FIGURE SUPPLEMENTS

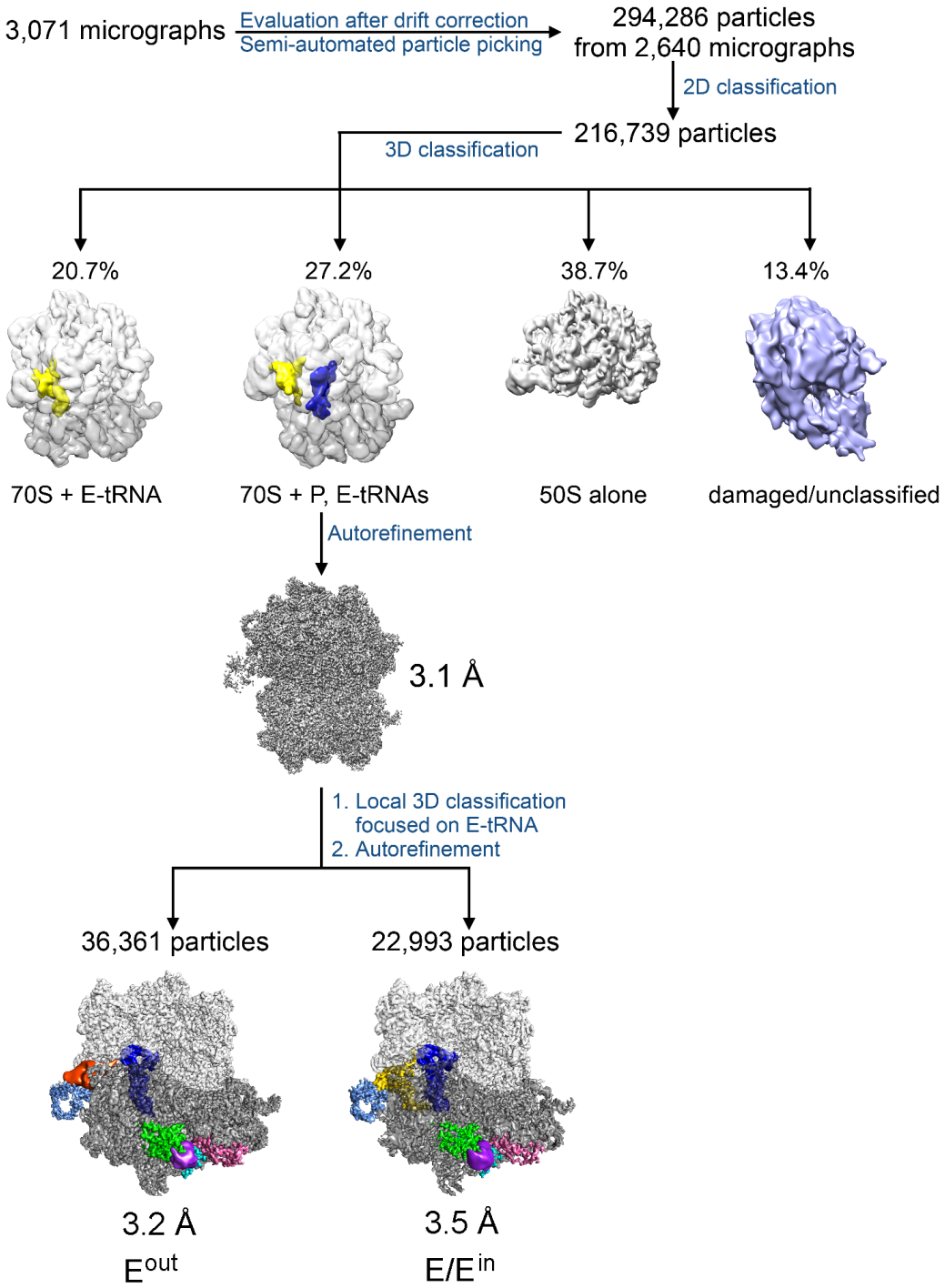

Figure S1- Related to Figure 1. Particle projection selection, classification, and 3D map reconstruction. Flow chart of cryoEM data processing of ribosome complexes. Details are provided in the Methods section**.**

