Data supplement:

Supplemental Methods.

Western Blotting with TGFBR3 recombinant proteins:

TGFBR3 recombinant proteins (G&P biosciences, catalog # FCL0239; R & D, catalog # 242-R3; and Sinobiological, catalog # 10778H08B) were electrophoresed at 0.2 µg/lane and transferred to nitrocellulose membranes under reducing and non-reducing conditions. The membranes were incubated with 1:50 patient sera in 2% bovine serum albumin (BSA) for 2 h, after blocking for 1 h in 5% BSA. The membranes were washed with PBS containing 0.1% Tween, incubated with anti-human IgG-HRP at 1:1000 for 1 h RT and developed using 3,3'-diaminobenzidine (DAB). A rabbit polyclonal anti-TGFBR3 antibody (Sigma, catalog # HPA008257) was used as a positive control.

Western blotting was also performed post-deglycosylation of TGFBR3 using recombinant PNGase F enzyme according to the manufacturer's protocol (New England Biolabs catalog # P07095).

Cell-based indirect immunofluorescence assay (IFA):

Human embryonic kidney-293 (HEK-293) cells (ATCC, catalog # CRL 1573) were grown in Eagle's minimal essential medium (EMEM) containing 10% fetal bovine serum, seeded at density of 200,000 cells/mL, and incubated at 37°C 5%CO_2 to reach 70-90% confluency. Cells were transfected with TGFBR3 plasmid DNA (OriGene catalog # RC216595) or pCMV6-entry vector plasmid (OriGene pCMV6 entry catalog# PS100-001) using lipofectamine LTX and plus reagent, according to the manufacturer's protocol (Invitrogen, catalog # 15338-100).

24 h-post transfection, cells were added to poly-L-lysine coated slides and incubated overnight at 37ºC. Cells were fixed using 4% paraformaldehyde in PBS and permeabilized with 0.2% Triton-X-100. Patient sera was added at concentration of 1:10 and incubated for 2 h, washed with PBS, and incubated with fluorescein-conjugated anti-serum to human IgG (Kent laboratories, catalog # 1912MJ6) at 1:100 dilution. Rabbit polyclonal TGFBR3 antibody was used as control for the antigen expression at 1:50 dilution; with a goat anti-rabbit Rhodamine Red X secondary antibody used at 1:100 dilution (Jackson Immunoresearch, catalog # 111-295-144). Slides were examined by standard immunofluorescence microscopy.

