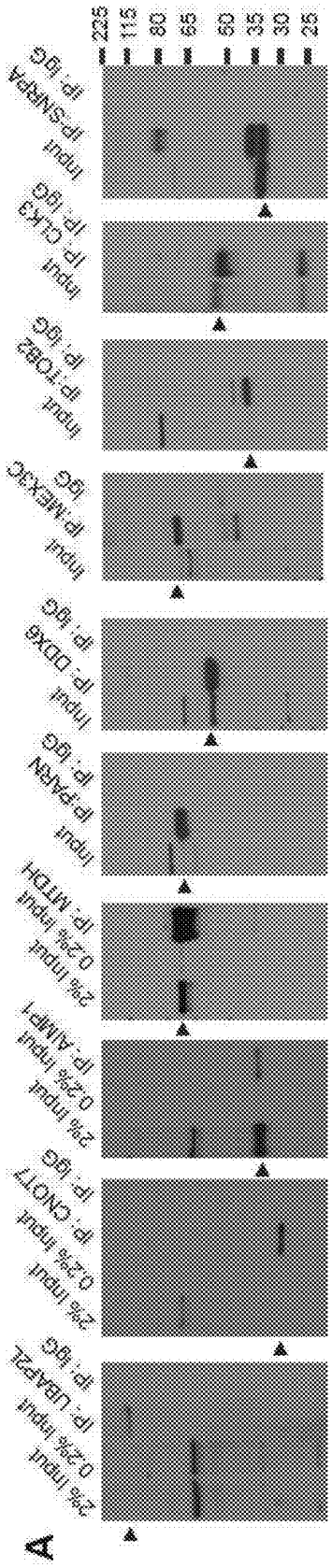

225
115
80
65
60
35
30
25
Input
IP: SNRPA
IP: IgG
Input
IP: CLK3
IP: IgG
Input
IP: TOB2
IP: IgG
Input
IP: MEX3C
IgG
Input
IP: DDX6
IP: IgG
Input
IP: PARN
IP: IgG
2% Input
0.2% Input
IP: MTDH
2% Input
0.2% Input
IP: AIMP1
2% Input
0.2% Input
IP: CNOT7
IP: IgG
2% Input
0.2% Input
IP: UBAP2L
IP: IgG
A

FIG. 8A

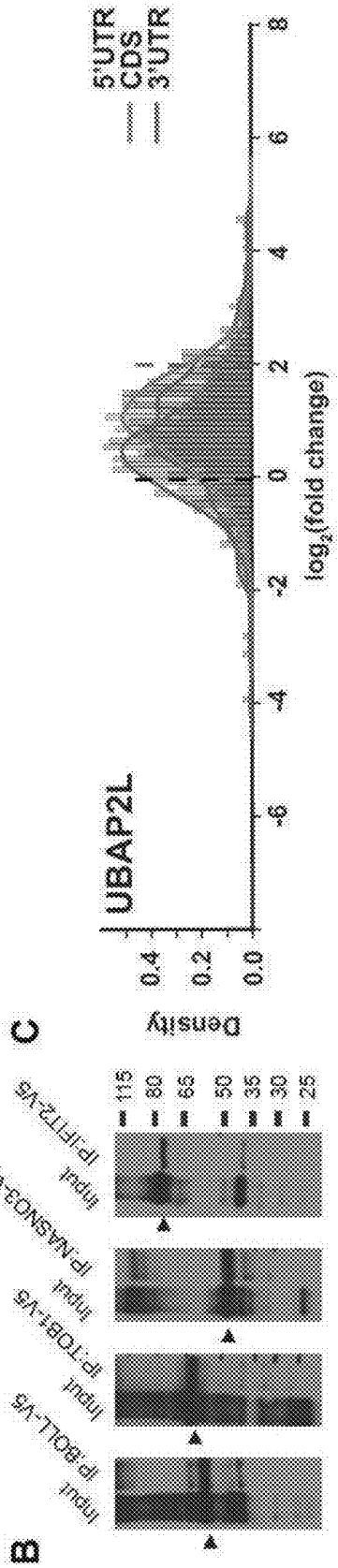

C
Density
0.4
0.2
0.0
UBAP2L
-6
-4
-2
0
2
4
6
8
log2(fold change)
5'UTR
CDS
3'UTR
B
Input
IP-BOLL-V5
Input
IP-TOB1-V5
Input
IP-NASNO3-V5
Input
IP-IFIT2-V5
115
80
65
50
35
30
25

FIG. 8C

FIG. 8B

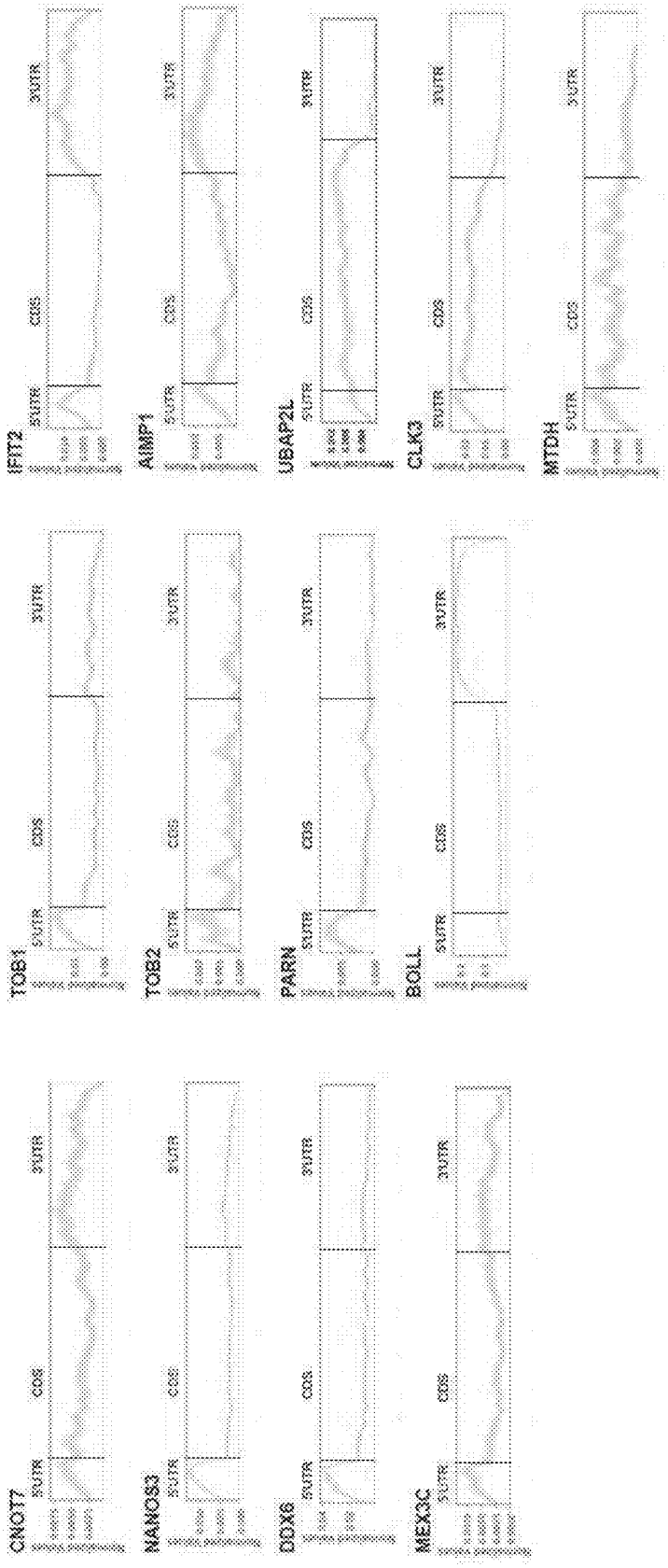

IFIT2
AIMP1
UBAP2L
CLK3
MTDH
TOB1
TOB2
PARN
BOLL
CNOT7
NANOS3
DDX6
MEX3C
5'UTR
CDS
3'UTR

FIG. 8D

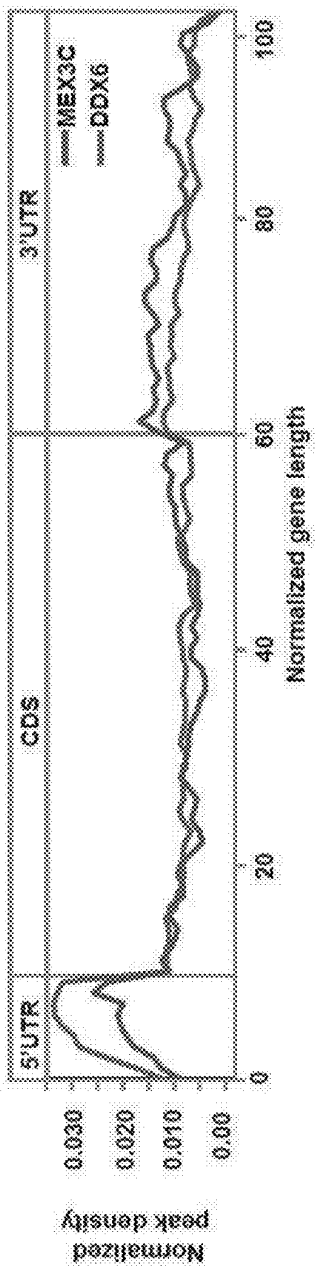

5'UTR
CDS
3'UTR
MEX3C
DDX6
Normalized peak density
0.030
0.020
0.010
0.00
0
20
40
60
80
100
Normalized gene length

FIG. 8F

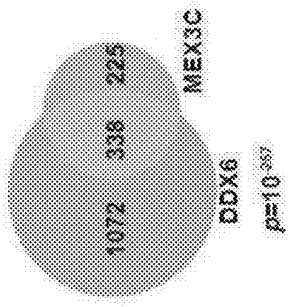

MEX3C
DDX6
225
339
1072
$p=10^{-257}$

FIG. 8E

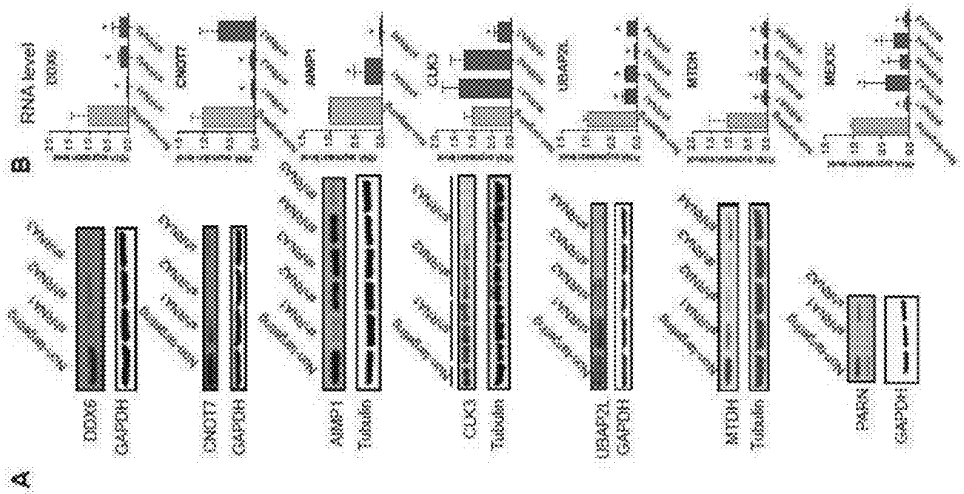

A
DDX6
GAPDH
DNOT7
GAPDH
AMP1
Tubulin
CLK3
Tubulin
UBAP2L
GAPDH
MTOR
Tubulin
PAPN
GAPDH
B
RNA level
DDX6
CNOT7
AMP1
CLK3
UBAP2L
MTOR
PAPN

FIG. 9A

FIG. 9B

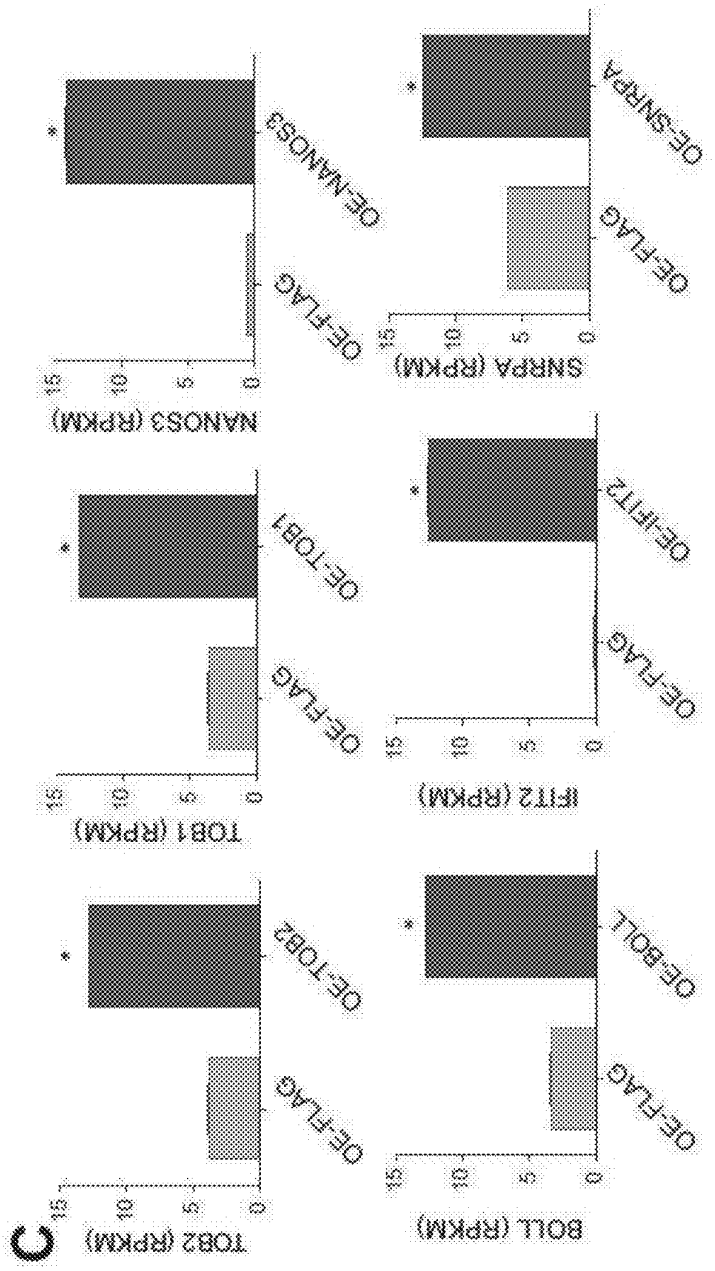

C
TOB2 (RPKM)
BOLL (RPKM)
TOB1 (RPKM)
IFIT2 (RPKM)
NANOS3 (RPKM)
SNRPA (RPKM)
OE-TOB2
OE-BOLL
OE-TOB1
OE-IFIT2
OE-NANOS3
OE-SNRPA
OE-FLAG
*
15
10
5
0

FIG. 9C

D

	MEX3C	DDX6	CNOT7	PARN
Up|Bound	701	704	51	114
Down|Bound	469	483	104	158
Unchanged|Bound	759	1454	707	345
Up|Not bound	3887	2334	777	1772
Down|Not bound	2139	1302	1724	2587
Unchanged|Not Bound	13034	14711	17625	15812

FIG. 9D

E

	SNRPA	TOB1	TOB2	NANOS3
Up|Bound	82	105	56	355
Down|Bound	267	71	82	212
Unchanged|Bound	1438	917	580	2655
Up|Not bound	1184	989	1147	858
Down|Not bound	1189	1139	1125	929
Unchanged|Not Bound	15812	17787	18018t	15979

FIG. 9E

F

	UBAP2L	CLK3	MTDH	AIMP1
Up|Bound	90	136	8	195
Down|Bound	519	205	7	144
Unchanged|Bound	843	2785	557	593
Up|Not bound	1656	384	66	1392
Down|Not bound	1910	758	176	2276
Unchanged|Not Bound	11923	16720	20176	16388

FIG. 9F

G

	BOLL	IFIT2
Up|Bound	949	111
Down|Bound	383	62
Unchanged|Bound	7519	1715
Up|Not bound	136	719
Down|Not bound	561	548
Unchanged|Not Bound	11440	17833

FIG. 9G

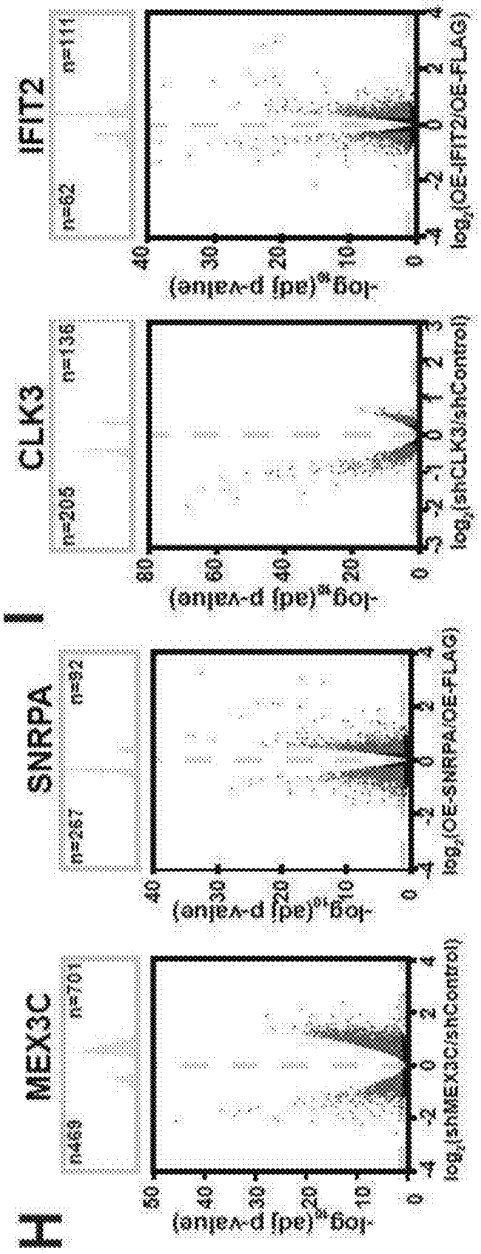

H
MEX3C
n=459
n=701
-log10(adj p-value)
log2(shMEX3C/shControl)
SNRPA
n=287
n=82
log2(OE-SNRPA/OE-FLAG)
I
CLK3
n=205
n=136
log2(shCLK3/shControl)
IFIT2
n=62
n=111
log2(OE-IFIT2/OE-FLAG)

FIG. 9H

FIG. 9I

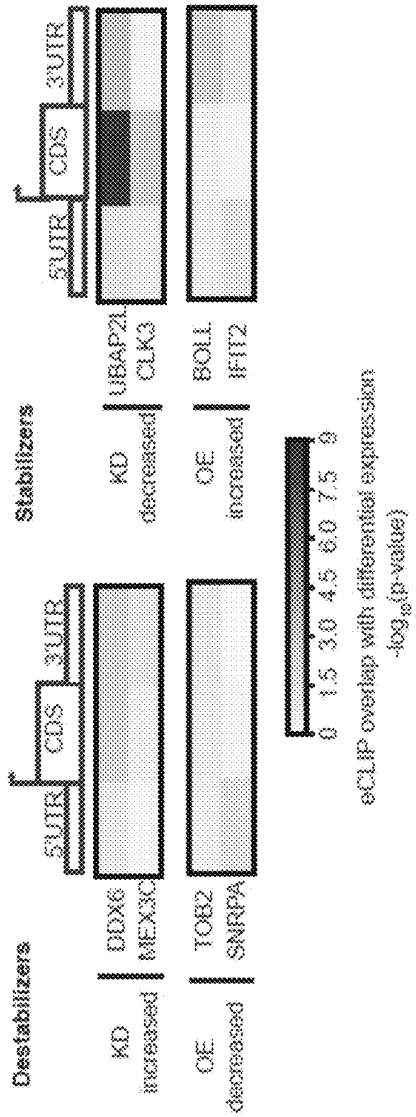

Destabilizers
KD increased
DDX6
MEX3C
OE decreased
TOB2
SNRPA
5'UTR
CDS
3'UTR
Stabilizers
KD decreased
UBAP2L
CLK3
OE increased
BOLL
IFIT2
5'UTR
CDS
3'UTR
0 1.5 3.0 4.5 6.0 7.5 8
eCLIP overlap with differential expression
-log$_e$(p-value)

FIG. 9J

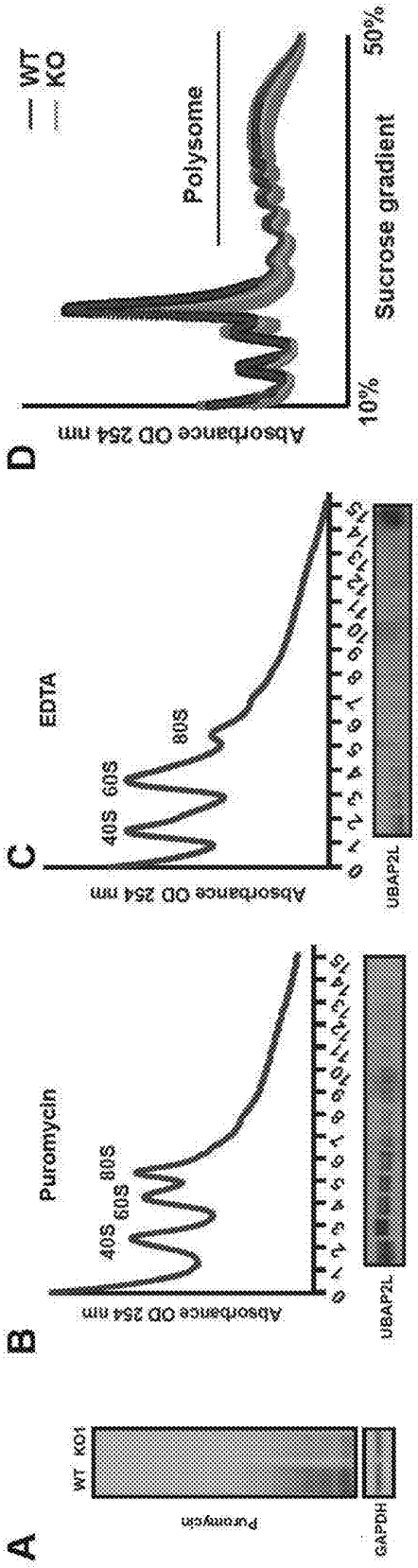


FIG. 10A

FIG. 10B

FIG. 10C

FIG. 10D

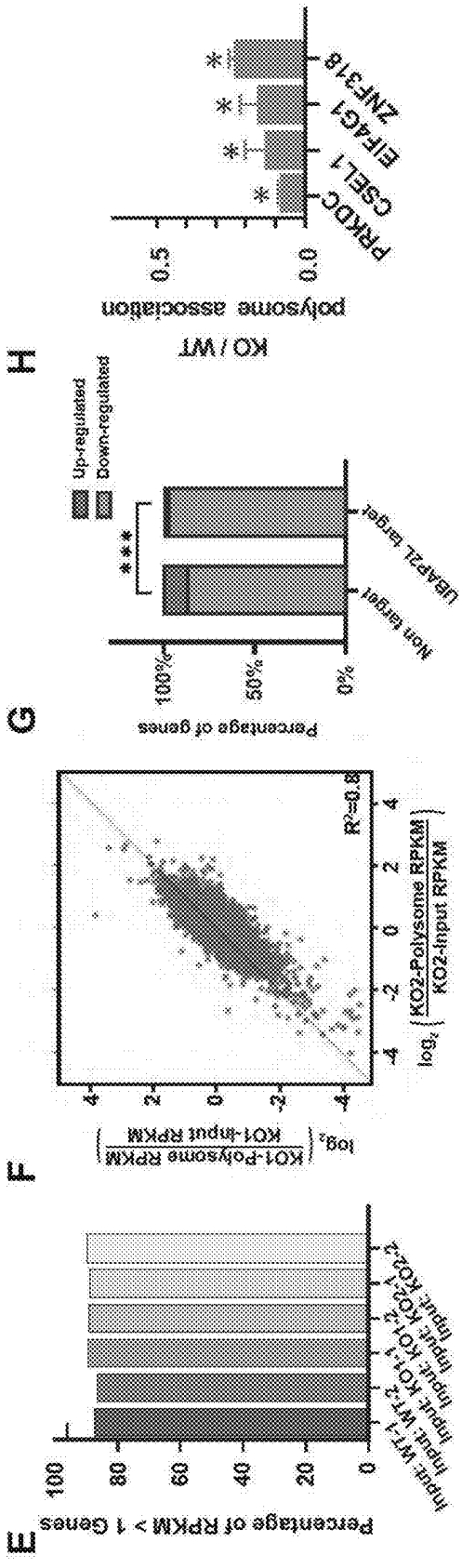
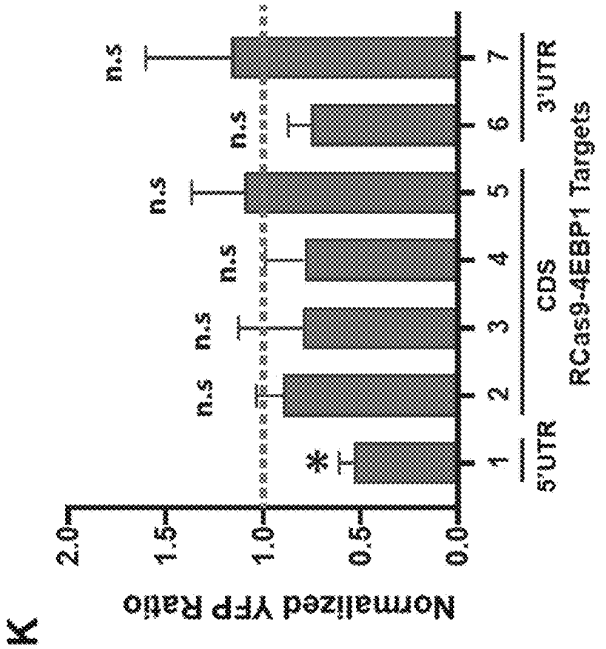


