Supplemental Materials

Molecular Biology of the Cell

Rey-Suarez *et al*.

Supplementary Information

Supplementary Table 1. Instantaneous speeds of EB3 in DMSO and SMIFH2 treated cells activated on CD3 or CD3+VCAM-1 coated coverslips.

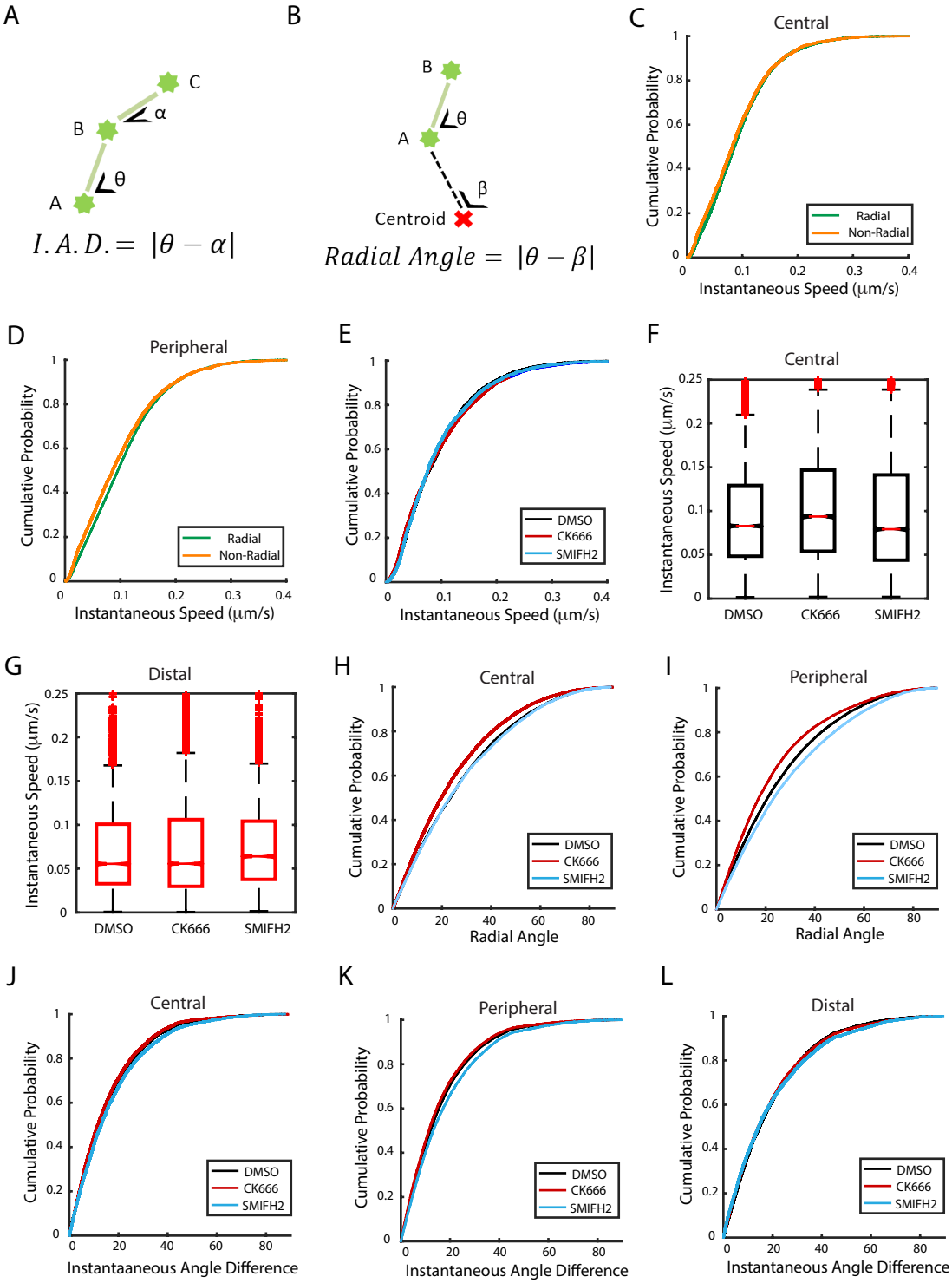
	Region	Median speed (μm/s)	Mean speed (μm/s)	SD (μm/s)
CD3				
DMSO	Central	0.0829	0.0973	0.0657
	Peripheral	0.0865	0.1029	0.0766
	Distal	0.0537	0.0711	0.0597
SMIFH2	Central	0.0793	0.1012	0.0755
	Peripheral	0.0794	0.1019	0.1041
	Distal	0.0615	0.0783	0.0620
CD3 + VCAM-1				
DMSO	Central	0.0933	0.1054	0.0718
	Peripheral	0.0846	0.1056	0.0825
SMIFH2	Central	0.0553	0.0716	0.0561
	Peripheral	0.0515	0.0686	0.0592

