C11orf70 Mutations Disrupting the Intraflagellar Transport-Dependent Assembly of Multiple Axonemal Dyneins Cause Primary Ciliary Dyskinesia

Fassad, Mahmoud R.; Shoemark, Amelia; le Borgne, Pierrick; Koll, France; Patel, Mitali; Dixon, Mellisa; Hayward, Jane; Richardson, Charlotte; Frost, Emily; Jenkins, Lucy; Cullup, Thomas; Chung, Eddie M. K.; Lemullois, Michel; Aubusson-Fleury, Anne; Hogg, Claire; Mitchell, David R.; Tassin, Anne-Marie; Mitchison, Hannah M.

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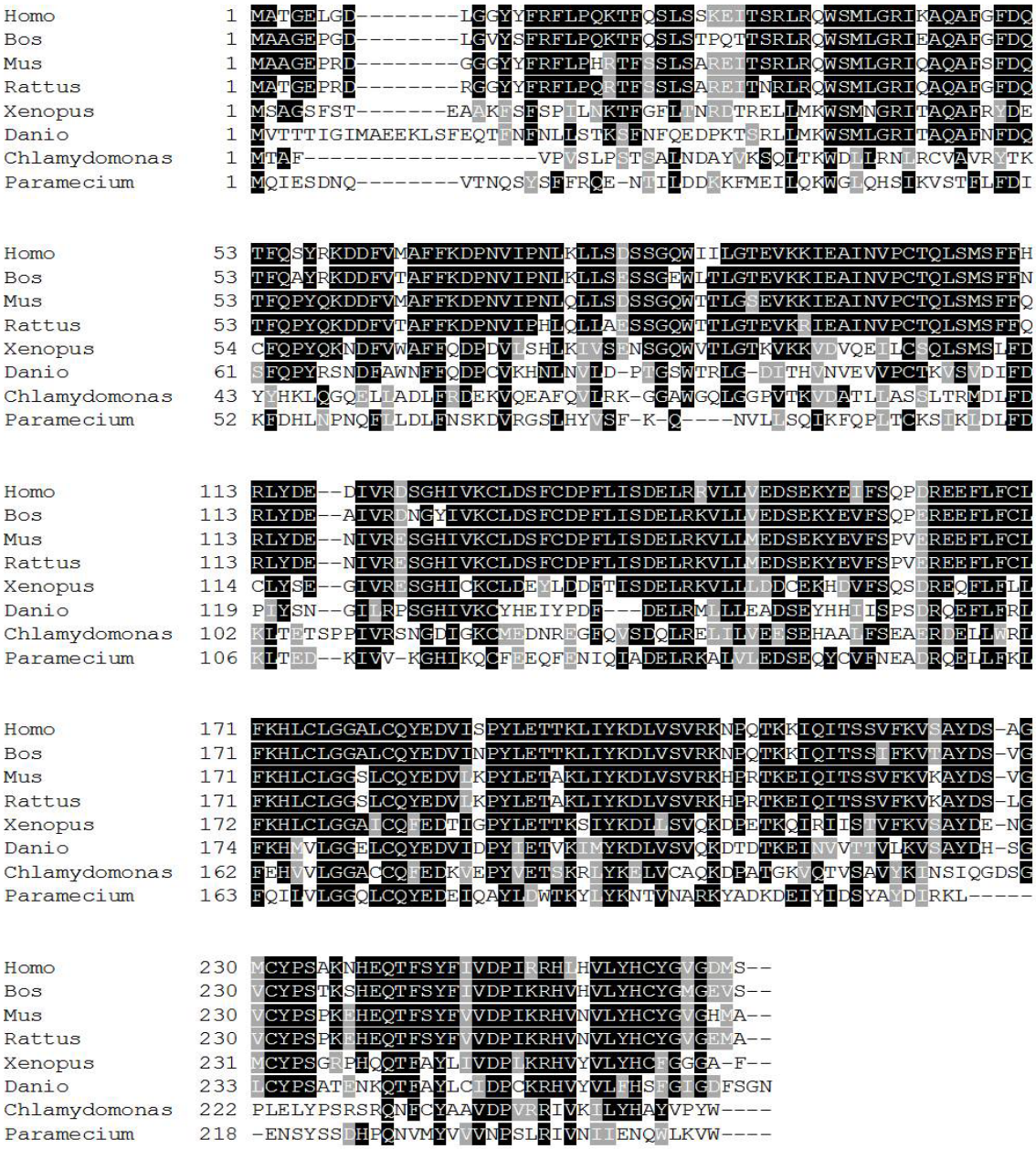

