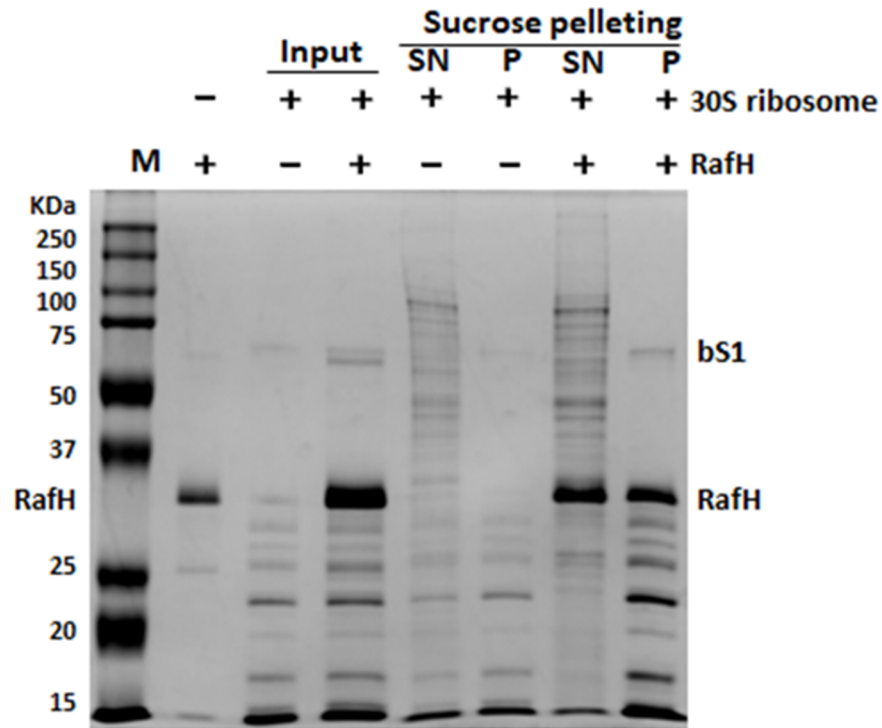
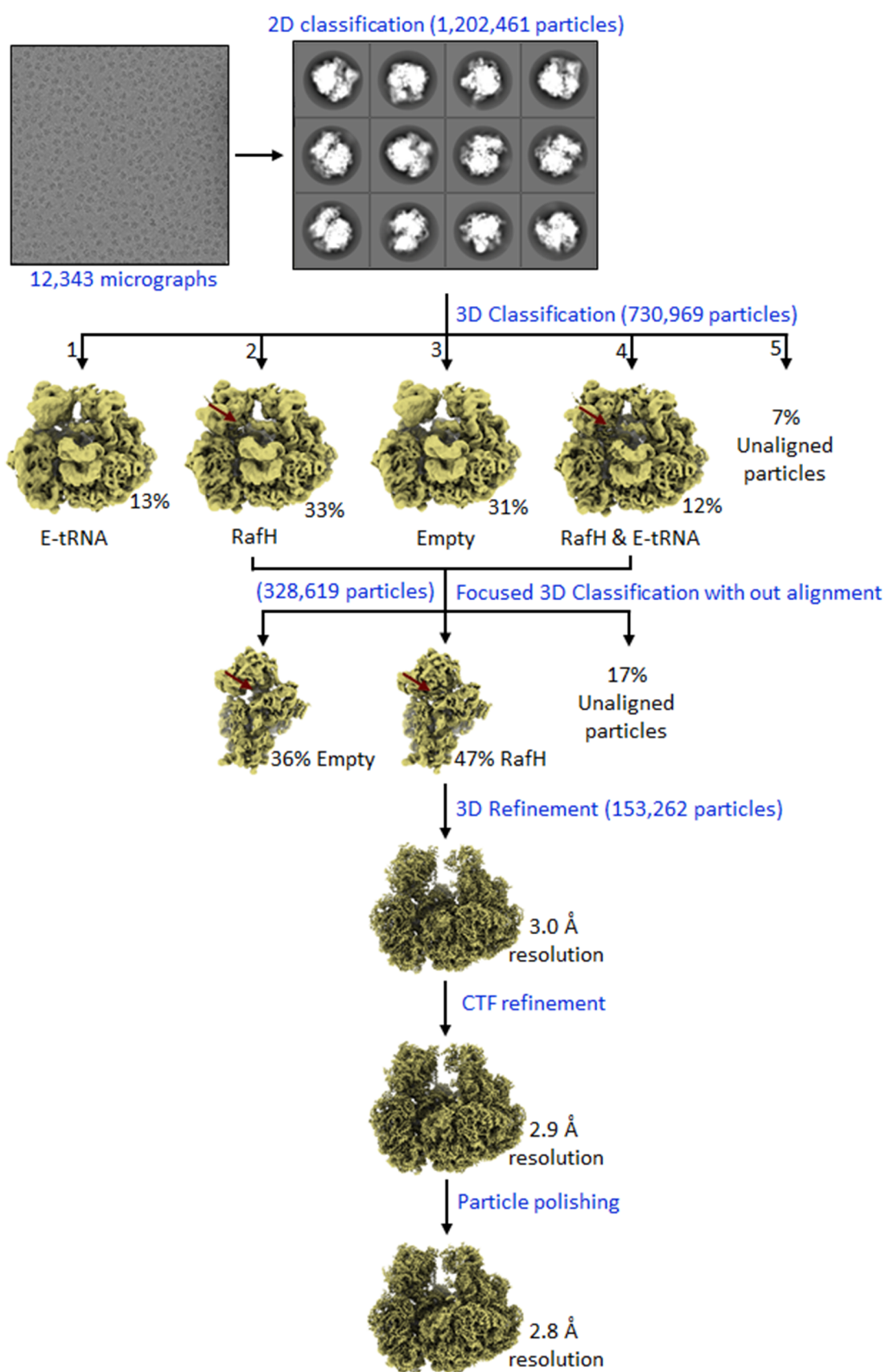
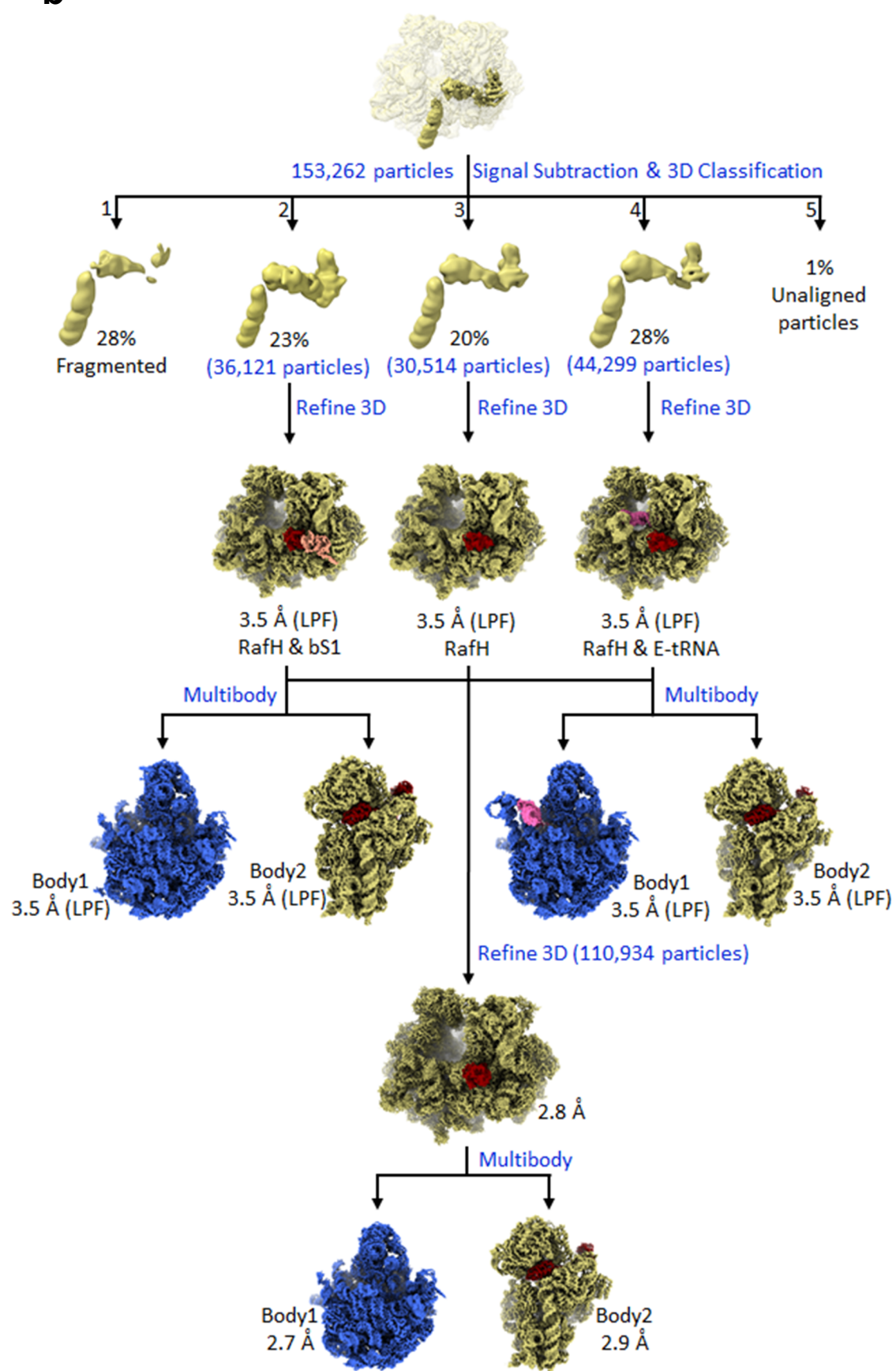


