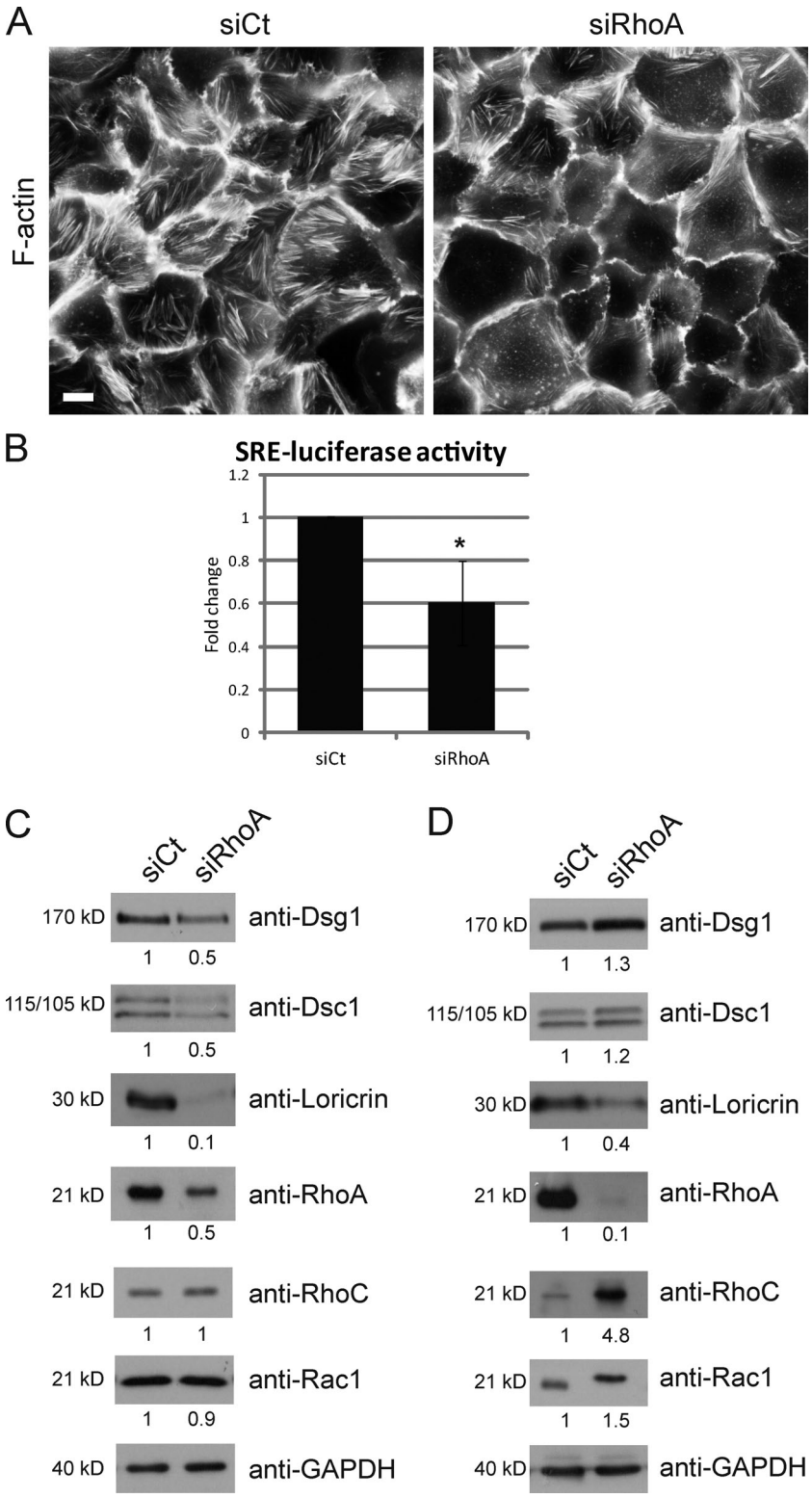
Dubash et al., http://www.jcb.org/cgi/content/full/jcb.201304133/DC1

Figure S1. **RhoA regulates stress fibers, SRF activity, and differentiation in epidermal keratinocytes.** (A) Control and RhoA KD NHEKs were grown on coverslips, subjected to calcium switch for 4 h, fixed, and processed for immunofluorescence to visualize F-actin. Bar, 20 µm. Images shown are representative of three independent experiments. (B) Control and RhoA KD NHEKs coexpressing an SRE-luciferase construct were induced to differentiate for 2 d, and luciferase activity measured using a standard reporter assay. Error bars represent SD. *, $P < 0.05$. (C and D) Control and RhoA KD NHEKs were induced to differentiate for 2 d, lysed, and samples blotted for Dsg1, Dsc1, loricrin, RhoA, RhoC, Rac1, and GAPDH. Western blots shown are representative of three independent experiments. Fold-change values over control are quantified by densitometry and noted below blots.



