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SUPPLEMENTARY ONLINE DATA

Chloroplast HCF101 is a scaffold protein for [4Fe-4S] cluster assembly

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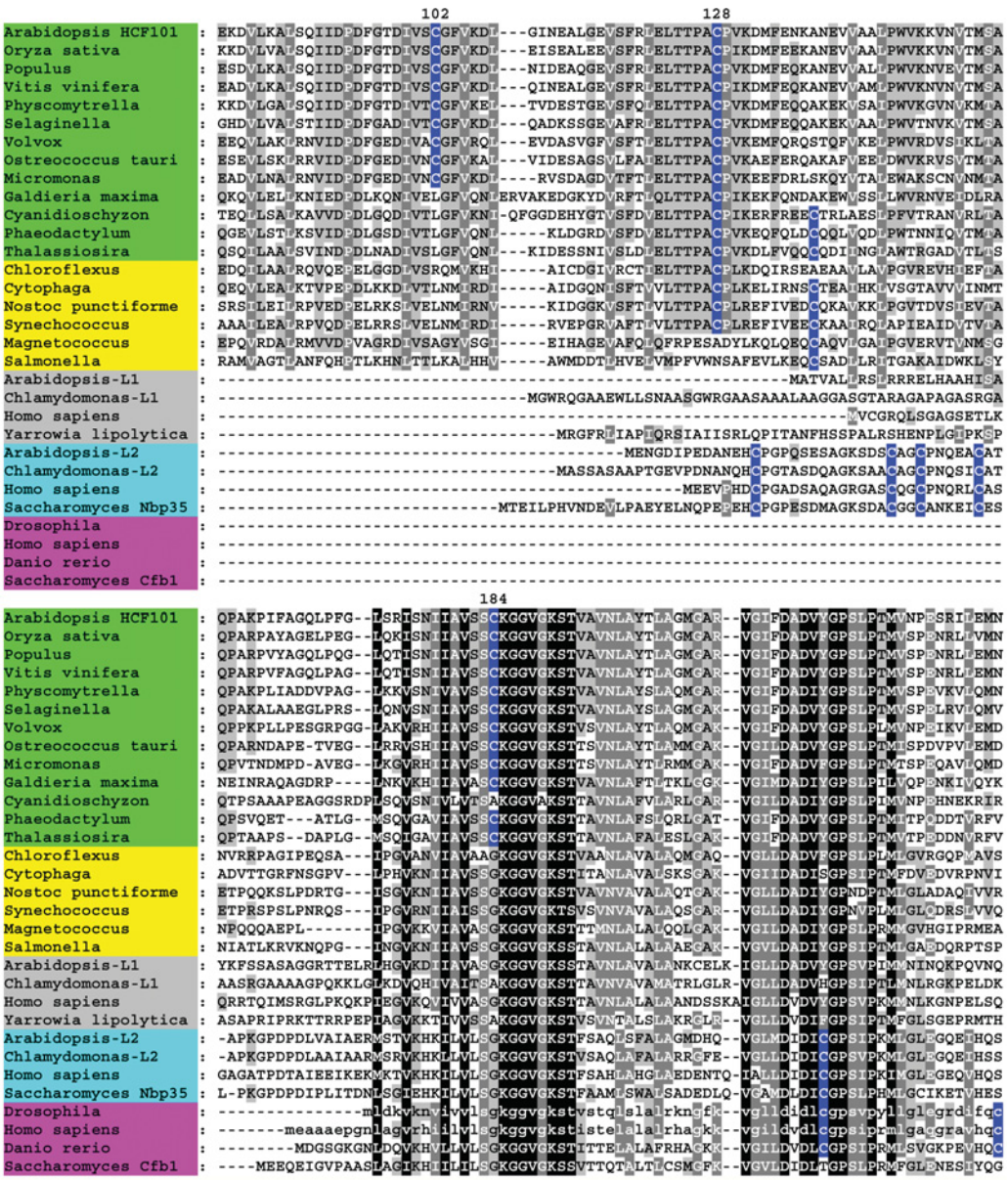

