PNAS
www.pnas.org

Supplementary Information for

Ciliogenesis Requires Sphingolipid-dependent Membrane and Axoneme Interaction

Dou Wu[1,2,3,4 *], Jingying Huang[1,2,3,4 *], Hao Zhu[1,2,3,4 *], Zhe Chen[1,2,3,4], Yongping Chai[1,2,3,4], Jingyi Ke[1,2,3,4], Kexin Lei[1,2,3,4], Zhao Peng[5,6], Ranhao Zhang[1,2], Xueming Li[1,2], Kaiyao Huang[5,6], Wei Li[7], Chengtian Zhao[8,9,10,11], and Guangshuo Ou[1,2,3,4†].

[*]These authors contributed equally to this work
†Guangshuo Ou

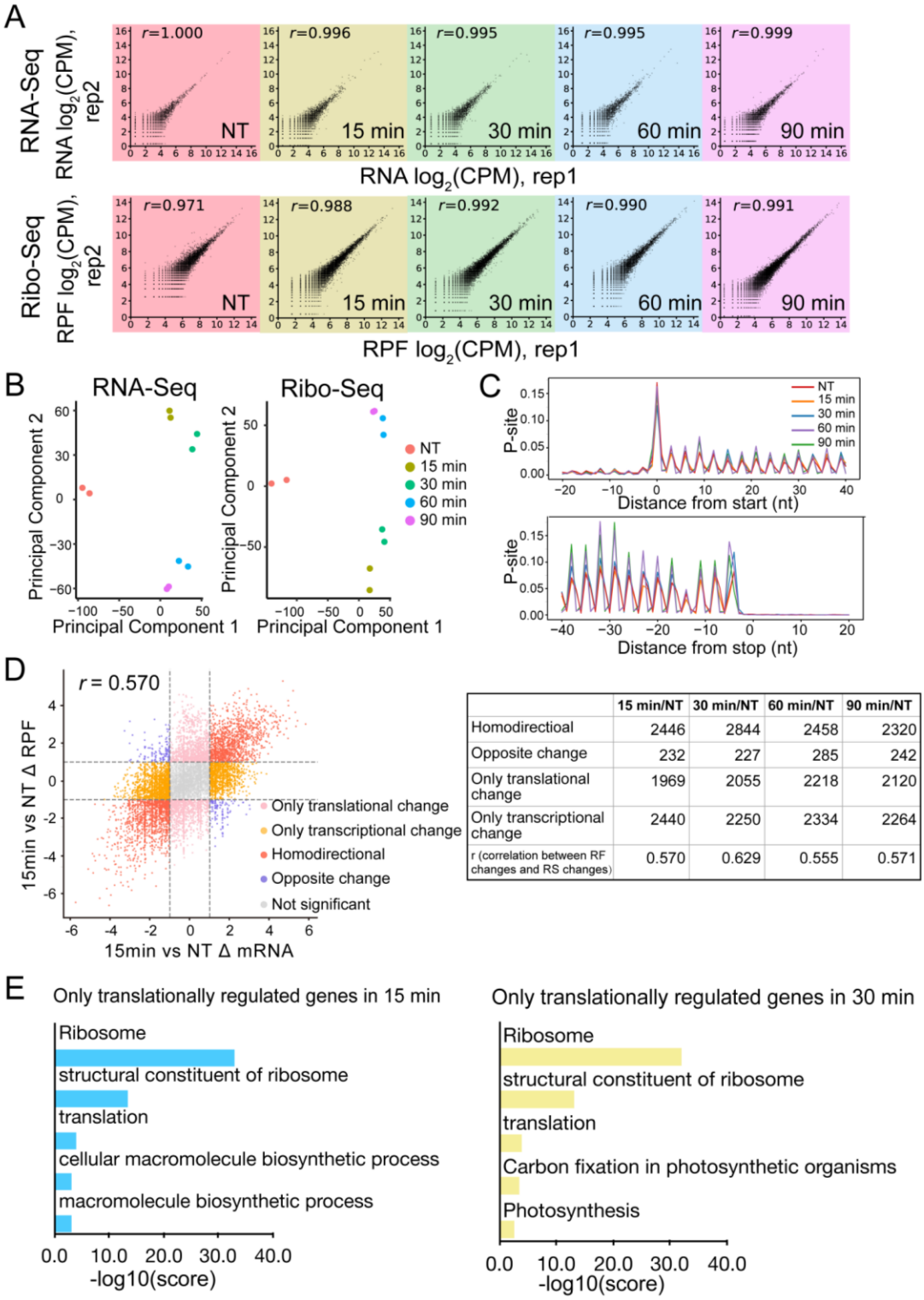
Email: guangshuoou@tsinghua.edu.cn

This PDF file includes:

Figures S1 to S6
Tables S1 to S2
Legends for Movie S1 to S2
Legends for Datasets S1 to S4

Other supplementary materials for this manuscript include the following:

Movies S1 to S2
Datasets S1 to S4



	15 min/NT	30 min/NT	60 min/NT	90 min/NT
Homodirectioal	2446	2844	2458	2320
Opposite change	232	227	285	242
Only translational change	1969	2055	2218	2120
Only transcriptional change	2440	2250	2334	2264
r (correlation between RF changes and RS changes)	0.570	0.629	0.555	0.571

Fig. S1. The quality and reproducibility of RNA-Seq and Ribo-Seq data. (***A***) Correlation between two replicates (rep1/2) of RNA-Seq or Ribo-Seq samples. Data are shown as the correlation of log2(CPM) in CDS (Ribo-Seq) or exon (RNA-Seq) for expressed genes with CPM >1.

