SUPPLEMENTAL INFORMATION

Clinical relevance of domain-specific PLA_2R1-ab levels in patients with membranous nephropathy

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Supplemental Methods

Expression of PLA_2R1 constructs

Transfection of the PLA_2R1 deletion and soluble constructs into HEK293 cells was performed using the Lipofectamine™ LTX Reagent with PLUS™ Reagent (Thermo Fisher Scientific; #15338100) according to the manufacturers protocol. For the PLA_2R1 deletion constructs, the cell lysate containing HA-tagged recombinant PLA_2R1 deletion constructs was prepared from approximately 5 million transfected HEK293 cells, which were lysed in 800 µL lysis-buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 1%(v/v) Triton X-100, 1x protease inhibitor), sonicated 3 x 10 sec and incubated for 1h at 4°C at 12 rpm on a lab rotator. The cell debris was removed by centrifugation for 15 min at 4°C at 14000 g. The supernatant of the cell lysate was collected and the protein concentration was measured with a DC-Assay (Bio-Rad Laboratories GmbH) following the procedure provided by the manufacturer. Since the CTLD7-8 and CTLD8 deletion constructs were only produced at very low levels, cell lysates containing these mutants were affinity-purified using Pierce™ anti-HA agarose (Thermo Scientific; #26182) according to the manufacturers protocol. The proteins were eluted directly in non-reducing Laemmli buffer. The deletion constructs were then stored in aliquots at -20°C until further use. For the PLA_2R1 soluble constructs, the cell medium containing the recombinant protein were stored in aliquots at -20°C and then directly used for Western blot analyses.

Deglycosylation of PLA_2R1 constructs

All PLA_2R1 deletion constructs were deglycosylated using neuraminidase, *N*-glycopeptidase F and *O*-glycopeptidase (Sigma-Aldrich; #10.269.611.001, #11.365.169.001 and #11.347.101.001, respectively). One µL of the enzyme solution was added to 20 µl of cell lysate and samples were incubated for 3h at 37°C and subsequently analyzed by Western blot.

Western blot analysis of PLA_2R1 constructs

Successful expression of the PLA_2R1 full-length, deletion and soluble constructs in transfected HEK293 cells was confirmed by Western blot analysis. Cell lysates (for the deletion constructs) or cell medium (for the soluble constructs) were separated by SDS-PAGE (4-15% Mini-PROTEAN® TGX™ Precast Protein Gels, 15-well; Biorad) and transferred to methanol activated polyvinylidine difluoride (PVDF) membranes

(Merck, # IVPH00010) under semi-dry conditions using Trans-blot Turbo (Biorad) at 25V for 35 min. The membrane was blocked with blocking buffer (3%(w/v) milk in Dulbecco´s phosphate buffered saline (DPBS; gibco by life technologies) supplemented with 0.1% Tween-20). As primary antibody rabbit polyclonal anti-HA-tag antibodies (Sigma-Aldrich; #SAB4300603; 1:1,000 dilution in blocking buffer) were used, followed by the horseradish peroxidase (HRP)–conjugated goat anti-rabbit IgG (Sigma-Aldrich; #A9169; 1:10,000 dilution in blocking buffer). The amount of protein applied in subsequent Western blot analysis was adjusted in order to compensate for differences in the transfection and expression rate of the individual PLA_2R1 constructs. The exact same amount of cell lysate containing recombinant protein (or cell medium for the soluble constructs) was loaded in all subsequent Western blot analyses of this study.

For the Western blot analyses with human sera, after the blocking step, the PVDF membrane was quickly dipped into washing buffer (PBS-T: PBS supplemented with 0.1% Tween-20) to remove excessed milk. Next, the membrane was incubated in 10 ml diluted human serum (unless otherwise indicated, a 1:100 dilution in 0.05% milk in PBS-T was used) for 18-20h at 4°C and 15 rpm on a vertical shaker. The membrane was then washed 4x with washing buffer for 5 min each and incubated for 1.5h with HRP-coupled mouse anti-human IgG (Southern Biotech; #9040-05; 1:24,000 dilution in blocking buffer) at room temperature (RT) on a vertical shaker. This secondary antibody exhibits an identical reactivity to all IgG subclasses (IgG1, IgG2, IgG3 and IgG4), as shown in https://www.southernbiotech.com/?catno=9040-05&type=Monoclonal#&panel1-2 (accessed on August 11th, 2019). Afterwards, the membrane was washed 4x in washing buffer, 5 min each. Excess liquid was removed from the membrane by a quick dip with the edge of the membrane onto a paper towel. The PVDF membrane was covered with 800 µl Clarity^{TM} Western ECL Blotting Substrate (Biorad) and incubated for 5 min in the dark. After removing access liquid from the PVDF membrane, chemiluminescent recording was performed in a Luminescent Detection Imager 600 (Amersham).

As negative controls, a control cohort of 35 individuals was analyzed at a 1:100 serum dilution on the deletion and soluble constructs (6 healthy individuals, 6 patients with FSGS, 7 patients with minimal change disease, 7 patients with MPGN and 9 patients with IgA nephropathy) (Supplemental Figure 1 and 2). A selected sub-group of 27 sera from the control cohort was further analyzed at a 1:25 serum dilution on the soluble constructs (Supplemental Figure 2D; 6 healthy donors; 5 FSGS patients; 6 IgA nephritis patients; 5 minimal change disease patients and 5 MPGN patients). In order

to exclude unspecific binding of human IgG to the constructs, serum from one healthy donor was analyzed undiluted on the soluble constructs (Supplemental Figure 2E). All experiments were assayed under non-reducing conditions, unless otherwise stated.

PLA_2R1 domain-specific ELISA

A lama nanobody – rabbit Fc fusion protein (control-rFc) was used as a negative control to compensate for the possible presence of heterophilic antibodies in human sera, which can give an unspecific background signal by recognizing the rFc portion.[1] This background absorption value of each specific serum was then subtracted from the PLA_2R1 domain-specific signal. All samples were analyzed in duplicates. Each plate also contained a standard curve composed of serial dilutions of a high-titer standard serum (Supplemental Figure 5).

