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**Supplemental Information**

**Protein Stability Buffers the Cost of  
Translation Attenuation following  
eIF2 $\alpha$  Phosphorylation**

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## Supplemental Information

**Table S1: Top 10 GO terms of translationally regulated mRNAs as determined by anota2seq, Related to Figure 1**

Top 10 GO terms of relative translationally down- or upregulated mRNAs as determined by anota2seq at 2 and 4 hours following Tm injection.

### Translationally downregulated mRNAs at 2 hours

Category	GO terms	count	%	p value	adjusted p value
GOTERM_BP_DIRECT	translation	136	8.2	1.90E-52	6.60E-49
GOTERM_BP_DIRECT	transport	232	14	2.50E-15	4.50E-12
GOTERM_BP_DIRECT	mitochondrial translation	21	1.3	1.00E-13	1.20E-10
GOTERM_BP_DIRECT	protein transport	99	6	2.40E-13	2.20E-10
GOTERM_BP_DIRECT	rRNA processing	36	2.2	1.10E-11	8.10E-09
GOTERM_BP_DIRECT	oxidation-reduction process	101	6.1	1.20E-10	7.30E-08
GOTERM_BP_DIRECT	glutathione metabolic process	21	1.3	1.50E-10	7.40E-08
GOTERM_BP_DIRECT	antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent	16	1	9.10E-10	4.00E-07
GOTERM_BP_DIRECT	proton transport	21	1.3	4.90E-09	1.90E-06
GOTERM_BP_DIRECT	vesicle-mediated transport	43	2.6	1.20E-08	4.20E-06

### Translationally downregulated mRNAs at 4 hours

Category	GO terms	count	%	p value	adjusted p value
GOTERM_BP_DIRECT	translation	105	10.	7.20E-50	1.80E-46
GOTERM_BP_DIRECT	transport	144	14.	4.90E-11	6.10E-08
GOTERM_BP_DIRECT	oxidation-reduction process	69	6.9	8.00E-10	6.60E-07
GOTERM_BP_DIRECT	proton transport	17	1.7	4.20E-09	2.60E-06
GOTERM_BP_DIRECT	ATP synthesis coupled proton	11	1.1	2.50E-08	1.30E-05
GOTERM_BP_DIRECT	ribosomal small subunit assembly	10	1	1.50E-07	6.20E-05
GOTERM_BP_DIRECT	cytoplasmic translation	12	1.2	1.90E-07	6.60E-05
GOTERM_BP_DIRECT	mitochondrial translation	12	1.2	3.60E-07	1.10E-04
GOTERM_BP_DIRECT	rRNA processing	20	2	4.50E-06	1.20E-03
GOTERM_BP_DIRECT	ATP metabolic process	11	1.1	9.20E-06	2.30E-03

### Translationally upregulated mRNAs at 2 hours

Category	GO terms	count	%	p value	adjusted p value
GOTERM_BP_DIRECT	mRNA processing	95	5.1	3.50E-25	1.60E-21
GOTERM_BP_DIRECT	metabolic process	116	6.2	6.20E-24	1.50E-20
GOTERM_BP_DIRECT	RNA splicing	70	3.8	2.40E-18	3.80E-15
GOTERM_BP_DIRECT	protein transport	110	5.9	1.00E-12	1.20E-09
GOTERM_BP_DIRECT	covalent chromatin modification	61	3.3	4.10E-11	3.80E-08
GOTERM_BP_DIRECT	transcription, DNA-templated	255	13.	6.70E-11	5.30E-08
GOTERM_BP_DIRECT	flavonoid biosynthetic process	15	0.8	1.80E-10	1.20E-07

GOTERM_BP_DIRECT	flavonoid glucuronidation	15	0.8	1.80E-10	1.20E-07
GOTERM_BP_DIRECT	oxidation-reduction process	113	6.1	4.30E-10	2.50E-07
GOTERM_BP_DIRECT	DNA repair	65	3.5	1.40E-09	7.40E-07

