

Spatial and Temporal Relationships between Actin-Filament Nucleation, Capping, and Disassembly

Janet H. Iwasa and R. Dyche Mullins

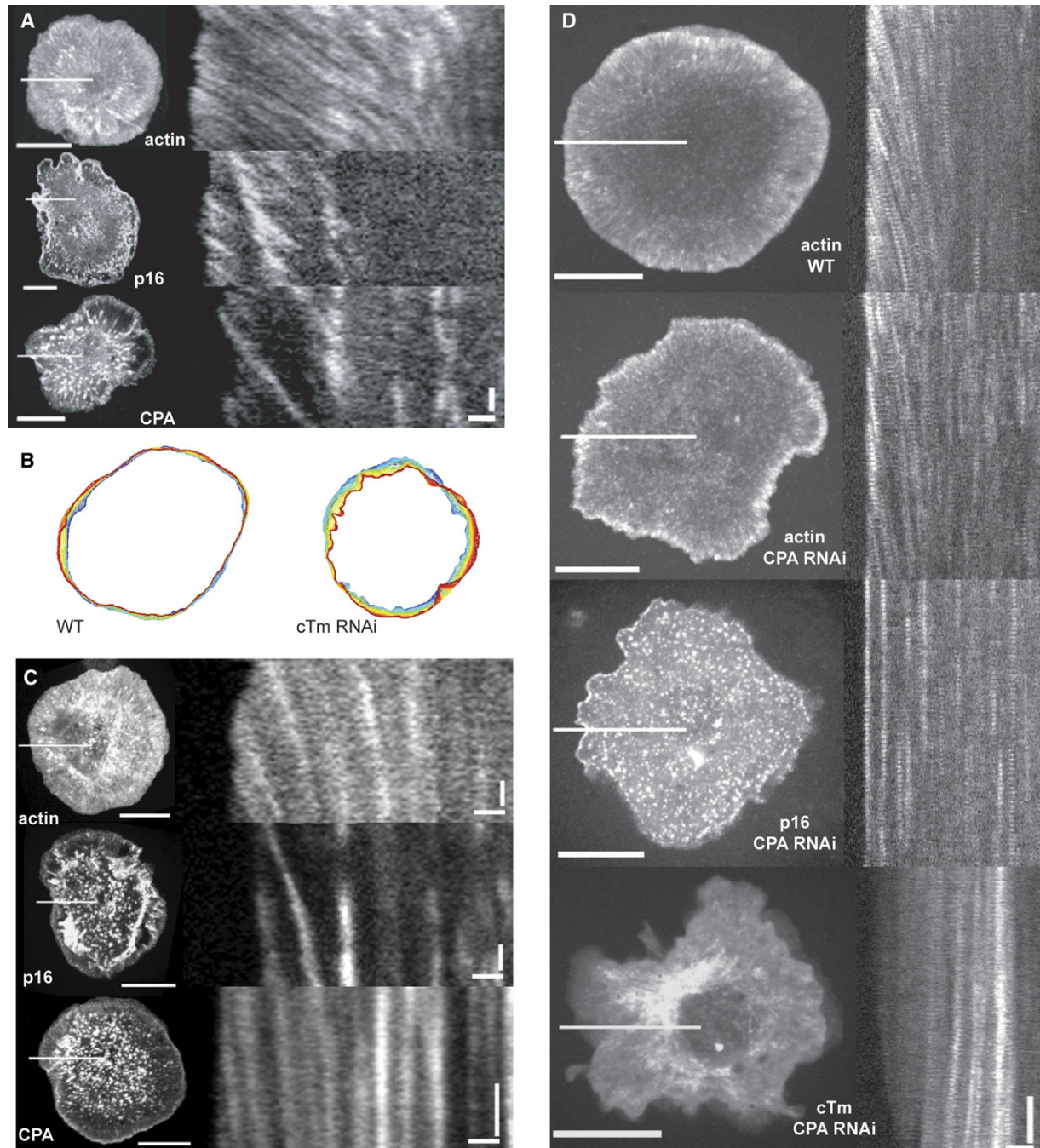


Figure S1. Representative Kymographs for dsRNA treated S2 cells

(A) Left, maximum-intensity projections of time-lapse movies of S2 cells treated with cTm dsRNA and expressing (from top to bottom) GFP-actin, GFP-p16, GFP-CPA, and GFP-cTm. The scale bar (A, C, and D) represents 10 μm . The white line at the 9 o'clock position (A, C, and D) indicates the cell area from which kymographs (right) were constructed. The kymograph x axis scale bar represents 1 μm ; the y axis scale bar represents 30 s.

