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Supporting Material

Actomyosin tension exerted on the nucleus through nesprin-1 connections influences endothelial cell adhesion, migration and cyclic strain induced reorientation

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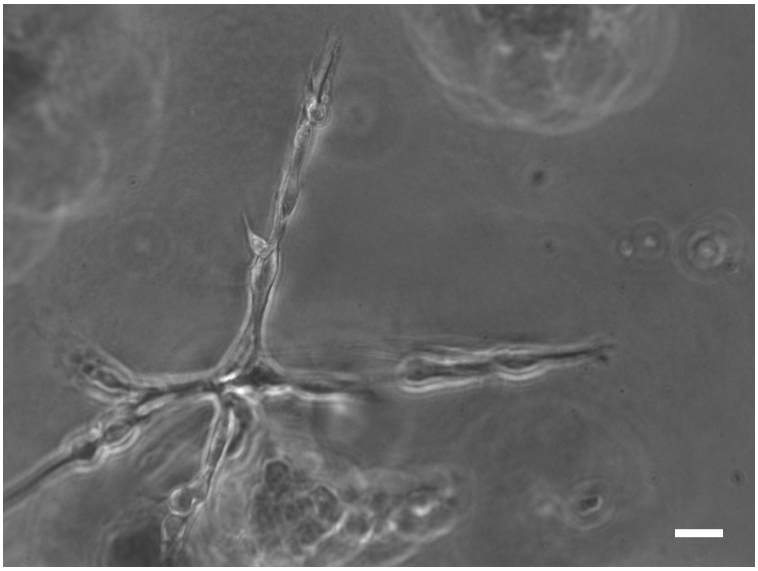
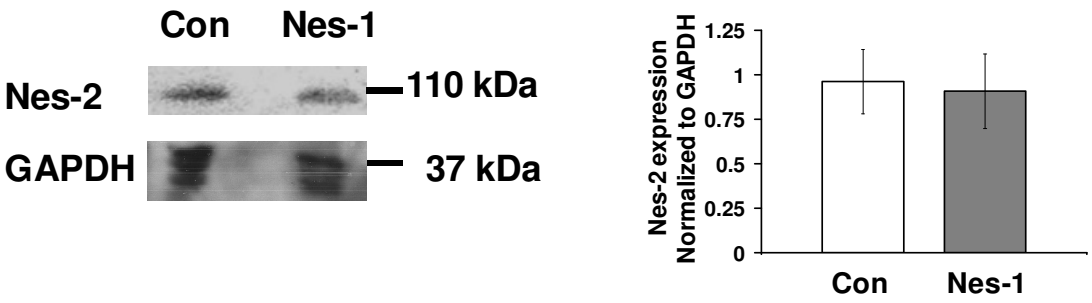
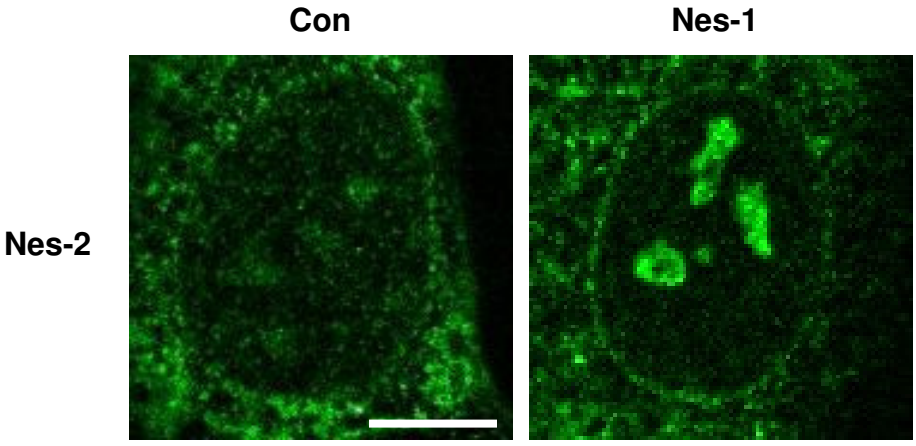


Supp. Fig. 1. In vitro 3D Angiogenesis assay of HUVEC cells: HUVECs were mixed with Matrigel and monitored for their ability make tubular structures (angiogenesis) for 2 weeks as described in Materials and Methods. The representative image shows tube formation by HUVECs in 3D matrigel confirming that these cells are functional endothelial cells.