Supplementary Figure 1. Hibernation promotion factor domain organization. The domain organization of HPF, N-terminus domain (black), C-terminus domain (grey), connecting linker (black), and ribosome modulation factor (RMF) (grey) are shown. The right side shows the ribosome hibernation state 100S (disome) or 70S (monosome). (a) HPF^{long}, (b) HPF^{short} and RMF, (c) YfiA, (d) MPY from *M. smegmatis* and *M. tuberculosis*, (e) RafH from *M. smegmatis* and *M. tuberculosis* are shown.



Supplementary Figure 2. Sucrose pelleting assay. The 30S ribosomes RafH complex formation by sucrose cushion pelleting assay analyzed on 12% SDS-PAGE, and stained with coomassie blue staining solution. Lane 1 - marker, lane 2 - pure RafH protein, lane 3, 4 - input, lane 5 to 8 - supernatant and pellet fraction after pelleting on a 0.8 M sucrose cushion. The source data for Supplementary Figure 2 is provided in the source data file.

a

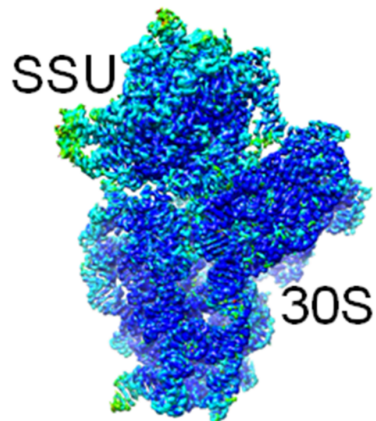
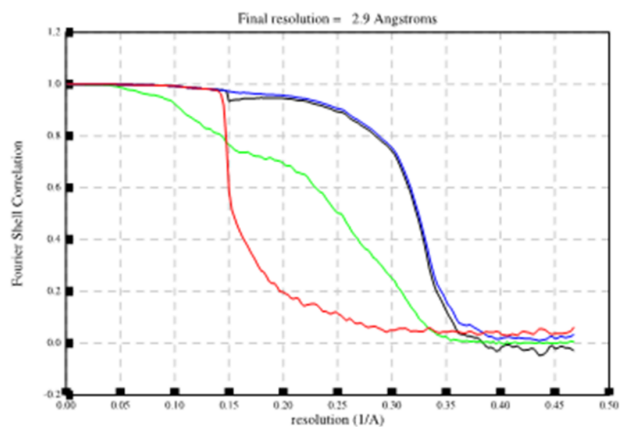
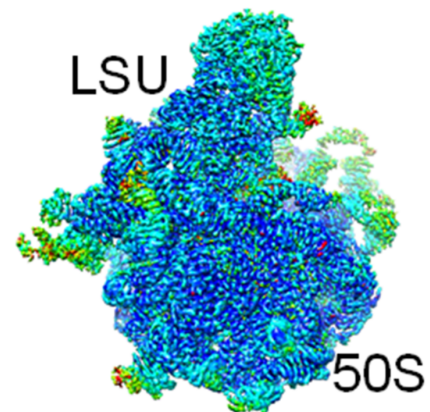
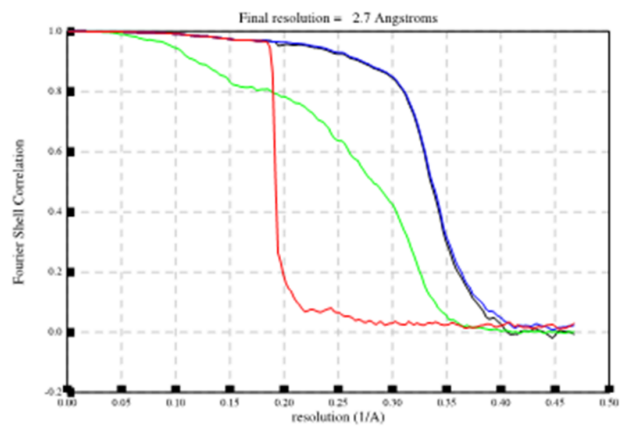
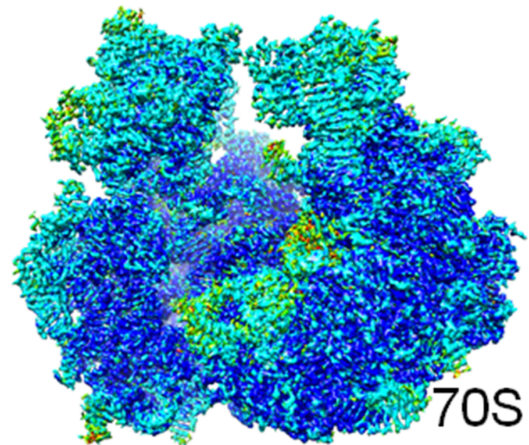
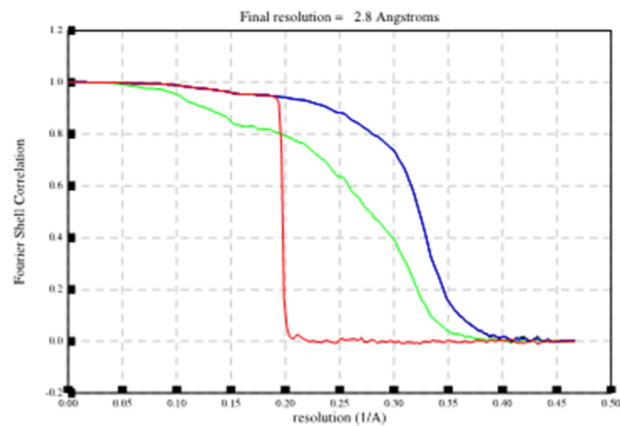
b

Supplementary Figure 3. Summary of single particle reconstruction. (a) 2D classification, 3D classification and initial consensus 3D maps and particle polishing are shown. The RafH NTD binding site is shown in a maroon arrow. (b) Signal subtraction with 3D classification without alignment and final multi-body refinement are shown. Maps were low pass filtered (LPF). All steps were performed using Relion 3.1.4.

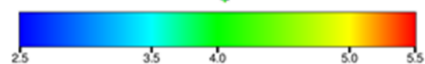
Supplementary Table 1. Cryo-EM data collection, refinement, and validation statistics

