Supplementary Information for

The eukaryotic translation initiation factor eIF4E elevates steady-state m\textsuperscript{7}G capping of coding and non-coding transcripts

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**Supplementary Information Text**

**Subhead.**

**Extended Materials and Methods**

**Reagents and Constructs**

*pcDNA2Flag-eIF4E* wildtype or S53A mutant constructs were previously described (1, 2). *pcDNA3.1-lacZ*, *LacZ-4ESE, 3'UTR-Full, 3'UTR-A and 3'UTR-B* (all from human *CCND1*) constructs were previously described (3). *pcDNA3.1-LacZ 3'UTR-C* (contains segment 1611-2459) and *pcDNA3.1-LacZ 3'UTR-D* (2091-2458) was amplified from *pcDNA3.1- LacZ-3'UTR-Full* construct using specific primers containing NotI (forward primer) or XbaI (reverse primer) restriction sites at their 5’ ends, and cloned downstream of LacZ. The first exon was amplified from *CCND1* cDNA (ATCC MGC-2316) and cloned using the HindIII site, upstream of AUG codon of *pcDNA3.1-LacZ* construct. All constructs were confirmed by sequencing.

**Cell Culture**

U2Os cells (obtained from ATCC, CA) were maintained in Dulbecco's modified Eagle's medium (DMEM) (ThermoFisher Scientific) supplemented with 10% fetal bovine serum (FBS) (ThermoFisher Scientific) and 1% penicillin-streptomycin (ThermoFisher Scientific), at 37°C and 5% CO₂. The identity of U2Os cell lines was authenticated using STR profiling (Montreal EpiTerapia Inc). All cell lines were routinely checked to ensure that there was no mycoplasma contamination by PCR (4). eIF4E-Flag wildtype, mutant or vector control stable cell lines were generated as previously described (1), using TransIT-LT1 Transfection Reagent (Mirus) as specified by the manufacturer and G418
(Wisent Bioproducts) at 1 mg/mL for selection. Selected clones were screened for the presence of Flag-eIF4E by Western blot.

Transient transfections with pcDNA3.1-lacZ constructs were carried out using TransIT-LT1 Transfection Reagent (Mirus) according to the manufacturer's instructions. RNAs were isolated 36h after transfection using Trizol (ThermoFisher Scientific) and Direct-zol RNA Miniprep Kit (Zymo Research).

**siRNA transfections**

For RNMT knockdowns, vector or eIF4E overexpressing cells were transfected with Lipofectamine 2000 (ThermoFisher Scientific, Cat# 11668019) and 25nM siRNA duplex according to the manufacturer's instructions. Cells were analyzed 48 hr after transfection. siRNA duplexes purchased from IDT Technologies CA, were as follows:
siRNMT duplex (hs.Ri.RNMT.13.4): sense: CAAGCUAGUUGUACUGA and antisense: GGCAAUAUCAGUACAAAC
siCtrl (siLuciferase): sense: CACGUACGCGGAAUACUUCGAAATG and antisense: CAUUUCGAAGUAUUCCGCGUACGUGUU

**Generation of CRISPR clones with eIF4E disruption**

The plasmid Lenticrisprv2 was purchased from Addgene (5, 6). Lenticrisprv2 plasmids with the following sgRNA inserts were kindly provided by the lab of Dr. Mike Tyers (IRIC, Montreal): for EIF4E (TTAAACATCCCCCTACAGAAC) and for Azami-Green protein as the control (GGCCACAACTTCTCGTAGTGAAGAAATG). U2OS cells (ATCC) were transfected with
these plasmids using TransIT-LT1 (Mirus) and selected with puromycin at 1µg/mL. Clones were screened for eIF4E expression by western blot. In clones with reduced protein expression, the area around the sgRNA sequence was amplified by PCR from genomic DNA using the following primers: Forward: 5’-GCT GTC ATA GAT GTG TTA AGA C-5’ and Reverse: 5’-CCT GGG TTC AAG CAA TTA TCT TCT C-3’. Gene disruption was confirmed by Sanger sequencing with the same forward primer as used for the PCR.

**Polysome profiling**

Polysome profiling was done as previously described (7, 8). Briefly, after 10 minutes treatment with cycloheximide (100 µg/mL), cells were harvested and lysed in Polysome lysis buffer (15mM Tris pH 7.4, 250mMNaCl, 15mM MgCl₂, 1% Triton X-100, 100µg/mL cycloheximide, 1mM dithiothreitol, 400 U/mL RNaseOUT (ThermoFisher Scientific), and protease inhibitors (Roche). Equal amounts (2 mg) of cell lysates were layered on a 20% to 50% linear sucrose gradient, and centrifuged in a Beckman SW41Ti rotor at 92 000xg for 3 hours at 4°C. Sucrose gradients were prepared by mixing 20% and 50% sucrose solutions (prepared in 15mM Tris pH 7.4, 15mM MgCl₂, 150mM NaCl, 1mM dithiothreitol, 100 µg/mL cycloheximide, and 20 U/mL RNAseOut) on Gradient Station IP (Biocomp). After centrifugation, polysome fractions were collected by continuously monitoring and recording the A₂₅₄ on a Gradient Station IP (Biocomp) attached to a UV-MII (GE Healthcare) spectrophotometer. RNAs were isolated from polysome fractions using Trizol reagent (ThermoFisher Scientific). RNAs were reversed transcribed using MMLV reverse transcription and analyzed by RT-qPCR. RNA content of individual fractions are displayed as a percentage of the given fraction compared to the
entire profile (area under the curve, calculated as a sum of all fractions) for each RNA analyzed.

**Cellular fractionation and RNA export assays**

Fractionation was carried out as described (7). Approximately $3 \times 10^7$ cells were collected and washed twice in ice cold PBS (1200 rpm, 5 min) and then re-suspended with slow pipetting in 1 ml of Lysis Buffer B (10mM Tris pH 8.4, 140mMNaCl, 1.5mM MgCl2, 0.5% Nonident P-40, 1mM DTT and 100 U/ml RNaseOUT (ThermoFisher Scientific). The lysates were centrifuged at 1000g for 3 min at 4°C and the supernatant (cytoplasmic fraction) was transferred into a fresh microtube. The pellet (nuclear fraction) was resuspended in 1 ml of Lysis Buffer B, transferred to round bottom polypropylene tube and 1/10 volume (100 µl) of detergent stock (3.3% (w/v) Sodium Deoxycholate, 6.6% (v/v) Tween 40 in DEPC H₂O) was added with slow vortexing, and incubated on ice for 5 min, transferred to a microtube and centrifuged at 1000 g for 3 min at 4°C. Supernatant (post-nuclear fraction) was transferred to a fresh tube and the pellet (nuclear fraction) was rinsed in 1 ml of lysis buffer B and centrifuged at 1000 g for 3 min at 4°C. The postnuclear and cytoplasmic fractions were combined. The RNA was extracted from the different fractions by adding TRIzol reagent (ThermoFisher Scientific) and Direct-zol RNA Miniprep Kit (Zymo Research) according to the manufacturer’s instructions.
**Immuno-dot blots**

For dot immunoblot analysis, purified RNAs (total or from nuclear or cytoplasmic fractions) were diluted to appropriate concentrations (125, 250 or 500 ng/μl), denatured for 15 min at 65°C and chilled on ice for additional 10 min. Then 2μl of each dilution was spotted onto the positively charged nylon membrane (BrightStar-Plus, Ambion). Membranes were dried, then UV crosslinked at 254 nm for 4 min (both sides).

