

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

	<i>Chlamydomonas</i> ^{a)}			<i>Tetrahymena</i>		
	<i>pWT</i> ^{b)}	<i>amiRNAi FAP61-KD</i>	<i>amiRNAi FAP91-KD</i>	<i>WT</i>	<i>FAP61-KO</i>	<i>FAP251-KO</i>
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.

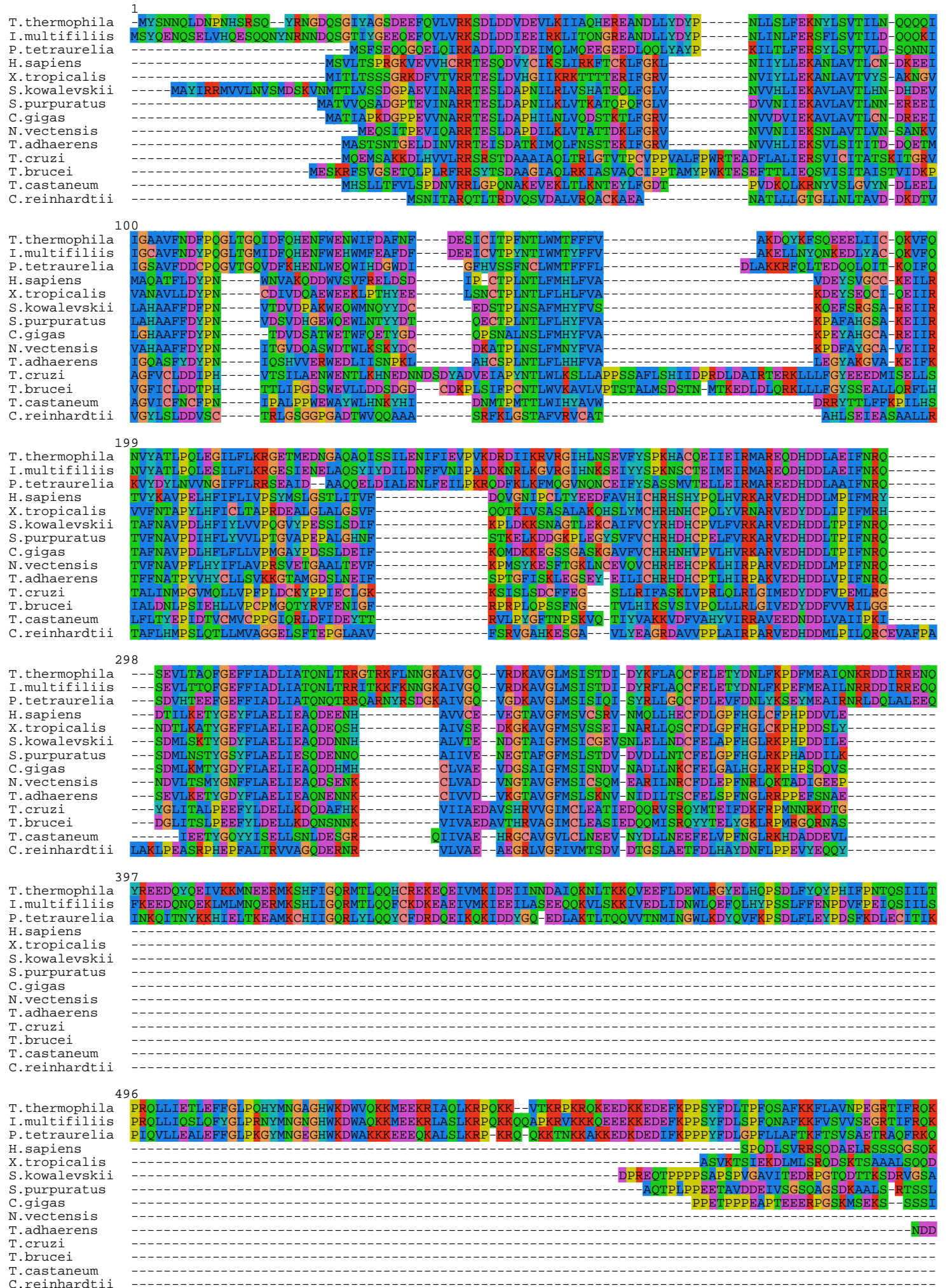
b) This pseudo wild-type strain (pWT) was used as the control for classification analysis in Heuser *et al.* 2012. It is a mutant rescue that is biochemically, structurally, and phenotypically indistinguishable from WT (Rupp and Porter, 2003; Heuser *et al.*, 2009; Nicastro *et al.*, 2011; Barber *et 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 <i>MTT1</i> promoter in <i>BTU1</i> locus	
FAP61-MluI-F	ATAT <u>ACGCGT</u> CATG TACTCCAACAATTAATTAG
FAP61-BamHI-R	ATT <u>GGATCC</u> CTTAATTT CAAT CTACATTTAC
FAP251-MluI-F	ATAT <u>ACGCGT</u> CATG TATTAGTAAGAAGAATACGAAG
FAP251-BamHI-R	ATAT <u>GGATCC</u> TCAAT CTTAATCATATTCTTCAT
FAP91-MluI-F	ATAT <u>ACGCGT</u> TATG GCCACAACAATAATTTCTTCATG
FAP91-BamHI-R	ATAT <u>GGATCC</u> TCAAT TTTAAACATTTGCGTGCTT
FAP91-BamHI-R (<i>MTT</i> locus)	ATAT <u>GGATCC</u> ATTTTAAACATTTGCGTGCTTATTTG
Domain analysis (overexpression under <i>MTT1</i> promoter in <i>BTU1</i> locus)	
FAP61-F330-MluI-F	ATAT <u>ACGCGT</u> CATG TTTAAGCCTGATTTTCATGGAAGC
FAP61-D1400-BamHI-R	ATAT <u>GGA TCC</u> TCA GTC AATATCAACTAAACCTGAAGTTAC
FAP251-G792-BamHI-R	TTAAG <u>GGATCC</u> TCAT CCATCTTCTCCACCTTC
FAP251-W179-MluI-F	ATAT <u>ACGCGT</u> TTGGGAGTGGAATAAAGATG
FAP251-A342-MluI-F	ATAT <u>ACGCGT</u> AGCTTGTTTGAAGATGCAATT
FAP251-E787-F	GATGAAGATGAAGGTGGAGAAGATGGA
FAP251-D370-R	TCCACCTTCATCTTCATCTTCATTGTCTTC
Native locus expression with C-terminal 3xHA tag	
FAP61-coding-ApaI-F	AATT <u>GGGCCC</u> GATAAGAGAATCAACGATCC
FAP61-3'UTR-PstI-F	AATT <u>CTGCAG</u> CCAACAAACCAATCTATCAATCAACC
FAP61-3'UTR-SacI-R	AATT <u>GAGCTC</u> <u>CTCGAG</u> TGAATGCATATATCCGTACCTGC
FAP91-coding-ApaI-F	AATT <u>GGGCCC</u> GGTATTACTCACGAA
FAP91-3'UTR-PstI-F	AATT <u>CTGCAG</u> CTATCCTAACTAACTAATCTATCTTTCT
FAP91-3'UTR-SacI-R	AATT <u>GAGCTC</u> <u>CTCGAG</u> TTAATACCAAGCAAAATCCAAATTTACT
FAP251-coding-ApaI-F	AATT <u>GGGCCC</u> GAAACAATACCTACTGC
FAP251-3'UTR-PstI-F	AATT <u>CTGCAG</u> TAAGTTGCTTACTTGCTTGTCATCT
FAP251-3'UTR-SacI-R	AATT <u>GAGCTC</u> <u>CTCGAG</u> GTCCTTTACAACCTTCTAAGAAGGTCCTTT
3'UTR <i>BTU1</i> -ClaI-R	AATT <u>ATCGAT</u> GTTTATCAATCTTGAAGTAATAAC
Knockout construct	
FAP61-5'UTR-ApaI-F	TTAT <u>GGGCCC</u> GAAGTTCCTGTGAAAGAC

