Supplementary information

Generation and characterization of an *Abcc1* humanized mouse model (*hABCC1*$^{flx/flx}$) with knockout capability

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Supplementary Table 1. Genotyping primer contributions to the possible occurring genotypes

genotype	contributing primers	band size (bp)
wt/wt	N351 + N352	149
wt/flx	N351 + N352	149 + 216
flx/flx	N351 + N352	216
-/flx	N351 + N352 + N353	216 + 318
wt/-	N351 + N353	149 + 318
-/-	N351 + N353	318

The primers contributing to the differentiation of genotypes and sizes of resulting PCR products are listed.

Supplementary Table 2. *Actb* Ct-values of analysed groups

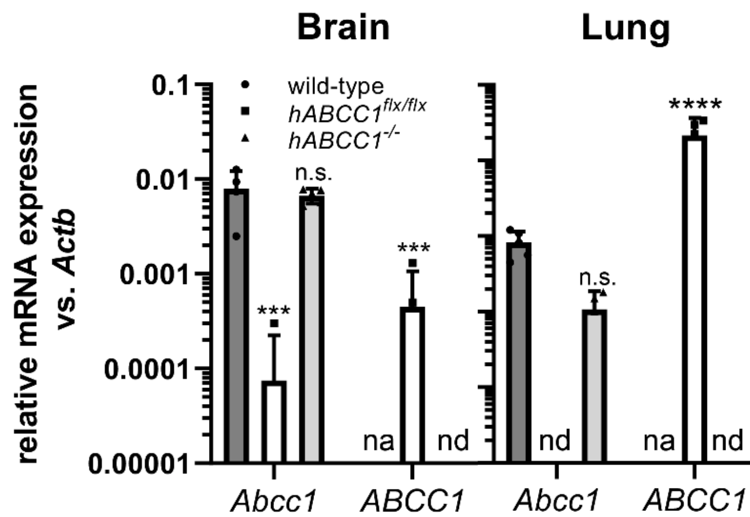
Strain	Tissue	mean Ct of *Actb* in *Abcc1* assays (±StD)	mean Ct of *Actb* in *ABCC1* assays (±StD)
wild-type	Brain	23.90 (0.27)	23.78 (0.33)
hABCC1$^{flx/flx}$		23.16 (0.69)	23.21 (0.31)
hABCC1$^{-/-}$		23.80 (0.47)	23.42 (0.30)
wild-type	Lung	27.00 (1.70)	26.23 (1.61)
hABCC1$^{flx/flx}$		25.08 (0.60)	25.13 (0.34)
hABCC1$^{-/-}$		25.15 (0.33)	25.04 (0.13)

Taqman® assays were performed using two colour detection of *Actb* as reference gene (VIC-labelled probes) along with the gene of interest (FAM-labelled probes).

Supplementary Table 3. Western blot luminescence signals of ATP1A2 bands

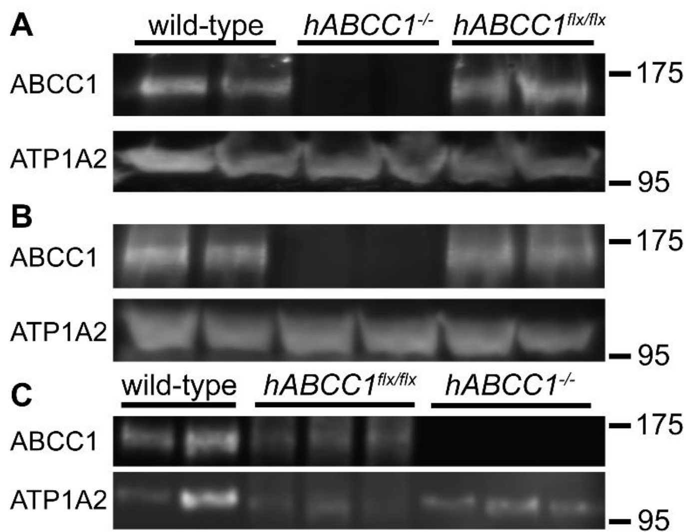
Strain	Tissue	mean ATP1A2 luminescence (±StD)
wild-type	Brain	18900 (3078)
hABCC1$^{flx/flx}$		16176 (6706)
hABCC1$^{-/-}$		14642 (5907)
wild-type	Lung	7555 (3841)
hABCC1$^{flx/flx}$		8448 (1560)
hABCC1$^{-/-}$		7782 (2302)

Luminescence of detected bands was captured using an Octoplus QPLEX system (DyeAGNOSTICS).



