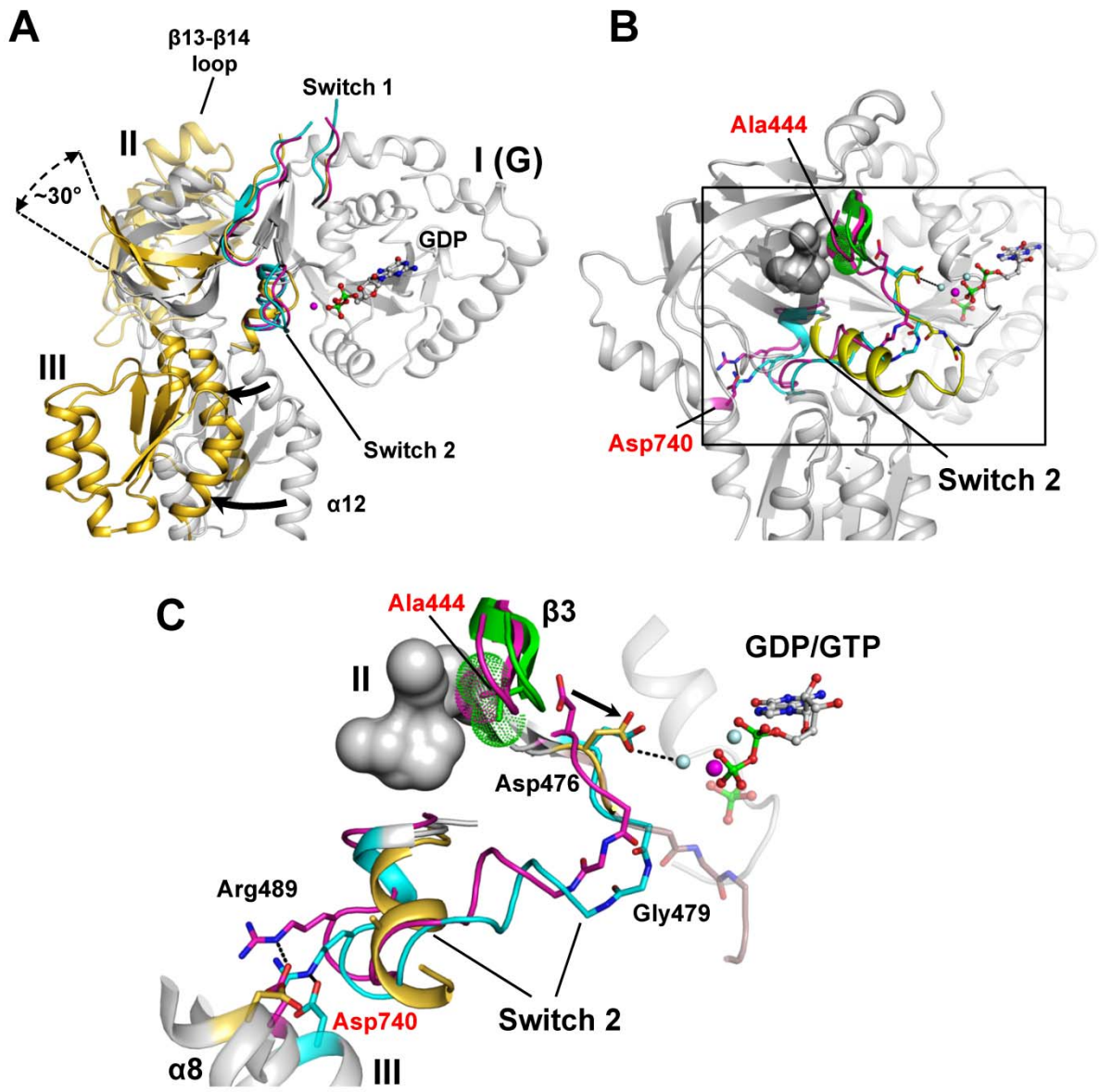

Figure S7. A) G domain-based superposition of eIF5B domains I-III from *S. cerevisiae* in its apo and GDP-bound states. The G domain is colored grey; the switch regions are colored pink for the apo state, cyan for molecule A of Sc-eIF5B·GDP and dark yellow for molecule B of Sc-eIF5B·GDP. While domains II and III of molecule A in the Sc-eIF5B·GDP structure superimpose well with domains II and III of the apo form (grey), domain III in molecule B (dark yellow) is moved away from the G domain (arrows) and has no contact to the partially disordered switch 2. Domain II of molecule B is in its rotated state with the β9-β10 loop moved upwards; switch 1 and the β13-β14 loop remain in their respective inactive conformations. **B)** Overview of apo eIF5B with the locations of Ala444 and Asp740 relative to the nucleotide binding pocket and switch 2. Switch 2 is shown in its apo (pink), GDP- (cyan) and GTP-bound forms (yellow). **C)** Detail of the area indicated in B) (box). Switch 2 is shown in four different states: i) The inactive apo state (pink), in which Asp476 and Gly479 point away from the nucleotide binding pocket and Arg489 forms a salt bridge to Asp740 of

domain III. ii) The partially activated GDP state in Ct-eIF5B·GDP (cyan), in which Asp476 interacts with the Mg^{2+} ion. Gly479 has undergone the peptide flip relative to the apo state. iii) The partially activated GDP state in molecule B of Sc-eIF5B·GDP (dark yellow). Asp476 interacts with the Mg^{2+} ion; switch 2 is shifted towards the GDP molecule and has lost its contacts to domain III (see also Fig. S5A). iv) The G3 motif of the activated switch 2 in Ct-eIF5B·GTP (brown; the rest of switch 2 is not shown). Gly479 has undergone the peptide flip relative to the apo state and contacts the γ-phosphate. Mutagenesis of Gly479 to Ala most likely prevents the peptide flip and thereby stabilizes the inactive conformation of switch 2. Mutagenesis of Asp740 to Arg would result in the steric and electrostatic repulsion of switch 2 in its inactive conformation. Mutagenesis of Ala444 to Val would result in a steric clash with domain II (grey surface), most likely causing strand β3 to move towards Asp476 (green model). To avoid a clash with the repositioned strand β3 Asp476 would have to retreat towards the nucleotide binding pocket, thereby facilitating the partial or full activation of switch 2 by GDP as well as GTP.