(B) Cell outlines from each frame of a movie of (left) an untreated cell and (right) a cTm-depleted cell. A blue outline indicates the first frame of each movie, and a red outline indicates the last frame.

(C) Left, maximum-intensity projections of time-lapse movies of S2 cells depleted of cofilin and expressing (top to bottom) GFP-actin, GFP-p16, and GFP-CPA.

(D) Left, maximum-intensity projections of time-lapse movies of (top to bottom) an untreated S2 cell expressing GFP-actin and CPA-depleted cells expressing GFP-actin, GFP-p16, and GFP-cTm.

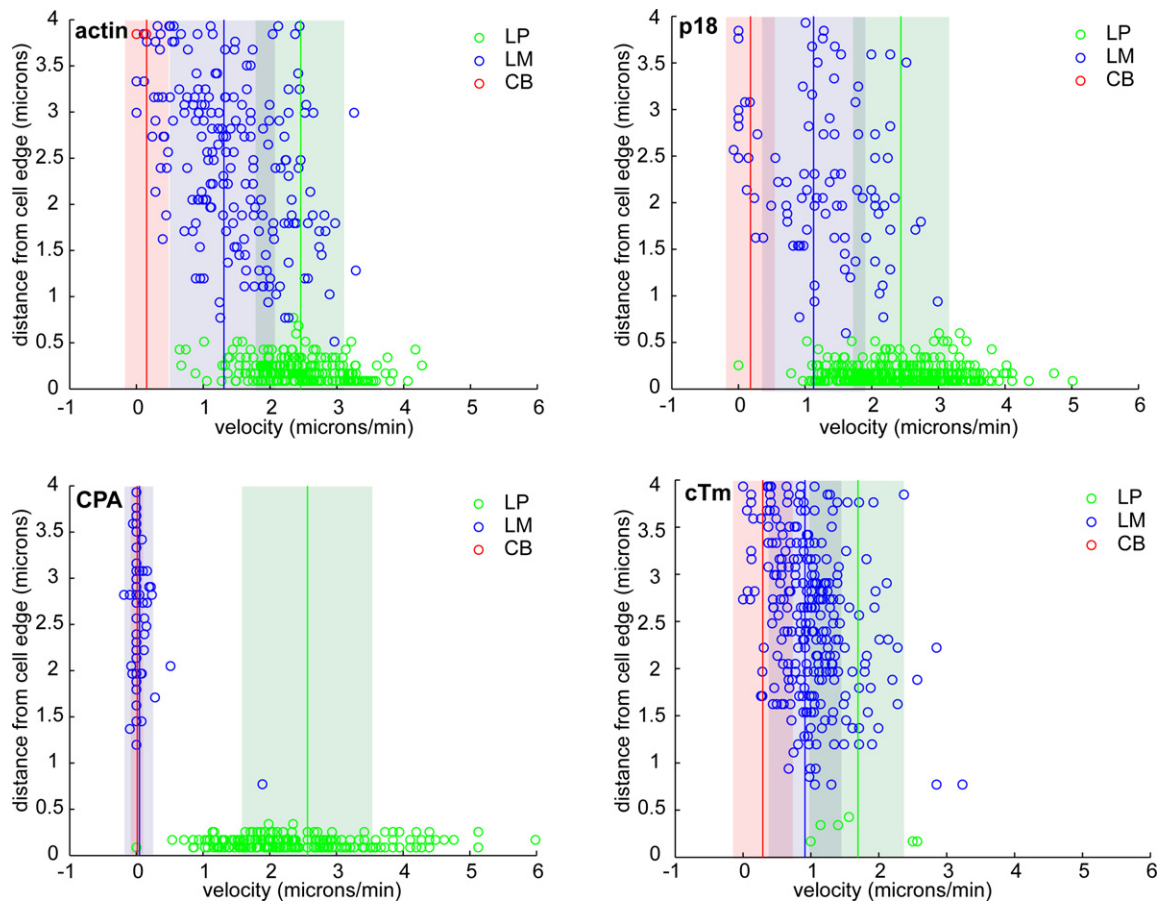


Figure S2. GFP-Tagged Actin and Actin-Regulatory Proteins Occupy Unique Compartments at the S2 Cell's Leading Edge

Scatter plots of the distance from the cell edge versus the velocity of speckles of (top left) actin-, (top right) p16-, (bottom left) CPA-, and (bottom right) cTm-expressing cells are identical to those in Figure 2C but focus on the lamellar and lamellipodial regions (0–4 μm from the cell edge).

