Schatton et al., https://doi.org/10.1083/jcb.201607019

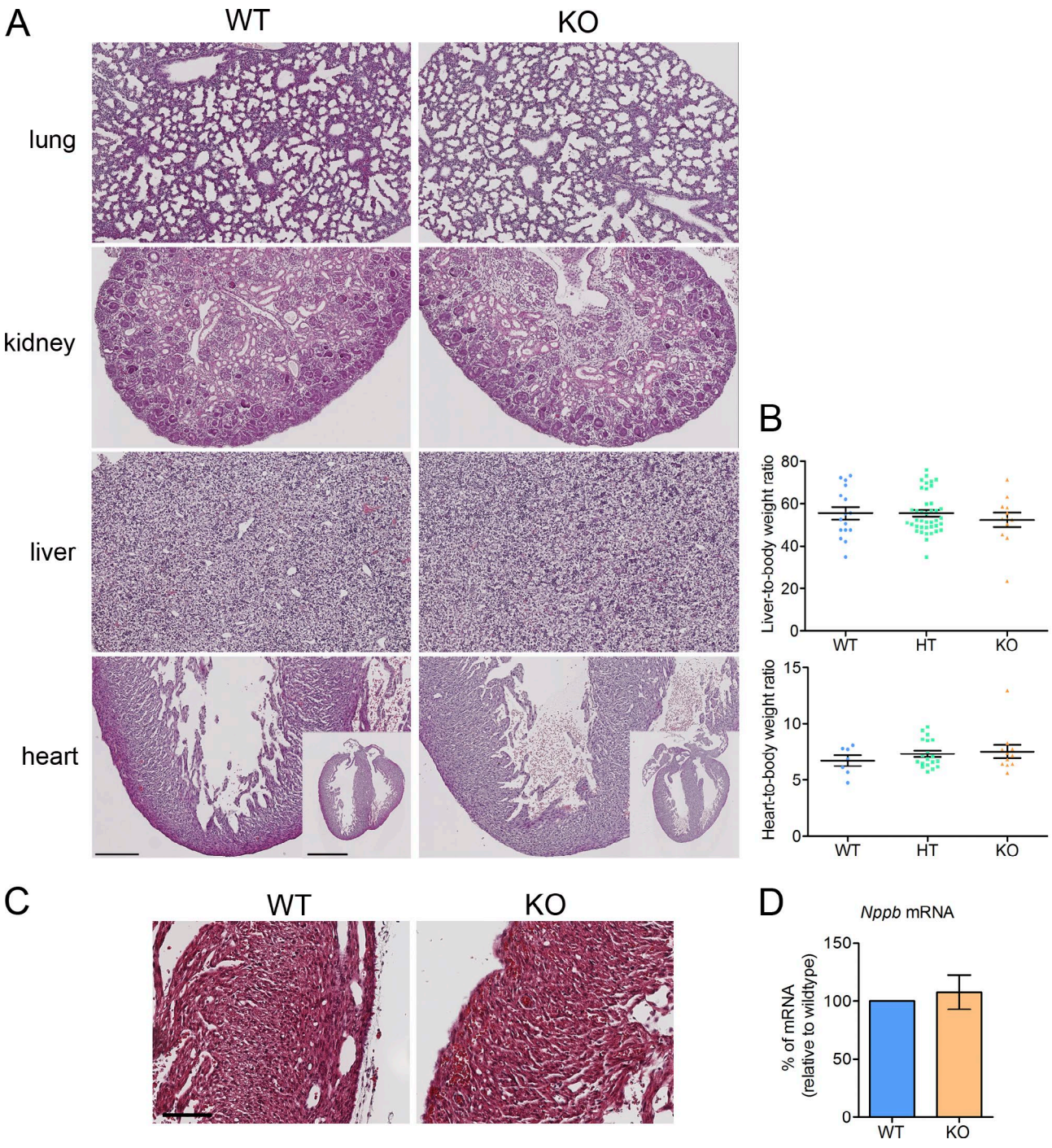

Figure S1. ***Cluh*$^{-/-}$ mice display no major organ malformation.** (A) Representative hematoxylin/eosin staining of different organs of WT and KO mice at E18.5. *n* = 5. Bars: (main images) 200 µm; (insets) 1 mm. (B) Heart and liver to body weight ratios of WT and KO mice at E18.5. Graphs show individual data points as scatter plots and means ± SEM. Top: WT, *n* = 16; HT, *n* = 40; KO, *n* = 12. Bottom: WT, *n* = 7; HT, *n* = 19; KO, *n* = 11. (C) Representative Masson Trichrome staining of hearts of WT and KO mice at E18.5. *n* = 5. Bar, 100 µm. (D) Quantitative RT-PCR of *Nppb*, a marker of hypertrophy, in hearts of KO mice at E18.5. Expression was normalized to GAPDH levels. Graph shows percentages of mRNA in KO relative to WT samples and represents means ± SEM. *n* = 4.

