Supplementary Figures:

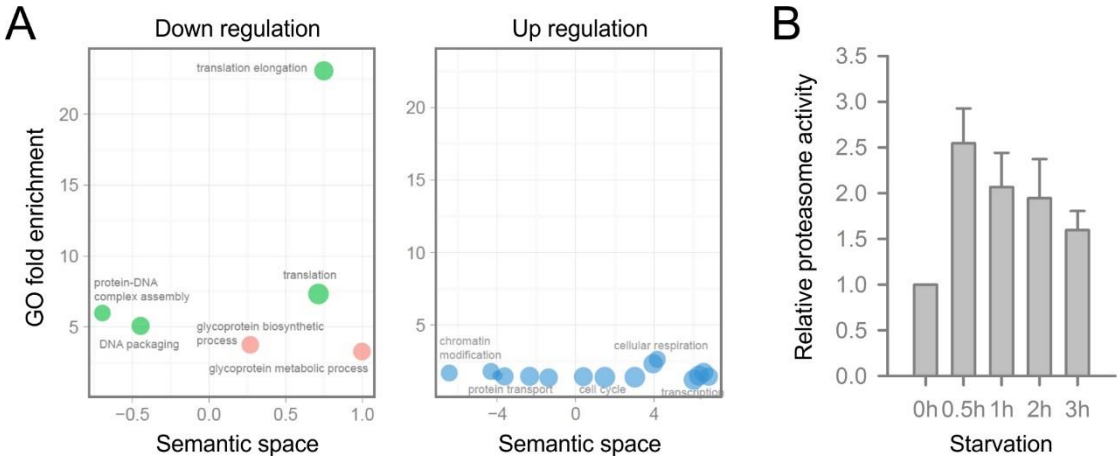

Fig. S1. Differential translational regulation in response to amino acid starvation.
(A) GO term enrichment in genes significantly downregulated (decrease > 3 fold) and upregulated (increase >3 fold) during amino acid starvation was summarized and visualized in semantic similarity based scatter plots using REViGO. x axis: likeliness of meaning between GO terms; y axis: Fold enrichment for GO category. Translation and protein DNA assembly related GO terms are shown in green, GO terms related to metabolic process are shown in red. (B) Relative Proteasome activity in HEK 293 cells during amino acid starvation. Equal number of HEK293 cells were plated and then subjected to amino acid starvation for varying periods of time. The chymotrypsin like activity of proteasome was measure using a luminescence based reagent.