Pearson correlation coefficient r is shown. (***B***) Principal Component Analysis (PCA) in the first two principal component spaces for RNA-Seq or Ribo-Seq data over all the time points. (***C***) Line plots of the distance from the 5' end of RPF reads to the annotated start or stop codons for Ribo-Seq. (***D***) (Left) Correlation between gene expression fold changes with RNA-Seq and Ribo-Seq data. Colored dots correspond to differentially expressed and translated genes. Pearson correlation coefficient *r* is shown. (Right) The number of differentially expressed and translated genes at different time points during flagellar regeneration. (***E***) Gene enrichment analysis for the translational upregulated genes in 15 min and 30 min algae after deflagellation compared with Non-treated algae.

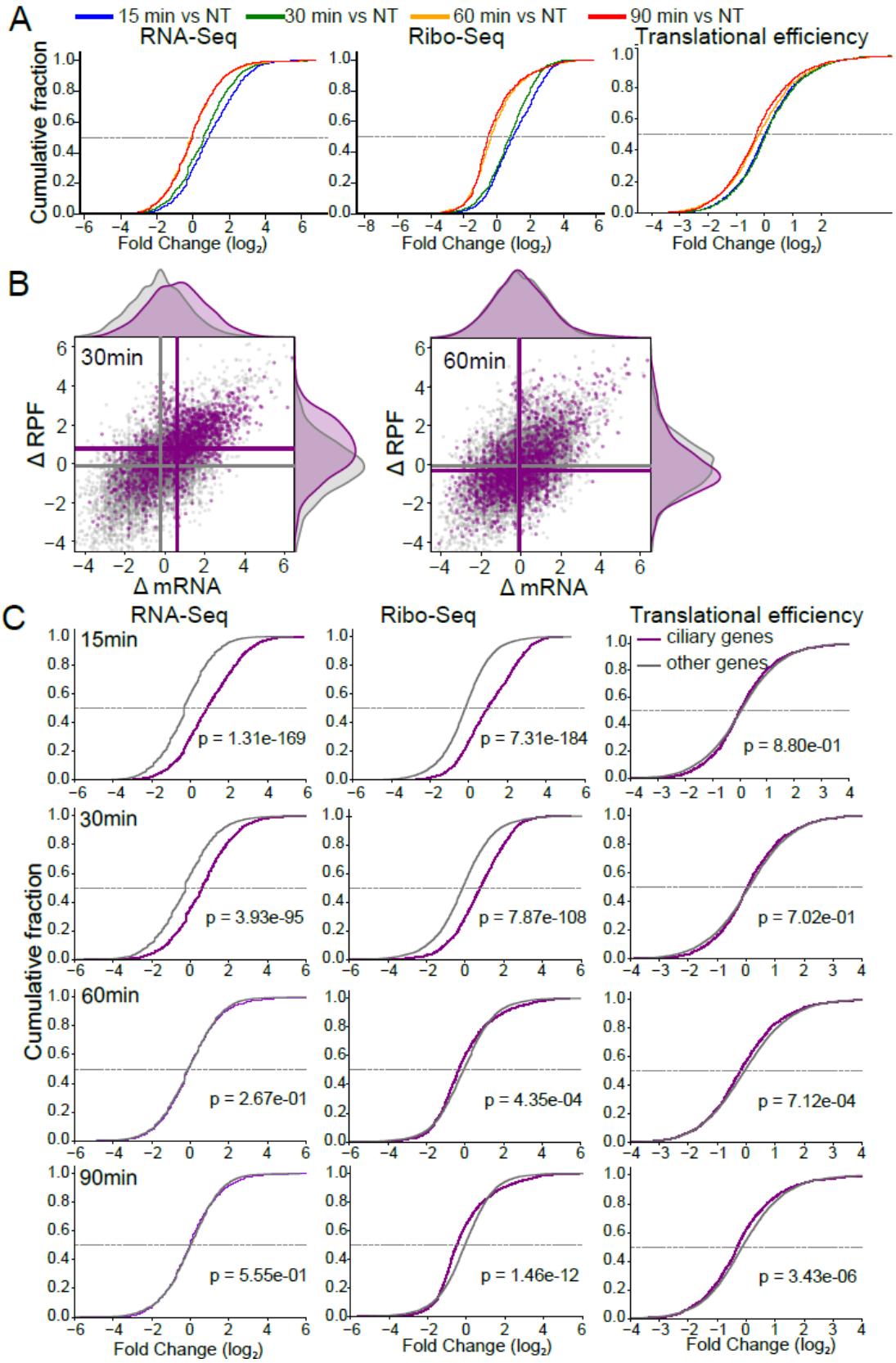
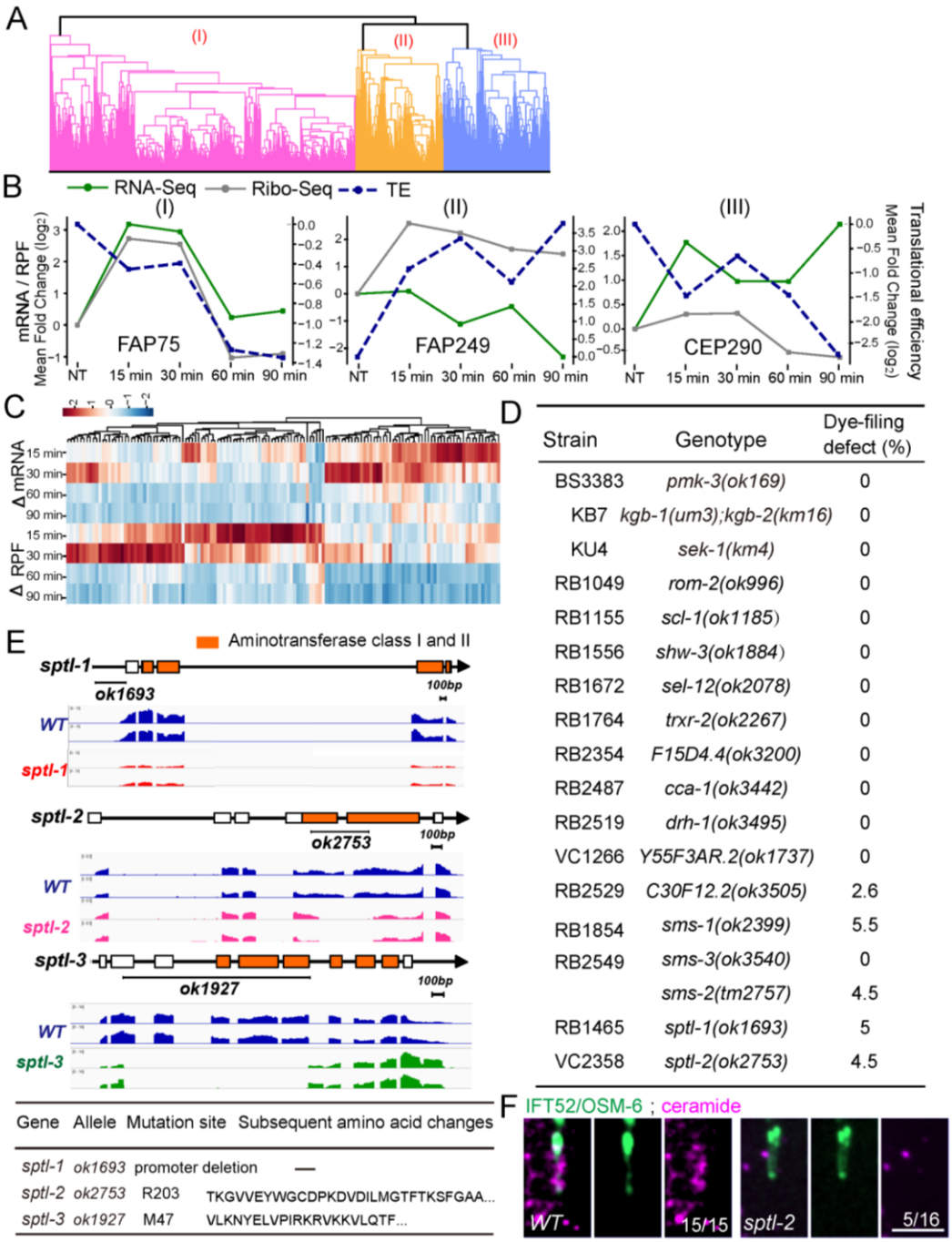

