

**Epitope Spreading in PLA2R1 is Associated with Bad Prognosis in  
Idiopathic Membranous Nephropathy**

By Seitz-Polski & Dolla et al

**Supplementary data**

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

1/500

1/50

1/100

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

1/100

1/100

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WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

1/200

1/200

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WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

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WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

1/50

1/100

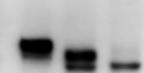
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**Figure S1.** Epitope profile of all 50 iMN sera and control sera against the deletion mutants of PLA2R1. The serum dilution is indicated. All western blots were revealed with anti-IgG4 secondary antibodies. Same legend as main Fig. 1.

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7



1/25



1/100

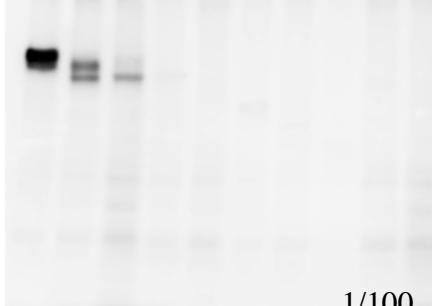


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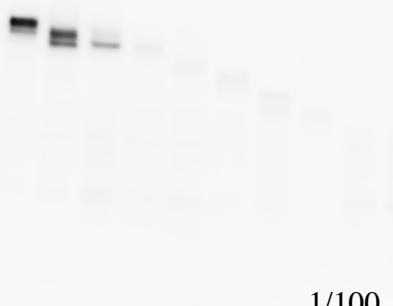
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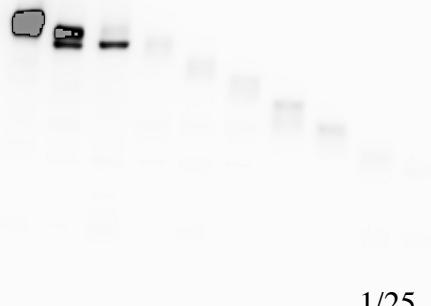
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1/100



1/100



1/25

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

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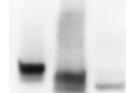
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1/100



1/100



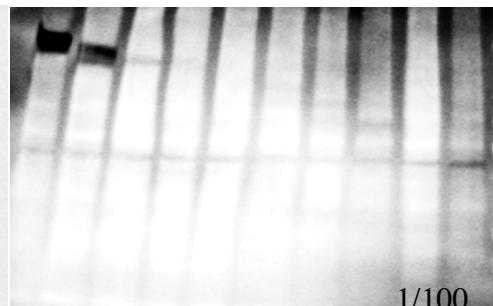
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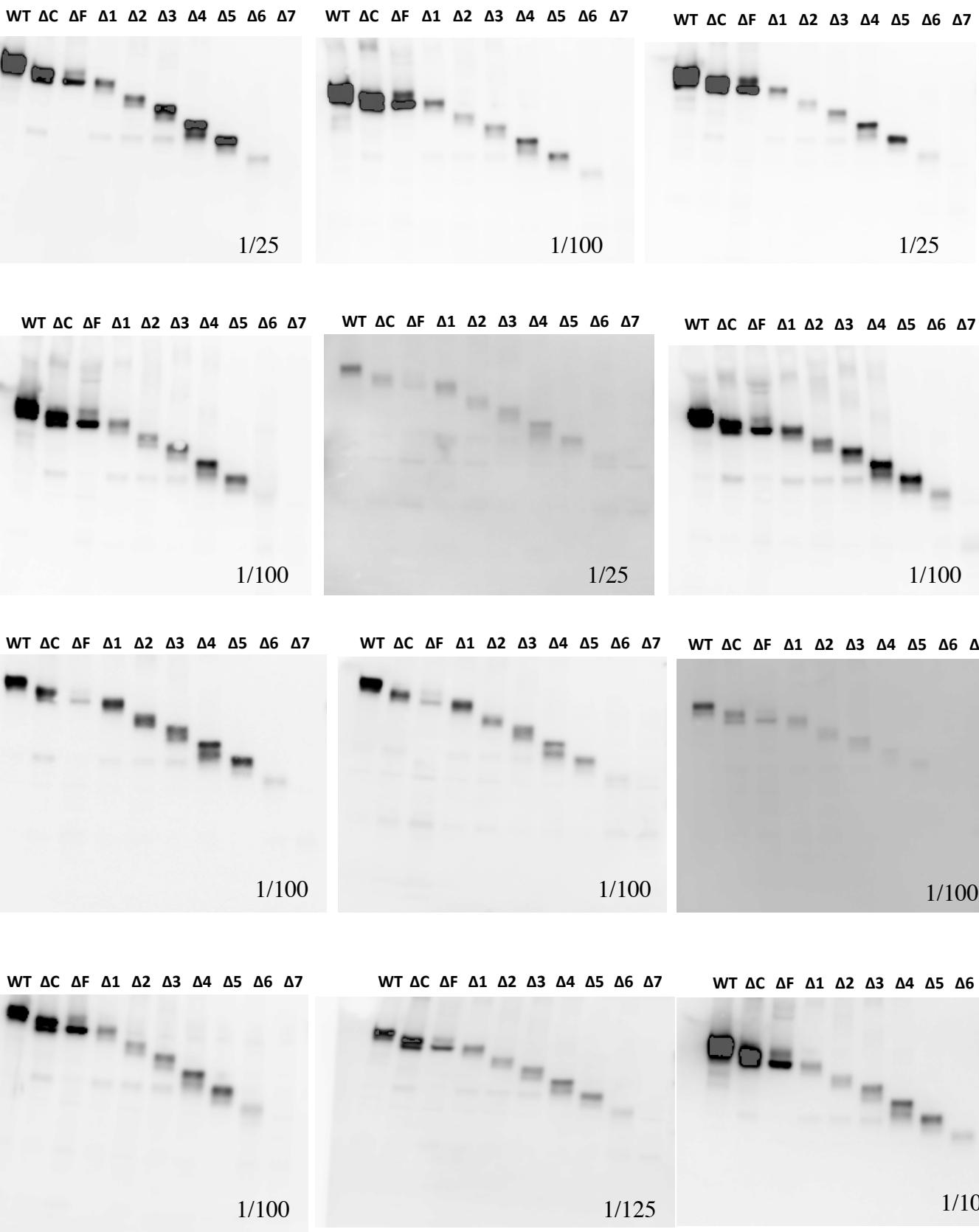


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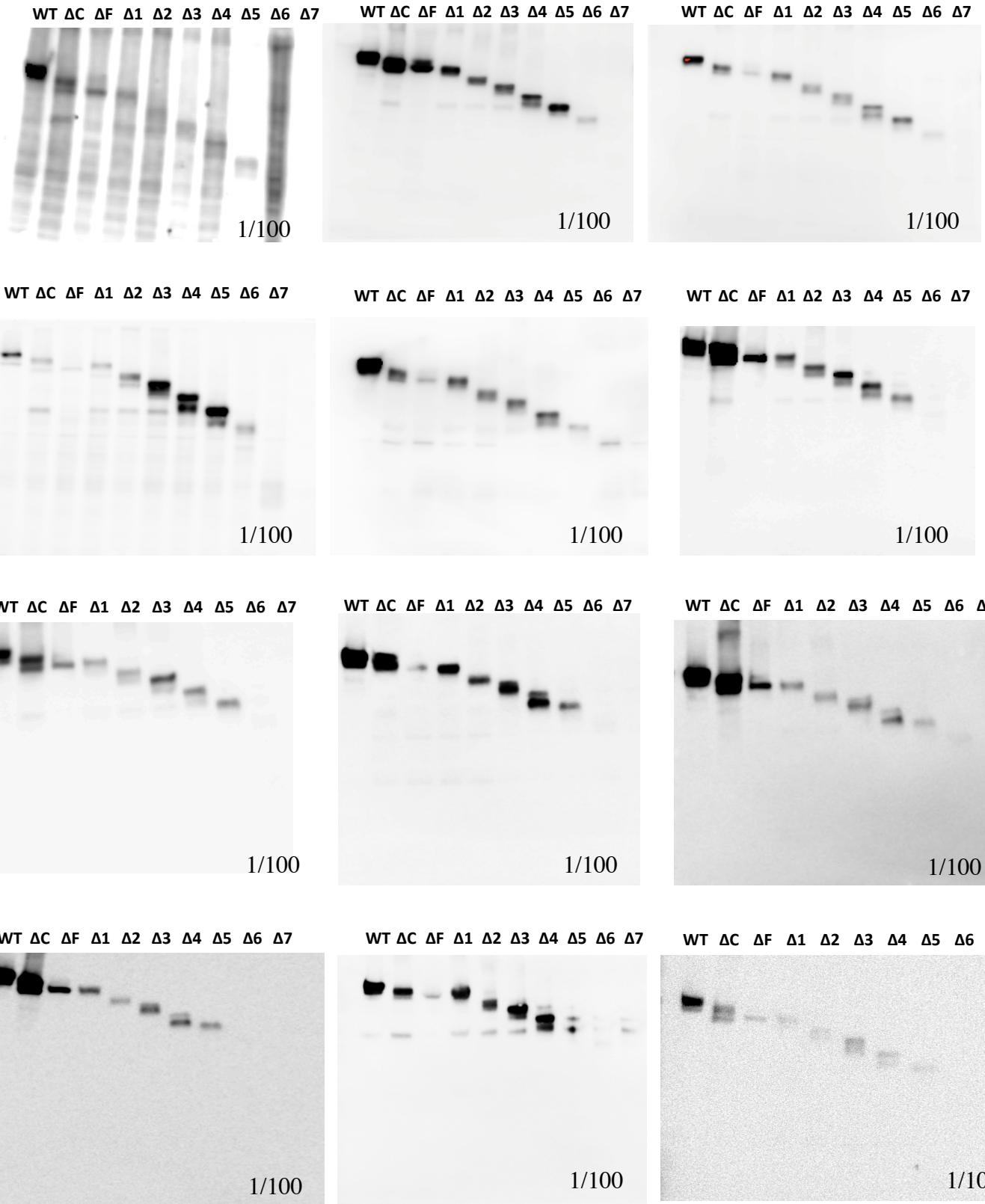


