mTOR-4EBP1/2-independent translational regulation of mRNAs encoding ribosomal proteins

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SI Appendix

Materials and Methods

Cell culture. MDA-MB-468 cells (ATCC) were cultured in RPMI-1640 (Gibco) supplemented with 10% fetal bovine serum (Gemini Bioproducts), 1X GlutaMAX (Gibco), 10 mM HEPES (Gibco), and 1X Antibiotic-Antimycotic (Gibco). *hTERT*-immortalized human mammary epithelial cell line HMEC-CT2 (Clontech) was cultured in DMEM/F12 (Gibco) supplemented with 0.6% fetal bovine serum (Gemini Bioproducts), 1 ng/ml cholera toxin (Sigma-Aldrich), 10 ng/ml recombinant human EGF (Sigma-Aldrich), 10 µg/ml recombinant human insulin (Gibco), 0.5 µg/ml hydrocortisone (Sigma-Aldrich), and 1X Antibiotic-Antimycotic (Gibco). MCF10A cells, with stable expression of ER-Src (1, 2), were cultured in DMEM/F12 without phenol-red (Gibco) supplemented with 5% charcoal-stripped fetal bovine serum (Sigma-Aldrich), 100 ng/ml cholera toxin (EMD Millipore), 20 ng/ml recombinant human EGF (Sigma-Aldrich), 10 µg/ml bovine insulin (Sigma-Aldrich), 0.5 µg/ml hydrocortisone (Sigma-Aldrich), 1X Penicillin-Streptomycin (Gibco), 0.5 µg/ml puromycin (Invivogen). All cells were cultured in a humidified chamber at 37°C and 5% CO_2.

Generation of CRISPR-Cas9 edited cell lines. LentiCRISPR v2 vectors targeting 4EBP1 and 4EBP2 (Genscript), and GFP (gift from Y. Wang), having the following target sequences were used to generate knock-down cell lines. Target sequences: GFP 5'-GAGCTGGACGGCGACGTAAA-3'; 4EBP1 5'-GTGAGTTCCGACACTCCATC-3'; 4EBP2 5'-CATGACTATTGCACCACGCC-3'. HEK-293T cells were transfected with packaging plasmids pCMVΔ8.91 and pMD2.G, and lentiCRISPR v2 vectors; viral supernatant was collected 48 h after transfection, filtered through 0.45 µm membranes and concentrated with Lenti-X Concentrator (Clontech). HMEC-CT2 cells were transduced with concentrated virus supplemented with 8 µg/ml polybrene, and selected with 2 µg/ml of puromycin. To achieve sufficient dual knockdown of 4EBP1 and 4EBP2, cells were subjected to multiple rounds of viral transduction and puromycin selection.

Gene expression knock-down by siRNA. HMEC-CT2 and MCF10 (Er-Src) cells were seeded into 6-well plates the previous day (HMEC-CT2 at 200,000 cells, and MCF10A (Er-Src) at 100,000 or 150,000 cells, per 9.6 cm^2 well in 4 ml of media), transfected with 30 pmol siRNA per well using Lipofectamine RNAiMAX (Invitrogen) per manufacturer's protocol, and incubated for 44–48 h under standard culture conditions before proceeding to subsequent steps. Transfections were carried out using FlexiTube siRNA (Qiagen) targeting GCN2 (sense strand 5'-AGGUUAAGUCUUUCGAGAATT-3'; anti-sense strand 5'-UUCUCGAAAGACUUAACCUTG-3'), EIF2S1 (sense strand 5'-GGCUGUAAAUCCUAGACUUTT-3'; anti-sense strand 5'-AAGUCUAGGAUUUACAGCCAG-3'), or AllStars negative control (Qiagen).

Chemical compounds. Kinase inhibitors for chemical screening were obtained from Harvard Medical School ICCB-Longwood Screening Facility, as 10 mM stocks in DMSO and diluted to 1 mM DMSO working stocks. Additional 10 mM DMSO stocks of Torin-1, Dabrafenib and MK1775 were purchased from Selleck Chemicals. Compounds were used in biological assays at 0.1% DMSO final concentration in supplemented media (as described above).

Targeted Profiling of RNA translation (TPRT). For chemical perturbation experiments, MDA-MB-468 and HMEC-CT2 cells were seeded into 6-well plates the previous day (500,000 cells per 9.6 cm^2 well in 4 ml of media) or as described above for siRNA-based experiments, and treated as indicated. For physiological perturbation experiments, MCF10A (ER-Src) cells were seeded into 6-well plates the previous day (200,000 cells per 9.6 cm^2 well in 4 ml of media) or as described above for siRNA-based experiments, then treated with fresh cell culture media supplemented with 1 µM 4-hydroxytamoxifen (Sigma-Aldrich) for 24 h prior to experimental perturbation to induce Src-mediated oncogenic transformation, before being subjected to perturbation conditions as indicated. Following treatment, cells were washed with ice-cold wash buffer (4 ml/well, comprising PBS supplemented with 100 µg/ml cycloheximide), keeping the buffer in contact with the cells for approximately 60 seconds on ice, followed by vacuum aspiration of the buffer. Lysis buffer (200 µl/well, comprising 20 mM Tris (pH 7.4), 150 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 1 mg/ml cycloheximide, 1% v/v Triton X-100) was gently dispersed over the surface of the dish, and samples were frozen in liquid nitrogen. Samples were thawed on ice, collected into a microcentrifuge tube by scraping and frozen in liquid nitrogen. After thawing on ice, samples were centrifuged (20,000*g*, 5 min, 4°C), supernatant was recovered as a clarified lysate, its RNA concentration measured using Qubit RNA HS assay kit (Invitrogen) according to manufacturer's protocol, and lysate diluted to 500 ng RNA in 100 µl lysis buffer. RNase I (Invitrogen) (1,000 U in 10 µl) and TurboDNase (Invitrogen) (10 U in 5 µl) were added to the diluted lysate and incubated (1 h, 4°C, with gentle agitation). TRIzol (Invitrogen) (460 µl) was added, mixed, incubated (5 min, room temperature), chloroform (92 µl) was added, mixed, incubated (3 minutes, room temperature), centrifuged (12,000*g*, 15 min, 4°C), and 250 µl of the upper aqueous phase was recovered. GlycoBlue (Invitrogen) (30 µg in 2 µl) was added, mixed, isopropanol (375 µl) was added, mixed and incubated (dry ice or cooler, at least 30 min). Samples were thawed (room temperature), centrifuged (20,000*g*, 30 min, 4°C), pellet was washed in ethanol (70%, ice-cold, 1 ml), centrifuged (20,000*g*, 10 min, 4°C), pellet was recovered and dried (10 min, room temperature), and dissolved in water (10 µl). Reverse transcription (RT) was carried out as follows. RT primer mix (2 µl of a mixture of all RT primers at 1 µM each in water) was added, mixed, incubated at 80°C for 2 minutes followed by a gradual decrease of 0.3°C per second until reaching a temperature of 30°C, and then cooled to 4°C. RT master mix (8 µl, comprising 4 µl of 5X SuperScript III first strand buffer (Invitrogen), 1 µl of dNTP mixture (10 mM each), 1 µl of DTT (0.1 M), 1 µl of SUPERase-In (20 U/µl), and 1 µl of SuperScript III reverse transcriptase (200 U/µl)

(Invitrogen)) was added, mixed, incubated using a temperature ramp of 40°C, 42°C, 44°C, 46°C, 48°C, and 50°C in this order for 10 minutes at each temperature, cooled to 4°C, NaOH (2.2 µl of 1 N stock) was added, mixed, incubated at 98°C for 20 min, and cooled to 4°C. Samples were added to mixture of water (156 µl) and NaOAc (20 µl of 3 M stock), GlycoBlue (30 µg in 2 µl) was added, mixed, isopropanol (300 µl) was added, mixed and incubated (dry ice or cooler, at least 30 min). Samples were thawed (room temperature), centrifuged (20,000*g*, 30 min, 4°C), pellet was washed in ethanol (70%, ice-cold, 1 ml), centrifuged (20,000*g*, 10 min, 4°C), pellet was recovered and dried (10 min, room temperature), and cDNA was dissolved in water (500 µl). For qPCR analysis, individual reactions were mixed by addition of cDNA sample (5 µl) to a mixture of SYBR-Select master mix (10 µl of 2X mix) and qPCR primer mix (5 µl of mixture of gene-specific forward primer and qPCR common reverse primer BR at 0.8 µM each in water). Each cDNA-probe pair was analyzed with 3 or 4 technical replicates on an Applied Biosystems 7300 Real Time PCR System with the following protocol: 95°C for 120 seconds and 40 cycles of [95°C for 15 seconds, 50°C for 15 seconds, 55°C for 15 seconds, 60°C for 15 seconds, and 65°C for 45 seconds], with fluorescence signal measured at the final step of each cycle. Plate set-up and qPCR fluorescence values, normalized against ROX control, were exported as CSV files and analyzed as below.

