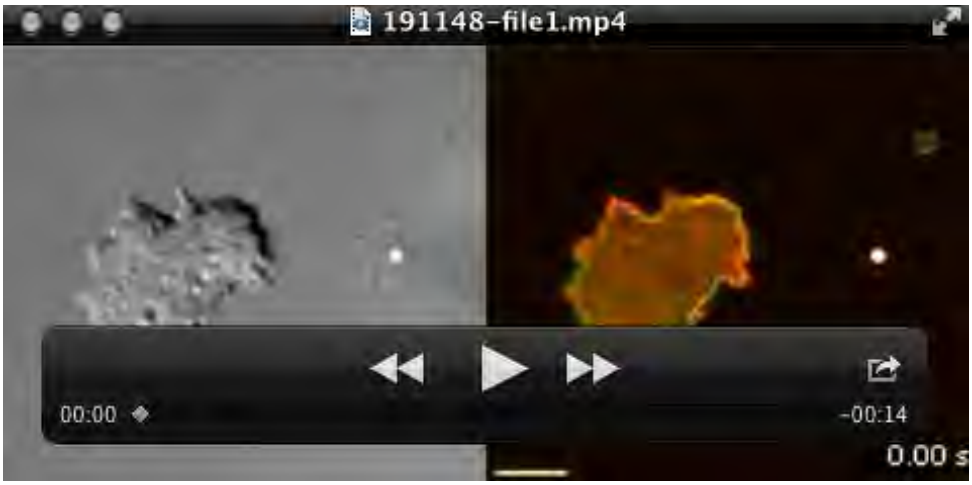
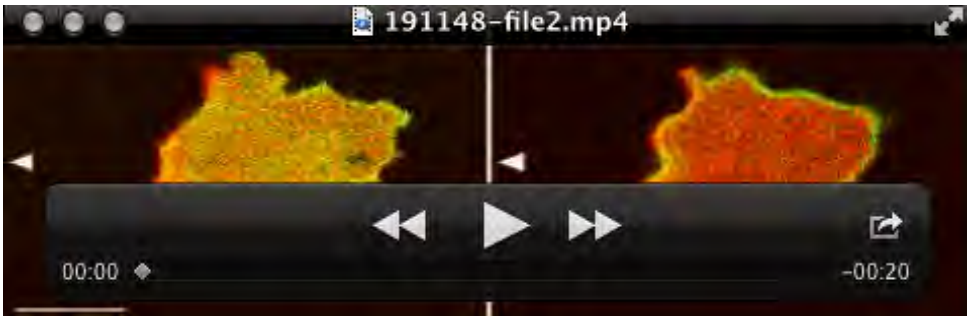
Supplementary Information

Movies

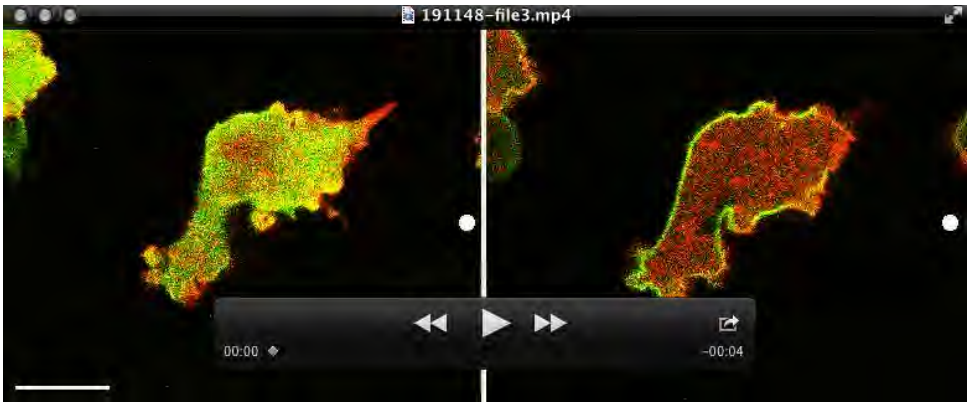
All movies show enlarged cells obtained by electric-pulse induced fusion. Tip positions of a micropipette filled with cyclic AMP are indicated by dots or arrowheads as in the figures. Seconds after the first frame of each sequence are indicated. The scale bars in the first frames indicate 10 µm.



