SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Diets and the ingredients

Calories provided by (%)	Regular chow	34% Fructose Diet	70% Fructose Diet
Protein	26.8	20	20
Fat	16.7	10	10
Carbohydrate	56.4 (0.24% fructose and 3.24% sucrose)	34 (34% free fructose)	70 (70% free fructose)

Generation of Plasmids and Adenovirus

pENTRY/U6-shChop and -shSrebp2 constructs were made by ligating dsDNA oligos with pEntry/U6 vector (Invitrogen). The targeting sequence for the mouse *Chop* and *Srebp2* coding sequence are 5'-CACCGCTCTCCAGATTCCAGTCAGACGAATCTGACTGGAATCTGGAGAAGC-3' and CTGGTAAATGGTGTGATTGT, respectively. AdBlockiT-sh*Chop* and AdBlockiT-sh*Srebp2* plasmid was generated through Gateway LR recombination between pEntry/U6-sh*Chop* or -shSrebp2 and pAdBlock-iT vector (Invitrogen). pDONR/Zeo-Flag-Chrebp vector was made through Gateway BP recombination with pDONR/Zeo using pCMV-Flag-Chrebp (a gift from Dr. Towle at the University of Minnesota) as PCR template. pAdEasy-Flag-Chrebp was generated through LR recombination with pAdCMV-GW (Invitrogen). All adenoviruses were produced in 293AD packaging cells (Agilent) after LipofectAMINE-mediated transfection and concentrated after ultracentrifuge in cesium chloride gradient solutions. pcDNA3.1-2xFlag-Srebp2 (mature form) from Addgene (Cat. 26807) was used as template to generate Myc-tagged Srebp2 PCR product for a Gibson reaction (NEB) with pDONR/Zeo PCR fragment. pQCXIP-Myc-Srebp2 was created through Gateway LR recombination between pQCXIP and pDONR/Zeo Myc-Srebp2. All constructs were verified by either direct sequencing or restrictive enzyme digestion.

Primary Mouse Hepatocyte Isolation and Culture

Primary mouse hepatocytes (PMHs) were isolated from C57BL/6 male mice (9–10 weeks) using the protocol described before (1). The liver was perfused with EBSS (Invitrogen) with 0.5 mM EGTA for 5 min, followed by perfusion with 100 U/ml type I collagenase (*Worthington*) via the inferior vena cava for 5 min. After dissection, hepatocytes were released by scattering, passed through a 100-μm cell strainer, and then spun at 50 × g for 1 min. The pellet was re-suspended in DMEM and then spun at 50 × *g* for 10 min in a Percoll gradient to remove dead hepatocytes. Viable cells were washed with DMEM at 50 × g for 10 min and checked by trypan blue staining. PMHs in DMEM with 5% FBS were seeded at a density of 2×10^5 cells/ well of 12-well-plate. Adenovirus was transduced 6 hours after seeding. The cells were harvested 24h after seeding for protein or RNA preparation.

Immunoblotting

For whole cell lysate preparation, the primary hepatocytes were washed once in 1 × PBS buffer and lysed in RIPA buffer supplemented with 1 × protease inhibitor (Roche Applied Science). The liver tissues were weighed and homogenized in RIPA buffer (8 ul/mg tissue weight). After whole cell lysates were precleared

at maximal speed at 4 °C in a microfuge, the protein concentration of each supernatant was measured using Bio-Rad reagent. Equal amounts of protein samples were separated in 9% SDS-poly-acrylamide gels and transferred to PVDF membrane (*Millipore*). The membranes were incubated in primary antibodies at 4°C overnight. HRP-conjugated secondary antibodies against mouse, rabbit, or goat IgG and Western Lightning ECL substrate were used for detecting chemi-luminescence on a HD2 AlphaImager (*Cell Biosciences*).

Primary antibodies against ChREBP (SC-33764), AKTα1/α2 (SC-1619), FASN (SC-20140), SCD1 (SC-14719), SREBP-1c (SC-366), GCK (SC-7908), ChREBP, ACC1 (SC-30212), FOXO1 (SC-11350), ATF6 (SC-22799), ATF4 (SC-200), phosphor-PERK (SC-32577), CHOP (SC-575), PERK (SC-13073), GRP78 (SC-13968), p-CaMKII (sc-32289), CamKII (sc-5306) and HMGCR (SC-27578) are all from *Santa Cruz*. Primary antibodies against phosphorylated AKT at Ser-473 (4060s), p53 and MYC (2276s) are from *Cell Signaling*. β-tubulin (T5201), FLAG (F1804) and PUMA (P4618), and HRP-conjugated secondary antibodies anti-rabbit (A6154), anti-mouse (A4416) and anti-goat (A5420) are from *Sigma*. SREBP2 (10007663) and Caspase3 (160745) is from *Cayman*. β-Actin is from the Developmental Studies Hybridoma Bank in Department of Biology, University of Iowa. Keratin18 antibody is a generous gift from Dr. Bishr Omary from the Department of Molecular & Integrative Physiology, University of Michigan Medical School.

