Allison et al., http://www.jcb.org/cgi/content/full/jcb.201211045/DC1

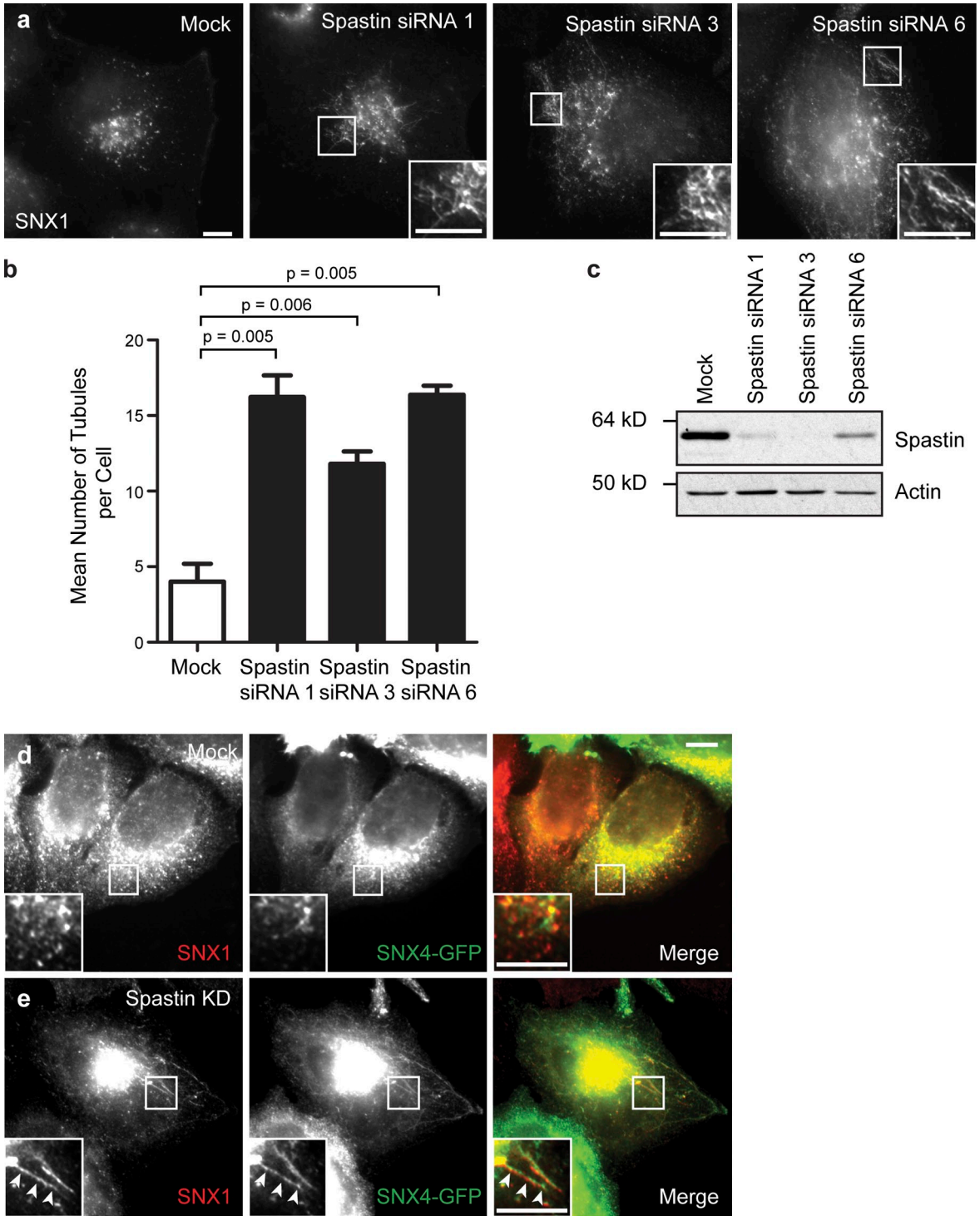


Figure S1. **Spastin depletion causes increased endosomal tubulation.** (a and b) HeLa cells were subjected to mock transfection or transfected with one of the three indicated siRNAs targeting spastin mRNA. The cells were then labeled for endogenous SNX1, and the mean number of SNX1 tubules per cell was quantified (b; *n* = 3 experiments, 30 cells per condition counted in each experiment). (c) Spastin depletion was confirmed by immunoblotting. (d and e) Mock-transfected HeLa cells (d) or cells depleted of spastin by transfection with an siRNA pool (e) were transfected with SNX4-GFP and labeled for endogenous SNX1. The arrowheads in the magnified insets in e indicate colocalization of the two sorting nexin proteins on tubules induced by spastin depletion. Insets are magnifications of boxed regions. Bars, 10 µm. Error bars show SEMs.