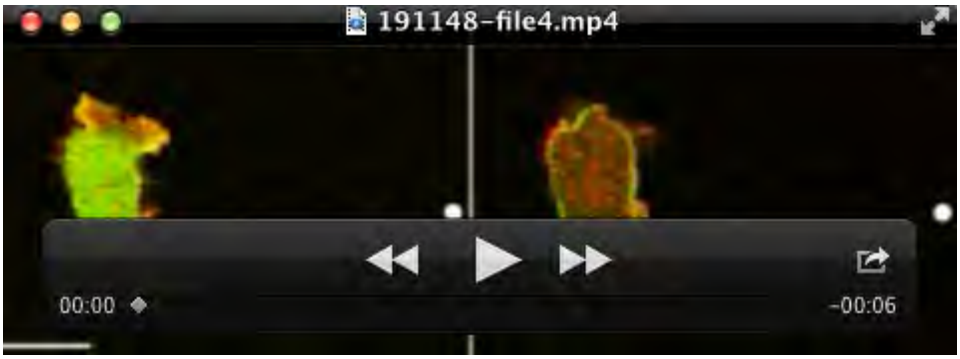
Movie 1. **Three cells of different sizes responding to changing positions of a micropipette filled with the chemoattractant.** The cells are labeled for activated Ras (red) and express GFP-PTEN (green). The left panels display bright-field DIC images, the right panels confocal cross-sections though the cell at a plane 1.5 µm beyond the substrate surface. The middle cell exemplifies that large cells may respond almost as fast as small ones: when the micropipette is moved from the right to the left at 235.44 s and 514.08 s, the first protrusions with activated Ras which are formed at the previous tail become detectable at 252.72 s and 529.20 s, respectively. In particular the large cell entering from top responds with a broad front, which is sub-divided into sections occupied with activated Ras and interspersed with PTEN-decorated zones. The same cells are shown in Figure 1A; the 170-s image of the Movie corresponding to the 0-s frame in the Figure. Frame-to-frame interval 2.16 s.



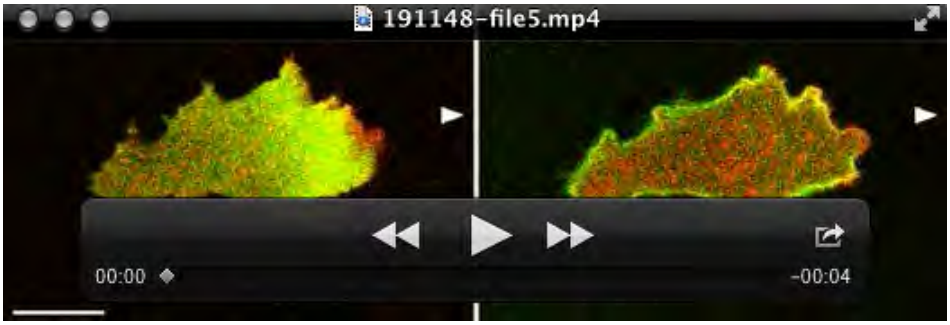
Movie 2. **Merged confocal images of a large cell labeled for activated Ras (red) and expressing PTEN-GFP (green).** The left panels are focused on the substrate-attached cell surface, the right panels display cross-sections through the cell bodies at a plane 1.5 µm beyond the substrate-attached surface. The cell re-orientates twice, showing various responses to reversal of the attractant gradient. In the periods of the 51.60-s to 94.60-s frames and of the 172.00 to 268.75-s frames the previous tail, now exposed to the source of attractant, retracts rather than protrudes toward the micropipette. In the 174.15-s to 180.60-s period, a thin protrusion emanating at the right border of the frame is seen in the left panel to turn into the direction of the gradient, with activated Ras at its tip. In the 184.90-s to 245.10-s frames, the formation of protrusions progresses at the bottom part of the cell from the previous front (opposite to the actual micropipette position) toward the newly established front. Frame-to-frame interval 2.15 s.



