Supplemental Results

Detailed description of the IFT and Western blot results

In the prospective Hamburg cohort, 250 of the 345 patients resulted positive for PLA_2R1-Ab and negative for THSD7A-Ab by the IFT (and/or ELISA in the case of PLA_2R1-Ab). The remaining 95 PLA_2R1-Ab negative sera were analyzed for PLA_2R1-Ab and THSD7A-Ab by both Western blot and IFT. In nine (9.5%) of the 95 cases PLA_2R1-Ab could be detected by Western blot, bringing the number of PLA_2R1-Ab positive patients in the cohort to 259. None of these patients was THSD7A-Ab positive. Eight of the 86 PLA_2R1-Ab negative patients tested positive for THSD7A-Ab by both the IFT and Western blot. The remaining 78 patients were negative for PLA_2R1-Ab and THSD7A-Ab by both Western blot and IFT (and also ELISA in the case of PLA_2R1-Ab). Sera from 31 patients, who tested THSD7A-Ab negative, but PLA_2R1-Ab positive by IFT, were analyzed by Western blot. The Western blot analyses (Figure 1B) showed identical results to the IFT (Figure 1E and 1F), as all patients were THSD7A-Ab negative, while the PLA_2R1-Ab positivity found by IFT was confirmed by Western blot.

In the retrospective Hamburg cohort, 57 of the 689 patients resulted positive for PLA_2R1-Ab and negative for THSD7A-Ab by the IFT. All 632 PLA_2R1-Ab negative sera were analyzed by Western blot for the presence of PLA_2R1-Ab or THSD7A-Ab. In 62 cases PLA_2R1-Ab, but not THSD7A-Ab could be detected, bringing the number of PLA_2R1-Ab positive patients in the cohort to 119. When analyzed by Western blot, 20 THSD7A-Ab positive patients could be detected. In 18 of these patients, THSD7A-Ab were detectable by IFT. The remaining 550 patients were negative for PLA_2R1-Ab and THSD7A-Ab by both Western blot and IFT.

In the Boston cohort, 133 of the 242 patients resulted positive for PLA_2R1-Ab by IFT. When analyzed by Western blot, 20 more patients were identified as PLA_2R1-Ab positive, bringing the whole number of PLA_2R1-Ab positive patients to 153. All these patients were negative for THSD7A-Ab by both IFT and Western blot. Of the 89 PLA_2R1-Ab negative patients eleven THSD7A-Ab positive patients were identified by Western blot. When

analyzed by IFT, THSD7A-Ab could be detected in ten of these patients. The remaining 78 patients were negative for PLA_2R1-Ab and THSD7A-Ab by both Western blot and IFT.

Glomerular PLA_2R and THSD7A immunohistochemical findings

Immunohistochemical analyses of PLA_2R1 expression in renal tissue were performed in 176 cases of the Hamburg prospective cohort; 136 PLA_2R1-Ab positive cases (in 131 of these patients PLA_2R1-Ab were detectable by both IFT and Western blot, in five cases PLA_2R1-Ab were detectable only by Western blot, but not by IFT), six THSD7A-Ab positive cases and 34 cases negative for both PLA_2R1-Ab and THSD7A-Ab. An enhanced glomerular PLA_2R1-staining was found in all 136 PLA_2R1-Ab positive cases, but in none of the six THSD7A-Ab positive cases, or the 34 cases who were negative for both PLA_2R1-Ab and THSD7A-Ab. Sixty-five renal biopsies, which were already analyzed for PLA_2R1-expression, were then stained for THSD7A. A normal, and non-enhanced THSD7A-staining was found in all 48 biopsies with enhanced PLA_2R1 staining (PLA_2R1-Ab positive, THSD7A-Ab negative cases). At the same time, all six biopsies from THSD7A-Ab positive patients showed an enhanced THSD7A staining. The remaining 11 biopsies were from patients negative for both PLA_2R1-Ab and THSD7A-Ab in the blood. In one of these biopsies, an enhanced THSD7A staining was identified (Patient 7 in Table 3).

Clinical follow-up of THSD7A-Ab positive patients

For eleven THSD7A-Ab positive patients in the Hamburg cohorts, follow-up data over a follow-up time of at least 12 months were available. The median follow-up time of these patients was 36.0 months (interquartile range 19.5 – 55.5 months). One patient progressed to end-stage renal disease nine months after study inclusion, despite treatment with calcineurin inhibitors and glucocorticoids. Four patients had a complete remission of proteinuria, THSD7A-Ab became negative in all of them. All these four patients received an immunosuppressive treatment (one patient calcineurin inhibitors; two patients cyclophosphamide and glucocorticoids – one of them later switched to calcineurin inhibitors

and glucocorticoids; one patient – Patient 1, table 3 – was treated with chemotherapy because of a gall bladder carcinoma). Five patients had a partial remission of proteinuria, four of them received immunosuppression (two patients calcineurin inhibitors – one of them later switched to cyclophosphamide and glucocorticoids; one patient cyclophosphamide and glucocorticoids; one patient MMF, rituximab and glucocorticoids). THSD7A-Ab had become negative in one of these patients, and remained positive in the other four patients. One patient had no remission of proteinuria and the THSD7A-Ab persisted, despite immunosuppression with calcineurin inhibitors, MMF, rituximab and glucocorticoids.