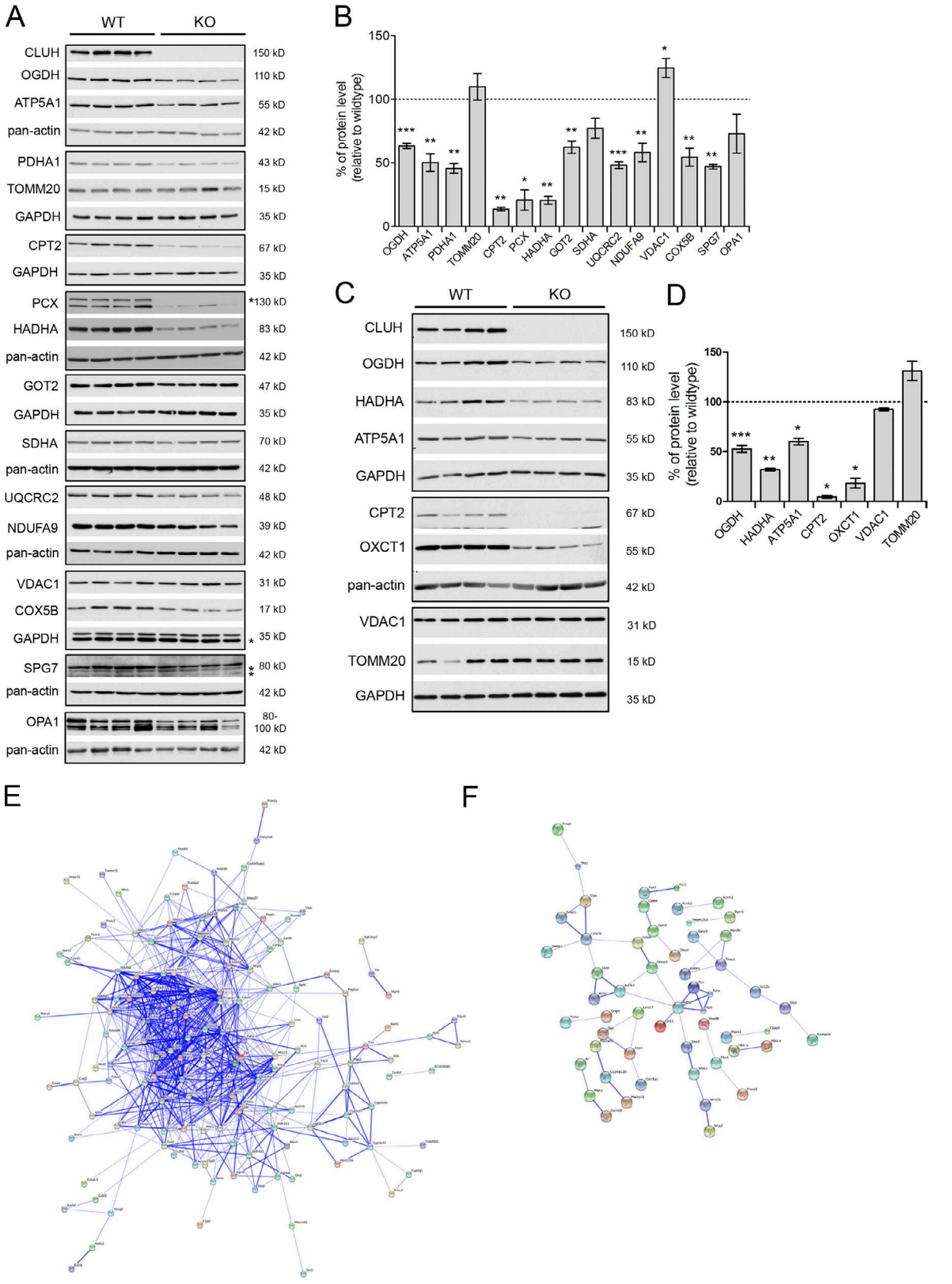

Figure S2. ***Cluh* deficiency decreases mitochondrial proteins encoded by target transcripts in the liver and heart.** (A) Immunoblots of whole-liver lysates of WT and KO mice at E18.5 probed for CLUH targets and control proteins. GAPDH or pan-actin was used as a loading control. Asterisks indicate residual or unspecific signals. (B) Quantification of immunoblots shown in A. The dotted line represents the value of WT. (C) Immunoblots of whole-heart lysates of WT and KO mice at E18.5 probed for CLUH targets and control proteins. GAPDH or pan-actin was used as a loading control. (D) Quantification of protein levels of KO samples relative to WT samples (dotted line) of the immunoblot shown in C. Graphs show means ± SEM. $n = 4$. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ (two-tailed *t* test). (E and F) String networks of down-regulated (E) and up-regulated (F) transcripts (FC ≥1.5) in transcriptomic analysis of WT and KO livers at E18.5.

