Supplementary materials

Figure Sup 1A

Serum dilutions were associated to decrease (dilution 1:3) or negative (dilution 1:10) cytotoxicity. (Figure Sup 1A). Standard rabbit complement (5 µl/well, Cedarlane©, Ontario, Canada) or OptiMEM were added and incubated for 1 hour at room temperature. Dead cells were revealed after adding 2.5 µl/well of Fluoroquench AO/EB staining/quench (Ingen©Chilly-Mazarin, France) for 10 minutes in the dark. The percentage of dead cells was estimated using a fluorescent microscope (Videomicroscope Zeiss LSM780).

Figure sup 1B and 1C

Two investigators read plates blindly with a good correlation ($rs=0.81$ $p<0.0001$) (Figure 1B) and an excellent concordance (ICC=0.89 [0.85; 0.92] (Figure 1C).

Figure Sup 2

Various versions of gelatin veronal buffer (GVB) were used to determine the complement pathway involved in anti-PLA2R1 cytotoxicity. GVB supplemented with EDTA (GVB-EDTA) (ComplementTech©) was used to inhibit all complement activation pathways, whereas GVB supplemented with magnesium and EGTA (Mg-EGTA) (ComplementTech©) was used to inhibit the classical and lectin pathways, but not the alternative one. Three microliters of three PLA2R1-positive sera were diluted in 5 µl of GVB supplemented with GVB-EDTA or Mg-EGTA and 5 µl of standard rabbit complement. Cytotoxicity was assessed as described above. We confirmed in these conditions the inhibition of the classical pathway activity by measuring CH50 (Total Haemolytic Complement Kits Binding Site©) (Figure Sup 2).

All cytotoxicity assays were performed using the same batch of HEK293 T-Rex cells. A minimal of 50 cells per well was necessary for reading.

Figure Sup 3. ROC Curve of cell cytotoxicity between MN patients and healthy donors

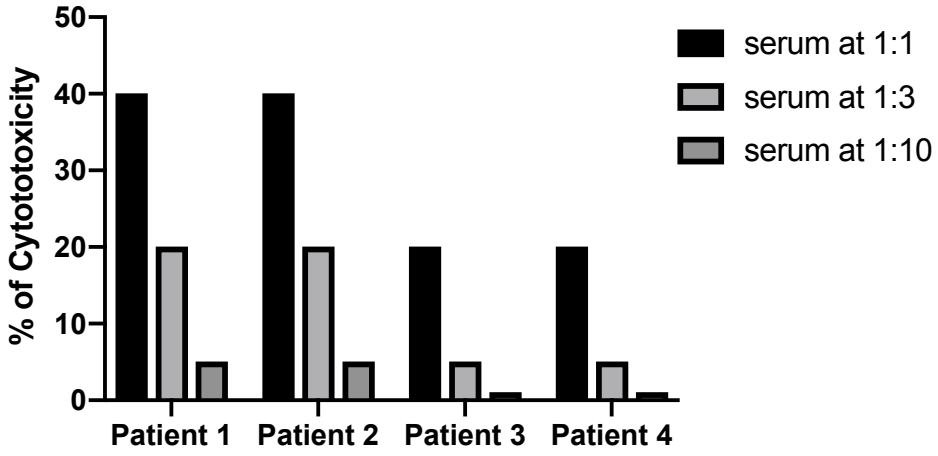
Using ROC curve analysis, we identified a threshold >10% of cytotoxicity associated with a positive test with a sensitivity at 87.5% and specificity at 84.6% (AUC=0.90 [0.83 to 0.98] $p<0.0001$)

Figure sup 4. Correlation between anti-PLA2R1 IgG1, 2, 3 and 4 titer and complement-mediated cytotoxicity.

