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Supplemental Information

The Structural Basis of FtsY Recruitment and GTPase Activation by SRP RNA

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Supplemental Online Materials

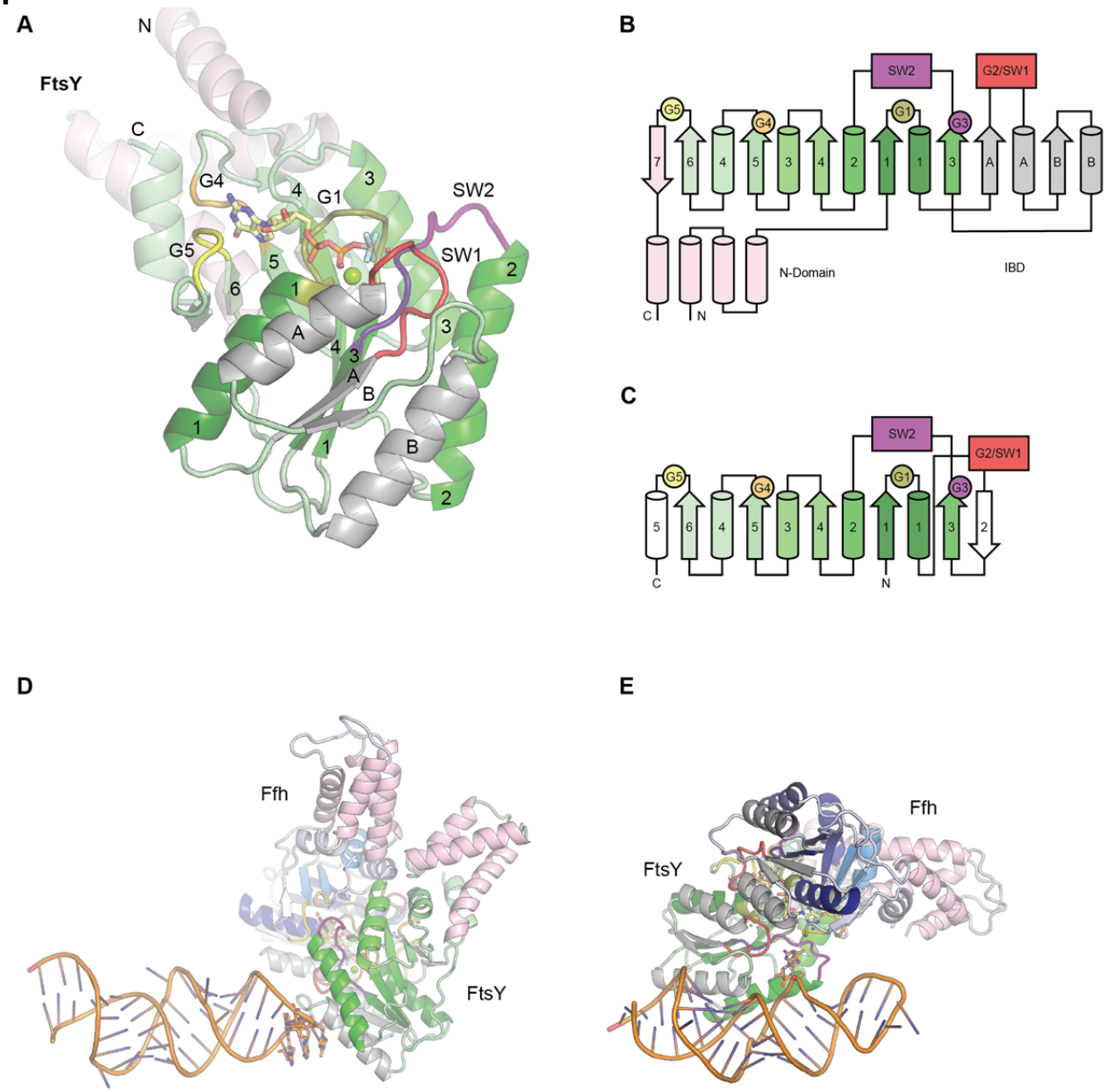

Figure S1: Organization of structural elements of FtsY, related to figure 2. a) The structure of FtsY shown as cartoon, with the G-domain core colored in green, the insertion box domain (IBD) is colored in grey and the N-domain is colored in light pink. The GDP and aluminum tetrafluoride ligand is shown as sticks colored by element and the magnesium ion is shown as a green sphere. The P-loop/G1 is colored in olive, the SW1/G2 region in red, the SW2/G3 region in purple, the G4 region in light orange and the G5 region (closing loop) in yellow. The G-protein nomenclature is adapted from (Wittinghofer and Vetter, 2011). **b and c)** Secondary structure diagram of FtsY and Ras, using the same color scheme. The