Supplemental data related to the manuscript

# Structural basis for RNA-genome recognition during bacteriophage Qβ replication

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**Supplemental Figure legends**

**Figure S1. Analysis of molecular weights and elution volumes of monomeric and dimeric forms of the Qβ replicase core complex.** Molecular weights and elution volumes were analysed after gel filtration over a Superdex 200 10/300 GL column coupled to static light scattering equipment. Gel filtration profile of monomeric (**A**) and dimeric (**B**) Qβ replicase core complex.

**Figure S2. Representative example of the electron density map.** The N-terminal α-helix of $OB_1$ is shown as a purple cartoon with side chains as sticks, while the rest of the structure is coloured as denoted in figure 3. The final, refined 2mFo-DFc σ-A weighted map is depicted as a black mesh contoured at 1.0 σ.

**Figure S3. Interaction of the $OB_{1-2}$ domains of ribosomal protein S1 with the β-subunit of the Qβ replicase complex. A**. $OB_{1-2}$ (in magenta) interacts with the fingers domain (green) of the β-subunit. Other subdomains are the palm domain in cyan and the thumb domain in pale pink. The active site residues Asp274, Asp359 and Asp360 are shown as red spheres. The RNA product and template strands are docked by superpositioning with the RNA-bound Qβ replicase core complex (PDB ID 3AVY). **B**. Zoom-in on details of the interaction between $OB_{1-2}$ and the β-subunit. In the following text, residues in the β-subunit, EF-Tu and S1 are indicated with the prefixes β, u and s, respectively. Panel a. The β-barrel of $OB_1$ forms two salt bridges to the β-subunit that define the outer boundary of the interface, sLys43 to βAsp202 and sAsp39 to βArg191 and βArg199. The bottom of the barrel is packed against residues 181-203 of the β-subunit via hydrophobic interactions. Panel b. The N-terminal helix of $OB_1$ engages in π-stacking interactions as follows: sPhe5–uPhe261, sPhe9–βLeu149 and sLeu13–βTyr186. Furthermore, sIle16 and βLeu188 form a van der Waals interaction and a hydrogen bond is present between sSer12 and βAla189. Salt bridges between sThr2 and uArg262 as well as sGlu17 and βLys185 stabilizes the N and C-terminal end of the helix, respectively. Panel c. The α-helix of the linker connecting OB domains 1 and 2 (residues 87-102) contributes to the binding of $OB_2$ to the β-subunit via a salt bridge between βGlu288 and sArg91 as well as hydrogen bonds between the backbone of βAla104 and sArg86. Further interactions between

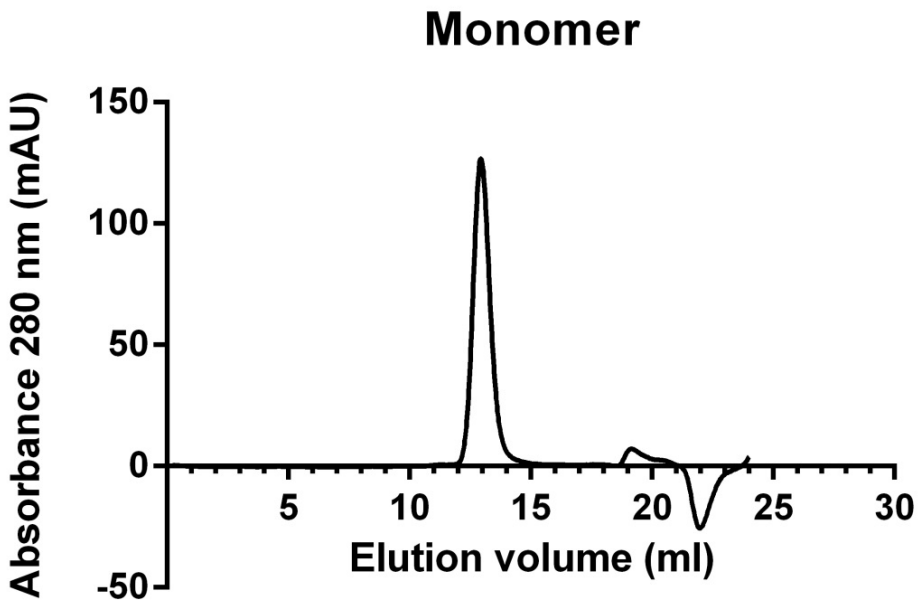
the β-subunit and $OB_2$ are as follows: sArg128 interacts with both βGlu125 and the main chain of βLeu110, sGlu123 and sAsn113 interact with βTyr115, sAsn165 interacts with βArg132, sVal116 and sThr121 stacks with βHis129, sArg163 interacts with βAsp141 and lastly, sAsn125 interacts with βArg112. Only a part of the mentioned interactions is shown in the figure for clarity.