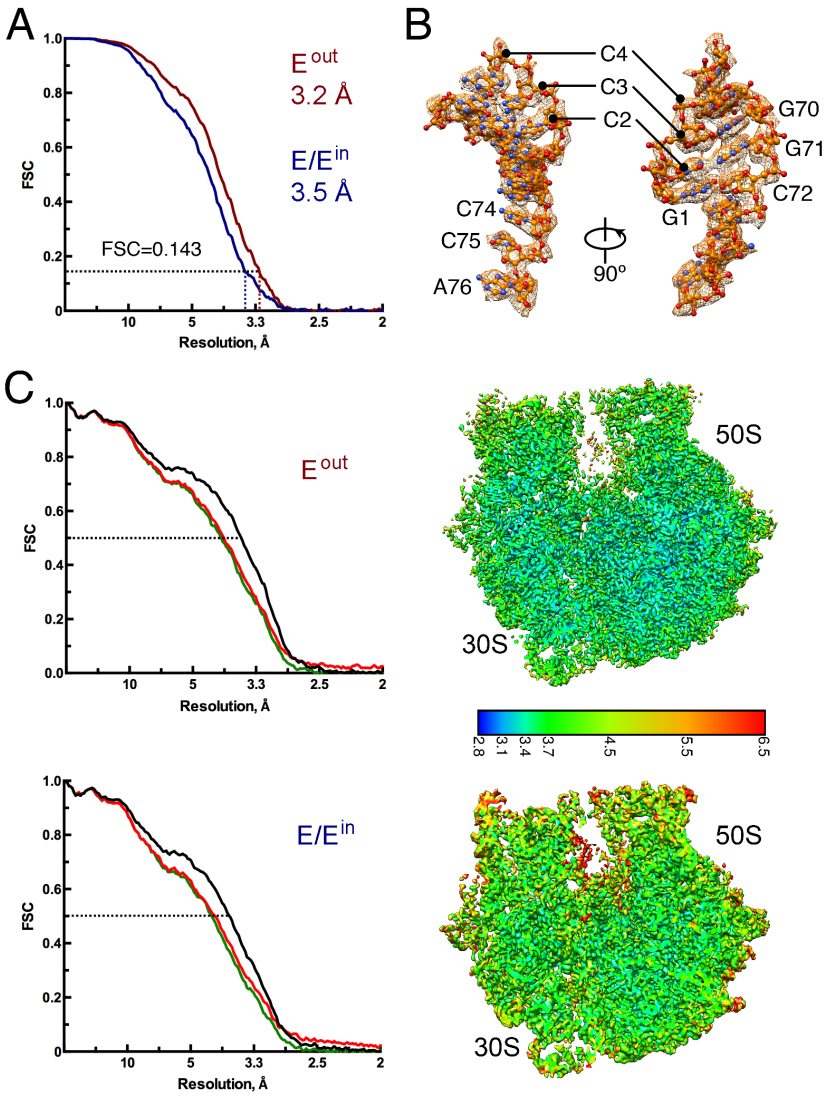

Figure S2- Related to Figure 1. Quality of maps and models. (A) Fourier shell correlation (FSC) curves for the EM maps of E^{out} and E^{in}. **(B)** Snapshots of map *vs.* model for the section of acceptor stem of E-site tRNA in the conformation of E^{out}. The EM density corresponding to acceptor stem of E-site tRNA in E^{out} is well defined, indicating the high occupancy of E-tRNA. **(C)** Cross-validation between maps and models. FSC curves of the final refined model versus the final cryoEM map (full dataset, black), of the outcome of model refinement with a half map versus the same map (red), and of the outcome of model refinement with a half map versus the other half map (green). The excellent agreement between red and green curves indicates lack of overfitting.

