

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.

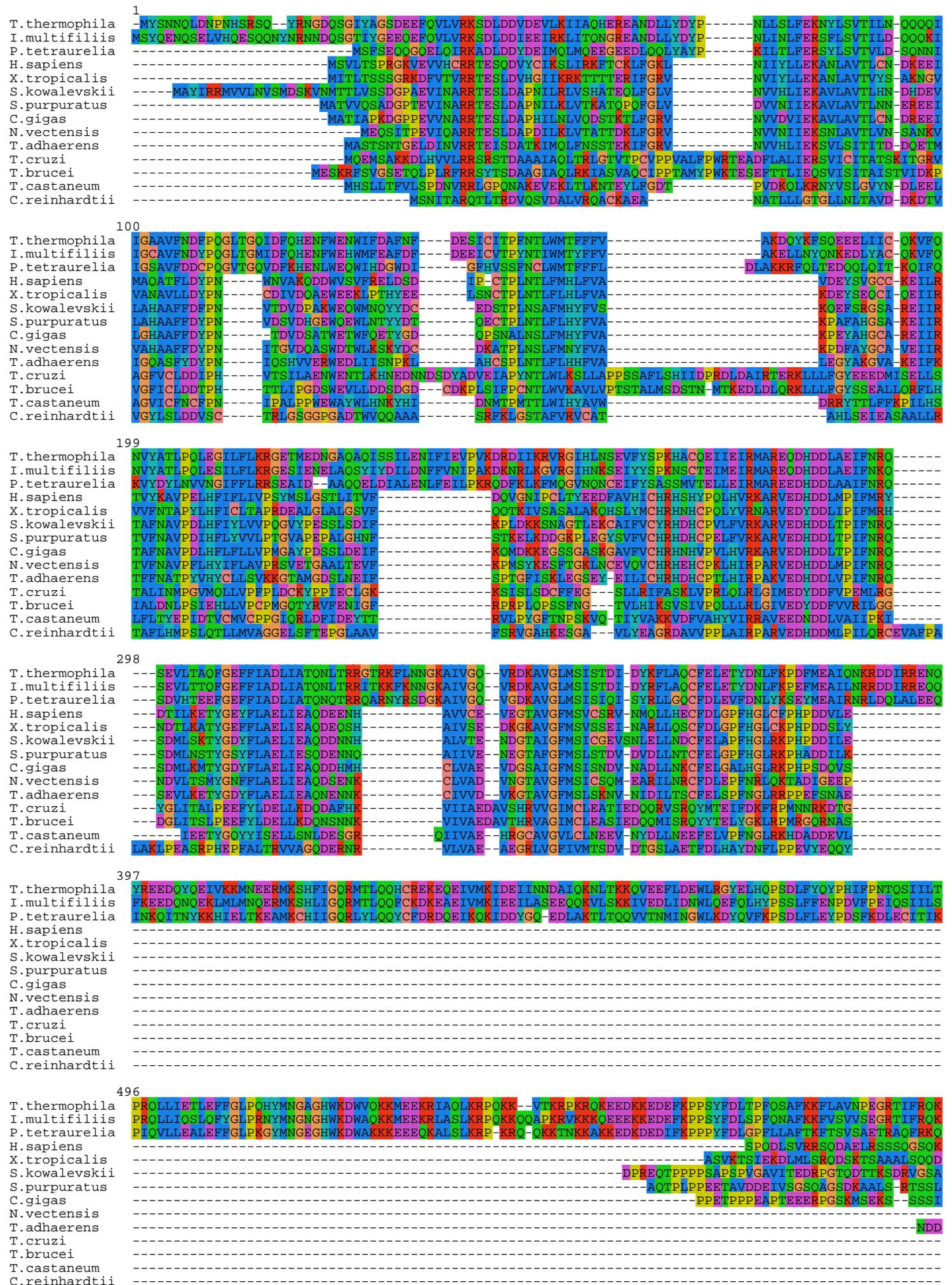
b) This pseudo wild-type strain (pWT) was used as the control for classification analysis in Heuser *et al.* 2012. It is a mutant rescue that is biochemically, structurally, and phenotypically indistinguishable from WT (Rupp and Porter, 2003; Heuser *et al.*, 2009; Nicastro *et al.*, 2011; Barber *et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



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T.thermophila IEASKMVKIKMMFCNENEMKKEERHVDLNDLGNELNKQKMDIAPEVCENLASFLECFGEIKYEEIIMVNPPEKEDTNONAKIAOTKKMFASEQTESKPIIP
I.multifiliis VEONLKFIKIMFCNENEMKKEERHVDLNDLGNELNNAKMDIPOEVCENLASFLECFGEIVYETETIMVVP-KEDDPD--KNTKNTKKFGNEOQEQKFWP
P.tetraurelia IESRVKELIMEFCTENEMDPSRHIDLEFPDVLNRKGFDIGAPMGENLAFILECFGELEVDNRIVOKIKEKDAKK--K DANLKKVGDAP--KPV D
H.sapiens IVEELQEPVSPDPTMENICGNI-----
X.tropicalis NIDHNTTDEKIDSTNDAITVS-----
S.kowalevskii GSRKDEDLDELEGGGAGKTPSVKGTPLPOOATPO-----SOPPASR
S.purpuratus SSIKKGDTGTAEPTPSTOPPACTPQOTKATPLPAKAATPMTAKSTPLPTKGTPKPGATPL-----NKPPTVA
C.gigas NSKAEQTDGQMDERKPGSOASSGO-----NSRA
N.vectensis
T.adhaerens GKQASETEEPTDTPAKTSSITITIG-----
T.cruzi
T.brucei
T.castaneum KTOVLSATAIPVLEDSTERLSNTSLOEETREESAAEQDDODNVSDDEFS
C.reinhardtii EAARDSVRGGKLA MLRHROQEQEAEEGEGAGGAGG

694

T.thermophila KLLKKTSTFOEFFDAVYKMKFEFIMNRLDITKSTTLKAEVSOIVKBEAQQEKAKQEKRRRIERSPNPYDDYVKNLKDLDSEIPEVPIAONAIVINLFCID
I.multifiliis KILKKTSTFIEFFDAIHMKMSFDOMNRLNFTKSGSLKKEVQOIIEDENLKEQOKKRRILRKSTPYDDYVKNLLEDSEIPEVPEAONAIVINLFCVD
P.tetraurelia VLQKMTSYSEFIFGAIQKLNKYDQMCRLQLVRSNTLQTEVEEIIQDEQNKITNLERAKNVERNOTQDEHVSOIOSIDOLPEVPKAAQNAVINLFCID
H.sapiens QSGNVSEPEDIEKLSDISTG-----YAQYHVSSRSLASLVLPEEPVHFPIYRIGASAAVFCIOLF CID
X.tropicalis TIGNDTMGPREEDTLOEPNDL-----VSRDPAADDAIOTRNCRAPESGSNAAEQHPHPVYKGDVTFASIOOLF CID
S.kowalevskii EAAESESEVKTIRKRLSDIOMGGSSCSLOSSESRPESAKSGASSKVSQVSKSPVOEEFEPEPPAPERPTPTPOLDRKMRITTFKSVYNGPNSCFICOLF CID
S.purpuratus GSIAESELQVEGKLSDSHMK--SSGSVMSDKDEVEVSKGMS--KRSGS-----IASNHDAEEAAPSLSVTSSEPSLPEPRFEPTTYGPNANAFSIOOLF CID
C.gigas GMSMGDPPKQSKLSDAQLT--GASLLSDGEQMEEVARDAE--DRESQ--ISSRKSIIKEEAVOSRLGSPPPPTMRTOKRFVPTYPNGPLNAFSIOOLF CID
N.vectensis
T.adhaerens
T.cruzi
T.brucei
T.castaneum
C.reinhardtii

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T.thermophila ENFESRSLDFVEYAFSIFGDRDYIILTOPFTVPEITLLOOFIKIPMKKNST-----FDHVLYIYHKDCLLSNT--LFI RKS KLEDMOYVAPLFSN
I.multifiliis ENFESRSLDFVEYAFNIFODRDYIILTOPFTCEPTLLOOFIKIOMKKNST-----FDHVLYIFHKDALLSNS--IFIRKSKLEDMONVLLFSN
P.tetraurelia ERFESRSLDFVEYAFELFPDREYLLITQPTVYIOTETLLSHFLOVSRKKHST-----FEHVLYIFHRNSLDSHL--IELRKFNOSEWNOCOFLVEN
H.sapiens EKYEARS LDFMNFVFLSFDKNFCVISLPHLTPEFFLIONFVKMVPFNTCT-----LEQDLYVFHRAGLLKS--INIRFATLLDTPGVENLVS T
X.tropicalis EKHESRSDVFLSYAFSLFPDKDFCVITVPHLTPEFPLLONFVRVPLSTCT-----LSQELYVFHRAALISS--IKVRAVRNSDIDNRSLLET
S.kowalevskii ERYEMRSLDFLPMADFDPDRDFCVITVPHLVPEFPLLOSFVRVTPRCPS T-----LAQELYVFHRNGLLON--FLVRPVCSTDMNSIONVIKS
S.purpuratus ERYEMRSODFLPMAFSLFPDRDFCILTVPHLVPEFPLLONFVRVTPRCPSV-----LPQELYVFHRAGLLOS--FTVRPACTSDVPAVTSLEIEG
C.gigas ERYEMRSGDFLATCFSLFPDLNFCVITVPHLVPEFPLLORFMRVTPRCPS T-----LPQELYVFYKAGLLKD--FKVRPACTKDTAGVENLVR
N.vectensis EKYEEMRSLDFLPAFAAFQDFDRDYCVITVPHLVPEFPLLOHFTRVTPKRST-----LSQELYVYHRWGLIES--FNVRPCISSDSDAIRKVIK
T.adhaerens EKYEEMRSLDFLPAFAAFQDFDRDYCVITVPHLVPEFPLLOHFTRVTPKRST-----FPQELYVFHHYGLINS--MKVRNVKVEDVETISKLVON
T.cruzi POYAMCADOPLPFVFOEFPYVEYAMILPHEKDEPPFLOEFHVLRRYOPRNARGEVITPPOGLWIYCRYAADPIWIPV LKNDVDESISAFNLPEP V
T.brucei PAYALRAKSLPLVYIKFEPVEYVILTPYDTEKPPDFGDHDIPLRKYYPNREGYLIPDPDGLWINCRYAADPVVATPVRSEKDTITSINFLDEPHM
T.castaneum DDHENCLYLLFOAAFECFPDREYLIVSLPTETOMCYLKKHFARVTPRPNV-----FNHELVLHKSAILGT--LWARVAVSEDKPLVSELVAT
C.reinhardtii PAFEAQAEIETLPAFAAYTKLYCVVTLPHDSREPALMGTRVAPNPGSL-----FPEVLFMFHRHALIPD--FAVRLGEPGLDVAVASLVAG

