SUPPLEMENTAL MATERIALS

Supplemental Figure legends

Supplemental Figure 1. Cos7 cells were co-transfected with dynactin subunit siRNAs plus pCAGIG (Control; left) or pCAGIG-p27 (p27; right) and harvested after 72 hours. Detergent lysates were analyzed by immunoblotting.

Supplemental Figure 2. siRNA-treated Cos7 cells were immunostained to evaluate the steady state distributions of the Golgi complex (giantin), LAMP1 positive compartments and transferrin receptor (TfR). (A) Representative images. Bar = 5 μm. (B) Quantification of cells exhibiting normal compartment morphology and distribution (mean ± SD).

Supplemental Figure 3. (A) Cos7 cells were transfected with expression constructs encoding GFP-p62 or GFP-Arp 11 (AA 23-418), then fixed and stained with antibodies to γ-tubulin or DIC to determine cell cycle stage (see (Quintyne and Schroer, 2002)). Cells were categorized as follows: those with one γ-tubulin focus or no detectable centrosomal spot of DIC were defined as being in G_1/early S and those with two γ-tubulin foci or a centrosomal spot of DIC were defined as being in late S/G_2. The two populations then were scored for GFP-p62 or -Arp11 on their nuclear envelopes. The percent of cells in which endogenous DIC (green bars) localizes to the nuclear envelope is provided for comparison (from Figure 1B of (Quintyne and Schroer, 2002): G_1/early S corresponds to cells scored at the end of the double thymidine block or 15 h after release; late S/G_2 corresponds to cells 10 h after release). (B) siRNA-treated Cos-7 cells were stained with Abs to phospho-histone 3 plus Arp1, $p150^{Glued}$ or DIC. Cells with homogenous nuclear labeling of phospho-histone 3 (i.e., prior to chromosome condensation) were chosen as the prophase population and their nuclear envelopes were outlined on the basis of DAPI staining. The fluorescence intensity of Arp1, $p150^{Glued}$ or DIC in a 1 μm line drawn perpendicular to the nuclear envelope was quantified, normalized to phospho-histone 3 pixel values, and expressed as percent of control (mean

± SD; n = 50 nuclear envelopes per condition). * indicates values that are significantly different from controls ($p < 0.05$).

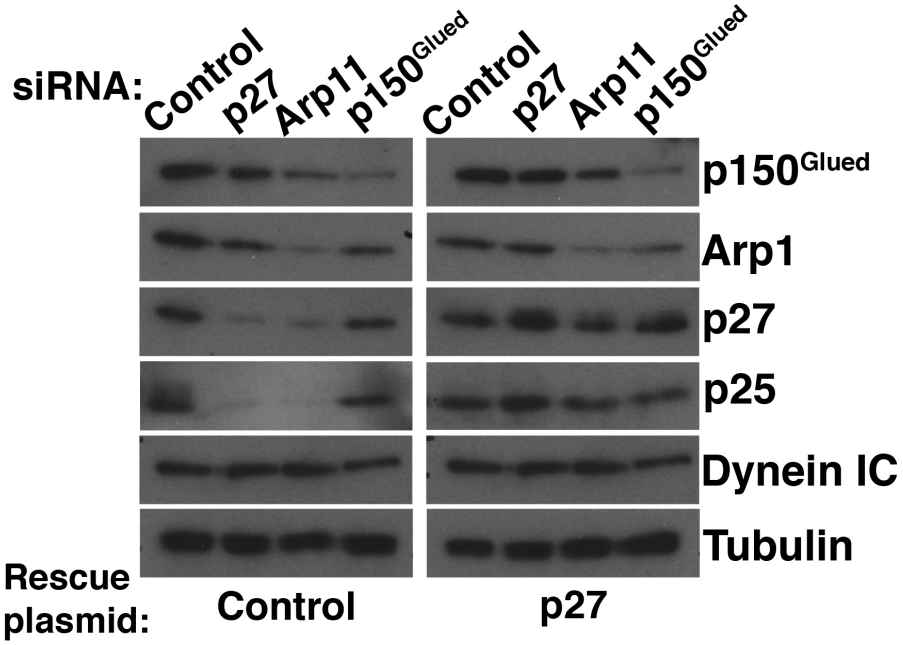
Supplemental Figure 4. (A) Representative images of Cos7 cells cotransfected with GFP-p62 and control (Ctrl) or Arp11 siRNA, then fixed and stained for Arp1 (red). (B) Representative images of Cos7 cells cotransfected with GFP-Arp11 (full length) and control or p62 siRNA, then fixed and stained for Arp1. Bar = 5 μm.

