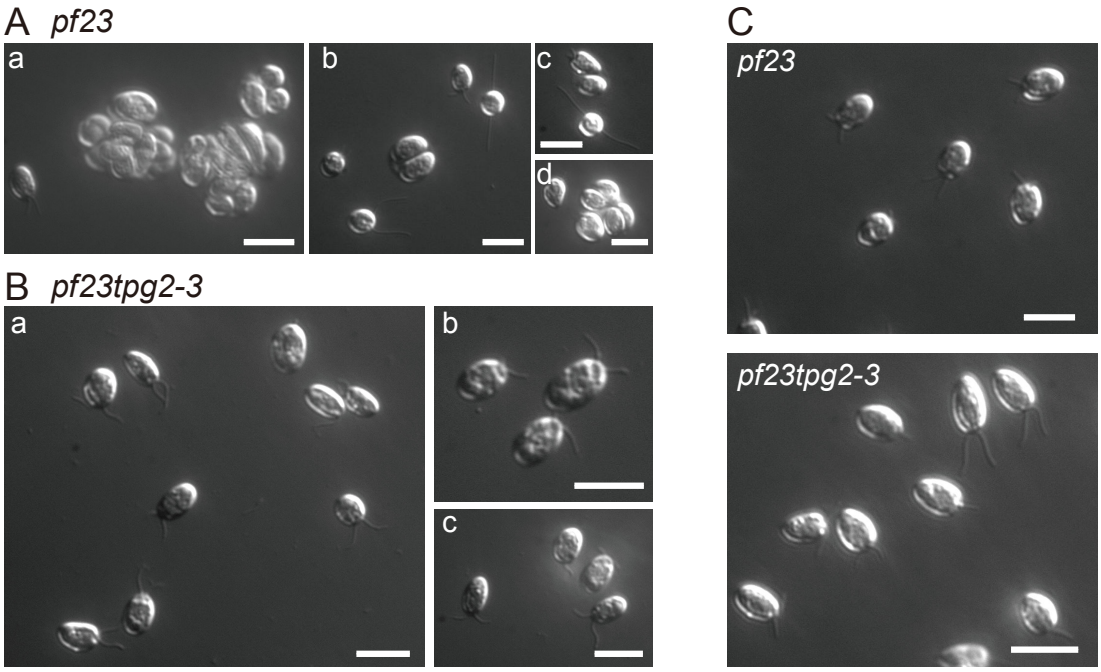
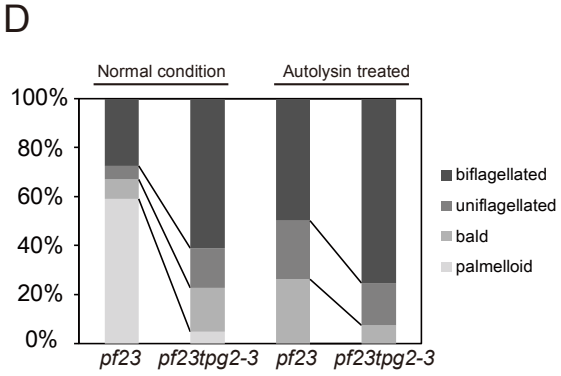
Supplemental Materials

