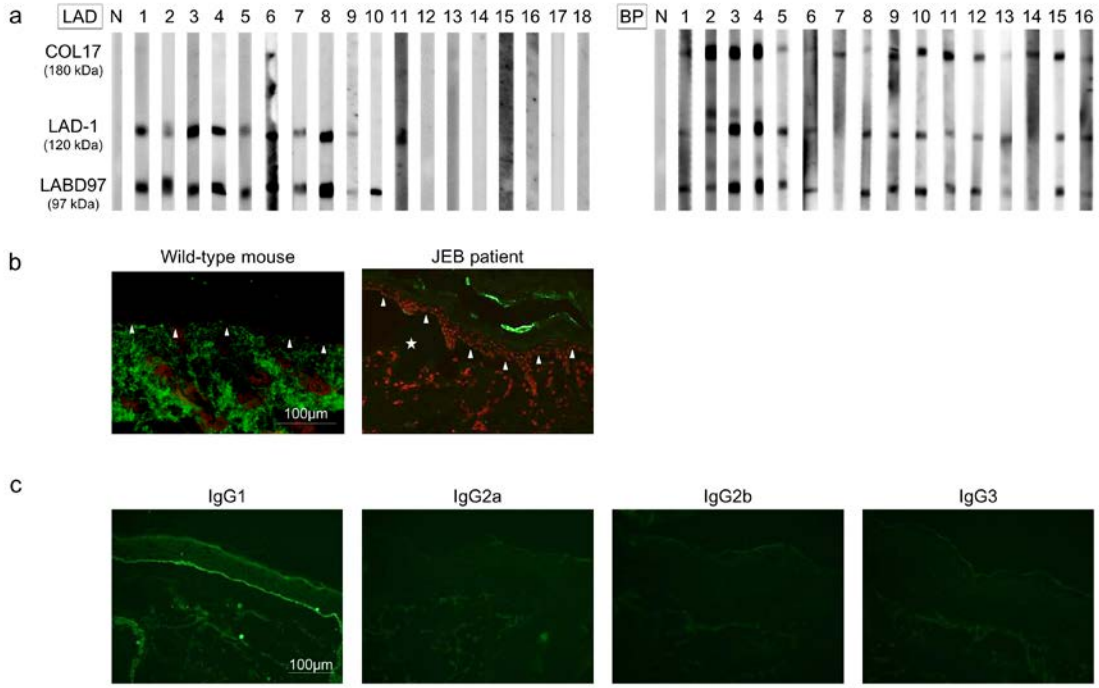
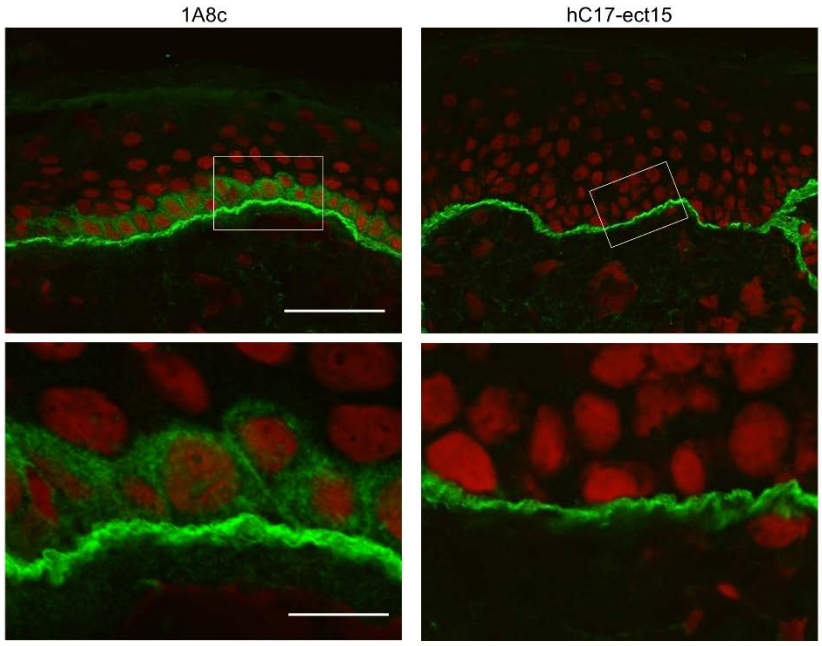


