

Supplementary Materials: Contribution of miRNAs, tRNAs and tRFs to Aberrant Signaling and Translation Deregulation in Lung Cancer

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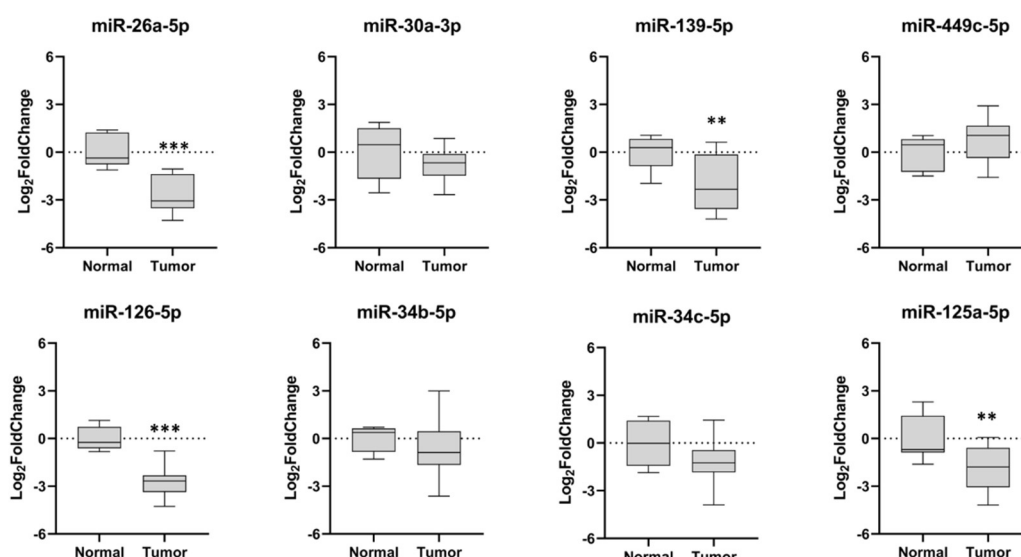


Figure S1. Validation of the expression levels of several miRNAs by RT-qPCR. A total of 8 samples were analysed (3 normal and 5 tumor tissue specimens) for validation of 8 miRNAs after the NGS analysis. Normalization and subsequent calculation of log₂FoldChange were performed using miR-103 as calibrators and $-\Delta\Delta C_t$ method. All experiments were performed in triplicates. Asterisks represent p -values after unpaired t test between the log₂fold expression of each miRNA in normal and tumor specimens (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

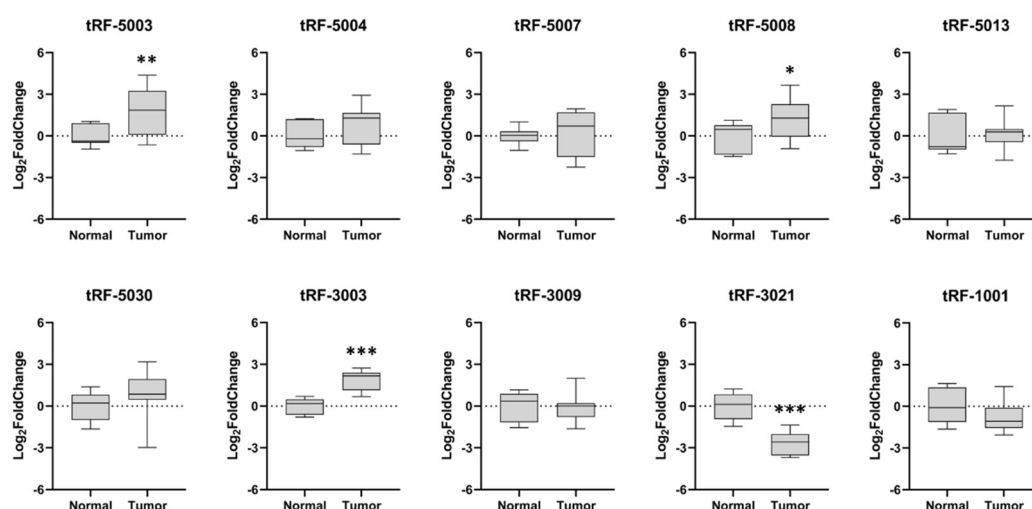


Figure S2. Validation of the expression levels of selected tRFs by RT-qPCR. A total of 8 samples were analyzed (3 normal and 5 tumor tissue specimens) for validation of 10 tRFs after the NGS analysis. Normalization and subsequent calculation of log₂FoldChange were performed using miR-103 as calibrators and $-\Delta\Delta C_t$ method. All experiments were performed in triplicates. Asterisks represent p -

values after unpaired t test between the log2fold expression of each tRF in normal and tumor specimens (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

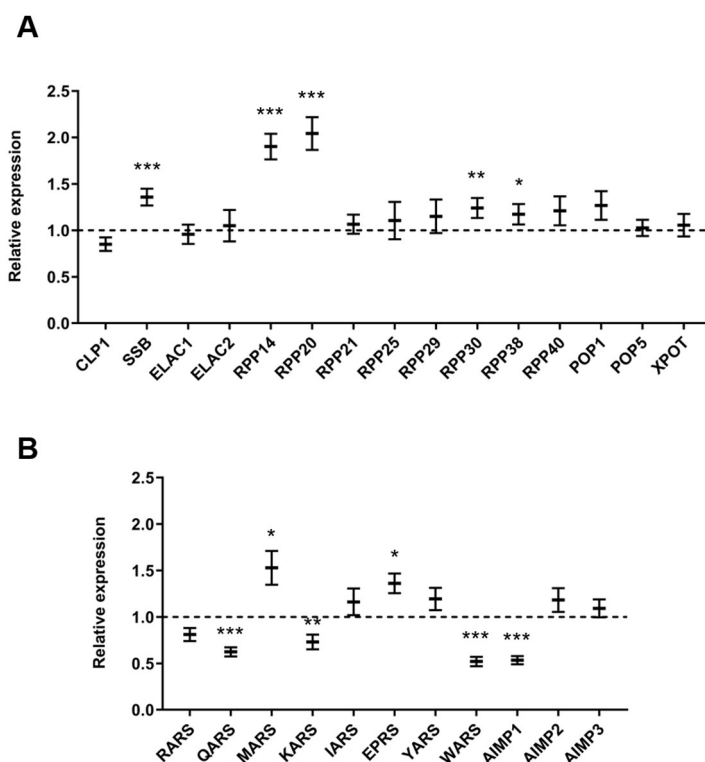


Figure S3. Expression level alterations of important RNA related genes. **(A)** tRNA biogenesis related genes were examined for their relative expression in lung cancer. *SSB*, *RPP14*, *RPP20*, *RPP30* and *RPP38* were found significantly upregulated in lung cancer biopsies. **(B)** mRNA expression levels of genes responsible for aminoacylation of tRNA molecules were also analyzed. *MARS* and *EPRS* expression were found elevated, whereas we observed downregulation of *WARS*, *QARS*, *KARS* and *AIMP1* expression in tumor specimens. All experiments were performed in triplicates, bar graphs represent mean \pm SEM (error bars) and one-way Anova was used for the statistical analysis (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

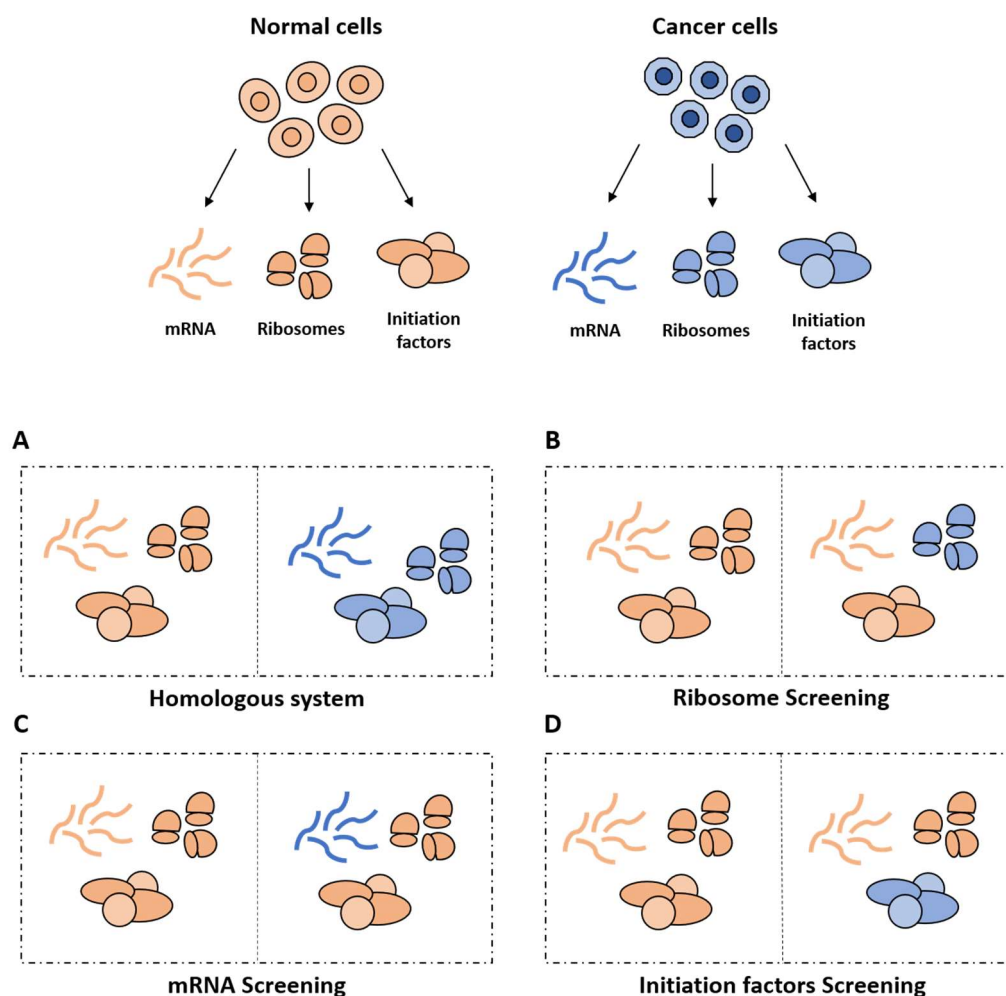


Figure S4. Explanatory illustration of the experimental procedure of the 43S pre-initiation complex formation assays indicating each examined condition. (A to D) the origin of the components which derived either from normal (brown color) or tumor specimens (blue color).