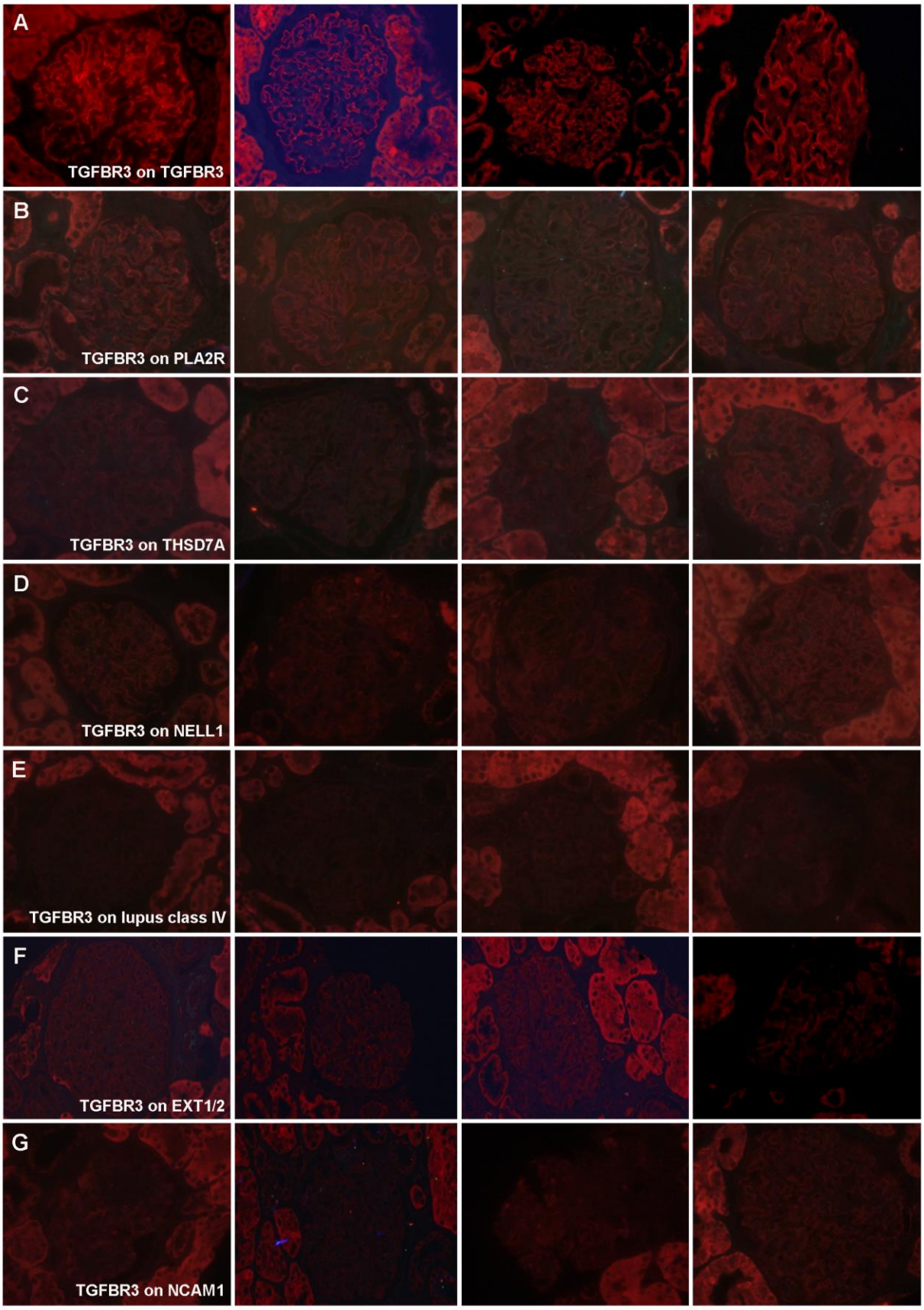
Immunoprecipitation with human glomerular extract:

Human sera from patients with TGFBR3-associated MN or PLA2R-positive MN were individually incubated (4 hr at 4°C) with Triton X-100 extracts of human glomerular proteins (HGE), and through addition of immobilized protein G-agarose beads (Santa Cruz Biotechnology). The immunoprecipitates were electrophoresed after the addition of beta-mercaptoethanol (reducing conditions) and then assayed for the presence of TGFBR3 or PLA2R with the corresponding commercial antibodies (Sigma/Prestige).

