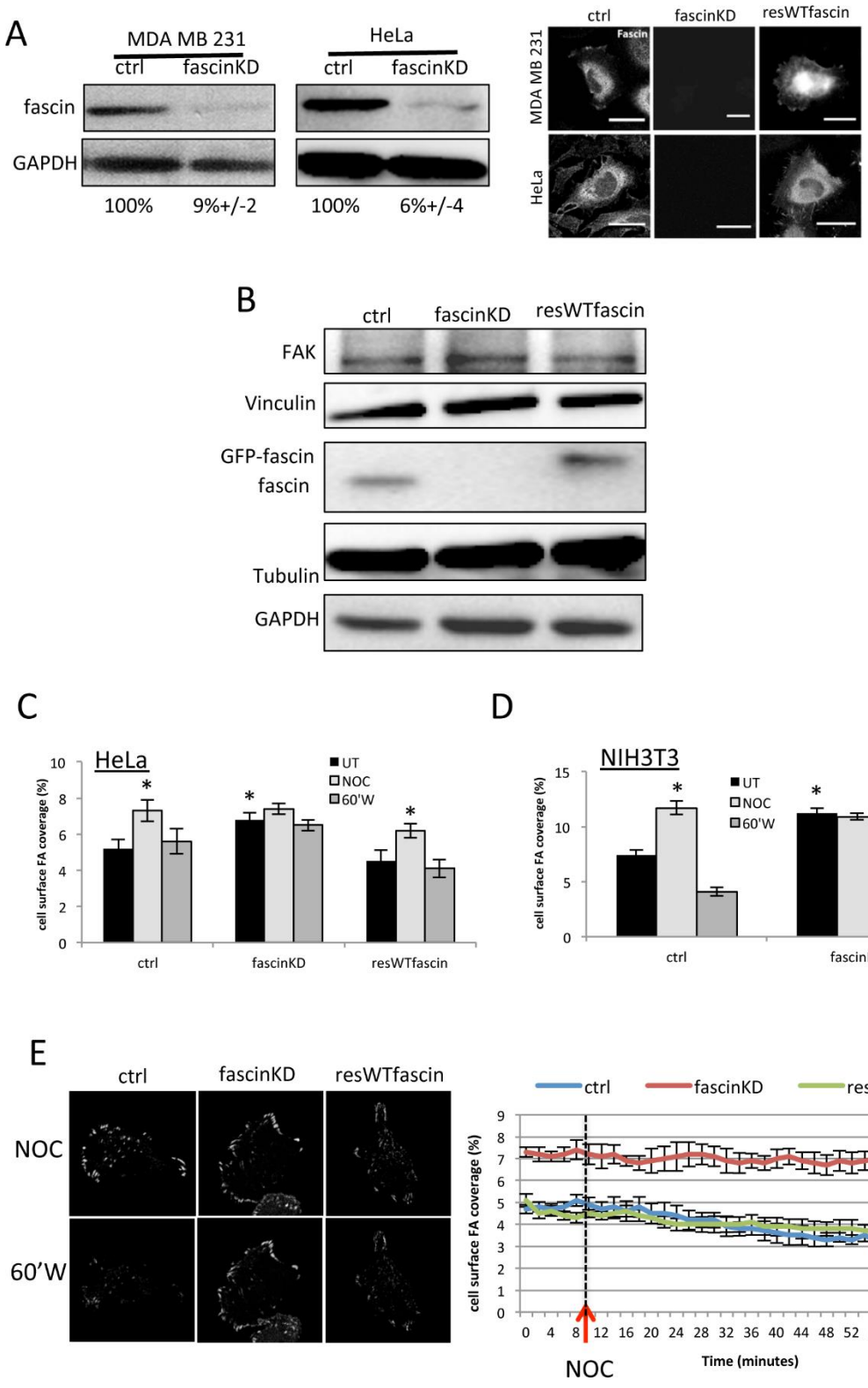
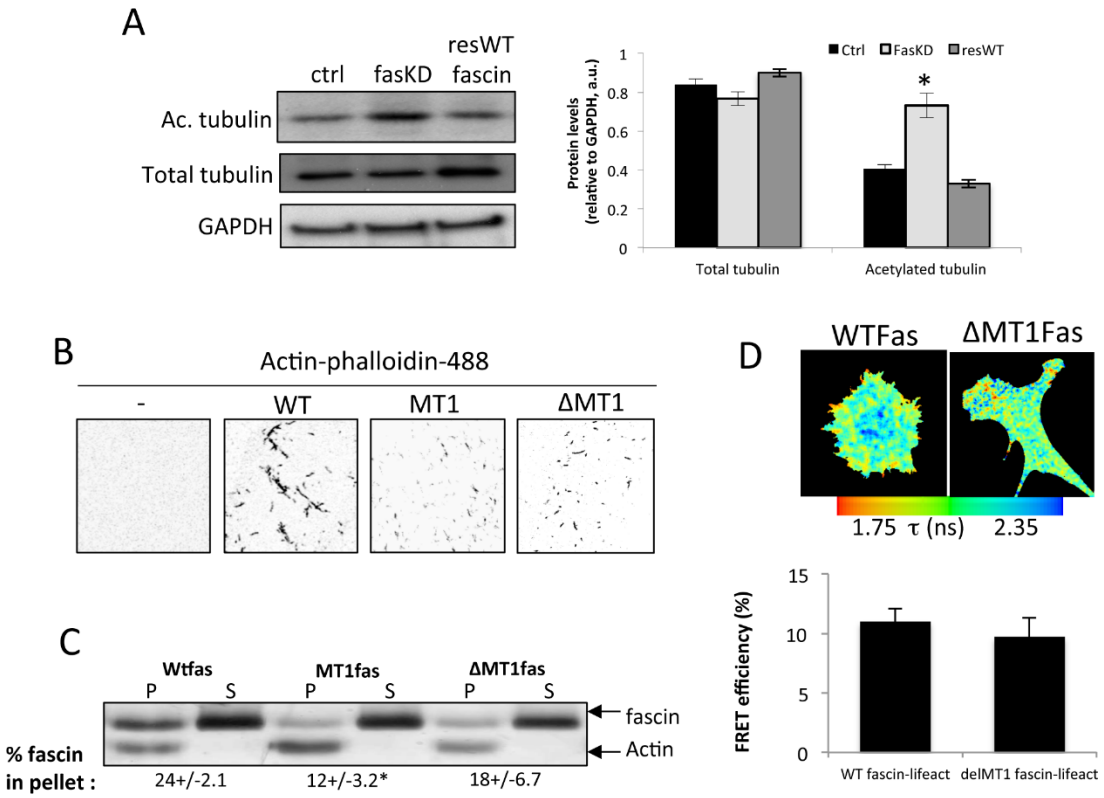
