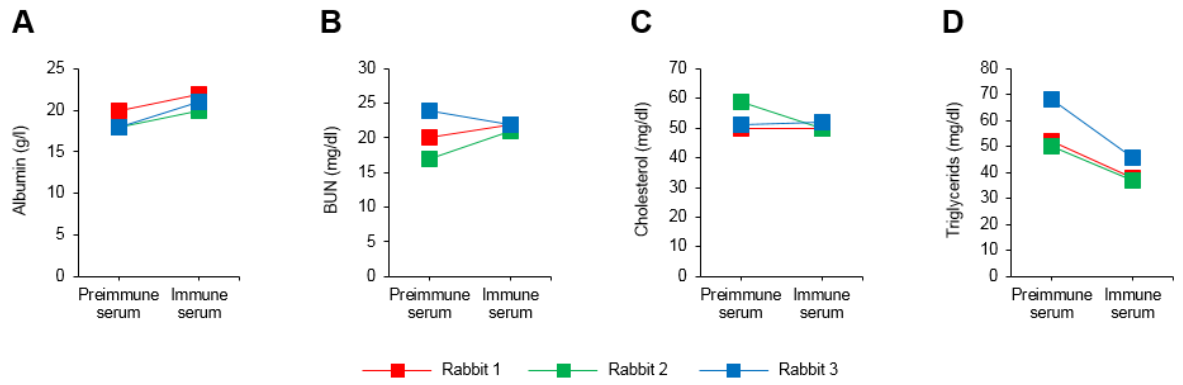
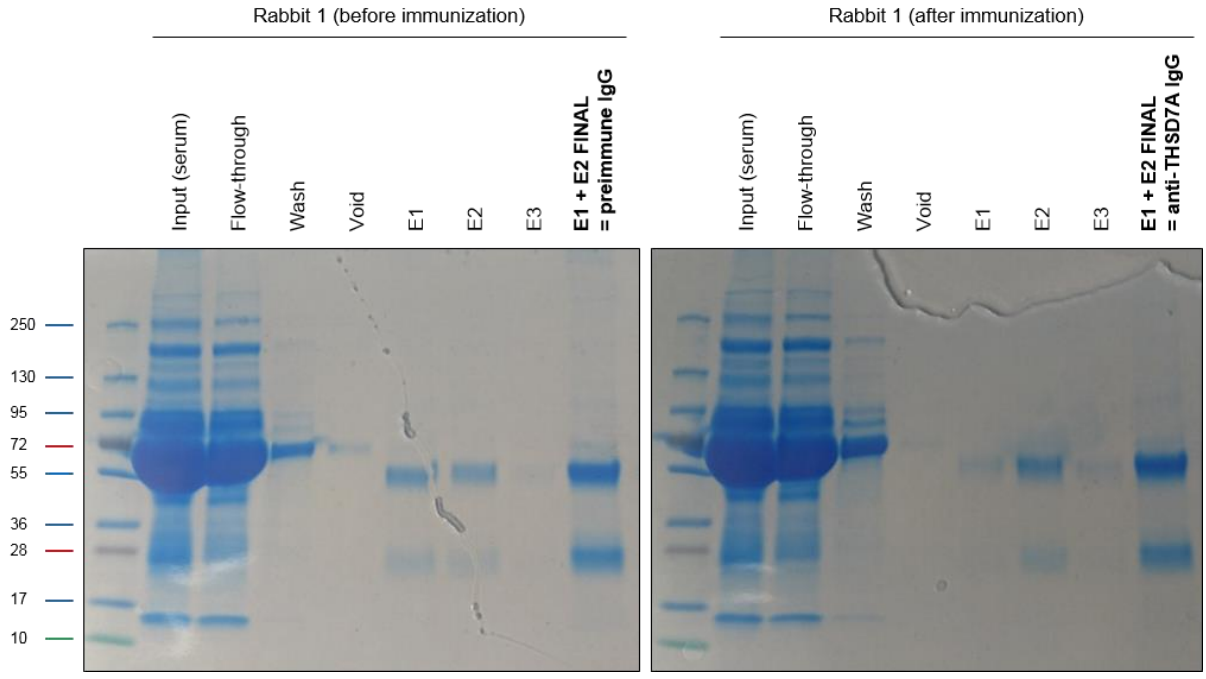
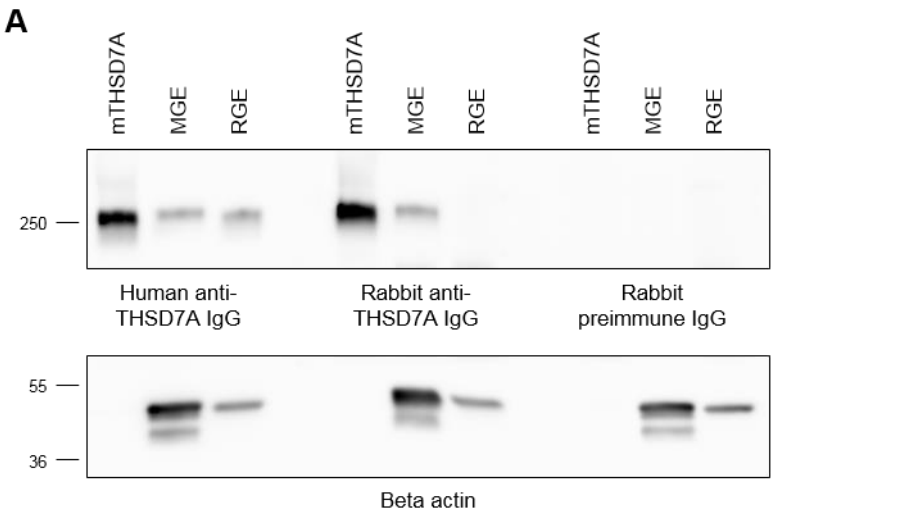
SUPPLEMENTAL APPENDIX

