Supplementary material

AMPK-activator ATX-304 reduces oxidative stress and improves MASLD via metabolic switching

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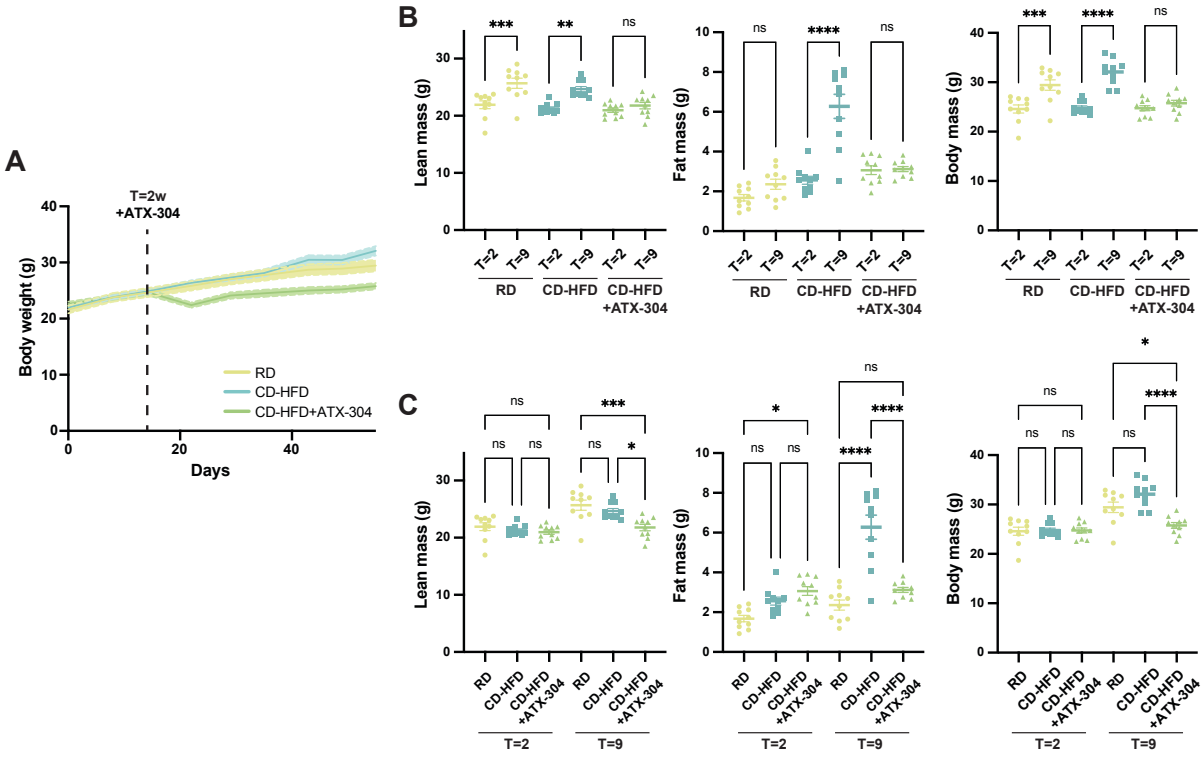
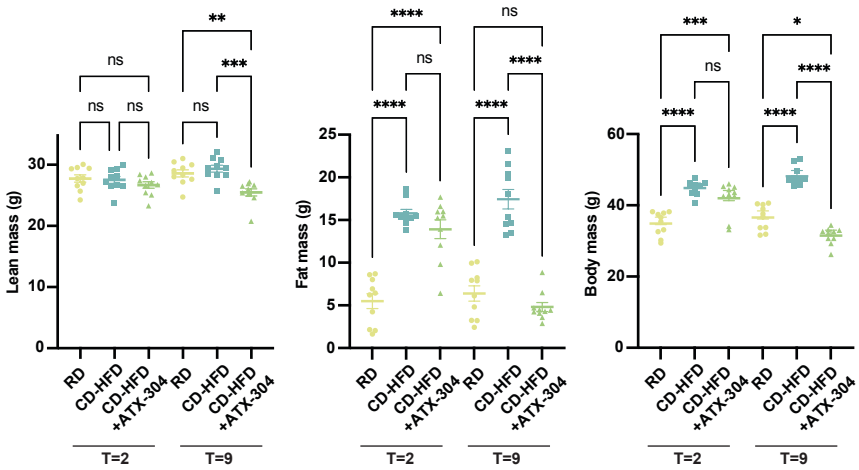
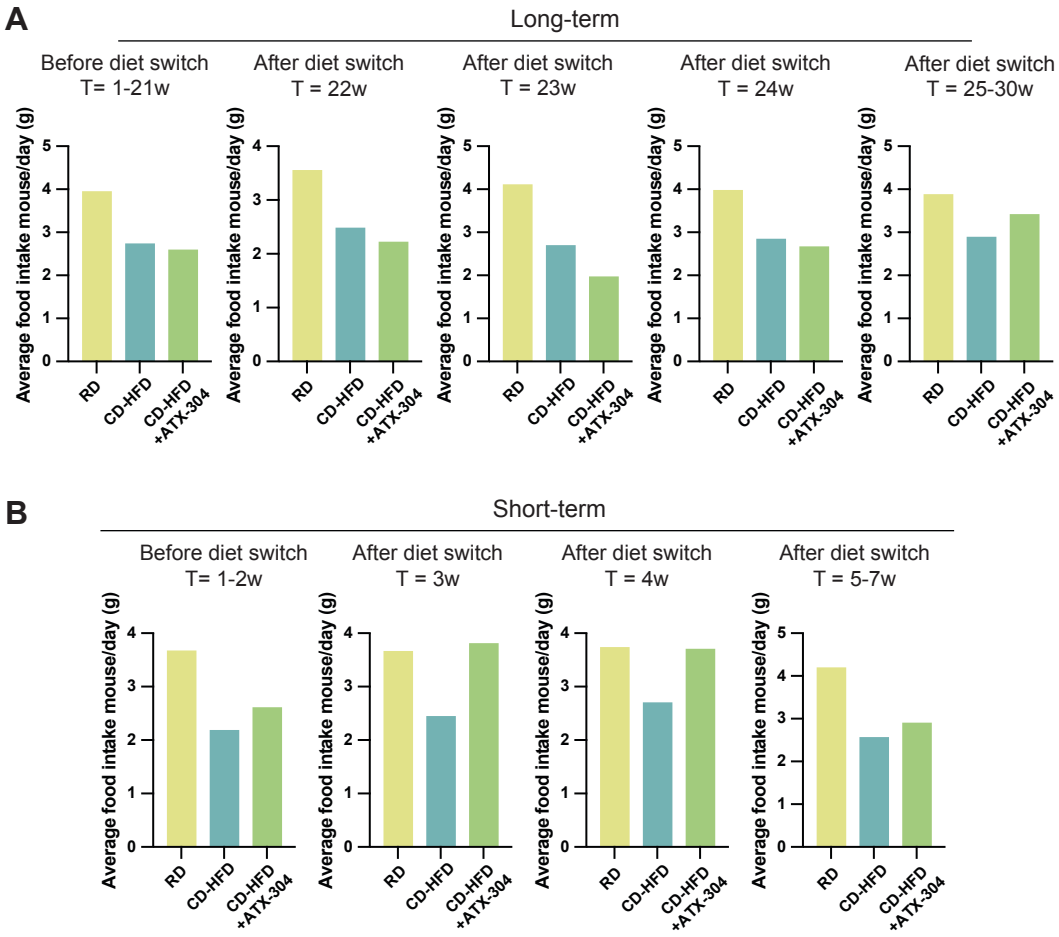


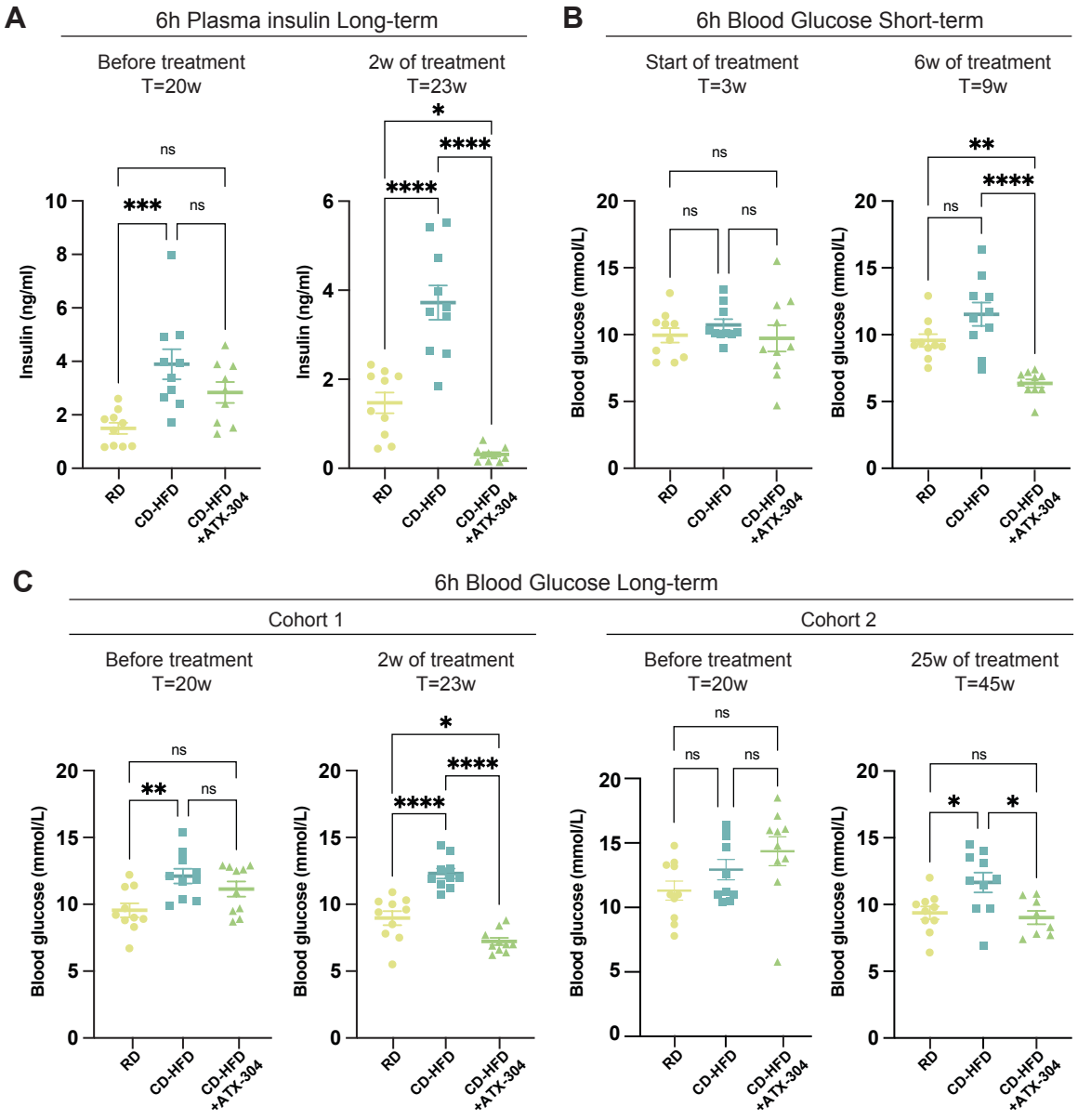
Supplementary figure 1. ATX-304 treatment reduces fat mass in CD-HFD mice. **A**) Weight curve for short-term RD, CD-HFD and CD-HFD+ATX-304 mice. Dashed line indicate start of ATX-304 treatment (T=2w). Colored shade depicts standard error of the mean (SEM). **B)** Lean, fat and total body mass in short-term cohort before (T=2w) and after (T=9w) ATX-304-treatment as measured by EchoMRI. **C**) Same data as in **B**) but depicting comparisons between RD, CD-HFD and CD-HFD+ATX-304 experimental groups before (T=2) and after treatment (T=9). **p<0.01,***p<0.001, ****p<0.0001 (One-way ANOVA with Tukey's multiple comparisons test). Individual data points, mean ± SEM are indicated (n=10 for all groups).



