# Supplemental Materials

***Molecular Biology of the Cell***

Urbanska et al.

**SUPPLEMENTARY MATERIALS:**

**TABLE S1:** A summary of the cilia and flagella structural defects in CSC subunits mutants

| | ***Chlamydomonas*** [a] | | | ***Tetrahymena*** | | |
|---|---|---|---|---|---|---|
| | *pWT* [b] | *amiRNAi FAP61-KD* | *amiRNAi FAP91-KD* | *WT* | *FAP61-KO* | *FAP251-KO* |
| Swimming velocity | normal | reduced | reduced | normal | reduced | reduced |
| RS1 structure | present in 100% of repeats | present in 100% of repeats | present in 100% of repeats | present in 100% of repeats | present in 100% of repeats | present in 100% of repeats |
| RS2 structure | present in 100% of repeats | missing in 49 % of repeats | missing in 27-55 % of repeats | present in 100% of repeats | present in 100% of repeats | present in 100% of repeats |
| RS3 structure | short (RS3S) | loss of the entire RS3S | reduction of the RS3S structure | full-length RS3, present in 100% of repeats | missing completely in 36 % of repeats; missing a part of the RS3 stem region in 100% of repeats | missing completely in 16 % of repeats; missing/incomplete arch-like structure of RS3 base in 100% of repeats |
| Additional or irregular RS | not observed | observed | observed | not observed | not observed | not observed |
| IDAs | (not analyzed) | reduction of dynein e (50%); slight reduction of dynein a/d (50%) | reduction of dynein e; slight reduction of dynein a/d | reduction of dynein d (10%) and g (10%) | reduction of dynein d (38%) and g (25%) | reduction of dynein d (13%) and g (13%) |
| N-DRC | normal | reduction of a small part of the fork; missing connection between N-DRC baseplate and the base of RS2 | reduction of a small part of the fork; missing connection between N-DRC baseplate and the base of RS2 | normal | normal | normal |
| Hole in the inner junction of the microtubule doublet | present | absent | absent | present | present | present |

**a)** Based on Dymek *et al*., 2011; Heuser *et al*., 2012**.**

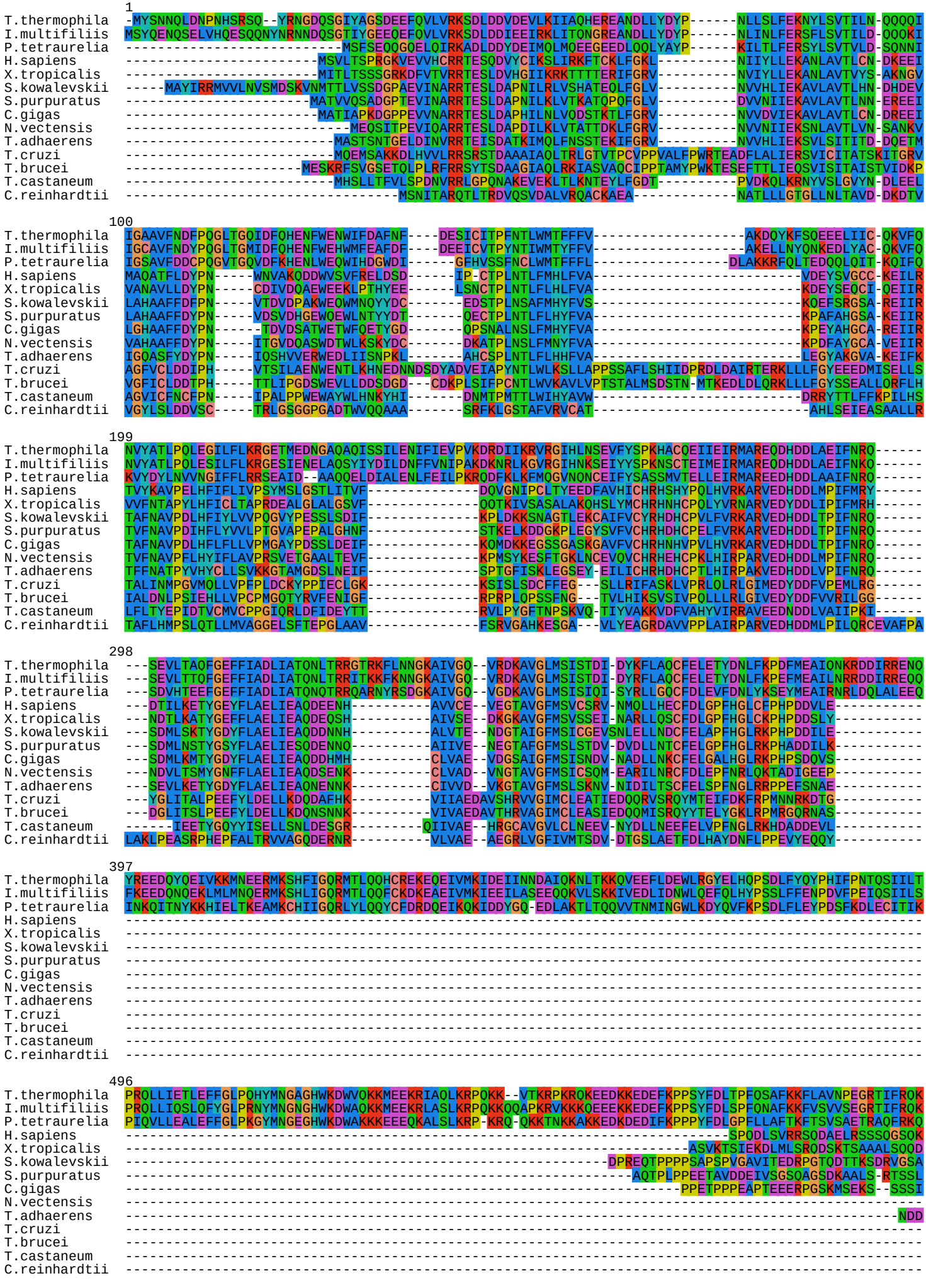
b) This pseudo wild-type strain (pWT) was used as the control for classification analysis in Heuser *et al*. 2012. It is a mutant rescue that is biochemically, structurally, and phenotypically indistinguishable from WT (Rupp and Porter, 2003; Heuser *et al.*, 2009; Nicastro *et al.*, 2011; Barber *et al.*, 2012; Heuser *et al*., 2012).

**TABLE S2:** Primers used to amplify DNA fragments used to prepare expression, overexpression and knockout constructs. Nucleotide sequence of the introduced restriction site is underlined. The ATG or TGA sequences are in bold.