| | 70S & RafH EMDB-37551 PDB-8WHX | 50S (body1) EMDB-37552 PDB-8WHY | 30 & RafH (body 2) EMDB-37565 PDB-8WIF | 70S, RafH & bS1 EMDB-37559 PDB-8WI7 | 50S (body1) EMDB-37560 PDB-8WI8 | 30S, RafH & bS1 (body2) EMDB-37561 PDB-8WI9 | 70S, RafH & tRNA EMDB-37562 PDB 8WIB | 50S & tRNA (body1) EMDB-37563 PDB-8WIC | 30S, RafH (body2) EMDB-37564 PDB- 8WID |
|--|--------------------------------------|---------------------------------------|---|--|---------------------------------------|--|---|---|---|
| Data collection & processing | | | | | | | | | |
| Magnification | 70,000 | | | | | | | | |
| Voltage (kV) | 300 | | | | | | | | |
| Electron exposure (e-/Å ²) | 1.34 | | | | | | | | |
| Defocus range (µm) | -0.5 – -3.0 | | | | | | | | |
| Pixel size (Å) | 1.07 | | | | | | | | |
| Symmetry imposed | C1 | | | | | | | | |
| Initial particle images (no.) | 1,202,461 | 1,202,461 | 1,202,461 | 1,202,461 | 1,202,461 | 1,202,461 | 1,202,461 | 1,202,461 | 1,202,461 |
| Final particle images (no.) | 110,934 | 110,934 | 110,934 | 36,121 | 36,121 | 36,121 | 44,299 | 44,299 | 44,299 |
| Map resolution (Å) | 2.8 | 2.7 | 2.9 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| FSC threshold | 0.143 | 0.143 | 0.143 | LPF | LPF | LPF | LPF | LPF | LPF |
| Map resolution range (Å) | 2.5-5.0 | 2.5-5.0 | 2.5-5.0 | 3.5 -5.5* | 3.5-5.5* | 3.5-5.5* | 3.5-5.5* | 3.5-5.5* | 3.5-5.5* |
| Refinement | | | | | | | | | |
| Initial model used (PDB code) | 6DZI | 8WHX | 8WHX | 8WHX | 8WHX | 8WHX | 8WHX | 8WHX | 8WHX |
| Map sharpening <i>B</i> factor (Å ²) | -66.2 | -55.98 | -67.15 | 2.88 | 2.68 | 3.73 | 0.35 | 0.56 | 2.4 |
| Model composition | | | | | | | | | |
| RNA nucleotides | 4760 | 3237 | 1523 | 4760 | 3237 | 1523 | 4836 | 3313 | 1523 |
| Protein residues | 11094 | 6918 | 4176 | 11263 | 6918 | 4345 | 11094 | 6918 | 4176 |
| R.m.s. deviations | | | | | | | | | |
| Bond lengths (Å) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Bond angles (°) | 1.56 | 1.33 | 1.21 | 1.64 | 1.41 | 1.25 | 1.73 | 1.42 | 1.31 |
| Validation | | | | | | | | | |
| MolProbity score | 1.95 | 1.82 | 2.11 | 2.2 | 2.0 | 2.4 | 2.4 | 2.3 | 2.6 |
| Clashscore | 3.96 | 3.22 | 4.1 | 3.9 | 3.1 | 4.1 | 3.8 | 2.5 | 3.9 |
| Poor rotamers (%) | 2.1 | 2.98 | 2.5 | 2.5 | 2.3 | 2.7 | 2.6 | 2.4 | 2.8 |
| Ramachandran plot | | | | | | | | | |
| Favored (%) | 95.5 | 96.0 | 95.5 | 95.0 | 97.0 | 95.7 | 96.0 | 96.7 | 96.0 |
| Allowed (%) | 4.5 | 4.0 | 4.5 | 5.0 | 3.0 | 4.3 | 4.0 | 3.3 | 4.0 |
| Disallowed (%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

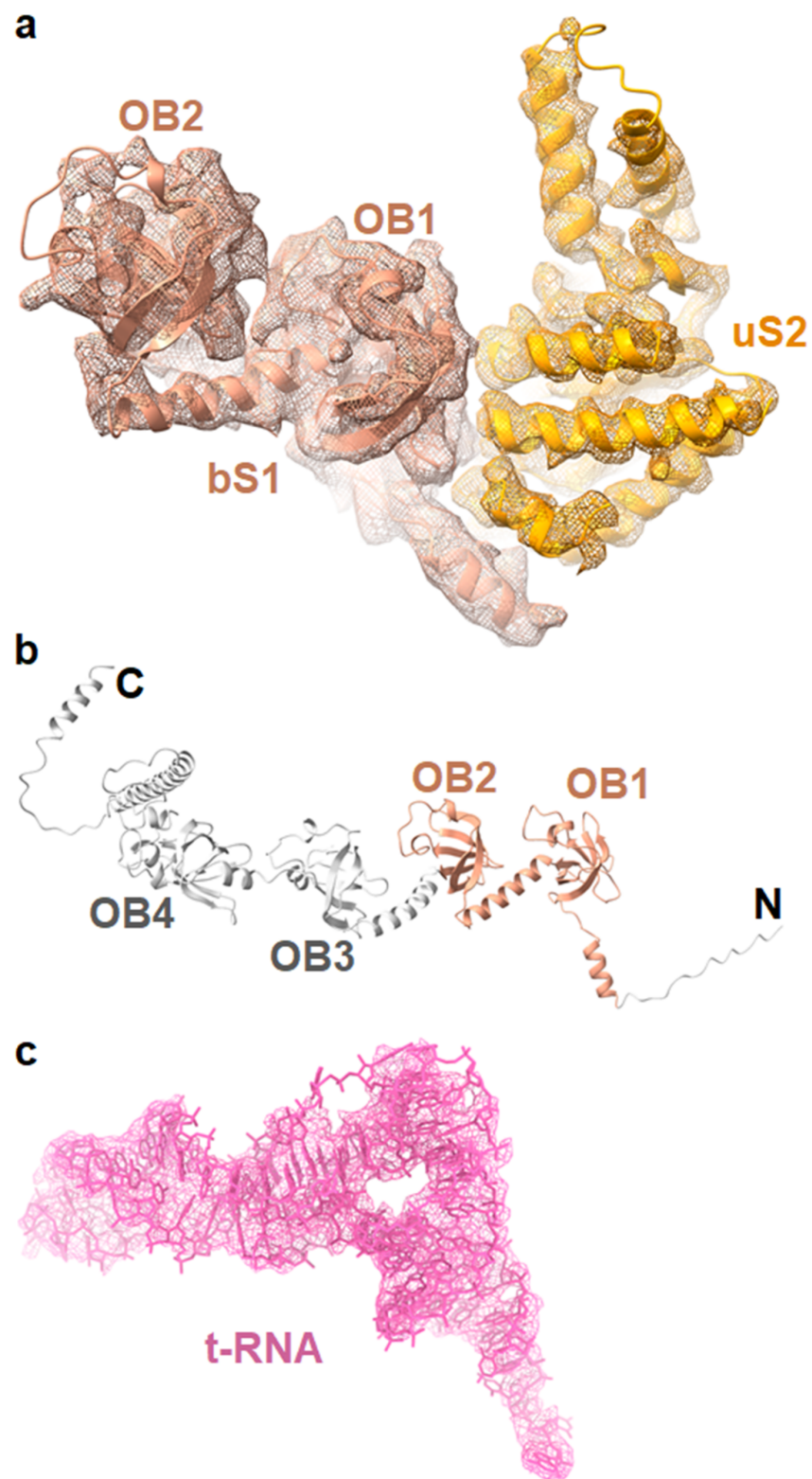
* Ad-hoc Low pass filter (LPF) 3.5 Å was applied to the final map



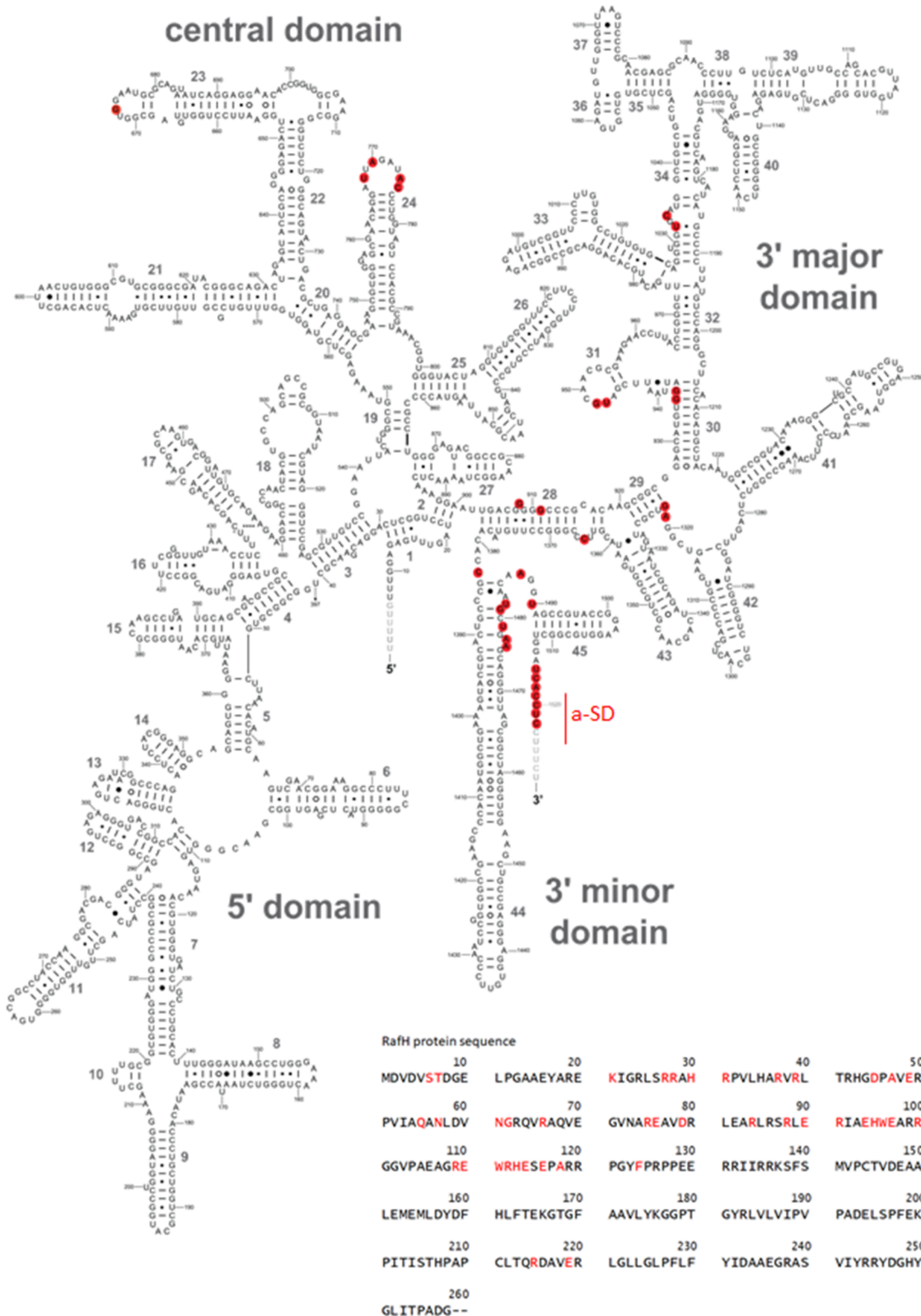
— Corrected
 — Unmasked Maps
 — Masked Maps
 — Phase Randomized Masked Maps



