SUPPLEMENTAL FIGURES

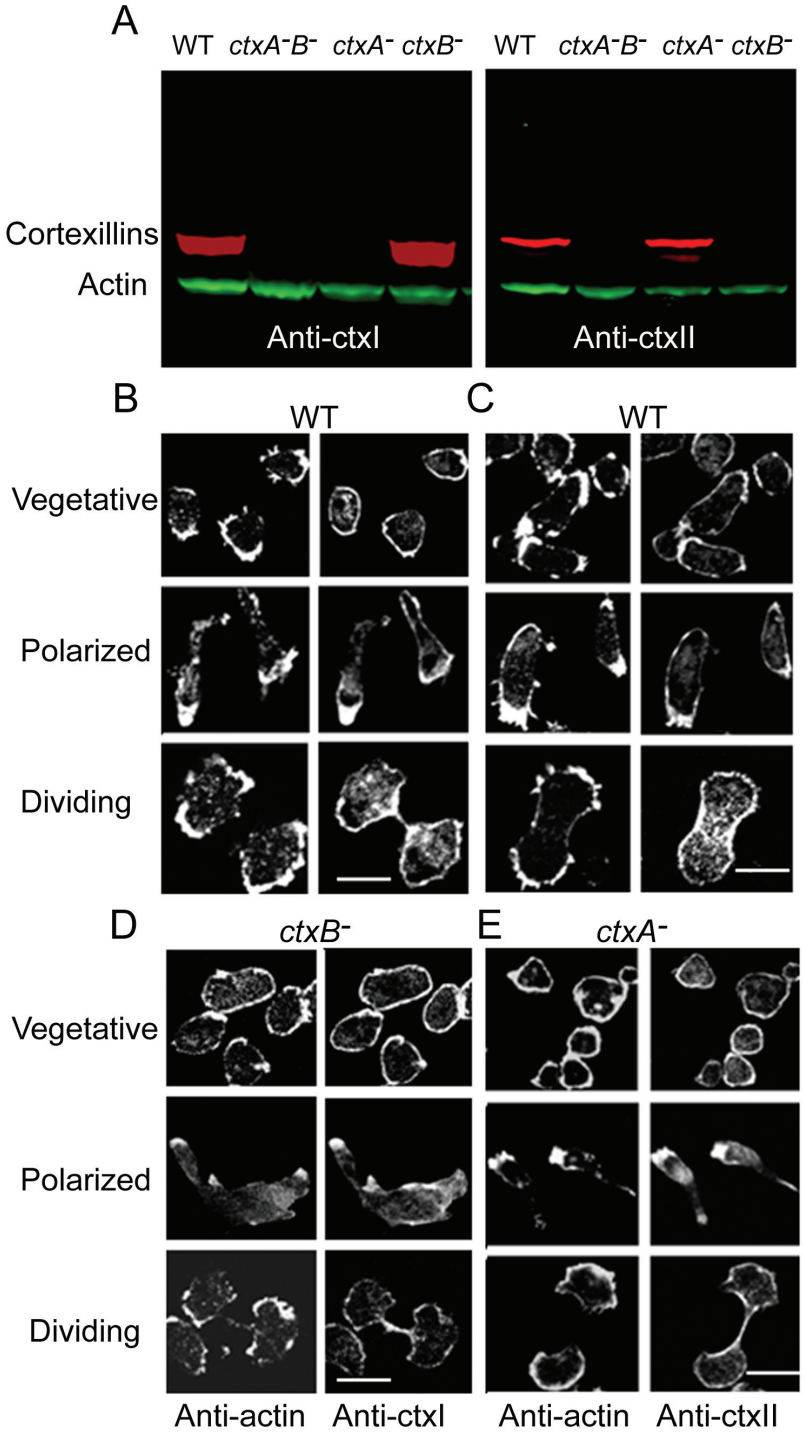

Figure S1. Confirmation of cortexillin knockouts and localization of cortexillins. (A) WT and cortexillin knockout cells were subjected to SDS-PAGE, blotted and incubated with anti-actin (green) and either anti-ctxI or anti-ctxII (red) antibodies. (B-E) Immunofluorescence labeling by actin and ctxI or ctxII antibodies. Fixed WT-cells (B and C), *ctxB*$^-$-cells (D) and *ctxA*$^-$-cells *(E)* were stained with anti-actin (green) and either anti-ctxI (D) or anti-ctxII (E) (red); vegetative cells (upper rows), polarized cells (middle rows) and dividing cells (lower rows). Scale bars are 10 µm.

