Supplementary Data

Table S1: Statistics of cryo-EM data collection, refinement and validation

Data collection and processing	
Magnification	100720x
Acceleration voltage (kV)	300
Electron exposure ($e^-/Å^2$)	35
Defocus range (μm)	-0.7 to -3.0
Pixel size (Å)	1.39
Initial particle images (no.)	857,260
Final particle images (no.)	165,073
Map resolution (Å)	3.1
FSC threshold	0.143
Refinement	
Model resolution (Å)	3.2
FSC threshold	0.5
Map sharpening B-factor ($Å^2$)	-141.3
Model composition	
Non-hydrogen atoms	34,436
Protein residues	1,730
RNA residues	964
Mg^{2+}	167
B-factors (min/max/mean)	
Protein	36/209/77
RNA	28/177/64
Ligand	24/103/51
R.m.s. deviations	
Bond lengths (Å)	0.002
Bond angles (°)	0.328
Validation	
MolProbity score	1.35
Clashscore	5.88
Poor rotamers (%)	0.98
Ramachandran plot	
Favored (%)	97.88
Allowed (%)	2.12
Disallowed (%)	0

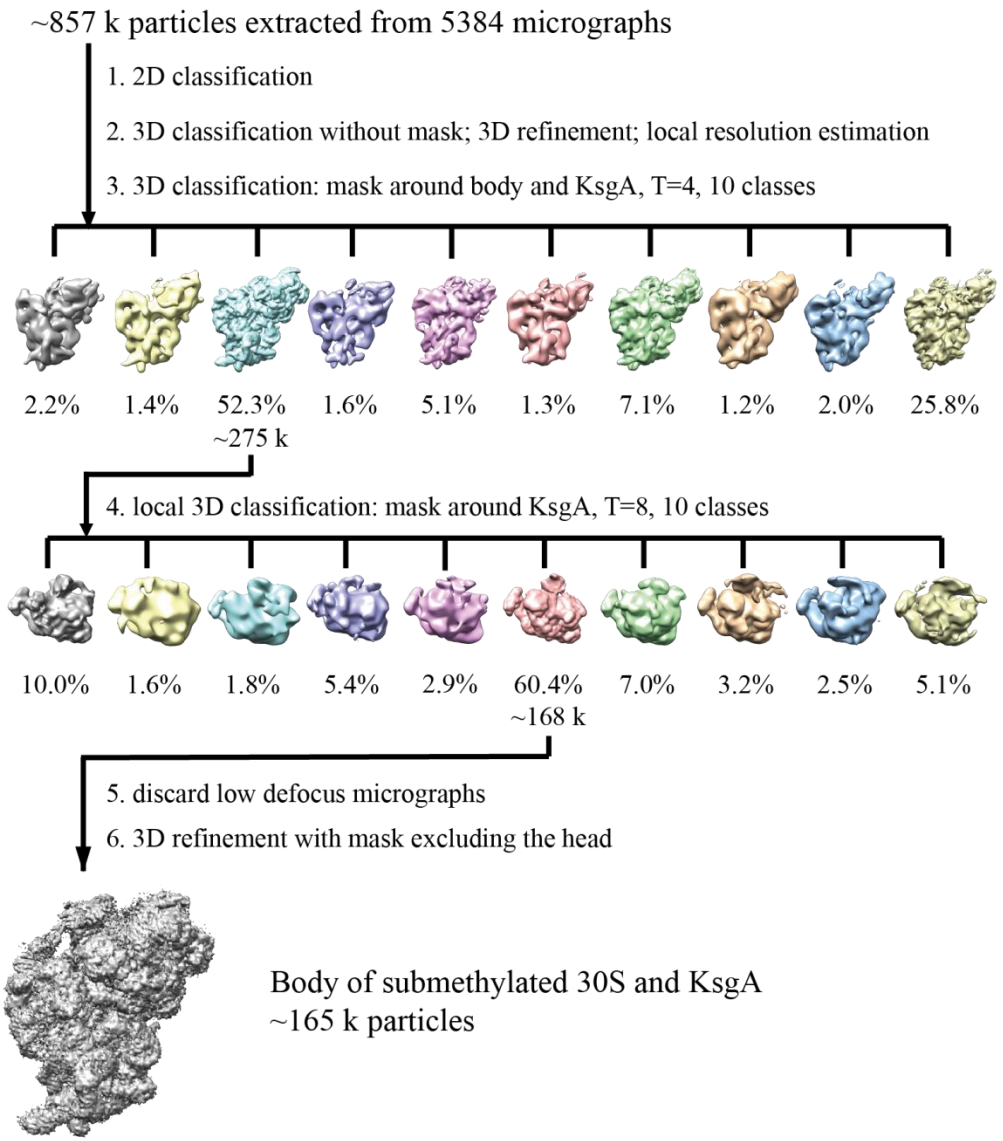

Figure S1. Data processing workflow

More than 850k particles were extracted from all images and subjected to 2D classification using Relion 2.1 (31). Projections showing small subunits were selected for 3D classifications. The first 3D classification was pooled and used for mask creation. A second 3D classification was focused on the body and KsgA. A third classification was focused on KsgA and its surrounding. All 3D classifications were carried out without image alignment following a 3D refinement of the 30S body. After sorting out micrographs with too low defocus the remaining 165073 particles were subjected to a final masked 3D refinement.

