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Title:

C-terminal processing of collagen XVII induces neoepitopes for linear IgA dermatosis autoantibodies

Running title:

Neoepitopes on COL17 for LAD autoantibodies

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Abbreviations

BP, bullous pemphigoid; COL17, collagen XVII; DEJ, dermal-epidermal junctions; DIF, direct immunofluorescence; ECM, extracellular matrix; IF, immunofluorescence; IIF, indirect immunofluorescence; IP, immunoprecipitation; JEB, junctional epidermolysis bullosa; LAD, linear IgA dermatosis; NC, non-collagenous; NHEK, normal human epidermal keratinocyte

Abstract

Transmembrane collagen XVII (COL17) is a hemidesmosomal component of basal keratinocytes that can be targeted by autoantibodies in autoimmune blistering disorders, including linear IgA dermatosis (LAD). The COL17 can be physiologically cleaved within the juxtamembranous extracellular NC16A domain, and LAD autoantibodies preferentially react with the processed ectodomains, indicating that the processing induces neoepitopes. However, the details of how neoepitopes develop have not been elucidated. In this study, we show that C-terminal processing of COL17 also plays a role in inducing neoepitopes for LAD autoantibodies. First, a monoclonal antibody (mAb) hC17-ect15 targeting the 15th collagenous domain of COL17 was produced, which showed characteristics similar to LAD autoantibodies. Interestingly, the mAbs preferentially reacted with C-terminally deleted (up to 682 amino acids) recombinant COL17, suggesting that C-terminal processing reveals neoepitopes on the 15th collagenous domain. The LAD autoantibodies also react with C-terminal deleted COL17. Therefore, neoepitopes for LAD autoantibodies also develop after C-terminal processing. Finally, the passive transfer of the mAbs hC17-ect15 into human COL17-expressing transgenic mice failed to induce blistering disease, suggesting that neoepitope-targeting antibodies are not always pathogenic. In summary, this study shows that C-terminal processing induces dynamic structural changes and neoepitopes for LAD autoantibodies on COL17.

Introduction

The processing of proteins is a crucial event in cell biological functions, and nearly 2% of mammalian genes encode proteases (Overall and Blobel, 2007). However, the impaired processing of proteins may be involved in the development of various disorders, such as Alzheimer's disease, sensory processing disorder, and TNF-receptor-associated periodic febrile syndrome (Overall and Blobel, 2007; Puts et al., 2014), which shows that the regulated processing of proteins is essential for mammals. Protein processing, regardless of the physiological or pathological setting, may induce new antigenic sites on cleaved polypeptides, and such sites are known as neoepitopes (Mort and Buttle, 1999). Neoepitopes are known to be targeted by autoimmunity in autoimmune disorders, such as systemic lupus erythematosus (Bigler et al., 2009). Moreover, it is well known that autoantibodies in autoimmune blistering disorders may target neoepitopes on processed epidermal proteins, including collagen XVII (COL17) (Zone et al., 1990, 1998; Marinkovich et al., 1996; Zillikens et al., 1999).

COL17, which is also known as BP180 or BPAG2, is a major component of hemidesmosomes, which adhere basal keratinocytes to the underlying basement membrane (Diaz et al., 1990; Giudice et al., 1992; Green and Jones, 1996; Jones et al., 1998). COL17 is associated with both congenital and acquired blistering disorders (McGrath et al., 1995; Gatalica et al., 1997; Franzke et al., 2003). Autoimmunity to COL17 induces various autoimmune blistering skin disorders, including bullous

pemphigoid (BP), mucous membrane pemphigoid, gestational pemphigoid, and linear IgA dermatosis (LAD) (Diaz et al., 1990; Giudice et al., 1992; Schmidt and Zillikens, 2013). LAD is a distinct IgA-class autoantibody-mediated autoimmune blistering disorder that commonly occurs in children and adolescents (Wojnarowska et al., 1988; Zone et al., 1998; Schmidt and Zillikens, 2013). LAD autoantibodies may target various proteins at the dermal-epidermal junction (DEJ), including collagens IV, VII, XVII, and laminin 332 (Zone et al., 1990; Fortuna and Marinkovich, 2012). In sharp contrast to IgG BP autoantibodies, which preferentially target full-length COL17 (Izumi et al., 2016), LAD autoantibodies preferentially react with processed COL17 LAD-1 and LABD97 (Zone et al., 1990, 1998; Marinkovich et al., 1996; Schumann et al., 2000; Hirako et al., 2003; Hofmann et al., 2009; Yamauchi et al., 2014).

COL17 can be physiologically cleaved within the juxtamembranous non-collagenous (NC) 16A domain by ADAM9/17 (Hirako et al., 1998; Franzke et al., 2002, 2004, 2009, Nishie et al., 2010, 2012), which yields 120-kDa COL17 ectodomains. This ectodomain shedding is thought to be associated with the migration, differentiation, and development of basal keratinocytes (Franzke et al., 2005). Regarding the pathological settings of blistering disorders, both plasmin and neutrophil elastase, which are major proteases present in BP lesional skin (Verraes et al., 2001; Schmidt et al., 2004), are known to cleave COL17 within the NC16A domain (Hofmann et al., 2009; Lin et al., 2012). Those 120-kDa COL17 ectodomains may be further processed into 97-kDa polypeptides by

Carboxyl-terminal (C-terminal) processing within the NC4 domain (Hirako et al., 2003; Hofmann et al., 2009). Although the physiological roles of C-terminal processing in COL17 remain obscure, our group has recently reported that impaired C-terminal processing due to R1303Q mutation induces duplications of the basement membrane in skin (Nishimura et al., 2016).