Supplementary Figure S1

IIF and Western blotting detected by LAD autoantibodies and mAbs

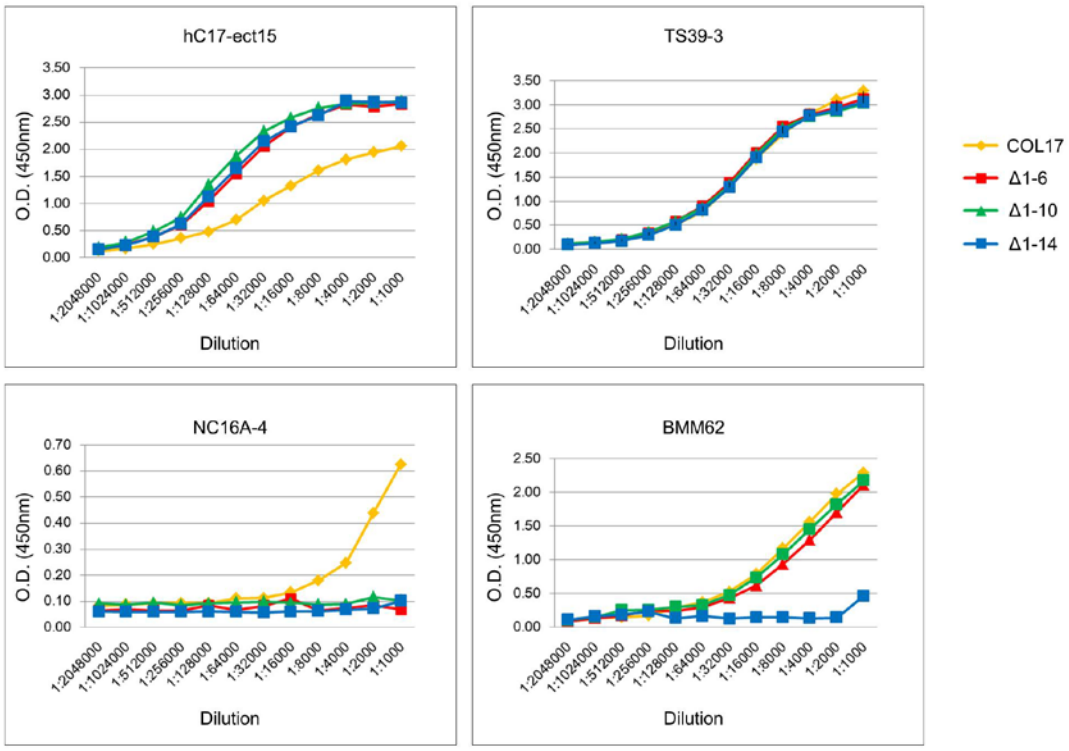
(a) Western blotting using a mixture of full-length COL17, LAD-1, and LABD97 was analyzed using LAD (n=18) and BP sera (n=16) followed by incubation with HRP-conjugated anti-human IgA and IgG, respectively. N: normal control sera. LAD No. 1, 2, 5, 7 and BP No. 3, 4 are shown in Figure 1b. **(b)** IIF staining of wild-type mouse skin and JEB patient skin using the mAb hC17-ect15. The mAb hC17-ect15 shows negative deposition at the DEJ in both sections. Arrowheads: DEJ. Star: subepidermal blister. Scale bar: 100 µm. **(c)** Identification of the mAb hC17-ect15 using subclass-specific secondary antibodies.



Supplementary Figure S2

IIF staining of normal human skin using the mAb 1A8c directing the intracytoplasmic domain of COL17 and the mAb hC17-ect15