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

Fig. S1 FAP61



595

T.thermophila IEASMKVVKMMFCNENEMKKEERHVDLNDLGNELNKKQMDIAPEVCENLASFLECFGEIKYEEIIMVNPPKEDTNONAKIAOTKKMFASEQTESKPIIP
I.multifiliis VEONLKFVKIMFCNENEMKKEERHVDLNDLGNELNNAKNDIPEVCENLASFLECFGEIVYETETIMVVP-KEDDPO--KNTKNTKKFGNEOQEQKFWP
P.tetraurelia IESRVKELIMEFCTENGENMDPSRHIDLEFPDVLNRKGFIDGAPMGENLAFILECFGELEVDRIVOKIKEKDAKK--K DANLKKVGDADP--KPV D
H.sapiens IVEELQEPVSPDPTMNTICGNI
X.tropicalis NIDHNTTDEKIDSTNDAITVS
S.kowalevskii GSRKDEDLDELEGGGAGKTPSVKGTPLPOOATPO
S.purpuratus SSIKKGDTGTAEPTPSTOPPACTPQOTKATPLPAKAATPMTAKSTPLPTKGTPKPGATPL
C.gigas NSKAEQTDGQMDERKPGSOASSGO
N.vectensis
T.adhaerens GKQASETEEPTDTPAKTSSITITIG
T.cruzi
T.brucei
T.castaneum KTOVLSATAIPVLEDSTERLSNTSLOEETREESAAEQDDODNVSDFEFS
C.reinhardtii EAARDSVVRGOKLMLRHRQOEKFAEGEGAGGAGG

694

T.thermophila KLLKKTSTFOEFFDAVYKMKFEFIMNRLDITKSTTLKAEVSOIVKBEAQQEKAKOEKRRRIERSPNPYDDYVKNLKDLDSEIPEVPIAONAIVINLFCID
I.multifiliis KILKKTSTFIEFFDAIHKKMSFDOMNRLNFTKSGSLKKEVQOIIEDENLKEQOKRROILRKSTPYDDYVKNLLEDSEIPEVPPAONAIVINLFCVD
P.tetraurelia VLQKMTSYSEFIFGAIQKLNKYDQMKRLQLVRSNTLQTEVEEIIQDEQNKITNLERAKNVERNOTQDEHVSOIOSIDOLPEVPKAAQNAVINLFCID
H.sapiens QSGNVSEPEDEIEKLSDISTG
X.tropicalis TGNDTIMGPREEDTLOEPNDL
S.kowalevskii EAAESESEVKIKRKLSDIQMGSSCSLOSSEGRPEAKSGASSKVSQVSPVVOEEFEPEPPAPERPTPTPOLDRKMRITTFKSVYNGPNSCFICLQFCID
S.purpuratus GSIAESELQVEGKLSDSHMK--SSGSVMSDKDEVEVSKSGMS--KRSGS--IASNHDAEEAAPSLSVTSSEPSLEPEPRFEPTYGPNANAFSICLQFCID
C.gigas GMSMGDPPKQSKLSDAQLT--GASLLSDGQEMEEVARDAE--DRESQ--ISSRKSSIKKEAVOSRLGSPPPPTPMRTOKRFVPTYNGPLNAFSICLQFCID
N.vectensis
T.adhaerens
T.cruzi
T.brucei
T.castaneum
C.reinhardtii

793

T.thermophila ENFESRSLDFVEYAFSIFGDRDYIILTOPFTVPEITLLOOFIKIPMKKNST
I.multifiliis ENFESRSLDFVEYAFNIFODRDYIILTOPFTCEITLLOOFIKIOMKKNST
P.tetraurelia EFESRSLDFVEYAFELFPDREYILITQPTVYIOTETLSSHLOVSRKKHST
H.sapiens EKYEARSDFMNFVPSLFDKNCFCVISLPHLTPEFFLIONFVKMVPFNTCT
X.tropicalis EKHESSRSVDFLSYAFSLFPDKFCVITVPHLTPPEFLLONFVRVPLSTCT
S.kowalevskii ERYEMRSLDFLPMADFDPDRDFCVVMTPHLVPEFPLLOQSFVRVTPRCPS
S.purpuratus ERYEMRSODFLPMAFSLFPDRDFCILTVPHLVPEFPLLOFVRGTPKCPSV
C.gigas ERYEMRSGDFLATCFSLFPDLNFCVITVPHLVPEFPLLOFMRVTPRCPS
N.vectensis EKYEEMRSLDFLPMAFDFPDRDYCVITVPHLVPEFPLLOHFTRVTPKRST
T.adhaerens EKYEEMRSLDFLPMAFDFPDRDYCVITVPHLVPEFPLLOHFTRVTPKRST
T.cruzi POYAMCADOPLPVFQEFYVEYAMILPHEKDEPPFLOFHEVPLRRYOPRNARGEVITPQGLWICYRYAADPIWIOPVLKNDVDESISAFNLPEPVP
T.brucei PAYALRAKSEPLVYIKFFPVEYVILITLPVDEKPPDFGDHDIPLRKYYPNSSEGLIPDPDGLWINCRYAADPVVATPVRSEKDTITSINFLDEPHM
T.castaneum DDHENCLYLLFOAAFECFPDRDYLVSLPTETOMCYLKKHFAVTRPNSV
C.reinhardtii PAFEAQAEIETLPAFAAYTKLYCVVTLPHDSREPALMGTRVAPNPGSL