| Name | Primer sequence |
|---|---|
| **Overexpression under *MTT1* promoter in *BTU1* locus** | |
| FAP61-MluI-F | ATAT ACGCGT C**ATG**TACTCCAACAATTAATTAG |
| FAP61-BamHI-R | ATT GGATCC CTTAATT**TCA**ATCTACATTTAC |
| FAP251-MluI-F | ATAT ACGCGT C**ATG**TATTAGTAAGAAGAATACGAAG |
| FAP251-BamHI-R | ATAT GGATCC **TCA**ATCTTAATCATATTCTTCAT |
| FAP91-MluI-F | ATAT ACGCGT T**ATG**GCCACAACAACTAATATTCTTCATG |
| FAP91-BamHI-R | ATAT GGATCC **TCA**ATTTTAAACATTTGCGTGCTT |
| FAP91-BamHI-R (*MTT locus*) | ATAT GGATCC ATTTTAAACATTTGCGTGCTTATTTG |
| **Domain analysis (overexpression under *MTT1* promoter in *BTU1* locus)** | |
| FAP61-F330-MluI-F | ATAT ACGCGT C**ATG**TTTAAGCCTGATTTCATGGAAGC |
| FAP61-D1400-BamHI-R | ATAT GGA TCC **TCA**GTCAATATCAACTAAACCTGAAGTTAC |
| FAP251-G792-BamHI-R | TTAAGGATCC **TCA**TCCATCTTCTCCACCTTC |
| FAP251-W179-MluI-F | ATATACGCGT TTGGGAGTGGAATAAAGATG |
| FAP251-A342-MluI-F | ATATACGCGT AGCTTGGTTTGAAGATGCAATT |
| FAP251-E787-F | GATGAAGATGAAGGTGGAGAAGATGGA |
| FAP251-D370-R | TCCACCTTCATCTTCATCTTCATTGTCTTC |
| **Native locus expression with C-terminal 3xHA tag** | |
| FAP61-coding-ApaI-F | AATT GGGCCC GATAAGAGAATCAACGATCC |
| FAP61-3'UTR-PstI-F | AATT CTGCAG CCAACAAACCAATCTATCAATCAACC |
| FAP61-3'UTR-SacI-R | AATT GAGCTC CTCGAG TGAATGCATATATCCGTACCTGC |
| FAP91-coding-ApaI-F | AATT GGGCCC GGTATTACTCACGAA |
| FAP91-3'UTR-PstI-F | AATT CTGCAG CTATCCTAACTAACTAACTATCTATCTTTCT |
| FAP91-3'UTR-SacI-R | AATT GAGCTC CTCGAG TTAATACCAAGCAAAATCCAAATTTACT |
| FAP251-coding-ApaI-F | AATT GGGCCC GAAACAATACCTACTGC |
| FAP251-3'UTR-PstI-F | AATT CTGCAG TAAGTTGCTTACTTGCTTGCATCT |
| FAP251-3'UTR-SacI-R | AATT GAGCTC CTCGAG GTCTTTTACAACTTCTAAGAAGGTCCTTT |
| 3'UTR BTU1-ClaI-R | AATT ATCGAT GTTTATCAATCTTGAAGTAATAAC |
| **Knockout construct** | |
| FAP61-5'UTR-ApaI-F | TTAT GGGCCC GAAGTTCCTGTGAAAGAC |

| | |
|---|---|
| FAP61-5'UTR-SmaI-R | TAAT CCCGGG GAACTTCTGGAATGCTATC |
| FAP61-3'UTR-PstI-F | AATA CTGCAG ACCTAACTGAAGTCTAAG |
| FAP61-3'UTR-SacII-R | AATA CCGCGG GTAAGATCTTCAGTCTTA |
| FAP251-5'UTR-ApaI-F | AATT GGGCCC GAGGTTGGTCATGCGTATCC |
| FAP251-5'UTR-SmaI-R | AATT CCCGGG CAATTCTTGTCTTACCAGTTGTTGC |
| FAP251-3'UTR-PstI-F | AATT CTGCAG GCATTGATTACTCTCCAGATGGAA |
| FAP251-3'UTR-SacII-R | AATA CCGCGG ACCATCAAATTCTTCATTAGTCATACG |
| **Knockout cells analysis** | |
| FAP251-del-KO-F | GTAACATTCCATCTCTTAAGGTT |
| FAP251-BamHI-R | ATAT GGATCC TCA ATC TTA ATC ATA TTC TTC AT |
| FAP61-4110-F | CTTGCATGTATTTCGAAGTTCAAG |
| FAP61-5650-R | CTGCCTGGAGACTAGTTAAAG |

