Supplemental Material

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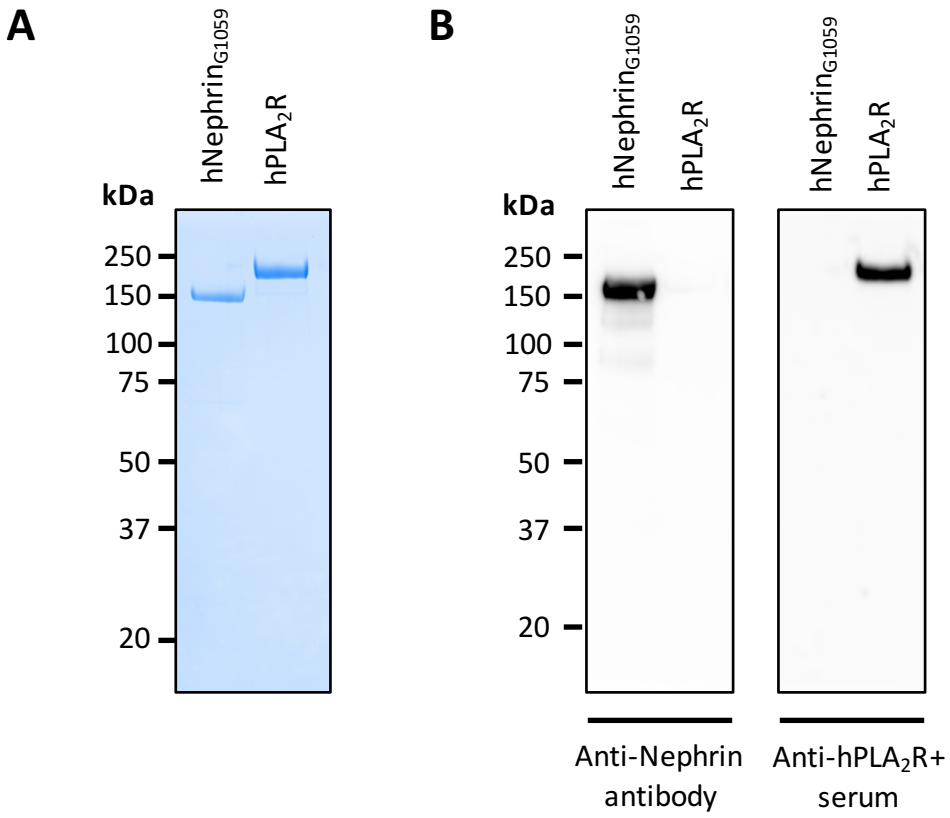
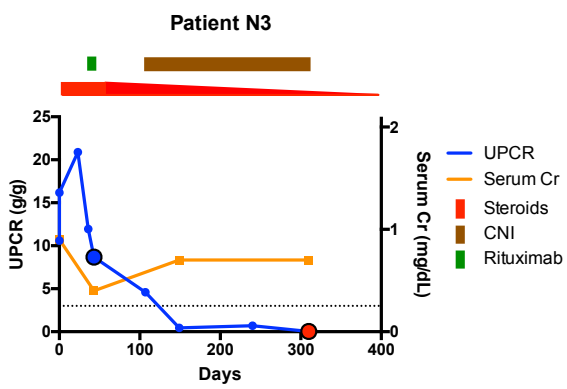
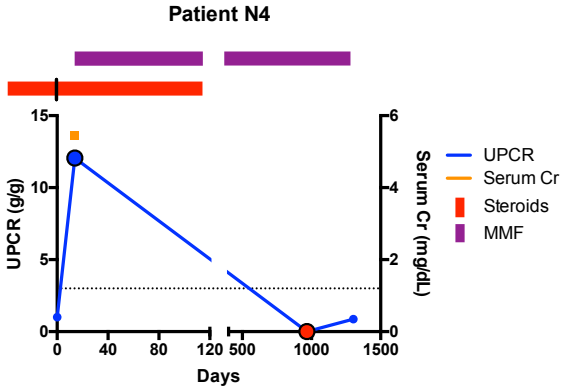


Figure S1. Generation and validation of purified recombinant extracellular domains of human nephrin and human phospholipase A_2 receptor (hPLA$_2$R). Recombinant human extracellular domain of nephrin (hNephrin$_{G1059}$) (~150 kDa) and hPLA$_2$R (~180kDa) both with C-terminal polyhistidine (6XHIS) tags were affinity purified with Nickel-NTA resin followed by size exclusion chromatography. **(A)** Purity was confirmed by Coomassie staining of 1 µg recombinant protein resolved by SDS-PAGE under reducing conditions. **(B)** Immunoreactivity of (Left) 0.5 µg nephrin, under reducing conditions, was confirmed using a primary sheep anti-human nephrin antibody and (Right) 0.5 µg hPLA$_2$R, under non-reducing conditions, using serum from a patient with anti-hPLA$_2$R antibodies diluted 1:1000. The appropriate HRP-conjugated donkey anti-sheep or anti-human IgG secondary antibody was used for detection.


Patient N3
UPCR (g/g)
Serum Cr (mg/dL)
Days
UPCR
Serum Cr
Steroids
CNI
Rituximab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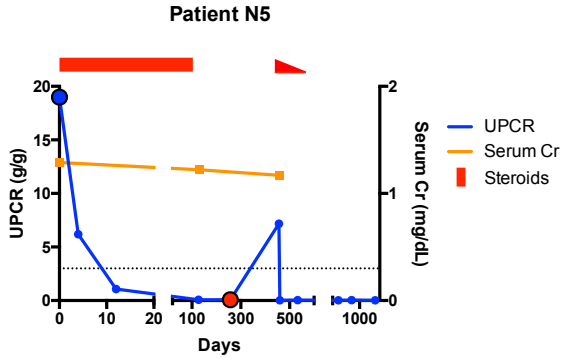
UPCR (g/g)
Serum Cr (mg/dL)
Days
UPCR
Serum Cr
Steroids
MMF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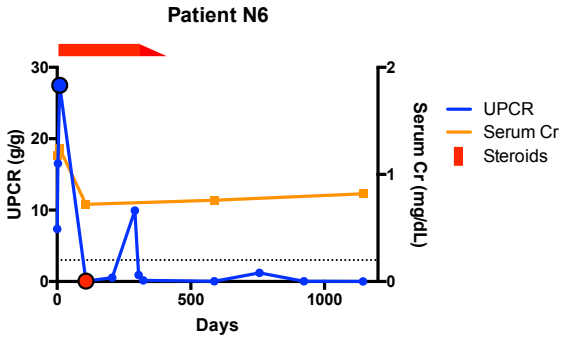
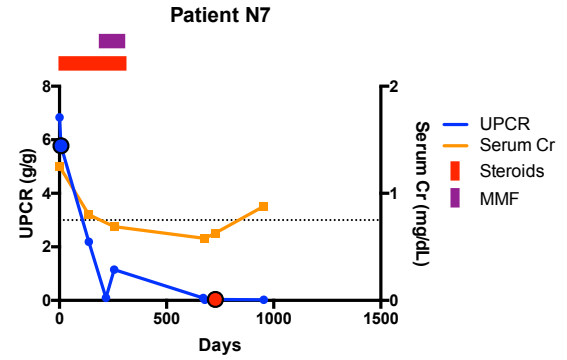
UPCR (g/g)
Serum Cr (mg/dL)
Days
UPCR
Serum Cr
Steroids


Patient N6
UPCR (g/g)
Serum Cr (mg/dL)
Days
UPCR
Serum Cr
Steroids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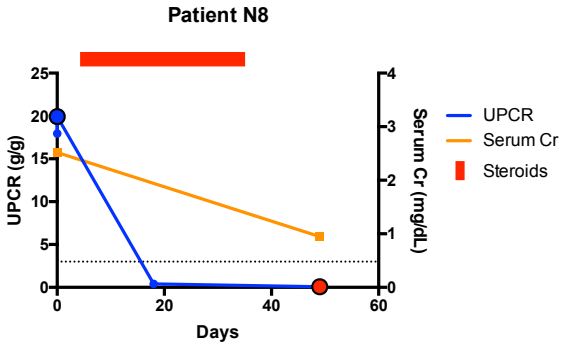
UPCR (g/g)
Serum Cr (mg/dL)
Days
UPCR
Serum Cr
Steroids
MMF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UPCR (g/g)
Serum Cr (mg/dL)
Days
UPCR
Serum Cr
Steroids