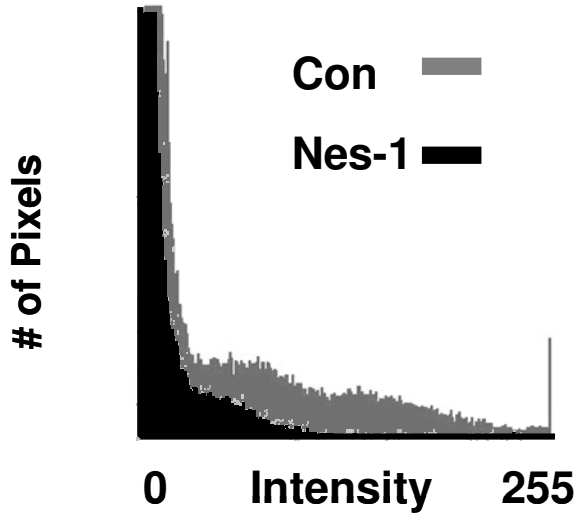


Supp. Fig. 2. Western blots (A) and their quantification (B) showing that Nesprin-2 (Nes-2) expression is unchanged in cells depleted of Nes-1. Error bars represent SEM; differences are not statistically significant.



Supp Fig 3: Nesprin-2 is present in the nuclear envelope in nesprin-1 deficient cells. Cells were transfected with control and nesprin-1 targeting siRNA and cultured for 72 hours and then fixed as described in the methods section. Cells were imaged using a Leica confocal microscope equipped with a 63X objective.

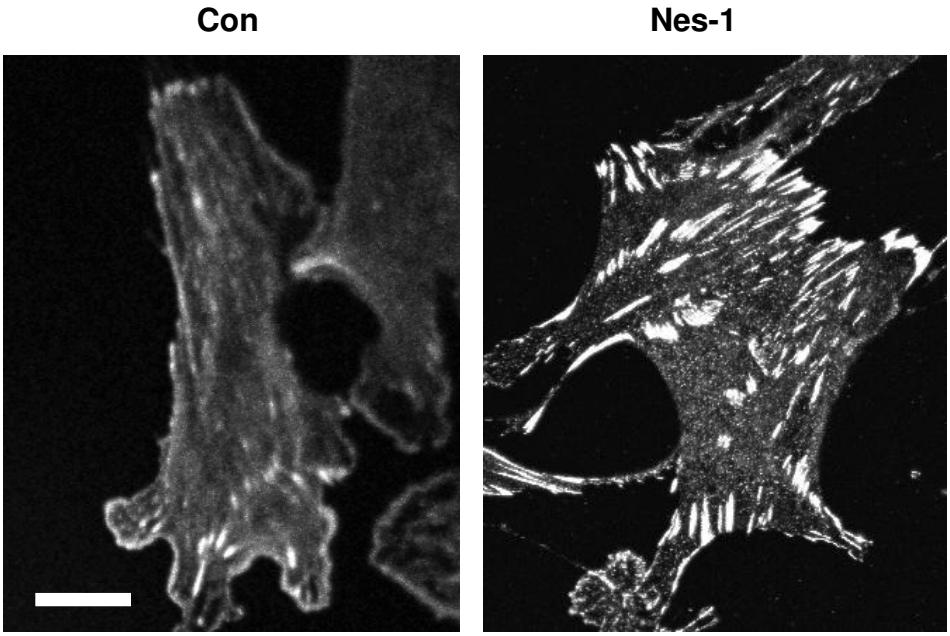
A



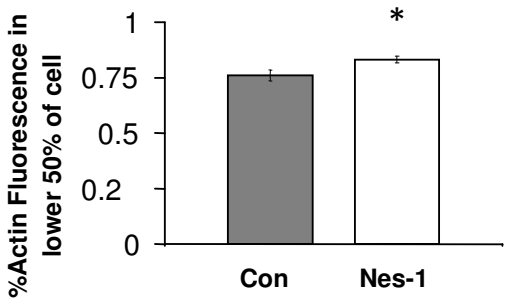
B

	Mean	Stdev
Con	50.1	59.3
Nes-1	15.3	20.1

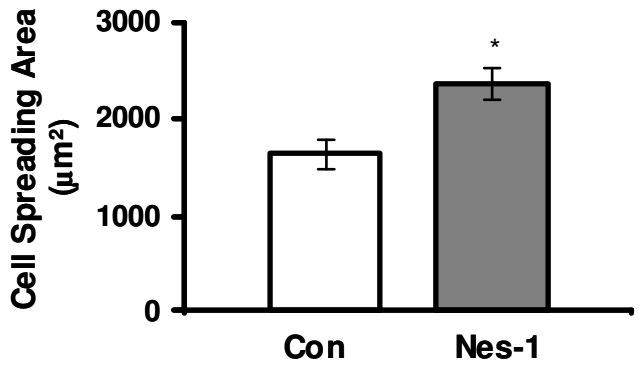
Supp. Fig. 4. A) Histogram comparison of pixel intensity of cells transfected with control and nesprin-1 siRNA immuostained for nesprin-1. B) Quantification of the pixel intensity shows a significant decrease in nesprin-1 expression in nes-1 siRNA treated cells compared to control siRNA treated cells.