THSD7A mRNA levels in a lymph node infiltrated by metastases of the endometrium carcinoma

We assessed the THSD7A mRNA levels, related to the expression of 18sRNA as reference gene. The ΔCT between THSD7A and 18sRNA was 14.30 (THSD7A 34.30 and 18sRNA 20.00) in the lymph node infiltrated by metastases of the endometrium carcinoma and 10.68 (THSD7A 36.96 and 18sRNA 26.28) in the control lymph node. The difference in ΔCT between the lymph node infiltrated by metastases of the endometrium carcinoma versus the control lymph node was therefore 3.62, which translates into a 12.3-fold higher expression of THSD7A mRNA in the lymph node infiltrated by metastases of the endometrium carcinoma. The control lymph node showed no pathologies and came from a patient with a prostate carcinoma.

Supplemental Tables

	Complete cohort	THSD7A-associated MN	THSD7A-Ab negative MN		P-value: THSD7A-associated MN vs.	
			PLA_2R1-Ab positive MN	PLA_2R1-Ab negative MN	PLA_2R1-Ab pos. MN	PLA_2R1-Ab neg. MN
Number of Patients (%)	689 (100%)	20 (2.9%)	119 (17.3%)	550 (79.8%)		
Age - years (median, IQR)	60, 46-70	67, 55.25-76	57, 46.5-65	60, 46-71	0.01	0.08
Male Gender (%)	464 (67.3%)	11 (55%)	82 (69%)	371 (67%)	0.22	0.24
Proteinuria - g/24h (median, IQR)	4.3, 1.5-8.0 (n=308)	7.2, 4.3-9.9 (n=16)	3.9, 1.9-8.1 (n=79)	4.3, 1.4-7.4 (n=213)	0.09	0.05
Serum creatinine - mg/dl (median, IQR)	1.3, 0.9-2.0 (n=319)	1.3, 1.0-2.0 (n=16)	1.2, 0.9-1.7 (n=75)	1.4, 1.0-2.0 (n=228)	0.61	0.77
Patients with malignancy (%)	13 (1.9%)	4 (20.0%)	2 (1.7%)	7 (1.3%)	<0.01	<0.01
Number of patients on immunosuppression (%)	260, 58% (n=452)	7, 44% (n=16)	40, 44% (n=91)	213, 62% (n=345)	0.99	0.15

Supplemental Table 1: Clinical baseline characteristics of patients from the retrospective Hamburg cohort

Data on proteinuria, serum creatinine and immunosuppressive treatment at the time of serum collection were available for 308, 319 and 452 patients, respectively.

	Complete cohort	THSD7A-associated MN	THSD7A-Ab negative MN		P-value: THSD7A-associated MN vs.	
			PLA_2R1-Ab positive MN	PLA_2R1-Ab negative MN	PLA_2R1-Ab pos. MN	PLA_2R1-Ab neg. MN
Number of Patients (%)	242 (100%)	11 (4.5%)	153 (63.2%)	78 (32.2%)		
Age - years (median, IQR)	51, 38-62	51, 33-66	51, 38-62.5	50.5, 38-61.25	0.98	0.92
Male Gender (%)	154 (64%)	3 (27%)	109 (71%)	42 (54%)	<0.01	0.10
Proteinuria - g/24h (median, IQR)	6.0, 3.5-9.3	5.9, 3.0-7.7	7.4, 4.0-10.5	4.0, 2,4-7.0	0.26	0.42
Serum creatinine - mg/dl (median, IQR)	1.1, 0.8-1.5 (n=232)	0.8, 0.8-1.5 (n=9)	1.1, 0.9-1.5 (n=149)	1.0, 0.8-1.5 (n=74)	0.24	0.69
Patients with malignancy (%)	18 (7.4%)	1 (9.1%)	8 (5.2%)	9 (11.5%)	0.59	0.81
Number of patients on immunosuppression (%)	71, 36% (n=196)	6, 55% (n=11)	45, 35% (n=127)	20, 34% (n=58)	0.21	0.21

