Supplemental File Legends

Supplemental Fig. S1**.** The impact of septin-depletion on HeLa cells. *A.* Western blot (WB) of HeLa cells transfected with siRNA against control (CTRL), SEPT2, SEPT9, or SEPT11. Cell lysates were separated by 10% SDS-PAGE before immunoblotting. The blots were probed with antibodies specific to GAPDH, SEPT2, SEPT9, SEPT11, and actin. GAPDH is shown as a loading control. The red box outlines depleted protein levels for targeted septins. *B.* Septin depletions have different effects on cell shape and actin and SEPT2 filament distribution. Endogenous F-actin and SEPT2 were visualized by immunostaining with anti-F-actin (green) and anti-SEPT2 antibodies (red) for control (CTRL)-, SEPT2-, SEPT9-, and SEPT11-depleted cells. Nuclei were marked with DAPI (blue). Scale bars indicate 10μm. *C.* Septin depletions have different effects on cell shape and actin and SEPT11 filament distribution. Endogenous F-actin and SEPT11 were visualized by immunostaining with anti-F-actin (green) and anti-SEPT11 antibodies (red) for control (CTRL)-, SEPT2-, SEPT9-, and SEPT11-depleted cells. Nuclei were marked with DAPI (blue). Scale bars indicate 10μm.

Supplemental Fig. S2. The morphological impact of SEPT11-depletion on HeLa cells**.** *A.* Representative scanning electron microscopy images of uninfected SEPT11-depleted HeLa cells to depict their membrane surface. Left image: magnification = 1500x, where the scale bar indicates 10μm. Right image: magnification = 10000x, where the scale bar indicates 1μm. *B***.** The range of surface areas for siRNA-treated HeLa cells. To quantify the surface area of siRNA-treated HeLa cells, confocal Z-stack images of cells were segmented using MetaMorph. Once segmented, the surface area of individual cells was measured as ranging from 0-6000μm^2. Cells were manually classified according to 200μm^2 intervals, where 200μm^2 was calculated to represent the largest interval minimizing the chance of putting together 2 'different' areas in the same range. Histograms depict the number of control (CTRL)- and SEPT11-depleted HeLa cells classified into a specific range of surface area values.

Supplemental Fig. S3. Septin-depletion in HeLa cells does not impact microtubules. In HeLa cells, septin depletions have different effects on cell shape and actin and septin filament distribution, but no noticeable effect on microtubules. Endogenous α–tubulin, F-actin, and SEPT9 were visualized for immunostaining with anti-α-tubulin (grey and in Merge blue), anti-F-actin (green), and anti-SEPT9 antibodies (red) for control (CTRL)-, SEPT9-, and SEPT11-depleted cells. Representative confocal images are shown here to present intact microtubules in septin-depleted cells. Scale bars indicate 10μm.

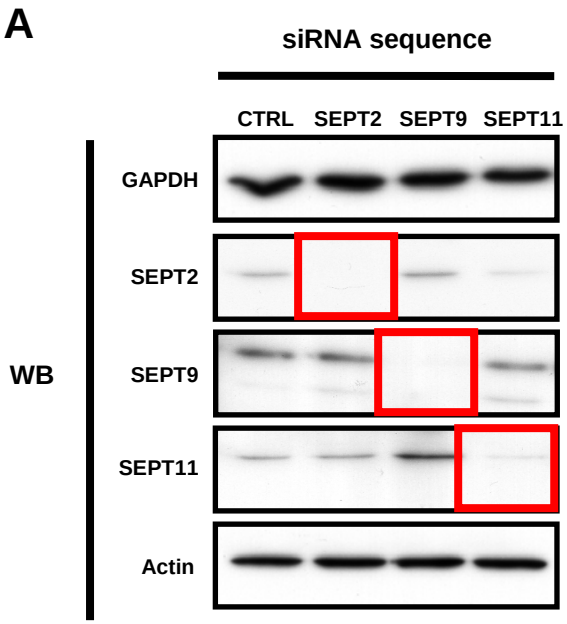
Supplemental Fig. S4. Investigating the relationship between infection and SEPT11-induced binucelation. *A.* Quantitative analysis for the percentage of binucleated HeLa cells counted for populations of siRNA-treated cells. Data represent the mean percentage of binucleated host cells ± SEM (where 50 or more binucleated cells were counted) for each of $n \geq 2$ separate experiments per siRNA treatment. *B*. Analysis comparing the percentage of HeLa cells infected with *L. monocytogenes* EGD that were binucleated, as counted for both siRNA treatments. The observed percentage of infected cells that were binucleated was normalized according to the expected percentage of binucleated cells within that siRNA treatment (where expected percentage values were derived from non-infected cells as plotted in Supplemental Fig. S4A). Graphed data represent this normalized value (i.e. observed/expected) ± SEM (where 100 or more infected host cells were counted) for each of $n \geq 2$ separate experiments per siRNA treatment.

