Cargo Tag	Condition	Anterograde %	Retrograde %	Stationary %
Rab7 (endosomes)	Control	6.2 ± 0.01	16.24 ± 0.01	77.56 ± 0.01
	dnCDK5	5.42 ± 0.01	13.86 ± 0.01	80.72 ± 0.02
	p25	7.86 ± 0.01	16.77 ± 0.01	75.37 ± 0.02
LAMP1 (lysosomes)	Control	18.67 ± 0.01	18.66 ± 0.01	62.67 ± 0.01
	dnCDK5	18.10 ± 0.01	22.18 ± 0.01	59.73 ± 0.01
	p25	15.86 ± 0.01	17.56 ± 0.01	66.58 ± 0.02
LC3 (autophagosomes)	Control	0.26 ± 0.00	55.44 ± 0.04	44.30 ± 0.04
	dnCDK5	1.08 ± 0.01	40.80 ± 0.05 *	58.11 ± 0.05 *
	p25	1.91 ± 0.01	26.92 ± 0.05 ***	71.17 ± 0.05 ***
Mito (mitochondria)	Control	13.42 ± 0.01	15.19 ± 0.01	71.38 ± 0.02
	dnCDK5	9.73 ± 0.01	9.36 ± 0.01	80.91 ± 0.02
	p25	8.15 ± 0.01	9.51 ± 0.01	82.35 ± 0.02
TrkB (signaling endosomes)	Control	16.55 ± 0.01	23.37 ± 0.01	60.08 ± 0.02
	dnCDK5	19.24 ± 0.02	21.95 ± 0.02	58.81 ± 0.03
	p25	19.04 ± 0.02	23.64 ± 0.02	57.32 ± 0.03

Table S1, Related to Figures 1-3**:** Varying CDK5 activity level has minimal effect on motility of cargos. Condition indicates expression of CDK5 construct. Error represents SEM. Anterograde and retrograde are defines as >10 microns traveled over the course of imaging. Values that differ significantly from control by two-way ANOVA with Tukey's post-hoc test are denoted in the table (*$p<0.05$; ***$p<0.001$).

Figure S1, Related to Figure 1

(A) Targeted reduction of endogenous CDK5 via siRNA, as assessed by western blot versus control neurons.

(B) Quantitative analysis of western blots of cell lysates suggest a lower limit for CDK5 knockdown of 60%, skewed by the overall transfection efficiency of 30-40% of neurons on the plate (graph shows means ± SEM).

(C) Expression of CDK5 by immunoflourescence in control DRG neurons, neurons transfected with siRNA targeted against CDK5, and neurons without primary antibody.

(D) siRNA targeted against CDK5 resulted in 90.4% knockdown of endogenous protein at the cellular level, as assessed by immunofluorescence (graph shows means ± SEM).

(E) Increasing (p25) or decreasing (dnCDK5) CDK5 activity had minimal effect on the peak velocity of Rab7-positive lysosomes.

(F) Expression of p25 increased the density of Rab7-positive lysosomes in the mid-axon.