Membranes were blocked for 1h at room temperature with 2%BSA (in TBS-Tween 20) and incubated with anti-Cap antibody (MBL, RN016M, 1μg/ml in 2% BSA) ON at 4°C. After washing in TBS-Tween 3x5min, membranes were incubated with HRP-conjugated anti-mouse secondary antibodies (Jackson Immuno Research) diluted 1:10 000 in 5% milk (TBS-Tween solution) for 1h at room temperature. After washing 3x 5min with TBS-Tween20, blots were visualized using SuperSignal™ West Pico PLUS Chemiluminescent Substrate (ThermoFisher Scientific) and X-ray films.

**Reverse transcription and quantitative PCR**

DNAse treated RNA samples (Direct-zol RNA Miniprep Kit (Zymo Research)) were reversed transcribed using MMLV reverse transcription (ThermoFisher Scientific) for RNA export assays, total RNA levels and polysome profiling experiments. SuperScript VILO cDNA synthesis kit (ThermoFisher Scientific) was used for RIP and CapIP experiments. RT-qPCR analyses were performed using SensiFast Sybr Lo-Rox Mix (Bioline) in Applied Biosystems QuantStudio thermal cycler using the relative standard curve method (Applied Biosystems User Bulletin #2). All conditions were described previously (7). The list of primers is given in Table S3.
**Western blot analysis**

Western blot analyses were performed as described (2). Briefly, ~70% confluent cells were washed in plates with ice-cold phosphate buffer saline and lysed with 0.5ml RIPA buffer (Tris-HCl 50mM, NaCl, 150mM, Nonident P-40 1%, sodium deoxycholate 0.25%, SDS 0.1%) containing fresh protease inhibitor cocktail. After sonication and centrifugation for 10 min 10 000rcf, lysate protein concentration was determined by BCA assay (Pierce) and 20 μg of protein samples were loaded and separated on 12% polyacrylamide electrophoresis gels, transferred onto PVDF membranes. Blots were blocked in 5% milk in TBS–Tween 20. Primary antibodies were diluted in 5% milk. Antibodies used for immunoblotting: mouse monoclonal anti-eIF4E (BD PharMingen), mouse monoclonal anti-β-actin (A5441Sigma Aldrich), rabbit polyclonal anti-Mcl1 (S19, sc819 Santa Cruz), rabbit polyclonal anti-Myc (ab32072 Abcam), rabbit polyclonal anti-CyclinD1 (ab134175 Abcam), mouse monoclonal anti-RNGTT (D3, sc377464 Santa Cruz), rabbit polyclonal anti-RNMT (06-1355Millipore), rabbit polyclonal anti-RAM (19422-I-AP Proteintech), and mouse monoclonal anti-Hsp90 (F8, sc13119 Santa Cruz).

**RNA-immunoprecipitation (RIP)**

Nuclei isolated using the Cellular Fractionation protocol were rinsed 2x with 1xPBS and fixed with 1% PFA for 10min at RT with rotation, quenched 5min with 0.15M Glycine (RT with rotation), then washed 3 times with 1xPBS and lysed in 0.5ml NT-2 buffer by 3 times 6 seconds bursts (with 30 second pause between each burst) using microtip at 25% power (Sonic Dismembrator Model 500, Fisher, Max Output 400W). NT-2 buffer: 150mM NaCl, 50mM Tris-HCl (pH 7.4), 2.5mM MgCl₂, 0.05% (v/v) NonidentP-40,
supplemented with 1mM DTT, 1x protease inhibitors without EDTA, 200U/ml RNaseOut. Nuclear lysates were centrifuged at 10 000rcf for 10min, and supernatants were transferred into fresh tubes. After adjusting the concentration to be no more than 1mg/ml, nuclear extracts were pre-cleared with sepharose-conjugated protein G beads (GE Healthcare) for 30 min at 4°C. 5% of pre-cleared extract (relative to the amount used for RIP) was set aside to isolate Input RNA (using Trizol and Direct-zol columns), and the remainder of the lysate was incubated with 7 μg of anti-eIF4E antibody (per 1mg of the lysate) or 7 μg of appropriate IgG as a control, and 0.5 mg/ml yeast tRNA (Sigma-Aldrich), and additional 100U/ml RNaseOUT, ON at 4°C with rotation. Rabbit anti-eIF4E (RN001P, MBL), mouse anti-eIF4E (A10, Santa Cruz) and rabbit anti-RNMT (Millipore) antibodies were used for RIPs in this study as indicated in the text. After ON incubation, sepharose-conjugated protein G beads (50μl) were added and incubated for additional 2-3h at 4°C with rotation. Beads were washed once with NT-2 buffer supplemented with 1mg/mL heparin (Sigma-Aldrich) for 5min at 4°C with rotation, and an additional six times with NT-2 buffer with 300mM NaCl. In all steps that included sepharose beads, centrifugation was 500rcf/4min/4°C. After washing, beads were re-suspended in Elution buffer: 100mM Tris-HCl (pH 6.8), 4% (w/v) SDS, 20% (v/v) glycerol, 12% (v/v) β-mercaptoethanol, and incubated for 5 min at 98°C. PFA fixation was reversed by incubating lysates for input and RIPs 20min at 65°C. RNA was extracted using Trizol and Direct-zol RNA Microprep Kit (Zymo Research). Input and RIP RNAs were reverse transcribed using SuperScript VILO cDNA synthesis kit (ThermoFisher Scientific), diluted in 40ng/ml Glycogen and used for qPCR analyses. Values obtained for RIP samples were normalized to values obtained from input samples (input samples
were set to 1). Flag-RIPs from nuclear and cytoplasmic fractions were performed as described in (2). Briefly, vector control, eIF4E-Flag wildtype or S53A-Flag eIF4E mutant U2Os cells were fractionated using the protocol described above. Cytoplasmic or nuclear lysates in NT-2 buffer were pre-cleared with sepharose-conjugated protein G beads (GE Healthcare) for 30 min at 4°C. 10% of pre-cleared lysates were set aside to isolate Input RNA, and 1mg of the lysate was incubated with 8μg of anti-Flag antibody (M2, Sigma-Aldrich), 0.5 mg/ml yeast tRNA (Sigma-Aldrich), and additional 100U/ml RNaseOUT, ON at 4°C with rotation, and processed the same as described above for other RIPs. To calculate enrichment of RNAs, Flag-IP samples were normalized to their respective input samples and finally presented as enrichment relative to the vector control (which served to monitor background binding to the Flag antibody).

