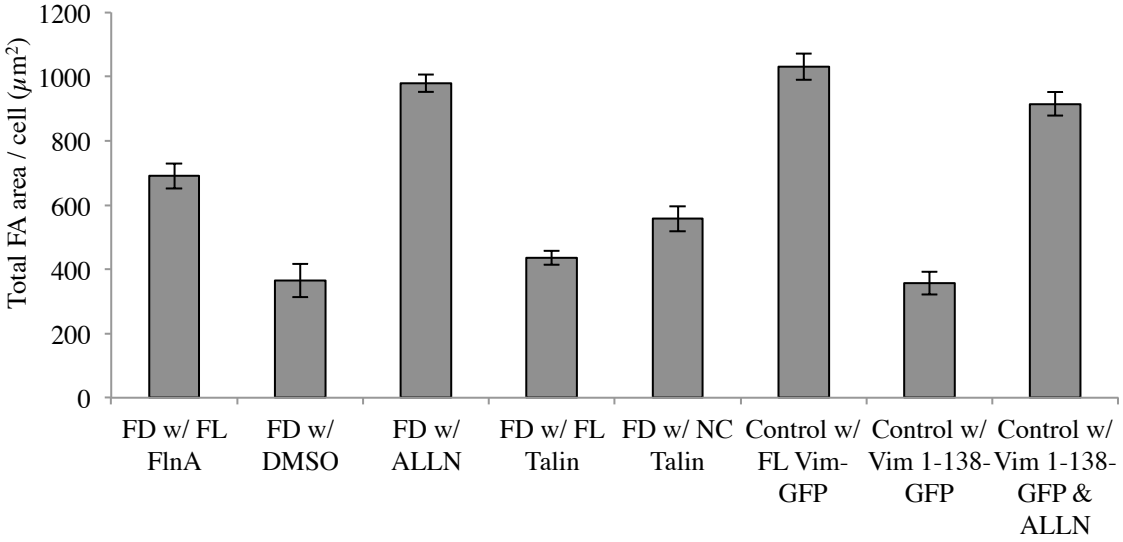
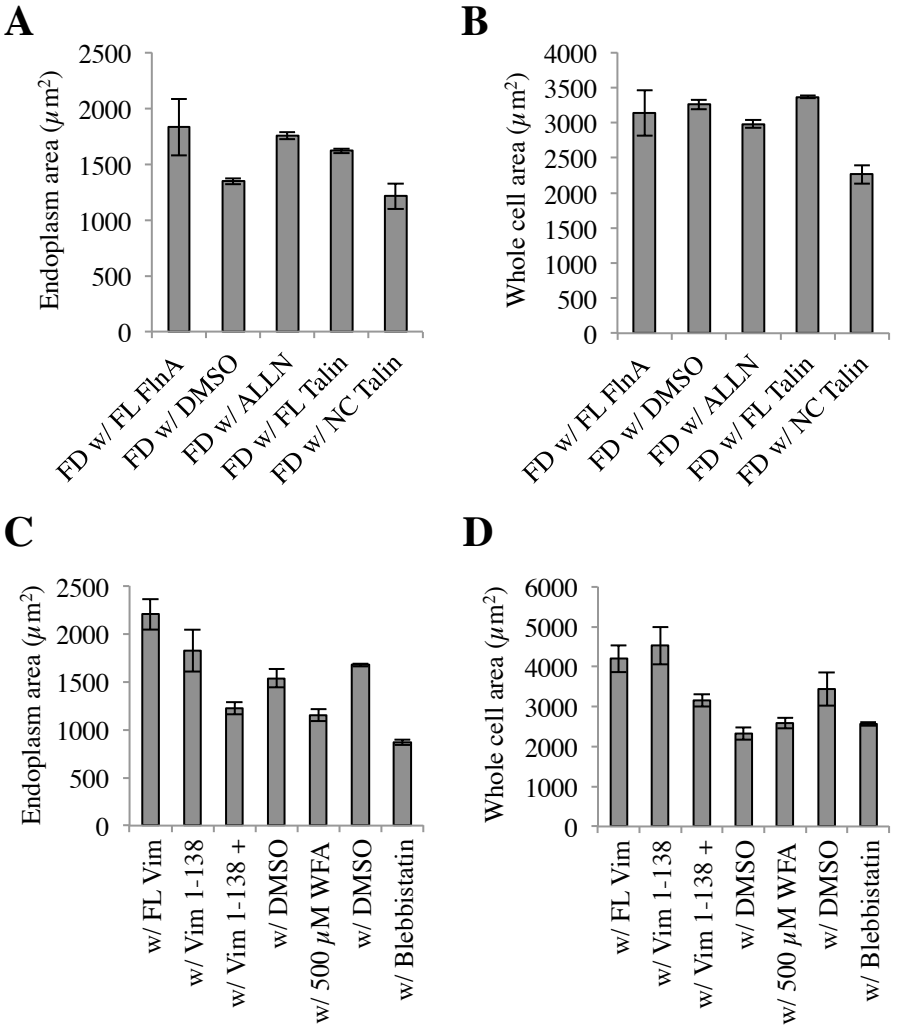
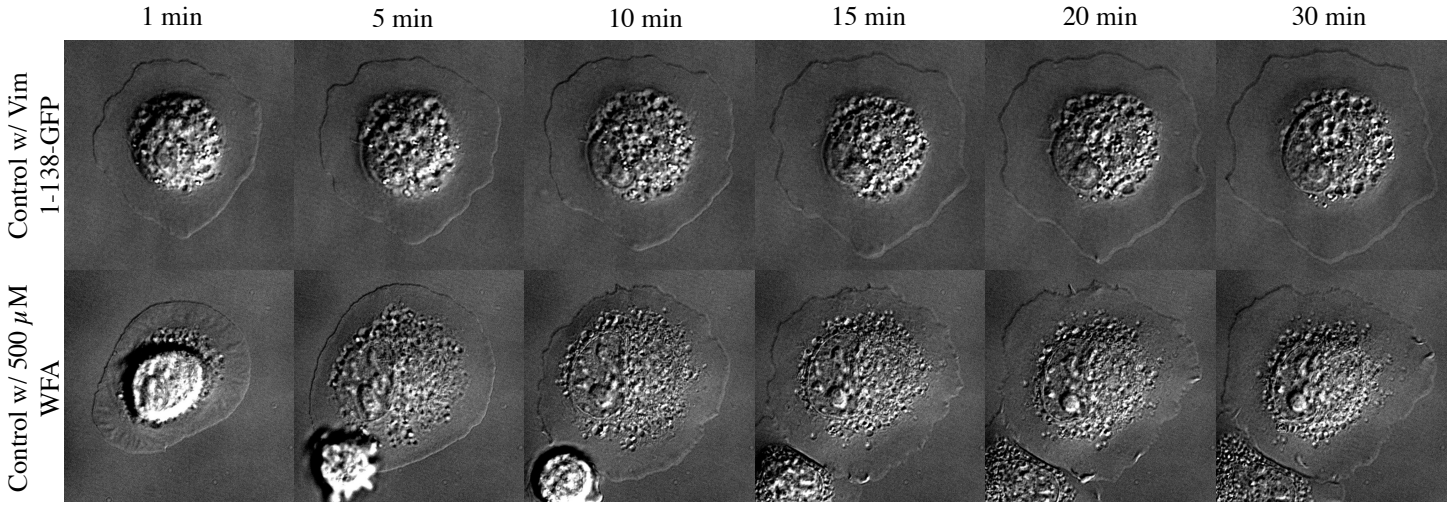