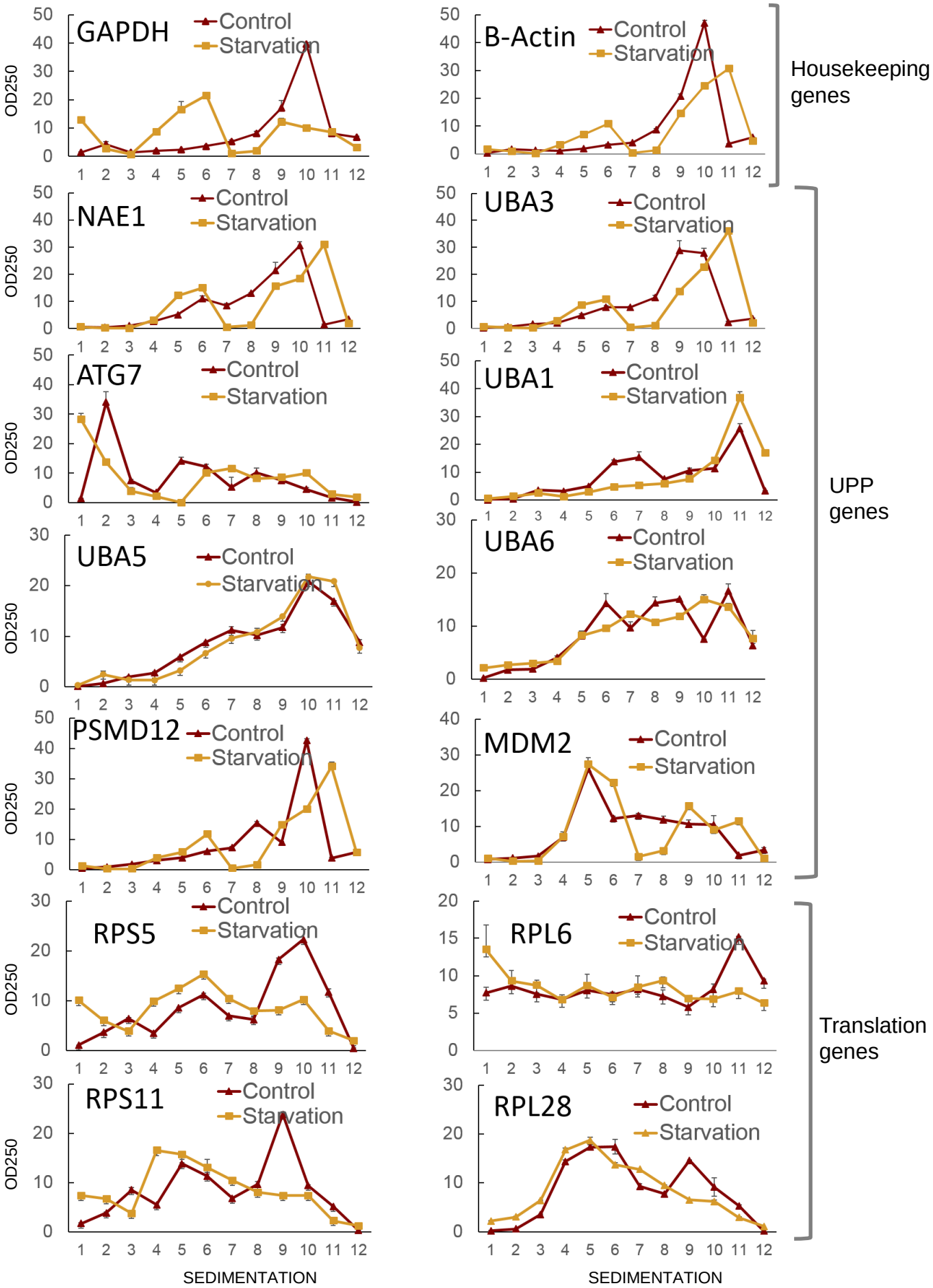

Fig. S2. Distribution of various mRNAs on sucrose gradient fractions obtained from HEK293 cells with or without amino acid starvation. qRT-PCR was used to determine the amount of target mRNAs present in all the sedimentation fraction. Values were normalized so that the sum of all fraction = 100

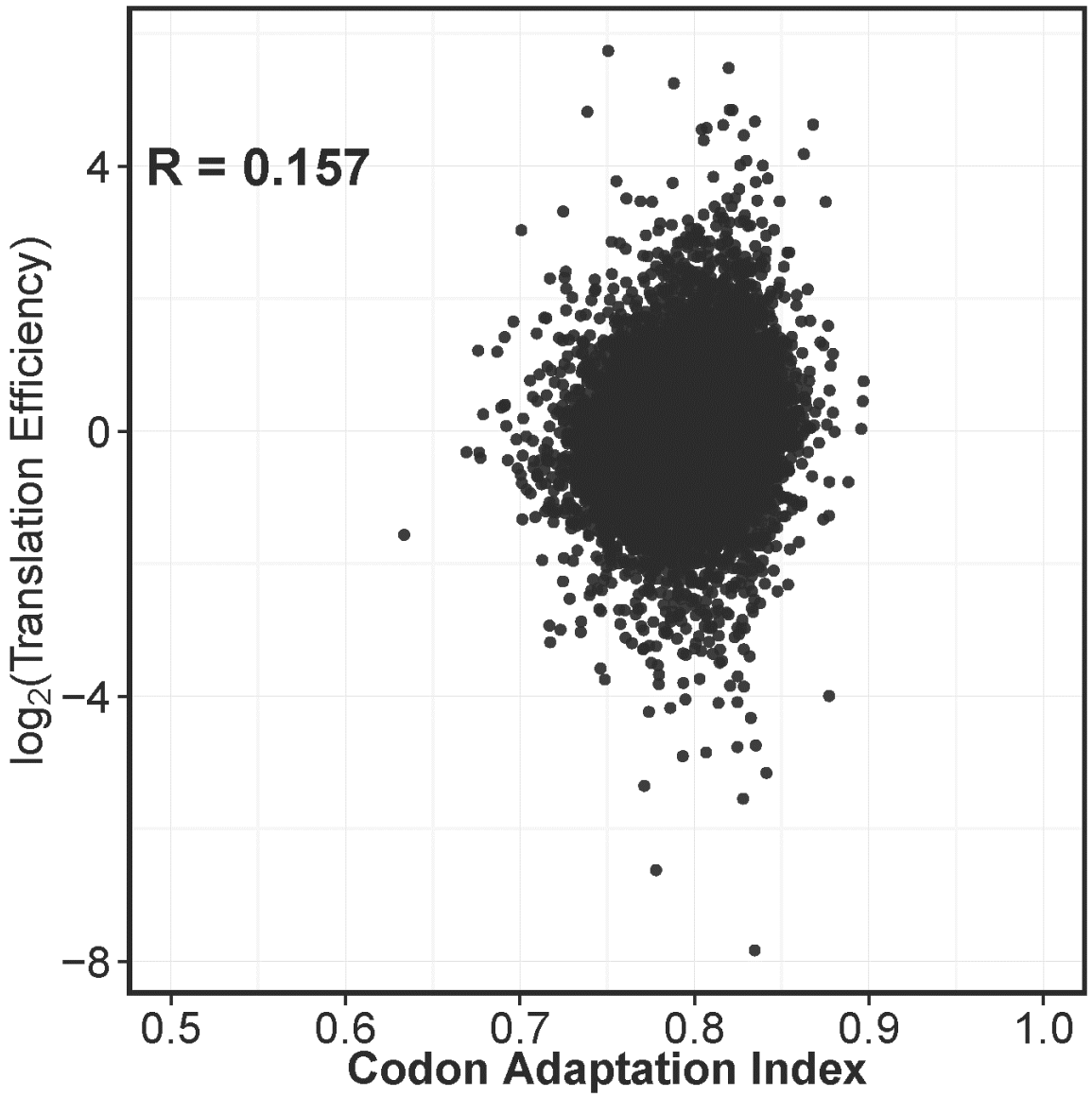

Fig. S3. Scatterplot showing the correlation between TE values of all genes with respective CAI values. The Pearson correlation coefficient is shown in the plot.

