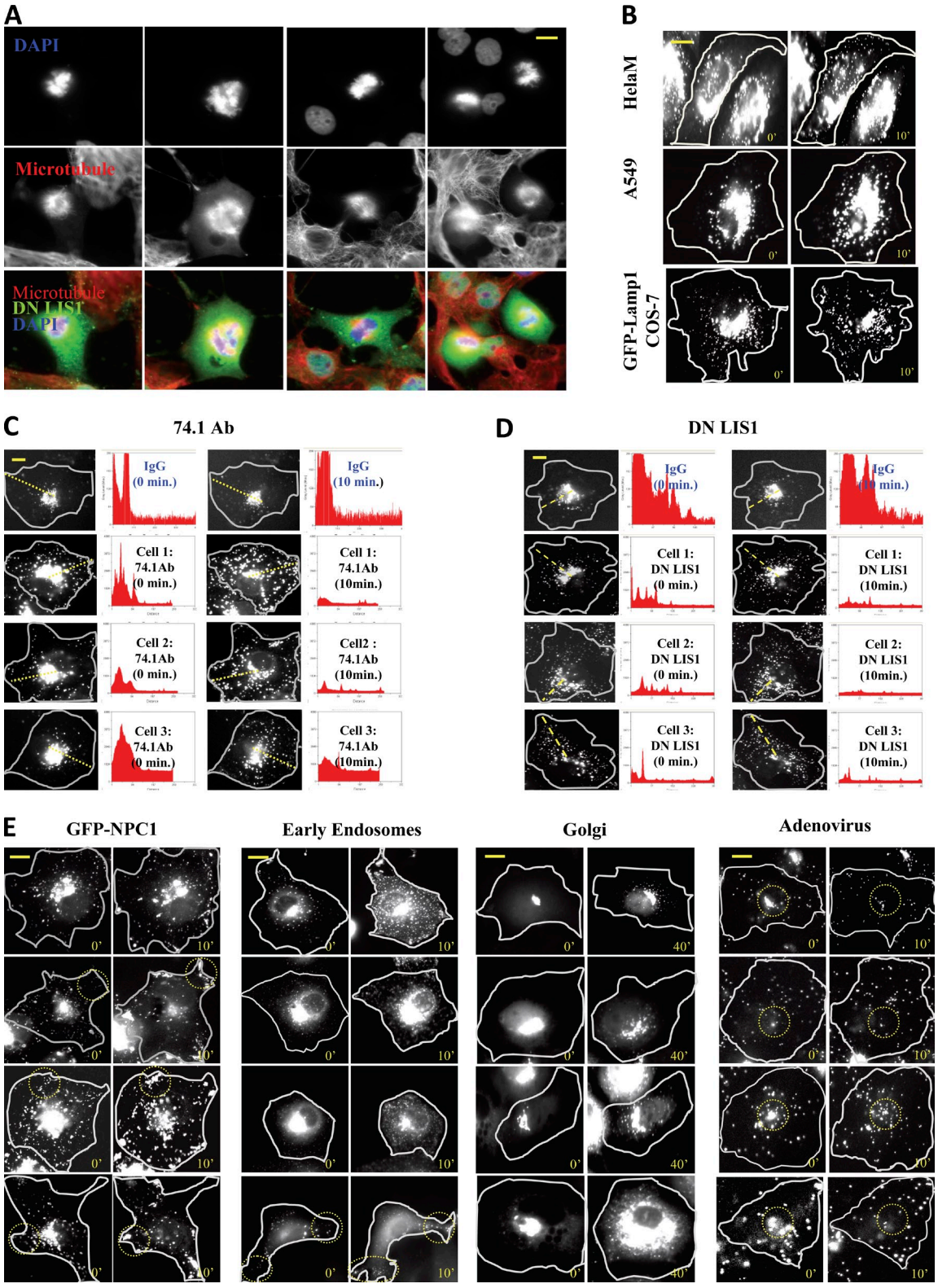
Yi et al., http://www.jcb.org/cgi/content/full/jcb.201104076/DC1



