Supplementary figure legends

Figure S1: TSS expression levels were obtained from the Wang et al. 2016 data (GEO accession GSE78241) and assigned to protein-coding genes to compute 5'UTR length as in Fig. 1A. 5'UTR lengths were then binned and the total expression level in each bin was tallied and normalized to compute the fraction of transcripts assigned to each bin, which is shown in the heatmap. The 5'UTR length and the ribosomal fraction are denoted on the right and bottom, respectively. The colors represent the % of transcript of the indicated 5'UTR length in each fraction.

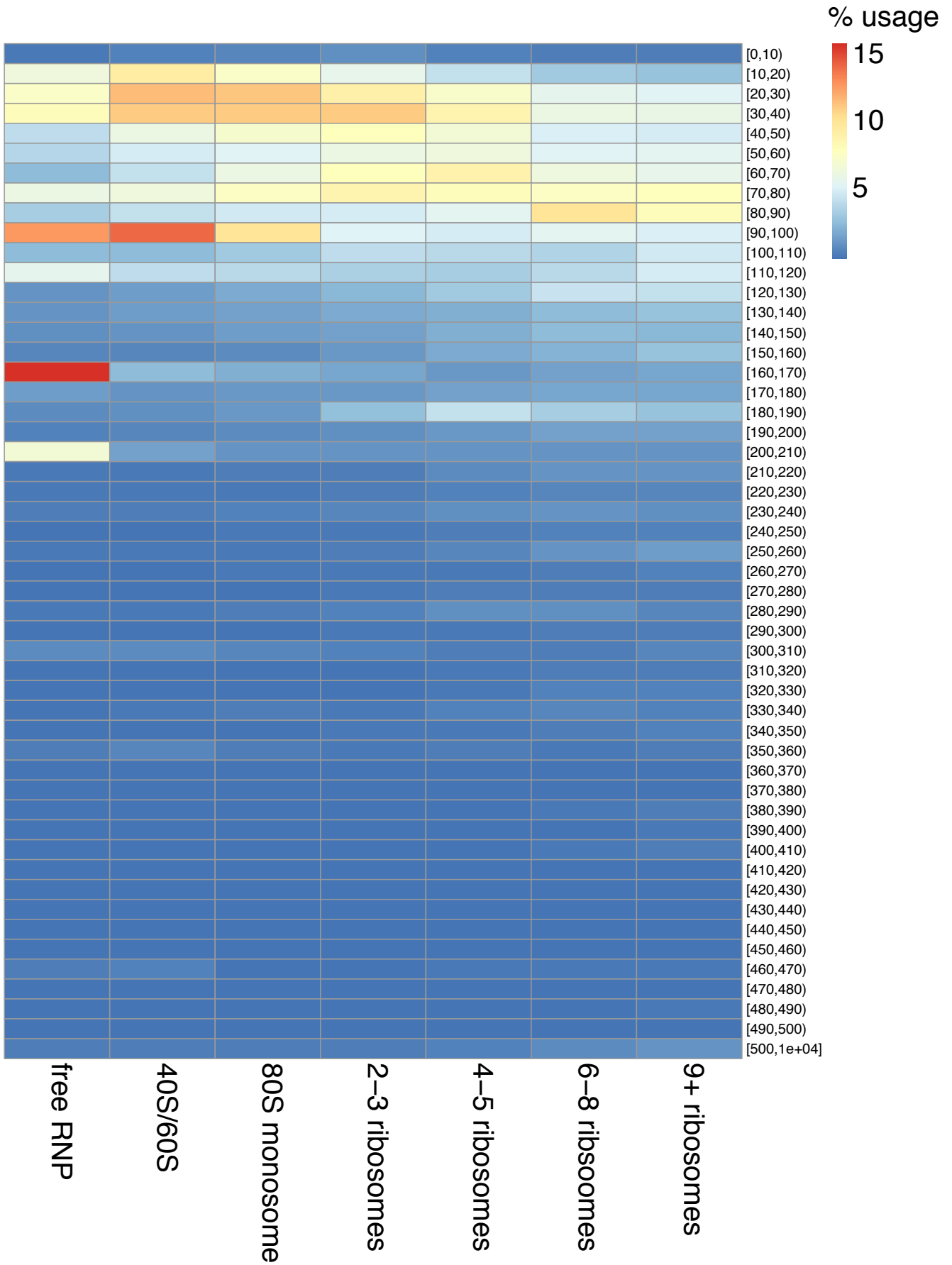
Figure S2: The integrity of the *in vitro* transcribed and capped mRNAs (TISU WT, mutants and Luciferase) that were used for the *in vivo* translation shown in Fig. 2, was analyzed by agarose-gel electrophoresis. M, denotes DNA size marker.

