

# Loss of the E3 ubiquitin ligase MARCHF6 alters hepatic lipid metabolism and drives spontaneous hepatosteatosis

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## Supplemental information

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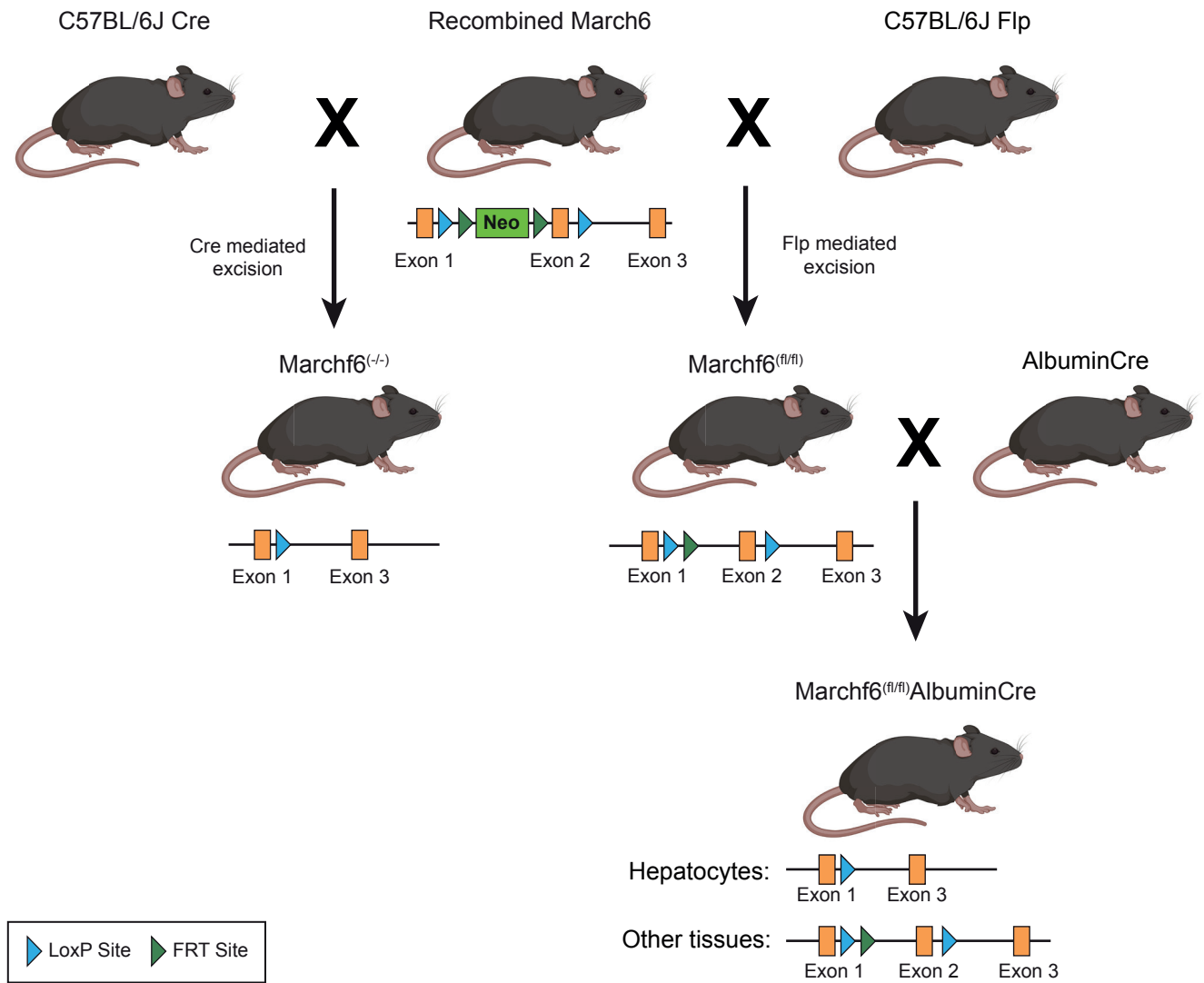
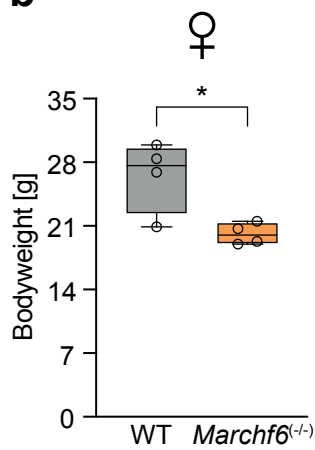
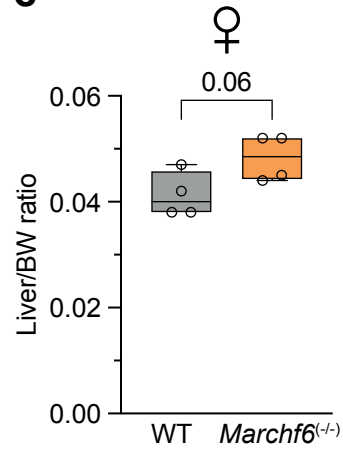
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# These authors contributed equally