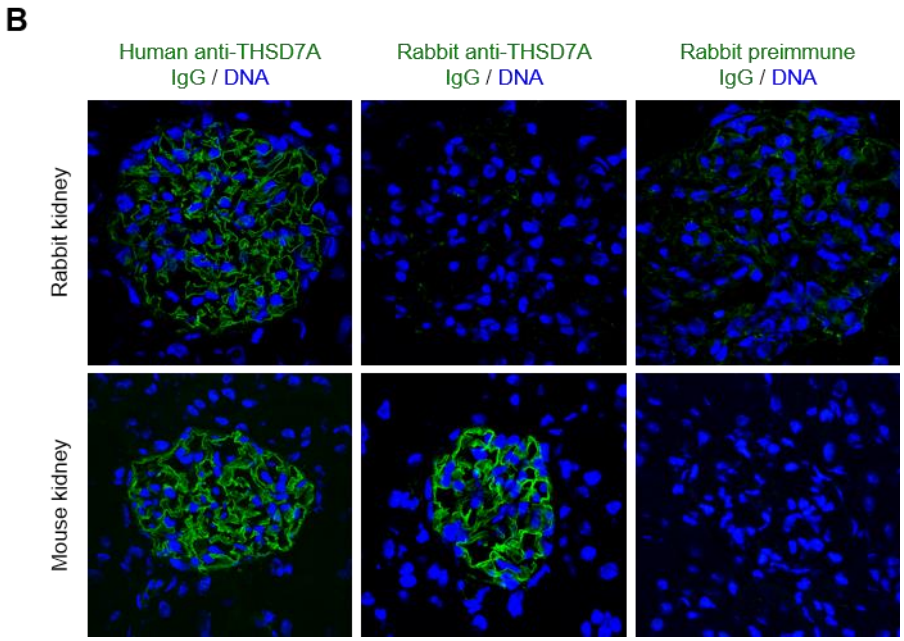


Supplemental Figure 1. Blood parameters in immunized rabbits. (**A-D**) Measurement of serum albumin (**A**), blood urea nitrogen (BUN, **B**), cholesterol (**C**), and triglycerids (**D**) before and after immunization with mouse and human THSD7A.