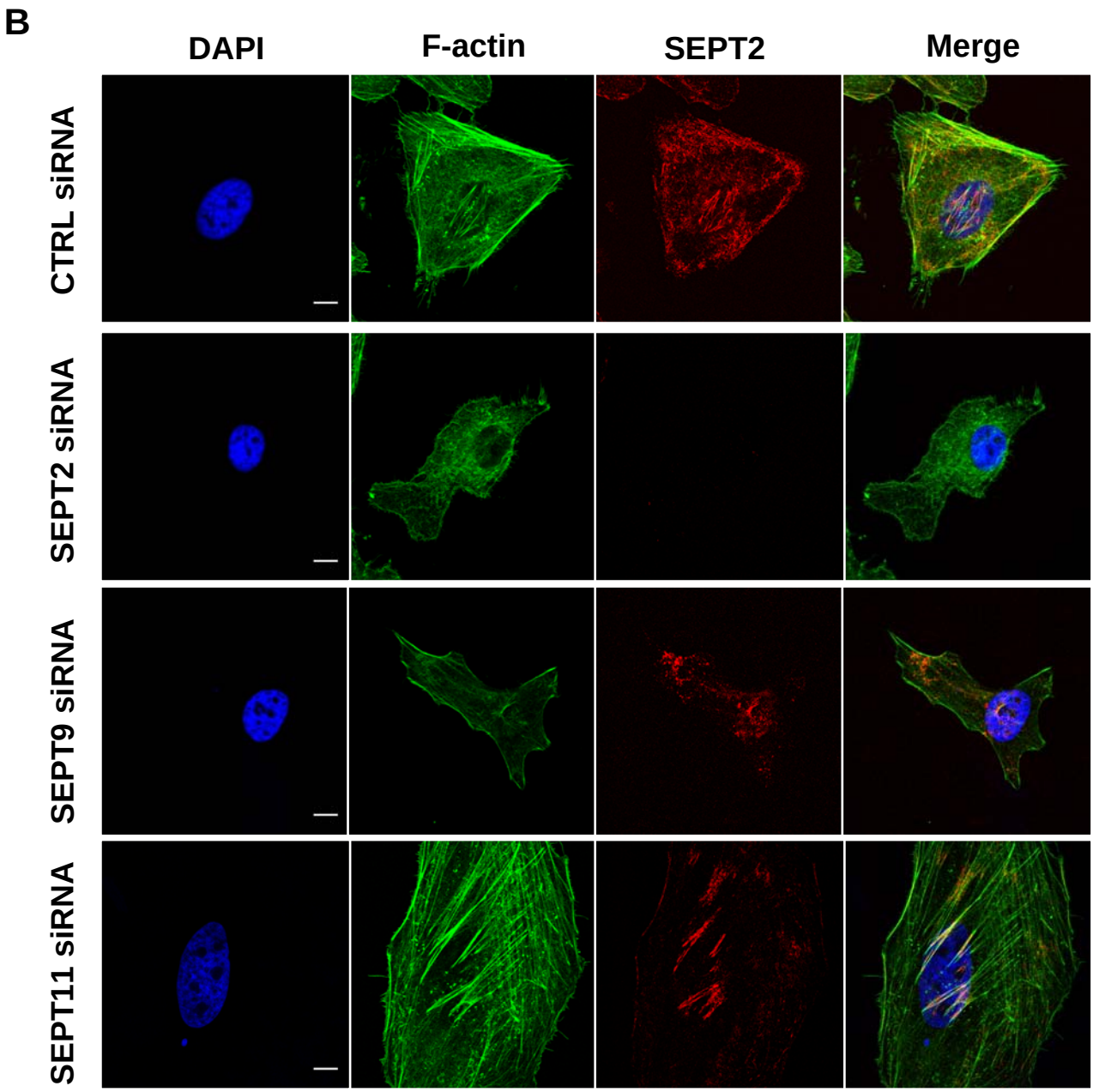
Supplemental Fig. S5. Investigating the impact of pharmacological agents and SEPT6-depletion on *Listeria* invasion. *A.* Gentamicin survival assays for *L. monocytogenes* EGD were performed for cells treated with pharmacological inhibitors of actin polymerization. Graphs represent the number of intracellular bacteria found inside treated HeLa cells after a 2 hour gentamicin survival assay. Cells were treated 30 minutes prior to infection, and throughout the 2 hour assay, with various concentrations

