# Supplementary Material

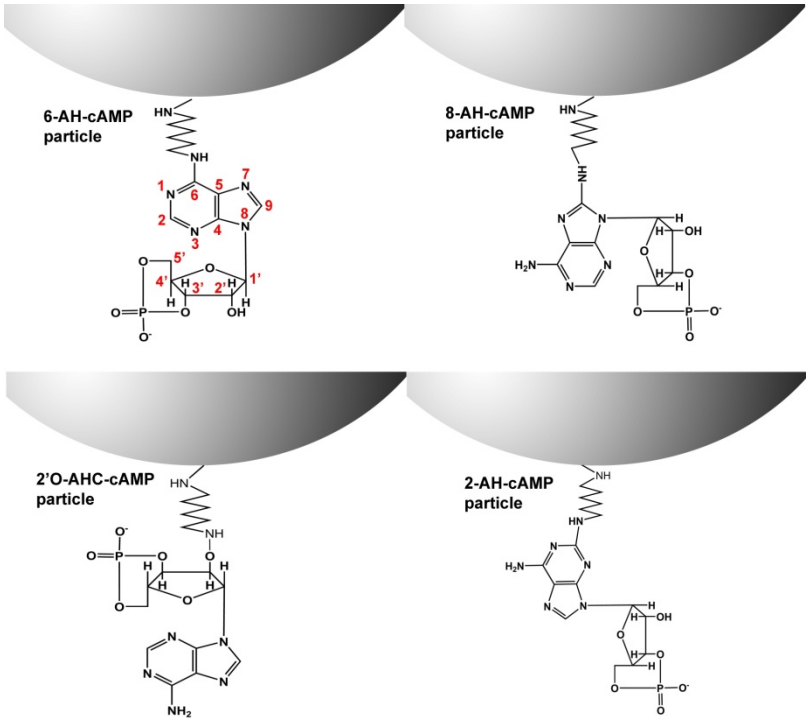



**Fig. S1. Particles used to stimulate differentiated *Dictyostelium* cells with immobilized cAMP molecules.** 8-AH-cAMP: 8-(6-aminohexylamino)-adenosine-3',5'-cyclomonophosphate; 6-AH-cAMP: N6-(6-aminohexyl)-adenosin-3', 5'-cyclomonophosphate; 2-AH-cAMP: 2-(6-aminohexylamino)-adenosin-3', 5'-cyclomonophosphate; 2'O-AHC-cAMP: 2'-O-(6-aminohexylcarbamoyl)-adenosine-3',5'-cyclomonophosphate.