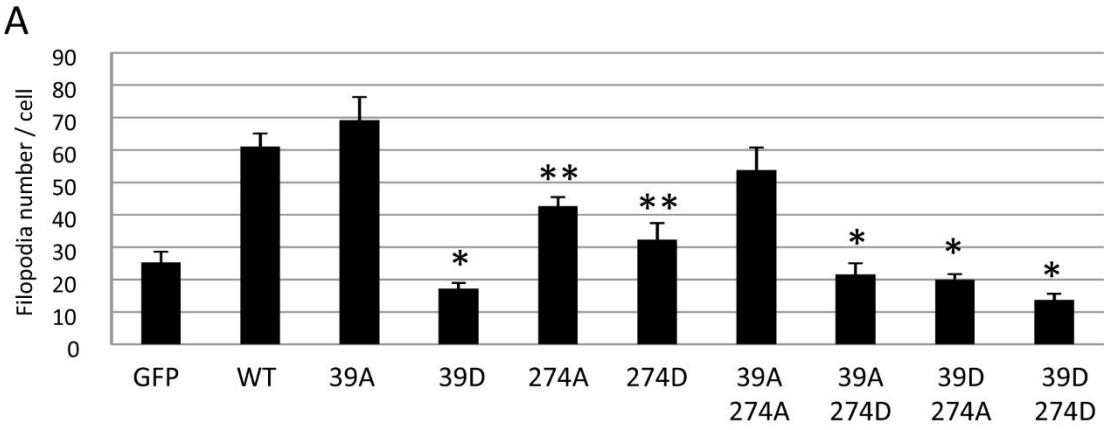
Supplemental Figure 1