**Fig. S1 FAP61**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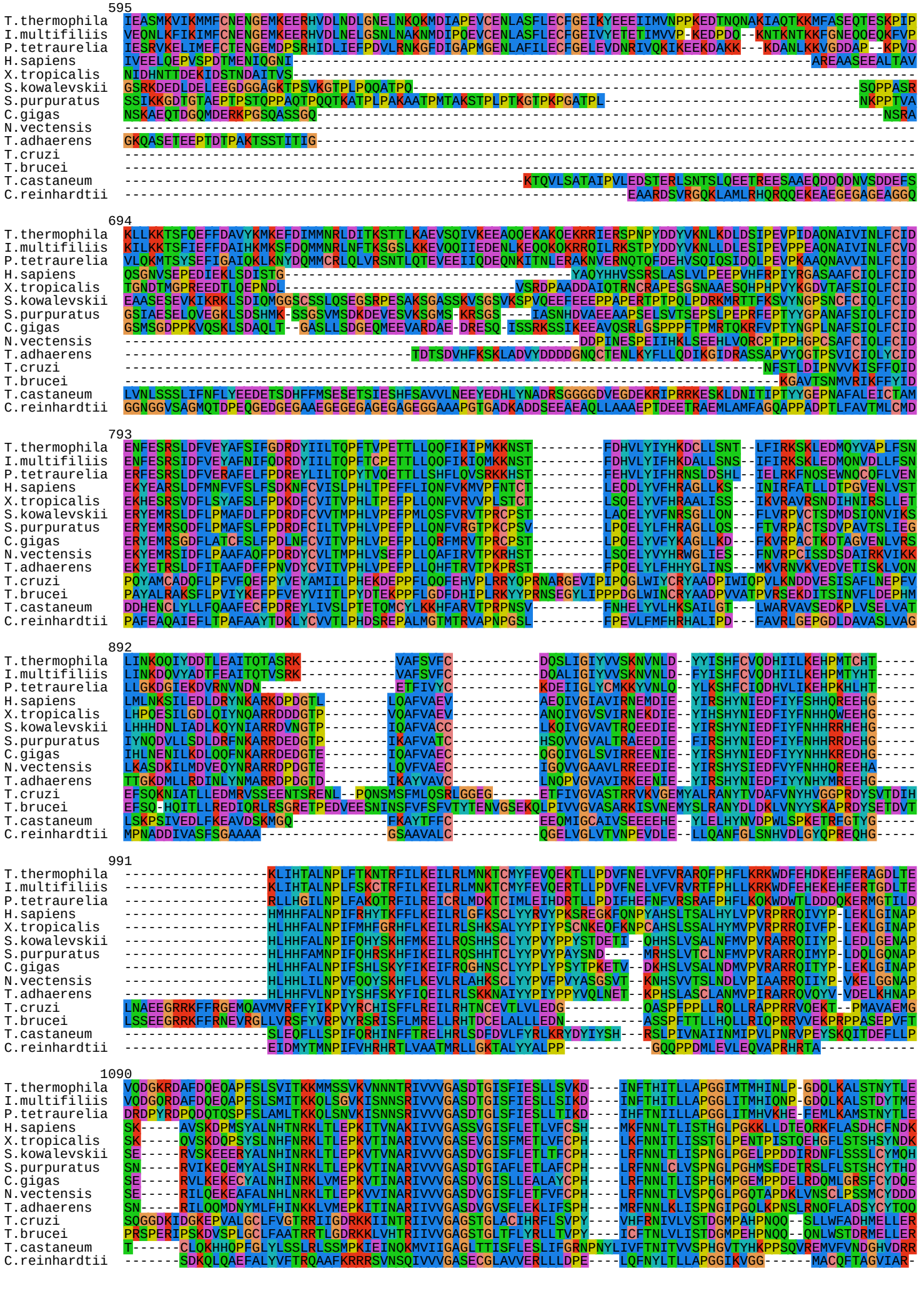
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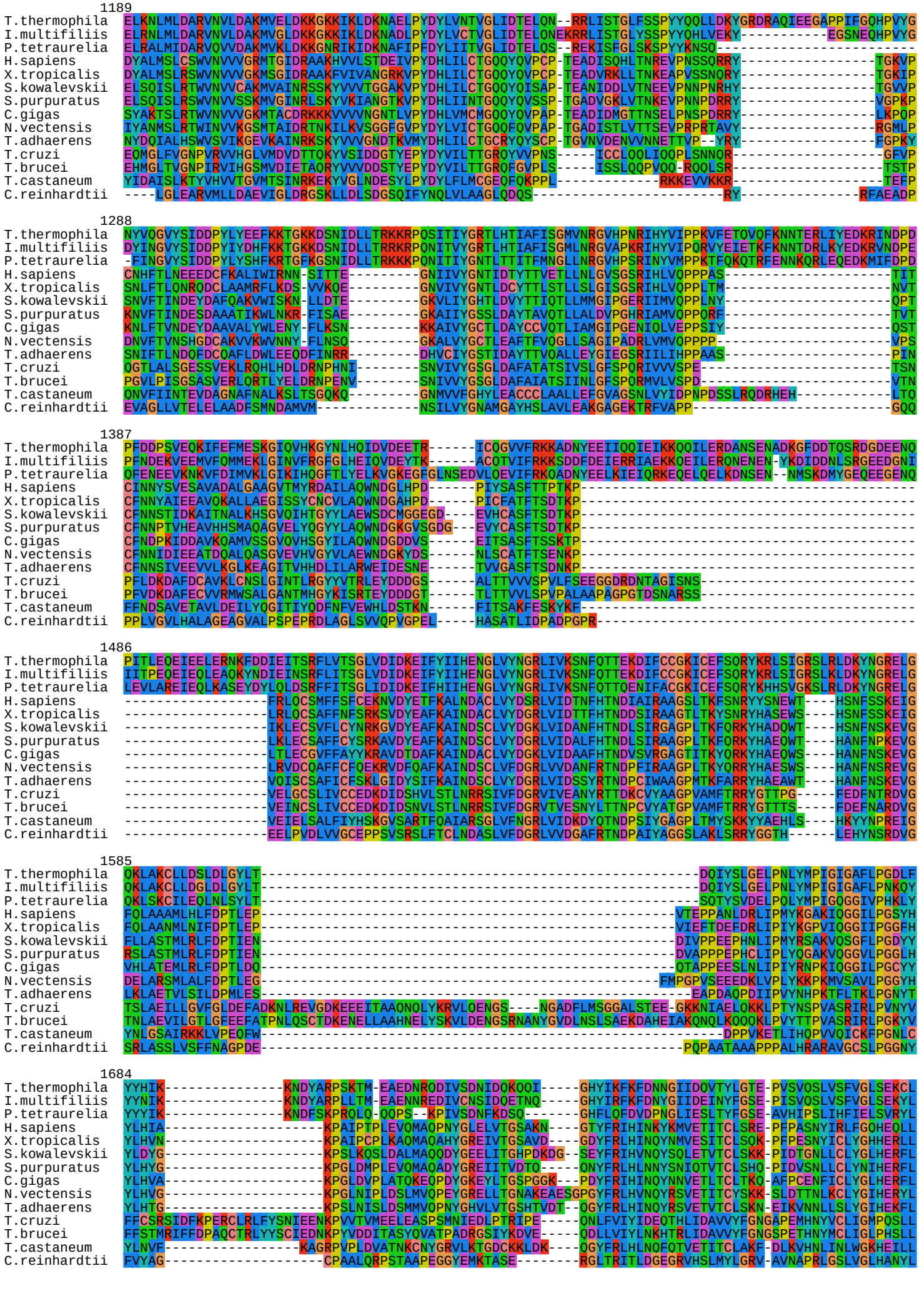