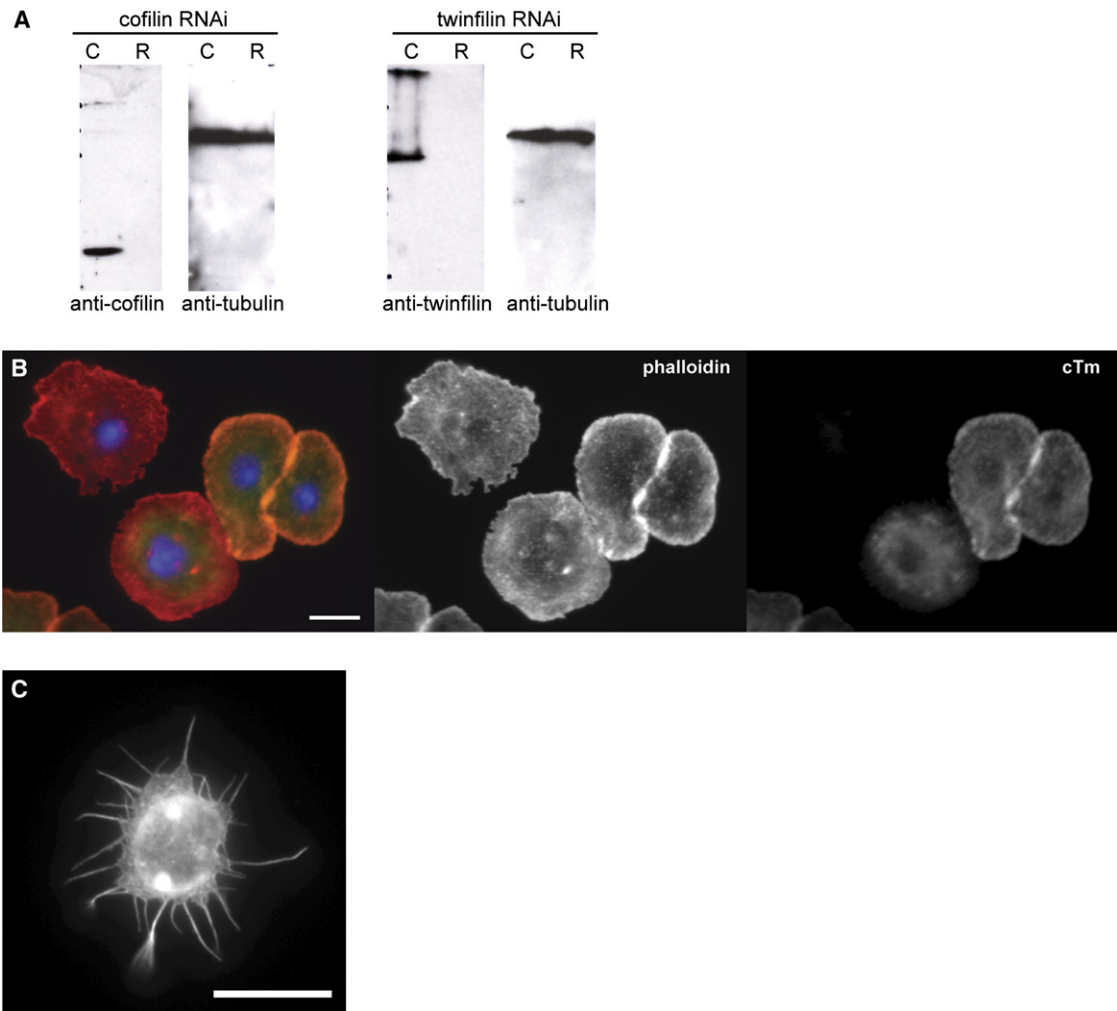


Figure S3. Depletion of Target Proteins in S2 Cells

(A) Western blot showing depletion of cofilin (left), twinfilin (right) in S2 cell extracts. The left lane ("C") shows control untreated extracts, and the right lane ("R") shows extracts from cells treated with dsRNA for 7 days. Alpha-tubulin antibodies were used as a loading control.