insertion box domain (IBD) of the SRP GTPases replaces sheet 3 of the G-domain found in Ras (PDB:5P21), resulting in an extension of the α-β repeat. In contrast to Ras, sheets A, B and 3 are in a parallel orientation and the direction of the G1 motif is reversed. **d)** Tetraloop binding is mediated by the Insertion Box Domain of FtsY. The NG heterodimer is shown as a cartoon and FtsY is colored as above. The core of the Ffh G-domain is shown in blue, SRP RNA in orange. Tetraloop bases shown as sticks and colored by element.

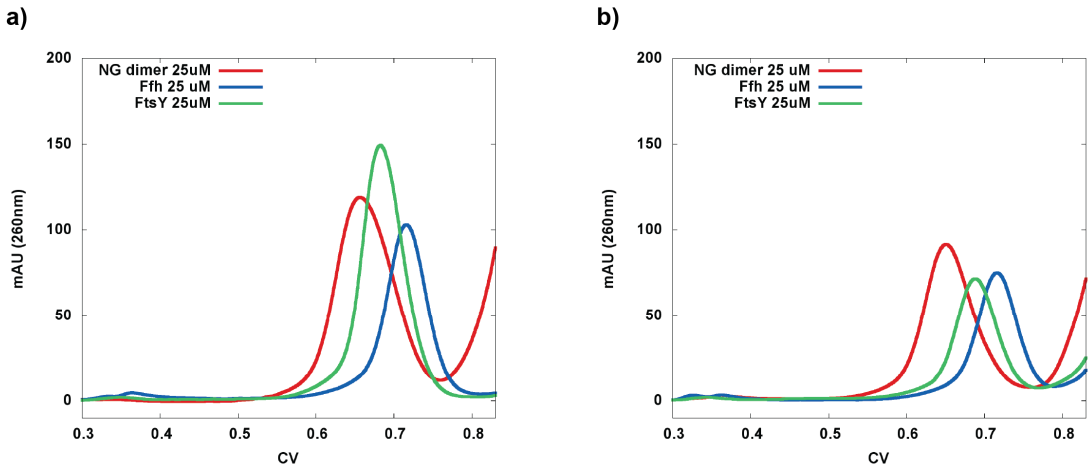

Figure S2:. Control experiments for the size exclusion chromatography of GTPase Dimer:RNA complexes, as shown in Figure 1. Analytical size exclusion chromatography of Ffh (blue), FtsY (green) and NG-heterodimer (red) (25 µM each). The Ffh:FtsY NG heterodimer is stable in buffer containing either (**a**) GDP:AlF_4 or (**b**) GMPPCP and elutes as a single, symmetric peak at lower elution volume than the individual proteins.

