

Gut microbiome modulates tacrolimus pharmacokinetics through the transcriptional regulation of ABCB1

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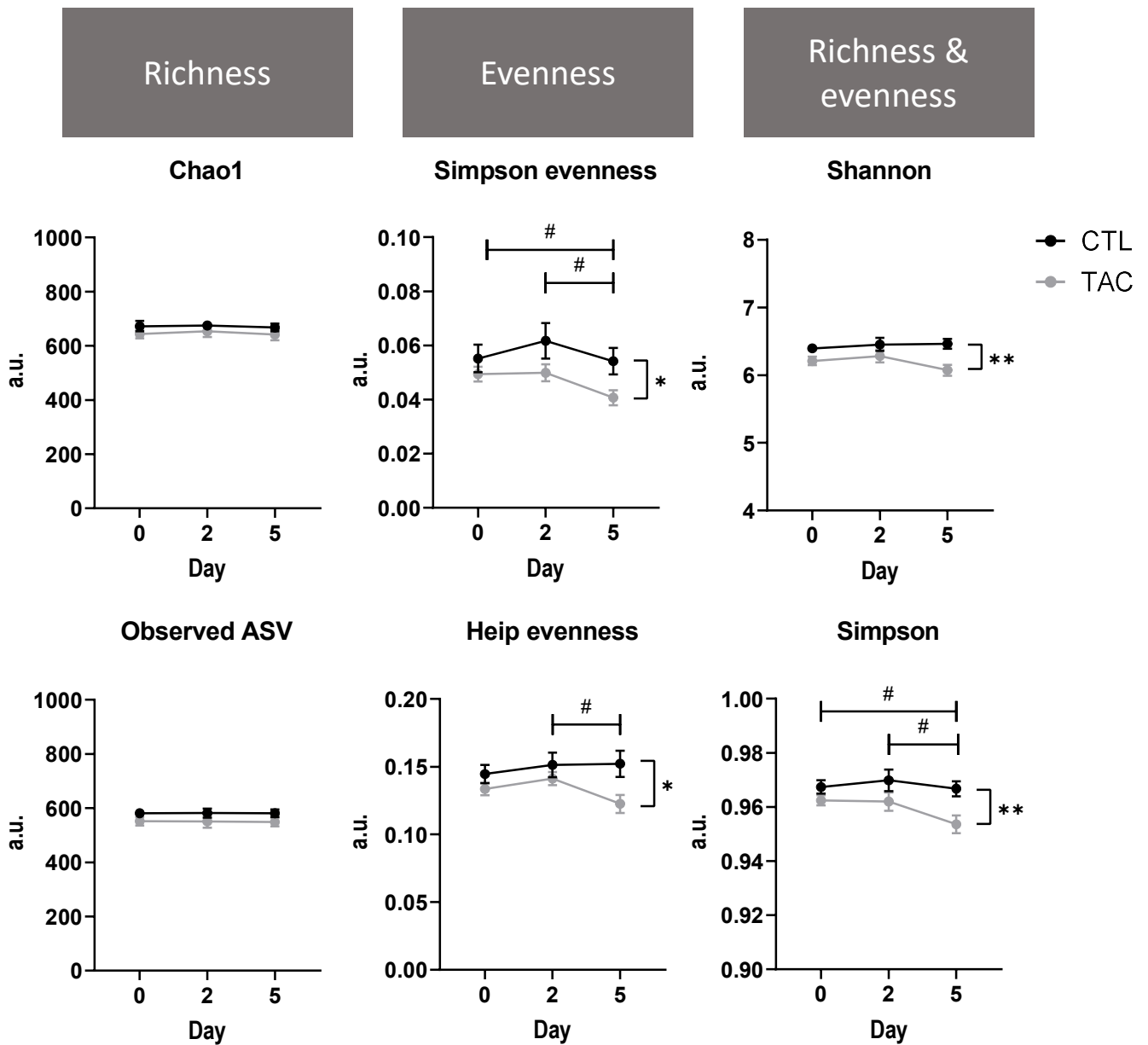


Figure S1. Tacrolimus (TAC) treatment affects the evenness but not the richness of the faecal microbiota. Evolution of α -diversity metrics (in arbitrary units, a.u.) of control (CTL) and TAC-treated mice along the treatment ($n = 7-8/\text{group}$). ** $p < 0.01$, * $p < 0.05$ (Mann-Whitney test, CTL vs TAC). # $p < 0.05$ (Friedman test with Dunn *post hoc* test, TAC repeated-measures).