The cleavage of COL17 within the NC16A domain can induce drastic changes of antigenicity around the cleavage sites (Nishie et al., 2010). In fact, previous studies have reported that approximately 20% of LAD autoantibodies target the NC16A domain of COL17 (Zillikens et al., 1999). In addition, the boundary region between the NC16A domain and the 15th collagenous domain of COL17 has been reported to be a candidate epitope for LAD autoantibodies (Yamauchi et al., 2014). Furthermore, the 15th collagenous domain of COL17 has been reported to be targeted primarily by LAD autoantibodies (Nie et al., 2000). These different observations indicate that processing-dependent neoepitope induction on COL17 may be more dynamic than expected.

By studying a unique monoclonal antibody (mAb) that preferentially targets LAD-1 and LABD97, and by using various recombinant COL17 proteins, we provide the evidence that C-terminal cleavage induces neoepitopes for LAD autoantibodies. Notably, the neoepitopes can develop on the 15th collagenous domain of COL17, which is a domain that is far from the processed regions, including the NC16A domain or the C-termini. The present study shows that the processing of COL17 may

induce dynamic structural changes that are involved in the autoimmunity of LAD.

Results

LAD autoantibodies preferentially react to LAD-1 and LABD97 derived from recombinant COL17

Eleven out of 18 LAD sera reacted with LAD-1 and/or LABD97 derived from recombinant human COL17 but not with the full-length form **(Figure 1b, Supplementary Figure S1a).** In contrast, BP sera (n=16) reacted not only with the full-length COL17 but also with LAD-1 and LABD97 **(Figure 1b, Supplementary Figure S1a).**

The mAb hC17-ect15 targets the 15th collagenous domain of processed COL17

The mAb hC17-ect15 targets human skin but not junctional epidermolysis bullosa (JEB) skin or mouse skin **(Figure 1d, Supplementary Figure S1b)**, and it preferentially reacted with LAD-1 and LABD97 but not with full-length human COL17. In contrast, the mAb BMM62 recognized all of them **(Figure 1d)**. IIF confirmed that the mAb hC17-ect15 was IgG1 **(Supplementary Figure S1c)**. As reported in previous studies, full-length COL17 presents not only at the DEJ but also at the apicolateral membrane of the basal cells (Hirako et al., 1998; Nishie et al., 2010), and the mAb 1A8c targeting the intracellular domain of COL17 showed apicolateral regions in addition to the DEJ. Meanwhile, the mAb hC17-ect15 showed linear deposition at the DEJ **(Supplementary Figure S2)**. Negative reactivity to the NC16A domain in the NC16A enzyme-linked immunosorbent assay (ELISA) (not shown) and positive reactivity to COL17 (Δ1-14) recombinant protein (Met^{1} to Met^{816}) in Western blotting **(Figure 2a)** suggest that the epitope is present on the 15th collagenous domain

(Gly^{567} to Met^{816}). As shown in **Figure 2b**, the antibody Mo-NC14A recognizes both recombinant mouse COL17 and partially humanized mouse COL17, whereas the mAb hC17-ect15 only recognizes the plasmin-digested and partially humanized COL17 (**Figure 2b,** arrowheads). The positive reactivity of the mAb hC17-ect15 to partially humanized mouse COL17 with the mouse 13th collagenous domain (Asp^{652} to Pro^{691}) replaced by a portion of the human 15th collagenous domain (Glu^{645} to Leu^{684}) **(Figure 1c, Figure 2b)** indicates that the mAb targets the swapped region.

C-terminal processing induces neoepitopes on the 15th collagenous domain of COL17

Interestingly, some LAD sera reacted more strongly to LABD97 than with LAD-1 **(Figure 1b, LAD cases No. 2, 5, 7, and 10)**. Since LABD97 is produced by the processing of LAD-1 within the C-terminal NC4 domain **(Figure 1a)** (Nishimura et al., 2016), the preferential reactivity of LAD autoantibodies to LABD97 indicates that the C-terminal cleavage of COL17 may play a role in the development of neoepitopes. To address this hypothesis, immunoprecipitation (IP) and ELISA were performed using mAbs and recombinant COL17 proteins for which C-termini had been serially deleted **(Figure 1a)**. The mAb hC17-ect15 immunoprecipitates COL17 (Δ1-6), COL17 (Δ1-10), and COL17 (Δ1-14) more efficiently than it immunoprecipitates full-length COL17 or COL17 (Δ1-2) **(Figure 2c).** Of note, the epitope of the mAb hC17-ect15 on the 15th collagenous domain (Glu^{645} to Leu^{684}) is far from the C-terminal ends **(Figure 1a)**, which suggests that C-terminal processing may induce structural changes in various domains of COL17. As expected, an ELISA using C-terminally

truncated COL17 proteins as substrates and mAbs recognizing different epitopes revealed that C-terminal modification induces structural changes in different regions on COL17 **(Supplementary Figure S3)**. These results reveal that some epitopes are exposed while others are hidden when the C-termini of COL17 have been deleted**.**

LAD autoantibodies target neoepitopes on C-terminal-deleted COL17

Western blotting using C-terminal-deleted recombinant COL17 showed similar results to IP **(Figure 2a, 2c)**, which suggests that the structural modifications on the recombinant proteins are at least partially conserved in the samples that were loaded onto SDS-PAGE gels. Therefore, 6 LAD patients' sera (LAD No.1-6 of **Figure 1b** and **Supplementary Figure S1a**) were tested by Western blotting using these recombinant proteins. LAD autoantibodies showed weak reactivity to both the full-length COL17 and the COL17 with the largest c-terminal deletion (Δ1-14). In contrast, they tended to react strongly with COL17 (Δ1-2), COL17 (Δ1-6), and COL17 (Δ1-10) **(Figure 2d, Supplementary Figure S4, Supplementary Table 1)**. These results indicate that the C-terminal processing of COL17 induces neoepitopes that are preferentially targeted by LAD autoantibodies.