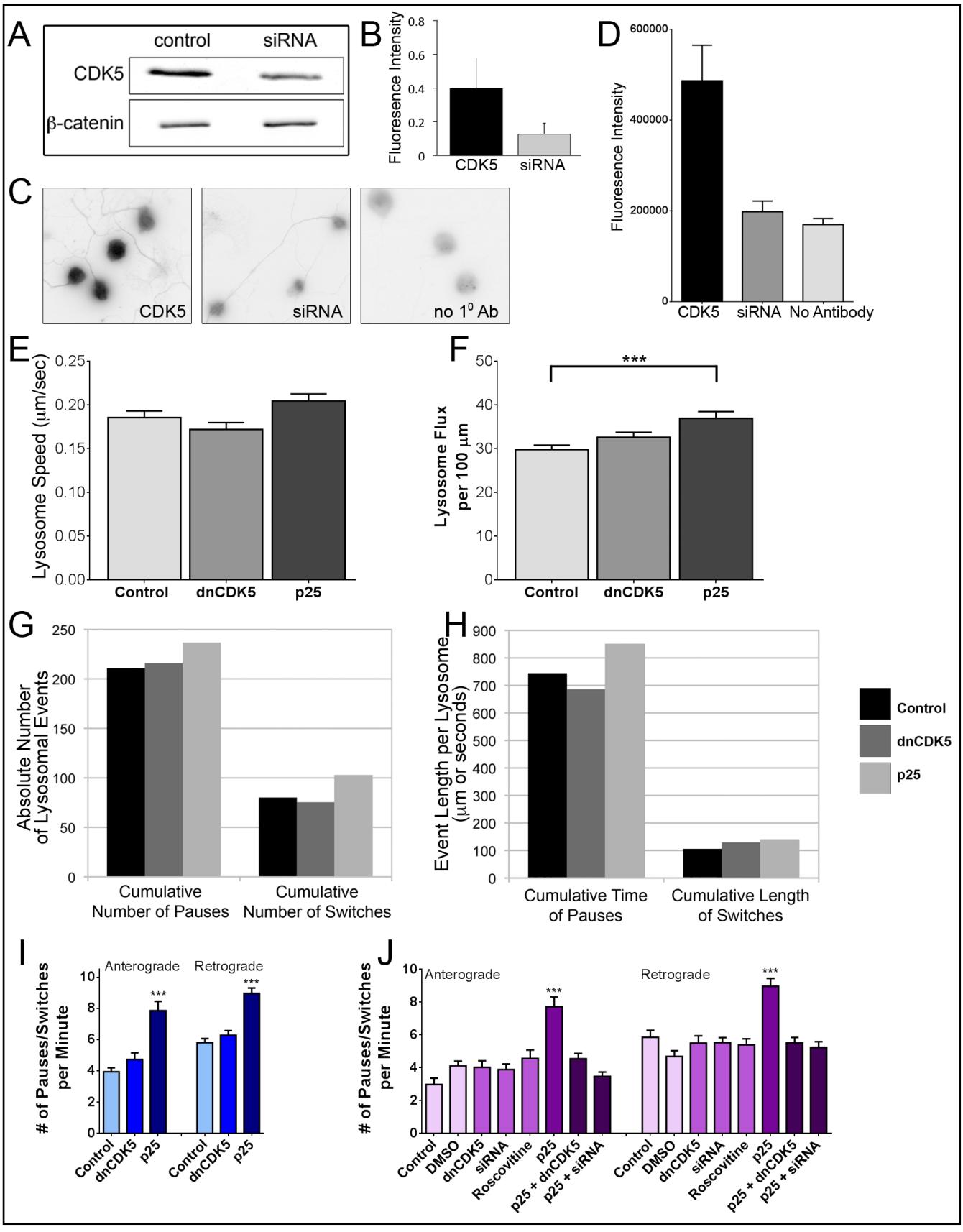
(G) Activation but not inhibition of CDK5 activity increased the absolute number of pauses and reversals undertaken by actively moving LAMP1-positive vesicles.

(H) LAMP1-positive vesicles spent only slightly more time paused or moving opposed to the direction of primary motility.

(I) Rab7-positive late endosomes display similar changes in both anterograde- and retrograde-directed trafficking events in response to changes in CDK5 activity.

(J) LAMP1-positive lysosomes exhibit decreased processivity when exposed to activated CDK5 in both directions. CDK5 inhibitors have no effect on processivity of motion.

For (I) and (J): graphs depict ± SEM; $n \geq 12$ neurons from ≥3 experiments. Values that differ significantly (one-way ANOVA with Tukey's post-hoc test) are denoted on graphs (***$p<0.001$).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control
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CDK5
β-catenin
B
Fluorescence Intensity
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0.6
0.4
0.2
0
CDK5
siRNA
D
600000
400000
200000
0
Fluorescence Intensity
CDK5
siRNA
No Antibody
C
CDK5
siRNA
no 1⁰ Ab
E
Lysosome Speed (μm/sec)
0.25
0.20
0.15
0.10
0.05
0.00
Control
dnCDK5
p25
F
***
50
40
30
20
10
0
Lysosome Flux per 100 μm
Control
dnCDK5
p25
G
Absolute Number of Lysosomal Events
250
200
150
100
50
0
Cumulative Number of Pauses
Cumulative Number of Switches
H
Event Length per Lysosome (μm or seconds)
900
800
700
600
500
400
300
200
100
0
Cumulative Time of Pauses
Cumulative Length of Switches
Control
dnCDK5
p25
I
# of Pauses/Switches per Minute
Anterograde
Retrograde
***
***
10
8
6
4
2
0
Control
dnCDK5
p25
Control
dnCDK5
p25
J
# of Pauses/Switches per Minute
Anterograde
Retrograde
***
***
10
8
6
4
2
0
Control
DMSO
dnCDK5
siRNA
Roscovitine
p25
p25 + dnCDK5
p25 + siRNA
Control
DMSO
dnCDK5
siRNA
Roscovitine
p25
p25 + dnCDK5
p25 + siRNA

Figure S2, Related to Figure 2

(A) Increasing (p25) or decreasing (dnCDK5) CDK5 activity had minimal effect on the peak velocity of dsRed2-labeled mitochondria.