Molecular Biology of the Cell

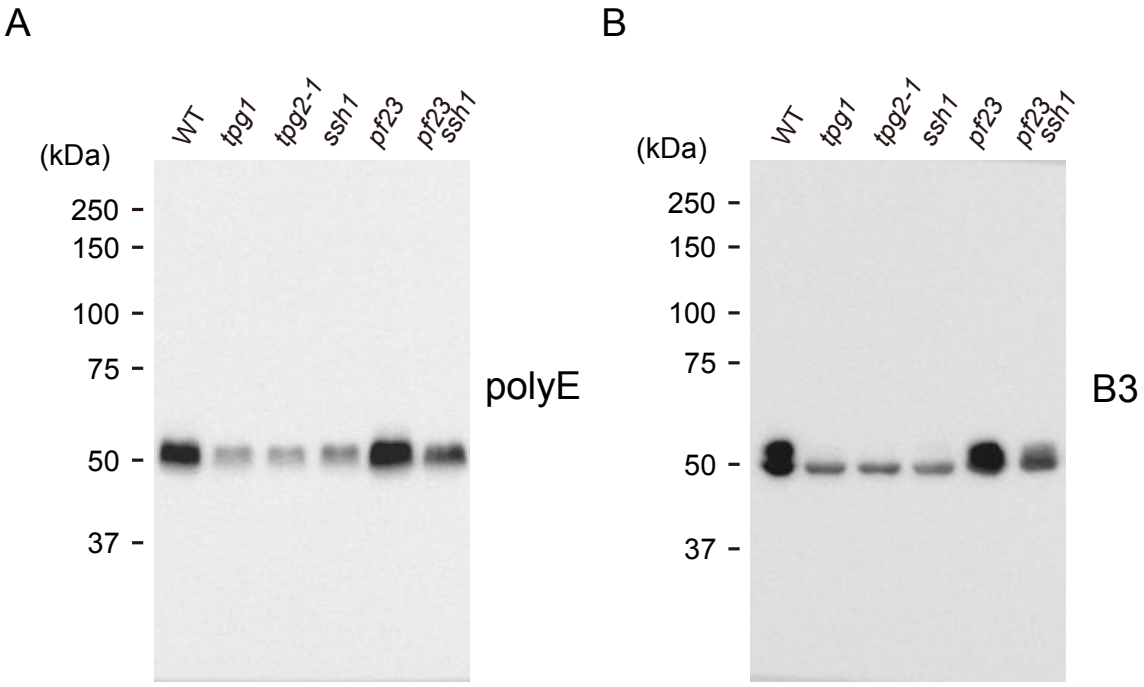
Kubo et al.





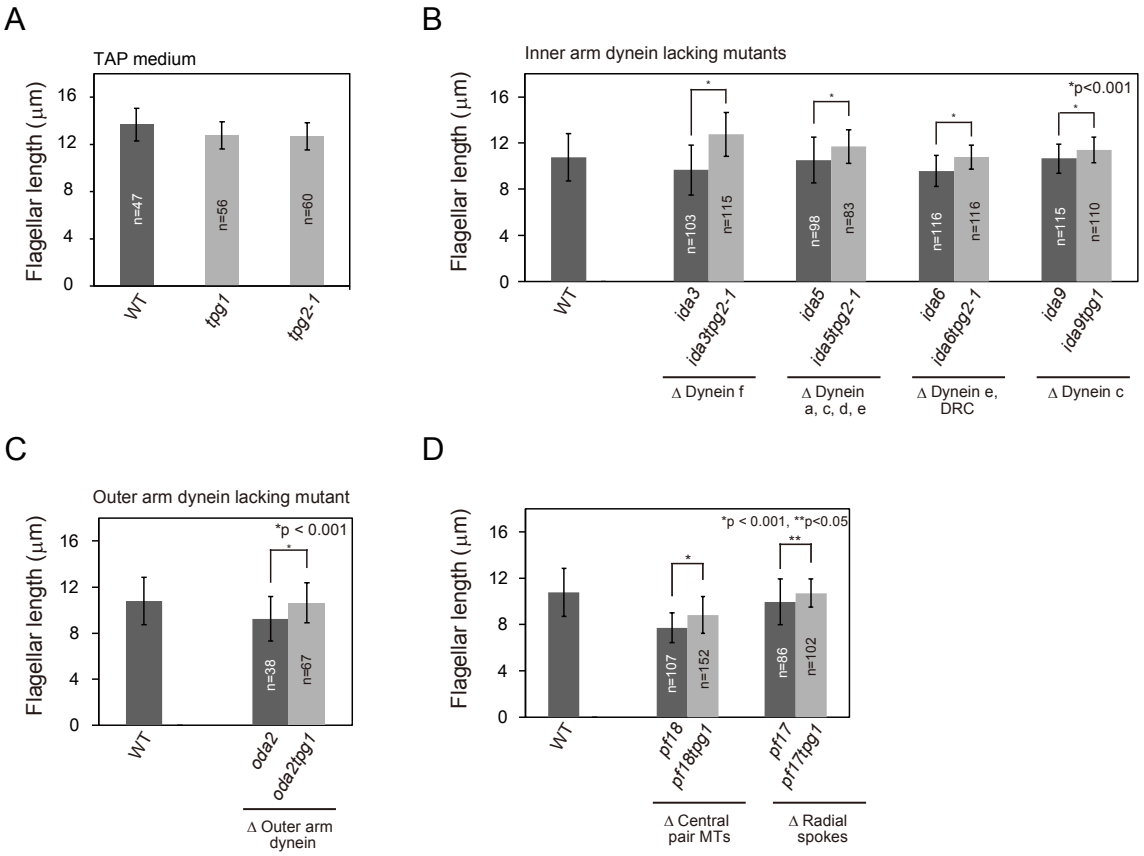
Supplemental Figure S1. The *tpg2-3* mutation facilitates the flagellar growth of *pf23*.

