Figure S1

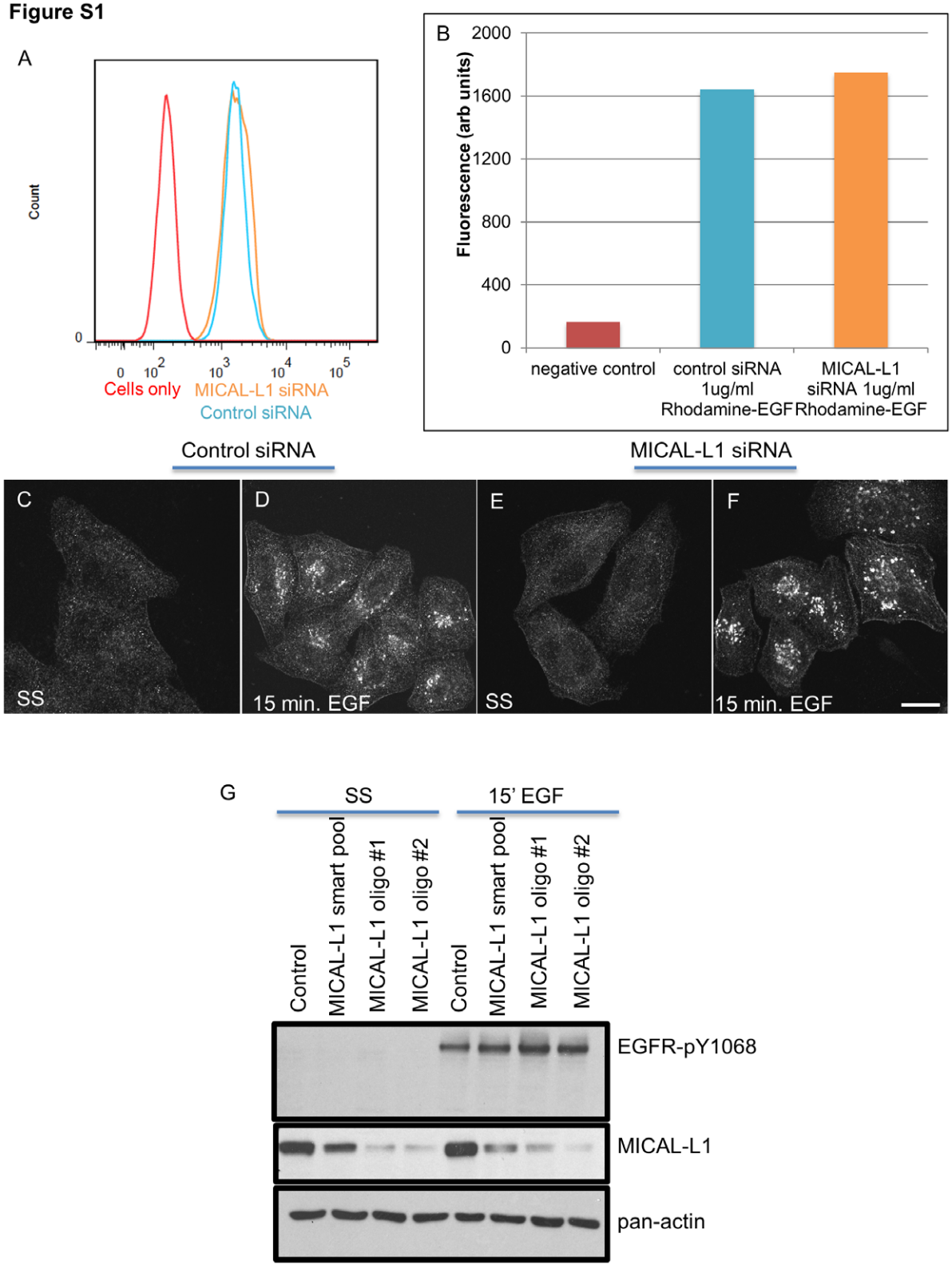

Fig. S1 MICAL-L1-depletion in HeLa cells does not effect EGF binding capacity, EGFR internalization or EGFR activation. (A,B) Control- (blue bar) and MICAL-L1-siRNA-treated (orange bar) HeLa cells were labeled with EGF-Rhodamine as described in the materials and methods and EGF surface binding was quantifed using flow cytometry (red=unlabeled, background control). The histogram and graph are representative ones from 3 experiments, each done with ~10,000 cells **(C-F)** Control (C,D) and MICAL-L1 KD (E,F) cells were serum starved (SS) and then pulsed with unlabeled EGF to stimulate EGFR internalization. Immunofluorescence demonstrates that EGFR internalization is similar in control (D) and MICAL-L1-siRNA cells (F). **(G)** Immunoblot analysis of EGFR autophosphorylation in control and MICAL-L1-siRNA cells (additional siRNA oligonucleotides are included to demonstrate specificity). MICAL-L1 KD has no effect on EGF-induced EGFR phosphorylation (compare lane 1-4 to 5-8).