For the CysR/FnII- and CTLD1-specific ELISA the fusion proteins including the control-rFc were diluted in PBS to a concentration of 1 ng/µl. Microtiter plates (Sarstedt; #82.1581) were then coated with 100 µl/well and plates were incubated over-night at 4°C. Wells were washed twice with 200 µl Dulbecco´s phosphate buffered saline (DPBS; gibco by life technologies). 300 µl of blocking buffer II (4% milk in DPBS supplemented with 0.05% Tween-20 (DPBS-T)) was applied to each well and incubated for 1.5h at RT with gentle agitation (15 rpm). In order to allow measurement in the linear range of the standard curve (Supplemental Figure 5), the sera had to be diluted. For the CysR/FnII-specific ELISA, human serum was diluted in blocking buffer II whereas the dilution factor was adjusted according to the total PLA_2R1-ab level: 1:100 dilution for sera with total PLA_2R1-ab <150 U/ml, 1:300 dilution for sera with PLA_2R1-ab 150-300 U/ml, 1:600 dilution for sera with PLA_2R1-ab 300-500 U/ml, 1:1,000 dilution for sera with PLA_2R1-ab 500-1,000 U/ml, 1:2,000 dilution for sera with PLA_2R1-ab >1,000 U/ml. For the CTLD1-specific ELISA a 1:100 dilution was used and if required, the experiment was repeated with a higher serum dilution (up to a dilution of 1:2,000). 100 µl of serum dilution was added per well and incubated for 2h at RT with gentle agitation. Wells were washed 3x with 300 µl DPBS-T with 2 min incubation time followed by 2x 300 µl DPBS-T and 15 min incubation at RT under gentle agitation. As secondary antibody HRP-coupled mouse anti-human IgG (Southern Biotech; #9040-05) was diluted 1:10,000 in blocking buffer II and 100 µl of the dilution were applied per well, followed by incubation for 1h at RT under gentle agitation. Wells were washed 5x with 300 µl DPBS-T and 2 min incubation time. For detection, 100 µl tetramethylbenzidine (TMB; Biozol) were added to the wells and incubated 3 min in

the dark, until the reaction was stopped by the addition of 100 µl 1M H_3PO_4. The absorption measurement was done at 450 nm in a plate reader Infinite M200 Pro (Tecan Life Sciences).

The CTLD7- and CTLD8-ab specific ELISA was less sensitive and hence certain adjustments had to be made. In a first established protocol, the proteins, including the control-rFc were diluted to 1.5 ng/µl in 50 mM carbonate buffer and 100 µl/well were used for coating over-night at 4°C. The rest of the protocol corresponds to the CysR/FnII- and CTLD1-ab specific ELISA, with a modification in the serum incubation step: 100 µl of serum dilution was added per well and incubated for 1h at RT. Wells were then re-loaded with 100 µl of the same serum dilution and incubated another 1.5h at RT. This re-loading approach was found to result in a better signal-to-noise ratio compared to a protocol of a single loading of a 1:50 serum dilution. The re-loading was also applied for the standard curve. For detection, the incubation time with TMB was increased to 10 min.

In the optimized protocol the following adjustments were made for the CTLD7 and CTLD8 specific ELISA: a 1:100 diluted serum was applied once (no re-loading) and incubated for 2h at RT. As secondary antibody a mixture of mouse anti-human IgG3 Hinge-HRP and IgG4 Fc-HRP (SothernBiotech; #9210-05 and #9200-05; 1:2500 for IgG3 and 1:5000 for IgG4) was used. The time for the development was increased to 30 min in the dark. This protocol produced better signal-to-noise ratios than the previous protocol and was chosen to be used for all further experiments.

For each serum the averaged absorption value of the control-rFc was subtracted from the averaged PLA_2R1 domain-specific absorption value, giving the corrected absorption value. For the standard curve, the corrected absorption values were used to fit the data with a Boltzmann sigmoid equation applying a four parameter fitting procedure (Equation 1). The resulting parameters were used to calculate the PLA_2R1 domain-specific antibody levels, also considering the serum dilution, which was applied in the experiment.

$$A_x = A_{min} + \frac{A_{max} - A_{min}}{1 + \exp\left(\frac{IP - x}{s}\right)} \qquad \text{(Equation 1)}$$

with A_x: Absorption at a given value x

x: PLA_2R1 specific antibody level

A_{min}: minimum Absorption value

A_{max}: maximum Absorption value

IP: inflection point

s: slope factor

The cut-off values for each ELISA were determined as follows: mean domain-specific antibody level of all negative controls (n=50 sera) plus 3 standard deviations for CysR/FnII-ab and CTLD1-ab. The cut-off value for CTLD7-ab was used at 4 standard deviations. For CTLD8-ab, the cut-off value was used at 4 standard deviations when detected with anti-total IgG, and 5 standard deviations when detected with anti-IgG3/IgG4. The obtained PLA_2R1 domain-specific ELISA values were plotted against the total PLA_2R1-ab level.

Supplemental Results

Epitope regions targeted by PLA_2R1-ab from patient sera tested positive only by IFT and Western blot in the initial screening

In 14 patients included in the study PLA_2R1-ab at the time of study inclusion were only detectable by IFT and Western blot, but not by the commercial ELISA (total PLA_2R1-ab level <20 U/ml). In the initial Western blot screening, ten (71.4%) of these patients showed only recognition of the full-length CysR-CTLD8 construct (pattern 1), while three (21.4%) patients had recognition down to the CTLD1-CTLD8 (pattern 2) and one patient (7.1%) to the CTLD7-CTLD8 (pattern 3) deletion construct.