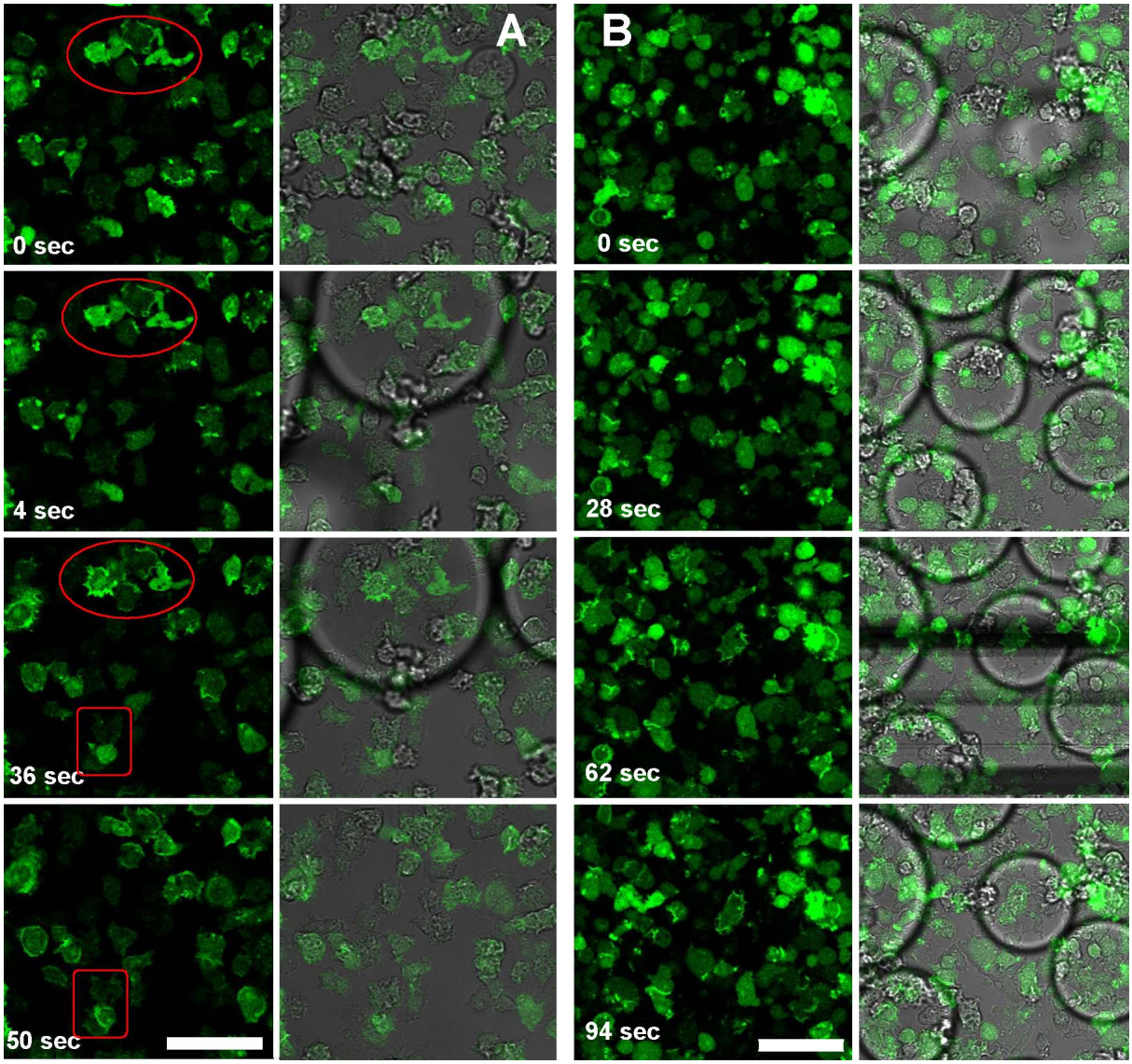



**Fig. S2. Particles that expose 6-AH-cAMP (A) and 2'O-AHC-cAMP (B) where let to sediment on to a layer of differentiated *Dictyostelium* cells expressing the PIP3 marker $PH_{CRAC}$-GFP**. (A) The green fluorescence of the marker increased in the membrane of such cells that were hit by the 6-AH-cAMP particles at a frame time of 4sec and indicates there for the presence of PIP3. Cells that had not been in contact with particles (red rectangle) did not display translocations of the marker indicated by no changes in the green fluorescence – only after 20 µM cAMP final were added at a frame time of 36 sec those cells also displayed an intracellular translocation of the marker which indicates for the presence of PIP3 in the membrane. (B) Upon collisions (7 - 24 sec) between the cells and 2'O-AHC-cAMP particles the intensity of green fluorescence did not changed. At a frame time of 62 sec 20 µM of cAMP final were added. Surprisingly also cells that had not been direct contact with the particles did not displayed any translocation of the marker intracellularly. Probably the presence of the 2'O-AHC-cAMP causes inhibition of the cAMP receptor causing no reaction in case of addition of free cAMP molecules. The size of the scale bar is 50 µm.

