Figure S1. β-galactosidase activity in aMHC-Cre;ROSA-LacZ hearts

Hearts from αMHC-Cre or C57/BL6 crossed with ROSA-LacZ mice were harvested and stained for β-galactosidase.

Figure S2. Conditional inactivation of *Mfrn1* in the liver

(**A**) Livers and spleens from the progeny of Alb-Cre and ROSA-LacZ mice were harvested and stained for β-galactosidase. (**B)**. PCR analysis of genomic DNA from liver of $Mfrn1^{flox/-}$ and *Alb-Cre* ;$Mfrn1^{flox/-}$ mice showing reduced levels of the *Mfrn1* due to *Alb-Cre*. (**C**) RT-PCR was performed on mRNA extracted from livers and spleens of $Mfrn1^{flox/-}$ mice and *Alb-Cre* ;$Mfrn1^{flox/-}$ mice. The primers used were specific for exon 1 and exon 4. (**D**) RT-PCR was performed for Mfrn2 mRNA and actin (control) and Western analysis was performed using antibodies to Mfrn2 and porin on livers from mice with the noted genotypes.

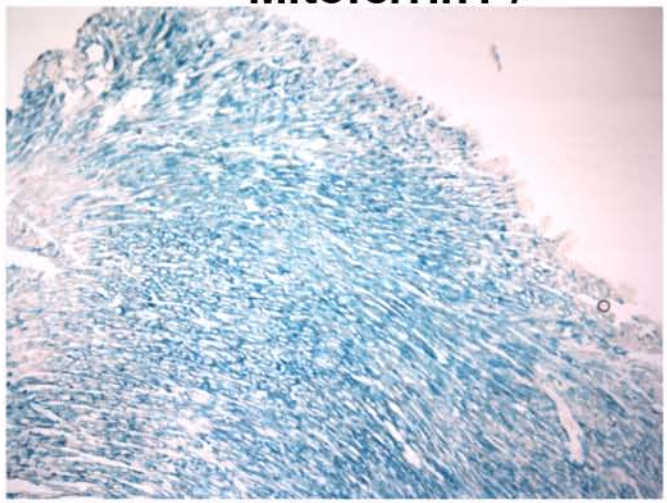
Figure S3. Liver enzyme activity from wild type and hepatocyte-specific *Mfrn1* deleted mice

Livers were dounce homogenized, a postnuclear supernatant obtained and cytosol and membrane fractions analyzed for enzyme activity and protein levels as described in Materials and Methods. **(A)** Xanthine oxidase activity and a representative Western blot of xanthine oxidase and tubulin, (**B)** Cytosolic aconitase activity. Asterisks denote P values of less than 0.05. (**C)** mitochondrial aconitase and (**D)** ferrochelatase were assayed in extracts of livers from mice of the noted genotypes.

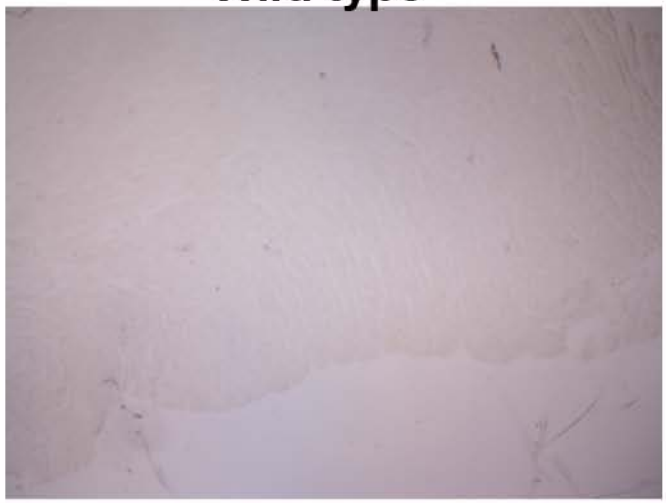
Figure S4. Liver cytochrome complex and malate dehydrogenase activity from wild type and hepatocyte-specific *Mfrn1* deleted mice

Mitochondria were isolated from the livers of mice with the noted genotypes as described in Materials and Methods. Enzyme activities and protein levels were determined on (**A**) Complex II, (**B**) Complex III, (**C**) Complex IV and (**D**) Malate dehydrogenase.

