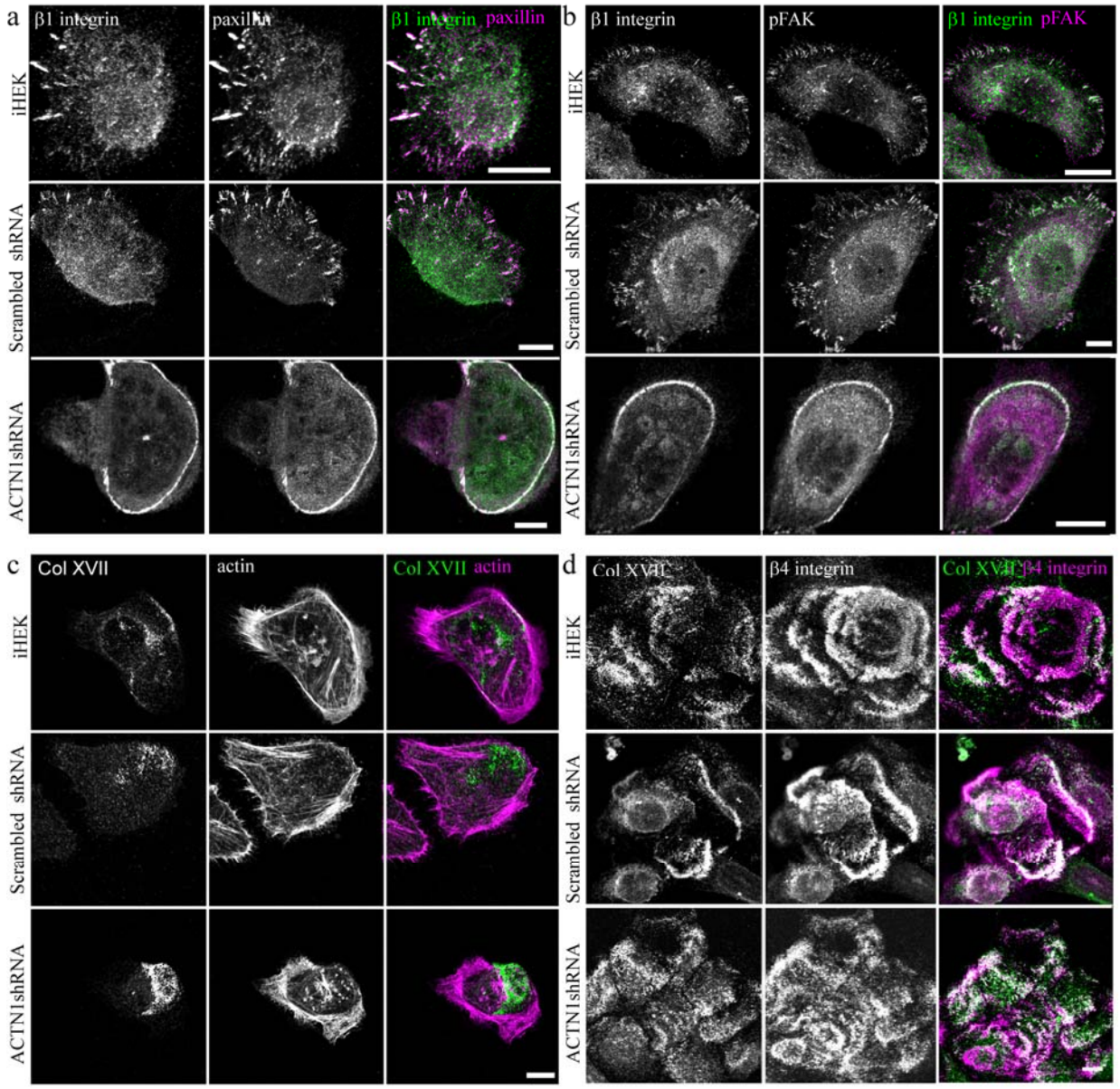
Supplemental Figure 1. Hemidesmosomal and focal adhesion protein localization in ACTN1-knockdown cells is perturbed. iHEKs, iHEKs expressing scrambled shRNA and iHEKs expressing ACTN1 shRNA were prepared for immunofluorescence staining with antibodies against β1 integrin and paxillin (**a**), β1 integrin and pFAK (**b**) or collagen XVII together with rhodamine conjugated

phalloidin (**c**) or β4 integrin (**d**), as indicated. Panels on right show overlays of the two images. Bars, 10 μm.

