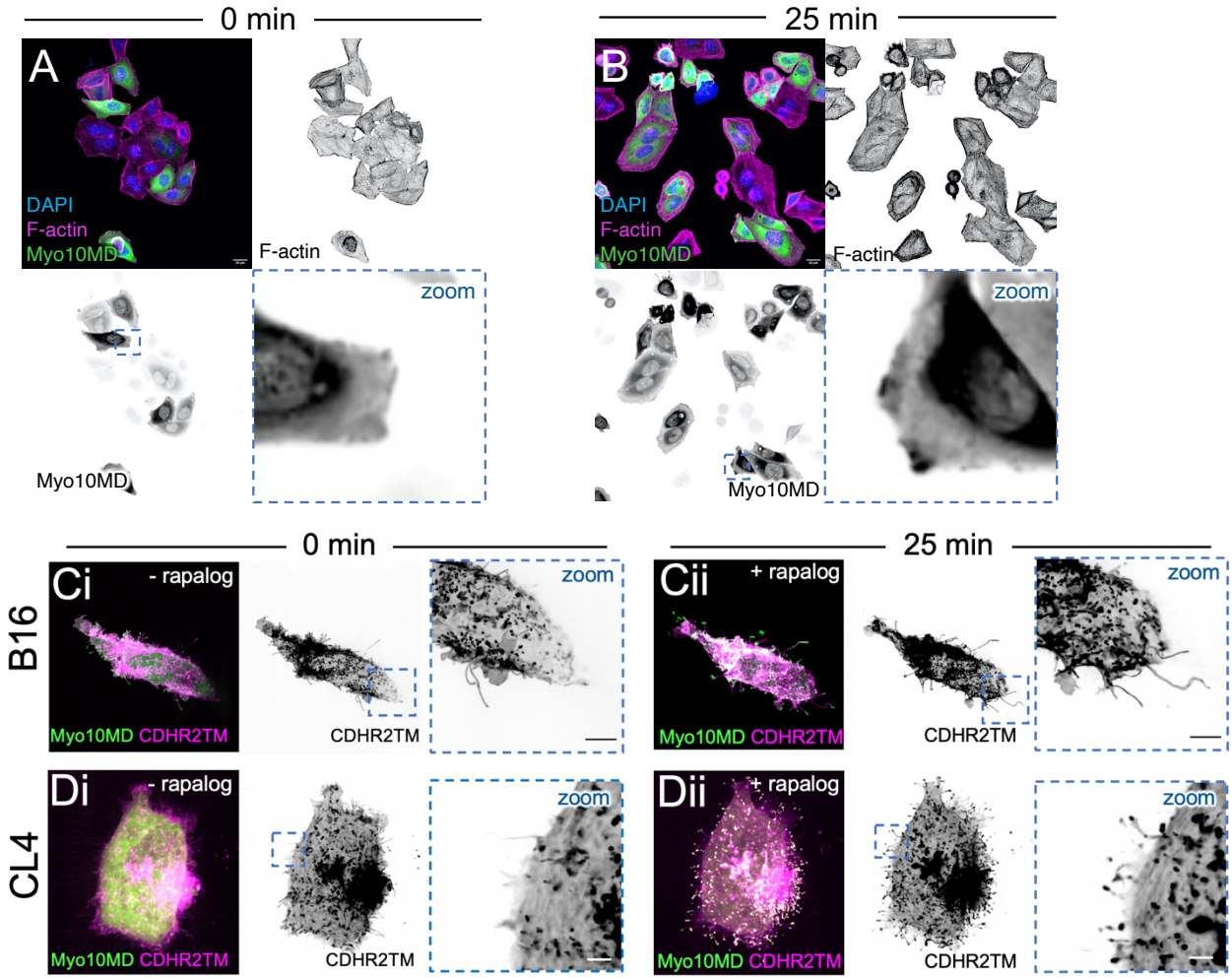

Figure S1, related to Figure 1: Myo10MD cannot elongate filopodia without the membrane attachment motif, and membrane bound Myo10MD can elongate filopodia in distinct cell types. Merged confocal maximum intensity projection of HeLa cells expressing only the Myo10MD (green) constructs treated without (**A**) or 25 min after (**B**) rapalog treatment. Inverted single channel image of F-actin to the right of merged images and inverted single channel image of Myo10MD and a zoom below. Scale bars = 20 μm. (**C**) Merged confocal maximum intensity projection of B16 cells expressing Myo10MD (green) and CDHR2TM (magenta) at 0 min (**Ci**) and 25 min after (**Cii**) rapalog treatment. (**D**) Merged confocal maximum intensity projection of CL4 cells expressing the Myo10MD (green) and CDHR2TM (magenta) at 0 min (**Di**) and 25 min after (**Dii**) rapalog treatment. Scale bars = 10 μm.

