

Supplemental material

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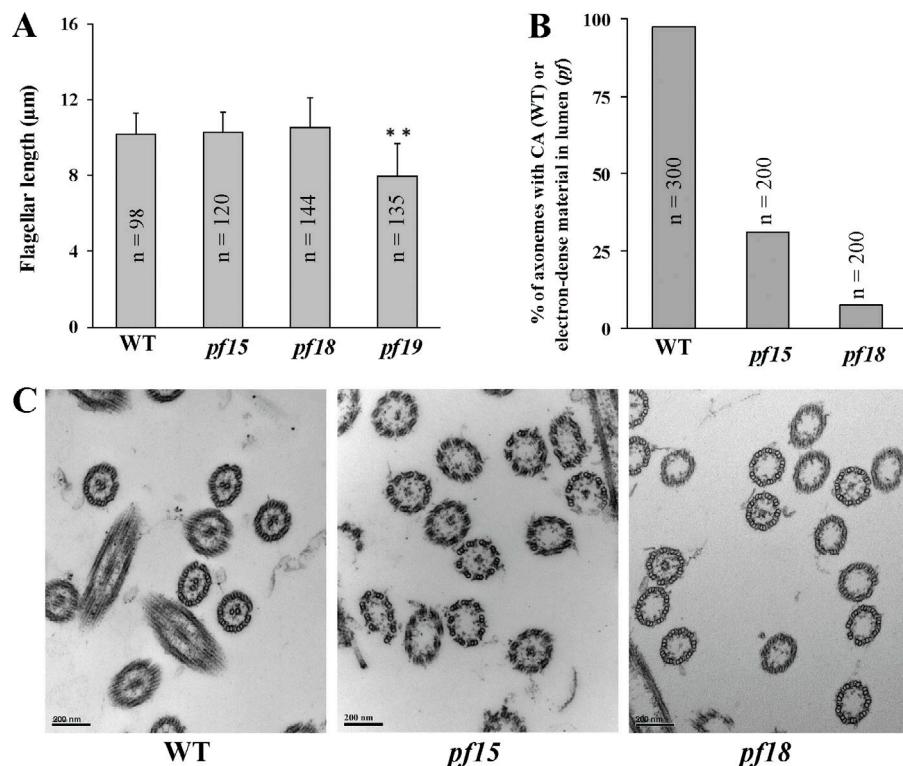


Figure S1. Selection of pf18 for comparative proteomic analysis to identify novel CA proteins. (A) Flagellar length of *pf* mutants. *pf15* and *pf18* have normal flagellar length, whereas flagellar length is reduced in *pf19*. n, number of cells scored; error bars indicate standard deviation; **, significant difference (Student's t test, $P < 0.001$) compared with WT cells. **(B and C)** Analysis of WT and *pf* mutant axonemes by TEM. 97% of WT axonemes contained a normal CA. *pf15* and *pf18* axonemes lacked the CA, which was replaced by electron-dense material in the lumens of 31% of *pf15* axonemes and 7.5% of *pf18* axonemes. Consequently, *pf18* axonemes were selected for the comparative proteomics analysis. Bars, 0.2 μm.

