

**Molecular mechanism of the RNA helicase DHX37 and its activation by
UTP14A in ribosome biogenesis**

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SUPPLEMENTAL MATERIAL

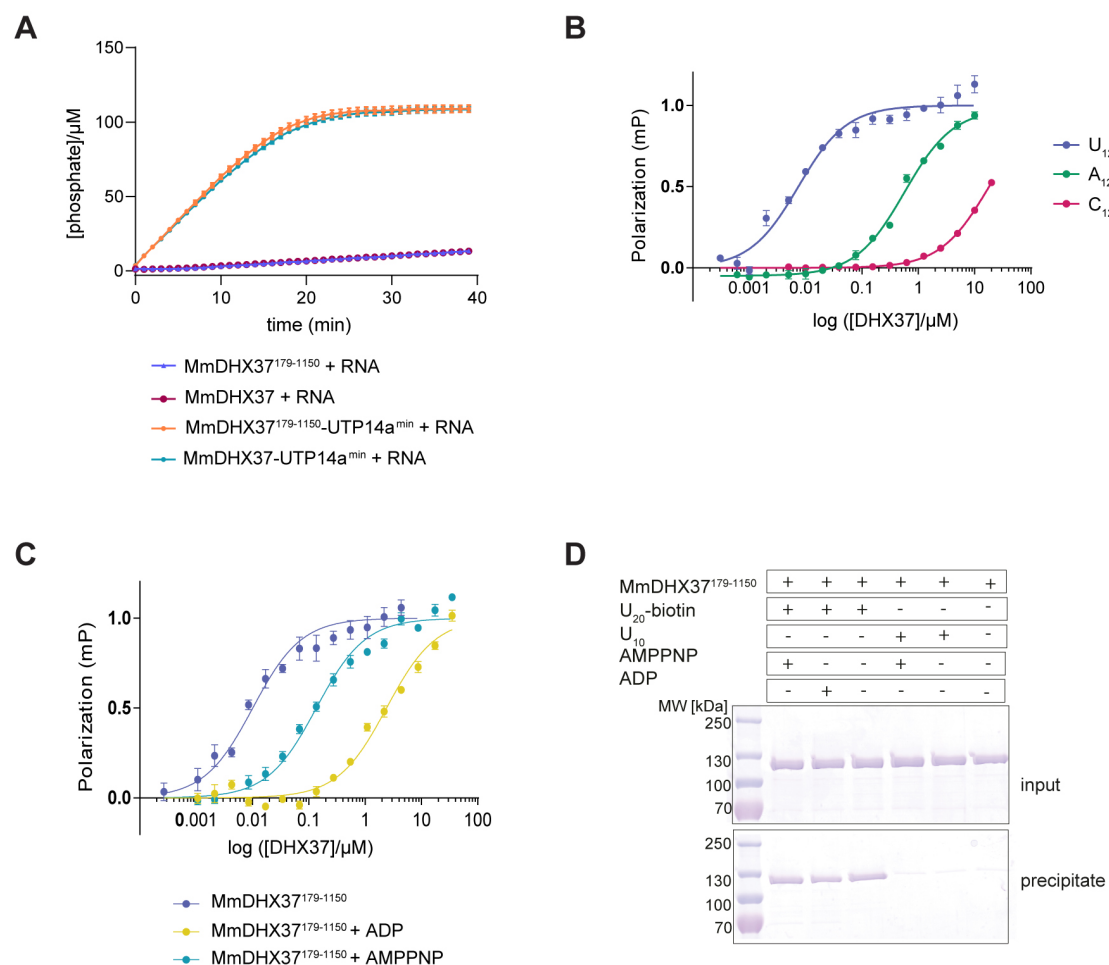


Figure S1. ATPase activity and RNA binding analysis of DHX37.

(A) RNA- and UTP14A-dependent ATPase activities of full-length MmDHX37 and the amino-terminally truncated MmDHX37¹⁷⁹⁻¹¹⁵⁰ construct used for crystallization and structure determination. Initial reaction rates are provided in Supplemental Table S1. (B) Fluorescence polarization RNA binding assays with MmDHX37¹⁷⁹⁻¹¹⁵⁰ and ATTO532 fluorophore-labeled U₁₂, A₁₂ and C₁₂ RNA nucleotides. (C) Fluorescence polarization RNA binding assays with MmDHX37¹⁷⁹⁻¹¹⁵⁰ and ATTO532 fluorophore-labeled U₁₂ RNA nucleotides in the absence of adenosine nucleotides and in the presence of ADP or the non-hydrolyzable ATP analog AMPPNP. (D) Pull-down experiment using biotin-labeled U₂₀ RNA immobilized on streptavidin beads as bait and purified MmDHX37¹⁷⁹⁻¹¹⁵⁰ as prey. Unlabeled U₁₀ RNA was used as a competitor.