TPRT with ribosome footprint purification. Cells were seeded, treated, and harvested as for TPRT. Following lysate harvest, two nuclease digest reactions were set up per sample, each with 500 ng RNA in 200 µl lysis buffer. RNase I (2,000 U in 20 µl) and TurboDNase (20 U in 10 µl) were added to the diluted lysate and incubated (1 h, 4°C, with gentle agitation). SUPERase-In (600 U in 30 ul) was added and mixed. The mixture was overlaid on a 1 M sucrose cushion, and centrifuged at 55,000 rpm overnight at 4°C using a TLS 55 rotor. The sucrose cushion was decanted, the pellets from the two reactions were resuspended and combined in TRIzol (500 ul total volume), mixed, incubated (5 min, room temperature), chloroform (92 µl) was added, mixed, incubated (3 minutes, room temperature), centrifuged (12,000*g*, 15 min, 4°C), and 250 µl of the upper aqueous phase was recovered. GlycoBlue (Invitrogen) (30 µg in 2 µl) was added, mixed, isopropanol (375 µl) was added, mixed and incubated (dry ice or cooler, at least 30 min). Samples were thawed (room temperature), centrifuged (20,000*g*, 30 min, 4°C), pellet was washed in ethanol (70%, ice-cold, 1 ml), centrifuged (20,000*g*, 10 min, 4°C), pellet was recovered and dried (10 min, room temperature), and dissolved in water (10 µl). 10 ul of 2X loading buffer (10 mM EDTA, 300 ug/ml bromophenol blue, in formamide) was added, mixed, incubated (80°C, 90 s), mixed, separated on a 15% TBE-urea gel, and visualized using 1X SYBR Gold (Invitrogen). Bands in the region of 26 nt to 34 nt were excised, RNA was purified, and suspended in 10 ul of water, as described by Ingolia *et al.*, using demarcation markers NI-NI-19 and NI-NI-20 (3). Subsequent steps, comprising reverse transcription, qPCR, and data analysis, were undertaken as described for the standard TPRT protocol above. For experiments directly comparing with standard TPRT (without ribosome footprint purification), the standard TPRT reactions were doubled in quantity from nuclease digest through to RNA resuspension (in 20 ul) following

TRIzol extraction, after which 10 ul was used for subsequent steps and 10 ul was used for gel analysis together with the samples above.

mRNA expression RT-qPCR. Changes in mRNA levels were measured using a variation on the TPRT protocol as described below, using the same reverse transcription and qPCR primers. Cells were seeded and treated as for TPRT. Media was vacuum aspirated, TRIzol (400 µl) was added directly to each well, lysate was collected, mixed, incubated (5 min, room temperature), chloroform (80 µl) was added, mixed, incubated (3 minutes, room temperature), centrifuged (12,000*g*, 15 min, 4°C), and 150 µl of the upper aqueous phase was recovered. GlycoBlue (30 µg in 2 µl) was added, mixed, isopropanol (225 µl) was added, mixed and incubated (dry ice or cooler, at least 30 min). Samples were thawed (room temperature), centrifuged (20,000*g*, 30 min, 4°C), pellet was washed in ethanol (70%, ice-cold, 1 ml), centrifuged (20,000*g*, 10 min, 4°C), pellet was recovered and dried (10 min, room temperature), dissolved in water (20 µl), RNA concentration measured using a NanoDrop instrument, and RNA samples diluted to 100 ng in 10 µl of water. Samples were subsequently processed for RT and qPCR as described for TPRT.

TPRT and mRNA expression qPCR data analysis. Due to the acute nature of drug and metabolic treatments under study, mRNA levels are not expected to vary substantially. However, measurement of changes in mRNA level presents with presents with some noise. Normalization of changes in translation with mRNA level would therefore introduce this additional noise. To avoid these effects, changes in translation are analyzed as "gross" changes without consideration of changes in mRNA levels, and mRNA levels are measured and analyzed separately, and any unexpected, substantial changes are noted.

ROX-normalized qPCR fluorescence values R_n were analyzed using MATLAB (version R2017a or R2018a) for background subtraction and determination of Ct values. Briefly, for each qPCR sample (i.e. individual well), a baseline value was selected that gives the most linear initial increase in $\log_{10}(R_n)$ against PCR cycle. Quantification cycle value C_q was determined by interpolation against $\log_{10}(R_n) = -0.5$ which is in the exponential phase of amplification, and for each set of cDNA-probe samples, mean C_q was calculated across technical replicates. Within each biological replicate, fold change FC for a particular gene of interest (goi) of drug-treated versus DMSO-treated sample was calculated using a variation on Hellemans *et al.* (4), as:

$$FC = \frac{2^{C_q(DMSO,goi)-C_q(drug,goi)}}{NF}$$

The normalization factor, for multiple internal control genes i,

$$NF = \sqrt[i]{\prod_i 2^{C_q(DMSO,i)-C_q(drug,i)}}$$

$\log_2(FC)$ values from each biological replicate is used to calculate their means and standard errors over the biological replicates.

Ribosome profiling and data analysis. Cells were treated as indicated, and subjected to ribosome profiling per Ingolia *et al.* (3, 5). Sequencing reads were preprocessed with the FASTX toolkit (version 0.0.13) to filter for read quality, remove adaptor sequences, remove short reads, and trim the 5' most nucleotide, with Bowtie2 (version 2.2.4) to remove rRNA, tRNA and mtRNA sequences, and filtered to keep reads of footprint lengths (26 to 32 nucleotides). Filtered reads were aligned using STAR (version 2.5.2b) to the hg19 human reference genome. Alignments were processed with the GenomicAlignments R library to filter for those compatible with known splice variants and replaced and represented by the nucleotide in the central-most position of the read, with Bedtools (version 2.25.0) to count number of alignments to the CDS regions of each gene (across only common regions between all isoforms, and excluding short regions spanning from -2 to +15 codons of each start codon, and -8 to +2 codons of each end codon), and RPKM was calculated. Quality of library preparation, sequencing and analysis was assessed using FastQC (version 0.11.3), Picard (version 1.139) and RiboseqR (version 1.4.0).

For TPRT protocol validation studies, ribosome profiling data was analyzed for translation changes without normalization against changes in mRNA levels, to provide equivalence to TPRT data analysis (see below under "TPRT and mRNA expression qPCR data analysis"). For global analysis (Fig. S2B), DESeq2 (version 1.10.1) was used to calculate fold changes (maximum *a posteriori* estimates) and adjusted *p*-values (Wald test), testing for deviations from zero change as the alternative hypothesis, with multiple testing correction by the Benjamini-Hochberg method, significance cutoff for independent filtering of 0.05, and other parameters as default values. For gene-focused analysis (Fig. 1B), fold change in translation for the ribosome profiling data was calculated as

$$FC = \left[\frac{RPKM(drug, goi)}{RPKM(DMSO, goi)}\right] / NF$$

where, by analogy to the analysis for TPRT qPCR data, for multiple internal control genes i,

$$NF = \sqrt[i]{\prod_i \frac{RPKM(drug, i)}{RPKM(DMSO, i)}}$$

For the metabolic perturbation study (Fig. 5B), translation efficiencies (TE) were calculated as the ratio of reads per million reads (RPM) values between ribosome footprint and total RNA samples, and fold changes in TE as the ratio in TE values for the respective samples.

$$FC(TE) = \left[\frac{RPKM(drug, footprint, goi)}{RPKM(drug, bulk, goi)}\right] \Big/ \left[\frac{RPKM(vehicle, footprint, goi)}{RPKM(vehicle, bulk, goi)}\right]$$

For these metabolic perturbation experiments, spike-in of *S. cerevisiae* sample was added during library preparation, and resulting reads were excluded during data analysis.

Western Blotting. Cells were seeded and treated as for TPRT, and harvested directly into Laemmli buffer (200 µl/well of 6 well plates). Samples were subjected to SDS-PAGE on 12% or 15% gels with 20 µl sample/lane (15 lane mini gels), and transferred to PVDF membranes under wet transfer conditions. Membranes were incubated in blocking buffer (room temperature, approximately 30 min), and probed with primary antibodies in blocking buffer at 4°C overnight at specified dilutions: 4EBP1 (Cell Signaling Technology (CST) 9452S, 1:500), 4EBP2 (CST 2845S, 1:500), phospho-4EBP1 (p-T37/T46) (CST 2855S, 1:1000), phospho-4EBP1 (S65) (CST 9451S, 1:1000), phospho-4EBP1 (p-T70) (CST 13396S, 1:1000), S6RP (CST 2217L, 1:1000), phospho-S6RP (p-S235/S236) (CST 2211L, 1:1000), GCN2 (CST 3302S, 1:1000), p-GCN2 (R&D Systems AF7605, 1:1000), eIF2α (Bethyl A300-721A-M, 1:1000), p-eIF2α (clone D9G8) (p-S51) (CST 3398, 1:500), p-eIF2α (clone 119A11) (p-S51) (CST 3597S), p-eIF2α (44-728G polyclonal) (p-S52) (Invitrogen 44-728G, 1:500), p-eIF2α (clone E90) (p-S51) (Abcam ab32157), and vinculin (Sigma Aldrich V9131, 1:5000). Membranes were probed with secondary antibodies in blocking buffer for 1 h at room temperature at specified concentrations: Alexa Fluor 680 goat anti-rabbit IgG (Life Technologies A21109, 1:5000), IRDye 800 goat anti-mouse IgG (Rockland 621-132-121, 1:5000), and visualized using the Odyssey CLx Imaging System (LI-COR). All antibodies use Odyssey TBS Blocking Buffer (LI-COR), except p-eIF2α (44-728G polyclonal) (p-S52) (Invitrogen 44-728G, 1:500) and its subsequent secondary antibody, which use 5% blotting-grade blocker (non-far dry milk) (Bio-Rad). Data was processed with Image Studio Lite (LI-COR) with linear mapping of detection values to output image within the signal range of interest (K=0).

KINOMEscan profiling. Chemical compounds were profiled by the KINOMEscan platform over 403 non-mutant kinases and 65 mutant kinases, at 1 μM in DMSO. Profiling was carried out by DiscoverX Corporation, to obtain percentage interaction between recombinant kinases and their ligands in the presence of each chemical compound compared to DMSO. Primary data was visualized using TREEspot (DiscoverX). Likelihood scores (*LS*) were calculated as described below.

Likelihood Score (LS) for mechanistic targets based on KINOMEscan profiles. The KINOMEscan assay measures the extent to which a chemical compound disrupts the interaction between recombinant kinases and their ligands. We subjected Dabrafenib, MK1775 and AZ628 to KINOMEscan profiling over 403 non-mutant kinases and 65 mutant kinases. We reasoned that the mechanistic target through which Dabrafenib and MK1775 act to suppress TOP-mRNA translation would likely be disrupted by both compounds, but not by AZ628, a BRAF inhibitor similar to Dabrafenib, but which did not affect TOP-mRNA translation in the chemical screen (Fig. 2A-B). We defined a likelihood score (LS) of an assayed kinase being the mechanistic target, as a function based on the Euclidean distance between interaction data for this assayed kinase and the ideal case, namely interaction with Dabrafenib (Dbr) = 0%, MK1775 (MK) = 0%, AZ628 (AZ) = 100%. Higher LS value indicates greater likelihood that a kinase is the mechanistic target.

$$LS = \left\{ \frac{\%Int(\mathrm{Dbr})^2 + \%Int(\mathrm{MK})^2 + [100 - \%Int(\mathrm{AZ})]^2}{3 \times (100)^2} \right\}^{-\frac{1}{2}}$$

where $\%Int(\mathrm{compound})$ is the percentage interaction of the recombinant kinase with its ligand, in the presence of the compound compared to DMSO control.