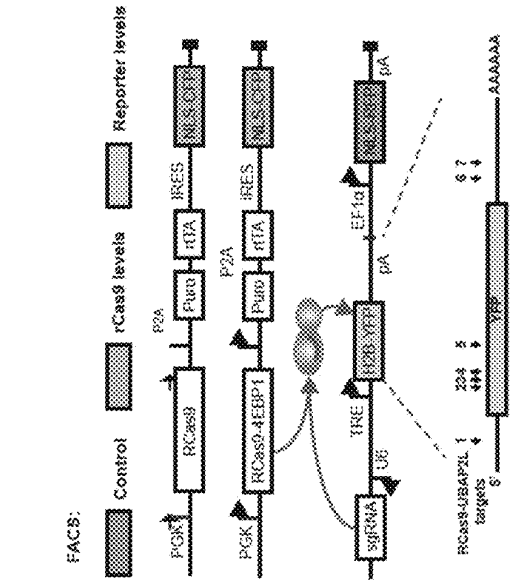
FIG. 10E

FIG. 10F

FIG. 10G

FIG. 10H





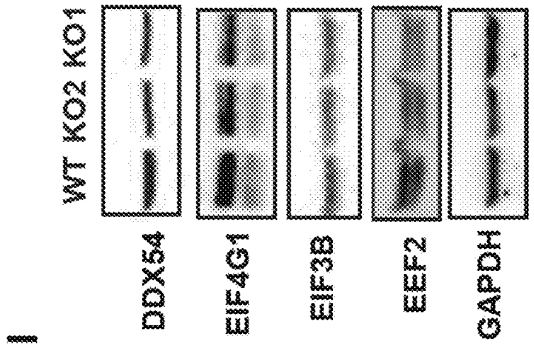


FIG. 10K

FIG. 10J

FIG. 10I

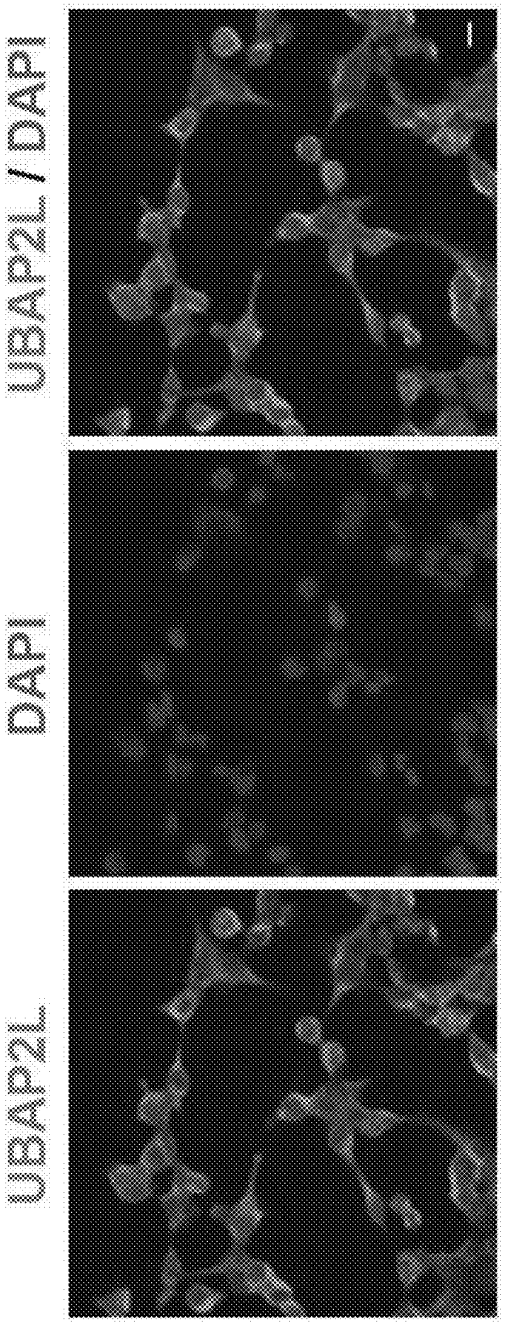


FIG. 11A

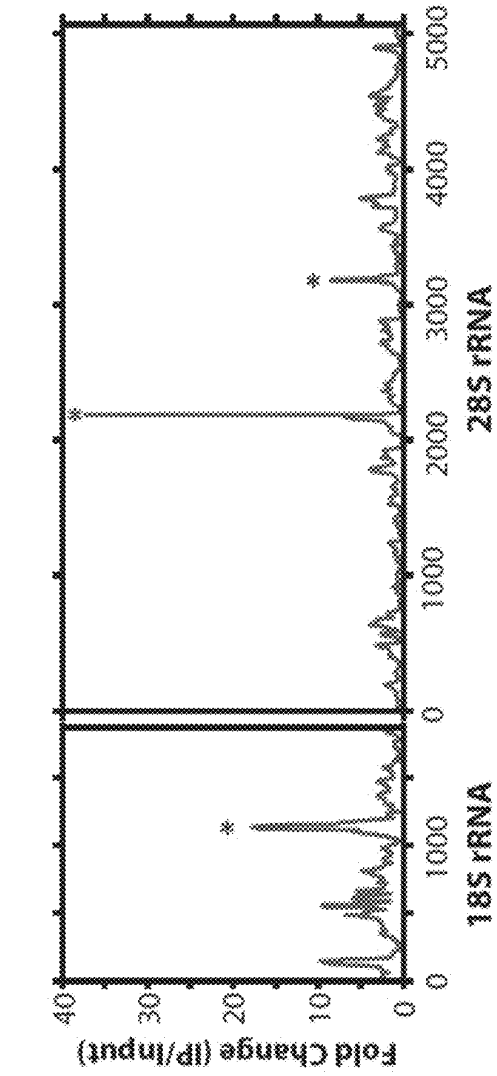


FIG. 11C

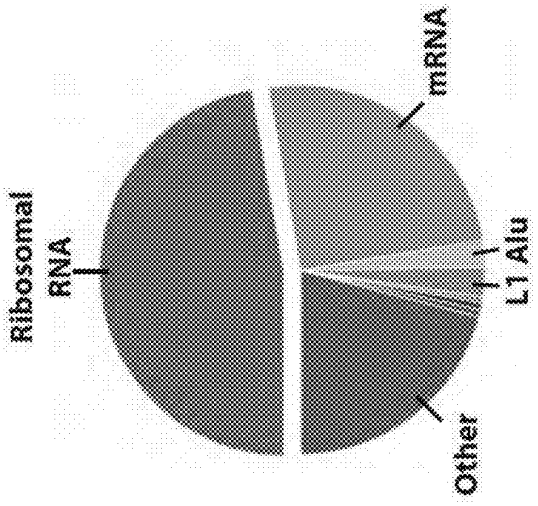


FIG. 11B

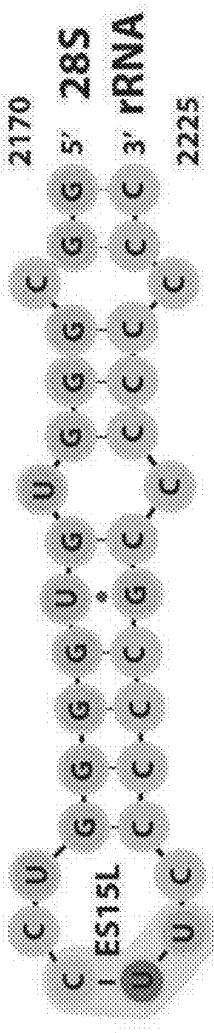


FIG. 11D

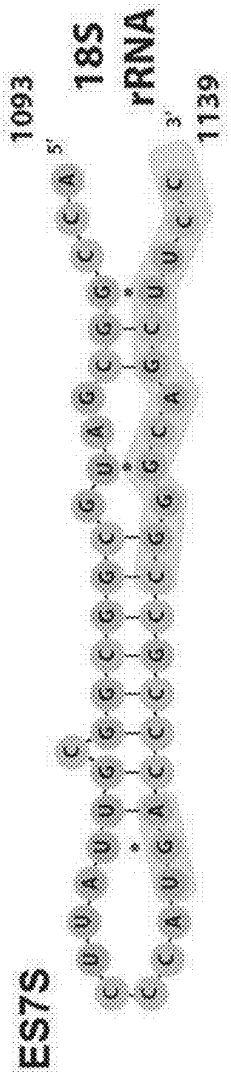


FIG. 11E

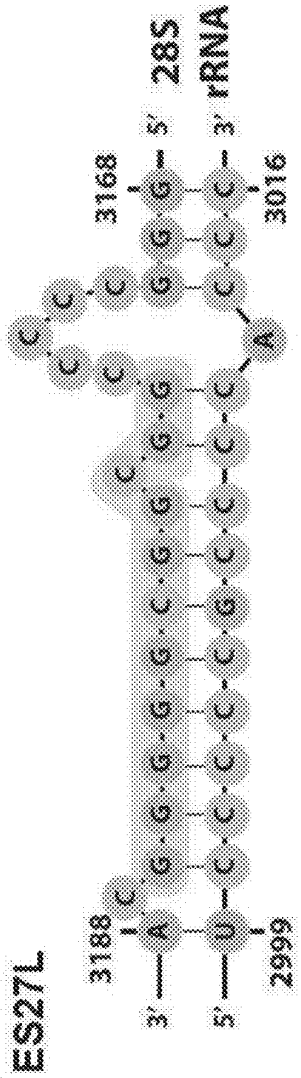


FIG. 11F

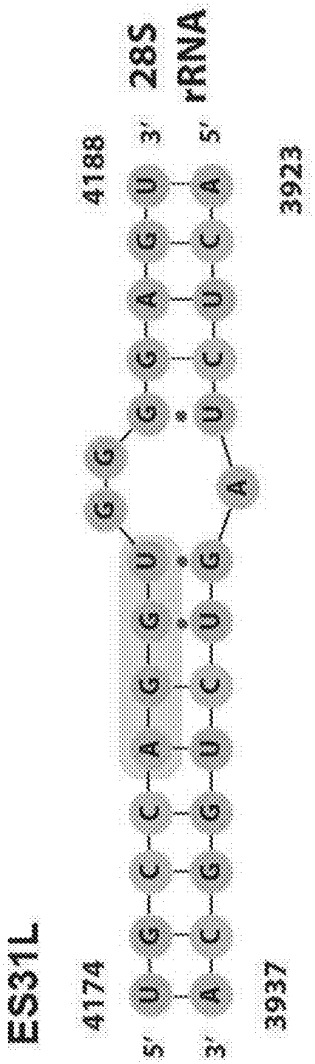


FIG. 11G

METHODS OF MODULATING RNA TRANSLATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Ser. No. 63/106,631, filed on Oct. 28, 2020. The disclosure of the prior application is considered part of the disclosure of this application, and is incorporated herein by reference in its entirety.

BACKGROUND

[0002] The fate of the transcriptome determines the status and health of a cell, and RNA-binding proteins (RBPs) control the post-transcriptional processing of these mRNA transcripts. Dysfunction of RBPs is linked to dozens of multisystemic diseases, cancer, and neurological disorders. However, despite their association with disease and although the importance of regulating gene expression at the cytoplasmic stages of an mRNA life cycle is well appreciated, only a small fraction of the over 1,500 RBPs identified thus far have known RNA targets and molecular roles. Rapid, large-scale assignment of molecular functions to more than a thousand uncharacterized and emerging RNA binding proteins (RBPs) is a critical bottleneck to a complete understanding of gene expression regulation.

SUMMARY

[0003] The present disclosure is based, at least in part, on modulating RNA translation in a cell. Provided herein are methods of modulating gene expression of a target RNA in a cell comprising (a) assembling a modulation unit, wherein the modulation unit comprises an RNA binding protein (RBP), an exogenous RNA binding moiety, and a gene-editing agent; (b) delivering the modulation unit into the cell; and (c) detecting change in the target RNA translation, wherein the modulation unit modulates gene expression of the target RNA in the cell.

[0004] In some embodiments, the exogenous RNA binding moiety comprises a MS2 bacteriophage coat protein (MCP). In some embodiments, the gene-editing agent comprises CRISPR components. In some embodiments, the gene-editing agent comprises shRNAs, siRNAs, ASOs, or microRNa mimics.

[0005] In some embodiments, the delivering step (b) comprises lipofection. In some embodiments, the delivering step (b) comprises a virus-based delivery. In some embodiments, the virus-based delivery comprises adeno-associated virus or lentivirus.

[0006] In some embodiments, the detecting step (c) comprises using a reporter mRNA. In some embodiments, the reporter mRNA comprises a luciferase mRNA. In some embodiments, the target RNA is an endogenous mRNA. In some embodiments, the target RNA is a non-coding RNA.

[0007] In some embodiments, the RBP is BTG1, CNOT2, CNOT4, CNOT7, CPSF5, DDX6, EWSR1, FUBP1, hnRNPA0, hnRNPC1/2, MEX3C, NANOS1, NANOS2, NOP56, PARN, PRR3, RBM14, RBM7, RPS6, SAMD4A, SNRPA, SRSF11, TOB1, TOB2, UTP11L, YTHDF2, ZC3H18, ZCCHC11, ZFP36, ZFP36L1, ZFP36L2, ABT1, AC004381.6, AIMP1, ALDH18A1, ANXA2, APOBEC3F, ASCC1, ATP5C1, BCCIP, BOLL, BYSL, BZW1, CELF5, CLK1, CLK2, CPSF1, DAZ2, DAZ3, DAZ4, DCN, DDX1, DDX19B, DDX20, DDX39A, DMPK, EEF1A1, EIF3G, ERAL1, XOSC4, FAM46A, FAM98A, FKBP3, FXR2, G3BP2, GLTSCR2, GSPT2, GTF2F1, GTPBP10, HADHB, HDGF, hnRNPE1, HNRPDL, HSPB1, KIAA1324, LARP1, LARP4, LARP4B, LIN28A, LUC7L, MAK16, MATR3, MBNL2, MEPCE, MRPL39, MTDH, NDUFV3, NUFIP2, NUSAP1, PABPC1, PABPC5, PCBP4, PEG10, PPAN, PPIL4, PRPF3, PRPF31, PRRC2B, PTRH1, PUS7, RBM33, RBM38, RBMX2, RPL10A, RPL14, RPL15, RPLP0, RPS20, RPUSD3, RPUSD4, RTN4, SERBP1, SF3A3, SFRS10, SFRS13A, SFRS2IP, SLC7A9, SMN1, SPATS2L, SRSF5, SRSF8, THOC1, TRA2A, TRIM39, TUFM, UBAP2L, UTP23, XPO5, XRN1, YWHAE, or ZRANB2.

[0008] In some embodiments, the gene expression of the target RNA is upregulated. In some embodiments, the gene expression of the target RNA is downregulated.

[0009] Also provided herein are methods of identifying a function of an RNA binding protein (RBP) comprising (a) contacting the RBP to an exogenous RNA binding moiety; (b) allowing the exogenous RNA binding moiety to interact with an RNA structural motif; and (c) profiling the RBP tethered to the RNA structural motif, thereby identifying a function of the RBP.

[0010] In some embodiments, the exogenous RNA binding moiety comprises a MS2 bacteriophage coat protein (MCP). In some embodiments, the RNA structural motif comprises a reporter mRNA. In some embodiments, the reporter mRNA comprises a MS2 genomic RNA stem-loop.

[0011] In some embodiments, the profiling comprises transcriptome analysis or gene expression analysis. In some embodiments, the profiling comprises enhanced cross-linking immunoprecipitation (eCLIP).

[0012] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

[0013] Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

[0014] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0015] FIG. **1**A shows a collection of 1,062 open reading frames (ORFs) for 888 unique RBPs and their overlap with those identified experimentally by Baltz et al. (Baltz et al., 2012) and Castello et al. (Castello et al., 2012).

[0016] FIG. **1**B shows an exemplary schematic of luciferase reporters. The coding region for either firefly (top) or Renilla (bottom) luciferase contain 6 MS2 stem-loop structures in the 3'UTR. The complementary reporters lacking MS2 hairpins were used as internal controls to normalize

reporter signals. RBPs fused C-terminally to the MS2 coat protein (MCP), which recognizes MS2 hairpins with high affinity, are co-expressed with the reporters in a HeLa cell line.

[0017] FIG. **1**C shows time-course analysis of the activity of the luciferase reporters in the presence of co-expressed known negative regulators of RNA stability (CNOT7 and ZFP36) or negative controls (EGFP and the FLAG peptide), with ('-MCP') or without MCP fusion. Values are expressed as ratio of the median luciferase activity of MS2-tagged over untagged reporters in the presence of the indicated RBPs, relative to that of the ratio of MS2-tagged over untagged FLAG controls at timepoint 0. Left and right panels in FIG. **1**C correspond to top and bottom reporter pairs in FIG. **1**B, respectively.

[0018] FIG. **1**D shows an exemplary experimental and analysis workflow. The screen was conducted on 888 MCP-tagged RBPs in two reporter contexts. Levels of MS2-tagged luciferase reporters were normalized to untagged co-transfected controls reporters. The effect of RBP recruitment was calculated as the ratio of normalized luciferase levels in the presence of MCP-tagged RBPs relative to that of MCP-FLAG control.

[0019] FIG. **1**E shows hit discovery, wherein RBPs with effects at estimated FDR <0.01 in both reporter assays were considered candidate regulators.

[0020] FIG. **1**F shows qPCR validation of reporter levels for 35 candidate RBP regulators. Means (n=3 independent measurements) of log 2-transformed fold-changes of reporter mRNA levels, calculated analogously to FIG. **1**D, were plotted against the corresponding log 2-transformed fold-changes of reporter luciferase levels. The line represents the least-squares linear regression fit. Shaded areas denote the 95% confidence interval. R^2, Pearson correlation coefficient.

[0021] FIG. **1**G shows examples of our 50 candidate RBP regulators that are known to affect RNA stability and translation.

[0022] FIGS. **1**H-**1**I shows validation of the FIG. **1**H 9 negative and FIG. **1**I 6 positive candidate regulators of RNA stability and/or translation by repeat luciferase and RT-PCR measurements. Values were calculated as in FIG. **1**F. Error bars denote mean±SD for n=4 replicate transfections. *p<0.05 (two-tailed Student's t-test) vs. FLAG control.

[0023] FIGS. **1**J-**1**K shows volcano plots showing the distribution of fold changes for 50 RBP hits from the FIG. **1**J Renilla and FIG. **1**K firefly reporter assays.

[0024] FIG. **1**L shows classification of candidate RBP regulators by manual curation.

[0025] FIG. **2**A shows domain structures of 14 candidate RBPs with RNA destabilizing (left) and stabilizing (right) effects in the tethering assay, with lengths of their polypeptide chains.

[0026] FIGS. **2**B-**2**D show histograms showing region-based fold-enrichment of read densities, normalized to paired SMInput controls for (FIG. **2**B) BOLL and IFIT2, which show read density enrichment in 3'UTRs; (FIG. **2**C) DDX6, MEX3C, TOB2, and TOB1, which show read density enrichment in 5'UTRs; and (FIG. **2**D) UBAP2L, which shows read density enrichment in CDS.

[0027] FIG. **2**E shows bar graphs showing eCLIP binding cluster distribution across transcript regions for the 8 destabilizers and 6 stabilizers. Peak assignment was performed using stringent enrichment criteria (≥4-fold-enrichment and $p \leq 10^{-3}$ versus SMInput). The average region distribution of the entire transcriptome annotated in GENCODE v19 is indicated at the top.

[0028] FIGS. **2**F-**2**H show example genome browser track views of eCLIP read densities (in reads per million, RPM) and corresponding SMInput read densities for (FIG. **2**F) BOLL and IFIT2, which show peak enrichment in 3'UTRs, (FIG. **2**G) DDX6 and MEX3C, which show peak enrichment in 5'UTRs, and (FIG. **2**H) UBAP2L, which shows peak enrichment across exons.

[0029] FIGS. **3**A-**3**B show volcano plots showing the distribution of fold changes in transcript levels upon modulation of (FIG. **3**A) destabilizers and (FIG. **3**B) stabilizers, with distribution histograms shown at the top. FIG. **3**A shows depletion of DDX6 (left) and overexpression of TOB2 (right). FIG. **3**B shows depletion UBAP2L (left), and overexpression of BOLL (right).

[0030] FIGS. **3**C-**3**D show bar plots showing the percentage of overlap between genes significantly up- or down-regulated [log 2(fold change) ≥1.23 and FDR-corrected $p \leq 0.05$] and significantly bound (≥4-fold-enriched and $p \leq 10^{-3}$ versus SMInput in eCLIP) upon knockdown (KD) or overexpression (OE) of candidate (FIG. **3**C) destabilizers and (FIG. **3**D) stabilizers.

[0031] FIGS. **3**E-**3**H show cumulative distribution plots of transcript $\log_2$-transformed fold changes of overexpression versus vector control or shRNA-mediated knockdown vs non-targeting control, as indicated, for the destabilizers (FIG. **3**E) DDX6 and (FIG. **3**F) TOB2, and the stabilizers (FIG. **3**G) UBAP2L and (FIG. **3**H) BOLL. p-values were calculated using a two-tailed Mann-Whitney U test.

[0032] FIGS. **3**I-**3**J show genome browser views from shRNA-mediated knockdowns showing RNA-seq reads and eCLIP reads for (FIG. **3**I) MEX3C at the NSMF locus and (FIG. **3**J) CLK3 at the NELFCD locus.

[0033] FIGS. **4**A-**4**B show translation monitoring using puromycin incorporation. FIG. **4**A shows Western blots of extracts from control (WT) HEK293T cells and two independent clonal isolates with CRISPR-mediated disruption of UBAP2L. FIG. **4**B (Left) representative anti-puromycin western blot of extracts from puromycin-treated WT and KO cells. GAPDH served as loading control. (Right) Densitometric quantitation of blots from of n=3 independent experiments.

[0034] FIG. **4**C shows a polysome profile of UBAP2L. (Top) Absorbance (at 260 nm) plot of a HEK293T cell lysate fractionated through a 10-50% a sucrose gradient. (Bottom) Western blots of UBAP2L from corresponding sucrose fractions.

[0035] FIGS. **4**D-**4**E show global transcript association with polysomes in UBAP2L knockout cells.

[0036] FIG. **4**D shows scatter plots of $\log_2$-transformed RPKM ratios of polysome transcript levels (y-axis) and input transcript levels (x-axis) between the UBAP2L knockout HEK293T lines and WT samples. The RPKM values from the two replicates were averaged prior to analysis and transcripts with average RPKM ≥1 were considered. Numbers and percentages of transcripts in each quadrant are indicated. FIG. **4**E shows cumulative distribution plots of $\log_2$-transformed transcript levels (RPKM ≥1) in pooled polysome fractions from the two UBAP2L knockout HEK293T lines and WT control, normalized to levels in the respective input lysates. p-values were calculated using a two-sample Kolmogorov-Smirnov test.

[0037] FIG. **4**F shows a bar graph showing log_2-transformed ratios of input-normalized polysome transcript levels (RPKM) between the two UBAP2L knockout lines (KO) and control (WT). Only transcripts with RPKM ≥1 in all three samples were considered (n=9,692). RPKM levels for the two KO lines were averaged.

[0038] FIG. **4**G shows gene ontology (GO) analysis for UBAP2L exon target transcripts (n=1,425). Significantly enriched GO terms were determined by Fisher's exact test at a false discovery rate of $p<0.01$. Shown are GO terms that are related to mRNA translation.

[0039] FIG. **4**H shows a heat map showing log_2-transformed polysome association ratio between UBAP2L knockout lines (KO) and control (WT) for the indicated translation regulators.

[0040] FIG. **4**I (Left) Representative western blots of UBAP2L, EIF4G1, EIF3B, DDX54, and EEF2 in UBAP2L knockout cells. GAPDH served as a loading control. (Right) Densitometric quantitation of blots from of n=3 independent experiments.

[0041] FIGS. **4**J-**4**K show quantitative fluorescence-activated cell sorting (FACS)-based reporter assay for mRNA translation using RCas9-fused UBAP2L. FIG. **4**J shows transgene expression constructs. RCas9 is expressed from a tetracycline responsive element (TRE) reporter. A constitutive promoter drives a polycistronic transcript containing puromycin N-acetyl transferase (Puro) and the reverse tetracycline (tet)-controlled transactivator (rtTA) separated by a P2A self-cleaving peptide, as well as CFP fused to a nuclear localization signal (NLS) preceded by an internal ribosome entry site (IRES). A second construct drives rCas9 fused to UBAP2L in same plasmid backbone. rCas9 and rCas9-UBAP2L constructs were integrated into the genome at random copy number to establish stable cell lines. A third reporter construct harbors a U6 promoter driven single guide (sg)RNA targeting the indicated sites in the YFP reporter, which contains of a YFP fused to histone H2B driven by a tet-inducible promoter, and NLS-fused RFP driven by the EF1a promoter. The reporter construct was transiently transfected into rCas9 and rCas9-UBAP2L-expressing lines, and the expression levels of the three reporters were measured by FACS. FIG. **4**K shows a bar graph showing mean YFP levels in rCas9-UBAP2L expressing cells, normalized to rCas9 expressing cells, on each targeting site.

