Supplementary Fig. 1A- Co-localization of NMMIIA with α2–integrin at collagen bead binding sites in gelsolin null and WT cells. **B-** Isolation of full-length NMMIIA from J744 macrophages. Lane 1 shows cell lysate, lane 2 is the crude actomyosin-enriched fraction, which was treated with MgATP to dissociate actin from myosin (lane 3). Lane 4 is the dialysed and concentrated NMMIIA sample. **C-** Myosin preparations immunoblotted for actin at different stages of purification. Middle lane is the crude preparation, left lane is the crude preparation treated with MgATP; right lane shows NMMIIA that has been treated with MgATP and this fraction shows no detectable actin contamination. **D-**Representative images obtained by electron microscopy of negatively-stained NMMIIA filaments diluted to 50 nM in buffer containing 150 mM KCl, 2 mM MgCl₂, 0.1 mM EGTA, 10 mM MOPS pH 7.0. **E-** Enhanced immunogold labeling of gelsolin on NMMIIA filaments compared with samples stained without primary antibody or NMMIIA filaments without gelsolin. **F -** Control experiment with GST alone shows no interaction with NMMIIA filaments. **G-** GST-Sepharose-bound gelsolin did not co-sediment with His-thioredoxin.

Supplementary Fig. 2A- Polymerization curves of G-actin (pyrene-labeled, 2 μ M actin) after addition of 0.05 μ M gelsolin or with varying concentrations of NMMIIA (0.025 μ M, 0.1 μ M, 0.2 μ M or 0.4 μ M) incubated with gelsolin (0.05 μ M). The fluorescence signal due to Cys-374bound pyrene is not altered by addition of the NMMIIA-gelsolin complex. **B-** NMMIIA alone had no effect on polymerization. **C-** End-point polymerization (overnight) due to capping of pyrene-labeled actin with 0.05 μ M gelsolin or with various concentrations of NMMIIA (0.025 μ M, 0.05 μ M, 0.1 μ M, or 0.2 μ M, 0.4 μ M) and gelsolin (0.05 μ M). **D-** NMMIIA alone had no effect on capping of pyrene-labeled actin.

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Supplementary Fig. 3 -**A**- Phase contrast images of cells plated on collagen-coated planar surfaces or on 2 μ m, 20 μ m and 45 μ M collagen-coated beads. **B**- Cells plated on collagen-coated beads of 20 μ m or 45 μ m diameter showed minimal collagen internalization. **C**- Gelsolin null or WT cells were plated on biotinylated collagen for 2 or 8 hours, trypsinized from plates and lysed. Cell lysates were separated by SDS-PAGE, transferred to membranes and the intracellular biotinylated collagen was probed with streptavidin peroxidase. The blots show more extensive collagen proteolysis after 2 and 8 hours in cells expressing gelsolin compared with gelsolin null cells. **D**-Treatment with blebbistatin (50 μ M) did not prevent targeting of NMMIIA and gelsolin to collagen bead sites.



Planar(no beads)

CCB2µm

ССВ20 µm

ССВ45 µm







Blebbistatin



A WΤ



100 nm

NMMIIA in high salt NMMIIA filaments

100 nm

purification of NMMIIA

Е

NMMIIA filaments plus gelsolin NMMIIA filaments only with primary antibody no primary antibody with primary antibody F G S Ρ S Ρ 250 -NMIIA 250 70 GST-gelsolin_ 130 (G1-6) 56 56 28 -GST 28 His-thio-redoxin-15

100



D