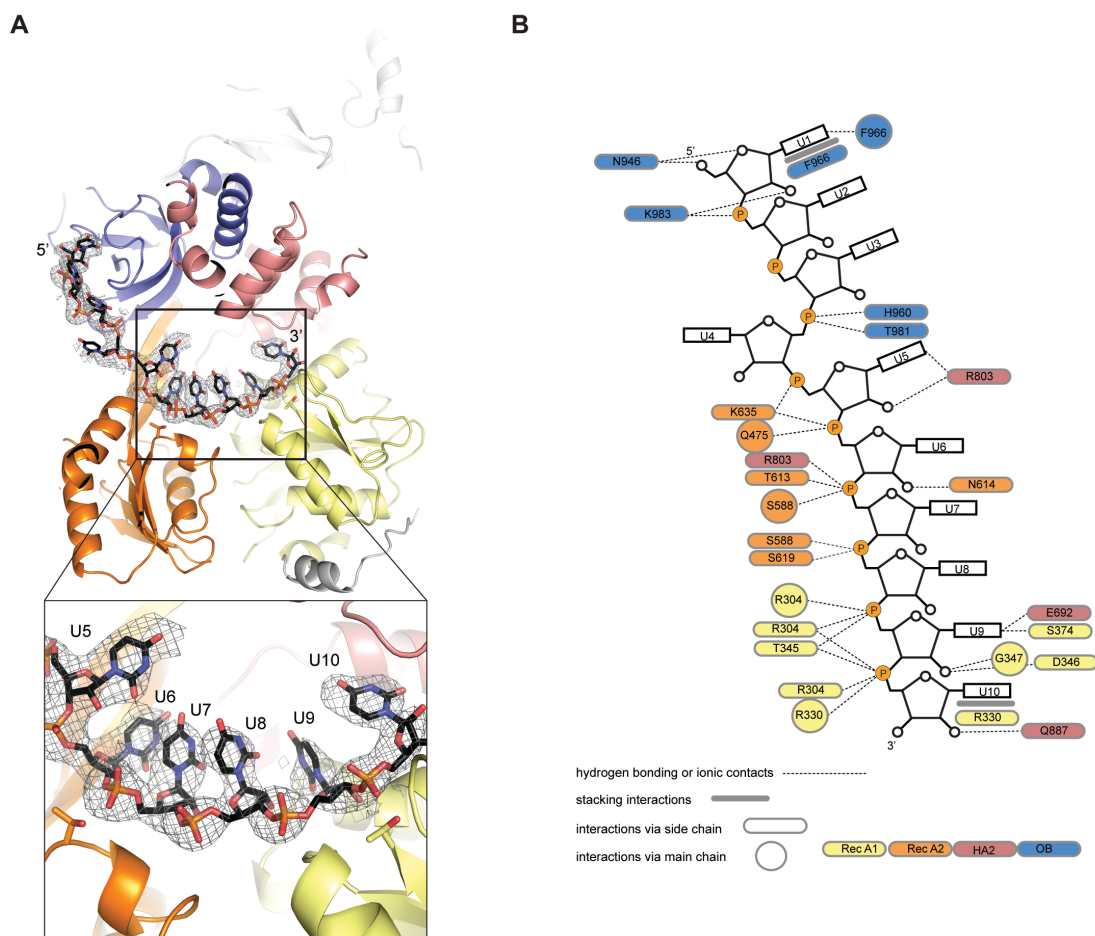


Figure S2. Structural details of RNA binding by DHX37.

(A) Top: Composite omit map of the electron density corresponding to U₁₀ RNA bound to MmDHX37¹⁷⁰⁻¹¹⁵⁰, superimposed with the final refined model. The map was calculated using Phenix (Adams et al. 2010) and is contoured at 1.0 s and displayed within 2.4 Å of the RNA. Bottom: Zoom-in view of the RNA binding channel in DHX37. (B) Schematic diagram of DHX37-RNA interactions. Hydrogen bonding and ionic interactions within 4 Å are indicated with dashed lines. Grey bars indicate aromatic stacking interactions. The amino acid residues are colored according to the color scheme in Fig. 1A.

