Scherer et al., http://www.jcb.org/cgi/content/full/jcb.201307116/DC1

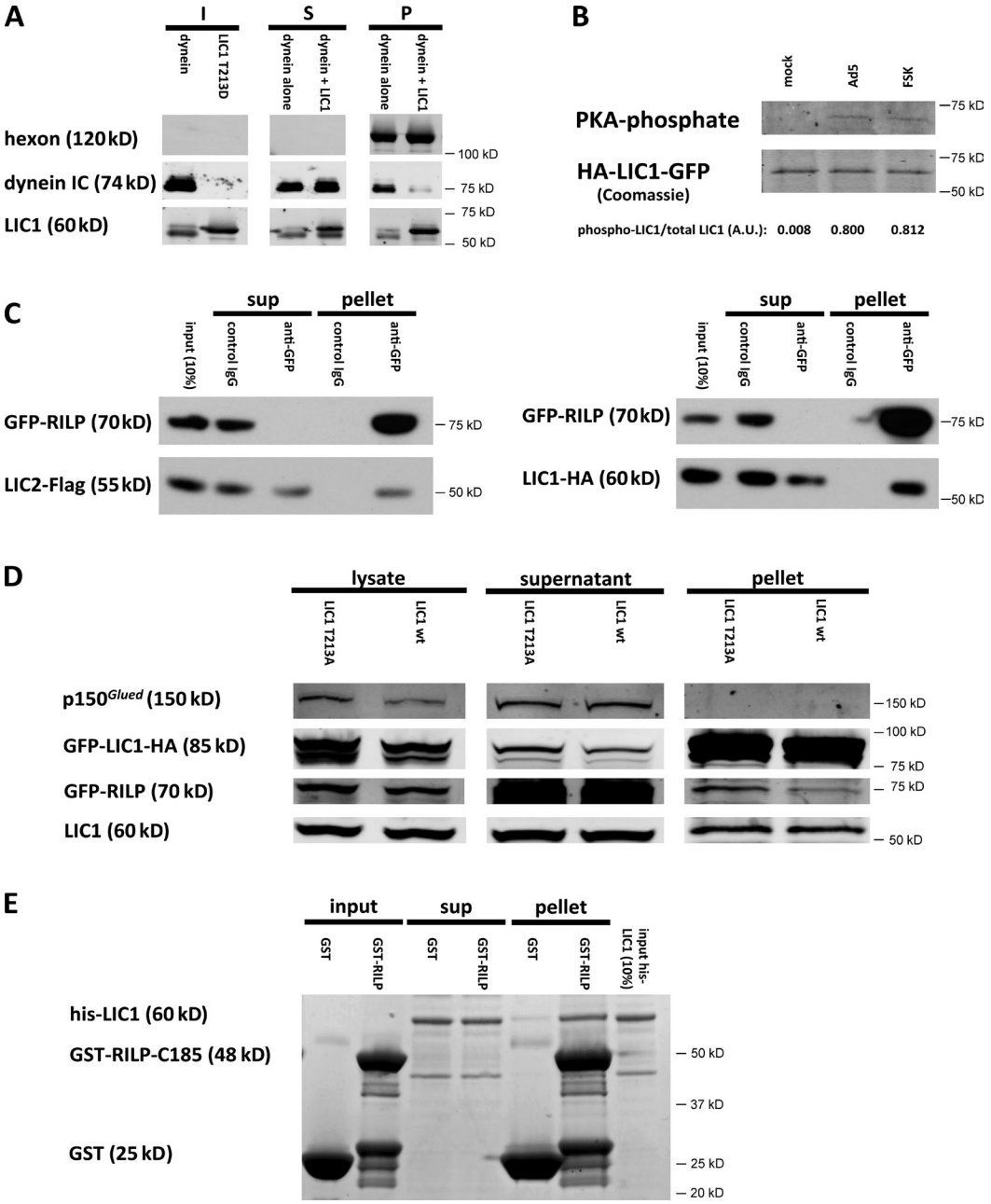


Figure S1. **Biochemical analysis of the dynein interactions with hexon and RILP.** (A) Competition of recombinant LIC1 for hexon binding by dynein complex. Purified rat brain cytoplasmic dynein was tested for pull-down with hexon in the presence of bacterially expressed LIC1-T213D by immunoblotting of the inputs (I), supernatants (S), and pellets (P) with antibodies against hexon and dynein IC and LIC1 subunits. LIC1-T213D strongly reduced sedimentable dynein, as indicated by a loss of IC signal, whereas LIC1-T213D can now be detected. (B) Dynein LIC1 becomes PKA phosphorylated during Ad5 infection. A549 cells were transfected with HA-LIC1-GFP wild type and 24 h later either mock or Ad5 infected or 20 µM forskolin (FSK) treated. Cell lysates were taken 30 min thereafter, overexpressed LIC1 was immunoprecipitated with an anti-HA mAb, and pellet samples were processed for SDS-PAGE and Coomassie staining or immunoblotting with PKA phosphate–specific antibody. The ratio of phospho-LIC1 over total immunoprecipitated LIC1 was determined to be ∼100-fold higher after Ad5 infection compared with mock control, an extent similar to forskolin treatment. A.U., arbitrary unit. (C) Both dynein LICs interact with RILP. GFP-RILP was coexpressed with LIC2-Flag (left) or LIC1-HA (right) in 293A cells and tested for coimmunoprecipitation with an anti-GFP antibody. LIC2-Flag and LIC1-HA coimmunoprecipitated with RILP. (D) The RILP–LIC interaction is independent of dynactin. Coimmunoprecipitations of GFP-RILP with coexpressed GFP-LIC1-HA wild type (wt) or T213A in 293A cell lysate. Anti-HA precipitates show increased binding of GFP-RILP to HA-tagged GFP-LIC1-T213A over GFP-LIC1 wild type. The dynactin subunit $p150^{Glued}$ was not detected in the LIC1 immunoprecipitations, suggesting that RILP coimmunoprecipitation did not involve dynactin. (E) Direct interaction of purified bacterially expressed proteins RILP N terminus (C185) and LIC1 by pull-down. His-tagged LIC1 was incubated with immobilized GST-RILP-C185 or GST alone. LIC1 was specifically pulled down by GST-RILP-C185 but not GST. Coomassie-stained SDS-PAGE gel. sup, supernatant.