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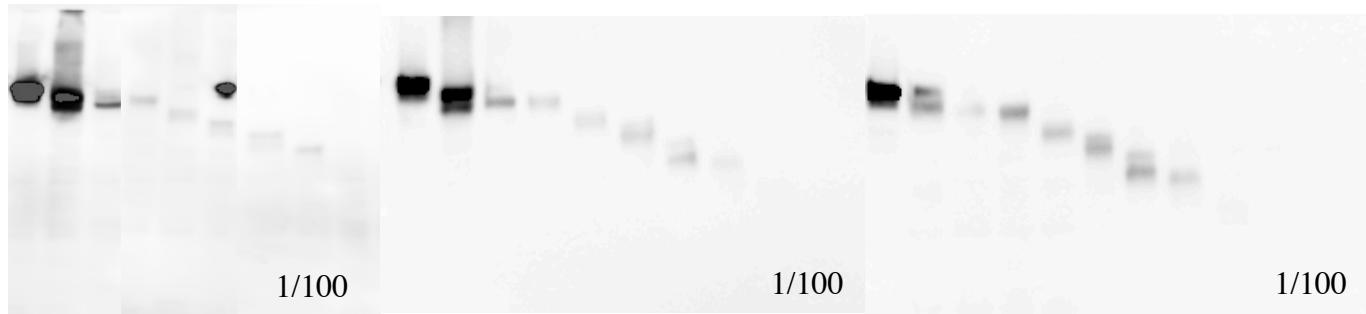


## Figure S1 (continued)



## Figure S1 (continued)

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1/100

1/100

1/100

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

Control serum 1/100

Control serum 1/100

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

WT ΔC ΔF Δ1 Δ2 Δ3 Δ4 Δ5 Δ6 Δ7

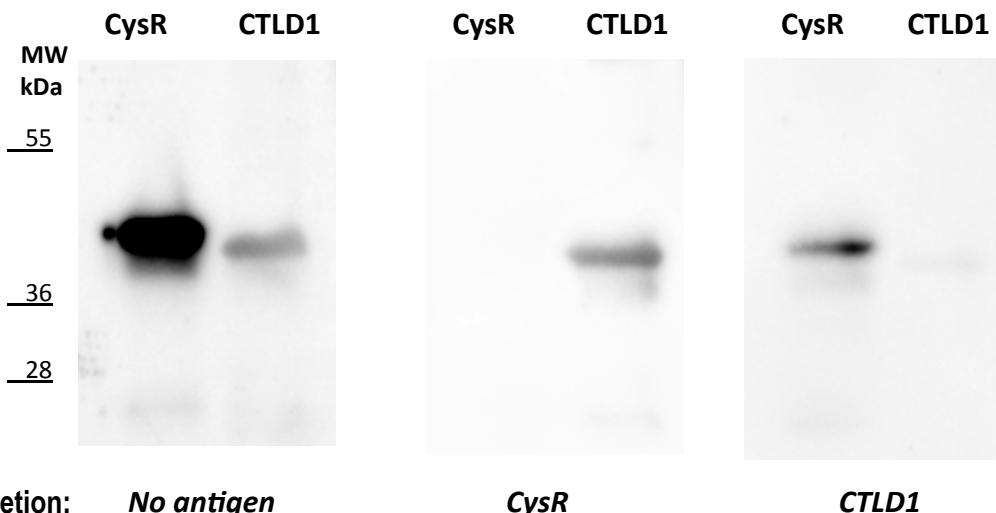
Control serum 1/100

Control serum 1/100

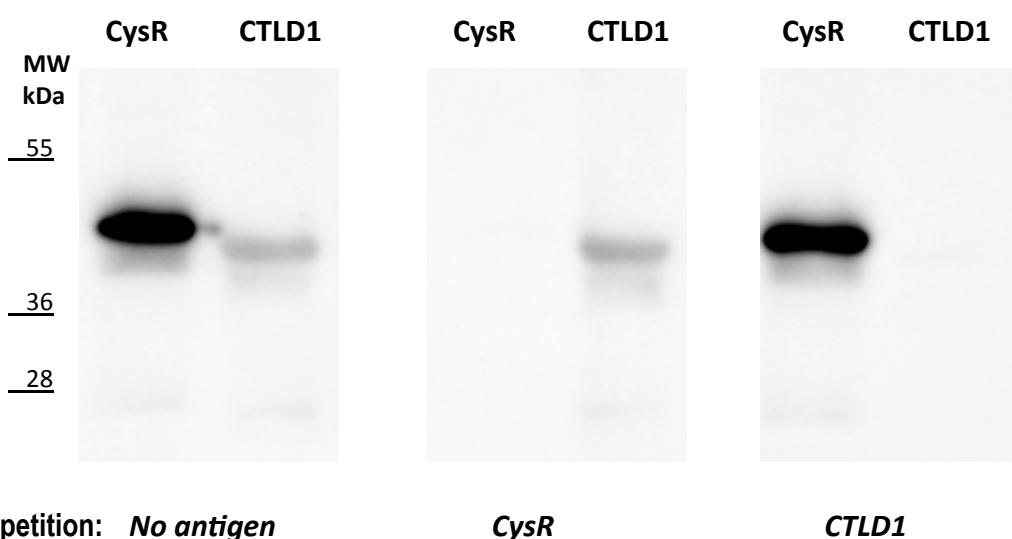
Control serum 1/100

**Figure S1 (continued)**

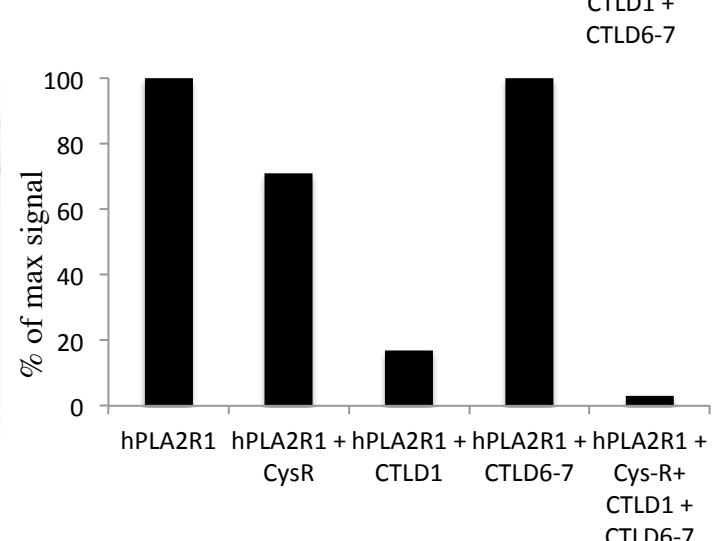
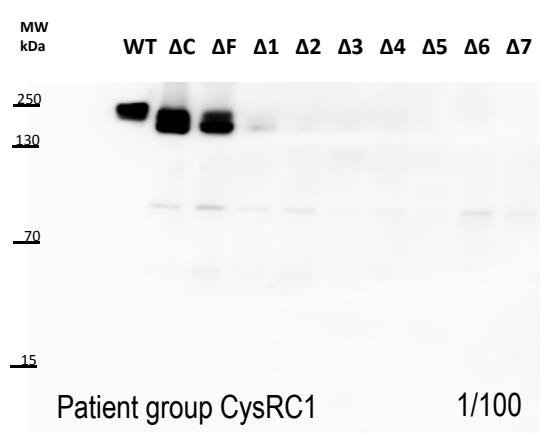
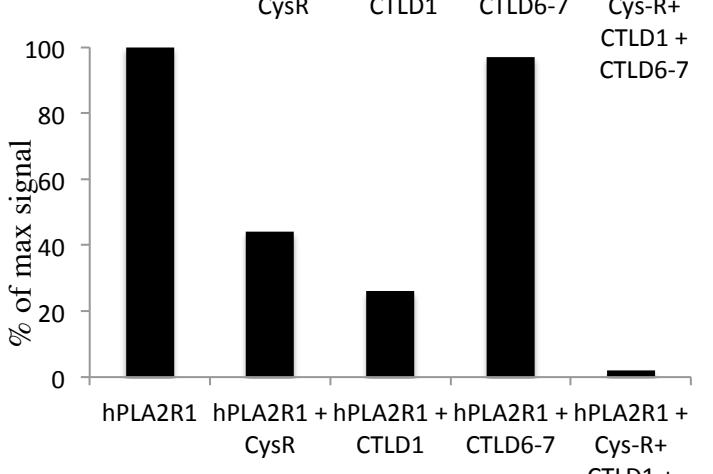
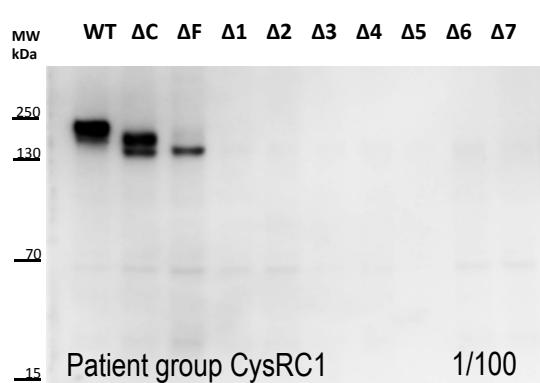
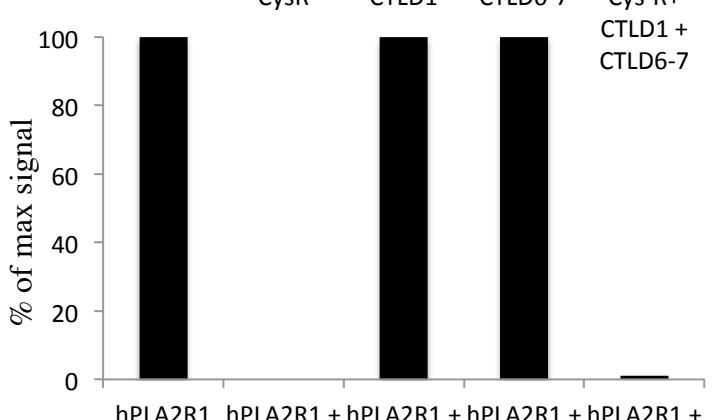
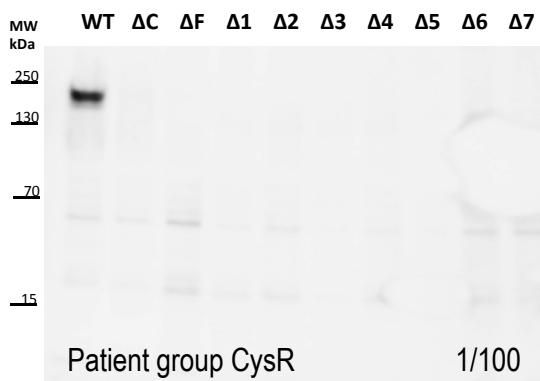
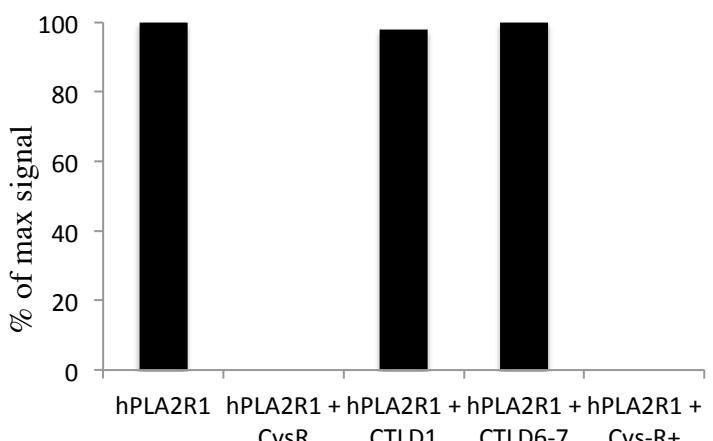
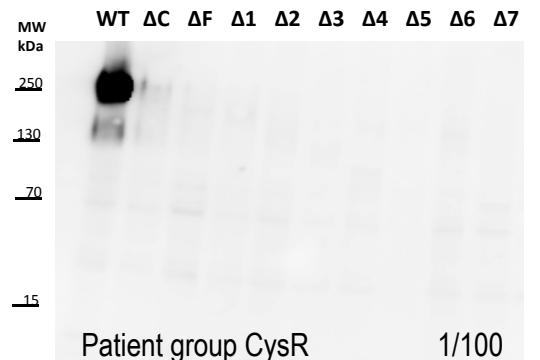
## Depletion with antigen bound to anti-HA beads