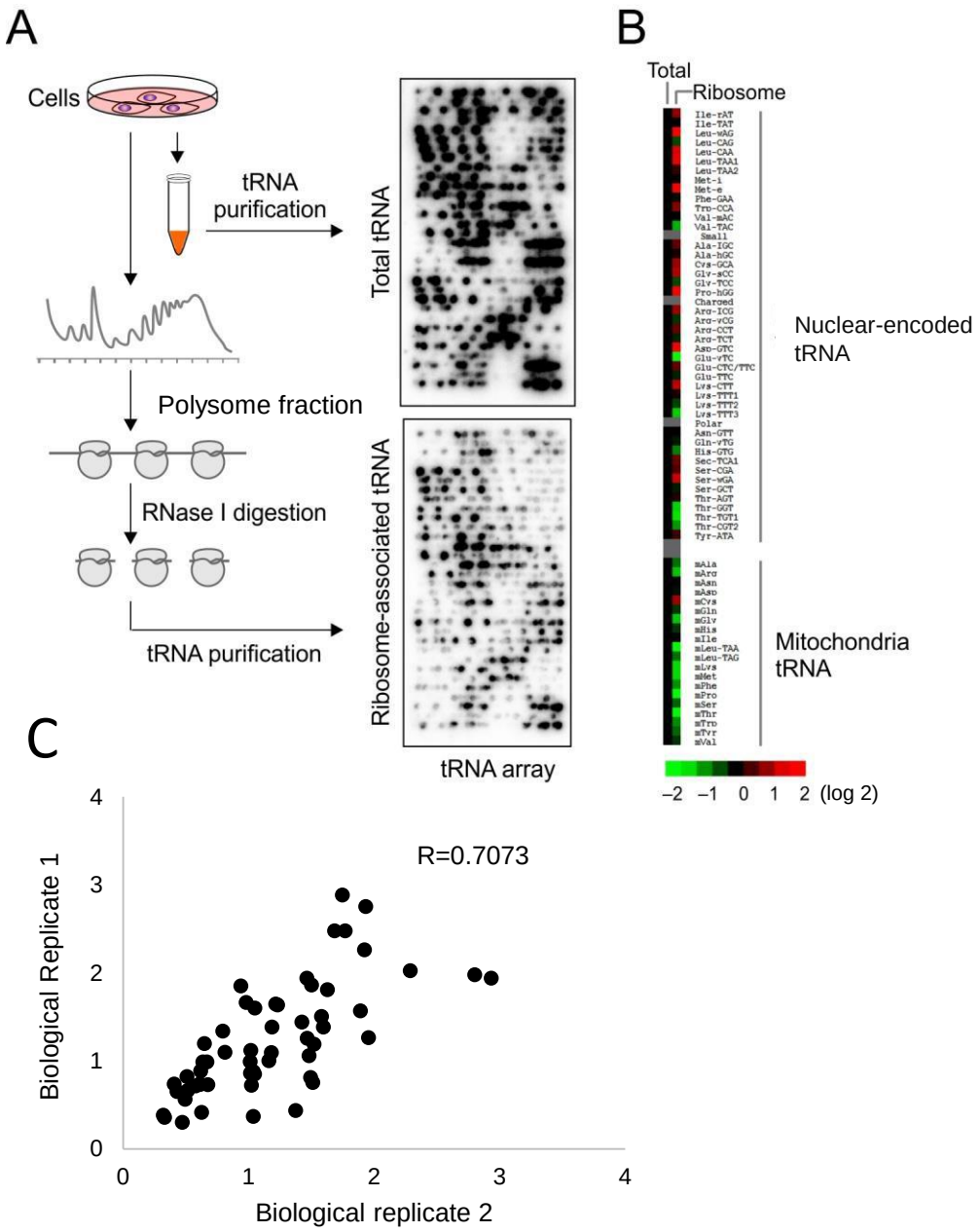

Fig. S4. Microarray analysis of ribosome-associated tRNA molecules.

(A)Schematic of the tRNA microarray method used to measure the tRNA levels in elongating ribosomes. (B)The relative levels of tRNA molecules in total cell lysates and ribosome associated samples are presented in a heatmap. The tRNAs are divided into groups based on the nature of amino acid they carry. The log 2 values of tRNA abundance is plotted. The color change from green to red signifies increasing abundance. The signal for mitochondrial $tRNA^{Cys}$ is most likely an experimental artifact arising due to high sequence similarity between cytoplasmic $tRNA^{Cys}$ and mitochondrial $tRNA^{Cys}$.