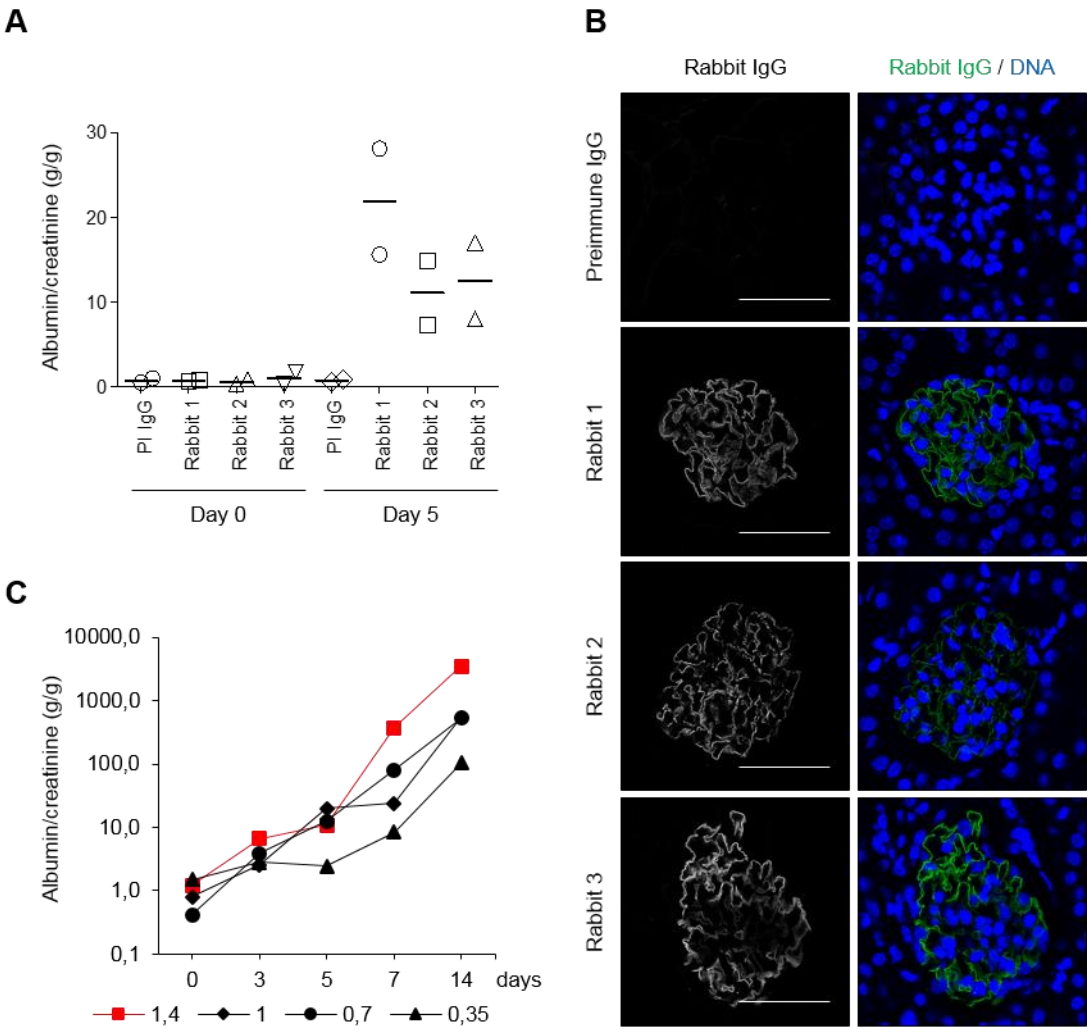


Supplemental Figure 2. Purification of rabbit IgG before and after THSD7A cDNA immunization. IgG purification from serum of one of the three rabbits (rabbit 1) before and after immunization is shown exemplarily. Whole serum (input) from rabbits before and after immunization with an expression construct coding for human and mouse THSD7A was given over columns containing Protein G resin. Columns were washed (wash) and then eluted three times with 100 mM glycine pH 3 (E1-3). E1 and E2 were buffered to PBS and concentrated, resulting in the final sample (E1+E2 FINAL) with a concentration of around 6-7 mg/ml.

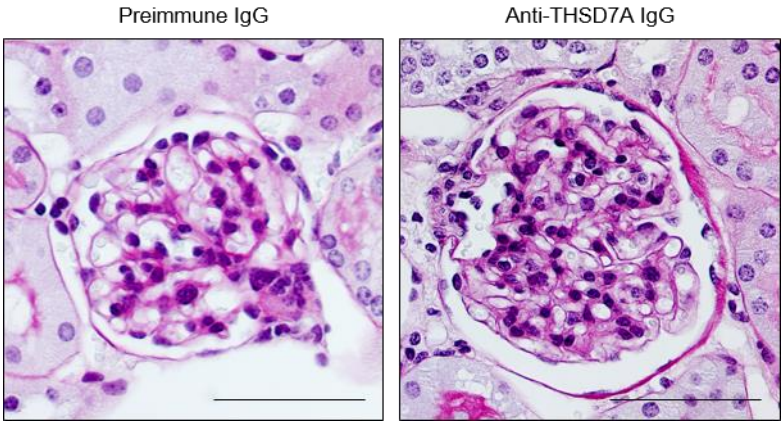




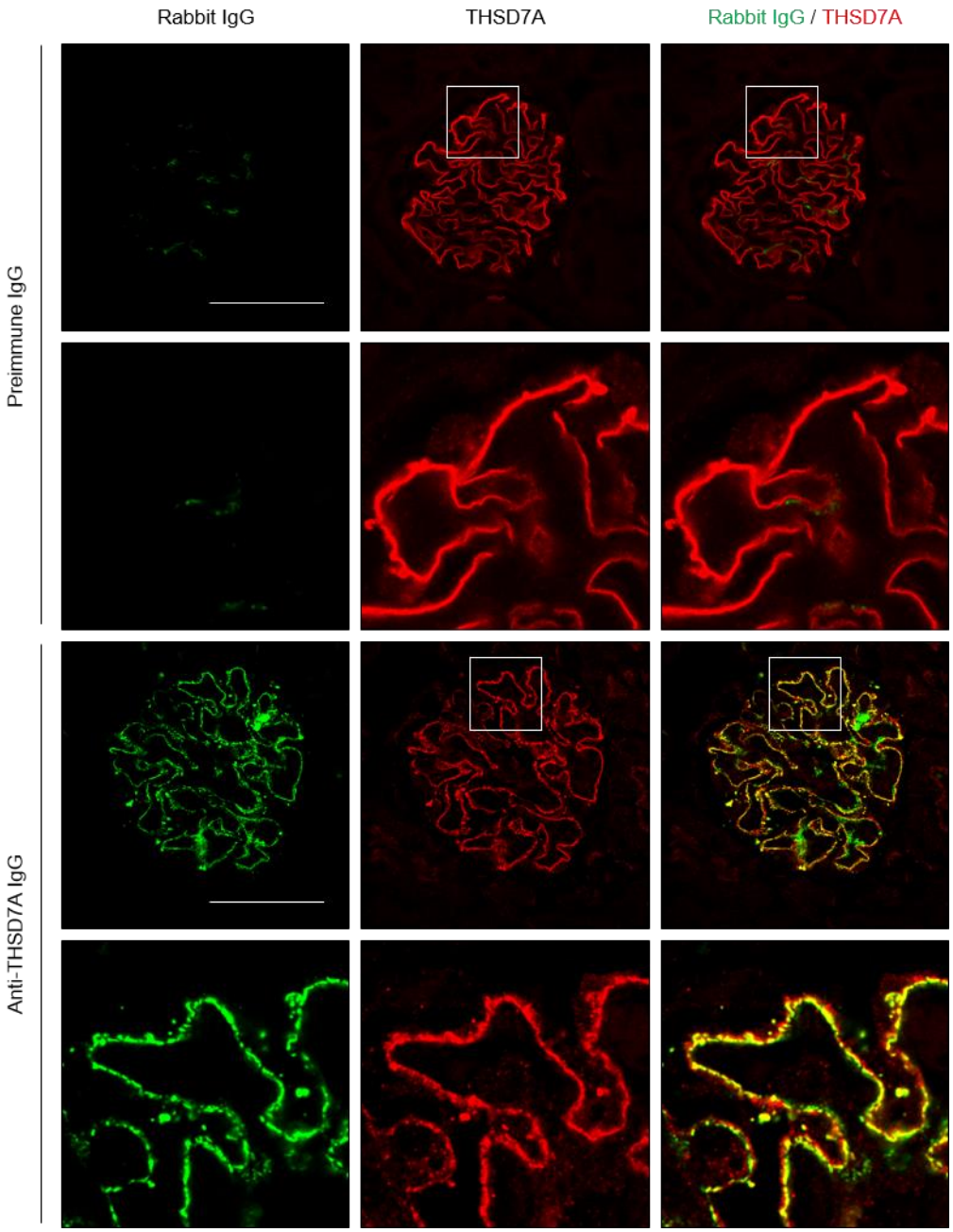
Supplemental Figure 3. Self-reactivity of rabbit anti-THSD7A IgG. (**A**) Reactivity of purified human anti-THSD7A IgG, rabbit anti-THSD7A IgG and rabbit preimmune IgG with recombinant mouse THSD7A (mTHSD7A), mouse glomerular extracts (MGE) and rabbit glomerular extracts (RGE). (**B**) Immunofluorescence staining of frozen kidney sections of wild-type rabbits using human anti-THSD7A IgG, rabbit anti-THSD7A IgG and rabbit preimmune IgG as primary antibodies.