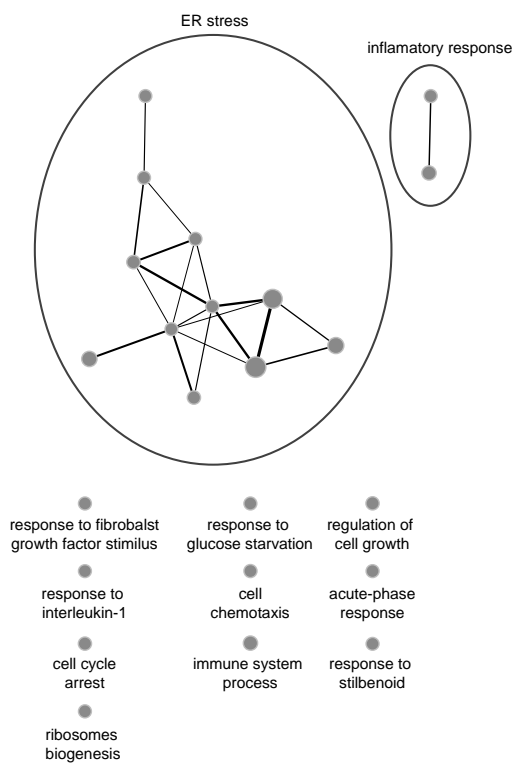
#### Translationally upregulated mRNAs at 4 hours

Category	GO terms	count	%	p value	<b>adjusted p value</b>
GOTERM_BP_DIRECT	flavonoid glucuronidation	12	1.8	6.20E-12	1.40E-08
GOTERM_BP_DIRECT	flavonoid biosynthetic process	12	1.8	6.20E-12	1.40E-08
GOTERM_BP_DIRECT	xenobiotic glucuronidation	9	1.4	7.20E-12	8.20E-09
GOTERM_BP_DIRECT	metabolic process	38	5.7	1.50E-07	1.20E-04
GOTERM_BP_DIRECT	transcription, DNA-templated	93	14	6.90E-06	3.90E-03
GOTERM_BP_DIRECT	mRNA processing	25	3.8	7.20E-05	3.20E-02
GOTERM_BP_DIRECT	protein ubiquitination involved in ubiquitin-dependent protein catabolic process	15	2.3	7.30E-05	2.80E-02
GOTERM_BP_DIRECT	regulation of transcription, DNA-	103	15.	7.40E-05	2.40E-02
GOTERM_BP_DIRECT	response to nutrient	10	1.5	4.10E-04	1.10E-01
GOTERM_BP_DIRECT	protein ubiquitination	25	3.8	4.20E-04	1.00E-01

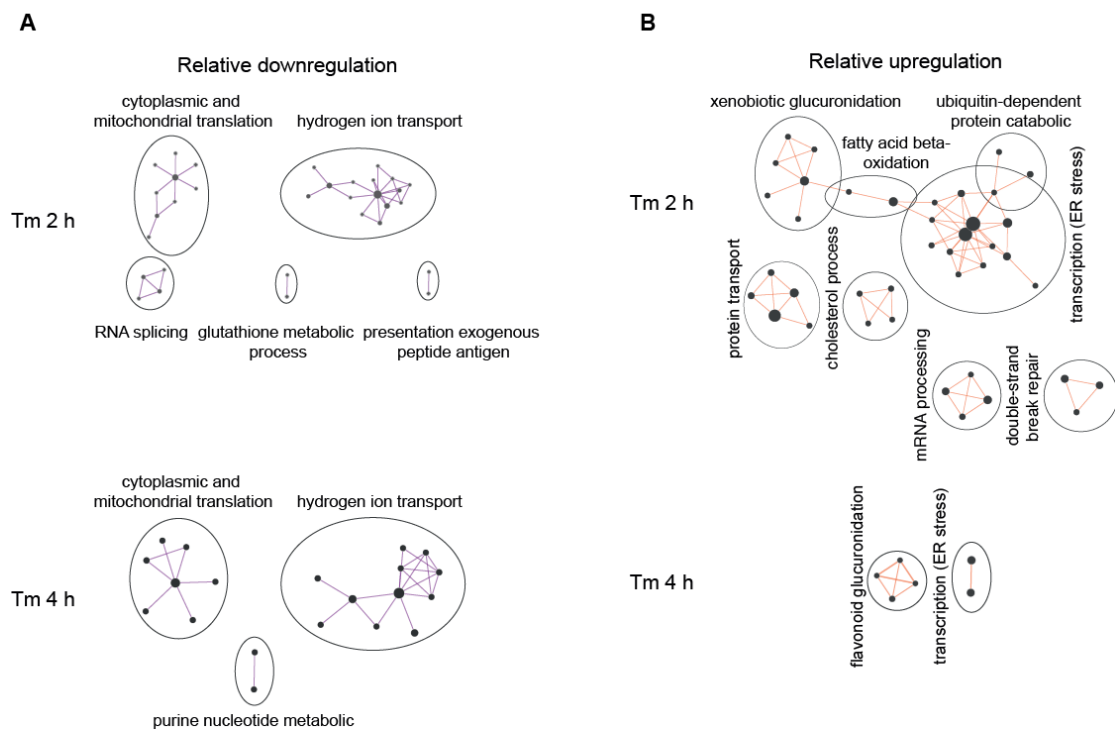
**Table S2: Oligonucleotides used in this study**