(C) Scatterplot showing correlation between two biological replicates for this experiment. The Pearson correlation coefficient is shown.

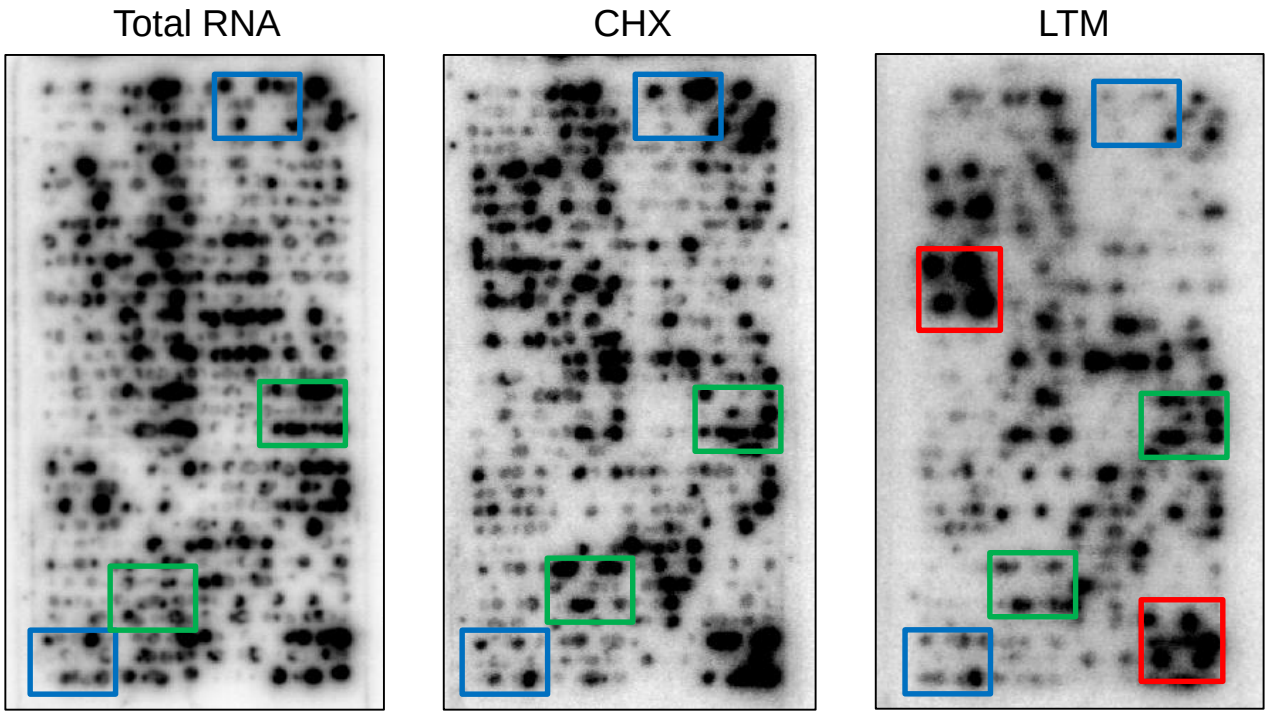

Fig S5 Microarray analysis of LTM treated sample shows an increase in initiator-tRNA^{Met} signal
Microarrays showing tRNAs isolated from total cell lysate (total RNA), Cycloheximide treated polysome fractions (CHX) and lactimidomycin (LTM) treated monosome fractions respectively. The 8 tRNA$_i^{Met}$ spots are in red boxes. The 8 *E.coli* tRNA^{Lys} spots are shown in blue boxes and the 8 *E.coli* tRNA^{Phe} are shown in green boxes.

