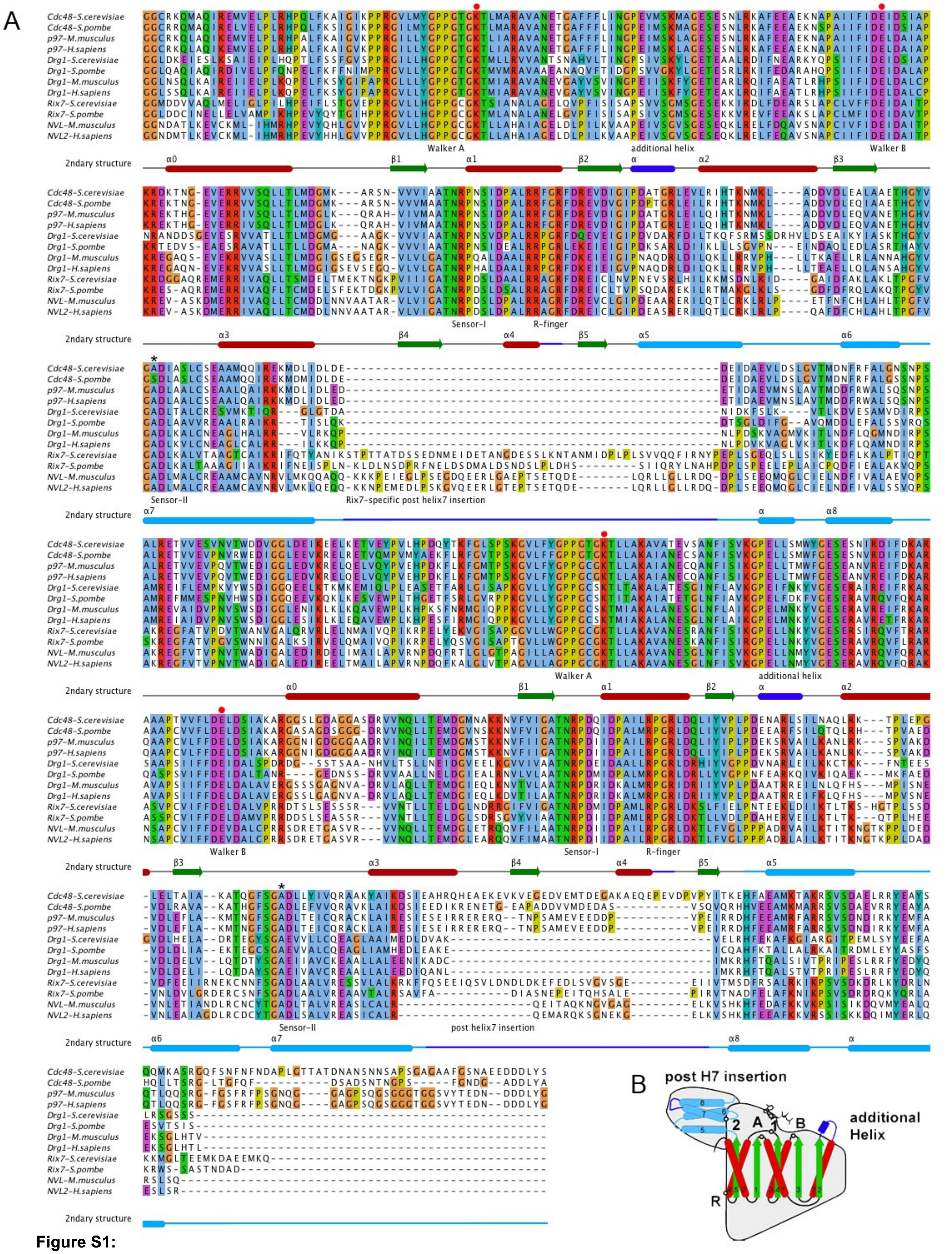

Figure S1:

A, Multiple sequence alignment of Cdc48/p97, Drg1, and Rix7/NVL over the D1 and D2 AAA-ATPase domains. The alignment was generated with ClustalW [76] and displayed with Jalview [77]. Secondary structure elements (α-helices and β-strands) are according to the crystal structure of p97 (pdb 3CF2) [20]. In the D2 domain, the positions of α-helix 8 and of the last α-helix are according to secondary structure prediction (PSIPRED) [78]. Numbering of α-helices and β-strands as well as nomenclature of sequence elements is according to [17]. Elements of the ATPase core are coloured in red (α-helices) and green (β-strands), the lid domain and its α-helices are depicted in light blue. The additional α-helix of classical clade AAA-ATPases and the post helix7 insertion are indicated in dark blue. Specific ATPase elements are labelled. The classical Walker A (K>A, defective nucleotide binding) and Walker B mutations (E>Q, impaired ATP hydrolysis) are also indicated [11]. Unique features of the classical clade are highlighted: (i) replacement of the generally conserved sensor-II arginine at the base of α-helix7 by an alanine; (ii) short insertion within the arginine finger region leading to the occurrence of two conserved arginines that are usually separated by proline and glycine. **B,** Representation of the secondary-structure distribution within each ATPase domain with the α/β domain depicted in green (β-strands) and red (α-helices) and the α-helical elements of the α-helical lid domain depicted in light blue. The classic-clade-specific additional α-helix and the post helix7 insertion are shown in dark blue.

