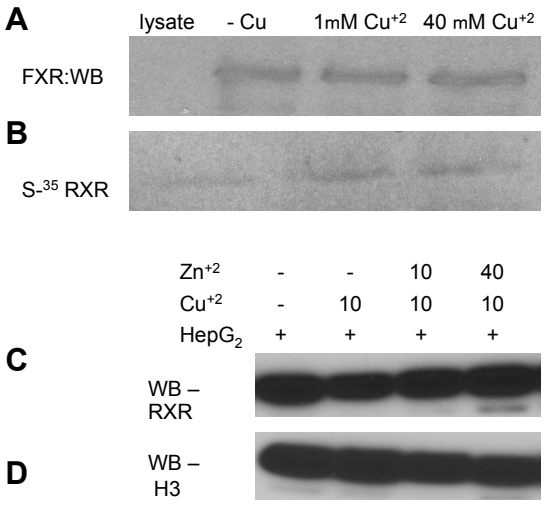
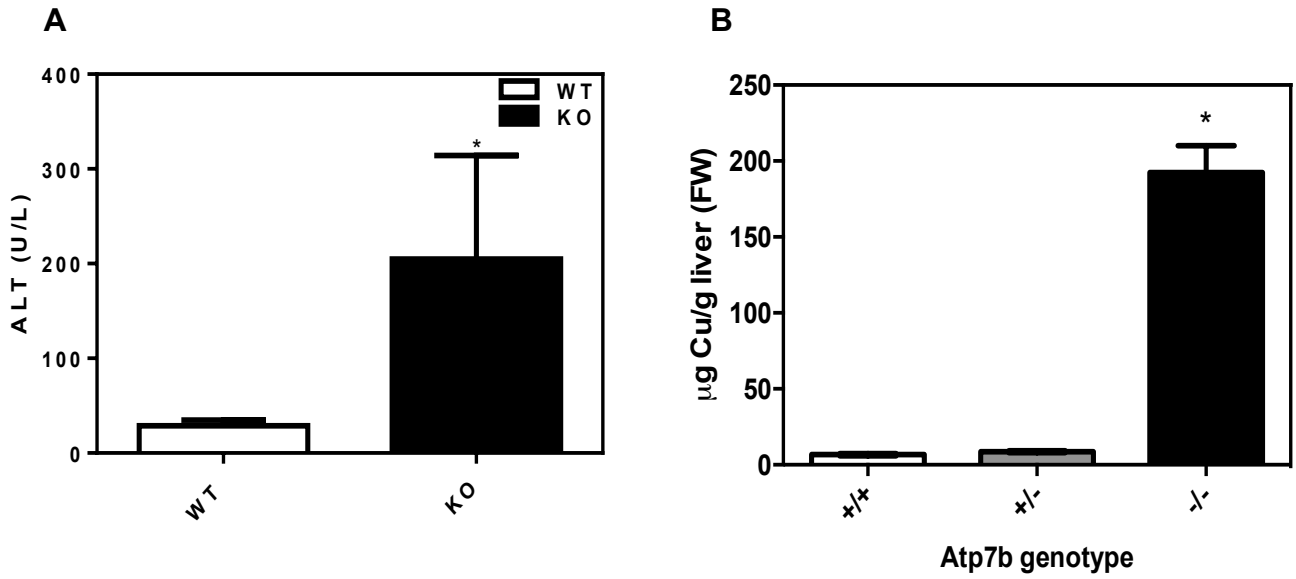
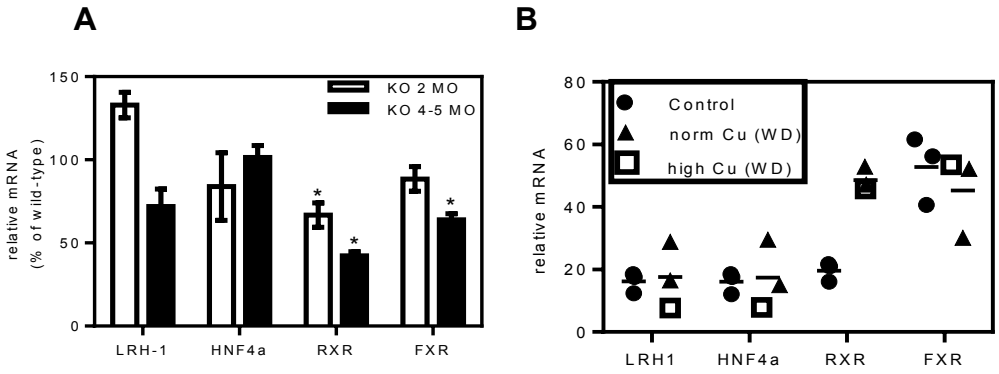