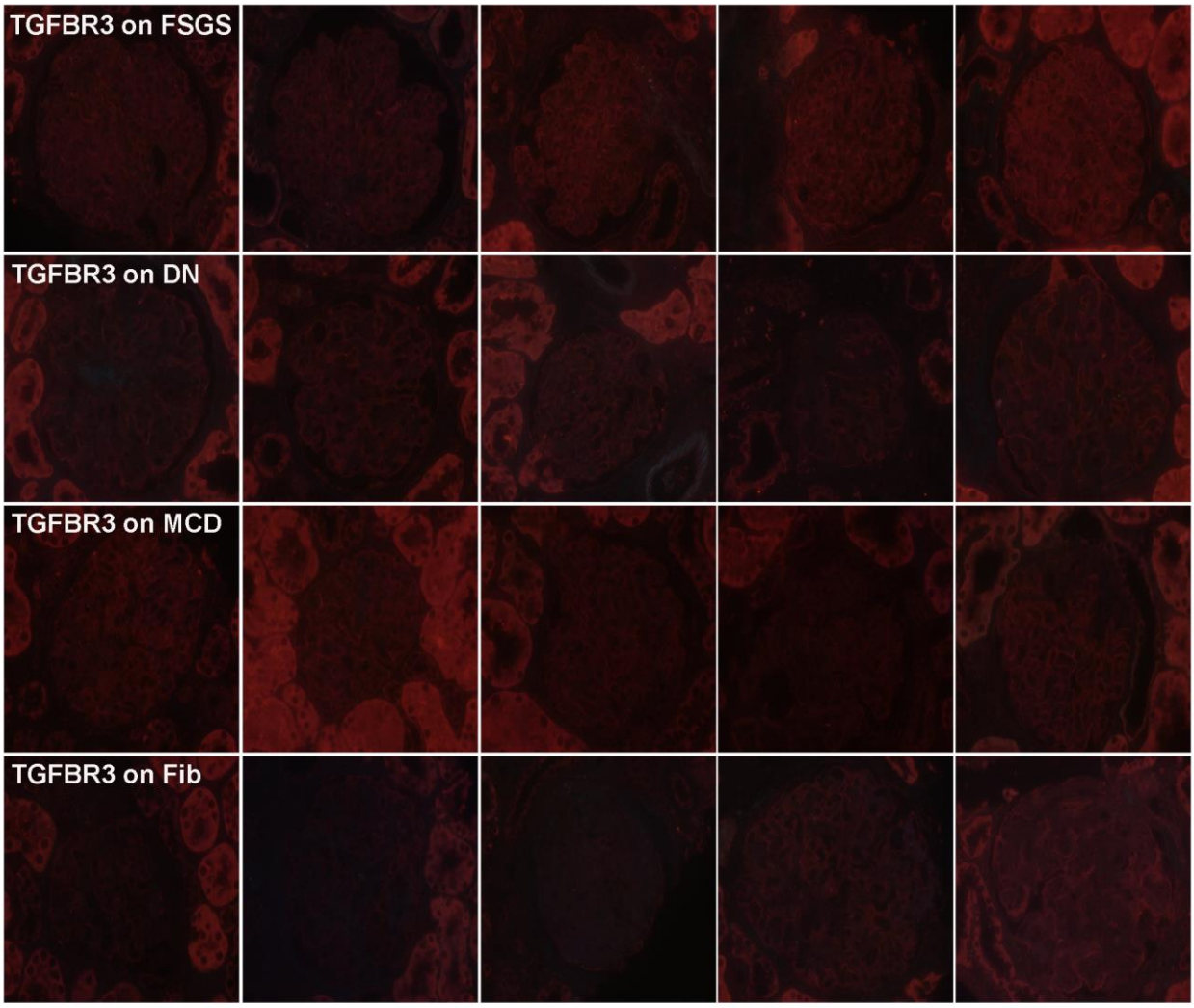
Supplemental Table 1. IgG subclass staining in 13 cases of TGFBR3-associated membranous lupus nephritis.

Case	IgG1	IgG2	IgG3	IgG4	Predominant subtype
1	3	1	0	3	1 and 4
2	2	3	3	0	2 and 3
3	3	3	3	0	1, 2, and 3
4	2	2	0	trace	1 and 2
5	0	2	0	1	2
6	2	3	3	0	2 and 3
7	3	1	3	0	1 and 3
8	2	2	1	0	1 and 2
9	0	1	0	3	4
10	3	2	1	0	1
11	3	3	3	trace	1, 2, and 3
12	3	3	0	0	1 and 2
13	2	2	trace	trace	1 and 2