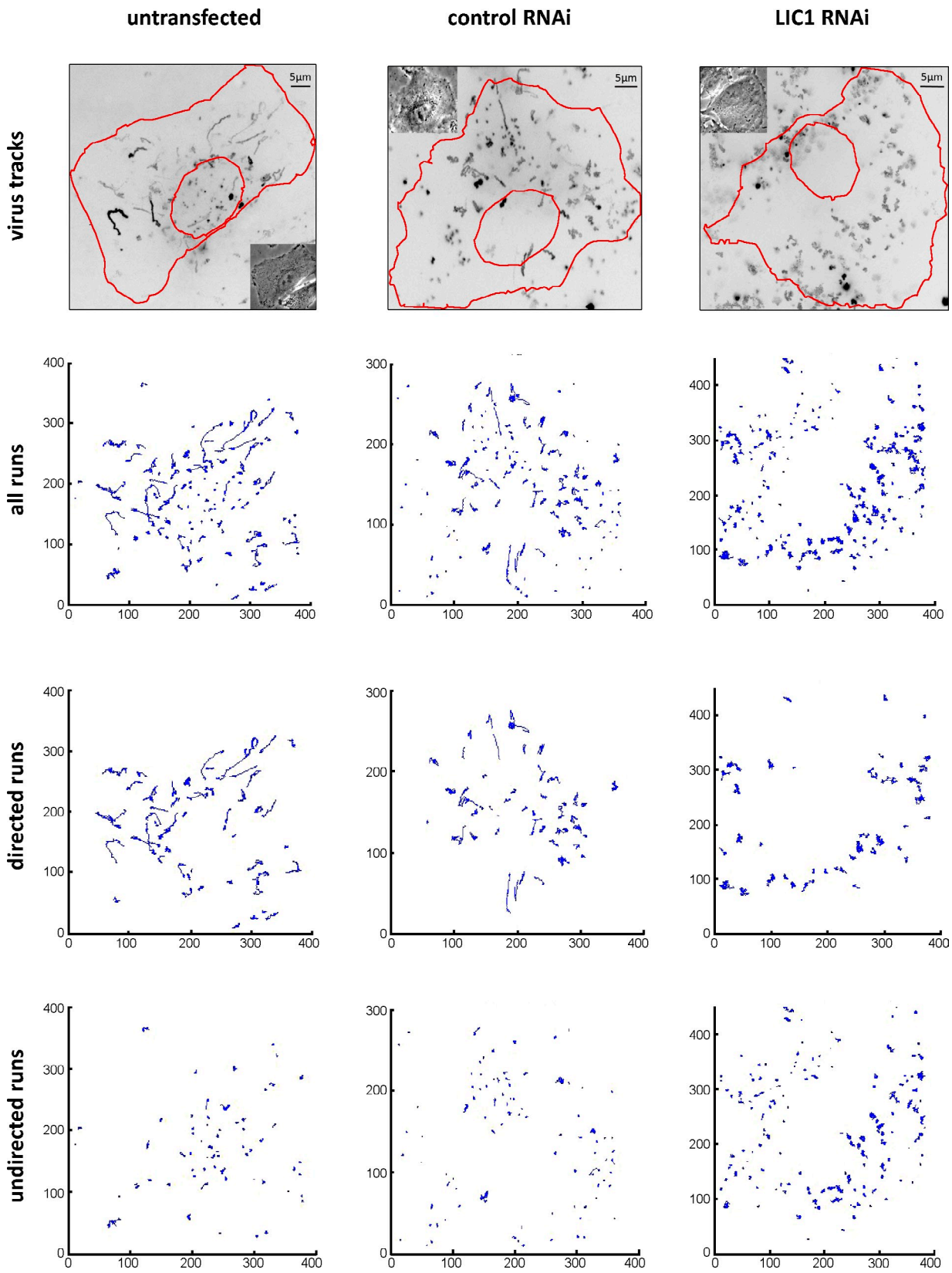


Figure S2. **Particle tracking of Ad5 capsids in A549 cells.** Cells were untransfected or transfected with control or LIC1 shRNA constructs, infected with Alexa Fluor 546–Ad5 capsids 48 h later, and processed for image acquisition beginning at between 15 and 45 min p.i. at ∼17 Hz (see Materials and methods). Top images (virus tracks) show time stacks illustrating movements of individual capsids over a 30-s period. (Outlines of cell and nucleus are shown in red, additional micrographs in corners show transmitted light images of the cells at reduced size). Second row (all runs) represents all tracks recognized by the automated tracking software (Bremner et al., 2009) containing directed (third row) and undirected (bottom row) runs. Only directed runs were analyzed further to extract capsid run length for Fig. 2 F. Figure corresponds to Videos 1, 2, and 3.