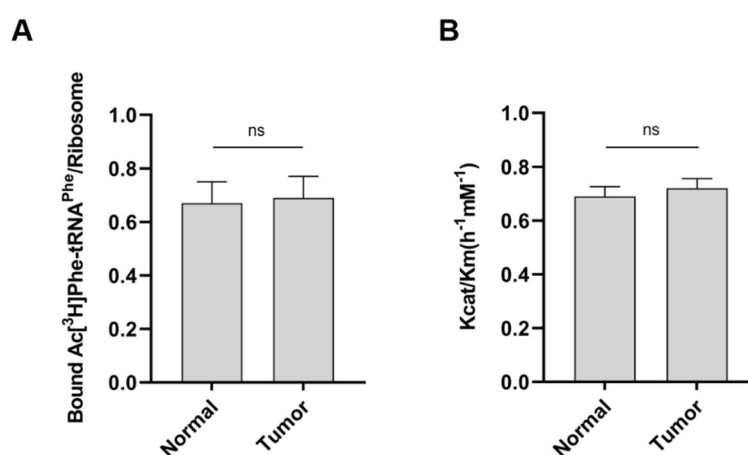


Figure S5. Translation-elongation assays in cell free systems derived from normal and lung cancer tissues. (A) Binding of Ac³H]Phe-tRNA^{Phe} to the A-site of poly(U)-programmed 80S ribosomes prefilled with tRNA^{Phe} at the *p*-site. (B) Catalytic activity of peptidyl transferase estimated from kinetic data that were collected from a reaction between complex C and excess of puromycin. Unpaired t test

between the relative values between normal and tumor specimens showed no statistically significant alterations (ns, not significant).

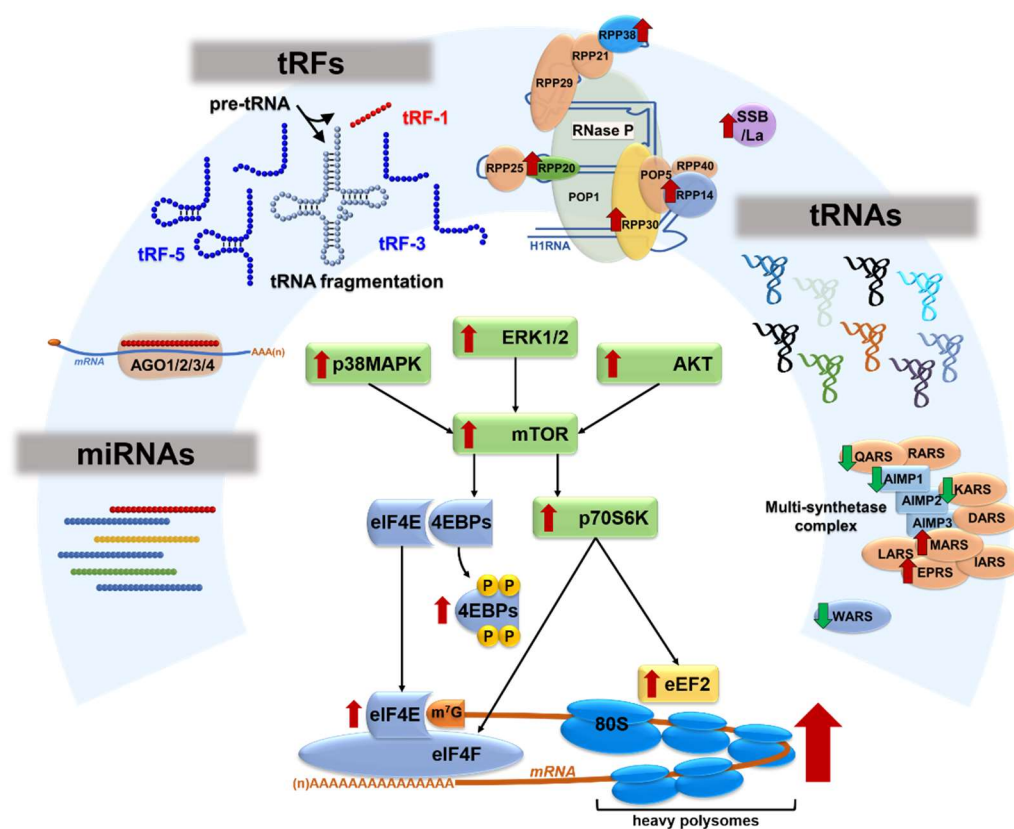


Figure S6. A schematic representation of events that link the expression patterns of miRNAs, tRFs and tRNAs with aberrant signaling and deregulated translation initiation, in lung cancer biopsy specimens.

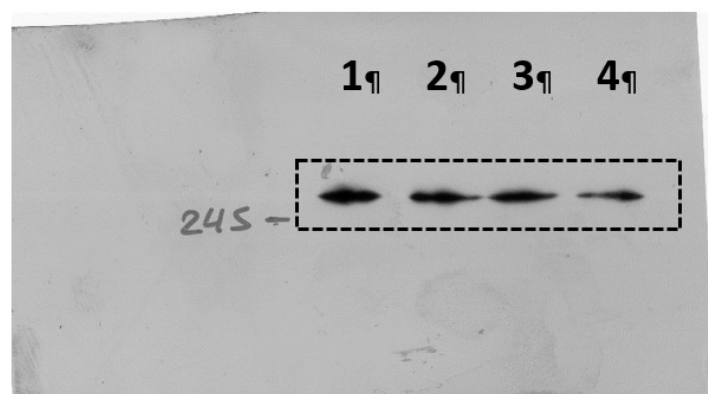


Figure S7. Western blot analysis of mTOR. Uncropped image used for the main Figure 4. Samples order: 1: si Luciferase; 2: tRF-5003b; 3: si Luciferase; 4: tRF-3021a.

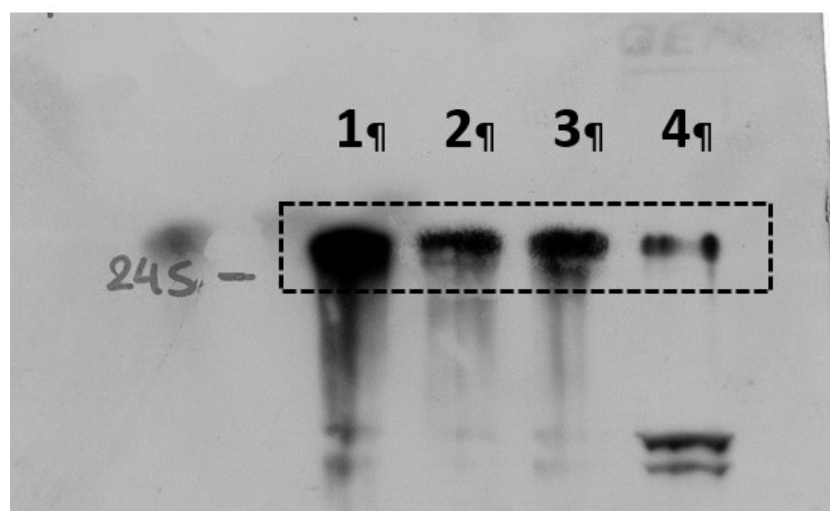


Figure S8. Western blot analysis of p-mTOR (Ser2448). Uncropped image used for the main Figure 4. Samples order: 1: si Luciferase; 2: tRF-5003b; 3: si Luciferase; 4: tRF-3021a.

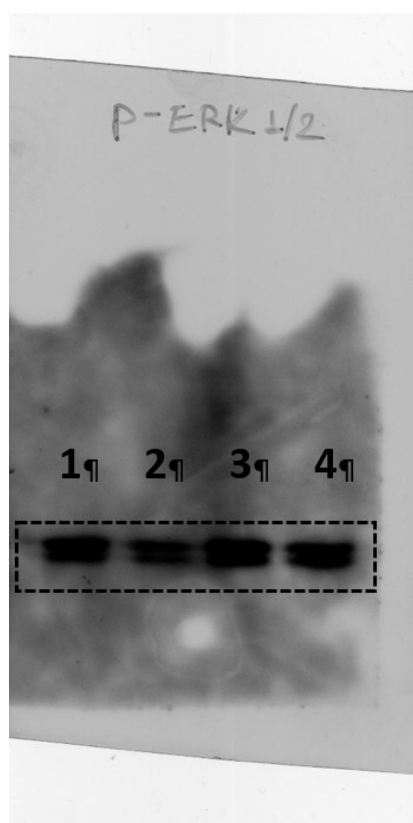


Figure S9. Western blot analysis of p-ERK1/2 (Thr202, Tyr204). Uncropped image used for the main Figure 4. Samples order: 1: si Luciferase; 2: tRF-5003b; 3: si Luciferase; 4: tRF-3021a.

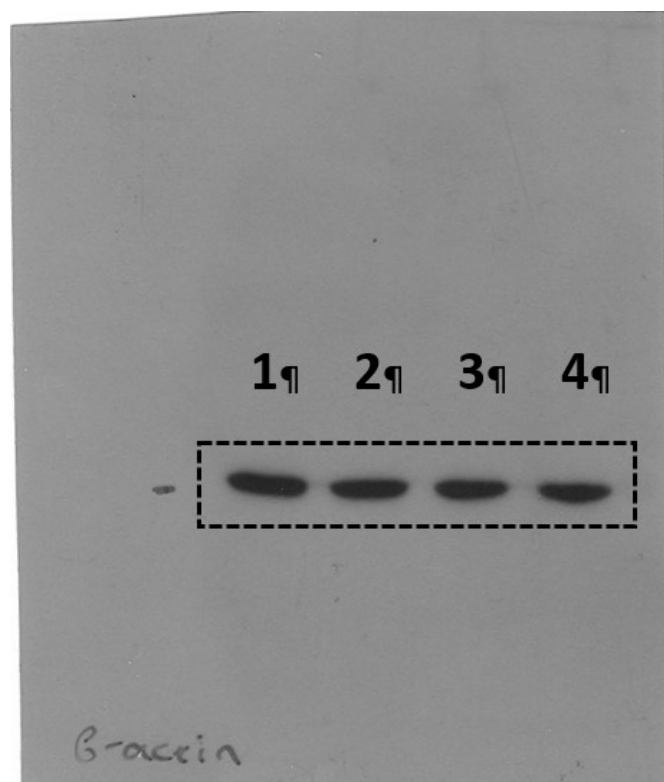


Figure S10. Western blot analysis of β -actin (Thr202, Tyr204). Uncropped image used for the main Figure 4. Samples order: 1: si Luciferase; 2: tRF-5003b; 3: si Luciferase; 4: tRF-3021a.

