Supplemental Material

Altered glycosylation of IgG4 promotes lectin complement pathway activation in anti-PLA2R1 associated membranous nephropathy

George Haddad, Johan Lorenzen, Hong Ma, Noortje de Haan, Harald Seeger, Christelle Zaghrini, Simone Brandt, Malte Kölling, Urs Wegmann, Bence Kiss, Gábor Pál, Péter Gál, Rudolf P. Wüthrich, Manfred Wuhrer, Laurence H. Beck, David J. Salant, Gérard Lambeau, Andreas D. Kistler

Supplemental Methods

List of reagents

RPMI1640, DMEM, Pierce® BCA protein assay kit, DH5α™ Competent Cells, CaptureSelect™ IgG4 affinity matrix, Pierce® protein concentrator 30K MWCO (Life Technologies, Switzerland), ITS (100x) media supplement, collagen, hexadimethrine bromide, cyclosporin A, C6 deficient serum, protease inhibitor cocktail, PNGase, cycloheximide, SB290157, W54011, and MTT (Sigma Aldrich, Switzerland), FuGene HD transfection reagent (Promega, Switzerland), human complement serum (Innovative Research, Novi, MI, USA), recombinant human TNFα (R&D System, Bio-Techne AG, Zug, Switzerland), ActinFarRed™ 647 stain (C055) (GeneCopoeia, BioCat GmbH, Heidelberg, Germany), FlexiTube PLA2R1, ITGAX, C3aR1, C5aR1, C5L2 siRNA, HiPerFect transfection reagent (Qiagen, Hombrechtikon, Switzerland), recombinant human MBL2 (Sino Biological, Lucerna-Chem AG, Luzern, Switzerland), E64 (Selleckchem, LuBio Science, Luzern, Switzerland), Gelatin veronal buffer (Boston BioProducts, Ashland, MA, USA), Leupeptin, AEBSF, and pepstatin A (Enzo Life Sciences, Lausen, Switzerland), T4 DNA ligase, BamHI, AflII, and glycosidase buffer pack (New England Biolabs, Bioconcept, Allschwil, Switzerland).

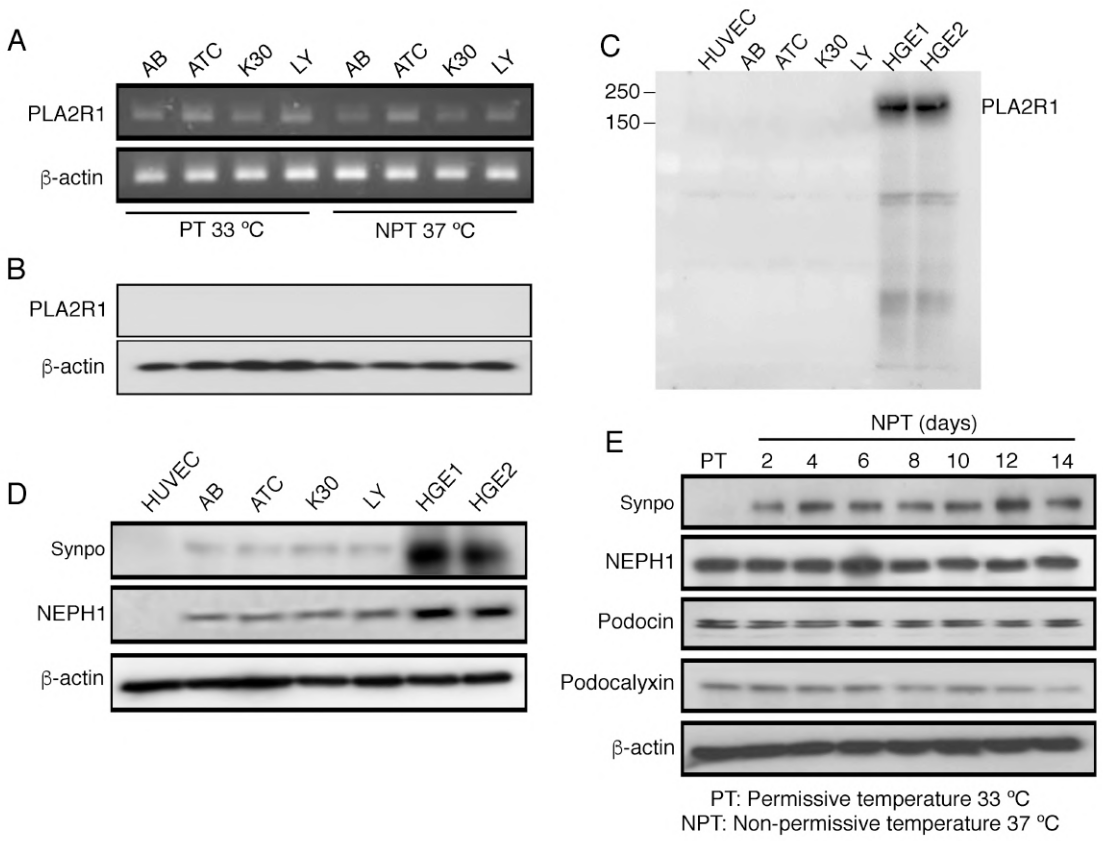
The following antibodies were used in this study: β-actin (A5441, mouse monoclonal, Sigma Aldrich, Switzerland); PLA2R (ab188028, mouse monoclonal, Abcam and AMAb90772, mouse monoclonal, Atlas antibodies), dynamin (sc-17807, mouse monoclonal, Santa Cruz Biotechnology); NEPH1 (sc373787 mouse monoclonal, Santa Cruz Biotechnology); podocalyxin (AF1556, goat polyclonal, R&D Systems); podocin (P0372, rabbit polyclonal, Sigma Aldrich, Switzerland); synaptopodin (sc-21537, goat polyclonal, Santa Cruz Biotechnology and PA5-80088, rabbit polyclonal, ThermoFisher Scientific); C4b (ab66791, rabbit polyclonal, Abcam); C3c (ab4212, rabbit polyclonal, Abcam); C5b-9 (ab55811, rabbit polyclonal, Abcam); ITGAX (sc-6619, goat polyclonal, Santa Cruz Biotechnology); C5L2 (sc-

30352, goat polyclonal, Santa Cruz Biotechnology); C3aR1 (ab126250, rabbit polyclonal, Abcam); C5aR1 (ab59390, rabbit polyclonal, Abcam); MBL (ab23457, mouse monoclonal, Abcam); AKT1 (ab194875, rabbit polyclonal, Abcam); anti-human IgG4-FITC (ab99821, mouse monoclonal, Abcam); phospho-Akt T308 (13038, rabbit monoclonal), phospho-Akt S473 (4060, rabbit monoclonal), phospho-p44/p42 (9101, rabbit monoclonal), p44/p42 MAPK (4695, rabbit monoclonal), all from Cell Signaling Technologies.

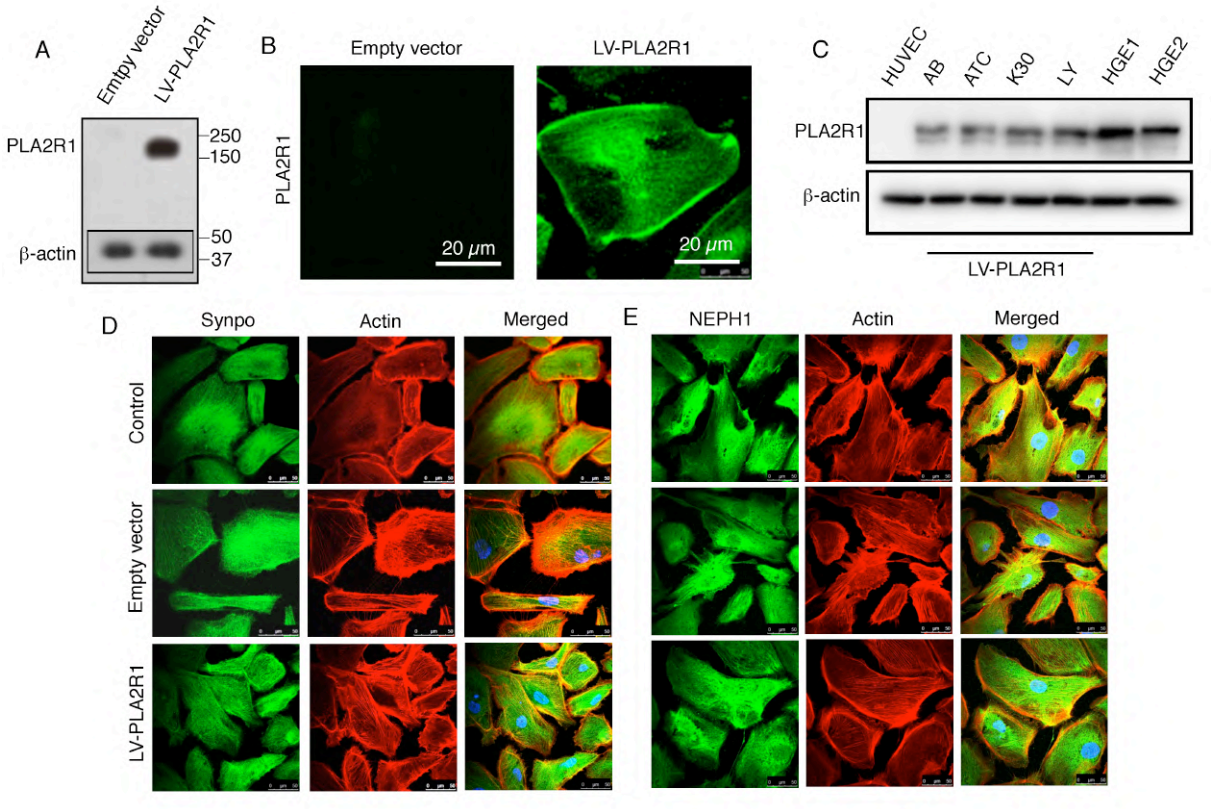
Gene	**Forward (5'-3')**	**Reverse (5'-3')**
C3aR1	GAAACCAGCCCACTGGATAA	TGGTAGCTCAGACTCGTAGAA
C5aR1	AGTGGTGGCCAGTTTCTTTAT	CACAGGGAGTCCAGCTTATTC
CR3	CAGAACAACCCTAACCCAAGAT	AACAGCTCTCGTACCACTTTG
CR4	GAACTTCGTGAGAGCTGTGATA	CCTCGAAAGTGAAGTGTGTTTG
C5L2	TGCTGTGCTGTTTGTCTCT	ACATGGTCAGCAGGATGATG
PLA2R1	CCAAGAGATGTGAAACCCAAGA	CCATTCTGAGGCAAAGGTATGA
nephrin	TCCATGTCCAACCTGACATTC	CAGTTTCTGGGCGGGATATT
podocin	GTACCAAATCCTCCGGCTTAG	TTACGCAGAACCAGATGGAAA
p-cadherin	GGATGATGCCATCTACACCTAC	TGCTCCGGTGAATTGTGAA

