Expanded View Figures

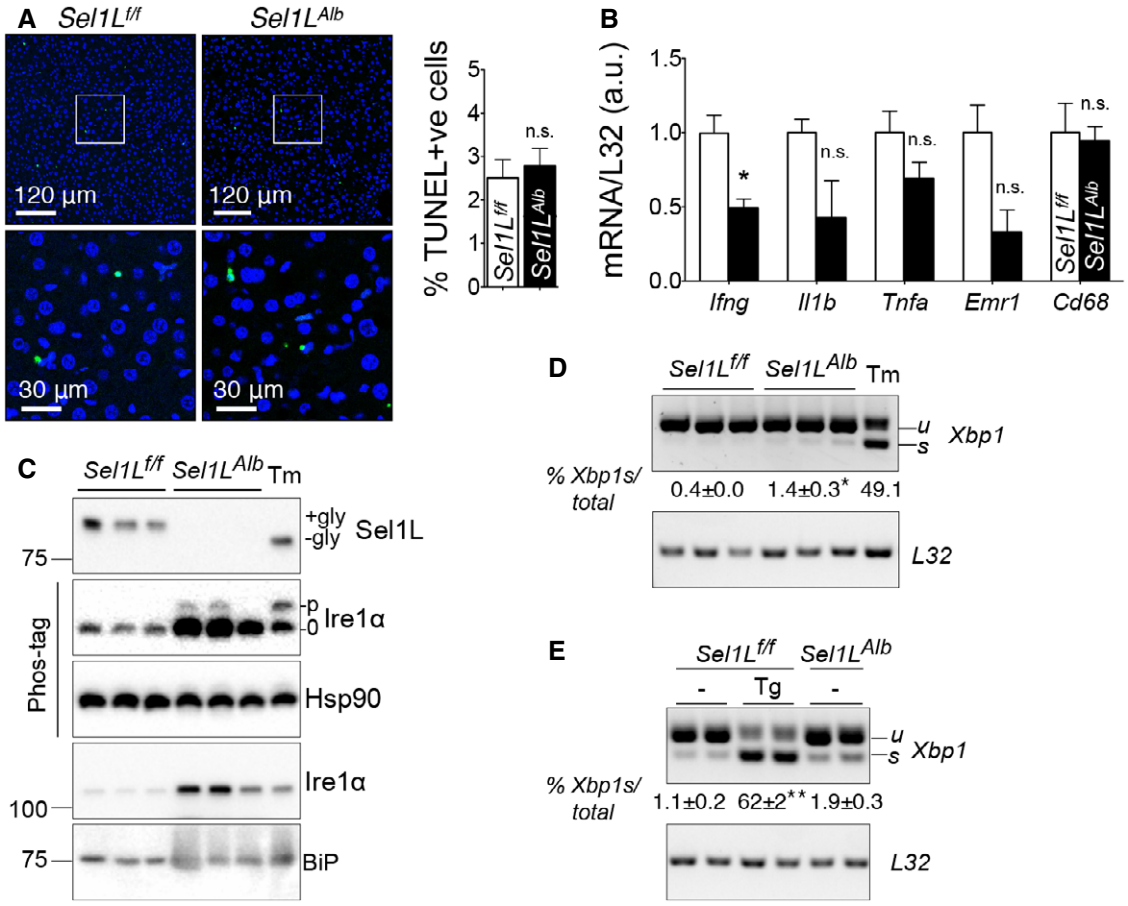

Figure EV1. Lack of cell death, inflammation, and overt ER stress in Sel1L-deficient hepatocytes.

A TUNEL staining of paraffin-embedded livers of 9-week-old mice with quantitation shown on the right (n = 4 per group, 2 independent repeats).

B qPCR analysis of inflammation associated hepatic gene expression in *Sel1L*$^{f/f}$ and *Sel1L*^{Alb} mice (n = 4 per group, 3 independent repeats).

C Western blot analysis of Sel1L and UPR proteins (Ire1α and BiP) in the livers of 9-week-old mice (n = 3 per group, 3 independent repeats). +/− Gly refers to proteins with or without glycosylation; and p/0 refers to phosphorylated or non-phosphorylated Ire1α. WT mice injected i.p. with tunicamycin (Tm, 1.5 µg/g body weight) for 72 h were included as a control.

