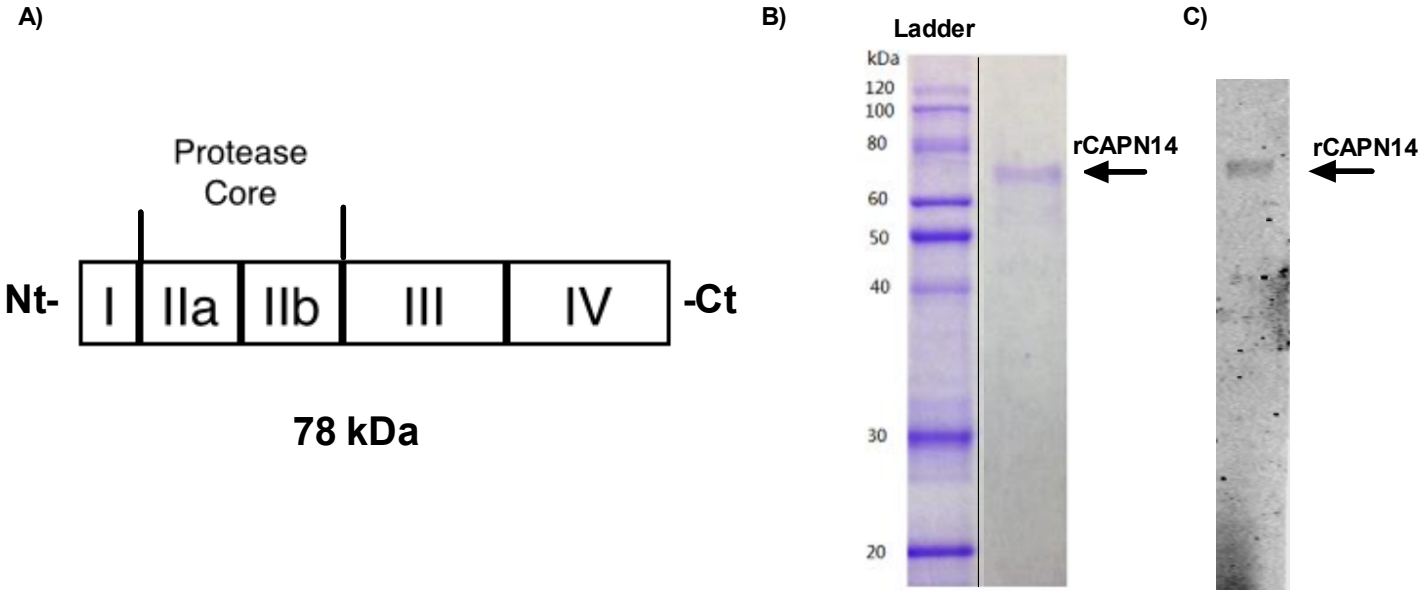
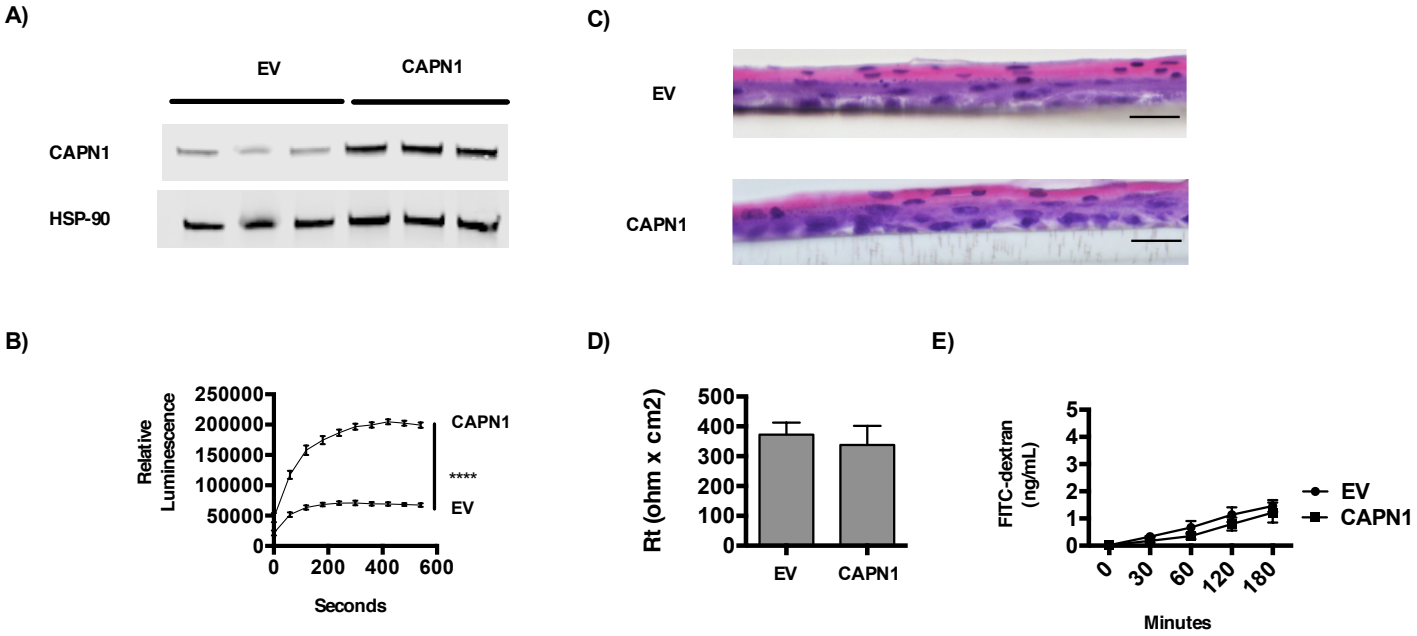