***z*-score calculations and threshold determination.** We found that the collection of kinase inhibitors profiled was enriched for translation inhibitors, and therefore skewed towards $\log_2 FC < 0$ (Fig. 2A, Fig. S). However, we propose that the population of all chemical compounds should not be enriched for such inhibitors. For the purposes of z-score calculations, we assumed population mean $\log_2 FC$ (μ) of zero. We also assumed that this population is Gaussian with distribution and standard deviation (σ) that can be approximated by the subset of compounds with $\log_2 FC > 0$. z-score was calculated as $z = (x - \mu)/\sigma$, where x is $\log_2 FC$ for a particular compound. Threshold for translation modulation was defined as $|\log_2 FC| > 2.58\sigma$, which corresponds to $p < 0.01$ (two-tailed).

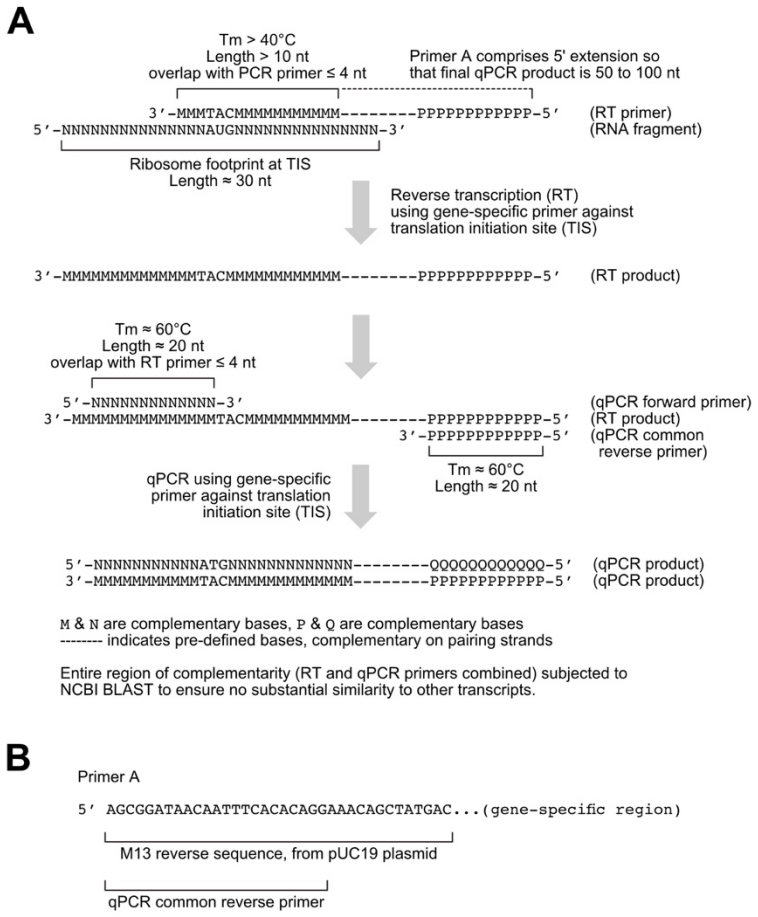

Figure S1. TPRT primer design principals. (A) Reverse transcription (RT) and qPCR primers designed to target ribosome footprint sequence at translation initiation site (TIS) of pre-determined genes, with Tm and length of complementary regions as specified. RT primers have extended 5' sequence to increase cDNA product length. Overlap between RT and qPCR forward primer is kept minimal. **(B)** Sequences of RT primer 5' region and qPCR common reverse primer.

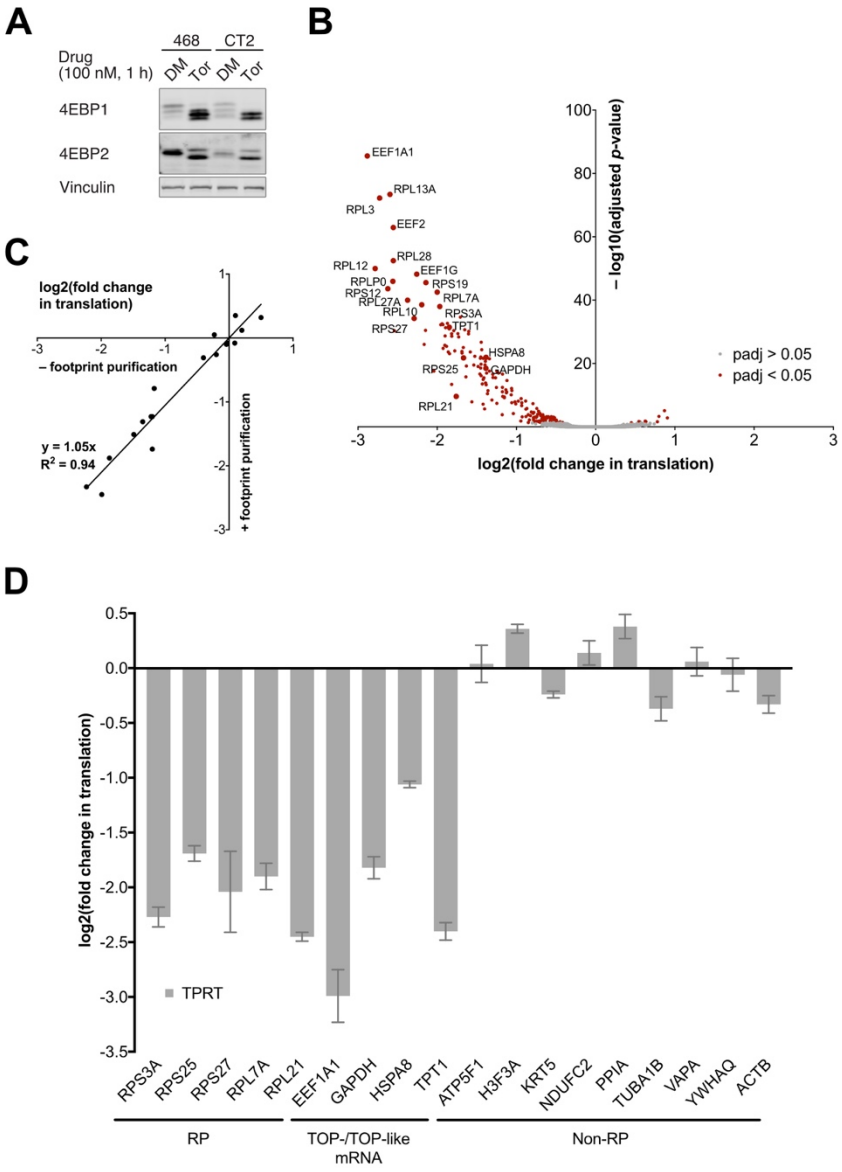

Figure S2. Experimental validation of the TPRT protocol. (Continued overleaf)

Figure S2. Experimental validation of the TPRT protocol. (A) mTOR signaling under Torin-1 treatment (100 nM, 1 h) in MDA-MB-468 and HMEC-CT2 cells. mTOR activity is measured by phosphorylation shifts in total 4EBP1 and 4EBP2 blots. **(B)** Changes in transcriptome-wide translation under Torin-1 treatment (100 nM, 1 h) in MDA-MB-468 cells, measured by ribosome profiling and calculated based on RPKM measurements across the coding sequence. For direct comparison to TPRT data, changes in translation are not normalized to mRNA levels, as described in the Materials and Methods. **(C)** Change in translation of RPs, TOP-/TOP-like and non-RP mRNAs examined in Fig. 1B as measured by TPRT ("– footprint purification"), or TPRT with the addition of ribosomal footprint purification ("+ footprint purification"). MDA-MB-468 cells were treated with Torin-1 (100 nM, 1 h) and processed to determine effect of including ribosomal footprint purification steps in TPRT protocol, as described in the Materials and Methods. Fold changes derived as mean of 3 biological replicates, each with 4 technical replicates. **(D)** Change in translation level of ribosomal protein (RP) mRNAs, other TOP- and TOP-like mRNAs, and non-RP internal control mRNAs, under Torin-1 treatment (100 nM, 1 h) in HMEC-CT2 cells. Translation changes, measured by TPRT, are relative to normalization factor of non-RP internal controls, and DMSO vehicle control, as described in the Materials and Methods. For direct comparison to TPRT data, changes in translation are not normalized to mRNA levels, as described in the Materials and Methods. Fold changes derived as mean of 4 biological replicates, each with 4 technical replicates; error bars denote standard errors.

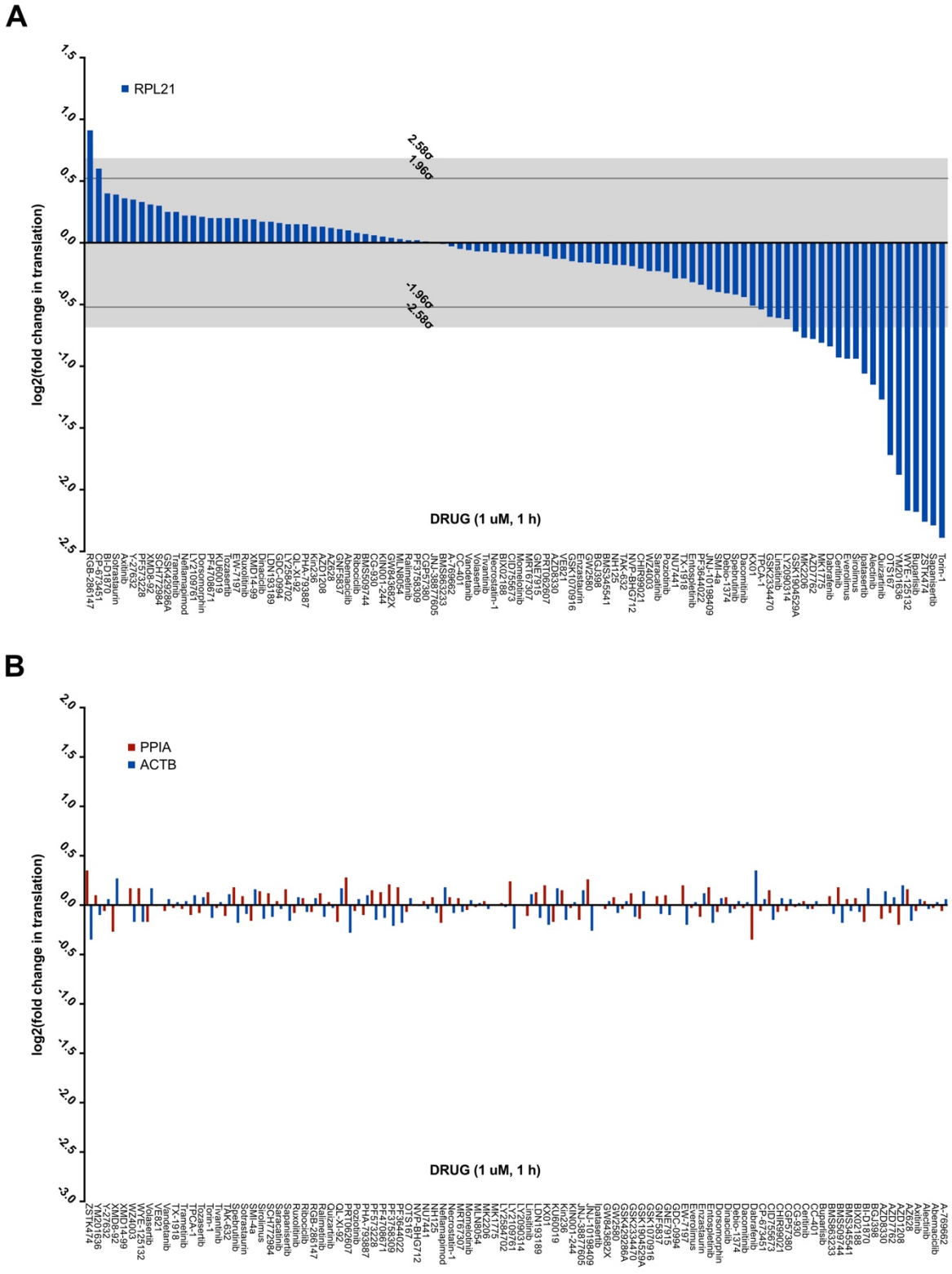

Figure S3. Supporting data for RP translation inhibitor chemical screen. (Continued overleaf)

