Evolution of the Cytosolic Iron/Sulfur cluster Assembly machinery in *Blastocystis* sp. and other microbial eukaryotes

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Supplementary materials list:

Supplementary figure legends:

Figure S1:

Schematic model of the CIA pathway in opisthokonts. Dre2 along with the diflavin reductase Tah18, form an electron transfer chain with the use of NADPH for Tah18-dependent reduction of one of the two Fe/S clusters of Dre2. The nuclear binding proteins Nbp35 and Cfd1 form a scaffold complex responsible for assembling of transient bound Fe/S cluster in an early step of the biosynthetic process. In a later stage the Fe/S clusters are transferred to apo-proteins via Cia1 and Nar1 proteins. Lastly, MMS19 and Cia2 form a complex with Cia1 and Nar1 that facilitates the transfer of mature clusters to apo-proteins.

Figure S2:

Identification of functionally important structures in eukaryotic Cia1 sequences. Sequence alignment of Cia1 protein sequences from microbial eukaryotes against the published structure of the yeast Cia1p (3). Cia1 are WD40 proteins that contain 28 β-sheet structures highlighted in green. Potential helices are highlighted in pink. We used the PSIPRED server (1) for the structure prediction of the different proteins.

Figure S3:

Conservation of functionally important residues in Nar1 sequences from microbial eukaryotes. Sequence alignment of Nar1 protein sequences against orthologous proteins from microbial eukaryotes have demonstrated the presence of a ferredoxin-like domain

(highlighted in blue) along with conserved residues forming an H-cluster domain (highlighted in yellow). Cysteines (potentially involvement in Fe/S cluster binding) are marked in red.

Figure S4:

Conservation of functionally important residues in Nbp35 and Cfd1 sequences from microbial eukaryotes. Sequence alignment of Nbp35 and Cfd1 protein sequences against bacterial and eukaryotic orthologous proteins. The conserved N-terminal CX13CX2CX5C motif (2) found only in the Nbp35 eukaryotic homologues is highlighted in blue. The Mrp family signature is indicated in green, the conserved Walker A motif is highlited in grey, whereas the C-terminal CPXC is highlighted in yellow. Cysteines (potentially involvement in Fe/S cluster binding) are marked in red. Cfd1 protein sequences are shaded in grey for comparison with the Nbp35 homologues.

Figure S5:

Western blot analyses of the expression of *Blastocystis* IscS, Nar1, Nbp35, SufCB and Tah18. Western blots demonstrating the cellular localization of the *Blastocystis* components of Fe/S cluster biosynthesis. The heterologous anti-yeast Nbp35, Nar1 and Tah18 antisera shows specific detection of *Blastocystis* Nbp35, Nar1 and Tah18 proteins with apparent relative molecular mass of 36 kDa, 83 kDa and 75 kDa respectively in the cytosolic fraction, but absent in the MRO fraction, that is consistent with the absence of this protein in the mitochondria. In the blot of Nbp35 protein, a double band appears, which could be a result of degradation of the protein. The full-sized western blots are

shown on the right panel. Noted that in the case of the Nbp35 protein, a second band is present in an apparent molecular weight of 72 kDa (indicated with "**") in the cytosolic fraction, which could be a result of dimmerisation of the aforementioned protein. The *Blastocystis* SufCB antiserum shows specific detection of *Blastocystis* SufCB with an apparent relative molecular mass of 77 kDa in the cytosolic fraction as well (positive control). The heterologous anti-*Trichomonas vaginalis* IscS antisera shows specific detection of *Blastocystis* IscS (4) with an apparent relative mass of 47 kDa in the MRO fraction (MRO positive control).

Figure S6:

Immunolocalization of Nbp35, Nar1 with MitoTracker in *Blastocystis* sp.

a. Cellular localization of the Nbp35 and MitoTracker in *Blastocystis* cells. i. Rabbit anti-yeast Nbp35 antibody (1:200) detects *Blastocystis* Nbp35 protein. ii. Localization of MitoTracker staining. iii. Overlapping of the previous images showing the localization pattern of Nbp35 and no co-localization with MitoTracker iv. Differential interference contrast (DIC) image of the cells used for immunofluorescence. **b.** Cellular localization of the Nar1 and MitoTracker in *Blastocystis* cells. i. Rabbit anti-yeast Nar1 antibody (1:100) detects *Blastocystis* Nbp35 protein. Localization of MitoTracker staining. iii. Overlapping of the previous images showing the localization pattern of Nbp35 and no co-localization with MitoTracker iv. Differential interference contrast (DIC) image of the cells used for immunofluorescence. Scale bar, 5 μm.

Figure S7:

Immunolocalization of Nbp35 in *Blastocystis*. Image demonstrating the cytosolic localization of Nbp35 in *Blastocystis* cells by transmission electron microscopy. Inset is enlargement of specific regions of the cell, focusing in the localization of Nbp35 within the different compartments of the cell but also the distribution of the protein within the cytosol.

Figure S8:

Immunolocalization of Nar1 in *Blastocystis*. Image demonstrating the cytosolic localization of Nar1 in *Blastocystis* cells by transmission electron microscopy. Inset is enlargement of specific regions of the cell, focusing in the localization of Nar1 within the different compartments of the cell but also the distribution of the protein within the cytosol.

Figure S9:

Immunolocalization of Tah18 in *Blastocystis*. Image demonstrating the cytosolic localization of Tah18 in *Blastocystis* cells by transmission electron microscopy. Insets are enlargement of specific regions of the cell, focusing in the localization of Tah18 within the different compartments of the cell but also the distribution of the protein within the cytosol.

Figure S10:

Identification of functionally important structures in eukaryotic Dre2 sequences. Sequence alignment of Dre2 protein sequences from microbial eukaryotes demonstrating

the highly conserved C-terminus that contains two Fe/S cluster-binding motifs (CX_2CXC and a $CX_2CX_7CX_2C$ in red), and the more divergent N-terminus with a methyltransferase domain.

Figure S11:

Phylogeny of the cytosolic Fe/S cluster assembly protein Dre2. The phylogenetic tree was inferred by Randomized Axelerated Maximum Likelihood (RAxML). A total of 78 taxa and 96 characters were used for the final phylogenetic analysis. Numerical values at the nodes represent branch support in the form of bootstrap (BP). Only bootstrap support values greater than 50 are shown.

Figure S12:

Phylogeny of the cytosolic Fe/S cluster assembly proteins Nar1 and iron hydrogenase. The iron hydrogenase protein sequences were used to root the tree. The phylogenetic tree was inferred by Randomized Axelerated Maximum Likelihood (RAxML). A total of 81 taxa and 233 characters were used for the final phylogenetic analysis. Numerical values at the nodes represent branch support in the form of bootstrap (BP). Only bootstrap support values greater than 50 are shown. Red line indicates the *Blastocystis* spp. homologues.

Figure S13:

Phylogeny of the cytosolic Fe/S cluster assembly protein Cia1. The phylogenetic tree was inferred by RAxML. A total of 59 taxa and 225 characters were used for the final

phylogenetic analysis. Numerical values at the nodes represent branch support in the form of bootstrap (BP). Only bootstrap support values greater than 50 are shown. Red line indicates the *Blastocystis* spp. homologues.

Figure S14:

Phylogeny of the cytosolic Fe/S cluster assembly protein MMS19. The phylogenetic tree was inferred by Randomized Axelerated Maximum Likelihood (RAxML). A total of 85 taxa and 53 characters were used for the final phylogenetic analysis. Numerical values at the nodes represent branch support in the form of bootstrap (BP). Only bootstrap support values greater than 50 are shown. Red line indicates the *Blastocystis* spp. homologues.

Figure S15:

Phylogeny of the cytosolic Fe/S cluster assembly protein Cia2. The phylogenetic tree was inferred by Randomized Axelerated Maximum Likelihood (RAxML). A total of 60 taxa and 98 characters were used for the final phylogenetic analysis. Numerical values at the nodes represent branch support in the form of bootstrap (BP). Only bootstrap support values greater than 50 are shown. The groups of the putative Cia2A and Cia2B are indicated. The *Homo sapiens* sequences are depicted in red and are the only Cia2A and Cia2B proteins that have been characterized to date.

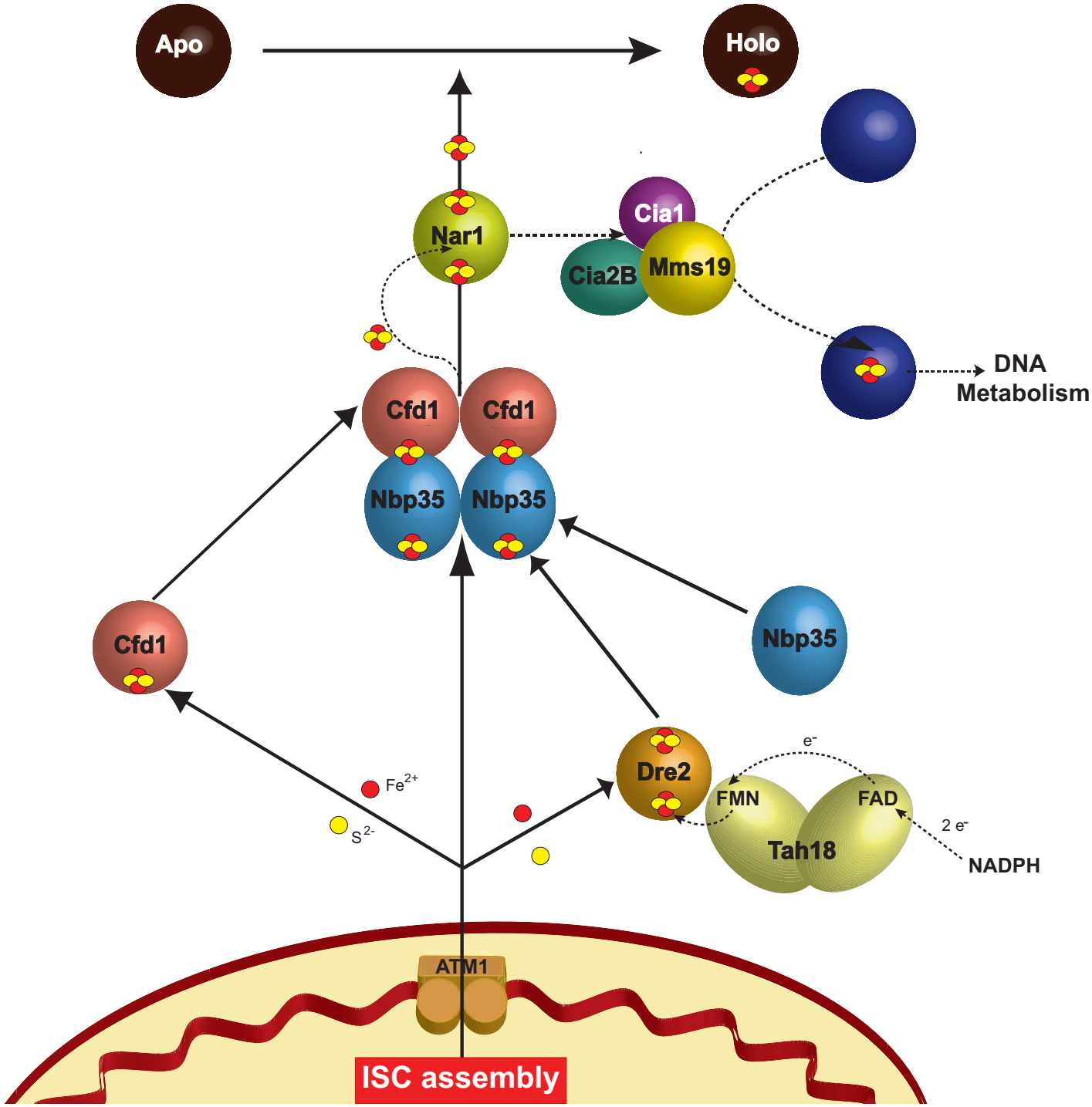
Table S1

Distribution of homologues of the CIA machinery among publicly available genomes or expressed sequence tag data from eukaryote taxa. Table includes the accession numbers for all proteins reported in this manuscript.

Supplementary References:

1. **McGuffin, L. J., K. Bryson, and D. T. Jones**. 2000. The PSIPRED protein structure prediction server. Bioinformatics **16**:404-405.
2. **Netz, D. J., A. J. Pierik, M. Stumpfig, E. Bill, A. K. Sharma, L. J. Pallesen, W. E. Walden, and R. Lill**. 2012. A bridging [4Fe-4S] cluster and nucleotide binding are essential for function of the Cfd1-Nbp35 complex as a scaffold in iron-sulfur protein maturation. J Biol Chem **287**:12365-12378.
3. **Srinivasan, V., D. J. Netz, H. Webert, J. Mascarenhas, A. J. Pierik, H. Michel, and R. Lill**. 2007. Structure of the yeast WD40 domain protein Cia1, a component acting late in iron-sulfur protein biogenesis. Structure **15**:1246-1257.
4. **Tsaousis, A. D., S. Ollagnier de Choudens, E. Gentekaki, S. Long, D. Gaston, A. Stechmann, D. Vinella, B. Py, M. Fontecave, F. Barras, J. Lukes, and A. J. Roger**. 2012. Evolution of Fe/S cluster biogenesis in the anaerobic parasite *Blastocystis*. Proc Natl Acad Sci U S A **109**:10426-10431.