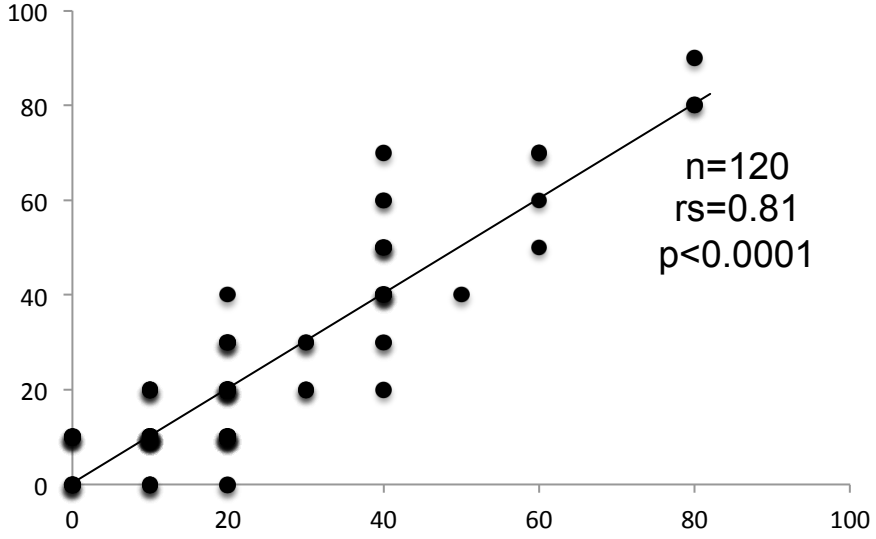
OD: Optic Density

Figure sup 5. Correlation between anti-PLA2R1 titer and epitope spreading

A



B



C

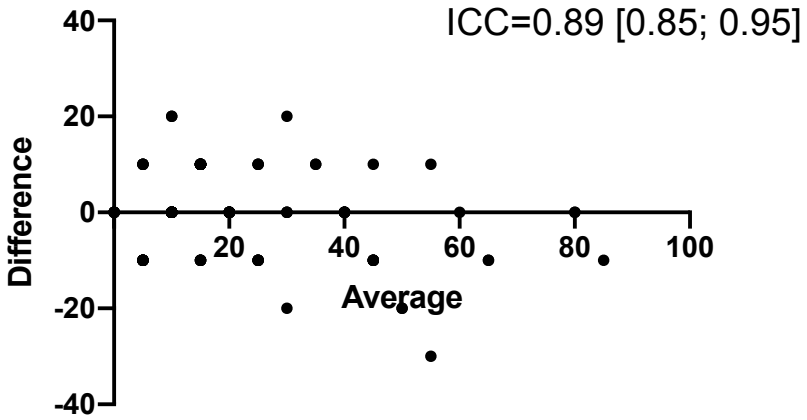
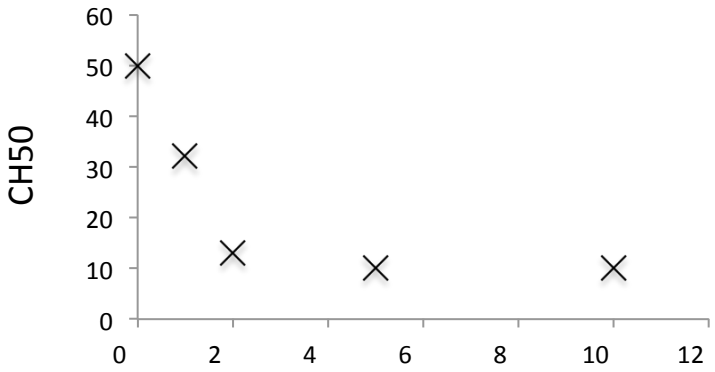
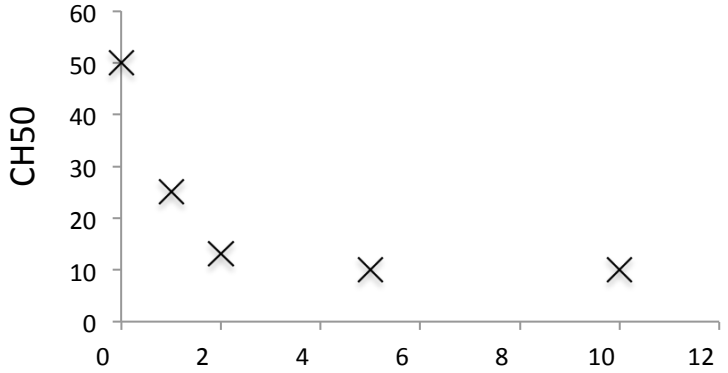