892

T.thermophila LINKOOIYDDTLEAITOTASRK
I.multifiliis LINKDOYADTFEAITOTVSRK
P.tetraurelia LLGKDGIEKDVRRVNDN
H.sapiens LMLNKSILEDLDRYNKARKDPDGLT
X.tropicalis LHPQESILGDLQIYNQARRDDGTP
S.kowalevskii LHHHDNLIADLKOYNIARRDVNGTP
S.purpuratus IYNOVDLLSDLDRFNKARDEDGTP
C.gigas IHLNENILKDLQFNKARDEDGTE
N.vectensis LKASDKILMDVEQYNRARRDPDGT
T.adhaerens ITGKDMLLRLINLYNMARRDPDGT
T.cruzi EFSOENIATLLEDMRVSSEENTSRLEN--PONSMSFMLSRLGGEG
T.brucei EFSO--HOITLLREDIQRLRSGRETPEDVEESNINSFVFSFVITYTENVGSEKOLPIVVGVASARKISVNEMYSLRANVDLKLNVNYSKAPRDYSETDVT
T.castaneum LSKPSIVEDLFKEAVDSKMGQ
C.reinhardtii MPNADDIVAFSFGAAAA

991

T.thermophila
I.multifiliis
P.tetraurelia
H.sapiens
X.tropicalis
S.kowalevskii
S.purpuratus
C.gigas
N.vectensis
T.adhaerens
T.cruzi
T.brucei
T.castaneum
C.reinhardtii

1090

T.thermophila VODGKRDAFDQEOAPFSLSVITKKMMSSVKVNNNTRIVVGASDTGISFIESLLSVKD
I.multifiliis VODGKRDAFDQEOAPFSLSMITKKOLSGVKISNNSRIVVGASDTGISFIESLLSFKD
P.tetraurelia DRDPYRDPDOTOSPLSAMLTKKLSNLSNNSRIVVGASDTGLSFIESLLTKD
H.sapiens SK--AVSKDPMYSALNHTNRKLTLEPKITVNAKIIVVGASSVGISFLETLVFCSH
X.tropicalis SK--OVSKDOPSYSLNHFNRKLTLEPKVTINARIVVGASDVGISFMETLVFCSH
S.kowalevskii SE--RVSKDEERYALNHINRKLTLPEKVTINARIVVGASDVGISFLETLVFCSH
S.purpuratus SN--RVLKEOEMYALSHINRKLTLPEKVTINARIVVGASDTGLAFLETLVFCSH
C.gigas SE--RVLKEKECEYALNHINRKLTLPEKVTINARIVVGASDVGISFLETLVFCSH
N.vectensis SE--RILQEKAEFALNHINRKLTLPEKVTINARIVVGASDVGISFLETLVFCSH
T.adhaerens SN--RILLOOMDNYMLFHNKLVMEPKVTINARIVVGASDVGVSLFLETLVFCSH
T.cruzi SOGGDKIDGKEPVALGCLFVGTTRIIIGDRKKIINTRIIVVGASDTGLACIHRFLSVPY
T.brucei PRSPERIPSKDVSPLGCLFAATRIRIVVGASDTGLFVYRLLTVPY
T.castaneum T--CLQKHHPFGLYLSSLRLSSMPKIEINOKMVIIGAGLTTISFLESIFGRNPNYLIVFTNITVSPHGVYHKPPSOVREMFVNDGHVDRR
C.reinhardtii

1189

T.thermophila ELKNLMLDARVNVLDAKMVELDKKGGKIKLDKNAELPYDYLNTVGLIDTELO...
I.multifiliis ELRNLMLDARVNVLDAKMVLGDKKGGKIKLDKNADLPYDYLNTVGLIDTELO...
P.tetraurelia ELRALMIDARVOVDAKVMKLDKKNRRIKIDKNAFIPFDYLIITVGLIDTELOS...
H.sapiens DYALMSLCSWVNVVGRMTGIDRAAKHVVLSTDEIVPYDHLILCTGOOYQVPCP...
X.tropicalis DYALMSLRSWVNVVYKMSGIDRAAKFVIVANGRKVPYDHLILCTGOOYQVPCP...
S.kowalevskii ELSQISLRTWVNVVCAKMAVAINRSSKYVVVTGGAKVPYDHLILCTGOOYQISAP...
S.purpuratus ELSQISLRSWVNVVSSKMVINRLSKYVKIANGTKVPYDHLIINTGOOYQVSSP...
C.gigas SYAKTSLRRTWVNVVYKMTACDRKKKVVVVNGTLVYDHLVMCGOOYQVPPAP...
N.vectensis IYANMSLRTWVNVVKSMTAIDRINKLKVSGGGFVVPYDYLVICTGOOYQVPPAP...
T.adhaerens NYDQIALHSWVSVIKGEVKAINRKSQYVVVGNDTKVMYDHLILCTGCRYOYSCP...
T.cruzi EOMGLFVGNPVRVHGLVMDVDTTKQYVSDIDGTEPYDYLITGROYVVPNS...
T.brucei EHMGLTVGNPVRVHIGSMVDIETAORYVVVDDSTYEPYDYLITGROYVPLS...
T.castaneum YIDATSLKTYVHVVTGVMVTSINRKEKYVGLNDESILPYDYLFLMCGEYQKPP...
C.reinhardtii ----LGLEARVMLLDAEIVGLDRGSKLLDLSGDSQIFYNQLVLAAGLQDOS...
-----RKKEVVKKR-----RVAEADP