A
15 min vs NT
30 min vs NT
60 min vs NT
90 min vs NT
RNA-Seq
Ribo-Seq
Translational efficiency
Cumulative fraction
Fold Change (log2)
B
30min
60min
Δ RPF
Δ mRNA
C
RNA-Seq
Ribo-Seq
Translational efficiency
ciliary genes
other genes
15min
30min
60min
90min
p = 1.31e-169
p = 7.31e-184
p = 8.80e-01
p = 3.93e-95
p = 7.87e-108
p = 7.02e-01
p = 2.67e-01
p = 4.35e-04
p = 7.12e-04
p = 5.55e-01
p = 1.46e-12
p = 3.43e-06
Cumulative fraction
Fold Change (log2)

Fig. S2. Transcriptional and translational patterns of the ciliary genes during flagellar regeneration. (***A***) Cumulative distributions of log2-fold change of RNA-Seq (left), Ribo-Seq (middle) and translational efficiency (right) at 15 (blue), 30 (green), 60 (orange), and 90 (red) min after flagellar regeneration for ciliary genes. (***B***) Scatter plots show log2-fold change of RPFs (y-axis) and mRNA (x-axis) at 30 min and 60 min after flagellar regeneration. Ciliary genes are in purple, and other genes are in gray. Mean values per group are indicated as lines. (***C***) Cumulative distributions log2-fold change of RNA-Seq (left), Ribo-Seq (middle), and translational efficiency (right) at 15, 30, 60, and 90 min after flagellar regeneration for ciliary genes (purple) and other genes (grey). P values from rank-sum tests for ciliary genes versus other genes.



Strain	Genotype	Dye-filing defect (%)
BS3383	*pmk-3(ok169)*	0
KB7	*kgb-1(um3);kgb-2(km16)*	0
KU4	*sek-1(km4)*	0
RB1049	*rom-2(ok996)*	0
RB1155	*scl-1(ok1185)*	0
RB1556	*shw-3(ok1884)*	0
RB1672	*sel-12(ok2078)*	0
RB1764	*trxr-2(ok2267)*	0
RB2354	*F15D4.4(ok3200)*	0
RB2487	*cca-1(ok3442)*	0
RB2519	*drh-1(ok3495)*	0
VC1266	*Y55F3AR.2(ok1737)*	0
RB2529	*C30F12.2(ok3505)*	2.6
RB1854	*sms-1(ok2399)*	5.5
RB2549	*sms-3(ok3540)*	0
	sms-2(tm2757)	4.5
RB1465	*sptl-1(ok1693)*	5
VC2358	*sptl-2(ok2753)*	4.5

Fig. S3. Sphingolipid metabolism genes regulate ciliogenesis. (***A***) Hierarchical clustering dendrogram of mRNA and RPFs fold changes in all-time points to divide ciliary genes into three clusters. (***B***) The representative plots show the log2-fold change of RNA-Seq (green, left axis), Ribo-Seq (grey, left axis), and transcriptional efficiency (TE, blue, right axis) of example genes in three ciliary clusters. (***C***) Heatmap shows mRNA and RPFs changes of genes found with a similar regulation pattern to ciliary genes cluster I during flagellar regeneration. Columns are genes, and rows are time points. The total signal is normalized per column to allow the comparison of patterns. (***D***) Summary of the visual screen for ciliary dye-filing defects in *C. elegans* mutant strains. N = 250