[0042] FIG. **4**L shows a bar graph showing ratios of YFP/RFP mRNA levels in rCas9-UBAP2L expressing cells, normalized to rCas9 expressing cells, in the presence of the gRNA targeting site 2. Transcript levels were measured by qRT-PCR and calculated with the ΔΔCT method.

[0043] FIG. **5**A shows domain structures of UBAP2L constructs inducibly expressed in UBAP2L knockout HEK293T cells. The ubiquitin-associated domain (UBA) and arginine-glycine-rich region (RGG) are indicated.

[0044] FIG. **5**B shows autoradiograph of UBAP2L-RNA complexes immunoprecipitated from UV cross-linked HEK293T cells treated with increasing concentrations of RNase I, radiolabeled and separated on SDS polyacrylamide gel. Arrow indicates the expected molecular weight of UBAP2L.

[0045] FIG. **5**C shows autoradiograph of UBAP2L-RNA complexes immunoprecipitated from lysates of UV-cross-linked UBAP2L knockout cells (KO-UBAP2L) expressing the indicated constructs, treated with RNase I, radiolabeled and separated on SDS polyacrylamide gel. Arrow indicates the expected molecular weight of UBAP2L.

[0046] FIG. **5**D shows a pie chart showing fractions of UBAP2L eCLIP reads from HEK293T cells unambiguously mapping to mRNAs, ribosomal RNAs, and other repeat families.

[0047] FIG. **5**E shows locations of UBAP2L binding sites on rRNAs. Line plots showing the Kullback-Leibler divergence (relative entropy) for UBAP2L in HEK293T cells and the mean of 446 other RBPs from the ENCODE consortium on 18S and 28S rRNA. Lines show the mean of relative entropy, with light areas indicating 10%-90% confidence intervals.

[0048] FIGS. **5**F-**5**H show models of the interactions of UBAP2L on the human ribosome structure. (FIG. **5**F) Surface view with 60S ribosomal subunits (RNA and protein). (FIG. **5**G) View as in (FIG. **5**F) with non-highlighted proteins removed. (FIG. **5**H) View as in (FIG. **5**G) rotated 90° around the z-axis.

[0049] FIG. **6**A shows an exemplary schematic of the role of UBAP2L in regulation of global protein synthesis. UBAP2L regulates translation of key genes involved in control of protein synthesis and degradation, including the indicated components the polyadenylation machinery, translation initiation and elongation factors, tRNA synthesis enzymes and members of the ubiquitin pathway.

[0050] FIG. **6**B shows an exemplary schematic where UBAP2L enhances global protein synthesis by increasing translation efficiency of its target transcripts, as demonstrated (i) by tethered function reporter assay, (ii) by rCas9-fused UBAP2L reporter assay, and (iii) endogenously in cells.

[0051] FIG. **7**A shows sources of RBP open reading frames (ORFs). The collection of 1062 ORFs for 888 RBPs were acquired from the Dana-Farber Cancer Institute (73.5%), DNASU Plasmid repository (17.5%), in-house cloning efforts (7.7%) and Promega (1.3%).

[0052] FIG. **7**B shows distribution of known classical and non-classical RNA-binding domains in the RBP library.

[0053] FIG. **7**C shows a summary of molecular categories for RNA-related functions of the RBP library.

[0054] FIG. **7**D shows a scatter plot of luciferase effect and RBP size.

[0055] FIG. **7**E shows luciferase activities from two different reporter constructs. Bar graphs showing log_2-fold changes of the activity of Renilla (top) or firefly (bottom) luciferase reporters in presence of the MS2-fusion ORFs over FLAG control. Each vertical line represents a tethered ORF.

[0056] FIG. **7**F shows a scatter plot of luciferase activities from the two reporter constructs.

[0057] FIGS. **8**A-**8**B show in-line western blots of eCLIP immunoprecipitations of candidate RBPs. (FIG. **8**A) Extracts from HEK293T cells or (FIG. **8**B) from HEK293T transfected with the indicated MCP-tagged RBP ORFs immunoprecipitated with non-immune (IgG) control antibodies, and western blot analysis using either RBP-specific (FIG. **8**A) or anti-VS (FIG. **8**B) antibodies. The molecular weight (in kDa) of standards are indicated on the right. Arrowheads indicate the calculated molecular weight for each RBP or RBP fusion protein.

[0058] FIG. **8**C shows histograms showing region-based fold-enrichment of read densities, normalized to paired SMInput controls for UBAP2L, which shows read density enrichment in CDS.

[0059] FIG. **8**D shows metagene maps showing the distribution of eCLIP peak densities at target transcripts. Lines indicate the average number of significantly enriched peaks (≥4-fold-enriched and $p \leq 10^{-3}$ versus SMInput) across transcripts.

[0060] FIG. **8**E shows a Venn diagram showing overlap in target transcripts between DDX6 and MEX3C in HEK293T cells.

[0061] FIG. **8**F shows a metagene map showing the distribution of DDX6 and MEX3C eCLIP peak densities at target transcripts. Lines indicate the average number of significantly enriched peaks (≥4-fold-enriched and $p \leq 10^{-3}$ versus SMInput) across transcripts.

[0062] FIGS. **9**A-**9**B show shRNA-mediated depletion of RBPs in HEK293T cells using 3-5 distinct shRNAs for each RBP, as indicated, compared to non-targeting shRNA control. FIG. **9**A shows Western blots, with GAPDH or tubulin serving as loading controls, as indicated. FIG. **9**B shows bar graphs indicating RBP transcript levels determined by qRT-PCR, normalized to levels of 18S rRNA.

[0063] FIG. **9**C shows overexpression of RBPs in HEK293T cells. Bar plots showing transcript levels (RPKM) for each RBP following transfection of RBP expression constructs or FLAG vector control.

[0064] FIGS. **9**D-**9**G show numbers of up- or downregulated or unchanged genes for transcripts bound or not bound by the indicated RBP, for (FIG. **9**D) knockdown and (FIG. **9**E) overexpression of destabilizing RBPs and (FIG. **9**F) knockdown and (FIG. **9**G) overexpression of stabilizing RBPs.

[0065] FIGS. **9**H-**9**I show volcano plots showing the distribution of fold-changes in transcript levels, with distribution histograms at the top, upon (FIG. **9**H) depletion of the destabilizer MEX3C (left), overexpression of the destabilizer SNRPA (right); and (FIG. **9**I) depletion of the stabilizer CLK3 (left), and overexpression of the destabilizer IFIT2 (right).

[0066] FIG. **9**J shows a heatmap showing significance in differential expression of genes significantly differentially expressed and significantly bound vs all unbound genes, upon knockdown (KD) or overexpression (OE) of candidate RBPs in each region.

[0067] FIG. **10**A shows translation monitoring using puromycin incorporation. Representative anti-puromycin western blot of extracts from puromycin-treated UBAP2L knockout (KO1) and parental (WT) HEK293T cell lines. GAPDH served as loading control.

[0068] FIGS. **10**B-**10**C show polysome profile of UBAP2L after (FIG. **10**B) treatment with 0.5 mM puromycin in vivo, and (FIG. **10**C) 30 mM EDTA in vitro. (Top) Absorbance (at 260 nm) plot of a HEK293T cell lysate fractionated through a 10-50% a sucrose gradient. (Bottom) Western blots of UBAP2L from the corresponding fractions.

[0069] FIG. **10**D shows polysome profiles of HEK293T cells (WT, n=2) and UBAP2L knockout HEK293T cells (KO, n=4) fractionated through 10-50% a sucrose gradients.

[0070] FIG. **10**E shows bar graphs showing percentages of transcripts with RPKM ≥1 of all transcripts with ≥10 reads per transcript, for two UBAP2L knockout lines and control samples (WT).

[0071] FIG. **10**F shows scatter plots showing correlation of $\log_2$-transformed ratios of input-normalized polysome transcript levels (RPKM) between the two UBAP2L knockout HEK293T lines.

[0072] FIG. **10**G shows a bar graph showing the percentage of regulated transcripts in UBAP2L targets, and nontargets.

[0073] FIG. **10**H shows quantitative qRT-PCR validation of reduced polysome association for the indicated transcripts. Transcript levels in inputs and polysome fractions were measured for KO and WT samples.

[0074] FIG. **10**I shows Western blots of EIF4G1, EIF3B, DDX54, and EEF2 in UBAP2L knockout cells (KO1, KO2). GAPDH served as a loading control.

[0075] FIGS. **10**J-**10**K show quantitative fluorescence-activated cell sorting (FACS)-based reporter assay for mRNA translation using RCas9-fused 4EBP1. (FIG. **10**J) Plasmid design for RCas9-4EBP1 experiment. (FIG. **10**K) Bar graph showing mean YFP levels in rCas9-4EBP1 expressing cells, normalized to rCas9 expressing cells, on each targeting site.

[0076] FIG. **11**A shows immunofluorescence images showing UBAP2L in HEK293T cells. DAPI is a nuclei marker.

[0077] FIG. **11**B shows a pie chart showing fractions of UBAP2L replicate 1 eCLIP reads unambiguously mapping to repeat families in HEK293T cells.

[0078] FIG. **11**C shows locations of UBAP2L binding sites on rRNAs. The line plot shows the fold enrichment of reads for IP over SMInput. Diagram for the expansion segment ES15L shows the nucleotide corresponding to the highest peak in 28S rRNA region.

[0079] FIGS. **11**D-**11**G show location of UBAP2L binding sites on rRNA. (FIG. **11**D) ES15L (FIG. **11**E) ES7S, (FIG. **11**F) ES27L, and (FIG. **11**G) ES31L.

DETAILED DESCRIPTION

[0080] Detailed herein are methods of modulating gene expression of a target RNA in a cell and methods of identifying a function of an RNA binding protein (RBP). In some embodiments, a method of modulating gene expression of a target RNA in a cell can include (a) assembling a modulation unit, wherein the modulation unit comprises an RNA binding protein (RBP), an exogenous RNA binding moiety, and a gene-editing agent; (b) delivering the modulation unit into the cell; and (c) detecting change in the target RNA translation, wherein the modulation unit modulates gene expression of the target RNA in the cell.

[0081] In some embodiments, a method of identifying a function of an RNA binding protein (RBP) can include (a) contacting the RBP to an exogenous RNA binding moiety; (b) allowing the exogenous RNA binding moiety to interact with an RNA structural motif; and (c) profiling the RBP tethered to the RNA structural motif, thereby identifying a function of the RBP.

[0082] Various non-limiting aspects of these methods are described herein, and can be used in any combination without limitation. Additional aspects of various components of methods for modulating gene expression of a target RNA, or identifying a function of an RNA binding protein are known in the art.

[0083] It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[0084] As used herein, "biological sample" can refer to a sample generally including cells and/or other biological material. A biological sample can be obtained from a mammalian organism. For example, a biological sample can be obtained from a human. A biological sample can be obtained from a non-human mammal (e.g., a dog, a cat, a monkey, a mouse, or a rat). A biological sample can be obtained from non-mammalian organisms (e.g., a plants, an insect, an arachnid, a nematode), a fungi, an amphibian, or a fish (e.g., zebrafish). A biological sample can be obtained from a prokaryote such as a bacterium, e.g., *Escherichia coli*, Staphylococci or *Mycoplasma pneumoniae*; an archaea; a virus such as Hepatitis C virus or human immunodeficiency virus; or a viroid. A biological sample can be obtained from a eukaryote, such as a patient derived organoid (PDO) or patient derived xenograft (PDX). Biological samples can be derived from a homogeneous culture or population of organisms or alternatively from a collection of several different organisms, for example, in a community or ecosystem.

[0085] The biological sample can include any number of macromolecules, for example, cellular macromolecules and organelles (e.g., mitochondria and nuclei). The biological sample can be a nucleic acid sample and/or protein sample. The biological sample can be a carbohydrate sample or a lipid sample. The biological sample can be obtained as a tissue sample, such as a tissue section, biopsy, a core biopsy, needle aspirate, or fine needle aspirate. The sample can be a fluid sample, such as a blood sample, urine sample, or saliva sample. The sample can be a skin sample, a colon sample, a cheek swab, a histology sample, a histopathology sample, a plasma or serum sample, a tumor sample, living cells, cultured cells, a clinical sample such as, for example, whole blood or blood-derived products, blood cells, or cultured tissues or cells, including cell suspensions.

[0086] In some embodiments, the biological sample can be a tissue sample. In some embodiments, the tissue sample can include live cells from a cell culture. In some embodiments, the tissue sample can be a fresh, frozen tissue sample. In some embodiments, the fresh, frozen tissue sample is cryoground into powder. In some embodiments, the biological sample can be live cells on standard tissue culture dishes. In some embodiments, the biological sample can be flash, frozen tissues that have been cryoground into powder and placed on tissue culture dishes, pre-chilled on dry ice.

[0087] As used herein, a "cell" can refer to either a prokaryotic or eukaryotic cell, optionally obtained from a subject or a commercially available source.

[0088] As used herein, "delivering", "gene delivery", "gene transfer", "transducing" can refer to the introduction of an exogenous polynucleotide into a host cell, irrespective of the method used for the introduction. Such methods include a variety of well-known techniques such as vector-mediated gene transfer (e.g., viral infection/transfection, or various other protein-based or lipid-based gene delivery complexes) as well as techniques facilitating the delivery of "naked" polynucleotides (e.g., electroporation, "gene gun" delivery and various other techniques used for the introduction of polynucleotides). The introduced polynucleotide may be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome.

[0089] In some embodiments, a polynucleotide can be inserted into a host cell by a gene delivery molecule. Examples of gene delivery molecules can include, but are not limited to, liposomes, micelles biocompatible polymers, including natural polymers and synthetic polymers; lipoproteins; polypeptides; polysaccharides; lipopolysaccharides; artificial viral envelopes; metal particles; and bacteria, or viruses, such as baculovirus, adenovirus and retrovirus, bacteriophage, cosmid, plasmid, fungal vectors and other recombination vehicles typically used in the art which have been described for expression in a variety of eukaryotic and prokaryotic hosts, and may be used for gene therapy as well as for simple protein expression.

[0090] As used herein, "detecting" can refer to a method used to discover, determine, or confirm the existence or presence of a compound and/or substance (e.g., DNA, RNA, a protein). In some embodiments, a detecting method can be used to detect a protein. In some embodiments, a detecting method can be used to detect an RNA binding protein bound to an RNA fragment. In some embodiments, detecting can include chemiluminescence or fluorescence techniques. In some embodiments, detecting can include immunological-based methods (e.g., quantitative enzyme-linked immunosorbent assays (ELISA), Western blotting, or dot blotting) wherein antibodies are used to react specifically with entire proteins or specific epitopes of a protein. In some embodiments, detecting can include immunoprecipitation of the protein.

[0091] As used herein, the term "expression" refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently translated into peptides, polypeptides, or proteins. In some embodiments, if the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell. The expression level of a gene may be determined by measuring the amount of mRNA or protein in a cell or tissue sample; further, the expression level of multiple genes can be determined to establish an expression profile for a particular sample.

[0092] As used herein, "modulating" can refer to modifying, regulating, or altering the endogenous gene expression in a cell. In some embodiments, modulating gene expression can include systematically influencing RNA stability and/or translation by activating or suppressing the gene expression. In some embodiments, modulation of gene expression can include stabilizing a target RNA. In some embodiments, stabilizing a target RNA can increase translation of the target RNA. In some embodiments, modulation of gene expression can include destabilizing a target RNA. In some embodiments, destabilizing a target RNA can suppress translation of the target RNA. In some embodiments, modulation of gene expression can include increasing translation of a target RNA. In some embodiments, modulation of gene expression can include suppressing translation of a target RNA. In some embodiments, the gene expression of the target RNA is upregulated. In some embodiments, the gene expression of the target RNA is downregulated.

[0093] As used herein, "nucleic acid" is used to include any compound and/or substance that comprise a polymer of nucleotides. In some embodiments, a polymer of nucleotides

are referred to as polynucleotides. Exemplary nucleic acids or polynucleotides can include, but are not limited to, ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs, including LNA having a β-D-ribo configuration, α-LNA having an α-L-ribo configuration (a diastereomer of LNA), 2'-amino-LNA having a 2'-amino functionalization, and 2'-amino-α-LNA having a 2'-amino functionalization) or hybrids thereof. Naturally-occurring nucleic acids generally have a deoxyribose sugar (e.g., found in deoxyribonucleic acid (DNA)) or a ribose sugar (e.g., found in ribonucleic acid (RNA)).

[0094] A nucleic acid can contain nucleotides having any of a variety of analogs of these sugar moieties that are known in the art. A deoxyribonucleic acid (DNA) can have one or more bases selected from the group consisting of adenine (A), thymine (T), cytosine (C), or guanine (G), and a ribonucleic acid (RNA) can have one or more bases selected from the group consisting of uracil (U), adenine (A), cytosine (C), or guanine (G).

[0095] In some embodiments, the nucleic acid is a messenger RNA (mRNA). As used herein, "messenger RNA" (mRNA) can refer to any polynucleotide which encodes a polypeptide of interest and which is capable of being translated to produce the encoded polypeptide of interest in vitro, in vivo, in situ, or ex vivo.

Methods of Modulating Gene Expression of a Target RNA

[0096] Provided herein are methods of modulating gene expression of a target RNA in a cell including (a) assembling a modulation unit, wherein the modulation unit comprises an RNA binding protein (RBP), an exogenous RNA binding moiety, and a gene-editing agent; (b) delivering the modulation unit into the cell; and (c) detecting change in the target RNA translation, wherein the modulation unit modulates gene expression of the target RNA in the cell. In some embodiments, a target RNA is an endogenous mRNA. In some embodiments, a target RNA is a non-coding RNA.

[0097] In some embodiments, a modulation unit can include an RNA binding protein (RBP), an exogenous RNA binding moiety, and a gene-editing agent. In some embodiments, the exogenous RNA binding moiety comprises a MS2 bacteriophage coat protein (MCP). In some embodiments, the gene-editing agent comprises CRISPR components. In some embodiments, the gene-editing agent comprises shRNAs, siRNAs, ASOs, or microRNa mimics.

RNA Binding Protein

[0098] RNA binding proteins (RBPs) are proteins that bind to the double or single stranded RNA in cells and have important roles in cellular processes (e.g., cellular transport, or localization). RBPs also play a role in post-transcriptional control of RNAs, such as RNA splicing, polyadenylation, mRNA stabilization, mRNA localization, and translation. In some embodiments, an RBP is a cytoplasmic protein. The term "RNA binding protein" can refer to a protein that interacts with RNA molecules (e.g., mRNA) from synthesis to decay to affect their metabolism, localization, stability, and translation. In some embodiments, an RBP is a nuclear protein. In some embodiments, RBPs can include, but are not limited to, splicing factors, RNA stability factors, histone stem-loop binding proteins, or ribosomes. For example, a eukaryotic ribosome can include a collection of RBPs that can interact directly with mRNA coding sequences. In some embodiments, an RBP is a cytoplasmic protein.

[0099] In some embodiments, an RNA binding protein comprises a ribosomal protein, wherein the ribosomal protein binds to a ribosome and an mRNA during translation. In some embodiments, an RNA binding protein comprises a ribosomal protein, wherein the ribosomal protein binds to a ribosome or an mRNA during translation. In some embodiments, the RNA binding protein comprises at least one of: SLTM, ZGPAT, PPARGC1B, PELP1, DCP2, CSTF3, TRA2B, ZNF638, SRSF9, LUC7L2, PTBP3, SF3B3, VCP, HNRNPA2B1, PTBP1, PCBP2, LSM14A, LSM12, DHX15, DDX27, DDX17, DDX21, IPO5, RPL22L1, RPL35, RPSA, MRPS34, NIFK, THUMPD1, RPUSD3, RRBP1, EEFSEC, UBAP2L, PUS7L, EIF4ENIF1, BICC1, EIF4E2, DARS2, TRDMT1, UPF3B, ZFP36L2, YTHDF2, EDC3, HNRNPR, UPF3A, ELAVL1, RBM27, XRN1, FUS, EXOSC7, PSPC1, CNOT7, CNOT6, CNOT4, CNOT3, AGO2, ENDOU, RBFOX1 (A2BP1), RBFOX2 (RBM9), RBFOX3 (NeuN), SLBP, RBM5, RBM6, PRBP1, ACO1, Adat1, PCBP1, PCBP3, PCBP4, RBM3, RBM4, APOBEC1, BTG1, CNOT2, CPSF5, DDX6, EWSR1, FUBP1, hnRNPA0, hnRNPC1/2, MEX3C, NANOS1, NANOS2, NOP56, PARN, PRR3, RBM14, RBM7, RPS6, SAMD4A, SNRPA, SRSF11, TOB1, TOB2, UTP11L, ZC3H18, ZCCHC11, ZFP36, ZFP36L1, ABT1, AC004381.6, AIMP1, ALDH18A1, ANXA2, APOBEC3F, ASCC1, ATP5C1, BCCIP, BOLL, BYSL, BZW1, CELF5, CLK1, CLK2, CPSF1, DAZ2, DAZ3, DAZ4, DCN, DDX1, DDX19B, DDX20, DDX39A, DMPK, EEF1A1, EIF3G, ERAL1, XOSC4, FAM46A, FAM98A, FKBP3, FXR2, G3BP2, GLTSCR2, GSPT2, GTF2F1, GTPBP10, HADHB, HDGF, hnRNPE1, HNRPDL, HSPB1, KIAA1324, LARP1, LARP4, LARP4B, LIN28A, LUC7L, MAK16, MATR3, MBNL2, MEPCE, MRPL39, MTDH, NDUFV3, NUFIP2, NUSAP1, PABPC1, PABPC5, PCBP4, PEG10, PPAN, PPIL4, PRPF3, PRPF31, PRRC2B, PTRH1, PUS7, RBM33, RBM38, RBMX2, RPL10A, RPL14, RPL15, RPLP0, RPS20, RPUSD3, RPUSD4, RTN4, SERBP1, SF3A3, SFRS10, SFRS13A, SFRS2IP, SLC7A9, SMN1, SPATS2L, SRSF5, SRSF8, THOC1, TRA2A, TRIM39, TUFM, UBAP2L, UTP23, XPO5, XRN1, YWHAE, or ZRANB2.

[0100] RNA-binding proteins (RBPs) have roles in controlling the fate of RNAs including the modulation of pre-mRNA splicing, RNA modification, translation, stability and localization. RBPs are a group of proteins that interact with RNA using an array of strategies from well-defined RNA-binding domains to disordered regions that recognize RNA sequence and/or secondary structures.

[0101] As used herein, "RNA-RBP complex" can refer to a ribonucleoprotein complex comprising an RNA-binding protein (RBP) bound to a double or single stranded RNA in a cell. In some embodiments, the RNA-RBP complex can include an RNA fragment bound by an RNA binding protein. In some embodiments, the RBP is crosslinked to an RNA in a biological sample. In some embodiments, the crosslinking can include UV crosslinking. In some embodiments, the RBP is covalently linked to the RNA in a biological sample. In some embodiments, crosslinking can be performed by any method including, but not limited to, thermal crosslinking, chemical crosslinking, physical crosslinking, ionic crosslinking, photo-crosslinking, free-radical initiation

crosslinking, an addition reaction, condensation reaction, water-soluble crosslinking reactions, irradiative crosslinking (e.g., x-ray, electron beam), or combinations thereof. As used herein, "ribosomal protein" can refer to a protein that is present in a ribosome (e.g., a mammalian ribosome) or a protein that binds to a ribosome and an mRNA during translation (e.g., a translation initiation factor, a translation elongation factor, and a translation termination factor). The eukaryotic ribosome is composed of 79 ribosomal proteins, large ribosomal proteins (RPLs) and small subunit proteins (RPSs) that interweave with 4 highly structured RNAs (5S, 5.8S, 18S, and 28S rRNAs) to form the final translation-capable ribonucleoprotein. Thus, quantification of ribosome-associated RNA is highly similar to profiling of RNAs associated with other RNA binding proteins.

[0102] In some embodiments, the ribosomal protein binds to a ribosome or an mRNA during translation. The term "translation initiation factor" can refer to a protein that binds to a ribosome, a subunit of a ribosome, and/or an mRNA during the start of translation of an mRNA. The term "translation elongation factor" can refer to a protein that binds to a ribosome, a subunit of a ribosome, and/or mRNA during translation of an mRNA. The term "translation termination factor" can refer to a protein that binds to a ribosome, a subunit or a ribosome, and/or mRNA during cessation of translation and/or release of an mRNA from a ribosome or a subunit of a ribosome. In a ribosome, ribosomal proteins can participate in the translation process and binding of translation factors (e.g., translation initiation factor, translation elongation factor, translation termination factor). In some embodiments, the ribosomal protein is selected from the group consisting of: RPS2, RPS3, RPS3A, RPS4X, RPS4Y1, RPS4Y2, RPS5, RPS6, RPS7, RPS8, RPS9, RPS10, RPS11, RPS12, RPS13, RPS14, RPS15, RPS15A, RPS16, RPS17, RPS18, RPS19, RPS20, RPS21, RPS23, RPS24, RPS25, RPS26, RPS27, RPS28, RPS29, RPS30, RSSA, RACK1, RPL3, RPL4, RPL5, RPL6, RPL7A, RPL7, RPL8, RPL9, RPL10A, RPL10, RPL11, RPL12, RPL13A, RPL13, RPL14, RPL15, RPL17, RPL18A, RPL18, RPL19, RPL21, RPL22, RPL23A, RPL23, RPL24, RPL26, RPL27A, RPL27, RPL28, RPL29, RPL30, RPL31, RPL32, RPL34, RPL35A, RPL35, RPL36, RPL37A, RPL37, RPL38, RPL39, RPL40, RPL41, RPLA0, RPLA1, and RPLA2. In some embodiments, the ribosomal protein is a translation initiation factor. In some embodiments, the ribosomal protein is a translation elongation factor. In some embodiments, wherein the ribosomal protein is a translation termination factor.

