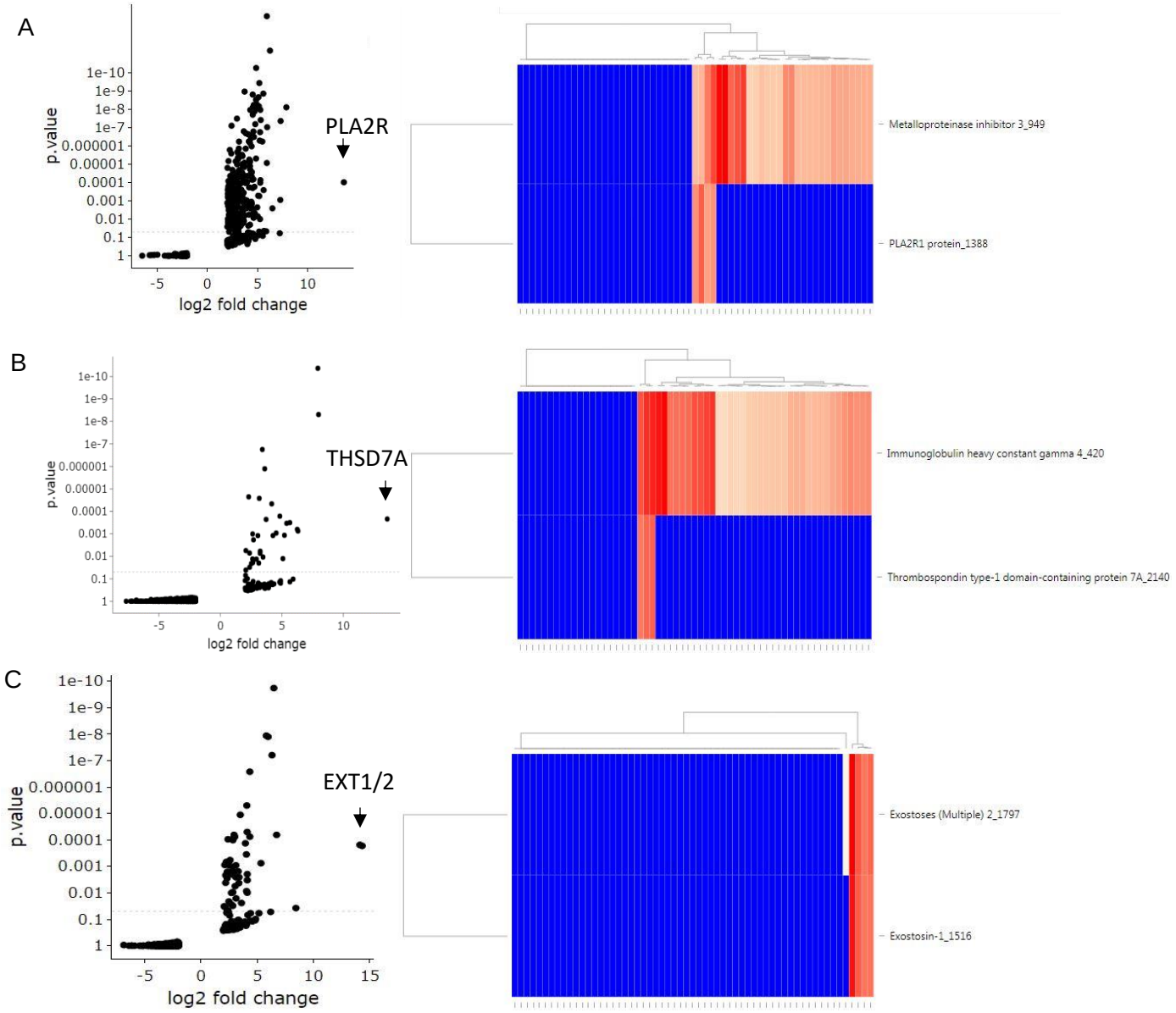
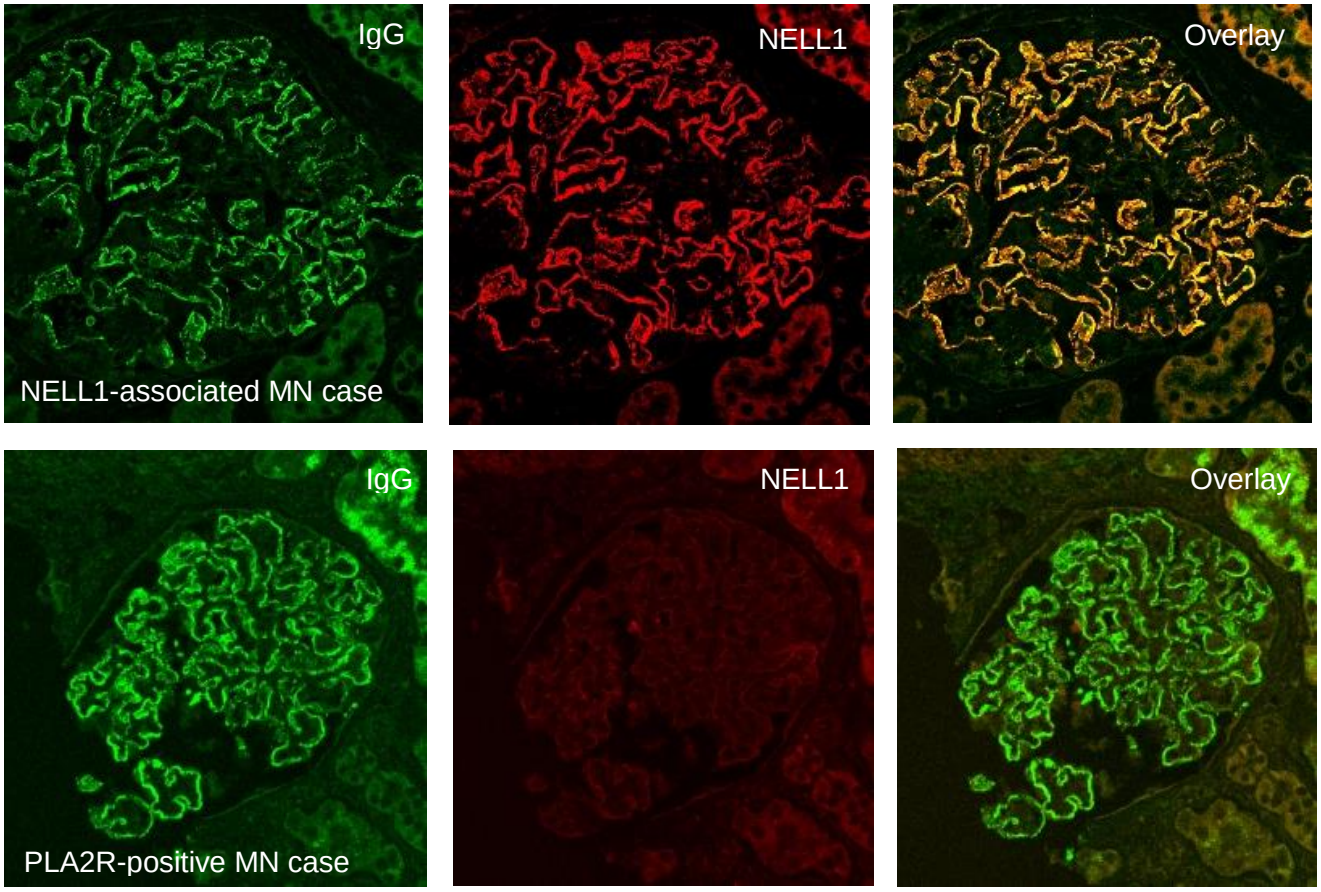
Supplemental Figures and Tables

