LECT2 deletion exacerbates liver steatosis and macrophage infiltration in a male mouse model of LPS-mediated NASH

Supplemental information

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Materials and Methods

RNA isolation and real-time PCR analysis

The total RNA was isolated from the epididymal white adipose tissue (eWAT) using the RNeasy Lipid Tissue Mini Kit (QIAGEN, Germany), according to the manufacturer's protocol. In addition, we used the high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA) to prepare cDNA from total RNA. Real-time PCR was used to assess the expression levels of target genes using qPCR MasterMix Plus (EUROGENTEC, Selaing, Belgium) and the StepOnePlus Real-Time PCR system (Applied Biosystems, CA, USA). The gene expression data are presented as $2^{\Delta\Delta CT}$. The primers used here, which were cataloged primers (Applied Biosystems) for mice, were as follows: *Ppara* (*Mm00440939_m1*), *Acox1* (*Mm01246834_m1*), *Srebf1* (*Mm00550338_m1*), *Fasn* (*Mm00662319_m1*). The relative mRNA expression levels were normalized to those of the endogenous control, *Gapdh* (#4351309) (Applied Biosystems, CA, USA).

Fatty acid composition analysis

The fatty acid composition was measured as described previously (Nakamura *et al*., *Cell Death Dis*, 2018., Matsuzaka *et al*., *Hepatology*, 2020., Muranaka et al., *Oncogenesis*, 2017.) with some modifications. Approximately 100 mg liver tissues were homogenized with 1 mL of methanol containing 1% acetic acid by a polytron homogenizer, followed by the addition of

6.7 ml of a 1:2:2 mixture of chloroform:methanol:ethanol and 333 µl of a 2:1:1:1 mixture of chloroform:methanol:ethanol:acetic acid. The mixture was vortexed at room temperature and centrifuged for 10 min at 4 °C; the organic layer was collected and evaporated under reduced pressure. For FA analysis, extracted lipids were methylated with 2.5% H_2SO_4 in methanol. The FA methyl esters were then extracted with n-hexane and quantified by gas chromatography-mass spectrometry (GC/MS) using a Clarus SQ 8 GC/MS (PerkinElmer Japan, Kanagawa, Japan).

Supplemental Results

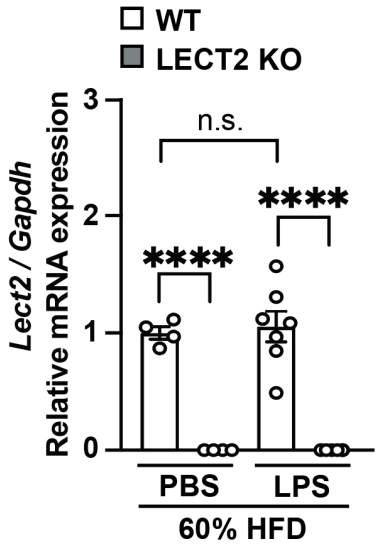

Figure S1. *Lect2* mRNA expression levels in the liver tissue. Fold change in the expression levels of the *Lect2* mRNA. (PBS = WT: n = 1.0) (PBS + WT: n = 1.0; PBS + WT: n = 4; PBS + LECT2 KO: n = 4; LPS + WT: n = 7; LPS + LECT2 KO: n = 7). Values are the mean ± SEM. ****$p < 0.0001$; n.s., not significant.