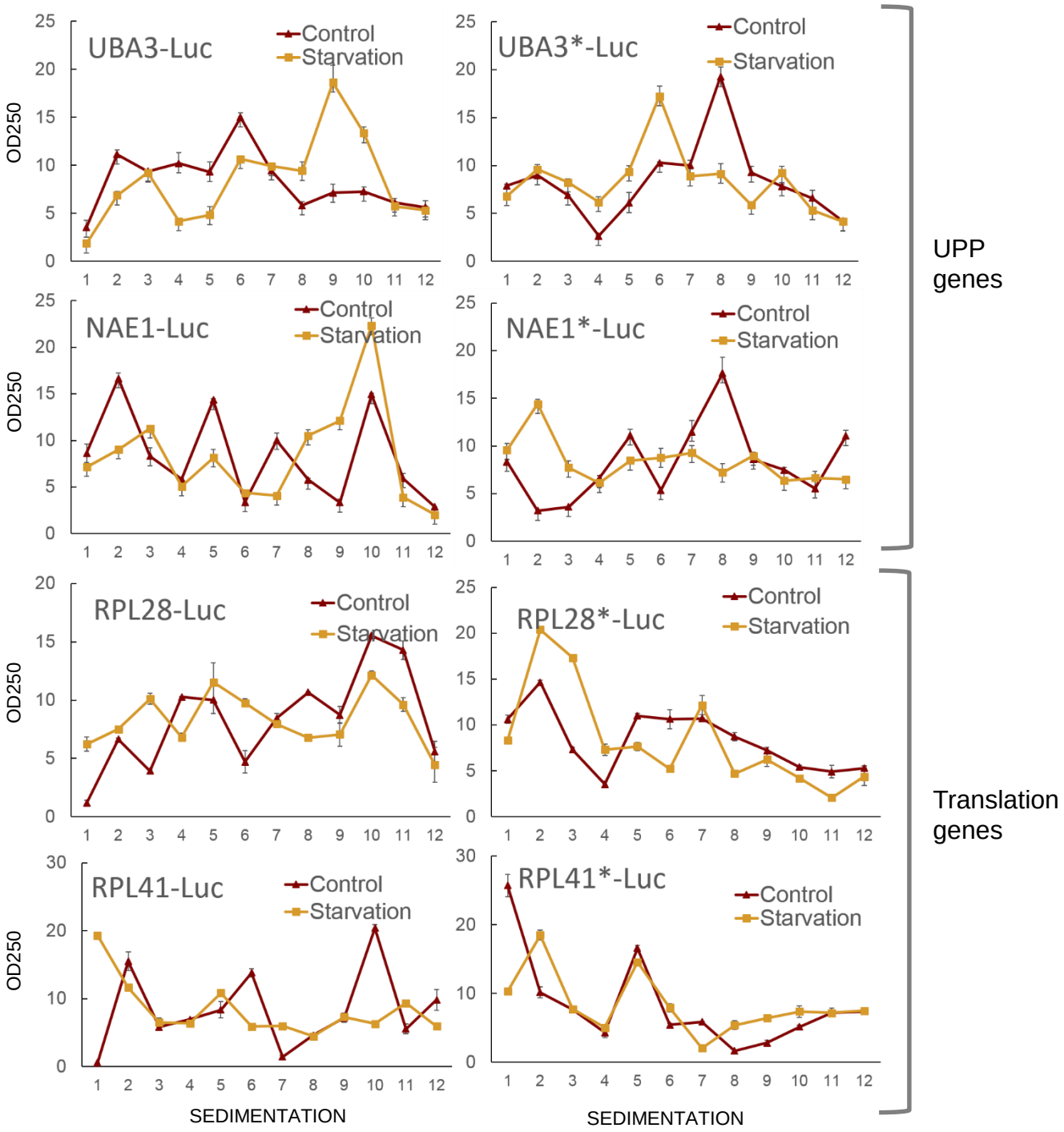

Fig. S6. Distribution of various Fluc reporter mRNAs on sucrose gradient fractions obtained from HEK293 cells with or without amino acid starvation. qRT-PCR was used to determine the amount of target mRNAs present in all the sedimentation fraction. Values were normalized so that the sum of all fraction = 100

Supplementary Tables:

RNA	Primers
GAPDH	Forward 5'- GAAGGTCGGAGTCAACGGAT Reverse 5'- TGAAGGGGTCATTGATGGCA
β-Actin	Forward 5'- ACGTTGCTATCCAGGCTGTG Reverse 5'- GAGGGCATACCCCTCGTAGA
UBA1	Forward 5'- AGCAGCCACTCAGTGCTATG Reverse 5'- CTCCATGGGCTGATTTCCGT
UBA3	Forward 5'- AGAATAGAGGAGCTGCTGGCT Reverse 5'- TCAGTGCTCGGTTCGAAATCA
NAE1	Forward 5'- TGCTCAAGGAGCAGAAGTACG Reverse 5'- TCAGTTCCTGTGGCTGTTGC
ATG7	Forward 5'- CAGCAGTGACGATCGGATGA Reverse 5'- GGCTGACGGGAAGGACATTA
RPS5	Forward 5'- AGTGGAGCACCGATGATGTG Reverse 5'- GCTCCACAATGGGACACTGA
RPS11	Forward 5'- AGAGGACCATTGTCATCCGC Reverse 5'- CGATCTGGACGTCCCTGAAG
RPL6	Forward 5'- GGCCAAGCTTATTGCTTATAGACCG Reverse 5'- GCATCCATGGCCATCTTGCAAGACGG
RPL28	Forward 5'- ACCATCAACAAGAATGCTCGC Reverse 5'- CTCCAGCCCATATTGCAACCA
Luciferase reporter mRNA primers: The same reverse primer was used for all the reporters.	Reverse 5'-CTTTATGTTTTTGGCGTCTTCCA NAE1-For 5'- atagaatctcatccagataatgcattagaggatctacgacta NAE1*-For 5'-atcgaatctcatccagataatgcactggaggatctgcggctg UBA3-For 5'-ggtgttacgtataggctcactcaaggggtagtaaaaagaatcatt UBA3*-For 5'-ggcgttacctataggctcactcagggggtggtgaaaagaatcatt RPL28-For 5'-gtcgtgcggaactgctccagtttcctgatcaagaggaataagcag RPL28*-For 5'-gtagtacgtaactgctcgtcgttcctaataaagcgtaataagcaa RPL41-For 5'-gccaagtggaggaagaagcgaatgcgcaggctgaagcgcaaaaga RPL41*-For 5'-gcgaagtggcgtaagaagcgaatgcgtcgtctaaagcgtaaacgt