1288

T.thermophila NYVQGVYSIDDPYLYEYFKKTKKDSNIDLLTRKKRPOSITIYGRTRLHTIAFISGMVNRGVHPNRIHYVIPPKVFE...
I.multifiliis DYINGVYSIDDPYIYDFHFKTKKDSNIDLLTRKKRONITIVYGRTRLHTIAFISGMLNRGVAPKRIHYVIPORVYE...
P.tetraurelia -FINGVYSIDDPYIYSHFKRTGFGKSNIDLLTRKKRONITIVYGNITLITITFMNGLLRGVHPNRIYVMPKTFQ...
H.sapiens CNHFTLNEEEDCFKALWIWRNN-SITTEGNIIVYGNITIDTYITVETLLNLGVSRSRIHLVOPPPAS...
X.tropicalis SNLFTLONRQDCLAAMRFLKDS-VVKQEGNVIVYGNITLDCYITLSTLLSLGISRSRIHLVOPPLTM...
S.kowalevskii SNVFTINDEYDAFAQAKVWISKN-LLDTEGKVLIVYGNITLDCYITLSTLLSLGISRSRIHLVOPPLNY...
S.purpuratus KNVFTINDEYDAFAATIKWLNKR-FISAEKAIIVYGNITLDCYITLSTLLSLGISRSRIHLVOPPPORF...
C.gigas KNLFTVNDYDAVALYWLENY-FLKSNKKAIVYGNITLDCYITLSTLLSLGISRSRIHLVOPPPSIY...
N.vectensis DNVFTINSHGDCAKVVKWNNY-FINSGKALVYGNITLDCYITLSTLLSLGISRSRIHLVOPPPP...
T.adhaerens SNIFTVNDQDFCAFLDWLEQDFINRRDHYIVYGNITIDAYITVQALLEGYEGSRIILIHPPAAS...
T.cruzi OGTLALSSESSVEKLRQHLHDLDRNPHNTSNVIVYGNITLDCYITLSTLLSLGISRSRIHLVOPPP...
T.brucei PGVILPISGSASVERLORTLYELDRNPENVSNIVYGNITLDCYITLSTLLSLGISRSRIHLVOPPP...
T.castaneum ONVFIINTEYDAGNAFNALKSLTSGKQKGNMVFYGHYLEACCLLAALLEGYEGSRIILIHPPAAS...
C.reinhardtii EVAGLLVTELELAADFSMNDAMVNSILVYGNAMGAYHSLAVLEAKGAGEKTRFVAPP...
-----GQQ

1387

T.thermophila PFDDPSVEQKIFEFMESKGIQVHKGYNLHIDVDEETR----ICQGVVFRKKADNYEEITOOEIKKOOILERDANSENADKGFDD...
I.multifiliis PFNDEKVEEMVFOOMEKLGINVRFGGLHETIOVDYTK----ACQTVFRKKSDDFDETERRIAEKKOEILLERONENEN...
P.tetraurelia CFENEVEVKNKVFDMVKLGIKIHOFTLYELKVGKEGFLNSSEDVLQEVIFRKKADNYEELKIEIQRKEQELQELKDNSEN...
H.sapiens CINNYSVESAVADALGAAGVTMYRDAILAOWNDGLHPDPIYSASFSTPTKPK...
X.tropicalis CFNNYAIEEAVOKALLAEGISSYCNVLAOWNDGAHPDPICFATFSTDTKPK...
S.kowalevskii CFNNSITDKAITNALKHSVGOIHTGYLLAEWSDCMGGEGDEVHCASFSTDTKPK...
S.purpuratus CFNNPVHEAVHMSMAOAGVELYQGYLLAOWNDGKGVSGDGEVYCSASFSTDTKPK...
C.gigas CFNDPKIDDAVKAQMVSSGVVHSGYLLAOWNDGDDVSEITSAFSTSSKTP...
N.vectensis CFNNIDIEEATDOALQASGEVHVGYVLAEWNDGKYDSNLSCAFSTSENKPK...
T.adhaerens CFNNSIVEEVVLKGLKEAGITVHHDLLARWEIDESNETVVGASFSTDNKPK...
T.cruzi PFLDKDAFDCAVKLCNSLGINTRLRGYVTRLEYYDDGSAITTVVSPVLFSEEGDRDNTAGISNS...
T.brucei PFVDFDAFECVVRMSALGANTMHGYKISRTEYDDGTTLTTVVSPVLAAPAGPGTDSNARSS...
T.castaneum FFDNSAVETAVALDEILYOGITIIYQDFNFVEWHLDSTKNFITSAKFESKYKF...
C.reinhardtii PPLVGVLHALAGEATVALPSPPEPRDLAGLWVQVGPPELHASATLIDPADPGPR

1486

T.thermophila PITLQEIIEELERNKFDIEITSRFLVTSGLVDDIKEIFYIIHENGLVYNGRLIVKSNFOTTEKIDIFCCGKICEFSORYKRLS...
I.multifiliis LIITEQEIIELEAQKYNDIEINSRFLITSGLVDDIKEIFYIIHENGLVYNGRLIVKSNFOTTEKIDIFCCGKICEFSORYKRLS...
P.tetraurelia LEVLAREIEQLKASEYDYLQDSRFFITSGLIDIDKEIFHIIHENGLVYNGRLIVKSNFOTTEKIDIFACGKICEFSORYKRLS...
H.sapiens ----FRLOCSMFFSFEKKNVDYETFKALNDACLVYDGRVLDITNFHTNDIAIRAAGSLTKFSNRYYSNEWT...
X.tropicalis ----LRLCCSAFFNFSRKSDVYEAFAKAINDACLVYDGRVLDITDFHTNDIAIRAAGSLTKFSNRYHASEWS...
S.kowalevskii ----IKLECSVFLCYNRKGVYEAFAKAINDSCLVYDGRVLDIDANFHTNDLSIRGAPLTKFORKYHADQWT...
S.purpuratus ----LKLECSAFFCYSRKAVYEAFAKAINDSCLVYDGRVLDIDAFHTNDLSIRAAGSLTKFORKYHAEQWT...
C.gigas ----LTLCEGFFAYYKRAVTDFAKAINDACLVYDGRVLDIDAAFHTNDVSVRGAGLITKYQRKYHAEQWS...
N.vectensis ----LRVDCQAFFCFQEKRVDFQAFKAINDSCLVYDGRVLDANFRNDPEIRAAGSLTKYQRYHAEQWS...
T.adhaerens ----VOISCAFICFKLGDYSIFKAINDSCLVYDGRVLDISSYRNDPCVLAAGPMTKFFARYHAEQWT...
T.cruzi ----VELGCSLIVCEDDKIDSHVLTNLNRRSIVDFGRVIVBEANRYTIDKCVYAGPVMFTFRYGTTPG...
T.brucei ----VEINCSLIVCEDDKIDSNVLTNLNRRSIVDFGRVIVBEANRYTIDKCVYAGPVMFTFRYGTTPG...
T.castaneum ----VEIELSALFIYHSGKVSARTFOAIRSGLVFNGLVLDKDYOTNDPSIYAGPLTMSKKKYAHEHLS...
C.reinhardtii ----EELPVLDLVVGCPEPPSVSRSLFTCLNDASLVFDGRVLDVDFGAFRTNDPAIYAGGSLAKLSRRYGGTH...
-----LEHYNSRDVG

