Manganese transporter Slc30a10 controls physiological manganese excretion and toxicity

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Supplemental Figures and Methods Video

Figure S1. Generation of mice with global and conditional Slc30a10 deficiency, related to Methods. (A) Generation of mice. Numbered boxes represent exons; unnumbered light blue boxes represent introns. Start (ATG) and stop codons (STOP) are noted. *loxP*-flanked region encodes 246 of 487 C-terminal residues. Slc30a10-deficient (*neo/neo*) mice were generated using targeted inactivation by homologous recombination (sites indicated by angled dashed lines). *Neo/neo* mice were bred to mice expressing beta-actin promoter-driven Flp recombinase to delete *lacZ* and neomycin resistance (*neo*) genes, resulting in a conditional (*lox*) allele. (B) *Lox/lox* mice were bred to mice expressing beta-actin promoter-driven Cre recombinase (*Act*). Progeny were intercrossed to generate mice with global Slc30a10 deficiency devoid of *Act* (*KO/KO*). (C-E) *Lox/lox* mice were bred to mice expressing Cre recombinase transgenes driven by various promoters to induce tissue-specific deficiencies: albumin promoter for hepatocytes (*Alb*) (C); villin promoter for enterocytes (*Vil*) (D); both for hepatocytes and enterocytes (*Alb Vil*) (E). (F) Mice expressing Slc30a10-GFP fusions (*Slc30a10*$^{GFP/GFP}$) were created by in-frame insertion of a sequence immediately before the stop codon of *Slc30a10* encoding an mClover3 cDNA flanked by $(GGGGS)_2$ linkers and a 3xFLAG epitope.

