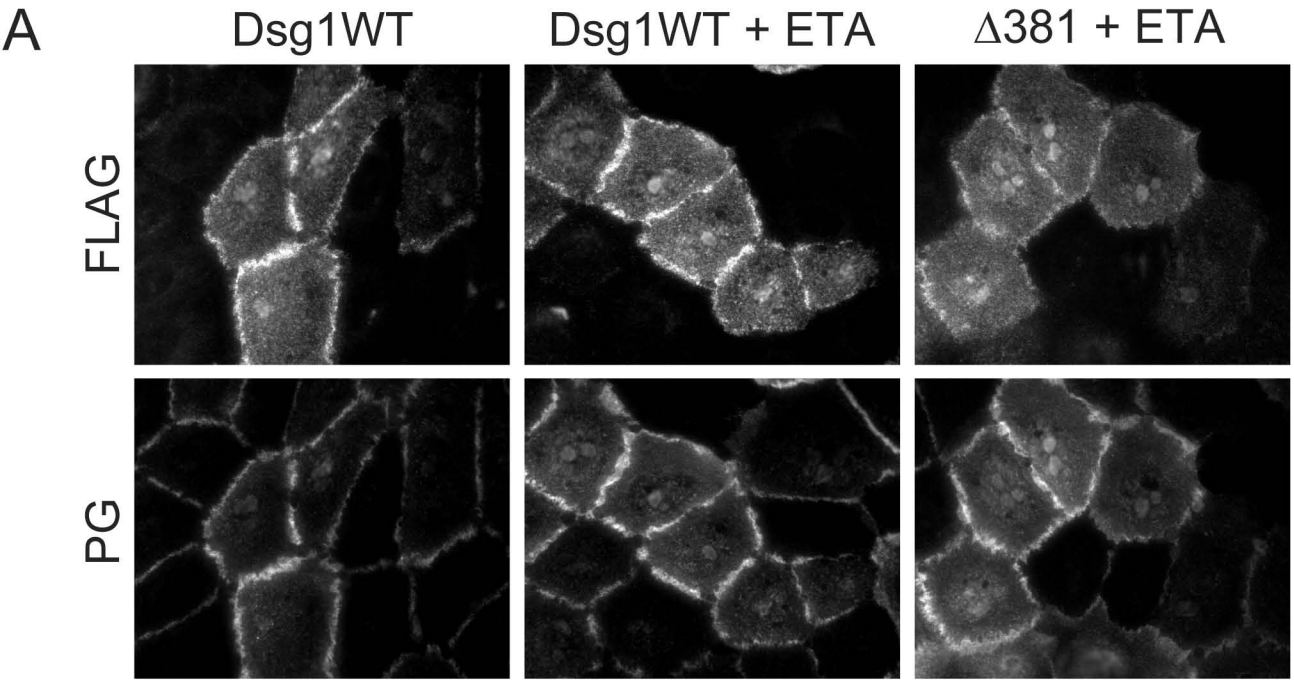
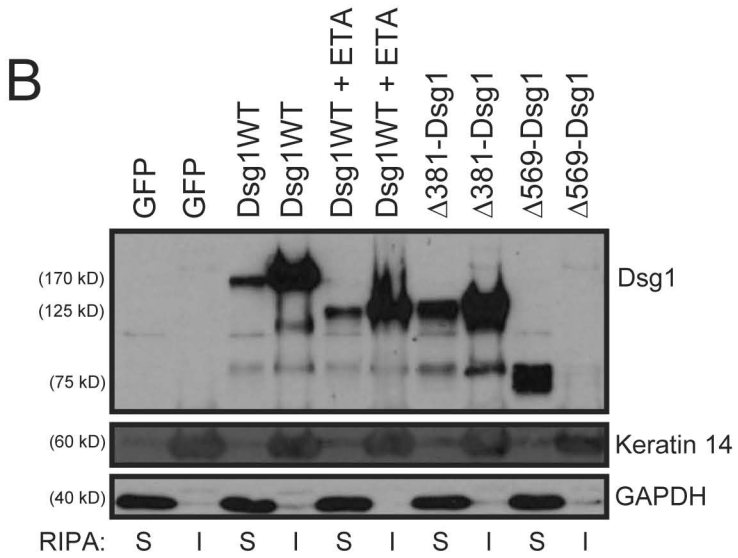
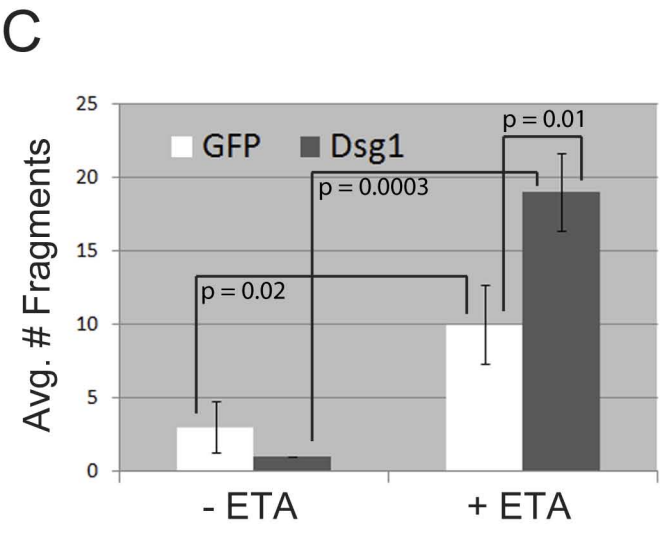
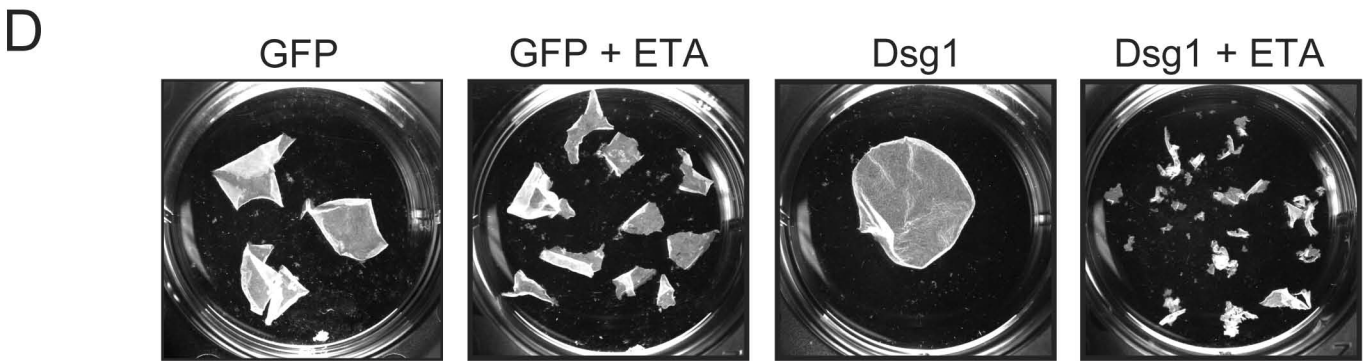
SUPPLEMENTARY FIGURE S1









SUPPLEMENTARY FIGURE S1: Δ381-Dsg1 and toxin-truncated Dsg1 exhibit similar localization, solubility, and effects on intercellular adhesion. To compare the characteristics of toxin-cleaved Dsg1 to the pre-truncated cadherin, NHEKs were transduced with Flag-tagged full-length Dsg1 (WT) or Δ381-Dsg1, then treated with ETA at 1 μM for 24 h in 1.2 mM calcium. (A) Immunofluorescence imaging was used to compare localization of Dsg1. Full-length Dsg1 (WT), toxin-cleaved Dsg1 (WT + ETA), and ectodomain-truncated Dsg1 (Δ381-Dsg1 + ETA) were all enriched at intercellular borders and were capable of recruiting PG to these areas of intercellular adhesion. (B) The solubility of Dsg1 was assessed by fractionation of transduced keratinocytes into RIPA-soluble (S, GAPDH-enriched) and RIPA-insoluble (I, Keratin 14-enriched) pools. WB of NHEK fractions showed that full-length Dsg1 (WT), toxin-cleaved Dsg1 (WT + ETA), and ectodomain-truncated Dsg1 (Δ381-Dsg1) all exhibited similar solubility, being highly concentrated in the RIPA-insoluble fraction, while the non-membrane-bound Δ569-Dsg1 construct was completely soluble in RIPA. (C) NHEKs were transduced with GFP or Dsg1, seeded as monolayers in 1.2 mM calcium for 24 h, treated with ETA at 1 μg/ml for an additional 24 h, then subjected to mechanical stress. Triplicate monolayers were fragmented and counted to show that while ectopic, full-length Dsg1 tends to promote adhesion, treatment with ETA converts the cadherin into a dominant-negative construct that greatly increases fragmentation compared to ETA-treated GFP control monolayers. Student's T-test p-values are shown. (D) Fragmented monolayers show that while ETA treatment increases the fragmentation of GFP-transduced monolayers, an even greater loss of adhesion is seen with toxin-cleaved ectopic Dsg1, which induces fragmentation similar to Δ381-Dsg1.