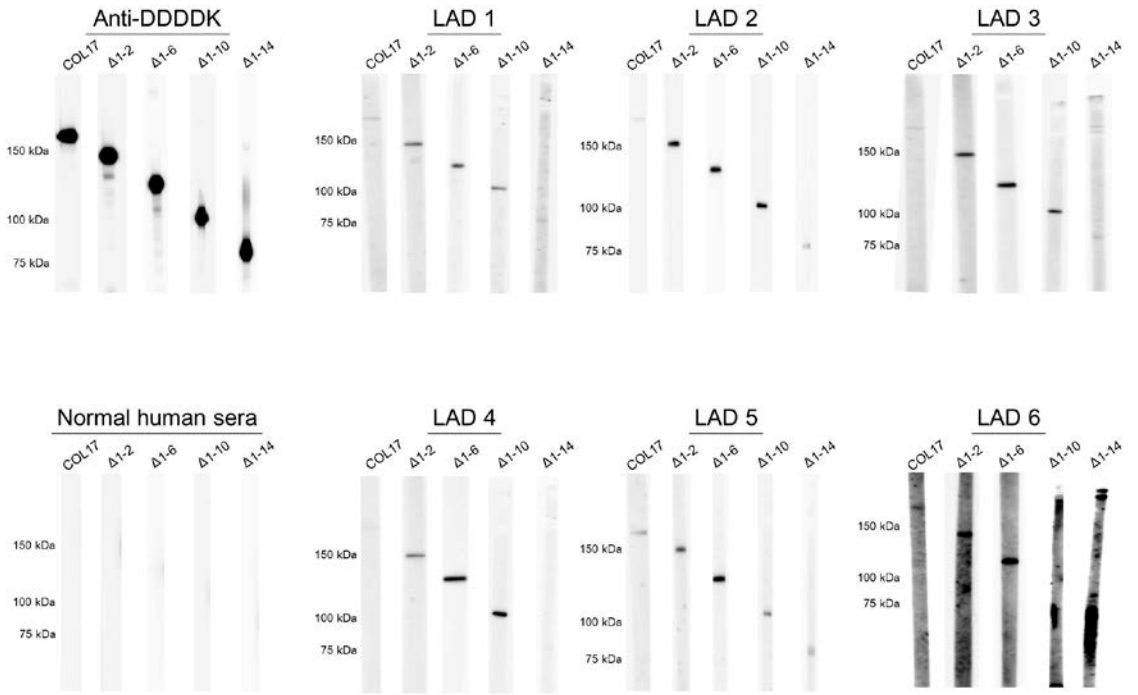
Upper panels show low magnification. Scale bar: 50 μm. Lower panels show high magnification. Scale bar: 15 μm. The mAb 1A8c detects COL17 in the apicolateral regions of basal keratinocytes as well as at the DEJ. In contrast, the mAb hC17-ect15 shows linear staining only at the DEJ.



