Supplemental Materials

Molecular Biology of the Cell

Connacher et al.

SUPPLEMENTAL MATERIAL
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SUPPLEMENTAL FIGURE LEGENDS:

Figure S1. MCAM is not artefact of cell retraction. Stills from confocal live-cell imaging with MCAM-GFP and mCherry in (**A**) WM239a cells and (**B**) A375 cells. Scale bars = 10 µm, time stamp (h:min).

Figure S2. Dynamics of movement of MCAM, F-actin and myosin IIB during WRAMP structure formation. Cells shown in live imaging experiments from Figure 2, plotting fluorescence intensities of MCAM-GFP, myosin IIB-mCherry, and LifeAct vs. distance from the rear position of the cell. (**A,B**) MCAM-GFP and LifeAct appear simultaneously at WRAMP structures, with little evidence for temporal separation. (**C,D**) In contrast, MCAM-GFP appears at WRAMP structures before myosin IIB, by 3-5 min. Thus, (**C**) MCAM appears at 1:15, while myosin IIB appears at 1:20. (**D**) MCAM appears at 1:15, while myosin IIB appears at 1:18.

Figure S3. Inhibition of WRAMP structures slows cell migration and wound healing, with no effect on cell proliferation. (**A,B**) Treatment with Box5 (Wnt5a inhibitor) or Wnt5a-siRNA blocks the ability of cells to form WRAMP structures in (**A**) WM239a and (**B**) A375 cells. Cells were treated with Box5 for 18 h or Wnt5a-siRNA for 48 h, then fixed and immunostained for MCAM to determine the number of cells with WRAMP structures after adding recombinant Wnt5a or in ligand-free control for 30 min. Values show the mean percentages and S.E.M. from 3 slides (>750 cells). Asterisks * indicate $p < 0.01$ and ** indicate $p < 0.005$ compared to NTC (non-targeting control) siRNA. The *p*-values were calculated using standard two-tailed Student T-test. **X** indicates no data was collected. (**C,D**) Western blot analysis performed to demonstrate the efficiency of siRNA to knock-down (**C**) Wnt5a or (**D**) MCAM. GAPDH and nucleolin were used as loading controls. (**E,F**) Cell proliferation assays of (**E**) WM239a cells and (**F**) A375a cells show no changes upon treatment with MCAM-siRNA, Box5, Wnt5a-siRNA, or recombinant Wnt5a (rWnt5a). Values show average cell counts and S.E.M. from 6 replicate wells in one experiment. One-way ANOVA indicates no significant differences in relative cell numbers across the treatments (for WM239a cells: $p = 0.192$ for 32 h, $p = 0.469$ for 50 h and for A375 cells: $p = 0.152$ for 13 h, $p = 0.433$ for 20 h, and $p = 0.818$ for 30 h).