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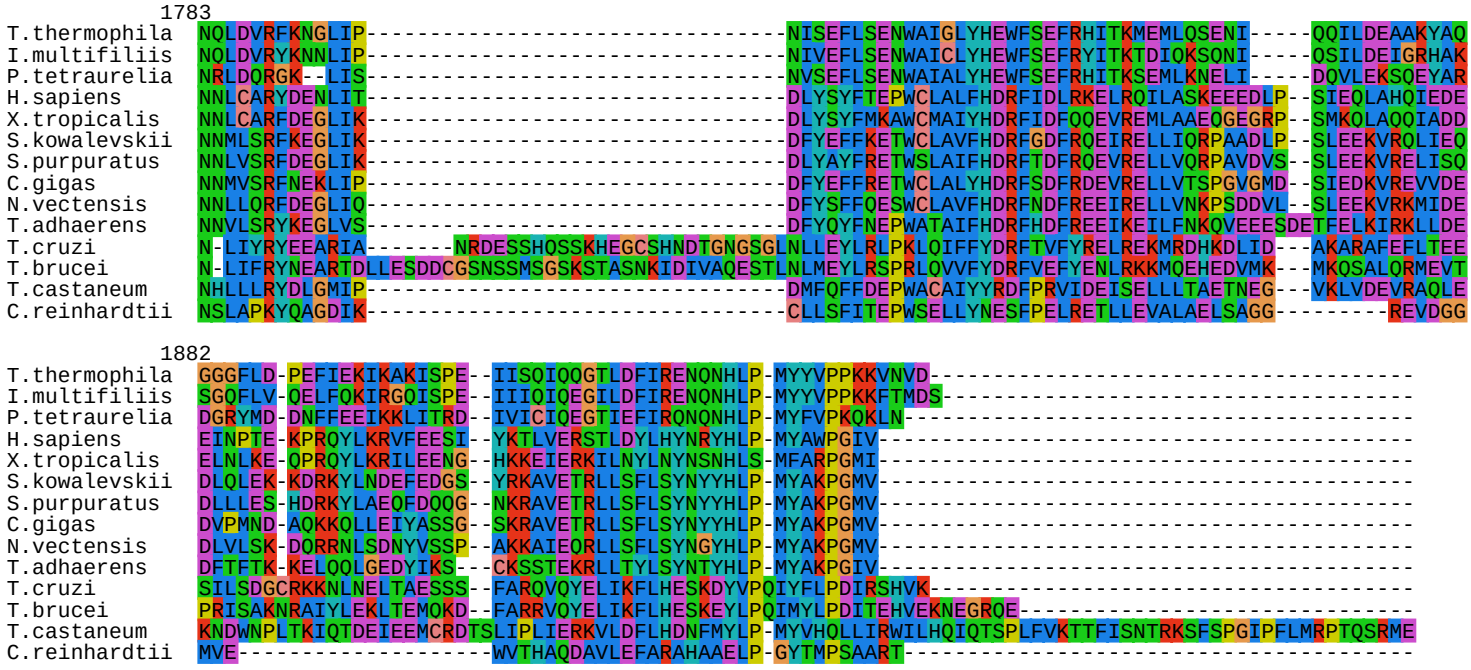
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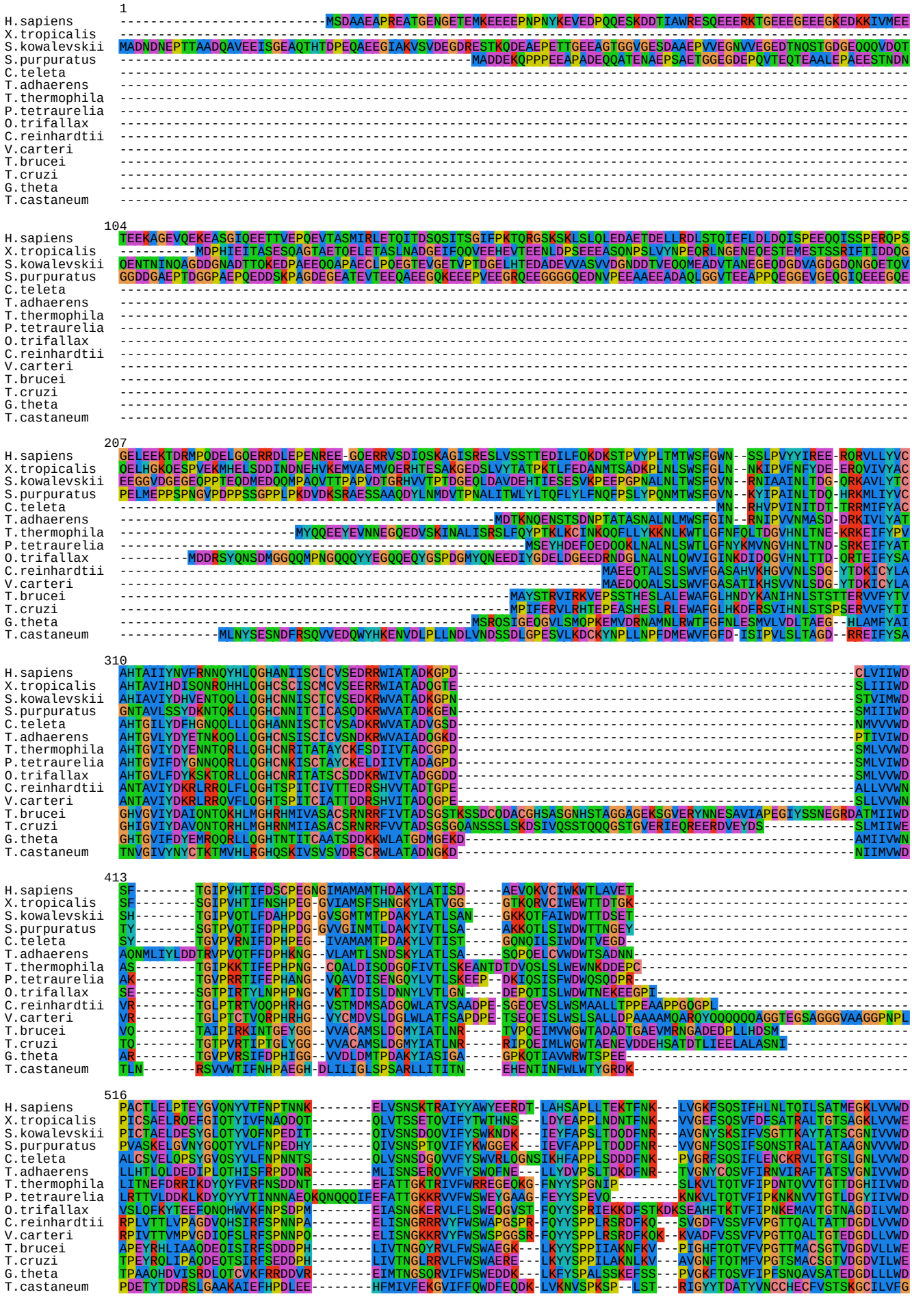
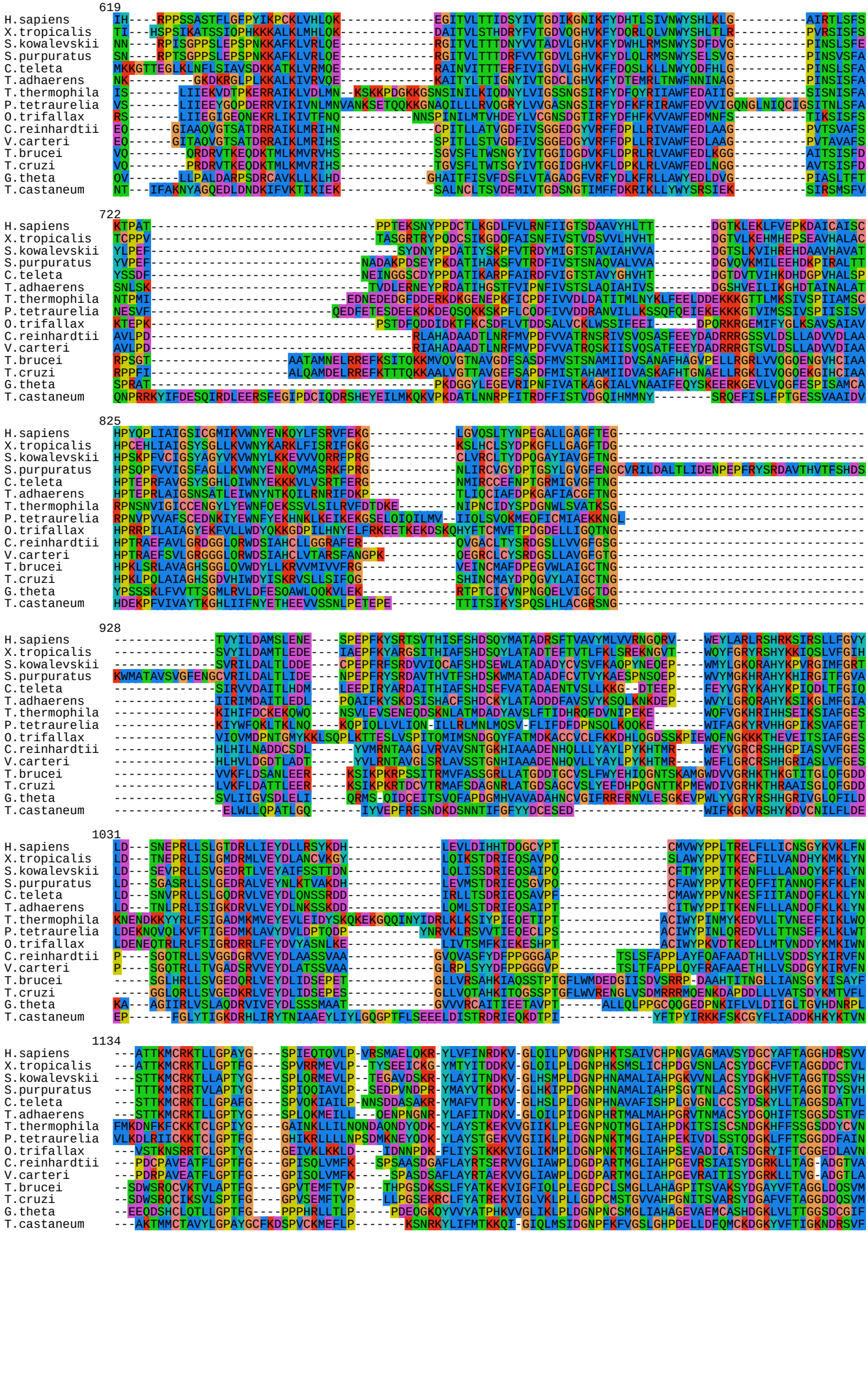
T.cruzi
T.brucei
T.castaneum
C.reinhardtii

