

SUPPLEMENT

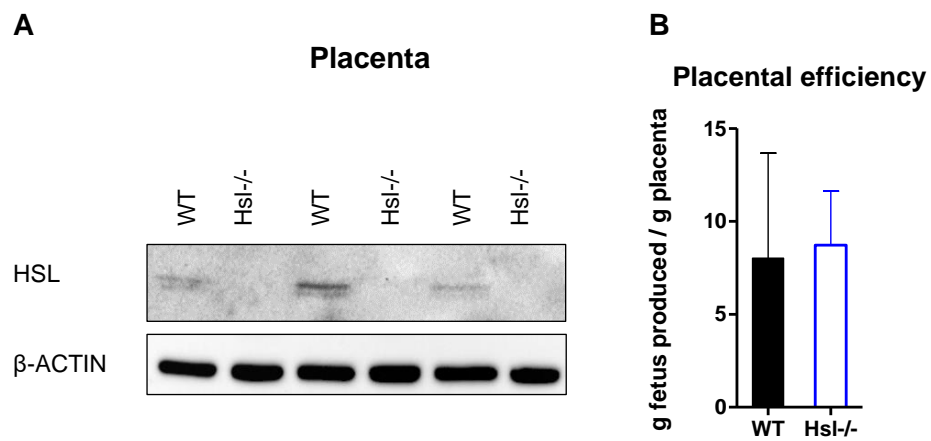


Figure S1. HSL deficiency does not alter placental efficiency. **(A)** Western blot analysis of placental tissue. **(B)** Placental efficiency of WT and Hsl^{-/-} mice (n = 34 WT / 24 Hsl^{-/-}). Data represent mean values + SD.

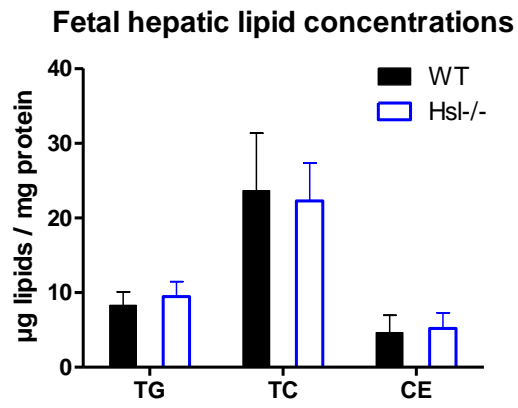
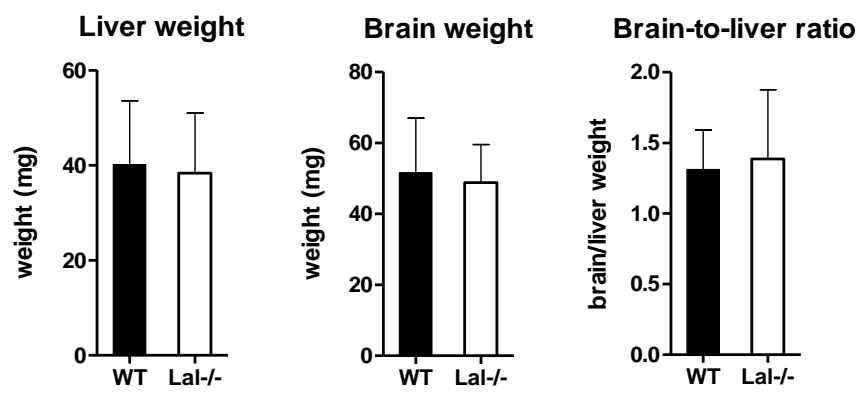
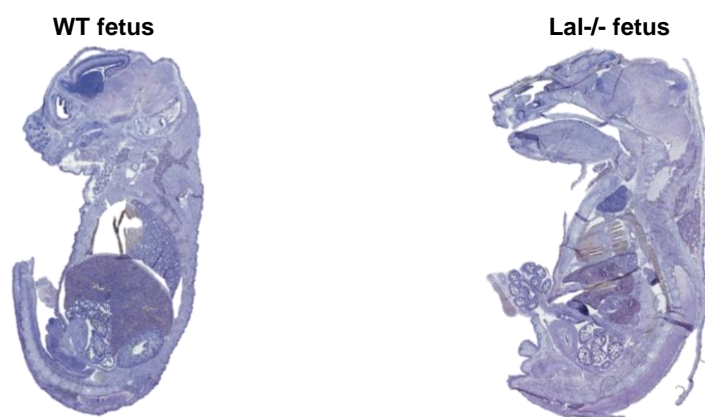


