SUPPLEMENTARY MATERIAL

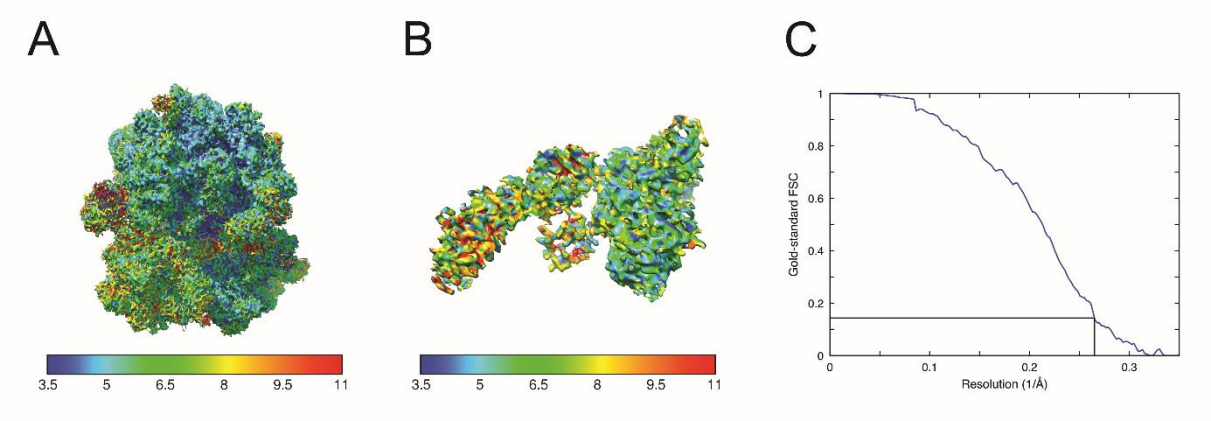

Figure S1: Global resolution of the EF-G-ribosome complex
(**A**) Surface view of the final map, colored according to the local resolution as calculated with ResMap (*9*). (**B**) Same as in a) but for EF-G. (**C**) Gold-standard Fourier shell correlation (FSC) curves for the EF-G ribosome complex. The resolution calculated using the 0.143 FSC cut-off criterion is 3.8 Å.

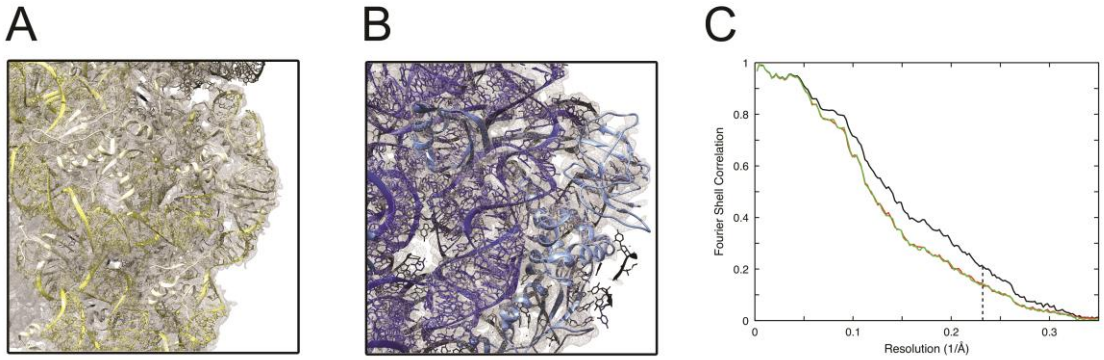

Figure S2: Structure refinement and local fit of atomic model in the cryo-EM map.
(**A**) Local fit of a portion of the 30S subunit, the sRNA is in yellow and the protein in light yellow. (**B**) Portion of the 50S subunit, the rRNA is in blue and the protein in light blue. (**C**) Analysis of overfitting by cross-validation of the atomic model. FSC_{work} curve (green) correspond to the refined model versus the half-map it was refined against. The FSC_{test} curve (red) is calculated between the refine model and the other half-map. The black curve shows the FSC between the refined model and the full unfiltered map. The dashed line represents the highest resolution (4.3 Å) used in refinement.