**CapIP and Cap-Sn**

25μl Sepharose-G beads (GE Healthcare) per sample were blocked in 0.2% BSA for 10min at RT°C with rotation, washed 4 times in 1xPBS and once in CapIP Buffer (0.05% BSA, 1mM DTT and 0.05% TritonX-100). 5μg anti-m7Cap antibody (MBL, RN016M) or the same amount of mouse IgG (as a control) per sample was coupled to Sepharose-G beads in 200μL CapIP Buffer for 2-3h at RT (or ON at 4°C) with rotation. Beads were washed in CapIP Buffer. Before CapIP, RNAs (total RNAs isolated from eIF4E-overexpressing and vector control cells) were denatured for 5min at 65°C and transferred immediately to ice for an additional 5 min. 4μg of RNAs were added to 200μl CapIP Buffer containing 200U/ml RNaseOUT and 0.01mg/ml PolyU, and incubated with prepared antibodies-coupled beads for 1h at RT. As a control, RIPs coupled with anti-m7G Cap antibody in the
presence of 100µM m7GpppG was used to compare capping efficiency between vector control and eIF4E overexpressing cells (CtrlIP and Ctrl-Sn). After incubation, tubes were centrifuged 5 min at 500 rcf, and supernatants were transferred to 1ml of Trizol for Cap-Sn analysis. Beads were washed 3 times with CapIP buffer containing 0.4 mM RVC (ribonucleotide-vanadyl complex, NEB), and additionally 3 times with CapIP buffer containing RVC and 50µM GTP. After the last wash, 1ml Trizol was added and RNA isolated using Direct-zol MicroPrep kit.

Equal amounts of RNAs used for CapIPs and RNAs obtained from the CapIP experiments were reverse transcribed using SuperScript VILO cDNA synthesis kit (ThermoFisher Scientific), diluted in 40ng/ml Glycogen and used for qPCR analyses, where all CapIPs were normalized to their corresponding inputs and control CapIPs, and finally normalized to the vector control CapIP (set to 1). For Cap-Sn experiments, equal amounts of RNAs were reverse transcribed using SuperScript VILO cDNA synthesis kit (ThermoFisher Scientific), diluted in 40ng/ml Glycogen and used for qPCR analyses. All samples were first normalized to 18S rRNA and the ratio of supernatant fractions after the CapIPs (Cap-Sn) and Control IPs (Ctrl-Sn) were calculated (this ratio provided the percent of uncapped RNA in the supernatant), and then converted to the percent of capped RNA.

**Cap Quantitation (CapQ)**

We used enzymes specific to different 5’ structures on RNAs leading to a 5’-monophosphate so that transcripts could be ligated to specific RNA oligonucleotides (in
order to prepare libraries for either capped or total RNAs (Supp Figure 3). Specifically, 10μg of RNAs were denatured for 5min at 65°C, chilled on ice for 5-10min, and treated with 20U FastAP Thermosensitive Alkaline Phosphatase (AP, Thermo Scientific) for 15 minutes at 37°C. This removes the 5’ phosphate group from uncapped RNAs, leaving a hydroxyl which cannot ligate to the RNA oligonucleotides. Importantly, AP has no effect on capped RNAs. AP treated RNAs were purified using Trizol and Direct-zol mini-prep columns. The RNA is then treated with the Tobacco Acid Pyrophosphatase decapping enzyme (TAP) which specifically hydrolyzes methylated capped RNAs to release m7GDP and leaves the 5’ monophosphorylated RNAs, which are then ligated to specific RNA oligonucleotides using T4 ssRNA ligase. In parallel, in order to obtain information about the total levels of RNAs, an aliquot of AP treated RNAs were additionally treated with T4 Polynucleotide Kinase in order to add a 5’ phosphate group to uncapped RNAs prior TAP treatment. A combination of all 3 enzymes converts all RNAs to 5’-monophosphorylated forms that can be ligated to RNA oligonucleotides. Thus, analyzed RNAs are divided into two groups: 1) AP and TAP treated RNAs which represents only capped RNAs, and 2) AP, T4 Polynucleotide Kinase and TAP treated RNAs, representing all RNAs, both capped and non-capped. Specifically, 4 μg of AP treated RNAs were incubated with 4U of T4 Polynucleotide Kinase (NEB) for 30 minutes at 37°C and purified using Direct-zol micro-prep columns. 1-2 μg of AP and AP+PNK RNAs were denatured (5min at 65°C, and chilled on ice for 5-10min) and treated with 15U of TAP (Enzymax LLC)/μg RNA for 1 hour at 37°C. 2ng of in vitro transcribed and m7GpppG capped Luciferase RNA (also denatured as described) was added per sample to monitor TAP efficiency among samples and later used as a loading control. After
purifying with RNA micro Kit (Enzymax LLC), RNAs were ligated to specific RNA oligonucleotides (Cap RNA linker), and subsequently reverse transcribed using random primers (with specific overhang RvTranscr, Table S3) under conditions that allow synthesis of long cDNAs using Sorbitol/Trechalose and Betaine mix(9). Specifically, equal amounts (0.8-1 μg) of RNAs were first mixed with 1μl of 100μM Cap RNA linker (Table S3), denatured for 5min at 65°C, chilled on ice for 5-10min and then incubated with 10U of T4 ssRNA ligase (NEB) in a final volume of 20μL for 16 hours at 16°C. After purifying the resulting RNA with RNA micro Kit (Enzymax LLC), RNAs were in vitro transcribed with MMLV reverse transcriptase (Invitrogen). Equal amounts of RNAs (600-700ng) were mixed with 1μl of 150μM Reverse Random Primer (RvTranscr Table S3) and 4.5μl of 3.3 M sorbitol/0.66 M trehalose mixture in a final volume of 11.5μl, denatured for 5min at 65°C, and chilled on ice for 5min. 13.5 μl of MMLV mix containing 200mM Betaine (Sigma), 500μM dNTPs and 250U of MMLV was added and samples were incubated 10min at 25°C and additional 2 h at 42°C. In order to amplify and subsequently analyze only RNAs ligated to specific Cap RNA linker, second strand synthesis was performed using a biotinylated primer matching the sequence from the Cap RNA linker (2ndStrandCapQ, Table S3) with High Fidelity Taq Polymerase (NEB) using only one cycle (3 min 97°C, followed by 5 sec 62°C and 10min 72°C) without further amplification. Biotinylated DNA strands were subsequently isolated using Streptavidin conjugated beads (Sigma) according to the manufacturer’s recommendations, purified using QIAquick PCR Purification Kit (Qiagen), and analyzed for selected transcripts by Real-Time PCR, where the ratio of Capped/Total RNA indicates the percentage of capped RNAs.
CapIP-Seq data collection and analyses

