**Supplementary Materials and Methods 1.**

The BP patient's skin was mounted and snap-frozen in OCT compound (Thermo Fisher Scientific), and 5-µm cryosections were prepared for direct immunofluorescence. The sections were incubated with FITC-conjugated anti-human IgG (Jackson Immunoresearch Laboratories) in a 1:100 dilution for 30 minutes at 37°C. Indirect immunofluorescence was performed on skin samples from normal healthy individuals, prepared as above. The sections were incubated with sera from BP patients for 1 hour at 37°C and detected by FITC-conjugated anti-human IgG.

**Supplementary Materials and Methods 2.**

First, human *COL17A1* cDNA (a gift of Professor Kim B. Yancey) expressing a DDDDK-tag on the N-terminus was inserted into the NotI site of pcDNA5/FRT (Invitrogen), and the resulting plasmid was designated as COL17-pcDNA5 (Nishie *et al.*, 2010). COL17-pcDNA5, or the pcDNA5/FRT empty vector as a control, was co-transfected with pOG44 (Invitrogen) into Flp-In 293 cells (Invitrogen) by Lipofectamine 2000 (Invitrogen). After stably transfected cells were selected under 200 μg/ml hygromycin B (Invitrogen), the transfected cells were cultured in DMEM (Invitrogen) containing 10% foetal calf serum (Invitrogen). Freshly prepared ascorbic acid was added to the culture medium at a final concentration of 50 μg/ml to allow prolyl and lysyl hydroxylation of collagen and proper triple helix formation (Franzke *et al.*, 2002). Confluent, stably human COL17-expressing Flp-In-293 cells were lysed for 30 minutes on ice in a buffer containing 1% Nonidet P-40, 0.1 M NaCl, 25 mM Tris-HCl (pH 7.4), 10 mM EDTA and 1:100 diluted protease inhibitor (P8340, Sigma-Aldrich). The cell lysate was immunoprecipitated with anti-DDDDK-tag-magnetic beads (MBL), then eluted with DDDDK peptide (Sigma-Aldrich). The concentration of the purified protein was determined by BCA Protein Assay (Thermo Fisher Scientific).

Reference

Franzke CW, Tasanen K, Schacke H, Zhou Z, Tryggvason K, Mauch C, *et al.* (2002) Transmembrane collagen XVII, an epithelial adhesion protein, is shed from the cell surface by ADAMs. *The EMBO J*

21:5026-35.

Nishie W, Lamer S, Schlosser A, Licarete E, Franzke CW, Hofmann SC, *et al.* (2010) Ectodomain shedding generates Neoepitopes on collagen XVII, the major autoantigen for bullous pemphigoid. *J Immunol* 185:4938-47.

**Supplementary Materials and Methods 3.**

PCR products that had been amplified using primers and COL17-pcDNA5 as a template were introduced into the NotI and ApaI sites of pcDNA5-FRT, which is designated as N-50K-pcDNA5. Forward (F) and reverse (R) primers were used for N-50K (F: 5’-CGCAAATGGGCGGTAGGCGTG-3’; R: 5’-GTTGGGCCCTCACTTCCACCAGCTGCAGCA-3’; underlined: ApaI). The N-50K-pcDNA5 was transfected into Flp-In 293 cells to generate stably expressing cells.

**Supplementary Materials and Methods 4.**

Following SDS-PAGE, proteins were transferred onto a nitrocellulose membrane for immunoblotting. Mouse monoclonal anti-DDDDK (M2, Sigma-Aldrich) targeting the DDDDK-tag, C17-C1 (Wada *et al*., 2016), rabbit polyclonal Abs 08003 and R7 (Natsuga *et al.*, 2012) directing to different portions of COL17 were used (**Figure 1a**). Epitopes of these Abs are $Met^{1}$ to $Ser^{204}$ (08003), $Asp^{522}$ to $Gln^{545}$ (R7) and $Gly^{1316}$ to $Gly^{1342}$ (C17-C1), which respectively correspond to the intracellular domain, the NC16A and the C-terminus region of COL17 (**Figure 1a**). After incubation with an HRP-conjugated secondary, Ab signals were visualised by ECL Prime (GE Healthcare).