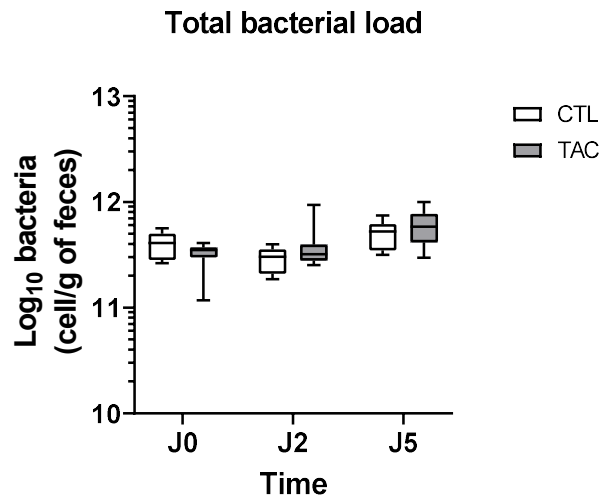


Figure S2. Total bacterial load is unaffected by tacrolimus (TAC) treatment. Evolution of the total bacterial load in faecal samples of control (CTL) and TAC-treated mice along the treatment (n = 7-8/group).

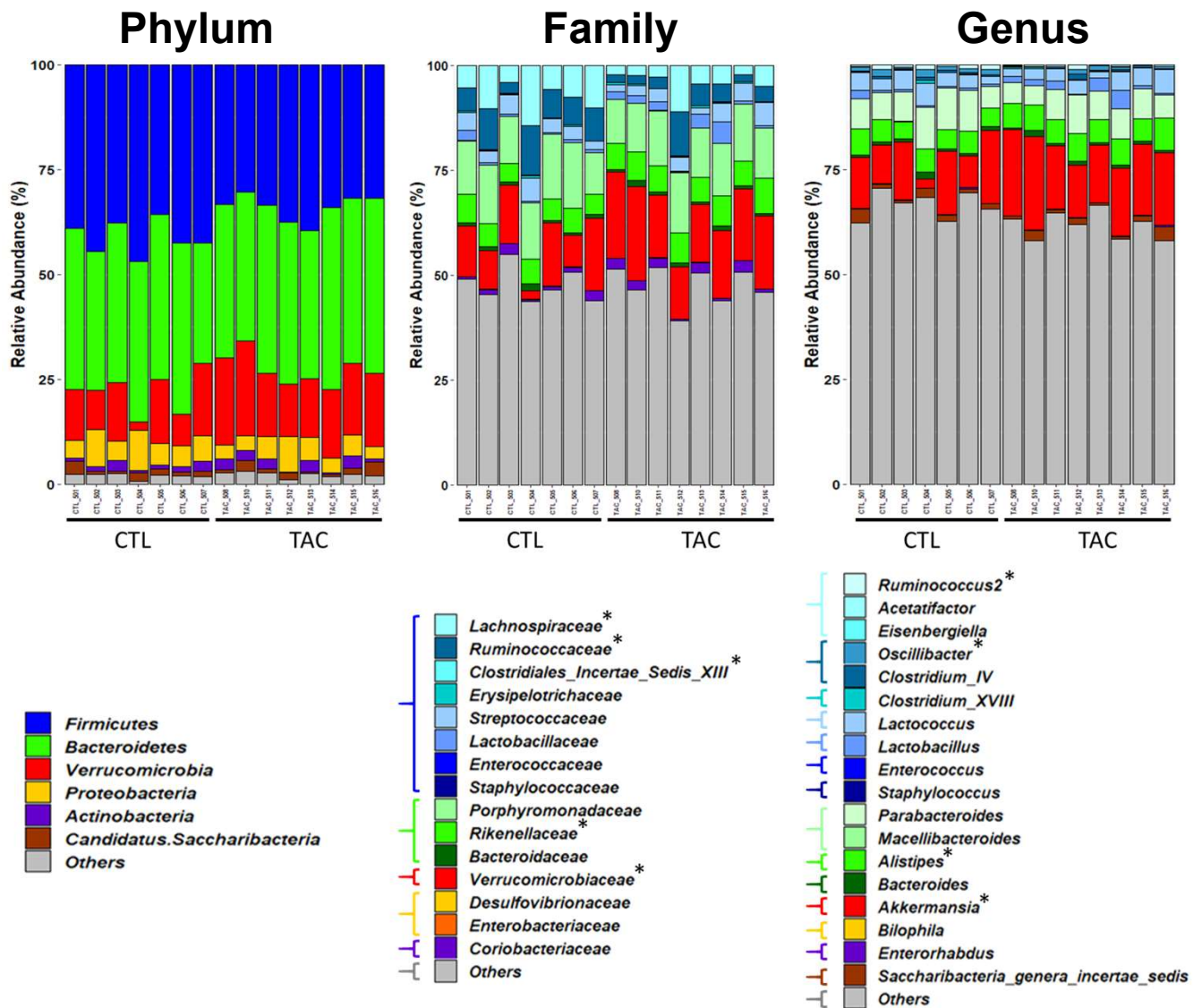


Figure S3. Faecal microbiota composition after tacrolimus (TAC) treatment. Stacked barplots showing the relative abundance of phyla, families, and genera of mice after 