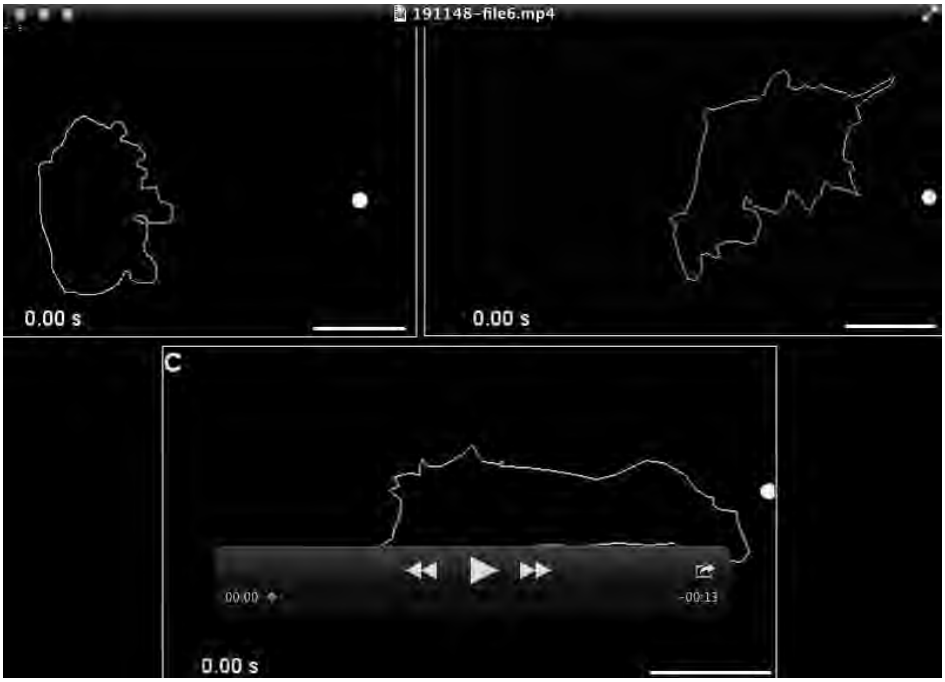
Movie 3. **Recording similar to Movie 2. The same cell is displayed in Figure 2A**; the 9.18-s image corresponding to the 0-s frame in Figure 2A. Frame-to-frame interval 3.06 s.



Movie 4. R**ecording similar to Movie 2 of the cell displayed in Figure 2B.** Frame-to-frame interval 3.06 s. The 15.30-s image corresponds to the 0-s frame in Figure 2B.

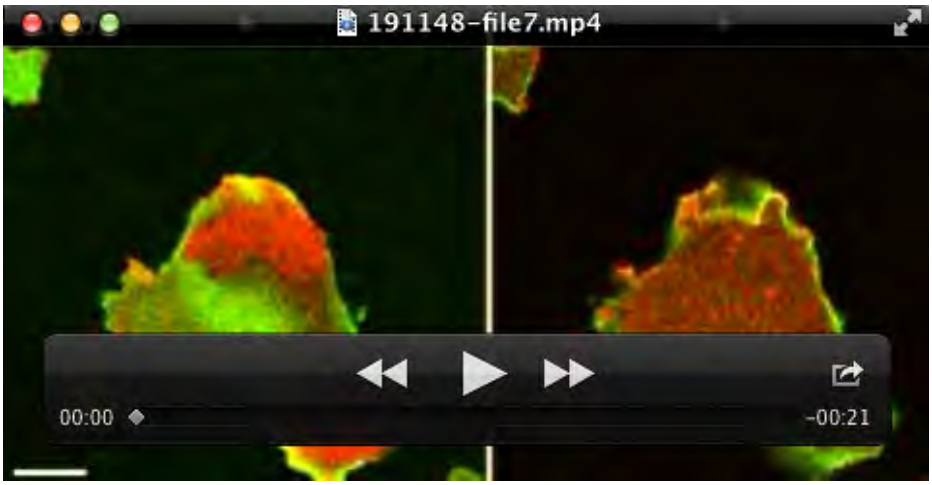


Movie 5. **Recording similar to Movie 2 of the cell displayed in Figure 2C.** Frame-to-frame interval 3.06 s. The 12.24-s image corresponds to the 0-s frame in Figure 2C.