(B) cTm knockdown by RNAi correlates with leading-edge morphological abnormalities. Epifluorescent microscopic images of a stably transfected GFP-tropomyosin cell line depleted of cTm. Left: overlay. Middle: Alexa 568 phalloidin staining. Right: GFP fluorescence. The scale bar represents 10 μm .

(C) cTm RNAi causes a minority of cells to adopt a stellate phenotype. The cell is stained with Alexa 488 phalloidin. The scale bar represents 10 μm .

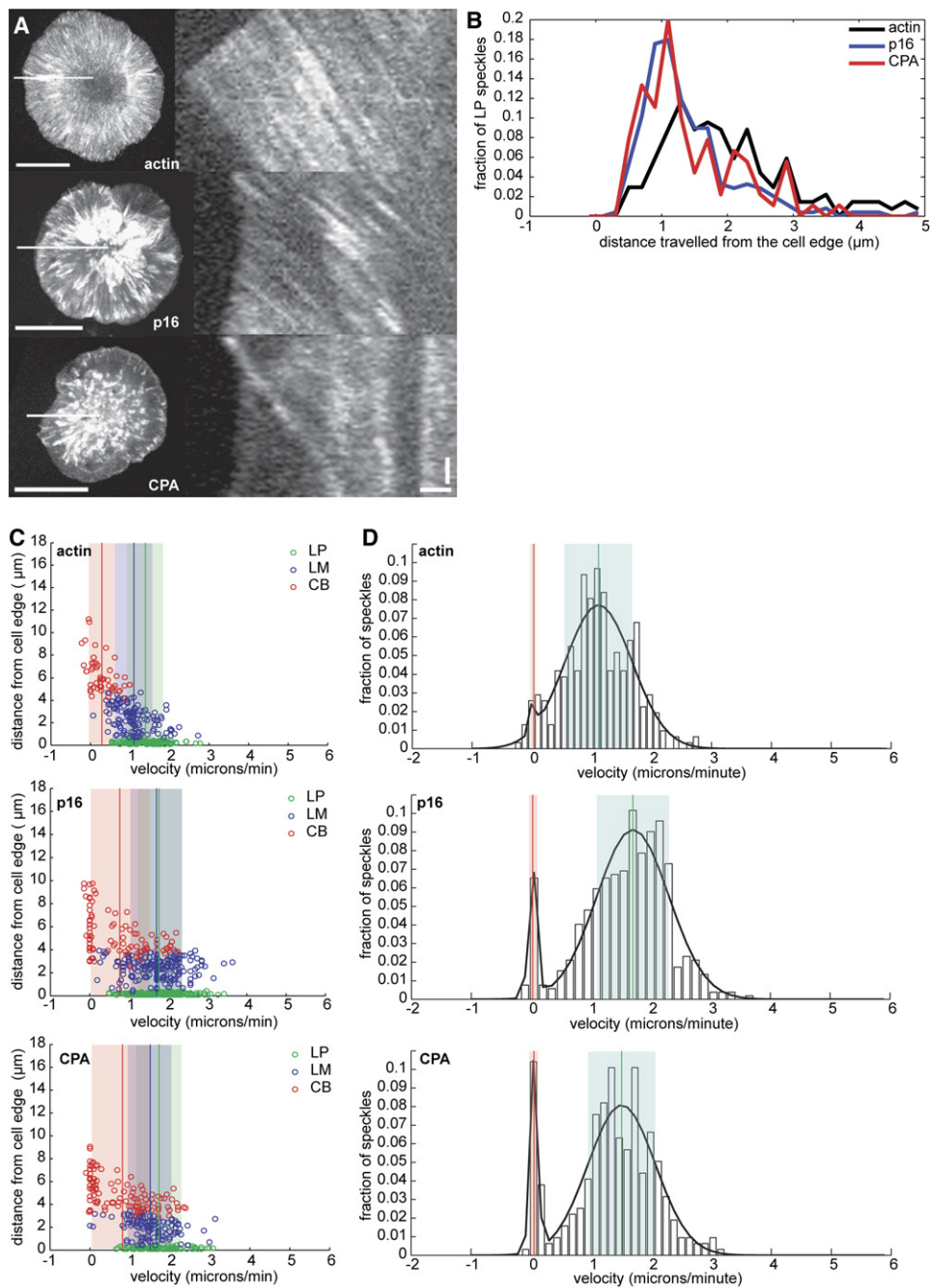


Figure S4. Slingshot RNAi Causes Expansion of the Lamellipod at the Expense of the Lamellum