Supplementary Figure 4 Fourier Shell Correlation and local resolution. FSC for the 70S, 50S and 30S at top, middle, and bottom, respectively, shown in the left panel. The local resolution for the 70S, 50S, and 30S at the top, middle, and bottom, respectively, is shown in the right panel. The color bar for resolution is shown at the bottom right.



Supplementary Figure 5 Cryo- EM density and models. (a) the cryo- EM density corresponds to the OB1 and OB2 domains of bS1, and uS2 in mesh, and their models in the ribbon are shown. (b) the full-length structure of bS1 predicted using AlphaFold2. The domains the OB1 and OB2 (dark salmon) and unmodeled regions (grey) are shown. (c) the E- tRNA cryo- EM density in mesh (pink) and model in the sticks (pink) is shown.

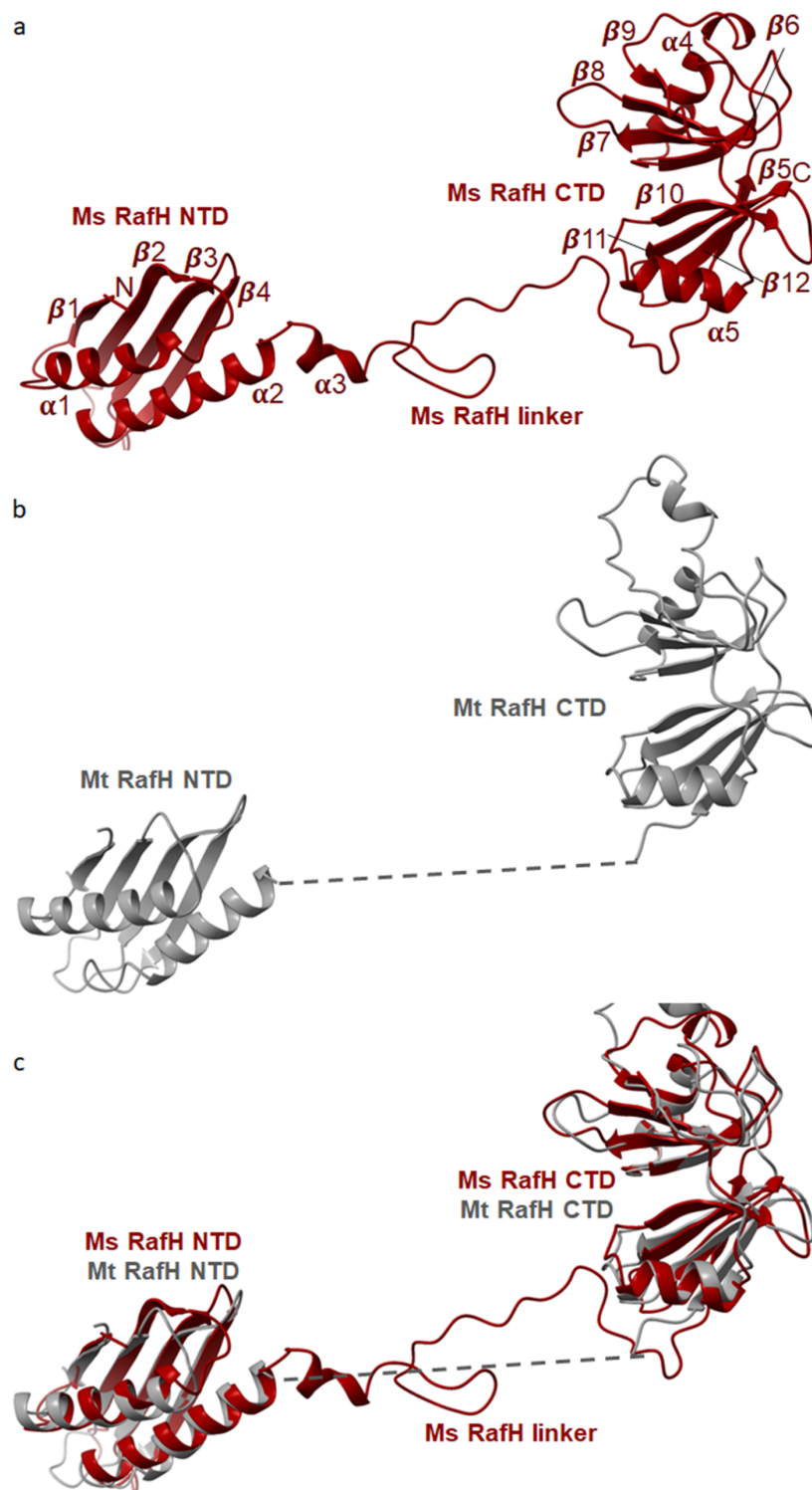


Supplementary Figure 6 16S rRNA and RafH interaction. The 16S rRNA 2D diagram and RafH sequence are shown. The RafH amino acid residues (red) and nucleotides (highlighted in red) are involved in interactions. The template for 16S rRNA 2D diagram was adopted from (Hentschel *et al.*, 2017)⁴⁰.

Supplementary Table 2 Interactions between RafH protein and rRNA & r-proteins and comparison with Mtb.

| S.NO | Ms_RafH (Mt_RafH) | Ms_16S rRNA (Mt_16S rRNA) | Ms_r-proteins (Mt_r-proteins) |
|------|-------------------|------------------------------------|--|
| 1 | SER 6 (PHE 27) | G935 (G946) | |
| 2 | THR 7 (SER 28) | G936 (G947) | |
| 3 | LYS 21 (ALA 43) | G1478 (G1487) | |
| 4 | ARG 24 (ARG 46) | U1479 (U1488) | |
| 5 | ARG 27 (ASP 50) | A770 (A781) | |
| 6 | ARG 28 (ARG 51) | G1481/A770 (G1490/A781) | |
| 7 | HIS 30 | A770 (A781) | |
| 8 | ARG 31 | A35 E-tRNA | |
| 9 | LEU 34 (GLY 55) | A1321 (A1331) | |
| 10 | HIS 35 (GLY 55) | C1211 (C1222) | uS9 ARG 150 (uS9 ARG 150) |
| 11 | ARG 37 (ARG 58) | U947 (U958) | |
| 12 | ARG 39 (ARG 58) | U947 (U958) | |
| 13 | ASP 45 | U1032/C1334 (U1043 /C1344) | |
| 14 | GLU 49 | G510 (G521) | |
| 15 | GLN 55 (GLN 74) | G948 (G959) | |
| 16 | ASN 57 (ASN 76) | G948 (G959) | |
| 17 | ASP 59 (GLN 78) | - | uS9 ARG 150 (uS9 ARG 150) |
| 18 | ASN 61 (GLY 80) | A1321 (A1331) | |
| 19 | GLY 62 (ASP 81) | G1322 (G1332) | |
| 20 | GLN 64 (PRO 83) | - | uS9 LYS 149 (uS9 LYS 149) |
| 21 | ARG 66 (ARG 85) | G948 (G959) | |
| 22 | ARG 75 (ASP 94) | A1477 (A1486) | |
| 23 | GLU 76 (ASP 95) | A1477 (A1486) | |
| 24 | ASP 79 (ARG 97) | A1477 (A1486) | |
| 25 | ARG 80 (PRO 98) | A1476 (A1485) | |
| 26 | ARG 84 (ARG 102) | G1384 (G1394) | |
| 27 | ARG 88 (GLN 106) | C1383 (C1393) | |
| 28 | GLU 90 (VAL 108) | U1482 (U1491) | |
| 29 | ARG 91 (ARG 109) | C1382/G908 (C1392/G919) | |
| 30 | GLU 94 (ALA 112) | C34 E-tRNA | |
| 31 | HIS 95 (GLN 113) | C34 E-tRNA /U769 (U780) | |
| 32 | TRP 96 (TRP 114) | G673 (G685) | |
| 33 | GLU 97 (CYS 115) | C775/A774/U768 (C786/A785/U779) | |
| 34 | ARG 100 | C775/U1490 (C786/U1499) | uS11 ARG 137, VAL 138 (uS11 ARG 137, VAL 138) |
| 35 | ARG 109 (ARG 117) | C1517 (C1526) | |
| 36 | GLU 110 (PRO 118) | U1516 (U1525) | |
| 37 | TRP 111 (TRP 119) | A1518 | |
| 38 | ARG 112 (PRO 120) | G909/A1487 (G920/A1496) | |