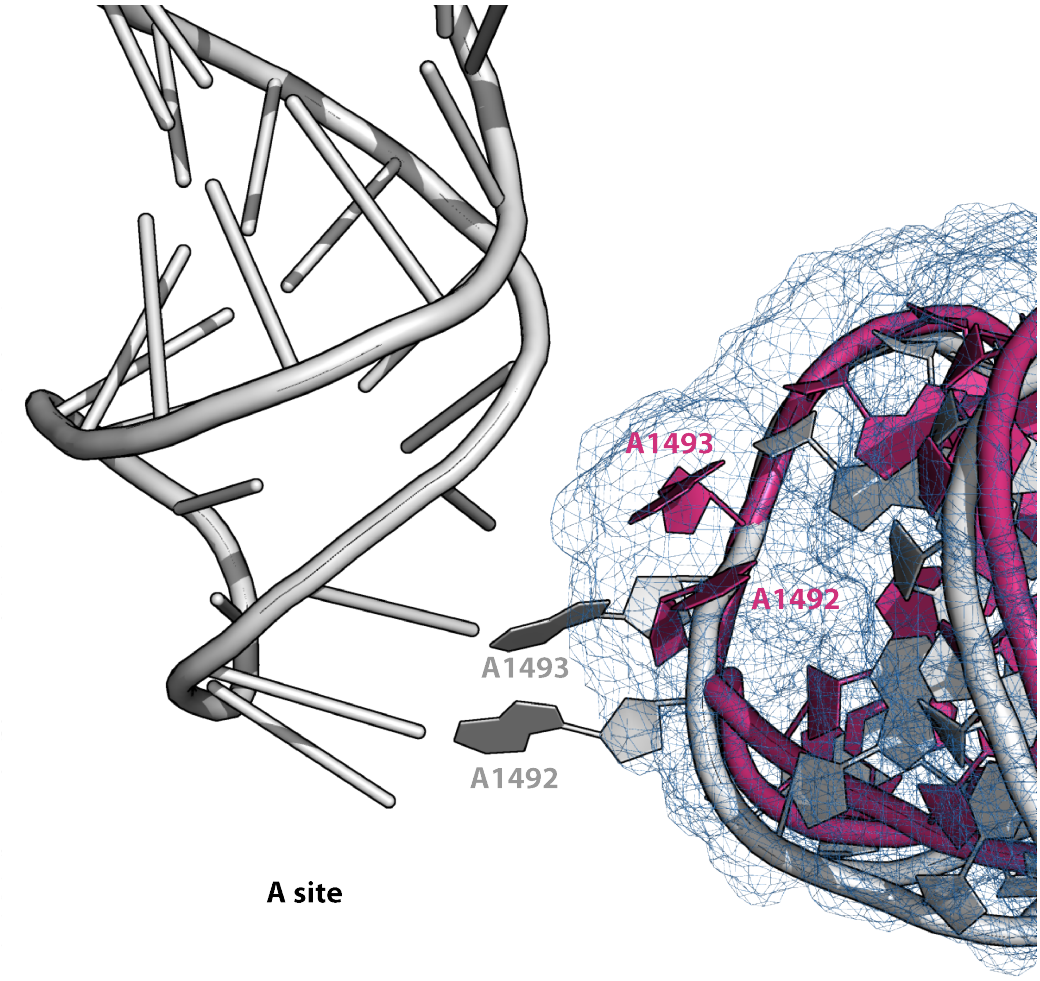

Figure S3- Related to Figure 1. Decoding center interactions. Upon A-site tRNA binding, 16S rRNA nucleotides A1492 and A1493 flip from h44 to interact with the mRNA-tRNA pair (shown in gray; PDB code 4Y4P). In the 70S-complex structure, the map indicates that A1492 remains inside h44 and A1493 is half removed from h44 (shown in magenta) indicative of a structure lacking A-site tRNA.

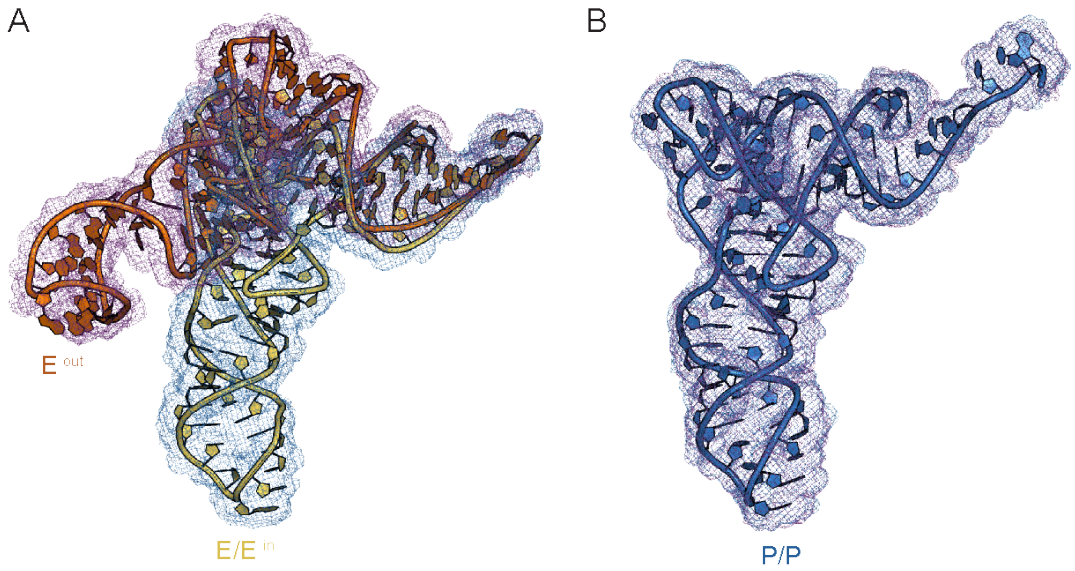

Figure S4- Related to Figure 1. Map quality for P-site and E-site tRNAs. EM maps corresponding to a 2.0 Å radius around E^{in} (light blue) and E^{out} (light purple) coordinates of the E-site tRNA (panel A) and P-site tRNA (panel B) are shown.