Supplemental Figure 1.



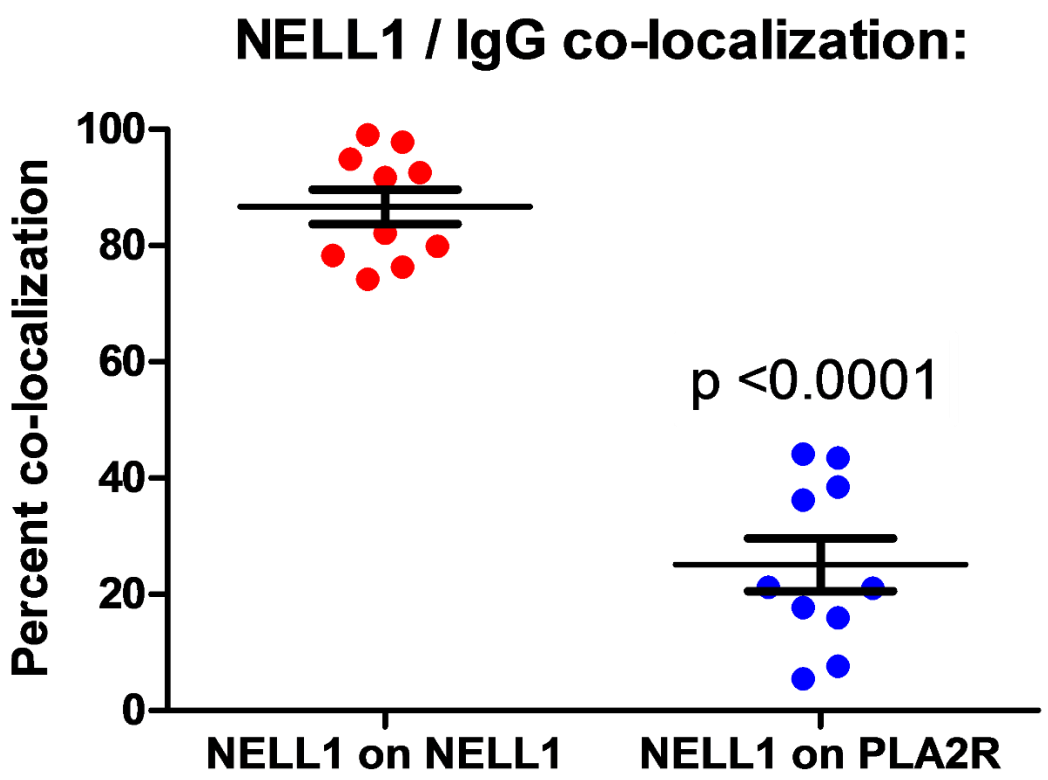
Supplemental Figure 1. Proof-of-principle mass spectrometry analysis, showing tissue IgG immunoprecipitation correctly identified the target antigens within glomeruli in known cases of membranous nephropathy, including PLA2R (A), THSD7A (B), and the EXT1/2 complex (C).