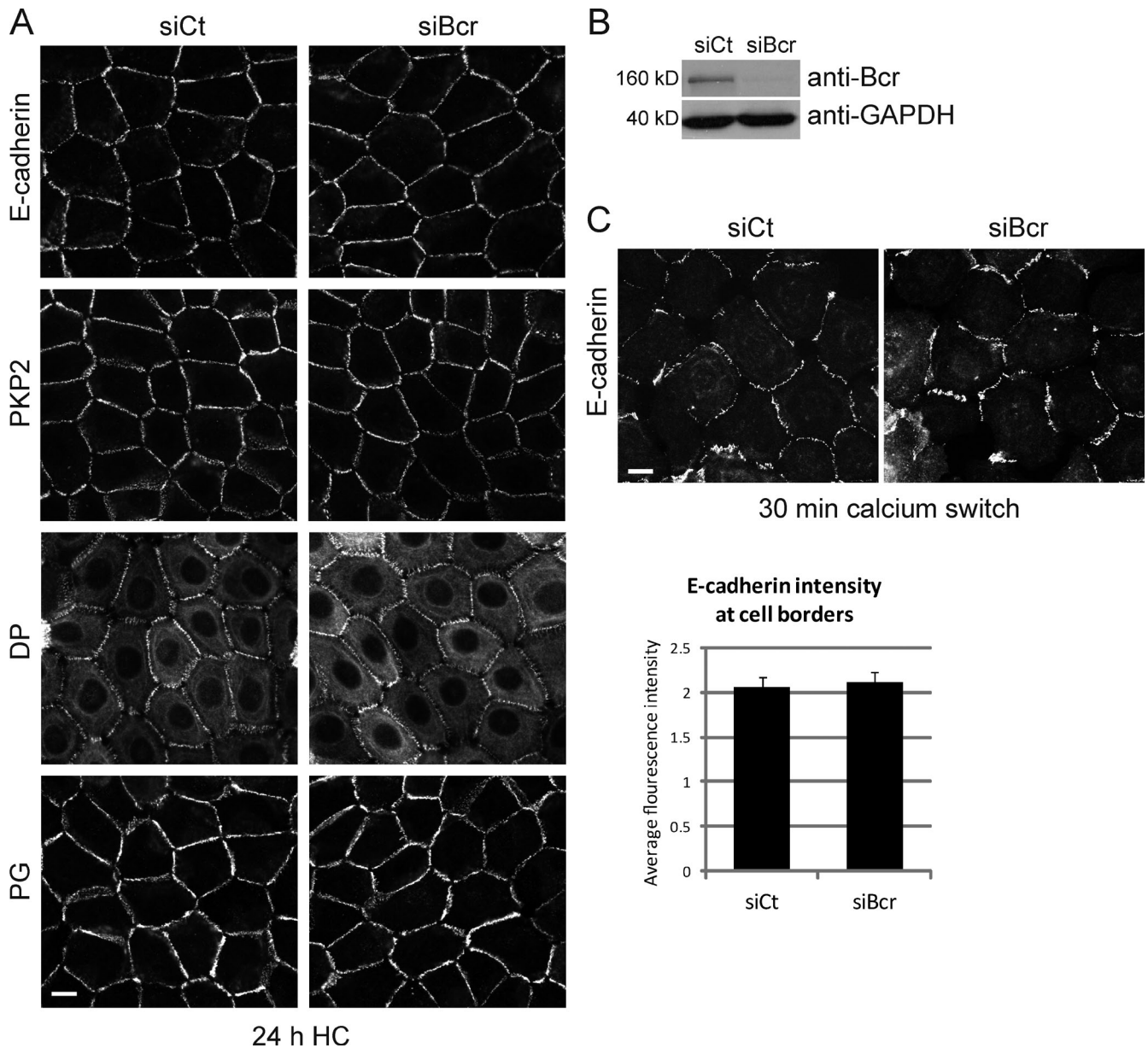


Figure S2. **Loss of Bcr does not affect junctional localization of adhesion proteins.** (A) Control and Bcr KD NHEKs were grown on coverslips, subjected to high calcium media for 24 h, fixed, and processed for immunofluorescence to visualize E-cadherin, PKP2, DP, and PG. Bar, 20 µm. (B) Lysates were prepared from duplicate samples of control and Bcr KD NHEKs, and Western blots performed for Bcr and GAPDH to indicate efficiency of KD. (C) Assembly of cell–cell junctions was induced in control and Bcr KD NHEKs by addition of high calcium media for 30 min, after which samples