Supplemental Figure 2. Motility of and focal adhesions in ACTN1 knockdown cells maintained on diverse substrates. (**a**) The processivity (linear distance migrated/total distance migrated) of iHEKs, iHEKs expressing scrambled shRNA and the three ACTN1 knockdown clones (ACTN1shRNA-A, -B and -C) is depicted graphically. The cells were viewed over a 1 hour period, 18-24 hours after plating onto uncoated substrates. * Denotes significant differences from iHEK and scambled shRNA expressing cells, $p<0.05$. (**b**) Scrambled shRNA or ACTN1shRNA expressing cells were plated on FN or LM332 coated dishes for 6h, then processed for indirect immunofluorescence microscopy with antibodies against paxillin and with rhodamine conjugated phallodin. (**c**) Quantification of paxillin staining shown in **b** (above) and Supplementary Figure 1a. Staining was assessed either as arc-like or focal adhesion-like. (**d**) The processivity of iHEKs, iHEKs expressing ACTN1 shRNA and the latter cells induced to express GFP-tagged ACTN1 mRNA refractory to ACTN1 shRNA are presented graphically. The cells were plated onto iHEK matrix and allowed to adhere for 6-10 hours prior to being assayed for 1 hour. The results of quantification of GFP-positive cells only are shown for the 'rescued' cells (ACTN1shRNA + ACTN1GFP). * Denotes significant differences from both iHEK and ACTN1 shRNA cells induced to express the GFP-tagged ACTN1 mRNA, $p<0.05$.

Supplemental Figure 3. The impact of ACTN1 knockdown on primary human keratinocytes (pHEK). (**a**) Untreated pHEK or pHEK infected with either virus encoding ACTN1 shRNA or virus encoding scrambled shRNA were viewed by phase contrast microscopy and the cells scored based on the number of lamellipodial protrusions projected and plotted as percentage of the population displaying 0, 1, 2, or 3+ lamellipodia. (**b,c**) Confluent monolayers of untreated pHEK or pHEK infected either with virus encoding ACTN1 shRNA or with virus encoding a scrambled shRNA were scratch wounded and then observed 6 hours after wounding. In **b**, scratch width relative to the initial width is plotted. In **a** and **b**, * denotes significant difference from both pHEK and pHEK+scrambled shRNA, $p<0.05$. Bar in **c**, 10 μm.



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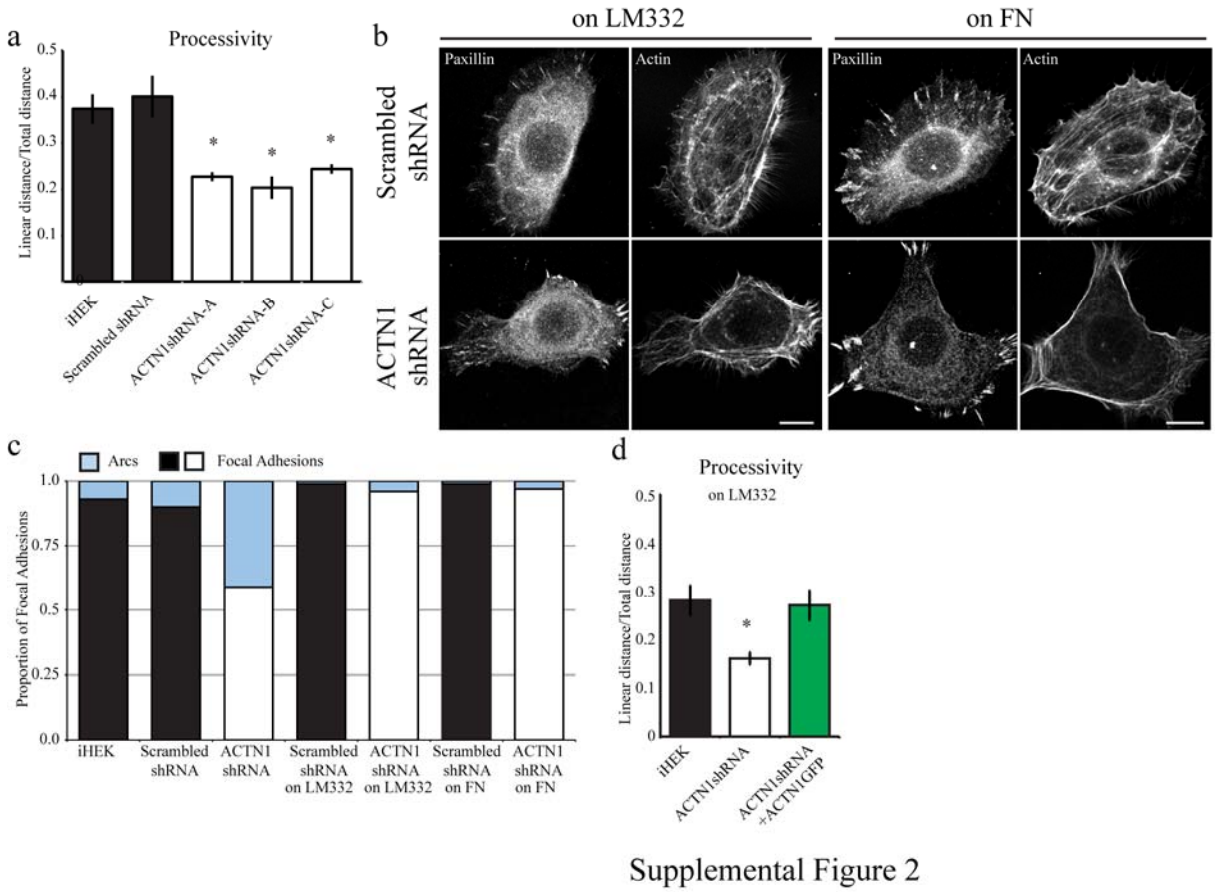