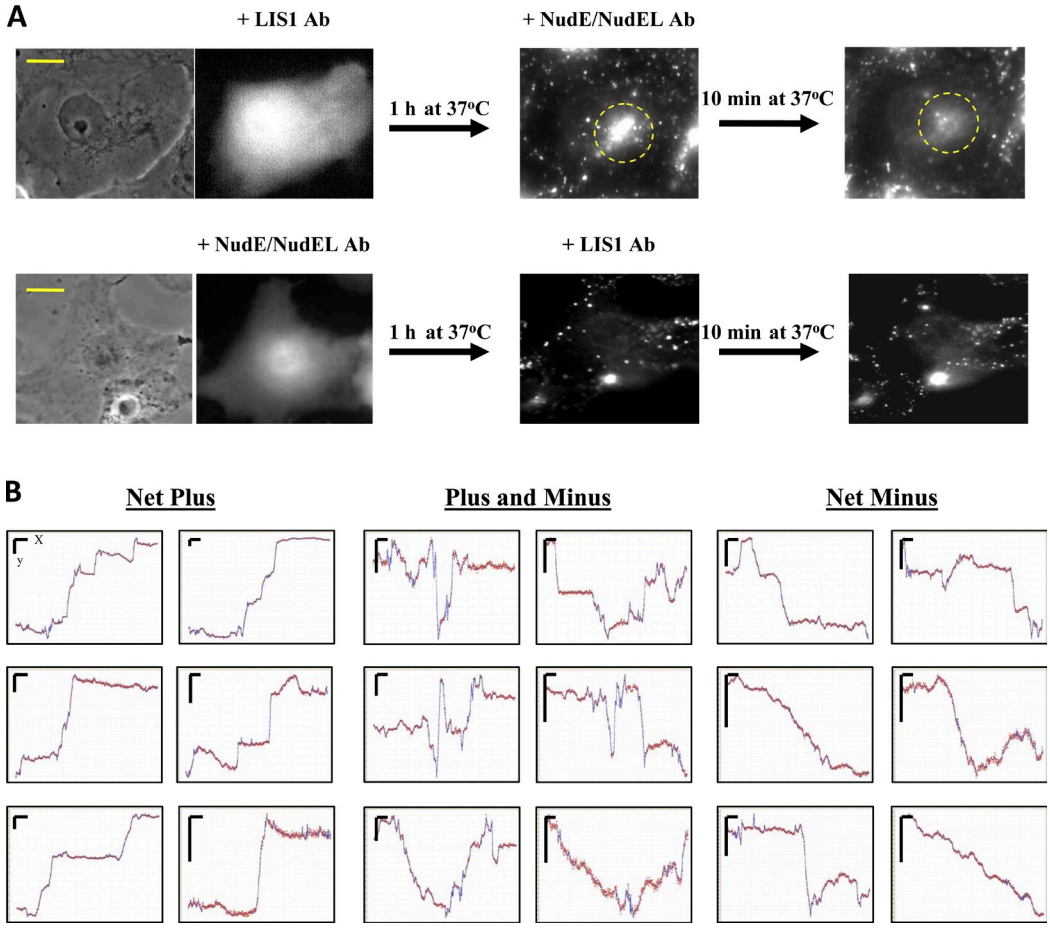


Figure S2. **Further characterization of acute dynein, LIS1, and NudE/L inhibition in nonneuronal cells.** (A) To test the effect of combined LIS1-NudE/L inhibition, COS-7 cells were sequentially injected with anti-LIS1 and anti-NudE/L antibodies or vice versa. NudE/L antibody dispersed lysosomes within 10 min regardless of prior LIS1 inhibition (see dashed circles). Bars, 10 µm. (B) Individual LysoTracker red tracks from a total of five different COS-7 cells made at 1 min after 74.1 injection showing examples of net plus-end–, net minus-end–, and plus- and minus-end–directed particles. Bars: (x) 5.0 s; (y) 1.0 µm.

Figure S1. **Characterization of acute dynein and LIS1 inhibition in nonneuronal cells.** (A) COS-7 cells were injected with DN LIS1 protein and incubated overnight at 37°C. 27 out of 35 cells were arrested in mitosis. (B) Rapid dispersal of lysos/LEs by 74.1 Ab injection in HeLa-M and A549 imaged using LysoTracker red and Lamp1-GFP expressing COS-7 cells. (C and D) Additional fluorescence images (Fig. 1) and linescans (along the dotted yellow straight line) of LysoTracker red–positive membranes in 74.1 Ab–injected (C) and DN LIS1–injected COS-7 (D) cells are shown. (E) Multiple examples (Fig. 1 B) of rapid dispersal of GFP-NPC1–labeled lysos/LEs, GFP-Rab5 early endosomes, GFP–*N*-acetylglucosaminyltransferase-labeled Golgi membranes, and Alexa Fluor 546–labeled adenovirus moved away from the microtubule-organizing center within 10 min of 74.1 Ab injection. Dotted circles denote dispersed particles. Bars, 10 µm.

