

Supplemental Files For

PakB Binds to the SH3 domain of Dictyostelium Abp1 and Regulates its Effects on Cell Polarity and Early Development

Yidai Yang, Marc de la Roche, Scott Crawley, Zhihao Li, Emilia Furmaniak-Kazmierczak, and Graham P. Côté

Video S1. Localization of GFP-PakB-1-276 in developed, migrating AX3 cells. GFP-PakB-1-276 localizes to the rear of the migrating cell. Bar, 10 μm .

Video S2. Localization of GFP-PakB-1-180 in developed, migrating AX3 cells. GFP-PakB-1-180 is enriched at the leading edge of the migrating cells. Bar, 10 μm .

Video S3. Localization of GFP-PakB-1-180 in growth phase AX3 cells. GFP-PakB-1-180 is enriched in pseudopodia and macropinocytic cups of growth phase AX3 cells. Bar, 10 μm .

Video S4. Localization of RFP-dAbp1 in developed, migrating AX3 cells. RFP-dAbp1 is enriched at the leading edge of the migrating cells. Bar, 10 μm .

Video S5. Localization of RFP-dAbp1 in developed, migrating PakB⁻ cells. RFP-dAbp1 exhibits a diffuse distribution in the absence of PakB.

Figure S1. Localization of PakB and PakB-1-180 in PakB⁻ cells. (A) Immunofluorescence localization of PakB and GFP-PH-Crac in AX3 cells. Cells expressing GFP-PH-Crac were fixed and stained with anti-GFP and anti-PakB antibodies. PakB localized to the cell cortex and within pseudopods. PH-Crac was largely cytosolic but some co-localization to the leading edge of pseudopods was apparent. Bar, 10 μm . (B) Time course images of a polarized, aggregation competent PakB⁻ cell expressing PakB-GFP. PakB was localized to the anterior of the cell and to pseudopods extended from the lateral edge. Arrow shows the direction of migration. Bar, 10 μm . (C) Time course images of randomly migrating PakB⁻ cells expressing GFP-PakB-1-180. Similar to PakB, PakB-1-180 localized to the anterior of cells and to extending pseudopods. The arrow indicates a cell that sharply changed direction. PakB-1-180 was lost from the existing leading edge and reappeared at the newly protruding leading edge. Bar, 10 μm .

Figure S2. Profile plots of polarized migrating cells expressing GFP, PakB-GFP and GFP-PakB-1-180.

Images of cells expressing GFP, PakB-GFP (from Fig. S1A) and GFP-PakB-1-180 (from Fig. S1B) were analyzed using the ImageJ Analyze>Plot Profile function. Profile plots through the centers of the cells (boxes) show that GFP is relatively evenly distributed throughout the length of the cell, whereas PakB and PakB-1-180 are enriched within the leading edge pseudopod. Arrows indicate the direction of migration. Bar, 10 μm .

Figure S3. Actin filament distribution in PakB⁻ cells. The time course images show aggregation competent PakB⁻/dAbp1^{OE} cells expressing GFP-ABD (the actin-binding domain from ABP-120) as a marker for filamentous actin. Recruitment of GFP-ABD to the cell periphery shows that PakB⁻ cells remain capable of assembling cortical actin filaments. The diffuse localization of RFP-dAbp1 is consistent with the conclusion that PakB is needed for it to localize to actin-rich regions. Arrow indicates the direction of migration. Bars, 10 μm .

Figure S4. Localization of PakB-1-180 in cells that cannot produce PIP₃. (A) GFP, GFP-ABD, GFP-dAbp1 and GFP-PakB-1-180 were expressed in a cell line that is unable to produce PIP₃ due to the loss of all PI3Ks and PTEN. Images of individual live cells show that GFP exhibits a diffuse distribution, whereas GFP-ABD, GFP-dAbp1 and GFP-PakB-1-180 localize to cortical regions and pseudopods. Bar, 10 μm .