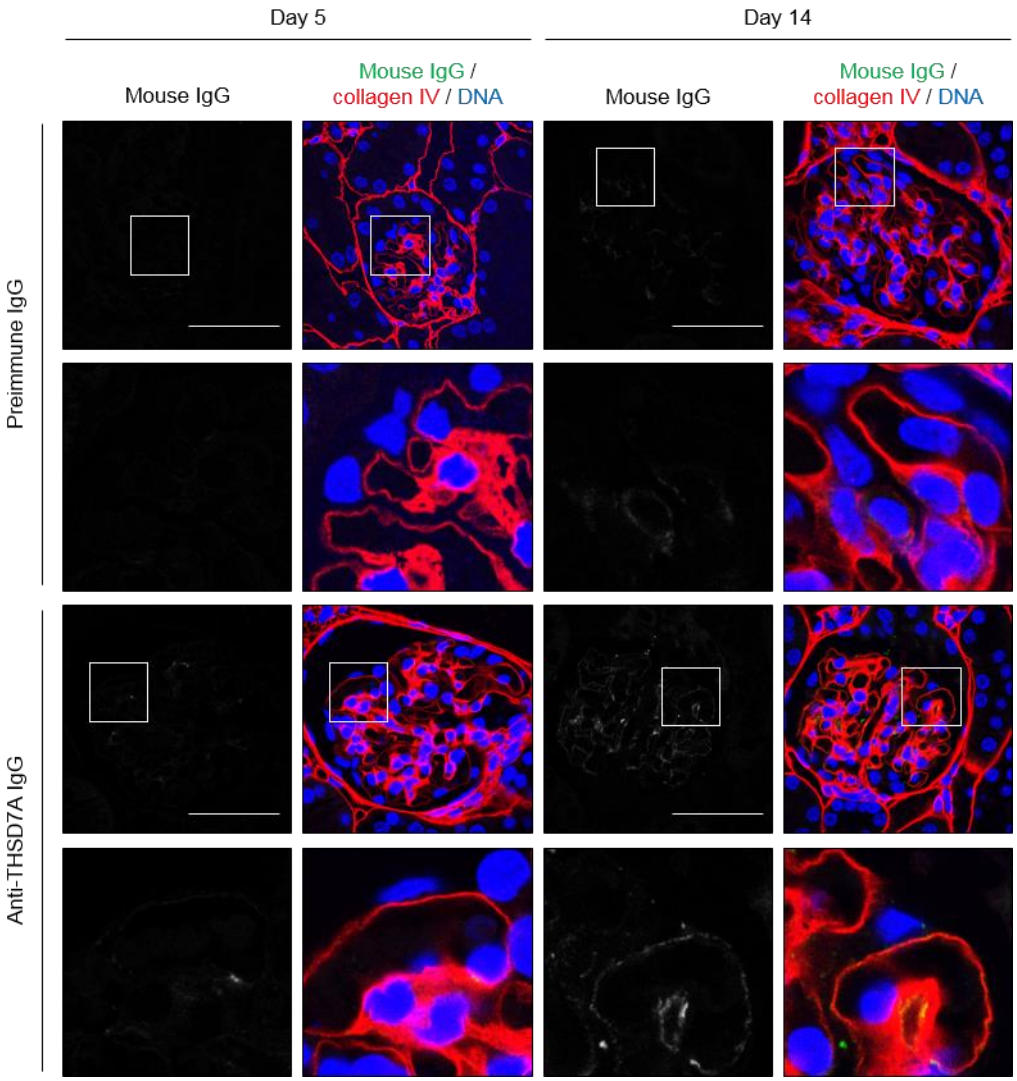
Supplemental Figure 4. Development of membranous nephropathy in mice after exposure to anti-THSD7A IgG. (**A**) Proteinuria as measured by albumin-to-creatinine ratio in mice 5 days after injection of 1,400 µg of preimmune (PI) or anti-THSD7A IgG. Purified anti-THSD7A IgG was available from three different immunized rabbits (rabbit 1-3). (**B**) Immunofluorescence staining for rabbit IgG and DNA in mice 5 days after exposure to preimmune or anti-THSD7A IgG from the three different rabbits. Scale bars indicate 50 µm. (**C**) Proteinuria as measured by albumin-to-creatinine ratio in mice injected with 1.4, 1, 0.7, and 0.35 mg of preimmune or anti-THSD7A IgG. Albumin-to-creatinine-ratios after 14 days were 3450, 540, 430, and 100 g/g, respectively.