Figure S2. HSL deficiency does not alter hepatic lipid concentrations in the fetus. Fetal hepatic triglyceride (TG), total cholesterol (TC), and cholesteryl ester (CE) levels. Data represent mean values + SD (n= 4).

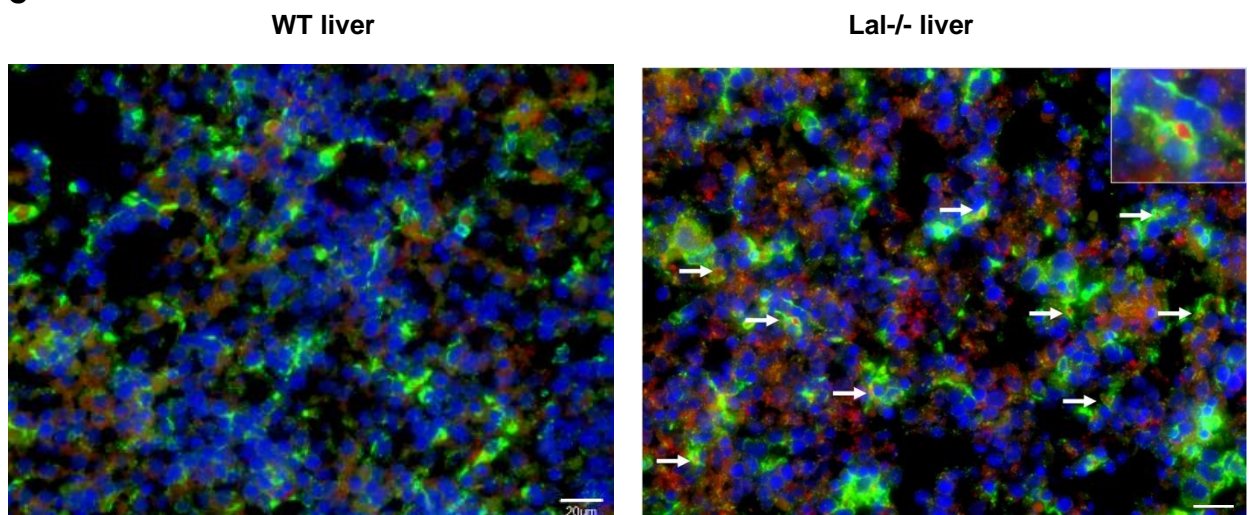
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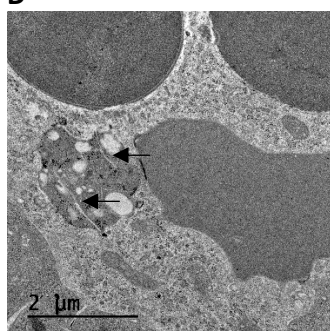
B



