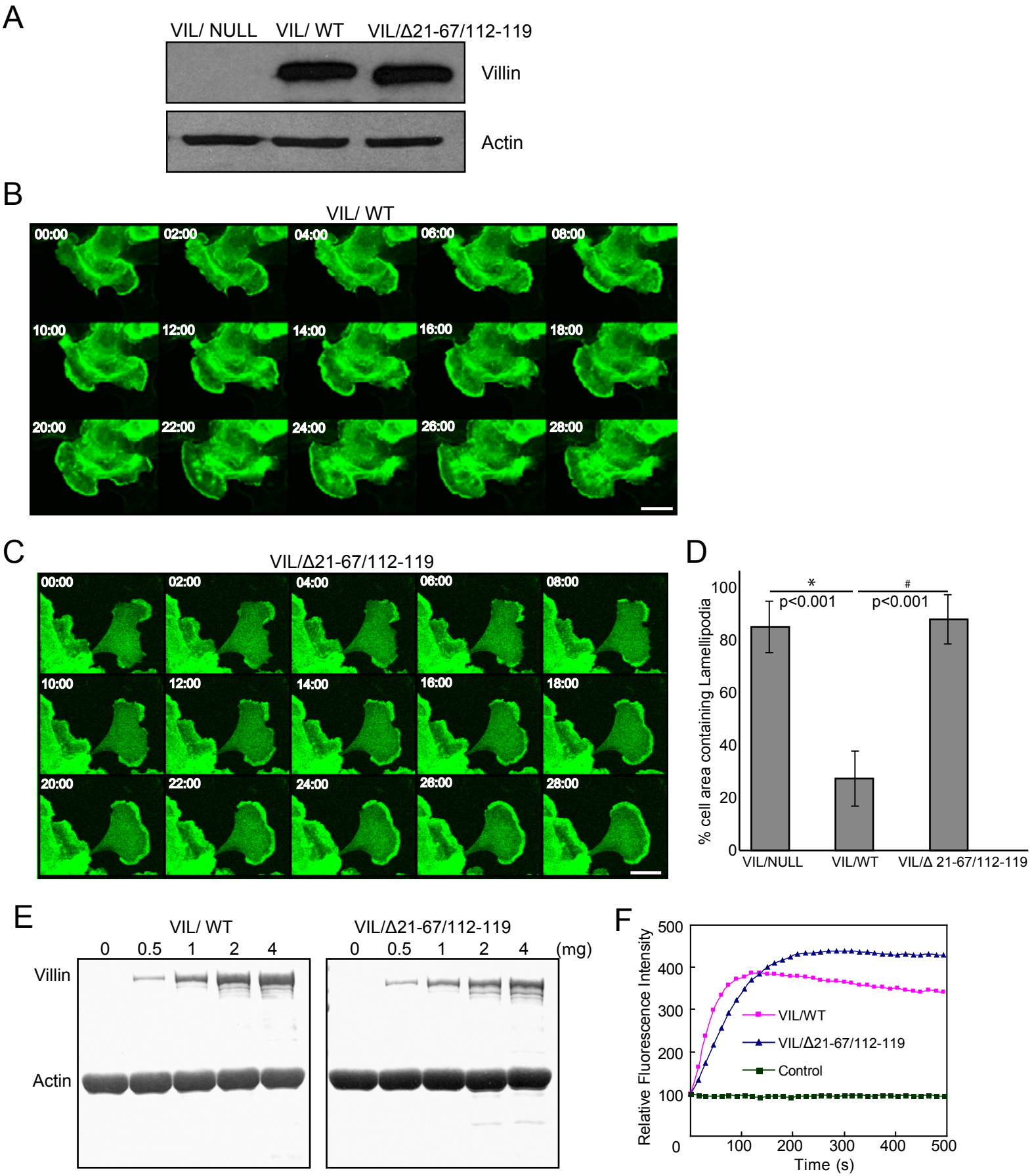
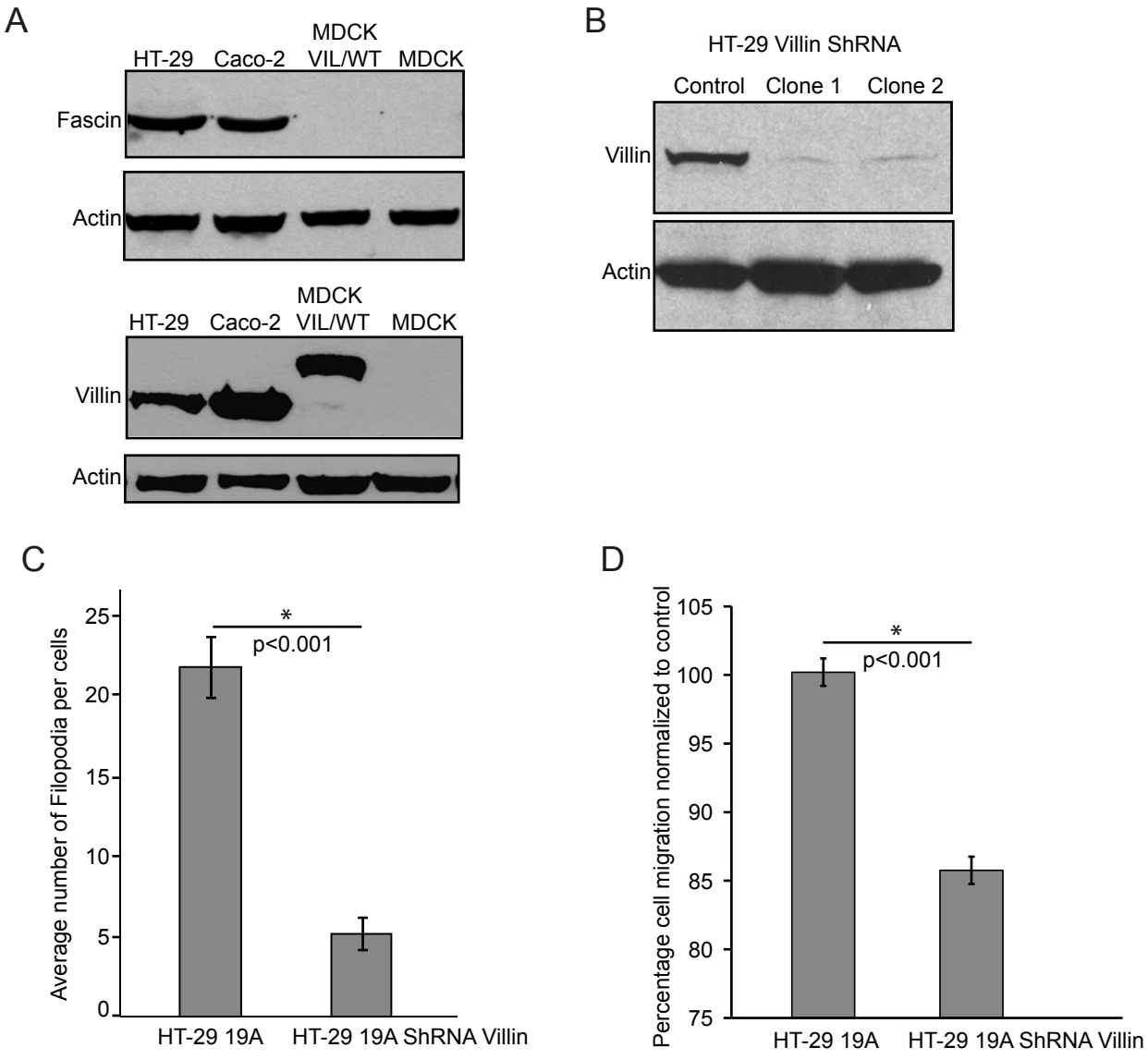
Supplementary Figure 1