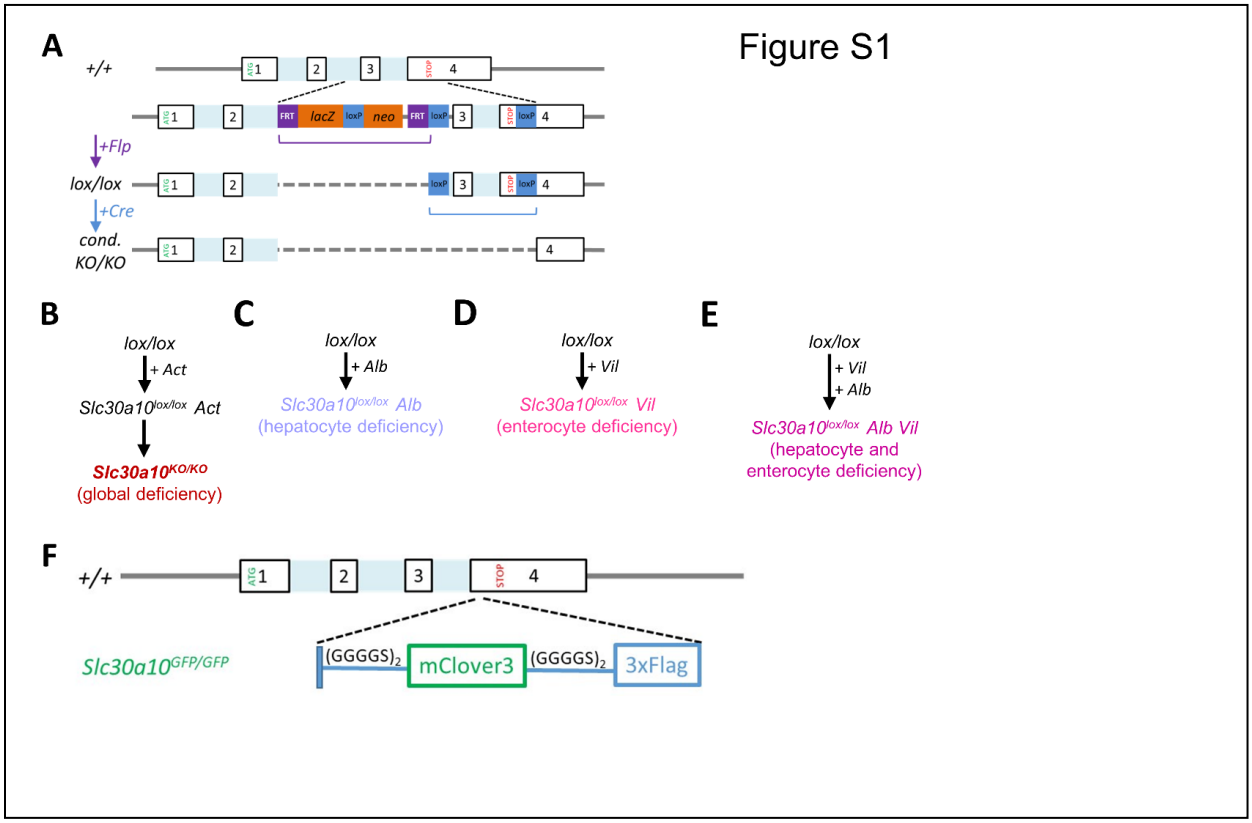


Figure S2. Tissue metal levels in eight-week-old *Slc30a10*$^{+/+}$ and *Slc30a10*$^{KO/KO}$ mice, related to Figure 1. Manganese (A), copper (B), iron (C), and zinc (D) levels in pancreas, heart, kidney, and spleen. Data presented as individual values; bars represent mean ± SEM. 5-9 replicates/group. Outliers identified by ROUT (Q=1%) (outliers not shown). Two-tailed P values calculated by unpaired t-tests. Removal of outlier in (A) (11.49 in male *Slc30a10*$^{+/+}$ pancreas) changed P value from >0.05 to <0.05. Removal of three other outliers in (A) and one each in (C,D) did not impact identification of comparisons with P values <0.05. No outliers identified in (B). (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001)