892

T.thermophila LINKOOIYDDTLEAITOTASRK-----VAFSVFC-----DOSLIGIYVSKNVNLD--YYISHFCVODHILKEHPMTCHT
I.multifiliis LINKDOYADTFEAITOTVSRK-----VAFSVFC-----DOALIGIYVSKNVNLD--FYISHFCVODHILKEHPMTYHT
P.tetraurelia LLGKDGIEKDVRRVNDN-----ETFIVYC-----KDEIIGLYCMKKYVNLQ--YLSKSHFCIQDHVLKEHPKHLHT
H.sapiens LMLNKSILEDLDRYNKARKDPDGLT-----LOAFVAEV-----AEQIVGIAVIRNEMDIE--YIRSHYNIEDPIYFNHHRDEHG
X.tropicalis LHPQESILGDLQIYNOARRDDGTP-----VOAFVAEV-----ANQIVGVS VIRNEKDIE--YIHSYNIEDPIYFNHHOWEHEG
S.kowalevskii LHHHDNLIADLKOYNIARRDVNGTP-----IOAFVACC-----LKOIVGVAVTROEDIE--YIRSHYNIEDPIYFNHHRREHG
S.purpuratus IYNOVLLSDLDRFNKARDEDGTP-----IKAFVATC-----HSQVGVVALTRAEDIE--FIRSHYNIEDPIYFNHHRDEHG
C.gigas IHLNENILKDLQOFNKARDEDGTE-----IOAFVAEC-----CGQIVGLSVIRREENIE--YIRSHYNIEDPIYFNHHRDEHG
N.vectensis LKASDKILMDVEQYNRARRDPDGT-----LOVFAEC-----IGOVGAAVLRREEDIE--YIRSHYSIEDFVYFNHHRREHA
T.adhaerens ITGKDMLLRLINLYNMARRDPDGT-----IKRAYVAC-----LNQVGVAVIRKEENIE--YIRSHYNIEDPIYFNHHRREHG
T.cruzi EFSQNIATLDEMRVSSSEENTSRNL--PONSMSFMLSRLGGEG-----ETFIVGVASTRRVKGEMALRANVTDFAFVNYHGGPRDYSVTDI H
T.brucei EFSQ--HOITLLREDIQRLRSRETPEDEVEENINSFVFSFVITYTENVGSEKOLPIVVGVASARKISVNEMYSLRANVDLKLNVNYSKAPRDYSETDVT
T.castaneum LSKPISIVEDLFKEAVDSKMGQ-----FKAYTFCC-----EEOIMGCAIVSEEEHE--YLELHYNVDPNLSPKETRFQYFG
C.reinhardtii MPNADDIVAFSFGAAAA-----GSAVAALC-----CGELVGLVTVNPEVDLE--LLOANFGLSNHVDLGYQPREQHG

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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 VODGKRDAFDOEOPFSLSVITKKMSSVKNMNTRIVVVGASDTGISFIESLLSVKD---INFTHITLLAPGGIMTMHINLP-GDOLKALSTNYTLE
I.multifiliis VODGKRDAFDOEOPFSLSMITKKOLSGVKISNNSRIVVVGASDTGISFIESLLS IKD---INFTHITLLAPGGITMHI ONP-GDOLKALSTDYTME
P.tetraurelia DRDPYRDPDOTOSPLAMLTQKLSNLSVINSNRIVVVGASDTGLSFIESLLT IKD---INFTHITLLAPGGITMHIHVE-FEMLKAMSTNYTLE
H.sapiens SK---AVSKDPMYSALNHTNRKLTLEPKITVNAKIVVGASSVGISFLETLVFCSH---MKFNNTLTI STHGLPGKLLDTEORRFLASDHCFNDK
X.tropicalis SK---OVSKDOPSYSLNHFNRKLTLEPKVITINARIVVVGASDVGISFMETLVF CPH---LKFNNITLISSTGLPENTLSTOEHGLFSTSHSYNDK
S.kowalevskii SE---RVSKDEERYALNHINRKLTPKPVTVNARIVVVGASDVGISFLETLVF CPH---LRFNNLTLISSTGLPELPPDDIRDNFLSSLSYCMQH
S.purpuratus SN---RVLKEQEMYSALNHNKLTLEPKVITINARIVVVGASDTGLAFLETLAF CPH---LRFNNLCLVSPNGLPGHMSDEDELRSFLSTSHCYTHD
C.gigas SE---RVLKEKECYALNHNKLVMEPKVITINARIVVVGASDVGISLLEALAY CPH---LRFNNLTLISPHGPMGEMPEDELROMLGRSFCYDOE
N.vectensis SE---RILQEKAEFALNHNKLTLEPKVITINARIVVVGASDVGISFLETLVF CPH---LRFNNLTLISVPOGLPGOTAPDKLVNSCLPSSMCKYDD
T.adhaerens SN---RILLOOMDNYMLFHINKLVMEPKVITINARIVVVGASDVGVSLFKLIF SPH---MRFNNTLKLISPNGLPGOLKPNLSRNOFLADSYCYTOQ
T.cruzi SOGGDKIDGKEPVALGCLFVGTTRIIIGDRKKIINTRIVVVGASDTGLACIHRFLSVPY---VHFRNIVLVS TDGMPAHPNOQ---SLLWFADHMELLER
T.brucei PRSPERIPSKDVSPLGFAATRRIIVVVGASDTGLTFLYRLLTVPY---ICFTNLVLISTDGMPEHPNOQ---ONLWSTDREMLLER
T.castaneum T---CLQKHHQPFGLYLSLRLSSMPKIEINOKMVIIGAGLTIISFLESIFGRNPNYLVFTNITVSPHGVYHKPPSOVREMVVFNVDGHVDRR
C.reinhardtii SDKLOAEFALYVFTROAAFKRRRSVNSQIVVVGASECGLAVVERLLLDPE---LOFNLYLTLAPGGIKVGG---MACQFTAGVIAR-

1189

T.thermophila ELKNLMLDARVNVLDAKMVELDKKGGKIKLDKNAELPYDYLNTVGLIDTELO...
I.multifiliis ELRNLMLDARVNVLDAKMVLGDKKGGKIKLDKNADLPYDYLNTVGLIDTELO...
P.tetraurelia ELRALMIDARVQVVDKAMVLDKGGNRKIDKNAFIPFDYLIITVGLIDTELOS...
H.sapiens DYALMSLCSWVNVVGRMTGIDRAAKHVVLSTDEIVPYDHLILCTGOOYQVPCP...
X.tropicalis DYALMSLRSWVNVVYKMSGIDRAAKFVIVANGRKVPYDHLILCTGOOYQVPCP...
S.kowalevskii ELSQISLRTWVNVVCAKMAVAINRSSKYVVVTGGAKVPYDHLILCTGOOYQISAP...
S.purpuratus ELSQISLRSWVNVVSSKMVINRLSKYVKIANGTKVPYDHLIINTGOOYQVSSP...
C.gigas SYAKTSLRRTWVNVVYKMTACDRKKKVVVVNGTLVYDHLVLMCGOYOVPAP...
N.vectensis IYANMSLRTWVNVVKSMTAIDRINKLKVSGGFGVYDYLVICTGOOYQVPPAP...
T.adhaerens NYDQIALHSWVSVIKGEVKAINRKSQYVVVNDTKVMYDHLILCTGCRYOYSCP...
T.cruzi EOMGLFVGNPVRVHGLVMDVDTTKQYVSDIDGTEYDYLITGROYVVPNS...
T.brucei EHMGLTVGNPVRVHIGSMVDIETAORYVVVDDSTYEPDYVILTTGROFVPLS...
T.castaneum YIDATSLKTYVHVVTGVMVTSINRKEKYVGLNDESILPYDYLFLMCGEYQKPP...
C.reinhardtii ----LGLEARVMLLDAEIVGLDRGSKLLDLSGDSQIFYNQLVLAAGLQDOS...
-----RKKEVVKKR-----RVAEADP