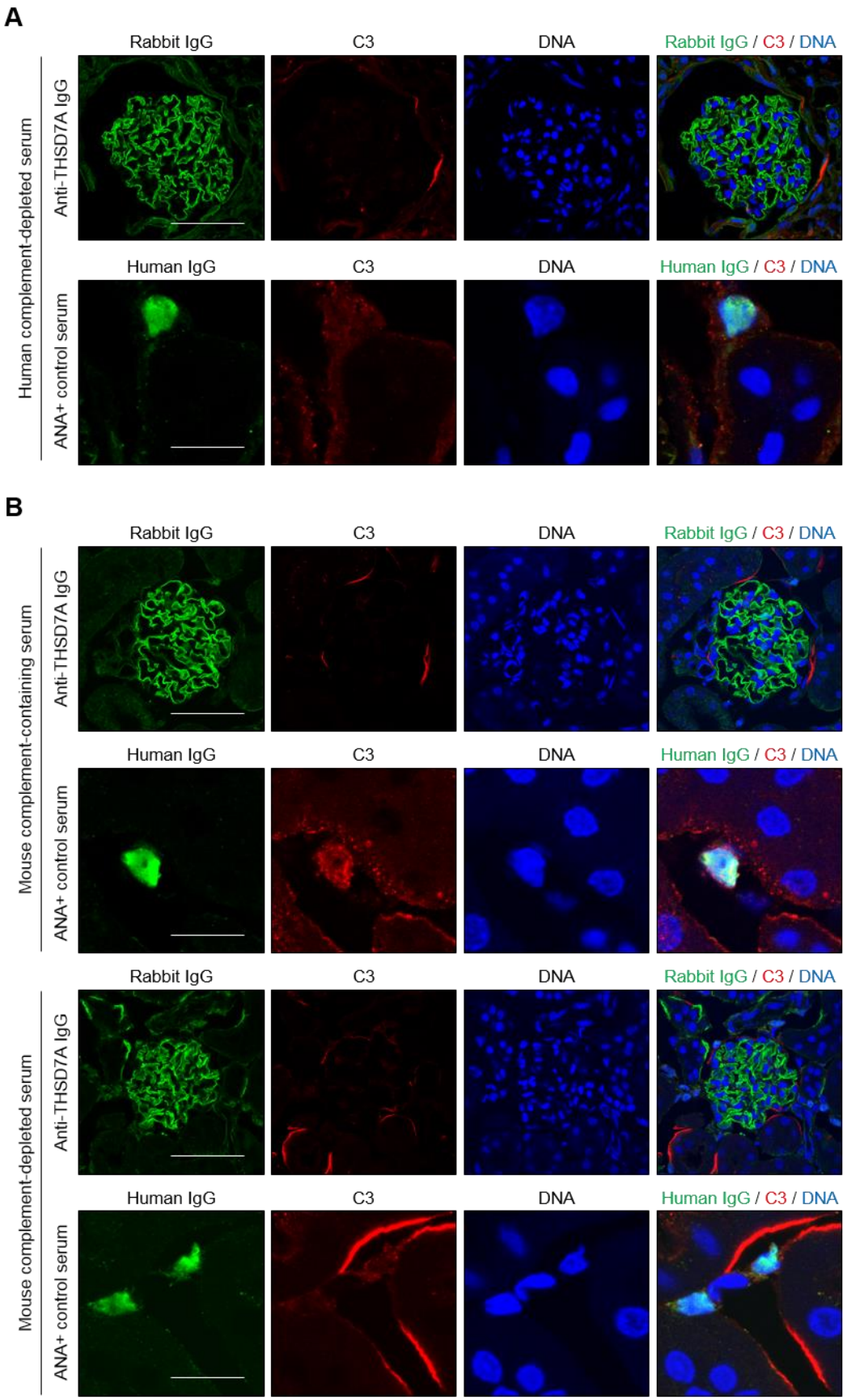
Supplemental Figure 5. PAS staining 14 days after injection of preimmune or anti-THSD7A IgG. Scale bars indicate 50 µm.



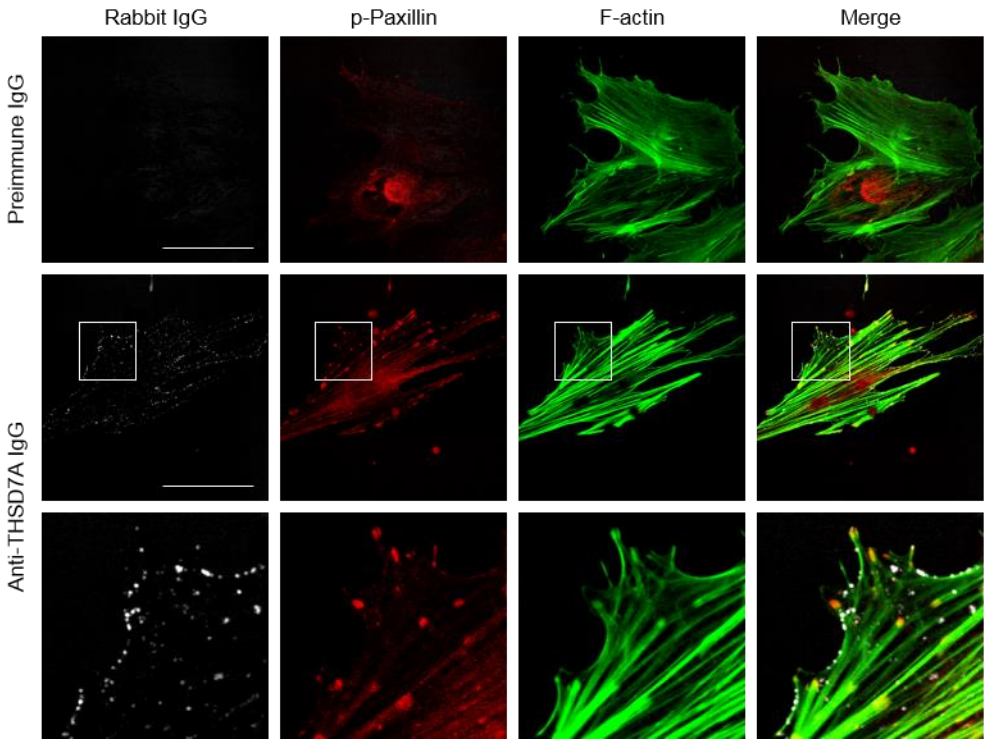
Supplemental Figure 6. Co-localization of rabbit IgG with THSD7A in immunofluorescence analysis. Fourteen days after injection of rabbit IgG, no rabbit IgG was seen in mice that received preimmune IgG. In contrast, mice that were exposed to anti-THSD7A IgG showed granular staining for rabbit IgG that strongly co-localized with granular THSD7A, suggesting presence of THSD7A-anti-THSD7A immune complexes at the glomerular filtration barrier. Scale bars indicate 50 µm. Lower panels represent 4x enlargements of the boxed areas in the upper panels.