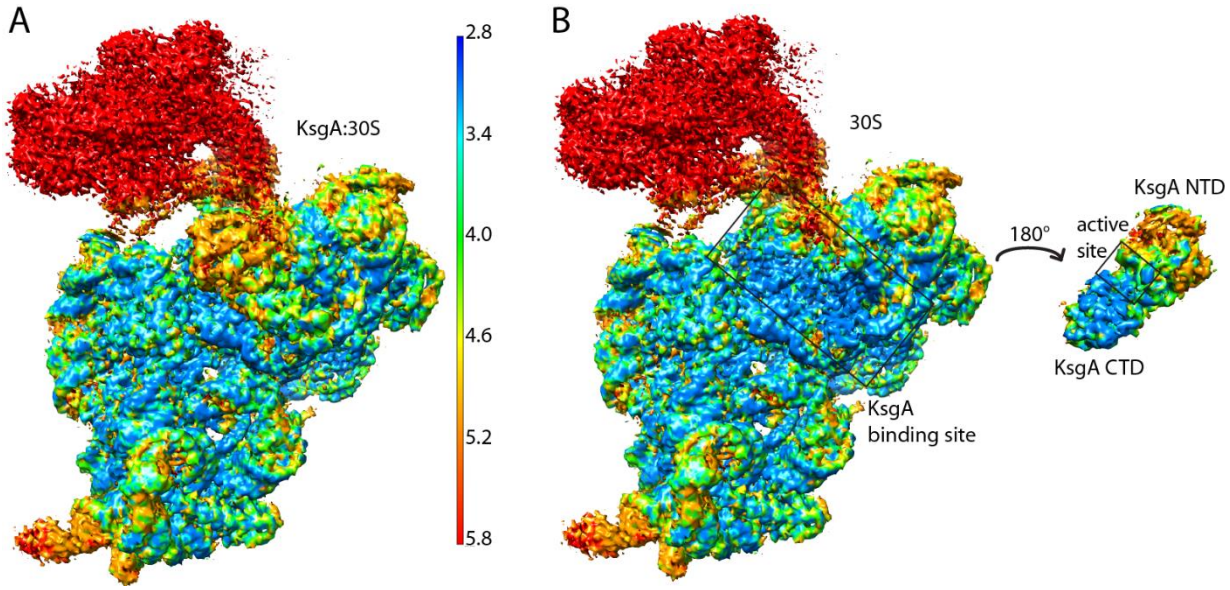

Figure S2. Local resolution EM map of KsgA:30S

(A) EM map of KsgA in complex with the hypomethylated 30S. Local resolution was calculated with Resmap (37). The head and toe of the 30S were due to high flexibility reconstructed only at low resolution and thus not built in the model. Legend connects colors to resolution values in Å. (B) EM map of hypomethylated 30S with KsgA density cut out and rotated by 180° to display local resolutions at the interacting surface areas of KsgA and the 30S. Color legend is the same as in (A).

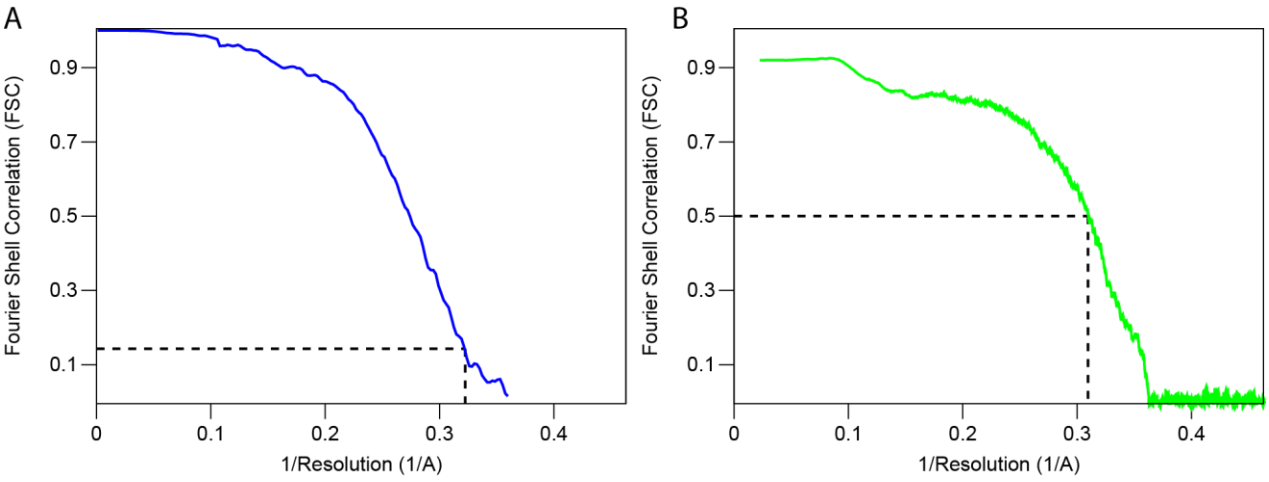

Figure S3. FSC curves

(A) Half map vs. half map FSC curve (blue) of KsgA in complex with the 30S, threshold 0.143.
(B) Map vs. model FSC curve of (green) KsgA in complex with the 30S, threshold 0.5

