Expanded View Figures

Figure EV1. Classification and refinement scheme for the pre-40S complex, the 80S-like particle, and the Dim1-containing complex.

2D classification from 594,910 particles that had been picked from 2,875 micrographs revealed two particle subpopulations of "40S-like" and "80S-like" particles, which where separately processed and subjected to 3D classification and auto-refinement yielding a 3D reconstruction of the pre-40S complex at an overall resolution of 3.4 Å and a 3D reconstruction of the 80S-like particle at an overall resolution of 3.1 Å. Subsequent rounds of focused 3D classification from this final set of pre-40S particles focusing on either Rio2 (blue circle), Enp1 (red circle), or Dim1 (green circle) yielded subpopulations of the pre-40S complex at overall lower resolution but with higher local resolution in the area of focus, further facilitating the model building and docking in these areas.

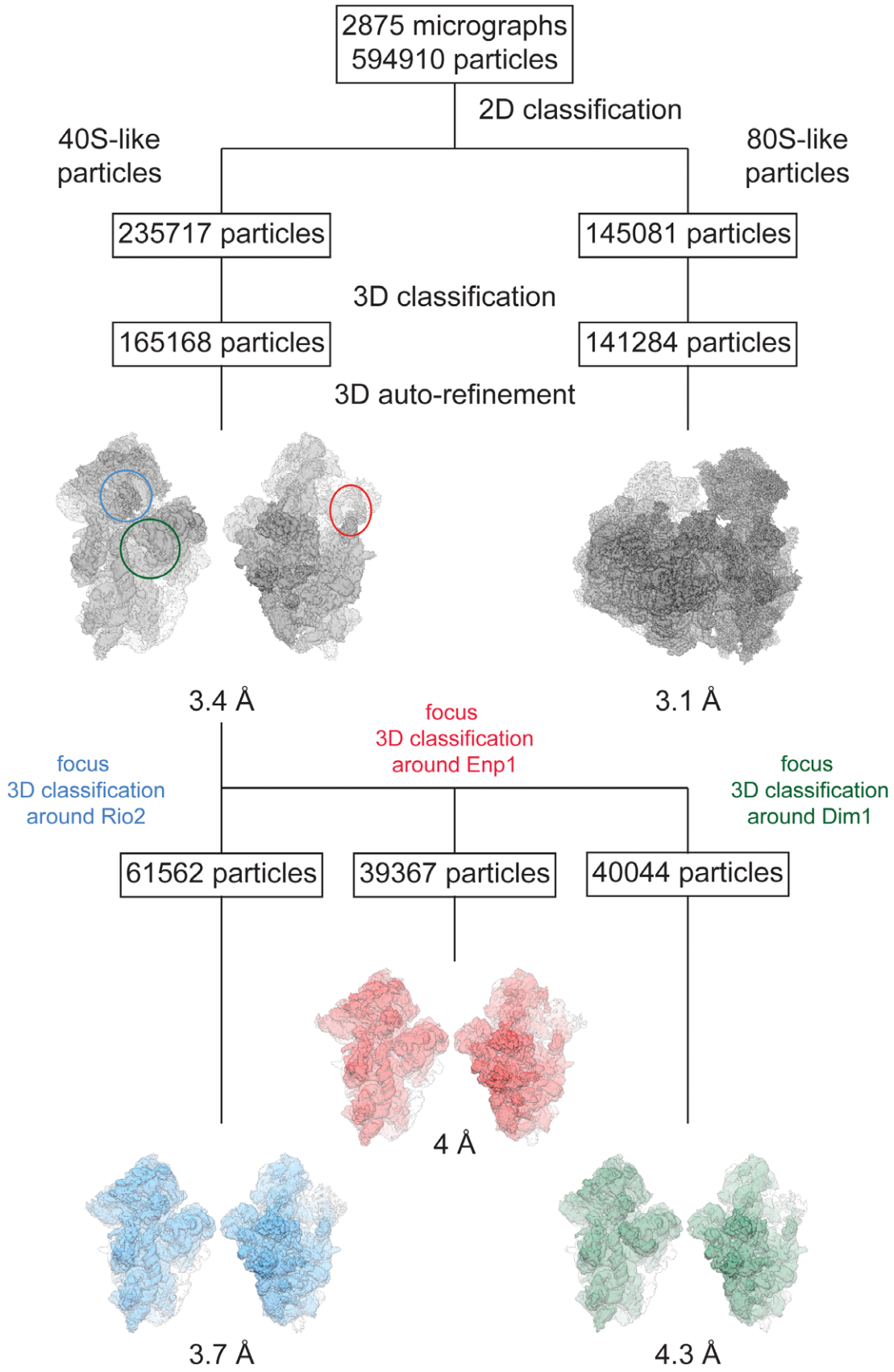
2875 micrographs
594910 particles
2D classification
40S-like particles
80S-like particles
235717 particles
145081 particles
3D classification
165168 particles
141284 particles
3D auto-refinement
3.4 Å
3.1 Å
focus 3D classification around Enp1
focus 3D classification around Rio2
focus 3D classification around Dim1
61562 particles
39367 particles
40044 particles
4 Å
3.7 Å
4.3 Å

Figure EV1.