Transfection, immunoblotting and Immunoprecipitation

U2OS cells (ATCC) were transfected with Flag-*Srebp2-n* and co-transduced with Ad-GFP or Ad-Flag-*Chrebp*. 24h later the cells were treated with 10 μM MG132 for 3 or 6 hrs. Flag-ChREBP and Flag-SREBP2-N expression levels were assessed by western blotting with anti-FLAG. For detecting the interaction of CBP-SBP-ChREBP and nuclear SREBP2 in over-expressed condition, 293A cells were transfected with Flag-Srebp2-n and co-transfected with pNTAP-CBP-SBP-Chrebp or pNTAP empty vector. 24h later, cells were then treated with 10nM MG132 for 6 hr before harvested. The pre-cleared protein lysates (1 mg of protein) were incubated with 20 μL of Streptavidin magnetic beads (GE Healthcare) for 16 hr. The beads were then washed with lysis buffer for 5 times and eluted in 20 μL of 2x SDS loading buffer. Western blotting was performed to detect the presence of targeted proteins with specific antibodies.

Cell-based Nuclear ChREBP Acetylation and SREBP2 Ubiquitination Assay

For acetylation assay, primary hepatocytes isolated from wildtype mice fed regular chow or 70% HFrD for 1 wk were transduced with Ad-Flag-*Chrebp*. A denaturing immunoprecipitation protocol was followed to pull down FLAG-tagged nuclear ChREBP with anti-FLAG M2 antibody. The lysis buffer was supplemented with histone deacetylase inhibitors (500 nM TSA, 10 μM EK547, and 100 mM nicotinamide) to preserve the acetylated ChREBP. The acetylated ChREBP was detected by anti Acetyl-Lysine (Abcam). To detect ubiquitination of nuclear SREBP2, 293A cells were transduced with pQCXIP-Myc-h*Srebp2* along with either Ad-GFP or Ad-*Chrebp*. 24h later, cells were then treated with 10nM MG132 for 6 hr before

harvested for denaturing immunoprecipitation with anti-MYC. The poly-ubiquitinated SREBP-2 was detected by anti-ubiquitin (Sigma).

cDNA Synthesis and qPCR

Total cellular RNA extraction was performed with TRIzol reagent (Invitrogen) and chloroform. cDNA was synthesized with the Verso cDNA kit (*ThermoFisher Scientific*, Surrey, UK) and subjected to qPCR using Radiant™ Green 2X qPCR Mix (*Alkali Scientific*) on an ABI 7900 HT thermal cycler (Applied Biosys-tems, Foster City, CA). The value of each cDNA was calculated using the ΔCt method and normalized to the value of the house-keeping gene control, 18S ribosomal RNA. The data were plotted as fold change. The primer sequences are listed below.