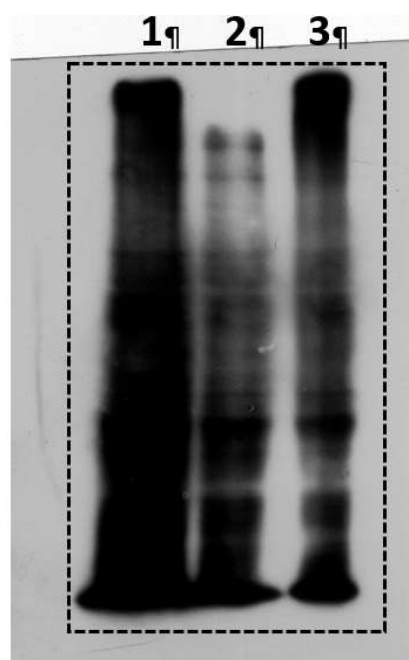


Figure S11. Western blot analysis using anti-puromycin to assess the levels of puromycylated nascent peptides. Uncropped image used for the main Figure 4. Samples order: 1: si Luciferase; 2: tRF-5003b; 3: tRF-3021a.

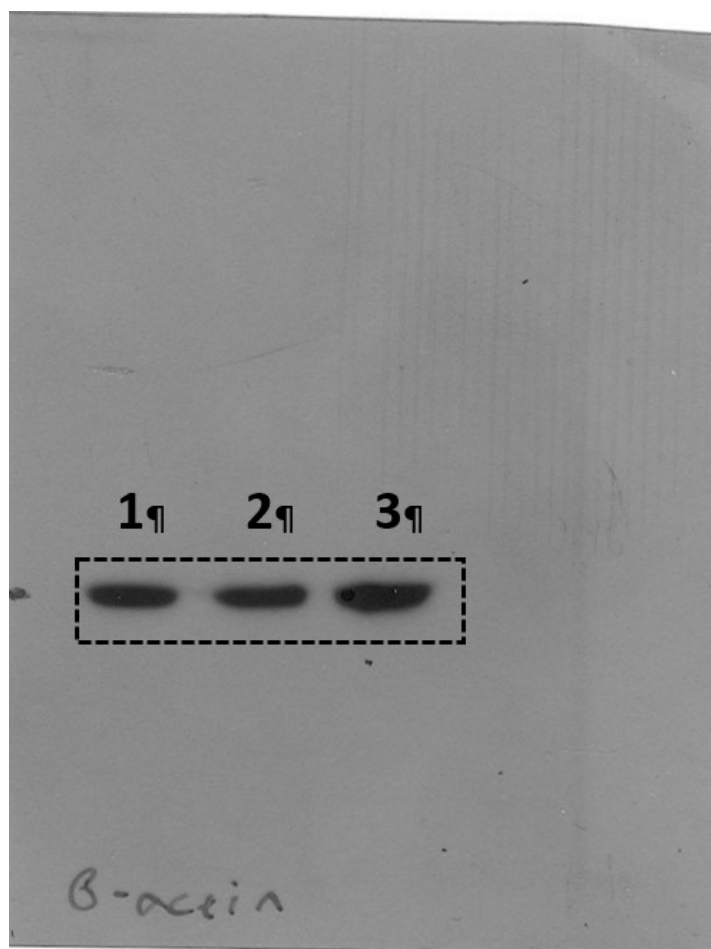


Figure S12. Western blot analysis of β -actin. Uncropped image used for the main Figure 4. Samples order: 1: si Luciferase; 2: tRF-5003b; 3: tRF-3021a.

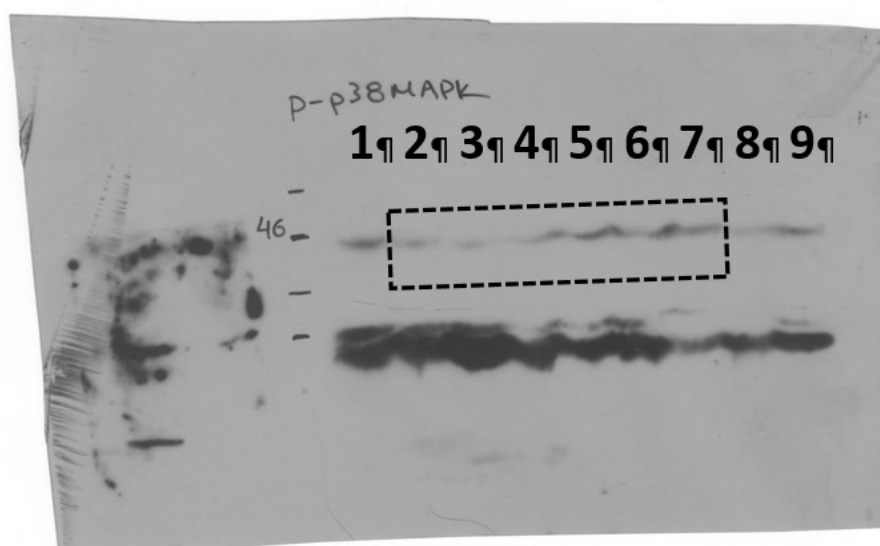


Figure S13. Western blot analysis of P-p38 MAPK (Thr180, Tyr182). Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: Normal 1; 3: Normal 2; 4: Normal 3; 5: Tumor 1; 6: Tumor 2; 7: Tumor 3; 8: Tumor 4; 9: CL1-5 cell extract (positive control).

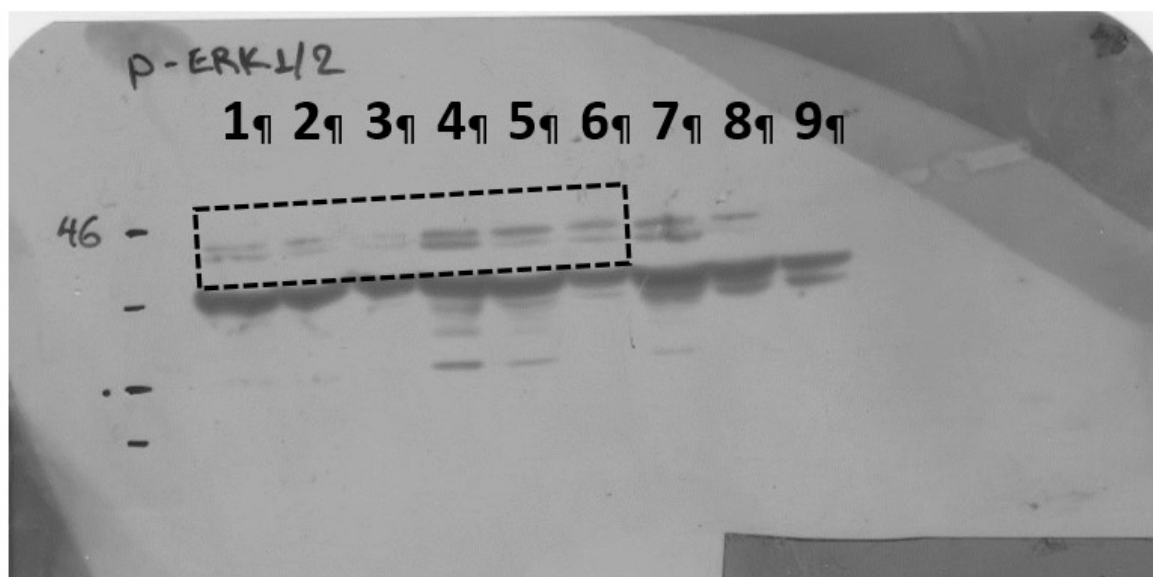


Figure S14. Western blot analysis of P-ERK1/2 (Thr202, Tyr204). Uncropped image used for the main Figure 5. Samples order: 1: Normal 1; 2: Normal 2; 3: Normal 3; 4: Tumor 1; 5: Tumor 2; 6: Tumor 3; 7: Tumor 4; 8: A549 cell extract (positive control); 9: CL1-5 cell extract (positive control).

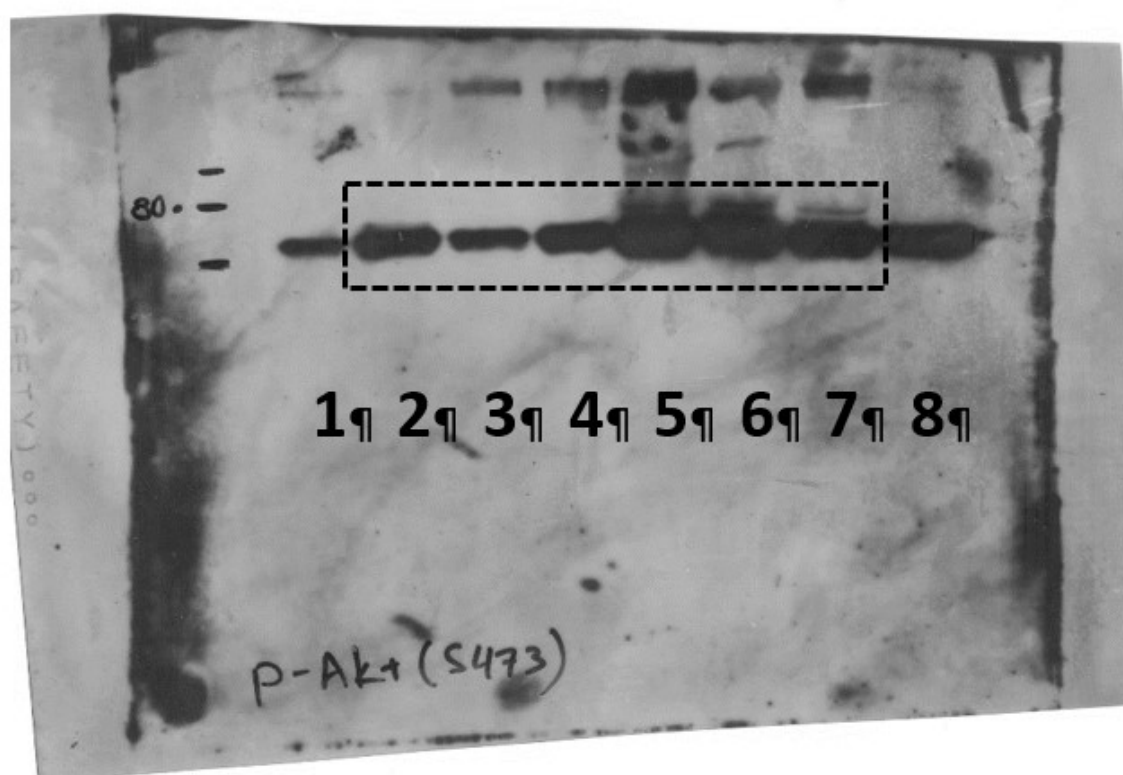


Figure S15. Western blot analysis of P-AKT (Ser473). Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: Normal 1; 3: Normal 2; 4: Normal 3; 5: Tumor 1; 6: Tumor 2; 7: Tumor 3; 8: Tumor 4.

