Appendix

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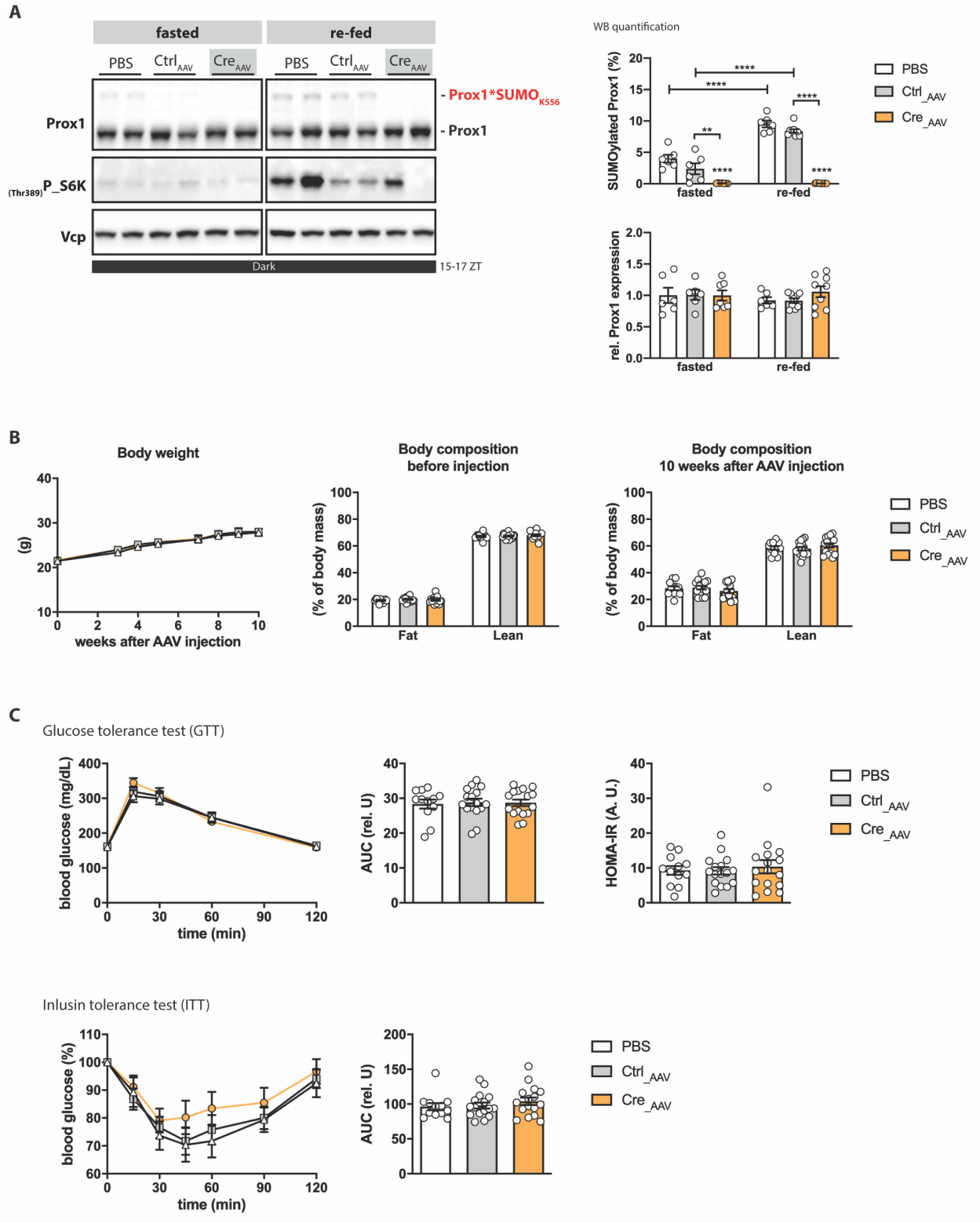
Appendix Figure S1



Appendix Figure S1 – Characterization of lean male mice expressing a Prox1$_{K556R}$ mutant in the liver (part 1)

8 weeks-old Prox1$_{K556R}$ K.I. (f/f) male mice were injected with phosphate-buffered saline (PBS), a control AAV (Ctrl_AAV) or with an AAV to overexpress Cre recombinase (Cre_AAV). 11 weeks after the AAV injection tissue samples were collected in the fasted (8 h) and re-fed (fasted 8h and re-fed 4-5 h) states between ZT 15 and 17 (n=6-9).

