Supporting information for:

The F_1-ATPase from *Trypanosoma brucei* is elaborated by three copies of an additional p18-subunit

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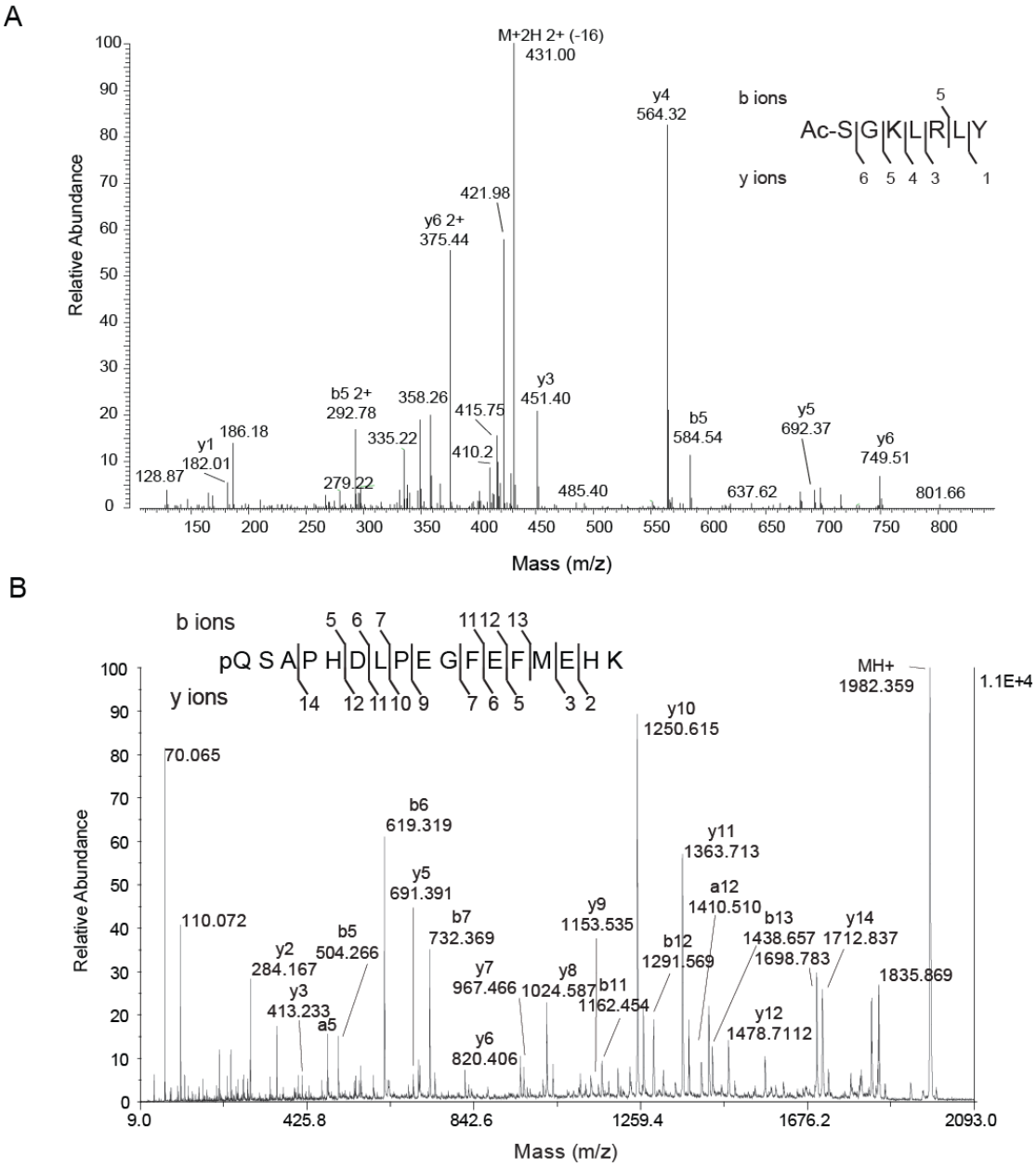

Figure S1: N-terminal sequences of the γ- and δ-subunits of the F_1-ATPase from *T. brucei*

(**A**) Tandem mass spectrum of a doubly charged ion of N-terminal chymotryptic peptide of the γ-subunit with m/z 439.75, with an N-terminal acetyl-serine residue. (**B**) Tandem mass spectra of a singly charged ion with m/z 1982.4 in which the N-terminal glutamine residue of the δ-subunit has been cyclised to pyroglutamic acid. Fragment ions have been mapped onto the sequences.