Exogenous RNA Binding Moiety and Gene-Editing Agent

[0103] As used herein, the term "exogenous RNA binding moiety" refers to a molecule or moiety capable of binding to an RNA (e.g., target RNA). In some embodiments, an exogenous RNA binding moiety can be fused to a protein (e.g., RNA binding protein). In some embodiments, an exogenous RNA binding moiety can include a reporter mRNA. In some embodiments, the exogenous RNA binding moiety can be attached to a protein through an artificial RNA-protein interaction. In some embodiments, an exogenous RNA binding moiety can include a MS2 bacteriophage coat protein (MCP). In some embodiments, an exogenous RNA binding moiety can be fused to an RNA binding protein (RBP).

[0104] As used herein, the term "gene-editing agent" can refer to an agent that allows for changing the DNA or RNA (e.g., mRNA) in the genome. In some embodiments, gene-editing can include insertion, deletion, modification, or replacement of the DNA or RNA. In some embodiments, a gene-editing agent can include a nuclease-based gene editing platform. In some embodiments, a gene-editing agent can include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), engineered meganucleases, or a clustered regularly interspaced short palindromic repeats (CRISPR) system. In some embodiments, a gene-editing agent can include RNA interference (e.g., short hairpin RNA (shRNA), small interfering RNA (siRNA), antisense oligonucleotide (ASO), or microRNA mimics). In some embodiments, the gene-editing agent can include CRISPR components. For example, in some embodiments, CRISPR components can include, but are not limited to, a guide RNA and a CRISPR-associated endonuclease (Cas protein). In some embodiments, the gene-editing agent can include a guide RNA (e.g., gRNA or sgRNA) and a CRISPR-associated endonuclease (Cas protein). In some embodiments, the gene-editing agent comprises shRNAs, siRNAs, ASOs, or microRNa mimics.

[0105] As used herein, the term "CRISPR" refers to a technique of sequence specific genetic manipulation relying on the clustered regularly interspaced short palindromic repeats pathway, which unlike RNA interference regulates gene expression at a transcriptional level. The term "gRNA" or "guide RNA" refers to the guide RNA sequences used to target specific genes for correction employing the CRISPR technique. Techniques of designing gRNAs and donor therapeutic polynucleotides for target specificity are well known in the art. For example, Doench, J., et al. Nature biotechnology 2014; 32(12):1262-7 and Graham, D., et al. Genome Biol. 2015; 16: 260. The term "Single guide RNA" or "sgRNA" is a specific type of gRNA that combines tracrRNA (transactivating RNA), which binds to Cas9 to activate the complex to create the necessary strand breaks, and crRNA (CRISPR RNA), comprising complimentary nucleotides to the tracrRNA, into a single RNA construct. Exemplary methods of employing the CRISPR technique are described in WO 2017/091630, which is incorporated by reference in its entirety.

[0106] In some embodiments, the single guide RNA can recognize a target RNA, for example, by hybridizing to the target RNA. In some embodiments, the single guide RNA comprises a sequence that is complementary to the target RNA. In some embodiments, the sgRNA can include one or more modified nucleotides. In some embodiments, the sgRNA has a length that is about 10 nt (e.g., about 20 nt, about 30 nt, about 40 nt, about 50 nt, about 60 nt, about 70 nt, about 80 nt, about 90 nt, about 100 nt, about 120 nt, about 140 nt, about 160 nt, about 180 nt, about 200 nt, about 300 nt, about 400 nt, about 500 nt, about 600 nt, about 700 nt, about 800 nt, about 900 nt, about 1000 nt, or about 2000 nt).

[0107] In some embodiments, a single guide RNA can recognize a variety of RNA targets. For example, a target RNA can be messenger RNA (mRNA), ribosomal RNA (rRNA), signal recognition particle RNA (SRP RNA), transfer RNA (tRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), antisense RNA (aRNA), long noncoding RNA (lncRNA), microRNA (miRNA), piwi-interacting RNA (piRNA), small interfering RNA (siRNA), short hairpin RNA (shRNA), retrotransposon RNA, viral

genome RNA, or viral noncoding RNA. In some embodiments, a target RNA can be an RNA involved in pathogenesis of conditions such as cancers, neurodegeneration, cutaneous conditions, endocrine conditions, intestinal diseases, infectious conditions, neurological conditions, liver diseases, heart disorders, or autoimmune diseases. In some embodiments, a target RNA can be a therapeutic target for conditions such as cancers, neurodegeneration, cutaneous conditions, endocrine conditions, intestinal diseases, infectious conditions, neurological conditions, liver diseases, heart disorders, or autoimmune diseases.

[0108] In some embodiments, a method described herein can include assembling a modulation unit, wherein the modulation unit comprises an RNA binding protein (RBP), an exogenous RNA binding moiety, and a gene-editing agent. In some embodiments, the assembling of the modulation unit can be performed outside of a host cell. In some embodiments, the assembling can include plasmid construction.

[0109] In some embodiments, a method described herein can include delivering a modulation unit into a cell. In some embodiments, the delivering step comprises lipofection. In some embodiments, the delivering step comprises a virus-based delivery. In some embodiments, the virus-based delivery comprises adeno-associated virus or lentivirus.

[0110] In some embodiments, a method described herein can also include detecting change in a target RNA stability and/or translation, wherein a modulation unit modulates gene expression of the target RNA in a cell. As used herein, a "reporter mRNA" can refer to an mRNA that can be attached to another gene of interest, wherein the reporter mRNA can express a protein that is easily measured and identified and can be used as a marker to indicate whether the gene of interest in expressed in a cell or organism. In some embodiments, the detecting step comprises using a reporter mRNA. In some embodiments, a reporter mRNA can include a luciferase mRNA. In some embodiments, a reporter mRNA can include chloramphenicol acetyltransferase, 3-galactosidase (GAL), β-glucuronidase, β-glucuronidase, firefly luciferase, Renilla luciferase, or green fluorescent protein (GFP).

Methods of Identifying a Function of an RNA Binding Protein (RBP)

[0111] Provided herein are methods of identifying a function of an RNA binding protein (RBP) including (a) contacting the RBP to an exogenous RNA binding moiety; (b) allowing the exogenous RNA binding moiety to interact with an RNA structural motif; and (c) profiling the RBP tethered to the RNA structural motif, thereby identifying a function of the RBP.

[0112] In some embodiments, a function of an RNA binding protein can include regulating target RNA translation and/or stability. In some embodiments, a function of an RNA binding protein can include controlling global protein homeostasis by affecting levels of RNA translation regulators. In some embodiments, a function of an RNA binding protein can include RNA splicing, modulating RNA stability, RNA transport, or RNA translation. In some embodiments, a function of an RNA binding protein can include stabilizing a target RNA. In some embodiments, a function of an RNA binding protein can include destabilizing a target RNA. In some embodiments, a function of an RNA binding protein can include enhancing translation of a target RNA. In some embodiments, a function of an RNA binding protein can include suppressing translation of a target RNA.

[0113] In some embodiments, the contacting step can include an exogenous RNA binding moiety being fused to a RNA binding protein. In some embodiments, the exogenous RNA binding moiety can be fused to a RNA binding protein through an artificial RNA-protein interaction. In some embodiments, an exogenous RNA binding moiety can include a reporter mRNA. In some embodiments, an exogenous RNA binding moiety comprises a MS2 bacteriophage coat protein (MCP). In some embodiments, an RNA structural motif comprises a reporter mRNA. In some embodiments, the reporter mRNA comprises a MS2 genomic RNA stem-loop. As used herein, an "RNA structural motif" can refer to a collection of residues that fold into a stable three-dimensional (3D) structure of an RNA molecule. In some embodiments, an RNA structural motif can include an RNA hairpin loop, RNA internal loop, a tetraloop, a sarcin-ricin loop, or a T-loop. In some embodiments, an RNA structural motif can includes a MS2 genomic RNA stem-loop.

[0114] As used herein, "profiling" can refer to the measurement of an activity (e.g., expression) of one or more genes, to create a global picture of cellular function. In some embodiments, the profiling comprises transcriptome analysis or gene expression analysis. In some embodiments, the profiling comprises enhanced cross-linking immunoprecipitation (eCLIP). As used herein, "Enhanced crosslinking and immunoprecipitation (eCLIP)" refers to a method to profile RNAs bound by an RNA binding protein of interest. In some embodiments, eCLIP can be modified and used to profile RNAs bound by specific ribosomal subunit proteins. In some embodiments, enhanced crosslinking and immunoprecipitation (eCLIP) recovers protein-coding mRNAs (with a particular enrichment for coding sequence regions).

EXAMPLES

[0115] The disclosure is further described in the following examples, which do not limit the scope of the disclosure.

Example 1—Generation of Resource of RBP Open-Reading Frames Fused to MS2 Coat Protein and Tethered Function Assays

[0116] A collection of RBP expression constructs was assembled using in-house bioinformatics tools to extract genes annotated to contain RNA-binding domains as predicted by PFAM and

[0117] PRINTS. This set was extended with mRNA-bound putative RBPs identified experimentally in two different studies which used UV-cross-linking and oligo(dT) capture followed by mass spectrometry. 888 unique RBPs with 1,062 RBP ORFS (FIG. **1**A) were acquired from both commercial sources and through in-house cloning efforts (FIG. **7**A; Table 1) and sub-cloned into two constructs using Gateway-mediated cloning: one that directs expression of the RBPs as fusion proteins with the V5 epitope tag C-terminally appended, and one with an additional bacteriophage MS2 coat protein (MCP) domain at the C-terminus. Overall, ~40% of the 69 RBPs in the collection contain known canonical RNA-binding motifs, while the remainder may associate with RNA through other interaction domains or binding modes (FIG. **7**B). Highlighting the need for assessing the roles of RBPs in RNA metabolism, Gene Ontology

(GO) analysis showed that ~60% of the RBPs in the collection have no known RNA-related functions (FIG. **7**C). Thus, a comprehensive resource of representative 'tethered' and 'untethered' RBP expression libraries was assembled comprising the majority of all predicted and/or experimentally identified RBPs.

[0118] Next, a set of tetracycline-repressible luciferase reporter plasmids were constructed that measure the effect of RBP recruitment to the 3'UTR on reporter expression. F-Luc-6MS2 encodes firefly luciferase followed by 6 MS2 hairpin sequences inserted into the 3'UTR context of HBB (β-globin). To address potential reporter context dependencies, a corresponding Renilla luciferase construct was also generated. Matched constructs lacking MS2 sequences served as negative controls (FIG. **1**B). To validate the system, each reporter was co-introduced into HeLa cells along with constructs expressing MCP-fused and unfused versions of ZFP36 (also known as Tristetraprolin, TTP), an RBP activator of AU-rich element (ARE)-mediated RNA decay, enhanced GFP (EGFP) or the FLAG peptide. As expected, ZFP36 but not enhanced GFP (EGFP) or the FLAG peptide, dramatically reduced protein levels of the luciferase reporter in a manner that depended on the presence of the tether but not the identity of the luciferase protein (FIG. **1**C). This demonstrated that tethered ZFP36 can recruit functional CCR4-NOT deadenylase complexes, which contain the Caf1 subunit CNOT7 (an RNase), to the reporter. Tethering of CNOT7 itself recapitulated this finding, indicating that productive recruitment is not limited to sequence-specific RBPs (such as ZFP36), but extends to effector RBPs (such as CNOT7) (FIG. **1**C).

TABLE 1

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
A1CF	BC054873.1	13.8	Modification		RRM	DFCI
ABT1	BC048812.1	31.1	Other	Baltz/Castello	RRM	DFCI
ABT1	BC066313.1	31.1	Other	Baltz/Castello	RRM	DFCI
ACAA2	BC001918.1	41.9	Other		Other	DFCI
ACOT9		48.9	Other		Other	In-house
ACTN4	BC005033.1	104.9	Other	Castesllo	Other	DFCI
ADAD1	BC040229	62.8	Other		dsRBD	In-house
ADAD2	BC033491.1	61.8	Other		dsRBD	DFCI
ADAR	BC038227	136.0	Modification	Baltz/Castello	dsRBD	DNASU
ADARB1	BC065545.1	76.6	Modification	Baltz	dsRBD	DFCI
ADD1	BC013393	44.0	Other		Other	DNASU
ADK	BC003568.1	38.7	Other	Castello	Other	DFCI
AGGF1	BC002828.2	12.5	Other		Other	DFCI
AGGF1	BC032844.1	80.9	Other		Other	DFCI
AHNAK	BC012477.1	16.1	Other	Castello	Other	DFCI
AHNAK	BC000926.1	16.2	Other	Castello	Other	DFCI
AIMP1	BC014051.2	34.4	Translation		tRNA	DFCI
AK8	BC034776.1 BC050576.1	54.9	Other		Ostler	DFCI
ALDH18A1	HQ268499	87.3	Other	Castello	Other	DNASU
ALDH6A1	BC004909.1, BC032371.1	57.8	Other	Castello	Other	DFCI
ANKHD1	BC040231.1	91.5	Other	Baltz/Castello	KH	DFCI
ANKHD1	BC004457	46.1	Other	Baltz/Castello	KH	In-house
ANXA2	BC052567.1	38.6	Other	Castello	Other	DFCI
ANXA2	BC009564.1	38.6	Other	Castello	Other	DFCI
ANXA2	BC023990.1	38.6	Other	Castello	Other	DFCI
APEH	BC000362.2	81.2	Other	Castello	Other	DFCI
APOBEC3A	BC126416.1	23.0	Other		Other	DFCI
APOBEC3B	BC053859.1	29.8	Other	Baltz	Other	DFCI
APOBEC3C	BC011739.2	22.8	Other	Baltz/Castello	Other	DFCI
APOBEC3B	BC017022.1	46.6	Other		Other	DFCI
APOBEC3F	BC038808.1	45.0	Other	Baltz	Other	DFCI
APOBEC3F	BC061914	9.4	Other	Baltz	Other	In-house
APOBEC3G	BC024268.1	46.4	Other		Other	DFCI
APOBEC3H	BC069023.1	21.5	Other		Other	DFCI
APOBEC4	BC021711	41.6	Other		Other	In-house
ARL6IP4	uc004dat.1	24.0	Splicing	Baltz/Castello	Other	DFCI
ARL6IP4	BC001958.1	24.6	Splicing	Baltz/Castello	Other	DFCI
ASCC1	BC012291.1	41.2	Other		KH	DFCI
ASCC3	BC050681.1	13.0	Other	Baltz	Other	DFCI
ASS1	BC021676.1	46.5	Other	Castello	Other	DFCI
ASS1	BC009243.2	46.5	Other	Castello	Other	DFCI
ATP5C1	BC000470.2, BC000931.3	33.0	Other	Castello	Other	DFCI
ATF5C1	BC016812.1	33.0	Other	Castello	Other	DFCI
ATXN1	BC117125	86.9	Localization		Other	In-house
BCCIP	BC009771.1	36.1	Other	Castello	Other	DFCI
BCDIN3D	BC053560.1	33.2	Modification		Other	DFCI
BCL7B	BC000956	22.2	Other		Other	In-house
BMS1	BC043345.1	145.8	Other	Baltz/Castello	Other	DFCI
BOLL	BC033874.1	31.3	Translation		RRM	DFCI
BST2	BC033873.1	19.8	Other	Castello	Other	DFCI
BTG1	BC016759	19.2	Other		Other	DNASU

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
BTG2	BC105949	17.4	Other		Other	DNASU
BTG3	BC011957.1	34.2	Other		Other	DNASU
BTG4	BC031045	24.0	Other		Other	DNASU
BTN3A3	BC015815.2	65.0	Other		Other	DFCI
BUD13	BC006350.2	70.5	Localization	Castello	Other	DFCI
BYSL	BC007340.2	37.1	Other	Castello	Other	DFCI
BZW1	BC026303.1	48.0	Other	Castello	Other	DFCI
C16ORF88	BC117562.1	51.6	Other	Castello	Other	DFCI
C1D	BC005235.1	16.0	Other		Other	DFCI
C1D	BC009584.1, BC0095891, BC016284.2	16.0	Other		Other	DFCI
C1ORF131	BC036800.1	31.4	Other	Baltz/Castello	Other	DFCI
C1ORF35	BC002843.2	29.4	Other	Baltz/Castello	Other	DFCI
C9ORF72	C9ORF72	54.3	Other		Other	In-house
CALR	BC002500.2, BC007911.1, BC020493.1	48.1	Other	Castello	Other	DFCI
CCDC137	BC009369.2	33.2	Other	Baltz/Castello	Other	DFCI
CCDC59	BC020647.1	28.7	Other	Castello	Other	DFCI
CCDC75	BC071798.1	18.6	Other		Other	DFCI
CCDC9	BC002787.2, BC009743.2	59.7	Other	Baltz/Castello	Other	DFCI
CCNL1	JF432881	59.6	Other		Other	DNASU
CCNL2	BC016333.1	24.6	Other		Other	DNASU
CCT4	BC106934.1	57.9	Other	Castello	Other	DFCI
CCT6A	BC106942.1	58.0	Other	Castello	Other	DFCI
CDC2L5	NM_003718.3	164.9	Other		Other	DNASU
CDC40	HQ258473	65.5	Localization	Castello	Other	DNASU
CDC42EP4	BC010451.1	38.0	Other		Other	DFCI
CDC42EP4	BC002774.1	38.0	Other		Other	DFCI
CELF3	BC052491.1	50.5	Splicing		RRM	DFCI
CELF4	BC001946.1	51.8	Translation		RRM	DFCI
CELF5	BC028101.1	52.4	Other		RRM	DFCI
CHAF1B		61.5	Other		Other	DNASU
CHAF1B	JF432525	61.5	Other		Other	DNASU
CHD2	BC007347.2	56.8	Other	Baltz/Castello	Other	DFCI
CHTOP	BC120961.1	26.5	Localization	Baltz	Other	DFCI
CIR1	BC015040.1	23.3	Other		Other	DNASU
CIRBP	BC000901.1	18.6	Stability	Baltz/Castello	RRM	DFCI
CIRBP	BC000403.2	18.6	Stability	Baltz/Castello	RRM	DFCI
CIRH1A	BC009348.2	76.9	Other	Castello	Other	DFCI
CISD2	BC032300.1	15.3	Other	Castello	Other	DFCI
CLASRP		77.2	Splicing		Other	DNASU
CLK1	BC031549.1	57.3	Splicing		Other	DNASU
CLK2	BC014067.2	60.0	Splicing		Other	DNASU
CLK3	BC019881.1	58.6	Splicing	Baltz/Castello	Other	DFCI
CLK3	BC002555.2	58.6	Splicing	Baltz/Castello	Other	DFCI
CLP1	BC000446.1	47.6	Splicing		Other	DNASU
CMSS1	BC006475.1	31.8	Other		Other	DFCI
CMBP	BC000288.2	18.7	Other	Baltz/Castello	zf-CCHC	DFCI
CNBP	BC014911.1	18.7	Other	Baltz/Castello	zf-CCHC	DFCI
CNOT1			Stability	Baltz/Castello	Other	In-house
CNOT10	BC002928.2	79.4	Stability		Other	DNASU
CNOT10	BC002931.2	82.3	Stability		Other	DNASU
CNOT2	BC011826	59.7	Stability		Other	DNASU
CNOT3	BC016474	81.9	Stability		Other	DNASU
CNOT4	BC035590.1	63.1	Stability	Baltz	RRM	DFCI
CNOT6	NM_015455.3	63.3	Stability		Other	DNASU
CNOT6	BC150174	10.3	Stability		Other	DNASU
CNOT6L	NM_144571	63.0	Stability		Other	DNASU
CNOT6L	BC152816	63.0	Stability		Other	DNASU
CNOT7	BC007315.2	28.4	Stability		Other	DFCI
CNOT7	BC060852.1	32.7	Stability		Other	DFCI
CNOT8	BC017366.2	33.5	Stability		Other	DFCI
COA6	BC116455.1	14.1	Other		Other	DFCI
CORO1A	BC126385.1, BC126387.1	51.0	Other		Other	DFCI
CPEB1	BC036348.1	53.6	Translation		RRM	DNASU
CPEB2	BC103939.1	61.3	Translation	Baltz/Castello	RRM	DFCI
CPEB4	BC036899.1	36.2	Other	Baltz/Castello	RRM	DFCI
CPEB4	BC117150	80.2	Other	Baltz/Castello	RRM	In-house
CPNE3	BC015734.1	5.0	Other	Castello	Other	DFCI
CPSF1	BC017232	160.9	Localization		Other	DNASU
CPSF2	BC070095.1	88.5	Localization		Other	DNASU

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
CPSF3	BC011654	77.4	Localization		Other	DNASU
CPSF3L	AM393218	44.1	Other		Other	DNASU
CPSF4	BC050738.1	27.4	Modification	Baltz	ZnF-CCCH	DNASU
CPSF4	BC003101.1	27.5	Modification	Baltz	ZnF-CCCH	DNASU
CPSF4L	BC157870.1	20.7	Other		ZnF-CCCH	DNASU
CPSF5	BC001403	26.2	Other		Other	DNASU
CPSF5	BX537360	26.2	Other		Other	DNASU
CPSF6	BC005000.1	52.3	Other	Baltz/Castello	RRM	DFCI
CPSF6	BC000714.2	63.5	Other	Baltz/Castello	RRM	DFCI
CPSF7	BC018135.1	52.0	Splicing	Baltz/Castello	RRM	DFCI
CRABP2		15.6	Other		Other	DNASU
CRKRS	NM_016507	164.2	Other		Other	DNASU
CSNK1G2		47.5	Other		Other	In-house
CSTF1	BC001011	48.4	Splicing	Baltz/Castello	Other	DNASU
CSTF2	BC017712	61.0	Splicing	Baltz/Castello	RRM	DNASU
CSTF2T	BC028239.1	64.5	Other	Baltz/Castello	RRM	DFCI
CSTF3	HQ447685	12.1	Splicing	Baltz	Other	DNASU
CTNNA1	BC031262.1	59.5	Other	Castello	Other	DFCI
CUGBP1	BC031079	51.6	Other		Other	DNASU
CWC15	BC040946.1	26.6	Splicing	Castello	Other	DFCI
DARS	BC000629.2	57.1	Translation	Castello	tRNA	DFCI
DAZ2	BC113006	60.4	Other		RRM	In-house
DAZ3	BC113005.1	49.5	Other		RRM	DFCI
DAZ4	BC047480.1, BC047617.1	44.1	Other		RRM	DFCI
DAZAP1	BC012062.1	43.4	Other	Baltz/Castello	RPM	DFCI
DBR1	BC009472.1	61.6	Splicing	Castello	Other	DFCI
DCD	BC062682.1	11.3	Other		Other	DFCI
DCN	BC005322.1	39.7	Other		Other	DFCI
DCP1A	BC007439.2	63.3	Other		Other	DNASU
DCP2	BC064593.1	44.4	Stability		Other	DNASU
DDX1	BC012132.1	82.4	Translation	Baltz/Castello	DEAD	DFCI
DDX1	BC012739.2	77.9	Translation	Baltz/Castello	DEAD	DFCI
DDX11	BC011264.1	108.3	Other		Other	DNASU
DDX11	BC111733.1	101.6	Other		Other	DNASU
DDX17	BC000595.2	72.4	Other	Baltz/Castello	DEAD	DFCI
DDX18	BC001238.1, BC003360.1, BC024739.1	75.4	Other	Baltz/Castello	DEAD	DFCI
DDX19A	BC005162.2, BC006544.2	54.0	Localization		DEAD	DFCI
DDX19B	BC010008.2	41.8	Localization		DEAD	DFCI
DDX19B	BC003626.2	53.9	Localization		DEAD	DFCI
DDX20	BC031062.1	92.2	Other		DEAD	DFCI
DDX20	BC011566	92.2	Other		DEAD	In-house
DDX21	BC004182	79.7	Other	Baltz/Castello	DEAD	In-house
DDX21	BC008071.2	87.3	Other	Baltz/Castello	DEAD	DNASU
DDX23	BC002366.2	95.6	Splicing	Baltz	DEAD	DFCI
DDX24	BC008847	96.3	Other	Baltz/Castello	DEAD	DNASU
DDX27	HQ253546	86.6	Other	Baltz/Castello	DEAD	DNASU
DDX27	HQ258508	89.6	Other	Baltz/Castello	DEAD	DNASU
DDX28	BC024273.1	59.6	Other	Baltz/Castello	DEAD	DFCI
DDX31	BC012726	75.5	Other	Baltz/Castello	DEAD	In-house
DDX31	BC158832 (NM_138620.1)	64.1	Other	Baltz/Castello	DEAD	DNASU
DPX39A	BC032128.2	36.6	Localization	Castello	DEAD	DFCI
DDX39A	BC001009.2	49.1	Localization	Castello	DEAD	DFCI
DDX39A	BC010455.2	35.1	Localization	Castello	DEAD	DFCI
DDX41	BC015476.1	69.8	Splicing	Baltz/Castello	DEAD	DFCI
DDX43	BC066938.1	72.9	Other		KH	DFCI
DDX47	BC009379.2	36.7	Splicing	Baltz/Castello	DEAD	DFCI
DDX49	BC002674.2	54.2	Other	Castello	DEAD	DFCI
DDX5	BC016027	69.1	Splicing	Baltz/Castello	DEAD	DNASU
DDX50	BC000272.1	82.6	Other	Baltz/Castello	DEAD	DFCI
DDX51	notBC040185	11.9	Other	Baltz/Castello	DEAD	In-house
DDX52	BC041785.1	67.5	Other	Baltz/Castello	DEAD	DFCI
DDX53	BC067878.1	71.2	Other		KH	DFCI
DDX54	BC001132.2	25.5	Other	Baltz/Castello	DEAD	DFCI
DDX55	BC035911.1	24.3	Other	Castello	DEAD	DFCI
DDX55	BC030020.2	68.5	Other	Castello	DEAD	DFCI
DDX56	BC001235	61.7	Other	Castello	DEAD	DNASU
DDX59	BC041801.1	68.9	Other		DEAD	DFCI
DDX6	BC085007.1	54.4	Stability	Baltz/Castello	DEAD	DFCI
DDX60	BC020601.1	20.8	Other		DEAD	DFCI
DGCR8	BC009323.2	55.6	Other		dsRSD	DFCI