- 300 worms from three independent experiments. (***E***) RNA-Seq analysis the transcription of *sptl-1*, *sptl-2*, *sptl-3*. Mutation sites, amino acid changes are shown below. The deleted sequences in *sptl-2* (*ok2753*) and *sptl-3* (*ok1927*) result in frameshifts that disrupt the aminotransferase motifs. The deletion of promoter sequence in *sptl-1* (*ok1693*) reduces the expression of *sptl-1* RNA level. (***F***) Representative immunocytochemistry images of phasmid cilia in WT *C. elegans* and *sptl-2* mutant labeled with IFT52/OSM-6::GFP. Magenta, ceramide; green, IFT52/OSM-6. Scale bar, 5 μm. The numbers of cilia used for quantification are shown in the images.

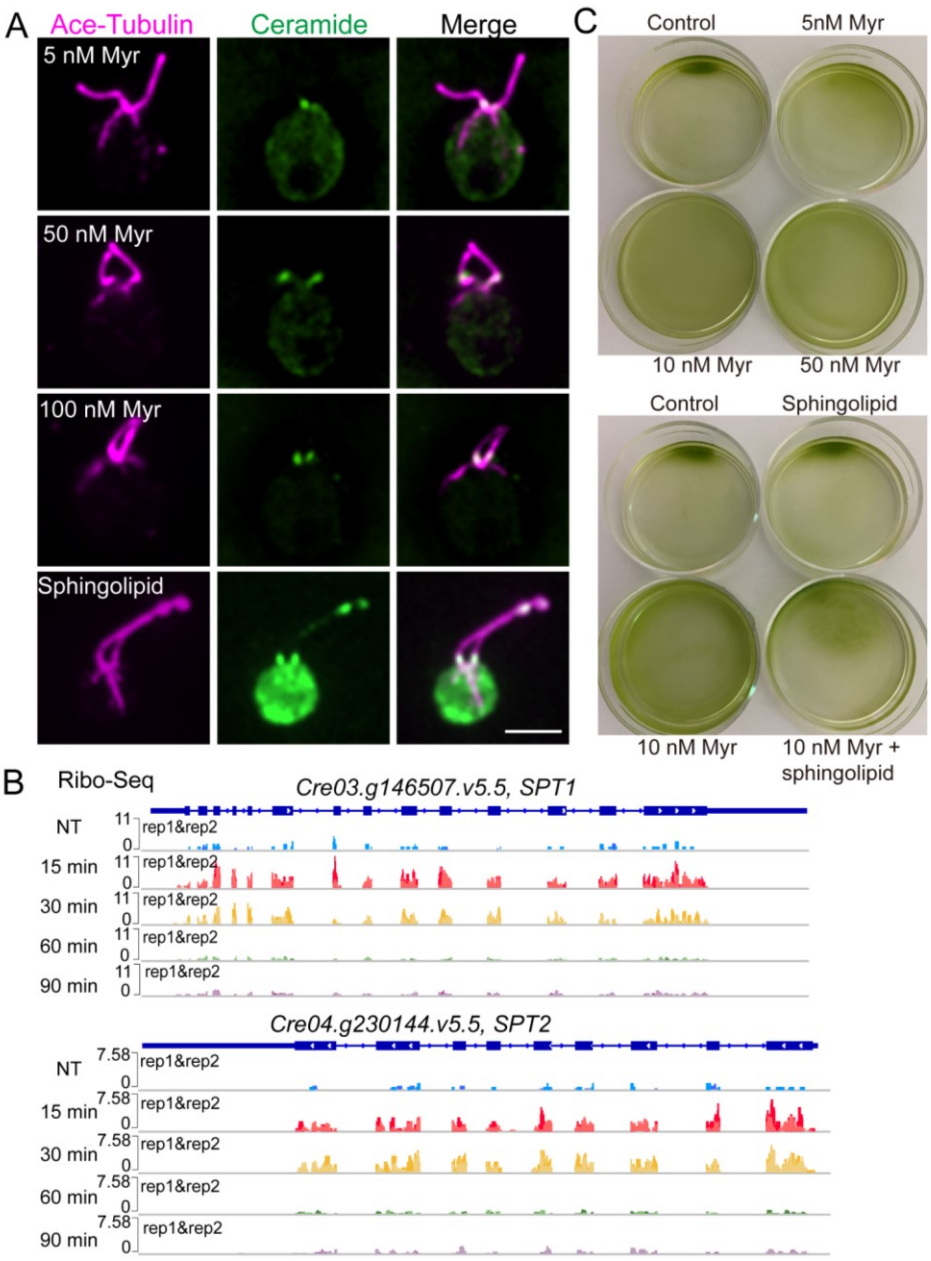


Fig. S4. Sphingolipids regulate ciliogenesis in *Chlamydomonas.* (***A***) Representative immunocytochemistry images of *Chlamydomonas* cells treated with ceramide biosynthesis inhibitors (5 nM -100 nM myriocin) or with exogenous 2 μM sphingolipid mixture (magenta, acetylated tubulin; green, ceramide). Scale bar, 5 μm. (***B***) IGV plots show RPFs levels for *SPT1* and *SPT2.* (***C***) Phototaxis motility assay with *Chlamydomonas* incubated with 0-50 nM myriocin (myr) with or without exogenous 2 μM sphingolipid mixture, N = 3 from three independent experiments.