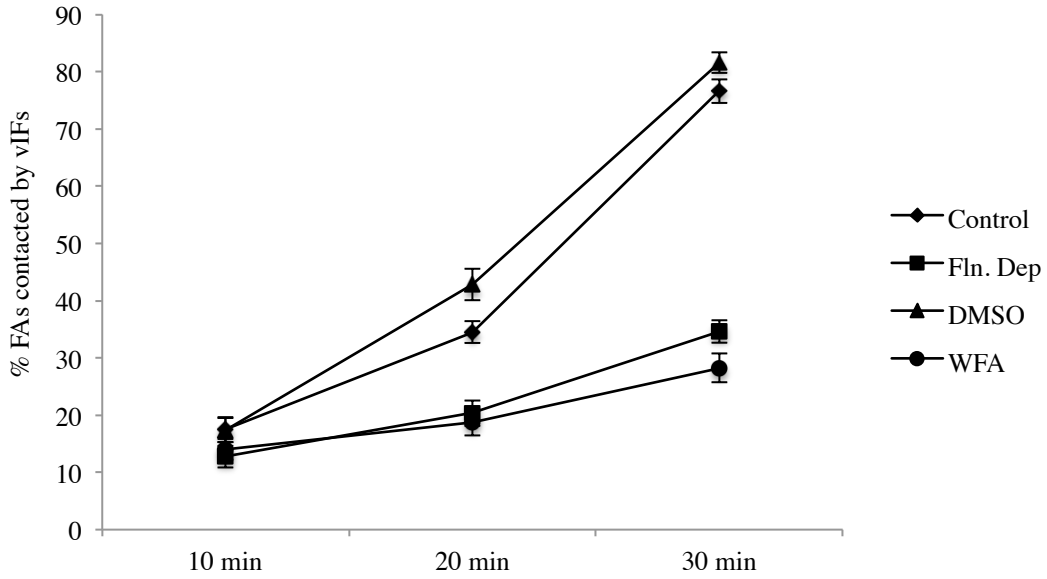
Supplemental Figure 1: Average areas of individual peripheral focal adhesions correlate with total focal adhesion areas. Total focal adhesion area measurements were generated for each cell type shown in Figures 1E, 2F, and 3I by thresholding paxillin signal and deleting the central non-adhesive background. For each cell type, n = >13 cells, at least 3 experiments per cell type.



Supplemental Figure 2: Absolute values of endoplasm and whole cell areas. A) Endoplasm areas that were used to compute endoplasm/whole cell area ratios from Figures 1I and 2C are shown. B) Same as A, but instead showing whole cell areas. C) Endoplasm areas that were used to compute endoplasm/whole cell area ratios from Figures 3H, 4D, and 6B are shown. D) Same as C, but instead showing whole cell areas. Values from A and B are from Fln-depleted MEFs, treated as labeled. Values from C and D are from control cells treated as labeled.



Supplemental Figure 3: Cells exposed to vimentin inhibitors exhibit continuous spreading of the edge without rounding up. A) Control MEFs were transfected with Vim 1-138-GFP overnight, trypsinized, resuspended, and spread on FN-coated glass for 30 minutes. B) Control MEFs were incubated for 22 hours in 500μM Withaferin A, trypsinized, resuspended, and spread on FN-coated glass for 30 minutes. Neither treatment resulted in cells rounding up during the spreading process.



Supplemental Figure 4: Vimentin intermediate filaments contact focal adhesions throughout cell spreading. Each cell type was plated on FN-coated glass for 30 minutes, fixed, and stained for vimentin and paxillin. FA plaques co-localizing with vIF tips were quantified as a percentage of total FA plaques (13 cells per cell type per time point).