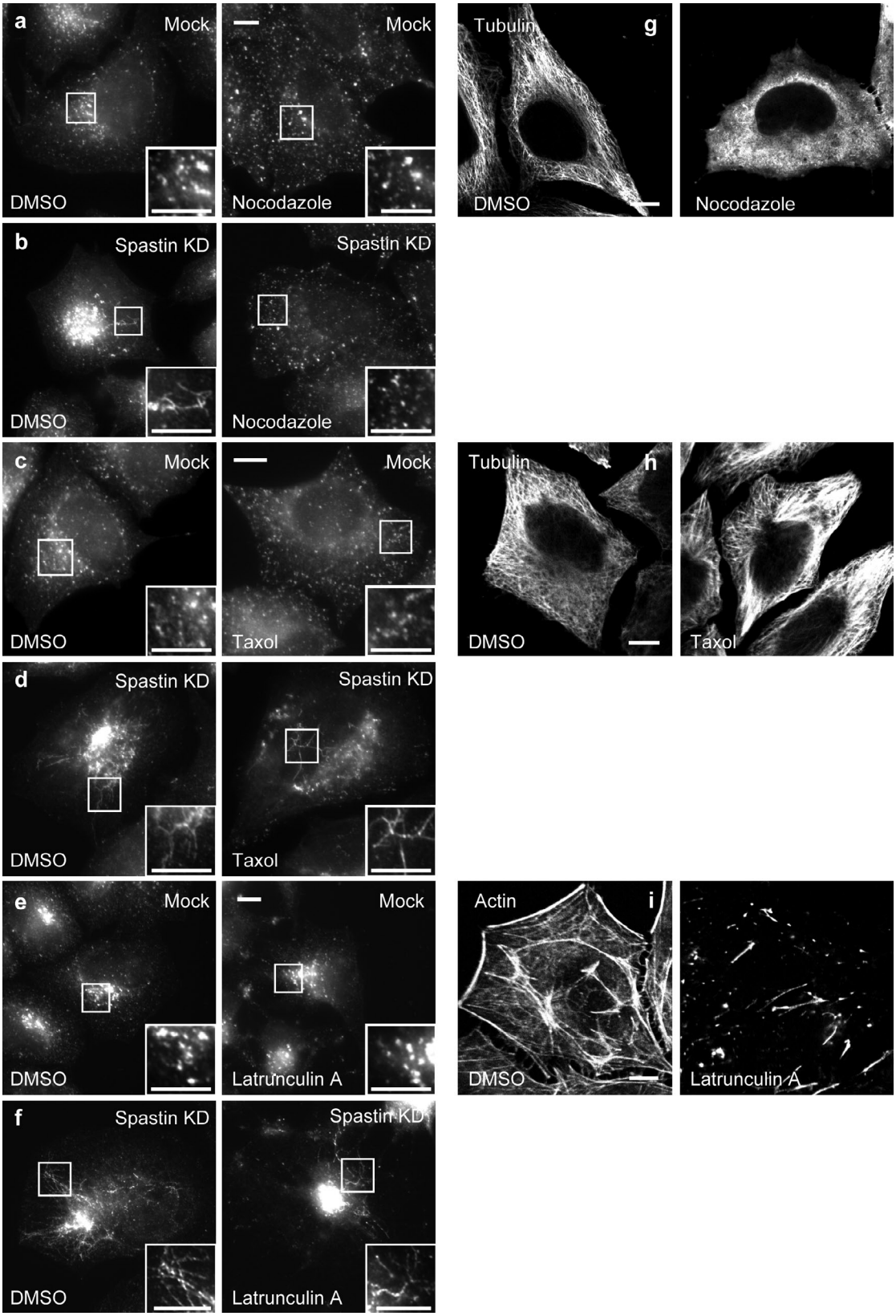


Figure S2. **Endosomal tubulation in spastin-depleted cells requires intact MTs.** (a–i) Mock-transfected cells (a, c, e, and g–i) or cells depleted of spastin by transfection with an siRNA pool (b, d, and f) were treated with vehicle (DMSO) or with nocodazole, taxol, or latrunculin A, as indicated. The cells were then labeled for endogenous SNX1 (a–f) and, to confirm the effect of the drugs, α-tubulin (g and h) or actin (with phalloidin; i). The corresponding SNX1 tubule counts are shown in Fig. 2. KD, knockdown. Insets are magnifications of boxed regions. Bars, 10 µm.