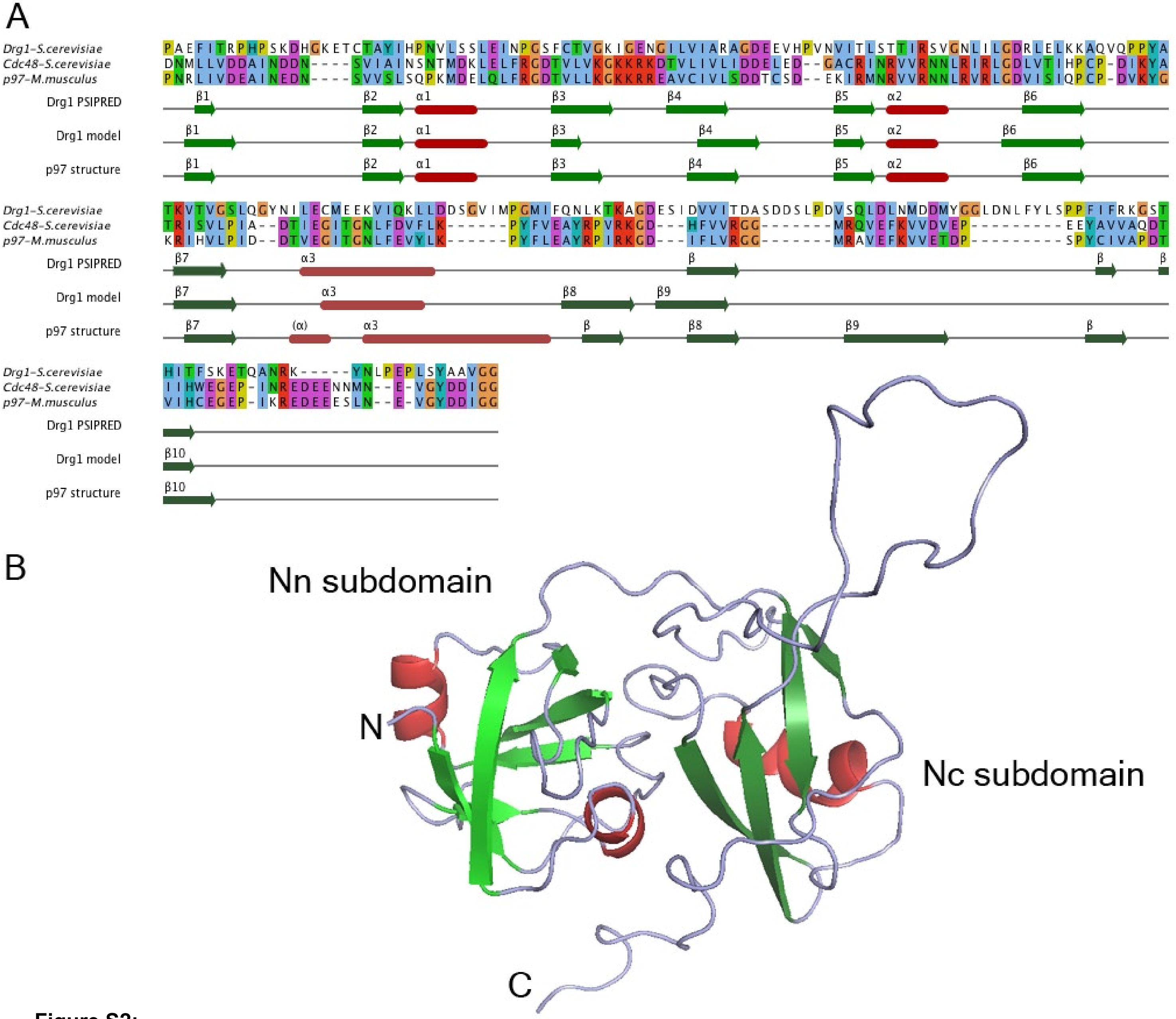

Figure S2:
A, The N-terminal domain of Drg1 is composed of two sub-domains: a double-ψ β barrel Nn-domain and a four-stranded β-barrel Nc-domain. A, Multiple sequence alignment of Drg1 (*S. cerevisiae*, amino acids 31-249), Cdc48 (*S. cerevisiae*, amino acids 33-218) and p97 (*M. musculus*, amino acids 23-208). The alignment was generated with ClustalW [76] and displayed with Jalview [77]. Secondary structure elements (α-helices in red and β-strands in green) are derived from secondary structure prediction (PSIPRED) [78], a Drg1 structure model, or crystal structures of p97 (pdb 3CF2, 1R7R, and 1E32) [20, 22, 23]. Numbering of α-helices and β-strands is according to [22]. Note that the alignment was refined in order to properly position the predicted β-strand 10 of Drg1. **B,** Structure model of the N-terminal domain of Drg1. The structure model was calculated, based on HHpred alignments [79], by the MODELLER software from a p97 reference structure (pdb 3CF2) [20]. The PyMOL program was used to display the structure [80]. As in A, the structural elements of the Nc-domain are distinguished from the ones of the Nn-domain by the use of darker colours.

