Expanded View Figures

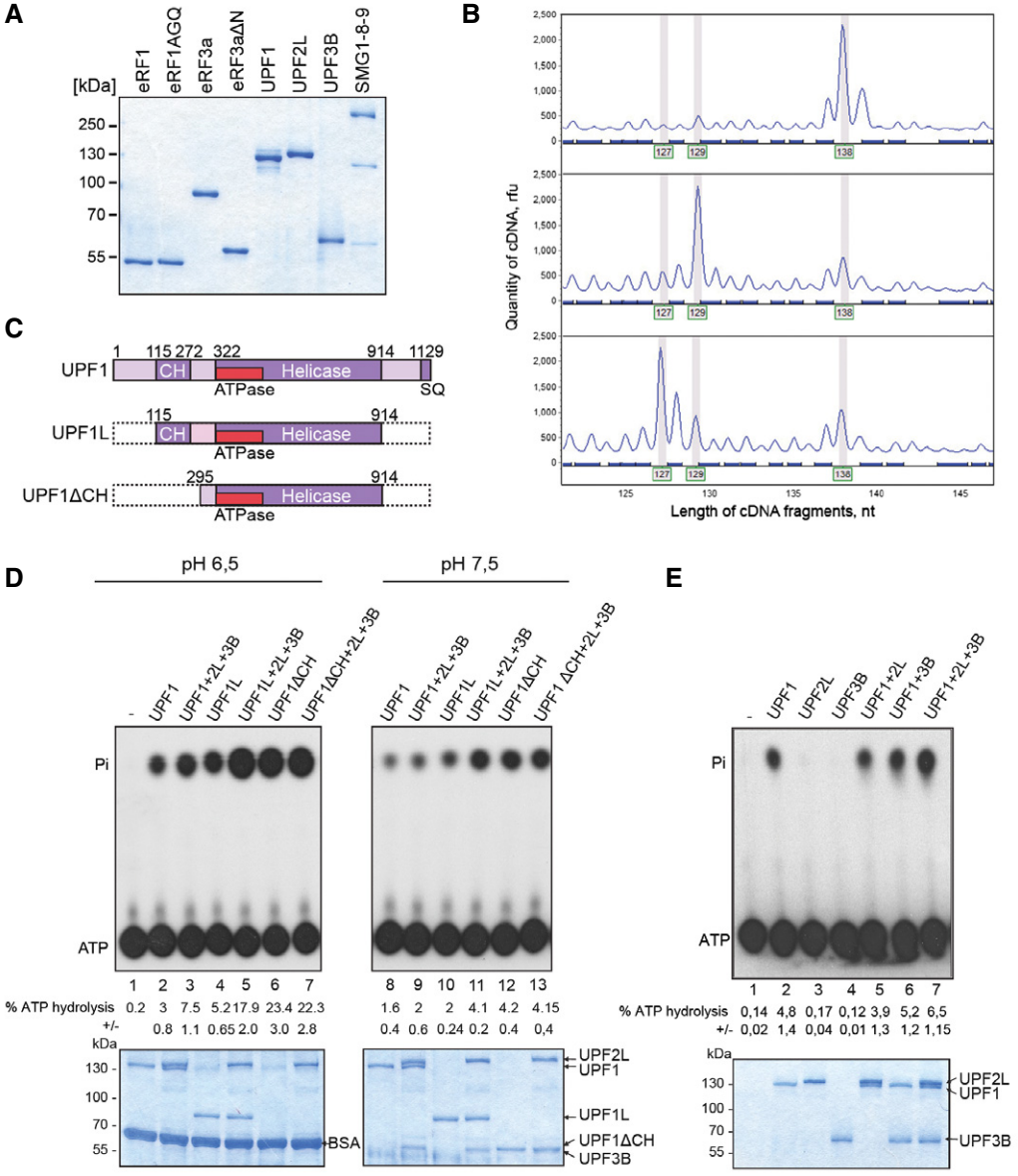

Figure EV1. Validation of the experimental system.

A SDS–PAGE analysis of purified recombinant proteins used in the toeprinting experiments as indicated.

B Toeprint analysis of translation complexes prepared by the reconstituted *in vitro* translation system. Above: 80S initiation complex; middle: pre-termination complex (pre-TC); below: termination complex formed in presence of eRF1, eRF3a and GTP. Peaks at 138 nt indicate the position of the 80S initiation complex on mRNA, peaks at 129 nt indicate the position of pre-TC and peaks at 127 nt correspond to the termination complex (post-TC). Rfu—relative fluorescence units.