(B) Mitochondria respond to increased CDK5 activity with an increase in pauses and switches in cargo moving both towards and away from the cell body. Inhibiting CDK5 activity does not affect motility (graph shows mean ± SEM; n≥12 neurons from ≥3 experiments. Values that differ significantly (one-way ANOVA with Tukey's post-hoc test) are noted (*p<0.05; **p<0.01).

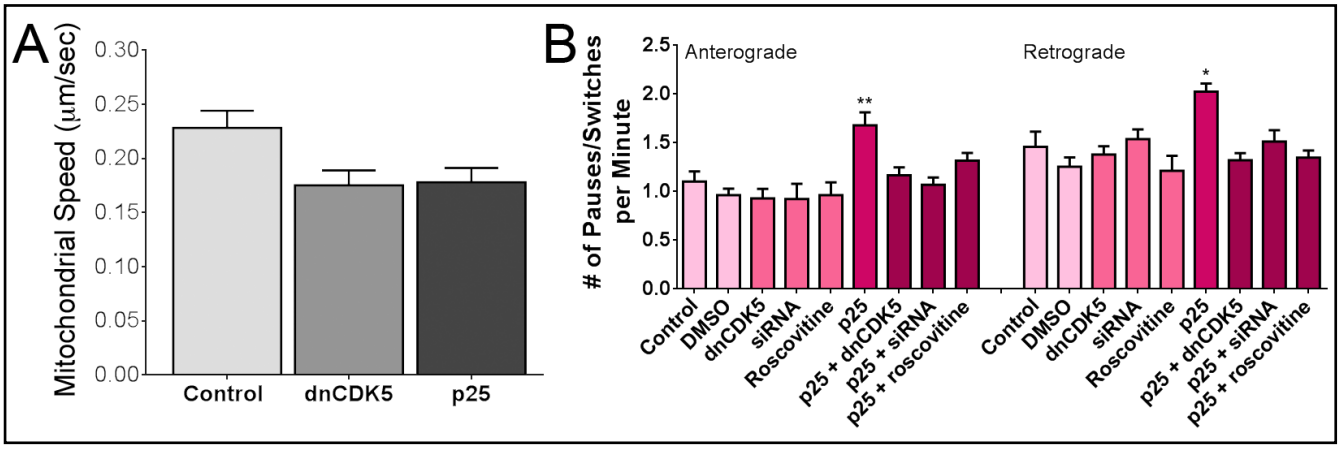


Figure S3, Related to Figure 3

(A) Expression of p25 increased the density of LC3-positive autophagosomes in the mid-axon.

(B) Activation of CDK5 by p25 increased the density of TrkB-positive signaling endosomes in the mid-axon.

Graphs showsmean ± SEM; n≥12 neurons from ≥3 experiments. Values that differ significantly (one-way ANOVA with Tukey's post-hoc test) are noted (**p<0.01).

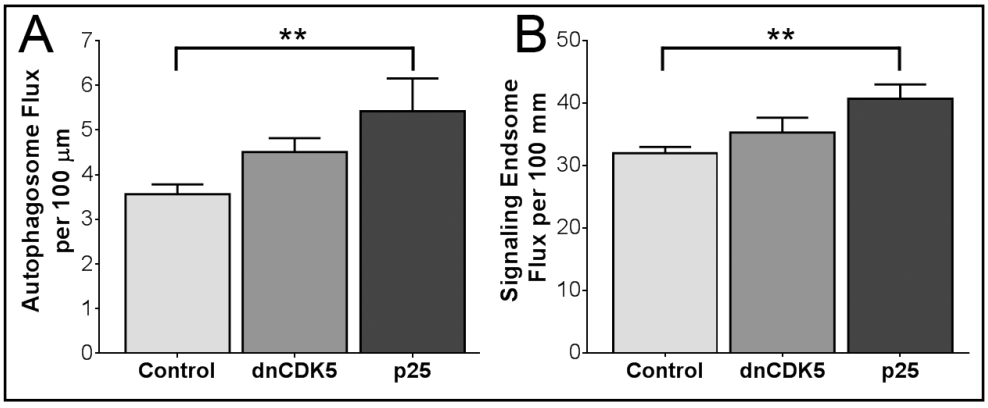


Figure S4, Related to Figure 5

Kymographs of lysosome and autophagosome motion in DRG neurites expressing LAMP1-RFP or GFP-LC3 and wildtype Lis1. Overexpression of wildtype Lis1 did not alter the appearance of lysosomal (Figure 1D) or autophagosome (Figure 3A) motility. Scale bars indicate 10 s and 10 μm.