<i>MmPpp1r15a_R</i> : tctcaggtcctccttctca	Sigma-Aldrich	N/A
<i>MmChop_F</i> : cctagcttgctgacagagg	Sigma-Aldrich	N/A
<i>MmChop_R</i> : ctgctccttctcctcatgc	Sigma-Aldrich	N/A
<i>MmBiP_F</i> : ctttgatcagcgggtcatgg	Sigma-Aldrich	N/A
<i>MmBiP_R</i> : agctctcaaattggcccg	Sigma-Aldrich	N/A
<i>MmGrp94_F</i> : agtcggaagcaacagagaa	Sigma-Aldrich	N/A
<i>MmGrp94_R</i> : tctccatgtgccagaccat	Sigma-Aldrich	N/A
<i>MmNedd4_F</i> : atgtggatgctgggagttga	Sigma-Aldrich	N/A
<i>MmNedd4_R</i> : ctgcacgctggaaggattc	Sigma-Aldrich	N/A
<i>MmRps6_F</i> : aaggaagaagcccaggacc	Sigma-Aldrich	N/A
<i>MmRps6_R</i> : ctgttcctggcgcttttctt	Sigma-Aldrich	N/A
<i>MmCul4b_F</i> : ccctgtcttcttgacac	Sigma-Aldrich	N/A
<i>MmCul4b_R</i> : aggaagcaaagcagagtctt	Sigma-Aldrich	N/A
<i>MmAsns_F</i> : aggagcatcgagaaaacca	Sigma-Aldrich	N/A
<i>MmAsns_R</i> : aagaagggagtggtggagtg	Sigma-Aldrich	N/A
<i>HsRpl4_F</i> : tgggacgtttctgcatttgg	Sigma-Aldrich	N/A
<i>HsRpl4_R</i> : cgatggatcttctgcgtgg	Sigma-Aldrich	N/A
<i>HsMrpl12_F</i> : ggaggcggtggaagaagata	Sigma-Aldrich	N/A
<i>HsMrpl12_R</i> : gactccaccagcttctttgc	Sigma-Aldrich	N/A
<i>HsMrpl28_F</i> : ggacctgtgctccaagttg	Sigma-Aldrich	N/A
<i>HsMrpl28_R</i> : ttgaacaggggtacagggtc	Sigma-Aldrich	N/A
<i>HsMrps15_F</i> : ggccaacaagaaggagatgc	Sigma-Aldrich	N/A
<i>HsMrps15_R</i> : ttgtgggctttgtcctttcg	Sigma-Aldrich	N/A
<i>HsMrps25_F</i> : caggaagaggaggaggaga	Sigma-Aldrich	N/A
<i>HsMrps25_R</i> : aacccttgggtgcagatcat	Sigma-Aldrich	N/A
<i>MmMrpl12_F</i> : tcctcctccttccctgag	Sigma-Aldrich	N/A
<i>MmMrpl12_R</i> : cctccaaggctgctttgatc	Sigma-Aldrich	N/A
<i>MmMrpl28_F</i> : ccccgggtactacaaacct	Sigma-Aldrich	N/A
<i>MmMrpl28_R</i> : tccacaccttctcacctc	Sigma-Aldrich	N/A
<i>MmMrps15_F</i> : caagcaggacgatgaaccac	Sigma-Aldrich	N/A
<i>MmMrps15_R</i> : attgcgatcctgacagtca	Sigma-Aldrich	N/A
<i>MmMrps25_F</i> : cccttctgcggttctatct	Sigma-Aldrich	N/A