Figure S1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Cia1
Cia2B
Mms19
DNA
Metabolism
Cfd1
Cfd1
Nbp35
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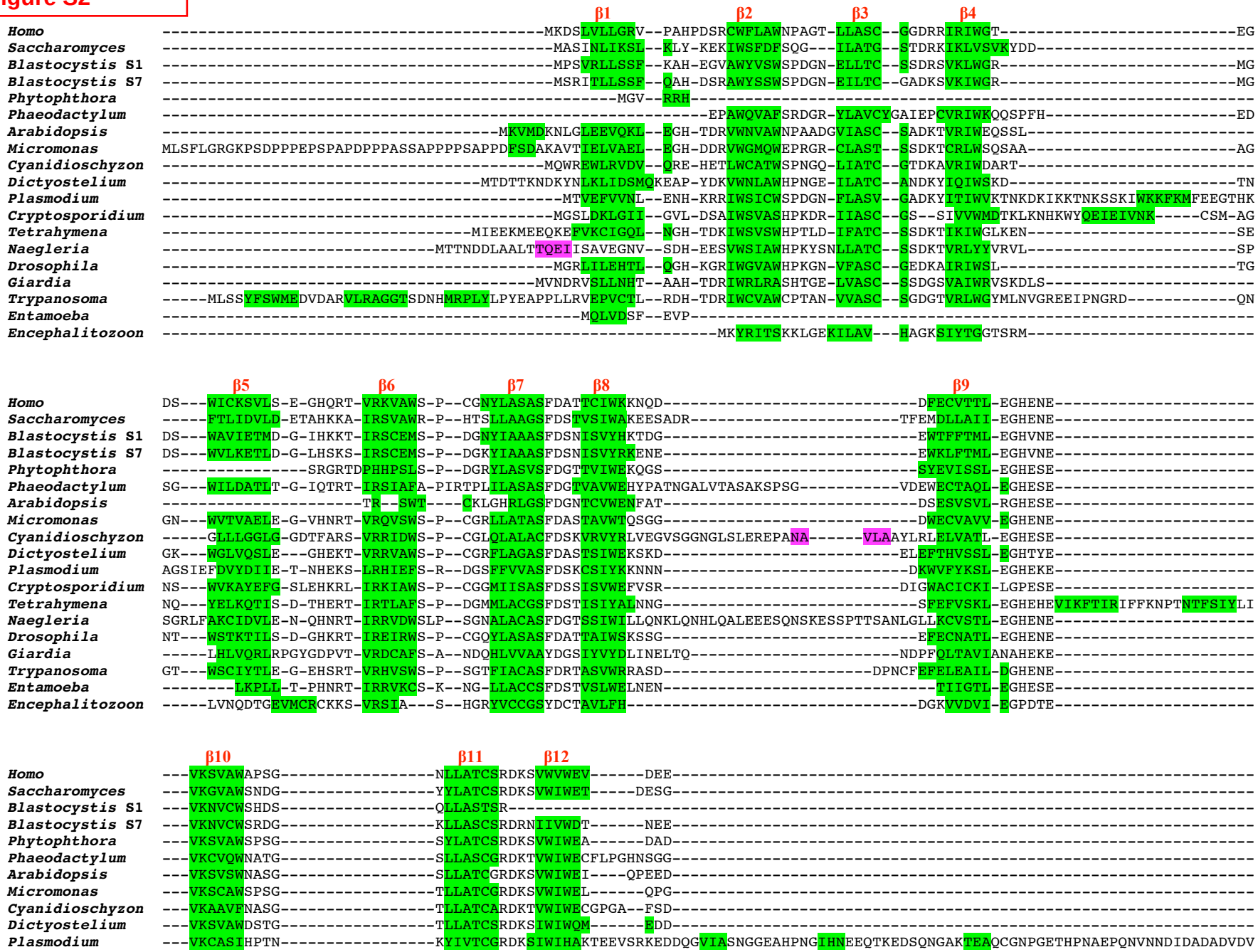
Dre2
e-
FMN
FAD
Tah18
2 e-
NADPH
Fe2+
S2-
ATM1
ISC assembly

Figure S2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Blastocystis S7
Phytophthora
Phaeodactylum
Arabidopsis
Micromonas
Cyanidioschyzon
Dictyostelium
Plasmodium
Cryptosporidium
Tetrahymena
Naegleria
Drosophila
Giardia
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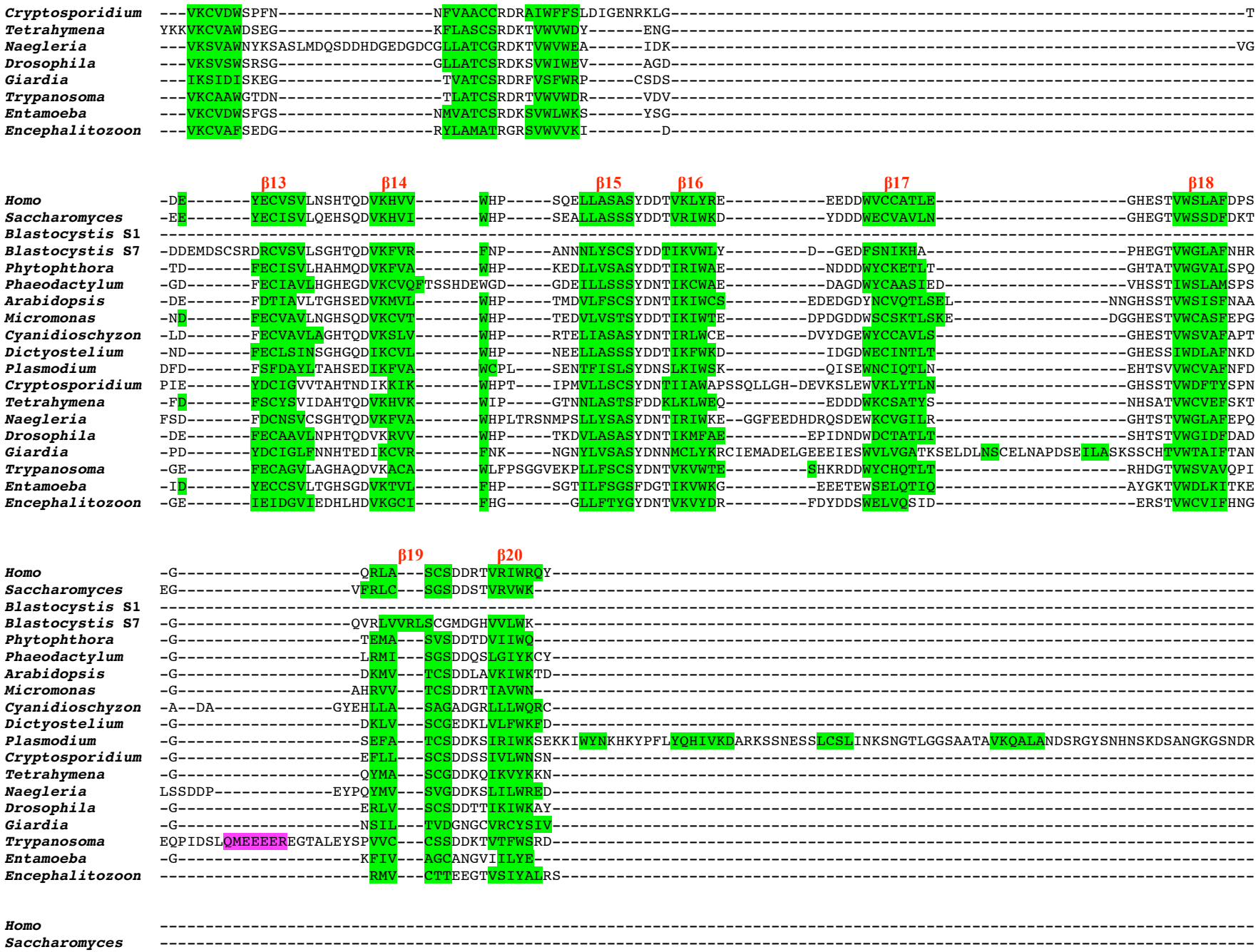
Encephalitozoon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β17
β18
β19
β20
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Entamoeba
Encephalitozoon
Homo
Saccharomyces
Blastocystis S1
Blastocystis S7
Phytophthora
Phaeodactylum
Arabidopsis
Micromonas
Cyanidioschyzon
Dictyostelium
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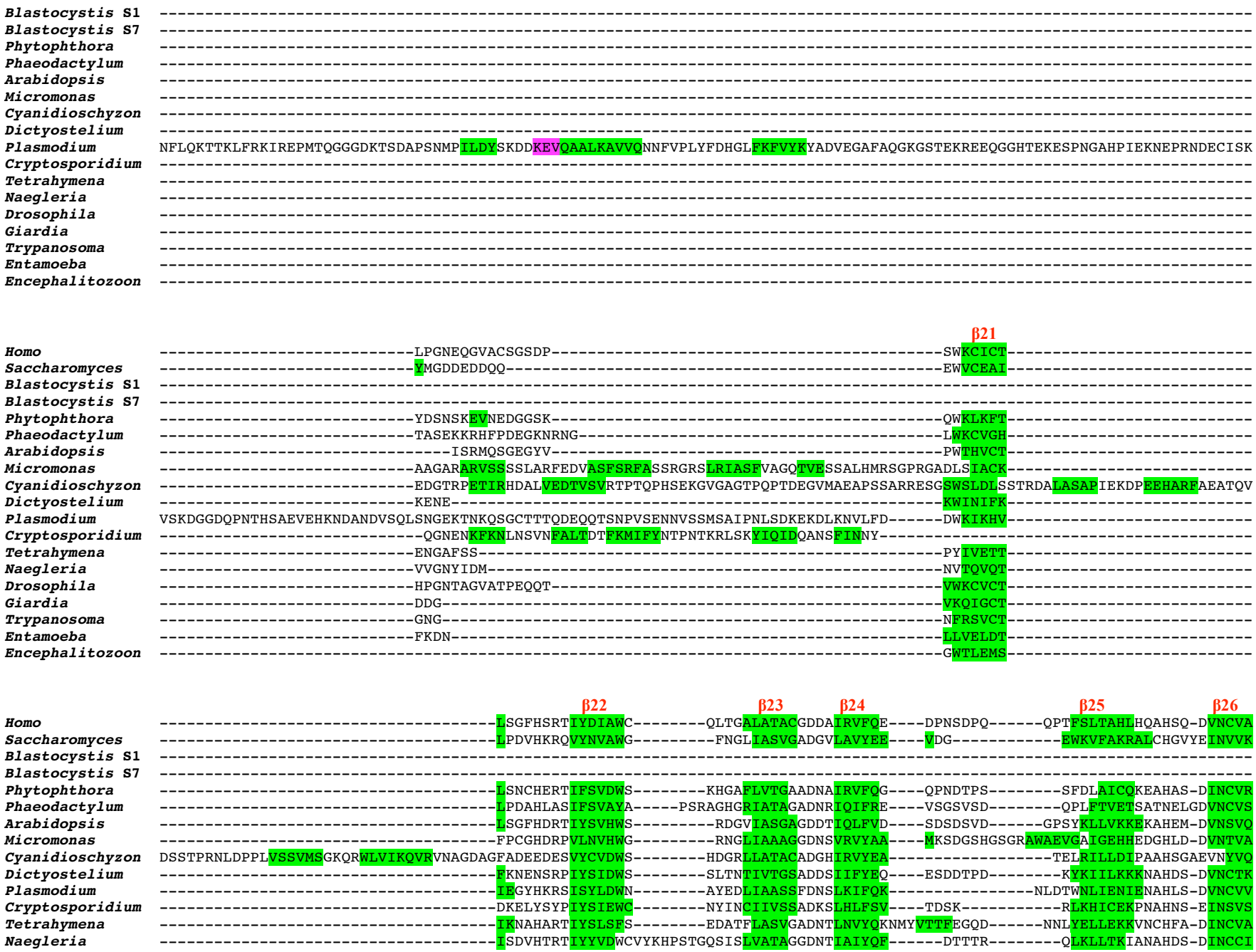
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Arabidopsis
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Cyanidioschyzon
Dictyostelium
Plasmodium
Cryptosporidium
Tetrahymena
Naegleria
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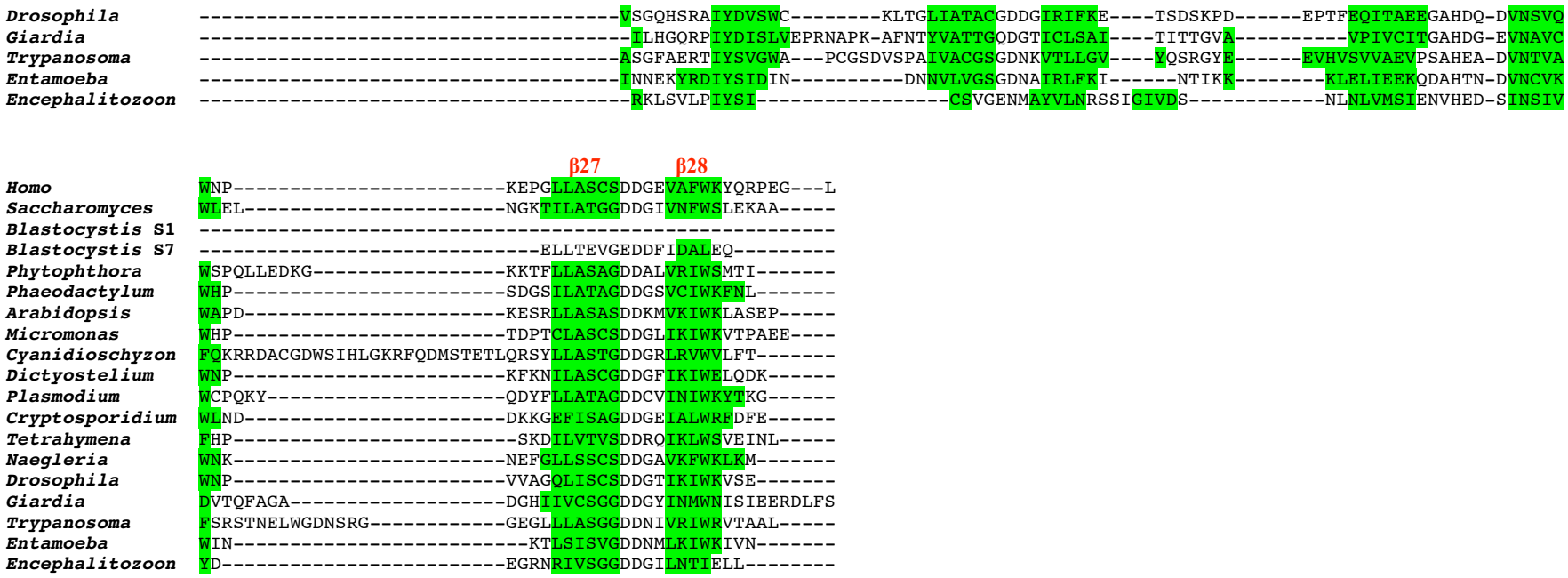
β23
β24
β25
β26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Phaeodactylum
Arabidopsis
Micromonas
Cyanidioschyzon
Dictyostelium
Plasmodium
Cryptosporidium
Tetrahymena
Naegleria
Drosophila
Giardia
Trypanosoma
Entamoeba
Encephalitozoon

