Fig. S1. PKBA/PKBR1 double null cells do not phosphorylate PKB substrates in response to uniform FA stimulation. PKBA/PKBR1 double null vegetative cells were grown on bacterial lawns overnight, washed and stimulated with 50 μM FA. 4×10^4 cells were loaded per well on an SDS-PAGE gel. Western blot was probed for phospho-PKB substrates. PKB substrates were not phosphorylated upon FA stimulation. These results demonstrate the specificity of the anti-phospho-PKB substrate antibody (Fig. 4C).

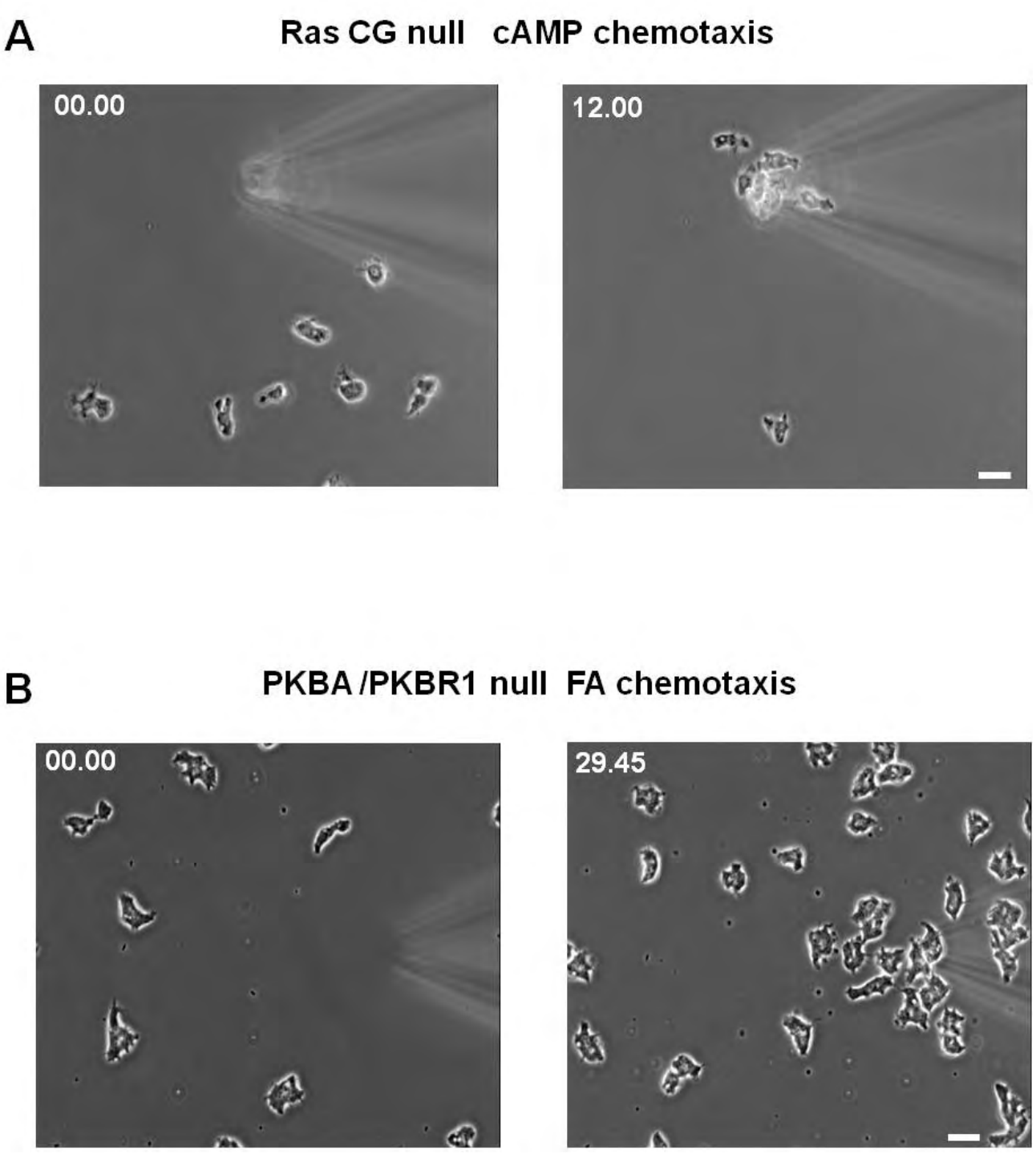


Fig. S2. Chemotaxis of Ras CG and PKBA/PKBR1 null cells. (A) Frames of Ras CG null cells at indicated time points in a cAMP gradient. Cells migrate up the concentration gradient and towards the camp-loaded micropipette. (B) Frames of PKBA/PKBR1 null cells at indicated time points in a FA gradient. Cells migrate up the concentration gradient and towards the FA-loaded micropipette. Scale bar: ~10 μm.