MmMrps25_R: ggcttctactctgcacatg	Sigma-Aldrich	N/A
<i>Cyclophilin B</i> _F: ggagatggcacaggaggaa	Sigma-Aldrich	N/A
<i>Cyclophilin B</i> _R: gcccgtagtgcttcagctt	Sigma-Aldrich	N/A
<i>Luciferase</i> _F: aaaggatatcaggtggcccc	Sigma-Aldrich	N/A
<i>Luciferase</i> _R: ccacaaacacaactcctccg	Sigma-Aldrich	N/A
<i>Atp5d</i> _5UTR_F: gatacaactagtccttcggagaatcctgtgc	Sigma-Aldrich	N/A
<i>Atp5d</i> _5UTR_R: gatacacatggggtggcggaaaagtcagtg	Sigma-Aldrich	N/A
<i>Atf4</i> _5UTR_F: gatacaactagtggttaggtgtcccaccc	Sigma-Aldrich	N/A
<i>Atf4</i> _5UTR_R: gatacagaattcgtgtggggcttggctggattcg	Sigma-Aldrich	N/A
<i>Mrps28</i> _5UTR_F: gatacagaattcagggaagctttaactaccggac	Sigma-Aldrich	N/A
<i>Mrps28</i> _5UTR_R: gatacacatgggacgcctgcgcgacctc	Sigma-Aldrich	N/A
<i>Mrps33</i> _5UTR_F: gatacaactagtccgggtgtccgcccac	Sigma-Aldrich	N/A
<i>Mrps33</i> _5UTR_R: gatacacatggttcttcaccagtagggaacaggattcc	Sigma-Aldrich	N/A
<i>Rps15a</i> _5UTR_F: gatacaactagtataattccgtgcttggcccggcccactctc tttctgccatcttccctcgcccacc	Sigma-Aldrich	N/A
<i>Rps15a</i> _5UTR_R: gatacacatggggtggcggcgaggggaagatggcagaa agagagtgggcccgggcaaacgcacggaattat	Sigma-Aldrich	N/A
<i>Rps18</i> _5UTR_F: gatacaactagtgctacttccgctctcttccacaggaggcc tacacgccgcccgtctgtgccgcccgc	Sigma-Aldrich	N/A
<i>Rps18</i> _5UTR_R: gatacacatggggcggcgccacagacggcggcgtgtag gcctcctgtggaagagagagcggaagtgc	Sigma-Aldrich	N/A
<i>Rpl35</i> _5UTR_F: ggactagtgaacagtggaagaagagaactc	Sigma-Aldrich	N/A
<i>Rpl35</i> _5UTR_R: catgccatgggtcggcggtgttcacg	Sigma-Aldrich	N/A
<i>Rps24</i> _5UTR_F: ggactagtgtggggtccttctt	Sigma-Aldrich	N/A
<i>Rps24</i> _5UTR_R: catgccatgggatggctacggcgcc	Sigma-Aldrich	N/A
<i>Rps15</i> _5UTR_F: gatacaactagtgacttcttccgagtaaccgccaag	Sigma-Aldrich	N/A
<i>Rps15</i> _5UTR_R: gatacacatggcttggcgttactcggaaaaggaagtac	Sigma-Aldrich	N/A



