Electronic Supplementary Material (ESI) for Soft Matter.

SUPPORTING MATERIALS for

Anisotropic mechanics and dynamics of a living mammalian cytoplasm

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Table S1

	p-value		
	β = Unrestricted and β = 10 μm	β = 10 μm and β = 20 μm	β = 20 μm and β = 40 μm
Actin	8.15184E-12	1.73186E-12	0.00168
Microtubules	1.82165E-5	5.00881E-9	0.009

Table S1. P-values from comparison of distribution of cytoskeletal fibers using Two-Sample Kolmogorov-Smirnov test.

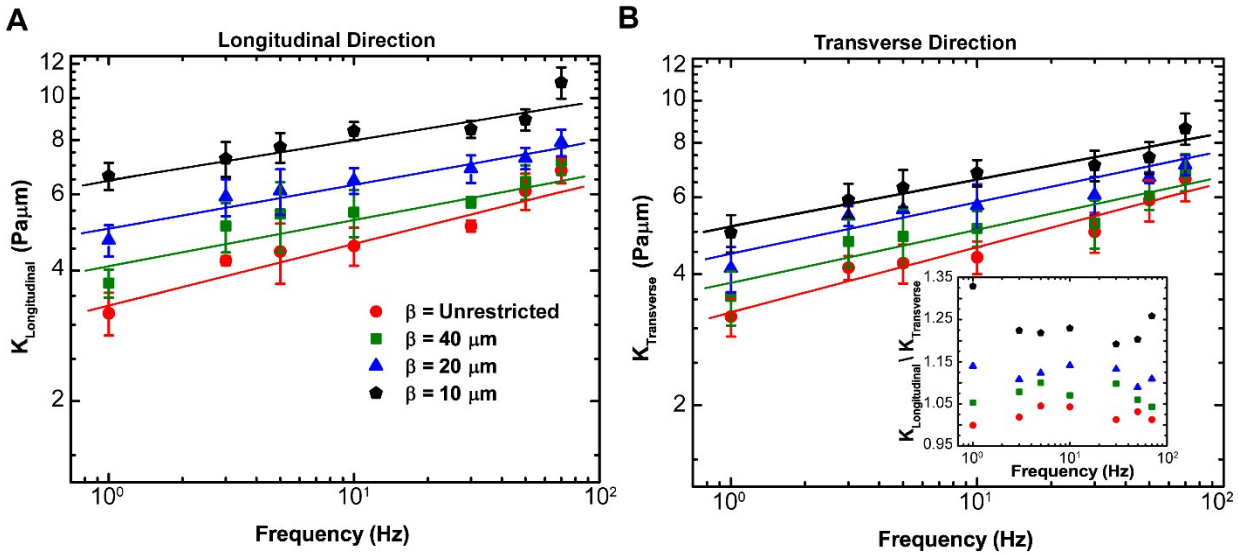

Figure S1. Active microrheology measurements of intracellular mechanics using optical tweezers. (A and B) 500 nm polystyrene beads are endocytosed into mEFs and are trapped and subjected to sinusoidally varying force F at frequency f. The frequency dependent complex intracellular spring constant, K is calculated by measuring the resultant displacement of bead, x. Longitudinal and transverse intracellular spring constant as a function of frequency follows power-law rheology different widths of the collagen strips (β = Unrestricted, 40μm, 20μm, 10μm). Inset shows the ratio of cytoplasmic stiffness in longitudinal and transverse direction, $K_{Longitudianal}/K_{Transverse}$ with aspect ratio across the entire frequency range measured in this study. Cells become mechanically anisotropic with increase in aspect ratio. Error bars represent standard deviation (n =15 cells). Solid lines are power law fits.

Table S2

	β = Unrestricted	β = 10 μm	β = 20 μm	β = 40 μm
Longitudinal Direction	0.14 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
Transverse Direction	0.15 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01

Table S2. Power law exponent obtained from power law fits of the intracellular mechanics varying with frequency.