Domain recognition pattern of the ELISA antibody standard used to calibrate the commercial PLA_2R1-ab ELISA (Euroimmun)

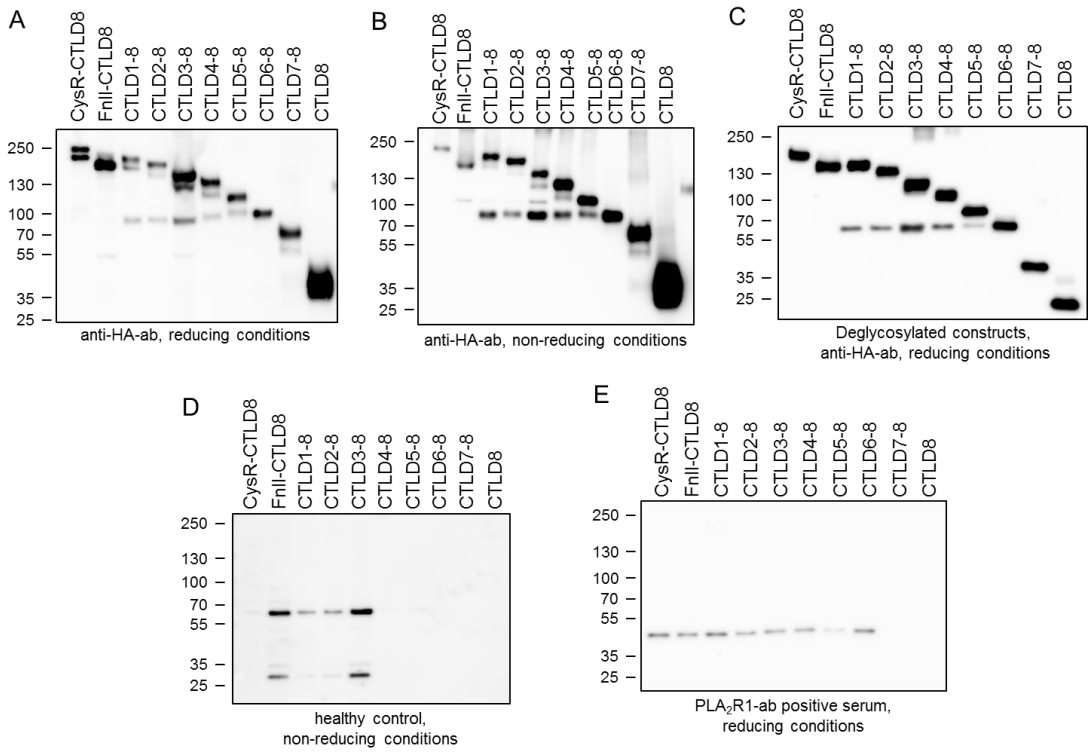
We analyzed the domain recognition pattern of the commercial ELISA standard (Euroimmun) (Supplemental Figure 7). Firstly, we analyzed the pattern on the PLA_2R1 deletion constructs and found that the standard recognizes all constructs down to the CTLD8-domain. For a more detailed characterization, we further analyzed the recognition of the soluble sCysR-CTLD1 and sCTLD1-CTLD2 constructs, which showed that the standard recognizes the N-terminal domain of PLA_2R1 and CTLD1. Since this experimental setup did not allow to characterize the domain recognition in detail (it was unclear, whether the ELISA Standard was able to recognize CysR or CTLD7), we analyzed the CysR and CTLD7 binding by the newly developed ELISA

and found that the ELISA Standard did recognize both CysR and CTLD7. These results demonstrate that the Euroimmun standard clearly recognizes N- and C-terminal domains, precisely, all four relevant domains: CysR, CTLD1, CTLD7 and CTLD8. Therefore, it can be used as a true calibrant for the N- and C-terminal recognition patterns, since all patients in our cohort have antibodies directed to the N- (CysR-CTLD1) and C-terminal region (CTLD7-CTLD8) of PLA_2R1.