A) Liver lysates were analyzed by immunoblotting using anti-Prox1 and anti-P_S6K(Thr389) antibodies; Vcp was detected as an input control. The quantification of SUMOylated Prox1 and total Prox1 protein expression are shown.

B) Body weight records and Echo-MRI measurements taken before and 10 weeks after the AAV injections are shown (n=12-18).

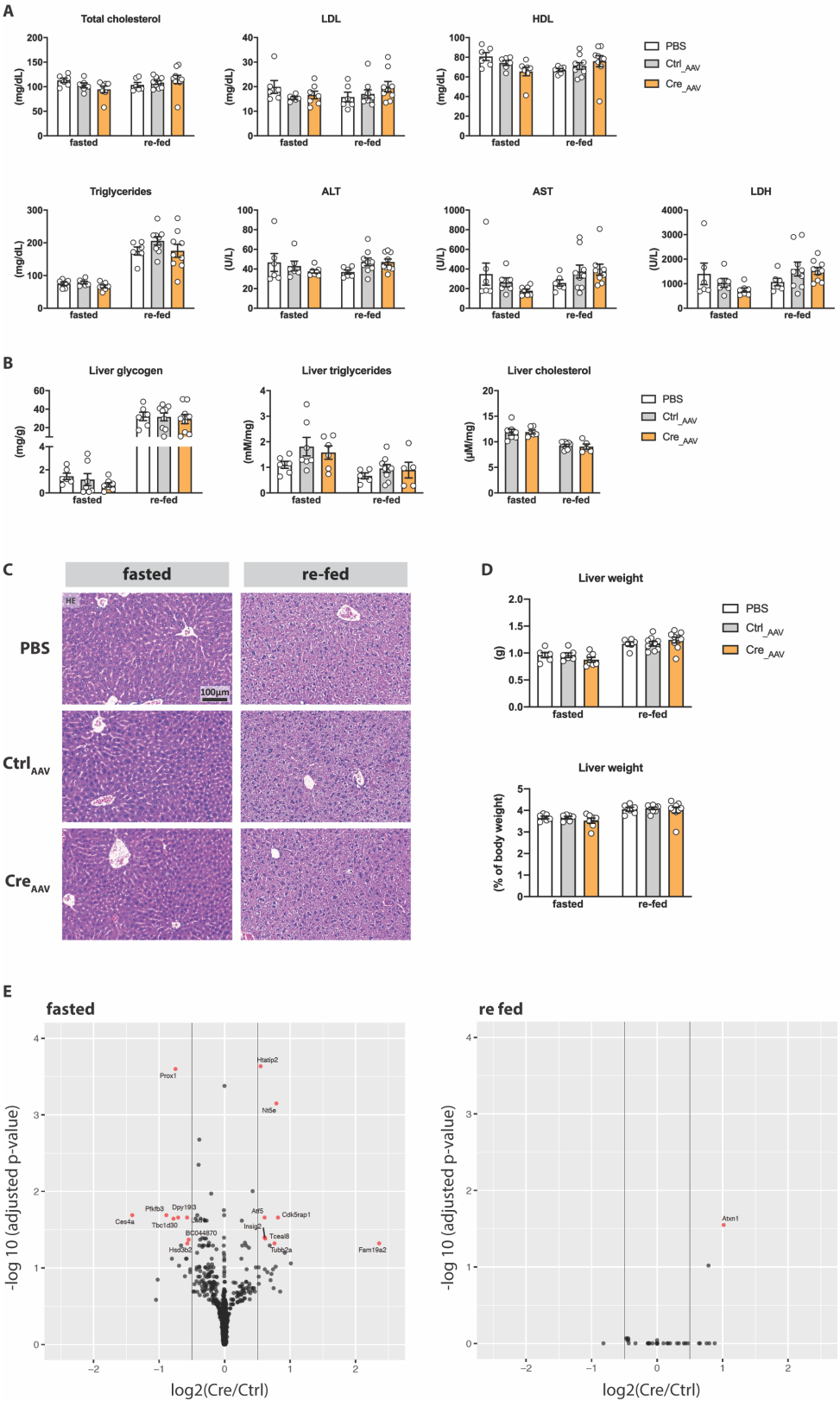
C) Top: GTT performed 8 weeks after the AAV injection. For the GTT mice were fasted for 5 to 6 h then challenged with glucose (1.5 g/kg) via an intraperitoneal injection. Blood samples were collected at time points: 0, 15, 30, 60 and 120 min after the glucose injection (n=12-18). Bottom: ITT performed 9 weeks after the AAV injection. For the ITT mice were fasted for 5 h then challenged with insulin (1.2 U/kg) via an intraperitoneal injection. Blood samples were collected at time points: 0, 15, 30, 45, 60, 90 and 120 min after the insulin injection (n=12-18). The area under the curve (AUC) and the calculated homeostatic model assessment for insulin resistance (HOMA-IR) are shown.