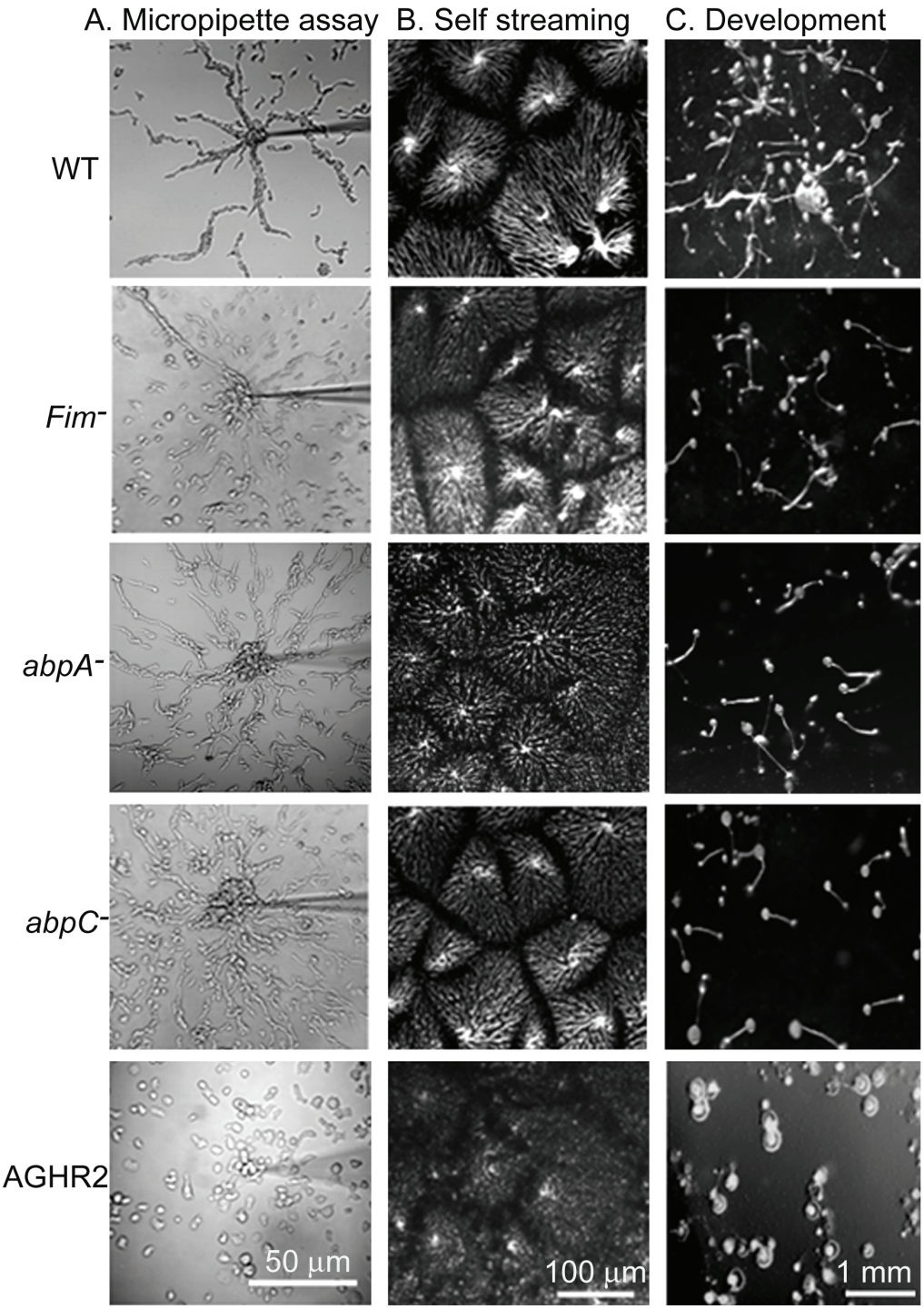

Figure S2. Chemotaxis, self streaming and development assays of WT-cells, fimbrin null cells (*Fim*$^-$), α-actinin null cells (*abpA*$^-$), filamin null cells (*abpC*$^-$), and α-actinin and filamin double null cells (AGHR2. (A) Micropipette assay with 1 μM cAMP, (B) self streaming assay and (C) development at 24 h.