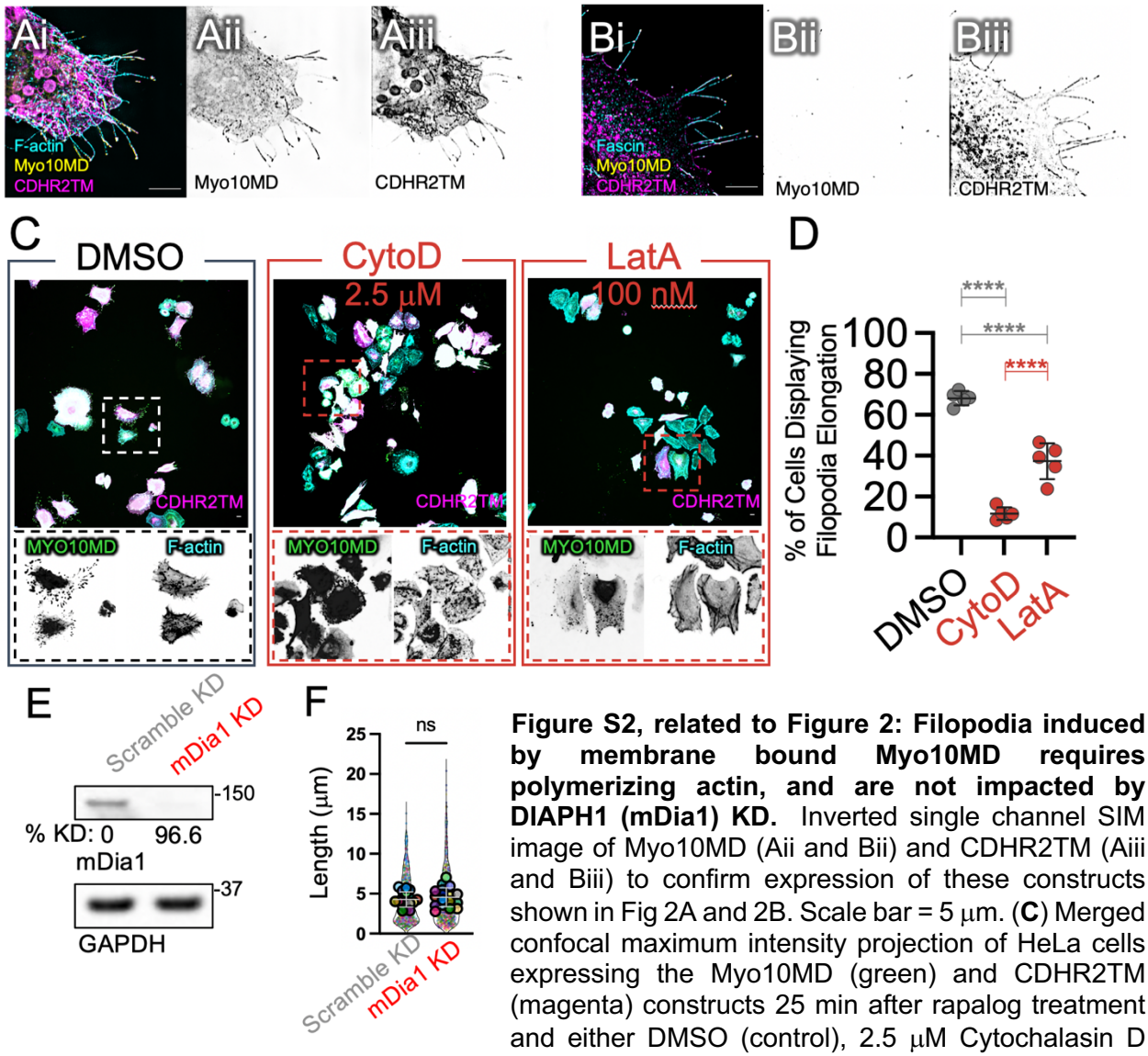

Figure S2, related to Figure 2: Filopodia induced by membrane bound Myo10MD requires polymerizing actin, and are not impacted by DIAPH1 (mDia1) KD. Inverted single channel SIM image of Myo10MD (Aii and Bii) and CDHR2TM (Aiii and Biii) to confirm expression of these constructs shown in Fig 2A and 2B. Scale bar = 5 μm. (**C**) Merged confocal maximum intensity projection of HeLa cells expressing the Myo10MD (green) and CDHR2TM (magenta) constructs 25 min after rapalog treatment and either DMSO (control), 2.5 μM Cytochalasin D (CytoD), or 100 nM Latrunculin A (LatA). Scale bar = 10 μm. (**D**) Quantification of the percentage of cells displaying filopodia elongation after treatment with rapalog and either DMSO, CytoD, or LatA; n = > 370 cells for each treatment; error bars represent the mean ± SD; ****p-value ≤ 0.0001 was calculated using an Ordinary one-way ANOVA followed by Tukey's multiple comparisons test to compare treatments with each other. (**E**) Representative western blot analysis of expression levels of mDia1 in lysates from HeLa cells siRNA scramble control (Scramble KD) and siRNA mDia1 KD (mDia1 KD). GAPDH was used as a loading control. Percent KD below mDia1 expression blot based on densitometry measurements normalized to GAPDH. (**F**) Length of individual protrusions measured from fixed samples 25 min post rapalog treatment in HeLa cells expressing a siRNA scramble control (Scramble KD) or siRNA mdia1 KD (mDia1 KD). *n* = > 650 individual