

Appendix for

LARP1 binds ribosomes and TOP mRNAs in repressed complexes

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Appendix Text

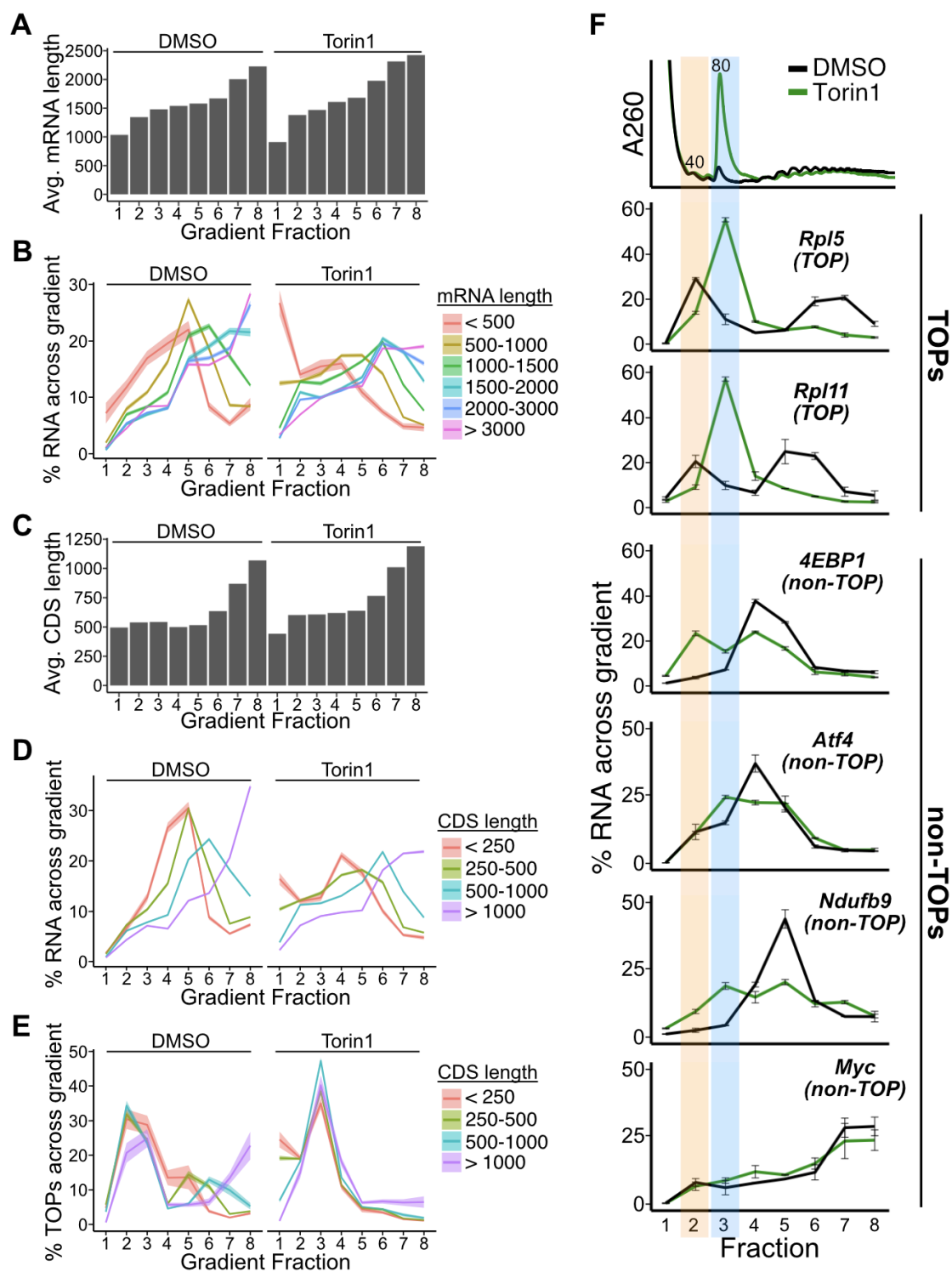
TOP-80S shift in high-K⁺ gradients

Previous studies have shown that vacant 40S and 60S subunit couples are held together more weakly than ribosomes actively engaged with mRNAs, peptidyl-tRNAs or initiation complexes (Infante & Baierlein, 1971; Beller & Lubsen, 1972; Noll *et al*, 1973). As a result, these “vacant couples” are more susceptible to splitting by high hydrostatic pressure as they travel through sucrose gradients. Hydrostatic pressure follows the following formula:

$$P = \rho gh$$

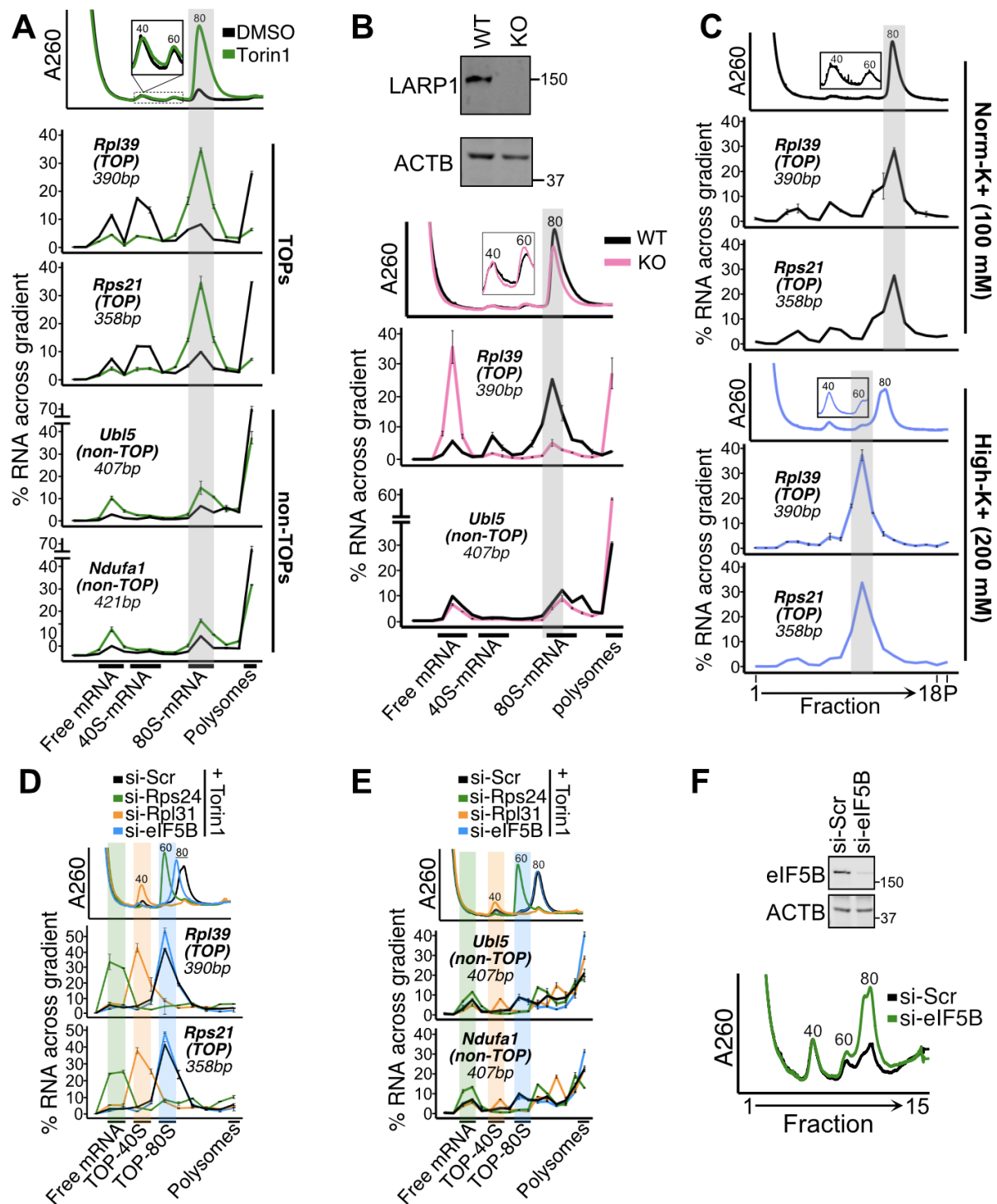
where P = hydrostatic pressure, ρ = fluid density, g = acceleration due to gravity, and h = fluid depth. From this formula it becomes clear that as depth in the gradient increases, the hydrostatic pressure proportionally increases. In high-K⁺ gradients, the high potassium concentration further weakens the general intersubunit interactions of vacant couples. As a result, they initially sediment as an 80S ribosome. Once they travel deep enough in the gradient such that the hydrostatic pressure overcomes the force of intersubunit attraction between the 40S and 60S subunits, they split and then begin sedimenting more slowly as independent 40S and 60S subunits. Ultimately this combination of initial migration as an 80S followed by migration as individual subunits causes them and the associated TOP mRNA to sediment in between a normal 40S and 80S distribution.

LARP1 isoforms: While the 1019 amino acid isoform of LARP1 (ENSEMBL LARP1-201) has been referenced extensively in past literature (Philippe *et al*, 2018; Fonseca *et al*, 2015; Kozlov *et al*, 2022; Jia *et al*, 2021; Lahr *et al*, 2017; Hong *et al*, 2017), the 1096 amino acid isoform (ENSEMBL LARP1-204) appears to be the major isoform expressed in most cell types (Schwenzer *et al*, 2021). Importantly, the two constructs differ only at their disordered N-termini (amino acids 1-144 of the LARP1-204 construct), are identical at characterized domains including the La/PAM2, RBR, and DM15 domains, and are thought to be functionally equivalent. We used the 1019 amino acid isoform (LARP1-201) for expression of WT-, RBRmut-, and LPDmut constructs. However, all of our annotations and numbering throughout the manuscript correspond to the 1096 amino acid isoform (LARP1-204) to maintain consistency with the isoform that appears to be majorly expressed in human cells (Schwenzer *et al*, 2021).