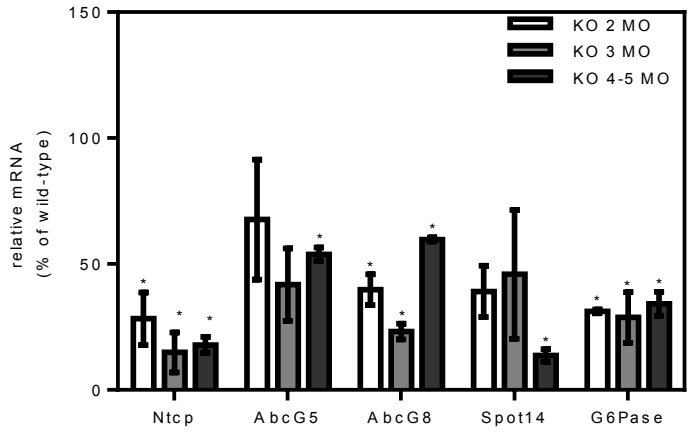
Supplemental Figure 1. FXR and RXR are not degraded with copper treatment. (**A**) Western blot of FXR or (**B**) S^{35}-labelled RXR from an in vitro translation reaction without or with 1 mM or 40 mM Cu in the synthetic reaction. (**C**) Western blot of nuclear extracts for RXR protein expression from HepG2 cells treated with 10 mM copper sulfate, 10 mM copper sulfate + 10 mM zinc sulfate, and 10 mM copper sulfate and 40 mM zinc sulfate. **(D)** Histone H3 was used as the loading control.



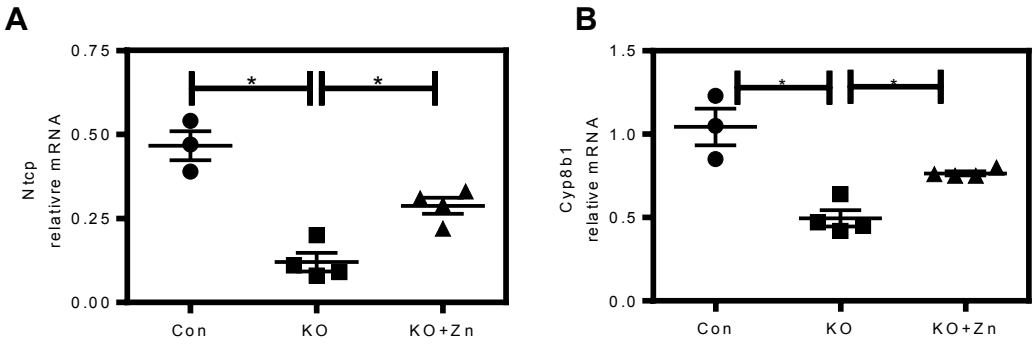
Supplemental Figure 2. (**A**) serum ALT and (**B**) hepatic copper measurements in WT and Atp7b$^{-/-}$ at 3 months of age. In panel (**B**) WT, Atp7b$^{+/-}$, and Atp7b$^{-/-}$ were included. Shown are the mean $\pm$ SEM for n= 4-11 samples, * P<0.05 as determined Student's t-test (**A)** or (**B**) One-Way ANOVA followed by Bonferroni's post-hoc test.