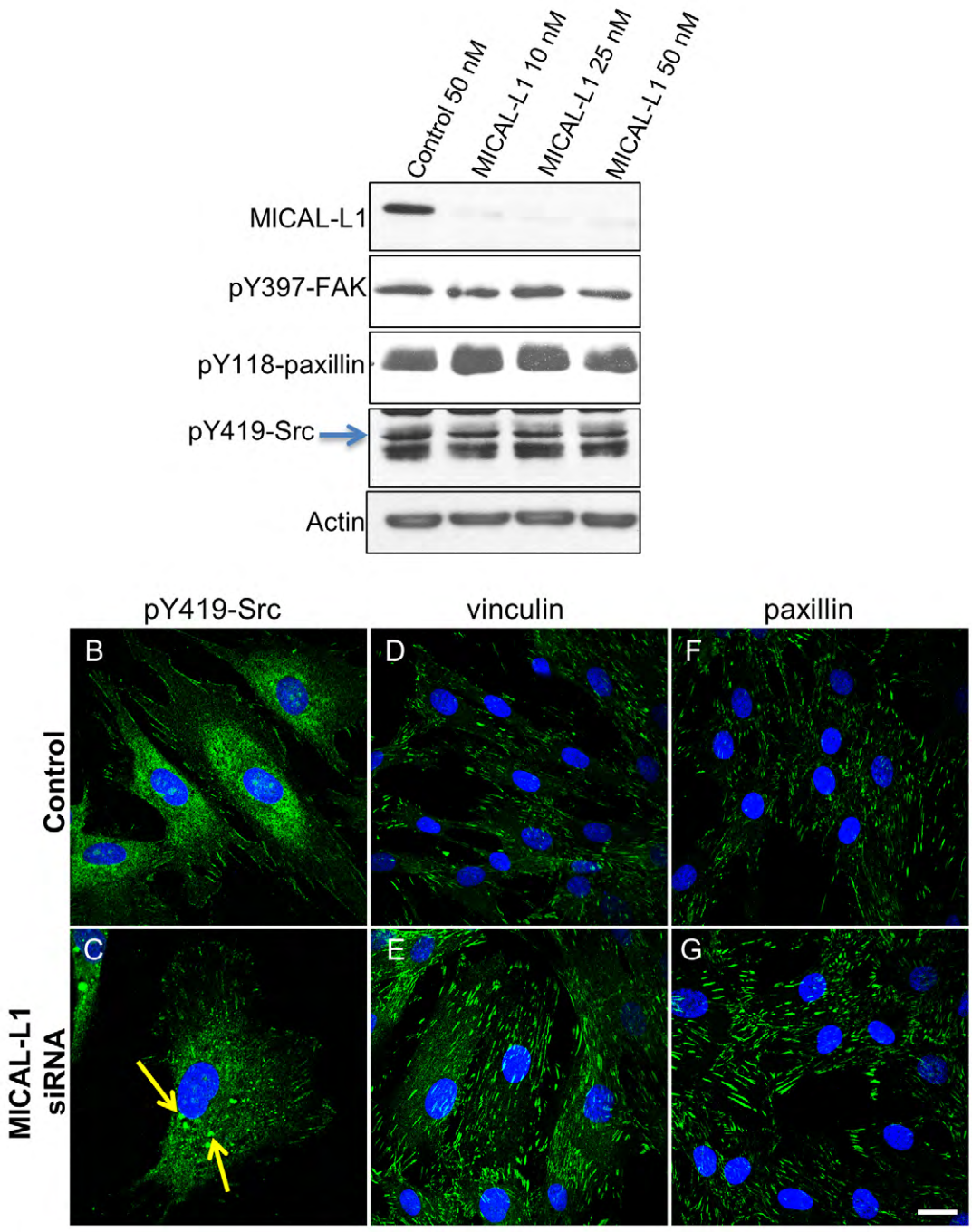

Fig. S2 MICAL-L1-depleted BJ cells show altered distribution but not expression of focal adhesion proteins at steady-state. (A) Immunoblot analysis demonstrating efficiency of MICAL-L1-depletion and expression of several focal adhesion proteins. **(B-G)** Distribution of active Src (B and C) vinculin (D and E) and paxillin (F and G) in MICAL-L1-depleted fibroblasts compared to control cells. Arrows mark the accumulation of Src in intracellular vesicles in MICAL-L1-depleted cells. Blue=DAPI Scale bar=10 μm.