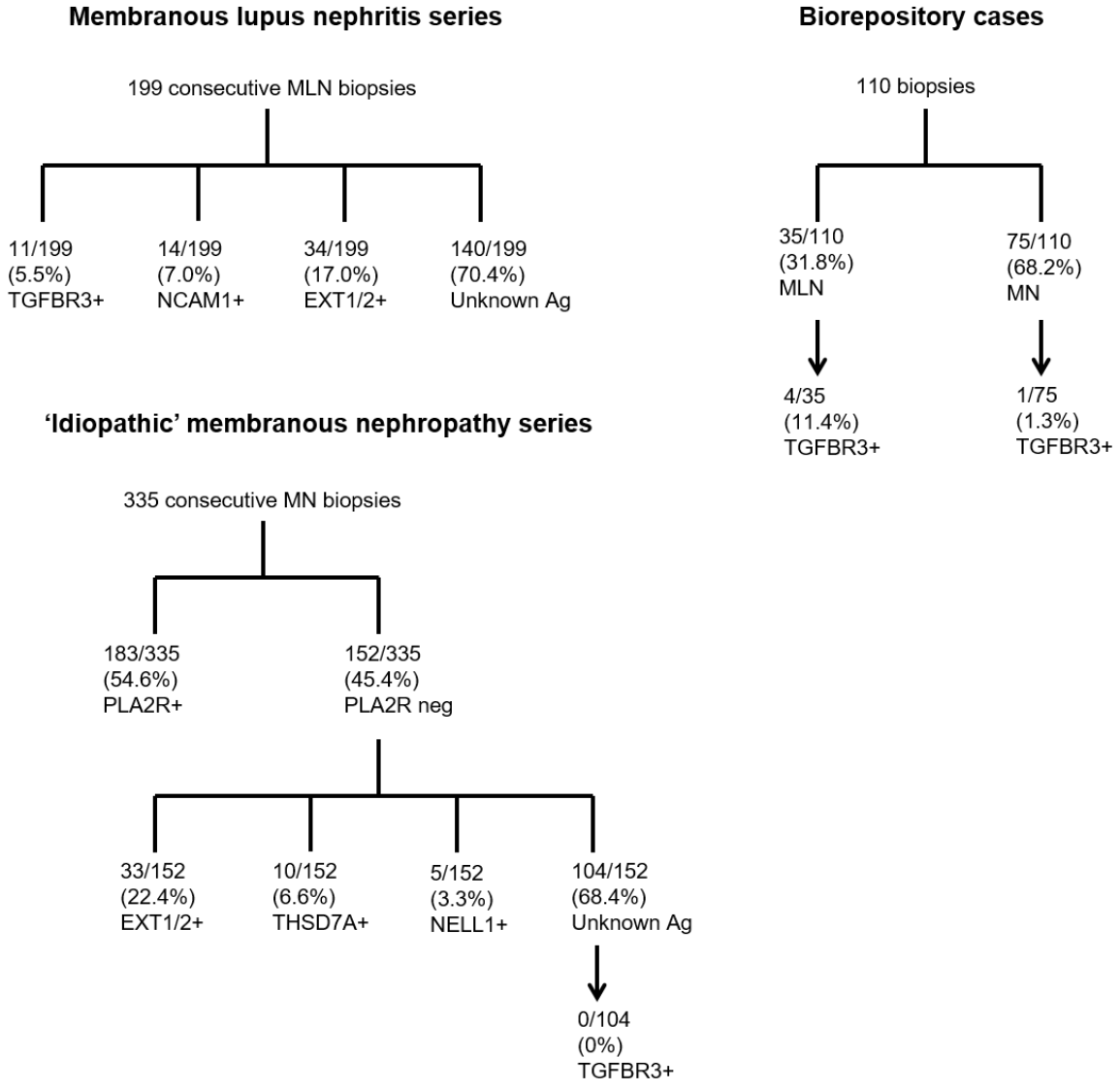
Supplemental Figure 1. Immunofluorescence staining on TGFBR3 compared to negative control cases. All images are at 400x magnification. A) TGFBR3 staining on TGFBR3-associated MN; B) TGFBR3 staining on PLA2R-positive MN; C) TGFBR3 staining on THSD7A-positive MN; D) TGFBR3 staining on NELL1-associated MN; E) TGFBR3 staining on diffuse lupus nephritis cases (ISN/RPS class IV); F) TGFBR3 staining on EXT-associated MLN; G) TGFBR3 staining on NCAM1-associated MLN. Glomeruli from four cases are shown, representative from 19 PLA2R-positive MN cases, 20 THSD7A-positive MN cases, 19 NELL1-positive MN cases, 40 diffuse lupus nephritis cases, 33 EXT-associated MLN cases, and 12 NCAM1-associated MLN cases.