Data information: (A) Every dot represents one individual mouse. Data: mean ±SEM. Significance was determined by two-way ANOVA with Sidak´s multiple comparison test between different groups and conditions. (B-C) Every dot represents one individual mouse. Data: mean ±SEM.

Significance was determined by one-way ANOVA with Tukey´s multiple comparison test between different groups.

** P≤0.01, **** P≤0.0001.

Appendix Figure S2



Appendix Figure S2 – Characterization of lean male mice expressing a Prox1K556R mutant in the liver (part 2)

A) Serum analysis using a colorimetric-based serum analyzer. Levels of total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) are shown.

B) Colorimetric measurement of glycogen, triglycerides and total cholesterol content within the liver.

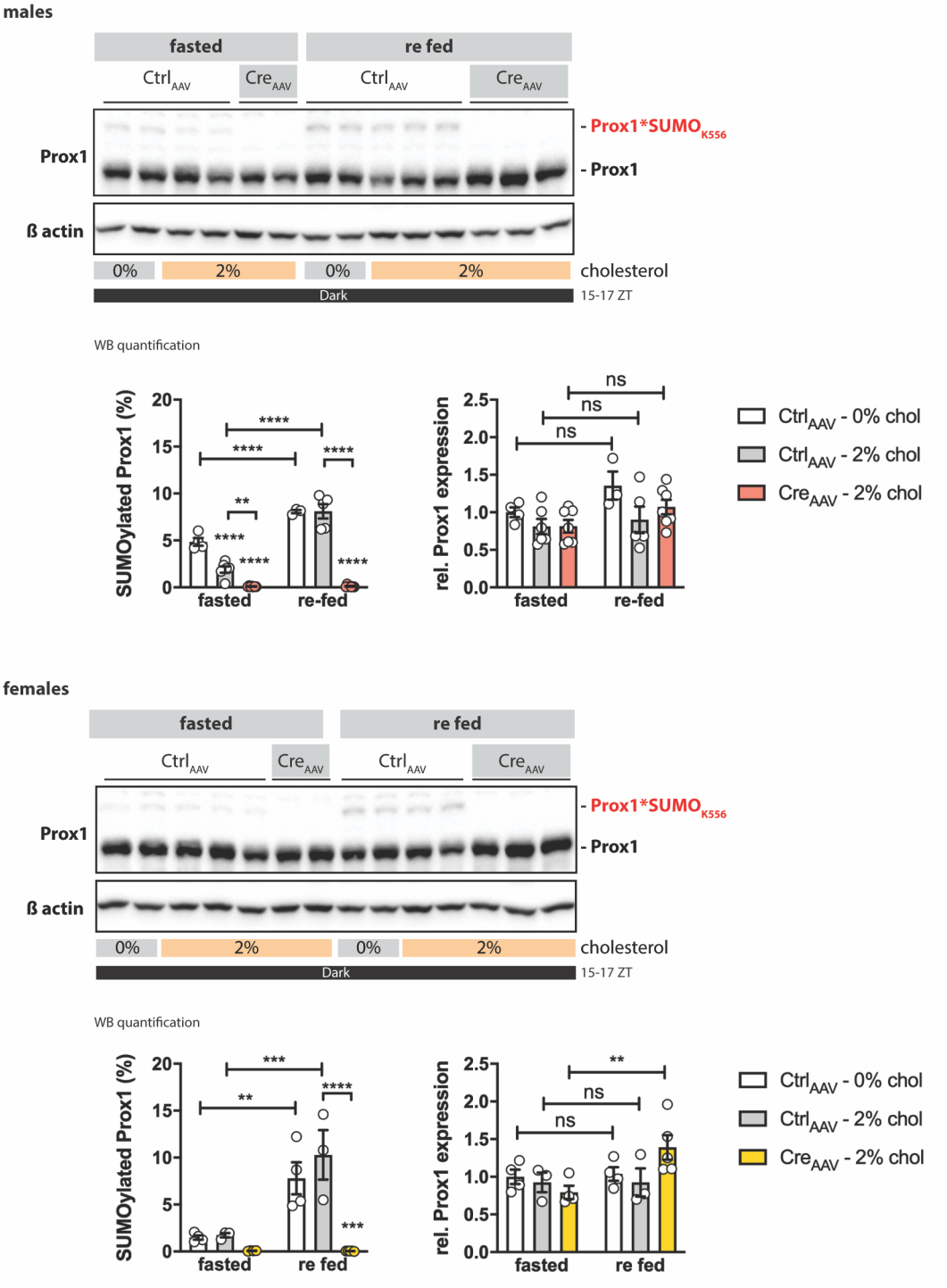
C) Liver morphology analyzed via hematoxylin and eosin staining (representative images; scale bar = 100 µm).

