Supplementary Information:

Materials and Methods:

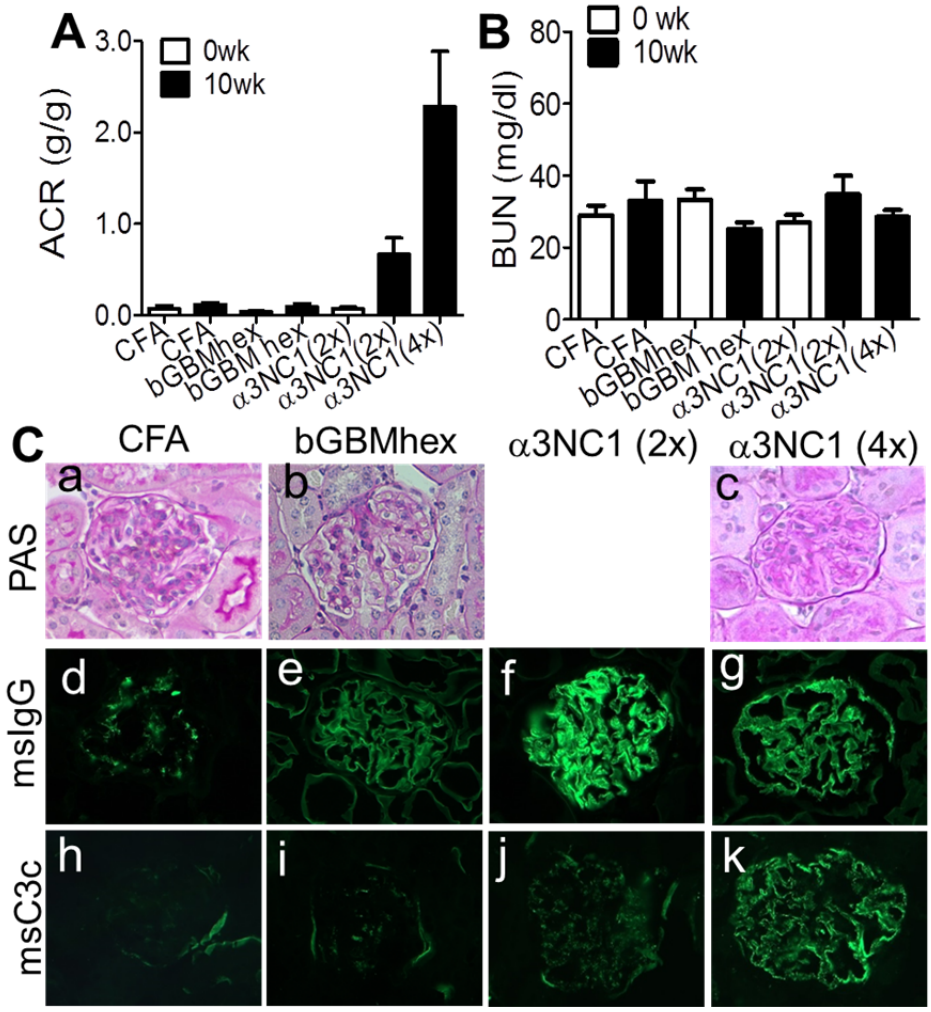
Susceptibility of C57Bl/6 mice to antibody-mediated glomerular injury induced by immunization with NC1 domains of GBM collagen IV and selection of a mouse model. Commercially available FcRn-/- mice were on the C57Bl/6 (B6) genetic background, which is among the most resistant to immune mediated nephritis.[1] Although B6 mice lacking the inhibitory IgG receptor FcγRIIB are highly susceptible to crescentic glomerulonephritis, for instance after passive immunization with human IgG anti-GBM alloantibodies from patients with Alport post-transplant nephritis, wild type B6 mice are resistant.[2] To avoid the need for time-consuming back-crosses, we sought a mouse model in which antibody-mediated glomerular injury could be induced in wild type B6 mice. Previously, antibody-mediated glomerulonephritis has been reported in B6 mice immunized four times with recombinant NC1 monomers of human α3(IV) collagen (α3NC1),[3] as well as mice immunized twice with α3-α5NC1 dimers from bovine GBM.[4] In preliminary experiments, we compared the susceptibility of B6 mice to develop kidney injury after active immunization with recombinant human α3NC1 monomers and total NC1 hexamers from bovine GBM (containing α345NC1 hexamers).