Supplemental Table 1. Primer sequences used for RT-PCR

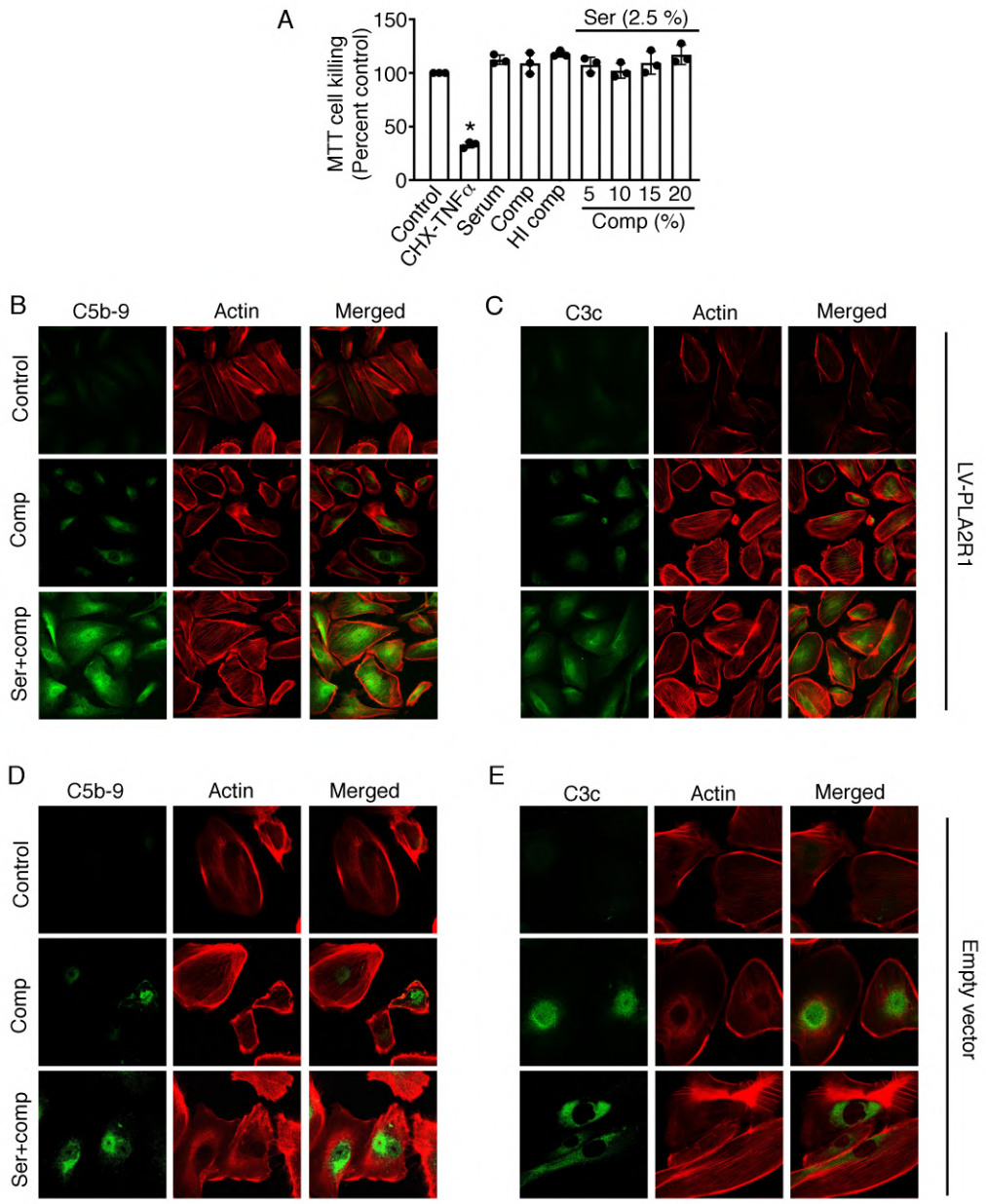
Supplemental Figures



Supplemental Figure 1. Endogenous PLA2R1 expression in cultured human podocytes and characterization of podocyte cell lines. The endogenous expression of PLA2R1 was assayed in four podocyte cell lines (ATC, AB, K30 & LY) under permissive and non-permissive conditions (i.e. growth at 33 and 37°C, respectively, the former allowing proliferation and the latter allowing differentiation of the cells) both at the RNA (A) and the protein (B) levels. PLA2R1 mRNA is found expressed in all four podocyte cell lines at similar levels in the cells cultured at permissive and non-permissive temperatures. However, endogenous PLA2R1 expression at the protein level was below the detection limits of the commercial anti-PLA2R1 antibody used (Abcam mouse monoclonal ab188028), which readily detected endogenous PLA2R1-expression in human glomerular extracts from undiseased kidneys (C). All four cell lines expressed synaptopodin and NEPH1 (D). Characterization of the LY podocyte cell line, which was used for most further experiments, by expression of four key podocyte markers over the timecourse of differentiation is shown in panel E. The podocytes shown in panels C and D were differentiated under non-permissive conditions for 14 days. HGE, human glomerular extracts; HUVEC, human umbilical vein endothelial cells; Synpo, synaptopodin.

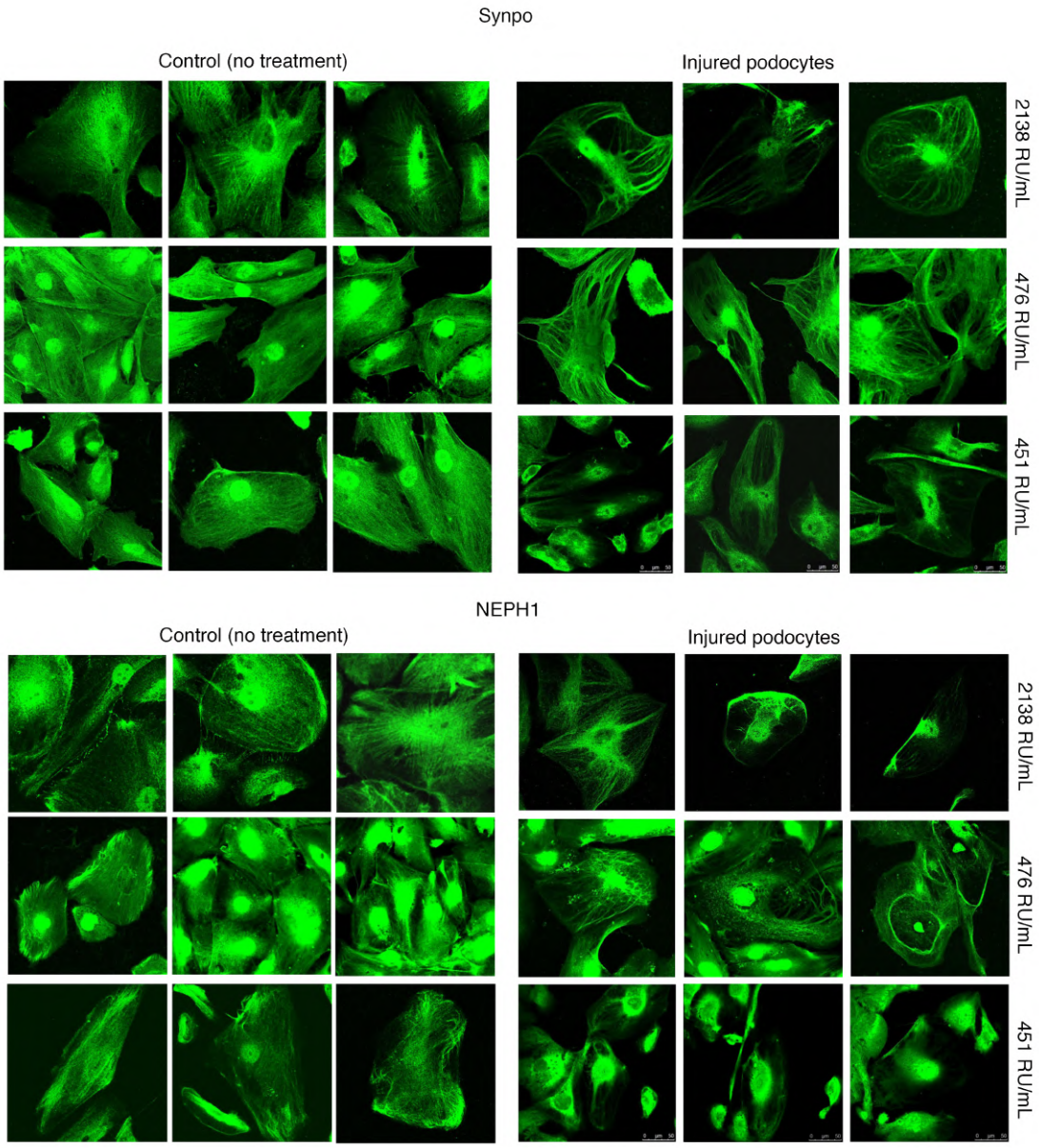


Supplemental Figure 2. PLA2R1 overexpression does not alter the expression levels of synaptopodin and NEPH1. Lentiviral gene transfer allowed to express PLA2R1 in differentiated podocytes as shown by western blot (A) and immunofluorescence (B) analysis. PLA2R1 was expressed in four podocyte cell lines, and protein levels of cell lysates were compared to human glomerular extracts (HGE), 30 µg total protein were loaded per lane (C). Differentiated podocytes grown on cover slips were treated with PLA2R1 lentivirus, empty vector, or were left untreated. 72 h post-infection the cells were fixed, permeabilized and stained for synaptopodin (D, green), NEPH1 (E, green) and actin (red). The representative fluorescent images show a normal actin filament structure and normal synaptopodin and NEPH1 expression levels as compared to controls. The representative images were captured using Leica SP8 upright confocal microscope and 63X objective lens.

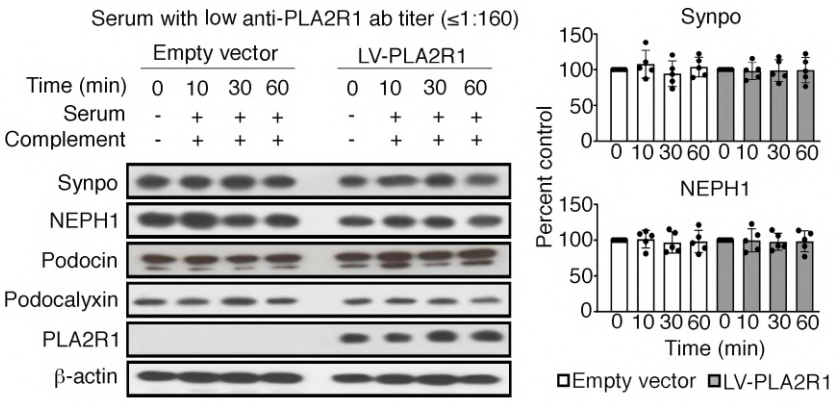


Supplemental Figure 3. Human podocytes are resistant to complement-mediated cell lysis despite C5b-9 and C3c deposition. (A) Differentiated human podocytes expressing PLA2R1 were pretreated with 2.5 % high-titer (1:1000) anti-PLA2R1-positive pMN serum for 30 min at 37°C followed by 1h incubation with various concentrations (5-20 %) of NHS as a source of functional complement (Comp) or heat inactivated NHS (HI Comp). Cycloheximide (CHX, 30 µg/mL) plus TNFα (25 ng/mL) was used as an apoptotic stimulus for the positive control. MTT was added for 4h, the formazan crystals were solubilized with DMSO and the plate was read at 540 nm. n=3, the bars represent mean ± SEM, * $P < 0.05$ by one-way ANOVA and Tukey's post hoc test for the comparison with control. (B-E) Differentiated human podocytes infected with PLA2R1-containing lentivirus (LV-PLA2R1, B and C) or empty vector (D and E) were

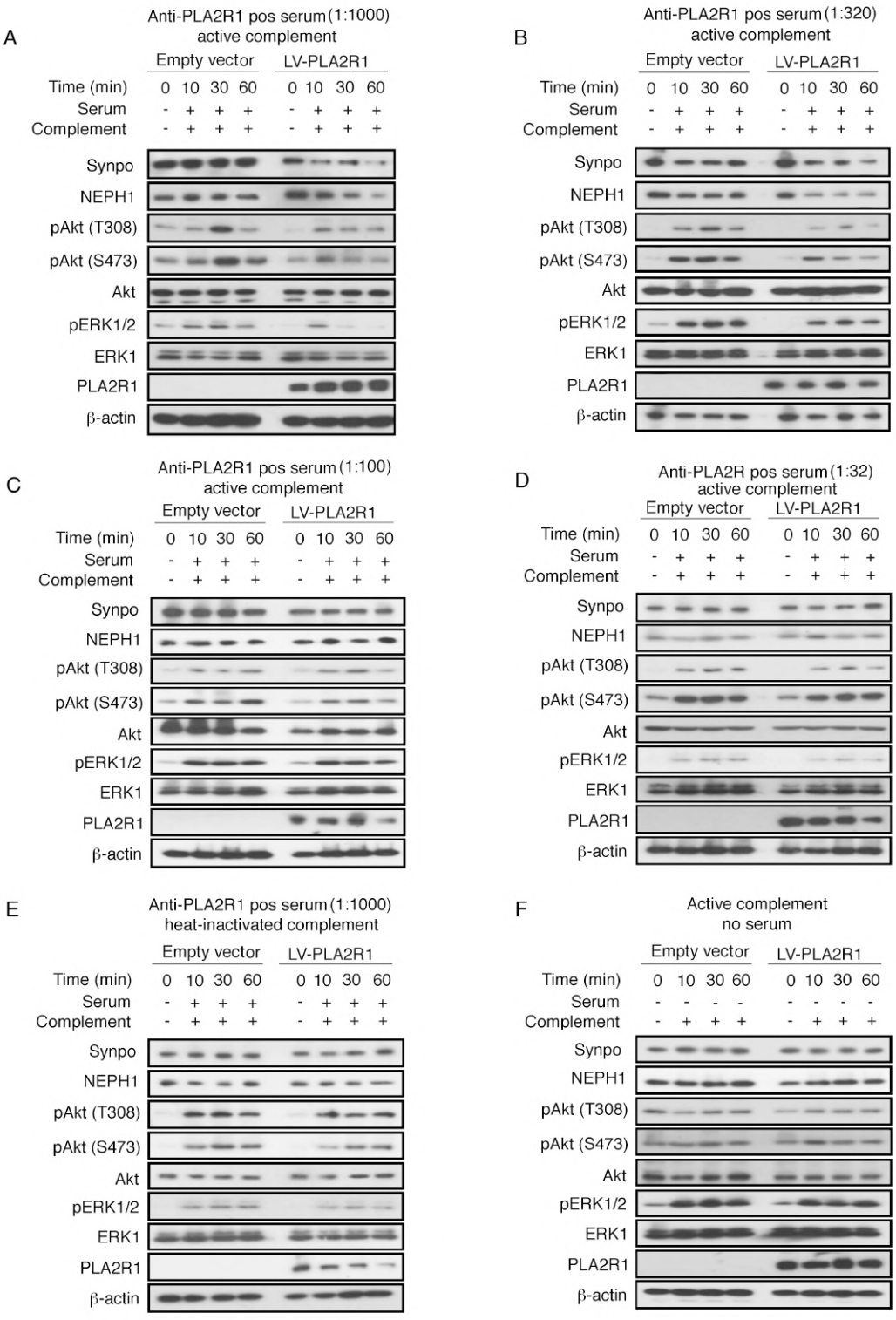
incubated for 1h at 37°C with 4% NHS as a complement source either without (Comp) or with (Ser+comp) preincubation with high-titer anti-PLA2R1-positive pMN patient serum (for 30 min) and C5b-9 (B and D) or C3c (C and E) deposition were visualized by immunofluorescence. The representative images were captured using Leica SP8 upright confocal microscope and 63X objective lens.