1288

T.thermophila NYVQGVYSIDDPYLYEYFKKTKKDSNIDLLTRKKRPOSITIYGRTRLHTIAFISGMVNRGVHPNRIHYVIPPKVFE...
I.multifiliis DYINGVYSIDDPYIYDFHFKTKKDSNIDLLTRKKRONITIVYGRTRLHTIAFISGMLNRGVAPKRIHYVIPORVYE...
P.tetraurelia -FINGVYSIDDPYIYSHFKRTGFGKSNIDLLTRKKRONITIVYGNITLITITFMNGLLNRGVHPNRIYVMPKTFQ...
H.sapiens CNHFTLNEEEDCFKALWIWRNN-SITTEGNIIYVGNITIDTYITVETLLNLGVSRSRIHLVOPPPAS...
X.tropicalis SNLFTLONRQDCLAAMRFLKDS-VVKQEGNVIVYGNITLDCYITLSTLLSLGISGRRIHLVOPPLTM...
S.kowalevskii SNVFTINDEYDAFAQAKVWISKN-LLDTEGKVLIIYGHITLDVYITITQTLMMGPGERIIMVQPPILNY...
S.purpuratus KNVFTINDESDAAATIKWLNKR-FISAEKAIYIGSSLDAYTAVOTLLALDVPGHRITAMVOPPORF...
C.gigas KNLFTVNDYDAVALYWLENY-FLKSNKKAIVYGCITLDAYCCVOTLIAMGIPGENIQLVEPPSIY...
N.vectensis DNVFTVNSHGDCAKVVKWNNY-FINSGKALVYGCITLEAFYVQGLLSAGIPADRLVMVOPPPP...
T.adhaerens SNIFTLNDQDFDCAFLDWLEQDFINRRDHCYIGSTIDAYTITVQALLEGSRRIILIHPPAAS...
T.cruzi OGTLALSSESSVEKLRQHLHDLDRNPHNTSNVIVYSGLDAFATATSIVSLGFSRORIVVVSPE...
T.brucei PGVILPISGSASVERLORTLYELDRNPENVSNIVVYSGLDAFATATSIIINLGFSPORMVLVSPD...
T.castaneum ONVFIINTEVDAGNAFNALKSLTSGKQKGNMVFVGHYLEACCLLAALLEGVSNLVIIDNPDSSSLRQDRHEH...
C.reinhardtii EVAGLLVTELELAADFSMNDAMVNSILVYGNAMGAYHSLAVLEAKGAGEKTRFVAPP...
-----GQQ

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T.thermophila PFDDPSVEOKIFEFMESKGIQVHKGYNLHOIDVDEETR----ICQGVVFRKKADNYEEITOOEIKKOOILERDANSENADKGFDD...
I.multifiliis PFNDEKVEEMVFOOMEKLGINVRFGGLHETIOVDYTK----ACQTVIFRKKSDDFDETERRIAEEKOEILLERONENEN...
P.tetraurelia QFENEVEVKNKVFDMVKLGIKIHOFTLYELKVGKEGFLNSSEDVLQEVIFRKKADNYEELKIEIQRKEQELQELKDNSEN...
H.sapiens CINNYSVESAVADALGAAGVTMYRDAILAOWNDGLHPDPIYASAFSTPTKPIYASAFSTPTK...
X.tropicalis CFNNYAIEEAVOKALLAEGISSYCNVLAOWNDGAHPDPICFATFTSDTKPICFATFTSDTK...
S.kowalevskii CFNNSITDKAITNALKHSVQIHTGYLLAEWSDCMGGEGDEVHCASFSTSDTKPEVHCASFSTSDTK...
S.purpuratus CFNNPVHEAVHMSMAOAGVELYQGYLLAOWNDGKGVSGDGEVYCASFTSDTKPEVYCASFTSDTK...
C.gigas CFNDPKIDDAVKAQMVSSGVVHSGYLLAOWNDGDDVSEITSAFSTSSKTPETITSAFSTSSKTP...
N.vectensis CFNNIDIEEATDOALQASGEVHVGYVLAEWNDGKYDSNLSCATFTSENKPNLSCATFTSENK...
T.adhaerens CFNNSIVEEVVLKGLKEAGITVHHDLLARWEIDESNETVVGASFSTSDNKPITVVGASFSTSDNKP...
T.cruzi PFLDKDAFDCAVKLCNSLGINTRLRGYVTRLEYDDGSAITTVVVSPLVFSVEGGDRDNTAGISNS...
T.brucei PFVDFDAFECVRRMMSALGANTMHGYKISRTEYDDGTTLTTVVLSVPVPAALAPGPGTDSNARSS...
T.castaneum FFDNSAVETAVALDEILYOGITIIYQDFNFVEWHLDSTKNFITSAKFESKYKFFITSAKFESKYK...
C.reinhardtii PPLVGLHALAGEATVALPSPPEPRDLAGLSVWQVGPPELLHASATLIDPADPGPRHASATLIDPADPGPR

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T.thermophila PITLQEIIEELERNKFDIEITSRFLVTSGLVDDIKEIFYIIHENGLVYNGRLIVKSNFOTTEKIDIFCCGKICEFSORYKRLS...
I.multifiliis LIITEQEIIELEAQKYNDIEINSRFLITSGLVDDIKEIFYIIHENGLVYNGRLIVKSNFOTTEKIDIFCCGKICEFSORYKRLS...
P.tetraurelia LEVLAREIEQLKASEYDYLQDSRFFITSGLIDIDKEIFHIHENGLVYNGRLIVKSNFOTTEQENIFACGKICEFSORYKHSV...
H.sapiens ----FRLOCSMFFSFCCKNVYDFTKALNDACLVDYDRLVIDTFNHTNDIAIRAAGSLTKFSNRYYSNEWT...
X.tropicalis ----LRLCCSAFFNFSRKSDVDEAFKAINDACLVYDGRLLVIDTFHTNDIAIRAAGSLTKFSNRYHASEWS...
S.kowalevskii ----IKLECSVFLCYNRKGVDEYAFKAINDSCLVYDGLVIDANFHTNDLSIRGAGPLTKFORKYHADQWT...
S.purpuratus ----LKLECSAFFCYSRKAVDYAFKAINDSCLVYDGRLLVIDALFHTNDLSIRAAGSLTKFORKYHAEQWT...
C.gigas ----LTLECGVFFAYYKRAVDTDAFKAINDACLVYDGLVIDAAFHTNDVSVRGAGLITKYQRKYHAEQWS...
N.vectensis ----LRVDCQAFFCFQEKRVDFQAFKAINDSCLVFDGRLLVDFANFRNDPEIRAAGPLTKYQRYHAEWS...
T.adhaerens ----VOISCAFICFKLGDYSIFKAINDSCLVYDGRLLVIDSSYRNDPCVWAAGPMTKFFARYHAEAWT...
T.cruzi ----VELGCSLIVCEDDKIDSHVLTNLNRRSIVFDGRVIVDEANRYTTDKCVYAGPVMFTFRYGTTPG...
T.brucei ----VEINCSLIVCCEDKIDSNVLTNLNRRSIVFDGRVIVESNYLTTNCPVYATGPMFTFRYGTITTS...
T.castaneum ----VEIELSALFIYHSGKVSARTFOAIRASGLVFNGLRLVIDKDYOTNDPSIYAGPLTMSKKKYAHEHLS...
C.reinhardtii ----EELPVDLVVGCEPPSVSRSLFTCLNDASLVFDGRLLVVDGAFRTNDPAIYAGGSLAKLSRRYGGTH...
-----LEHYNSRDVG

1585

T.thermophila OKLAKCLLDSLDLGYLT-----DOIYSLGELPNLYMPIGIGAFPLPGDLF...
I.multifiliis OKLAKCLLDGLDLGYLT-----DOIYSLGELPNLYMPIGIGAFPLPKQY...
P.tetraurelia OKLSKCILEOLNLSYLT-----SOTYSVDELPMOLYMPIGOGGIVPHKLY...
H.sapiens FOLAAMHLFDPTLEP-----VTEPPANLDRLIPLYKGPVIOGGIIPGGFH...
X.tropicalis FOLAANMLNIFDPTLEP-----VIEFTDFDRLIPIYKGPVIOGGIIPGGFH...
S.kowalevskii FLLASTMLRFLDPTIEN-----DIVPPEPHNLIPIYRSKAVOSGFLPGDY...
S.purpuratus RSLASTMLRFLDPTIEN-----DVAPPPPEHCLIPLYOGAKVCGVLPGLH...
C.gigas VHLATEMLRFLDPTLDQ-----QTAPPEESLNLPIYRNPKIQGGIIPGCYY...
N.vectensis DELARSMLALFDPTLEG-----FMPGPPVEEEDKLVPLYKPKMVASVLPGGYH...
T.adhaerens LKLAETVLSILDPMLES-----EAPDAQPDIIIPVYNHPTFLTKLPNGYT...
T.cruzi TSLAEIILLGVFLDEFADKNLREVGDKEEETIAAONQLYKRVLOENGSG---NGADFLMSGGALSTEE...
T.brucei TNLAEVILGTGFEFATPNLOSCTDKENELLAHNELYSKVLDENGSRNANYGVDLNSLSAEKDAHEIAKONQLK...
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-----KPAIPPLPLEVOMAQPNYGRELVTGSAKN-----GTYFRIRHINKYKMMVETITCLSR...
X.tropicalis YLHVM-----KPAIPPLPLEVOMAQPNYGRELVTGSAKN-----GDYFRIRHINKYKMMVETITCLSR...
S.kowalevskii YLDYG-----KPSLQKSDALMAQDYGEEELITGHPDKDG-----SEYFRIRHINKYKMMVETITCLSR...
S.purpuratus YLHYG-----KPGLDMPLEVOMAQADYGRELITVDTEQ-----ONYPFRIRHINKYKMMVETITCLSR...
C.gigas YLHVA-----KPGLDVPLATOKEOPYGKEYLTSNPGGK-----PDYFRIRHINKYKMMVETITCLSR...
N.vectensis YLHYG-----KPGLDMPLEVOMAQADYGRELITVDTEQ-----ONYPFRIRHINKYKMMVETITCLSR...
T.adhaerens YLHTG-----KPSLNIISLDSMMVQPNYGHVLTGSHVDTI-----OGYFRIRHINKYKMMVETITCLSR...
T.cruzi FFSRSIDFKPERCLRLFYSNIEENKPVVTVMEELASPSMNIEDLPTRIPE-----ONLQVYIYIDEOTHLIDAVVYFGNGAPEMHNVCILGMPSSL...
T.brucei FFSRTRIFFDPAQCTRLRYSYCIEDNKPVYDDITASYOVATPADRSIYKDV-----ODLVIYLNKHTRLIDAVVYFGNGAPEMHNVCILGMPSSL...
T.castaneum YLNVH-----KAGRPVPLDVATNKNYGRVLTGDCCKLKD-----OGYFRIRHINKYKMMVETITCLSR...
C.reinhardtii FVYAG-----CPAALORPSTAAPEGGYEMKTASE-----RGLTRITLDGEGRVHSLMYLGRV...
-----AVNAPRLGSLVGLHANYL

