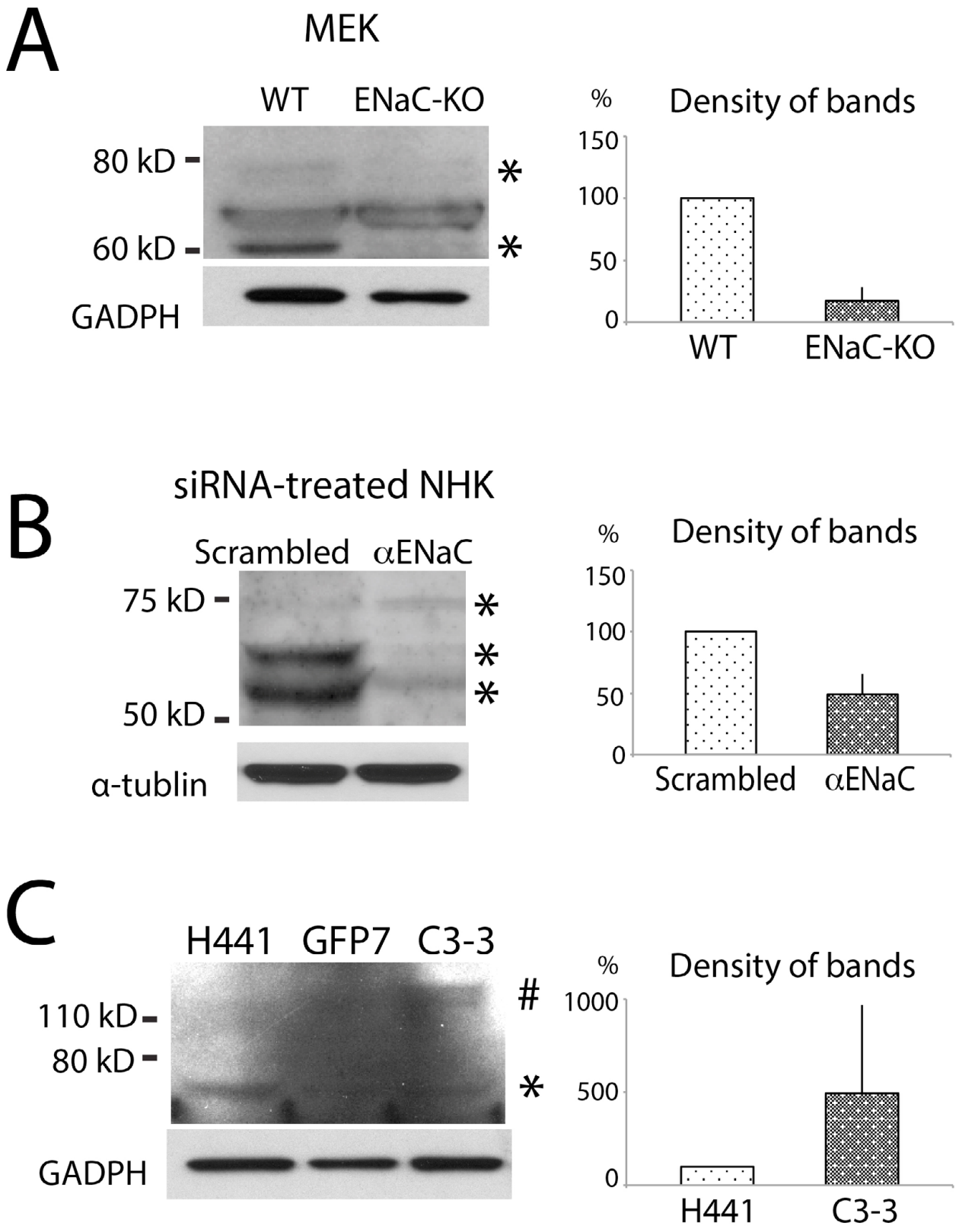


Fig. S1. Alpha-ENaC expressed in cells. Immunoblots of cell lysates for alpha-ENaC expression. (**A**) αENaC in MEK. Alpha-ENaC protein (labeled with *) is depleted in αENaC-KO cells (the density of the bands on the right panel). The middle bands about 65kDa appearing in both wild type and αENaC-KO cells are non-specific. (**B**) αENaC in siRNA-treated NHK. The densitometric analysis showed that the αENaC-siRNA treatment knocked down the protein level by 50% in NHK (right lane) when it is compared to the scrambled-siRNA treatment. (**C**) αENaC in H441, GFP7 and C3-3 cells. Endogenous αENaC is visualized as the lower molecular weight bands (labeled with *) present in the lysates of the H441, GFP7 and C3-3 cells, and a higher molecular weight band of the GFP-tagged-αENaC band (labeled with #) is expressed in the C3-3 cells. The total ENaC in C3-3 increased by 5-fold compare to the parental H441 cells (right panel). GAPDH and tubulin were used as loading controls.