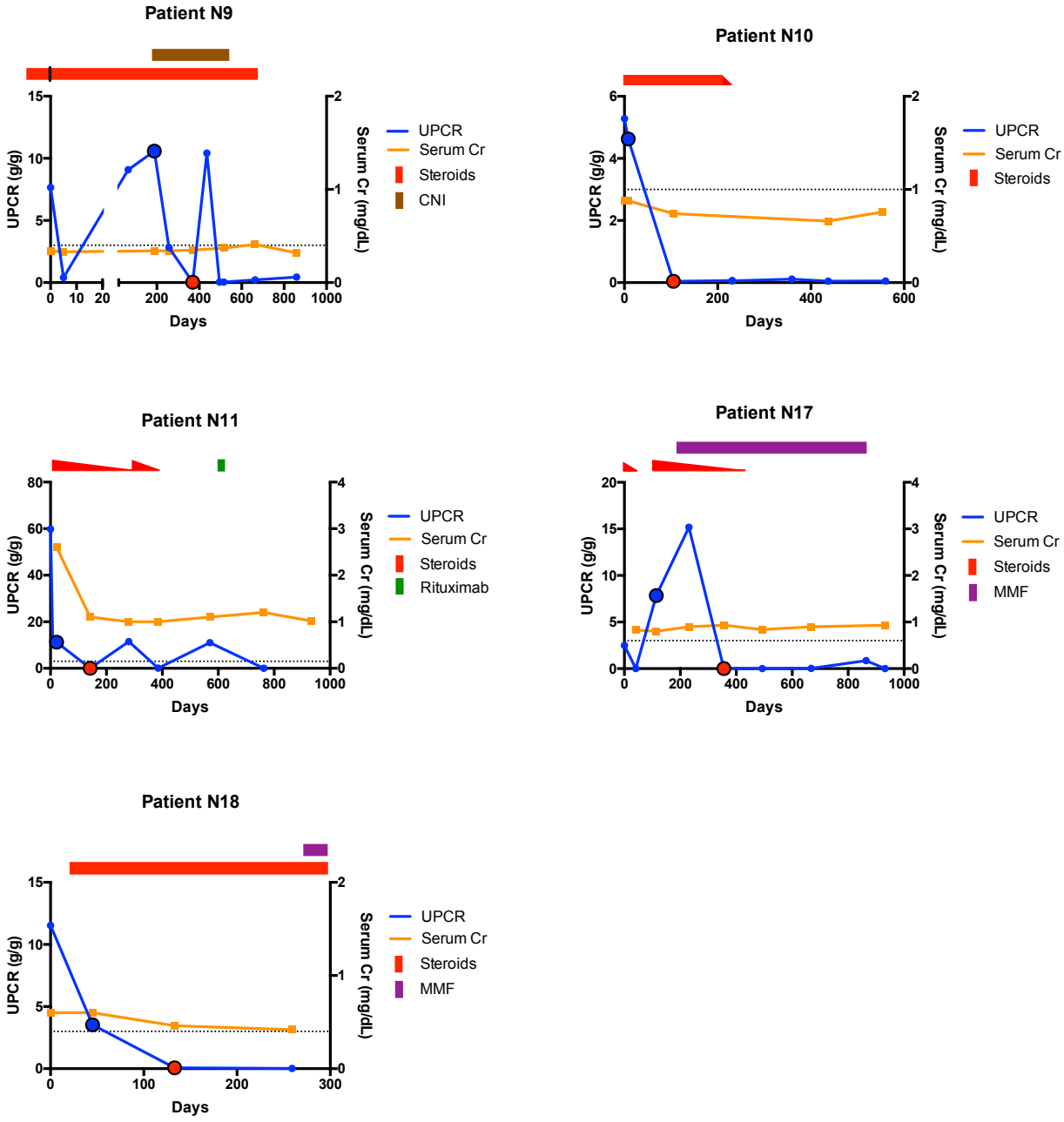

Figure S2. The clinical course of patients from the NEPTUNE cohort with samples obtained during active disease and subsequent complete remission. UPCR (g/g) and serum creatinine (Cr) are shown for each patient with serum samples available during active disease (large blue circle with black border) and during subsequent complete remission (large red circle with black border). The serum samples were evaluated for anti-nephrin antibodies and refer to the data shown in Figure 1B/C. Therapy is shown above each graph and for steroid treatment a dose reduction is indicated by a downward sloping wedge (red line). The dotted line indicates a UPCR of 3 g/g and complete remission was defined as UPCR < 0.3 g/g. Days, days following enrollment.

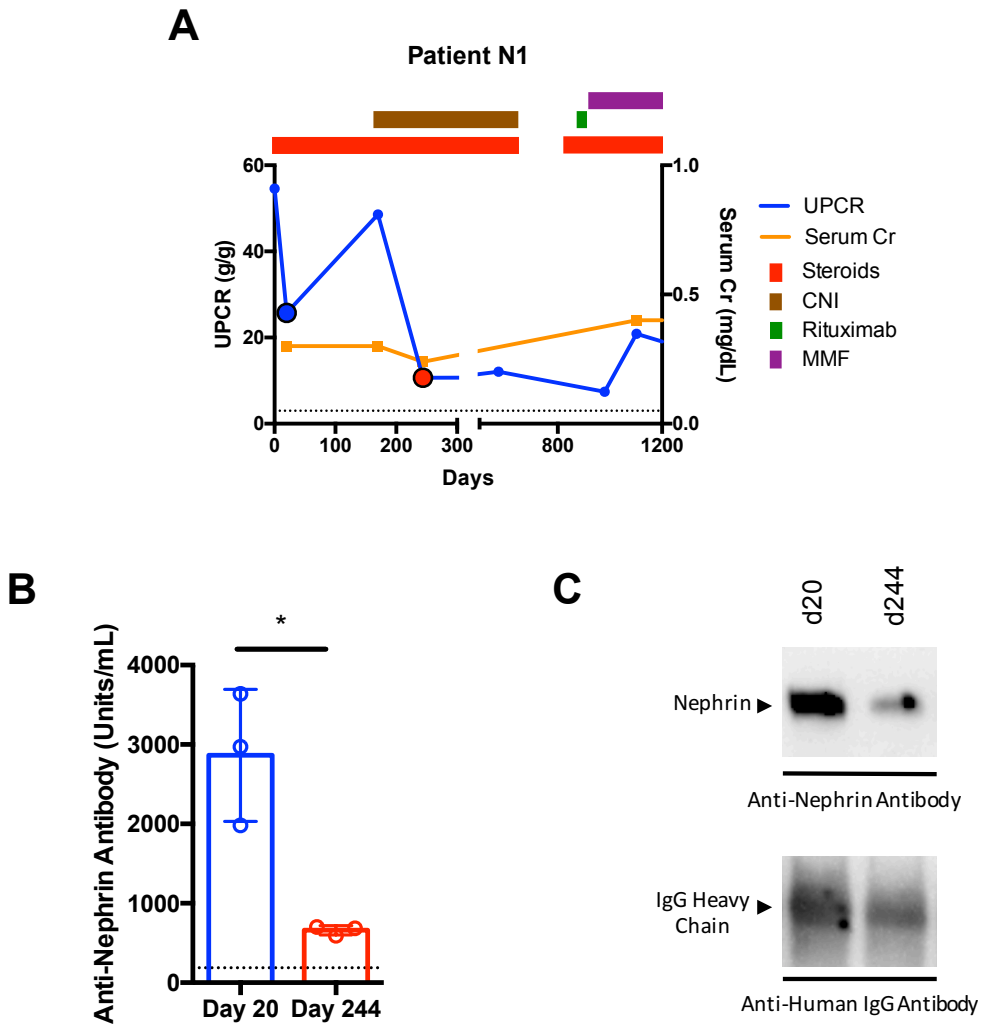

Figure S3. Clinical and anti-nephrin antibody data for a single patient from the NEPTUNE cohort with active proteinuria and a partial response to therapy. (A) Clinical course of patient with a serum sample available during active disease (large blue circle with black border) and partial remission (large red circle with black border) showing UPCR (g/g) (dotted line indicates UPCR of 3 g/g), serum creatinine (Cr) and treatment (shown above graph). **(B)** Significant reduction in anti-nephrin antibody titers associated with partial remission (*p=0.01) (dotted line indicates threshold for positive antibody titer of 187 U/ml based on a healthy control population). **(C)** The serum samples taken on day 20 (d20) and day 244 (d244) both immunoprecipitated nephrin from HGE derived from disease-free human kidneys, with the band intensity mirroring the antibody titers by ELISA. Days; days following enrollment. Partial remission was defined as a >50% reduction in UPCR between samples.

