Supplemental Methods

Expression Vectors.

The cDNA encoding the N-terminus of mouse c-Moesin was amplified from purified P6 CGN first strand cDNA using the following primers: Moe 5' Eco 5'-ggggaattcaccatggcccagagtgaggccgaaaagctagccaag-3' and Moe 3' GFP Fus 5'-cagctcctcgcccttgctcacgctcccgccgccgctcttgtatttgtctcgtccca-gtcgcatgtt-3'. The PCR product was then fused in frame with the Cyan fluorescent protein cDNA via a joining PCR reaction and sub-cloned into the EcoRI and NotI restriction sites of pCIG2.

The cDNA encoding full-length mouse MLC9 was amplified from purified P6 CGN first strand cDNA using the following primers: MLC9 5' EcoRI 5'-ggggaattcaccatgtcgagcaagagagccaaggcc-3' and MLC9 Venus Fus 5'-cagctcctcgcccttgctcaccatgctcccgccgccgctgtcgtccttgtcctt-ggcgccgtg-3'. The PCR product was then fused in frame with the Venus cDNA via a joining PCR reaction and subcloned into the EcoRI and NotI restriction sites of pCIG2.

The cDNA encoding full-length mouse Map2C was amplified from purified P6 CGN first strand cDNA using the following primers: Map2C 5'ATG 5'-atggccgacgagcggaaagatgaaggaaag-3' and Map2C 3' GFP Fus Not 5'-cccgcggccgctcacaagccctgcttagcaagcgccgcagt-3'. The PCR product was then fused in frame with the mRFP1 cDNA via a joining PCR reaction and subcloned into the EcoRI and NotI restriction sites of pCIG2.

Venus-MLCK cDNA was generated by amplifying the Venus cDNA with the following primers: EGFP 5' Xho 5'-ggggaattcaccatggtgagcaagggcgaggagctg-3' and Ven MLCK Fus 5'-ccgcggccgcttaagctttgagggatccacactgagggaggttttggaaatgtgtgacgaggcaaccagcttcacatcccccatatggtacagctcgtccatgcc-3'. The subsequent PCR product was subcloned into the XhoI and NotI sites of pBS SK. A 5.6 kb BamHI and NotI restriction fragment of the full-

length human MLCK cDNA (the kind gift of Anne Bresnick, Albert Einstein College of Medicine) was then subcloned into the pBS Venus vector. The resulting full-length Venus-MLCK cDNA was then subcloned into the XhoI and NotI sites of pCIG2.

The cDNA encoding full-length human Arp3 (Open Biosystems cDNA clone 5261640) was amplified using the following primers: Arp3 5' Eco 5'-ggggaattcaccatggcgggacggctgccggcctgtgtggtg-3' and Arp3 3' GFP Fus 5'-cagctcctcgcccttgctcaccatgctcccgccgccgctggacatgac-tccaaacactggattgtgacg-3'. The PCR product was then fused in frame with the Venus cDNA via a joining PCR reaction and subcloned into the EcoRI and NotI restriction sites of pCIG2.

The cDNA encoding full-length chicken Paxillin (Kind gift of Dr. Ken Jacobson, UNC-Chapel Hill) was amplified using the following primers: Pax 5' Eco 5'-ggggaattcaccatggacgacctcgatgccctgctggcagac-3' and Pax GFP Fus 5'-ctcctcgcccttgctcaccatgctcccgccgccgctgcagaagagcttcacgaa-gcagctctg-3'. The PCR product was then fused in frame with the Venus cDNA via a joining PCR reaction and subcloned into the EcoRI and NotI restriction sites of pCIG2.

Immunocytochemistry of primary CGNs

CGNs that were cultured *in vitro* for various times were washed with PBS, permeablized with Triton X-100 and blocked with normal donkey serum. Primary and secondary antibody staining was carried out in PBS plus 1% normal donkey serum. The primary antibodies used for this study were: anti-GFP (rabbit IgG, Invitrogen) and anti-MLC pSer19 (mouse IgG, Cell Signaling Technologies). After confocal microscopy, the fluorescence intensity of the resulting images was quantified using the Laserpix software associated with the Radiance 2000 suite of programs.

Transfection and Western Blotting of HEK293T cells.