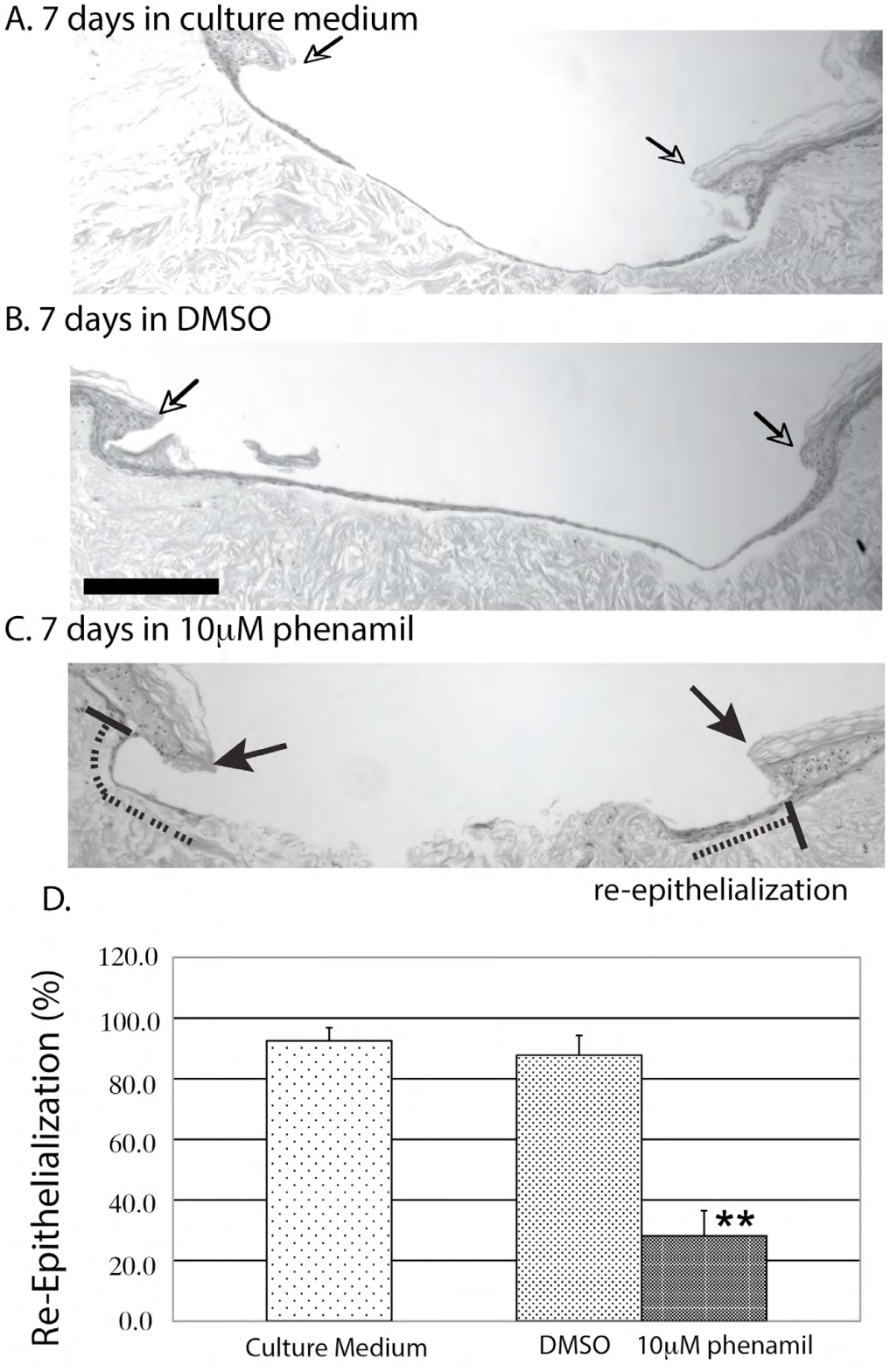