Figure Sup 1

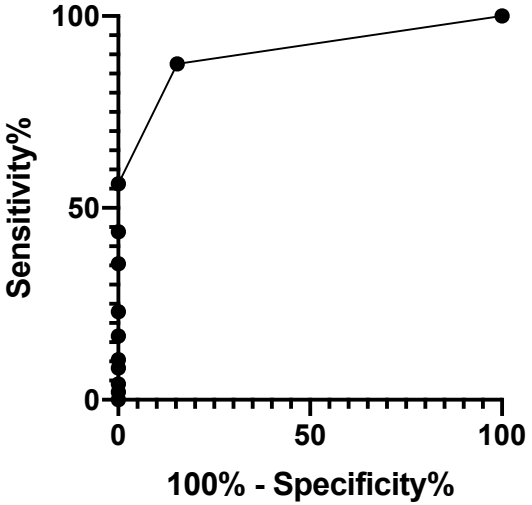


Volume (µl) of GVBE added in 3 µl of serum and 5 µl of complement



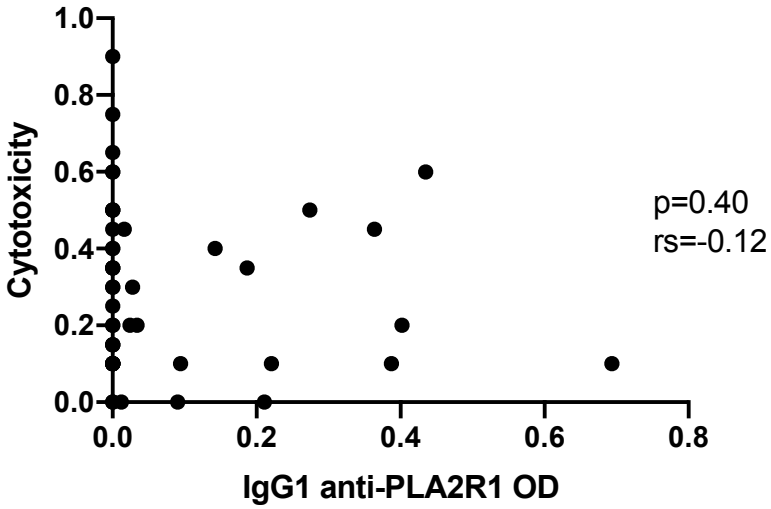
Volume (µl) of Mg-EGTA added in 3 µl of serum and 5µl of complement