Supplementary Figure S3

ELISA using different lengths of recombinant COL17 proteins detected by mAbs

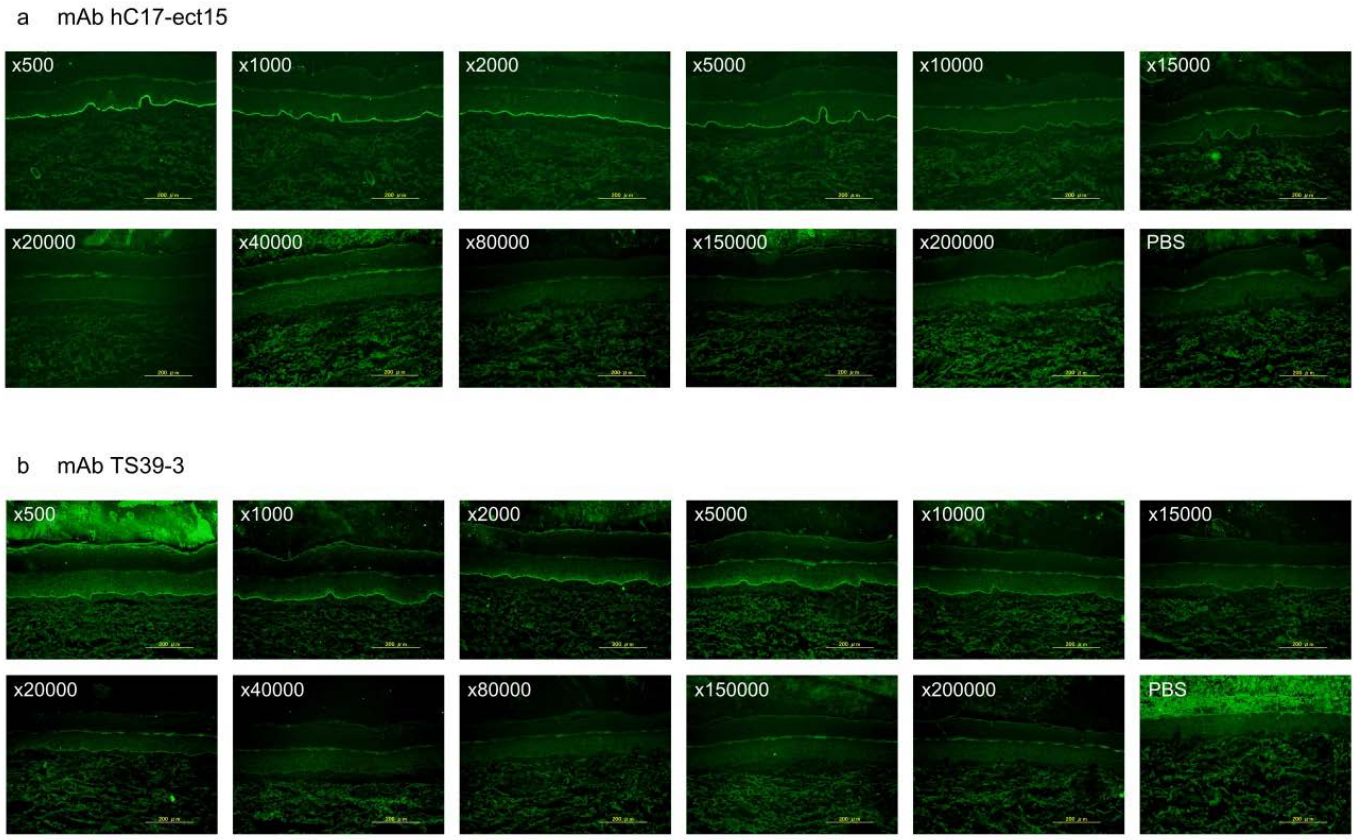
ELISA using different mAbs, including hC17-ect15, TS39-3, NC16A-4, and BMM62 on full-length human COL17 (COL17), COL17(Δ1-6), COL17(Δ1-10), and COL17(Δ1-14). Note that the mAb hC17-ect15 weakly reacts with full-length COL17, whereas the mAb NC16A-4 reacts strongly with the full-length form. The mAbs TS39-3 and BMM62 react equally with recombinant COL17. To adjust the molecular amount of each protein, the absorbance was normalized by the mAb anti-DDDDK-tag.



Supplementary Figure S4

Western blotting using 5 different recombinant COL17 proteins detected by LAD autoantibodies

Western blotting using recombinant full-length human COL17 (COL17) and C-terminal-deleted COL17 (Δ1-2), COL17 (Δ1-6), COL17 (Δ1-10), and COL17 (Δ1-14) proteins and LAD sera with preferential reactivity to the 97-kDa and 120-kDa ectodomains (n=6, No. 1-6 in **Figure 1b**). The mAb anti-DDDDK-tag shows that the amount of full-length COL17, COL17 (Δ1-2), COL17 (Δ1-6), COL17 (Δ1-10) and COL17 (Δ1-14) were equally loaded. LAD No. 2 is shown in Figure 2d.



Supplementary Figure S5

IIF using normal human skin detected with mAb hC17-ect15 and mAb TS39-3

The results show that the **(a)** mAbs hc17-ect15 (1 mg/ml) and **(b)** TS39-3 (1 mg/ml) react with the DEJ of normal human skin up to a dilution of 1:40,000. Scale bar: 200 µm.

Supplementary Materials and Methods 1

To produce mouse full-length COL17, mouse *Col17a1* cDNA (NM_007732) was amplified by polymerase chain reaction (PCR) using the following primers 5’-CCTGAATTCCATCCGAGGGAGGAAGAGAG-3’ and 5’-TGAGACCAAATCACCCAGCGACACTAGT-3’ (underlined: EcoRI; double-underlined: SpeI, restriction sites) on mRNA isolated from wild-type mouse skin. The PCR products were then inserted into the restriction sites of EcoRI and SpeI in pBC SK(-) (Agilent Technologies). After being cloned, DDDDK-tag sequences were inserted into the N-terminus of the mouse *Col17a1* cDNA and inserted into the HindIII and NotI sites of pcDNA5/FRT (Invitrogen), which was designated mCol17-pcDNA5.