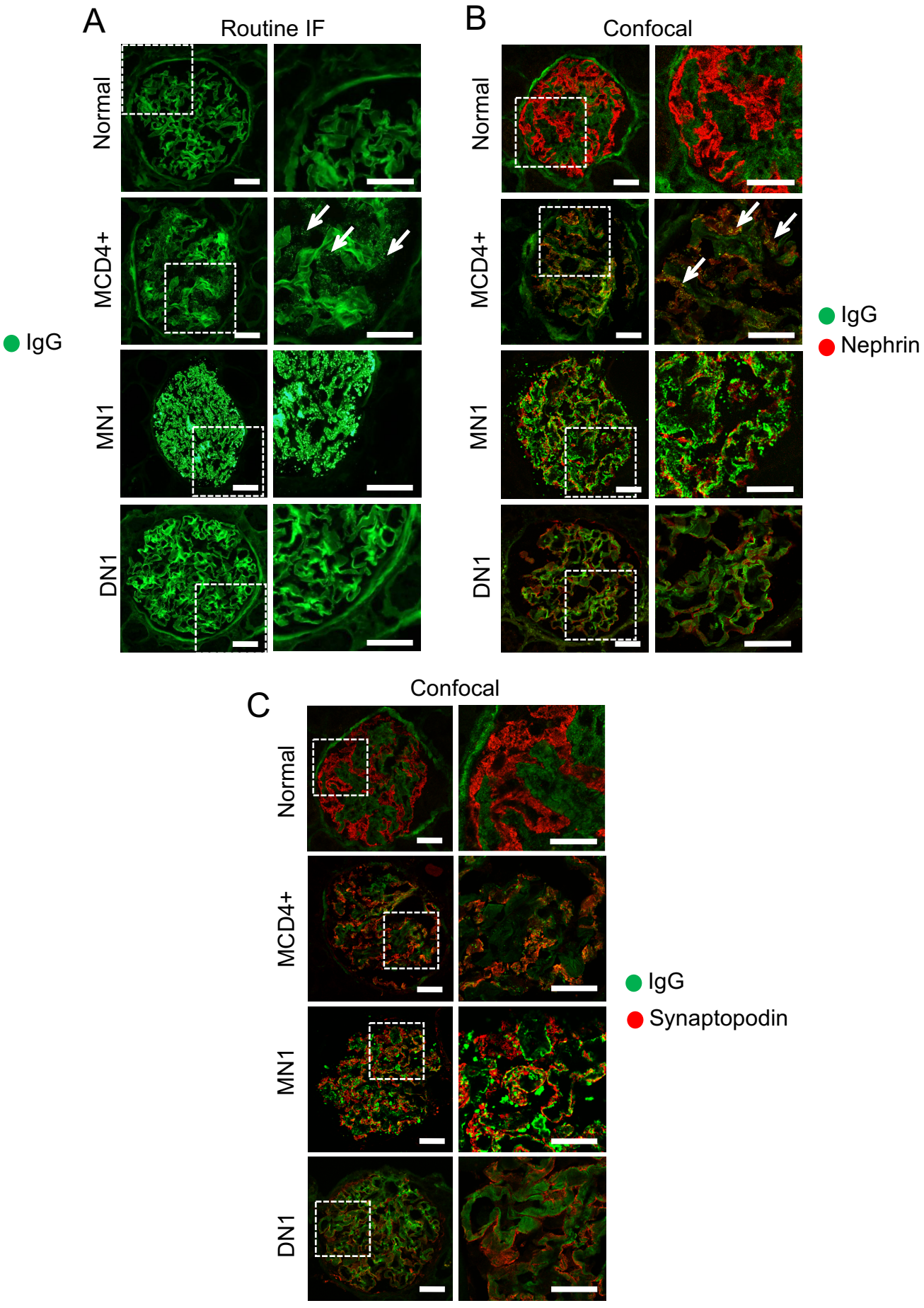
A
Routine IF
Normal
MCD4+
MN1
DN1
IgG
B
Confocal
Normal
MCD4+
MN1
DN1
IgG
Nephrin
C
Confocal
Normal
MCD4+
MN1
DN1
IgG
Synaptopodin

Figure S4: Immunofluorescence microscopy images of IgG, IgG/Nephrin and IgG/Synaptopodin staining. (A) Clinical epifluorescence images of glomeruli stained for IgG using FITC-conjugated anti-human IgG $(Fab)_2$ antibodies (ab). Right panel shows larger magnification of dotted square. A normal glomerulus shows low intensity linear staining along all basement membranes. In a subset of MCD (here shown in MCD4+), a delicate punctate staining for IgG is observed in the extracapillary compartment, closely associated with GBMs (white arrows). In contrast, granular IgG staining in MN (here shown in MN1) is of much stronger intensity. No granular or punctate staining is observed in other proteinuric conditions, including in patients with DN (here shown in DN1). **(B)** Confocal microscopy images of the same cases stained for IgG and nephrin using primary unconjugated mouse anti-human IgG (green) and sheep anti-human nephrin (red) antibodies. Right panel shows larger magnification of dotted square. While reduction of nephrin staining intensity can be seen in all proteinuric conditions, co-localization of IgG with nephrin is only observed in MCD+ (white arrows). **(C)** Confocal microscopy images of same cases stained for IgG and synaptopodin using primary unconjugated mouse anti-human IgG (green) and guineapig anti-human synaptopodin (red) antibodies. Right panel shows larger magnification of dotted square. No appreciable co-localization of IgG staining with the intracellular actin-associated synaptopodin is seen in the normal kidney, MCD+, MN or DN. Scale bars: 20μm.

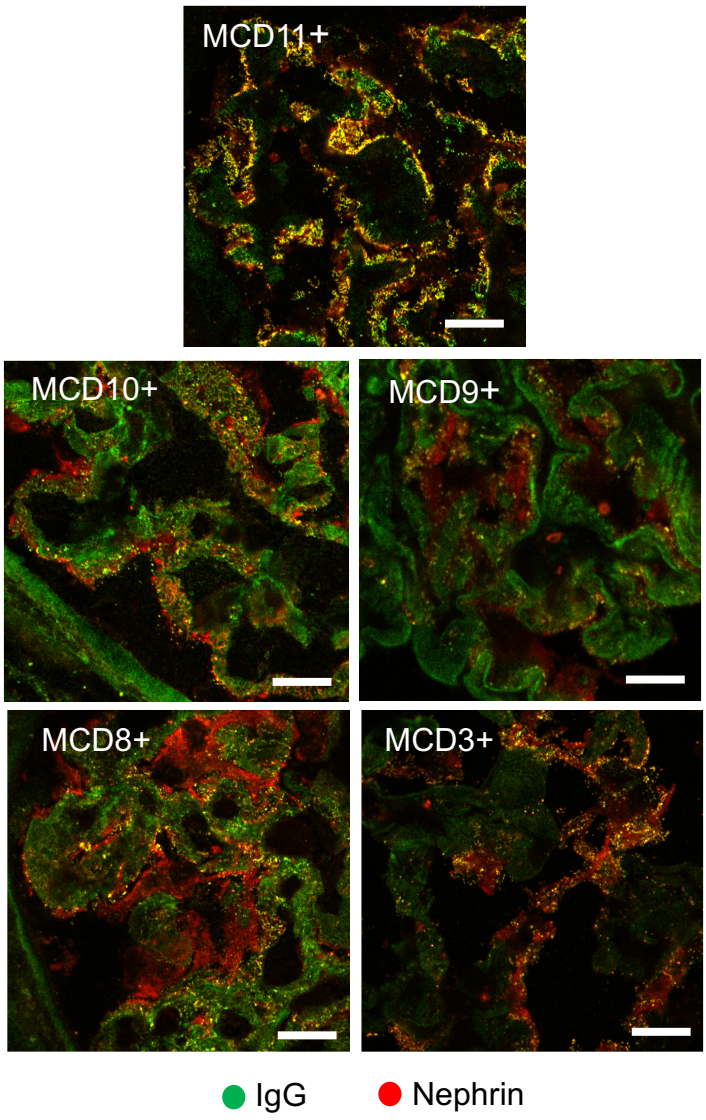

Figure S5. Representative confocal microscopy images of 5 additional IgG-positive MCD renal biopsies showing colocalization between IgG and nephrin. Immunofluorescence staining showing colocalization (yellow) between IgG (green) and nephrin (red) in renal biopsies from patients presenting with acute onset nephrotic syndrome (NS) and a histological diagnosis of minimal change disease (MCD) with diffuse foot process effacement and absence of electron-dense deposits by electron microscopy. Scale bar: 10μm