Figure S5. Expression and localization of RFP-dAbp1- Δ SH3. (A) The immunoblot shows lysates of PakB⁻ cells expressing RFP-dAbp1- Δ SH3 or RFP-dAbp1 probed using an anti-dAbp1 antibody. (B) Time course images of aggregation competent PakB⁻ cells expressing GFP-ABD and RFP-dAbp1- Δ SH3. GFP-ABD localized to the cell cortex, the leading edge pseudopod and the rear of the cell, whereas RFP-dAbp1- Δ SH3 exhibited a diffuse localization. Arrow indicates the direction of migration. Bars, 10 μm .

Figure S1

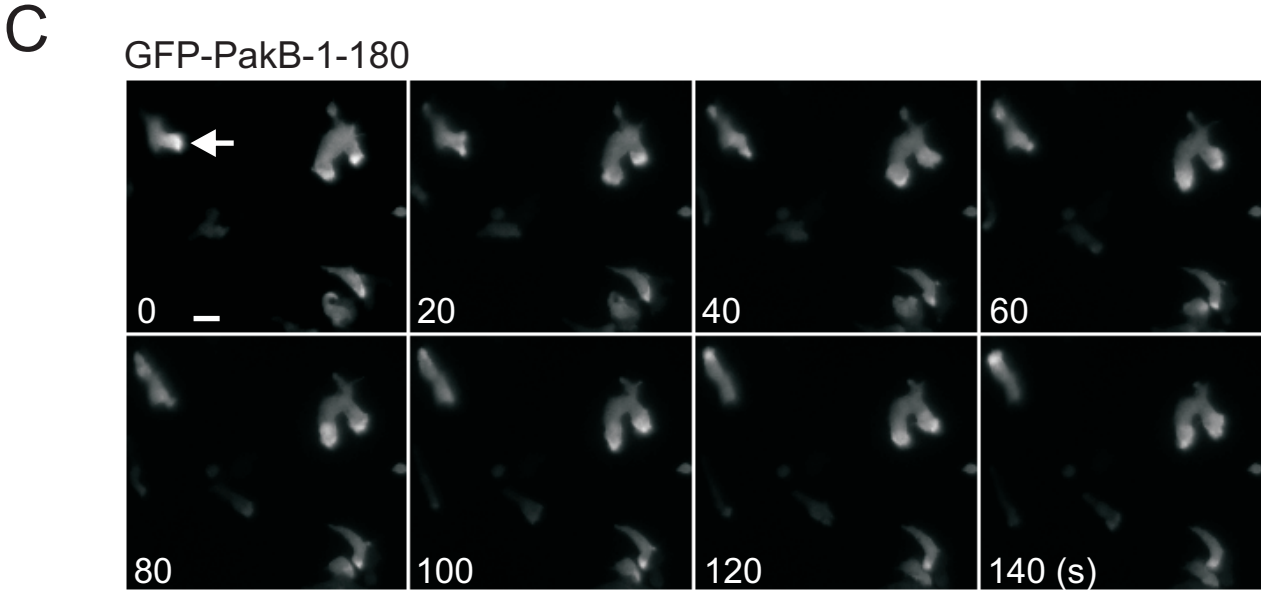
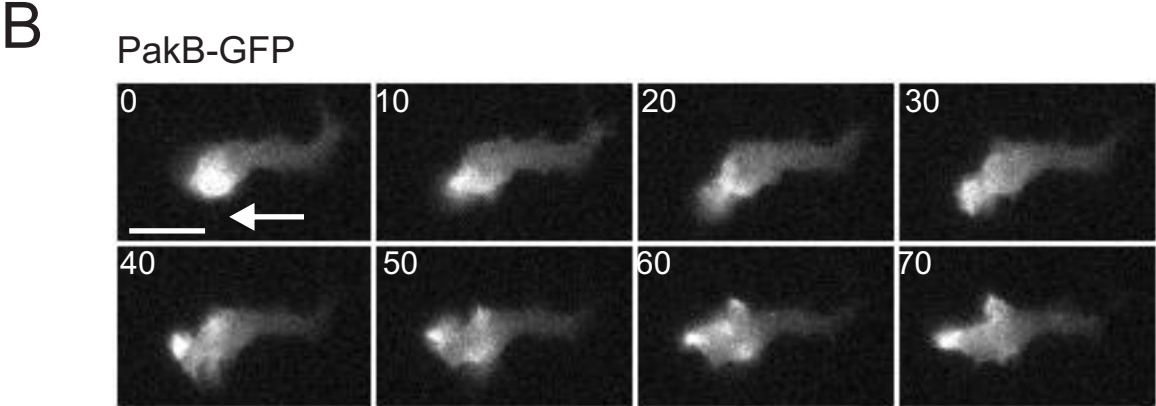
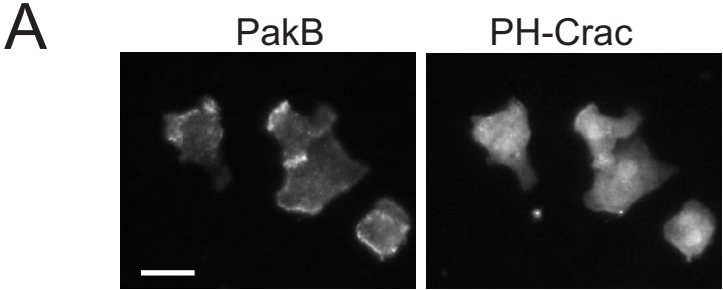