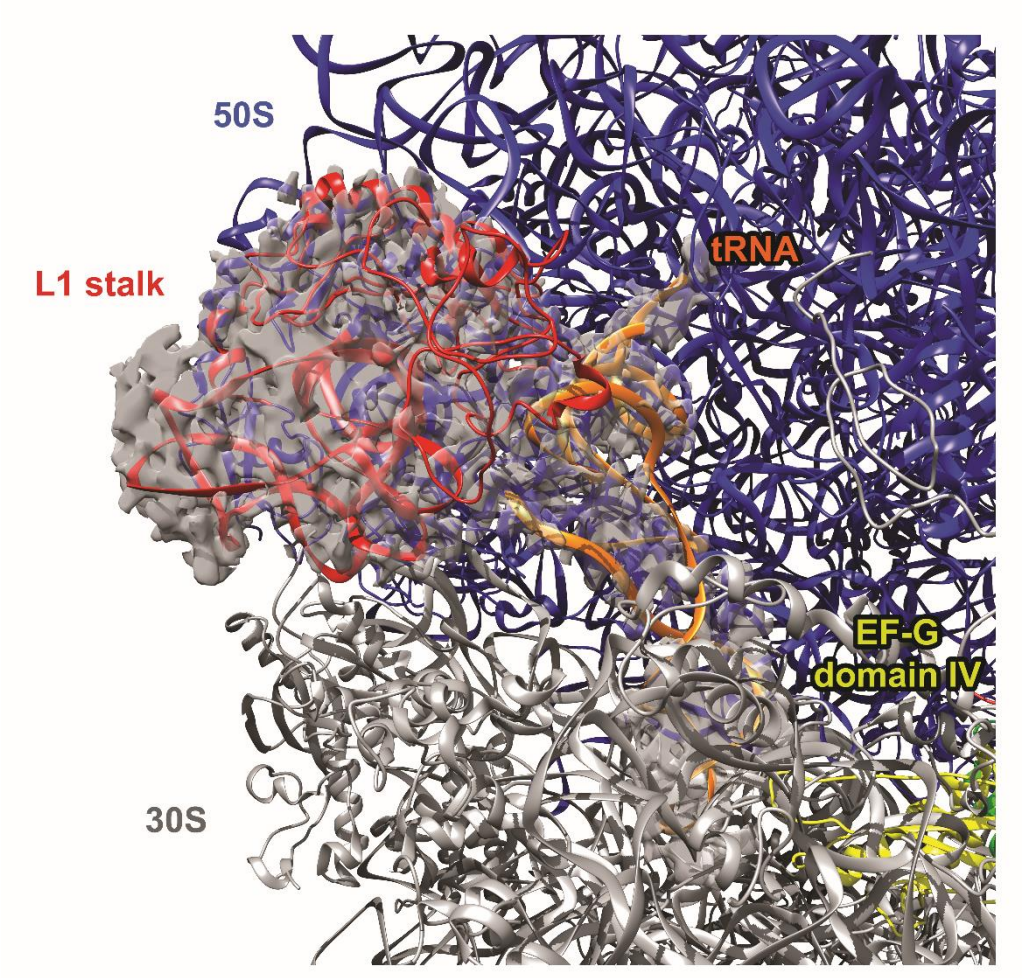

Figure S3: Interaction between tRNA and the ribosomal L1 stalk characteristic of a translocation intermediate state.
The 50S subunit is blue, the 30S subunit is grey, the L1 stalk is red, the tRNA is orange, and the EF-G domain IV is yellow. The portion of the cryo-EM map corresponding to the tRNA and L1 stalk is shown as a dark gray surface.