(A) Left panel, maximum-intensity projections of time-lapse movies of S2 cells treated with slingshot dsRNA and expressing (from top to bottom) GFP-actin, GFP-p16, and GFP-CPA. The scale bar represents 10 μm . The white line at the 9 o'clock position indicates the cell area from which kymographs (right panel) were constructed. Right panel: the x axis scale bar represents 1 μm ; the y axis scale bar represents 30 s.

(B) Line histogram showing the distance traveled by GFP-actin, GFP-p16, and GFP-CPA speckles originating in the LP in slingshot-depleted cells. Speckles used in this histogram are shown as green circles in each of the scatter plots in (C).

(C) Scatter plots were created as described in Figure 2C. Slingshot-depleted cells expressing (from top to bottom) GFP-actin, GFP-p16, and GFP-CPA were analyzed.

(D) Probability density functions were created as described in Figure 2D. Slingshot-depleted cells expressing (from top to bottom) actin, p16, and CPA were analyzed.

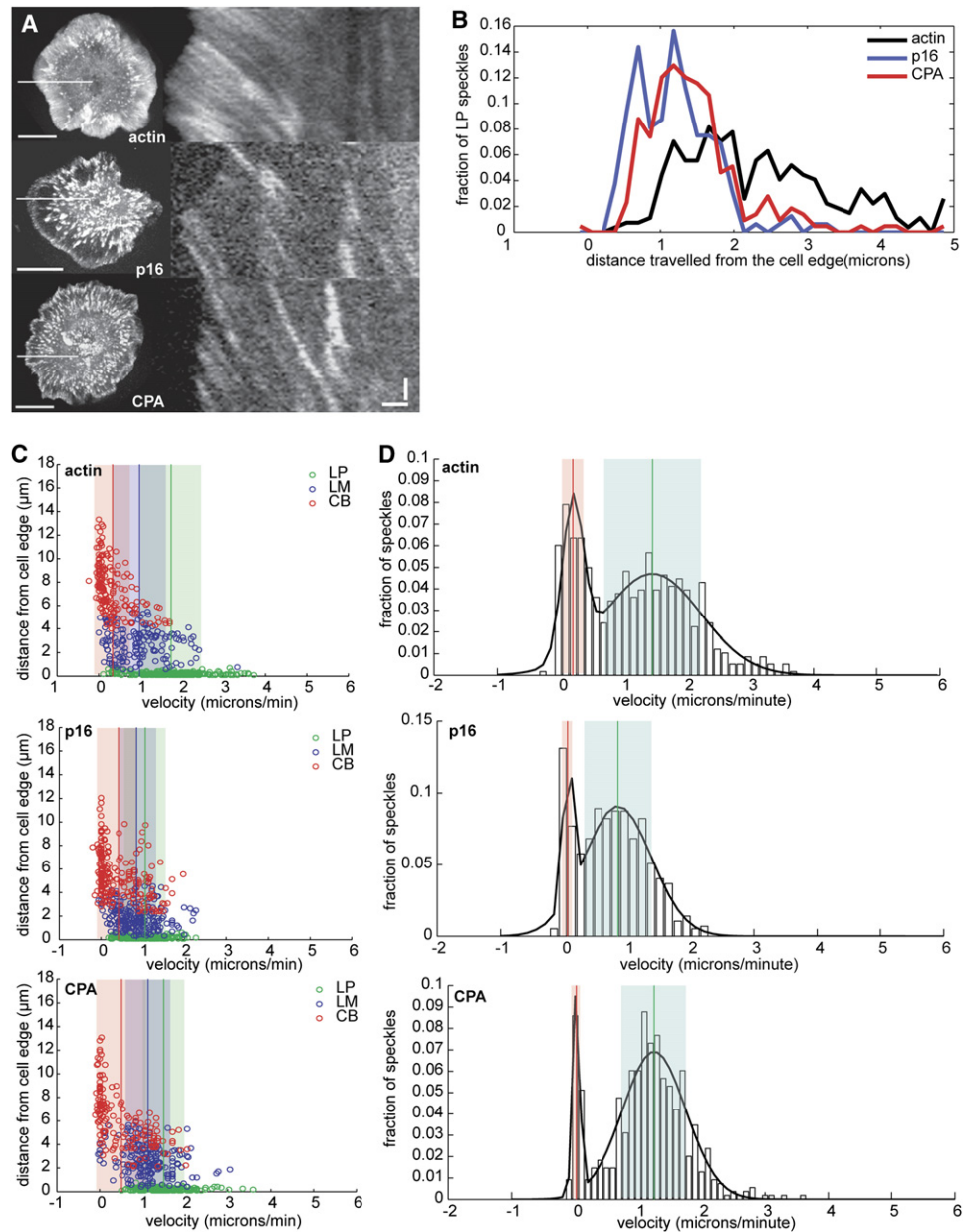


Figure S5. Twinfilin RNAi Causes Expansion of the Lamellipod at the Expense of the Lamellum

(A) Left panel, maximum-intensity projections of time-lapse movies of S2 cells treated with twinfilin dsRNA and expressing (from top to bottom) GFP-actin, GFP-p16, and GFP-CPA. The scale bar represents 10 μm . The white line at the 9 o'clock position indicates the cell area from which kymographs (right panel) were constructed. Right panel: the x axis scale bar represents 1 μm ; the y axis scale bar represents 30 s.

(B) Line histogram showing the distance traveled by GFP-actin, GFP-p16, and GFP-CPA speckles originating in the LP in twinfilin-depleted cells. Speckles used in this histogram are shown as green circles in each of the scatter plots in (C).

(C) Scatter plots were created as described in Figure 2C. Twinfilin-depleted cells expressing (from top to bottom) GFP-actin, GFP-p16, and GFP-CPA were analyzed.

(D) Probability density functions (PDF) were created as described in Figure 2D. Twinfilin-depleted cells expressing (from top to bottom): actin, p16, and CPA were analyzed.

Table S1. Summary of Values for Speckle Trajectories Based on Kymograph Analyses of GFP-Labeled Proteins in S2 Cells