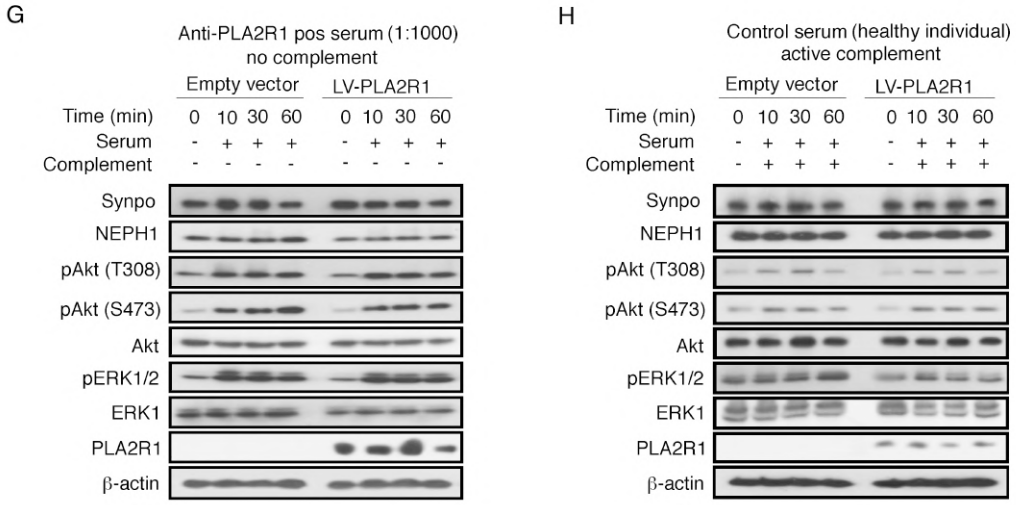
Supplemental Figure 4. Effect of anti-PLA2R1-positive MN sera and complement on synaptopodin and NEPH1 levels and their subcellular distribution. Differentiated human podocytes overexpressing PLA2R1 were pretreated in three separate experiments with 2.5 % serum from three different pMN patients with high titer anti-PLA2R1 antibodies for 30 min at 37°C followed by 24h incubation with 5% NHS. The cells were stained for synaptopodin (A) or NEPH1 (B). Three image examples of treated cells and three image examples of untreated control cells from the same experiment are shown. The images in the lower row of both figure panels are from the same experiment as the images shown in Figure 4 of the main paper.



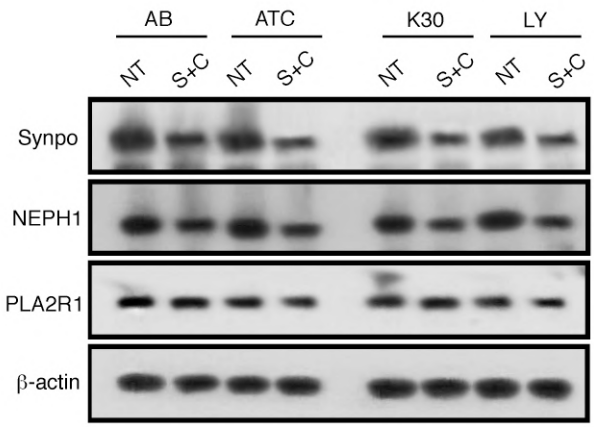
Supplemental Figure 5. Low-titer anti-PLA2R1 positive sera do not induce degradation of synaptopodin and NEPH1. The same experiment as shown in Figure 1A was performed using 2.5% anti-PLA2R1-positive patient sera with low antibody titer (≤ 1:160). Bars represent mean ± SEM of the average of 3 independent experiments in each of 5 patients.



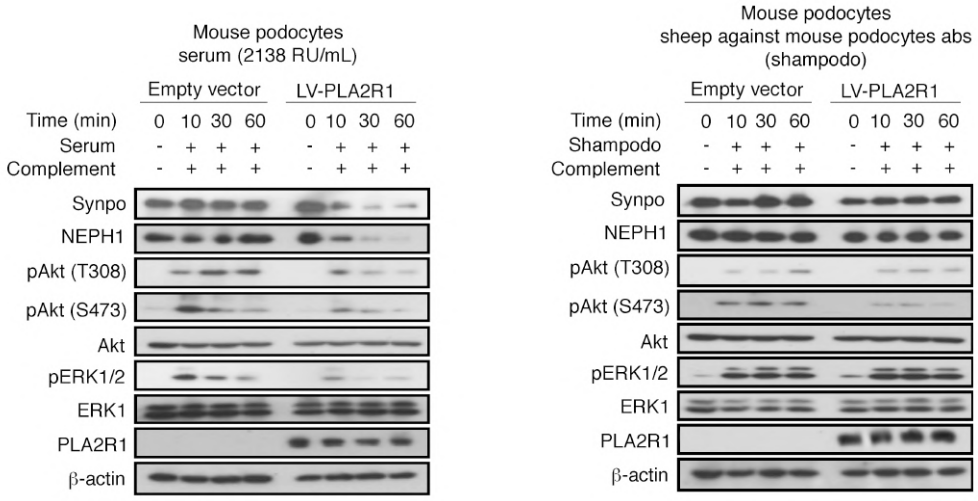
Supplemental Figure 6, continued on next page



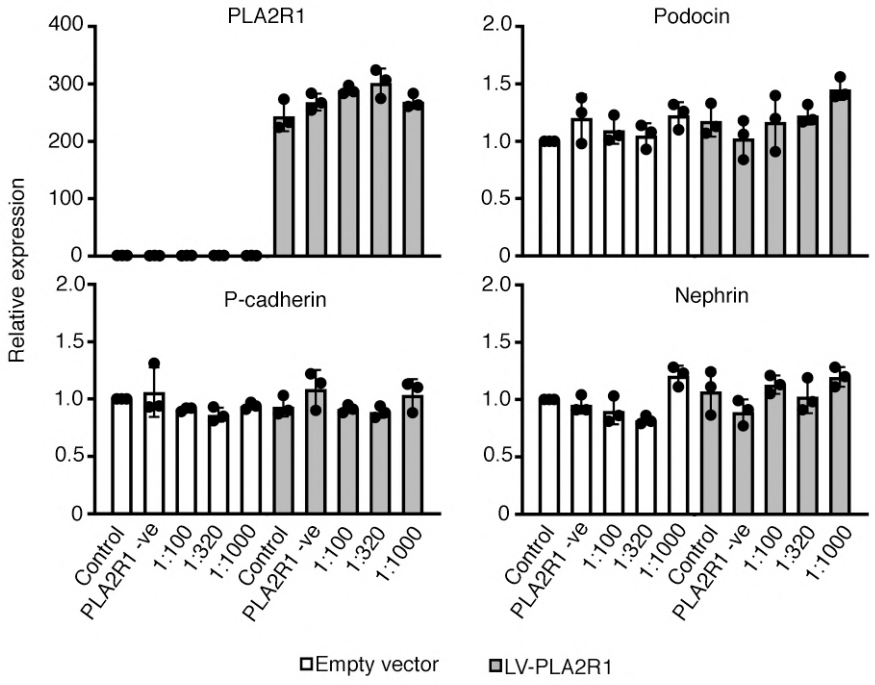
Supplemental Figure 6. Dependence of synaptopodin and NEPH1 degradation as well as ERK and Akt phospohorylation on PLA2R1-expression, anti-PLA2R1-positive serum and complement. Differentiated podocytes infected with PLA2R1-containing lentivirus (LV-PLA2R1) or empty vector were preincubated for 30 min with 2.5% serum from pMN patients with the indicated anti-PLA2R1 antibody titers (A-E, G) or a healthy individual (H) and cryopreserved (A-D, F, H) or heat-inactivated (E) NHS (5%). The decrease of synaptopodin and NEPH1 was dependent on PLA2R1 expression, high titer anti-PLA2R1 positivity of serum and active complement. ERK and Akt phosphorylation was induced upon addition of serum (patient serum or NHS) independent on complement activity and anti-PLA2R1 positivity.



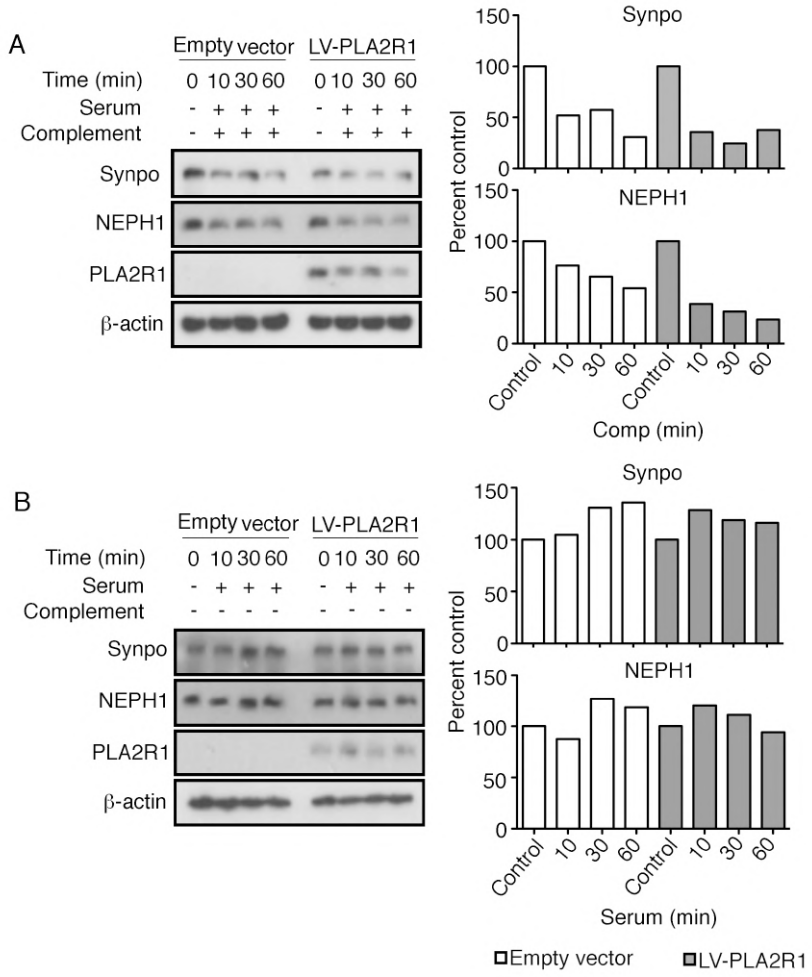
Supplemental Figure 7. Effects of anti-PLA2R1-positive MN sera and complement on various human podocyte cell lines. Four different human podocyte cell lines grown under nonpermissive conditions (i.e. differentiated) for 14 days and expressing PLA2R1 by lentiviral transfer were pretreated with 2.5 % high-titer anti-PLA2R1-positive pMN serum for 30 min at 37°C followed by incubation with 5 % of NHS for 60 min (S+C) and compared to untreated cells (NT) by western blot of synaptopodin and NEPH1 levels.



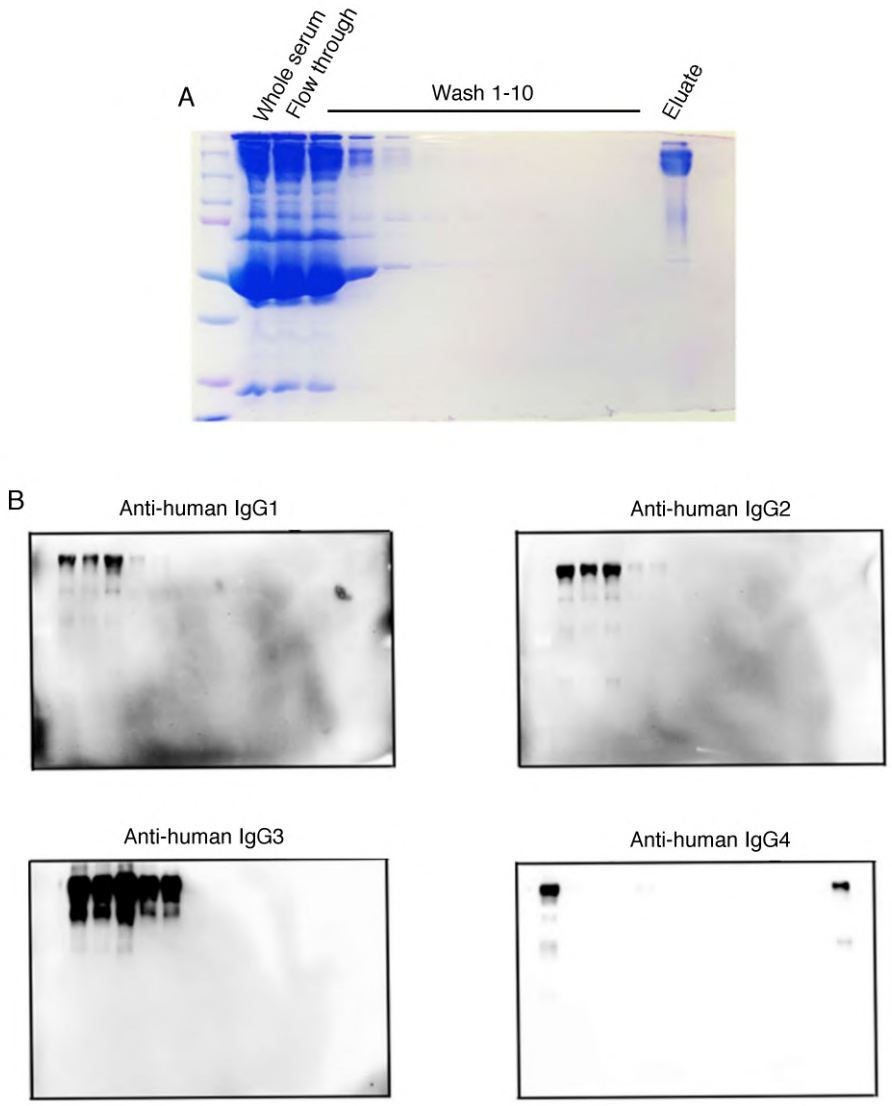
Supplemental Figure 8. Effects of anti-PLA2R1-positive MN sera and sheep anti mouse podocyte serum on cultured mouse podocytes. Differentiated conditionally immortalized mouse podocytes were infected with lentivirus containing PLA2R1 (LV-PLA2R1) or empty vector and either pretreated with 2.5 % high-titer anti-PLA2R1-positive pMN serum (A) or with sheep anti mouse podocyte serum (shampodo, B) for 30 min at 37°C followed by incubation with 5 % of NHS for the indicated time periods and the levels of the indicated proteins / phosphoproteins were analyzed by western blot.