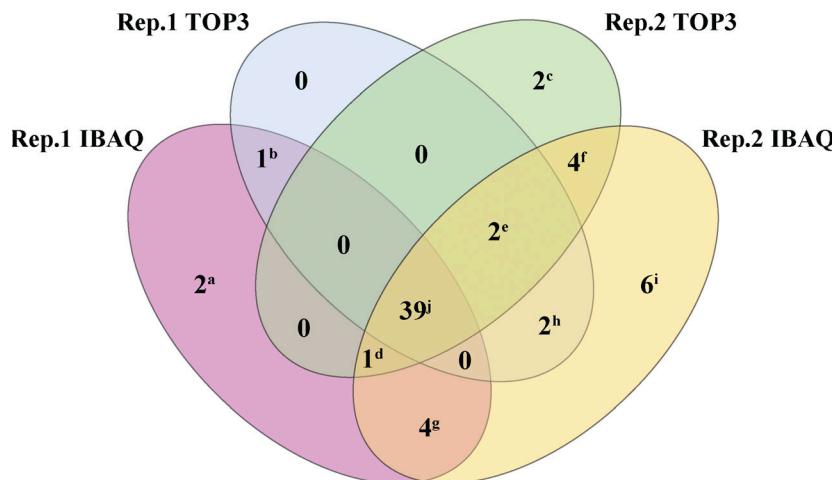


Figure S2. Venn diagram of known and candidate CA proteins in replicates 1 and 2. The diagram shows the number of proteins from each of the two biological replicates (Rep.) that met our criteria for candidate CA proteins based on *pf18:WT* ratio and protein abundance as determined by IBAQ and Top3 methods. A total 63 proteins were identified by these criteria. 19 (including PP1c) are previously known CA proteins, and 44 are candidates for being novel CA proteins. 48 (76%) of the proteins met both criteria in both replicates. Proteins in each subgroup are as follows: (a) FAP348, FAP415; (b) FAP72; (c) FAP99, FAP412; (d) FAP312; (e) FAP105, FAP123; (f) FAP108, FAP139, FAP275, FAP289; (g) FAP125, FAP345, FAP413, KLP1; (h) FAP194, FAP411; (i) FAP39, FAP380, FAP414, FAP416, FAP417, DPY30; and (j) all other proteins. Protein abundance and ratios are detailed in Tables S2 and S3.

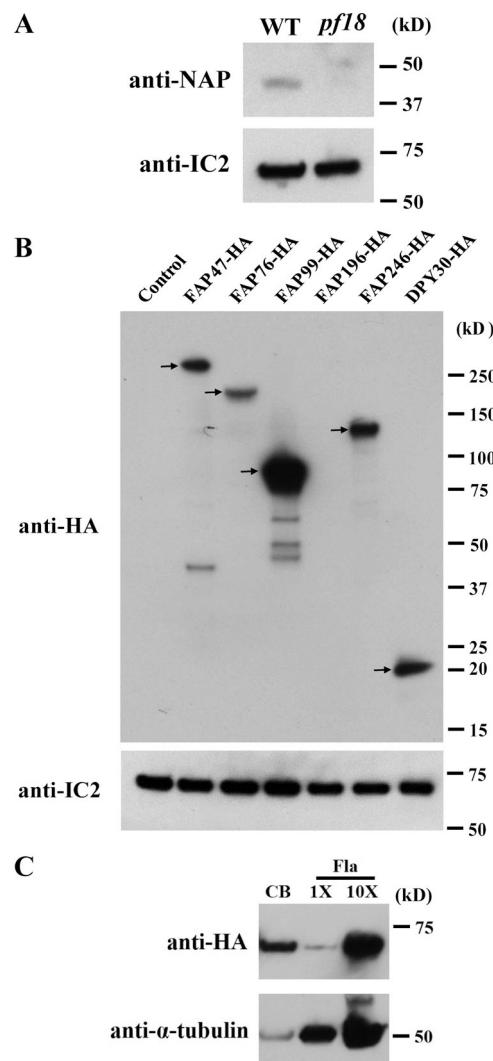


Figure S3. Confirmation that NAP is present in WT axonemes and absent from *pfl18* axonemes, and HA-tagged proteins are expressed in the axonemes of rescued strains. **(A)** Western blot of axonemes isolated from WT and the mutant *pfl18* probed with an antibody to NAP. NAP is greatly decreased in *pfl18* axonemes relative to WT axonemes. The outer arm dynein intermediate chain IC2 was used as a loading control. 5 µg of protein were loaded in each lane. **(B)** Western blot of axonemes isolated from WT and mutant strains rescued with constructs designed to express the indicated proteins. The blot was probed with anti-HA antibody and with an antibody to the outer arm dynein intermediate chain IC2 as a loading control. 1 µg of sample was loaded in each lane. Arrows indicate proteins of the expected masses. **(C)** Western blot with samples from the FAP196-HA strain. Cell bodies (CB) and flagella (Fla) were probed with anti-HA antibody; anti- α -tubulin was used for the loading control. Lanes were loaded with protein from an equal number of cells or 10 \times that number as indicated.