C Schematic representation of UPF1 variants used in (D).

D Thin layer chromatography (TLC) analysis of the ATPase activity of UPF1 variants in the absence or presence of UPF2L and/or UPF3B at 30°C in MES buffer (pH 6.5, lanes 1–7) or translation buffer (pH 7.5, lanes 8–13), respectively. 1.5 μl of the samples was spotted on the TLC plates, and the residual 18.5 μl was analysed on SDS–PAGE gels for loading control (lower panels). The positions of γ^{32}P-ATP and γ^{32}Pi are indicated. % ATP hydrolysis in (D and E) was calculated using a phosphoimager and displays the means ± SEM of four independent experiments.

E ATP hydrolysis experiment as in (D) at 37°C in translation buffer.

Source data are available online for this figure.

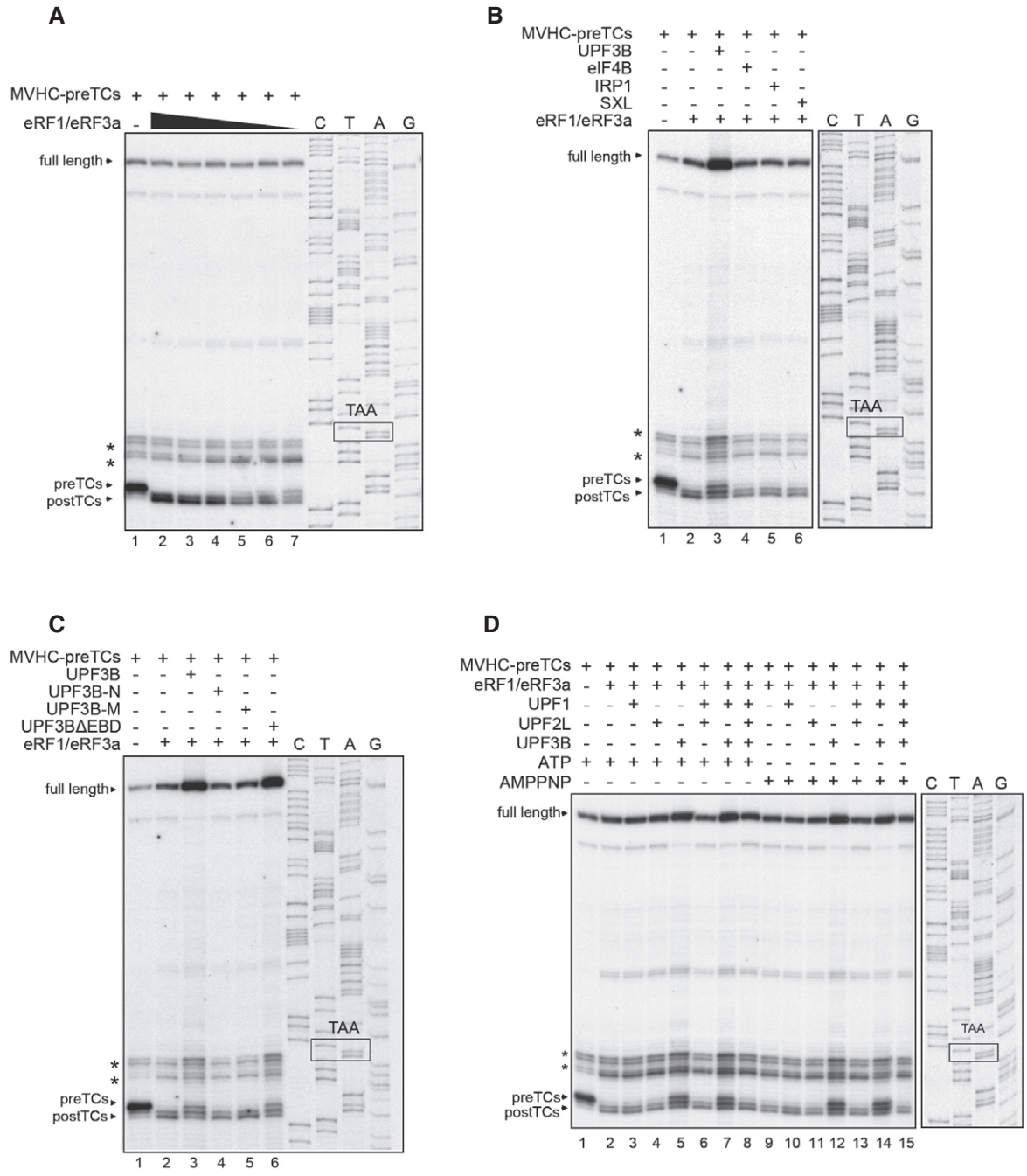

Figure EV2. Validation of the termination-delaying effect of UPF3B.