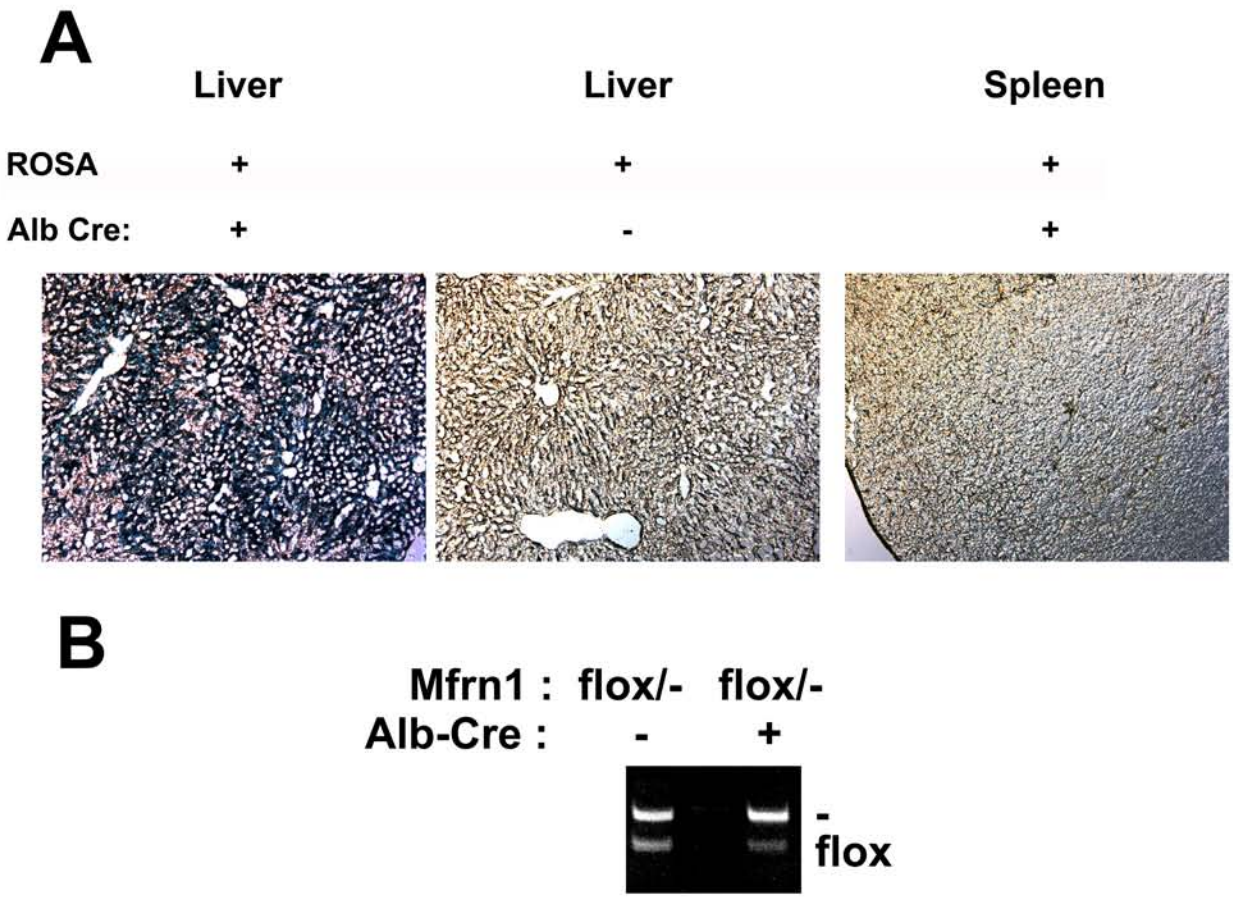
ROSA x αMHC Cre
Mitoferrin1-/-



