<u>Supplementary Figures</u> for "Collagen VII Expression is Required in Both Keratinocytes and Fibroblasts for Anchoring Fibril Formation in Bilayer Engineered Skin Substitutes," by Supp DM, Hahn JM, Combs KA, McFarland KL, Schwentker A, Boissy RE, Boyce ST, Powell HM, Lucky AW
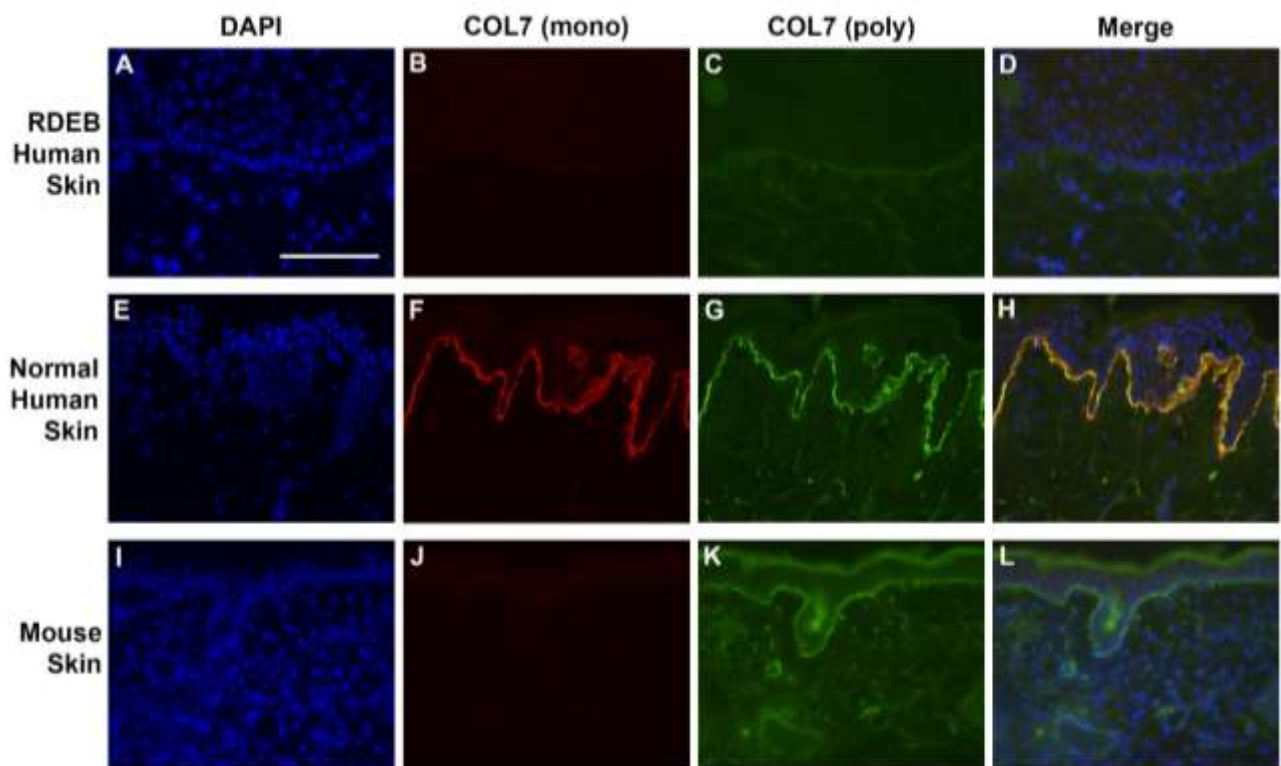
Supplementary Figure S1:

Figure S1. Immunohistochemistry demonstrating specificity of collagen VII (COL7) antibodies. Immunohistochemistry was used to localize COL7 in sections of RDEB patient skin (top; **A**-**D**), normal human skin (middle; **E**-**H**) and immunodeficient mouse skin (bottom; **I**-**L**). Staining was performed using two different antibodies: a monoclonal antibody (**B**, **F**, **J**; red), specific for human COL7 and a polyclonal antibody (**C**, **G**, **K**; green) against the full-length COL7 protein that cross-reacts with mouse COL7. Nuclei were counterstained using DAPI (**A**, **E**, **I**; blue). Merged images are shown in right column (**D**, **H**, **L**). Scale bar in **A** is same for all panels (100 μm).