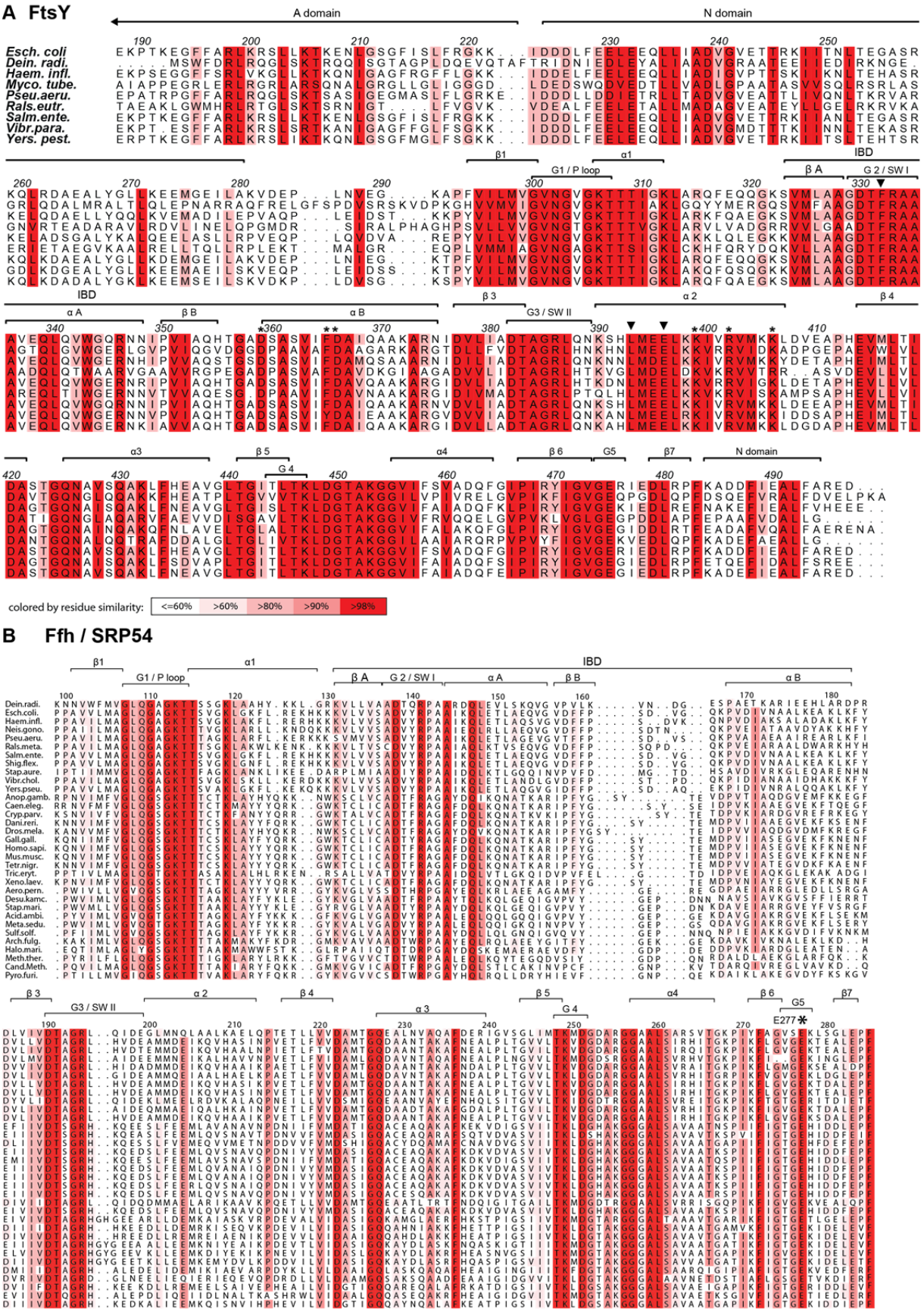

Figure S3. Color coded sequence alignment of bacterial FtsY proteins and Ffh/SRP54 proteins across all kingdoms of life, related to figures 3, 4 and 6. a) Alignment of Bacterial FtsY sequences. Residue numbers are based on the *E. coli* sequence. RNA interacting regions: IBD strand B 350-355, IBD helix B 359-375, Helix 2 390-407 (important residues: K399, R402, K407, F365, D366. Colors are based on sequence similarity, as indicated in the color key. **b)** Alignment of Ffh/SRP54 across all kingdoms. The universally conserved E277 (E. *coli* gene numbering) is indicated by (*) and has 100% sequence identiy. Colors as in panel (a).