Supplemental Figure S1

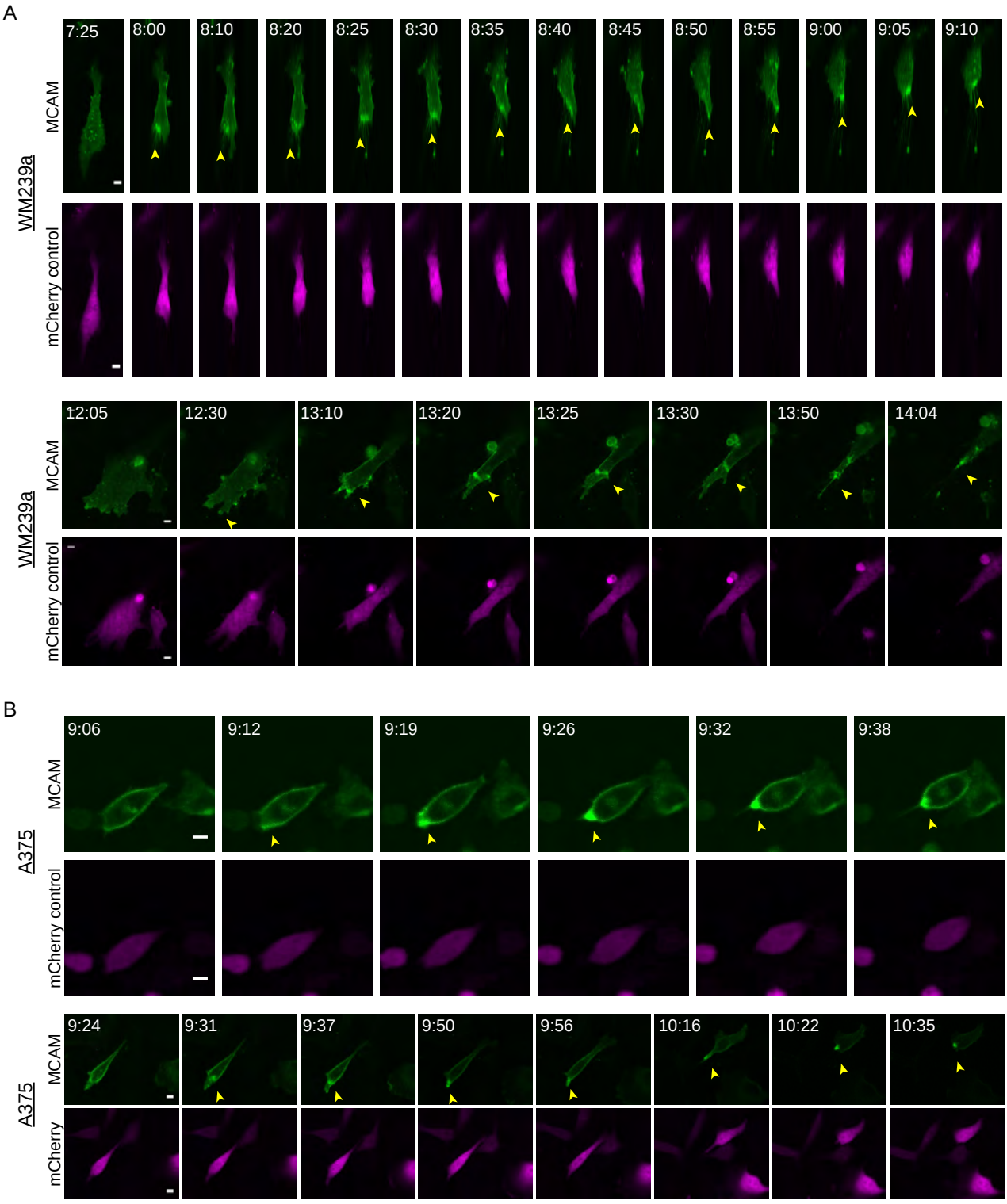


Figure S1. MCAM is not artefact of cell retraction. Stills from confocal live-cell imaging with MCAM-GFP and mCherry in (**A**) WM239a cells and (**B**) A375 cells. Scale bars = 10 µm, time stamp (h:min). Panels A and B correspond to Movies 6 and 7.