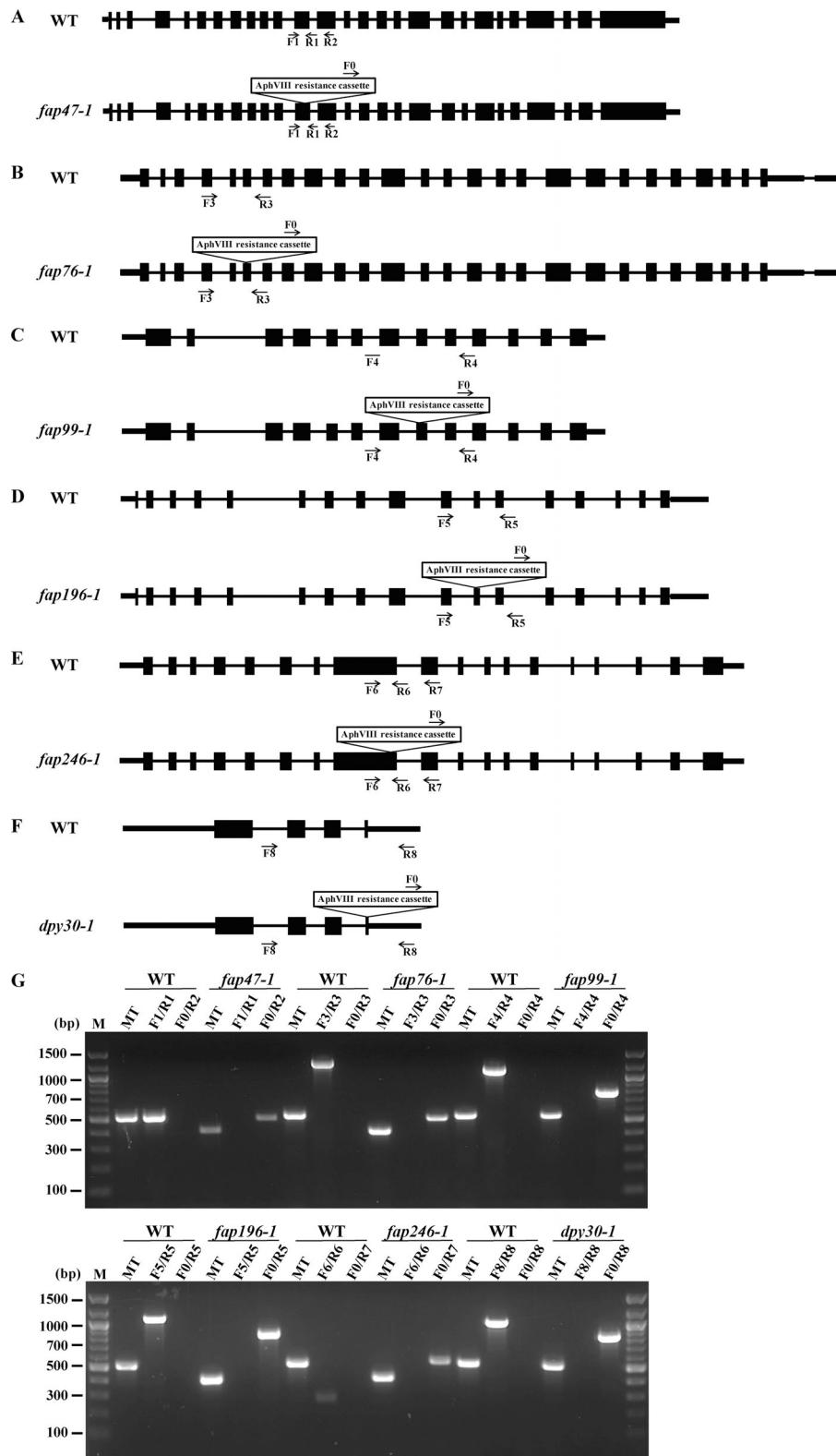


Figure S4. Confirmation of mutant insertion sites by PCR. **(A-F)** Models for the indicated genes in WT and the *Chlamydomonas* Library Project mutants; the models are based on Phytozome gene predictions (<https://phytozome.jgi.doe.gov/pz/portal.html>). The strain names, numbers, and mating types of the original strains and the progenies used for further study are listed in Table S7. **(G)** Mutant insertion sites were confirmed by PCR with the primers indicated in A-F; mating-type primers (MT) were used as the positive amplification control. Primer sequences are detailed in Table S8.

