Oda et al., http://www.jcb.org/cgi/content/full/jcb.201312014/DC1

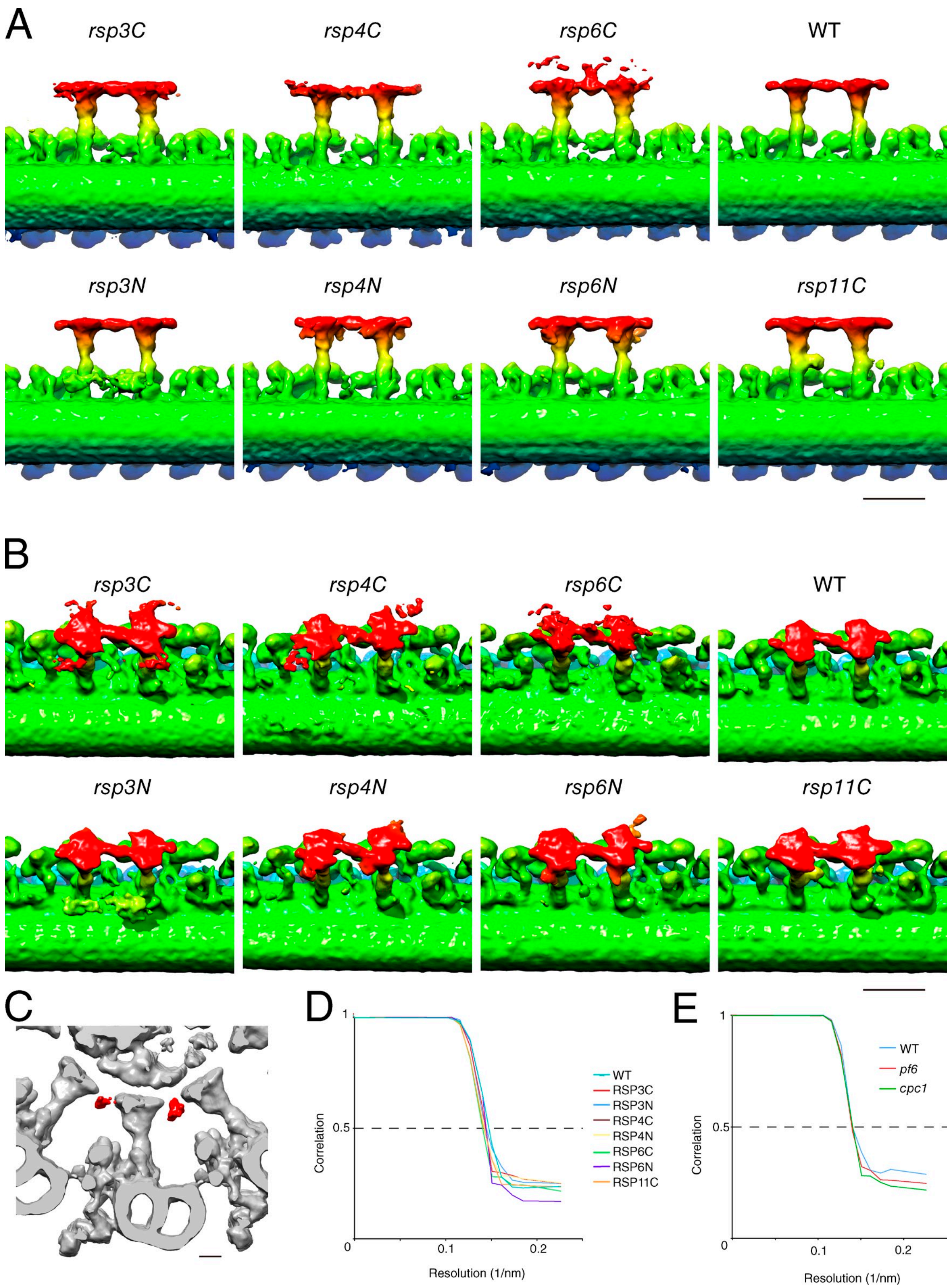


Figure S1. **Averaged tomograms of DMT used for Student's *t* test.** Related to Fig. 2. (A and B) Individual averaged tomograms of the wild-type and streptavidin–cytochrome *c*-labeled BCCP-RSP mutant axonemes. Bars, 20 nm. (C) Relationship between the label densities of *rsp3C* (red) and the neighboring RS on the adjacent DMT. Bar, 10 nm. (D and E) Fourier shell correlation curves of the averaged DMT (D) and CP (E) tomograms. The intersection between each curve and the horizontal line at 0.5 was taken as the effective resolution. The effective resolutions of all the structures were ~7 nm. WT, wild type.