Number of interframe displacements N: For DMSO (CD3 only) $N_{central}$ = 12930, $N_{peripheral}$ = 23962, N_{distal} = 9579. For SMIFH2 (CD3 only) $N_{central}$ = 10268, $N_{peripheral}$ = 21128, N_{distal} = 10345. For DMSO (CD3+VCAM-1) $N_{central}$ = 16672, $N_{peripheral}$ = 53854. For SMIFH2 (CD3+VCAM-1) $N_{central}$ = 11921, $N_{peripheral}$ = 42267.

Supplementary Table 2. Persistence length l_p obtained from fitting the curves of cosine correlation decay with distance.

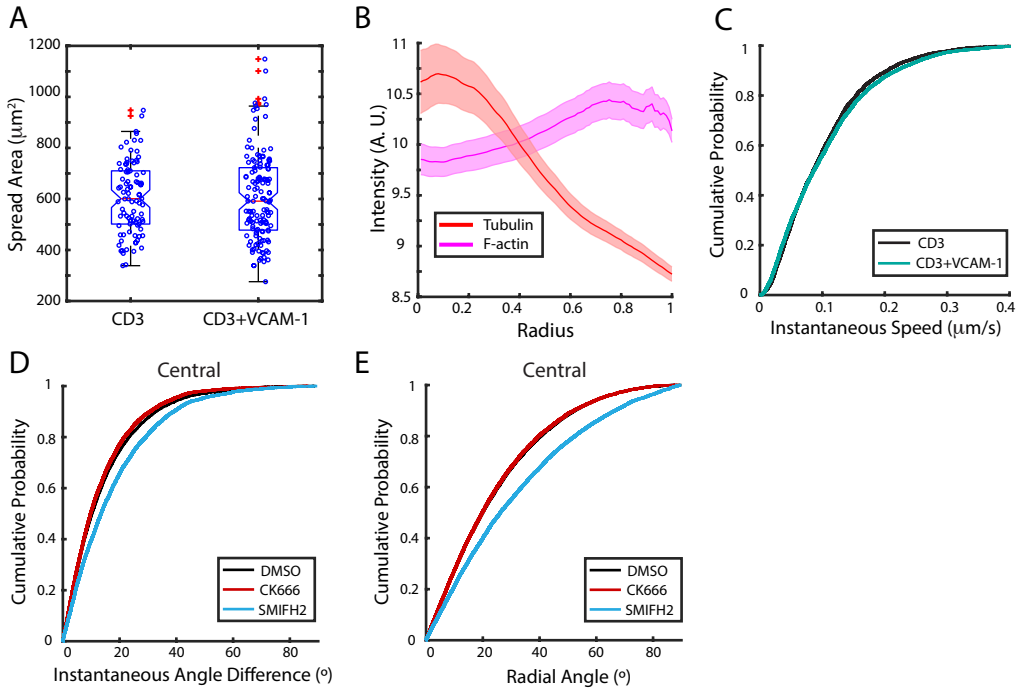
Condition	DMSO Periphery	DMSO Center	CK666 Periphery	Ck666 Center	SMIFH2 Periphery	SMIFH2 Center	Y27632 Periphery	Y27632 Center
l_p	6.65 μm	14.23μm	11.58 μm	16.11μm	9.79 μm	15.41μm	11.50 μm	27.30μm
95% Lower bound	5.41 μm	10.57μm	10.60 μm	14.39μm	8.84 μm	10.99μm	10.66 μm	25.46μm
95% Upper bound	7.89 μm	17.9 μm	12.57 μm	17.83μm	10.75 μm	19.84μm	12.34 μm	29.14μm

