## **Supplementary Information for**

## DYF-5/MAK-dependent phosphorylation promotes ciliary tubulin unloading.

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## This PDF file includes:

Figures S1 to S3 Table S1

	10	20	30		
MERPS	STASSRPRTSTG	RAPSARARPPS	AMRAPPPQP		
40	50	60	70		
YENRF	PTTGMSMRNGGF	PVPP <b>S</b> RSGMIP	VPPSRSGGP		
	80 9	90 1	00		
PAPMF	VSRAGGPPRAP	TSMGGRPM <b>T</b> GI	MARPPTAG		
110	120	130	140		
LRPVT	QQGLRAPPSRM	GTGNSRQVFDK	SYYIGV		
	IFT-74 phospho-dead (PD) mutant				
	10	20	30		
MERPS	SAASSRPRASTG				
40	50	60	70		
 YENRE					
			00		
PAPMF	VARAGGPPRAP	L TSMGGRPMAG	MARPPAAG		
110	120	130	140		
	QQGLRAPPARM	GTGNSRQVFD	(SYYIGV		
;	IFT 74 phoon	he mimie (DM) n	utont		
	IFT-74 phospho-mimic (PM) mu		30		
MERPS	SEASSRPRESTG				
40	50	60	70		
			I		
		1			
	VDRAGGPPRAP	TSMGGRPMFGI			
PAPMP	VDRAGGPPRAP	TSMGGRPMEGI 130	140		

Fig. S1. Amino acid mutations of in IFT-74 phospho-mimic (PM) and phospho-dead (PD) mutants.

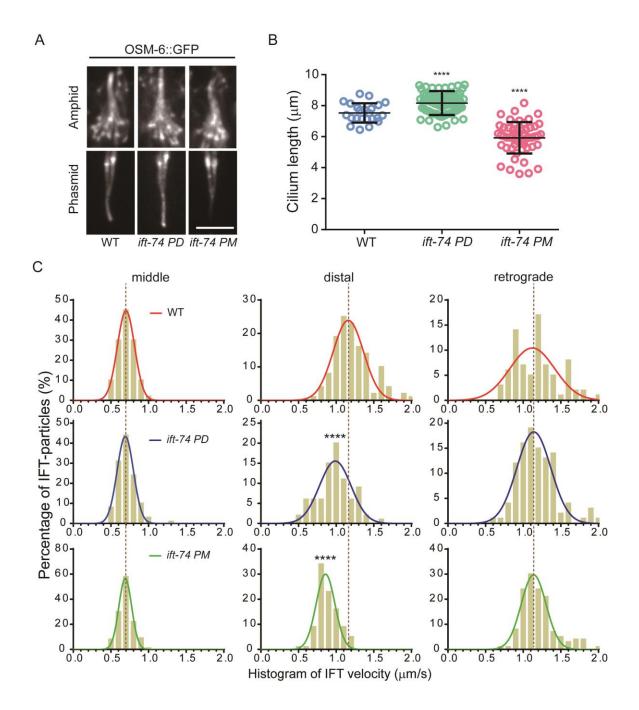


Fig. S2. Cilium morphology and IFT velocity in *ift-*74 *PM* and *PD* mutant animals based on OSM6::GFP. (A) Amphid (top) and phasmid (bottom) cilia morphology in ift-74 PM and PD mutants in comparison with wild-type (N2) animals. Cilia were imaged with GFP-tagged OSM-6. (B) Cilium length. Scale bar: 5  $\mu$ m. (B) Cilium length in wild-type and *ift-74 PM/PD* animals (mean ± SD; n = 30; \*\*\*\**P* < 0.0001). OSM-6::GFP was used as the cilium marker. (C) Histogram of IFT velocities in wild-type and *ift-*74-mutant animals. Left: anterograde IFT along the middle segments. Middle: anterograde IFT along the distal segments. Right: retrograde IFT. OSM-6::GFP was used as the IFT marker for the measurement. Each plot was fit by a Gaussian distribution. Comparisons were performed between the wild-type and mutants. \*\*\*\**P* < 0.0001.