Supplemental Figure 2.



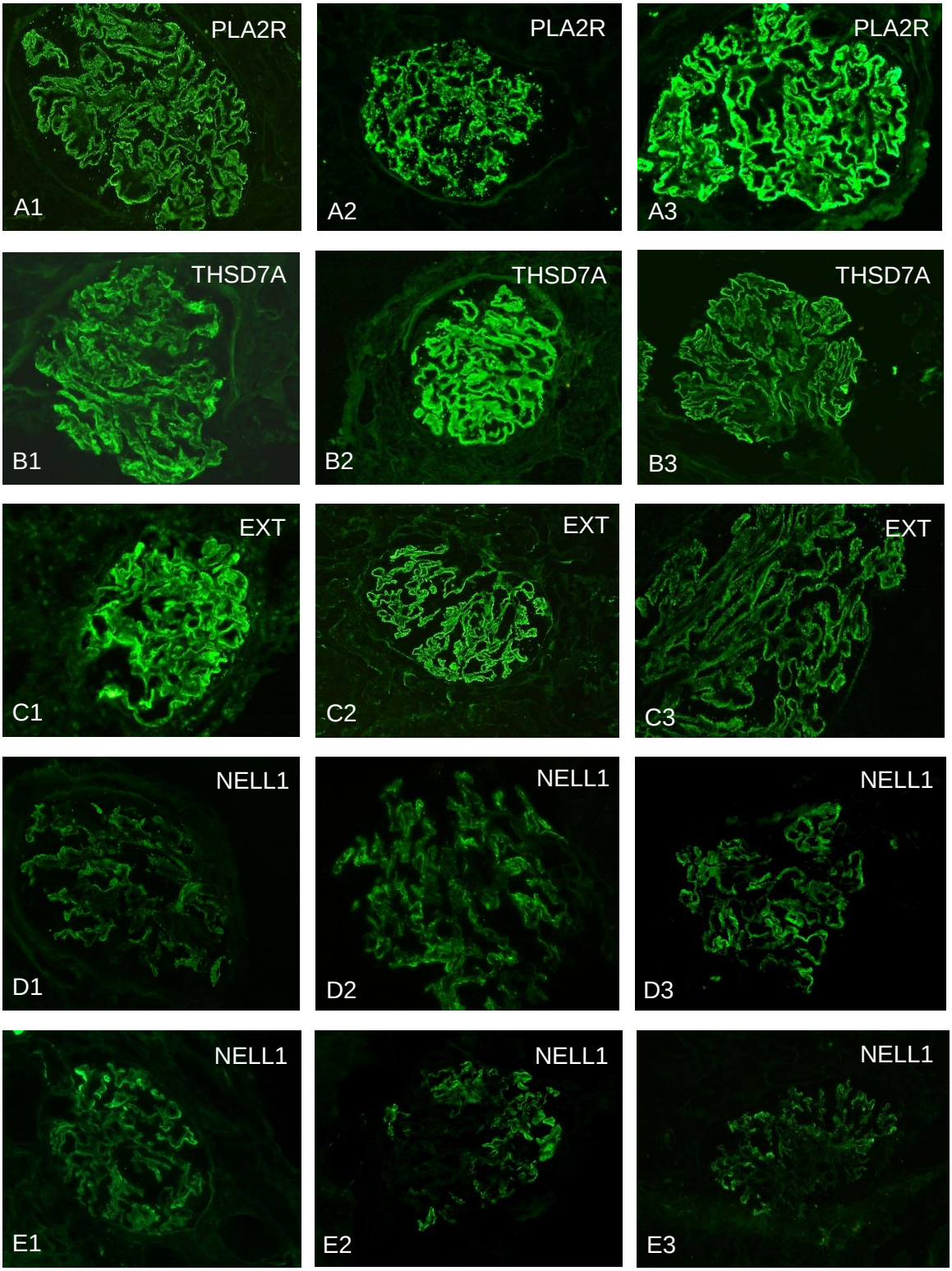
Supplemental Figure 2. NELL1 co-localizes with IgG in NELL1-associated MN cases (top panels), but not in PLA2R-positive MN controls (bottom panels).

Supplemental Figure 3.



Supplemental Figure 3. NELL1 co-localizes with IgG in NELL1-associated MN cases, but not PLA2R-positive MN controls. Results represent 10 glomeruli per group. p-value represents student's t-test.