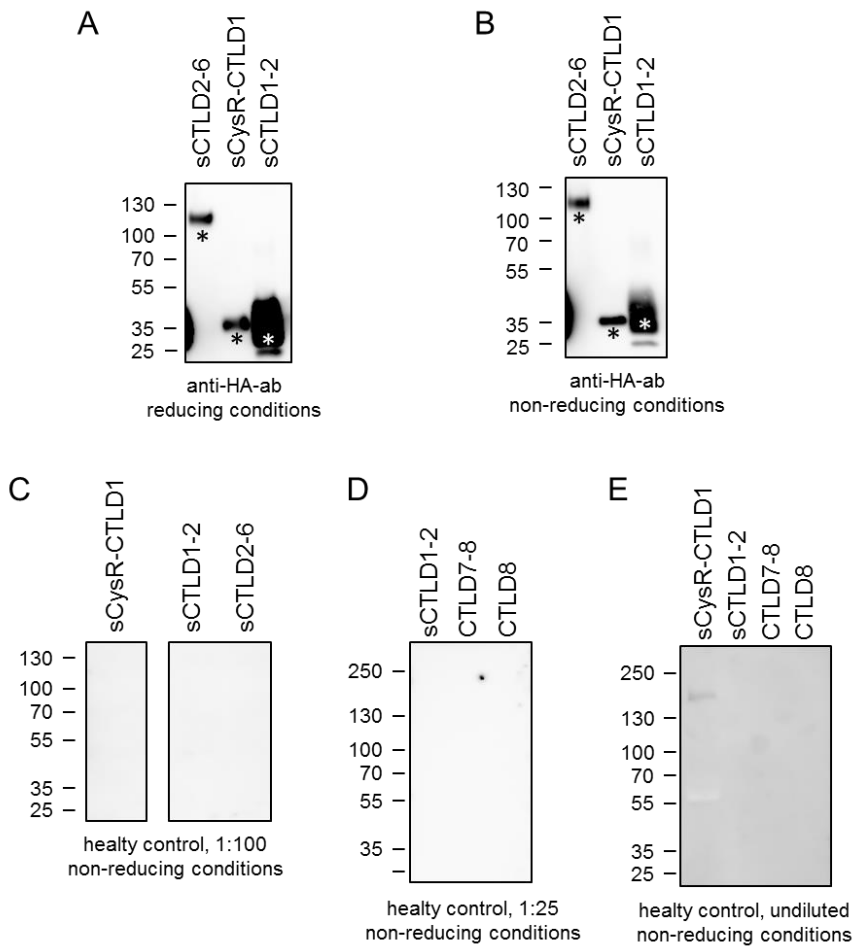
Supplemental References

1. Boscate LM, Stuart MC: Heterophilic antibodies: a problem for all immunoassays. *Clin Chem* 34: 27-33, 1988

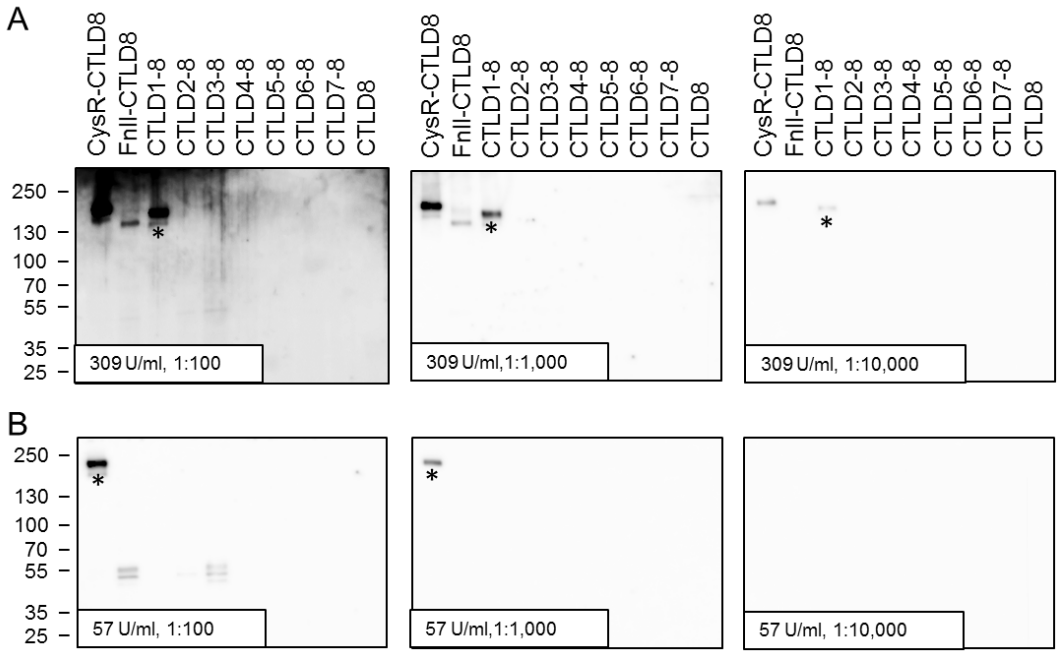
Supplemental Figures



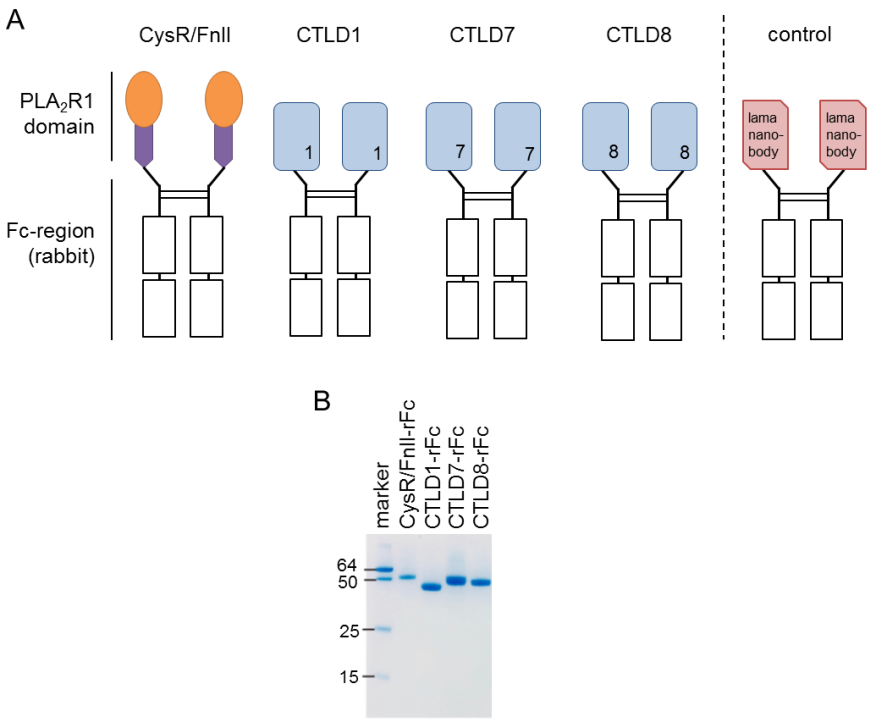
Supplemental Figure 1: Quality control of recombinant PLA_2R1 deletion constructs by Western blot. A,B) The protein amount for each construct applied in the analyses was adjusted for the expression level of the construct in the HEK293 cell lysate using anti-HA antibodies (anti-HA-ab) under reducing (A) and non-reducing (B) conditions. C) The double band seen in most constructs disappeared after deglycosylation of the constructs (see Supplemental Methods). D) Serum from a healthy donor failed to recognize any of the constructs (analysis performed with n=35 control sera at a 1:100 serum dilution). E) PLA_2R1-ab positive serum did not react with any of the constructs when the Western blot analysis was performed under reducing conditions (analysis performed with n=3 sera).