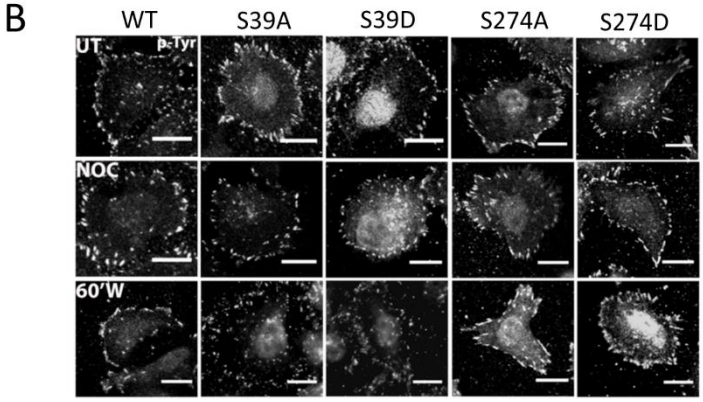


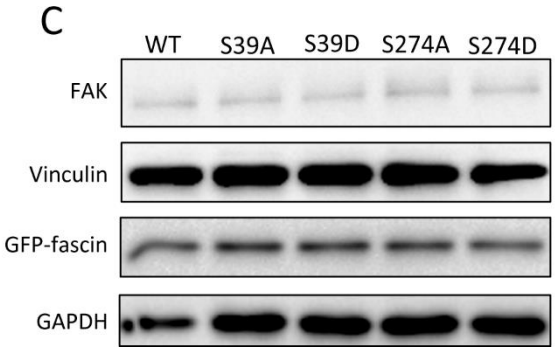
Supplemental Figure 2



Supplemental Figure 3

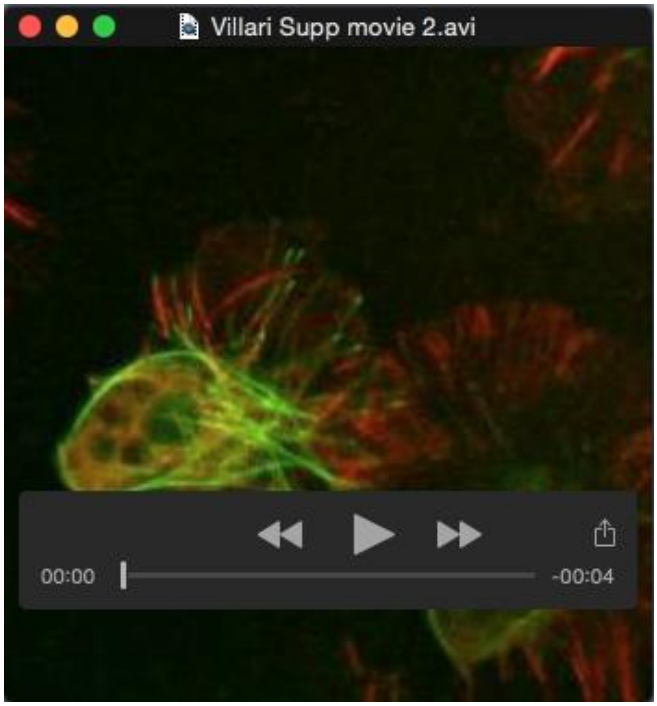






Movie 1

Movie 2



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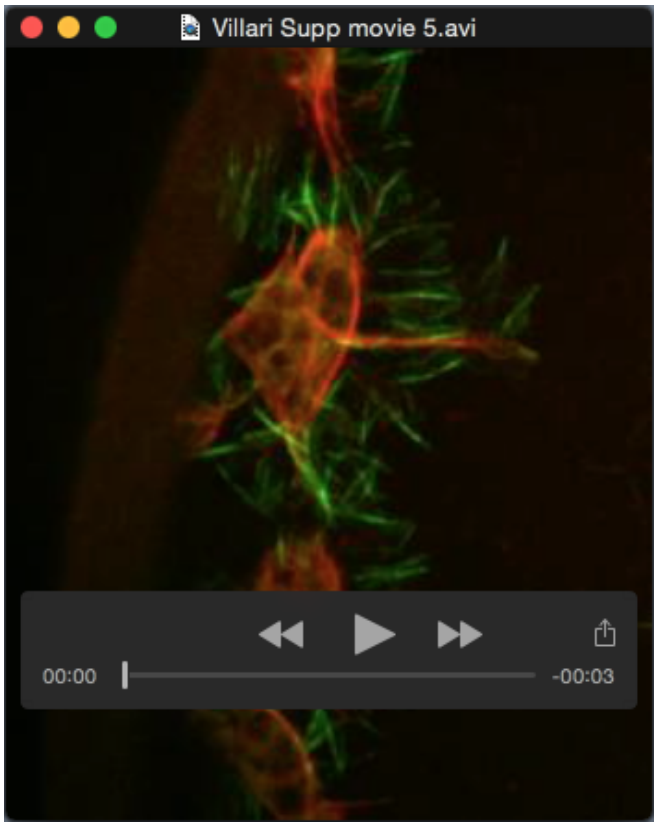
Movie 3