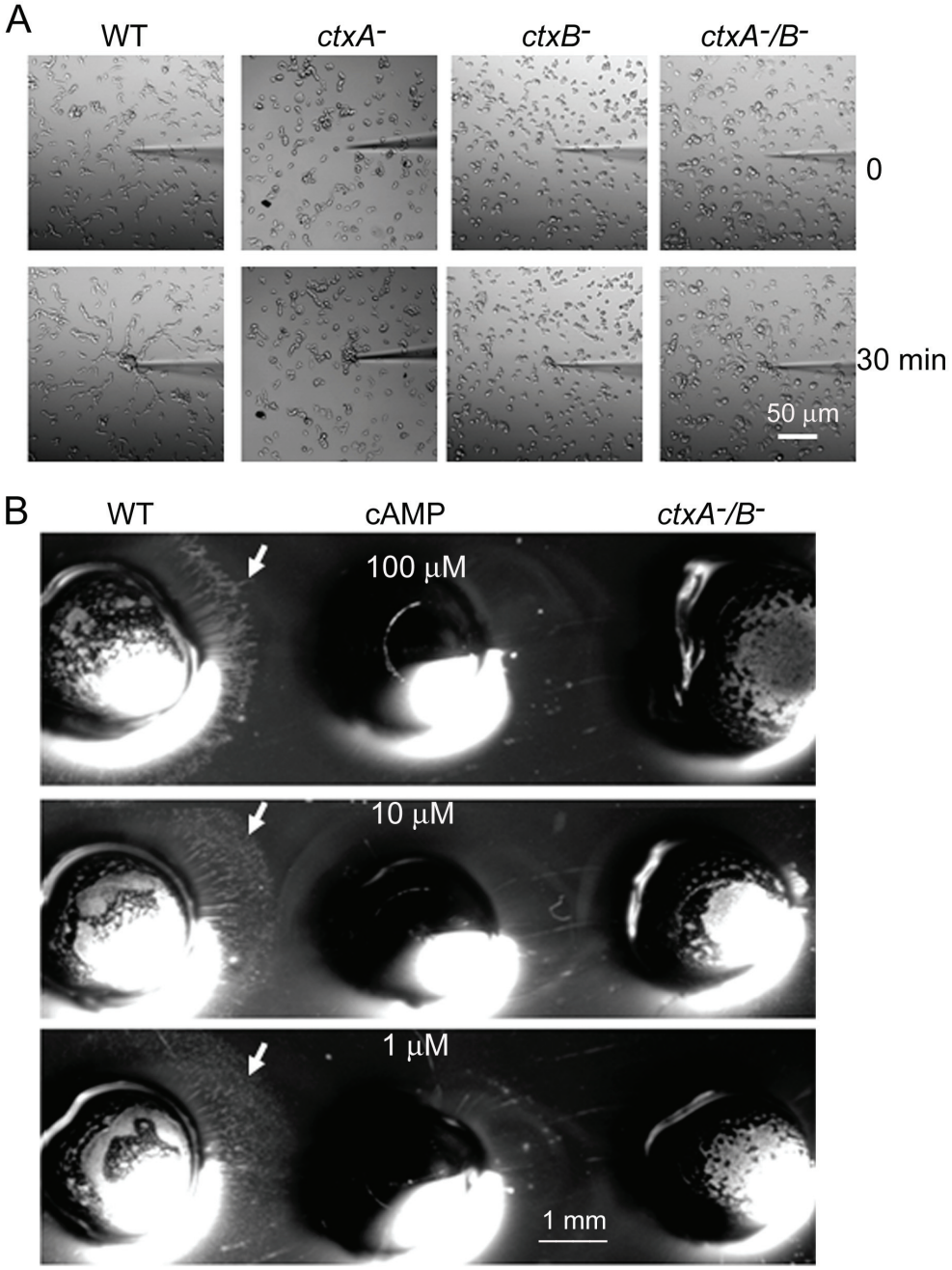

Figure S3. Chemotaxis of WT-cells and cortexillin knockout-cells at different cAMP concentrations. (A) Micropipette assay with 0.5 μM cAMP (usual cAMP concentration is 1-10 μM). (B) Under agar chemotaxis assay. Chemotaxis-competent WT- and *ctxA*$^-$/B$^-$-cells were placed in wells in agar equidistant from wells containing the indicated concentration of cAMP and images were taken with a stereo microscope. WT-cells streamed at the lowest concentration of cAMP (1 μM) but *ctxA*$^-$/B$^-$-cells did not stream even at 100 μM cAMP.

SUPPLEMENTAL MOVIES

Movies S1-S4. Self streaming of WT-cells (Movie S1), ctxA$^-$-cells (Movie S2), *ctxB*$^-$-cells (Movie S3) and *ctxA*$^-$*/B*$^-$-cells (Movie S-4). Cells were placed on plates in starvation buffer and cells streaming and aggregating were recorded with a stereo microscope. Images were captured at 1 frame/min and are played back at 30 frames/s. Movies S1-S4 correspond to Figure 4A.

Movies S5-S8. Micropipette Assay of Individual Cell Chemotaxis**.** WT-cells (Movie S-5), *ctxA*$^-$-cells (Movie S-6), *ctxB*$^-$-cells (Movie S-7) and *ctxA*$^-$*/B*$^-$-cells (Movie S-8) were exposed to a micropipette containing 10 µM cAMP and the migration of individual cells visualized by time-lapse confocal microscopy. Images were captured at 1 frame/10 s and are played back at 10 frames/s. Movies S5-S8 correspond to Figure 4C.

