Expanded View Figures

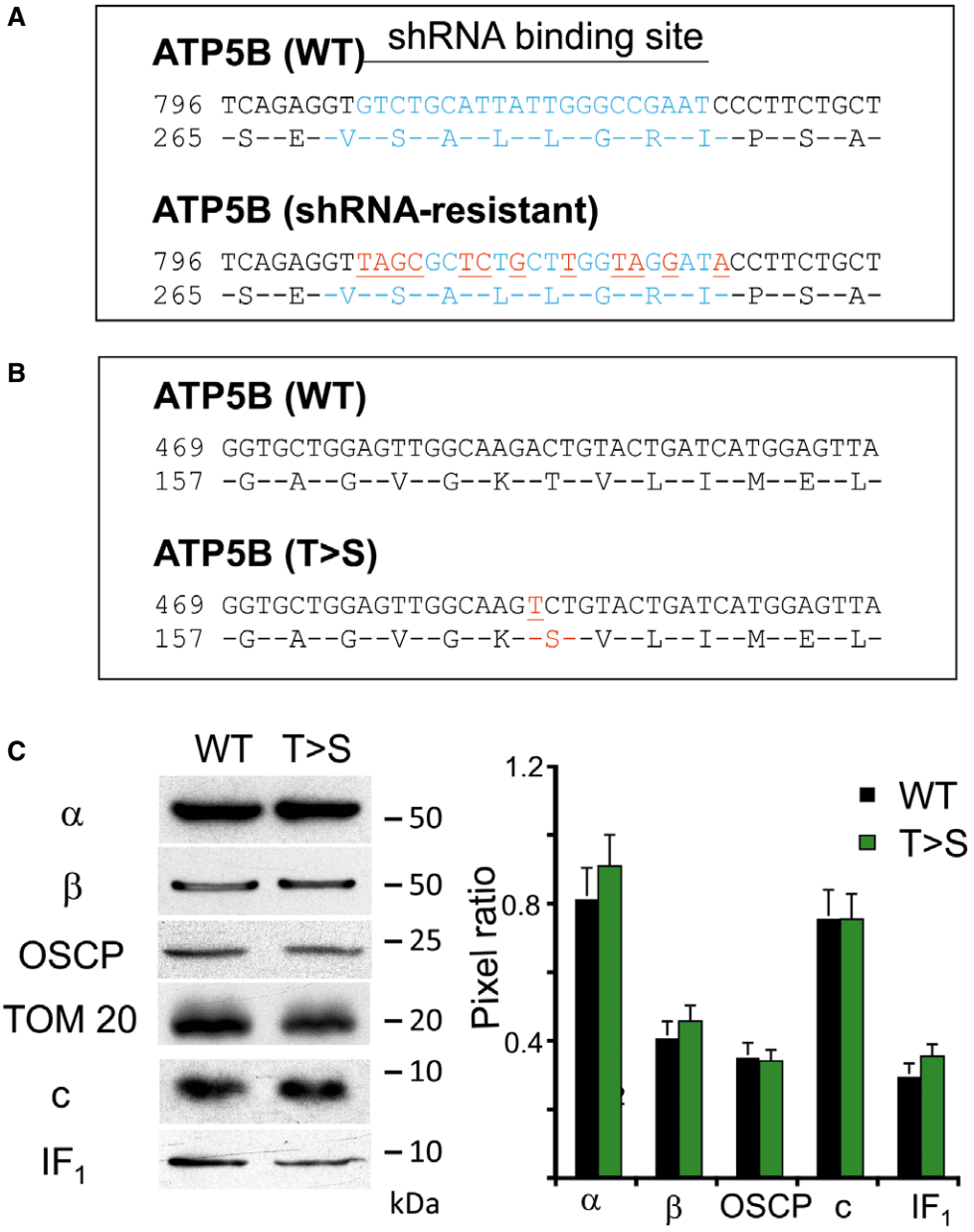

Figure EV1. Strategy followed in transient co-transfection of HeLa cells: silencing of endogenous β subunit of F-ATP synthase and rescue with wild-type or T163S mutated sequences.