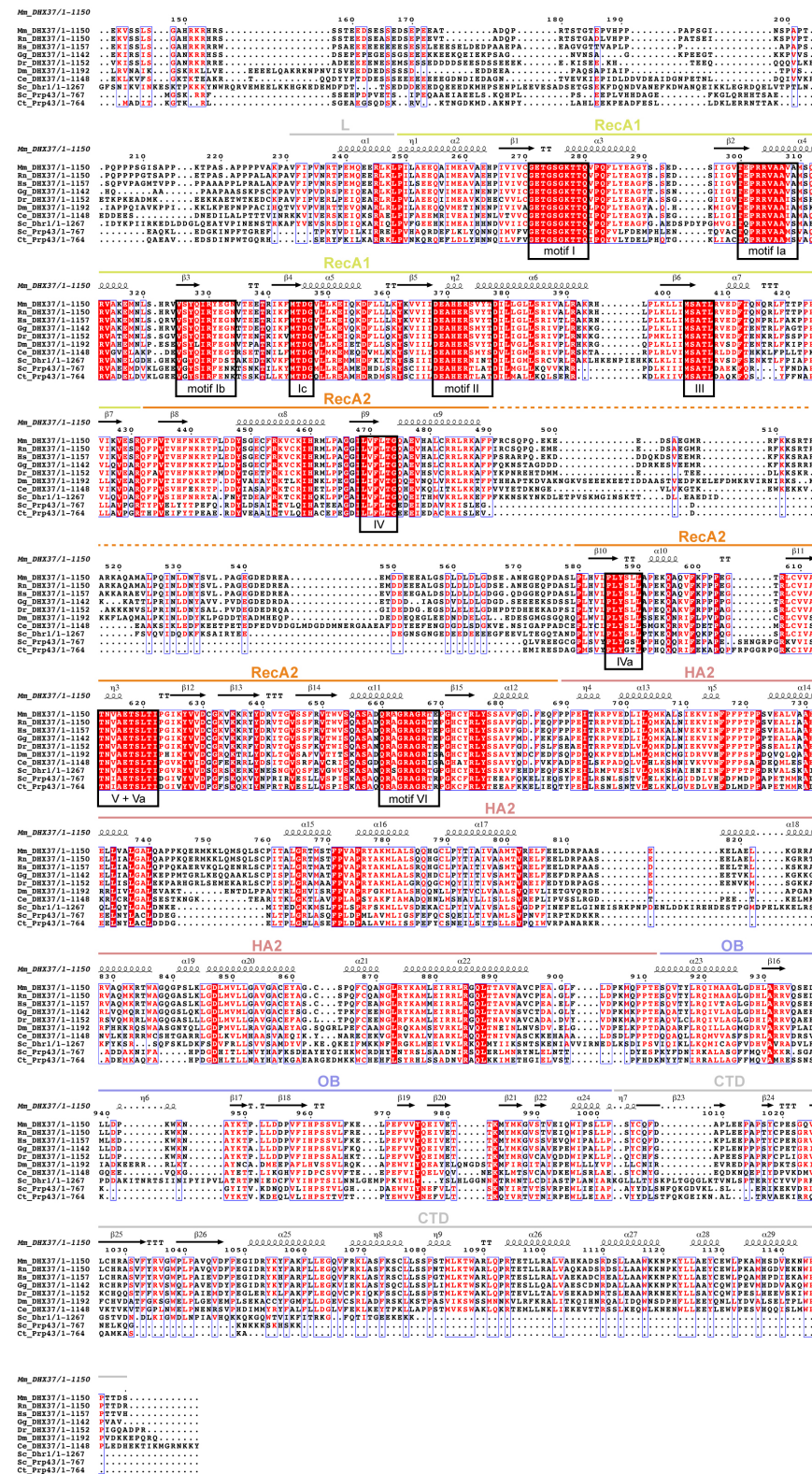


Figure S3. Multiple sequence alignment of DHX37 and Prp43 orthologs.

The alignment was generated with Clustal Omega (Sievers et al. 2011) and visualized using Espritt (Gouet et al. 1999). The secondary structure of MmDHX37 is depicted above the sequence and colored according to the scheme in Fig. 1A.

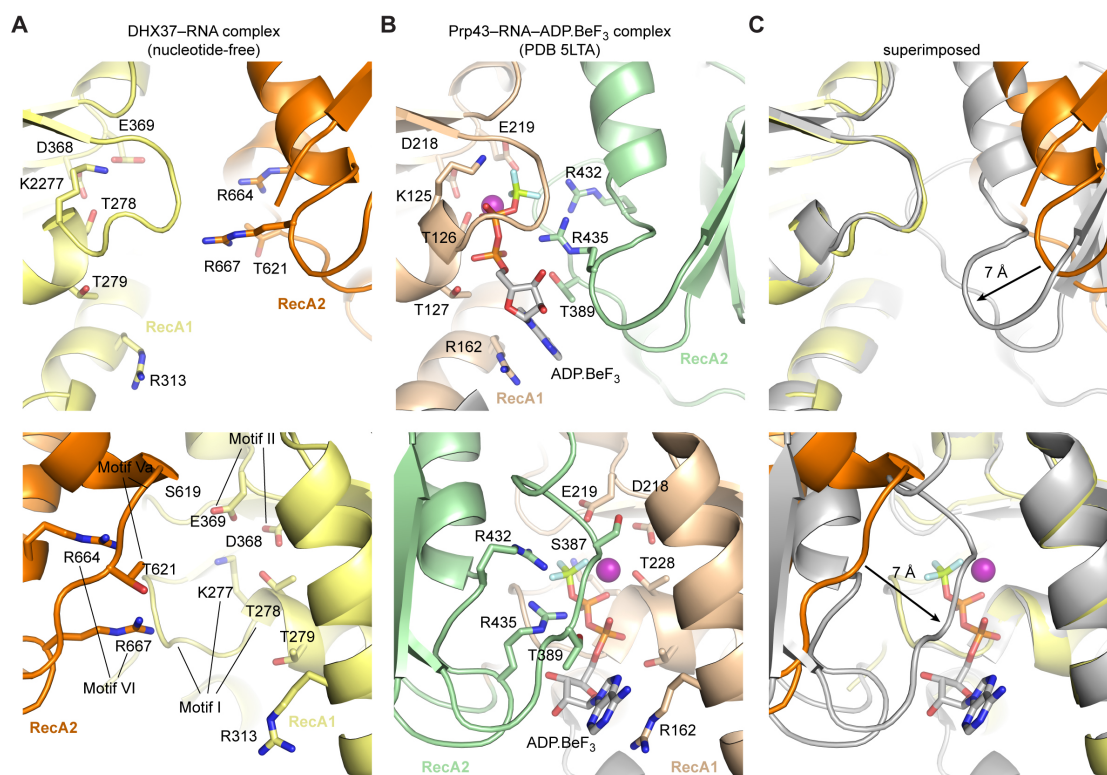


Figure S4. The ATPase active site in the DHX37–RNA complex adopts an open conformation.

(A) Top and Bottom: Zoom-in views of the ATPase active site in the MmDHX37^{179–1150}–U₁₀ RNA complex. Amino acid residues involved in ATP binding are shown in stick format. (B) Zoom-in views of the ATPase active site in *Chaetomium thermophilum* Prp43 in complex with U₇ RNA and the transition state mimic ADP.BeF₃[–]. Amino acid residues involved in ADP.BeF₃[–] binding are shown in stick format; the Mg²⁺ ion is shown as purple sphere. (C) Superposition of the active sites in the DHX37 and Prp43 complexes. The helicases were superimposed on their RecA1 domains. The arrow indicates a 7 Å displacement of the ATP binding motif Va in the nucleotide-free conformation relative to the ATP-bound state.

