

## Supplementary Figure Legends

### **Figure S1. Sequence and structure alignment of Lamtor1-3, associated with Figure**

2. Structure based sequence alignments of Lamtor 1-3 were carried out by Clustal Omega.

### **Figure S2. Sequence and structure alignment of Lamtor4-5, associated with Figure**

2. Structure based sequence alignments of Lamtor 4-5 were carried out by Clustal Omega.

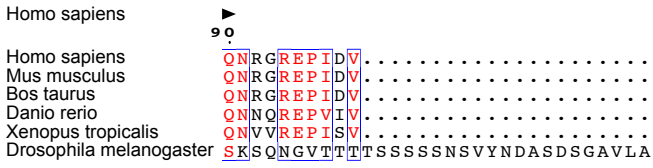
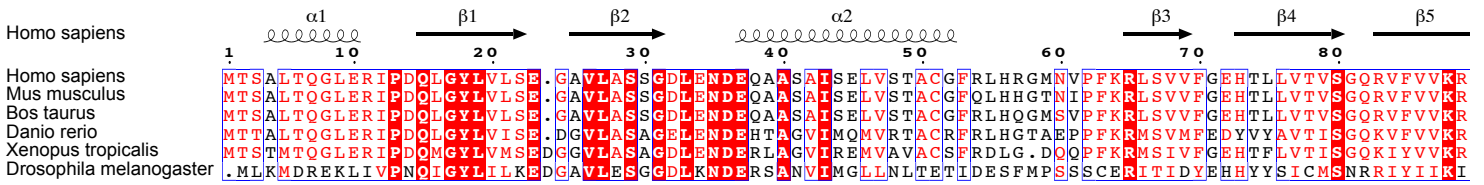
**Figure S3. EM Validation, associated with Figure 5.** RagA/C:Ragulator complex experimental averages (A) were compared to RagA/C pre-reconstitution (B) confirming the identity of the heterodimeric GTPases in the whole complex to inform model construction. (B) The gold-standard Fourier shell correlation for the 16.2 Å structure of the RagA/C:Ragulator complex obtained by negative stain EM.

**Figure S4. Preparation of active and inactive RagA/C:Ragulator complexes, associated with Figure 6.** A. Purification of the RagA/C:Ragulator complex. B. Nucleotide loading of active and inactive RagA/C complexes.