HEK293 cells were transfected with 1 µg of pCIG2 Venus-MLCK construct or 1 µg of pCAG myc-Rock1 in the presence or absence of 5 µg pRK5 or pCIG2 myc-Par6α. 18 hours post-transfection, cells were harvested and lysed. Cell extracts were then loaded on SDS-PAGE gels (Nupage, Invitrogen), electrophoresed, and transferred to nylon membranes (Immobilon, Millipore). Blots were then blocked with non-fat milk and probed with antibodies recognizing phospho-Ser18,19 of MLC (mouse IgG, Cell Signaling Technologies), Thr696 of Mypt1 (rabbit IgG, Upstate), or p150 dynactin (mouse IgG, Transduction Labs) as a loading control. Blots were developed using an ECL Plus Western Blotting Kit (GE Healthcare) and exposed to X-ray film (Kodak).

ORNL Automated Centrosome Motion Analysis Approach

Our approach to automated centrosome motion analysis may be divided into two stages: (1) detection, and (2) tracking. The goal of the detection stage is to locate all centrosome-like objects in each frame of the time sequence. The tracking stage then attempts to connect these detections to form coherent tracks. The details of each stage are illustrated in Figure 1 and outlined below.

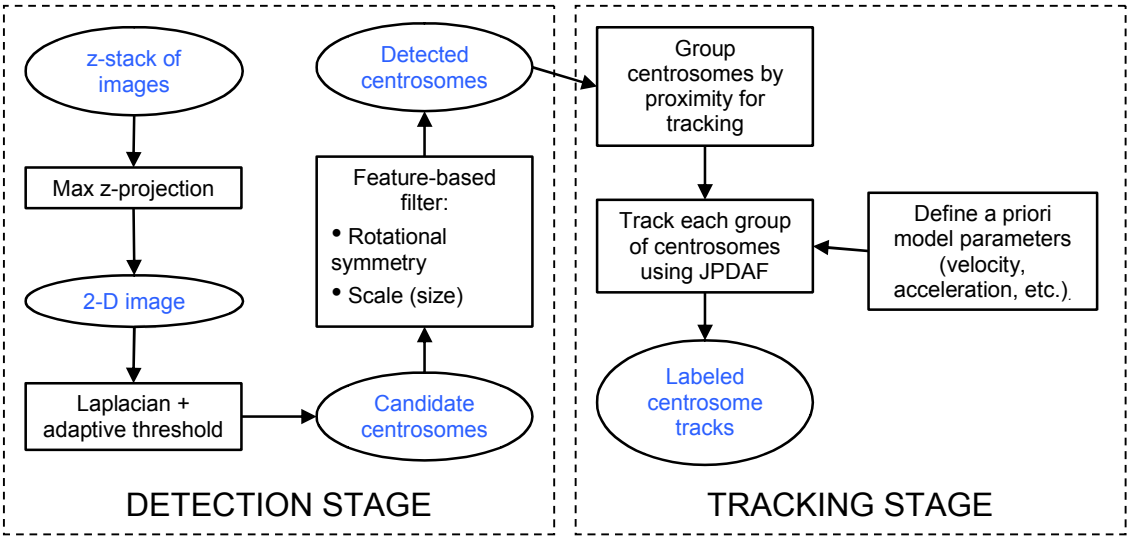


Figure 1. Flowchart of centrosome detection/tracking algorithm.

In the detection stage, we first compress the 3-D volume at each time step into a single 2-D image by computing the maximum intensity along the *z*-dimension at each (*x*,*y*) location. We then apply a Laplacian filter to this image, which helps to accentuate small, bright, circularly

shaped objects in the image. We apply an adaptive threshold to the filtered image by first computing the mean and standard deviation of the intensity values in a small window surrounding each pixel. Each pixel whose intensity is greater than a certain number of standard deviations above the mean is considered to be a potential centrosome location. We refine this set of detections by computing the size of the objects at these pixel locations as well as a roundness metric and rejecting objects for which these measurements fall outside of a predetermined range. For each object in this refined set, we compute the z-position of the object by extracting the column of intensity values at the corresponding (x,y) location from the original volume, interpolating this signal, and locating the maximum intensity value along z.

Once we have the detections for each frame, we then take the following approach to tracking the objects. Based on the assumption that no centrosome will jump by more than a predefined distance d between any two consecutive frames, we first partition the set of all detections over the entire sequence such that the Euclidean distance between any two detections from different groups is greater than d. We then apply a well-known tracking algorithm called the *joint probabilistic data association filter* (JPDAF) [1] to each group of detections, which is designed to handle missed detections, false alarms, and measurement noise. The JPDAF algorithm relies on a predefined motion model that includes *a priori* knowledge of velocity and acceleration parameters. We provide the tracker with starting locations by selecting (at most) the six brightest objects from each group in the first frame of the sequence; the JPDAF algorithm then produces a track for each of these starting locations. While most computed tracks run the entire duration of the sequence, some may terminate prematurely if the tracker loses the object before the end of the sequence. Examples of computed tracks in 3-D space are shown in Figure 2.

