Supplementary materials and methods

Antisense oligonucleotide, 2'MOE, and siRNA treatment

Lipofectamine RNAiMax (Invitrogen, USA) was used for transfecting ASOs and siRNAs as per manufacturer's instructions. For DNM3OS depletion, 5-10-5 MOE DNA gapmer antisense oligonucleotides (ASOs) with phosphorothioate internucleosidic linkages were designed and synthesized by Ionis Pharmaceuticals, Inc. They were modified with five 2'-O-methoxyethyl nucleotides on the 5' and 3'ends and ten consecutive deoxynucleotides to support RNase H activity. ASOs were transfected at 40nM, for two consecutive days. Cells were collected two days after the 2nd round transfection. ASO Ionis #: Scramble ASO: #559631, ASO70: #1432970, ASO73: 1432973.

For blocking the interaction between Drosha/DGCR8 (MOE51,53)/SRSF1 (MOE60) and *MIR222HG* transcripts, uniform MOE with 2'-phosphodiester (phosphate backbone) were designed and synthesized by Ionis Pharmaceuticals, Inc. MOEs were transfected at 40nM for one round. Cells collected the next day after transfection. MOE Ionis#: Scramble MOE: #1064923, MOE51: #1023351, MOE53: #1023353, MOE60: 1022860. The siRNAs for Drosha and DGCR8 depletion were from IDT predesigned siRNA pool (HSS.RNAI.N013235.12.1 for Drosha, HSC.RNAI.N022720.12.1 for DGCR8). siRNAs were transfected at 40nM for two consecutive days. Cells were collected days after 2nd round transfection. The sequences of antisense oligonucleotides (ASO), Uniform MOE, and siRNAs are upon request.

Reverse transcription and quantitative PCR (RT-qPCR):

Total cellular RNA was extracted from the cells using Trizol^{TM} (Invitrogen, Cat# 15596018) as per manufacturer's instructions and reverse transcribed into cDNA using Multiscribe Reverse transcriptase and Random hexamers (Applied Biosystems, Cat# 4368813). RT-qPCRs were performed using the StepOne Plus system (Applied Biosystems). Transcript levels were quantitated against a standard curve by Real-time RT-PCR using the SYBR Green I fluorogenic dye and data analyzed using the StepOne Plus system software. Primer sets used for RT-qPCR were listed in the primer table. 18s rRNA is used as reference gene to normalize gene expression in most experiments unless stated otherwise.

Northern blot

For northern blot to detect MIR222HG, polyA$^+$ RNA was purified using NucleoTrap® mRNA Mini kit (MACHEREY-NAGEL, Cat# 740655). 1.2µg polyA$^+$ RNAs and ssRNA marker (NEB, N0362S) were separated by 1% formaldehyde agarose gel. The gel was prepared using NorthernMax™ Denaturing Gel Buffer (Ambion, AM8676) and run using NorthernMax™ MOPS Gel Running Buffer (Ambion, AM8671). RNA gel was washed in nucleotide-free water 30min, 2 times, followed by transfer with upward capillary transfer method in 10x SSC buffer to Amersham Hybond-N+ blot (GE Healthcare, RPN1520B). RNA was then fixed by UV crosslinking with 120 mJ/cm2. Linear DNA fragments were released from plasmids for random priming reactions. Probes were prepared using dCTP, [α-32P] (PerkinElmer, NEG013H100UC) with Prime-It II Random Primer Labeling Kit (Agilent, Cat#300385) as per the manufacturer's instructions. Hybridization was done overnight at 42 degrees in ULTRAhyb hybridization buffer (Ambion, AM8670) as per the manufacturer's instruction. Blots were washed at 42 degrees using 2X SSC+0.1%SDS and 0.1xSSC+0.1%SDS. Blot was developed using x-ray files.

To detect miRNAs miR-221 and miR-222, 20 µg total RNAs were separated by 12% polyacrylamide/8M urea denaturing gel electrophoresis and electroblotted to Hybond N+ membrane (GE Healthcare). RNA was then fixed by UV crosslinking with 120 mJ/cm2. Oligo probes were labeled with [γ-32P] ATP with T4 polynucleotide kinase (NEB). Decade Markers were used as a ladder (Ambion, AM7778). ULTRAhyb-Oligo Hybridization Buffer was used as per the manufacturer's instruction (Ambion, AM8663).