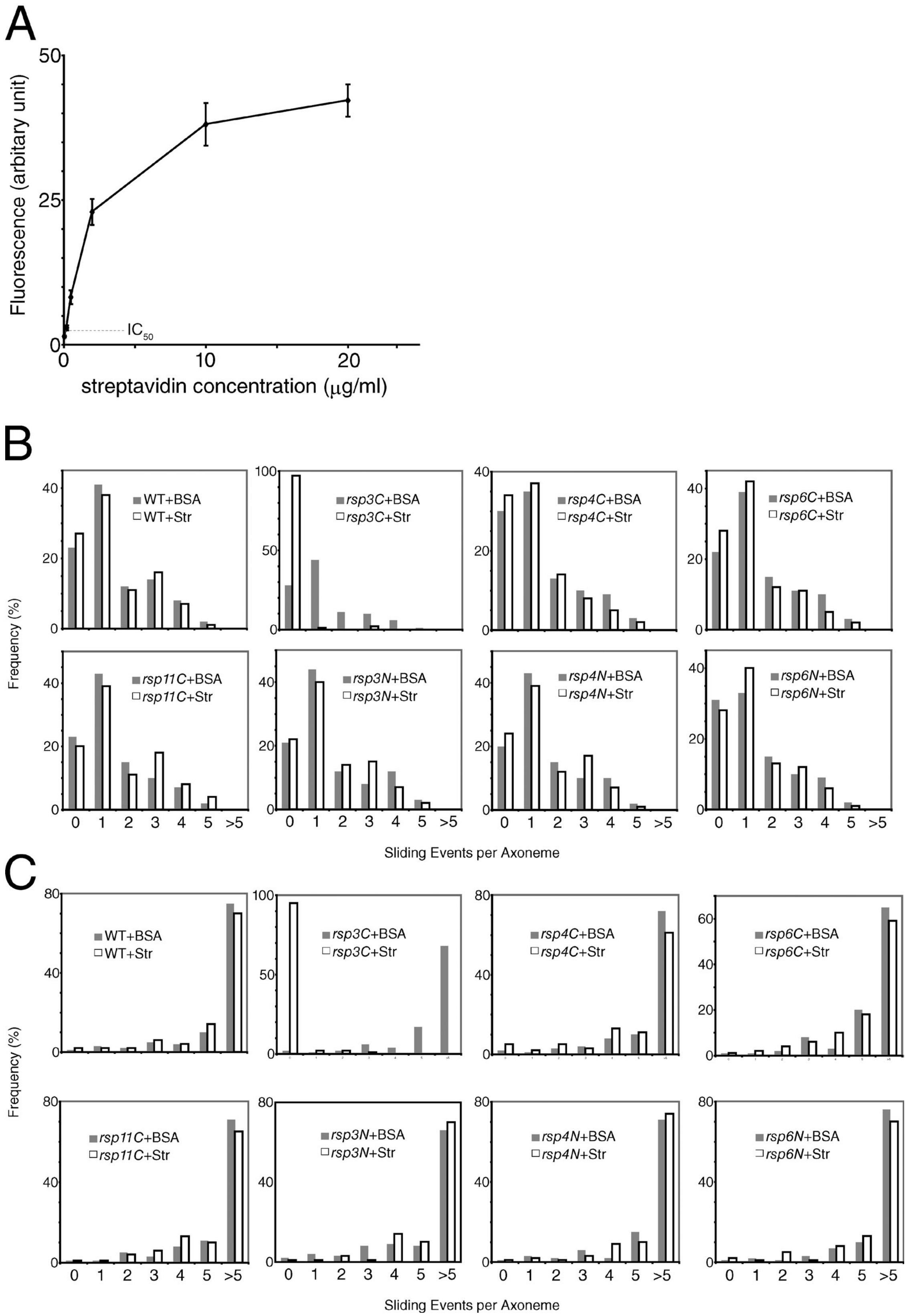


Figure S2. **Effect of streptavidin on the motor activity of dyneins.** Related to Fig. 3. (A) Binding of Alexa Fluor–conjugated streptavidin to *rsp4C* axonemes was quantified by measuring the intensity of the fluorescent signals. Binding of streptavidin appears to saturate at 20 μg/ml. The binding ratio of streptavidin at the concentration of IC_{50} is ∼5% of the maximum. Means ± SEM were calculated from 10 axonemes. (B and C) Sliding disintegration assays of intact axonemes. Axonemes were incubated with streptavidin (Str) or BSA, and microtubule sliding was induced with ATP and trypsin treatment. Sliding events per axoneme were counted and presented as histograms. (B) 1 mM ATP; (C) 50 μM ATP. *rsp3C* axonemes showed complete inhibition of sliding in the presence of streptavidin even with 50 μM ATP. Each of the frequencies was determined by observation of >100 axonemes. WT, wild type.

