

ARTICLE

Mechanically induced septin networks protect nuclear integrity

Margaret E. Utgaard¹, Alexia Caillier¹, Shreya Chandrasekar¹, Joseph J. Tidei¹, Harikrushnan Balasubramanian², Rachel M. Lee², Owen Puls², Satya Khuon², Jesse Aaron², Teng-Leong Chew², Jordan R. Beach¹, and Patrick W. Oakes¹

The cytoskeleton is a key mediator of mechanical interactions in cells, but specific contributions of septins remain unclear. Septins preferentially localize with a subset of actin stress fibers positioned under the nucleus, where they are situated between the membrane and stress fibers. Removing the nucleus from the cell results in the loss of these subnuclear septin-decorated stress fibers. Surprisingly, however, their formation can be rescued using a large glass bead in place of the nucleus. Similarly, applying a compressive force to the cell via confinement, whether externally or through internally generated actomyosin forces, results in increased septin accumulation in regions where the nucleus engages the cell cortex. Finally, loss of septin filaments via knockdown of SEPT7 increases nuclear membrane rupture during confinement. Together, these data argue that septins act as a dynamic mechanosensitive protective network to buffer mechanical forces on the nucleus.

Introduction

Physiological environments are crowded. Cells are surrounded by other cells and dense extracellular matrix, whose mechanical properties change throughout development and disease. When coupled with the internally generated forces from the actomyosin cytoskeleton, these confined architectures apply significant stress and strain on cells, even when cells appear stationary (Yamada and Sixt, 2019). On account of its size and stiffness, the nucleus is particularly sensitive to mechanical forces and is often the limiting factor for navigating through confined spaces (Lammerding, 2011; Denais et al., 2016; Lele et al., 2018; Renkawitz et al., 2019). While deformation of the nucleus can contribute to downstream mechanotransduction (Lomakin et al., 2020; Venturini et al., 2020), excessive deformation can cause DNA damage, loss of nuclear integrity, and ultimately apoptosis (Denais et al., 2016; Lindenboim et al., 2020). Cells therefore must employ defenses to buffer these mechanical forces.

The cytoskeleton is largely responsible for regulating both a cell's morphology and its mechanical properties. As dynamic networks of filaments, the organization and architecture of the cytoskeleton are responsive to a variety of biochemical and mechanical cues. Generally, myosin motors pulling on the actin cytoskeleton create contractile networks that keep cells globally under tension, while microtubules and intermediate filaments aid in resisting compressive forces (Pegoraro et al., 2017). Each of these cytoskeletal networks has unique mechanical properties that can change both spatially and temporally in response to

changing environmental conditions (Weber et al., 2012; Pegoraro et al., 2017). Complicating the matter, they do not operate independently, and interactions with one another can further modulate their behavior and mechanics (Esue et al., 2006; Pelletier et al., 2009; Farhadi et al., 2020; Conboy et al., 2026).

The septin cytoskeleton is most well-known for its role in cell division but also prominently localizes to subnuclear stress fibers and other cytoskeletal structures during interphase, where they likely scaffold signaling molecules (Kinoshita et al., 2002; Kremer et al., 2007; Calvo et al., 2015; Martins et al., 2023; Sturgess et al., 2024). Septins largely form heteromeric palindromic subunits, comprised of monomers from three or four different families of septin genes (Cavini et al., 2021; Martins et al., 2023). Like other cytoskeletal elements, septin subunits dynamically assemble into filaments and can bundle and crosslink into larger networks (Kinoshita et al., 2002; Cavini et al., 2021). Septins can also directly bind and bundle both actin and microtubules, providing a clear connection between these networks (Bai et al., 2013; Mavrikakis et al., 2014; Spiliotis, 2018; Nakos et al., 2019; Tomasso et al., 2025). Intriguingly, septins are found at many sites where there are large applied forces, such as the contractile ring, sperm annulus, cilia base, and stress fibers (Ihara et al., 2005; Mostowy and Cossart, 2012; Lam and Calvo, 2018). Despite these localizations and interactions, however, septins' direct role in mechanotransduction remains ill-defined.

¹Department Cell & Molecular Physiology, Stritch School of Medicine, Loyola University Chicago, Maywood, IL, USA; ²Advanced Imaging Center and Integrative Imaging, Howard Hughes Medical Institute Janelia Research Campus, Ashburn, VA, USA.

Correspondence to Patrick W. Oakes: poakes@luc.edu; Jordan R. Beach: jbeach1@luc.edu.

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Here, we set out to investigate why septins preferentially localize to the subset of actin stress fibers located around the nucleus. We first created an endogenously tagged SEPT2 fibroblast cell line to visualize septins in live imaging under various perturbations. We observe that perinuclear septin localization is dynamic, moving with the nucleus during migration, and that it is present in both 3D and on substrates of physiological stiffness. Using a combination of super-resolution microscopy approaches, we find that septins are typically positioned below actin stress fibers, closer to the membrane. Enucleating cells results in the loss of perinuclear septin fibers but can be partially recovered by introducing large glass beads into the cytoplasm, indicating a role for mechanics in septin formation. Applying a compressive force to the cells via confinement results in increased septin accumulation under the nucleus, and this behavior is mimicked as cells squeeze themselves through constrictions in a microfluidic device. In each case, septins accumulate in regions where the nucleus is pressed against the cortex. Lastly, we knock down SEPT7 and show that without these septin networks, nuclei are more susceptible to deformation under strain and ultimately more likely to rupture in confinement. Together, these findings argue that septins have a novel role in mechanotransduction, specifically by playing a dynamic protective role buffering mechanical forces on the nucleus.

Results

Septins form a dynamic network around the nucleus

As perturbation of individual septin genes has been previously reported to affect expression levels of other septin monomers that comprise the heteromeric subunit (Kinoshita et al., 2002), we used CRISPR/Cas9 to endogenously tag SEPT2 with a C-terminal HaloTag to visualize septins in a fibroblast cell line (SEPT2-Halo; Fig. S1 A). SEPT2-Halo perfectly colocalized with antibody staining of SEPT2, SEPT7, and SEPT9, indicating that it can successfully incorporate into filaments, while western blots revealed no impact on protein levels (Fig. S1, B and C). Similar to previous reports (Kinoshita et al., 2002; Kremer et al., 2007; Calvo et al., 2015), these stainings reveal septin accumulation along portions of the cortex and a robust subnuclear septin network that aligns with actin stress fibers (Fig. 1 A and Fig. S1 B). This nuclear network is not exclusive to the ventral surface, as enrichment also occurs along actin stress fibers above the nucleus on the dorsal surface (Fig. 1 A). Comparable accumulations are seen around the nucleus when cells are plated on elastic substrates or embedded in 3D collagen matrices, indicating that this localization occurs regardless of the substrate stiffness and architecture (Fig. S2).

During migration, this perinuclear septin network dynamically reorganizes as the cell changes morphology (Fig. 1 B and Video 1). Specifically, as the cell moves, the septin network reorganizes to remain primarily under the nucleus, although extensions beyond the nuclear periphery occur. To quantify this spatial correlation, we measured the cell centroid, nuclear centroid, and center of intensity of the prominent perinuclear septin network (Fig. 1 C). For all cells, the center of the septin

network was positively biased toward the nucleus relative to the cell centroid, supporting a model that the ventral septin network and the nucleus are spatially coupled (Fig. 1 C).

Septin networks form between the actin and plasma membrane

Given the clear relationship between septins, actin, and the nucleus, we sought to gain a better understanding of the organization of septins and actin in these structures. We performed 3D structured-illumination microscopy (SIM) and found that the prominent septin filaments formed both below and above, but not along the sides of the nucleus (Fig. 2 A). Further underscoring this point, the subnuclear actin and septin fibers often extended beyond the boundary of the nucleus along the cortex (Fig. 1 A and Fig. 2 A). These observations suggest that these septin and actin networks are situated at the cortex and not the nuclear envelope.

To explore this hypothesis further, we used interference photoactivation localization microscopy (iPALM) to further resolve the spatial organization of the actin and septin networks under the nucleus. iPALM imaging, which provides nanometer-scale lateral and axial resolution, reveals that despite having overlapping X-Y localizations, septins are consistently positioned below actin filaments (Fig. 2, B and C). To quantify this separation, we isolated individual subnuclear fibers and measured the difference in the median position of the septin and actin localization events to be ~ 40 nm (42 ± 22 nm; Fig. 2 D). While this distribution was broad, it was consistently positive. This distinct separation of the septin and actin network suggests septins could potentially have a unique role in cortical and nuclear mechanics.

Externally applied forces induce septin network formation under the nucleus

To investigate the role of mechanical signaling in stimulating septin network formation, we used a previously developed dynamic confinement system to observe septin assembly in response to external compression (Berre et al., 2012). We chose a confinement height of 5 μ m to apply compressive pressure to the nucleus without causing catastrophic mechanical damage. Upon compression, septin intensity accumulates under the nucleus (Fig. 3, A and B; and Video 2). While the compression occurs rapidly (< 1 min), the changes in septin distribution occur over tens of minutes (Fig. 3 A), indicating that the subnuclear signal increase is not due to pushing pre-assembled septin networks down into the ventral imaging plane. Upon release of compression, the septin accumulation under the nucleus returns to pre-compression levels (Fig. 3, A and B; and Video 2). Similar results were obtained for septin accumulation above the nucleus on the dorsal surface (Fig. 3, C and D). Imaging of actin, using EGFP-Ftractin, and myosin, using EGFP-non-muscle myosin 2A (EGFP-NM2A), reveals similar increases in these cytoskeletal components under the nucleus (Fig. S3, A and B). These data demonstrate that nuclear compression into the cortex via extracellularly applied force elicits a septin assembly response.

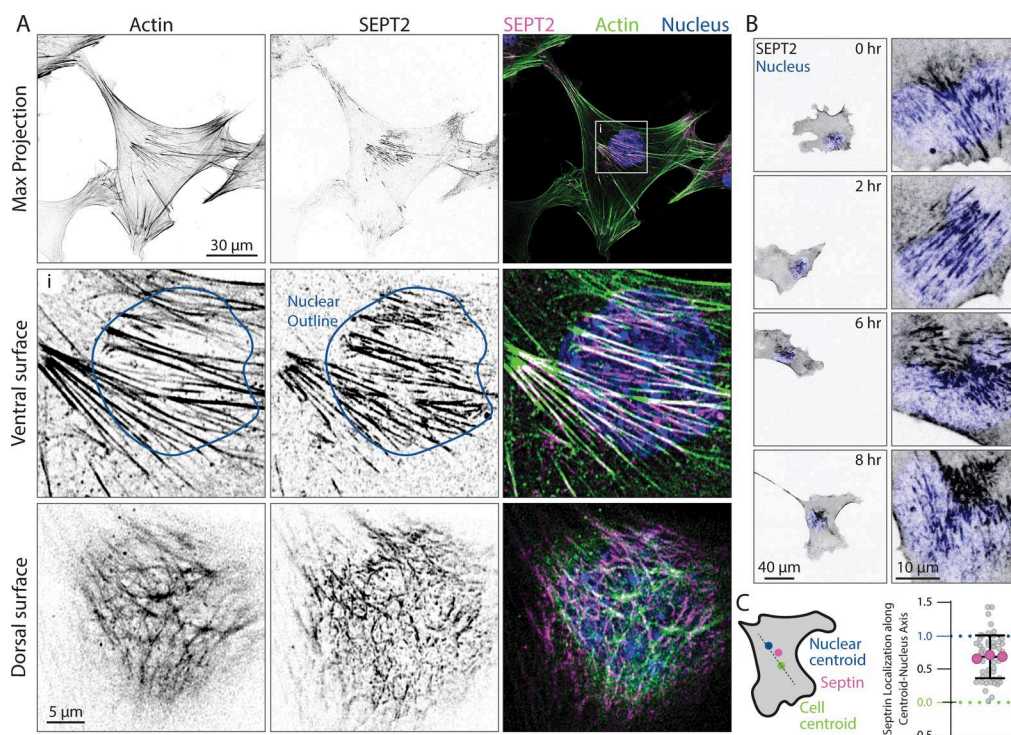


Figure 1. Septins both coincide with and move with the nucleus. (A) Fibroblasts were fixed, immunolabeled with SEPT2 antibody (magenta), and stained for actin (phalloidin; green) and nuclei (DAPI; blue). The top row is a max projection of the whole cell, while the middle and bottom rows are single confocal slices of cropped regions (white box) above and below the nucleus. (B) SEPT2-Halo cells stained with Hoechst nuclear dye were imaged for 12 h (Video 1). Selected time points are shown of the whole cell in the left column and cropped regions near the nucleus in the right column. (C) The center of intensity of the ventromedial septin network along the axis running from the cell centroid, defined as 0, to the nuclear centroid, defined as 1, was identified in SEPT2-Halo cells stained for nuclei (DAPI). Positive values indicate a septin preference toward the nucleus relative to the cell centroid. Data were collected from 48 cells (gray dots) from three independent experiments (means plotted as pink dots), with error bars indicating mean $\pm$ SD.

