Expanded View Figures

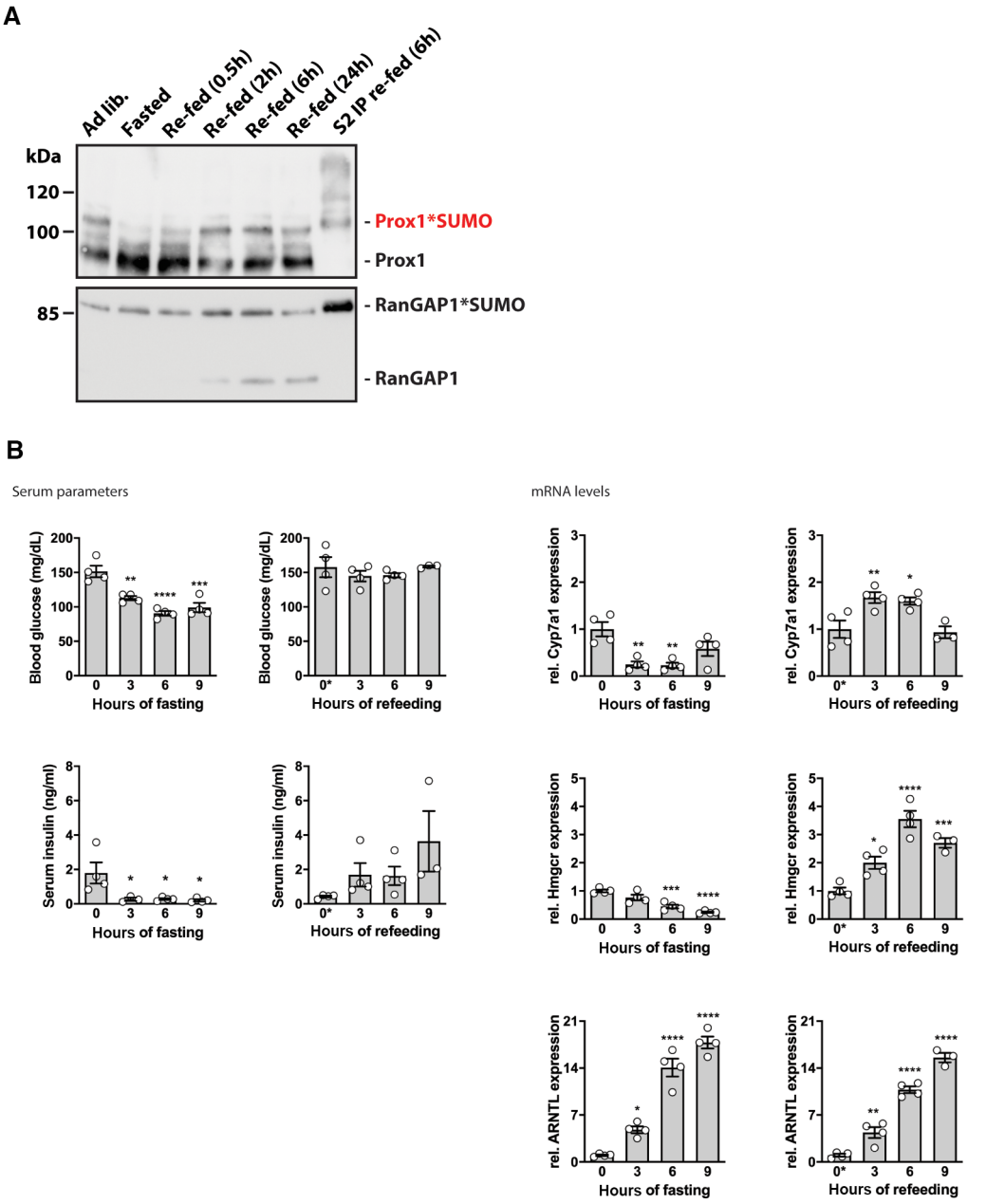

Figure EV1. Hepatic Prox1 is modified by SUMOylation in response to nutrient availability. (Related to Fig 1).