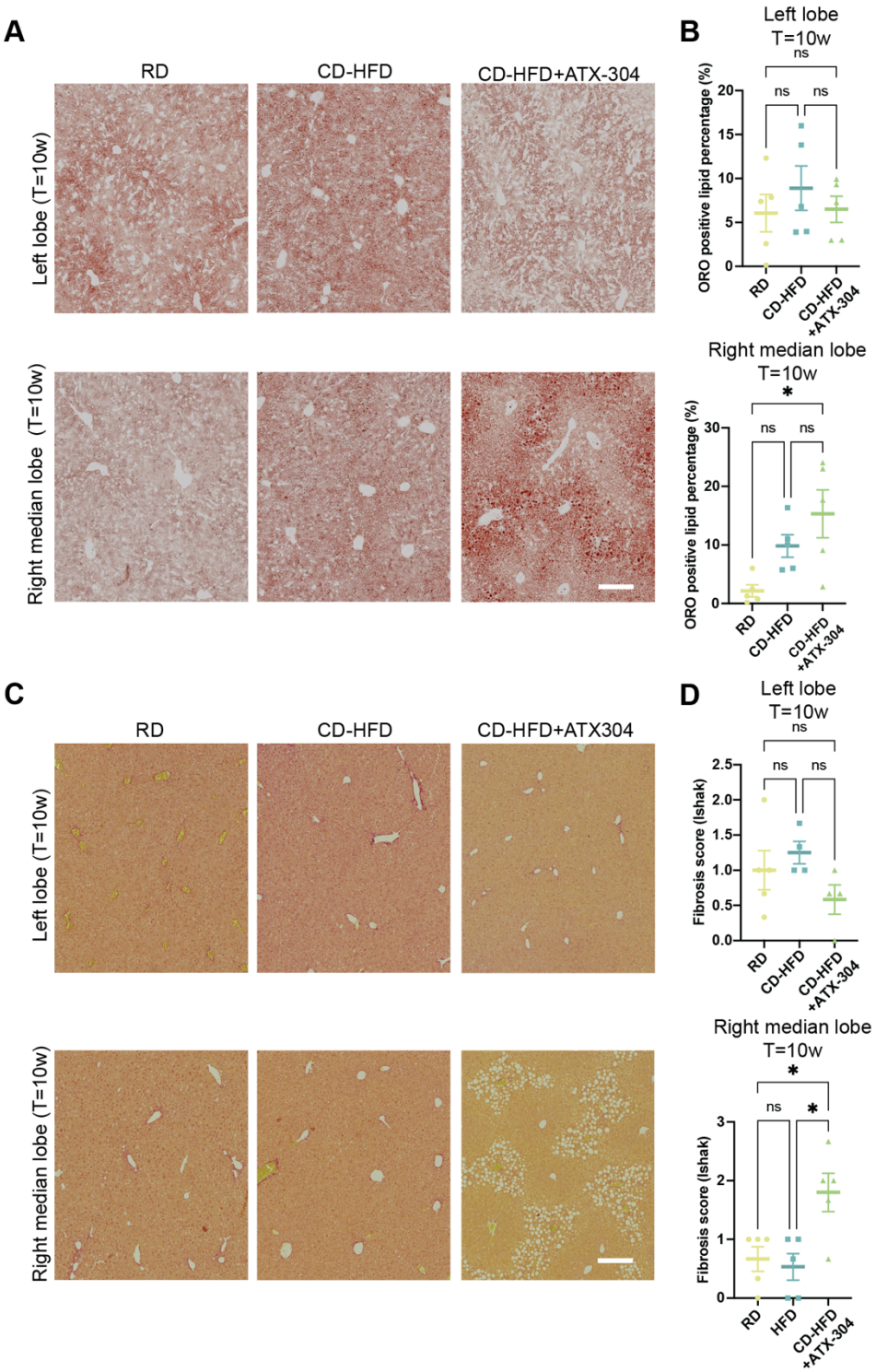
Supplementary figure 2. ATX-304 treatment reduces fat mass in CD-HFD mice. Lean, fat and total body mass as measured by EchoMRI. Same data as in Figure 1C-E but graphs depict the comparisons between RD, CD-HFD and CD-HFD+ATX-304 experimental groups in the long-term cohort before (T=20w) and after (T=30w) ATX-304-treatment . **p<0.01,***p<0.001, ****p<0.0001 (One-way ANOVA with Tukey's multiple comparisons test). Individual data points, mean ± SEM are indicated (n=10 for all groups).



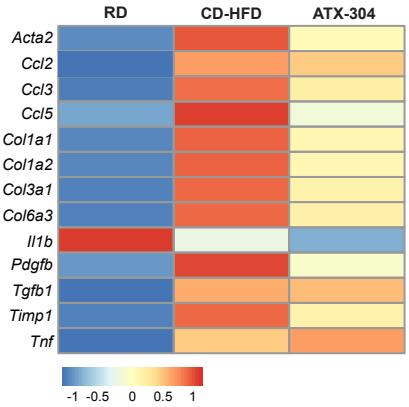
Supplementary figure 3. ATX-304 increase long-term food intake in C57Bl/6J male mice. Average food intake per day and mouse for RD, CD-HFD and CD-HFD+ATX-304 in **A**) long-term and **B**) short-term cohorts. Graphs depict the average food intake per mouse and day at indicated time periods before and after diet-switch. Average food intake is increased