1585

T.thermophila OKLAKCLLDSLDLGYLT-----DOIYSLGELPNLYMPIGIGAFPLPGDLF...
I.multifiliis OKLAKCLLDGLDLGYLT-----DOIYSLGELPNLYMPIGIGAFPLPKQY...
P.tetraurelia OKLSKCILOLNLSYLT-----SOTYSVDELPMOLYMPIGOGGIVPHKLY...
H.sapiens FOLAAMHLFDPTLEP-----VTEPPANLDRILPMYKGAIKOGGILPGSYH...
X.tropicalis FOLAANMLNIFDPTLEP-----VIEFTDFEFDRLIPIYKGPVIOGGIIPGGFH...
S.kowalevskii FLLASTMLRFLDPTIEN-----DIVPPEPHNLIPIYRSKAVOSGFLPGDY...
S.purpuratus RSLASTMLRFLDPTIEN-----DVAPPPPEPHCLIPLYOGAKVCGVLPGLH...
C.gigas VHLATEMLRFLDPTLDQ-----QTAPPEESLNLPIYRNPKIIOGGIIPGCYY...
N.vectensis DELARSMLALFDPTLEG-----FMPGPPVEEEDKLVPLYKPKMVAVLPGGYH...
T.adhaerens LKLAETVLSILDPMLES-----EAPDAQPDIIIPVYNHPTFLTKLPNGYT...
T.cruzi TSLAEILLGVFLDEFAKLNREVGDKEEIIITAAONLYKRVLOENGSG---NGADFLMSGGALSTEE...
T.brucei TNLAEVILGTGFEFATPNLOSCTDKENELLAHNELYSKVLDENGSRNANYGVDLNSLSAEKDAHEIAKONQLKOOOKL...
T.castaneum YNLGSAIRKLVPEQFW-----DPVVKETLIHQPVVQICKFPGNLIC...
C.reinhardtii SRLASSLVSFNAGPDE-----PQPAATAAPPALHRRARAVGCSLPGGNY

1684

T.thermophila YYHIK-----KNDYARPSKTM-EAEDNRDIDVSDNIDOKOOI-----GHYIKFKFDNNGIIDOVTYLGT...
I.multifiliis YYNIK-----KNDYARPLLTM-EAENNRDIDVSDNIDQETNQ-----GHYIRFKFDNNGIIDEINFGSE...
P.tetraurelia YYYIK-----KNDYARPSKTM-EAEDNRDIDVSDNIDOKOOI-----GHYIRFKFDNNGIIDEINFGSE...
H.sapiens YLHIA-----KPAIPPLEVOMAQPNYGRELVTGSAKN-----GTYFRIRHINKYKMMVETITCLSR...
X.tropicalis YLHVM-----KPAIPPLKQAOMAQAHYGREIVTGSAVD-----GDYFRRLHINQYNNVETITCLSK...
S.kowalevskii YLDYG-----KPSLQSDALMAQDYGEEELITGHPDKDG-----SEYFRHINQYNNVETITCLSK...
S.purpuratus YLHYG-----KPGLDMPLEVOMAQADYGRELITVDTQ-----ONYPRLHLLNYSNIQVTVCLSHO...
C.gigas YLHVA-----KPGLDVPLATOKEOPYGKEYLTSNPGGK-----PDYFRIRHINQYNNVETITCLTKO...
N.vectensis YLHVG-----KPLNIPLDLSLMVQPEYGRELLTGNKAEEAG-----GPGYFRHINQYNNVETITCLSK...
T.adhaerens YLHTG-----KPSLNLISLDSMMVQPNYGHVLTGSHVDT-----OGYFRHINQYNNVETITCLSK...
T.cruzi FFSRSIDFKPERCLRLFYSNIEENKPVVTVMEELASPSMNIEDLPTRIPE-----ONLFWIYIDEOTHLIDAVVYFGNGAPEMHNVCILGMPSSL...
T.brucei FFSRTRIFFDPAQCTRLYSYSCIEDNKPVDDITASYOVATPADRSIYKDV-----ODLVIYLNKHTRLIDAVVYFGNGAPEMHNVCILGMPSSL...
T.castaneum YLNVH-----KAGRPVPLDVATNKNYGRVLTGDCCKLKD-----OGYFRHINQYNNVETITCLAKF...
C.reinhardtii FVYAG-----CPAALORPSTAAPEGGYEMKTASE-----RGLTRITLDGEGRVHSLMYLGRV...
-----AVNAPRLGSLVGLHANYL