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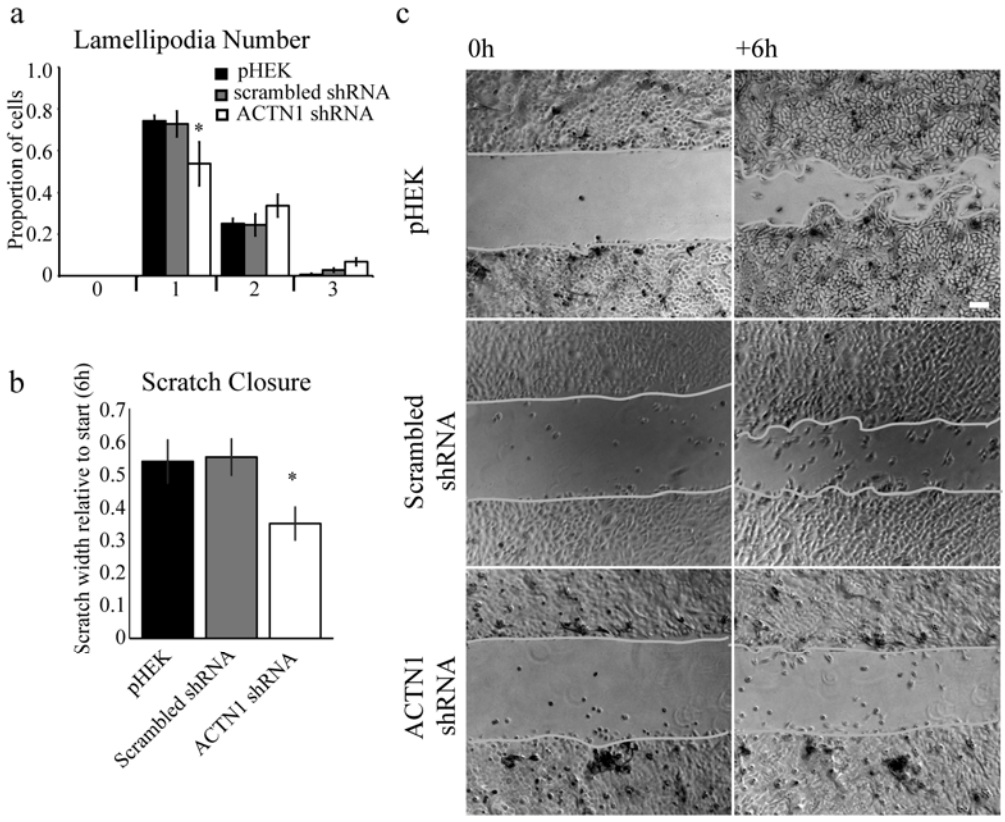

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