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
DHX18	BC008825.2, BC009392.2	119.4	Splicing	Baltz/Castello	DEAD	DFCI
DHX29	BC056219.1	155.3	Translation	Baltz	DEAD	DFCI
DHX30	BC014237.2	56.5	Other	Baltz/Castello	dsRBD	DFCI
DHX32	DQ895902	84.4	Other		Other	DNASU
DHX33	BC042040.1	54.6	Other	Castello	DEAD	DFCI
DHX34	BC172389	128.1	Other	Baltz	DEAD	DNASU
DHX35	HQ258480	78.9	Splicing		DEAD	DNASU
DHX36	BC036035.1	111.5	Other	Baltz/Castello	DEAD	DFCI
DHX37	BC004463.1	3.9	Other		DEAD	DNASU
DHX38	BC004235	140.5	Localization	Baltz	DEAD	DNASU
DHX40	BC024187.2	88.6	Other		DEAD	DNASU
DHX57	BC060778.1	63.2	Other	Baltz/Castello	DEAD	DFCI
DHX58	BC014949	76.6	Other		Other	In-house
DHX58	JF432115	76.6	Other		Other	DNASU
DHX8	BC044586.1	138.8	Splicing	Baltz/Castello	DEAD	DFCI
DIAPH1	BC007411	141.4	Other	Castello	Other	DNASU
DICER1	BC150287.1	218.7	Other		PIWI/PAZ	DFCI
DIEXF	BC022964.1	87.1	Other	Castello	Other	DFCI
DIMT1	BC010874.2	35.2	Modification		Other	DFCI
DIS3	BC038101.1	30.8	Stability		Other	DNASU
DIS3L	BC022089.2	111.1	Other		Other	DNASU
DKC1	BC010015.2	57.7	Modification	Castello	Other	DFCI
DMGDH	BC156312	96.8	Other	Castello	Other	DNASU
DMPK	BC062553	69.4	Other		Other	DNASU
DNAJC17	BC000048.2	34.7	Other		RRM	DFCI
DNAJC2	BC056682.1	19.0	Other	Castello	Other	DFCI
DNAJC5	BC053642.1	22.1	Other		Other	DFCI
DND1	BC033496.1	38.7	Other		RRM	DFCI
DNTTIP2	BC130622.1	84.5	Other	Baltz/Castello	Other	DFCI
DPPA5	BC137549.1	13.5	Other		Other	DFCI
DUS2L	BC006527.2	55.0	Other		Other	DFCI
DUT	BC033645.1	17.7	Other	Castello	Other	DFCI
DYNC1H1	BC064521.1	22.2	Other	Baltz/Castello	Other	DFCI
DYNC1LI1	BC131620.1	58.6	Other	Castello	Other	DFCI
DZIP3	BC083882.1	138.6	Other	Castello	Zn-Finger	DFCI
EBNA1BP2	BC009175.2	34.9	Other	Baltz/Castello	Other	DFCI
EDC3	BC011534.1, BC021271.2	56.1	Stability		LSM	DNASU
EDF1	BC015500.1	16.4	Other	Baltz/Castello	Other	DFCI
EEF1A1	BC008557.1, BC009733.1, BC009875.2, BC010735.1, BC012891.1, BC014224.2, BC018150.1, BC018641.2, BC010669.1, BC021686.1, BC028674.1, BC038339.1, BC066893.1	50.1	Translation	Baltz/Castello	Other	DFCI
EEF1A1	notBC131708	50.1	Translation	Baltz/Castello	Other	In-house
EEF2	BC126259.1	95.3	Translation	Baltz/Castello	Other	DFCI
EFTUD2	BC002360.2	109.5	Splicing	Baltz/Castello	Other	DFCI
EIF2AK2	BC101475.1	62.1	Translation	Baltz	dsRBD	DFCI
EIF2C1	BC083275.1	97.2	Other	Baltz	Other	DFCI
EIF2C2	BC007633.1	42.4	Other	Baltz/Castello	Other	DFCI
EIF2C2	BC018727.1	66.3	Other	Baltz/Castello	Other	DFCI
EIF2C3	BC066888.1	25.6	Other	Baltz/Castello	Other	DFCI
EIF2S2	BC000934.2	38.4	Translation	Baltz/Castello	Other	DFCI
EIF2S2	BC000461.2	38.4	Translation	Baltz/Castello	Other	DFCI
EIF3C	BC001571.1	105.3	Translation	Baltz/Castello	Other	DFCI
EIF3D	BC080515.1	64.0	Translation	Baltz/Castello	Other	DFCI
EIF3G	BC000733.2	35.6	Translation	Baltz/Castello	RRM	DFCI
EIF3H	BC000386.2	39.9	Translation	Castello	Other	DFCI
EIF3L	BC001101.2, BC007510.2	66.7	Translation	Baltz/Castello	Other	DFCI
EIF4A1	BC009585.1	46.2	Stability	Baltz/Castello	DEAD	DFCI
EIF4A2	BC015842.1	46.4	Stability	Baltz/Castello	DEAD	DFCI
EIF4A2	BC012547	46.5	Stability	Baltz/Castello	DEAD	In-house
EIF4A2	BC016295	21.0	Stability	Baltz/Castello	DEAD	In-house

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
EIF4A2	BC048105	46.5	Stability	Baltz/Castello	DEAD	In-house
EIF4A3	BC004386.1, BC011151.1	46.9	Stability	Baltz/Castello	DEAD	DFCI
EIF4A3	BC003662	46.9	Stability	Baltz/Castello	DEAD	In-house
EIF48	BC073139.1	69.2	Stability	Baltz/Castello	RRM	DFCI
EIF4H	BC021214.2, BC066928.1	25.2	Translation	Baltz/Castello	RRM	DFCI
EIF5B	BC032639.1	138.8	Translation	Baltz/Castello	Other	DFCI
ELAC2	BC001939.1	92.2	Other	Baltz/Castello	Other	DFCI
ELAVL1	BC003376	36.1	Stability	Baltz/Castello	RRM	In-house
ELAVL2	BC030692.1	38.0	Other	Baltz/Castello	RRM	DFCI
ELAVL3	BC014144	39.5	Other		RRM	In-house
ELAVL4	BC036071.1	40.4	Other		RRM	DFCI
ELMOD3	BC001942.1	44.3	Other		Other	DNASU
EMG1	BC055314.1	26.7	Other	Baltz/Castello	Other	DFCI
ENOX1	BC024178	73.3	Other		RRM	In-house
ENOX2	BC019254.1	36.9	Other		RRM	DFCI
ERAL1	BC019094.2	48.3	Other	Baltz/Castello	KH	DFCI
ERCC3	BC008820.2	89.3	Other		Other	DFCI
ERI3	BC001072.2	14.5	Other	Baltz/Castello	Other	DFCI
ESRP1	BC067098.1	75.6	Splicing		RRM	DFCI
EWSR1	BC000527	37.8	Other	Baltz/Castello	RRM	In-house
EWSR1	BC004817	68.5	Other	Baltz/Castello	RRM	In-house
EWSR1	BC011048	68.4	Other	Baltz/Castello	RRM	In-house
EWSR1	BC072442	68.4	Other	Baltz/Castello	RRM	In-house
EXOSC1	BC022067.2	21.5	Stability		Other	DNASU
EXOSC10	BC073788.1	100.8	Stability	Baltz/Castello	Other	DNASU
EXOSC2	BC000747	32.9	Stability		Other	DNASU
EXOSC3	BC008880.2	29.6	Stability		Other	DNASU
EXOSC3	BC002437.2	29.6	Stability		Other	DNASU
EXOSC4	BC002777.2	26.4	Stability		Other	DNASU
EXOSC5	BC007742.2	25.3			Other	DNASU
EXOSC7	BC012831.2	31.8	Stability		Other	DNASU
EXOSC8	BC020773.1	30.0	Stability		Other	DNASU
EXOSC9		48.9	Stability	Castello	Other	DNASU
EZR	BC013903.2	89.4	Other	Baltz/Castello	Other	DFCI
FAM120A	BC111736.1	121.9	Other	Baltz/Castello	Other	DFCI
FAM120A	BC098584.1	121.9	Other	Baltz/Castello	Other	DFCI
FAM208A	BC129986.1	125.4	Other		Other	DFCI
FAM32A	BC090639.1, BC017286.1	13.2	Other	Castello	Other	DFCI
FAM46A	BC000683.2	49.7	Other	Castello	Other	DFCI
FAM98A	CCSB53266.1	55.3	Other	Baltz/Castello	Other	DFCI
FAM98A	BC060860.1	55.2	Other	Baltz/Castello	Other	DFCI
FANCM	BC036056.1	75.6	Other		Other	DFCI
FASN	BC007909.1	48.3	Other	Baltz/Castello	Other	DFCI
FASTK	BC011770.2	61.1	Splicing		Other	DFCI
FASTKD1	BC032687.2	77.2	Other	Baltz/Castello	Other	DFCI
FASTKD2	BC001544.1	81.5	Other	Baltz/Castello	Other	DFCI
FASTKD3	BC113563.1	75.7	Other	Castello	Other	DFCI
FASTKD5	BC007413.2	88.8	Other	Baltz	Other	DFCI
FBL	BC019260.1	33.8	Modification	Baltz/Castello	Other	DFCI
FCF1	BC080600.1	12.8	Other	Castello	Other	DFCI
FDPS	BC010004.2	48.3	Other	Castello	Other	DFCI
FGF17	BC113489.1	24.9	Other		Other	DFCI
FGF17		24.9	Other		Other	DNASU
FGF19	BC017664.1	24.0	Other		Other	DFCI
FGF19	JF432499	24.0	Other		Other	DNASU
FIP1L1	AL136310	58.4	Other	Baltz/Castello	Other	DNASU
FKBP3	BC016288.1, BC020809.1	25.2	Other	Castello	Other	DFCI
FKBP4	BC001786.1, BC007924.2	51.8	Other	Castello	Other	DFCI
FLYWCH2	BC014089.2	14.6	Other	Castello	Other	DFCI
FMR1	BC038998	34.1	Localization	Baltz/Castello	KH	In-house
FNDC3B	BC012204.1	7.3	Other	Castello	Other	DFCI
FRG1	BC053397.1	29.2	Splicing	Castello	Other	DFCI
FSCN1	BC000521.2, BC007348.2	54.5	Other	Castello	Other	DFCI
FTO	NM_001080432	58.3	Modification		Other	DNASU
FTSJ3	BC000131.1	65.7	Other	Baltz/Castello	Other	DFCI
FUBP1	BC017247	68.7	Other	Baltz/Castello	KH	DNASU
FUBP3	BC007874.2	28.5	Other	Baltz/Castello	KH	DFCI
FUS	BC000402.2, BC082459.1	53.4	Splicing	Baltz/Castello	RRM	DFCI

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
FUSIP1	BC010074	21.0	Other		Other	In-house
FXR2	BC020090.1	74.2	Other	Baltz/Castello	KH	DFCI
FXR2	BC051907.1	74.2	Other	Baltz/Castello	KH	DFCI
FZD10	BC074997.2	66.3	Other		Other	DFCI
FZD3	NM_017412	76.3	Other		Other	DNASU
FZD4	BC114527.1	59.9	Other		Other	DFCI
FZD4		59.9	Other		Other	DNASU
FZD7	BC015915.1	63.8	Other		Other	DFCI
FZD8	BC111845	73.3	Other		Other	DNASU
FZD9	BC026333	64.5	Other		Other	DNASU
G3BP1	BC000997.1	52.2	Other	Baltz/Castello	RRM	DFCI
G3BP2	BC011731.2	50.8	Localization	Baltz/Castello	RRM	DFCI
GANAB	BC065266.1	96.2	Other	Castello	Other	DFCI
GAFDH	BC001601.1, BC004109.2, BC009081.1, BC013310.2, BC023632.2, BC025925.1, BC026907.1, BC029618.1	36.1	Other		Other	DNASU
GAR1	BC003413.1	22.3	Other	Baltz/Castello	Other	DFCI
GFM1	BC049210.1	83.5	Translation	Castello	Other	DFCI
GLE1	BC030012.1	79.9	Localization		Other	DNASU
GLRX3	BC014372.1	21.5	Other	Castello	Other	DFCI
GLRX3	BC005289.1	37.4	Other	Castello	Other	DFCI
GLTSCR2	BC006311.2, BC010095.2	54.4	Other	Baltz/Castello	Other	DFCI
GNB2L1	BC014788.1	35.1	Other	Baltz/Castello	Other	DFCI
GNL2	BC000107.2	83.7	Other	Baltz/Castello	Other	DFCI
GNL2	BC009250.2	83.7	Other	Baltz/Castello	Other	DFCI
GNL3	BC001024.2	62.0	Other	Baltz/Castello	Other	DFCI
GNL3L	BC011720.2	65.6	Other	Castello	Other	DFCI
GPANK1	BC008783.1	39.3	Other		Other	DFCI
GPATCH2	BC063474.1	42.6	Other		Other	DFCI
GPATCH4	BC056904.1	50.4	Other	Castello	G-patch	DFCI
GPKOW	BC090397.2	52.2	Other		G-patch	DPCl
GRB2	BC000631.2	25.2	Other	Castello	Other	DFCI
GRN	BC000324.2	47.0	Other	Castello	Other	DFCI
GRN	BC010577.2	63.5	Other	Castello	Other	DFCI
GSPT1	BC009503.2	68.4	Stability	Baltz/Castello	Other	DFCI
GSPT2	BC036077.1	69.0	Stability	Baltz/Castello	Other	DFCI
GTF2E2	BC030572.2	33.0	Other	Castello	Other	DFCI
GTF2F1	BC000120.1	58.2	Splicing	Baltz/Castello	Other	DFCI
GTPBP10	BC021573.1	39.7	Other	Castello	Other	DFCI
GTPSP4	BC038975.2	74.0	Other	Baltz/Castello	Other	DFCI
GTSF1	BC021179.1	19.2	Other		Other	DFCI
GTSF1L	BC040049.1	16.9	Other		Other	DFCI
HADHB	BC014572.1	51.4	Other		Other	DFCI
HADHB	BC017554.2, BC030824.1, BC066963.1	51.4	Other		Other	DFCI
HDGF	BC018991.1	26.8	Other	Castello	Other	DFCI
HEATR1	BC062442.1	13.5	Other	Baltz/Castello	Other	DFCI
HEATR1	BC011983.1	39.9	Other	Baltz/Castello	Other	DFCI
HELQ	BC011863.2	30.5	Other		Other	DFCI
HERC5	BC140716.1	116.8	Other	Castello	Other	DFCI
HFM1	BC132823.1	53.6	Other		Other	DFCI
HIST1H1C	BC002649.1	21.4	Other	Baltz/Castello	Other	DFCI
HIST1H4H	BC120939.2	11.4	Other	Castello	Other	DFCI
HMGB1	BC003378.1	24.9	Other	Baltz/Castello	Other	DFCI
HMGB2	BC001063.2	24.0	Other	Baltz/Castello	Other	DFCI
HNRNPA0	hnRNPA0	30.8	Other		RRM	Promega
HNRNPA1	BC002335.2, BC009800.1, BC812158.1, BC033714.1	34.2	Localization	Baltz/Castello	RRM	DFCI
HNRNPA1	hnRNPA1	34.2	Other		RRM	Promega
HNRNPA2B1	BC000506.2	28.4	Localization	Baltz/Castello	RRM	DFCI
HNRNPC	BC008423.1	33.6	Splicing	Baltz/Castello	RRM	DFCI
HNRNPC	BC003394	32.3	Splicing	Baltz/Castello	RRM	In-house
HNRNPC	BC008364	32.4	Splicing	Baltz/Castello	RRM	In-house
HNRNPC1/2	hnRNPC1/2	33.7	Other		Other	Promega
HNRNPCL1	BC137258.1	32.1	Other	Baltz	RRM	DFCI
HNRNPD	BC002401.1	38.4	Stability	Baltz/Castello	RRM	DFCI

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
HNRNPD0	hnRNPD0	32.8	Other		Other	Promega
HNRNPE1	hnRNPE1	37.5	Other		Other	Promega
HNRNPE2	hnRNPE2	38.2	Other		Other	Promega
HNRNPF	BC004254.1	45.7	Splicing	Baltz/Castello	RRM	DFCI
HNRNPF	BC001432	45.7	Splicing	Baltz/Castello	RRM	
HNRNPF	BC016736	45.7	Splicing	Baltz/Castello	RRM	In-house
HNRNPF	hnRNPF	45.7	Other		RRM	Promega
HNRNPH	hnRNPH	51.2	Other		Other	Promega
HNRNPH1	BC001348.2	49.2	Splicing	Baltz/Castello	RRM	DFCI
HNRNPH2	BC130345.1	49.3	Splicing	Baltz/Castello	RRM	DFCI
HNRNPH2			Splicing	Baltz/Castello	RRM	In-house
HNRNPI	hnRMPI	57.2	Other		Other	In-house
HNRNPK	BC000355.2	51.0	Splicing	Baltz/Castello	KH	DFCI
HNRNPK	BC014980	51.0	Splicing	Baltz/Castello	KH	In-house
HNRNPK	hnRNPK	48.6	Other		KH	Promega
HNRNPL	hnRNPL		Other		RRM	Promega
HNRNPM	BC000138	77.5	Splicing	Baltz/Castello	RRM	In-house
HNRNPP2	hnRNPP2	53.4	Other		Other	Promega
HNRNPQ	hnRNPQ	69.6	Other		Other	Promega
HNRNPR	BC001449.2	71.2	Splicing	Baltz/Castello	RRM	DFCI
HNRNPR	hnRNPR	70.9	Other		RRM	Promega
HNRNPU	BC003367	89.0	Stability	Baltz/Castello	Other	In-house
HNRNPU	hnRNPU	89.0	Other		Other	In-house
HNRNPUL1	BC027713.2	90.3	Splicing	Baltz/Castello	Other	DFCI
HNRNPUL1	hnRNPUL1	95.7	Other		Other	Promega
HNRNPUL2	NM_001079559.1	85.1	Other	Baltz/Castello	Other	DNASU
HNRPDL	BC007392.2	33.6	Other	Castello	Other	DFCI
HNRPLL	BC008217.1	30.8	Other	Baltz/Castello	Other	DFCI
HNRPLL	BC017480	80.1	Other	Baltz/Castello	Other	In-house
HRSP12	BC010280.1, BC012592.1	14.5	Translation	Castello	Other	DFCI
HSP90AA1	BC121062.2	84.7	Other	Baltz/Castello	Other	DFCI
HSP90AB1	BC004926.1, BC012807.2	83.3	Other	Baltz/Castello	Other	DFCI
HSPA8	BC007276.1	64.6	Other	Baltz/Castello	Other	DFCI
HSPA8	BC018179.1, BC016680.1	70.9	Other	Baltz/Castello	Other	DFCI
HSPA9	BC000478.2	73.7	Other	Baltz/Castello	Other	DFCI
HSPB1	BC073768, BC000510	22.9	Other	Castello	Other	DNASU
HSPD1	BC003030.1	61.1	Other	Castello	Other	DFCI
HSPD1	BC002676.2	61.1	Other	Castello	Other	DFCI
HTATSF1	BC009896.2	85.9	Other	Castello	RRM	DFCI
HTATSF1-DPF3	nolBC009896	85.9	Other		Other	In-house
HYPE	BC001342	50.8	Other		Other	In-house
IFI16	BC017059.1	82.0	Other	Castello	Other	DFCI
IFIH1	BC111750.1	116.7	Other		Other	DFCI
IFIH1	BC046206.1	25.1	Other		Other	DFCI
IFIT2	BC032839.2	56.2	Other	Castello	Other	DFCI
IGF2BP1	NM_006546.3	63.5	Stability	Baltz/Castello	RRM	DNASU
IGF2BP2	BC021290.2	66.0	Translation	Baltz/Castello	RRM	DFCI
IGF2BP3	BC065269.1	63.7	Translation	Baltz/Castello	RRM	DFCI
IGF2BP3	BC051296.1	11.3	Translation	Baltz/Castello	RRM	DFCI
IGFBP6		25.3	Other		Other	In-house
ILF2	BC000382.2	43.1	Other	Baltz/Castello	Zn-Finger	DFCI
ILF3	BC064838.1	76.5	Other	Baltz/Castello	dsRBD	DFCI
ILF3	BC003086.1	17.8	Other	Baltz/Castello	dsRBD	DFCI
INTS6	BC039829.1	100.4	Other		Other	DNASU
ISY1	BC004442.1, BC019849.1	33.0	Splicing	Castello	Other	DFCI
KHDRBS2	BC034043.1	38.9	Other		KH	DFCI
KHDRBS3	BC032606	38.8	Other	Baltz	KH	In-house
KIAA0020	BC016137.2	73.8	Other	Baltz/Castello	Other	DFCI
KIAA1324	BC125208.1	102.0	Other		Other	DFCI
KIAA1967	BC018269.1	40.8	Other	Baltz/Castello	Other	DFCI
KIF1C	BC034993.1	122.9	Other	Baltz/Castello	Other	DFCI
KIF1C	BC111736.1	121.9	Other	Baltz/Castello	Other	DNASU
KLKL3	BC034035	34.1	Other		Other	In-house
KRR1	BC026107.2	43.8	Other	Baltz/Castello	KH	DFCI
KRR1	BC033867.2	43.8	Other	Baltz/Castello	KH	DFCI
KRR1	BC016778.1	43.8	Other	Baltz/Castello	KH	DFCI
KRT18	BC000698.2	48.0	Other	Castello	Other	DFCI
LARP1	BC001460.2	116.5	Other	Baltz/Castello	Other	DFCI
LARP1	BC010144	5.8	Other	Baltz/Castello	Other	In-house
LARP1	BC033856	32.9	Other	Baltz/Castello	Other	In-house