**Figure S5. HDX-MS of RagA/C, associated with Figure 6.** The first five residues in RagC are derived from the tag.



# Lamtor 4



# Lamtor 5

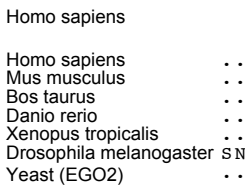
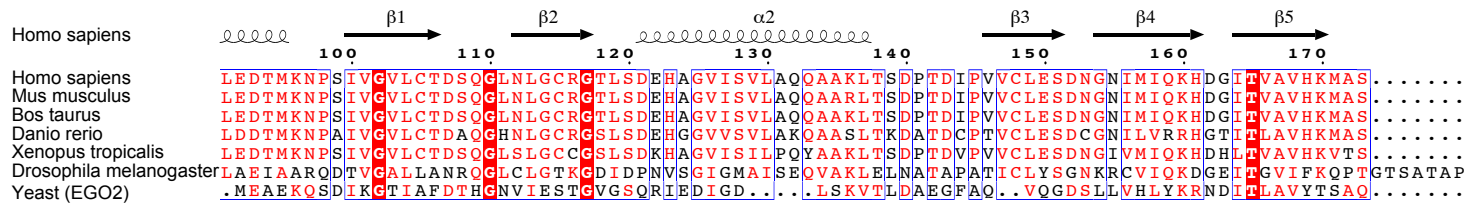