Movies 1-4. Representative time-lapse movies illustrating the movement of GFP-EEA1 labeled structures (as in Figure 4C). Cos7 cells were cotransfected with (1) control, (2) p27, (3) Arp11, or (4) $p150^{Glued}$ siRNA. Movies were made in ImageJ with Sorensen normal compression. Playback is set at 10X real time.

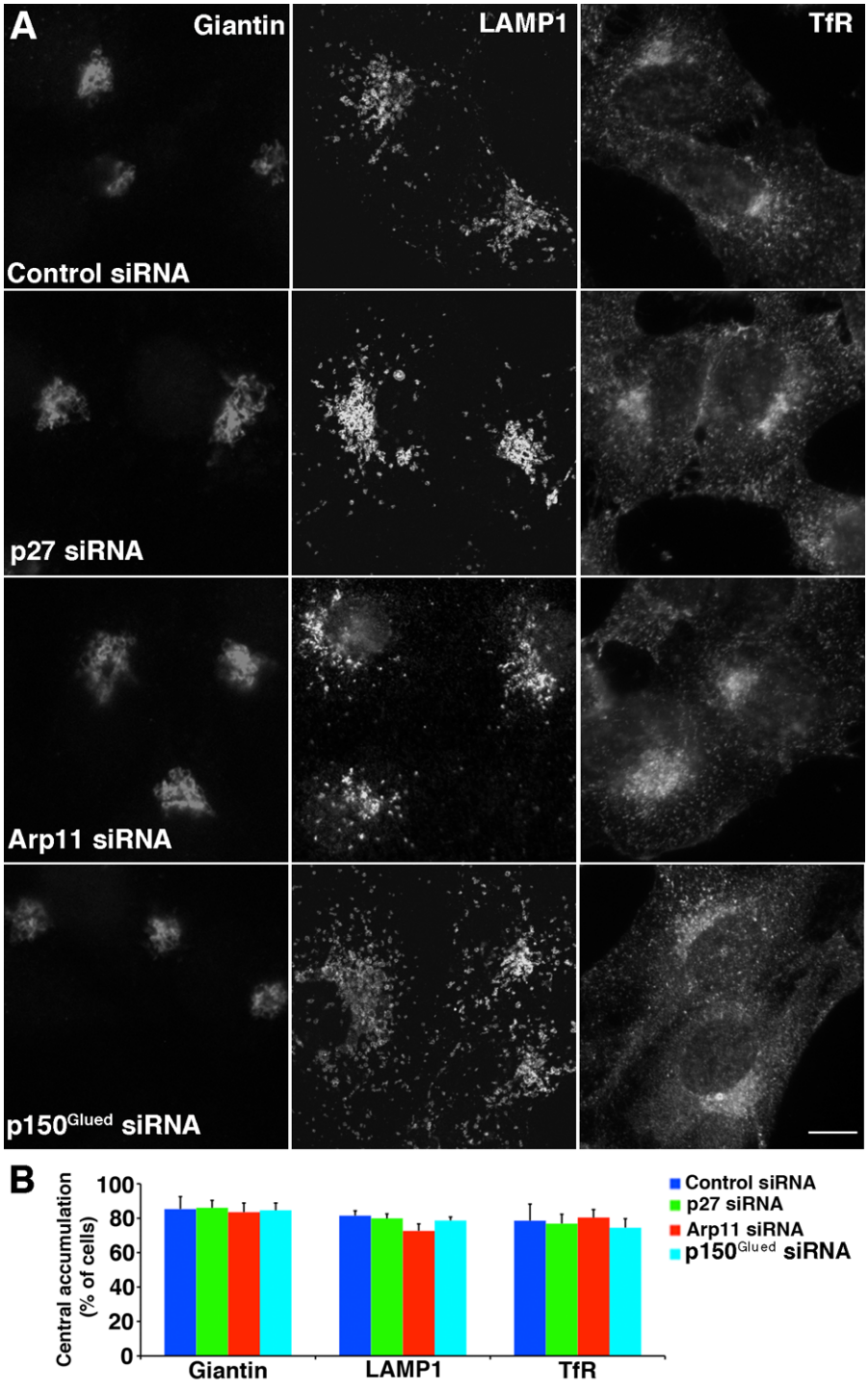
Movies 5-8. Representative time-lapse movies illustrating the movement of recycling endosome (as in Figure 5E). Cos7 cells transfected with (5) control, (6) p27, (7) Arp11, or (8) $p150^{Glued}$ siRNA were labeled with Alexa 555-Tfn for 2 min, then imaged during a 10 to 15 min chase interval. Movies were made in ImageJ with Sorensen normal compression. Playback is set at 10X real time.