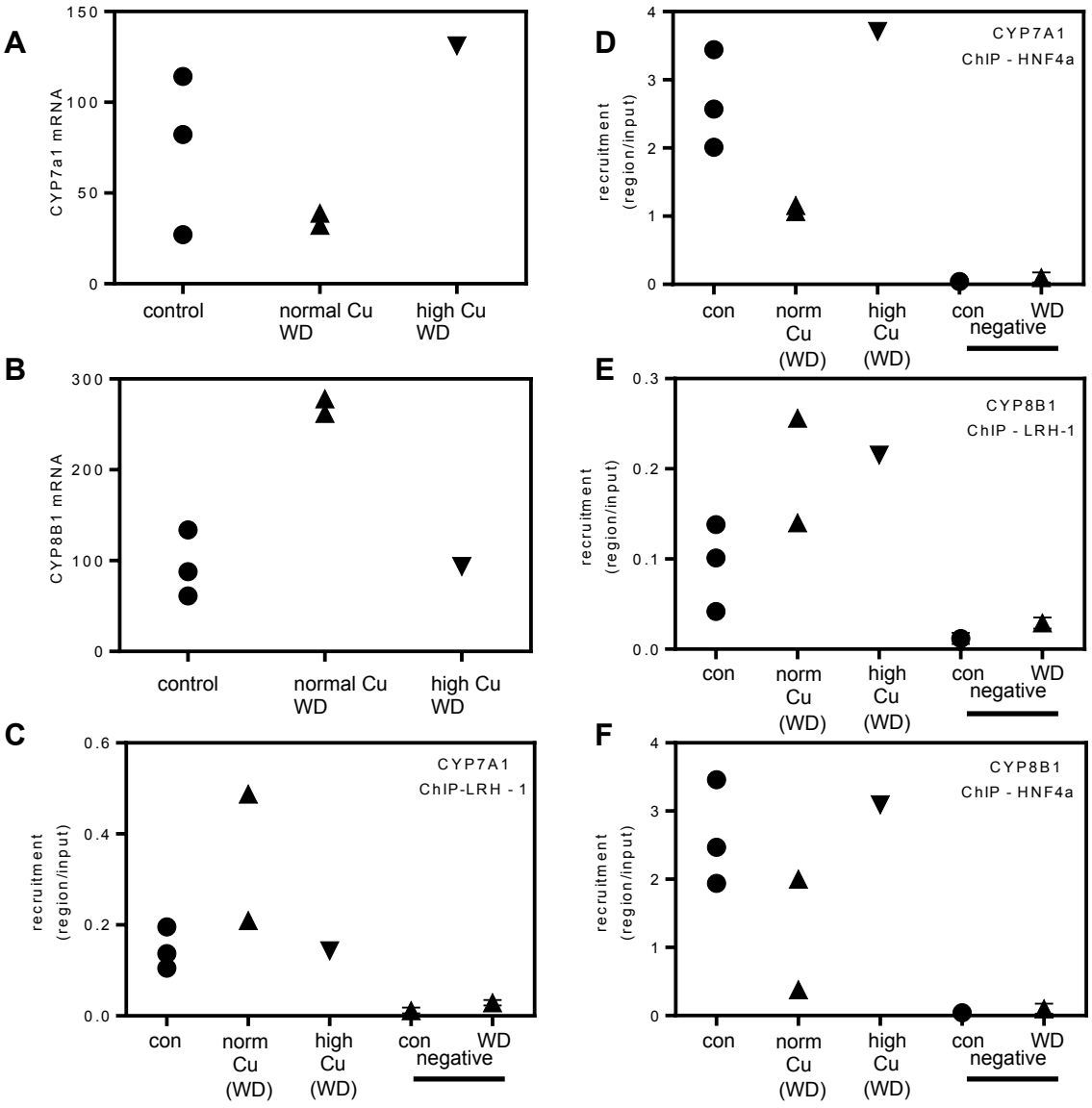
Supplemental Figure 3. Nuclear receptor expression in Atp7b$^{-/-}$ mice (**A)** and adult human (**B**) samples. LRH-1, HNF4a, RXR, and FXR mRNA expression was measured by real-time PCR or Western blot. Shown are the mean ± SEM for panel **A**, n=4-6 samples and * P<0.05 as determined by One-Way ANOVA followed by Bonferroni's post-hoc test. For panel **B**, samples were measured in duplicate and the average plotted for the individual sample, and we performed each experiment 3 times.



Supplemental Figure 4. LRH-1, HNF4a, PPARa, and TR target gene mRNA expression is altered in the Atp7b$^{-/-}$ mice. Ntcp, AbcG5, AbcG8, Spot14, and G6Pase mRNA was measured by real-time PCR for wild-type and Atp7b$^{-/-}$ mice at 2, 3, and 4-5 months of age. For each time point, the Atp7b$^{-/-}$ target gene expression is set as % of the age - matched wild-type control. Shown are the mean $\pm$ SEM for n=4-6 samples, * indicates $P<0.05$ relative to wild-type control by One-Way ANOVA test followed by Bonferroni's post-hoc test.