| | | | |
|-------------|--------------------------|----------------------------------|-----------------------------|
| 39 | HIS 113 (ASP 121) | G911 (G922) | |
| 40 | GLU 114 (ARG 122) | C1365 (C1375) | |
| 41 | ARG 120 (ARG 125) | C1519/C1520 | |
| 42 | PRO 121 | U1521 | |
| 43 | PHE 124 | C1522 | |
| 43 | ARG 215 (ALA 223) | - | bS18 GLU 44 (bS18 GLU 44) |
| 44 | GLU 219 (ASP 227) | - | bS18 ARG 45 (bS18 ARG 45) |
| S.NO | Ms_RafH (Mt_RafH) | Ms_23S rRNA (Mt_23S rRNA) | Inter-subunit bridge |
| 1 | ARG 75 (GLU 94) | A2137 (A2151) | B2a |



Supplementary Figure 7. *M. smegmatis* RafH full-length model and its structure comparison with *M. tuberculosis* RafH model. (a) the RafH full-length structure in ribbon style with labeled secondary structures, domains, and linker is shown. (b) the *M. tuberculosis* RafH N- and C- terminus domain fold predicted by AlphaFold2 and connecting linker shown in dotted line. (c) the N- and C- terminus domains of *M. smegmatis* RafH (red) superimposed with the N- and C- terminus domains of the *M. tuberculosis* RafH (grey).

```

Ms_RafH 1 .....MDVDVSTDGELP.GAAEYAREKIGR.LS 26
Mt_RafH 1 MEPKRSRLVVCAPESHAREFPDVAVFSGGRANASQAERLARAVGRVLA 49
          ** * :.* . ** . : ** * :

Ms_RafH 27 RRAHRPVLHARVRLTRHGDPAPER....PVIAQANLDVNGRQVRAQVEGV 72
Mt_RafH 50 DRG..VTGGARVRLTMAN.....CADGPTLVQINLQVGDTPLRQAATA 91
          *. . ***** . * : . * * : * . : : ** .

Ms_RafH 73 NAREAVDRLEARLRSRLERIAEHWEARRGGVPAEAGREWRHESEPARRPG 122
Mt_RafH 92 GIDD.LRPALIRLDROIVRASAWC.....PRPWPDR..P.RR.. 125
          . : : * : : * : * : * * * * *

Ms_RafH 123 YFPRPPEERRIIRRSFSMVPCTVDEAALEMMLDYDFHLFTEKGTGFAA 172
Mt_RafH 126 RLITTPAE.ALVTRRKPVVLRATPLQAIAMDAMDYDVLFTDAETGEDA 174
          . * . * : * * . : . * : * : : * * . * * * : * * *

Ms_RafH 173 VLYKGGPTGYRLVLPVPADELSPF.....EKPITISTHPAPCLT 213
Mt_RafH 175 VVYRAGPSGLRLARQHVF..PGW.SRCRAPAGPPVPLIVNSRPTVLT 221
          * : * : * * : * * . * . . * : : : * * : * *

Ms_RafH 214 QDAVERLGLLGLPFLFYIDAAEGRASVIYRRYDGHYGLITPADG..... 258
Mt_RafH 222 EAAAVDRAREHGLPFLFFTDQATGRGQLLYSRYDGNLGLITPT.GDGVAD 270
          : * * : * * * * * : * * * * . : : * * * * : * * * * : *

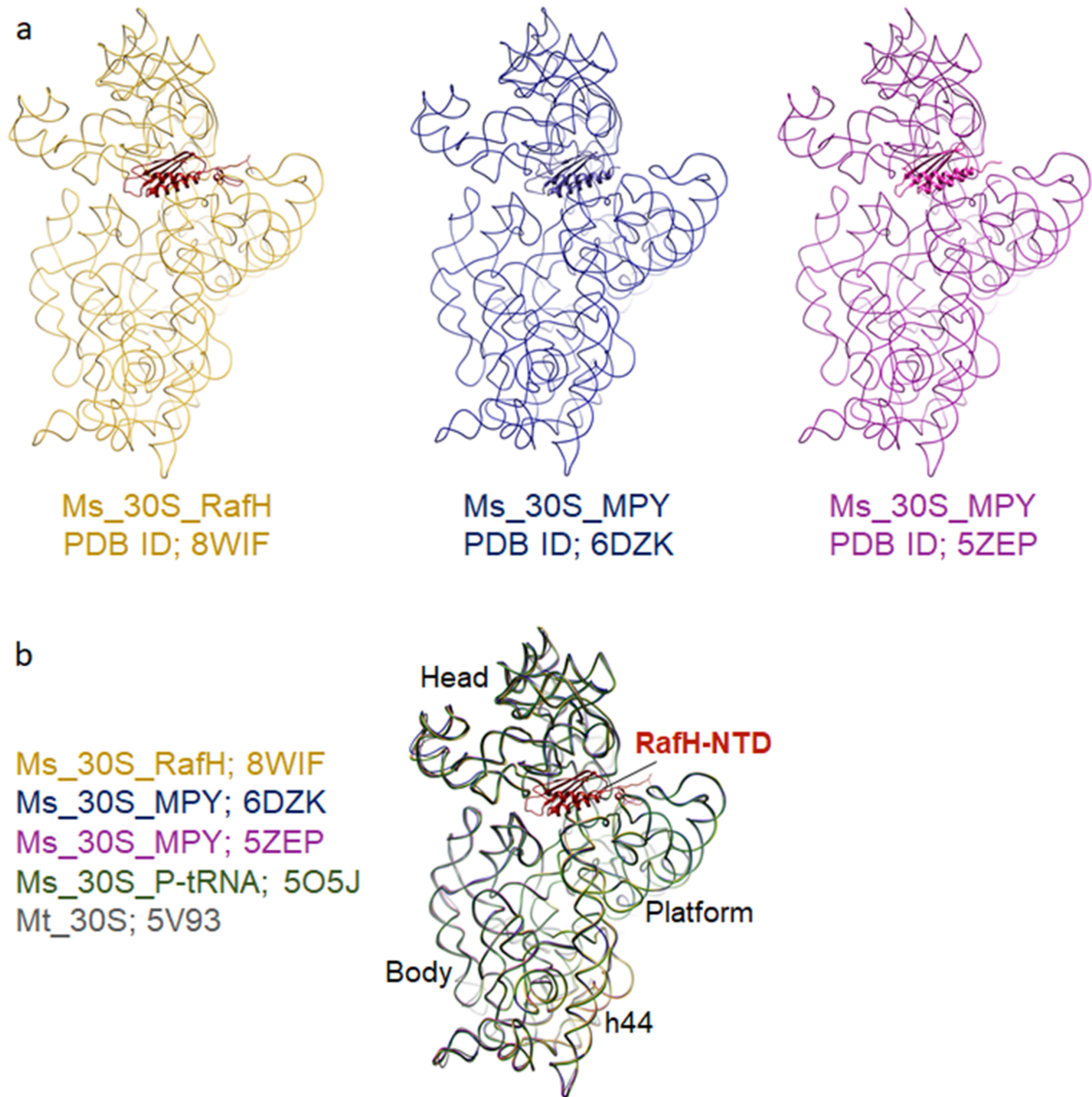
Ms_RafH ...
Mt_RafH 271 GLA 273