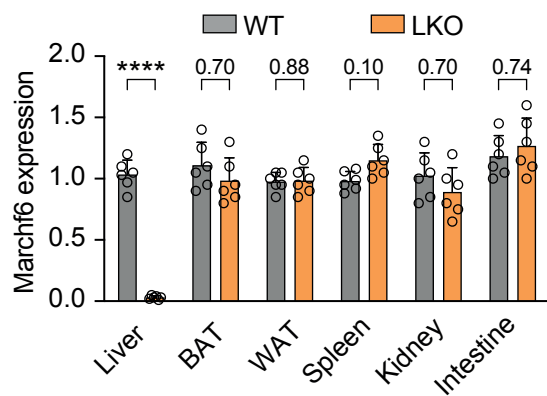
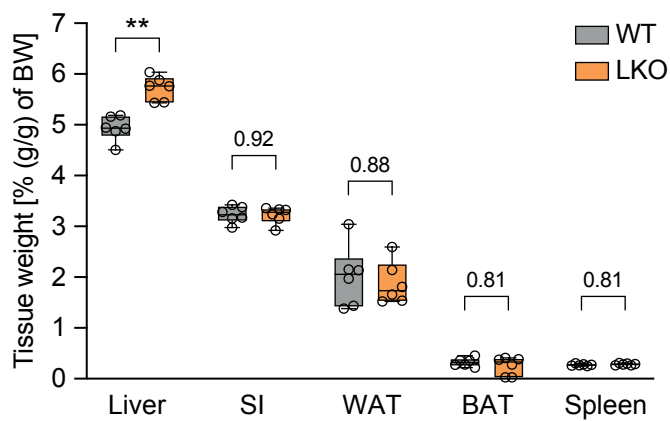
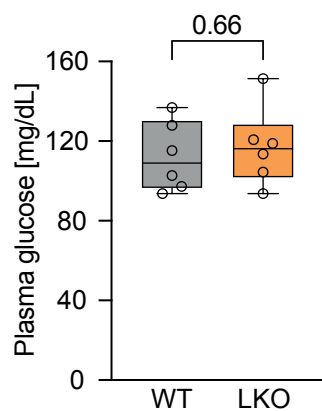
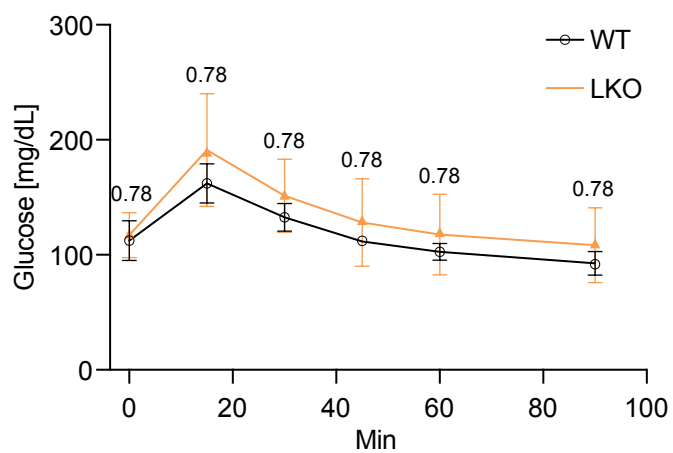
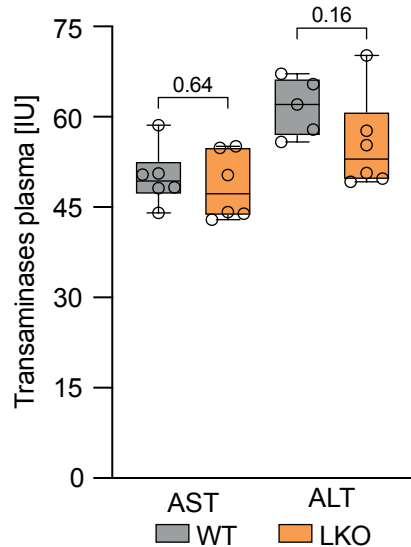
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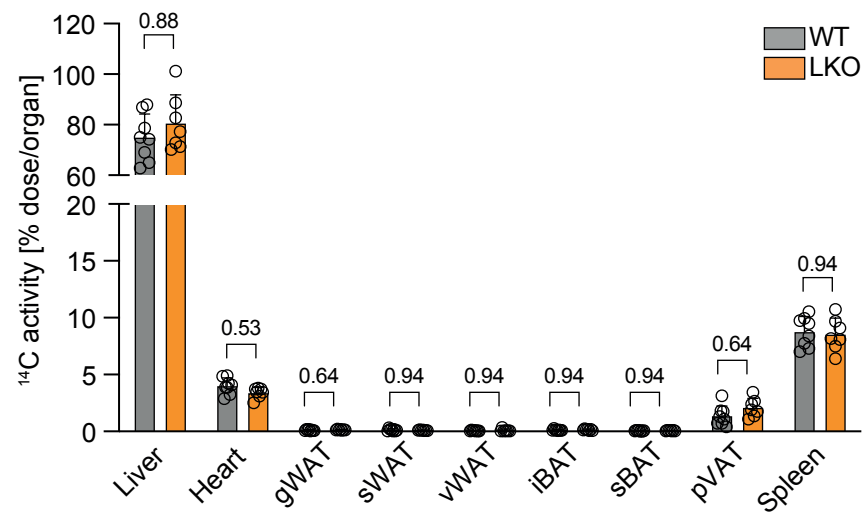
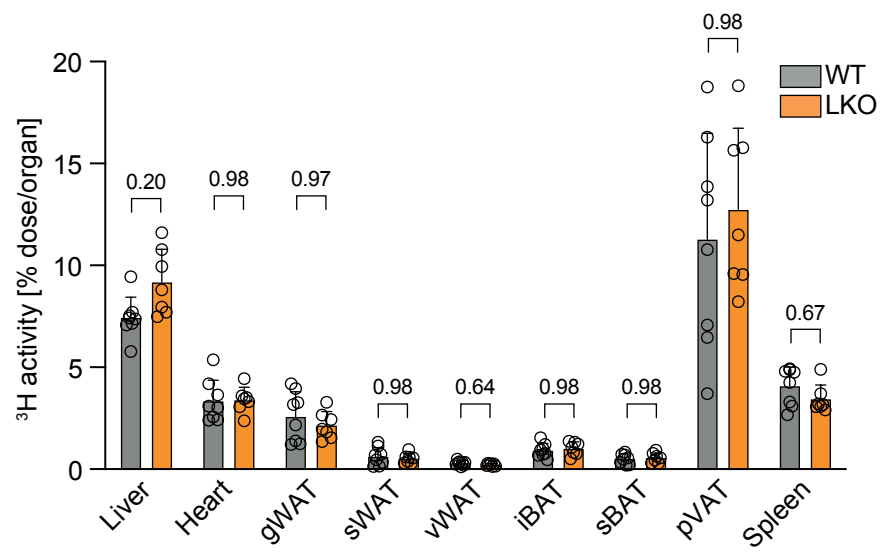
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**Supplementary Figure 1. Mouse model generation and characterization of female *Marchf6*<sup>(-/-)</sup> mice.** (a) Schematic representation of the generation of constitutive and hepatocyte-specific *Marchf6* knockout mouse models. (b) Bodyweight and (c) liver/bodyweight ratio of female WT and *Marchf6*<sup>(-/-)</sup> mice (n=4/group). Box plots show the median (middle line) 25<sup>th</sup>, 75<sup>th</sup> percentile (box), minimum and maximum values (whiskers). \* p<0.05 as analyzed by two-tailed Welch's t-test.