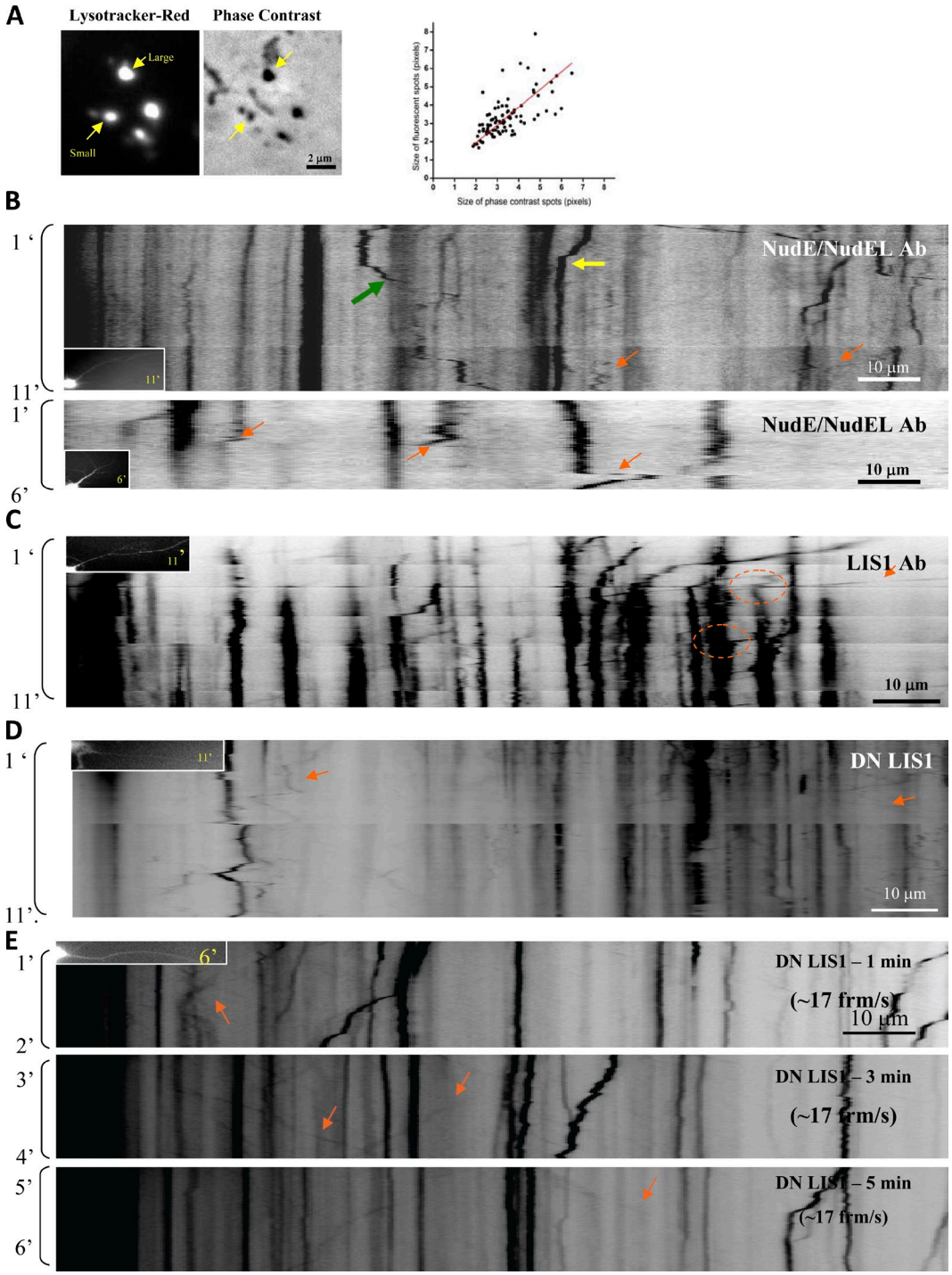
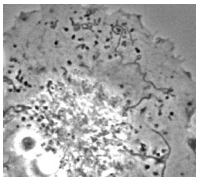
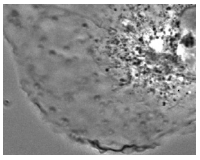