A C-terminal-deleted COL17 (Δ1-6) detects LAD autoantibodies more efficiently than full-length COL17 in ELISA

Based on these results, we hypothesized that C-terminal-deleted COL17 may be useful for detecting

LAD autoantibodies by ELISA. Full-length COL17 (Met^{1} to Pro^{1497}) and COL17 (Δ1-6) (Met^{1} to Arg^{1174}) proteins were used as substrates for the ELISA. Sera from 9 LAD patients who reacted with LAD-1 and/or LABD97 derived from recombinant human COL17 but not the full-length form were analyzed. The LAD sera showed greater reactivity to both full-length COL17 and COL17 (Δ1-6) than that of normal control sera **(Figure 3, left and middle panels)**. As expected, LAD autoantibodies were found to have significantly greater reactivity to COL17 (Δ1-6) protein than to full-length COL17. **(**p=0.017, **Figure 3, right panel)**. These results indicate that the C-terminal-deleted protein COL17 (Δ1-6) detects LAD autoantibodies more efficiently than full-length COL17 does.

In vivo and in vitro pathogenicity of the mAb hC17-ect15

The final question is whether the mAb hC17-ect15 is pathogenic for blister formation. IIF studies showed that the mAbs hc17-ect15 and TS39-3 (1 mg/ml) both react with the DEJ of normal human skin up to a dilution of 1:40,000 **(Supplementary Figure S5)**. The passive transfer of the mAbs hC17-ect15 (100 µg/ear) and TS39-3 (50 µg/ear) into the ears of adult COL17-humanized mice showed that the mAb TS39-3 induces skin fragility. In contrast, the mAb hC17-ect15 and the mouse IgG1 isotype control both failed to induce skin fragility **(Figure 4a)**. DIF showed that mouse IgG1 and the mAbs TS39-3 and hC17-ect15 were linearly deposited at the DEJ **(Figure 4b)**. The positive reactivity of the mouse IgG1 isotype control was attributed to the high pressure of locally injected

mAbs in the dermis, as observed in a previous study (Tonra and Mendell, 1997). Consistent with the *in vivo* data, NHEKs treated with the pathogenic mAb TS39-3 reduced the expression of COL17. In contrast, the mAb hC17-ect15 showed no effect **(Figure 4c)**.

Discussion

This study has demonstrated that cleavage of COL17 not only within the NC16A domain but also in the C-termini induce neoepitopes on the 15th collagenous domain. Notably, the neoepitopes on the 15th collagenous domains are far from the processed regions, including the NC16A domain and the C-terminus, which indicates that processing induces dynamic conformational changes in COL17 and neoepitopes in different regions. Although it is uncertain as to whether LAD autoantibodies also target the same neoepitopes of the mAb hC17-ect15, the current data are consistent with a study reporting that the main epitopes of LAD autoantibodies are present in the 15th collagenous domain (Nie et al., 2000). Taken together, the findings suggest that collagenous domains of COL17 may be involved in the development of neoepitopes for LAD autoantibodies.

We initially wondered why it is possible for LAD autoantibodies to detect LAD1 and LABD-97 in Western blotting because SDS-PAGE in a denatured setting is generally believed to cause proteins to lose their secondary structures. In this study, we happened to find that the preferential reactivity of the mAb hC17-ect15 to C-terminal-deleted COL17, including COL17 (Δ1-6), COL17 (Δ1-10) and

COL17 (Δ1-14), was observed not only by IP but also by Western blotting. These findings suggest that conformation-dependent neoepitopes on the 15th collagenous domain can be retained even in a denatured setting. The phenomenon may be explained, at least in part, by the fact that proteins containing polyproline II structures, such as collagens, can retain their secondary structures even in denatured settings induced by dithiothreitol, 4 M urea, or SDS (Rath et al., 2005; Lopes et al., 2014). This notion is in line with the findings that neoepitopes for the mAb hC17-ect15 and LAD autoantibodies were both identified on the 15th collagenous domain of COL17 (Nie et al., 2000), which will be presumably detected by Western blotting in denatured settings. However, the mAb hC17-ect15 can weakly react with native full-length COL17 by IP and ELISA, which suggests that neoepitopes for the mAb hC17-ect15 are not completely masked on the unprocessed form.

Because neoepitopes on the C-terminal-deleted COL17 can be retained in a denatured setting of SDS-PAGE, we revealed that LAD autoantibodies also target the neoepitopes on the deleted mutants. Interestingly, LAD autoantibodies preferentially target the C-terminal-deleted COL17 (Δ1-2) proteins, whereas the mAb hC17-ect15 does not. This observation indicates that other neoepitopes in addition to the mAb hC17-ect15 develop when the C-terminus has been processed in COL17. Since COL17 has 15 collagenous domains in its extracellular domain (Giudice et al., 1992), it may be possible that other neoepitopes can develop in various domains of the molecule and can be targeted by LAD autoantibodies.

Concerning clinical applications, this study may provide a clue and serve as an ELISA using C-terminal-deleted COL17 protein COL17 (Δ1-6) (Met^{1}-Arg^{1174}). Previous attempts to detect LAD autoantibodies by ELISA used a bacterial NC16A domain polypeptide (Zillikens et al., 1999) or the 120-kD entire ectodomain of COL17 produced from HEK293 cells (Csorba et al., 2011). Since the NC16A domain contains a portion of epitopes for LAD autoantibodies, only 20% of LAD autoantibodies were detectable by an ELISA using NC16A domain polypeptides (Zillikens et al., 1999). In contrast, an ELISA using the entire COL17 ectodomain (Glu^{490} to Pro^{1497}) enabled detection of 83.3% of LAD autoantibodies (Csorba et al., 2011). It should be noted that the entire ectodomain of COL17 used in this study is not the same as LAD-1 because the recombinant protein was produced from the first amino acid of the extracellular domain of COL17 (Glu^{490}), whereas Leu^{524} is the N-terminus of LAD-1 (Hirako et al., 2003). The fact that the recombinant 120-kD ectodomain of COL17 can detect LAD autoantibodies regardless of having a different N-terminus from LAD-1 indicates that the loss of the intracytoplasmic and transmembrane domain also induces neoepitopes on the ectodomains.