pf23 (A(a-d)) and *pf23tpg2-3* (B(a-c)) in M-medium, or in M-medium containing autolysin (C) observed by DIC microscopy. Bars, 10 μm. (D) Presence or absence of flagella on *pf23* and *pf23tpg2-3* cells in M-medium (Normal condition) or M-medium containing autolysin. Biflagellated (cells with two flagella), uniflagellated (one flagella), bald (no flagella), and palmelloid cells were counted.



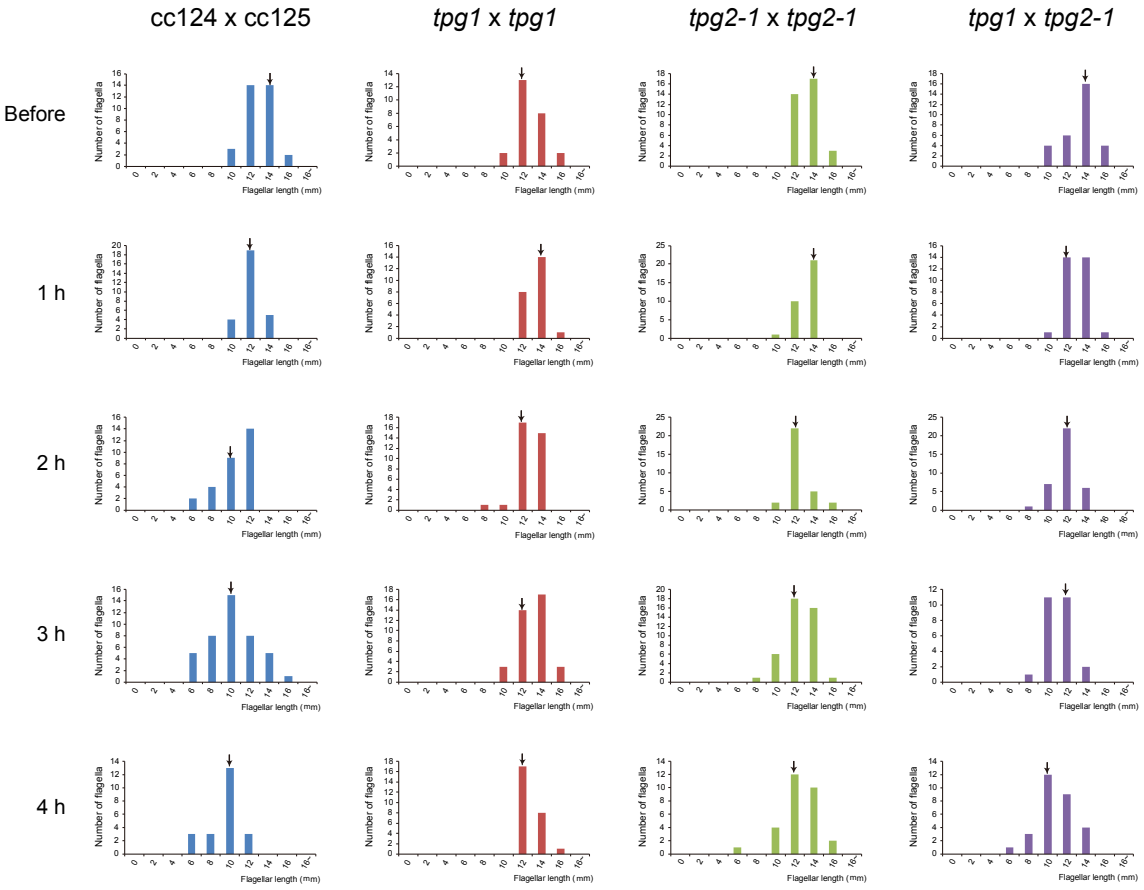
Supplemental Figure S2. The polyE antibody recognizes only tubulins in the axoneme.

Western blot analyses of axonemes using polyE antibody (A) and B3 antibody (B). Images obtained from the same membranes were used in Figure 1B except that the entire blots are shown here and the membranes were exposed for a longer time to detect any minor signals that might be present. The polyE antibody was produced against Cys-E_9 [1] and should recognize polyglutamate side chains of at least three or more glutamates [2], whereas the B3 antibody recognizes tubulins with two or more glutamate side chains [3]. The result that the polyE antibody recognized only tubulin indicates that there are no other major substrates of polyglutamylation in the axoneme.