in both long and short-term cohorts for ATX-304-treated mice at most time points, with the exception of the initial weeks after diet switch in the long-term cohort, where food intake is reduced.



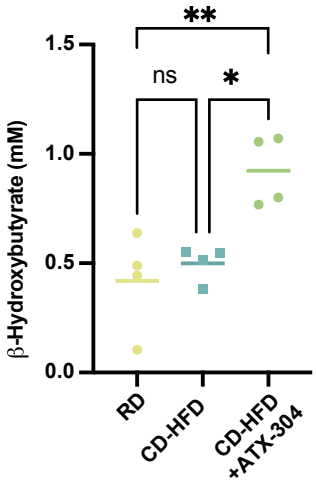
Supplementary figure 4. ATX-304 treatment significantly lowers insulin and blood glucose in C57Bl/6J male mice. A) Fasted insulin for RD, CD-HFD and CD-HFD+ATX-304 before start (T=20 weeks) and after 2w (T=23 weeks) of ATX-304 treatment. **B**) Fasted blood glucose for RD, CD-HFD and CD-HFD+ATX-304 at start (T=3 weeks) and after 6w (T=9 weeks) of ATX-304 treatment. **C**) Fasted blood glucose for RD, CD-HFD and CD-HFD+ATX-304 at start (T=20 weeks) and after 2w (T=23) or 25w (T=45w) of ATX-304 treatment. *p<0.05, ***p<0.001, ****p<0.0001 (One-way ANOVA with Tukey's multiple comparisons test). Individual data points, mean ± SEM are indicated in all graphs (n=10 for all groups).