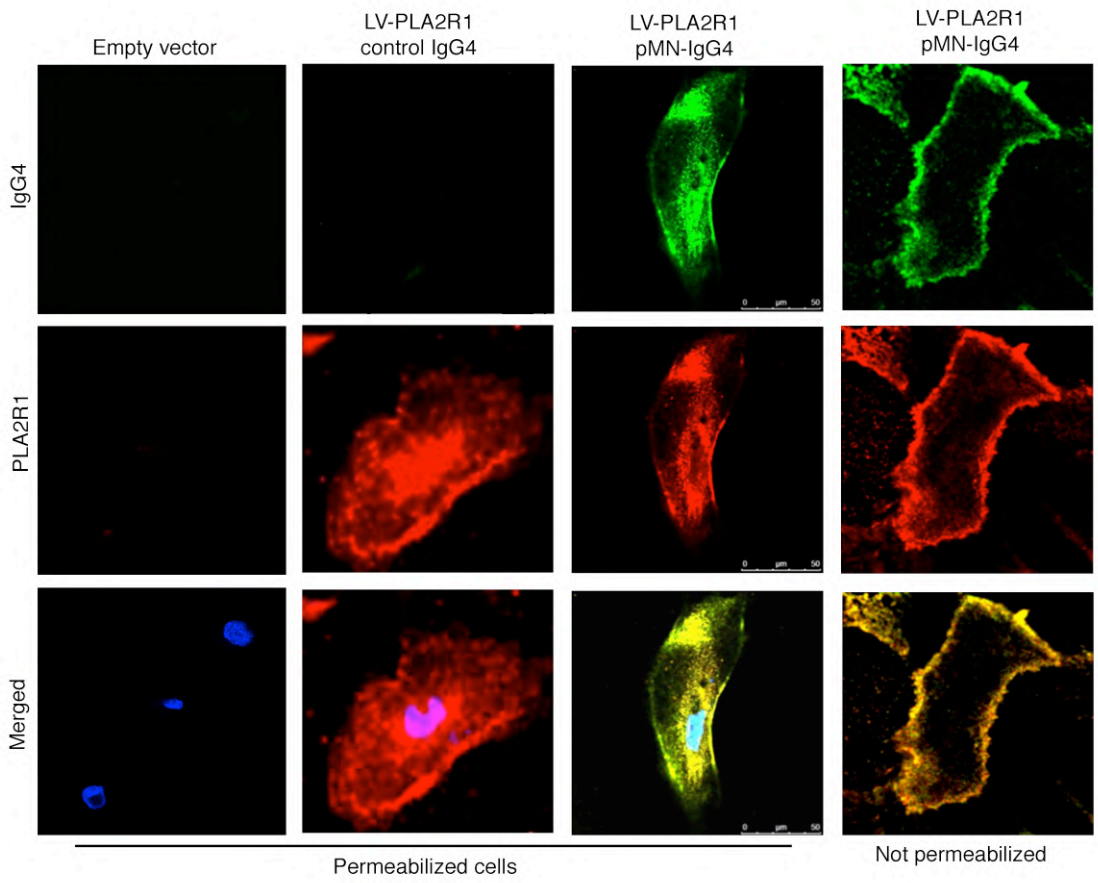
Supplemental Figure 9. Nephrin, podocin and P-cadherin transcription are unaffected by pMN serum and complement. Human podocytes infected with PLA2R1-containing lentivirus (LV-PLA2R1) or empty vector pretreated with 2.5% anti-PLA2R1-positive pMN serum with the indicated titer for 30 min followed by the addition of 5% NHS for 60 min. The cells were then lyzed, RNA was extracted and reverse transcribed into cDNA and analyzed by quantitative PCR (n=3, The bars represent mean ± SEM). We did not find any significant difference in the expression of any of the three proteins for the comparison of treated vs. untreated cells expressing PLA2R1 or for the comparison of PLA2R1-expressing vs. empty virus infected cells treated with the same serum.



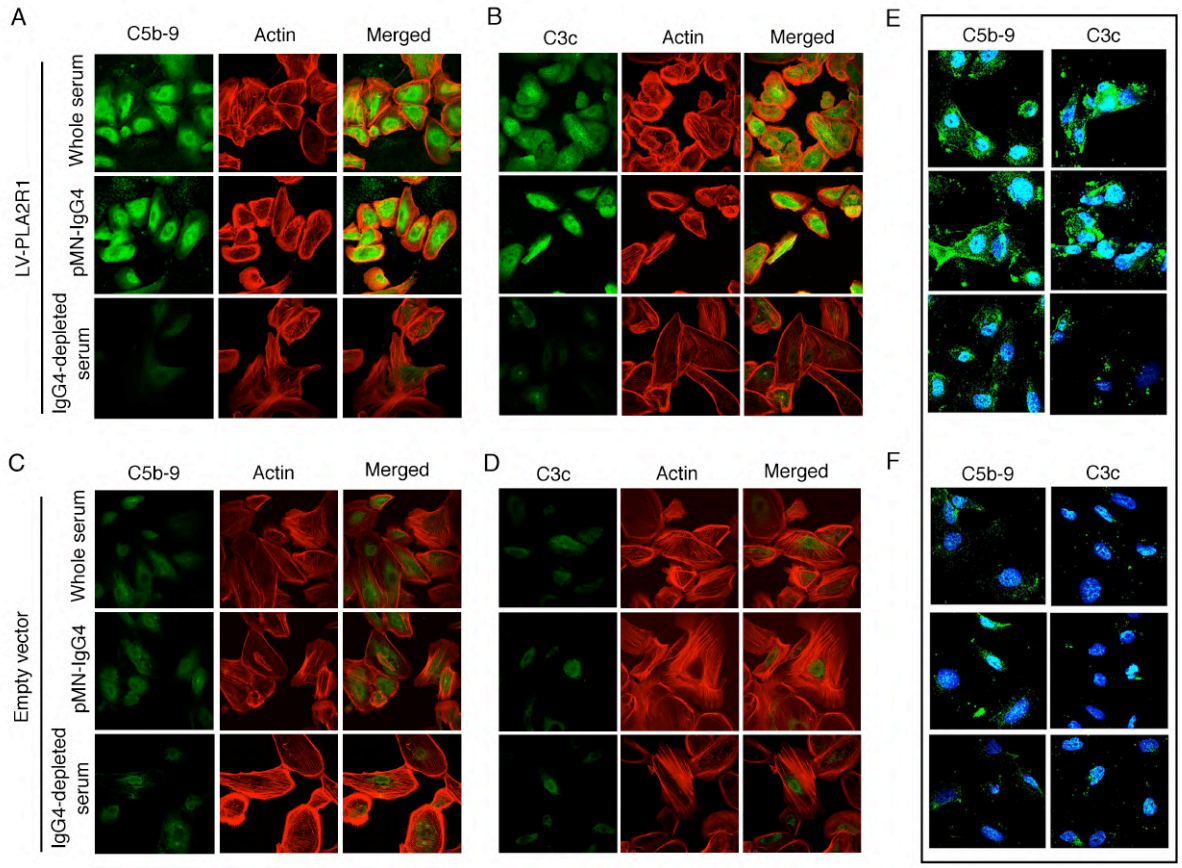
Supplemental Figure 10. Serum from an FSGS patient decreases synaptopodin and NEPH1 expression independent of PLA2R1-expression. Human podocytes infected with PLA2R1-containing lentivirus or with empty vector, pretreated with 2.5% FSGS serum for 30 min followed by the addition of 5% NHS for 60 min both showed a reduction of synpatopodin and NEPH1 in the presence of NHS as a source of complement (A) but not without complement (B).



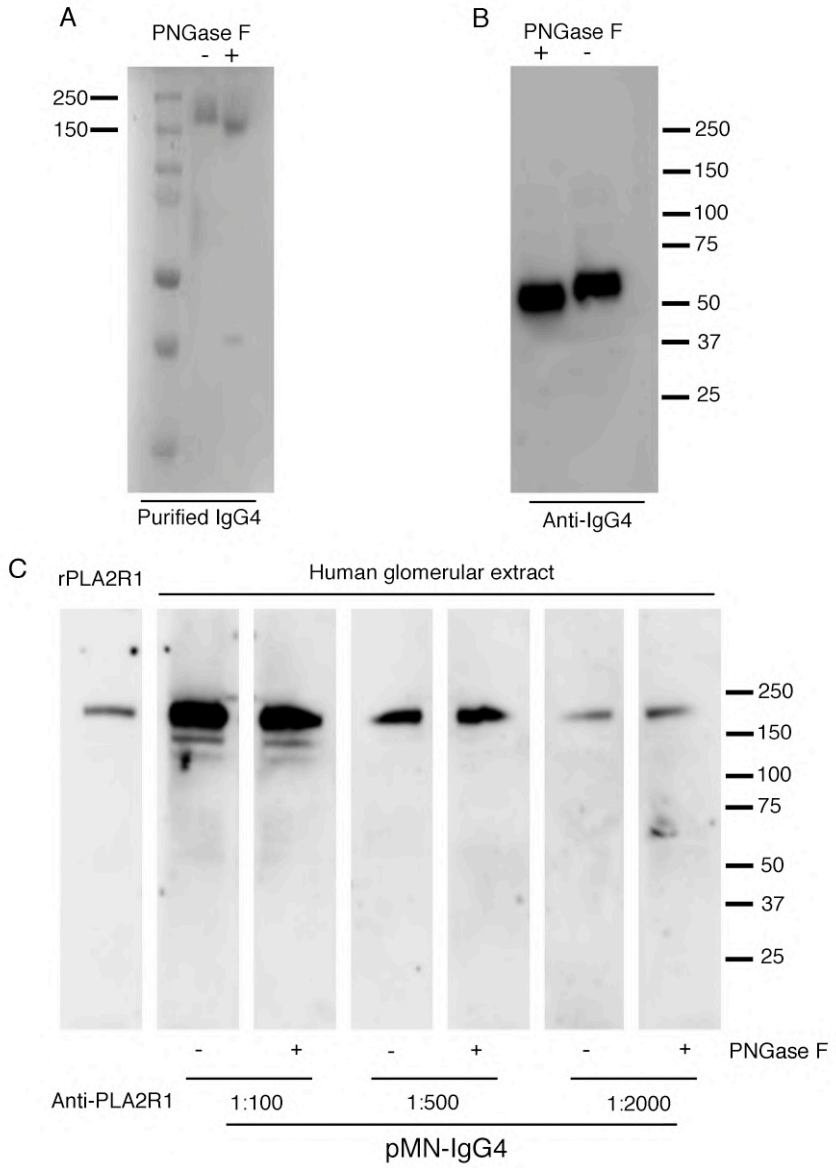
Supplemental Figure 11. Analysis of the IgG4 purification process. Sera from pMN patients or from healthy individuals were loaded onto a column filled with CaputreSelect™ IgG4 affinity matrix bead. After several washes, IgG4 was eluted from the column and concentrated using 30 kDa MWCO protein concentrator. The purification process in a representative patient is shown. Whole serum, flow through and all wash fractions as well as the eluted, concentrated IgG4 fraction were resolved using SDS-PAGE and (A) stained with Coomassie Brilliant Blue or (B) transferred to a PVDF membrane followed by staining with isotype-specific antibodies (IgG1-4). Virtually no IgG4 was lost in the flow-through and wash fractions, whereas all IgG1-3 appeared in the flow-through and wash fractions with no residual contamination of the eluate.



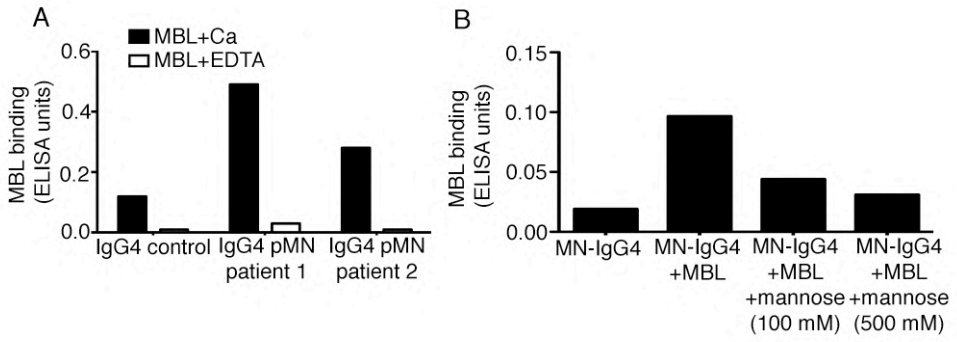
Supplemental Figure 12. Co-localization of PLA2R1 and IgG4. Human podocytes infected with PLA2R1-containing lentivirus (LV-PLA2R1) or with empty vector were treated with 100 µg/mL purified IgG4 from a pMN patient with high anti-PLA2R1 antibody titer or from a healthy control. The cells were fixed and either permeabilized or not permeabilized, and anti-human PLA2R1 (red) and anti-human IgG4 (green) antibodies were added. The representative images were captured using Leica SP8 upright confocal microscope and 63X objective lens.