## Competition with antigen



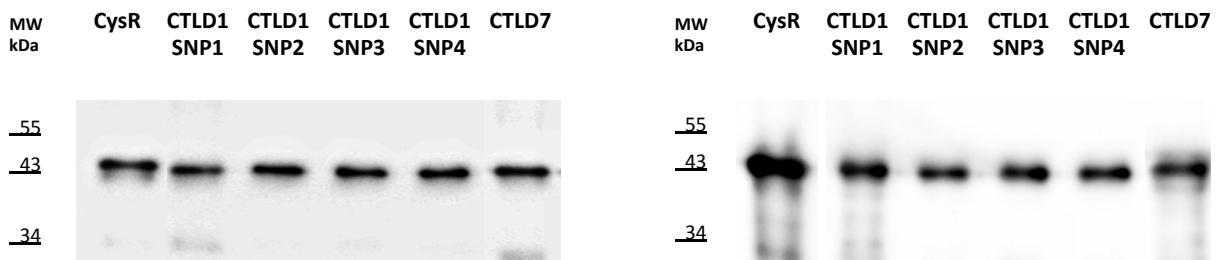
**Figure S2.** Top panel : Depletion of a serum with CysR and CTLD1 antibodies by adsorption of the serum on CysR or CTLD1 domains bound to anti-HA beads and then probing on the two antigens by western blot. Bottom panel: Competition with CysR or CTLD1 domains for antigen detection by western blot. Depletion : Serum dilutions from a patient with CysR and CTLD1 antibodies were loaded on three parallel anti-HA affinity columns preloaded with no antigen or purified *E. coli* DsbC-HA-CysR or DsbC-HA-CTLD1 antigens (5 µg each). The breakthrough fractions were collected and probed by western blot on the respective DsbC-HA-CysR or DsbC-HA-CTLD1 antigens (5 ng each). Competition : Serum dilution from the same above patient was preincubated with DsbC-HA-CysR or DsbC-HA-CTLD1 antigens (5 µg each) for one hour and then probed by western blot as above. Detection was done with anti-IgG4 secondary antibodies.



**Figure S3 : competition with specific antigens on full-length PLA2R1 by ELISA**

**Figure S3 (legend): Competition by ELISA with CysR, CTLD1 or CTLD6-7 domains (5 µg each, from *E. coli*) against the full-length PLA2R1 for two patients with a CysR profile and two patients with a CTLD1 profile.** Patients with a CysR profile: preincubation of serum with the CysR antigen induced a total loss of signal but no change with CTLD1 and CTLD6-7. Patients with a CTLD1 profile: competition with CysR or CTLD1 antigens alone induced a partial loss of signal, while competition with CTLD6-7 had no effect. However, competition with all antigens induced a total loss of signal.

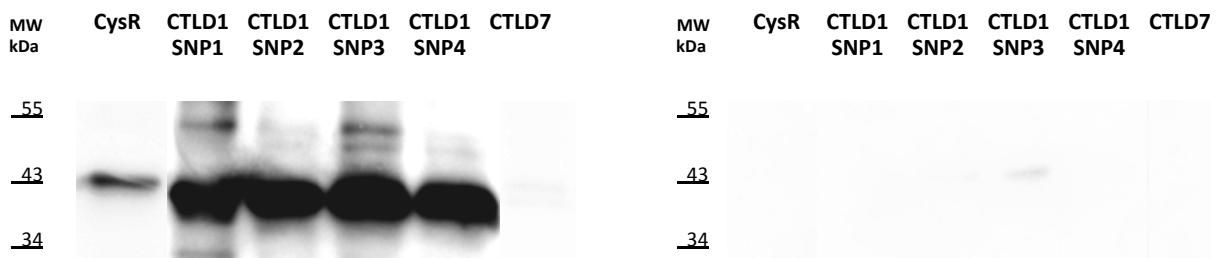
## Anti-HA



*Non reducing*

*Reducing*

## Patient group CysRC1



*Non reducing*

*Reducing*

## Patient group CysRC1C7

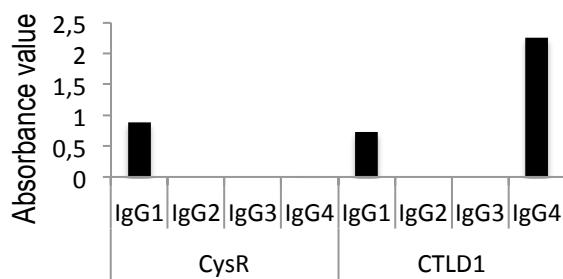


*Non reducing*

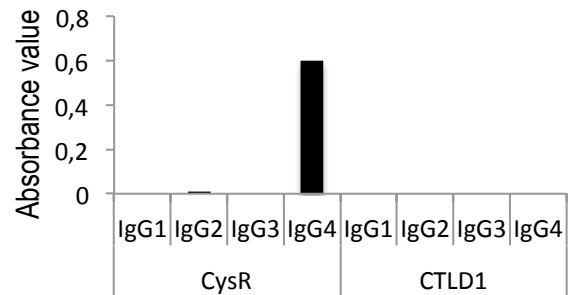
*Reducing*

**Figure S4: All three epitopes in CysR, CTLD1 and CTLD7 domains are sensitive to reducing conditions and CTLD1 domain with the 4 different SNPs are similarly recognized by patients' antibodies.** Western blots were loaded with 10-50 ng of the indicated E. coli DsbC-HA-antigens under non reducing and reducing conditions, and probed with anti-HA or serum of patients from the CysRC1 or CysRC1C7 groups, both having also anti-CysR autoantibodies. All CTLD1 SNP variants were equally recognized.