Figure S2

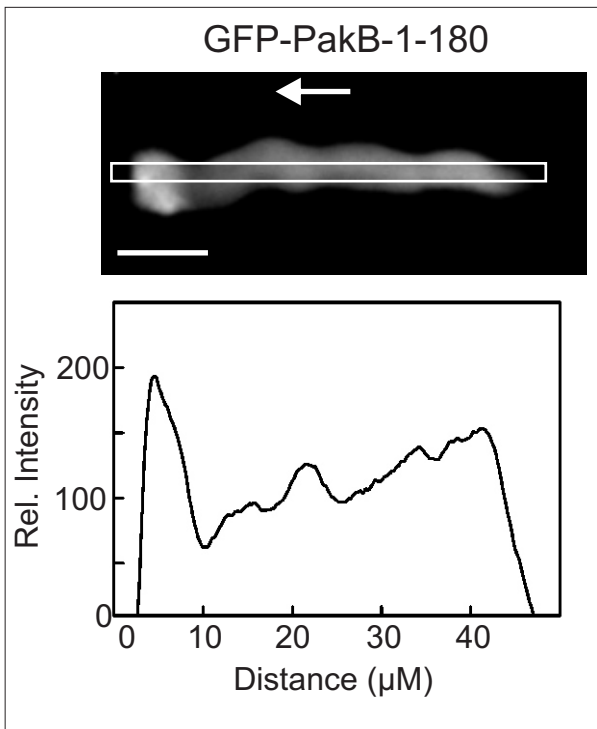
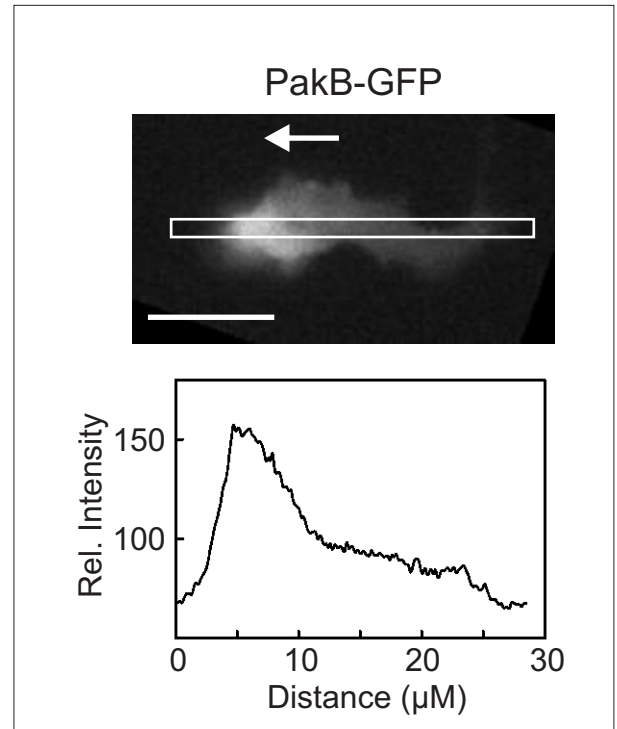
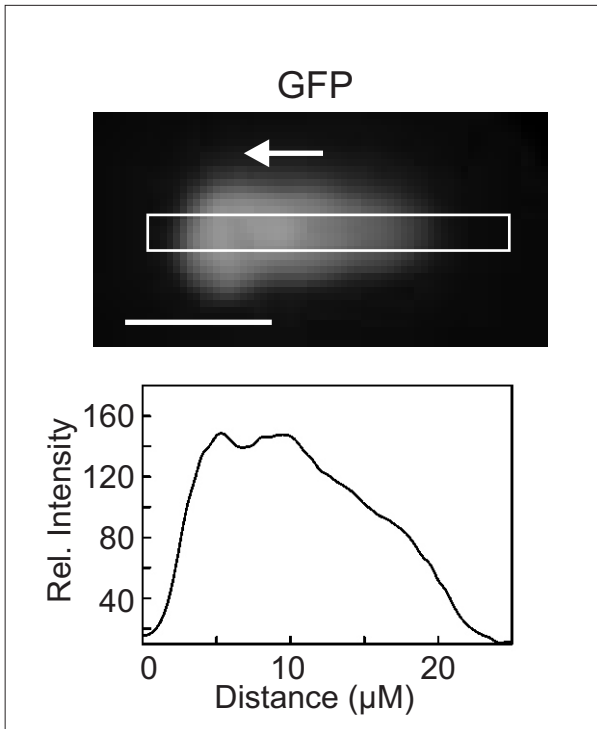