A Liver samples from C57BL/6J mice in the ad libitum, fasted (16 h) and refed (16 h fasted and refed for 0.5, 2, 6 and 24 h) states were analyzed by immunoblotting using anti-Prox1 and RanGAP1 antibodies. The SUMO2 immunoprecipitation eluate was used for size verification. SUMOylated RanGAP1 was used as a loading control.

B Eight weeks old C57BL/6N male mice were fasted: food was removed at ZT 12 or re-fed: all groups were fasted for 8 h during the light phase (ZT 4–12) for synchronization, the food was reintroduced at ZT 12. Blood and liver samples were collected at ZT 12, 15, 18 and 21 (n = 4). Blood glucose and insulin levels (left) as well as liver expression analysis at the mRNA level by qPCR (right) are shown. qPCR data presented as relative fold change normalized to the housekeeping gene TBP.

Data information: (B) Every dot represents one individual mouse. Data: mean ± SEM. Significance was determined by one-way ANOVA with Dunnett's multiple comparison test relative to samples collected at ZT 12. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$.
Source data are available online for this figure.

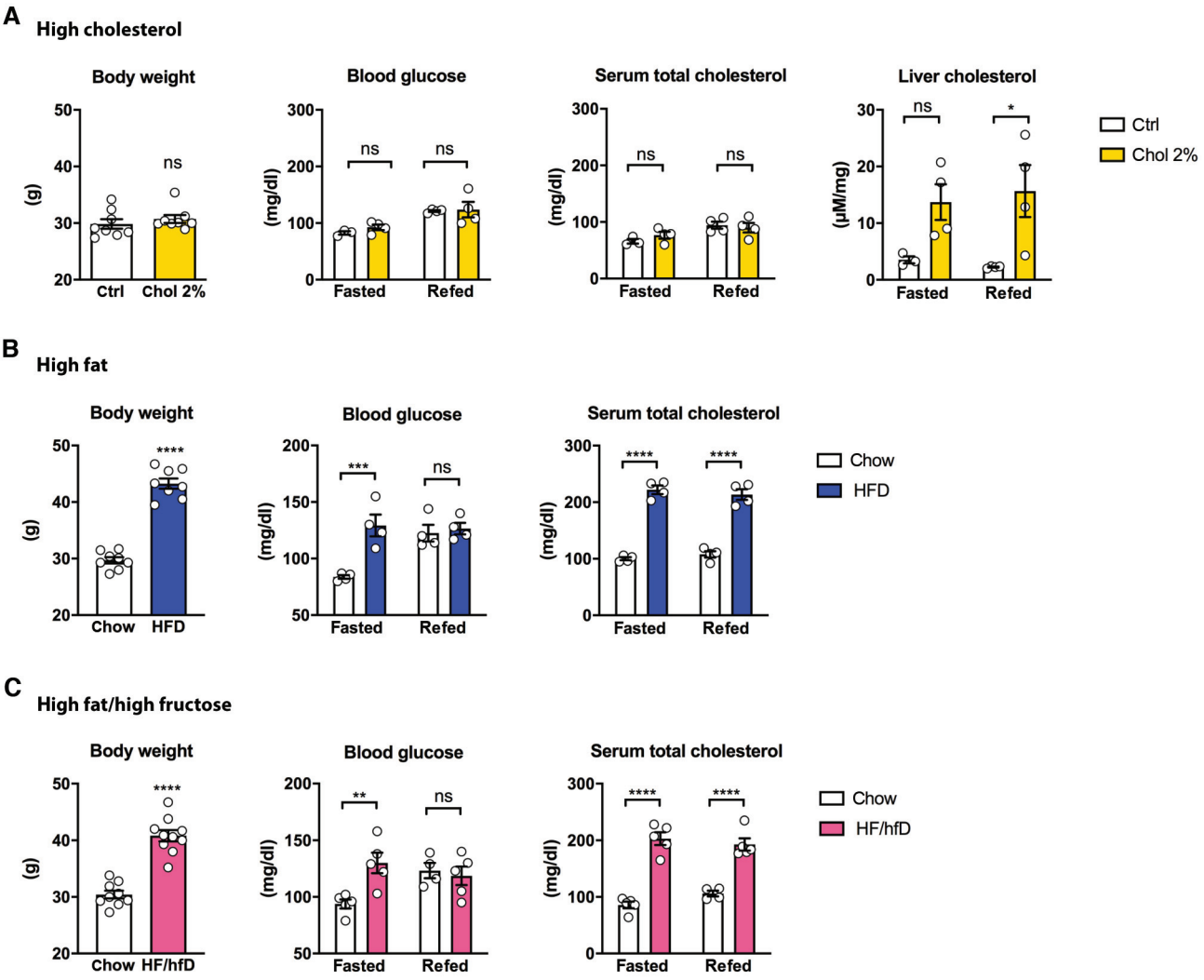

Figure EV2. Diet-induced obesity prevents Prox1 de-SUMOylation during fasting. (Related to Fig 3).

A Six-week-old C57BL/6N male mice were fed either a 0% cholesterol diet (ctrl) or a 2% high-cholesterol diet (chol 2%) for 6 weeks.

B Six-week-old C57BL/6N male mice were fed either a standard chow diet or a 60% high fat diet (HFD) for 8 weeks.

C Six-week-old C57BL/6N male mice were fed either a standard chow diet or a 45% high fat +20% w/v fructose diet (HF/hfD) for 12 weeks.

(A–C) Blood and liver samples were collected at ZT 15 in the fasted (11 h) and re-fed (fasted 8 h and refed 3 h) states (n = 4–5). Body weight records taken a day before tissue collection (n = 8–10), blood glucose, serum levels of total cholesterol and liver content of total cholesterol (for A) 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. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$.
Source data are available online for this figure.

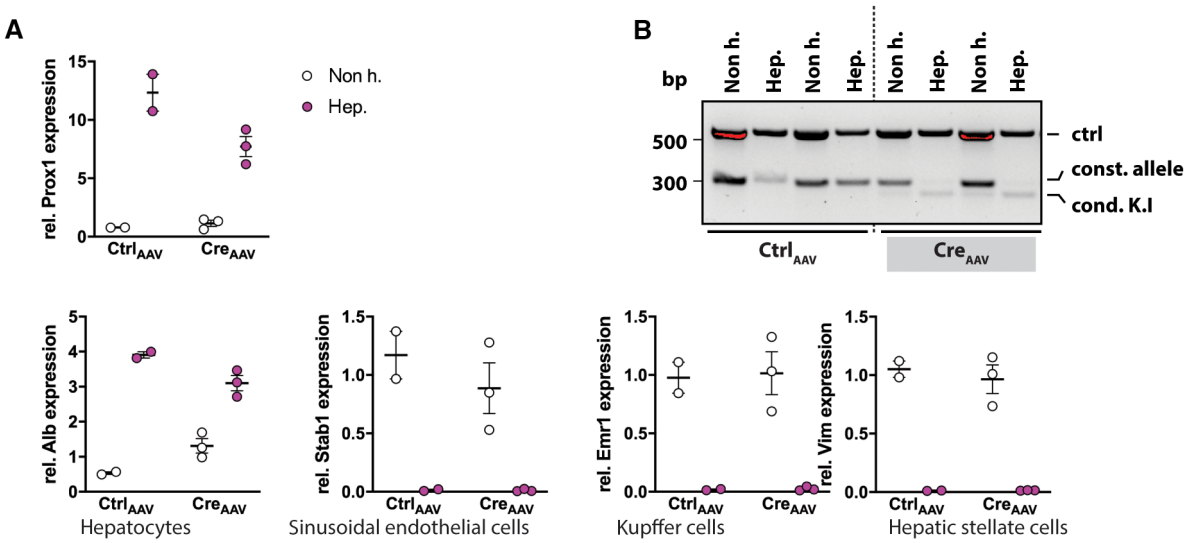

Figure EV3. Conditional SUMO-deficient Prox1 knock-in mouse model. (Related to Fig 4).

