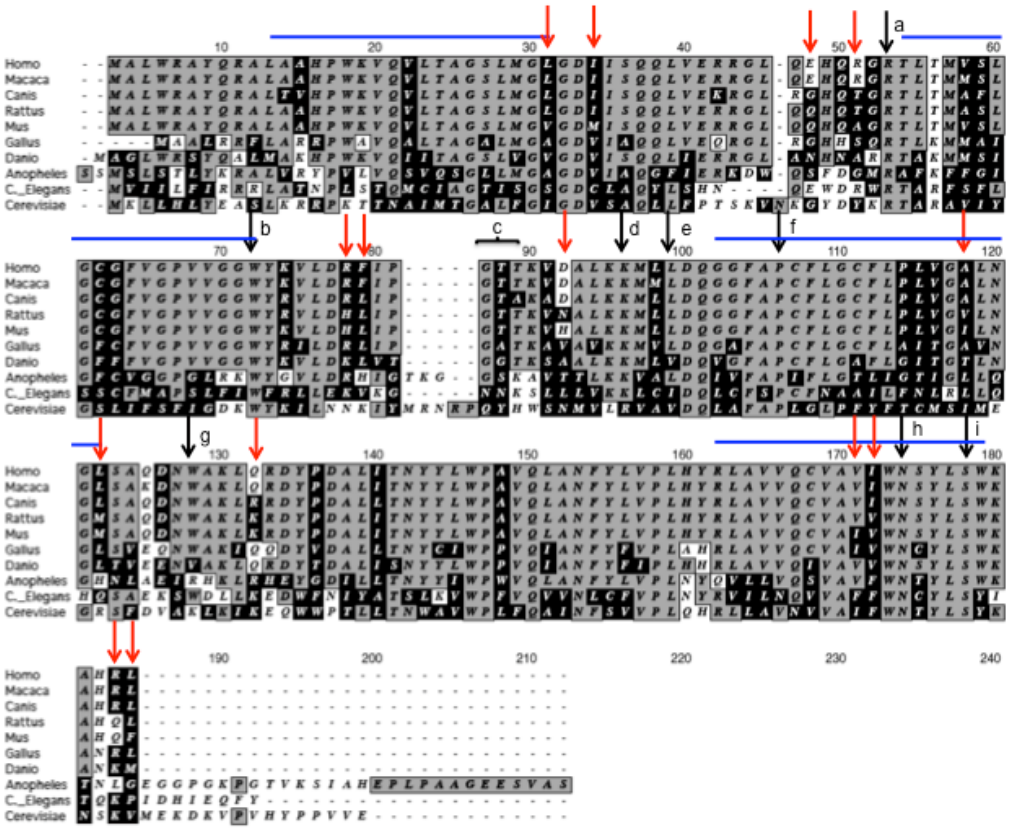
Supplemental Material Online

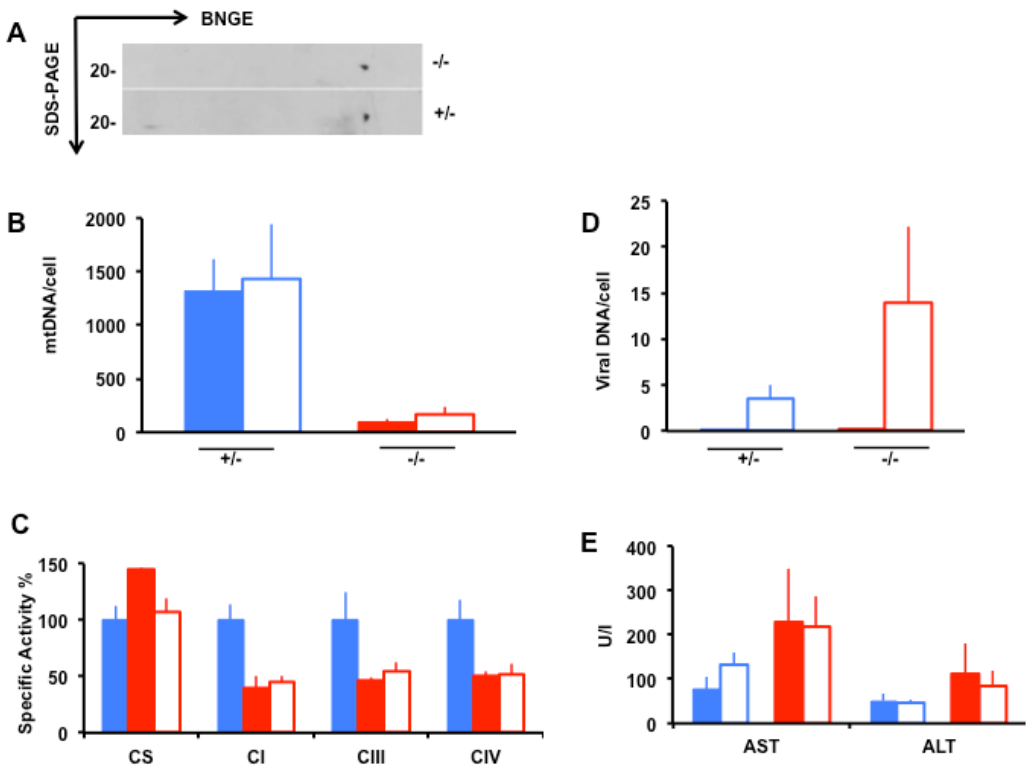
Supplemental Figure 1



Alignment of eukaryotic Mpv17 orthologues. The blue lines indicate the transmembrane domains predicted with TMpred (Hofman and Stofel, 1993). *Shaded grey*: identities; *letters on black background*: similarities; *letters on white background*: mismatches. Note that none of the mismatches between humans and mice was predicted to be