Supplementary figures

Reduced spontaneous itch in mouse models of cholestasis

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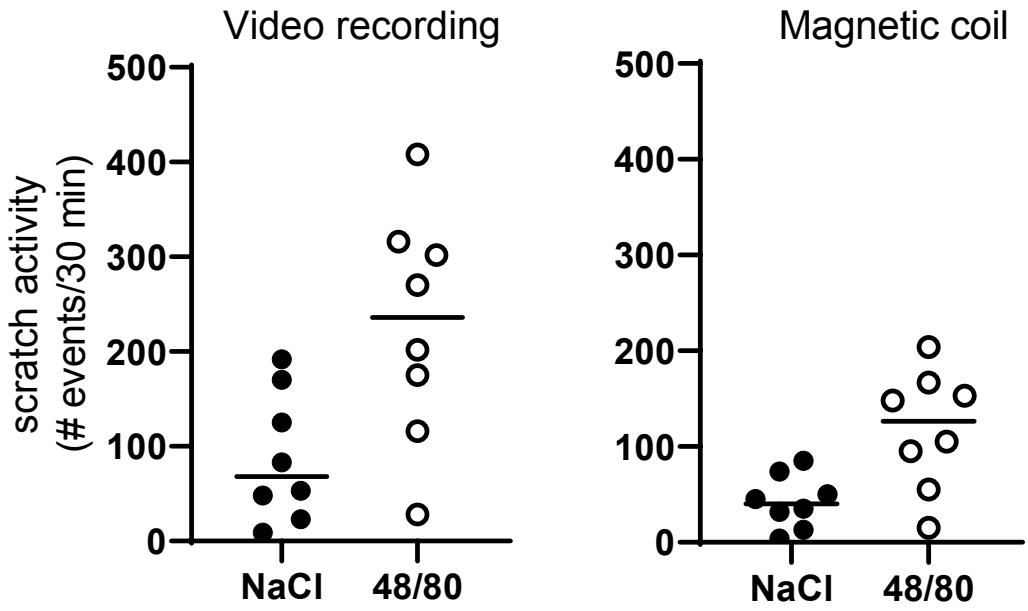
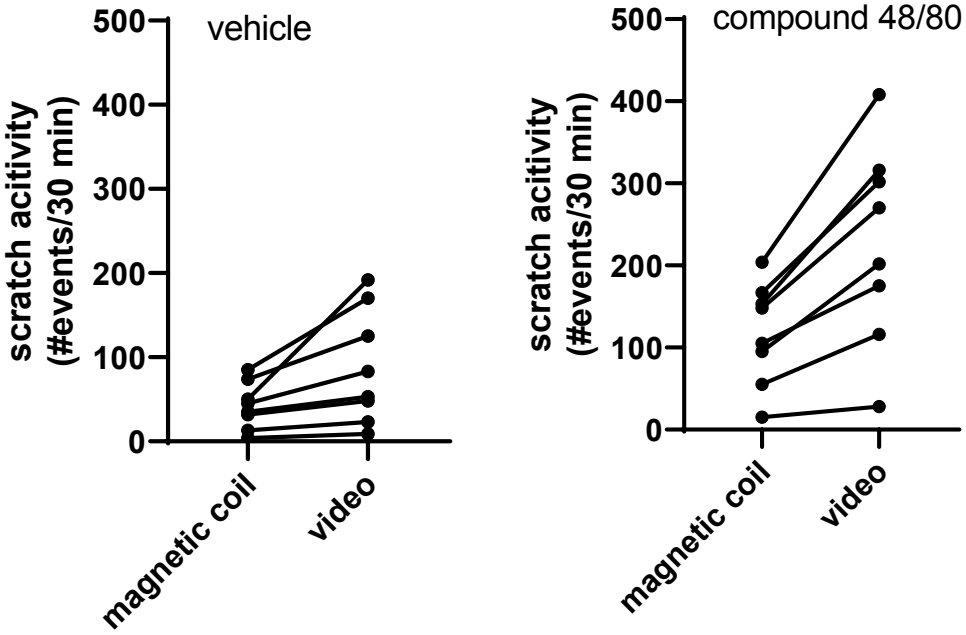
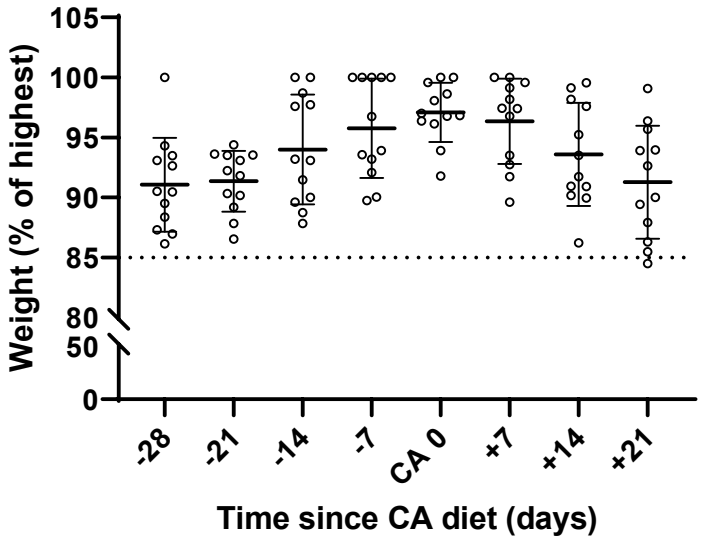