Figure S3. Supporting data for RP translation inhibitor chemical screen. Change in translation of **(A)** RPS27, and **(B)** PPIA and ACTB, under acute treatment by compound library (1 μM, 1 h) in HMEC-CT2 cells. Translation changes, measured by TPRT, are relative to normalization factor of non-RP internal controls (PPIA, ACTB), and DMSO vehicle control; normalization and *z*-value thresholds are described in the Materials and Methods.

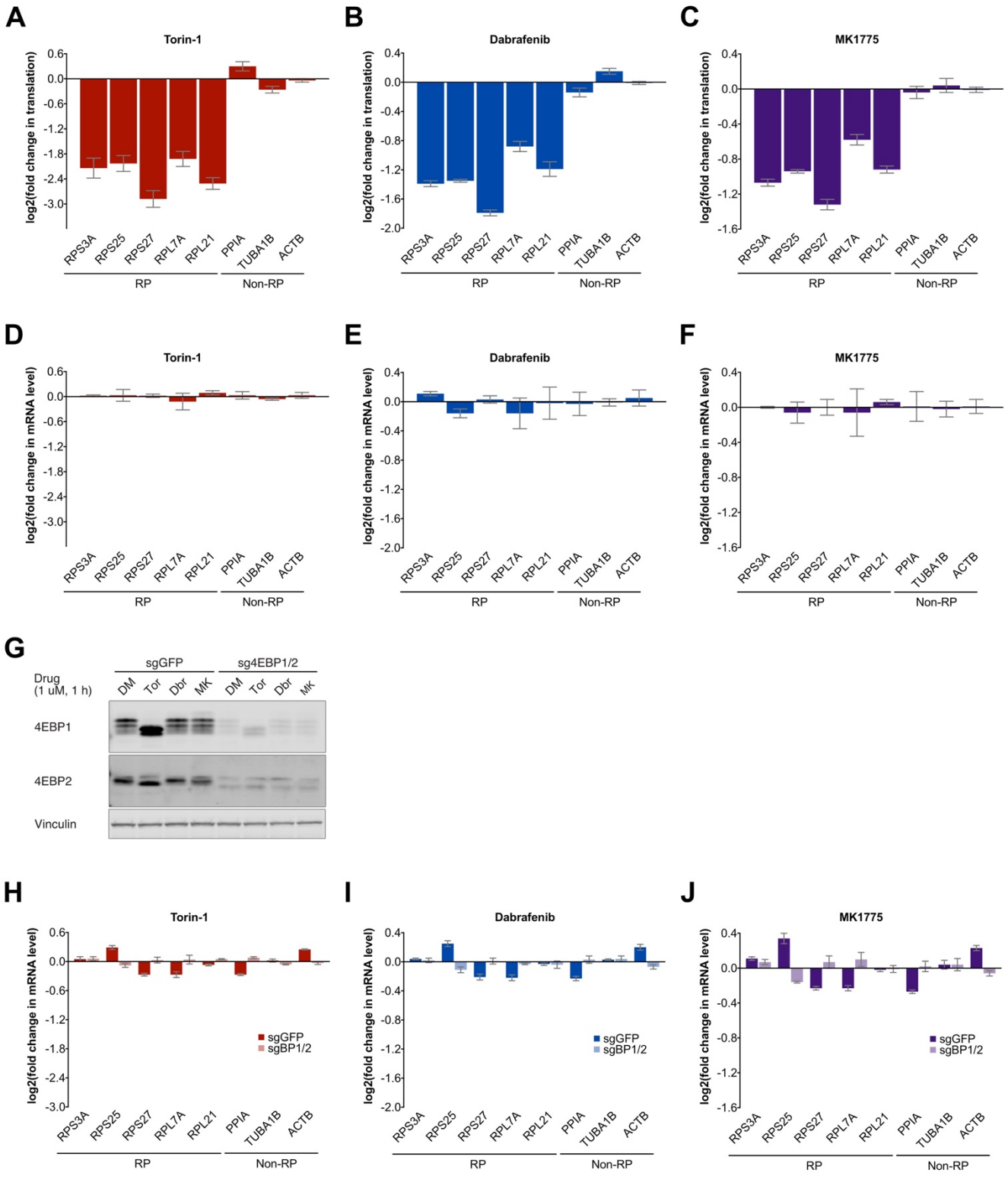

Figure S4. Supporting data for validation of Dabrafenib (Dbr) and MK1775 (MK) as mTOR-4EBP1/2-independent translation inhibitors. (Continued overleaf)

Figure S4. Supporting data for validation of Dabrafenib (Dbr) and MK1775 (MK) as mTOR-4EBP1/2-independent translation inhibitors. Changes in **(A-C)** RP translation, and **(D-F)** RP mRNA levels, under acute drug treatment (1 μM, 1 h) in HMEC-CT2 parental cells. **(G)** mTOR signaling and 4EBP1/2 expression, and **(H-J)** Changes in RP mRNA levels, under acute drug treatment (1 μM, 1 h) in HMEC-CT2 subjected to CRISPR-Cas9 targeting GFP or 4EBP1/2. Translation changes, measured by TPRT, and mRNA level changes are relative to normalization factor of non-RP internal controls, and DMSO vehicle control, as described in the Materials and Methods. Fold changes derived as mean of 3 biological replicates, each with 3 technical replicates; error bars denote standard errors.

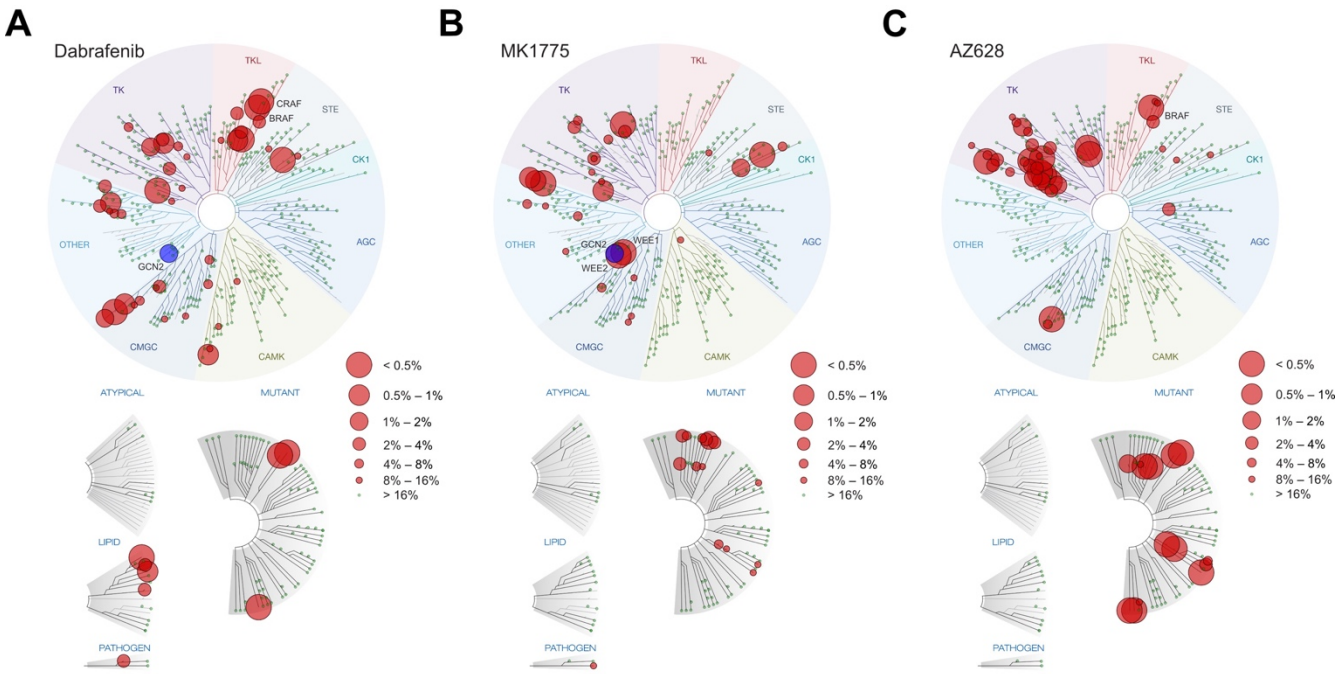

Figure S5. KINOMEscan profiling data for Dabrafenib, MK1775 and AZ628. TREEspot visualization indicates percentage interaction of each recombinant kinase to its ligand in the presence of the compound versus DMSO control (SI Table 5).