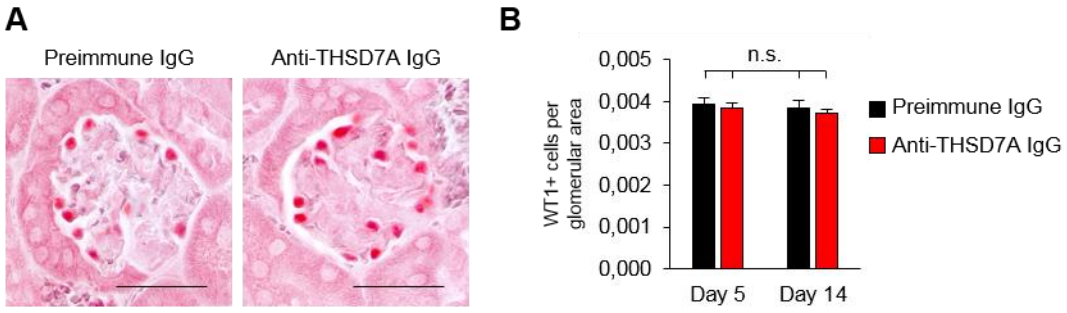
Supplemental Figure 7. Development and glomerular binding of mouse IgG in mice after exposure to anti-THSD7A IgG. Panels depict immunofluorescence staining for mouse IgG, collagen type IV, and DNA in mice 5 and 14 days after exposure to 1,400 µg of preimmune or anti-THSD7A IgG. Faint binding of mouse IgG along the glomerular filtration barrier was exclusively seen 14 days after injection of anti-THSD7A IgG. Scale bars indicate 50 µm. Lower panels represent 4x enlargements of the boxed areas in the upper panels.


A
Rabbit IgG
C3
DNA
Rabbit IgG / C3 / DNA
Human complement-depleted serum
Anti-THSD7A IgG
Human IgG
C3
DNA
Human IgG / C3 / DNA
ANA+ control serum
B
Rabbit IgG
C3
DNA
Rabbit IgG / C3 / DNA
Mouse complement-containing serum
Anti-THSD7A IgG
Human IgG
C3
DNA
Human IgG / C3 / DNA
ANA+ control serum
Rabbit IgG
C3
DNA
Rabbit IgG / C3 / DNA
Mouse complement-depleted serum
Anti-THSD7A IgG
Human IgG
C3
DNA
Human IgG / C3 / DNA
ANA+ control serum

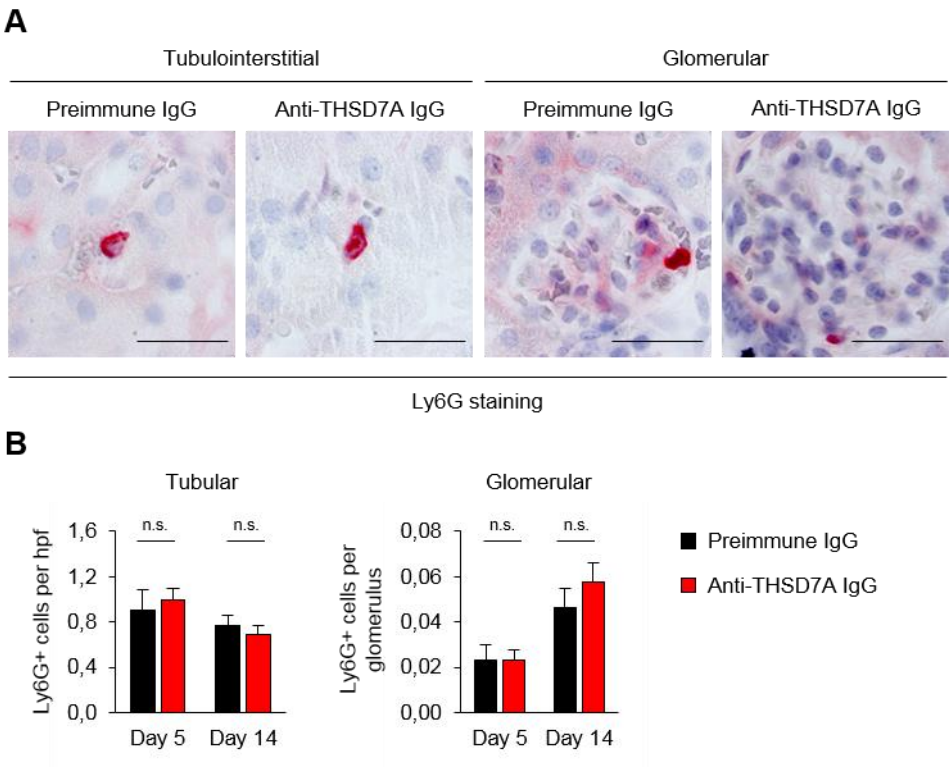
Supplemental Figure 8. In vitro complement fixation assay. (**A**) Immunofluorescence staining for rabbit IgG (upper panels), human IgG (lower panels), complement component C3 and DNA in kidney sections from naïve mice that were incubated with rabbit anti-THSD7A IgG or anti-nuclear antibody (ANA)–containing human serum diluted in complement activation buffer supplemented with human complement-depleted serum. Linear binding of rabbit IgG but no positivity for complement component C3 was observed in cryo sections of a naïve mouse kidney exposed to anti-THSD7A IgG. In line, nuclear positivity for human IgG without detection of complement component C3 was seen in paraffin sections exposed to serum with a high ANA titer. Scale bars indicate 50 µm (upper panels) and 200 µm (lower panels). (**B**) The experiment was repeated with complement-containing or complement-depleted whole mouse serum with identical results. Graph depicts immunofluorescence staining for rabbit IgG or human IgG, complement component C3 and DNA. Rabbit anti-THSD7A IgG and ANAs bound to their targets and were detected in the expected area, but complement activation was exclusively seen where ANAs bound in the presence of complement-containing serum. No complement activation was detected with anti-THSD7A IgG in the presence of complement-containing or –depleted serum and no complement activation was seen with ANA-containing serum in the presence of complement-depleted serum. Scale bars indicate 50 µm (upper panels) and 200 µm (lower panels).



