Supplement Figures

Supplemental Figure 1. **PKL RNAi using a second oligonucleotide**. (A) Normal MEFs were transfected with mouse specific siRNA for PKL (siPKL #2). At 60 hours post-transfection, cells were lysed and subjected to Western immunoblotting. Cell lysates were blotted with PKL specific antibody and α-actinin as a loading control. (B) Effect of PKL RNAi #2 on cell migration in a wound-healing assay. Time-lapse images were captured every 10min for 6 hours. Images taken at 10min, 3h and 6h post wounding are shown. (C) Quantification of migration post wound healing. Migration of individual cells at the wound edge was determined by centroid tracking. Representative tracks of control RNAi versus PKL RNAi are shown. Cell migration velocity and directionality persistence were also calculated from six cells. Error bars represent S.E.M. from two independent experiments. (D) Modified Boyden chamber assays were performed on control versus PKL RNAi #2 cells to evaluate chemotaxis and chemokinesis. Migration index of control cells was set at 100%. n=1. (E) Kymograph analysis of membrane protrusion. Control and PKL RNAi #2 cells were plated for the scrape wound healing assay and images of cells at the wound edge were captured every minute for 1 hour. Kymograph analysis of membrane protrusion was processed using NIH ImageJ software. (F) Role of PKL in cell polarization at the wound edge. Four hours post wounding, cells were fixed and stained with a Golgi marker (anti-Giantin, red), nucleus (Hoechst 33342, blue) and F-actin (Alexa633-phalloidin, grey). Percentage of cells exhibiting reoriented Golgi into the 120° sector facing the wound was quantified. Random distribution of Golgi is predicted to be ~33% (red line). Error bars represent S.E.M. from two independent experiments. Scale bar, 10μm.

Supplemental Figure 2. **Quantification of PKL tyrosine-phosphorylation by Src and FAK in response to PDGF**. (A) MEFs expressing GFP-PKL WT were incubated in the absence and presence of Src inhibitor (10μM PP2) for 30min. Quantification of relative PKL tyrosine phosphorylation was calculated from three independent experiments. (B) GFP-PKL WT was expressed alone or with Src (WT, kinase dead K295R, or constitutively active Y527F) in $SYF^{-/-}$ MEFs and PKL phosphorylation was evaluated. Quantification of relative PKL tyrosine phosphorylation was calculated from three

independent experiments. (C) GFP-PKL WT was expressed alone or with FAK (WT, KD, Y397F, superFAK) in $FAK^{-/-}$ MEFs. PKL tyrosine phosphorylation was evaluated by blotting the GFP precipitates with 4G10. Quantification of relative PKL tyrosine phosphorylation was calculated from three independent experiments. Error bar represents S.E.M.

Supplemental Figure 3. **PKL tyrosine phosphorylation is required for polarized localization of βPIX to the leading edge**. Quantification of βPIX polarized localization to adhesions at the leading edge of migrating cells in parental, GFP-PKL or GFP-PKL 3YF transfected cells. The number of cells demonstrating paxillin localization to focal adhesions for each condition was also determined. Over thirty cells of each cell population from three independent experiments were counted. Error bar represents S.E.M. Student's t-test, **p<0.01.

Supplemental Movie 1. Control siRNA cell migration in the scrape wound assay. Control RNAi cells were plated for the scrape wound healing assay and images of cells at the wound edge were captured every minute for 1 hour.

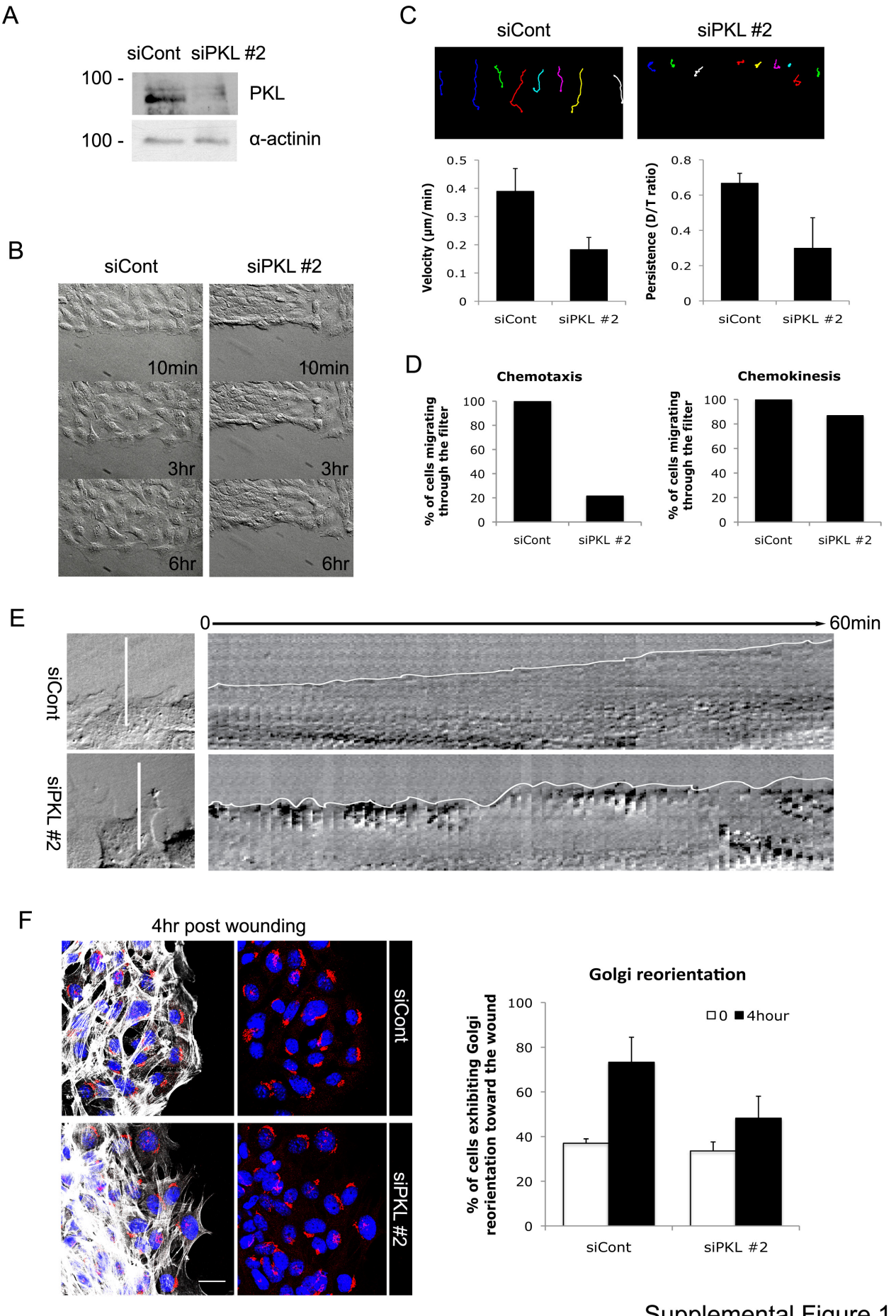
Supplemental Movie 2. PKL siRNA cell migration in the scrape wound assay. PKL RNAi cells were plated for the scrape wound healing assay and images of cells at the wound edge were captured every minute for 1 hour.