A Toeprinting analysis of ribosomal complexes obtained by incubating MVHC-pre-TCs at 1 mM free Mg^{2+} with decreasing amounts of eRFs. Representative example for the titration of eRF1 and eRF3a to identify concentrations slowing down the pre-TC to post-TC transition. The amount used for the sample in lane 5 was chosen for further experiments with this batch of pre-TCs.

B Toeprinting analysis of ribosomal complexes obtained by incubating MVHC-pre-TCs with UPF3B, eIF4B, IRP1, SXL or BSA at 1 mM free Mg^{2+} and 1 mM ATP followed by termination with limiting amounts of eRF1 and eRF3a.

C Toeprinting analysis of ribosomal complexes obtained as in (B) by incubating MVHC-pre-TCs with UPF3B, UPF3B-N, UPF3B-M, UPF3BΔEBD or BSA.

D Toeprinting analysis of ribosomal complexes obtained as in Fig 1B in the presence of 1 mM ATP or AMPPNP, respectively.

Data information: Asterisks mark initiation and elongation complexes. Panels (A, B) each represent two independent experiments. Panel (D) represents three independent experiments.
Source data are available online for this figure.

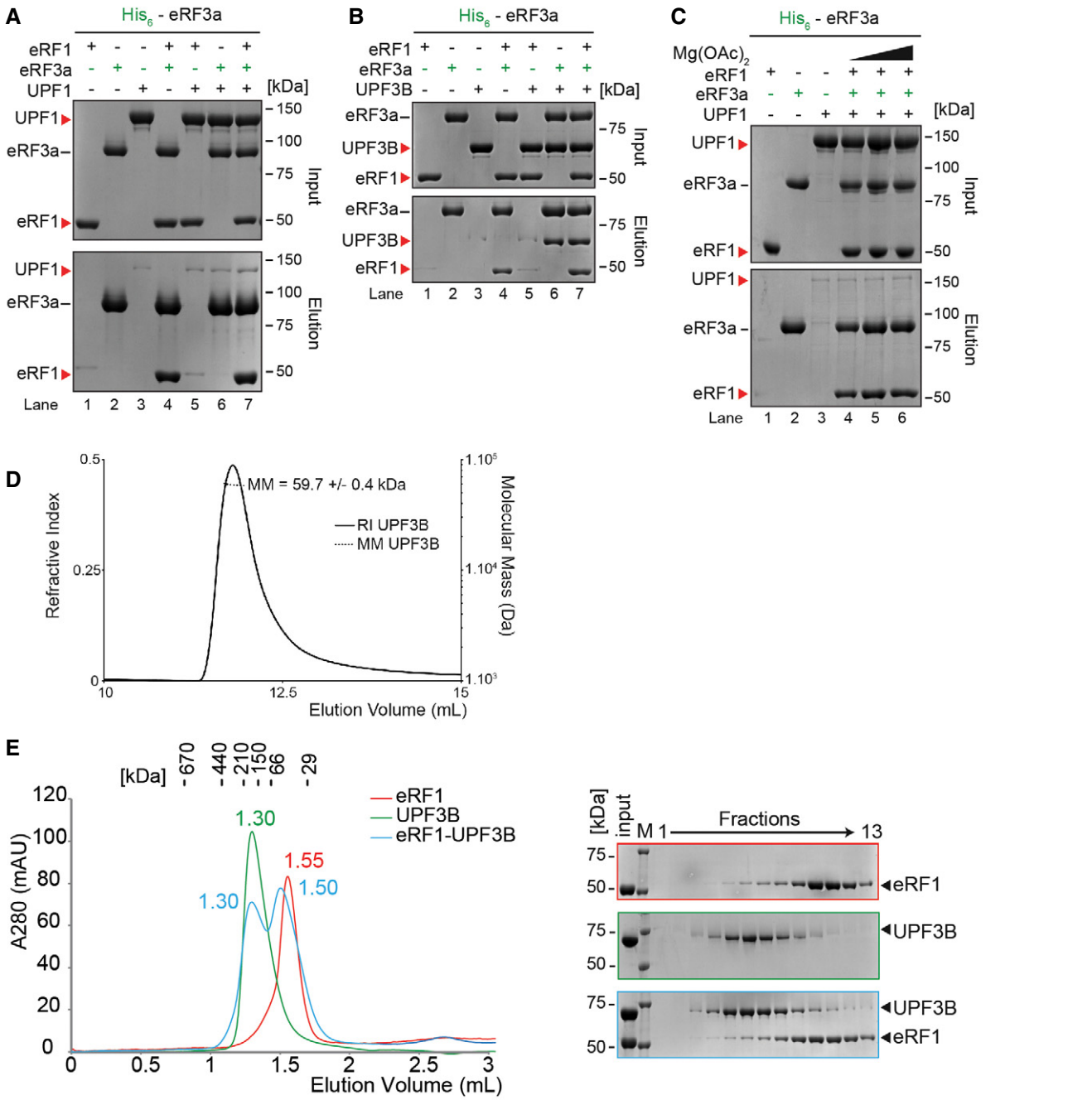

Figure EV3. Validation of UPF3B's complex formation with eRF1 and eRF3a.

A *In vitro* pulldown of eRF1 and/or UPF1 with His-eRF3a. Protein mixtures before loading onto the beads (input) or after elution (eluate) were separated by SDS–PAGE.

B Pull-down experiment as in (A) with eRF1, UPF3B and His-eRF3a.

C Pull-down experiment as in (B) with eRF1, UPF1 and His-eRF3a in buffer containing 0, 2.5 or 5 mM Mg^{2+} (lanes 4, 5, or 6 respectively).

D Molecular mass of UPF3B determined by size-exclusion chromatography using a Superdex 200 column combined with detection by multiangle laser light scattering and refractometry (SEC-MALLS-RI). The SEC elution profiles as monitored by refractometry (RI) are represented for UPF3B. The molecular mass (MM) of UPF3B calculated from light scattering and refractometry data is indicated.

E Left: SEC elution profile of eRF1 (red), UPF3B (green) or both (blue). The elution volume (in ml) is indicated for each experiment. Calibration of the column was performed with globular proteins (shown above). Right: SDS–PAGE analysis of eluate fractions. M: protein molecular weight standards (kDa).

Data information: Panels (A–C) each represent three independent experiments. Panels (D, E) each represent two independent experiments. (A–C) Bands in lanes 1, 3 and 5 of the eluate panels (A, B) and bands in lanes 1 and 3 of the eluate panel in (C) represent background binding of untagged proteins to the Ni-NTA resin.
Source data are available online for this figure.