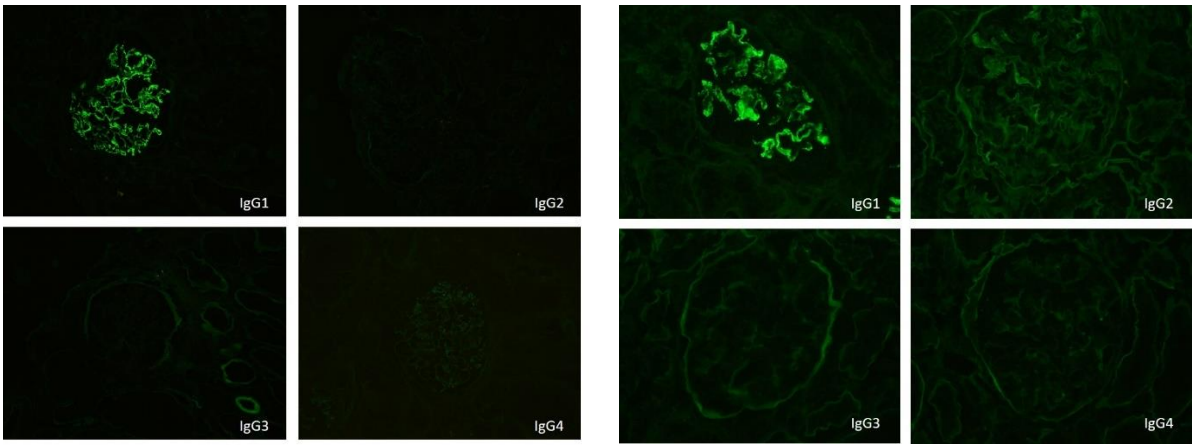
Supplemental Figure 4.



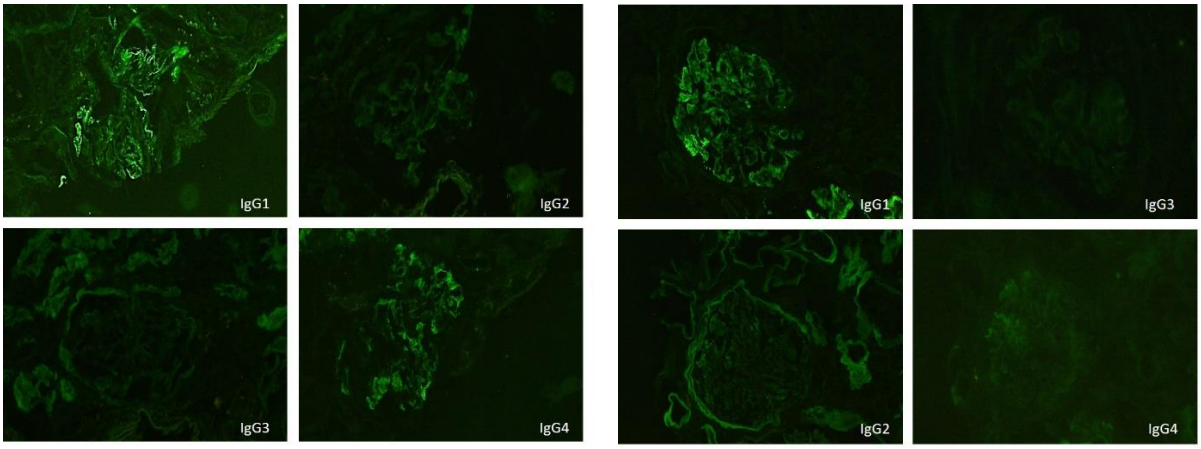
Supplemental Figure 4. IgG immunofluorescence staining shows a complete, global staining pattern in PLA2R-positive MN (A1-A3); IgG immunofluorescence staining shows a complete, global staining pattern in THSD7A-positive MN (B1-B3); EXT-associated MN contained global capillary loop and often mesangial staining for IgG (C1-C3); NELL1-associated MN displays incomplete global (D1-D3) to segmental (E1-E3) capillary loop staining for IgG.

Supplemental Figure 5.