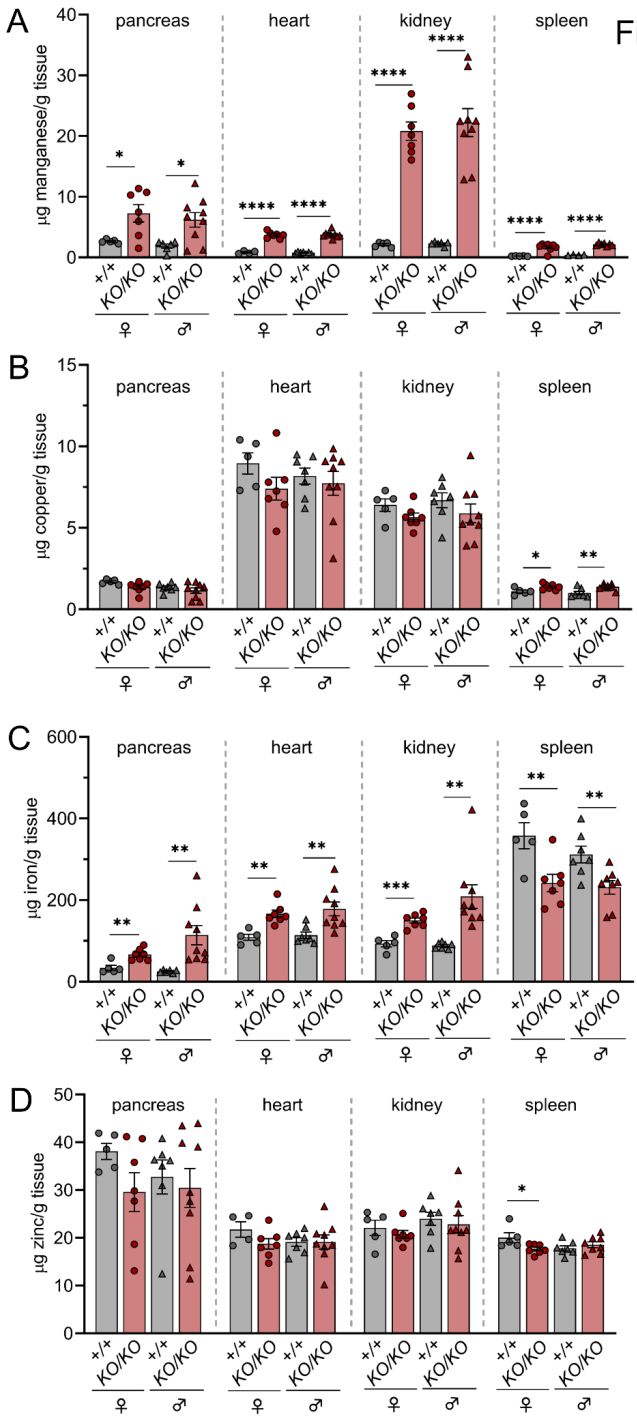


Figure S3. Tissue metal levels in eight-week-old *Slc30a10*$^{+/+}$, *Slc30a10*$^{+/KO}$, and *Slc30a10*$^{KO/KO}$ mice, related to Figure 1. Copper (A,B), iron (C,D), and zinc (E,F) levels in blood (A,C,E) and tissues (B,D,F). Data presented with individual values; bars represent mean ± SEM. 5-9 replicates/group except female *Slc30a10*$^{+/KO}$ (4). Outliers identified by ROUT (Q=1%) (outliers not shown). P values calculated by one-way ANOVA with Tukey's multiple comparisons test. No outliers identified for (A-C,E,F). Removal of outlier in (D) did not impact designation of comparisons with P <0.05. (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001)