Table S1. Primers used for RT-qPCR

Ensembl gene ID	Gene	Gene Description
ENSG00000008988	RPS20	ribosomal protein S20
ENSG00000037241	RPL26L1	ribosomal protein L26 like 1
ENSG00000063177	RPL18	ribosomal protein L18
ENSG00000071082	RPL31	ribosomal protein L31
ENSG00000083845	RPS5	ribosomal protein S5
ENSG00000089009	RPL6	ribosomal protein L6
ENSG00000089157	RPLP0	ribosomal protein
ENSG00000100316	RPL3	ribosomal protein L3
ENSG00000105193	RPS16	ribosomal protein S16
ENSG00000105640	RPL18A	ribosomal protein L18a
ENSG00000108107	RPL28	ribosomal protein L28
ENSG00000108298	RPL19	ribosomal protein L19
ENSG00000109475	RPL34	ribosomal protein L34
ENSG00000110700	RPS13	ribosomal protein S13
ENSG00000112306	RPS12	ribosomal protein S12
ENSG00000115268	RPS15	ribosomal protein S15
ENSG00000122026	RPL21	ribosomal protein L21
ENSG00000122406	RPL5	ribosomal protein L5
ENSG00000124614	RPS10	ribosomal protein S10
ENSG00000125691	RPL23	ribosomal protein L23
ENSG00000130255	RPL36	ribosomal protein L36
ENSG00000131469	RPL27	ribosomal protein L27
ENSG00000134419	RPS15A	ribosomal protein S15a
ENSG00000136942	RPL35	ribosomal protein L35
ENSG00000137154	RPS6	ribosomal protein S6
ENSG00000137818	RPLP1	ribosomal protein
ENSG00000137876	RSL24D1	ribosomal L24 domain containing 1
ENSG00000138326	RPS24	ribosomal protein S24
ENSG00000140988	RPS2	ribosomal protein S2
ENSG00000142534	RPS11	ribosomal protein S11
ENSG00000142541	RPL13A	ribosomal protein L13a
ENSG00000142676	RPL11	ribosomal protein L11
ENSG00000142937	RPS8	ribosomal protein S8
ENSG00000143947	RPS27A	ribosomal protein S27a
ENSG00000144713	RPL32	ribosomal protein L32
ENSG00000145425	RPS3A	ribosomal protein S3A
ENSG00000145592	RPL37	ribosomal protein L37
ENSG00000147604	RPL7	ribosomal protein L7
ENSG00000149273	RPS3	ribosomal protein S3
ENSG00000149806	FAU	Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV) ubiquitously expressed

ENSG00000156482	RPL30	ribosomal protein L30
ENSG00000161016	RPL8	ribosomal protein L8
ENSG00000162244	RPL29	ribosomal protein L29
ENSG00000163584	RPL22L1	ribosomal protein L22-like 1
ENSG00000163682	RPL9	ribosomal protein L9
ENSG00000164587	RPS14	ribosomal protein S14
ENSG00000165502	RPL36AL	ribosomal protein L36a like
ENSG00000166441	RPL27A	ribosomal protein L27a
ENSG00000167526	RPL13	ribosomal protein L13
ENSG00000168028	RPSA	ribosomal protein SA
ENSG00000171858	RPS21	ribosomal protein S21
ENSG00000171863	RPS7	ribosomal protein S7
ENSG00000172809	RPL38	ribosomal protein L38
ENSG00000174444	RPL4	ribosomal protein L4
ENSG00000174748	RPL15	ribosomal protein L15
ENSG00000182899	RPL35A	ribosomal protein L35a
ENSG00000185088	RPS27L	ribosomal protein S27 like
ENSG00000186468	RPS23	ribosomal protein S23
ENSG00000188846	RPL14	ribosomal protein L14
ENSG00000197728	RPS26	40S ribosomal protein S26
ENSG00000197728	RPS26	ribosomal protein S26
ENSG00000197756	RPL37A	ribosomal protein L37a
ENSG00000197958	RPL12	ribosomal protein L12
ENSG00000198242	RPL23A	ribosomal protein L23a
ENSG00000198755	RPL10A	ribosomal protein L10a
ENSG00000198918	RPL39	ribosomal protein L39
ENSG00000213741	RPS29	ribosomal protein S29
ENSG00000221983	UBA52	ubiquitin A-52 residue ribosomal protein fusion product 1
ENSG00000227794	RPS18	ribosomal protein S18
ENSG00000229117	RPL41	ribosomal protein L41
ENSG00000233927	RPS28	ribosomal protein S28
ENSG00000265681	RPL17	ribosomal protein L17