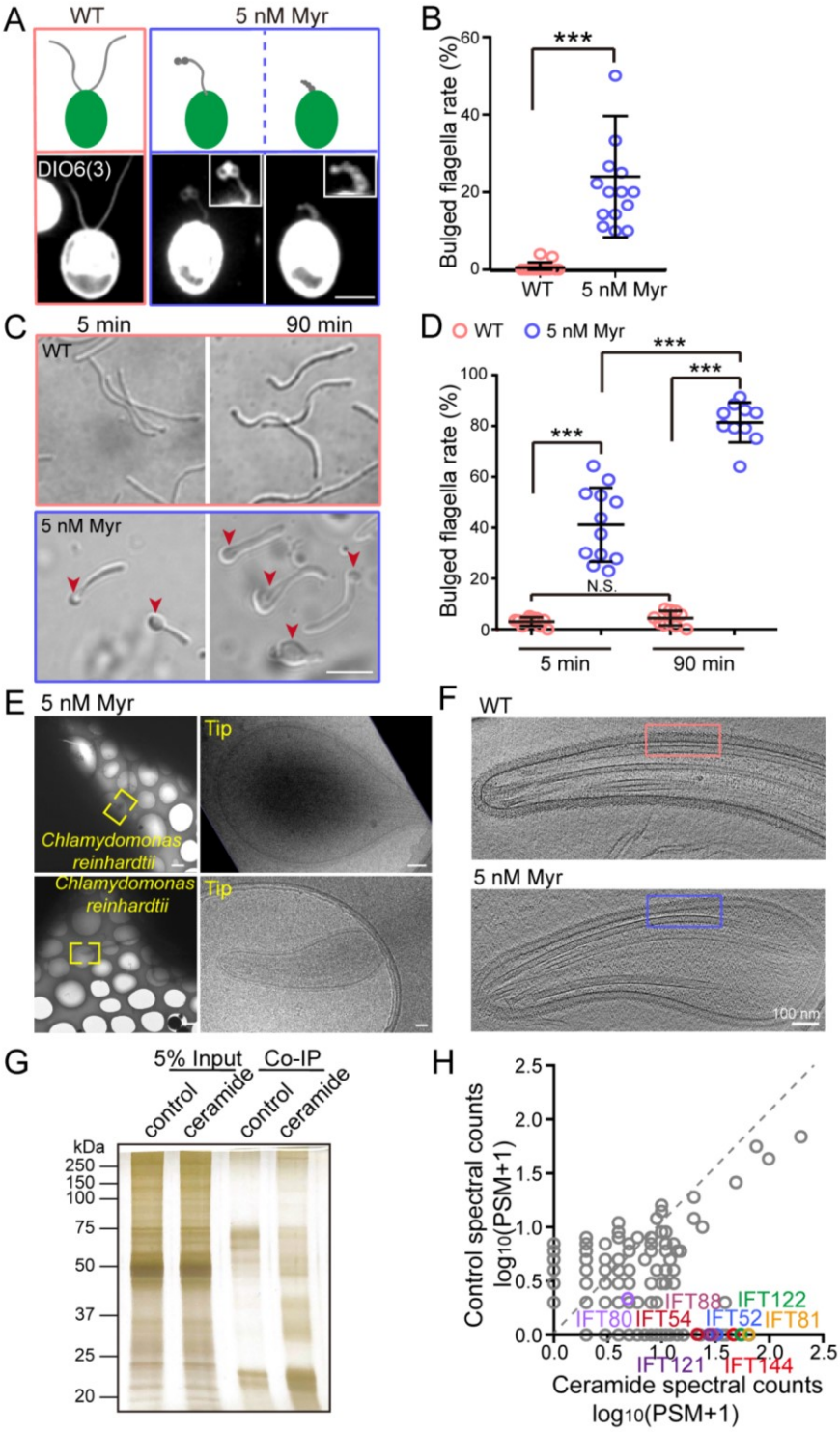


Fig. S5. Loss of ceramide caused bulged flagella. (**A**) Representative images of *Chlamydomonas* cells treated with or without ceramide biosynthesis inhibitors (5 nM myriocin) labeled with the lipid dye Dioc6(3). Scale bar, 5 μm. (**B**) Quantifications in *Chlamydomonas* cells

treated with or without ceramide biosynthesis inhibitors (5 nM myriocin) labeled with the lipid dye Dioc6(3). N= 14 independent fields from three independent experiences were used for statistical analysis. Values, mean ± SD for combined cell populations. Statistical significances were calculated by the Student's t-test using the means of of biological replicates, ***p < 0.001. (***C***) Representative images of *Chlamydomonas* flagella treated with or without ceramide biosynthesis inhibitors (5 nM myriocin). Scale bar, 5 μm. (***D***) Bulged flagella rates in *Chlamydomonas* cells treated with or without ceramide biosynthesis inhibitors (5 nM myriocin). N > 10 independent fields from three independent experiences were used for statistical analysis. Values, mean ± SD. Statistical significances were calculated by the Student's t-test using the means of of biological replicates, ***p < 0.001. (**E**) Cryo-TEM images show the representative flagella in 5 nM Myr (Left). Scale bars, 500 nm. High-magnification view of flagellar tips using cryo-TEM (right). Scale bars, 100 nm. (***F***) Cryo-ET images of the representative cilia in WT and 5 nM Myr. Scale bars, 100 nm. The boxes show the high-magnification images in Fig. 5*E* and *F*. (***G***) Protein interacting with ceramide was isolated using ceramide beads as shown in Fig. 6*A*. Protein was then separated by SDS-PAGE and silver-stained. (***H***) Mass spectrometric analysis of potential ceramide binding proteins by biotin-linked ceramide streptavidin agarose beads using the lysate from purified *Chlamydomonas* flagella. The plot compares proteins co-precipitated with control beads (y-axis) and ceramide beads (x-axis), the colorful dots represent IFT proteins (see Dataset S4 and Fig. 6*B*).