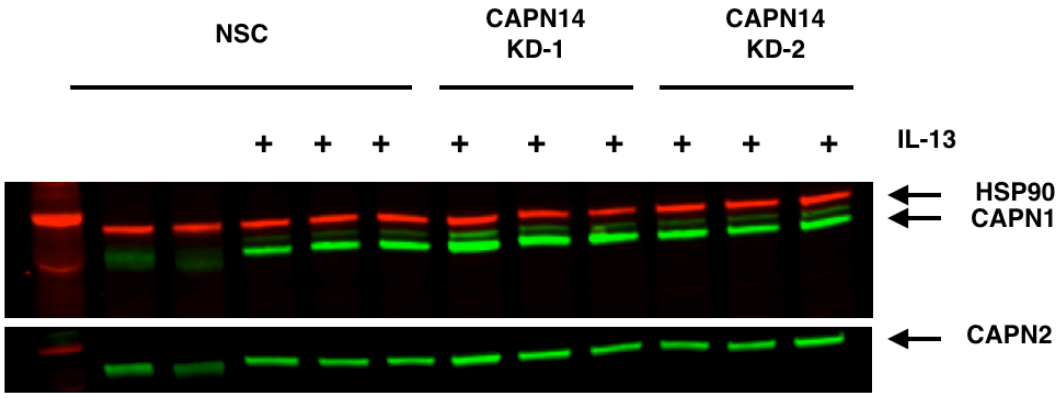