(ranging from 10-0.01μM) of cytochalasin D (cytoD) or latrunculin B (latB). Graphs represent the relative number of intracellular bacteria found inside treated cells after the survival assay, where CFU counts obtained from cytoD- or latB-treated cells were normalized to dimethyl sulfoxide (DMSO)-treated cells. On the graph DMSO treatment is figuratively presented as 1, and therefore does not have error bars. Data represent the mean ± SEM from n ≥ 3 experiments per cytoD or latB concentration. ***B.*** Gentamicin survival assays for *L. monocytogenes* EGD were performed for cells treated with pharmacological disruptors of microtubules. Graphs represent the number of intracellular bacteria found inside treated HeLa cells after a 2 hour gentamicin survival assay. Cells were treated 30 minutes prior to infection, and throughout the 2 hour assay, with various concentrations (ranging from 20-0.05μM) of nocodazole (noco) or paclitaxel (pacl). Graphs represent the relative number of intracellular bacteria found inside treated cells after the survival assay, where CFU counts obtained from noco- or pacl-treated cells were normalized to dimethyl sulfoxide (DMSO)-treated cells. On the graph DMSO treatment is figuratively presented as 1, and therefore does not have error bars. Data represent the mean ± SEM from n ≥ 3 experiments per noco or pacl concentration. ***C.*** Forchlorfeneuron (FCF) creates ring-like structures at the surface of treated HeLa cells (uninfected). Scanning electron microscopy images of FCF-treated HeLa cells at a molar concentration of 20μM for 30 minutes. Magnification = 1400x (left panel) and 4300x (right panel), where scale bars indicate 10μm and 1μm, respectively. ***D.*** Gentamicin survival assays for *L. monocytogenes* EGD were performed for cells treated with FCF. Graphs represent the number of intracellular bacteria found inside treated HeLa cells after a 2 hour gentamicin survival assay. Cells were treated 30 minutes prior to infection, and throughout the 2 hour assay, with various concentrations of FCF (ranging from 200-0.2μM). Graphs represent the relative number of intracellular bacteria found inside treated cells after the survival assay, where CFU counts obtained from FCF-treated cells were normalized to dimethyl sulfoxide (DMSO)-treated cells. On the graph DMSO treatment is figuratively presented as 1, and therefore does not have error bars. Data represent the mean ± SEM from n ≥ 6 experiments per FCF concentration. ***E.*** SEPT6 inactivation increases *Listeria* invasion. Gentamicin survival assays for *L. monocytogenes* EGD were performed in HeLa cells treated with control (CTRL) siRNA, or siRNA targeted against SEPT6. Graphs represent the relative number of intracellular bacteria found inside siRNA-treated cells after the survival assay, where CFU counts obtained from SEPT6-depleted cells were normalized to CTRL siRNA-treated cells. On the graph CTRL siRNA is figuratively presented as 1, and data represent the mean from n ≥ 9 experiments. Results were analyzed for statistical significance using the Student's t-test.

