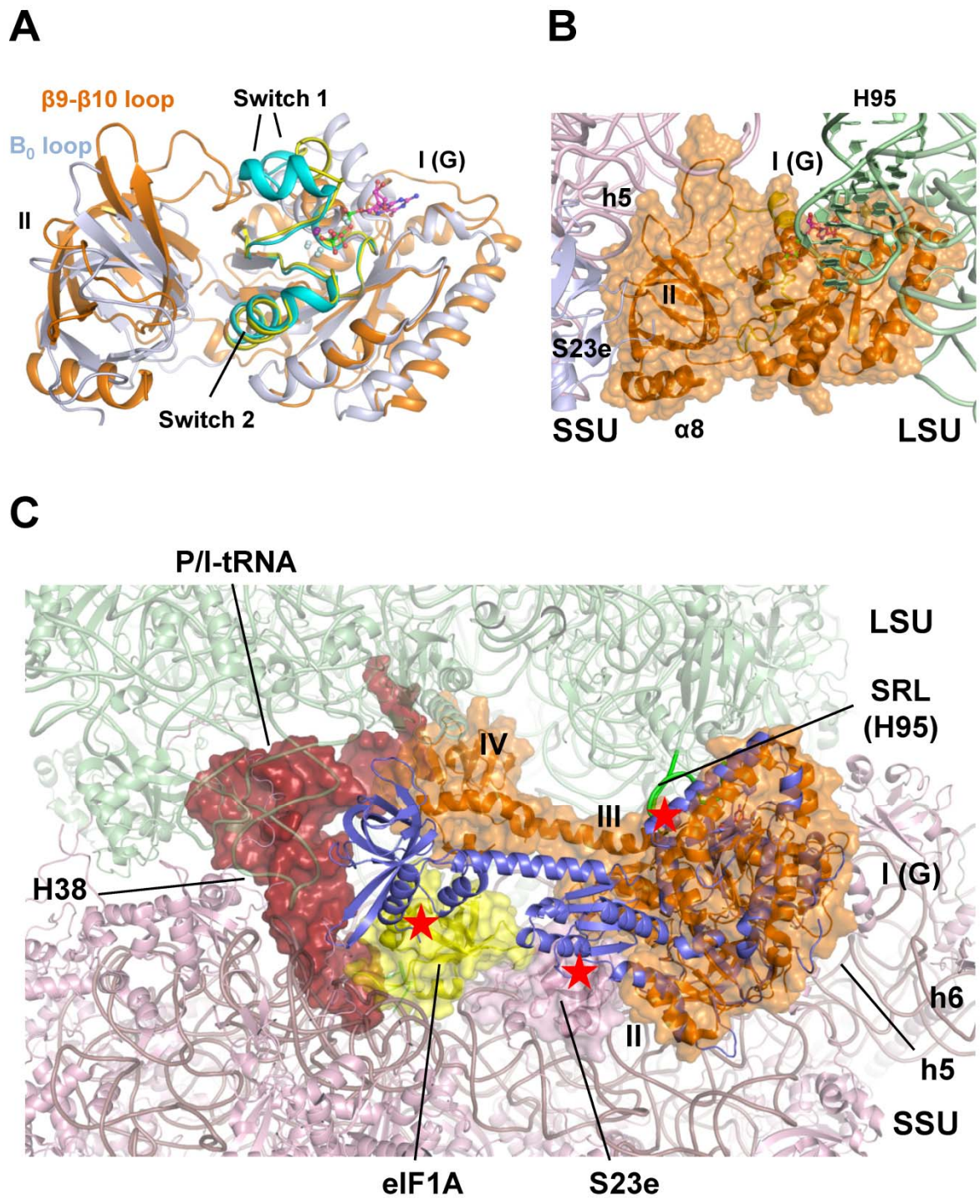

Figure S5. A) Superposition of eIF5B·GTP (orange with P loop, switch 1 and switch 2 in yellow) and free EF-Tu·GDPNP (light blue with P loop, switch 1 and switch 2 in cyan; PDB code: 1EXM), based on their respective catalytic centers. Domains I and II from eIF5B and EF-Tu show the same overall dimensions and exhibit very similar arrangements in regions involved in nucleotide binding (switch 1 and switch 2) or in interactions with the small ribosomal subunit (e.g. the β9-β10 loop in eIF5B and the B_0 loop in EF-Tu). **B)** Cryo-EM model of domains I and II of eIF5B·GDPCP (orange) on the 80S ribosome (Fernandez et al, 2013). The large ribosomal subunit (LSU) is shown in green; the small ribosomal subunit

(SSU) is shown in light pink. **C)** Comparison of eIF5B·GDPCP in the 80S IC (orange; (Fernandez et al, 2013)) with inactive eIF5B (blue). The structure of inactive eIF5B was modeled by superposition of its domain II onto domain II of eIF5B·GDPCP. According to this model, GTP hydrolysis and subsequent P_i release causes domain IV to move away from the acceptor end of the tRNA (dark red) as domain III associates with domains I and II. Simultaneously, the G domain rotates with respect to domain II by ~30° towards the SRL. These movements result in clashes (red stars) of domains I, III and IV with the SRL, ribosomal protein S23e and A site-bound eIF1A (yellow; modeled according to the structure of eIF1 and eIF1A on the 40S subunit (PDB: 4BPE)), respectively, which reduce the affinity of eIF5B·GDP to the ribosome and facilitate its dissociation.