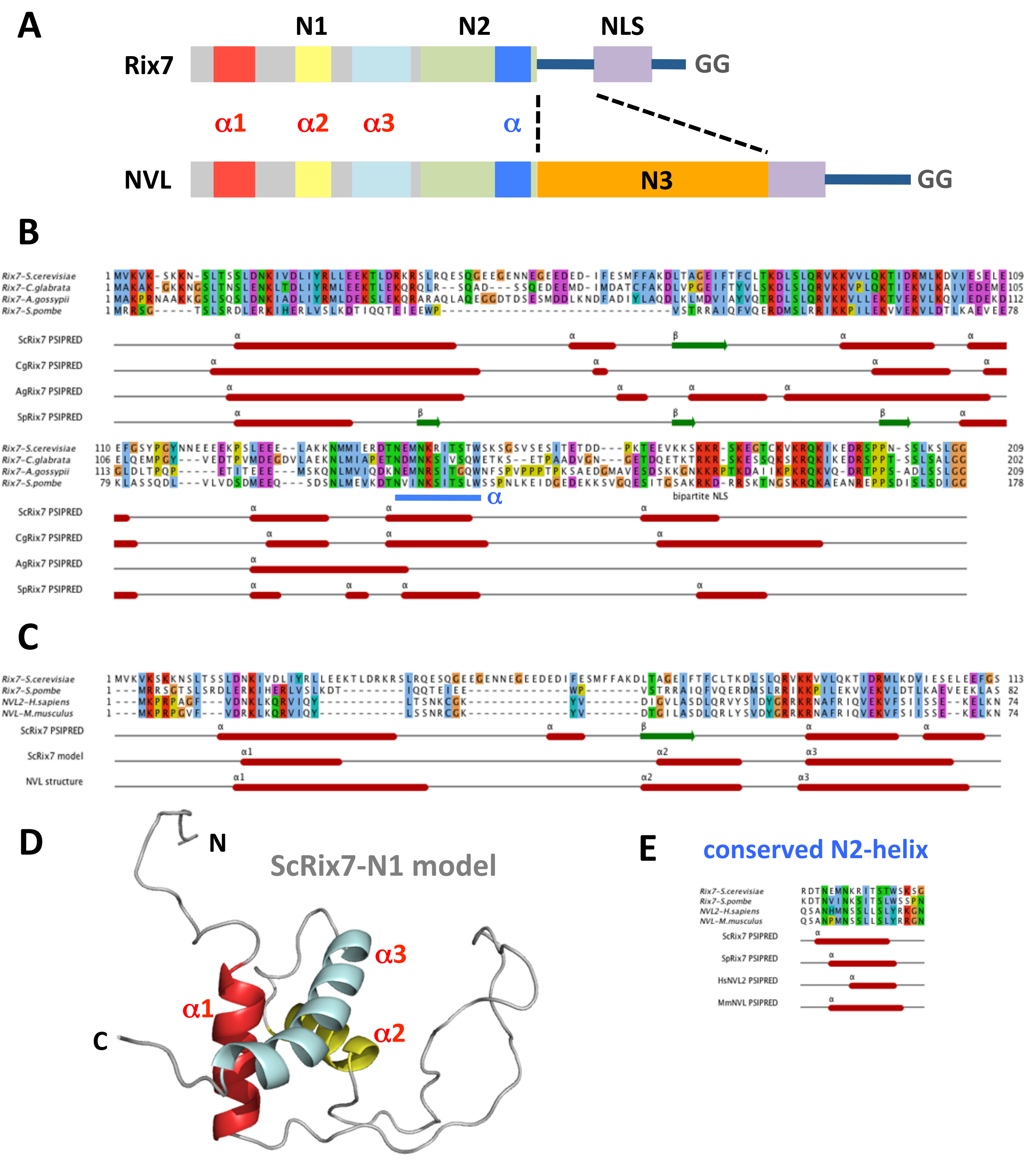

Figure S3:
Structural analysis of the N-terminal domain of Rix7. **A,** Schematic representation of the N-terminal domains of Rix7 and NVL. The distinct N-terminal regions are denoted by N1, N2 and N3 and they are followed by a bipartite nuclear localization signal (NLS). Relevant predicted α-helices are indicated. The two glycines (GG) indicate the start of the AAA-domain D1. **B,** Multiple sequence alignment of the N-terminal domains of fungal Rix7 proteins (*S. cerevisiae*, *C. glabrata*, *A. gossypii* and *S. pombe*). The alignment was generated with ClustalW [76] and displayed with Jalview [77]. Secondary structure elements (α-helices in red and β-strands in green) are derived from secondary structure prediction (PSIPRED) [78]. The conserved α-helix at the end of the N2 region is underlined in blue. **C,** Multiple sequence alignment of the N1 regions of fungal Rix7 (*S. cerevisiae* and *S. pombe*) and mammalian NVL (*M. musculus*). Secondary structure elements are derived from secondary structure prediction (PSIPRED) [78], a Rix7 (*S. cerevisiae*) structure model, or an NMR structure of mouse NVL (pdb 2RRE) [29]. **D,** Structure model of the N1 region of Rix7 (*S. cerevisiae* amino acids 1-113). The structure model was calculated, based on HHpred alignments [79], by the MODELLER software from a mouse NVL reference structure (pdb 2RRE) [29]. The PyMOL program was used to display the structure [80]. The colour of the α-helices is as in A. **E,** Multiple sequence alignment of the conserved α-helix at the end of the N2 region of fungal Rix7 (*S. cerevisiae* and *S. pombe*) and mammalian NVL (*H. sapiens* and *M. musculus*). The position of this α-helix (red) is derived from secondary structure prediction (PSIPRED) [78].

