Movie Figure Legends

Chapter 3 – The function of spastin in endosomal tubule fission

Movie 1 – **GFP-SNX1 dynamics in mock and spastin siRNA depleted MRC5 cells**. MRC5 cells stably expressing GFP-SNX1 were treated with either mock or spastin siRNA and imaged using live-cell spinning disk microscopy to visualise endosomal tubule fission dynamics. Imaging was performed at 400ms per frame for 3 minutes. The large left image shows an overview of a typical mock cell, and the right image shows an overview of a typical spastin siRNA depleted cell. The insets in each show a zoom of the region highlighted by the white box in each overview image respectively. Note the difference in time for tubule fission in the mock and spastin siRNA treated cell. Scale bars for both overview images represent 10μm. 1 minute of GFP-SNX1 dynamics is shown in total. The video is displayed at 15 frames per second.

Movie 2 – **GFP-SNX1 dynamics in mock and spastin siRNA depleted COS7 cells**. COS7 cells stably expressing GFP-SNX1 were treated with either mock or spastin siRNA and imaged using live-cell spinning disk microscopy to visualise endosomal tubule fission dynamics. Imaging parameters and movie as in Movie 1. Note, the mock and spastin siRNA depletion parts of the movie shows an endosomal tubule fission and endosomal tubule collapse events respectively. Note the difference in time for tubule fate resolution in the mock and spastin siRNA treated cell.

Movie 3 – **GFP-SNX1 dynamics in mock and IST1 siRNA depleted MRC5 cells**. MRC5 cells stably expressing GFP-SNX1 were treated with either mock or IST1 siRNA and imaged using live-cell spinning disk microscopy to visualise endosomal tubule fission dynamics. Imaging parameters and movie as in Movie 1. Note, the mock and IST1 siRNA depletion parts of the movie both show endosomal tubule fission events, but the mock cell shows fission at the endosome body, and the siRNA depletion cell shows fission along the tubule. Note the difference in time for tubule fate resolution in the mock and IST1 siRNA treated cell.

Movie 4 – **GFP-SNX1 dynamics in spastin$^{WT/WT}$ (WT), spastin$^{WT/N384K}$ (HET), spastin$^{N384K/N384K}$ (HOM) immortalised mouse embryonic fibroblasts (MEFS).** GFP-SNX1 stably expressing wildtype, heterozygous, and homozygous spastin ATPase mutant SV-40 immortalised MEFs were imaged using live-cell spinning disk microscopy to visualise endosomal tubule fission dynamics. Imaging parameters and movie as in Movie 1. Note the difference in time for tubule fission fate resolution between the different spastin mutants.

Movie 5 – **4-colour fixed cell Airyscan imaging of GFP-M1 spastin, IST1, and α-tubulin on an mCherry-SNX1 endosomal tubule in an MRC5 cell**. MRC5 cells stably expressing GFP-M1 spastin and mCherry-SNX1 were fixed and stained with IST1 and α-tubulin antibodies, before being the entire Z-dimension of the cell was imaged using Airyscan microscopy. This movie shows a 3D reconstruction of a z-stack of a single SNX1 tubule, with 3D reconstruction performed using Imaris 9.1.0 software. Note the

apposition of GFP-M1 spastin and IST1 at endosomal tubule constrictions, and the tubule aligning along the α-tubulin labelled microtubule.

Movie 6 – **Live cell 2 colour imaging of the spatio-temporal association between GFP-M1 spastin and mCherry-SNX1 during endosomal tubule fission**. MRC5 cells stably expressing GFP-M1 spastin and mCherry-SNX1 were imaged using live cell Airyscan microscopy. Imaging was performed at 2.88 seconds per frame for 3 minutes in both channels simultaneously. The large image on the left shows an overview of the highlighted cell during the selected endosomal tubule fission event, with the white box showing the zoom region. The top right and bottom right show a zoom of the highlighted endosomal tubule during fission, with the top right panel showing mCherry-SNX1 alone, and the bottom right panel showing both mCherry-SNX1 and GFP-M1 spastin. The movie is displayed at 10 frames a second.