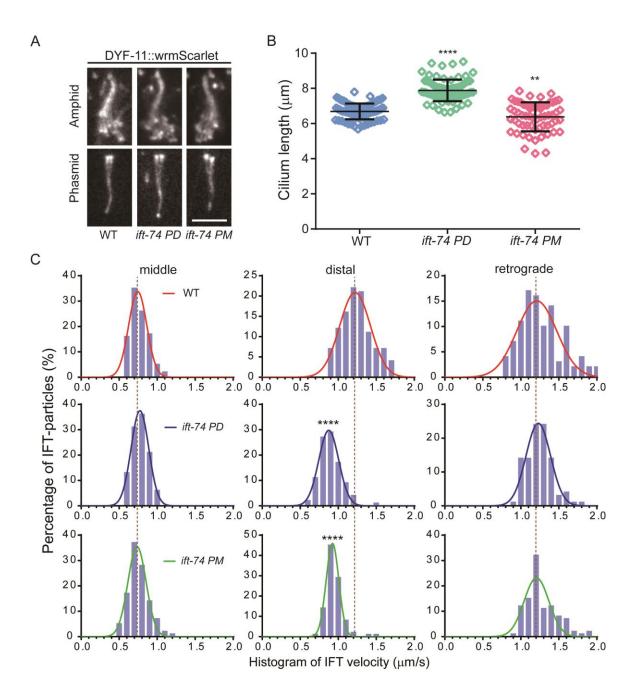


Fig. S3. Cilium morphology and IFT velocity in *ift*-74 *PM* and *PD* mutant animals based on DYF-11::wrmScarlet. (A) Amphid (top) and phasmid (bottom) cilia morphology in ift-74 PM and PD mutants in comparison with wild-type (N2). Cilia were imaged with wrmScarlet-tagged DYF-11. (B) Cilium length. Scale bar: 5  $\mu$ m. (B) Cilium length in wild-type and *ift-74 PM/PD* animals (mean  $\pm$  SD; n = 30; \*\**P* < 0.01, \*\*\*\**P* < 0.0001). DYF-11::wrmScarlet was used as the cilium marker. (C) Histogram of IFT velocities in wild-type and *ift-*74-mutant animals. Left: anterograde IFT along the middle segments. Middle: anterograde IFT along the distal segments. Right: retrograde IFT. DYF-11::wrmScarlet was used as the IFT marker for the measurement. Each plot was fit by a Gaussian distribution. Comparisons were performed between the wild-type and mutants. \*\*\*\**P* < 0.0001.

Strain name	Genotype	Method	Resouce	
N2	Wild type	/	CGC	
GOU2362	cas499 [ift-74::gfp knock-in]	Microinjection	This study	
SP1745	dyf-5 (mn400)	/	CGC	
GOU4775	dyf-5 (mn400); ift-74 (cas499)	Genetic cross	This study	
PHX4679	syb4679 [ift-74 PD knock-in]	Microinjection	Suny Biotech	
PHX4674	syb4674 [ift-74 PM knock-in]	Microinjection	Suny Biotech	
GOU4784	ift-74 (syb4679); mnIs17 [osm-6::gfp unc-36(+)]	Genetic cross	This study	
GOU4785	ift-74 (syb4674); mnIs17 [osm-6::gfp unc-36(+)]	Genetic cross	This study	
GOU4772	cas1086 [dyf-11::wrmScarlet knock-in]	Microinjection	This study	
GOU4786	ift-74 (syb4679); dyf-11 (cas1086)	Genetic cross	This study	
GOU4787	ift-74 (syb4674); dyf-11 (cas1086)	Genetic cross	This study	
PHX4963	syb4963 [ift-74 PD::gfp knock-in]	Microinjection	Suny Biotech	
PHX4964	syb4964 [ift-74 PM::gfp knock-in]	Microinjection	Suny Biotech	

Table S1. C. elegans strains used in this study