Supplementary figure legends

Figure S1. Graph showing the intensities of bands based on scanned lanes of the gels presented in Figures 3A and 3B. The upper graph shows intensity of DMS modifications for newly synthesized RNA after 2 and 3 minutes of labeling (corresponding to the gel presented in Fig. 3A). The shape of the curves is similar for shorter and longer labeling times, showing that the relative reactivity of nucleotides is unaltered. The lower graph presents the intensity of DMS modifications for RNA present in purified late, cytoplasmic pre-40S complexes, co-purified with Tsr1 or Nob1 (Fig. 3B). Peak 2 is relatively elevated in the pre-40S fraction compared with 4TU RNA. A peak resulting from degradation is marked by an asterisk. Site D is marked by arrows on each graph.

Figure S2. Graph showing the intensities of bands based on scanned lanes of the gel presented in Figure 5A.

Figure S3. DMS modification pattern of the H28/H44 hinge region in total and newly synthesized RNA. The nucleotide positions are indicated on both sides of the gel. To visualize modifications in 4TU labeled RNA a longer exposure of the gel is presented. The strong stop at C1639 represents a site of endogenous 2'-*O*-methylation. Lane $6_{(C+)}$ – additional control lane with 6 min 4TU labeling without DMS addition.

Figure S4. Structure of a region of the Rps23-18S rRNA H18 interface (data from NDB ID: NA0650, (Ben-Shem et al. 2010; Ben-Shem et al. 2011))

Supplementary references

Ben-Shem A, Garreau de Loubresse N, Melnikov S, Jenner L, Yusupova G, Yusupov M. 2011. The structure of the eukaryotic ribosome at 3.0 A resolution. *Science* **334**: 1524-1529.

Ben-Shem A, Jenner L, Yusupova G, Yusupov M. 2010. Crystal Structure of the Eukaryotic Ribosome. *Science* **330**: 1203-1209.

