Appendix

Hepatic Sel1L-Hrd1 ER-Associated Degradation (ERAD) manages FGF21 levels and systemic metabolism via CREBH

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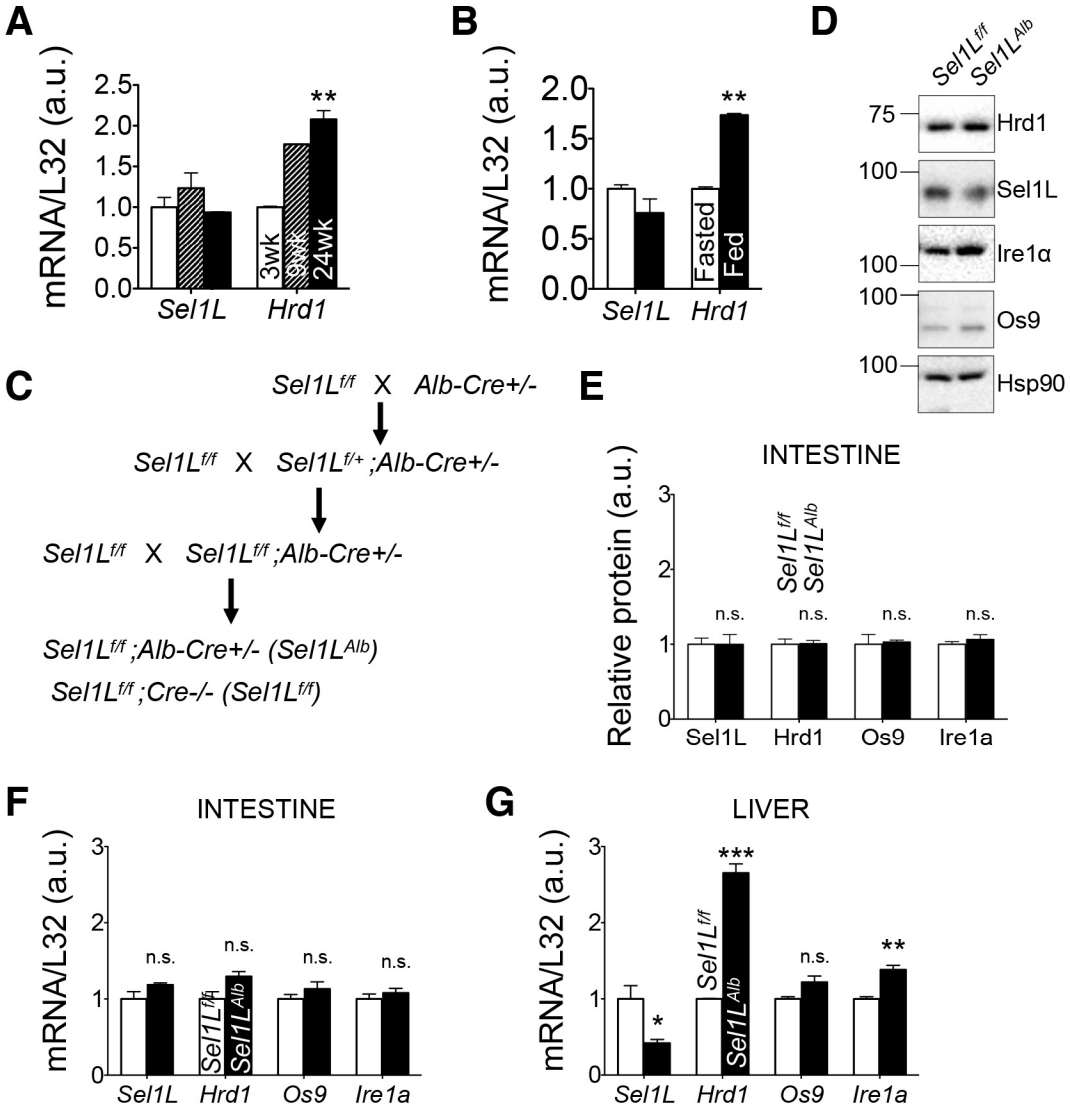
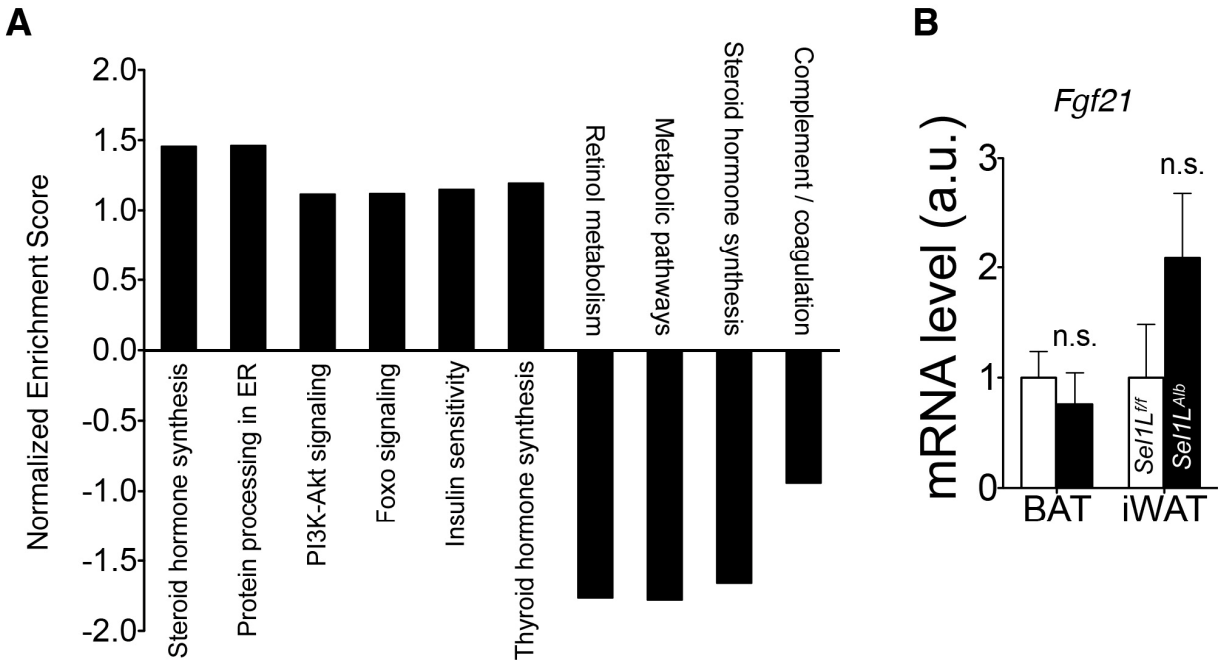
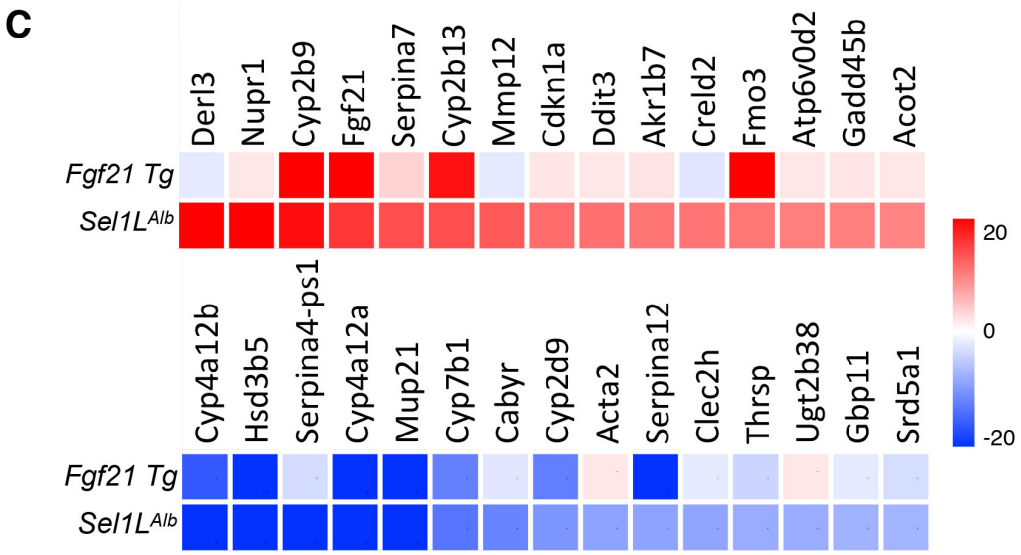
Appendix Figure S1. Generation of liver-specific Sel1L-Hrd1 ERAD deficient mouse model. (A) mRNA levels of ERAD genes (*Sel1L, Hrd1*) in the livers of WT mice at 3, 9 and 24 weeks of age (n=3-4 per group, 2 independent repeats). (B) mRNA levels of Sel1L-Hrd1 ERAD genes in the livers of 10-week-old WT mice under fasted (overnight) or fed conditions (n=3-4 per group, 2 independent repeats). (C) Schematic breeding plan for generation of liver-specific Sel1L-knockout mouse model. (D-F) Western blot analysis (D), quantitation (E) and qPCR analysis (F) of ERAD genes (*Hrd1, Sel1L, Ire1α, Os9*) in small intestines of *Sel1L*$^{f/f}$ and *Sel1L*^{Alb} mice (n=3 per group, 2 independent repeats). (G) qPCR analysis of ERAD genes (*Hrd1, Sel1L, Ire1α, Os9*) in the livers of 9-week-old *Sel1L*$^{f/f}$ and *Sel1L*^{Alb} mice (n=6 per group, 2 independent repeats). Values, mean ± SEM; *, p<0.05; **, p<0.01; ***, p<0.001, n.s., not significant by Student's t test.