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
LARP1B	BC030516.1	24.1	Other	Baltz	Other	DFCI
LARP1B	BC062606.1	29.0	Other	Baltz	Other	DFCI
LARP4	BC022377.1	21.5	Other	Baltz/Castello	RRM	DFCI
LARP4	BC083479.1	42.0	Other	Baltz/Castello	RRM	DFCI
LARP4B	BC131630.1	80.6	Translation	Baltz/Castello	RRM	DFCI
LARP8	BC006082.1, BC009446.1, BC014018.2	54.7	Translation		Other	DFCI
LARP7	BC066945.1	86.9	Other	Baltz/Castello	RRM	DFCI
LGALS1	BC001693.1, BC020675.1	14.7	Other	Castello	Other	DFCI
LGALS3	BC053667.1	26.2	Other	Castello	Other	DFCI
LIN28A	BC028566.2	22.7	Translation		zf-CCHC	DFCI
LIN28B	BC137526.1	27.1	Other	Baltz	zf-CCHC	DFCI
LLPH	BC006002.1	15.2	Other	Baltz/Castello	Other	DFCI
LSM1	BC001767.1	15.2	Stability	Baltz/Castello	LSM	DFCI
LSM10	BC007623.1	14.1	Splicing		LSM	DFCI
LSM11	BC126449	39.5	Other		LSM	In-house
LSM2	BC009192.2	10.8	Stability	Baltz/Castello	LSM	DFCI
LSM3	BC007055.1	11.8	Stabihty	Baltz/Castello	LSM	DFCI
LSM4	BC000387.2, BC003652.2, BC022198.2, BC023665.2	15.3	Stability	Baltz/Castello	LSM	DFCI
LSM5	BC005938.1	9.9	Stability		LSM	DFCI
LSM6	BC018026.1	9.1	Stability	Baltz	LSM	DFCI
LSM7	BC018621.1	11.8	Stability		LSM	DFCI
LSMD1	BC033861.1	18.7	Other		LSM	DFCI
LSMD1	BC059944.1	13.5	Other		LSM	DFCI
LUC7L	HQ448098	41.9	Other		Other	DNASU
LUC7L2	BC017163.2, BC050708.2, BC056886.1	46.5	Other	Baltz/Castello	Other	DFCI
LUC7L3	BC056409.1	9.2	Splicing	Baltz/Castello	Other	DFCI
LYAR	BC015796.2	43.6	Other	Baltz/Castello	Other	DFCI
MAGOHB	BC010905	17.3	Localization		Other	In-house
MAK16	BC050528.1	35.4	Other	Castello	Ribosomal	DFCI
MAP4	BC008715.2, BC012794.2	102.9	Other	Baltz/Castello	Other	DFCI
MATR3	BC015031	94.6	Other	Baltz/Castello	RRM	DNASU
MA2	BC041629.1	28.6	Other	Baltz/Castello	Zn-Finger	DFCI
MBNL1	BC043493.1	41.0	Splicing	Baltz/Castello	ZnF-CCCH	DFCI
MBNL2	BC104040.1	39.3	Splicing	Baltz/Castello	ZnF-CCCH	DFCI
MDH2	BC001917.1	35.5	Other	Baltz/Castello	Other	DFCI
MECP2	BC011612.1	52.4	Other	Castello	Other	DFCI
MBPCE	BC000556.2, BC018396.1	25.0	Modification	Baltz/Castello	Other	DFCI
METYL16	BC050603.1	63.6	Other	Baltz	Other	DFCI
METTL3	BC052244	64.4	Modification		Other	DNASU
MEX3C	NM_018626	89.4	Other	Baltz/Castello	KH	DNASU
MFAP1	BC023557.2, BC050742.1	52.0	Other	Baltz/Castello	Other	DFCI
MKI67IP	BC022990.1	34.2	Other	Baltz/Castello	Other	DFCI
MKI67IP	BC024238.2	34.3	Other	Baltz/Castello	Other	DFCI
MOV10	BC002548.1, BC009312.2	113.7	Other	Baltz/Castello	Other	DFCI
MPHOSPH6	BC031017.1	19.0	Other		Other	DNASU
MRM1	BC072411.1	38.6	Other	Baltz/Castello	Other	DFCI
MRPL1	BC014356.1, BC032595.1	34.5	Translation	Baltz/Castello	Ribosomal	DFCI
MRPL11	BC005002.1	20.7	Translation	Baltz/Castello	Ribosomal	DFCI
MRPL13	BC009190.2, BC021744.2	20.7	Translation	Baltz/Castello	Ribosomal	DFCI
MRPL3	BC003375.2	38.6	Translation	Baltz/Castello	Ribosomal	DFCI
MRPL30	BC022391.1	18.5	Other		Ribosomal	DFCI
MRPL32	BC013147.1	21.4	Translation	Castello	Ribosomal	DFCI
MRPL37	BC000041.2	48.1	Translation	Castello	Other	DFCI
MRPL39	BC004896.2	38.2	Other	Castello	Other	DFCI
MRPL4	BC009856.2	34.9	Translation	Baltz/Castello	Ribosomal	DFCI
MRPL41	BC040035.1	15.4	Translation	Baltz/Castello	Other	DFCI
MRPL42	BC040240.2	18.7	Translation	Castello	Other	DFCI
MRPL43	BC041165.1	23.4	Translation	Castello	Other	DFCI
MRPL45	BC006235.2	28.8	Translation	Baltz/Castello	Other	DFCI
MRPL45	BC130382.1, BC130384.1	35.4	Translation	Baltz/Castello	Other	DFCI

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
MRPS11	BC012489.1	20.5	Translation	Baltz/Castello	Ribosomal	DFCI
MRPS11	BC032378.1	20.6	Translation	Baltz/Castello	Ribosomal	DFCI
MRPS15	BC031336.1	29.8	Translation	Castello	Ribosomal	DFCI
MRPS23	BC000242.1	21.8	Translation	Castello	Other	DFCI
MRPS24	BC012167.1	19.0	Translation	Baltz/Castello	Other	DFCI
MRPS24	BC054865.1	19.0	Translation	Baltz/Castello	Other	DFCI
MRPS30	BC007735.2	50.4	Translation	Castello	Other	DFCI
MRPS31	BC022045.1	45.3	Other	Baltz/Castello	Other	DFCI
MRPS35	BC015862.1	26.4	Other	Baltz	Other	DFCI
MRPS5	BC014172.2	48.0	Translation	Baltz/Castello	Ribosomal	DFCI
MRPS7	BC000241.1	28.2	Translation	Baltz/Castello	Ribosomal	DFCI
MRTO4	BC003013.1	27.6	Other	Baltz/Castello	Ribosomal	DFCI
MSI2	BC017560.2	17.2	Other	Baltz/Castello	RRM	DFCI
MSI2	BC001526	35.2	Other	Baltz/Castello	RRM	In-house
MTDH	BC045642.1	63.3	Other	Baltz/Castello	Other	DFCI
MTPAP	BC061703.1	66.2	Other	Baltz/Castello	Other	DFCI
MUSK	GQ129313	86.4	Other		Other	DNASU
MYEF2	BC014533	22.4	Other	Baltz	RRM	In-house
NAA15	BC104806.1	101.3	Other	Castello	Other	DFCI
NANOS1	BC156179	30.2	Translation		Zn-Finger	DNASU
MANOS2	BC117484.1, BC117486.1	15.1	Translation		Zn-Finger	DFCI
NANOS3	BC101209.2	20.7	Translation		Zn-finger	DFCI
NAP1L3	BC034954	57.6	Other		Other	DNASU
NAT10	BC035558.1	115.7	Other	Baltz/Castello	tRNA	DFCI
NCL	BC002343	51.0	Other	Baltz/Castello	RRM	In-house
NDUFV3	BC033766.1	11.9	Other	Baltz/Castello	Other	DFCI
NDUFV3	BC021217.2	51.0	Other	Baltz/Castello	Other	DFCI
NGDN	BC030817.1	35.9	Translation	Baltz/Castello	Other	DFCI
NGRM	BC001682.2, BC007222.1, BC009389.2, BC017192.2	24.4	Other	Baltz/Castello	Other	DFCI
NHP2	BC000009.2, BC006387.2	17.2	Other	Castello	Ribosomal	DFCI
NHP2L1	BC019282.2	14.2	Splicing	Baltz/Castello	Ribosomal	DFCI
NIP7	BC015941.1	20.5	Other	Baltz/Castello	Other	DFCI
NKRF	BC047878.2	77.7	Other	Baltz/Castello	dsRBD	DFCI
NMD3	BC013317.1	57.6	Other	Castello	Other	DFCI
NOA1	BC004894.2	78.5	Translation		Other	DFCI
NOB1	BC064630.1	46.7	Other		Other	DFCI
NGC2L	BC003555.1	84.9	Other	Baltz/Castello	Other	DFCI
NOL10	BC005125.2	80.3	Other	Baltz/Castello	Other	DFCI
NOL12	BC002808.1	24.7	Other	Baltz/Castello	Other	DFCI
NOL7	BC023517.2	29.4	Other	Castello	Other	DFCI
NOL8	BC146810.1	123.8	Other	Baltz/Castello	RRM	DFCI
NOLC1	BC006769.2	44.2	Other	Baltz/Castello	Other	DFCI
NONO	BC010049.2	39.0	Splicing	Baltz/Castello	RRM	DFCI
NONO	BC002364.1, BC003129.1, BC012141.1, BC028299.1, BC069639.1	54.2	Splicing	Baltz/Castello	RRM	DFCI
NOP10	BC008866.2	7.7	Other		Other	DFCI
NOP16	BC040106.1	21.2	Other	Baltz/Castello	Other	DFCI
NOP16	BC032424.2	26.6	Other	Baltz/Castello	Other	DFCI
NOP2	BC106072.1	92.9	Other	Baltz/Castello	Other	DFCI
NOP56	BC004937.1	19.5	Other	Baltz/Castello	Other	DFCI
NOP58	BC032592.2	59.6	Other	Baltz/Castello	Other	DFCI
NOP9	BC025332.1	58.2	Other		Other	DFCI
NOSIP	BC011249.1	33.2	Other	Castello	Other	DFCI
NOSIP	BC009299.2, BC010077.2	33.2	Other	Castello	Other	DFCI
NOVA1	BC075038.2	51.7	Splicing	Baltz	KH	DFCI
NPM1	BC009623.2	29.5	Translation	Baltz/Castello	Other	DFCI
NPM1	BC002398.2, BC008495.1, BC014349.1, BC016716.1, BC018824.1, BC021668.1, BC050628.1	32.6	Translation	Baltz/Castello	Other	DFCI
NPM1	BC012566.1	32.6	Translation	Baltz/Castello	Other	DFCI
NPM3	BC054868.1	19.3	Other	Baltz/Castello	Other	DFCI
NR5A1	BC032501	51.6	Other		Other	In-house

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
NSA2	BC005288.1	30.1	Other	Baltz/Castello	Ribosomal	DFCI
NSUN2	BC001041.2	63.3	Modification	Baltz/Castello	Other	DFCI
NSUN2		63.3	Modification	Baltz/Castello	Other	DNASU
NSUN5	BC008084.2	50.4	Other	Baltz/Castello	Other	DFCI
NUDT16	BC031215.1	17.8	Other		Other	DNASU
NUDT16L1	BC006223.2	23.3	Other	Baltz	Other	DNASU
NUFIP2	BC129990.1	76.1	Other	Baltz/Castello	Other	DFCI
NUFIP2	BC108307.1	76.1	Other	Baltz/Castello	Other	DFCI
NUP35	BC047029.1, BC061896.1	34.8	Other		Other	DFCI
NUSAP1	BC010838.1	24.9	Other	Castello	Other	DFCI
NUSAP1	BC024772.1	49.2	Other	Castello	Other	DFCI
NVL	BC012105.1	72.7	Other	Castello	Other	DFCI
NXF1	BC004904.2, BC028041.1	70.2	Localization	Baltz/Castello	Other	DFCI
NXF2	BC015020.1	71.6	Localization		Other	DFCI
NXF3	BC031616.1	60.1	Localization		Other	DFCI
NXF5	BC131708.1	42.2	Localization		Other	DFCI
OASL	BC117406.1, BC117410.1	59.2	Other	Castello	Other	DFCI
P4HB	BC010859.1, BC029617.1	57.1	Other	Baltz/Castello	Other	DFCI
PA2G4	BC001951.1, BC007561.1, BC069786.1	43.8	Translation	Castello	Other	DFCI
PABPC1	BC015958	70.7	Stability	Baltz/Castello	RRM	DNASU
PABPC1P2	BC068242.1	29.9	Other		Other	DNASU
PABPC3	BC027617.2	70.0	Other		RRM	DFCI
PABPC5	BC063113.1	43.3	Other		RRM	DFCI
PABPN1L	BC148673	31.5	Other		RRM	DNASU
PAN3	BC128179.1	62.0	Stability		ZnF-CCCH	DNASU
PAPD5		63.3	Other		Other	In-house
PAPD7	HQ258305	59.9	Other		Other	DNASU
PAPOLA	BC000927.1	32.6	Splicing		Other	DFCI
PAPOLG	BC111701.1	82.8	Other		Other	DFCI
PARN	BC050029.1	73.5	Modification	Castello	Other	DFCI
PARP12	BC081541.1	79.1	Other	Castello	ZnF-CCCH	DFCI
PATL1	BC085264.1, BC109038.1	70.6	Stability	Baltz/Castello	Other	DFCI
PCBP1	BC039742.1	37.5	Splicing	Baltz/Castello	KH	DFCI
PCBP2	BC001155	38.2	Splicing	Baltz/Castello	KH	In-house
PCBP3	BC012061.1	33.3	Other	Castello	KH	DFCI
PCBP4	BC017098.1	37.1	Other	Castello	KH	DFCI
PCSK9	NM_174938.2	74.3	Other	Castello	Other	DNASU
PDIA3	BC038000.1	56.8	Other	Castello	Other	DFCI
PDIA3	BC014433.1	56.8	Other	Castello	Other	DFCI
PDIA4	BC000425.2	72.9	Other	Castello	Other	DFCI
PEG10	BC060659.2	37.0	Other	Baltz/Castello	Other	DFCI
PES1	BC032489.1	68.0	Other	Baltz/Castello	Other	DFCI
PHF5A	BC007321.2	12.4	Splicing	Baltz/Castello	Other	DFCI
PHF6	BC005994.1	35.3	Other	Castello	Other	DNASU
PINX1	BC015479.1	37.0	Other		G-patch	DFCI
PIWIL1	BC028581.2	93.5	Translation		PIWI/PAZ	DFCI
PIWIL2	BC025995.1	109.8	Translation		PIWI/PAZ	DFCI
PIWIL4	BC031080.1	96.6	Translation		PIWI/PAZ	DFCI
PKM	BC007640.1	57.9	Other		Other	DFCI
PLRG1	BC020786.1	56.3	Splicing		Other	DNASU
PNLDC1	BC112246.1	60.1	Other		Other	DFCI
PNN	BC062602.1	81.6	Stability	Baltz/Castello	Other	DFCI
PNO1	BC008304.1	27.9	Other	Baltz	KH	DFCI
PNRC2	BC001959.1	15.6	Stability		Other	DNASU
POLDIP3	BC019643.1	24.8	Translation	Baltz/Castello	RRM	DFCI
POLK	BC050718.1	64.1	Other		Other	DFCI
POLR2G	BC112162.1	19.3	Splicing		S1	DFCI
POLR3E	BC000285.1	74.8	Other		Other	DFCI
PORCN	BC895869	51.8	Other		Other	DNASU
POU5F1	BC117437.1	38.6	Other	Castello	Other	DFCI
PPAN	BC033202.1	53.2	Splicing	Baltz/Castello	Other	DFCI
PPAN	BC009833.2	53.2	Splicing	Baltz/Castello	Other	DFCI
PPAPDC1A	BC101268.2	23.5	Other		Other	DNASU
PPAPDC1A	BC132787.1	30.4	Other		Other	DNASU

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
PPAPDC1B	BC033025.1	26.2	Other		Other	DNASU
PPAPDC2	BC038108.2	32.3	Other		Other	DNASU
PPIA	BC000689.2, BC003026.1, BC005320.1, BC013915.1	18.0	Other	Baltz/Castello	Other	DFCI
PPIA	BC005982.1	18.0	Other	Baltz/Castello	Other	DFCI
PPIB	BC001125	23.7	Other	Baltz/Castello	Other	DNASU
PPIE	BC004898.2, BC008451.1	33.4	Splicing	Castello	RRM	DFCI
PPIG	BC111693.1	87.1	Other	Baltz/Castello	Other	DFCI
PPIG	BC001555.1	40.3	Other	Baltz/Castello	Other	DFCI
PPIL4	BC016984	23.1	Other	Baltz/Castello	RRM	In-hoase
PPIL4	BC018984.1	23.2	Other	Baltz/Castello	RRM	DFCI
PPIL4	BC020986.1	57.2	Other	Baltz/Castello	RRM	DFCI
PPP1CA	BC001888.1, BC004482.2, BC008010.1	37.6	Other		Other	DNASU
PPP1CB	AM392772	37.2	Other		Other	DNASU
PRKRA	BC008470.1	34.4	Other	Baltz	dsRBD	DFCI
PRMT1		39.4	Other	Baltz	Other	DNASU
PRPF18	BC000794	39.9	Splicing		other	DNASU
PRPF19	BC008719.2	55.2	Splicing		Other	DNASU
PRPF3	BC000184.2, BC001954.1	77.5	Splicing	Baltz	Other	DNASU
PRPF31	BC117389.1	55.5	Splicing	Baltz/Castello	Other	DFCI
PRPF38B	BC053838.1	21.1	Splicing	Castello	Other	DFCI
PRPF4	BC001588.2	58.4	Splicing		Other	DNASU
PRPF4	BC007424.2	58.3	Splicing		Other	DNASU
PRPF40A	BC027178.1	25.5	Splicing	Baltz	Other	DNASU
PRPF4B	BC034969	117.0	Splicing	Baltz	Other	DNASU
PRPF6	BC001666.2	108.9	Localization	Castello	Other	DFCI
PRPF8		273.6	Splicing	Baltz/Castello	RRM	DNASU
PRR3	BC126455.1	20.6	Other	Baltz/Castello	ZnF-CCCH	DFCI
PRR3	BC126457.1	20.7	Other	Baltz/Castello	ZnF-CCCH	DFCI
PRRC2B	BC012289.1	34.4	Other	Baltz/Castello	Other	DFCI
PSMA3	BC005265	28.4	Other		Other	In-house
PSMC1	BC000512.2	49.2	Other		Other	DFCI
PSMC1	BC016368.1	49.2	Other		Other	DFCI
PSMD4	BC002365.2	40.7	Other	Castello	Other	DFCI
PSPC1	BC014184.2	45.6	Other	Baltz/Castello	RRM	DFCI
PTBP1	BC004383.1	67.2	Splicing	Baltz/Castello	RPM	DFCI
PTBP1	BC002397	59.6	Splicing	Baltz/Castello	RRM	In-house
PTBP2	BC018582	67.6	Splicing	Baltz/Castello	RRM	In-house
PTBP3	BC044585.1	60.4	Splicing		RRM	DFCI
PTCD1	BC103495.1	78.9	Other	Baltz/Castello	Other	DFCI
PTCD2	BC018720.1	26.7	Other	Baltz/Castello	Other	DFCI
PTCD3	BC011832.2	63.1	Translation	Baltz/Castello	Other	DFCI
PTRF	BC065123.1	43.5	Other		Other	DFCI
PTRH1	BC047012.1	22.9	Other	Castello	tRNA	DFCI
PUF60	BC009734.1	54.0	Splicing	Baltz/Castello	RRM	DFCI
PUF60	BC011265.1, BC011879.1	55.7	Splicing	Baltz/Castello	RRM	DFCI
PUF60	BC008875.2	58.2	Splicing	Baltz/Castello	RRM	DFCI
PUM1	BC013398.2	126.5	Translation	Baltz/Castello	Pumilio	DFCI
PURB	BC101735.1	33.2	Other	Baltz/Castello	Other	DFCI
PURG	BC106708.2	39.6	Other	Baltz	Other	DFCI
PUS7	BC005209.2	29.8	Modification	Baltz/Castello	Other	DFCI
PUS7	BC011398	29.8	Modification		Other	In-house
PUS7L	BC068502.1	80.7	Modification		Other	DFCI
PUS7L	BC033621.2	80.7	Modification		Other	DFCI
PWP2	BC013309.2, BC014986.1	102.4	Other	Baltz/Castello	Other	DFCI
OARS	BC001772.1	69.8	Translation		tRNA	DFCI
OARS	BC016634.1	69.8	Translation		TRNA	DFCI
OKI	BC019917.2	37.7	Translation	Baltz	KH	DFCI
RALY	BC103753.1	32.6	Splicing	Baltz/Castello	RRM	DFCI
RALYL	BC031090.1	32.3	Other	Castello	RRM	DFCI
RALYL	HQ447147	32.3	Other	Castello	RRM	DNASU
RAN	BC014518.2, BC014901.2, BC016654.1, BC051908.2	24.4	Localization	Baltz/Castello	Other	DFCI

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
RBBP6	BC029352.1	13.2	Other	Baltz	zf-CCHC	DNASU
RBBP6	BC172357	201.6	Other	Baltz	zf-CCHC	DNASU
RBFOX1	BC113691.1	42.4	Localization		RRM	DFCI
RBFOX2	BC025281.1	40.4	Splicing	Baltz/Castello	RRM	DFCI
RBFQX2	BC013115.1	37.9	Splicing	Baltz/Castello	RRM	DFCI
RBFOX3	RBFOX3	33.9	Splicing		RRM	In-house
RBM10	BC004181.2, BC008733.2, BC024153.2	103.5	Stability	Baltz/Castello	RRM	DFCI
RBM11	BC030196.1	32.2	Splicing		RRM	DFCI
RBM12	BC012787.2, BC013981.2	97.4	Other	Castello	RRM	DFCI
RBM12B	BC039260.1	87.4	Other	Baltz/Castello	RRM	DFCI
RBM14	BC000488.2	69.5	Other	Baltz/Castello	RRM	DFCI
RBM15	BC103493.1	106.4	Other	Baltz/Castello	RRM	DFCI
RBM15B	BC139836.1	63.1	Splicing	Baltz/Castello	RRM	DFCI
RBM17	BC039322.1	46.0	Splicing		RRM	DFCI
RBM18	BC008942.2	21.8	Other		RRM	DFCI
RBM19	BC004289.1, BC006137.1	107.3	Other	Baltz/Castello	RRM	DFCI
RBM22	AL136933	46.9	Splicing	Baltz/Castello	RRM	DNASU
RBM23	BC002586.2	46.8	Other		RRM	DFCI
RBM24	BC104810.1	19.8	Stability		RRM	DFCI
RBM25	BC136775.1	100.2	Splicing	Baltz/Castello	RRM	DFCI
RBM26	BC000791.2	7.3	Other	Baltz/Castello	RRM	DFCI
RBM26	BC111697.1	111.0	Other	Baltz/Castello	RRM	DFCI
RBM26	BC041655.1	110.7	Other	Baltz/Castello	RRM	DFCI
RBM28	BC013889.2	85.7	Splicing	Baltz/Castello	RRM	DFCI
RBM3	BC006825.1	17.2	Translation	Baltz/Castello	RRM	DFCI
RBM33	BC011923.2	30.2	Other	Baltz/Castello	RRM	DFCI
RBM34	BC029451.1	48.1	Other	Baltz/Castello	RRM	DFCI
RBM38	BC018711	23.4	Stability	Baltz/Castello	RRM	DNASU
RBM4	BC021120.1	20.0	Splicing	Baltz/Castello	RRM	DFCI
RBM4	BC032735.1	40.3	Splicing	Baltz/Castello	RRM	DFCI
RBM41	BC006986	47.1	Other		RRM	DNASU
RBM42	BC002868.2	47.4	Other	Castello	RRM	DFCI
RBM42	BC004204.2	50.4	Other	Castello	RRM	DFCI
RBM43	BC136411.1	40.7	Other		RRM	DNASU
RBM45	BC086549.1	53.3	Other	Baltz/Castello	RRM	DFCI
RBM46	BC028588.2	60.0	Other		RRM	DFCI
RBM47	BC126261.1	64.1	Other	Baltz/Castello	RRM	DFCI
RBM48	BC003503.1, BC004951.1	40.1	Translation	Baltz/Castello	RRM	DNASU
RBM5	BC002957.1	81.5	Splicing	Baltz	RRM	DFCI
RBM6	BC046643.1	69.2	Other	Baltz/Castello	RRM	DFCI
RBM7	BC034381.1	30.5	Other	Baltz/Castello	RRM	DFCI
RSM8A	GQ0120283	19.9	Stability	Baltz	RRM	DNASU
RBMS1	BC085192.1, BC080620.1	6.7	Other	Baltz/Castello	RRM	DFCI
RBMS1	BC018951.2	44.5	Other	Baltz/Castello	RRM	DFCI
RBMS2	BC027863.1	44.0	Other	Baltz/Castello	RRM	DFCI
RBMX	BC006550.2	42.3	Splicing	Baltz/Castello	RRM	DFCI
RBMX2	BC033750.1	37.3	Other	Castello	RRM	DFCI
RBMX2	BC125126.1	74.0	Other	Castello	RRM	DNASU
RBMY1A1	X76059	40.7	Splicing		RRM	DNASU
RBMY1A1	BC070298.1	51.3	Splicing		RRM	DNASU
RBMY1F	BC030018.2	55.7	Splicing		RRM	DFCI
RBPMS	BC003608.2	24.3	Other	Caatello	RRM	DFCI
RC3H2	BC044642.1	56.9	Other	Baltz/Castello	RRM	DFCI
RDBP	BC025235.1, BC050617.2	43.2	Other	Castello	Other	DFCI
RDX	BC047109.1	68.6	Ottrer	Castello	Other	DFCI
REPIN1	BC001760.1	63.6	Other	Castello	Zn-Finger	DFCI
REXO4	BC009274.2	46.7	Other	Baltz/Castello	Other	DFCI
REXO5	BC007646	86.9	Other		Other	In-house
RNMTL1	BC050614.1	47.0	Other	Baltz/Castello	Other	DFCI
RNMTL1	BC011550.1	47.0	Other	Baltz/Castello	Other	DFCI
RNPC3	BC010697.1	30.1	Splicing		RRM	DFCI
RNPS1	BC001659.2	34.2	Stability	Baltz/Castello	RRM	DFCI
RPGR	BC031624.1	52.6	Other	Castello	Other	DFCI
RPL10A	BC006791.1, BC011366.1	24.8	Stability	Baltz/Castello	Ribosomal	DFCI
RPL13A	BC000514.2, BC001675.2, BC065236.1	23.6	Stability	Castello	Ribosomal	DFCI