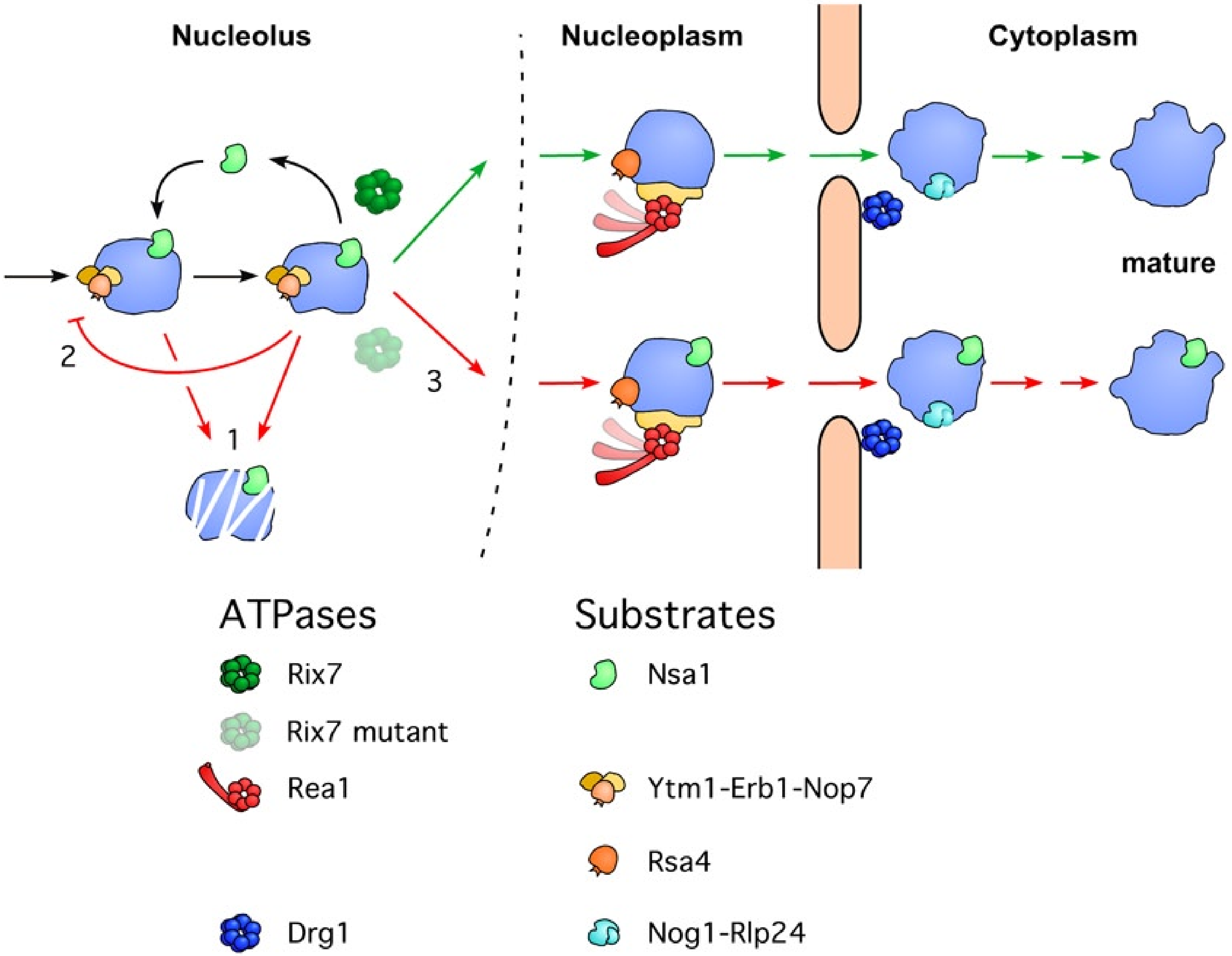

Figure S4:
Effects of mutational inactivation of Rix7 on the fate of Nsa1-containing pre-60S particles. In wild-type cells (RIX7, upper green pathway), Rix7 releases Nsa1 from late nucleolar pre-60S particles. Upon mutational inactivation of Rix7 (rix7, lower red pathway), the majority of pre-60S particles do not further evolve and are degraded (1), while a fraction of pre-60S particles gain an earlier composition and are retained in the nucleolus (2). Nsa1 remains associated with pre-60S particles that have escaped from disassembly or nucleolar retention, and accumulates over time on ‘aberrant’ cytoplasmic 60S subunits (3). [15, 19]. For simplicity, the Rea1-mediated release of the trimeric Ytm1-Erb1-Nop7 sub-complex has been omitted.