A HeLa cells were transfected with shRNA directed to the nucleotide sequence represented in light blue of endogenous β subunit of F-ATP synthase (ATP5B WT). To rescue β subunit expression, HeLa cells were simultaneously transfected with shRNA and with *ATP5B* harboring silent mutations (red) in the shRNA targeted region (ATP5B shRNA-resistant) in order to avoid its degradation. The corresponding amino acid sequence (ATP5B shRNA-resistant) is identical to that of the wild-type (WT) nucleotide sequence (ATP5B WT).

B Two different species of shRNA-resistant β sequences were used to rescue β subunit in HeLa cells: shRNA-resistant *ATP5B* (WT) which generates an amino acid sequence that does not differ from that of the endogenous β subunit and shRNA-resistant *ATP5B* (T>S) that generates a β subunit with a point mutation (in red) that causes the T163S substitution.

C The effect of silencing and reexpression of WT or T>S mutant *ATP5B* on expression of F-ATP synthase subunits and of its inhibitory protein IF_1 was assessed by Western blotting. TOM20 was included as a loading control. Molecular size is indicated on the right. The bar graph shows the ratio (± s.e., three independent experiments) between the quantified bands corresponding to F-ATP synthase subunits and TOM20 in WT or T>S mutants.

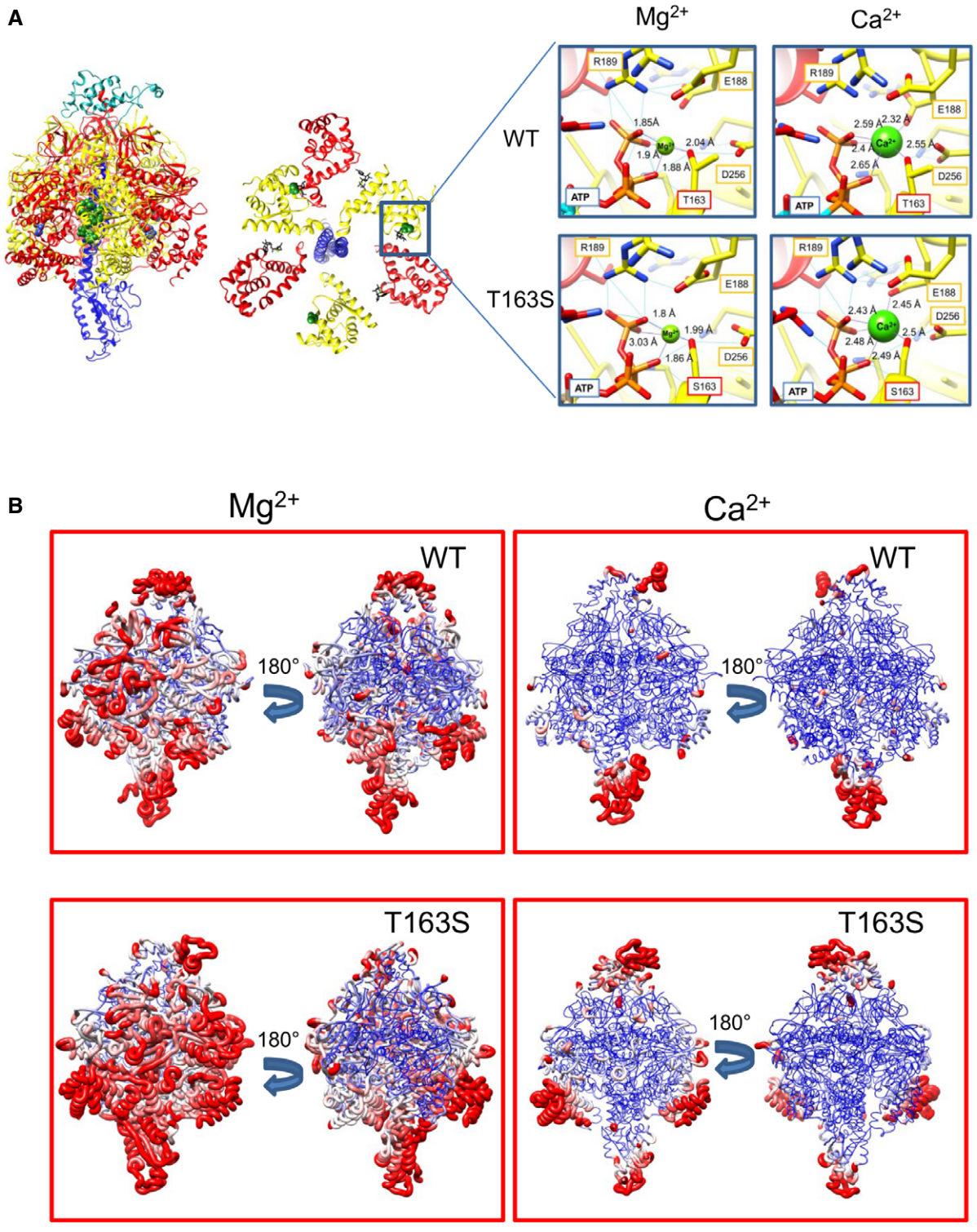

Figure EV2. Molecular dynamics simulation of wild-type and T163S β mutant F-ATP synthase with Mg^{2+} and Ca^{2+} bound to the catalytic site.

A Lateral and top view of F1; location of β T163 is marked by green spheres. Red, α subunits; yellow, β subunits; blue, γ subunit; turquoise, OSCP (lateral view only). The enlargement shows Mg^{2+} and Ca^{2+} bound to the Me^{2+} sites and their coordination distances. Hydrogen bonds and ion contacts are shown in blue and purple, respectively, with the distance between ion and coordinating atoms in Å.

B F1-OSCP backbone with coloring and thickness representing RMSF. Blue and red denote residues with RMSF ≤ 4 Å and ≥ 5.5 Å, respectively. The same RMSF range is used to draw backbone thickness. Rotation is along the *y*-axis.

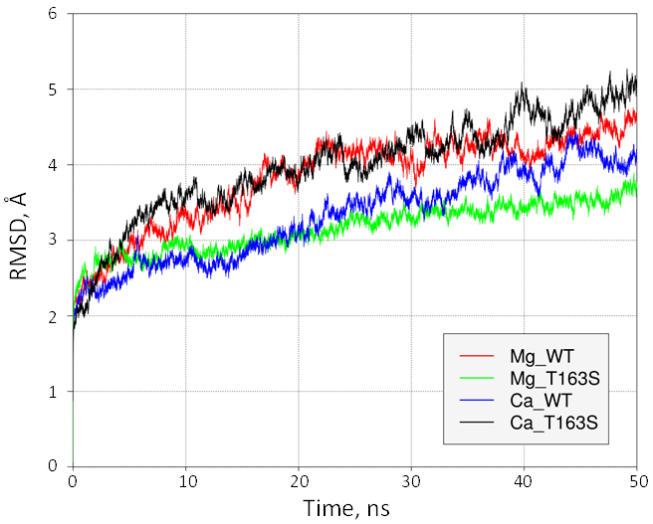

Figure EV3. Root mean square deviation of coordinates for non-hydrogen atoms as a function of simulation time.
Calculation was performed during 50 ns of molecular dynamics simulation. The *x*-axis represents the simulation time (ns) and the *y*-axis the backbone movement (Å).