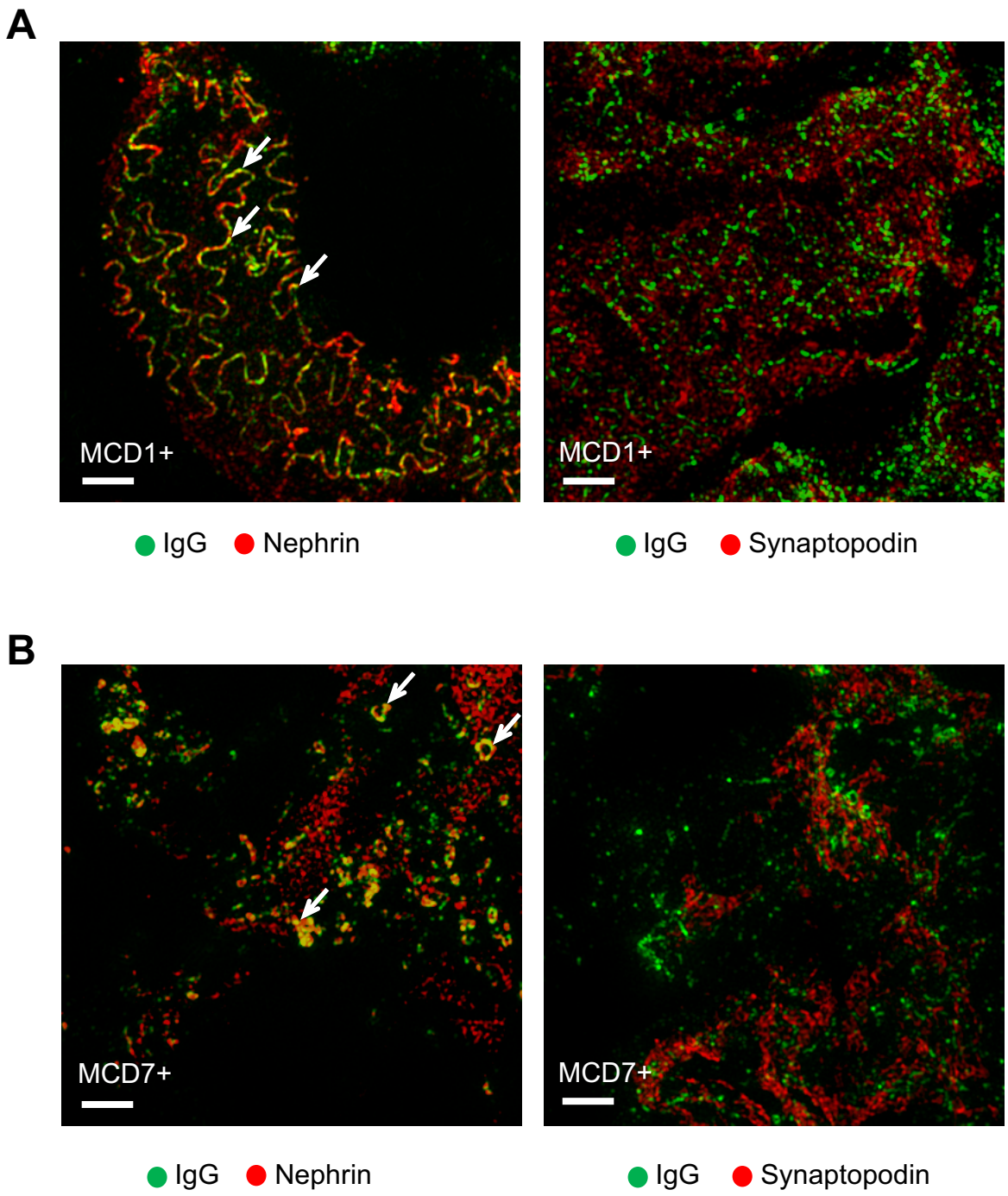

Figure S6. Structured Illumination Microscopy images of representative MCD+ biopsies illustrating the two different patterns of IgG distribution observed in all MCD+ cases. Complete Z-stack of multiple images taken at different focal distances (z-stack) combined to show greater depth of field for the biopsies shown in Figure 3 C,D. The two cases shown are representative of the two different patterns of IgG distribution observed in all MCD+ cases. **(A)** MCD1+ illustrates the co-localization (yellow) of IgG (green) with nephrin (red) in a pattern reflecting their presence along cell-cell junction (white arrows) (left). Essentially no overlap of the IgG (green) with the actin binding protein synaptopodin (red) is observed (right). **(B)** MCD7+ shows overlap (yellow) of IgG (green) with nephrin (red) in a more scattered granular, vaguely vesicular pattern (white arrows) (left). There is no overlap with the actin-binding protein synaptopodin (right). Scale bar: 2 μm