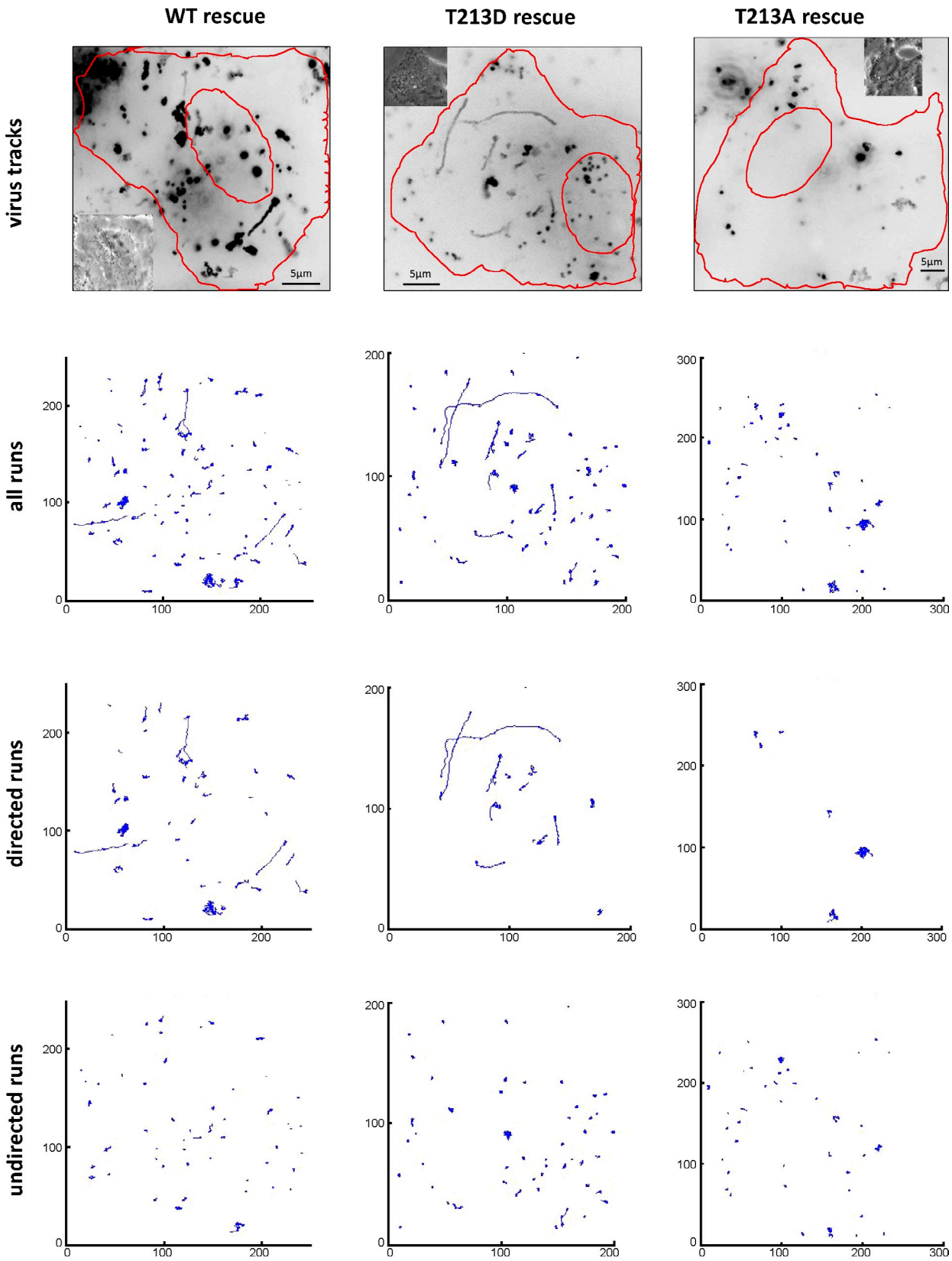


Figure S3. **Particle tracking of Ad5 capsids in A549 cells.** Same as Fig. S2 except cells were transfected with LIC1 siRNA and rescue constructs LIC1 wild type (WT), -T213D, and -T213D, and run lengths were extracted for Fig. 3 C. Figure corresponds to Videos 4, 5, and 6.