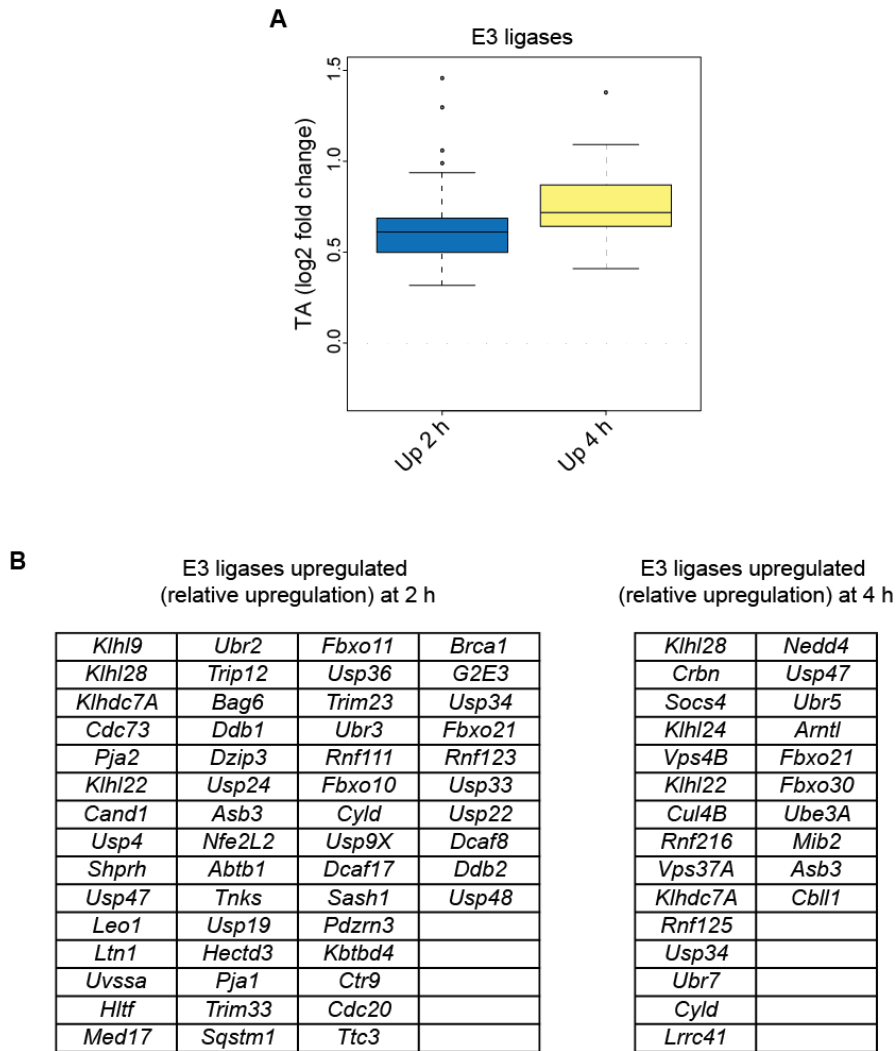
**Figure S1. Gene ontology enrichment map of mRNAs transcriptionally upregulated after Tm, Related to Figure 1**  
 Significant ( $P < 0.001$ ) gene ontology enrichment maps of the 900 mRNAs transcriptionally upregulated (2-fold cut-off) 4 hours after Tm injection.



**Figure S2. GO analysis of mRNA translationally affected following Tm, Related to Figure 3**

(A) Significant ( $P < 0.001$ ) GO enrichment maps of translationally downregulated mRNAs as determined by anota2seq at 2 and 4 hours following Tm injection.

(B) Same as (A) for genes resistant to translational attenuation (relative upregulation).

