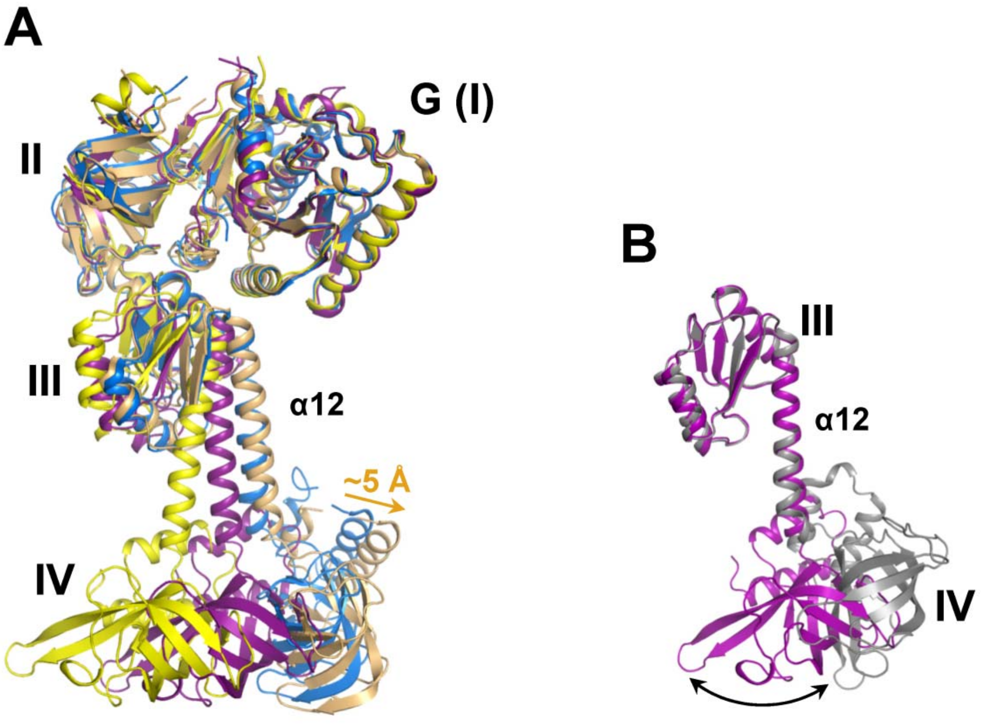

Figure S2. G domain based superposition of the apo structures of eIF5B from *C. thermophilum* (purple), *S. cerevisiae* (yellow) and aIF5B from *M. thermoautotrophicum* (blue; PDB: 1G7R), showing the conformational flexibility of domain III relative to domain I in the absence of a bound nucleotide. The C-terminus of helix α12 in apo aIF5B lies 12 and 22 Å apart from that in Ct-eIF5B and Sc-eIF5B, respectively. The GDPNP-bound form of aIF5B is also shown (wheat; PDB: 1G7T), highlighting the relatively changes observed in the archeal ortholog upon binding of the GDPNP (see also Fig. S8). As the structure of the *S. cerevisiae* ortholog does not contain the second half of helix α12 and domain IV, domains III and IV of the Ct-eIF5B structure were superimposed onto domain III of the Sc-eIF5B structure. **B)** Superposition of domains III and IV of the apo Ct-eIF5B(517C) structure (purple) with the structure of Ct-eIF5B(870C) (grey), based on domain III. Domain III and the following helix α12 are nearly identical in both structures; in contrast, the orientation of domain IV relative to domain III differs significantly, indicating a high degree of conformational flexibility in the linker between helix α12 and domain IV.