Although it remains uncertain whether LAD autoantibodies play direct pathogenic roles in blister formation, a previous study using the passive transfer of mouse IgA mAb against LABD97 into SCID mice with human skin transplanted on the back induced the deposition of IgA and

inflammation at the basement membrane zone (Zone et al., 2004). To verify the pathogeneses of our LAD-like mAb hC17-ect15, we performed passive transfer with COL17-humanized mice. The mAb failed to induce skin fragility, which could be explained by the inability of the mAb to deplete COL17 expression in the *in vitro* experiment. In contrast, BP autoantibodies and pathogenic/non-pathogenic mAbs directing the NC16A domain of COL17 can deplete COL17 expression in normal human skin and HaCaT keratinocytes (Iwata et al., 2009; Natsuga et al., 2012; Wada et al., 2016). In line with these reports, the mAb hC17-ect15 did not reduce the expression of COL17 in cultured NHEKs. Since mAb hC17-ect15 is IgG1, different isotypes may induce different pathogenicity. Thus, we are now trying to induce class-switching of the mAbs (*e.g.*, from IgG1 to IgA), which will be reported in future studies.

In conclusion, it is not only cleavage within the NC16A domain that plays a role in the development of neoepitopes for LAD autoantibodies in COL17; C-terminal processing affects neoepitopes as well, and this processing may be associated with LAD autoimmunity.

Materials and Methods

LAD and BP patients

LAD was diagnosed based on linear IgA deposition at the DEJ as determined by direct immunofluorescence (DIF) and on clinical manifestations, including blistering or erosions on the

skin and/or mucosa (Schmidt and Zillikens, 2013). BP criteria were defined as previously reported (Izumi et al., 2016). Sera samples from 18 LAD patients and 16 BP patients were used. This study was approved by the Ethics Committee of Hokkaido University (15-025) and fully written informed consent was obtained from all patients and healthy volunteers for the use of their materials. All the studies using human materials were performed according to the principles of the Declaration of Helsinki.

Production of recombinant COL17

Full-length human COL17 (NM_000494, Met^{1} to Pro^{1497}) (Izumi et al., 2016) and 4 different truncated forms, COL17 (Δ1-2) (Met^{1} to Asp^{1340}), COL17 (Δ1-6) (Met^{1} to Arg^{1174}), COL17 (Δ1-10) (Met^{1} to Pro^{977}), and COL17 (Δ1-14) (Met^{1} to Met^{816}), whose C-termini were serially deleted were produced as previously reported (Nishie et al., 2011) **(Figure 1a)**. The production of mouse full-length COL17 was described in **Supplementary Materials and Methods 1**. In the partially humanized mouse COL17, a portion of the mouse 13^{th} collagenous domain (Asp^{652} to Pro^{691}) was replaced by a portion of the human 15^{th} collagenous domain (Glu^{645} to Leu^{684}) **(Figure 1c, Supplementary Table 2, Supplementary Materials and Methods 2).** The limited digestion of recombinant human COL17 by plasmin was performed as previously reported (Hofmann et al., 2009; Yamauchi et al., 2014; Izumi et al., 2016; Nishimura et al., 2016).

Antibodies targeting different epitopes on COL17, cell culture, immunofluorescence (IF) studies, Western blotting, immunoprecipitation (IP) and ELISA

Methods for these experiments were described in **Supplementary Materials and Methods 3 to 8**.

Passive transfer of mAbs into COL17-humanized mice

COL17-humanized mice, whose skin expresses human COL17 and not mouse COL17, were generated as previously described (Nishie et al., 2007). A dose of 100 μg/50 μl of the mAb hC17-ect15, 50 μg/50 μl of TS39-3 and 100 μg/50 μl of control mouse IgG1 was injected once into the dermis of the ears of adult mice. At 48 hours after injection, skin detachment was evaluated by gentle rubbing (Nishie et al., 2007; Ujiie et al., 2014; Wada et al., 2016). Briefly, the ear skin was gently stretched and mechanical shearing forces were applied by the same investigator repeatedly (three times). After the evaluation of skin detachment, the ears were sampled for DIF.

Treatment of cultured NHEK with mAbs

NHEKs were treated with the mAbs and lysed for Western blotting **(Supplementary Materials and Methods 9)**.

Statistical analyses

All statistical analyses were performed using R version 3.1.2. P-values of < 0.05 were considered

significant. The correlation of optical density (OD) absorbance by ELISA for full-length and COL17 (Δ1-6) proteins was assessed by a Welch t-test and a paired t-test.

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Conflicts of interest

The authors declare there are no conflicts of interest associated with this manuscript.

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Figure legends

Figure 1

IIF and Western blotting detected by LAD autoantibodies and mAbs

(a) Schematic of full-length human COL17 (COL17), LAD-1, LABD97, and recombinant C-terminal-deleted COL17. The epitope of each antibody is shown at the bottom. **(b)** Western blotting using a mixture of full-length COL17, LAD-1, and LABD97 was analyzed using LAD (No. 1, 2, 5, 7, and 10) and BP sera (No. 3 and 4) followed by incubation with HRP-conjugated anti-human IgA and IgG, respectively. N: normal control sera. **(c)** Schematic of recombinant mouse COL17 and partially humanized mouse COL17. **(d)** IIF staining of normal human skin using hC17-ect15. Scale bar: 100 μm. **(e)** Western blotting using full-length COL17 with versus without digestion by plasmin. The right panel shows the Coomassie blue staining.