RNAs isolated from CapIP experiments obtained from three biological replicates of vector and eIF4E-overexpressing cell lines were used for library generation. Libraries were generated using KAPA RNA Hyperprep with RiboErase Kit (Roche) following the manufacturer’s instructions. All libraries were subjected to Flowcell High Output 75 cycles Single-End sequencing on an Illumina NextSeq 500 sequencer at the Genomic platform of IRIC, University of Montreal. Reads were aligned to the human reference genome (GRCh38.p5) using STAR (version 2.5.1b) (10). To improve accuracy of the mapping, the genome was created with a splice junction database based on the Gencode human v24 basic annotation [http://www.gencodegenes.org/]. Sequences that mapped to more than one locus were excluded from downstream analysis, since they cannot be confidently assigned. Other options used include: –outFilterMismatchNmax 3 –outFilterMultimapNmax 20 –alignSJoverhangMin 8 –alignSJDBoverhangMin 1 –alignIntronMin 20 –alignIntronMax 100000.

Uniquely mapped sequences were intersected with composite gene models from Gencode human v24 basic annotation using feature Counts (version 1.4.5-p1)(11). Composite gene models for each gene consisted of the union of exons of all transcript isoforms for that gene. Uniquely mapped reads that unambiguously overlapped with no more than one Gencode composite gene model were counted for that gene model; the remaining reads were discarded. The counts for each gene model correspond to gene expression values, and were used for subsequent analyses.
To compare the ratios of IP/Input between cell types, we used both limma’s (12) ratio contrast and DESeq2’s (13) LRT comparison (comparing a more complex model including cell type, assay and combination of cell type and assay, versus a simpler model including just the cell type and assay variable). DESeq2 was also used to perform differential gene expression analyses on each cell population separately, identifying genes differentially expressed between eIF4E-Flag and vector-containing cells. The complete sample set was used to estimate gene expression variances, and then the contrasts for input samples only, and IP samples only, were extracted. An adjusted p-value of 0.05 was used as the threshold for significance.
**Fig. S1.** A) The enrichment of RNAs in eIF4E-RIPs versus input RNAs from the nuclear fractions of eIF4E-Flag overexpressing U2Os cells monitored by RT-qPCR. Data were normalized to input samples and presented as a fold change. The mean, standard deviations and p-values (**p<0.01, ***p<0.001) were derived from three independent experiments (each carried out in triplicate). 