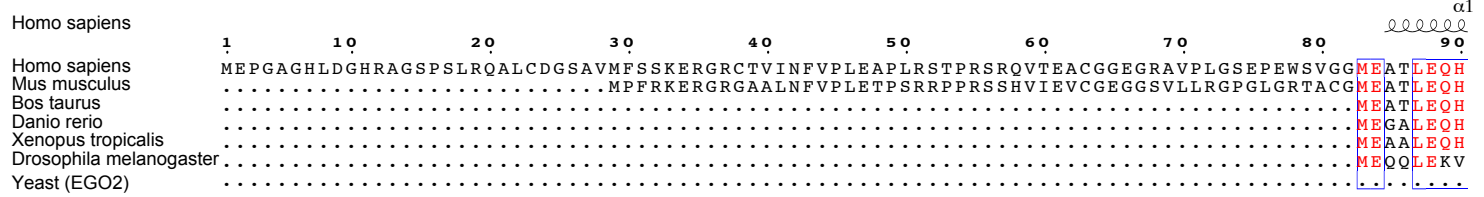
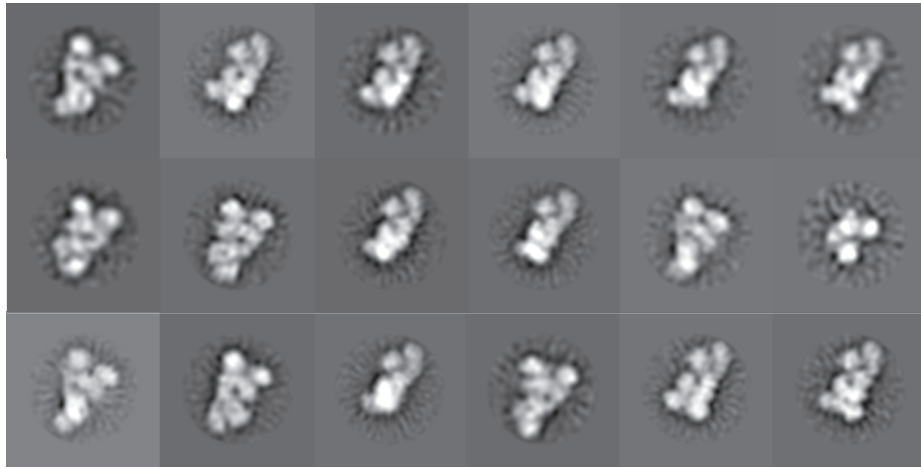
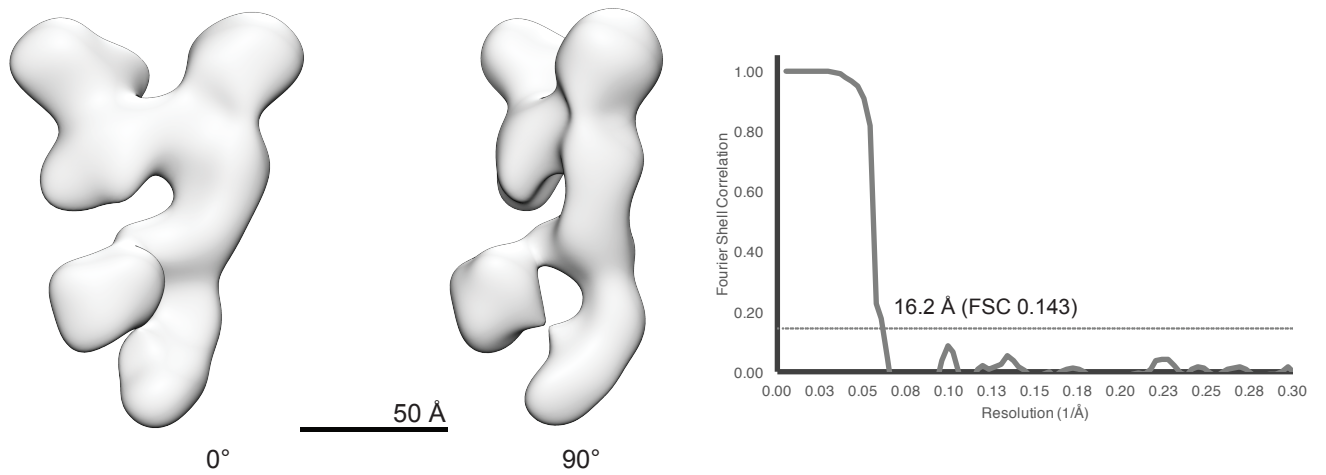
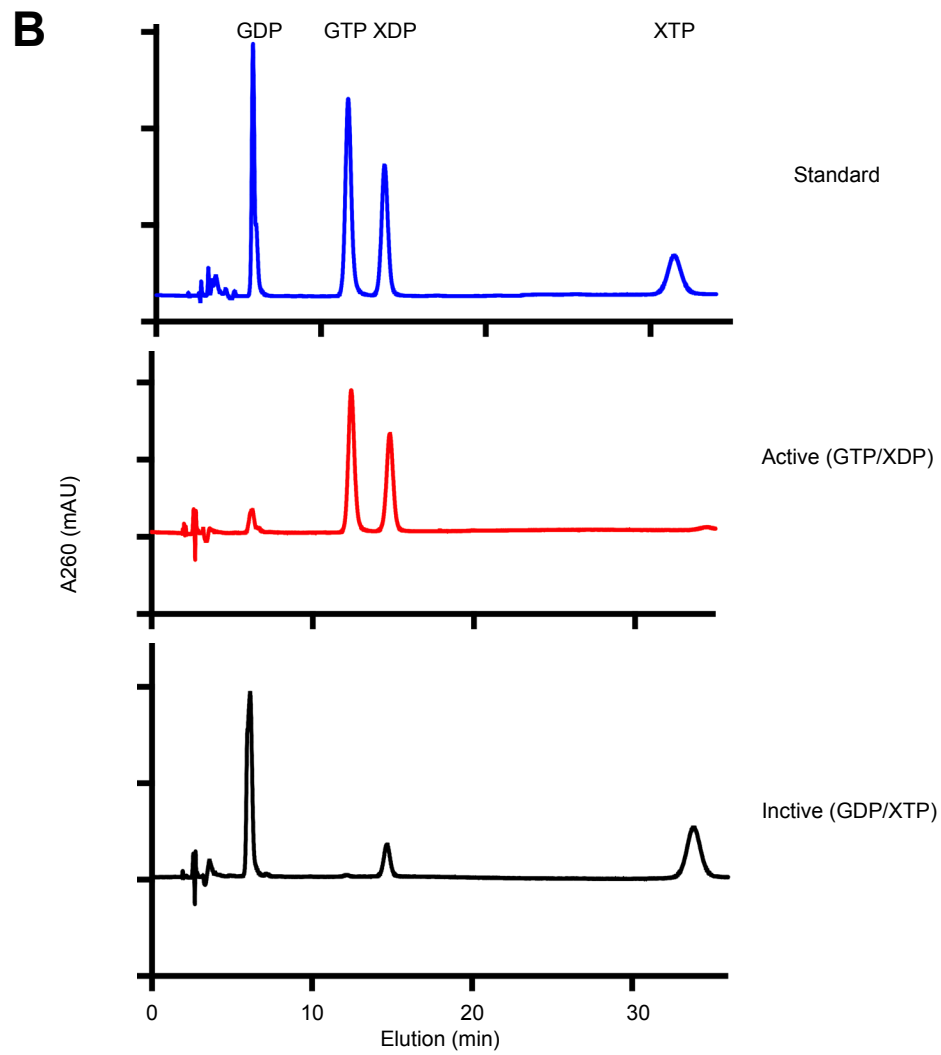
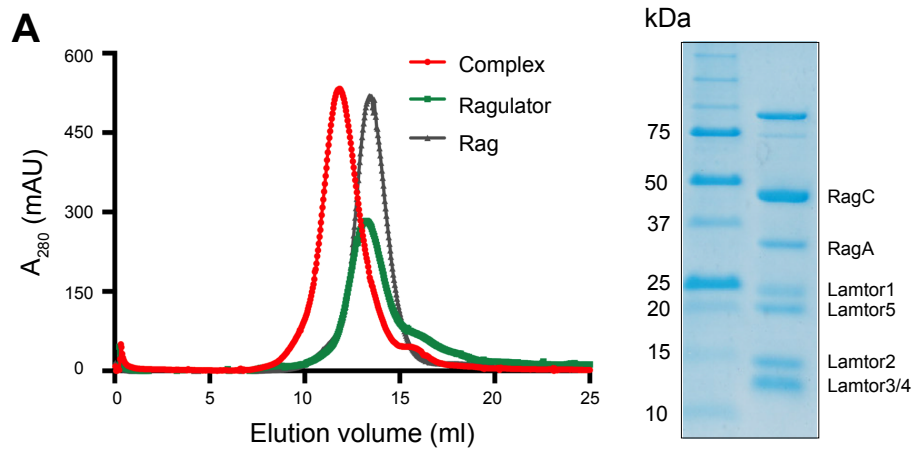


