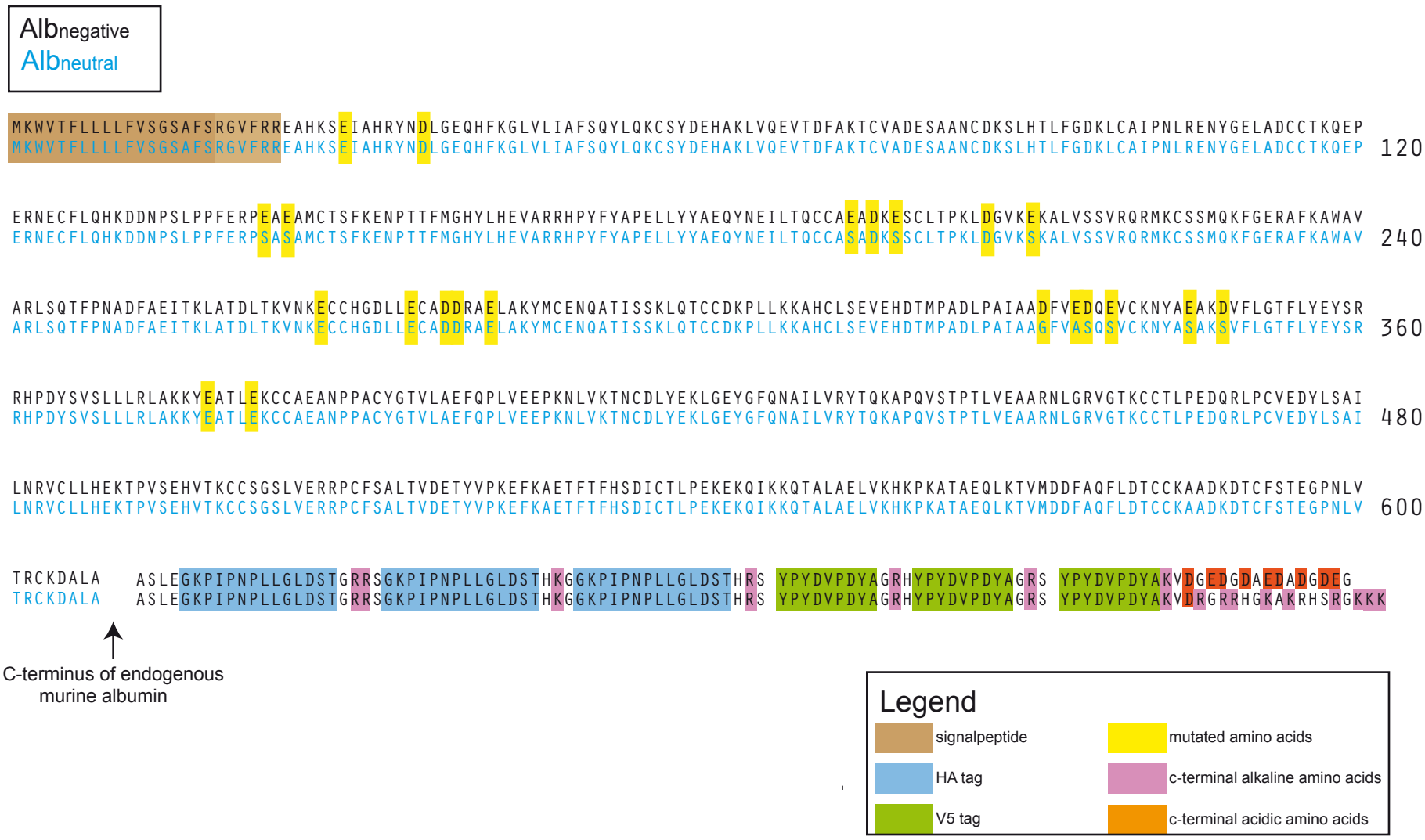