1783

T.thermophila NQLDVRFKNGLIP-----NISEFLSENWAIGLYHEWFSFRHITKMEMLQSENI---OOILDEAAKYAQ
 I.multifiliis NQLDVRYKNNLIP-----NIVEFLSENWAICLYHEWFSFRYITKTDIOKSONI---OSILDEIGRHAK
 P.tetraurelia NRLDORGG--LIS-----NVSEFLSENWAIALYHEWFSFRHITKSEMLKNELI---DOVLEKSOEYAR
 H.sapiens NNLCAARYDENLIT-----DLYSYFTEPWCLALPHDRFIDLKRELROILASKEEEDLP---SIEQLAHQIIDE
 X.tropicalis NNLCA RFDEGLIK-----DLYSYFMKAWCMAYHDFRFDFOEVREMLAAEQEGRP---SMKOLAQOIADD
 S.kowalevskii NNMLSRFKEGLIK-----DFYEFFKETWCLAVFHDRFGDFRQETRELLIORPAADLP---SLEEKVROLIEO
 S.purpuratus NNLVSRFDEGLIK-----DLYAYFRETWSLAIFHDFRFTDFROEVRELLVORPAVDVS---SLEEKVRELISQ
 C.gigas NNMVSRFNEKLIP-----DFYEFFRETWCLALYHDFRSDFRDEVRELLVTS PGVGMD---SIEDKVREVVDE
 N.vectensis NNLLORFDEGLIO-----DFYSFFOESWCLAVFHDFRNDFREEIRELLVNKPSDDVL---SLEEKVRKMIDE
 T.adhaerens NNVLSRYKEGLVS-----DFYOYFNEPWATAIFHDFRFDFREEIKELLFNKOVEESDET---FELKTRKLLDE
 T.cruzi N-LIYRYEEARIA-----NRDESSHOSKHEGCSHNDTGNGSGLNLLLEYLRLPKLOIFFYDRFTVFYRELREKMRDHKDLID---AKARAFEFLEETEE
 T.brucei N-LIFRYNEARTDLLESDDCGSNSSMSGSKSTASNKIDIVAQESTLNLMEYLRSPRLOVVFYDRFVFEYENLRKKMOEHEDVMK---MKOSALORMEVT
 T.castaneum NHLLLRIDLGMIP-----DMFOFFDEPWACAIYRDFPRVIDEISELLLTAEETNEG---VKLVDEVRAOLE
 C.reinhardtii NSLAPKYQAGDIK-----CLLSFITEPWSSELLYNESFPELRETLLEVALAELSAGG-----REVDGG

1882

T.thermophila GGGFLD-PEFIEKIKAKISPE--IISQIQOGLDFIRENONHLP-MYYVPPKKNVD-----
 I.multifiliis SGOFLV-QELFOKIRGOISPE--IIIOIQEGILDFIRENONHLP-MYYVPPKKTMDSE-----
 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-DORRNLSDNVSSP--AKKAIEORLLSPLSYNGYHLP-MYAKPGMV-----
 T.adhaerens DFTFTK-KELOQLGEDYIKS--CKSSTEKRLLTYSNTYHLP-MYAKPGIV-----
 T.cruzi SILSDGCRKKNLNELTAESSS--FARVOYELIKFLHESKDYVPOIYFLPDIRSHVK-----
 T.brucei PRISAKNRAIYLEKLTTEMKRD--FARVOYELIKFLHESKEYLPQIMYLPDI TEHVKEKNEGROE-----
 T.castaneum KNDWNPLTKIQTDEIEEMCRDTSLIPLIERKVLDLFDNFMYLPMYVHOLLIRWILHQIQTSPLFVKTTTFLSNTRKSFSPGIPFLMRPTQSRME
 C.reinhardtii MVE-----WVTHAQDAVLEFARAHAAELP-GYTMP SAART-----

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.

619
H.sapiens ---RPPSSASTFLGFPYIKPKCKLVHLOR---EGITVLTITDSYIVTGDIKGNIKFYDHTLSIVNWYSHLKLG---AIRTLSFS
X.tropicalis ---HSPSIKATSSIOPHKKKALKMLHLOK---DAITVLSHTRDYFVTDVVOGHVKFYDORLQLVNWNYSHLMLR---PVRISFSF
S.kowalevskii ---RPIISGPPSLPSPNKKAFKLVRLQE---RGITVLTITIDNVYVADVLGHVKFYDWHLRMSNWNYSDFDVG---PINSLSFE
S.purpuratus ---RPTSSEPPSLPSPENKAFKLVRLQE---RGITVLTITIDNVYVADVLGHVKFYDWHLRMSNWNYSDFDVG---PINSLSFE
C.teleta ---MKKGTTEGLKLNFLSIAYSDKKATKLVRLMOE---RAINVTITTEPRFIVAGDVLGHVKFYDWSLKLNLNWDHFLG---PINSLSFA
T.adhaerens ---TK---GKDKRRLPKKALKIVRVOE---KAITYLTTIGNYIVAGDCLGHVKFYDTEMLRNWNFNINAG---PINSLSFA
T.thermophila ---LS---LTIIEKVDTPKERRAIKLVLDLNM---RKKPKDGGKNGSINILKIDNLYVIGSSNGSIRFYDFOYRIIAWFEDAIIG---SISNISFA
P.tetraurelia ---VS---LIIIEVGGDERRVIVKLVNLMVANKSETQOKKNGOAILLRVVOGRYLVVGSANSGIRFYDFKPRIIAWFEDVVG---GNCINIOCGISITNLSFA
O.trifallax ---RS---LIIIEGEOEKRLKIVTFNO---NNSPINILMTVHDEYLVVCGNSDGTIRFYDFHFKVVAWFEDMNFSS---TIKISFSF
C.reinhardtii ---EO---GIAAOVTSATDRRAIKLMRIHN---CPITLLATVGFDFIVSGGGEDGYVRFDFPLLRIVAWFEDLAAG---PVTSAVFS
V.carteri ---EO---GITAOVTSATDRRAIKLMRIHS---SPITLLSTVGFDFIVSGGGEDGYVRFDFPLLRIVAWFEDLAAG---PVTSAVFS
T.brucei ---VQ---QRDFVTKEQDKTMLKVMRVHS---SGVSPFLTWSNGYIVTGGIDGDKVFLDPLLRVAVWFEDLKGK---AITSISFD
T.cruzi ---VQ---PRDFVTKEQDKTMLKVMRIHS---TGVSPFLTWSNGYIVTGGIDGDKVFLDPLLRVAVWFEDLKGK---AITSISFD
G.theta ---OV---LLPALDARPSDRCAVKLLKLDH---GHAITPISVTFDSFLVTAGADGQVRFYDLKFRLLAWFEDLDVG---PTASLTFT
T.castaneum ---NT---IFAKNYAGQEDLDNDRKIFVKTIKIEK---SALNCLTSVDEMIVTGDSNGTIFMFFDKRIKLLYVYRSIEK---SIRSMSFV

722
H.sapiens ---KTPAT---PPTPEKSNYPDPDCTLKGDLFVLRNFIIIGTSDAAVYHLTT---DGTLEKLFVPEPKDAICALS
X.tropicalis ---KCPPV---TASGRTRYPODCSIRKGDFAISNFIVSTVDSVVLHVHT---DGTVLEKHEMHPSEAVHALAC
S.kowalevskii ---YLPEF---SYDNYPPDATIYSKPFVTRDYMIQTSTAVIAHVVA---DGTSLKVIHREHDAAVHAVAT
S.purpuratus ---YVPEF---NADAKPDSSEYPKDATIHKASFVTRDFIVSTSNAOVALVVA---DGOVKMILEHDKPIRALTT
C.teleta ---YSSDF---NEINGGSCDYPDPAIIRKFAIRDFVIGTSTAVYGHVHT---DGTDVTVIHKDHDGPHVHALSP
T.adhaerens ---SNLSK---IVDLERNEYPRDAITHGSTVFIPIFVSTSLAQIAHIVS---DGSHEILIKGHDTAINALAT
T.thermophila ---NTPMI---EDNEDEDDGFDDEKDKGENEPKFCDFIVVDLDAITIMLYKLFEEIDDEKKGKTKMLKSIIVSPIIAMS
P.tetraurelia ---NESVE---QEDFFETESDEEKDKDEOSOKKSKPFLCQDFIVVDDRANVILLKSSQFOEIEKEKKGKTIIMSSIVSPLISIS
O.trifallax ---KTEPK---PSTDFODDIDDKTFKSDFLVTDSSALVCLWSSIFEEI---DPORRKGEMIFYGLRSVAIAIV
C.reinhardtii ---AVLPD---RLAHADAADTLNRFMVPDFVATRNRSIVSVOSASFEEDADRRRGSVLDLSDADVDVLA
V.carteri ---AVLPD---RLAHADAADTLNRFMVPDFVATRNRSIVSVOSATFEEDADRRRGSVLDLSDADVDVLA
T.brucei ---RPSGI---AATAMNELRRFESITQKKMVOGYNVAGDFASDFMSTSNAMIIDVSAFAHAGVPELLRGRVVGQENGHVICAA
T.cruzi ---RPFIT---ALQAMDELRRFESITQKKMVOGYNVAGDFASDFMSTSNAMIIDVSAFAHAGVPELLRGRVVGQENGHVICAA
G.theta ---SPRAT---PKDGGYLEGEVRIPNFIVATKAGKIALVNAIFEOYSKEERKGEVLVGFESPIAMCA
T.castaneum ---QNPRRKYIFDESQIRDLERSFEGIPDCIQDRSHEYELLMKQKVPKDATLNNRPFITRDFIISTVDGQIHMNY---SROEFISLFPTESSVAADIV