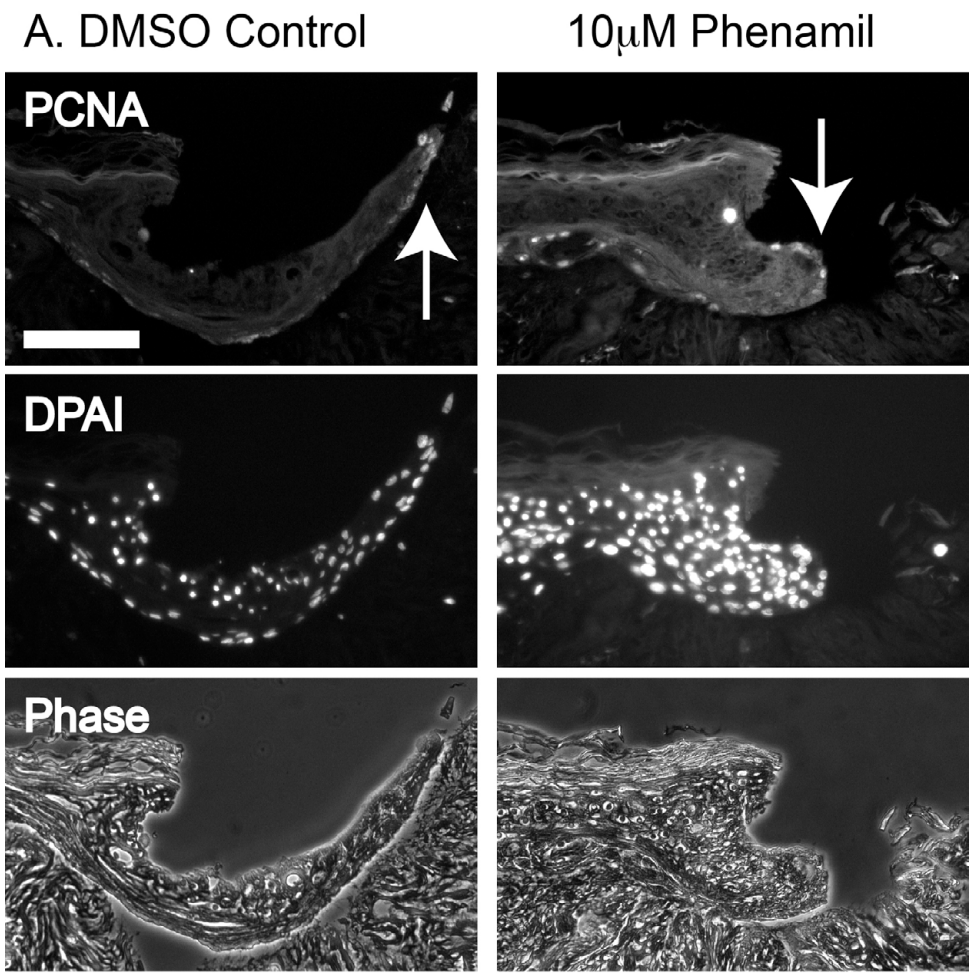