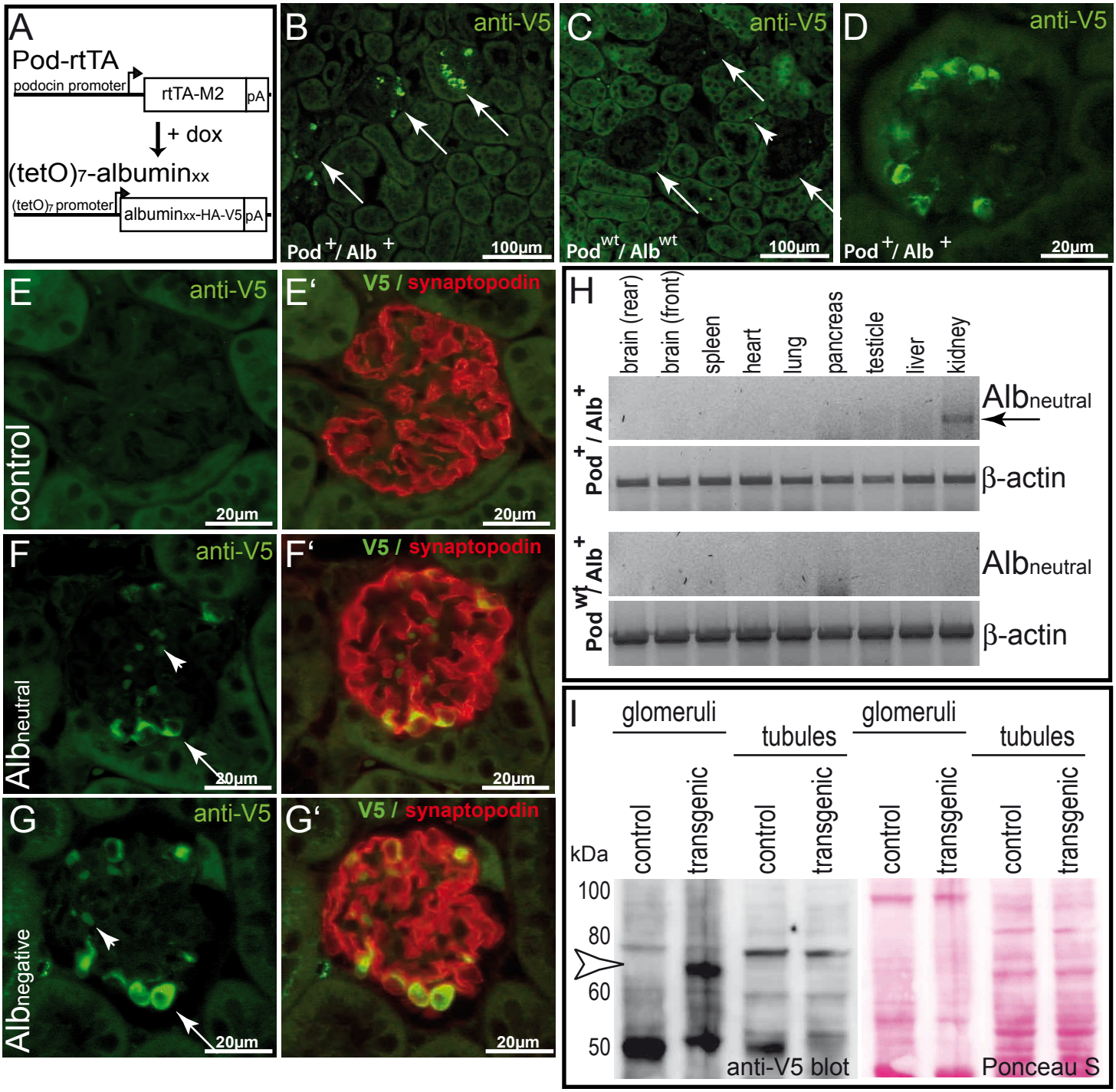