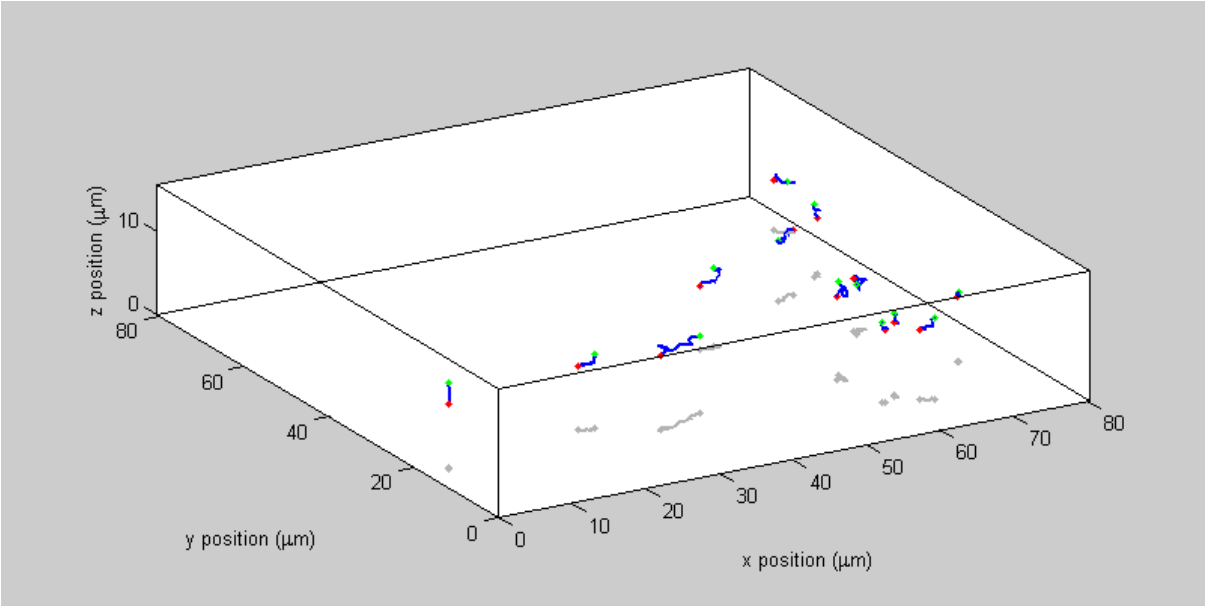


Figure 2. 3-D centrosome tracks computed using the JPDAF algorithm. The path of each centrosome is shown in blue, and the beginning and ending positions are shown as green and red dots, respectively. Track shadows on the *x-y* plane are shown in gray.

References

[1] Y. Bar-Shalom and T. Fortmann, Tracking and Data Association, Academic Press, 1988

Supplemental Figure Legends

Figure S1. Schematic of fusion proteins used in these studies, and the cellular compartments that they label.

Figure S2. Arp3 and Paxillin localize to the proximal leading process. (A) Arp3 (labeled by Arp3-Venus) is concentrated at the proximal leading process just before somal translocation. **(B)** Paxillin-Venus is concentrated at the proximal leading process just before somal translocation, a finding consistent with the localization of a diffuse adhesion plaque previously observed in electron micrographs of migrating CGNs (Gregory et al., 1988). **(C)** Paxillin-Venus is arrayed in focal adhesions at the leading edge of a cultured fibroblast-like cell.

Figure S3. Comparison of Myosin IIB and Myosin Va localization in cultured cerebellar CGNs. Cultured CGNs were fixed and stained with antibodies recognizing Myosin IIB or Myosin Va in combination with a Par6α antibody to serve as a normalization control. After immunostaining, respective images were collected with a spinning disk confocal microscope and the fluorescent intensity of Myosin II, Myosin Va and Par6α signals were analyzed by the line intensity scanning feature of Slidebook (ver. 4). **(A)** Representative image of Myosin II localization. Line intensity scanning reveals that Myosin II is enriched in the leading process as described the Results section. Par6α staining is more intense in the leading portion of the soma. **(B)** Representative image of Myosin Va localization. Line intensity scanning reveals that Myosin Va is evenly distributed along the leading and trailing processes. Par6α staining is more intense in the leading portion of the soma.