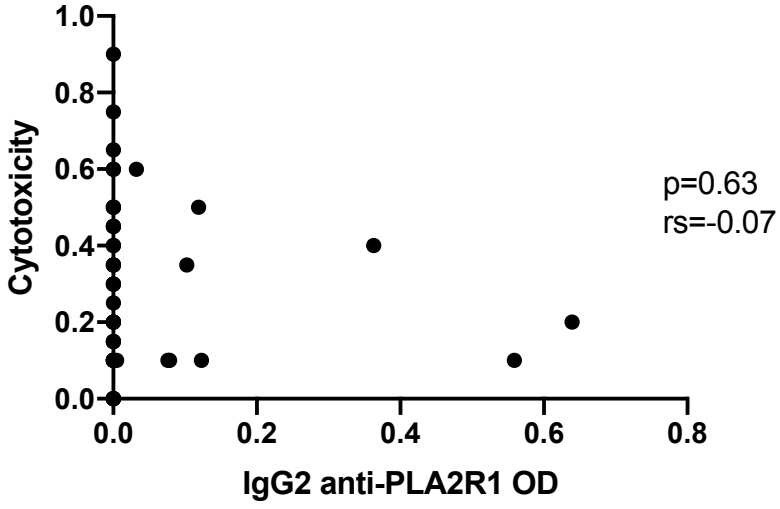
Figure Sup 2

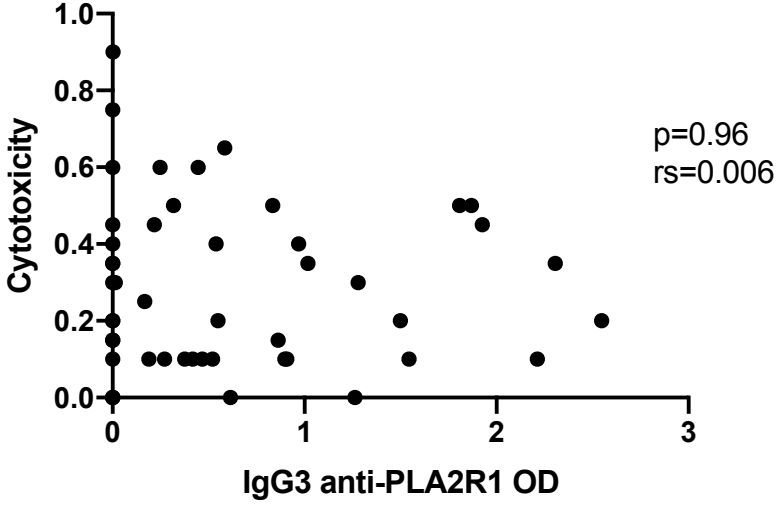


AUC=0.90
p<0.0001
Threeshold<10% Se=87.50 Spe=84.62

Figure Sup 3


Cytotoxicity
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
IgG1 anti-PLA2R1 OD
p=0.40
rs=-0.12


Cytotoxicity
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
IgG2 anti-PLA2R1 OD
p=0.63
rs=-0.07


Cytotoxicity
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
IgG3 anti-PLA2R1 OD
p=0.96
rs=0.006

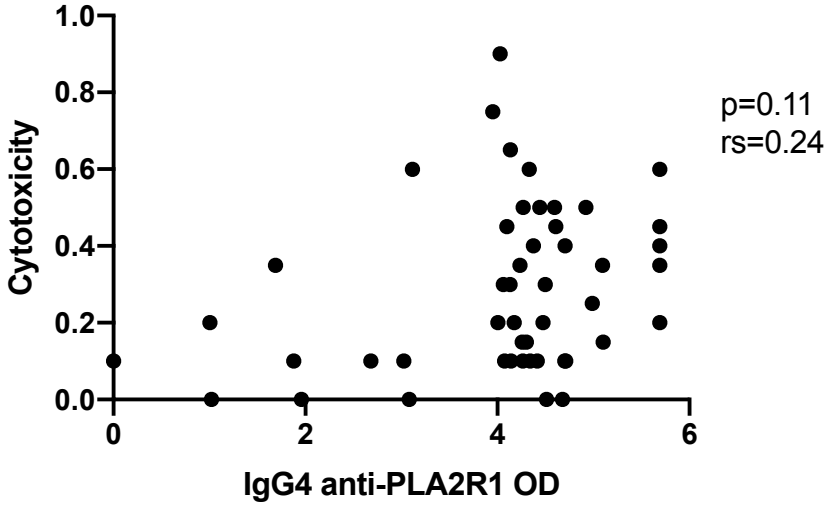
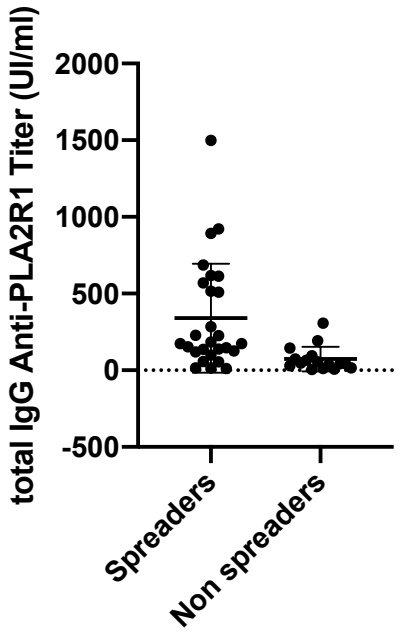

Cytotoxicity
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
IgG4 anti-PLA2R1 OD
p=0.11
rs=0.24

Figure sup 4



p=0.0004

Figure sup 5