1783

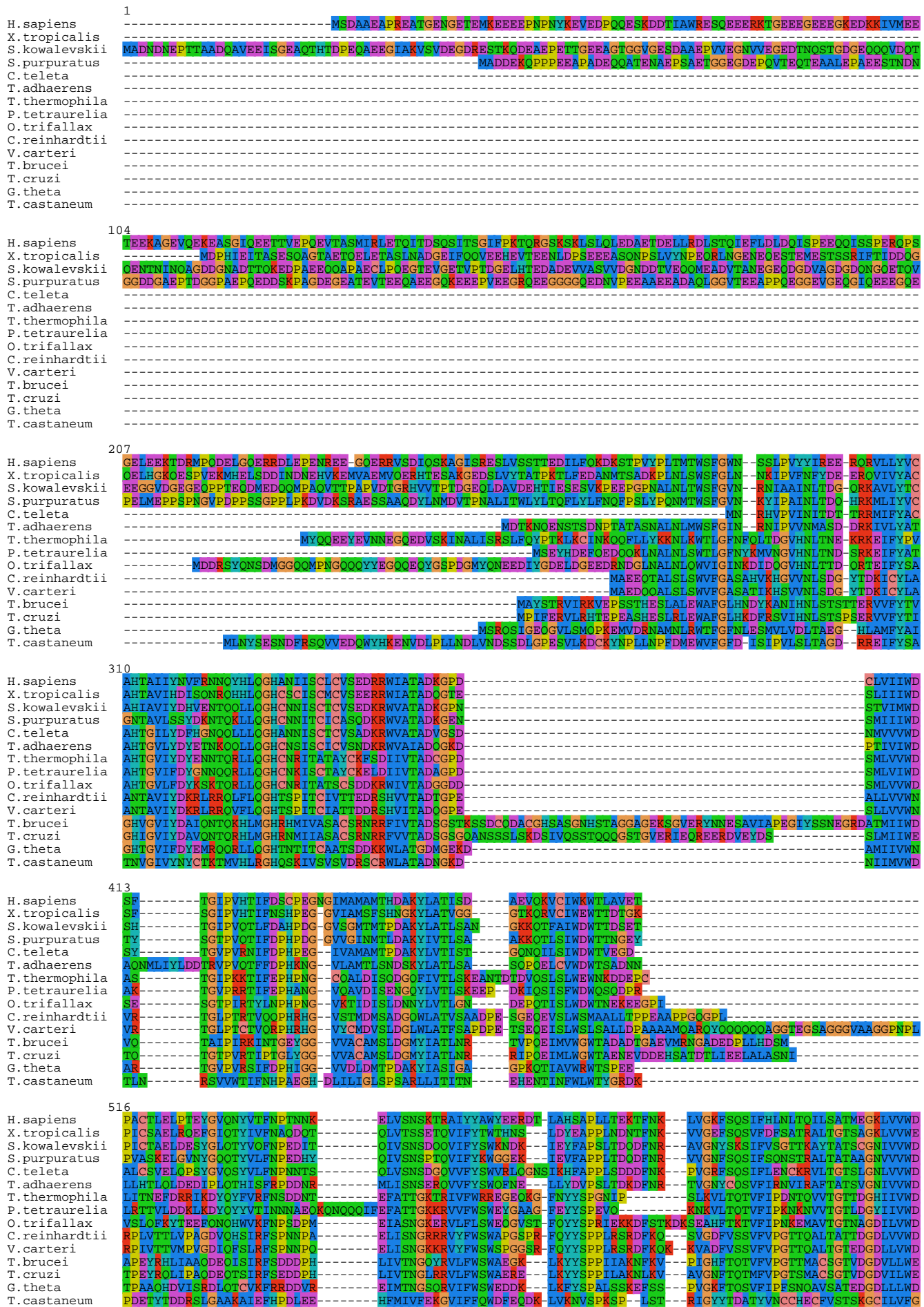
T.thermophila NQLDVRFKNGLIP-----NISEFLSENWAIGLYHEWFSFRHITKMEMLOSENI---OOILDEAAKYAQ
I.multifiliis NQLDVRYKNNLIP-----NIVEFLSENWAICLYHEWFSFRYITKTDIOKSONI---OSILDEIGRHAK
P.tetraurelia NRDLORGG--LIS-----NVSEFLSENWAIALYHEWFSFRHITKSEMLKNELI---DOVLEKSOEYAR
H.sapiens NNLCAARYDENLIT-----DLYSYFTEPWCLALPHDRFIDLKELROILASKEEEDLP---SIEQLAHQIEDE
X.tropicalis NNLCARFDEGLIK-----DLYSYFMKAWCMAYHDFRFDFOEVREMLAAEOGEGRP---SMKOLAQOIADD
S.kowalevskii NNMLSRFKEGLIK-----DFYEFFKETWCLAVFHDRFGDFROEIRELLIORPAADLP---SLEEKVROLIEO
S.purpuratus NNLVSRFDEGLIK-----DLYAYFRETWLAIFHDFRFTDFROEVRELLVORPAVDVS---SLEEKVRELISQ
C.gigas NNMVSRFNEKLIP-----DFYEFFRETWCLALYHDFRSDFRDEVRELLVTSPGVGMD---SIEDKVREVVDE
N.vectensis NNLLORFDEGLIO-----DFYSFFOESWCLAVFHDFRNDFREEIRELLVNKPSDDVL---SLEEKVRKMIDE
T.adhaerens NNVLSTRYKEGLVS-----DFYOYFNEPWATAIFHDFRFDFREEIKELLFNKOVEESDET---FELKTRKLLDE
T.cruzi N-LIYRYEARIA-----NRDESSHOSKHEGCSHNDTGNGSGLNLLLEYLRPLKLOIFFYDRFTVFYRELREKMRDHKDLID---AKARAFEFLEETEE
T.brucei N-LIFRYNEARTDLLESDDCGSNSSMSGSKSTASNKIDIVAQESTLNLMEYLRSPRLOVVFYDRFVFEYENLRKKMOEHEDVMK---MKOSALORMEVT
T.castaneum NHLLLRDYDLGMIP-----DMFOFFDEPWACAIYRDFPRVIDEISELLLTAEETNEG---VKLVDEVRAOLE
C.reinhardtii NSLAPKYQAGDIK-----CLLSFITEPWSSELLYNESFPELRETLLEVALAELSAGG-----REVDGG

1882

T.thermophila GGGFLD-PEFIEKIKAKISPE--IISQIQOGLDFIRENONHLP-MYYVPPKKNVD-----
I.multifiliis SGOFLV-QELFOKIRGOISPE--IIIOIQEGILDFIRENONHLP-MYYVPPKKTMDG-----
P.tetraurelia DGRYMD-DNFFEEIKKLITRD--IVICIQEGTIEFIRONONHLP-MYFVPPKOKLN-----
H.sapiens EINPTE-KPROYLKRVFEESI--YKTLVERSTLDYLHYNRYHLP-MYAWPGIV-----
X.tropicalis ELNLKE-OPROYLKRILEENG--HKKEIERKILNYLNYSNHLNLS-MFARPGMI-----
S.kowalevskii DLQLEK-KDRKYLNDEFEDGS--YRKAVETRLLSPLSYNYHLP-MYAKPGMV-----
S.purpuratus DLLLES-HDRKYLAEQFDQOG--NKRAVETRLLSPLSYNYHLP-MYAKPGMV-----
C.gigas DVPMD-AOKKOLLEIYASSG--SKRAVETRLLSPLSYNYHLP-MYAKPGMV-----
N.vectensis DLVLSK-DORRNLSDNYVSSP--AKKAIEORLLSPLSYNGYHLP-MYAKPGMV-----
T.adhaerens DFTFTK-KELOQLGEDYIKS--CKSSTEKRLTYLSYNTYHLP-MYAKPGIV-----
T.cruzi SILSDGCRKKNLNETAESSG--FARVOYELIKFLHESKDYVPOIYFLPDIRSHVK-----
T.brucei PRISAKNRAIYLEKLTTEMKRD--FARVOYELIKFLHESKEYLPQIMYLPDI TEHVKEKNEGROE-----
T.castaneum KNDWNPLTKIQTDEIEEMCRDTSLIPLIERKVLDLFDNFMYLP-MYVHOLLIRWILHQIQTSPLFVKTTTFLSNTRKSFSPGIPFLMRPTQSRME
C.reinhardtii MVE-----WVTHAQDAVLEFARAHAALP-GYTMPAART-----