Figure S4. F-actin accumulates at the centrosome when this organelle moves toward the proximal leading process. Successive images of a migrating neuron expressing Centrin2-Venus and RFP-UTRCH-ABD, note the centrosome moves toward the leading process (left of image) before somal translocation. Adjacent to the time-lapse images are line scan profiles of RFP-UTRCH-ABD (red) and Centrin2-Venus (black), the left of the curve is the tip of the leading process while right is the tip of the trailing process. Note that the centrosome appears a peak within the black somal peak as cytoplasmic centrin2-venus signal fills the cytoplasm within the neuronal soma.

Figure S5. Activation of Myosin II motor activity spurs centrosome and somal translocation. 10 μM Calyculin A (CalA) spurs centrosome and somal motility in stationary CGNs. Green arrow: relative centrosome position. White arrow: relative position of rear of the soma before CalA addition.

Figure S6. Model of Par6α interaction with the Myosin II Motor Complex. Par6α binds to both MLC and MLCK, key signaling nodes regulating acto-myosin contractility.

Inset: The Par6-MLC interaction may be mediated by the IQ domain of Par6α (IQ Motif (aa 104-120)=AFASNSLQRRKKGLLLRPV) and the EF hand domains of MLC.

Supplemental Movies

Supplemental Movie 1. Actin, labeled with Moesin C-terminus-CFP, accumulates in the proximal leading process during glial-guided migration. Total elapsed time is 40 minutes.

Supplemental Movie 2. F-actin (EGFP-UTRCH-ABD) localizes to the leading process during glial guided neuronal migration. Note the nucleus (Histone 2B-mCherry) remains in the rear of the soma at all times.

Supplemental Movie 3. MLC-Venus accumulates in the leading process during Glial-guided neuronal migration *in vitro*. Expansion and contraction of this domain indicates this region of the leading process is a contractile domain. Total elapsed time is 15 minutes.

Supplemental Movie 4. MLC-Venus accumulates in the leading process during glial-guided neuronal migration *in situ*. Contraction precedes somal translocation. Total elapsed time is 44 minutes.

Supplemental Movie 5. Venus-MLCK accumulates in the leading process during glial-guided neuronal migration. As MLCK phosphorylates MLC, the presence of this kinase indicates Myosin II is active in the leading process. Total elapsed time is 40 minutes.

Supplemental Movie 6. Venus-MLCK (green) co-localizes with the f-actin contractile domain (red, labeled by moesin C-terminus-CFP) just before somal translocation. As

MLCK phosphorylates MLC, this suggests Myosin II is activated prior to somal translocation. Total elapsed time is 12 minutes.

Supplemental Movie 7. The centrosome (green, labeled with Centrin2-Venus) is embedded within the f-actin contractile domain (red, labeled by moesin C-terminus-CFP) at all stages of the migration cycle. The centrosome translocates when the leading process f-actin dilates. Total elapsed time is 58 minutes.

Supplemental Movie 8. Active Myosin II (green, labeled with Venus-MLCK) is located forward of the centrosome (red, labeled with Centrin2-mRFP) in the leading process of migrating neurons.

Supplemental Movie 9. Active Myosin II (green, labeled with Venus-MLCK) accumulates at the centrosome (red, labeled with Centrin2-mRFP) when it translocates during the initial phase of the migration cycle. Total elapsed time is 3 minutes.

Supplemental Movie 10. Microtubules (green, labeled with YFP-MAP2C) are embedded within the f-actin contractile domain (red, labeled by moesin C-terminus-CFP) at all stages of the migration cycle. The microtubule network translocates when leading process f-actin dilates. Total elapsed time is 35 minutes.

Supplemental Movie 11. 50 μM Blebbistatin halts randomized centrosomal motion (labeled with Centrin2-Venus) in a stationary neuron.

Supplemental Movie 12. 20 μM Blebbstatin inhibits centrosomal (labeled with Centrin2-Venus) and somal translocation in a migrating neuron.

Supplemental Movie 13. 10 μM CalA (added at the 10 minute time point) stimulates first centrosomal (labeled with Centrin2-Venus) then somal translocation.

