Supplementary Information

Movie 1 - NM2 Filament appearance tracking. Sum intensity Z-projection of time lapse of EGFP-NM2A knockin cell. Lamellar protrusion imaged with a 5 second frame rate. The leading edge is in the top right corner with retrograde flow to the bottom left. Magenta circles mark NM2 filaments in the first frame of appearance. Scale bar = 5 um. Time = mm:ss.

Movie 2 - Leading edge retraction precedes NM2 filament appearance. Sum intensity Z-projection of time lapse of EGFP-NM2A knockin cell. Lamellar protrusion imaged with a 1 second frame rate. The leading edge is in the top right corner with retrograde flow to the bottom left. Scale bar = 5 um. Time = mm:ss.

Movie 3 - JL treatment stalls actin dynamics. Sum intensity projection of Z-stack time lapse of 3x-mScarlet-FTractin tagged lamellar protrusion with a 5 second frame rate. The leading edge is in the top right corner with retrograde flow to the bottom left. Scale bar = 5 um. Time = mm:ss.

Movie 4 - DMSO treatment with NM2 appearance. Sum intensity Z-projection of time lapse of EGFP-NM2A knockin cell expressing 3x-mScarlet-FTractin. Lamellar protrusion imaged with a 5 second frame rate. The leading edge is in the top right corner with retrograde flow to the bottom left. Actin is in grey LUT and NM2A in blue LUT. Drug treatment indicated in orange text at the first frame of treatment.Scale bar = 5 um. Time = mm:ss.

Movie 5 - JL treatment with NM2 appearance. Sum intensity Z-projection of time lapse of EGFP-NM2A knockin cell expressing 3x-mScarlet-FTractin. Lamellar protrusion imaged with a 5 second frame rate. The leading edge is in the top right corner with retrograde flow to the bottom left. Actin is in grey LUT and NM2A in blue LUT. Drug treatment indicated in orange text at the first frame of treatment. Scale bar = 5 um. Time = mm:ss.

Movie 6 - Actomyosin dynamics during long term cell migration. EGFP-NM2A knockin cells expressing 3x-mScarlet-FTractin imaged every minute for 8 hours. Actin is in grey LUT and NM2A in blue LUT. Scale bar = 50 um. Time = hh:mm.

Movie 7 - Tail retraction precedes NM2 filament appearances. Sum intensity Z-projection of time lapse of EGFP-NM2A knockin cells imaged with a 20 second frame rate. Initial tail outline in orange and initial protrusion outline in green. Scale bar = 20 um. Time = mm:ss.

Movie 8 - Y27 treatment results in increased NM2 filament appearances. Time lapse of EGFP-NM2A knockin cell with a 5 second frame rate. Drug treatment indicated in orange text at the first frame of treatment. Scale bar = 50 um. Time = hh:mm.

Movie 9 - JLY Treatment with NM2 Appearance. Sum intensity Z-projection of time lapse of EGFP-NM2A knockin cell expressing 3x-mScarlet-FTractin. Lamellar protrusion imaged with a 5 second frame rate. The leading edge is in the top right corner with retrograde flow to the bottom left. Actin is in grey LUT and NM2A in blue LUT. Drug treatment indicated in orange text at the first frame of treatment. Scale bar = 5 um. Time = mm:ss.

Movie 10 - NM2 Monomer Recruitment Jumpstarts NM2 Filament Assembly. Sum intensity Z-projection time lapse of Halo-NM2A knockin cell expressing GFP-Stargazin-Lov-SsrA and SspB-mApple-NM2A. Lamellar protrusion imaged with a 10 second frame rate. The leading edge is in the top right corner with retrograde flow to the bottom left. Recruitable (SspB-mApple) NM2 shown in the left panel, endogenous (Halo-646-NM2) in the middle panel, and an overlay with recruitable in blue and endogenous in purple in the right panel. Orange circle marks the activation ROI that was stimulated after each acquisition frame. Scale bar = 5 um. Time = mm:ss.

Movie 11 - NM2 Filament mediated amplification. Sum intensity Z-projection of time lapse of EGFP-NM2A knockin cell. Lamellar protrusion imaged with a 5 second frame rate. The leading edge is in the top right corner with retrograde flow to the bottom left. Scale bar = 5 um. Time = mm:ss.

Movie 12 - NM2 Filament Partitioning. TIRF-SIM time lapse of EGFP-NM2A knockin cell with a 2 second frame rate. Green circles indicate the first frame with 2-puncta $\sim$300 nm apart, or pre-partitioning. Blue circles indicate the first frame with 3-puncta $\sim$300 nm apart, or mid-partitioning. Purple circles indicate the first frame with 4-puncta $\sim$300 nm apart, or post-partitioning. Scale bar = 300 nm. Time = mm:ss.