D RT–PCR analysis of *Xbp1* splicing in the livers of 9-week-old mice (n = 3 per group, 3 independent repeats); *u*/*s*/*t* refers to unspliced/spliced/total *Xbp1*. WT mice injected i.p. with tunicamycin (Tm, 1.5 µg/g body weight) for 72 h were included as a control.

E RT–PCR analysis of *Xbp1* splicing in primary mouse hepatocytes (n = 2 per group, 2 independent repeats). WT primary hepatocytes treated with 200 nM thapsigargin (Tg) for 6 h were included as a control. Quantitation of the percent of Xbp1s in total Xbp1 mRNA is shown below.

Data information: Hsp90, loading control for Western blot analysis. Ribosomal *L32*, loading control for qPCR and RT–PCR analysis. Values are mean ± SEM; *P < 0.05; **P < 0.01; n.s., not significant by Student's t-test.

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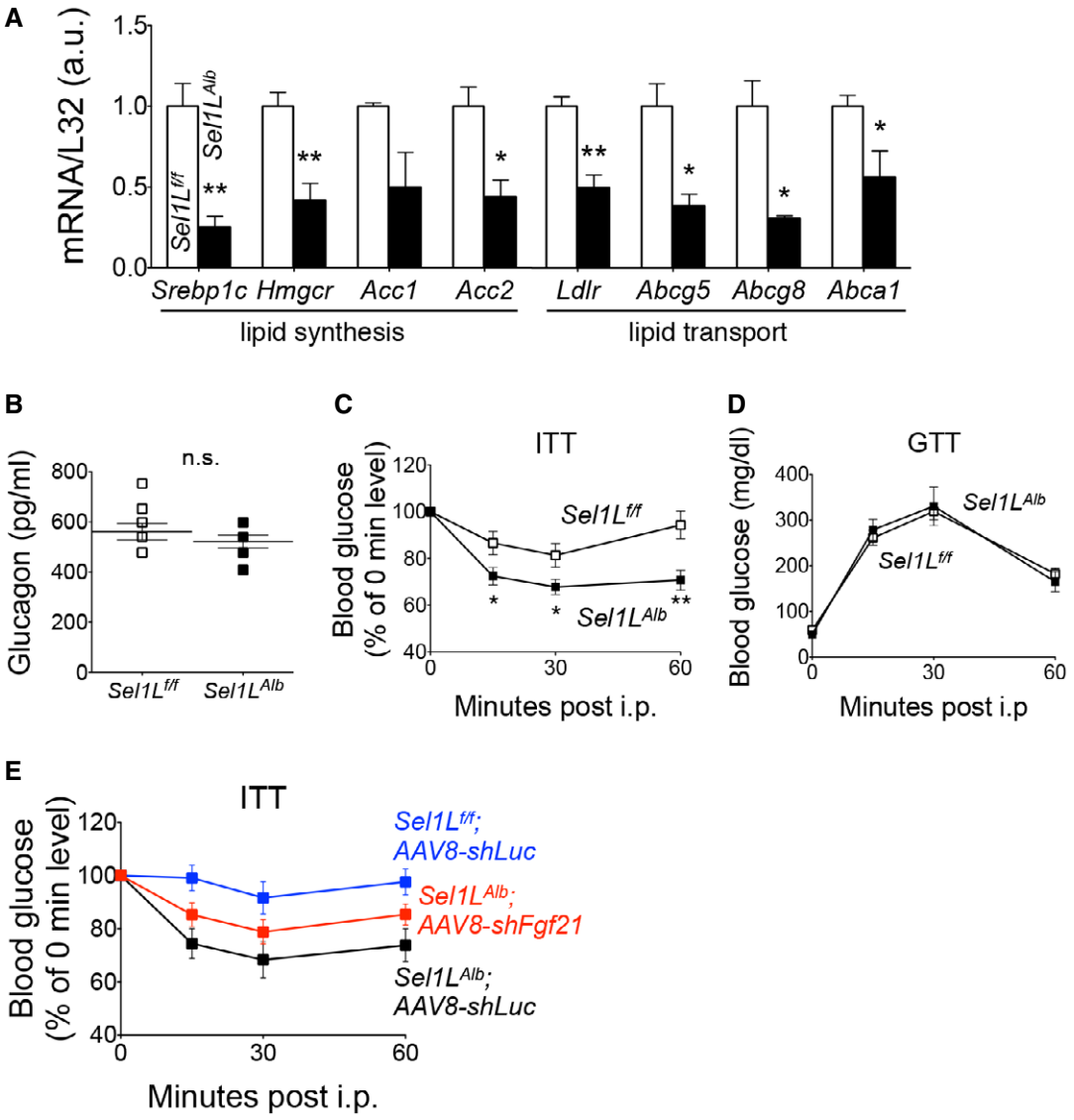