Table S2: Translation related genes

Ensembl gene ID	Gene	Gene Description
ENSG00000003096	KLHL13	kelch-like family member 13 [Source:HGNC Symbol;Acc:HGNC:22931]
ENSG00000008853	RHOBTB2	Rho-related BTB domain containing 2 [Source:HGNC Symbol;Acc:HGNC:18756]
ENSG00000009335	UBE3C	ubiquitin protein ligase E3C [Source:HGNC Symbol;Acc:HGNC:16803]
ENSG00000012048	BRCA1	breast cancer 1, early onset [Source:HGNC Symbol;Acc:HGNC:1100]
ENSG00000033178	UBA6	ubiquitin like modifier activating enzyme 6 [Source:HGNC Symbol;Acc:HGNC:25581]
ENSG00000044090	CUL7	cullin 7 [Source:HGNC Symbol;Acc:HGNC:21024]
ENSG00000049759	NEDD4L	neural precursor cell expressed, developmentally down-regulated 4-like, E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:7728]
ENSG00000055130	CUL1	cullin 1 [Source:HGNC Symbol;Acc:HGNC:2551]
ENSG00000069869	NEDD4	neural precursor cell expressed, developmentally down-regulated 4, E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:7727]
ENSG00000072401	UBE2D1	ubiquitin-conjugating enzyme E2D 1 [Source:HGNC Symbol;Acc:HGNC:12474]
ENSG00000072803	FBXW11	F-box and WD repeat domain containing 11 [Source:HGNC Symbol;Acc:HGNC:13607]
ENSG00000078043	PIAS2	protein inhibitor of activated STAT, 2 [Source:HGNC Symbol;Acc:HGNC:17311]
ENSG00000078140	UBE2K	ubiquitin-conjugating enzyme E2K [Source:HGNC Symbol;Acc:HGNC:4914]
ENSG00000078747	ITCH	itchy E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:13890]
ENSG00000079999	KEAP1	kelch-like ECH-associated protein 1 [Source:HGNC Symbol;Acc:HGNC:23177]
ENSG00000081307	UBA5	ubiquitin like modifier activating enzyme 5 [Source:HGNC Symbol;Acc:HGNC:23230]
ENSG00000094880	CDC23	cell division cycle 23 [Source:HGNC Symbol;Acc:HGNC:1724]
ENSG00000100023	PPIL2	peptidylprolyl isomerase (cyclophilin)-like 2 [Source:HGNC Symbol;Acc:HGNC:9261]
ENSG00000100387	RBX1	ring-box 1, E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:9928]
ENSG00000101966	XIAP	X-linked inhibitor of apoptosis, E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:592]
ENSG00000103657	HERC1	HECT and RLD domain containing E3 ubiquitin protein ligase family member 1 [Source:HGNC Symbol;Acc:HGNC:4867]
ENSG00000104343	UBE2W	ubiquitin-conjugating enzyme E2W (putative) [Source:HGNC Symbol;Acc:HGNC:25616]
ENSG00000105229	PIAS4	protein inhibitor of activated STAT, 4 [Source:HGNC Symbol;Acc:HGNC:17002]
ENSG00000105325	FZR1	fizzy/cell division cycle 20 related 1 [Source:HGNC Symbol;Acc:HGNC:24824]
ENSG00000108395	TRIM37	tripartite motif containing 37 [Source:HGNC Symbol;Acc:HGNC:7523]
ENSG00000109332	UBE2D3	ubiquitin-conjugating enzyme E2D 3 [Source:HGNC Symbol;Acc:HGNC:12476]
ENSG00000109670	FBXW7	F-box and WD repeat domain containing 7, E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:16712]
ENSG00000110330	BIRC2	baculoviral IAP repeat containing 2 [Source:HGNC Symbol;Acc:HGNC:590]
ENSG00000110395	CBL	Cbl proto-oncogene, E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:1541]
ENSG00000113558	SKP1	S-phase kinase-associated protein 1 [Source:HGNC Symbol;Acc:HGNC:10899]
ENSG00000114062	UBE3A	ubiquitin protein ligase E3A [Source:HGNC Symbol;Acc:HGNC:12496]
ENSG00000114423	CBLB	Cbl proto-oncogene B, E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:1542]
ENSG00000115392	FANCL	Fanconi anemia, complementation group L [Source:HGNC Symbol;Acc:HGNC:20748]
ENSG00000117399	CDC20	cell division cycle 20 [Source:HGNC Symbol;Acc:HGNC:1723]
ENSG00000123124	WWP1	WW domain containing E3 ubiquitin protein ligase 1 [Source:HGNC Symbol;Acc:HGNC:17004]
ENSG00000126261	UBA2	ubiquitin-like modifier activating enzyme 2 [Source:HGNC Symbol;Acc:HGNC:30661]
ENSG00000130985	UBA1	ubiquitin like modifier activating enzyme 1 [Source:HGNC Symbol;Acc:HGNC:12469]
ENSG00000132388	UBE2G1	ubiquitin-conjugating enzyme E2G 1 [Source:HGNC Symbol;Acc:HGNC:12482]