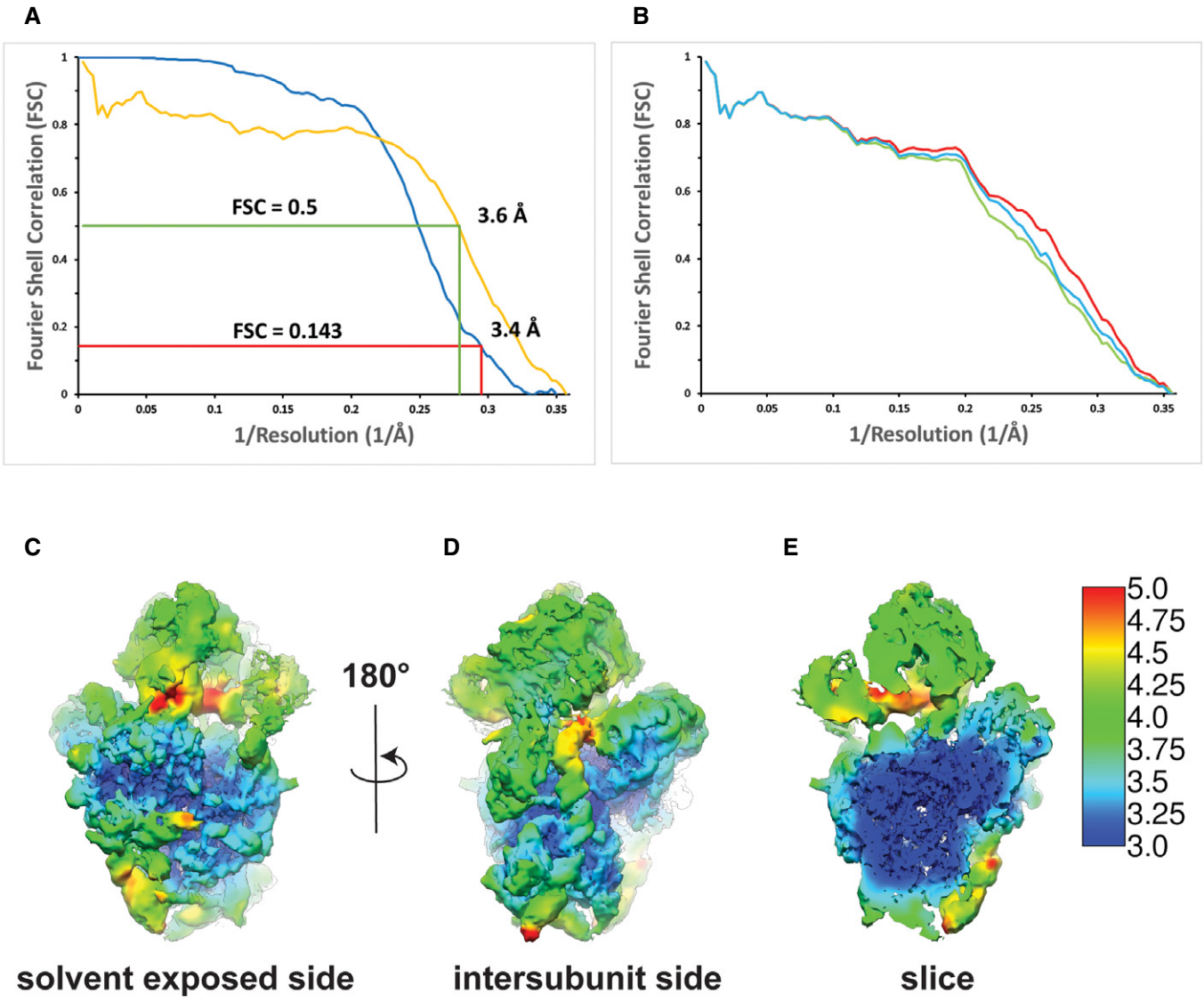

Figure EV2. Local resolution heat map of the high-resolution pre-40S complex and comparison of the Fourier shell correlation curves calculated for the experimental 3D-EM reconstruction and the refined model of the pre-40S complex.

A Fourier shell correlation (FSC) curves for the high-resolution EM map (blue) and the one calculated from the model versus the map (yellow). At the FSC = 0.143 criterion, the overall resolution of the high-resolution EM map was estimated to be 3.4 Å, which coincides well with the 3.6 Å resolution obtained for the model versus map FSC at a value of 0.5.