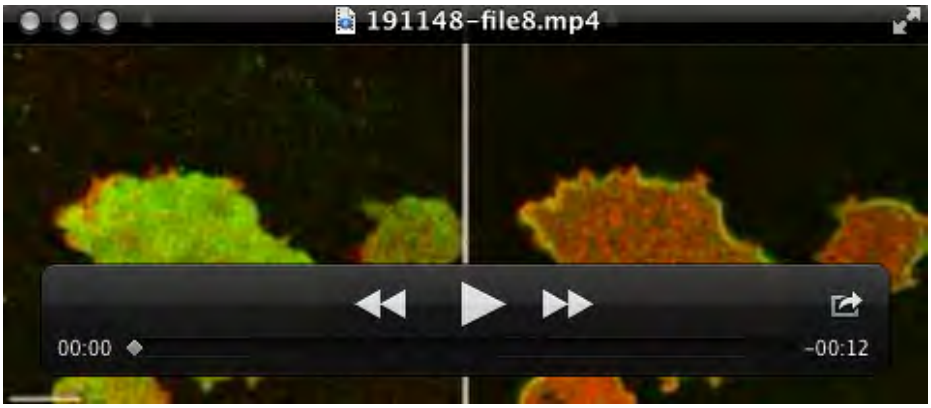
Movie 6. **Gain (red) and loss (blue) of cell area from one frame to the next in three cells responding to reversal of an attractant gradient.** The cells in A, B, and C are the same as in Figure 3A, B, and C. Micropipette positions are indicated by white dots.

Frame-to-frame intervals are for A and B 9.18 s, for C 8.60 s.

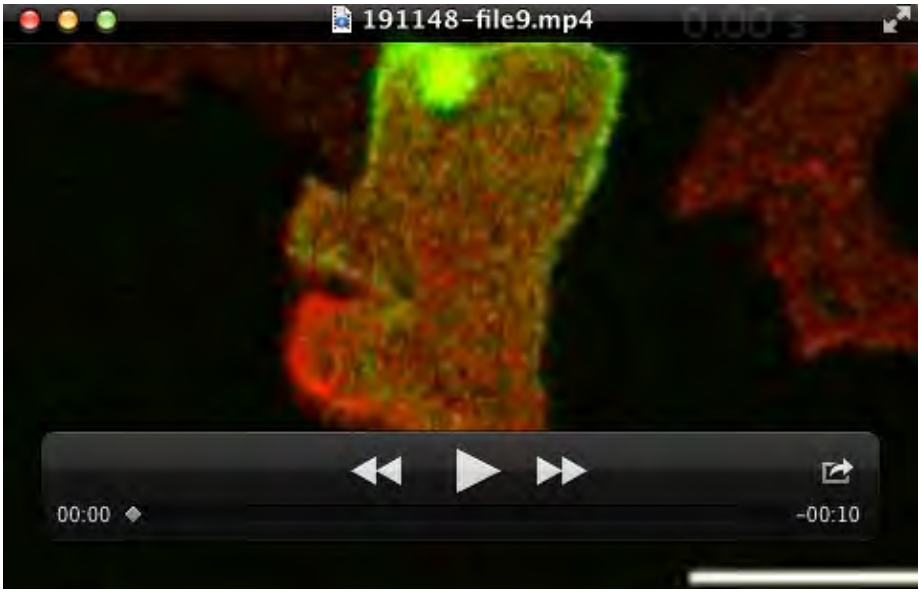


Movie 7. **A very large cell that forms waves on the substrate-attached membrane and responds to gradients of chemoattractant.** The cell recorded similar to Movie 2, forms propagating waves of activated Ras (red) on the substrate-attached membrane and simultaneously responds to changing directions of chemoattractant.