825
H.sapiens ---HPYOLPIAIGSICGMKIVWNYENKOYLFVSRVFEKQ---LGVOSLTYNPEGALIGAGFTEG---
X.tropicalis ---HPCEHLIAIGSYSGLLKIVWNYKARLFISRIKPKG---KSLHCLSYDPKGFLLGAGFTDG---
S.kowalevskii ---HPSKPFVIGSAGYLLKIVWNYENKQVVRORRFRG---CLVRCVLYDPOGAYIAVGFTEG---
S.purpuratus ---HPSOPFVIVIGSAGYLLKIVWNYENKQVVRORRFRG---NLRICRVDYDPTGSGYLVGFENGCVRILDALTLIDENPEPPFRYSRDAVTHVTFSHDS
C.teleta ---HPTEPRFAVGSYSGLHLOWNYENKQVVRORRFRG---NMRRCCEFPNTRGMIYVGFTEG---
T.adhaerens ---HPTEPRFAVGSYSGLHLOWNYENKQVVRORRFRG---NMRRCCEFPNTRGMIYVGFTEG---
T.thermophila ---RPNNSVIGICENGYLWNYENKQVVRORRFRG---NMRRCCEFPNTRGMIYVGFTEG---
P.tetraurelia ---RPNVPPVAFSCEDNKIYENWYENKQVVRORRFRG---NMRRCCEFPNTRGMIYVGFTEG---
O.trifallax ---HPRRPLAIAIGSICGMKIVWNYENKQVVRORRFRG---NMRRCCEFPNTRGMIYVGFTEG---
C.reinhardtii ---HPTRAFAVGLRGGGLORWDSIAHCLVGRAPFER---OVGACLYSRDGSLLVGFSGG---
V.carteri ---HPTRAFAVGLRGGGLORWDSIAHCLVGRAPFER---OVGACLYSRDGSLLVGFSGG---
T.brucei ---HPKLSRLAVAGHSGGLVWMDYLLKRVVMIIVPRG---VEINCMAPDFEGLVLAIGCTNG---
T.cruzi ---HPKLPOLAIAIGHSGDVHWDYLSKRVVMIIVPRG---SHINCMAYDPOGVYLAIGCTNG---
G.theta ---YPSSSKLFVVTITSGMLRVLDFEISOAWLOOKVLEK---RFTPTICVMPNGOELVIGCTDG---
T.castaneum ---HDEKPFVIVAYTKGHLLIFNYETHEEVVSSNIPETEPE---TITISIKYSPQSLHLACGRNG

928
H.sapiens ---IVYILDAMSLENE---SPEPFKYSRVSVTHISFSDHSDQYMATADRSFTVAVYMLVVRNGORV---WEYLARLRSHRKSIRSLFLGVY
X.tropicalis ---SVYILDAMTLEDE---TAEPPKYARSGIITHAFSDHSDQYMATADRSFTVAVYMLVVRNGORV---WQYFGRYRSHYKIOQSLVFGI
S.kowalevskii ---SVRILDALTLDEE---SPEPFKYSRVSVTHISFSDHSDQYMATADRSFTVAVYMLVVRNGORV---WYMLGKORAHYKIPVGRIMGRIT
S.purpuratus ---KWMATAVSVGFENGVCVRILDALTLIDE---NPEPPFRYSRDAVTHVTFSHDSKWMATAADAFCVTVYKAEPSNQEPE---WVMYMGKRAHYKHIRGITFCVA
C.teleta ---SIRVVDATLTHDM---LEEBIRYARDAITHAFSDHSEFVATADAENTVSLKKG---DTEEP---FEYVGRYKAHYKIPDLTFLGIC
T.adhaerens ---TIRIMDAITLBDL---POALFKYKSDSISAHCFSDHCKYLATADDDPAVSVYKRSOLKNDPEP---WVYLGROARAHYKIPDLTFLGIC
T.thermophila ---KIHFIDCKEKQWO---NSVLEFSENEQDSKALMTADADYVSLFTIDHROFDVNIPEKE---WQFVGRYKAHYKIPDLTFLGIC
P.tetraurelia ---KIYFOKLTKLNO---KOPIQLLVLIION---LILLMLNLMOSV---FLIFDFDPSNLSKOOKE---WIFACKYRHHGKIKSVAFGET
O.trifallax ---VIOVMDPNTGMYKKSQPLKTTESLVSPITOMIMSNDGQYFATMDKACCVCLFKKDHLOQDSSKPLEWOFNGKCRSHHGKIPASVFCES
C.reinhardtii ---HLHILNADDCSDL---VYMRNTAAGLRLVAVSNTGKHIAAADENHOLLLYAYLPYKHTMR---WEYVGRYKAHYKIPDLTFLGIC
V.carteri ---HLHVLDDGTLDAT---YVLRNTAAGLRLVAVSNTGKHIAAADENHOLLLYAYLPYKHTMR---WEYVGRYKAHYKIPDLTFLGIC
T.brucei ---VVKPLDANLEER---KSIKPKRPSITIRMFASGRLLATGDDTGCVSLFWYEHQGNSTSKAMGDVWVGRKHTRKGTITGLQFDD
T.cruzi ---SGLFLDATTLEER---KSIKPKRPTDCVTRMAFDAGNRLATGDSAGCVSLYFEDHPQGNITKPMEDWIVGRKHTRKGTITGLQFDD
G.theta ---SVLIIGVSDLELLI---GRMS---QIDCEIITSVOFAPDGMHVAVADAHNCVGFRRERNVLESCKEVPWLYVGRYRSHHGKIPVGLQFILD
T.castaneum ---ELWLLQPATLGO---IYVEPFRFSNDKDSNNTIFGFYDCESED---WLFKGVRSYKIDVCNMLFLDE

1031
H.sapiens ---LD---SNEPRLLSLGTDRLLIYDLLRSYKDH---LEVLDIHHTDQCCYPT---CMVWYPPITRELFLIICNSGYKVKLFN
X.tropicalis ---LD---TNEPRLLSLGMDRMLVEYDLANCVKGY---LQIKSTDRIEQSAVPO---SLAWYPPVTRKECFLLVANDHYKMKLYN
S.kowalevskii ---LD---SEVPRLLSVGEDRTLVEYAIFFSTTND---LQISSDRIEQSAVPO---CFMTYPPITKENFLVANDHYKMKLYN
S.purpuratus ---LD---SGASRLLSLGEDRALVEYENLKTVAKDH---LEVMDTRIEQSAVPO---CFWYPPVTRKEOFFITANNOQFKKLFN
C.teleta ---LD---SNVPRLLSLGQDRVLYVEYDLNQSRRDD---LRLLSDRIEQSAVPO---CMAWYPPVTRKEOFFITANNOQFKKLYN
T.adhaerens ---LD---TNLPRLISIGKDRVLYVEYDLNKSQKDD---LQMLSTRIEQSAIPT---CITWYPPITKENFLLANDQFKKLYN
T.thermophila ---KNENDKRYRFLSIGADMVMVEYVLEIDYSKQKQKQOINYLIRLKLKSYVPIEOTETPT---ACIWPINMYKEDVLLTNEEFKIKLWO
P.tetraurelia ---LDEKNOVOLKVFITIGEDMKLAVYDLDPTDDP---YNRVKLSRVPIEOTECPLS---ACIWPINLQREDDVLLTNSFVKLKLWT
O.trifallax ---LDENBQTRRLRFLSIRDRRLFEYDVYASNLKE---LIVTSMFKIEKESHT---ACIWPYKVDTRKEDLLMTVNDYKMKIWN
C.reinhardtii ---P---SGOTRLLSVGGDGRVVEYDLAASSVAA---GVOVASFYDFPPGGGAP---TSLSFAPPLAYFOAFAADTHLLVSDSYKIRVFN
V.carteri ---P---SGOTRLLTVGADSRVVEYDLATSSVAA---GLRPLSYDFPPGGGAP---TSLSFAPPLAYFOAFAADTHLLVSDSYKIRVFN
T.brucei ---SGLHRLLSVGEDORLVEYDLIDSEPEP---GLLVRSAAHIAQSSPTGFLWDEDGIIISDVSRP---DAAHITINGLLIANSYKISAYF
T.cruzi ---SGLORLLSVGEDORLVEYDLIDSEPEP---GLLVRSAAHIAQSSPTGFLWDEDGIIISDVSRP---DAAHITINGLLIANSYKISAYF
G.theta ---KA---AGIIRVLSLAQDRVVEYDLSSMAAT---GVVVRCAITIEETAVPT---ALLQIPPGCQOGEDNPKIPVLVDIIGLTVGHVNRPL
T.castaneum ---EP---FGLYTIKDRHLIRYTNIAAEYLIYLGGQPTFLSEELDISTRDRIEQKDTPI---YFTPYIRKFKSKCGYFLIADDKHYKTYN