Movie 4

Movie 5



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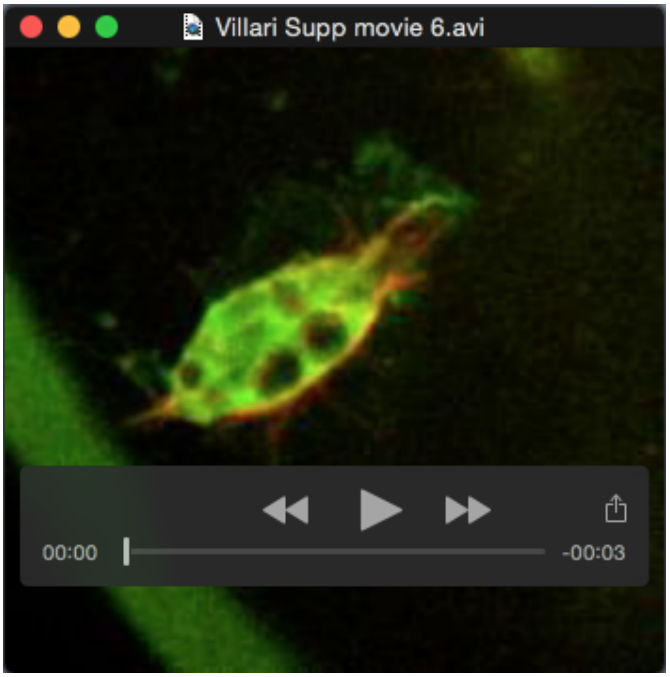
Movie 6



Supplemental Figure legends:

Supplemental Figure 1:
(A) Left panel: Western blots of lysates from MDA MB 231 or HeLa cells stably expressing control (ctrl) or fascin specific shRNA (fascinKD). Representative of 3 experiments. Right hand panels show example images of control MDA MB 231 (top) and HeLa cells (bottom), fascinKD or fascinKD expressing WTfascin-GFP, fixed and stained for fascin. Scale bars are 20μm. **(B)** Western blots of lysates from control, fascinKD or WTfascin-GFP rescued MDA MB 231 cells probed for specified proteins. Representative of 4 independent experiments. **(C)** Quantification of FA coverage (as also performed in Figure 1) in Control, fascinKD or fascinKD HeLa expressing WTfascin-GFP either untreated (UT), treated with Nocodazole (NOC) or 30/60 minutes post-NOC washout. Cells were stained for phosphotyrosine. Mean values shown +/- SEM, *=p<0.01. N=40 cells per condition over three independent experiments. **(D)** Quantification of cell surface FA coverage area in Control and fascin KD NIH-3T3 mouse fibroblasts either untreated (UT), treated with Nocodazole (NOC) or 30/60 minutes post-NOC washout. Mean values shown +/- SEM; *=p<0.01**.** N=45 cells per condition over three independent experiments. **(E)** Example stills taken from movies of MDA MB 231 cells expressing control shRNA, fascin shRNA or re-expressing WTfascin-GFP transfected with vinculin-mRFP. Cells were treated with Nocodazole for 10 minutes followed by an additional 10 minutes during imaging and washout for up to 60 minutes. Cells were monitored over the 60 min washout period and percentage of focal adhesion coverage per cell quantified as in Figure 1 at each time point. Data from 14 cells per condition over 3 experiments were plotted in the graph (right). Data points shown are mean +/- SEM. Red arrow head and dotted line indicates point of addition of Nocodazole to live cells during imaging period.

