SUPPLEMENTAL DATA

PYK2 ACTIVATION TRIGGERS EGF RECEPTOR SIGNALING AND CELL MOTILITY AFTER WOUNDING SHEETS OF EPITHELIAL CELLS

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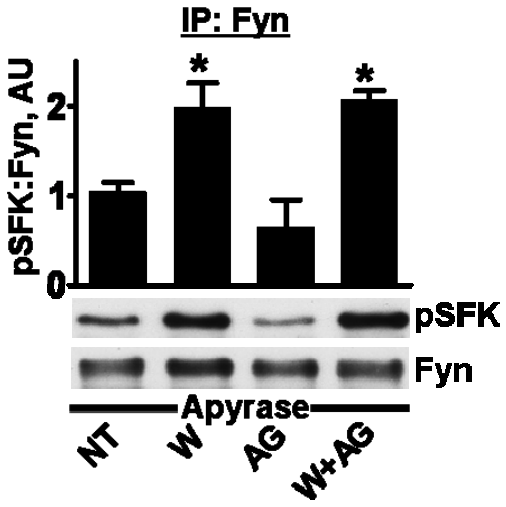
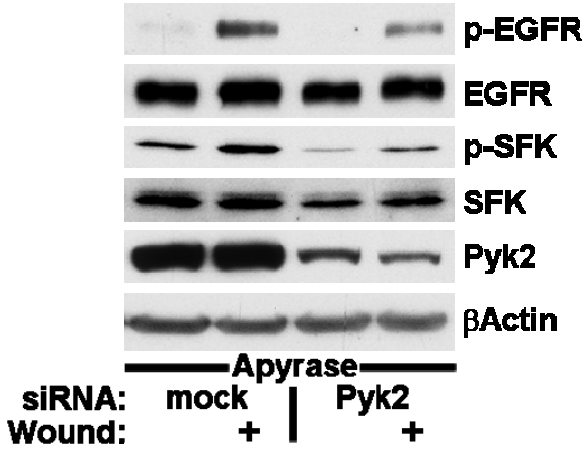
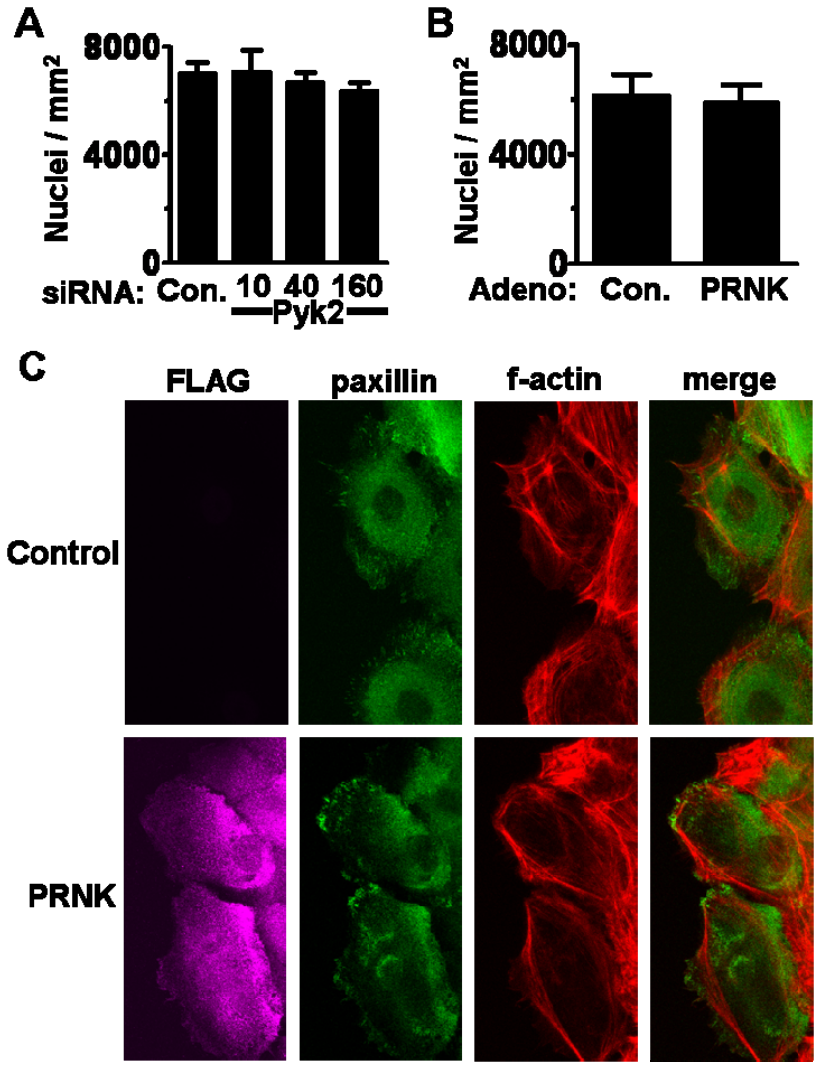