Figure 2

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

(a) Western blotting using recombinant human full-length COL17, COL17 (Δ1-2), COL17 (Δ1-6), COL17 (Δ1-10), and COL17 (Δ1-14) proteins were detected by the mAbs anti-DDDDK-tag, hC17-ect15, and BMM62. **(b)** Western blotting using mouse COL17 (upper panels) and partially humanized COL17 (lower panels) detected by the mAb hC17-ect15 and the polyclonal antibody

Mo-NC14A as a positive control. **(c)** The full-length COL17 and COL17 (Δ1-2), COL17 (Δ1-6), COL17 (Δ1-10), and COL17 (Δ1-14) were immunoprecipitated by the mAbs TS39-3, hC17-ect15, and control mIgG1 followed by Western blotting using anti-DDDDK-tag mAbs. **(d)** Western blotting using COL17, COL17 (Δ1-2), COL17 (Δ1-6), COL17 (Δ1-10), and COL17 (Δ1-14) detected by the mAb anti-DDDDK-tag, normal human sera and LAD sera. The mAb anti-DDDDK-tag shows that the amounts loaded for the five proteins were equal.

Figure 3

ELISA using full-length COL17 and COL17 (Δ1-6)

Sera from normal controls (n=10) and LAD patients (n=9, No. 1-9 in **Figure 1b, Supplementary Figure S1a**) were assayed in an ELISA using full-length COL17 (COL17) (left) and COL17 (Δ1-6) (middle) as substrates without normalization using the mAb anti-DDDDK-tag. Note that COL17 (Δ1-6) and full-length COL17 (COL17) can detect LAD autoantibodies. LAD autoantibodies have significantly greater binding affinity to COL17 (Δ1-6) than to COL17 (right). OD was normalized by the mAb anti-DDDDK-tag. Dotted lines indicate +2SD from the normal control.

Figure 4

In vivo and in vitro pathogenicity of the mAb hC17-ect15

(a) Normal control mouse IgG1, the mAbs TS39-3, and hC17-ect15 were injected into the ears of

adult COL17-humanized mice (n=4/group) 48 hours before rubbing. TS39-3 causes skin detachment, whereas hC17-ect15 fails to induce skin fragility. **(b)** DIF shows linear deposition at the DEJ in all the injected mice (yellow arrows). Epi: epidermis. Scale bar: 100 μm. **(c)** NHEKs were treated with mouse IgG1, the mAbs TS39-3 or hC17-ect15 for 1.5, 3 and 6 hours, respectively. Total cell lysates were subjected to Western blotting detected with anti-COL17 (mAb NC16A-3) and β-tubulin. The expression level of collagen XII is reduced in the mAb TS39-3-treated NHEKs. However, control mouse IgG1 and the mAb hC17-ect15 fail to deplete the expression of COL17.