Figure S3


Ferredoxin-like domain
Homo
Saccharomyces
Blastocystis S1
Blastocystis S7
Phaeodactylum
Phytophthora
Arabidopsis
Micromonas
Dictyostelium
Tetrahymena
Naegleria
Encephalitozoon
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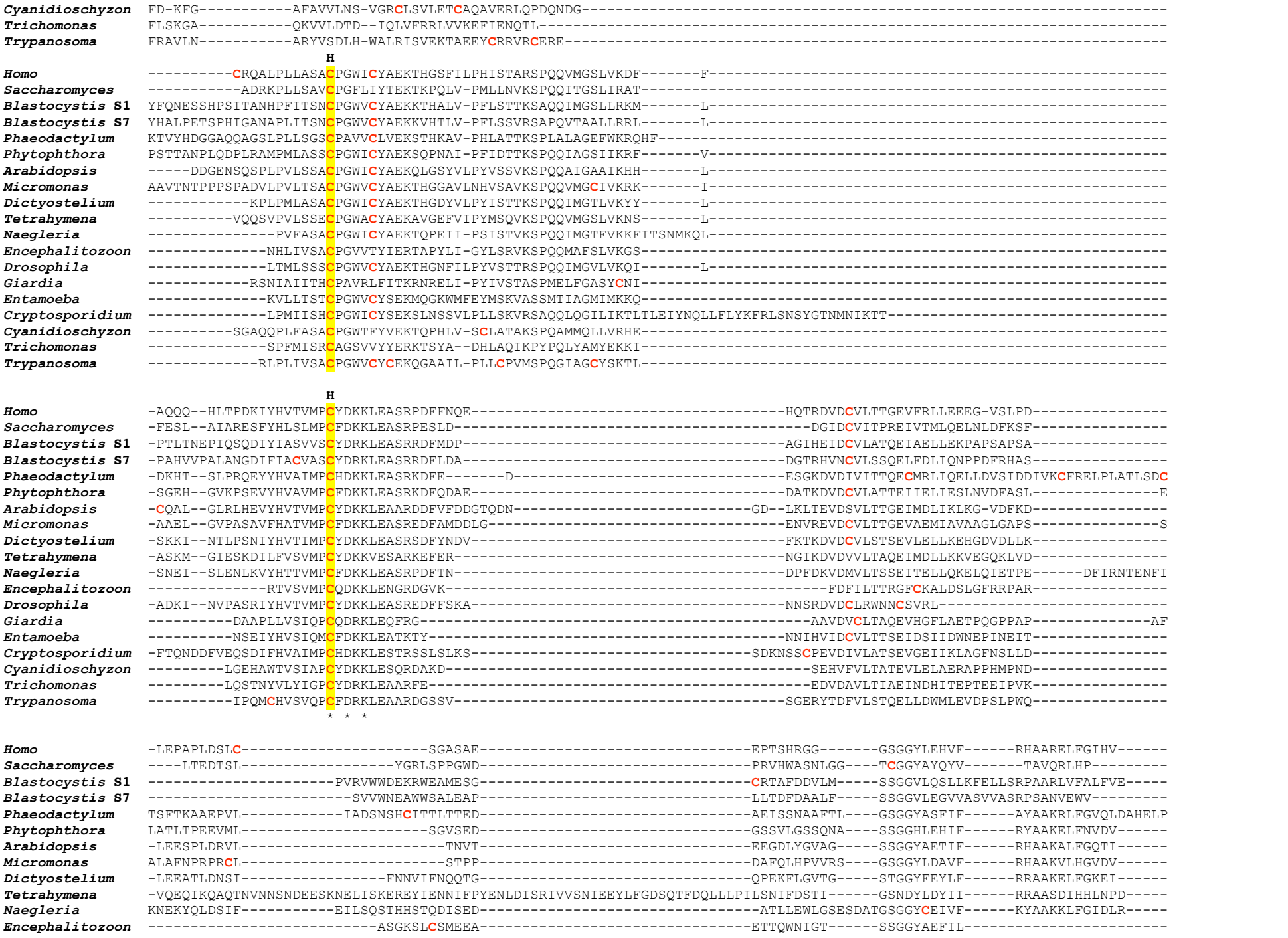
Trypanosoma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Trypanosoma FRAVLN----------ARYVSDLH-WALRISVEKTAEEYCRRVRCERE----------
H
Homo ----------CRQALPLLASACPGWICYAEKTHGSFILPHISTARSPQQVMGSLVKDF-------F----------
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Blastocystis S7 YHALPETSPHIGANAPLITSNCPGWVCYAEKKVHTLV-PFLSSVRSAPQVTAALLRRL-------L----------
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Trichomonas ----------SPFMISRCAGSVVYYERKTSYA--DHLAQIKPYPQLYAMYEKKI----------
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Blastocystis S1 -PTLTNEPIQSQDIYIASVVSCYDRKLEASRRDFMDP----------AGIHEIDCVLATQEIAELLEKPAPSAPSA----------
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Phytophthora -SGEH--GVKPSEVYHVAVMPCFDKKLEASRKDFQDAE----------DATKDVDCVLATTEIIELIESLNVDFASL----------E
Arabidopsis -CQAL--GLRLHEVYHVTVMPCYDKKLEAARDDFVFDDGTQDN----------GD--LKLTEVDSVLTTGEIMDLIKLKG-VDFKD----------
Micromonas -AAEL--GVPASAVFHATVMPCFDKKLEASREDFAMDDLG----------ENVREVDCVLTTGEVAEMIAVAAGLGAPS----------S
Dictyostelium -SKKI--NTLPSNIYHVTIMPCYDKKLEASRSDFYNDV----------FKTKDVDCVLSTSEVLELLKEHGDVDLLK----------
Tetrahymena -ASKM--GIESKDILFVSVMPCYDKKVESARKEFER----------NGIKDVDVVLTAQEIMDLLKKVEGQKLVD----------
Naegleria -SNEI--SLENLKVYHTTVMPCFDKKLEASRPDFTN----------DPFDKVDMVLTSSEITELLQKELQIETPE------DFIRNTENFI
Encephalitozoon ----------RTVSVMPCQDKKLENGRDGVK----------FDFILTTRGFCKALDSLGFRRPAR----------
Drosophila -ADKI--NVPASRIYHVTVMPCYDKKLEASREDFFSKA----------NNSRDVDCLRWNNCSVRL----------
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Blastocystis S7 ----------SVVWNEAWWSALEAP----------LLTDFDAALF-----SSGGVLEGVVASVVASRPSANVEWV---------
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Phytophthora LATLTPEEVML----------SGVSED----------GSSVLGSSQNA----SSGGHLEHIF------RYAAKELFNVDV------
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Micromonas ALAFNPRPRCL----------STPP----------DAFQLHPVVRS----GSGGYLDAVF------RHAAKVLHGVDV------
Dictyostelium -LEEATLDNSI----------FNNVIFNQQTG----------QPEKFLGVTG-----STGGYFEYLF------RRAAKELFGKEI------
Tetrahymena -VQEQIKQAQTNVNNSNDEESKNELISKEREYIENNIFPYENLDISRIVVSNIEEYLFGDSQTFDQLLLPILSNIFDSTI------GSNDYLDYII------RRAASDIHHLNPD-----
Naegleria KNEKYQLDSIF----------EILSQSTHHSTQDISED----------ATLLEWLGSESDATGSGGYCEIVF------KYAAKKLFGIDLR-----
Encephalitozoon ----------ASGKSLCSMEEA----------ETTQWNIGT------SSGGYAEFIL----------


H-cluster
Drosophila
Giardia
Entamoeba
Cryptosporidium
Cyanidioschyzon
Trichomonas
Trypanosoma
Homo
Saccharomyces
Blastocystis S1
Blastocystis S7
Phaeodactylum
Phytophthora
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Dictyostelium
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Naegleria
Encephalitozoon
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Giardia
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Cryptosporidium
Plasmodium
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Trypanosoma
Homo
Saccharomyces
Blastocystis S1
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Homo
Saccharomyces
Blastocystis S1
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Phaeodactylum
Phytophthora
Arabidopsis