	Forward	Reverse
18s RNA	5'-TTGACGGAAGGGCACCACCAG-3'	5'-GCACCACCACCCACGGAATCG-3'
Acc1	5'-GAAGCCACAGTGAAATCTCG-3'	5'-GATGGTTTGGCCTTTCACAT-3'
Acox1	5'-TGCTGCAGACGGCCAGGTTC-3'	5'-GGCCAGACTGCCACCTGCTG-3'
Bcl2-l1	5'-GCTGCATTGTTCCCGTAGAG-3'	5'-GTTGGATGGCCACCTATCTG-3'
Bim	5'-CGGTCCTCCAGTGGGTATTT-3'	5'-TATGGAAGCCATTGCACTGAGA-3'
Camk2d	5'-CGGAGGAGGGCTTCCATTAC-3'	5'-CAGTGTAGCACAGCCTCCAG-3'
Camk2g	5'-GGTCTGTCAACGGTCTACGG-3'	5'-CTTGACACCGCCATCTGACT-3'
Cd36	5'-CCAAGCTATTGCGACATGATT-3'	5'-CCGAACACAGCGTAGATAGACC-3'
Chrebp	5'-CTGGGGACCTAAACAGGAGC-3'	5'-GAAGCCACCCTATAGCTCCC-3'
Chrebpβ	5'-TCTGCAGATCGCGTGGAG-3'	5'-CTTGTCCCGGCATAGCAAC-3'
Cpt1a	5'-TCTGCATGTTTGACCCAAAA-3'	5'-TTGCTGGAGATGTGGAAGAA-3'
Fasn	5'-TTGGCCCAGAACTCCTGTAG-3'	5'-CTCGCTTGTCGTCTGCCT-3'
Fbw7	5'-TGTCCACGTTAGAATCTGTGACAT-3'	5'-ACCATGGTCCAACTTTCTTTTCA-3'
Fgf19	5'-AGGAGGACCAAAACGAACGA-3'	5'-CAGTCTTCCTCCGAGTAGCG-3'
Gck	5'-CCCTGAGTGGCTTACAGTTC-3'	5'-ACGGATGTGGAGTGTTGAAGC-3'
Hmgcr	5'-CACAATAACTTCCCAGGGGT-3'	5'-GGCCTCCATTTAGATCCG-3'
Hmgcs1	5'-TTCAAAGGAAGTGACCCAGG-3'	5'-GGTCTGATCCCCTTTGGTG-3'
Insig1	5'-TCACAGTGACTGAGCTTCAGCA-3'	5'-TCATCTTCATCACACCCAGGAC-3'
Insig2a	5'-CCCTCAATGAATGTACTGAAGGATT-3'	5'-TGTGAAGTGAAGCAGACCAATGT-3'
Insig2b	5'-CCGGGCAGAGCTCAGGAT-3'	5'-GAAGCAGACCAATGTTTCAATGG-3'
Itch1a	5'-CCATCTTAGCGGAGGGACTG-3'	5'-AGACAGCAAACCTGAAGTTCTCA-3'
Lats2	5'-AGCTGAAGGTGATCAACTGGGA-3'	5'-CGGGAAAAGTCGATGGTGTTG-3'
L-PK	5'-CTGGAACACCTCTGCCTTCTG-3'	5'-CACAATTTCCACCTCCGACTC-3'
Mcl-1	5'-TTCTTTCGGTGCCTTTGTGGC-3'	5'-AACCCATCCCAGCCTCTTTGTT-3'
Mttp1	5'-CTCCACAGTGCAGTTCTCACA-3'	5'-AGAGACATATCCCCTGCCTGT-3'
Noxa	5'-TGGAGTGCACCGGACATAAC-3'	5'-TCGTCCTTCAAGTCTGCTGG-3'
Pparα	5'-CCTTCTACGCTCCCGACCCA-3'	5'-CCATGTCCATAAATCGGCACCA-3'

Puma	5'-TACGAGCGGCGGAGACAAG-3'	5'-GTGTAGGCACCTAGTTGGGC-3'
Scd1	5'-GCCGAGCCTTGTAAGTTCTG-3'	5'-CCTCCTGCAAGCTCTACACC-3'
Shp	5'-AGTTCAGTGGCTGCAGCGCT-3'	5'-GAGGCCTGGCACATCTGGGTT
Sirt6	5'-GGGTTGTCGCCTTACGCGGAT-3'	5'-CATGGGGGCCTCTGAAGTCGG
Sqle	5'-GATGGGCATTGAGACCTTCT-3'	5'-TTTAAAAGAGCCCGACAGGA-3'
Srebp-1c	5'-AACGTCACTTCCAGCTAGAC-3'	5'-CCACTAAGGTGCCTACAGAGC-3'
Srebp-2	5'-CCCTATTCCATTGACTCTGAGC-3'	5'-GAGTCCGGTTCATCCTTGAC-3'

***De Novo Lipogenesis* Assay**

The assay protocol was adopted from the protocol described previously (2). In brief, primary hepatocytes were seeded on 12-well plates (2×10^5 cells/well) and cultured overnight. The cells were switched to serum-free medium 199 (Invitrogen) the next day and 6 hr later they were incubated with medium 199 with 100 nM insulin and 25 mM glucose overnight to activate *de novo* lipogenesis. On the third day, cells were incubated in phenol-free DMEM medium containing both cold acetate and 1 µCi H^3-labeled acetate (*Moravek Biochemicals*) for 4 hr. After 1 x PBS wash, cells were lysed in 200 µL of 0.1 N HCl. Protein concentration was measured by BioRad reagent with 2 µL of lysate. Lipids were extracted by adding 800 µL of chloroform/methanol (2:1, v/v). The organic fraction was transferred to a fresh tube and let air dry at room temperature overnight. The pellet was dissolved in hexane and 5% H_2SO_4 in methanol and heated at 100 °C for 30 min. The final radiolabeled lipids were extracted by 500 µL of petroleum and the H^3 radioactivity was measured on a Beckman scintillation counter. *De novo* lipogenesis rate was normalized by protein amount for each sample.