Cell-generated forces induce septin network formation around the nucleus

While our data indicate that extracellular forces can induce septin network formation, we sought to test whether forces generated by the actomyosin cytoskeleton could elicit a similar response. We imaged SEPT2-Halo cells migrating through a microfluidic device with different features, including narrow gaps that would induce nuclear compression as the cell squeezed through (Keys et al., 2018). We observe robust septin accumulation along the membrane as the nucleus is squeezed through constrictions (Fig. 4, A and B; and Videos 3 and 4). Interestingly, this behavior is not restricted to these narrow passages, as we observe septin enrichment whenever the nucleus is pushed against the walls of any features in the device (Fig. 4, C–E and Video 4). To quantify this, we measured the average fold increase in septin intensity along the wall as a function of the cortex distance from the nucleus. We found an average septin intensity increase of $\sim 30\%$ when the nucleus was pushed against any wall in the device. While previous studies have shown that septins preferentially recognize and assemble on micron-scaled curved membranes (Bridges et al., 2016; Cannon et al., 2019; Nakazawa et al., 2023), we found no difference in accumulation based on the geometry of the wall in our device (Fig. S3, C–F), although our smallest scale features were on the order of $3\ \mu\text{m}$. Together, these data indicate that intracellular force production

is sufficient for nuclear-induced septin assembly even in the absence of high local plasma membrane curvature.

Mechanical signaling drives septin network formation

We next sought to test whether the nucleus is specifically required for eliciting this septin accumulation. Cells were centrifuged in a density gradient to remove the nucleus (Graham et al., 2018), and the resulting cytoplasts were plated and imaged. Cytoplasts exhibit dynamic actin polymerization, including being able to migrate, but lack central septin and actin networks like their cell counterparts (Fig. 5 A). To quantify this difference, we measured septin assembly using local intensity and septin organization using a fiber alignment tool (Marcotti et al., 2021). We found that cytoplasts failed to assemble and organize septin networks in the cell center where the perinuclear filaments typically form (Fig. 5 B). Previous work used internalized glass beads to mimic organelle-scale mechanical stimuli in migrating dendritic cells (Thiam et al., 2016). Therefore, to test whether these central septin networks are dependent on signaling molecules associated with the nucleus or dependent on a mechanical stimulus, we added large glass beads with a diameter between $8\text{--}12\ \mu\text{m}$ into both cells and cytoplasts (Fig. 5 C). In cells, internalized beads often accumulate in the cell center near the nucleus, with the septin network assembling under both the nucleus and beads (Fig. S4, A and B). In cytoplasts, the glass

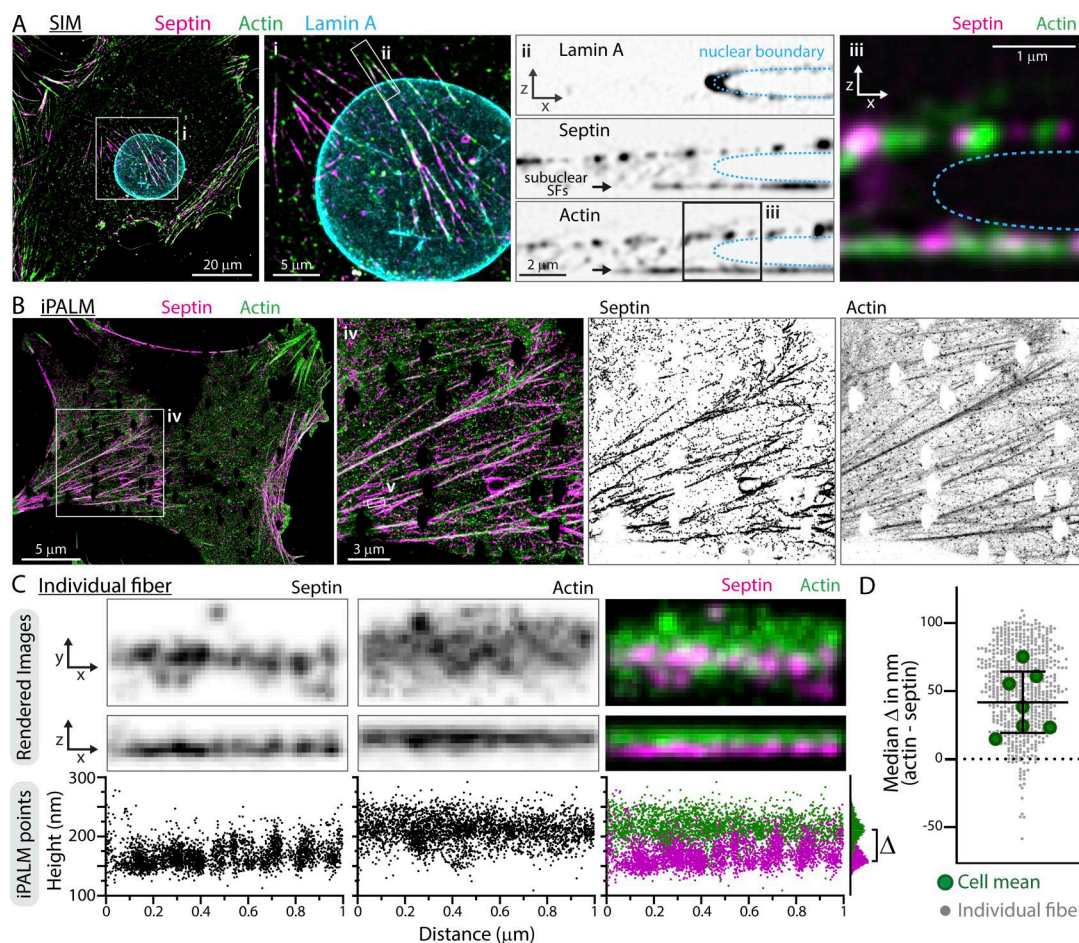


Figure 2. Septins are positioned along the cortex below actin. (A) SIM imaging of SEPT2-Halo, actin (FastAct_X), and mEmerald-Lamin A. Whole-cell and nuclear zooms are mean projections of short ventral z-stacks for actin and septin, and a mean projection of the entire z-stack for lamin A. Individual inverted grayscale and merged orthogonal images in (ii and iii) are mean projections, with the nuclear boundary indicated by a dashed cyan line. **(B)** Whole-cell and subnuclear regions of rendered iPALM images. **(C)** Rendered individual fiber (top), rendered orthogonal view (middle), and orthogonal plot of iPALM points from the same fiber (bottom). **(D)** The difference (Δ) between the median actin and median septin height was calculated for 599 fibers from 7 cells and plotted for each fiber (gray dots) and cell means (green dots), with error bars indicating the mean $\pm$ SD of cell means.

beads at least partially rescued both the assembly and organization of the septin network (Fig. 5, B and C). Disruption of the actin cytoskeleton with cytochalasin D, however, abolishes this response to internalized beads, indicating that actin is also necessary (Fig. S4, C and D). Together, these results indicate that perinuclear septin network formation is not entirely dependent on nuclear signals and can be at least partially induced via a physical object. These findings demonstrate a clear role for mechanical signaling in mediating the formation of ventral septin networks.

Septins buffer the nucleus during mechanical strain

As our data suggest that septin network formation is responsive to mechanical stimuli, we sought to test the contribution of septins during confined migration. We therefore knocked down SEPT7 (SEPT7-KD) in our SEPT2-Halo cells, as SEPT7 is required for septin octamer and hexamer formation (Cavini et al., 2021). Relative to non-targeting (NT) controls, SEPT7-KD cells have decreased expression of both SEPT7 and SEPT2 (Fig. 6, A and B). Imaging of SEPT2-Halo also reveals minimal remnant septin

signal and no apparent actin-septin ventral network, suggesting broad septin network disruption. We plated SEPT2-Halo and SEPT7-KD cells on transwells with 3-μm pores and compared nuclear morphology between groups before and after migration through the pores. Nuclear morphology was indistinguishable in control cells on either side of the transwell (Fig. 6, C and D). In contrast, nuclei of SEPT7-KD cells that migrate through the pores exhibit deformed morphologies and significantly increased aspect ratios (Fig. 6, C and D).

While we detected differences in the transwell assay, we suspected that many cells traversed overlapping pores $>3\ \mu\text{m}$ due to the random distribution of pores (Fig. S5). We therefore sought to test the response of the SEPT7-KD cells to direct compression. We exogenously expressed a 3xNLS-GFP to visualize the integrity of the nuclear envelope and measured the rate of nuclear rupture when the cells were compressed to a height of 2.5 μm. While only ~20% of control nuclei rupture at this level of compression, ~50% of SEPT7-KD nuclei rupture (Fig. 6, E and F). These data thus reveal a critical role for septins in buffering mechanical forces on the nucleus.

