

С (i) cilia

OAD complex







В



Fig S2





Α

βHC-C-hGFP





Fig S4

50 nm



В







Fig S6



CC1 85 22 50 * VGLRKIKETVDQVEELRRDLRIKSQELEVKNAAANDKLKKMVKDQQEAEKKKVMSQEIQEQLHKQQEVIADKQMSVKEDLDKVEP NGLHKLHKVQADVDILVEEAKVKAVEVEHKVASANIFAEQVGVEKEKVNAENAAAQVEAEKCAVIAKEVSEKQASCEKDLAAAEP NGIDKIAQAAAQVTDLQRVLKEEQIVVDEKKAQTDELIVSIGKEKAIVDQAVEAGREDEEAATALQTEVSAFQAECERDLLEAEP **GGLQKMFEAKADVNKMKAELAVKNQDLAVSAKEAEALLKQISESTAIAEKEKQKVAVIVDAVTKKASEIATVKDDAERDLAAAKP** IGLNKIQEATITINQMEISLKEEEIQLNEATEKTNQLLANLDKESKKANQKGEEVAATNKQCEIQAEQISKEKEEAERELEAALP TGLEKLKEASESVAALSKELEAKEKELQVANDKADMVLKEVTMKAQAAEKVKAEVQKVKDRAQAIVDSISKDKAIAEEKLEAAKP IGLDKLMEASESVAKLSQDLAVKEKELAVAS I KADEVLAEVTVSAQASAK I KNEVQEVKDKAQK I VDE I DSEKVKAESKLEAAKP IGLDKLQVTEESVTGMKEELIALQPQLEESTRQTEAAMEVISKESVEADKVKQVVSKEEATASAEAATVKAIKDECEADLAEALP

	MTBD	H1	H2		H3
mouse_cyto	AVIE <mark>AQNAVKSI</mark> K	K <mark>qhlvevrs</mark> man	PPAAVKLALESICLL	LGESTTD	<mark> KQIRSIIM</mark> REN-
Chlamydomonas_ α	LVAEAMAALETVT	KKDLGEAKSLKK	(PPPGVDDITAVVIIL	.Lennpkd <mark>ks</mark>	WQAAQKLMNNVDK
Chlamydomonas_ β	I I AQAEAALNSLN	KK <mark>el</mark> selksfgs	PAAEIVQVAAACLVL	TCGGK I P <mark>KDRD</mark>	WNAGKKMMADVNS
<mark>Chlamydomonas_y</mark>	ALDAALEALNSIK	DG <mark>DI</mark> KNLKALKK	PPQIITRIFDCVLVL	.RMLPVTK <mark>aeytdekgrmvqvgn</mark> —	YPEA-QKMMNQMS
Tetrahymena_DYH3	ALRRAQEAVDSIE	SK <mark>DI</mark> VEL <mark>K</mark> ANKK	PLDIIKYIMDAVLVF	FKARLIP <mark>IQIEERVFNKKEGKAV</mark>	LFLKES <mark>YDESGIQTLGDMN</mark>
Human_DNAH5	ALEEAEAALQTIR	PSDIATVRTLGR	PPHLIMRIMDCVLLL	FQRKVSA <mark>vkidleksctmps</mark>	WQES-LKLMTAGN
Human_DNAH8	ALEEAEAALNTIK	PND I ATVRKLAK	PPHLIMRIMDCVLLL	FQKKIDP <mark>vtmdpekscckps</mark>	WGES-LKLMSATG
Chlamydomonas_dynein-c	LLEAALKALDTLK	P <mark>aditev (</mark> GMKS	PP <mark>AGVRRVLEAICIM</mark>	<mark>K</mark> GVKPAR <mark>vKDTASGRMVDDY</mark>	<mark> eask-k</mark> mlmefd
	H4	H5	H6		
mouse_cyto	FIPTIVNFS	AE I S <mark>da i rekmk</mark>	KNYMSNPSYN <mark>yei</mark>	<mark>VN</mark> RASLACGPMVKWA I AQLNYAD ⁱ	MLKRVE
Chlamydomonas_ α	FLERVKSFKSVID	AGQVARKTVDAC	RPYLALEWFNREA	I GKKSAAAAGLCEWAVN I I KYYD	VVQEVE
Chlamydomonas_ β	FLSSLMNFDK	DNVPVVCVEVVE	KDYISNPGFTPDN	IKGKSAACAGLCSWVINICKYFR	IYQVVA
Chlamydomonas_y	FLQDLKDFA	KEQINDETVELL	.EPYFMSEDFTFEN	AQKASGNVAGLCNWAESMAKYHN	VAKVVE

mouse_cyto $Chlamydomonas_{\alpha}$ **Chlamydomonas_**B Chlamydomonas_y Tetrahymena_DYH3 Human_DNAH5 Human_DNAH8

B

Chlamydomonas_dynein-c

Tetrahymena_DYH3

Human_DNAH5

Human_DNAH8

Chlamydomonas_dynein-c

FLUULNUFA FIFENAURAJUNVAULUNVAEJMARTIINVARV -KDSINEETIELLEPYLNQSEDWFNDTFATKASKAAAGILKWAFAIYEYHQKSKIVK FMKKLKEFE-FLQNLQQFP----KDTINEEVIEFLSPYFEMPD--YNIETAKRVCGNVAGLCSWTKAMASFFSINKEVL FLWSLQQFP----KDTINEETVELLQPYFNMDD--YTFESAKKVCGNVAGLLSWTLAMAIFYGINREVL -KDHIPP<mark>EVIVKIRPFA</mark>QDPE--FQ<mark>PKVIEK</mark>QSVACAGLCSWVIALEKYDKVIKEVE DSLRKFD-

CC2

mouse_cyto $Chlamydomonas_{\alpha}$ Chlamydomonas_8 Chlamydomonas_y Tetrahymena_DYH3

Human_DNAH5

Human_DNAH8

Chlamydomonas_dynein-c

19 47 82 PLRNELQKLEDDAKDNQQKANEVEQMIRDLEASIARYKEEYAVLISEAQAIKADLAAVEAKVNRSTALLKSLSAERERWEKT PKRQELAAANAKLEEANVTLAAVEEKVALLNAKVQELEQQYKEANDDKEAAIRESERCQRKLELANRLINALASEGERWALT PKRAALAEANKKLDTANKKLKVIRDEVKRLQDRVALLEQSLMKATEDKNAAIAQADRTARKAQMAERLINGLSGENTRWGAE PKIAKLREAEAELKLATKEKNAAEERMAKVQAKLDEMQAQFDAAMAHKQALEDDAAATQRKMDSANALIGALAGEEARWTAQ PKRIQVAIAEGRQAIALKELEKAREDLAQIQAYIKNLKDVYTKQMEEKNELEMKAAKTKKKINTARTLITSLSGEKDRWGKG PLKANLVVQENRHLLAMQDLQKAQAELDDKQAELDVVQAEYEQAMTEKQTLLEDAERCRHKMQTASTLISGLAGEKERWTEQ PLKANLAKQEGRLAVANAELGKAQALLDEKQAELDKVQAKFDAAMNEKMDLLNDADTCRKKMQAASTLIDGLSGEKIRWTQQ PKRQKLREAEAQLEVVMAALRAKQAELKVVMDKLSRLDADLQEKKRRKEKLEHDVHMCTVKLERAEKLISGLGGEKTRWTAA