C57Bl/6J mice were purchased from The Jackson Laboratory and maintained in specific-pathogen free facilities with access to water and food. For active immunization experiments, 4-5 mice in each group were immunized at 8-10 weeks of age with α3NC1 monomers (25 μg) and NC1 hexamers from bovine GBM (100 μg), prepared as previously described.[5] The antigens were emulsified in complete Freund's adjuvant (Sigma, St. Louis, MO) and injected subcutaneously at two sites. Mice received one (for both antigens) or three (for α3NC1) booster immunizations with antigen in incomplete Freund's adjuvant. Measurements of blood urea nitrogen and urinary albumin-creatinine ratio, and analysis of kidney histopathology by light microscopy and immunofluorescence microscopy were performed as described in the main text. All mouse studies were performed in accordance with the principles for humane treatment of lab animals and were approved by the IACUCs at Vanderbilt University.

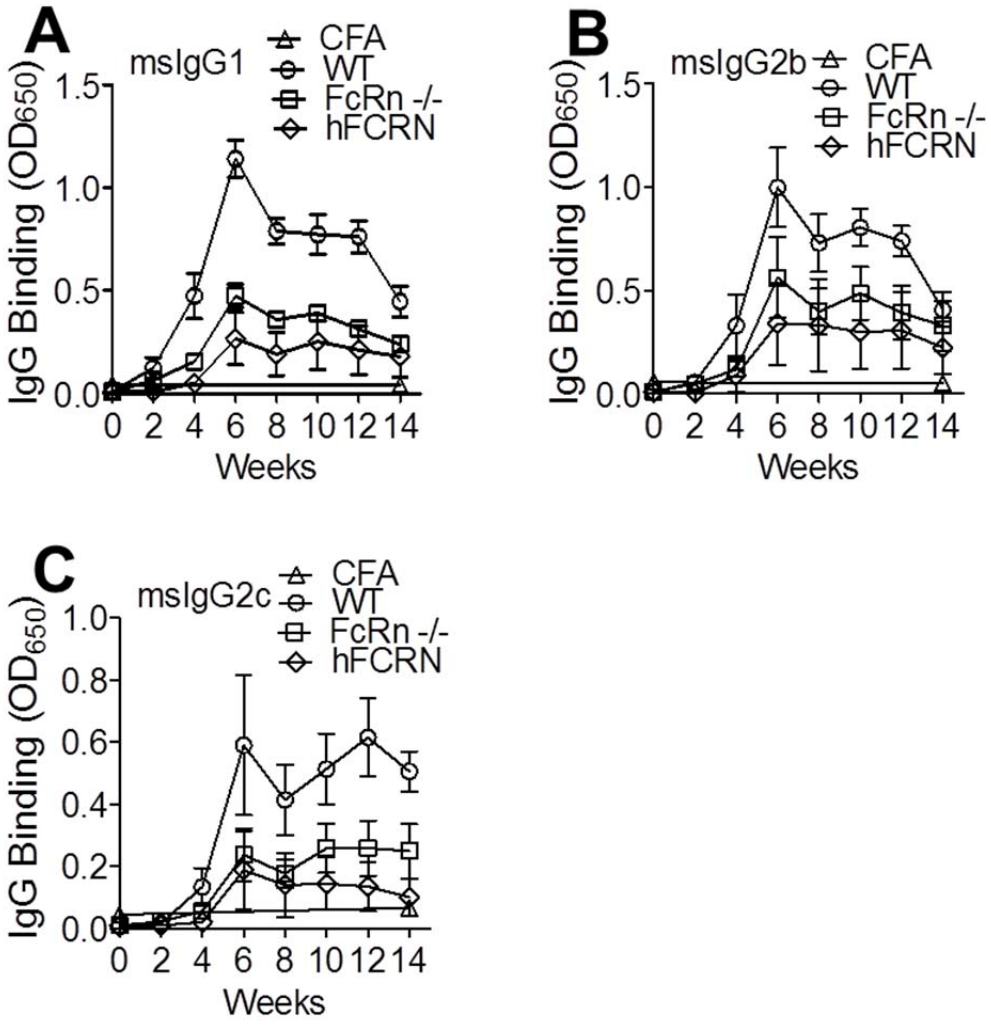
References:

1. Xie C, Sharma R, Wang H, Zhou XJ, Mohan C: Strain distribution pattern of susceptibility to immune-mediated nephritis. *J Immunol* 172**:** 5047-5055, 2004.
2. Luo W, Wang XP, Kashtan CE, Borza DB: Alport alloantibodies but not Goodpasture autoantibodies induce murine glomerulonephritis: protection by quinary crosslinks locking cryptic alpha3(IV) collagen autoepitopes in vivo. *J Immunol* 185**:** 3520-3528, 2010.
3. Hopfer H, Maron R, Butzmann U, Helmchen U, Weiner HL, Kalluri R: The importance of cell-mediated immunity in the course and severity of autoimmune anti-glomerular basement membrane disease in mice. *Faseb J* 17**:** 860-868, 2003.
4. Kitching AR, Turner AL, Semple T, Li M, Edgtton KL, Wilson GR, Timoshanko JR, Hudson BG, Holdsworth SR: Experimental autoimmune anti-glomerular basement membrane glomerulonephritis: a protective role for IFN-gamma. *J Am Soc Nephrol* 15**:** 1764-1774, 2004.
5. Boutaud A, Borza DB, Bondar O, Gunwar S, Netzer KO, Singh N, Ninomiya Y, Sado Y, Noelken ME, Hudson BG: Type IV collagen of the glomerular basement membrane: Evidence that the chain specificity of network assembly is encoded by the noncollagenous NC1 domains. *J Biol Chem* 275**:** 30716-30724, 2000.

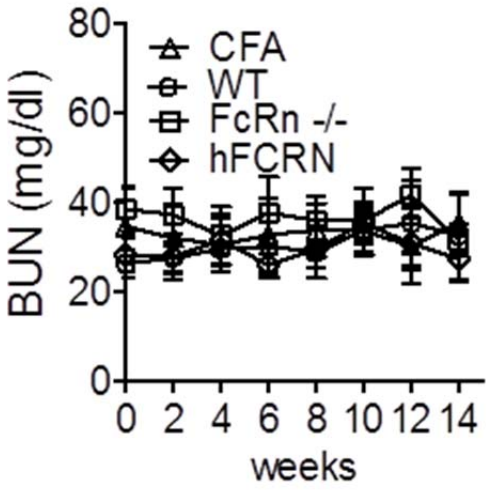
Supplementary Figures:



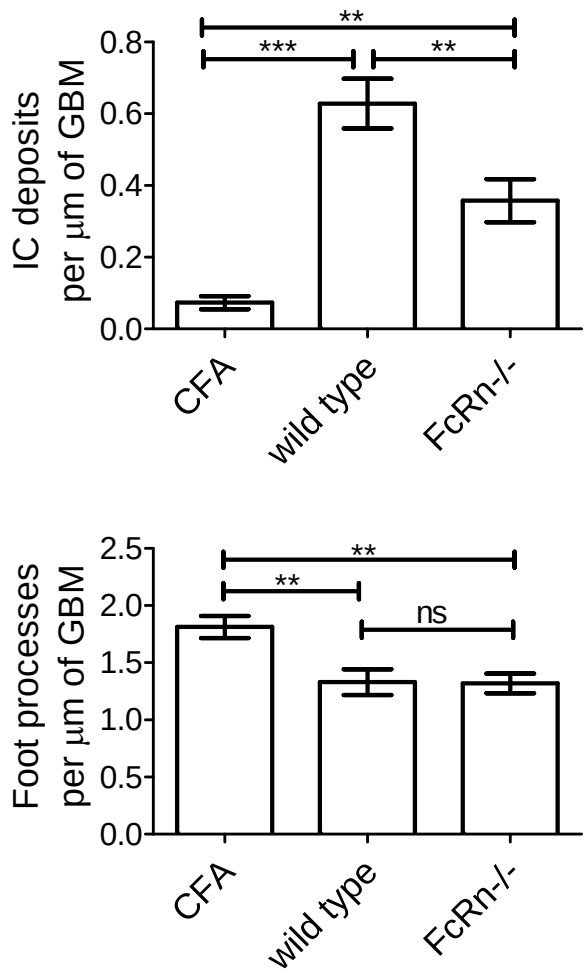
Supplemental Figure 1. Susceptibility of wild type B6 mice to kidney disease induced by immunization with α3NC1 monomers and bovine GBM NC1 hexamers. A. At 10 weeks, urinary albumin creatinine ratio (ACR) was significantly increased in mice immunized twice (2x) or four times (4x) with α3NC1 monomers, but not in mice immunized with bovine GBM NC1 hexamers or CFA alone (control). B. Blood urea nitrogen levels remained in the normal range in all groups of mice. C. Kidneys from mice sacrificed at 10 weeks after the first immunization were observed by light microscopy and immunofluorescence microscopy. Light microscopy revealed minimal glomerular injury in mice immunized with CFA (a), bovine GBM NC1 hexamers (b) or α3NC1 monomers (c) (periodic acid-Schiff staining, magnification 400x). Immunofluorescence showed linear GBM deposition of mouse IgG in mice immunized with bovine GBM NC1 hexamers (e) and linear-granular GBM staining for IgG in mice immunized with α3NC1 (f, g). Weak mesangial IgG deposition was observed in CFA control mice (d). Granular C3c deposition along the GBM was only observed in mice immunized with α3NC1 monomers, more intense after four immunizations (k) than after two immunizations (j). GBM deposition of C3c was absent in mice immunized with CFA (h) and bovine GBM NC1 hexamers (i). Magnification 400x. Acid-eluted antibodies from the kidneys of mice immunized with bovine GBM contained mouse IgG1, IgG2a and IgG2b autoantibodies binding to α345NC1 hexamers from mouse GBM (not shown). Based on these preliminary results, in the experiments described in the main text, wild type B6 mice were immunized with α3NC1 four times.



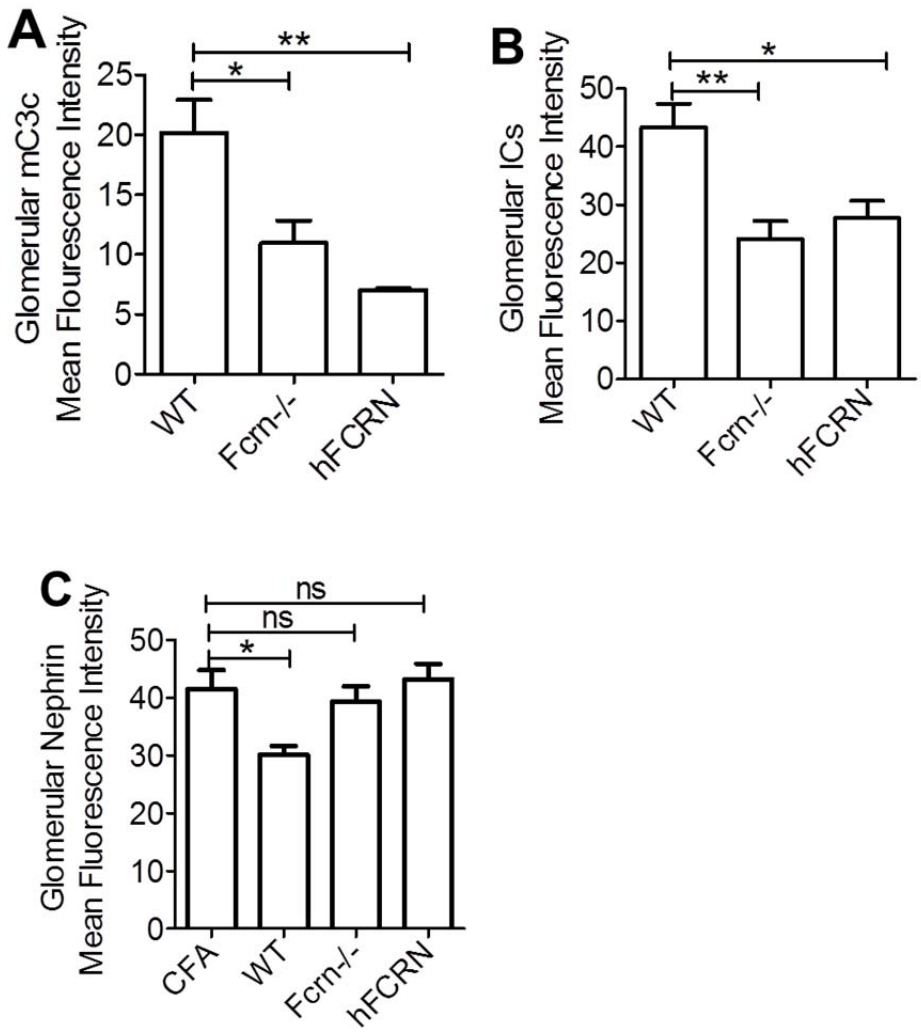
Supplemental Figure 2. Serum levels of mouse IgG1, IgG2b and IgG2c anti-α3NC1 antibodies in α3NC1-immunized mice. Circulating mIgG1 (**A**), mIgG2b (**B**) and mIgG2c (**C**) autoAbs from C57Bl/6 wild type mice (circles), FcRn -/- mice (squares), hFCRN mice (diamonds) and control CFA group (triangles) were assayed by indirect ELISA in plates coated with α3NC1 (100 ng/well). Mouse sera were diluted 1/5000.