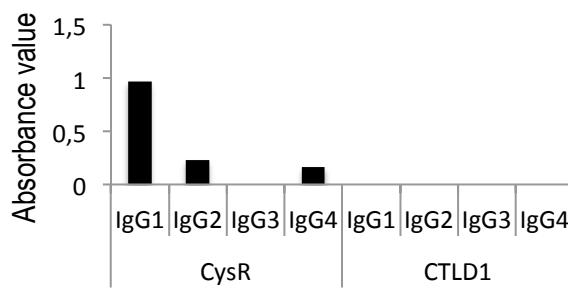
A. Patient IgG4 CysR-CTLD1+CTLD7-



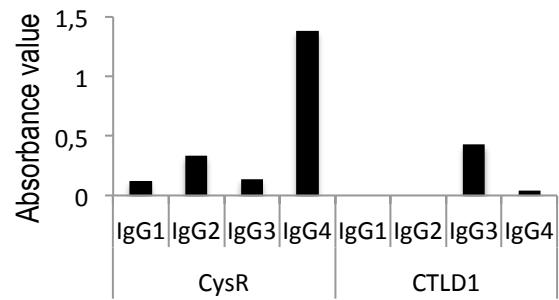
B. Patient IgG4 CysR+CTLD1-CTLD7+



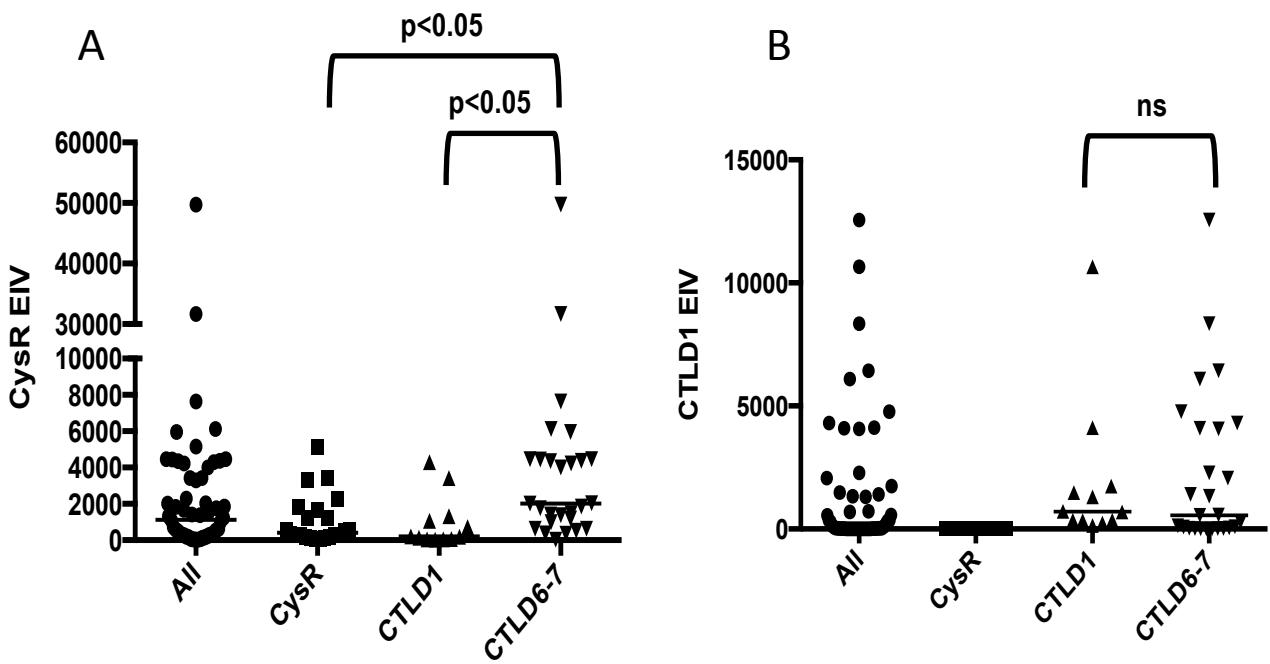
C. Patient IgG4 CysR+CTLD1-CTLD7+



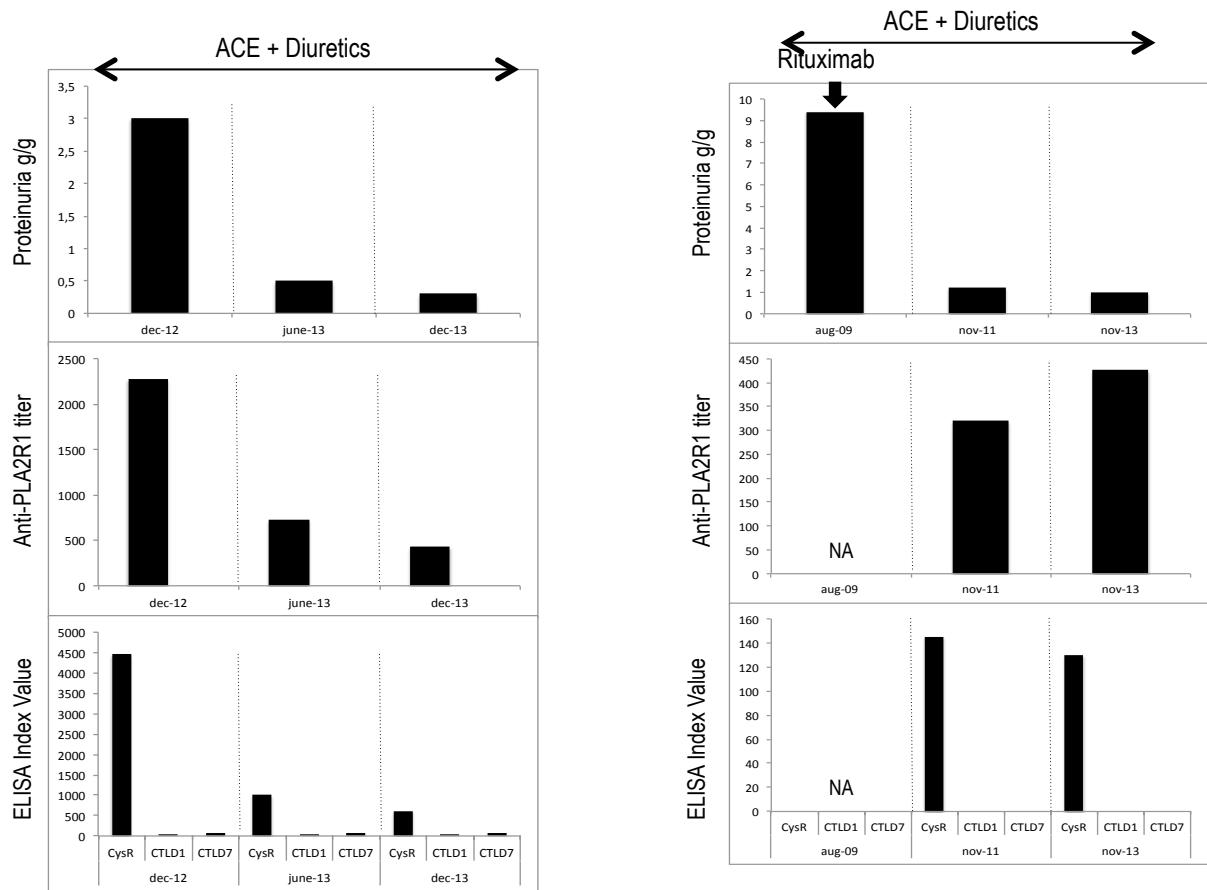
D. Patient IgG4 CysR+CTLD1-CTLD7+



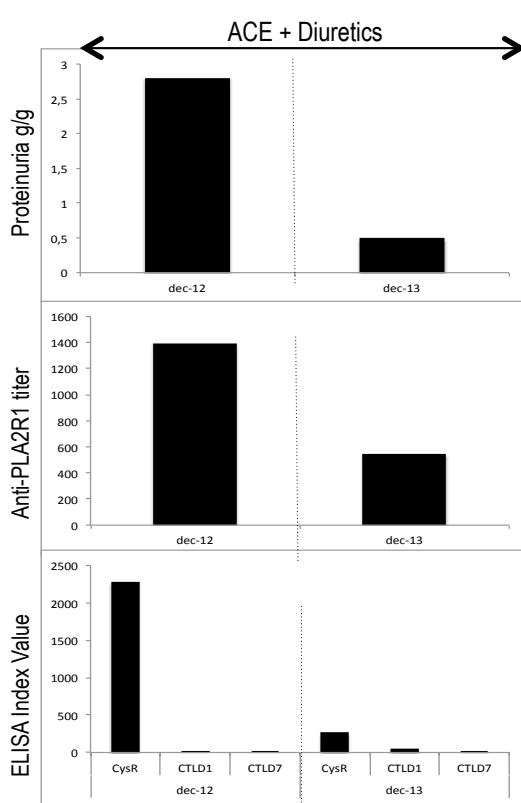
**Figure S5. IgG subclasses of anti-PLA2R1 autoantibodies against CysR and CTLD1 antigens for a few iMN patients negative for IgG4 anti-CysR or anti-CTLD1.** Detection of the different subclasses of anti-PLA2R1 autoantibodies against CysR and CTLD1 by epitope-specific ELISA for one patient negative for IgG4 anti-CysR (CysR-CTLD1+CTLD7-, panel A) and three patients negative for IgG4 anti-CTLD1 (CysR+CTLD1-CTLD7+, panels B, C and D). Patient A has IgG1 anti-CysR antibody while patient D has IgG3 anti-CTLD1 antibody. No anti-CTLD1 subclasses could be detected for patients B and C.



**Figure S6. Relationship between epitope-specific titers (CysR or CTLD1) in the different groups of patients CysR, CysRC1 (here indicated as CTLD1) and CysRC1C7 (here indicated as CTLD6-7). (A)** Anti-CysR titer is significantly higher in CysRC1 (CTLD1) and CysRC1C7 (CTLD6-7) groups than in CysR group. Titers measured for the CysR epitope for the 68 positive patients were sorted by epitope-specific groups and displayed. The highest CysR titers were observed for the CysRC1C7 group. **(B)** On the other hand, anti-CTLD1 titer is not significantly different in the CysRC1 and CysRC1C7 groups. EIV, ELISA index value.