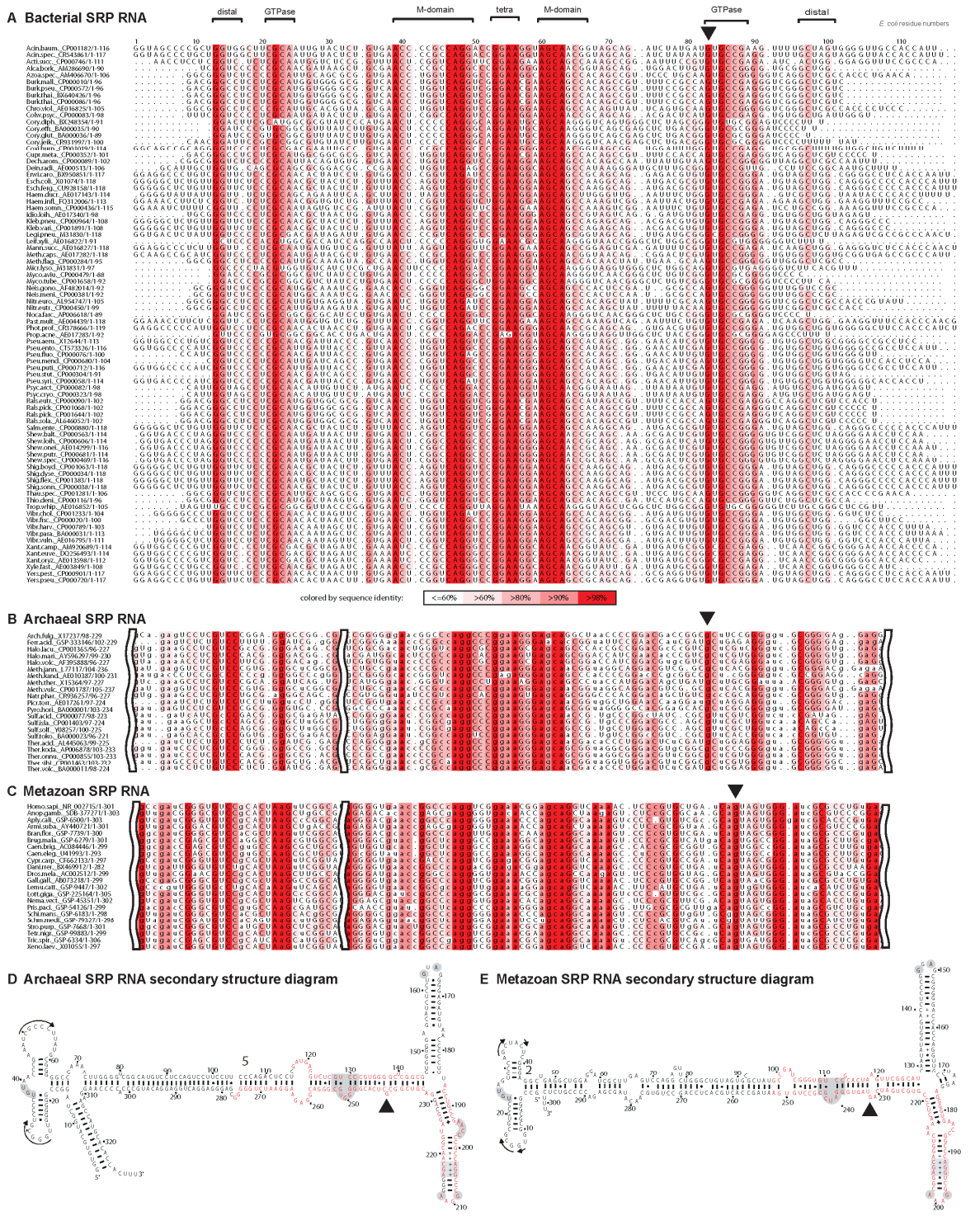

Figure S4. Color coded sequence alignments for SRP RNA across kingdoms, related to figure 3 and 6.a) Sequence alignment of bacterial 4.5S RNAs(Andersen et al., 2006), generated with Clustal(Larkin et al., 2007) and colored by conservation(Bond and Schüttelkopf, 2009). Highly conserved regions symmetrically distributed around the tetraloop correspond to the M-domain binding site, the GTPase activating site and the distal docking site. The position of the conserved unpaired guanine (G83 in E. coli) is marked by an arrow. **b**) Sequence alignment of archaeal and **c**) metazoan SRP RNAs, reproduced from the SRPDB(Andersen et al., 2006). Only regions