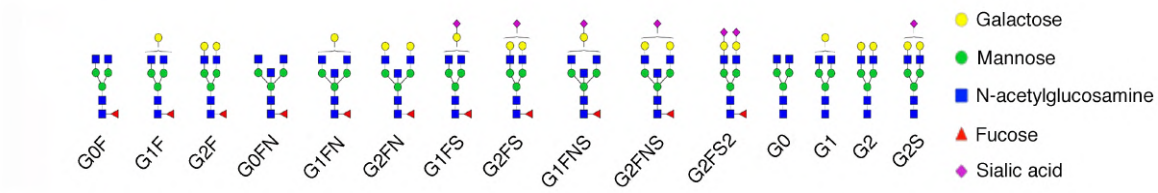
Supplemental Figure 13. Complement deposition in podocytes in response to pMN-IgG4 treatment. Differentiated human podocytes infected with PLA2R1-containing lentivirus (LV-PLA2R1, panels A, B and E) or with empty vector (panels C, D and F) were incubated for 1h at 37°C with 5% NHS as a complement source after preincubation with 2.5% high titer (1:1000) anti-PLA2R1-positive pMN patient serum, 100 µg/mL purified pMN-IgG4, or 2.5% IgG4 depleted serum. C5b-9 or C3c deposition were visualized in permeabilized cells (A-D). The same experiment was repeated without cellular permeabilization before staining (E, F). The representative images were captured using a Leica SP8 upright confocal microscope and 63X objective lens.



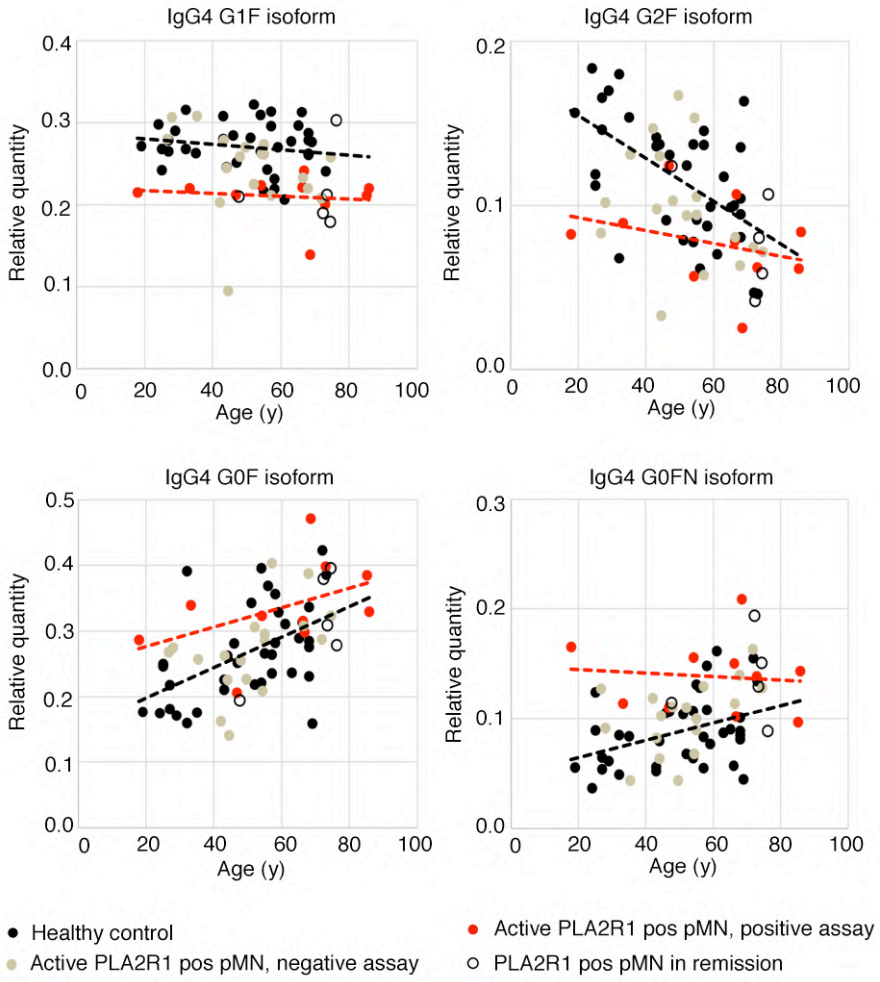
Supplemental Figure 14. Deglycosylated IgG4 retains its immunoreactivity against PLA2R1. Total IgG4 isolated from an anti-PLA2R1-positive pMN patient was deglycosylated using PNGase F under non-denaturing conditions. IgG4 prior to and after deglycosylation was electrophoresed under non-reducing (A) and under reducing (B) conditions and Coomassie-stained (A) or blotted and probed with anti-IgG4 antibody (B) to visualize the band-shift. To confirm preserved immunoreactivity of deglycosylated IgG4, human glomerular extracts (HGE) were run on a non-reducing gel and transferred to a nitrocellulose membrane. The individual lanes were cut out and probed with IgG4 either treated or untreated with PNGase F at the indicated dilutions. As a positive control, recombinant PLA2R1 was run and probed with anti-PLA2R1 monoclonal antibody (ATLAS AMAb90772).



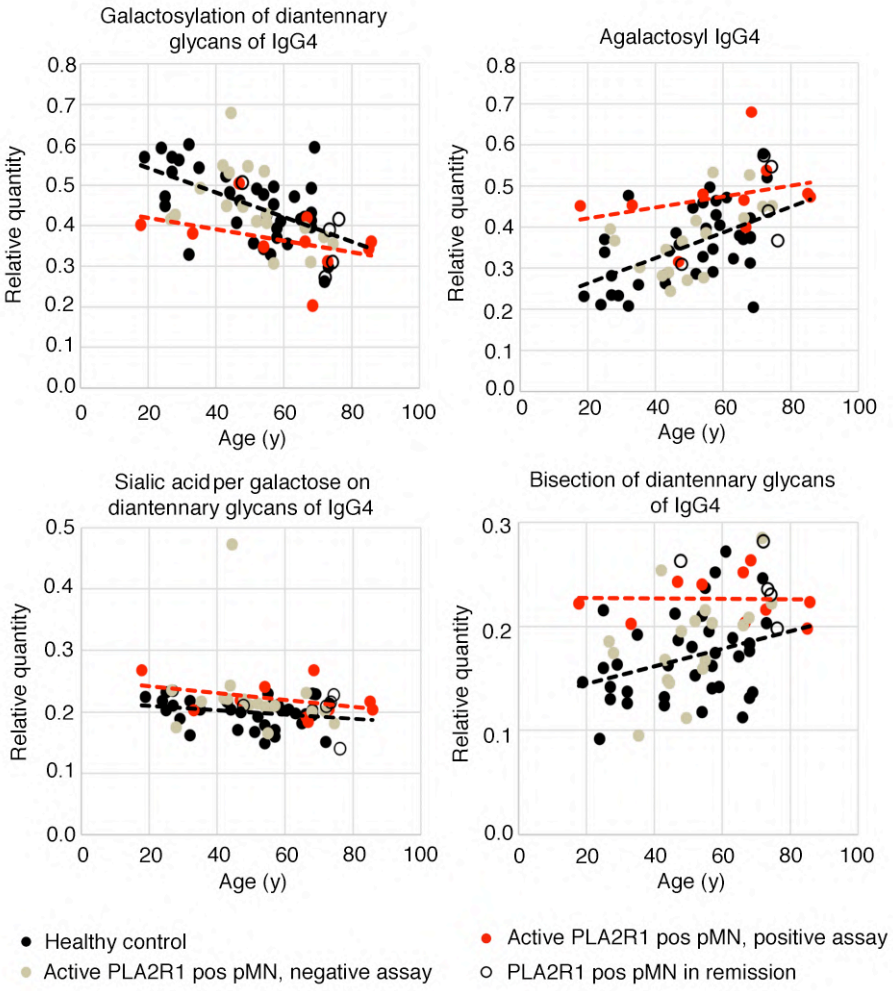
Supplemental Figure 15. Calcium-dependent interaction between pMN-IgG4 and MBL. (A) Purified total IgG4 from two pMN patients was coated onto an ELISA plate and recombinant human MBL was added in the presence of calcium or EDTA. (B) In a separate experiment, purified total IgG4 from another pMN patient was coated onto an ELISA plate and recombinant human MBL was added in the presence of various concentrations of mannose. Binding of MBL was detected with a mouse monoclonal anti-MBL antibody and anti-mouse IgG-HRP antibody.



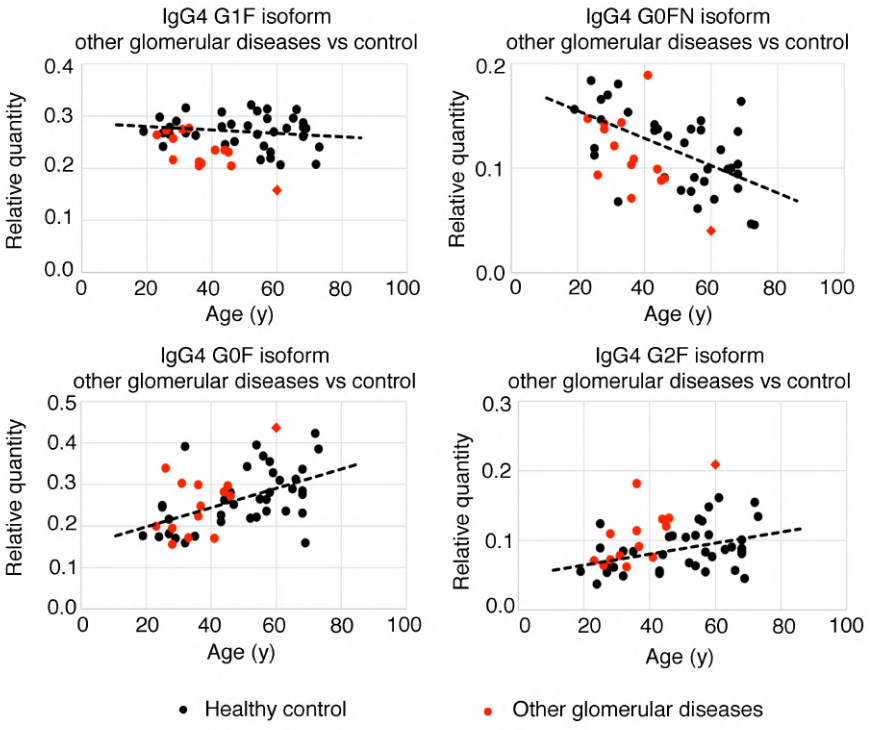
Supplemental Figure 16. Schematic representation of the various glycan side chains found on IgG molecules and their nomenclature.



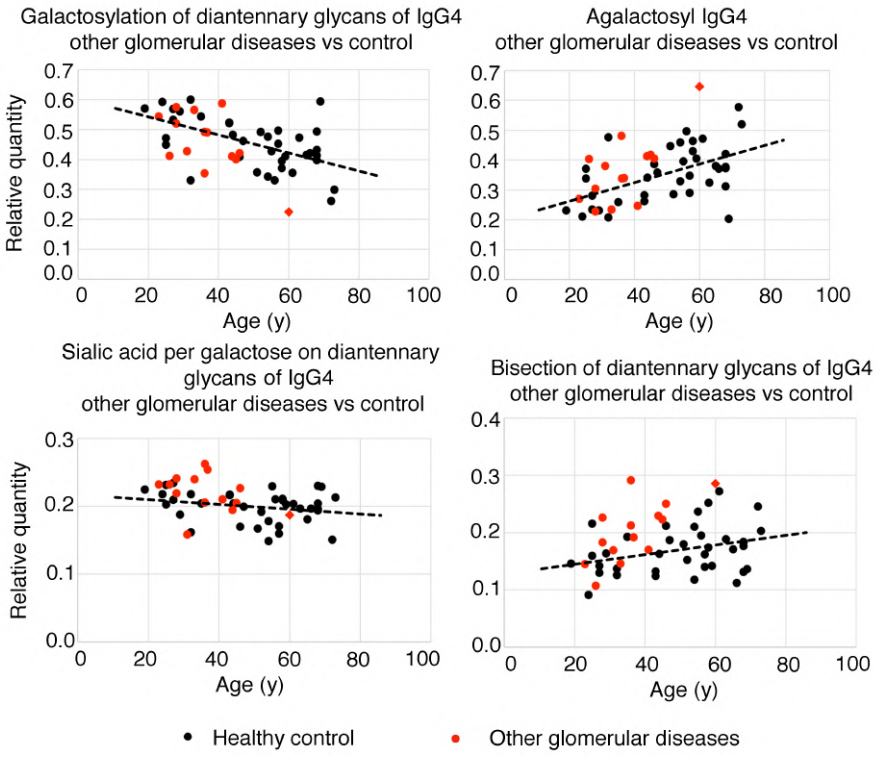
Supplemental Figure 17. Individual IgG4 glycoforms in pMN patients compared to healthy controls according to age. The fraction of the four IgG4 glycoforms that were significantly altered in pMN patients is shown for every patient according to age. Red dots denote anti-PLA2R1-positive pMN patients with a positive cellular assay (i.e. a statistically significant decrease of synaptopodin and NEPH1 levels by >25% after exposure to PLA2R1-positive serum and complement compared to baseline values in three independent experiments), grey dots anti-PLA2R1-positive pMN patients with a negative cellular assay, circles anti-PLA2R1-positive pMN patients in partial or complete remission and black dots healthy controls. For the definition of active disease and remission, see Table S1.