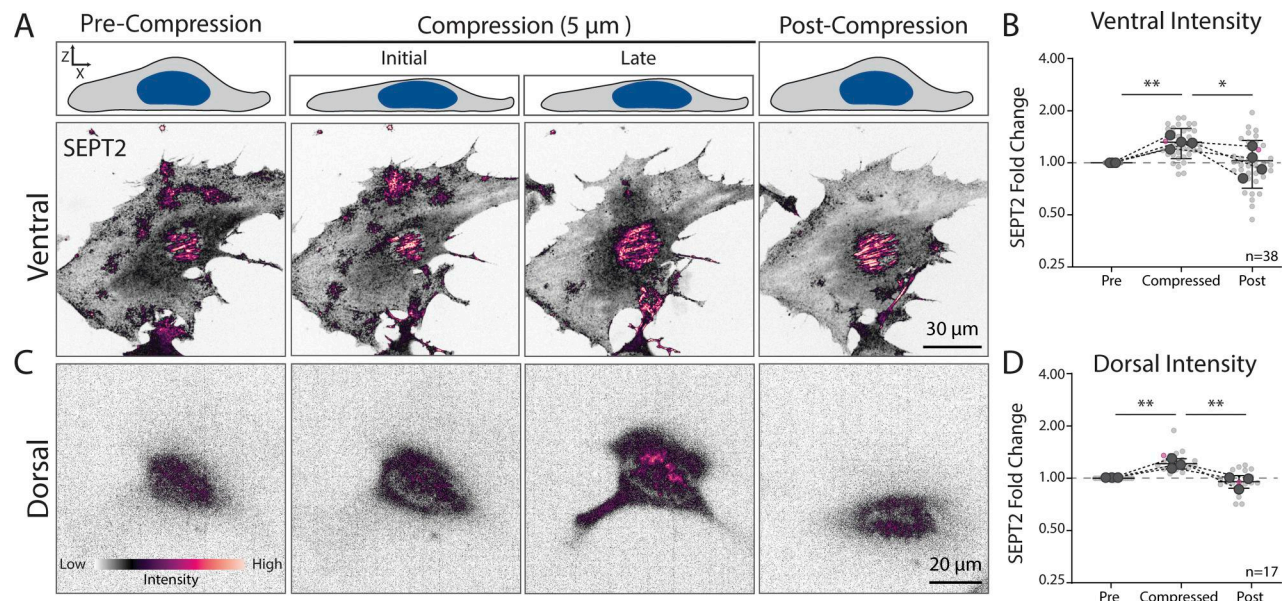


Figure 3. Compression increases perinuclear septin network assembly. (A) Representative single confocal slices of the ventral surface of SEPT2-Halo before, during, and after compression (Video 2). Initial compression images were taken immediately after confinement, while late images were taken after 30 min of confinement. (B) The fold intensity change for septin in the subnuclear regions of the cell. (C) Representative max projections of three confocal slices of the dorsal surface of SEPT2-Halo before, during, and after compression. (D) The fold intensity change for septin in the dorsal regions of the cell. Larger dots represent replicate means and smaller dots represent single cells. Representative image data points are indicated as pink dots. Bars indicate mean $\pm$ SD (ventral, $n = 38$; dorsal, $n = 17$; $N = 3$ biological replicates for each). The pink dots indicate the representative images shown in A and C. A nested 1 way ANOVA was used to determine significance, with $*$ = $P < 0.05$, $**$ = $P < 0.01$.

Discussion

The septin cytoskeleton has been implicated in mechanotransduction, but specific contributions remain poorly defined. Here, we used endogenously labeled SEPT2 fibroblasts to investigate the role of septins in responding to applied forces. Septins associate with a subpopulation of the actin cytoskeleton, with specific enrichment between the actin and membrane of perinuclear stress fibers (Figs. 1 and 2). We find that this septin accumulation is the result of the nucleus pushing on the cortex, and that this response can result from both internally or externally generated forces (Figs. 3 and 4). Strikingly, this response can be partially replicated simply by swapping the nucleus for a glass bead, indicating a clear role for mechanics in mediating this reaction (Fig. 5). Septin knockdown subsequently perturbs nuclear morphology following migration through constricted spaces and increases nuclear rupture upon compression (Fig. 6). Together, these data suggest that local septin accumulation reinforces the actin cytoskeleton in these regions to buffer forces applied on the nucleus.

It is intriguing that glass beads only partially rescue septin assembly and organization following enucleation (Fig. 5 B). One possibility is that other features of the nucleus besides its physical presence, such as the LINC complex, are contributing to recruiting or organizing the actin-septin network. Alternatively, this could simply be an experimental limitation. The glass beads we used are both smaller and less compressible than the nucleus, making their footprint of engagement with the cortex relatively smaller. Supporting this line of thinking, we often observe larger septin networks forming with multiple internalized beads (Fig. S4). Future experiments disrupting nuclear components and

using larger deformable beads (Vorselen et al., 2020) may clarify these results.

Interestingly, there is a subpopulation of cytoplasts that exhibit a robust ventral septin network (Fig. 5, B and C). Additionally, some cells migrating through microfluidic channels display cortical septin enrichment before the nucleus actually approaches the wall of the device (Fig. 4). We speculate that these septin networks are induced by other organelles being pushed into the cortex (i.e., ER, Golgi, etc.). This idea aligns with our bead data, arguing that the septin response is not a nuclear-specific response, but a general phenomenon to buffer intracellular material from mechanical forces.

This mechanoprotective role for septins is consistent with previous reports showing that knockdown of septins reduces global cortical stiffness (Tooley et al., 2009; Gilden and Krummel, 2010; Mostowy et al., 2011; Park et al., 2015) and that loss of SEPT9_i1 in particular resulted in nuclear deformation (Oklety et al., 2023). Our data, however, suggest a dynamic spatial component to this response, helping to target septins to specific regions of the cortex that are tensed. While actin appears to be a requisite collaborator in this mechanoprotective response, septins display more distinct localization than the omnipresent actin cytoskeleton, likely imparting unique mechanochemical properties on the actin-septin networks. A local reinforcement role could also explain why septins localize to other structures in the cell, such as the base of cilia (Hu et al., 2010), the sperm annulus (Ihara et al., 2005), neuronal spines (Ewers et al., 2014), and even the contractile ring—all regions where the cortex is likely under increased local stress. Similarly, it could explain septin accumulation at the base of blebs, where

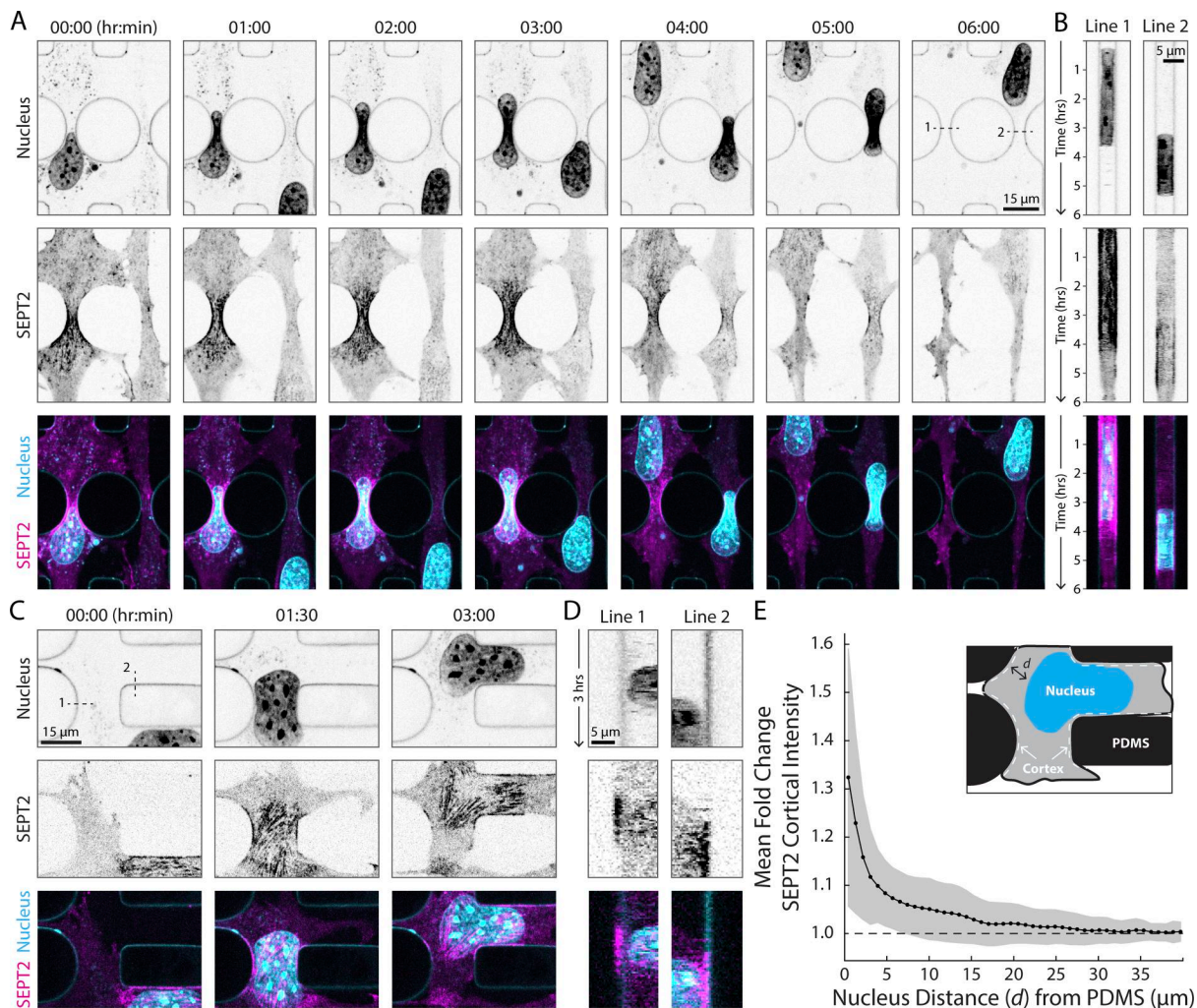


Figure 4. Cell generated forces induce septin assembly at the cortex when the nucleus is near. (A) Single confocal slices from a timelapse (Video 3) of SEPT2-Halo cells migrating through a microfluidic device showing septin accumulation as the cells squeeze the nucleus through narrow openings. (B) Kymographs of the dotted lines indicated in A showing septin accumulation around the nucleus during migration through the constriction point. (C) Single confocal slices from a timelapse (Video 4) showing septin accumulation occurs anywhere the nucleus pushes against the cortex. (D) Kymographs from dotted lines indicated in C showing septin accumulation coincides with the nucleus nearing the PDMS features. (E) Mean fold change in septin cortical assembly along the cortex as a function of distance from the nucleus. All data is normalized to the average SEPT2 intensity at the 30 μm distance (dashed line), representing the average cortex intensity in the absence of the nucleus. Points and shaded regions represent mean $\pm$ SD of all points along the cortex taken from 31 different cells, across 19 fields of view, from 3 different experiments. Time in hr:min.

septins are thought to help stabilize the ruptured cortex as it rebuilds (Gilden et al., 2012; Vadjnal et al., 2022; Weems et al., 2023; Stjepić et al., 2024).

Septin's role in buffering mechanical forces is most immediately reminiscent of a similar role ascribed to intermediate filaments (Herrmann and Aebi, 2016). Keratin, an intermediate filament, helps to maintain the mechanical integrity of epithelial sheets by anchoring the cell to the extracellular matrix through desmosomes, and disruption of this network results in blistering diseases (Coulombe, 2016). Similarly, vimentin is an intermediate filament expressed in mesenchymal cells that contributes to cell stiffness and buffers mechanical forces to protect the nucleus (Patteson et al., 2019). Like intermediate filaments, septins are nonpolar filaments that build larger networks and architectures through lateral association, but they also exhibit

key differences. While intermediate filaments are distributed throughout the cytosol, with some enrichment around the nucleus (Dupin et al., 2011), septins are primarily cortical. This localization, orchestrated by localized signaling (e.g., Rho family GTPases [Joberty et al., 2001; Calvo et al., 2015; Chandrasekar et al., 2026]) and binding partners (actin, membrane, phosphoinositides, etc. [Kinoshita, 2003; Bertin et al., 2010; Bridges et al., 2016; Karasmanis et al., 2019; Omrane et al., 2019]), could create heterogeneous subcellular mechanical support by septins, as opposed to broader cell-scale mechanical support provided by intermediate filaments. As our data further demonstrate that both extracellular forces upon the cell and intracellular forces generated within the cell can initiate cortical septin assembly, this suggests this mechanism can act at multiple force scales. In some ways, this multimodal force response is both reminiscent