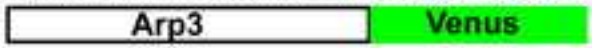
Supplemental Figures S1

A) Mouse C-Moesin C-term CFP (reporter for f-actin)

B) Venus-Myosin Light Chain Kinase (long isoform cDNA provided by A. Bresnick, reporter for active Myosin II)

C) Myosin Light Chain 9-Venus (reporter for Myosin II motors)

D) Actin related protein 3-Venus (reporter for actin nucleation and branching)



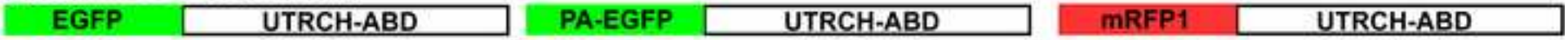
E) Paxillin-Venus (reporter for focal adhesion complexes)

F) Venus-Microtubule associated protein 2C (reporter for microtubules, construct provided by G. Banker)

G) Centrin2-Venus (reporter for centrosomes and centrioles)

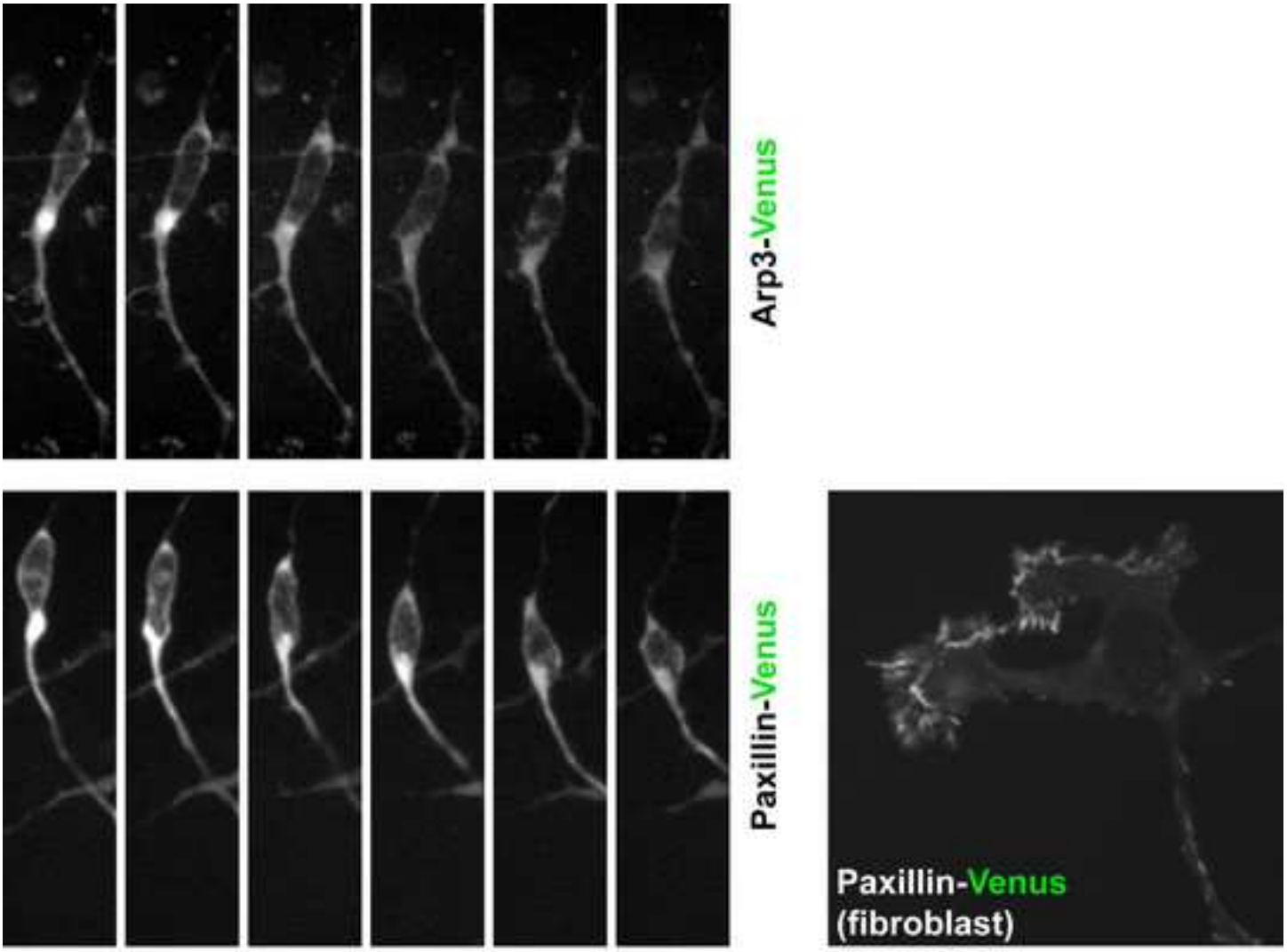
H) Centrin2-mRFP1 (reporter for centrosomes and centrioles)

I) Utrophin ABD Constructs (f-actin)