Fig. 1

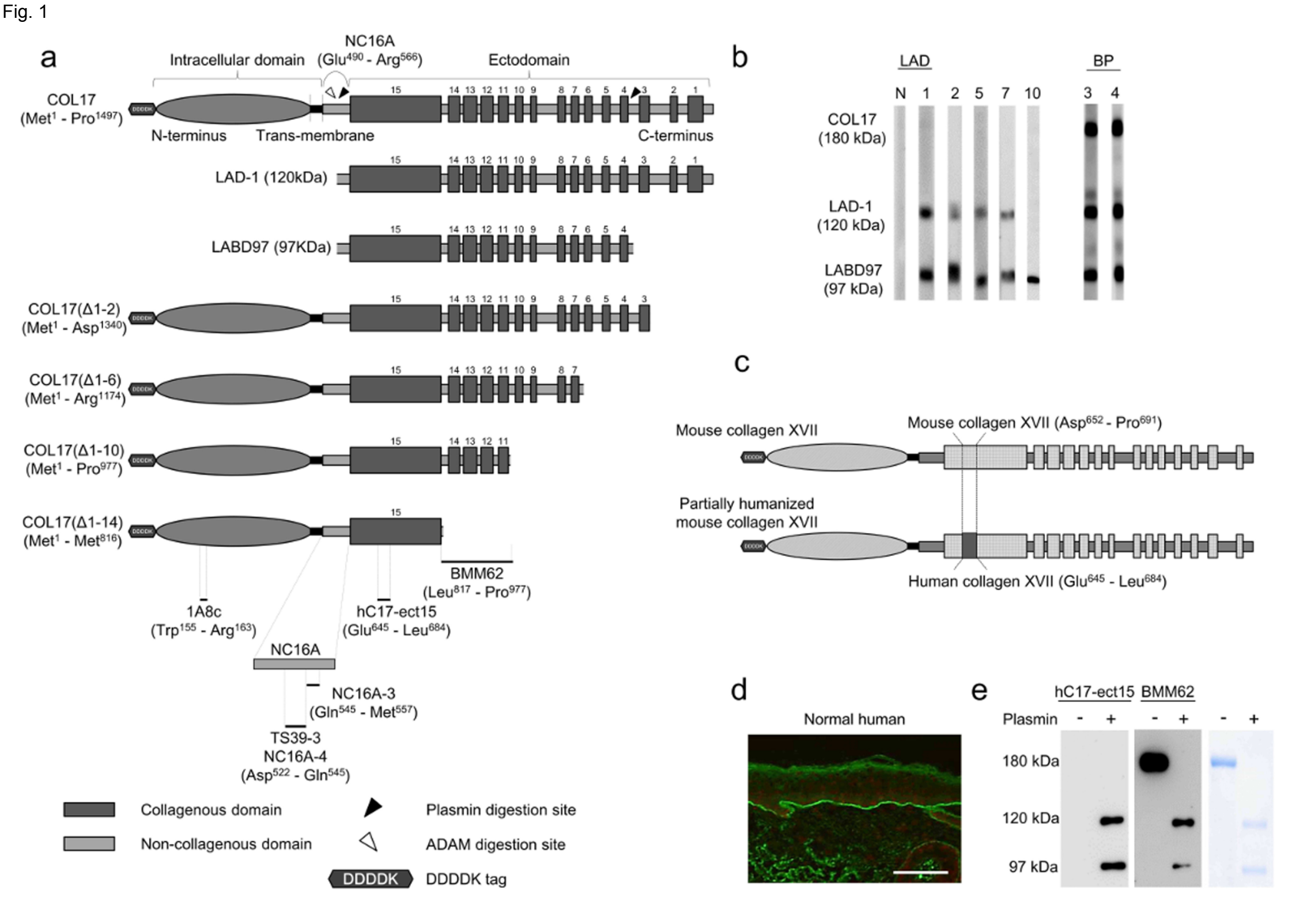

a
NC16A
Intracellular domain
(Glu490 - Arg566)
Ectodomain
COL17
(Met1 - Pro1497)
N-terminus
Trans-membrane
C-terminus
LAD-1 (120kDa)
LABD97 (97KDa)
COL17(Δ1-2)
(Met1 - Asp1340)
COL17(Δ1-6)
(Met1 - Arg1174)
COL17(Δ1-10)
(Met1 - Pro977)
COL17(Δ1-14)
(Met1 - Met816)
BMM62
(Leu817 - Pro977)
1A8c
(Trp155 - Arg163)
hC17-ect15
(Glu645 - Leu684)
NC16A
NC16A-3
(Gln545 - Met557)
TS39-3
NC16A-4
(Asp522 - Gln545)
Collagenous domain
Non-collagenous domain
Plasmin digestion site
ADAM digestion site
DDDDK
DDDDK tag
b
LAD
BP
N 1 2 5 7 10 3 4
COL17
(180 kDa)
LAD-1
(120 kDa)
LABD97
(97 kDa)
c
Mouse collagen XVII
Mouse collagen XVII (Asp652 - Pro691)
Partially humanized
mouse collagen XVII
Human collagen XVII (Glu645 - Leu684)
d
Normal human
e
hC17-ect15
BMM62
Plasmin - + - + - +
180 kDa
120 kDa
97 kDa

Fig. 2

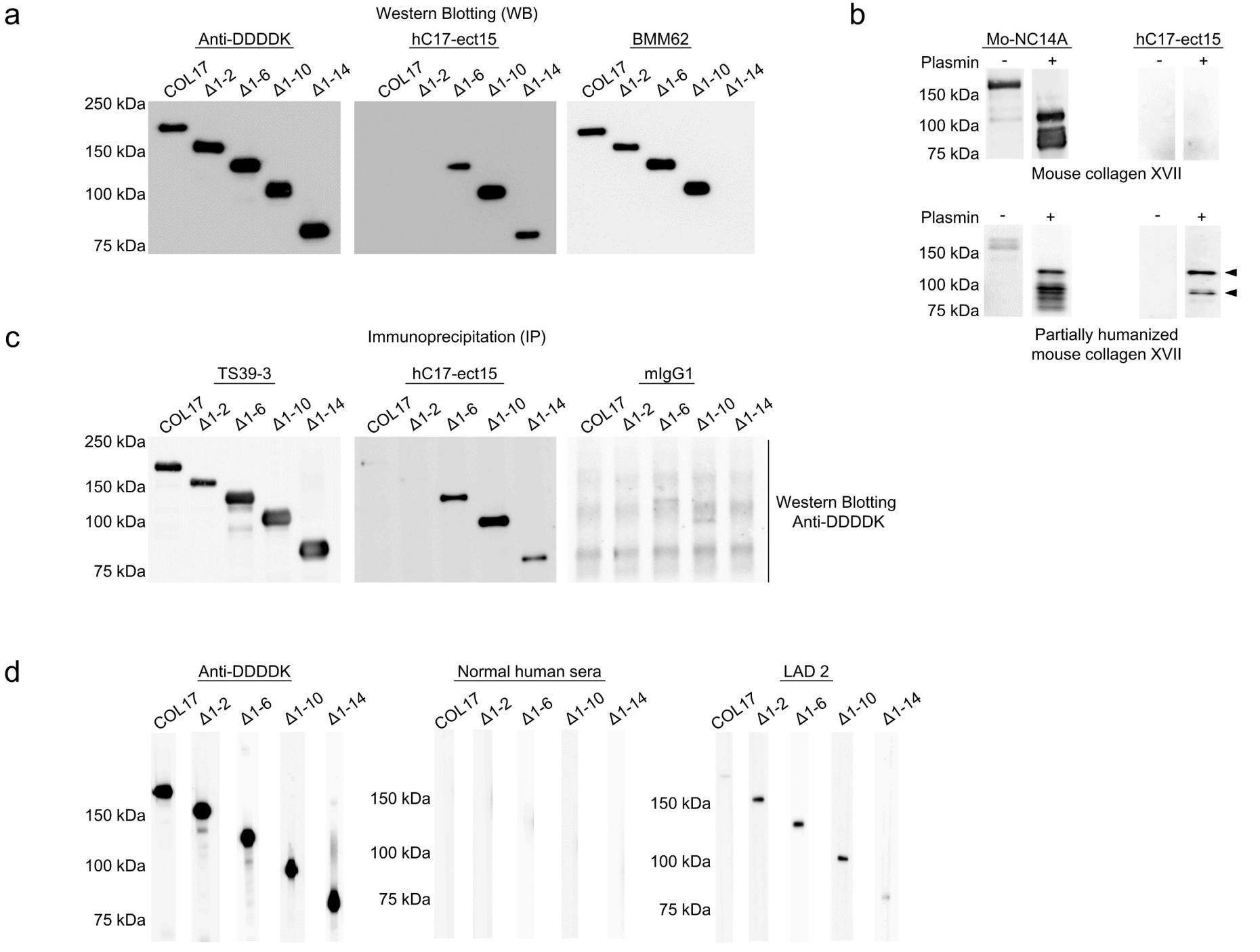

a
Western Blotting (WB)
Anti-DDDDK
hC17-ect15
BMM62
COL17
Δ1-2
Δ1-6
Δ1-10
Δ1-14
250 kDa
150 kDa
100 kDa
75 kDa
b
Mo-NC14A
hC17-ect15
Plasmin
-
+
150 kDa
100 kDa
75 kDa
Mouse collagen XVII
Partially humanized
mouse collagen XVII
c
Immunoprecipitation (IP)
TS39-3
hC17-ect15
mIgG1
Western Blotting
Anti-DDDDK
d
Anti-DDDDK
Normal human sera
LAD 2

