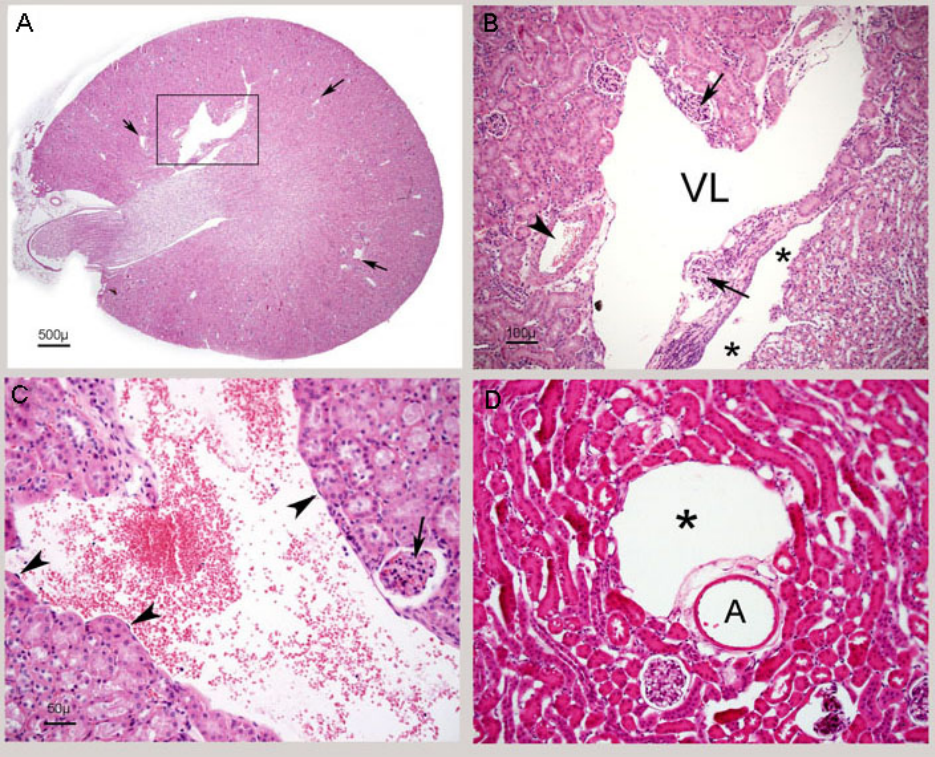
Supplementary Fig. 1. The lack of kidney lesions in CerS2 null mice. (A) Transverse section of the kidney of a 13.5 month-old WT mouse. With the exception of one large venous space (boxed, shown in B), most of the veins (indicated by arrows) included in this section are small. Note that the boxed space is much bigger than any shown in the CerS2 null mouse (Fig. 8A). (B) The shape of the venous lumen (VL) is irregular. As expected, an artery is present next to it (arrowhead). Several glomeruli are located just outside the lining cells (arrows). The adjacent space (asterisks) is the urinary space. (C) A sample from the kidney of another 13.5 month-old WT mouse. In this section, blood is present in the venous lumen. The space is lined by a monolayer of flattened endothelial cells (arrowheads). An adjacent glomerulus is identified (arrow). These histological features are identical with those described by Imgrund et al. (1), which we believe were misidentified as abnormal tissue clefts (see Supplemental Fig. 2C-F in Ref. (1)). (D) A sample from the kidney of a WT mouse which has undergone perfusion with excessive pressure. As a result the lumen of the artery (A) and vein (asterisk) are markedly dilated. The dilated vein compresses the surrounding renal tubules. This image is practically identical to that shown by Imgrund et al. in Supplemental Figure 2F, taken from a CerS2 null mouse. The spaces identified by Imgrund et al with an arrow are most compatible with a dilated vein compressing surrounding renal tubules.



Discussion of Supplementary Fig. 1

We could not identify any consistent macroscopic or microscopic pathological changes in the kidneys of CerS2 null mice. Lymphoplasmacytic interstitial nephritis and pyelonephritis was present in several CerS2 null and WT mice, a common background lesion. One older CerS2 null mouse had intratubular casts of eosinophilic and partly mineralized material in the renal papilla.

In contrast, Imgrund et al. (1) reported histological changes in the kidney of CerS2 null mice at 7-9 months, which consisted of several 'gaps of 100 μm width' within the parenchyma, mainly in the vicinity of blood vessels and glomeruli, which were interpreted as a 'discrete loss of renal parenchyma' or 'tissue clefts'. Based on their description and images, we believe that the spaces they describe as abnormal and located 'apposed to larger veins' are rather normal renal veins. Imgrund et al. (1) also describe the 'clefts' to be most common at the corticomedullary junction, the level where the interlobar veins branch into the arcuate veins (2), giving rise to some irregularity in their contours in histologic preparations. We have examined serial sections of the kidney of old CerS2 null and WT mice (n = 3 for each) to determine if any 'abnormal spaces' were observed and to check the possibility that we failed to detect a consistent change in the diameter of the renal veins between the two groups. We did not detect any abnormal spaces and no significant differences were observed in the size of renal veins. Thus, we believe that the histological lesions reported by Imgrund et al. are normal renal veins, with the size difference due to the vagaries of histological sectioning.

1. Imgrund, S., Hartmann, D., Farwanah, H., Eckhardt, M., Sandhoff, R., Degen, J., Gieselmann, V., Sandhoff, K., and Willecke, K. (2009) *J. Biol. Chem.* **284**, 33549-33560

2. Khan, Kanwar, Nasir M. Alden, Carl L. Kidney. In: Handbook of Toxicologic Pathlogy. 2nd ed. vol.2 p.238. editors: Wanda M Hasckek, Colin G. Rousseaux and Matthew A. Wallig. Academic Press, (San Diego etc.) 2002.