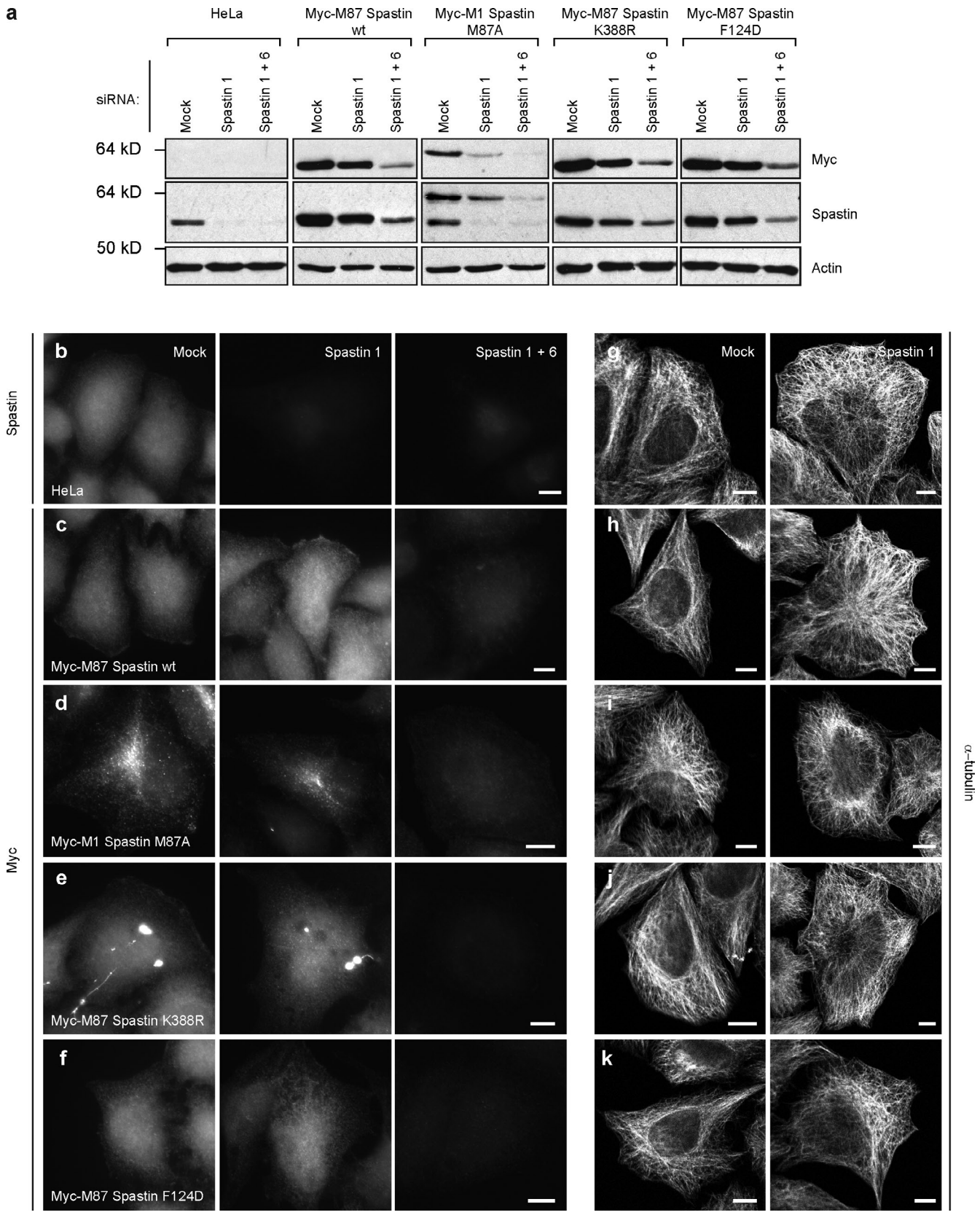


Figure S3. **Depletion of endogenous and exogenous spastin in cell lines expressing siRNA-resistant spastin proteins.** (a–f) Depletion of endogenous or exogenous spastin in the experiments shown in Figs. 3 and 4 was verified by immunoblotting with the antibodies shown (a) and at the individual cell level by immunofluorescence labeling with the anti-spastin (b) or anti-myc antibody (c–f). (g–k) Representative images showing the appearance of the MT cytoskeleton in each cell line, with and without endogenous spastin depletion, are shown, in which cells have been labeled with α-tubulin. wt, wild type. Bars, 10 μm.