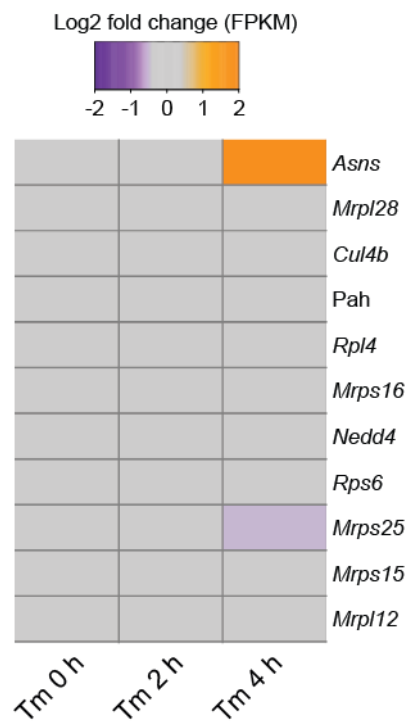


**Figure S3. Relative translational regulation of E3 ligases following Tm, Related to Figure 3**

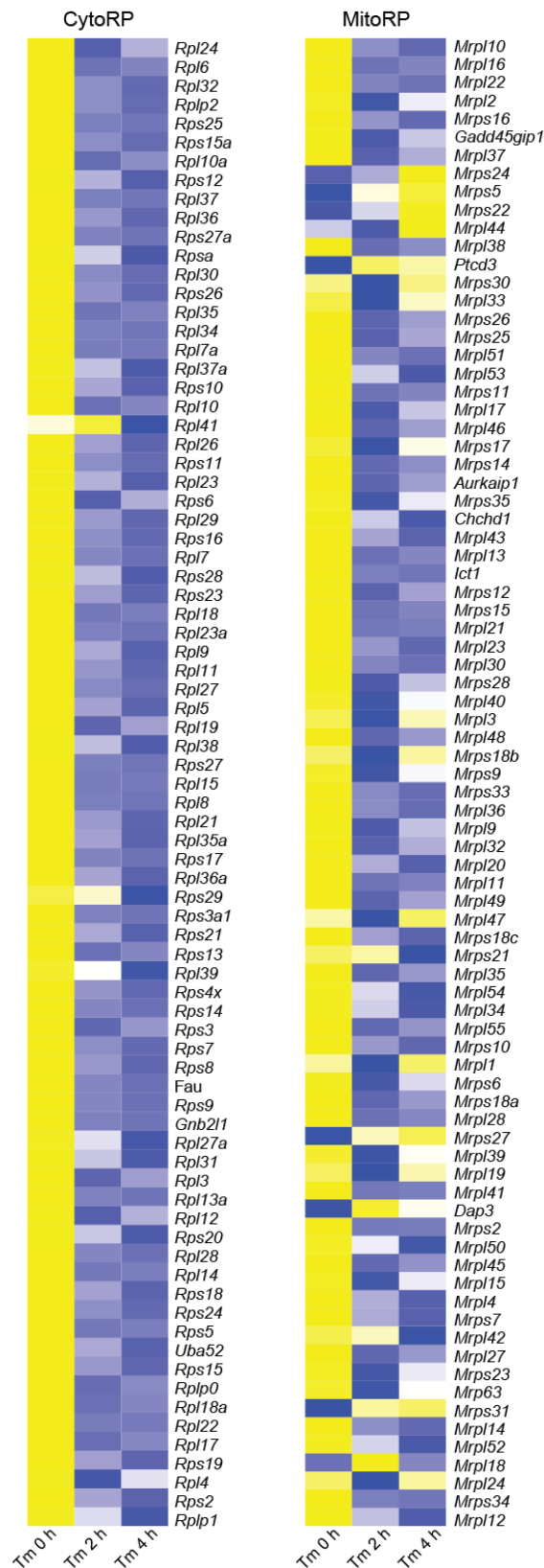
(A) Boxplot showing the median translation efficiency (TA) as determined by anota2seq at 2 and 4 hours following Tm treatment.

(B) List of E3 ligases translationally upregulated (relative upregulation) at 2 and 4 hours following Tm, respectively.





