Thomas et al., http://www.jcb.org/cgi/content/full/jcb.201502039/DC1

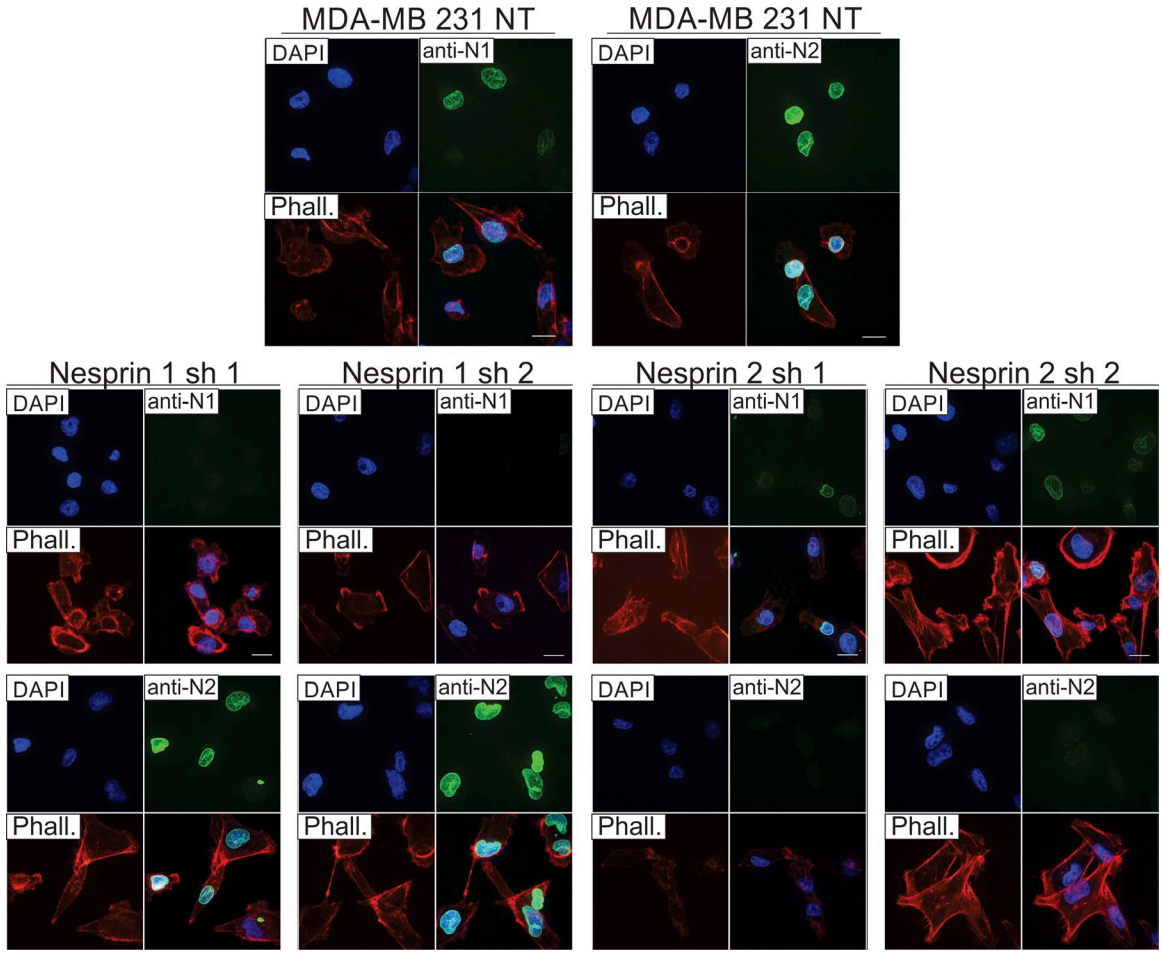
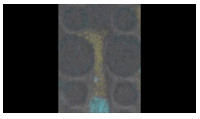
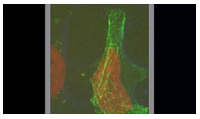


Figure S1. **Immunofluorescent imaging of nesprin isoform shRNA.** MDA-MB 231 cells were stably infected with lentivirus expressing shRNA constructs for nesprin-1 or -2, with two distinct shRNA sequences for each. Cells were stained using the mAbs MANNES1E or MANNES2G (MDA Monoclonal Antibody Resource) to label nesprin-1 or -2, respectively. Cells were colabeled with Alexa Fluor 568 Phalloidin (red) to visualize F-actin and DAPI (blue) to visualize nuclei. Bars, 20 µm.



Video 1. **Time-lapse video of MDA-MB 231 cell migrating through a 5-µm-wide pore of the 3D migration device.** MDA-MB 231 cells were infected with lentivirus for stable expression of histone 2B–CFP fusion to mark the nucleus (blue). The cells are also expressing YPF to label the cytoplasm (yellow). Images were analyzed using time-lapse spinning disk confocal microscopy (UltraView VOX laser system [PerkinElmer]; DM1 6100 base [Leica]; 63× oil/NA 1.47 at 37°C and 5% CO_2). Images were acquired every 5 min. Length of time-lapse is ∼3 h.



Video 2. **Formation of NMIIB stress fibers around nucleus.** MDA-MB 231 NMIIB shRNA cells were infected with lentivirus for stable expression of histone 2B–RFP fusion to mark the nucleus (red). The cells are also transiently transfected with NMIIB-GFP construct to label myosin IIB fibers (green). Images were analyzed using time-lapse spinning disk confocal microscopy (UltraView VOX laser system [Perkin-Elmer]; DM1 6100 base [Leica]; 63× oil/NA 1.47 at 37°C and 5% CO_2). Images were acquired every 5 min. Length of time-lapse is ∼4 h.