Myc, Mcl1 and CCND1 are known eIF4E nuclear targets and served as positive controls, while ACTB, GAPDH, POLR2A and 18S rRNA served as negative controls. B and C) The enrichment of RNAs in eIF4E-RIPs versus input RNAs from the nuclear fractions of vector control (B) and eIF4E-Flag (C) U2Os cells using a different antibody, a mouse monoclonal anti-eIF4E antibody. Data were normalized to input samples and presented as a fold change. Myc, Mcl1 and CCND1 are known eIF4E nuclear targets and served as positive controls, whereas ACTB, GAPDH, and 18S rRNA served as negative controls. D) Total RNA levels monitored by RT-qPCR corresponding to RNA export assays shown in Figure 1B. Data were normalized to vector control to calculate fold change. The mean and standard deviation, as well as p-values were derived from three independent experiments (each carried out in triplicate). E) Total RNA levels monitored by RT-qPCR corresponding to RNA export assays shown in Figure 1E. Data were normalized to CRISPR-Ctrl cell line and presented as fold change. The mean and standard deviation, as well as p-values were derived from three independent experiments (each carried out in triplicate). F) Semi-qPCR for tRNA\textsuperscript{Met} and U6 snRNA as control for the cytoplasmic and nuclear fractions, respectively, corresponding to the export assay shown in Figure 1B; n=nuclear fraction, c=cytoplasmic fraction. Representative experiment (out of 3 independent replicates) is shown. G) Semi-qPCR for tRNA\textsuperscript{Met} and U6 snRNA as control for the cytoplasmic and nuclear fractions, respectively, corresponding to the export assay shown in Figure 1E. n=nuclear fraction, c=cytoplasmic fraction. Representative experiment (out of 3 independent replicates) is shown. H) Polysome profiles of U2Os vector control and eIF4E-Flag cells (OD\textsubscript{264} nm).
**Fig. S2. A and B**) eIF4E but not the S53A mutant increased m\(^7\)G cap levels in both nucleus (Nc) (A) and the cytoplasm (Cyt) (B). Top: immunoblot analysis of RNAs isolated from nuclear or cytoplasmic fractions of vector control, eIF4E-Flag and S53A-Flag U2Os stable cell lines using anti-m\(^7\)G cap antibody. Amounts of loaded RNAs are indicated at the top of the blots (left) and silver-stained membranes shown for loading (right). Dot blots were quantified using ImageJ, and presented as a fold change compared to vector control (bottom). Means and standard deviations from three independent experiments are shown. **C**) Optimization of quantitative CapIP conditions. Titrations with different ratios of anti-m\(^7\)G cap antibody to purify RNA isolated from eIF4E-Flag cells. Enrichment of bound transcripts versus mouse IgG was compared for different ratios of anti-m\(^7\)G cap antibody to purified RNA (isolated from eIF4E-overexpressing cells), assessed by RT-qPCR. **D**) RNAs isolated from corresponding supernatant fractions from the CapIP shown in Figure S2D were analyzed by RT-qPCR to assess the efficiency of the CapIP. All samples were normalized to 18S rRNA and used to calculate ratios of Cap-Sn to Ctrl-Sn. **E**) m\(^7\)GpppG but not GpppG washes eliminate CapIP interactions in eIF4E-Flag cells. CapIPs using anti-m\(^7\)G cap antibody were performed with or without m\(^7\)GpppG or GpppG analogs, and analyzed by RT-qPCR. Data were normalized to the CapIP sample and presented as fold change. Experiments were carried out two independent times, means and standard deviations are shown from one representative experiment (carried out in triplicate). **F**) Schematic diagram of experimental designs for CapIP and % Capping from Cap-Sn. Note that CtrlIP represents sample where RNAs were incubated simultaneously with the Cap antibody and 100\(\mu\)M of m\(^7\)GpppG to determine the extent of non-specific (i.e. cap-independent) binding of RNAs to the Cap antibody or beads as described in the main text. **G**) Relative RNA enrichment in CapIPs in eIF4E-Flag to vector controls relative to vector CtrlIP (m\(^7\)G cap antibody with excess of m\(^7\)GpppG). Data were first normalized to input samples for each vector and eIF4E sets and normalized to vector CtrlIP, and presented as a fold change for each analyzed RNA.
Fig. S3. Overview of CapQ Method. In this method, differential sensitivity of capped and uncapped RNAs to Tobacco Acid Pyrophosphatase (TAP) and Alkaline Phosphatase (AP) was used to prepare libraries for either capped or total RNAs. Specifically, while capped RNAs are unaffected by AP, their treatment with TAP leaves a 5’ monophosphate on the RNA which then can be ligated to RNA oligonucleotides and used for library preparation. The uncapped RNAs are not affected by TAP, and AP treatment leaves a hydroxyl group on their 5’ end making them incompatible for ligation to the RNA oligonucleotides. In order to determine total RNA levels for the same samples, separate aliquots of RNAs were treated with AP followed by Polynucleotide kinase (PNK) and TAP. This way, all RNAs, regardless of their initial 5’ end status will have a single phosphate group which can be used for library preparation allowing measurement of total RNA levels from parallel samples.
Figure S4. Global assessment of increased capping efficiency as a function of eIF4E overexpression. A) Heatmap showing significantly differentially expressed genes in CapIP samples from RNAs derived from eIF4E-overexpressing and vector control cells, scaled by row. B) Validation of capping targets obtained from Cap-Seq. Comparison of RNA enrichment in CapIPs on RNAs isolated from eIF4E-overexpressing and vector control U2Os cells monitored by RT-qPCR. Data were first normalized to input samples for respective RNAs, and then normalized to vector values and presented as a fold change for each analyzed RNA. The mean, standard deviations and p-values (*p<0.05, **p<0.01, ***p<0.001) were derived from three independent experiments (each carried out in triplicate). C) IPA analysis of Molecular and Cellular Functions for RNAs that had increased capping in eIF4E-Flag cells.
Fig. S5. Some but not all capping targets are eIF4E nuclear targets. RT-qPCR analysis was used to monitor the enrichment of RNAs in eIF4E-RIPs versus input RNAs from the nuclear fractions of vector control (A) and eIF4E-Flag (B and C) U2Os cells. Data were normalized to input samples and presented as a fold change. The mean, standard deviations and p-values (*p<0.05, **p<0.01, ***p<0.001) derived from five (vector cells) and three (eIF4E-Flag cells) independent experiments (each carried out in triplicate) are shown. Note that the same data from Figure 1A were used here and thus values for Myc, Mcl1, CCND1, ACTB, GAPDH, POLR2A, 18S rRNA, RNMT, RNGTT and RAM are the same as in Figure 1A (repeated here for easier visual comparison with CapIP data). The analysis was done for all transcripts from the same RNA isolation/cDNA preparation and thus can be compared in this manner. D) Enrichment of RNAs in Flag-RIPs from the cytoplasmic fractions of eIF4E-Flag wildtype and S53A-Flag mutant U2Os cells. The mean and standard deviation, and p-values, were derived from three independent experiments (each carried out in triplicate). E) RNA export assays for Mdm4 transcript in eIF4E-Flag wildtype, S53A mutant overexpressing and vector control U2Os cell lines. F) Polysome profile of Mdm4 transcript in eIF4E-Flag and vector control U2Os cell lines. G) Total RNA levels monitored by RT-qPCR. Data were normalized to vector control to calculate fold change. The mean and standard deviation, as well as p-values, were derived from three independent experiments (each carried out in triplicate).
Figure S6. eIF4E RIPs with LacZ-4ESE RNAs but not LacZ or LacZ-CapSE from nuclear fractions of eIF4E-Flag cells (transfected with LacZ, LacZ-CapSE or LacZ-4ESE chimeric constructs) monitored by RT-qPCR. Data were normalized to inputs and presented as fold change relative to LacZ. The mean, standard deviation and p-values were derived from three independent experiments (each carried out in triplicate).
Fig. S7. Increased capping of selected transcripts in high-eIF4E AML specimens. A) WB analysis of RNGTT and RNMT levels in primary AML samples with high eIF4E levels and bone marrow mononuclear cells from healthy volunteers (Norm). Numbers indicate samples are derived from different individuals. ACTB was used as a loading control. B) The enrichment of capped transcripts among AML and Normal groups in CapIPs presented in Figure 6B. RNA enrichment in CapIPs using RNAs isolated from high-eIF4E primary AML cells (samples AML 1-4) vs Normal bone marrow mononuclear (Normal samples 1-3) or CD34+ cells (Normal 4), monitored by RT-qPCR. CapIPs were first normalized to input for each sample, then normalized to values obtained for sample Normal 1 and presented as fold change. The mean and standard deviation calculated from triplicates of each AML and Normal samples are shown.
Table S1. List of significantly differentially expressed genes in input samples only, isolated from eLF4E-Flag and vector control cells.