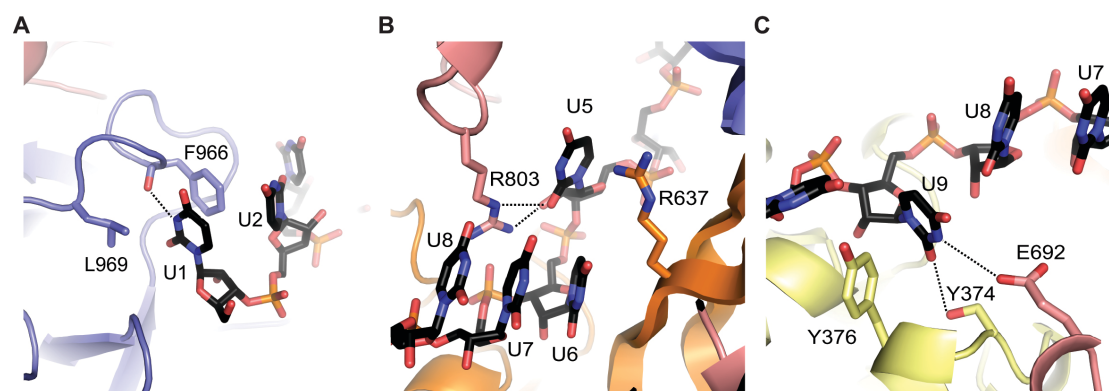


Figure S5. RNA base interactions in the DHX37-RNA complex.

Zoom-in views of interactions with RNA bases U1 (A), U5 (B) and U9 (C). Hydrogen bonding interactions are indicated with dashed lines.

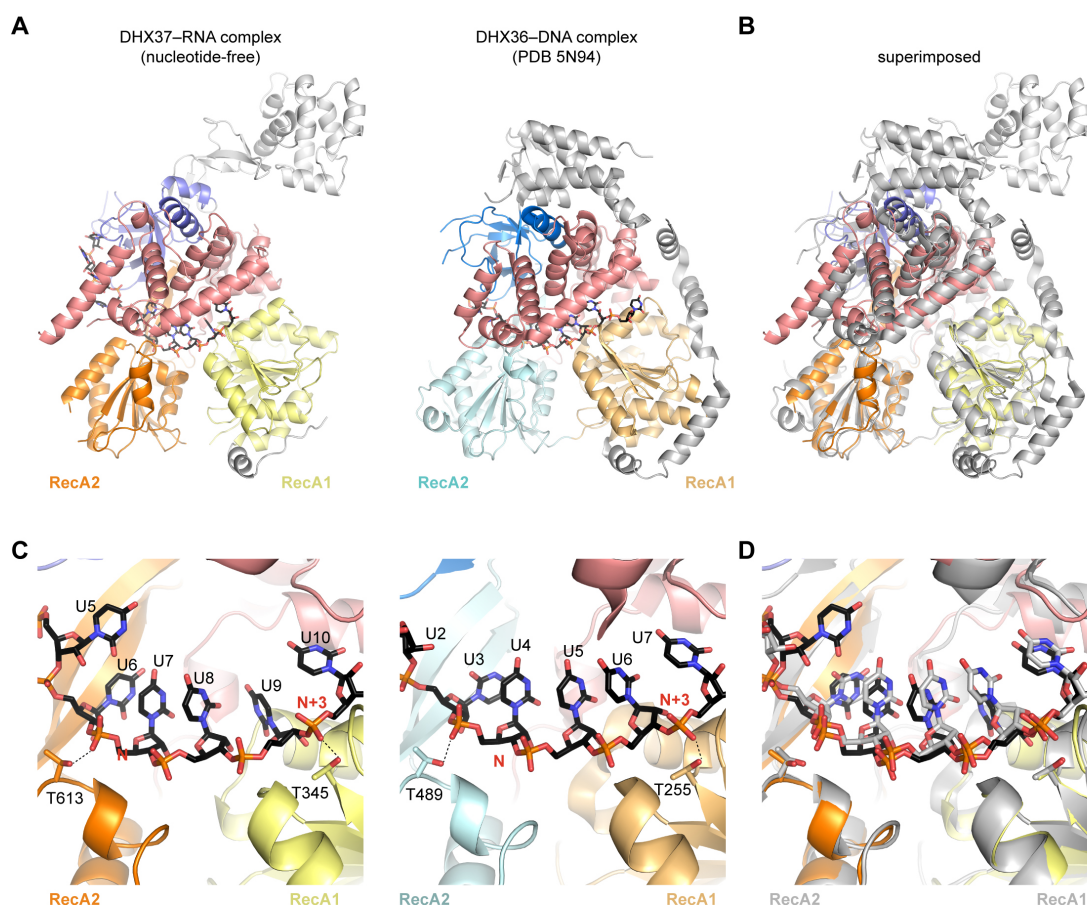


Figure S6. Structural comparisons of RNA-bound DHX37 and DHX36 helicases in their ATP-free states.

(A) Side-by-side comparison of the structures of the DHX37-RNA (left) and DHX36-polyU RNA (PDB entry 5N94, right) complexes. The helicases were superimposed using their RecA1 domains and are shown in identical orientations. (B) Superposition of DHX37 (colored as in A) and DHX36 (colored grey). (C) Side-by-side views of the RNA binding channels in the DHX37-RNA (left) and DHX36-RNA (right) complexes. Both DHX37 and DHX36 accommodate five-nucleotide stacks (U6-U10 and U3-U7) in their ATP-free states. Motif V has been omitted from the figures to give unobstructed view of the RNA. (D) Superposition of the RNA binding channels in DHX37 (colored as in A) and DHX36 (grey).