Reference

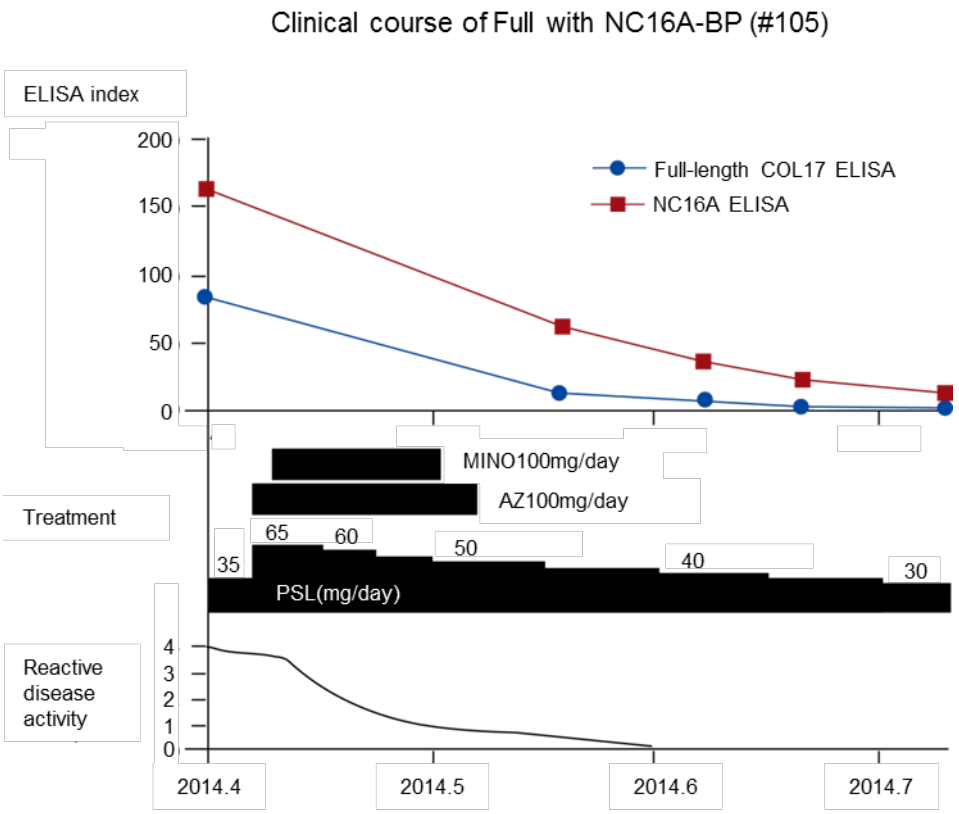
Natsuga K, Nishie W, Shinkuma S, Ujiie H, Nishimura M, Sawamura D, *et al.* (2012) Antibodies to pathogenic epitopes on type XVII collagen cause skin fragility in a complement-dependent and -independent manner. *J Immunol* 188:5792-9.

Wada M, Nishie W, Ujiie H, Izumi K, Iwata H, Natsuga K, *et al.* (2016) Epitope-Dependent Pathogenicity of Antibodies Targeting a Major Bullous Pemphigoid Autoantigen Collagen XVII/BP180. *J Invest Dermatol* 136:938-46.

