

# Supplemental Figure 1

Alb<sup>negative</sup>  
Alb<sup>neutral</sup>

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RHPDYSVSLLLRLAKKYEATLEKCCAEANPPACYGTVLAEFQPLVEEPKNLVKTNC DLYEKLGEYGFQNAILVRYTQKAPQVSTPTLVEAARNLGRVGT KCCTLPEDQRLPCVEDYLSAI

LNRVCLLHEKTPVSEHVTKCCSGSLVERRPCFSALTVD ETVVPKEFKAETFTFHS DICTLPEKEKQIKKQTALAE LVKHKPKATAEQLKTVMD DFAQFLDTCCAADKDTCFSTEGPNLV 600  
LNRVCLLHEKTPVSEHVTKCCSGSLVERRPCFSALTVD ETVVPKEFKAETFTFHS DICTLPEKEKQIKKQTALAE LVKHKPKATAEQLKTVMD DFAQFLDTCCAADKDTCFSTEGPNLV

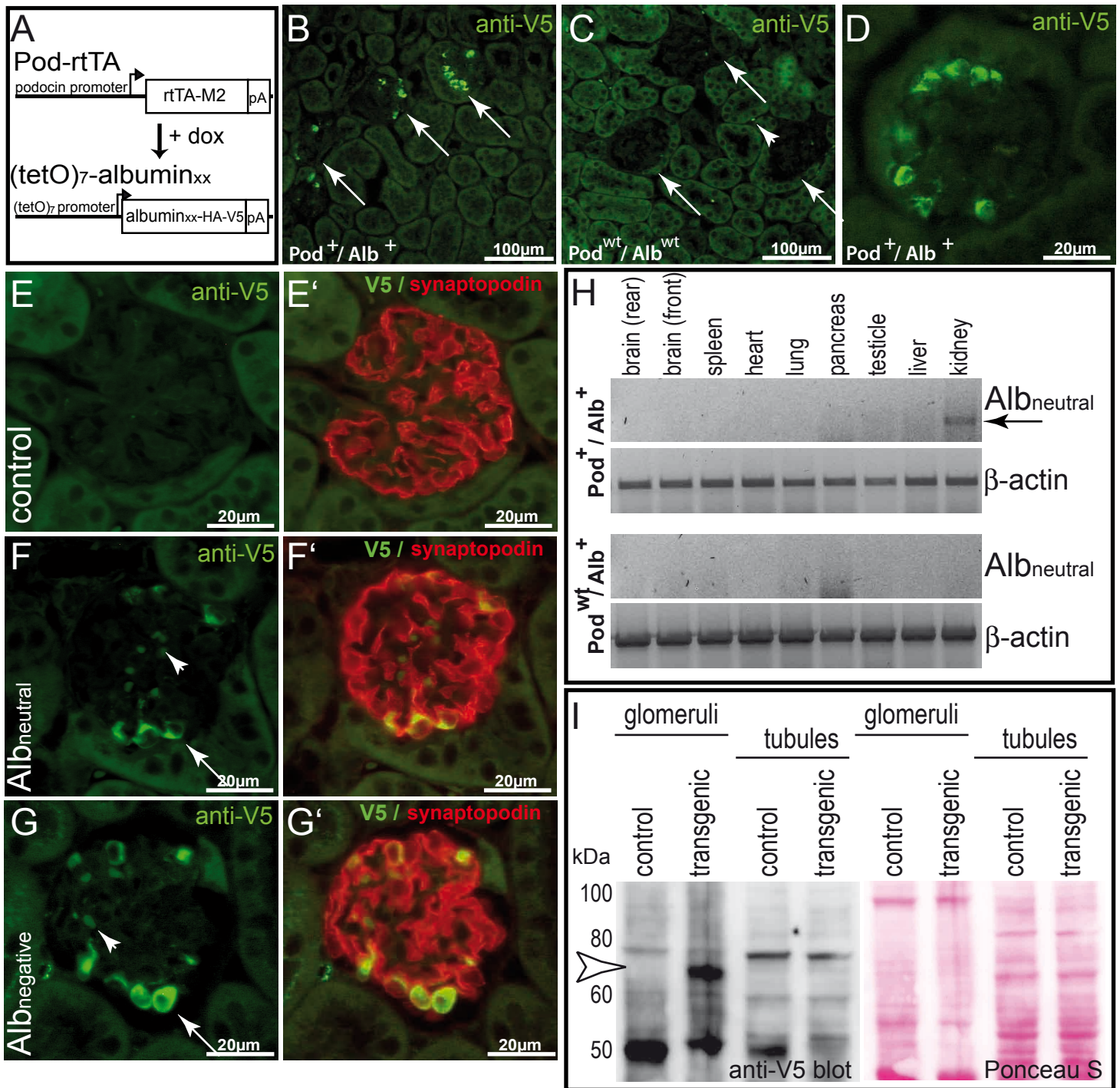
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TRCKDALA ASLEGGKPIPNPLLGLDSTGRRSGGKPIPNPLLGLDSTHKGGKPIPNPLLGLDSTHRS YPYDVPDYAGRHYPYDVPDYAGRS YPYDVPDYAKVDRGRRHGKAKRHSRGKKK