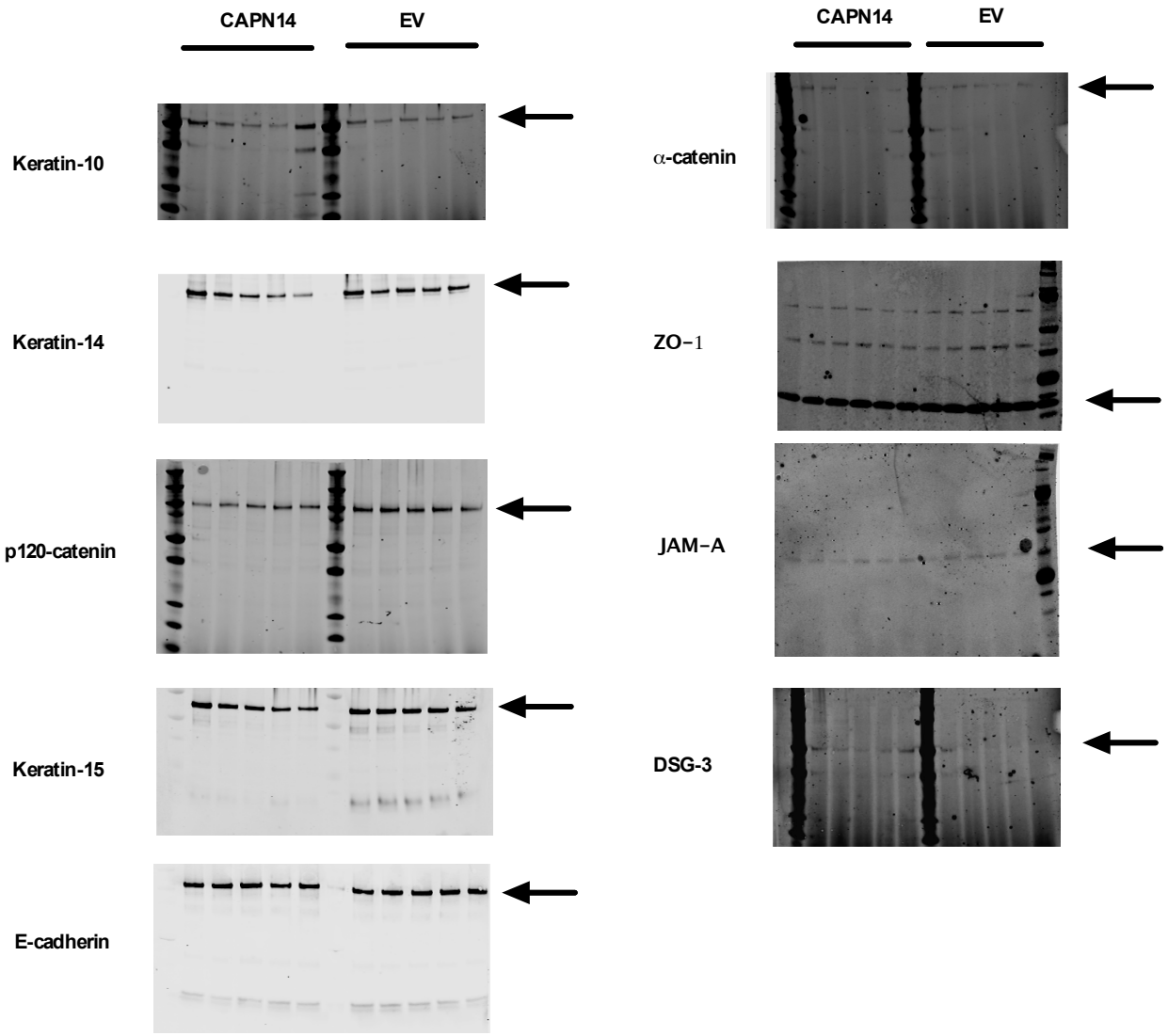
Supplementary Figure 1. Recombinant calpain 14 purification analysis. A) The general domain structure of classical calpains is shown. The protease core consists of two domains, IIa and IIb. Calpain 14 (CAPN14) is 78 kDa. B) Coomassie stain of SDS PAGE gel of purified recombinant CAPN14 (rCAPN14). Lanes were run on the same gel but were noncontiguous. C) Western blot analysis of purified rCAPN14 are shown. Ct, carboxyl-terminus; Nt, amino-terminus.