[γsta	alk•I	LC1]						
1.8	μM	2.4	μM	3.0	μM	3.6	μM	4
S	Ρ	S	Ρ	S	Ρ	S	Ρ	





S: supernatant P: precipitate











Fig S9

В

1 Table S1. Summary of the primers used in this study

Columphonemer tagging 10:15-P 9: CANTERCARTICAGECATACA LC1:N=Rational 9: CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	purpose	primer name	sequence
Interview ICISAS STGGAGCACAATTATTGGGGATGGATGGA Interview SCGCACCACCACCACCACCACCACCACGGGG ICISAS Interview SCGCACCACCACCACCACCACCACCACGGGG ICISAS Interview SCGCACCACCACCACCACCACCACCACCACCACGGGG ICISAS Interview SCGCACCACCACCACCACCACCACCACCACCACGGGGGGGG	Chlamydomonas tagging	LC1-5p-F	5'-CAATCTCAGTTTCACCGCATCAAC
I.I.N.Maile FCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC		LC1-3p-R	5'-TGGAGCACAAATTTTGAGGAATGA
LC1NABLAS \$-COCCONGRIGATION CONGRIGATION CONCONCONCONCONCONCONCONCONCONCONCONCONC		LC1-N-8xHis-1	5'-CCCACCACCACCACCACCACCACGGCG
LCH9 S-MACAAGCCCCCAACACCAA LCH9 S-CCCATCATCATCAGCGGGGGGGGGGGGGGGGGGGGGGGG		LC1-N-8xHis-2	5'-CGCCGTGGTGGTGGTGGTGGTGGTGGTGGG
LC1-CASH.Sept 5-COCCLAGECCOCCCACCGACCTTCCCCCCCCCCCCCCCCCCCCCCCCC		LC1-F1	5'-AGACAAGCCCCCAACACCAA
Claphane SicelertadeAccectrateContrectingGetad pGPP modicements pGPP modicements SicelertadeContrectingGetadeTicAdTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTC		LC1-C-8xHis-SpeI-R	5'-CGCACTAGTGGTGGTGGTGGTGGTGGTGGTGGTCCTCCGCGGGCCACGTT
Tarabay Scattartartartartartartartartartartartarta		LC1-3p-XbaI-F	5'-CGCTCTAGACACCGCTAGCTTTGGGCA
phGP#-mod_cosmucion phGP#-mone.Prime S-CATCATCATCATCATCATCATCATCATCATTAGCTAAT phGFP-mone.Prime S-ATCATGATAGATGAACAAAACATCATCATTGATAATATAGCTAAT phGFP-mone.Prime S-ACCATGATGATGACAAAACATCATCATGATAAGTGATAGATGATAGCTGATCATTTAGATGATGATTAGATGATGATTAGATGATGATTAGATGAT	Tetrahymena tagging		
phGPP-novi-construintin phGPP-nove-less phGP-nove-less phGP-nove-less phGPP-nove-less phGPP-nove-less phGPP-nove-less phGP-nove-less phGP-nove-	phGFP-neo4 construction	phGFP-inverse-F	5'-CATCATCATGGTTCTGGTGGTTCAGTTCAATTAGCTGAT
phoRPseudonaution phoRPseudonaution concretorCretor		phGFP-inverse-R	5'-ATGATGATGATGACCAGAACCATCTTCAATATTATGTCTAATTTTA
phoOP+work.psc phoOP+work.psc	phsGFP-neo4 constrcution	phsGFP-inverse-1st-F	5'-CCTTAAGGTTAAAGAGAACCTTCTCATGGTTCTGGTTCAGTTCAATTAGCTGATCATT
phoofPinnene2adP 3140AA1AATAA0ACACACATACACACATCACCTATAGAAQAACATCT MGPPacol. hGPPacol-faguest GPPacol. hGP-acol. hGP-acol. Control Co		phsGFP-inverse-1st-R	5'-ACCTCTCCAACCAGTAGTCTTTTCATCCATAGAACCATGATGATGATGATGATGAT
Both Preschingument 6 Presching 5 GGTGGTGTGTGGTGGAGAACTTUCATGGTGTGTGGT Bolb Preschingument 6 Presching 5 GGTGGTGGTGGGAGACCCTGGGGGGGAGAGTGGAGTGGGTGG		phsGFP-inverse-2nd-F	5'-TAGAATAATTAAGAGCTAGATTAGAACATCATCCTTAAGGTTAAAGAGAACCTTCTC
bGPP acol. HorfP. GPP acol. A GPP acol. A GGT GGT AT GGT TTCT CA AGGGT GGT AGAT TTT CAC GGT GGT GGT TTT CAC GGT GGA AGA AGA AGA AGA AGA AGA AGA AGA		phsGFP-inverse-2nd-R	S-ATTCACCAGCTAAACCTTCAACAACATGACCACCTCTCCAACCAGTAGTCTTTTCA
GPB-eed-R 5-AAGCTTGATATCGAATCGAATCGACCGCGGCGCGCGCGCG	hGFP-neo4, hsGFP-neo4-fragment	GFP-neo4-F	5'-GGTGGTATGGTTTCTAAGGGTGAAGAACTTTTCACTGGTGTTGTT
LC1 LT1 LT1ACCAGICAGATICAATICACCAGGATICAATICACCAGGATICAATICA		GFP-neo4-R	5'-AAGCTTGATATCGAATTCAGATCCCCCGGGCTGCATTTTTCCAGT
Li 1-28 5-MAD INCLICALCUI MARAAA LA MACAA LA MACAAA LA MACAA LA MACAAA LA MACAAA LA MACAAA LA MACAAA LA MACAAAA LA MACAAAAAAAAAA	LCI	LCI-C-F	5'-GCIGAIGGCGAIGAAIGAACACIGIAIIIIACCAGICAAICAA
LC 3-7 3-3-CCOMMONGAN LIANAL TARGAGATTEGTUD, LTA LANDA ANALANAL TARGA pHC HB C-7 3-4-CCOMMONGAN LIANAL TARGAGATTEGTUD, LTA LANDA ANALANAL TARGA pHC 3-R 5-AAGTTCTTAGACACTTGAGATAGACCTTAGATAGACATTGAGAGATAGAT		LCI-C-R	
pHC Fig.C-F S-CCTGATGGGGGGATGAATCAACCATGGTTTCCTACAGACATATGTAA HIC-S-F S-CCCGGGGGATGAATCAACCAGCCCCCCGACCACCCTTTTATCTTTACTGATGAGACCA HIC-S-F S-CCCGGGGGATGAATCAATCACACCTGCACCCCTTTTAACTTTAATCAAAAAAAA		LC1-3-F	
pine pine seader for a sea	внс	BHC-C-F	S-GCTGATGGCGATGAACGACCGGGCTTCCGGACTACAGACTAA
image: space \$-CCCCGGGGGATCTAATTCGATTCCAAATTCGATAATCAAAATCATAAAATCATAAAAACAATTTAAAATCATAAAAACAATTTAAAATCAATAAT	pric	BHC-C-R	S-AAGTTCTTCACCCTTAGAAACCATACCATCCCTTTTTATCTTCTTAACTTCATCAGAGAC
overlapoing PCR9:-GCGAGCACCAGATTATATCGACTCCTCATAAACCTCAGAATAAGGPCR clock9:-GCGAGCACCAGATTAATACGACTPCR clock9:-GCGAGCACCAGATTAATACGACTPCR clock9:-GCGAGCACCAGATTAATACGACTConstruction for biochemical experimers9:-GCGAGCACCAGATTAATACGACGACGAGATGAGAGCGACConstruction for biochemical experimers9:-GCGAGCACCAGAGTCGCAC AACGGCCCCACGAGCGCACAGGGTGCACAGGGTGCACAGGGTGCACAGGGTGCACAGGGTGCACAGGGTGCACAGGGTGCACAGGGTGCACAGGGTGCACAGGGCAGCAGGGAGTGGAGAGAGGAGTGGAGAGGAGGAGGAGGAGGAG		внс-3'-F	5'-CCCGGGGGGATCTGAATTCGATATCAAGCTTGAAAAATCATAAAAAATTTAAAATAATAATAAACATTTCTATA
overlapping PCRverlapponer-RS-CACTGAGCAGTGAAACACTG overlapping PCRPCR checkPCG-gnome-check RS-CAACTAGAACATTATACAGCAGGT CALE-gnome-check RPCR checkPCG-gnome-check RS-CAACTAGACATTATATCAGCAAGGGorathe-conserverCABD-8582-XhoBIM <br< td=""><td></td><td>βHC-3'-R</td><td>5'-GCGAGCACAGAATTAATACGACTTCCTGATAAACCTTCAGAATAAGG</td></br<>		βHC-3'-R	5'-GCGAGCACAGAATTAATACGACTTCCTGATAAACCTTCAGAATAAGG
PCR ebck weinsponner-Res 5-CGCAGCACGAATTAATACCAGCT DPC ebck 5-CGCTCAAATCATAAACAGGCAAGG Construction for biochemical experime 5-CGCTCAAATCATAAACAGGCCCCCAAGGCTGCAC Mather 0-ADa-85-82-Nubsille 5-CGCAACTCGGCCCAAGGCTGCACAAGGTGCAC Bather 0-ADa-85-82-Nubsille 5-CGAACTCGGCCCCAGCCCCAGGC Mather 0-ADB-85-82-Nubsille 5-CGAACTCGGCCCCAGCCCCAGGC Mather 0-ADB-85-82-Nubsille 5-CGAACTCGGCCCCCCCGCCGCAGGAGGTGGCCCAGGGCAGGCCAGGCAAGGCAGGC	overlapping PCR	overlap-outer-F	5'-GCTGATGGCGATGAATGAACACTG
PCR check BKC-genome-check-R S ¹ CAACTAAAACAGCAAGG Construction for biochenical experiments S ¹ CAACTCAAGGATAATTATCGGC castalk ADDe-85:82-MoBILF S ¹ CAACTCGAGGTCGACAACGGCCACAGCGCCA ADDB-85:82-MoBILF S ¹ CAACTCGAGGTCGACAACGGCCACAGGCG ADDB-85:82-MoBILF S ¹ CAACTCGAGGTCGACAACGGCTGCACAGGCG ADDP-85:82-MoBILF S ¹ CAACTCGAGGTCGACACGGCCACCAGGC ADDP-85:82-MoBILF S ¹ CAAGATTCGAGGTCGACAGGCCACCAGCG ADDP-85:82-MoBILF S ¹ CAGAATTCGAGGTCGACGACGCCACGGCG Yatalk ADP-85:82-MoBILF S ¹ CAGAATTCGAGGCCCACGCGCCAGGCG LC1 LC1-BanHF S ¹ CAGAATTCGAGGCCCACGCGCCACGGCG LC1 LC1-BanHF S ¹ CAGAATTCGAGGCCCTCCGCGCGCC LC1 LC1-BanHF S ¹ CTGGCGCGCCTCCTCCAGGCG B3535-y50:47 B5355-y50:47 S ¹ CTGGCGCGCCCTCGTGGCGAATTAG B3535-y50:47 B5355-y50:47 S ² CTGGAGGCGCCCTCCTCATCCT B3535-y50:47 B5355-y50:47 S ² CTGGCGCGCCCTCGTGGCGAATTGG B3535-y50:47 B5355-y50:47 S ² CTGGGCGCCCCTCGGCGAGAGTGG B3535-y50:47 S ² CTGGCGCACCCAGCGGCGGCGGCGGGGGGGCGCCCCGGAGAGA S ² CTGGGCGCCCCCGGGGGGCGCCCCGGAGAGA B3535-y50:47 S ² S3-y50:47 S ² S3-y50:47 S ² S3-y50:47 B3535-y50:47 S ² S3-y50:47 S ² S3-y50:47 S ² S3-y50:47 <td< td=""><td></td><td>overlap-outer-R</td><td>5'-GCGAGCACAGAATTAATACGACT</td></td<>		overlap-outer-R	5'-GCGAGCACAGAATTAATACGACT