FigureS1

Supplemental Figures S2



Solecki Figure S2

Supplemental Figures S3

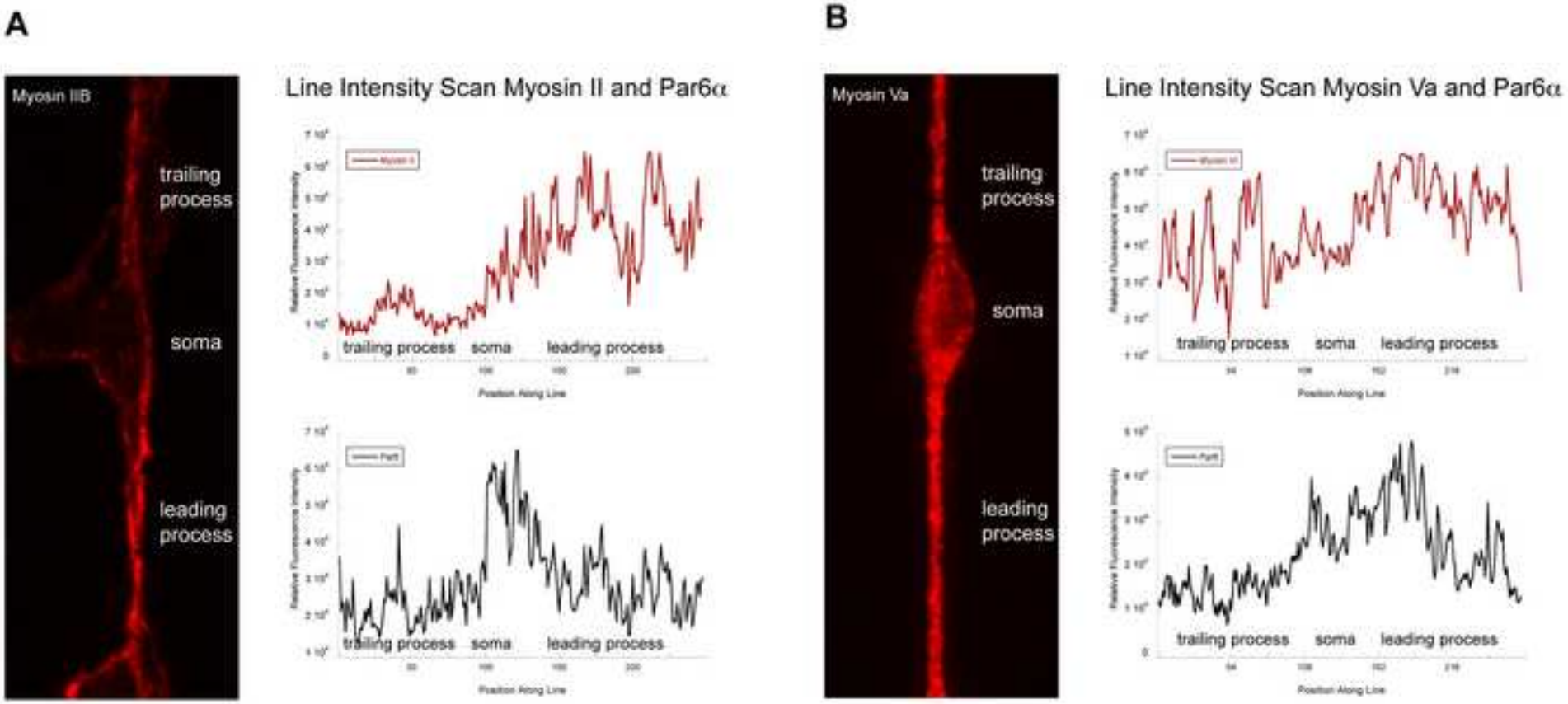


Figure S3