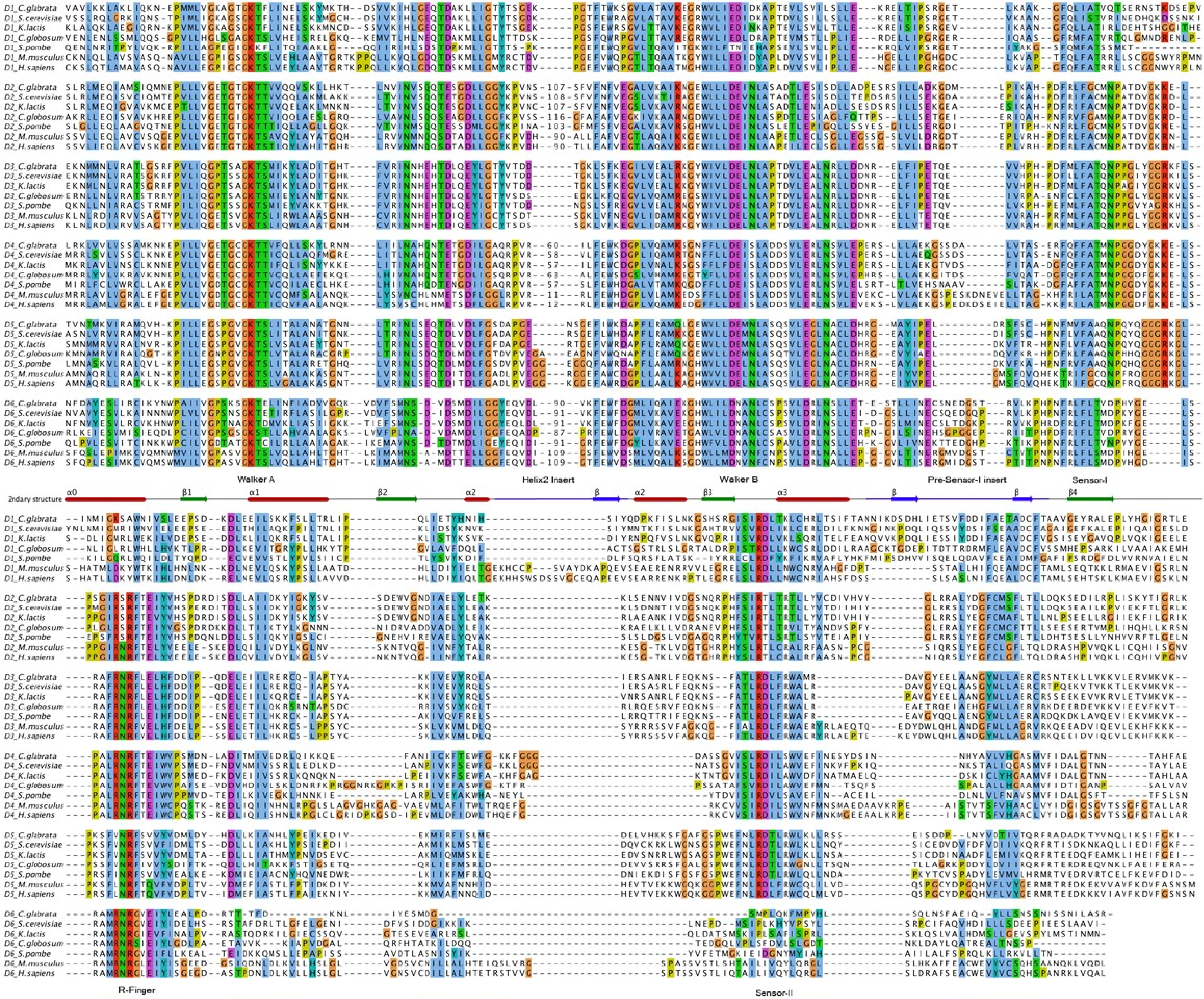

Figure S5:

Multiple sequence alignment of the Rea1 ATPase domains was done with ClustalW [76] and displayed with Jalview [77]. Secondary structure elements are depicted below (see also Figure S6). Elements of the ATPase core are coloured in red (α-helices) and green (β-strands), the lid domain is depicted in light blue and clade-specific insertions are indicated in dark blue. Specific ATPase elements are labelled.