**FIGURE S1:** A multiple alignment of FAP61 homolog sequences. *Chlamydomonas reinhardtii* (XP_001703513.1), *Crassostrea gigas* (EKC26516.1), *Homo sapiens* (NP_056400.3), *Ichthyophthirius multifiliis* (EGR32779.1), *Nematostella vectensis* (XP_001629331.1), *Paramecium tetraurelia* (XP_001443527.1), *Saccoglossus kowalevskii* (XP_002731664.1), *Strongylocentrotus purpuratus* (XP_783801.2), *Tetrahymena thermophila* (XP_001015337.1), *Tribolium castaneum* (XP_967262.1), *Trichoplax adhaerens* (XP_002107865.1), *Trypanosoma brucei* (XP_845755.1), *Trypanosoma cruzi* (XP_807700.1), *Xenopus (Silurana) tropicalis* (NP_001072512.1). Some of the predicted protein sequences were manually corrected to include the most probable coding sequence based on the homologies to the predicted ORFs; *Paramecium tetraurelia* FAP61 (XP_001443527.1) was corrected based on the analysis of the data from http://www.genoscope.cns.fr. Corrections in the translation of the predicted coding region (scaffold_95) were between nucleotides of the analyzed scaffold_95: 2251-2421 (reading frame prediction between I687 and Q745, see alignment, 3027-3039 (Y950-P953 aa were missing), 3582-3642 (S1136-T1142 aa were missing due to intron prediction), 4911-5026 (from L1597, intron and stop codon were predicted). Inspection of the mRNA sequence of *Ichthyophthirius multifiliis* (XM_004036717.1) suggested translation of possibly non-processed introns (2598-2641bp, 2837-2880bp, 4756-4803bp) during protein prediction.