**Figure S4. Crystallographic dimers of Qβ replicase complexes.** The crystallographic dimers of the Qβ replicase core complex (PDB ID 3MMP; upper panel), the $OB_{1\text{-}2}$-bound Qβ replicase complex (this study; middle panel) and the Qβ replicase core complex crystallized with $OB_{1\text{-}3}$ (PDB ID 4Q7J; lower panel) are presented from two different views (left and right, respectively). The darker coloured monomers are presented in identical views, whereas the lighter coloured monomers are positioned relative to their dimer counterpart. Orange: β-subunit, purple: OB domains, blue: EF-Tu and green: EF-Ts.

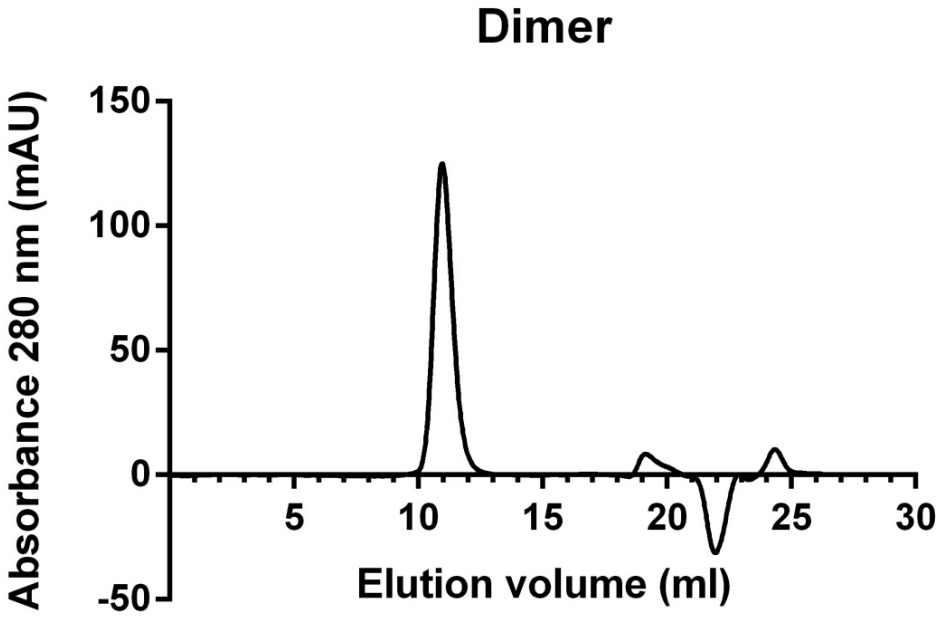
**Figure S5. Structural changes occurring in the Qβ replicase core complex upon binding of the $OB_{1\text{-}2}$ or $OB_{1\text{-}3}$ protein.** The β-subunits of the unbound (turquoise, PDB ID 3MMP), RNA-bound (grey, PDB ID 3AVY), $OB_{1\text{-}2}$ bound (yellow, this study) and $OB_{1\text{-}3}$ bound (purple, PDB ID 4Q7J) Qβ replicase core complexes were superimposed. The arrow indicates a rotation of the EF-Tu-EF-Ts subcomplex towards the cavity forming the RNA exit channel. The largest displacement caused by this rotation is found in EF-Ts in which helices are displaced by up to 9.4 Å ($OB_{1\text{-}3}$ bound complex). The displacement of a flexible loop in the β-subunit caused by $OB_{1\text{-}2}$ binding is enlarged for clarity. Major distance changes are indicated.