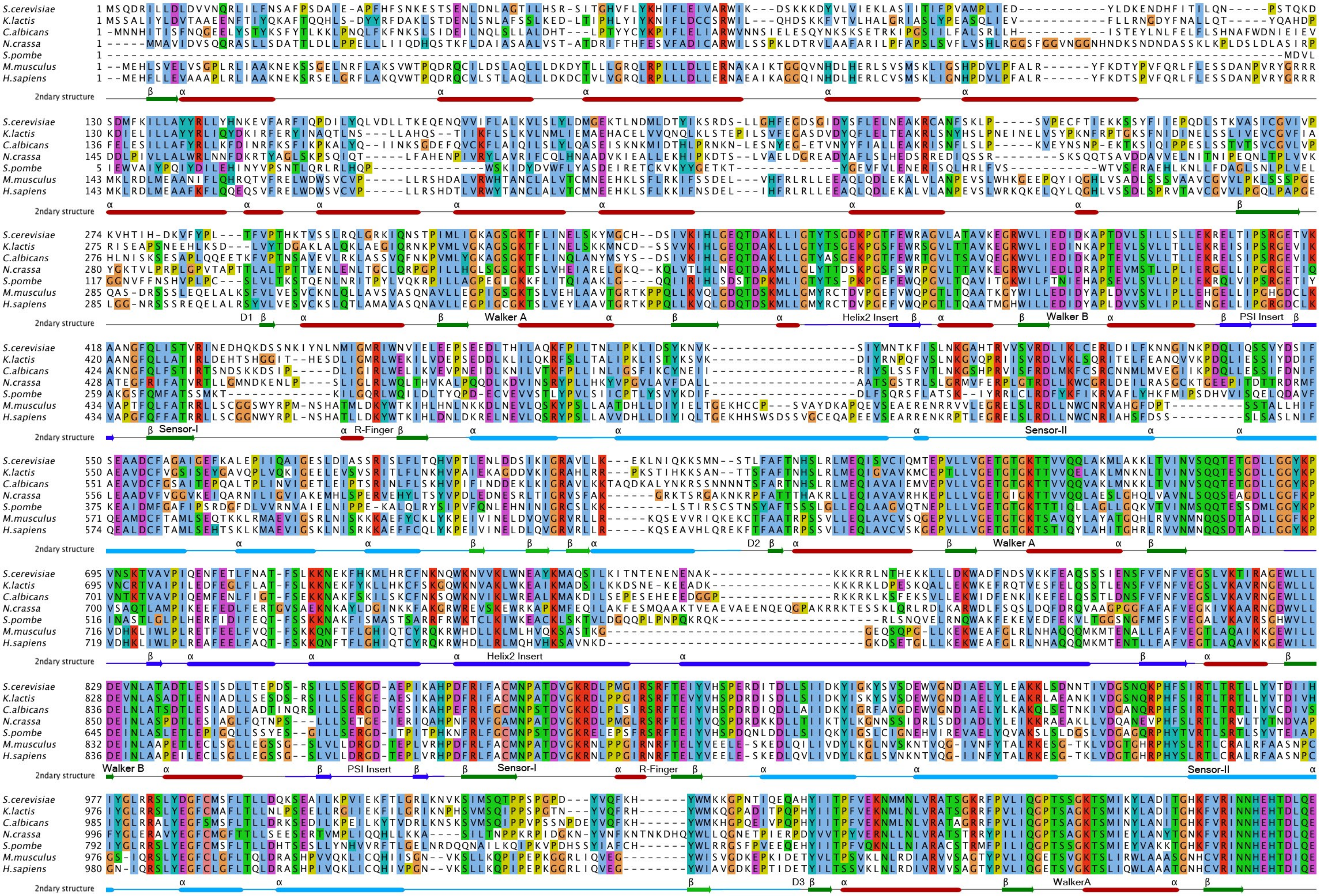

Figure S6, page 1

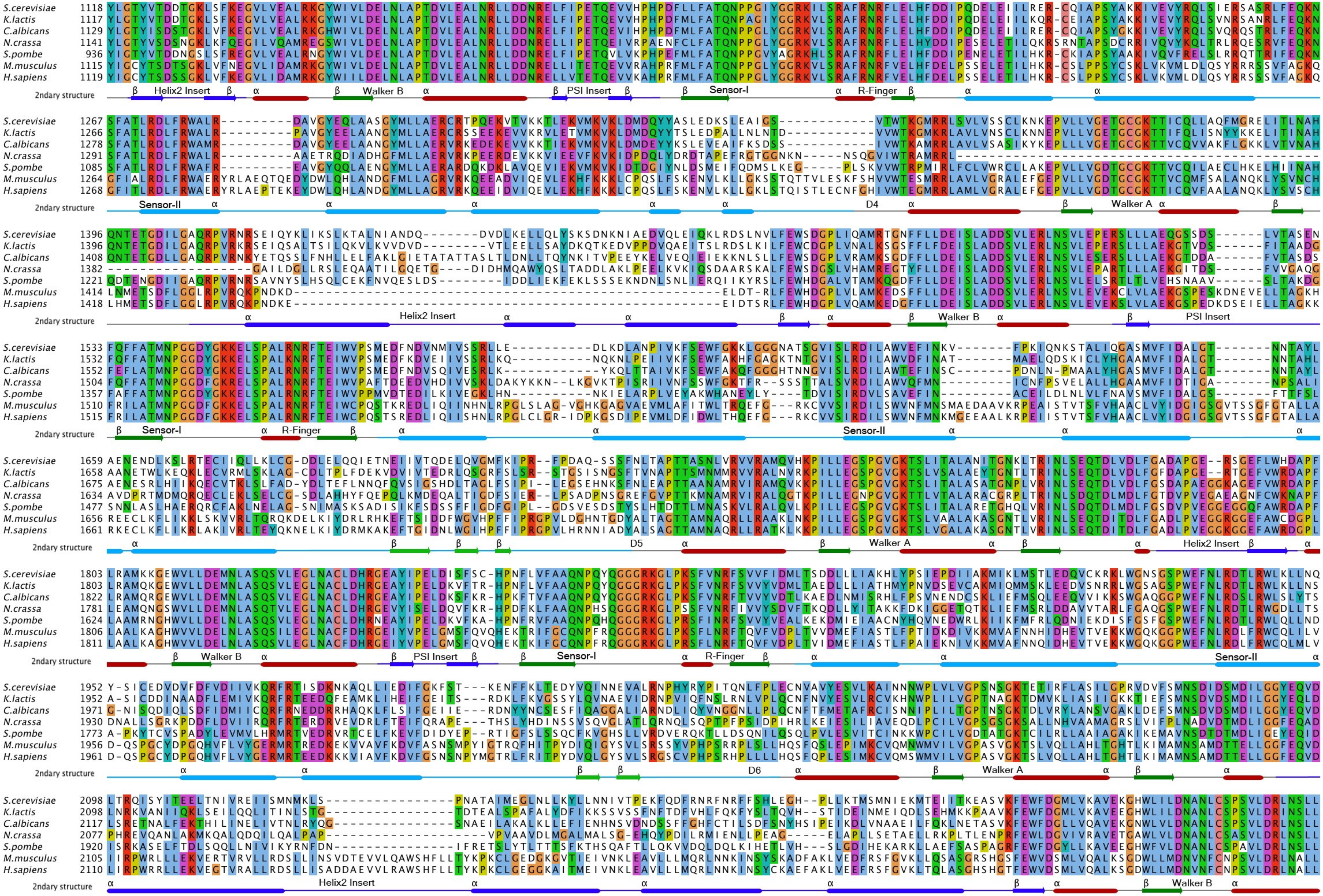

Figure S6, page 2

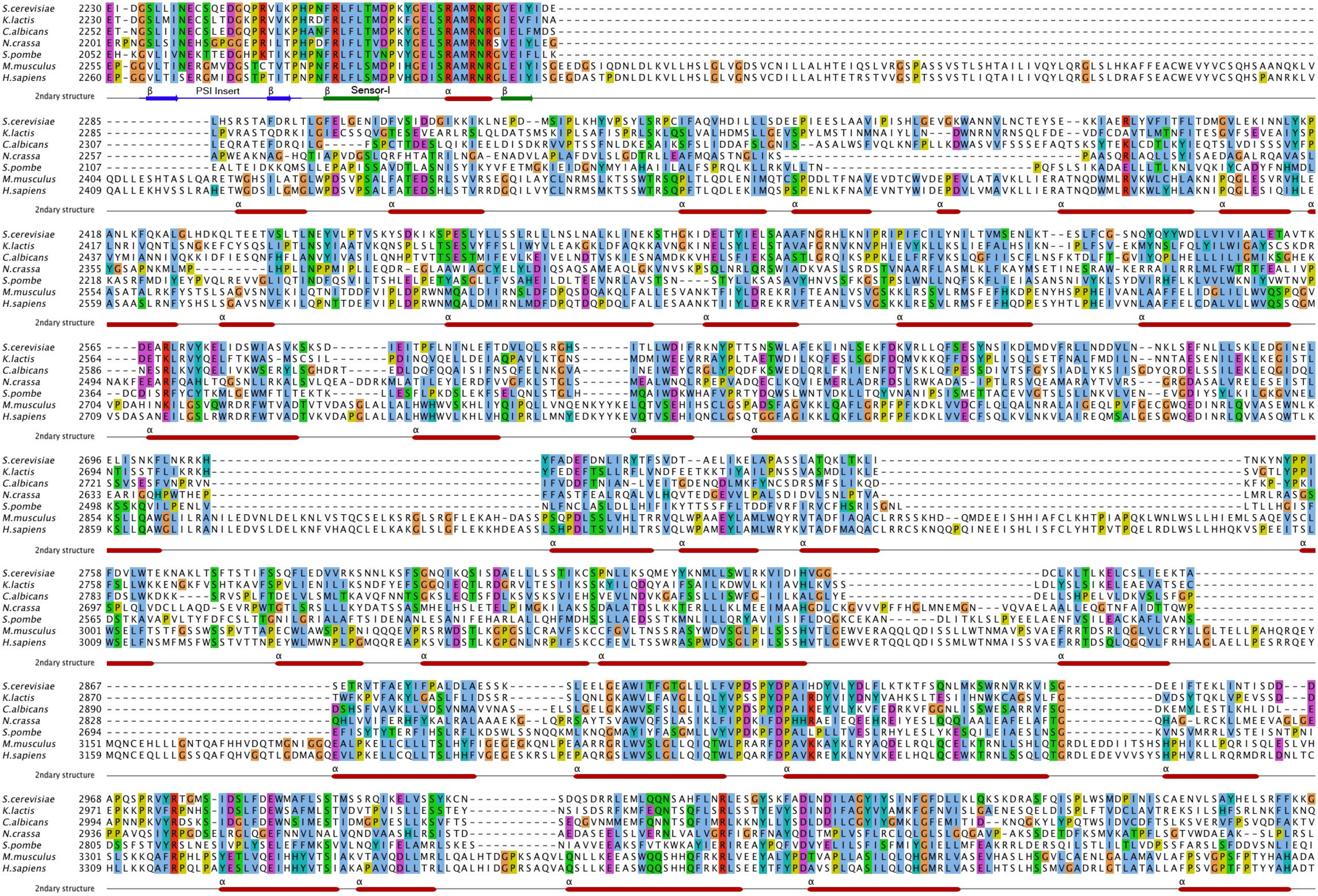

Figure S6, page 3

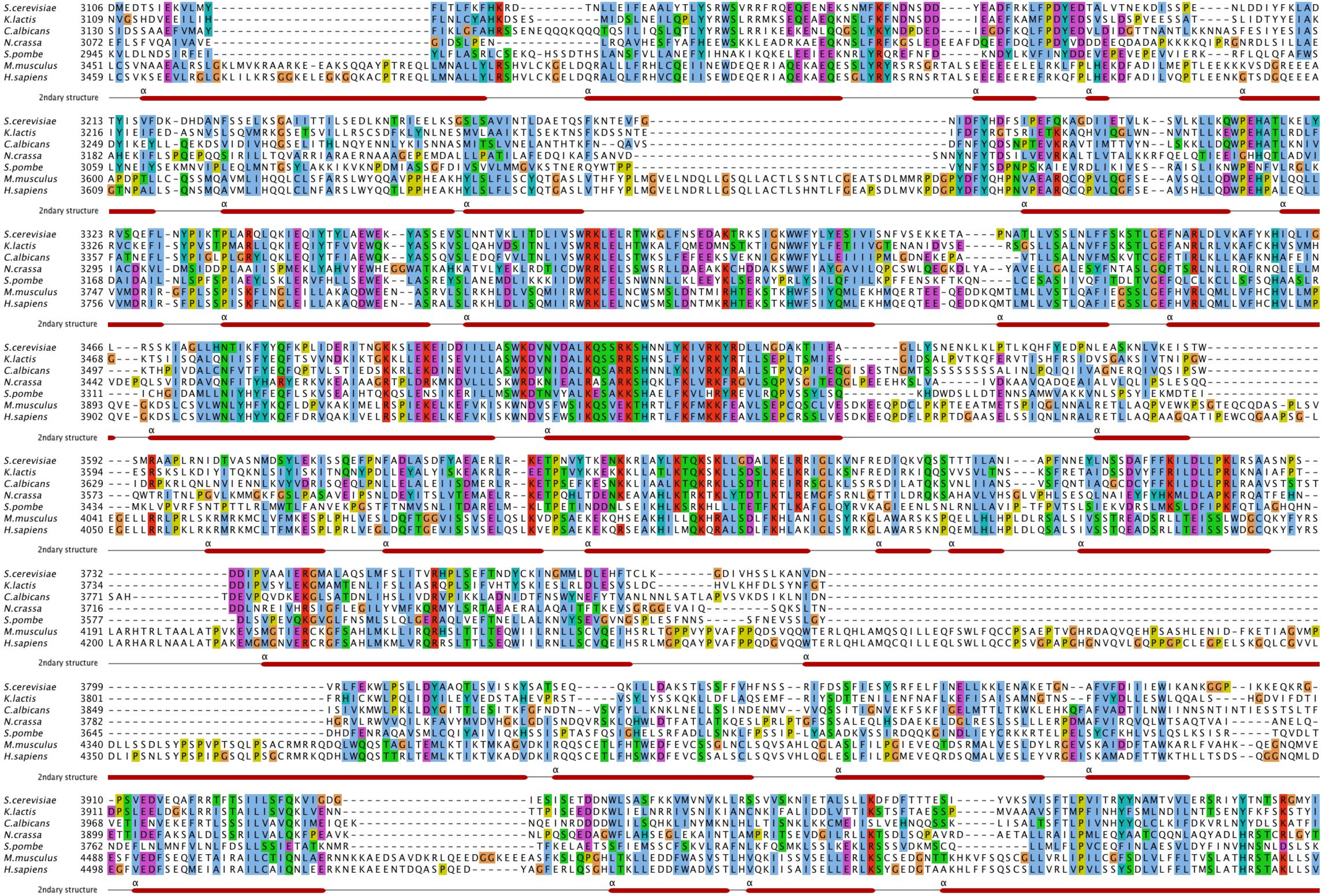

Figure S6, page 4