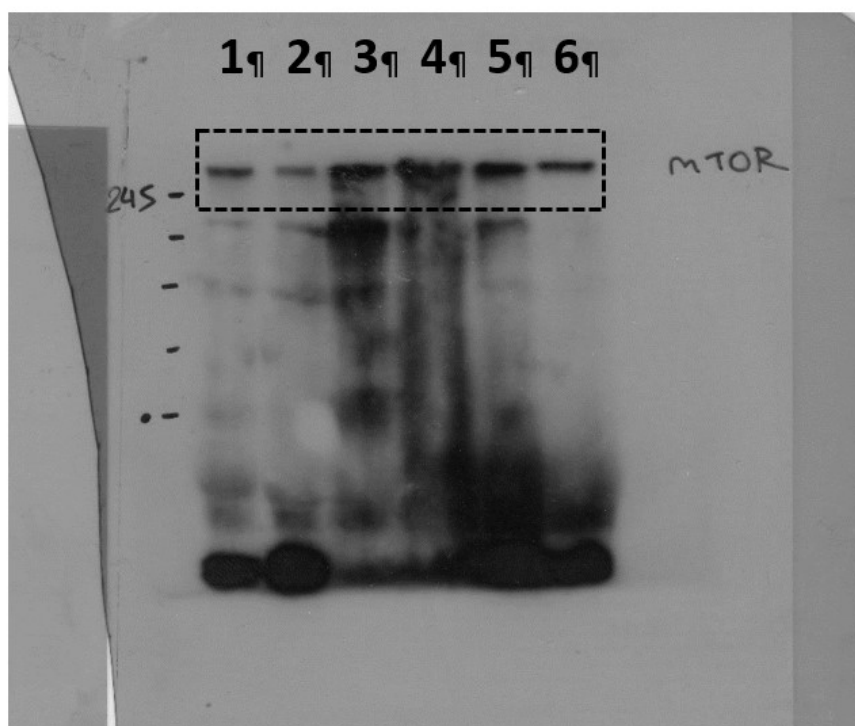


Figure S16. Western blot analysis of mTOR. Uncropped image used for the main Figure 5. Samples order: 1: Normal 1; 2: Normal 2; 3: Normal 3; 4: Tumor 1; 5: Tumor 2; 6: Tumor 3.

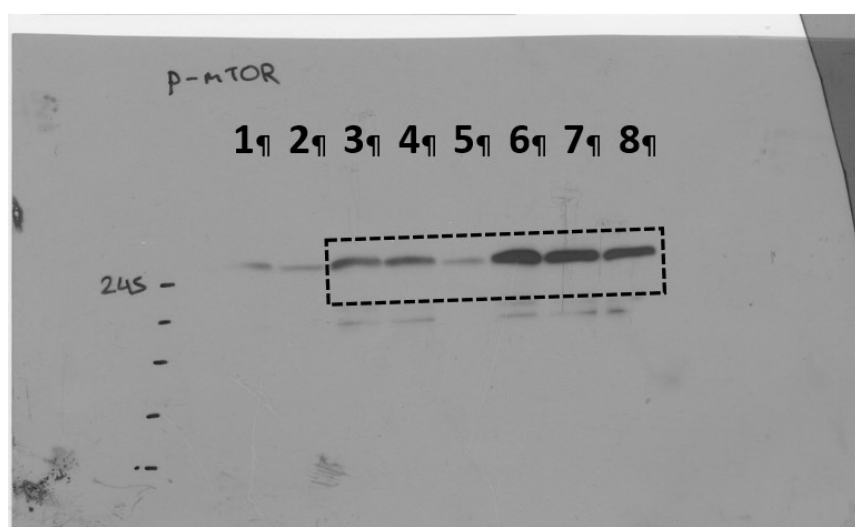


Figure S17. Western blot analysis of P-mTOR (Ser2448). Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: CL1-5 cell extract (positive control); 3: Normal 1; 4: Normal 2; 5: Normal 3; 6: Tumor 1; 7: Tumor 2; 8: Tumor 3.

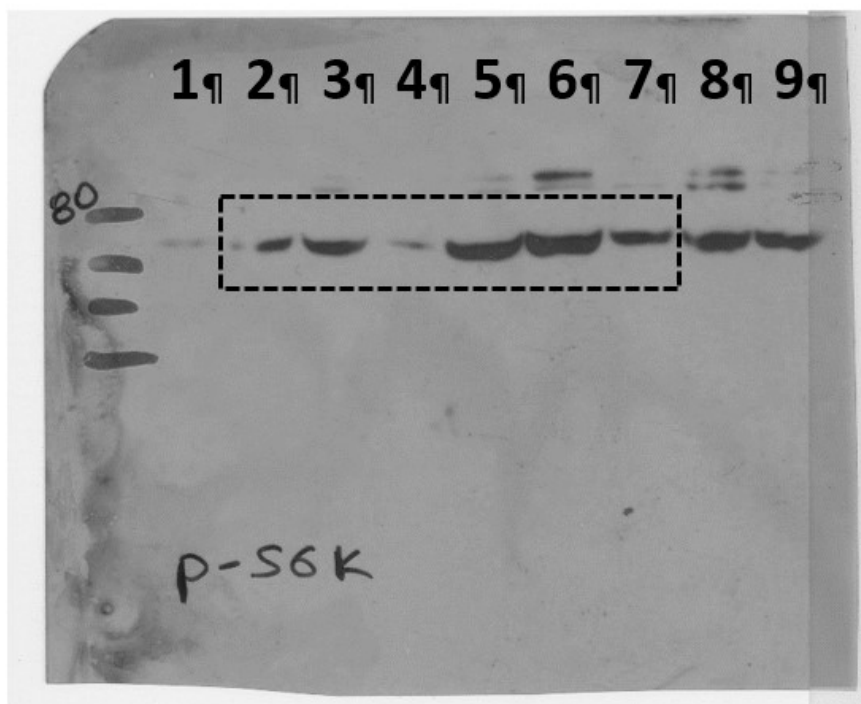


Figure S18. Western blot analysis of P-p70 S6 kinase (Thr389). Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: Normal 1; 3: Normal 2; 4: Normal 3; 5: Tumor 1; 6: Tumor 2; 7: Tumor 3; 8: Tumor 4; 9: CL1-5 cell extract (positive control).

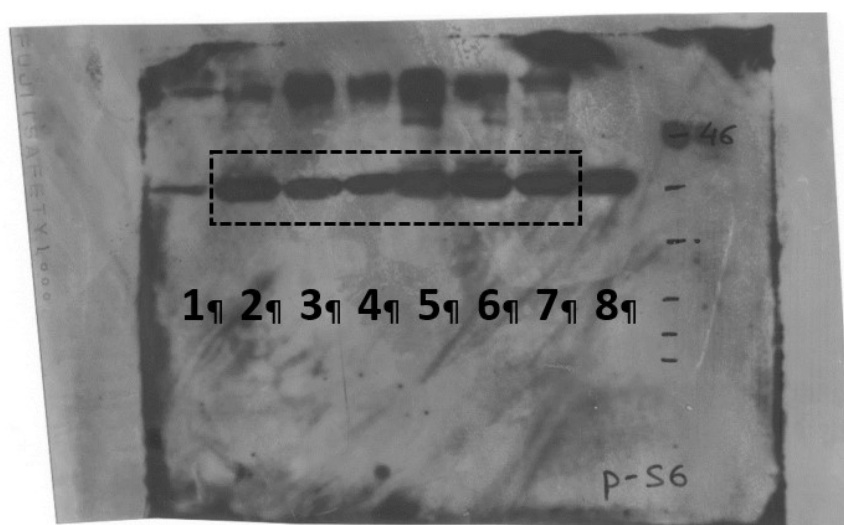


Figure S19. Western blot analysis of P-S6 (Ser235/236). Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: Normal 1; 3: Normal 2; 4: Normal 3; 5: Tumor 1; 6: Tumor 2; 7: Tumor 3; 8: Tumor 4.

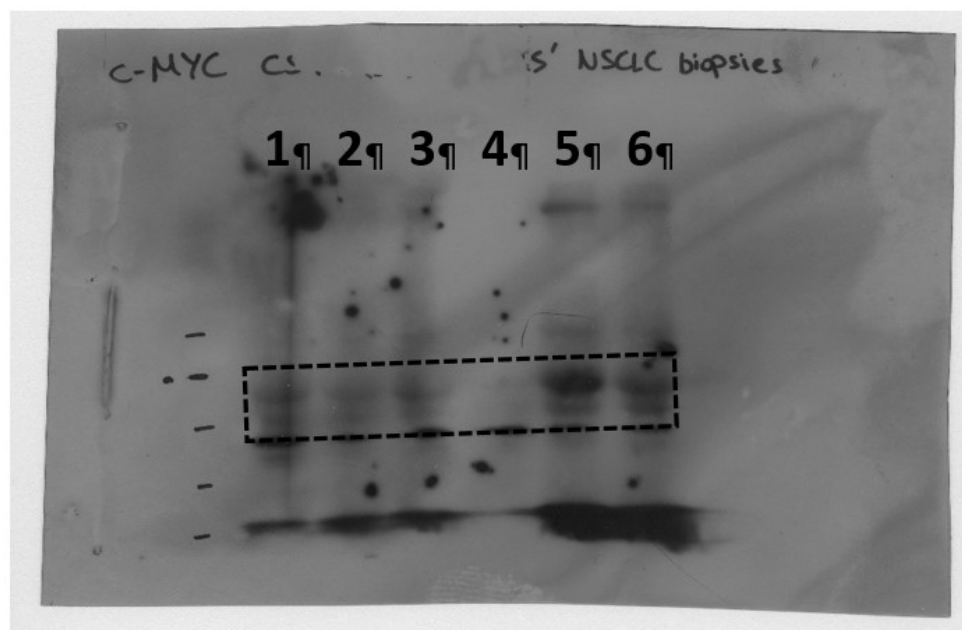


Figure S20. Western blot analysis of c-MYC. Uncropped image used for the main Figure 5. Samples order: 1: Normal 1; 2: Normal 2; 3: Normal 3; 4: Tumor 1; 5: Tumor 2; 6: Tumor 3.