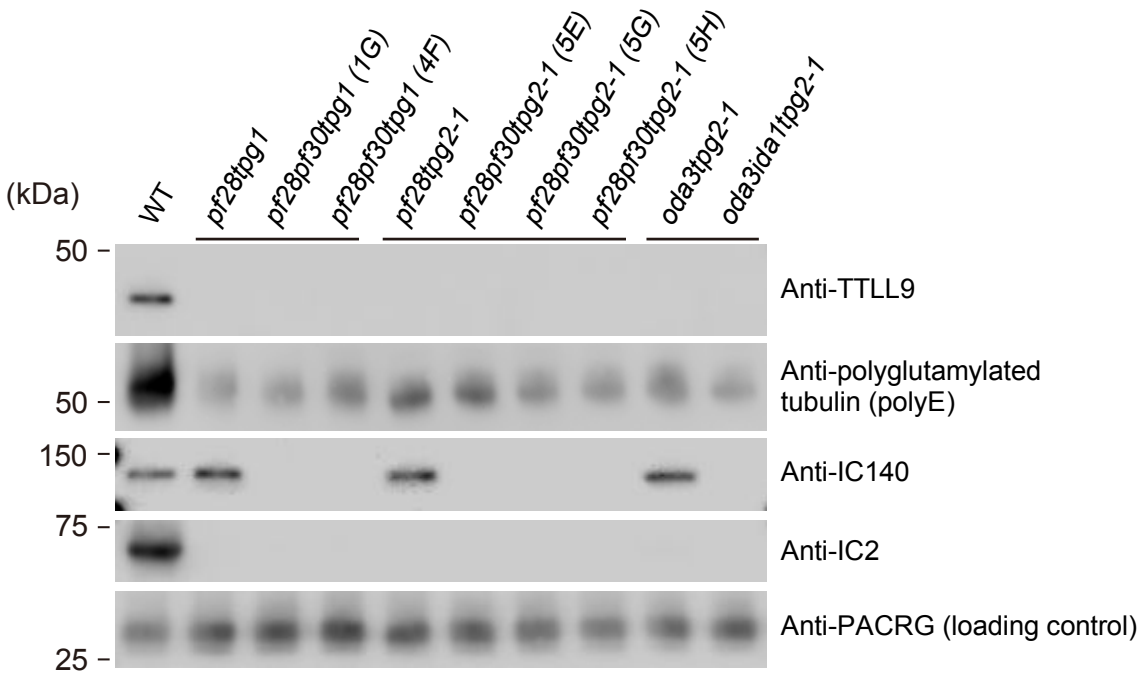
Supplemental Figure S3. Flagellar length of mutants lacking various components of the axoneme.

(A) Flagellar length of wild type, *tpg1*, and *tpg2-1* cultured in TAP medium. (B) Flagellar length in wild type (WT), *ida3*, *ida3tpg2-1*, *ida5*, *ida5tpg2-1*, *ida6*, *ida6tpg2-1*, *ida9*, and *ida9tpg1*. (C) Flagellar length in *oda2* and *oda2tpg1*. (D) Flagellar length in *pf18*, *pf18tpg1*, *pf17*, and *pf17tpg1*. Cells in B, C, and D were cultured in M-medium. Data for wild type are from Figure 3. Standard deviations for each measurement are shown as bars. Asterisks indicate statistically significant differences (t-test, *P<0.001 or **P<0.05).



Supplemental Figure S4. Tubulin polyglutamylation deficiency inhibits flagellar shortening after mating.

Histograms of dikaryon flagellar lengths after the mating between CC124 and CC125, *tpg1* and *tpg1*, *tpg2-1* and *tpg2-1*, and *tpg1* and *tpg2-1*. The same data sets were used in Figure 4B. Non-flagellated cells were excluded from the flagellar length measurements. Note that the flagellar-length peaks of wild-type and *tpg1-tpg2-1* dikaryons gradually shift toward shorter lengths during the time course, reflecting the rate of the slow flagellar-shortening phase. Those of the *tpg1* and *tpg2-1* dikaryons did not undergo this shift. The arrows indicate the average lengths of the flagella at each time point.