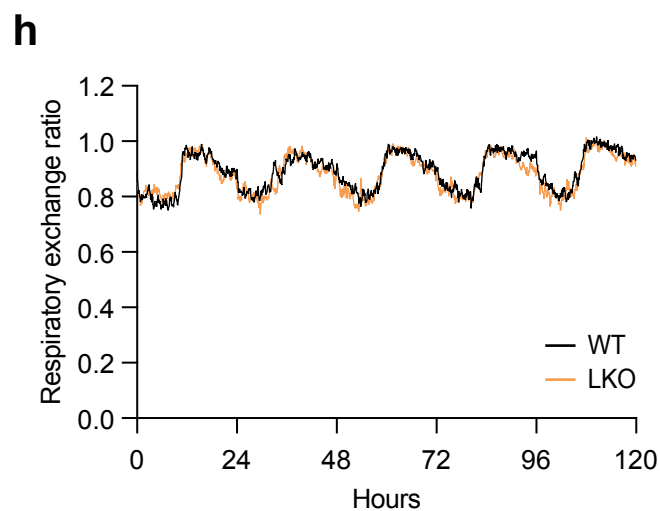
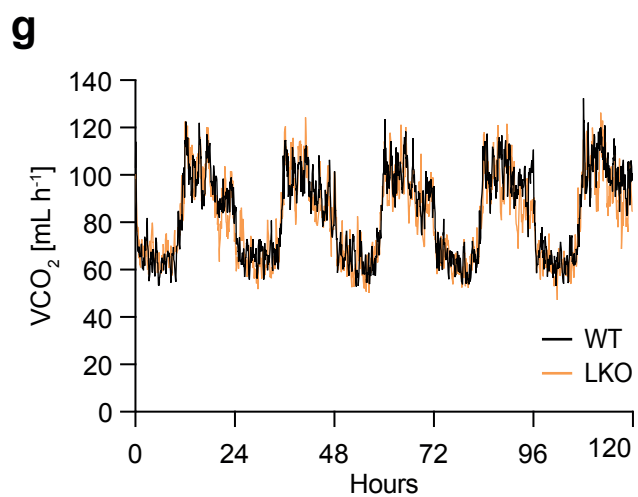
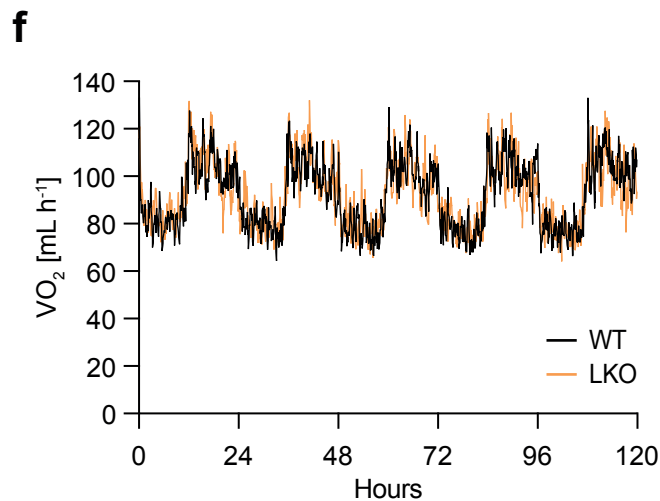
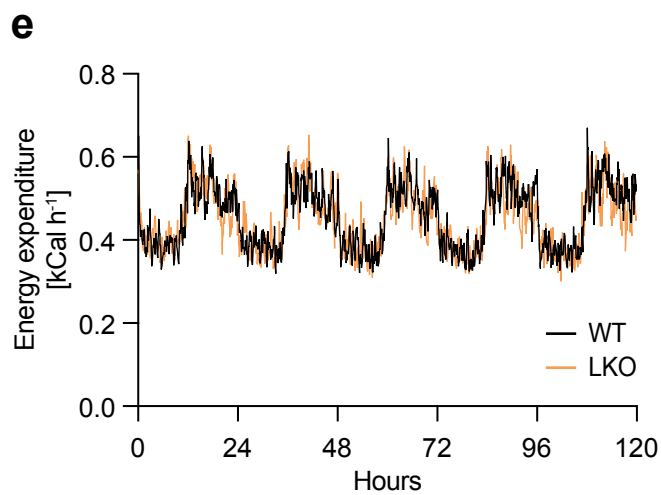
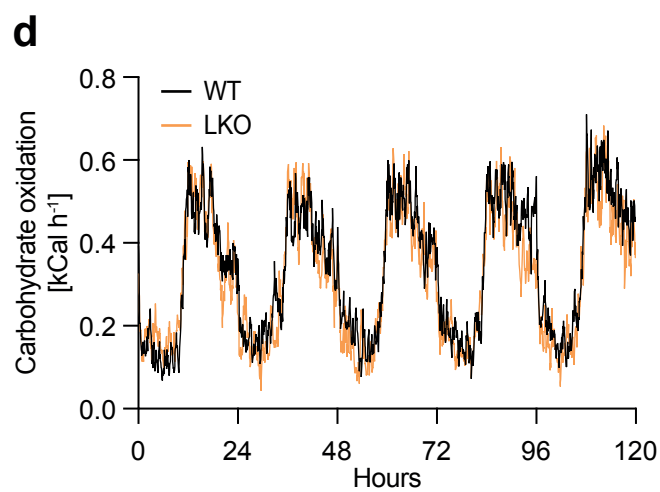
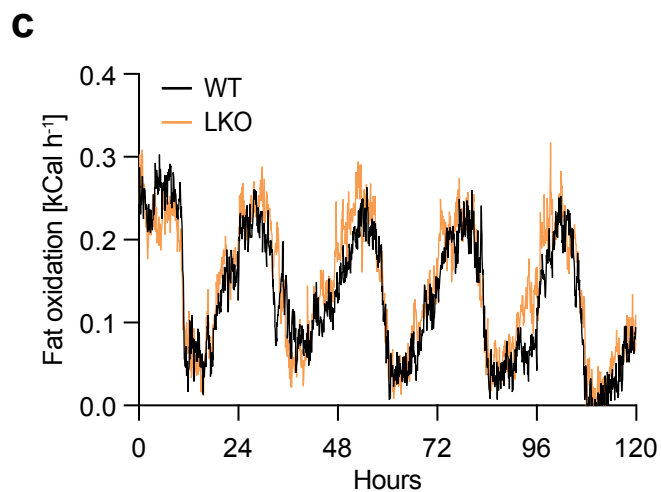
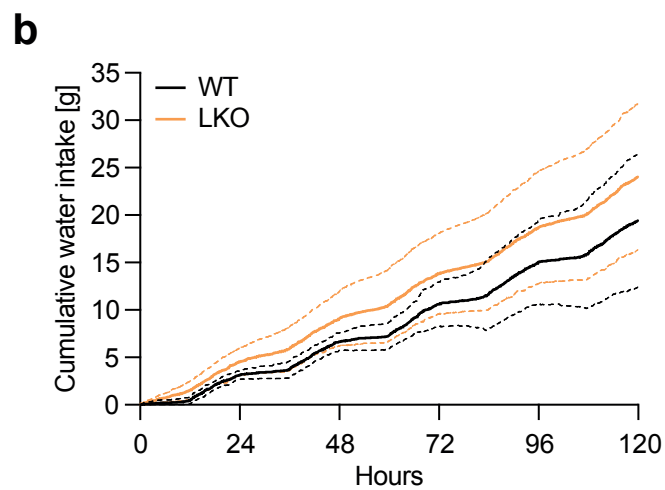
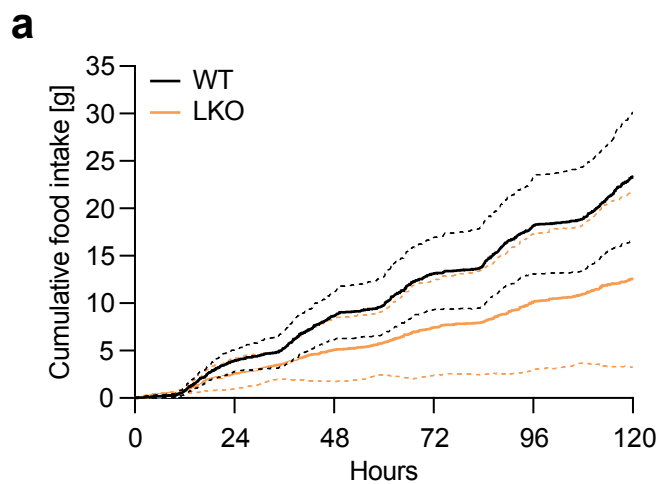
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**Supplementary Figure 2. Characterization of liver-specific *Marchf6* knockout mouse model. (a)**

Male *Marchf6*<sup>LKO</sup> (n=6) and control littermates (n=6) on standard chow diet were sacrificed at 12 weeks of age and gene expression of *Marchf6* was determined in the indicated tissues. (b) Tissue weight of same mice as in (a) was determined relative to the total bodyweight of the mice. (c) Plasma glucose levels in same mice as in (a) were determined after 4 hours of fasting. (d) 19-weeks-old female WT and *Marchf6*<sup>LKO</sup> mice (n=6/group) were fasted for 4 hours and then given a glucose bolus (2g/kg). Blood glucose