Movie 7 – **Live cell 2 colour imaging of GFP-SNX1 endosomal tubule dynamics in relation to RFP-KDEL ER tubule dynamics in mock and spastin siRNA depleted MRC5 cells**. MRC5 cells stably expressing GFP-SNX1 were treated with mock or spastin siRNA and transfected with RFP-KDEL and imaged using live-cell spinning disk microscopy to visualise the relationship between endosomal tubule fission and ER tubule dynamics. Imaging was performed at 0.4 seconds per frame for three minutes, with both channels acquired simultaneously. Left and right figures show mock and spastin siRNA treated cells respectively. Top images show GFP-SNX1 alone, and bottom images show both GFP-SNX1 and RFP-KDEL. In each large image the inset shows a zoom of an endosomal tubule fission event, with the location highlighted by the white box in each overview image. T = 0 for both mock and spastin siRNA treated images is the formation of the endosomal tubule, and the end of each sets of movies is several frames after the fission of the endosomal tubule. Note the location of fission and duration for fission in relation to ER tubules for endosomal tubules in both mock and spastin siRNA treated cells. The scale bar for all overview images represents 10µm, and the movie displays at 15fps.

Movie 8 – **Dilution series of SiR-tubulin in MRC5 cells**. MRC5 cells were treated with 100nM, 50nM, 25nM, 10nM, 5nM, and 1nM for 8hrs before being imaged using live cell spinning disk microscopy to determine the lowest concentration of SiR-tubulin dye that would give good microtubule labelling. Imaging was performed at 0.45 seconds per frame for three minutes. The scale bar in all images represents 10µm. The movie shows 100 seconds of SiR-tubulin labelled microtubule dynamics and displays at 90 frames per second. 25nM was chosen as an appropriate concentration to use the SiR-tubulin dye.

Movie 9 – **The effect of increasing Emerald-M87 spastin on SiR-tubulin labelled microtubules in MRC5 cells.** MRC5 cell were treated with 25nM SiR-tubulin dye for 8 hours, and after 2 hours during the dye treatment were transfected with Emerald-M87 spastin. 6 hours post-transfection, the Emerald-M87 spastin and SiR-tubulin were imaged using live cell spinning disk imaging, with images taken at 2 minutes per frame for 24 hours. The left panel shows both Emerald-M1 spastin, and the middle and right panels show Emerald-M87 spastin and SiR-tubulin alone respectively. Note the loss of SiR-tubulin signal as the expression of Emerald-M87 spastin increases.

Movie 10 – **GFP-SNX1 endosomal tubule dynamics in relation to SiR-tubulin labelled microtubules**. MRC5 cells stably expressing GFP-SNX1 were treated with 25nM SiR-tubulin dye for 8 hours and imaged using live cell spinning disk microscopy to observed endosomal tubule dynamics in SiR-tubulin treated cells. Imaging was performed at 0.22 seconds per frame for three minutes, with both channels imaged simultaneously. The movie shows an individual endosomal tubule fission event in relation to the microtubule network. The left, middle, and right images show the GFP-SNX1 and SiR-tubulin channels combined, the GFP-SNX1 alone, and SiR-tubulin alone respectively. The scale bar for all images represents 1µm. The total movie length displays 30 seconds and is shown at 25 frames per second.

Movie 11 – **EB3-mCherry labelled microtubule plus-end dynamics in mock and spastin siRNA treated MRC5 cells**. MRC5 cells were treated with mock or spastin siRNA and transfected with EB3-mCherry before being imaged using widefield live cell imaging to visualise EB comet formation rates. Imaging was performed at 500ms per frame for three minutes. The movie displays a typical mock cell (left) and a spastin siRNA depleted cell (right) for 1 minute. The scale bar in each image represents 20µm. The movie is displayed at 25 frames per second. Note the rate of comet formation between the mock and spastin siRNA treated cells.

Movie 12 – **EB3-mCherry labelled microtubule plus-end dynamics in mock and spastin siRNA treated HeLa cells**. HeLa cells were treated with mock or spastin siRNA and transfected with EB3-mCherry before being imaged using widefield live cell imaging to visualise EB comet formation rates. Imaging and movie details as in Movie 11.