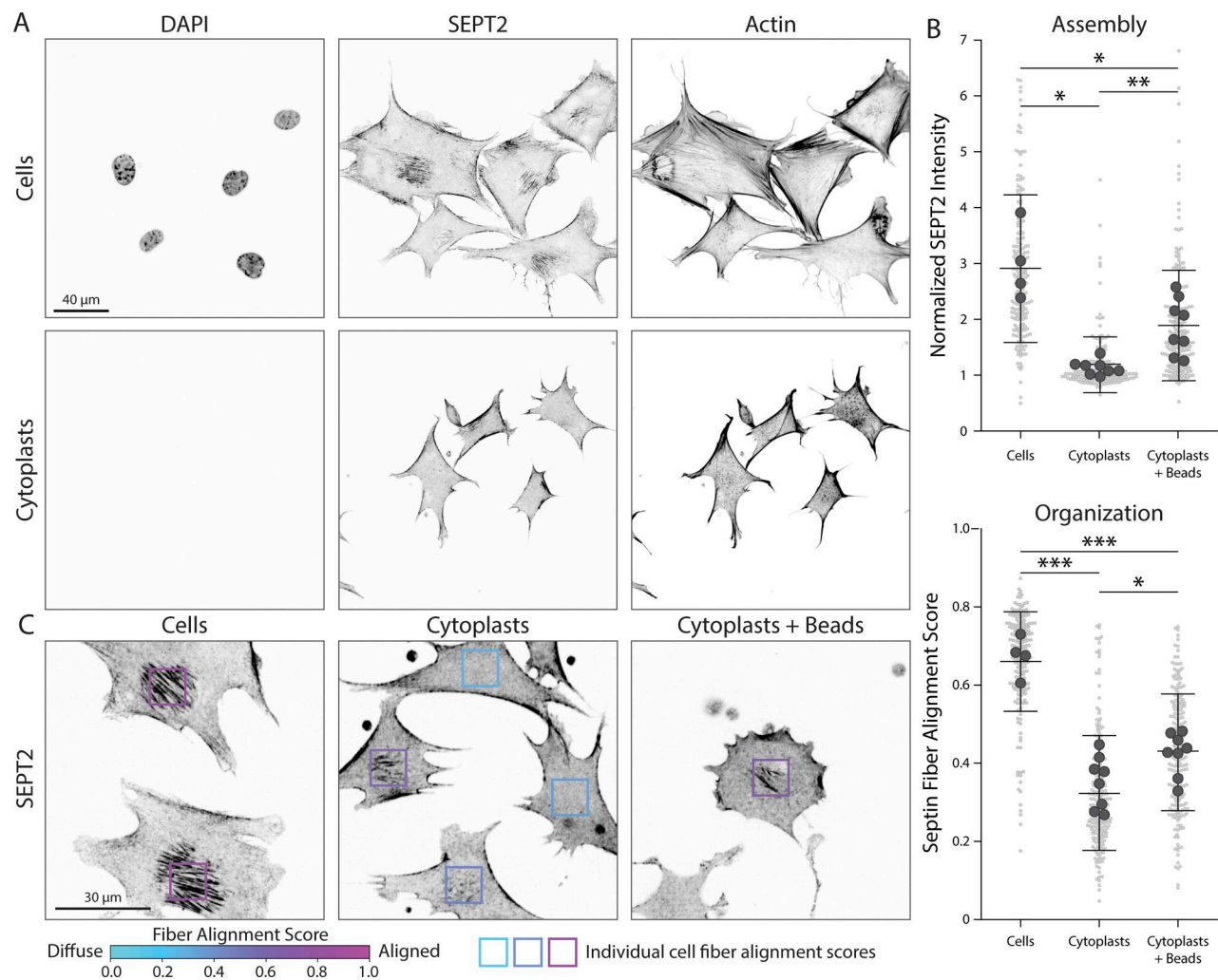


Figure 5. Mechanical signaling mediates ventral septin network formation. (A) Single confocal slices of SEPT2-Halo cells and cytoplasts fixed 2 h after plating and stained for actin (phalloidin) and nuclei (DAPI). (B) Quantification of septin assembly and organization in SEPT2-Halo cells, cytoplasts, or cytoplasts with beads. Assembly was measured by comparing the intensity of the central septin network to the surrounding cytoplasm. Organization was measured by determining the eccentricity of the FFT of the central septin network. (C) Single confocal slices of SEPT2-Halo cells, cytoplasts, or cytoplasts containing 8–12- μ m glass beads. Boxes indicate the regions analyzed for septin fiber formation. The box color indicates the magnitude of alignment from diffuse (0) to aligned (1) according to the range indicator below. Each gray dot represents a single cell, and bars indicate mean $\pm$ SD (cells $n = 196$, cytoplasts $n = 170$, cytoplasts + beads $n = 198$). Black dots represent the mean for each data set (cells, $N = 4$; cytoplasts, $N = 8$; cytoplasts + beads, $N = 8$). A one-way ANOVA was used to determine significance, with * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$. FFT, Fast Fourier Transform.

of and implicative of mechanosensitive ion channels, like Piezo, that respond to a range of mechanical forces (Lacroix and Wijerathne, 2025). Thus, while both intermediate filaments and septins may be mechanical buffers, we theorize the two networks are more likely parallel than redundant systems with unique responses to the spatial distribution, magnitude, direction, and kinetics of the applied force.

Exactly how septins enable nuclear mechanical resilience remains to be determined, but we speculate on some possible mechanisms and additional contributions. A simple “bed of nails” model is that perinuclear actomyosin and septin networks distribute the load more uniformly and broadly across the nucleus. Disruption of these networks results in asymmetric forces upon the nucleus, like squeezing a balloon at a single cross-section point, and leads to nuclear membrane rupture.

Alternatively, our iPALM data (Fig. 2) place septins between the actin fibers and the membrane, consistent with previous data and known interaction partners (Bertin et al., 2010; Bridges et al., 2016; Omrane et al., 2019; Martins et al., 2023). Therefore, septins could be acting as a type of lubricant during nuclear translocation, limiting nuclear engagement with cortical and plasma membrane components while allowing the actin fibers to couple to the nucleus and LINC complexes (Luxton et al., 2010; Antoku et al., 2015), similar to a role recently proposed for the endoplasmic reticulum (Janota et al., 2022). Septins in this capacity could also potentially alter local contractility to prevent a disrupted actin network from being shredded by myosin motors, or could be scaffolding, corraling, or sequestering cortical and transmembrane components for local signaling and structure (Caudron and Barral, 2009; Hu et al., 2010). Importantly, these

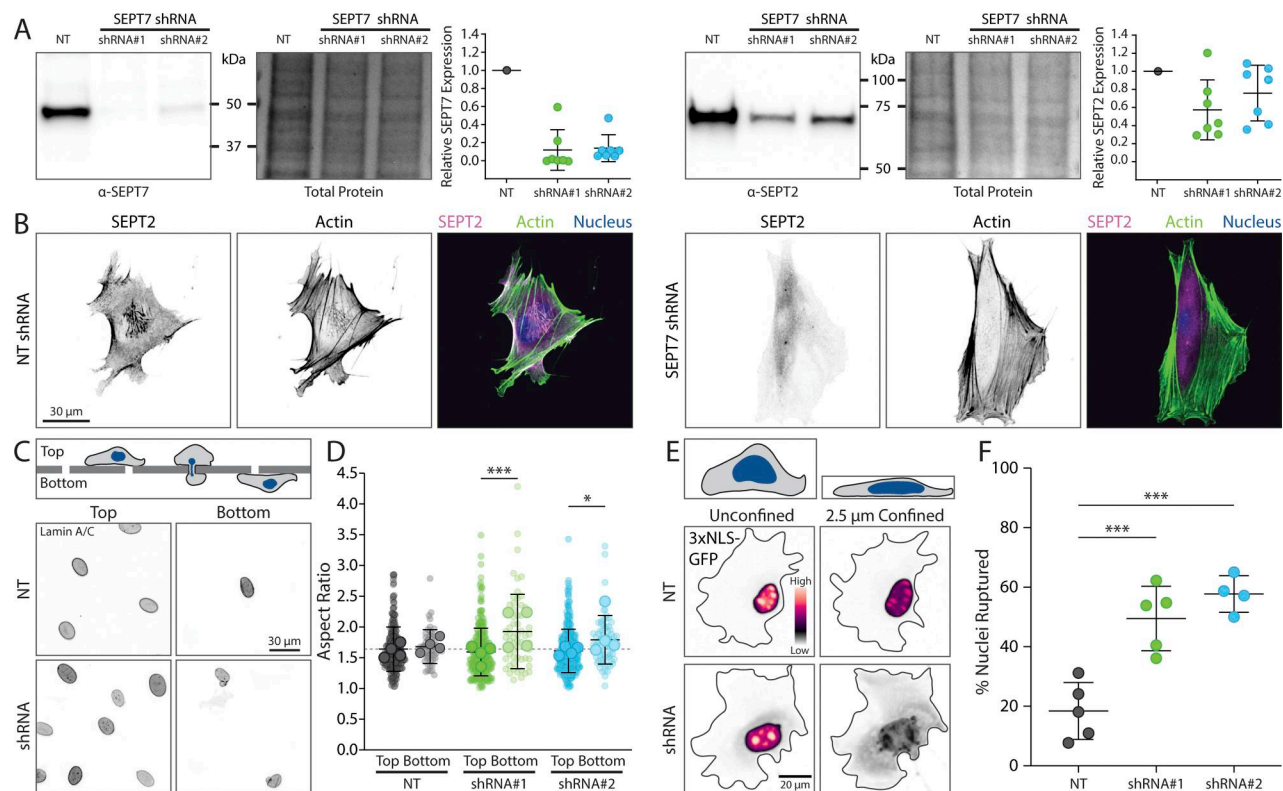


Figure 6. Septins buffer the nucleus during mechanical strain. (A) SEPT2-Halo cells were treated with non-targeting (NT) or SEPT7 shRNAs. Whole-cell lysates were probed with SEPT7 (left) or SEPT2 (right) antibodies via western blot. The right panels include individual repeats and mean $\pm$ SD. **(B)** Single confocal slices of SEPT2-Halo NT (left) and SEPT7 (right) shRNA cells labeled with Halo dye and stained for actin (phalloidin). **(C)** NT and SEPT7 shRNA cells were plated on a transwell with 3- μ m pores and allowed to migrate overnight. Transwells were extracted, fixed, and immunostained for lamin A/C. **(D)** Nuclear aspect ratio on the top and bottom of the transwell was measured. Experimental replicates and total cells imaged for NT were 4 and top = 250 and bottom = 54; for shRNA#1 were 4 and top = 278 and bottom = 59; and for shRNA#2 were 4 and top = 329 and bottom = 77. A two-way ANOVA was used to determine significance between top and bottom for each condition. **(E)** Single confocal slices of NT and SEPT7 shRNA cells exogenously expressing 3xNLS-GFP when unconfined and confined to 2.5 μ m. **(F)** The percentage of nuclei that ruptured upon confinement was measured. Circles indicate replicate means and bars indicate mean $\pm$ SD. Experimental replicates (N) and total cells imaged (n) for NT were N = 5 and n = 474, for shRNA#1 were N = 5 and n = 310, and for shRNA#2 were N = 4 and n = 189. A one-way ANOVA was used to determine significance. For all statistics * = $P < 0.05$, and *** = $P < 0.001$. Source data are available for this figure: SourceData F6.

septin functional models are not exclusive to the nucleus and could be happening with smaller organelles impinging upon the cortex and plasma membrane that are less apparent due to the magnitude of response. More than likely, multiple of these potential mechanisms are occurring in parallel.

Functionally, septins are strongly implicated in regulating immune cell migration (Tooley et al., 2009; Zhovmer et al., 2024). Immune cell nuclei are constantly being squeezed as they navigate dense tissue and extracellular matrix throughout the body, and therefore immune cell cortical septin enrichment is consistent with our findings. It is thus also similarly unsurprising that upregulation of septin expression is associated with a number of cancers (Scott et al., 2006; Stanbery et al., 2010; Gilad et al., 2015; Jędrzejczak et al., 2024), and methylation of the SEPT9 gene that enhances SEPT9 expression is a biomarker for colorectal cancer (De Vos et al., 2020; Krausewitz et al., 2022; Zhang et al., 2024). In work submitted concurrently with this manuscript, Mayca-Pozo and colleagues flesh this idea out explicitly (Mayca-Pozo et al., 2026), showing that septins protect against large-scale genomic alterations (or excessive genomic

instability) during confined migration and that overexpression of septins could be mechanoprotective for cancer cells that would otherwise succumb to apoptotic signals. These results, combined with our data, suggest that increases in septin expression could therefore facilitate metastasis by specifically enabling cells to migrate resiliently through confined spaces.