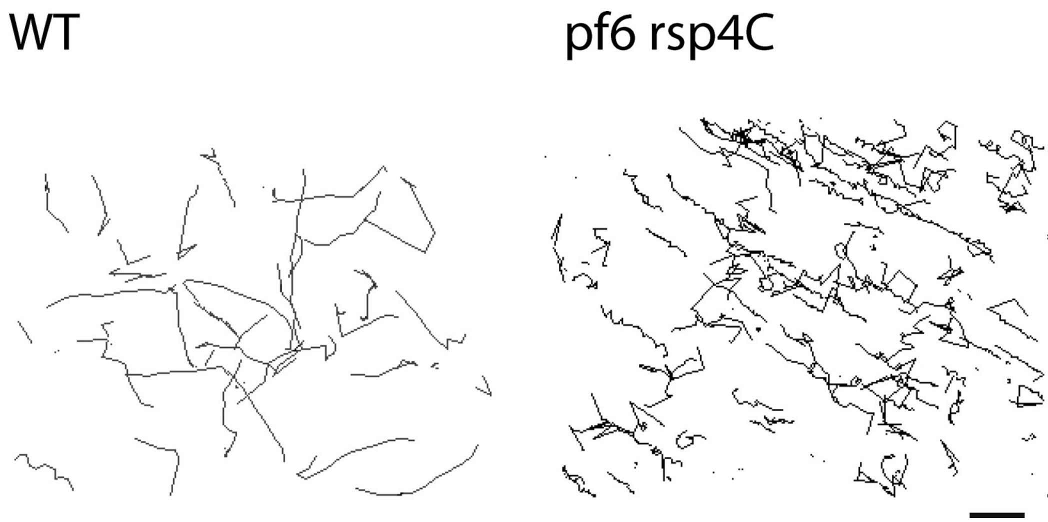


Figure S3. **The swimming paths of wild-type and *pf6 rsp4C* cells.** Related to Fig. 4. Images were recorded using an inverted microscope (IX70; Olympus) and a CCD camera (ORCA-R2; Hamamatsu Photonics). The trajectories of the swimming cells were detected using an ImageJ plugin, Particle Tracker (Sbalzarini and Koumoutsakos, 2005). WT, wild type. Bar, 50 μm.