| Protein Tagged | RNAi Treatment | Cellular Compartment | Number of Trajectories | Velocity ($\mu\text{m}/\text{min}$) | Distance Traveled (μm) | Lifetime (min) | |
|----------------|----------------|----------------------|------------------------|---------------------------------------|-------------------------------------|------------------------|------------------------|
| actin | none | LP | 244 | 2.46 \pm 0.04 [0.65] | 1.40 \pm 0.03 [0.50] | 0.59 \pm 0.01 [0.20] | |
| | | LM | 251 | 1.31 \pm 0.04 [0.76] | 0.77 \pm 0.03 [0.45] | 0.63 \pm 0.02 [0.24] | |
| | | CB | 193 | 0.15 \pm 0.02 [0.33] | 0.11 \pm 0.02 [0.22] | 0.85 \pm 0.03 [0.43] | |
| | CPA | LP | 281 | 1.76 \pm 0.06 [0.97] | 0.70 \pm 0.02 [0.32] | 0.48 \pm 0.01 [0.25] | |
| | | LM | 306 | 0.94 \pm 0.04 [0.71] | 0.50 \pm 0.02 [0.34] | 0.62 \pm 0.02 [0.29] | |
| | | CB | 164 | 0.24 \pm 0.03 [0.38] | 0.16 \pm 0.02 [0.25] | 0.88 \pm 0.04 [0.50] | |
| | cofilin | LP | 147 | 0.88 \pm 0.05 [0.63] | 1.00 \pm 0.05 [0.61] | 1.45 \pm 0.07 [0.80] | |
| | | LM | 239 | 0.48 \pm 0.03 [0.47] | 0.67 \pm 0.03 [0.48] | 1.83 \pm 0.06 [0.89] | |
| | | CB | 187 | 0.14 \pm 0.01 [0.18] | 0.24 \pm 0.02 [0.27] | 1.90 \pm 0.06 [0.75] | |
| | cTm | LP | 502 | 2.08 \pm 0.03 [0.57] | 1.82 \pm 0.03 [0.78] | 0.92 \pm 0.02 [0.45] | |
| | | LM | 260 | 1.70 \pm 0.04 [0.65] | 1.46 \pm 0.05 [0.81] | 0.89 \pm 0.02 [0.38] | |
| | | CB | 241 | 0.51 \pm 0.03 [0.54] | 0.46 \pm 0.03 [0.45] | 1.14 \pm 0.03 [0.50] | |
| | slingshot | LP | 136 | 1.39 \pm 0.04 [0.44] | 1.71 \pm 0.08 [0.94] | 1.32 \pm 0.07 [0.77] | |
| | | LM | 123 | 1.09 \pm 0.04 [0.46] | 1.50 \pm 0.08 [0.84] | 1.45 \pm 0.07 [0.77] | |
| | | CB | 51 | 0.29 \pm 0.04 [0.32] | 0.36 \pm 0.06 [0.40] | 1.57 \pm 0.11 [0.77] | |
| | twinfilin | LP | 270 | 1.75 \pm 0.04 [0.71] | 2.13 \pm 0.06 [1.04] | 1.33 \pm 0.04 [0.61] | |
| | | LM | 148 | 0.98 \pm 0.05 [0.63] | 1.25 \pm 0.06 [0.76] | 1.47 \pm 0.05 [0.61] | |