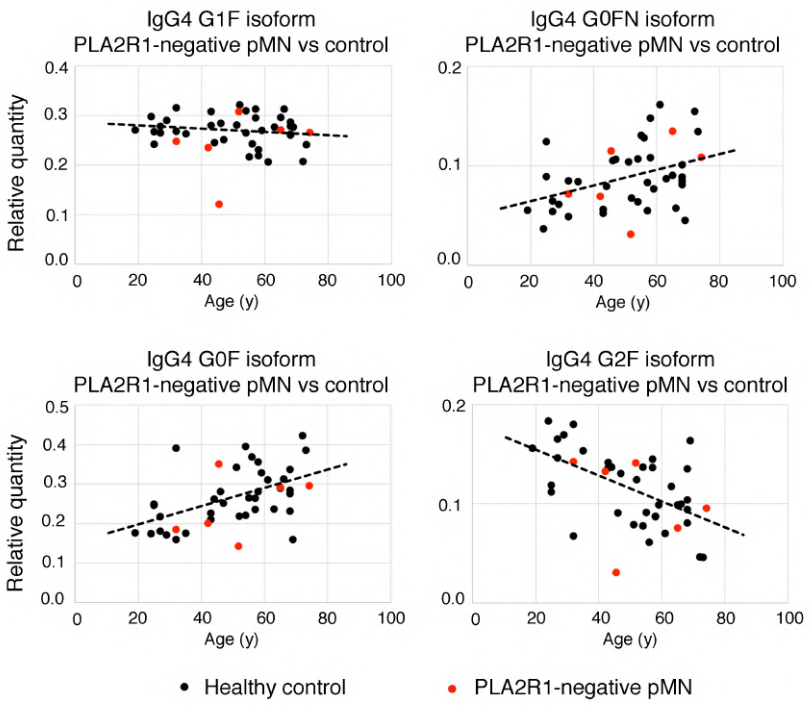
Supplemental Figure 18. Derived glycosylation traits in pMN patients compared to healthy controls according to age. Glycosylation characteristics of IgG4 are shown for every patient according to age. Red dots denote anti-PLA2R1-positive pMN patients with a positive cellular assay (i.e. a statistically significant decrease of synaptopodin and NEPH1 levels by >25% after exposure to PLA2R1-positive serum and complement compared to baseline values in three independent experiments), grey dots anti-PLA2R1-positive pMN patients with a negative cellular assay, circles anti-PLA2R1-positive pMN patients in partial or complete remission and black dots healthy controls. For the calculation of the derived traits, see Methods section.



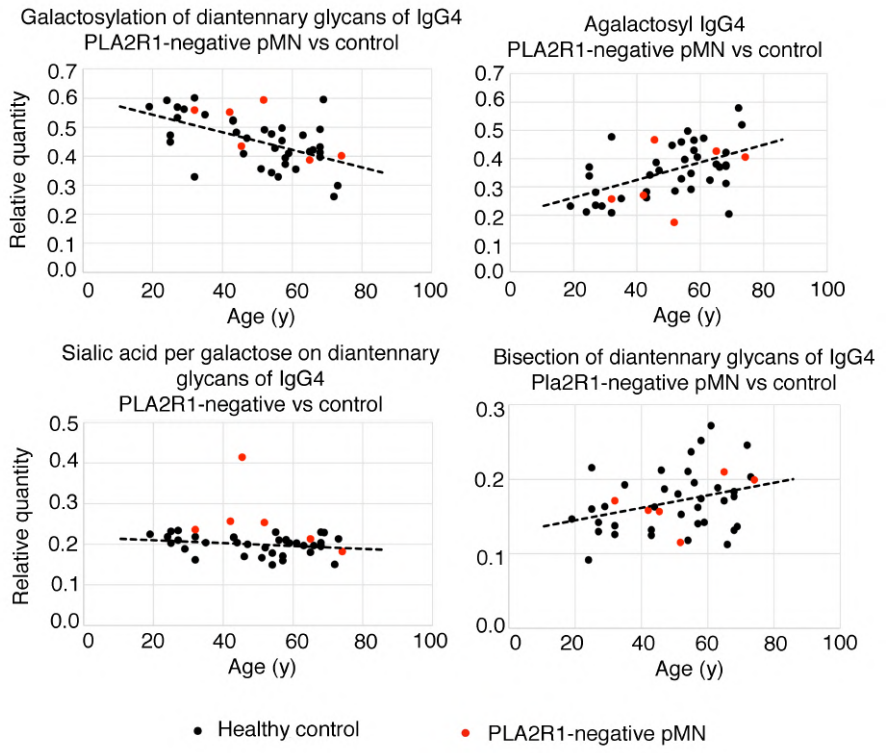
Supplemental Figure 19. Individual IgG4 glycoforms in patients with other glomerular diseases compared to healthy controls according to age. The fraction of the four IgG4 glycoforms that were significantly altered in pMN patients is shown for every patient with a variety of glomerular diseases (red dots) and healthy controls (black dots) according to age. The red diamond denotes a the patient with primary FSGS shown in Supplemental Figure 10, who showed a reduction of synaptopodin and NEPH1 in the cellular assay.



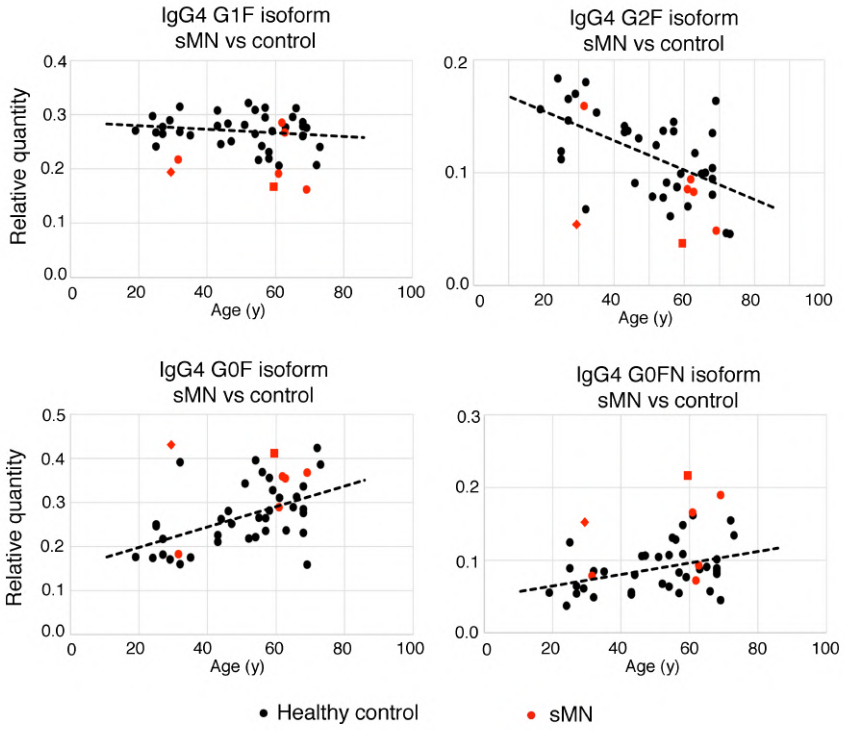
Supplemental Figure 20. Derived glycosylation traits in patients with other glomerular diseases compared to healthy controls according to age. Glycosylation characteristics of IgG4 are shown for every patient with a variety of glomerular diseases (red dots) and healthy controls (black dots) according to age. The red diamond denotes a the patient with primary FSGS shown in Supplemental Figure 10, who showed a reduction of synaptopodin and NEPH1 in the cellular assay. For the calculation of the derived traits, see Methods section.



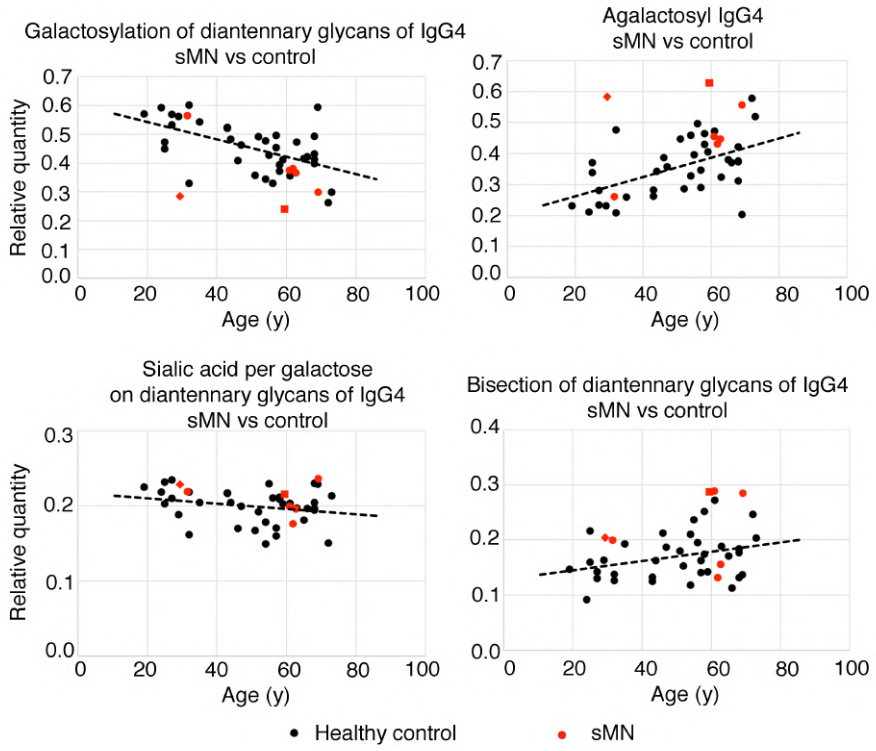
Supplemental Figure 21. Individual IgG4 glycoforms in patients with anti-PLA2R1-negative pMN compared to healthy controls according to age. The fraction of the four IgG4 glycoforms that were significantly altered in pMN patients is shown for every patient with PLA2R1-negative pMN (red dots) and healthy controls (black dots) according to age.



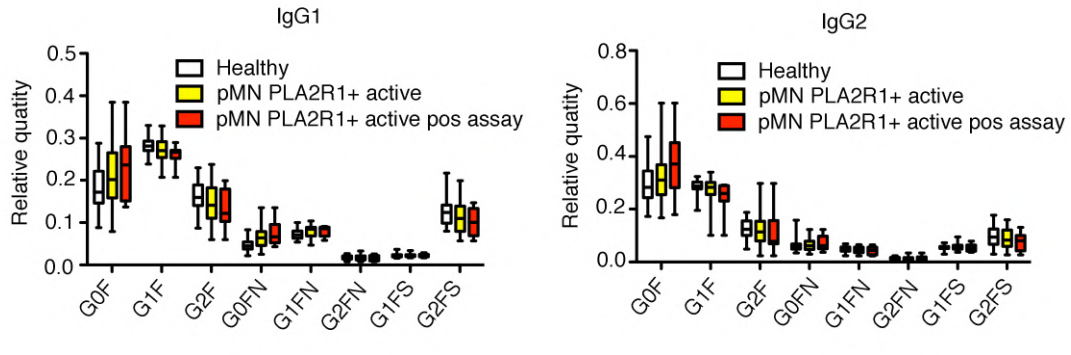
Supplemental Figure 22. Derived glycosylation traits in in patients with anti-PLA2R1-negative pMN compared to healthy controls according to age. Glycosylation characteristics of IgG4 are shown for every patient with PLA2R1-negative pMN (red dots) and healthy controls (black dots) according to age. For the calculation of the derived traits, see Methods section.



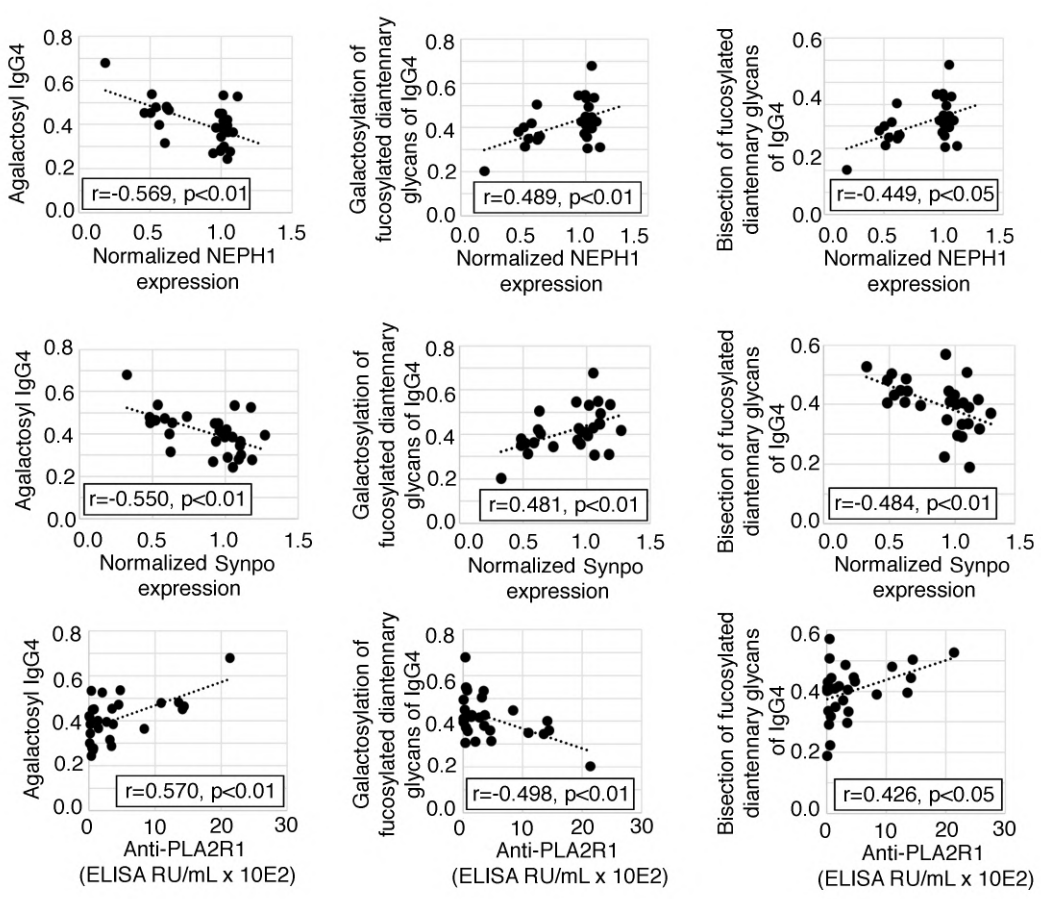
Supplemental Figure 23. Individual IgG4 glycoforms in patients with secondary MN compared to healthy controls according to age. The fraction of the four IgG4 glycoforms that were significantly altered in pMN patients is shown for every patient with secondary MN (red dots) and healthy controls (black dots) according to age. The red diamond and the red square denote two patients with Hodgkin's disease and polycythemia vera, respectively – both neoplasms that are not typically associated with MN. Moreover, the former patient showed a positive glomerular PLA2R1-staining in immunohistochemistry. Thus, these two patients may represent primary MN coinciding with hematologic neoplasms.