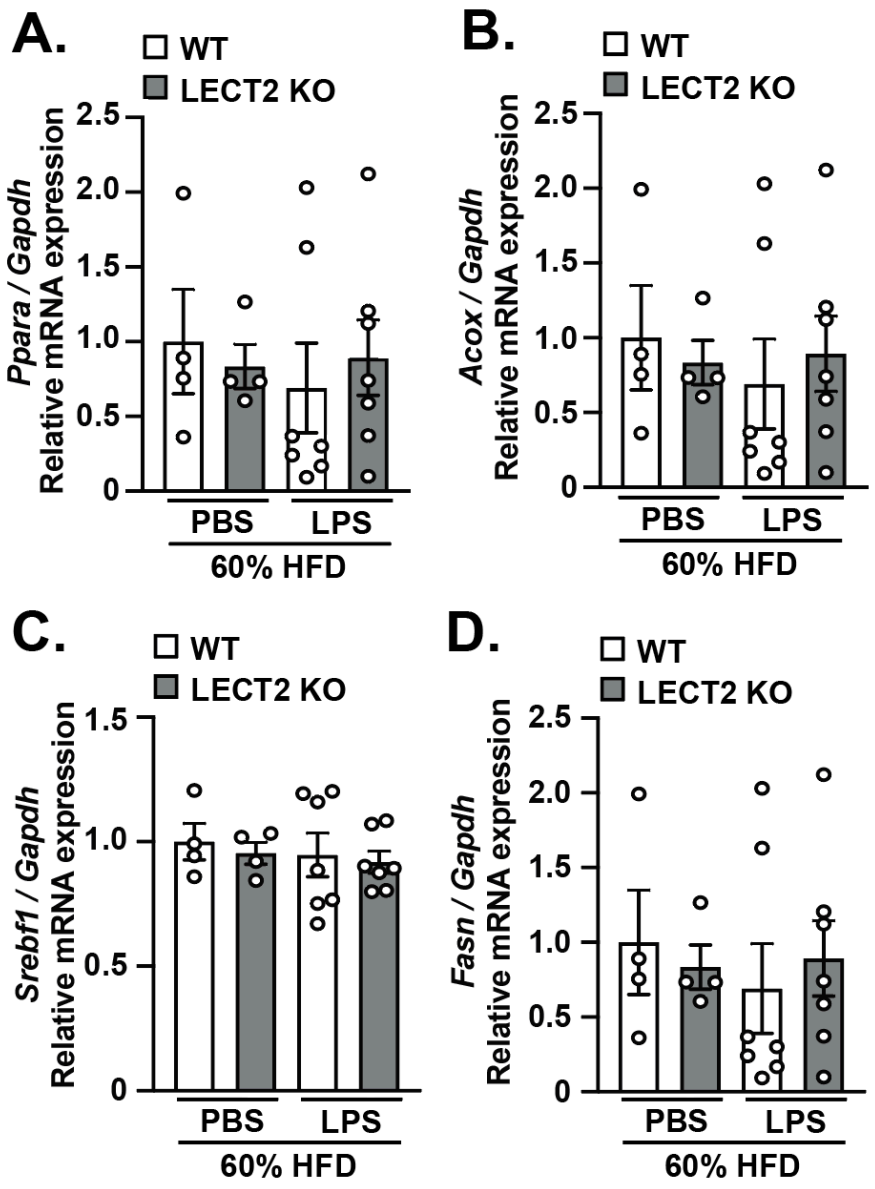

Figure S2. Lipid metabolism-related gene expression levels. A-D: Fold change in the expression levels of the *Pparα*, *Acox*, *Srebf1*, and *Fasn* (PBS + WT = 1.0) (PBS + WT: n = 1.0; PBS + WT: n = 4; PBS + LECT2 KO: n = 4; LPS + WT: n = 7; LPS + LECT2 KO: n = 7). Values are the mean ± SEM. There were no significant changes among all conditions.

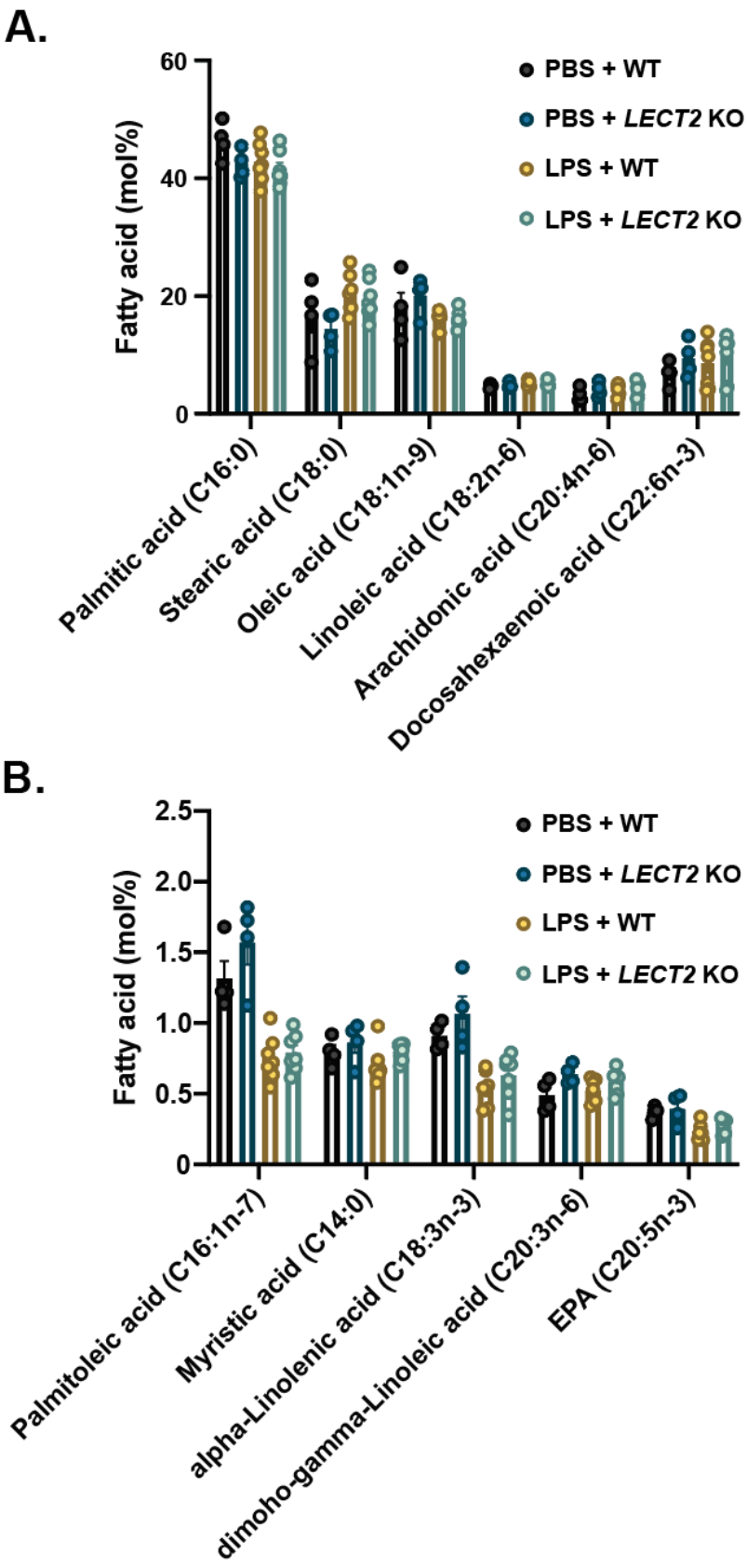

Figure S3. Intergroup comparison of major and minor fatty acid content in the liver. A: large amount, and B: small amount lipid kinds among the whole detected lipid profile data. (PBS + WT: n = 1.0; PBS + WT: n = 4; PBS + *LECT2* KO: n = 4; LPS + WT: n = 7; LPS + *LECT2* KO: n = 7). Values are the mean ± SEM. All gene expressions showed no significant alteration.