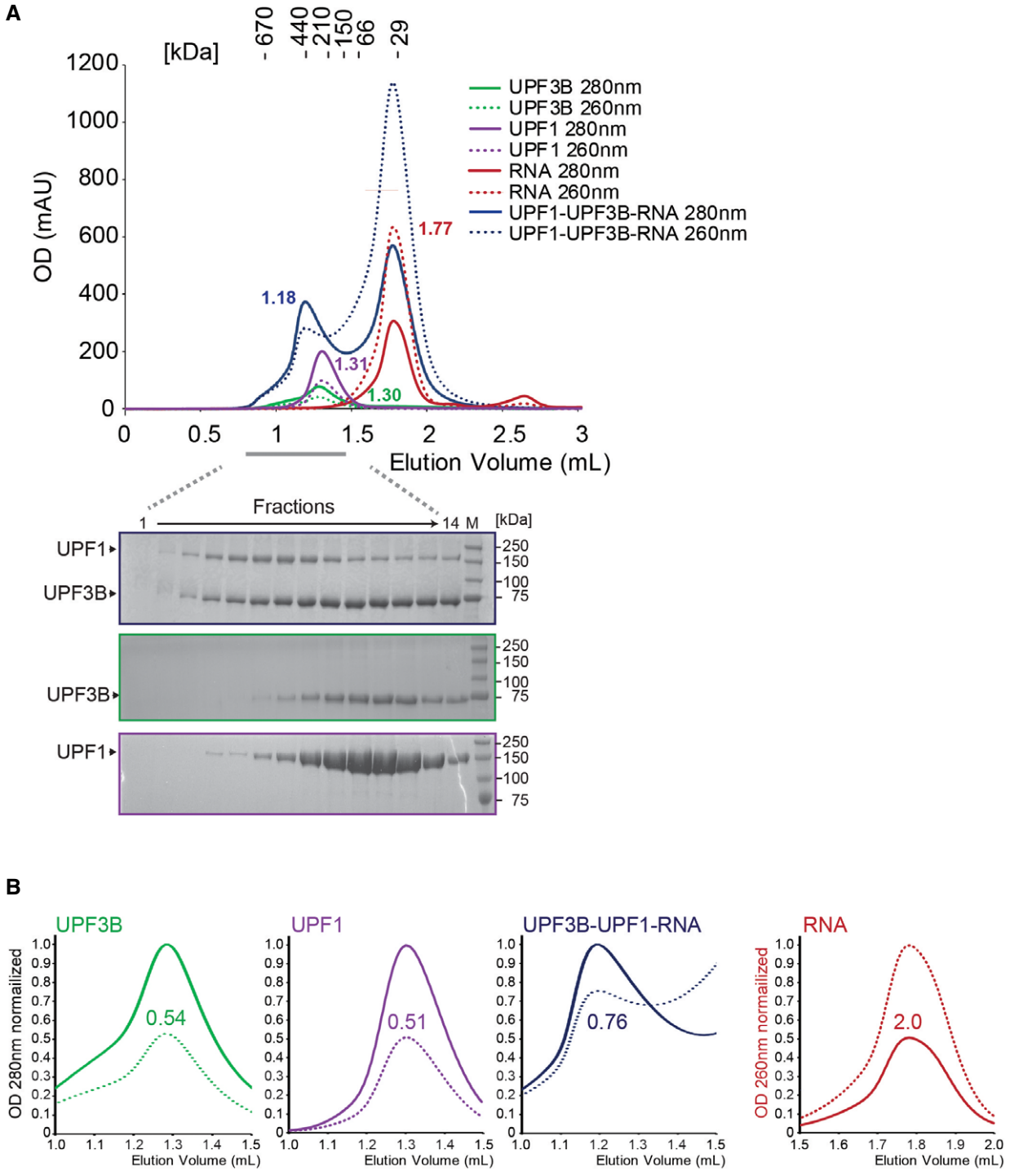

Figure EV4. UPF1-UPF3B complex formation is not prevented by RNA.

A SEC elution profile of UPF3B (green), UPF1 (purple), RNA (red) and a mix of UPF3B, UPF1 and a threefold excess of RNA (blue). Below: SDS–PAGE analysis of eluate fractions.

B Analysis of SEC peak fractions. Peaks representing UPF3B (green) and UPF1 (purple), elute with an OD 260 nm/280 nm ratio of 0.54 and 0.51, respectively, whereas the RNA oligomer (red) elutes with an OD 260 nm/280 nm ratio of 2.0. The peak containing the UPF3B-UPF1 complex after incubation of UPF1, UPF3B and RNA (blue) has a higher OD 260 nm/280 nm ratio of 0.76 due to the presence of RNA in this peak.

Source data are available online for this figure.