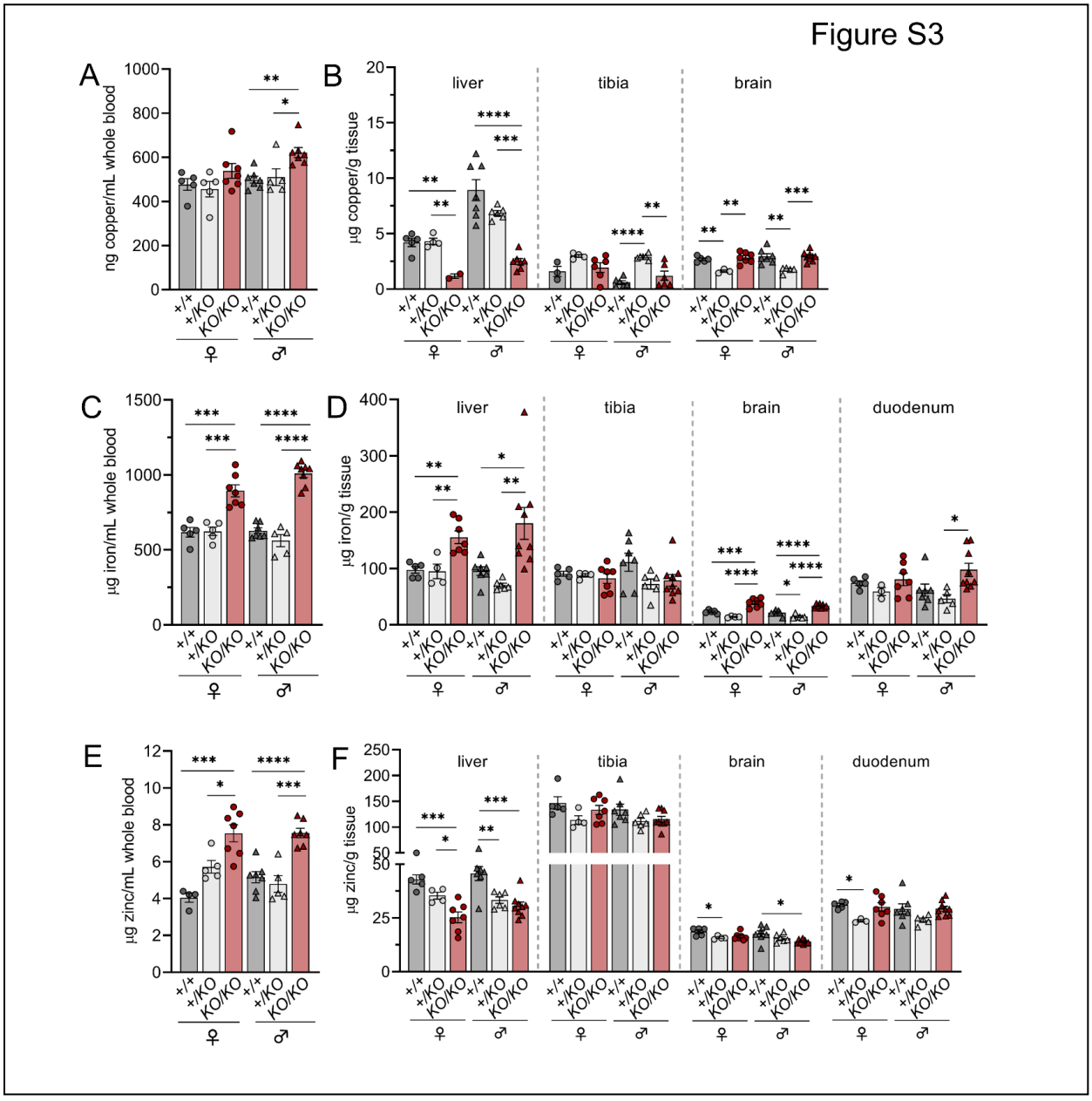


Figure S4. *Slc30a10*$^{+/+}$ and *Slc30a10*$^{KO/KO}$ organ histology, related to Figure 1. Organs were harvested from eight-week-old mice, fixed, embedded, sectioned, and stained with hematoxylin and eosin. Images, representative of sections from several female and male mice, shown at 20x (liver, duodenum, pancreas) or 10x (spleen). 100 or 200 µm scale bar.