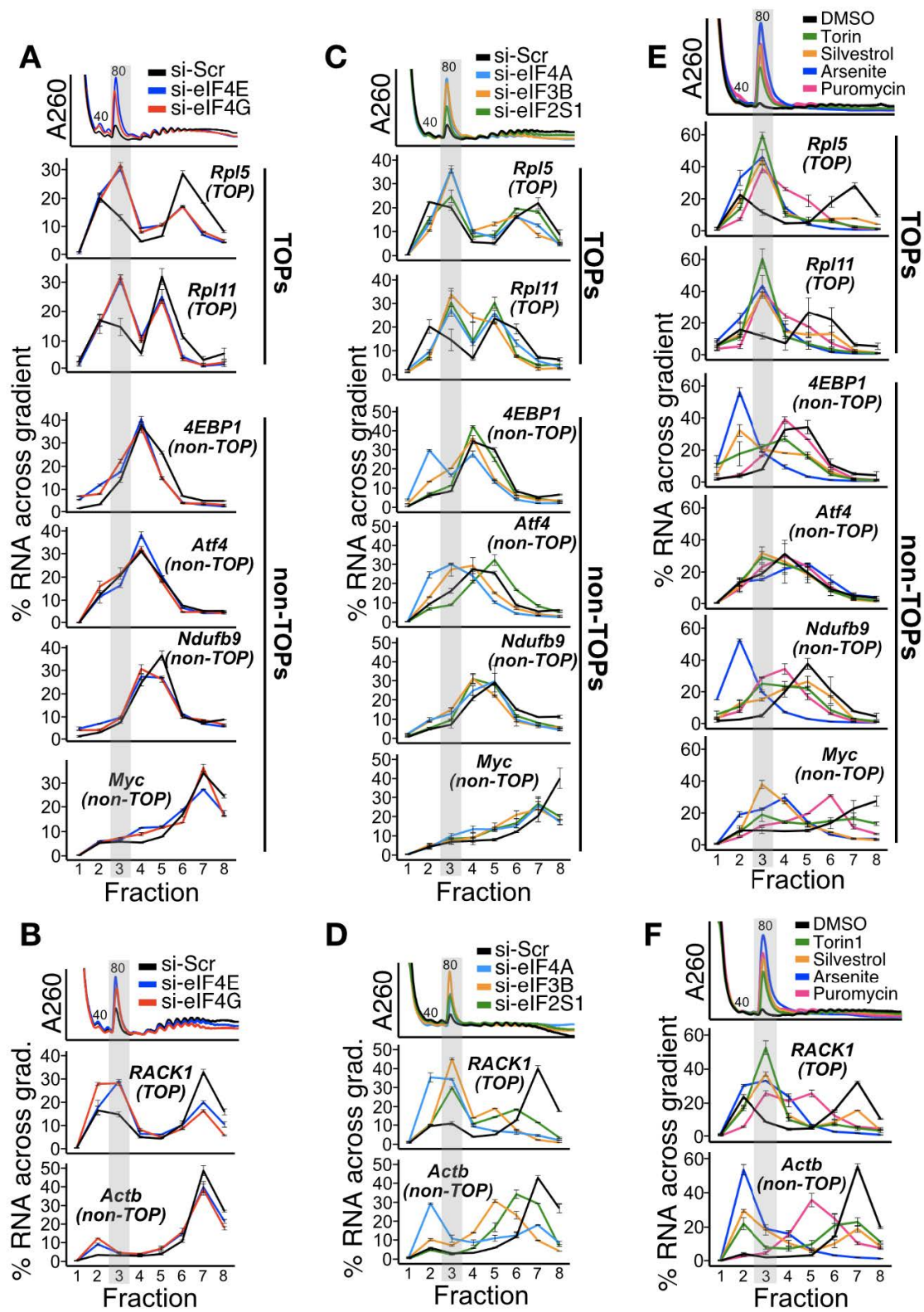
Appendix Figure S1. Analysis and validation of nanopore sequencing data.

(A) Average mRNA length from each sample of the nanopore sequencing data. Each read was weighted according to the annotated transcript length (ENSEMBL). (B) Average distribution across the gradient for mRNAs, binned by their annotated transcript length. Values are centered at the average $\pm$ SEM (lighter shade). (C) Same as (A) except that reads were weighted according to their annotated CDS length. (D) Same as (B) except that reads were binned by their annotated CDS length. (E) Same as (D), except for TOPs only. (F) qPCR for additional TOPs and non-TOPs from Fig 1C. For clarity, the A260 trace is reproduced from Fig 1C. Error bars are centered at the average and represent the SD from 2-4 technical replicates. For (A-E), only reads aligning to the ENSEMBL canonical transcript were used in the analysis. Annotated transcript and CDS lengths are for the ENSEMBL canonical transcript.



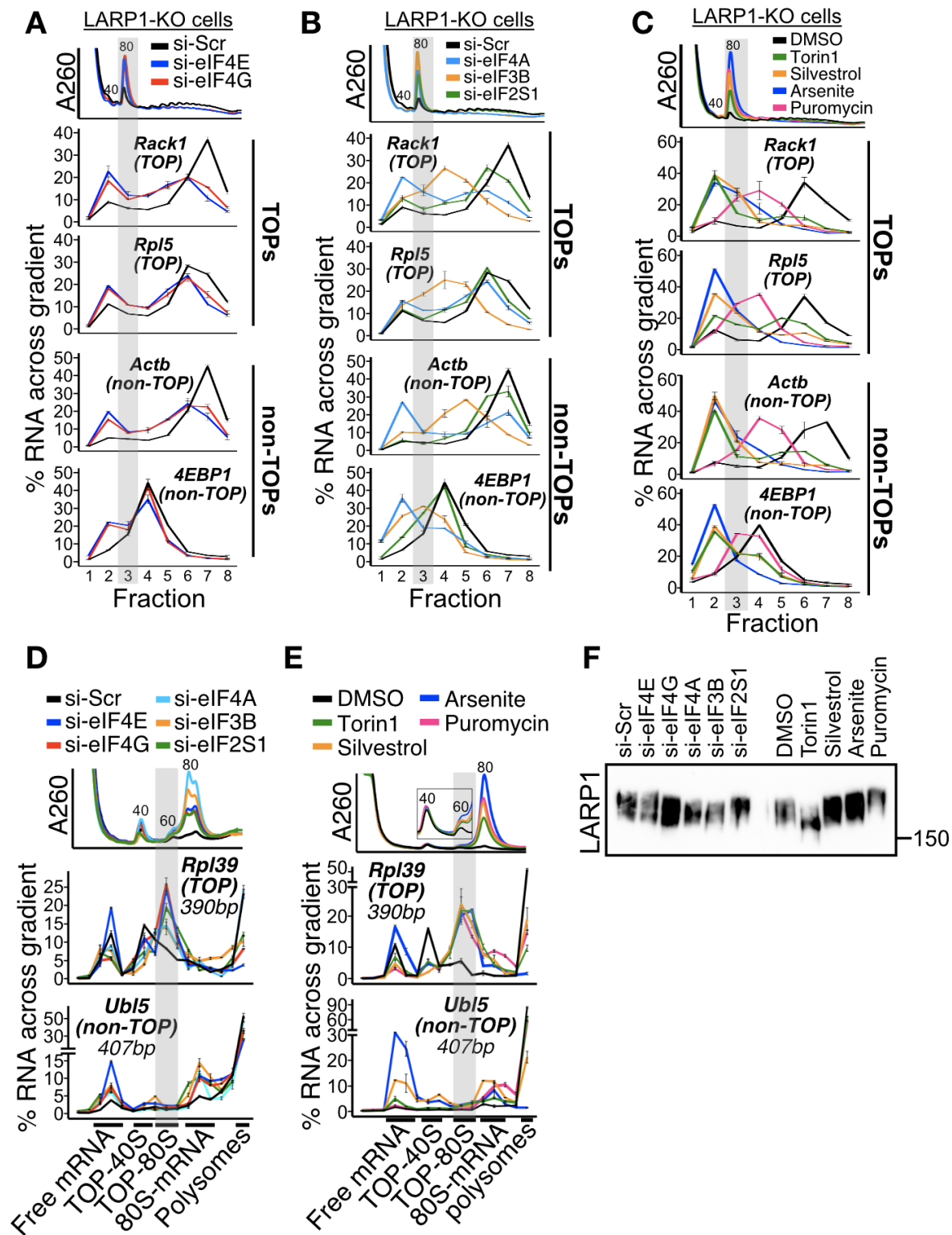
Appendix Figure S2. Characterization of the TOP-80S complex (related to Figure 2).