LCI-genome-check-R \$-TCGTCAAATCATAGCATAACTATAGCATAACTATCGC Construction for biochemical experiments 0ADe-85:82-MonSILF \$-GAACTCGGAGGCACAACGGGCCTGCACAAGCTGCAC balk 0ADe-85:82-MonSILF \$-GAACTCGGGGCCCACGGGCCCAGGGC \$-GAACTGGGGGCCCAGGGCCCAGGGCCCAGGGCCCAGGGCCCAGGGCCCAGGGCCCAGGGCCCTGGCAGAAGGTTTGGACGGGGCGCCTGGCAGAAGGCTTTGGAGGCAGGGCGGCCTGCAGAAGGCTTTGGGGGGCCCTGGCAGAAGGCCTGCAGACGGCCTGCAGAAGGCCTGCAGAGGGCCTGGCAGACGGGCCTGGCAGACGGGCCTGGCAGACGGGCCTGCAGACGAGCTGGGCCGCCGGCAGACGACTAC LC1 CLI-EscaRLR \$-CTGAAGCTGGCCGCCGCCGCCGCGCCCGGCGCCCGGCGCCGGCGCCGGGCCCTGCGGGCCTGCAGAGGGCCTGGGGCCTGCAGACGAGCTGG Dimeric stalk constructs B3535-inverse-F \$-AAGAACGCCGCCGCCATCGGCGCA B3535-inverse-F \$-GTGGGGCCGCGCCTCCTCTCTCT 75047-R \$-OTGCGGCTGGGGCCTCGGGCAGCTGGA B3535-inverse-F \$-OTGCGGCCTGCGGCAGCTGGA 75047-R \$-OTGCGGCCTGCGCGCAGACTGGA B3535-inverse-F \$-OTGCGGCCTGGCGGCAGCTGGAGGCGGGAGGAGGA 75047-R \$-OTGCGGCTGCGCGCGCCGGCGGCGGGGGGGGGGGGGGGG	PCR check	βHC-genome-check-R	5'-CAACTAAGACATATAAACAGCAAGG
Construction for biochemical experiments UADbe-855 82-NinoBial F S-GACTCGAGGCTGGACAAGGCTGCACA Batak OADbe-855 82-NinoBial F S-GAACTCGAGGCTGGACAAGGCTGCACAAGGTGGACAAGGTGGCAGAGAAGATGCACAAGGC ystak OADbe-855 82-NinoBial F S-GAACTCGAGGTGGACGACGAGGGACGACAAGGTGTGTGACAAGGATGGCAGGAAGAAGTGTTGAG LC1 CL-Bamill F S-GAACTCGAGGCCGCAAGGCAAGGAAGATGGTTGAG LC1 LC1-Bamill F S-GAAGCCCCACGGGC B3535-19047 B3535-inverse-F S-AAGAACGCCGCCATGGCGCAAGGCAATAG 79047A S-GGCGTGCTGCAAGGCAATAG 79047A S-GGGCGCCTGCACTGGGCCATGGCGAAGGAAGAAGATTAG 79047A S-GGGCGCCTGCAAGGCAAGTAG 98536-j902 PG3-G3-inverse-F S-CTGAAGCCACTTGGTGCACGCAGGAAGTAG 7219A PG3-G3-inverse-F S-CTGAAGCCACTTGGTGCCACGCTGA P85382-jMTBD PG3-S9-inverse-F S-CTGACCCCCGCGCGAAGGCAGGAAGTGC 73535-j50-47 Y219FA S-CTCACCCTCCGCCAGA 74363-inverse-F S-CACACGCGGCGCTGGCAAGGCAGA 74363-inverse-F S-CACACGCGGCCTGCCAAATGC 73535-j50-47 Y3535-inverse-F S-CACACGCGGCCTGGCAAATGC 74363-inverse-F S-AAGCAGCGCGCCTGCCAAATGC 74		LC1-genome-check-R	5'-TCGTCAAATCATAGCATAATTTATCGC
estalk OADe-\$\$782-Xbb/SallF \$'-GAACTCGAGGCCACAGCGCCACGGC pstalk OADP-\$\$782-Xbb/SallF \$'-GGAACTCGGCCCACGGC pstalk OADP-\$\$782-Xbb/SallF \$'-GGAACTCGGCCCCCACGG ystalk OADP-\$\$782-Xbb/SallF \$'-GGAACTCGGCCCCCACGC pstalk OADP-\$\$782-Xbb/SallF \$'-GGAACTCGGCCCCCACGC LC1 LC1-BanHlF \$'-GTGAACCTCTGGCCCAGCGCCACACAC LC1 LC1-BanHlF \$'-GTGATCCTACGCCCACGCC S355-5y0-617 \$''''''''''''''''''''''''''''''''''''	Construction for biochemical experiments		
OADess 82-HindleR [9stalkOADess 82-HindleR CoGAGCTCGCACGCCCAGGC CGAGGTGGACAGGGTTGCACAGGCACAGGC CGAGGTGGACAGGGACGGCCCCAGGC CGGGCCCCAGGGAGGTGGCCCGAGAGATGTTGAG CGAGGTGCCCCCCCGCCCCAGCG CCCCCCCCCCCCCGCGGGGC CCCCCCCCCCCCCGGGGC121OADP-85.82-HindleR CCGAGGCGCCCCCCCCCCCCCCGGGGC121CI-BamHF CCGGAGCGCCCCCCCCCCCCCCCGGGGC121CI-BamHF CCGGAGCGCCCCCCCCCCCCCCGGGGC121S535-inverseF S-CGGGCGCCCCCCCCCCGGGGC123535-y50.47B535-inverseF S-GGCGCCCTGGGCCCTCCGCCCCCCGGGGC123535-y50.47B535-inverseF S-GGCGCCCCCCCCCCCCCCCCCCCCCCGGGG123535-y50.47B535-inverseF S-GGCGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	αstalk	OADα-85:82-XhoISalI-F	5'-GAACTCGAGGTCGAC AACGGCCTGCACAAGCTGCAC
jstalkOADJS-SS2-MouSail-BS-GAACTCGAGCTCGACAACGGATTTGCACAGATTGCACAGGCGyatalkOADy-SS2-MouSail-BS-CTGAAGCTTCTGCGCCCCCCCCGAGAAGATGTTTGAGLC1CAB-#SS2-MouSail-BS-CTGAAGCTCCTGCGCCCCCCCCGCAGAAGATGTTTGAGLC1LC1-BeoRLRS-CTGAAGCTCCTGCGCCCCCCCCCGCGGCCChineric stalk constructsS-STGGATCCATGCCCAAGGCCAACGCCACTGCGCAj3535-inverse-FS-AGGACCCCCCCCCCCCCGCGCGCAj3535-inverse-FS-GTGGCCCCCCCCCCCCCCCCCGCGAj3535-inverse-FS-GTGGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC		OADa-85:82-HindIII-R	5'-CTGAAGCTTCGTCAGCGCCCAGCGC
OAD9-8582-bindlikeS-CTGAAGCTTTTGGCGCCCCCGGAAGATGTTTGAGystalkOAD9-8582-bindlikeS-CGAAGCTGCCGCGGCGCCCGGAGAGATGAGLC1LC1-BamHl-FS-GTGGATCCTATGCCGCGGCGCLC1LC1-BamHl-FS-GTGGATCCTATGCCGCGGGCChineric stalk constructsS-GTGGGTCGCGCCATCGCGCAB35:35-inverse-RS-GTGCGTCTGCGCCTTCTTCTy50:47B35:35-inverse-RB35:35-y50:47B35:35-inverse-RB35:35-y50:47S-GGCGCCCCATGGCGCATCGGCAB35:35-inverse-RS-GTCGGCCCATGCGCGCATCGAB63:63-inverse-RS-GTGGGCCCCTCTCTCTy50:47-RS-GGCGCCCCCCTCCTCATCCTy21:19-FS-AGGAGCCAGCGAGATTGGB35:32-inverse-RS-CTCGAGGCCAGCGCAGATTGGy21:19-FS-AGGAGCCCAGCGAGATTGCy21:19-FS-AGGCGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	βstalk	OADβ-85:82-XhoISalI-F	5'-GAACTCGAGGTCGACAACGGTATTGACAAGATTGCACAGGC
ystak ODP-x8522-bindfullGAACTCCAAGGTGGACGGTGGACGAAGTGTTTGAG CAAGTCATGGCCAAGGCAACTAC LC1-BamHLFS-CGAGAGCTTCTGCGCCACGCGC CCAGGCCACGGGACTAC LC1-BamHLFLC1LC1-BamHLFS-CGGGCGCCGCCGCCGCGCGGGCChineric stalk constructsS5:35-y50-47B5:35-y50-47B5:35-inverse-FS'-AAGAACGCCGCCCATCGCGCA CGCGCGCTCTCTCT y50-47-FB5:35-y50-47B5:35-inverse-FS'-AGGACGCCGCCATCGCGCA CGCGCGCCTCCTCTCT y50-47-FB6:36-inverse-RS'-GTGGGCCGCCTCGCGCA y50-47-FB6:36-inverse-RS'-GTGGCCCCCCCCTCATCGC PG:36-inverse-RP6:36-inverse-RS'-GTGGCCCCCCTCCATCCT y2:19-Fy2:19-FS'-AAGAGGCCCAGCAGATTGG y2:19-RP6:36-inverse-RS'-CCCGCCGCCTCCTCATCGC y2:19-Ry3:53-y50-47B5:82-inverse-Fy3:53-y50-47S:S2-inverse-Fy3:53-y50-47S:S2-inverse-Fy3:53-y50-47S:S2-inverse-Fy3:53-y50-47S:S2-inverse-Fy3:53-y50-47S:S2-inverse-Fy3:53-y50-47S:S2-inverse-Fy3:53-y50-47S:S2-inverse-Fy3:53-y50-47S:S2-inverse-Fy3:53-y50-47S:GGGCCCCCACACCTTGGCCGACA y5:35-inverse-Fy4:53:5-inverse-FS'-AAGCGCCCCCAGGGGG y5:35-inverse-Fy5:35-y50-47S:S2-inverse-Fy5:35-y50-47S:GGGCCCCCCACCCTGGGGGCG y5:35-inverse-Fy5:35-y50-47S:GGGCCCCCCACCCTGGGGGCGA y5:35-inverse-Fy5:35-y50-47S:GGGCCCCCCCCCCCGGGGGGGGGGGGGGGGGGGGGGG		OADβ-85:82-HindIII-R	5'-CTGAAGCTTTTCGGCGCCCCAGCG
OAD-95.35.2-Hindlink 5'-CTGAAGCTTCTGCCCCCCAGGC LC1 LC1-BamHFF 5'-CAGAATTCCTATCCTCCGCGGGC Chineric stalk constructs 5'-AAGAATCCTATCCTCCGCGGCA β5:35-ry50.47 β5:35-inverse-F 5'-AAGAACCCCCGCCATCGCGCA β5:35-inverse-F 5'-GCGGTCTGCGCCTTCTTCT ry50:47-R 5'-GCGCCATGGCGCAAGCAGATTAG β6:36-inverse-F 5'-CTGAAGCTCTCTGCGCCTCTCTCT ry20:47-R 5'-GCGCCCTGCGCAGCGCGCAGA β6:36-inverse-F 5'-CTGAAGGCCATCGGCGCATCGA β6:36-inverse-F 5'-CTCATGGGCGCAGCTGGCAGATTGC ry20:47-R 5'-GCGCGCGCGCGCCTGCCCCC ry20:47-R 5'-CTCCTTGGGCCAGCTGGAGATTGC ry20:47-R 5'-CTCCTGGGCCAGCTGGCAGATTGC ry20:47-R 5'-CTCCTGGGCCCTGGCAGATTGC ry20:47-R 5'-CTCCGGTCGCAGCAGCTGGCAGATTGC ry35:35-inverse-F 5'-ACGCGCCTCCTGGCCA ry35:35-inverse-F 5'-ACGCGCCTCTGGCCA ry35:35-inverse-F 5'-ACGCGCCTCGCGCCAGGGCGA ry35:35-inverse-F 5'-ACGCGCCTCGCGCCAGGCGCA ry35:35-inverse-F 5'-CCCCCCCCTGGCGCAA ry35:35-inverse-F 5'-CCCCCCCCTGGCGCAA ry35:35-invers	γstalk	OADγ-85:82-XhoISalI-F	5'-GAACTCGAGGTCGACGGTGGCCTGCAGAAGATGTTTGAG
LC1 LC1-BanHrl- LC1-EcoRIR S-OLIGGATECTATECTATECTATECTATECTATECTATECTATE		OADγ-85:82-HindIII-R	5'-CTGAAGCTTCTGCGCCGTCCAGCGC
Chimeric stalk constructs Societation β35:35-y50:47 β35:35-inverse-F S'-AAGAACGCCGCCATCGCGCA β35:35-y50:47 β35:35-inverse-R S'-GTCGGCTGCAGCGCCTTCTCT y50:47-F S'-GTCGGCCATGGCGGCATCGCA β63:63-inverse-R S'-GTCGCCCATGGCGGCATCGCA β63:63-inverse-F S'-CTCAAGGTCATTCGTGACGA β63:63-inverse-F S'-AAGAAGGCCCAGCGAGAATTGC γ22:19-F S'-AAGAAGGCCCTCCTCATCCT γ22:19-R S'-CTCATGGTGGCCGCCTTGA β85:82-inverse-F S'-AAGCAGGCCCTGGCAGA β85:82-inverse-F S'-AAGCAGCCCCTGGCAGA γ22:19-R S'-CTCAGCCTCTGAGCGCCCTGGCA γ35:35-inverse-R S'-CTCAGCCTCTGGCCAA γ35:35-j550:47 Y3:35-inverse-R S'-GTCCCTCGACACCCTTGGCCAA γ35:35-j550:47 Y3:35-inverse-R S'-GTCCCCGCGCGCAA γ35:35-j550:47 Y3:35-inverse-R S'-GTCCCCGCGCGCAA γ3:47-F S'-GGCCTCCACACCTTGGCCAA J3:47-F γ3:53-inverse-R S'-GTCCCCGCGCCGCAA J3:47-F γ3:36-j2:19 Y3:36-inverse-R S'-GTCCCCGCGCGAACCAA γ3:63-jnverse-R S'-GTCCCCGCGCGAACGAGGCG <td>ICI</td> <td>LC1-BamHI-F</td> <td>5°-GIGGAICCAIGGCCAAGGCAACIAC 5°-CAGAATTCCTATCCTCCGCGGGC</td>	ICI	LC1-BamHI-F	5°-GIGGAICCAIGGCCAAGGCAACIAC 5°-CAGAATTCCTATCCTCCGCGGGC