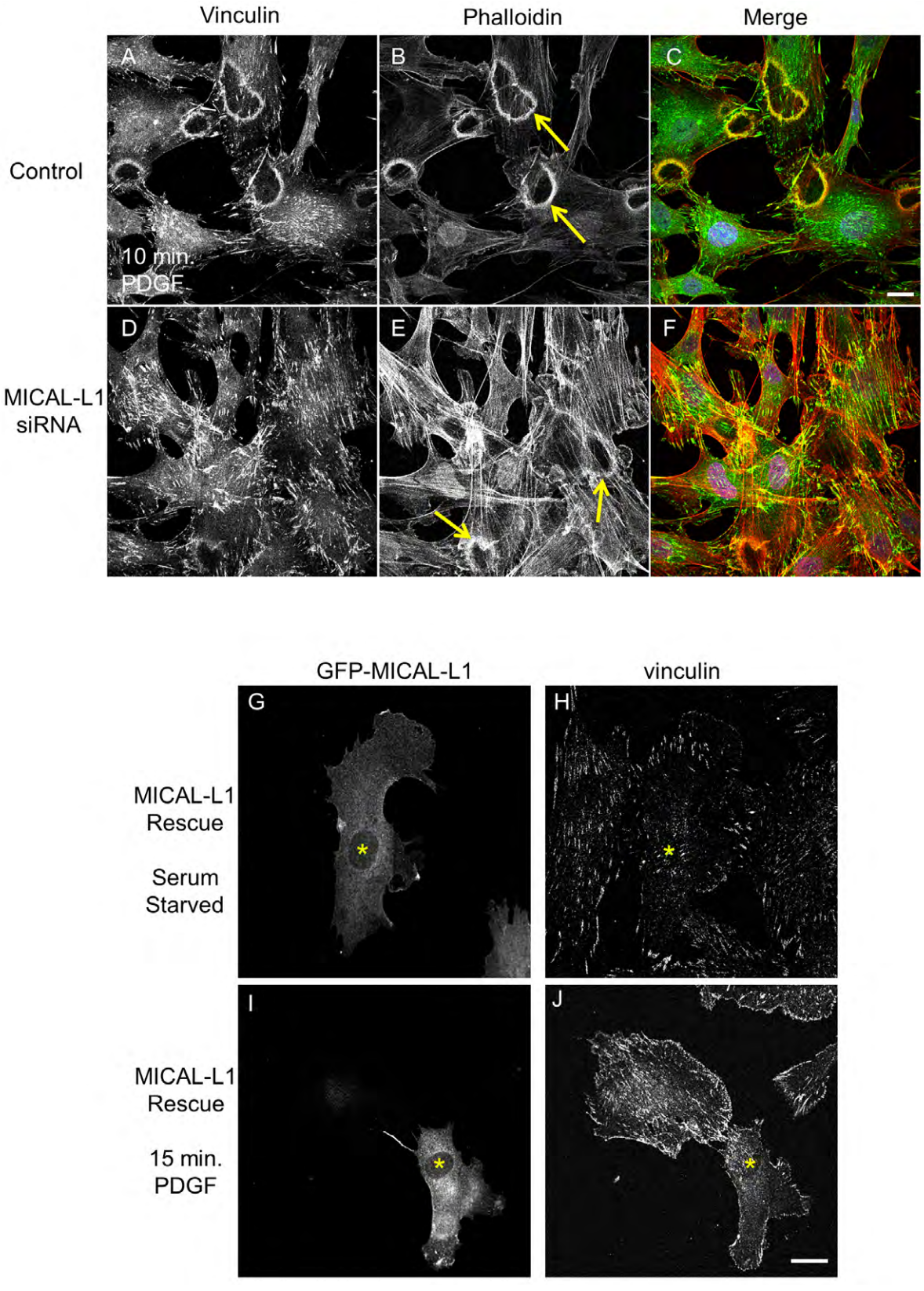

Fig. S3 Vinculin and phalloidin double-staining of CDR in MICAL-L1 KD cells and introduction of siRNA-resistant GFP-MICAL-L1 into MICAL-L1-depleted fibroblasts partially rescues focal adhesion number and turnover. (A-F) Control (A-C) and MICAL-L1 KD cells (D-F) were stimulated with PDGF for 10 minutes, fixed and stained with vinculin (green) and phalloidin (red) to mark CDR (yellow arrows). **(G-J)** MICAL-L1-depleted cells were electroporated with an siRNA-resistant construct, plated onto fibronectin, serum starved (G,H) and fixed or stimulated with PDGF for 15 min. (I,J) prior to fixation. GFP fluorescence denotes transfected serum-starved (G) and PDGF-stimulated (I) cells. Focal adhesions were identified by vinculin (H,J) staining and GFP-MICAL-L1 transfected cells are marked by an asterisk. Scale bar=10 μm.