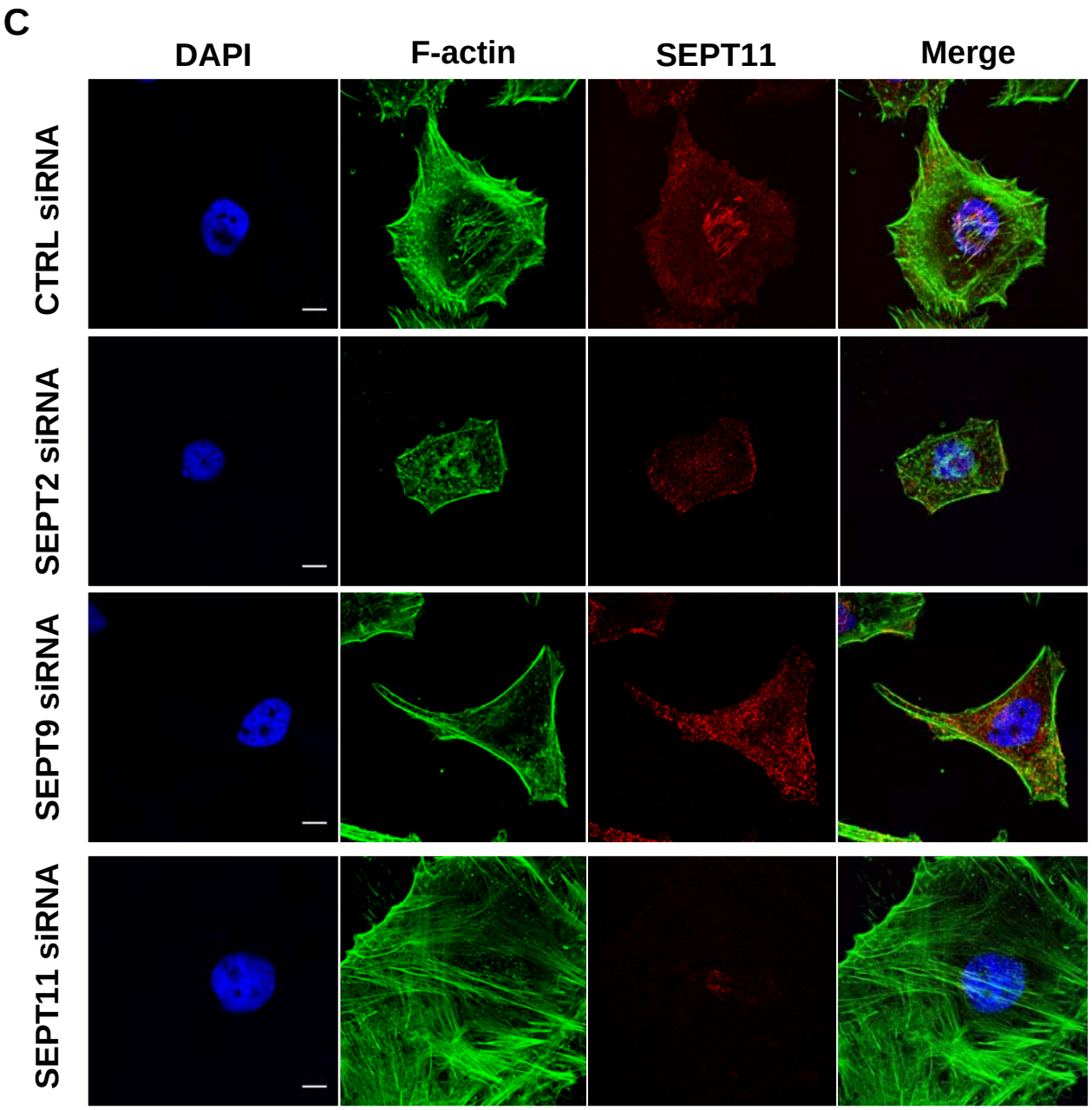
Supplemental Movie S1. Representative FRET movies for siRNA-treated cells. *A & B.* HeLa cells treated with (A) control (CTRL) siRNA or (B) siRNA targeted against SEPT11 were transfected with YFP-AktPH and CFP-AktPH. Transfected siRNA-treated cells were placed on the microscope stage at 37°C and image series were collected every 15 seconds for 15 minutes. Purified InlB was added to a final concentration of 5nM after frame 15 (i.e. time point just prior to 4 minutes) after the start of imaging. Movies for the entire timecourse of InlB stimulation for cells depicted in Fig. 4A are here presented (i.e. frame 0 to 60), showing the collected images (phase-contrast, Akt recruitment, and FRET efficiency) of siRNA-treated cells. Pseudocolor scale represents the range of FRET efficiency values from original signal ranging from blue (low) to red (high).