homologous to the bacterial SRP RNA are shown. **d**) Secondary structure diagrams of archaeal and **e**) metazoan SRP RNAs, adapted from the SRPDB(Andersen et al., 2006). Regions depicted in red correspond to the alignments above. Both eukaryotes and archaea have a conserved unpaired guanine in a position corresponding to the bacterial G83 (black arrows).

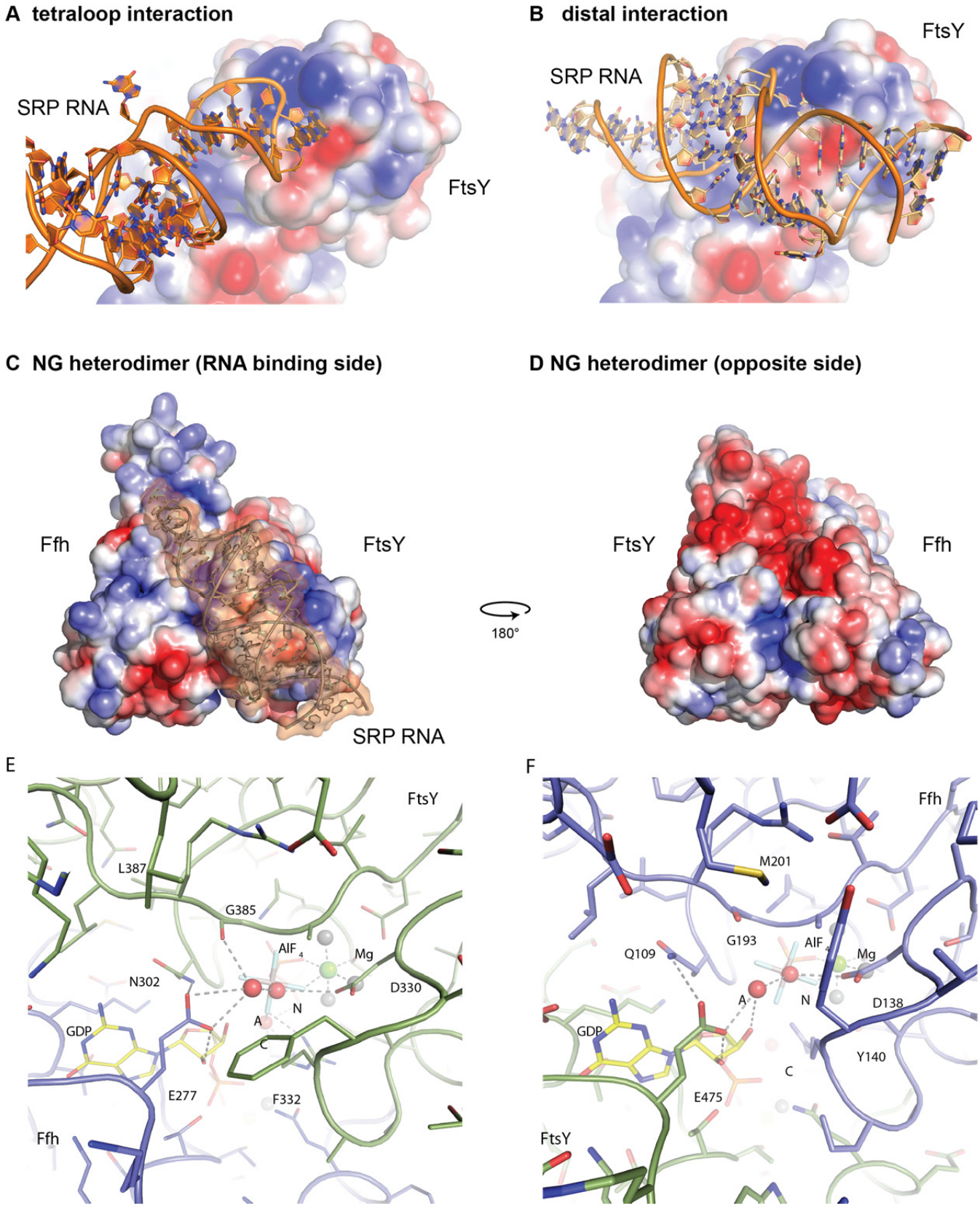
A tetraloop interaction
SRP RNA
FtsY
B distal interaction
FtsY
SRP RNA
C NG heterodimer (RNA binding side)
Ffh
FtsY
SRP RNA
180°
D NG heterodimer (opposite side)
FtsY
Ffh
E
FtsY
L387
G385
AlF4
Mg
N302
D330
N
A
GDP
C
E277
F332
Ffh
F
Ffh
M201
G193
AlF4
Q109
Mg
A
N
D138
GDP
Y140
C
E475
FtsY