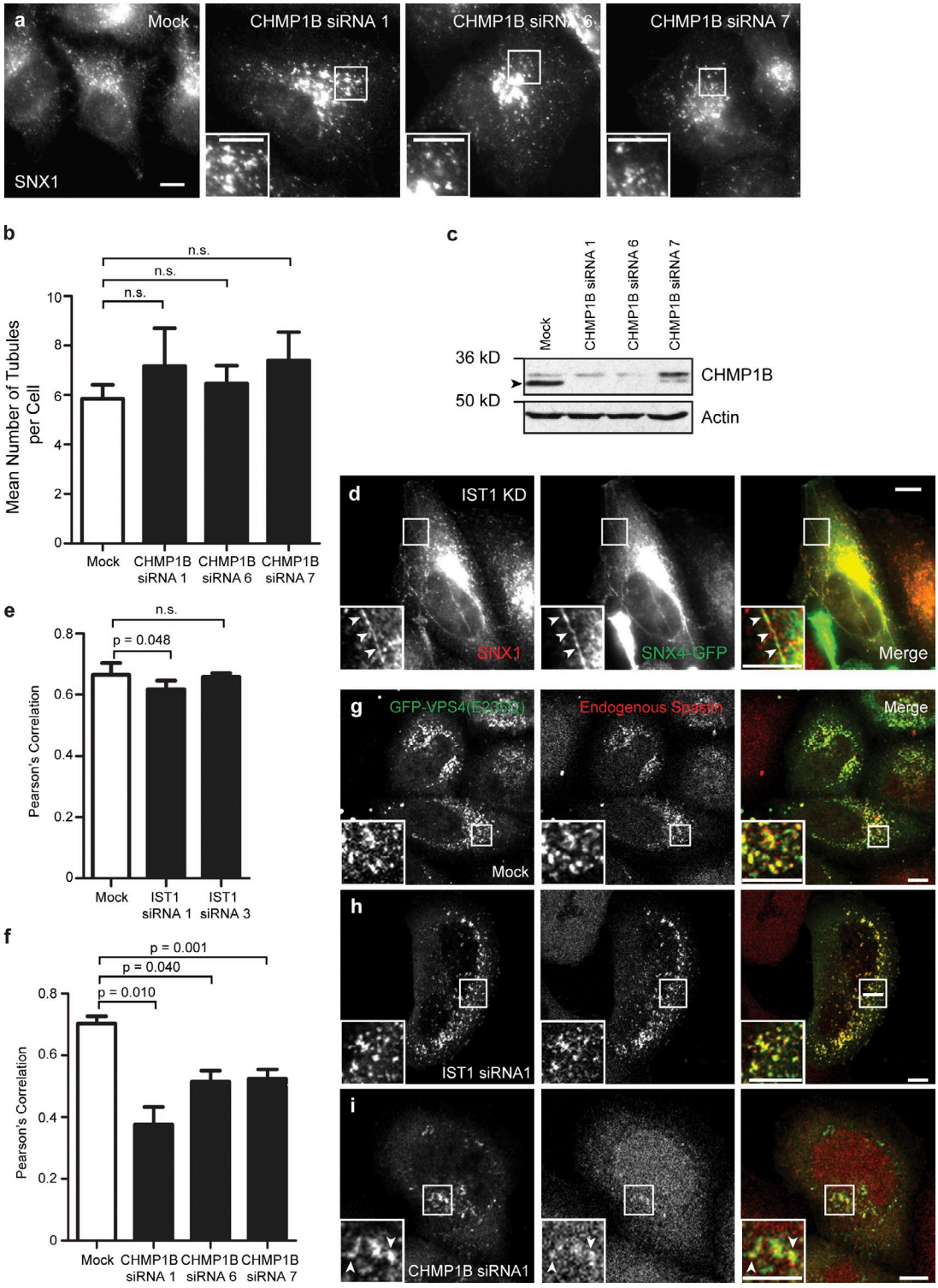


Figure S4. **The role of CHMP1B and IST1 in endosomal tubulation and recruitment of spastin to VPS4-EQ endosomes.** (a and b) HeLa cells were subjected to mock transfection or transfection with three different siRNA oligonucleotides directed against CHMP1B. The cells were labeled with endogenous SNX1 (a), and the mean number of tubules per cell was counted (b; *n* = 3 experiments, 30 cells per condition counted in each experiment). (c) CHMP1B (arrowhead) depletion was confirmed by immunoblotting. No increased tubulation was seen. (d) In contrast, HeLa cells depleted of IST1 by siRNA transfection show increased tubulation of SNX1 and SNX4-GFP, with the two sorting nexins colocalizing on the same tubules (see arrowheads in insets). See Fig. S1 d for a corresponding image from a control cell. (e–i) HeLa cells were subjected to mock