Supplemental Figure S1



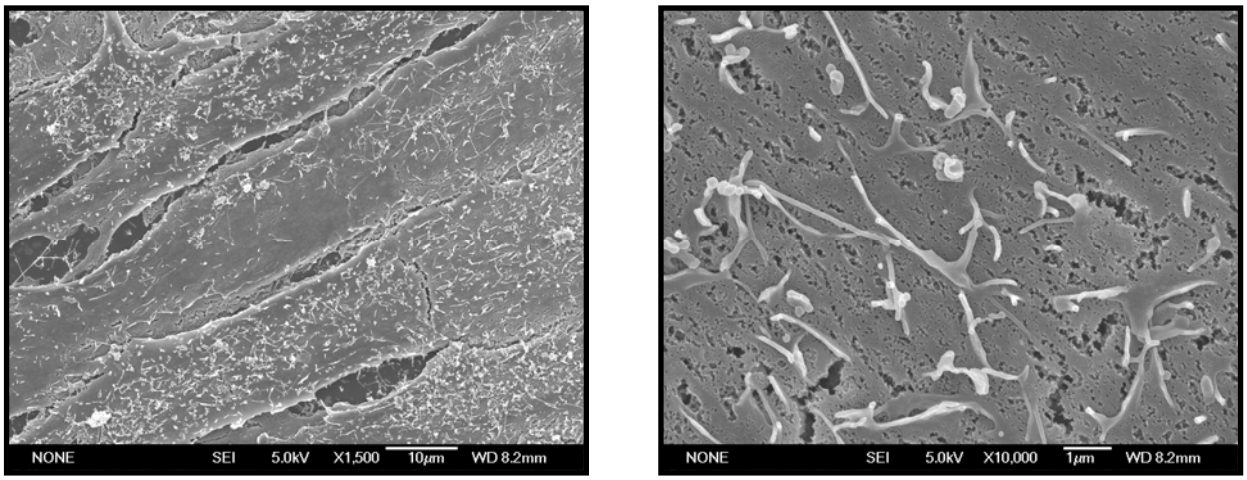


Supplemental Figure S1



Supplemental Figure S2

A



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