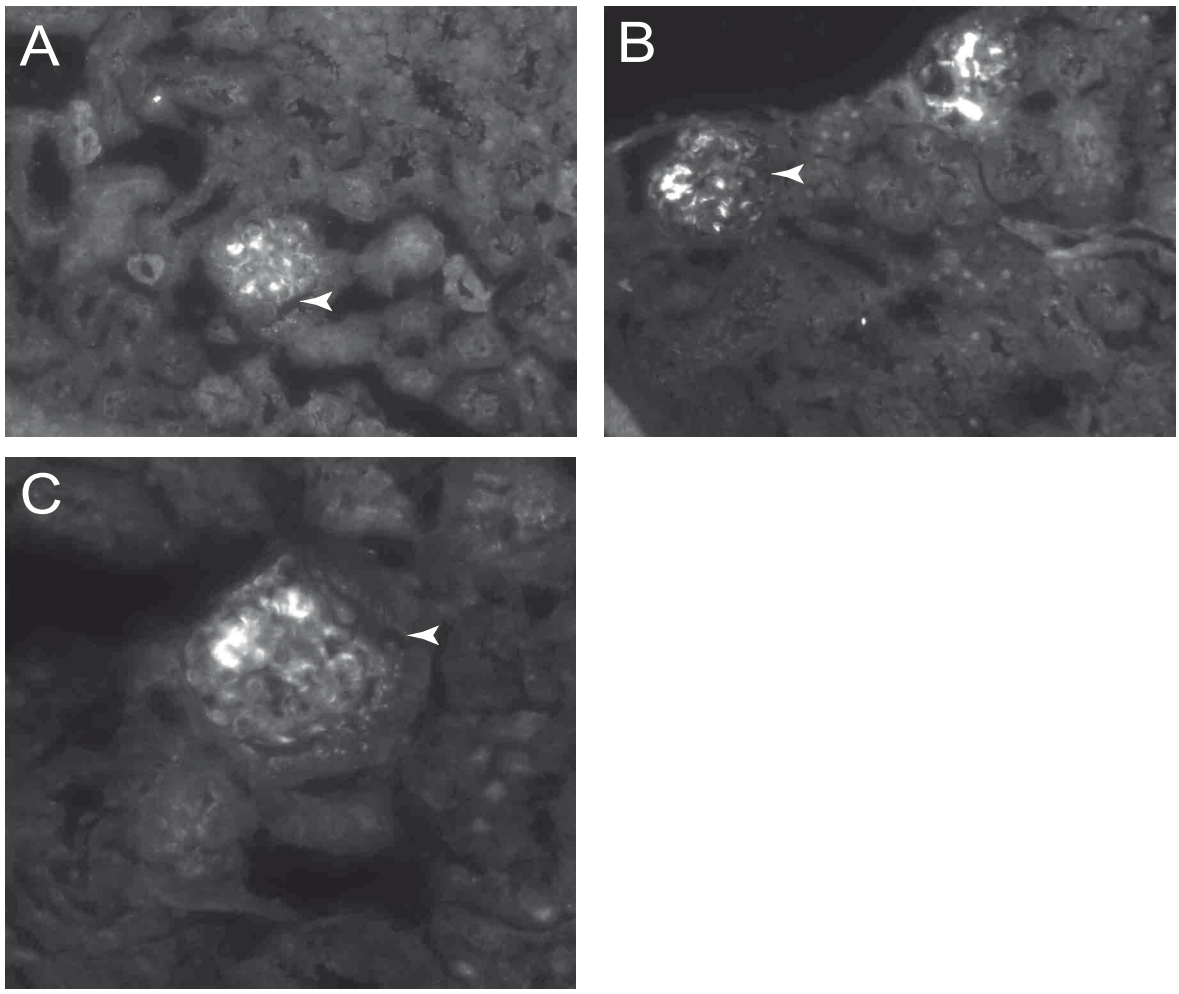
Supplemental Figure 1