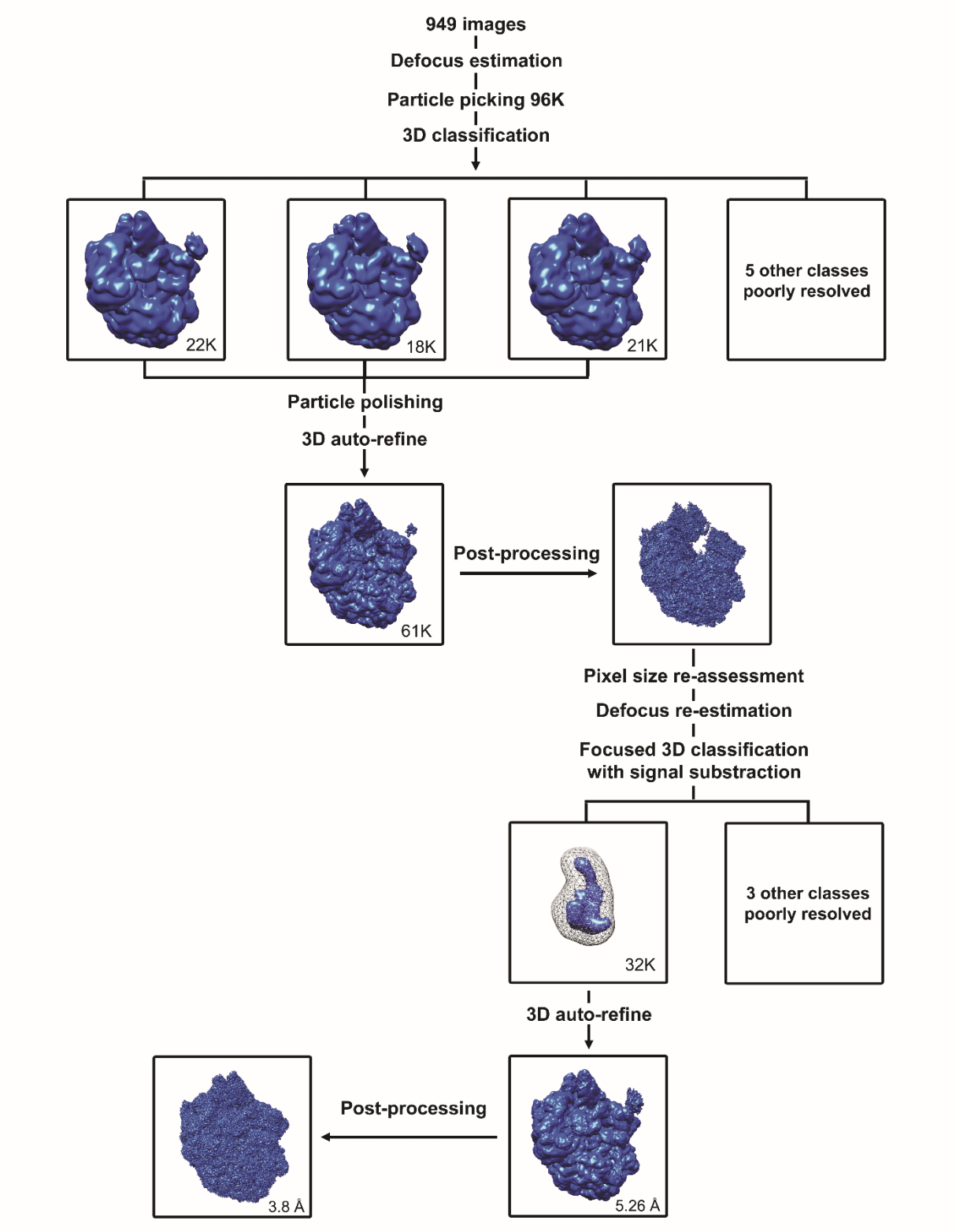

Figure S4: Maximum-likelihood 3D classification scheme

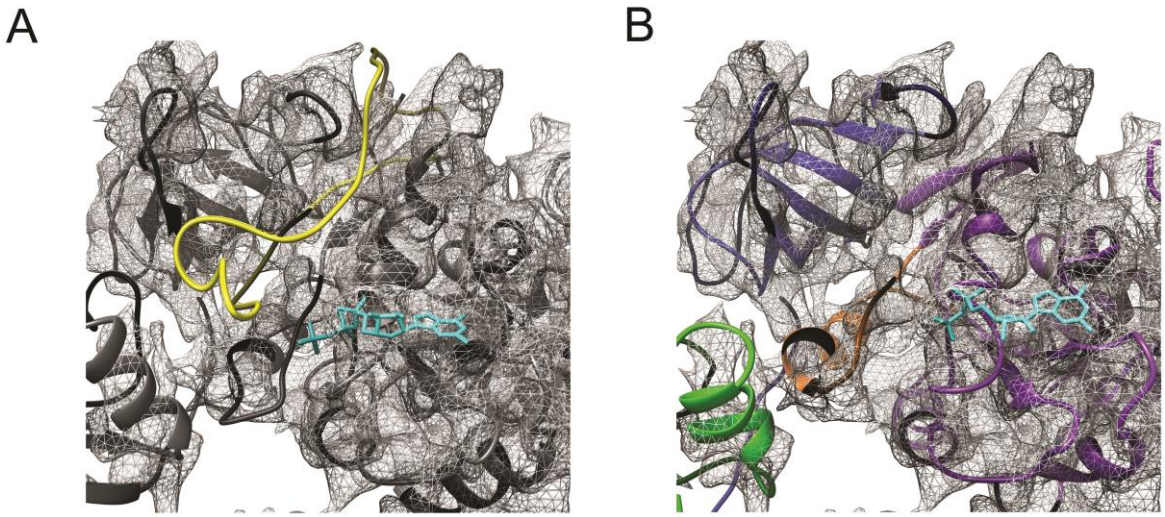

Figure S5: Graphical justification for EF-G•GDP state
(**A**) Rigid body fit of the atomic model of EF-G•GDPNP (from PDB accession code 4V9K) in our cryo-EM map. Switch I is yellow and the GDPNP is cyan. (**B**) Local fit of our EF-G•GDP model in the same map. EF-G domains I, II and III are purple, blue and green, respectively. Switch II is orange and the GPD is cyan. The catalytic domain of EF-G is well resolved and the GDP perfectly fits in the electron density map. On the contrary, no density can be attributed to the third pyrophosphate or to the switch I, which must be disordered. Thus, they were not included in our atomic model.

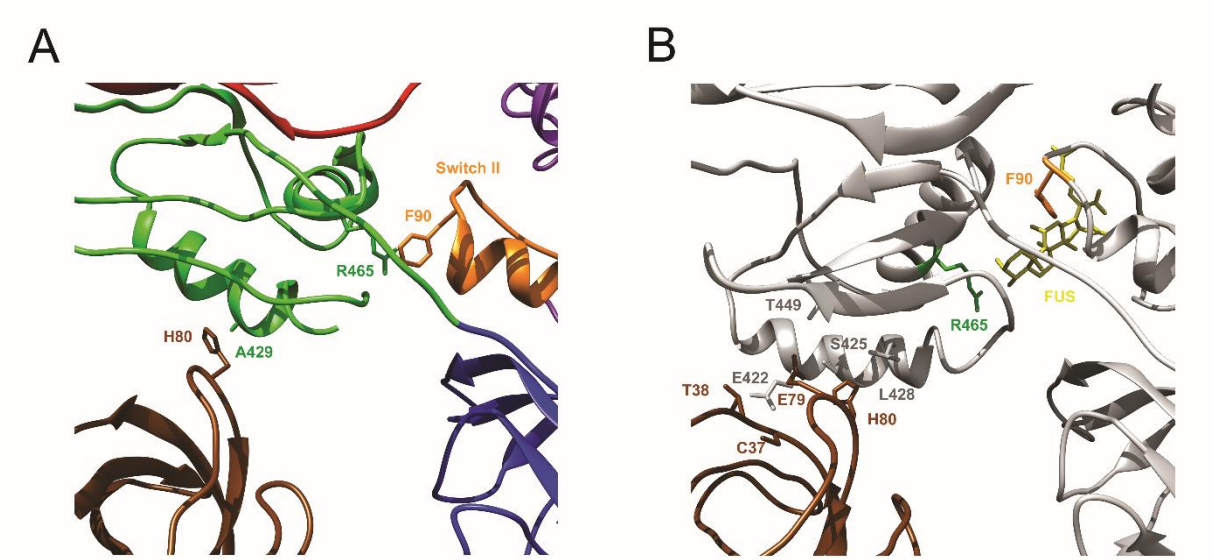

Figure S6: Loss of the interactions between domain III and uS12 ribosomal protein, after GTP hydrolysis.
(**A**) Cartoon representation of the interactions between EF-G·GDP domain III and uS12. EF-G domain I is purple, II is blue, III is green and V is red. The switch II is orange and uS12 is brown. (**B**) Cartoon representation of the same interactions in a 70S ribosome translocation intermediate state trapped with fusidic acid (PDB accession code 4V9L). EF-G is gray, uS12 is brown and fusidic acid is yellow. The two figures also include R465, F90 and the residues at the interface (distance < 3Å) between domain III and uS12. This illustrates the loss of interactions in our structure after GTP hydrolysis. Only one interaction between uS12 H80 and EF-G A465 is observed, while the interface between uS12 and EF-G is made of several residues (uS12 C37, T38, E79, H80 and EF-G E422, S425, L428, T449) when the ribosome is trapped with fusidic acid.