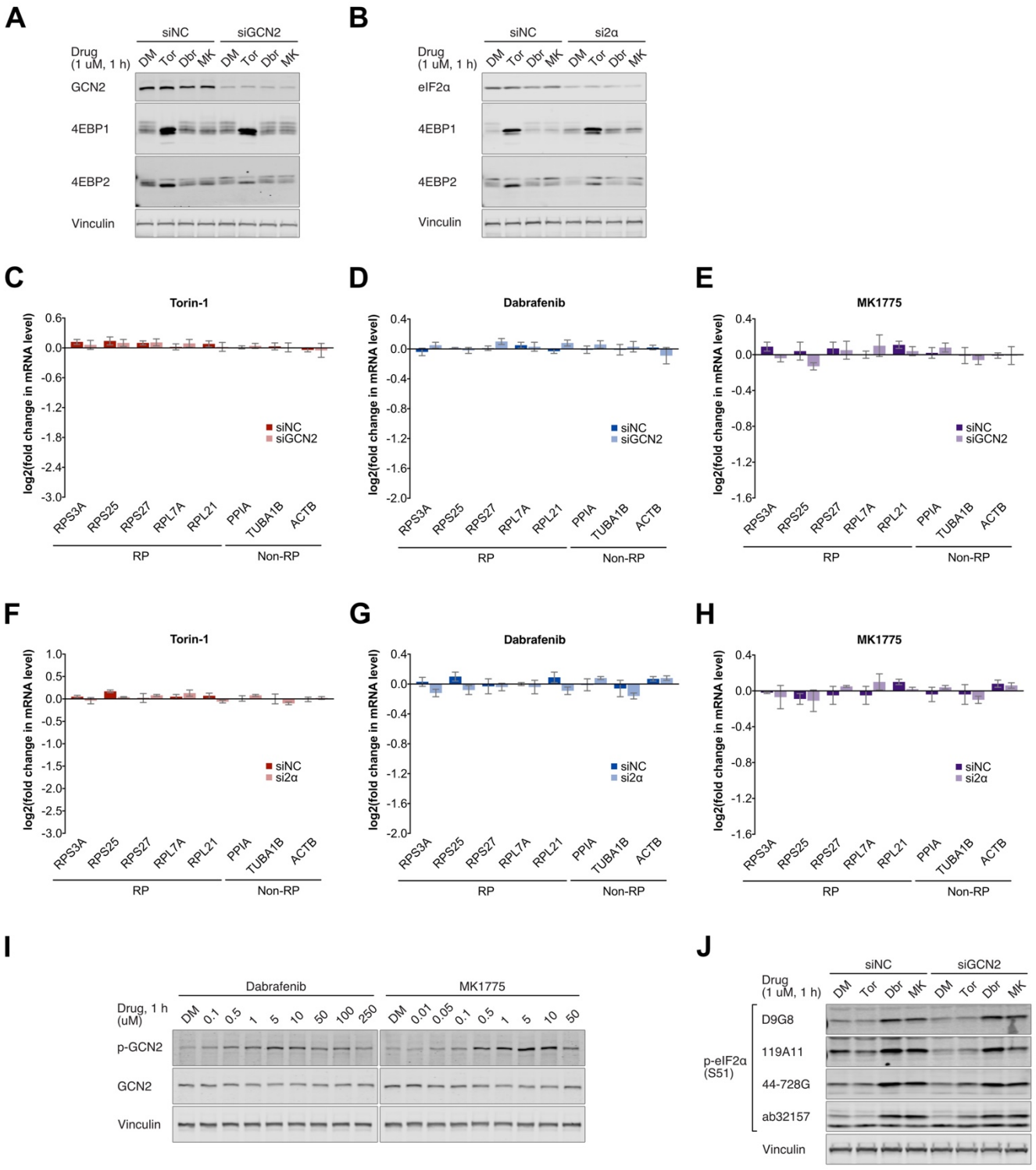

Figure S6. Supporting data for examining dependency of Dabrafenib (Dbr) and MK1775 (MK) translation effects on GCN2 and eIF2α. (Continued overleaf)

Figure S6. Supporting data for examining dependency of Dabrafenib (Dbr) and MK1775 (MK) translation effects on GCN2 and eIF2α. (A, B) mTOR signaling, and GCN2 or eIF2α expression, and **(C-H)** changes in RP mRNA levels, under acute drug treatment (1 μM, 1 h) in HMEC-CT2 subjected to negative control siRNA, or siRNA targeting GCN2 (C-E) or eIF2α (F-H). mRNA level changes are relative to normalization factor of non-RP internal controls, and DMSO vehicle control, as described in the Materials and Methods. Fold changes derived as mean of 4 (C-E) or 3 (F-H) biological replicates, each with 3 technical replicates; error bars denote standard errors. **(I)** GCN2 phosphorylation level under acute drug treatment (1 h) under varying drug doses in HMEC-CT2 parental cells. **(J)** eIF2α phosphorylation level under acute drug treatment (1 μM, 1 h) in HMEC-CT2 subjected to negative control siRNA, or siRNA targeting GCN2. p-eIF2α antibody clones or catalog numbers are indicated, and described in the Material and Methods.

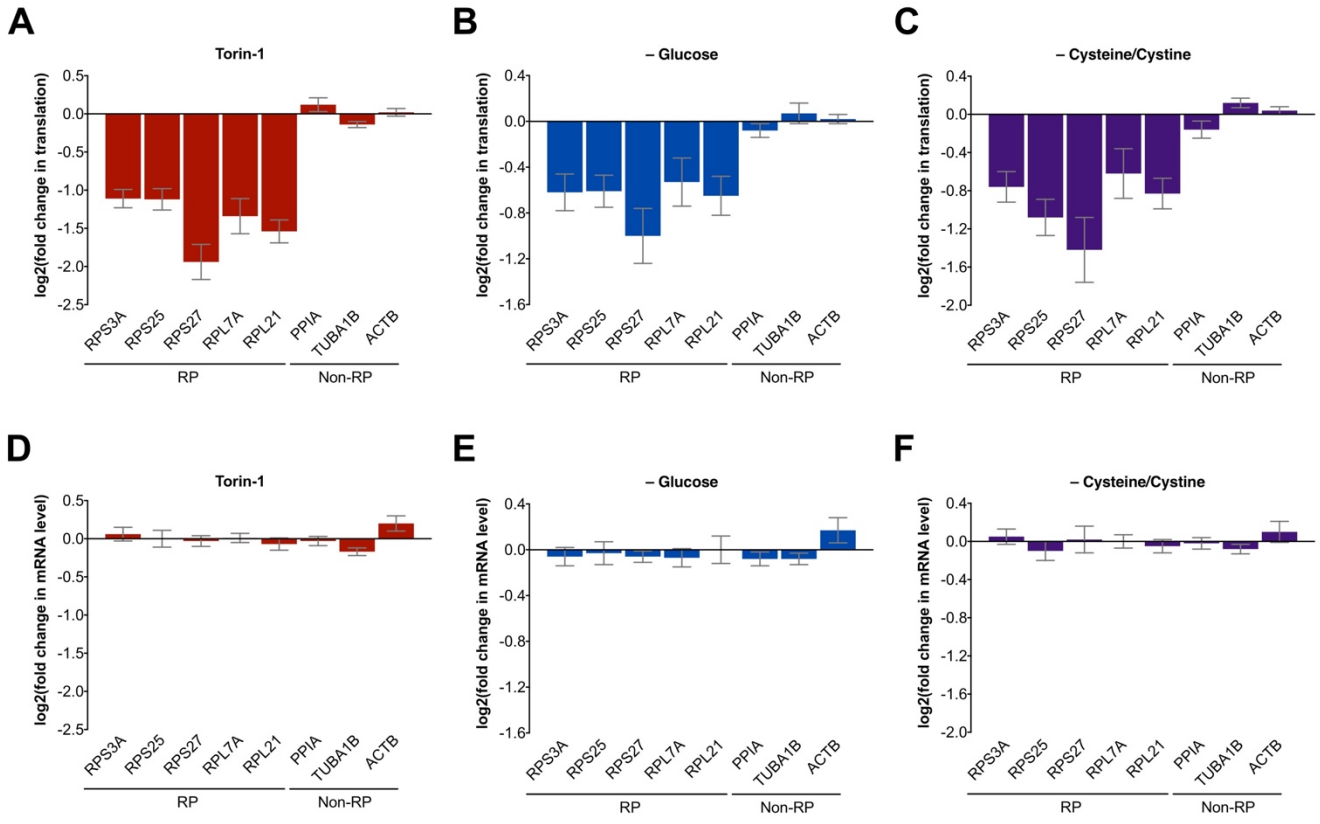

Figure S7. Supporting data for examining RP translation under limitation of glucose (– Gluc) or cysteine/cystine (– C/C). Changes in **(A-C)** RP translation, and **(D-F)** RP mRNA levels, under Torin-1 treatment (1 µM, 30 min), or acute metabolic perturbations (30 min), in Src-transformed MCF10A cells. Translation changes, measured by TPRT, and mRNA level changes are relative to normalization factor of non-RP internal controls, and DMSO vehicle control, as described in the Materials and Methods. Fold changes derived as mean of 3 biological replicates, each with 3 technical replicates; error bars denote standard errors.

