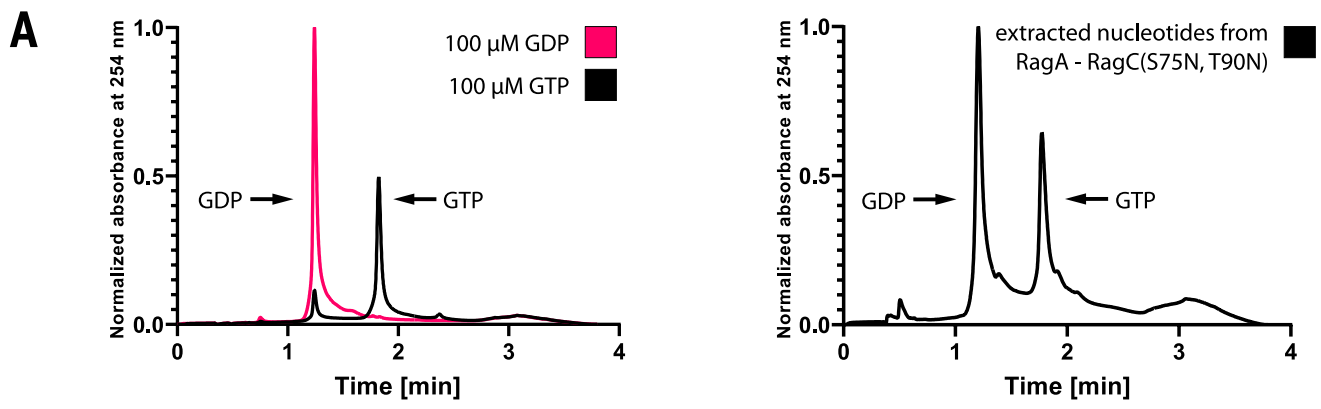
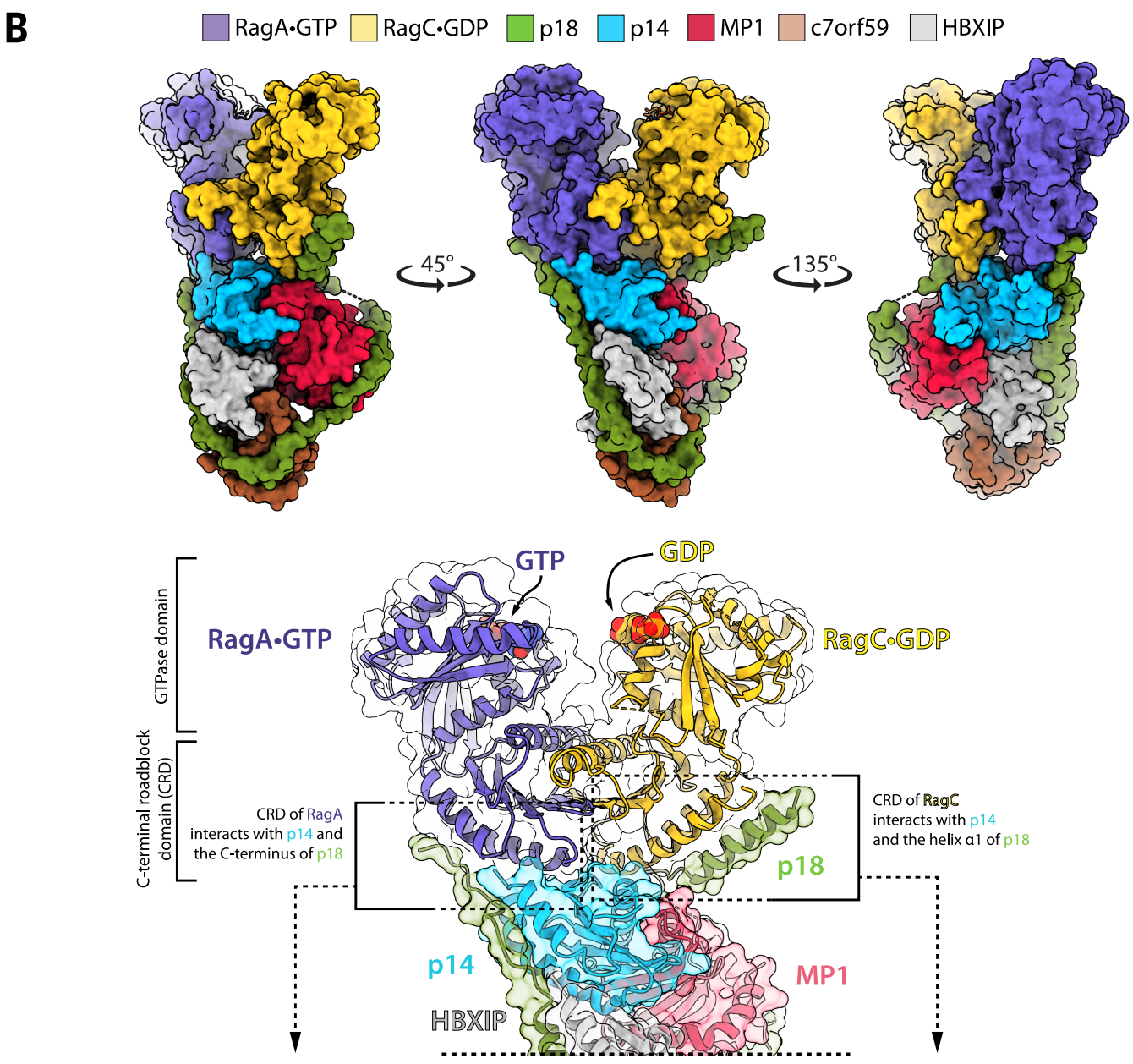
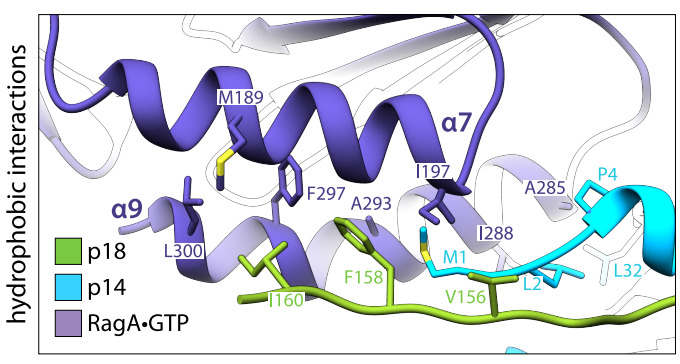
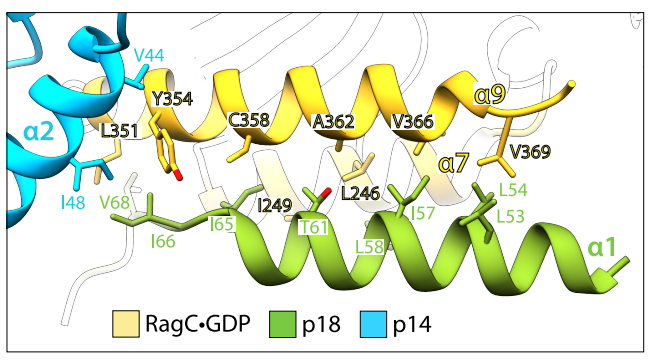
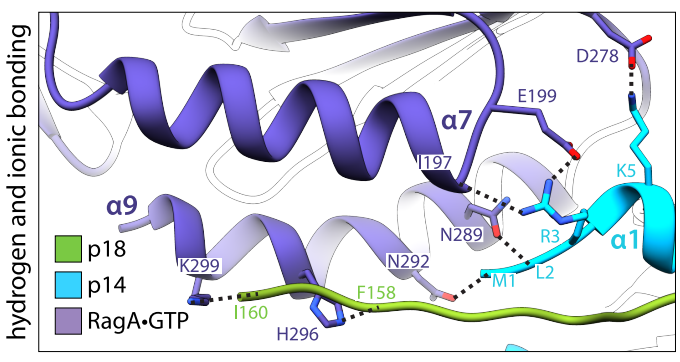
FIGURE S1

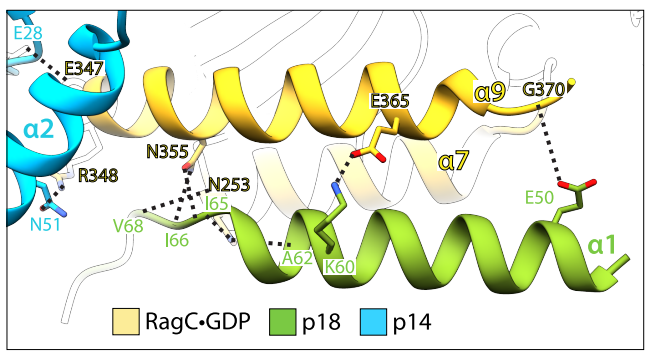












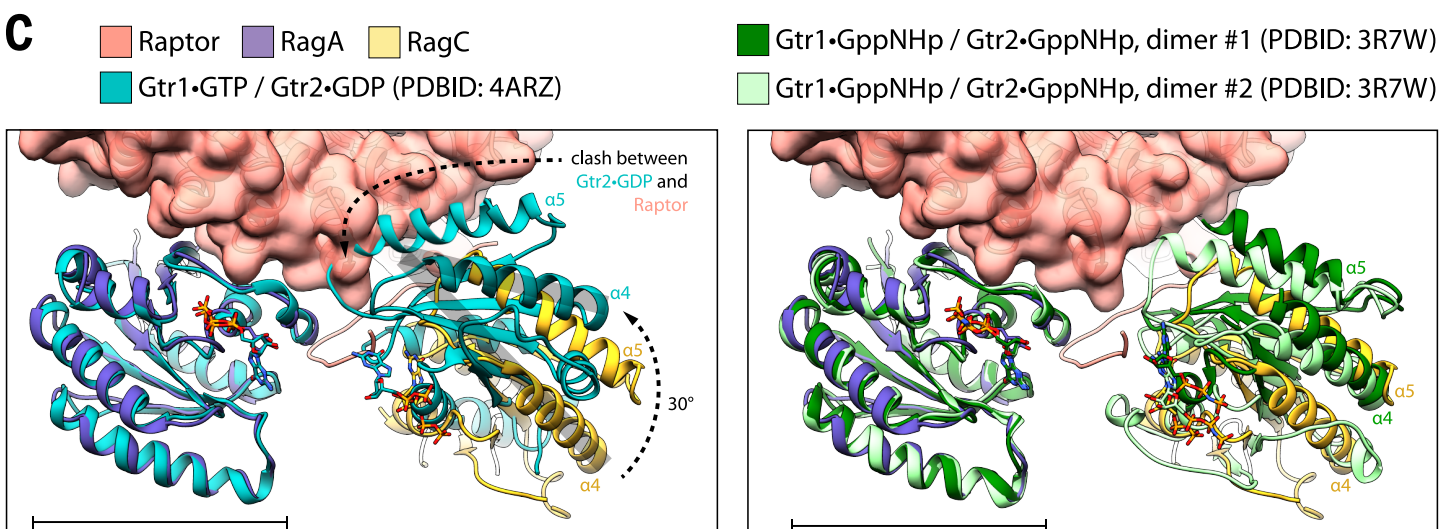


Fig. S1. (A) Chromatographic analysis of nucleotides extracted from purified Rag GTPases. A difference of a single phosphate group is enough to separate GTP from GDP with high confidence. The nucleotide standards eluted from a C18 column at 1.24 min for GDP, and at 1.82 min for GTP (left panel). Please note that the GTP standard used in this study contains a small amount of contaminating GDP. Also, in our chromatographic assay, GTP appears to elute with a diminished signal compared to GDP at an equal molar concentration. Nucleotides extracted from the RagA-RagC(S75N, T90N) mutant, despite their lower concentration, provide enough signal for a clear separation of GTP and GDP. Note that the amount of GDP in this mutant appears higher than that of GTP. Given the fact that the relative signal strength of equimolar GTP and GDP in this assay is not identical, we can assume that the stoichiometry between the two extracted nucleotides in our mutant Rag GTPases is in fact close to 1:1 (right panel).