| Transcripts with elevated expression in eLF4E-Flag cells compared to vector control cells (p_{adj} < 0.05) |
|--------------------------------------------------------------------------------------------------|
| KRT15 | CYP24A1 | NQO2 | ETV4 | BRD9 | PPP6R1 |
| TGM2  | IMPDH1P4 | EFN1 | LRRC8A | MISP | MCM7 |
| C3    | RP13-895J2.3 | EPHA2 | TUBB6 | ITGA3 | AC006116.27 |
| RPL13AP20 | TOR4 | LTBR | SDHA | GNB1 | CCDC137 |
| CTC-241F20.4 | B3GALT6 | MAP2K3 | RP3-416H24.1 | MRPL20 | SERPINE1 |
| RP11-388M20.2 | FAM129B | PTGFRN | SDF4 | MYBBP1A | ID1 |
| ST13P12 | WWC1 | AURKAIP1 | SDC4 | ABALON | THBS1 |
| TNFRSF14 | COL27A1 | CDK6 | AP006621.9 | MAFK | YBX3 |
| AC004510.3 | BCL2L1 | ATAD3A | NFKB2 | TNIP1 | HSPG2 |
| CATSPER1 | RPUSD1 | NOP2 | CPTP | MYC | LA16c-366D1.3 |
| RP3-395M20.8 | SGK223 | AC068134.8 | SFN | ERCC2 | KRT18 |
| ARHGEF16 | TRAF2 | MAGEB17 | SSU72 | BAIAP2L1 | BCAM |
| GDF15 | RPL12P14 | CAPN15 | MTCL1 | TRIM28 | TRIP6 |
| HIST1H4D | PERM1 | SAMD11 | RP4-758J18.2 | AC006116.24 | NOC2L |
| SFTA1P | KCNN4 | C11orf68 | CTPS1 | FDXR | NFE2L1 |
| ATAD3B | COL13A1 | RP11-350N15.6 | SDC1 | GADD45A | PRKCG |
| HCG4 | ABT1 | PMPCA | C15orf52 | CCDC86 | KRT8 |
| C9orf142 | AGRN | NUP153 | AFAP1 | AEN | FLII |
| RP11-334E6.3 | TRIM47 | ZNF395 | ST3GAL1 | LASP1 | MLF2 |
| RP13-895J2.6 | PLXNA2 | NOTCH1 | FAM212B | SLC2A1 | HMGA1 |
| ISG15 | FAM207A | WRNIP1 | PVR | CPSF3L | PDAP1 |
|     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|
| IL32 | UNC13D | LAMB3 | SKI | SURF6 | TMEM120B |
| SLCO4A1-AS1 | DVL1 | CFLAR | NEDD4L | PTPN1 | LMNA |
| KLHL17 | EPS8L2 | FAM83H | FOSL1 | TBC1D9B | PKM |
| CGN | EXTL3 |     |     |     |     |

Transcripts with decreased expression in eIF4E-Flag overexpressing compared to vector control cells ($p_{adj} < 0.05$).

|     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|
| RP11-575L7.8 | CTBP2 | DAAM2 | PRICKLE1 | GALNT16 | RP4-678D15.1 |
| SEMA3F-AS1 | WDFY3-AS1 | AC007620.3 | NCAM1-AS1 | TTC28-AS1 | LINC00662 |
| RAB15 | RBM5-AS1 | RP4-665N4.8 | ZBED3 | COL5A2 | MOCS1 |
| AC002116.7 | FAN1 | MN1 | UTS2 | EDIL3 | MAP3K7CL |
| SPTB | CTD-2002J20.1 | AC015971.2 | RP11-328C8.4 | RP11-586K2.1 | AC093690.1 |
| RP11-135A24.4 | RP11-484D2.2 | MMP2 | AC000068.9 | LRRN1 | RP11-295I5.4 |
| ATG7 | AF230666.2 | RP11-423P10.2 | RP11-344N10.2 | CXCL14 | RP11-611J3.3 |
| RP11-471B22.2 | AC025165.8 | CASK-AS1 | RP11-166N17.1 | RP11-211G3.2 | ZXDA |
| PTCHD3P1 | KIAA0196-AS1 | SH3BP5-AS1 | RP11-269F20.1 | ZNF503 | FSIP2 |
| C14orf119 | CTA-125H2.2 | SDC2 | RP11-728F11.4 | RP11-488C13.6 | RP5-1021J20.1 |
| RP13-270P17.2 | APOBEC3B-AS1 | NCAM1 | AC005330.2 | MAF | DQX1 |
| STK24 | PPHLN1 | CEBPZ | IAH1 | RP11-538J12.2 | RP11-270M14.5 |
| COL16A1 | TTC28 | PTGR2 | AC104134.2 | LARGE | RP11-363J20.2 |
| RP3-323A16.1 | RP11-73M7.6 | COQ6 | RP11-49884.5 | ITFG1-AS1 | RP11-681H18.2 |
| RP11-218E20.5 | PCSK7 | AC007906.1 | RP4-724E13.2 | AGAP2-AS1 | CBR3-AS1 |
| LINC00987 | RP11-632L2.2 | FUT8 | RP11-521C20.3 | ZXDB | RP11-517I3.1 |
| RP11-632F7.3 | ARG2 | NNT-AS1 | CTD-2384B11.2 | GLB1L3 | TBPL1 |
| CTB-175E5.7 | STBD1 | RP11-50E11.2 | SYTL4 | PRICKLE2-AS1 | RP11-701H16.4 |
| Gene   | Gene   | Gene   | Gene   | Gene   | Gene   |
|--------|--------|--------|--------|--------|--------|
| RP11-244F12.3 | RP11-15L13.4 | RP3-430N8.11 | PIGF | RP5-1172A22.1 | RP11-1069G10.2 |
| SIAE   | RP11-732A19.5 | ABLIM1 | AC007098.1 | MSC-AS1 | MEIS1-AS2 |
| RP11-287D1.3 | SCUBE3 | CTD-2196E14.7 | PLPP1 | RP11-651K21.1 | C10orf142 |
| CTB-43P18.1 | NEMF | INHBE | RP4-613B23.1 | KIF26B | RP11-145A3.1 |
| KLHDC2 | RP11-278A23.4 | RSPH10B | RP11-1151B14.4 | HECW1 | SLC5A9 |
| RP11-223P11.3 | RP11-47I22.2 | LINC01465 | ZNF503-AS2 | RP11-488C13.5 | CDR1 |
| RP11-27M24.1 | CTA-221G9.12 | CEP68 | CTC-564N23.2 | ARNTL2-AS1 | HLA-DQB1-AS1 |
| CCDC73 | RP11-19P22.8 | RP11-290012.2 | DENND6A-AS1 | ZMAT5 | RBPMS-AS1 |
| RFFL   | RRM1-AS1 | PIK3IP1 | ITPR1 | DYSF | PTPRB |
| RP11-481J2.4 | AC107081.5 | AC007899.3 | MT-TE | DACT1 | RNF150 |
| RP11-256I6.2 | PCNXL4 | SYNC | AL022476.2 | CCDC121 | RP11-53B2.3 |
| MTIF3  | MSH6 | RP3-430N8.10 | AC010136.2 | LINC00565 | HOTAIR |
| TFIP11 | RP4-735C1.4 | ZP3 | ANKRD18EP | COMTD1 | NBAT1 |
| RBBP4  | BBOF1 | FANCD2OS | CREB3L1 | CTD-2269F5.1 | PAGE2B |
| RP11-589M4.1 | TLDC2 | PTPRG-AS1 | GGACT | PIEZO2 | PKIB |
| ST7-AS1 | CTD-2047H16.5 | PPP3CB-AS1 | RP11-3L21.2 | RP11-264B14.2 | RP11-300E4.2 |
| CTA-363E6.7 | LOXL1 | PRICKLE2-AS3 | AP000619.5 | SEPP1 | CCDC152 |
| IL10R8-AS1 | SPHK2 | AC007308.7 | STARDA-AS1 | LOXL1-AS1 | CTC-459F4.1 |
| FANCL  | AC239868.1 | AC007563.5 | SEMA3B-AS1 | GLIPR2 | CTA-373H7.7 |
| PIGCP1 | PYROXD1 | CTD-2319H12.2 | AC009480.3 | RP11-477D19.2 | SEMA6A-AS1 |
| PPP1R12B | RP11-500G22.5 | BTD | SPINK5 | ZNF503-AS1 | TRPA1 |
| RP11-34A14.3 | CADM3-AS1 | SVIL-AS1 | FOS | CTA-407F11.9 | PALM3 |
| CTD-2154I11.2 | CTC-428H11.2 | ATP8B1 | RP11-244F12.2 | RP11-712B9.2 | RP11-856M7.2 |
| ASPHD2 | RBP1 | CMC1 | DHFRL1 | LTBP1 | CH507-513H4.6 |
| RP11-57H14.2 | RP11-2E11.9 | RP11-9N12.2 | | | |
Table S2. List of significantly differentially expressed genes in CapiP samples from eIF4E-Flag relative to vector control cells.