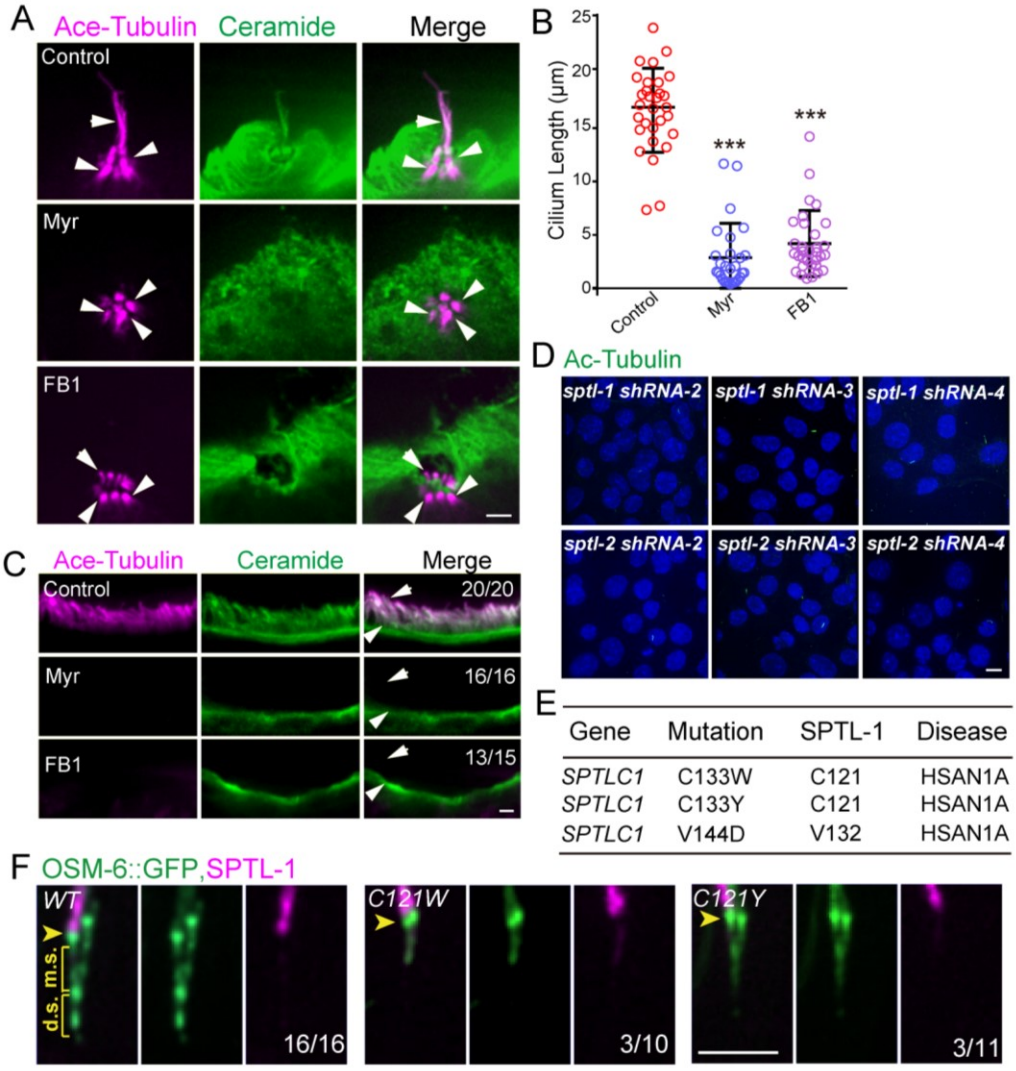


Fig. S6. Sphingolipids regulate ciliogenesis in vertebrate cells. (***A***) Representative immunocytochemistry images in zebrafish olfactory placode treated with ceramide biosynthesis inhibitors (25 μM myriocin, 250 μM FB1, or DMSO) (magenta, acetylated tubulin; green, ceramide) for four days. Arrowheads, cilia. Scale bar, 5 μm. (***B***) Quantifications in zebrafish olfactory placode treated with ceramide biosynthesis inhibitors (25 μM myriocin, 250 μM FB1, or DMSO) (magenta, acetylated tubulin; green, ceramide) for four days. N = 29-32 animals from three independent replicates. Statistical significances were calculated by the Student's t-test using the means of biological replicates, *** P < 0.001. (***C***) Representative immunocytochemistry and quantifications of zebrafish lateral line neuromasts that were treated for four days with ceramide biosynthesis inhibitors (25 μM myriocin, 250 μM FB1 or DMSO) (magenta, acetylated tubulin; green, ceramide). Arrowheads, olfactory epithelium. Arrow, cilia. Scale bar, 5 μm. The numbers of zebrafish used for quantification are shown in the bars. (***D***) Representative immunocytochemistry images in IMCD3 cells transferred with *sptlc1* or *sptlc2* siRNA (green, acetylated tubulin; blue, DAPI) for 48 hours. Scale bar, 5 μm. N = 115 - 200 cells from three independent experiments. (***E***) Disease-associated mutations in SPTLC1. Both mutations are dominant in human diseases. (***F***) Phasmid cilia in *C. elegans* were labeled with OSM-6::GFP and SPTL-1(C121W):: wrmScarlet (a

123 red fluorescence protein), SPTL-1(C121Y)::wrmScarlet. Arrowheads, the ciliary base, and
124 transition zone. Scale bar, 5 μm. N = 10-16 animals from two independent replicates.