1134
H.sapiens ---ATTKMCRKTLGLPAY---SPIEOTOVLP---VRSMAELORR---YLVFINRDKV---GLOILPVDGNPHKTSIVCHPNVAGMAVSYDGCYAFATAGGHRDRSV
X.tropicalis ---ATTKMCRKTLGLPTFG---SPVRRMEVLP---TYSBEICCKG---YMTYITDDKV---GLOILPLDGNPHKSMLSLICHDPGVSNLACSVDGCFVFTAGGDDCTVL
S.kowalevskii ---STTKMCRKTLGLPTFG---SPLORMEVLP---TEGAVDSKR---YLAITITNDKV---GLHSMPLDGNPHNMAALIAHPGVNVLACSVDGKHVFTAGGDSVSH
S.purpuratus ---TTTKMCRKTLGLPTFG---SPIQOIAVLP---SEDPVNDPR---YMAVYTKDKV---GLHSPDGNPHNMAALIAHPGVNVLACSVDGKHVFTAGGDSVSH
C.teleta ---STTKMCRKTLGLGPAFG---SPVOKIAIPL---NNSDDASAKR---YMAVYTKDKV---GLHSLPLDGNPHNMAVAFISHPLGVNLCSSYDGLITAGGSDATVFL
T.adhaerens ---STTKMCRKTLGLPTFG---SPLQKMEILL---OENPNMNR---YLAFTITNDKV---GLOILPLDGNPHRTMAMLAHPGVNVMACSVDGQHIPTSGGSDTVF
T.thermophila ---FMKDNFKCKTKCLGPIYG---GAINKLLILNDAONDYODK---YLAYSTKEKVV---GIKPLLEGPNMOTMGLIAHPDKITISCSNDGKHFPSSGDDYCVN
P.tetraurelia ---VLRDLRIICKTKCLGPIYG---GHIKRLLILNPSDMKNEYODK---YLAYSTKEKVV---GIKPLDGNPNRTMGLIAHPKIVLDLSSDQDGLKFTSGGDDPAIN
O.trifallax ---VSTKMRNRKCLGPIYG---GETIVLKLKLD---IDNNDPK---FLIYTSKTKVV---GLTKMPLDGNPNRTMGLIAHPSEVADICATSDGRYIFTCGGEDLAVN
C.reinhardtii ---PDCPAVEATFLGPTFG---GPISOLVMPK---SPSAAASDGAFLAYRTSERVGLTAWPLDGDPAITMGLIAHPGEVRAITISYDGRKLLTAG---ADGTVA
V.carteri ---PDRPAVEATFLGPTFG---GPISOLVMPK---SPASDSAFSLYATREKVVGLTAWPLDGDPAITMGLIAHPGEVRAITISYDGRKLLTAG---ADGTVA
T.brucei ---SDNSROCKIVLAPTFG---GPVTEMFTVP---THPGSDKSLFYATREKVI---GLTAWPLDGDPAITMGLIAHPGEVRAITISYDGRKLLTAG---ADGTVA
T.cruzi ---SDNSROCKIVLAPTFG---GPVTEMFTVP---LLPGSEKRCFLYATREKVI---GLVCLPLDGDPCMTGTVVAHPGNITSVARSYDGAFAVFTAGGDDQSM
G.theta ---EEDSHCLOTLLGPTFG---PPPHRLTLIP---PDEGQYVYVATPKVV---GLTKLPLDGNPNCSMGLIAHAGEVAEMCASDHGKLVLTGGSDCGIF
T.castaneum ---AKTMCTAVLGPAYGCFKDSVCKMEFLP---KSNRKYIYMPHTKKOIT---GILQMSIDGNPFVKGSLGHPDELDFOMCKDQKYVFTIGKNDRSVF

1237

H.sapiens **QWKIT**-----**LSVLEAAVSLGGEDLTPFYGLI**-----**SGGREGKIFYRELEDYFYYSOLRSOGIDTME****TRKVS****EHI****CLSEL****PVVMRAIGFYPS****EEKI****IDDIFNE**
X.tropicalis **MWKTN**-----**LOALESVSSLGGDGLTPFYGLI**-----**GGREGLLFKELENYFYAOLRHOGIDTME****TROVSTH****IPLKEV****PFIMRALGYPS****EOEVEN****MLNE**
S.kowalevskii **MWNVN**-----**LMVLEAGSKLGGEDLTPFYGLI**-----**EGGREGELFAELEDYFYAOLRSOGVDTMDSREI****STK****IPLTEV****PFVVMRALGFYPS****EOEIED****MLNE**
S.purpuratus **MWDTH**-----**FNALEAAVKLGGDDLTPFYGLM**-----**EGGREGELFKELENYFYAOLRSOGVDTT****DROVSTL****IPLSEI****PFVVMRAMGHYPS****EOEVED****MLNE**
C.teleta **MWAIN**-----**PAALEAAKLLGGEDLTPFYGLI**-----**DGGREGDLYKELEDYFYAOLRSOGVDTME****TRKVASSI****PVTEI****PFVVMRAMGFYPS****EOEIED****MLNE**
T.adhaerens **MWKTN**-----**IEALEAAVHLGGEDMVPFYNLM**-----**DGGREGELFAELENYFYAOLRQIDTME****SRKVS****TKVALR****QIPYIMRALGFYPS****EOEIDD****MVNE**
T.thermophila **MWSVN**-----**VOALEQIFATNPNE****DPYPKLI**-----**EGGEDGOTHQDLKNFFYYSOLRSKDEHTT****KARKL****DGKVP****LIAIPDMMR****SMGYYP****INKEI****ENMNE**
P.tetraurelia **IWSVD**-----**FAALEDNFOTETEYEIFSNLI**-----**DGGREGOTLRDLKDFYYSOLRSKDEHTT****KARKL****DGLVPL****TAIADMMR****SMGYFP****INOEI****ENMNE**
O.trifallax **MWSVD**-----**VNPIDQAIMGGEGLEPPFNLI**-----**EGGRDGGOTFQDMKDFFYAMIRAKDENTT****KRKL****NGTVPL****DEL****PNL****MRAMGYYP****TEQEV****TNMKDE**
C.reinhardtii **SWDIN**-----**TAPLERSATAAEGAGGEARWAIVL**-----**GDPDLLREMRDYFVYAOIK****TGEGDALE****PRDVP****GTVP****VDL****VP****DL****MR****SAGFYPS****ESD****ID****NLLHH**
V.carteri **SWDIN**-----**TSPLERSAAAASVSEGEARWSEVIL**-----**GDPDLLREMRDYFVYAOIK****TGEGES****LQPR****AV****PGL****VP****VE****IV****P****DL****LR****AA****GFYPS****SEAD****ID****H****L****T****N****H**
T.brucei **QWRVNGN****ILVPEE****ASK****L****S****A****S****V****A****E****G****N****G****V****P****L****D****H****L****L****A****V****E****G****G****R****E****G****E****F****M****R****E****I****V****D****Y****F****Y****A****O****I****R****L****Q****G****E****E****T****T****A****K****R****E****L****L****G****A****V****P****F****S****O****V****N****L****F****R****A****L****G****Y****P****S****E****M****E****L****G****R****L****T****Y****E**
T.cruzi **QWRVNGEG****I****A****P****P****D****E****I****A****L****G****S****A****F****I****E****G****G****D****V****P****L****D****H****L****L****A****A****V****E****G****G****R****E****G****F****M****R****E****I****V****D****Y****F****Y****A****O****I****R****L****Q****G****E****E****T****T****A****K****R****E****L****L****G****A****V****P****F****S****O****V****N****L****F****R****A****L****G****Y****P****S****E****L****E****I****G****R****L****T****Y****E**
G.theta **LWEVD**-----**PSALEASAALGSGTEPFEALTI**-----**PGGRGGELYDEMDYFYLAOLRAOGEDT****TE****ER****K****V****T****G****L****A****P****V****E****A****V****P****E****L****M****O****A****F****G****Y****P****S****K****F****E****I****R****D****M****V****H****E**
T.castaneum **MWRVK**-----**TASVETMHLGGVLELQPYCLTI**-----**EGGRAGFLYQEMRDLFYMQILQOGE****Q****I****C****A****P****R****V****V****S****N****C****I****N****L****G****E****L****P****D****L****M****R****A****C****G****Y****P****S****E****Y****E****I****E****N****L****L****T****D**