A
TGFBR3 on TGFBR3
B
TGFBR3 on PLA2R
C
TGFBR3 on THSD7A
D
TGFBR3 on NELL1
E
TGFBR3 on lupus class IV
F
TGFBR3 on EXT1/2
G
TGFBR3 on NCAM1

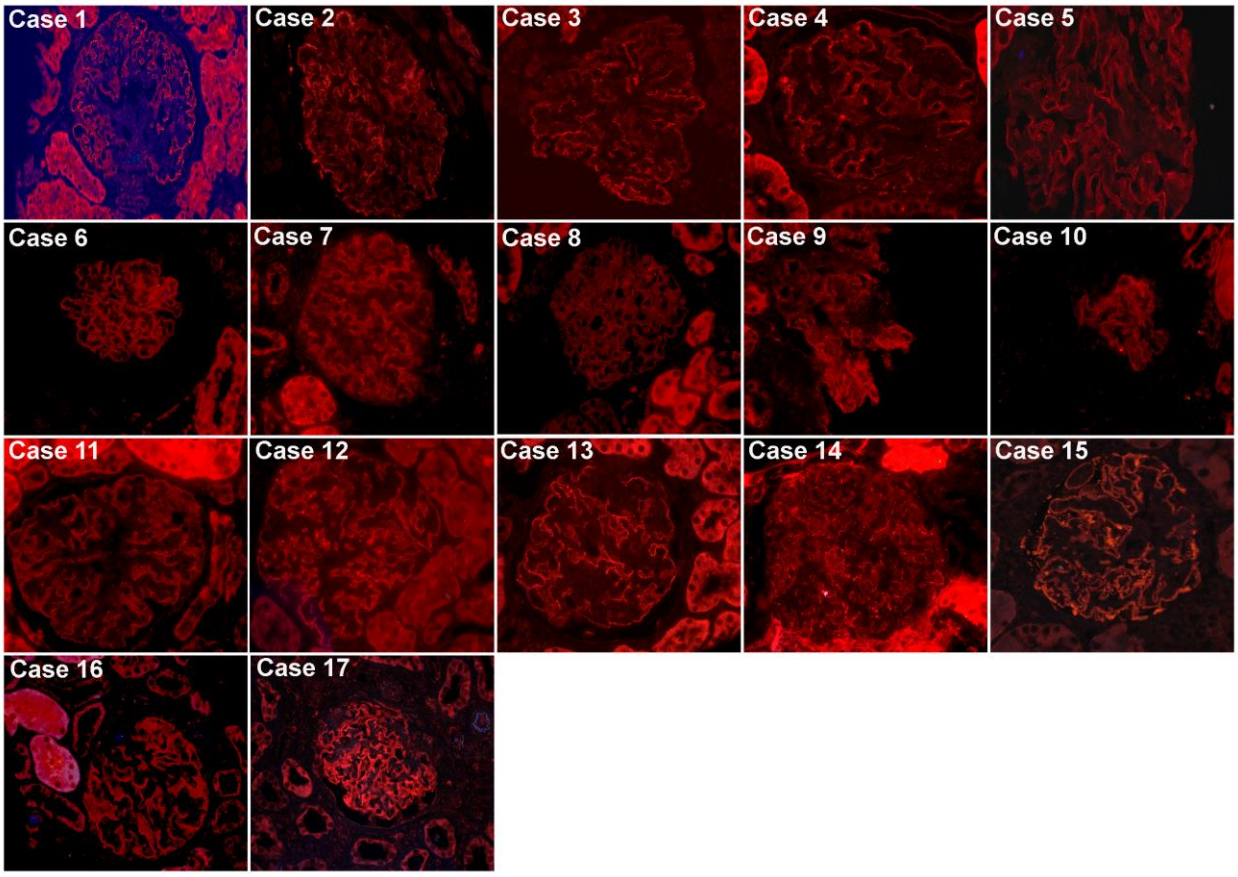
Supplemental Figure 2. TGFBR3 immunofluorescence staining on non-membranous proteinuric kidney diseases. TGFBR3 staining of a representative glomerulus of five cases of primary focal segmental glomerulosclerosis (FSGS), diabetic nephropathy (DN), minimal change disease (MCD), and fibrillary glomerulopathy (Fib) are shown. All images are at 400x magnification.