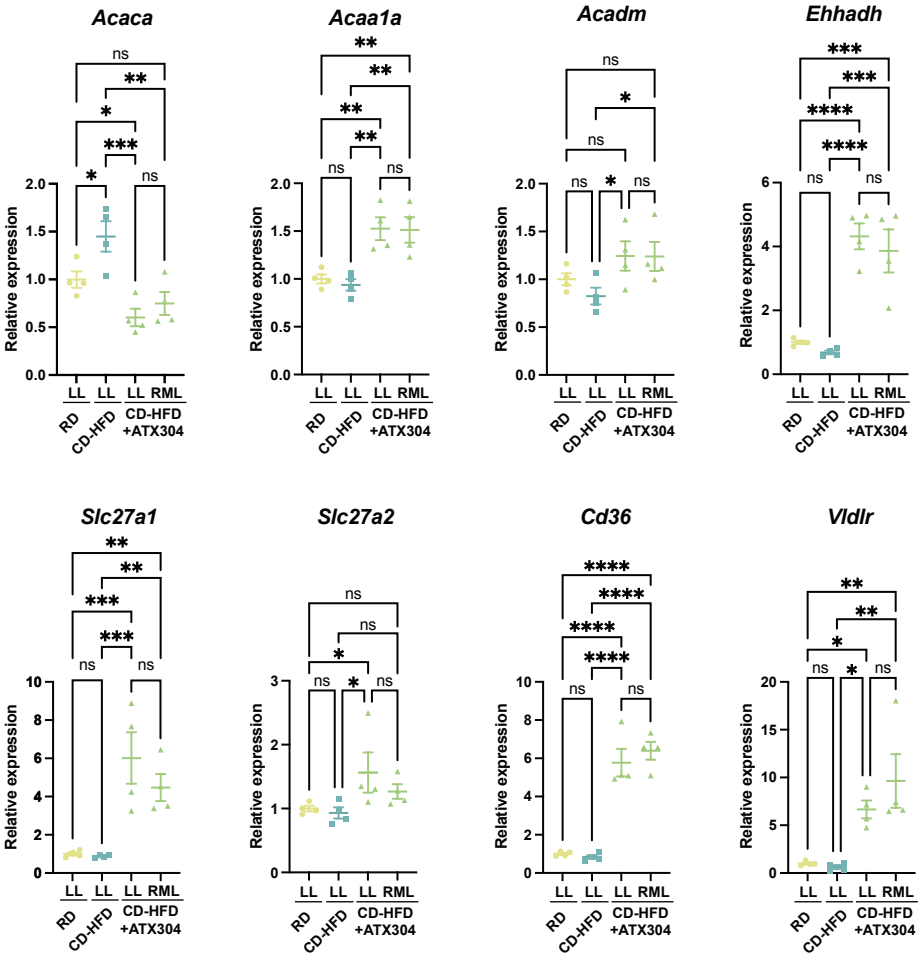
Supplementary figure 5. Lobular heterogeneities in distribution of lipids and fibrosis in ATX-304-treated livers. A) Oil red O-stained liver sections from the left lobe (upper row) and the right median lobe (bottom row) of RD, CD-HFD and ATX-304 treated mice at T=10w. Scalebar corresponds to 200um. Percentage of ORO positive area for the left (top) and right median lobe (bottom) is displayed in **B**) (n=5 for all groups). **C**) Representative images depicting picrosirius red (PSR) staining of sections from the left lobe (top row) and right median lobe (bottom row) from RD, CD-HFD and CD-HFD+ATX-304 livers at T=10w. Scalebar corresponds to 200um. **D**) Fibrosis score based on PSR staining for the left (top) and right median lobe (bottom). *p<0.05, ***p<0.001, ****p<0.0001 (One-way ANOVA with Tukey's multiple comparisons test). Individual data points, mean ± SEM are indicated in all graphs (n=5 for RD and CD-HFD, and n=4 for CD-HFD+ATX-304).



