

Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

Constructs and cell lines

COL17A cDNA was amplified from human skin and cloned into the expression vector, pME18S with a FLAG-tag at the C-terminus. HEK293T cells (RCB2202, RIKEN Bio Resource Center, Tsukuba, Japan) were transfected with vectors expressing *COL17A* and green fluorescent protein (GFP) at the equal mass ratio using a transfection reagent (PEI MAX[®]; Polyscience, Warrington, PA, USA). The mean transfection efficacy was $48.6 \pm 0.63\%$, as determined by the expression levels of GFP.

Flow cytometry analysis

The double transfected cells were analysed 2 days after transfection, when gene expression levels are considered to reach the maximum. The cell suspension (1×10^5 cell count) was incubated with serum at a dilution ratio of 1:100 and stained with the secondary antibody (allophycocyanin (APC) anti-human IgG; Jackson ImmunoResearch, West Grove, PA, USA). An APC anti-FLAG monoclonal antibody (L5, BioLegend, San Diego, CA, USA)

was used for probing FLAG peptide. Flow cytometric analysis was performed using a FACSCelesta (BD Biosciences, Franklin Lakes, NJ, USA). After excluding dead cells by propidium iodide (PI) staining, specific binding to BP180 protein was determined by APC-mean fluorescence intensity (MFI) in the GFP-positive population, and the background signals from the GFP-negative population were subtracted. Arbitrary units were determined by generating a standard curve, as described previously (S1–S3) using serum samples from patients with BP.

REFERENCES

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