Movie 13 – **EB3-mCherry labelled microtubule plus-end dynamics in spastin$^{WT/WT}$ (WT), spastin$^{WT/N384K}$ (HET), spastin$^{N384K/N384K}$ (HOM) mouse embryonic fibroblasts (MEFs)**. SV-40 immortalised MEFs were transfected with EB3-mCherry and imaged using spinning disk live cell microscopy to visualise EB comet formation rates. Imaging and movie details as in Movie 11.

Movie 14 – **GFP-SNX1 endosomal tubule dynamics in relation to EB3-mCherry labelled microtubule plus-end generation in MRC5 cells**. MRC5 cells stably expressing GFP-SNX1 were transfected with EB3-mCherry and imaged using live cell spinning disk microscopy. Imaging was performed at 0.42 seconds per frame for three minutes, with both channels acquired simultaneously. The top left panel shows an overview of the whole cell, with the white box indicating the zoom region displayed in the other three panels. The other panels show a zoom of an endosomal tubule fission event, with top right showing GFP-SNX1 alone, bottom left showing EB3-mCherry alone, and bottom right showing both channels. The scale bar for the overview image and the zoom images represent 10µm and 1µm respectively. The movie shows 50 seconds of GFP-SNX1 and EB3-mCherry dynamics and is displayed at 15 frames per second. Note the lack of spatio-temporal association between endosomal tubule fission and microtubule plus-end generation.

Chapter 4 – The function of spastin in the early secretory pathway

Movie 1 – **Live cell 2 colour imaging of GFP-M1 spastin and mCherry-Sec23 in MRC5 cells**. MRC5 cells stably expressing GFP-M1 spastin were transfected with mCherry-Sec23 and imaged using live cell spinning disk microscopy. Imaging was performed at 420ms per frame for 3 minutes simultaneously in both channels. Left panel shows an overview of two cells with both GFP-M1 spastin and mCherry-Sec23 displayed. The right panels show a zoom of the region indicated by the white box on the overview image, with the top panel showing GFP-M1 spastin alone, the middle showing mCherry-Sec23 alone, and the bottom showing both channels merged. The scale bars for the overview and zoom images show 10µm and 2µm respectively. The video is displayed at 15 frames per second.

Movie 2 – **Live cell imaging of TNFα-GFP release and trafficking in mock, spastin depleted, IST1 depleted, and H89 treated TNFα-SBP-GFP KDEL-Streptavidin RUSH HeLa cells**. HeLa cells stably coexpressing TNFα-SBP-GFP RUSH reporter and KDEL-Streptavidin RUSH hook were transfected with mock, spastin siRNAs, IST1 siRNAs, or treated with 100µM H89 and transfected with SiT-FusRed to label the Golgi apparatus. Cells were treated with 40µM biotin to stimulate TNFα-GFP reporter release and imaged using live cell confocal microscopy at ~40 seconds per frame for 90 minutes. Time 0 corresponds to the time of biotin addition. The top left, top right, bottom left, and bottom right panels display mock, spastin depleted, IST1 depleted, and H89 treated cells respectively. All images show both TNFα-GFP and SiT-FusRed. All scale bars represent 20µm. The video is displayed at 20 frames per second.

Movie 3 **- Live cell imaging of GFP-GPI release and trafficking in mock, spastin depleted, and H89 treated SBP-GFP-GPI KDEL-Streptavidin RUSH HeLa cells**. HeLa cells stably coexpressing SBP-GFP-GPI RUSH reporter and KDEL-Streptavidin RUSH hook were transfected with mock, spastin siRNAs, or treated with 100µM H89 and transfected with SiT-FusRed to label the Golgi apparatus. Cells were treated with 40µM biotin to stimulate GFP-GPI reporter release and imaged using live cell confocal microscopy at ~40 seconds per frame for 90 minutes. Time 0 corresponds to the time of biotin addition. The left, middle, and right panels display mock, spastin depleted, and H89 treated cells respectively. All images show both GFP-GPI and SiT-FusRed. All scale bars represent 20µm. The video is displayed at 20 frames per second.