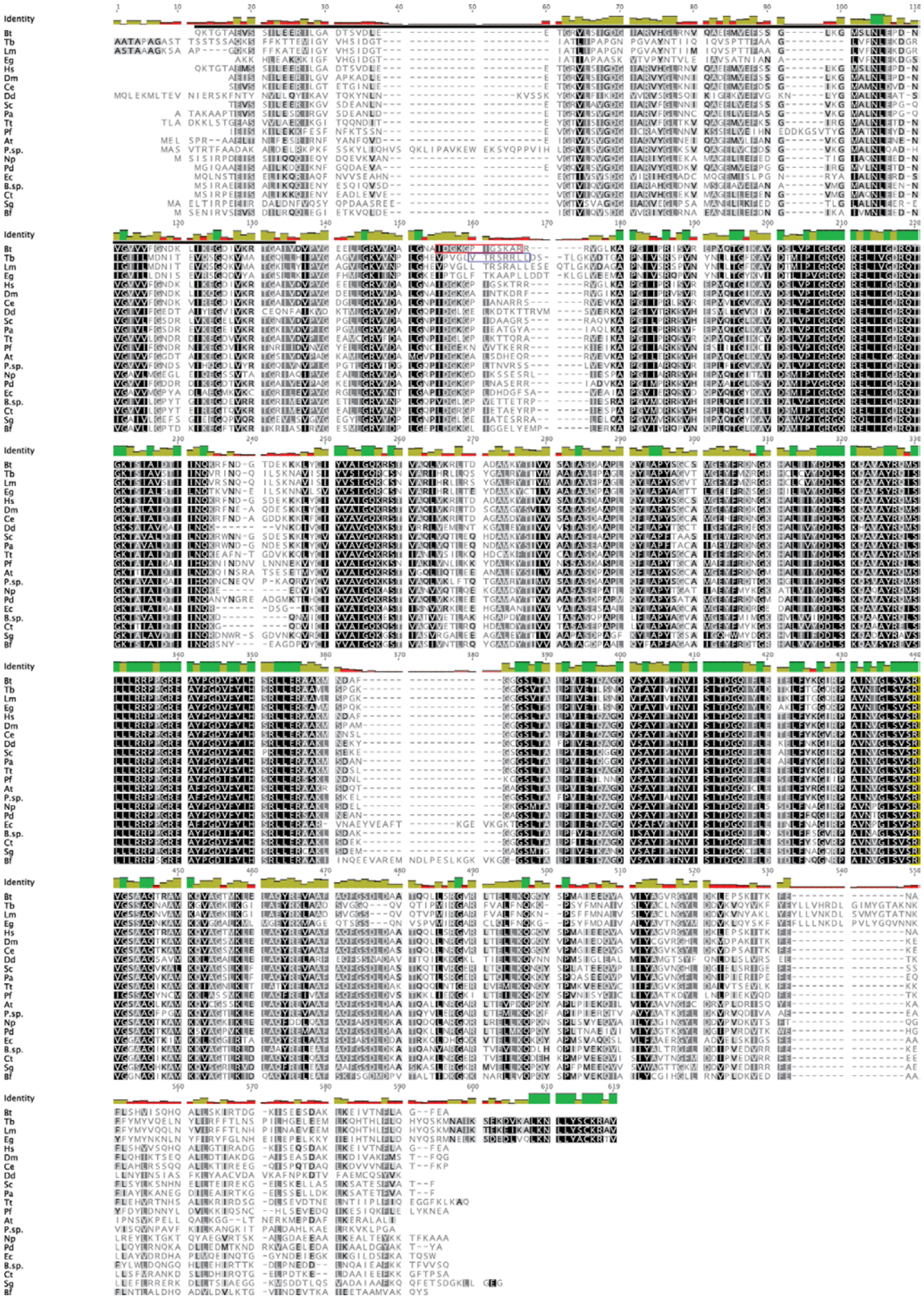

Figure S2: Sequences of α-subunits from major eubacterial and eukaryotic groups Mitochondrial targeting signals have been excluded. The eight *T. brucei* amino acids excised by proteolysis are outlined by a blue box. In the bovine α-subunit, the region in a red box forms an exposed surface loop linking the N-terminal and nucleotide binding domains. The black bar directly under the identity graph marks the N-terminal crown region of the bovine α-subunit. The Arg-373 and Arg-386 in the bovine and *T. brucei* α-subunit, respectively, and the corresponding arginine residues in other homologs are highlighted in yellow. Bt, *Bos taurus*; Tb, *T. brucei*; Lm, *Leishmania major*; Eg, *Euglena gracilis*; Hs, *Homo sapiens*; Dm, *Drosophila melanogaster*; Ce, *Caenorhabditis elegans*; Dd, *Dictyostelium discoideum*; Sc, *Saccharomyces cerevisiae*; Pa, *Pichia angusta*; Tt, *Tetrahymena thermophila*; Pf, *Plasmodium falciparum*; At, *Arabidopsis thaliana*; P. sp, *Polytomella* sp Pringsheim; Np, *Nostoc punctiforme*; Pd, *Paracoccus denitrificans*; Ec, *Escherichia coli*; B. sp, *Bacillus* strain PS3; Ct, *Caldoalkalibacillus thermarum* strain TA2; Sg, *Streptomyces griseus*; Bf, *Bacteroides fragilis*. The green, yellow, and red bars above the alignment correspond to 100%, <100% and ≥30%, and <30% identities at the respective position.