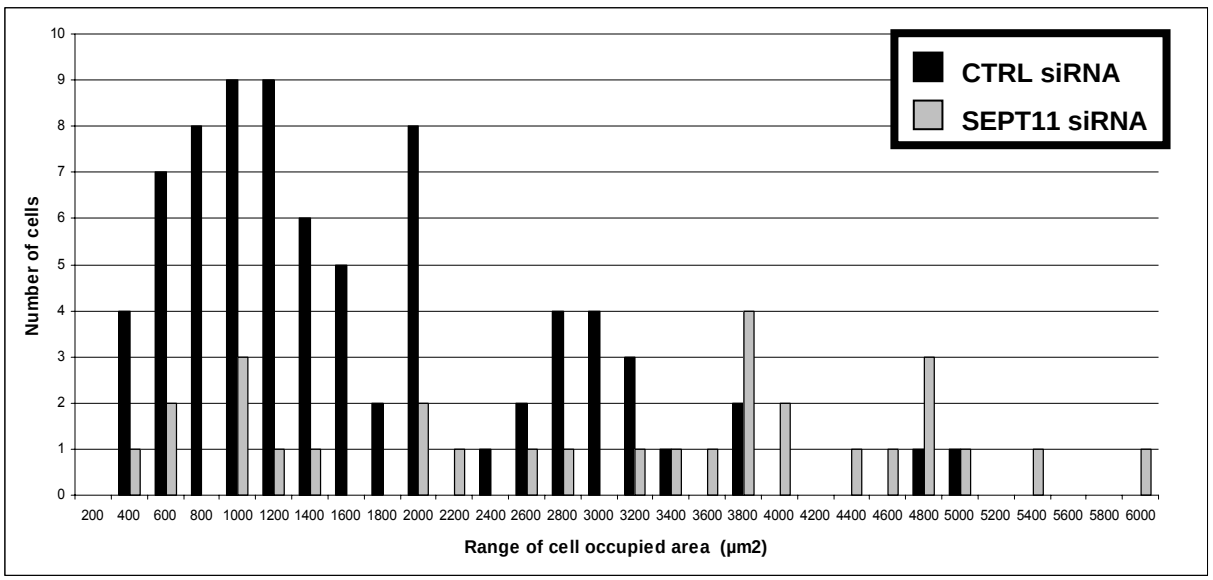
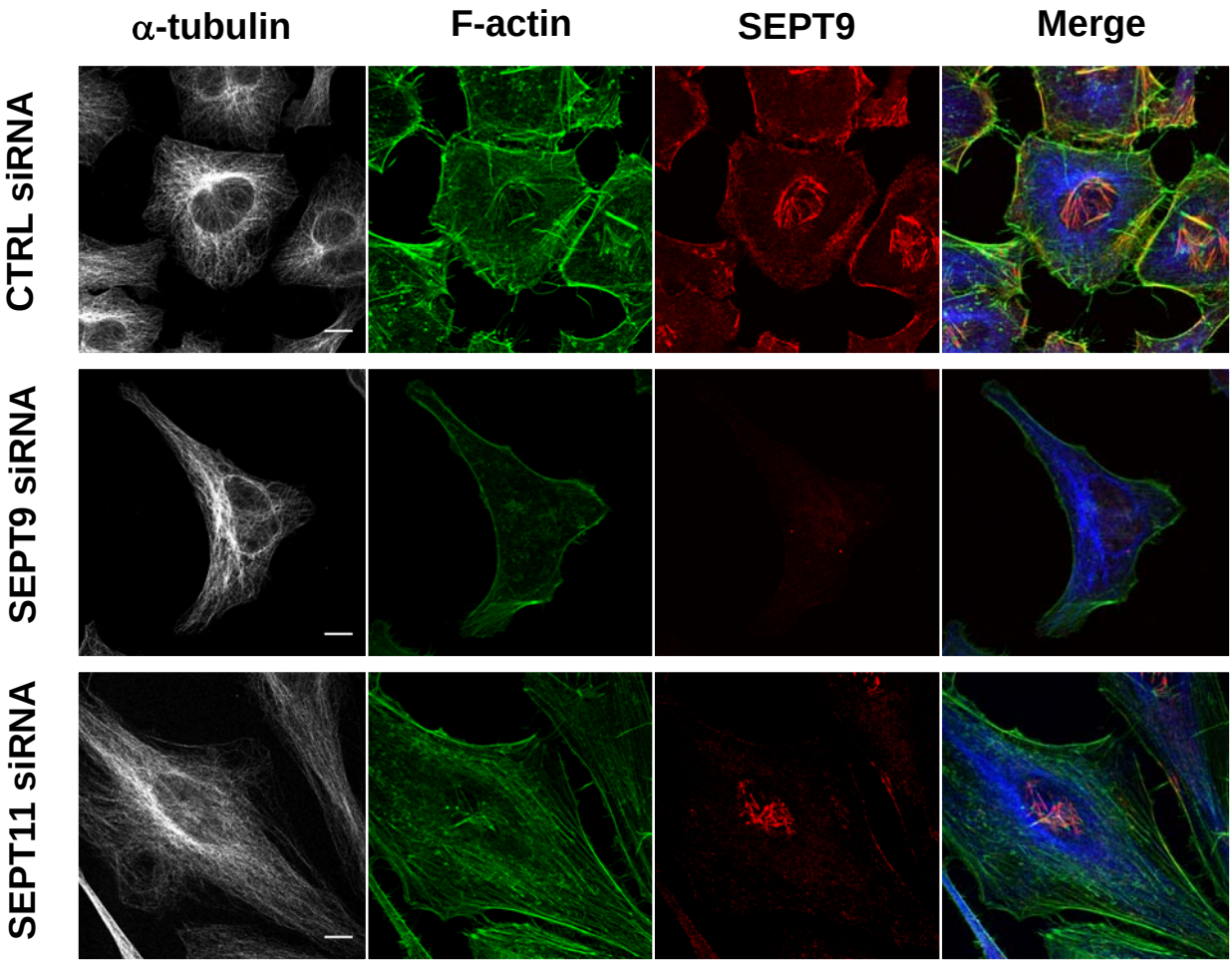
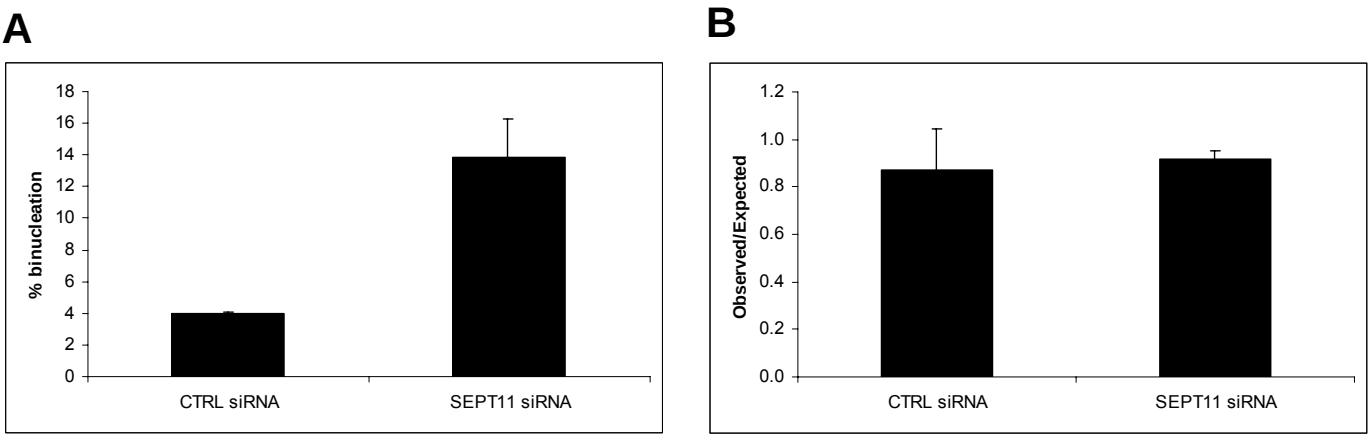