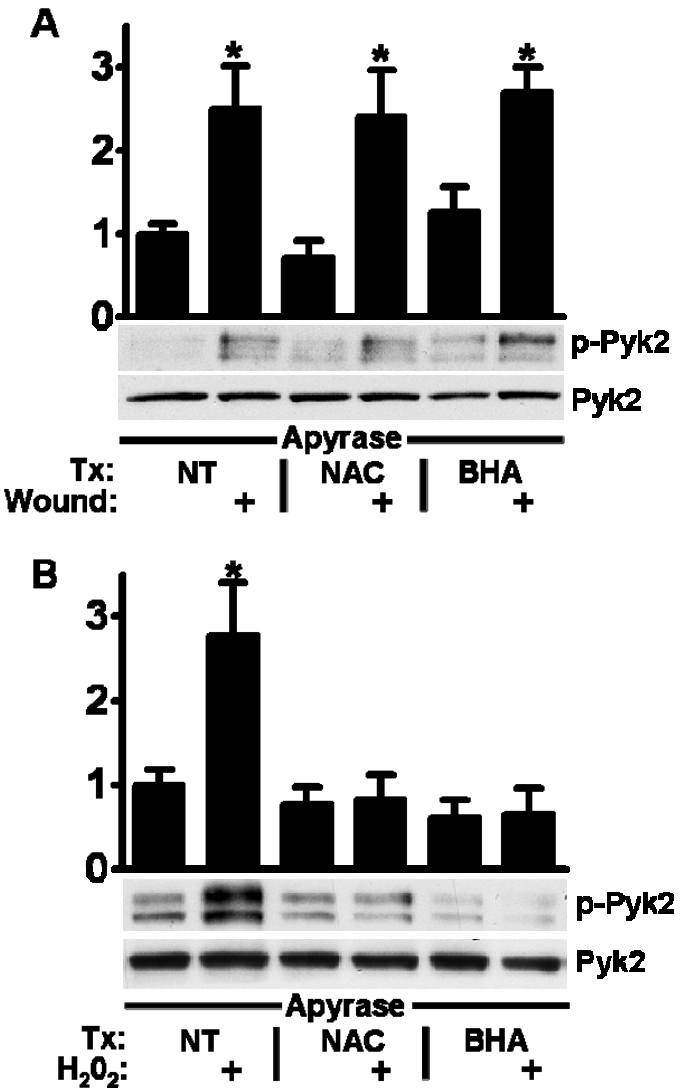
Supplemental Fig. 1 **The Fyn isoform is activated after wounding.** In the presence of 25 U/ml apyrase, cells received media change with no additional treatment (NT), or stimulation by wounding and culturing for 5 min (W). Where indicated, cells were treated with 100 nM of tyrphostin AG 1478 (AG) for 30 min prior to and during wounding. Lysates were subjected to immunoprecipitation with anti-c-Fyn antibodies that depleted about 90% of the c-Fyn in the extracts (not shown). The precipitates were subjected to Western Blotting for SFK phosphorylated on tyr-419 (p-SFK) followed by stripping and re-probing for total levels of Fyn (Fyn). The ratio of p-SFK:Fyn was determined by densitometry and * denotes significant differences from unwounded groups ($p<0.01$).



Supplemental Fig. 2 **Knockdown of Pyk2 with siRNA inhibits SFK and EGFR activation after wounding.** HCLE cells mock-transfected or transfected with a pool of Pyk2 siRNA oligonucleotides were wounded where indicated. All cells received 25 U/ml apyrase for the five minutes prior to lysis following medium change or wounding. Western Blots were probed for SFK phosphorylated on tyr-419 (p-SFK) and EGFR phosphorylated on tyr-1173 (p-EGFR) and were then stripped and re-probed for total levels of EGFR, SFK non-phosphorylated on tyr-419 (SFK), total levels of Pyk2, and for βActin.



Supplemental Fig. 3 **Pyk2 siRNA or dominant negative does not alter cell density or localization of paxillin.** **A**. HCLE cells were subjected to wound healing assays following transfection with 10, 40, or 160 nM of control or Pyk2 siRNA. Cells were fixed, stained with DAPI, and the average number of nuclei/mm^2 in ten random fields was determined. **B**. HCLE cells were subjected to wound healing assays following infection with control or PRNK-expressing adenovirus. Nuclei/mm^2 were determined as for **A**. **C**. HCLE cells were subjected to wound healing assays following infection with control or PRNK-expressing adenovirus. Cells were fixed, stained as indicated, and confocal immunofluorescence images were obtained with a 60X objective.



Supplemental Fig. 4 **Pyk2 activation after wounding occurs independently of signaling by reactive oxygen species.** HCLE cells were pre-treated for 30 minutes with 1 mM of N-acetyl cysteine (NAC) or butylated hydroxyanisole (BHA) and were (**A**) wounded or (**B**) treated with 41 μM H_2O_2 for five minutes as indicated. All cells received 25 U/ml apyrase for the five minutes prior to lysis following medium change or wounding. Western Blots were probed for Pyk2 phosphorylated on tyr-402 (p-Pyk2) and for total levels of Pyk2. Ratios were determined by densitometry and * denotes significant differences from controls ($p<0.001$).