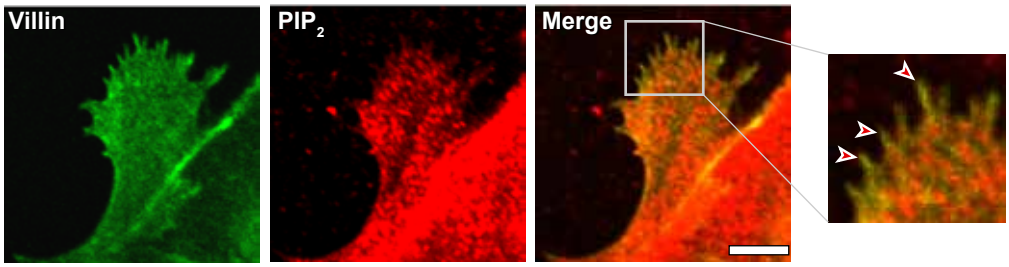
Supplementary Figure 1 (A) Similar levels of expression of full-length (VIL/WT) and mutant (VIL/Δ21-67/112-119) villin in MDCK Tet-Off cell clones. Full-length (B) and mutant (C) villin localize to the lamellipodia. (D) Cells expressing full-length villin express lamellipodia over significantly lower cell surface area compared to cells expressing the villin mutant VIL/Δ21-67/112-119 or the VIL/NULL cells. Comparable actin binding (E) and actin nucleating property of recombinant full-length and VIL/Δ21-67/112-119 mutant villin proteins. These are a representative of three experiments with similar results.