Supplemental Figure 2: Quality control of recombinant soluble PLA_2R1 constructs by Western blot. A,B) The protein amount for each construct applied in the analyses was adjusted for the expression level of the construct in the HEK293 cell lysate using anti-HA antibodies (anti-HA-ab) under reducing (A) and non-reducing (B) conditions. The amount of sample loaded is identical in both conditions. An asterisk marks the recognized soluble constructs. C,D) Representative results of sera from the control cohort at a serum dilution of 1:100 (n=35) and 1:25 (n=27). E) As a high total-IgG sample, undiluted serum from a healthy donor was analyzed, whereas still no specific signal for the PLA_2R1 domain constructs was obtained.

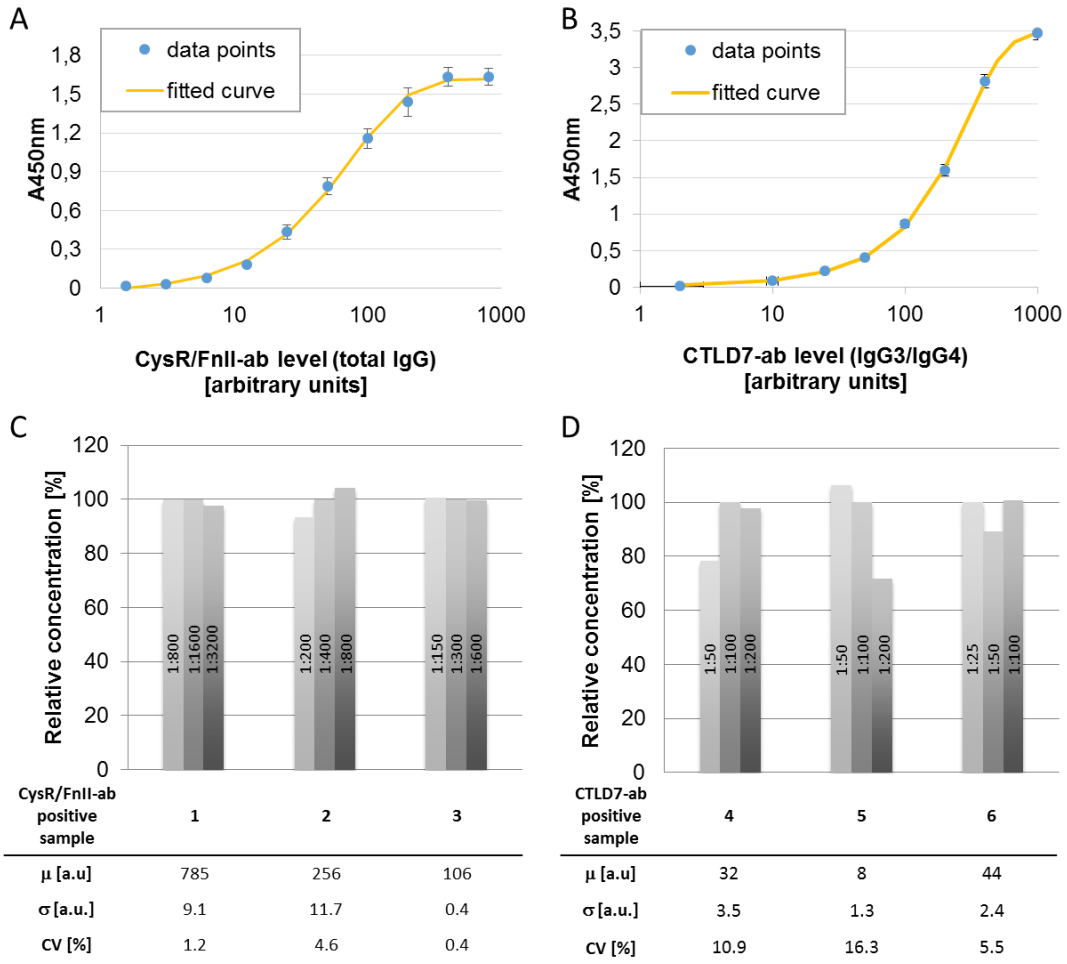


Supplemental Figure 3: Influence of serum dilution on the recognition of N-terminal PLA_2R1 domains. Representative Western blots of serum dilution experiments of pattern 2 (A) and pattern 1 (B) (total n=2 for each pattern). An increase in serum dilution to 1:10,000 results in decreased but detectable signal for the CysR-CTLD8 and CTLD1-8 deletion constructs (in A), while a complete loss of signal is observed for patterns 1 (in B). An asterisk marks the last recognized deletion construct.



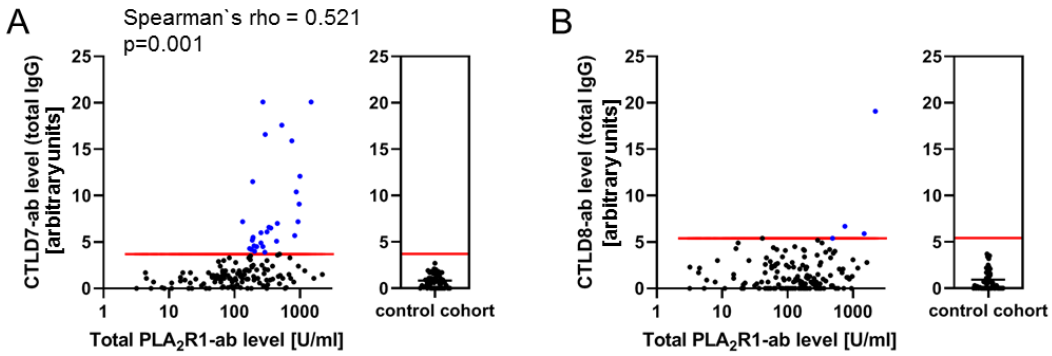
Supplemental Figure 4: PLA_2R1 domain rFc fusion proteins.