deleterious in humans, suggesting preservation of protein function. The *red arrows* show the differences between human and murine proteins. The black arrows and the bracket show the position of residues mutated in patients (note that the numbers in the alignment are NOT referred to the position in humans):

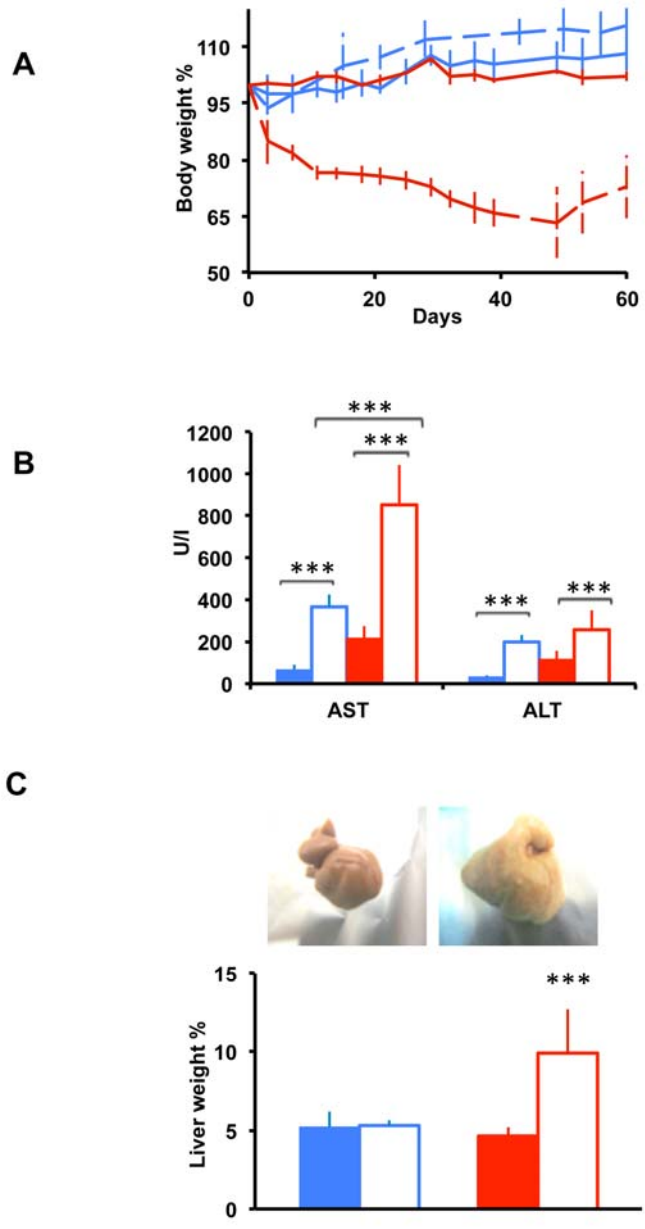
a) R50Q[8]
b) W69X: Wong et al, 2007
c) G79_T81Del[10]
d) K88E; K88Del[13]
e) L91Del[13]
f) P98L[13]
g) W120X[8]
h) N166K[8]

