Supplemental Data

Supplemental Figure 1. *Klf5* and *ILK* were each inhibited with a second siRNA. (A-C) A second *Klf5* siRNA hairpin with a core sequence of 5'-CCACAGGAGGTGAACAATA-3' was utilized for these experiments. The control siRNA contained point mutations of the targeting sequence, as indicated: 5'-CTACCGAAGTTGCACGATA-3' (mutated bases underlined). (D-F) A second *ILK* siRNA hairpin with a core sequence of 5'-CCGCTGGCAGGGCAATGAT-3' was utilized. Control siRNA contained the sequence 5'-CAGTTGTCACGGTAACGAT-3' (mutated bases underlined). (B, E) Migration assays were performed in Transwell plates as described in Experimental Procedures. Migrating cells (purple) were stained with 0.005% crystal violet. (C, F) Results were compiled from three individual experiments and expressed as mean±SEM. Student's *t*-test was used for statistical analyses.

Supplemental Figure 2. Induction of *Klf5* siRNA did not significantly increase apoptosis in primary esophageal keratinocytes. (A) TUNEL assays were performed using the *In Situ Cell Death Detection Kit* (Roche). Apoptotic cells were stained in red, with DAPI stain in blue. Magnification was 200x. (B) The number of positive cells as a percentage of total number of cells was determined in five 200x fields. By Student's *t*-test, there were no significant differences in the percentage of apoptotic cells when primary esophageal keratinocytes were infected with siRNA against *Klf5*, compared to those infected with mismatch controls. Results were expressed as mean±SEM.

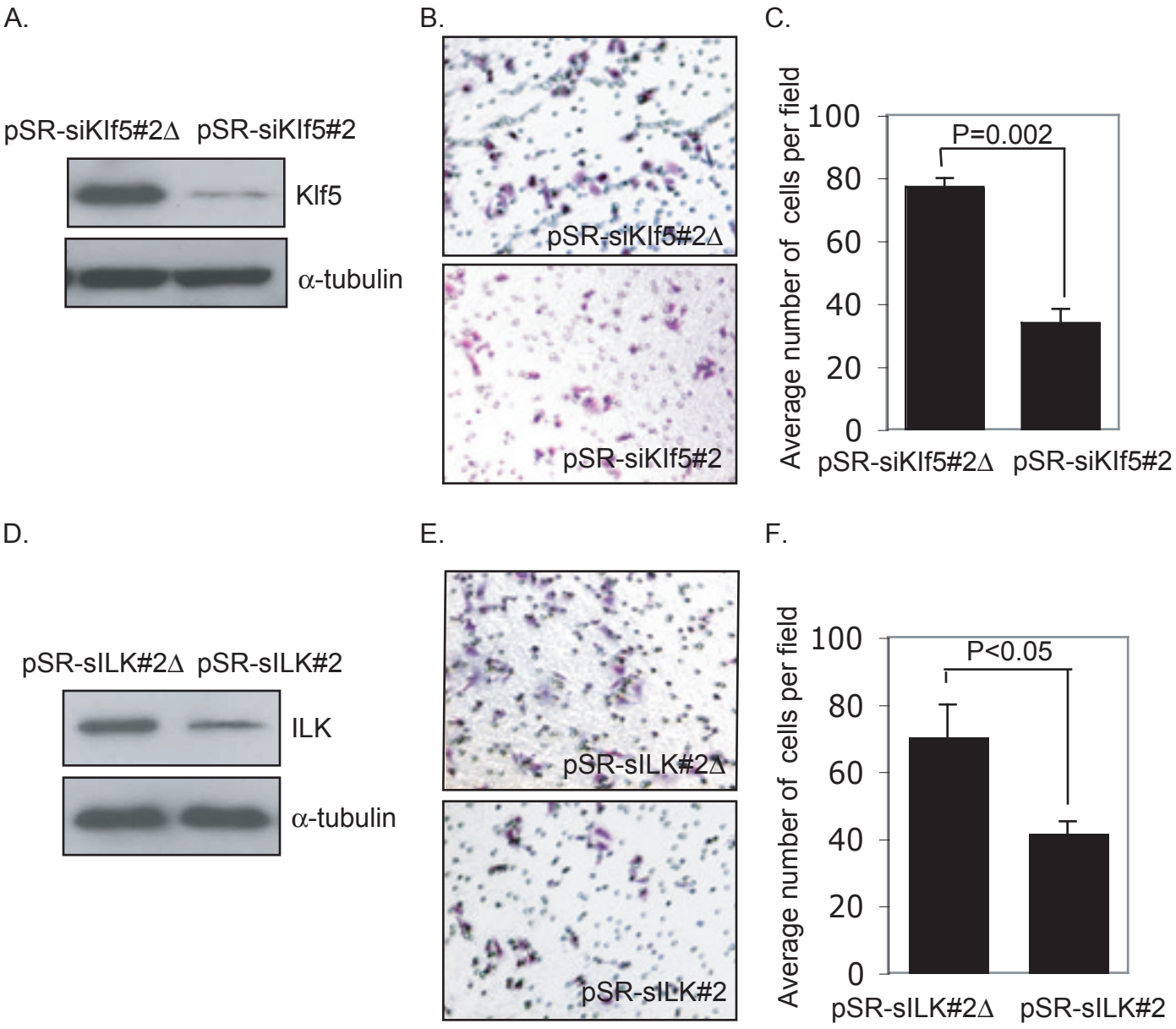
Supplemental Figure 3. Klf5 and ILK were both expressed in keratinocytes migrating across the wound in scratch assays. Wounds, indicated by diagonal lines, were generated using a plastic pipet tip when cells reached 90% confluence. Cells were grown for an additional 24 hours and then fixed with 4% paraformaldehyde in PBS for 10 minutes at room temperature and stained for Klf5 and ILK as described in Experimental Procedures.