siRNA transfection or were transfected with siRNA oligonucleotides directed against IST1 (e) or CHMP1B (f). The cells were then transfected with GFP-VPS4-E235Q and labeled for endogenous spastin. The extent of colocalization between GFP-VPS4-E235Q and spastin was estimated by calculating the Pearson's correlation coefficient for red and green pixels in each cell, using Volocity software (e and f; *n* = 3 experiments for each set of siRNAs, 20 cells per condition quantified in each experiment). Representative images are shown in g–i. Note that some recruitment of spastin to the VPS4-E235Q endosomes is retained in cells lacking CHMP1B (arrowheads in magnified box in i). Insets are magnifications of boxed regions. Bars, 10 µm. Error bars show SEMs.

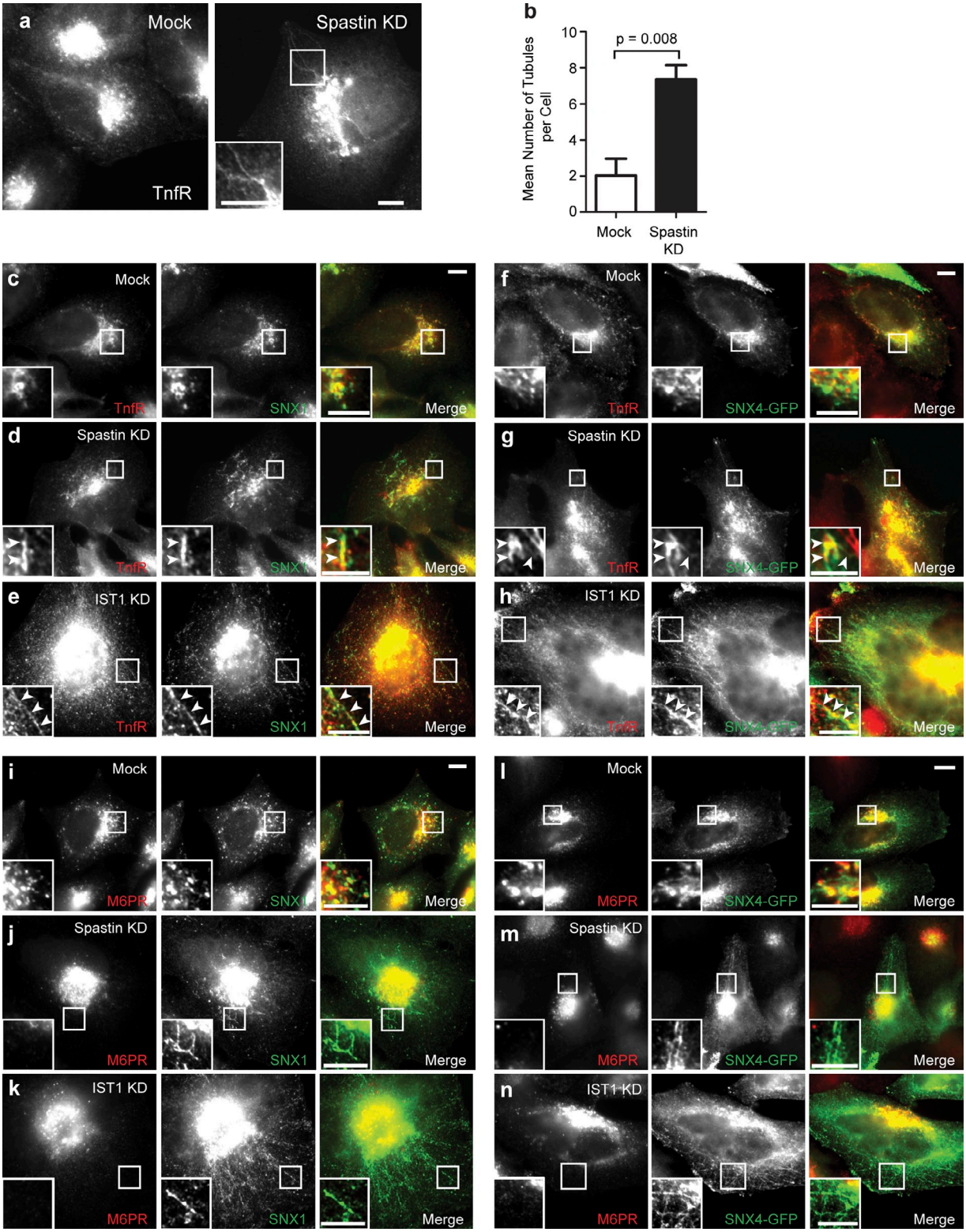
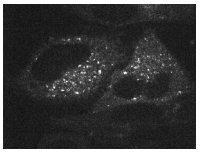
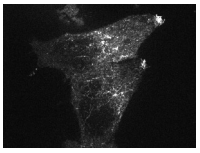