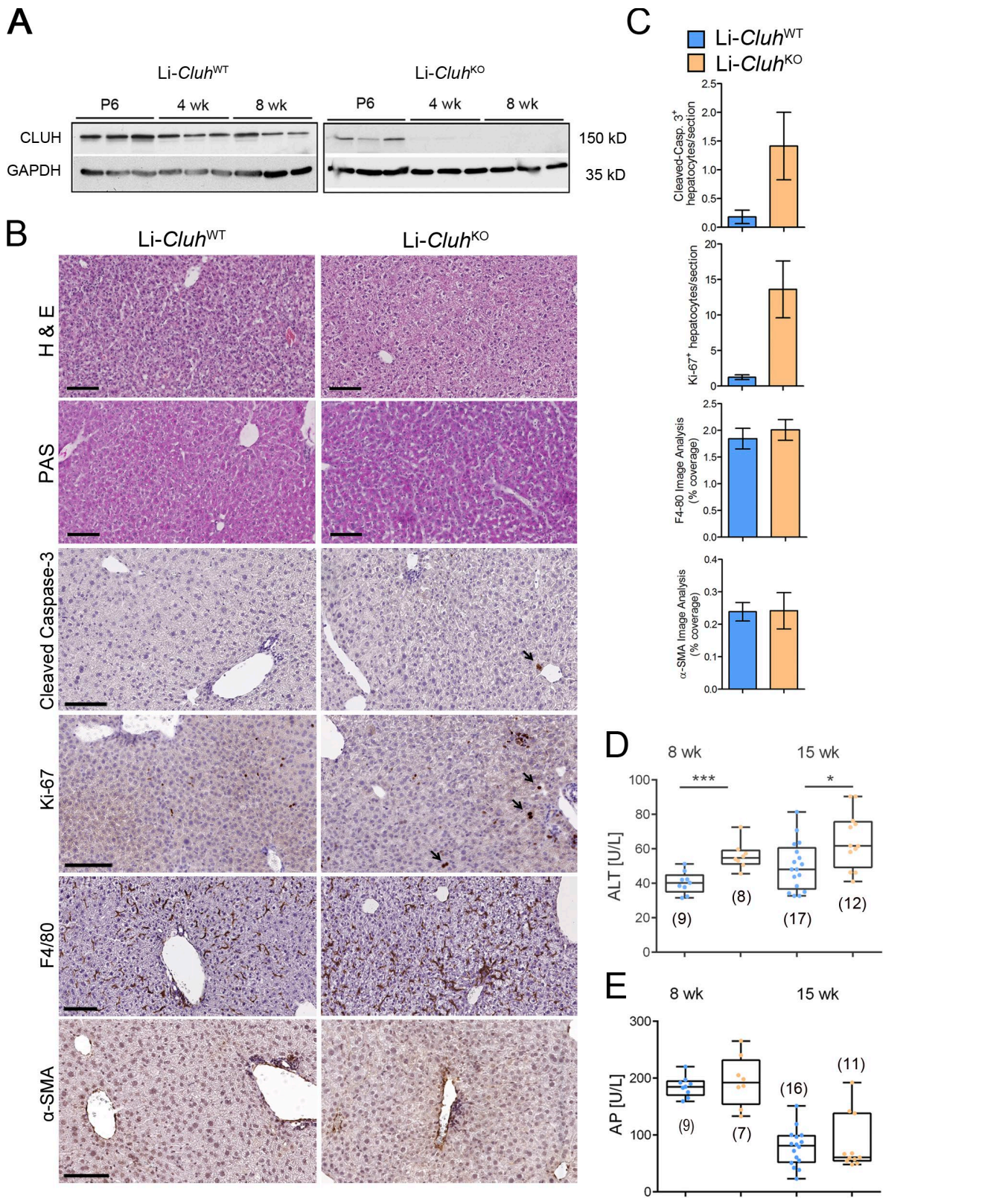

Figure S3. **Liver-specific deletion of *Cluh* using the *Alb-Cre* transgenic line.** (A) CLUH immunoblots in liver lysates at the indicated time points. GAPDH was used as a loading control. (B) Representative images of liver sections from 8-wk-old mice of liver-specific WT and KO mice immunostained for cleaved caspase-3 (arrow, apoptosis), Ki-67 (arrows, proliferation), F4/80 (macrophage infiltration), and α-SMA (fibrosis). Bars, 100 µm. (C) Quantification of immunohistochemistry stainings in B. $n = 3$. (D and E) Enzymatic activity of ALT (D) or AP (E) measured in serum from 8- and 15-wk-old mice. Graphs show individual data points as boxplots. Error bars represent means ± SEM. *n* values are indicated in parentheses. *, $P \leq 0.05$; ***, $P \leq 0.001$ (two-tailed *t* test).