**Supplementary Materials and Methods 5.**

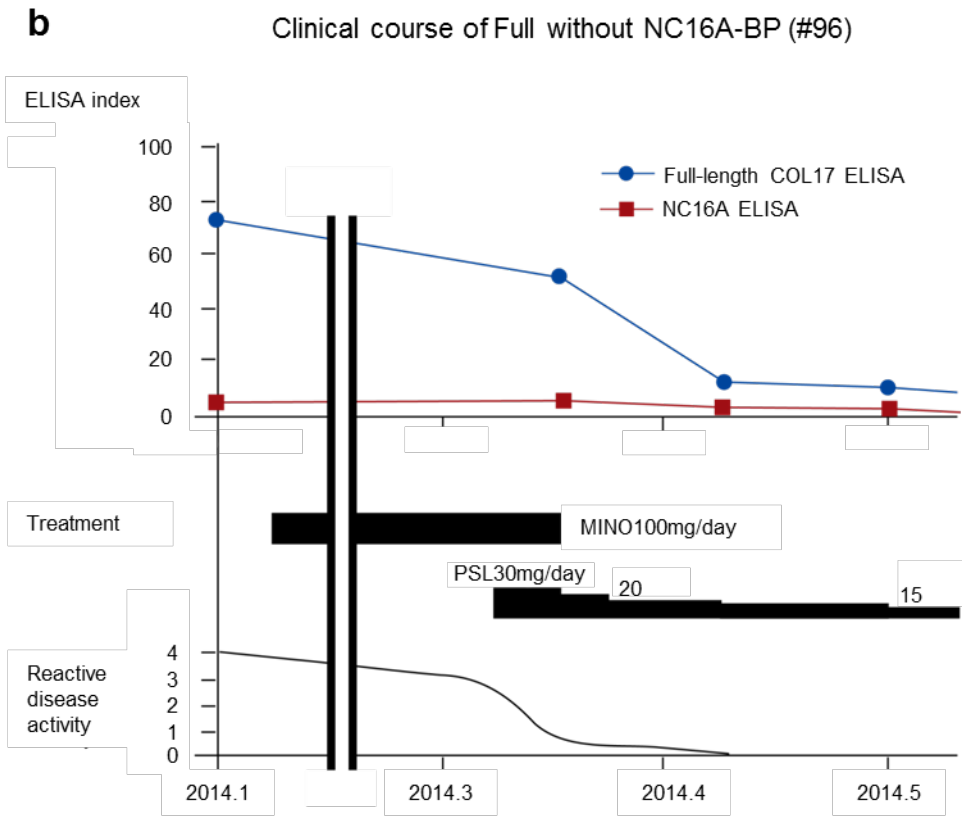
For the full-length COL17 ELISA, 96-well microplates (BD Biosciences) were coated with 1 µg/well of full-length recombinant COL17 in 50mM carbonate buffer with a pH of 9.5 overnight at 4°C, and subsequently blocked with 2% BSA in phosphate-buffered saline for 2 hours at RT. The plate was incubated with 1:100 diluted sera for 1 hour at RT, washed and then incubated with 1:5000 diluted HRP-conjugated rabbit polyclonal anti-human IgG Abs (DAKO) for 1 hour at RT. After washing, the colour development was achieved by substrate solution [3, 3', 5, 5'-tetramethylbenzidine dihydrochloride/hydrogen peroxide; TMB/$H_2O_2$ (MBL)] for 30 minutes at RT and stopped by adding 1.0 N sulfuric acid. Absorbance was measured at 450 nm, with the correlation wavelength set at 620 nm by a microplate reader (Tecan Austria GmbH). Each serum was assayed in duplicate. The ELISA index value was defined by the following formula: index = (OD450 of tested serum - OD450 of negative control) / (OD450 of positive control - OD450 of negative control) ×100.

The ability to discriminate between BP patients and control subjects for one parameter (reactivity with full-length human COL17) was estimated using a receiver operating characteristic curve. The receiver operating characteristic analysis was used to determine a cut-off value for the full-length COL17 ELISA. Based on the maximisation of the Youden index (J=sensitivity+specificity-1), the cut-off value for the ELISA with the full-length COL17 was set at 4.64.