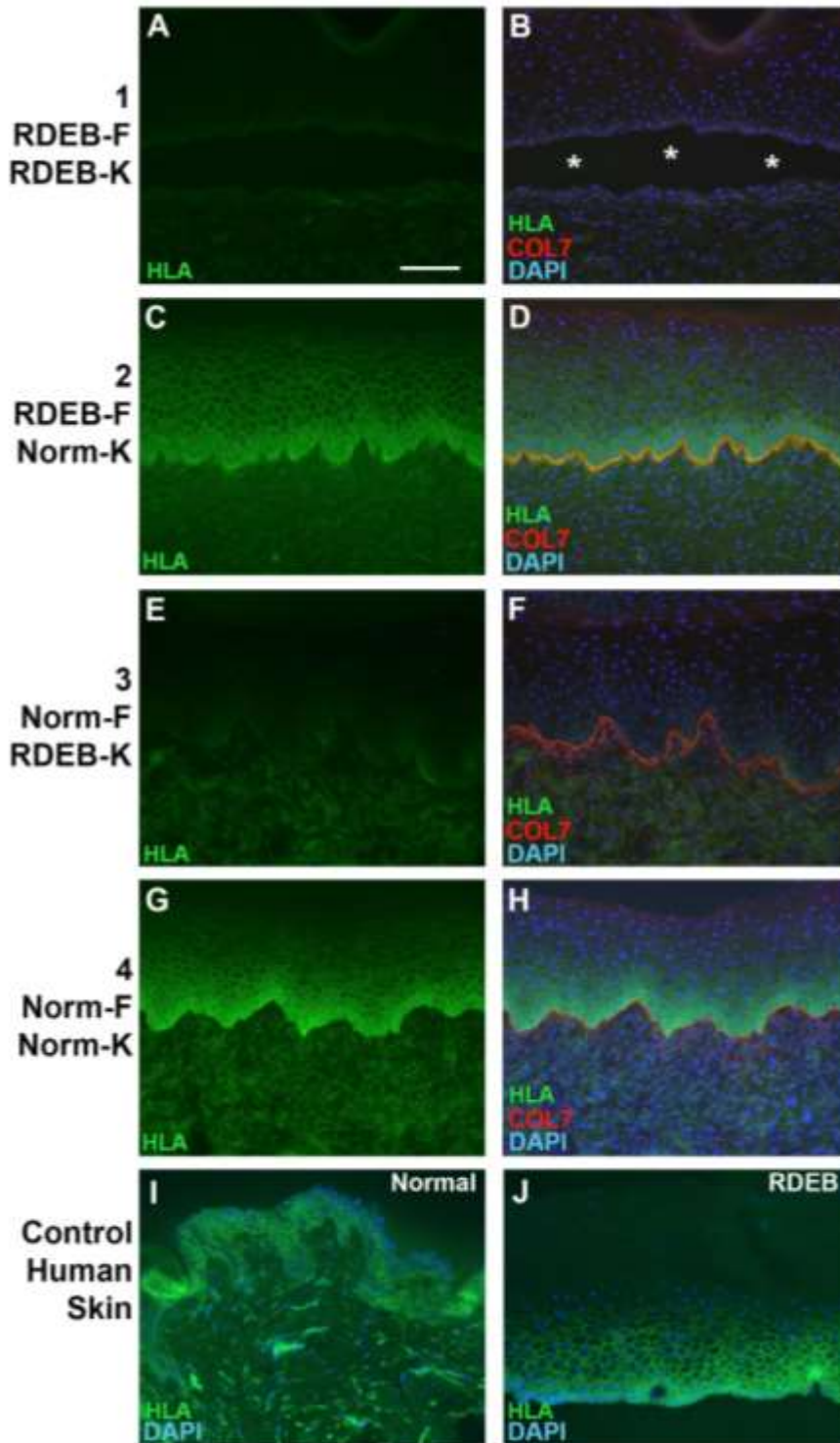
Supplementary Figure S2:

Figure S2. Localization of human leukocyte antigen (HLA) in ESS and human skin. **A-H**, sections of ESS 3 weeks after grafting. ESS were prepared with RDEB fibroblasts and RDEB keratinocytes (group 1; **A**, **B**), RDEB fibroblasts and normal keratinocytes (group 2; **C**, **D**), normal fibroblasts and RDEB keratinocytes (group 3; **E**, **F**), and normal fibroblasts and normal keratinocytes (group 4; **G**, **H**). **I**, Normal human skin; **J**, RDEB patient skin. Immunostaining was performed with an anti-HLA-ABC antibody (green) and an anti-human COL7 antibody (**A-H**; red). Nuclei were counterstained using DAPI (**B**, **D**, **F**, **H-J**; blue). Note that HLA staining is deficient in ESS prepared with RDEB keratinocytes, but is observed in native RDEB patient skin. Asterisks (**B**) indicate epidermal blistering in ESS prepared with all RDEB cells. Scale bar in **A** (100 μm) is same for all panels.