D) Liver weight in grams and in % relative to the total body weight.

E) Volcano plots illustrating the genes that are significantly up- or downregulated in the liver of mice expressing the Prox1 K556R mutant in comparison to mice expressing wild-type Prox1 in the fasted and re-fed states (n=5).

Data information: (A, B and D) Every dot represents one individual mouse. Data: mean ±SEM. Significance was determined by two-way ANOVA with Sidak´s multiple comparison test between different groups and conditions.

Appendix Figure S3



Appendix Figure S3 – Characterization of male and female Prox1K556R K.I. mice fed a high cholesterol diet (part 1)

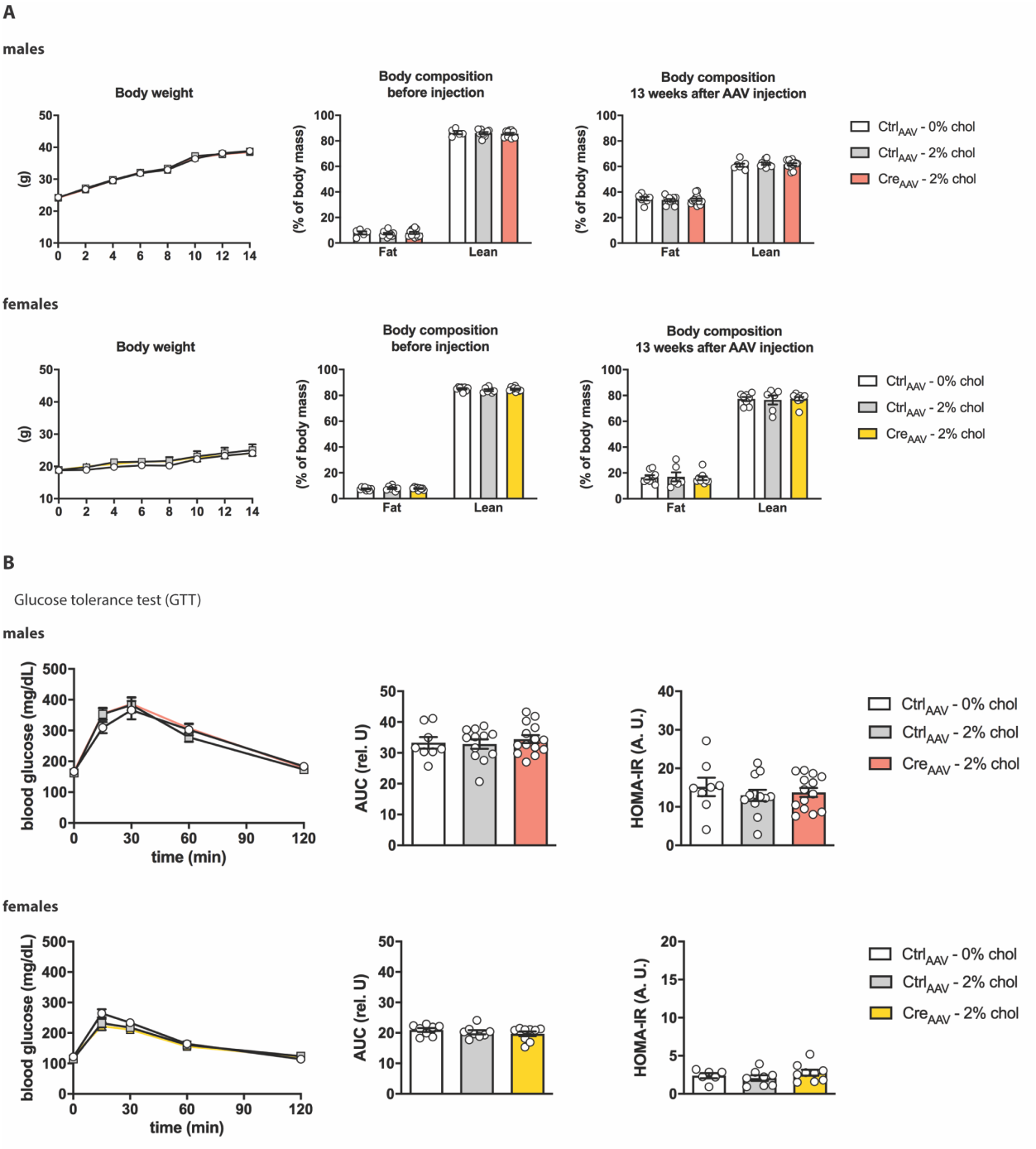
8 weeks-old $Prox1_{K556R}$ K.I. (f/f) male and female mice were injected with a control AAV (Ctrl_AAV) or with an AAV to overexpress Cre recombinase (Cre_AAV). Mice were fed a 2% high cholesterol for 14 weeks, a 0% cholesterol diet was used as control. Tissue samples were collected in the fasted (8 h) and re-fed (fasted 8h and re-fed 4-5 h) states between ZT 15 and 17 (n=3-7).

Liver lysates were analyzed by immunoblotting using anti-Prox1 antibodies; β actin was detected as an input control. The quantification of SUMOylated Prox1 and total Prox1 protein expression are shown.

Data information: Every dot represents one individual mouse. Data: mean ±SEM. Significance was determined by two-way ANOVA with Sidak´s multiple comparison test between different groups and conditions.