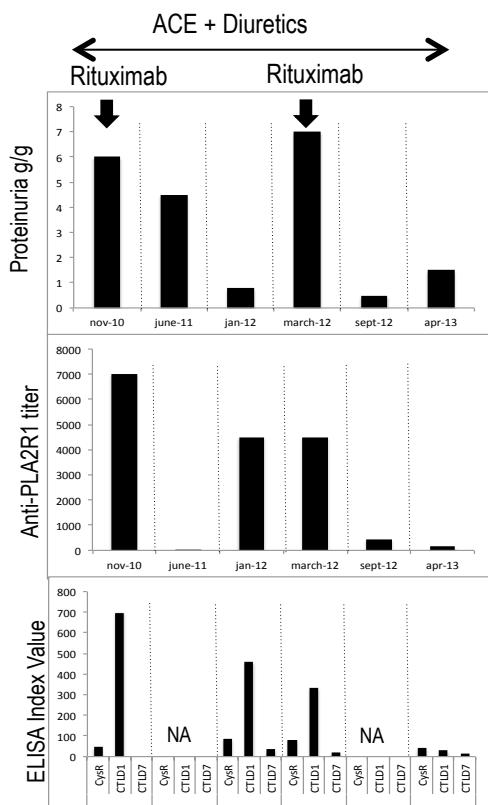


**Patient 9**

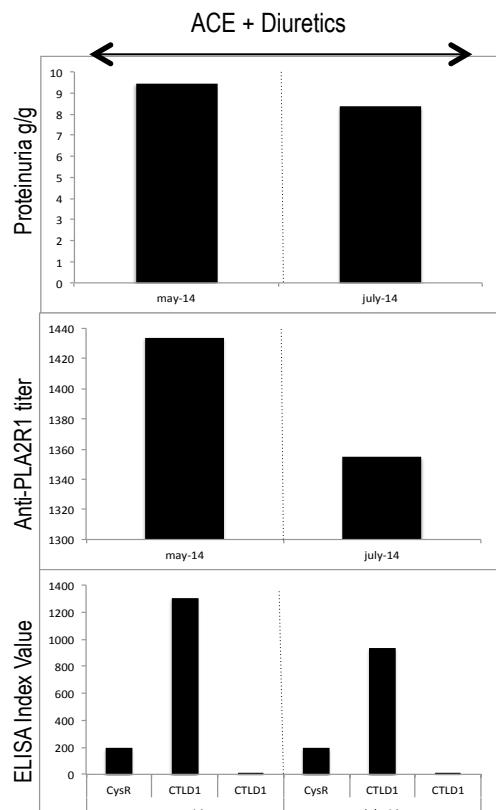


**Patient 10**

**Figure S7A: Patients showing a decrease of CysR antibodies associated with remission**

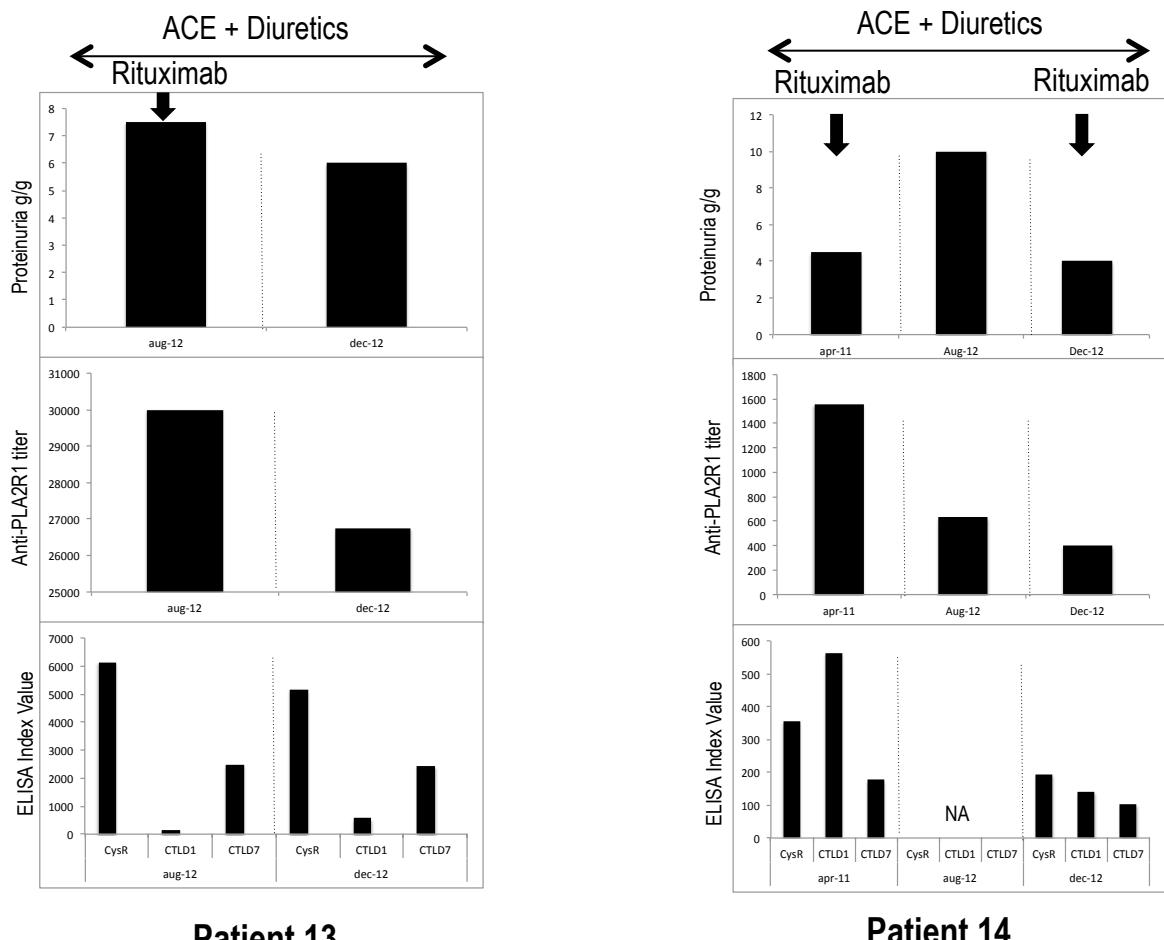


**Patient 11**

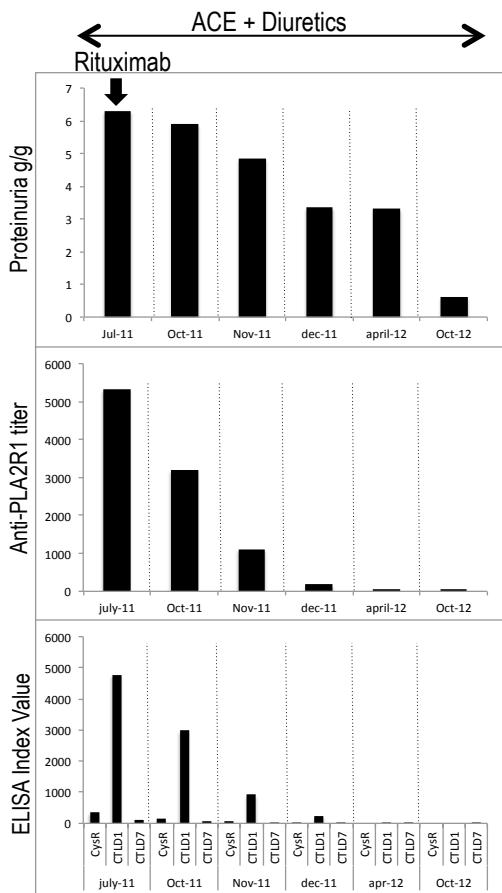


**Patient 12**

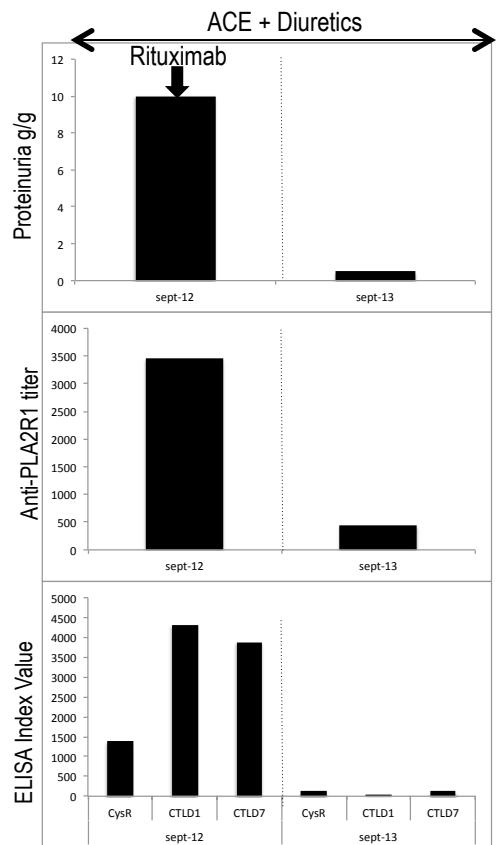
**Figure S7B: Patients showing a stable CysRC1 profiling**



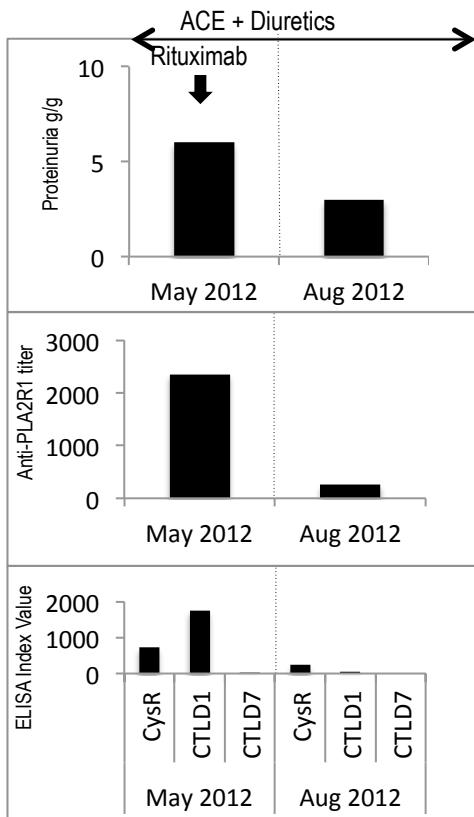
**Figure S7C: Patients showing a stable CysRC1C7 profiling**



**Patient 19**



**Patient 20**

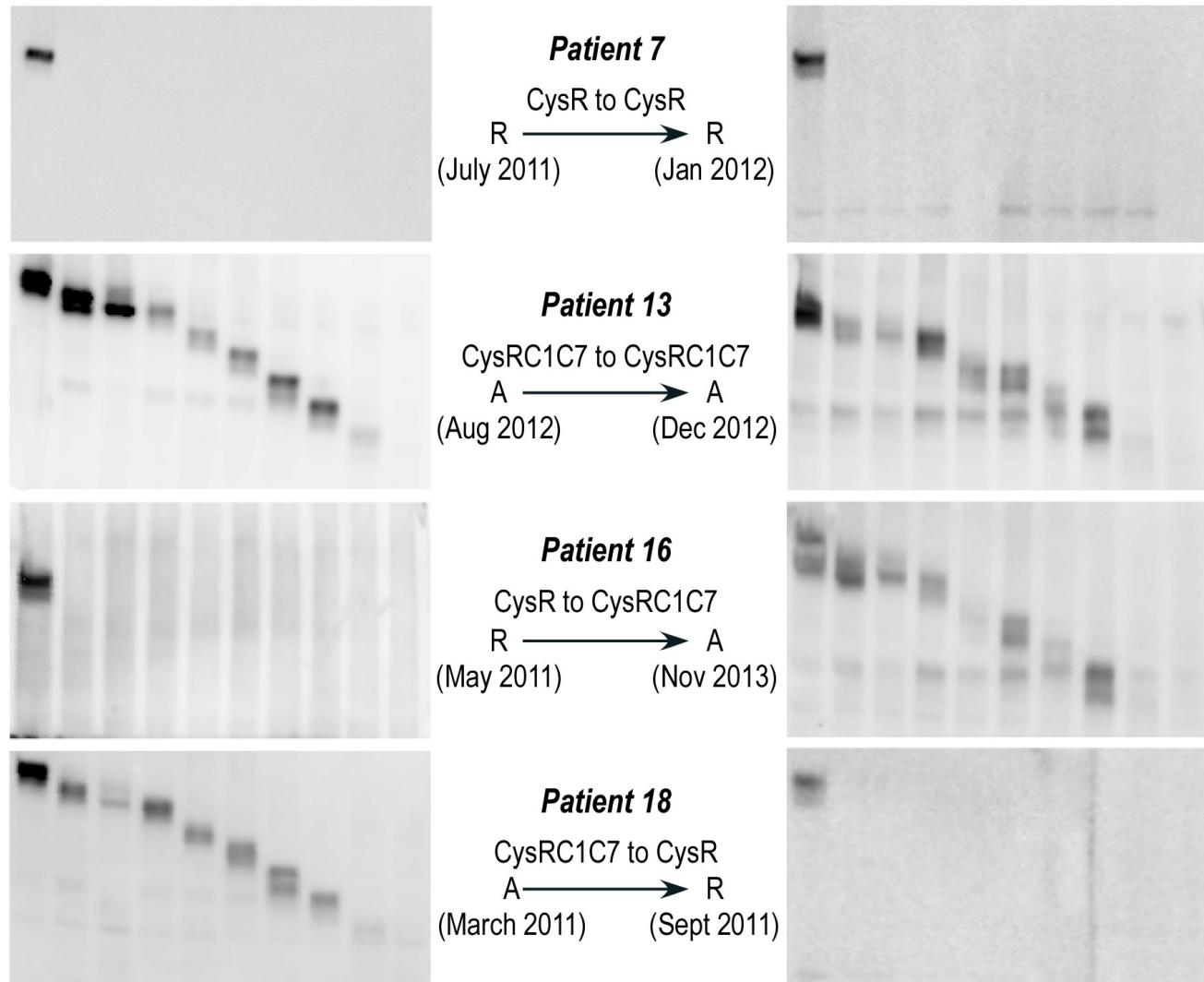


**Patient 21**

**Figure S7D: Patients showing a progressive decrease of each antibody with remission**

WT  $\Delta$ C  $\Delta$ F  $\Delta$ 1  $\Delta$ 2  $\Delta$ 3  $\Delta$ 4  $\Delta$ 5  $\Delta$ 6  $\Delta$ 7

WT  $\Delta$ C  $\Delta$ F  $\Delta$ 1  $\Delta$ 2  $\Delta$ 3  $\Delta$ 4  $\Delta$ 5  $\Delta$ 6  $\Delta$ 7



**Figure S7E: Epitope profiling of 4 patients shown in Fig. 10B, 10D, 10F, and in Fig S7C using the deletion mutants of PLA2R1 (IgG4 detection).**

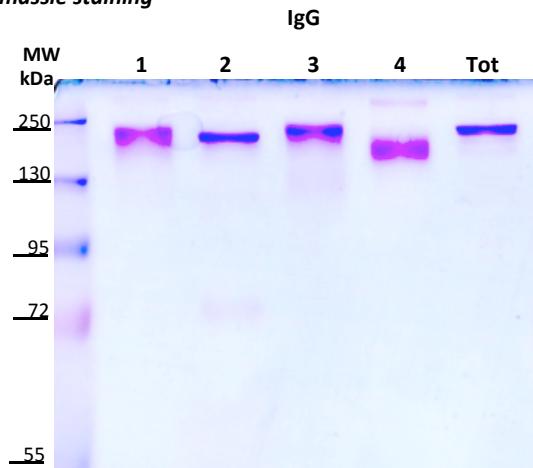
**Legend to Fig. S7: Epitope profiling during follow-up of patients** (A) Three patients presented a decrease of CysR antibodies while entering into remission. (B) Two patients with a stable level of CTLD1 antibodies and active disease. (C) Two patients with stable CTLD7 activity and active disease. (D) Three patients with a progressive decrease of several antibodies and remission. (E) Epitope profiling of the 4 patients shown in Fig. 10B, 10D and 10F, and in Fig S7C using the deletion mutants of PLA2R1 (IgG4 detection).