Fig. 3

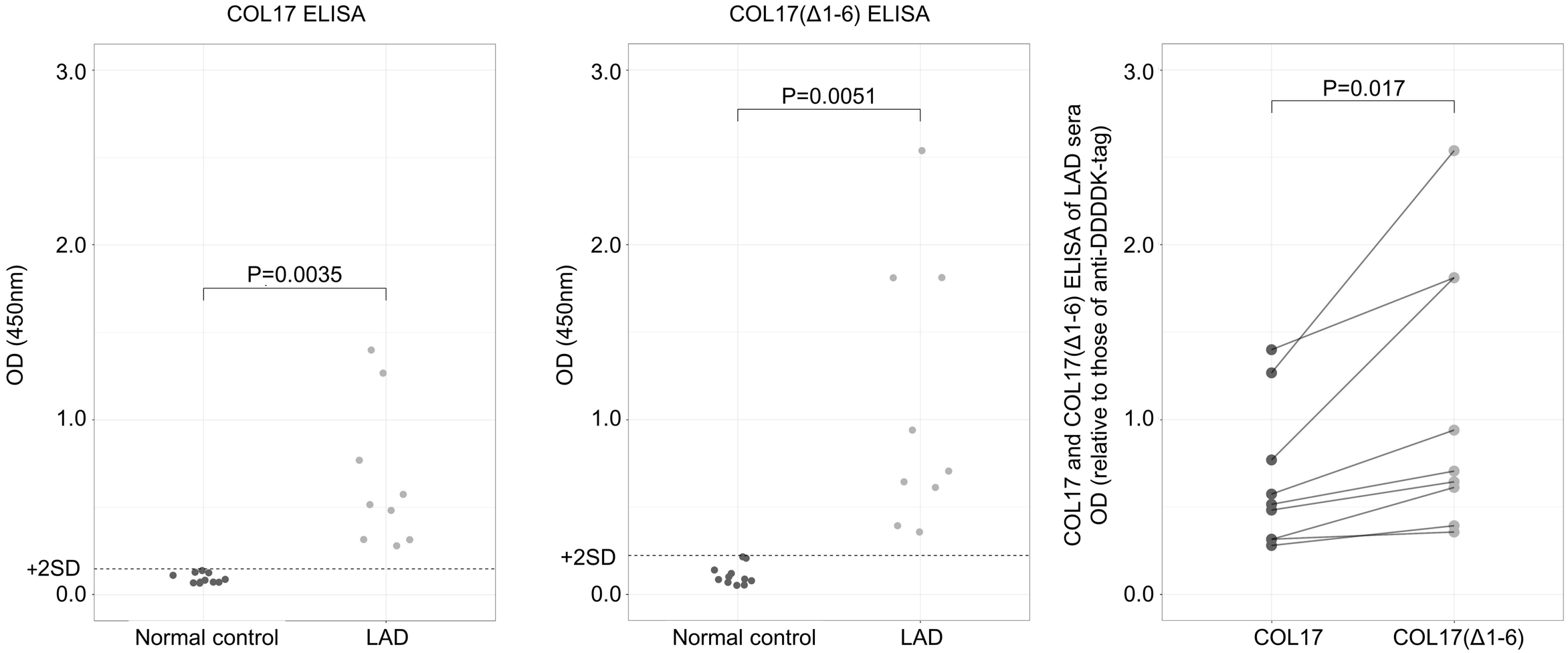
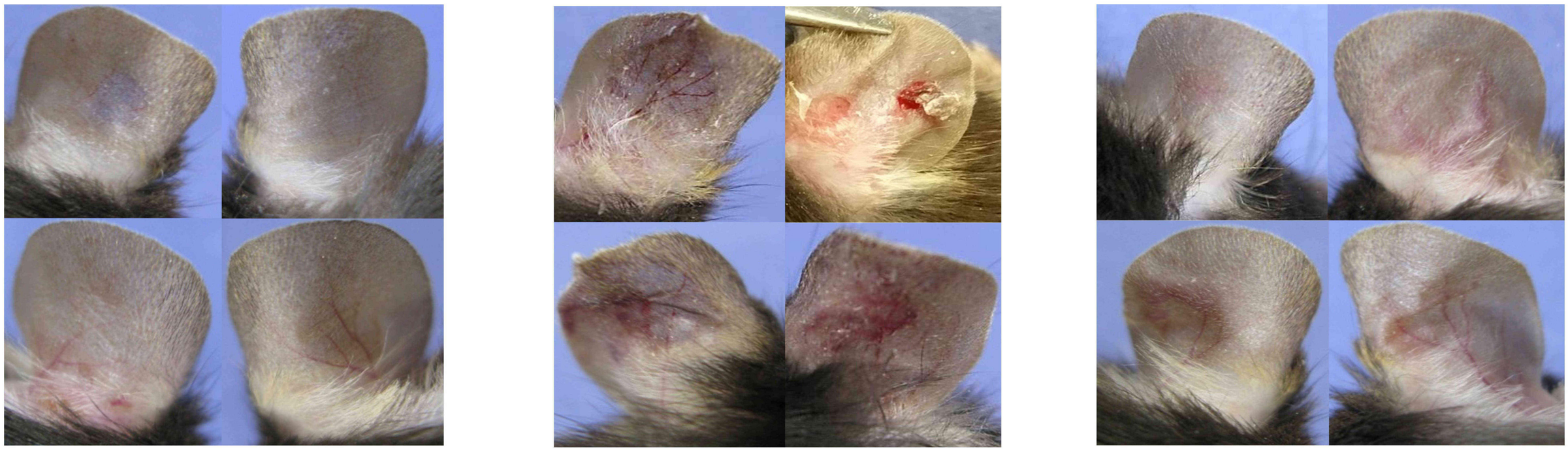
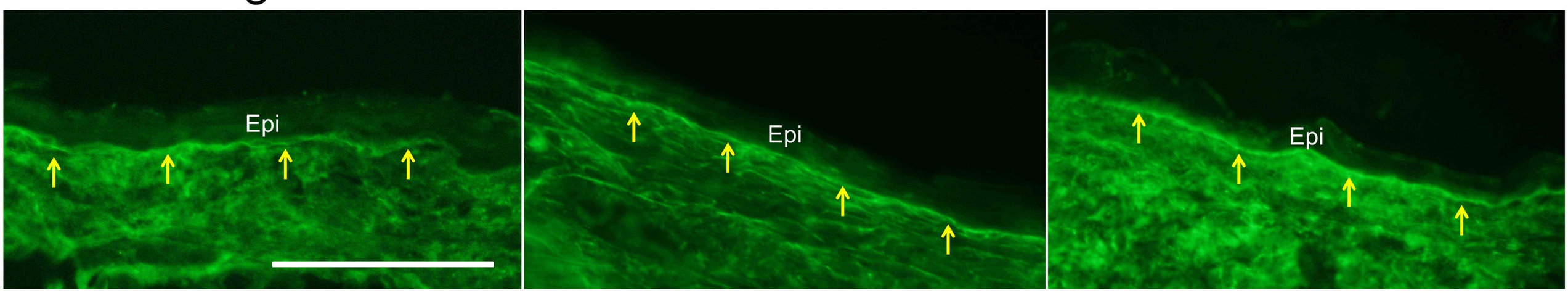