| | | CB | 164 | 0.33 \pm 0.03 [0.42] | 0.45 \pm 0.04 [0.48] | 1.76 \pm 0.05 [0.61] | |
| | p16/Arp3 | none | LP | 468 | 2.44 \pm 0.03 [0.72] | 0.83 \pm 0.01 [0.27] | 0.36 \pm 0.01 [0.13] |
| | | | LM | 116 | 1.13 \pm 0.07 [0.77] | 0.44 \pm 0.03 [0.33] | 0.49 \pm 0.03 [0.27] |
| | | | CB | 67 | 0.18 \pm 0.04 [0.36] | 0.09 \pm 0.02 [0.14] | 0.77 \pm 0.06 [0.53] |
| CPA | | LP | 102 | 0.45 \pm 0.04 [0.39] | 0.31 \pm 0.02 [0.22] | 0.89 \pm 0.05 [0.48] | |
| | | LM | 268 | 0.20 \pm 0.02 [0.25] | 0.14 \pm 0.01 [0.16] | 0.97 \pm 0.04 [0.61] | |
| | | CB | 293 | 0.08 \pm 0.01 [0.16] | 0.06 \pm 0.01 [0.13] | 1.06 \pm 0.04 [0.63] | |
| cofilin | | LP | 184 | 1.11 \pm 0.04 [0.49] | 0.91 \pm 0.04 [0.49] | 0.91 \pm 0.03 [0.46] | |
| | | LM | 113 | 0.60 \pm 0.05 [0.51] | 0.58 \pm 0.05 [0.50] | 1.30 \pm 0.07 [0.76] | |
| | | CB | 117 | 0.07 \pm 0.02 [0.16] | 0.08 \pm 0.02 [0.17] | 1.99 \pm 0.08 [0.82] | |
| cTm | | LP | 387 | 1.76 \pm 0.03 [0.65] | 1.01 \pm 0.03 [0.51] | 0.68 \pm 0.02 [0.49] | |
| | | LM | 374 | 1.33 \pm 0.04 [0.72] | 0.86 \pm 0.02 [0.47] | 0.83 \pm 0.03 [0.57] | |
| | | CB | 216 | 0.59 \pm 0.05 [0.80] | 0.31 \pm 0.02 [0.37] | 1.33 \pm 0.06 [0.91] | |
| slingshot | | LP | 245 | 1.76 \pm 0.04 [0.55] | 1.22 \pm 0.04 [0.69] | 0.71 \pm 0.02 [0.33] | |
| | | LM | 186 | 1.70 \pm 0.05 [0.65] | 1.05 \pm 0.04 [0.59] | 0.66 \pm 0.02 [0.31] | |
| | | CB | 90 | 0.76 \pm 0.08 [0.76] | 0.53 \pm 0.06 [0.53] | 1.19 \pm 0.09 [0.82] | |
| twinfilin | | LP | 160 | 1.06 \pm 0.04 [0.49] | 0.98 \pm 0.04 [0.49] | 1.03 \pm 0.04 [0.48] | |
| | | LM | 204 | 0.85 \pm 0.03 [0.47] | 0.77 \pm 0.03 [0.38] | 1.12 \pm 0.04 [0.63] | |
| | | CB | 208 | 0.42 \pm 0.03 [0.50] | 0.36 \pm 0.03 [1.38] | 1.38 \pm 0.05 [0.70] | |
| CPA | | none | LP | 319 | 2.57 \pm 0.05 [0.97] | 0.58 \pm 0.01 [0.25] | 0.25 \pm 0.01 [0.11] |
| | | | LM | 101 | 0.05 \pm 0.02 [0.20] | 0.03 \pm 0.01 [0.87] | 0.87 \pm 0.04 [0.44] |