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Figure S1

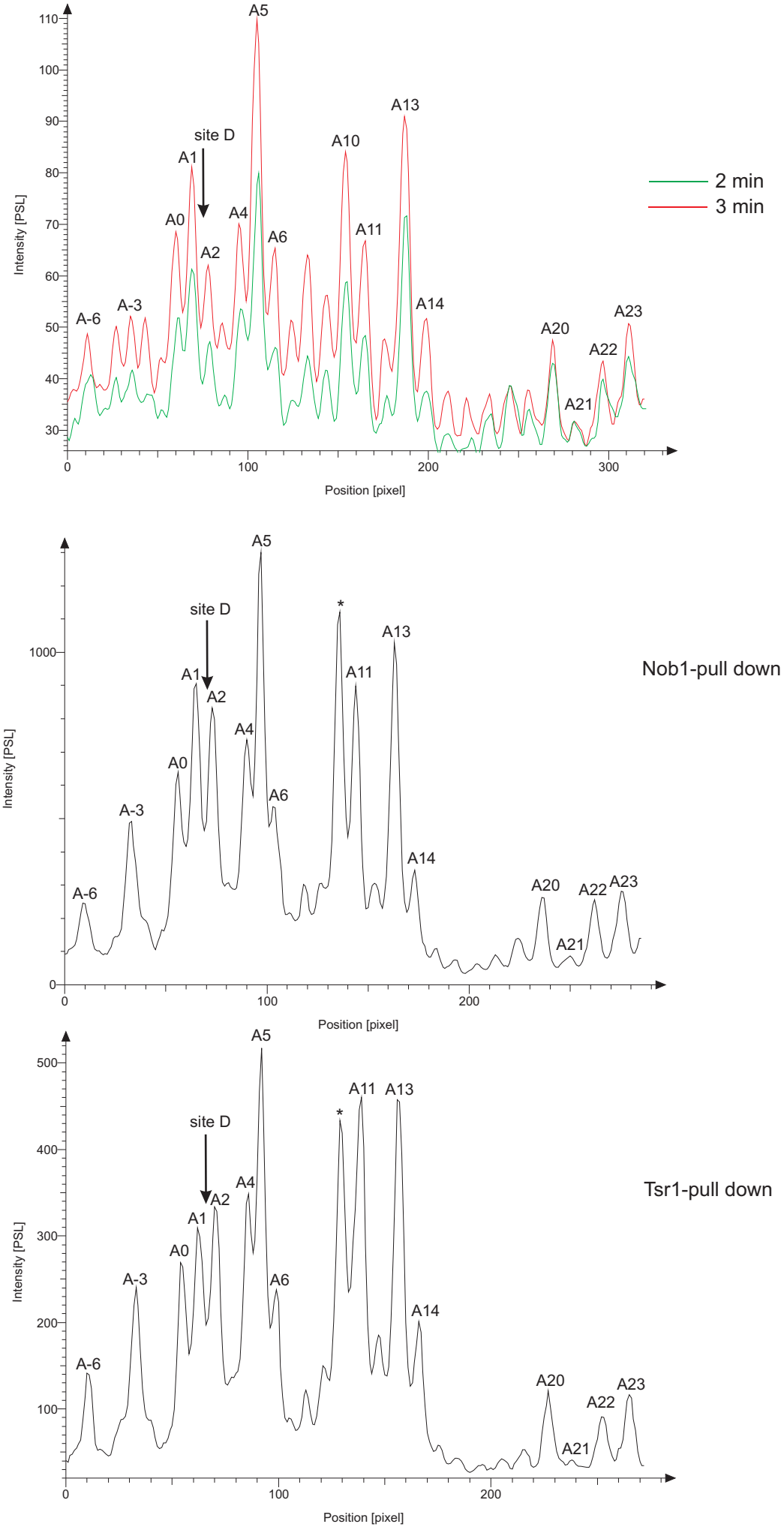

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Figure S2