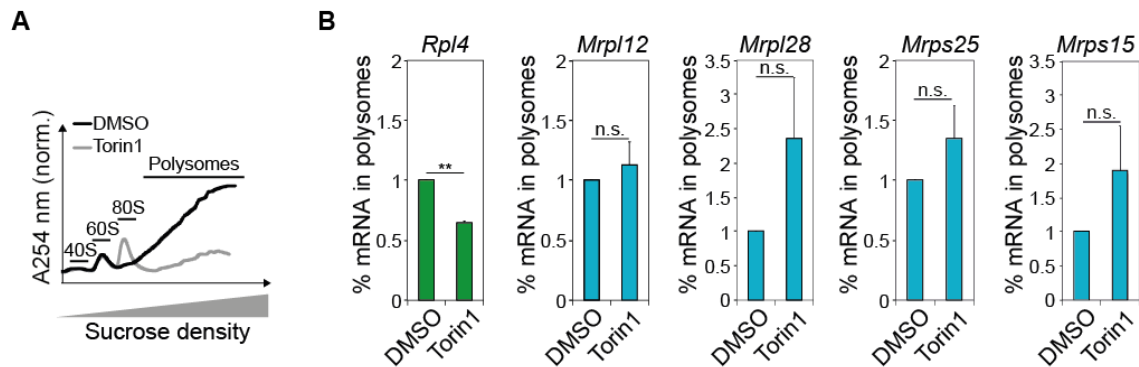
**Figure S4. mRNAs abundance of selected transcripts, Related to Figure 3**  
 Log2 FPKM values for indicated genes at 0, 2 and 4 hours following Tm.



**Figure S5. Cytosolic and mitochondrial ribosomal proteins are translationally downregulated by Tm, Related to Figure 3**

(A) Heat map showing z-scores of anota2seq translation efficiencies of all 79 cytosolic ribosomal proteins (CytoRP) subunit-encoding mRNAs at 0, 2 and 4 hours following Tm injection.

(B) Same as (A) but with all 82 mitochondrial ribosomal proteins (MitoRP) subunits.

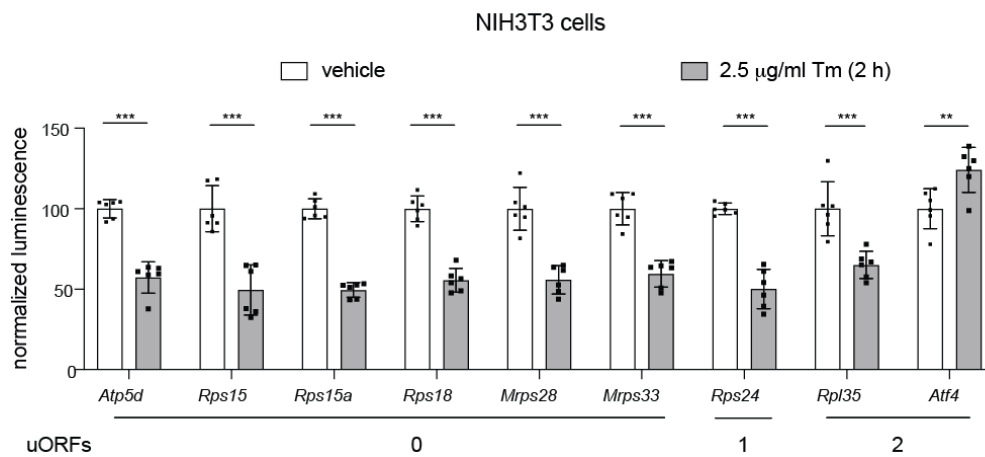


**Figure S6. Translation of mitochondrial ribosomal proteins is not sensitive to acute mTORC1 inhibition, Related to Figure 4**

(A) Representative polysome profile (optical density of the fractions at 254 nm) on 5-50% sucrose gradients of HeLa cells treated with DMSO or 250 nM of Torin1 for 2 hours.

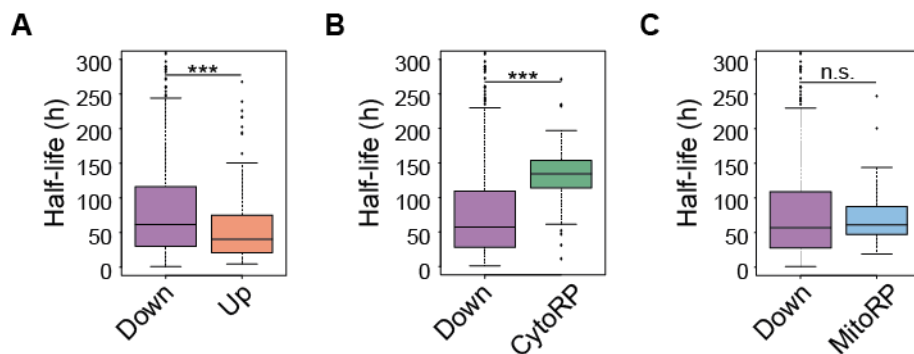
(B) *Rpl4*, *Mrpl12*, *Mrpl28*, *Mrps25* and *Mrps15* mRNA abundance from heavy polysomal fractions (more than 3 ribosomes) from (A) assessed by qPCR.