| | | | CB | 204 | 0.01 \pm 0.01 [0.086] | 0.01 \pm 0.01 [1.05] | 1.05 \pm 0.03 [0.45] |
| | cofilin | LP | 120 | 1.25 \pm 0.08 [0.88] | 0.81 \pm 0.04 [0.49] | 0.85 \pm 0.04 [0.46] | |
| | | LM | 120 | 0.67 \pm 0.07 [0.81] | 0.49 \pm 0.04 [0.47] | 1.04 \pm 0.04 [0.49] | |
| | | CB | 260 | 0.11 \pm 0.02 [0.25] | 0.10 \pm 0.02 [0.23] | 1.43 \pm 0.03 [0.47] | |
| | cTm | LP | 125 | 1.86 \pm 0.07 [0.81] | 0.97 \pm 0.05 [0.55] | 0.59 \pm 0.03 [0.34] | |
| | | LM | 132 | 1.92 \pm 0.08 [0.89] | 0.94 \pm 0.04 [0.45] | 0.59 \pm 0.03 [0.34] | |
| | | CB | 135 | 0.60 \pm 0.07 [0.83] | 0.34 \pm 0.04 [0.44] | 1.26 \pm 0.07 [0.76] | |
| | slingshot | LP | 90 | 1.73 \pm 0.06 [0.56] | 1.25 \pm 0.07 [0.70] | 0.76 \pm 0.05 [0.45] | |
| | | LM | 119 | 1.51 \pm 0.05 [0.53] | 1.06 \pm 0.05 [0.56] | 0.75 \pm 0.03 [0.36] | |
| | | CB | 108 | 0.81 \pm 0.07 [0.74] | 0.51 \pm 0.04 [0.45] | 1.08 \pm 0.08 [0.79] | |
| | twinfilin | LP | 215 | 1.50 \pm 0.03 [0.48] | 1.24 \pm 0.04 [0.84] | 0.84 \pm 0.02 [0.34] | |
| | | LM | 177 | 1.13 \pm 0.04 [0.52] | 0.94 \pm 0.04 [0.92] | 0.92 \pm 0.04 [0.48] | |
| | | CB | 154 | 0.51 \pm 0.05 [0.57] | 0.39 \pm 0.03 [1.28] | 1.28 \pm 0.06 [0.72] | |
| | cTm | none | LP | 6 | 1.69 \pm 0.28 [0.68] | 1.52 \pm 0.22 [0.55] | 0.98 \pm 0.18 [0.43] |
| | | | LM | 334 | 0.91 \pm 0.03 [0.53] | 1.07 \pm 0.03 [0.57] | 1.32 \pm 0.03 [0.54] |
| | | | CB | 227 | 0.29 \pm 0.03 [0.44] | 0.35 \pm 0.03 [0.49] | 1.75 \pm 0.04 [0.66] |
| | | CPA | LP | 0 | n/a | n/a | n/a |
| | | | LM | 49 | 0.29 \pm 0.04 [0.29] | 0.45 \pm 0.05 [0.36] | 1.77 \pm 0.10 [0.72] |
| | | | CB | 170 | 0.24 \pm 0.02 [0.23] | 0.37 \pm 0.03 [0.37] | 1.63 \pm 0.05 [0.69] |

Trajectories were categorized as lamellipodial (LP), lamellar (LM), or cell body (CB) based on their starting distance from the cell edge. The error is calculated as the standard error of the mean, and the standard deviation is indicated in square brackets.