Supplemental Figures S4

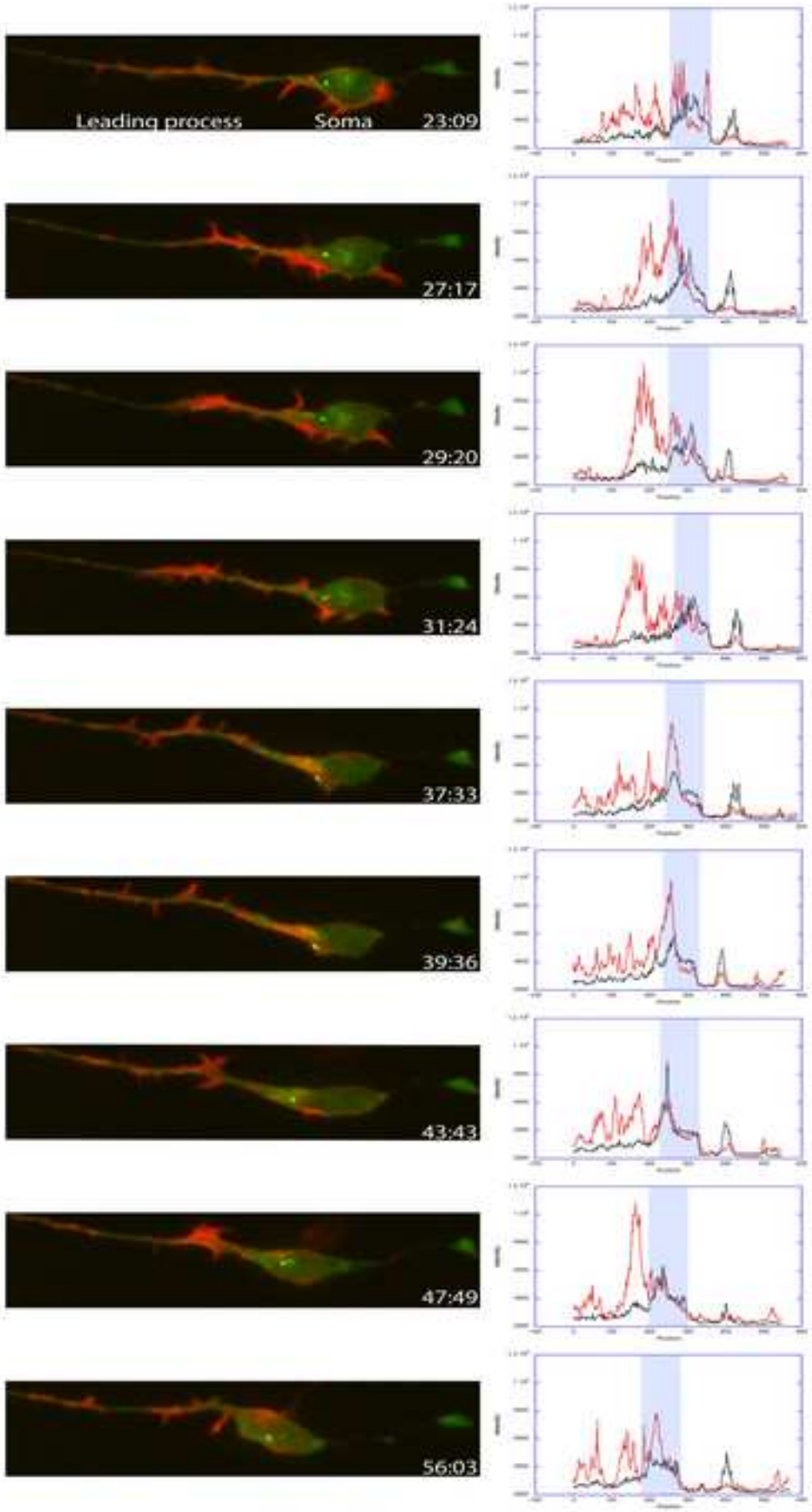


Figure S4

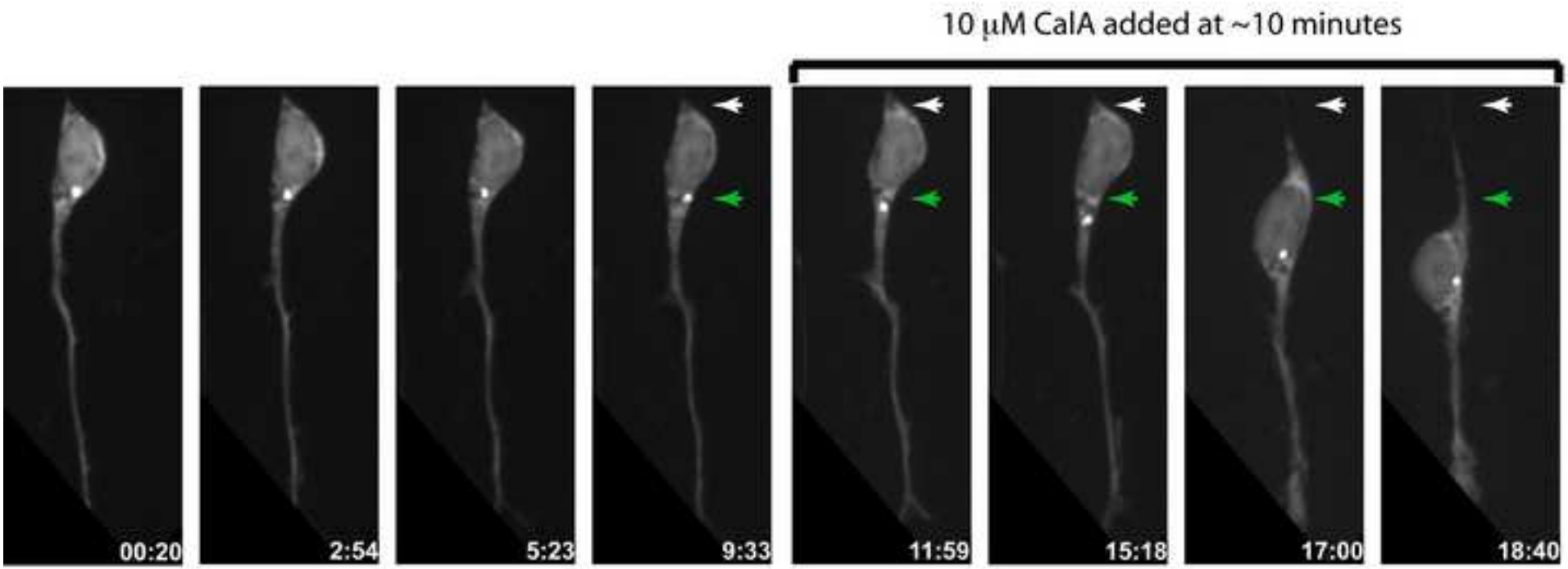

10 μM CalA added at ~10 minutes