**Figure S6. The N-terminal α-helix of S1.** Presentation of the helix-binding site on the Qβ replicase core complex and the ribosome, same view in all. **A.** Binding of the PEP compound (green) in the Qβ replicase core complex (PDB ID 3MMP). The β-subunit is coloured orange and EF-Tu is coloured blue. The PEP compound binds the same hydrophobic groove on the Qβ replicase core complex as the N-terminal helix. **B.** Binding of the N-terminal helix of $OB_1$ (purple) in the on the Qβ replicase core complex. **C.** Binding of the N-terminal helix of $OB_1$ (purple) to the ribosomal protein S2 (green) (PDB ID 4TOI).

A


Monomer
Absorbance 280 nm (mAU)
150
100
50
0
-50
5
10
15
20
25
30
Elution volume (ml)

B


Dimer
Absorbance 280 nm (mAU)
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100
50
0
-50
5
10
15
20
25
30
Elution volume (ml)

Figure S1

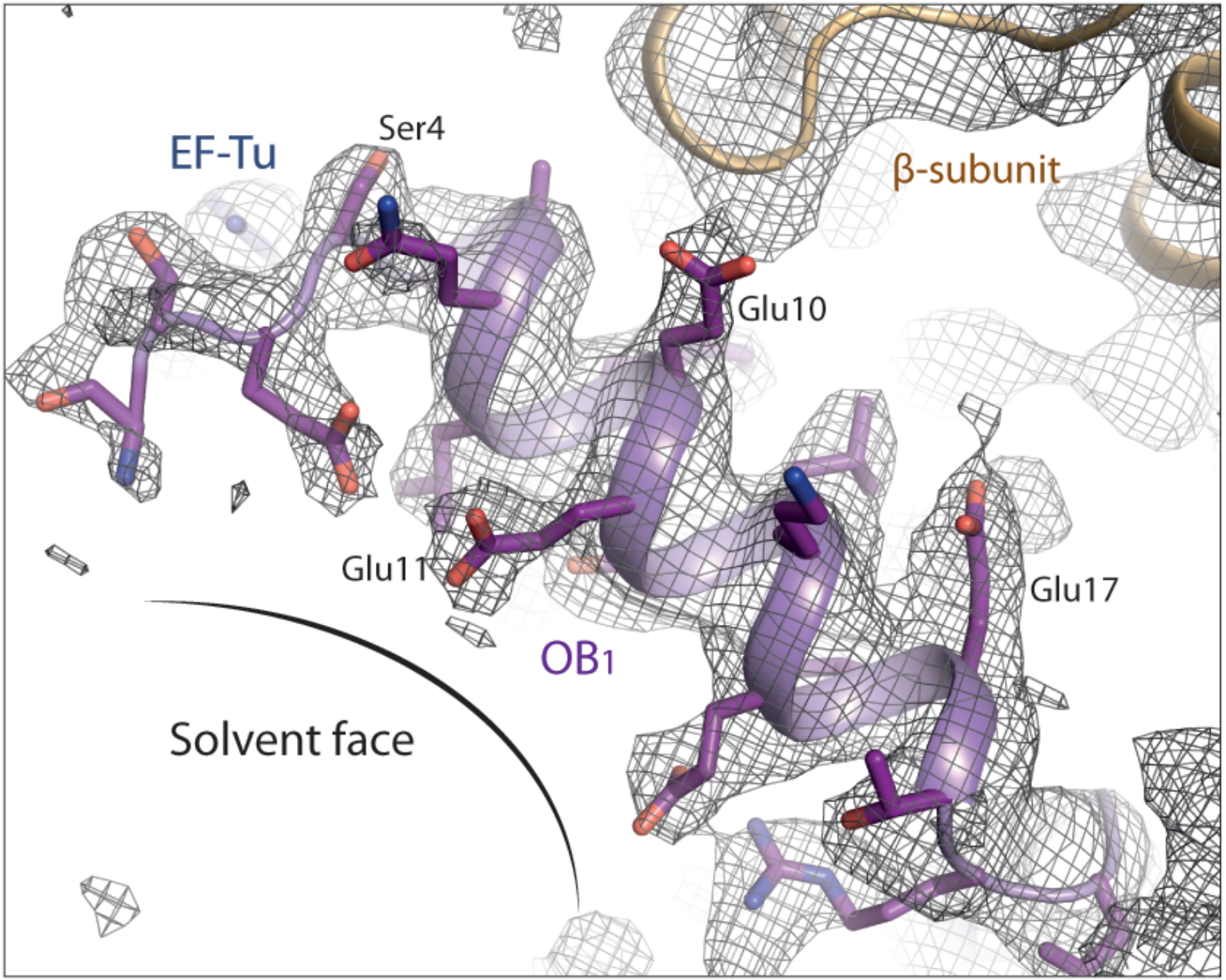


Figure S2

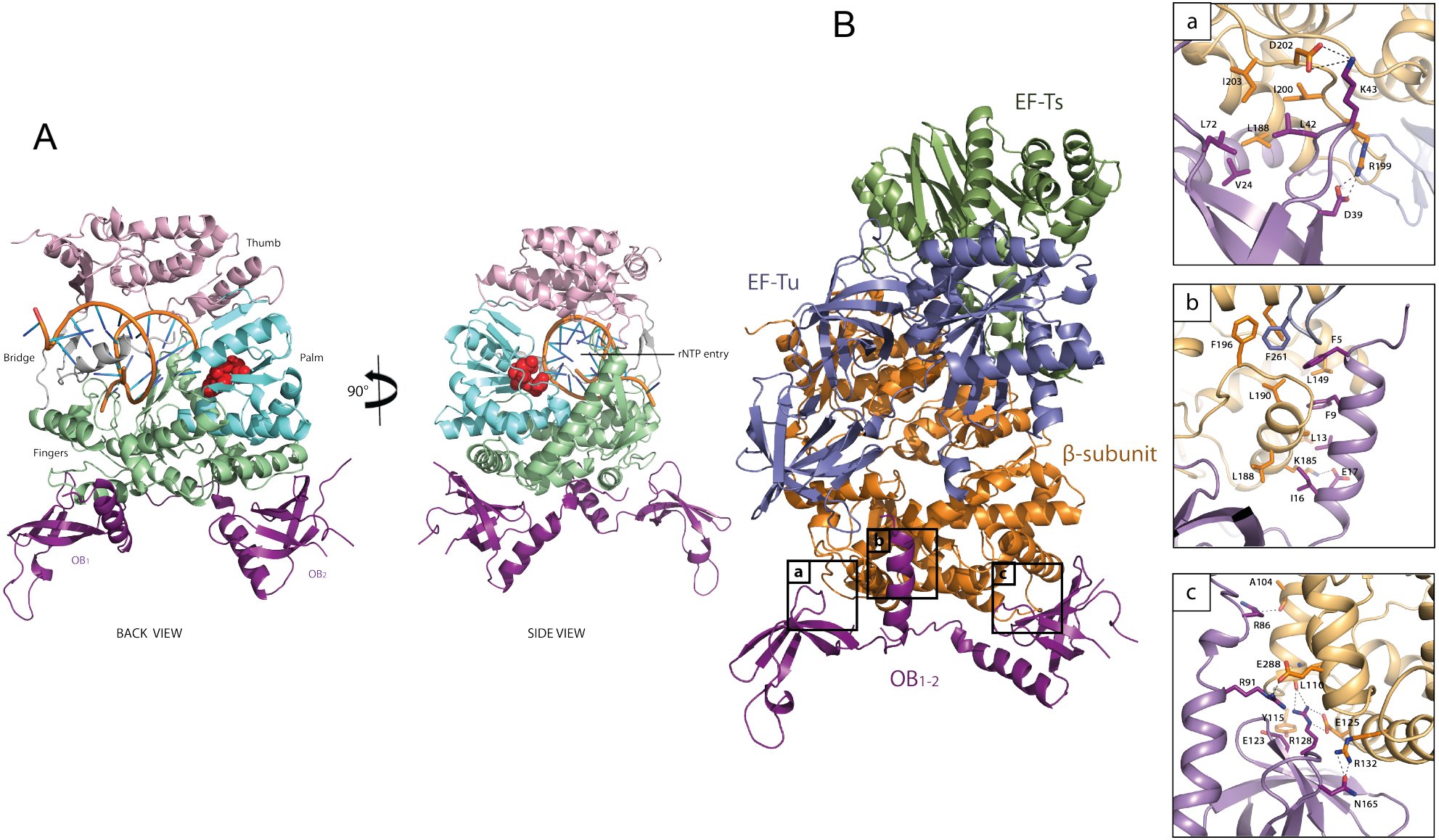
A
Thumb
Bridge
Palm
Fingers
OB1
OB2
BACK VIEW
90°
rNTP entry
SIDE VIEW
B
EF-Ts
EF-Tu
β-subunit
OB1-2
a
b
c
D202
I203
I200
K43
L72
L188
L42
R199
V24
D39
F196
F261
F5
L149
L190
F9
L13
K185
E17
L188
I16
A104
R86
E288
R91
L110
Y115
E125
E123
R128
R132
N165