Immunoblotting

Immunoblotting was performed as previously described (1). Antibodies used in this study include: SRSF1 (1:1000, sc-33652), ILF3 (also called NF90/110, 1:1000, Bethyl, A303-120A), ILF2 (also called NF45, 1:1000, Santa Cruz, sc-365283), α-tubulin (1:10000, Sigma-Aldrich, T5168), P27 (1:200, Santa Cruz, sc-528), p-Rb (Ser780) (1:300, Cell Signaling), CyclinA (1:100, Santa Cruz, sc-751), CyclinD1 (1:500, 2922s, Cell Signaling), EIF4E(1:400, Santa Cruz, sc-9976).

Propidium Iodide (PI) Flow Cytometry

PI flow was performed as described previously (1).Quantification was performed using FCS Express.

BrdU labeling

WI-38 cells were incubated in BrdU (10µM for 45 min) (Sigma, USA), staining was performed as previously described (1).

shRNA knockdown

All shRNA sequences were listed in the 'shRNA sequences' table below. *MIR222HG* shRNA constructs were cloned from plko.1 construct using protocol from Addgene (http://www.addgene.org/protocols/plko/). The control construct was from Sigma (MISSION® pLKO.1-puro Non-Mammalian shRNA Control Plasmid DNA, SHC002). For lentivirus packaging, HEK293T cells were transfected with Plko.1 construct, pCMV-VSV-G, and pCMV-dR8.2 plasmids. For 10cm^2 culture, 3µg Plko.1, 300ng pCMV-VSV-G, and 2.7µg pCMV-dR8.2 plasmids were transfected using lipofectamine 2000 (Invitrogen, Cat# 11668019). After overnight transfection, fresh 30% FBS contained medium was replaced for cells followed by 48 hrs incubation. Then, the medium was collected, centrifuged (to remove cell debris), aliquoted, and stored at -80°C. The thawed virus was added to WI-38 cells for 24 hrs, followed by replacing the puromycin-contained medium (2.5µg/mL) to select the infected cells. For most of serum starvation-serum stimulation experiments, WI-38s were infected on day 0, and then the medium was placed to 0.1% FBS medium with puromycin to induce cellular quiescence for three days, followed by serum stimulation. Lentiviral shRNA infected cells were never passaged and kept as stable cells.

RNA IP

RNA IP were performed based on published protocol from (https://www.epigenesys.eu/images/stories/protocols/pdf/20111026164810_p28.pdf). 2µg SRSF1 antibody was used (sc-33652). Pulldown RNA was treated by DNAse1 followed by reverse transcription. Percentage of input was calculated by comparing the relative RNA levels from pulldown samples and input.

RNA affinity assay

RNA affinity assay was performed as described previously (2) with minor modifications when detecting interacting proteins. Specifically, RIPA buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) was used for extracting whole cell lysis from 10^7 WI-38 cells (at 6hr serum release condition). Then cell lysis was diluted 10 times using NT2 buffer (50 mM Tris-HCl, pH 7.4, 150mM NaCl, 1mM MgCl2, 0.05% Nonidet P-40). For pulldown, 40µL Dynabeads M-280 Strepatavidin was washed 2 times with Buffer A (0.1M NaOH, 0.05M NaCl), and 1 time with Buffer B (0.1M NaCl). 1mL of nuclear extract was added to beads for pre-clearing at 4°C for 1hr. Pre-cleared lysate was mixed with 1.5µg biotin-labeled RNA probe and incubated for 2-3 hrs. Another 40 µL washed beads were blocked in 1mL of NT2 buffer with 10uL 50mg/mL BSA, 5µL 10mg/mL Yeast t-RNA (Sigma, Cat# R5636) for 3-hrs. Then, lysate with the biotin RNA probe mix was added to the blocked beads for overnight pulldown. Beads then washed with high salt buffer (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl pH 8.0, 500mM NaCl), low salt buffer (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl pH 8.0, 150 mM NaCl) and TE buffer (1mM EDTA, 10mM Tris-HCl pH 8.0). 40µL protein loading buffer was then added to the beads and heated at 95°C for 10min for protein samples in western blotting.