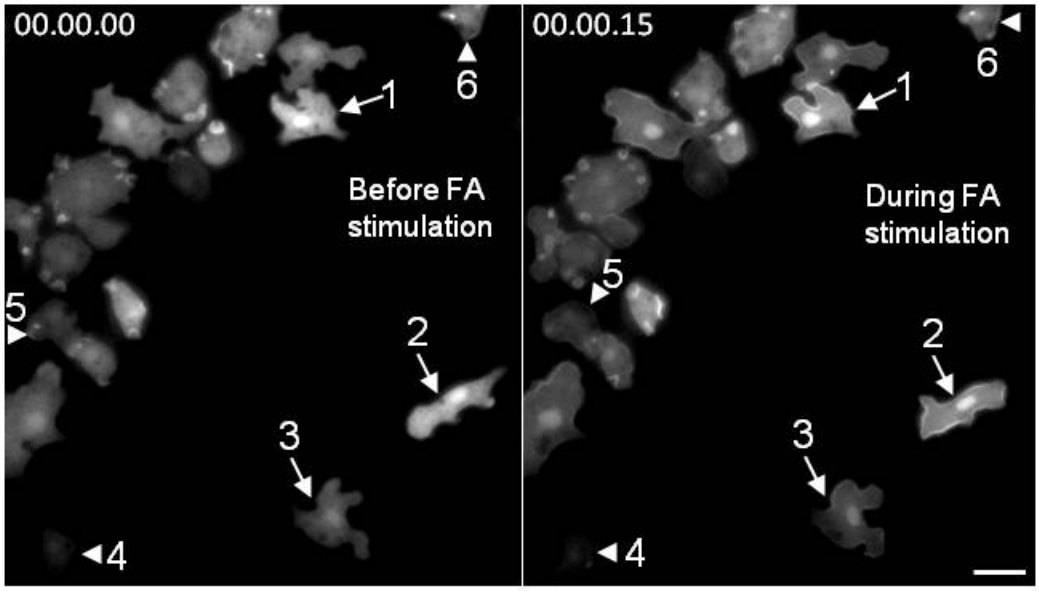
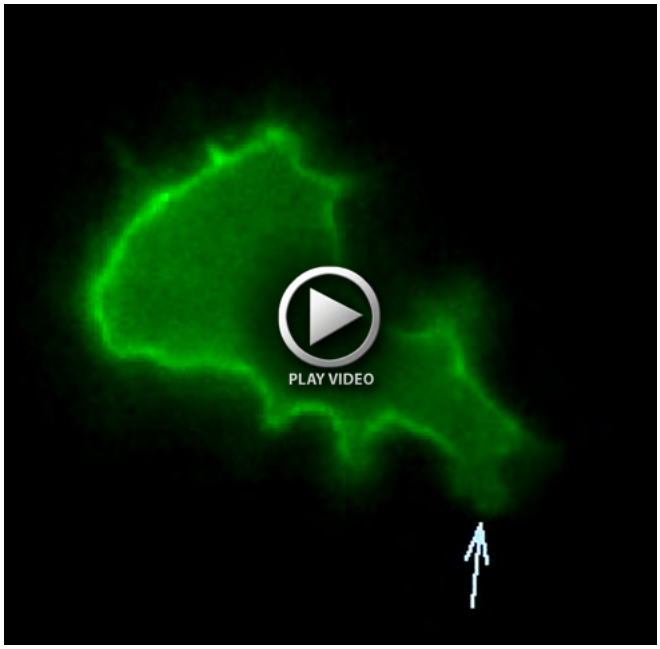
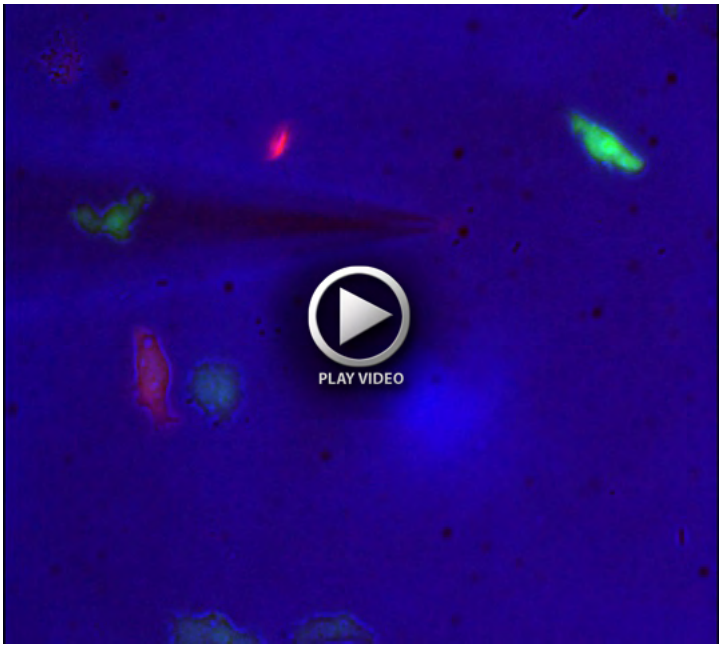