(A) Biological replicate experiment for Fig 2A. **(B)** WT and LARP1-KO cells (HEK293T) were treated with 300 nM Torin1 for 1 h and lysates spread along 15-35% sucrose gradients containing 100 mM KOAc followed by qPCR against genes of interest. Western blot validating the LARP1-KO is shown. **(C)** Biological replicate experiment for Fig 2B. **(D)** Biological replicate experiment for Fig 2C. **(E)** qPCR for additional genes from Fig 2C. For clarity, the A260 trace is reproduced from Fig 2C. **(F)** HEK293T cells were treated with siRNAs targeting scrambled (Si-Scr) or eIF5B for 48 hours and lysates fractionated along 15-35% sucrose gradients containing 200 mM KOAc. Equal RNA was loaded for each sample. Western blot demonstrating knockdown efficiency is shown. For (A-C), gray highlights correspond to the TOP-80S. For (D-E), green, orange, and blue highlights correspond to free mRNA, the TOP-40S and the TOP-80S, respectively. For qPCR plots, error bars are centered at the average and represent the SD from 2-4 technical replicates.



Appendix Figure S3. Increases in free ribosomes drive TOP- 80S formation (related to Figure 3).

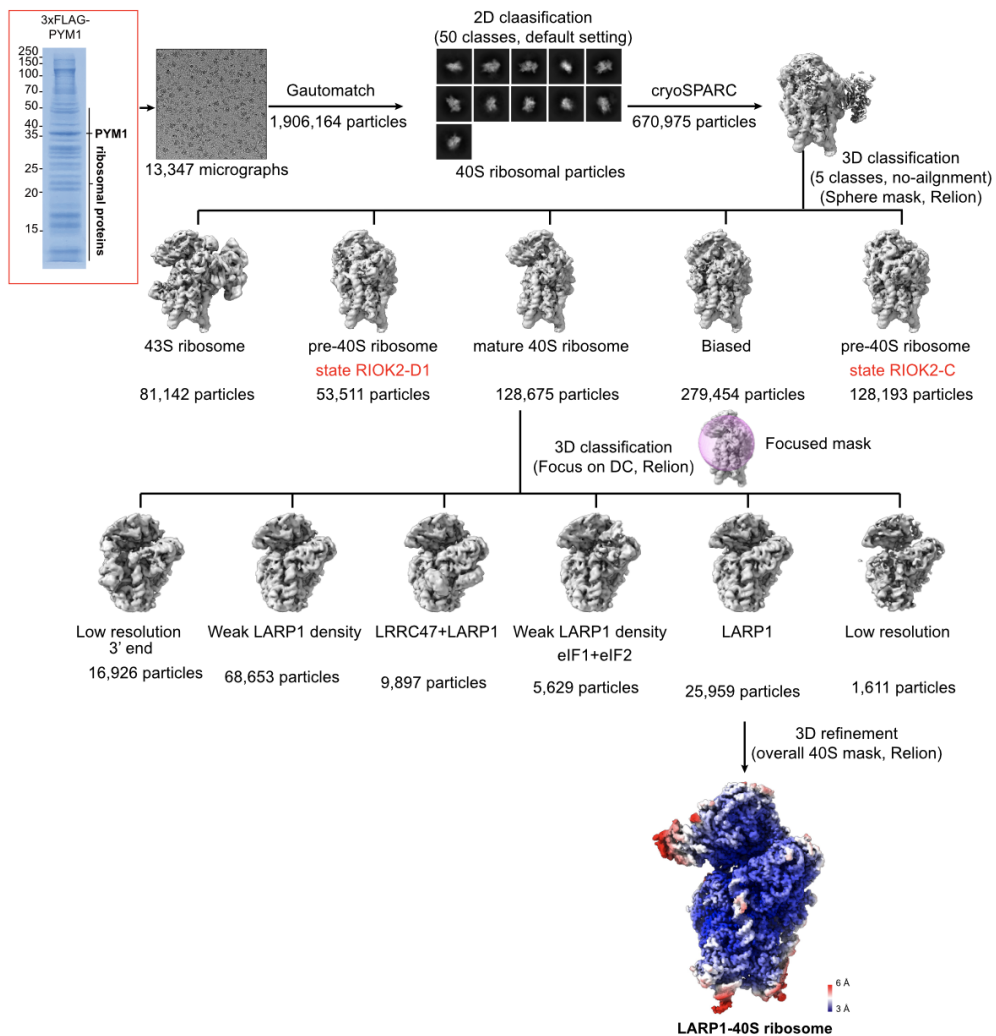
(**A-B**) qPCR for additional TOPs and non-TOPs (A) and biological replicate experiment (B) for Fig 3A. (**C-D**) qPCR for additional TOPs and non-TOPs (C) and biological replicate experiment (D) for Fig 3B. (**E-F**) qPCR for additional TOPs and non-TOPs (E) and biological replicate experiment (F) for Fig 3C. For (A-F), gray highlights correspond to the TOP-80S. For qPCR plots, error bars are centered at the average and represent the SD from 2-4 technical replicates. For (A, C, and E), A260 traces are reproduced from their respective main figures for clarity.



