Supplementary Information Table 1. Antibody characteristics.

Hyperlinks are partially available.

* this study (epitope mapping);

** kind gift from dr. Alexander Nyström from the Department of Dermatology, Medical Center - University of Freiburg, Germany;

*** personal communication Merck: targets the amino-terminal (datasheets not corrected for Col VII polarity shift in 1991 by Parente MG et al.

Same antibody as ‘NC-1 mAb’ from Sakai LY et al. (1986) and Keene DR et al. (1987);

**** personal communication US Biologics: targets the amino-terminal (datasheets were also not corrected for Col VII polarity shift)

Same antibody as ‘LH7.2 mAb’ from Lapiere JC et al. (1994) and Tanaka T et al. (1994).

Supplementary Information Figure 1. Report on pAb(16) epitope mapping by PEPperPRINT GmbH (Heidelberg, Germany).
Material and Methods

Microarray Content: The sequence of human type VII collagen was elongated by neutral GSGSGSG linkers to avoid truncated peptides. The elongated antigen sequence was translated into 10 and 15 amino acid peptides with peptide-peptide overlaps of 9 and 14 amino acids. The resulting peptide collection was split in two different peptide microarrays with 2,949 different 10 aa and 2,944 different 15 aa peptides printed in duplicate (5,898 and 5,888 peptide spots, respectively); both microarrays were framed by Flag (DYKDDDDKAS, 158 spots) and HA (YPYDVPDYAG, 158 spots) control peptides.

Sample: Rabbit Polyclonal IgG Antibody (Merck Cat. 234192)

Washing Buffer: PBS, pH 7.4 with 0.05% Tween 20 (3x1 min after each assay)

Blocking Buffer: Rockland blocking buffer MB-070 (30 min before the first assay)

Incubation Buffer: Washing buffer with 10% blocking buffer

Assay Conditions: Antibody concentrations of 1 µg/ml and 10 µg/ml in incubation buffer; incubation for 16 h at 4°C and shaking at 140 rpm

Secondary Antibodies: Sheep anti-rabbit IgG (H+L) DyLight680; 45 min at RT and a dilution of 1:5000 in incubation buffer

Control Antibody: Monoclonal anti-HA (12CA5)-DyLight800; 45 min at RT and a dilution of 1:2000 in incubation buffer

Scanner: LI-COR Odyssey Imaging System; scanning offset 0.65 mm, resolution 21 µm, scanning intensities of 7/7 (red = 700 nm/green = 800 nm)

Microarray Data: Microarray Data Wullink (PEP201558111).xlsx

Microarray Identifier: 001080_02 (15 amino acid peptides), 001080_03 (10 amino acid peptides)
Experimental and Data Analysis

After 15 min pre-swelling in standard buffer and 30 min in blocking buffer, both human type VII collagen microarrays were initially incubated with the secondary antibody sheep anti-rabbit IgG (H+L) DyLight680 at a dilution of 1:5000 in the presence of the control antibody monoclonal anti-HA (12CA5)-DyLight800 at a dilution of 1:2000 for 45 min at room temperature to analyze background interactions with the collagen-derived peptides. Subsequent incubation of both peptide microarrays with the rabbit polyclonal IgG antibody at concentrations of 1 µg/ml and 10 µg/ml was followed by staining with the secondary antibody sheep anti-rabbit IgG (H+L) DyLight680 in the presence of the monoclonal anti-HA (12CA5)-DyLight800 control antibody and read-out at scanning intensities of 7/7 (red/green). HA control peptides were simultaneously stained as internal quality control to confirm the assay quality and to facilitate grid alignment for data quantification.

Quantification of spot intensities and peptide annotation were based on 16-bit gray scale tiff files that exhibit a higher dynamic range than 24-bit colorized tiff files; microarray image analysis was done with PepSlide® Analyzer and summarized in Excel file Microarray Data Wullink (PEP201558111).xlsx. A software algorithm breaks down fluorescence intensities of each spot into raw, foreground and background signal (see “Raw Data” tabs), and calculates the standard deviation of median foreground intensities (see “Mapping Summary” tab). Spots with a deviation of 40% were zeroed to yield corrected averaged foreground intensities. Based on corrected averaged median foreground intensities, intensity maps were generated and binders in the peptide maps highlighted by an intensity color code with red for high and white for low spot intensities.