**Fig. S2 FAP251**

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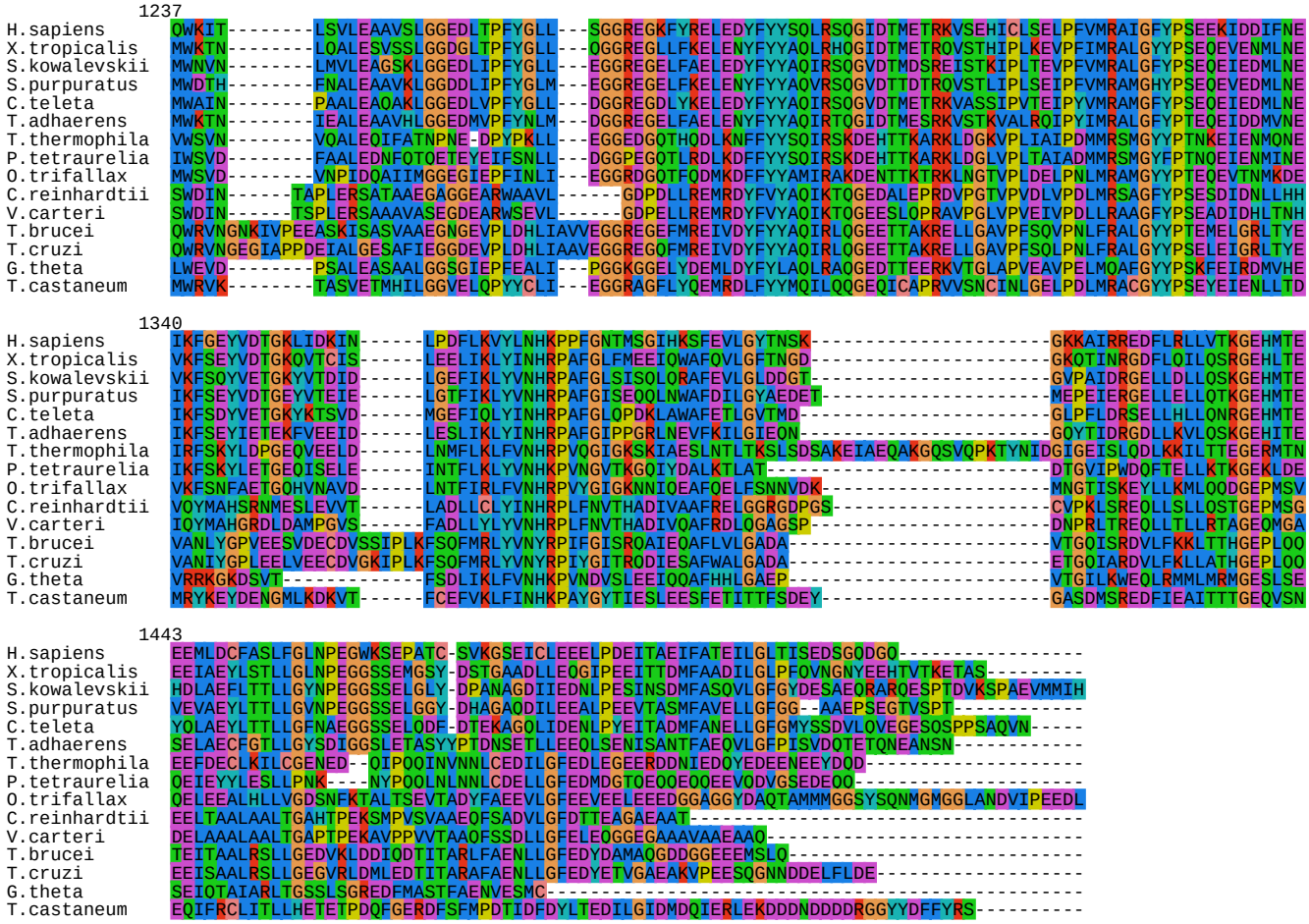
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X.tropicalis MWKTN--------LQALESVSSLGGDGLTPFYGLL---QGGREGLLFKELENYFYYAQLRHQGIDTMETROVSTHIPLKEVPFIMRALGYYPSEQEVENMLNE
S.kowalevskii MWNVN--------LMVLEAGSKLGGEDLIPFYGLL---EGGREGELFAELEDYFYYAQIRSQGVDTMDSREISTKIPLTEVPFVMRALGFYPSEQEIEDMLNE
S.purpuratus MWDTH--------FNALEAAVKLGGDDLIPFYGLM---EGGREGELFKELENYFYYAQVRSQGVDTTDTRQVSTLIPLSEIPFVMRAMGHYPSEQEVEDMLNE
C.teleta MWAIN--------PAALEAQAKLGGEDLVPFYGLL---DGGREGDLYKELEDYFYYAQIRSQGVDTMETRKVASSIPVTEIPYVMRAMGFYPSEQEIEDMLNE
T.adhaerens MWKTN--------IEALEAAVHLGGEDMVPFYNLM---DGGREGELFAELENYFYYAQIRTQGIDTMESRKVSTKVALRQIPYIMRALGFYPTEQEIDDMVNE
T.thermophila VWSVN--------VQALEQIFATNPNE-DPYPKLL---EGGEDGQTHQDLKNFFYYSQIRSKDEHTTKARKLDGKVPLIAIPDMMRSMGYYPTNKEIENMQNE
P.tetraurelia IWSVD--------FAALEDNFQTQETEYEIFSNLL---DGGPEGQTLRDLKDFFYYSQIRSKDEHTTKARKLDGLVPLTAIADMMRSMGYFPTNQEIENMINE
O.trifallax MWSVD--------VNPIDQAIIMGGEGIEPFINLI---EGGRDGQTFQDMKDFFYYAMIRAKDENTTKTRKLNGTVPLDELPNLMRAMGYYPTEQEVTNMKDE
C.reinhardtii SWDIN------TAPLERSATAAEGAGGEARWAAVL------GDPDLLREMRDYFVYAQIKTQGEDALEPRDVPGTVPVDLVPDLMRSAGFYPSESDIDNLLHH
V.carteri SWDIN------TSPLERSAAAVASEGDEARWSEVL------GDPELLREMRDYFVYAQIKTQGEESLQPRAVPGLVPVEIVPDLLRAAGFYPSEADIDHLTNH
T.brucei QWRVNGNKIVPEEASKISASVAAEGNGEVPLDHLIAVVEGGREGEFMREIVDYFYYAQIRLQGEETTAKRELLGAVPFSQVPNLFRALGYYPTEMELGRLTYE
T.cruzi QWRVNGEGIAPPDEIALGESAFIEGGDEVPLDHLIAAVEGGREGQFMREIVDYFYYAQIRLQGEETTAKRELLGAVPFSQLPNLFRALGYYPSELEIGRLTYE
G.theta LWEVD--------PSALEASAALGGSGIEPFEALI---PGGKGGELYDEMLDYFYLAQLRAQGEDTTEERKVTGLAPVEAVPELMQAFGYYPSKFEIRDMVHE
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X.tropicalis VKFSEYVDTGKQVTCIS------LEELIKLYINHRPAFGLFMEEIQWAFQVLGFTNGD----------GKQTINRGDFLQILQSRGEHLTE
S.kowalevskii VKFSQYVETGKYVTDID------LGEFIKLYVNHRPAFGLSISQLQRAFEVLGLDDGT----------GVPAIDRGELLDLLQSKGEHMTE
S.purpuratus IKFSEYVDTGEYVTEIE------LGTFIKLYVNHRPAFGISEQQLNWAFDILGYAEDET----------MEPEIERGELLELLQTKGEHMTE
C.teleta IKFSDYVETGKYKTSVD------MGEFIQLYINHRPAFGLQPDKLAWAFETLGVTMD----------GLPFLDRSELLHLLQNRGEHMTE
T.adhaerens IKFSEYIETEKFVEEID------LESLIKLYINHRPAFGIPPGRLNEVFKILGIEQN----------GQYTIDRGDLLKVLQSKGEHITE
T.thermophila IRFSKYLDPGEQVEELD------LNMFLKLFVNHRPVQGIGKSKIAESLNTLTKSLSDSAKEIAEQAKGQSVQPKTYNIDGIGEISLQDLKKILTTEGERMTN
P.tetraurelia IKFSKYLETGEQISELE------INTFLKLYVNHKPVNGVTKGQIYDALKTLAT----------DTGVIPWDQFTELLKTKGEKLDE
O.trifallax VKFSNFAETGQHVNAVD------LNTFIRLFVNHRPVYGIGKNNIQEAFQELFSNNVDK----------MNGTISKEYLLKMLQQDGEPMSV
C.reinhardtii VQYMAHSRNMESLEVVT------LADLLCLYINHRPLFNVTHADIVAAFRELGGRGDPGS----------CVPKLSREQLLSLLQSTGEPMSG
V.carteri IQYMAHGRDLDAMPGVS------FADLLYLYVNHRPLFNVTHADIVQAFRDLQGAGSP----------DNPRLTREQLLTLLRTAGEQMGA
T.brucei VANLYGPVEESVDECDVSSIPLKFSQFMRLYVNYRPIFGISRQAIEQAFLVLGADA----------VTGQISRDVLFKKLTTHGEPLQQ
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G.theta VRRKGKDSVT----------FSDLIKLFVNHKPVNDVSLEEIQQAFHHLGAEP----------VTGILKWEQLRMMLMRMGESLSE
T.castaneum MRYKEYDENGMLKDKVT------FCEFVKLFINHKPAYGYTIESLEESFETITTFSDEY----------GASDMSREDFIEAITTTGEQVSN
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H.sapiens EEMLDCFASLFGLNPEGWKSEPATC-SVKGSEICLEEELPDEITAEIFATEILGLTISEDSGQDGQ----------
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C.teleta YQLAEYLTTLLGFNAEGGSSELQDF-DTEKAGQLIDENLPYEITADMFANELLGFGMYSSDVLQVEGESQSPPSAQVN-----
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**FIGURE S2:** A multiple alignment of FAP251 homolog sequences. *Capitella teleta* (ELU04166.1), *Chlamydomonas reinhardtii* (XP_001691834.1), *Guillardia theta* (EKX39032.1), *Homo sapiens* (NP_653269.3), *Oxytricha trifallax* (EJY70697.1), *Paramecium tetraurelia* (XP_001457947.1), *Saccoglossus kowalevskii* (XP_002741792.1), *Strongylocentrotus purpuratus* (XP_785437.2), *Tetrahymena thermophila* (XP_001026044.1), *Tribolium castaneum* (XP_967922.1), *Trichoplax adhaerens* (XP_002113637.1), *Trypanosoma brucei* (CBH09811.1), *Trypanosoma cruzi* (XP_816807.1), *Volvox carteri* (XP_002946746.1), *Xenopus (Silurana) tropicalis* (XP_002937749.2). Two of the predicted protein sequences were manually corrected to include the most probable coding sequence based on the homologies to the predicted ORFs; *Chlamydomonas reinhardtii* FAP251 (XP_001691834.1) was corrected based on the analysis of the data from http://genome.jgi-psf.org/Chlre4/Chlre4.home.html. Corrections in the translation of the predicted coding region (chromosome3:4483344-4484109) resulted in identification of highly conserved fragment between D594 and A688, see alignment. *Trichoplax adhaerens* FAP251 (XP_002113637.1) was corrected based on the analysis of the strain Grell-BS-1999 TRIADscaffold_6, whole genome shotgun sequence (http://genome.jgi-psf.org). Corrections in the translation of the predicted coding region (scaffold 6:1632061-1632488) resulted in identification of highly conserved region between W307 – T328, see alignment).