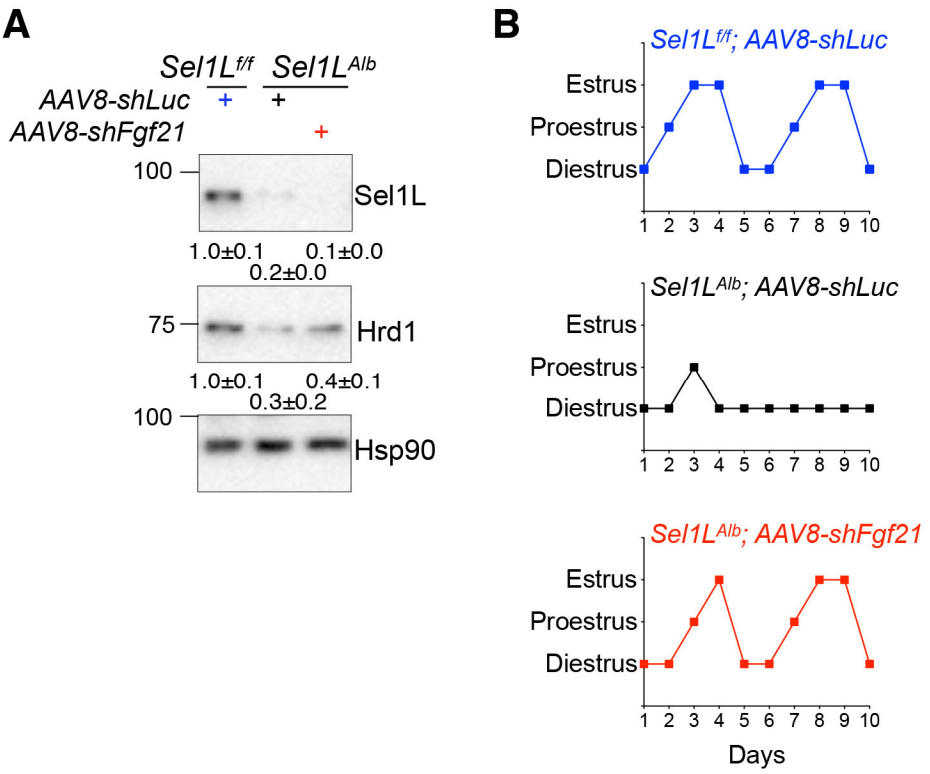


Appendix Figure S2. Transcriptomics analysis of Sel1L-Hrd1 ERAD deficient liver. (A) KEGG pathway Gene Set Enrichment Analysis (GSEA) of differentially expressed hepatic genes of p<0.05 and fold change >1.9. (B) qPCR analysis of *Fgf21* in brown and inguinal white adipose tissues (BAT, iWAT) of 9-week-old mice (n=3 per group, 3 independent repeat). (C) Heatmaps of top 15 significantly upregulated and downregulated genes in *Sel1L*^{Alb} livers and their expression levels in *Fgf21 Tg* livers (n=3 per group). Ribosomal *L32*, loading control for qPCR and RT-PCR analysis. Values, mean ± SEM; *, p<0.05; **, p<0.01; ***, p<0.001, n.s., not significant by Student's t test.





Appendix Figure S3. Fgf21 knockdown partially reverses the phenotypes of *Sel1L*^{Alb} mice. (A-B) Data from the rescue experiments where 5-week-old *Sel1L*$^{f/f}$ and *Sel1L*^{Alb} mice were injected i.v. with AAV8-*shFgf21* or control AAV8-*shLuc*: (A) Western blot analysis of hepatic Sel1L and Hrd1, 3 weeks after injection (n=3 mice each). (B) Representative estrus cycles 3 weeks after injection (n=5-6 per group). Hsp90, loading control for Western blots. Values, mean ± SEM; *, p<0.05; **, p<0.01; ***, p<0.001, n.s., non-significant by 2-way ANOVA analysis.



Appendix Figure S4. Hepatic ERAD deficient mice show resistance to diet induced weight gain. (A-B) Confocal immuno-stained images (A) and quantitation (B, 100 droplets per image) of perilipin in inguinal white adipose tissues (iWAT) of 8-week-old mice (n=3 per group, 2 independent repeats). (C) Representative images of adipose tissues in male mice (n=4 per group) depicting difference in color and amount of WAT after 9 weeks of HFD feeding. (D) Insulin tolerance test (ITT) after 6 weeks of HFD (n=3-4 per group, 2 independent repeats). Values, mean ± SEM; *, p<0.05; **, p<0.01; ***, p<0.001, n.s., non-significant by Student's t test.