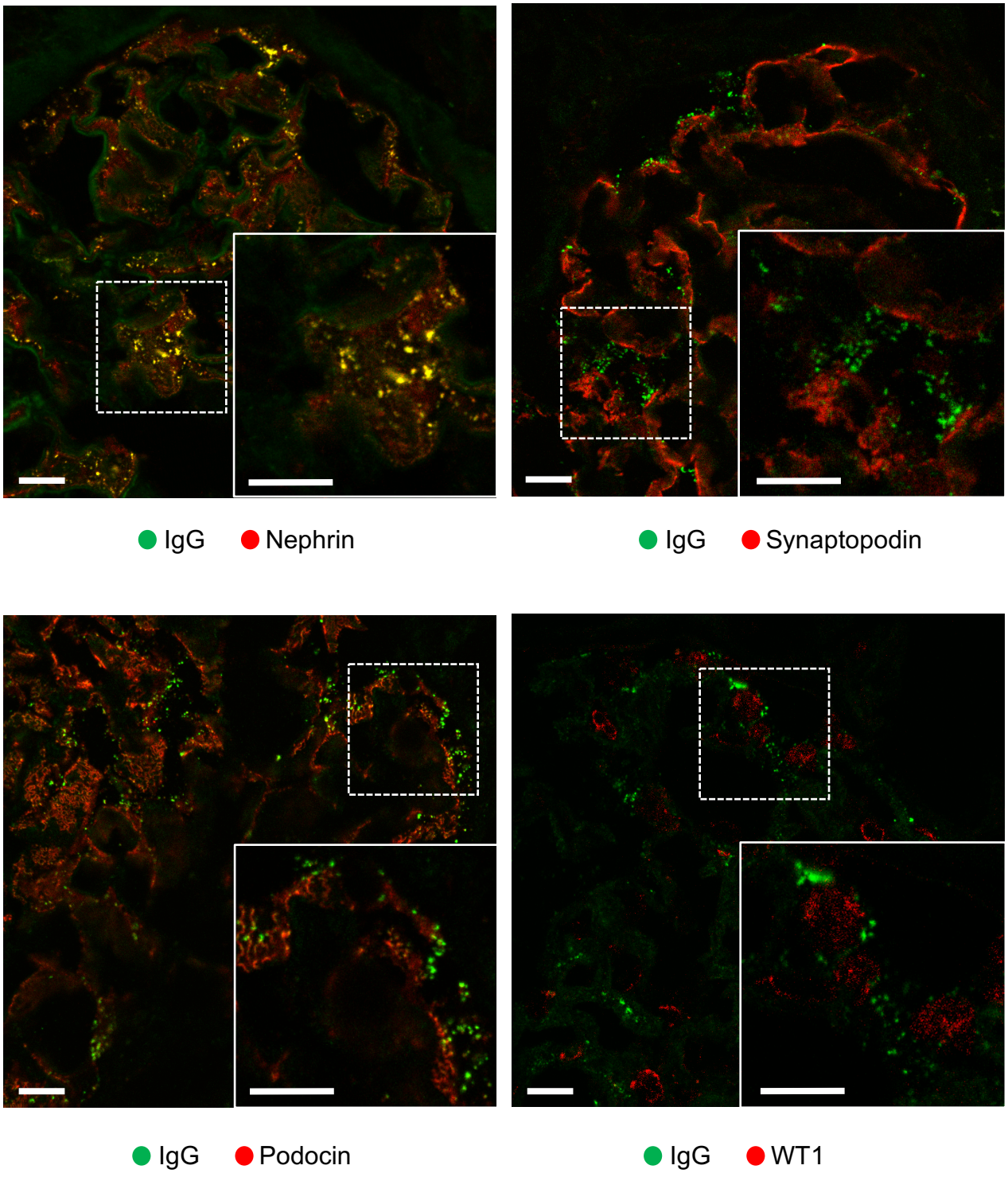

Figure S7. Consistent co-localization of granular IgG with nephrin but not with other podocyte specific proteins in IgG-positive MCD. Confocal microscopy imaging of a representative MCD+ biopsy (MCD12+) with granular IgG staining (green) illustrating substantive overlap (yellow) of IgG (green) with nephrin (red), but not with the cytoplasmic actin-associated foot process component synaptopodin (red), the intracellular slit diaphragm protein podocin (red) or the nuclear transcription factor WT1 (red). A magnified image of the boxed area (dotted line) is shown at the bottom right of each image (box with solid line). Scale bars: 5 μm

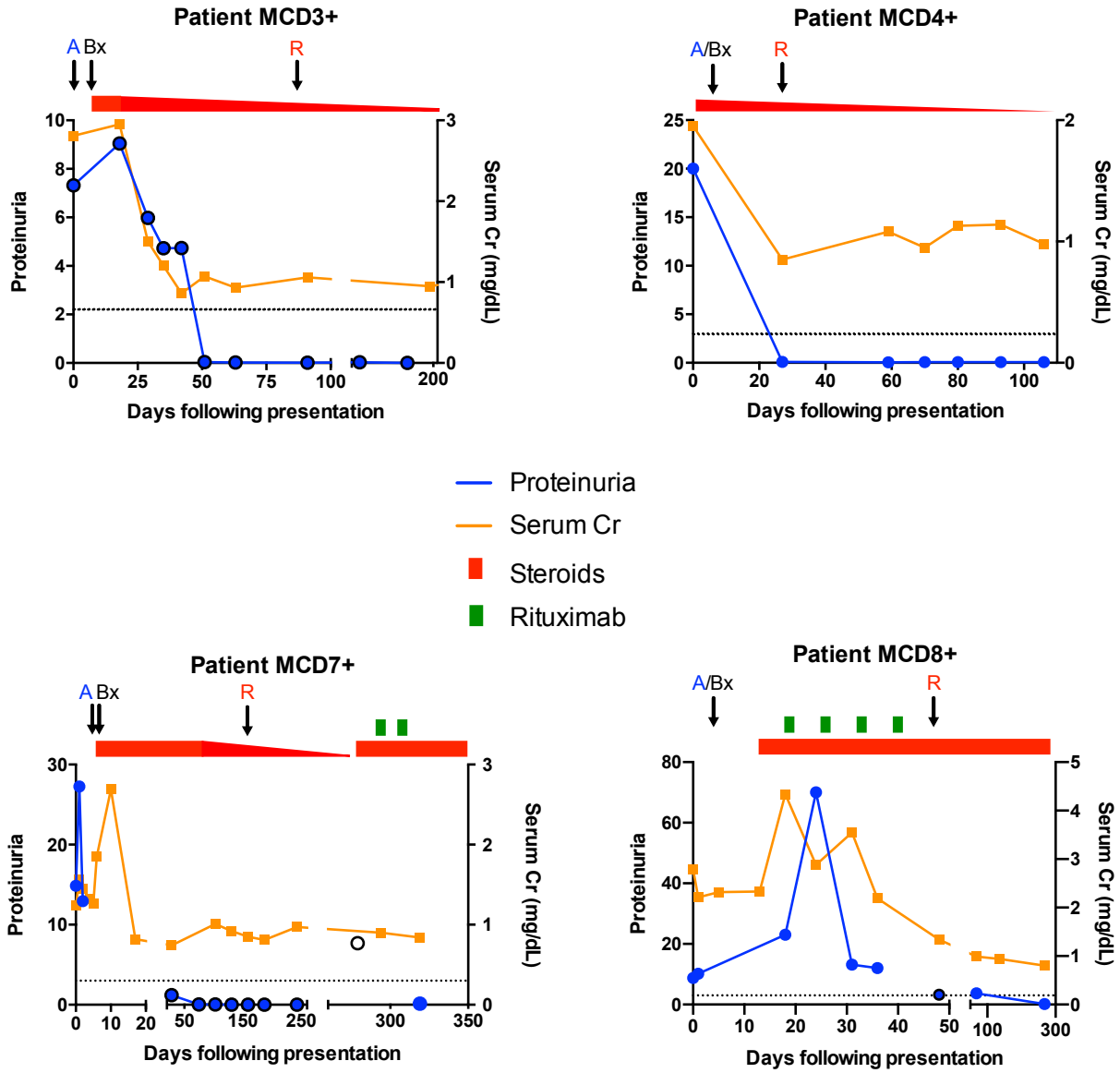

Figure S8. Clinical course of biopsy IgG-positive MCD patients evaluated for nephrin autoantibodies during active disease and following response to treatment. Proteinuria (Urine protein creatinine ratio, UPCR (g/g), blue circle; Urine albumin creatinine ratio, UACR (g/g), blue circle with black border; 24hr urine protein (g/24hr), clear circle with black border) and serum creatinine (Cr) are shown for patients with biopsy IgG-positive MCD (MCD+) whose serum was evaluated for anti-nephrin antibodies during active disease and during treatment response. Active samples (A) were obtained within 7-days of clinical presentation and response samples (R) were obtained when the patients were in complete (MCD4+, MCD7+) or partial remission (MCD8+), defined by UPCR < 0.3 g/g (UACR < 0.2 g/g) or 50% reduction in proteinuria respectively, on the day of sample collection. For MCD3+, the responsive sample was collected approximately 3 weeks after entering a period of sustained clinical remission. Treatment is shown at the top of the graph, with a dose reduction in steroids indicated by a downward sloping wedge. The dotted line indicates a proteinuria level of 3. Bx, renal biopsy.