↑  
C-terminus of endogenous  
murine albumin

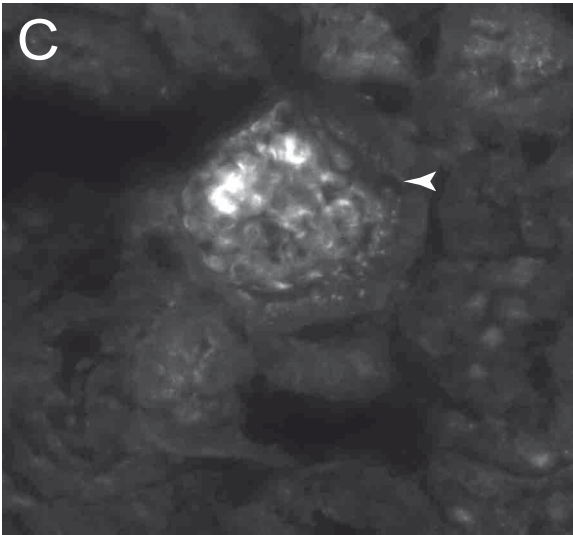
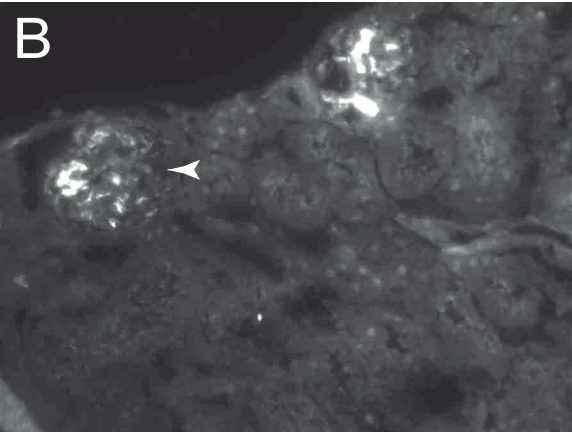
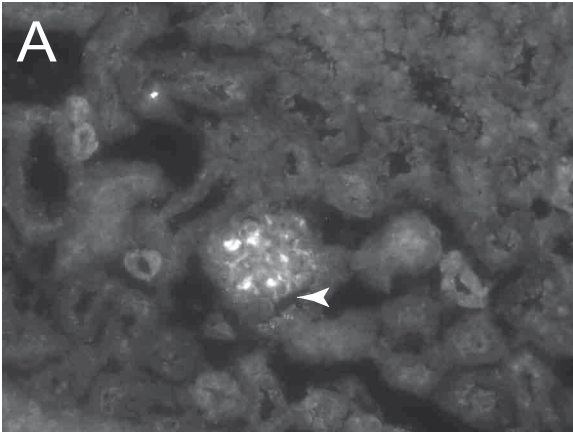
## Legend

signalpeptide	mutated amino acids
HA tag	c-terminal alkaline amino acids
V5 tag	c-terminal acidic amino acids