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



1237

Sequence alignment for position 1237 across species: H. sapiens, X. tropicalis, S. kowalevskii, S. purpuratus, C. teleta, T. adhaerens, T. thermophila, P. tetraurelia, O. trifallax, C. reinhardtii, V. carteri, T. brucei, T. cruzi, G. theta, T. castaneum. The table shows amino acid residues with gaps represented by dashes.

1340

Sequence alignment for position 1340 across species: H. sapiens, X. tropicalis, S. kowalevskii, S. purpuratus, C. teleta, T. adhaerens, T. thermophila, P. tetraurelia, O. trifallax, C. reinhardtii, V. carteri, T. brucei, T. cruzi, G. theta, T. castaneum. The table shows amino acid residues with gaps represented by dashes.

1443

Sequence alignment for position 1443 across species: H. sapiens, X. tropicalis, S. kowalevskii, S. purpuratus, C. teleta, T. adhaerens, T. thermophila, P. tetraurelia, O. trifallax, C. reinhardtii, V. carteri, T. brucei, T. cruzi, G. theta, T. castaneum. The table shows amino acid residues with gaps represented by dashes.

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).

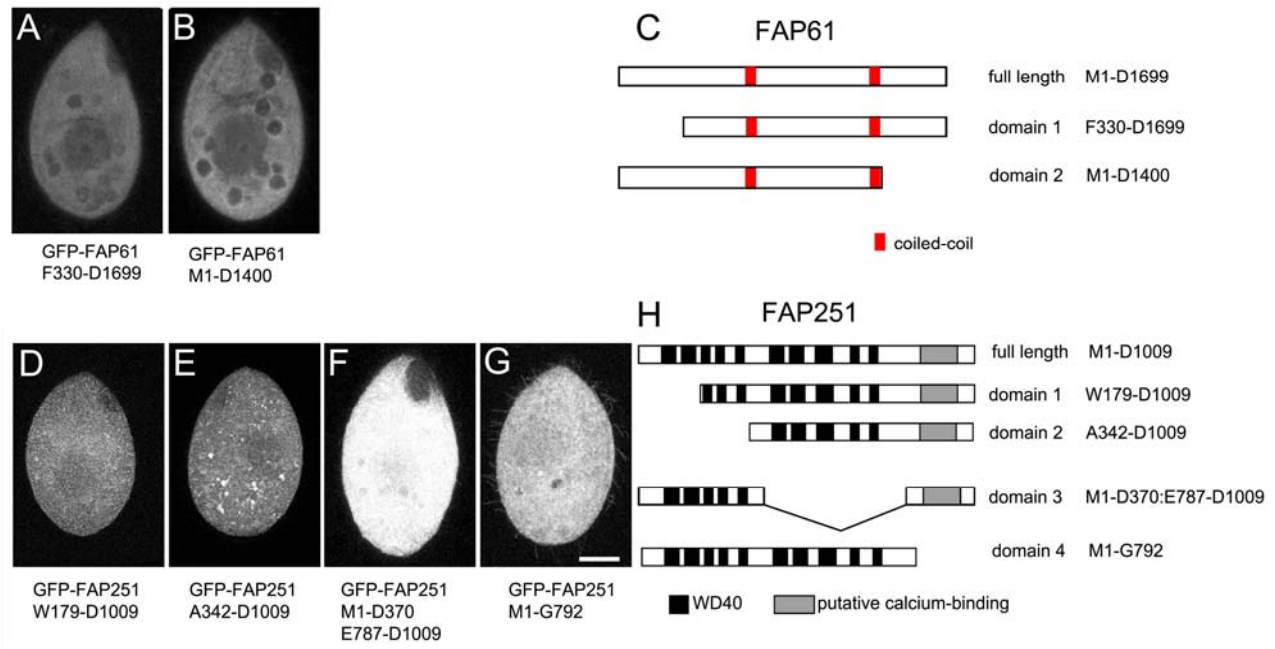


FIGURE S3: Domain analysis of FAP61 and FAP251. (A and B) Localization of overexpressed GFP-tagged truncations of FAP61; FAP61 that lacks either N- (A) or C-terminal (B) fragment accumulates in the cell body and is not targeted to cilia. (C) Schematic representation of the domains of *Tetrahymena* FAP61 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 .