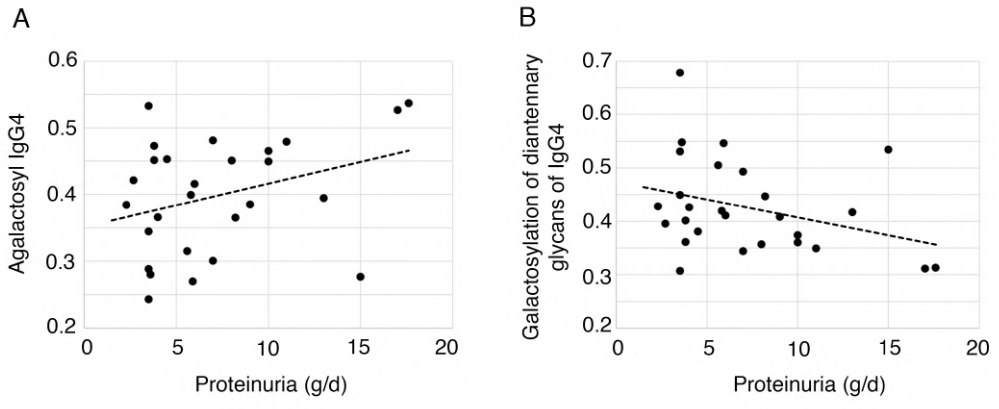
Supplemental Figure 24. Derived glycosylation traits in in patients with secondary MN compared to healthy controls according to age. Glycosylation characteristics of IgG4 are shown for every patient with secondary MN (red dots) and healthy controls (black dots) according to age. The red diamond and the red square denote two patients with Hodgkin's disease and polycythemia vera, respectively – both neoplasms that are not typically associated with MN. Moreover, the former patient showed a positive glomerular PLA2R1-staining in immunohistochemistry. Thus, these two patients may represent primary MN coinciding with hematologic neoplasms. For the calculation of the derived traits, see Methods section.



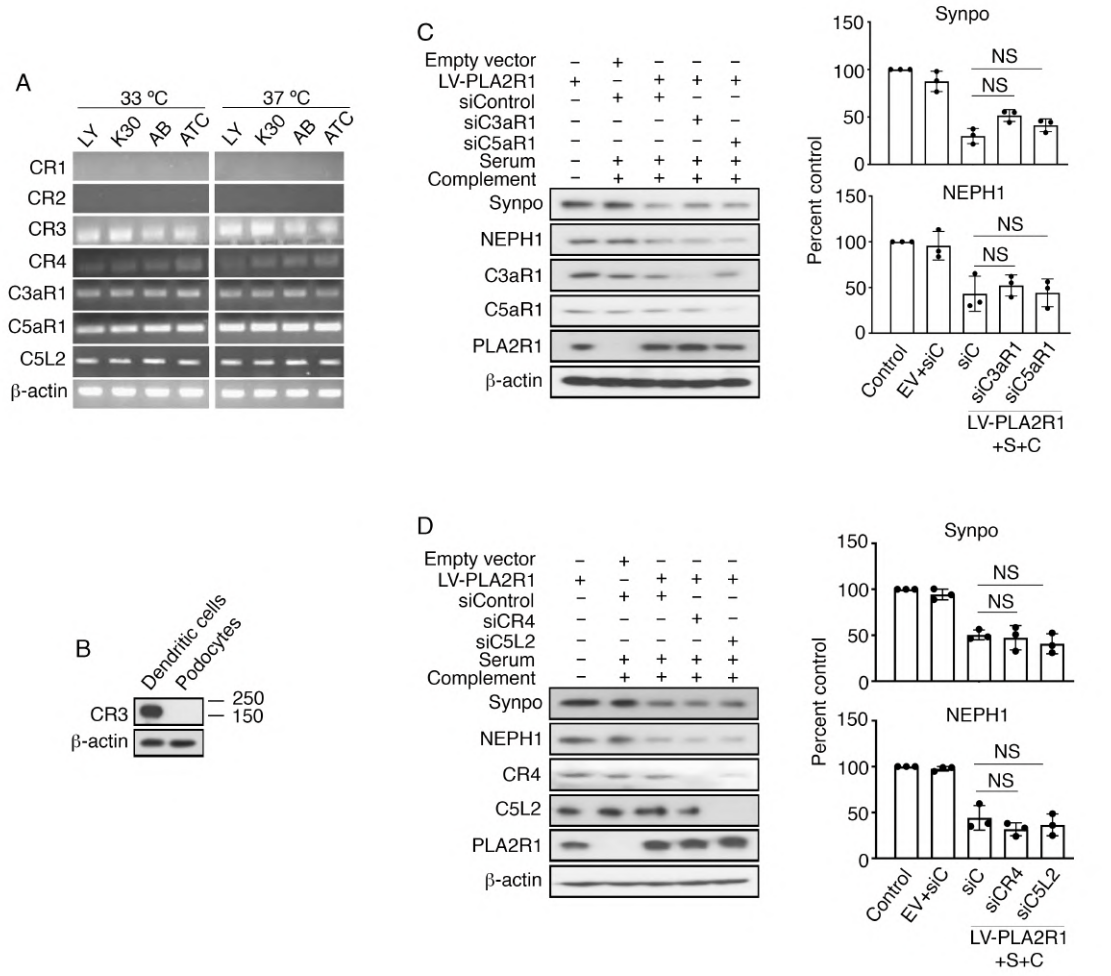
Supplemental Figure 25. Glycosylation patterns of IgG1 and IgG2 in pMN. The same analysis shown for IgG4 in Figure 6B is shown here for IgG1 and IgG2.



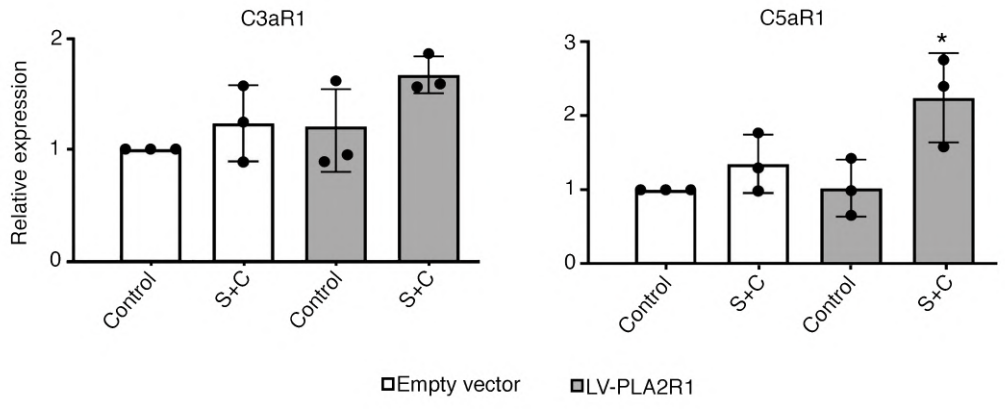
Supplemental Figure 26. IgG4 glycosylation patterns, PLA2R1-antibody levels and serum effects in the cellular complement assay. Shown is the correlation of agalactosyl IgG4, overall IgG4 galactosylation and bisection with anti-PLA2R1-antibody levels and with synaptopodin and NEPH1 levels after exposure to patient sera and complement in all patients with active pMN. r, Pearson's correlation coefficient.



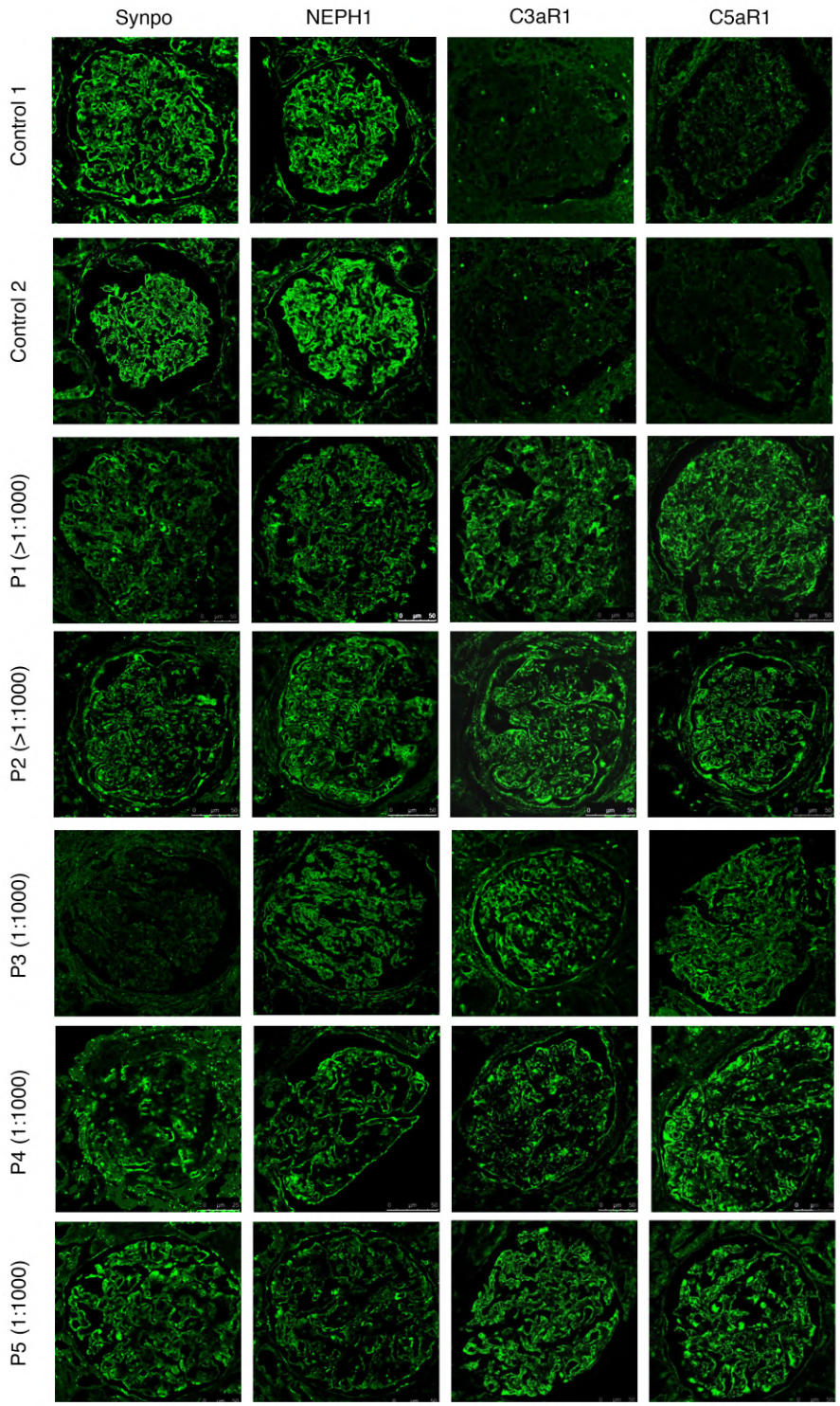
Supplemental Figure 27. Correlation of IgG4 glycosylation patterns with proteinuria. Shown is the correlation of the fraction of agalactosyl IgG4 (A) and of overall IgG4 galactosylation (B) with proteinuria in all patients with active anti-PLA2R1-positive pMN. Pearson's correlation coefficients were 0.330 ($P = 0.092$) and -0.342 ($P = 0.081$), respectively.