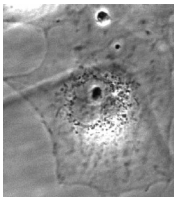
Figure S3. **Size dependence of lysosome transport in neurons.** (A, left) Selected field showing comparison of LysoTracker red fluorescence and phase-contrast images of lysos/LEs in COS-7 cells. (right) A correlation plot showing the linear relationship between imaging methods for lysos/LEs in primary rat cortical neurons. For analysis of Fig. 4, particles ≥1 µm in diameter (4 pixels) were scored as large. (B–F) Additional videos of antibody-injected axons of primary rat cortical neurons at 1 frame (frm)/2 s, except for E, which is at 17 frames/s. Time-dependent breaks in kymographs are a result from refocusing. Diffusion of the dextran marker is indicated in the insets. (B) Kymographs of two NudE/L antibody–injected neurons. Examples of arrest (yellow arrow), directional reversal (green arrow), and bidirectional movement (orange arrows) are shown. (C–E) Kymographs of acute LIS1-inhibited neurons showing severe inhibition of large versus small lysos/LEs (orange circles and arrows). Individual kymographs were taken at 1, 3, and 5 min after injection.



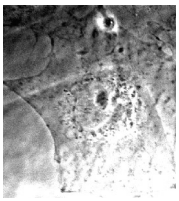
Video 1. **Live recording of lysos/LEs in COS-7 cells injected with 74.1 Ab.** The original recording was made at 1 frame/2 s using a 40× objective, but, to reduce the file size, the video is played for ~2 s for a 10-min elapsed time period.



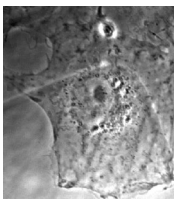
Video 2. **Live recording of lysos/LEs in COS-7 cells injected with IgG.** The original recording was made at 1 frame/2 s using a 40× objective, but, to reduce the file size, the video is played for ~2 s for a 10-min elapsed time period.



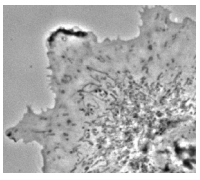
Video 3. **High-temporal resolution recording (~17 frames/s) of lysos/LEs in a COS-7 cell for a 1-min duration at 1 min after injection with 74.1 Ab.** The high-temporal resolution recording was used for motility analysis. The 1-min-duration video is played for 2 s.



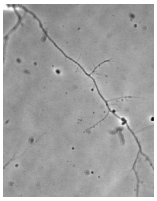
Video 4. **High-temporal resolution recording (~17 frames/s) of lysos/LEs in a COS-7 cell for a 1-min duration at 5 min after injection with 74.1 Ab.** The high-temporal resolution recording was used for motility analysis. The 1-min-duration video is played for 2 s.



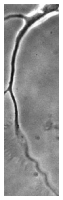
Video 5. **High-temporal resolution recording (~17 frames/s) of lysos/LEs in a COS-7 cell for a 1-min duration at 10 min after injection with 74.1 Ab.** The high-temporal resolution recording was used for motility analysis. The 1-min-duration video is played for 2 s.



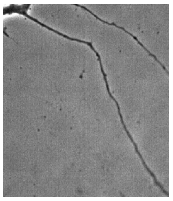
Video 6. **Live recording of lysos/LEs in COS-7 cells injected with DN LIS1 protein.** The original recording was made at 1 frame/2 s using a 40× objective, but, to reduce the file size, the video is played for ~2 s for a 10-min elapsed time period.



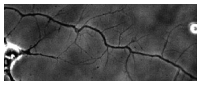
Video 7. **Live recording of lysos/LEs in uninjected rat cortical neurons.** The original recording was made at 1 frame/2 s. The final video was constructed from every fifth frame to match Video 9. The video is played for 2 s, representing a 10-min elapsed time period.



Video 8. **Live recording of lysos/LEs in 74.1 Ab–injected rat cortical neurons.** The original recording was made at 1 frame/2 s. The final video was constructed from every fifth frame to match Video 10. The video is played for 2 s, representing 10 min elapsed time.



Video 9. **Live recording of lysos/LEs in NudE/L antibody–injected rat cortical neurons.** The original recording was made at 1 frame/10 s. The video is played for 8 s, representing a 40-min elapsed time period.



Video 10. **Live recording of lysos/LEs in LIS1 antibody–injected rat cortical neurons.** The original recording was made at 1 frame/10 s. The video is played for 8 s, representing a 40-min elapsed time period.