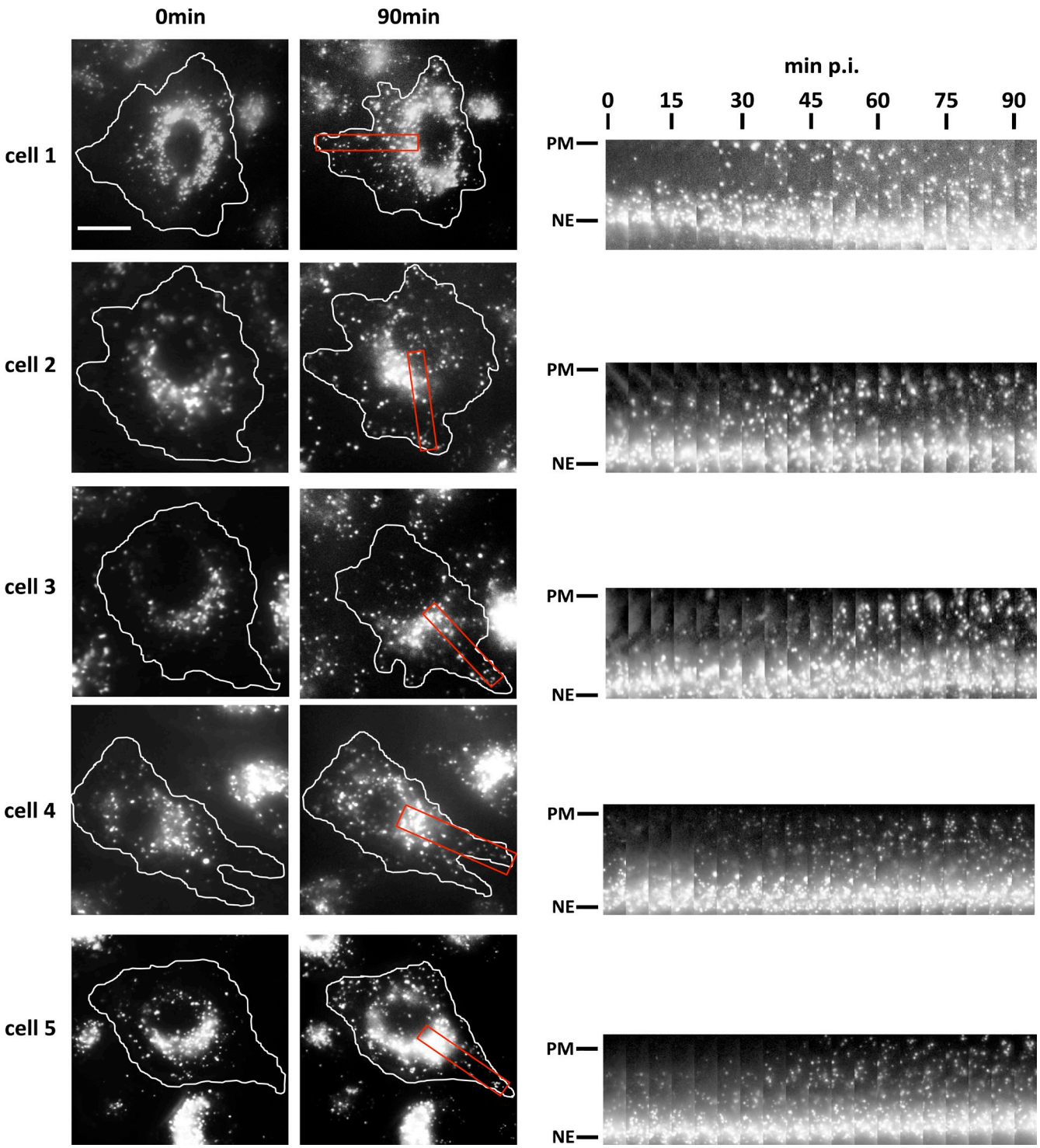


Figure S4. **Lysosome dispersal induced by Ad5 infection.** Still images from Ad5-infected LysoTracker-treated A549 cells at 0 and 90 min p.i. showing initial absence of lyso/LEs in peripheral region of the cell and subsequent invasion of this region by the lyso/LEs. Boxed regions were monitored in detail and are shown as kymographs on the right (frames every 5 min), again revealing clear migration of lyso/LEs into the