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Figure S5

Supplemental Figures S6

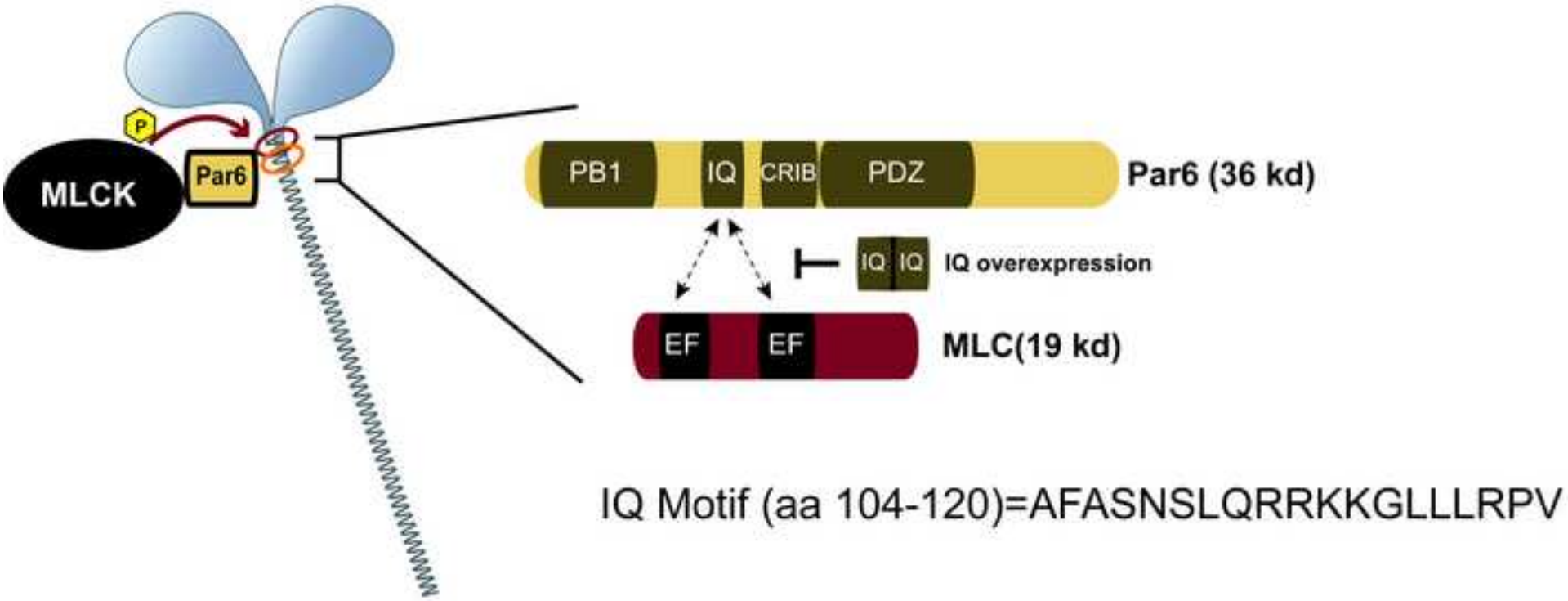

FIgure S6