Supplemental Figure S3

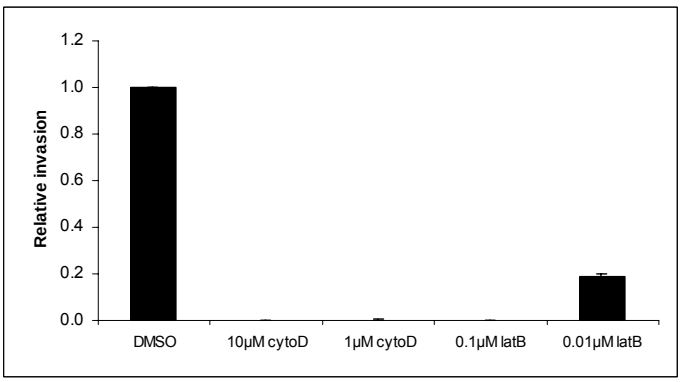


Supplemental Figure S4

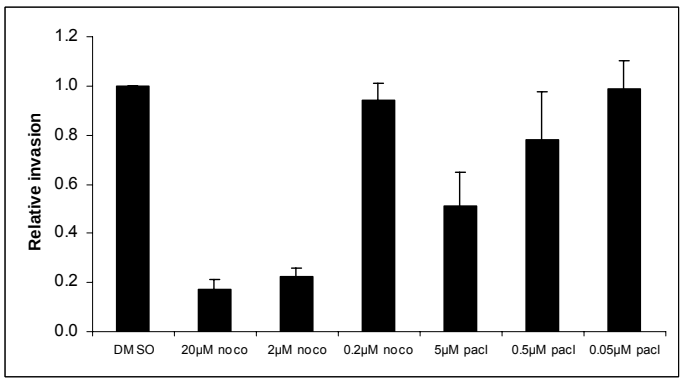


Supplemental Figure S5

A (Inhibition of actin)

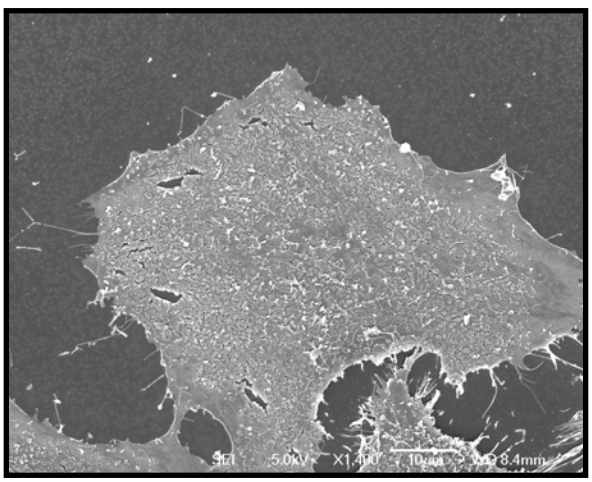


B (Disruption of microtubules)