B FSC curves calculated between the model refined in the density of the halfset1 of the data and the map calculated with the full dataset (red), the halfset1 map (green, half used for refinement), or the halfset2 map (blue, half not used for refinement). The agreement between the three FSCs curves indicates the absence of overfitting (Fernandez *et al*, 2014; Greber *et al*, 2014).

C, D Local resolution heat map of two different orientations of the reconstruction of the full pre-40S complex. The local resolution varies between 3 Å for the core of the ribosomal subunit and 4–5 Å for the peripheral parts of the rRNA expansion segments as well as the head of the pre-40S and the assembly factors. Local resolution was calculated using the "relion_postprocess" from Relion 2.1 (Kimanius *et al*, 2016).

E Cut-through of the view in (D).

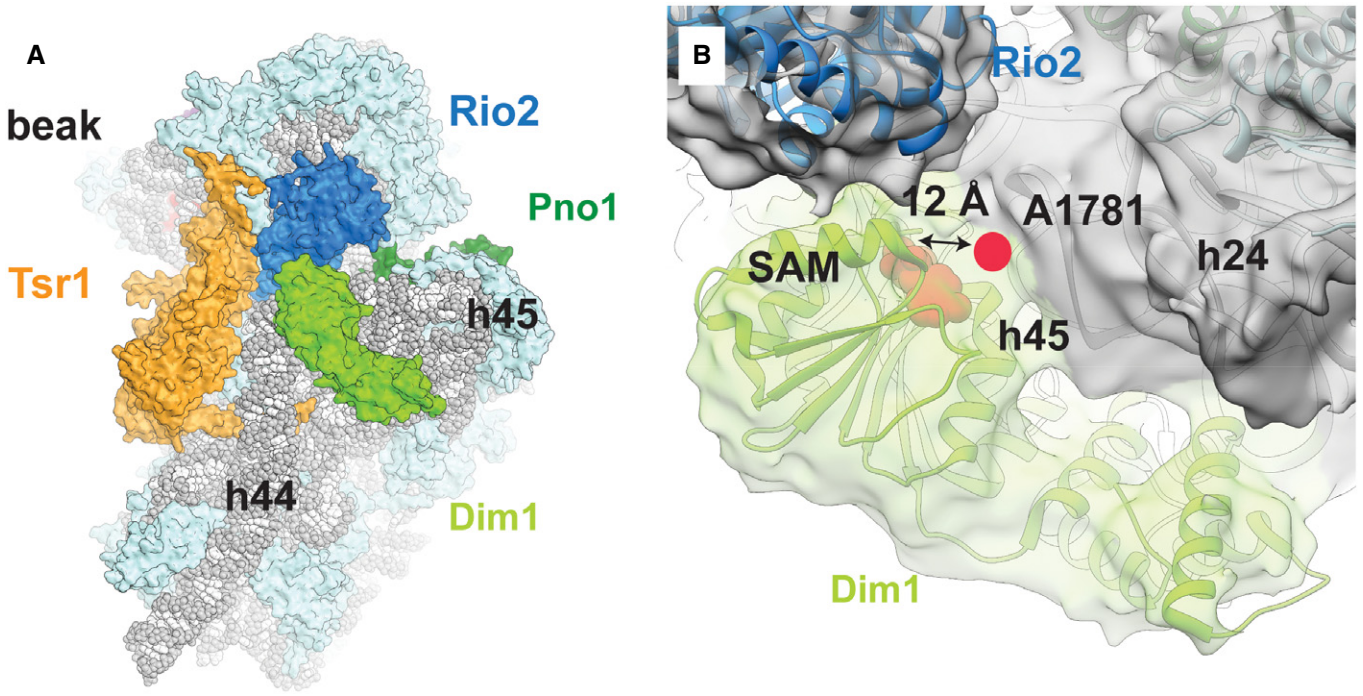

Figure EV3. 3D reconstruction of the Dim1-containing subclass of cytoplasmic pre-40S particles.

A Overview over the Dim1-containing complex (r-proteins in light blue, rRNA in gray) from the intersubunit face. The rRNA methylase Dim1 (bright green) binds to the complex between the platform and the top of the displaced helix 44 in close vicinity of Rio2 (blue), Tsr1 (orange), and the decoding center.

B Dim1 (bright green) binds not only to helix 44, but also helices 45 and 24. Rigid-body fitting of the available crystal structure of human Dim1 in complex with S-adenosyl methionine (SAM) (pink) (PDB: 1ZQ9) into the cryo-EM density shows that the previously described active site of Dim1 would be located too distant from its methylation target, A1781 (pink circle), to allow the methylation reaction to take place. Additionally, the unmethylated helix 45 has been reported to be rotated upward to allow access for Dim1 (Johnson *et al*, 2017). Thus, Dim1 has likely already methylated A1781 and A1782, as shown previously by primer extension analysis of the Nob1-D15N particle (Lebaron *et al*, 2012).