```

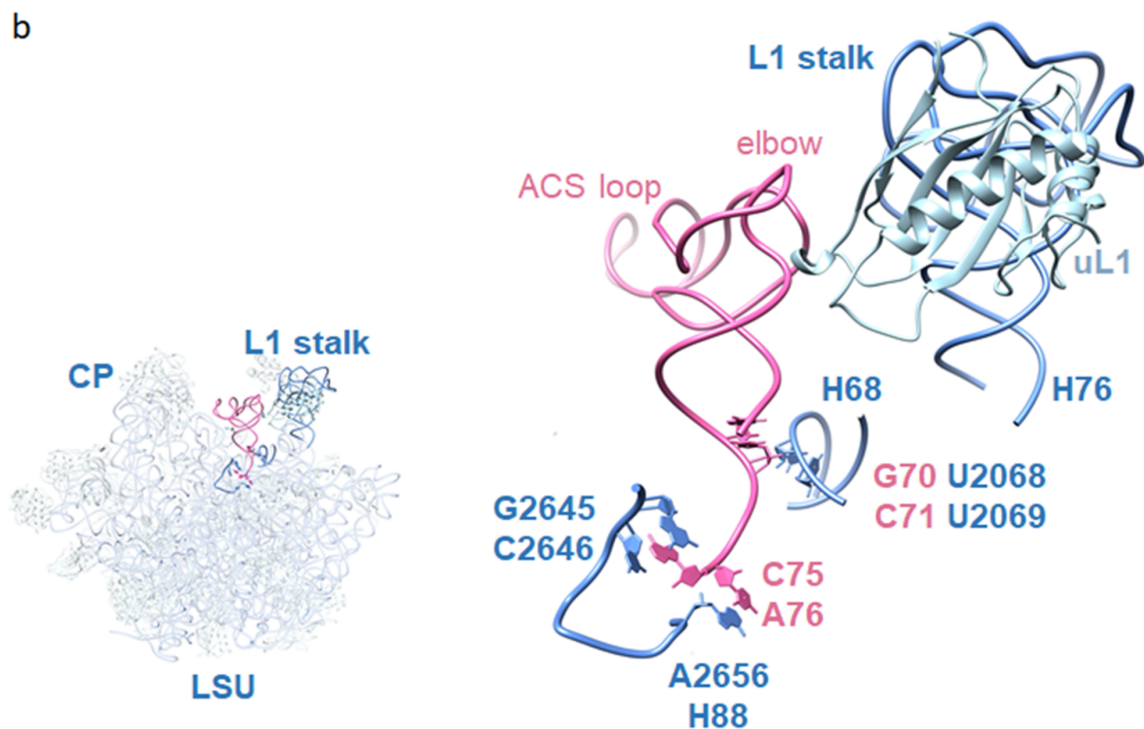
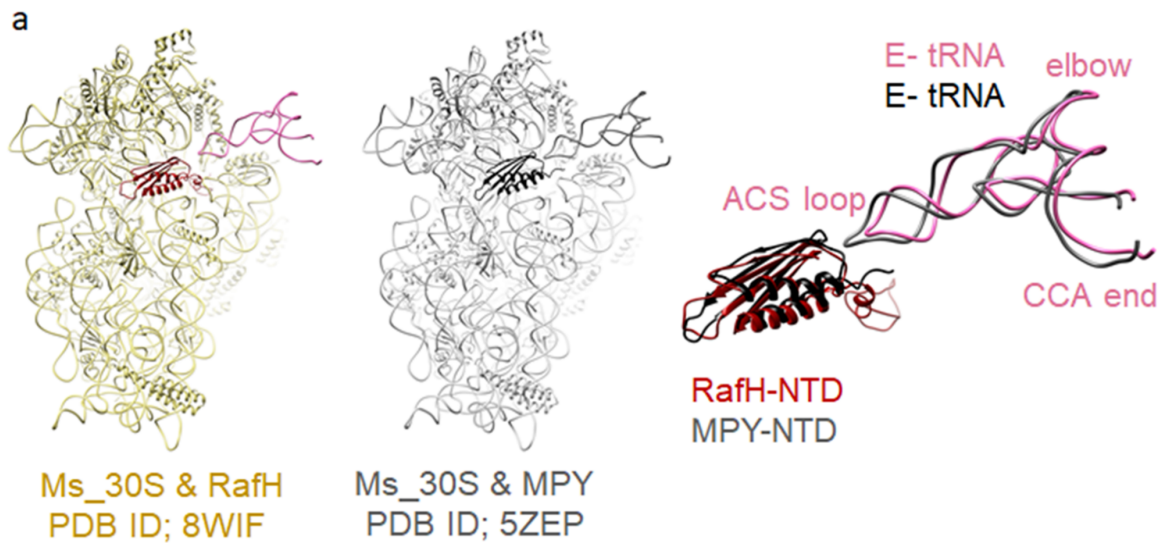
Supplementary Figure 8. Sequence comparison between *M. smegmatis* and *M. tuberculosis* RafH. The alignment carried out using Clustal Omega, between *M. smegmatis* and *M. tuberculosis* RafH sequences. The * and . represents the identical and similar amino acid residues, respectively. The *M. smegmatis* amino acid residues interacting with 30S ribosomal subunit and corresponding residues in *M. tuberculosis* are boxed with a red background.



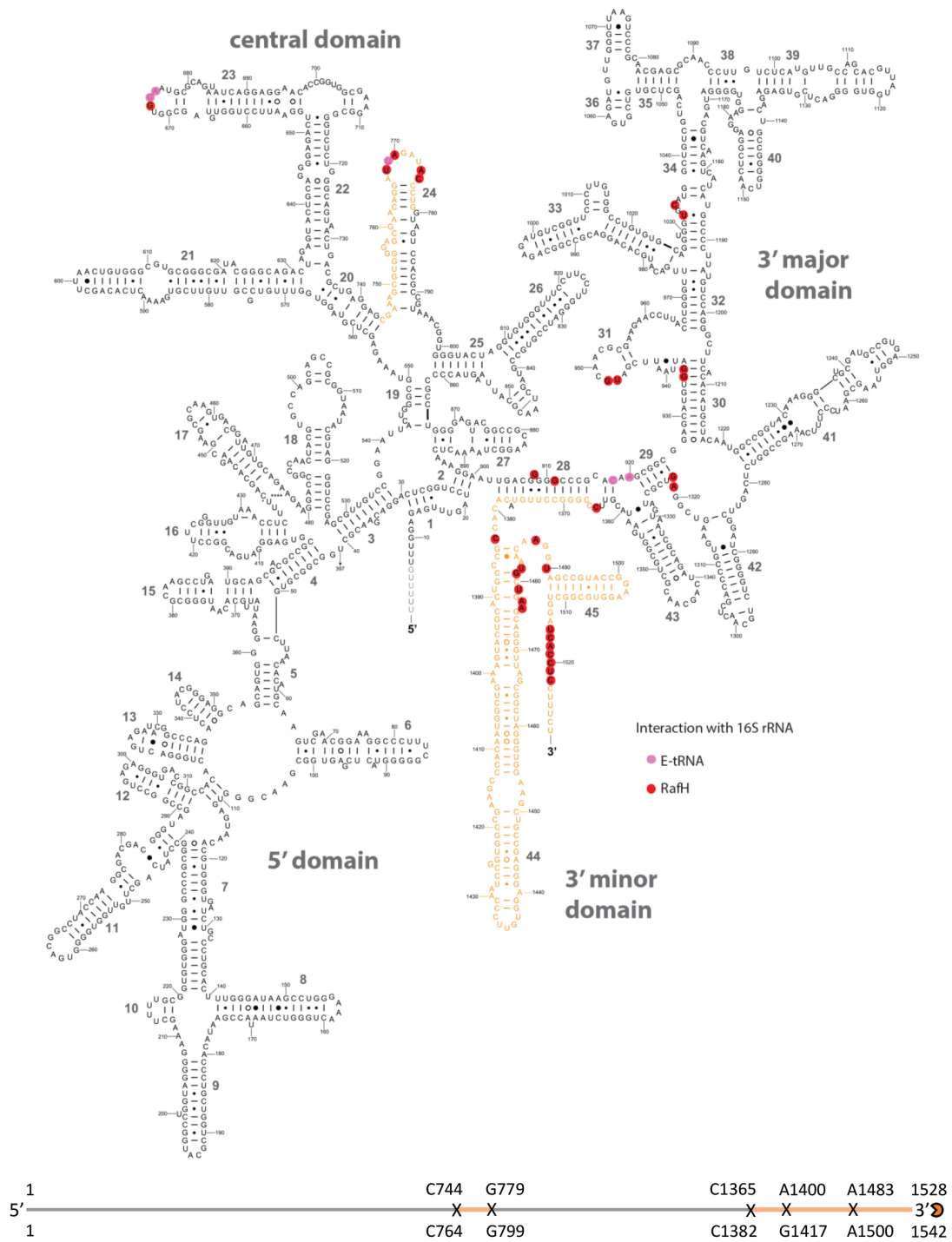
Supplementary Figure 9 Multiple sequence alignments. The multiple sequence alignment for *M. smegmatis* RafH (Ms_RafH), *M. tuberculosis* RafH (Mt_RafH), *M. smegmatis* MPY (Ms_MPY), *S. aureus* HPF^{long} (Sa_1-HPF), *E. coli* YfiA (Ec_YfiA) and *E. coli* HPF^{short} (Ec_s-HPF) with the secondary structures present in RafH (on top) is shown.