Figure S5. Distribution of surface charges on FtsY highlights RNA interaction regions and asymmetry of the GTPase complex, related to figure 3, 4 and Scheme 1. a) The distribution of surface charges on FtsY mirrors the surface charges of an RNA helix. Positively charged residues compensate the negative charges of the RNA backbone phosphates, while a negatively charged patch forms the interface for G54 at the top of the tetraloop base stack. FtsY is shown as a surface and colored by charge distribution, the SRP RNA backbone is shown as an orange cartoon, and bases are shown as sticks, with non-carbon atoms colored by element (generated with the ABPS Pymol (Schrödinger) plugin). **b)** The same region of FtsY (shown in the same orientation as in panel **a)**) also mediates binding to the distal docking site of the SRP RNA. Positively charged residues on the surface of FtsY compensate the negative charges of the RNA backbone phosphates while negatively charged and hydrophobic residues are lined up along the minor groove. **c)** Surface charge distribution on the RNA-bound side of the Ffh-FtsY heterodimer and **d)** on the opposite side. The surface residues on the RNA bound side of the protein heterodimer form a positively charged to hydrophobic surface, mirroring the charges of the SRP RNA helix. In contrast, the surface that points away from the RNA is dominated by negatively charged residue. Thus, the interaction with the SRP RNA is asymmetric and specifically occurs on one side of the heterodimer. Compared to the corresponding F332 of FtsY **(e)**, Ffh Y140 partially obstructs the cleft between the two proteins **(f)**, which would prevent base insertion. This conformation is stabilized by interactions with the conserved Ffh E204 (FtsY S396) and M201 (FtsY L393). Between the two nucleotide binding sites of the heterodimer, the relative position of the auxiliary water molecule differs by 1.1 Å: On the RNA-facing side, it is 0.8 Å closer to the main chain carbonyl group of the G3 motif glycine and further away from the 3' OH group of the nucleotide. As a result, the auxiliary water molecule is within hydrogen bonding distance of three carbonyl/carboxyl groups on the RNA-facing side, compared to one carbonyl/carboxyl group and one hydroxyl group on the opposite side. Ffh is shown in blue, FtsY in green, GDP in yellow, magnesium ions in bright green and the central (C), nucleophilic (N) and auxiliary water molecules are depicted in red. Water molecules in the coordination shell of the magneisum ion are shown as grey spheres. Panel (f) shows a 180° rotation of the NG heterodimer, relative to (e). The core domains of Ffh and FtsY superimpose with an RMS of ~0.8Å, with the N-domain, the RMS rises to ~1.3 Å. For the figure, the proteins were aligned via one of the two nucleotides.