Liver Triglyceride and Cholesterol Measurements

Hepatic total lipids were extracted according to Bligh and Dyer (3). The liver tissues were weighed and homogenized in 1% acetic acid (7uL acetic acid/mg liver tissue). After quick spinning, 200 µL of supernatant was transferred to a 1.5 mL tube containing 800 µL of chloroform/methanol (2:1, v/v). After centrifuge for 10 min at 10000xg at room temperature, 500 µL of organic phase was transferred to a new tube from the bottom layer, and the tubes were left in a fume hood overnight. For triglyceride level, total lipids were dissolved in 200 µL 3M KOH and incubated at 70°C for 1 hour. Then 600 µL of 1M $MgCl_2$ was added. After spinning for 10000 xg for 10 min at room temperature, 8 µL were used for measurement with the Free Glycerol Reagent (*Sigma*, F6428). For total cholesterol level measurement, total lipids were resuspended in 200 µL of isopropanol. 20 µL was used for total cholesterol measurement with the total cholesterol testing kit from *Pointe Scientific* (Cat. A7510).

For Oil Red O (ORO) staining, frozen section slides were fixed in pre-chilled 10% formalin for 10 minutes, air dried for 10min and incubated in absolute propylene glycol for 5 min to avoid carrying water into ORO. Then the slides were stained in pre-warmed 0.5% ORO solution for 10 min in 60°C water bath, differentiated in 85% propylene glycol solution for 2-5 minutes, counter stained with hematoxylin and eosin for 5 seconds, and visualized under microscope.

Liver free cholesterol levels were determined with Filipin staining. Frozen section slides were rinsed three times with PBS, and then fixed with 10% formaldehyde for 1 hr at room temperature. Fixed sections were washed twice with PBS with 1.5 mg/ml glycine for 5 min to quench formaldehyde, and then were incubated with 0.1 mg/ml Filipin (Filipin Complex, Sigma, F-9765) solution at 4 °C overnight. Lastly, all slides were washed with PBS twice, and checked under microscope with a UV filter set.

ELISA

Serum FGF21 level were determined by the ELISA kit (MF2100, R&D Systems) according to the manufacturer's instructions.

References:

1. Tong X, Zhang D, Buelow K, Guha A, Arthurs B, Brady HJ, and Yin L. Recruitment of histone methyltransferase G9a mediates transcriptional repression of Fgf21 gene by E4BP4 protein. *J Biol Chem.* 2013;288(8):5417-25.
2. Zhang D, Tong X, Arthurs B, Guha A, Rui L, Kamath A, Inoki K, and Yin L. Liver clock protein BMAL1 promotes de novo lipogenesis through insulin-mTORC2-AKT signaling. *Journal of Biological Chemistry.* 2014;289(37):25925-35.
3. Bligh EG, and Dyer WJ. A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology.* 1959;37(8):911-7.

Supplemental Figure Legends:

Figure S1 (Related to Figure 1) Metabolic responses of mice to regular chow (Chow) and short-term high fructose diet (HFrD) feeding.

8-wk male mice were fed chow or 70% HFrD for 3-wk before dissection (n = 7). Body weight (**A**), non-fasting blood glucose (**B**), and insulin tolerance (**C**) were measured before dissection. Serum insulin (**D**), serum triglyceride (**E**) and liver weight (**F**) were measured after dissection. **p* < 0.05. These data are presented as mean ± SEM.

Figure S2 (Related to Figure 1) HFrD increases hepatic de novo lipogenesis

8-wk mice were fed regular chow or 70% HFrD for 3 wks (n = 7, both male and female). (**A-B**) HFrD induces hepatic steatosis assessed by liver triglyceride assay (**A**), Oil Red O staining and H&E staining (**B**). (**C**) Quantification of gene expression levels in the major lipid metabolic pathways by RT-qPCR. (**D-E**) HFrD feeding induces hepatic lipogenic genes. The levels of lipogenic enzymes and lipogenic transcription factors in the liver were measured by RT-qPCR (**D**) and western blotting (**E**). **p* < 0.05, ***p* < 0.01, ****p* < 0.001 by two-tailed Student's *t* test. These data are presented as mean ± SEM. Scale bar = 100 μm.

Figure S3 (Related to Figure 1) HFrD feeding elevates *de novo* lipogenesis in hepatocytes.