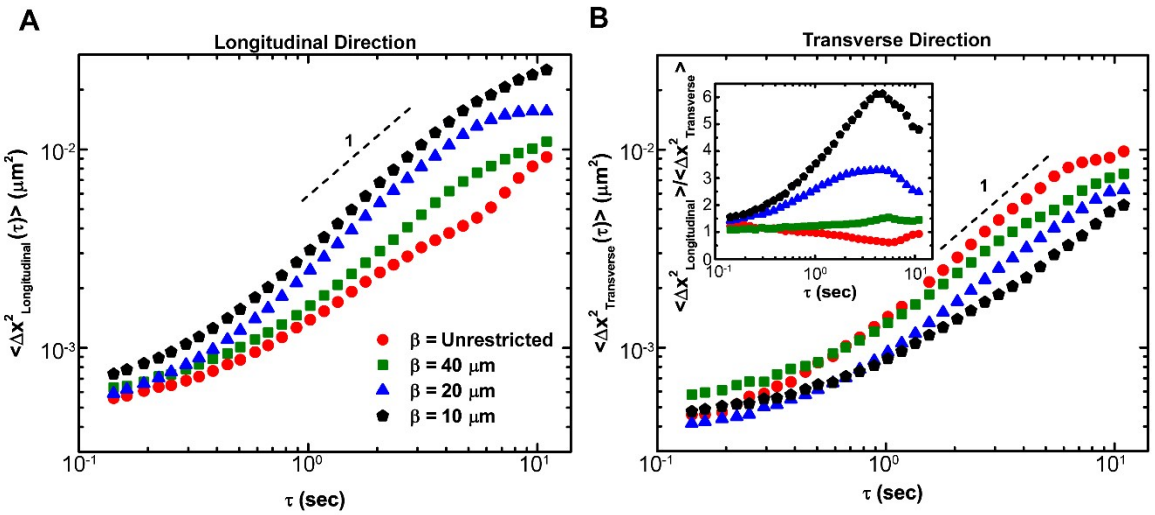

Figure S2. Intracellular movement of 500 nm endocytosed particles. (A and B) MSD $\langle \Delta x^2(\tau) \rangle$, *in longitudinal and transverse direction of tracer particles are plotted against lag time on a log-log scale for different aspect ratio cells. Inset shows the ratio of MSDs in the longitudinal and transverse directions,* $\langle \Delta x^2{}_{Longitudinal}(\tau) \rangle / \langle \Delta x^2{}_{Transverse}(\tau) \rangle$, *for different aspect ratios with lag time. Anisotropy in intracellular movement calculated in terms of ratio of MSDs in longitudinal direction and transverse direction increases with decrease in the width of the rectangular collagen island particularly when the lag time increases (n=15 cells).*