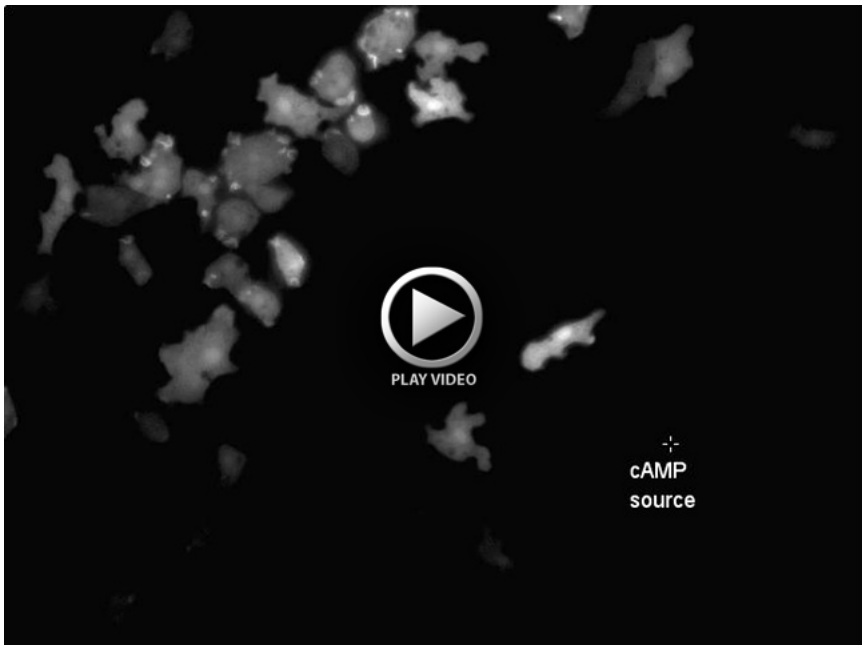
Fig. S3. Response of 4-hour-starved PH_{Crac}-GFP/WT cells to cAMP. Four-hour-starved cells contain mixed population of semi-polarized (arrow, 1-3) and unpolarized cells (arrowhead, 4-6), (left panel). During uniform cAMP stimulation PH_{Crac}-GFP translocated to membrane in both semi and unpolarized cells (right panel).



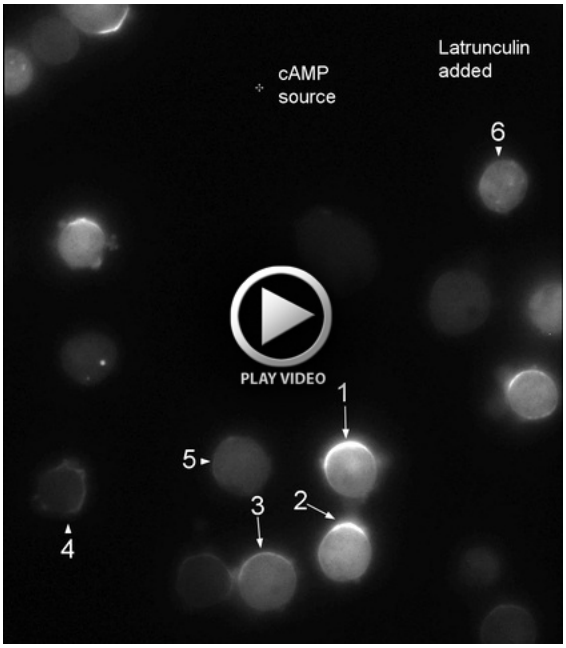
Movie 1. Random migration of a PTEN-GFP/PLC null cell. Time-lapse movie of PTEN-GFP expressed in PLC null cells was captured for 28.5 seconds with interval of 1.5 seconds. Arrow indicates absence of PTEN-GFP on the periphery of pseudopods.