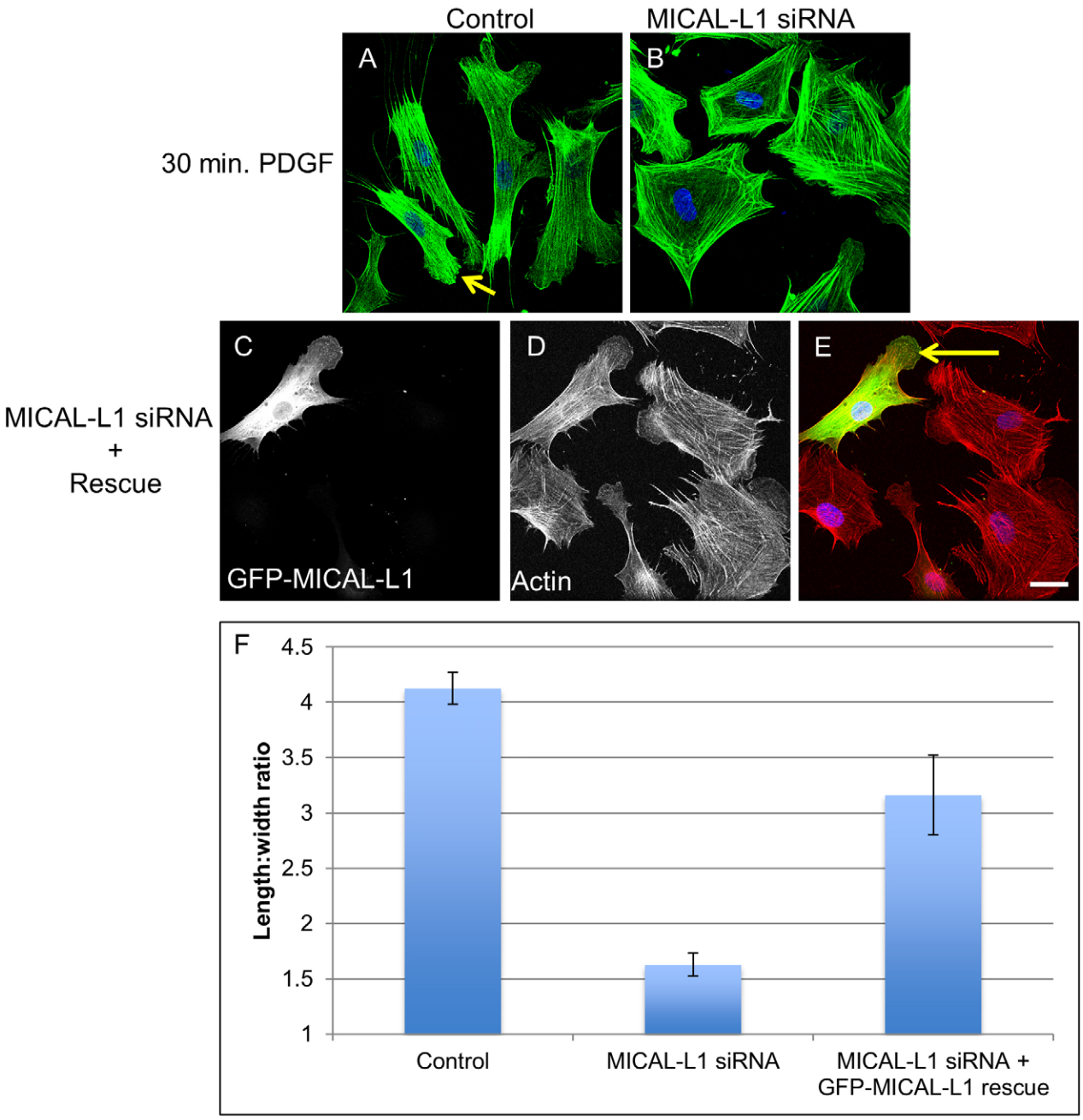

Fig. S4 MICAL-L1-depletion impairs PDGF-induced cell elongation that is partially rescued by siRNA-resistant GFP-MICAL-L1. (A,B) Control and MICAL-L1-depleted cells were stimulated with PDGF for 30 min., fixed and stained with phalloidin-488 to mark the actin cytoskeleton (green). **(C-E)** MICAL-L1-depleted cells were electroporated with siRNA-resistant GFP-MICAL-L1, stimulated with PDGF, fixed and stained with phalloidin-Rhodamine (red). Arrows denote formation of single lamellipodia in migrating cells and transfected cell in E. Scale bar=10 μm. **(F)** Cell elongation was quantified from three independent experiments as described in materials and methods. N=45 cells/experiment/treatment. Error bar=S.E.M. Tukey Test demonstrated cell elongation was significantly impaired in MICAL-L1 KD cells ($p<0.01$). GFP-MICAL-L1 partially rescued cell elongation phenotype (significantly more elongated than MICAL-L1 KD cells but significantly different than control cells, $p<0.01$).