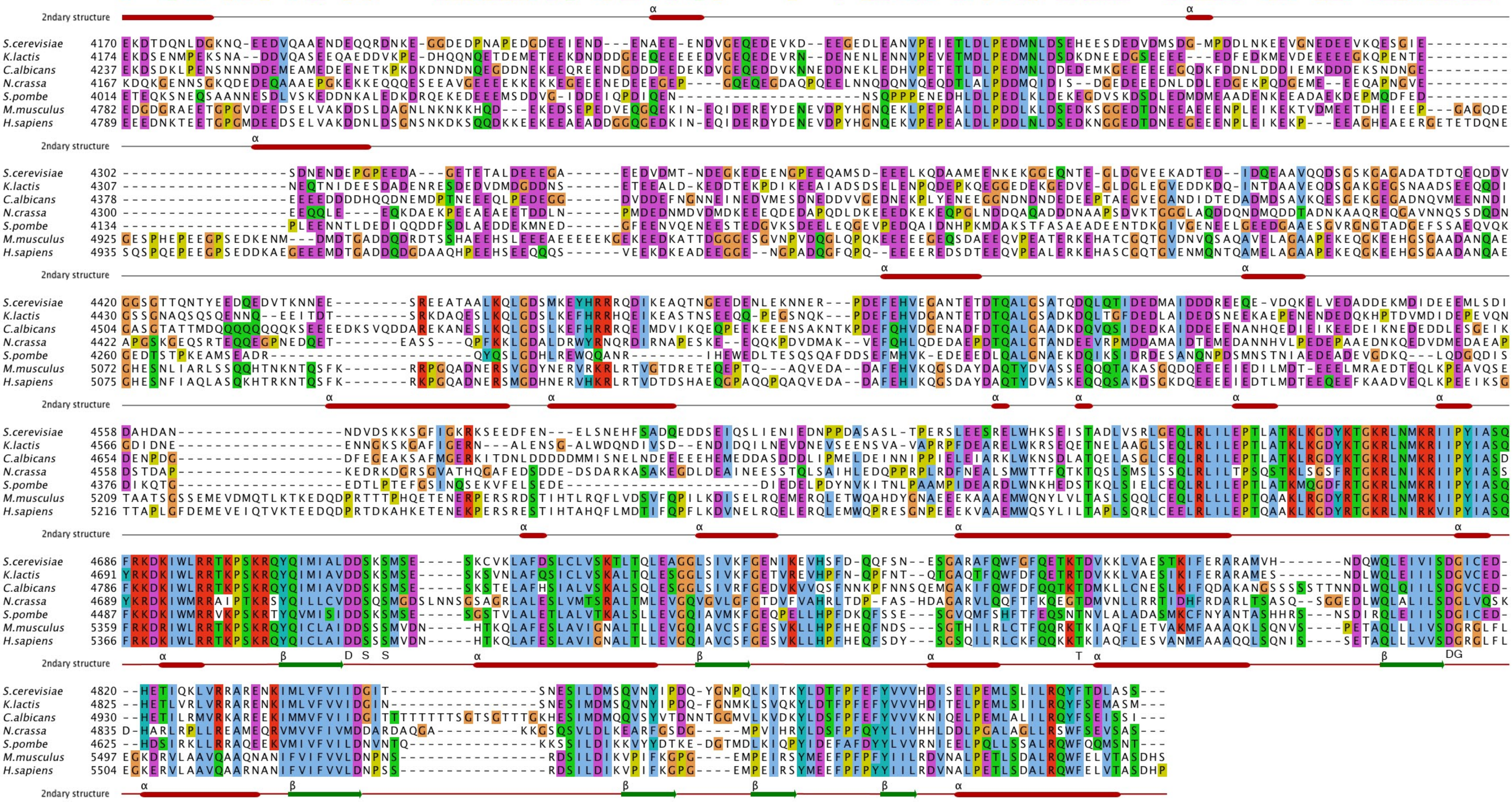

Figure S6:

Multiple sequence alignment of Rea1 full-length was done with ClustalW [76] and displayed with Jalview [77]. Secondary structure prediction of S. cerevisiae Rea1, done with PSIPRED [78], is depicted below and ATPase specific elements are labelled (α-helices are shown in red, β-strands in green, the AAA-ATPase specific lid domain is indicated in light blue and the clade-specific helix2 insert and pre-sensor-I insert (PSI) are indicated in dark blue. A red line marks the MIDAS domain.