

Supplemental Material

Figure S1 **Characterization of SV40-immortalized wild type and DGK ζ -null fibroblasts**

(A) Schematic showing the location of various protein domains within DGK ζ . The N-terminal (open box), C1 domains, MARCKS (filled box) and catalytic domains are indicated. Also shown are the four ankyrin repeats (I-IV) at the C-terminus. The extreme C-terminus contains a class I PDZ-binding motif. (B) Immunoblot analysis of wt (+/+) and DGK ζ -null (-/-) fibroblasts. Equivalent protein amounts from cell lysates of (+/+) and (-/-) fibroblasts were immunoblotted with an antibody to the N-terminus of DGK ζ (top panel) or to the closely related isoform DGK ι (bottom panel). (C) Replicate blots were probed with antibodies to syntrophin (Syn), Rac1, and several cytoskeletal and focal adhesion proteins as indicated. Pax, paxillin.

Figure S2 **PAK localization in wt and DGK ζ -null cells**

Indirect immunofluorescence localization of PAK in wt and DGK ζ -null fibroblasts. Wt fibroblasts were fixed and double stained with an antibody to PAK1 followed by AlexaFluor 594-conjugated secondary antibody (red) and with AlexaFluor 488-conjugated phalloidin (green) to visualize actin. The pattern of PAK immunoreactivity was indistinguishable between wt and null cells. Note the numerous filopodia present in the DGK ζ -null cells. Scalebars = 10 μ m.

Figure S3 **Focal adhesion formation in wt and DGK ζ -null cells.**

(A) Immunofluorescence localization of actin (green) and phosphotyrosine (pY, red) after replating DGK ζ -null cells on FN for 60 min. Arrowheads indicate focal adhesions. Scalebar: 20 μ m. (B) Wt MEFs expressing HA-DGK ζ were double stained with antibodies to HA and to tyrosine-phosphorylated FAK (FAK-pY861). The lower panels are magnified images of the area boxed area

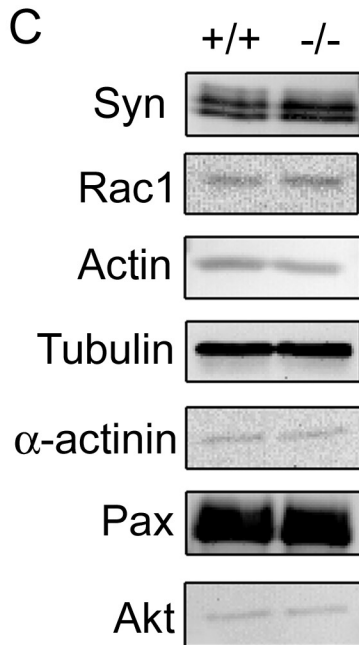
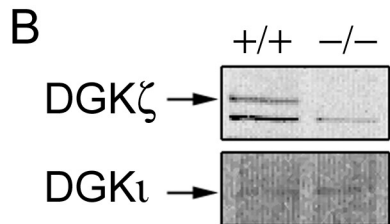
in the merged image. The *arrow* indicates a region of signal overlap. Scalebars = 10 μm . (C) A cell stained as in (B) showing an area of intense DGK ζ immunoreactivity that overlaps with diffuse FAK-pY861 staining. The lower panels are magnified images of the area boxed area in the merged image. The *arrowheads* delineate the area of intense staining. Scalebars = 10 μm .

Figure S4 **DGK ζ and syntrophin are present in circular ruffles**

Wt fibroblasts were fixed and stained for the indicated endogenous proteins then visualized by indirect immunofluorescence. Arrows indicate circular ruffles. Scalebars: 20 μm .

Video 1 Time-lapse observation of wild type MEFs immediately following stimulation with 50 ng/ml PDGF. The cells were viewed using differential interference microscopy. Circular ruffles appear as dark rings that begin at the cell edge and progress inwards. Images were acquired at 15 s intervals over a period of 15 min and are displayed at 3.1 frames/s. Scalebar: 50 μm

Video 2 Time-lapse observation of DGK ζ -null MEFs following stimulation with 50 ng/ml PDGF. The cells were viewed using differential interference microscopy. Images were acquired at 15 s intervals over a period of 15 min and are displayed at 3.1 frames/s. Scalebar: 50 μm .