Figure S3

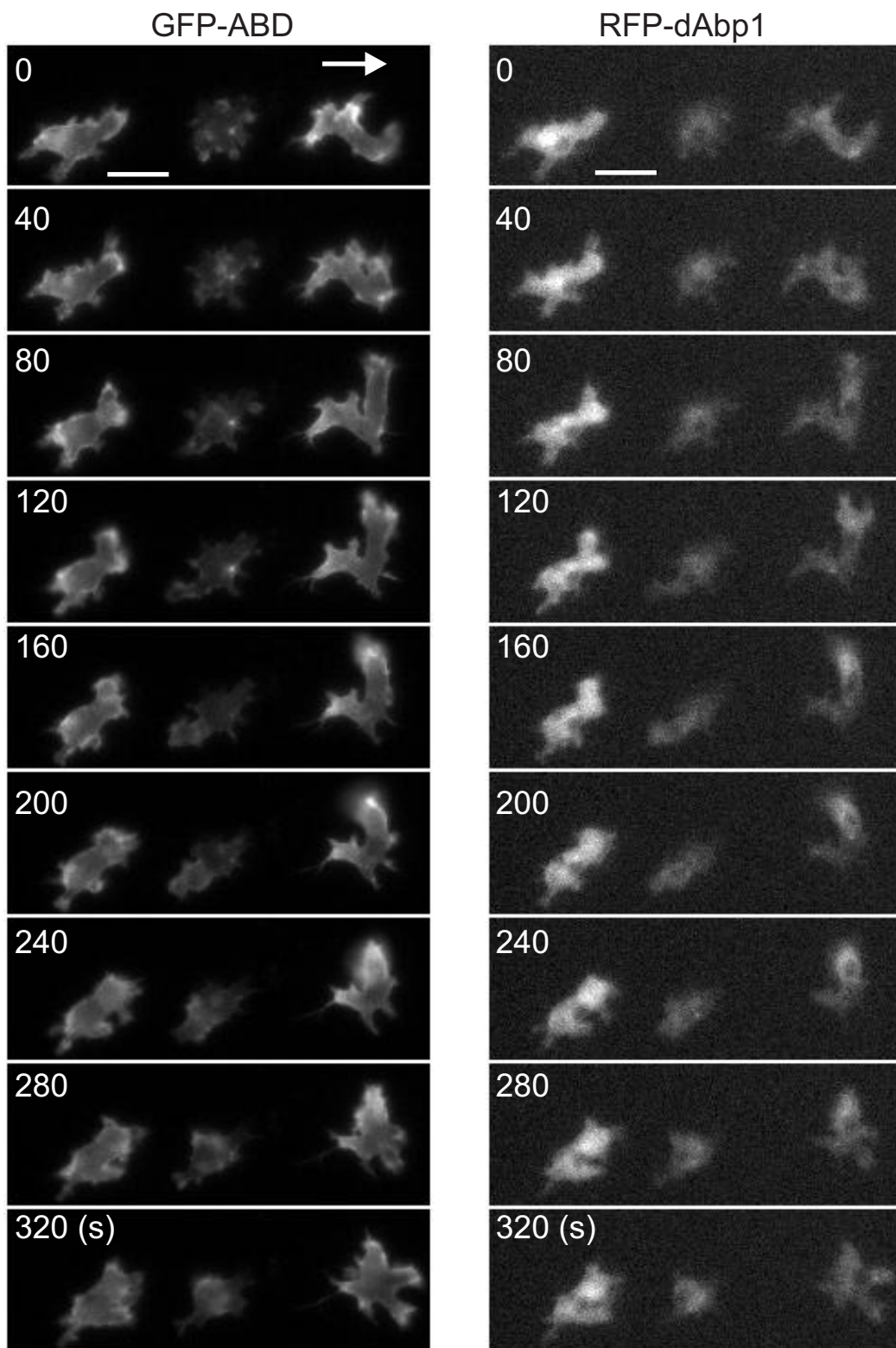


Figure S4