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D



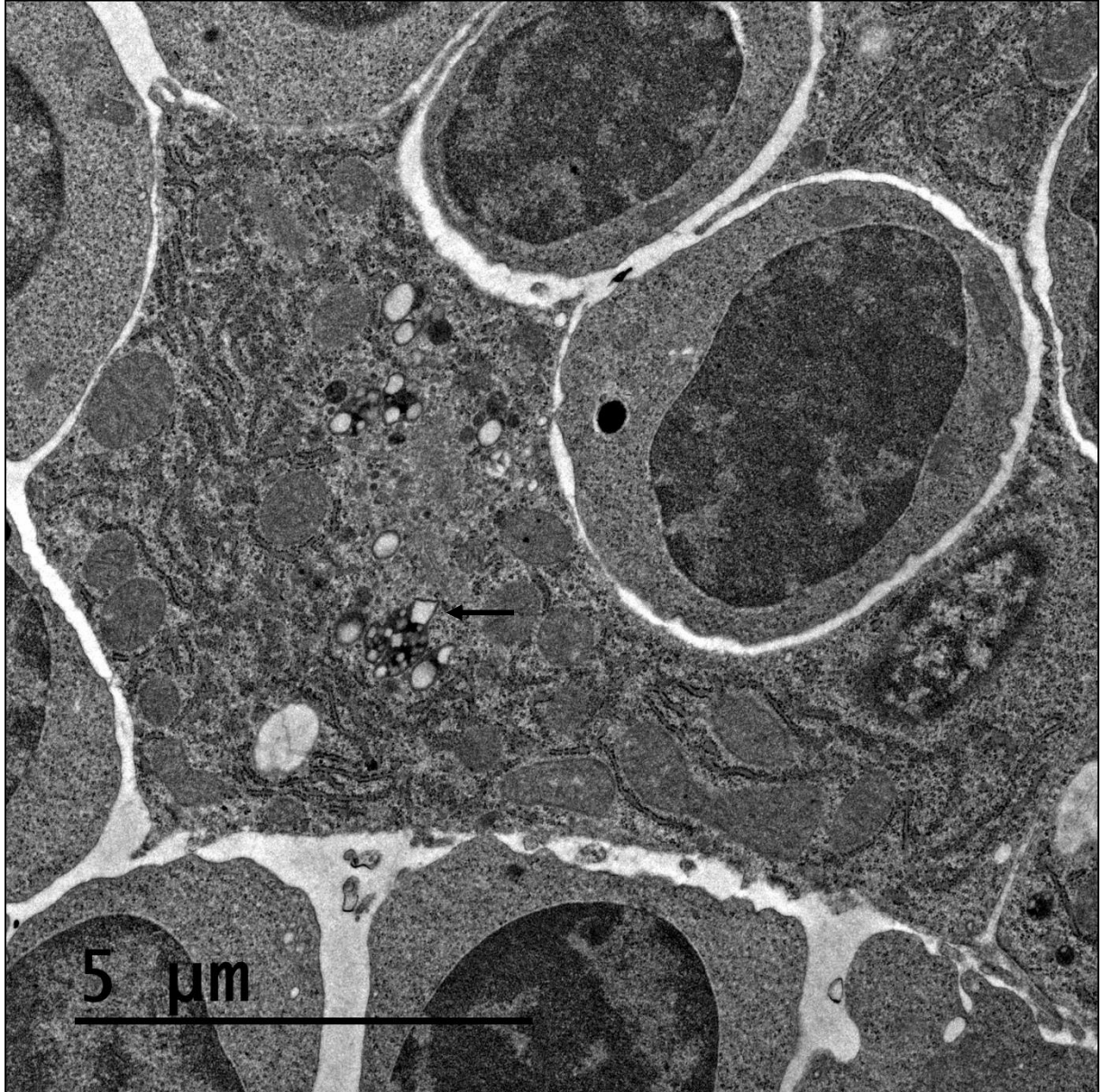
E

Figure S3. Increased abundance of lysosomal lipids in fetal *Lal*^{-/-} livers. Fetuses were isolated from WT and *Lal*^{-/-} mice on day 19 of pregnancy. (A) Fetal liver and brain weights and brain-to-liver ratio. Data represent mean values + SD (n = 25 WT / 29 *Lal*^{-/-}). (B) ORO staining of a whole-body WT and *Lal*^{-/-} fetus. (C) Immunofluorescent staining of lysosomes (LAMP1, green), neutral lipids (ORO, red), and nuclei (DAPI, blue) in fetal liver sections; white arrows indicate lipids co-localizing with lysosomes. Magnification, 40x; Scale bar, 20 μm. (D, E) Electron micrographs of *Lal*^{-/-} fetal liver; black arrows indicate CE crystals. Scale bars, (D) 2 μm, (E) 5 μm.