Supplemental Figure S2

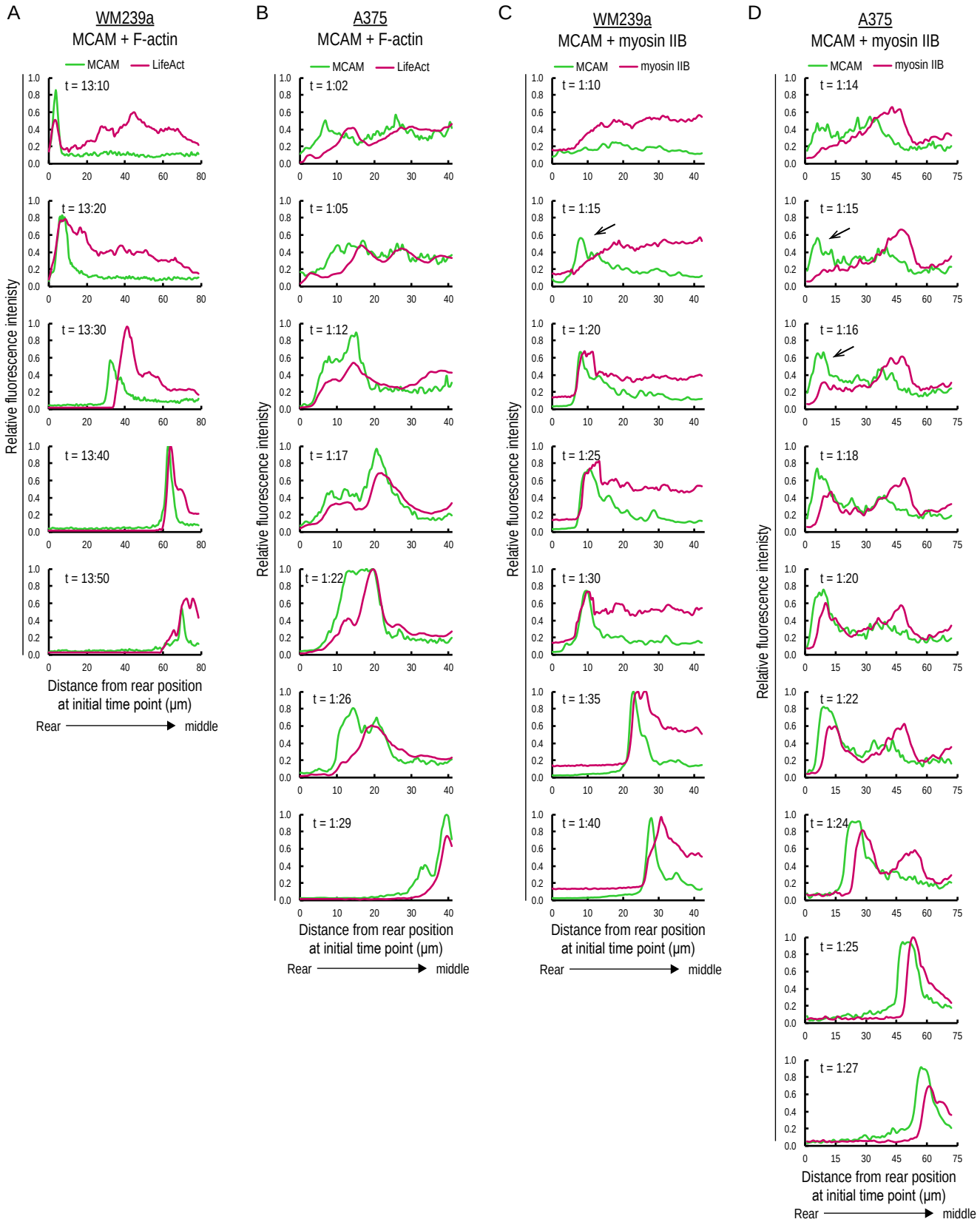
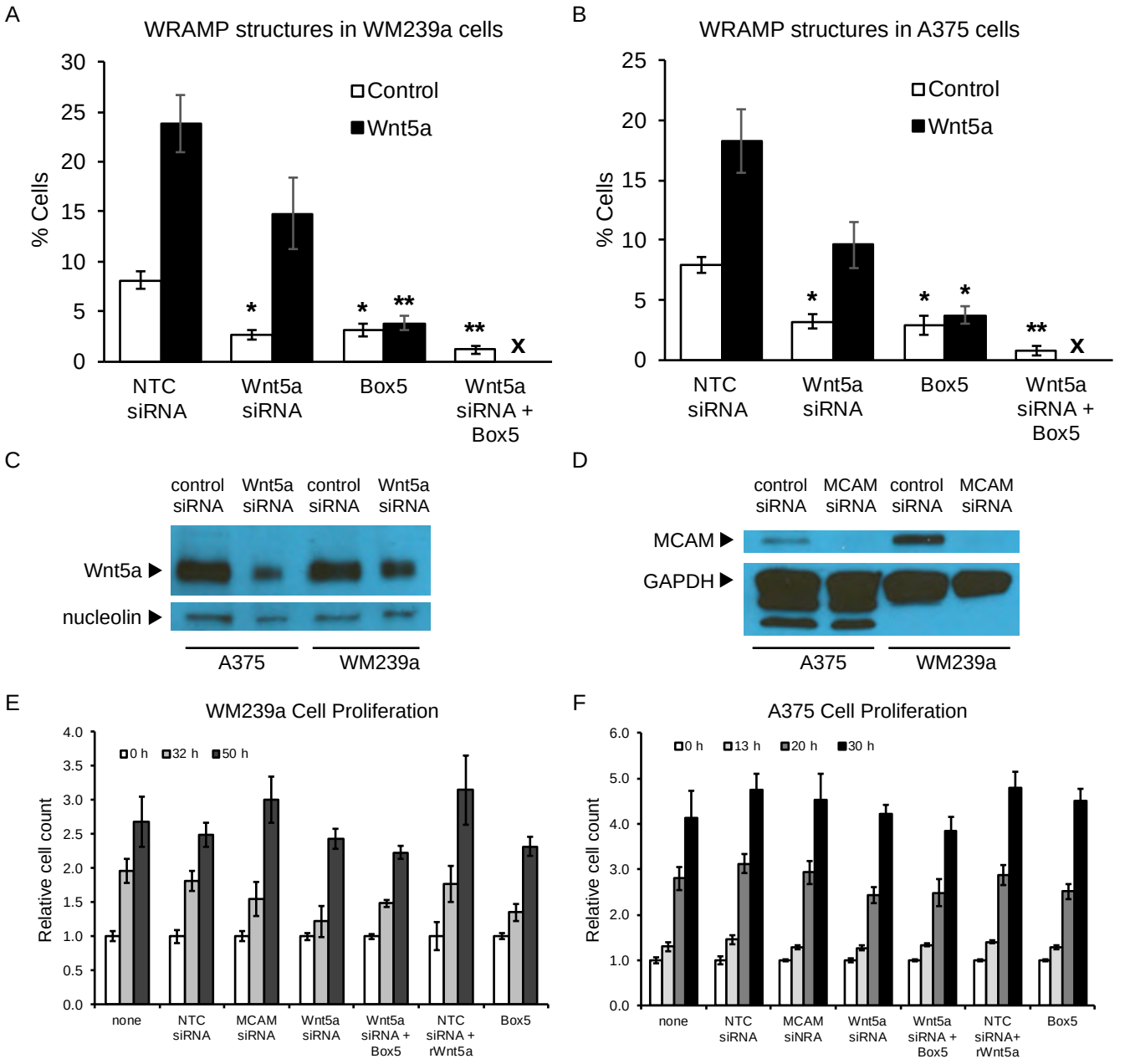


Figure S2. Dynamics of MCAM-GFP and myosin IIB-mCherry in WRAMP structure formation shows that the recruitment of myosin IIB is delayed compared to MCAM. Plots for fluorescence intensities vs. the distance from the rear position of the cell at the initial time point show that the recruitment of myosin IIB follows MCAM while F-actin is coordinated with MCAM. Plots correspond to the panels in Figure 2, respectively. (C) MCAM appears at 1:15, myosin IIB does not appear until 1:20. (D) MCAM appears at 1:15, myosin IIB does not appear until 1:18.

Supplemental Figure S3



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