A) Schematic presentation of the specific PLA_2R1-domain rFc fusion proteins. Importantly, the FnII-domain was never shown to be a target of PLA_2R1 autoantibodies and is hence assumed not to interfere with the detection system. As a control-rFc, a lama nanobody rFc fusion protein was used. B) The PLA_2R1-domain rFc fusion proteins after purification and concentration were analyzed by SDS-PAGE with Coomassie staining.



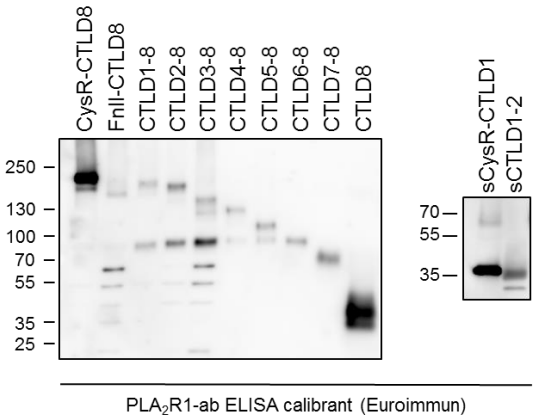
Supplemental Figure 5: Linearity of PLA_2R1 domain-specific ELISA.

In both ELISA setups used in our experiments (total IgG for CysR/FnII- and CTLD1-specific PLA_2R1-ab; IgG3/IgG4 for CTLD7- and CTLD8-specific PLA_2R1-ab) calibration curves were established using highly positive index sera. A) Representative example of a 10-point calibration curve for the total-IgG based ELISA. The curve is based on four independent experiments. B) Representative example of a 8-point calibration curve for the IgG3/IgG4 based ELISA. The curve is based on three independent experiments. C) Serial dilution experiments of three PLA_2R1-ab positive patient sera for evaluation of linearity of the total-IgG based ELISA. For each patient serum, three dilutions were analyzed (as indicated on the bars) and presented as relative concentrations, setting the median concentration to 100%. Each serum dilution was analyzed in duplicates. The resulting averaged concentrations μ, standard deviation σ (both in arbitrary units, a.u.) and coefficient of variation (CV) are depicted below the graph. D) Serial dilution experiments of three PLA_2R1-ab positive patient sera for evaluation of linearity of the IgG3/IgG4 based ELISA. All parameters are as discussed in (C).



Supplemental Figure 6: Results of the ELISA screening for CTLD7-ab and CTLD8-ab detected with anti-human total IgG.

The CTLD7-ab (A) and CTLD8-ab (B) levels based on anti-human total IgG detection are plotted against the total PLA_2R1-ab level. The cut-off values are presented by red lines. In the MN patient cohort (n=149), positive results are colored in blue and negative in black. The determined values for the control cohort (n=50) are presented in nested graphs. CTLD7-ab and CTLD8-ab were detected in 29 (19.5%) and 4 (2.7%) PLA_2R1-ab positive patients, respectively.



Supplemental Figure 7: Western blot using the Euroimmun standard as primary antibody.

When analyzed on the PLA_2R1 deletion constructs, the standard recognizes all constructs down to the CTLD8-domain. For a more detailed characterization, the recognition of the soluble sCysR-CTLD1 and sCTLD1-CTLD2 constructs was analyzed, showed that the standard recognizes the N-terminal domain of PLA_2R1 and CTLD1.

Supplemental Tables

Supplemental Table 1: Protein sequence information of the PLA_2R1 deletion constructs.

All PLA_2R1 deletion constructs exhibit the PLA_2R1 signal peptide (Met-1 to Ala-20) and the linker sequence (Ala-21 to Trp-35), followed by the appropriate PLA_2R1 domain sequence, the transmembrane domain and the intracellular domain (Ile-1398 to Gln 1463). All constructs were sequenced to confirm correct cloning (Mycrosynth, Balgach, Switzerland).

Construct name	**Deleted region**
Full length (CysR-CTLD8)	no deletion
FNII-CTLD8	deletion of CysR (Gln-36 to Asp-165)
CTLD1-8	deletion of CysR-FnII (Gln-36 to Thr-223)
CTLD2-8	deletion of CysR-CTLD1 (Gln-36 to Tyr-357)
CTLD3-8	deletion of CysR-CTLD2 (Gln-36 to Ala-504)
CTLD4-8	deletion of CysR-CTLD3 (Gln-36 to Pro-660)
CTLD5-8	deletion of CysR-CTLD4 (Gln-36 to Lys-805)
CTLD6-8	deletion of CysR-CTLD5 (Gln-36 to Lys-947)
CTLD7-8	deletion of CysR-CTLD6 (Gln-36 to His-1105)
CTLD8	deletion of CysR-CTLD7 (Gln-36 to Pro-1235)

Supplemental Table 2: **Protein sequence information of the soluble PLA_2R1 domain constructs.**

All soluble constructs included the CD8 leader peptide (MALPVTALLLPLALLLHAARP) followed by the appropriate PLA_2R1 wild type sequence. A C-terminal HA-tag was added via the reverse-primer. All constructs were sequenced to confirm correct cloning (Mycrosynth, Balgach, Switzerland).

Construct name	**Region**
sCysR-CTLD1	Gln-36 to Tyr 357
sCTLD1-CTLD2	Ser-224 to Ala-504
sCTLD2-CTLD6	Leu-358 to His-1105

Supplemental Table 3: **Regions of the PLA_2R1 protein sequence fused to the rabbit Fc.**

The specific PLA_2R1 domains were cloned into the pCSE2.5 vector, which adds a rabbit antibody Fc (rFc) unit to the C-terminus. Correct cloning was verified by sequencing. As a control (control-rFc) a lama nanobody-rFc fusion protein was used.