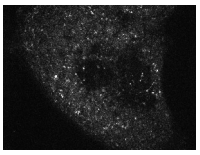
Figure S5. **TfnR distribution in cells lacking spastin and IST1.** (a and b) HeLa cells were subjected to mock transfection or transfected with a pool of spastin siRNA oligonucleotides. The cells were then labeled for endogenous TfnR (a), and the mean number of TfnR-positive tubules per cell was quantified (b; n = 3 experiments, 30 cells per condition counted in each experiment). (c–n) HeLa cells were subjected to mock transfection or transfected with a pool of spastin siRNA oligonucleotides or with IST1 siRNA1. In f–h and l–n, cells were also transfected with SNX4-GFP. Cells were then labeled with the endogenous markers indicated. Although partial tubular colocalization was observed between TfnR and both SNX1 and SNX4 (see arrowheads in inset magnified boxes), no tubular colocalization was observed between the sorting nexin proteins and M6PR. In a and c–f, the TfnR exposure settings in the spastin and IST1 knockdown (KD) images have been increased to compensate for the reduced TfnR levels caused by depletion of these proteins. Insets are magnifications of boxed regions. Bars, 10 µm. Error bars show SEMs.



Video 1. **HeLa cells were subjected to mock siRNA transfection and then transfected with SNX4-mCherry.** The SNX4 signal was visualized by time-lapse microscopy with a spinning-disc live-cell microscope (Axio Observer.Z1). Frames were taken every 2 s for 70 s.



Video 2. **HeLa cells were transfected with a pool of siRNA against spastin and then transfected with SNX4-mCherry.** The SNX4 signal was visualized by time-lapse microscopy with a spinning-disc live-cell microscope (Axio Observer.Z1). Frames were taken every 2 s for 58 s. Obvious tubulation of SNX4 is visible, with the tubules forming a relatively static network.



Video 3. **HeLa cells were transfected with a pool of siRNA against spastin and then transfected with SNX4-mCherry.** The SNX4 signal was visualized by time-lapse microscopy with a spinning-disc live-cell microscope (Axio Observer.Z1). Frames were taken every 2 s for 82 s. In this video, there are several examples of moving tubules remaining connected to a spherical structure, suggesting a failure of tubule fission from a parent endosome.