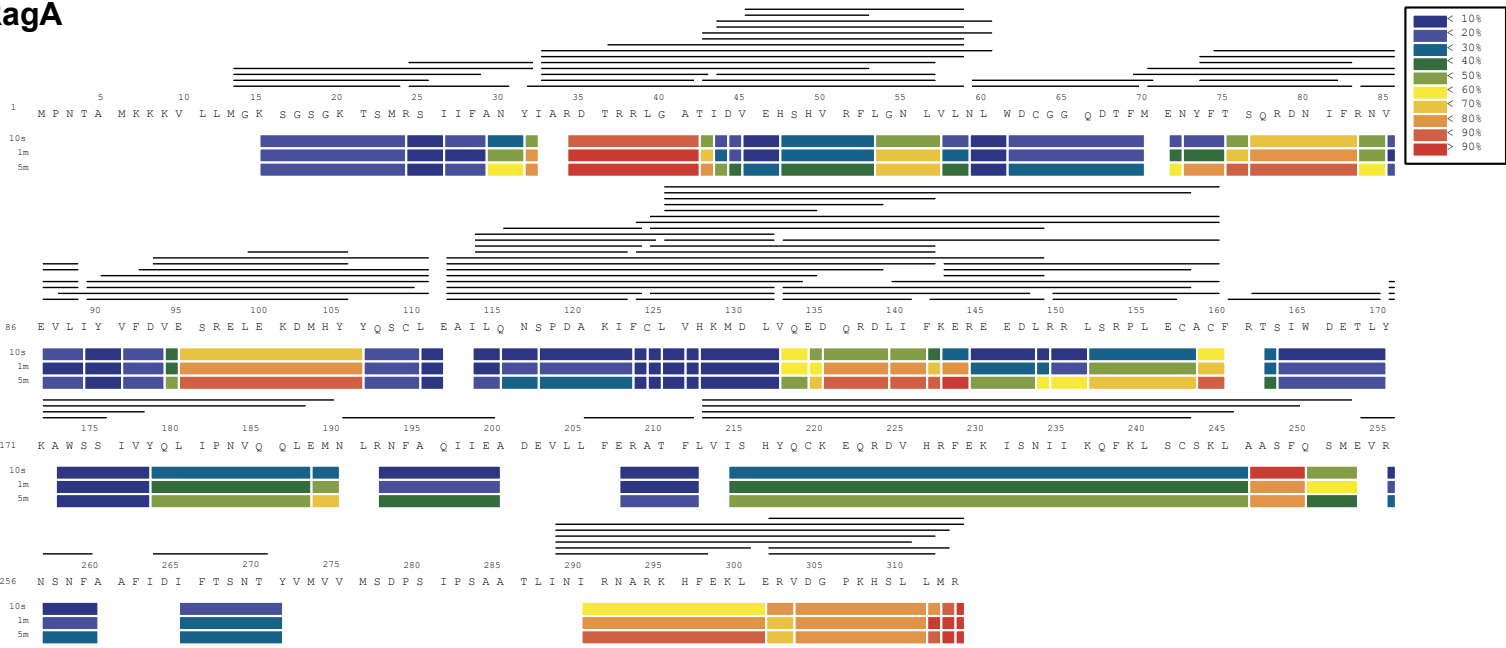
Figure S2

**A****B****C****Figure S3**