Figure S1. Cross species protein alignment for C11orf70

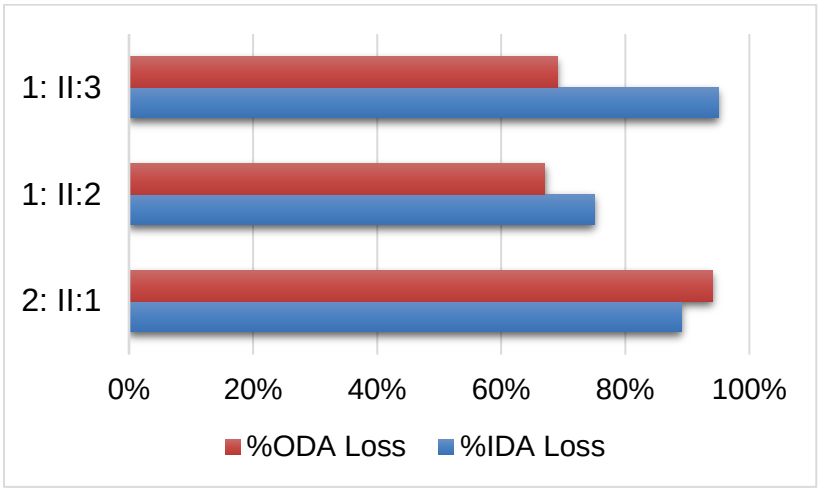

Figure S2. Quantification of TEM analysis of outer and inner dynein arm loss in affected individuals with *C11orf70* mutations

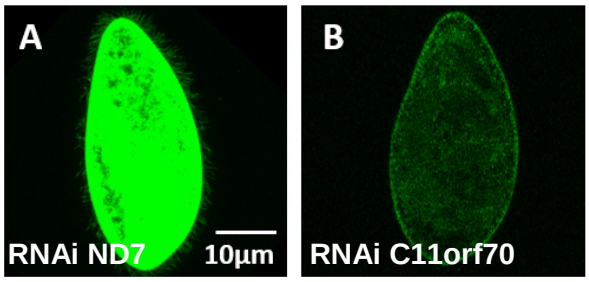

Figure S3. Successful RNAi ablation of *C11orf70* shown in *Paramecium* transformed with GFP-tagged C11orf70. *ND7*- (control) and *C11orf70*- silenced Paramecia were fixed and visualised for C11orf70-GFP protein expression after 72h of RNAi. Scale bars, 10 µm.

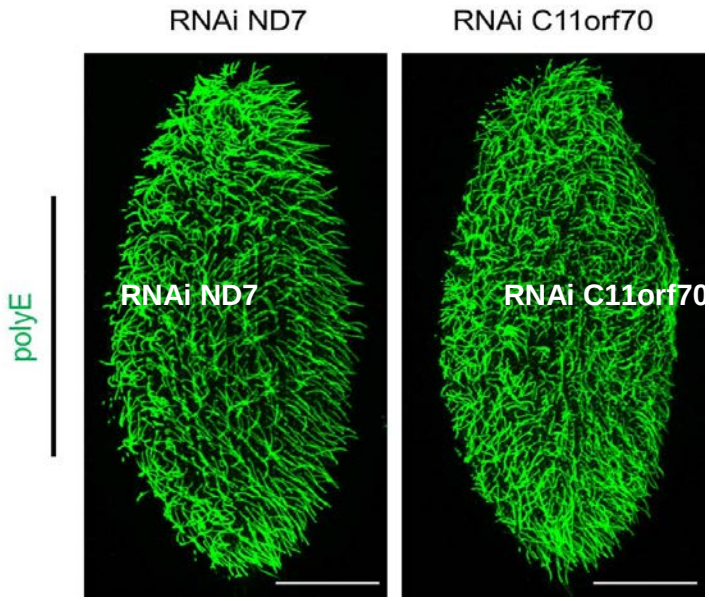

Figure S4. Depletion of *C11orf70* in *Paramecium* does not affect cilia number and length. *ND7* (control) and *C11orf70* silenced Paramecia were fixed and stained for cilia after 72h of RNAi. Cilia were stained using anti - polyglutamylated tubulin antibodies to indicate the cilia (PolyE). Scale bars, 20 µm.

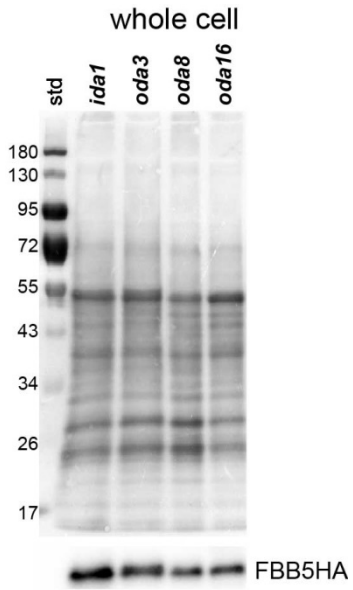

Figure S5. *Chlamydomonas* FBB5 is expressed at similar levels in a number of mutant *Chlamydomonas* strains. Amido black stained gels shows equivalent protein in strains with mutations in proteins shown in Figure 5, main text.