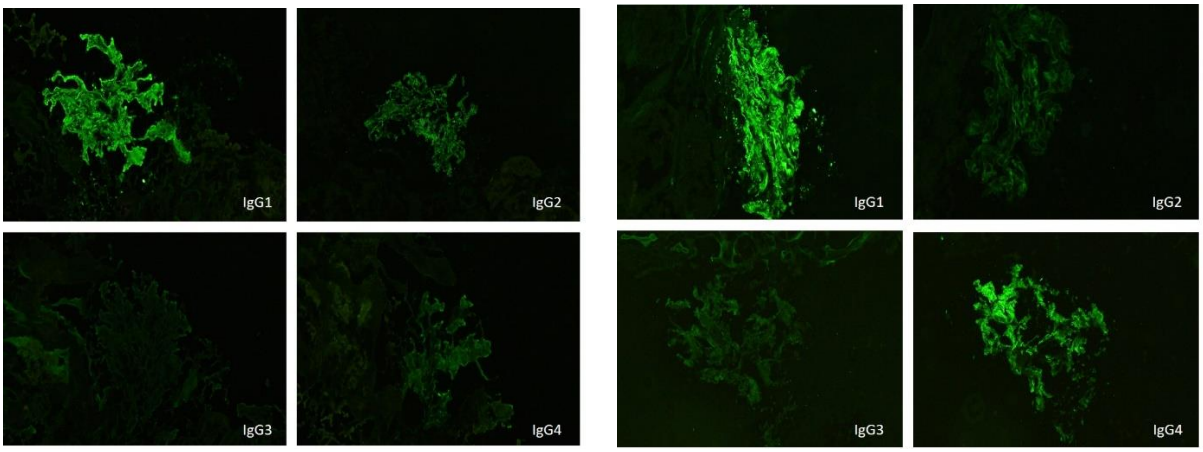
A. Incomplete global staining pattern



B. Segmental staining pattern

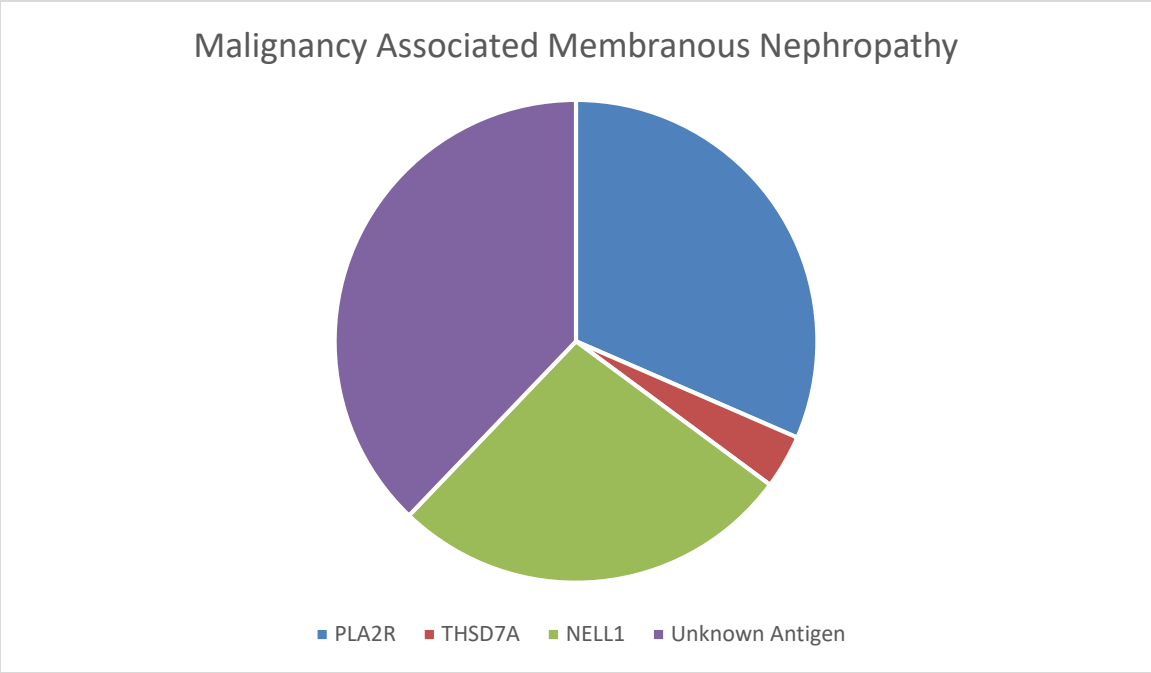


C. Complete global staining pattern



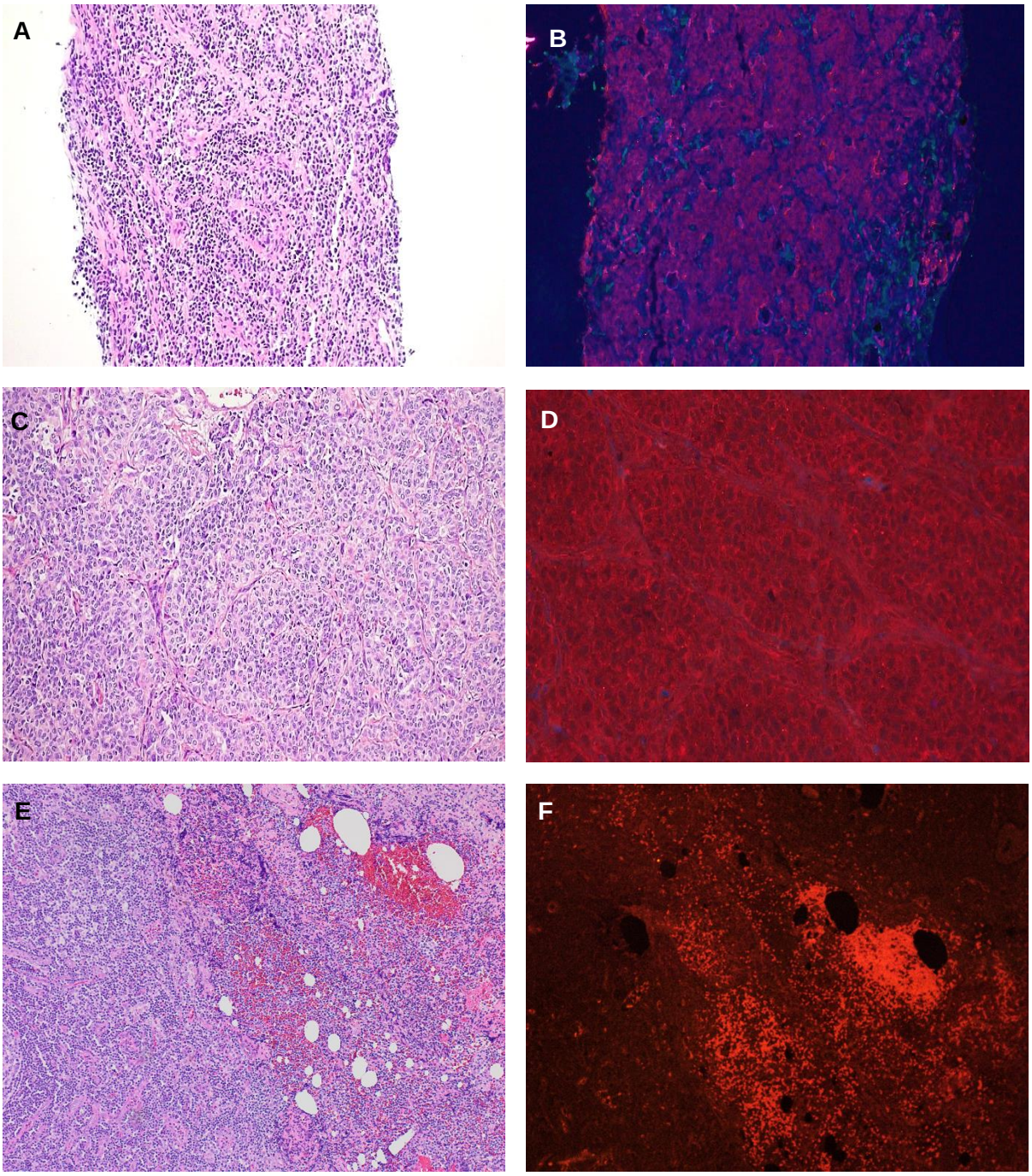
Supplemental Figure 5. Additional examples of IgG subclass staining. A) Two cases with an incomplete global staining pattern, B) Two cases with a segmental IgG staining pattern, C) Two cases with a complete global IgG staining pattern.

