#### **Supplementary Figures**

#### Figure 1

### Liver damage occurs abruptly in $Atp7b^{-/-}$ rats.

A sudden increase of serum bilirubin occurs in  $Atp7b^{-/-}$  animals at around 90 days of age.

#### Figure 2

### Structural alterations are retained in liver mitochondria isolated from *Atp7b<sup>-/-</sup>* rats.

Transmission electron micrographs from:

(A) Liver tissue of an  $Atp7b^{-/-}$  rat (age 70 days) demonstrating severe alterations in mitochondrial ultrastructure with enlarged intermembrane spaces (arrows).

(B-E) Isolated liver mitochondria from a control rat (B), displaying "normal" mitochondria, an  $Atp7b^{-/-}$  rat (C, age 50 d) comprising mitochondria with "altered" morphology, an  $Atp7b^{-/-}$  rat (D, age 70 d) and an  $Atp7b^{-/-}$  rat (E, age 90 d) with "severely condensed" mitochondria comprising strongly enlarged intermembrane spaces and shape alterations.

Scale bar in A-E equals 1 µm.

#### Figure 3

### Clinically apparent *Atp7b<sup>-/-</sup>* livers contain severely damaged mitochondria.

(A) Electron micrograph of an  $Atp7b^{-/-}$  pellet fraction comprising destroyed mitochondria with matrix remnants gathering at cristae relicts at the membrane (arrowheads) and small mitochondrial remnants (arrow). Scale bar equals 1  $\mu$ m.

(B) Immunoblotting analysis of the mitochondrial and associated pellet fraction of three clinically apparent *Atp7b<sup>-/-</sup>* animals (1-3). By detection of mitochondrial marker proteins for the outer membrane (VDAC), intermembrane space (Cyt C), inner membrane (COX IV) and matrix (CYP D, HSP 60) a mitochondrial content was confirmed in all pellet fractions but differed in amount.

#### Figure 4

#### Differential mitochondrial volume changes upon copper challenge.

(A) Copper induces mitochondrial swelling determined in mitochondrial suspensions at 540 nm at non-reducing conditions. In contrast to copper, calcium dependent swelling could be inhibited by CsA. Each data curve represents the average of two to four individual measurements.

(B) In the presence of DTT, repetitive copper dosing induced a "staircase like" increase of light refraction, indicative of mitochondrial contraction. This increase could be reversed by repetitive equimolar doses of the copper chelator methanobactin. Measurement of mitochondrial suspensions was performed at 540 nm in a fluorescence spectrometer.

(C-F) Electron micrographs of one mitochondrial preparation repeatedly treated with copper doses in the presence of DTT, as in Figure 5C-G. (C) 100  $\mu$ M copper /1 mM DTT (D) 200  $\mu$ M copper/1 mM DTT (E) 300  $\mu$ M copper /1 mM DTT, (F) 400  $\mu$ M copper /1 mM DTT. Mitochondria with progressively enlarged cristae and intermembrane spaces and smaller mitochondria with depositions at their membranes (arrows) were found. Scale bars equal 1  $\mu$ m.

#### Figure 5

Representative silver stained BAC/SDS PAGE of mitochondrial membrane proteins enriched by carbonate extraction. Protein load was 200 µg.

#### Figure 6

### *Atp7b<sup>-/-</sup>* animals subjected to copper chelating therapies largely remain healthy.

In contrast to D-PA or methanobactin treated male and female  $Atp7b^{-/-}$  rats, untreated  $Atp7b^{-/-}$  animals develop WD as indicated by (A) a loss of weight and (B) an increase in aspartate aminotransferase (AST). D-PA (C) or methanobactin (D) treated  $Atp7b^{-/-}$  animals showing a liver with preserved architecture and unremarkable cell profiles. (E, F) Liver section from one  $Atp7b^{-/-}$  rat

that did not respond to the methanobactin treatment and developed WD as indicated by necrotic areas (arrows) and single cell apoptoses (stars). Bar equals  $200 \,\mu m$  in C-E and  $50 \,\mu m$  in F.







# В

Mitochondrial Pellet Fraction Fraction						
1	2	3	1	2	3	Animal No.
=	=	=				VDAC
-	-	-		-	-	CYTC
-	-	-		-	-	COX IV
-	-	-	-	-	-	CYP D
<u> </u>	-	-		-	-	HSP 60