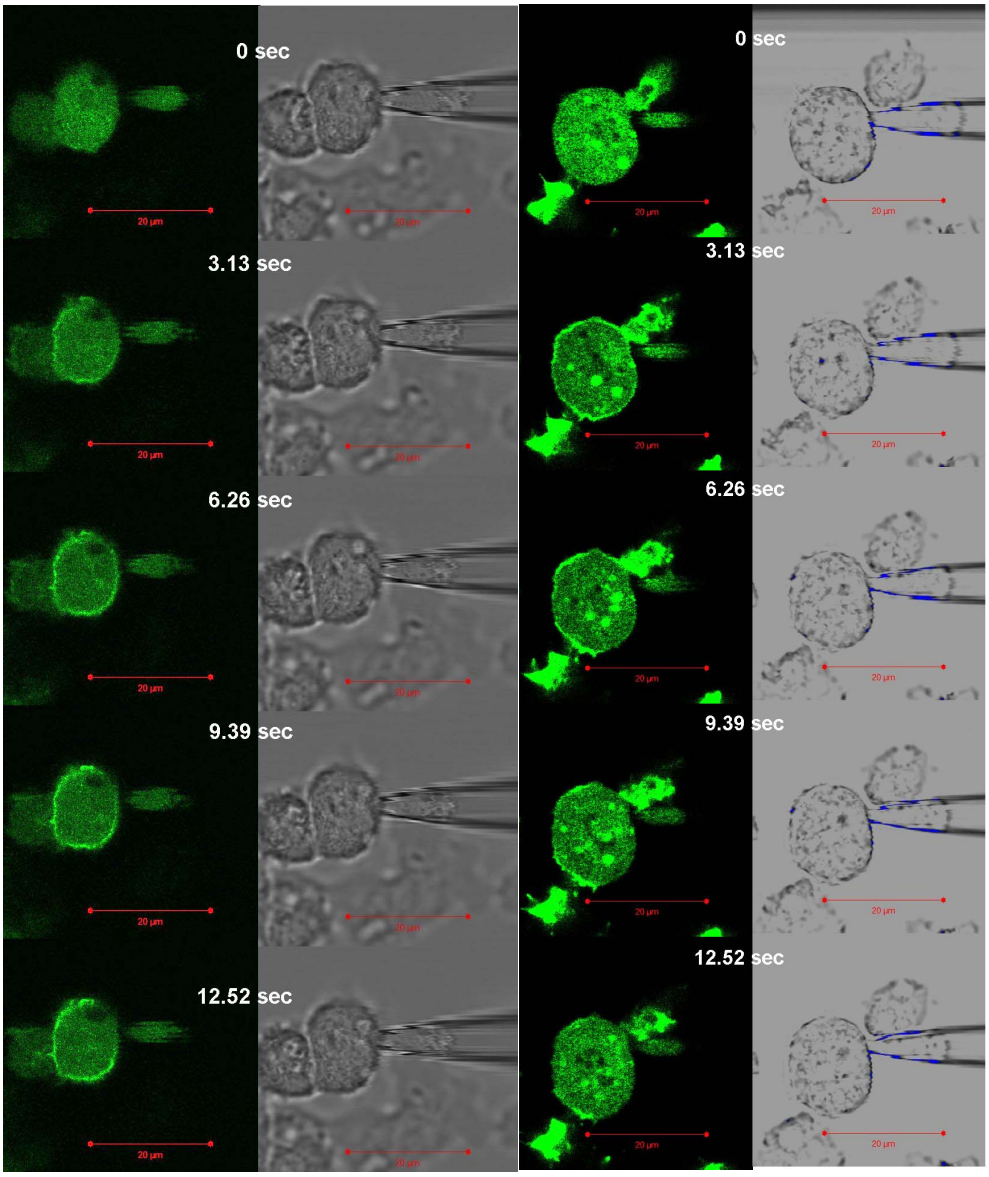



**Fig. S3. Two examples of a shielding experiment, where a differentiated $PH_{CRAC}$-GFP expressing *Dictyostelium* cell is exposed to cAMP.** A patch of the cell membrane was aspirated into the open tip of a glass micropipette and sealed with a seal resistivity higher than 50 MΩ by applying negative pressure. At a frame time of 0 sec, 20 µM cAMP final were added, which resulted in increased fluorescence intensity at the inner membrane while the fluorescence in the cytsol decreased. The fluorescence intensity in the aspirated patch stayed unchanged.