ENSG00000134086	VHL	von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:12687]
ENSG00000135679	MDM2	MDM2 proto-oncogene, E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:6973]
ENSG00000138641	HERC3	HECT and RLD domain containing E3 ubiquitin protein ligase 3 [Source:HGNC Symbol;Acc:HGNC:4876]
ENSG00000139842	CUL4A	cullin 4A [Source:HGNC Symbol;Acc:HGNC:2554]
ENSG00000140367	UBE2Q2	ubiquitin-conjugating enzyme E2Q family member 2 [Source:HGNC Symbol;Acc:HGNC:19248]
ENSG00000140464	PML	promyelocytic leukemia [Source:HGNC Symbol;Acc:HGNC:9113]
ENSG00000143207	RFWD2	ring finger and WD repeat domain 2, E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:17440]
ENSG00000144744	UBA3	ubiquitin-like modifier activating enzyme 3 [Source:HGNC Symbol;Acc:HGNC:12470]
ENSG00000145604	SKP2	S-phase kinase-associated protein 2, E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:10901]
ENSG00000151148	UBE3B	ubiquitin protein ligase E3B [Source:HGNC Symbol;Acc:HGNC:13478]
ENSG00000153107	ANAPC1	anaphase promoting complex subunit 1 [Source:HGNC Symbol;Acc:HGNC:19988]
ENSG00000154582	TCEB1	transcription elongation factor B (SIII), polypeptide 1 (15kDa, elongin C) [Source:HGNC Symbol;Acc:HGNC:11617]
ENSG00000158290	CUL4B	cullin 4B [Source:HGNC Symbol;Acc:HGNC:2555]
ENSG00000159202	UBE2Z	ubiquitin-conjugating enzyme E2Z [Source:HGNC Symbol;Acc:HGNC:25847]
ENSG00000159593	NAE1	NEDD8 activating enzyme E1 subunit 1 [Source:HGNC Symbol;Acc:HGNC:621]
ENSG00000160087	UBE2J2	ubiquitin-conjugating enzyme E2, J2 [Source:HGNC Symbol;Acc:HGNC:19268]
ENSG00000160714	UBE2Q1	ubiquitin-conjugating enzyme E2Q family member 1 [Source:HGNC Symbol;Acc:HGNC:15698]
ENSG00000162298	SYVN1	synovial apoptosis inhibitor 1, synoviolin [Source:HGNC Symbol;Acc:HGNC:20738]
ENSG00000163743	RCHY1	ring finger and CHY zinc finger domain containing 1, E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:17479]
ENSG00000166167	BTRC	beta-transducin repeat containing E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:1144]
ENSG00000166266	CUL5	cullin 5 [Source:HGNC Symbol;Acc:HGNC:2556]
ENSG00000174989	FBXW8	F-box and WD repeat domain containing 8 [Source:HGNC Symbol;Acc:HGNC:13597]
ENSG00000175104	TRAF6	TNF receptor-associated factor 6, E3 ubiquitin protein ligase [Source:HGNC Symbol;Acc:HGNC:12036]
ENSG00000176248	ANAPC2	anaphase promoting complex subunit 2 [Source:HGNC Symbol;Acc:HGNC:19989]
ENSG00000176386	CDC26	cell division cycle 26 [Source:HGNC Symbol;Acc:HGNC:17839]
ENSG00000177889	UBE2N	ubiquitin-conjugating enzyme E2N [Source:HGNC Symbol;Acc:HGNC:12492]
ENSG00000184182	UBE2F	ubiquitin-conjugating enzyme E2F (putative) [Source:HGNC Symbol;Acc:HGNC:12480]
ENSG00000185019	UBOX5	U-box domain containing 5 [Source:HGNC Symbol;Acc:HGNC:17777]
ENSG00000185651	UBE2L3	ubiquitin-conjugating enzyme E2L 3 [Source:HGNC Symbol;Acc:HGNC:12488]
ENSG00000186591	UBE2H	ubiquitin-conjugating enzyme E2H [Source:HGNC Symbol;Acc:HGNC:12484]
ENSG00000196510	ANAPC7	anaphase promoting complex subunit 7 [Source:HGNC Symbol;Acc:HGNC:17380]
ENSG00000197170	PSMD12	proteasome 26S subunit, non-ATPase 12 [Source:HGNC Symbol;Acc:HGNC:9557]
ENSG00000197548	ATG7	autophagy related 7 [Source:HGNC Symbol;Acc:HGNC:16935]
ENSG00000198373	WWP2	WW domain containing E3 ubiquitin protein ligase 2 [Source:HGNC Symbol;Acc:HGNC:16804]
ENSG00000198742	SMURF1	SMAD specific E3 ubiquitin protein ligase 1 [Source:HGNC Symbol;Acc:HGNC:16807]
ENSG00000198833	UBE2J1	ubiquitin-conjugating enzyme E2, J1 [Source:HGNC Symbol;Acc:HGNC:17598]

Table S3: UPP genes.