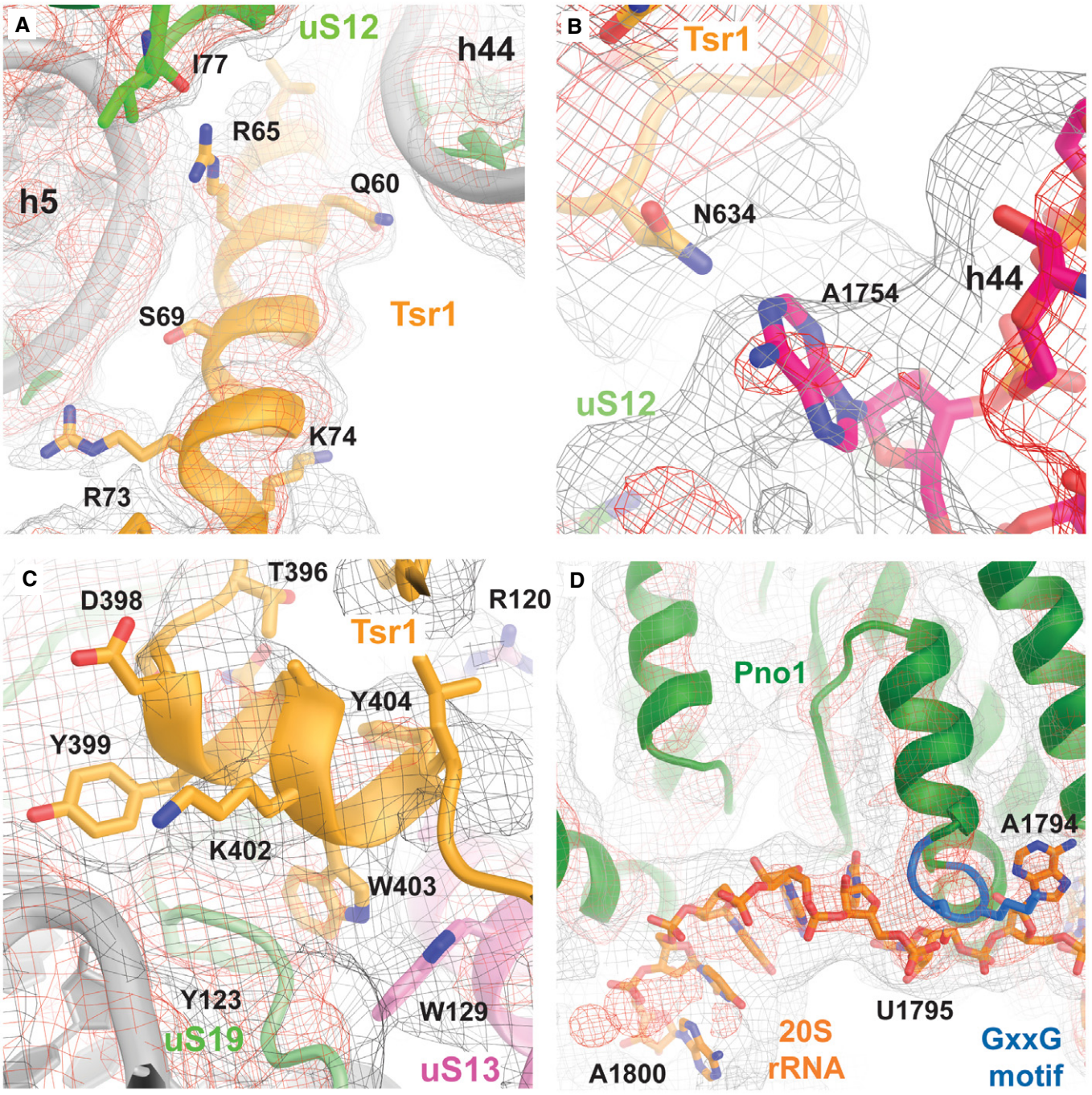

Figure EV4. Details of the functionally important assembly factors' interactions in the cryo-EM map of the cytoplasmic pre-40S.
The unfiltered cryo-EM map is shown at two different contour levels (gray and red mesh).

A N-terminal α-helix of Tsr1 (orange) interacting with helix 44.
B Visualization of the flipped out position of the decoding center base A1754 at the top of helix 44 (pink).
C Interactions of the conserved loop of Tsr1 with uS19 (green) and uS13 (pink).
D Interaction of the Pno1 (green) C-terminal KH domain (containing a GxxG RNA binding motif shown in blue) with the 3′ terminal bases of the rRNA (orange).