Supplemental Figure 6.



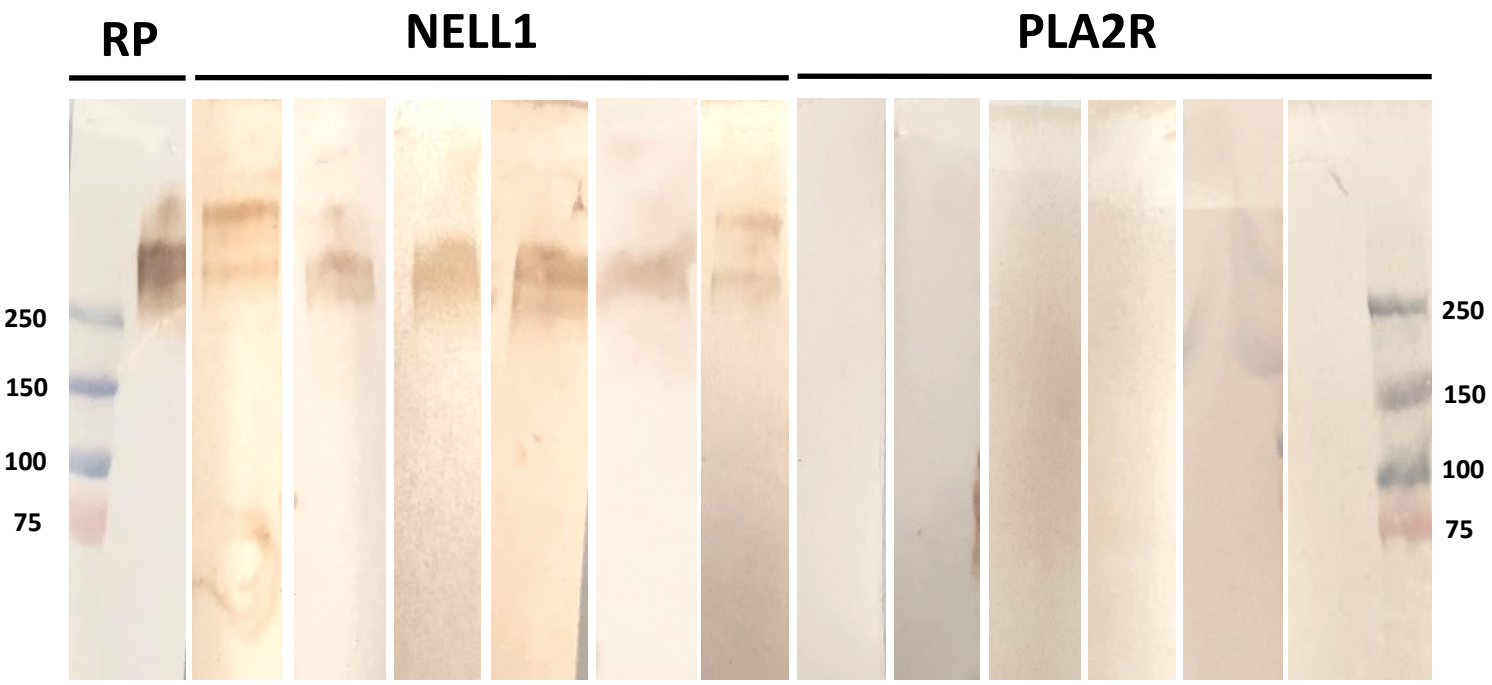
Supplemental Figure 6. Pie graph depicting frequencies of antigen expression in malignancy-associated membranous nephropathy.

Supplemental Figure 7.