Supplemental Figure 2:
(A) Western blots of lysates from MDA MB 231 cells stably expressing control (ctrl) or fascin specific shRNA (fascinKD) probed for total or acetylated tubulin. Graph in right panel shows quantification of densitometry analysis of western blots of total and acetylated tubulin levels over three independent experiments normalised to GAPDH levels. **(B)** Confocal images of phallodin-488 labelled F-actin bundles formed following incubation and co-sedimentation with purified WT, MT1 or ΔMT1 fascin. Images are inverted for clarity. **(C)** Representative silver stained gel of co-sedimentation with purified WT, MT1 or ΔMT1 fascin and F-actin. Numbers beneath blots are normalized densitometry values from 3 different experiments +/-SEM. *=p<0.05 compared to WTfascin levels. **(D)** Example images of fluorescence lifetime of WT or ΔMT1 fascin-GFP co-expressed in MDA MB 231 cells with lifeact-mRFP and subjected to FRET/FLIM analysis. Pseudocolour scale shows low lifetime pixels as red indicating regions of high FRET. Graph shows cumulative FRET efficiency data poled for 15 cells across 3 different experiments. Data shown is mean FRET efficiency +/-SEM.

Supplemental Figure 3:
(A) Graph showing filopodia formed in fascin knockdown MDA MB 231 cells re-expressing single or double mutant S39 and S274 forms of GFP-fascin. Data is shown as filopodia number/cell quantified from 50 cells per condition across 3 independent experiments. Mean+/-SEM is shown; *=p<0.01. **(B)** Representative images of phosphotyrosine (p-Tyr) stained fascinKD MDA MB 2-1 cells re-expressing GFP-fascin constructs as specified. Cells were either untreated (UT), treated with Nocodazole for 20 minutes (NOC) or 60 minutes post-nocodazole washout (60'W). Quantification is presented in Figure 4A. Scale bars are 15 μm. **(C)** Western blots of lysates from MDA MB 231 cells stably expressing control fascin specific shRNA and rescued with WT or mutant fascin-GFP as specified. Blots were probed for FAK, vinculin and fascin. GAPDH serves as a loading control.

Movie 1:
Example movies of tubulin-mCherry expressed in control (SCR) or fascinKD HeLa cells used for analysis of MT dynamics as shown in Figure 1. Arrows and arrow-heads denote growing or stable MT respectively. Asterisks denote catastrophe events. Fluorescence is inverted to black on white for clarity. Movies acquired at 1fr/sec, playback rate is 30fr/sec.

Movie 2:
Live imaging of WT fascin-mCherry expressing hemocytes co-expressing a fluorescently tagged microtubule probe (GFP-Clip170). Note that most of the microtubules polymerize around the cell body, and into a single microtubule bundle ('MT arm') within the lamella. A number of MT co-localise or co-align with MT. Movie was acquired at a rate of 10 seconds/frame.

Movie 3:
Example movies of tubulin-mCherry expressed in fascinKD HeLa cells re-expressing WT GFP-fascin-(WT), delMT1-fascin or MT1-fascin as used for analysis of MT dynamics as shown in Figure 2F. Fluorescence is inverted to black on white for clarity. Movies acquired at 1fr/sec, playback rate is 30fr/sec.

Movie 4:
Example movies of tubulin-mCherry expressed in fascinKD HeLa cells re-expressing WT GFP-fascin-(WT), or S39a, S39D, S274A or S274D GFP-fascin variant as used for analysis of MT dynamics as shown in Figure 3D. Fluorescence is inverted to black on white for clarity. Movies acquired at 1fr/sec, playback rate is 30fr/sec.

Movie 5:
Live imaging of WT fascin-mCherry expressing hemocytes co-expressing a fluorescently tagged microtubule probe (GFP-Clip170). Note that most of the microtubules polymerize around the cell body, and into a single microtubule bundle ('MT arm') within the lamella. Movie was acquired at a rate of 10 seconds/frame.

Movie 6:
Live imaging of fascin S289D-mCherry expressing hemocytes co-expressing a fluorescently tagged microtubule probe (GFP-Clip170). Note the co-bundling of fascin and MT around the cell body and lack of dynamic protrusions containing MT. Movie was acquired at a rate of 10 seconds/frame.