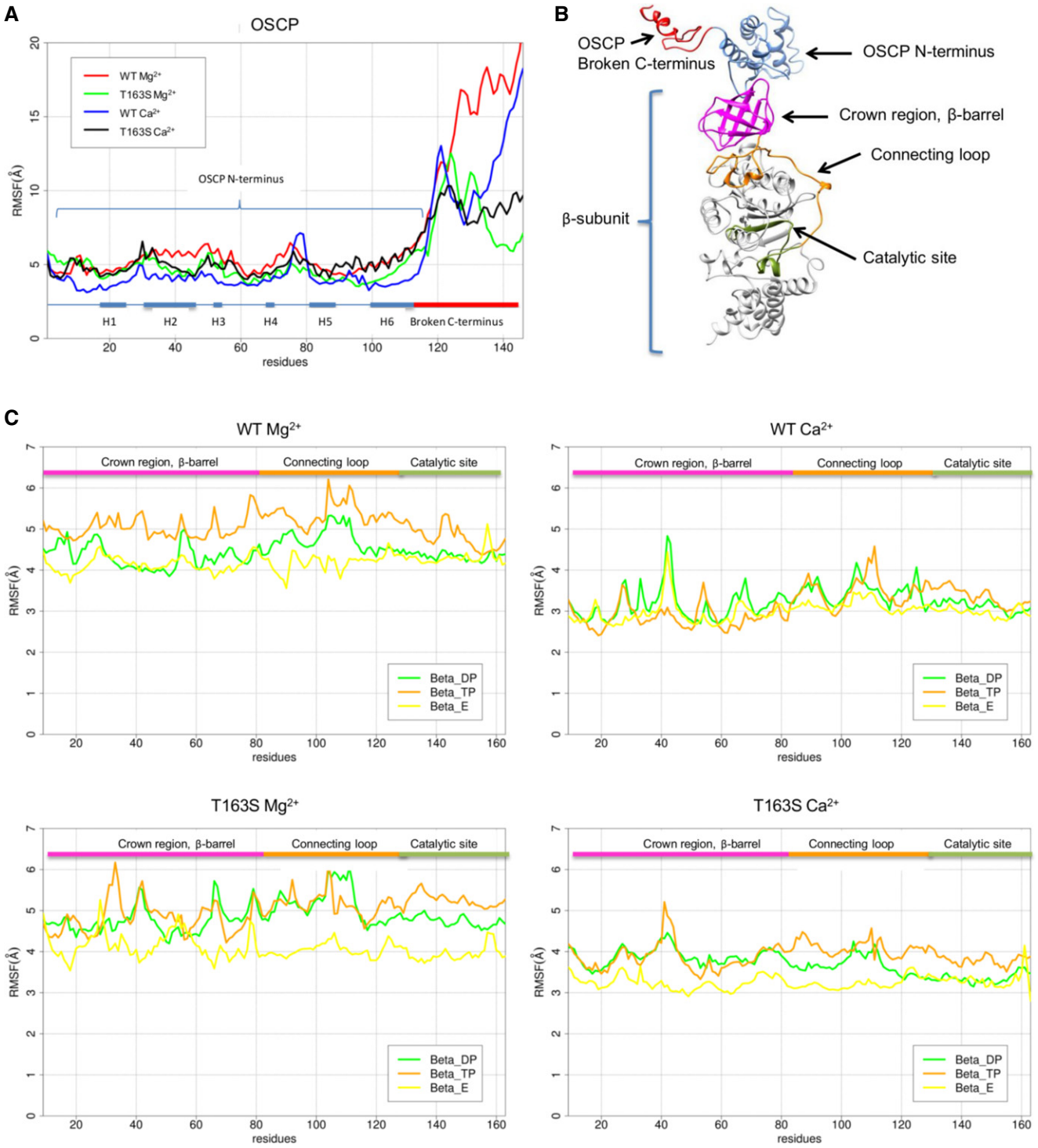

Figure EV4. Root mean square fluctuations of OSCP 1–146 and β subunit region 9–163.

A The plot shows the RMSF values of OSCP interacting with wild-type (WT) and T>S mutant β subunits binding Mg^{2+} and Ca^{2+}. Rectangles below the graphs indicate the position of the α helices (H) of OSCP and the C-terminal region.

B Structure of subunit β and OSCP.

C The plots show the RMSF values of wild-type (WT) and T>S mutant of subunit β region 9–163 binding Mg^{2+} and Ca^{2+}.