(B) The structure of the Rag-Ragulator sub-complex. Top panel: three views of the RagA•GTP-RagC•GDP-Ragulator sub-complex structure, in surface representation. Ragulator forms a compact structure of two roadblock dimers: p14-MP1 and c7orf59-HBXIP. These are tied together by the p18 subunit which wraps around them, and engages the Rag GTPases with its termini. The overall fold of Ragulator is similar to previously reported structures [PDBID: 5X6U, 6EHP, 5Y3A, 5YK3, 6B9X (25, 26, 74, 75, 76)], and its binding to Rag GTPases closely resembles that of the Ragulator-RagCRD structures [PDBID: 5X6V, 6EHR (25, 26)]. Middle panel: the CRD domains of Rag GTPases make extensive contacts with the p18 and p14 subunits of Ragulator. MP1, despite being in proximity, makes no contacts with Rag GTPases. While p14 engages the middle part of the CRD domain, p18 flanks it from both sides, and forms contacts with each Rag GTPase. Bottom-left panel: p14 (M1, L2, P4, L32) and the C-terminus of p18 (V156, F158, I160) make a number of hydrophobic contacts with the bottom-face helices α7 and α9 of the RagA CRD (M189, I197, A285, I288, A293, F297, L300). These are supported by a number of side-chain-side-chain and side-chain-main-chain hydrogen bonds and salt bridges between p18 (F158, I160), p14 (M1, L2, R3, K5) and RagA (I197, E199, D278, N289, N292, H296, K299). Bottom-right panel: hydrophobic interactions dominate the binding interface between the bottom-face helices α7 and α9 of the RagC CRD (L246, I249, L351, Y354, C358, A362, V366, V369), p14 (V44, I48) and the helix α1 of p18 (L53, L54, I57, L58, I65, I66, V68). A network of salt bridges and hydrogen bonding further supports this interaction: side-chain-side-chain [RagC (R348, E365), p18 (K60), p14 (N51)], side-chain-main-chain [RagC (N253, E347, N355, G370), p18 (E50, A62, I65, I66), p14 (E28)], main-chain-main-chain [RagC (N253), p18 (V68)].
(C) Spatial arrangement of GTPase domains in yeast Gtrs is different than in human Rags. Structures of Rag GTPase homologs in yeast, Gtr1 and Gtr2, are superimposed with our cryo-EM structure of the Raptor-Rag-Ragulator supercomplex. The GTPase domains of RagA•GTP and Gtr1•GTP were used for structural alignment. The relevant Raptor-binding configuration Gtr1•GTP-Gtr2•GDP (PDBID: 4ARZ, left panel) shows a major Gtr2 domain shift relative to human RagC. Such a shift will cause a clash with Kog1 if the yeast interaction were to resemble that of human Raptor-Rag. The crystal structure of the Gtr1•GTP-Gtr2•GTP configuration (PDBID: 3R7W, right panel) has two Gtr heterodimers in the asymmetric unit. These two heteromdimers significantly differ in the relative position of their Gtr2 GTPase domains, which demonstrates a degree of plasticity that those domains can adapt in a crystal.