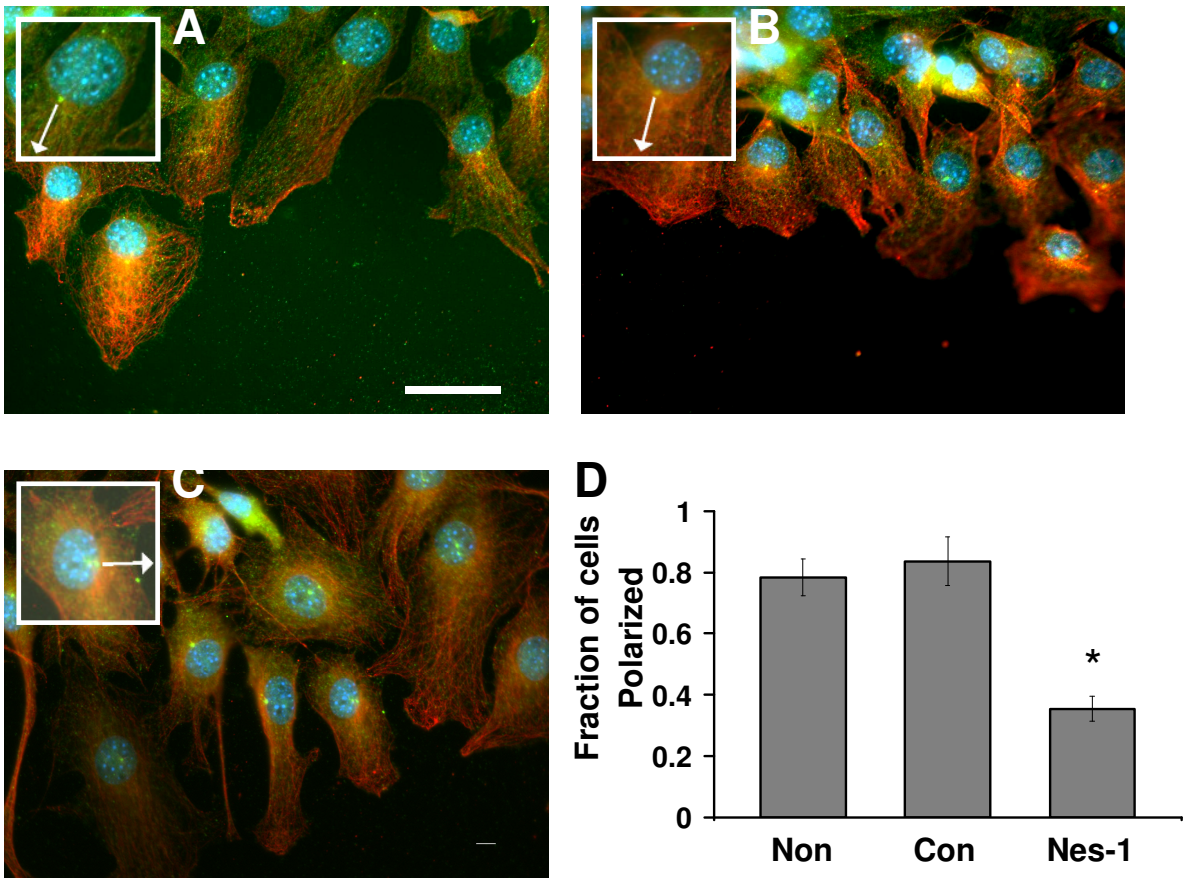
Supp. Fig. 5. Nesprin-1 deficient cells have an increased number of focal adhesions. Cells were cultured on fibronectin coated glass bottomed dishes (MatTek) for 24 hours then fixed and stained for vinculin. Confocal images at the base of each cell were taken to focal adhesion number and area. Scale bar is 15 um