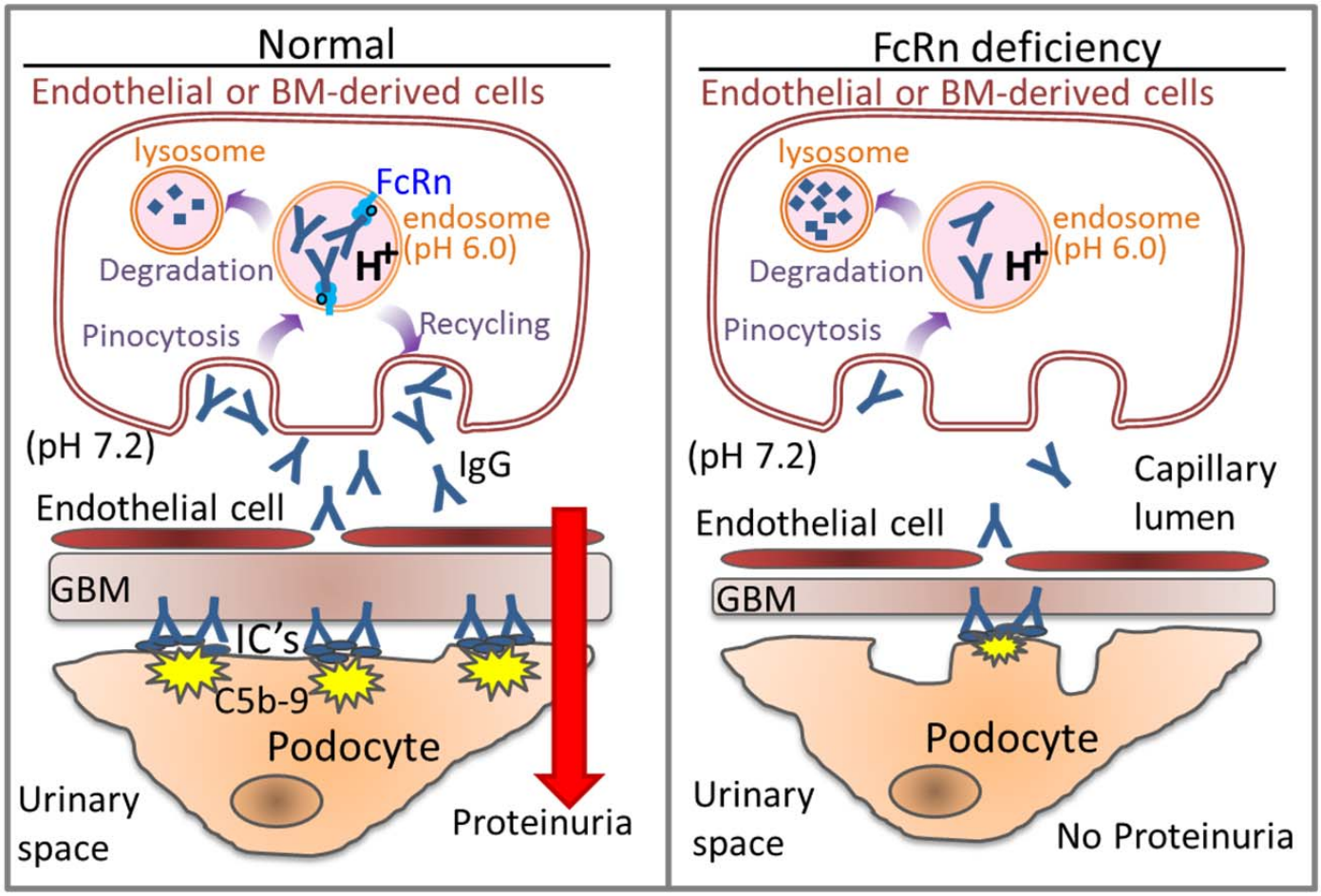
Supplemental Figure 3. Normal levels of blood urea nitrogen (BUN) in α3NC1-immunized mice. Renal function was evaluated by measuring blood urea nitrogen levels (mg/dl) in α3NC1-immunized C57Bl6 wild type mice (circles), FcRn -/- mice (squares), hFCRN mice (diamonds) and control CFA-immunized mice (triangles), which remained in the normal range for the duration of the experiment.