Fig. S2. Inhibiting ENaC blocks re-epithelialization of ex vivo skin wound. Normal adult human skin tissue obtained from elective plastic surgical abdominal reduction procedures, with a protocol approved by the UC Davis IRB, was trimmed to remove subdermal and adipose tissue. A 3 mm wide linear lesion was created on the epidermis and the wounded skin tissue was incubated in DMEM containing 10% FBS for 7 days. The tissue was fixed, embedded in paraffin for sectioning and staining with H&E. Wound images were taken, the length of the whole wound and re-epithelialized areas were measured to calculate the percentage of re-epithelialization. The arrows indicate the original wound edge. (**A**) In the control wound, re-epithelialization occurred and keratinocytes resurfaced the wound (the fine line of stained epithelium between 2 arrows). (**B**) In the DMSO solvent control wound, keratinocytes also migrated and resurfaced the wound. (**C**) In the presence of phenamil, re-epithelialization (the dotted line) was limited and keratinocytes migrate only a short distance into the wound. (**D**) Re-epithelialization of the *ex vivo* wound is blocked by phenamil by 70% on day 7 (P<0.05, tissue from 4 patients) when compared with the DMSO control treatment. Scale bar: 250 μm (panels A,B,C).



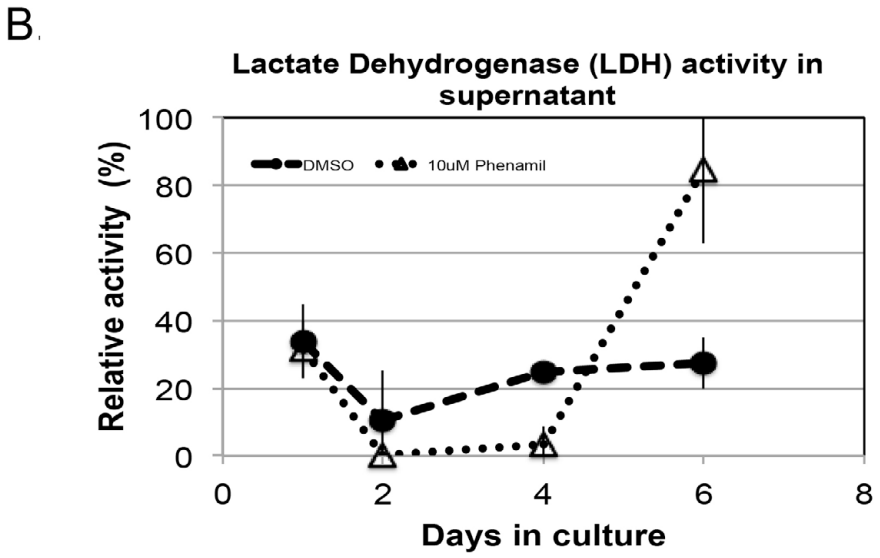


Fig. S3. Viability of keratinocytes in the *ex vivo* wound and in culture with phenamil. (**A**) Human wounded skin tissue that was treated with either DMSO or 10 μM phenamil, was paraffin-embedded and sectioned on day 7. Immunohistochemistry with anti-PCNA to label proliferating cells was performed. The migrating epithelial tongue was observed at the wound edge of the tissue. In both groups, PCNA stained keratinocyte nuclei at the leading edge of migrating epidermis (arrows), demonstrating that cells proliferated during the period of wound re-epithelialization, whether in control solvent DMSO or 10 μM phenamil. The nuclear staining is shown in the DAPI images and the wound edge is shown in the phase contrast images. Scale bar: 100 μm. (**B**) Using the activity of extracellular lactate dehydrogenase (LDH) as an indicator of cell damage, the viability of human cultured keratinocytes exposed to 10 μM phenamil in DMSO was compared to control cells exposed to the same concentration of DMSO alone. LDH levels are similar in treated and control cells through day 4 of exposure, and then rise on day 6. Thus, there is cytotoxicity to the cultured keratinocytes from the prolonged phenamil exposure at this dose, that may be limiting migratory ability of the cells in the *ex vivo* model at later times of incubation.

Movie 1. Galvanotaxis of mouse keratinocytes. MEK were exposed to an EF of 100 mV/mm. Cell migration was filmed for 60 min by time-lapse capture of images using phase contrast microscopy. The direction of the EF is from the bottom (anode) to the top (cathode) of the frames and galvanotaxic keratinocytes migrated directionally to the cathode. Track lines and direction of cell migration are shown in the end of the movie. Scale bar: 100 μm.

Movie 2. Galvanotaxis of αENaC-KO-MEK. Alpha-ENaC-KO-MEKs were exposed to an EF of 100 mV/mm. Cell migration was filmed for 60 min by time-lapse capture of images using phase contrast microscopy. The direction of the EF is from the bottom (anode) to the top (cathode) of the frames and keratinocytes lost the directionality and migrated randomly. Track lines and direction of cell migration are shown in the end of the movie. Scale bar: 100 μm.

Movie 3. Galvanotaxis of human keratinocytes with scrambled siRNA treatment. Scrambled siRNA treated NHKs were exposed to an EF of 100 mV/mm. Cell migration was filmed for 60 min by Hoffman modulation contrast microscopy. The direction of the EF is from the bottom (anode) to the top (cathode) of the frames and galvanotaxic keratinocytes migrated directionally to the cathode. Track lines and direction of cell migration are shown in the end of the movie. Scale bar: 100 μm.

Movie 4. Galvanotaxis of human keratinocytes with αENaC siRNA treatment. Alpha-ENaC siRNA treated NHKs were exposed to an EF of 100 mV/mm. Cell migration was filmed for 60 min by Hoffman modulation contrast microscopy. The direction of the EF is from the bottom (anode) to the top (cathode) of the frames and keratinocytes lost the directionality and migrated randomly. Track lines and direction of cell migration are shown in the end of the movie. Scale bar: 100 μm.