Supplementary Materials and Methods 2

The partially humanized mouse COL17 was produced by the insertion of synthesized DNA **(Supplementary Table 2)** into the mCol17-pcDNA5, which was designated Ph-mCol17-pcDNA5/FRT. The mCol17-pcDNA5 and Ph-mCol17-pcDNA5 were each co-transfected with pOG44 (Invitrogen) into Flip-In 293 cells (Invitrogen) using the Lipofectamine 2000 transfection reagent (Thermo Fisher Scientific) according to the manufacturer’s protocol. The transfected cells were cultured in DMEM (Invitrogen) containing 10% fetal bovine serum (FBS) followed by selection under 200 μg/ml of

hygromycin B (Invitrogen). Twenty-four hours before the cell lysates were collected, freshly prepared ascorbic acid was added to the culture medium at a final concentration of 50 μg/ml. Cell lysates were prepared using lysis buffer containing Nonidet P-40 (Nacalai Tesque), 25 mM Tris-HCl (pH 7.4), protease inhibitor cocktail (Sigma-Aldrich) and 10 mM ethylenediaminetetraacetic acid, followed by the purification of recombinant proteins by using anti-DDDDK-tag mAb-magnetic beads (M185-9; MBL).

Supplementary Materials and Methods 3

Preparation of antibodies targeting different epitopes on COL17

BALB/cCrSlc mice were immunized with full-length COL17 together with LAD-1 and LABD97 (Yamauchi et al., 2014; Izumi et al., 2016; Nishimura et al., 2016). Then, splenocytes from the immunized mice were fused with P3U1 cells (Ujiie et al., 2014) to obtain hybridoma cells secreting the mAbs hC17-ect15 and NC16A-4. The mAbs were affinity-purified from serum-free medium (Hybridoma-SFM, Gibco) using Ab-Capcher Extra (ProteNova) according to the manufacturer's instructions followed by concentration using Amicon Ultra Centrifugal Filters (10 K, Millipore). Mouse IgG1 mAb targeting Asp^{522} to Gln^{545} of the NC16A domain of human COL17 (mAb TS39-3) was produced as previously reported (Ujiie et al., 2014). Mouse mAb NC16A-3

directing Gln^{545} to Met^{557} within the NC16A domain (kindly provided by Prof. Leena Bruckner-Tuderman) (Hofmann et al., 2009), the mAb BMM62 targeting the ectodomain (Hirako et al., 2014), and the mAb *1A8C* targeting the intracellular domain (Trp^{155} to Arg^{163}, which is a kind gift from Dr. K. *Owaribe*, Nagoya, Japan) (Hirako et al., 1998) of COL17 were used. Rabbit polyclonal antibody Mo-NC14A targeting the mouse NC14A domain (Glu^{499} to Arg^{573}) of mouse COL17 was produced as previously reported (Nishie et al., 2015). The epitope of the mAb NC16A-4 (Asp^{522} to Gln^{545}) was mapped within the NC16A domain using recombinant R7 polypeptides as previously reported (Natsuga et al., 2012; Ujiie et al., 2014).

Supplementary Materials and Methods 4

Cell culture

Primary normal human keratinocytes (NHEKs) isolated from normal human skin obtained from an uninvolved skin from surgical specimens for benign skin tumors were cultured with CnT-Prime Epithelial Culture Medium (CELLnTEC) containing 0.03 mM calcium and supplements. Cells with no more than four passages were used.

Supplementary Materials and Methods 5

Immunofluorescence (IF)

Indirect immunofluorescence (IIF) was performed using different substrates, including normal human skin, normal mouse skin and JEB patient skin lacking COL17 expression. Briefly, 5-µm frozen sections were incubated with primary antibodies and then stained using 1:1,000 diluted FITC or Alexa488-conjugated anti-mouse IgG (Jackson ImmunoResearch Laboratories,). The 1:100 diluted IgG1, IgG2a, IgG2b, and IgG3 (BD Biosciences) were used as the secondary antibodies to identify the IgG subclasses. The images were collected using immunofluorescence microscopy (BX51, Olympus) or confocal laser scanning microscopy (FLUOVIEW FV1000-D, Olympus).