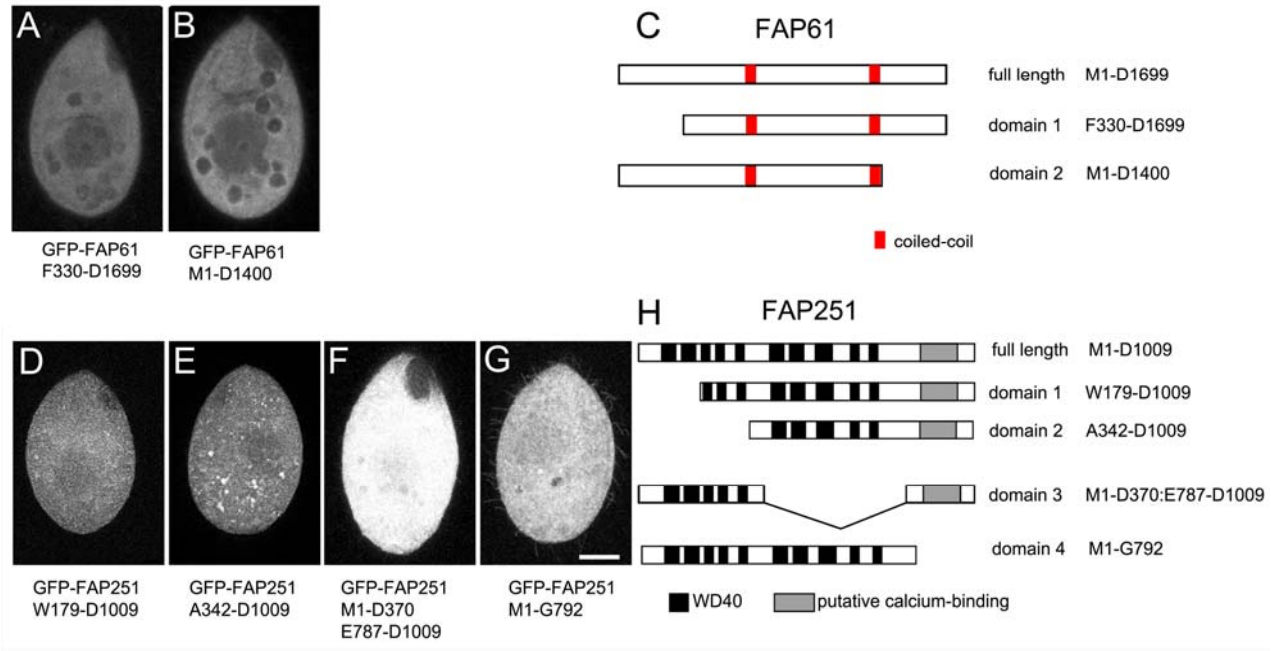


**FIGURE S3:** Domain analysis of FAP61 and FAP251. (A and B) Localization of overexpressed GFP-tagged truncations of FAP61; FAP61 that lacks either N- (A) or C-terminal (B) fragment accumulates in the cell body and is not targeted to cilia. (C) Schematic representation of the domains of *Tetrahymena* FAP61 and its truncated variants. Red rectangle represents coiled-coil region as predicted by the SMART program. (D-G) Localization of GFP-tagged truncations of FAP251. FAP251 truncations that lack WD40 domain(s) are not targeted to cilia (D-F) whereas the deletion of a C-terminal fragment that contains the putative calcium binding region, does not affect ciliary localization (G). (H) Schematic representation of the domains of *Tetrahymena* FAP251 and its truncated variants. Black rectangle represents WD-40 motif and grey rectangle marks position of the putative calcium-binding domain as predicted by SMART and WDSP programs. Bar, 10 μm.

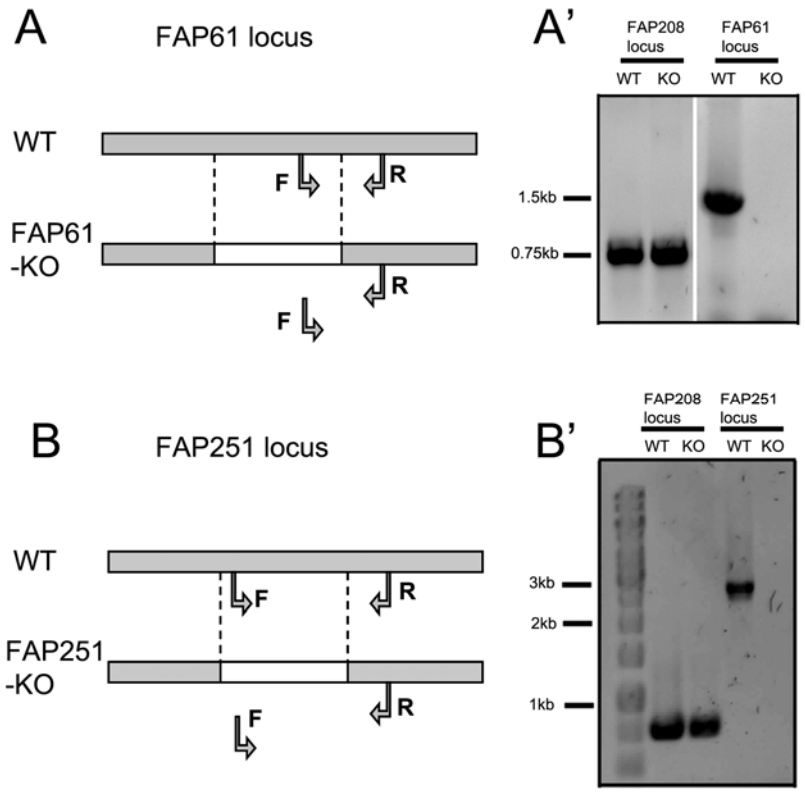


**FIGURE S4:** PCR analysis of the FAP61 and FAP251 loci in wild type, FAP61-KO and FA251-KO cells. (A and A') Analysis of the germ line FAP61-KO cells. (A) Scheme of FAP61 locus in wild type and knockout cells. (A') PCR analysis of FAP61 locus with primers indicated in (A) showing that part of FAP61 coding region is removed in FAP61-KO cells. (B and B') Analysis of germ line FAP251-KO cells. (B) Scheme of FAP251 locus in wild type and knockout cells. (B') PCR analysis of FAP251 locus with primers indicated in (B) showing that part of FAP251 coding region is removed in FAP251-KO cells. Amplification from FAP208 locus was used as a control of the quality of the obtained genomic DNA.

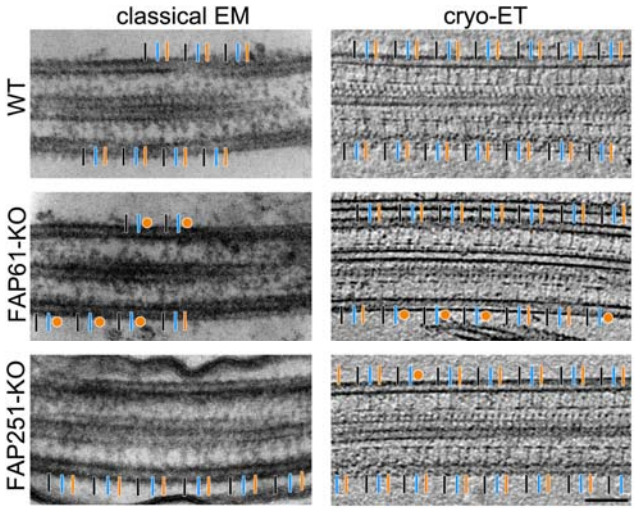


**FIGURE S5:** RS3 is lost in a subset of FAP61-KO repeats based on both classical electron microscopy (EM) and cryo-electron tomography (cryo-ET). Longitudinal sections of chemically fixed cilia (left column) and longitudinal tomographic slices of rapidly frozen axonemes (right column) from wild type and the two CSC-knockouts, FAP61-KO and FAP251-KO show the presence (orange bars) and absence (orange dots) of RS3. Radial spoke triplets have a highly conserved spacing (between RS1-RS2: 32nm, RS2-RS3: 24nm, RS3-RS1: 40 nm), allowing RS1 (black bars), RS2 (blue bars) and RS3 (orange bars) to be easily identified. Both classical EM data and cryo-ET show that RS3 is missing from many axonemal repeats from FAP61-KO, but rarely from FAP251-KO repeats. Bar, 100 nm.