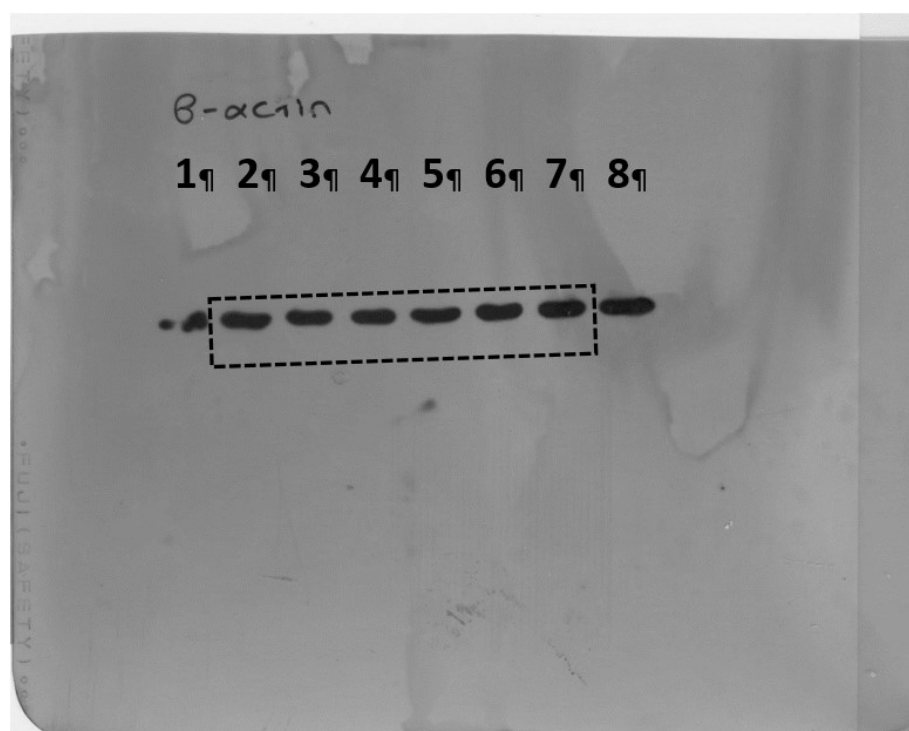


Figure S21. Western blot analysis of β -actin. Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: Normal 1; 3: Normal 2; 4: Normal 3; 5: Tumor 1; 6: Tumor 2; 7: Tumor 3; 8: Tumor 4.

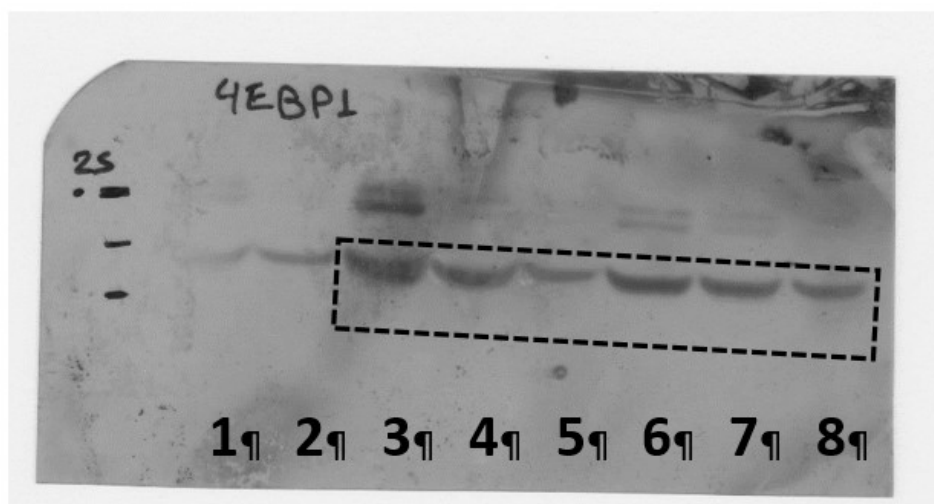


Figure S22. Western blot analysis of 4EBP1. Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: CL1-5 cell extract (positive control); 3: Normal 1; 4: Normal 2; 5: Normal 3; 6: Tumor 1; 7: Tumor 2; 8: Tumor 3.

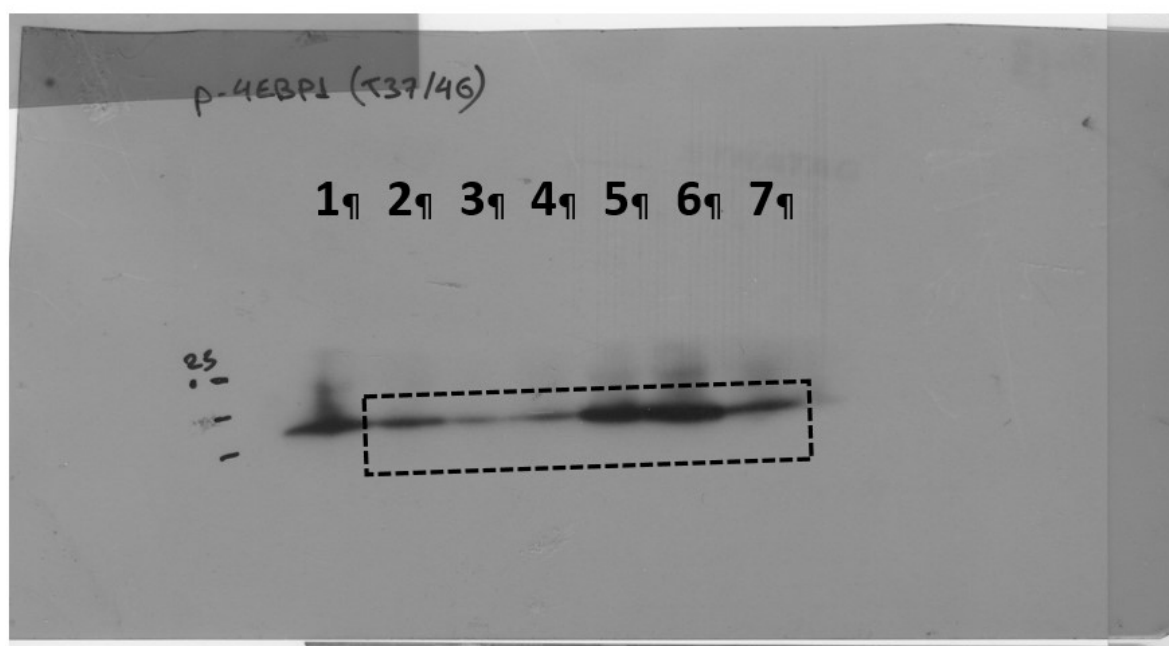


Figure S23. Western blot analysis of P-4EBP1 (Thr37/46). Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: Normal 1; 3: Normal 2; 4: Normal 3; 5: Tumor 1; 6: Tumor 2; 7: Tumor 3.

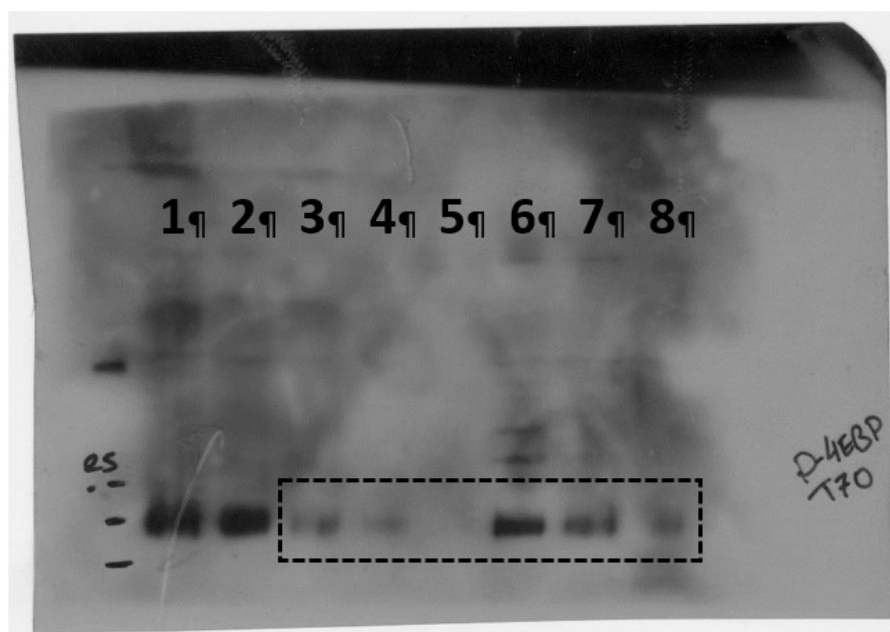


Figure S24. Western blot analysis of P-4EBP1 (Thr70). Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: CL1-5 cell extract (positive control); 3: Normal 1; 4: Normal 2; 5: Normal 3; 6: Tumor 1; 7: Tumor 2; 8: Tumor 3.