Supplemental Figure 2



AAV-mediated expression of hMPV17-HA in *Mpv17*$^{-/-}$ mice.
A) 2D-BNGE using anti-HA in *Mpv17*$^{-/-}$ and *Mpv17*$^{+/-}$ mice
B) mtDNA analysis. Note that there is no significant difference between AAV-*h.MPV17-HA* treated vs. untreated samples.
C) Biochemical analysis
D) Viral DNA content
E) AST and ALT transaminases levels in plasma
Colour codes: *solid blue:* untreated *Mpv17*$^{+/-}$ mice; *blue outline:* AAV-treated *Mpv17*$^{+/-}$ mice; *solid red:* untreated *Mpv17*$^{-/-}$ mice; *red outline:* AAV-treated *Mpv17*$^{-/-}$ mice. Bars indicate the standard deviation (SD).

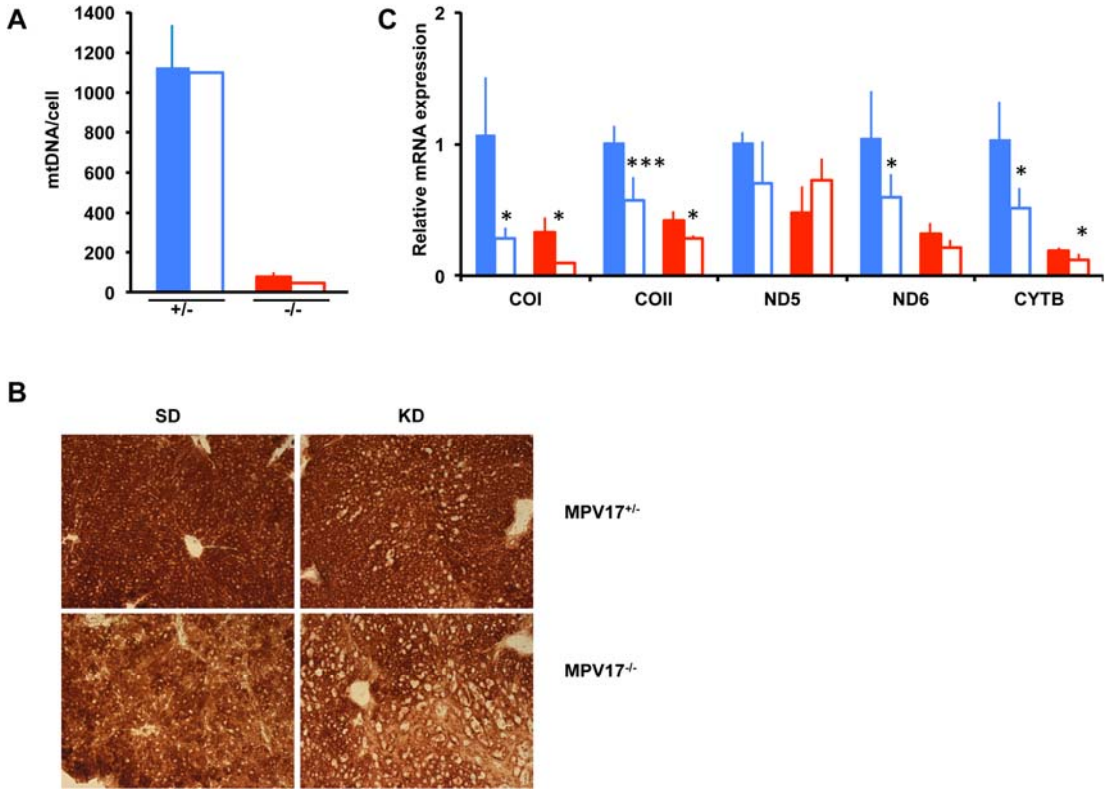
Supplemental Figure 3



Effects of KD on *Mpv17*$^{-/-}$ and control littermates.
A) Body weight changes during 2-months of SD and KD. *Solid blue*: SD-fed Mpv17$^{+/-}$; *dashed blue*: KD-fed Mpv17$^{+/-}$; *solid red*: SD-fed Mpv17$^{-/-}$; *dashed red*: KD-fed Mpv17$^{-/-}$.