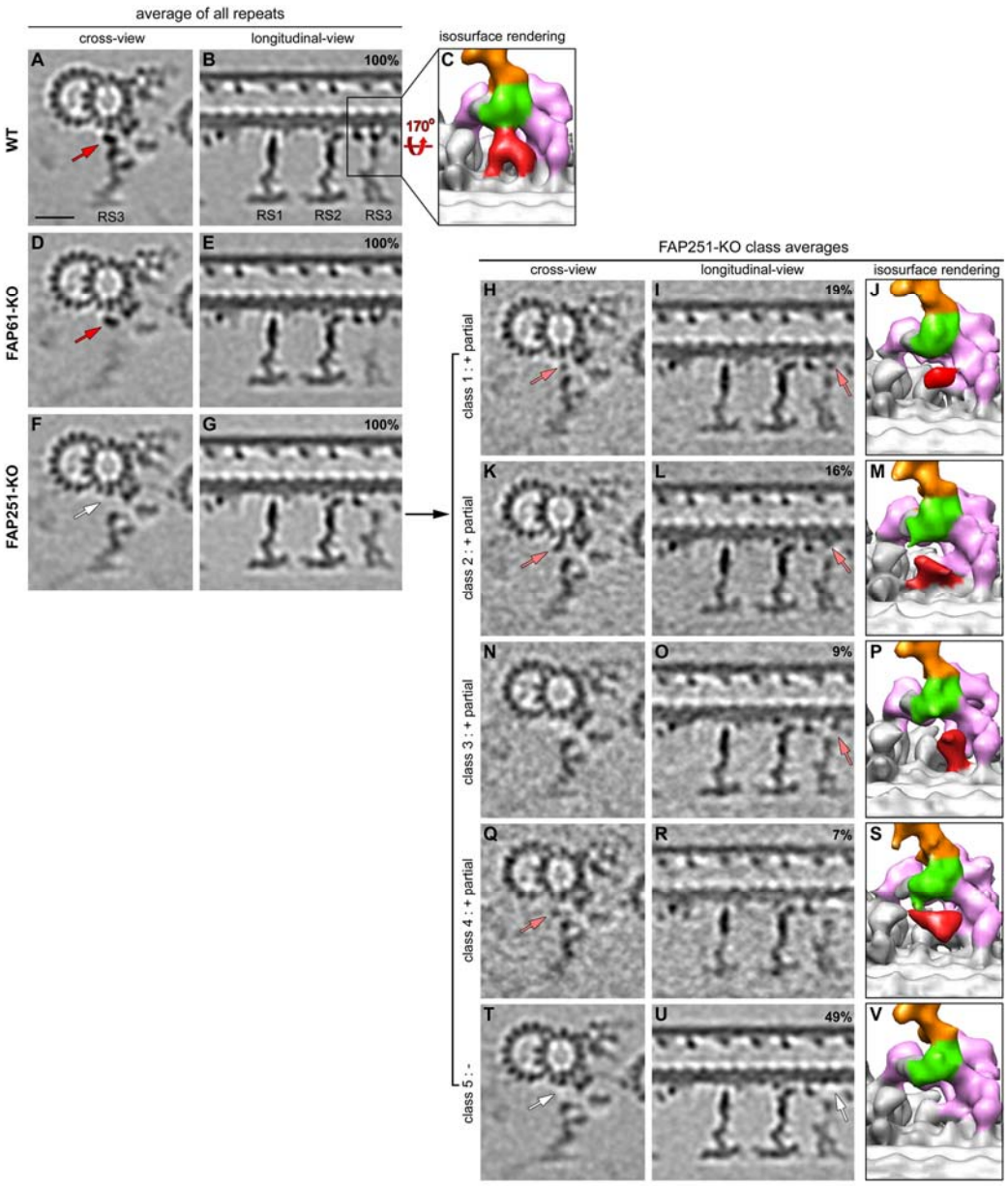


**FIGURE S6:** Classification analysis of the arch-like structure at the RS3 base. Cross-sectional (A, D, F, H, K, N, Q and T), longitudinal tomographic slices (B, E, G, I, L, O, R and U), and isosurface renderings (C, J, M, P, S and V) of the averaged 96-nm repeats show the defects of the arch-like structure at the RS3 base in FAP251-KO (F-V). Subtomogram averages of all 96-nm repeats from WT (A-C), FAP61-KO (D and E) and FAP251-KO (F and G) showed that the arch-like structure at the RS3 base is missing only from the FAP251-KO axonemes (F and G). A classification analysis focused on the RS3 base region in the 96-nm repeats from FAP251-KO, revealed that the arch is missing from only half of all repeats (49%, T-V; white arrows), whereas small parts of the arch-

like structure remained visible in a total of 51% of FAP251-KO repeats; these small remaining densities are found in different positions (light red arrows or red structures in classes 1-4; H-S). Bar, 20 nm.

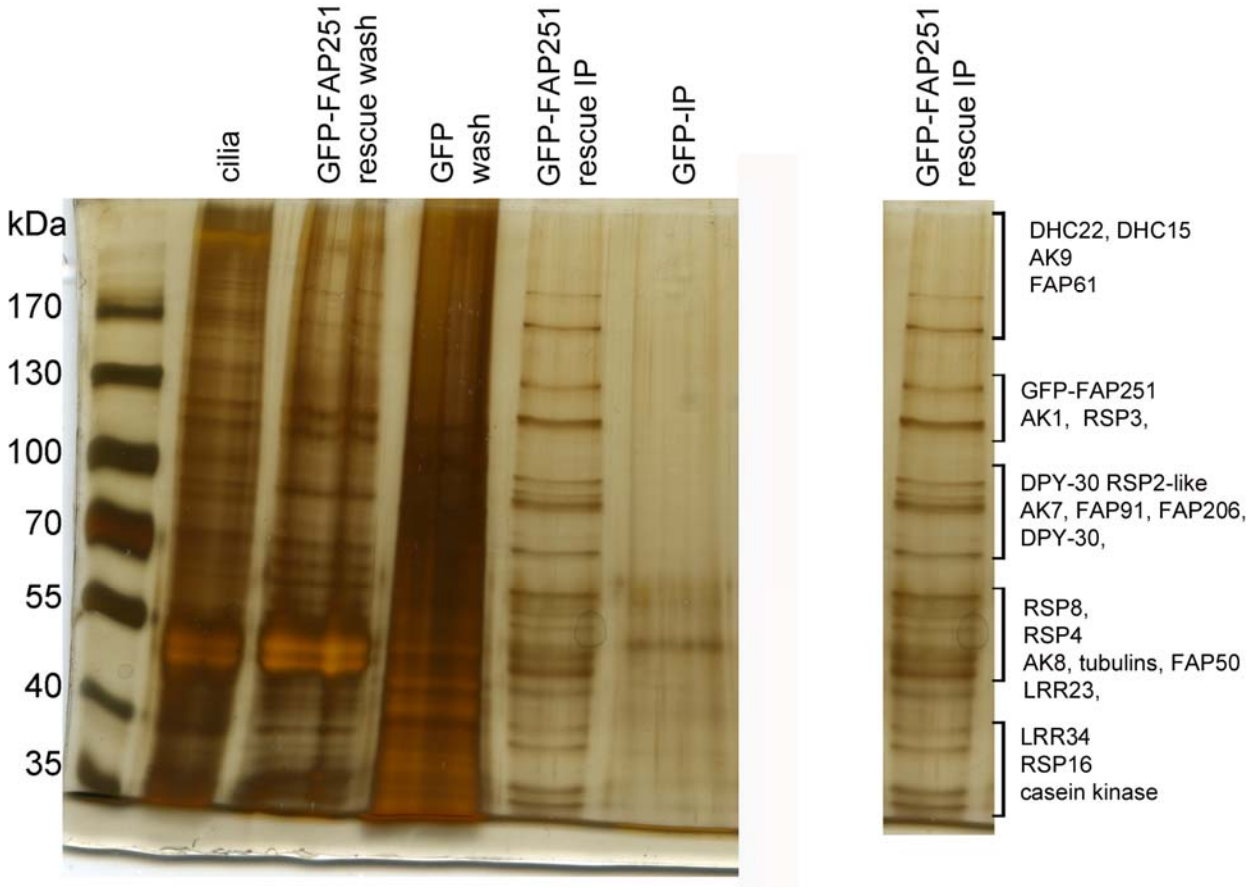


**Figure S7:** GFP-FAP251p co-immunoprecipitates with numerous proteins**.** Silver-stained gel showing cilia isolated from FAP251-KO cells rescued with GFP-FAP251 (line 1), proteins that were washed out from the resin (unbound proteins) in the experimental (FAP251-KO cells rescued with GFP-FAP251) and control (wild type expressing GFP) samples (lines 2 and 3, respectively) and proteins that co-immunoprecipitated with GFP-FAP251 or GFP using GFP-Trap resin in the experimental and control samples (lines 4 and 5, respectively). The precipitates were resolved on a 8% acrylamide gel. Indicated are proteins that were identified by ten or more peptides.

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