Table S1. **Summary of the strains used in this study**

Strain	Description	Swimming speed	Beat frequency	References
		μm/s	*Hz*	
Wild-type 137c		180.8 ± 12.8	70 ± 5	
pf14	Missing RSP3, paralyzed flagella	–	–	Witman et al., 1978
pf14-rsp3-c-bccp (*rsp3C*)	Wild-type motility, sensitive to streptavidin[a]	167.1 ± 8.8	68 ± 5	This study
pf14-rsp3-n-bccp (*rsp3N*)	Wild-type motility, insensitive to streptavidin[a]	176.9 ± 9.3	70 ± 5	This study
pf1	Missing RSP4, paralyzed flagella	–	–	Piperno et al., 1977
pf1-rsp4-c-bccp (*rsp4C*)	Wild-type motility, sensitive to streptavidin	180.5 ± 8.2	65 ± 8	This study
pf1-rsp4-n-bccp (*rsp4N*)	Wild-type motility, insensitive to streptavidin	179.5 ± 14.9	70 ± 5	This study
pf1-rsp4-c-3×HA (*rsp4C3HA*)	Wild-type motility	177.1 ± 10.5	70 ± 5	This study
pf1-rsp4-c-GFP (*rsp4CGFP*)	Slightly slow swimming	141.7 ± 7.2	55 ± 4	This study
pf26	Mutation in RSP6, temperature-sensitive motility defect	ND	ND	Huang et al., 1981
pf26-rsp6-c-bccp (*rsp6C*)	Wild-type motility, sensitive to streptavidin	176.8 ± 6.5	68 ± 6	This study
pf26-rsp6-n-bccp (*rsp6N*)	Wild-type motility, insensitive to streptavidin	182.9 ± 10.8	70 ± 6	This study
pf25	Missing RSP11, culture medium-dependent motility defect	ND	ND	Yang and Yang, 2006
pf25-rsp11-c-bccp (*rsp11C*)	Wild-type motility, insensitive to streptavidin	170.2 ± 4.4	68 ± 8	This study
pf6	Missing C1a projection, jiggling on the bottom of culture dish	–	–	Dutcher et al., 1984; Rupp et al., 2001
pf6 pf1-rsp4-c-bccp (*pf6 rsp4C*)	Slow swimming, jaggy paths, sensitive to streptavidin	96.3 ± 9.9	35 ± 10	This study
pf6 pf1-rsp4-c-3×HA (*pf6 rsp-4C3HA*)	Slow swimming, jaggy paths	56.8 ± 9.4	30 ± 12	This study
pf6 pf1-rsp4-c-GFP (*pf6 rsp4C-GFP*)	Slow swimming, jaggy paths	64.2 ± 10.8	36 ± 11	This study
pf6 pf14-rsp3-c-bccp (*pf6 rsp3C*)	Slow swimming, jaggy paths, sensitive to streptavidin	61.7 ± 5.4	31 ± 9	This study
pf6 pf26-rsp6-c-bccp (*pf6 rsp6C*)	Slow swimming, jaggy paths, sensitive to streptavidin	57.9 ± 7.3	30 ± 10	This study
pf6 pf14-rsp3-n-bccp (*pf6 rsp3N*)	Similar to *pf6*, insensitive to streptavidin	–	–	This study
pf6 pf1-rsp4-n-bccp (*pf6 rsp4N*)	Similar to *pf6*, insensitive to streptavidin	–	–	This study
pf6 pf26-rsp6-n-bccp (*pf6 rsp6N*)	Similar to *pf6*, insensitive to streptavidin	–	–	This study
cpc1	Missing C1b and C2b, slow swimming	83.6 ± 5.8	40 ± 9	Mitchell and Sale, 1999
cpc1 pf1-rsp4-c-bccp (*cpc1 rsp4C*)	Slow swimming, similar to *cpc1*, sensitive to streptavidin	92.7 ± 15.7	41 ± 8	This study
oda1	Missing the docking complex 2 protein and entire ODA	63.1 ± 9.8	35 ± 4	Kamiya, 1988; Takada et al., 2002
oda1-rsp4-c-bccp (*oda1 rsp4C*)	Slow swimming, similar to *oda1*, insensitive to streptavidin.	61.8 ± 10.5	35 ± 4	This study
ida1	Missing IDA f, slow swimming	86.5 ± 17.1	51 ± 8	Kamiya et al., 1991
ida1 pf26-rsp6-c-bccp (*ida1 rsp6C*)	Slow swimming, similar to *ida1*, sensitive to streptavidin	86.3 ± 11.5	51 ± 6	This study
ida3	Missing IDA f, slow swimming	89.3 ± 15.6	52 ± 6	Kamiya et al., 1991
ida3 pf14-rsp4-c-bccp (*ida3 rsp4C*)	Slow swimming, similar to *ida1*, sensitive to streptavidin	88.1 ± 12.8	52 ± 7	This study
ida5	Missing actin and IDAs a, c, d, and e, slow swimming	62.5 ± 8.1	48 ± 5	Kato et al., 1993
ida5 pf1-rsp4-c-bccp (*ida5 rsp4C*)	Slow swimming, similar to *ida5*, sensitive to streptavidin	64.4 ± 10.2	49 ± 4	This study
pf6 oda1	Paralyzed flagella	–	–	This study
pf6 oda1 pf1-rsp4-c-GFP (*pf6od-a1rsp4C-GFP*)	Paralyzed flagella	–	–	This study
pf6 ida1	Paralyzed flagella	–	–	This study
pf6 ida1 pf1-rsp4-c-GFP (*pf6id-a1rsp4C-GFP*)	Rotating cells	–	–	This study
pf6 ida5	Paralyzed flagella, small number of jiggling cells	–	–	This study
pf6 ida5 pf1-rsp4-c-GFP (*pf6id-a5rsp4C-GFP*)	Paralyzed flagella, small number of jiggling cells	–	–	This study
pf18	Missing whole CP, paralyzed flagella	–	–	Adams et al., 1981