Figure S3: A. *In vitro* transcribed and capped canonical and TISU mRNAs modified with Thio-UTP at the indicated positions and labeled with ^{32}P-CTP were analyzed by 10% denaturing polyacrylamide gel followed by autoradiography. These mRNAs were used for the site-specific UV cross-linking experiments. **B&C** TISU and Canonical AUG and mRNAs modified with a Thio-U in the indicated positions were incubated with rabbit reticulocyte lysate in the presence of GMP-PNP (**B**) or Cycloheximide (**C**) and were then subjected to 365nm UV irradiation followed by RNase treatment. The cross-linked proteins were analyzed by 15% SDS-PAGE followed by autoradiography. Red arrows denote specific cross-linked polypeptides.

Figure S4: TISU mRNA was incubated with RRL in the presence of GMP-PNP or CHX as indicated. The mRNA-ribosomal complexes were then subjected to 8-32% sucrose gradient sedimentation and fraction collection. The fractions were TCA precipitated and analyzed by western blot using RPL11 and RPS5 antibodies as indicated.

Figure S5: Analysis of RPS10e, RPS3 and RPS19 antibodies in immunoprecipitation assay using RRL and the indicated antibodies. The immune complexes were analyzed by immunoblot with the corresponding antibodies.

Figure S1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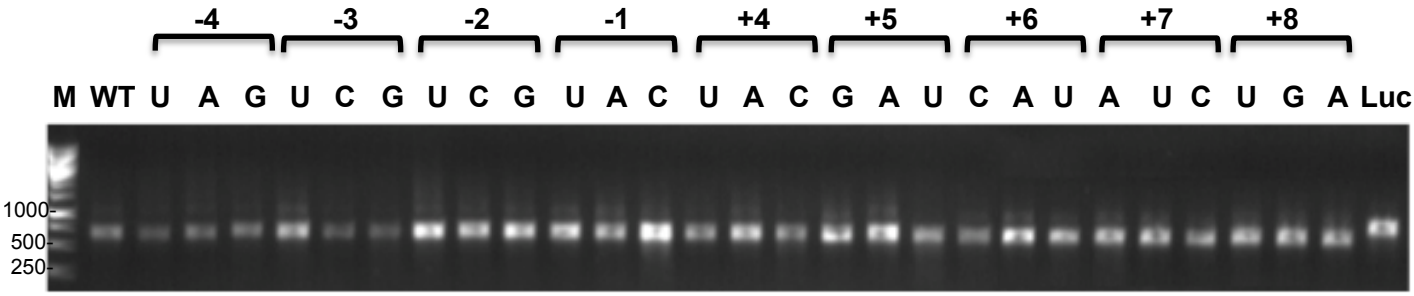
4–5 ribosomes
6–8 ribsoomes
9+ ribosomes

Figure S2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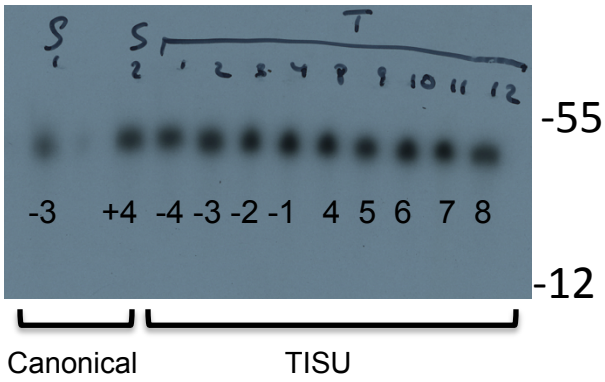
250

Figure S3

A.