** P≤0.01, *** P≤0.001, **** P≤0.0001.

Appendix Figure S4



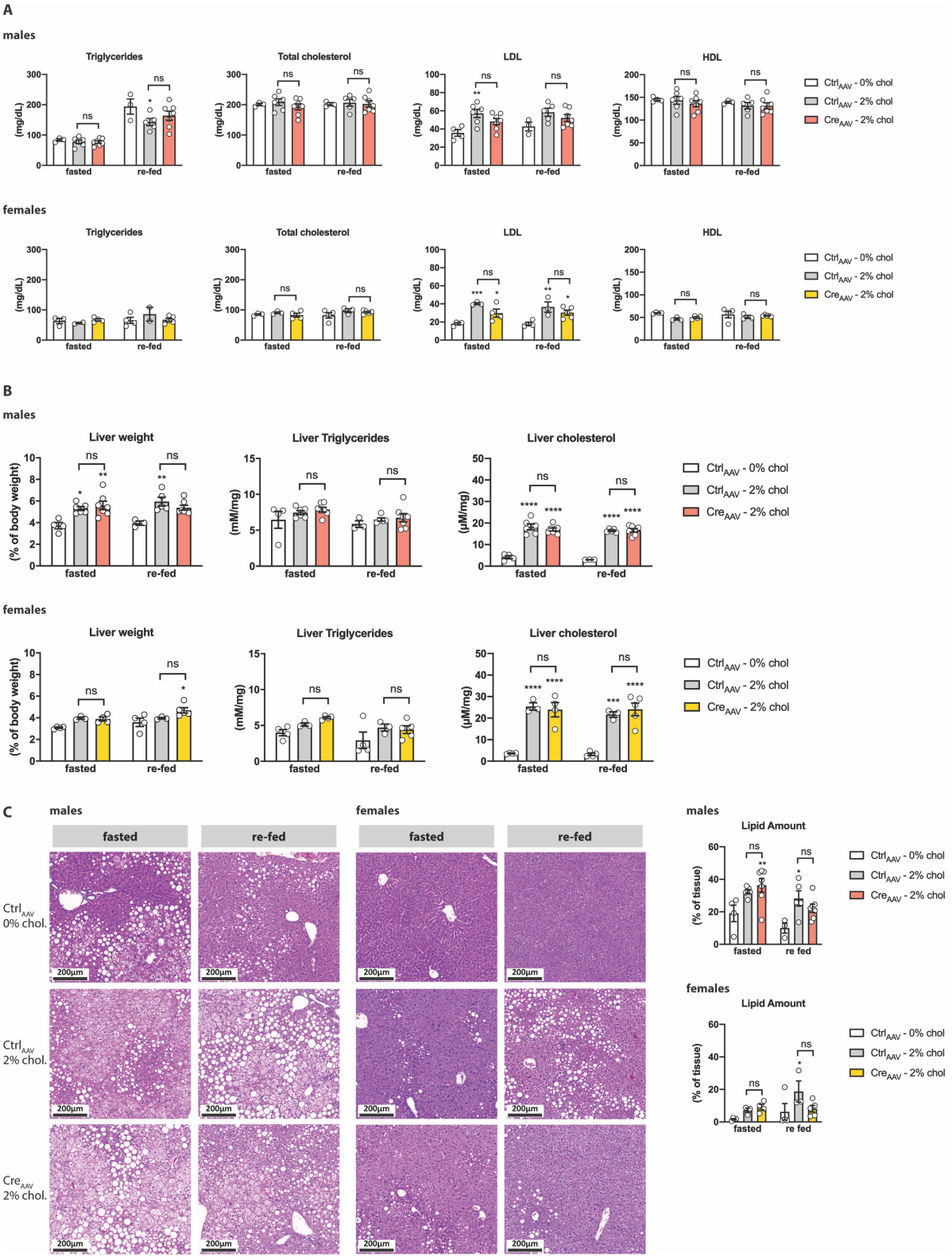
Appendix Figure S4 – Characterization of male and female Prox1K556R K.I. mice fed a high cholesterol diet (part 2)

A) Body weight records and Echo-MRI measurements taken before and 13 weeks after the AAV injections and diet start are shown (n=6-14).

B) GTT performed 4 weeks after the AAV injection and diet start. For the GTT mice were fasted for 5 to 6 h then challenged with glucose (2 g/kg) via an intraperitoneal injection. Blood samples were collected at time points: 0, 15, 30, 60 and 120 min after the glucose injection (n=6-14). The area under the curve (AUC) and the calculated homeostatic model assessment for insulin resistance (HOMA-IR) are shown.

Data information: Every dot represents one individual mouse. Data: mean ±SEM. Significance was determined by one-way ANOVA with Tukey´s multiple comparison test between different groups.

Appendix Figure S5



Appendix Figure S5 – Characterization of male and female Prox1K556R K.I. mice fed a high cholesterol diet (part 3)

A) Serum analysis using a colorimetric-based serum analyzer. Levels of triglycerides, total cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL) are shown.

B) Liver weight in % relative to the total body weight and colorimetric measurement of triglycerides and total cholesterol content within the liver.

C) Liver morphology analyzed via hematoxylin and eosin staining (representative images; scale bar = 200 μm). The digital quantification of lipid vacuoles per tissue area are shown.

Data information: Every dot represents one individual mouse. Data: mean ±SEM. Significance was determined by two-way ANOVA with Sidak´s multiple comparison test between different groups and conditions. *P≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001.