B) AST and ALT transaminases levels in plasma
C) Liver weight (as a percentage of body weight). Note that Mpv17$^{-/-}$ liver is yellowish and hugely increased.
Asterisks indicate significance (p) calculated by Mann-Whitney test for unpaired samples: *p < 0.05; **p < 0.01; ***p < 0.0001.
Colour codes: *solid blue:* SD-fed *Mpv17*$^{+/-}$ mice; *blue outline:* KD-fed *Mpv17*$^{+/-}$ mice; *solid red:* SD-fed *Mpv17*$^{-/-}$ mice; *red outline:* KD-fed *Mpv17*$^{-/-}$ mice. Bars indicate the

standard deviation (SD).

Supplemental Figure 4



KD does not induce mitochondrial biogenesis in liver

A) MtDNA content analysis in SD- and KD-fed *Mpv17*$^{+/-}$ and *Mpv17*$^{-/-}$ mice.

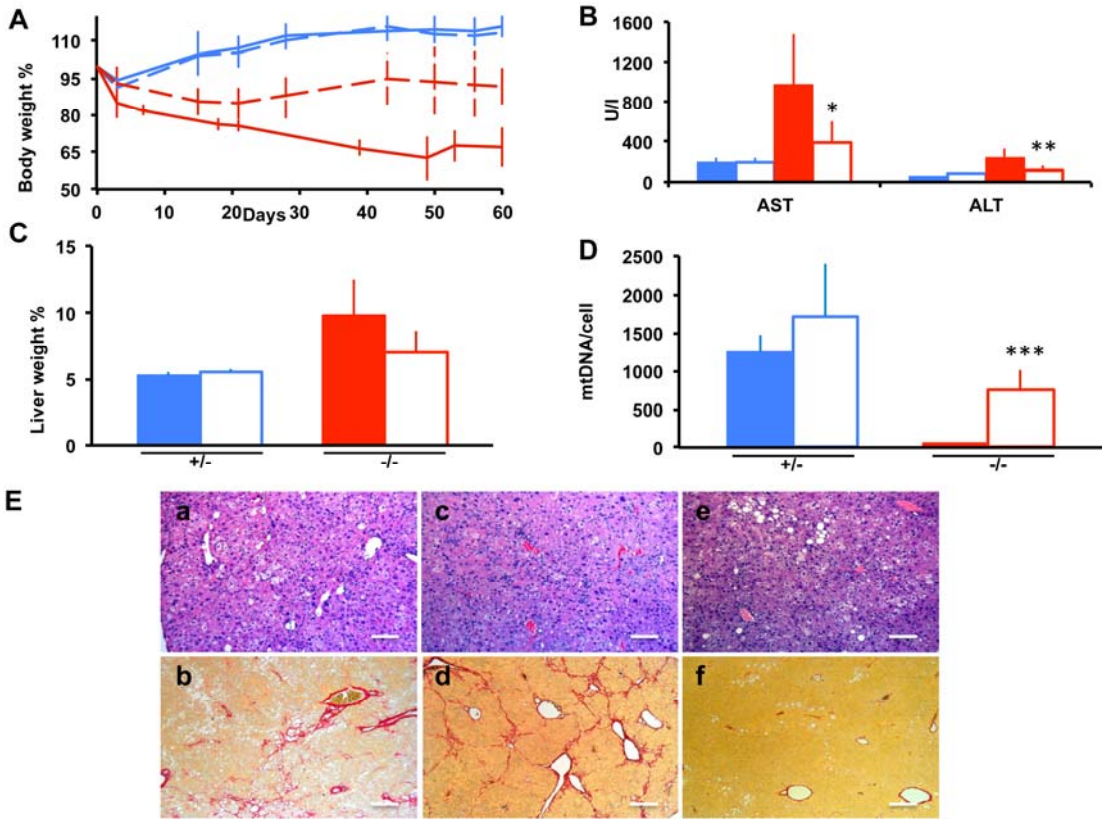
B) COX histochemical staining. Note that COX activity is as much reduced in KD- as in SD-fed animals.