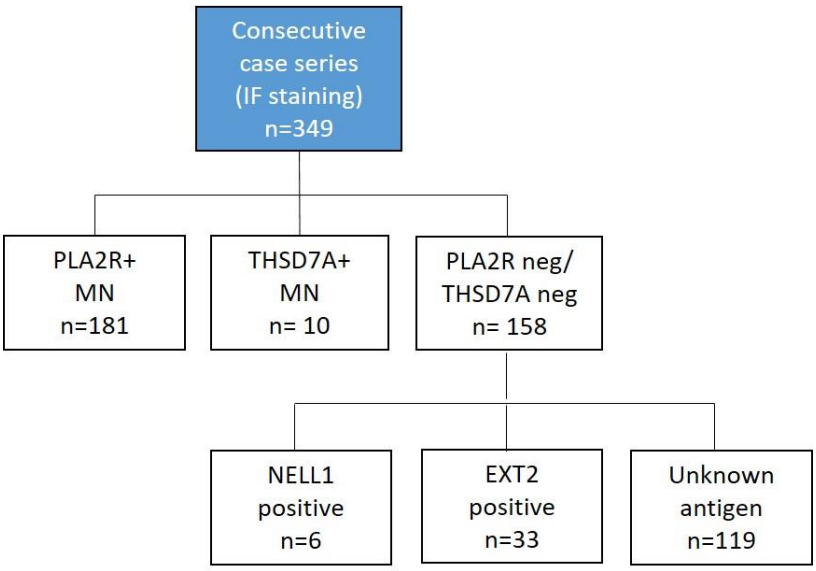
Supplemental Figure 7. NELL1 expression in an invasive ductal carcinoma of the breast in a patient with concurrent NELL1-associated MN. A) Hematoxylin & eosin (H & E) stained section of breast biopsy; B) NELL1 positivity in breast biopsy; C) H & E stained section of mastectomy showing invasive ductal carcinoma; D) NELL1 positivity within breast carcinoma, mastectomy specimen; E) H & E stained section of a follicular lymphoma, showing infiltration into fat; F) NELL1 positivity within follicular lymphoma.

Supplemental Figure 8.



Supplemental Figure 8. Serum reactivity to NELL1 recombinant protein in patients with NELL1-associated MN, but not PLA2R+ MN controls. Although the molecular weight was larger than the predicted molecular weight of 140 kDa, it corresponded to the same molecular weight produced when reacting a rabbit polyclonal antibody against the NELL1 recombinant protein (labeled RP). Seroreactivity was only achieved under non-reducing conditions and was not seen under reducing conditions. Representative western blot strips are shown here, from a total of 28 NELL1-associated MN patients and 28 PLA2R positive MN controls studied in total.

Supplemental Figure 9. Cohort diagram to show our algorithm for identification of NELL1-associated MN cases in a consecutive case series of 9 months' of idiopathic membranous nephropathy and membranous lupus nephritis cases.



Supplemental Tables.

Supplemental Table 1.

Prevalence of NELL1, PLA2R. THSD7A, and EXT2 staining in a consecutive case series of kidney biopsies with membranous nephropathy

Associated disease	NELL1 positive (n=91)	PLA2R positive (n=181)	THSD7A positive (n=10)	EXT2 positive (n=33)
Idiopathic	54/91 (59%)	177/181(97.7%)	7/10 (70%)	4/33 (12%)
SLE	2/91 (2%)	1/181 (1%)	3/10 (30%)	26/33 (79%)
ANCA	0	0	0	0
Rheumatoid arthritis	1/91 (1%)	0	0	0
Crohn's disease	2/91 (2%)	0	0	0
IgG4-related	0	0	0	0
Neoplasia	30/91 (33%)	3/181 (1.7%)	0	0
Hepatitis C virus	2/91 (2%)	0	0	2/33 (6%)
Syphilis	0	0	0	0
HIV	0	0	0	1/33 (3%)
Graft versus host disease	0	0	0	0
Ankylosing spondylitis	0	0	0	0

Supplemental Table 2. Timing between diagnosis of NELL1-positive MN and malignancy and response to therapy in patients with suspected malignancy-associated MN. Complete remission was defined as <300 mg proteinuria per 24 h, partial remission >50% reduction of proteinuria but >300 mg proteinuria per 24 h, and no remission <50% reduction of proteinuria and/or >3.5 g proteinuria per 24 h.

Abbreviations: TAH-BSO = total abdominal hysterectomy with bilateral salpingo-oophorectomy; TURBT = transurethral resection of bladder tumor; ESKD = end stage kidney disease; SCC = squamous cell carcinoma; ACEI = angiotensin-converting enzyme inhibitor; GIST = gastroesophageal stromal tumor; ARB = angiotensin receptor blocker; CT = computerized tomographic imaging.

Case	Age	Sex	Cancer type	Interval between dx of cancer and MN	Change in proteinuria and treatment of malignancy
1	59	F	Ovarian	Concurrent	Complete remission after TAH-BSO.
2	65	F	Breast, Lung (metastatic)	Concurrent	Patient deceased at follow-up
3	68	F	Breast, Lung, Bladder (metastatic)	Concurrent	Complete remission after anastrozole for metastatic breast cancer and TURBT for bladder carcinoma
4	64	M	Prostate	Prostate cancer diagnosed 8 months prior to membranous	Prostatectomy led to partial remission
5	77	F	SCC, head & neck	Concurrent	Radiation therapy led to partial remission
6	77	F	Thyroid	Concurrent	Thyroidectomy near time of biopsy, patient progressed to ESKD
7	74	M	Metastatic melanoma	Concurrent	Unknown, no clinical follow-up
8	68	F	Lung	Concurrent	Patient deceased before follow-up.
9	54	F	Cervical	Unknown	Complete remission with mycophenolate + prednisone therapy.
10	79	F	Colon	Colon cancer preceded MN diagnosis	Patient deceased at follow-up.
11	71	M	Prostate	Concurrent, on chemotherapy	Partial remission on bicalutamide anti-androgen therapy and ACEI.
12	61	F	Kidney	Concurrent	No remission, but no treatment of underlying tumor.
13	63	F	Thymoma	Thymoma diagnosed 3 months prior to membranous	Patient lost to follow-up.
14	71	F	Lung, metastatic	Concurrent	Patient lost to follow-up.
15	76	F	Skin	Unknown	Partial remission.
16	60	M	Bladder and nasopharyngeal	Concurrent	Unknown, patient lost to follow-up.