## **Epitope profiling and anti-PLA2R1 IgG subclasses**

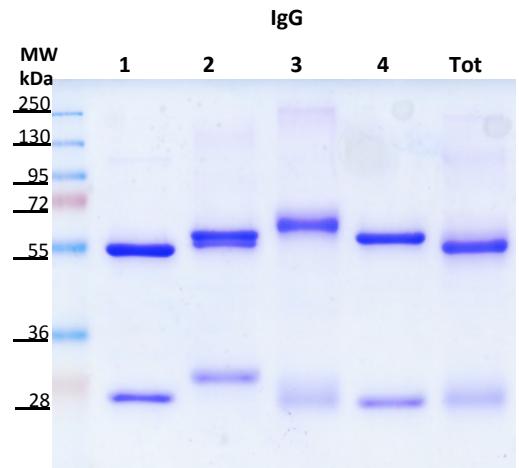
**Figure S8: Epitope profiling of different iMN patients probed with the series of deletion mutants of PLA2R1 by western blot and with the epitope specific ELISAs with detection for the different subclasses of anti-PLA2R1 IgGs.** (S8A) Validation of specificity of the commercially-available secondary anti-IgG subclasses used for detection. Purified human IgG subclasses were quantified by SDS-PAGE analysis followed by Coomassie staining. They were then probed with secondary antibodies specific for the different sulasses or total anti-IgGs. (S8B) Validation of expression of the HA-tagged deletion mutants of PLA2R1 by western blot with anti-HA antibodies under non reducing conditions. (S8C to E) Western blot results obtained for two patients from each group (CysR, CysRC1 and CysRC1C7) showing different subclasses of anti-PLA2R1 antibodies. (S8F) Detection by epitope-specific ELISA of 6 patients with the different IgG subclasses. In both western blot and ELISA assays, IgG1, IgG2, IgG3 and IgG4 autoantibodies reactive against CysR and CTLD1 were detected in some patients. However, only IgG4 antibodies could be detected against CTLD7, and IgG4 was always the most prominent anti-PLA2R1 IgG subclass. Detection with anti-total IgG provided the same epitope profile as anti-IgG4.

## SDS PAGE

### Coomassie staining



Non reducing conditions  
Amount IgG: 1 $\mu$ g



Reducing conditions  
Amount IgG: 5 $\mu$ g

## WESTERN BLOT

Non reducing conditions  
Amount IgG: 50ng

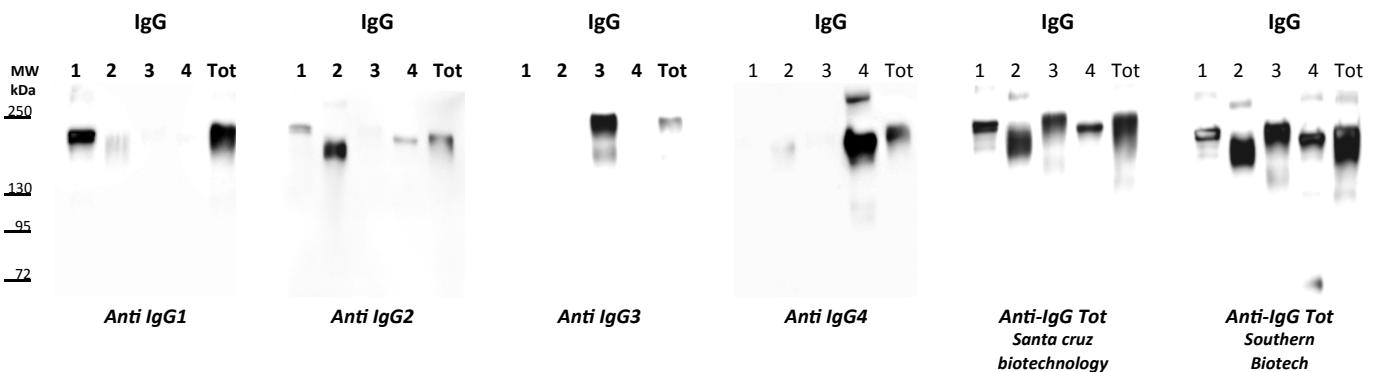


Figure S8A: Validation of secondary anti-IgG subclasses used for detection

# IgG subclasses (continued)

## PLA2R1 Deletion mutants

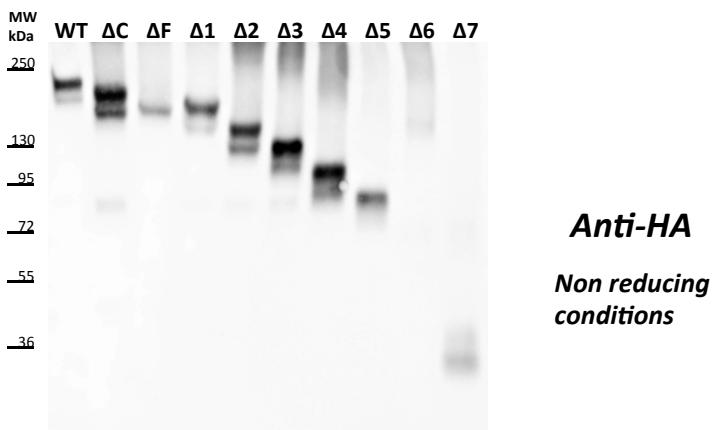
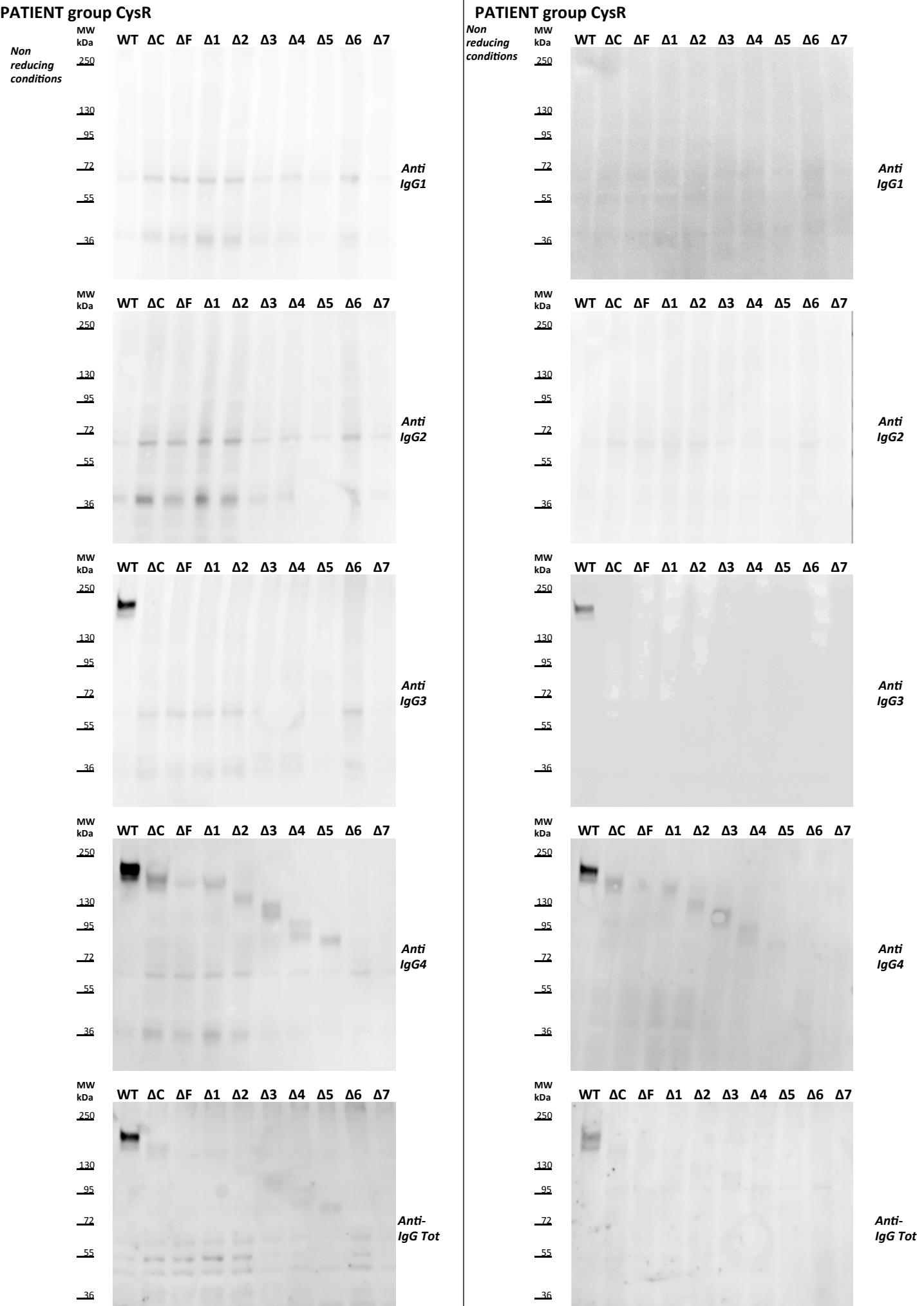