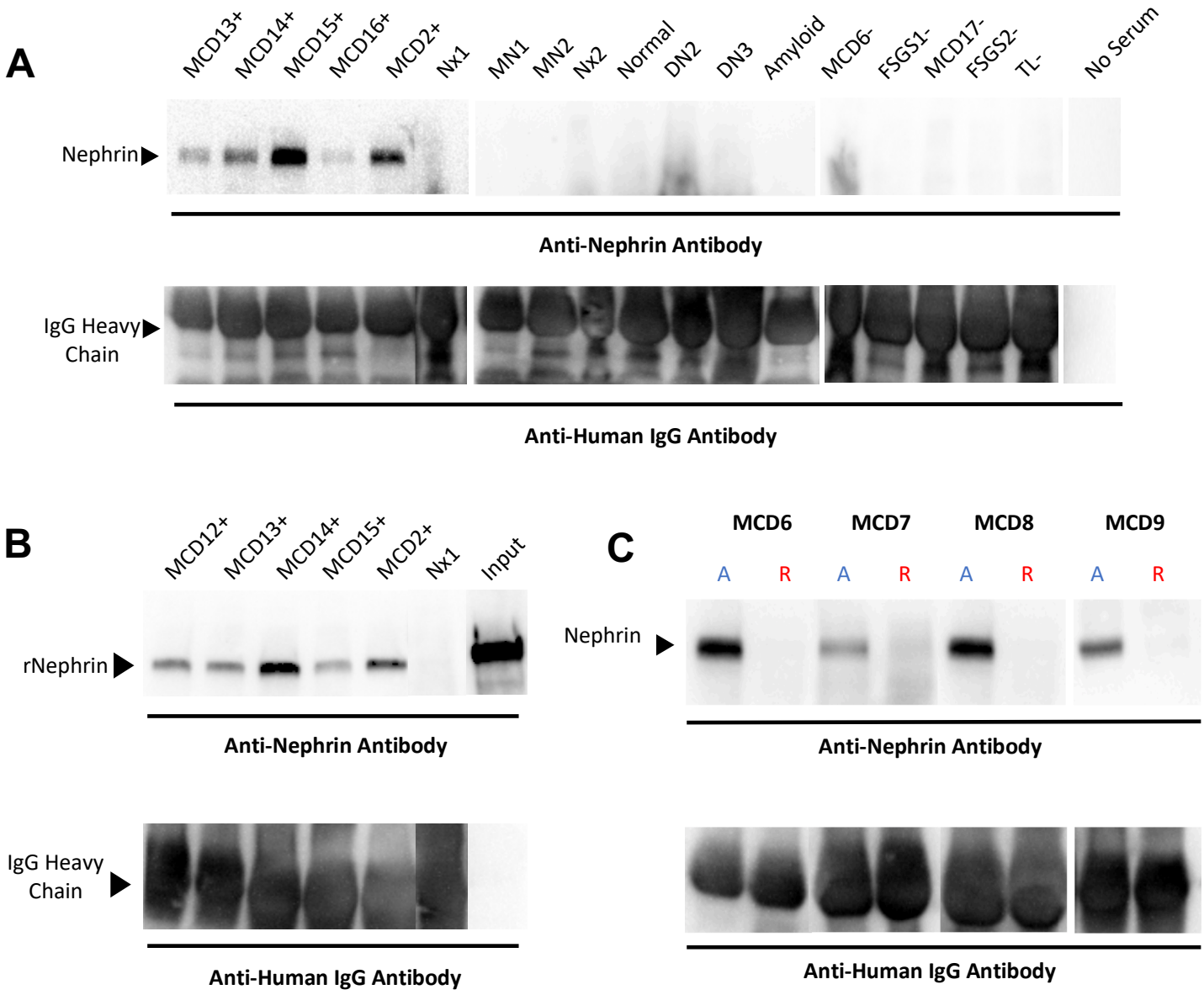

Figure S9. Biopsy IgG+ MCD patient serum or plasma immunoprecipitates both nephrin from human glomerular extract (HGE) and affinity-purified recombinant extracellular domain of human nephrin. (A) Nephrin was immunoprecipitated from human glomerular extract (HGE), derived from healthy donor kidney, with serum from patients with biopsy IgG positive MCD (+) and not from control patients lacking IgG on renal biopsy. **(B)** Purified recombinant extracellular domain of human nephrin ($hNephrin_{G1059}$) was immunoprecipitated by serum or plasma from patients with MCD and punctate IgG in their renal biopsies (MCD2+, 13+, 14+, 15+, 16+), but not by a control patient without IgG deposition in the biopsy (Nx1, disease-free area of tumor nephrectomy). The input lane shows the starting amount of recombinant $hNephrin_{G1059}$ protein used for the immunoprecipitation (not incubated with serum or Protein G beads). **(C)** Nephrin was precipitated from HGE in four MCD+ patients during active disease, but not following remission. Immunoprecipitates were electrophoresed under reducing conditions and subjected to Western blot analysis with a primary sheep anti-human nephrin antibody and secondary HRP-conjugated donkey anti-sheep IgG antibody (top) or a primary HRP-conjugated donkey anti-human IgG alone (bottom).

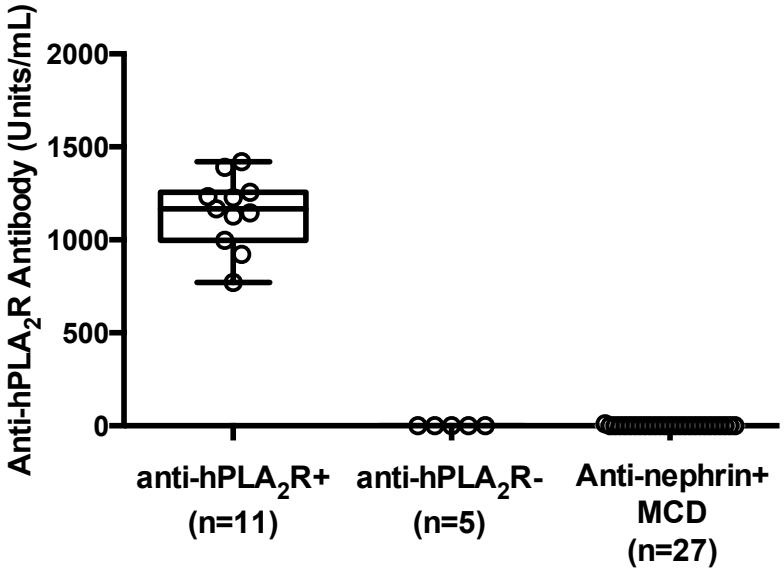

Figure S10. Antibodies to nephrin in MCD patients do not cross react with hPLA$_2$R. Serum from patients with anti-nephrin antibodies were evaluated for antibodies to the extracellular domain of recombinant affinity purified human PLA$_2$R (hPLA$_2$R) by ELISA. Control serum was obtained from patients screened for anti-hPLA$_2$R antibodies by two validated routine clinical assays; ELISA and IIFT (Euroimmun). The standard curve was generated using a single high titer patient sample with a 1:40,000 dilution defined to contain 1000 Units/ml.

NEPTUNE cohort

Patient	Age	Sex	Race/ Ethnicity	UPCR (g/g)	Peak sCr (mg/dL)	α–Nephrin Ab (U/ml)	Follow up (months)	Remission	Relapse	Treatment
N1	4	Male	White*	25.70	0.5	2864 ± 832	53	Partial	N/A	P/CNI/RIT/MMF
N2	54	Male	Black	7.04	4.53	1271 ± 101	23	Complete	Yes	P/CNI/MMF
N3	17	Male	White	8.68	0.9	963 ± 72	10	Complete	No	P/CNI/RIT
N4	14	Female	White	12.06	5.45	745 ± 99	43	Complete	No	P/MMF
N5	39	Male	Asian	19.00	1.29	736 ± 26	29	Complete	Yes	P
N6	36	Male	Asian	27.49	1.24	512 ± 48	38	Complete	Yes	P
N7	24	Female	Black	5.78	1.25	442 ± 62	31	Complete	No	P/MMF
N8	51	Female	White*	19.93	2.52	423 ± 53	1	Complete	No	P
N9	3	Male	Black	10.58	0.41	392 ± 62	28	Complete	Yes	P/CNI
N10	22	Male	White*	3.43	0.88	381 ± 38	18	Complete	No	P
N11	59	Female	White*	11.22	2.6	320 ± 67	31	Complete	Yes	P/RIT
N12	2	Male	Asian	4.28	0.83	305 ± 115	56	Partial	N/A	P/CNI/MMF
N13	6	Male	Asian	9.60#	0.34	293 ± 57	14	Complete	Yes	P/CNI/RIT
N14	37	Female	White	7.77	0.8	273 ± 135	6	Complete	Yes	P/MMF
N15	18	Male	Asian	9.57	0.84	253 ± 62	60	Complete	Yes	P/CNI
N16	4	Male	Multi	3.49	0.3	235 ± 19	12	Partial	N/A	P/CNI/MMF
N17	16	Male	Black	7.85	0.93	193 ± 22	38	Complete	Yes	P/MMF
N18	30	Female	White*	3.54	0.6	191 ± 71	19	Complete	No	P/MMF
N19	7	Male	White	4.80	0.64	172 ± 18	33	Complete	No	P/CNI/MMF
N20	48	Female	White	8.42	0.91	172 ± 32	3	Complete	Yes	P/MMF
N21	17	Male	Black	8.41	0.7	165 ± 35	80	Complete	Yes	P/CNI
N22	24	Male	Black	6.17	3.16	156 ± 30	21	Complete	No	P/CNI
N23	12	Female	Black	10.38	10	137 ± 15	57	Complete	Yes	P/CNI/MMF
N24	15	Female	Black	6.66	1.02	134 ± 69	18	Complete	Yes	P
N25	55	Male	White	3.28	1.10	129 ± 76	55	Complete	No	CTX/RIT/FLU
N26	3	Female	Multi	4.71	0.52	129 ± 48	53	Complete	Yes	P/CNI
N27	15	Male	White	7.34	0.86	117 ± 18	30	Complete	Yes	P/CNI/RIT
N28	4	Female	White	7.58	0.51	116 ± 36	60	Complete	No	P/CTX/CNI
N29	29	Male	White	13.84	0.7	98 ± 80	47	Complete	No	P
N30	4	Male	Black	16.24	0.40	86 ± 40	48	Complete	Yes	P/CNI
N31	15	Male	Asian	10.03	0.88	85 ± 45	25	Complete	Yes	P/CNI
N32	36	Female	White*	3.00	0.71	82 ± 18	20	Complete	No	P
N33	1	Male	White*	20.93	0.32	80 ± 11	20	Complete	Yes	P/CNI/RIT
N34	18	Male	White	5.25	0.8	78 ± 12	30	Complete	Yes	P/MMF/RIT
N35	17	Female	Black	5.18	0.7	74 ± 54	13	Complete	No	P/CNI
N36	6	Female	Unknown*	10.51	0.5	72 ± 85	54	Complete	Yes	P/CTX
N37	1	Female	Black	40.38	0.33	71 ± 110	28	Complete	No	P/CNI
N38	13	Female	Black	4.71	1.1	70 ± 12	57	Complete	No	P/MMF
N39	11	Male	Multi	5.54	1.82	59 ± 40	53	Complete	Yes	P/CNI
N40	17	Male	White	7.66	0.91	55 ± 5	25	Complete	No	P
N41	15	Female	White	12.53	0.57	52 ± 51	23	Complete	No	P
N42	57	Female	Black	3.46	1.77	38 ± 30	13	Complete	Yes	P/CNI
N43	16	Male	White*	3.52	0.59	35 ± 12	46	Complete	No	P/CNI/MMF
N44	9	Female	Asian	5.18	0.47	33 ± 13	37	Partial	N/A	P/CNI/MMF
N45	16	Female	Unknown*	3.07	1.2	31 ± 31	47	Complete	No	P
N46	14	Male	White	3.01	0.9	29 ± 58	60	Complete	Yes	P
N47	39	Male	White*	6.94	3.62	25 ± 30	55	Complete	No	P