# Supplemental Figure 2



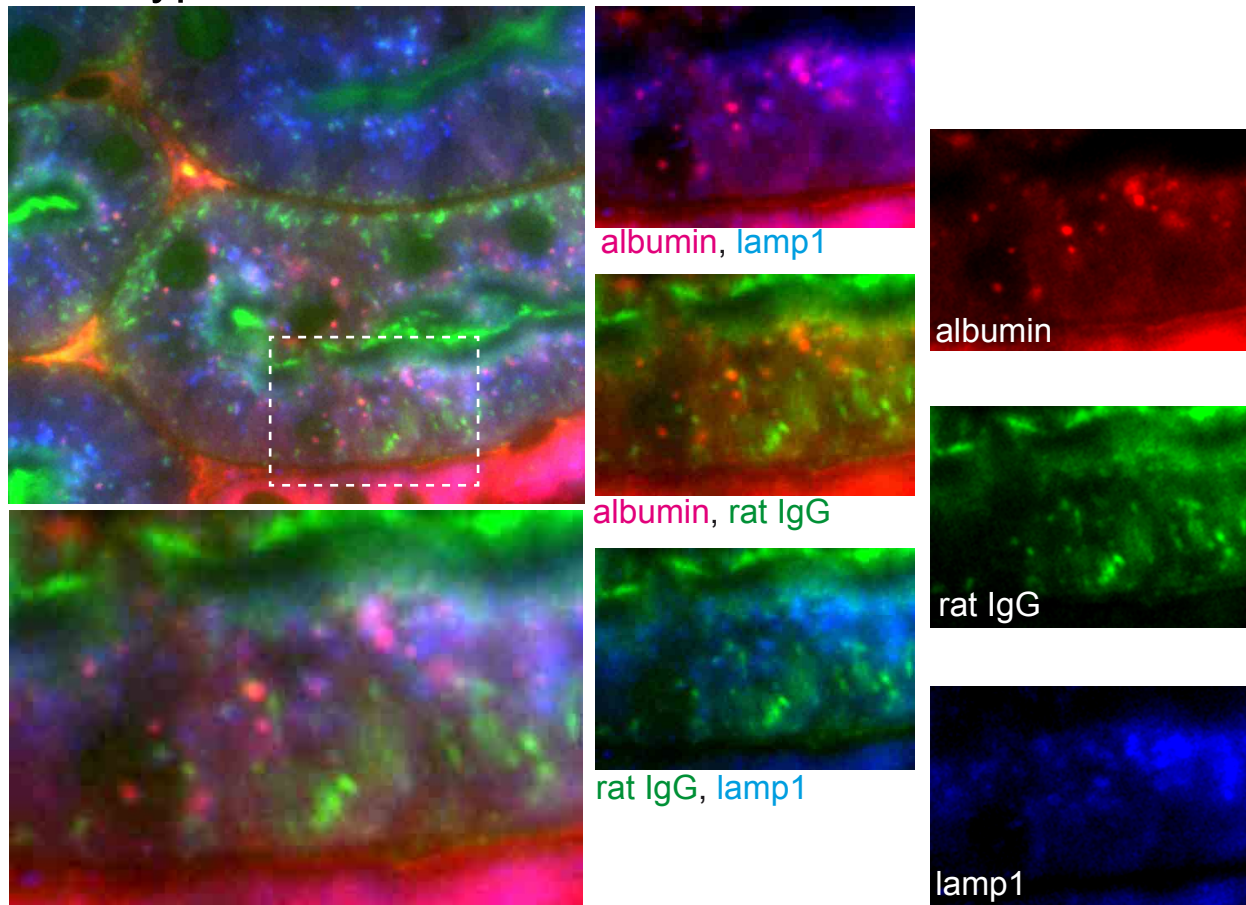
# Supplemental Figure 3



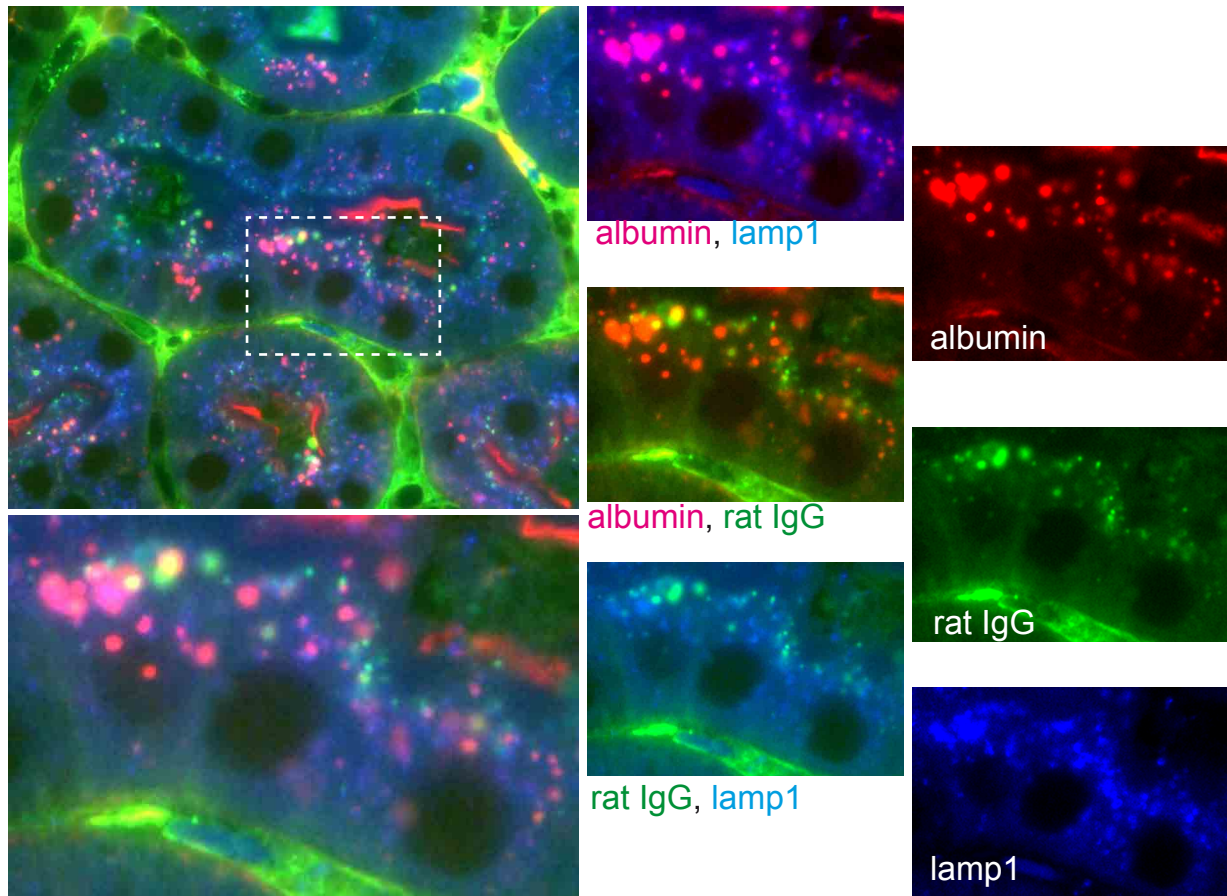


# Supplemental Figure 4

FcRn wild-type



FcRn knock-out



## Supplemental Figure 1

**Aminoacid sequence of Alb<sub>negative</sub> and Alb<sub>neutral</sub>.** 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<sub>neutral</sub>) or 5.42 (Alb<sub>negative</sub>), 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<sub>neutral</sub> (yellow).

## Supplemental Figure 2

**Characterization of transgenic mice. A:** Transgenic map of Pod-rtTA/Alb<sub>xx</sub> 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)<sub>7</sub>-Alb<sub>xx</sub>. **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<sup>neg</sup>/Alb<sub>neutral</sub> (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<sub>neutral</sub> mice (F, F') was similar to Pod-rtTA/Alb<sub>negative</sub> mice (G, G'). **H:** Kidney specific transgene expression. Upper panel: rt-PCR of Pod-rtTA/Alb<sub>neutral</sub> mice (arrow, similar results for Pod-rtTA/Alb<sub>negative</sub> mice, not shown); lower panel; single-transgenic littermate (both after administration of doxycycline). Equal loading was verified by beta-actin rt-PCR. **I:** By immunoblot, transgene expression within the kidney is detected exclusively within glomerular lysates (representative image for Pod-rtTA/Alb<sub>neutral</sub> 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)<sub>7</sub>-Alb<sub>neutral</sub> and Pod-rtTA/(tetO)<sub>7</sub>-Alb<sub>negative</sub> mice were induced with doxycycline for 5 days. Next, the kidneys were removed from the anaesthetized 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 exhibited nephrotic range proteinuria (+++ on dipstick). As a consequence of proteinuria, proximal tubular cells contained in general larger lamp1-positive vesicles with albumin. **FcRn knock-out mice.** Basolateral extracellular accumulations of rat IgG were absent in FcRn knock-out mice, as shown above.