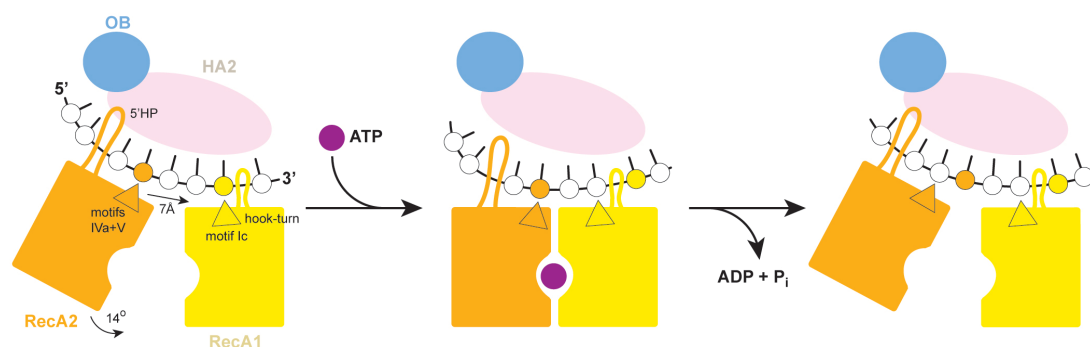
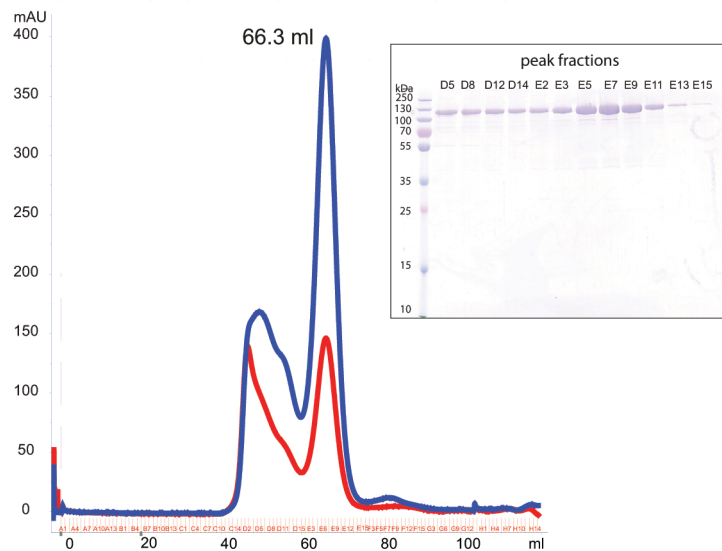


Figure S7. Mechanistic model for ATP-dependent RNA translocation of DEAH helicases

RNA translocation occurs by ATP-driven conformational cycling between open and closed states. In the nucleotide-free state, DEAH helicases adopt an open conformation, in which a five-nucleotide stack is bound within the RNA binding channel, bookended by the 5'HP and hook-turn motifs at the 5' and 3' ends, respectively. Binding of ATP at the interface of the RecA1 and RecA2 domains induces closure of the ATPase active site by a 14° rotation of the RecA2 domain, coupled with a 7 Å translational shift. By maintaining interactions with motifs IV, IVa and V and the 5'HP in RecA2, the RNA is shifted by one nucleotide past the RecA1 domain and the 3'-terminal nucleotide previously contacted by motif Ic exits past the hook-turn motif Ib. In the resulting ATP-bound state, a four-nucleotide stack is accommodated in the RNA binding channel. Upon ATP hydrolysis and ADP/Pi release, the helicase reverts to the open conformation in which motifs Ic and Ib in RecA1 remain tightly bound to the 3'-terminal part of the RNA, while the RecA2 domain undergoes a register shift and engages the next 5'-terminal nucleotide.

A



B

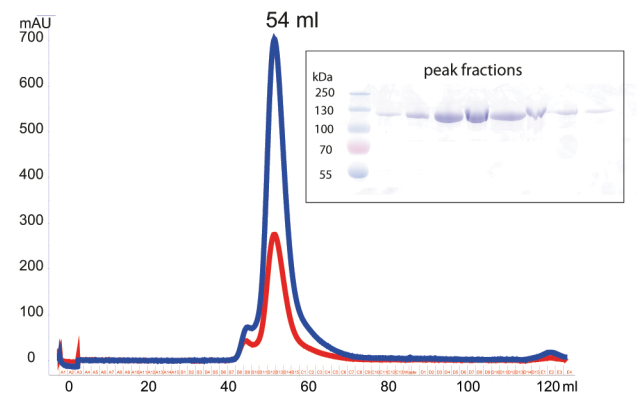


Figure S8. MmDHX37 physically interacts with human UTP14A in vitro.

(A) Size exclusion chromatography analysis of MmDHX37¹⁷⁹⁻¹¹⁵⁰ on a Superdex 200 (16/60) column. The protein elutes at a volume of 63.9 ml. Inset shows SDS-PAGE gel analyzing peak fractions. (B) Size exclusion chromatography analysis of the MmDHX37¹⁷⁹⁻¹¹⁵⁰-HsUTP14A complex on a Superdex 200 (16/60) column. The complex elutes at a volume of 54 ml. Inset shows SDS-PAGE gel analyzing peak fractions. Note that the bands corresponding to MmDHX37¹⁷⁹⁻¹¹⁵⁰ and full length HsUTP14A overlap due to the proteins having similar molecular masses.