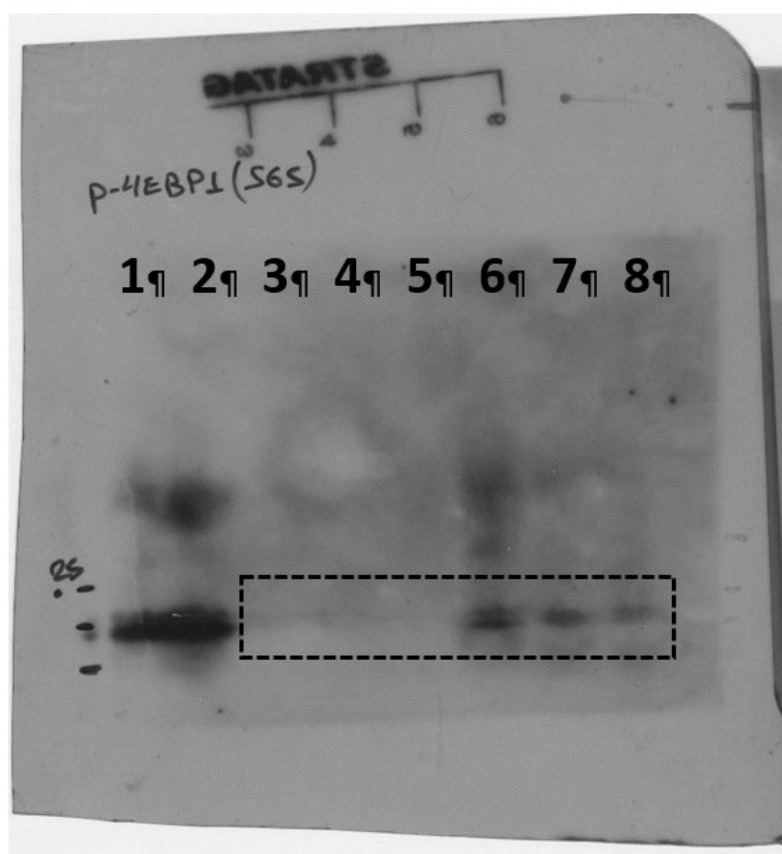


Figure S25. Western blot analysis of P-4EBP1 (Ser65). Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: CL1-5 cell extract (positive control); 3: Normal 1; 4: Normal 2; 5: Normal 3; 6: Tumor 1; 7: Tumor 2; 8: Tumor 3.

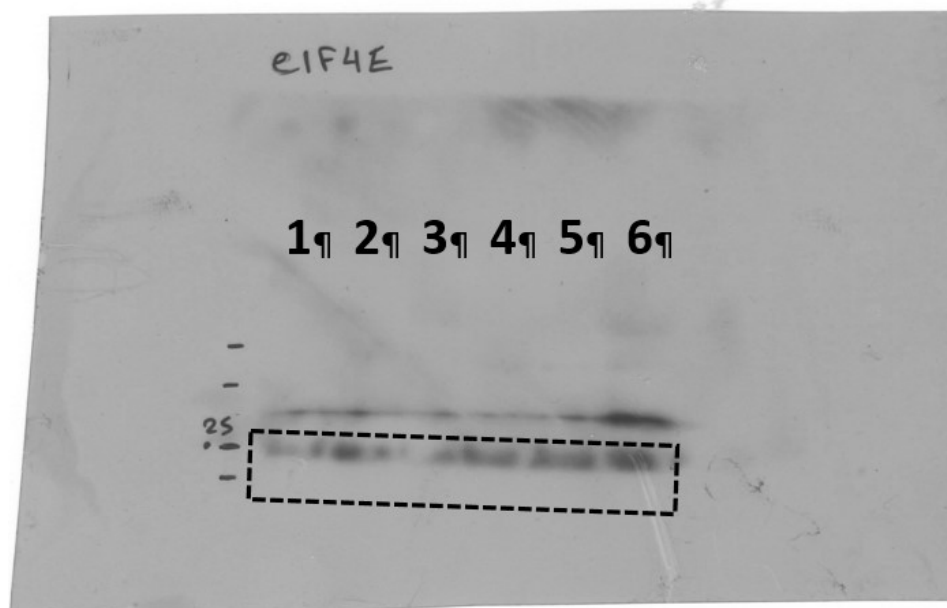


Figure S26. Western blot analysis of eIF4E. Uncropped image used for the main Figure 5. Samples order: 1: Normal 1; 2: Normal 2; 3: Normal 3; 4: Tumor 1; 5: Tumor 2; 6: Tumor 3.

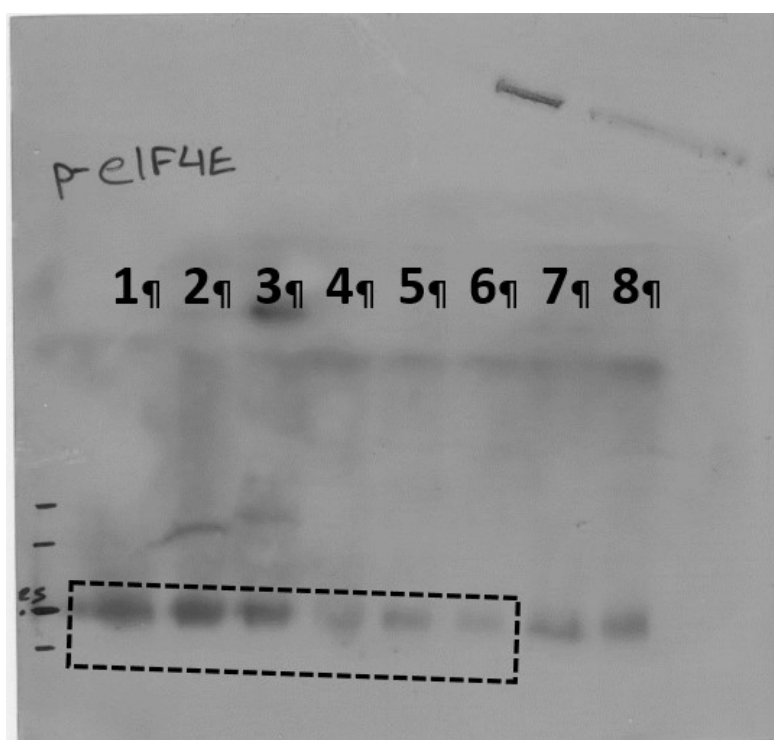


Figure S27. Western blot analysis of P-eIF4E (Ser209). Uncropped image used for the main Figure 5. Samples order: 1: Normal 1; 2: Normal 2; 3: Normal 3; 4: Tumor 1; 5: Tumor 2; 6: Tumor 3; 7: A549 cell extract (positive control); 8: CL1-5 cell extract (positive control).

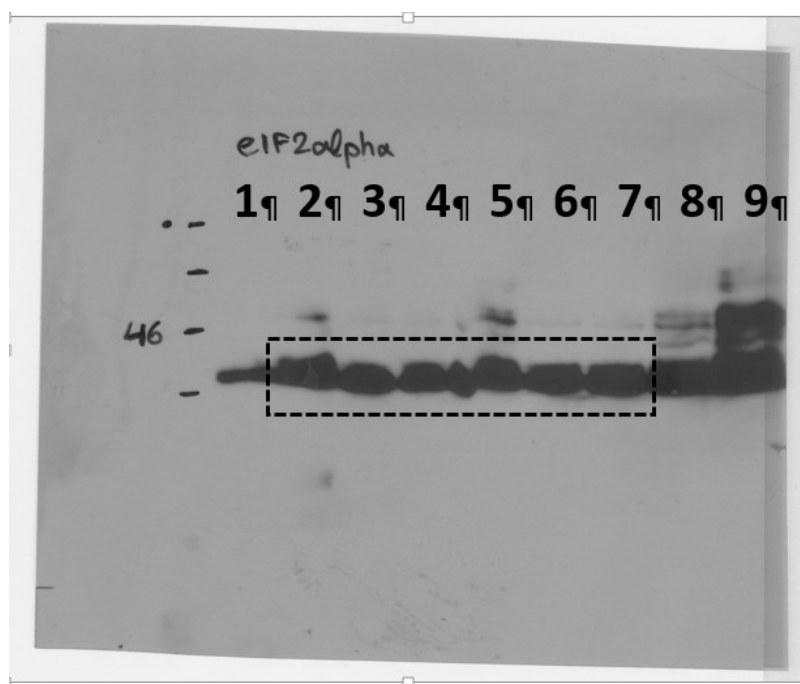


Figure S28. Western blot analysis of eIF2α. Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: Normal 1; 3: Normal 2; 4: Normal 3; 5: Tumor 1; 6: Tumor 2; 7: Tumor 3; 8: Tumor 4; 9: CL1-5 cell extract (positive control).

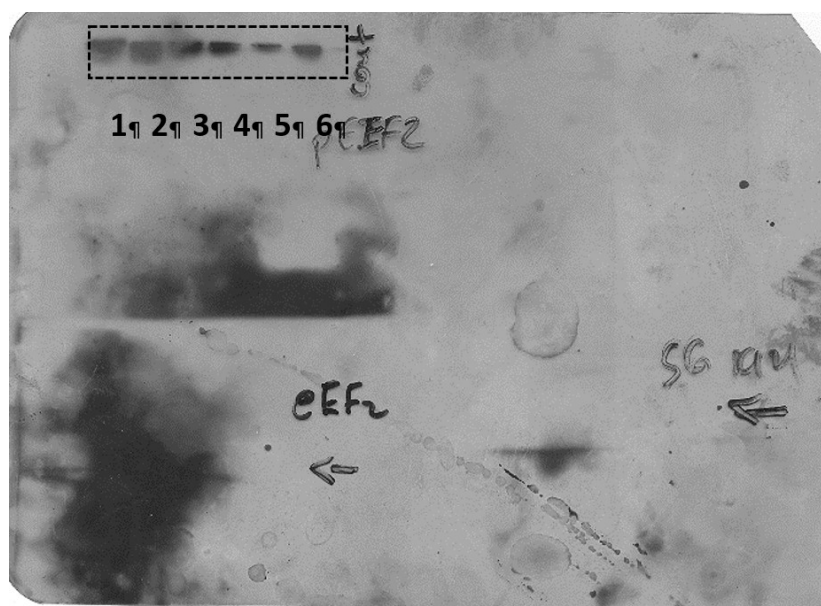


Figure S29. Western blot analysis of P-eIF2α (Ser51). Uncropped image used for the main Figure 5. Samples order: 1: Normal 1; 2: Normal 2; 3: Normal 3; 4: Tumor 1; 5: Tumor 2; 6: Tumor 3.