| RNA with increased capping in eIF4E-Flag cells relative to vector controls (p_{adj} < 0.05) |
|---------------------------------|---------------------------------|---------------------------------|
| RP11-1114I9.1 | ZNF114 | ZSCAN5A | MAGEB17 | AGRN |
| ST13P12 | B3GALT6 | SLFN1 | HMMR-AS1 | ERCC2 |
| RP11-885L14.1 | AC006116.27 | RP11-15A1.4 | MAP3K14-AS1 | RP11-352G18.2 |
| KIF1A | HRNR | RPL12P14 | AP006621.9 | LGALS8 |
| TNFRSF14 | AC006116.24 | RP13-895J2.6 | SPAG17 | MRPL20 |
| CTC-241F20.4 | NUP153 | CYP24A1 | TM4SF19-AS1 | RP11-500C11.3 |
| RP3-395M20.8 | RP11-806L2.6 | CTD-2544N14.3 | BDNF-AS | CTD-2033D15.1 |
| AC004510.3 | RP11-15A1.3 | PERM1 | AL391730.2 | RP11-56B16.5 |
| RP11-334E6.3 | SLCO4A1-AS1 | ZNF45 | CPSF3L | RP11-670E13.5 |
| TGM2 | RP11-463I20.1 | NUDT6 | HIST1H4I | CTD-2012K14.3 |
| HCG4 | RP11-55J15.2 | CATSPER1 | PUSL1 | AL365181.2 |
| TRHDE | PDK1 | AC068134.8 | BAIAP2L1 | RP11-69L16.4 |
| IMPDH1P4 | RPE65 | SAMD11 | PIAS1 | CTD-2033D15.3 |
| TNFSF8 | RP13-895J2.3 | RP11-178H8.7 | RP11-78I14.1 | DNMBP-AS1 |
| RP11-388M20.2 | CCDC148-AS1 | CFLAR | TUBB6 | SRR |
| COL27A1 | PLXNA2 | RP11-350N15.6 | ABALON | TMEM120B |
| KRT15 | NQO2 | RP11-611O2.5 | FAM129B |
### RNAs with reduced capping in eIF4E-Flag relative to vector cells (p<sub>adj</sub> < 0.05)

| Gene          | Gene          | Gene          | Gene          | Gene          |
|---------------|---------------|---------------|---------------|---------------|
| CTA-221G9.12  | MDFI          | RNF187        | RP11-488C13.5 | PAGE2B        |
| CADM3-AS1     | ZXDB          | RP11-145A3.1  | SCN5A         | RPS-1021I20.1 |
| TMSB4X        | PIK3IP1       | AGAP2-AS1     | HOTAIR        | LMCD1         |
| COL1A1        | ZNF503-AS2    | MN1           | RP11-701H16.4 | CXCL14        |
| LOXL1         | MOCS1         | CTD-2319I12.2 | RP11-1069G10.2| MAP3K7CL      |
| RP11-728F11.4 | MMP2          | RCN3          | ZXDA          | AC069363.1    |
| PCSK7         | EDIL3         | CTD-2269F5.1  | CNN1          | OLFML3        |
| ZP3           | SYNPO         | ITPR1         | GALNT16       | CTC-459F4.1   |
| IER2          | HPS4          | LOXL1-AS1     | RP11-363J20.2 | CHD5          |
| MAGEA4        | FUT8          | FOS           | COMTD1        | SEMA6A-AS1    |
| CDR1          | DAAM2         | RBP1          | LRRN1         | RP11-116G8.5  |
| MMP14         | NBAT1         | ZNF503        | C10orf142     | PALM3         |
| DHR52         | COL9A2        | ZNF503-AS1    | VAT1L         | AC055736.1    |
| RP11-611I13.3 | LINC00662     | RP5-1172A22.1 | PKIB          | CTB-118N6.2   |
Table S3. List of oligonucleotide and primers