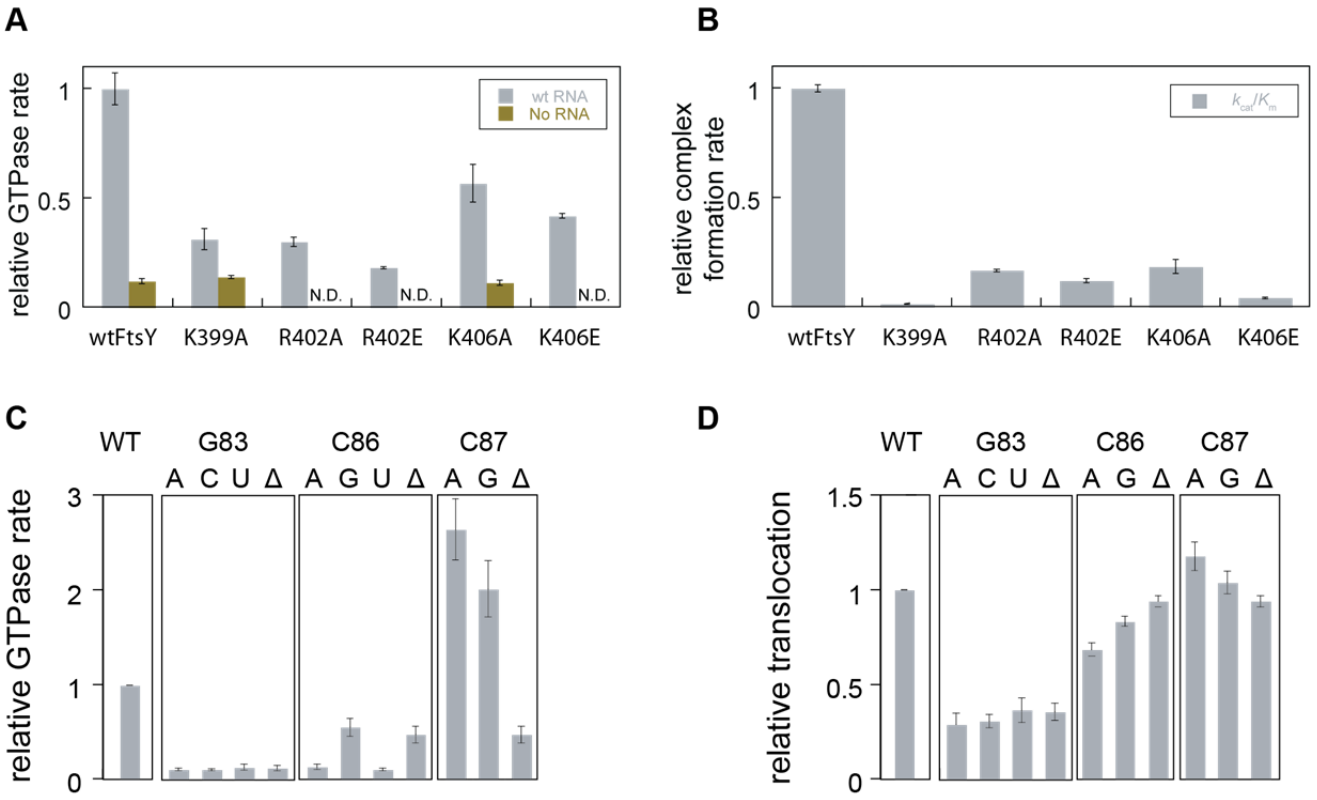

Figure S6. Mutational analysis of FtsY's RNA binding region and RNA base substitutions, related to figure 3,4 and 5. a) Relative GTPase rate for mutations in the RNA binding region of FtsY. All tested mutants can be efficiently stimulated by full length SRP RNA. **b)** NG-domain dimer complex formation rates observed for FtsY mutants in the RNA binding region. The complex formation rate of the K399A mutation is similar to the rates observed in the absence of RNA. (Shen and Shan, 2010). Complex formation rates determined as in (Peluso et al., 2001).
c) Stimulation of GTP hydrolysis and **d)** translocation by SRP RNA. Substitutions or deletions of G83 have a marked effect on both GTPase activation and translocation. Substitutions of C86 or C87 have opposed effects on GTPase activation, decreasing or increasing the observed GTPase rate, respectively. However, substitutions or deletions of C86 or C87 do not have a strong effect on the observed translocation rate. Data represent mean +/- SD (A,B: n=2, C:n=3, D: n=4).