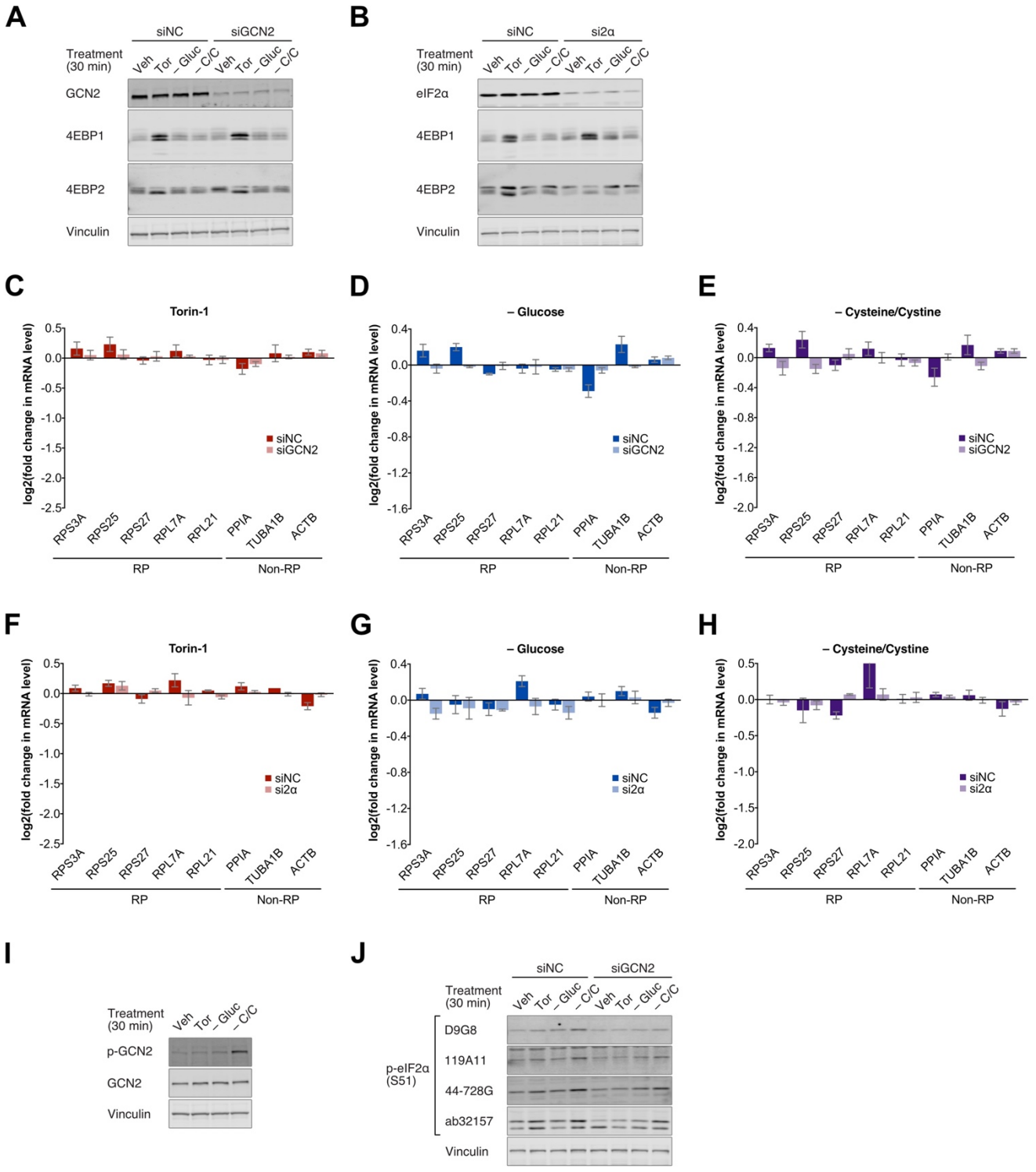

Figure S8. Supporting data for examining dependency of glucose (– Gluc) or cysteine/cystine (– C/C) limitation-mediated translation effects on GCN2 and eIF2α. (Continued overleaf)

Figure S8. Supporting data for examining dependency of glucose (– Gluc) or cysteine/cystine (– C/C) limitation-mediated translation effects on GCN2 and eIF2α. (A, B) mTOR signaling, and GCN2 or eIF2α expression, and **(C-H)** changes in RP mRNA levels, under Torin-1 treatment (1 μM, 30 min), or acute metabolic perturbations (30 min), in Src-transformed MCF10A cells subjected to negative control siRNA, or siRNA targeting GCN2 (A, C-E) or eIF2α (B, F-H). mRNA level changes are relative to normalization factor of non-RP internal controls, and DMSO vehicle control, as described in the Materials and Methods. Fold changes derived as mean of 4 (C-E) or 3 (F-H) biological replicates, each with 3 technical replicates; error bars denote standard errors. Phosphorylation levels of **(I)** GCN2, and **(J)** eIF2α, under Torin-1 treatment (1 μM, 30 min), or acute metabolic perturbations (30 min), in Src-transformed MCF10A cells (I), or Src-transformed MCF10A cells subjected to negative control siRNA or siRNA targeting GCN2 (J).

Group	Gene Symbol	Gene Name	Function	5' Sequence
RP	RPS3A	ribosomal protein S3A	RNA translation	CCCTTTTGGCTCTCTGACCAGCACCATGGCGGTTGGCAAGAACAAGCGCC
	RPS25	ribosomal protein S25	RNA translation	CTTTTTGTCCGACATCTTGACGAGGCTGCGGTGTCTGCTGCTATTCTCCG
	RPS27	ribosomal protein S27	RNA translation	CTTTCCGGCGGTGACGACCTACGCACACGAGAACATGCCTCTCGCAAAGG
	RPL7A	ribosomal protein L7A	RNA translation	CTCTCTCCTCCCGCCGCCCAAGATGCCGAAAGGAAAGAAGGCCAAGGGAA
	RPL21	ribosomal protein L21	RNA translation	CCTTTCGGCCGGAACCGCCATCTTCCAGTAATTCGCCAAAATGACGAACA
TOP-/TOP-like mRNA	EEF1A1	eukaryotic translation elongation factor 1 alpha 1	RNA translation	CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTGTCGTGAAAACTACC
	TPT1	tumor protein, translationally-controlled 1	immunity & proliferation	CTTTTCCGCCCGCTCCCCCCTCCCCCCGAGCGCCGCTCCGGCTGCACCGC
	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	metabolism	GCTCTCTGCTCCTCCTGTTCGACAGTCAGCCGCATCTTCTTTTGCGTCGC
	HSPA8	heat shock protein family A (Hsp70) member 8	heat shock response	CTCATTGAACTCGCCTGCAGCTCTTGGGTTTTTTGTGGCTTCCTTCGTTA
Non-RP	ATP5F1	ATP synthase, H+ transporting, mitochondrial Fo complex subunit B1	metabolism	ATCGGGGTCACAGGGACGCTAAGATTGCTACCTGGACTTTCGTTGACCAT
	H3F3A	H3 histone family member 3A	nucleosome structure	AATTGTGTTCGCAGCCGCCGCCGCGCCGCCGTCGCTCTCCAACGCCAGCG
	KRT5	keratin 5	cell structure	AACAGAGCCACCTTCTGCGTCCTGCTGAGCTCTGTTCTCTCCAGCACCTC
	NDUFC2	NADH:ubiquinone oxidoreductase subunit C2	metabolism	GAGTCCGGCGCGCAGAGGAGGAGGAGAAAGCTGACCGCTTAGGCCGGGGT
	PPIA	peptidylprolyl isomerase A	metabolism	GTTTTGCAGACGCCACCGCCGAGGAAAACCGTGTACTATTAGCCATGGTC
	TUBA1B	tubulin alpha 1b	cell structure	AGTGCGTTACTTACCTCGACTCTTAGCTTGTCGGGGACGGTAACCGGGAC
	VAPA	VAMP associated protein A	vesicle transport	GTCTCTCCGATGGCGTCCGCCTCAGGGGCCATGGCGAAGCACGAGCAGAT
	YWHAQ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein theta	signal transduction	AAAGCCAAAAGCAGATCAAAGTGGTGGGACTCGCGTCGCGGCCGCGGAGA
	ACTB	actin beta	cell structure	ACCGCCGAGACCGCGTCCGCCCCGCGAGCACAGAGCCTCGCCTTTGCCGA

Table S1. 5' Sequences of TPRT panel genes. Transcription start site co-ordinates obtained from NCBI RefSeq database under 'major' or 'predominant' transcription initiation site annotation if available (RPL7A, RPL21, EEF1A1, KRT5), otherwise obtained from dbTSS (*dbtss.hgc.jp*) based on the predominant TSS tag signal. Terminal oligopyrimidine (TOP) motif sequences shown in red. TOP- and TOP-like mRNAs defined according to Yamashita *et al.* (6).

Group	Gene	Isoform target	Translation initiation site sequence	Primer type	Primer sequence	Tm	Length
RP	RPS3A	All isoforms	TCTCTGACCAGCACC ATG GCGGTTGGCAAGAAC	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC GTTCTTGCCAACCG	49.8	14
				qPCR forward	TCTCTGACCAGCACCATGG	61.0	19
	RPS25	All isoforms	TCTCCGAGCTTCGCA ATG CCGCCTAAGGACGAC	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC TCGTCCTTAGGCG	49.2	13
				qPCR forward	TCTCCGAGCTTCGCAATGC	62.2	19
	RPS27	All isoforms	TACGCACACGAGAAC ATG CCTCTCGCAAAGGAT	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC ATCCTTTGCGAGAGG	49.4	15
				qPCR forward	TACGCACACGAGAACATGCC	62.3	20
	RPL7A	All isoforms	CTCCCGCCGCCCAAG ATG CCGAAAGGAAAGAAG	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC CTTCTTTCCTTTCGGC	56.0	16
				qPCR forward	CCCGCCGCCCAAGAT	61.3	15
	RPL21	All isoforms	CAGTAATTCGCCAAA ATG ACGAACACAAAGGGA	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC TCCCTTTGTGTTCG	50.9	14
				qPCR forward	CAGTAATTCGCCAAAATGACGAA	59.8	23
TOP-/TOP-like mRNA	EEF1A1	All isoforms	CCCCTAAAAGCCAAA ATG GGAAAGGAAAAGACT	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC AGTCTTTTCCTTTCCC	58.4	16
				qPCR forward	CCCCTAAAAGCCAAAATGGG	58.9	20
	GAPDH	Isoforms 1,3,4	CATCGCTCAGACACC ATG GGGAAGGTGAAGGTC	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC GACCTTCACCTTCC	54.3	14
				qPCR forward	CATCGCTCAGACACCATGG	59.9	19
	HSPA8	All isoforms	TACACCCCAGCAACC ATG TCCAAGGGACCTGCA	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC AGGTCCCTTGGAC	52.3	13
				qPCR forward	TACACCCCAGCAACCATGT	61.1	19
	TPT1	Isoforms 1,2	CCTTCAGTCGCCATC ATG ATTATCTACCGGGAC	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC GTCCCGGTAGATAATC	50.3	16
				qPCR forward	CCTTCAGTCGCCATCATGAT	59.5	20
Non-RP	ATP5F1	All isoforms	GGACTTTCGTTGACC ATG CTGTCCCGGGTGGTA	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC CCACCCGGGACA	52.1	12
				qPCR forward	GGACTTTCGTTGACCATGCTG	61.1	21
	H3F3A	All isoforms	GGAGGTCTCTGTACC ATG GCTCGTACAAAGCAG	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC CTGCTTTGTACGAGC	49.0	15
				qPCR forward	GGAGGTCTCTGTACCATGGC	61.2	20
	KRT5	All isoforms	CAGGAACAAGCCACC ATG TCTCGCCAGTCAAGT	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC ACTTGACTGGCGAG	44.9	14
				qPCR forward	CAGGAACAAGCCACCATGT	59.9	19
	NDUFC2	All isoforms	GGAAACGGCGTCACC ATG ATCGCACGGCGGAAC	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC CGCCGTGCGAT	45.5	11
				qPCR forward	GGAAACGGCGTCACCAT	59.4	17
	PPIA	Isoforms 1	CGTGTACTATTAGCC ATG GTCAACCCCACCGTG	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC CGGTGGGGTTGA	46.6	12
				qPCR forward	CGTGTACTATTAGCCATGGTCA	59.3	22
	TUBA1B	All isoforms	TAATCCCTAGCCACT ATG CGTGAGTGCATCTCC	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC GAGATGCACTCACG	43.7	14
				qPCR forward	TAATCCCTAGCCACTATGCGT	60.4	21
	VAPA	All isoforms	TGCGCTGTCTCTCCG ATG GCGTCCGCCTCAGGG	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC CTGAGGCGGACG	43.1	12
				qPCR forward	CGCTGTCTCTCCGATGGC	61.7	18
	YWHAQ	All isoforms	GGCCCCGCGCCCGCC ATG GAGAAGACTGAGCTG	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC CAGCTCAGTCTTCTC	54.7	15
				qPCR forward	GCGCCCGCCATGG	59.7	13
	ACTB	All isoforms	CGCCGCCAGCTCACC ATG GATGATGATATCGCC	RT	AGCGGATAACAATTTCACACAGGAAACAGCTATGAC GGCGATATCATCATC	45.1	15
				qPCR forward	CGCCAGCTCACCATGGA	61.3	17
qPCR common reverse					AGCGGATAACAATTTCACACAGG	61.1	23