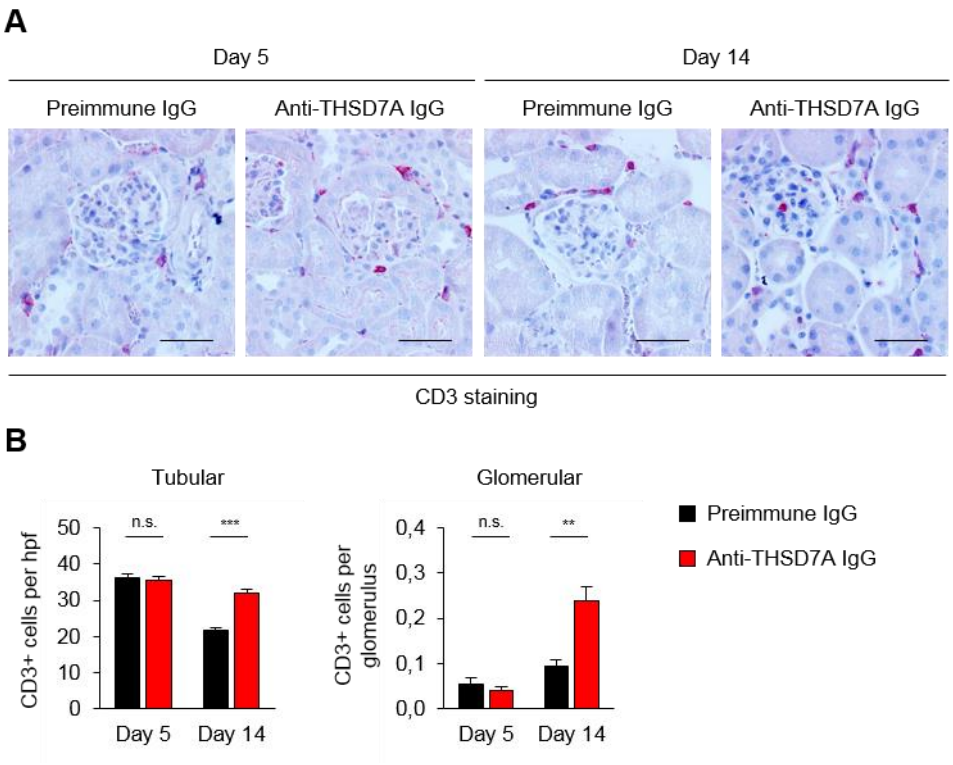
Supplemental Figure 9. Signaling at focal adhesions after exposure to anti-THSD7A IgG. Immunofluorescence staining for phosphorylated paxillin (p-paxillin) in primary cultured glomerular epithelial cells (GECs) after treatment with preimmune or anti-THSD7A IgG. Scale bars indicate 50 µm. Lower panels represent enlargements of the boxed areas in the middle panels.