peripheral region of the cell. Lyso/LEs beyond cell boundary lines (white lines) are in adjacent cells and in several cases approach the circumscribed cell by the end of the video. Cell 1 corresponds to Video 10 (first part), and cells 2 and 3 correspond to Video 10 (second part). NE, nuclear envelope; PM, plasma membrane. Bar, 10 µm.

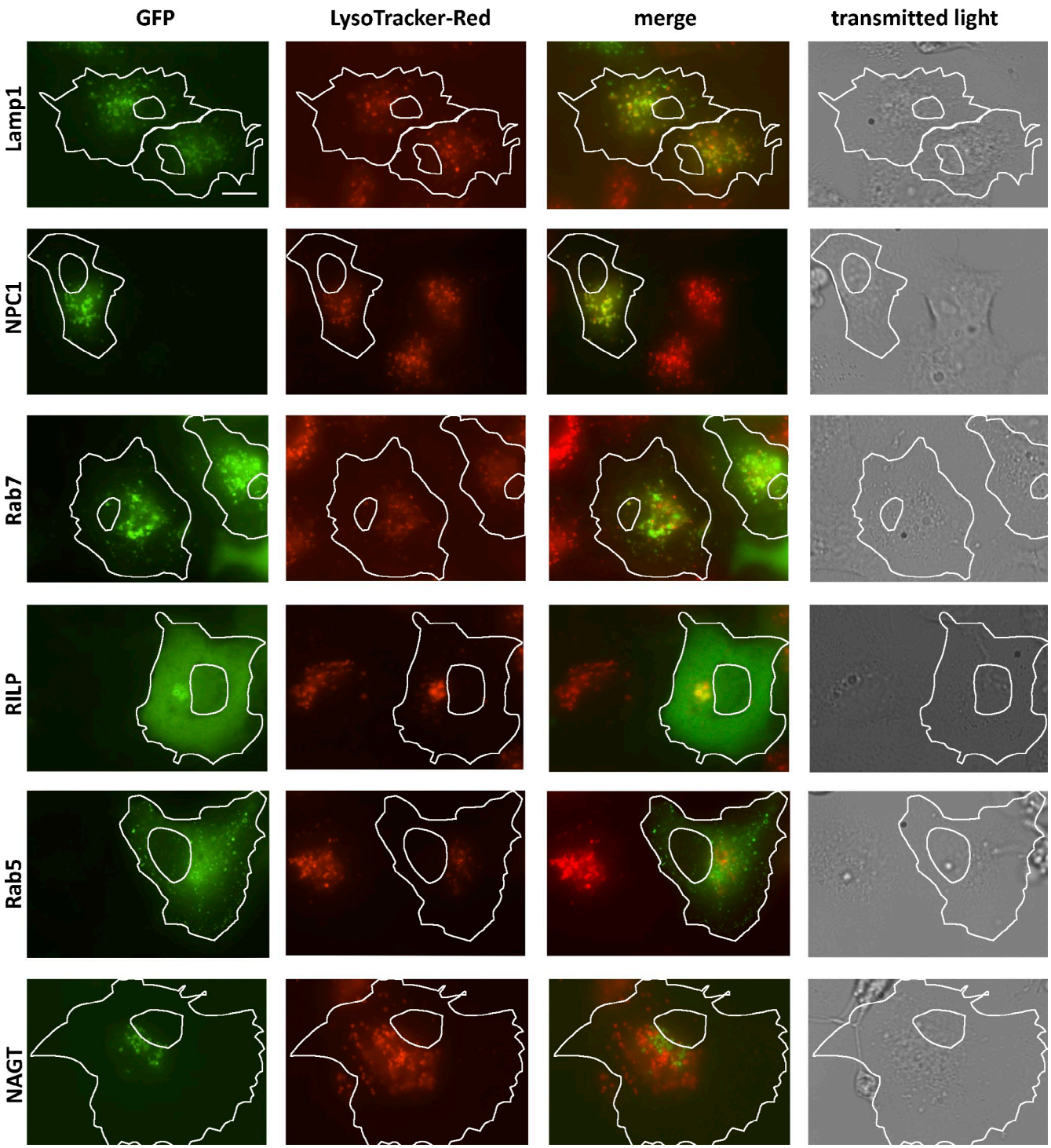
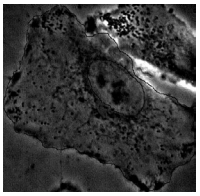
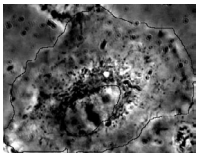