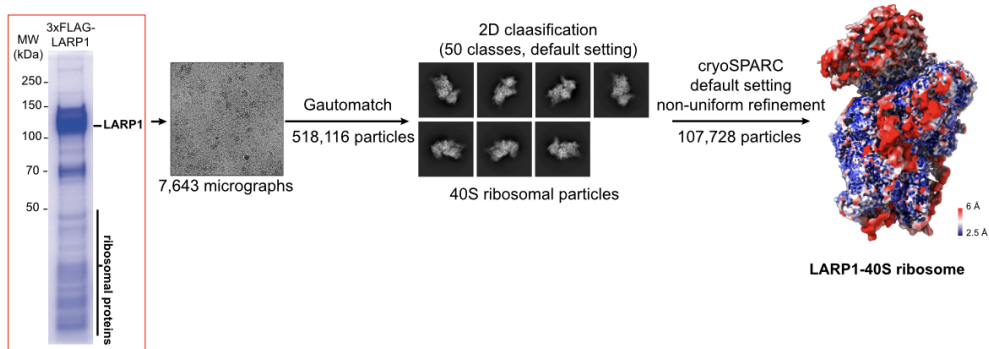
Appendix Figure S4. TOP-80S complexes are LARP1-dependent and weakly-associated.

(A) LARP1-KO cells were treated with siRNAs targeting scrambled (si-Scr), eIF4E, or eIF4G and lysates fractionated along 10-50% sucrose gradients followed by qPCR against genes of interest. (B) Identical to (A) except with siRNAs targeting scrambled (si-Scr), eIF4A1/2, eIF3B, or eIF2S1. (C) Identical to (A) except cells were treated with Torin1 (300 nM, 1 h), Silvestrol (30 nM, 1 h), Sodium arsenite (Arsenite; 100 μ M, 1 h), or Puromycin (250 μ M, 30 min). (D) WT HEK293T cells were treated with siRNAs targeting scrambled (si-Scr), eIF4E, eIF4G, eIF4A1/2, eIF3B, or eIF2S1 and lysates fractionated along 15-35% sucrose gradients containing 200 mM KOAc (High-K⁺) followed by qPCR against genes of interest. (E) Identical to (C) except cells were treated with Torin1 (300 nM, 1 h), Silvestrol (30 nM, 1 h), Sodium arsenite (Arsenite; 100 μ M, 1 h), or Puromycin (250 μ M, 30 min). (F) Biological replicate experiment for Fig 3D. For (A-E), gray highlights correspond to the TOP-80S fraction. For qPCR plots, error bars are centered at the average and represent the SD from 2-4 technical replicates.

A

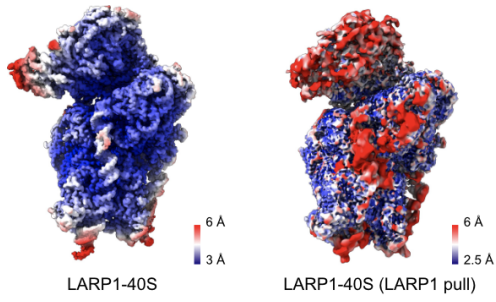
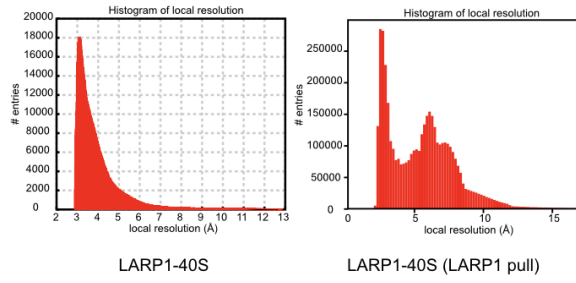
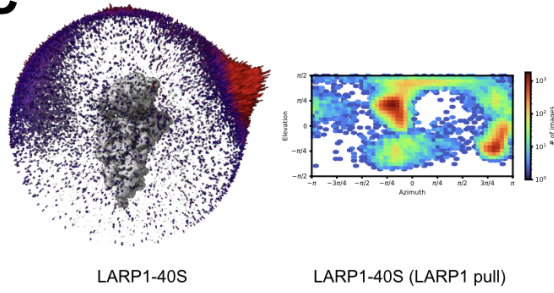
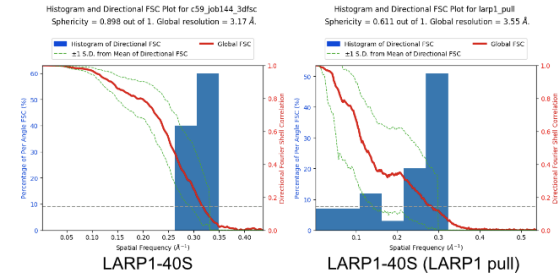
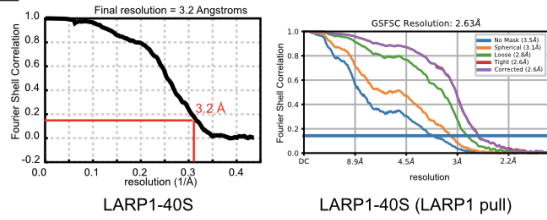
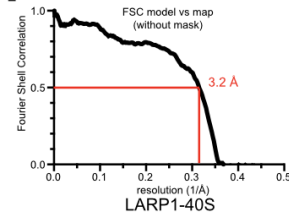