Luciferase assay

Luciferase Assay was performed as described previously (3). Plasmids carrying 3'-UTR sequences of p27 (psiCHECK2-p27Kip1 3'UTR was transfected into WI-38 cells, then subject to dual-luciferase reporter assay using Dual-Luciferase® Reporter Assay System (Promega, #TM040), as per manufacturer's instructions.

RNA Stability Analysis

RNA stability analysis was carried out by incubating 5 µg/ml of Actinomycin D cells. Samples were then collected at indicated time points, and the relative RNA levels at specific time points were normalized to the 0-hour time point (right before the addition of Actinomycin D) when analyzing RT-qPCR results.

Nuclear and cytoplasmic fractionation

For nuclear and cytoplasmic fractionation, cells were washed with PBS once, then resuspended in 250 µL of lysis buffer (10mM Tris-HCl pH7.4, 100 mM NaCl, 2.5 mM $MgCl_2$, 40 µg/mL digitonin, RNase inhibitor, protein inhibitors) and incubated on ice for 10 min. Centrifugation was done for 10 min, at 2000g, 4°C and supernatant was collected as cytoplasmic fraction. The cytoplasmic fraction was then mixed with 750 µL Trizol™ LS (Invitrogen, Cat# 10296028) for RNA extraction as per the manufacturer's instructions. The pellet was resuspended and lysed in Trizol™ (Invitrogen, Cat# 15596018) for RNA extraction as per the manufacturer's instructions.

RNA Complementarity analysis

Complementarity of human MIRR222HG and target transcripts was analyzed using methods we reported in previous studies (4-6). We utilized the BLAST for "Optimize for Somewhat similar sequences" with "alignment parameters": Expect threshold = 1000000, word size = 7, and filter masker = none. We parsed the results to obtain a predetermined sufficient number of hits: E value <=200, Word Size >= 15, and match reverse/complementary strand.

Copy number analysis

Copy number of human *MIR222HG* transcripts were analyzed as described previously (2).

RACE

Rapid amplification of cDNA ends (RACE) was performed using GeneRace kit (Invitrogen) as per the manufacture's protocol. Both 5' RACE and 3' RACE were performed, using gene-specific-primers (listed in the primer table) and the GeneRacer primers provided with the kit. Clones from RACE were sequenced and analyzed to determine the 5' and 3' of *MIR222HG* transcript.

Polysome fractionation

Polysome fractionation experiments were performed as previously described (7, 8).

Proximity Ligation Assay (PLA)

PLA was performed using Duolink In Situ Red (Sigma) as per the manufacture's protocol. Cells on coverslips were fixed in 2% paraformaldehyde for 15 min at room temperature and permeabilized on ice with PBS containing 0.5% Triton X-100 for 5 min. Coverslips were then blocked in blocking solution at 37 °C for 1h and incubated with primary antibodies (ILF3: 1:1000, ILF2: 1:50) overnight at 4 °C. After washing twice in buffer A, coverslips were incubated with Duolink PLA probes anti–mouse MINUS and anti–rabbit PLUS at 37 °C for 1h. Coverslips were then washed twice in buffer A and preceded to ligation and amplification. Ligation was done by incubating the coverslips in the ligation reaction mixture (1 µl of ligase in 40 µl of 1x ligation buffer per coverslip) at 37 °C for 30 min. Coverslips were next washed twice in buffer A and incubated in the amplification mixture (0.5 µl polymerase in 40 µl of 1x amplification buffer per coverslip) in the dark for exact 100 min at 37 °C. After washing twice in buffer B and once in 0.01x buffer B, coverslips were stained with DAPI and mounted.