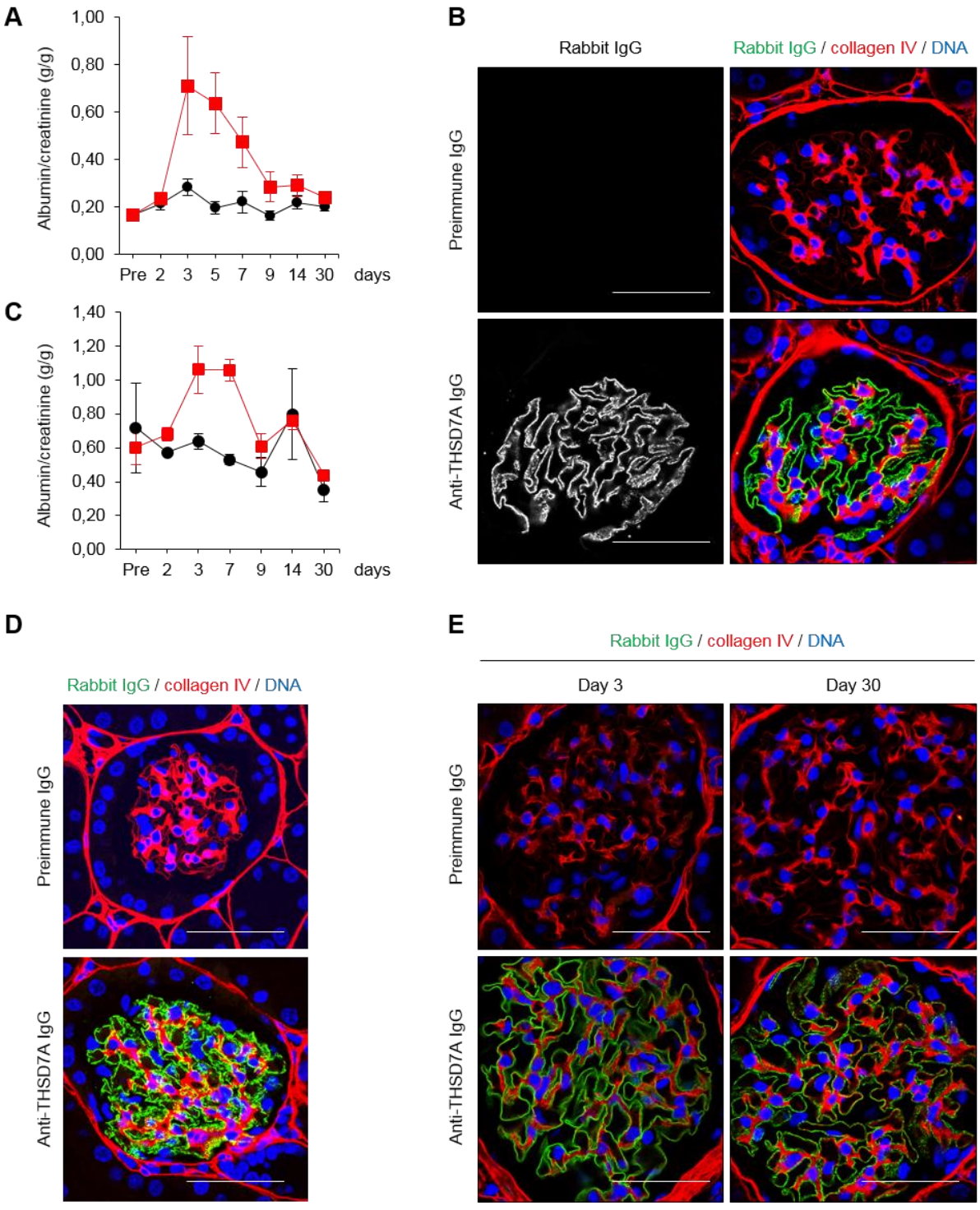
Supplemental Figure 10. (**A**) Immunohistochemical staining for WT1 in mice 14 days after injection of preimmune or anti-THSD7A IgG. Scale bars indicate 50 µm. (**B**) Quantification of WT1-positive cells 5 and 14 days after antibody injection. Data show mean ± s.e.m; n.s., not significant.



Supplemental Figure 11. Immunohistochemical evaluation of inflammatory cell infiltration after induction of membranous nephropathy with anti-THSD7A IgG. (**A**) Immunohistochemical staining for Ly6G as a marker of granulocytes in the tubulointerstitial and glomerular compartment 14 days after injection of preimmune or anti-THSD7A IgG. Scale bars indicate 50 µm. (**B**) Quantification of Ly6G-positive granulocytes in the glomerular and tubular compartments of experimental mouse kidneys 5 and 14 days after exposure to preimmune or anti-THSD7A IgG. Data depict mean ± s.e.m. $^{**}P < 0.01$; $^{***}P < 0.001$; n.s., not significant; one-way ANOVA with Bonferroni's post-test for multiple comparisons.



Supplemental Figure 12. (**A**) Immunohistochemical staining for CD3 as a marker of T-cells 5 and 14 days after injection of preimmune or anti-THSD7A IgG. Scale bars indicate 50 µm. (**B**) Quantification of CD3-positive T cells in the glomerular and tubular compartments of experimental mouse kidneys 5 and 14 days after exposure to preimmune or anti-THSD7A IgG. Data depict mean ± s.e.m. ***P* < 0.01; ****P* < 0.001; n.s., not significant; one-way ANOVA with Bonferroni's post-test for multiple comparisons.



Supplemental Figure 13. Injection of anti-THSD7A IgG in C57BL/6 mice, DBA/J1 mice and Sprague Dawley rats. (**A**) Proteinuria as measured by albumin-to-creatinine ratio in male C57BL/6 mice after injection of 1.4 mg of preimmune or anti-THSD7A IgG. Data depict mean ± s.e.m. (**B**) Immunofluorescence staining for rabbit IgG, collagen type IV and DNA in C57BL/6 mice 30 days after exposure to preimmune or anti-THSD7A IgG. Scale bars indicate 50 µm. (**C**) Proteinuria as measured by albumin-to-creatinine ratio in male DBA/J1 mice after injection of 1.4 mg of preimmune or anti-THSD7A IgG. Data depict mean ± s.e.m. (**D**) Immunofluorescence staining for rabbit IgG, collagen type IV and DNA in DBA/J1 mice 30 days after exposure to preimmune or anti-THSD7A IgG. Scale bars indicate 50 µm. (**E**) Immunofluorescence staining for rabbit IgG, collagen type IV and DNA in Sprague Dawley rats 3 and 30 days after exposure to preimmune or anti-THSD7A IgG. Scale bars indicate 50 µm.