Construct name	**Region within PLA_2R1**
CysR/FnII-rFc	Asp-38 to Thr-223
CTLD1-rFc	Thr-223 to Glu367
CTLD7-rFc	Asp-1101 to Glu-1237
CTLD8-rFc	Glu-1237 to Lys-1392

Supplemental Table 4: Intra- and inter-assay coefficients of variation for the total-IgG and IgG3/IgG4 based ELISA. Four representative samples for each ELISA were used to determine the CysR/FnII- and CTLD7-ab levels. For the intra-assay coefficients of variation, each serum was incubated 16-fold in one microtiter plate. For the inter-assay coefficients of variation, each serum was incubated 3-fold on ten different microtiter plates. μ: mean; a.u.: arbitrary units; σ: standard deviation; CV: coefficient of variation = σ/μ expressed in %; ØCV: mean of the CV.

	Total IgG (CysR/FnII)				**IgG3/IgG4 (CTLD7)**			
Sample	1	2	3	4	5	6	7	8
Intra-assay								
μ [a.u.]	631	151	125	33	26	26	25	15
σ [a.u.]	32.7	10.7	9.7	1.3	3.4	1.6	2.4	2.0
CV [%]	5.2	7.1	7.8	4.0	12.8	6.1	9.6	13.4
	ØCV 6.0%				ØCV 10.5%			
Inter-assay								
μ [a.u.]	625	194	148	38	30	27	25	14
σ [a.u.]	51.2	15.2	12.4	2.4	4.0	2.4	2.0	1.8
CV [%]	8.2	7.9	8.4	6.3	13.3	8.7	8.2	12.4
	ØCV 7.7%				ØCV 10.7%			

Supplemental Table 5: Clinical characteristics and follow-up of patients with exclusive N-terminal PLA_2R1 domain recognition (CysR+CTLD1) and patients with N- and C-terminal PLA_2R1 domain recognition (CTLD7+CTLD8) from the initial Western blot screening.
The recognition patterns were identified in the initial Western Blot screening on the PLA_2R1 deletion constructs at a 1:100 serum dilution. CysR+CTLD1: Patients with domain recognition pattern 1 or 2: sera recognized only the CysR-CTLD8 full-length PLA_2R1 protein (pattern 1) or showed a binding to the CysR-CTLD8, FnII-CTLD8 and CTLD1-CTLD8 constructs (pattern 2). CTLD7+CTLD8: Patients with domain recognition pattern 3 or 4: sera recognized all constructs containing CTLD7-CTLD8 (pattern 3); some of these sera also recognized CTLD8 (pattern 4). PLA_2R1-ab: PLA_2R1-antibody.

	Complete cohort	CysR + CTLD1	CTLD7 + CTLD8	P-value
Number of patients (%)	150	26 (17.3%)	124 (82.7%)	na
Age - years (median, 1st – 3rd quartile)	55, 43 – 64	59, 44 – 64	55, 43 – 64	0.533
Male sex (%)	109 (72.7%)	17 (65.4%)	92 (74.2%)	0.468
Proteinuria - g/24h (median, 1st – 3rd quartile)	6.9, 4.8 – 10.6	4.6, 3.4 – 6.8	7.7, 5.0 – 10.7	<0.001
Serum creatinine - mg/dl (median, 1st – 3rd quartile)	1.0, 0.8 – 1.2	1.0, 0.9 – 1.5	1.0, 0.8 – 1.2	0.343
eGFR, CKD-EPI - mL/min/1.73 m^2 (median, 1st – 3rd quartile)	85, 62 – 103	83, 42 – 97	86, 62 – 103	0.296
PLA_2R1-ab level, U/ml (median, 1st – 3rd quartile)	151, 64 – 294	19, 9 – 67	175, 86 – 325	<0.001
Time renal biopsy to study inclusion - months (median, 1st – 3rd quartile)	0.8, 0.3 – 1.7	0.5, 0.3 – 1.8	0.8, 0.3 – 1.6	0.966
% tubular atrophy and interstitial fibrosis (median, 1st – 3rd quartile)	5.0, 5.0 – 20.0	10.0, 5.0 – 20.0	5.0, 5.0 – 20.0	0.184
Immunosuppressive therapy (%)	116 (77.3%)	11 (42.3%)	105 (84.7%)	<0.001
First line immunosuppression: CYC / CNI / RTX / other	48 / 56 / 10 / 2	5 / 5 / 1 / 0	43 / 51 / 9 / 2	
Second line immunosuppressive therapy was initiated	49 (42.2%)	4 (36.4%)	45 (42.9%)	0.758
PLA_2R1-ab negative during follow-up (%)	124 (82.7%)	22 (84.6%)	102 (82.3%)	>0.999
Remission of proteinuria during follow-up (%)	133 (88.7%)	23 (88.5%)	110 (88.7%)	>0.999
Complete remission of proteinuria during follow-up (%)	90 (60.0%)	19 (73.1%)	71 (57.3%)	0.186
Spontaneous remission of proteinuria during follow-up (%)	31 (20.7%)	15 (57.7%)	16 (12.9%)	<0.001
Time to remission of proteinuria - months (median, 1st – 3rd quartile)	18, 9 – 30	15, 6 – 21	18, 12 – 30	0,181
Follow-up time - months (median, 1st – 3rd quartile)	54, 36 – 66	51, 32 – 68	54, 38 – 66	0.935

Supplemental Table 6: Clinical characteristics and follow-up of patients depending on the epitope recognition pattern in the initial Western blot screening on the PLA_2R1 deletion constructs.

CysR: Sera recognized only the CysR-CTLD8 full-length PLA_2R1 protein, but failed to detect any of the other constructs (pattern 1); CTLD1: Sera showed a binding only to the CysR-CTLD8, FnII-CTLD8 and CTLD1-CTLD8 constructs (pattern 2); CTLD7: Sera recognized all constructs including CTLD7-CTLD8, but failed to react with CTLD8 (pattern 3); CTLD8: Sera recognized all constructs, including CTLD8 (pattern 4). PLA_2R1-ab: PLA_2R1-antibody. CYC: alkylating agents. CNI: Calcineurin inhibitors. RTX: Rituximab.

