



**Figure 5 - supplement 2. RHOV Regulates Epidermal Cell Adhesion and Junction Dynamics.** (A) Formation of adherens junctions is delayed in the absence of RHOV. Primary mouse keratinocytes transfected with shRNAs were cultured in low-Ca<sup>2+</sup> (growth) media, which prevents adherens junction formation. Cultures were switched to high-Ca<sup>2+</sup> (differentiation) media to induce adherens junction formation. Cultures were immunolabeled for E-CADHERIN. DAPI denotes nuclei. Note that without RHOV, the active formation of adherens junction, which requires associated actin dynamics (Vasioukhin et al., 2000), is impaired. At later times, passive adherens junction formation not requiring actin dynamics still occurs. (B-D) RHOV regulates focal adhesion dynamics. Cultured primary keratinocytes transfected with shRNAs were plated for 24 hr on fibronectin before being processed for immunofluorescence microscopy. (B) Representative immunofluorescence images for each condition from n=3 experiments. Note that F-ACTIN stress fibers, stained by phalloidin, are accentuated and focal adhesions are enlarged when RHOV is depleted. Both are indicative of a reduction in focal adhesion dynamics. (C) Quantifications of focal adhesion areas as measured by VINCULIN immunolabeling. Error bars shows SEM from *shScr* n=2196, *shRhou-504* n=2046 and *shRhou-505* n=1792 focal adhesions from 3 independent experiments. Parametric, unpaired independent two-tailed t-test. (D) Quantifications of focal adhesion lengths as measured by VINCULIN immunolabeling. Error bars show SEM from *shScr* n=2196 *shRhou-504* n=2046 and *shRhou-505* n=1792 focal adhesions from 3 independent experiments. Parametric unpaired independent two-tailed t-test.