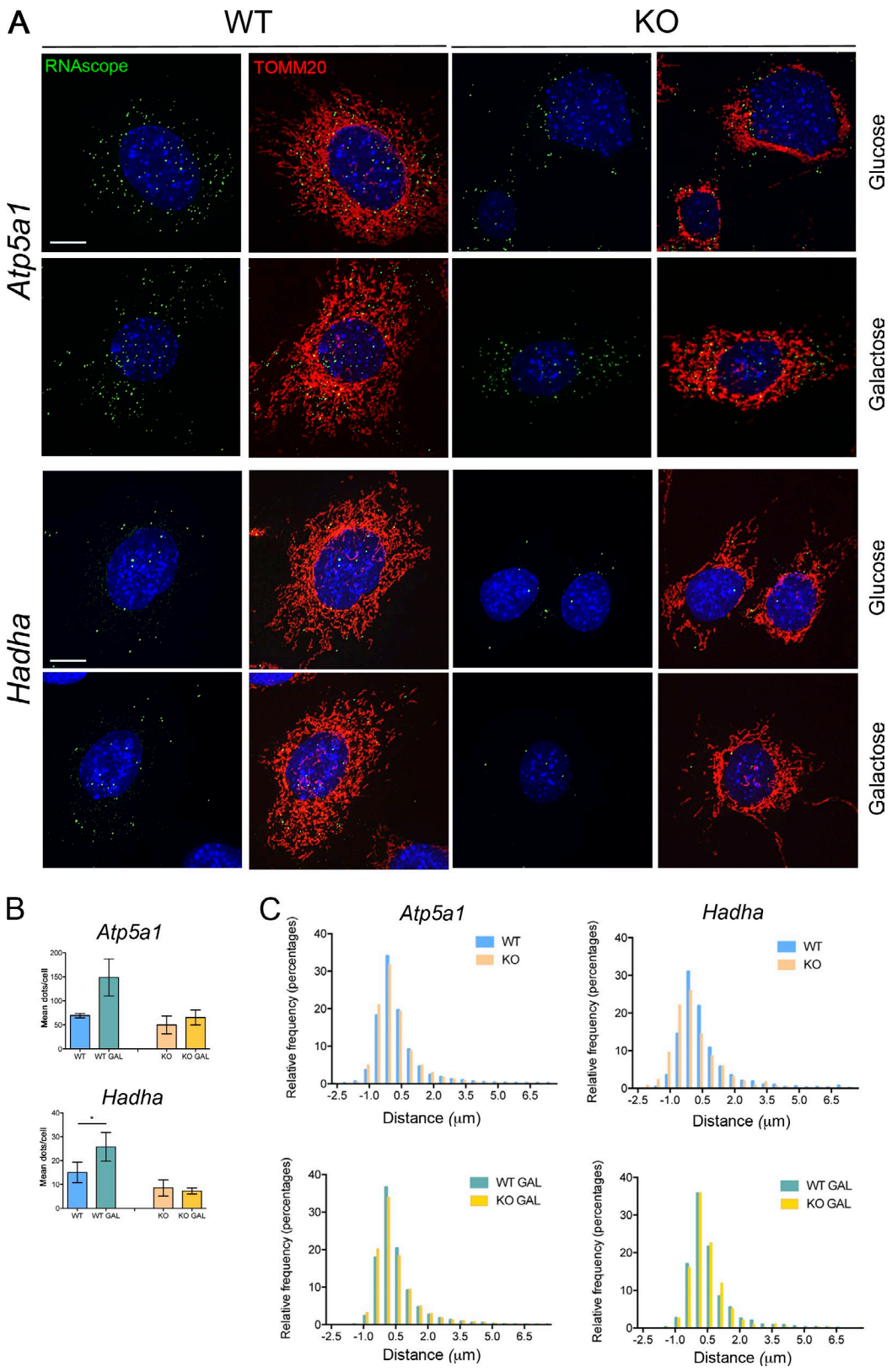

Figure S4. ***Atp5a1*and *Hadha* mRNA molecules are reduced in *Cluh*-deficient MEFs, but show unaltered distribution in relation to mitochondria.** (A) Single-molecule FISH of *Atp5a1* and *Hadha* combined with TOMM20 immunofluorescence in WT and KO MEFs. Cells were cultured either in glucose- or galactose-containing medium, as indicated. mRNA molecules are shown as green dots and TOMM20 as red. Panels on the left depict the green channel only, and merged channels are shown on the right. Bars, 12 µm. (B) Quantification of the mean cell numbers of mRNA dots in each condition. Graphs show means ± SEM (≥80 cells per experiment). *, $P \leq 0.05$ (two-tailed *t* test). (C) Relative frequency distribution of the distances between each mRNA dot and its closest mitochondria for experiments in glucose and galactose media (≥80 cells per experiment). $n = 3$.

Table S1 is an Excel file that shows an analysis of proteomics data.
Table S2 is an Excel file that reports transcripts up-regulated or down-regulated in the liver of *Cluh* KO mice in respect to WT mice.
Table S3 is an Excel file that contains a list of measured metabolites.