Primer ID	(5'- Sequence -3')
Sanger sequencing for c.776A>G mutation	
Fwd	GATCCTATCAGGCGTCACCTT
Rev	ATCCCCGGTTAGTATTCCACAC
Sanger sequencing for c.154C>T mutation	
Fwd	GCCAGCCCCCAGATGATTT
Rev	GAGACCCAGGGAACTCTCCG
Sanger sequencing for c.361C>T mutation	
Fwd	GTTCCTTGCACACAGCTTTCAA
Rev	TCCTGAAATAAAATTCCACTGCGA
q(RT-PCR) for expression profiling of C11orf70 in ALI culture	
C11orf70-Fwd	TGGGTGGCTACTACTTCAGGT
C11orf70-Rev	GCCTTGATTCTGCCCAGCAT
DNAH5-Fwd	TGCAGATGCCATGGTTCACT
DNAH5-Rev	ATGAAGCCAACCTCGTCAGG
GAPDH-Fwd	TGCACCACCAACTGCTTAGC
GAPDH-Rev	GGCATGGACTGTGGTCATGAG
561bp segment of *Paramecium* GSPATG00011350001 for RNAi design	
Fwd	TAGAACTAGTATGTAAATTGAATCAGACAATTAAGTTAC
Rev	TTTTTTCTCGAGCTTTGTCCAATCTAAATAGGCTTG
q(RT-PCR) for GSPATG00011350001 knockdown in *Paramecium*	
GSPATG00011350001-F	TGCTCGAAAATATGCAGATAAGGA
GSPATG00011350001-R	TGATGGGTTCACAACGACAT
GAPDH-Fwd	GAGAGCCGGAAGAGCTGCTA
GAPDH-Rev	TGGTGGAACTCTGAAGGCCATA
Amplification of *Paramecium* GSPATG00011350001 (C11orf70) for GFP expression vector cloning	
Fwd	TAGAACTAGTATGTAAATTGAATCAGACAATTAAGTTAC
Rev	TTTTTCTCGAGTCCTCCTCCCCATACTTTTAACCATTAGTTTTCT
Amplification of *Paramecium* GSPATG00024708001 (IFT46) for GFP expression vector cloning	
Regulator-Fwd	ATACTTGGATCCTATTTAATATAATTAAATAAATACTGTTATTC
Regulator-Rev	GACAAAACAATGATGAAATTTAAGGCATGCAAGTAT
GSPATG00024708001-F	ATACTTGGTACCTGAATATTCAATAATATAACATTATTTTC
GSPATG00024708001-R	GGAATATTTATTATAATTATTAA

Table S1. Primers used in the study

Antigen	Antibody	Host species	Source	Application
DNAH5	HPA037470	Rabbit	Sigma-Aldrich	IF (1:800)
DNALI1	HPA053129	Rabbit	Sigma-Aldrich	IF (1:200)
RSPH4A	HPA031196	Rabbit	Sigma-Aldrich	IF (1:400)
GAS8	HPA041311	Rabbit	Sigma-Aldrich	IF (1:500)
Acetylated Tubulin	T7451	Mouse	Sigma-Aldrich	IF (1:500)
Acetylated Tubulin	YF488	Mouse	Proteintech	IF (1:500)
Polyglutamylated Tubulin	PolyE	Rabbit	A gift from J. Cohen	IF (1:500)
GFP	IgG	Rabbit	Interchim, France	IF (1:500)
HA	McAb 3F10	Rat	Roche, Indianapolis	IB (1:1000)
Chlamydomonas IFT46C	α-IFT46C404	Guinea Pig	A gift from G. Witman	IB (1:10000)
Chlamydomonas IFT46N	α-IFT46-17601	Rabbit	A gift from H. Qin	IB (1:5000)
Chlamydomonas IFT81	81.3	Mouse	A gift from D. Cole	IB (1:10000)
Chlamydomonas IFT139	139.1	Mouse	A gift from D. Cole	IB (1:10000)
Chlamydomonas IC2	C11.4	Mouse	DRM lab	IB (1:2000)
Chlamydomonas ODA16	α-ODA16-927	Rabbit	DRM lab	IB (1:100)

Table S2: Primary antibodies used in the study

Antibody	Host Species	Source	Application
Alexa 488, 594 – conjugated anti-rabbit IgG (H+L)	Goat	Invitrogen (Molecular Probes)	IF (1:1000)
Alexa 488, 594 – conjugated Anti-mouse IgG1	Goat	Invitrogen (Molecular Probes)	IF (1:1000)
HRP-conjugated Anti-mouse IgG	Goat	BioRad	IB (1:2000)
HRP-conjugated Anti-rabbit IgG	Goat	Sigma-Aldrich	IB (1:2000)
HRP-conjugated Anti-guinea Pig IgG	Goat	Rockland	IB (1:1000)
HRP-conjugated Anti-rat IgG	Goat	Sigma-Aldrich	IB (1:5000)

Table S3: Secondary antibodies used in the study