C) mRNA transcription analysis. Note that some of the mitochondrial transcripts are significantly reduced in both control and knockout KD-fed mice.

Asterisks indicate significance (p) calculated by Mann-Whitney test for unpaired samples: *$p < 0.05$; ***$p < 0.0001$

Colour codes*: solid blue:* SD-fed *Mpv17*$^{+/-}$ mice; *blue outline:* KD-fed *Mpv17*$^{+/-}$ mice; *solid red:* SD-fed *Mpv17*$^{-/-}$ mice; *red outline:* KD-fed *Mpv17*$^{-/-}$ mice. Bars indicate the standard deviation (SD).

Supplemental Figure 5



AAV2/8-h*MPV17* 4 x 10^{12} vg/Kg partially rescues KD-induced liver damage in *Mpv17*$^{-/-}$ mice

A single retro-orbital injection of 4x10^{12} vg/Kg was performed in two-month old *Mpv17*$^{+/-}$ and *Mpv17*$^{-/-}$ mice. KD was started three weeks later.

A) Body weight changes during 2 months of KD in AAV-treated and untreated mice. *Solid blue*: untreated *Mpv17*$^{+/-}$; *dashed blue*: AAV-treated *Mpv17*$^{+/-}$; *solid red*: untreated *Mpv17*$^{-/-}$; *dashed red*: AAV-treated *Mpv17*$^{-/-}$.

B) AST and ALT transaminases levels in plasma.

C) Liver weight (% of body weight). Note the non-significant reduction in AAV-treated *Mpv17*$^{-/-}$ mice.