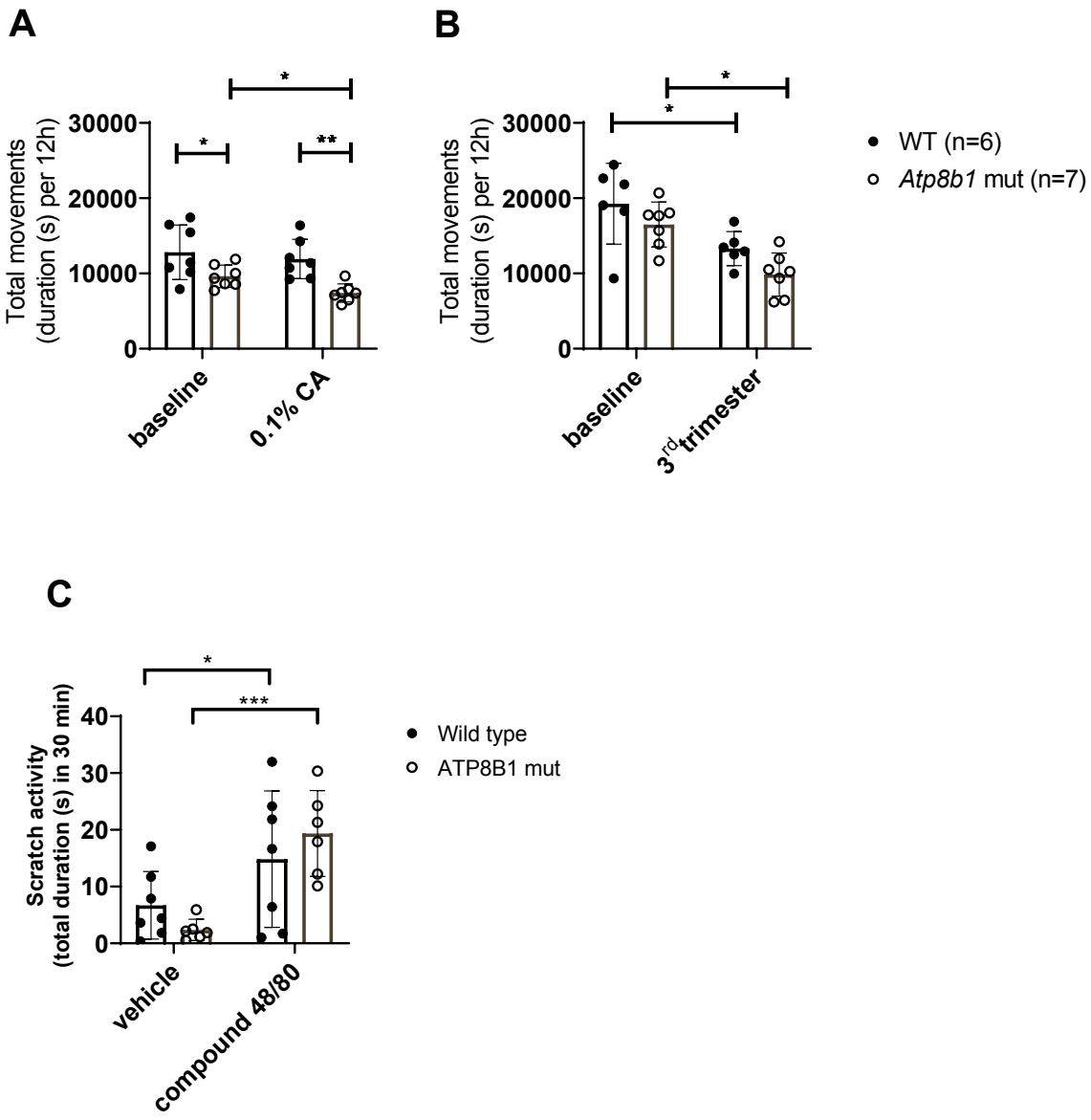
Supplementary figure 1A: Scratch activity (in seconds) measured during 30 minutes immediately after intradermal injection of 0.9% NaCl (50µl) or C48/80 (7µg in 50µl) in WT mice (n=7) or *Atp8b1* mutant mice (n=6) that where on a 0.1% CA diet for 17 days. Bars depict mean ± SD. Statistics: paired t-test; * p-value <0.05, ** p-value <0.01.