A Eight-week-old $Prox1_{K556R}$ K.I. (f/f) male mice were injected with a control AAV (Ctrl_AAV) or an AAV to overexpress Cre recombinase (Cre_AAV) (n = 2–4). 3 weeks later, hepatocytes (hep.) were isolated from the nonhepatocyte (non.h.) fraction. Expression analysis at the mRNA level by qPCR, data presented as relative fold change normalized to the housekeeping gene TBP.

B Amplification reaction by PCR detecting the constitutive allele (280 bp) or the conditional K.I. allele after Cre-mediated recombination (235 bp); control product (585 bp).

Data information: (A) Every dot represents one individual mouse. Data: mean ± SEM.
Source data are available online for this figure.

Figure EV4. Obese mice expressing SUMO-deficient Prox1 in the liver show reduced serum cholesterol (Related to Fig 5).

Eight-week-old $Prox1_{K556R}$ K.I. (f/f) male mice were injected with a control AAV (Ctrl_AAV) or with an AAV to overexpress Cre recombinase (Cre_AAV) and placed on a high-fat (45%) high-fructose (20% w/v) diet for 18 weeks. Serum and tissues were collected between ZT 16 and 17 in the fasted (8 h) and refed (fasted 8 h and re-fed 4–5 h) states (n = 7–8).

A Body weight records and fasting glucose levels recorded 12 weeks after the AAV injection are shown (n = 14).

B Serum analysis using a colorimetric-based serum analyzer. Levels of triglycerides, total bile acids, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) are shown (n = 7–8).

C Colorimetric measurement of total cholesterol and cholesteryl ester within the liver as well as liver weight (in % relative to the total body weight) are shown (n = 7–8).

D Liver morphology analyzed via hematoxylin and eosin staining and detection of connective collagenous tissue via Sirius red staining (representative images; scale bar = 100 μm). The digital quantification of lipid vacuoles per tissue area is shown.

Data information: (A) Every dot represents one individual mouse. Data: mean ± SEM. Significance was determined by two-tailed, unpaired t-test. (B–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.
Source data are available online for this figure.