Number of filaments N: For DMSO N_{center} = 113, $N_{periphery}$ = 128. For CK666 N_{center} = 143, $N_{periphery}$ = 98. For SMIFH2 N_{center} = 73, $N_{periphery}$ = 149. For Y27632 N_{center} = 171, $N_{periphery}$ = 170.

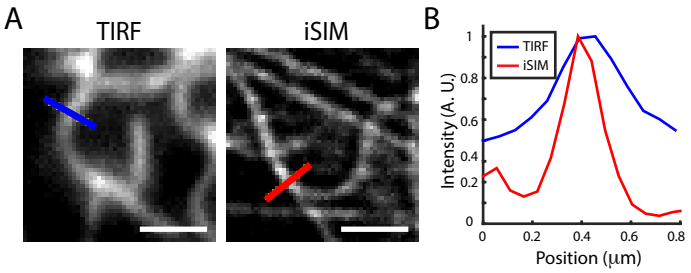


Supplementary Figure 1. A) Diagram illustrating how the instantaneous angle difference (I.A.D.) is calculated from two consecutive inter-frame displacements within a trajectory (point A is the starting point and C the ending point). B) Diagram illustrating how the radial angle is calculated for an inter-frame displacement with respect to the cell centroid (represented by the red cross). Cumulative distributions of instantaneous EB3

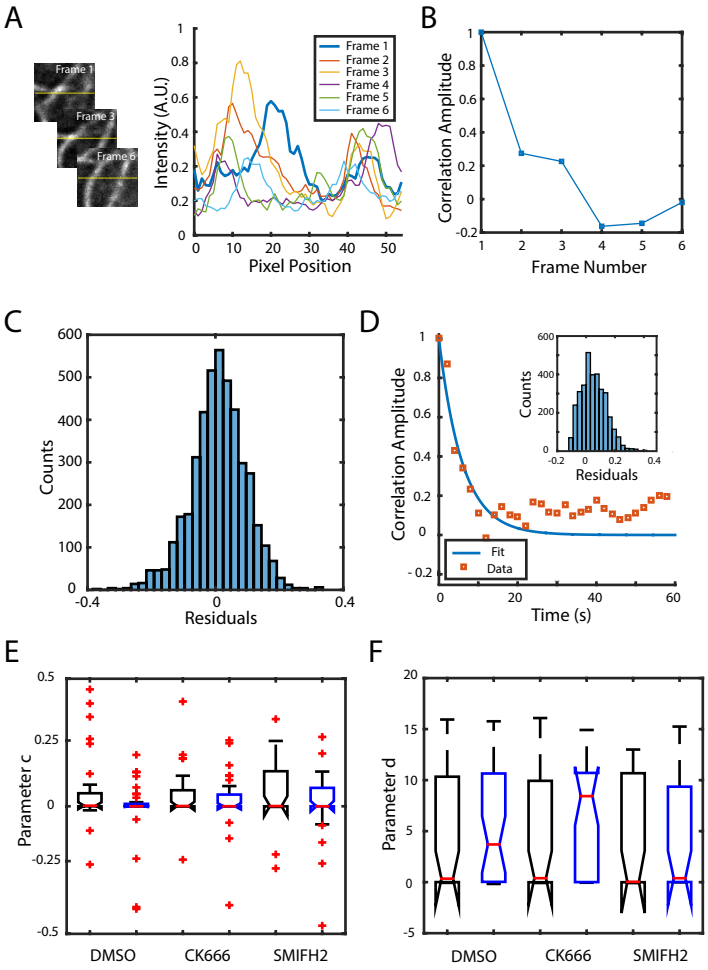
speeds classified as radial (radial angle ≤ 45°) or non-radial (radial angle > 45°) for central (C) and peripheral (D) regions in DMSO control cells. E) Cumulative distribution of EB3 instantaneous speeds for all regions in cells treated with the actin nucleation inhibitors CK666 (Arp2/3) or SMIFH2 (formin) compared with DMSO control. N = 11 cells for DMSO, N = 11 for CK666 and N = 16 for SMIFH2. F) Comparison of EB3 instantaneous speed measured in the central and G) peripheral region for cells treated with actin nucleation inhibitors CK666 or SMIFH2 with DMSO control. Cumulative distribution of radial angles for cells treated CK666 and SMIFH2 as compared to DMSO control in the central (H) and peripheral (I) regions. J, K, L) Cumulative distribution plots of instantaneous angle differences for central, peripheral and distal regions respectively. Significance of differences was tested using the Kruskal-Wallis test. For H) CK666 was significantly different from DMSO and SMIFH2 (p<0.01) and for I) DMSO, CK666 and SMIFH2 were significantly different from each other (p<0.01).



