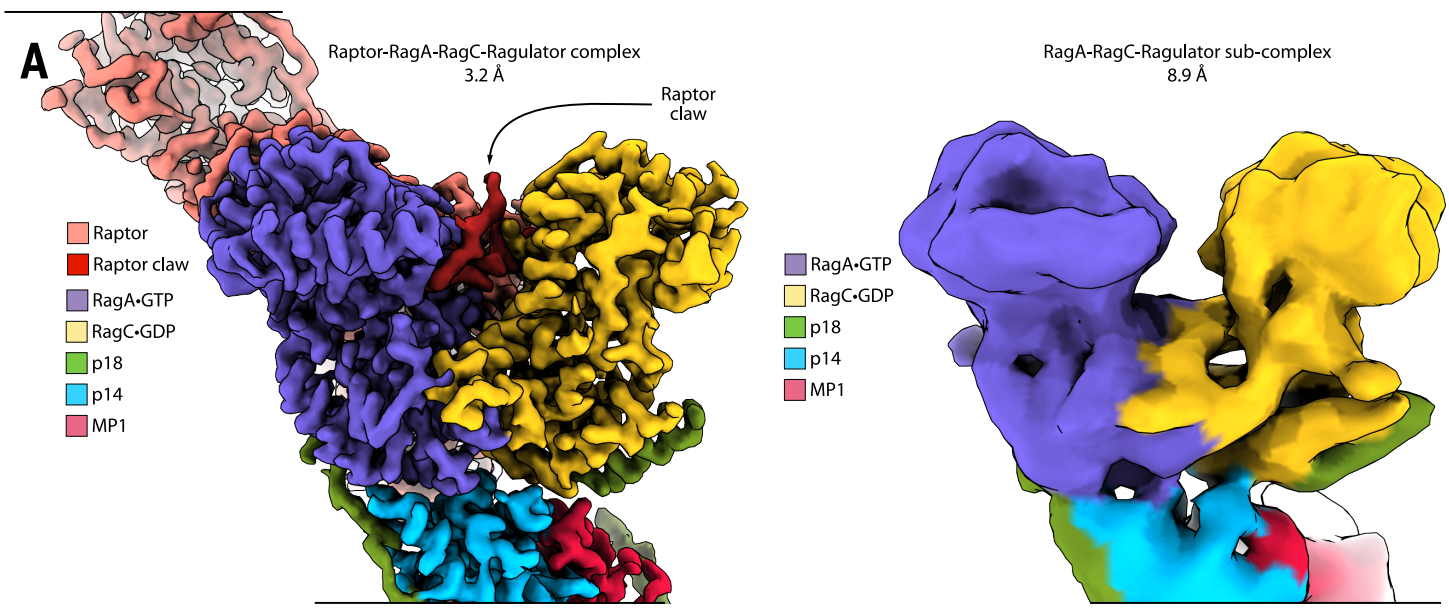
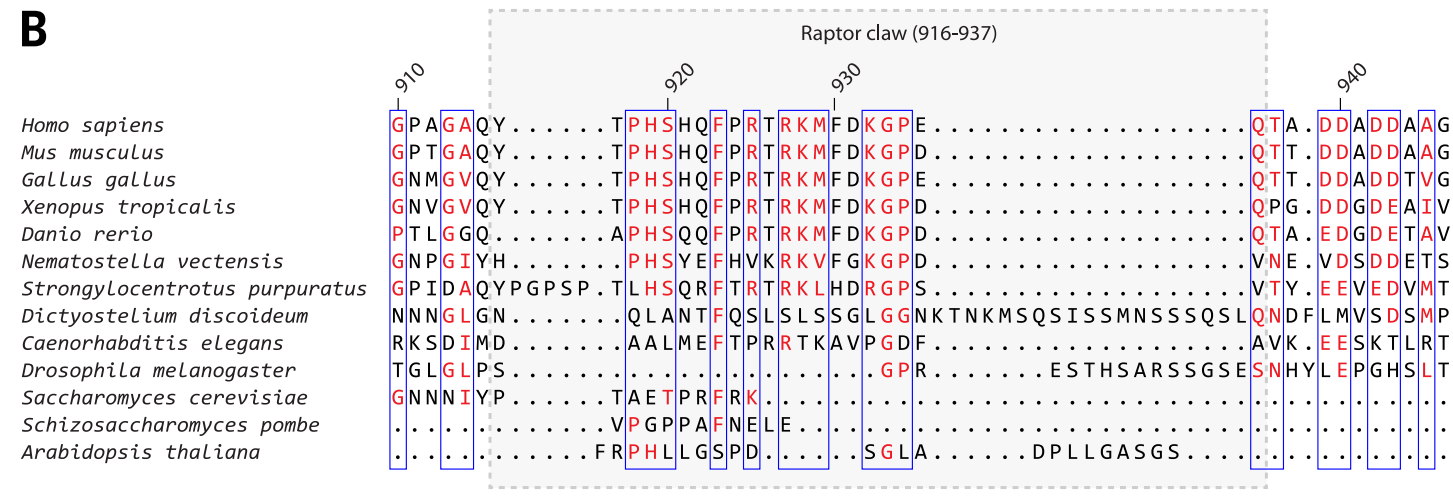
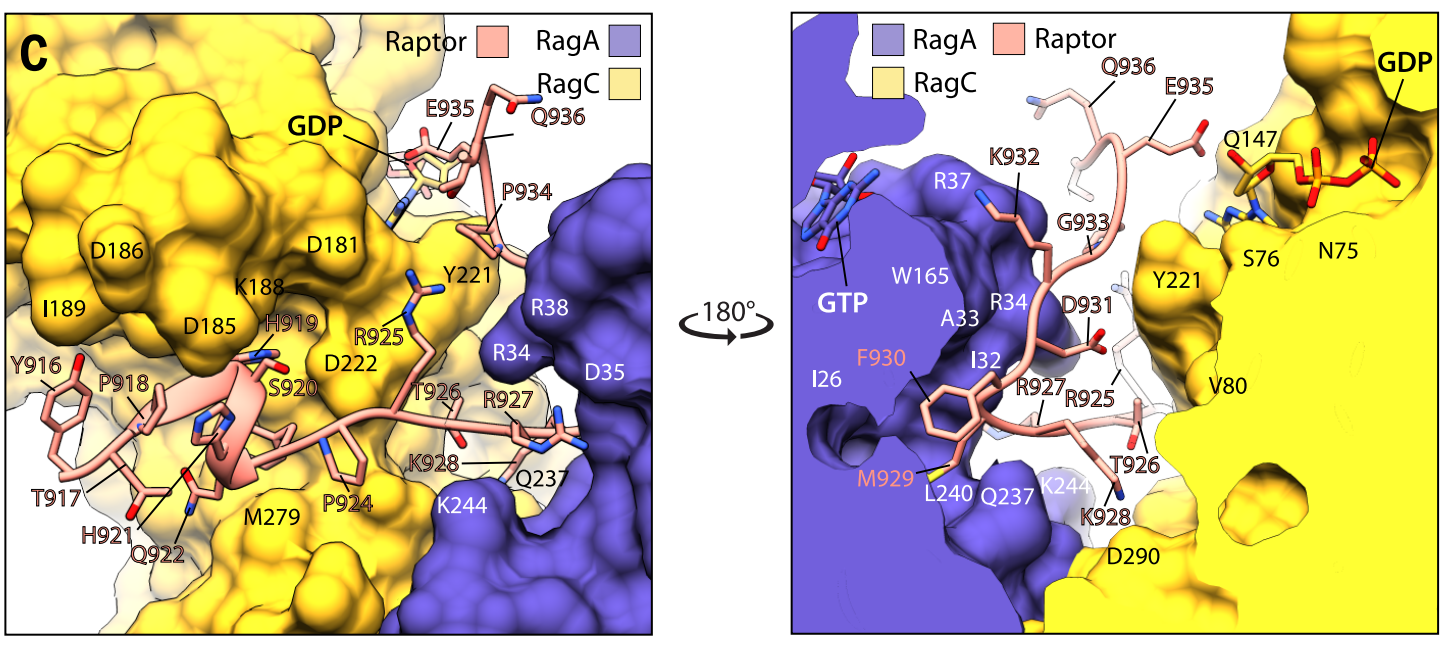
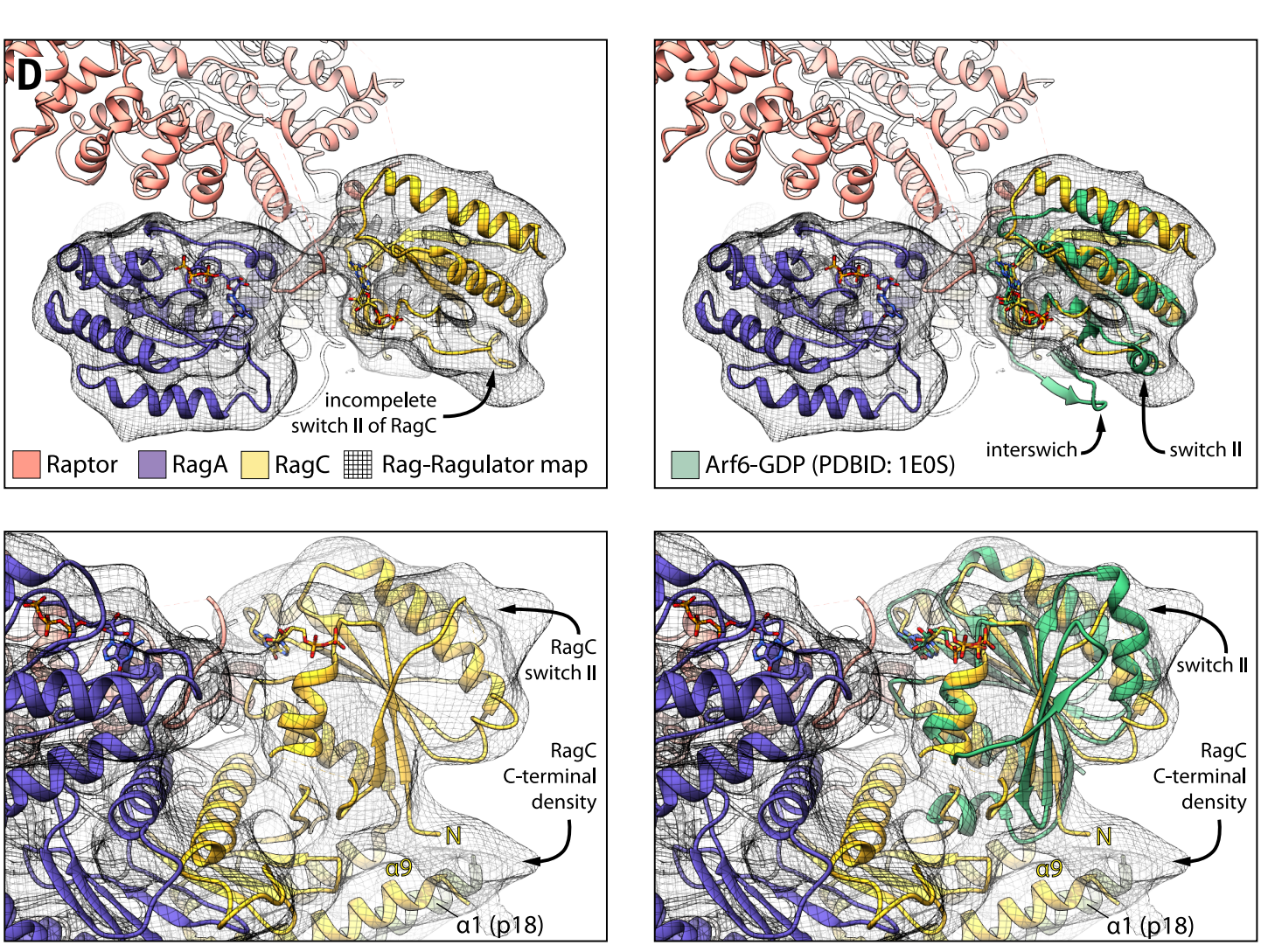
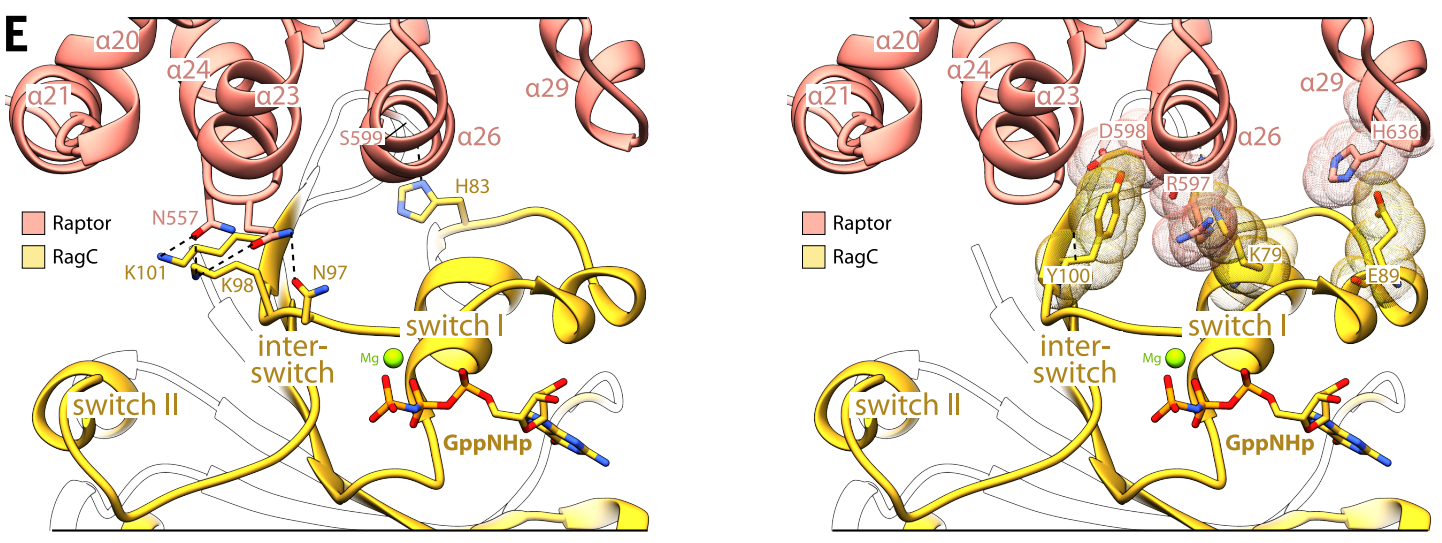
FIGURE S3











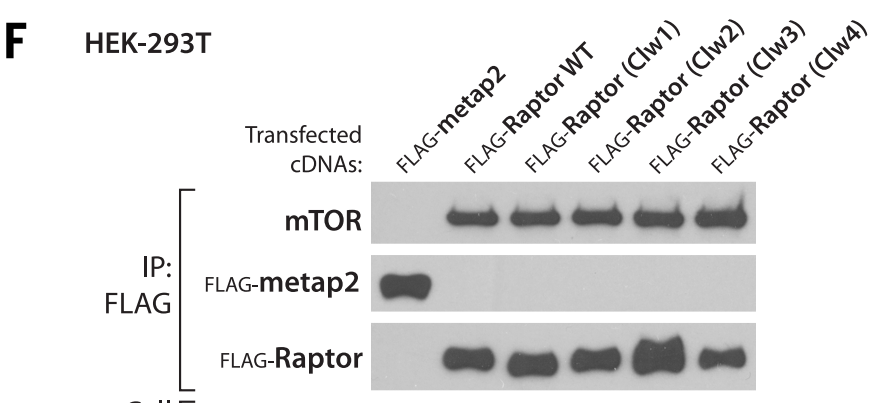


Fig. S3. (A) Side-by-side comparison of an unsharpened Raptor-Rag-Ragulator supercomplex map at 3.2 Å (left) with that of the Rag-Ragulator sub-complex at 8.9 Å (right). Both maps were generated from the same protein sample, and the same set of electron micrographs. Note the extra electron density (red) in between the two Rag GTPases in the map of the Raptor-Rag-Ragulator supercomplex. This density is absent from the map of the Rag-Ragulator sub-complex, and therefore must originate from the Raptor subunit.