| **18S Fw** | CGG CGA CGA CCC ATT CGA AC |
| **18S Rv** | GAA TCG AAC CCT GAT TCC CCG TC |
| **Mdm2 Fw** | ACATGGTCCCGACCTAGGTTCA |
| **Mdm2 Rv** | TTCACATGCTGACCCGTCTATTCT |
| **U6 Fw** | CGCTTCCGCGACATATAC |
| **U6 Rv** | AAAATATGGAACGCTTCACGA |
| **tRNA Met Fw** | 5'-AGC AGA GTG GCG CAG CGG-3', |
| **tRNA Met Rv** | 5'-GAT CCA TCG ACC TCT GGG TTA-3'; |
| **Actin BF** | GCAAGAGGAGACCTCTGGGACAGG |
| **Actin BR** | GGTGTAACGCAACTAAGTCTAG |
| **Mcl1 Fw** | TTTACCGCAGCGGGTAGAACACTG |
| **Mcl1 Rv** | TGTTTCGATGCAGCTTCTTGTT |
| **POL2 AR Fw** | TGACTGCAAACACAGCCATCTACT |
| **POL2 AR Rv** | GGCGACATCAAAGTCGGGATT |
| **ABALON Fw** | TCTCCGATTCAGTCCCTTCT |
| **ABALON Rv** | GGTGTTTGACTCTTCCTCCTAC |
| **AC006116.27 Fw** | TGTTCAATCAGGGGAAGACAGG |
| **AC006116.27 Rv** | AAGTGCCCAGCTCATTAGAC |
| **AC006116.24 Fw** | CCTCTTTTGCAGATAAATTCCTT |
| **AC006116.24 Rv** | GGTGCTAAAGTGGGAGTTGA |
| **Luc Fw** | GCCAAAGC ACT CTGATTGAC |
| **Luc Rv** | CCA TAT CCT TGC CTG ATA CCT |
| **GAPDH Fw** | GAAGGTGAAGGTTCGGAGTC |
| **GAPDH Rv** | GAAGATGGTGATGGGAGTT |
| **Fbl Fw** | GACCCCTGAGCCATATGAAAGA |
| **Fbl Rv** | CAACACATCTCTCGCAATCC |
| **eIF4A1 Fw** | TTGTACTGGATGAAGCTGACG |
| **eIF4A1 Rv** | TCACCTCAAGCACCACATCAG |
| **CDK4 Fw** | ATGTGGAGTGGTGGCTGTATC |
| **CDK4 Rv** | CAGCCCAATCAGGGCTTGAAG |
| **CCND1 Fw** | CACTTCTCTCAAAAATGCCA |
| **CCND1 Rv** | CCTGGGCCAGGCTCTGACTC |
| **eIF4E Fw** | AGGAGGTGGCTAACCCAGAACACT |
| **eIF4E Rv** | AAAGTGAGTAGTCAAGCGACGCA |
| Gene   | Forward Sequence | Reverse Sequence |
|--------|------------------|------------------|
| cMycFw | CTTCTCTGAAAGGCTCTCTTTG | GTCGAGGTACATAGTCTCCTGTG |
| cMycRv | GTCGAGGTACATAGTCTCCTGTG | CTTCTCTGAAAGGCTCTCTTTG |
| Mcl1 R6 | GGCAGTCCGCTGGAGATTAT | CAACCCGCTCGTAAGGTCTC |
| MINARv | GTCCTCAGGAAGCCACTTAAATC | CTTCACCTGACAGATCAAAGTC |
| Cdc25aFw | GGAAGTACAAAGAGGAGAAGAG  | GGGAAGATGCCAGGAGGAAA |
| Cdc25aRv | GGGAAGATGCCAGGAGGAAA  | GGAAGTACAAAGAGGAGAAGAG |
| Mcm5Fw | CAATGAGGAGAGGATGTATG  | CACTCGGAGATGCAATAA |
| Mcm5Rv | CACTCGGAGATGCAATAA  | CAATGAGGAGAGGATGTATG |
| CTNNBFw | CTTCACCTGACAGATCAAAGTC | CTTCCACCTCCCTCCTGTGTTAG |
| CTNNBRv | CTTCCACCTCCCTCCTGTGTTAG  | CTTCACCTGACAGATCAAAGTC |
| RuvBL1Fw | GGGTGTGCTGTGTGTGTGATG  | TGACGATGGGAGCGGATAG |
| RuvBL1Rv | TGACGATGGGAGCGGATAG  | GGGTGTGCTGTGTGTGTGATG |
| CDK2Fw | ACCACTGGAGGAGCTCTATT  | TCTTCAAGCTCCAGGCTATT |
| CDK2Rv | TCTTCAAGCTCCAGGCTATT  | ACCACTGGAGGAGCTCTATT |
| eIF2B1Fw | GTCCGGCCTCTTTCCACTAA | ATGCTCCTCCTTGGACTTT |
| eIF2B1Rv | ATGCTCCTCCTTGGACTTT  | GTCCGGCCTCTTTCCACTAA |
| MALAT1Fw | GTTCTGATCCCGCTGGCTATT  | TCCTCAACACTCAGCCCTTATC |
| MALAT1Rv | TCCTCAACACTCAGCCCTTATC  | GTTCTGATCCCGCTGGCTATT |
| NEAT1Fw | TGGAGGAGTCAGGAGGAATAG  | GGCATTGGCAAGTGGGAAGTAG |
| NEAT1Rv | GGCATTGGCAAGTGGGAAGTAG  | TGGAGGAGTCAGGAGGAATAG |
| RNMTFw | CAGGGTGACTCTCCAAAAGAAA  | TCTCAGTGCTGGCAATCTA |
| RNMTRv | TCTCAGTGCTGGCAATCTA  | CAGGGTGACTCTCCAAAAGAAA |
| TNFRSF14Fw | CCAAGTGCAGTCCAGGTTAT  | ATGGAGGTGGGCAATGTAG |
| TNFRSF14Rv | ATGGAGGTGGGCAATGTAG  | CCAAGTGCAGTCCAGGTTAT |
| TGM2Fw | ATCACCACACACTCAAAATACC  | ATGCCCTGTCTCCTCTTCTC |
| TGM2Rv | ATGCCCTGTCTCCTCTTCTC  | ATCACCACACACTCAAAATACC |
| TNFSF8Fw | CGAGGCCCGAGCTATTTTCTAT  | TGTCAGTGGATTTGGGAATAG |
| TNFSF8Rv | TGTCAGTGGATTTGGGAATAG  | CGAGGCCCGAGCTATTTTCTAT |
| HRNRFw | CAGCACCAGAGGAAACAAGA  | CCTTCAAGCTTTTGTGAATAG |
| HRNRRv | CCTTCAAGCTTTTGTGAATAG  | CAGCACCAGAGGAAACAAGA |
| PDK1Fw | ACGCTGGGTAATGAGGATT TG  | GAGGTCTTGGTGCAGTTGAATA |
| PDK1Rv | GAGGTCTTGGTGCAGTTGAATA  | ACGCTGGGTAATGAGGATT TG |
| CFLARFw | GAGGTGTATGGGTGGGATCAG  |
| **CFLARRv:**  | CTTTGCTTCTCCCTGCTAGATAA |
| **RNMTFw:**   | CTGATATTGCGGATTTTCTGTC |
| **RNMTTRv:**  | GGGTCACGAAAATTGTCAATCAG |
| **RNGTTFw:**  | GGCAAAAAAGGAAAAAGAAGCGG |
| **RNGTTTRv:** | TCCCCAGCCACAGAATTGATG |
| **RAMFw:**    | CCT CAAACCTTTTGGGATT |
| **RAMRv:**    | TTCTTGATACTCCTTGATCA |
| **Mdm4Fw:**   | GACCCCTATCAGCCCTTGTAAC |
| **Mdm4Rv:**   | TTCCCCATCTACCGCCTAACT |
| **LacZFw:**   | TTG CGC AGC CTG AAT GGC GAA |
| **LacZRv:**   | ATC TGA ACT TCA GCC TCC AGT |
| **CapRNAlinker:** | rArCrArCrUrCrUrUrCrUrArCrArCrGrArCrGrCrUrCrUrCrUrCrGrArUrCrUr |
| **RvTranscr:** | TAGTCGAACTGAAGGTCTCCGAACCGCTCTTCCGATCNNNNNNNNN |
| **2ndStrandCapQ:** | 5′Biotin-ACACTCTTCTCCCTACACGACGCTCTTCCGATC |
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