Figure S8B: Validation of PLA2R1 deletion mutants expression with anti-HA



**Figure S8C: Western blot of two CysR patients with detection for all IgG subclasses**

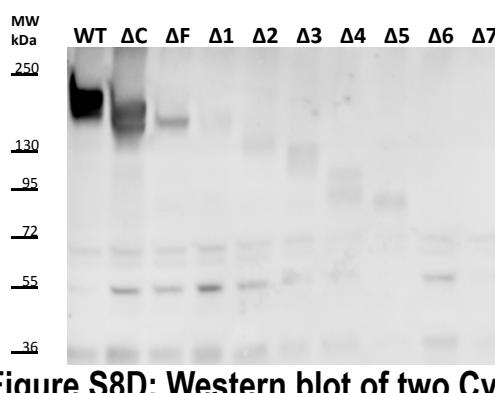
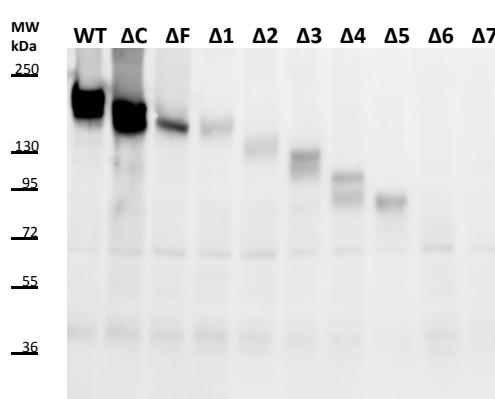
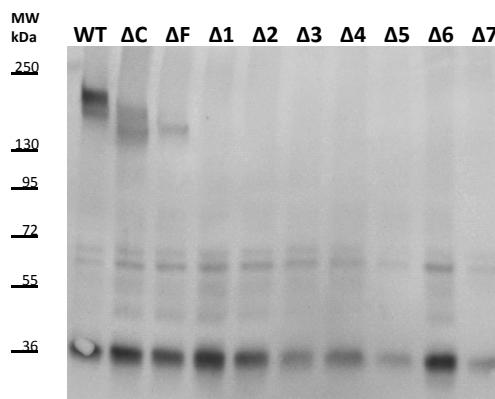
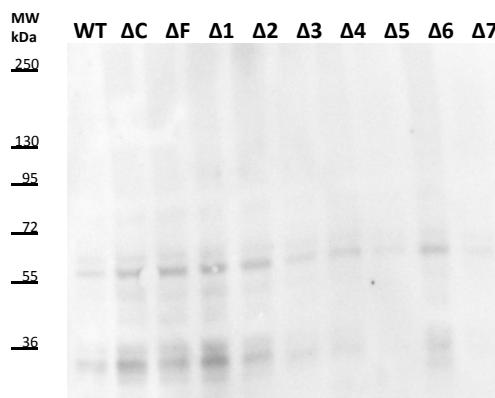
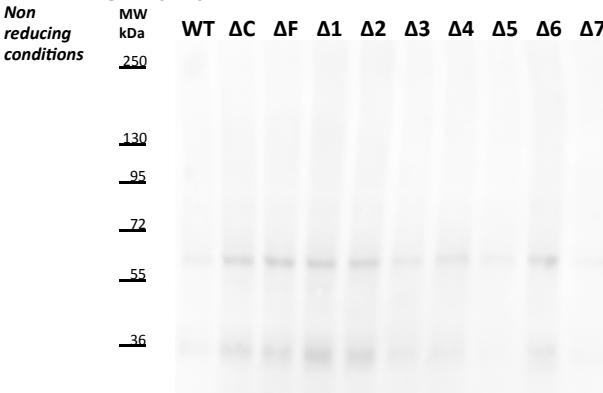
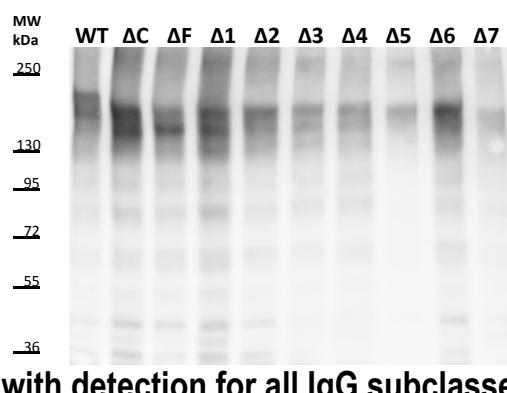
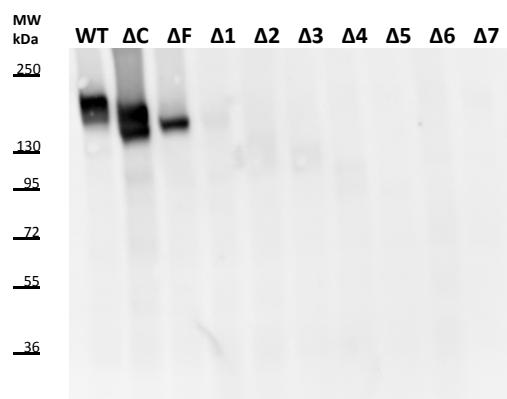
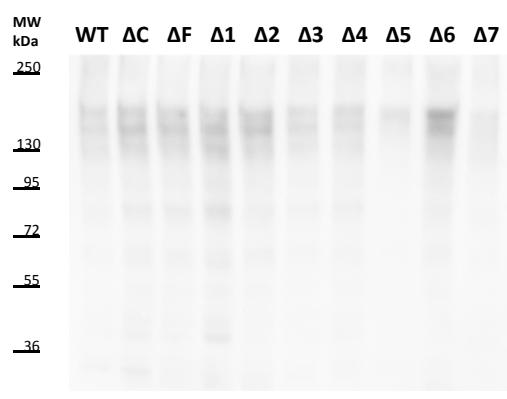
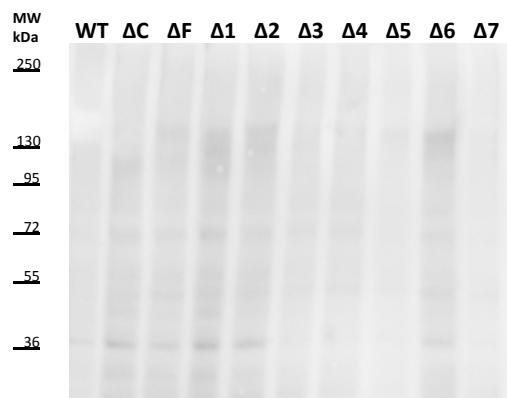
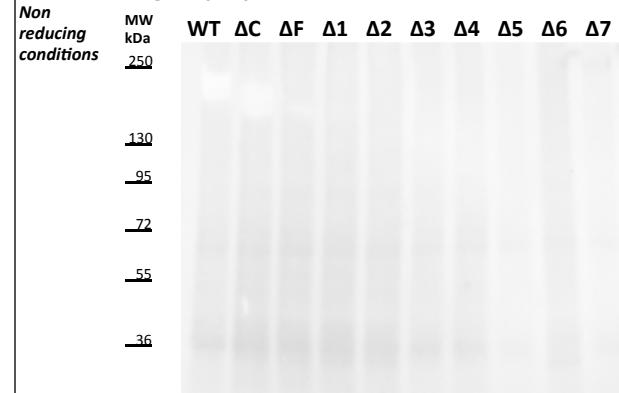
**PATIENT group CysRC1****PATIENT group CysRC1**

Figure S8D: Western blot of two CysRC1 patients with detection for all IgG subclasses

