

S7 Fig. S-F Cryo-EM processing workflow. (A) 3,054 micrographs were processed and classified to give rise to an initial particle pool of 648,714 particles. **(B)** *ab initio* classification gave rise to initial classes. **(C)** A modified build-and-retrieve (BaR) approach was used to generate the full particle set. Complete 70S classes were combined and refined. **(D)** Full particle set was separated based off of tRNA population using 3D variability analysis in cryoSPARC. **(E)** Particles were extracted at full resolution and refined with homogeneous refinement. **(F)** Final maps refined with local/global CTF correction and focused local refinements and composite maps generated using Relion postprocessing.