			carcinoma (two primaries)		
17	67	M	Prostate	Prostate carcinoma diagnosed prior to proteinuria	Radiation therapy, as well as immunosuppression prednisone and tacrolimus attempted for treatment, no remission.
18	65	F	Recurrent breast + renal mass (two primaries)	Concurrent	Mastectomy performed to treat breast cancer, partial remission.
19	67	M	Prostate, metastatic	Diagnosed 9 years prior, now with lymphadenopathy	No remission
20	85	F	Breast, metastatic	Concurrent	Complete remission
21	78	M	Prostate	Concurrent	No remission, active malignancy and nephrotic syndrome (8 g proteinuria)
22	90	M	Melanoma, GIST (2 primaries)	Concurrent with large abdominal GIST	No remission. Tumor was not resected or treated.
23	61	F	Lung cancer	Concurrent	Currently receiving chemotherapy and radiation therapy, no remission at follow-up.
24	81	M	Follicular lymphoma	Follicular lymphoma diagnosed 2 years prior to presentation, but not treated	Surveillance/supportive care for follicular lymphoma, no remission.
25	75	F	Breast carcinoma, benign brain tumor (two primaries)	Breast carcinoma recognized after membranous nephropathy	Patient underwent mastectomy and resection of intracranial mass, as well as ARB therapy, entered complete remission
26	68	M	Skin cancer	Preceded membranous 4 years, resection with orbitotomy and skin resection / grafting	Resection and concurrent immunosuppressive therapy (prednisone + azathioprine), partial remission.
27	76	M	Prostate, metastatic	Concurrent	Prostate cancer treated with bicalutamide and enzalutamide, ACEI, no remission
28	62	F	Soft tissue tumor, chest wall	Concurrent	Chest tumor excised (same month as diagnosis of membranous), ACEI, partial remission
29	65	M	Kidney cancer	Concurrent, identified on CT at time of biopsy	Complete remission after surgery
30	77	F	Skin cancer (SCC)	Prior to MN diagnosis, but several recurrences	Multiple excisions and partial remission

Supplemental Table 3. Treatment and clinical follow-up of patients with NELL1-associated membranous nephropathy. Of the 91 patients in the study, 59 had available clinical follow-up data, 8 were deceased or on dialysis at the time of follow-up, 6 were lost to follow-up, and 18 patients did not have sufficient time since diagnosis for clinical follow-up data to be available. A majority of patients were treated with angiotensin-converting enzyme inhibitor or angiotensin receptor blockade (n=32), and other treatments included calcineurin inhibitors (either cyclosporine or tacrolimus, n=8), cyclophosphamide + prednisone / Ponticelli protocol (n=3), mycophenolate mofetil (n=3), rituximab (n=1), or resection and/or chemoradiation of an underlying malignancy (n=12). The values provided include medians ± interquartile range or percentages of total. Abbreviations: Prot = proteinuria, CR = complete remission, PR = partial remission, NR = no remission.

Treatment	**n=**	**Mean follow up (months)**	**Follow up Cr (median ± IQR)**	**Prot (g) (median ± IQR)**	**Follow up Prot (g) (median ± IQR)**	**% CR**	**% PR**	**% NR**
RAS blockade only	32	8.7	1.1± 0.4	4.9 ± 4.7	1.9 ± 4.0	9/32 (28%)	12/32 (38%)	11/32 (34%)
Calcineurin inhibitor	8	8.8	1.8 ± 3.0	10 ± 9.1	6.4 ± 10.4	2/8 (25%)	1/8 (12.5%)	5/8 (62.5%)
Cyclophosphamide	3	2.7	1.4 (N/A)	9.6 (N/A)	3.9 (N/A)	1/3 (33%)	0	2/3 (67%)
Mycophenolate +/- prednisone	3	19.3	0.9 (N/A)	8 (N/A)	0.1 (N/A)	2/3 (67%)	0	1/3 (33%)
Rituximab	1	4	1 (N/A)	10 (N/A)	10 (N/A)	0	0	1 (100%)
Treatment of malignancy *	12	8.3	1.0 ± 0.9	5 ± 7.4	0.1 ± 2.1	6/12 (50%)	3/12 (25%)	3/12 (25%)
Total	59	10.4	1.1 ± 0.6	5.4 ± 6.6	1.9 ± 4.1	20/59 (34%)	16/59 (27%)	23/59 (39%)

* A total of 14 patients were being treated for an underlying malignancy. 12 of these patients were not receiving concurrent immunosuppression. There is one patient treated for malignancy in the calcineurin inhibitor group as well as the cyclophosphamide group.