Supplementary Figure 2



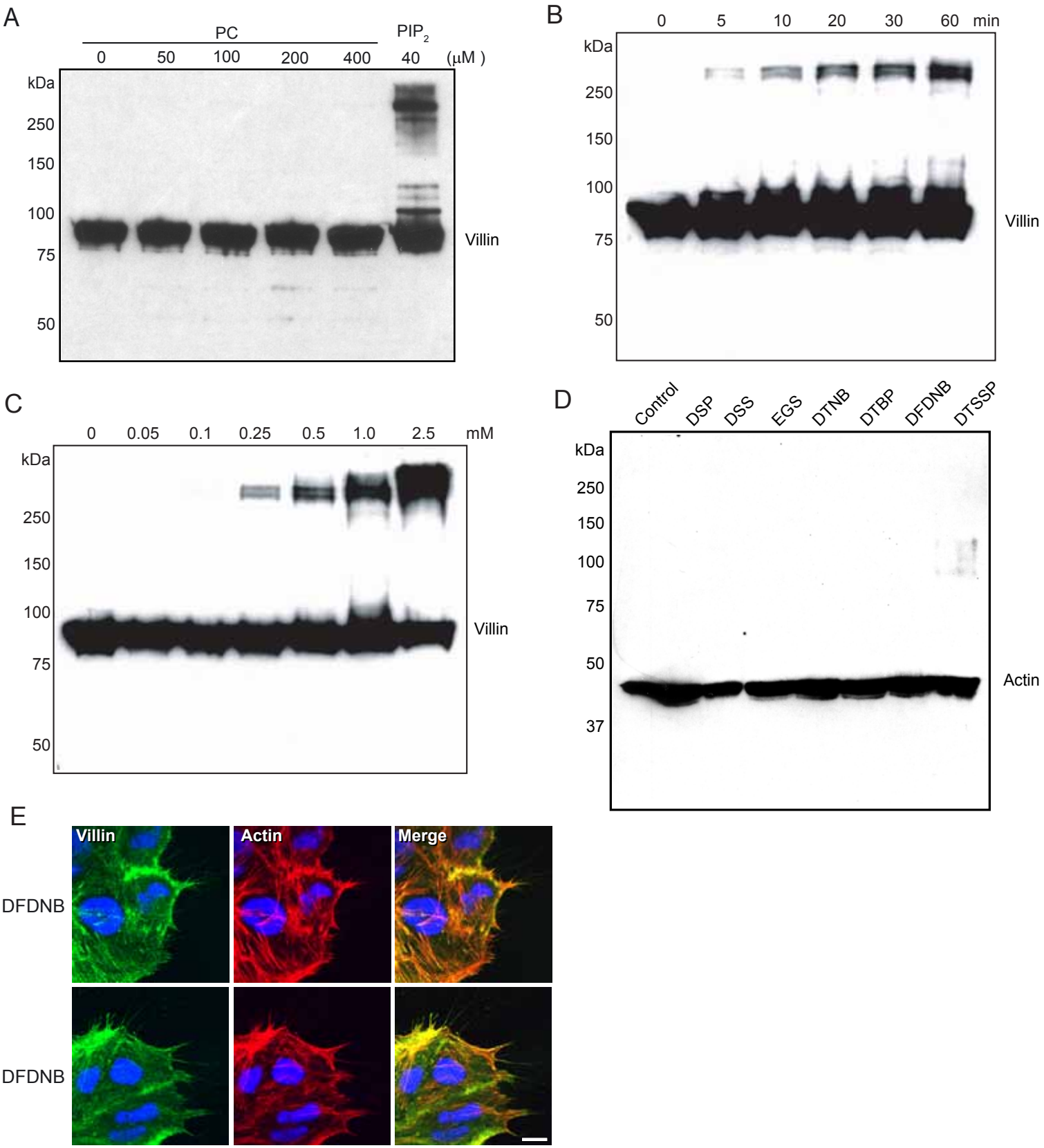
Supplementary Figure 2 (A) Colon adenocarcinoma cell lines HT-29/19A and Caco-2 BBe1 cells express villin and fascin. HT-29 cells were grown in DMEM media supplemented with 10% Fetal bovine serum. Also shown in this figure are MDCK Tet-Off cells transfected with SEYFP-tagged full-length human villin. These are a representative of three experiments with similar results. (B) HT-29/19A cells were infected with non-targeting shRNA (control) and shRNA against villin (clones 1 and 2). Both clones of HT-29/19A show greater than 95% knockdown of villin. Villin shRNA did not change the relative amounts or distribution of fascin in these cells (data not shown). This is a representative of three experiments with similar results. (C) Quantification of filopodia in control cells and mutant clones of HT-29/19A that lack villin. Values are means ± S.E. (n=20). Data shown are avearage of both clones. (D) Rates of cell migration in parental HT-29/19A and mutant (HT-29/19A shRNA villin) cell lines was determined by using wound healing assay. The error bars are the measured means ± S.E. Data shown are average of both clones.