Figure S3

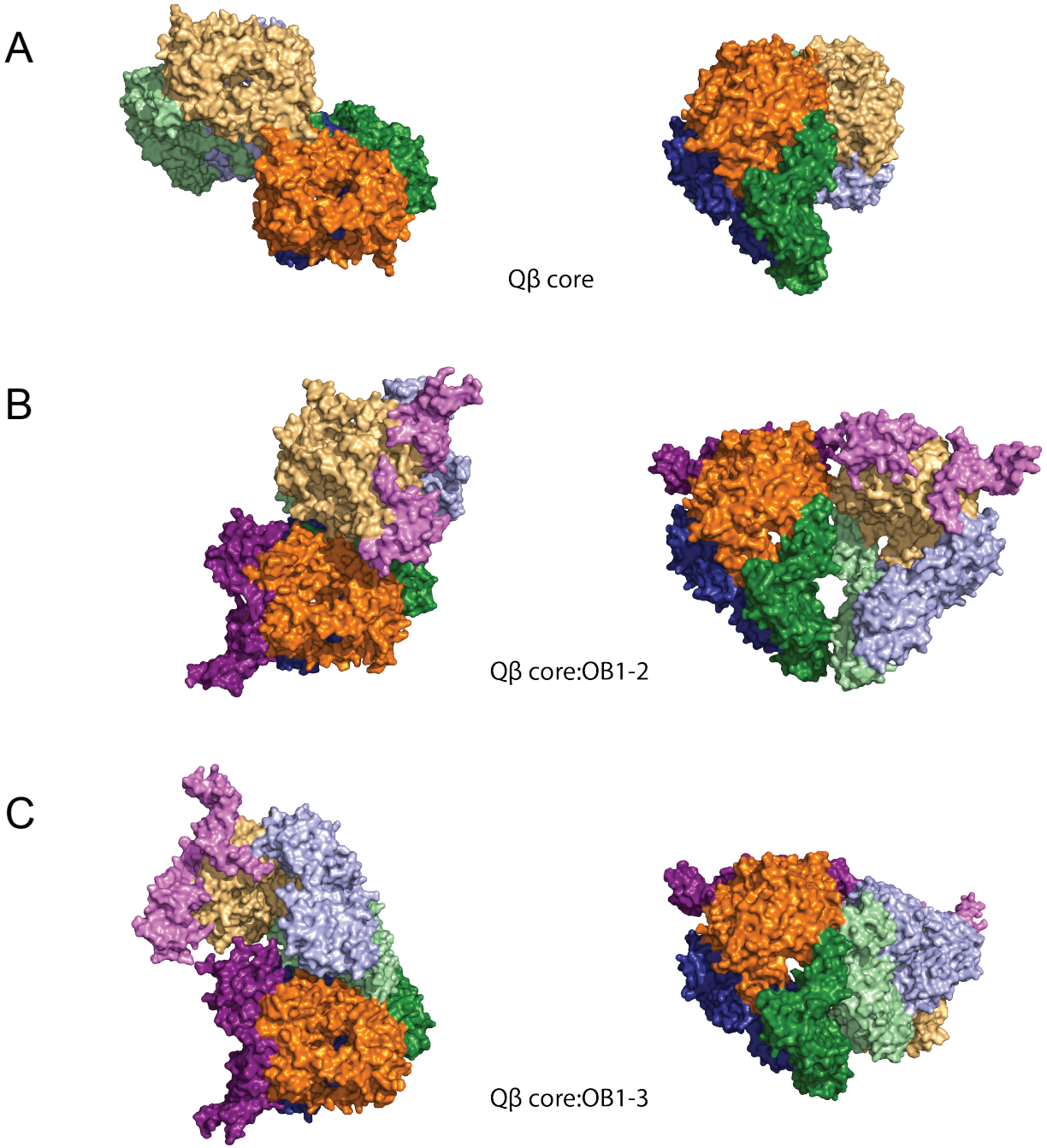