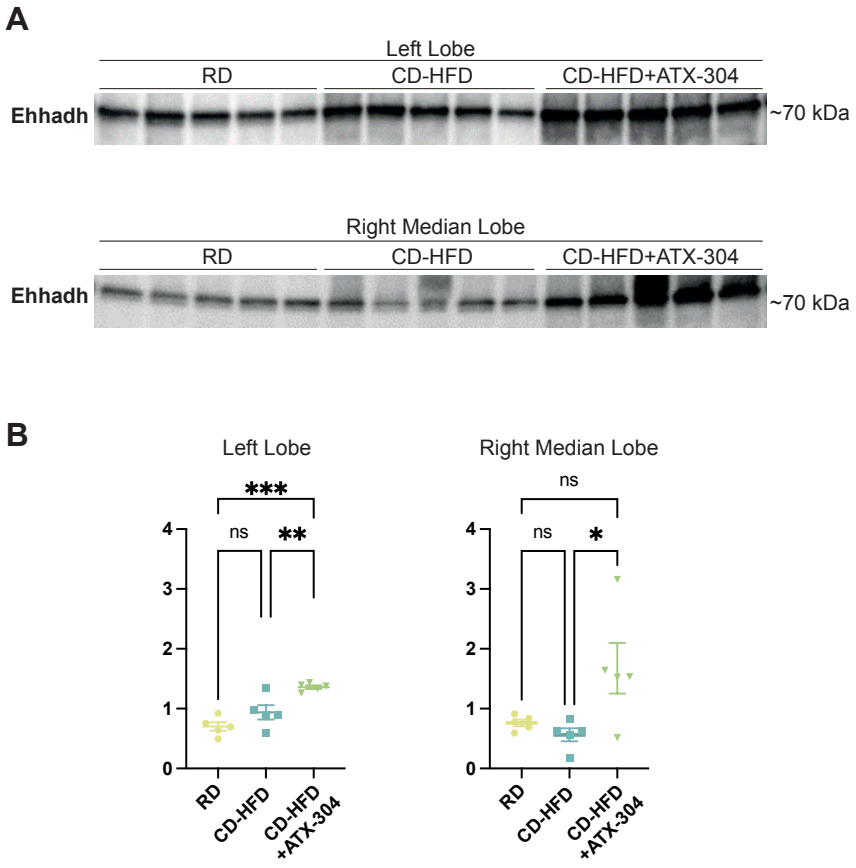
Supplementary figure 6. Expression of liver fibrosis and hepatic stellate cell activation markers in long-term treated mice. Heatmap of normalized RNA-seq read counts for markers of fibrosis and activated hepatic stellate cells in RD, CD-HFD and CD-HFD+ATX-304 from long-term cohort at T=31. Scale from blue to red indicates fold change over average read count for each row.



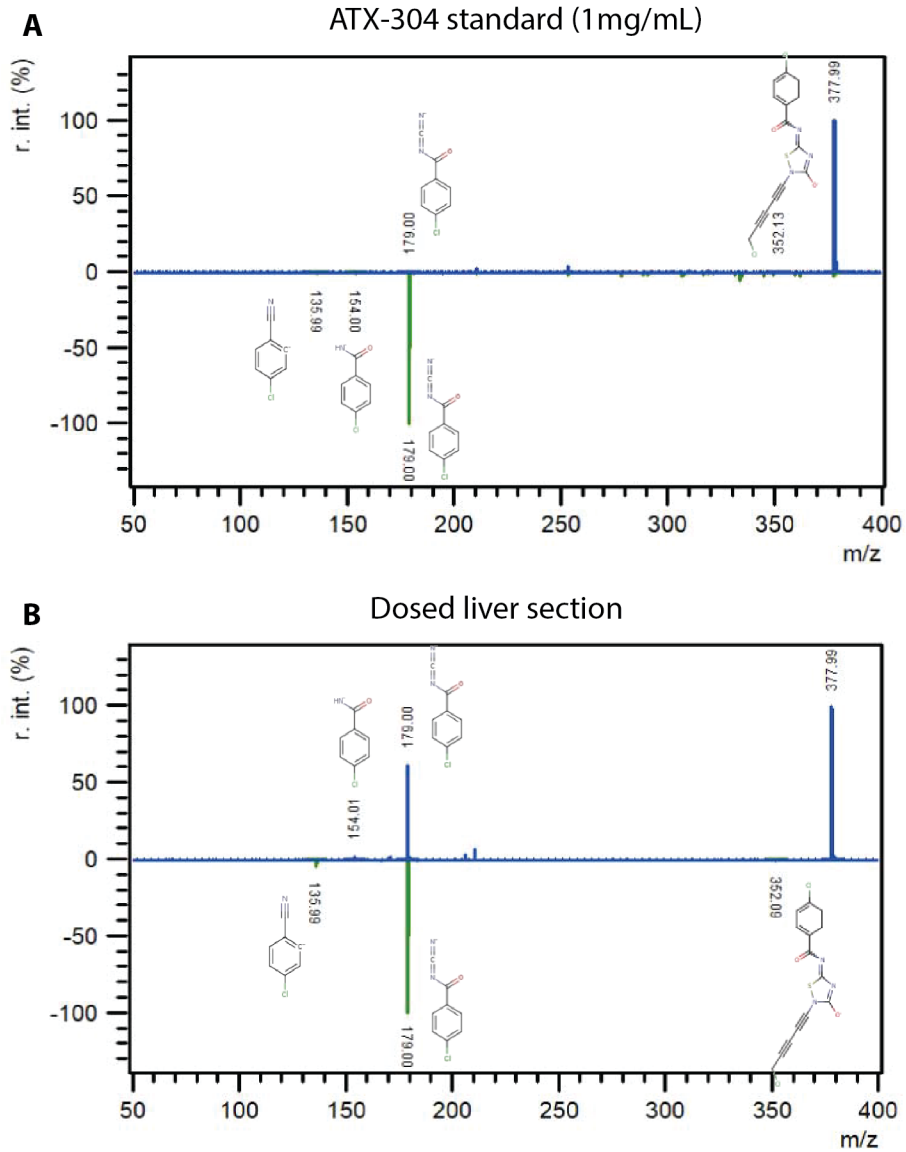
Supplementary figure 7. ATX-304 induces increased ketogenesis in short-term treated mice. Blood ß-Hydroxybutyrate levels in RD, CD-HFD and CD-HFD+ATX-304 mice at T=10. *p<0.05, **p<0.01 (One-way ANOVA with Tukey's multiple comparisons test). Individual data points, mean ± SEM are indicated in all graphs (n=4 for all groups).



Supplementary figure 8. ATX-304 induce expression changes in liver lipid metabolism genes. Relative expression of genes involved in fatty acid synthesis (*Acaca*), beta-oxidation (*Acaa1a*, *Acadm, Ehhadh*), and lipid transport (*Slc27a1*, *Slc27a2, Cd36, Vldr*) for RD, CD-HFD and CD-HFD+ATX-304 mice at T=10. *p<0.05, **p<0.01 (One-way ANOVA with Tukey's multiple comparisons test). Individual data points, mean ± SEM are indicated in all graphs (n=4 for all groups).



Supplementary figure 9. Protein levels of beta-oxidation enzyme EHHADH is increased in long-term treated mice. A) Immunoblot of EHHADH protein for the left lobe (upper panel) and right median lobe (lower panel) of the liver in RD, CD-HFD and CD-HFD+ATX-304 mice at T=45. **B)** Graph depicting relative protein expression normalised to total protein. *p<0.05, **p<0.01, ***p<0.001 (One-way ANOVA with Tukey's multiple comparisons test). Individual data points, mean ± SEM are indicated in all graphs (n=5 for all groups).



Supplementary figure 10. Identification of ATX-304 MS/MS spectrum in liver tissue sections. MS/MS spectra from **A**) ATX-304 drug standard (1mg/mL) spotted on ITO slide and **B**) dosed liver section.