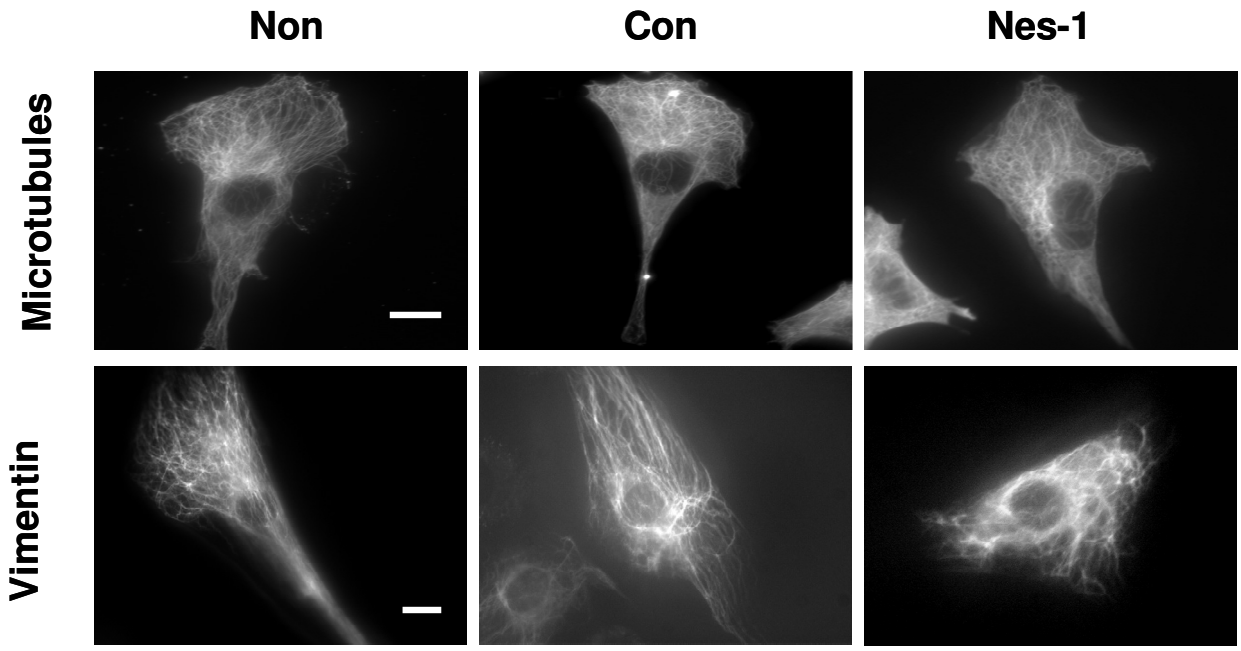
Supp. Fig. 6. Reduction of nesprin-1 expression shifts the distribution of F-actin toward the base of the cell. Cells transfected with control siRNA and siRNA targeting nesprin-1 were stained for F-actin and z-stack images were collected. The distribution of F-actin was measured along a single line through the highest point of the cell. The distribution was normalized and the sum of the fluorescent intensity was compared for the lower 50% of the cell.



Supp. Fig. 7: Cell spreading area increases in nesprin deficient cells. HUVECs were cultured on fibronectin coated glass bottomed dishes for 8 hrs and were then imaged on a Nikon E2000 microscope equipped with a 10X planar objective. Nikon Elements software was used to measure 15 cells in each condition. * Indicates $p<0.05$.



Supp. Fig. 8. Actomyosin forces are decoupled from the nucleus in the absence of nesprin-1. HUVEC cells serum-starved overnight were wounded and treated with LPA. LPA treatment triggers rearward actomyosin flow that moves the nucleus away from the wounded edge(29). To quantify this, the cell polarization vector in non-transfected HUVECs (A) and cells transfected with control siRNA(B) was measured and found to be approximately perpendicular to the wounded edge. White arrows indicate the direction of polarization of each cell calculated by drawing a vector from the nuclear centroid to the γ-tubulin stained centrosome . C) Nesprin-1 deficent cells are unable to polarize as is clear from the random directions of the cell polarization vector with respect to the wounded edge. Scale bar is 50 μm. All cells were stained for γ-tubulin (green) , F-actin (red) and for chromatin (blue). D) Quantification of cell polarization. Cells which were oriented within 30 degrees of a line perpendicular to the leading edge were quantified as polarized cells according to the approach in (7). The polarization defects in nesprin-1 deficient cells are statistically significant (*$p < 0.01$).



Supp. Fig. 9. Cytoskeletal organization in nes-1 deficient cells. There were no visible differences in microtubule and intermediate filament assembly in nes-1 deficient cells. Scale bars for both sets of images are 10 μm.