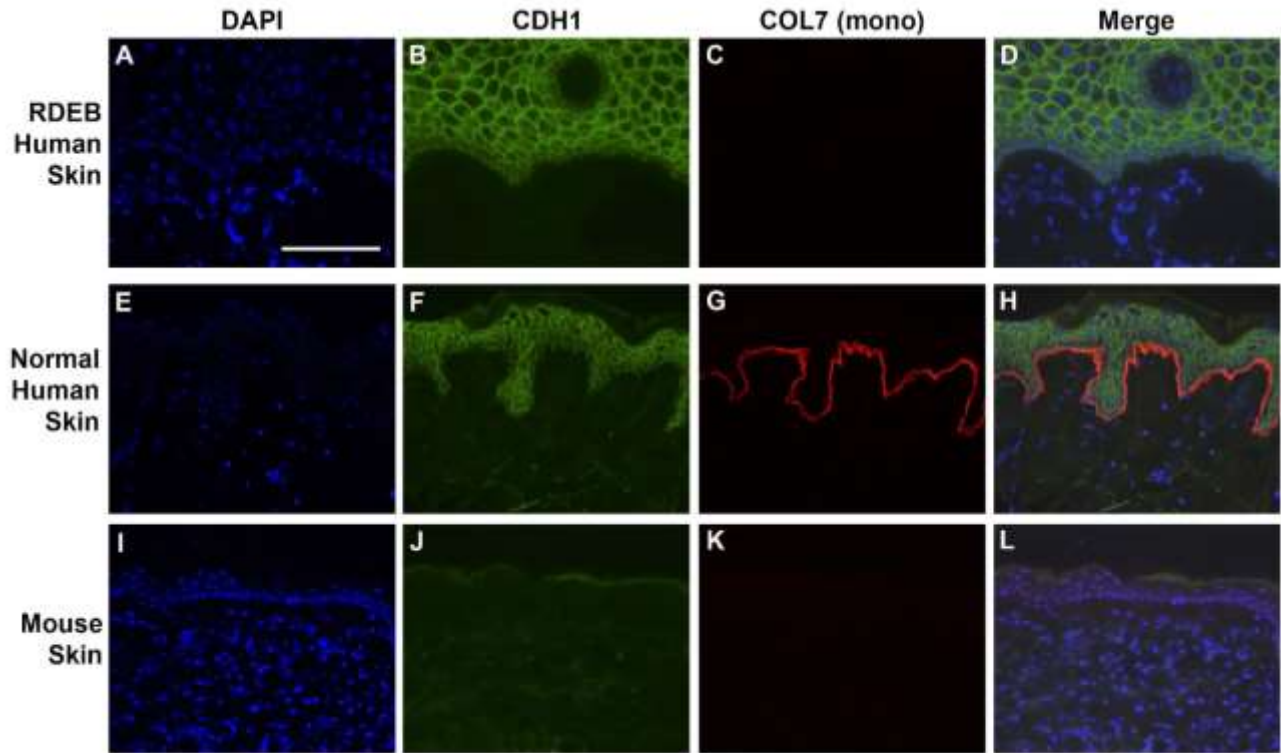
Figure S3. Immunohistochemistry demonstrating specificity of E-cadherin (CDH1) and collagen VII (COL7) antibodies. Immunohistochemistry was used to localize human CDH1 (**B**, **F**, **J**; green) and COL7 (**C**, **G**, **K**; red) in sections of RDEB patient skin (top; **A-D**), normal human skin (middle; **E-H**) and immunodeficient mouse skin (bottom; **I-L**). Nuclei were counterstained using DAPI (**A, E**, **I**; blue). Merged images are shown in right column (**D**, **H**, **L**). Scale bar in **A** is same for all panels (100 µm).