Supplemental Figure 2

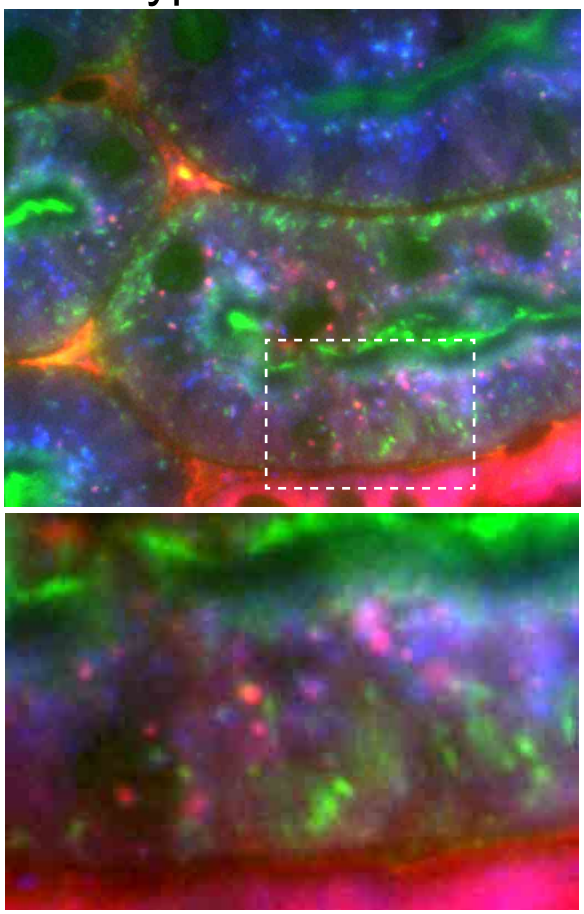


Supplemental Figure 3

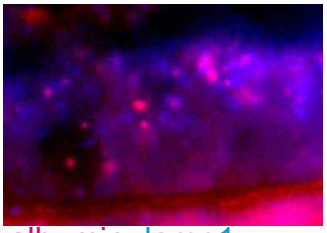


Supplemental Figure 4

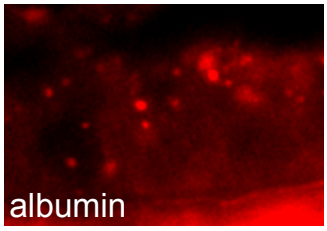
FcRn wild-type



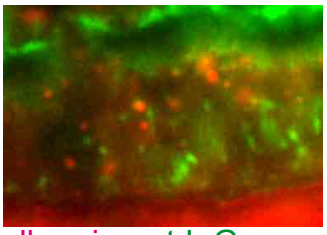
albumin, lamp1



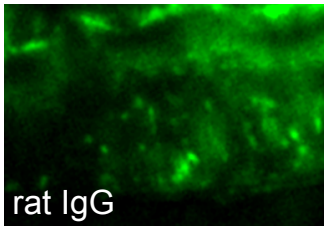
albumin



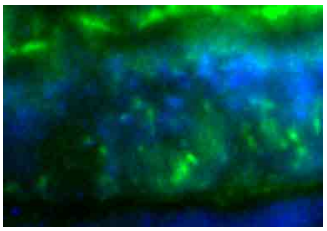
albumin, rat IgG



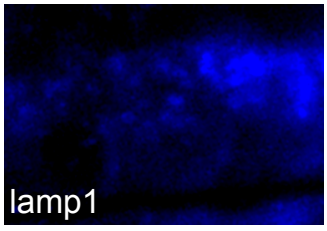
rat IgG



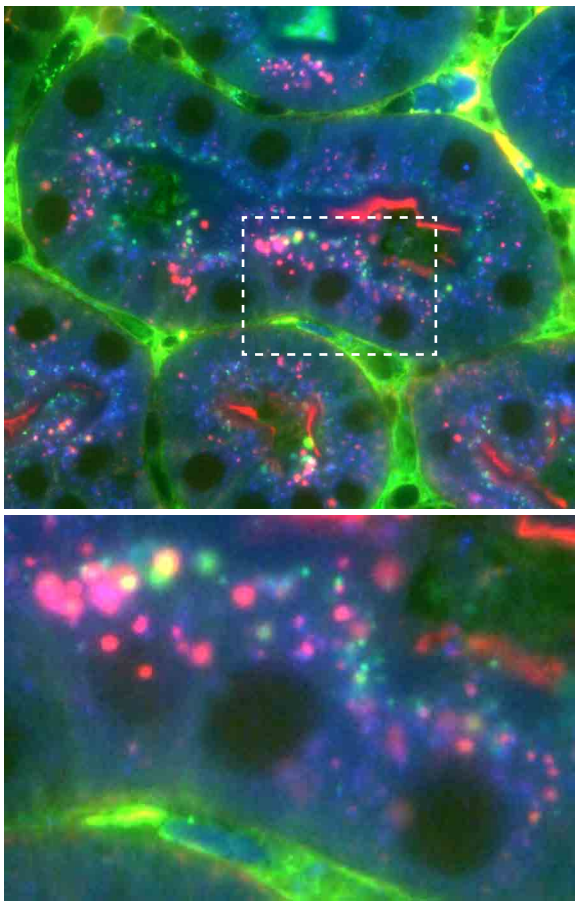
rat IgG, lamp1



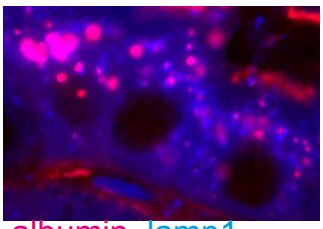
lamp1



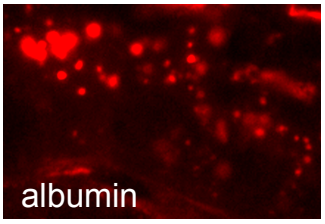
FcRn knock-out



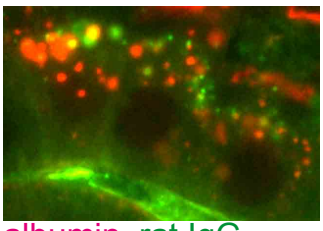
albumin, lamp1



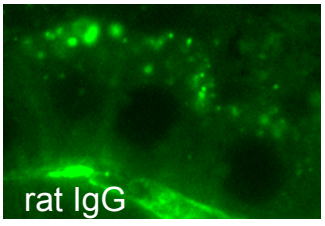
albumin



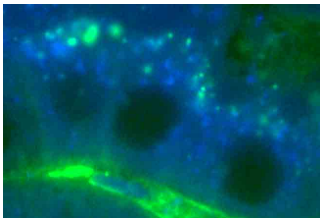
albumin, rat IgG



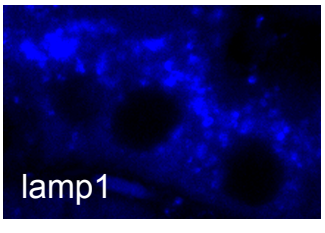
rat IgG



rat IgG, lamp1



lamp1



Supplemental Figure 1

Aminoacid sequence of $Alb_{negative}$ and $Alb_{neutral}$. Murine full-length cDNA for albumin was derived from IMAGE clone IRAVp968A0855D. At the C-terminus, 3xHA (red) and 3xV5 tags (green) were added including basic or acidic aminoacids (aa, pink or orange, respectively) to adjust the isoelectric point to 7.52 ($Alb_{neutral}$) or 5.42 ($Alb_{negative}$), respectively. The tag sequence is identical in neutralized and negative transgenic albumin to assure equal affinity for the respective V5 and/or HA antibodies. In addition, 11 acidic aa were mutated in two patches on the surface of the albumin molecule in $Alb_{neutral}$ (yellow).

Supplemental Figure 2

Characterization of transgenic mice. A: Transgenic map of Pod-rtTA/Alb_{xx} mice. The reverse tetracycline-inducible transactivator (rtTA) is expressed specifically in podocytes (27). Upon administration of doxycycline (dox), transgenic 3xHA/3xV5-tagged albumin (with a negative or neutral charge) is expressed by the second transgene $(TetO)_7$-Alb_{xx}. **B-G':** Immunofluorescent stainings of paraffin-embedded kidney sections show transgene expression exclusively within the podocytes of glomeruli (arrows, anti-V5, green) of transgenic animals (B, D, F, G and F', G'; co-staining with synaptopodin). Non-transgenic (C) or single transgenic litter mates Pod-$rtTA^{neg.