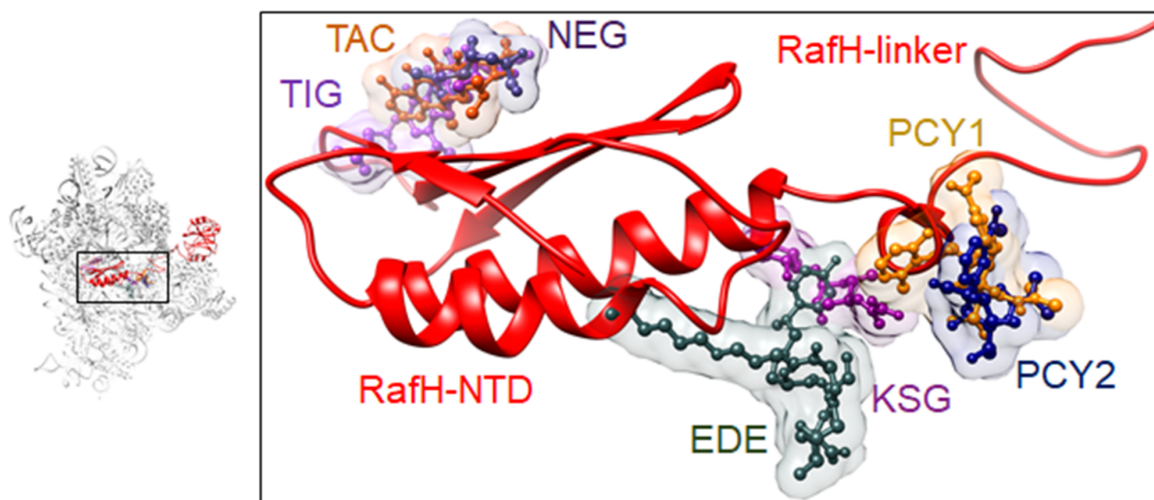
Supplementary Figure 10 Ribosomal small subunit comparison. (a) the structures of *M. smegmatis* SSU (Khaki) bound with RafH (red) (PDB ID; 8WIF) (left panel), bound with MPY (navy blue) (PDB ID; 6DZK), ribosome purified from Zinc starvation (middle panel), and bound with MPY (magenta) (PDB ID; 65ZEP), ribosome purified from stationary phase (right panel) are shown. For comparison, only RafH N- terminus domain is shown. (b) the structures shown in panel ‘a’ are superimposed with other structures of SSU from *M. smegmatis* bound with P- tRNA, translating ribosome, (green) (PDB ID; 5O5J) and SSU from *M. tuberculosis* (grey) (PDB ID; 5V93) are shown. For clarity, the MPY is not shown in superimposed structures.



Supplementary Figure 11. E- site tRNA binding analysis. (a) The structures of SSU (Khaki) bound with RafH (red) and E- site tRNA (hot pink) (PDB ID; 8WIF) in left panel, SSU bound with MPY and E- tRNA (grey) (PDB ID; 5ZEP) in the middle panel, and after superimposing the RafH/MPY and tRNAs in the right panel is shown. For clarity, the SSU is not shown in the right panel. (b) E- site tRNA interactions on LSU is shown. The tRNA CCA end-binding pocket, helix H68, tRNA elbow, H76, uL1 r-protein and L1 stalk are labeled. A thumbnail is shown in left panel where CP (central protuberance) and L1 stalk are labeled.



Supplementary Figure 12. 16S rRNA and RNAase degradation sites. (Top panel) the 16S rRNA 2D diagram. The corresponding nucleotide residues prone to RNAase degradation in *M. smegmatis*, as predicted by Prossliner *et al.*, 2022 in *E. coli*, are shown in orange and other nucleotide residues are shown in grey. The RafH and E- tRNA interacting nucleotides are highlighted in red and pink, respectively. The template for 16S rRNA 2D diagram was adopted from Hentschel *et al.*, 2017. (bottom panel) 16S rRNA is shown in a bar diagram. The RNAase endonuclease site in *E. coli* 16S rRNA and the corresponding site in *M. smegmatis* are labeled below and above the bar, respectively. The regions prone to RNAase degradation are shown in orange. 3' to 5' exonuclease RNase PH/RNase R are shown in an orange Pie shape.



Supplementary Figure 13 RafH NTD and antibiotic binding. The RafH and antibiotic binding in its vicinity on 16S rRNA is shown in the thumbnail on the left side. A magnified view is shown on the right side. The antibiotics were docked onto RafH ribosome 30S. The antibiotics; Tigecycline (TIG) (PDB ID; 4V9B), tetracycline (TAC) (PDB ID; 4V9A), Negamycin (NEG) (PDB ID; 4WF1) Edeine (EDE) (PDB ID; 1I95), Plectamycin1 (PCY1) (PDB ID; 4KHP), Plectamycin2 (PCY2) (PDB ID; 4W2H) and Kasugamycin (KSG) (PDB ID; 4V4H) are shown.

Supplementary Notes 1. List of reagents used in this study.

| Reagent | Manufacturer | Catalogue number |
|--|----------------------|-------------------------|
| HEPES Sodium salt | Sigma aldrich | H3784-500G |
| TRIS hydrochloride (TRIS-HCl) | Himedia | MB030-1Kg |
| Tris(hydroxymethyl)aminomethane (Tris, Free Base) | Himedia | MB029-1Kg |
| Ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate | Himedia | MB011-500G |
| Glycine | Himedia | MB013-500G |
| Acetic acid | Sigma aldrich | A6283 |
| Lauryl sulfate sodium salt(SDS) | Himedia | MB010-500G |
| Imidazole | Sigma aldrich | I2399-500G |
| Isopropyl β - d-1-thiogalactopyranoside (IPTG) | Himedia | MB072 |
| Kanamycin sulfate | Sigma aldrich | 60615-5G |
| Magnesium chloride hexahydrate | Sigma aldrich | M2393-500G |
| Ammonium chloride | Himedia | MB054-500G |
| Protease Inhibitor cocktail tablets | Roche(Sigma-aldrich) | O5056489001 |
| Phenylmethanesulfonyl – fluoride(PMSF) | Sigma aldrich | P726-25G |
| D2-Dithiothreitol(DTT) | SRL | 17315 |
| Diethyl pyrocarbonate(DEPC) | Himedia | MB076-100ml |
| Sucrose | Sigma aldrich | S1888-1Kg |
| Agarose special, low EEO | Himedia | MB002-100G |
| Sauton's fluid medium base | Himedia | M1276-500G |
| Luria Broth (LB) medium | Himedia | M1245-500G |
| Glycerol | Himedia | MB060-1L |
| Agar powder | Himedia | GRM026-500G |
| Bis-acrylamide | Himedia | MB005-250G |
| Acrylamide | Himedia | MB068-1Kg |
| Ammonium persulfate (APS) | Sigma aldrich | A3678-100G |
| Ethidium Bromide solution(EtBr) | Sigma aldrich | E-1510-10ml |
| Coomassie brilliant blue | Sigma aldrich | 27816 |
| Bromophenol blue | Himedia | MB123 |
| N,N,N',N'-Tetramethylethylenediamine(TEMED) | Himedia | MB026-100ml |
| Ni-NTA His-Bind Resin | Merck | 7066-5 |
| 2-Mercaptoethanol | Sigma-aldrich | M6250-250ml |
| Tween-20 | Himedia | MB067-100mL |
| Tween-80 | Himedia | PCT1513-500mL |
| Ribonuclease Inhibitor | Sigma | R1158-10KU |
| DNaseI | Thermo scientific | EN0521 |
| Ethanol | Merck | E1570 |
| Spermidine | Sigma aldrich | S2626 |

Supplementary Notes 2. Site-directed mutagenesis.

(A) Forward primer W96A- 5' TATTGCGGAGCAC**GCG**GGAAGCGCGTTCG 3'
 Reverse primer W96A- 5' CGACGCGCTTCC**GCG**GTGCTCCGCAATA 3'