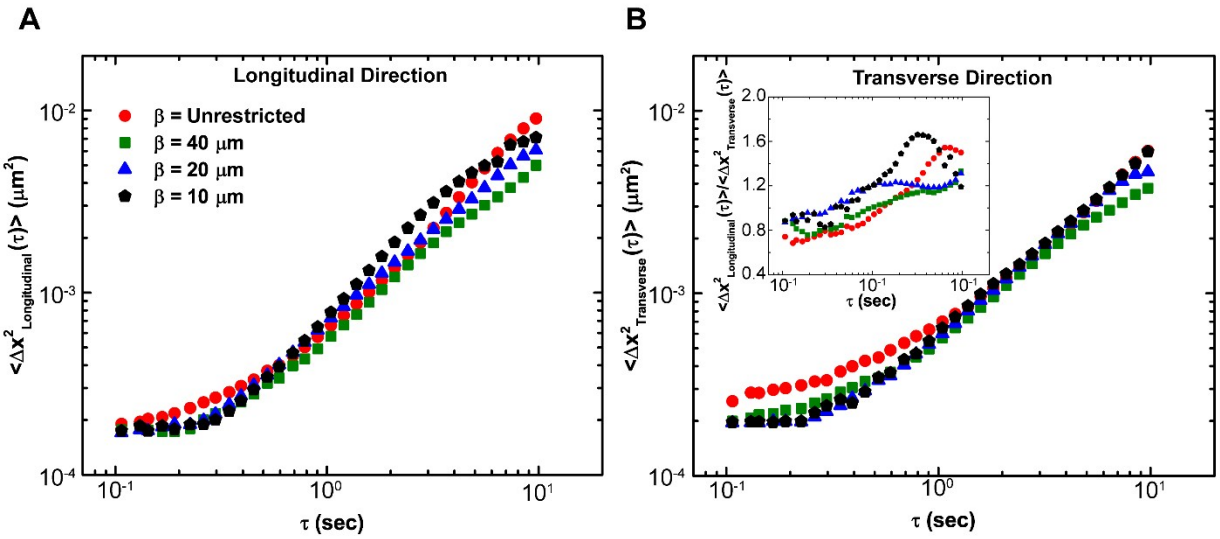

Figure S3. (A and B) MSDs $\langle \Delta x^2(\tau) \rangle$, *in longitudinal and transverse directions of tracer particles are plotted against lag time on a log-log scale for cytochalasin D treated cells. Inset shows the ratio of MSDs in the longitudinal and transverse directions,* $\langle \Delta x^2_{Longitudinal}(\tau) \rangle / \langle \Delta x^2_{Transverse}(\tau) \rangle$, *for different aspect ratios with lag time for cytochalasin D treated cells. The MSD curves collapse for different aspect ratio in both directions with similar magnitude suggesting anisotropy in intracellular movement can possibly be attributed to alignment of actin and related forces in longitudinal direction (n=15 cells).*

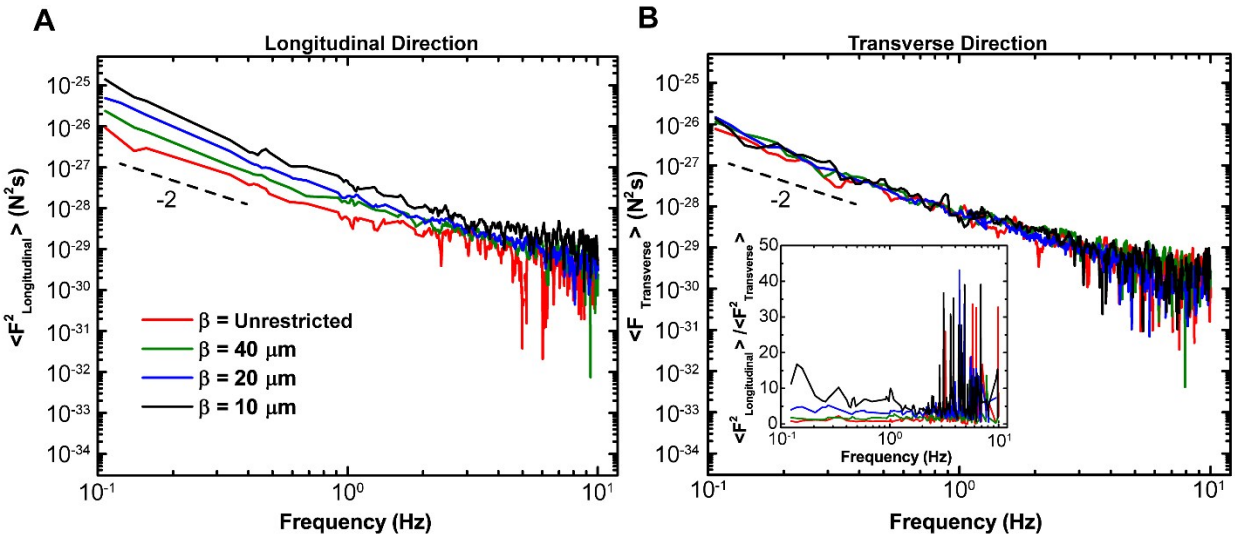

Figure S4. (A and B) Cytoplasmic force spectrum calculated from intracellular movement and active microrheology measurement using $\langle F^2(f)\rangle = |K(f)|^2 \langle x^2(f)\rangle$ *in longitudinal and transverse direction. Inset shows the ratio of forces in the longitudinal and transverse directions,* $\langle F^2{}_{Longitudinal}(f)\rangle / \langle F^2{}_{Transverse}(f)\rangle$ *for different aspect ratios with frequency (n=15 cells).*