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Micromonas       ------------------
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Tetrahymena      ---------------LNW
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Encephalitozoon  ---------------VDW
Drosophila       ------------------
Giardia          RIQD-----------LAW
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Cryptosporidium  ---------------LKW
Cyanidioschyzon  DIKSQVTVSERHRLVHQW
Trichomonas      ---------------FAW
Trypanosoma      ---------------LKW
```

Figure S4


Homo sapiens-nbp35
Saccharomyces cerevisiae-nbp35
Dictyostelium purpureum-nbp35
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Guillardia theta-nbp35
Encephalitozoon cuniculi-nbp35
Trypanosoma vivax
Giardia lamblia-nbp35
Blastocystis-nbp35
Blastocystis sp. NandII-nbp35
Phytophthora infestans-nbp35
Phaeodactylum tricornutum-nbp35
Arabidopsis thaliana-nbp35
Micromonas sp.-nbp35
Cyanidioschyzon merolae-nbp35
Cryptosporidium hominis-nbp35
Tetrahymena thermophila-nbp35
Metallosphaera cuprina
Pyrococcus furiosus
Methanococcus maripaludis
Homo sapiens-cfd1
Saccharomyces cerevisiae-cfd1
Dictyostelium purpureum-cfd1
Entamoeba dispar-cfd1
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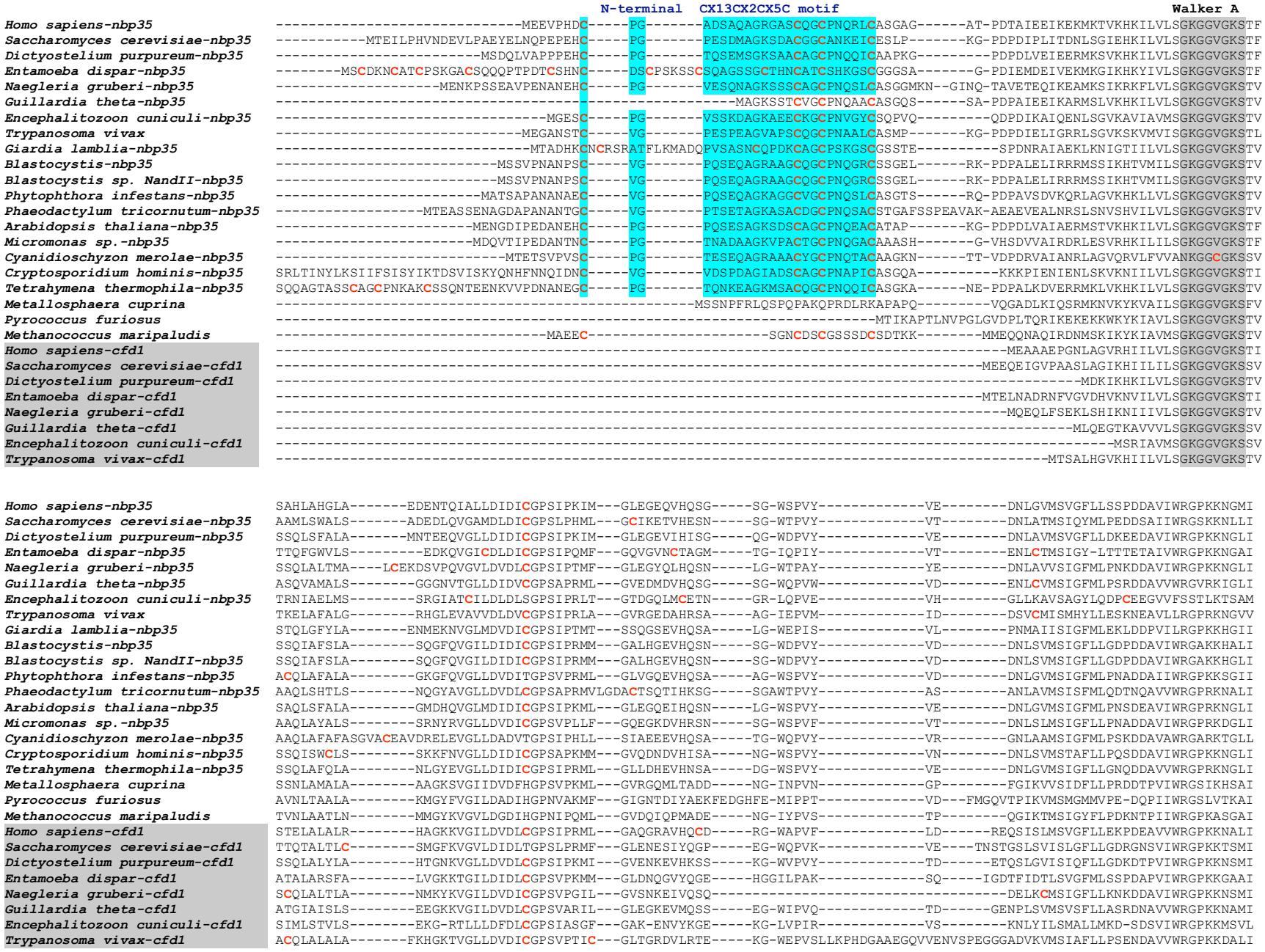
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Giardia lamblia-nbp35
Blastocystis-nbp35
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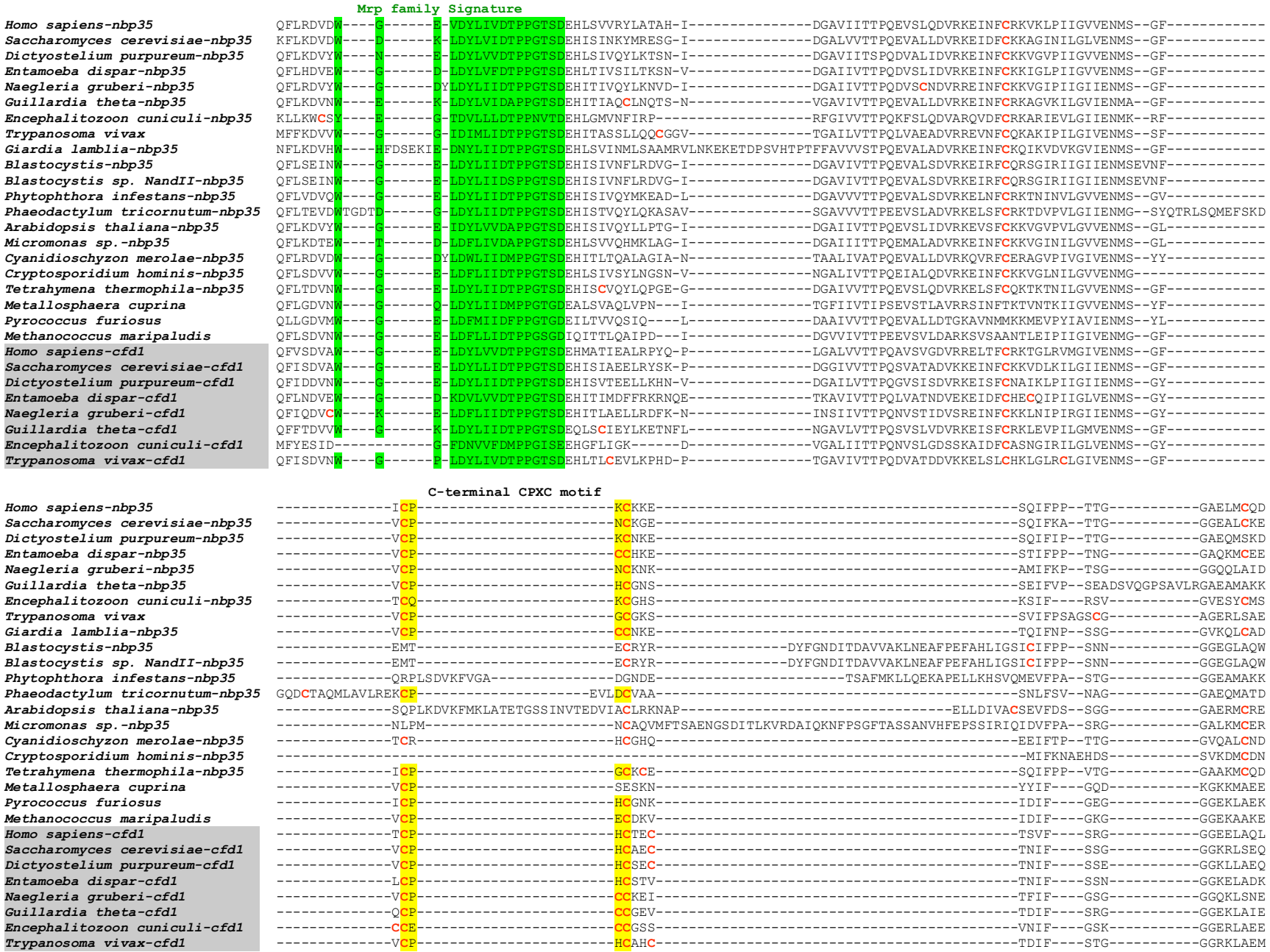
Trypanosoma vivax-cfd1


Mrp family Signature
C-terminal CPXC motif
Homo sapiens-nbp35
Saccharomyces cerevisiae-nbp35
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Entamoeba dispar-nbp35
Naegleria gruberi-nbp35
Guillardia theta-nbp35
Encephalitozoon cuniculi-nbp35
Trypanosoma vivax
Giardia lamblia-nbp35
Blastocystis-nbp35
Blastocystis sp. NandII-nbp35
Phytophthora infestans-nbp35
Phaeodactylum tricornutum-nbp35
Arabidopsis thaliana-nbp35
Micromonas sp.-nbp35
Cyanidioschyzon merolae-nbp35
Cryptosporidium hominis-nbp35
Tetrahymena thermophila-nbp35
Metallosphaera cuprina
Pyrococcus furiosus
Methanococcus maripaludis
Homo sapiens-cfd1
Saccharomyces cerevisiae-cfd1
Dictyostelium purpureum-cfd1
Entamoeba dispar-cfd1
Naegleria gruberi-cfd1
Guillardia theta-cfd1
Encephalitozoon cuniculi-cfd1
Trypanosoma vivax-cfd1

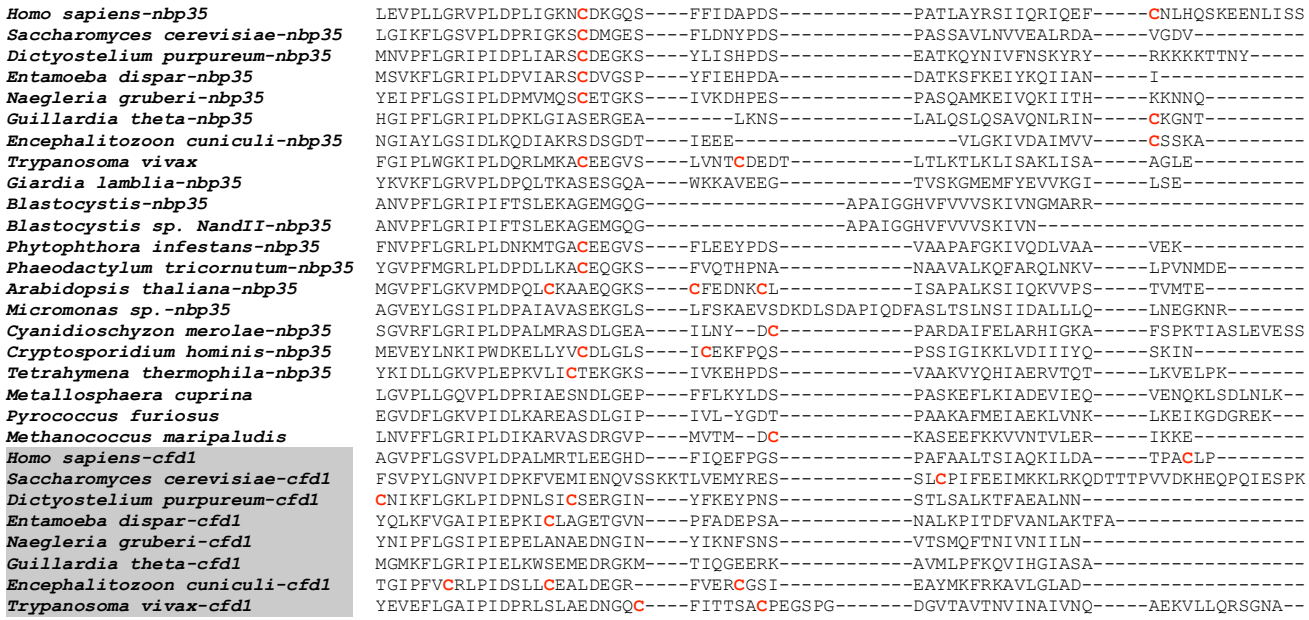

Homo sapiens-nbp35 LEVPLLGRVPLDPLIGKNCDKGQS----FFIDAPDS----------PATLAYRSIIQRIQEF-----CNLHQSKEENLISS
Saccharomyces cerevisiae-nbp35 LGIKFLGSVPLDPRIGKSCDMGES----FLDNYPDS----------PASSAVLNVVEALRDA-----VGDV----------
Dictyostelium purpureum-nbp35 MNVPFLGRIPIDPLIARSCDEGKS----YLISHPDS----------EATKQYNIVFNSKYRY-----RKKKKTTNY-----
Entamoeba dispar-nbp35 MSVKFLGRIPLDPVIARSCDVGSP----YFIEHPDA----------DATKSFKEIYKQIIAN-----I----------
Naegleria gruberi-nbp35 YEIPFLGSIPLDPMVMQSCETGKS----IVKDHPES----------PASQAMKEIVQKIITH-----KKNNQ---------