Supplementary figure 1B: Comparison of scratch activity recording by magnetic coil or videorecording. Scratch activity (in events) was measured during 30 minutes immediately after intradermal injection of vehicle (saline) or C48/80 (7µg) in WT mice (n=8) and scratch events were monitored simultaneously by magnetic oil and by video recording

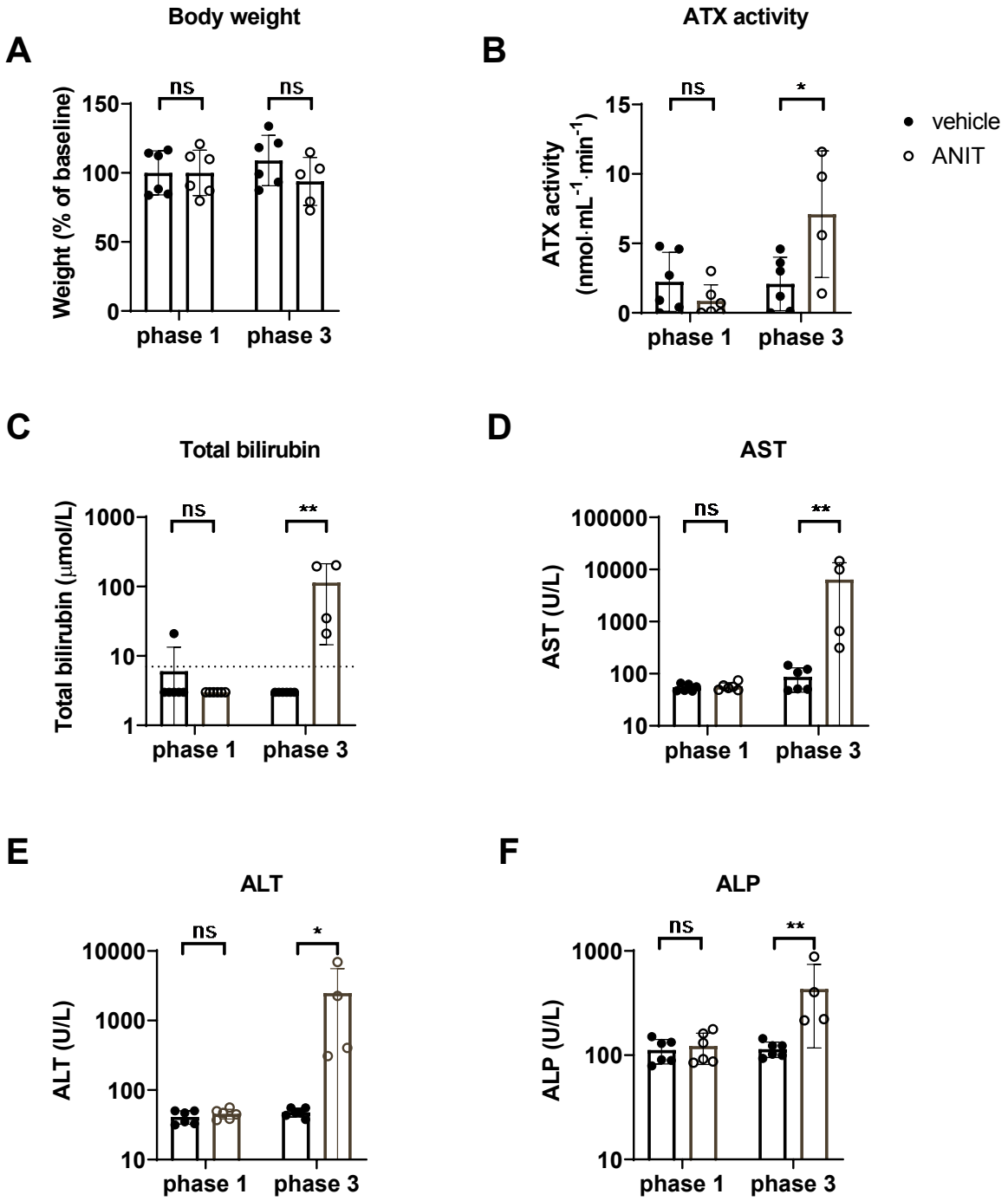


Supplementary figure 2: Weight (in percentage of highest measured weight of each animal) of *Atp8b1* mutant mice (n=12) before and after 0.1% CA diet (8-10 weeks old at start of measurements). Time in days relative to the start of CA diet. The dotted line indicates the critical humane end point of 15% weight loss. Bars depict mean ± SD.



Supplementary figure 3: Total movements (as mean duration in seconds over four consecutive nights per experimental condition) in wild type mice on a semi-synthetic reference diet (n=7 females; n=6 pregnant females) and *Atp8b1* mutant mice on a semi-synthetic reference diet with supplementation of 0.1% CA from day 12 of pregnancy (n=7 females; n=7 pregnant females), before (**A**) and during pregnancy (**B**).

Wild type and cholestatic Atp8b1 mice are equally sensitive to the pruritogen compound 48/80 (**C**). Mice were fed a 0.1% CA-supplemented diet as indicated in Fig. 1. Subsequently, the animals received an intradermal injection of compound 48/80 (7µg in 50 µl) and scratch activity was measured for 30 min. Bars depict mean ± SD. Statistics: two-way ANOVA followed by Sidak's post hoc test; within genotypes all symbols are shown, between genotypes only symbols are shown when significant; ns: not significant, * p-value <0.05, ** p-value <0.01.



Supplementary figure 4: (**A**) Body weight (in percentage of baseline), (**B**) plasma ATX activity (in nmol·mL^{-1}·min^{-1}), (**C**) plasma total bilirubin (µmol/L), (**D**) plasma aspartate aminotransferase (AST) (U/L), (**E**) plasma alanine aminotransferase (ALT) (U/L), (**F**) plasma alkaline phosphatase (ALP) (U/L). All measured in WT mice during baseline scratch measurement (phase 1) and after oral treatment of vehicle (n=8) or ANIT (n=8) (phase 3). Bars depict mean ± SD. Statistics: two-way ANOVA followed by Sidak's post hoc test; ns not significant, * p-value <0.05, ** p-value <0.01.