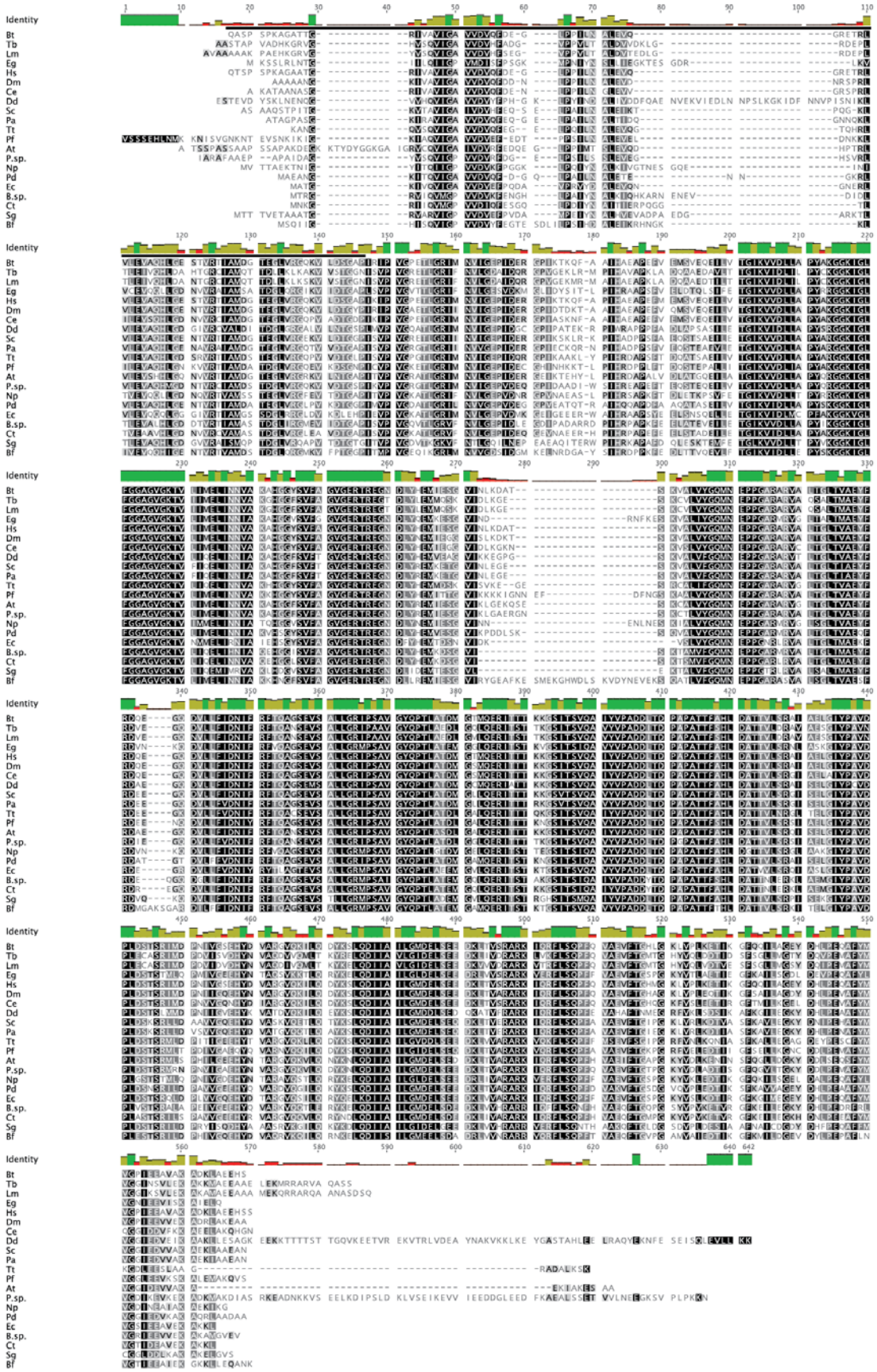

Figure S3: Sequences of β-subunits from major eubacterial and eukaryotic groups

Mitochondrial targeting signals have been excluded. The black bar marks the N-terminal crown domain in the β subunit of *B. taurus*. Names of the species are abbreviated as in the Fig. S4. The green, yellow, and red bars above the alignment correspond to 100%, <100% and ≥30%, and <30% identities at the respective position.