B



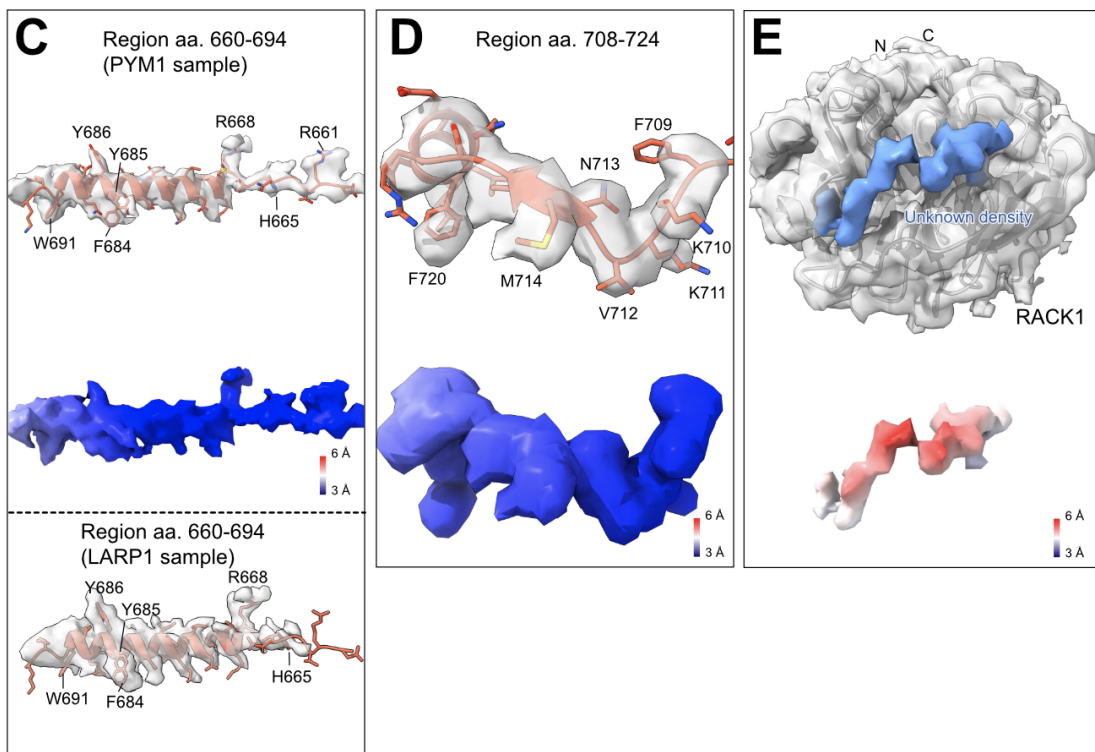
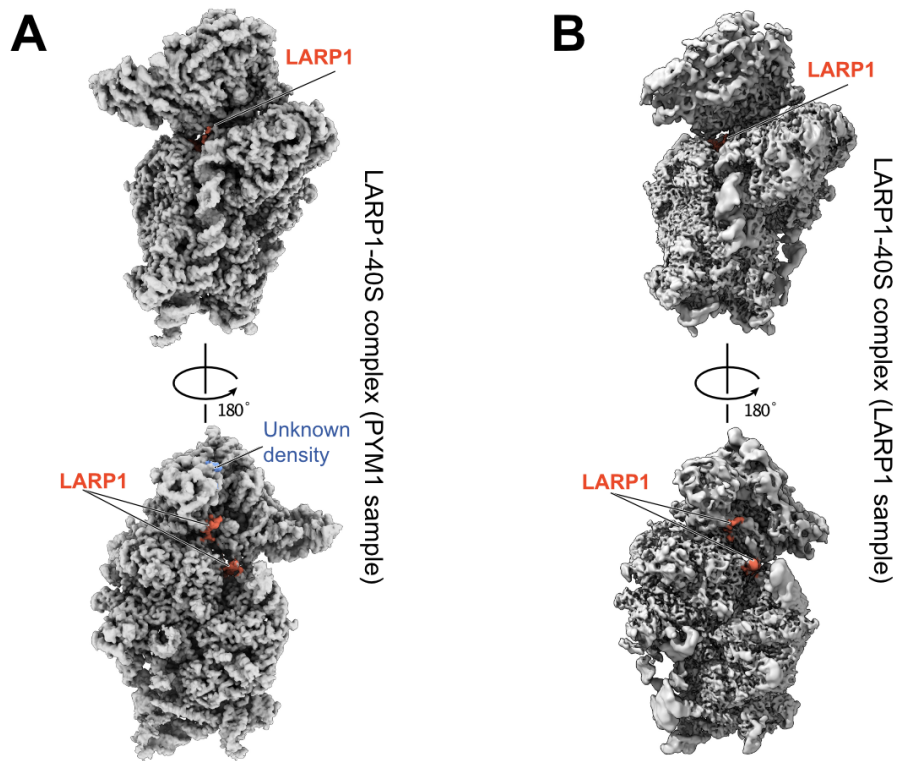
Appendix Figure S5. The sorting scheme of the cryo-EM dataset.

(A-B) Sorting schemes of the cryo-EM datasets from the PYM1 (A) and LARP1 (B) immunoprecipitation samples. Masks and software used during data processing are shown. Maps are colored according to their local resolution distribution.