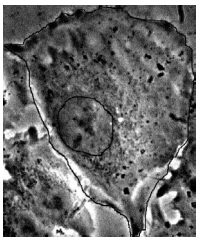
Figure S5. **LysoTracker red colocalization with vesicular markers.** Still images of live A549 cells incubated with LysoTracker red and GFP-tagged vesicular markers for lyso/LEs (GFP-Lamp1, GFP-NPC1, and GFP-Rab7), RILP (GFP-RILP), early endosomes (GFP-Rab5), and Golgi elements (GFP-NAGT). LysoTracker red–positive particles highly colocalize with lyso/LE markers Lamp1 (92 ± 12.6% [mean± SD]; eight cells), NPC1 (93 ± 11.6%; six cells), Rab7 (97 ± 6.1%; 10 cells), and RILP (close to 100%, but strong organelle aggregation obscures clear counts; 14 cells) but do not colocalize with GFP-Rab5 (22 ± 21.3%; 13 cells) and GFP-NAGT (12 ± 18.9%; 10 cells). White lines depict cell outlines. Bar, 10 µm.



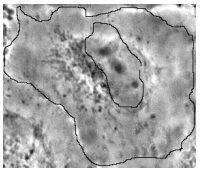
Video 1. **Alexa Fluor 546–Ad5 motility in untreated cells.** A549 cells were infected with Alexa Fluor 546–labeled Ad5 at 10 MOI and imaged at 37 min p.i. at 17-Hz frame rate for 30 s using an epifluorescence microscope (DM IRBE; Leica). Playback is 10× speed of real time. First frame shows transmitted light channel; all subsequent frames show Alexa Fluor 546–Ad5 (100 frames, every fifth frame of the original acquisition). Many capsids are mobile during this period, with some showing long-range bidirectional movement. Time stamp indicates seconds after start of the video. Cell corresponds to Fig. S2 (untransfected). Black lines depicted cell outlines.



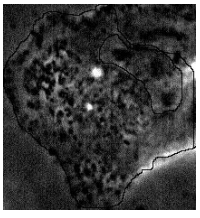
Video 2. **Alexa Fluor 546–Ad5 motility in control siRNA-treated cells.** A549 cells were infected with Alexa Fluor 546–labeled Ad5 at 10 MOI and imaged at 37 min p.i. at 17-Hz frame rate for 30 s using an epifluorescence microscope (DM IRBE; Leica). Playback is 10× speed of real time. First frame shows transmitted light channel; all subsequent frames show Alexa Fluor 546–Ad5 (100 frames, every fifth frame of the original acquisition). Cells were transfected with control siRNA and imaged 48 h later. Note the long-range motility of viral capsids especially in the top part of the cell. Video was taken at 28 min p.i. Time stamp indicates seconds after start of the video. Cell corresponds to Fig. S2 (control RNAi). Black lines depicted cell outlines.