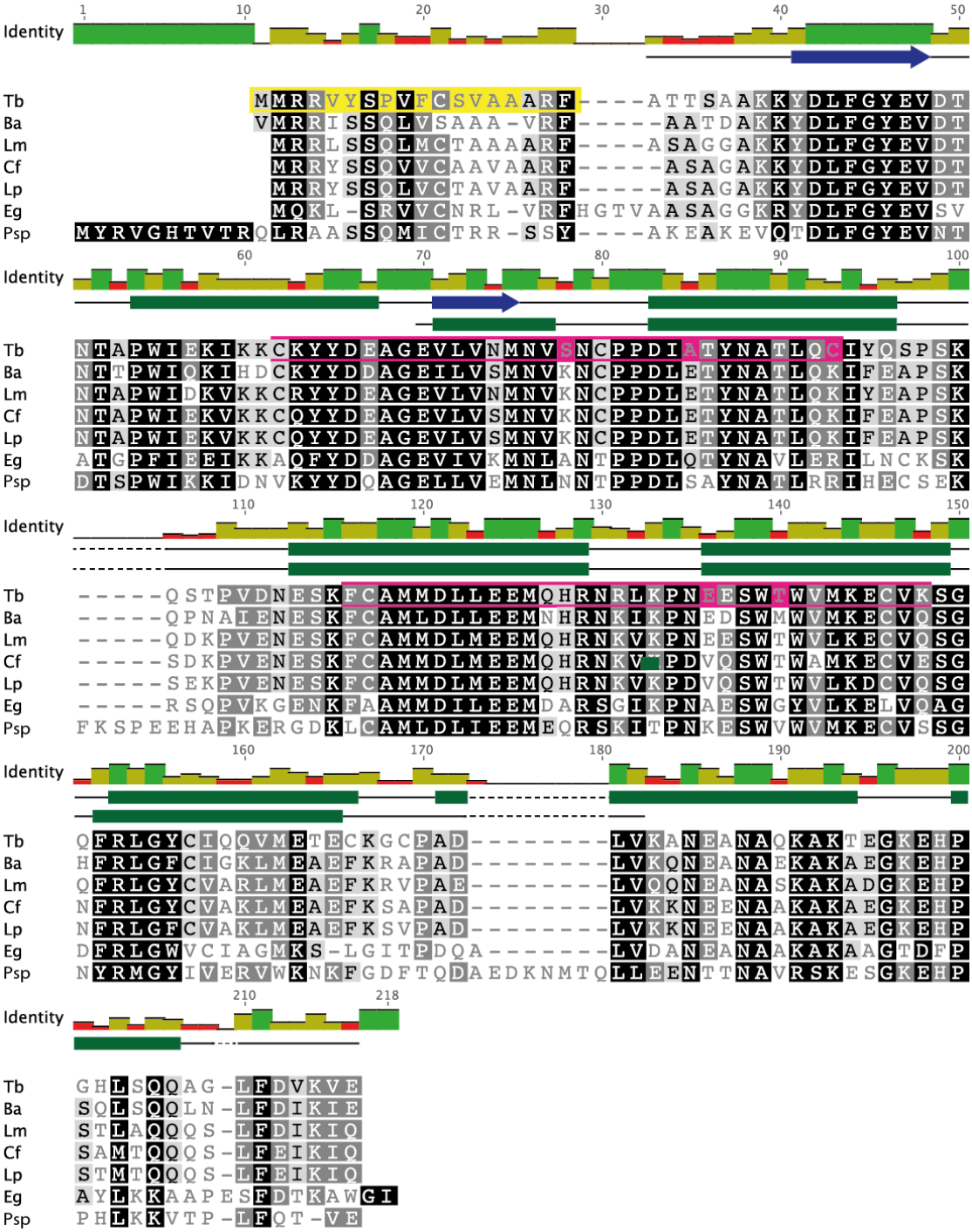

Figure S4: Sequences of p18-subunits from representatives of *Euglenozoa* and secondary structure prediction of *T. brucei* p18-subunit

The experimentally determined mitochondrial import sequence and predicted PPR repeats in the *T. brucei* sequence are highlighted in yellow and magenta, respectively. Tb, *T. brucei*, Ba, *Blechomonas ayali*, Lm, *Leishmania major*, Cf, *Crithidia fasciculata*, Lp, *Leptomonas pyrrhocoris*, Eg, *Euglena gracilis*, Psp, *Perikinsela sp*. The green,

yellow, and red bars above the alignment correspond to 100%, <100% and ≥30%, and <30% identities at the respective position. The tracks above the alignment show α-helices (green rectangles) and β-strands (blue arrows) in the *T. brucei* p18-subunit as predicted by secondary structure (upper track) and template-assisted secondary structure prediction (lower track) by the Phyre2 tool.