Table S2. TPRT primer information. Sequences of translation initiation site (TIS), and TIS-targeting reverse transcription (RT) and qPCR primers, with Tm and length of primer complementary regions. Tm calculated using IDT OligoAnalyzer 3.1 (*idtdna.com/calc/analyzer*) with parameters: for RT, target type is RNA, 0.1 µM oligo, 75 mM monovalent cation, 3 mM divalent cation, 2 mM dNTPs total; for qPCR, target type is DNA, 0.2 µM oligo, 50 mM monovalent cation, 1.5 mM divalent cation, 0.8 mM dNTPs. Complementary regions on transcript sequence: RT primer, blue; qPCR forward primer, yellow; RT & qPCR primer overlap, red.

Group	Drug	Nominal target(s)	Nominal target family	log2(fold change)			
				RPS27	RPL21	PPIA	ACTB
1	Tozasertib	Aurora A/B/C	Aurora	0.14	0.20	-0.08	0.08
1	Saracatinib	Src family	SRC	0.01	-0.23	0.04	-0.04
1	Torin-1	mTOR	mTOR	-2.38	-2.39	0.13	-0.13
1	Ruxolitinib	JAK1/2	JAK	0.05	0.19	-0.08	0.08
1	Trametinib	MEK1/2	MEK1/2	0.34	0.25	-0.04	0.04
1	Buparlisib	pan-PI3K	PI3K	-2.84	-2.18	-0.01	0.01
1	BGJ398	FGFR1/2/3/4	FGFR	-0.47	-0.17	0.00	0.00
1	CC-401	JNK1/2/3	JNK	-0.18	-0.05	-0.04	0.04
1	Sapanisertib	mTOR	mTOR	-2.61	-2.29	0.16	-0.16
1	Everolimus	mTOR(FKBP12)	mTOR(FKBP12)	-1.25	-0.94	-0.03	0.03
1	Ipatasertib	AKT1/2/3	AKT	-1.59	-1.06	0.01	-0.01
1	CP-673451	PDGFRa/b	PDGFR	0.77	0.60	-0.06	0.06
1	AZD1208	PIM1/2/3	PIM	0.02	0.13	-0.20	0.20
1	LY2584702	p70S6K1	p70S6K1	0.09	0.15	0.02	-0.02
1	Abemaciclib	CDK4/6	CDK	-0.04	0.10	-0.03	0.03
1	Poziotinib	ERBB1/2/4	ERBB	-0.50	-0.24	-0.06	0.06
1	CGP57380	MNK1	MNK	-0.21	0.01	-0.06	0.06
1	BI-D1870	RSK1/2/3/4	RSK	0.42	0.40	-0.17	0.17
1	Axitinib	VEGFR1/2/3	VEGFR	0.34	0.36	-0.06	0.06
1	RGB-286147	CDK1/2/3/5/7/9	CDK	0.99	0.91	-0.07	0.07
2	GSK429286A	ROCK1/2	ROCK	0.22	0.25	-0.04	0.04
2	Quizartinib	FLT3	FLT3	-1.41	-1.27	0.03	-0.03
2	NU7441	DNA-PK	DNA-PK	-0.20	-0.29	0.04	-0.04
2	Enzastaurin	pan-PKC	PKC	-0.36	-0.16	-0.12	0.12
2	QL-XI-92	DDR1	DDR1	0.09	0.15	-0.17	0.17
2	XMD8-92	ERK5	ERK5	0.39	0.31	-0.27	0.27
2	Tivantinib	MET	MET	-0.16	-0.07	-0.03	0.03
2	Linsitinib	IGF-1R, IR	IGF-1R, IR	-1.15	-0.61	-0.11	0.11
2	KU60019	ATM	ATM	0.04	0.20	-0.17	0.17
2	Neflamapimod	p38a/b	p38	0.11	0.22	-0.18	0.18
2	CHIR99021	GSK3a/b	GSK3	-0.31	-0.21	-0.07	0.07
2	Alectinib	ALK	ALK	-1.48	-1.15	0.04	-0.04
2	Dabrafenib	b/c-Raf	RAF	-1.51	-0.84	-0.35	0.35
2	Volasertib	PLK1/2/3	PLK	-0.32	-0.07	-0.17	0.17
2	Dorsomorphin	AMPK	AMPK	0.13	0.21	-0.07	0.07
2	GW2580	CSF-1R	CSF-1R	-0.38	-0.16	0.08	-0.08
2	CID755673	PKD1/2/3	PKD	-0.17	-0.09	0.15	-0.15
2	BIX02188	MEK5	MEK5	-0.33	-0.08	0.07	-0.07
2	NH125	eEF2K	eEF2K	-0.43	-0.18	0.08	-0.08
2	PF3644022	MK2, MK3	MK2, MK3	-0.54	-0.34	0.18	-0.18
3	BMS345541	IKK2	IKK	0.12	-0.17	0.06	-0.06
3	XMD14-99	EPHB3	EPHB	0.25	0.19	0.00	0.00
3	YM201636	PIKfyve	PIKfyve	-2.42	-1.88	0.10	-0.10
3	LDN193189	ALK2/3	BMP	0.31	0.17	0.13	-0.13
3	BMS509744	ITK	ITK	-0.09	0.07	0.18	-0.18
3	MK1775	WEE1	WEE1	-1.40	-0.81	0.00	0.00
3	Kin236	Tie2	TIE2	-0.03	0.13	0.15	-0.15
3	PF573228	FAK	FAK	0.23	0.33	0.15	-0.15
3	GSK2334470	PDK1	PDK	-1.15	-0.60	0.12	-0.12
3	PF3758309	PAK family	PAK	-0.09	0.02	0.21	-0.21
3	VE821	ATR	ATR	-0.77	-0.13	-0.01	0.01
3	MRT67307	IKBKE, TBK1	IKBKE, TBK1	-0.44	-0.09	0.07	-0.07
3	GNF5837	TrkA/B/C	TRK	0.11	0.11	0.09	-0.09
3	Entospletinib	Syk	SYK	-0.74	-0.32	0.18	-0.18
3	BMS863233	Cdc7	Cdc7	-0.47	-0.01	0.09	-0.09
3	Necrostatin-1	RIP1	RIP	-0.22	-0.08	0.08	-0.08

3	Spebrutinib	BTK	BTK	-1.05	-0.42	0.18	-0.18
3	GNE7915	LRRK2	LRRK2	-0.55	-0.09	0.10	-0.10
3	WZ4003	NUAK1/2	NUAK	-0.65	-0.23	0.17	-0.17
3	EW-7197	ALK4/5	TGFB	-0.11	0.20	0.20	-0.20
4	AZD7762	CHK1/2	CHK	-1.04	-0.78	-0.08	0.08
4	MK2206	AKT1/2/3	AKT	-0.94	-0.77	0.04	-0.04
4	CG-930	JNK1/3	JNK	-0.10	0.06	-0.02	0.02
4	GSK1070916	Aurora B/C	Aurora	-0.39	-0.15	0.00	0.00
4	WYE-125132	mTOR	mTOR	-2.33	-2.17	0.17	-0.17
4	GSK1904529A	IGF-1R, IR	IGF-1R, IR	-0.97	-0.72	-0.14	0.14
4	TPCA-1	IKK2	IKK	-0.77	-0.54	-0.10	0.10
4	Momelotinib	JAK1/2	JAK	-0.24	-0.09	-0.05	0.05
4	JNJ-38877605	MET	MET	-0.09	0.00	-0.15	0.15
4	AZD8330	MEK1/2	MEK1/2	-0.25	-0.13	-0.14	0.14
4	NVP-BHG712	EPHB4	EPHB	-0.31	-0.19	0.01	-0.01
4	Dacomitinib	pan-ERBB	ERBB	-0.75	-0.44	-0.03	0.03
4	OTS167	MELK, others	MELK, others	-2.26	-1.72	-0.07	0.07
4	GDC-0994	ERK1/2	ERK1/2	0.05	0.16	0.00	0.00
4	LY2090314	GSK3a/b	GSK3	-0.85	-0.62	0.01	-0.01
4	Ceritinib	ALK	ALK	-1.30	-0.93	0.04	-0.04
4	TAK-632	b/c-Raf	RAF	-0.38	-0.18	-0.11	0.11
4	SMI-4a	PIM1/2	PIM	-0.67	-0.40	-0.16	0.16
4	Debio-1374	FGFR1/2/3	FGFR	-0.69	-0.41	-0.04	0.04
4	TX-1918	eEF2K	eEF2K	-0.68	-0.29	-0.03	0.03
5	GW843682X	PLK1/3	PLK	-0.05	0.04	-0.04	0.04
5	AZ628	pan-Raf	RAF	0.30	0.12	0.16	-0.16
5	Sirolimus	mTOR(FKBP12)	mTOR(FKBP12)	-0.96	-0.94	0.14	-0.14
5	ZSTK474	pan-PI3K	PI3K	-1.54	-2.26	0.35	-0.35
5	MLN8054	Aurora A/B	Aurora	-0.04	0.03	-0.02	0.02
5	PHA-793887	CDK1/2/4/5/7/9	CDK	-0.23	0.15	-0.10	0.10
5	KIN001-244	PDK1	PDK	-0.36	0.05	-0.03	0.03
5	A-769662	AMPK	AMPK	-0.37	-0.03	-0.06	0.06
5	Y-27632	ROCK1/2	ROCK	0.08	0.35	-0.06	0.06
5	Vandetanib	VEGFR2/3	VEGFR	-0.56	-0.06	-0.06	0.06
5	Dinaciclib	CDK1/2/5/9	CDK	0.31	0.17	0.08	-0.08
5	KX01	Src	SRC	-0.26	-0.51	0.20	-0.20
5	Ribociclib	CDK4/6	CDK	0.41	0.08	0.07	-0.07
5	PF4708671	p70S6K1	p70S6K1	0.11	0.20	0.13	-0.13
5	PRT062607	Syk	SYK	0.28	-0.11	0.28	-0.28
5	Sotrastaurin	pan-PKC	PKC	0.77	0.39	0.09	-0.09
5	LY2109761	TbRI/II	TGFB	0.54	0.22	0.24	-0.24
5	Ralimetinib	p38a/b	p38	0.36	0.02	0.12	-0.12
5	SCH772984	ERK1/2	ERK1/2	0.59	0.30	0.12	-0.12
5	JNJ-10198409	PDGFRa/b	PDGFR	0.03	-0.38	0.26	-0.26