levels were sampled at the indicated timepoints via the tail vein. (e) Plasma transaminases were measured in the same mice as in (a) as an indicator for liver damage. (a) Bars and (d) open circles represent the mean value and the error bars represent the SD. \*\*\*\* p < 0.0001 as analyzed by unpaired, two-tailed Welch's t-test with Holm-Šídák correction for multiple comparison. (b,c,e) Box plots show the median (middle line) 25<sup>th</sup>, 75<sup>th</sup> percentile (box), minimum and maximum values (whiskers). \*\* p < 0.01, as analyzed by unpaired, two-tailed Welch's t-test with (b) Holm-Šídák correction for multiple comparison.

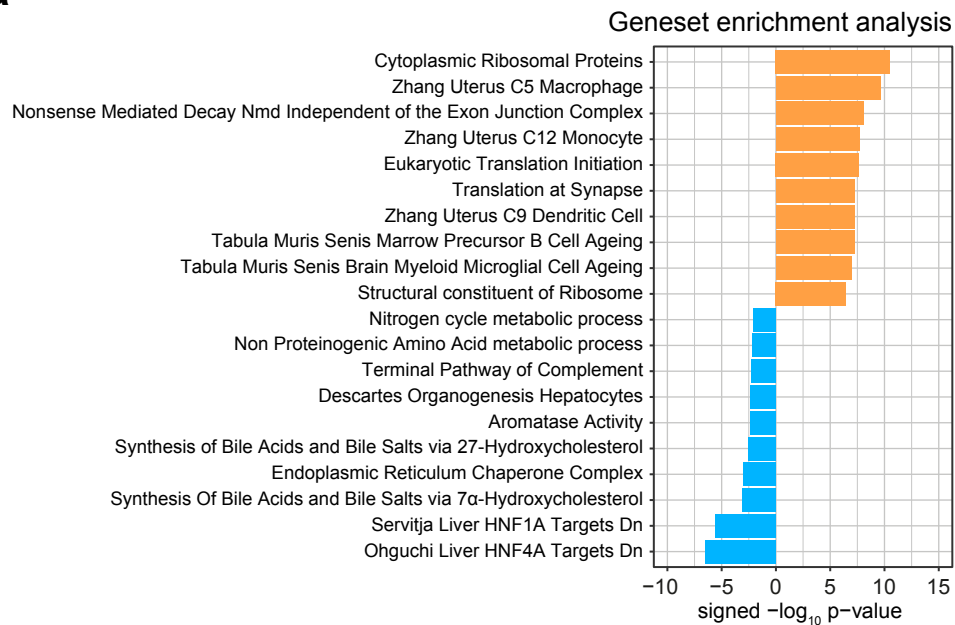
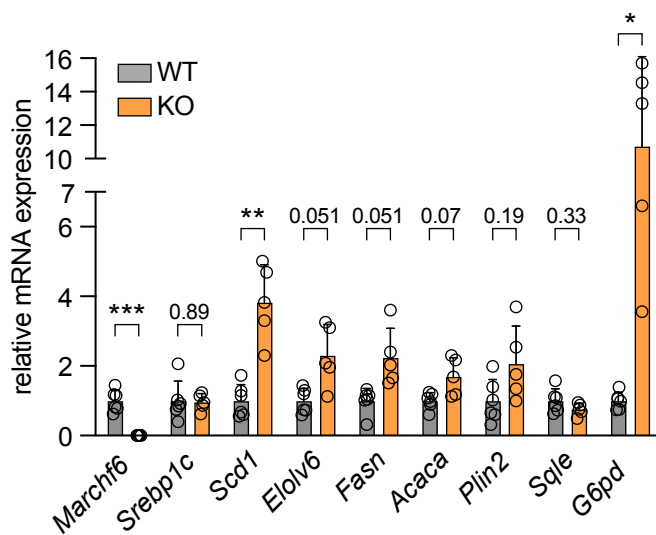
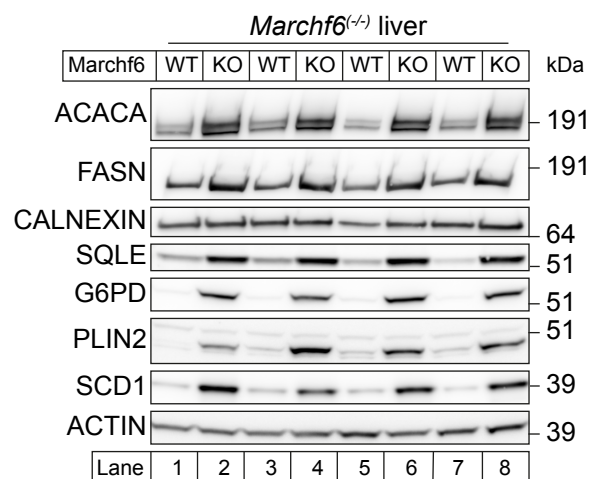
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**Supplementary Figure 3. Uptake of lipoprotein-like particles in different tissues.