In conclusion, our data reveal a novel role for septins in mediating mechanical signaling in the cell, specifically around the nucleus. This behavior is reminiscent of, but distinct from, the role of intermediate filaments, and appears focused on dynamically and locally reinforcing the cell cortex. This mechanism acts to protect the nucleus from damage and facilitates cell migration through confined spaces. Coupled with the work of Mayca-Pozo et al. (2026), our results shine a spotlight on a critical role for septins in mechanotransduction, where they coordinate mechanical signaling in the cortex and prevent damage to the nucleus. There is still much to learn about the various functions of septins, as they continue to demonstrate that their role extends well beyond their function in cell division.

Materials and methods

Cell culture and transfections

Immortalized mouse embryonic fibroblasts (MEFs) were a kind gift from Dr. Mary Beckerle's lab (University of Utah, Salt Lake City, UT, USA). HEK293FT cells (CRL-3216; ATCC) were cultured for making lentivirus. All cell lines were cultured in Dulbecco's modified Eagle's Medium (MT10013CV; Corning) supplemented with 10% FBS (10437-028; Gibco), 1% Antibiotic-antimycotic (#15240062; Thermo Fisher Scientific), and 5 µg/ml prophylactic Plasmocin (ant-mpp; InvivoGen). All cells were grown in uncoated plastic tissue culture dishes (229661, 229621; CELL-TREAT). For imaging, cells were plated on #1.5 coverslips (Corning) or glass bottom dishes (Cellvis) coated with 10 µg/ml fibronectin (FC010; EMD Millipore) for 1 h at 37°C. For EGFP-Fractin (Johnson and Schell, 2009), EGFP-NM2A (Plasmid #11347; Addgene), 3x-NLS-GFP (Plasmid #58468; Addgene), and mEmerald-LaminA (Plasmid #54139; Addgene) exogenous transfections were performed using Neon electroporation (Invitrogen) with 1 × 20 ms pulses of 1600V and 3.5 µg DNA per 200,000 cells in 100 µl of reaction.

For lentiviral production, HEK293FT cells were plated in 6 cm dishes at 60–80% confluent. The transfection complex was generated using LipoD293 (SL100668; SigmaGen), packaging plasmid psPax (Plasmid #12260; Addgene), envelope plasmid pmD2.G (Plasmid #12259; Addgene), and lentiviral constructs (shRNAs TRCN0000101845, TRCN0000101847, SHC016; Sigma-Aldrich). The complex was added dropwise to the HEK cells and allowed to incubate 24 h. Media was changed and discarded at 24 h and collected at 48 and 72 h. Viral media was collected and filtered using a 0.45 µm Basix syringe filter (13-100-107; Thermo Fisher Scientific). SEPT2-Halo cells were infected with viral media. Viral media was removed after 24 h. Cells were selected with 2 µg/ml Puromycin (61-385-RA; Corning).

Generation of CRISPR knock-in cell line

SEPT2-Halo knock-in cells were derived from the immortalized MEF line using CRISPR/Cas9 as described previously (Chandrasekar et al., 2026). Briefly, we generated pSpCas9(BB)-2A-Puro (PX459) V2.0 (Plasmid #62988; Addgene) with target sequence 5'-AAACTTCA-TCAATAACCCGC-3' using established protocols (Ran et al., 2013). To generate donor plasmids, pUC57 was digested with EcoRI and StuI and purified. A four-piece Gibson assembly was then performed with three gBlocks (IDT): (1) a 794 bp 5'HDR of genomic sequence immediately upstream of the endogenous start codon, (2) HaloTag with an 18-amino acid GS-rich linker, (3) an 802 bp 3'HDR of genomic sequence immediately downstream of the endogenous start codon with a silent PAM site mutation. Fibroblasts were transfected with donor and target-Cas9 plasmids and single-cell sorted. Individual clones were evaluated for knock-in via western blotting (WB) and microscopy.

Microscopy

Unless stated otherwise (e.g., SIM, iPALM) all imaging was performed with a Plan-Apochromat 63X/1.4 NA objective or a Plan-Apochromat 63X/1.46 NA TIRF objective on Axio Observer 7 microscopes (Zeiss) equipped with a CSU-W1 Spinning Disk

(Yokogawa), Prime 95B sCMOS cameras (Photometrics), and enclosed in a temperature and humidity-regulated environmental chamber (Okolabs) controlled by SlideBook Software (Intelligent Imaging Innovations). For excitation of fluorophores, 405, 488, 561, and 637 nm lasers were used.

Fixation

Cells were fixed using a 4% paraformaldehyde solution (15710; Electron Microscopy Sciences) in cytoskeletal buffer (CB) (0.1 M MES, 0.03 M MgCl₂, 1.38 M KCl, and 0.02 M EGTA in 1L H₂O) for 15 min at 37°C. The PFA solution was removed, cells were rinsed with 1xPBS, permeabilized with a solution of 0.5% Triton X-100 (Fisher BioReagents) in CB for 15 min, rinsed in 1x PBS, and blocked with a solution of 1.5% BSA (CAS 9048-46-8; Research Products International) in CB. Fixed samples were incubated with primary antibodies at 4°C overnight, washed in 0.05% Tween 20 (AAJ20605AP; Thermo Fisher Scientific) in 1x PBS three times for 10 min with gentle rocking, incubated with secondary antibodies diluted in blocking solution for 1 h at RT, and washed in 0.05% Tween 20 in PBS three times for 10 min before imaging or mounting. Cells were mounted using ProLong Glass antifade mounting medium (P36980; Thermo Fisher Scientific). Mounted samples were allowed to cure for 18 h in a dark drawer at RT prior to imaging.

Antibodies and dyes

The following antibodies and dyes were used for WB, IF, and live imaging: Anti Lamin A/C (39087; Active Motif; IF 1:500). Anti SEPT2 (11397-1-AP; Protein Tech; IF and iPALM 1:500). Anti SEPT2 (60075-1-Ig; Protein Tech; WB 1:2,000). Anti SEPT7 (13818-1-AP; Protein Tech; WB 1:2,000). Anti SEPT7 (18991; IBL America; IF 1:500). Anti SEPT9 (A8657; ABclonal; IF 1:500 and WB 1:2,000). Goat Anti-Rabbit Alexa Fluor 488 (A32731; Thermo Fisher Scientific; IF 1:1,000). Goat Anti-Mouse Alexa Fluor 488 (A32723; Thermo Fisher Scientific; IF 1:1,000). Goat Anti-Rabbit Alexa Fluor 647 (A21244; Thermo Fisher Scientific; IF 1:1,000). Phalloidin, CF(R)583R (BOT-00064-T; Biotium; iPALM 1:200). Phalloidin-Atto 647N (65906; Sigma-Aldrich; IF 1:5,000). DAPI (D1306; Thermo Fisher Scientific; IF 1:5,000). Hoechst (H3570; Invitrogen; live imaging 1:5,000 for 10 min). SPY650-FastAct_X (CY-SC502; Cytoskeleton; live imaging 1:1,000). Janelia Fluor JFX554 HaloTag Ligand (HT103A; Promega; live imaging 10–50 nM); Janelia Fluor 646 HaloTag Ligand (HT106A; Promega; live imaging 10–50 nM).

Collagen gels and polyacrylamide hydrogels

Collagen gels were generated using a protocol adapted from (Doyle, 2016). 3.0 mg/ml rat tail collagen (354,236; Corning) was mixed with cell culture DMEM and reconstitution buffer on ice. 200–250 µl of the collagen mixture was added to pre-chilled 35-mm glass-bottom dishes (Cellvis). Dishes were pre-coated with Poly-D-Lysine (A3890401; Gibco) for 10 min at RT. Gels were allowed to polymerize at 18°C overnight. Cells were plated on top of gels in media with Halo dye and allowed to invade for 24 h before imaging. Halo dye was removed 1 h before imaging.

Polyacrylamide gels were made as previously described (Oakes et al., 2018). Briefly, gels were polymerized on an

activated coverslip with 7.5% acrylamide and 0.3% bis-acrylamide for a shear modulus of 8 kPa. The gels were covalently coupled with fibronectin (1 mg/ml) via sulfo-SANPAH (NC2028693; Thermo Fisher Scientific).

Septin localization along nucleus and cell centroid axis

WT MEFs were fixed and immunostained for SEPT2 and DAPI-stained for nuclei. The SEPT2 channel was used to generate a cell mask and identify the cell centroid. The nuclear channel was used to generate a nuclear mask and identify a nuclear centroid. To subtract background in the SEPT2 channel, the intensity of a large region outside of the cell was measured and subtracted from the entire image. A bounding box was manually traced around the prominent septin medio-ventral network, and the center of mass was measured in the background-subtracted SEPT2 channel. The distance of the septin center of mass along the nuclear-cytoplasmic axis was calculated and presented as a fraction of the distance between the cell centroid (value of 0) and the nuclear centroid (value of 1), where positive values indicate a septin preference towards the nucleus and negative numbers indicate a septin preference away from the nucleus.

Structured illumination microscopy

SEPT2-Halo cells were transiently transfected with mEmerald-LaminA and stained with Spy650-FastActin_X (CY-CS502; Cytoskeleton). SIM imaging was performed on a Zeiss Lattice SIM 5 with a Plan-Apochromat 63x/1.40 NA oil objective and Hamamatsu Fusion BT cameras. Z-stacks were collected in Leap mode with optimal step size. Processing was performed in ZEN BLUE 3.9 with SIM² and the following parameters: result sampling = 2; processing sampling = 2; auto sharpness = on; order combination = Wiener filter; regularization = none; iterations = 3 for actin and septin, 2 for laminA; fitting = fast fit; histogram = scaled to raw.

iPALM

iPALM imaging and analysis were performed similarly to previous work (Shtengel et al., 2009; Kanchanawong et al., 2010; Case et al., 2015; Stubb et al., 2019; Kumari et al., 2024). For sample preparation, MEFs were plated on 25-mm diameter round coverslips containing gold nanorod fiducial markers (A12-40-600-CTAB; Nanopartz, Inc.), passivated with a ~50 nm layer of SiO₂, deposited using a Denton Explorer vacuum evaporator, except that the coverslips were not coated with any ECM protein before plating the cells. Coverslips were incubated in complete cell culture media for 1 h at 37°C prior to plating cells. Cells were plated for ~18 h before fixation as above. Rinse steps are different than listed above, six sequential washes in PBS were performed (three times for 5 min, two times for 10 min). Actin was labeled using phalloidin-CF583R (#00064-T; Biotium). Septins were labeled with either anti-SEPT2 or a combination of anti-SEPT2 and anti-SEPT7 to achieve higher labeling density, followed by goat-anti-rabbit Alexa Fluor 647 secondary antibody. The coverslips were then covered with STORM buffer, prepared as follows: 17 mg/ml catalase (C100-50 MG; Sigma-Aldrich) and 70 mg/ml Glucose oxidase (G-2133-50KU; Sigma-Aldrich) solutions were prepared in Buffer A (10 mM Tris, pH 8.0 + 50 mM NaCl). Both were mixed in a 1:4 volume ratio to

prepare GLOX solution. This was centrifuged, and only the supernatant was used. 1 M cysteamine (30070-50 G; Sigma-Aldrich) solution was prepared in 0.25 N HCl. The STORM buffer was prepared by combining GLOX solution, 1 M cysteamine, and Buffer B (50 mM Tris, pH 8.0 + 10 mM NaCl +10% Glucose) in a 1:10:90 volume ratio. Following the coverslips being covered with STORM buffer, an 18-mm diameter round coverslip (CS-18R17, #1.5 thickness; Warner Instruments) was placed centrally on top of the sample, and the boundary was sealed with Valap (1:1:1 of vaseline, lanolin, and paraffin) to create a sandwich mount. The mounted samples were then imaged on the iPALM.