Mass spectrometry

Interacting proteins were fractionated by SDS-PAGE and each lane was cut into 10 slices. The protein bands were then in-gel digested with trypsin (Thermo) overnight at 37 °C, as described (9). The peptides were extracted following cleavage and lyophilized. The dried peptides were solubilized in 2% acetonitrile, 0.5% acetic acid, 97.5% water for mass spectrometry analysis. They were trapped on a trapping column and separated on a 75 µm x 15 cm, 2 µm Acclaim PepMap reverse phase column (Thermo Scientific) using an UltiMate 3000 RSLCnano HPLC (Thermo Scientific). Peptides were separated at a flow rate of 300 nL/min followed by online analysis by tandem mass spectrometry using a Thermo Orbitrap Fusion mass spectrometer using a linear gradient from 96% mobile phase A (0.1% formic acid in water) to 55% mobile phase B (0.1% formic acid in acetonitrile) over 30 minutes. Parent full-scan mass spectra were collected in the Orbitrap mass analyzer set to acquire data at 120,000 FWHM resolution; ions were then isolated in the quadrupole mass filter, fragmented within the HCD cell (HCD normalized energy 32%, stepped ± 3%), and the product ions analyzed in the ion trap. Proteome Discoverer 2.1 (Thermo) was used to search the data against human proteins from the UniProt database using SequestHT. The search was limited to tryptic peptides, with maximally two missed cleavages allowed. Cysteine carbamidomethylation was set as a fixed modification, and methionine oxidation set as a variable modification. The precursor mass tolerance was 10 ppm, and the fragment mass tolerance was 0.6 Da. The Percolator node was used to score and rank peptide matches using a 1% false discovery rate. The mass spectrometry proteomics data have been

deposited to the ProteomeXchange Consortium via the PRIDE (10) partner repository with the dataset identifier PXD017585 and 10.6019/PXD017585.

Primers and shRNA sequences
RT-qPCR primers:

MIR222HG-Exonic-F	TGCAGAGAAAGGACCGCAAG
MIR222HG-Exonic-R	GGCTGGTGTGTGAGACCATT
MIR222HG-Intron-F	GCAGCCCCCTGTCATTACTT
MIR222HG-Intron-R	CTCACCCGTGCAATGTCTCT
MIR222HG-Intron-2-F	ACATTATCAGCTGGGGCTTGG
MIR222HG-Intron-2-R	CAGATTCCTCCCCCTTGTAGT
pri-MIR222HG-F	GTCTCACCAATGCTACCCCT
pri-MIR222HG-R	ATCAGCTTTCTTGCGGTCCT
MIR222HG-Spliced-F	AAGCAGCAGCATTCTGTCAC
MIR222HG-Spliced-R	GCGGTCCTTTCTCTGCACTC
MIR222HG-Spliced-2-F	TATCAAGCTGCGAAGAGGGAG
MIR222HG-Spliced-2-R	GAGGTGATCAGCTTTCTTGCG
SRSF1-RIP-F	AGAAGGCAAAGGATCACCCAG
SRSF1-RIP-R	CTCTCAGGACACTGAAGCAGAA
DNM3OS-F	ATTTATGGGAGAGAGAGGAAAAGT
DNM3OS-R	GCATTCAGTGCAACAGAAAGTA
DNM3OS-F2	GCATGCATCATTCCTTTAATTGAGC
DNM3OS-R2	AACTTTTCCTCTCTCTCCCATAAA
EIF4E-F	CCCCTACTCCTAATCCCCCG
EIF4E-R	GCCCATCTGTTCTGTAGGGG
EIF4E-3'UTR-F	GGCTAGAGTTTCCACTATCCCA
EIF4E-3'UTR-R	TTACAGAGTGGGCCAGGAAC
NORAD-F	AGCGAAGTCCCGAACGACGA
NORAD-R	TGGGCATTTCCAACGGGCCAA
GAPDH-F	GAAACTGTGGCGTGATGGC
GAPDH-R	CACCACTGACACGTTGGCAG
ACTB-F	TGCGTTACACCCTTTCTTGA
ACTB-R	AAAGCCATGCCAATCTCATC
MALAT1-F	TTCCGGGTGTTGTAGGTTTC
MALAT1-R	TCTCCAGGACTTGGCAGTCT
18SrRNA-F	CGCCGCTAGAGGTGAAATTCT
18SrRNA-R	CGAACCTCCGACTTTCGTTCT

shRNA sequences:

Name	Sequence
MIR222HG-sh1	CCGGAACCAGAGATAAGGTGTAATGCTCGAGCATTACACCTTATCTCTGGTTTTTTTG
MIR222HG-sh2	CCGGAACCAGTGGAGTTTATCATTGCTCGAGCAATGATAAACTCCACTGGTTTTTTTG
SRSF1-sh1	CCGGAAGGATTGTGGAGCACATTTCCTCGAGGAAATGTGCTCCACAATCCTTTTTTTG
SRSF1-sh2	CCGGGAAGCAGGTGATGTATGTTATCTCGAGATAACATACATCACCTGCTTCTTTTT *SRSF1-sh2 is a commercial shRNA clone from Sigma: TRCN0000001095