Supplemental Figure 4. Inhibition of MEK/ERK signaling did not alter ILK expression or ILK kinase activity in keratinocytes with overexpression of *Klf5*. Cells with overexpression of Klf5 were treated for 24 hours with DMSO or with the MEK inhibitor PD98059 at 50μM in DMSO. (A) ILK expression, as determined by Western blots, and ILK kinase activity, assessed as described in Experimental Procedures, were not changed by treatment with PD98059. (B) ILK kinase assays (n=3) were quantified to confirm that inhibition of MEK/ERK signaling did not alter ILK kinase activity. Results were expressed as mean±SEM. Student's *t*-test was used for statistical analyses.

Supplemental Figure 5. Inhibition of *Cdc42* decreased cell migration. pFB-Klf5 infected keratinocytes were transduced with pGIPZ lentiviral vector (OpenBiosystem) containing siRNA targeted against the sequence 5'-CACAGCTGGACAAGAAGATTA-3' from *Cdc42* or a control sequence (5'-CATAGATGAACGAGCAGAGTA-3', mismatches underlined). (A-B) pGIPZ-siCdc42 decreased expression of Cdc42 as well as activated Cdc42 (Cdc42-GTP). (B) Inhibition of *Cdc42* decreased migration in *Klf5* overexpressing keratinocytes. Migration assays were performed in Transwell plates as described in Experimental Procedures. (C) Data from three

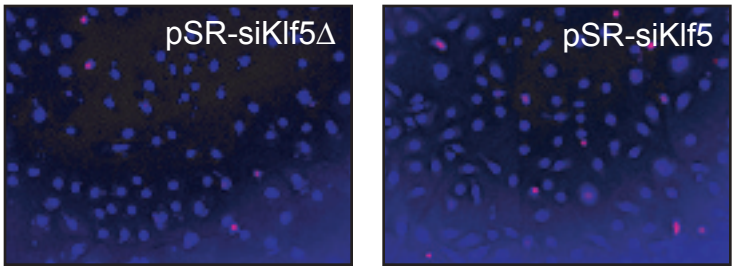
individual experiments (mean±SEM) confirmed that suppression of *Cdc42* decreased migration in *Klf5* overexpressing keratinocytes. Student's *t*-test was used for statistical analyses.

Supplementary Figure 1



Supplementary Figure 2

A.



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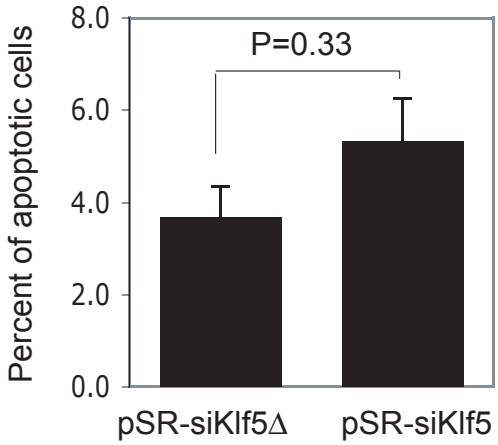
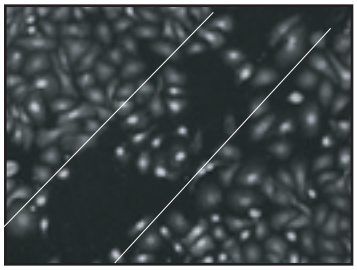
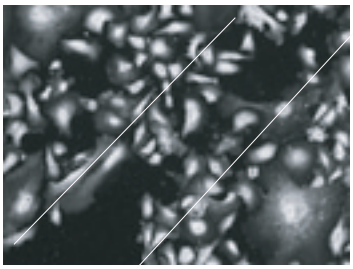


Supplementary Figure 3

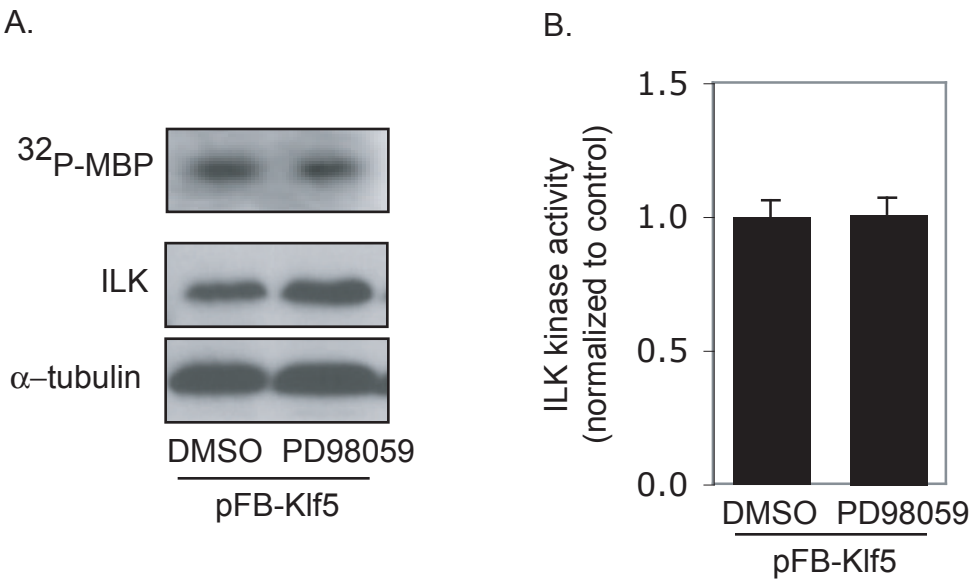


Klf5



ILK

Supplementary Figure 4



Supplementary Figure 5