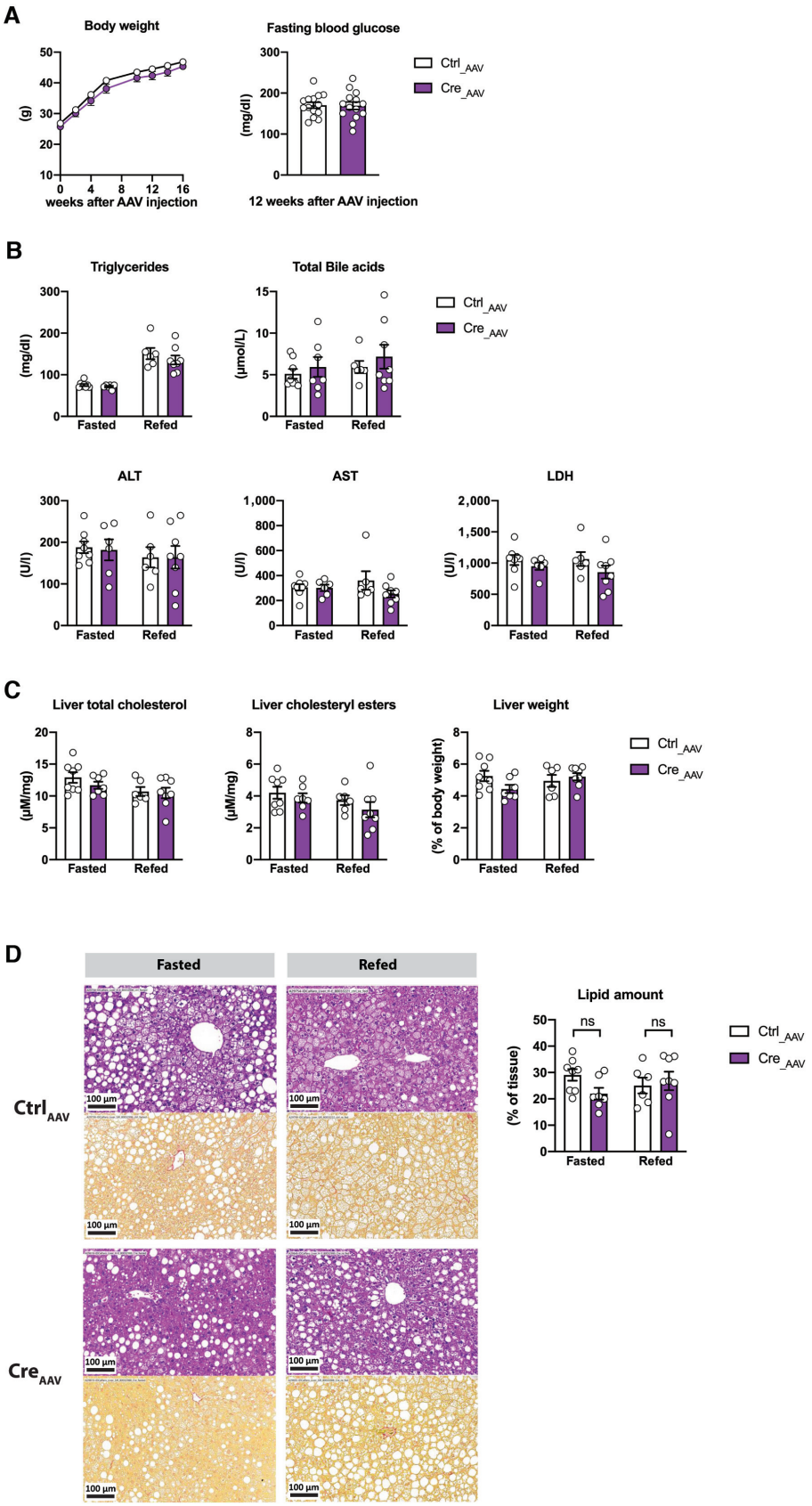

Figure EV4.