Supplemental Figure S5. Generation of triple mutants lacking outer arm dynein, inner arm dynein f/I1, and tubulin polyglutamylation.
Western blot analysis of isolated axonemes to confirm the production of triple mutants. Western blots of axonemes of wild type (WT), the double mutants *pf28tpg1*, *pf28tpg2-1*, and *oda3tpg2-1*, and candidate strains for *pf28pf30tpg1*, *pf28pf30tpg2-1*, and *oda3ida1tpg2-1*, probed with anti-TTLL9, anti-polyglutamylated tubulin (polyE), anti-IC140 (dynein f/I1 intermediate chain), anti-IC2 (outer arm dynein intermediate chain), and anti-PACRG (for loading control) antibodies. For experiments with *pf28pf30tpg1* and *pf28pf30tpg2-1* (Figure 2), 1G and 5E strains were used respectively.

Table S1

Name	Strain	Missing component/protein	Mutated protein	Mutated gene	Reference
Wild type	CC124, CC125				
pf23		Inner-arm dynein a, c, d, f	DYX1C1	*DYX1C1*	[4]
ssh1 (*tpg2-3*)	A kind gift from Dr. Gianni Piperno	FAP267 (TTLL9), FAP234	FAP234	*TPG2*	[5]
tpg1		FAP267 (TTLL9), FAP234	FAP267	*TPG1*	[6]
tpg2-1		FAP267 (TTLL9), FAP234	FAP234	*TPG2*	[7]
pf28pf30ssh1	W4, a kind gift from Dr. David Mitchell	Inner arm dynein f/I1, outer arm dynein, FAP267 (TTLL9), FAP234	DHC1, γHC, FAP234	*DHC1*, *DHC15*, *TPG2*	[8]
oda2	CC2230, CC2231	Outer-arm dynein	γHC	*DHC15*	[9-11]
ida1	CC2664, CC2665	Dynein f	DHC1	*DHC1*	[12-17]
ida3	CC2668, CC2669	Dynein f			[12]
ida4	CC2670, CC2671	Dynein a, c, d	p28	*DII1*	[12], [13], [18]
ida5	CC3420, CC3421	Dynein a, c, d, e	Actin	*DII4*	[19], [20]
ida6	CC3090, CC3091	Dynein e			[20]
ida9	CC4074	Dynein c	DHC9	*DHC9*	[21]
pf17	CC262	RSP1, 4, 6, 9, 10	RSP9	*RSP9*	[22]
pf18	CC1036	Central pair			[23], [24]
fla10-1	CC1919	Anterograde IFT lost at 32°C	FLA10	*FLA10*	[25-27]

Table S2.

Antibody	Dilution for WB	Dilution for IFM	Animal/type	Source
Anti-α-tubulin (B-5-1-2)	1:5,000	N/A	Mouse-monoclonal IgG1	Sigma-Aldrich
Anti-acetylated tubulin (6-11B-1)	N/A	1:500	Mouse-monoclonal IgG2b	Abcam
Anti-polyglutamylated tubulin (B3)	1:2,000	N/A	Mouse-monoclonal IgM	Abcam
Anti-polyglutamylated tubulin (polyE)	1:2,000	N/A	Rabbit-polyclonal	[1]
Anti-FAP234C	1:1,000	N/A	Rabbit-polyclonal	[7]
Anti-TTLL9	1:1,000	N/A	Rabbit-polyclonal	[6]
Anti-IFT172	1:20	N/A	Mouse-monoclonal IgG1	[28]
Anti-IFT139	1:100	N/A	Mouse-monoclonal IgG2a	[28]
Anti-IFT81	1:100	N/A	Mouse-monoclonal IgG1	[28]
Anti-IFT57	1:20	N/A	Mouse-monoclonal IgG2a	[28]
Anti-IFT46	N/A	1:2000	Rabbit-polyclonal	[29]
Anti-DHC1b	1:2,000	N/A	Rabbit-polyclonal	[30]
Anti-D1bLIC	1:2,000	N/A	Rabbit-polyclonal	[31]
Anti-IC2	1:50	N/A	Mouse monoclonal IgG	[32]
Anti-IC140	1:10,000	N/A	Rabbit-polyclonal	[33]
Anti-PACRG	1:5,000	N/A	Rabbit-polyclonal	[34]
Anti-HA tag (3F10)	1:2,000	1:100	Rat-monoclonal IgG1	Roche
Anti-βF1-ATPase	1:80,000	N/A	Rabbit-polyclonal	Agrisera

N/A; not applicable

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