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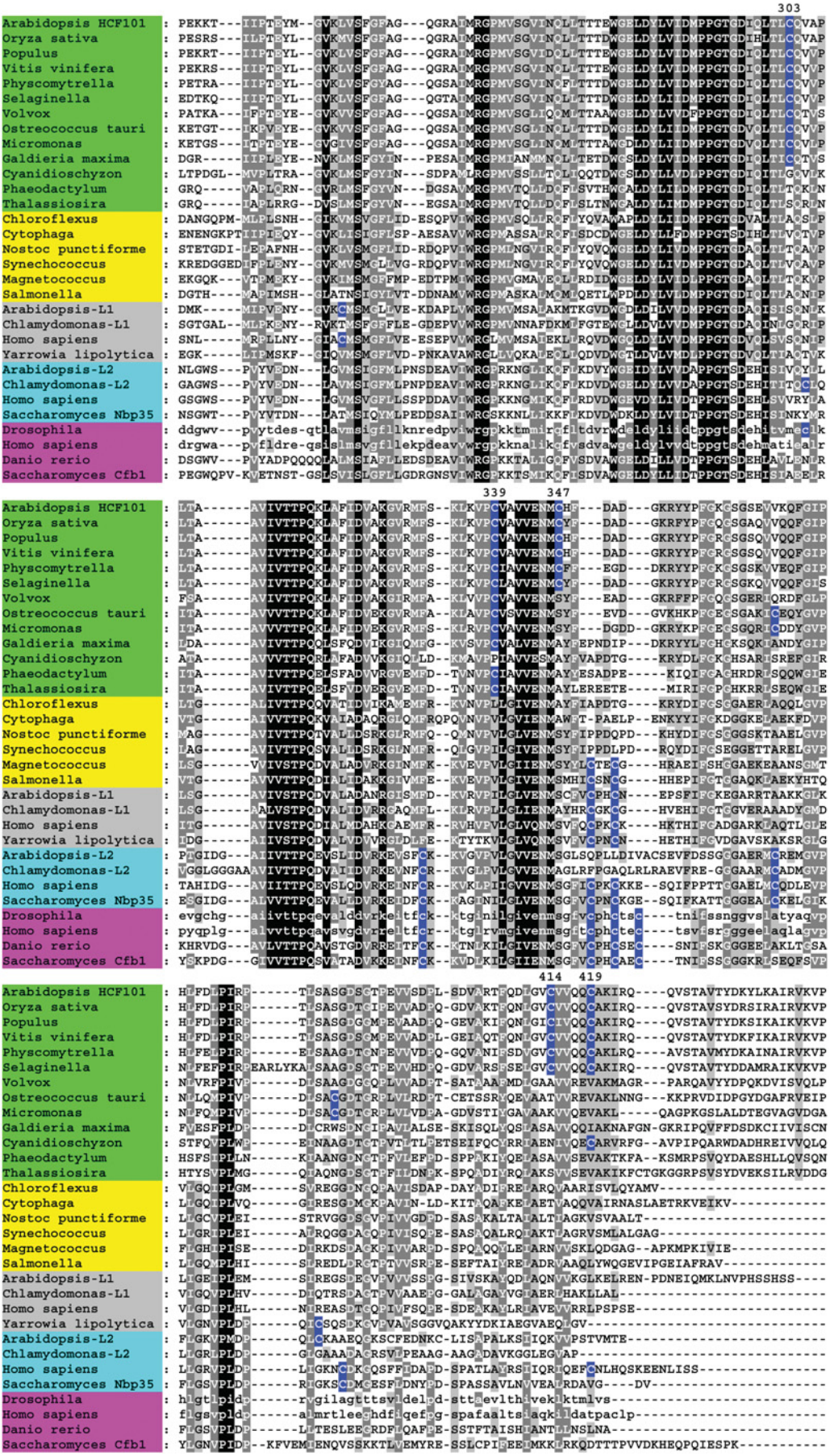


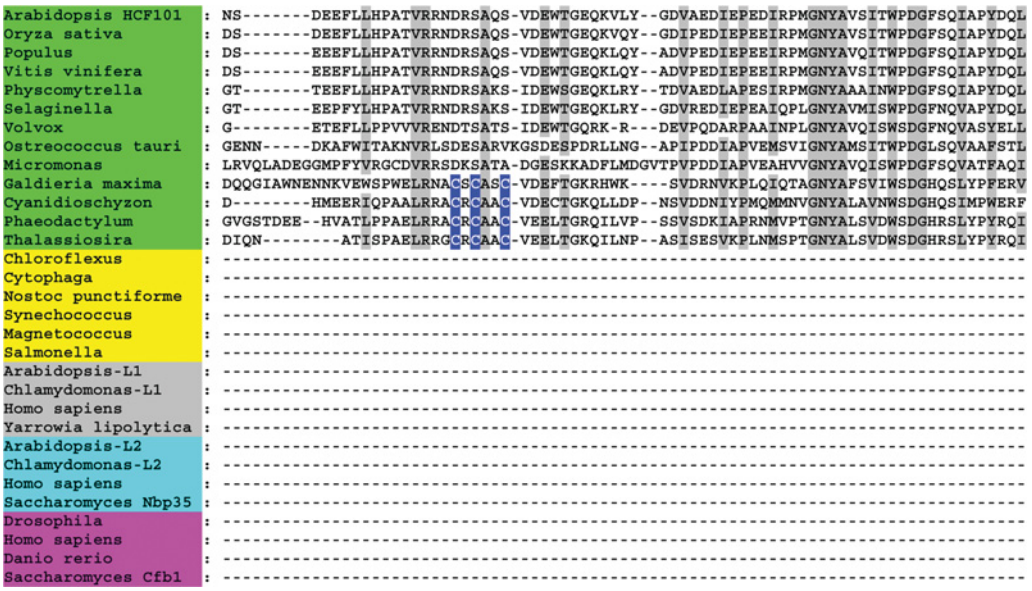
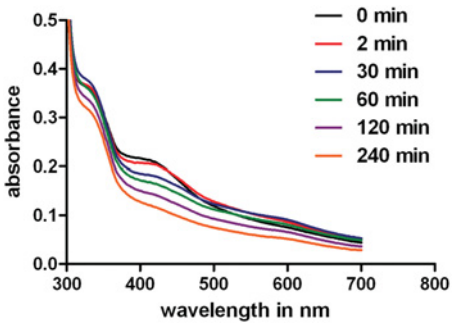