Figure EV2. Hepatic Sel1L-Hrd1 ERAD-deficient mice have altered metabolism.

A qPCR analysis of lipid synthesis and transport genes in 9-week-old mice (n = 6 per group, 2 independent repeats).

B Serum glucagon levels after 6 h of fast in the morning (n = 5–6 per group).

C, D Insulin tolerance test (ITT) (C) and glucose tolerance test (GTT) (D) of 10-week-old male mice (n = 6 per group).

E Insulin tolerance test (ITT) 3 weeks after i.v. injection (n = 5–6 per group) with AAV8-*shFgf21* or control AAV8-*shLuc*.

Data information: Ribosomal *L32*, loading control for qPCR analysis. Values are mean ± SEM; *$P < 0.05$; **$P < 0.01$; n.s., non-significant by Student's t-test.

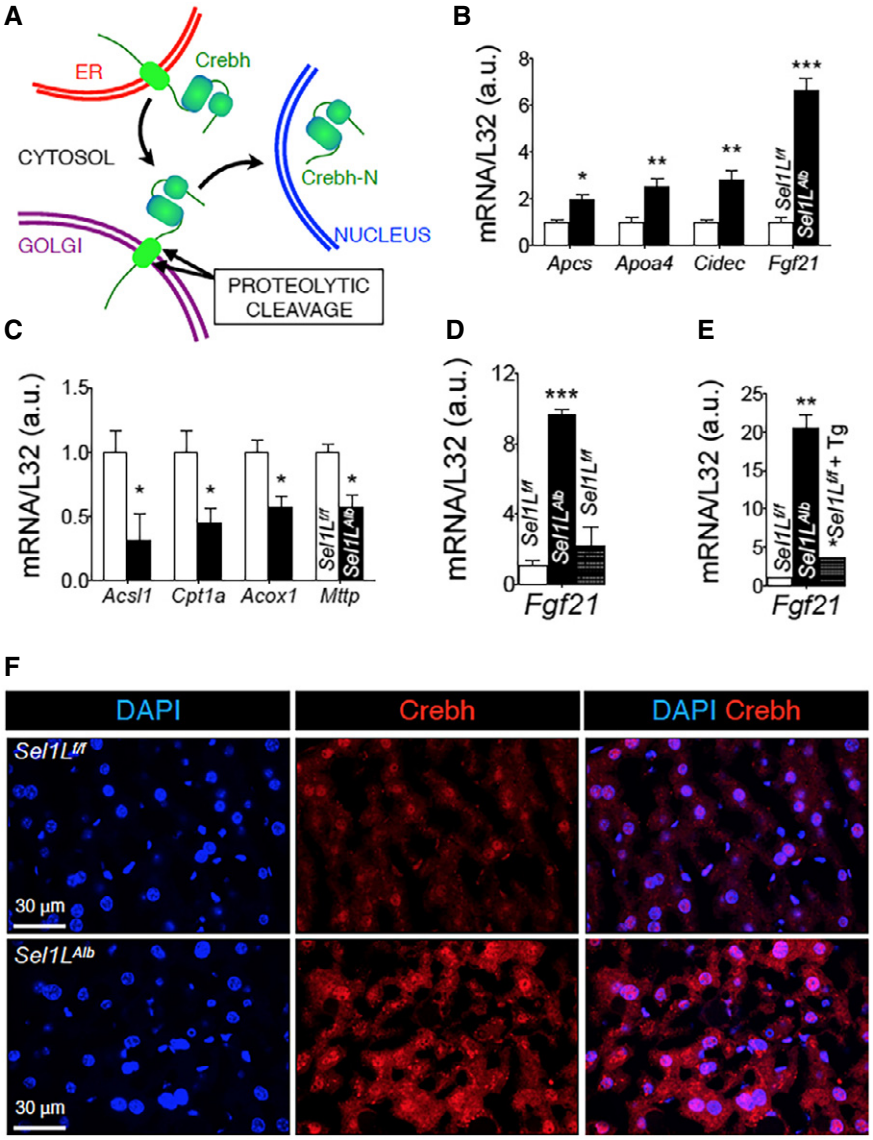

Figure EV3. Hepatic Sel1L regulates protein stability and activity of Crebh, not Pparα.

A Schematic diagram showing the intracellular trafficking of ER-resident Crebh protein to the Golgi for proteolysis, leading to the generation of Crebh-N. Crebh-N subsequently translocates into the nucleus to activate gene transcription.

B, C qPCR analysis of Crebh (B) and Pparα (C) target genes in WT and *Sel1L*^{Alb} livers of 9-week-old mice (n = 4 per group, 2 independent repeats).

D qPCR analysis of hepatic *Fgf21* expression in the livers of 9-week-old *Sel1L*$^{f/f}$ and *Sel1L*^{Alb} mice (n = 3 per group, 2 independent repeats). WT mice injected i.p. with tunicamycin (Tm, 1.5 µg/g body weight) for 72 h were included as a control.

E qPCR analysis of *Fgf21* expression in primary hepatocytes (n = 2 per group, 2 independent repeats). WT primary hepatocytes treated with 200 nM thapsigargin (Tg) for 6 h are included as a control.

F Representative immunostaining images (zoomed out from Fig 4H) from 8-week-old liver cryosections.

Data information: Ribosomal *L32*, loading control for qPCR analysis. Values are mean ± SEM; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ by Student's t-test.