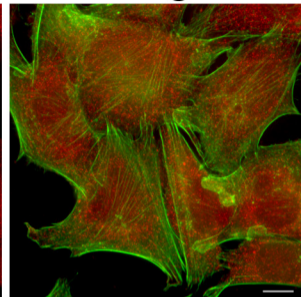
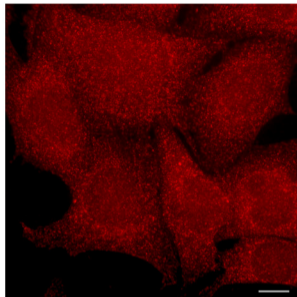
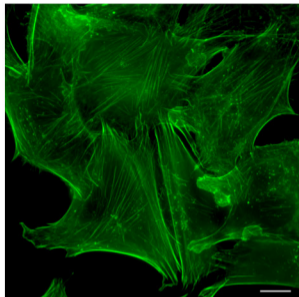


Actin

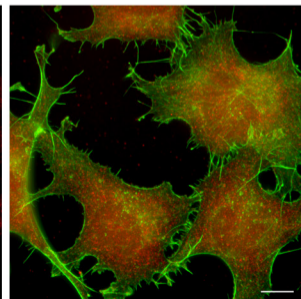
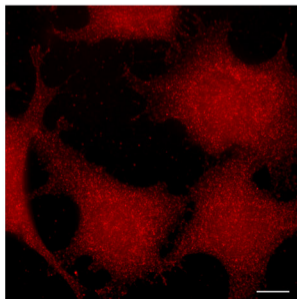
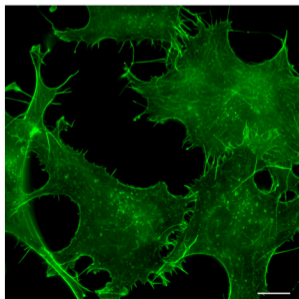
PAK1

