SUPPLEMENTARY DATA

Table S1. Summary of kinetic data for neurofilament movement in WT, KO and rescue experiments

Summary of the kinetic data in Fig. 2. Note that the proportion of neurofilaments moving anterogradely in cultured mouse SCG neurons is about 50%, which differs from cultured rat SCG neurons of comparable age in culture, in which anterograde movements predominate (Wang *et al.*, 2000). For the small number of neurofilaments that reversed direction, the anterograde and retrograde phases of movement were considered to be separate bouts of movement and were analyzed separately. n.d. = not determined

Table S2. Summary of statistical analysis for neurofilament movement in WT, KO and rescue experiments

Summary of the p-values obtained in our statistical comparisons of the kinetic data shown in Fig. 2 using the Mann-Whitney test. P values less than 0.05 are highlighted in yellow. A significant difference between the knockout and rescue (KO vs. 1A, 1B or 1C rescue) and no significant difference between the wild type and rescue (WT vs. 1A, 1B or 1C rescue) indicates complete rescue. In contrast, a significant difference between the wild type and rescue and no significant difference between the knockout and rescue indicates no rescue. A significant difference between both the wild type and rescue and the knockout and rescue indicates partial rescue.

Table S3. Summary of kinetic data for neurofilament movement in the dynein inhibition experiments

Summary of the kinetic data in Fig. 6B-F for SCG neurons (Table S3A) and cortical neurons (Table S3B). Note that the proportion of neurofilaments moving anterogradely in cultured mouse SCG and cortical neurons is about 50%, which differs from cultured rat SCG neurons of comparable age in culture, in which anterograde movements predominate (Wang *et al.*, 2000). For the small number of neurofilaments that reversed direction, the anterograde and retrograde phases of movement were considered to be separate bouts of movement and were analyzed separately. For the p150-CC1 experiment, the analysis was performed at 2 and 4 days after transfection with 25 μg/ml or 75 μg/ml p150-CC1 construct (labeled “low” and “high” respectively).

Table S4. Summary of statistical analysis for neurofilament movement in the dynein inhibition experiments

Summary of the p-values obtained in our statistical comparisons of the kinetic data shown in Fig. 6B-F using the Mann-Whitney test. P values less than 0.05 are highlighted in yellow. The DHC siRNA is compared to the control siRNA (cells injected with scrambled siRNA) as well as to the control (untreated). The control siRNA is also compared to the untreated control. The DIC antibody (DIC Ab) is compared to the control antibody (control Ab) as well as to the untreated control. The control antibody is also compared to the untreated control. The p50 and p150-CC1 are compared to the corresponding untreated controls. For the p150-CC1 experiment, the analysis was performed at 2 and 4 days after transfection with 25 μg/ml or 75 μg/ml p150-CC1 construct (labeled "low" and "high" respectively).

Movie 1: Neurofilament movement in a wild type neuron

A typical time-lapse movie of a gap in the neurofilament array of a wild type SCG neuron. The movie was 15 minutes long and the images were acquired at 4 second intervals. Several neurofilaments of different lengths can be seen to move, some anterogradely and some retrogradely. Note that one retrograde neurofilament appears to fold and then unfold while it is pausing, which is not unusual. On average, we observed about 3 moving neurofilaments per 15 minute movie. Proximal is left and distal is right. Time compression= 40:1.

Movie 2: Low frequency of neurofilament movement in a kinesin-1A knockout neuron

A typical time-lapse movie of a gap in the neurofilament array of a kinesin-1A knockout SCG neuron. The movie was 15 minutes long and the images were acquired at 4 second intervals. Note that the neurofilaments flanking the gaps exhibit some jiggling movements, but no neurofilaments enter the gap. On average, we observed less than one moving neurofilaments per 15 minute movie. Proximal is left and distal is right. Time compression= 40:1.

Movie 3: An anterogradely moving neurofilament in a kinesin-1A knockout neuron

An excerpt from a time-lapse movie of a gap in the neurofilament array of a kinesin-1A knockout SCG neuron showing an anterogradely moving neurofilament. The images were acquired at 4 second intervals. Though the frequency of movement in the knockout neurons was low, those neurofilaments that did move were capable of rapid and persistent movement. Proximal is left and distal is right. Time compression= 40:1.

Movie 4: A retrogradely moving neurofilament in a kinesin-1A knockout neuron

An excerpt from a time-lapse movie of a gap in the neurofilament array of a kinesin-1A knockout SCG neuron showing an retrogradely moving neurofilament. The images were acquired at 4 second intervals. Though the frequency of movement in the knockout neurons was low, those neurofilaments that did move were capable of rapid and persistent movement. Proximal is left and distal is right. Time compression= 40:1.

Movie 5: Kinesin-1A knockout neuron rescued by expressing kinesin-1A

A typical time-lapse movie of a gap in the neurofilament array of a kinesin-1A knockout SCG neuron expressing kinesin-1A. The movie was 15 minutes long and the images were acquired at 4 second intervals. Several neurofilaments of different lengths can be seen to move, some anterogradely and some retrogradely. On average, we observed about 3 moving neurofilaments per 15 minute movie, which is comparable to the wild type. Proximal is left and distal is right. Time compression= 40:1.