Guillardia theta-nbp35 HGIPFLGRIPLDPKLGIASERGEA--------LKNS----------LALQSLQSAVQNLRIN-----CKGNT---------
Encephalitozoon cuniculi-nbp35 NGIAYLGSIDLKQDIAKRSDSGDT----IEEE--------------------VLGKIVDAIMVV-----CSSKA---------
Trypanosoma vivax FGIPLWGKIPLDQRLMKACEEGVS----LVNTCDEDT----------LTLKTLKLISAKLISA-----AGLE----------
Giardia lamblia-nbp35 YKVKFLGRVPLDPQLTKASESGQA----WKKAVEEG----------TVSKGMEMFYEVVKGI-----LSE----------
Blastocystis-nbp35 ANVPFLGRIPIFTSLEKAGEMGQG----------APAIGGHVFVVVSKIVNGMARR----------
Blastocystis sp. NandII-nbp35 ANVPFLGRIPIFTSLEKAGEMGQG----------APAIGGHVFVVVSKIVN----------
Phytophthora infestans-nbp35 FNVPFLGRLPLDNKMTGACEEGVS----FLEEYPDS----------VAAPAFGKIVQDLVAA-----VEK----------
Phaeodactylum tricornutum-nbp35 YGVPFMGRLPLDPDLLKACEQGKS----FVQTHPNA----------NAAVALKQFARQLNKV-----LPVNMDE-------
Arabidopsis thaliana-nbp35 MGVPFLGKVPMDPQLCKAAEQGKS----CFEDNKCL----------ISAPALKSIIQKVVPS-----TVMTE---------
Micromonas sp.-nbp35 AGVEYLGSIPLDPAIAVASEKGLS----LFSKAEVSDKDLSDAPIQDFASLTSLNSIIDALLLQ-----LNEGKNR-------
Cyanidioschyzon merolae-nbp35 SGVRFLGRIPLDPALMRASDLGEA----ILNY--DC----------PARDAIFELARHIGKA-----FSPKTIASLEVESS
Cryptosporidium hominis-nbp35 MEVEYLNKIPWDKELLYVCDLGLS----ICEKFPQS----------PSSIGIKKLVDIIIYQ-----SKIN----------
Tetrahymena thermophila-nbp35 YKIDLLGKVPLEPKVLICTEKGKS----IVKEHPDS----------VAAKVYQHIAERVTQT-----LKVELPK-------
Metallosphaera cuprina LGVPLLGQVPLDPRIAESNDLGEP----FFLKYLDS----------PASKEFLKIADEVIEQ-----VENQKLSDLNLK--
Pyrococcus furiosus EGVDFLGKVPIDLKAREASDLGIP----IVL-YGDT----------PAAKAFMEIAEKLVNK-----LKEIKGDGREK---
Methanococcus maripaludis LNVFFLGRIPLDIKARVASDRGVP----MVTM--DC----------KASEEFKKVVNTVLER-----IKKE----------
Homo sapiens-cfd1 AGVPFLGSVPLDPALMRTLEEGHD----FIQEFPGS----------PAFAALTSIAQKILDA-----TPACLP---------
Saccharomyces cerevisiae-cfd1 FSVPYLGNVPIDPKFVEMIENQVSSKKTLVEMYRES----------SLCPIFEEIMKKLRKQDTTTPVVDKHEQPQIESPK
Dictyostelium purpureum-cfd1 CNIKFLGKLPIDPNLSICSERGIN----YFKEYPNS----------STLSALKTFAEALNN----------
Entamoeba dispar-cfd1 YQLKFVGAIPIEPKICLAGETGVN----PFADEPSA----------NALKPITDFVANLAKTFA----------
Naegleria gruberi-cfd1 YNIPFLGSIPIEPELANAEDNGIN----YIKNFSNS----------VTSMQFTNIVNIILN----------
Guillardia theta-cfd1 MGMKFLGRIPIELKWSEMEDRGKM----TIQGEERK----------AVMLPFKQVIHGIASA----------
Encephalitozoon cuniculi-cfd1 TGIPFVCRLPIDSLLCEALDEGR-----FVERCGSI----------EAYMKFRKAVLGLAD----------
Trypanosoma vivax-cfd1 YEVEFLGAIPIDPRLSLAEDNGQC----FITTSACPEGSPG-------DGVTAVTNVINAIVNQ-----AEKVLLQRSGNA--

Figure S5

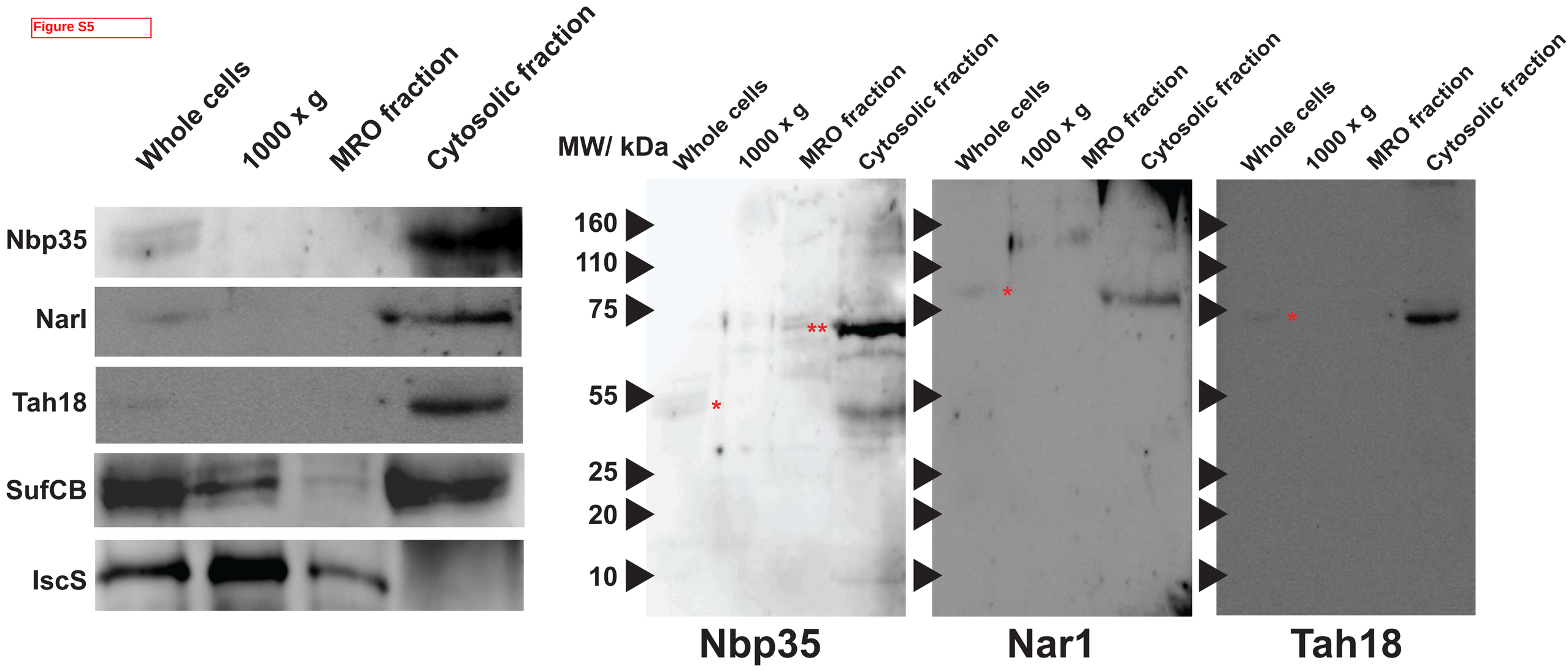

Whole cells
1000 x g
MRO fraction
Cytosolic fraction
Nbp35
Narl
Tah18
SufCB
IscS
MW/ kDa
160
110
75
55
25
20
10
*
**
Nbp35
Nar1
Tah18

Figure S6

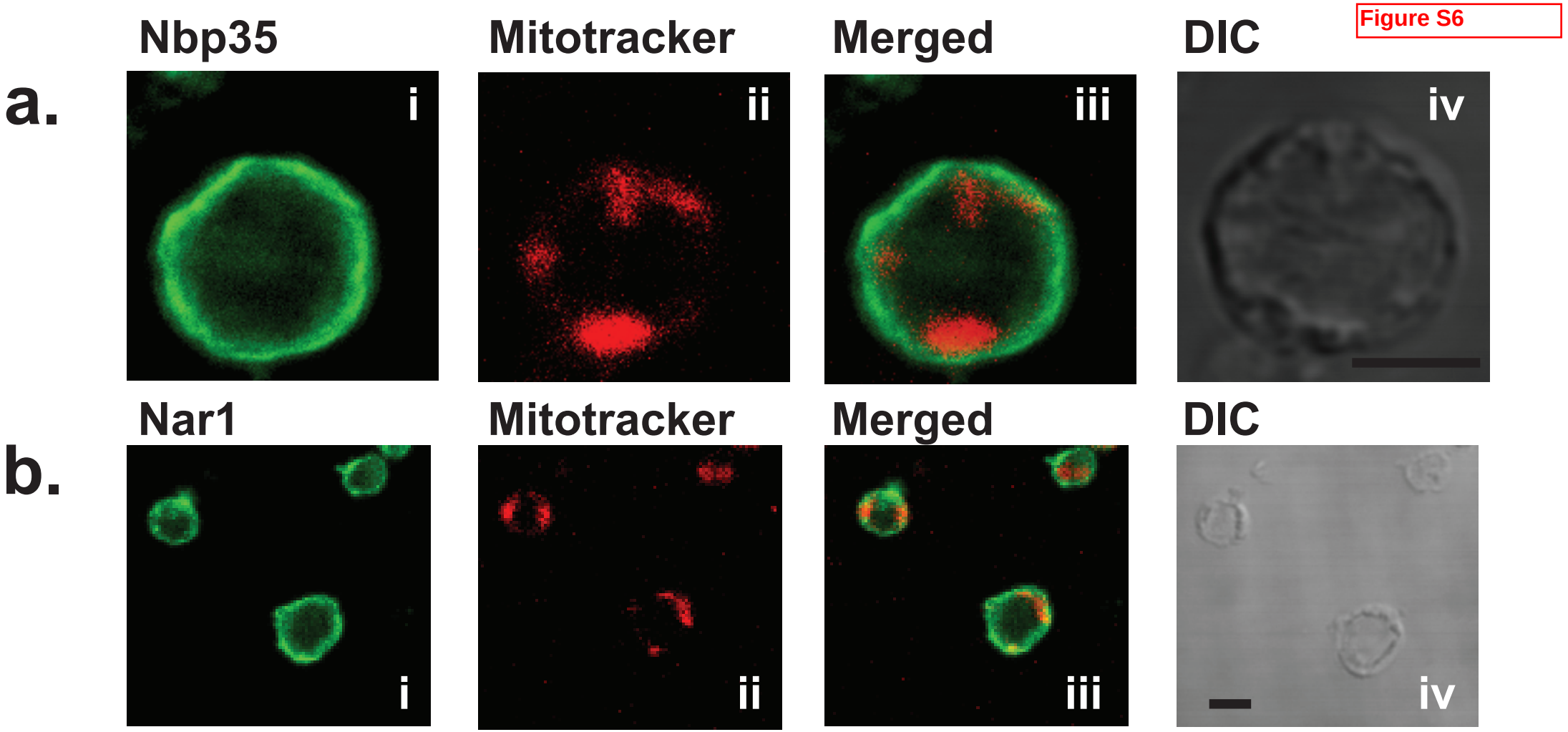

Nbp35
Mitotracker
Merged
DIC
a.
i
ii
iii
iv
Nar1
Mitotracker
Merged
DIC
b.
i
ii
iii
iv

Figure S7 - Localization of Nbp35

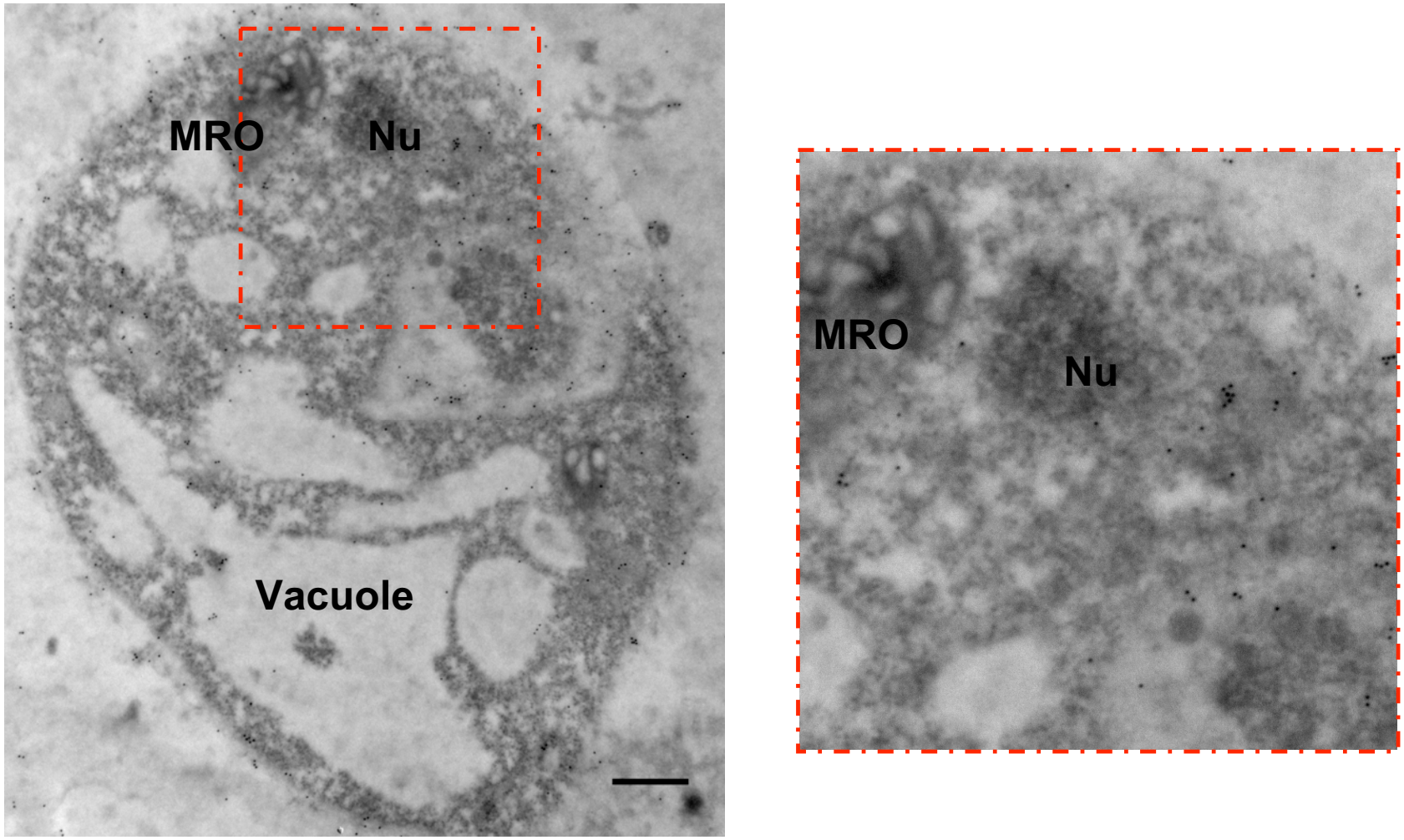