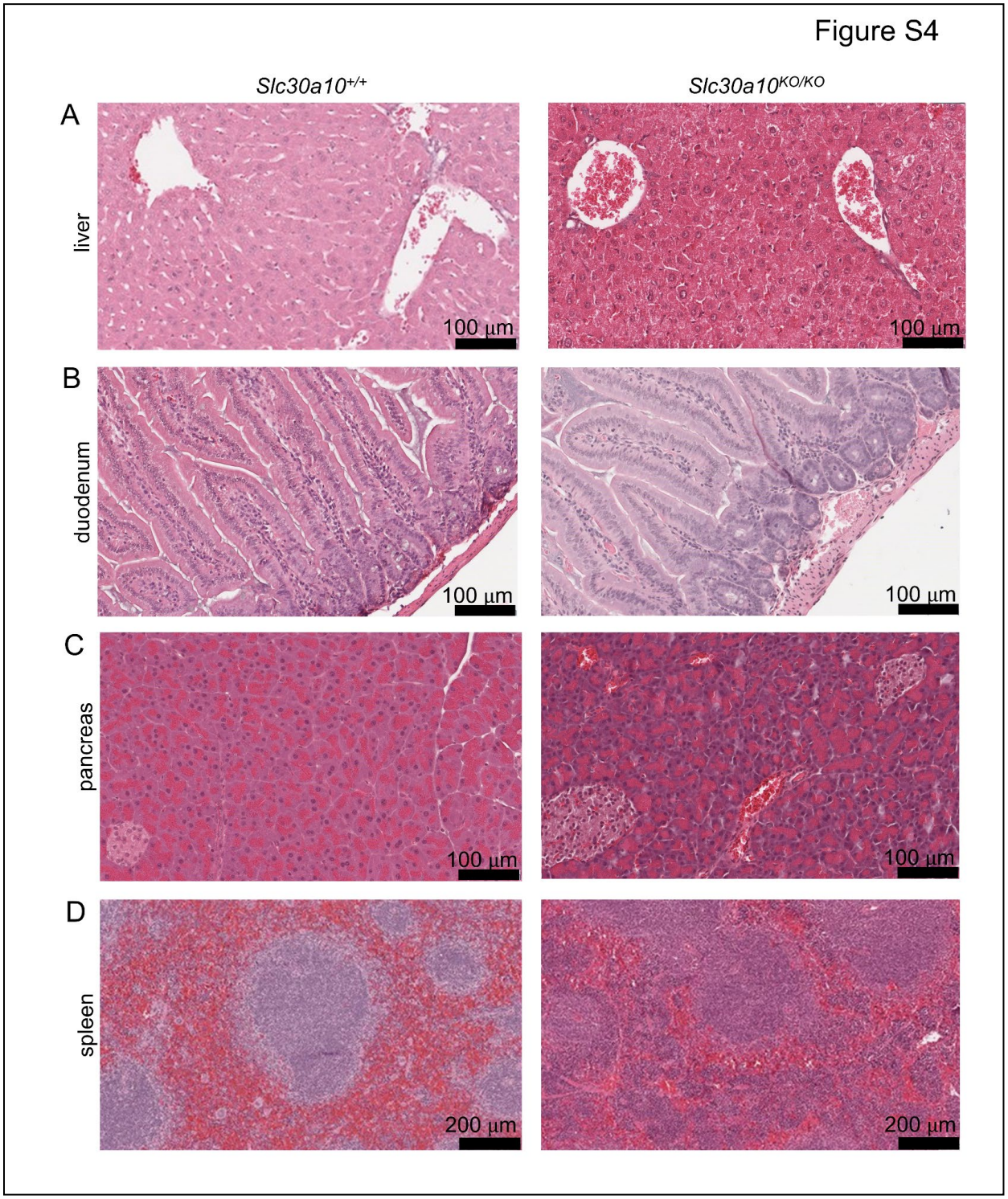


Figure S5. Transporter RNA levels in liver, brain, and duodenum of wild-type mice raised on control or high manganese (Mn) diets and *Slc30a10*$^{+/+}$ and *Slc30a10*$^{KO/KO}$ mice, related to Figures 1-5. (A, C, E) *Slc30a10*, *Slc39a8*, and *Slc39a14* RNA levels normalized to *Hprt1* in liver (A), brain (B), and duodenum (C) of eight-week old C57BL/6NJ mice raised on control or high Mn diets. (B, D, E) *Slc39a8* and *Slc39a14* RNA levels normalized to *Hprt1* in liver (B), brain (D), and duodenum (E) of eight-week *Slc30a10*$^{+/+}$ and *Slc30a10*$^{KO/KO}$ mice. Data presented with individual values; bars represent mean ± SEM. 4-7 replicates/group. No outliers were identified by ROUT (Q=1%). Two-tailed P values calculated by unpaired t-test. (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001)