COL17 ELISA
OD (450nm)
3.0
2.0
1.0
+2SD
0.0
P=0.0035
Normal control
LAD
COL17(Δ1-6) ELISA
OD (450nm)
3.0
2.0
1.0
+2SD
0.0
P=0.0051
Normal control
LAD
COL17 and COL17(Δ1-6) ELISA of LAD sera
OD (relative to those of anti-DDDDK-tag)
3.0
2.0
1.0
0.0
P=0.017
COL17
COL17(Δ1-6)

Fig. 4

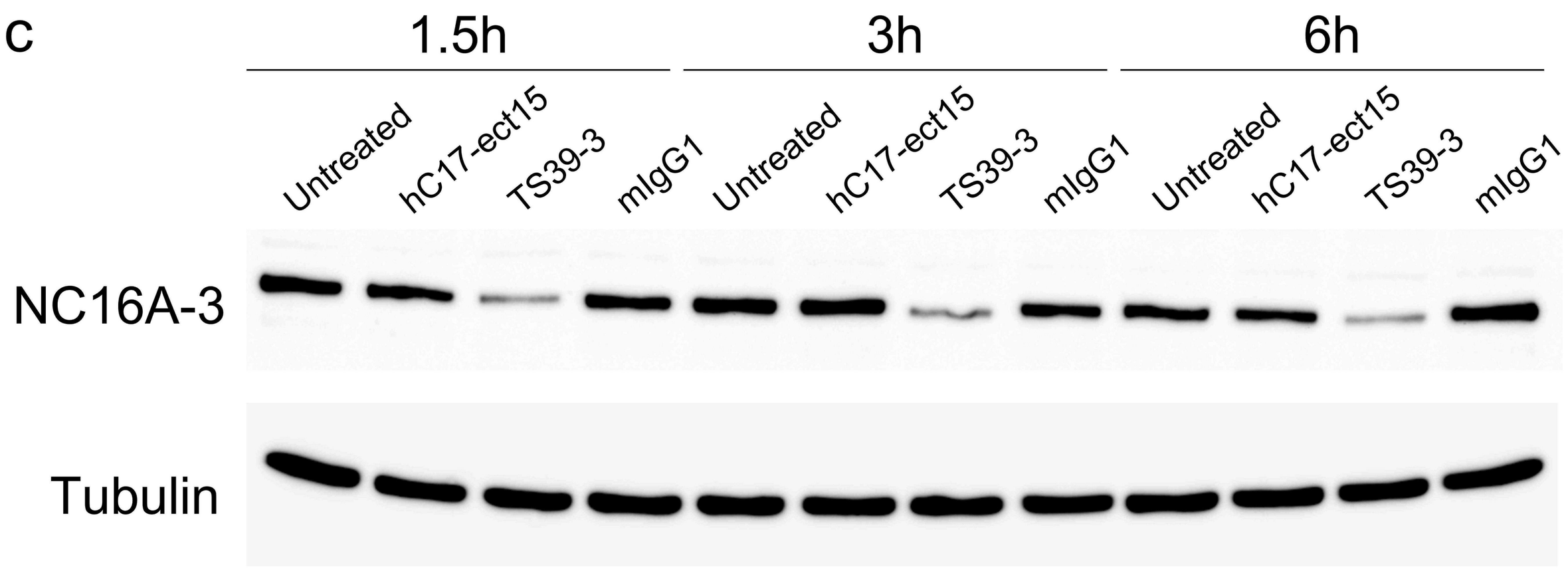
a


mIgG1
TS39-3
hC17-ect15

b


mIgG1
TS39-3
hC17-ect15
Epi
Epi
Epi

c


1.5h
3h
6h
Untreated
hC17-ect15
TS39-3
mIgG1
Untreated
hC17-ect15
TS39-3
mIgG1
Untreated
hC17-ect15
TS39-3
mIgG1
NC16A-3
Tubulin