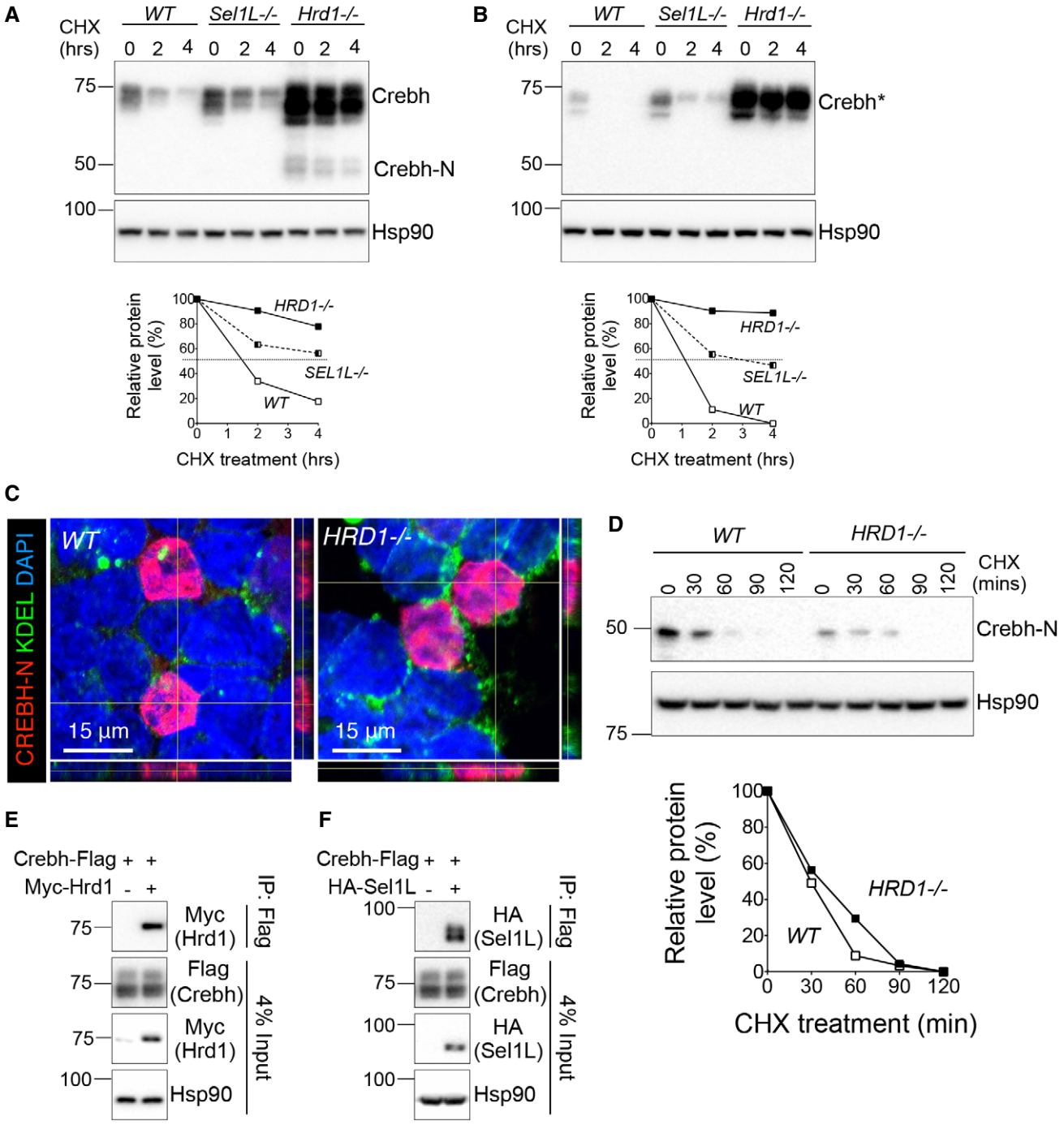

Figure EV4. Crebh, and not Crebh-N, is an ERAD substrate.

A, B Western blot analysis of Crebh (A) and cleavage-defective-Crebh (B, Crebh*) half-life in transfected *WT*, *Sel1L*$^{-/-}$, and *Hrd1*$^{-/-}$ N2a cells treated with cycloheximide (CHX) for indicated times. The decay of Crebh proteins is shown below.

C Representative immunostaining images of transfected Crebh-N-Flag protein 24-h posttransfection into *WT* and *HRD1*$^{-/-}$ HEK293T cells.

D Western blot analysis and quantitation of Crebh-N protein decay in Crebh-N-Flag-transfected *WT* and *HRD1*$^{-/-}$ HEK293T cells with cycloheximide (CHX) treatment for the indicated times, with quantitation shown below.

E, F Co-immunoprecipitation analysis of Crebh with Hrd1 (E) and Sel1L (F) when co-expressed in HEK293T cells.

Data information: All cell culture experiments were done in 2–3 independent repeats with cells passaged less than three times. Hsp90, loading control for Western blot analysis.