Chinario stalle constructs β35:35-rjob.47 β35:35-inverse-F 5'-AGAAAGCGCGCATGGGCGATGGGGCA β35:35-rjob.47 β35:35-inverse-R 5'-GTCGGTCTGCACGCGCTTCTCT γ50:47-F 5'-GCGCGCGCCTGCGAAGCAGATTAG β36:35-inverse-R 5'-GTGGGCCGCGCCTCTCATAG β36:36-inverse-R 5'-GTGGGCCGCCCTCTCATCCT γ2:19-F 5'-AAGCAGCCGCGCGCCTCTCATCCT γ2:19-F 5'-AAGCAGCCGCAGCAGGAGATTGC γ2:19-R 5'-CTCCTGGTGGCCCAGCAGCAGA β85:82-inverse-R 5'-CTCAGCTCCCACGCAGGAGA γ2:19-R 5'-CCCAGCCTGGAGCCGCCCTGGCAGA γ85:82-inverse-R 5'-CCCAGCCTGGAGCCCGCGCAGA γ85:82-inverse-R 5'-CCCAGCCTCGGAGACA γ35:35-inverse-R 5'-GCCGCCTGGAGGCAGA γ35:35-inverse-R 5'-GCCGCCTGGAGAGCA γ35:35-inverse-R 5'-GCCGCCCAGCACA γ35:35-inverse-R 5'-GCCCCCCGAGCAGA γ35:35-inverse-R 5'-GCCCCCGCGCGAGCA γ35:35-inverse-R 5'-GCCCCCGCGCGAGCA γ35:35-inverse-R 5'-GCCCCCGCGCGAGCCA γ35:35-inverse-R 5'-GCCCCCCGCGCGAGCCA γ35:35-inverse-R 5'-GCCCCCCCCACACCCCCCCGAG <t< td=""><td></td><td>ECT-ECORFR</td><td>5 CAGAATICCTATCCTCCCCCCCCC</td></t<>		ECT-ECORFR	5 CAGAATICCTATCCTCCCCCCCCC
β35:35-γ50:47 β35:35-inverse-F \$'-AAGAACGCCGCCATCGCGCA β35:35-inverse-R \$'-GTCGGCCTTGCGCCATCTCT γ50:47-R \$'-GTCGGCCCATGCGGCCATCGA β63:63-γ22:19 β63:63-inverse-F \$'-GTGAGCCGCCATCGAAGCAGATGA β63:63-inverse-R \$'-GGTGGCCGCCTCATTCCT γ2:19-F \$'-AAGAAGCCGCGCCACGCAGCAGA β8:82-inverse-R \$'-GGTGGCCGCCTCATCCT γ2:19-F \$'-AAGAAGCCGCGCCGCCGCCTGAAGCAGA β8:82-inverse-R \$'-GCCTGGGCGCCGCCGCCGCGCGCGAGA β8:82-inverse-F \$'-AAGAAGCCCCCCCCCGCGCCGCGCCGC γ35:35-inverse-R \$'-CCCCGCGCGGAGCCCCCCCCCCCCCCCCCCCCCCCCCC	Chimeric stalk constructs		
β53:35-inverse-R 5'-GTCGGTCTGCGCCTTCTTCT y50:47-F 5'-GCGGTGCTGAAGCAGATTAG y50:47-R 5'-GTGGCGCCTTGTACGA β63:63-inverse-F 5'-CTGAAGGTCATTCGTGACGA β63:63-inverse-F 5'-CAGAGGCCCTCTCATCCT y22:19-F 5'-AAGAAGGCCAGCGAGATTGC y22:19-R 5'-CTCCTTGGTGGCCAGCGAGAT β85:82-yMTBD β85:82-inverse-F β85:82-inverse-R 5'-CTCCAGCGCCCTGGCCGC yMTBD-R 5'-GCGCCCCACCACCTTGGCCA yMTBD-R 5'-GCGCCCCCTCGCGCGC y35:35-β50:47 y35:35-inverse-F y35:35-inverse-R 5'-GTCCCGCCTCCTGGGCGCA y35:35-inverse-R 5'-GTCCCGCTCCTGGGCGCA y35:35-inverse-R 5'-GTCCCGCTCCTGGGCGCA y35:35-inverse-R 5'-GTCCCGCTCCTGGGCGCA y35:35-inverse-R 5'-GTCCCGCTCCTGGGGCA y35:35-inverse-R 5'-GTCCCGCTCCTGGGGCA y35:35-inverse-R 5'-GTCCCGCGCCGCA y35:35-inverse-R 5'-GCTCCCGGGCGCCGCGA y63:63-inverse-R 5'-GCTCCCGGGCTCCACAATCA y21:19-F 5'-GCGCTCCCACAGGGTGCCA y85:82-inverse-R 5'-GTCGCGCCCGCGGAGGCGGCGGA y85:82-inverse-R 5'-GCTCAGCGGCGCCAGGGTGCC	β35:35-γ50:47	β35:35-inverse-F	5'-AAGAACGCCGCCATCGCGCA
$ \begin{array}{llllllllllllllllllllllllllllllllllll$		β35:35-inverse-R	5-GTCGGTCTGCGCCCTTCTTCT
β63:63-γ22:19 β63:63-inverse-F 5'-CTGAGGGCATTGCGGACGA β63:63-inverse-R 5'-GGGGCCGCCTCCTATCCT γ22:19-R 5'-AAGAGGCCAGCGGGCCTGGAGA β85:82-γMTBD β85:82-inverse-F 5'-AAGCGTGGCGGCCTGGCAGA β85:82-inverse-R 5'-CTCCACCCCCCGGCAGA γ35:35-β50:47 γ35:35-inverse-F 5'-AGCGCTCCACCACCTTGGCCGA γ35:35-β50:47 γ35:35-inverse-F 5'-AAGCAGCGCCTGGAGGACGA γ35:35-β50:47 γ35:35-inverse-F 5'-AGCGCTCCACCACCTTGGCCA γ36:36-β22:19 γ63:63-inverse-R 5'-CTCCGCGCGGAGGCG γ63:63-inverse-R 5'-CTCCGCTCCTTGGCCGACA γ63:63-inverse-R 5'-CTCCGTAGCCTCCATCGG γ63:63-inverse-R 5'-CTCCGTAGCCTCCATGG γ63:63-inverse-R 5'-CTCCGTAGCCTCCATGG γ63:63-inverse-R 5'-CTCCGTAGGCTCCACAATCA β22:19-F 5'-GCGCTCCACAGCTGGCGGAGCG γ85:82-jNTBD γ85:82-inverse-R 5'-CTTGGCGCGCAGCCAGGTGCC γ85:82-jNTBD γ85:82-inverse-R 5'-CTTGGCGCGCAGCCAGGCGGA γ85:82-inverse-R 5'-CTTGGCGCGCAGCCAGCCAGGCGGA γ85:82-inverse-R 5'-CTTGGCGCGCAGCCAGCCAGGCGGA γ85:82-inverse-R 5'-CTTGGCGCGCAGCCAGCCAGGCGGA <		γ50:47-F	S'-GCGCIGCIGAAGCAGAIIAG
pb3:03-j22.19pb3:03-jmerse-R5'-GTGAGCCGCCTCCTCATCCT Y22:19-Fβ63:63-inverse-R5'-GTGGCGCCAGCGCAGCAGAγ2:19-F5'-AAGAAGCCCACCGCGCGCAGAβ85:82-inverse-F5'-ACGCGGCCGCGCCAGCAGAβ85:82-inverse-R5'-CTCAGCCTCCAGCAGGCCCTγMTBD-F5'-CCCGCCCGGCAGAGCGCCCAγ35:35-j50:47γ35:35-inverse-R5'-CTCCGCCTCCGTGGCCGCAGAj50:47-Fγ35:35-inverse-R5'-CTCCGCCTCCTTGGCCGACAβ5:42-j9γ36:35-inverse-Rγ36:35-j22:19γ36:35-inverse-Rγ35:35-j195'-GGCTCCCCCCCGCGCGGAGAGCGγ36:35-j22:19γ36:35-inverse-Rγ35:35-j195'-GCGCTGCACACCCCCCGAGAGCGγ35:35-inverse-R5'-CCTCCGGCGCCGCAGAGAGCGγ36:35-j22:19γ36:35-inverse-Rγ36:35-j22:19γ36:35-inverse-Rγ35:35-j195'-GCCCTCCCGCAGCAGCAGCGγ35:35-j195'-GCCCTCCCGCAGACTGAGCTGCCAγ35:35-j195'-GCCCTCCCGCAGACTGAGCTGCCAγ36:35-j22:19γ36:35-inverse-Rγ35:35-j195'-CCCCACGCCGCGCGGCGCGCγ35:35-j195'-CCCCACGCCGCCGCCGCGCGCGCGCGγ35:35-j195'-CCCACGCCCGCCGCGCGCGCGCGCGCGCGCGCGCGAγ35:35-j19γ35:35-inverse-Rγ35:35-j195'-CCCACGCCGCCGCCGCGCGCGCGCGCGCGAγ35:35-j19γ35:35-inverse-Rγ35:35-j19γ35:35-inverse-Rγ35:35-j195'-CCCACGCCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG	863-63	γ50:47-K 863:63 invorce E	
γ22:19-F5'-AAGAAGGCCAGCGAGATTGCγ22:19-R5'-CTCCTTGGTGGCCAGCTGGγ22:19-R5'-CTCCTGGTGGCCAGCTGAβ85:82-inverse-F5'-AAGCGTCCAGCCTGGCAGAβ85:82-inverse-F5'-CCCAGCTCCAGCAGCGCCγMTBD-F5'-CCCGGCGCGGAGGCGCCγMTBD-F5'-CCCGCCCCCCTTGGCCAγ35:35-β50:47γ35:35-inverse-Rγ35:35-inverse-R5'-CTCCGCCTCCTTGGCCGACAβ50:47-F5'-GAGCTGCATCGGγ35:35-inverse-R5'-CTCCGCCTCCTTGGCCGACAβ50:47-F5'-GAGCTGCATCGGγ63:63-β22:19γ3:63-inverse-Rγ63:63-β22:19γ3:82-inverse-Rγ85:82-βMTBDγ85:82-inverse-Fγ85:82-βMTBDγ85:82-inverse-Rγ85:82-βMTBDOADy-MTBD-KhOLF5'-CCCCGCGCGCCCCACACTTGGCCGCAγ0HBD-F5'-CCCCGCTCGAGGCCGCAγ0HBD-F5'-CCCCGCTGGAGCCGCCCACACTTGGCGCAγ0HBD-F5'-CCCCGCTCGAGGCCGAγ0HBD-F5'-CCCCGCTCGAGGCCGAγ0HBD-F5'-CCCCGCCGCGCCCACACTTGGCCGCAγ0HBD-F5'-CCCCGCCGCGCCCACACCTGGCCGAγ0HBD-F5'-CCCCGCCGCGCCCCACACCTGGCCAγ0HBD-F5'-CCCCGCCGCGCCGCGCAγ0HBD-F5'-CCCCGCCGCGCCGCGCCCCCγ0HBD-F5'-CCCCGCCCGCCCCCCCCCCCCCCCCCCCCCCCCCCCC	p03.03-722.17	B63:63-inverse-R	S-GRAGOCGCCTCTCATCCT
$\begin{array}{llllll} & \begin{array}{llllll} & \begin{array}{lllll} & \begin{array}{llllll} & \begin{array}{lllllllllllllllllllllllllllllllllll$		γ22:19-F	5-AAGAAGGCAGCAGGATTGC
β85:82-γMTBDβ85:82-inverse-F5'-AAGCGTGCGGCCCTGGCAGAβ85:82-inverse-R5'-CTCAGCCTCCAGCAGGTCGCγMTBD-F5'-CCGGCTCCACCACCTTGGCCCAγ35:35-β50:47γ35:35-inverse-Fγ35:35-inverse-R5'-CTCCGCCTCCTTGGCCGACβ50:47-F5'-GGCTCCTCCGTAGCCTCCTGGCCGACβ50:47-R5'-GTCCTCCGTAGCCTTCATGGγ36:63-β22:19γ36:63-inverse-Fγ35:35-inverse-F5'-AAGAGCGGCGCCGAGGAGGGγ85:82-βMTBDγ35:32-inverse-Fγ85:82-βMTBDγ35:32-inverse-Fγ85:82-βMTBD5'-CTGGCGCCCACACCCGGCGCGCGAGGCGCGγ47BD-R5'-CCCATCATGGCCACCGCGAGCGCGγ85:82-βMTBDγ35:32-inverse-Fγ63:63-merse-R5'-CTTCTGTGGCGGCCCACACCCGGAGCGGγ85:82-βMTBDγ35:32-inverse-Fγ85:82-βMTBD5'-CTGCGCGCGCCACACCCGGCGCGAGCGγ85:82-βMTBDγ35:32-inverse-Fγ85:82-βMTBD5'-CCCATCATGGCGAGCCGCGGAGCGGγ9TBD-R5'-CCGCTGCAGGCCGCAγ9TBD-R5'-CCGCTCGAGCCCGGCGGCGCGAγ9TBD-R5'-CCGCTCGAGCCCGGCGGAGCGGγ9TBD-R5'-CCGCTCGAGCCCGGCGGAGCGGAGCGGAGCGAγ9TBD-R5'-CCGCTCGAGCCCGGCGGCGGCGGCGGAGCGGAGCGGAGC		γ22:19-R	5'-CTCCTTGGTGGCCAGCTTGA