Supplemental Movie 3. GFP-PKL WT cell migration in the scrape wound assay. Cells were transiently transfected with GFP-PKL WT. Cells were plated for the scrape wound healing assay and images of GFP positive cells (marked with a color dot) at the wound edge were captured every 10 minutes for 6 hours.

Supplemental Movie 4. GFP-PKL 3YF cell migration in the scrape wound assay. Cells were transiently transfected with GFP-PKL 3YF. Cells were plated for the scrape wound healing assay and images of

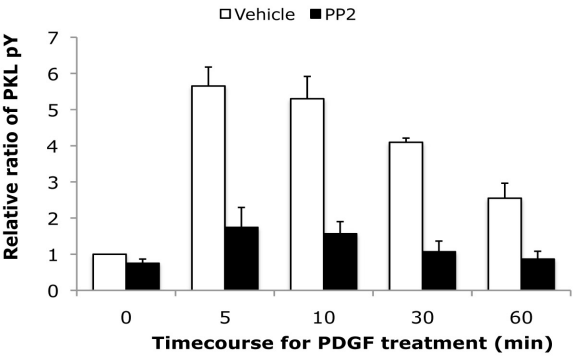
GFP positive cells (marked with a color dot) at the wound edge were captured every 10 minutes for 6 hours.

Supplemental Movie 5. PP2 treated GFP-PKL WT cell migration in the scrape wound assay. GFP-PKL WT transfected cells were treated with PP2 (10μM) for 30 minutes. Cells were scraped and images of GFP positive cells (marked with a color dot) at the wound edge were captured every 10 minutes for 6 hours.



Supplemental Figure 1

A



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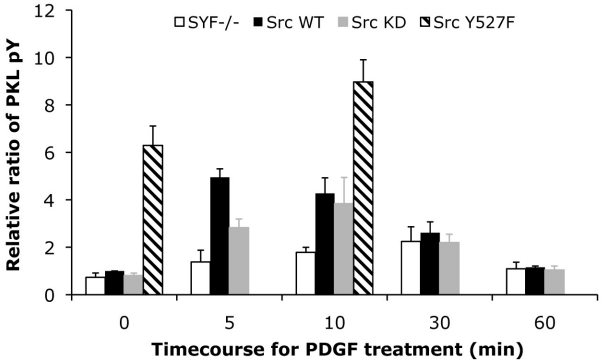

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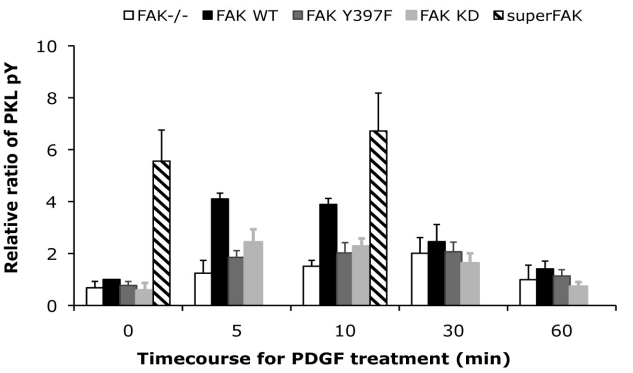
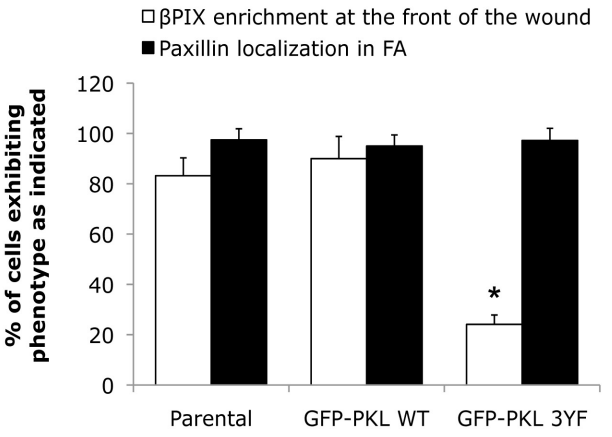


Supplemental Figure 2



Supplemental Figure 3