Table S3. Chemical screening data. List of kinase inhibitors tested for TOP-mRNA translation inhibition, their nominal targets and kinase family of nominal targets, and their effects on translation of TOP-mRNA transcripts (RPS27, RPL21), relative to non-TOP-mRNA transcripts (PPIA, ACTB), and DMSO vehicle control, under acute treatment (1 μM, 1 h) in HMEC-CT2 cells. Screening was carried out in 5 groups of 20 compounds each; group numbers are shown.

Drug	Nominal target(s)	Clinical indication	Clinical trial progress	RPS27		RPL21	
				log2(FC)	*z*-score	log2(FC)	*z*-score
Torin-1	mTOR	-	Preclinical	-2.38	-6.52	-2.39	-8.97
Sapanisertib	mTOR	various cancers	Phase 2	-2.61	-7.15	-2.29	-8.61
ZSTK474	pan-PI3K	various cancers	Phase 1	-1.54	-4.22	-2.26	-8.49
Buparlisib	pan-PI3K	breast cancer	Phase 3	-2.84	-7.77	-2.18	-8.18
WYE-125132	mTOR	-	Preclinical	-2.33	-6.39	-2.17	-8.16
YM201636	PIKfyve	-	Preclinical	-2.42	-6.62	-1.88	-7.07
OTS167	MELK	liquid cancers	Phase 1/2	-2.26	-6.20	-1.72	-6.46
Quizartinib	FLT3	AML	Phase 3	-1.41	-3.86	-1.27	-4.79
Alectinib	ALK	NSCLC	FDA approved	-1.48	-4.05	-1.15	-4.31
Ipatasertib	AKT1/2/3	prostate cancer	Phase 3	-1.59	-4.37	-1.06	-3.99
Everolimus	mTOR (FKBP12)	breast, pancreatic, RCC, SEGA	FDA approved	-1.25	-3.43	-0.94	-3.54
Sirolimus	mTOR (FKBP12)	various cancers	Phase 3/4	-0.96	-2.63	-0.94	-3.53
Ceritinib	ALK	NSCLC	FDA approved	-1.30	-3.56	-0.93	-3.48
Dabrafenib	b/c-Raf	melanoma	FDA approved	-1.51	-4.14	-0.84	-3.17
MK1775	WEE1	head and neck, ovarian cancers	Phase 2	-1.40	-3.84	-0.81	-3.05
AZD7762	CHK1/2	various solid cancers	Phase 1	-1.04	-2.86	-0.78	-2.94
GSK1904529A	IGF-1R, IR	-	Preclinical	-0.97	-2.65	-0.72	-2.69

Table S4. List of RP-mRNA translation inhibitors identified in chemical screen. RP translation inhibitors, defined as those that meet inhibition threshold for RPS27 and RPL21, are listed with their nominal targets, clinical indication and clinical trial status (*clinicaltrials.gov*, retrieved January 15, 2018).

DiscoveRx Gene Symbol	Entrez Gene Symbol	% DMSO control			Likelihood score (LS)
		Dabrafenib	MK1775	AZ628	
GCN2(Kin.Dom.2,S808G)	EIF2AK4	1.40	1.50	100.00	84.41
NEK11	NEK11	3.30	13.00	100.00	12.91
HIPK1	HIPK1	13.00	14.00	79.00	6.10
SYK	SYK	14.00	23.00	80.00	5.16
CDK4-cyclinD1	CDK4	41.00	5.50	100.00	4.19
TRKA	NTRK1	32.00	27.00	92.00	4.06
SIK2	SIK2	15.00	40.00	96.00	4.04
PRKD3	PRKD3	13.00	41.00	86.00	3.83
JAK2(JH1domain-catalytic)	JAK2	34.00	3.90	67.00	3.64
TYK2(JH2domain-pseudokinase)	TYK2	3.20	4.60	52.00	3.58
PCTK2	CDK17	1.40	48.00	89.00	3.52
ULK2	ULK2	33.00	31.00	78.00	3.44
CDK7	CDK7	22.00	33.00	68.00	3.40
PCTK1	CDK16	0.20	52.00	94.00	3.31
HIPK3	HIPK3	46.00	27.00	87.00	3.15
STK36	STK36	0.75	59.00	100.00	2.94
HIPK4	HIPK4	51.00	30.00	96.00	2.92
PIK4CB	PI4KB	3.70	57.00	84.00	2.92
CSNK2A2	CSNK2A2	63.00	12.00	96.00	2.70
ERBB3	ERBB3	6.90	64.00	93.00	2.67
CDK4-cyclinD3	CDK4	65.00	5.80	93.00	2.64
SLK	SLK	66.00	15.00	94.00	2.55
GAK	GAK	68.00	7.20	100.00	2.53
TBK1	TBK1	59.00	19.00	71.00	2.53
JAK1(JH2domain-pseudokinase)	JAK1	0.85	69.00	77.00	2.38
STK33	STK33	68.00	16.00	79.00	2.37
PKNB(M.tuberculosis)	pknB	72.00	12.00	96.00	2.37
PLK3	PLK3	64.00	0.55	63.00	2.34
PRKD2	PRKD2	39.00	63.00	86.00	2.30
PRKD1	PRKD1	27.00	71.00	100.00	2.28
MAP3K3	MAP3K3	75.00	6.20	87.00	2.27
CDK3	CDK3	13.00	75.00	91.00	2.26
AAK1	AAK1	54.00	38.00	61.00	2.26
EGFR	EGFR	50.00	43.00	60.00	2.25
TYK2(JH1domain-catalytic)	TYK2	61.00	36.00	69.00	2.24
IKK-epsilon	IKBKE	67.00	35.00	83.00	2.24
ABL1(Q252H)-phosphorylated	ABL1	52.00	2.10	42.00	2.22
FLT3(ITD,F691L)	FLT3	66.00	40.00	89.00	2.22
JAK3(JH1domain-catalytic)	JAK3	78.00	0.00	100.00	2.22
ERBB2	ERBB2	30.00	36.00	37.00	2.21
PLK2	PLK2	75.00	0.90	76.00	2.20
CASK	CASK	66.00	29.00	67.00	2.18
NEK9	NEK9	11.00	79.00	97.00	2.17
TIE1	TIE1	48.00	52.00	63.00	2.17
PIP5K2C	PIP4K2C	26.00	45.00	38.00	2.14
NEK3	NEK3	65.00	6.60	52.00	2.14
ABL1(M351T)-phosphorylated	ABL1	52.00	5.70	38.00	2.14
PCTK3	CDK18	0.60	82.00	100.00	2.11
CAMK2A	CAMK2A	68.00	44.00	85.00	2.10
MAP3K2	MAP3K2	59.00	17.00	45.00	2.10
EGFR(L747-E749del, A750P)	EGFR	62.00	12.00	47.00	2.10
TESK1	TESK1	0.00	81.00	84.00	2.10
ADCK3	CABC1	28.00	73.00	73.00	2.09
CAMKK2	CAMKK2	60.00	31.00	52.00	2.09
ABL1(T315I)-phosphorylated	ABL1	62.00	48.00	73.00	2.09

CSNK2A1	CSNK2A1	73.00	23.00	68.00	2.09
DYRK1B	DYRK1B	42.00	56.00	55.00	2.08
CAMKK1	CAMKK1	63.00	35.00	57.00	2.06
DCAMKL3	DCLK3	57.00	62.00	100.00	2.06
PIK3CA	PIK3CA	0.00	85.00	97.00	2.04
ERK3	MAPK6	83.00	14.00	87.00	2.03
PIK3C2G	PIK3C2G	54.00	63.00	79.00	2.02
ERK4	MAPK4	71.00	44.00	81.00	2.02
PAK4	PAK4	48.00	71.00	96.00	2.02
MYLK	MYLK	44.00	69.00	74.00	2.02

Table S5. KINOMEscan profiling for Dabrafenib, MK1775 and AZ628. Profiling data of percentage loss of interaction of each recombinant kinase to its ligand in the presence of the compound, at 1 μM, versus DMSO control. Likelihood score (LS) of a kinase as the mechanistic target for the observed mTOR-independent TOP-mRNA translation suppression is shown. LS is calculated as described in the Material and Methods. Table shows kinase targets with $LS > 2$.

References

1. Iliopoulos D, Hirsch HA, & Struhl K (2009) An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell* 139(4):693-706.
2. Hirsch HA*, et al.* (2010) A transcriptional signature and common gene networks link cancer with lipid metabolism and diverse human diseases. *Cancer Cell* 17(4):348-361.
3. Ingolia NT, Brar GA, Rouskin S, McGeachy AM, & Weissman JS (2012) The ribosome profiling strategy for monitoring translation in vivo by deep sequencing of ribosome-protected mRNA fragments. *Nat Protoc* 7(8):1534-1550.
4. Hellemans J, Mortier G, De Paepe A, Speleman F, & Vandesompele J (2007) qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biol* 8(2):R19.
5. Ingolia NT, Lareau LF, & Weissman JS (2011) Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes. *Cell* 147(4):789-802.
6. Yamashita R*, et al.* (2008) Comprehensive detection of human terminal oligo-pyrimidine (TOP) genes and analysis of their characteristics. *Nucleic Acids Res* 36(11):3707-3715.