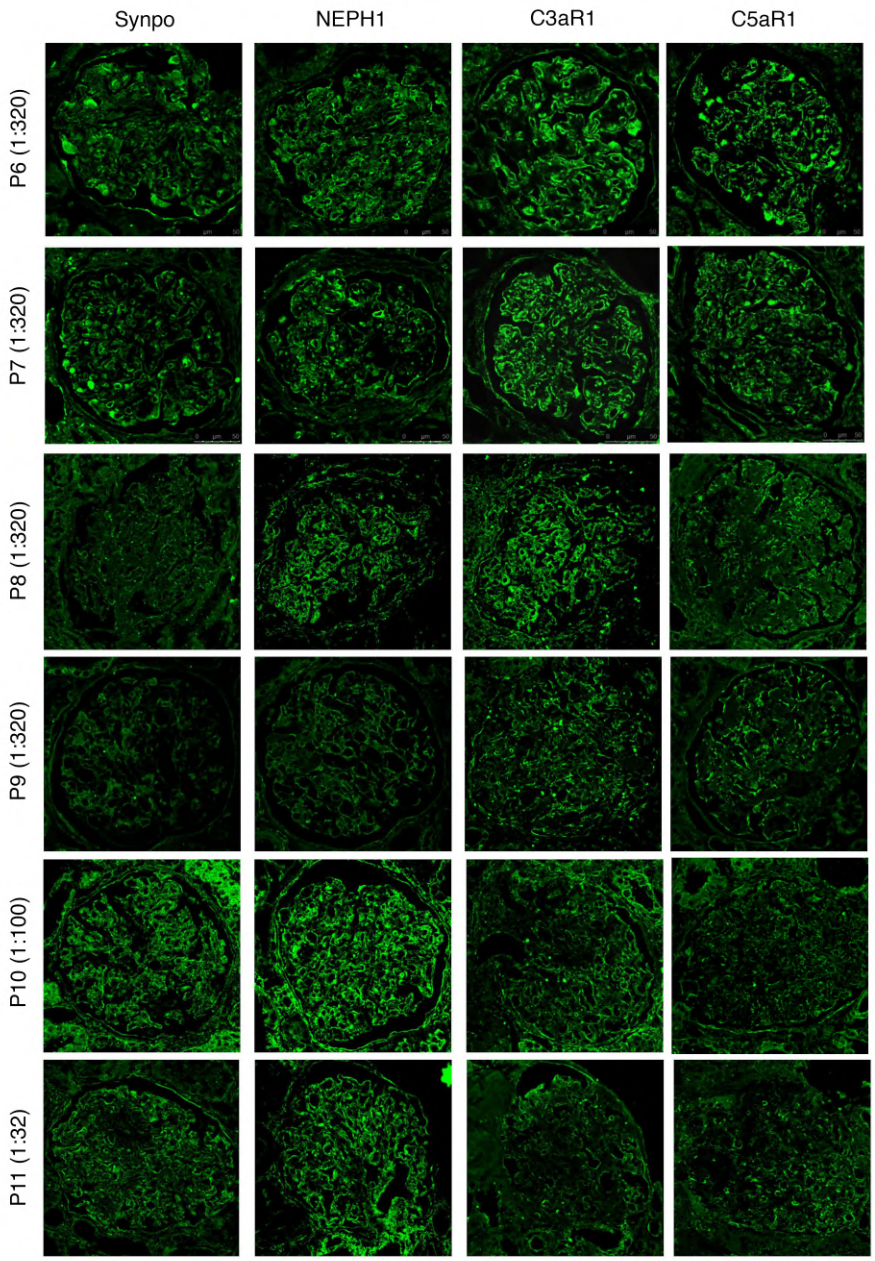
Supplemental Figure 28. Complement receptor expression and their effect in podocytes. The various complement receptors were analyzed for expression in four human podocyte cell lines at the mRNA level under permissive and non-permissive temperatures. CR1 and CR2 are not expressed in the podocyte cell lines (A). CR3 was expressed at the mRNA level but not on the protein level in an amount detectable by the anti-human CR3 antibody, which is able to detect CR3 in human isolated dendritic cells (B). To evaluate the role of C3aR1, C5aR1, CR4, and C5L2 in sublytic complement injury to podocytes, the receptors were knocked down individually in differentiated podocytes infected with PLA2R1-containing lentivirus (LV-PLA2R1) (C, D). After 72h of siRNA-mediated knockdown, the cells were treated with 2.5% high titer anti-PLA2R1 positive pMN patient serum for 30 min followed by incubation with 5% NHS for 60 min and analysis of the cell lysates by western blot. n=3, the bars represent mean ± SEM; synaptopodin and NEPH-1 were significantly ($P < 0.05$) reduced in all conditions with PLA2R1-expressing cells, serum and complement compared to controls, but knock down of C3aR1, C5aR1, CR4, and C5L2 did not rescue either protein compared to control siRNA (siControl / siC) by one-way ANOVA and Tukey's post hoc test.



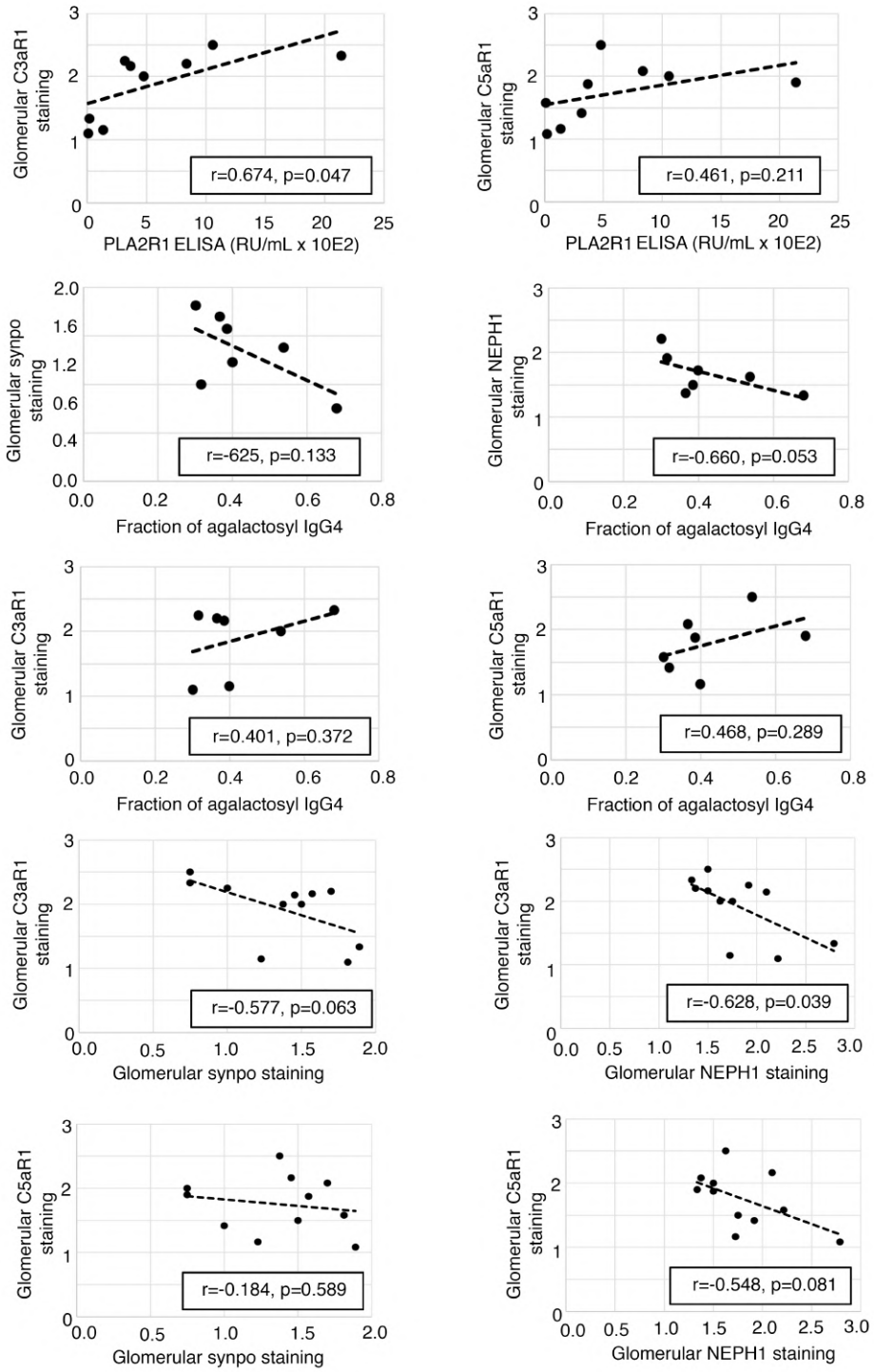
Supplemental Figure 29. Complement receptor C3aR1 and C5aR1 expression in human podocytes in response to pMN serum treatment. Differentiated human podocytes infected with PLA2R1-containing lentivirus (LV-PLA2R1) or empty vector were preincubated for 30 min with 2.5% high titer (1:1000) anti-PLA2R1 positive pMN serum followed by treatment for 60 min with 5% human NHS as a source of complement. The cells were then lyzed, RNA was extracted and reverse transcribed into cDNA and analyzed by quantitative PCR. n=3, The bars represent mean ± SEM, $*P < 0.05$ by one-way ANOVA and Tukey's post hoc test for the comparison with both, untreated empty vector and LV-PLA2R1 cells.



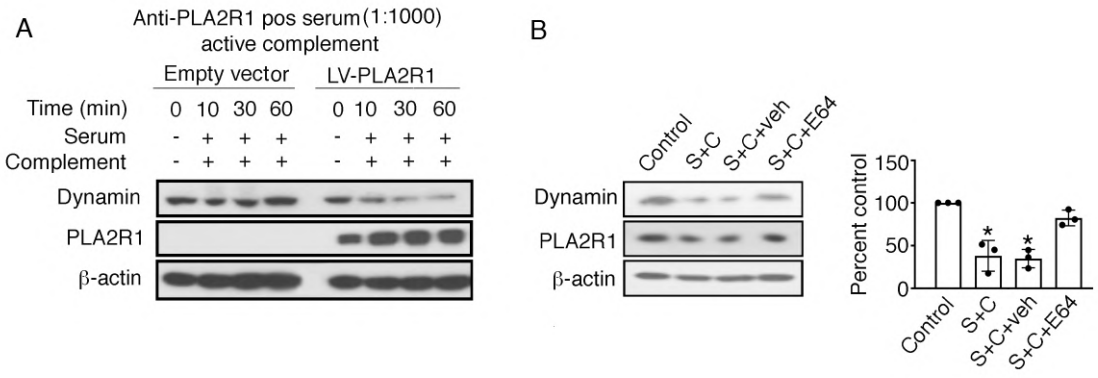
Supplemental Figure 30, continued on next page



Supplemental Figure 30. Levels of synaptopodin, NEPH1, C3aR and C5aR protein in human biopsies of anti-PLA2R1-positive pMN patients. Representative images of glomerular stainings for the mentioned proteins are shown for two control kidneys and 11 biopsies in 10 patients with anti-PLA2R1-positive pMN (one patient was rebiopsied after 10 months due to rapidly progressive renal failure). The images were captured using Leica SP8 upright confocal microscope and 63X objective lens. Additional images from patients 3 and 9 as well as additional controls are shown in Figure 8 of the main paper.



Supplemental Figure 31. Correlation of glomerular synaptopodin, NEPH1, C3aR and C5aR stainings among each other and with anti-PLA2R1 titer and fraction of agalactosyl IgG4.



Supplemental Figure 32. Degradation of dynamin and its rescue by E64. (A) In the same experiment shown in Supplemental Figure 6A, dynamin levels were assayed by western blot. (B) Differentiated podocytes expressing PLA2R1 were treated with 2.5% high titer anti-PLA2R1 positive human MN serum and 5% NHS, in the presence of the cysteine protease inhibitor E64 or vehicle and the levels of dynamin were assayed by western blot (B). n=3 for all experiments, the bars represent mean ± SEM, **P* < 0.05 by one way ANOVA and Tukey's post hoc test for the comparison with control.

Legend to Supplemental Table 2: Patient characteristics, summary of results of the in vitro assay and biopsy stainings.

eGFR was calculated according the CKD-EPI formula and is given in ml/min/1.73m^2.
Proteinuria was mostly estimated from spot urine and values are g/g creatinine (corresponding to proteinuria in g/day).
The effect in the cellular complement assay is given as the protein levels of synaptopodin and NEPH1 determined by western blot densitometry normalized to β-actin. Values for sera that induced a statistically significant decrease of synaptopodin and NEPH1 levels by >25% compared to untreated cells in three independent experiments are indicated with bold letters.

Abbreviations: CA, chlorambucil; CsA, cyclosporin A; CYC, cyclophosphamide; eGFR, estimated glomerular filtration rate according to the CKD-EPI formula; FSGS, focal segmental glomerulosclerosis; IgAN, IgA nephropathy; IS, immunosuppression; Leva, levamisole; LN, lupus nephritis (with ISN/RPS class in roman numbers); MCD, minimal change disease; MMF, mycophenolate mofetil; MN, membranous nephropathy; n/a, not applicable or not available (i.e. test not performed or results not available); Neg, negative
PCV, polycythaemia vera; PDN, prednisone / prednisolone; pMN, primary membranous nephropathy; Pos, positive; RTX, rituximab; seropos RA, seropositive rheumatoid arthritis; synpo, synaptopodin; Tac, tacrolimus; Tpl, transplantation.

Partial and complete remission were defined according to the 2012 KDIGO Glomerulonephritis Guidelines (complete remission: urinary protein excretion <0.3g/d, normal serum albumin and normal serum creatinine; partial remission: urinary protein excretion <3.5g/d and >50% reduction from peak values and improvement or normalization of the serum albumin concentration). Patients not in complete or partial remission were termed as “active disease” (mostly either new diagnosis of pMN by biopsy, persistent nephrotic syndrome / persistent proteinuria >50% of peak values or relapse of nephrotic syndrome).