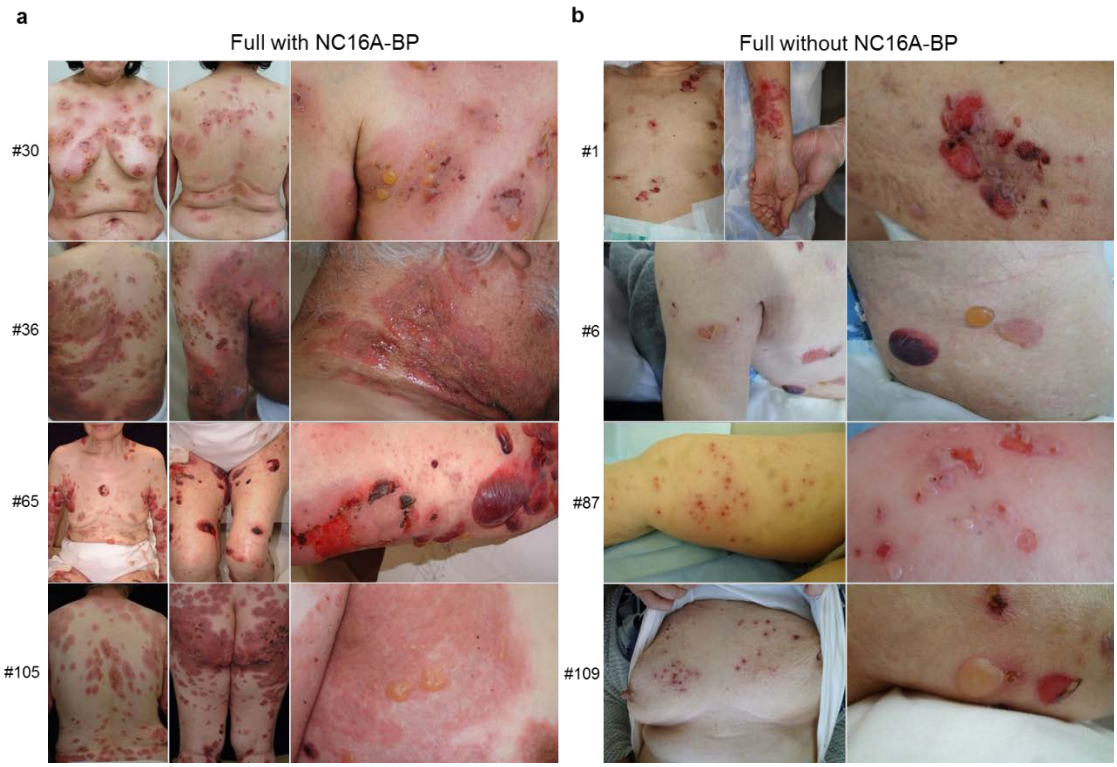

**Supplementary Figure 1. The clinical course of a representative 'inflammatory' 'Full with NC16A-BP' case (#105).** The BP patient was a 61-year-old Japanese woman. She presented with four months of itchy eruptions and about two weeks of oedematous erythema with tense blisters on the scalp, face and extremities. Physical examination showed well-defined oedematous erythema and tense blisters on the whole body. The histopathology of a skin lesion showed subepidermal separation. Eosinophils were observed in the upper dermis of a peri-blister lesion. DIF revealed linear deposits of IgG and C3 at the BMZ. IIF revealed IgG binding to the epidermal side of a 1M NaCl-split skin section. The results of both NC16A ELISA (ELISA index=163.8) and full-length COL17 ELISA (ELISA index=83.6) were positive at the diagnosis. Oral prednisolone at 35 mg/day was administered but did not improve the skin lesions, so we increased the dosage to 65 mg/day and added the administration of oral azathioprine at 100 mg/day and minocycline at 100 mg/day. After these treatments, new blistering lesions ceased to

occur. Both of the ELISA indices decreased in parallel with disease activity. The blue line indicates the full-length COL17 ELISA index. The red line indicates the NC16A ELISA index. PSL, prednisolone; MINO, minocycline; AZ, azathioprine. Disease activity was arbitrarily evaluated on a scale of 0–4. The score of 4 was set as the highest activity for each patient during the observation period, and relative activity was assessed arbitrarily.