were fixed and processed for immunofluorescence to visualize E-cadherin. Bar, 20 µm. *, $P < 0.05$. Loss of Bcr does not affect the assembly of E-cadherin in nascent cell–cell junctions.

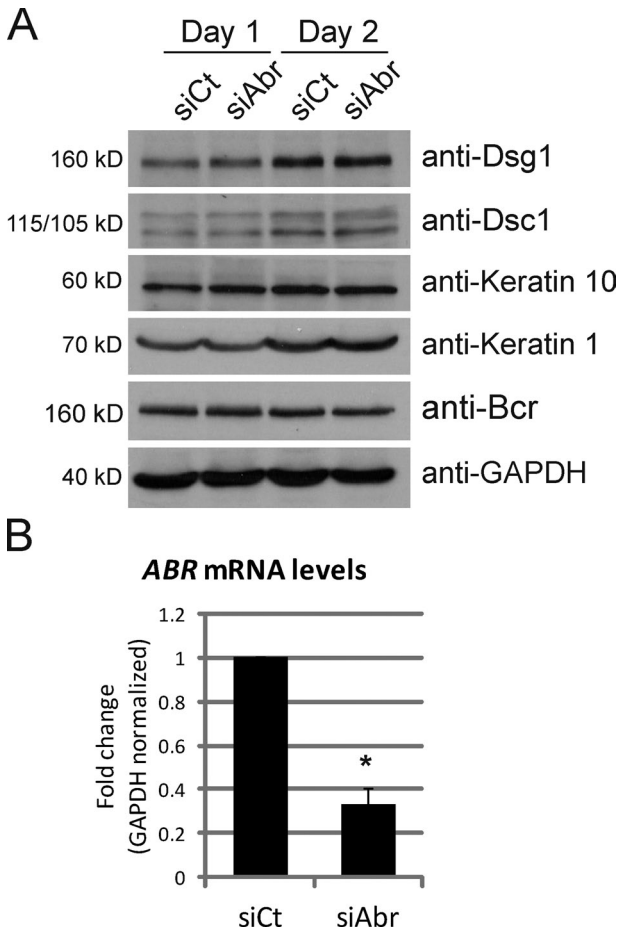


Figure S3. **Abr KD does not affect keratinocyte differentiation.** (A) Control and Abr KD NHEKs were induced to differentiate for 1–2 d, and samples blotted for Dsg1, Dsc1, keratin 10, keratin 1, Bcr, and GAPDH. (B) KD of Abr was confirmed via qPCR. Data from three independent samples are normalized to GAPDH and represented as fold-change over control. Error bars represent SD. *, $P < 0.05$.