Figure S4

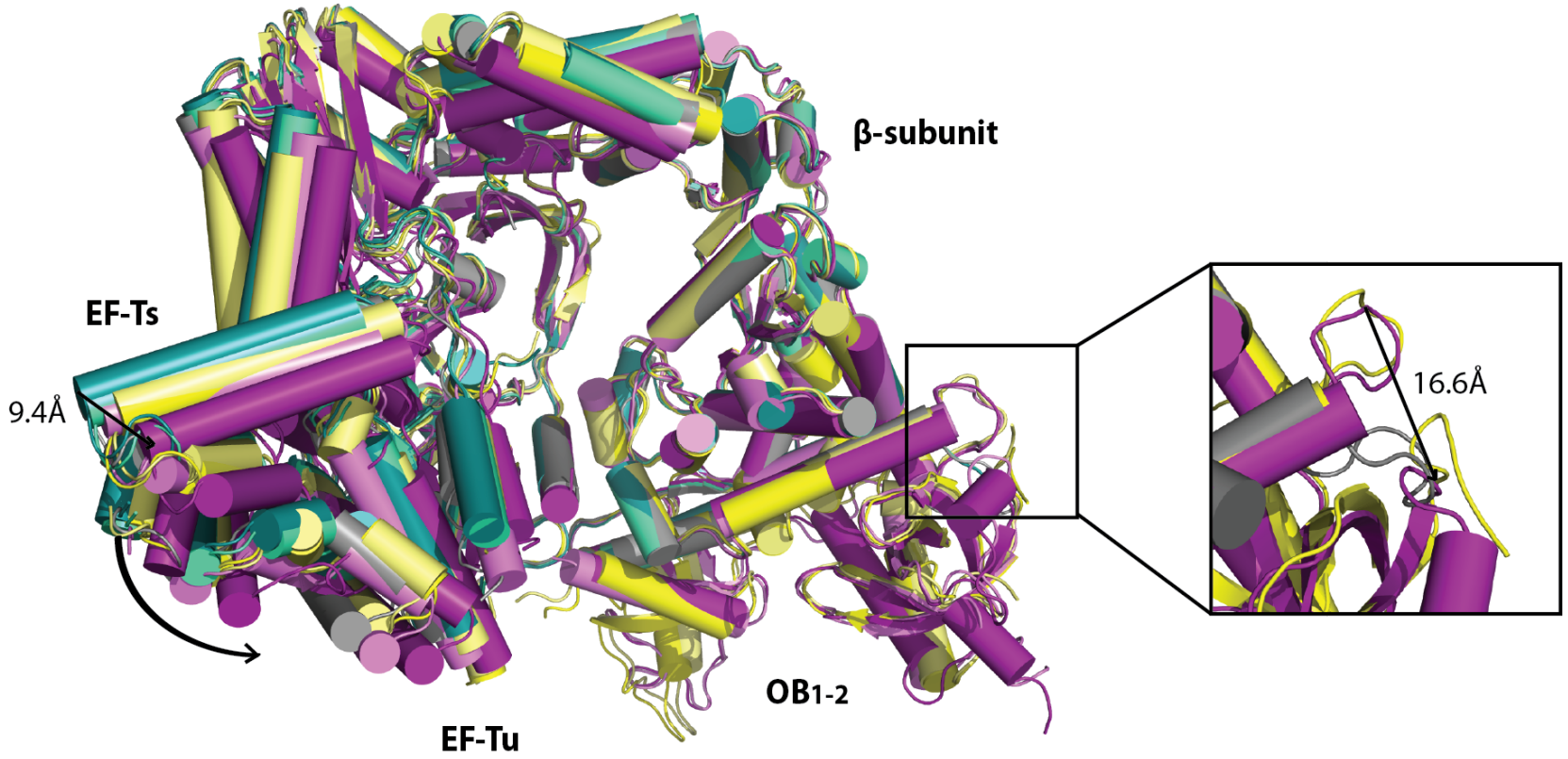


Figure S5