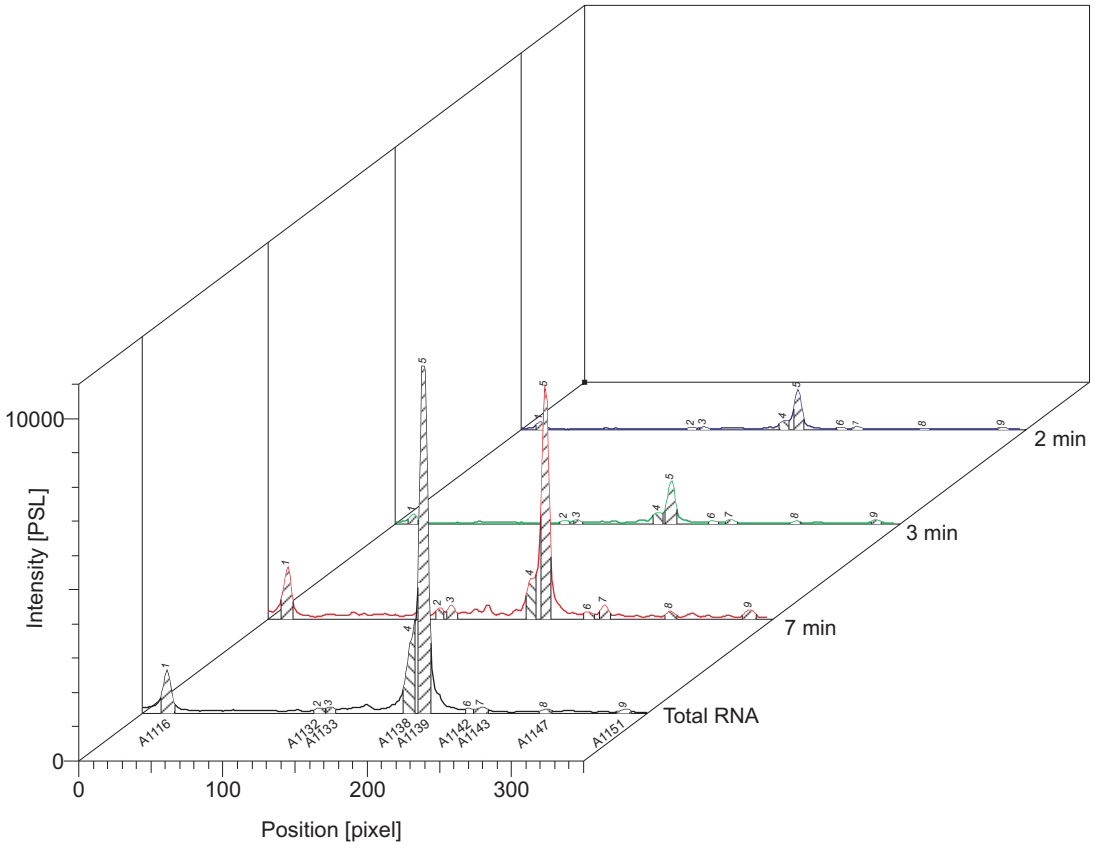

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Figure S3

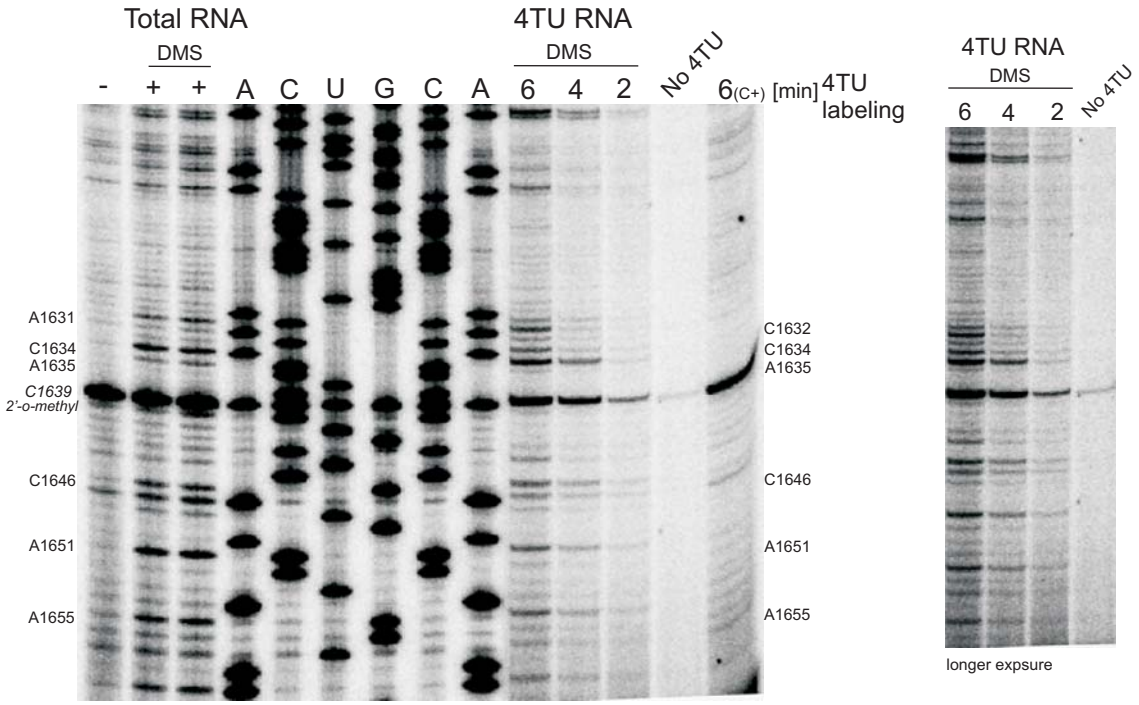

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Figure S4