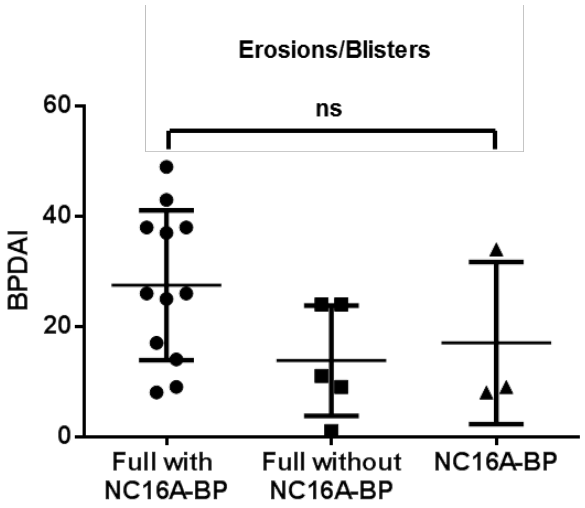



**Supplementary Figure 2. The clinical course of a representative 'non-inflammatory' 'Full without NC16A-BP' case (#96).** The BP patient was a 93-year-old Japanese woman. She was referred to our department with a one-month history of recurrent blisters and erosions on the trunk and extremities. Physical examination revealed erosions and flaccid blisters on the trunk and extremities, but few erythematous lesions were found. The histopathology of a skin lesion showed subepidermal separation. Only a few eosinophils were observed in the upper dermis of a peri-blister lesion. DIF revealed linear deposits of IgG and C3 at the BMZ. IIF revealed IgG binding to the epidermal side of a 1M NaCl-split skin section. The results of the full-length COL17 ELISA were positive (ELISA index=76.2), although those of the conventional NC16A and BP230 ELISAs were negative. After oral administration of minocycline and the initiation of a topical steroid, the skin erosions showed re-epithelialisation. However,

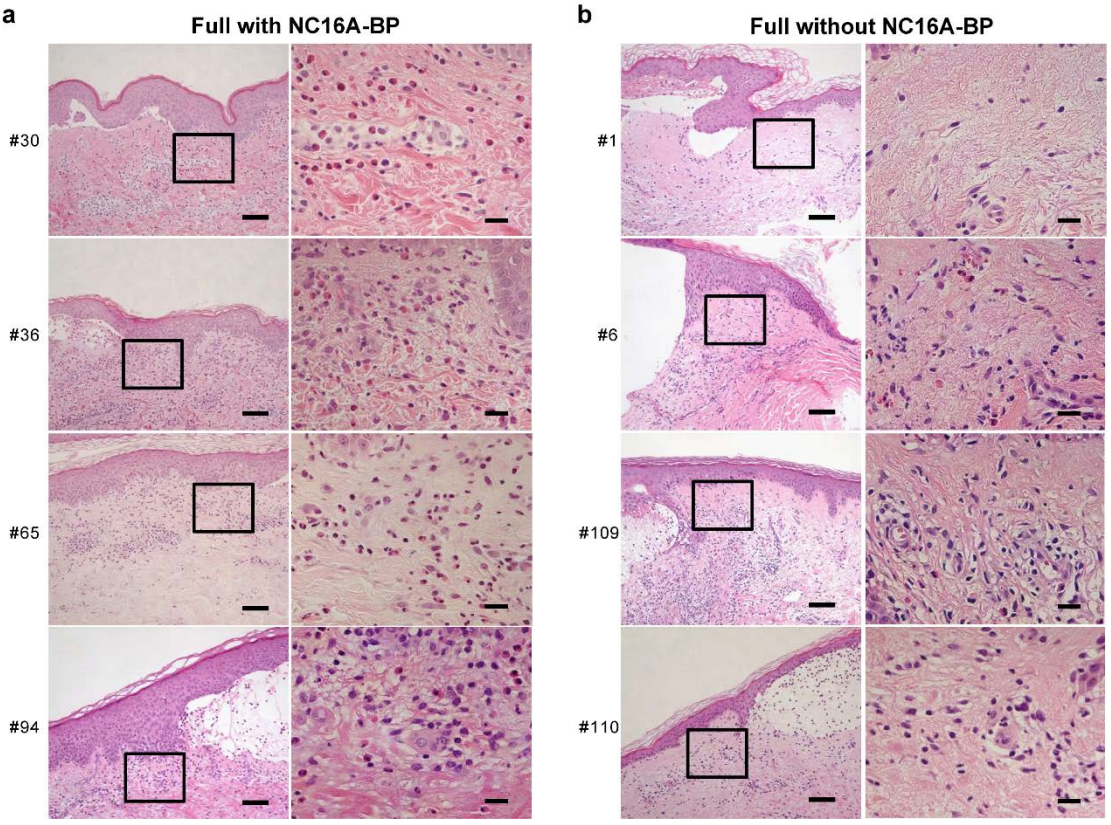
because itchiness and erosions due to rubbing persisted, oral prednisolone (30 mg/day) was added. A week after the administration of oral prednisolone, the skin lesions disappeared and the full-length COL17 ELISA index rapidly decreased. The blue line indicates the full-length COL17 ELISA index. The red line indicates the NC16A ELISA index. The double bold line indicates a skip in the plot for February 2014. PSL, prednisolone; MINO, minocycline.