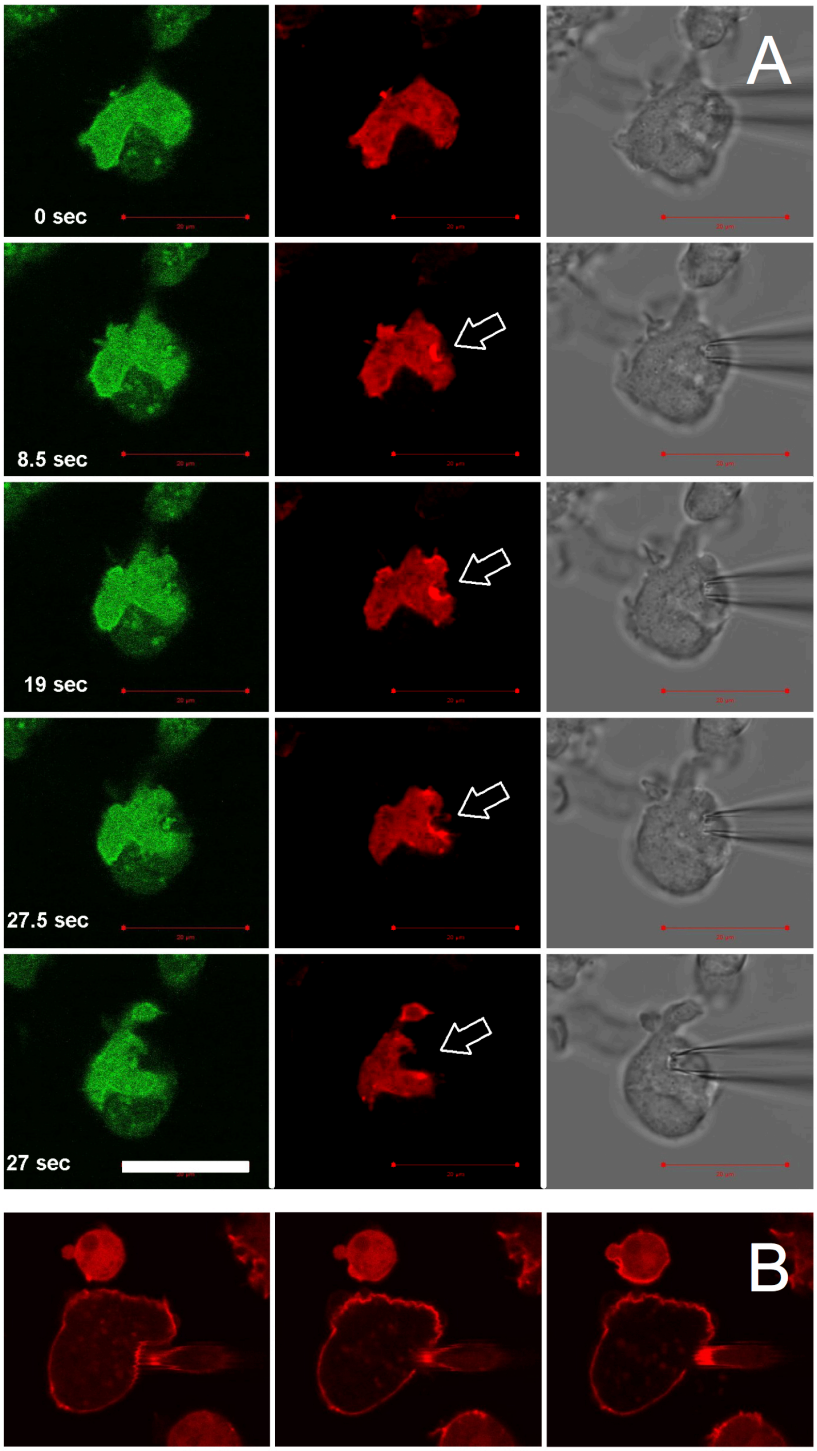



**Fig. S4. Effects of mechanical stress.** (A) Mechanical stimulation of a PTEN-GFP and DdLimEΔ-RFP coexpressing cell. Mechanical stress was induced by touching the surface of a differentiated cell with the tip of a glass pipette. The PTEN-GFP marker indicted no significant response of the cell whereas the fluorescence of the DdLimEΔ-RFP strongly increased at the membrane site in contact with the pipette tip (see arrow). After 28 sec, the DdLimEΔ-RFP fluorescence vanished, while the pipette tip was still in contact with the cell (scale bar 20 µm). (B) cAMP response of a DdLimEΔ-RFP expressing cell in the region of maximal mechanical stress. (left, middle) Cell with aspirated membrane patch before application of the cAMP