For imaging, actin samples were excited using a 561 nm laser (Opto Engine LLC) at 1–2 kW/cm² intensity in TIRF conditions. 100,000 images were collected through dual Nikon Apo TIRF 60x/1.49NA objective lenses coupled to a 593/40 nm bandpass filter (Semrock) and acquired via three EMCCD cameras (DU987U; Andor) with 50 ms exposure. Images were XYZ drift and tilt-corrected, channel-merged, and checked for alignment quality using the PeakSelector software (Janelia Research Campus80). Alexa Fluor 647-labeled septin was imaged similarly, but with 2–3 kW/cm² intensity 647 nm laser excitation (Opto Engine, LLC.), 647-nm long-pass filter (Semrock), and 30–40 ms exposure time.

For analysis, the iPALM data were processed/localized using PeakSelector software (Janelia Research Campus). The gold fiducials embedded in the coverslip were used for drift correction. iPALM data were analyzed using iPALM plotter (Advanced Imaging Center, Janelia Research Campus; <https://github.com/aicjanelia/ipalmplotter>), and images were rendered using the PeakSelector software. iPALM localization data record both the fluorescent molecules localized within the fibers as well as molecules in the cytoplasmic fraction. iPALM images were rendered using PeakSelector. To quantify the spatial distribution of the proteins within individual fibers, we identified fibers using custom MATLAB code (<https://github.com/aicjanelia/iPALM-fiberAnalysis>). After the gold nanorod fiducial markers were removed from the rendered images using custom MATLAB code (<https://github.com/aicjanelia/iPALM-beadRemoval>), localizations were roughly pruned by excluding localizations at unreasonably large z-positions—likely ghosting artifacts. A rendered image was produced by counting localizations within pixels of a given size. Fibers were detected in this rendered image through a series of morphological processing steps: MATLAB's fibermetric filter, binary opening and erosion, and skeletonization. Connected component analysis of the resulting binary image detected fibers. Small fiber segments were discarded at this step. Fibers were then iteratively segmented into short linear pieces. Localizations within a constant width of these segments were assigned to the fiber. By iterating over all segments for each fiber, complete sets of fiber-associated localizations were determined. The z-position of these localizations was subsequently recorded along the length of the fiber. This process was repeated for each channel. Fibers with low or nonuniform density and fibers not parallel with the coverslip were removed. For analysis of protein distributions in fibers, the 3D molecular coordinates for each region (individual fibers) were analyzed to extract the median axial

height for actin and septin, with the delta of actin minus septin plotted accordingly.

WB

Cells were collected, counted, and pelleted. Complete Laemmli buffer was made using 2x Tris-Glycine Laemmli sample buffer (1610737; Bio-Rad) with 10% β -mercaptoethanol and protease and phosphatase inhibitor cocktail (78442; Thermo Fisher Scientific). 100 μ l of complete buffer was added per 500,000 cells in a centrifuged pellet. Samples were boiled for 5 min at 95°C, and cell lysates were loaded into 4–15% Mini-PROTEAN TGX Stain-Free Gels (4568085; Bio-Rad). Protein was transferred using a Trans-Blot Turbo system (Bio-Rad) onto a polyvinylidene fluoride (PVDF) membrane. Blots were stained with Amido Black (CAS 1064-48-8; Bio Basic) for 10 min and rinsed in de-stain buffer (5% acetic acid in water) three times for 5 min. Total protein from blots was imaged on a ChemiDoc (Bio-Rad). Blots were blocked for 1 h at RT in 3% BSA in PBS with 0.1% Tween (PBS-T). Primary antibodies were added to the blocking buffer and rocked at 4°C overnight. Blots were washed in 0.1% PBS-T three times for 10 min, incubated in secondary antibodies for 1 h at RT, washed in 0.1% PBS-T 3 times for 10 min, incubated in Clarity Western ECL Substrate (170561, Bio-Rad) for 1 min, and imaged. Blots were analyzed using FIJI; samples were background subtracted and normalized to total protein. Normalized values were then compared with WT to determine relative expression values.

Microfluidic devices

The microfluidic chamber design was adapted from previously used designs (Keys et al., 2024), and a corresponding SU8 wafer for making the molds was fabricated by the UIC Nanotechnology Core Facility. Briefly, a negative of the wafer was cast in PDMS (SYLGARD 184 Silicone Encapsulant, 50-366-794; Thermo Fisher Scientific) and then used to create an epoxy mold. The mold was cleaned with isopropanol and dried with compressed air before use. A 10:1 mixture of SYLGARD 184 base and curing agent was poured into the epoxy mold, vacuum-degassed for 30 min, and baked at 70°C overnight. The PDMS was allowed to cool before demolding. Holes were then punched in the PDMS for the media container (2.5 mm) and cell port (1 mm). The features were cleaned with Scotch tape, washed twice with 70% ETOH, twice with Milli-Q water, and dried with compressed air. To assemble the microfluidic device, the PDMS slab was coupled immediately to a 35-mm glass-bottom dish (Cellvis) after both were plasma cleaned (Harrick Plasma) for 30 s. The coupled device was then heated at 70°C for 1 h to strengthen the bond. Before use, the microfluidic device was cooled to RT, plasma treated for 2 min, and coated with 20 μ g/ml fibronectin at 37°C for 20 min. It was then rinsed with PBS, filled with cell media, and kept at 37°C for 2 h. Cells were resuspended at 2.5 million/ml, and 10 μ l of the cell suspension was inserted into the cell port. The cells were allowed to attach for 5–10 min, prior to filling the media port with media. An additional 4 ml of media containing 1 μ g/ml Hoechst was added to flood the device and prevent evaporation. The device with cells was incubated overnight at 37°C with 5% CO₂,

then moved to the microscope and imaged every 2 min under the same conditions.

To quantify septin enrichment at the cortex, images were registered using the bright-field channel and the calculated translation was applied to the other channels. A binary mask of the PDMS features in the microfluidic device was made, and regions of interest that defined an area over a single cell were defined manually. Objects in the feature mask were then outlined to create a list of pixels representing the surfaces of the objects. For each frame, the nucleus image was thresholded to define the nucleus boundary, and a distance map from the binary nucleus mask was made, indicating the distance of each pixel in the image from the nucleus. For each frame, the septin intensity and the distance from the closest pixel of the nucleus were recorded for the list of points defining the outlines of the PDMS objects. Points were binned by distance with a bin width of five pixels and then averaged over all points in the movie. Intensity values were normalized to the average intensity at 30 μ m, and plotted as a function of the distance of the nucleus from the PDMS. The plot in Fig. 4 E represents the average of 31 cells from 19 fields of view, taken across three different experiments. To break the data down by the curvature of the PDMS features (Fig. S3, C–F), masks of only the flat edges of the rectangular pillars ($\kappa \sim 0$), the large round pillars ($\kappa \sim 0.1 \mu\text{m}^{-1}$), or the corners of the rectangular pillars ($\kappa \sim 0.35 \mu\text{m}^{-1}$) were used to create the point list. All analysis was performed in Python and is available at <https://github.com/OakesLab>.

Dynamic confinement

5- and 2.5- μ m pillars were made from SU8 wafer masks fabricated in the UIC Nanotechnology Core Facility. Masks were cleaned with water, dried with an air gun, and rinsed in isopropanol. 5 g of PDMS (SYLGARD 184 Silicone Encapsulant, 50-366-794; Thermo Fisher Scientific) and its accompanying curing agent were mixed at a ratio of 10:1 and poured over the cleaned mask. PDMS was degassed in a vacuum chamber to remove bubbles for 15 min. 10-mm glass coverslips were plasma cleaned (Harrick Plasma) for 5 min on high. Coverslips were pushed into the PDMS-covered mask and cured at 98°C for 30 min. PDMS was allowed to cool to RT for 10 min before peeling excess PDMS from the mask. Isopropanol was sprayed on the mask to help remove coverslips. Pillars were then dried, rinsed in PBS, and placed in a dish with complete cell media overnight in the cell culture incubator. Cells were plated in a 35-mm dish with a 28-mm glass bottom of #1.5 glass (Cellvis) coated with fibronectin (10 μ g/ml) for 1 h at 37°C. Cells were placed in 700 μ l of media for imaging at 37°C and 5% CO₂. The dynamic confinement cup was a kind gift from the Piel Lab (Institut Curie). The cup was cleaned with ethanol, allowed to dry, and then tape was used to remove any dust. The pillars were attached to the piston in the center of the cup, and the entire device was coupled to the vacuum (Flow EZ; Fluigent). The cup was placed on the dish, and pressure was applied to the outer edge to couple the cup to the dish. Cells were imaged at steady-state vacuum ($\sim$ –15 mBar) for 8 min; vacuum was increased ($\sim$ –90 mBar) to bring down the piston, and cells were imaged for 30 min. The confinement was then released by

reducing the vacuum, and cells were imaged for an additional 30 min.

To quantify changes in subnuclear intensity of cytoskeletal elements, a standard-sized circular region of interest (ROI) 25 μm in diameter was marked at the centroid of the nucleus. The background-subtracted mean intensity of the ROI was measured at each time point, and the pre-compression intensity was taken as the average of the pre-compression values. A rolling average of eight frames (to match the length of the pre-compression data) was calculated, and the maximum intensity during compression was determined. The last eight frames of the postcompression time points were averaged to create the postcompression mean. All three means were then normalized to the precompression mean, providing a relative fold change.

To quantify nuclear envelope integrity upon 2.5- μm confinement, nuclear and cytoplasmic masks were generated using the EGFP-3xNLS signal at varying threshold levels. These masks were applied to the raw images, and nuclei were considered to rupture if the cytoplasmic signal increased following compression.

Enucleating cells

Enucleation was performed as previously described (Graham et al., 2018). A stock Ficoll solution was formed by dissolving Ficoll-400 (26873-85-8; BioBasic) at 50% (wt/vol) in sterile PBS without $\text{Ca}^{2+}/\text{Mg}^{2+}$ overnight while being rotated at RT. The solution was filtered (0.4 μm) and stored at 4°C. This stock Ficoll was used to prepare Ficoll-DMEM stocks of 30, 20, 18, and 15%. Cytochalasin-D (100-0556; STEMCELL Technologies) was added to each stock at a final concentration of 10 μM . A discontinuous isoosmotic gradient was poured into a 13.2-ml centrifuge tube (C14277; Beckman Coulter) from the Ficoll-DMEM stocks in the following order: 2 ml of 30%, 20%, and 18%, then 1 ml of 15%. The gradient was allowed to equilibrate overnight in the tissue culture incubator. SW41 Ti rotor buckets were also incubated at 37°C overnight. 2×10^7 dye-labeled SEPT2-Halo cells were lifted, pelleted, resuspended in 1 ml of pre-warmed 15% Ficoll-DMEM, and layered on top of the gradient. DMEM was added to fill the tube. The gradient was placed into the SW41 Ti rotor bucket and incubated in the cell culture incubator for 45 min. The rotor was centrifuged at 125,000 $\times g$ for 1 h at 30°C in a Beckman Coulter Optima XL-100K Ultracentrifuge and stopped with minimal breaking. Following centrifugation, a thin band containing cytoplasts could be observed in the gradient, and fractions around this band were collected. Collected fractions were washed and centrifuged twice with PBS and twice in DMEM. Control cells were incubated in 15% Ficoll-DMEM stock in a cell culture incubator for the duration of the centrifugation and washed the same as the cytoplast fractions. Control cells and cytoplasts were plated in 4-well glass bottom dishes and allowed 1 h to recover and adhere to the dish. Cells were then fixed as above. For experiments using the solid glass 8.5-12- μm microspheres (P2015SL; Cospheric), microspheres were sterilized in a UV oven (UVP Crosslinker CL-1000; Analytik Jena) at max power for 5 min. Beads were further sterilized with 70% ethanol, rinsed three times with 1x PBS, coated in

Poly-D-Lysine for 10 min at RT with gentle agitation, and rinsed three times with 5 mL of sterile 1x PBS. Beads were added on top of adhered cells or cytoplasts, centrifuged (5810R; Eppendorf) in a custom holder for four well dish for 20 min at 200 rpm, 7 RCF. Media was exchanged to remove excess beads, and cells were incubated for 2 h prior to fixation as above. Cells were imaged using Z-stacks with steps of 0.5 μm to capture the entire cell or cytoplast.

