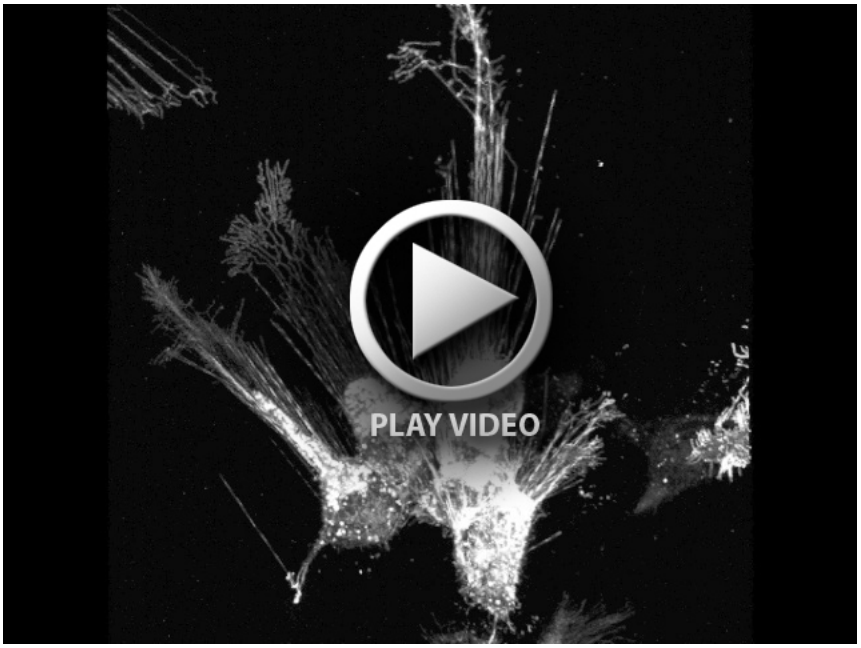
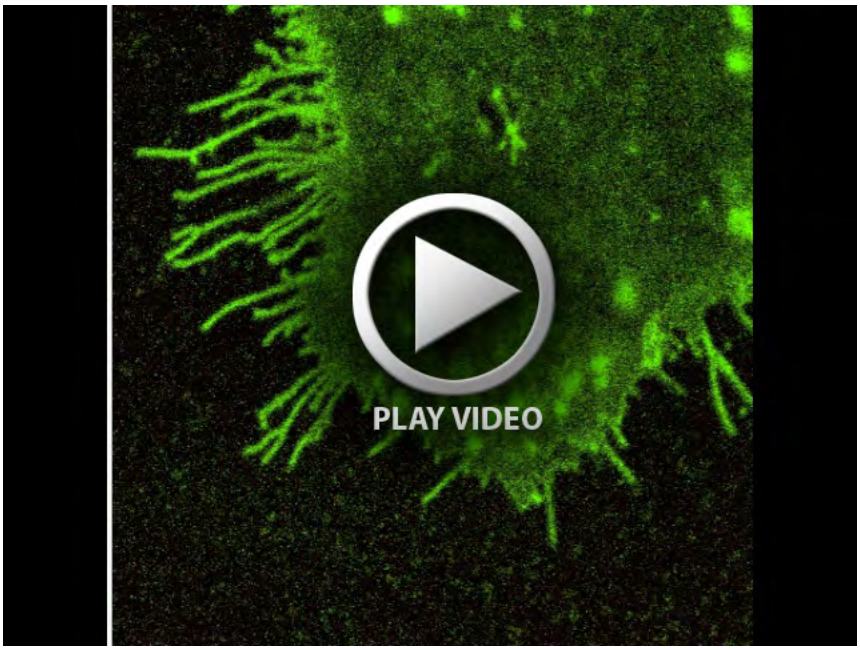


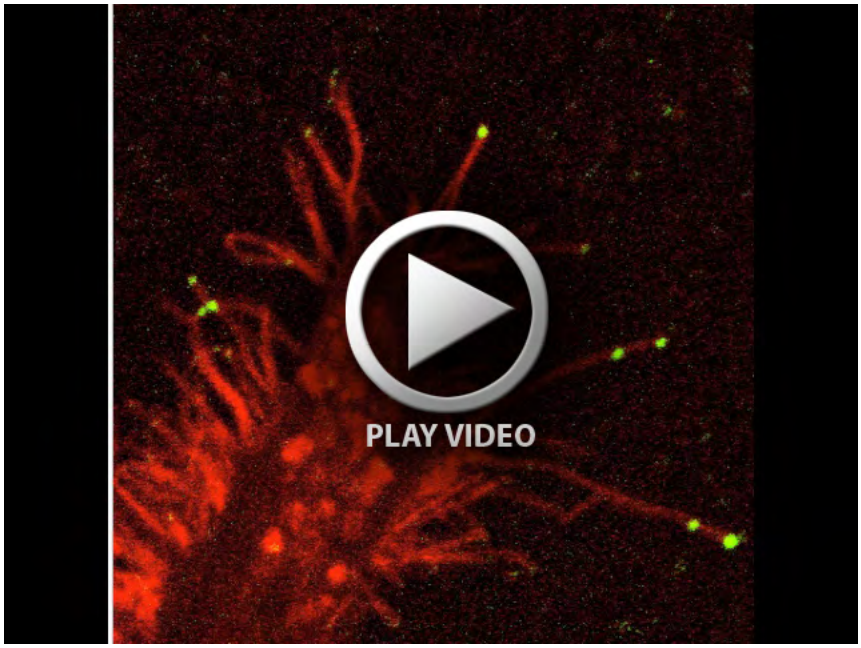
Movie S1. Lgr5 membrane protrusions are arborized at their contact with a substrate. Lgr5 (834del)-EGFP was transfected and imaged (See Fig 1I). Presented in this movie is a 3-dimensional reconstruction of a confocal Z-stack demonstrating protrusions that project downward toward the cell culture plate where they become arborized.

Movie S2. Short-term cytochalisin B treatment eliminates Lgr5 (834del)-EGFP membrane protrusions. HEK cells were Genecellin transfected with Lgr5 (834del)-EGFP. The next day cell media was replaced with serum free staining media (see materials and methods) containing 10μM cytochalisin B. Cells were optically sectioned and imaged live on a confocal microscope with a 37°C heated stage, beginning at time 0 and continuing every 30 minutes for a total of five hours. Z-sections were compressed into a maximum image projection and the time-course presented (2 frames/second).

Movie S3. Lgr5 (834del)-EGFP membrane protrusions rapidly reform after cytochalisin B washout. Lgr5 (834del)-EGFP transfected HEK cells were incubated with 10μM cytochalisin B overnight. Cells were washed five times with staining media (see materials and methods) and then optically sectioned and imaged live on a confocal microscope with a 37°C heated stage, beginning at time 0 and continuing every 30 minutes for a total of 2.5 hours. Z-sections were compressed into a maximum image projection and the time-course presented (2 frames/second). (Stage drift gives the false appearance of cell movement).



Movie S4. Concerted elongation of nascent membrane protrusions and retraction of plasma membrane forms elongated and branched cytonemes. The movie depicts data from Fig. 6A and represents 60 images (5 frames/second).



Movie S5. Lgr5-induced cytonemes as a platform for MyosinX transit. The movie depicts data from Fig. 6B and represents 60 images (5 frames/second).