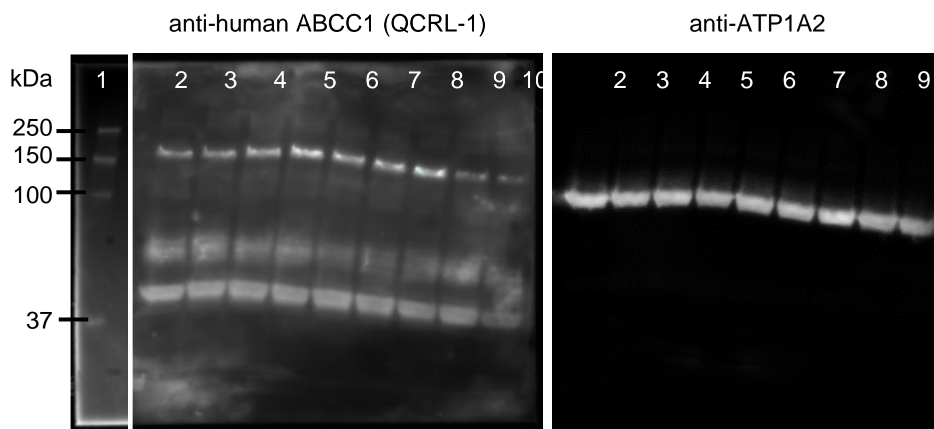
Supplementary Figure 1. mRNA expression analyses.

Expression level of murine *Abcc1* mRNA and human *ABCC1* mRNA are shown for brain and lung tissue, respectively. In both organs, *ABCC1* mRNA is expressed in *hABCC1*$^{flx/flx}$ mice (n=4f), but not detectable (nd) in *hABCC1*$^{-/-}$ mice (n=4f/1m). In *hABCC1*$^{flx/flx}$ mice, *ABCC1* mRNA abundance was at significantly lower levels than wild-type mRNA (p=0.0005) while significantly stronger expression was found in lung tissues (p<0.0001). As expected, murine *Abcc1* mRNA became re-expressed to wild-type (n=4f/1m) levels in brain (p=0.644) and lungs (p=0.974) of *hABCC1*$^{-/-}$ mice. In the Taqman assay used, the probe is situated at the exon-exon boundary of exons 5 and 6, which is behind the additional stop-codons we introduced into exon 3. In contrast to the translational machinery, the transcriptional process is insensitive for these stop-codons. Hence, it is reasonable to assume that deletion of the human CDS restores transcription of the remaining endogenous gene without resulting in translation of the same (see protein expression and PET data). In the brain of 1 out of 4 *hABCC1*$^{flx/flx}$ mice, *Abcc1* mRNA was amplified. Within each organ, one-way ANOVA of wild-type *Abcc1* mRNA expression vs. all other groups was used followed by Dunnett correction for multiple comparison. na – not applicable; *** p<0.001, **** p<0.0001; error bars = SD.



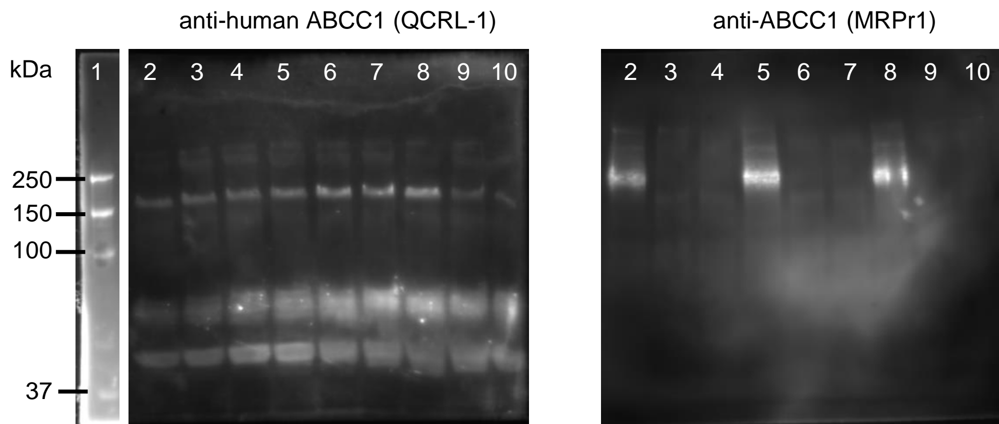
Supplementary Fig. 2. ABCC1 expression in spleen (A), kidney (B) and CP(C).

mABCC1 and hABCC1 protein expression in (A) spleen, (B) kidney and (C) choroid plexus of wild-type, *hABCC1*$^{flx/flx}$ and *hABCC1*$^{-/-}$ mice. No expression of ABCC1 could be detected in *hABCC1*$^{-/-}$ mice.



Supplementary Fig. 3. Antibody clone QCRL-1 is not specific for human ABCC1.

The antibody clone QCRL-1 is the only antibody reported to be specific for human ABCC1 and not to detect murine ABCC1. However, in our hands the antibody produced detection bands regardless of strain (wild-type – lanes 2-4, *hABCC1*$^{flx/flx}$ – lanes 5-7, *hABCC1*$^{-/-}$ - lanes 8-10) and fraction (membrane (shown here), cytosolic (not shown)).



Supplementary Fig. 4. Comparison of antibody clones QCRL-1 and MRPr1.

To verify selectivity of the used antibodies (QCRL-1, MRPr1), we blotted samples of conventional *Abcc1*$^{-/-}$ (lanes 4, 7, 10) mice together with wild-type (lanes 2, 5, 8) and *hABCC1*$^{-/-}$ (lanes 3, 6, 9) samples. As can be seen, the QCRL-1 antibody produced bands in all samples including conventional *Abcc1*$^{-/-}$ samples and can therefore be considered species-unspecific. The MRPr1 antibody only produced bands in the wild-type samples, as would be expected.