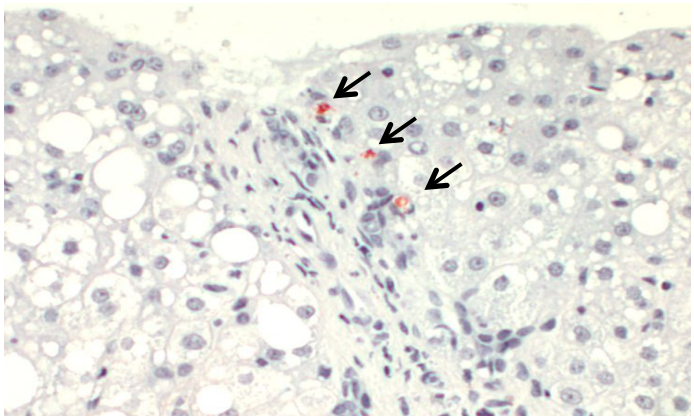


Supplemental Figure 5. In vivo effects of zinc therapy on nuclear receptor activity in Atp7b$^{-/-}$ mice. (**A**) Ntcp, (**B**) Cyp8b1 mRNA expression was measured by real-time PCR for wild-type and Atp7b$^{-/-}$ (KO mice on chow diet or a diet supplemented with 1000 ppm zinc acetate). Shown are the mean $\pm$ SEM for n=3-4 samples per group, * P<0.05 by One-Way ANOVA test followed by Sidak's post-hoc test .

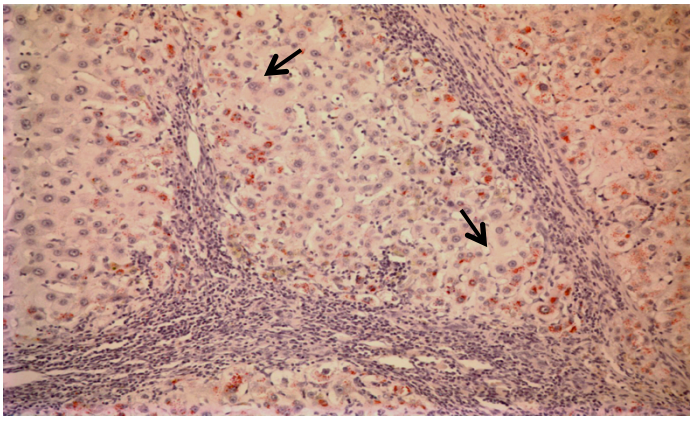


Supplemental Figure 6. LRH-1 and HNF4a activity is not changed in the adult WD samples. **(A)** Real-time PCR analysis for CYP7a1 (**B**) CYP8b1 from adult liver tissue obtained from the NDRI (Philadelphia, PA). ChIP analysis was performed with (**C,E**) LRH-1 (**D**,**F**) HNF4a antibodies. The response elements on each promoter were amplified by real-time PCR and normalized to input. Primers designed for a gene desert region of human chromosome 12 was used as a negative control. For each experiment, samples were measured in duplicate and the average plotted for the individual sample, and we performed each experiment 3 times. All graphs shown are representative figures for 3 separate experiments.