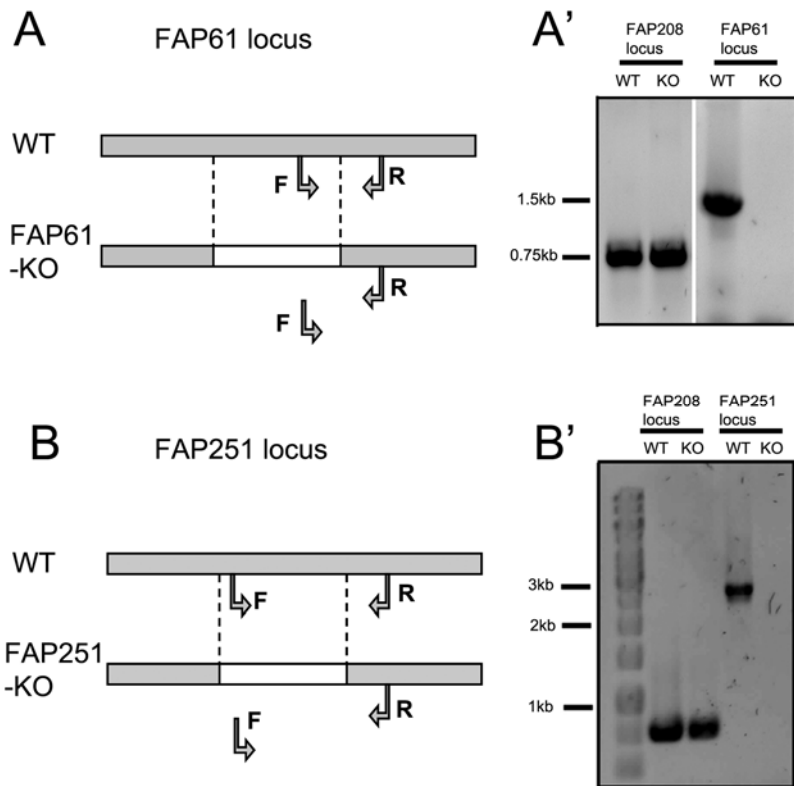


FIGURE S4: PCR analysis of the FAP61 and FAP251 loci in wild type, FAP61-KO and FA251-KO cells. (A and A') Analysis of the germ line FAP61-KO cells. (A) Scheme of FAP61 locus in wild type and knockout cells. (A') PCR analysis of FAP61 locus with primers indicated in (A) showing that part of FAP61 coding 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.

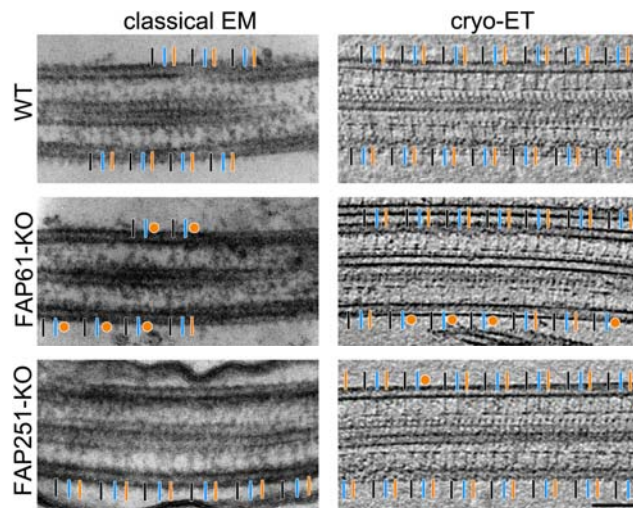


FIGURE S5: RS3 is lost in a subset of FAP61-KO repeats based on both classical electron microscopy (EM) and cryo-electron tomography (cryo-ET). Longitudinal sections of chemically fixed cilia (left column) and longitudinal tomographic slices of rapidly frozen axonemes (right column) from wild type and 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.

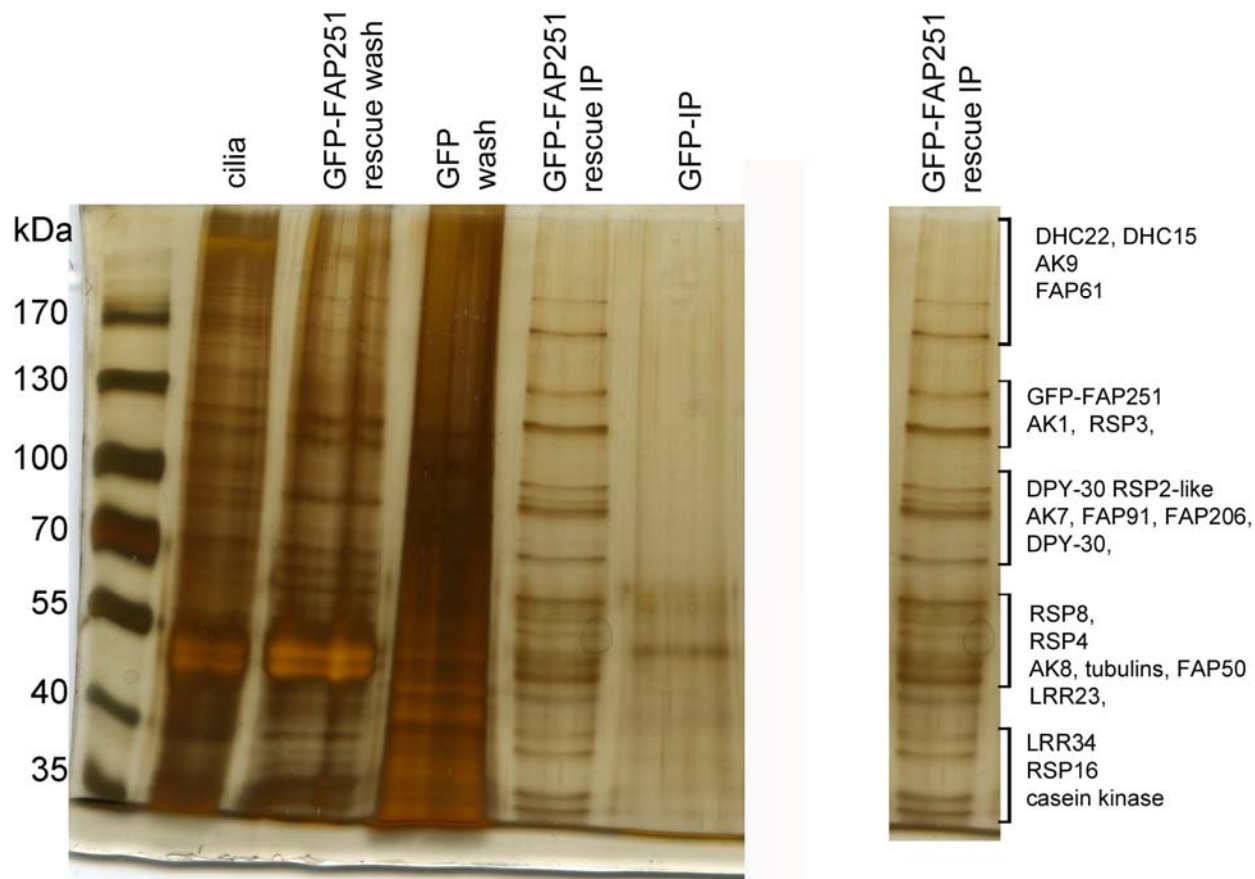


Figure S7: GFP-FAP251p co-immunoprecipitates with numerous proteins. Silver-stained gel showing cilia isolated from FAP251-KO cells rescued with GFP-FAP251 (line 1), proteins that were washed out from the resin (unbound proteins) in the experimental (FAP251-KO cells rescued with GFP-FAP251) and control (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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