A**B****C****D****E****F**

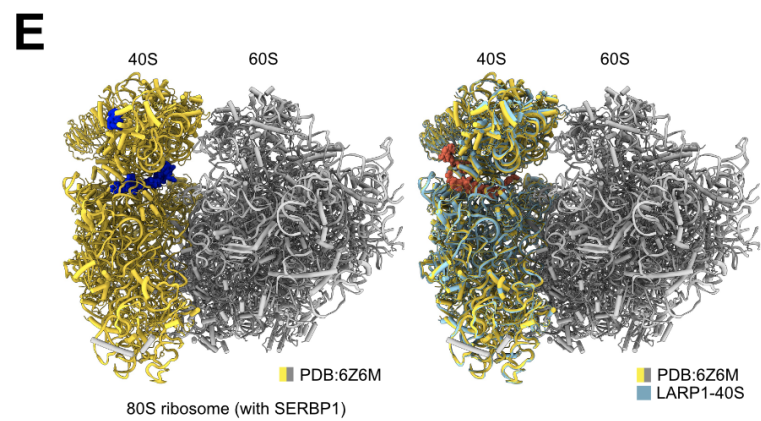
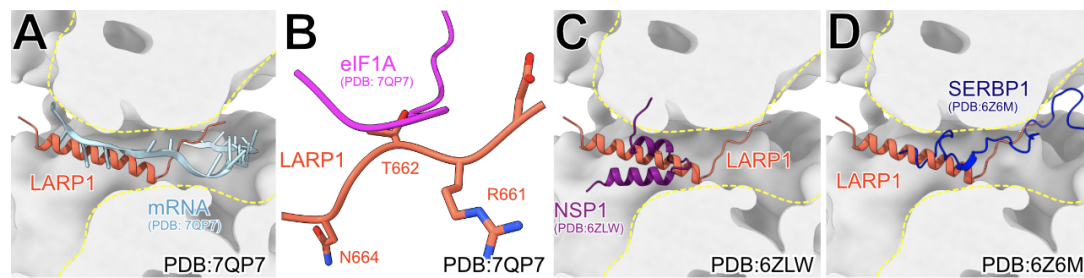
Appendix Figure S6. Structural analysis and validation of the LARP1-40S structures.

(A) Cryo-EM maps of the LARP1-40S ribosomes from the PYM1 (left) and LARP1 (right) samples, colored according to their local resolution estimation. (B-C) Local resolution distribution plots (B) and angular distributions of the particles (C) used for the final LARP1-40S (left) and LARP1-40S (LARP1 pull, right) reconstructions. The LARP1-40S plots were generated using Relion, while the LARP1-40S (LARP1 pull) plots were generated using cryoSPARC. (D) Corresponding 3D Fourier Shell Correlation (FSC) curves calculated in 3DFSC. (E-F) Standard FSC curves of the maps (E) and model-to-map correlation curves (F) calculated in Relion. Resolutions were estimated using either the 0.143 or 0.5 cutoff criterion (red dotted line).



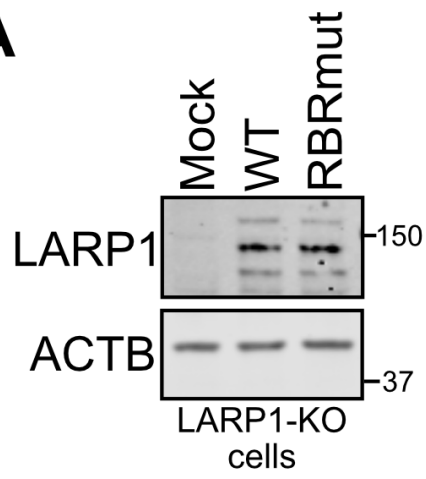
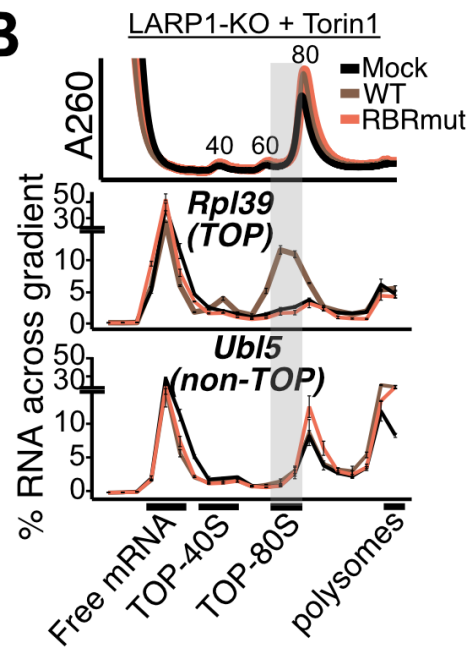
Appendix Figure S7. Detailed analysis of the interaction between LARP1 and the 40S ribosome.

(A-B) Two different views of the density maps of the LARP1-40S ribosome structures obtained from the PYM1 sample (A) and the LARP1 sample (B). The maps are either filtered by DeepEMhancer (A) or cryoSPARC (B) according to their local resolution estimation. LARP1 (tomato red) is indicated. (C-E) Molecular models for LARP1 residues 660-694 (C), 708-724 (D), and the unknown density on RACK1 (E) are shown with density maps to support the assignment. The density map for region 660-694 is derived from the unsharpened map. Their relative maps are colored according to the local resolution estimated by Relion. Region 660-694 from the structure obtained from the LARP1 sample is also shown (C, bottom)



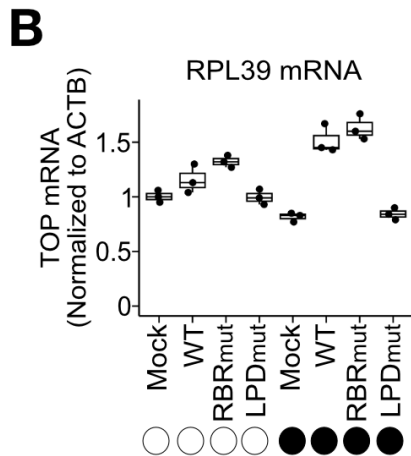
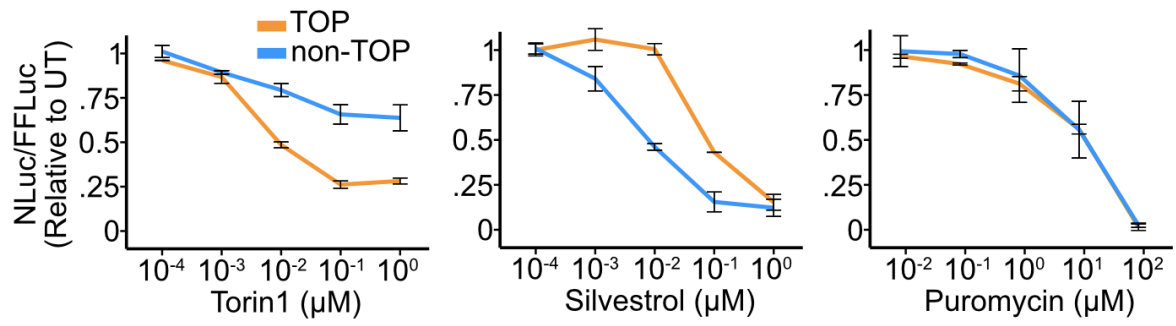
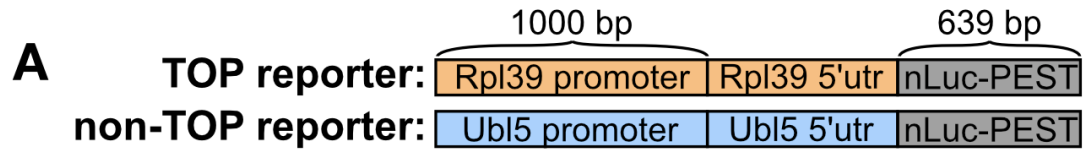
Appendix Figure S8. The LARP1 RBR occludes mRNA binding.

