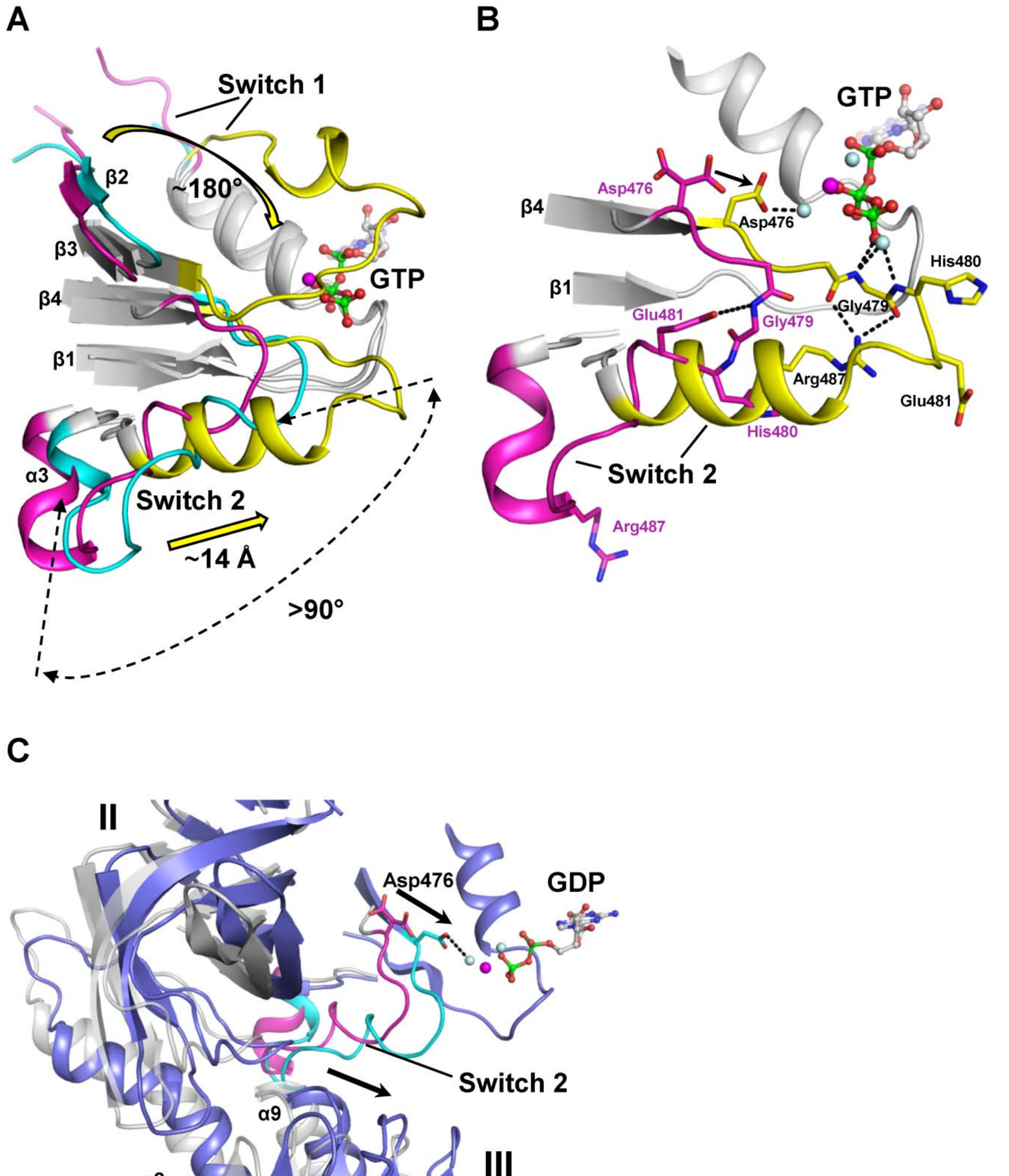

Figure S4. Nucleotide-dependent conformational changes in switch 1 and switch 2 of eIF5B. **A)** Overview of the GTP-induced conformational changes in the G domain. P loop, switch 1 and switch 2 are colored pink for apo eIF5B, cyan for eIF5B·GDP and yellow for eIF5B·GTP; the Mg^{2+} ion is shown as sphere in magenta; GTP is shown in balls and sticks; GDP is omitted for clarity. In the apo state switch 1 points away from the nucleotide binding pocket and is partially disordered. Switch 2 adopts a loop conformation with most residues positioned at the backside of the G domain (left). Upon GTP binding the switch regions become reorganized (yellow arrows) to form the catalytic center around the GTP-γ-phosphate. Switch 1 flips over by ~180° relative to its position in the apo state and the residues forming strand β2 are repositioned to form part of the coordination sphere for the Mg^{2+} ion. Switch 2 moves up to 15 Å in some residues, resulting in an extended helix α3, which is reoriented relative to α3 in the apo state by more than 90°. The transition from the GTP- to GDP-bound state reverses these changes and results in conformations of switch 1 and 2 like for the apo form, however, with switch 2 shifted ~3 Å towards the nucleotide binding pocket. **B)** Details of the transition between apo and GTP-bound forms of switch 2. The color code is the same as in A); water molecules are shown as grey spheres; hydrogen bonds indicated as dashed lines. Upon GTP binding Asp476 moves ~3 Å towards the GTP molecule (arrow). Gly479 moves towards the γ-phosphate (meanwhile the peptide bond to the preceding Pro478 flips by ∼160°) resulting in a reorganization of the rest of switch 2 including His480 (His^{cat}) and Arg487, which is involved in interactions with domain III in the apo and GDP-bound states of the factor (see also Fig. 3A/B). **C)** As shown in A), switch 2 in Ct-eIF5B·GDP (blue with switch 2 in cyan) remains ~3 Å closer to the nucleotide binding pocket than in the apo form (grey with switch 2 in pink) but forms the same contacts to domains II and III as found in apo eIF5B. As a consequence, also domain III and the ventral side of domain II are shifted in the same direction as switch 2 (arrows).