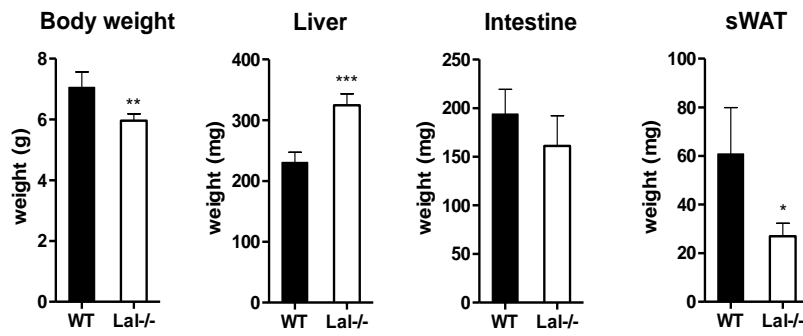
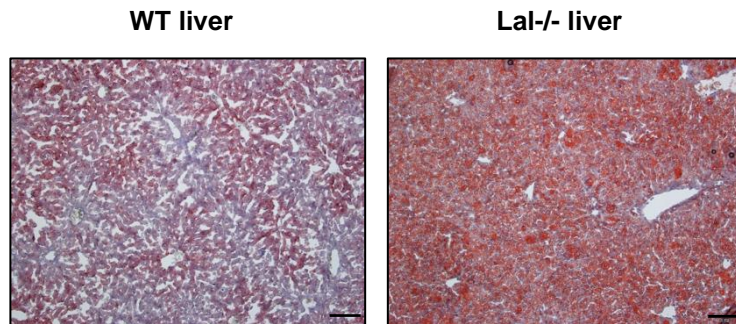
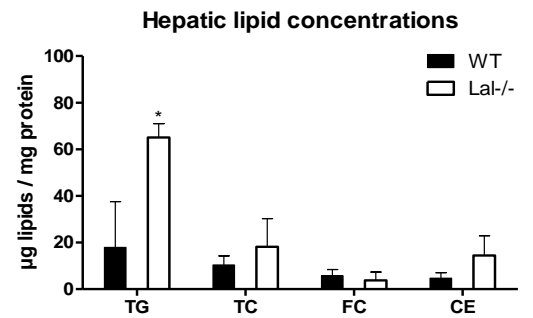
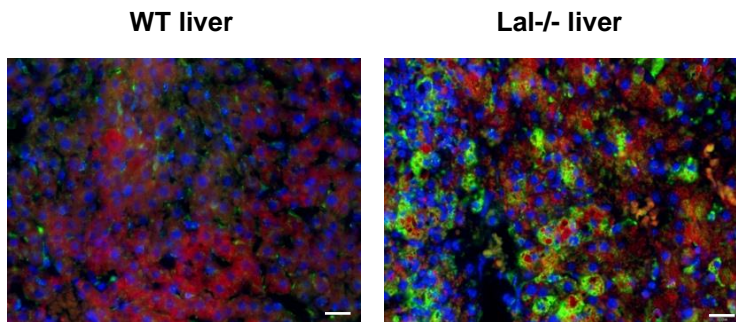
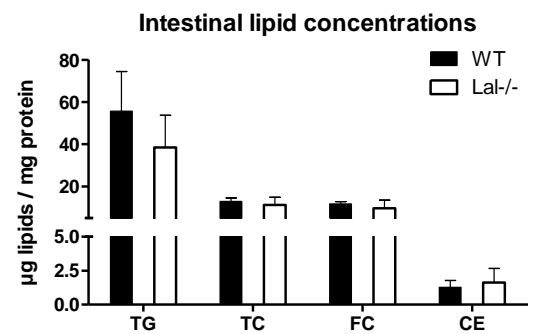
A**B****C****D****E**

Figure S4. *Lal*^{-/-} mice are severely affected by hepatic lipid accumulation already 2 weeks after birth. (A) Body and organ weights from 2-week-old WT and *Lal*^{-/-} mice (n = 5 WT / 4 *Lal*^{-/-}). (B) ORO staining of liver cryosections and (C) hepatic lipid concentrations (n = 4). (D) Immunofluorescent double staining for lysosomes (LAMP1, green), neutral lipids (ORO, red), and nuclei (DAPI, blue) on hepatic cryosections. Magnification, 10x; Scale bar, 100 µm. (E) Intestinal lipid concentrations (n = 3). Data represent mean values + SD. Statistically significant differences were calculated by two-tailed Student's t-test; * $p < 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