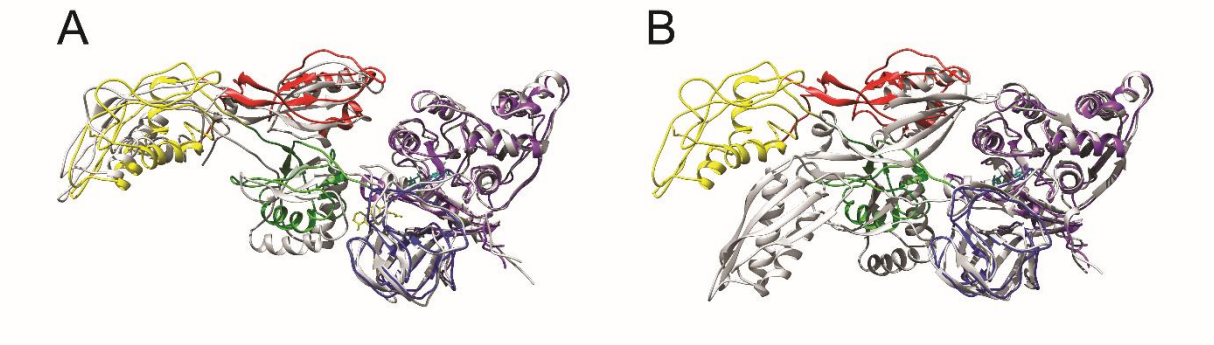

Figure S7: Comparison between EF-G•GDP on the translating ribosome and in solution
(**A**) Comparison between EF-G•GDP in our structure (domain I is purple, II is blue, III is green, IV is yellow, V is red and GDP is cyan) and trapped on the translating ribosome with fusidic acid (EF-G is gray and GDP is cyan and fusidic acid is yellow) (PDB accession code 4V9L). (**B**) Comparison between EF-G•GDP in our structure and in solution (EF-G is gray and GDP is cyan) (PDB accession code 2BM0). The three structures are aligned using EF-G domains I and II.

Data collection / processing	
Microscope	Titan Krios
Camera	Falcon II
Voltage (kV)	300
Defocus range (μm)	-0.4, -2,7
Exposure time per image (s)	1
Dose rate per image ($e^{-1}Å^{-2}$)	45
Original Pixel size (Å)	1.1624
Reassessed Pixel size (Å) used in processing	1.1025
Number of images used in processing	949
Particles processed	96509
Particles refined	32383
Resolution (unmasked, Å)	5.26
Resolution (masked and sharpened, Å)	3.80
Map sharpening B-factor ($Å^2$)	-64.5692
Refinement / model quality assesment	
Cell dimensions	
a = b = c (Å)	420
a = b = g (°)	90
Resolution (Å)	4.3
Overall score	1.97
Clashscore	6.45
Ramachandran favored (%)	87.23
Ramachandran outliers (%)	0.27
Rotamer outliers (%)	0.54
Bad bonds proteins (%)	0.01
Bad angles proteins (%)	0.12
Bad bonds RNA (%)	0.01
Bad angles RNA (%)	0.02
Bad RNA backbone conformations (%)	21.85
Correlation coefficient (model vs map %)	77.9 (3.8 Å resolution)
Overall FSC (model vs map)	0.782

Table S1: Data processing and refinement statistics. Values for model geometry were calculated with MolProbity (33)**.**

Movie S1: Conformational changes of EF-G upon GTP hydrolysis
Comparison between EF-G•GDP in our structure and trapped on the translating ribosome with fusidic acid (PDB accession code 4V9L). The five (I-V) domains of EF-G are purple, blue, green, yellow, and red, respectively.

Movie S2: Conformational change of EF-G upon GTP hydrolysis in the context of the translating ribosome
Same as supplementary movie 1, but with electron density map of the translating ribosome (gray).