Supplementary Materials and Methods 6

SDS-PAGE and Western blotting

The limited digested COL17 proteins and C-terminally deleted recombinant COL17 proteins were separated by 7% SDS-PAGE gel electrophoresis. The gels were stained with Coomassie blue or were transferred onto nitrocellulose membranes (Bio-Rad). The membranes were blocked at room temperature for 30 minutes in 2% skimmed milk in TBS, followed by incubation with primary antibodies targeting different epitopes on COL17, anti-β tubulin (ab6046, Abcam), and 1:100 diluted patient sera with 2% skimmed milk in TBS at 4°C overnight. Subsequently, horseradish peroxidase

(HRP)-conjugated secondary anti-mouse IgG, anti-human IgG or anti-human IgA in the same buffer were reacted at room temperature for 1 hour. Signals were visualized by Clarity Western ECL Substrate (Bio-Rad).

Supplementary Materials and Methods 7

Immunoprecipitation (IP)

IP was performed using Dynabeads Protein G (Novex, Life Technologies) according to the manufacturer's protocol. Briefly, purified recombinant full-length human collagen XVII, COL17 (Δ1-2), COL17 (Δ1-6), COL17 (Δ1-10), and COL17 (Δ1-14) proteins were immunoprecipitated by the mAbs hC17-ect15 and TS39-3, and control mouse IgG1 (MBL). Immunoprecipitated proteins were loaded on a 7% SDS-PAGE followed by Western blotting using a mAb against DDDDK-tag (M2, Sigma-Aldrich) as the primary antibody.

Supplementary Materials and Methods 8

Enzyme-linked immunosorbent assay (ELISA)

Collagen XVII NC16A ELISA (MESACUP BP180 ELISA kit, MBL) was performed using a 1:10 diluted culture medium of hybridomas and 1:20,000 diluted

HRP-conjugated anti-mouse IgG as the secondary antibody. ELISAs using different recombinant collagen XVII proteins, including full-length human collagen XVII, COL17 (Δ1-6), COL17 (Δ1-10), and COL17 (Δ1-14) proteins were performed as previously described with modification (Izumi et al. 2016). Then, 96-well plates (Thermo Fisher Scientific) were coated with 1 μg/well of recombinant proteins in 50-mM carbonate buffer pH 9.5, and non-specific binding was reduced by blocking plates with 2% bovine serum albumin in PBS at room temperature for 2 hours. LAD sera from patients were diluted to 1:100 and incubated at room temperature for 1 hour. After extensive washing, the plates were incubated with 1:5,000 diluted HRP-conjugated anti-human IgA at room temperature for 1 hour following by color development using a substrate solution (3, 3', 5, 5'-tetramethylbenzidine dihydrochloride/ hydrogen peroxide; TMB/H_2O_2 (KPL). After the color development was stopped by adding 0.12 N hydrochloric acid, the absorbance was measured at 450 nm with the correlation wavelength set at 620 nm by a microplate reader (TECAN Austria GmbH). In the ELISAs using different substrates, the absorbance was normalized to the mAb directing the DDDDK-tag, unless otherwise specified.

Supplementary Materials and Methods 9

Twenty-four hours after the medium was changed from CnT-Prime to DMEM with 10% FBS, the NHEKs were treated with 2.5 μg/ml of the mAb hC17-ect15, mouse IgG1, and TS39-3 for 1.5, 3.0, and 6.0 hours, respectively (Iwata et al., 2009).

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Supplementary Table 1

Summary of Western blotting (WB) and immunoprecipitation (IP) results

	Antibodies	COL17	Δ1-2	Δ1-6	Δ1-10	Δ1-15
WB	Anti-DDDDK	+	+	+	+	+
	hC17-ect15	-	-	+	+	+
	BMM62	+	+	+	+	-
	LAD1	very weak	+	+	+	very weak
	LAD2	very weak	+	+	+	very weak
	LAD3	very weak	+	+	+	very weak
	LAD4	very weak	+	+	+	very weak
	LAD5	very weak	+	+	+	very weak
	LAD6	very weak	+	+	+	very weak
	Normal human sera	-	-	-	-	-
IP	Anti-DDDDK	+	+	+	+	+
	hC17-ect15	very weak	very weak	+	+	+
	mIgG1	-	-	-	-	-