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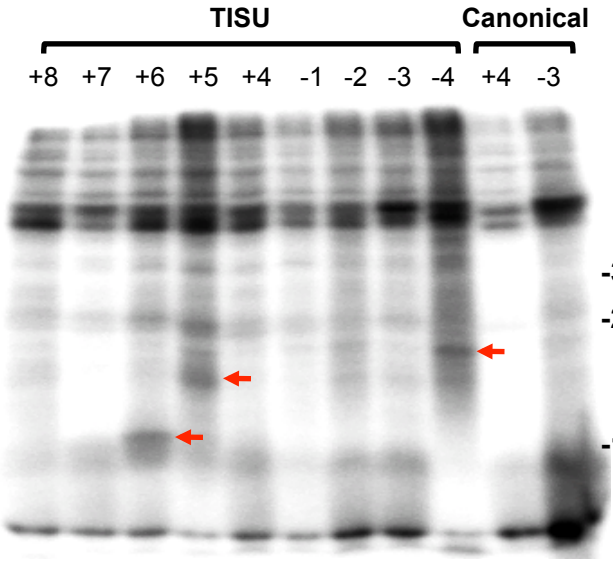

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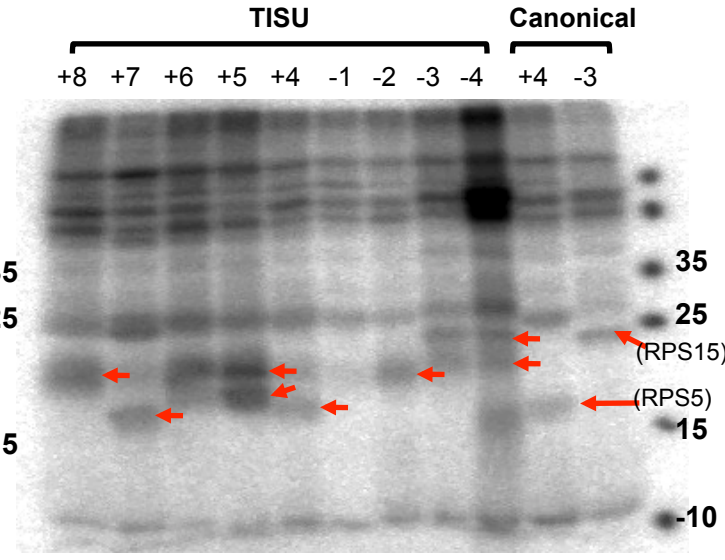