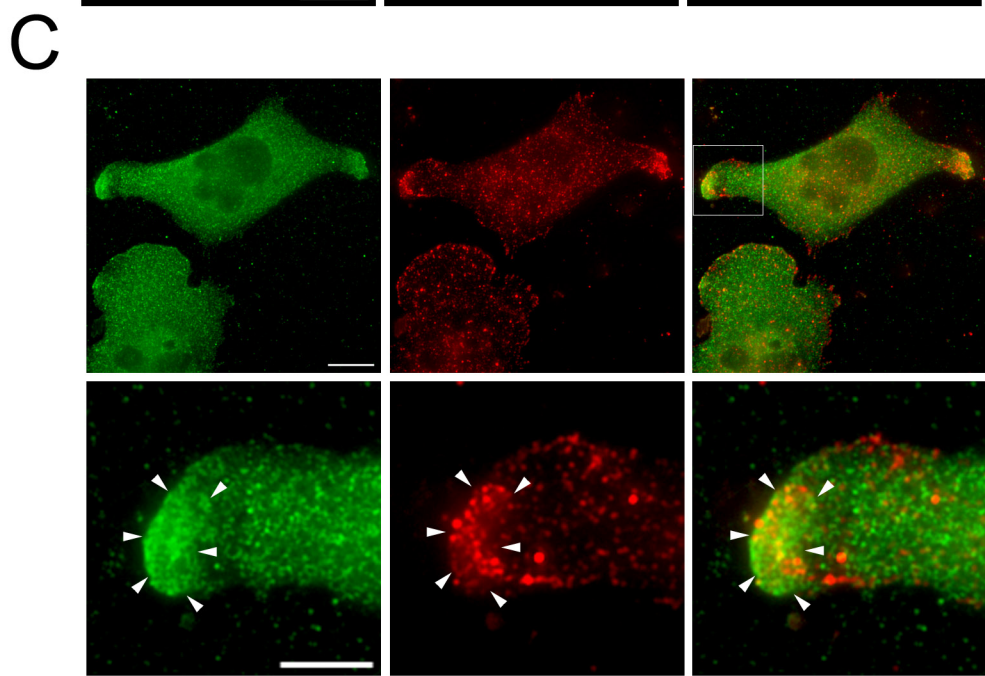
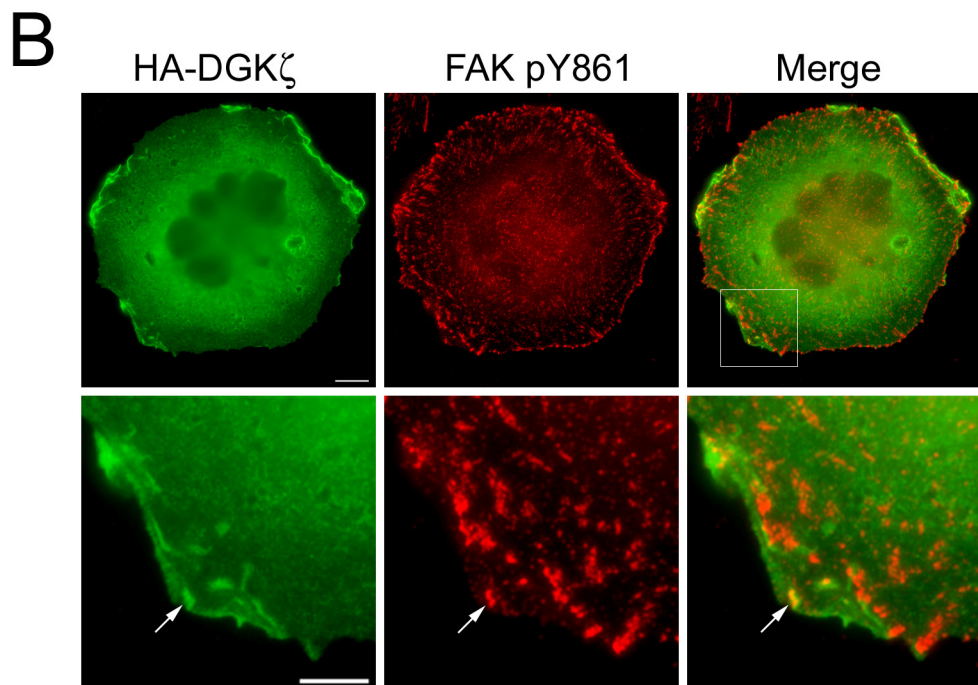
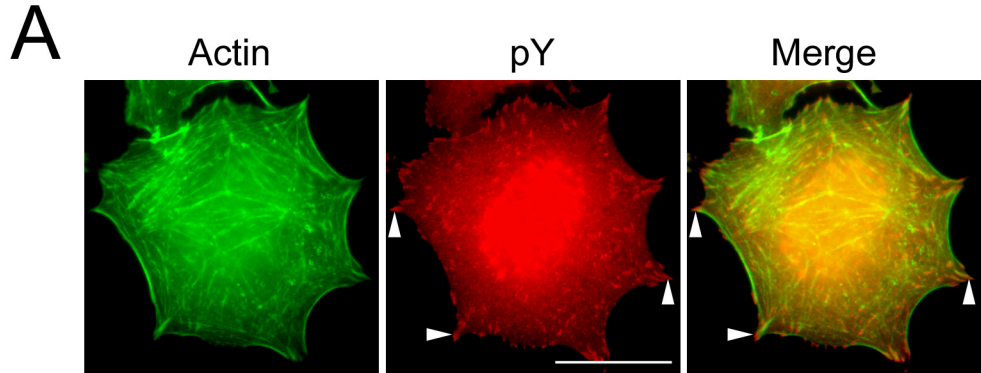
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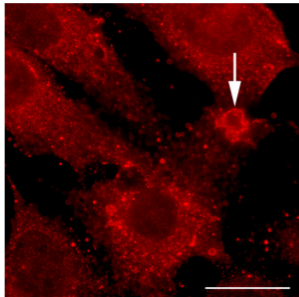


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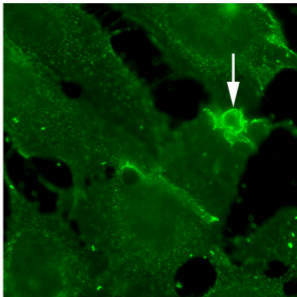




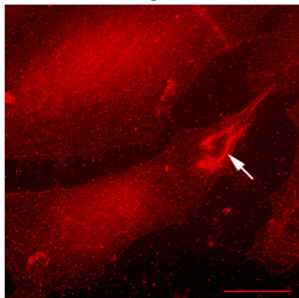
DGK ζ



Rac1



Syn



Actin