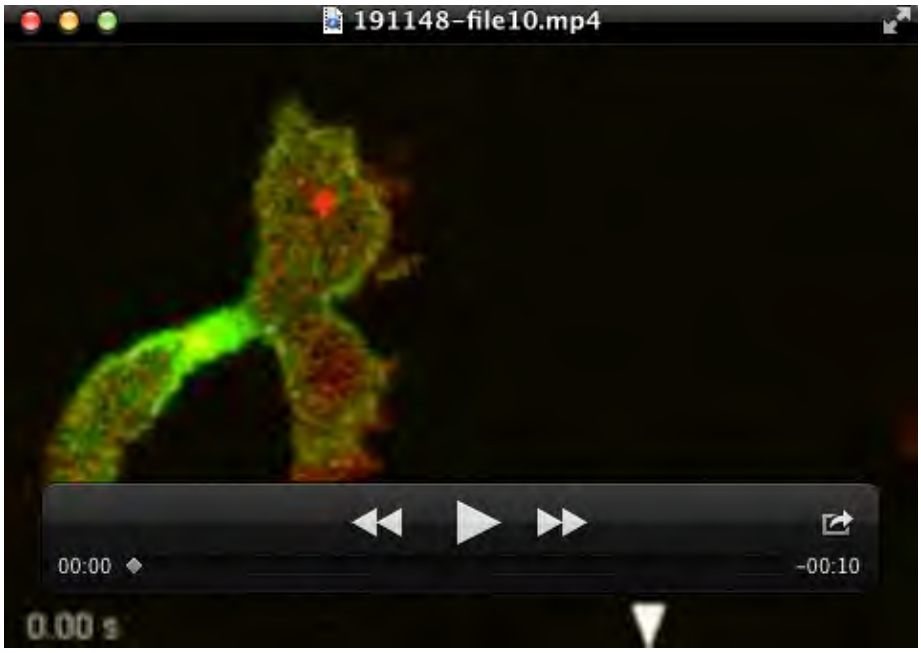
Frame-to-frame interval 2.16 s.



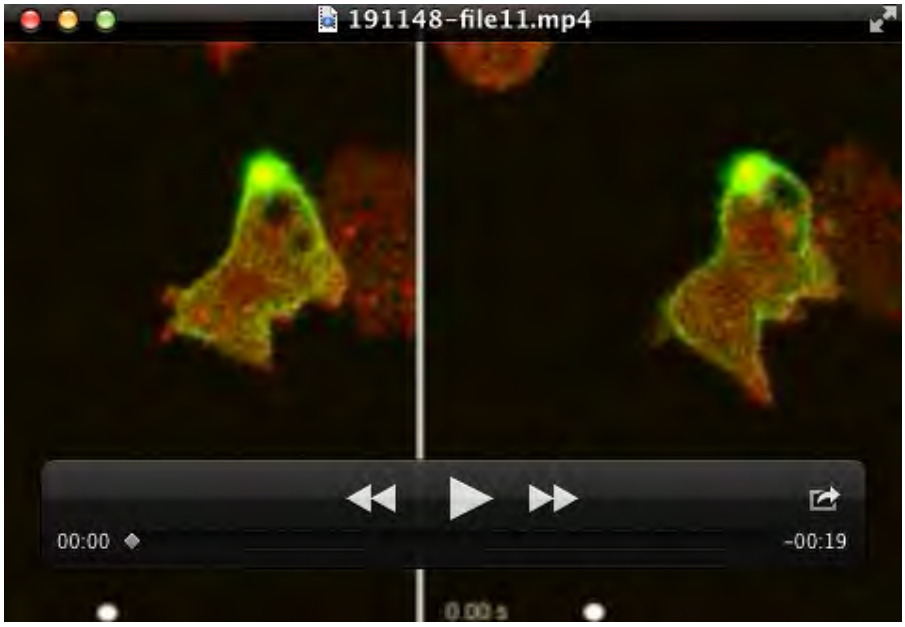
Movie 8. **Recording similar to Movie 2 of the cell displayed in Figures 6D and E.** Frame-to-frame interval 2.09 s. The 0-s image corresponds to the 0-s frame in Figure 6E.



Movie 9. **Confocal section through a cell expressing mRFP-RBD to label activated Ras (red), and GFP-talC63 (green) that accumulates in the direction of actin flow.** The focus is placed 1.5 µm above the substrate surface. The same cell is shown in Figure 7A. Frame-to-frame interval 2.50 s. The 30.00-s image corresponds to the 0-s frame in Figure 7A.



Movie 10. **Recording similar to Movie 9 of the cell displayed in Figure 7B.** Frame-to-frame interval 2.53 s. The 17.71-s image corresponds to the 0-s frame in Figure 7B.



Movie 11. **Recording of the cell displayed in Figure 7C, labeled as in Movie 9.** Left and right panels represent two planes of focus 1.5 µm apart of each other. Frame-to-frame interval 2.54 s. The 15.24-s image corresponds to the 0-s frame in Figure 7C.