\$\beta\$5:82-inverse-R\$'-CTCAGCCTCCAGCAGTCGC\mtBD-F\$'-CCCGCCCTGATCCTGCCCT\mtBD-R\$'-GGCCTCCACCACCTTGGCCA\mtBD-R\$'-GGCCTCCACCACCTTGGCCA\mtBD-R\$'-CTCCGCCTCCTTGGCCGACGA\mtBD-R\$'-CTCCGCCTCCTTGGCCGACA\mtBD-R\$'-CTCCGCCTCCTTGGCCGACA\mtBD-R\$'-GGCTCCACCACCTCCTGGCGACA\mtBD-R\$'-GTCCTCCGCCGCGAGGACG\mtBD-R\$'-GTCCTCCTCTGGCCGACA\mtBD-R\$'-GCCTCCTCTGGCCGACGACA\mtBD-R\$'-CTCTCTGCCGCCGCGGAGGCG\mtBD-R\$'-CCCCACCTCCTTGGCCGACA\mtBD-R\$'-CCCCACCCCGCGCGAGGCGA\mtBD-R\$'-CCCCACCCCGCGCGAGGCGACA\mtBD-R\$'-CCCCACCCCGCGCGAGGCGACA\mtBD-F\$'-CCCCCGCCGCGCGCGCGAGGCGACA\mtBD-F\$'-CCCCACCCCGCGCGGAGCCGACA\mtBD-R\$'-CCCCACCCCGCGCGGAGGCCGA\mtBD-F\$'-CCCACCCCGCGCGGAGGCCGA\mtBD-R\$'-CCCACCTCGGAGCCGCGCGAGAGCCGCCGA\mtBD-F\$'-CCCACCTGGAGCCGCGCGAGGCCGA\mtBD-F\$'-CCCACCTGGAGCCGCGCGAGAGCCGCCGA\mtBD-R\$'-CCCACCTGGAGCCGCGCGAGGCCGA\mtBD-HindIII-R\$'-CCCAAGCTTGGCCACCCCCCGCCTGGATGCCCC\mtBD-HindIII-R\$'-CCCAAGCTTGGCCCACCCCCCCCCCCCCCCCCCCCCCCC	β85:82-γMTBD	β85:82-inverse-F	5'-AAGCGTGCGGCCCTGGCAGA
γMTBD -F5'-CCCGCGCTGGATGCTGCCCTγMTBD-R5'-GGGCTCCACCACCTTGGCCAγ35:35-inverse-F5'-AAGCAGGCGCTGGAGGACGAγ35:35-inverse-F5'-CTCCGCCTCTTGGCCACAγ35:35-inverse-F5'-CTCCGCCTCCTTGGCCGACAβ50:47-F5'-GAGCTGATTGTGTCCATCGGβ50:47-R5'-GTCCTCCCTACCTAGAγ63:63-inverse-F5'-CCCCACCACCACCAγ63:63-inverse-F5'-CGTCACGGCGTCCACAATCAβ22:19-F5'-GGCTGCCAGCGCGGAGGGGGTβ22:19-F5'-CGTCATGTGGCCGAGCTGCAγ85:82-βMTBD85:82-inverse-Fγ85:82-inverse-F5'-CCTTGTGGCGGCGCCACAGTCGCGAγ85:82-inverse-F5'-CCCATCATTGCCCAGCTGCCGAγ85:82-inverse-F5'-CCCATCATTGCGCAGGCGAγ85:82-inverse-F5'-CCCATCATTGCCCAGGCCGAγ85:82-inverse-F5'-CCCATCATTGCGCAGGCCGAγ85:82-inverse-F5'-CCCATCATTGCGCAGGCCGAγ85:82-inverse-F5'-CCCATCATTGCGCAGGCCGAγ85:82-inverse-F5'-CCCATCATTGCGCAGGCCGAγ85:82-inverse-F5'-CCCATCATTGCGCAGGCCGAγ85:82-inverse-F5'-CCCATCATTGCGCAGGCCGAγ85:82-inverse-F5'-CCCATCATTGCCCAGCCGGCGAγ85:82-inverse-F5'-CCCATCATTGCCCAGCCGGCGAγ85:82-inverse-F5'-CCCATCATTGCCCAGCCGGCGAγ85:82-inverse-F5'-CCCATCATTGCCAGCCGGCGAγ85:82-inverse-F5'-CCCATCATTGCCAGCCGGCGAγ85:82-inverse-F5'-CCCAAGCTTGGCCACCCCGGCTGGATGCTGCCCTγ85:82-inverse-F5'-CCCAAGCTTGGGCTCCACCACCTTGGCCAγ85:82-inverse-F5'-CCCAAGCTTGGGCTCCACCACCTTGGCCAγ85:82-inverse-F5'-CCCAAGCTGGGCTCACCACCTTGGCCAγ8		β85:82-inverse-R	5'-CTCAGCCTCCAGCAGGTCGC
γMTBD-R5'-GGGCTCCACCACCTTGGCCAγ35:35-inverse-F5'-AAGCAGGCGCTGGAGGACGAγ35:35-inverse-R5'-CTCCGCCTCCTTGGCCGACAβ50:47-F5'-GAGCTGATTGTGTCCATCGGβ50:47-R5'-GTCCTCCCTACCTAGAγ63:63-inverse-F5'-AAGAACGCCGCCGAGGAGCGγ63:63-inverse-F5'-CGTCACGGGTCCACAATCAβ22:19-F5'-GCGCTGCAGCTGCAGAGCTGCAβ22:19-F5'-CGTCATGGCGGTGTCCAγ85:82-βMTBDβ5:82-inverse-Fγ5:82-inverse-R5'-CTTGTGTGGCGGCGCAγ85:82-inverse-R5'-CTTGGCGGCGCCACAGTCGCγ85:82-inverse-F5'-CCCATCATTGCGCGGCGAγ85:82-inverse-R5'-CTTGGCGGCGCCACAGTCGCβMTBD-F5'-CCCATCATTGCGCAGGCCGAβMTBD-F5'-CCCGTCGAGCCCGGCTGGAAGCConstruction of γMTBD fragmentOADγ-MTBD-XhoI-FS'-CTGGGGCTCACCCCTGGGGCGA5'-CCCAAGCTTGGGCTCACCACCTTGGCCAOADγ-MTBD-HindIII-R5'-CCCAAGCTTGGGCTCACCACCTTGGCCA		γMTBD-F	5'-CCCGCGCTGGATGCTGCCCT
$\begin{array}{llllllllllllllllllllllllllllllllllll$		γMTBD-R	5'-GGGCTCCACCACCTTGGCCA
γ5:35-inverse-R 5 -CICCGCCICCITGGCCCACA β50:47-F 5 -GAGCTGATTGTGCCACGG β50:47-R 5 '-GTCCTCCGTAGCCTTCATGA γ63:63-β22:19 γ63:63-inverse-F 5'-ACGACGCGCCCGAGGAGCG γ63:63-inverse-R 5'-CGTCACGGCGTCCACAATCA β22:19-F 5'-GCGCTGCAGACTGAGGTGTC β22:19-R 5'-CTTCTTGTTGGCGGTGTCCA γ85:82-βMTBD γ85:82-inverse-F 5'-ACAGTTGCCAAGCTGCGCGA γ85:82-inverse-F 5'-ACGGTGCCAGCAGCTGCCGCGA γ85:82-inverse-R 5'-CTTGGCGGCGCCACGGCTGGCGA γ85:82-inverse-R 5'-CCCATCATTGGCCAGGCCGC γ85:82-inverse-R 5'-CCCATCATTGGCCAGGCCGCA γ85:82-inverse-R 5'-CCCATCATTGGCCAGGCCGCA γ85:82-inverse-R 5'-CCCATCATTGGCCAGGCCGA γ85:82-inverse-R 5'-CCCATCATGGCGAGCCGA γ85:82-inverse-R 5'-CCCATCATTGGCCAGGCCGA γ85:82-	γ35:35-β50:47	γ35:35-inverse-F	5'-AAGCAGGCGCIGGAGGACGA
β50:47-F 5-GAGCTGATGGCCTACGG β50:47-R 5'-GTCCTCCGTAGCCTTCATGA γ63:63-inverse-F 5'-AAGAACGCCGCCGAGGAGCG γ63:63-inverse-R 5'-CGTCACGGCGTCCACAATCA β22:19-F 5'-GGCGCGCACGACTGAGGTGTC β22:19-R 5'-CTTCTTGTGGCGGTGTCCA γ85:82-βMTBD γ85:82-inverse-F γ85:82-inverse-F 5'-AAGATTGCCAAGCTGCCGCA γ85:82-inverse-F 5'-CCCATCATTGCCGCGCA γ85:82-inverse-F 5'-CCCATCATTGCGCAGCCGC βMTBD-F 5'-CCCATCATTGCGCAGCCGA γ85:82-inverse-R 5'-CCCATCATTGCGCAGCCGCA βMTBD-R 5'-CCCATCGTGGAGCCGCA βMTBD-R 5'-CCCATCGTGGAGCCGCA βMTBD-R 5'-CCCAAGCTTGGGCTGCCCT OADy-MTBD-HindIII-R 5'-CCCAAGCTTGGGCTCCACCACCTTGGCCA		γ35:35-inverse-K	
γ63:63-β22:19 γ63:63-inverse-F 5'-AGAACGCCGCCGAGGAGGCG γ63:63-inverse-R 5'-CGTCACGCCGAGGAGGGG β22:19-F 5'-GCGCTGCAGAGTGTCC β22:19-R 5'-CTTCTTGGCGGTGTCCA γ85:82-βMTBD γ85:82-inverse-F 5'-AGATTGCCAGCCGCGGCGCAG γ85:82-inverse-F 5'-CCCATCATTGCGCGGCGCA γ85:82-inverse-R 5'-CTTGTGCGCGCGCGCGC γ85:82-inverse-F 5'-CCCATCATTGCGCAGCCCGC γ85:82-inverse-R 5'-CTTGGCGCAGCCAGCCCGC βMTBD-F 5'-CCCATCATTGCGCAGGCCGA βMTBD-R 5'-GGGCGCCACCACCTGGTAGA		рэ0:47-F 850:47 Р	S GEOCHEREN ACCENTEN
γ63:53 inverse-R 5'-CGTCACGGCGTCCACAATCA β22:19-F 5'-GGCGCGCAGACTGAGGTGTC β22:19-R 5'-CTTCTTGTGGCGGTGCCA γ85:82-βMTBD γ85:82-inverse-F 5'-AGATTGCCAGGCTGCCA γ85:82-inverse-R 5'-CTTGTGCGCGAGCCAGGCTCGC βMTBD-F 5'-CCCATCATTGCGCAGGCCGA βMTBD-R 5'-GGGCGCCACCACCTGGTAGA Construction of γMTBD fragment OADγ-MTBD-XhoI-F 5'-CCCAAGCTGGCGCAGCCCACCTGGATGCTGCCCT OADγ-MTBD-HindIII-R 5'-CCCAAGCTTGGGCTCCACCACCTTGGCCA	v63:63-822:19	v63:63-inverse-F	S-AAGACGCGCGAGGAGCG
β22:19-F 5'-GCGCTGCAGACTGAGGTGTC β22:19-R 5'-CTTCTTGTTGGCGGTGTCCA γ85:82-βMTBD γ85:82-inverse-F 5'-ACAGTTGCCAGCTGCGCGA γ85:82-inverse-R 5'-CTTGGCGGCGCCAGCTGGCGCG βMTBD-F 5'-CCCATCATTGCGCAGCCCGC βMTBD-R 5'-GCGCTGCAGCCCGCGA βMTBD-R 5'-CCGCTCGAGCCCGCGCGCGGCTGGATGCTGCCCT OADγ-MTBD-HindIII-R 5'-CCCAACTTGGCGCACCACCTTGGCCA	105.05 pm. 17	y63:63-inverse-R	5'-CGTCACGGCGTCCACAATCA
β22:19-R 5'-CTTCTTGTTGGCGGTGTCCA γ85:82-βMTBD γ85:82-inverse-F 5'-AAGATTGCCAAGCTGCGCGA γ85:82-inverse-R 5'-CTTGGCGGCAGCCAGGTCGC βMTBD-F 5'-CCCATCATTGCGCAGGCCGA βMTBD-R 5'-GGGCGCCACCACCTGGTAGA Construction of γMTBD fragment OADγ-MTBD-XhoI-F 5'-CCCAAGCTCGCCGCGCTGGATGCTGCCCT OADγ-MTBD-HindIII-R 5'-CCCAAGCTTGGGCTCCACCACCTTGGCCA		β22:19-F	5'-GCGCTGCAGACTGAGGTGTC
γ85:82-βMTBD γ85:82-inverse-F γ85:82-inverse-R γ85:82-inverse-R γ5'-CTTGGCGGCAGCCAGGTCGC βMTBD-F γ5'-CCCATCATTGCGCAGGCCGA βMTBD-R γ-GGGCGCCACCACCTGGTAGA Construction of γMTBD fragment OADγ-MTBD-XhoI-F 5'-CCCAAGCTGGCCGCGCGGATGCTGCCCT OADγ-MTBD-HindIII-R 5'-CCCAAGCTTGGGCTCCACCACCTTGGCCA		β22:19-R	5'-CTTCTTGTTGGCGGTGTCCA
y85:82-inverse-R β'-CTTGGCGGCAGCCAGGTCGC βMTBD-F β'-CCCATCATTGCGCAGGCCGA βMTBD-R β'-GGGCGCCACCACCTGGTAGA Construction of yMTBD fragment OADy-MTBD-HindIII-R 5'-CCCAAGCTCGGCCGCAGGCCGCAGCCCCCCCTGGCAGCCCCCCCC	γ85:82-βMTBD	γ85:82-inverse-F	5'-AAGATTGCCAAGCTGCGCGA
βMTBD-F 5'-CCCATCATTGCGCAGGCCGA βMTBD-R 5'-GGGCGCCACCACCTGGTAGA Construction of γMTBD fragment OADγ-MTBD-XhoI-F 5'-CCGCTCGAGCCCGCGGCTGGATGCTGCCCT OADγ-MTBD-HindIII-R 5'-CCCAAGCTTGGGCTCCACCACCTTGGCCA		γ85:82-inverse-R	5'-CTTGGCGGCAGCCAGGTCGC
βMTBD-R 5'-GGCGCCACCACCTGGTAGA Construction of γMTBD fragment OADγ-MTBD-Xhol-F 5'-CCGCTCGAGCCCGCGGCTGGATGCTGCCCT OADγ-MTBD-HindIII-R 5'-CCCAAGCTTGGGCTCCACCACCTTGGCCA		βMTBD-F	5'-CCCATCATTGCGCAGGCCGA
Construction of yMTBD fragment OADy-MTBD-Xhol-F 5'-CCGCTCGAGCCCGCGGCTGGATGCTGCCCT OADy-MTBD-HindIII-R 5'-CCCAAGCTTGGGCTCCACCACCTTGGCCA		βMTBD-R	5'-GGGCGCCACCACCTGGTAGA
OADy-MTBD-Hindlik 5'-CCCAAGCTTGGGCTCCACCACCTTGGCCA	Construction of vMTBD fragment	OADy-MTBD-XhoI-F	5'-CCGCTCGAGCCCGCGCTGGATGCTGCCCT
	inghome provide inghom	OADy-MTBD-HindIII-R	5'-CCCAAGCTTGGGCTCCACCACCTTGGCCA