Movies 9-12. Representative time-lapse movies illustrating the movement of late endosomes (as in Figure 6C). Cos7 cells transfected with (9) control, (10) p27, (11) Arp11, or (12) $p150^{Glued}$ siRNA were labeled with Alexa 555-α_2M for 30 min, then imaged during a 90 – 120 min chase interval. Movies were made in ImageJ with Sorensen normal compression. Playback is set at 6X real time.

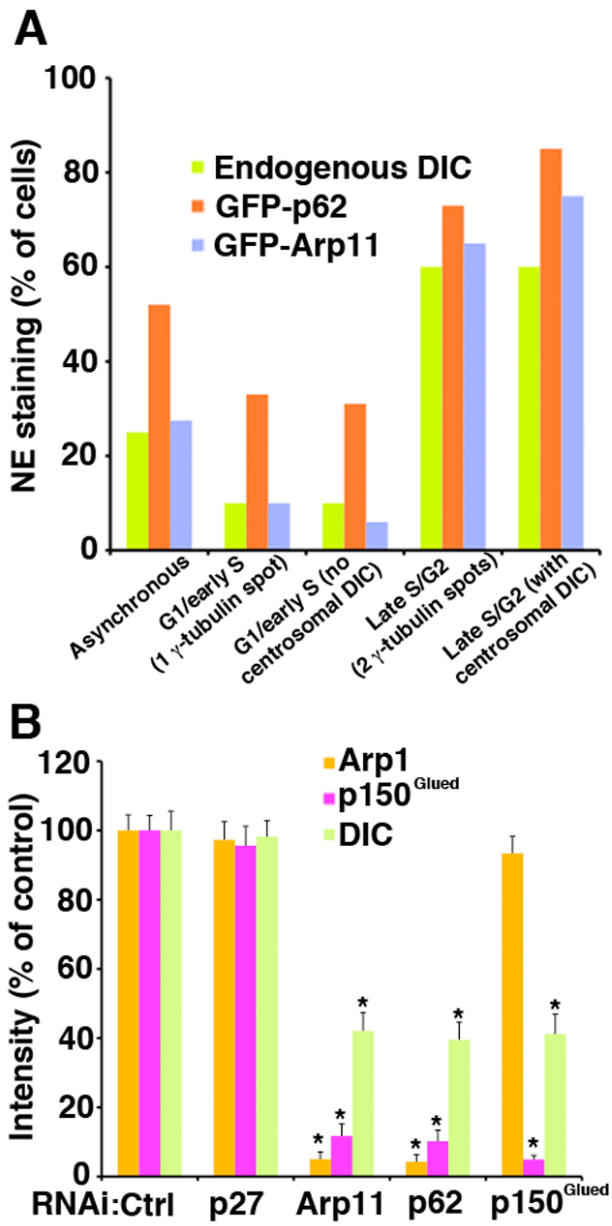
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4