		WT		KIF5A KO		KIF5A rescue		KIF5B rescue		KIF5C rescue	
		Antero	Retro	Antero	Retro	Antero	Retro	Antero	Retro	Antero	Retro
Frequency	filaments/min	0.105	0.116	0.027	0.029	0.104	0.112	0.056	0.048	0.043	0.030
	filaments/movie	1.5	1.7	0.4	0.4	1.6	1.7	0.8	0.7	0.7	0.5
Filament length (μm)	Average	6.7	7.9	9.2	9.2	8.4	8.3	8.9	7.8	7.3	10.3
	Maximum	23.2	24.7	23.0	23.0	44.8	42.0	26.1	28.2	25.2	35.6
	Minimum	1.4	1.4	1.4	2.0	1.6	1.4	1.7	1.3	1.5	1.3
Peak velocity (μm/s)	Average	1.22	1.29	0.93	1.21	1.15	1.46	n.d.	n.d.	n.d.	n.d.
	Maximum	2.58	3.19	1.94	3.46	2.68	2.83	n.d.	n.d.	n.d.	n.d.
	Minimum	0.22	0.25	0.28	0.16	0.24	0.19	n.d.	n.d.	n.d.	n.d.
Average velocity *excluding* pauses (μm/s)	Average	0.54	0.49	0.26	0.41	0.40	0.47	n.d.	n.d.	n.d.	n.d.
	Maximum	1.44	1.79	0.82	0.96	1.11	1.25	n.d.	n.d.	n.d.	n.d.
	Minimum	0.13	0.13	0.13	0.13	0.13	0.13	n.d.	n.d.	n.d.	n.d.
Average velocity *including* pauses (μm/s)	Average	0.37	0.29	0.13	0.24	0.20	0.26	n.d.	n.d.	n.d.	n.d.
	Maximum	1.25	1.25	0.52	0.88	1.04	1.07	n.d.	n.d.	n.d.	n.d.
	Minimum	0.00	0.00	0.00	0.00	0.01	0.01	n.d.	n.d.	n.d.	n.d.
Number of filaments		93	103	28	31	72	77	45	39	30	21
Total number of filaments		196		59		149		84		51	
Number of movies		61		72		46		54		46	
Total observation time (min)		885		1056		690		810		690	

Table S1

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	Neurofilament length		Frequency		Peak velocity		Average velocity *excluding* pauses		Average velocity *including* pauses	
	Antero	Retro	Antero	Retro	Antero	Retro	Antero	Retro	Antero	Retro
WT vs. KO	0.052	0.285	0.000	0.000	0.013	0.183	0.000	0.220	0.000	0.258
WT vs. 5A rescue	0.132	0.980	0.382	0.483	0.280	0.030	0.007	0.672	0.000	0.509
KO vs. 5A rescue	0.471	0.244	0.000	0.000	0.043	0.020	0.017	0.425	0.051	0.449
WT vs. 5B rescue	n.d.	n.d.	0.052	0.000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
KO vs. 5B rescue	n.d.	n.d.	0.004	0.024	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
WT vs. 5C rescue	n.d.	n.d.	0.009	0.000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
KO vs. 5C rescue	n.d.	n.d.	0.037	0.655	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Key:

$p<0.05$
$p \geq 0.05$

Table S2

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		Control siRNA		DHC siRNA		Control Ab		DIC Ab		Control		p50	
		Antero	Retro	Antero	Retro	Antero	Retro	Antero	Retro	Antero	Retro	Antero	Retro
Frequency	filaments/min	0.069	0.087	0.023	0.040	0.065	0.063	0.010	0.017	0.072	0.085	0.032	0.032
	filaments/movie	1.04	1.30	0.35	0.60	0.97	0.95	0.15	0.25	1.08	1.27	0.48	0.48
Number of filaments		24	30	7	12	37	36	3	5	64	75	21	21
Total number of filaments		47		18		69		8		125		40	
Number of movies		23		20		38		20		59		44	
Total observation time (min)		345		300		570		300		885		660	

Table S3A
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		Control (2 days)		p150-CC1 (low, 2 days)		p150-CC1 (high, 2 days)		Control (4 days)		p150-CC1 (low, 4 days)		p150-CC1 (high, 4 days)	
		Antero	Retro	Antero	Retro	Antero	Retro	Antero	Retro	Antero	Retro	Antero	Retro
Frequency	filaments/min	0.108	0.105	0.058	0.073	0.037	0.030	0.130	0.140	0.025	0.035	0.038	0.041
	filaments/movie	1.62	1.58	0.87	1.09	0.55	0.45	1.95	2.10	0.38	0.52	0.57	0.62
Number of filaments		42	41	20	25	11	9	39	42	8	11	12	13
Total number of filaments		83		45		20		81		19		25	
Number of movies		26		23		20		20		21		21	
Total observation time (min)		390		345		300		300		315		315	

Table S3B
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	Frequency	
	Antero	Retro
DHC siRNA vs. control siRNA	0.038	0.035
DHC siRNA vs. control	0.034	0.042
control siRNA vs. control	0.917	0.962
DIC Ab vs. control Ab	0.006	0.014
DIC Ab vs. control	0.001	0.001
control Ab vs. control	0.463	0.171
p50 vs. control	0.017	0.001
p150-CC1 (low, 2 days) vs. control	0.115	0.337
p150-CC1 (high, 2 days) vs. control	0.008	0.025
p150-CC1 (low, 4 days) vs. control	0.014	0.001
p150-CC1 (high, 4 days) vs. control	0.016	0.002

Key:

$p<0.05$
$p\geq 0.05$

Table S4
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