(A-B) Structural comparison of the LARP1-40S ribosome with the 48S initiation complex (PDB: 7QP7). The LARP1 RBR spatially clashes with mRNA in the mRNA channel (A) and with eIF1A near the decoding center (B). (C-D) Structural comparison of the LARP1 RBR with NSP1 (C) and SERBP1 (D) when bound in the mRNA channel. (E) Structural comparison of SERBP1-80S and LARP1-40S structures. Left: overall structure of the SERBP1-80S complex (PDB: 6Z6M). SERBP1 is colored in blue, while the 40S and 60S subunits are colored in yellow and gray, respectively. eEF2 and E-tRNA are not shown. Right: superimposition of the LARP1-40S structure (40S: light blue) with the SERBP1-80S structure (40S: yellow; 60S: gray), indicating that LARP1 does not spatially impede 80S formation. LARP1 is colored in tomato red.

A**B**

Appendix Figure S9. RBRmut-LARP1 does not form the TOP-80S.

(A) Biological replicate experiment for Fig 5B showing equal protein expression of WT- and RBRmut-LARP1 constructs expressed in LARP1-KO cells. (B) Biological replicate experiment for Fig 5C showing RBRmut-LARP1 is incapable of forming the TOP-40S or TOP-80S complexes. Gray highlights correspond to the TOP-80S fraction. Error bars are centered at the average and represent the SD from 2-4 technical replicates.



Appendix Figure S10. Free ribosomes do not drive TOP repression or stabilization.

(A) Top: Schematic (reproduced from Fig 6A) showing reporter constructs. FFLuc expressed from a CMV promoter on the same plasmid served as a transfection control. Bottom: NLuc-PEST expression (normalized to FFLuc) from HEK293T cells treated with the indicated treatments at the indicated concentrations for 90 minutes. Values were normalized to untreated. Error bars are centered at the average and represent the SD of three biological replicate experiments (B) Steady state mRNA levels (qPCR) from three biological replicates for RPL39 normalized to ACTB mRNA. Values were normalized to the mock-transfected, untreated sample. Torin1 treatment was 300 nM for 24 hours. For box plots: whiskers represent minima and maxima; bounds of box represent the quartiles (25th and 75th percentile); center line represents the median.

Appendix Table S1. Cryo-EM data collection, refinement, and validation statistics.

	LARP1+40S ribosome (PDB 8XP2) (EMDB 38548)	LARP1+40S ribosome (LARP1 pull) (EMDB 38550)
Data collection and Processing (for each dataset)		
Microscope	Titan Krios G4	Titan Krios G4
Voltage (keV)	300	300
Camera	Falcon IV	Falcon IV
Magnification	105,000	130,000
Pixel size at detector (Å/pixel)	1.146	0.932
Total electron exposure (e ⁻ /Å ²)	~ 50	~58
Exposure rate (e ⁻ /pixel/sec)	~ 12	~12
Number of frames collected during exposure	1737 (EER format)	1080 (EER format)
Defocus range (μm)	-1 to -2.5	-1 to -2.5
Phase plate (if used)	N/A	N/A
- phase shift range (in degrees)		
- number of images per phase plate position		
Automation software (EPU, SerialEM or manual)	EPU 2	EPU 2
Tilt angle (if grid was tilted)	N/A	N/A
Energy filter slit width (if used)	20 eV	20 eV
Micrographs collected (no.)	15,497	7,768
Micrographs used (no.)	13,347	7,643
Total extracted particles (no.)	1,906,164	518,116
For each reconstruction:		
Refined particles (no.)	25,959	107,728
Final particles (no.)	25,959	107,728
Point-group or helical symmetry parameters	C1	C1
Estimated error of translations/rotations (if available)	N/A	N/A
Resolution (global, Å)	9.3/3.9	8.2/3.0
FSC 0.5 (unmasked/masked)	4.7/3.2	3.5/2.6
FSC 0.143 (unmasked/masked)	2.9-13	2.5-15
Resolution range (local, Å)	2.9-3.6	2.2-13
Resolution range due to anisotropy (Å)	-66	N/A
Map sharpening B factor (Å ²)	Local resolution	N/A
Map sharpening methods	filter	
Model composition (for each model)		N/A
Protein	4,957	
Ligands	2	
RNA	1,740	
Model Refinement (for each model)		
Refinement package	Phenix	

- real or reciprocal space	Real
- resolution cutoff	3.2
Model-Map scores	
-CC	0.78
- Average FSC	0.70
<i>B</i> factors (Å ²)	72.98
Protein residues	71.54
Ligands	120.53
RNA	74.52
R.m.s. deviations from ideal values	
Bond lengths (Å)	0.003
Bond angles (°)	0.786
Validation (for each model)	
MolProbity score	1.63
CaBLAM outliers	2.92
Clashscore	5.87
Poor rotamers (%)	0.05
C-beta deviations (%)	0.02
EMRinger score (if better than 4 Å resolution)	2.39
Ramachandran plot	
Favored (%)	95.61
Outliers (%)	0.10

Appendix References

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