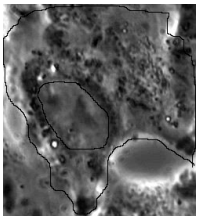
Video 3. **Alexa Fluor 546–Ad5 motility in LIC1 siRNA-treated cells.** A549 cells were infected with Alexa Fluor 546–labeled Ad5 at 10 MOI and imaged at 37 min p.i. at 17-Hz frame rate for 30 s using an epifluorescence microscope (DM IRBE; Leica). Playback is 10× speed of real time. First frame shows transmitted light channel; all subsequent frames show Alexa Fluor 546–Ad5 (100 frames, every fifth frame of the original acquisition). Cells were transfected with LIC1 siRNA. Note the drastically changed motility pattern of the capsids with very short bidirectional motions, which do not allow persistent movement toward the nucleus. Video was taken at 30 min p.i. Time stamp indicates seconds after start of the video. Cell corresponds to Fig. S2 (LIC1 RNAi). Black lines depicted cell outlines.



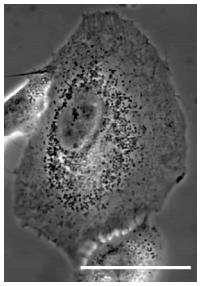
Video 4. **Alexa Fluor 546–Ad5 motility in LIC1 wild-type rescue cells.** A549 cells were infected with Alexa Fluor 546–labeled Ad5 at 10 MOI and imaged at 37 min p.i. at 17-Hz frame rate for 30 s using an epifluorescence microscope (DM IRBE; Leica). Playback is 10× speed of real time. First frame shows transmitted light channel; all subsequent frames show Alexa Fluor 546–Ad5 (100 frames, every fifth frame of the original acquisition). Cells were transfected with LIC1 siRNA 48 h before imaging and transfected with LIC1 wild-type rescue constructs 24 h before imaging. First frame shows the transmitted light channel, second frame shows GFP channel, and all subsequent frames show Alexa Fluor 546–Ad5. Note the rescue of long-range motility of several virus capsids. Video was taken at 44 min p.i. Time stamp indicates seconds after start of the video. Cell corresponds to Fig. S3 (WT rescue). Black lines depicted cell outlines.



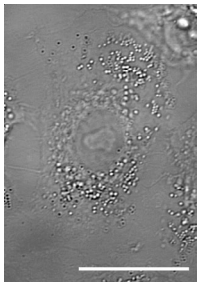
Video 5. **Alexa Fluor 546–Ad5 motility in LIC1-T213D rescue cells.** A549 cells were infected with Alexa Fluor 546–labeled Ad5 at 10 MOI and imaged at 37 min p.i. at 17-Hz frame rate for 30 s using an epifluorescence microscope (DM IRBE; Leica). Playback is 10× speed of real time. First frame shows transmitted light channel; all subsequent frames show Alexa Fluor 546–Ad5 (100 frames, every fifth frame of the original acquisition). Cells were transfected with LIC1 siRNA 48 h before imaging and transfected with LIC1-T213D rescue constructs 24 h before imaging. Long-range motility is restored for several capsids, indicating rescue of LIC1 RNAi. Video was taken at 45 min p.i. Time stamp indicates seconds after start of the video. Cell corresponds to Fig. S3 (T213D rescue). Black lines depicted cell outlines.



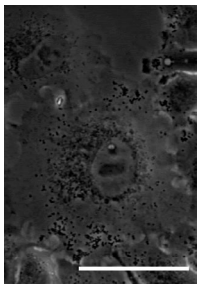
Video 6. **Alexa Fluor 546–Ad5 motility in LIC1-T213A rescue cells.** A549 cells were infected with Alexa Fluor 546–labeled Ad5 at 10 MOI and imaged at 37 min p.i. at 17-Hz frame rate for 30 s using an epifluorescence microscope (DM IRBE; Leica). Playback is 10× speed of real time. First frame shows transmitted light channel; all subsequent frames show Alexa Fluor 546–Ad5 (100 frames, every fifth frame of the original acquisition). Cells were transfected with LIC1 siRNA 48 h before imaging and transfected with LIC1-T213A rescue constructs 24 h before imaging. No rescue of LIC1 RNAi occurs because capsids fail to regain long-range motility patterns. Video was taken at 37 min p.i. Time stamp indicates seconds after start of the video. Cell corresponds to Fig. S3 (T213A rescue). Black lines depicted cell outlines.