Figure S8 - Localization of Nar1

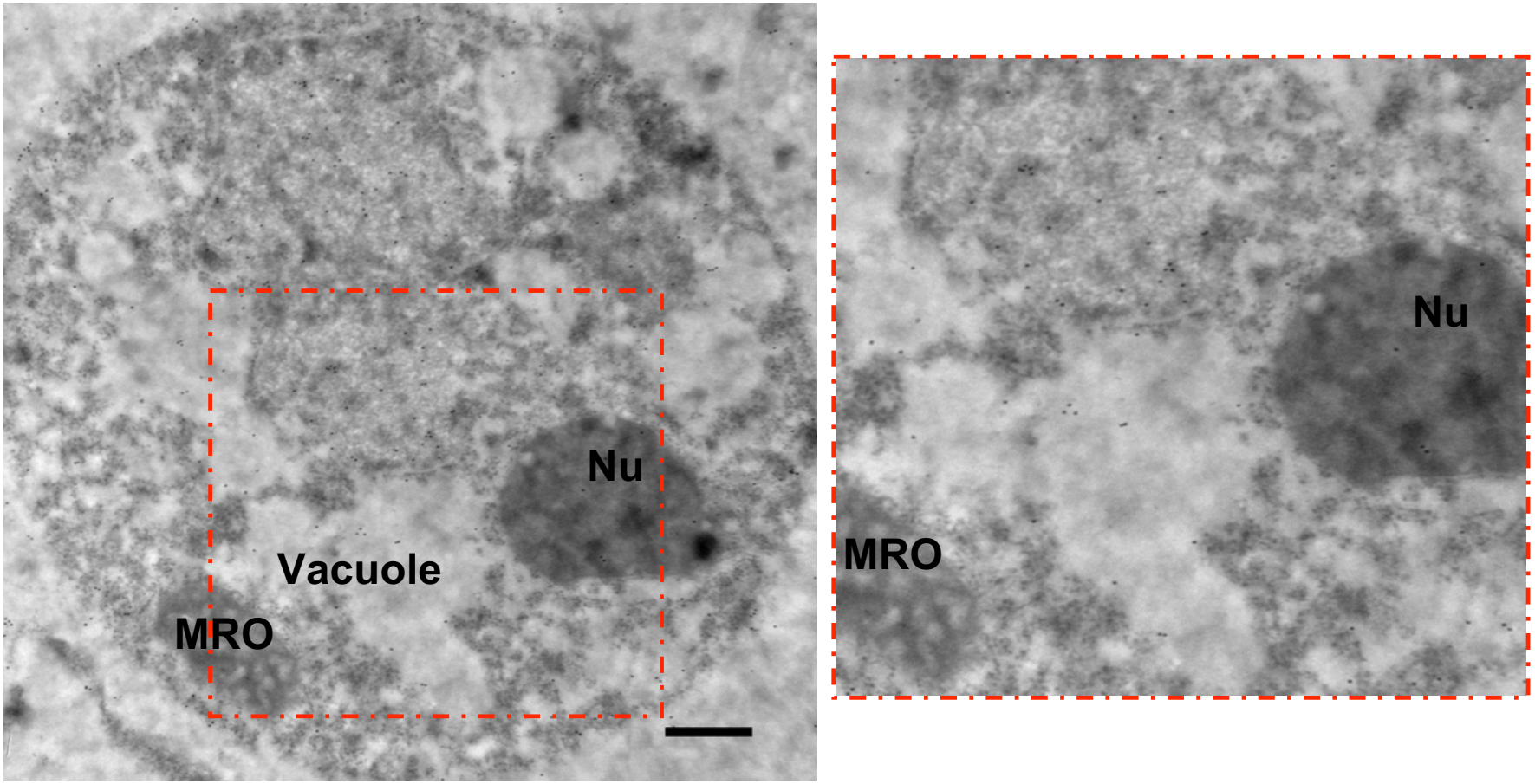
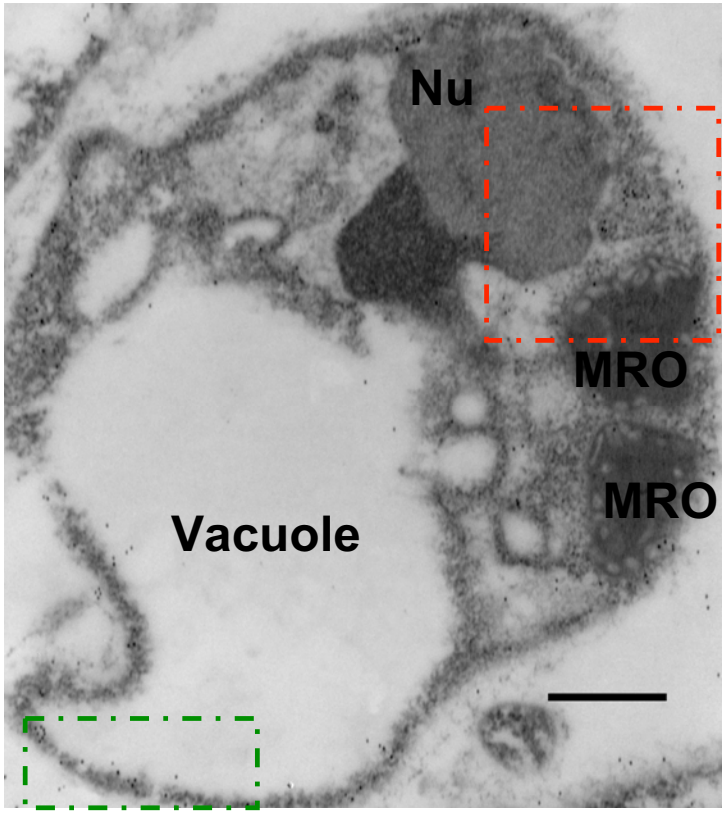


Figure S9 - Localization of Tah18



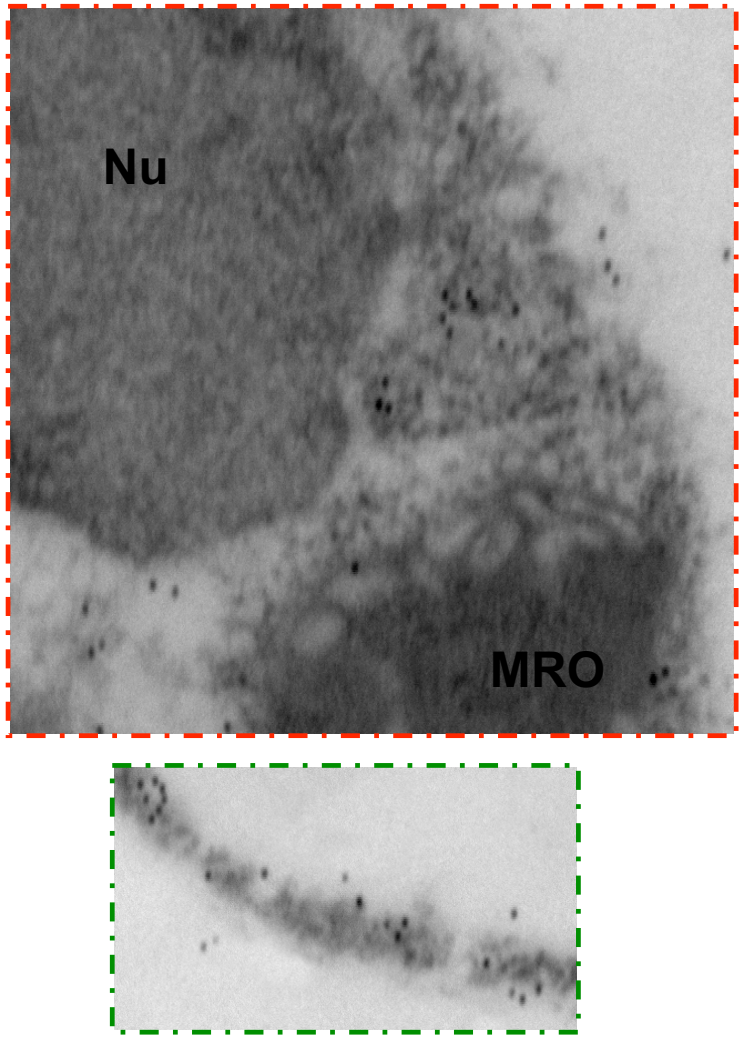
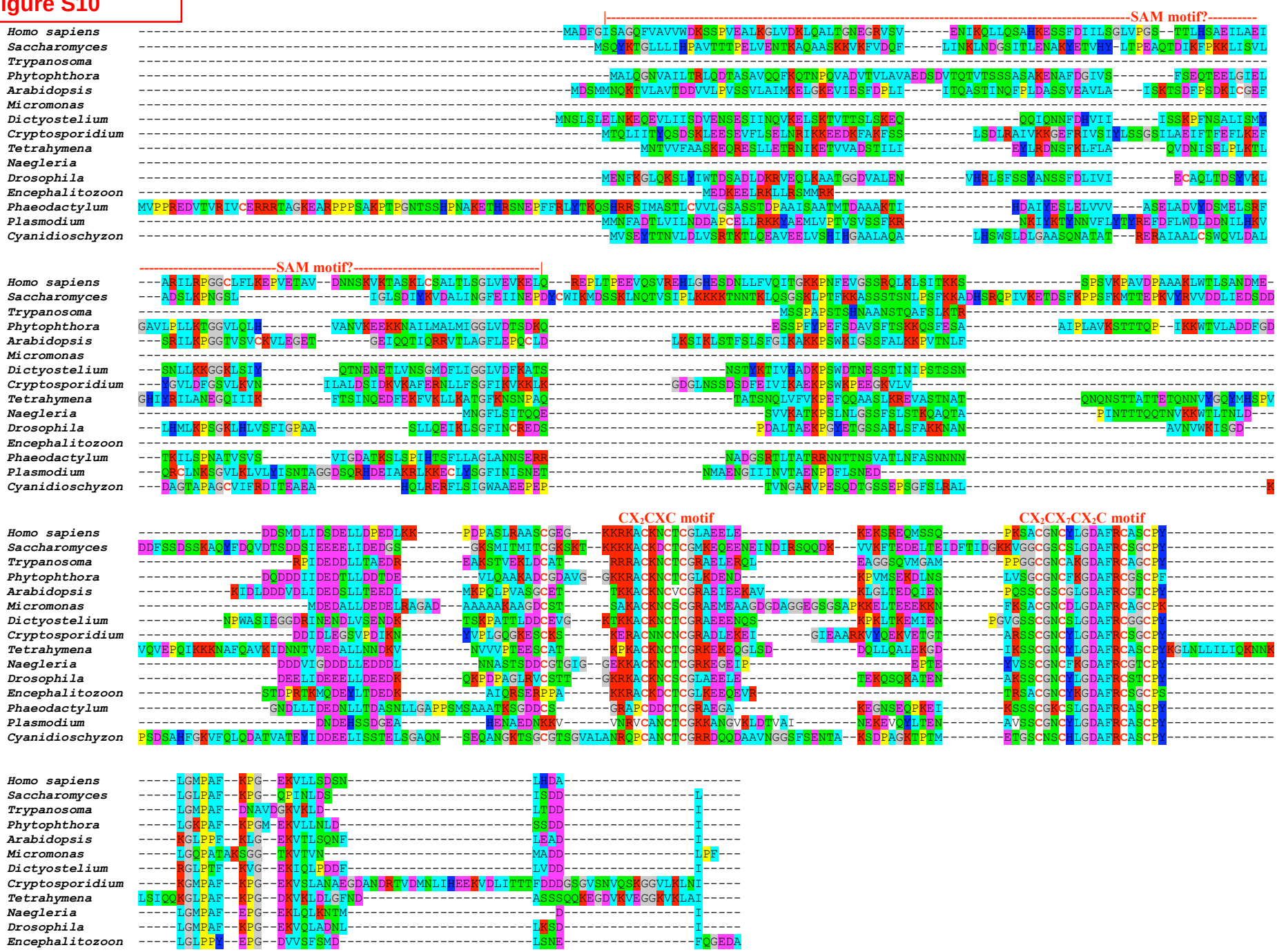


Figure S10


SAM motif?
SAM motif?
CX2CXC motif
CX3CX7CX2C motif
Homo sapiens
Saccharomyces
Trypanosoma
Phytophthora
Arabidopsis
Micromonas
Dictyostelium
Cryptosporidium
Tetrahymena
Naegleria
Drosophila
Encephalitozoon
Phaeodactylum
Plasmodium
Cyanidioschyzon

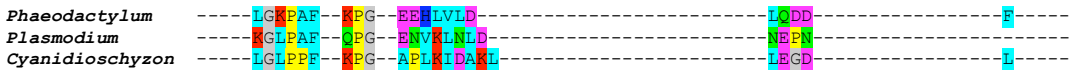

Phaeodactylum -----LGKPAF--KPG--EEHLVLD----------LQDD----------F-----
Plasmodium -----KGLPAF--QPG--ENVKLNLD----------NEPM----------
Cyanidioschyzon -----LGLPPF--KPG--APLKIDAKL----------LEGD----------L-----

Figure S11

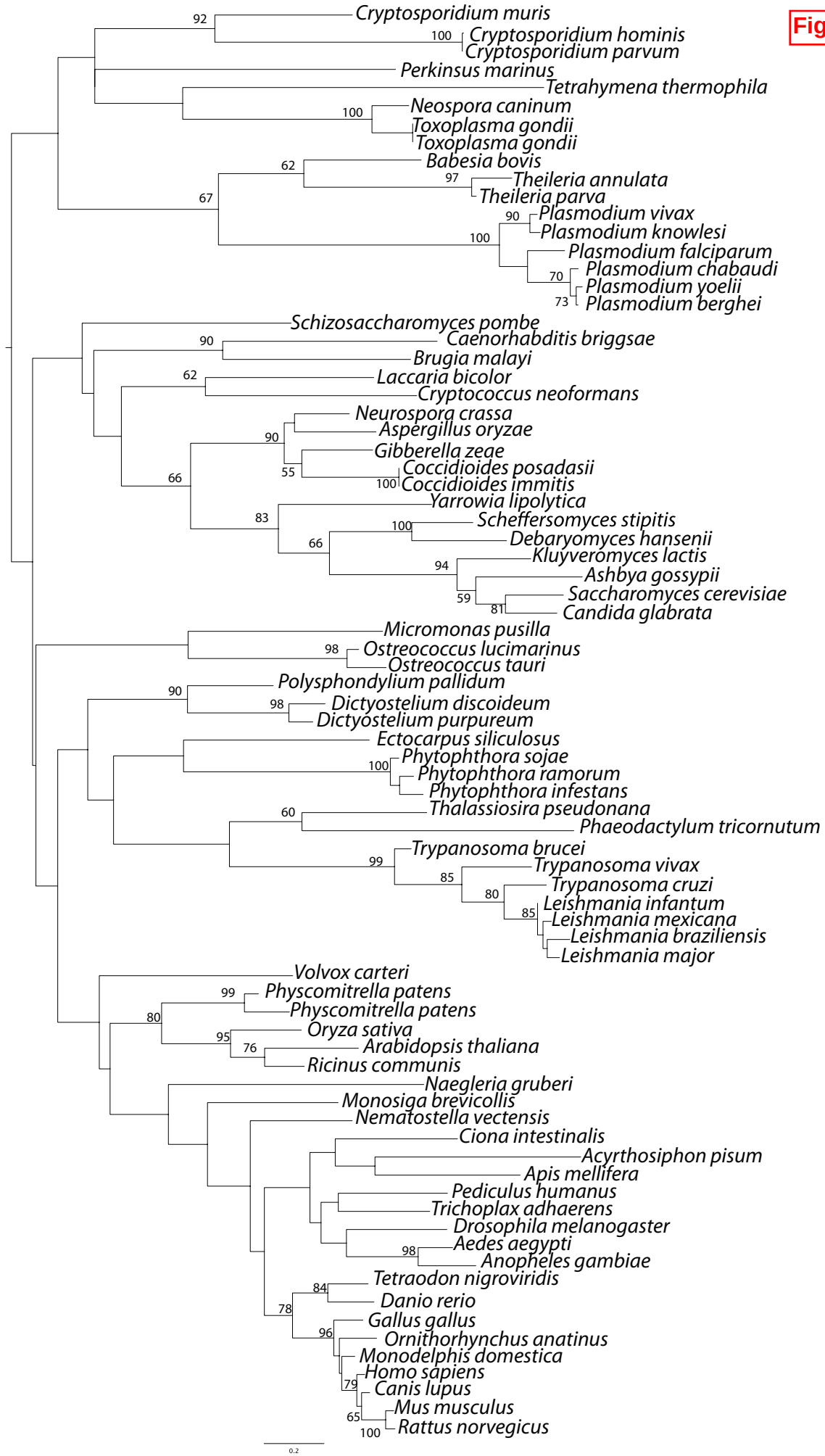

Cryptosporidium muris
Cryptosporidium hominis
Cryptosporidium parvum
Perkinsus marinus
Tetrahymena thermophila
Neospora caninum
Toxoplasma gondii
Toxoplasma gondii
Babesia bovis
Theileria annulata
Theileria parva
Plasmodium vivax
Plasmodium knowlesi
Plasmodium falciparum
Plasmodium chabaudi
Plasmodium yoelii
Plasmodium berghei
Schizosaccharomyces pombe
Caenorhabditis briggsae
Brugia malayi
Laccaria bicolor
Cryptococcus neoformans
Neurospora crassa
Aspergillus oryzae
Gibberella zeae
Coccidioides posadasii
Coccidioides immitis
Yarrowia lipolytica
Scheffersomyces stipitis
Debaryomyces hansenii
Kluyveromyces lactis
Ashbya gossypii
Saccharomyces cerevisiae
Candida glabrata
Micromonas pusilla
Ostreococcus lucimarinus
Ostreococcus tauri
Polysphondylium pallidum
Dictyostelium discoideum
Dictyostelium purpureum
Ectocarpus siliculosus
Phytophthora sojae
Phytophthora ramorum
Phytophthora infestans
Thalassiosira pseudonana
Phaeodactylum tricornutum
Trypanosoma brucei
Trypanosoma vivax
Trypanosoma cruzi
Leishmania infantum
Leishmania mexicana
Leishmania braziliensis
Leishmania major
Volvox carteri
Physcomitrella patens
Physcomitrella patens
Oryza sativa
Arabidopsis thaliana
Ricinus communis
Naegleria gruberi
Monosiga brevicollis
Nematostella vectensis
Ciona intestinalis
Acyrthosiphon pisum
Apis mellifera
Pediculus humanus
Trichoplax adhaerens
Drosophila melanogaster
Aedes aegypti
Anopheles gambiae
Tetraodon nigroviridis
Danio rerio
Gallus gallus
Ornithorhynchus anatinus
Monodelphis domestica
Homo sapiens
Canis lupus
Mus musculus
Rattus norvegicus
0.2

Figure S12

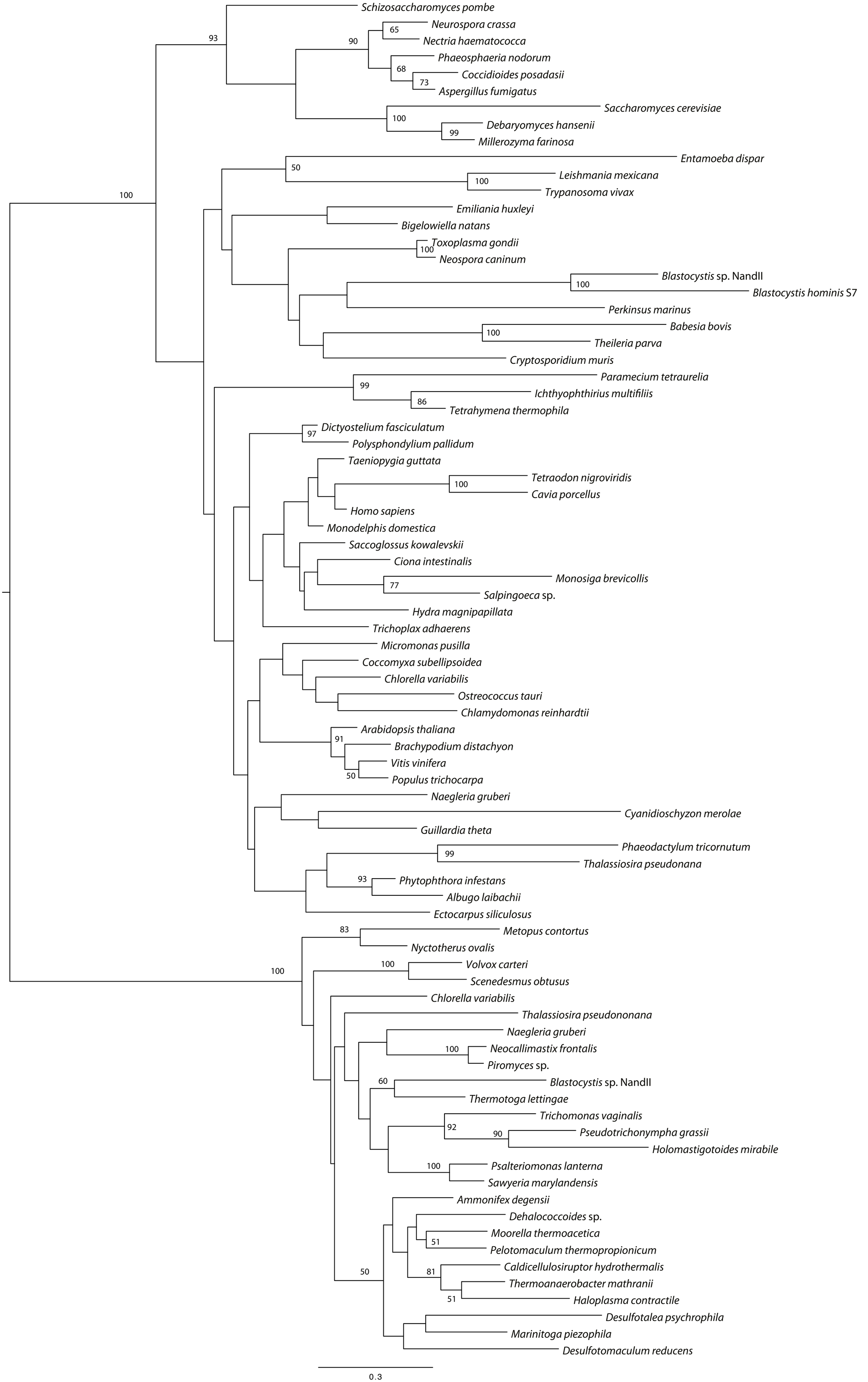

Schizosaccharomyces pombe
Neurospora crassa
Nectria haematococca
Phaeosphaeria nodorum
Coccidioides posadasii
Aspergillus fumigatus
Saccharomyces cerevisiae
Debaryomyces hansenii
Millerozyma farinosa
Entamoeba dispar
Leishmania mexicana
Trypanosoma vivax
Emiliania huxleyi
Bigelowiella natans
Toxoplasma gondii
Neospora caninum
Blastocystis sp. NandII
Blastocystis hominis S7
Perkinsus marinus
Babesia bovis
Theileria parva
Cryptosporidium muris
Paramecium tetraurelia