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H.sapiens **IKFGEYVD****TG****K****L****D****K****I****N**-----**LPDFLKVYLNHKPPFGNTMSGI****K****S****F****E****V****L****G****Y****T****N****S****K**-----**G****K****A****I****R****R****E****D****F****L****R****L****V****T****K****G****E****H****M****T****E**
X.tropicalis **VKFS****EYVD****TG****K****O****V****T****C****I****S**-----**LEELIKLYINHRRPAFLG****F****M****E****I****O****A****F****O****V****L****G****F****T****N****G****D**-----**G****K****O****T****I****N****R****G****D****F****L****O****I****L****O****S****R****G****E****H****L****T****E**
S.kowalevskii **VKFS****OYV****TG****K****Y****V****T****D****I****D**-----**LGEFIKLYINHRRPAFLG****S****I****S****O****L****R****A****F****E****V****L****G****D****D****G****T**-----**G****V****P****A****I****D****R****G****E****L****L****D****L****O****S****K****G****E****H****M****T****E**
S.purpuratus **IKFS****EYVD****TG****E****V****T****E****I****E**-----**LGTFIKLYINHRRPAFGI****S****E****O****O****L****N****W****A****D****I****L****G****A****E****D****E****T**-----**M****E****P****E****I****R****G****E****L****L****E****L****O****T****K****G****E****H****M****T****E**
C.teleta **IKFS****DYV****TG****K****Y****K****T****S****V****D**-----**MGEFIQLYINHRRPAFLG****O****P****D****K****L****A****W****A****F****E****T****L****G****V****T****M****D**-----**G****L****P****F****L****D****R****S****E****L****L****H****L****L****O****N****R****G****E****H****M****T****E**
T.adhaerens **IKFS****EYI****E****T****E****K****F****V****E****I****D**-----**LESLIKLYINHRRPAFGI****P****P****R****L****N****E****V****F****K****I****L****G****I****E****O****N**-----**G****O****Y****T****I****D****R****G****D****L****L****K****V****L****O****S****K****G****E****H****M****T****E**
T.thermophila **IRFS****KY****L****E****P****G****E****V****E****L****D**-----**LNMFILKLYINHRRPVQ****G****I****G****K****S****K****I****A****E****S****L****N****T****L****K****S****L****S****D****S****A****K****E****I****A****E****O****A****K****G****S****V****O****P****K****T****Y****N****D****I****G****I****G****E****I****S****L****O****D****L****K****K****L****I****T****E****G****E****R****M****T****N**
P.tetraurelia **IKFS****KY****L****E****T****G****E****O****I****S****E****L****E**-----**INTFLKLYINHRRPVNG****V****T****K****O****I****Y****D****A****L****K****T****L****A****T**-----**D****T****G****V****I****P****W****D****O****F****T****E****L****L****K****T****K****G****E****K****L****D**
O.trifallax **VKFS****N****F****A****E****T****G****O****H****V****N****A****V****D**-----**LNTFIRLFVNHRRPVY****G****I****G****K****N****N****I****O****E****A****F****O****E****L****F****S****N****N****V****D****R****K**-----**M****N****G****T****I****S****K****E****Y****L****L****K****M****L****O****O****D****G****E****P****M****S****V**
C.reinhardtii **VOY****M****A****H****S****R****N****M****E****S****L****E****V****V****T**-----**LADLLCLYINHRRPLF****N****V****T****H****A****D****I****V****A****A****F****R****E****L****G****G****R****D****G****P****G****S**-----**C****V****P****K****L****S****R****E****O****L****L****S****L****O****S****T****G****E****P****M****S****G**
V.carteri **IQY****M****A****H****G****R****D****L****D****A****M****P****G****V****S**-----**FADLLLYVNHRRPLF****N****V****T****H****A****D****I****V****O****A****F****R****D****L****O****G****A****G****S****P**-----**D****N****P****R****L****T****R****E****O****L****L****T****L****R****T****A****G****E****O****M****G****A**
T.brucei **VAN****L****Y****G****P****L****E****E****L****V****E****C****D****V****S****S****I****P****L****K****S****O****F****M****R****L****Y****N****R****P****I****G****I****S****R****O****A****I****E****O****A****F****L****V****G****A****D****A**-----**V****T****G****O****I****S****R****D****V****L****F****K****K****L****T****H****G****E****P****L****O**
T.cruzi **VAN****L****Y****G****P****L****E****E****L****V****E****C****D****V****G****K****I****P****L****K****S****O****F****M****R****L****Y****N****R****P****I****G****I****T****R****O****D****I****E****S****A****F****W****L****G****A****D****A**-----**E****T****G****O****I****A****R****D****V****L****F****K****L****L****A****T****H****G****E****P****L****O**
G.theta **VRR****K****G****K****D****S****V****T**-----**F****S****D****L****I****K****L****F****V****N****H****K****P****V****N****D****V****S****L****E****I****O****A****F****H****H****L****G****A****E****P**-----**V****T****G****I****L****K****W****E****O****L****R****M****L****M****R****M****G****E****S****L****S**
T.castaneum **MRY****K****E****Y****D****E****N****G****M****L****K****D****R****V****T**-----**F****C****E****F****V****K****L****F****I****N****H****K****P****A****Y****G****T****I****E****S****L****E****S****F****E****T****I****T****F****S****D****E****Y**-----**G****A****S****D****M****S****R****E****D****F****I****E****A****T****I****T****G****E****O****V****S****N**

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H.sapiens **EEML****D****C****F****A****S****L****F****L****N****P****E****G****K****S****E****P****A****T****C**-----**S****V****K****G****S****E****I****C****L****E****E****L****P****D****E****I****T****A****E****I****F****A****T****E****I****L****G****L****T****I****S****E****D****S****G****O****D****G****O**-----
X.tropicalis **EEI****A****E****Y****L****S****T****L****L****G****L****N****P****E****G****S****S****E****M****G****S****Y**-----**D****S****T****G****A****A****D****L****L****E****O****G****I****P****E****I****T****T****D****M****F****A****A****D****I****L****G****L****P****F****O****V****N****G****N****Y****E****E****H****T****V****T****K****E****T****A****S**
S.kowalevskii **HD****L****A****E****F****L****T****L****L****G****N****P****E****G****S****S****E****L****G****L****Y**-----**D****P****A****N****A****G****D****I****I****E****D****N****L****P****E****S****I****N****S****D****M****F****A****S****O****V****L****G****F****Y****D****E****S****A****E****O****R****A****R****O****E****S****P****T****D****V****K****S****P****A****E****V****M****M****I****H**
S.purpuratus **VE****V****A****E****Y****L****T****L****L****G****V****N****P****E****G****S****S****E****L****G****G****Y**-----**D****H****A****G****A****D****I****L****E****E****A****L****P****E****E****V****T****A****S****M****F****A****V****E****L****L****G****F****G****G****-****A****A****E****P****S****E****G****I****V****S****P****T**
C.teleta **Y****O****L****A****E****Y****L****T****L****L****G****N****P****E****G****S****S****E****L****O****D****F**-----**D****T****E****K****A****G****O****L****I****D****E****N****L****P****V****E****I****T****A****D****M****F****A****N****E****L****L****G****F****M****Y****S****S****D****V****L****O****V****E****G****E****S****O****S****P****P****S****A****O****V****N**
T.adhaerens **S****E****L****A****E****C****F****G****T****L****L****G****Y****S****D****I****G****S****L****E****T****A****S****Y****P****T****D****N****S****E****T****L****L****E****O****L****S****E****N****I****S****A****N****T****F****A****E****O****V****L****G****F****I****S****V****D****O****T****E****T****O****N****E****A****N****S****N**
T.thermophila **E****F****F****D****E****C****L****K****I****L****G****E****N****E****D****-****-****O****I****P****O****O****I****N****V****N****N****L****C****E****D****L****L****F****E****D****L****E****G****E****R****D****D****N****I****E****D****O****Y****E****D****E****N****E****Y****D****O**
P.tetraurelia **Q****E****I****E****Y****L****E****S****L****L****P****N****K****-****-****-****N****Y****P****O****O****L****N****L****N****L****C****D****E****L****L****G****F****E****D****M****D****G****T****O****E****O****O****E****O****E****V****O****D****V****G****S****E****D****E****O**
O.trifallax **Q****E****L****E****A****L****H****L****L****V****G****D****S****N****F****K****T****A****L****T****S****E****V****T****A****D****Y****F****A****E****V****L****G****F****E****V****E****E****L****E****E****D****G****G****A****G****G****Y****D****A****O****T****A****M****M****G****G****S****Y****S****O****N****M****G****M****G****L****A****N****D****V****I****P****E****E****D**
C.reinhardtii **E****E****L****T****A****A****L****A****A****L****T****G****A****P****T****P****E****K****S****M****P****V****S****V****A****A****E****O****F****S****A****D****V****L****G****F****D****T****E****A****G****A****E****A****A****T**
V.carteri **D****E****L****A****A****L****A****A****L****T****G****A****P****T****P****E****K****A****V****P****P****V****V****T****A****A****O****F****S****S****D****L****L****G****F****E****L****O****G****G****E****G****A****A****V****A****E****A****A****O**
T.brucei **T****E****I****T****A****A****L****R****S****L****L****G****E****D****V****K****L****D****D****I****O****D****T****I****T****A****R****L****F****A****E****N****L****L****G****F****E****D****Y****D****A****M****A****O****G****D****D****G****G****E****E****M****S****L****O**
T.cruzi **E****E****I****S****A****A****L****R****S****L****L****G****E****V****R****L****D****M****L****E****D****T****I****T****A****R****A****F****A****E****N****L****L****G****F****E****D****Y****T****V****G****A****E****A****K****V****P****E****S****S****O****G****N****D****D****E****L****F****L****D****E**
G.theta **S****E****I****O****T****A****I****A****R****L****L****T****G****S****S****L****S****G****R****E****D****M****A****S****T****F****A****E****N****V****S****M****C**
T.castaneum **E****Q****I****F****R****C****L****I****L****L****H****E****T****P****D****O****F****G****E****R****D****F****S****F****M****P****D****T****I****D****F****D****Y****L****T****E****D****I****L****G****I****D****M****D****O****I****E****R****L****E****K****D****D****D****N****D****D****D****R****G****G****Y****D****F****F****Y****R****S**

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).