(B) Multiple sequence alignment of the Raptor claw across different species. This element appears to be conserved in vertebrates and some invertebrates, but appears weak or potentially even absent in plants and yeast. The sequences of the claw region were extracted from the full-length protein alignment using MAFFT (77) in the E-INS-i mode. This mode is particularly suited for difficult alignments, where multiple conserved regions are interspersed by long gaps that are often unalignable globally, but contain short conserved motifs that can be aligned locally. The claw is an example of such a region. The alignment was evaluated with ESPript (78). Conservatively substituted residues are shown in red, and the most conserved columns are boxed in blue.
(C) Additional views of the Raptor claw, entering the inter-Rag space.
(D) The molecular model of the Raptor-Rag-Ragulator supercomplex was fit into the 8.9 Å resolution map of the Rag-Ragulator sub-complex. There are no major rearrangements in the relative position of the GTPase domains of Rags upon binding to Raptor (top-left panel). It is possible that RagC was already primed into the optimal orientation to receive Raptor by the two oncogenic mutations in RagC that were introduced to stabilize this supercomplex (S75N, T90N). The Rag-Ragulator map revealed extra densities (top-left and bottom-left panels) belonging to the switch machinery of RagC, which in the GDP-loaded state appear highly dynamic, and do not average well to a higher resolution – as evidenced by their absence in the 3.2 Å resolution map of the Raptor-Rag-Ragulator complex. Superimposition of the structure of the Arf6 GTPase loaded with GDP (PDBID: 1E0S) on top of RagC•GDP from this study (top-right and bottom-right panels), shows that one of the two extra densities belongs to switch II (switch I was still disordered). The other extra density is likely an extension of the well-resolved features: the N-terminal residues of RagC, and of the pair of interacting helices – α9 of RagC and α1 of p18.
(E) Rag GTPases are heterodimers and their primary sequences matter. We modeled a theoretical RagC•GTP-Raptor interaction by superimposing a previously reported RagC•GTP structure (3LLU) with our structure of RagA•GTP bound to Raptor. The interacting surfaces are not identical (compare with Fig. 2A). While some new contacts will be formed in such an arrangement (left), the key Raptor residues important for binding to RagA will ultimately clash with switch I and interswitch of RagC•GTP (right), and repel the interaction. Therefore, a rigidified switch I of RagC•GTP cannot fill in for its absence in RagA•GDP.
(F) Mutations in the Raptor claw have no effect on the ability of Raptor to co-immunoprecipitate endogenous mTOR. Flag-metap2 was used as a negative control protein.