Supplementary Figure 2. Analysis of calpain 1 overexpression in air-liquid interface–cultured EPC cells. Air-liquid interface (ALI) cultures of EPC2 cells transduced with empty vector (EV) or calpain 1 overexpression vector (CAPN1) were analyzed by A) western blot analysis (HSP-90 loading control is shown); B) calpain activity assay (n = 3); C) hematoxylin and eosin staining at 40x magnification. Scale bars represent 20 μm. D) transepithelial resistance (Rt, n = 3), and E) fluorescein isothiocyanate (FITC)-dextran flux (n = 3). (b, d, e) Data are expressed as the mean ± SEM; ****$p < 0.0001$; statistical significance determined using a two-tailed t-test.

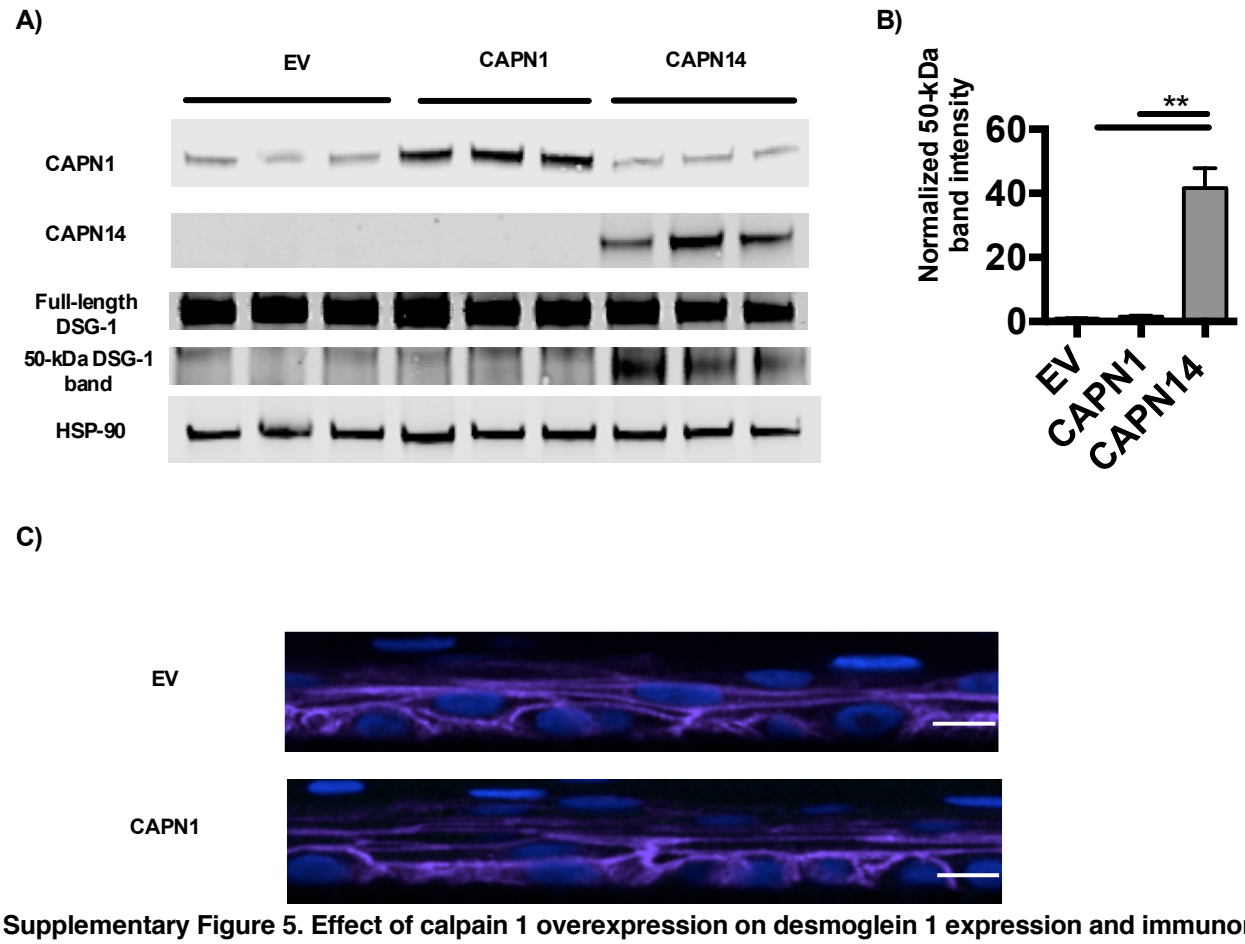


Supplementary Figure 3. CAPN14 knockdown does not affect other calpain protein expression. EPC2 cells transduced with either a non-silencing control (NSC) or calpain 14 (*CAPN14*) gene silencing (KD-1 and KD-2) vector were grown at air-liquid interface (ALI) with and without interleukin 13 (IL-13) and analyzed by western blot of ALI cultured cell lysate. HSP-90 is the loading control.