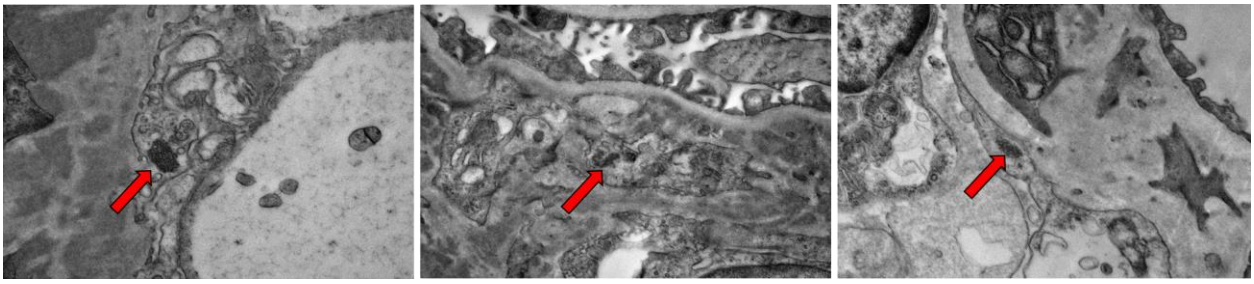
Supplemental Figure 3. Diagram of study cohorts. TGFBR3-associated MN cases were identified through immunofluorescence staining of three cohorts. These included a six-month consecutive membranous lupus nephritis series (n=199), a nine-month consecutive series of 'idiopathic' PLA2R negative / THSD7A negative / NELL1 negative MN cases (n=152), as well as 110 biorepository cases to identify cases with matched serum samples. Screening of these cohorts accounts for 15 of 17 TGFBR3-associated MN cases. The remaining two cases are derived from the mass spectrometry discovery cohort.



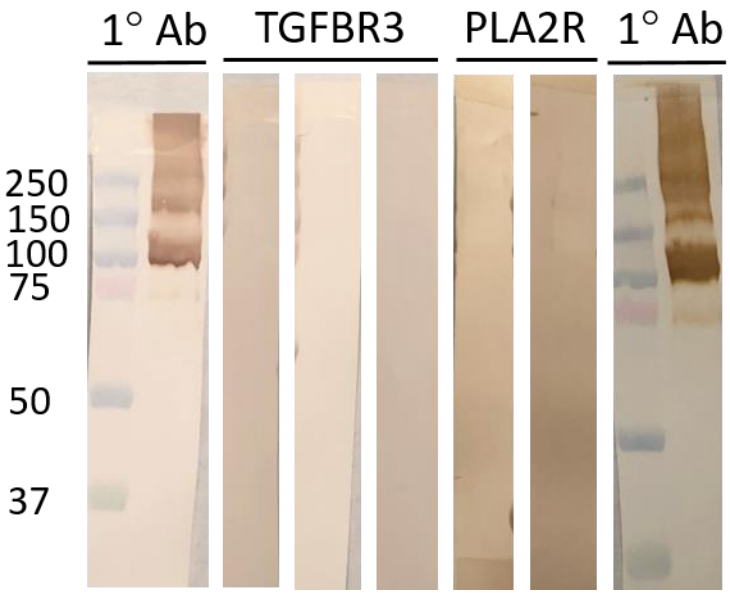
Supplemental Figure 4. TGFBR3 immunofluorescence staining of a representative glomerulus from each of the 17 cases included in the series. Images are at 400x magnification.