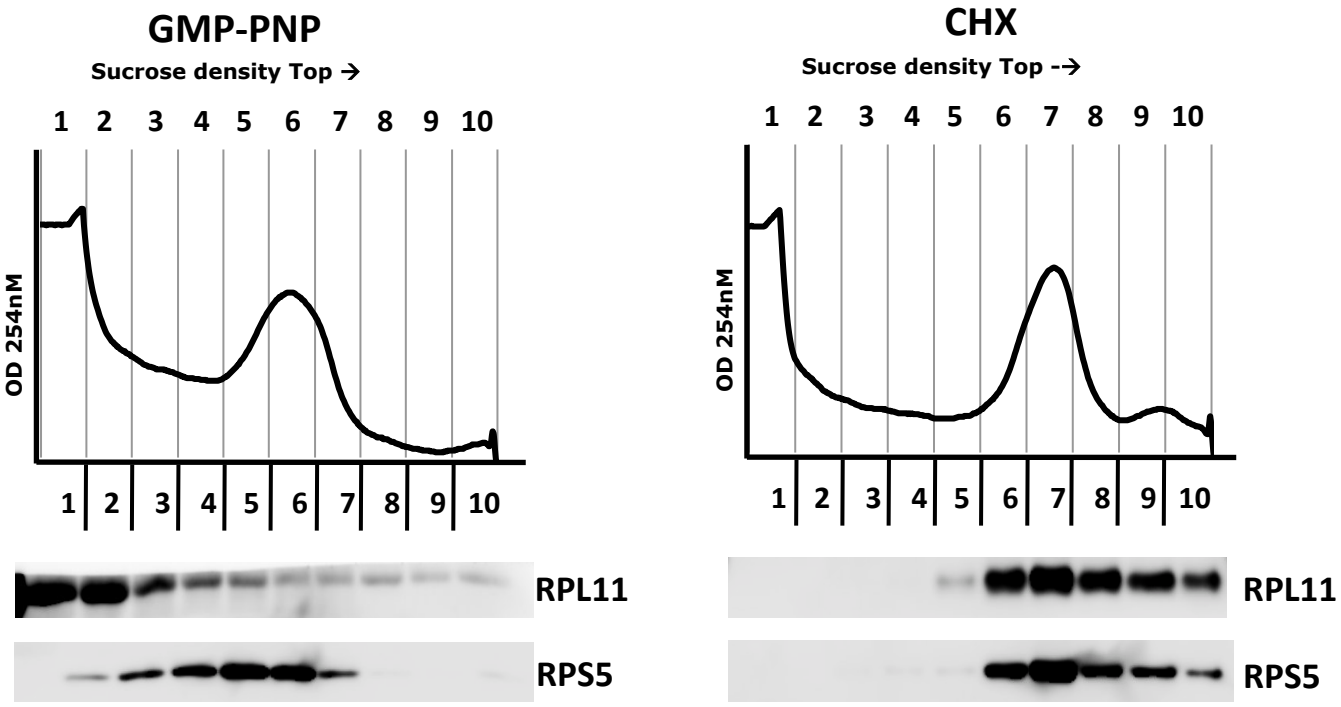
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RPL11
RPS5
CHX
Sucrose density Top -→
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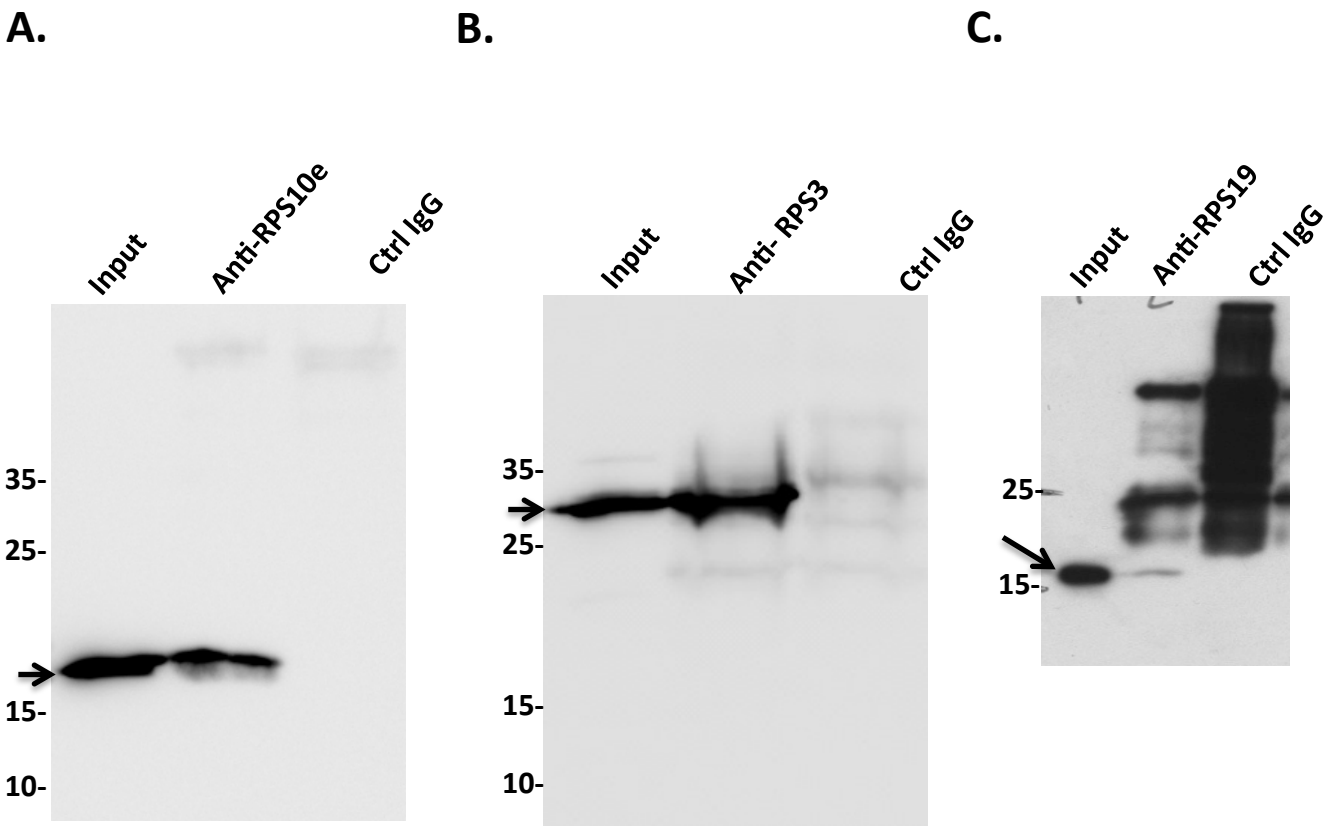
OD 254nM
1 2 3 4 5 6 7 8 9 10
RPL11
RPS5

Figure S5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C.
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