** (a,b) 15-week-old male WT and *Marchf6*<sup>LKO</sup> (n=8/group) were injected with [<sup>3</sup>H]triolein and [<sup>14</sup>C]cholesteryl oleate labeled TG-rich lipoprotein-like particles. After 20 minutes the mice were euthanized, perfused with ice-cold PBS and organs were harvested. The indicated organs were dissolved and (b) <sup>3</sup>H and (c) <sup>14</sup>C activity measured by liquid scintillation. Bars represent the mean value and the error bars represent the SD. Statistical significance was determined by unpaired, two-tailed Welch's t-test with Holm-Šídák correction for multiple comparison.

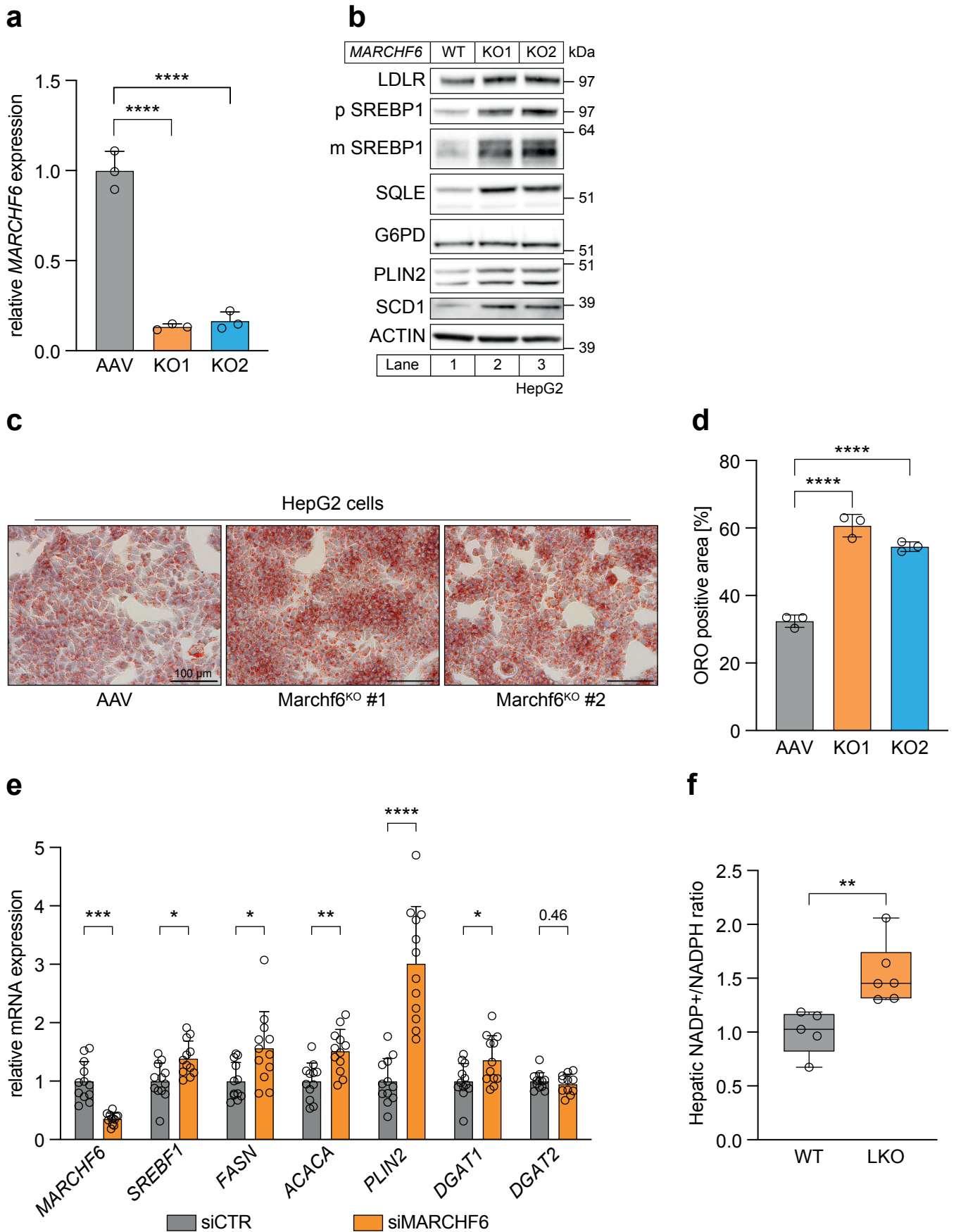




**Supplementary Figure 4. Loss of hepatic *Marchf6* does not affect indirect calorimetric parameters of mice.** 11-week-old male WT and *Marchf6*<sup>LKO</sup> (n=8/group) were individually housed in metabolic cages for 1 week and (a) cumulative food intake, (b) cumulative water intake, (c) fat and (d) carbohydrate oxidation, (e) energy expenditure, (f) O<sub>2</sub> and (g) CO<sub>2</sub> consumption as well as (h) respiratory exchange ratio were continuously measured.