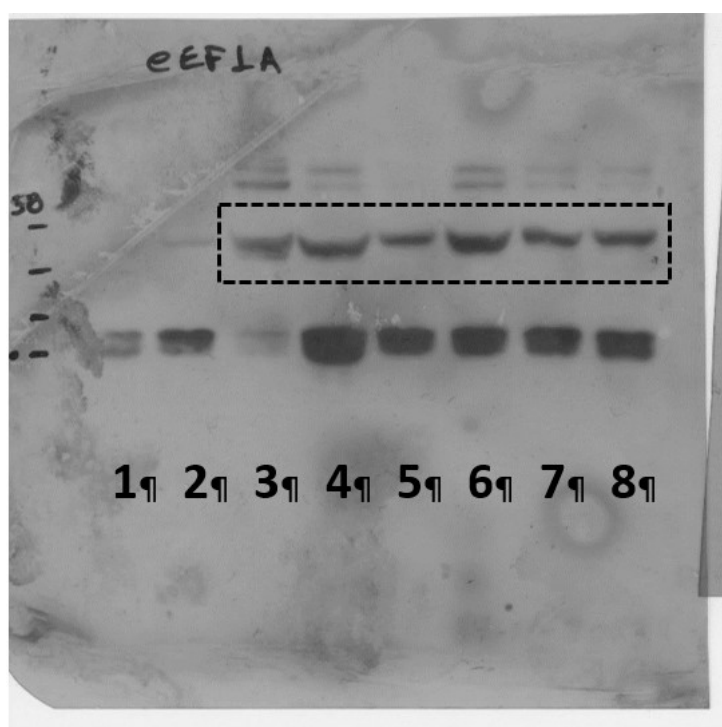


Figure S30. Western blot analysis of eEF1A. Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: CL1-5 cell extract (positive control); 3: Normal 1; 4: Normal 2; 5: Normal 3; 6: Tumor 1; 7: Tumor 2; 8: Tumor 3.

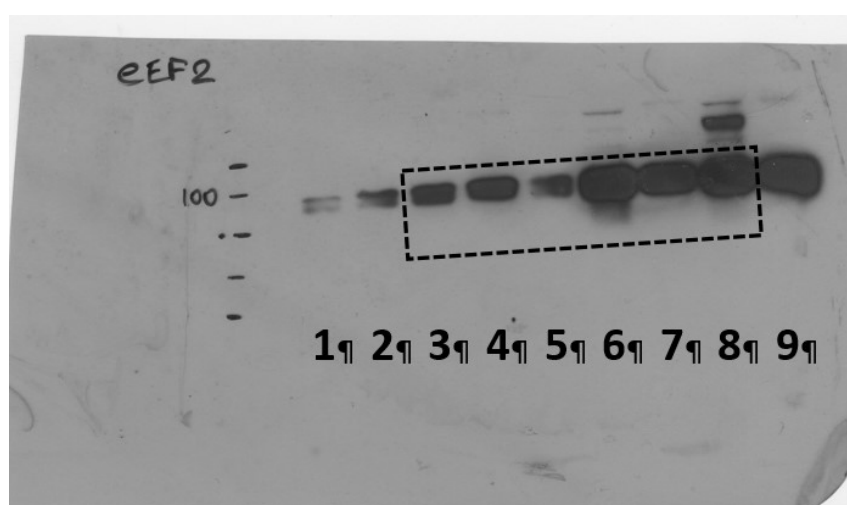


Figure S31. Western blot analysis of eEF2. Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: CL1-5 cell extract (positive control); 3: Normal 1; 4: Normal 2; 5: Normal 3; 6: Tumor 1; 7: Tumor 2; 8: Tumor 3.

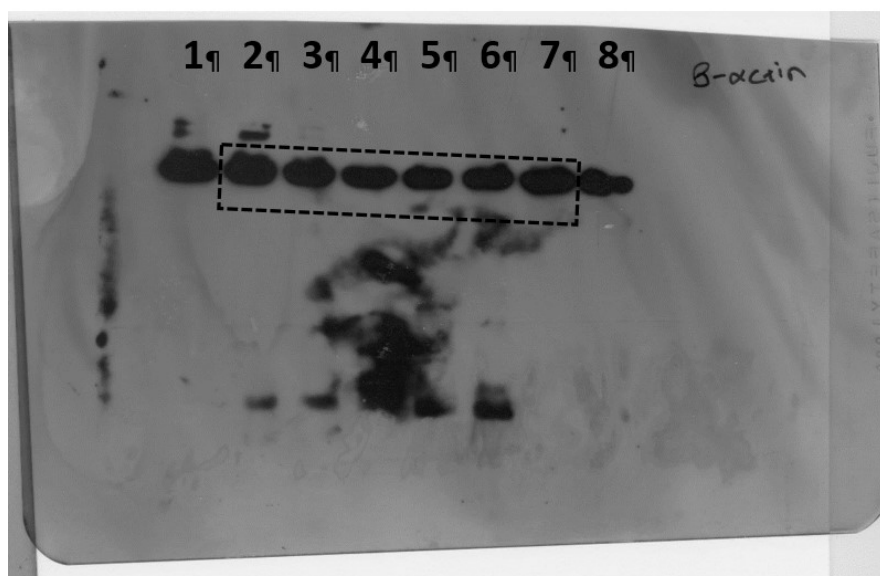


Figure S32. Western blot analysis of β -actin. Uncropped image used for the main Figure 5. Samples order: 1: A549 cell extract (positive control); 2: Normal 1; 3: Normal 2; 4: Normal 3; 5: Tumor 1; 6: Tumor 2; 7: Tumor 3; 8: Tumor 4.

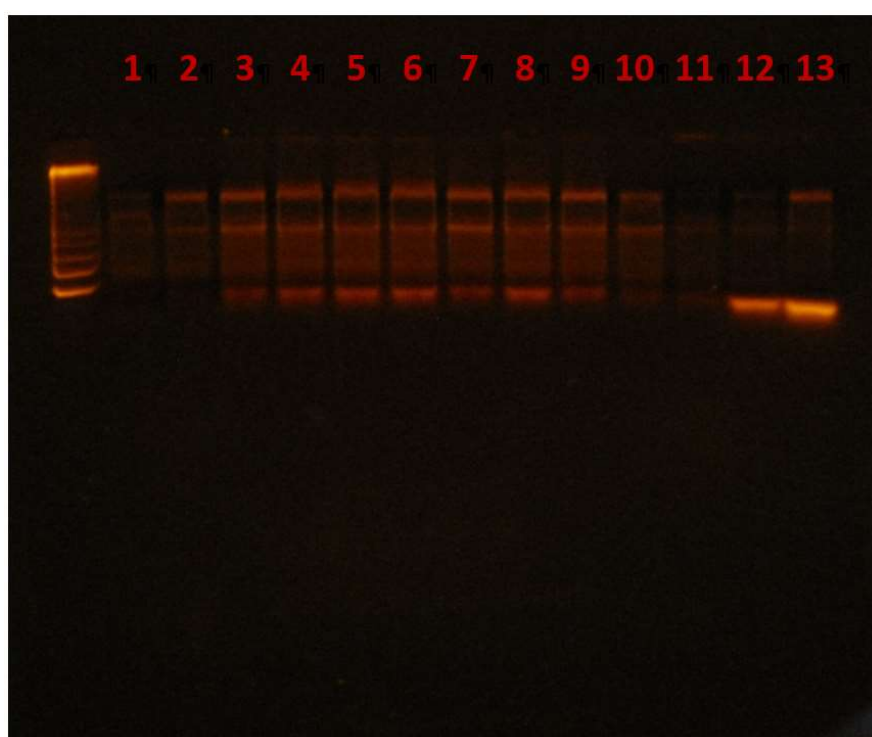


Figure S33. Polysome profiling Normal fractions (2% agarose gel). RNA extracted from fractions 1-13.



Figure S34. Polysome profiling Tumor fractions (2% agarose gel). RNA extracted from fractions 1–13.

Table S1. List of most significantly altered miRNAs in NSCLC is provided as a separate file (.xlsx format).

Table S2. List of most significantly altered tRFs in NSCLC is provided as a separate file (.xlsx format).

Table S3. List of identified tRFs along with their predicted targets is provided as a separate file (.xlsx format).

Table S4. List of most significantly altered tRNAs in NSCLC is provided as a separate file (.xlsx format).

Table S5. List of most significantly affected genes after transfection of tRF-5003b in A549 cells is provided as a separate file (.xlsx format).

Table S6. List of most significantly affected genes after transfection of tRF-3021a in A549 cells is provided as a separate file (.xlsx format).

Table S7. Baseline characteristics of lung adenocarcinoma patients.

| Patient | Gender | Age | Adjacent Normal Tissue | Staging | Smoker |
|---------|--------|-----|------------------------|---------|--------|
| 1 | F | 56 | Yes | pT4N2 | N/A |
| 2*# | M | 45 | Yes | pT1bN0 | Yes |
| 3* | M | 59 | Yes | pT2aN1 | Former |
| 4*# | F | 52 | Yes | pT2aN0 | Yes |
| 5 | F | 77 | — | pT2aN0 | No |
| 6 | F | 54 | Yes | pT3N1 | Yes |
| 7 | F | 77 | Yes | pT2N0 | Former |

| | | | | | |
|------|---|----|-----|--------|--------|
| 8* | F | 57 | Yes | pT2aN1 | Former |
| 9 | M | 71 | — | pT3N2 | Former |
| 10 | F | 76 | — | pT1bN1 | No |
| 11 | M | 72 | Yes | pT2aN1 | No |
| 12 | M | 68 | — | pT2aN0 | N/A |
| 13*# | M | 48 | Yes | pT1bN0 | Former |
| 14 | M | 62 | — | pT2aN1 | Former |
| 15 | M | 74 | Yes | pT2aN0 | Former |
| 16 | M | 64 | Yes | pT4N2 | Yes |
| 17 | M | 69 | — | pT2Nx | Yes |
| 18 | M | 58 | Yes | pT2aN0 | Yes |

* Specimens from patients 2, 3, 4, 8 and 13 were used for the NGS analyses. # Specimens from patients 2, 4 and 13 were used for Western blot analyses and their adjacent normal tissues were used as controls for all.