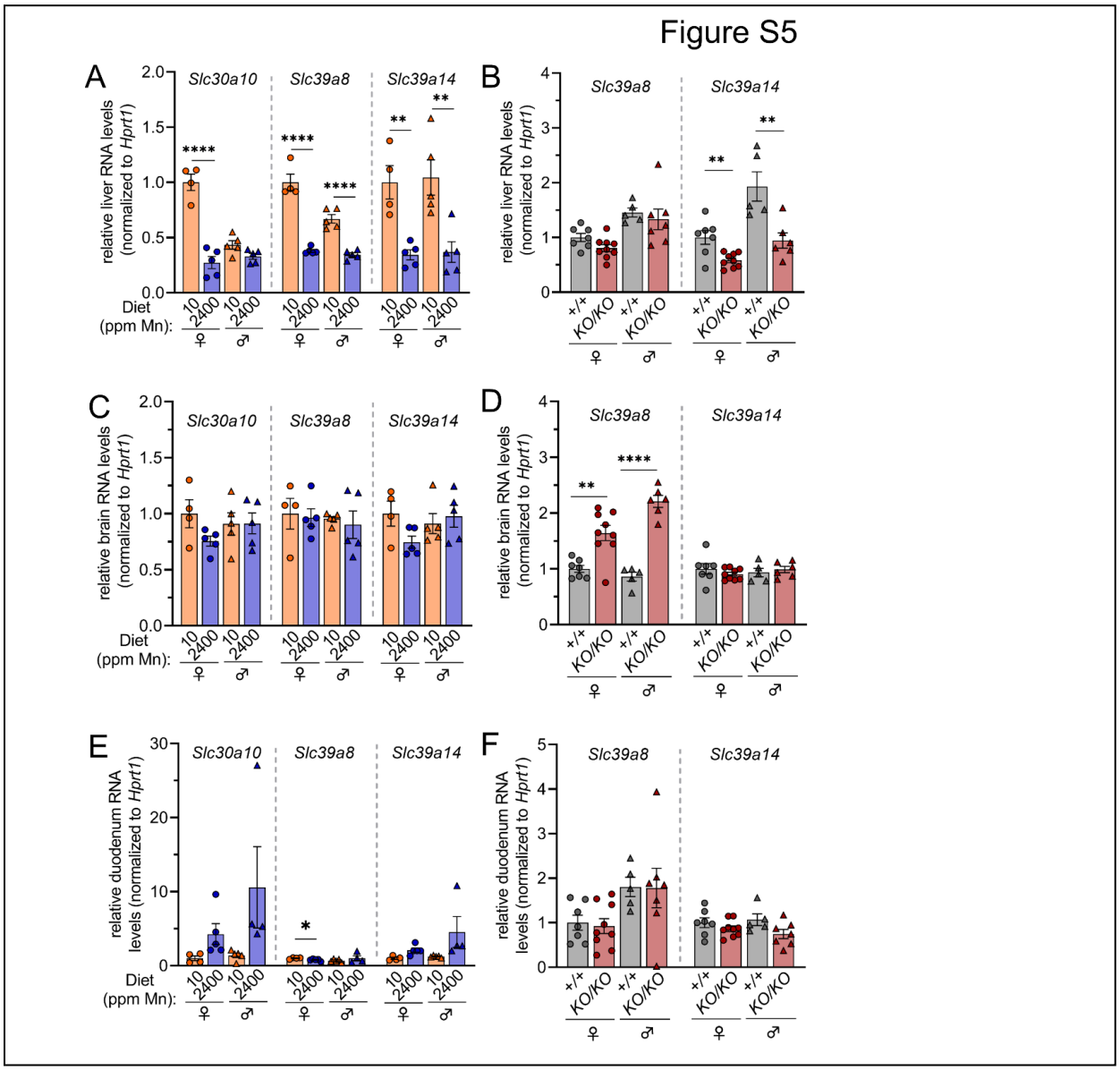
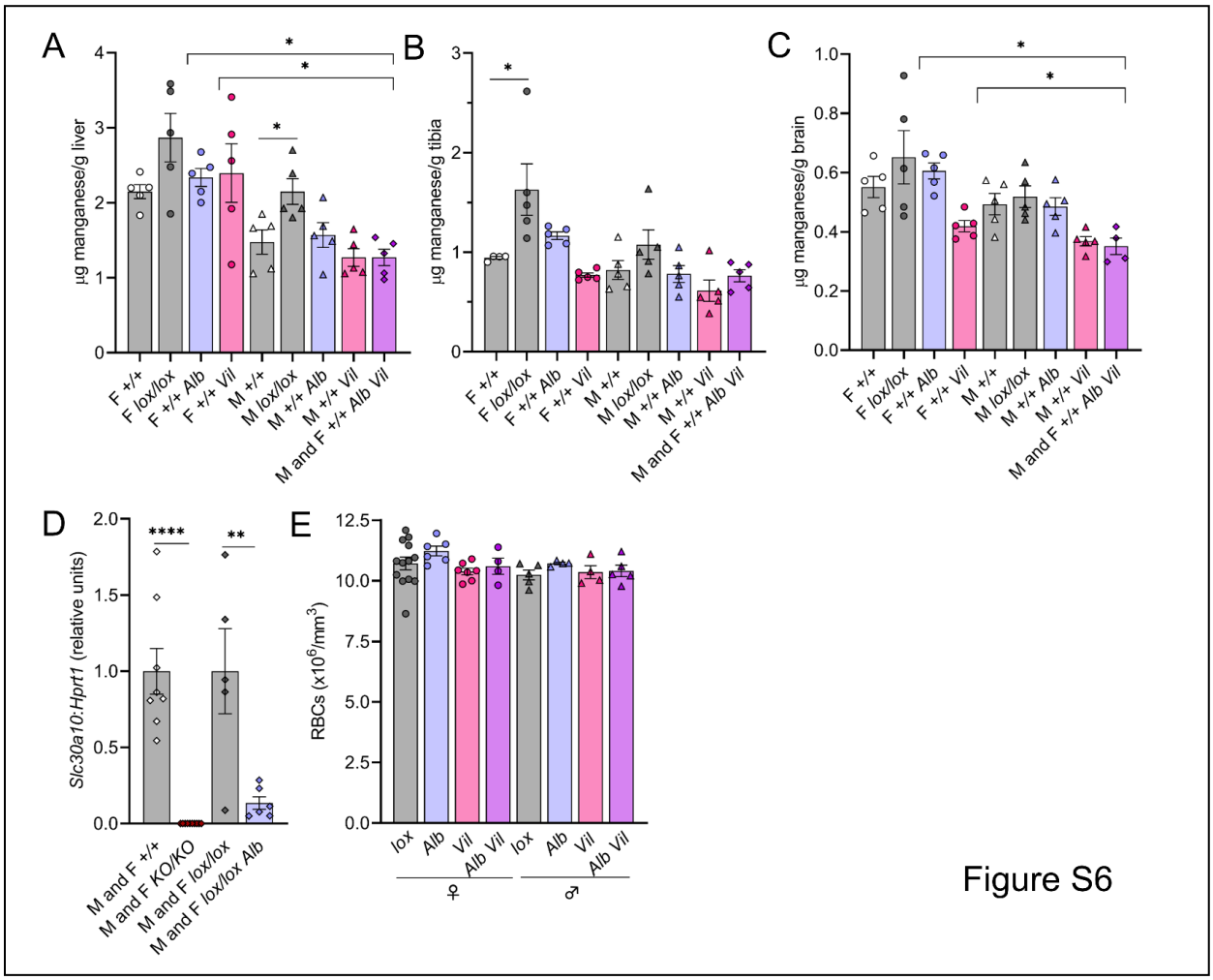