protrusions from 17 individual cells from 3 biological repeats. Data are represented as a SuperPlot, where transparent circles represent the length of individual protrusions, opaque circles represent the average length of protrusions of individual cells, violin plots show the distribution of the data, and all color matched circles represent measurements from the same cell. All graph error bars represent the mean ± SD; ns, p-value > 0.05.

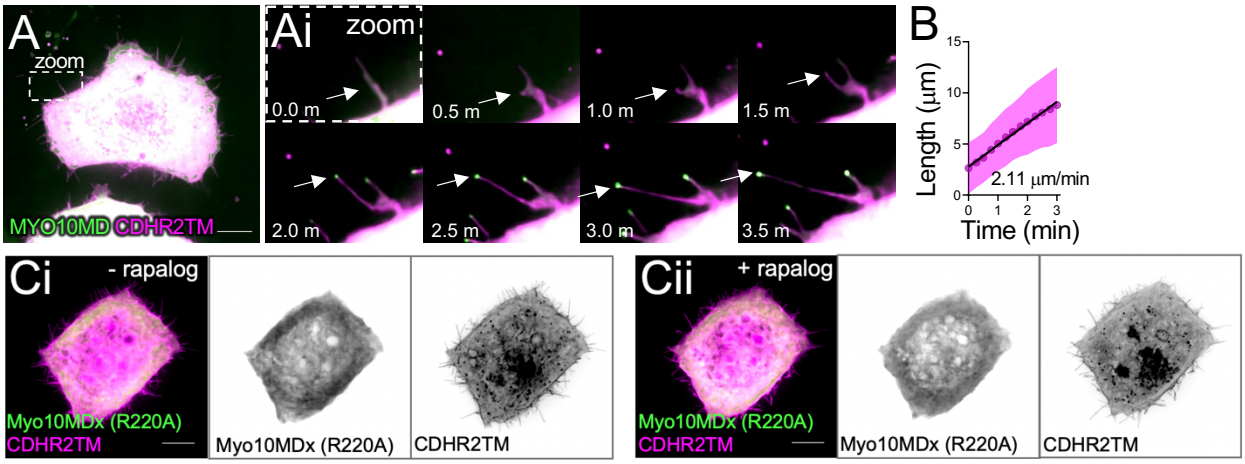

Figure S3, related to Figure 3: Methylcellulose does not alter the growth rates of filopodia induced by membrane bound Myo10MD, and the myosin-10 motor domain switch I dead (R220A) construct does not elongate filopodia. (**A**) Merged confocal maximum intensity projection of HeLa cell expressing Myo10MD (green) and CDHR2TM (magenta) after the addition of rapalog. Scale bar = 10 μm. (**Ai**) Montage of zoomed in region (**A**) highlighting the growth of a single filopodium (white arrow). (**B**) Quantification of the elongation rate of single filopodium induced by rapalog treatment in media containing 0.5% methylcellulose. Rate was calculated via the slope using a simple linear regression; *n* = 15 individual filopodia from n = 3 separate cells. Error bars represent the SD. Merged confocal maximum intensity projection of HeLa expressing Myo10MD-R220A (green) and CDHR2TM (magenta) at 0 min (**Ci**) and 25 min after (**Cii**) rapalog treatment. Inverted single channel images shown to the right of the merged. Scale bar = 10 μm.