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
RPL13A	BC070223.1	23.6	Stability	Castello	Ribosomal	DFCI
RPL14	BC005134.2	23.9	Stability	Baltz/Castello	Ribosomal	DFCI
RPL14	BC009294.2	23.6	Stability	Baltz/Castello	Ribosomal	DFCI
RPL15	BC014837.1	24.1	Stability	Baltz/Castello	Ribosomal	DFCI
RPL15	BC071672.1	24.1	Stability	Baltz/Castello	Ribosomal	DFCI
RPL15	BC081585.1	16.7	Stability	Baltz/Castello	Ribosomal	DFCI
RPL18A	BC066319.1	20.8	Stability	Baltz/Castello	Ribosomal	DFCI
RPL19	BC000530.2, BC013016.2	23.5	Stability	Castello	Ribosomal	DFCI
RPL21	BC091603.1, BC007505.2, BC071902.1	18.6	Stability	Baltz/Castello	Ribosomal	DFCI
RPL22	BC058887.1	14.8	Stability	Baltz/Castello	Ribosomal	DFCI
RPL23	BC010114.2	14.9	Stability	Castello	Ribosomal	DFCI
RPL23	BC062716.1	14.9	Stability	Castello	Ribosomal	DFCI
RPL23A	BC014459.1	17.7	Stability	Baltz/Castello	Ribosomal	DFCI
RPL27	BC001700.2, BC002588.2, BC007273.1, BC010026.2	15.8	Stability	Baltz/Castello	Ribosomal	DFCI
RPL28	BC010173.2, BC011582.1	15.7	Stability	Castello	Ribosomal	DFCI
RPL23	BC010182.2	15.8	Stability	Castello	Ribosomal	DFCI
RPL3	BC004323.1	26.8	Stability	Baltz/Castello	Ribosomal	DFCI
RPL3	BC006483.1, BC008003.1, BC012786.2, BC014017.2, BC063662.1	48.1	Stability	Baltz/Castello	Ribosomal	DFCI
RPL30	BC032700.2	12.8	Stability	Baltz/Castello	Ribosomal	DFCI
RPL31	BC017343.2	14.5	Stability	Castello	Ribosomal	DFCI
RPL32	BC011514.1	15.9	Stability	Castello	Ribosomal	DFCI
RPL35	BC010918.1	14.6	Stability	Castello	Ribosomal	DFCI
RPL35	BC000348.2	14.6	Stability	Castello	Ribosomal	DFCI
RPL35A	BC001037.2, BC010949.1, BC017093.1, BC081890.1	12.5	Stability	Baltz/Castello	Ribosomal	DFCI
RPL36	BC004971.1	12.3	Stability	Castello	Ribosomal	DFCI
RPU	BC001365.2, BC005817.2, BC007748.2, BC007996.1, BC009888.2, BC010151.2, BC014653.1, BC068925.1	47.7	Stability	Baltz/Castello	Ribosomal	DFCI
RPL5	BC001882.1	12.1	Stability	Baltz/Castello	Ribosomal	DFCI
RPL6	BC004138.2, BC032299.1	32.7	Stability	Castello	Ribosomal	DFCI
RPL6	BC022444.1	32.7	Stability	Castello	Ribosomal	DFCI
RPL7	BC006095.1, BC008850.2, BC009599.1	29.2	Stability	Baltz/Castello	Ribosomal	DFCI
RPL7L1	BC073890.1	28.7	Other	Baltz/Castello	Ribosomal	DFCI
RPL8	BC00077.2	28.0	Stability	Baltz/Castello	Ribosomal	DFCI
RPL8	BC012197.1, BC013104.1	28.0	Stability	Baltz/Castello	Ribosomal	DFCI
RPLP0	BC000087.2, BC008092.1, BC008594.1, BC009867.2, BC015173.1, BC015690.1	34.3	Stability	Baltz/Castello	Ribosomal	DFCI
RPLP0	BC000345.2, BC000752.2, BC003655.2	34.3	Stability	Baltz/Castello	Ribosomal	DFCI
RPN1	BC010839.1	68.6	Other	Castello	Other	DFCI
RPP21	BC011730.2	17.6	Other		Other	DFCI
RPP25	BC002497.2, BC007270.1	20.6	Other	Castello	Other	DFCI
RPP30	BC006991.1	29.3	Other	Castello	Other	DFCI
RPS10	BC001032.2, BC001955.1, BC005012.1	18.9	Stability	Baltz/Castello	Other	DFCI

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
RPS11	BC007283.1, BC007603.1, BC007945.2, BC010028.2, BC016378.1					
RPS15A	BC001697.2	14.8	Stability	Baltz/Castello	Ribosomal	DFCI
RPS15A	BC030569.1, BC048113.1	14.8	Stability	Baltz/Castello	Ribosomal	DFCI
RPS19BP1	BC037573.1, BC047711.1	15.4	Other	Baltz/Castello	Other	DFCI
RPS2	BC018178.1	31.3	Stability	Baltz/Castello	Ribosomal	DFCI
RPS2	BC001795.1, BC008862.2, BC010165.2, BC016951.2, BC021545.1, BC023354.1	31.3	Stability	Baltz/Castello	Ribosomal	DFCI
RPS20	BC007507.2	13.4	Stability	Baltz/Castello	Ribosomal	DFCI
RPS21	BC027976.1	7.1	Stability	Castello	Ribosomal	DFCI
RPS24	BC000523.2	15.1	Stability	Baltz/Castello	Ribosomal	DFCI
RPS28	BC000354.1, BC021239.2	7.8	Stability	Baltz/Castello	Ribosomal	DFCI
RPS3	BC003137.1, BC034149.1	26.7	Stability	Baltz/Castello	KH	DFCI
RPS3A	BC000204.1, BC001708.1, BC004981.1, BC006298.2, BC009219.2, BC009404.2, BC017123.2, BC019072.2, BC030161.2	29.9	Stability	Baltz/Castello	Ribosomal	DFCI
RPS4X	BC007308.1	22.2	Stability	Baltz/Castello	Ribosomal	DFCI
RPS4X	BC100903.1	29.6	Stability	Baltz/Castello	Ribosomal	DFCI
RPS6	BC013296.2	28.7	Stability	Castello	Ribosomal	DFCI
RPS6	BC027620.1	28.7	Stability	Castello	Ribosomal	DFCI
RPS7	BC002886.2, BC061901.1	22.1	Stability	Baltz/Castello	Ribosomal	DFCI
RPS8	BC070875.1	24.2	Stability	Baltz/Castello	Ribosomal	DFCI
RPUSD3	BC065741.1	37.6	Modification	Baltz/Castello	Other	DFCI
RPUSD3	BC032135.2	37.5	Modification	Baltz/Castello	Other	DFCI
RPUSD4	BC014131.2	42.2	Modification	Baltz/Castello	Other	DFCI
RRP36	BC011933.2	29.8	Other	Baltz/Castello	Other	DFCI
RRP7A	BC073834.1, BC121118.1	32.3	Other	Castello	RRM	DFCI
RRP8	BC001071.2	50.7	Other	Baltz/Castello	Other	DFCI
RRS1	BC001811.2, BC013043.2	41.2	Other	Baltz/Castello	Other	DFCI
RSL1D1	BC113899.1	55.0	Other	Baltz/Castello	Ribosomal	DFCI
RSRC1	HQ448170	38.7	Splicing		Other	DNASU
RTCA	BC012804.1	40.7	Other		Other	DFCI
RTN4	BC016165.1	42.3	Other	Castello	Other	DFCI
RTN4	BC012619.1	40.3	Other	Castello	Other	DFCI
RY1	BC017890.1	18.9	Other		Other	DNASU
S100A4	BC018300.1	11.7	Other	Castello	Other	DFCI
SAFB2	BC025279.1	57.2	Other	Baltz/Castello	RRM	DFCI
SAMD4A	BC057836.1	27.6	Translation	Castello	SAM	DFCI
SAMD4A	BC121173.1	70.0	Translation	Castello	SAM	DFCI
SAMSN1	BC029112.1	41.7	Other	Castsllo	Other	DFCI
SARNP	BC007099.1	23.7	Translation	Baltz/Castello	Other	DFCI
SART3	BC111883.1	109.9	Other	Baltz/Castello	RRM	DFCI
SBDS	BC065700.1	28.8	Other	Castello	Other	DFCI
SCAF8	BC070071.1	140.5	Splicing		RRM	DFCI
SCD5	BC004936.1	14.3	Other		Other	DFCI
SCD5		14.3	Other		Other	DNASU
SCG3	BC014539.1	53.0	Other	Castello	Other	DFCI
SEC61B	BC001734.1	10.0	Other	Cassette	Other	DFCI
SEC63	BC048287.1	88.0	Other	Castello	Other	DFCI
SEC63	BC047221.1	88.0	Other	Castello	Other	DFCI
SECISBP2	BC001189.1	95.5	Translation	Castello	Ribosomal	DFCI
SECP43		32.5	Other		Other	In-house
SENP5	BC030705	86.7	Other		Other	In-house
SERBP1	BC020555.1	44.3	Stability	Baltz/Castello	Other	DFCI
SERBP1	BC002488.2	44.2	Stability	Baltz/Castello	Other	DFCI

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
SERBP1	BC003049.1, BC008045.2, BC019273.1, BC026918.1	45.0	Stability	Baltz/Castello	Other	DFCI
SERPINH1	BC014623	46.4	Other	Castello	Other	DNASU
SF1	BC008080.2, BC020217.1	59.7	Splicing	Baltz/Castello	KH	DFCI
SF3A1	BC001976.1, BC097684.2	88.9	Splicing	Baltz/Castello	Other	DFCI
SF3A2	BC004434	49.3	Splicing	Baltz/Castello	Zn-finger	DNASU
SF3A3	BC011523	58.8	Splicing	Baltz/Castello	Other	DNASU
SF3B14	BC015413	13.6	Splicing	Baltz	RRM	In-house
SF3B14	BC016483.1	14.6	Splicing	Baltz	RRM	DNASU
SF3B2	BC007610.1	72.6	Splicing	Baltz/Castello	Other	DFCI
SF3B3	JF432652	30.2	Splicing		Other	DNASU
SF3B3	BC009463.1	44.8	Splicing		Other	DNASU
SF3B4	BC004273.1, BC013886.2	44.4	Splicing	Baltz/Castello	RRM	DFCI
SFPQ	BC051192.1	76.2	Splicing	Baltz/Castello	RRM	DFCI
SFRS10	BC000160	33.7	Other		Other	DNASU
SFRS13A	HQ448699	22.2	Other		Other	DNASU
SFRS16	BC013178.1	23.3	Other		Other	DNASU
SFRS17A	BC028151.1	51.5	Other		Other	DNASU
SFRS17A	BC110496.1	80.7	Other		Other	DNASU
SFRS2IP	NM_004719	164.7	Other		Other	DNASU
SFSWAP	BC136678.1	104.8	Splicing		Other	DFCI
SKIV2L	BC015758	137.8	Other		DEAD	DNASU
SKIV2L2	BC028604.2	117.8	Splicing	Castello	DEAD	DFCI
SLBP	BC014908.1, BC015703.1	31.3	Localization	Castello	Other	DFCI
SLC25A5	BC058160.1	32.9	Other	Castello	Other	DFCI
SLC3A2	BC001061.2, BC003000.1	57.9	Other	Castello	Other	DFCI
SLC7A9	BC017962.1	53.5	Other		Other	DFCI
SLIRP	BC017895.1	12.3	Other	Baltz	RRM	DFCI
SLU7	BC010634.1	68.3	Splicing		Other	DNASU
SMG6	BC148373	57.3	Stability		Other	DNASU
SMN1	BC015308	31.8	Other		Other	DNASU
SMN1	BC000908	30.4	Other		Other	DNASU
SMNDC1	BC011234.1	26.7	Splicing	Castello	Other	DFCI
SNIPI	BC027040.1	45.8	Other	Castello	Other	DFCI
SNRNP35	BC009622.1	30.0	Splicing		RRM	DFCI
SNRNP40	BC001494.2	39.3	Splicing	Castello	Other	DFCI
SNRNP70	BC001315.1	51.6	Splicing	Baltz/Castello	RRM	DFCI
SNRPA	BC000405.2, BC008290.1	31.3	Splicing	Baltz	RRM	DFCI
SNRPB	BC080516.1	23.7	Splicing	Baltz	LSM	DFCI
SNRPB2	BC018022.1, BC038737.2	25.5	Splicing		RRM	DFCI
SNRPD2	BC000488.2	13.5	Splicing	Castello	LSM	DFCI
SNRPE	BC002639.2	10.8	Splicing		LSM	DFCI
SNRPF	BC063397.1, BC128453.1	9.7	Splicing		LSM	DFCI
SNRPG	BC000070.2, BC022432.1, BC086302.1	8.5	Splicing	Castello	LSM	DFCI
SNRPN	BC003180.1, BC010057.1, BC024777.1, BC025178.1	24.6	Splicing		LSM	DFCI
SNURF	BC024777.1	24.6	Other		Other	DNASU
SNW1	BC108903.1	81.6	Splicing	Baltz	Other	DNASU
SOX21	BC111584	28.6	Other		Other	DNASU
SPATS2	BC048299.1	59.5	Other	Castello	Other	DFCI
SPATS2L	BC018738.1	64.0	Other	Baltz/Castello	Other	DFCI
SR140	BC111692.1	72.5	Other		Other	DNASU
SRBD1	BC032538.1	69.7	Other		S1	DFCI
SREK1	BC067770.1, BC112343.1	69.4	Splicing	Baltz	RRM	DFCI
SRFBP1	BC031222.1	48.6	Other	Baltz/Castello	Other	DFCI
SRP14	BC030495.2	14.6	Translation	Baltz/Castello	Other	DFCI
SRP68	BC020238.1	67.3	Translation	Castello	Other	DFCI
SRPK2	BC035214.1	77.6	Splicing	Baltz/Castello	Other	DFCI
SRPK2	BC068547.1	77.6	Splicing	Baltz/Castello	Other	DFCI

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
SRPR	BC001162.1, BC009110.1, BC013583.1	69.8	Translation	Castello	Other	DFCI
SRPR	BC008077.2	63.2	Translation	Castello	Other	DFCI
SRSF1	BC010264.1	27.7	Localization	Baltz/Castello	RRM	DFCI
SRSF10	BC001107.1	22.2	Localization	Baltz	RRM	DFCI
SRSF10	BC005039.1	31.3	Localization	Baltz	RRM	DFCI
SRSF11	BC040436.1	53.4	Localization	Castello	RRM	DFCI
SRSF12	BC021715.1	30.5	Splicing	Baltz	RRM	DFCI
SRSF2	BC066958.1	19.4	Localization	Castello	RRM	DFCI
SRSF3	BC000914.1, BC069018.1	19.3	Localization	Baltz/Castello	RRM	DFCI
SRSF4	BC002781.2	56.7	Localization	Castello	RRM	DFCI
SRSF5	BC018823.2	31.3	Localization	Castello	RRM	DFCI
SRSF6	BC006832.2	39.6	Localization	Castello	RRM	DFCI
SRSF7	BC000997.2, BC017369.2, BC022328.1	27.4	Localization	Castello	RRM	DFCI
SRSF8	BC057783.1	31.4	Other	Bahz/Castello	RRM	DFCI
SRSF9	BC093971.1	25.5	Localization	Castello	RRM	DFCI
SSBP1	BC000895.1	17.3	Other	Baltz/Castello	Other	DFCI
SSRP1	BC005116.1	81.1	Other	Baltz/Castello	Other	DFCI
STAU1	BC050432.1	63.2	Localization	Baltz	dsRBD	DFCI
STAU2	BC008370.1	11.8	Other	Baltz/Castello	dsRBD	DFCI
STAU2	BC110447.1	52.8	Other	Baltz/Castello	dsRSD	DFCI
STAU2	BC008369.1	59.0	Other	Baltz/Castello	dsRBD	DFCI
STIP1	BC002987.1	62.6	Other	Castello	Other	DFCI
STRBP	BC017732.1	73.7	Other	Baltz/Castello	dsRBD	DFCI
STXBP1	BC015749.1	67.6	Other	Castello	Other	DFCI
SUCLG1	BC000504.2	35.0	Other	Castello	Other	DFCI
SUGP1	BC142988.1	72.5	Splicing		G-patch	DFCI
SUGP2	BC020586.1	110.4	Splicing	Baltz/Castello	G-patch	DFCI
SUMO1	BC006462.1, BC053528.1	11.8	Other	Castello	Other	DFCI
SUMO1	BC066308.1	11.5	Other	Castello	Other	DFCI
SUPT5H	BC024203.2	121.0	Other	Baltz/Castello	Other	DFCI
SUPV3L1	BC036112.1	88.0	Other	Baltz/Castello	Other	DFCI
SURF6	BC014878.1	41.5	Other	Baltz/Castello	Other	DFCI
SYF2	BC010882.1	28.7	Splicing	Baltz/Castello	Other	DFCI
SYMPK	BC006567	126.6	Other		Other	DNASU
SYNCRIP	BC032843.1	58.7	Stability	Baltz/Castello	RRM	DFCI
TAF15	BC046099.1	81.8	Other	Baltz/Castello	RRM	DFCI
TAF15	TAF15(1)	61.8	Other		Other	In-house
TAf15	TAF15(5)	61.3	Other		Other	In-house
TARDBP	BC001487	29.5	Stability	Baltz/Castello	RRM	In-house
TARDBP	BC095435	44.9	Stability	Baltz/Castello	RRM	DNASU
TBRG4	BC014918.1, BC017235.2	70.7	Other	Baltz/Castello	Other	DFCI
TCOF1		96.8	Other	Baltz/Castello	Other	DNASU
TDRD3	BC030514.1	73.2	Other	Baltz/Castello	Other	DFCI
TDRD9	BC128057.1	99.5	Other		DEAD	DFCI
TDRKH	BC032890.1	82.1	Other		KH	DFCI
TEFM	BC024328.1	26.3	Other	Baltz	Other	DFCI
TERT	BC172541	125.7	Other		Other	DNASU
TES	BC001451.1	48.0	Other	Castello	Other	DFCI
TFB1M	BC017788.1	39.5	Modification	Castello	Other	DFCI
TFIP11	BC011599.2	96.8	Splicing		G-patch	DFCI
THOC1	BC010381	75.7	Localization		Other	DNASU
THOC3	BC006849	38.8	Localization		Other	DNASU
THOC4	BC052302.1	27.6	Other	Baltz/Castello	Other	DNASU
THOC5	CU013430	78.5	Localization		Other	DNASU
THOC6		37.5	Localization		Other	DNASU
THRAP3	BC112330.1	108.7	Stability	Baltz/Castello	Other	DFCI
THUMPD1	BC000448.2	39.3	Other	Castello	Other	DFCI
TIA1	BC015944	24.1	Splicing	Baltz/Castello	RRM	In-house
TIAL1	BC030025.1	27.9	Other	Baltz/Castello	RRM	DFCI
TOB1	DQ893993	38.1	Other		Other	DNASU
TOB2	BC038957	36.6	Other		Other	DNASU
TOE1	JF432161	56.5	Other		Other	DNASU
TOE1		56.5	Other		Other	In-house
TOP3B	BC002432.2	96.7	Other	Baltz/Castello	Other	DFCI
TPD52L2	BC008804.1	22.2	Other	Castello	Other	DFCI
TFT1	BC022436.1	15.6	Other	Castello	Other	DFCI
TPT1	BC003352.2, BC052333.1	19.6	Other	Castello	Other	DFCI

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
TRA2A	BC017094.2	32.7	Splicing	Baltz/Castello	RRM	DFCI
TRAP1	BC018950.2	80.1	Other	Castello	Other	DFCI
TRIM39	BC034985.1	56.4	Other		Other	DFCI
TRIM39	BC097661.2	56.4	Other		Other	DFCI
TRIM56	BC048194.1	81.5	Other	Baltz/Castello	Zn-Finger	DFCI
TRIP6	BC004999.1, BC028985.1	50.3	Other	Castello	Other	DFCI
TRIP6	BC002680.2	50.1	Other	Castello	Other	DFCI
TRMT10C	BC035967.1	46.7	Other		tRNA	DFCI
TRMT1L	BC045535.1	81.7	Other	Castello	Other	DFCI
TRMT2A	BC013352.2, BC017184.2	68.7	Modification	Castello	RRM	DFCI
TRMT6	BC001262.1	55.8	Translation	Castello	Other	DFCI
TRMU	BC080631.1	25.2	Other		tRNA	DFCI
TRNAU1AP	BC039879.1	26.4	Other	Baltz/Castello	RRM	DFCI
TRNAU1AP	BC000680.2	32.5	Other	Baltz/Castello	RRM	DFCI
TROVE2	BC038658.2	60.7	Other		Other	DFCI
TRUB2	BC001457.2	36.7	Modification	Baltz/Castello	Other	DFCI
TSR1	BC125110.1	91.8	Other	Castello	Other	DFCI
TSR1	BC019090.2	75.0	Other	Castello	Other	DFCI
TTYH1	BC019358	49.1	Other		Other	In-house
TUFM	BC001633.1, BC010041.2	49.9	Translation	Castello	Other	DFCI
TUT1	BC005013.1	57.9	Other		RRM	DFCI
TUT2	uc010jaf.1	56.0	Other		Other	In-house
TUT3	uc010vgo.2	75.8	Other		Other	In-house
TUT5	uc003jdx.1	59.9	Other		Other	In-house
TUT7	uc004aoq.3	171.2	Other		Other	In-house
TWF2	BC016452.1	39.5	Other	Castello	Other	DFCI
U2AF1	BC001177.1, BC001923.1	27.9	Localization	Baltz/Castello	RRM	DFCI
U2AF1	BC005915.1	19.8	Localization	Baltz/Castello	RRM	DFCI
U2AF1L4	BC021186.1	22.0	Splicing		RRM	DNASU
U2AF2	BC008740.2	53.1	Localization	Baltz/Castello	RRM	DFCI
UBAP2L	BC003170.1	114.5	Other	Baltz/Castello	Other	DFCI
UBE2I	BC000427.2, BC051289.1	18.0	Other	Castello	Other	DFCI
UCHL5	BC015521.1	37.5	Other	Castello	Other	DFCI
USO1	BC032654.1	107.8	Other	Castello	Other	DFCI
USF32	BC054344.1	44.7	Other		Other	DFCI
USP36	BC038983.1	31.8	Other	Castello	Other	DFCI
UTP11L	BC005182.1	30.4	Other	Baltz/Castello	Other	DFCI
UTP14A	BC001149.1, BC009649.1, BC014987.1	88.0	Other	Baltz/Castello	Other	DFCI
UTP15	BC013064.1	32.4	Other	Baltz/Castello	Other	DFCI
UTP23	BC006955.1, BC022441.1	28.4	Other	Castello	Other	DFCI
UTP3	BC004546.1	54.6	Other	Baltz/Castello	Other	DFCI
WBSCR16	BC007823	49.9	Other	Baltz/Castello	Other	DNASU
WDR3	BC058836.1	43	Other	Castello	Other	DFCI
WDR33	BC013990.2	38.3	Other	Baltz	Other	DNASU
WDR36	BC133025.1	105.3	Other	Castello	Other	DFCI
WDR6	BC002826.2	32.1	Other	Castello	Other	DFCI
XPO1	BC032847.2	123.4	Localization		Other	DFCI
XPO5	BC000129.1	31.5	Other	Baltz/Castello	Other	DFCI
XPO5	BC009969.2	75.8	Other	Baltz/Castello	Other	DFCI
XRCC6	BC008343.1, BC012154.2	69.8	Other	Baltz/Castello	Other	DFCI
XRCC6	BC010034.2, BC018259.2	69.8	Other	Baltz/Castello	Other	DFCI
XRN1	NM_019001	194.1	Stability	Baltz/Castello	Other	DNASU
XRN2	BC006417.1	63.8	Other	Baltz/Castello	Other	DFCI
YARS	BC001933.1, BC016689.1	59.1	Translation	Castello	tRNA	DFCI
YTHDC1	BC041119.1	84.7	Splicing	Baltz/Castello	Other	DFCI
YTHDF1	BC050284.1	60.9	Other	Baltz/Castello	Other	DFCI
YTHDF2	BC002559.2	82.3	Other	Baltz/Castello	Other	DFCI
YTHDF3	BC052970.1	63.9	Other	Baltz/Castello	Other	DFCI
YWHAE	BC000179.1, BC001440	29.2	Other	Castello	Other	DNASU
YWHAG	BC020963.2	28.3	Other		Other	DFCI
ZC3H11A	BC014268.2	89.1	Localization	Baltz/Castello	ZnF-CCCH	DFCI
ZC3H14	BC027807.2	34.9	Other	Baltz/Castello	ZnF-CCCH	DFCI
ZC3H18	BC050463.1	106.4	Other	Castello	ZnF-CCCH	DFCI

TABLE 1-continued

Gene symbol	Accession number	MW (kDa)	GO Term	Group	Domain	Source
ZC3H7A	BC012575.1	19.8	Other	Baltz/Castello	ZnF-CCCH	DFCI
ZC3H8	BC032001.1	34.3	Other	Baltz/Castello	ZnF-CCCH	DFCI
ZC3HAV1	BC040956.1	77.9	Other	Baltz/Castello	Other	DFCI
ZCCHC11		81.1	Other		Other	In-house
ZCCHC11		81.1	Other		Other	In-house
ZCCHC11		81.1	Other		Other	In-house
ZCCHC11	BC131734.1	185.3	Other	Baltz/Castello	zf-CCHC	DFCI
ZCCHC17	BC007446.2, BC050609.1	27.6	Other	Baltz/Castello	S1	DFCI
ZCCHC6	AL832026	144.5	Other	Baltz/Castello	zf-CCHC	DNASU
ZCCHC7	BC034022.1	34.5	Other	Castello	zf-CCHC	DFCI
ZCCHC7	BC036940.1	62.9	Other	Castello	zf-CCHC	DFCI
ZCCHC9	BC014841.1	30.9	Other	Castello	zf-CCHC	DFCI
ZCRB1	BC022543.1	24.6	Splicing	Baltz/Castello	RRM	DFCI
ZFC3H1	BC073843.1	37.8	Other	Castello	Zn-Finger	DFCI
ZFC3H1	BC015679.2	39.1	Other	Castello	Zn-Finger	DFCI
ZFP36 (TTP)	BC009693	34.0	Other		Other	In-house
ZFP36L1	BC018340.1	36.3	Stability	Castello	ZnF-CCCH	DFCI
ZFP36L2	BC005010	51.6	Stability	Castello	ZnF-CCCH	DNASU
ZGPAT	BC019338.1	54.7	Other		ZnF-CCCH	DFCI
ZMAT3	BC002896.2	32.1	Other	Castello	Zn-Finger	DFCI
ZNF9	NM_003418	19.5	Other		Other	DNASU
ZRANB2	BC039814.1	36.2	Splicing	Baltz/Castello	Zn-Finger	DFCI
ZRSR1	BC113599.1	67.6	Other		RRM	DFCI
ZRSR2	BC113454.1, BC113480.1	58.0	Splicing		RRM	DFCI
ZRSR2	BC050451.11	58.0	Splicing		RRM	DFCI
ZYX	BC003743.2, BC009360.2, BC010031.2	61.3	Other	Castello	Other	DFCI
AC004381.6	AC004381.6 (LOC81691 exonuclease NEF-sp)	88.9	Other		Other	In-house

Example 2—Large-Scale Tethered Function Screen Assigns RBPs to Roles in RNA Stability and Translation

[0119] 961 ORFs, representing 888 RBPs, were screened in triplicate. Two dual luciferase reporter systems were used as described above, and the FLAG expression construct was used as a negative control (FIG. **1**D, left). The effect of RBP recruitment to the tethering reporter was calculated as the fold change in luciferase activity relative to FLAG control, after normalization of each to the untethered reporter (FIG. **1**D, right). Supporting the validity of the screening approach, it was confirmed that the effect was not correlated with RBP size, indicating that steric hindrance is unlikely to account for these observations (FIG. **7**D). The magnitude of the effect on reporter transcript abundance generally depended on the reporter systems (FIG. **7**E) but for 97% of ORFs (961), both reporter systems agreed on the direction of regulation (FIG. **7**F), indicating high reproducibility.