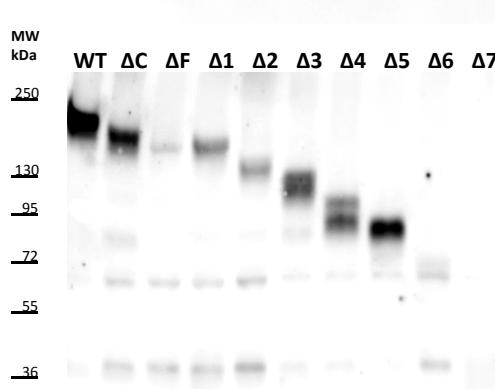
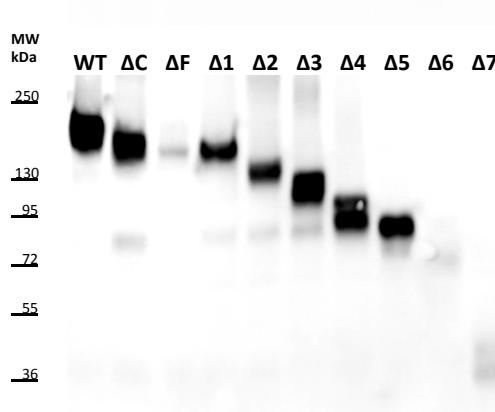
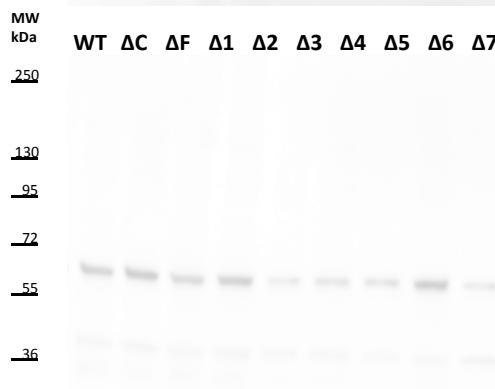
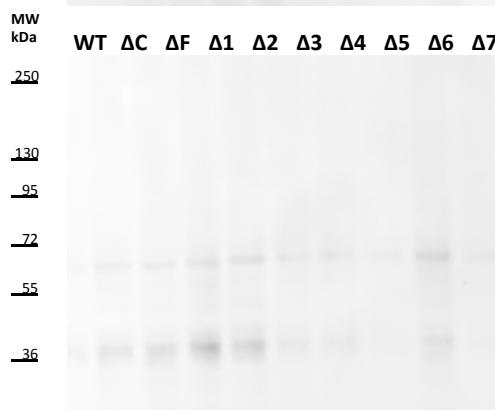
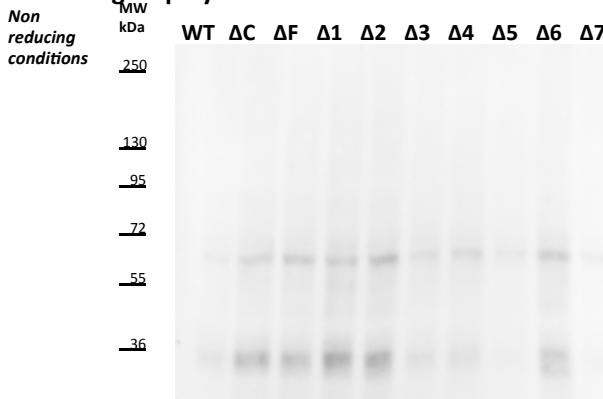
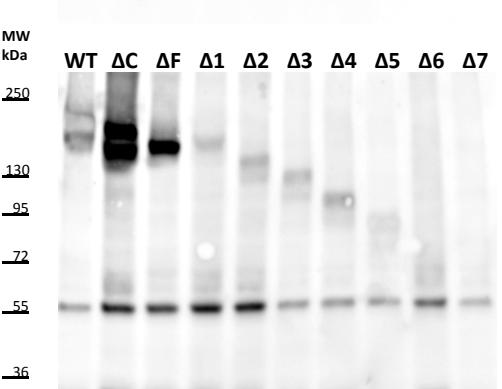
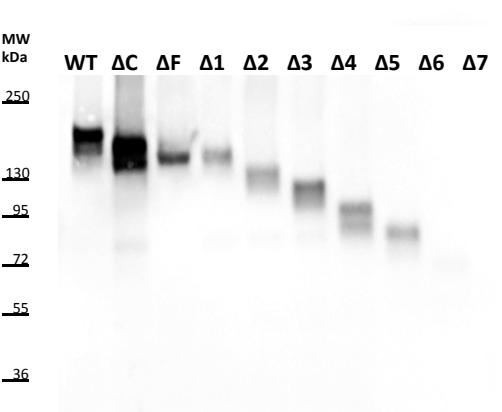
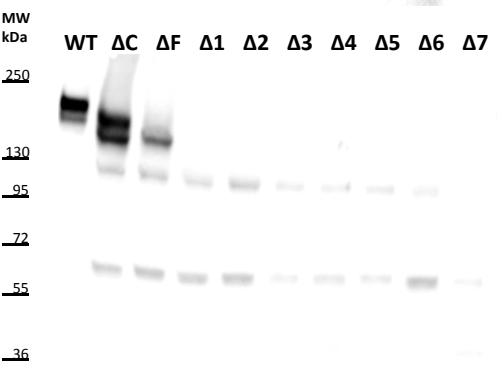
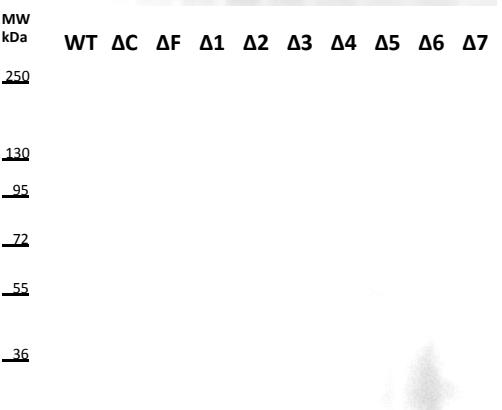
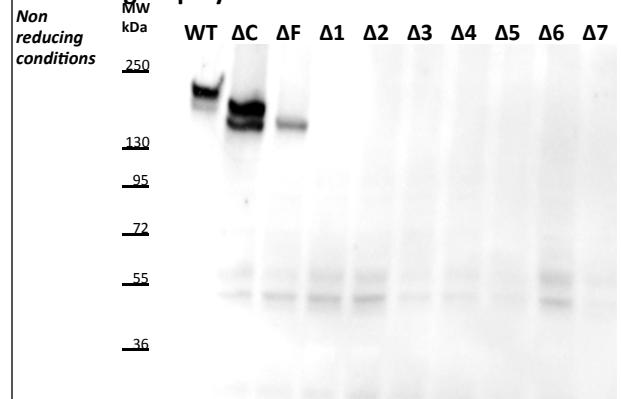
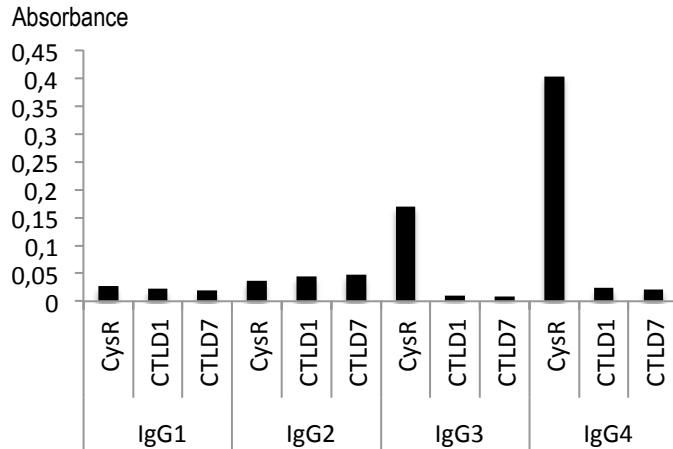
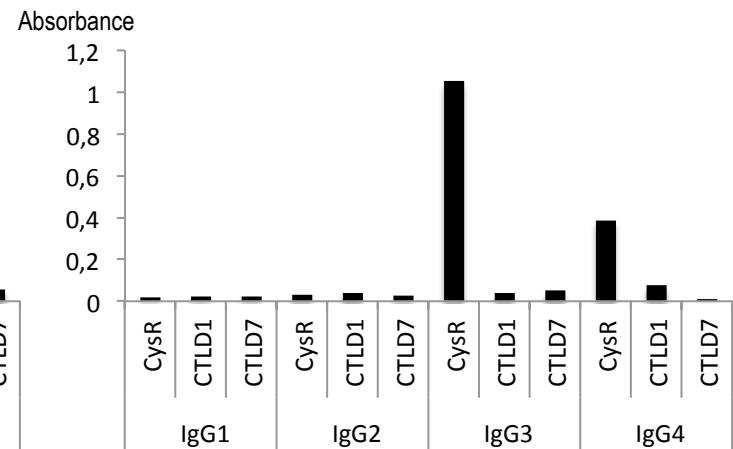
**PATIENT group CysRC1C7****PATIENT group CysRC1C7**

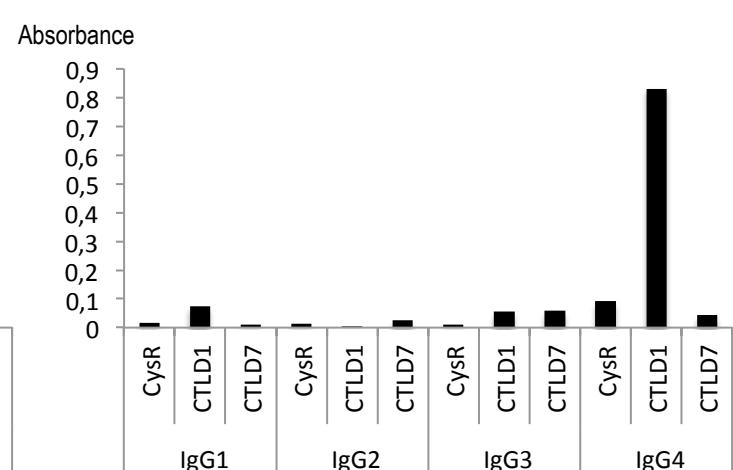
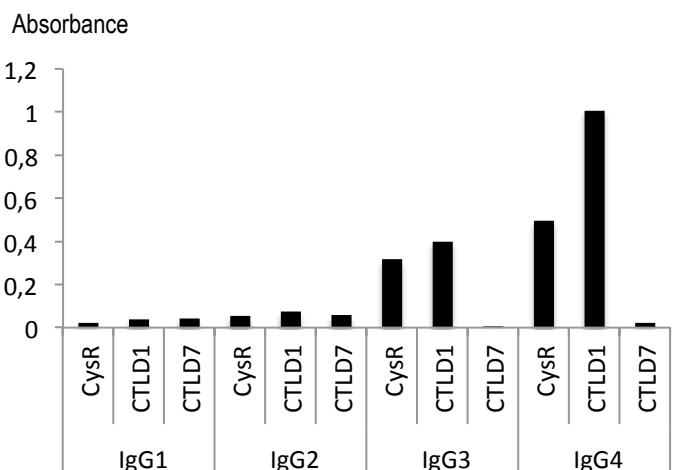
Figure S8E: Western blot of two CysRC1C7 patients with detection for all IgG subclasses



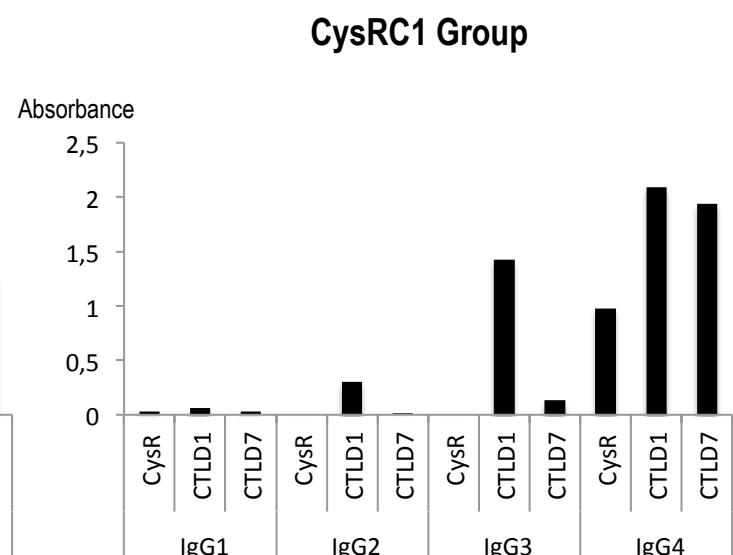
**CysR Group**



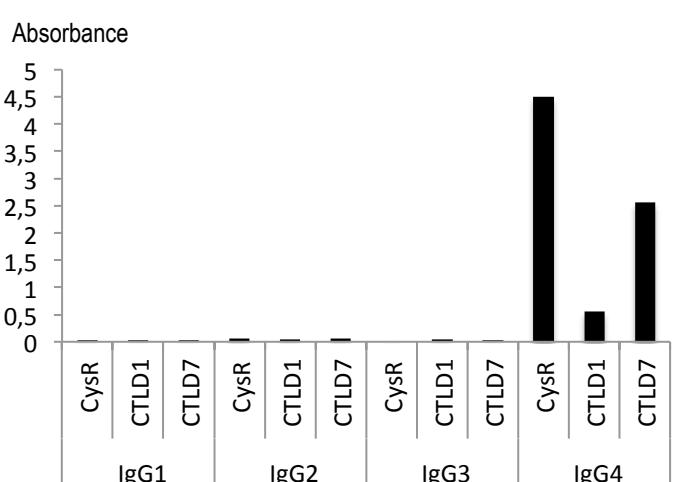
**CysR Group**



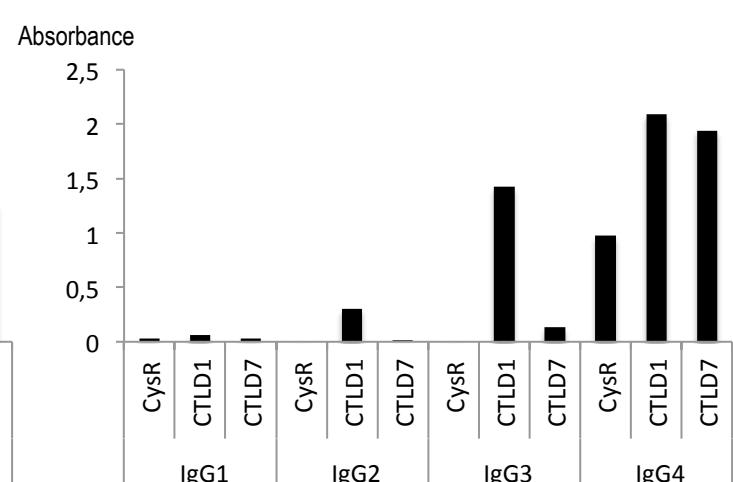
**CysRC1 Group**



**CysRC1 Group**



**CysRC1C7 Group**



**CysRC1C7 Group**

**Figure S8F: Detection of the different IgG subclasses of anti-PLA2R1 autoantibodies by epitope-specific ELISA for 6 patients belonging to the three different groups.**