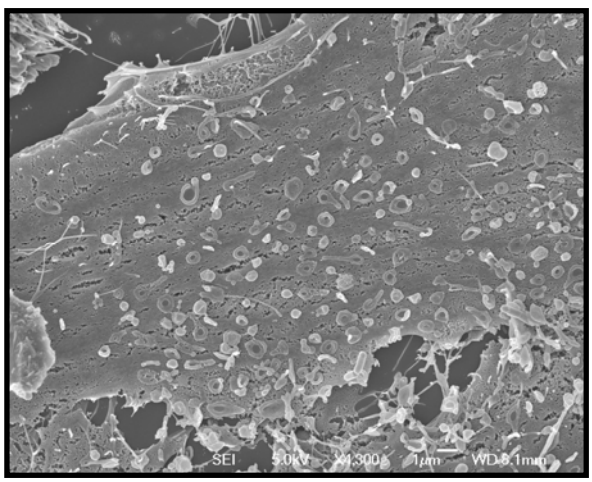


C

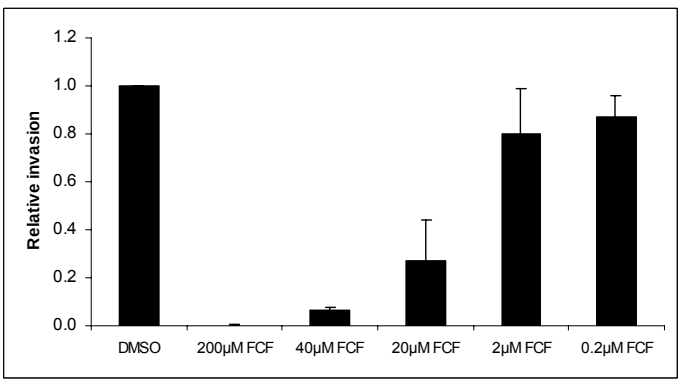
1400x



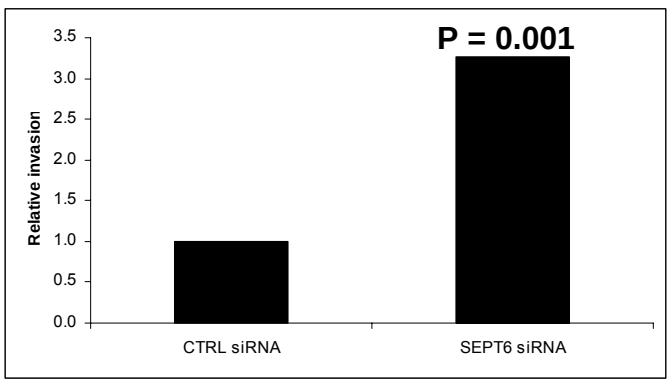
4300x



D (FCF-treated cells)



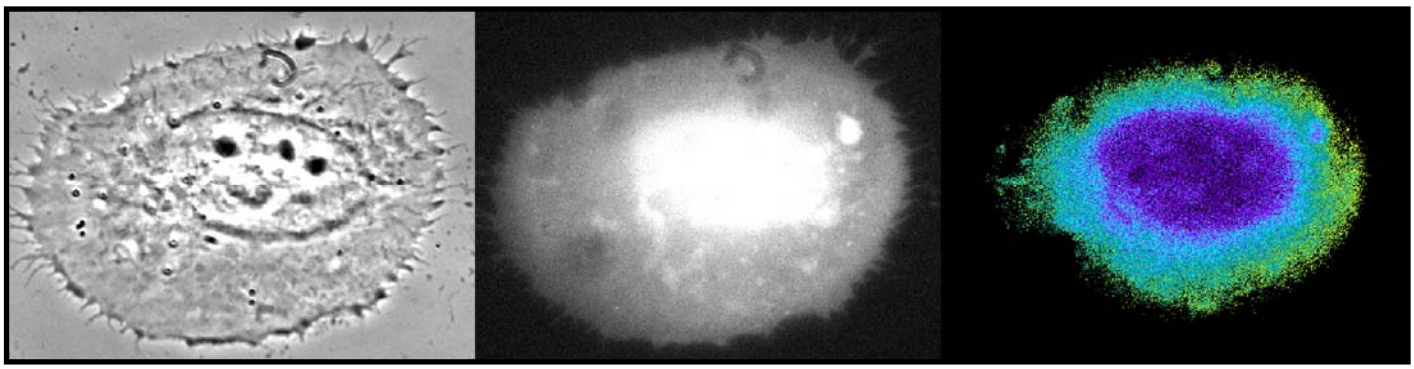
E (SEPT6-depleted cells)



Supplemental Movie S1

A (CTRL siRNA)

Phase | Akt recruitment | FRET efficiency



B (SEPT11 siRNA)

Phase | Akt recruitment | FRET efficiency