Ichthyophthirius multifiliis
Tetrahymena thermophila
Dictyostelium fasciculatum
Polysphondylium pallidum
Taeniopygia guttata
Tetraodon nigroviridis
Cavia porcellus
Homo sapiens
Monodelphis domestica
Saccoglossus kowalevskii
Ciona intestinalis
Monosiga brevicollis
Salpingoeca sp.
Hydra magnipapillata
Trichoplax adhaerens
Micromonas pusilla
Coccomyxa subellipsoidea
Chlorella variabilis
Ostreococcus tauri
Chlamydomonas reinhardtii
Arabidopsis thaliana
Brachypodium distachyon
Vitis vinifera
Populus trichocarpa
Naegleria gruberi
Cyanidioschyzon merolae
Guillardia theta
Phaeodactylum tricornutum
Thalassiosira pseudonana
Phytophthora infestans
Albugo laibachii
Ectocarpus siliculosus
Metopus contortus
Nyctotherus ovalis
Volvox carteri
Scenedesmus obtusus
Chlorella variabilis
Thalassiosira pseudononana
Naegleria gruberi
Neocallimastix frontalis
Piromyces sp.
Blastocystis sp. NandII
Thermotoga lettingae
Trichomonas vaginalis
Pseudotrichonympha grassii
Holomastigotoides mirabile
Psalteriomonas lanterna
Sawyeria marylandensis
Ammonifex degensii
Dehalococcoides sp.
Moorella thermoacetica
Pelotomaculum thermopropionicum
Caldicellulosiruptor hydrothermalis
Thermoanaerobacter mathranii
Haloplasma contractile
Desulfotalea psychrophila
Marinitoga piezophila
Desulfotomaculum reducens
0.3

Figure S13

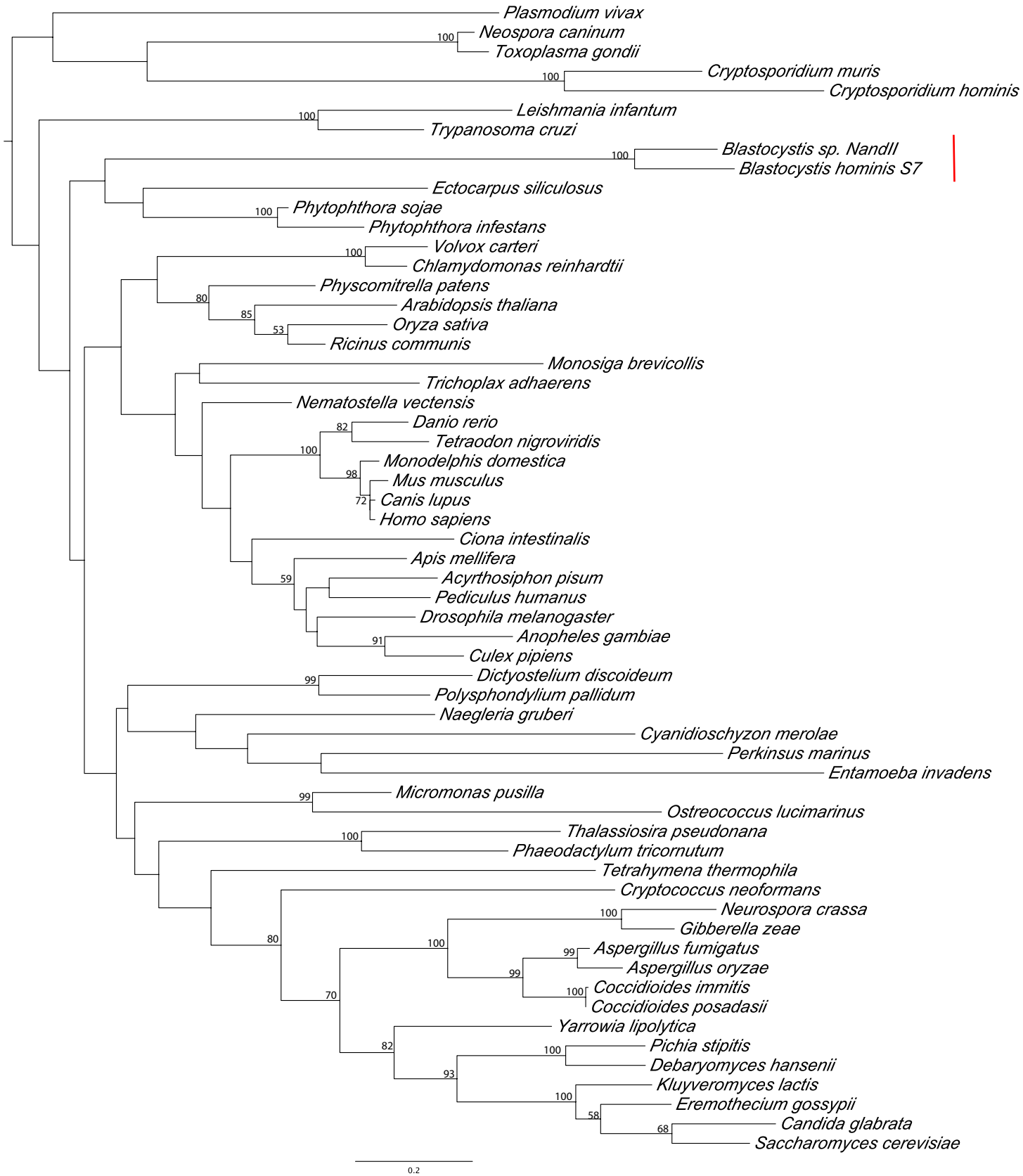

Plasmodium vivax
Neospora caninum
Toxoplasma gondii
Cryptosporidium muris
Cryptosporidium hominis
Leishmania infantum
Trypanosoma cruzi
Blastocystis sp. NandII
Blastocystis hominis S7
Ectocarpus siliculosus
Phytophthora sojae
Phytophthora infestans
Volvox carteri
Chlamydomonas reinhardtii
Physcomitrella patens
Arabidopsis thaliana
Oryza sativa
Ricinus communis
Monosiga brevicollis
Trichoplax adhaerens
Nematostella vectensis
Danio rerio
Tetraodon nigroviridis
Monodelphis domestica
Mus musculus
Canis lupus
Homo sapiens
Ciona intestinalis
Apis mellifera
Acyrthosiphon pisum
Pediculus humanus
Drosophila melanogaster
Anopheles gambiae
Culex pipiens
Dictyostelium discoideum
Polysphondylium pallidum
Naegleria gruberi
Cyanidioschyzon merolae
Perkinsus marinus
Entamoeba invadens
Micromonas pusilla
Ostreococcus lucimarinus
Thalassiosira pseudonana
Phaeodactylum tricornutum
Tetrahymena thermophila
Cryptococcus neoformans
Neurospora crassa
Gibberella zeae
Aspergillus fumigatus
Aspergillus oryzae
Coccidioides immitis
Coccidioides posadasii
Yarrowia lipolytica
Pichia stipitis
Debaryomyces hansenii
Kluyveromyces lactis
Eremothecium gossypii
Candida glabrata
Saccharomyces cerevisiae
0.2

Figure S14

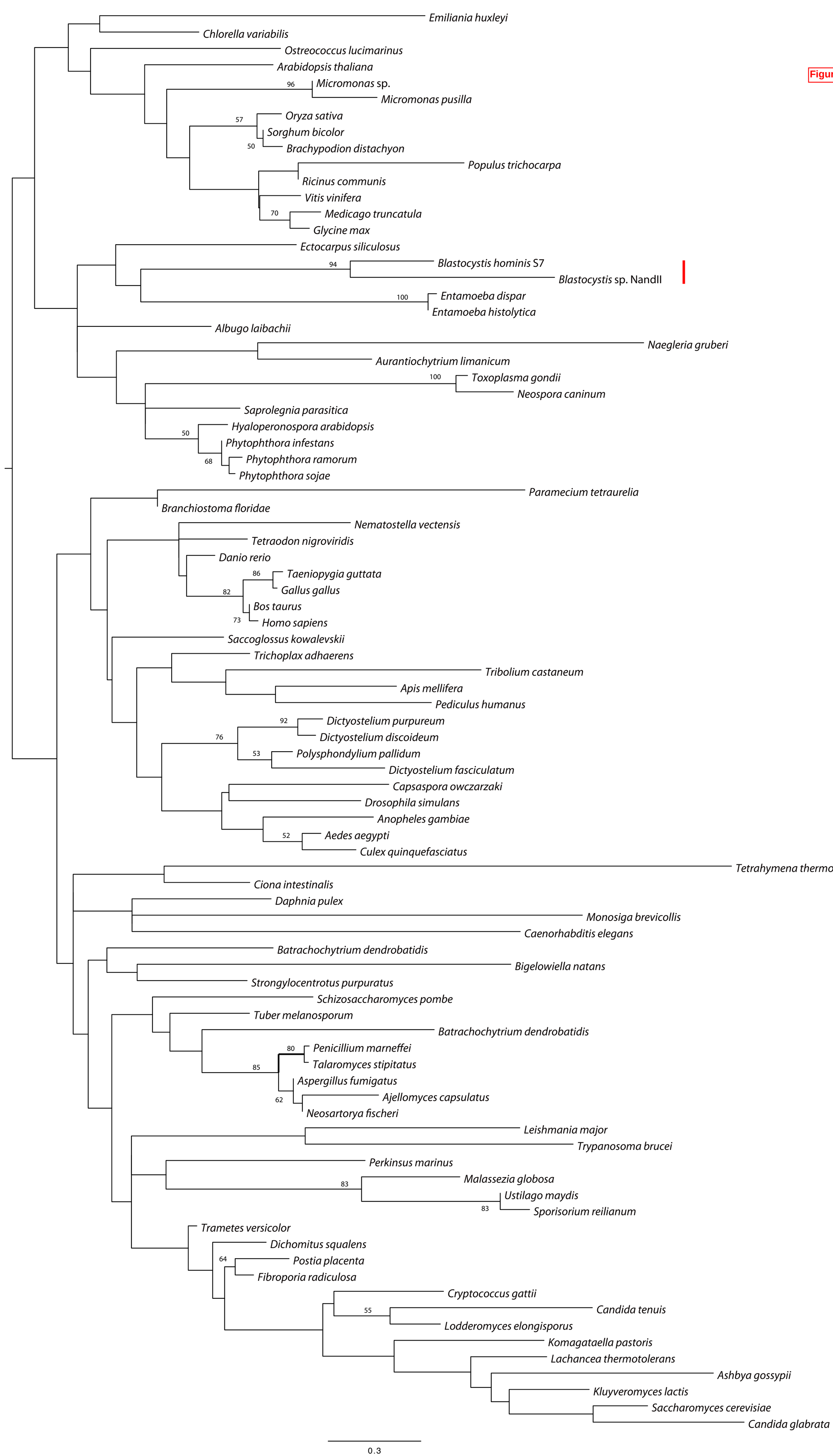
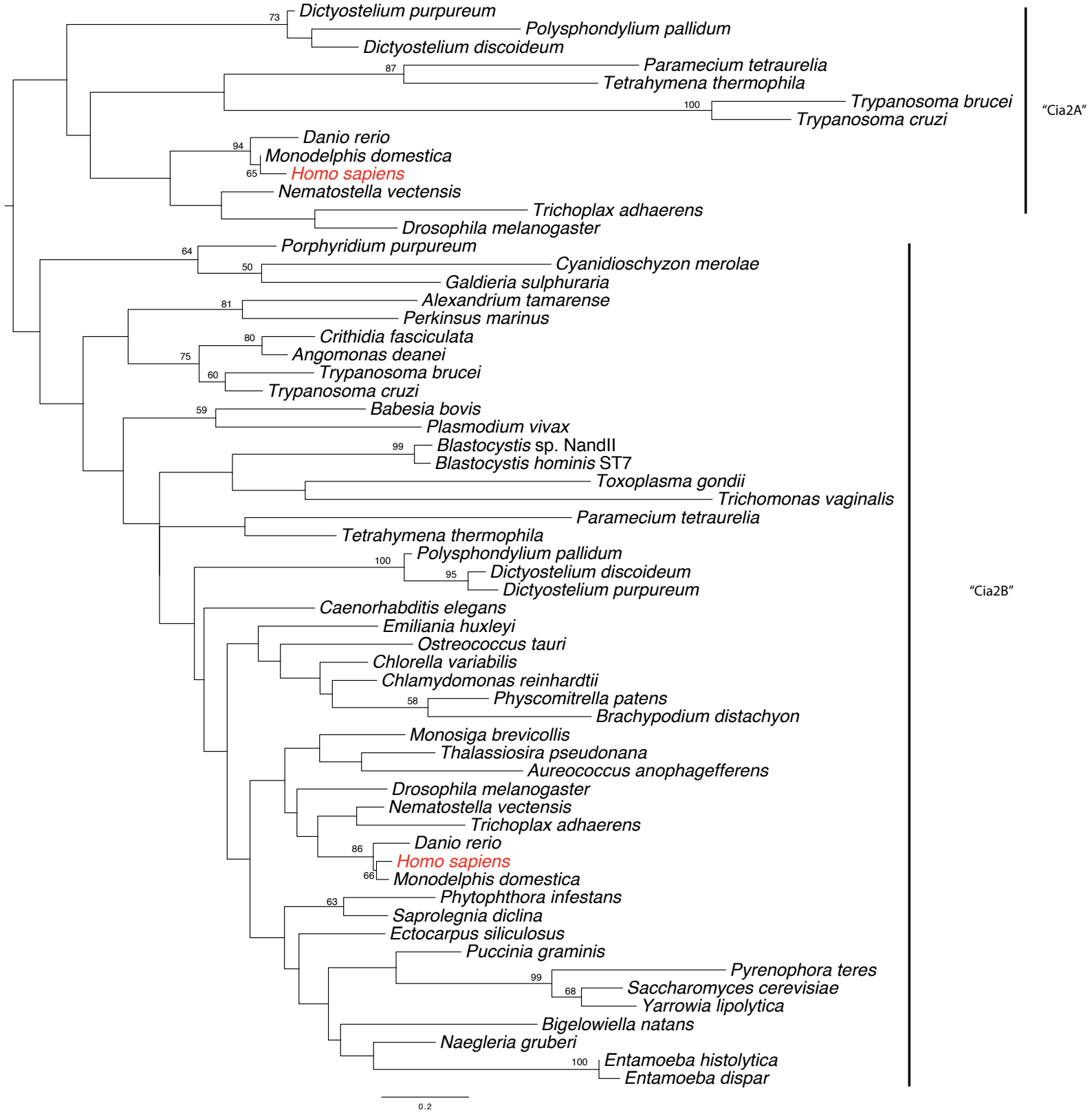

Emiliania huxleyi
Chlorella variabilis
Ostreococcus lucimarinus
Arabidopsis thaliana
96
Micromonas sp.
Micromonas pusilla
57
Oryza sativa
Sorghum bicolor
50
Brachypodion distachyon
Populus trichocarpa
Ricinus communis
Vitis vinifera
70
Medicago truncatula