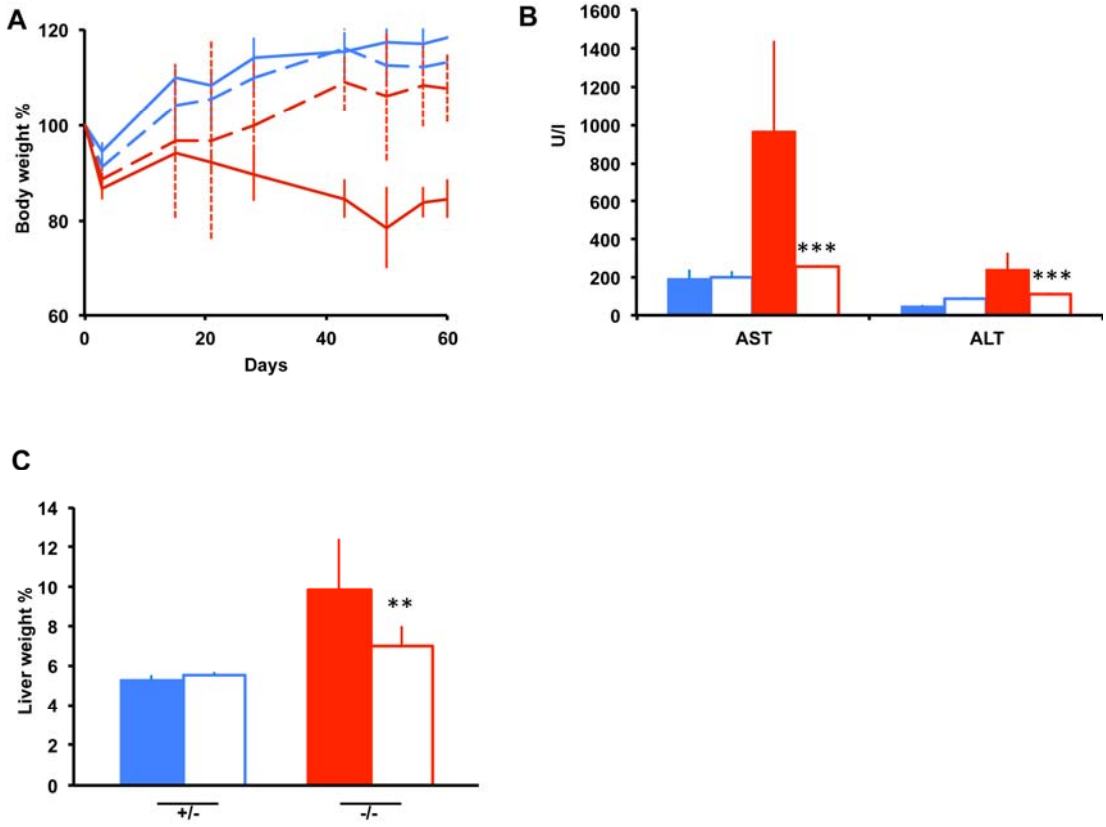
D) MtDNA analysis. Note that mtDNA content is AAV-treated *Mpv17*$^{-/-}$ is higher than in untreated littermates, but remains lower than in control littermates.

Asterisks indicate significance (p) calculated by the Mann-Whitney test for unpaired samples: *p < 0.05; **p < 0.01; ***p < 0.0001

Colour codes: *solid blue:* SD-fed *Mpv17*$^{+/-}$ mice; *blue outline:* KD-fed *Mpv17*$^{+/-}$ mice; *solid red:* SD-fed *Mpv17*$^{-/-}$ mice; *red outline:* KD-fed *Mpv17*$^{-/-}$ mice. Bars indicate the standard deviation (SD).

E) Histological features on hematoxylin-eosin (a, c, e); picrosirius red (b, d, f) staining. **a,b**: AAV-treated *Mpv17*$^{-/-}$ show liver steatosis and focal inflammatory infiltrates. There is only a mild increase in fibrosis, without overt cirrhosis. **c,d**: untreated *Mpv17*$^{-/-}$ with liver steatosis, moderate inflammatory infiltrates and cirrhosis. **e,f**: Untreated *Mpv17*$^{+/-}$ show only hepatocyte steatosis, in absence of cirrhosis. Scale bars: a, c, e: 150 m, b, d, f: 300 m.

Supplemental Figure 6



AAV2/8-h*MPV17* 4 x 10^{13} vg/Kg rescues KD-induced liver damage in *Mpv17*$^{-/-}$ mice

A single retro-orbital injection of 4x10^{13} vg/Kg was performed in two months old *Mpv17*$^{+/-}$ and *Mpv17*$^{-/-}$ mice. KD was started three weeks later.

A) Body weight changes during 2-months of KD in AAV-treated and untreated mice. *Solid blue*: untreated *Mpv17*$^{+/-}$; *dashed blue*: AAV-treated *Mpv17*$^{+/-}$; *solid red*: untreated *Mpv17*$^{-/-}$; *dashed red*: AAV-treated *Mpv17*$^{-/-}$.