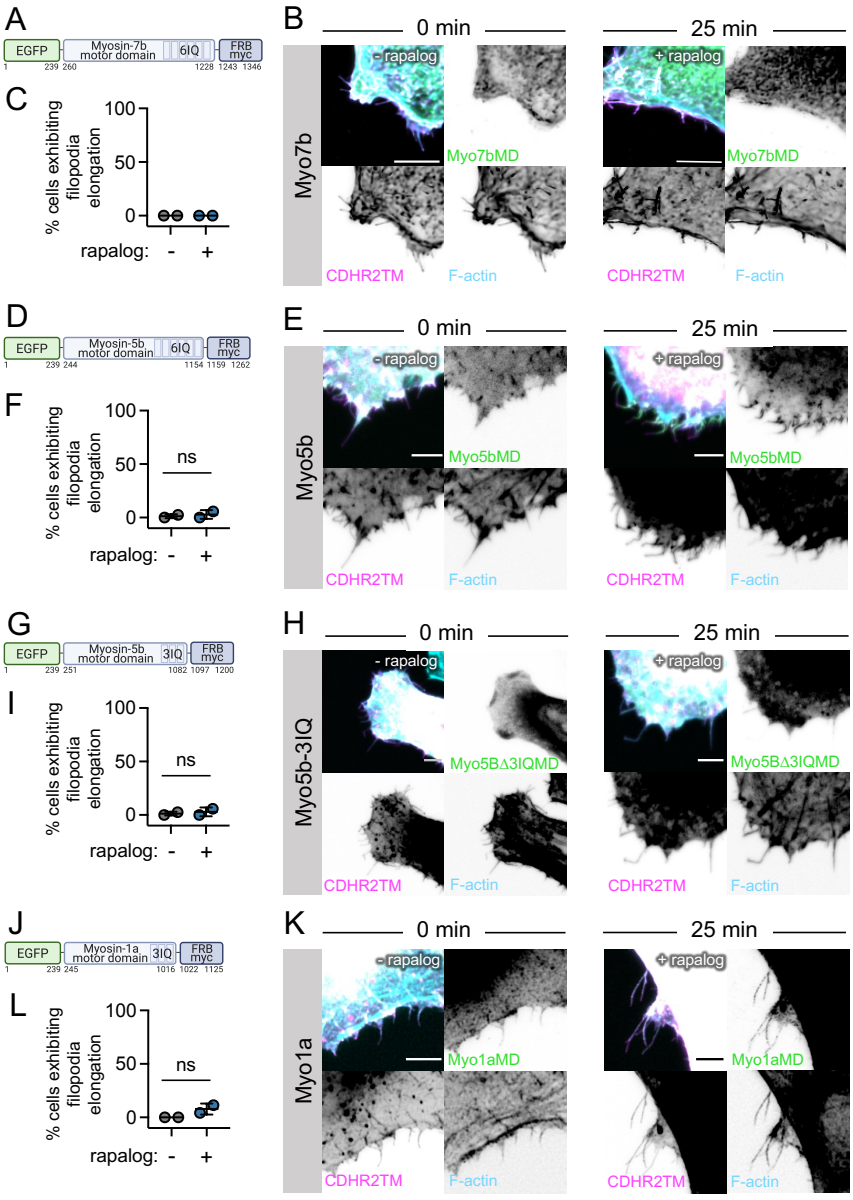

Figure S4, related to Figure 5: Myosin-7b, -5b, -5bΔ3IQ and -1a motor domains do not elongate filopodia. (**A**) Cartoon diagram showing the EGFP-Myosin-7b Motor Domain (Myo7bMD)-FRB-myc construct. (**B**) Merged confocal maximum intensity projection of HeLa cells expressing Myo7bMD (green) and CDHR2TM (magenta) at 0 min and 25 min after rapalog treatment. (**C**) Quantification of the percentage of cells displaying filopodia elongation without and after treatment with rapalog. (**D**) Cartoon diagram showing the EGFP-Myosin-5b Motor Domain (Myo5bMD)-FRB-myc construct. (**E**) Merged confocal maximum intensity projection of HeLa cells expressing Myo5bMD (green) and CDHR2TM (magenta) at 0 min and 25 min after rapalog treatment. (**F**) Quantification of the percentage of cells displaying filopodia elongation without and after treatment with rapalog. (**G**) Cartoon diagram showing the EGFP-Myosin-5b Motor Domain with 3 IQ domains deleted (Myo5bdel3IQMD)-FRB-myc construct. (**H**) Merged confocal maximum intensity projection of HeLa cells expressing Myo5bdel3IQMD (green) and CDHR2TM (magenta) at 0 min and 25 min after rapalog treatment. (**I**) Quantification of the percentage of cells displaying filopodia elongation without and after treatment with rapalog. (**J**) Cartoon diagram showing the EGFP-Myosin-1a Motor Domain (Myo1aMD)-FRB-myc construct. (**K**) Merged confocal maximum intensity projection of HeLa cells expressing Myo1aMD (green) and CDHR2TM (magenta) at 0 min and 25 min after rapalog treatment. (**L**) Quantification of the percentage of cells displaying filopodia elongation without and after treatment with rapalog. Inverted single channel images shown to the right and below of the merged. All images were also stained for F-actin (cyan). Scale bar = 5 μm; n = > 57 cells for each treatment from two biological repeats; error bars represent the mean ± SD; ns p-value > 0.05 was calculated using a Student's t-test.