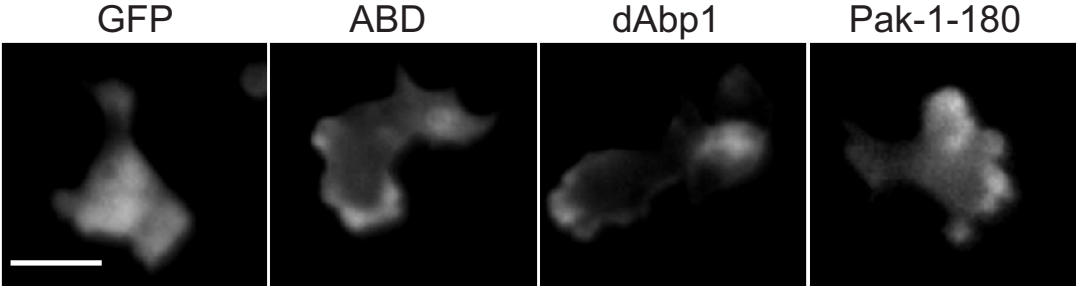
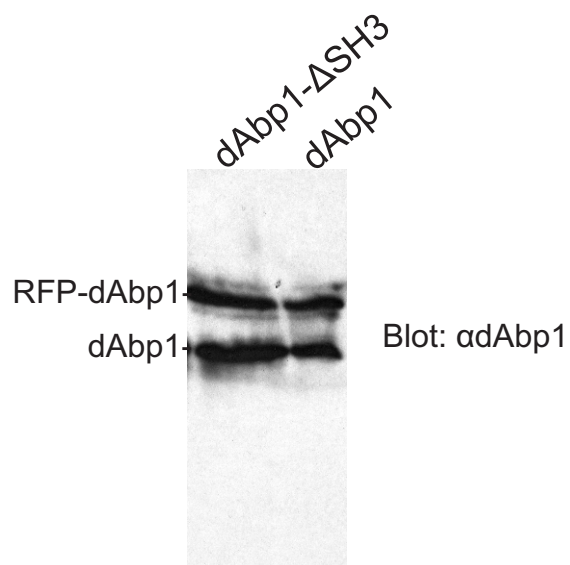


Figure S5

A



B