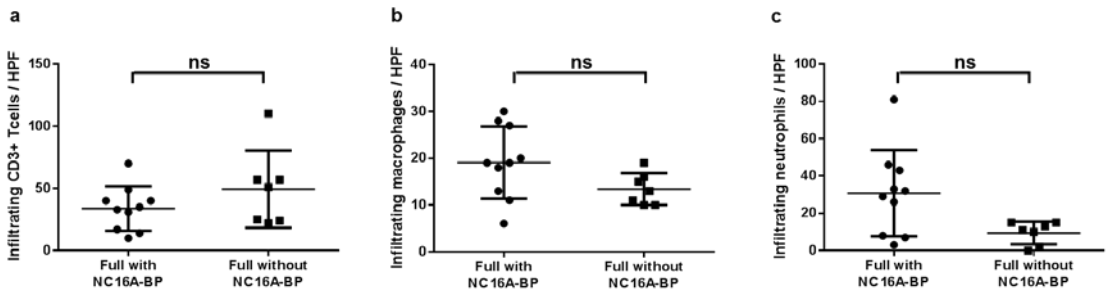

**Supplementary Figure 3. Clinical features of 'Full with NC16A-BP' and 'Full without NC16A-BP'.** (**a**) Clinical features of 'Full with NC16A-BP' (#30, 36, 65 and 105). Each column corresponds to each 'Full with NC16A-BP' case. 'Full with NC16A-BP' cases represent the 'inflammatory' subtype. (**b**) Clinical features of 'Full without NC16A-BP' (#1, 6, 87 and 109). Each column corresponds to each 'Full without NC16A-BP' case. 'Full without NC16A-BP' cases represent the 'non-inflammatory' subtype.



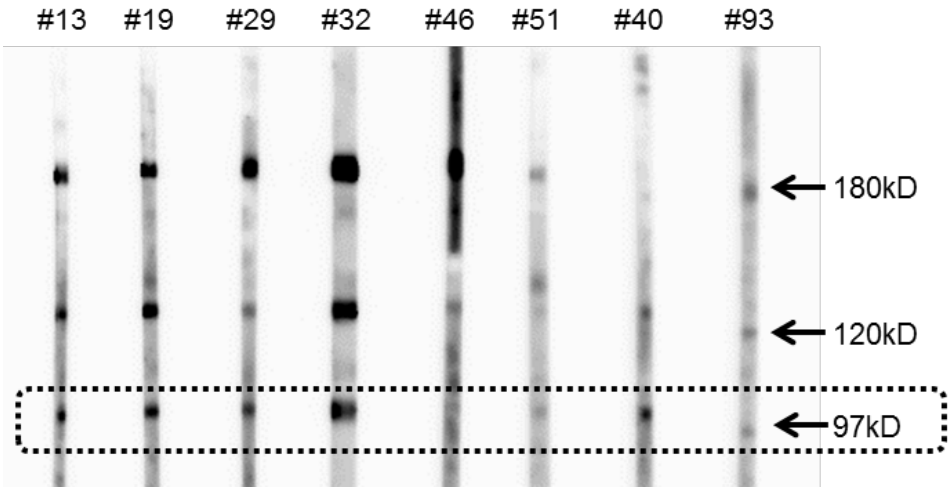

**Supplementary Figure 4. Comparison of BPDAI scores among BP cohorts.** Each plot shows the BPDAI (erosions/blisters) for 'Full with NC16A-BP' (n=12), 'Full without NC16A-BP' (n=5) and NC16A-BP (n=3). There are no significant differences (P=0.0984) using the Kruskal-Wallis test.