Data are means  $\pm$  SEM ( $n = 5-9$ ). \*\* $P < 0.01$  as determined by the unpaired Student t-test.



**Figure S7. Composition of UTRs regulates the translational response to eIF2 $\alpha$  phosphorylation, Related to Figure 5**

Firefly luciferase activity in NIH3T3 cells transfected with reporter mRNAs containing the 5'UTRs of indicated mRNAs and treated with either vehicle or 2.5  $\mu$ g/ml Tunicamycin (Tm) for 2 hours. Firefly luciferase activity was normalized against a transfection control (see Methods) and vehicle-treated cells. Data are means  $\pm$  SEM (n=6). Statistical analysis was carried out in Prism 8 using a two-way ANOVA test.



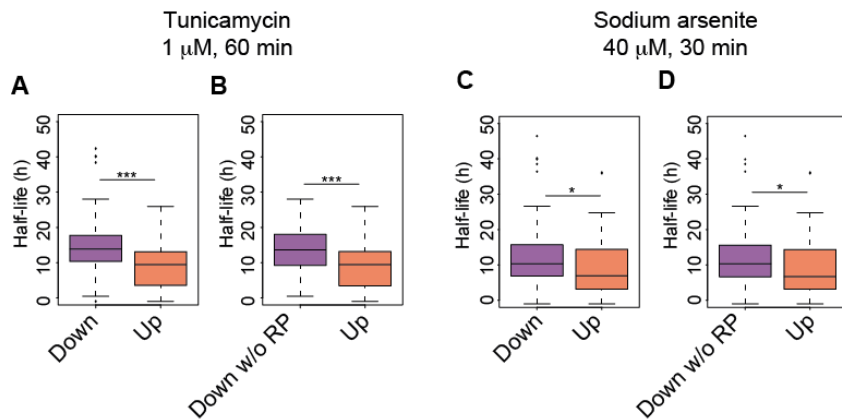
**Figure S8. Translationally downregulated mRNAs encode for highly stable proteins, Related to Figure 6**

Same as in Figure 6E with protein half-lives analyzed from (Schwanhäusser et al, 2013).

(A) Boxplot of half-lives of proteins encoded by down- or upregulated (Down or Up) mRNAs at 2 hours following Tm injection.

(B) Same as (A) comparing the half-lives of proteins encoded by translationally downregulated mRNAs with all 79 CytoRP subunits 2 hours after Tm.

(C) Same as (B) with all 82 MitoRP subunits. (A-C) \*\*\*P<0.001 as determined by the unpaired Wilcoxon test.



**Figure S9. eIF2 $\alpha$  phosphorylation induced by different stressors downregulates translation of mRNAs encoding for stable proteins, Related to Figure 6**

(A) Boxplot of half-lives of proteins encoded by the translationally down or upregulated (Down or Up) mRNAs using data from (Sidrauski et al, 2015) 1 hours following Tm treatment of HEK293T cells.

(B) Same as (A) but excluding the RPs from the category of translationally downregulated mRNAs (Down w/o RP).

(C) Same as (A) but with data from (Andreev et al, 2015) acquired 30 minutes following arsenite treatment of HEK293T cells.

(D) Same as (C) but excluding the RPs from the category of translationally downregulated mRNAs (Down w/o RP).

(A-D) Protein half-lives were analyzed from (Ly et al, 2018)

\*P<0.05, \*\*\*P<0.001 as determined by the unpaired Wilcoxon test.