Other primers and probes:

Customized Oligo dT RT primer	CGTACGATACGCTACGTAACGGCATGACA GTGTTTTTTTTTTTTTTTT
U6-3tail-qPCR-F	CGGCAGCACATATACTAAAATTG
222-3tail-qPCR-F	GCCAGTGTAGATCCTGTCTTT
3tail-qpcr-R	GCTACGTAACGGCATGACA
MIR222-Northern-probe	GAGACCCAGTAGCCAGATGTAGCT
MIR221-Northern-probe	GAAACCCAGCAGACAATGTAGCT
U6-Northern-probe	GCTAATCTTCTCTGTATCGTTCCAATTTTAGTATATGTGCTGCCG
Race-for-5'-end	GTTATCTGCATTTCATTCAAATGTTCCTTGTTTGG
Race-for-5'-end-nested	TCTCCCCTCCCCCGACATTT
Race-for-3'-end	TCTCACTTACCTGCAGAAACCCAGT
Race-for-3'-end-nested	GAATGACCCTCAATCTCACTGGAATC

Full-length spliced *MIR222HG* sequence:

Color-coding represents: Exon1-Exon2-Exon3-Exon4

GTTCTGGGCTTGCCTGGGAGAAGGAGCTGTTGCAGTCTTGCACGATCAAAATAGGGCCTTTAGGTTT
GAAGGATGAGGTTGAGCCCATATTTTGACTCCAAATGTCGGGGGAGGGGAGAAGAAGTCGACAGAT
CCAAACAAGGAACATTTGAATGAAATGCAGATAACTCTTTAGAAAACTTTTGTAGGAAAAGCGCCTCG
TGTGATCTGGAACAAAGACGTTTTATAATCAGATTTATCAAGCTGCGAAGAGGGAGTTTTAGTGAACG
GAAGCAGCAGCATTCTGTCACCAGGAAATACTCCTTTATCCAGAATTGAGCCCCTCCCCAGAAGGCA
AAGGATCACCCAGCTGCTGGAAGGTGAGGTATAAACCAGTGGAGTTTATCATTGAATAGAGTGCAGA
GAAAGGACCGCAAGAAAGCTGATCACCTCAGCAATGAATGATTTCAATACGATTTCACCCAAATGGT
CTCACACACCAGCCTAGTAAACCAGAGATAAGGTGTAATGTCATCCTTCCTTCCTTCTCCCTGCCTCA
ACTGTCAAAGATTGACAACTTACATGTTGTTTACTTTTTGGCTTCTATTTCTGTTCATAATTATTATCAG
AAGGCATAGCACCCACAAGCCTAATCATACTGCCTTACAACCTTATTCCTCTTCATTTTGTCTTCTCAC
TTACCTGCAGAAACCCAGTTTATTTTTGTTCTCCCTTTACCACACCAGCCTTGTAACTGTTATGAATGA
CCCTCAATCTCACTGGAATCTGAATCACTCAGGAATTCAAGCAAACTGGATGTTTTAACCACTGTTCA
GCTTTCTTATGGAATGACAGAGAACTTGTAAAGATAAAACACCAGTTTGCAGGAAGAAAGGAAGAGA
ATGGAAATTGCTTCTGGAAAATACTAGTTTTACAATATGTTTTGTTTGTCTGCTCTCTTAAATAAACTTA
ATCCTATAAACATTTTTTAAGAACTAGCCATTAAGACTGTTAAGTTCTCAATTATAAAGGAATAAAATGT
TTTAAGGAGGATTTATTTGCCCTTGCTTATATGAACTGCATATTTTTAGTAGGAAATCATGTAATTTAAA
TTAGTATTGGTTAACAGATTTAACGTAGAAAATGGACATATAAAGAATACAAGGATAAATGTCTACATG
TAATAATGGATGGTAGTGATATAATGCATTTTTGTCATCCCATGCTGAATTCAGTAAAAATAAAGCTAA
TGACTTGGAGCTTTTAAAAGAT

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