To quantify septin assembly, the images were first background subtracted and then the ratio of the average septin intensity using a 15-pixel-radius circle in the subnuclear region to the surrounding cytoplasm was calculated for each cell. For cytoplasts with beads, the region under the bead was analyzed, while in cytoplasts without beads, a central region away from the cortex was measured. To quantify organization of the septin network, we adapted an approach we developed previously (Marcotti et al., 2021). Briefly, a standard square ROI ($\approx 11 \times 11 \mu\text{m}$) was placed under the centroid of the nucleus, the center of the cytoplast, or under the centroid of the bead for analysis. The Fast Fourier Transform of this region was then calculated, and the central moments were calculated. The eccentricity was determined as $\sqrt{1 - \lambda_1/\lambda_2}$, where $\lambda_{1,2}$ represent the eigenvectors calculated from the central moments. An eccentricity value of 1 represents aligned linear features, while a value of 0 represents a homogeneous distribution of intensity.

Transwell

Transwell inserts (3402; Corning) were coated in 50 $\mu\text{g}/\text{ml}$ collagen (354236; Corning) for 1 h at 37°C and then rinsed in 1x PBS. Media with 0.1% FBS (Fetal Bovine Serum) and Halo dye were loaded into the top and bottom of the transwell. Cells were lifted and resuspended in the 0.1% FBS media and plated on the top of each transwell. Cells were allowed to adhere for 6 h before the media in the bottom of the well was swapped for media with 10% FBS and Halo dye to generate an FBS gradient. Cells were allowed to migrate through the pores overnight. Transwells were fixed the next morning following the fixation protocol above. After blocking, the transwell membrane was cut out from the device and placed in primary antibody solution overnight. The insert was rinsed, placed in secondary antibody solution, and mounted between two #1.5 22 $\times$ 30 mm coverslips following the mounting protocol above. Samples were either imaged as a full stack all the way through the transwell membrane or each side of the membrane was imaged separately using smaller stacks. Max projections of the Lamin A/C channel for the top and bottom of the well were created in FIJI and thresholded to create a mask of all the nuclei. The Analyze Particles feature in FIJI was used to identify nuclei and measure aspect ratio.

Online supplemental material

Fig. S1 shows the validation of the SEPT2-Halo knock-in cell line. Fig. S2 shows septins are recruited around the nucleus on soft substrates and in 3D environments. Fig. S3 shows actin and myosin responses to dynamic confinement and septin accumulation as a function of curvature in the microfluidic devices. Fig.

S4 shows glass beads inducing septin recruitment in both cells and cytoplasts. Fig. S5 shows that transwell pores are heterogeneous in size. Video 1 shows the data from Fig. 1 of septins moving with the nucleus in a migrating cell. Video 2 shows the data from Fig. 3 of septins accumulating in response to externally applied forces. Video 3 shows septins accumulating around the nucleus as it squeezes through a gap in a microfluidic device from Fig. 4. Video 4 shows septins accumulating wherever the nucleus pushes against the cortex from Fig. 4.

Data availability

The data are available from the corresponding author upon reasonable request.

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Supplemental material

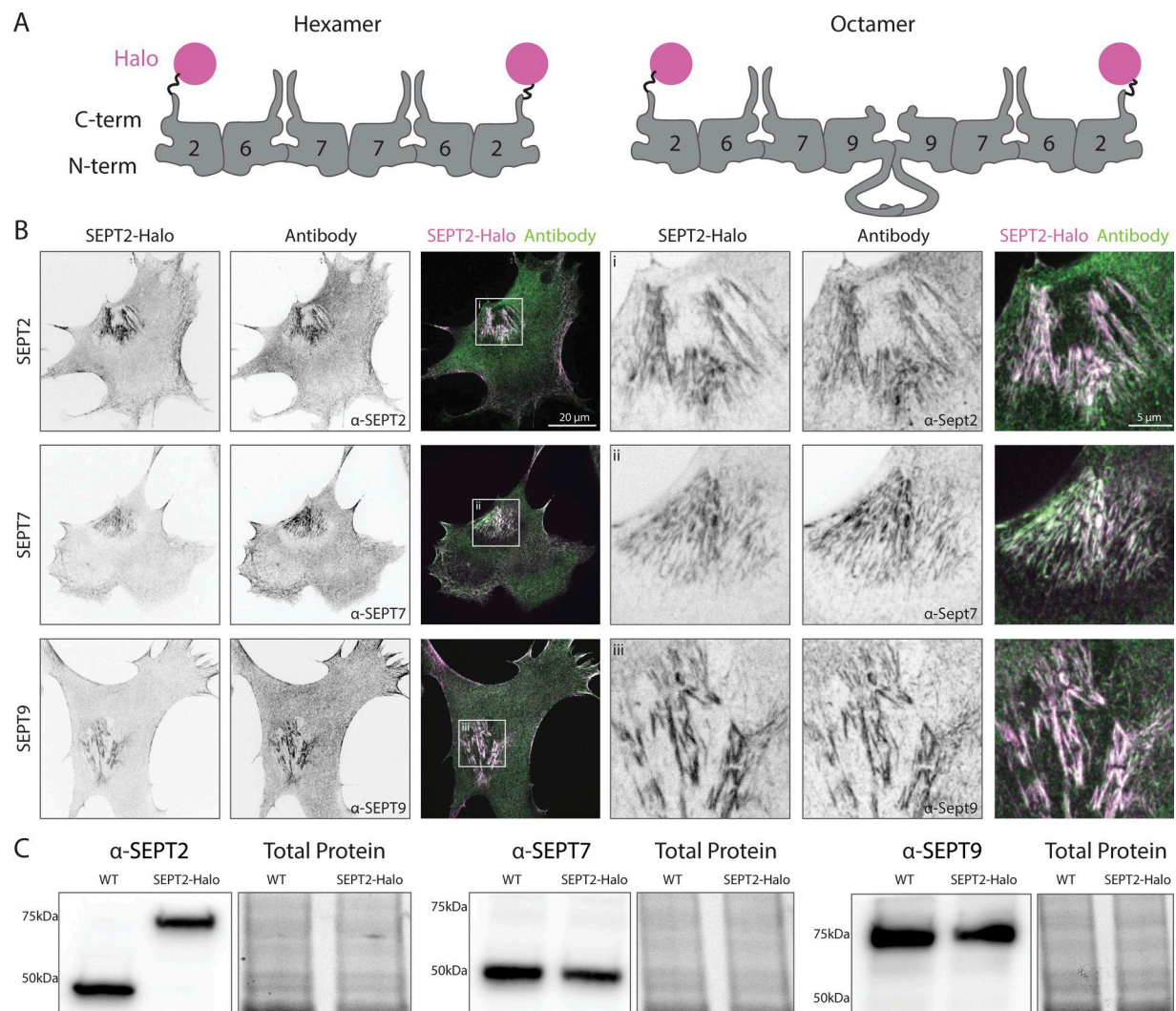


Figure S1. **SEPT2 knock-in cells have normal septin localization and expression.** (A) Cartoon depicting a septin hexamer and octamer with an approximation of the HaloTag and linker. (B) Single confocal slices of SEPT2-Halo knock-in cells fixed and labeled with Halo dye (magenta) and immunostained for either SEPT2, SEPT7, or SEPT9 (green). Zoomed insets of the subnuclear region are shown to the right. (C) Western blots of the parental MEF line and the SEPT2-Halo cells validating that the knock-in did not change septin expression. The SEPT2-Halo band is shifted ~30 kDa larger, consistent with the approximate size of a HaloTag. Source data are available for this figure: SourceData FS1.

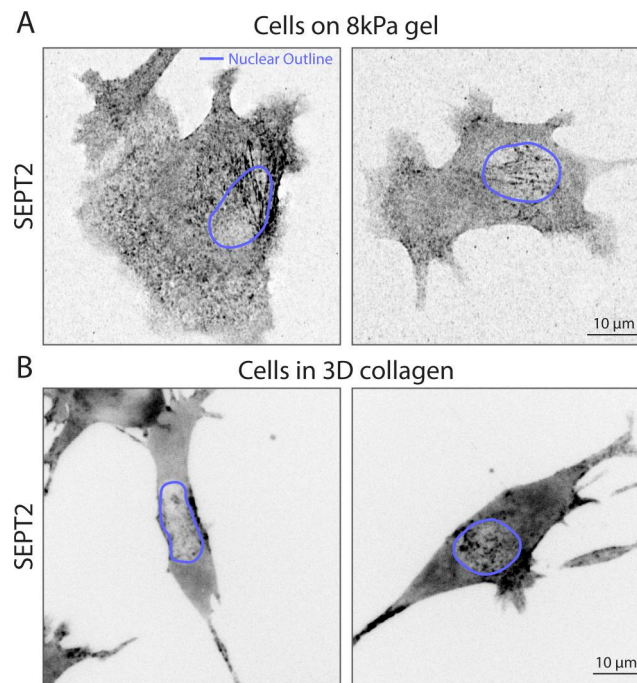


Figure S2. **Septins colocalize with the nucleus in physiological contexts.** (A) Single confocal slices of SEPT2-Halo cells labeled with Halo dye plated on a polyacrylamide hydrogel with a shear modulus of 8 kPa. (B) Single confocal slices of SEPT2-Halo cells labeled with Halo dye embedded in a 3D collagen matrix. The nucleus is outlined in blue in each image.