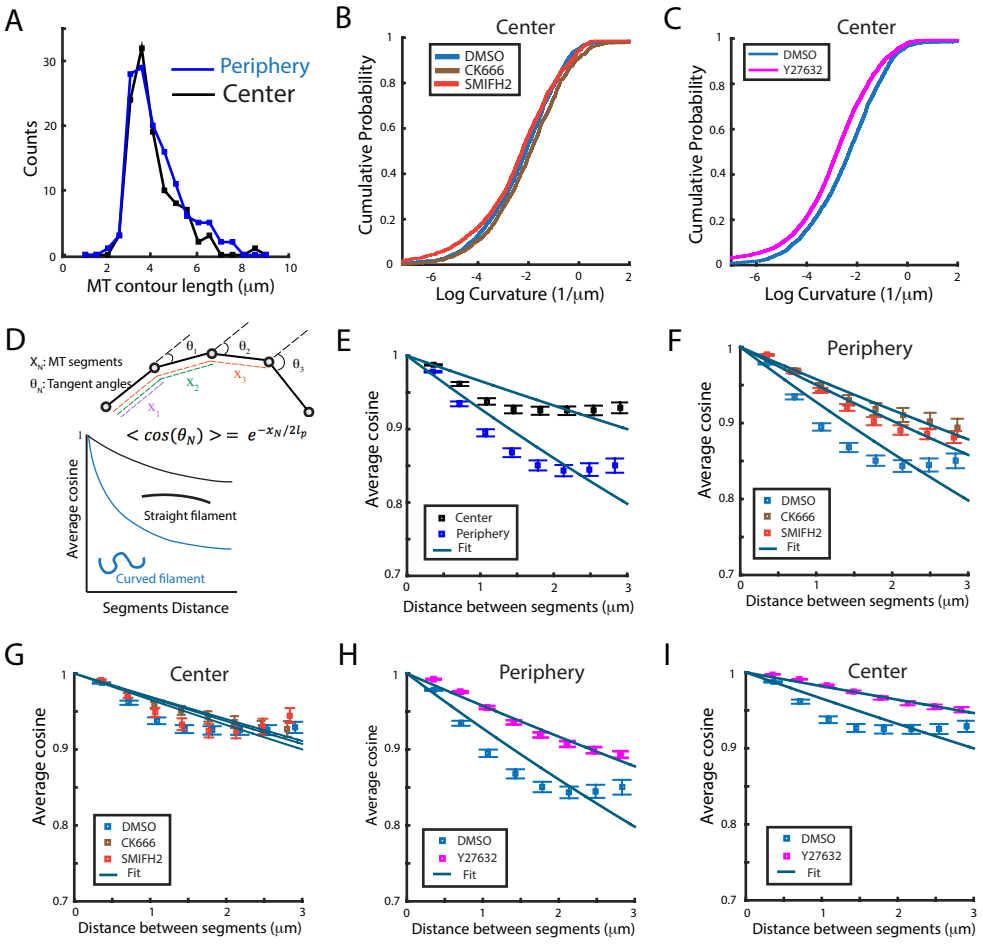
Supplementary Figure 2. A) Swarm box plots of the spread area measured with IRM of cells fixed on anti-CD3 and on glass coated with anti-CD3 + VCAM-1. B) Intensity profile for tubulin and F-actin for cells activated on anti-CD3 + VCAM-1 coated glass. The plot shows the average intensity profile obtained from N-82 cells. The shaded region represents the standard error. C) Cumulative distribution of instantaneous speeds of EB3 across all regions for cells activated on CD3 or CD3+VCAM-1 substrates. D) Cumulative distribution of instantaneous angle difference and E) radial angles in the central region for cells on CD3+VCAM-1 substrates treated with CK666 and SMIFH2 as compared to DMSO (carrier control). Significance of differences was tested using the Kolmogorov-Smirnoff test. SMIFH2 was significantly different from DMSO and CK666 (p<0.001) for both, radial and instantaneous angle differences.