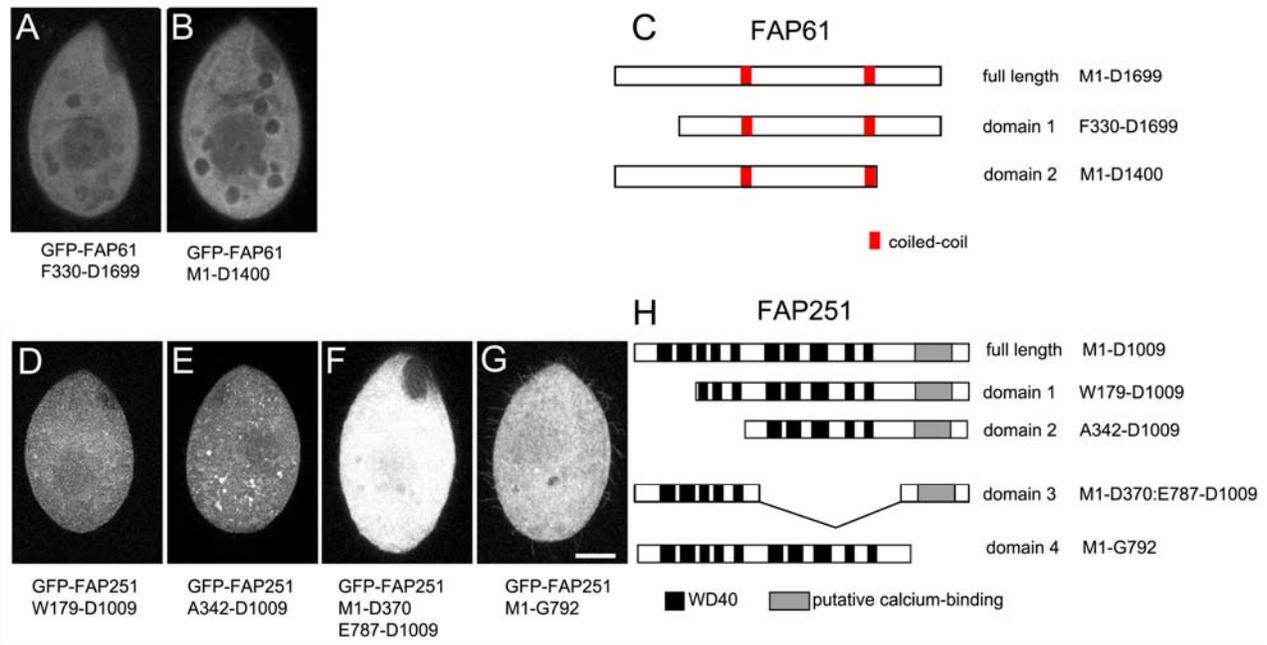


FIGURE S3: Domain analysis of FAP61 and FAP251. (A and B) Localization of overexpressed GFP-tagged truncations of FAP61; FAP61 that lacks either N- (A) or C-terminal (B) fragment accumulates in the cell body and is not targeted to cilia. (C) Schematic representation of the domains of *Tetrahymena* FAP61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

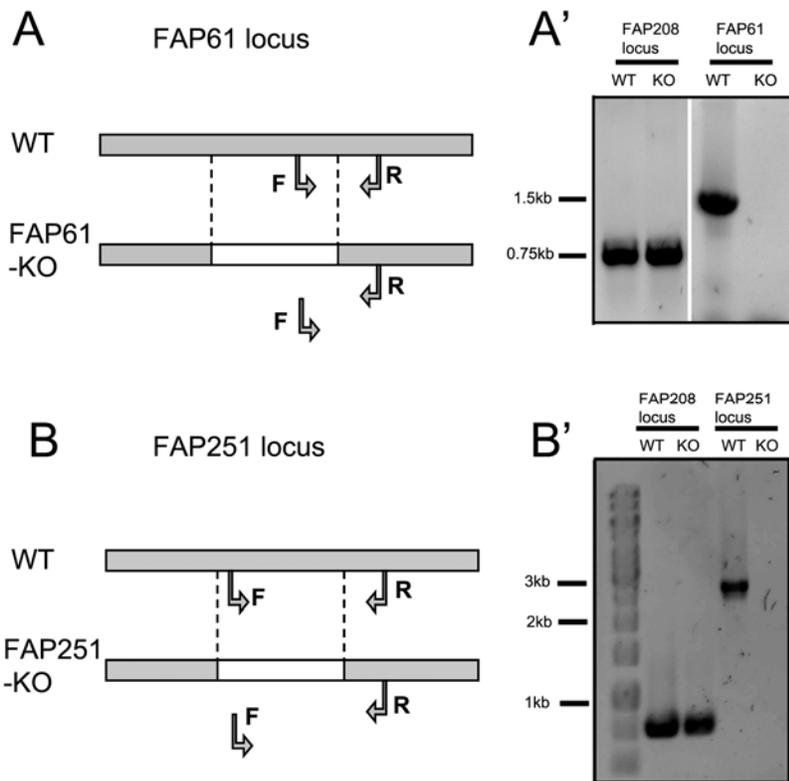


FIGURE S4: PCR analysis of the FAP61 and FAP251 loci in wild type, FAP61-KO and FA251-KO cells. (A and A') Analysis of the germ line FAP61-KO cells. (A) Scheme of FAP61 locus in wild type and knockout cells. (A') PCR analysis of FAP61 locus with primers indicated in (A) showing that part of FAP61 coding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

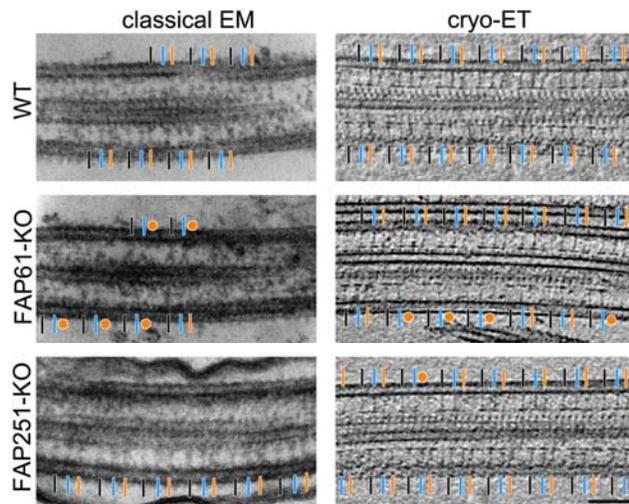


FIGURE S5: RS3 is lost in a subset of FAP61-KO repeats based on both classical electron microscopy (EM) and cryo-electron tomography (cryo-ET). Longitudinal sections of chemically fixed cilia (left column) and longitudinal tomographic slices of rapidly frozen axonemes (right column) from wild type and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

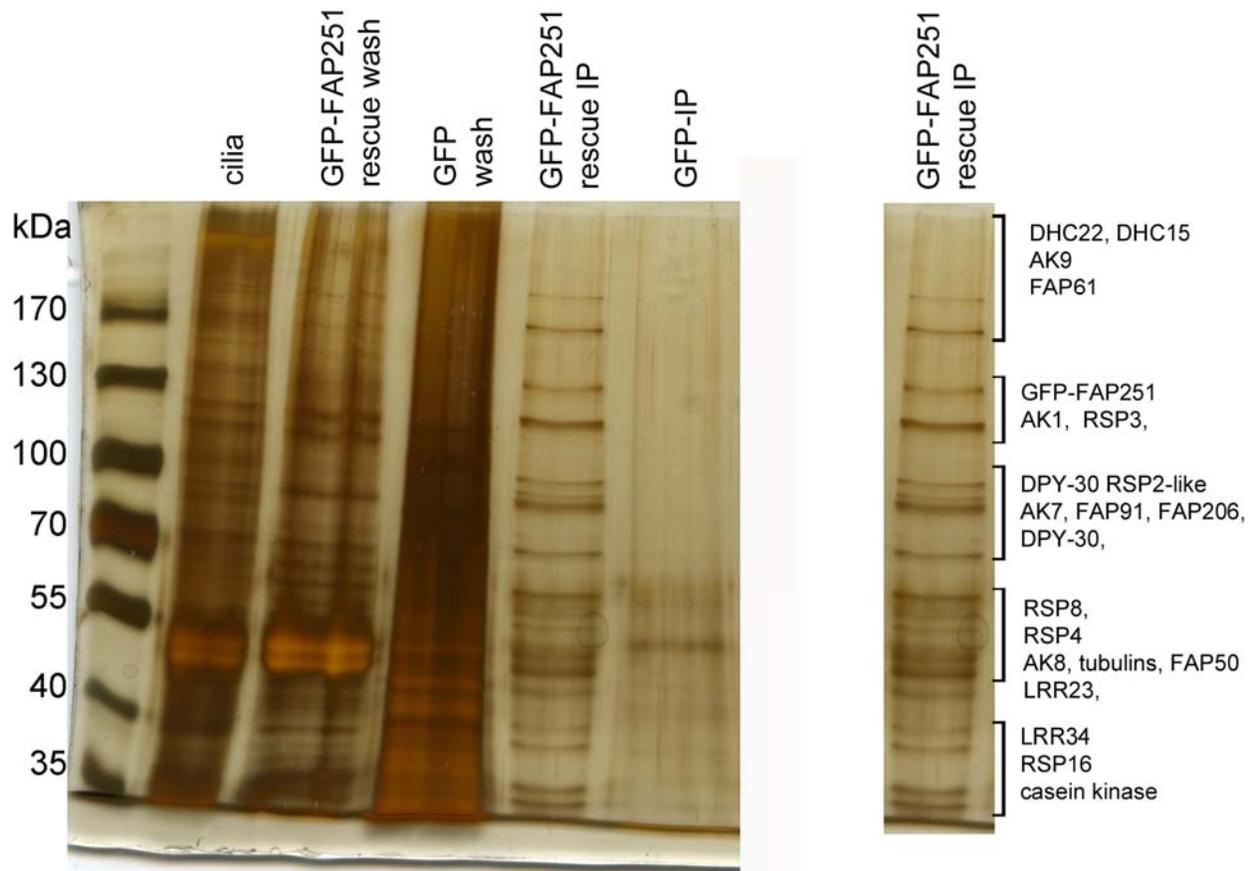


Figure S7: GFP-FAP251p co-immunoprecipitates with numerous proteins. Silver-stained gel showing cilia isolated from FAP251-KO cells rescued with GFP-FAP251 (line 1), proteins that were washed out from the resin (unbound proteins) in the experimental (FAP251-KO cells rescued with GFP-FAP251) and control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