Table S7. Strains used in this study

Strain name/allele	Strain number	Mating type	Source
CC-124		mt-	Strains from Witman laboratory or Chlamydomonas Resource Center
g1		mt+	
<i>pf15</i>	CC-1033	mt+	
<i>pf16</i>	CC-1034	mt+	
<i>pf18</i>	CC-1036	mt+	
<i>pf19</i>	CC-1037	mt+	
<i>fap47-1</i>	LMJ.RY0402.172093	mt-	
<i>fap47-2</i>	LMJ.RY0402.112485	mt-	
<i>fap70-1</i>	LMJ.RY0402.051955	mt-	
<i>fap76-1</i>	LMJ.RY0402.089534	mt-	
<i>fap99-1</i>	LMJ.RY0402.183993	mt-	
<i>fap196-1</i>	LMJ.RY0402.146355	mt-	
<i>fap246-1</i>	LMJ.RY0402.135524	mt-	
<i>dpy30-1</i>	LMJ.RY0402.074963	mt-	
<i>wnk1-1</i>	LMJ.RY0402.151678	mt-	
<i>fap47-1</i>	172093-6H	mt-	Strains generated by mating or rescue
<i>fap47-2</i>	112485-2B	mt+	
<i>fap76-1</i>	089534-2G	mt-	
<i>fap99-1</i>	183993-5A	mt+	
<i>fap196-1</i>	146355-1B	mt-	
<i>fap246-1</i>	135524-8A	mt-	
<i>dpy30-1</i>	074963-9F	mt+	
<i>fap47-1 FAP47-HA</i>	172093-6H-HA-B81	mt-	
<i>fap76-1 FAP76-HA</i>	089534-2G-HA-20	mt-	
<i>fap99-1 FAP99-HA</i>	183993-5A-HA-70	mt+	
<i>fap196-1 FAP196-HA</i>	146355-1B-HA-87	mt-	
<i>fap246-1 FAP246-HA</i>	135524-8A-HA-2	mt-	
<i>dpy30-1 DPY30-HA</i>	074963-9F-HA-7F	mt+	

Table S8. Primers used in this study

Name	Sequence (5'-3')	Note
F0	CTGCTGAGGCTGAATCTACTC	Confirmation of mutant insertion site
F1	GCCGTGGAGAAGCTGCTCAT	
R1	ATGGGTTGGAGTGATGAAAC	
R2	ATCTGCATCATCACGCTCTG	
F3	TGGACTCGTTGTGAGCACAC	
R3	CTGATGCGATCCACAAACAC	
F4	GGCAGTCATTGCTTGCCTTC	
R4	ACACTGTCTCGCTAGCCAT	
F5	AACAGACGGGTCCATCTACG	
R5	GGCTGATAACCGAAAACAC	
F6	TGTTGTGCCGCCGCGAG	
R6	TCTTGGCTCAGCGGCTTTG	
R7	GATGGGCAGCAGAGTGTGA	
F8	CTTCCCTCTACCTGCGACTG	
R8	ATTTGTAAGCCGTGCGCC	
Mid1	ACCGGTGTTTACCGTCGAGT	Mating type
Mid2	CCTTCTGTAGGGCCACCTG	
Fus3	TCCAACGCATAGCCATCAAC	
Fus4	TGTTTGCTAGGGGTGCAATG	
F9	CGGCTGGCGGCCGGGAGGCCTGTCGCGATACC	Gene cloning and rescue constructs
R9	GTCGGGGCGCGCCCTGCGAGTACTGCTAGCG	
F10	AGCCGCGCGCCGGGAGGCCTGTCGCGATAC	
R10	ACTCGCGCGGGCCGGCGAGTACTGCTAGCG	
F11	GGCATATGTTGAGCTGCAGCCCTTCGAAC	
R11	GACATGCCGTATGTCAGATC	
F12	AGCTGGCGCGCGGCCGGAGGCCTGTCGCGA	
R12	CTTGGCCGCGCGCGGGGGCGCGAGTACTGCTAGC	
F13	CGGGTCCAGGAGCTGTCGCGATACCCCTACGACGT	
R13	TGGGCTCTGGACCGGGGGCGCGAGTACTGCTA	
F14	GCTTAATTAACTAGCTGCGCTGGACATGTG	
R14	CTCACCATGCTGGTTGGAAG	
F15	AACGGATCCTAGAGACATCAGGGCGTT	
R15	CGGAAGCTTCCGCATGAGCCCCACTC	
F16	TTCGCCCCGGCCGGAGGCCTGTCGCGAT	
R16	GCACCGCGGGCCGGCGAGTACTGCTA	
F17	CCCCCGGGGCCATATCTCGCGCAATTAC	
R17	CCTTAATTAAACGGTATCCGCCAAGGTATG	
F18	GCGGGCGCGCCGGAGGGCTGTCGCGATACC	
R18	GCGGGCGCGCCGAGTACTGCTAGCGCGTA	

Provided online are six tables in Excel. Table S1 lists all the proteins quantified in two independent biological replicates comparing WT and *pf18* axonemes. Table S2 contains the MS data for the proteins in Fig. 2. Table S3 contains the MS data for the candidate CA proteins. Table S4 contains the MS data for Fig. 3. Table S5 contains the MS data for the immunoprecipitation experiments of Table 3. Table S6 contains the MS data for the comparative proteomics experiments of Table 4.