Supplementary Figure 3



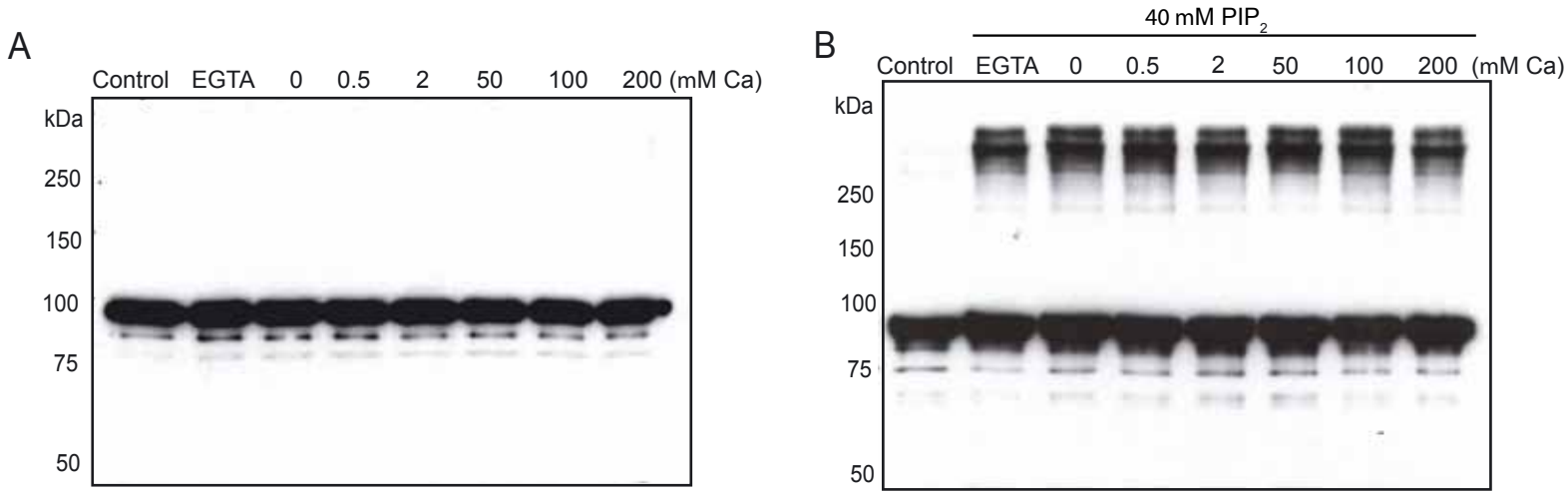
Supplementary Figure 3 Colocalization of villin and PIP_2 in filopodia of epithelial cells. Intracellular distribution of villin and PIP_2 was determined in MDCK Tet-Off cells stained with villin and PIP_2 antibodies. Merged image shows co-localization of villin and PIP_2 in filopodia. Bar, 10 μm.

Supplementary Figure 4



Supplementary Figure 4 (A) Recombinant villin was incubated with increasing concentrations of PC (0-400 μM) followed by cross-linking with sub-optimal concentration (10 fold molar excess) of 1,5-Difluoro-2,4-dinitrobenzene (DFDNB) for 30 min at room temperature. (B) Caco-2 cells were incubated with 1 mM DFDNB for different time intervals (0-60min) or with different concentrations of DFDNB (0-2.5 mM) for 30 minutes at room temperature (C). (D) Caco-2 cells were incubated with different crosslinkers (2.5 mM) for 30 minutes. The samples in B-D were prepared in SDS sample buffer, cross-linked proteins were analyzed by 8% SDS-PAGE and Western analysis. (E) Caco-2 cells were incubated in the presence of 2.5 mM DFDNB for 30 minutes and no change in the distribution of actin or villin was observed after treatment with DFDNB. These are a representative of four experiments with similar results.

Supplementary Figure 5



Supplementary Figure 5 (A) Recombinant villin protein was incubated with EGTA (1mM) or increasing concentrations of calcium (0-200μM) for 30 min at room temperature, followed by cross-linking with 10 fold molar excess of DFDNB for 30 min at room temperature. (B) Villin protein was incubated with EGTA (1mM) or increasing concentrations of calcium (0-200μM) and PIP_2 (40μM) for 30 min at room temperature, followed by cross-linking with 10 fold molar excess of DFDNB for 30 min at room temperature. Cross-linked proteins were analyzed by 8% SDS-PAGE and Western analysis. These are a representative of four experiments with similar results.