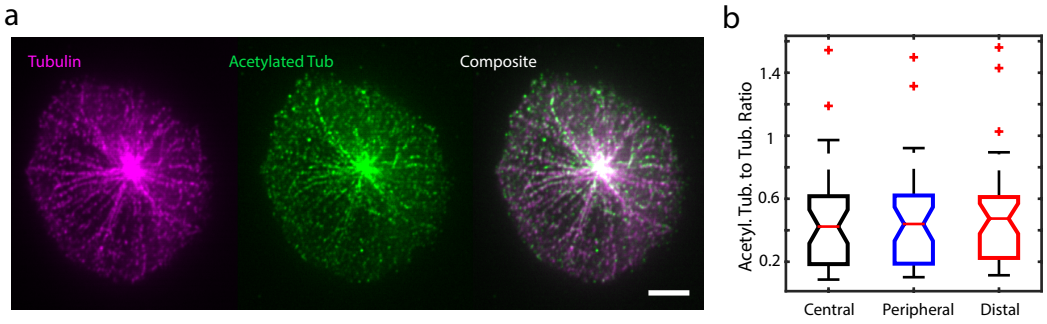
Supplementary Figure 3. A) Snapshot of microtubules labeled with EMTB-EGFP imaged with TIRF (left) and iSIM (right). B) Intensity profiles along the blue (TIRF) and red (iSIM) lines which highlights the higher resolution obtained with iSIM. Scale bar in both images is 1 μm.



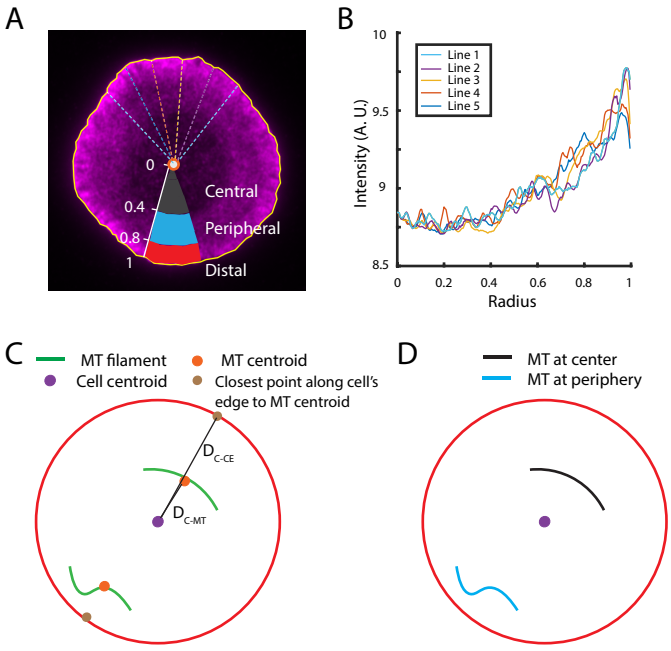
Supplementary Figure 4. A) Diagram illustrating the workflow to obtain the temporal correlation decay of the microtubule fluorescence intensity signal. The intensity profile plots correspond to the same yellow line for different frames/time points. B) Temporal correlation decay obtained from comparing the intensity profile of frame 1 with itself and consecutive frames (using the MATLAB function *corrcoef*). C) Distribution of residuals from double-exponential fit to correlation curves from DMSO cells. D) Sample single exponential fit for a correlation curve from the peripheral region of a DMSO-treated cell. Inset plot shows the distribution of residuals from single-exponential fit to correlation curves from DMSO cells. E) Plots of the amplitude of the second component (Parameter c) of the double exponential fit for central (black) and peripheral (blue) regions for cells treated with DMSO, CK666 and SMIFH2. F) Plots of the second correlation time (Parameter d) for the conditions described above.