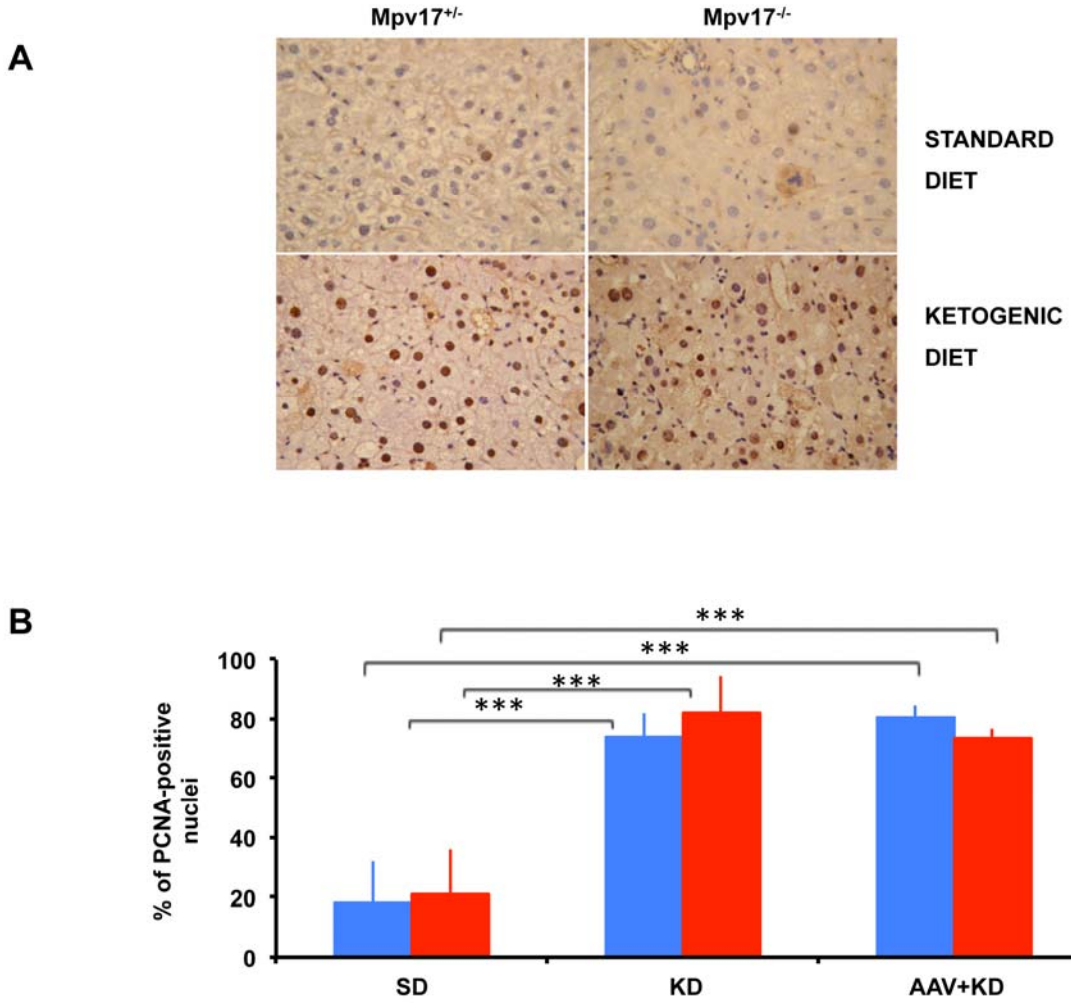
B) AST and ALT levels in plasma in AAV-treated and untreated groups.

C) Liver weight (% of body weight).

Asterisks indicate significance (p) calculated by the Mann-Whitney test for unpaired samples: **p < 0.01; ***p < 0.0001.

Colour codes: *solid blue:* untreated *Mpv17*$^{+/-}$; *blue outline:* AAV-treated *Mpv17*$^{+/-}$; *solid red:* untreated *Mpv17*$^{-/-}$; *red outline:* AAV-treated *Mpv17*$^{-/-}$. Bars indicate the standard deviation (SD).