stimulus. (right) Cell with aspirated membrane patch at the peak of the cAMP response. A strong LimEΔ translocation can be seen in the region of maximal mechanical stress.

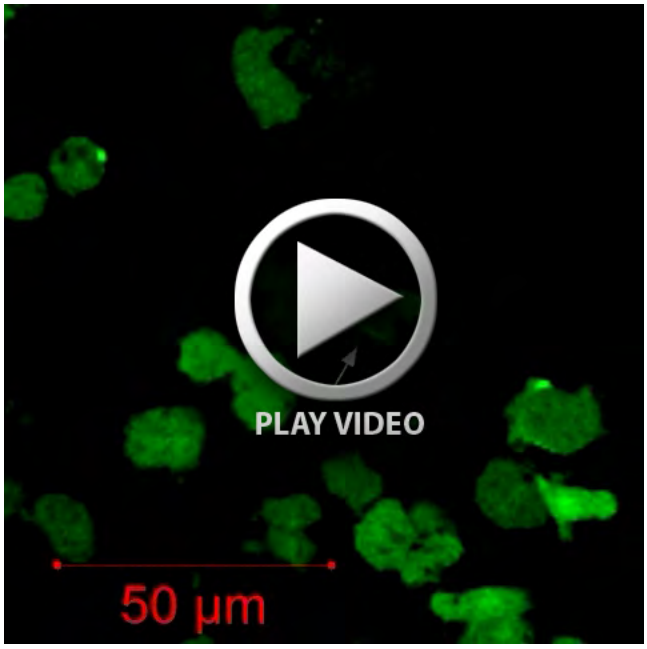
## Supplementary Movie Legends

**Movie 1. Autonomous PIP3 signaling versus cAMP induced responses.** $PH_{CRAC}$-GFP expressing cells are stimulated with 20 µM cAMP final (see the Discussion for a detailed concentration estimate). It can be seen that autonomous signaling exhibited by the cells prior to cAMP exposure is weakened but not wiped out by the cAMP stimulus (a cell displaying autonomous signaling is indicated by an arrow in the movie). The cAMP-triggered $PH_{CRAC}$-translocation is comparable in strength to the spontaneously occurring translocation patterns.