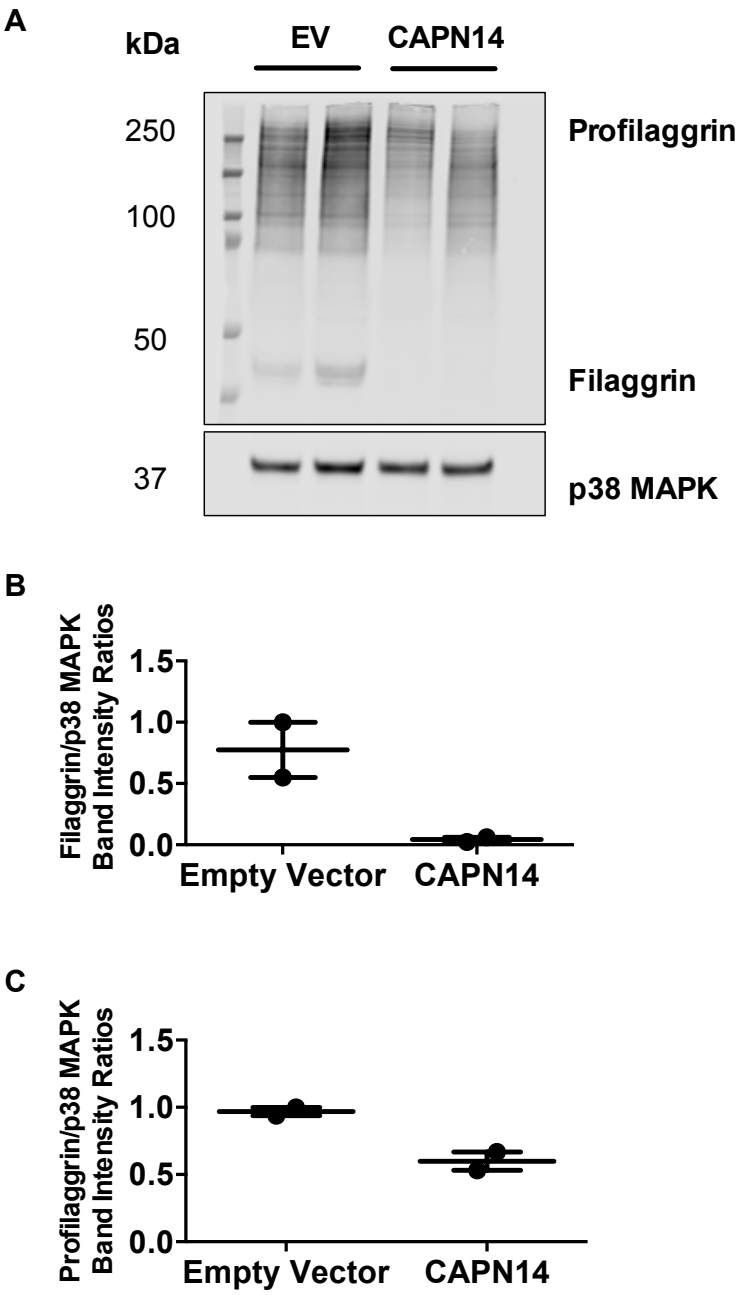


Supplementary Figure 4. Analysis of CAPN14 overexpression on junctional proteins in air-liquid interface culture.

EPC2 cells transduced with either an empty vector (EV) or calpain 14 (*CAPN14*) overexpression vector were grown at air-liquid interface (ALI) and analyzed by western blot. Arrow indicates full length protein. Abbreviations: ZO-1, Zona occludens protein 1; JAM-A, Junctional adhesion molecule-A; DSG-3, Desmoglein-3.



Supplementary Figure 5. Effect of calpain 1 overexpression on desmoglein 1 expression and immunoreactive molecular species. Air-liquid interface (ALI) culture of EPC2 cells transduced with empty vector (EV), calpain 1 (CAPN1), or calpain 14 (CAPN14) overexpression vectors were analyzed by A) western blot of lysates from ALI-cultured cells; HSP-90 is a loading control B) Quantitation of the 50-kDa DSG1 band intensity normalized to full-length DSG1 band intensity is shown. C) Immunofluorescent microscopy for DSG1 (purple) in the transduced ALI cultures is shown. Scale bars represent 20 μm. Images were taken at 20x magnification. Data in B) are expressed as the mean ± SEM; $^{**}p < 0.01$; statistical significance determined using a two-tailed t-test. Data is an extension of experiments performed in Supplementary Figure 2A as there is a duplicate component in Supplementary Figure 5A.



Supplementary Figure 6. CAPN14 overexpression effects epithelial de-differentiation. A, western blot of air-liquid interface cultured cells for calpain-14 (CAPN14); p38 mitogen-activated protein kinase (MAPK) is loading control. B and C, western blot band intensity in A.

Supplementary Table 1. Primers used for quantitative polymerase chain reaction analysis

Gene	Amplicon size (bp)	Forward sequence	Reverse sequence
CAPN14	320	TCTGAGCCAGCCTGATAGGT	GTCTGCTCCCCAGACTTGAC
GAPDH	351	TGGAAATCCCATCACCATCT	GTCTTCTGGGTGGCAGTGAT
CCL26 (Eotaxin-3)	151	AACTCCGAAACAATTGTACTCAGCTG	GTAACTCTGGGAGGAAACACCCTCTCC

Abbreviations: *CAPN14*, calpain 14; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; *CCL26*, chemokine (C-C motif) ligand 26.