We further plotted averaged spot intensities of the assays with the antibody sample against the elongated human type VII collagen sequence from the N- to the C-terminus to visualize overall spot intensities and signal-to-noise ratios (see “Intensity Plot” tab). For a better data overview, the baselines of the intensity plots were leveled and main responses annotated next to the corresponding signal. The intensity plots were correlated with peptide and intensity maps as well as with visual inspection of the microarray scan to identify the epitopes of the rabbit polyclonal IgG antibody. In case it was not clear if a certain amino acid contributed to antibody binding, the corresponding letters were written in gray.
After 15 min pre-swelling in standard buffer and 30 min in blocking buffer, both human type VII collagen microarrays were incubated with the secondary antibody sheep anti-rabbit IgG (H+L) DyLight680 (1:5000) in the presence of the monoclonal anti-HA (12CA5)-DyLight800 control antibody (1:2000) for 45 min at room temperature to analyze background interactions of secondary and control antibodies with the printed human type VII collagen peptides.

At scanning intensities of 7/7 (red/green), we did not observe any background interaction of the human type VII collagen peptides due to a cross-reaction or non-specific binding of secondary or control antibodies even upon significant increase of brightness and contrast (see adjusted scans). Data quantification with PepSlide® Analyzer was hence omitted. In the green channel at 800 nm, the monoclonal anti-HA (12CA5)-DyLight800 control antibody gave rise to the expected and well-defined control spot pattern and validated the overall peptide microarray integrity.
incubation with rabbit polyclonal IgG antibody at concentrations of 1 µg/ml (scan not shown) and 10 µg/ml followed by staining with control and secondary antibodies and read out at scanning intensities of 7/7 (red/green)

weak to moderate IgG antibody responses with two main epitope-like spot patterns formed by well-defined spots based adjacent peptides with a consensus motif (white frames)

some additional single peptide interactions and epitope-like spot patterns with rather blurry spot morphologies indicate less specific or non-specific interactions

intensity plot with 10 aa peptides on left and 15 aa peptides on right highlighted moderate signal to noise ratios
the intensity plots of the 10 aa (red) and 15 aa (blue) peptides were separated and leveled to provide a clearer data overview

clear epitope-like spot patterns were based on peptides with the consensus motifs RGIEGFRGP, GPGLSGEQ and PKVSVDE

the response against peptides with the consensus motif RGIEGFRGP was significantly less clear with the 10 aa peptides

the 10 aa peptides highlighted that the main peak was actually based on two adjacent epitopes with the consensus motifs GPGLSGEQ and PKVSVDE; the corresponding single signal of the 15 aa peptides resulted from an overlap of the corresponding epitopes

atypical responses against single peptides like SSQTLPPDST, QTLPPDSTAT, SGLNVVMLGMAGADP and VMLGMAGADP likely resulted from less specific cross-reactions or non-specific antibody binding
<table>
<thead>
<tr>
<th>clear epitope-like spot patterns</th>
<th>aa peptides</th>
<th>precursor (X1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGIEGFRGP</td>
<td>15</td>
<td>1754-1762 (3H)</td>
</tr>
<tr>
<td>GPGLSGEQ</td>
<td>10/15</td>
<td>2100-2107 (3H)</td>
</tr>
<tr>
<td>PKVSVDE</td>
<td>10/15</td>
<td>2092-2098 (3H)</td>
</tr>
<tr>
<td>SSQTLPPDST</td>
<td>10</td>
<td>639-648 NC1 (FN3)</td>
</tr>
<tr>
<td>QTLPPDSTAT</td>
<td>15</td>
<td>641-650 NC1 (FN3)</td>
</tr>
<tr>
<td>SGLNVVMLGMAGADP</td>
<td>15</td>
<td>1192-1206 NC1 (vWFA)</td>
</tr>
<tr>
<td>VMLGMAGADP</td>
<td>10</td>
<td>1197-1206 NC1 (vWFA)</td>
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Supplementary Information Figure 2. A. The superficial plane of the lens capsule appears fibrillar (*black arrow*), but is more lucent and practically unlabeled compared to over/underlying zonules and intracapsular densities. B. A deeper intracapsular density indents the lens epithelium surface, but intracellular gold labeling is not seen. C. Some fine, looping fibrils can be distinguished in between the labeled structures of zonule and underlying intracapsular densities (*white arrows*).
Supplementary Information Figure 3. Anti-Col VII antibody comparison on skin cryosections. All anti-Col VII antibodies stain the dermal-epidermal basement membrane specifically. Bars 50 µm.