Table S1. **Summary of the strains used in this study** *(Continued)*

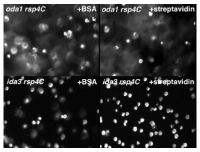
Strain	Description	Swimming speed	Beat frequency	References
pf18 pf1-rsp3-c-bccp (*pf18 rps3C*)[b]	Paralyzed flagella, similar to *pf18*	–	–	This study
pf18 pf14-rsp4-c-bccp (*pf18 rsp4C*)[b]	Paralyzed flagella, similar to *pf18*	–	–	This study
pf18 pf26-rsp6-c-bccp (*pf18 rsp6C*)[b]	Paralyzed flagella, similar to *pf18*	–	–	This study

Minus signs indicate these are unmeasurable due to the immotility of the cells.
[a]Sensitive (insensitive) to streptavidin means that the demembranated cells of the strain show (or do not show) streptavidin-dependent inhibition of motility.
[b]*pf18 rsp* mutants were created to confirm that the recovery of swimming in *pf6 rsp4C* does not result from a CP-independent suppressor activity of the *rsp4C* mutation.

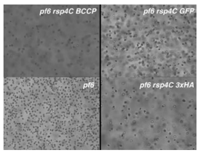
Table S2. **The ATPase activities and sliding speed of axonemes**

Strains and treatments	ATPase activity[a]	Sliding speed[b]
		µm/s
WT + BSA	0.49 ± 0.03	19.9 ± 2.6
WT + streptavidin	0.48 ± 0.01	19.2 ± 3.1
rsp3C + BSA	0.44 ± 0.02	18.8 ± 2.5
rsp3C + streptavidin	0.44 ± 0.01	No sliding
rsp3N + BSA	0.44 ± 0.04	20.1 ± 4.1
rsp3N + streptavidin	0.43 ± 0.03	19.3 ± 3.5
rsp4C + BSA	0.44 ± 0.03	18.3 ± 2.1
rsp4C + streptavidin	0.47 ± 0.04	17.9 ± 4.5
rsp4N + BSA	0.43 ± 0.05	19.2 ± 1.2
rsp4N + streptavidin	0.42 ± 0.05	18.8 ± 3.3
rsp6C + BSA	0.46 ± 0.03	16.7 ± 4.8
rsp6C + streptavidin	0.45 ± 0.02	15.9 ± 3.7
rsp6N + BSA	0.49 ± 0.03	18.5 ± 4.4
rsp6N + streptavidin	0.44 ± 0.05	18.0 ± 3.2
rsp11C + BSA	0.45 ± 0.03	19.8 ± 4.0
rsp11C + streptavidin	0.46 ± 0.05	19.1 ± 2.2

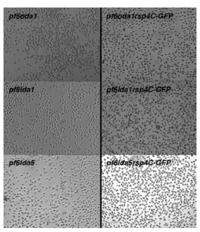
The means ± SEM were calculated from 10 and 20 measurements for ATPase activities and sliding speeds, respectively. WT, wild type.
[a]The unit of ATPase activity is micromoles P_i/minute/milligram of axoneme.
[b]Sliding speed of DMTs slid out of disintegrated axonemes.



Video 1. **Streptavidin-dependent inhibition of motility in *rsp4* tag mutant cells.** Related to Fig. 3. Swimming of demembranated, ATP-reactivated *rsp4C*, *rsp4N*, *oda1 rsp4C*, and *ida3 rsp4C* cells was observed using a dark-field microscope (BX51; Olympus). Image sequences were recorded using a high speed CCD camera (MC1362; Mikrotron) at 30 frames per second (fps; *rsp4C* and *rsp4N*) or 10 fps (*oda1 rsp4C* and *ida3 rsp4C*) and shown at 30 fps. Demembranated cells were incubated with 1 mM ATP and 2 μg/ml BSA or streptavidin for 1 min before recording.



Video 2. **Suppression of the motility defect in the *pf6* mutant by *rsp4C*.** Related to Fig. 4. Impaired motility of *pf6* mutant and the recovered motility of *pf6 rsp4C* live cells were recorded using an inverted microscope (IX70; Olympus). Image sequences were recorded using a high speed CCD camera (MC1362; Mikrotron) at 30 fps and shown at 30 fps.



Video 3. **ODA is required for suppression of the motility defect in *pf6* mutant by *rsp4C-GFP*.** Related to Fig. 4. Motility of *pf6 oda/ida* double mutants and *pf6 oda/ida rsp4C-GFP* quadruple mutants was recorded using an inverted microscope (IX70; Olympus). Image sequences were recorded using a high speed CCD camera (MC1362; Mikrotron) at 10 fps and shown at 30 fps.

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