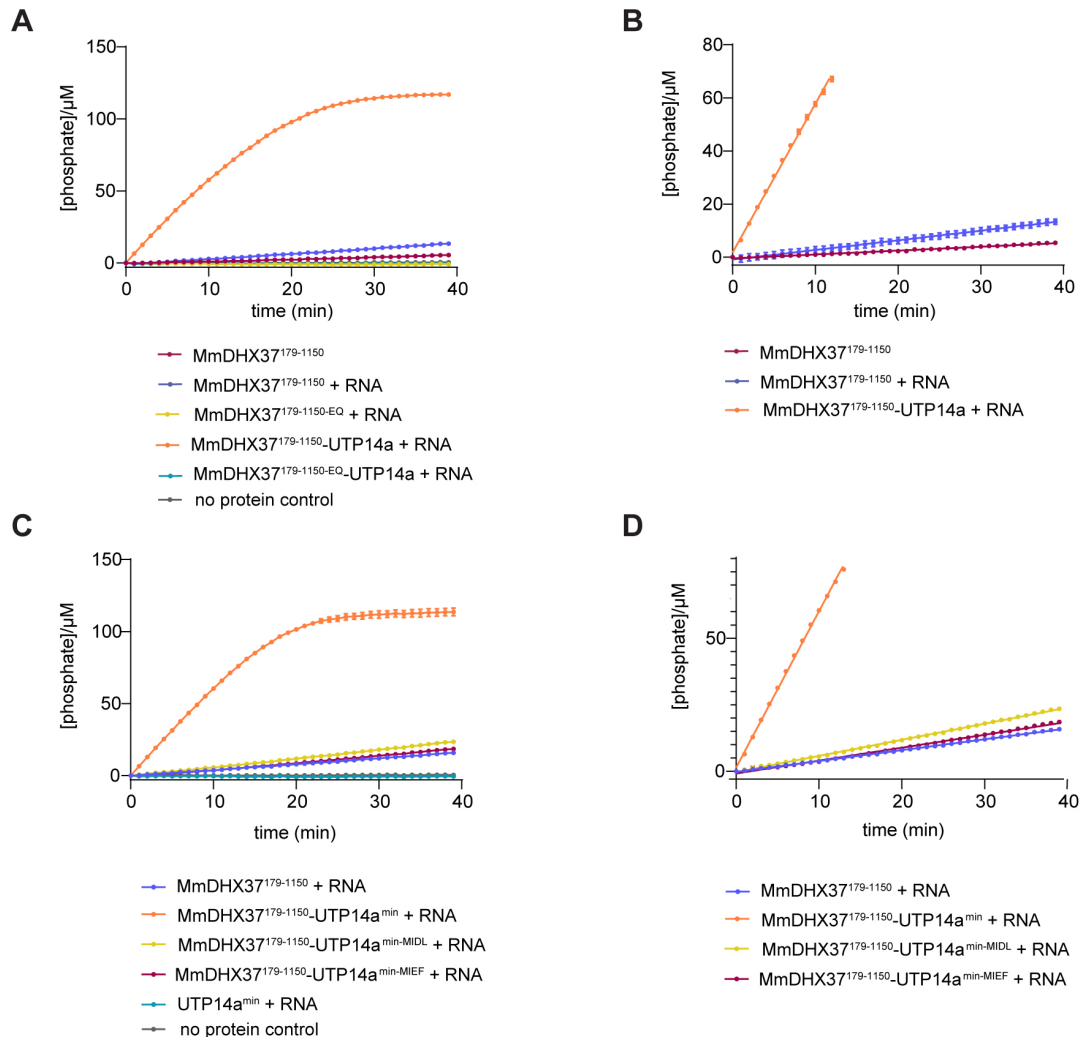


Figure S9. ATPase activities of DHX37 and the DHX37-UTP14A complex.

(A) ATPase activity of MmDHX37¹⁷⁹⁻¹¹⁵⁰ or the MmDHX37¹⁷⁹⁻¹¹⁵⁰-HsUTP14A complex in the absence or presence of U₁₀ RNA. The MmDHX37¹⁷⁹⁻¹¹⁵⁰ E369Q catalytic mutant serves as negative control. (B) Initial linear phase of a subset of the reactions in A used for the calculation of the initial reaction rates. (C) RNA-dependent ATPase activities of MmDHX37¹⁷⁹⁻¹¹⁵⁰ and the MmDHX37¹⁷⁹⁻¹¹⁵⁰-HsUTP14A^{min} wild-type and mutant complexes. (D) Initial linear phase of a subset of the reactions in C used for the calculation of the initial reaction rates.