Supplemental Figure 1.

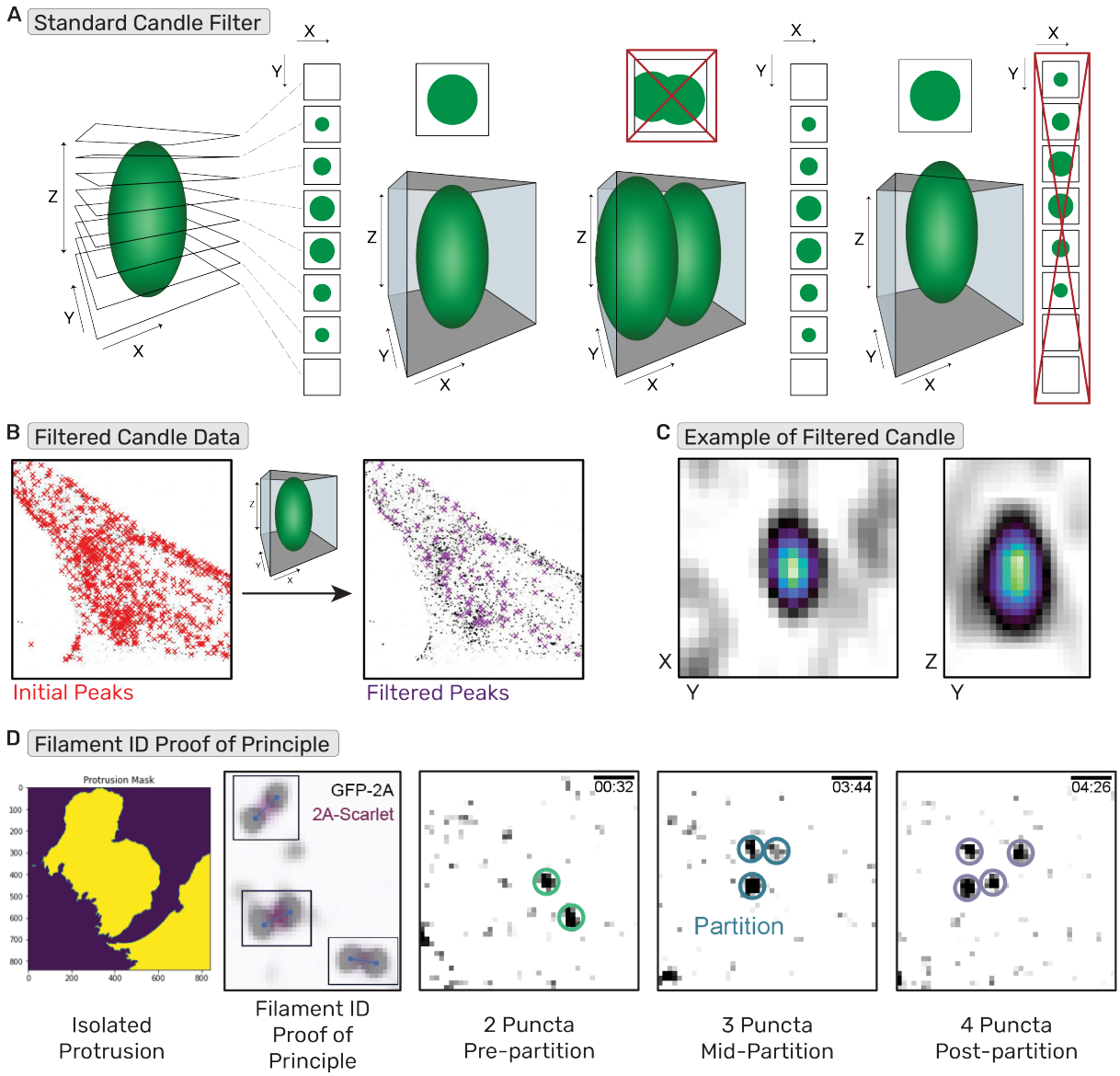

Fig. S1. Molecular Counting Workflow. (a) Depiction of filter used to identify candles unsuitable for analysis (i.e. too close to another candle or partially out of Z-range). (b) Example of standard candle image before and after filter. (c) Example image of passable filter in XY and ZY. (d) Depiction of NM2 filament identification workflow with (1) lamella isolation, (2) filament identification by puncta ∼300nm apart with proof of principle NM2-tail tag to confirm filament, and (3) examples of the 2-puncta, 3-puncta, and 4-puncta identifications.