Supplementary Figure 5. A) Histogram of the distribution of the MT contour lengths for the traces used for analysis. B) Cumulative probability plots of local curvature distribution measured for central MT filaments in cells treated with DMSO (control), CK666 (brown) and SMIFH2 (red). C) Cumulative distribution plot comparing the local curvature of central filaments in cells treated with Rho-kinase inhibitor Y27632 (magenta) with DMSO control cells (blue). D) Diagram describing how the tangent angles and segments are defined and used to compute the decay of the average of the cosines (of the tangent angles) with segment distance. E) Plots comparing center and periphery average cosines decay with distance along the filaments for cells from DMSO control condition. Solid line corresponds to the fit as described in methods. F) Comparison of average cosines decay for DMSO (blue), CK666 (brown) and SMIFH2 (red) for filaments located at the periphery and G) center. H) Plots average cosines decay with distance along periphery filaments and I) center filaments for cells from DMSO (blue) control compared with cells treated with Y27632 (magenta).



Supplementary Figure 6. A) iSIM images of cells fixed 10 minutes after activation and immunostained for tubulin (magenta) and acetylated tubulin (green). B) Comparison of the fluorescence intensity ratio of acetylated-tubulin to tubulin in each region (central, peripheral and distal) of the cell-substrate contact zone for cells as described in a. N=50 cells. Scale bar is 5 μm.



Supplementary Figure 7. A) Diagram showing the centroid (orange circle), cell contour (yellow line around the cell), sample lines to obtain radial intensity profiles (dashed lines) and the criteria used to assign pixels to a specific region of the cell based on their radial position. B) Plots of sample radial intensity profiles for the dashed lines in A. C) Schematic illustrating the process to assign a microtubule (MT) filament to a given region. The distance between the centroid of the cell and the filament centroid $D_{C\text{-}MT}$ is calculated. Then the distance between the centroid of the cell to the closest point to the MT centroid along the cell edge $D_{C\text{-}CE}$ is calculated. If the ratio $D_{C\text{-}MT}/ D_{C\text{-}CE}$ is less or equal to 0.4 the filament is assigned to the central region and D) plotted in black otherwise it is assigned to the peripheral region and plotted in blue.

Supplementary Movie Captions

Supplementary Movie 1. Time lapse movie of an activated Jurkat T cell double transfected with tdTomato-F-tractin (red) and EGFP-EB3 (green). Scale bar is 5 μm.

Supplementary Movie 2. Time lapse showing EB3 trajectories (labeled in red) detected using the MATLAB routine Utrack.

Supplementary Movie 3. Activated Jurkat T cells double transfected with tdTomato-F-tractin (red) and EGFP-EB3 (green) treated with DMSO (left), CK666 50 μM (center) and SMIFH2 25 μM (right). Scale bar is 5 μm.

Supplementary Movie 4. Jurkat T cells double transfected with tdTomato-F-tractin (red) and EGFP-EB3 (green) activated on glass slide coated with anti-CD3 and VCAM-1 treated with DMSO (left) and SMIFH2 25 μM (right). Scale bar is 5 μm.

Supplementary Movie 5. Instant structured illumination microscopy (iSIM) timelapse of an activated Jurkat T cell transfected with EMTB-EGFP. Scale bar is 5 μm.