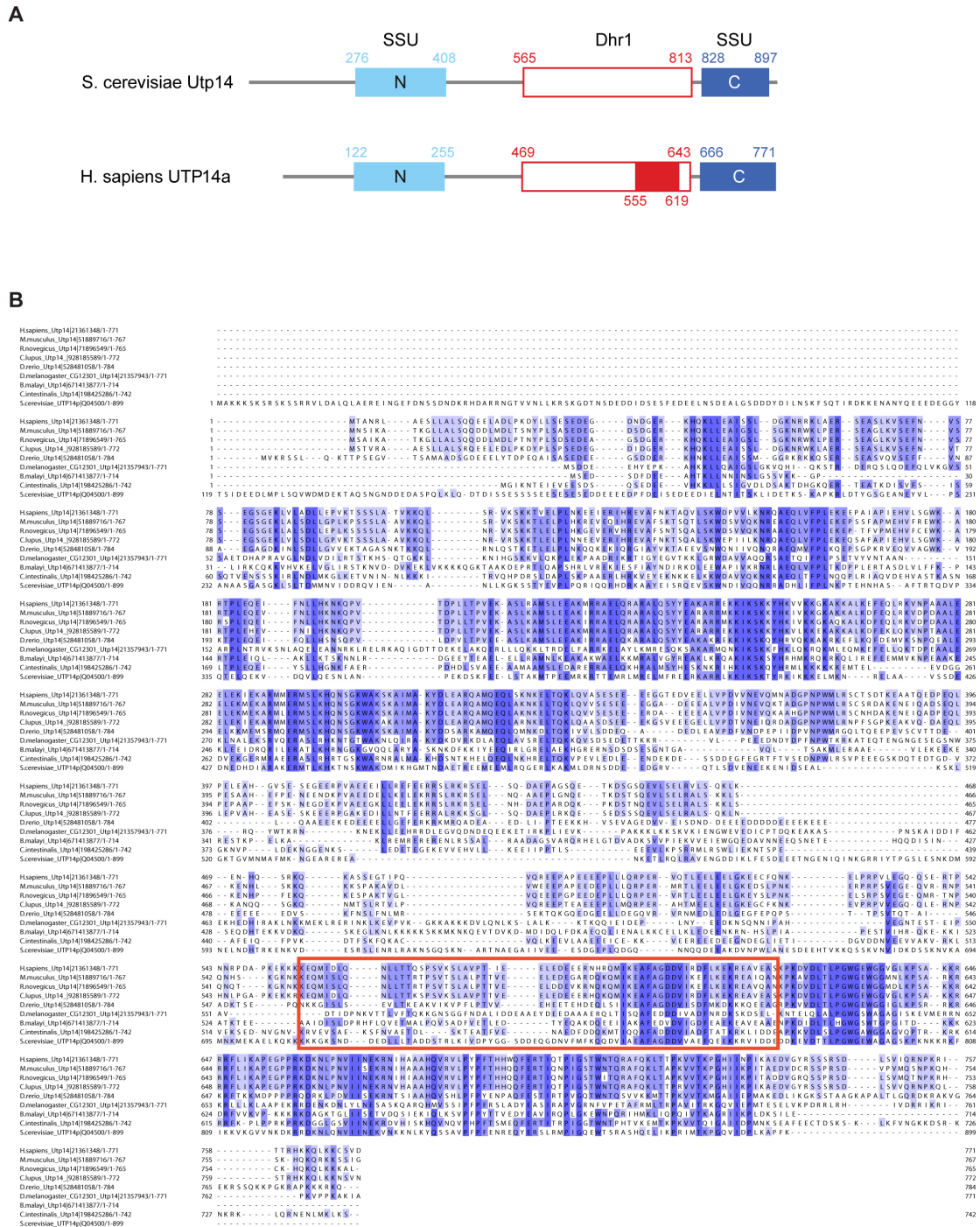


Figure S10. Multiple sequence alignment of UTP14 orthologs.

(A) Schematic diagram of human UTP14A and yeast Utp14 proteins. SSU-N and SSU-C denote the N- and C-terminal regions corresponding to the regions of Utp14 bound in the yeast SSU processome structure. Red boundary denotes region corresponding to the Dhr1 interacting region in yeast Utp14. Solid red box denotes minimal region in HsUTP14A required for interaction with DHX37. (B) Multiple sequence alignment of UTP14 orthologs. The alignment was generated using Clustal Omega (Sievers et al. 2011). Amino acid residues are colored in shades of blue according to the degree of conservation. The minimal region of UTP14A necessary and sufficient for interaction with DHX37 is indicated by a red box.