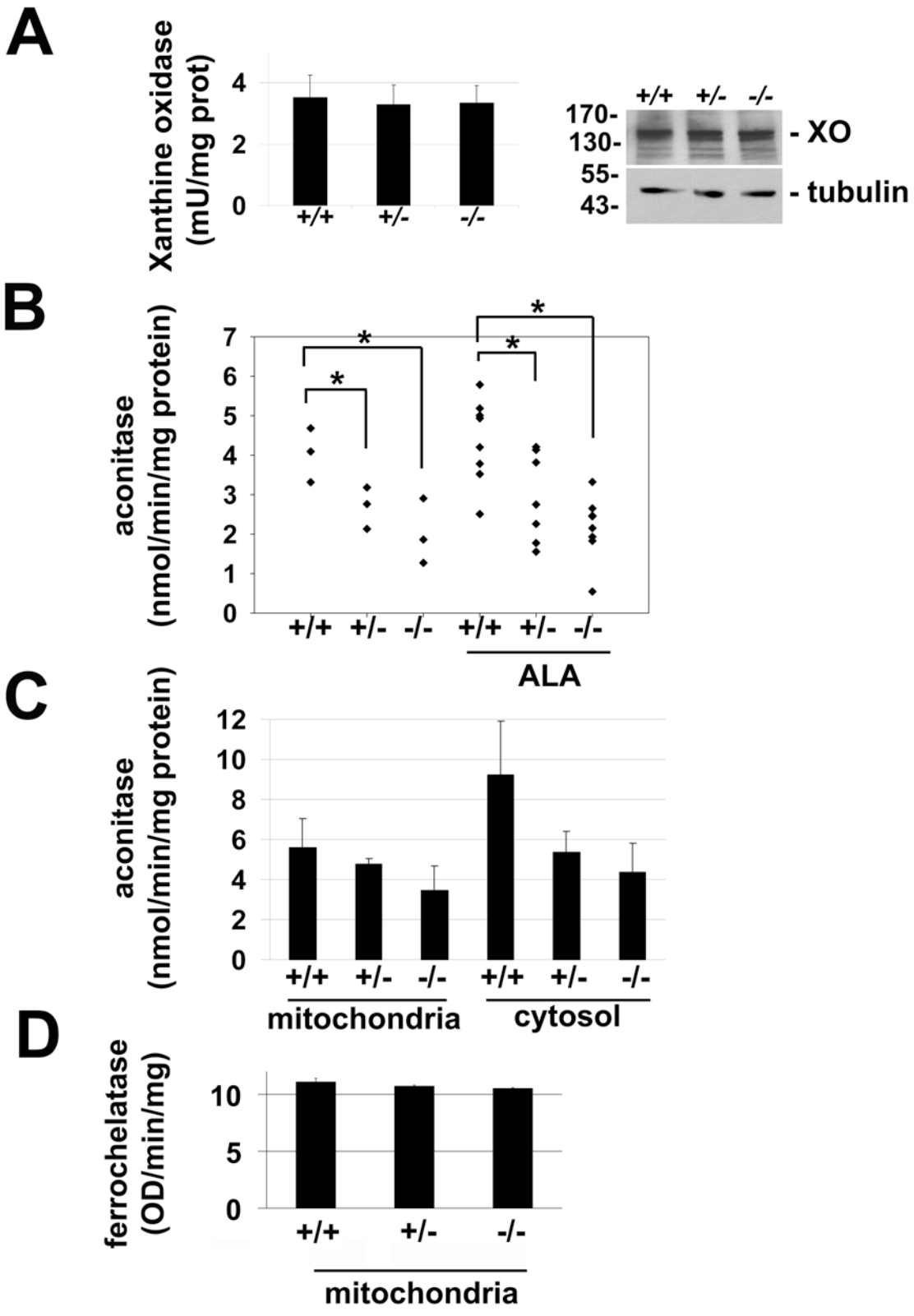
ROSA x C57/BL6
Wild type