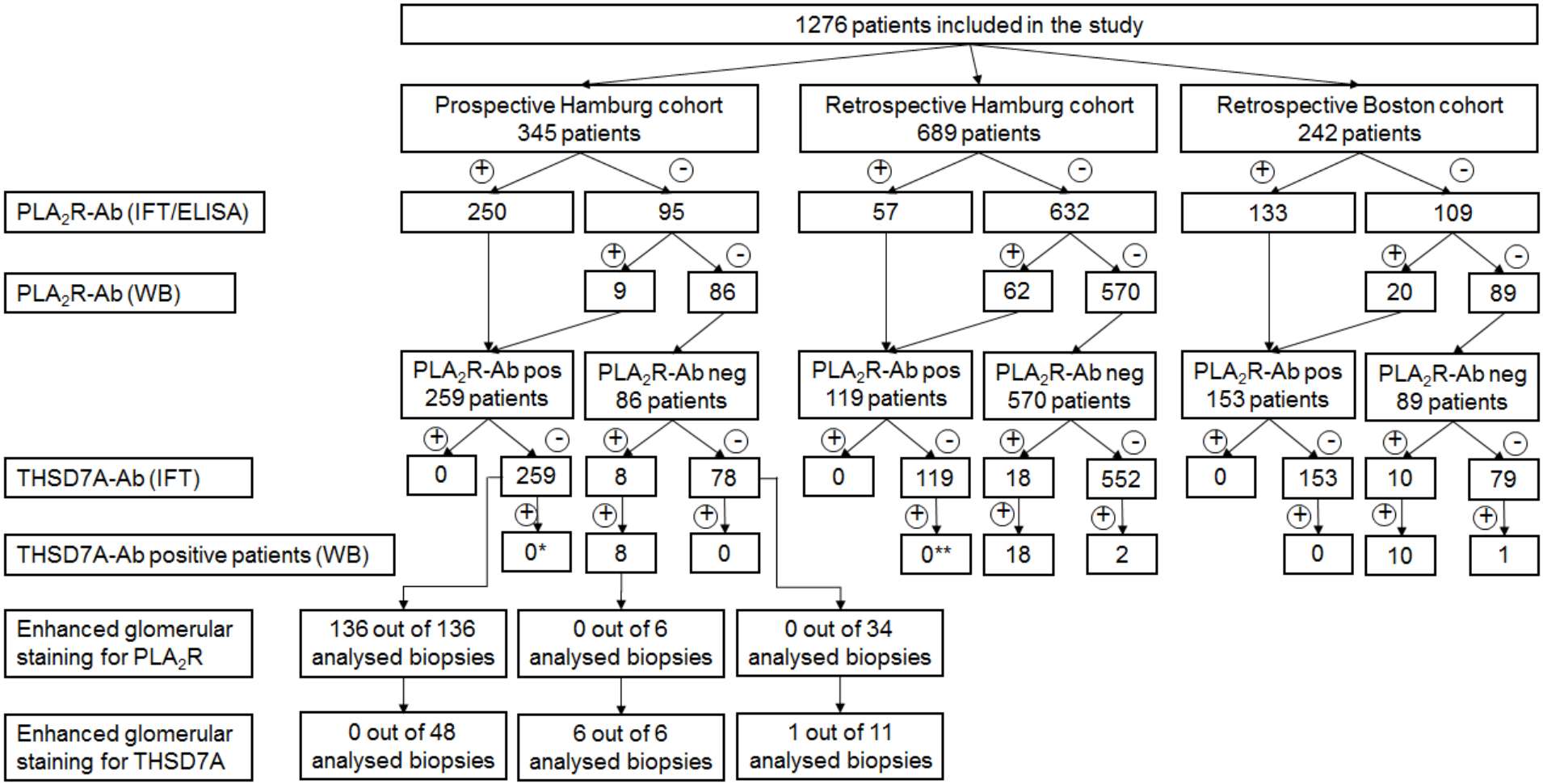
Supplemental Table 2: Clinical baseline characteristics of patients from the Boston cohort

Data on proteinuria, serum creatinine and immunosuppressive treatment at the time of serum collection were available for 242 (all), 232 and 196 patients, respectively.

Number of Patients	112
Age - years (median, IQR)	46.5, 35.75-61
Male Gender (%)	73 (65%)
Proteinuria - g/24h (median, IQR)	3.0, 0.9-6.0 (n=97)
Serum creatinine - mg/dl (median, IQR)	1.4, 1.1-2.1 (n=100)
Number – percentage of patients on immunosuppression	49, 51% (n=96)

Supplemental Table 3: Clinical baseline characteristics of control patients

In the control group 112 patients were included: 24 patients with minimal change disease, 24 patients with IgA-nephropathy, 23 patients with focal segmental glomerulosclerosis, 14 patients with lupus nephritis, 13 patients with membranoproliferative glomerulonephritis, six patients with ANCA-vasculitis, six patients with amyloidosis, and two patients with cryoglobulinemia. Data on proteinuria, serum creatinine and immunosuppressive treatment at the time of serum collection were available for 97, 100 and 96 patients, respectively. All these patients were negative for both PLA_2R1-Ab and THSD7A-Ab.



Supplemental Figure 1: Detailed analysis of the correlation of THSD7A-Ab and PLA_2R-Ab measurement results by IFT and Western blot and immunohistochemical analyses of renal biopsies for THSD7A and PLA_2R.

***** In the prospective Hamburg cohort, 40 of the 259 PLA_2R1-Ab positive sera were tested for THSD7A-Ab by Western blot.

** In the retrospective Hamburg cohort, 62 of the 119 PLA_2R1-Ab positive sera were tested for THSD7A-Ab by Western blot.