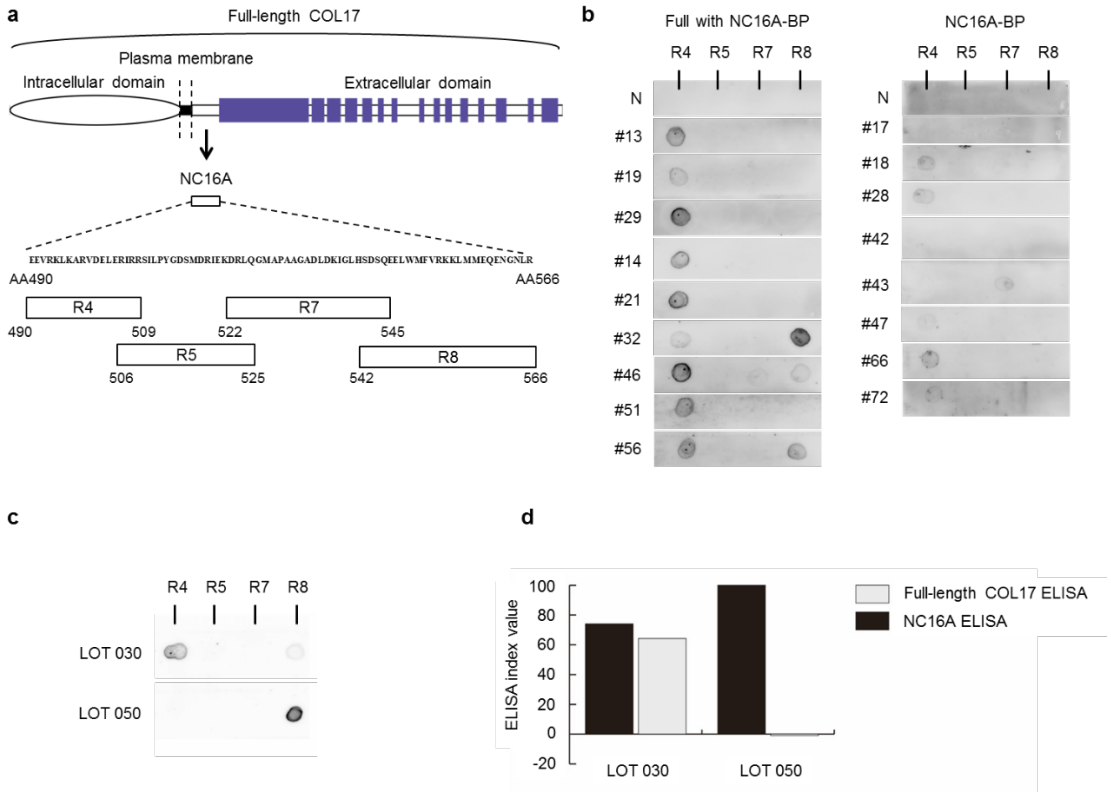

**Supplementary Figure 5. Histological findings of 'Full with NC16A-BP' and 'Full without NC16A-BP'.** (**a**) Histopathological findings of 'Full with NC16A-BP' (#30, 36, 65 and 94). Haematoxylin-eosin staining, original magnification x100, scale bar = 100μm (left). The black square indicates the area of HPF, original magnification x400, scale bar = 20μm (right). More eosinophils infiltrate in the upper dermis of peri-blister lesions than in 'Full without NC16A-BP' skin. (**b**) Histopathological findings of 'Full without NC16A-BP' (#1, 6, 109 and 110). Haematoxylin-eosin staining, original magnification x100, scale bar = 100μm (left). The black square indicates the area of HPF, original magnification x400, scale bar = 20μm (right).




**Supplementary Figure 6. Comparison of the number of infiltrating inflammatory cells between 'Full with NC16A-BP' and 'Full without NC16A-BP'.** (**a**) Comparison of the number of infiltrating $CD3^+$ T cells between 'Full with NC16A-BP' (n=10) and 'Full without NC16A-BP' (n=7). (**b**) Comparison of the number of infiltrating macrophages between 'Full with NC16A-BP' (n=10) and 'Full without NC16A-BP' (n=7). (**c**) Comparison of the number of infiltrating neutrophils between 'Full with NC16A-BP' (n=10) and 'Full without NC16A-BP' (n=7).