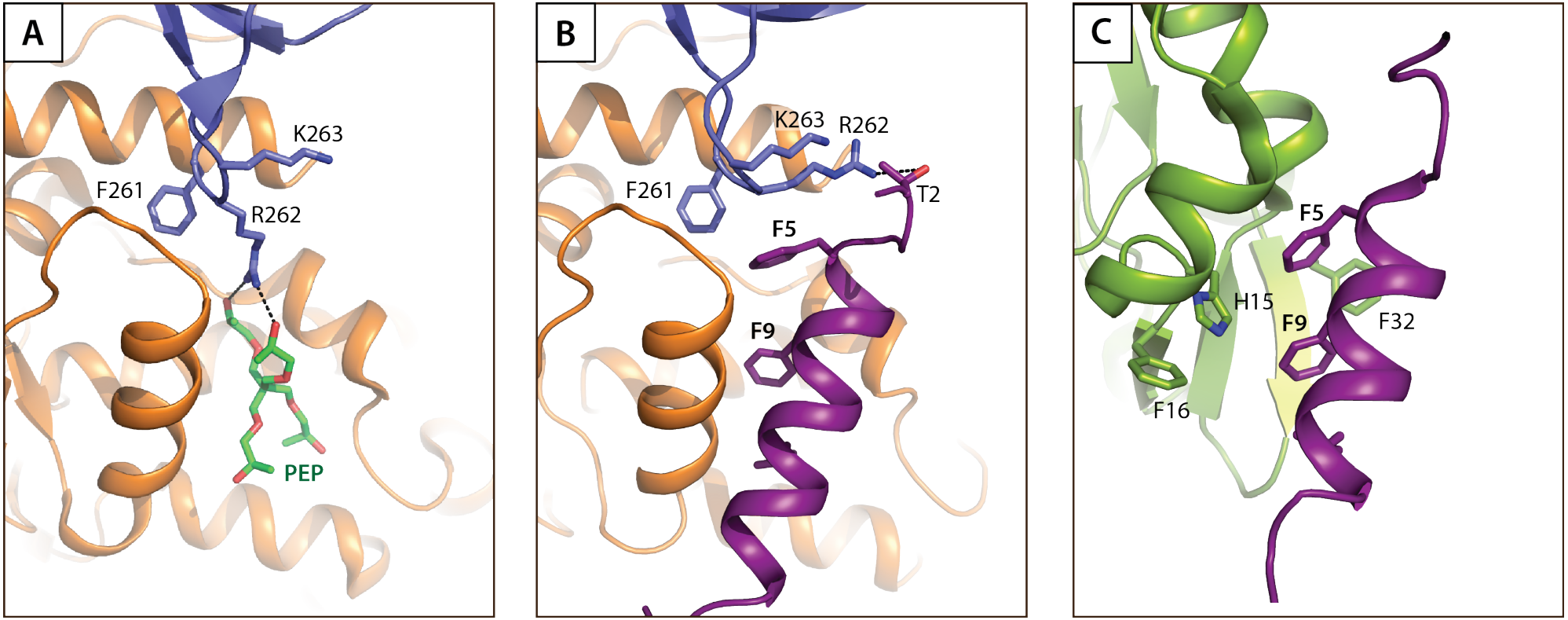


Figure S6