5 days of oral gavage with TAC or vehicle (CTL) (n = 7-8/group). * indicated taxa significantly impacted by TAC.

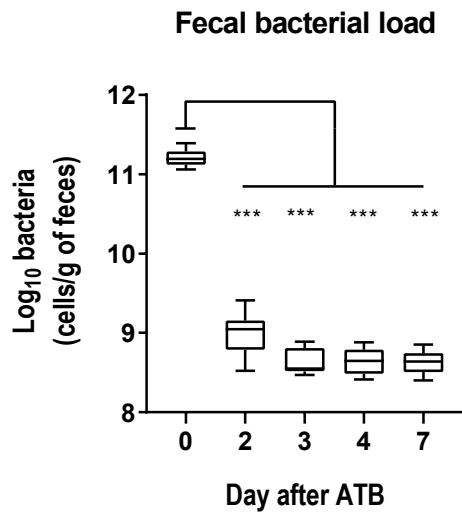
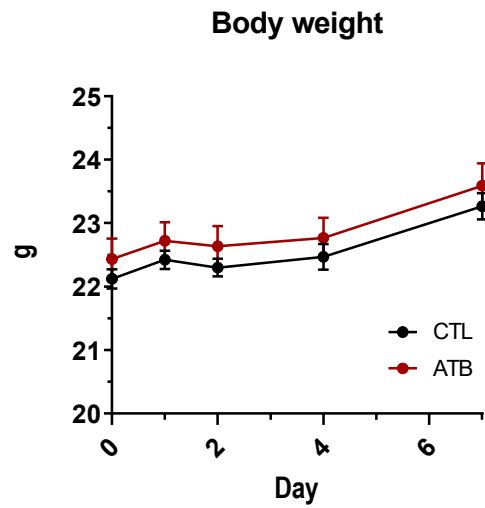
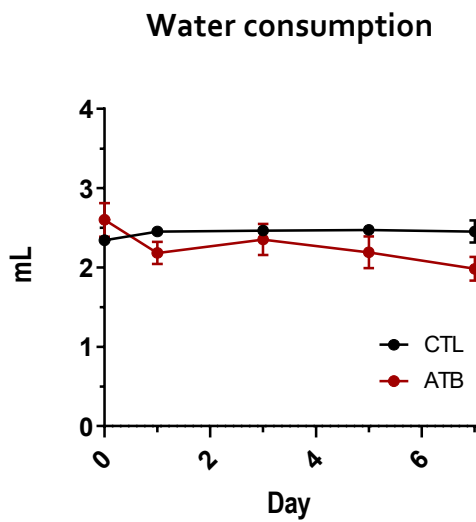
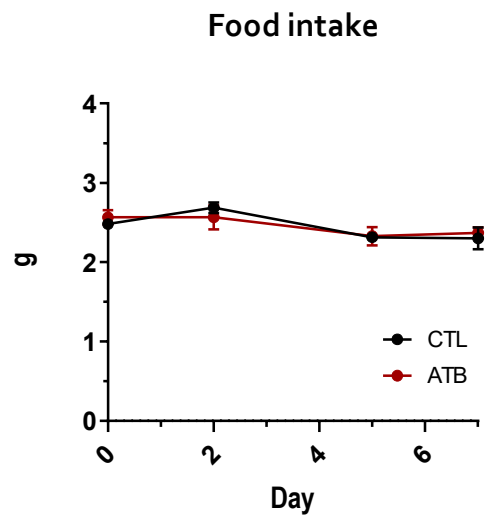
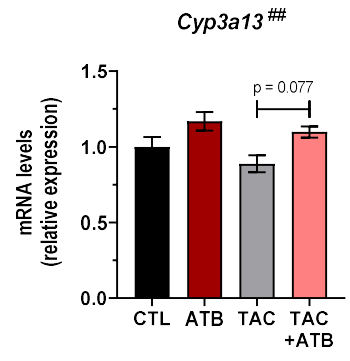
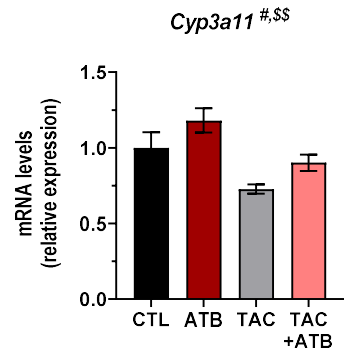
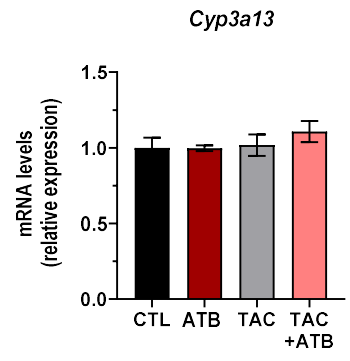
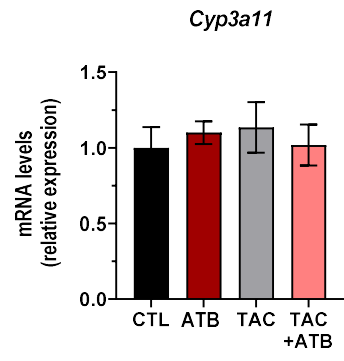
A**B****C****D**