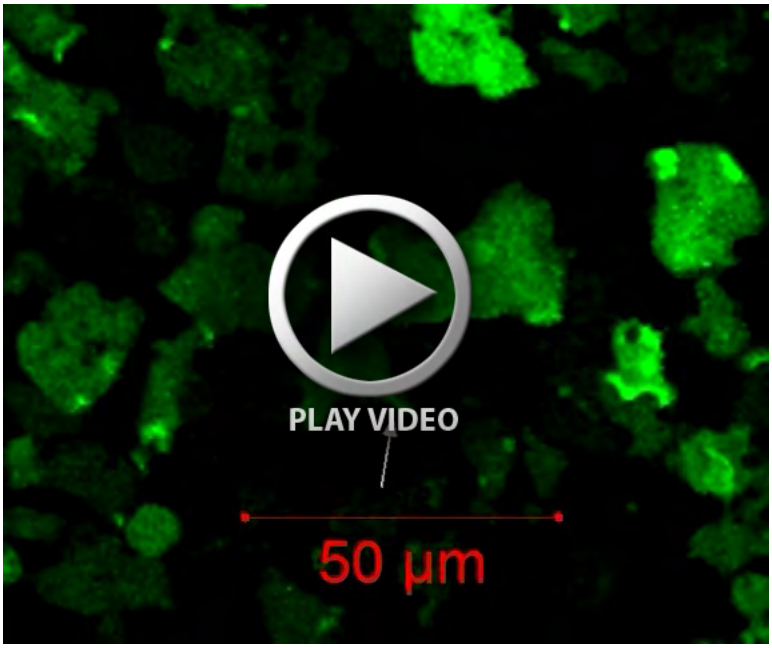
**Movie 2. Autonomous PIP3 signaling versus cAMP induced responses.** A second example for the same behavior, see caption of Movie 1 for further details.

**Movie 3. Responsiveness of non-stimulated membrane areas to a subsequent cAMP stimulus.** Example of a cell (indicated by a white arrow) in contact with a cAMP coated bead. The bead can be distinguished as a shadow in the bright field channel (a white outline is inserted during parts of the movie to guide the eye). Those parts of the cell membrane that did not touch the bead and thus did not exhibit any PIP3 signaling, are less responsive to a subsequent external cAMP stimulus. The cAMP stimulus can be observed indirectly through the response of the surrounding cells (indicated by yellow arrows).

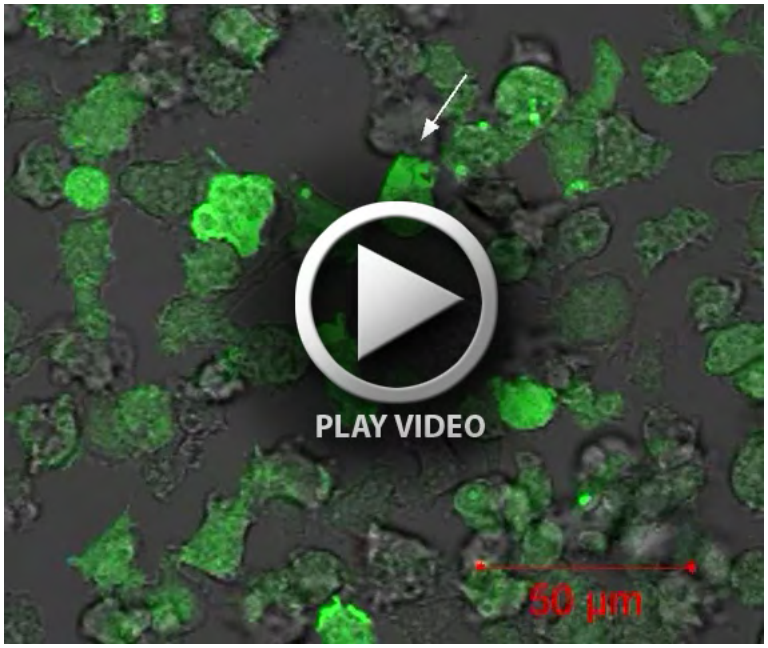
**Movie 4. Responsiveness of non-stimulated membrane areas to a subsequent cAMP stimulus.** A second example for the same behavior, see caption of Movie 3 for further details. In addition, a red arrow points to the less responsive parts of the membrane.




**Movie 1.**




**Movie 2.**




**Movie 3.**


**Movie 4.**