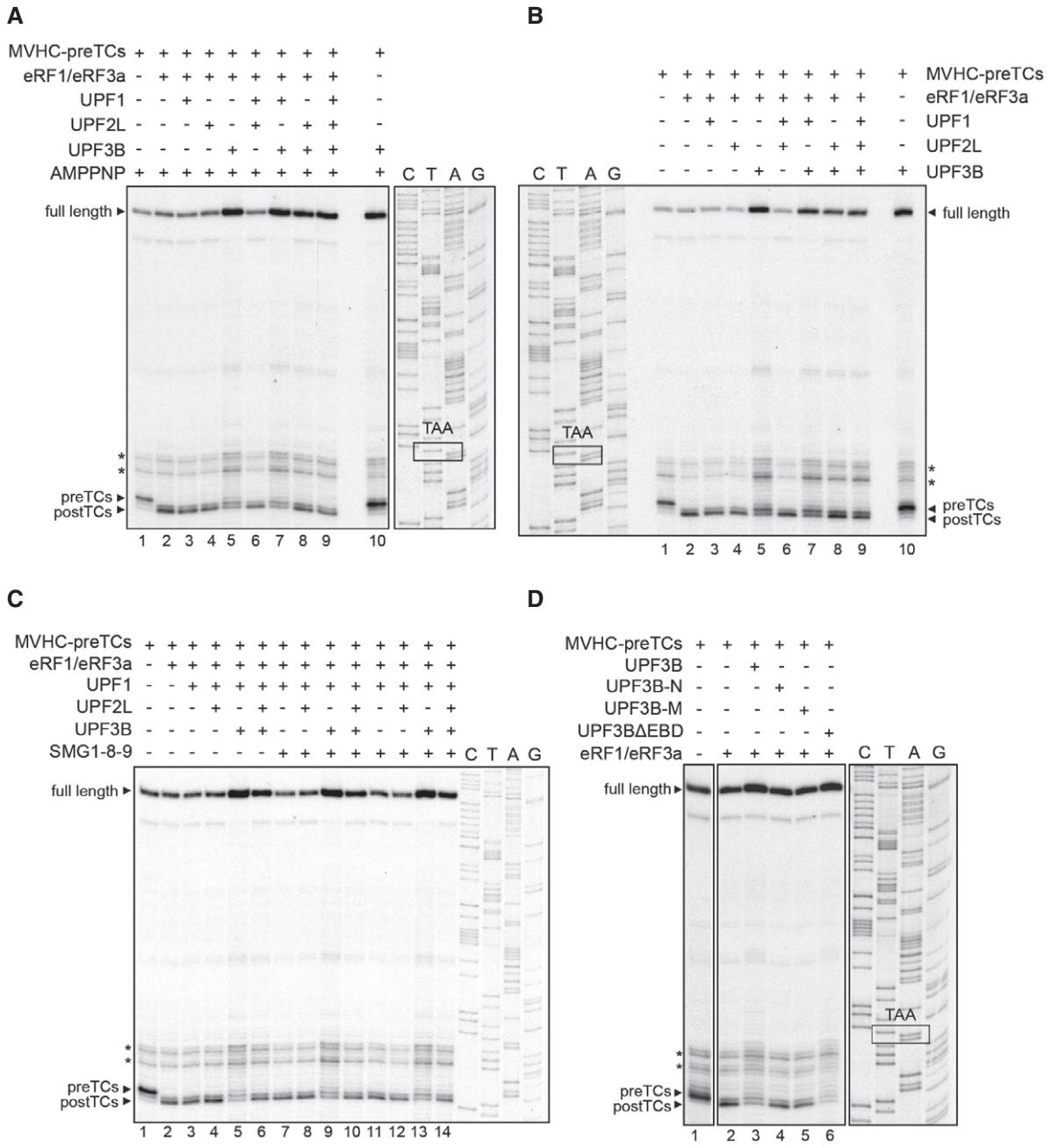

Figure EV5. UPF3B's post-TC-dissolving activity is independent of ATP and SMG1-8-9 and requires both the RRM and the middle domain.

A, B Toeprinting analysis of ribosomal complexes as in Fig 6A, but in the presence of 1 mM AMPPNP (A) or without adenosine nucleotide (B).

C Impact of UPF1-phosphorylation on efficient translation termination and on ribosome dissociation by UPF3B. Toeprinting analysis of ribosomal complexes obtained by incubating pre-TCs formed on MVHC-STOP mRNA (MVHC-pre-TCs) with UPF1, UPF2L, UPF3B or BSA at 1 mM free Mg^{2+} and 1 mM ATP. In lanes 7–10, UPF1 was incubated with SMG1-8-9 and ATP for 30 min at 37°C either alone (lane 7) or in the presence of UPF2L, UPF3B, or both (lanes 8–10) before pre-TCs were added to the mixture and again incubated for 10 min. In lanes 11–14, UPF1 was incubated with ATP and SMG1-8-9 for 30 min. Then, UPF2L and/or UPF3B were added for additional 15 min (lanes 12–14). Finally, MVHC-pre-TCs were added to the mixtures for 10 min followed by translation termination by eRF1 and eRF3a.

D Toeprinting analysis of ribosomal complexes obtained by incubating MVHC-pre-TCs with UPF3B, UPF3B-N, UPF3B-M, UPF3BΔEBD or BSA at 1 mM free Mg^{2+} followed by termination with saturating amounts of eRF1 and eRF3a.

Data information: Asterisks mark initiation and elongation complexes. Panel (A) represents three independent experiments. Panels (B, C) each represent two independent experiments.
Source data are available online for this figure.