Figure S4. Impact of antibiotic (ATB) treatment on mice monitoring parameters. (A) Faecal bacterial load over ATB treatment. *** $p < 0.001$. (B-D) The ATB cocktail does not affect the body weight (B), the water consumption (C), or the food intake (D) of the mice ($n = 6/\text{group}$).

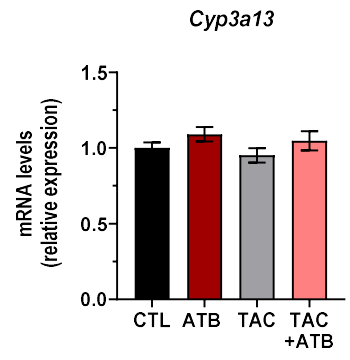
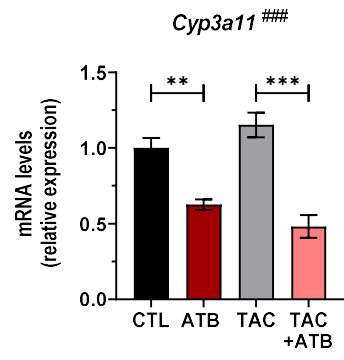
Proximal
small intestine



Median
small intestine

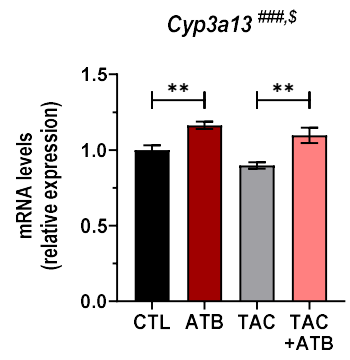


Distal
small intestine



Colon

Not
detected



Liver

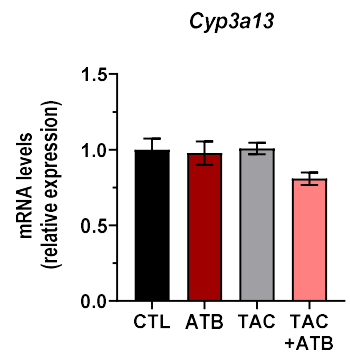
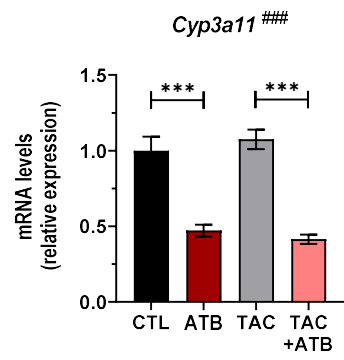