Table S8. List of primers used for miRNAs expression analysis.

| Primer | Sequence |
|----------------|---|
| oligoT adapter | GCGAGCACAGAATTAATACGACTCACTATAGGTTTTTTTTTTTTTVN |
| Outer primer | GCGAGCACAGAATTAATACGACT |
| miR-103 | AGCAGCATTGTACAGGGCTATGA |
| miR-26a-5p | TTCAAGTAATCCAGGATAGGCT |
| miR-30a-3p | CTTTCAGTCGGATGTTTGCAGC |
| miR-34b-5p | TAGGCAGTGTCTATTAGCTGATTGAA |
| miR-34c-5p | AGGCAGTGTAGTTAGCTGATTGC |
| miR-125a-5p | TCCCTGAGACCCCTTAACCTGTGA |
| miR-126-5p | CATTATTACTTTTGGTACGCGAAAAA |
| miR-139-5p | TCTACAGTGCACGTGTCTCCAGT |
| miR-449c-5p | TAGGCAGTGTATTGCTAGCGGCTGT |

Table S9. List of primers used for tRFs expression analysis.

| Primer | Sequence |
|----------|------------------------------|
| tRF-5003 | GCGTGGGTGGTTCAGTGGTAGAATTC |
| tRF-5004 | GCATTGGTGGTTCAGTGGTAGAATTCTC |
| tRF-5007 | CGCCGCTGGTGTAGTGGTATC |
| tRF-5008 | GCGTTGGTGGTATAGTGGTGAGC |
| tRF-5013 | GCTCGTTGGTCTAGGGGTATGATTC |
| tRF-5030 | TCCCTGGTGGTCTAGTGGTTAGGAT |
| tRF-3003 | TCAAATCCGGGTGCCCCCTC |
| tRF-3009 | TCGAACCCCACTCCTGGTACC |
| tRF-3021 | TCCCCGGCATCTCCACCA |
| tRF-1001 | GAAGCGGGTGCTCTTATTTTAAAAAA |

Table S10. List of primers used for gene expression analysis.

| Gene | Forward | Reverse |
|----------|------------------------|--------------------------|
| ACTB | AGCGAGCATCCCCAAAGTT | GGGCACGAAGGCTCATCATT |
| AGO1 | GGGTATATGGGATGGAAGCGG | TCAAAGTCGACCCGTTTCGTT |
| AGO2 | GTGGAAACATTCCAGCAGGC | CCCAGAGGACGTGATAGTGC |
| AGO3 | CCTCCATGAATTTCTGCCACTG | TTCTCTCCACAGTTTGGCCG |
| AGO4 | TTGCTGCTTCCAAAGGTGATG | TTCCCACTTGAAGGGTAGGC |
| AIMP1 | CGAGCCAAGTCAGCCAAGGTT | ATCTGCCTCTGCACCCTTCTGC |
| AIMP2 | CACGCGCTACCCCTTTTGCT | CGATGTCTTTCAGCGCCCCGT |
| AIMP3 | GCAATCGTTCAGCAGTGTT | ACAGATGTTGCCTGATGCCT |
| AKT1 | ATGCAGCATCGCTTCTTTGC | TGATCATCTGGGCGGTGAAC |
| ARAF | GGATGGCATGAGTGTCTACGA | GGTCAGCGGGACATCTTCA |
| ARIH1 | ACGAGGTCATCCAGAATCCA | ATCTGGCGTGTTCGAGACTT |
| CLP1 | ACGGGAGGACCTTCTGAGTT | CCTCCACCTCAAAGCGAAGT |
| DICER | GTACGACTACCACAAGTACTTC | ATAGTACACCTGCCAGACTGT |
| DROSHA | ATCGGTTGTTCTGAACCTGC | GGTTGTCACTCCAACGGTCT |
| EPRS | CAGTCTGTGTTTTCGAGC | TGCTGTCAACTATGGGGCAG |
| EIF4EBP1 | TCCTTGTCCTCCACTGATG | CTAAGGAAGGGTGGTTGCG |
| EIF4E | AGGACTGAAACCACCTCCAGCA | TCGGGGGATTAGGAGTAGGGGTGG |
| EIF6 | TCTTCAGCAATCAGGGAGGG | ATCCCAGCAGCAATCACCTC |

| | | |
|--------|--------------------------|---------------------------|
| ELAC1 | TGTGGTCCTTCGGTGTGAAG | AGAGTTCCATGGTTCGCCAG |
| ELAC2 | GCTCACCAGTTTCCGCTGTA | GACACAAACACAGCAGCCAG |
| GAPDH | GAAAGCCTGCCGGTGACTAA | TGGAATTTGCCATGGGTGGA |
| IARS | AGGCCCTCAGCAACAAAAT | AAACCCACTCTGGTGAGCAT |
| KARS | ACAGGGAACGCGACTGGGTCA | TGGGTCCACGCTCTCTTCCTCA |
| MAPK13 | GCATGAGAACGTCATTGGGC | CACGTAGCCAGTCATCTCGG |
| MAPK1 | CGCCGAAGCACCATTCAAG | AGAACACCGATGTCTGAGCA |
| MAPK3 | CTAAGGAGCGGCTGAAGGAG | GCCTCAGCAAAGGAGAGAGG |
| MARS | AGATTGTGTGGTCCCGTTCC | TGGTCAATGTGAGTCAGGGC |
| mTOR | AGCTGCCAAGTGCCGAGCAT | AGCAGCCATCCGGGCCATCTT |
| MYC | TCCATGAGGAGACACCGCCCA | TGGGGCTGGTGCAATTTTCGGT |
| PABPC1 | CTCCAGAGTCACTCCGTTCT | TCGGTGAAGCACAAGTTTCTT |
| PAQR4 | GAGGTTCCGAGGCTCAAAGG | CTGGTTTCATCCGGCACTCC |
| POP1 | TGCCTTGGGGAGAGGCCAACA | GGTTCCACACATCCGACCGT |
| POP5 | AGCTTGTGTGGTCAGCTCTTCCCT | AAGTGGGCCTGAAGCCTGAGCA |
| PTEN | GTGGAGTTTACCGGCAGCAT | CTGGCAGGTAGAAGGCAACT |
| QARS | ACTCCCTGTGCTCTTCACT | GGGCTCAGTGTGGATCTTCTT |
| RARS | CGGTGCATTTTACGGTTCCC | ACGCCACATGTTACCTTCA |
| RICTOR | TTTTCGGGGATTTCTGGATGC | GACAGCAAATGGGAAACCTCA |
| RPP14 | TGCGCCTTGGGTAGCTTGTCTT | GCGGCATCAACCTCCCCAAACA |
| RPP20 | TCCGTGATATGGCTCTTCGC | ACCGCATACACACACTCAGG |
| RPP21 | TCCTGTACCAGGCCGCCATT | TGGCAAGGGTTGTAGTGGTTTGA |
| RPP25 | TGACCGCGCACTAGCTGTCTT | GCCAACCCTCACAAACCGGCA |
| RPP29 | CACCAAAGAAGACCGCCTGA | TGCGACCTTCCCATGTTGTT |
| RPP30 | CTGAAAGTGACGCCAAGGCTGC | AAGTGACTCTCATCAGCCCAGGACA |
| RPP38 | ACCACGGATTTCGCCATCGTGAG | GCTCCAGCGGATGATGTATGGGT |
| RPP40 | CCGCGGCACTTACTGGTTTGC | TCCCATTTGGTAGCAGGGCAACAG |
| RPS6 | GCCCCAAAAGAGCTAGCAGA | TGCAGCAGCCTACTGAGAAC |
| RPS6KB | TTGGTCAGGCAGTCAACACT | TGCAGGACACGTGGAGTAAC |
| RPTOR | ATTCCCACCGCTCACTCATC | TTGACGCTCACACTCACGAT |
| SSB | AGTCGTTGCTGTTGCTGTTTG | AGGTAGGGGTTTGTCTGGAG |
| WARS | ATCAGGAAGGATTACACCAGCG | TGCAATGTTAGCCAGCACCA |
| XPO5 | ATGGCAGAGCCTTTCACCAA | CACGAAGCATGGGTCTGAGT |
| YARS | AAGCAGGGGTAGCGGAGCCAT | TGCGTGGAGGTCCGCAAACA |

Table S11. List of antibodies used for Western blot analysis.

| Antibody | Catalog # | Supplier |
|---------------------------------|------------|----------------|
| β -Actin | 4967 | Cell Signaling |
| phospho-p38 MAPK (T180/Y182) | 4631 | Cell Signaling |
| phospho-p44/42 MAPK (T202/Y204) | 4376 | Cell Signaling |
| phospho-AKT (S473) | 3586 | Cell Signaling |
| mTOR | 2983 | Cell Signaling |
| phospho-mTOR (S2448) | 5536 | Cell Signaling |
| phospho-p70 S6 Kinase (T389) | 9234 | Cell Signaling |
| phospho-S6 | 4857 | Cell Signaling |
| c-MYC | 5605 | Cell Signaling |
| 4E-BP1 | 9644 | Cell Signaling |
| phospho-4E-BP1 (T37/46) | 2855 | Cell Signaling |
| phospho-4E-BP1 (T70) | 9455 | Cell Signaling |
| phospho-4E-BP1 (S65) | 9456 | Cell Signaling |
| eIF4E | 2067 | Cell Signaling |
| phospho-eIF4E (S209) | 9741 | Cell Signaling |
| eIF2-alpha | 9722 | Cell Signaling |
| phospho-eIF2-alpha (S51) | 9721 | Cell Signaling |
| eEF1A | 3586 | Cell Signaling |
| eEF2 | 2332 | Cell Signaling |
| anti-puromycin (12D10) | AB_2566826 | Millipore |

Table S12. List of siRNAs for overexpression experiments.

| Table 5003. | Target Sequence |
|---------------|------------------------|
| si Luc (+) | CGUACGCGGAUACUUCGA |
| si Luc (-) | UCGAAGUAUUCGCGUACG |
| tRF 5003b (+) | GCAUGGGUGGUUCAGUGGUAGA |
| tRF 5003b (-) | UCUACCACUGAACCACCCAUGC |
| tRF 3021a (+) | UCCCCGGCAUCUCCACCA |
| tRF 3021a (-) | UGGUGGAGAUGCCGGGGA |



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