Figure S1 Multiple sequence alignment of the FSC-NTPase family

Representative protein sequences of different organisms are shown subdivided according to the proposed four FSC-NTPase classes [23]. Organism names are coloured according to the class affiliation. Green background represents plants and algae taxa belonging to the class I. Yellow background represents eubacterial class I proteins. Grey background corresponds to class II members. Blue and magenta congregate taxa belonging to the class III and class IV respectively. Note that algae and plants class I proteins have a C-terminal extension corresponding to the DUF971 domain (COG3536). The very N-terminal and C-terminal regions are not shown in the alignment as they show little conservation. Amino acids coloured with a black background are 100 % conserved. Amino acids coloured with a grey background and with white characters are >60 % conserved. Amino acids coloured with a grey background and written with black characters are >40 % conserved. Cysteine residues conserved in at least two sequences are depicted with a blue background. Numbers above the conserved cysteine residues in plants and algae correspond to the position of the residue in the sequence of the *Arabidopsis* HCF101 protein.



Supplementary Figure S2 Oxygen sensitivity of the reconstituted HCF101 protein

Purified HCF101 protein was reconstituted as described in the Experimental section in the main paper. Decay of the shoulder at 420 nm in the presence of oxygen was monitored by UV–visible spectroscopy.

Supplementary Table S1 Oligonucleotides used for site-directed mutagenesis and cloning

For details please see the Experimental section in the main paper. for, forward primer; rev, reverse primer.

Designation	Sequence
C102S-for	5′-GGAGCTGACAACACCCGCAT*c*TCCAGTCAAAGAC-3′
C102S-rev	5′-GAAACAATATCTGTCCCAAAATCAGGATC-3
C128S-for	5′-CATCATCGCTGTTTCTAGTT*ct*AAGGGTGGTG-3′
C128S-rev	5′-GTGTTGTCAGCTCCAAACGGAACGAAACCTC-3′
C184S-for	5′-GATATACAACTGACCTTAT*ct*CAGGTTGCGC-3′
C184S-rev	5′-CTAGAAACAGCGATGATGTTCGAAATTC-3′
C303S-for	5′-CTCAAAACTTAAGGTGCCTT*ct*GTTGCTGTTGTG-3′
C303S-rev	5′-TAAGGTCAGTTGTATATCACCAGTTCCAG-3′
C339S-for	5′-CTCAAAACTTAAGGTGCCTT*ct*GTTGCTGTTGTG-3′
C339S-rev	5′-GCACCTTAAGTTTTGAGAACATCCTTACAC-3′
C347S-for	5′-GCTGTTGTGGAGAATATGT*ct*CACTTTGACGC-3′
C347S-rev	5′-CATATTCTCCACAACAGCAACGCAAGGC-3′
C414S-for	5′-CGTTCCAGGATCTTGGTGTAT*c*TGTAGTGCAAC-3′
C414S-rev	5′-CACCAAGATCCTGGAACGTTCTGGCAACG-3′
C419S-for	5′-GTGTATGTGTAGTGCAACAAT*ct*GCCAAGATAC-3′
C419S-rev	5′-TGTTGCACTACACATACACCAAGATCCTGGA-3′
INCORP-for	5′-GTAGAATTCTCAGCTCAAGCTAGTAGTAGTGTTGG-3′
INCORP-rev	5′-GTAGAATTCGACTTCGACTGGAGACAATGGAGG-3′

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