Supplemental Figure 7



Effect of KD on hepatocytes proliferation

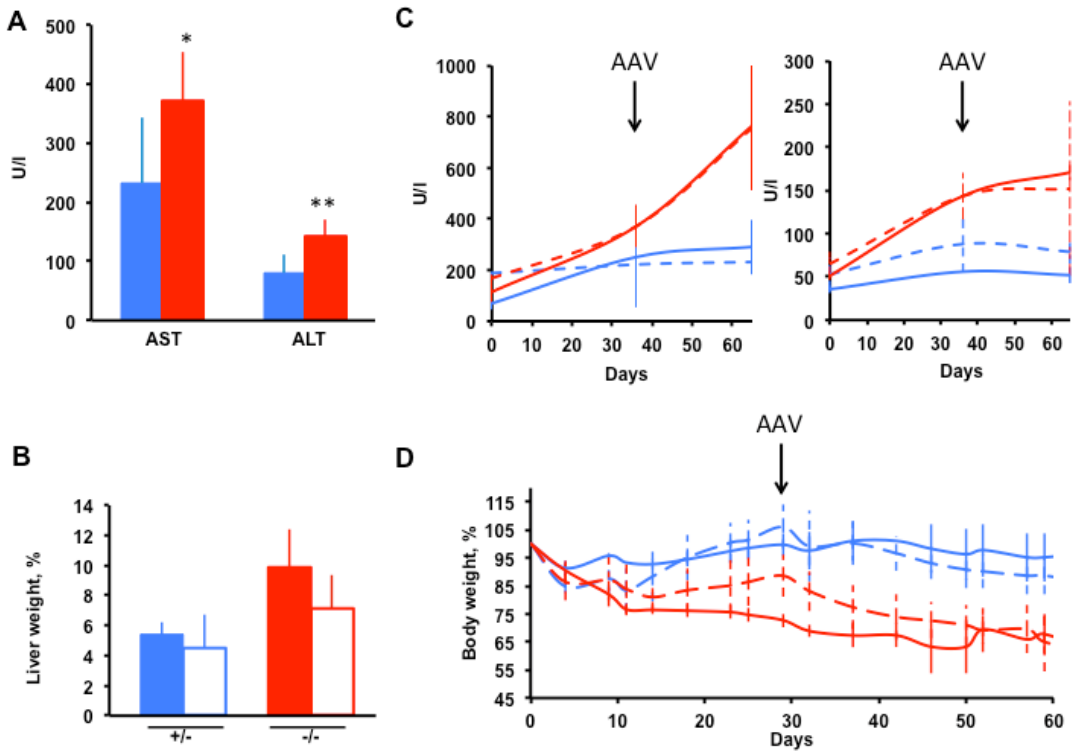
A) PCNA immuno-histochemical staining of SD and KD fed livers

B) quantitation of PCNA-positive nuclei.

Colour codes*: Solid blue: Mpv17*$^{+/-}$ mice; *solid red: Mpv17*$^{-/-}$ mice. The bars represent the standard deviation (SD).

Asterisks indicate significance (p) calculated by unpaired Student's two-tailed *t* test: ***p < 0.005.

Supplemental Figure 8



Effects of administration of AAV-*hMPV17* in mice pre-treated with KD

A) AST and ALT levels in plasma of *Mpv17*$^{+/-}$ and *Mpv17*$^{-/-}$ animals after one month of KD, just before AAV administration. Asterisks indicate significance (p) calculated by the Mann-Whitney test for unpaired samples: **p < 0.01; ***p < 0.0001.

B) Liver weight (% of body weight). *Solid blue*: untreated *Mpv17*$^{+/-}$, *blue outline*: AAV-treated *Mpv17*$^{+/-}$, *solid red*: untreated *Mpv17*$^{-/-}$, *red outline*: AAV-treated *Mpv17*$^{-/-}$.

C) AST (*left*) and ALT (*right*) trend during the experimental protocol. The arrow indicates the time point of AAV administration.

D) Body weight changes during 2-months of KD in AAV-treated and untreated mice. The arrow indicates the time point of AAV administration. *Solid blue*: untreated *Mpv17*$^{+/-}$; *dashed blue*: AAV-treated *Mpv17*$^{+/-}$; *solid red*: untreated *Mpv17*$^{-/-}$; *dashed red*: AAV-treated *Mpv17*$^{-/-}$.