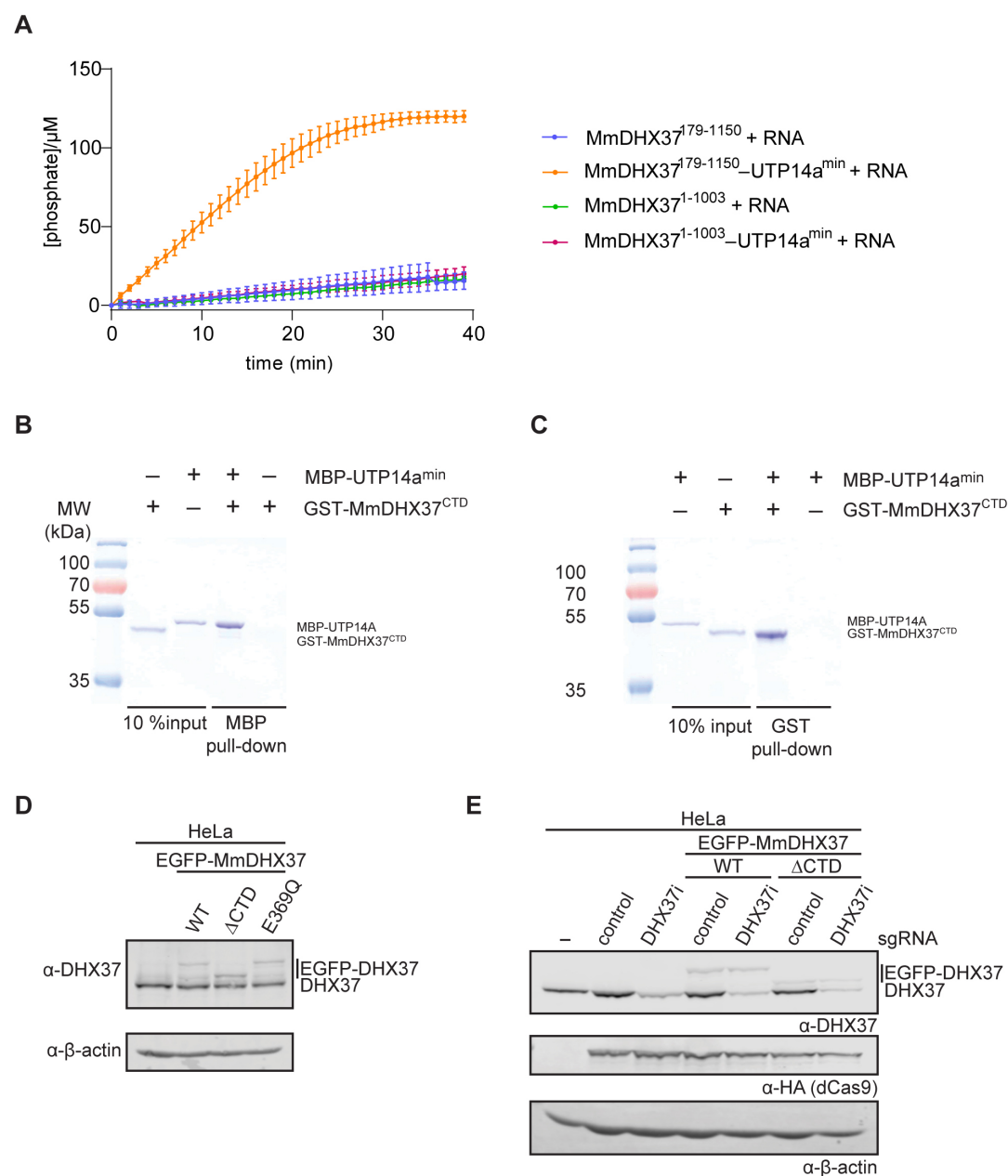


Figure S11. The C-terminal domain of DHX37 is required for interaction with UTP14A and for ribosome biogenesis.

(A) RNA- and UTP14A-dependent ATPase activities of MmDHX37¹⁷⁹⁻¹¹⁵⁰ and a truncated protein (MmDHX37¹⁷⁹⁻¹⁰⁰³) lacking the C-terminal domain (CTD). (B) Pull-down experiment using maltose-binding protein (MBP)-tagged UTP14A^{min} immobilized on amylose beads as bait and glutathione-S-transferase (GST)-tagged MmDHX37^{CTD} as prey. (C) Pull-down experiment using glutathione-S-transferase (GST)-tagged MmDHX37^{CTD} immobilized on glutathione sepharose beads as bait and MBP-tagged UTP14A^{min} as prey. (D) Expression of EGFP-tagged MmDHX37 constructs (WT, Δ CTD and E369Q) in HeLa cells used in experiment shown in Fig. 4B. Protein expression was detected by Western blotting using an anti-DHX37 antibody. (E) CRISPRi knock-down of endogenous human DHX37 and expression of EGFP-tagged MmDHX37 constructs (WT, Δ CTD) in HeLa cells used in experiment shown in Fig. 4C. DHX37 expression was detected by Western blotting using an anti-DHX37 antibody. dCas9 expression was detected by Western blotting using an anti-HA antibody.

Table S1. ATPase activities of DHX37 and DHX37–UTP14A complexes.

Enzyme	RNA	v [$\mu\text{M}/\text{min}$]	Figure
MmDHX37 (0.25 μM)	U ₁₀ (0.25 μM)	0.341 $\pm$ 0.003	Fig. S1A
MmDHX37 + UTP14A ^{min} (0.25 μM)	U ₁₀ (0.25 μM)	5.67 $\pm$ 0.066	Fig. S1A
MmDHX37 ¹⁷⁹⁻¹¹⁵⁰ (0.25 μM)	U ₁₀ (0.25 μM)	0.354 $\pm$ 0.005	Fig. S1A
MmDHX37 ¹⁷⁹⁻¹¹⁵⁰ + UTP14A ^{min} (0.25 μM)	U ₁₀ (0.25 μM)	5.914 $\pm$ 0.063	Fig. S1A
MmDHX37 ¹⁷⁹⁻¹¹⁵⁰ (0.25 μM)	–	0.146 $\pm$ 0.002	Fig. S9B
MmDHX37 ¹⁷⁹⁻¹¹⁵⁰ (0.25 μM)	U ₁₀ (0.25 μM)	0.364 $\pm$ 0.008	Fig. S9B
MmDHX37 ¹⁷⁹⁻¹¹⁵⁰ + UTP14A (0.25 μM)	U ₁₀ (0.25 μM)	5.60 $\pm$ 0.051	Fig. S9B
MmDHX37 ¹⁷⁹⁻¹¹⁵⁰ (0.25 μM)	U ₁₀ (0.25 μM)	0.409 $\pm$ 0.002	Fig. S9D
MmDHX37 ¹⁷⁹⁻¹¹⁵⁰ + UTP14A ^{min} (0.25 μM)	U ₁₀ (0.25 μM)	5.877 $\pm$ 0.037	Fig. S9D
MmDHX37 ¹⁷⁹⁻¹¹⁵⁰ + UTP14A ^{min_MIDL} (0.25 μM)	U ₁₀ (0.25 μM)	0.606 $\pm$ 0.002	Fig. S9D
MmDHX37 ¹⁷⁹⁻¹¹⁵⁰ + UTP14A ^{min_MIEF} (0.25 μM)	U ₁₀ (0.25 μM)	0.486 $\pm$ 0.003	Fig. S9D
MmDHX37 ¹⁷⁹⁻¹¹⁵⁰ (0.25 μM)	U ₁₀ (0.25 μM)	0.543 $\pm$ 0.086	Fig. S11A
MmDHX37 ¹⁷⁹⁻¹¹⁵⁰ + UTP14A ^{min} (0.25 μM)	U ₁₀ (0.25 μM)	5.144 $\pm$ 0.251	Fig. S11A
MmDHX37 ¹⁻¹⁰⁰³ (0.25 μM)	U ₁₀ (0.25 μM)	0.450 $\pm$ 0.016	Fig. S11A
MmDHX37 ¹⁻¹⁰⁰³ + UTP14A ^{min} (0.25 μM)	U ₁₀ (0.25 μM)	0.492 $\pm$ 0.036	Fig. S11A

Supplemental References

Gouet P, Courcelle E, Stuart DI, Métoz F. 1999. ESPript: analysis of multiple sequence alignments in PostScript. *Bioinformatics* **15**: 305–308.

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, et al. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology* **7**: 539–539.