125

126 **Table S1. Strains in this study.**

Experimental Models: Organisms/Strains	**Source**	**Identifier or Catalog Number**
IMCD3 cells (*M. musculus*)	ATCC	CRL-2123
CC-125 wild type mt+ (*C. reinhardtii*)	Chlamydomonas Resource Center	N/A
CrIFT46-YFP (*C. reinhardtii*)	Kaiyao Huang Lab	N/A
Tübingen (TU) wild type (*D. rerio*)	ZFIN	N/A
N2 (*C. elegans*)	Caenorhabditis Genetics Center	N/A
RB1465: sptl-1(ok1693) II (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB1465; WormBase: WBVar00092899
VC2358: sptl-2(ok2753) V (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: VC2358; WormBase: WBVar00093853
RB1579: sptl-3(ok1927) V (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB1579; WormBase: WBVar00093122
RB1036: hyl-1(ok976) IV (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB1036; WormBase: WBVar00092247
RB1498: hyl-2(ok1766) X (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB1498; WormBase: WBVar00092968
VC747: lagr-1(gk327) I (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: VC747; WormBase: WBVar00145734
VC765: lagr-1(gk331) I (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: VC765; WormBase:
RB782: asah-2 (ok564) II (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB782; WormBase: WBVar00091850
asah-1(tm495) I (*C. elegans*)	National Bioresource Project of Japan	WormBase: WBVar00249537

VC916: sphk-1(ok1097) II (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: VC916; WormBase: WBVar00092366
VC242: spl-2(ok490) V (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: VC242; WormBase: WBVar00091778
RB1203: cerk-1 (ok1252) I (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB1203; WormBase: WBVar00092498
asm-1(tm5023) II (*C. elegans*)	National Bioresource Project of Japan	WormBase: WBVar00317302
asm-2(tm3746) X (*C. elegans*)	National Bioresource Project of Japan	WormBase: WBVar00252354
RB1487: asm-3(ok1744) IV (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB1487; WormBase: WBVar00092946
ttm-5(tm6585) I (*C. elegans*)	National Bioresource Project of Japan	WormBase: WBVar02125250
RB1854: sms-1(ok2399) IV (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB1854; WormBase: WBVar00093548
sms-2(tm2757) X (*C. elegans*)	National Bioresource Project of Japan	WormBase: WBVar00251598
RB2549: sms-3(ok3540) III (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB2549; WormBase: WBVar00094520
BS3383: pmk-3(ok169) IV (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: BS3383; WormBase: WBVar00091488
KB7: kgb-1(um3) kgb-2(km16) IV (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: KB7; WormBase: WBVar00274912
KU4: sek-1(km4) X (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: KU4; WormBase: WBVar00088241

RB1049: rom-2(ok996) III (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB1049; WormBase: WBVar00092267
RB1155: scl-1(ok1185) IV (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB1155; WormBase: WBVar00092441
RB1556: shw-3(ok1884) V (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB1556; WormBase: WBVar00093080
RB1672: sel-12(ok2078) X (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB1672; WormBase: WBVar00093263
RB1764: trxr-2(ok2267) III (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB1764; WormBase: WBVar00093428
RB1919: sms-5(ok2498) II (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB1919; WormBase: WBVar00093634
RB2354: F15D4.4(ok3200) II (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB2354; WormBase: WBVar00094267
RB2487: cca-1(ok3442) X (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB2487; WormBase: WBVar00094445
RB2519: drh-1(ok3495) IV (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB2519; WormBase: WBVar00094484
VC1266: Y55F3AR.2(ok1737) IV (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: VC1266; WormBase: WBVar00092940
RB2529: C30F12.2(ok3505) I (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: RB2529; WormBase: WBVar00094494
SP2101: ncl-1(e1865) unc-36(e251) III; osm-6(p811) V; mnIs17[Posm-6::osm-6::GFP unc-36(+)] (*C. elegans*)	Caenorhabditis Genetics Center	WB Strain: SP2101; WormBase: WBTransgene00001004
GOU3788: sptl-1(ok1693) II; mnIs17[Posm-6::osm-6::GFP unc-36(+)](*C. elegans*)	This paper	N/A

GOU3897: sptl-2(ok2753) V; mnIs17[Posm-6::osm-6::GFP unc-36(+)](*C. elegans*)	This paper	N/A
GOU3789: sptl-1(ok1693) II; sptl-2(ok2753) V; mnIs17[Posm-6::osm-6::GFP unc-36(+)](*C. elegans*)	This paper	N/A
GOU3787: sptl-3(ok1927) V; mnIs17[Posm-6::osm-6::GFP unc-36(+)](*C. elegans*)	This paper	N/A
GOU3813: sptl-1(ok1693) II; sptl-3(ok1927) V; mnIs17[Posm-6::osm-6::GFP unc-36(+)](*C. elegans*)	This paper	N/A
GOU3798: hyl-1(ok976) IV; mnIs17[Posm-6::osm-6::GFP unc-36(+)](*C. elegans*)	This paper	N/A
GOU3795: hyl-2(ok1766) X; mnIs17[Posm-6::osm-6::GFP unc-36(+)](*C. elegans*)	This paper	N/A
GOU3800: lagr-1(gk331) I; mnIs17[Posm-6::osm-6::GFP unc-36(+)](*C. elegans*)	This paper	N/A
GOU3824: lagr-1(gk331) I; hyl-2(ok1766) X; mnIs17[Posm-6::osm-6::GFP unc-36(+)](*C. elegans*)	This paper	N/A
GOU3796: asah-2 (ok564) II; mnIs17[Posm-6::osm-6::GFP unc-36(+)](*C. elegans*)	This paper	N/A
GOU3799: sphk-1(ok1097) II; mnIs17[Posm-6::osm-6::GFP unc-36(+)](*C. elegans*)	This paper	N/A
GOU3794: cerk-1 (ok1252) I; mnIs17[Posm-6::osm-6::GFP unc-36(+)](*C. elegans*)	This paper	N/A
GOU4202: mnIs17[Posm-6::osm-6::GFP unc-36(+)];casEX6126[Pdyf-1::sptl-1(C121W)::wrmScarlet] (*C. elegans*)	This paper	N/A
GOU4203: mnIs17[Posm-6::osm-6::GFP unc-36(+)];casEX6127[Pdyf-1::sptl-1(C121Y)::wrmScarlet] (*C. elegans*)	This paper	N/A

129 **Table S2.** Plasmids and Primers in this study.