	CysR	CTLD1	CTLD7	CTLD8	P value
Number of Patients (%)	19 (12.7%)	7 (4.7%)	100 (66.7%)	24 (16.0%)	
Age - years (median, 1st – 3rd quartile)	58, 42 – 64	61, 59 – 64	55, 44 – 63	55, 41 – 69	0.505
Male sex (%)	12 (63.2%)	5 (71.4%)	72 (72.0%)	20 (83.3%)	0.522
Proteinuria - g/24h (median, 1st – 3rd quartile)	3.9, 2.8 – 5.6	6.7, 5.5 – 7.3	7.6, 5.0 – 10.7	8.6, 5.9 –10.3	0.002
Serum creatinine - mg/dl (median, 1st – 3rd quartile)	1.0, 0.8 – 1.2	1.2, 1.0 – 2.4	1.0, 0.8 – 1.2	1.0, 0.8 – 1.2	0.279
eGFR, CKD-EPI - mL/min/1.73 m^2 (median, 1st – 3rd quartile)	91, 53 – 104	66, 27 – 80	84, 64 – 103	88, 62 – 104	0.224
PLA_2R1-ab level, U/ml (median, 1st – 3rd quartile)	18, 7 – 62	34, 14 – 72	173, 83 – 315	188, 105 – 554	<0.001
Time renal biopsy to study inclusion - months (median, 1st – 3rd quartile)	0.5, 0.3 – 1.0	0.5, 0.8 – 3.5	0.8, 0.3 – 1.6	0.8, 0.3 – 1.3	0.694
% tubular atrophy and interstitial fibrosis (median, 1st – 3rd quartile)	10.0, 5.0 – 12.5	25.0, 10.0 – 60.0	5.0, 5.0 – 20.0	5.0, 0.0 – 10.0	0.193
Immunosuppressive therapy (%)	6 (31.6%)	5 (71.4%)	84 (84.0%)	21 (87.5%)	<0.001
First line immunosuppression: CYC / CNI / RTX / other	3 / 3 / 0 / 0	2 / 2 / 1 / 0	36 / 41 / 6 / 1	7 / 10 / 3 / 1	
Second line immunosuppressive therapy was initiated	2 (33.3%)	2 (40.0%)	35 (41.7%)	10 (47.6%)	0.146
Remission of proteinuria during follow-up (%)	17 (89.5%)	6 (85.7%)	89 (89.0%)	21 (87.5%)	0.990
Complete remission of proteinuria during follow-up (%)	13 (68.4%)	6 (85.7%)	60 (60.0%)	11 (45.8%)	0.215
Spontaneous remission of proteinuria during follow-up (%)	13 (68.4%)	2 (28.6%)	13 (13.0%)	3 (12.5%)	<0.001
PLA_2R1-ab negative during follow-up (%)	17 (89.5%)	5 (71.4%)	82 (82.0%)	20 (83.3%)	0.738
Follow-up time - months (median, 1st – 3rd quartile)	51, 30 – 62	69, 57 – 72	54, 38 – 63	56, 35 – 72	0.122

Supplemental Table 7: P-values for all differences of clinical characteristics of patients depending on the epitope recognition profile shown in Supplemental Table 6.

CysR: Sera recognized only the CysR-CTLD8 full-length PLA_2R1 protein, but failed to detect any of the other constructs (pattern 1); CTLD1: Sera showed a binding only to the CysR-CTLD8, FnII-CTLD8 and CTLD1-CTLD8 constructs (pattern 2); CTLD7: Sera recognized all constructs including CTLD7-CTLD8, but failed to react with CTLD8 (pattern 3); CTLD8: Sera recognized all constructs, including CTLD8 (pattern 4). PLA_2R1-ab: PLA_2R1-antibody.

	CysR vs. CTLD1	CysR vs. CTLD7	CysR vs. CTLD8	CTLD1 vs. CTLD7	CTLD1 vs. CTLD8	CTLD7 vs. CTLD8
Age	0.231	0.885	0.922	0.121	0.216	0.982
Male sex	>0.999	0.425	0.170	>0.999	0.596	0.308
Proteinuria	0.030	0.001	0.001	0.420	0.216	0.552
Serum creatinine	0.135	0.847	0.695	0.060	0.139	0.498
eGFR, CKD-EPI	0.073	0.957	>0.999	0.036	0.085	0.947
PLA_2R1-ab level	0.497	<0.001	<0.001	0.004	0.005	0.277
Time renal biopsy to study inclusion	0.209	0.613	0.639	0.312	0.444	0.926
Tubular atrophy and interstitial fibrosis	0.135	0.786	0.340	0.058	0.061	0.391
Immunosuppressive therapy	0.095	<0.001	<0.001	0.335	0.562	>0.999
Second line immunosuppressive therapy was initiated	0.287	0.056	0.039	>0.999	0.676	0.638
PLA_2R1-ab negative during follow-up	0.287	0.738	0.678	0.613	0.596	>0.999
Remission of proteinuria during follow-up	>0.999	>0.999	>0.999	0.576	>0.999	0.733
Complete remission of proteinuria during follow-up	0.629	0.610	0.217	0.247	0.094	0.253
Spontaneous remission of proteinuria during follow-up	0.190	<0.001	0.001	0.254	0.562	>0.999
Follow-up time - months	0.025	0.334	0.259	0.036	0.167	0.588

Supplemental Table 8: Correlation of CTLD1-ab positivity to the presence of epitopes in CTLD7 and CTLD8.

		CTLD1-ab	
		Positive	**Negative**
CTLD7-ab	**Positive**	70%	64%
	Negative	30%	36%
CTLD8-ab	**Positive**	18%	14%
	Negative	82%	86%