Figure S5. Antibiotic (ATB) -mediated gut microbiota depletion impacts the mRNA expression of key tacrolimus (TAC) -processing genes. Comparison of the mRNA expression of *Cyp3a11* and *Cyp3a13* in the proximal, median and distal small intestine, in the colon, and in the liver of control and ATB-treated mice, with or without TAC treatment (n = 7-8/group). #significant ATB effect; \$significant TAC effect; ***p < 0.001, **p < 0.01 and *p < 0.05.

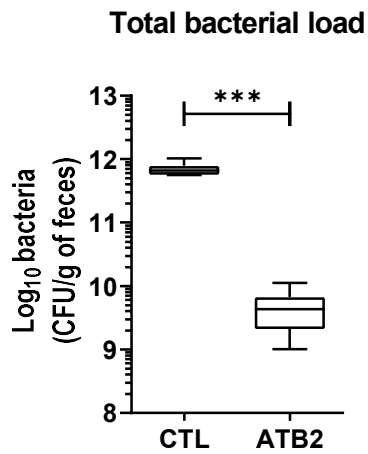
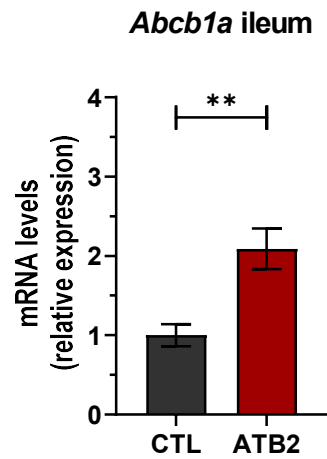
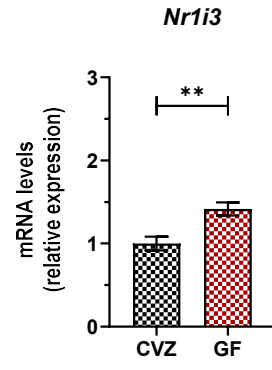
A**B**

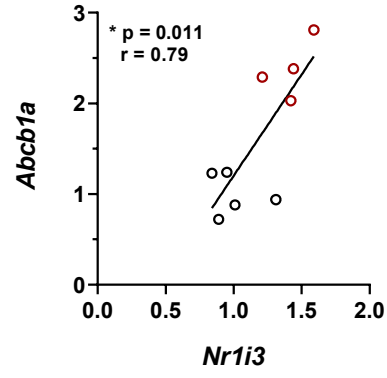
Figure S6. Intestinal *Abcb1a* expression is induced by an alternative antibiotic cocktail (ATB2) used to deplete the gut microbiota. (A) Efficient reduction of the total bacterial load by the ATB2 (neomycin 0.5g/L, ampicillin 1g/L) in BALB/c mice. (B) Comparison of the mRNA expression of *Abcb1a* in the ileum of control and ATB-treated mice (n = 7-8/group). ***p < 0.001 and **p < 0.01.

Proximal
small intestine

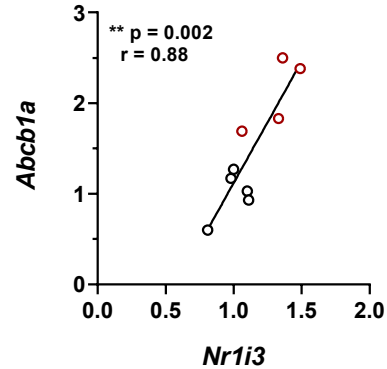
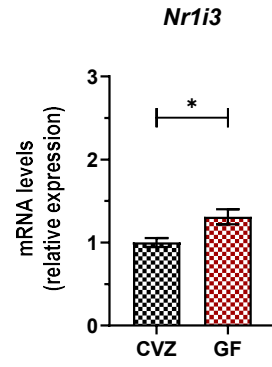
A