 $\mathbf{5}$

6 Figure S1. Recombinant Chlamydomonas and Tetrahymena, and recombinant OAD complex.

- 7 (A) Assessment of His-tagged *Chlamydomonas* LC1.
- 8 (i) Western blots of axonemes of WT and LC1-N-His recombinant strains by Chlamydomonas LC1 antibody (Ch
- 9 LC1 antibody).
- 10 (ii) Western blots of the OAD complexes of WT and LC1-C-His by Chlamydomonas LC1 antibody (Ch LC1
- 11 antibody). The OAD complex in (ii) was prepared by the ATP extraction. Both His-tagged and wild-type LC1 were
- 12 detected in (i) and (ii).
- 13 (iii) The OAD complex was extracted from recombinant *Chlamydomonas* axonemes under high salt conditions and
- 14 further affinity purified using a Ni-NTA affinity.
- 15 (B) Assessment of *Tetrahymena* transformants.
- 16 (i) PCR was performed using genomic DNA purified from the WT strain or transformants (strains βHC-C-hGFP and
- 17 LC1-C-hsGFP) using primer sets βHC-C-F/βHC-genome-check-R or LC1-C-F/LC1-genome-check-R. The positions
- 18 of the primer annealing sites are shown in the schematic diagram. By phenotypic assortment process, the wild type
- 19 genes were replaced by recombinant genes. For the β HC-C-hGFP strain, single cell lines were obtained (strains-1, 2,
- and 3). All ~45 copies of genes in strain-3 were thought to be replaced by recombinant genes because no wild-type
- 21 genes were detected in cells cultured without paromomycin (unlike for the strains-1 and 2).
- 22 (ii) Both the recombinant β HC and LC1 were detected in *Tetrahymena* cilia by GFP fluorescence. Arrowheads
- 23 indicate the oral apparatus. Bar, 20 μm.
- 24 (C) Assessment of the recombinant *Tetrahymena* OAD constructs.
- (i) βHC-C-hGFP was detected in cilia by Western blots using anti *Tetrahymena* β HC antibody (*Te* βHC antibody)
 and anti GFP antibody. The βHC-C-hGFP protein was purified using Ni-NTA resin.
- 27 (ii) LC1-C-hsGFP was detected in cilia and in the OAD complex by Western blots using anti GFP antibody and anti
- 28 Tetrahymena LC1 antibody (Te LC1 antibody). The LC1-C-hsGFP OAD complex was extracted in high salt condition
- and further purified by SBP-tag on the LC1.
- 30

31 Figure S2. Characterization of motility of recombinant *Chlamydomonas*.

- The beat frequencies (A) and swimming velocities (B) of wild type and the recombinant *Chlamydomonas*. The mean values for swimming velocities are as follows: Wild type, $154.8 \pm 15.8 \mu m/sec$; LC1-N-His, $151.4 \pm 17.4 \mu m/sec$; LC1-C-His, $151.5 \pm 17.8 \mu m/sec$ (mean \pm SD, n = 20 each).
- 35