Supplemental Figure 4. Quantitation of electron-dense subepithelial IC deposits and podocyte foot process effacement in wild type mice and Fcrn-/- mice immunized with α3NC1. The number of subepithelial electron-dense deposits per unit of GBM length was about 1.8-fold greater in α3NC1-immunized wild type mice than in Fcrn-/- mice (top). Compared to control mice immunized with CFA alone, the number of podocyte foot processes per unit of GBM length was decreased by about 30% in wild type mice immunized with α3NC1 as well as in Fcrn-/- mice immunized with α3NC1 (bottom). To quantitate the ultrastructural features, the numbers of foot processes and electron dense deposits per length of GBM in capillary loops from at least three glomeruli per mouse were determined. The graphs depict the means and SEM. The significance of differences among groups was assessed by one-way ANOVA followed by Bonferroni post tests for pairwise comparisons (ns, not significant; ** $p< 0.01$; ***$p< 0.001$).



Supplemental Figure 5. Glomerular deposition of C3c, exogenous antigen forming immune complexes and nephrin staining in α3NC1-immunized wild type and FcRn-deficient mice. ImageJ software was used to determine glomerular mean fluorescence intensity of C3c deposited in α3NC1-immunized wild type and FcRn-deficient mice (A). To evaluate the glomerular deposition of exogenous α3NC1 antigen, mean fluorescence intensity was determined on kidney sections stained by mAb H31 using native conditions (B). Wild type mice immunized with α3NC1 monomers show glomerular nephrin loss compared to CFA controls, but not FcRn-deficient mice (C). Means and SEM are shown for approximately 15 gloms (n=3-5 mice in each group). The significance of differences among groups was assessed by one-way ANOVA followed by Bonferroni post tests for pairwise comparisons (ns, not significant; * $p<0.05$; ** $p< 0.01$).



Supplemental Figure 6. The role of FcRn in immune complex-mediated glomerular disease. *Left*: Recycling of IgG by endothelial and bone marrow (BM)-derived cells maintains high serum IgG levels. IgG (as well as albumin, not shown) taken up into cells by fluid-phase pinocytosis binds to FcRn at pH 6.0-6.5 in endosomes and is then recycled to the plasma membrane, where it is released upon exposure to pH 7.4. In contrast, IgG not bound to FcRn enters a default lysosomal pathway and is degraded. In IgG-mediated kidney disease, FcRn maintains high serum level of pathogenic IgG antibodies, promoting the development of glomerular ICs which can activate various effector pathways, damaging the glomerular filtration barrier and causing albuminuria. *Right*: In the absence of FcRn, IgG catabolism is accelerated, decreasing the serum levels of pathogenic IgG antibodies, which limits formation of subepithelial ICs and associated glomerular pathology.