Movie S9. Localization of GFP-Myosin II Expressed in *ctxA*$^-$*/B*$^-$-cells. GFP-myosin II accumulates at the contractile ring and cleavage furrow in dividing cell visualized by time-lapse confocal microscopy. Left, phase-contrast; right, GFP-myosin II. Images were captured at 1 frame/10 s and are played back at 5 frames/s. Movie S9 corresponds to Figure 8B (middle row).

Movies S10-S11. Self Streaming of ACA-YFP/*ctxA*$^-$*/B*$^-$-cells (Movie S10) and cAR1-YFP/*ctxA*$^-$*/B*$^-$-cells (Movie S11). Live images recorded as described for Movies S-1-S4. Movies S10-S11 correspond to Figure 9E.

Movies S12-S13. Micropipette Chemotaxis Assay of ACA-YFP/*ctxA*$^-$*/B*$^-$-cells (Movie S12) and cAR1-YFP/*ctxA*$^-$*/B*$^-$-cells (Movie S13). Live images sequences were converted to movies and processed as described in Movies S5-S8. Movies S12-S13 correspond to Figures 10A and B).

Movie S14. Chemotaxis Assay of YFP-ACA/*ctxB*$^-$-cells. YFP-ACA translocates to the rear of a migrating cell and releases ACA-containing vesicles and the secreted cAMP attracts a neighboring cell. Images were captured at 1 frame/10 s and are played back at 5 frames/s. Movie S14 corresponds to Figure 10D.