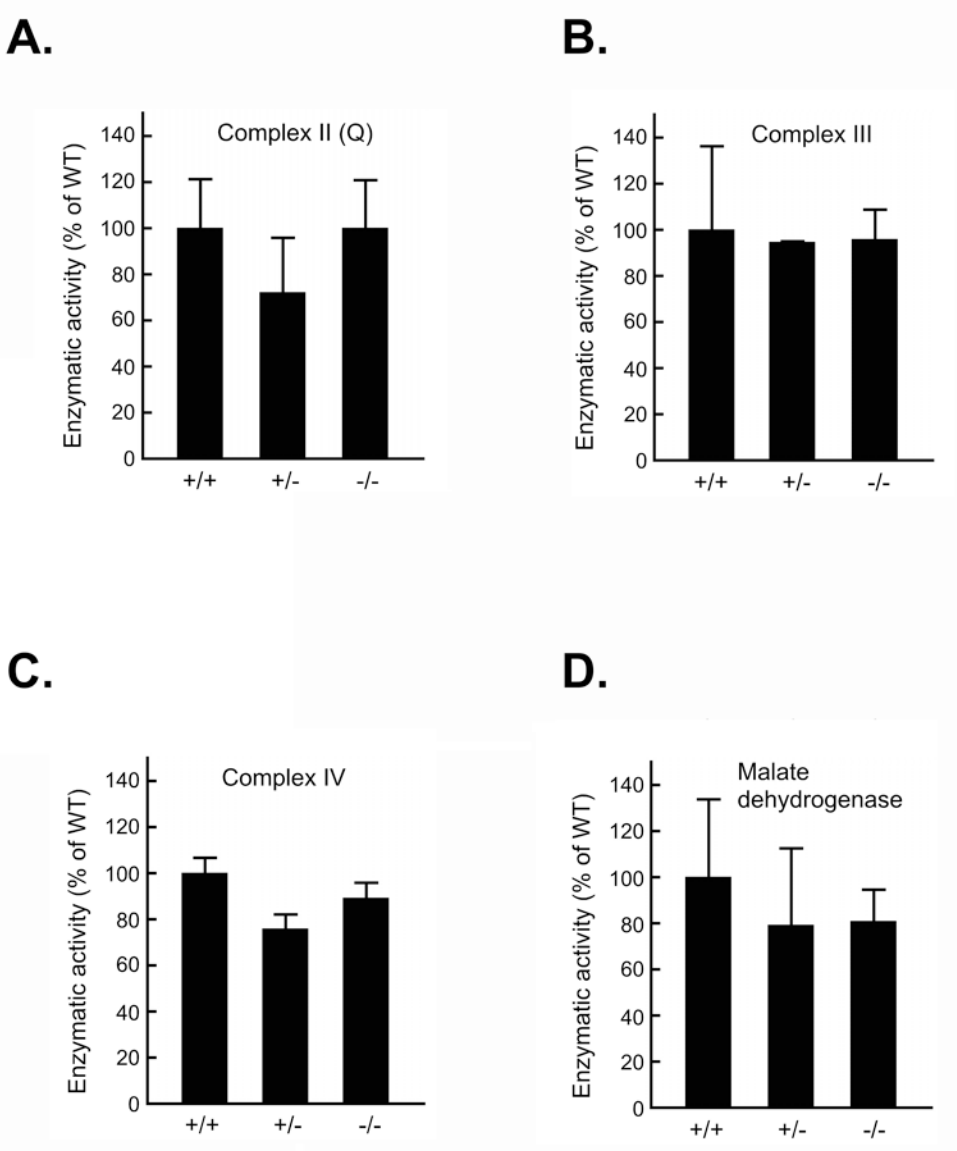
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4