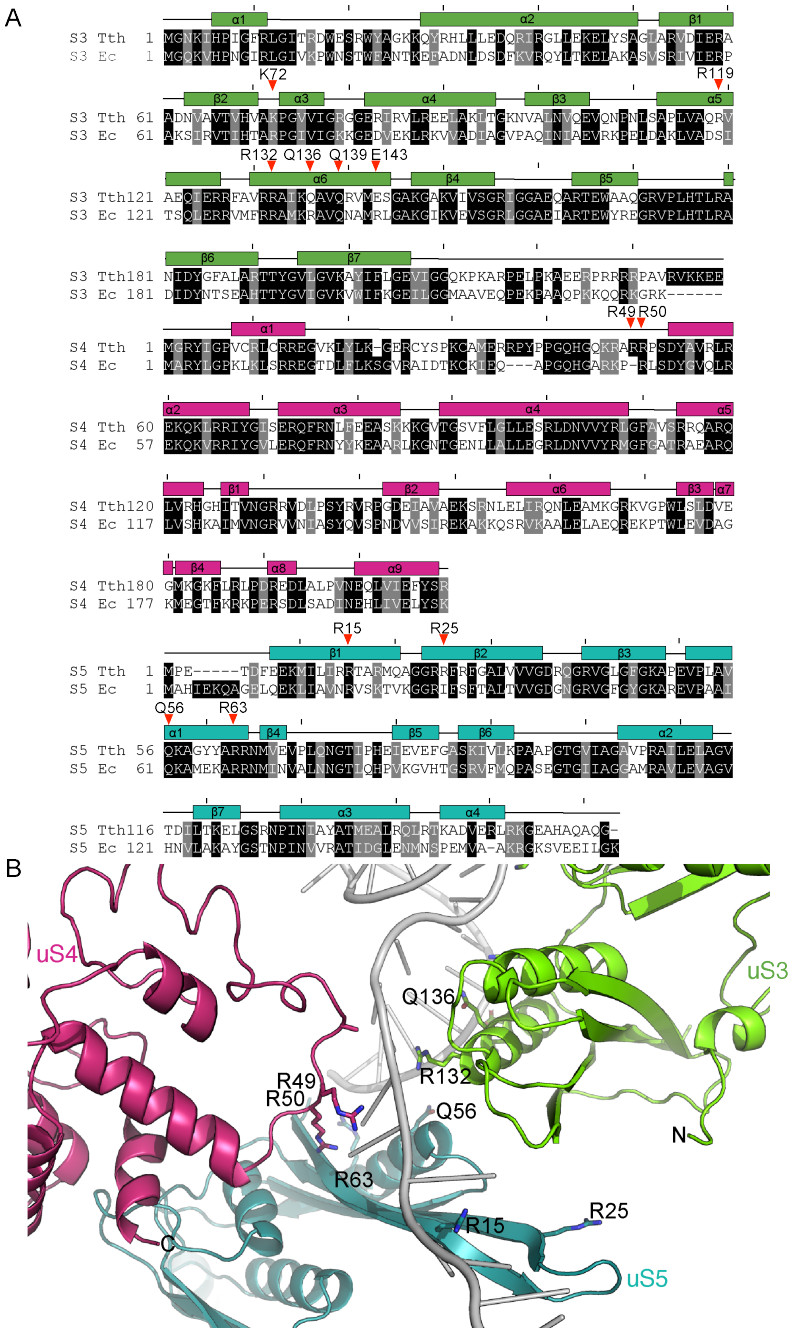

Figure S5- Related to Figure 2. uS3, uS4, and uS5 sequences and interactions with the mRNA. (A) Sequence alignments of *Thermus thermophilus* (*Tth*) and *E. coli* (*Ec*) uS3, uS4 and uS5 proteins. Residues implicated in interactions with mRNA as seen in our structure are shown with red triangles. **(B)** uS3, uS4, and uS5 interactions with the mRNA entrance channel looking towards the solvent-exposed stem-loop in the background.