Supplementary Table 2

The sequence of the synthesized partially humanized mouse collagen XVII DNA fragment (1,620 bp)

GCGCATCGATCATATGCCGCTGACACCTGCCTGAAGGCAGATGTGAATGGAGACCTAAAT

ACCGTGTCCACAAAGAGCAAGATGACCTCGGCAGAAAACCATGGCTACGACCGAGGTG

GCGGTGGTGGCAGAGGCAAAGGCGGAGGTGCTGGTGGTGGCGGTGGTGGCGGTGGCGC

CAGTGGCGGTGGAGGAGCATGGGGGGCTGCACCAGCCTGGTGCCCCTGCGGCTCCTGCT

GCAGCTGGTGGAAGTGGCTGCTGGGCCTGCTGCTCACCTGGCTGCTGCTGCTGGGTCTG

CTCTTCGGCCTCATTGCTCTGGCGGAGGAGGTAAGAAAGCTGAAGGCCCGCGTGGAGGA

GCTGGAAAAGACCAAGGTGCTATATCATGACGTCCAGATGGACAAAAGCAACAGGGACC

GCCTCCAGGCCGAGGCACCCAGCCTGGGACCTGGATTAGGCAAGGCTGAGCTGGACGG

CTACAGCCAGGAGGCCATCTGGCTGTTTGTAAGGAACAAGCTGATGACCGAGCAGGAGA

ACGGGAATCTCAGAGGAAGTCCTGGTCCAAAAGGTGACATGGGGAGTCAAGGACCTAA

AGGAGACCGAGGCCTTCCTGGGACCCCAGGTATCCCTGGGCCCCTGGGCCACCCTGGCC

CGGAAGGACCAAAGGGACAAAAAGGCAGCATTGGAGATCCTGGCATGGAAGGACCCAT

AGGCCAGAGAGGACTAGCAGGCCCCATGGGACCTCGTGGTGAACCCGGGCCTCCTGGG

TCTGGAGAGAAAGGGGAAAGAGGGGCTGCTGGTGAACCAGGTCCTCATGGCCCACCTG

GTGTCCCAGGTTCTGTGGGTCCCAAAGGTTCCAGCGGCTCTCCTGGCCCACAGGGCCCT

CCAGGTCCTGTAGGTCTCCAAGGGCTCCGAGGTGACGTGGGACTTCCTGGTGTCAAAGG

TGACAAAGGACTCATGGGACCACCAGGACCCAAAGGTGACCAGGGTGAGAAGGGACCC

AGAGGCCTCACAGGGGAGCCTGGCATTCGAGGTTTGCCTGGAGCTGTGGGTGAACCCG

GAGCCAAAGGCGCAATGGGTCCGGCTGGCGCTGATGGACAGCAAGGTTCCAGAGGTGA

ACAAGGCTTGACAGGGATGCCTGGAACCCGGGGCCCCCCAGGACCCGCTGGAGACCCA

GGAAAGCCAGGTCTCACAGGACCCCAGGGACCTCAGGGACTTCCTGGTAGCCCTGGCC

GACCAGGGACTAAAGGCGAACCCGGCGCTCCCGGCAGAGTCATGACTTCAGAGGGATCA

TCAACAATCACTGTGCCCGGACCTCCCGGACCTCCTGGTGCCATGGGTCCCCCAGGACC

TCCAGGGACGCCAGGTCCAGCTGGCCCTGCTGGTCTCCCAGGACAACAAGGCCCACGA

GGGGAGCCAGGACTTGCTGGTGACTCATTCCTAAGCAGTGGCAGCTCCATCTCTGAGGT

CCTCTCTGCCCAAGGTGTTGACTTACGAGGTCCCCCTGGCCCACCTGGCCCACGAGGGC

CACCAGGGCCTTCCATCCCAGGCCCGCCAGGACCCAGAGGTCCACCAGGGGAAGGCGT

ACCAGGCCCACCCGGGCCACCAGGATCCGCGGCCGCACCG

The ClaI restriction site is underlined, and the NdeI restriction site is doubled-underlined. The BamHI restriction site is marked with a dotted line, and the NotI restriction site is marked with a wavy line. The human sequence is highlighted in gray.