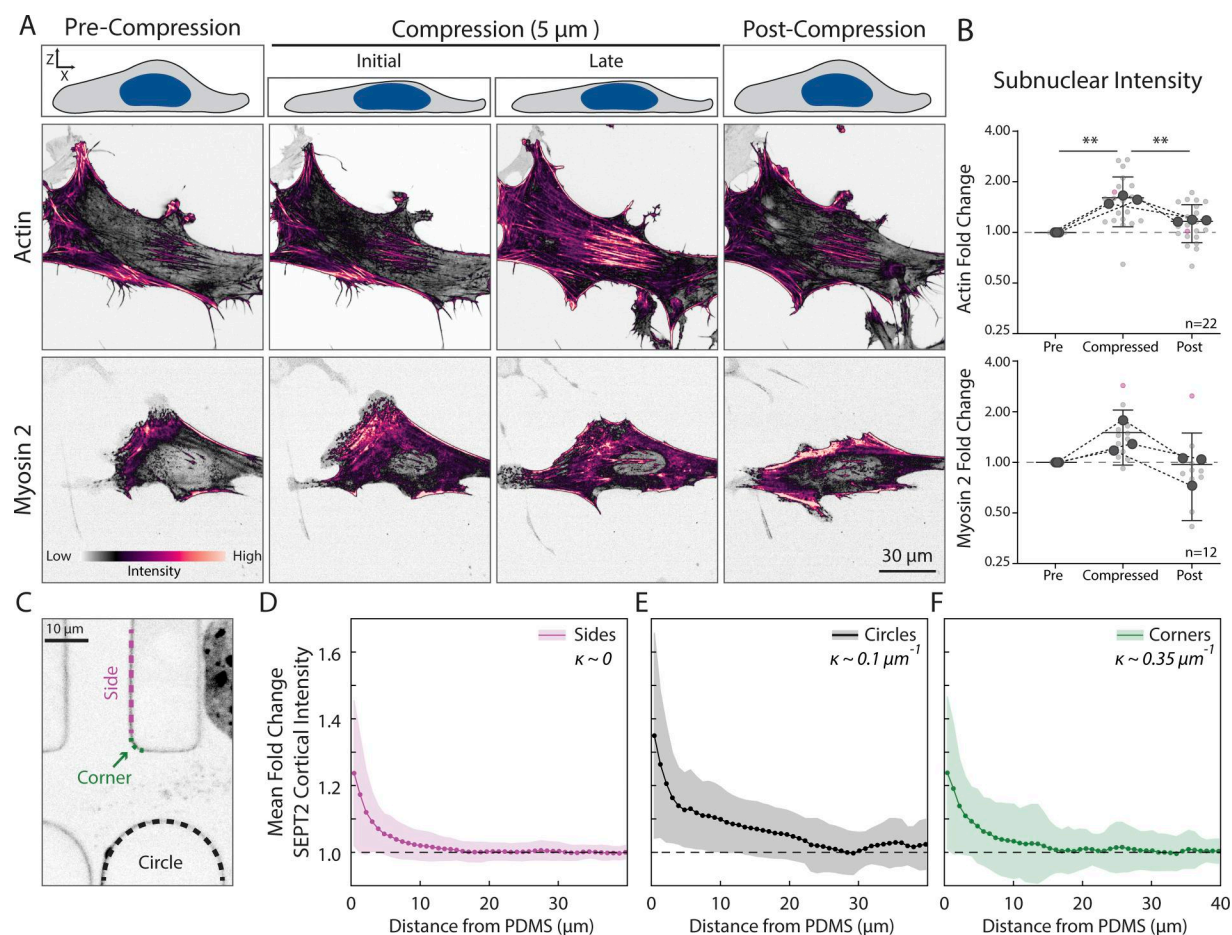


Figure S3. Cytoskeletal reorganization in response to forces. **(A)** Representative single confocal slices of the ventral surface SEPT2-Halo cells expressing EGFP-FTractin or EGFP-NM2A before, during, and after compression. Initial compression images were taken immediately after confinement, while late images were taken after 30 min of confinement. **(B)** The fold intensity change for actin and myosin in the subnuclear regions of the cell. A nested 1 way ANOVA was used to determine significance, with * = $P < 0.05$, ** = $P < 0.01$. **(C)** A representative image of the microfluidic device with labels identifying example surfaces with different curvature ($\kappa = 1/R$). **(D–F)** Mean fold change in SEPT2 cortical assembly along flat sides (D; $\kappa \sim 0$), circles (E; $\kappa \sim 0.1 \mu\text{m}^{-1}$), or corners (F; $\kappa \sim 0.35 \mu\text{m}^{-1}$) of features as a function of distance from the nucleus. Data for D–F represent the normalized average of all points along the cortex taken from 31 different cells, across 19 fields of view, from three different experiments. All data are normalized to the average SEPT2 intensity at the 30 μm distance (dashed line), representing the average cortex intensity in the absence of the nucleus. Points represent the mean value, and the shaded regions are the standard deviation.

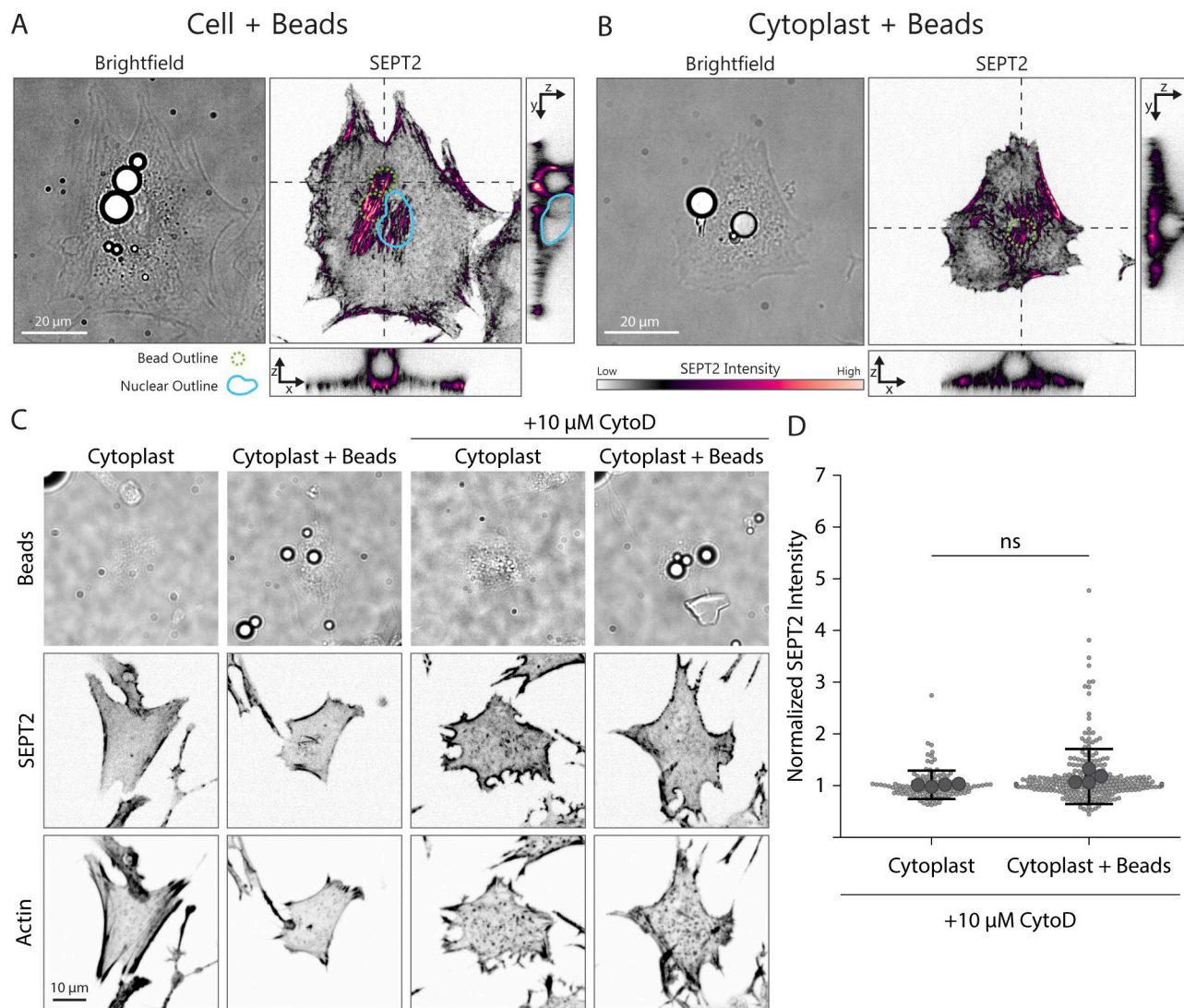


Figure S4. Glass beads induce septin filament assembly. (A) A brightfield image shows two large beads internalized by the cell (and multiple smaller beads not internalized). A single confocal slice of the ventral surface of a SEPT2-Halo cell shows septins accumulate under the large internalized beads (outlined in green dashed line) in addition to under the nucleus (outlined in blue). (B) A bright-field image shows two beads internalized in a cytoplasm. A single confocal slice of the ventral surface of a SEPT2-Halo cytoplasm shows septins still accumulate under the beads in the absence of the nucleus. X-Z and Y-Z projections in both panels show the beads fully internalized in the cell. (C) Single confocal slices of SEPT2-Halo cytoplasts and cytoplasts with beads, with and without treatment with 10 μ M cytochalasin D, were fixed and stained with phalloidin to visualize the actin cytoskeleton. (D) Quantification of septin assembly and organization in SEPT2-Halo cytoplasts or cytoplasts with beads treated with cytochalasin D. Each small gray dot represents a single cell, and bars indicate mean $\pm$ SD (cytoplasts $n = 120$, cytoplasts + beads $n = 253$). Large gray dots represent the mean for each data set ($N = 4$).

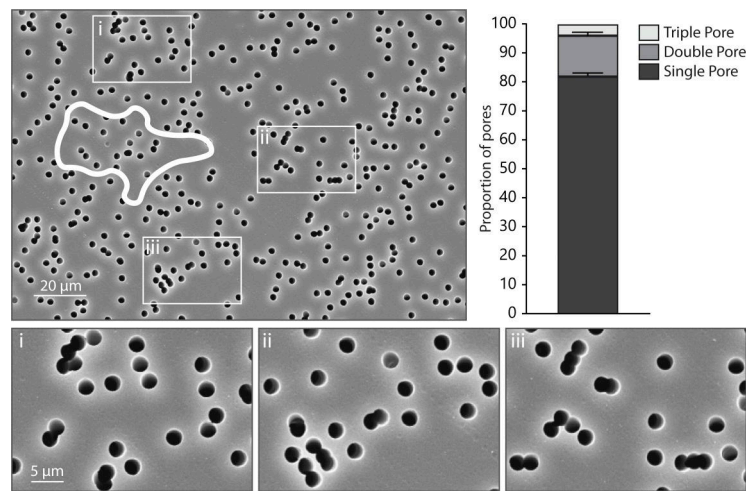


Figure S5. The distribution of pores in transwell plates is not uniform. An SEM image of a transwell insert showing non-uniform distribution of pores. Insets show multiple examples of pores overlapping each other, making pores significantly larger than the stated 3 μm diameter, and possibly adding noise to the assay. The white solid line shows an outline of a representative cell on the transwell for scale. The bar plot shows a quantification of the pore size. Approximately 20% of pores are $>3 \mu\text{m}$ in diameter.

Video 1. Septin networks coincide and move with the nucleus during migration. A SEPT2-Halo cell stained with Hoechst nuclear dye migrating on a glass coverslip as shown in Fig. 1. The septin network under the nucleus is dynamically reorganized to remain under the nucleus as the cell migrates. The movie is 13 h in length and is played at a frame rate of 10 fps. Time is in hr:min.

Video 2. Septin networks assemble under the nucleus in response to externally applied compression. The ventral surface of a SEPT2-Halo cell is imaged prior to, during, and after 5 μm confinement, which applies compression to the nucleus as shown in Fig. 3. Images were acquired every minute for 8 min (pre-confinement), 33 min (during confinement), and 15 min (after confinement). The movie plays at 12 fps, and time is in min:sec.

Video 3. Septin accumulates around the nucleus during confined migration. A SEPT2-Halo cell migrating through a constriction in a microfluidic shows strong septin accumulation around the nucleus as it is squeezed between the pillars in the device, as shown in Fig. 4 A. The nucleus is stained with Hoechst dye. The movie is 6 h in length and is played at a frame rate of 15 fps. Time is in hr:min.

Video 4. Septin accumulates where the nucleus pushes on the cortex. A SEPT2-Halo cell migrating through a microfluidic device without being constricted still shows septin accumulation along the cortex, as shown in Fig. 4 C. The nucleus is stained with Hoechst dye. The movie is 1.75 h in length and is played at a frame rate of 15 fps. Time is in hr:min.