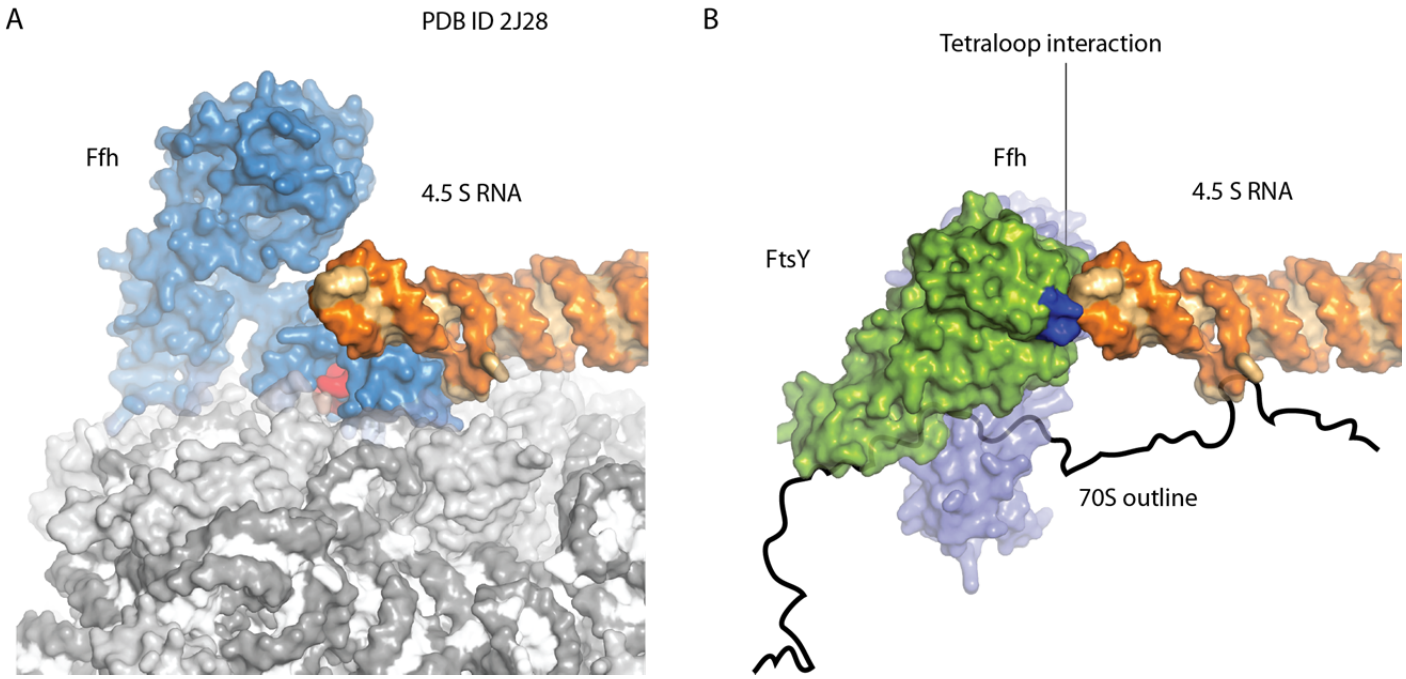

Figure S7: Comparison of the orientation of the tetraloop-bound SRP NG domain bound to the ribosome and in the closed SRP:FtsY complex structure, related to scheme 1. a) Cryo-EM structure of the bacterial SRP bound to a translating ribosome (PDB 2J28). SRP RNA is shown in orange, Ffh in blue and the ribosome is shown in grey). **b)** The structure of the closed NG domain heterodimer on the tetraloop determined here reveals that the position of Ffh overlaps partially with the ribosome if the SRP RNA adopts the same orientation as shown in panel **a)**. FtsY is shown in green. Superposition is based on the coordinates of the SRP RNA tetraloop.