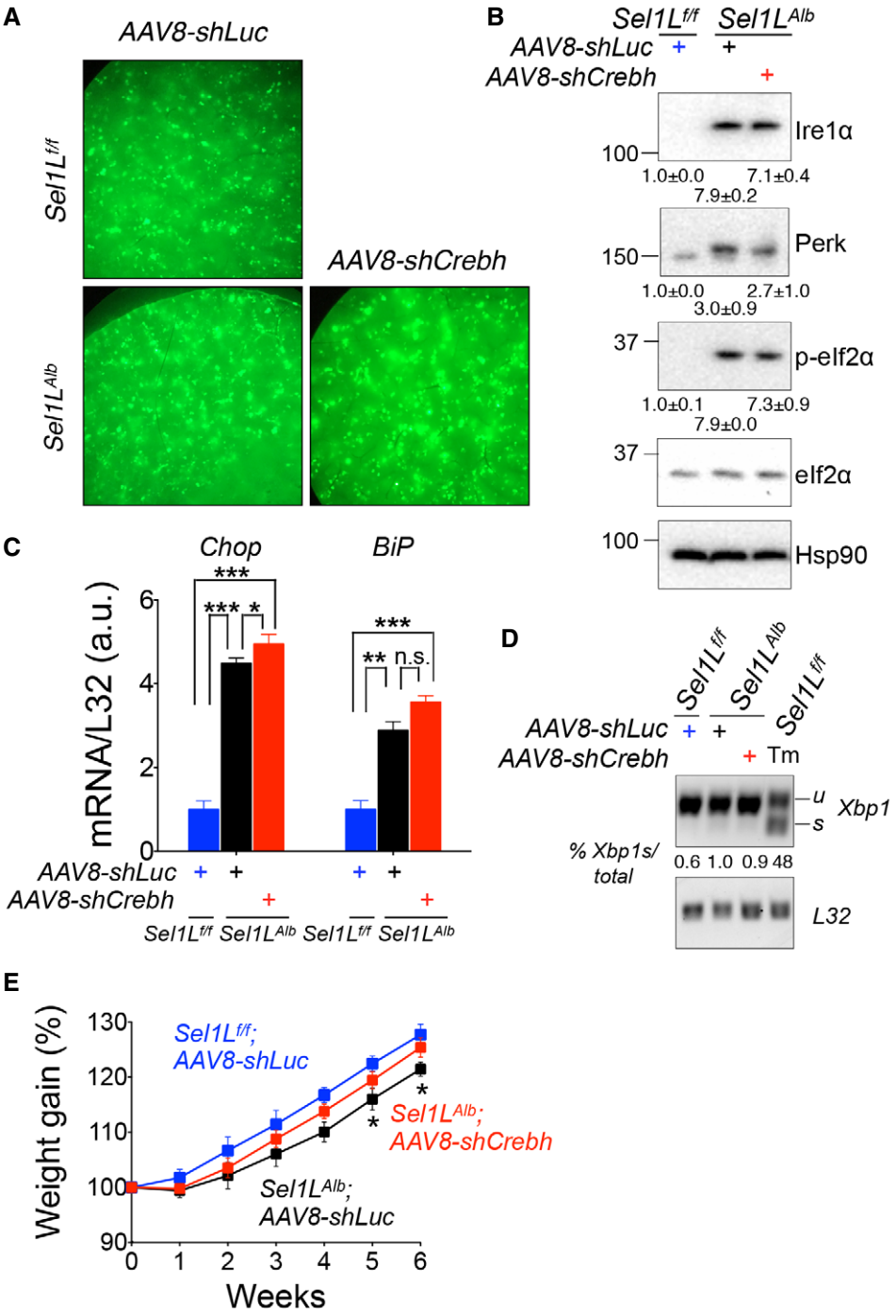

Figure EV5. Crebh deletion does not affect ER stress level in *Sel1L*^{Alb} liver.

A Representative images of livers from *Sel1L*$^{f/f}$ and *Sel1L*^{Alb} mice (n = 6 per group) post-AAV-shRNA-GFP injection showing green (GFP positive) livers.

B–D Western blot analysis (B), qPCR analysis (C), and RT–PCR analysis of *Xbp1* mRNA splicing (D) of hepatic UPR markers in *Sel1L*$^{f/f}$ and *Sel1L*^{Alb} mice 5 weeks post-one i.v. AAV8-*shCrebh* or control AAV8-*shLuc* injection (n = 3 per group, 2 independent repeats). Quantitation of protein levels (B) and the percent of Xbp1s in total Xbp1 mRNA (D) is shown below. WT mice injected i.p. with tunicamycin (Tm, 1.5 μg/g body weight) for 72 h were included as a control.

E Weekly weight gain post-i.v. injection (n = 10 per group) with AAV8-*shFgf21* or control AAV8-*shLuc*.

Data information: Hsp90, loading control for Western blot analysis. Ribosomal *L32*, loading control for qPCR analysis. Values are mean ± SEM; *P < 0.05; **P < 0.01; ***P < 0.001, n.s., non-significant by two-way ANOVA analysis.