**Supplementary Figure 7. Immunoblotting of 'Full with NC16A-BP' using the mixture of full-length COL17 and limited digestion fragments with plasmin.** The dotted frame indicates the plasmin-digested 97-kDa COL17.




**Supplementary Figure 8. Epitope mapping of 'Full with NC16A-BP' and NC16A-BP within the NC16A of COL17.** (**a**) Schematic of COL17 and four different peptides covering NC16A, including R4 ($Glu^{490}$ to $Ile^{509}$), R5 ($Arg^{506}$ to $Gln^{525}$), R7 ($Asp^{522}$ to $Gln^{545}$) and R8 ($Ser^{542}$ to $Arg^{566}$). (**b**) Dot blotting using four different peptides (R4, R5, R7, R8) covering NC16A for 'Full with NC16A-BP' and NC16A-BP. (Left panel) N: normal human serum, #: serum samples of 'Full with NC16A-BP'. (Right panel) N: normal human serum, #: serum samples of NC16A-BP. (**c**) Epitope mapping of two different human BP serum samples (LOT 030 and 050) provided as positive controls in the NC16A ELISA kit (MBL). While LOT 030 reacts with both the R4 and the R8 peptides, LOT 050 specifically recognises the R8 peptide. (**d**) The results of NC16A ELISA and full-length COL17 ELISA using two positive BP control serum samples (LOT 030 and 050). Though the human sera both show high titration of NC16A, LOT 050 fails to react with full-length COL17.

**Supplementary Table 1. Background clinical information of BP and controls.**

| | Age (Mean±SD) | Sex | | Diabetes |
|---|---|---|---|---|
| | | Male | Female | |
| BP (n=121) | 71.5±14.3[a] | 54 | 67[b] | 23/106[c] |
| Controls (n=122) | 50.5±20.7 | 65 | 57 | 18/122 |
| Pemphigus vulgaris (n=30) | | | | |
| Pemphigus foliaceus (n=14) | | | | |
| Non-autoimmune blistering disease (n=48) | | | | |
| Normal healthy individuals (n=30) | | | | |

Controls include not only normal healthy individuals but also pemphigus vulgaris, pemphigus foliaceus and non-autoimmune blistering disease. [a]$P<0.0001$ using the Mann-Whitney test. [b]There are no significant differences ($P=0.18$) using the chi-square test. [c]There are no significant differences ($P=0.15$) using the chi-square test. Clinical information about diabetes was not obtained for 15 of the BP cases.

**Supplementary Table 2. The treatment of BP cohorts at the evaluation of clinical manifestation in Figure 3b.**

| Full with NC16A-BP | topical steroid | anti-histamine | minocycline | nicotinic acid | systemic steroid |
|---|---|---|---|---|---|
| #2 | + | + | - | - | - |
| #30 | + | + | - | - | - |
| #44 | N/A | N/A | N/A | N/A | N/A |
| #88 | + | - | - | - | PSL 10 mg/day |
| #89 | - | - | - | - | - |
| #93 | + | + | - | - | PSL 25 mg/day |
| #105 | + | + | - | 600 mg/day | - |
| #111 | + | + | - | 600 mg/day | - |
| #113 | + | - | - | - | - |
| #114 | + | + | - | - | PSL 15 mg/day |
| #117 | - | - | - | - | - |
| #119 | - | - | - | - | PSL 10 mg/day |

| Full without NC16A-BP | topical steroid | anti-histamine | minocycline | nicotinic acid | systemic steroid |
|---|---|---|---|---|---|
| #3 | + | - | - | - | - |
| #110 | + | + | + | - | - |
| #115 | - | - | - | - | - |
| #118 | - | - | - | - | - |
| #119 | + | + | 100 mg/day | - | - |

| NC16A-BP | topical steroid | anti-histamine | minocycline | nicotinic acid | systemic steroid |
|---|---|---|---|---|---|
| #18 | + | + | - | - | - |
| #43 | + | + | - | - | - |
| #112 | + | + | - | - | - |

PSL: prednisolone; N/A: Data are not available