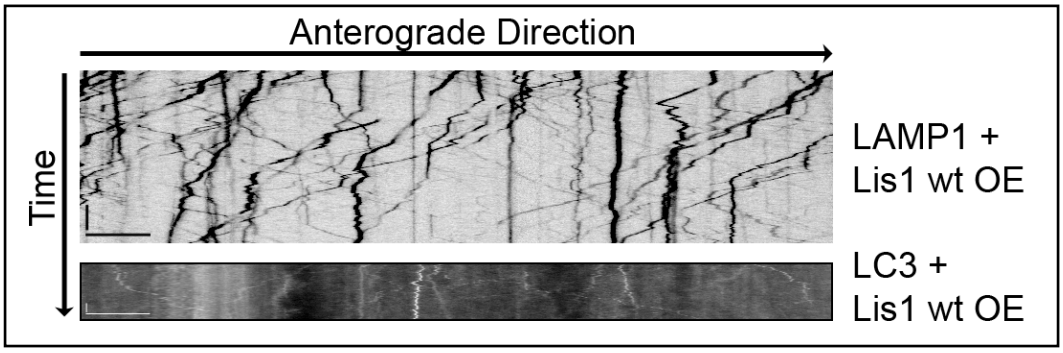
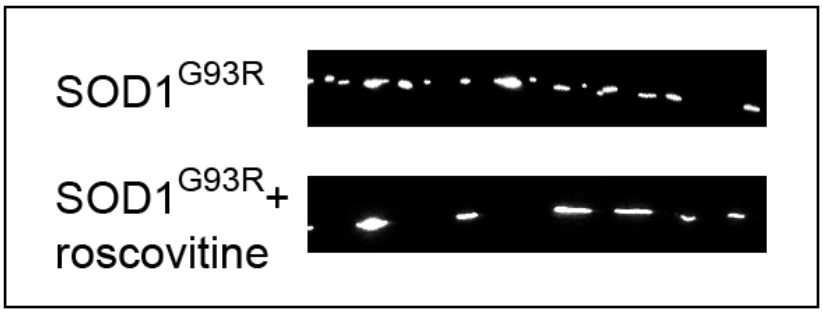


Figure S5, Related to Figure 6

Fragmented mitochondria were found along the neurites of DRG neurons in mutant SOD1 mice, but were returned to stereotypic long tubular mitochondria when CDK5 activity is inhibited.



Supplemental Experimental Procedures

Reagents

GFP-LC3 transgenic mice (Mizushima et al., 2004) were obtained from RIKEN. Transgenic mice overexpressing $SOD1^{G93A}$ [B6SJL-Tg(SOD*G93A)1Gur/J] were from Jackson Laboratory. Constructs include DsRed2-mito (T. Schwarz, Harvard Medical School, Boston, MA), LAMP1-RFP (Addgene), mRFP-TrkB (Addgene) and GFP-Rab7 (Addgene). Plasmids encoding, Ndel1, Ndel1 -5, Lis1, Lis1-K147A, hCDK5, dnCDK5, and p25 were from D.S. Smith, University of South Carolina. dnCDK5 and p25 were recloned into a bicistronic BFP-expressing vector to identify transfected cells. siRNA against CDK5 (Thermo Scientific) was generated based on Xing et al. (2012). Antibodies include polyclonals to CDK5 (Santa Cruz), p35/p25 (Santa Cruz), and phospo-Ndel1 (Biorbyt), and monoclonals to β-catenin (BD), dynein intermediate chain (Millipore), actin (Millipore), and β-III tubulin (R&D Systems).

Extraction of Spinal Cord Lysates

Spinal cord tissues were extracted from mice and homogenized in RIPA buffer (50 mM Tris [pH 8.0], 1 mM EDTA, 2 mM K^+ EGTA, 150 mM NaCl, 10% IGEPAL, 0.5% deoxycholate, 0.1 SDS) supplemented with protease inhibitors. Low speed extracts were spun at 1,000 *g*, and high speed extracts were then centrifuged at 100,000 *g*. Lysates were resuspended in denaturing buffer and boiled prior to analysis by SDS-PAGE and Western blot.

CDK5 Depletion

Endogenous CDK5 was depleted by siRNA. As an initial estimate of the extent of knockdown, transfected DRG cells were lysed two days post transfection in 100 mM PIPES, 1 mM EGTA, 2 mM $MgCl_2$, 25 mM NaCl, 0.5 mM DTT, 1% Triton X-100, and protease inhibitors (as above). Protein was analyzed by SDS-PAGE and Western blot. However, transfection efficiency in DRG cultures is 30-40%, so we used immunofluorescence to assess the extent of knockdown in individual neurons. Transfected DRG cells were fixed and stained two days post transfection in 4% PFA and 4% sucrose. Fixed cells were washed with PBS followed by blocking solution (2g BSA and 0.1g Saponin in 100 mL

PBS) for 1 hour, then incubated with primary antibodies to CDK5 and tubulin, and secondary fluorescent antibodies. Knockdown was determined by comparing fluorescence intensities using ImageJ.