[0120] Candidates from each reporter assay were prioritized by using multiple t-tests at a threshold $p<0.05$ and calculated false discovery rates (FDR) for each comparison using the Benjamini, Krieger & Yekutieli procedure. 344 and 87 RBPs were identified with an estimated FDR <0.01 in Renilla and firefly reporters, respectively, of which 50 RBPs were recovered from both reporter contexts (FIG. **1**E). In order to distinguish those RBPs that affect reporter mRNA stability from those regulating its translation, both luciferase transcripts were measured by reverse transcription quantitative PCR (rt-qPCR) for 35 RBPs of the 50 RBPs that significantly modulated luciferase activity in both reporter contexts. In general, the change in reporter translation levels was positively correlated with changes in reporter transcript levels (FIG. **1**F). Among the strongest candidate negative regulators were RBP components of both deadenylation-dependent and -independent exonuclease decay pathways, including ZFP36, as well as members of the CCR4-NOT deadenylase complex (CNOT2, CNOT4, CNOT7, TOB1, and TOB2), the 3'-to-5' exonuclease PARN, and the decapping activator DDX6, which is recruited to the 5' cap via interaction with the CCR4-Not complex. As another positive control, it was also confirmed that YTHDF2, a member of the YTH domain family of N6-methyladenosine binding proteins, which recruit target RNAs to degradation bodies, exerts a negative effect on target mRNA levels. The results of the screen also confirmed several known negative regulators of translation, such as NANOS3 specific to germ cells, and CPEB4, which binds polyadenylation elements in the 3' UTR and negatively regulates translation initiation by interacting with the translation initiation factor eIF3. Interestingly, EIF2S2, with roles in promoting translation initiation, emerged as positive regulator of translation when recruited to the 3' UTR. It was speculated that recruitment of this protein to the 3' UTR brings it into proximity to the mRNA cap and 5'UTR, similar to DDX6 and CPEB4 and consistent with the closed-loop model of translation (FIG. **1**G).

[0121] To verify these RBPs hits are not false positive in the large screen assay, reporter protein and transcript level changes were re-confirmed by luciferase assay and qRT-PCR and chose 14 RBPs with significant effects (8 candidate stabilizers and 6 candidate destabilizers) for further investigation. Focus was put on RBPs with known roles in RNA stability and translation but where transcriptome-wide binding sites and preferences have not been described (e.g.

CNOT7, DDX6, NANOS3, TOB1/2, MEX3C) and RBPs for which such roles are not known (e.g. UBAP2L, AIMP1, MTDH, IFTI2) (FIGS. **1**H-**1**I).

[0122] In summary, the screen revealed RBPs previously annotated to be implicated in metabolic processes, cell cycle, cell differentiation (BOLL, DAZ2, DAZ4, DAZAP1, NANOS3), stress granule regulators (UBAP2L), translation machinery (EIF2S2, LARP1, PABPC1, CPEB4), ER proteins (SRPR), and heat shock proteins (HSPB1). Eight annotated splicing factors (CLK3, CPSF5, PLRG1, PRPF3, RBFOX1, F3B3S, NRNP27, and SNRPA) and three nuclear export complex proteins (HNRNPD, THOC1, and YWHAE) were identified (FIGS. **1**J-**1**L).

Example 3—Enhanced CLIP Identifies Endogenous RNA Targets of Candidate Stabilizers and Destabilizers

[0123] In order to begin elucidating the physiological functions of candidate RBP regulators (FIG. **2**A), their endogenous mRNA targets and their transcriptome-wide binding sites were investigated using enhanced cross-linking immunoprecipitation followed by sequencing (eCLIP). HEK293T cells were subjected to UV-crosslinking, lysis and RNA fragmentation, and protein-RNA complexes were immunoprecipitated using validated RBP-specific antibodies (FIG. **8**A). Also, cells were transiently transfected with plasmids expressing VS-tagged fusions of those candidate RBPs which are not expressed in HEK293T cells or do not have RBP-specific antibodies (FIG. **8**B). In total, eCLIP datasets for 14 candidate proteins were generated, each consisting of an RBP eCLIP (IP) library and a paired size-matched input (SMInput) library. Libraries were sequenced to at least 15M (million) reads (average of 24M), of which at least 1M (average of 5M) mapped uniquely to the human genome.

[0124] Next, transcript binding region specificities were determined using two distinct metrics, namely read density enrichment and binding cluster enrichment. Read density enrichment within 5' and 3'UTRs and coding regions (CDS) of annotated protein coding genes were computed by the fold enrichment in the IPs normalized to their paired SMInputs. To illustrate, BOLL, a germ-cell specific RBP with some documented roles in mRNA stabilization and translation enhancer activity, displayed a strong preference for 3'UTR association (FIG. **2**B). Surprisingly, IFIT2 (Interferon Induced Protein With Tetratricopeptide Repeats 2), which is known to inhibit expression of viral messenger RNAs, robustly displays a strong 3'UTR preference in human mRNAs. The helicase DDX6 was enriched for binding within 5'UTRs, consistent with its role in the assembly of the decapping complex. A novel candidate 170 MEX3C, an RNA-binding E3 ubiquitin ligase that associates with the CCR4-NOT deadenylation complex to ubiquitinate CNOT7, unexpectedly exhibited preferential binding to 5'UTRs. Similarly, TOB family members TOB1 and TOB2, which recruit the catalytic subunits of the CCR4-NOT deadenylase complex to target mRNAs, showed a surprising preference for 5'UTRs, suggesting unexpected roles for this family of proteins (FIG. **2**C). Distinct from all these RBPs, UBAP2L (Ubiquitin-associated protein 2-like) showed strong enrichment across exons, especially in CDS, and 5' UTR (FIG. **2**D; FIG. **8**C).

[0125] To identify binding sites at higher resolution, binding clusters were discovered by the CLIPper algorithm. Cluster enrichment was computed by calculating the ratio of read densities between IPs and SMInputs within a cluster and significant clusters were defined as $p \leq 10^{-3}$ (Fisher's exact test for read numbers <5; χ^2 test for read numbers ≥5) and ≥4-fold enriched over SMInput. The significant clusters were generally located within the same enriched regions from the lower resolution gene region analysis (FIG. **2**E; FIG. **8**D). For example, the clusters for BOLL and IFIT2 were most enriched in 3'UTRs (FIG. **2**F). Interestingly, DDX6's and MEX3C's target genes (FIG. **8**E) and binding clusters (FIG. **2**G; FIG. **8**F) strongly overlap, suggesting that both proteins may be functionally linked and may act on the same mRNA targets. In contrast to the other candidate RBPs, the UBAP2L clusters were dispersed across exonic regions, rather than present as discrete binding sites (FIG. **2**H). Overall, the analyses not only revealed previously unrecognized binding maps and preferences for RBPs known to affect mRNA stability and translation (CNOT7, DDX6), but also revealed novel RNA interactomes of candidate RBPs.

Example 4—Integration of eCLIP and RNA-Seq Data Defines Regulatory Classes of RBPs and Transcripts

[0126] To gain insight into how the candidate RBPs affect transcriptome-wide mRNA levels, they were depleted or exogenously expressed in HEK293T cells and RNA-seq analysis was performed. Specifically, RBPs were either depleted by lentiviral transduction of short-hairpin RNAs (shRNAs) (FIGS. **9**A-**9**B), or ectopically expressed ORFs of those candidate RBPs which are not expressed in HEK293T cells or which do not have RBP-specific shRNAs (FIG. **9**C). For each RBP, either two independent transductions of two different targeting shRNAs and two non-targeting shRNAs were performed, or two independent transfections with a plasmid directing expression of the RBP as a V5-tagged fusion were performed, with the FLAG construct as a control. PolyA$^+$ RNA was selected, sequencing libraries were prepared and sequenced to a depth of >32 (or >26 uniquely mapped)$\times 10^6$ reads.

[0127] To assess the effect of a candidate RBP on transcript levels, the number of significantly up- or down-regulated genes were measured upon knockdown or overexpression (FIGS. **9**D-**9**G). In general, the manipulations of RBP levels resulted in a largely unperturbed population of transcripts, typically 80% at threshold of statistical significance [≥1.23-fold, false discovery rate (FDR)-corrected $p \leq 0.05$ versus non-targeting shRNA or FLAG control]. This indicates that the candidate RBPs affect specific sets of target transcripts, instead of having effects on global transcript stability. When only considering those transcripts that were bound by the respective RBP, as measured by eCLIP (≥1 significantly enriched cluster per transcript), higher numbers of targets were observed that change in the direction anticipated by the tethering assays, than in the opposite direction, for candidate destabilizers, MEX3C, DDX6, SNRPA, and TOB2 (FIG. **3**A; FIG. **9**H) and candidate stabilizers UBAP2L, CLK3, BOLL, and IFIT2 (FIG. **3**B; FIG. **9**I). In other words, knockdown of destabilizers led to more up-regulated genes, whereas overexpression of destabilizers led to more down-regulated genes. Expectedly, reciprocal effects are observed in the alterations of stabilizing RBPs.

[0128] It was also confirmed that the fraction of bound targets in the genes changing in the anticipated direction was

statistically significantly enriched relative to unbound targets (FIGS. **3**C-**3**D). In fact, significant correlation was observed between different eCLIP cluster fold enrichments IP over SMInput and change in transcript levels, for both candidate destabilizers (e.g. DDX6 and TOB2; FIGS. **3**E-**3**F) and candidate stabilizers (e.g. UBAP2L and BOLL; FIGS. **3**G-**3**H). This indicates that the candidate RBPs directly engage hundreds of previously unknown target endogenous mRNAs to affect transcript levels in the predicted direction. For example, knockdown of the destabilizer MEX3C increased transcript levels of NSMF mRNA, a MEX3C-bound transcript (FIG. **3**I). Conversely, depletion of the stabilizer CLK3 reduced the abundance of its target NELFCD mRNA (FIG. **3**J). Interestingly, when it was further evaluated which genic regions bound by the RBP are most correlated with transcript levels, UBAP2L binding within CDS was the most enriched (FIG. **9**J). In general, it was concluded that the majority of the candidate RBPs affect mRNA levels of their endogenous RNA targets, in agreement with the tethering results.

Example 5—UBAP2L Increases mRNA Polysome Association and Promotes Translation

[0129] Among the 13 candidates that were analyzed, UBAP2L had the highest CDS read density enrichment (FIG. **2**D and FIG. **9**J), suggesting a direct role in translation. However, such a function for UBAP2L had not been described. Global protein synthesis rates were measured in cells lacking UBAP2L with the SUnSET assay, which uses incorporation of puromycin (a structural analog of aminoacyl-transfer RNA) to label newly synthesized proteins. HEK293T cells biallelically deleted for UBAP2L by CRISPR/Cas9-mediated genome editing showed a ~40% reduction in protein synthesis (FIGS. **4**A-**4**B; FIG. **10**A), indicating that UBAP2L promotes global translation. Next, sucrose gradient centrifugation of HEK293T lysates was performed to examine the association of UBAP2L with ribosomes. UBAP2L from HEK293T cell lysates co-fractionated with monosomes and polysomes on sucrose gradients, suggesting a role for UBAP2L in translation (FIG. **4**C). In order to rule out the possibility that this observation is due to the presence of UBAP2L in non-ribosomal complexes of similar buoyant density, cells were treated with puromycin to release polysomes from transcripts. Puromycin treatment led to accumulation of 80S monosomes, as expected, and levels of UBAP2L in fractions corresponding to polysomes were strongly reduced (FIG. **10**B). Cell lysates were also treated with EDTA to disassemble 80S monosomes into 40S and 60S ribosomal subunits and found that, similarly, UBAP2L was depleted from fractions corresponding to monosomes (FIG. **10**C). These results strongly suggest that UBAP2L directly interacts with translating ribosomes.

[0130] To identify specific transcripts subject to UBAP2L-mediated translational regulation, polysome profiling was performed in cell lysates from two independent UBAP2L knockout clonal isolates and from two control samples (FIG. **10**D). From two independent fractionations per line, polyA$^+$ mRNA was isolated from a portion of the input lysates and from pooled polysome fractions, and RNA-seq libraries were prepared and sequenced. All transcripts with RPKM ≥1 in inputs were considered (FIG. **10**E). It was found that UBAP2L knockout resulted in a larger number of transcripts with changes in pooled polysome fractions compared to changes in input RNA abundance (FIG. **4**D), suggesting that UBAP2L predominantly acts at the translational level. As a measure of ribosome association, the ratio of transcript RPKMs in polysome pools over input for all transcripts was computed. A significant decrease was found ($p<10^{-307}$; Mann-Whitney U test, two-tailed) in mean transcript polysome-enrichment in both UBAP2L knockout lines compared to the controls (FIG. **4**E). Replicate analyses showed excellent correlation between the cell lines (FIG. **10**F). When isolated those genes that changed in the same direction in both knockout lines were isolated, it was found that overall nearly 10-fold more transcripts were reduced in translation (90.6%; n=8,784) than enhanced (9.4%; n=908) (FIG. **4**F). Even more strikingly, 99% of the 1,425 UBAP2L target transcripts, identified by eCLIP, showed significant downregulation in polysome association upon UBAP2L knockout (FIG. **10**G). A subset of target transcripts were also measured by quantitative RT-PCR, which confirmed the magnitude of translational downregulation (FIG. **10**H).

[0131] To investigate how depletion of UBAP2L affected global translation, the gene function attributes of UBAP2L direct targets were evaluated where a significant enrichment (FDR <0.05) was observe in protein translation and ribosome biogenesis terms by Gene ontology (GO) analysis (FIG. **4**G). It was also revealed that UBAP2L depletion decreased polysome association on mRNAs encoding translation initiation factors, elongation factors, tRNA synthesis proteins, and poly(A) binding proteins (FIG. **4**H). In fact, western blot analysis of these UBAP2L targets confirmed decreased protein levels of translation and elongation factors, such as Eukaryotic Translation Initiation Factor 4 Gamma 1 (EIF4G1), DEAD-Box Helicase 54 (DDX54), and Eukaryotic Translation Elongation Factor 2 (EEF2) in cells lacking UBAP2L (FIG. **4**I and FIG. **10**I). Taken together, these results suggest that UBAP2L enhances translation by directly binding mRNA substrates and also increasing translation of genes involved in global protein synthesis.

Example 6—Programmable RNA-Targeting CRISPR-Mediated Recruitment of UBAP2L Promotes Translation

[0132] In order to assess the dependence of UBAP2L-mediated translational regulation on direct binding to its target mRNA, a FACS-based reporter assay was employed using UBAP2L fused to RNA-targeting RCas9 (RCas9) (FIG. **4**J). As a control, the assay was performed with RCas9-fused 4EBP1, an inhibitor of translational initiation (FIG. **10**J). HEK293T cell lines expressing a RCas9-UBAP2L fusion, RCas9-4EBP1 fusion, or Cas9 only were derived via transposase-mediated piggyBAC genomic integration of plasmid constructs. A second construct harboring a reporter that stably expresses RFP transcripts not regulated by RCas9, a guide RNA, and tetracycline-inducible YFP transcripts was then transfected with the guide RNA target sequences. 7 different guide RNAs were designed, targeting locations across the YFP transcript (5' UTR, CDS, and 3'UTR), and a non-targeting guide RNA. Post transcriptional regulation was then measured as changes in the normalized YFP/RFP fluorescence ratio between Cas9-fusion and Cas9 only cells by using analytical flow cytometry. Due to the random nature of piggyBAC-mediated integration in terms of construct integration sites and numbers, regulation for various rCas9 construct levels (CFP) and reporter construct levels (RFP) can be quantified across thousands of data points (cells). With this highly sensitive and quantitative

assay, it was observed that the extent of the most strongly enhanced effect of UBAP2L on YFP reporter expression was dependent on UBAP2L directed to targeting sites within the 3'UTR and coding regions (FIG. **4**K). In contrast, significant 4EBP1-mediated reporter repression was only observed when 4EBP1 was targeted to the 5' UTR, as expected (FIG. **10**K). Normalized YFP mRNA levels were not significantly different between RCas9-UBAP2L and RCas9 expressing cells transfected with gRNA 2 (which elicited the strongest increase) (FIG. **4**L). These results indicate 305 that UBAP2L's positive effect on reporter expression was not due to upregulation of reporter mRNA. The UBAP2L-RCas9 results indicate a programmable means to enhance translation and further corroborate the observations from eCLIP and tethering in another orthogonal manner.

Example 7—UBAP2L Binds to RNA Via the RGG Domain and Crosslinks to the Expansion Segments of the Ribosome

[0133] To gain molecular insight into the mechanisms by which UBAP2L enhances mRNA translation, it was determined which protein domains mediate UBAP2L's interaction with RNA. UBAP2L is predicted to contain only two structured domains: a ubiquitin-associated (UBA) domain and an Arg-Gly-Gly repeat (RGG) domain, a common RNA and protein binding domain. Using inducible lentiviral vectors, UBAP2L was expressed, or truncated versions lacking the UBA domain (DUBA), the RGG domain (DRGG), or both (FIG. **5**A), in UBAP2L knockout HEK293T cells. Then, UV-crosslinking, immunoprecipitation, RNA fragmentation and radiolabeling was performed to visualize RNA bound to UBAP2L (FIG. **5**B). Deletion of the RGG domain resulted in dramatically reduced recovery of RNA, indicating that the interaction between UBAP2L and RNA is mainly mediated by the RGG domain (FIG. **5**C).

[0134] Given that UBAP2L cofractionated with monosomes and polysomes in sucrose gradients, it was reasoned that UBAP2L may interact directly with functional ribosomes. It was confirmed that UBAP2L is localized to the cytoplasm (FIG. **11**A). Two UBAP2L eCLIP datasets were next examined using a repeat-family centric mapping strategy, which maps reads to consensus transcripts from repetitive and recurrent genomic loci, including ribosomal RNA (rRNA) genes. Remarkably, reads from rRNAs constituted the largest fraction (47%-72%) in both replicates, while mRNA reads totaled 22-25% (FIG. **5**D; FIG. **11**B). Closer inspection showed that reads were most highly enriched over SMInput at the expansion segments (ES) 15L, 27L of 28S rRNA, and ES7S of 18S rRNA (FIGS. **11**C-**11**F), which are located at the solvent-exposed surface of ribosomes and are thought to engage with RBPs and mRNAs to modulate translation. As a further measure of the confidence of fold-enrichment, an information theoretic metric was utilized, relative entropy, which scales each enrichment with the strength of evidence (i.e. read depth) at each peak. It was confirmed that the peaks at ES15L, ES27L and ES7S (and an additional peak at ES31L) contained high information content (FIG. **5**E; FIG. **11**G). In contrast, the mean of 446 other RBPs shows very limited information content as a reflection of their specificity for binding the rRNAs. These crosslinking results indicate that UBAP2L directly interacts with ribosomes. This is consistent with a previous UBAP2L IP-mass spectrometry study that recovered peptides from 15 ribosomal proteins, further supporting a UBAP2L-ribosome interaction.

[0135] To assess the spatial arrangement of UBAP2L and the ribosome, these interactions were mapped onto the cryo-electron microscopy structure of the mammalian ribosome. The top ribosomal proteins that co-immunoprecipitate with UBAP2L cluster in the 60S subunit (FIG. **5**F). In addition, ES31L, which is highly enriched for UBAP2L binding, lies close to the region of the 60S subunit, which is normally occupied by tRNA in the peptidyl site (P site) during protein synthesis (FIGS. **5**G-**5**H). Collectively, these data support a model in which UBAP2L's function is associated with interactions with the ribosome.

[0136] Furthermore, the transcriptome-wide analyses reveal that UBAP2L affects a significant number of mRNA targets, wherein mRNAs targeted by UBAP2L are themselves enriched for central regulators of translation, and protein synthesis (FIG. **6**A), revealing a role for UBAP2L in modulating protein homeostasis in a global manner. The current working model proposes that UBAP2L is dynamically recruited to translating ribosome-mRNP complexes to enhance translation on many targets, including translational regulators to affect global protein synthesis (FIG. **6**B).

OTHER EMBODIMENTS

[0137] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is:

1. A method of modulating gene expression of a target RNA in a cell comprising:

(a) assembling a modulation unit, wherein the modulation unit comprises an RNA binding protein (RBP), an exogenous RNA binding moiety, and a gene-editing agent;

(b) delivering the modulation unit into the cell; and

(c) detecting change in the target RNA translation, wherein the modulation unit modulates gene expression of the target RNA in the cell.

2. The method of claim **1**, wherein the exogenous RNA binding moiety comprises a MS2 bacteriophage coat protein (MCP).

3. The method of claim **1**, wherein the gene-editing agent comprises CRISPR components.

4. The method of claim **1**, wherein the gene-editing agent comprises shRNAs, siRNAs, ASOs, or microRNa mimics.

5. The method of claim **1**, wherein the delivering step (b) comprises lipofection.

6. The method of claim **1**, wherein the delivering step (b) comprises a virus-based delivery.

7. The method of claim **6**, wherein the virus-based delivery comprises adeno-associated virus or lentivirus.

8. The method of claim **2**, wherein the detecting step (c) comprises using a reporter mRNA.

9. The method of claim **8**, wherein the reporter mRNA comprises a luciferase mRNA.

10. The method of claim **1**, wherein the target RNA is an endogenous mRNA.

11. The method of claim **1**, wherein the target RNA is a non-coding RNA.

12. The method of claim **1**, wherein the RBP is BTG1, CNOT2, CNOT4, CNOT7, CPSF5, DDX6, EWSR1, FUBP1, hnRNPA0, hnRNPC1/2, MEX3C, NANOS1, NANOS2, NOP56, PARN, PRR3, RBM14, RBM7, RPS6, SAMD4A, SNRPA, SRSF11, TOB1, TOB2, UTP11L, YTHDF2, ZC3H18, ZCCHC11, ZFP36, ZFP36L1, ZFP36L2, ABT1, AC004381.6, AIMP1, ALDH18A1, ANXA2, APOBEC3F, ASCC1, ATP5C1, BCCIP, BOLL, BYSL, BZW1, CELF5, CLK1, CLK2, CPSF1, DAZ2, DAZ3, DAZ4, DCN, DDX1, DDX19B, DDX20, DDX39A, DMPK, EEF1A1, EIF3G, ERAL1, XOSC4, FAM46A, FAM98A, FKBP3, FXR2, G3BP2, GLTSCR2, GSPT2, GTF2F1, GTPBP10, HADHB, HDGF, hnRNPE1, HNRPDL, HSPB1, KIAA1324, LARP1, LARP4, LARP4B, LIN28A, LUC7L, MAK16, MATR3, MBNL2, MEPCE, MRPL39, MTDH, NDUFV3, NUFIP2, NUSAP1, PABPC1, PABPC5, PCBP4, PEG10, PPAN, PPIL4, PRPF3, PRPF31, PRRC2B, PTRH1, PUS7, RBM33, RBM38, RBMX2, RPL10A, RPL14, RPL15, RPLP0, RPS20, RPUSD3, RPUSD4, RTN4, SERBP1, SF3A3, SFRS10, SFRS13A, SFRS2IP, SLC7A9, SMN1, SPATS2L, SRSF5, SRSF8, THOC1, TRA2A, TRIM39, TUFM, UBAP2L, UTP23, XPO5, XRN1, YWHAE, or ZRANB2.

13. The method of claim **1**, wherein the gene expression of the target RNA is upregulated.

14. The method of claim **1**, wherein the gene expression of the target RNA is downregulated.

15. A method of identifying a function of an RNA binding protein (RBP) comprising:

(a) contacting the RBP to an exogenous RNA binding moiety;

(b) allowing the exogenous RNA binding moiety to interact with an RNA structural motif; and

(c) profiling the RBP tethered to the RNA structural motif, thereby identifying a function of the RBP.

16. The method of claim **15**, wherein the exogenous RNA binding moiety comprises a MS2 bacteriophage coat protein (MCP).

17. The method of claim **15**, wherein the RNA structural motif comprises a reporter mRNA.

18. The method of claim **17**, wherein the reporter mRNA comprises a MS2 genomic RNA stem-loop.

19. The method of claim **15**, wherein the profiling comprises transcriptome analysis or gene expression analysis.

20. The method of claim **15**, wherein the profiling comprises enhanced cross-linking immunoprecipitation (eCLIP).

* * * * *