| | | |
|--|---|-------------|
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | TTANGGNANCNATTTCCCTCTANAATAATTTTGTTTAACTTTAAGAAGGAGATATACCA ----- 0 | 60 0 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | TGGGCAGCAGCCATCATCATCATCACAGCAGCGCCGTGGTGCCGC GCGCAGCCATA -----A * | 120 1 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | TGGATGTGGATGTTAGCACC GATGGCGAAC TGCCGGTGC GCGCGGAGTATGCGC GTGAGA TGGATGTGGATGTTAGCACC GATGGCGAAC TGCCGGTGC GCGCGGAGTATGCGC GTGAGA ***** | 180 61 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | AGATTGGTCGTC TGAGCCGTC GTGCGCACCGTCCGGTGC TGACGCGCGTGTTCGTCGTA AGATTGGTCGTC TGAGCCGTC GTGCGCACCGTCCGGTGC TGACGCGCGTGTTCGTCGTA ***** | 240 121 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | CCCGTACGGTGACCCGGCGGTGGAACGTC CGGTTATCGCGCAGGCGAACCTGGATGTGA CCCGTACGGTGACCCGGCGGTGGAACGTC CGGTTATCGCGCAGGCGAACCTGGATGTGA ***** | 300 181 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | ACGGTCGTCAGGTTCTGTGCGCAAGTGGAGGGCGTTAACGCGCGTGAAGCGGTGGACC GTC ACGGTCGTCAGGTTCTGTGCGCAAGTGGAGGGCGTTAACGCGCGTGAAGCGGTGGACC GTC ***** | 360 241 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | TGGAGGCGCGTC TGC GTAGCCGTC TGGAGCGTATTGCGGAGCAC GCG GAA GCGCGTCGTG TGGAGGCGCGTC TGC GTAGCCGTC TGGAGCGTATTGCGGAGCAC TGG GAA GCGCGTCGTG ***** | 420 301 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | GTGGCGTTC CGGCGGAGGCGGGTGTGAATGGCGTCATGAGAGCGAGCGCGCGCGTCGTC GTGGCGTTC CGGCGGAGGCGGGTGTGAATGGCGTCATGAGAGCGAGCGCGCGCGTCGTC ***** | 480 361 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | CGGGCTACTTCCCGCTCCCGCGGAGGAACGTCGTATCATTCGTCGTAAGAGCTTTAGCA CGGGCTACTTCCCGCTCCCGCGGAGGAACGTCGTATCATTCGTCGTAAGAGCTTTAGCA ***** | 540 421 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | TGGTCCCGTGCACC GTTGAC GAAGCGGCGCTGGAGATGGAATGCTGGACTATGATTTCCT TGGTCCCGTGCACC GTTGAC GAAGCGGCGCTGGAGATGGAATGCTGGACTATGATTTCCT ***** | 600 481 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | ACCTGTTTACC GAGAAGGGCACCGGTTTTGCGGCGGTGCTGTACAAAGGTGGCCCGACCG ACCTGTTTACC GAGAAGGGCACCGGTTTTGCGGCGGTGCTGTACAAAGGTGGCCCGACCG ***** | 660 541 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | GTTATCGTC TGGTGC TGGTTATCCCGTTCCGGCGGATGAGCTGAGCCCGTTTGAAAAAC GTTATCGTC TGGTGC TGGTTATCCCGTTCCGGCGGATGAGCTGAGCCCGTTTGAAAAAC ***** | 720 601 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | CGATCACCATTAGCACCCCGGCGCGTGCCTGACCAACGTGACGCGGTGGAACGTC CGATCACCATTAGCACCCCGGCGCGTGCCTGACCAACGTGACGCGGTGGAACGTC ***** | 780 661 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | TGGTCTGCTGGGCC TGCCGTTCTGTTTTACATTGATGCGGCGGAGGGCCGTGCGAGCG TGGTCTGCTGGGCC TGCCGTTCTGTTTTACATTGATGCGGCGGAGGGCCGTGCGAGCG ***** | 840 721 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | TTATTTACC GTCGTTATGATGGTCACTATGGTCTGATTACCCCGGCGGATGGTCTC GAGC TTATTTACC GTCGTTATGATGGTCACTATGGTCTGATTACCCCGGCGGATGGT----- ***** | 900 774 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | ACCACCACCACCACCTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAGCTGAGTTGG ----- 774 | 960 774 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | CTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGCCCTCTAAACGGTTCTT ----- 774 | 1020 774 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | GGAGGGGTTTTTTTGTGAAAGGGAGGGAAC TATATCCCGGATNGCCGAAATGGGGAC ----- 774 | 1080 774 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | GCGNCCCTGTTAGCGGGCGCATTTAAAGCGCGGNGGNGTGGGTGGNNTACGCGNCAG ----- 774 | 1140 774 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | CGNGACNNCTACANNNNNAAGCGCCNNTNGCGCCGCCCTCTCNTATNAGCNTNTACT ----- 774 | 1200 774 |
| Sequencing-Msmeg_3935 (w96A) Msmeg_3935 | TACNACTNCT 1212 ----- 774 | |

(B) Forward primer W111A- 5' GCGGGTCGTGAAGCGCGTCATGAGAGC 3'
 Reverse primer W111A- 5' GCTCTCATGACGGCTTTCACGACCCGC 3'

| | | |
|---|---|-------------|
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | TGCGTCATCCCTCTAGAATAATTTTGTTAACTTTAAGAAGGAGATATACCATGGGCAG ----- | 60 0 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | CAGCCATCATCATCATCACAGCAGCGCCGTGGTCCGCGCGGCAGCCATATGGATGT -----ATGGATGT ***** | 120 8 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | GGATGTTAGCACCGATGGC GAAC TGCCGGGTGCGGCGGAGTATGCGCGTGAGAAGATTGG GGATGTTAGCACCGATGGC GAAC TGCCGGGTGCGGCGGAGTATGCGCGTGAGAAGATTGG ***** | 180 68 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | TCGTC TGAGCC GTCGTGCGCACGTCGGTGC TGAC GCGCGTGTTCTGACCCGTCA TCGTC TGAGCC GTCGTGCGCACGTCGGTGC TGAC GCGCGTGTTCTGACCCGTCA ***** | 240 128 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | CGGTGACCCGGCGGTGGAACGTCGGTTATCGCGCAGGCGAACCTGGATGTGAACGGTCCG CGGTGACCCGGCGGTGGAACGTCGGTTATCGCGCAGGCGAACCTGGATGTGAACGGTCCG ***** | 300 188 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | TCAGGTTCTGCGCAAGTGGAGGCGTTAACGCGCGTGAAGCGGTGGACCCTGCGAGGC TCAGGTTCTGCGCAAGTGGAGGCGTTAACGCGCGTGAAGCGGTGGACCCTGCGAGGC ***** | 360 248 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | GCGTC TGC GTAGCCGTC TGGAGCGTATTGCGGAGC ACTGGGAAGCGCGTCTGGTGGCGT GCGTC TGC GTAGCCGTC TGGAGCGTATTGCGGAGC ACTGGGAAGCGCGTCTGGTGGCGT ***** | 420 308 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | TCCGGCGGAGGCGGGTCTGAA <u>GCGC</u> GTCATGAGAGCGAGCCGGCGCGTCTCCGGGCTA TCCGGCGGAGGCGGGTCTGAA <u>TGCG</u> GTCATGAGAGCGAGCCGGCGCGTCTCCGGGCTA ***** | 480 368 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | CTTCCGCGTCCGCGGAGGAACGTCGTATCATTCGTCGTAAAGAGCTTTAGCATGGTGCC CTTCCGCGTCCGCGGAGGAACGTCGTATCATTCGTCGTAAAGAGCTTTAGCATGGTGCC ***** | 540 428 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | GTGCACCGTTGACGAAGCGGCGCTGGAGATGGAATGCTGGACTATGATTTCACCTGTT GTGCACCGTTGACGAAGCGGCGCTGGAGATGGAATGCTGGACTATGATTTCACCTGTT ***** | 600 488 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | TACC GAGAAGGGCACCGTTTTCGCGCGGTGCTGTACAAAGGTGGCCGACCGGTTATCG TACC GAGAAGGGCACCGTTTTCGCGCGGTGCTGTACAAAGGTGGCCGACCGGTTATCG ***** | 660 548 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | TC TGGTGC TGGTTATCCCGGTTCCGGCGGATGAGC TGAGCCCGTTTGAAAAACCGATCAC TC TGGTGC TGGTTATCCCGGTTCCGGCGGATGAGC TGAGCCCGTTTGAAAAACCGATCAC ***** | 720 608 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | CATTAGCACCCACCGGCGCGTGC TGACCC AACGTGAC GCGGTGGAACGTC TGGGTCT CATTAGCACCCACCGGCGCGTGC TGACCC AACGTGAC GCGGTGGAACGTC TGGGTCT ***** | 780 668 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | GCTGGGCC TGCCGTTC TGTTTTACATTGATGCGGCGGAGGCGCGTGCGAGCGTTATTTA GCTGGGCC TGCCGTTC TGTTTTACATTGATGCGGCGGAGGCGCGTGCGAGCGTTATTTA ***** | 840 728 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | CCGTCGTATGATGGTCATATGGTCTGATTACCCGCGGATGGTCTGAGCACCA CCGTCGTATGATGGTCATATGGTCTGATTACCCGCGGATGGT----- ***** | 900 774 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | CCACCACCTGAGATCCGGCTGCTAACAAAGCCC GAAAGGGAAGCTGAGTTGGCTGCT ----- | 960 774 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | GCCACCGCTGAGCAATAAC TAGCATAAACCCCTTGGGGCCTCTAAACGGTCTTGAGGGGT ----- | 1020 774 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | TTTTGCTGAAAGGAGGACTATATCTGATGCGAATGGGACGCGCCCTGTAGCGCAATA ----- | 1080 774 |
| Sequencing-Msmeg_3935 (w111A) Msmeg_3935 | GCGCGCATGTGTAAACGGCAAGCGTTGAACGGCTACACTTGC CAAGGC GCGCCCTTAAAG ----- | 1140 774 |