N48	5	Female	White*	12.70	0.5	21 ± 18	54	Complete	Yes	P/CNI/MMF
N49	14	Female	White	8.34	0.61	19 ± 14	3	None	N/A	P/CNI/MMF
N50	8	Female	White	17.92	0.42	18 ± 19	54	None	N/A	P/CNI
N51	12	Male	Black	12.14	0.6	15 ± 13	51	Complete	Yes	P/MMF/CNI
N52	22	Female	White*	11.31	0.83	12 ± 21	36	Complete	No	P
N53	6	Male	Black	10.32	0.7	10 ± 14	57	Complete	Yes	P/CNI
N54	11	Male	White	9.70	0.52	9 ± 8	26	Complete	Yes	P/CNI
N55	2	Female	Black	8.89	0.58	7 ± 12	51	Complete	Yes	P/CNI
N56	28	Female	White*	7.12	1.06	6 ± 8	55	Partial	N/A	P/CNI/MMF
N57	2	Male	Multi	8.94	0.4	6 ± 7	52	Complete	Yes	P/CNI/MMF/CTX
N58	9	Male	White	6.48	0.6	3 ± 8	32	Complete	Yes	P/MMF
N59	3	Female	White	9.69	0.46	0 ± 0	52	Complete	Yes	P/CNI
N60	12	Male	White	5.64	0.6	0 ± 0	53	Complete	Yes	P/MMF
N61	48	Male	White	5.63	1.11	0 ± 0	43	Complete	Yes	P/RIT
N62	16	Male	Multi	10.76	1.01	0 ± 0	27	Complete	Yes	P/CNI

Healthy Controls

Patient	**Age**	**Sex**	**Race**	**α–Nephrin Ab (U/ml)**
CNT1	69	Male	White	187 ± 45
CNT2	32	Female	White	174 ± 84
CNT3	23	Male	White	100 ± 60
CNT4	65	Female	White	57 ± 35
CNT5	56	Female	White	49 ± 11
CNT6	17	Male	White	43 ± 21
CNT7	67	Male	White	41 ± 28
CNT8	52	Male	White	41 ± 34
CNT9	33	Male	White	39 ± 28
CNT10	62	Female	White	36 ± 52
CNT11	44	Female	White	33 ± 35
CNT12	82	Female	White	27 ± 26
CNT13	81	Male	White	23 ± 34
CNT14	60	Male	White	19 ± 14
CNT15	40	Male	White	18 ± 19
CNT16	58	Male	White	16 ± 13
CNT17	20	Male	White	12 ± 15
CNT18	22	Male	White	12 ± 11
CNT19	21	Male	White	8 ± 8
CNT20	61	Female	White	6 ± 8
CNT21	48	Male	White	6 ± 8
CNT22	11	Female	White	6 ± 11
CNT23	45	Male	White	4 ± 9
CNT24	56	Female	White	3 ± 7
CNT25	41	Male	White	0 ± 0
CNT26	64	Male	White	0 ± 0
CNT27	49	Male	White	0 ± 0
CNT28	8	Male	White	0 ± 0
CNT29	30	Male	White	0 ± 0
CNT30	22	Male	White	0 ± 0

hPLA$_2$R+ cohort

Patient	Age	Gender	α–Nephrin Ab (U/ml)
PLA$_2$R1	75	Female	196 ± 89
PLA$_2$R2	70	Female	184 ± 119
PLA$_2$R3	72	Male	162 ± 141
PLA$_2$R4	63	Female	160 ± 71
PLA$_2$R5	75	Female	132 ± 101
PLA$_2$R6	70	Female	128 ± 83
PLA$_2$R7	70	Male	101 ± 80
PLA$_2$R8	76	Male	80 ± 22
PLA$_2$R9	37	Male	61 ± 13
PLA$_2$R10	70	Male	53 ± 61
PLA$_2$R11	85	Male	52 ± 28
PLA$_2$R12	37	Male	45 ± 16
PLA$_2$R13	71	Male	42 ± 14
PLA$_2$R14	77	Female	37 ± 57
PLA$_2$R15	59	Male	36 ± 30
PLA$_2$R16	55	Female	30 ± 45
PLA$_2$R17	33	Male	27 ± 13
PLA$_2$R18	55	Male	25 ± 44
PLA$_2$R19	49	Male	21 ± 8
PLA$_2$R20	60	Male	20 ± 17
PLA$_2$R21	58	Male	20 ± 30
PLA$_2$R22	52	Male	17 ± 14
PLA$_2$R23	38	Female	10 ± 7
PLA$_2$R24	40	Female	10 ± 17
PLA$_2$R25	37	Male	10 ± 9
PLA$_2$R26	59	Male	10 ± 17
PLA$_2$R27	72	Male	9 ± 16
PLA$_2$R28	45	Female	6 ± 8
PLA$_2$R29	70	Male	5 ± 9
PLA$_2$R30	57	Female	5 ± 8
PLA$_2$R31	44	Male	4 ± 7
PLA$_2$R32	58	Male	3 ± 6
PLA$_2$R33	60	Male	2 ± 4
PLA$_2$R34	86	Female	2 ± 4
PLA$_2$R35	77	Male	2 ± 3
PLA$_2$R36	69	Male	1 ± 2
PLA$_2$R37	64	Male	0 ± 0
PLA$_2$R38	64	Male	0 ± 0
PLA$_2$R39	56	Female	0 ± 0
PLA$_2$R40	41	Female	0 ± 0
PLA$_2$R41	61	Female	0 ± 0
PLA$_2$R42	61	Female	0 ± 0
PLA$_2$R43	61	Male	0 ± 0
PLA$_2$R44	56	Female	0 ±0
PLA$_2$R45	45	Male	0 ± 0
PLA$_2$R46	75	Male	0 ± 0
PLA$_2$R47	84	Female	0 ± 0

PLA_2R48	65	Male	0 ± 0
PLA_2R49	83	Female	0 ± 0
PLA_2R50	53	Female	0 ± 0
PLA_2R51	56	Female	0 ± 0
PLA_2R52	75	Male	0 ± 0
PLA_2R53	90	Male	0 ± 0
PLA_2R54	81	Male	0 ± 0