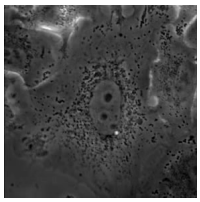
Video 7. **Lysosome motility in an uninfected A549 cell.** Cells were preincubated with LysoTracker green dye to label lyso/LE before being processed for imaging at 2 frames/min for 90 min using an epifluorescence microscope (DM IRBE; Leica). Playback is 450× speed of real time. First frame shows transmitted light image of the cell, and subsequent frames show LysoTracker signal (180 frames, all frames of the original acquisition). Time stamp indicates hours, minutes, and seconds after start of the video. Cell corresponds to Fig. 4 A (control). Bar, 20 μm.



Video 8. **Lysosome motility in Ad5-infected A549 cells.** Cells were preincubated with LysoTracker green dye to label lyso/LE before being processed for imaging at 2 frames/min for 90 min using an epifluorescence microscope (DM IRBE; Leica). Playback is 450× speed of real time. First frame shows a transmitted light image of the cell, and subsequent frames show LysoTracker signal (180 frames, all frames of the original acquisition). Cells were infected with 100 MOI Ad5 shortly before imaging (Materials and methods). Note the outward movement of lyso/LEs starting at around 30 min, leading to complete dispersal at around 60 min with LysoTracker signal in the cell periphery. Time stamp indicates hours, minutes, and seconds after start of the video. Cell corresponds to Fig. 4 A (Ad5). Bar, 20 μm.



Video 9. **Lysosome motility in Ad5-infected A549 cells treated with PKA inhibitor.** Cells were preincubated with LysoTracker green dye to label lyso/LE before being processed for imaging at 2 frames/min for 90 min using an epifluorescence microscope (DM IRBE; Leica). Playback is 450× speed of real time. First frame shows a transmitted light image of the cell, and subsequent frames show LysoTracker signal (180 frames, all frames of the original acquisition). Cells were infected with 100 MOI Ad5 shortly before imaging (Materials and methods) and pretreated with the PKA inhibitor H-89 (5 μM), leading to inhibition of Ad5-induced lyso/LE dispersal. Time stamp indicates hours, minutes, and seconds after start of the video. Cell corresponds to Fig. 4 A (Ad5 + H-89). Bar, 20 μm.



Video 10. **Lysosome motility in Ad5-infected A549 cells.** Same as in Video 8, showing additional examples. Cells were preincubated with LysoTracker green dye to label lyso/LE before being processed for imaging at 2 frames/min for 90 min using an epifluorescence microscope (DM IRBE; Leica). Playback is 540× speed of real time. First frame shows a transmitted light image of the cell, and subsequent frames show LysoTracker signal (180 frames, all frames of the original acquisition). Cells were infected with 100 MOI Ad5 shortly before imaging (Materials and methods). Time stamp indicates hours, minutes, and seconds after start of the video. The first part of this video corresponds to Fig. S4 (cell 1), and the cells in the center of the second half of this video correspond to Fig. S4 (cell 2 and cell 3).

Table S1. **Summary of statistical parameters of live cell imaging data**

Condition	Number of cells	Number of particles	Moving particles	Number of particles for run length analysis	Number of runs
			%		
Untransfected	11	1,151	35.0 ± 8.65	487	3,319
Control siRNA	28	1,859	45.3 ± 16.95	712	5,708
LIC1 siRNA	31	1,573	35.7 ± 10.36	505	16,869
LIC1 wild-type rescue	12	1,256	31.4 ± 15.53	278	3,365
LIC1-T213A rescue	8	746	22.1 ± 12.97	146	1,761
LIC1-T213D rescue	13	974	23.4 ± 8.73	211	2,682

Virus particles were tracked in infected A549 cells with the same algorithm described in Bremner et al. (2009), and the number of cells, particles, moving particles, and particles for run length analysis and of runs are indicated. Not all moving particles were used for run length analysis because of additional filtering (e.g., directionality).

Reference

Bremner, K.H., J. Scherer, J. Yi, M. Vershinin, S.P. Gross, and R.B. Vallee. 2009. Adenovirus transport via direct interaction of cytoplasmic dynein with the viral capsid hexon subunit. *Cell Host Microbe*. 6:523–535. http://dx.doi.org/10.1016/j.chom.2009.11.006