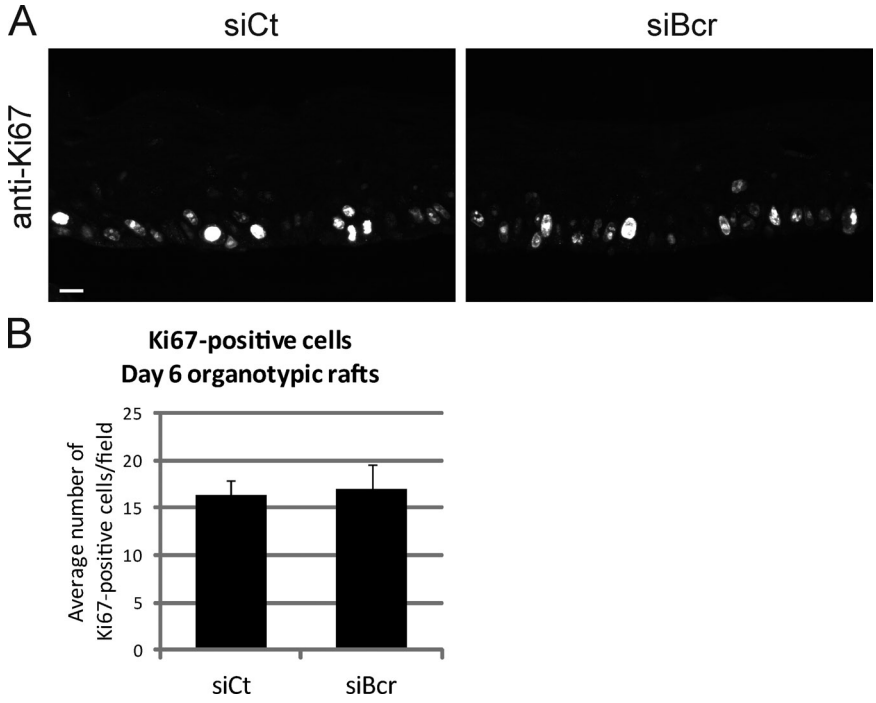


Figure S4. **Bcr KD does not affect proliferation of keratinocytes.** (A) Sections from control and Bcr KD organotypic rafts were stained with an anti-Ki67 antibody. Bar, 20 µm. (B) The average number of Ki67-positive cells per field was counted from three independent rafts and represented in the bar graph.

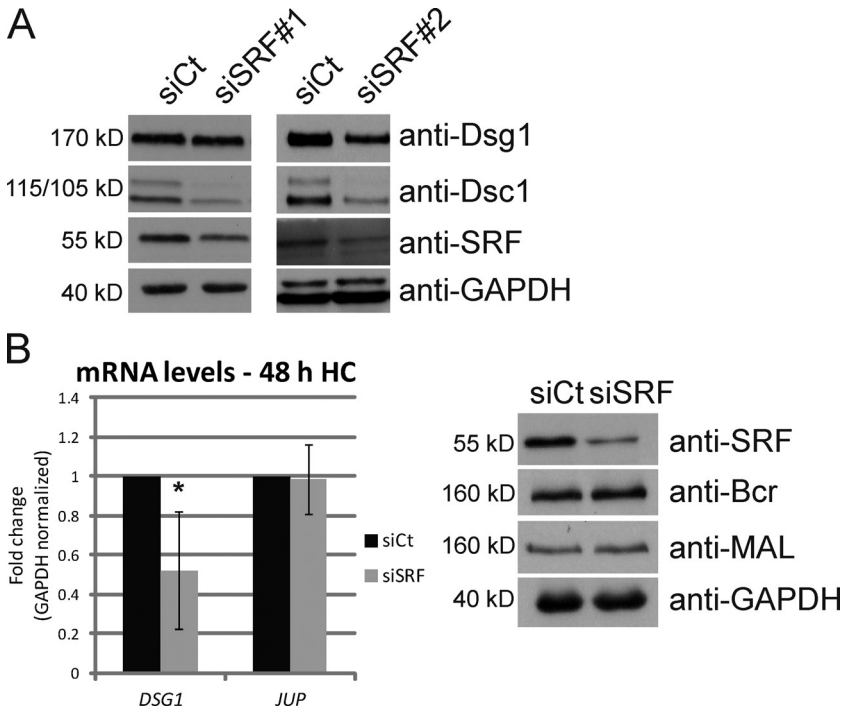


Figure S5. **KD of SRF inhibits differentiation in 2D submerged model.** (A) KD of SRF was achieved using two different oligonucleotides (siSRF#1 and siSRF#2). Samples from day 2 differentiating keratinocytes were blotted for Dsg1, Dsc1, SRF, and GAPDH. All blots shown are representative of three independent experiments. (B) qPCR analysis for *DSG1* and *JUP* mRNA levels from three independent experiments are normalized to GAPDH and represented as fold-change over control. Error bars represent SD. *, $P < 0.05$. Duplicate samples prepared from a representative experiment were lysed and Western blots performed to analyze protein levels of SRF, Bcr, MAL, and GAPDH.