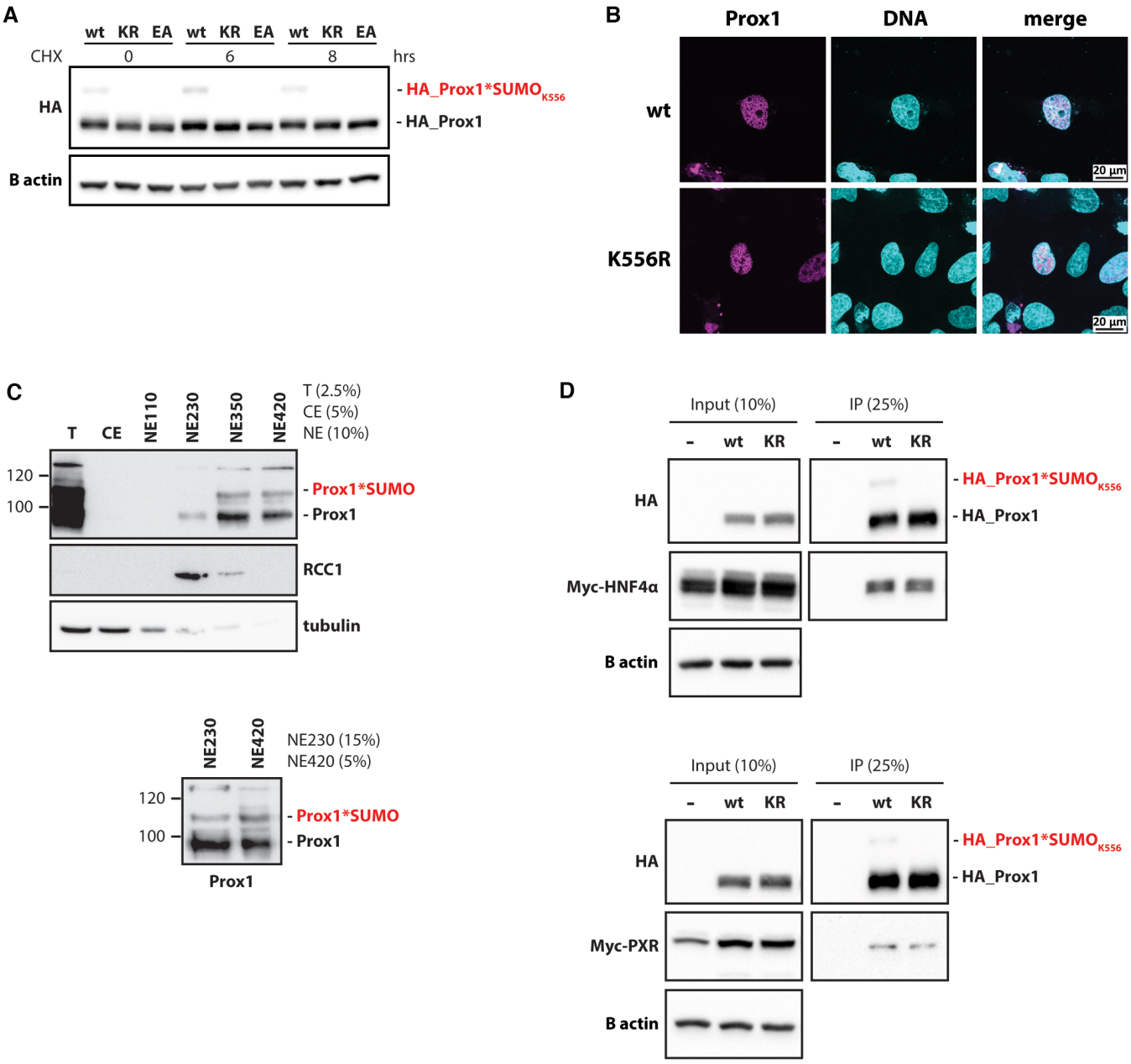

Figure EV5. SUMOylation does not affect the stability nor nuclear localization of Prox1.

A HEK293A cells overexpressing mouse HA-tagged wild-type Prox1 (wt), K556R mutant (KR) or E558A mutant (EA) were treated with cycloheximide (CHX) for 6 and 8 h. The cell lysates were analyzed by immunoblotting using anti-HA and anti-β actin antibodies.

B HepG2 cells overexpressing mouse YFP-tagged wild-type Prox1 (wt) or K556R mutant were analyzed by fluorescence microscopy (magenta), DNA was stained with DAPI (blue); (representative images; scale bar = 20 µm).

C The nuclei from HepG2 cells were isolated and used for protein extraction. Multiple nuclear extracts were prepared using different concentrations of NaCl (110, 230, 350 or 420 mM). Total samples collected before cell lysis (T), the cytoplasmic extracts (CE) and the nuclear extracts (NE) were analyzed by immunoblotting using anti-Prox1 antibodies. The chromatin binding factor RCC1 and tubulin were used as controls.

D Prox1/PXR and Prox1/HNF4α co-immunoprecipitation in HEK293A cells overexpressing HA-tagged Prox1 wild-type (wt) or K556R mutant (KR) and Myc-tagged PXR or HNF4α.