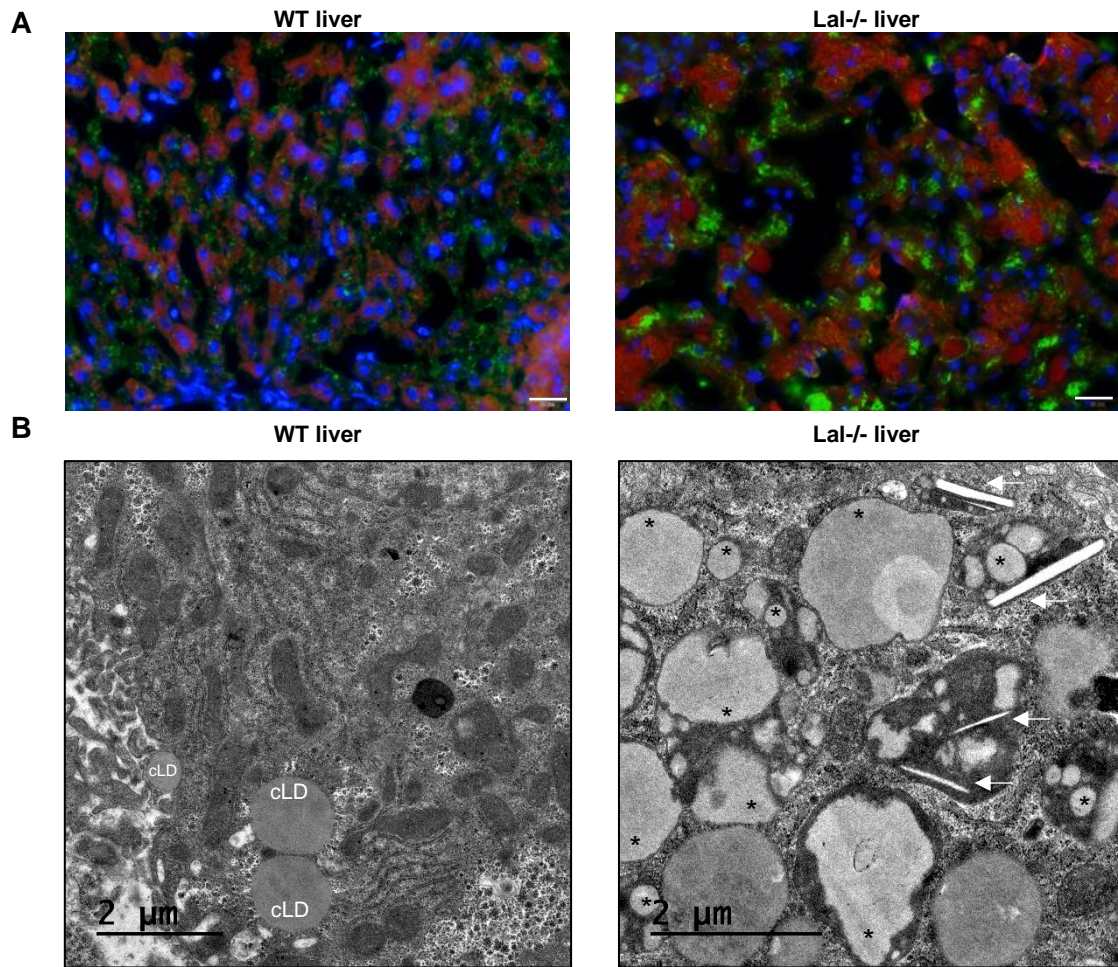


Figure S5. Lysosomal lipid accumulation in livers of 4-week-old *Lal*^{-/-} mice. **(A)** Immunofluorescent staining of lysosomes (LAMP1, green), neutral lipids (ORO, red), and nuclei (DAPI, blue) on hepatic cryosections. Magnification, 20x; Scale bar, 50 μm. **(B)** Increased image size of electron micrographs of WT and *Lal*^{-/-} livers; cLD, cytosolic lipid droplet; white arrows indicate lysosomal CE crystals, * indicate lysosomal lipids. Scale bar, 2 μm.