36 Figure S3. EM images of *Tetrahymena* OAD complex.

- (A) Schematic diagram of the LC1-C-hsGFP construct and EM images of the Ni-NTA-nanogold labeled *Tetrahymena*LC1-C-hsGFP OAD complex. Gold particles (orange arrowheads) bound at one of the stalk tips. Note that the
 distribution of the gold particles was wider than in the labeling of the *Chlamydomonas* LC1 (Figure 1A), possibly
 because of the flexibility of the hsGFP-tag.
- 41 (B) Ni-NTA-nanogold labeling of the *Tetrahymena* βHC-C-hGFP OAD complex.
- 42 The gold particles (orange arrowheads) were found at the edge of the AAA+ ring. Scale bars, 50 nm.
- 43

44 Figure S4. Supplemental results for the large stalk tip.

- 45 (A) EM images of the native *Tetrahymena* OAD complex.
- 46 (B) Class averages of the head domains of *oda11 Chlamydomonas* OAD.
- Representative class averages with (left side) or without (right side) extra density (red arrowhead) are shown. The
 numbers of EM images are shown.
- 40 numbers of EW images are shown.
- 49 (C) Ni-NTA-nanogold labeling of *oda11*×LC1-C-His *Chlamydomonas* OAD.
- 50 One of the stalk tips in the $\beta\gamma$ two-headed structure was labeled with Ni-NTA-nanogold (orange arrowheads).
- 51

52 Figure S5. Data related to the single particle analysis of *Tetrahymena* DYH3 head fragment.

- 53 (A) SDS-PAGE and Western blots of purified *Tetrahymena* DYH3 head fragment.
- 54 The main band at the top of the gel represents the DYH3 head fragment. The minor bands are thought to correspond
- to degradation products due to chymotryptic digestion. The purified DYH3 head fragment was found to associate
- 56 with endogenous LC1 by Western blots using *Tetrahymena* LC1 antibody (*Te* LC1 antibody).
- 57 (B) Single particle analysis of *Tetrahymena* DYH3 head and stalk region.
- 58 Class averages of *Tetrahymena* DYH3 head fragments showing the large stalk tip (top row). Similar EM images in
- 59 the major group (indicated by orange asterisk) were sub-classified into five classes by K-means clustering (middle
- 60 row). Subsequently, the images were aligned according to the stalk region by applying a mask shown (bottom row).
- 61 The mask was applied to most of the AAA+ ring except the base of the stalk, so that information on the stalk angle
- 62 was retained. Orange asterisks denote the class averages shown in Figure 3, and the numbers of images are indicated.
- 63 (C) Single particle analysis of the stalk-tip region.
- 64 Class averages of the stalk-tip region are shown in the upper two rows. In some classes, the large stalk tip image was 65 composed of two sub-structures. One class average was sub-classified and the representative class average is shown 66 in Figure 3B-iii (orange asterisk). The numbers of images are shown.
- 67

68 Figure S6. Supplemental results for Figure 4.

- 69 (A) Relationship between molar concentration and band intensities.
- 70 Known concentration of His-γ stalk and GST-LC1 (1-4 μM, 11.25 μl each) were assessed by SDS-PAGE and the
- 51 band intensities were measured using ImageJ (NIH). Mean value of the band intensity ratios (His-γ stalk /GST-LC1)
- 72 was 1.0, therefore, band intensity ratio (His-γ stalk/GST-LC1) can be considered as molar ratio. Co-purified His-γ
- stalk and GST-LC1 in Figure 3C-(i) is within this range.
- 74 (B) GST pull-down assay using γ MTBD region fragment.
- 75 Schematic diagram of γ MTBD region fragment sequence and result of GST pull-down assay. γ MTBD fragment was
- detected in bound fraction together with GST-LC1. Here, GST pull-down assay was performed basically same as in
- Figure 5, except that concentration of γ MTBD fragment was 450 nM.
- 78

79 Figure S7. Comparison of the stalk sequences.

- 80 (A) Schematic diagrams of the γ (yellow) and β (gray) stalk region sequences. The numbers of the amino acid residues
- 81 used as junction sites for the chimeric stalk constructs are shown.

- 82 (B) Amino acid sequence of the stalk region of mouse cytoplasmic dynein HC, *Chlamydomonas* α , β and γ HCs,
- 83 Tetrahymena DYH3 HC, human DNAH5 HC, human DNAH9 HC, and Chlamydomonas inner arm dynein-c. The
- regions corresponding to amino acids 3,047-3,350 of the *Chlamydomonas* γ HC were aligned (identical residues, red;
- similar residues, blue). The *Chlamydomonas* γ HC and its homologues are indicated in yellow, and *Chlamydomonas*
- 86 β HC is indicated in gray. The MTBD is defined as the region between two highly conserved proline residues
- 87 (magenta asterisks). The numbers of amino acid residues in CC1 and CC2, counted from the proline residues, are
- 88 shown above. Residues shown in magenta were used for the junction sites for the chimeric constructs. Known
- 89 structural information of the MTBD, helix 1-6 (H1-H6), is highlighted, based on Carter *et al.* (2008) and Kato *et al.*
- 90 (2014). The insert sequences between H2 and H3 are highlighted in magenta.
- 91

92 Figure S8. SDS-PAGE images of the microtubule co-pelleting assay.

SDS-PAGE results of the microtubule co-pelleting assay of γ stalk, γ stalk-LC1 complex, and LC1. Fixed concentration of microtubule (5 μ M) was incubated with increasing concentrations of γ stalk (0.97-5.8 μ M), γ stalk-LC1 complex (1.8-4.8 μ M), and LC1 (1.0-6.3 μ M). After centrifugation, the supernatant fraction and precipitation fraction were analyzed by SDS-PAGE. The images of gels were digitized and the intensities of the bands were quantified.

98

99 Figure S9. Homology models of the MTBD region.

- 100 Homology models were built from the NMR structure of dynein-c MTBD (PDB: 2RR7) for α HC (A), β HC (B), and
- 101 γ HC (C). Only the γ MTBD was predicted to have the "flap" structure (red arrowheads). Note that the flap structure
- 102 alone cannot explain the size of the additional structure in Figure 3B.
- 103

104 Supplemental materials and methods

105 Strains and culture conditions

- 106 Chlamydomonas reinhardtii strains used were WT (137C), oda11 (Sakakibara et al., 1991), oda4-s7 (Sakakibara et
- al., 1993), LC1-N-His, LC1-C-His, LC2-C-His (Furuta et al., 2009) and oda11×LC1-C-His. Cells were grown in
- 108 liquid Tris-acetate-phosphate medium (Gorman and Levine, 1965) with aeration on a 12 h light and 12 h dark cycle,
- 109 or on solid medium containing 1.5% agar.
- 110 Tetrahymena thermophila strains used were WT SB255, WT B2086, βHC-C-hGFP, and LC1-C-hsGFP. Cells were
- 111 cultivated in SPP media (1% proteose peptone No.3, 0.2% glucose, 0.1% yeast extract, 0.003% Fe-EDTA) or in PYD
- 112 media (1% proteose peptone No.3, 0.87% glucose, 0.5% yeast extract). Appropriate concentration of Cd²⁺ and
- 113 paromomycin for selection were added to the media if necessary.
- 114

115 Antibodies

- To generate antibodies against *Chlamydomonas* LC1's 104-198 aa region, the cDNA sequence coding this region was amplified by PCR from wild type cDNA and subcloned into the pCold proS2 DNA vector (Takara) between *NdeI* and *BamHI* sites. In brief, the plasmid was transformed into *E. coli* BL21-CodonPlus (DE3) RIL (Stratagene) and induced protein was purified using the His-tag. After the His-tag and proS2-tag were removed by Thrombin digestion (Sigma), the relevant peptide fragment was separated by SDS-PAGE and used as the antigen to generate rabbit polyclonal antibody. For the production of antibody against *Tetrahymena* LC1, a peptide fragment corresponding to the 131-144 aa region (NWEELDKLKDLPEL) was synthesized as the antigen to generate rabbit polyclonal antibody.
- 123

124 **Purification of** *Chlamydomonas* **OAD complex**

- 125Isolation and demembranation of Chlamydomonas flagella were performed by standard methods (Witman et al., 1261986). Chlamydomonas OAD complexes were extracted from the axonemes either by high salt extraction (0.6 M 127KCl, 30 mM HEPES pH 7.4, 1 mM EGTA, 5 mM MgSO₄) or ATP extraction (5 mM ATP, 75 mM PIPES pH 6.8, 1 128mM MgCl₂, 1 mM EGTA, 1 mM GTP, 1 mM DTT, 10 µg/ml leupeptin, 1 mM PMSF, 10 µM paclitaxel) (Goodenough and Heuser, 1984). The extracted OAD complex was further fractionated by 10-40% (w/v) sucrose density gradient 129130centrifugation in 0.1 mM ATP, 75 mM PIPES pH 6.8, 1 mM MgCl₂, 1 mM EGTA, 1 mM GTP, 1 mM DTT, 10 µg/ml 131leupeptin, 1 mM PMSF and 10 µM paclitaxel. For EM observation, the OAD complex purified by ATP extraction 132was used, since the three-headed structure was more readily observed in this condition.
- 133

134 **Purification of** *Tetrahymena* **OAD complex**

135*Tetrahymena* cells were deciliated by the addition of 2 mM Ca²⁺ (Rosenbaum and Carlson, 1969). Demembranation136of cilia was performed by adding 0.1% NP-40. *Tetrahymena* OAD complexes were isolated from the axoneme by137high salt extraction (0.6 M NaCl, 10 mM HEPES-NaOH pH7.4, 4 mM MgSO₄, 5 mM EGTA, 0.1 mM PMSF). The138extracted recombinant OAD complex containing LC1-C-hsGFP was purified by SBP-tag using Strep-Tactin139Sepharose resin (IBA), according to Ichikawa et al. (2011). Recombinant OAD complex containing β HC-C-hGFP140was purified by His-tag using Profinity IMAC Ni-Charged Resin (Bio-Rad), and imidazole was removed from eluted141protein with a NAP5 column (GE Healthcare). Purification of the wild-type OAD complex and chymotryptic

142 143 digestion to obtain the DYH3 head fragment was performed as in Yamaguchi et al. (2015).