Table S1. Clinical information for the NEPTUNE patients and controls evaluated for nephrin autoantibodies. The table provides relevant clinical information for the patients or controls. All patients in the NEPTUNE cohort had biopsy proven minimal change disease (MCD); however, the renal biopsy IgG deposition status was not reported and neither immunofluorescence images nor biopsy material were available for further assessment. Proteinuria values (Urine Protein Creatinine ratio (UPCR)) are from the same day (or within one day) that the serum sample was collected for anti-nephrin antibody (α-Nephrin Ab) testing during active disease. #For patient N13, the UPCR was calculated to be 323 g/g on the day of serum collection and so the value for the next available UPCR (assessed 20 days later) is given. Peak sCr (serum creatinine) was the highest serum creatinine reached during the follow-up period. Partial remission was defined as > 50% reduction in the UPCR and complete remission (CR) as UPCR < 0.3 g/g. A patient was deemed to have relapsed with a UPCR > 3 g/g after first reaching CR. In those patients not reaching CR, the relapse status is not applicable (N/A). Serum was obtained from a randomly selected healthy control cohort from Partners Healthcare Biobank. The threshold for a positive anti-nephrin antibody titer was based on the maximum value for the healthy cohort of 187 U/ml. Antibody titer is given as the mean ± S.D. of replicate samples (n≥3) for each patient. Serum from patients who tested positive for anti-human PLA_2R antibodies ($hPLA_2R$+) by two clinically validated assays, ELISA and IIFT (Euroimmun), were obtained from MGH Immunopathology Laboratory. Designations: P, prednisolone; CNI, calcineurin inhibitor; MMF, mycophenolate mofetil; CTX, cyclophosphamide; RIT, rituximab; FLU, flucytosine; * indicates Hispanic or Latino ethnicity.

BWH/MGH/BMC/Mayo Clinic cohort

Patient	Diagnosis	Age	Sex	Race/ Ethnicity	Proteinuria	Serum Cr (mg/dL)	α–Nephrin Ab (U/ml)	Punctate IgG on biopsy
MCD1 (+)	Minimal change disease	61	Female	White	28	2.2	No serum	Yes (IgG1)
MCD2 (+)	Minimal change disease	26	Male	White *	22.4	1.93	705 ± 103	Yes (IgG1)
MCD3 (+)	Minimal change disease	65	Male	White	7.32	2.81	5450 ± 1558	Yes (IgG4)
MCD4 (+)	Minimal change disease	28	Male	Asian	20	1.95	1375 ± 253	Yes (IgG1)
MCD5 (-)	Minimal change disease	71	Female	White	4.95	0.99	No serum	No
MCD6 (-)	Minimal change disease	52	Male	White	2.7	4.4	10 ± 12	No
MCD7 (+)	Minimal change disease	61	Female	White	12.95	1.4	330 ± 18	Yes (IgG1)
MCD8 (+)	Minimal change disease	61	Male	White	10.21	2.3	1427 ± 145	Yes (IgG4)
MCD9 (+)	Minimal change disease	42	Female	Black	18	2.45	No serum	Yes (IgG1)
MCD10 (+)	Minimal change disease	47	Male	White	8.33	1.2	No serum	Yes (ND)
MCD11 (+)	Minimal change disease	10	Female	White	3+	0.5	No serum	Yes (IgG4)
MCD12 (+)	Minimal change disease	30	Male	Unknown	5.8	0.95	No serum	Yes (Ig4)
MCD13 (+)	Minimal change disease	48	Male	White	15	2.4	649 ± 30	Yes (ND)
MCD14 (+)	Minimal change disease	22	Female	White	8.71	1.3	270 ± 50	Yes (IgG4)
MCD15 (+)	Minimal change disease	68	Male	White	7.2	2.55	911 ± 83	Yes (IgG4)
MCD16 (+)	Minimal change disease	81	Male	White	2	1.3	271 ± 39	Yes (ND)
MCD17 (-)	Minimal change disease	35	Female	White	2[#]	0.95	36 ± 11	No
MCD18 (-)	Minimal change disease	81	Male	White	2.14	3.22	21 ± 3	No
FSGS1-	Primary FSGS	38	Female	White	4.19	0.51	0 ± 0	No
FSGS2-	Primary FSGS	53	Male	White	8	3.5	8 ± 6	No
TL-	Podocytopathy with TL	39	Male	White	10	1.02	94 ± 100	No
Amyloid	Amyloidosis	44	Male	White	12	3.01	7 ± 7	No
MN1	PLA_2R+ MN	68	Male	White	7.6	0.87	24 ± 33	ND
MN2	PLA_2R- MN	40	Male	White	0.83	0.87	16 ± 15	ND
DN1	Diabetic Nephropathy	67	Male	Unknown	3.5	0.8	No serum	No
DN2	Diabetic Nephropathy	42	Male	White	7.41	4.6	0 ± 0	No
DN3	Diabetic Nephropathy	49	Male	Black	10	2.8	0 ± 0	No
Nx1	Nephrectomy for RCC	79	Male	White	0.07	1.46	47 ± 32	No
Nx2	Nephrectomy for RCC	61	Male	White	negative	1.51	19 ± 33	No
Normal	Normal	24	Female	White	0.2	0.74	32 ± 37	No

Table S2. BWH/MGH/BMC/Mayo Clinic cohort clinical characteristics. The BWH/MGH/BMC/Mayo Clinic cohort consists of patients whose renal biopsy was evaluated for IgG by immunofluorescence staining (IF) and a concurrent serum sample, where available, was evaluated for anti-nephrin antibodies. For the control patients that had a tumor nephrectomy for RCC (Nx1, Nx2) an area of non-neoplastic renal parenchyma was evaluated by IF. Proteinuria values are given as either UPCR (g/g) or urine dipstick (negative, 3+, 4+) unless otherwise stated ([#]For patient MCD17-, proteinuria is given as urine albumin creatinine ratio (UACR) (g/g)). Serum Creatinine (Serum Cr) and proteinuria values are those closest to the time of serum sampling for patients evaluated for anti-nephrin antibodies and closest to the biopsy for those who were not. The predominant IgG subclass is given in parenthesis where known (ND indicates that the IgG subclass was not determined due to lack of additional biopsy material). FSGS, focal segmental glomerulosclerosis; TL, tip lesion; MN, membranous nephropathy; RCC, renal cell cancer. * indicates Hispanic or Latino ethnicity. MCD+ indicates presence of punctate IgG in the biopsy and MCD- indicates absence of punctate IgG in the biopsy.

CLINICAL CASE DETAILS

To illustrate a potential role of pre-transplant nephrin autoantibodies in early post-transplant massive proteinuria recurrence, we present the case of a 27-year-old woman with an initial diagnosis of steroid-responsive MCD at age 2 who became steroid dependent (SDNS) and then developed FSGS at age 16. During this period, she had a total of four renal biopsies, with the first three showing MCD and the fourth one showing FSGS. Podocyte associated punctate IgG was noted in the two most recent biopsies. Importantly, clinical whole exome sequencing (Prevention Genetics) found no known NS disease causing variants. She eventually developed ESKD and initially underwent hemodialysis for 5 years and then received a pediatric deceased donor kidney (cold ischemia time 19 hours) with immediate graft function (Fig. 6A). Calculated panel reactive antibodies (cPRA) were zero and induction therapy consisted of basiliximab with maintenance therapy of mycophenolate mofetil, tacrolimus and prednisolone. In the setting of early massive post-transplant proteinuria recurrence, associated with lower limb edema, she was treated with five episodes of plasmapheresis (x5) and two doses of rituximab. Her proteinuria rapidly improved and she did not require an allograft biopsy (Fig. 6A). Two serum samples obtained 17 and 20 months prior to the transplant, together with the first plasmapheresate sample obtained during treatment for the post-transplant proteinuria recurrence, all tested positive for anti-nephrin antibodies by ELISA (Fig. 6B) and immunoprecipitated nephrin from healthy human kidney derived HGE (Fig. 6C). Serum samples evaluated following treatment response at day 27 and during complete remission at 1 year post transplant both tested negative for nephrin autoantibodies by ELISA (Fig. 6B).