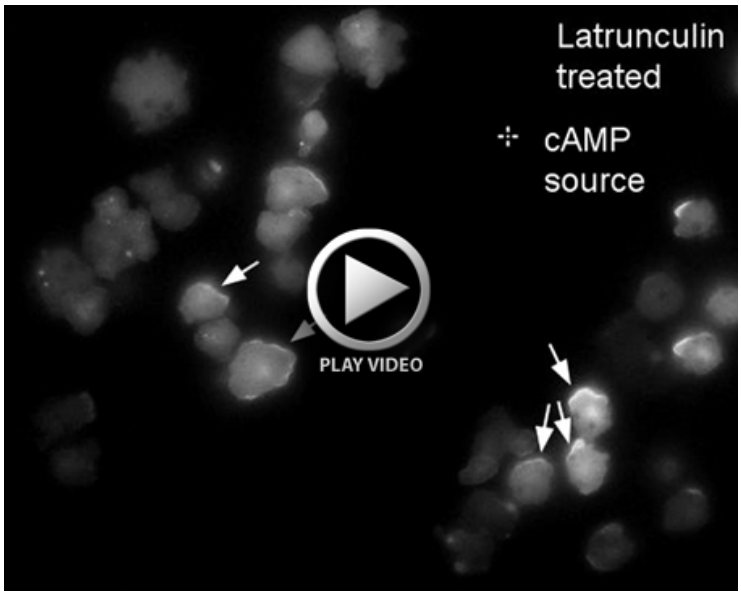
Movie 2. Unpolarized cells switch directions while polarized cells do U-turns when gradients are reversed. Time-lapse movie of polarized and unpolarized cells migrating towards a micropipette filled with both FA and cAMP. Unpolarized cells are only expressing PH_{Crac}-GFP and are green. Polarized cells starved for 6 hours are expressing PH_{Crac}-GFP and LimE-RFP and are orange in color. Polarized cells make a U-turn while unpolarized cells can quickly switch directions when micropipette is moved.



Movie 3. Semi-polarized and unpolarized cells respond to cAMP. Frames of 4-hour-starved wild type cells expressing PH_{Crac}-GFP were captured every 15 seconds as the cells migrated towards a cAMP-filled micropipette. A mixed population of semi-polarized (arrow, cells 1-3) and unpolarized cells (arrow head, cells 4-6) were observed prior to and during migration. When the cAMP filled micropipette was brought down, a uniform translocation of PH_{Crac}-GFP to the membrane was observed (frame 2). Both semi-polarized and unpolarized cells migrate towards the cAMP filled micropipette.



Movie 4. Unpolarized cells do not form a stable PH_{Crac}-GFP crescent in a cAMP gradient. Frames of the same 4-hour-starved wild-type cells expressing PH_{Crac}-GFP in the presence of Latrunculin-A were captured every 15 seconds 72 minutes later. The camp-loaded micropipette was moved to a 2nd position, and again, the semi-polarized cells (labeled 1-3) formed PH_{Crac}-GFP crescents toward the side of the new cAMP source while unpolarized cells (labeled 4-6) made unstable PH_{Crac}-GFP crescents and in some instances were able to extend pseudopods since these cells are more resistant to the Latrunculin-A treatment.latrunculin for immobilization as compared to cells that are semi-polarized.



Movie 5. Latrunculin-treated unpolarized cells migrate to cAMP source while polarized cells are immobilized. Time-lapse movie of latrunculin-treated unpolarized and semi-polarized cells. Arrow indicates semi-polarized cells that are still immobilized after 30 minutes of latrunculin treatment and making crescent towards cAMP source. Unpolarized cells, on the other hand, begin to resume motility towards the cAMP source as the latrunculin presumably degrades, and levels drop. Unpolarized cells require higher levels of latrunculin for immobilization as compared to cells that are semi-polarized.