}$/$Alb_{neutral}$ (E, E') showed no V5 staining. Weak non-specific green staining was observed in erythrocytes (arrowheads, C, F and G). Expression levels of Pod-rtTA/$Alb_{neutral}$ mice (F, F') was similar to Pod-rtTA/$Alb_{negative}$ mice (G, G'). **H:** Kidney specific transgene expression. Upper panel: rt-PCR of Pod-rtTA/$Alb_{neutral}$ mice (arrow, similar results for Pod-rtTA/$Alb_{negative}$ mice, not shown); lower panel; single-transgenic littermate (both after administration of doxycyline). Equal loading was verified by beta-actin rt-PCR. **I:** By immunoblot, transgene expression within the kidney is detected exclusively within glomerular lysates (respresentative image for Pod-rtTA/$Alb_{neutral}$ mice). Tubular lysates were prepared from kidney homogenate depleted of

glomeruli by differential sieving. Single-transgenic littermates show no expression of transgenic albumin.

Supplemental Figure 3

Transgenic albumin concentrations are low within the primary filtrate. A-C. Double-transgenic Pod-rtTA/$(tetO)_7$-$Alb_{neutral}$ and Pod-rtTA/$(tetO)_7$-$Alb_{negative}$ mice were induced with doxycycline for 5 days. Next, the kidneys were removed from the anaesthesized animals and immediately shock-frozen in liquid nitrogen within less than 3 seconds to minimize diffusion of transgenic albumin between the glomerular compartments. When staining transgenic albumin using a polyclonal rabbit anti-HA antibody, low fluorescent signals were detected within the Bowman's space (arrow heads) compared to the endocapillary compartment of the respective glomeruli.

Supplemental Figure 4

Co-staining endogenous albumin, rat IgG and lamp1 in proteinuric wild-type or FcRn knock-out mice.

Mice were injected with rat anti-APA 16 hours prior to sacrifice. All mice exhibeted nephrotic range proteinuria (+++ on dipstick). As a consequence of proteinuria, proximal tubular cells contained in general larger lamp1-positive versicles with albumin. **FcRn knock-out mice.** Basolateral extracellular accumulations of rat IgG were absent in FcRn knock-out mice, as shown above.