Figure S6. Effects of various *Slc30a10* genotypes and Cre transgenes on metal levels, *Slc30a10* RNA levels, and red blood cell counts, related to Figures 6-10. (A-C) Manganese levels in liver (A), bone (B), and brain (C) in four-month-old *Slc30a10*$^{+/+}$, *Slc30a10*$^{lox/lox}$, *Slc30a10*$^{+/+}$ *Alb,* *Slc30a10*$^{+/+}$ *Vil,* and *Slc30a10*$^{+/+}$ *Alb Vil* mice. (D) Liver *Slc30a10*:*Hprt1* RNA ratios in *KO/KO* mice normalized to +/+ mice, and *lox/lox Alb* mice normalized to *lox/lox* mice at three weeks of age. (A) Red blood cell (RBC) counts in four-month-old *Slc30a10*$^{lox/lox}$ mice with and without transgenes. Data presented with individual values; bars represent mean ± SEM. 5-13 replicates/group. Outliers identified by ROUT (Q=1%) (outliers not shown). For (A-C, E), P values calculated by one-way ANOVA with Tukey's multiple comparisons test. For (D), two-tailed P values calculated by unpaired t-tests. No outliers were identified for (A,C-E). Removal of one outlier in (B) did not alter identification of P values <0.05. (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001)



Methods Video. Common bile duct ligation, gallbladder cannulation, and portal vein injection, related to Figures 4, 5, 8, and 11. 0 seconds: Needle with suture is passed between bile duct and portal vein. One end of suture is passed through and tied in place using an instrument tie. Suture is clipped. 6 seconds: Gallbladder is mobilized by snipping connective tissue. 15 seconds: Suture, with knot pre-tied, is placed around gallbladder and partially tightened. 21 seconds: With gallbladder held in place with forceps, incision is made in tip of gallbladder. 26 seconds: Tubing is inserted into gallbladder. 32 seconds: Suture around gallbladder and tubing is tightened. 45 seconds: A 35g needle is used to inject fluid into portal vein.