144 Tagging of Chlamydomonas LC1

145To make *Chlamydomonas* LC1 expression constructs, a ~3.8 kb fragment containing the LC1 gene was amplified 146 from wild-type Chlamydomonas genomic DNA using primers LC1-5p-F and LC1-3p-R. Primers used in this study 147are shown in Table S1. The PCR product was cloned into the *Eco*RV site of pBluescript II (Agilent Technologies) to 148create the construct pLC1. For N-terminal tagging with 8 × His, two oligo nucleotides, LC1-N-8×His-1 and LC1-N-1498×His-2, were annealed and inserted into the MscI site of pLC1 to create the construct pLC1-N-His. For C-terminal 150tagging with $8 \times$ His, two LC1 genomic fragments were amplified using primer sets LC1-F1/LC1-C-8×His-SpeI-R 151and LC1-3p-XbaI-F/LC1-3p-R. The two amplification products were digested with SpeI and XbaI, ligated together, 152and the product was digested with NcoI and AatII and used to replace the untagged LC1 gene in pLC1 to create 153pLC1-C-His. Each of the two His-tagged LC1 plasmids was linearized with EcoRI and co-transformed with pSI103 154(PMID: 11602359) into wild-type Chlamydomonas cells by electroporation. Cells expressing His-tagged LC1 were 155screened by Western blots. A cross between the LC1-C-His strain and strain oda11 was produced using standard 156methods (Harris, 1989).

157

158 **Tagging of** *Tetrahymena* **OAD subunits.**

159pEGFP-neo4 vector (Kataoka et al., 2010) was modified so that an 8 × His-tag was introduced in a loop region of 160 EGFP as in Kobayashi et al. (2008) by inverse PCR using primer set phGFP-inverse-F and phGFP-inverse-R (phGFP-161 neo4 vector). The phGFP-neo4 vector was further modified so as to carry an SBP-tag by inverse PCR using primer 162sets phsGFP-inverse-1st-F/phsGFP-inverse-1st-R and phsGFP-inverse-2nd-F/phsGFP-inverse-2nd-R (phsGFP-neo4 163vector). Homologous recombination into the Tetrahymena macronucleus was performed as in Kataoka et al. (2010). 164 In brief, an hsGFP-neo4-fragment was amplified from phsGFP-neo4 vector with primers GFP-neo4-F and GFP-neo4-165R. The DNA sequences encoding the C-terminal region of LC1 (LC1-C-fragment; 1,263 bp) and the 3' flanking 166 region (LC1-3'-fragment; 1.475 bp) were amplified from *Tetrahymena* genomic DNA with primer sets LC1-C-F/LC1-167 C-R and LC1-3'-F/LC1-3'-R. These three fragments were integrated by overlapping PCR using primers overlap-168outer-F and overlap-outer-R. Transformation was performed using a PDS-1000/He biolistic delivery system (BioRad). 169 To select transformants, a drug resistance gene was induced with 1 μ g/ml Cd²⁺, and 100 μ g/ml paromomycin was 170 added for selection. hGFP-tagging to Tetrahymena BHC was performed similarly. Since the Tetrahymena macronucleus holds ~45 copies of genes, a phenotypic assortment process was performed to obtain transformants 171172with a high copy number of the recombinant gene (Wood et al., 2007). The concentration of Cd²⁺ was reduced 173gradually to 0.01 µg/ml, and then the paromomycin concentration was increased gradually to 850 µg/ml for the LC1-174C-hsGFP strain and 600 µg/ml for the βHC-C-hGFP strain. The increase of the ratio of recombinant genes was 175assessed by GFP fluorescence and PCR analysis (Supplemental Figure S1B). For the β HC-C-hGFP strain, a single 176cell line whose β HC genes were completely replaced with recombinant genes was obtained and used for the study 177(strain-3 in Supplemental Figure S1B). GFP fluorescence images were acquired using a CCD camera (iXonEM 178DV860, Andor) attached to an IX70 microscope (Olympus) equipped with a confocal scanner unit (CSU10, 179Yokogawa).

180

181 Measurements of swimming velocity and beat frequency.

Swimming of *Chlamydomonas* was recorded using BX53 microscope (Olympus) equipped with a CCD camera (ADT-33S, FLOVEL). Swimming velocities of *Chlamydomonas* cells were analyzed using ImageJ MTrack2 plug-in (http://valelab.ucsf.edu/~nstuurman/ijplugins/MTrack2.html). Beat frequencies of *Chlamydomonas* cells were measured by fast Fourier transform (FFT) analysis of vibrations of the cells (Kamiya, 2000).

187

188 Construction of proteins for biochemical experiments.

189 The cDNA regions encoding the stalk regions of α , β and γ HC were amplified from *Chlamydomonas* cDNA with 190 appropriate primer pairs (Supplemental Table S1) so that the numbers of amino acid residues in CC1 and CC2 would 191 be 85 and 82 respectively. The fragments were subcloned into pColdI vector between XhoI and HindIII sites, and 192pColdI- α stalk, pColdI- β stalk and pColdI- γ stalk were obtained. pColdI- γ MTBD was constructed similary. The 193 cDNA region coding Chlamydomonas LC1 was amplified and inserted into vector pGEX-6P-2 between BamHI and 194*Eco*RI sites to produce pGEX-6P-2-LC1. To create chimeric stalk constructs with a β HC base and a γ HC tip (β35:35γ50:47, β63:63-γ22:19, β85:82-γMTBD), pColdI-β stalk vector was linearized by inverse PCR and fragments 195196 encoding the γ stalk-tip region or the γ MTBD region were amplified from pColdI- γ stalk by PCR. These fragments were integrated by blunt-end ligation and pColdI-β35:35-y50:47, pColdI-β63:63-y22:19 and pColdI-β85:82-yMTBD 197 198vectors were obtained. pColdI-β35:35-γ50:47, pColdI-β63:63-γ22:19 and pColdI-β85:82-γMTBD vectors were 199 created vice versa. All PCR products were verified by sequencing.

200

201 **Purification of proteins for biochemical experiments.**

202The resultant plasmids were transformed into E. coli BL21-CodonPlus (DE3) RIL. Cells were cultured in 500 ml LB 203medium supplemented with 50 μ g/ml ampicillin at 37°C until the optical density OD₆₀₀ reached 0.4-0.5. For 204 constructs in pColdI, cells were cultured for a further 24 h after the temperature was lowered to 15°C and 0.1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added to the media for induction. Cells containing pGEX-6P-2-205206LC1 were cultured for a further 3 h at 20°C after 0.4 mM IPTG was added. For the stalk constructs and γ MTBD 207 fragment, purification was performed using the His-tag derived from the pColdI vector. The cells were lysed with 208His-tag lysis buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 20 mM imidazole), sonicated, and ultracentrifuged. 209 The supernatant obtained was left to bind to Profinity IMAC Ni-Charged Resin (BIO-RAD) for 30 min, 4°C. The 210resin was washed three times with His-tag wash buffer (50 mM Tris-HCl pH 8.0, 250 mM NaCl, 20 mM imidazole) 211and bound protein was eluted with His-tag elution buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 300 mM 212imidazole). LC1 construct was purified using the GST-tag from vector pGEX-6P-2. For the purification of GST-213tagged LC1 (GST-LC1), the cell pellet was resuspended, sonicated, and ultracentrifuged in PBS (10 mM Na₂HPO₄, 2141.8 mM KH₂PO₄, 140 mM NaCl, 2.7 mM KCl, pH 7.3) containing 1 mM DTT. GST•Bind Resin was added to the 215supernatant and incubated for 60 min at 4°C. The resin was washed three times with PBS containing 1 mM DTT. To 216obtain GST-tagged LC1 (GST-LC1), protein was eluted with GST elution buffer (50 mM Tris-HCl pH8.0, 100 mM 217NaCl, 10 mM glutathione). To purify LC1 without a GST-tag, PreScission base buffer (50 mM Tris-HCl pH 7.5, 150 218mM NaCl, 1 mM EDTA) was used instead of PBS, and the GST-tag was removed by incubating with PreScission 219base buffer containing 1 mM DTT and PreScission protease (GE Healthcare) overnight at 4°C. To perform tandem 220affinity purification of His-ystalk and GST-LC1, the supernatants of *E.coli* expressing either His-y stalk or GST-LC1 221were mixed for 1 h at 4°C prior to sequential purification using the His-tag and the GST-tag. For all the purified 222proteins, buffer was exchanged to NAP5 buffer by using NAP5 columns (GE Healthcare). Protein concentrations were determined according to Read and Northcote (1981) using BSA as the standard. 223224225**Computational analysis** 226To quantify band intensities, ImageJ (NIH) or Quantity One software (BioRad) were used. Data analysis was 227performed using Excel, Sigmaplot and GraphPad Prism 6. Statistical analyses were performed using GraphPad Prism 2286. Alignment of sequences was performed by Clustal W (Thompson et al., 1994). For homology modeling of the 229MTBD structure, Swiss Model Server (Arnold et al., 2006) was used. 230231232233234References 235Arnold, K., Bordoli, L., Kopp, J., & Schwede, T. (2006). The SWISS-MODEL workspace: a web-based environment 236for protein structure homology modelling. *Bioinformatics*, 22(2), 195-201. 237Gorman, D. S., & Levine, R. P. (1965). Cytochrome f and plastocyanin: their sequence in the photosynthetic electron 238239transport chain of Chlamydomonas reinhardi. Proceedings of the National Academy of Sciences of the United States 240of America, 54(6), 1665. 241242Harris, E. H. (1989). The chlamydomonas sourcebook (Vol. 2). San Diego: Academic Press. 243244Ichikawa, M., Watanabe, Y., Murayama, T., & Toyoshima, Y. Y. (2011). Recombinant human cytoplasmic dynein 245heavy chain 1 and 2: observation of dynein-2 motor activity in vitro. FEBS letters, 585(15), 2419-2423. 246247Kamiya, R. (2000). Analysis of cell vibration for assessing axonemal motility in Chlamydomonas. 248Methods. 22:383-387. http://dx.doi.org/10.1006/ meth.2000.1090 249250Kataoka, K., Schoeberl, U. E., & Mochizuki, K. (2010). Modules for C-terminal epitope tagging of Tetrahymena 251genes. Journal of Microbiological Methods, 82(3), 342-346. 252253Read, S. M., & Northcote, D. H. (1981). Minimization of variation in the response to different proteins of the 254Coomassie blue G dye-binding assay for protein. Analytical biochemistry, 116(1), 53-64. 255

8

256	Rosenbaum, J. L., & Carlson, K. (1969). Cilia regeneration in Tetrahymena and its inhibition by colchicine. The
257	Journal of Cell Biology, 40(2), 415-425.

258

Sakakibara, H., Mitchell, D. R., & Kamiya, R. (1991). A Chlamydomonas outer arm dynein mutant missing the alpha
heavy chain. *The Journal of Cell Biology*,113(3), 615-622.

- Sakakibara, H., Takada, S., King, S. M., Witman, G. B., & Kamiya, R. (1993). A Chlamydomonas outer arm dynein
 mutant with a truncated beta heavy chain. *Journal of Cell Biology*, 122(3), 653-661.
- 264

261

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive
multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22), 4673-4680.

268

271

Wood, C. R., Hard, R., & Hennessey, T. M. (2007). Targeted gene disruption of dynein heavy chain 7 of Tetrahymena
thermophila results in altered ciliary waveform and reduced swim speed. *Journal of Cell Science*, 120(17), 30753085.

275

^{Witman, G. B. (1986). Isolation of Chlamydomonas flagella and flagellar axonemes.} *Methods in enzymology*, 134,
280-290.