**Figure S4**

# RagA



# RagC

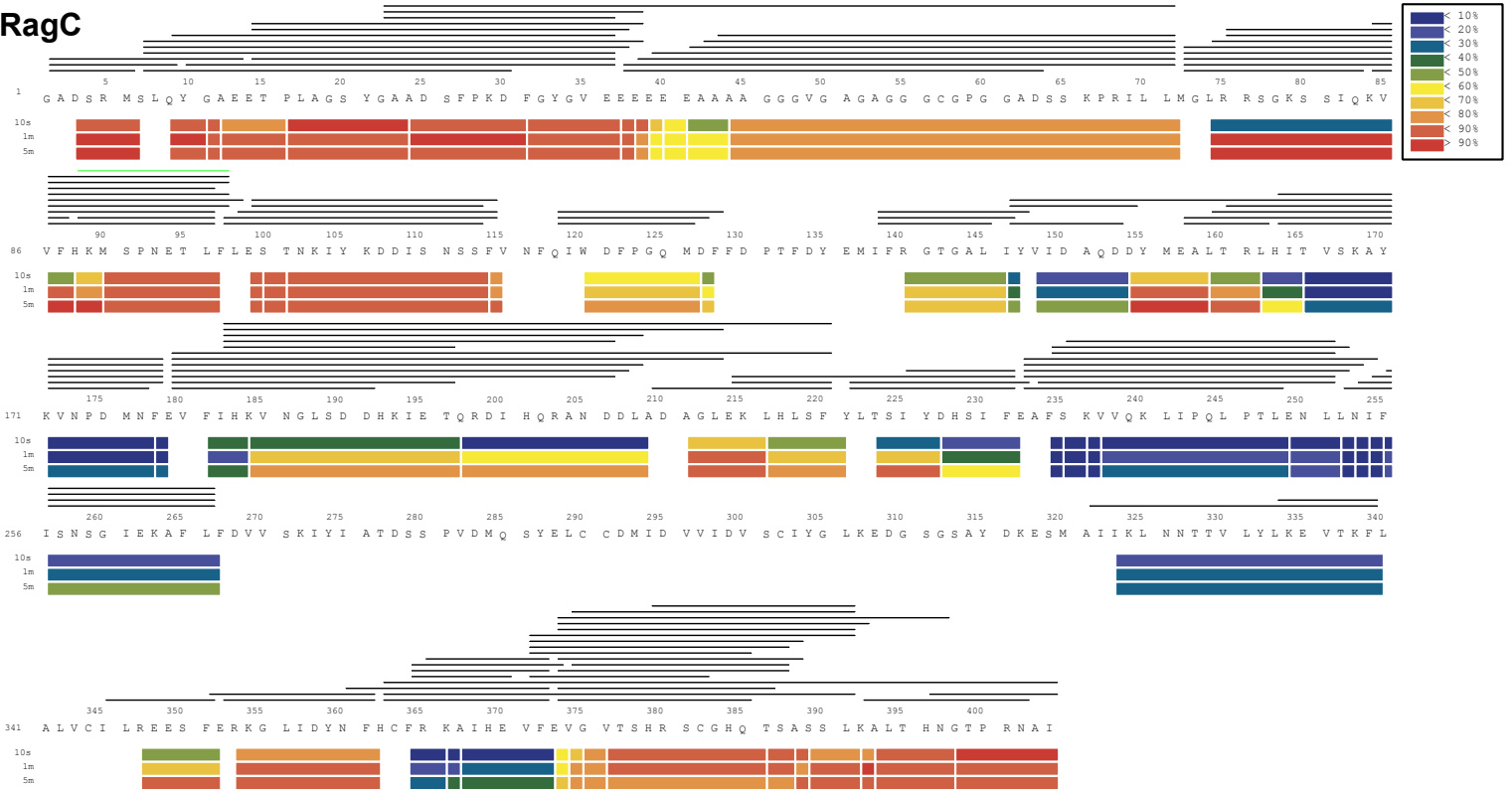


Figure S5