Experimental Models: Organisms/Strains	**Source**	**Identifier or Catalog Number**
Plasmid: pcDNA3.1-SPTLC1::HA	This paper	N/A
Plasmid: pcDNA3.1-SPTLC1(C133W)::HA	This paper	N/A
Plasmid: pcDNA3.1-SPTLC1(C133Y)::HA	This paper	N/A
Plasmid: pcDNA3.1-SPTLC1(V144D)::HA	This paper	N/A
Plasmid: pDONR-Pdyf-1::sptl-1::wrmScarlet	This paper	N/A
Plasmid: pDONR-Pdyf-1::sptl-1(C121W)::wrmScarlet	This paper	N/A
Plasmid: pDONR-Pdyf-1::sptl-1(C121Y)::wrmScarlet	This paper	N/A
Custom Glycerol-mouse shRNA library: pLKO mouse SPTLC1 shRNA	Sigma	TRCN0000103400
Custom Glycerol-mouse shRNA library: pLKO mouse SPTLC1 shRNA	Sigma	TRCN0000103402
Custom Glycerol-mouse shRNA library: pLKO mouse SPTLC1 shRNA	Sigma	TRCN0000103403
Custom Glycerol-mouse shRNA library: pLKO mouse SPTLC2 shRNA	Sigma	TRCN0000103170
Custom Glycerol-mouse shRNA library: pLKO mouse SPTLC2 shRNA	Sigma	TRCN0000103171
Custom Glycerol-mouse shRNA library: pLKO mouse SPTLC2 shRNA	Sigma	TRCN0000103173
Ribosome profiling: Preadenylated and 3′-blocked linker: 5rApp/CTGTAGGCACCATCAAT/3ddC/ (IDT)	This paper	N/A
Ribosome profiling: Reverse transcription primer: 5′-(Phos)-AGATCGGAAGAGCGTCGTGTAGGGAAAGA GTGTAGATCTCGGTGGTCGC-(SpC18)-CACTCA-(SpC18)-TTCAGACGTGTGCTCTTCCGATCTATTGAT GGTGCCTACAG	This paper	N/A

Ribosome profiling: library amplification: AATGATACGGCGACCACCGAGATCTACAC	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATTGTTGACTGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATACGGAACTGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A

Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATTCTGACATGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATCGGGACGGGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATGTGCGGACGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATCGTTTCACGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCG	This paper	N/A

Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATGTAGC CGTGACTGGAGTTCAGACGTGTGCTCTTC CG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATAAGGC CACGTGACTGGAGTTCAGACGTGTGCTCT TCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATTCCGA AACGTGACTGGAGTTCAGACGTGTGCTCTT CCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATTACGT ACGGTGACTGGAGTTCAGACGTGTGCTCT TCCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATATCCA CTCGTGACTGGAGTTCAGACGTGTGCTCTT CCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATATATC AGTGTGACTGGAGTTCAGACGTGTGCTCTT CCG	This paper	N/A
Ribosome profiling: library amplification index primer: CAAGCAGAAGACGGCATACGAGATAAAGG AATGTGACTGGAGTTCAGACGTGTGCTCTT CCG	This paper	N/A
Primer：mouse SPTLC1(C133W) forward: TACCTGGGGTCCTCGAGGGTTCTATGGCA CATTTGA	This paper	N/A
Primer：mouse SPTLC1(C133W) reverse: CGAGGACCCCAGGTACCCACTCCGTACTT CTTTAA	This paper	N/A
Primer：mouse SPTLC1(C133Y) forward: GGTACCTATGGTCCTCGAGGGTTCTATGG CACATT	This paper	N/A

Primer：mouse SPTLC1(C133Y) reverse: AGGACCATAGGTACCCACTCCGTACTTCTTTAAAG	This paper	N/A
Primer：mouse SPTLC1(V144D) forward: TTTGATGACCATCTGGATTTAGAAGAGCGCCTG	This paper	N/A
Primer：mouse SPTLC1(V144D) reverse: CAGATGGTCATCAAATGTGCCATAGAACCCTCGAG	This paper	N/A
Primer：*C. elegans* SPTL-1(C121W) forward: GATCGTGGGGGCCACGTGGATTCTACGGAACTGTT	This paper	N/A
Primer：*C. elegans* SPTL-1(C121W) reverse: GTGGCCCCCACGATCCTACGCCGTACTTGAAAATCG	This paper	N/A
Primer：*C. elegans* SPTL-1(C121Y) forward: GGATCGTACGGGCCACGTGGATTCTACGGAACTGTT	This paper	N/A
Primer：*C. elegans* SPTL-1(C121Y) reverse: GTGGCCCGTACGATCCTACGCCGTACTTGAAAATC	This paper	N/A

130

131

Movie S1 (separate file). The 3D reconstruction model of WT *Chlamydomonas* flagellum.

Movie S2 (separate file). The 3D reconstruction model of 5 nM Myr treated *Chlamydomonas* flagellum.

Dataset S1 (separate file). Log2FC of RNA-seq and Ribo-seq and TE for all genes during deflagellation.

Dataset S2 (separate file). Log2FC of RNA-seq and Ribo-seq and TE for the ciliary genes during deflagellation.

Dataset S3 (separate file). Genes with a similar translational pattern to the annotated ciliary genes and their orthologs.

Dataset S4 (separate file). Mass spec data analysis.