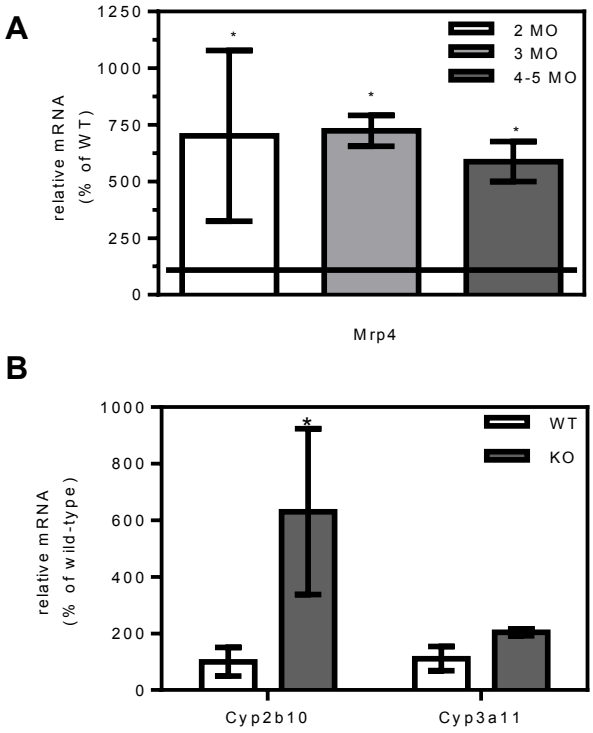
A



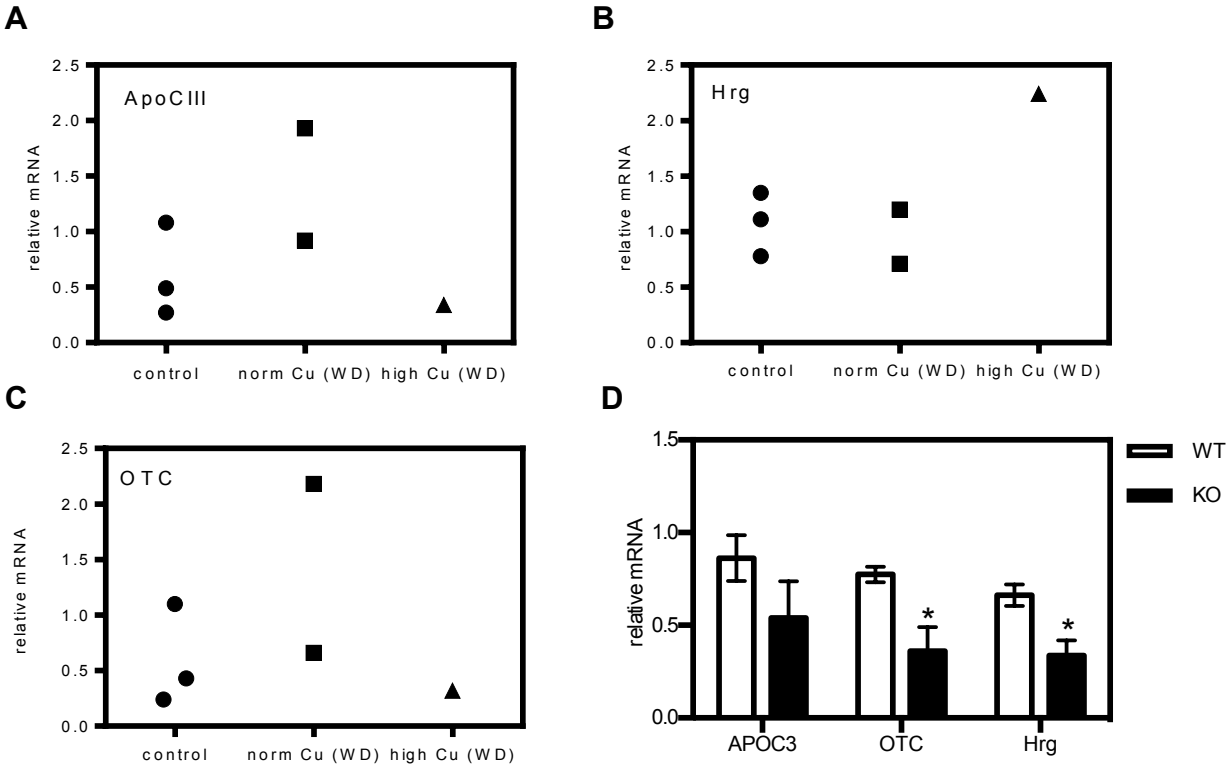
B



Supplemental Figure 7. (A) hepatic copper is demonstrable as granular red deposits in the cytoplasm of zone 1 hepatocytes in a 12 year old with Wilson's disease (x200 magnification). (**B**) A 10 year old boy with PFIC3 has biliary cirrhosis with abundant copper in the same zone 1 hepatocyte distribution (x125 magnification).



Supplemental Figure 8. (**A)** Mrp4 mRNA expression was measured by real-time PCR for wild-type and Atp7b$^{-/-}$ mice at 2, 3, and 4-5 months of age and set as % of control, which is depicted as a solid bar at 100%. (**B**) Cyp2b10 and Cyp3a11 mRNA was also measured by real-time PCR of 2 month old animals. For each time point, the Atp7b$^{-/-}$ target gene expression is set as % of the age-matched wild-type control. Shown are the mean $\pm$ SEM for n=4-6 samples, * indicates $P<0.05$ relative to wild-type control by One-Way ANOVA test followed by Bonferroni's post-hoc test (**A**) or $P=0.0557$ by Student's t-test (**B**).



Supplemental Figure 9. mRNA expression of HNF4a target genes. (**A-C**) mRNA expression of APOCIII, OTC1, and Hrg in adult control and Wilson's disease patients (**D**) and 2 month old wild-type and Atp7b$^{-/-}$ mice. Shown are the mean $\pm$ SEM for n=4-6 samples, * indicates P<0.05 by Student's t-test.