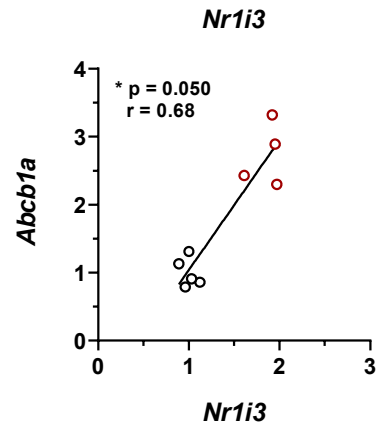
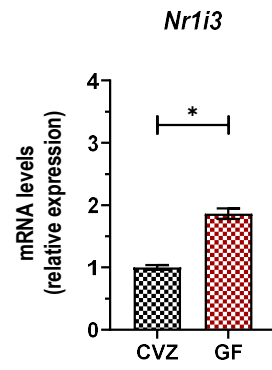
B



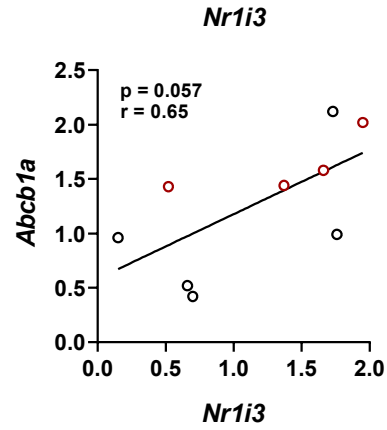
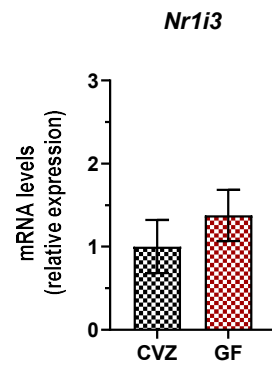
Median
small intestine



Distal
small intestine



Colon



Liver

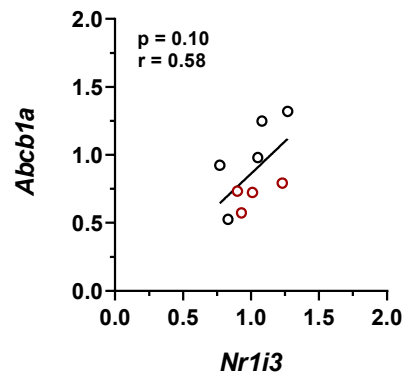
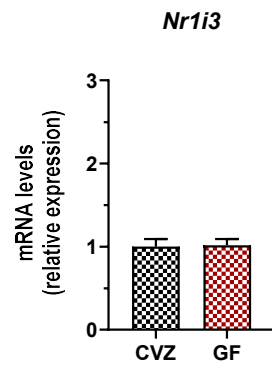


Figure S7. mRNA expression of CAR correlates with *Abcb1a* levels in mouse intestine. In mice, the transcription factor CAR is encoded by the *Nr1i3* gene. (A) Comparison of the mRNA expression of *Nr1i3* in the proximal, median and distal small intestine, in the colon, and in the liver of conventionalized (CVZ) and germ-free (GF) mice (n = 4-5/group). **p < 0.01 and *p < 0.05. (B) Spearman's correlation between *Nr1i3* and *Abcb1a* mRNA expression levels in the different tissues.

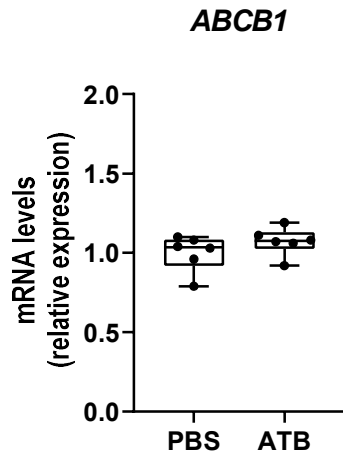


Figure S8. Antibiotics (ATB) do not affect *ABCB1* expression *in vitro*. Comparison of the mRNA expression of *ABCB1* in LS174T cells treated with ATB or vehicle (PBS) for 48 hours (n = 6/group, representative of two independent experiments).