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**Supplementary Figure 5. Expression of lipogenic genes is increased in *Marchf6*<sup>(-/-)</sup> mice.** (a) Gene set enrichment analysis of RNAseq experiment shown in main Figure 5A. (b) Expression of the indicated genes was assessed by RT qPCR in livers of male WT (n=6) and *Marchf6*<sup>(-/-)</sup> (n=5) mice. Bars represent the mean value and the error bars represent SD. \* p<0.05, \*\* p<0.01 and \*\*\* p <0.001 as analyzed by unpaired, two-tailed Welch's t-test with Holm-Šídák correction for multiple comparison. (c) Liver lysates from the same mice as in (b) were immunoblotted for the proteins indicated.



**Supplementary Figure 6. Loss of MARCHF6 causes neutral lipid accumulation in HepG2 cells.**

(a) Gene expression of *Marchf6* was determined in AAVS (control) and two independent *Marchf6*<sup>KO</sup> HepG2 clones. (b) Cell lysates of indicated HepG2 clones were lysed and immunoblotted as shown. (c) Representative images of ORO-stained HepG2 clones and (d) the quantification of ORO positive area is shown. (b,c) Representative images from at least 3 independent biological experiments is shown. (e) qPCR analysis of the indicated genes in primary human hepatocytes. (f) NADP<sup>+</sup> and NADPH were measured in hepatic tissue of WT and *Marchf6*<sup>LKO</sup> mice (n=6/group) and the NADP<sup>+</sup>/NADPH ratio was calculated. (a,d) Bars represent the mean value and the error bars represent the SD. \*\*\*\* p<0.0001 as analyzed by ordinary one-way ANOVA with Holm-Šídák post hoc analysis to correct for multiple comparisons. (e) Bars represent the mean value and the error bars represent SD. \* p<0.05, \*\* p<0.01, \*\*\* p <0.001 and \*\*\*\* p<0.0001 as analyzed by unpaired, two-tailed Welch's t-test with Holm-Šídák correction for multiple comparison. (f) Box plots show the median (middle line) 25<sup>th</sup>, 75<sup>th</sup> percentile (box), minimum and maximum values (whiskers). \*\* p<0.01 as analyzed by unpaired, two-tailed Welch's t-test.