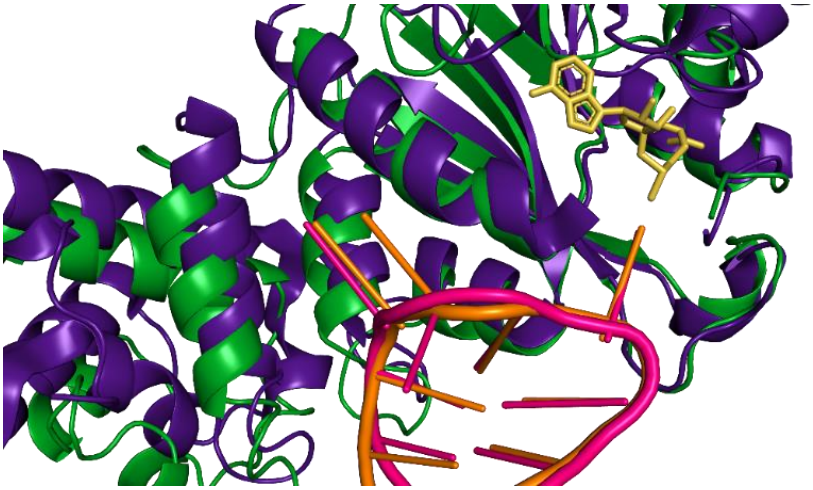

Figure S4. Overlay of KsgA – helix 45 structure with human mitochondrial mtTFB1 – helix 45 structure

The structure presented in this publication overlaid with the crystal structure of mtTFB1 bound to helix 45 (PDBID: 6AAX, (29)) using the helix 45 apical loop in both structures for reference. KsgA green, KsgA-helix 45 orange, mtTFB1 purple, mtTFB1-helix 45 pink, mtTFB1-SAM yellow. Position and conformation of both helices 45 are very similar except for the position of G1517 and its homolog, which underlines the potential flexibility of this base.

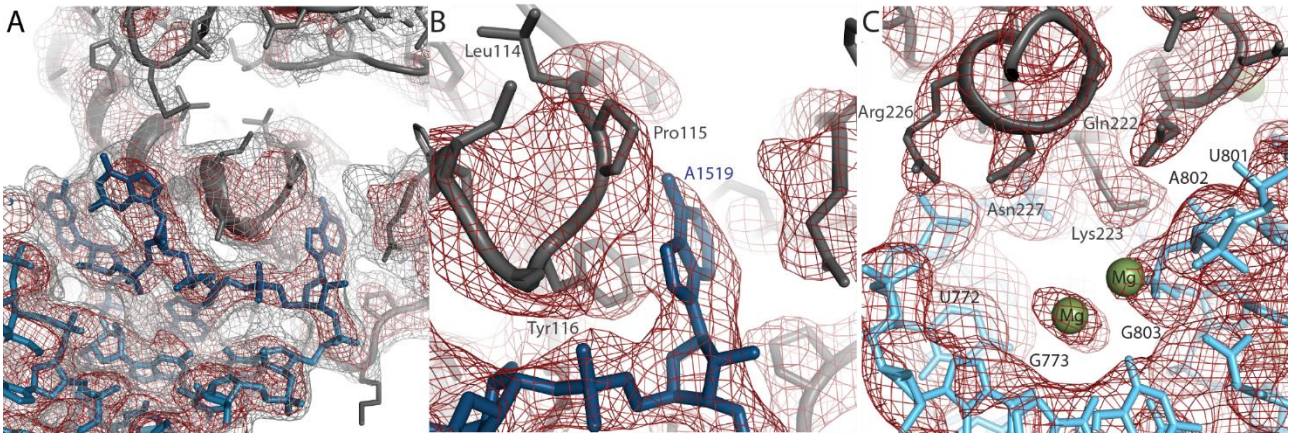

Figure S5. EM density of KsgA:h45 and KsgA:h24

(A) EM density of the helix 45 tetraloop (dark blue), rRNA in the same region (medium blue) and KsgA (grey). EM density is displayed at two levels: Red = 5.3σ, grey = 3.5σ. View is identical to figure 3.

(B) EM density (red) of A1519 (dark blue) in KsgA catalytic site (grey). EM density level is 4.8σ.

(C) EM density (red) of KsgA (grey) and helix 24 (light blue) with magnesium ions displayed as green spheres. EM density level is 5.3σ.