Glycine max
Ectocarpus siliculosus
94
Blastocystis hominis S7
Blastocystis sp. NandII
100
Entamoeba dispar
Entamoeba histolytica
Albugo laibachii
Naegleria gruberi
Aurantiochytrium limanicum
100
Toxoplasma gondii
Neospora caninum
Saprolegnia parasitica
Hyaloperonospora arabidopsis
50
Phytophthora infestans
68
Phytophthora ramorum
Phytophthora sojae
Paramecium tetraurelia
Branchiostoma floridae
Nematostella vectensis
Tetraodon nigroviridis
Danio rerio
86
Taeniopygia guttata
Gallus gallus
82
Bos taurus
73
Homo sapiens
Saccoglossus kowalevskii
Trichoplax adhaerens
Tribolium castaneum
Apis mellifera
Pediculus humanus
92
Dictyostelium purpureum
Dictyostelium discoideum
76
53
Polysphondylium pallidum
Dictyostelium fasciculatum
Capsaspora owczarzaki
Drosophila simulans
Anopheles gambiae
52
Aedes aegypti
Culex quinquefasciatus
Tetrahymena thermophila
Ciona intestinalis
Daphnia pulex
Monosiga brevicollis
Caenorhabditis elegans
Batrachochytrium dendrobatidis
Bigelowiella natans
Strongylocentrotus purpuratus
Schizosaccharomyces pombe
Tuber melanosporum
Batrachochytrium dendrobatidis
80
Penicillium marneffei
85
Talaromyces stipitatus
Aspergillus fumigatus
62
Ajellomyces capsulatus
Neosartorya fischeri
Leishmania major
Trypanosoma brucei
Perkinsus marinus
Malassezia globosa
83
Ustilago maydis
83
Sporisorium reilianum
Trametes versicolor
Dichomitus squalens
64
Postia placenta
Fibroporia radiculosa
Cryptococcus gattii
55
Candida tenuis
Lodderomyces elongisporus
Komagataella pastoris
Lachancea thermotolerans
Ashbya gossypii
Kluyveromyces lactis
Saccharomyces cerevisiae
Candida glabrata
0.3

Figure S15


Dictyostelium purpureum
Polysphondylium pallidum
Dictyostelium discoideum
Paramecium tetraurelia
Tetrahymena thermophila
Trypanosoma brucei
Trypanosoma cruzi
Danio rerio
Monodelphis domestica
Homo sapiens
Nematostella vectensis
Trichoplax adhaerens
Drosophila melanogaster
"Cia2A"
Porphyridium purpureum
Cyanidioschyzon merolae
Galdieria sulphuraria
Alexandrium tamarense
Perkinsus marinus
Crithidia fasciculata
Angomonas deanei
Trypanosoma brucei
Trypanosoma cruzi
Babesia bovis
Plasmodium vivax
Blastocystis sp. NandII
Blastocystis hominis ST7
Toxoplasma gondii
Trichomonas vaginalis
Paramecium tetraurelia
Tetrahymena thermophila
Polysphondylium pallidum
Dictyostelium discoideum
Dictyostelium purpureum
Caenorhabditis elegans
Emiliania huxleyi
Ostreococcus tauri
Chlorella variabilis
Chlamydomonas reinhardtii
Physcomitrella patens
Brachypodium distachyon
Monosiga brevicollis
Thalassiosira pseudonana
Aureococcus anophagefferens
Drosophila melanogaster
Nematostella vectensis
Trichoplax adhaerens
Danio rerio
Homo sapiens
Monodelphis domestica
Phytophthora infestans
Saprolegnia diclina
Ectocarpus siliculosus
Puccinia graminis
Pyrenophora teres
Saccharomyces cerevisiae
Yarrowia lipolytica
Bigelowiella natans
Naegleria gruberi
Entamoeba histolytica
Entamoeba dispar
"Cia2B"
0.2