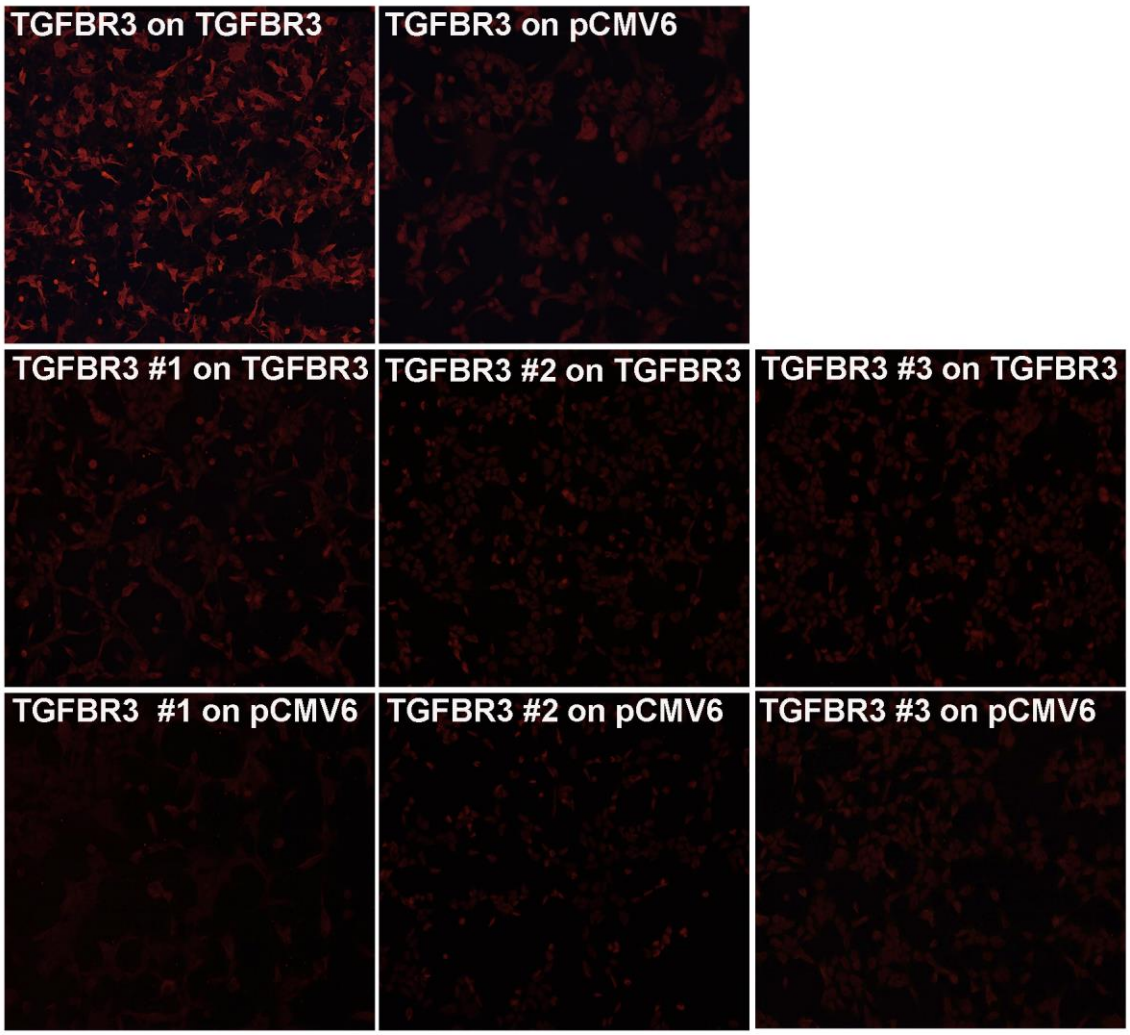
Supplemental Figure 5. Subendothelial tubuloreticular inclusions were present within 3 of 17 TGFBR3-associated MN cases. Images are at 4,000 x direct magnification.



Supplemental Figure 6. Serum from TGFBR3-associated MN patients does not react with TGFBR3 recombinant protein. Western blot strips under non-reducing conditions reacting serum from patients with TGFBR3-associated MN or PLA2R-positive MN controls against anti-TGFBR3 recombinant protein are shown. Identical results (without seroreactivity) were seen under reducing conditions or after de-glycosylation of the TGFBR3 protein (data not shown). 1° Ab shows reactivity with a rabbit polyclonal anti-TGFBR3 antibody.



Supplemental Figure 7. A cell-based indirect immunofluorescence assay through transient transfection of HEK-293 cells failed to detect anti-TGFBR3 antibodies within serum from patients with positive TGFBR3 staining within glomeruli. Serum samples from 3 patients are shown. An anti-TGFBR3 primary antibody was used as a positive control to confirm TGFBR3 expression post-transfection. Images are shown at 200x magnification.



Supplemental Figure 8. Sera from TGFBR3-associated MN patients does not immunoprecipitate with human glomerular extract. A) TGFBR3 is expressed within human glomerular extract (HGE) under reducing and non-reducing conditions. This occurred with and without the addition of Triton-X-100 (TX100) to enrich for lipid-raft containing proteins within HGE. B) Sera from TGFBR3-associated MN patients failed to immunoprecipitate with HGE. Sera from PLA2R-positive MN patients shown appropriate reactivity to HGE. There is no reactivity with normal human serum.