(**A-B**) HFrD feeding increases lipogenenic gene expression in hepatocytes. Lipogenic enzyme levels were assessed by RT-qPCR (**A**) and western blotting (**B**) in primary hepatocytes isolated from WT mice fed regular chow or HFrD for 1 wk and 2 wks. (**C**) HFrD feeding induces ChREBP acetylation in hepatocytes. Primary hepatocytes isolated from WT mice fed regular chow or HFrD for 1 wk were transduced with Ad-Flag-Chrebp and subjected to immunoprecipitation against FLAG and western blotting against Acetyl-Lysine (Ac-K). (**D**) *De novo* lipogenesis rate was determined in primary hepatocytes isolated from mice fed regular chow or HFrD for 1 wk (**I**). **p* < 0.05 by two-tailed Student's *t* test for (**D**). **p* < 0.05, ***p* < 0.01 by one-way ANOVA with Dunnett's test for (**A** and **B**). These data are presented as mean ± SEM.

Figure S4 (Related to Figure 1) Metabolic responses of *Chrebp*$^{-/-}$ mice to HFrD feeding.

8-wk WT and *Chrebp*$^{-/-}$ mice fed 70% HFrD for 2-wk (n = 4 for WT, n = 6 for *Chrebp*$^{-/-}$ mice). (A) Body weight was measured before and after 2-wk HFrD feeding. (**B and C**) WT and *Chrebp*$^{-/-}$ mice were kept in individual cages. Food intake of regular chow or HFrD by both groups was measured for 3 successive days. Food intake values were normalized to body weight. Liver weight (**D**) and hepatic glycogen content **(E**) were measured after dissection. (**F**) mRNA levels of lipogenic genes were quantified with RT-qPCR. (**G**) Quantification for western blotting in **Figure 1C**. **p* < 0.05 by two-tailed Student's *t* test. These data are presented as mean ± SEM.

Figure S5 (Related to Figure 1) Serum FGF21 level in WT or *Chrebp*$^{-/-}$ mice following HFrD.

8-wk WT and *Chrebp*$^{-/-}$ mice fed 70% HFrD for 2-wk (n = 4 for WT, n = 6 for KO). Serum FGF21 was assessed by ELISA. The data are presented as mean ± SEM.

Figure S6. 34% fructose diet feeding induces liver injury in *Chrebp*$^{-/-}$ mice.

8-wk WT and *Chrebp*$^{-/-}$ mice were fed 34% fructose diet for 4 wks (n = 4, both males and females). (**A**) Body weight was measured before and after 4-wk 34% fructose diet feeding. After dissection, liver injury was assessed with ALT assay (**B**), H&E staining (**C**). Hepatocytes apoptosis was tested by TUNEL staining (**D**). Apoptotic markers were assessed by RT-qPCR and western blotting (**E** and **F**). **p* < 0.05, *** p < 0.001 by two-tailed Student's *t* test. These data are presented as mean ± SEM. Scale bar = 100 μm.

Figure S7 (related to Figure 3) High-fructose diet induces ER stress in the liver.

(**A-B**) Mice were fed chow or 70% HFrD for 3-wk before dissection (n = 6 for chow and n = 7 for HFrD). The protein levels of ER stress markers in the liver were assessed by western blotting (**A**) and the mRNA level of spliced *Xbp1* by RT-qPCR (**B**). (**C**) Mice were fed chow or HFrD for 1-wk or 2-wk and then used for primary hepatocyte isolation. The protein levels of ER stress markers were assessed by western blotting (**C**) and the mRNA level of spliced *Xbp1* was assessed by RT-qPCR (**D**). **p* < 0.05 by two-tailed Student's *t* test for (**B)**. **p* < 0.05 by *ANOVA* with Dunnett's test for (**D**). These data are presented as mean ± SEM.

Figure S8 Quantification of protein levels in Figure 3B.

**p* < 0.05; ***p* < 0.01; ****p* < 0.001 by two-tailed Student's *t* test. These data are presented as mean ± SEM.

Figure S9 (related to Figure 3) ChREBP does not directly regulate *Chop* transcription.

Chop mRNA level was measured in the livers of regular chow fed WT and *Chrebp*$^{-/-}$ mice (**A**), and in the primary hepatocytes transduced with Ad-GFP *vs.* Ad-*Chrebp* (**B**). Data are presented as mean ± SEM.

Figure S10 (related to Figure 3) HFrD feeding induces Calcium signaling in *Chrebp*$^{-/-}$ mice.

8-wk WT and *Chrebp*$^{-/-}$ mice fed 70% HFrD for 2-wk (n = 4 for WT, n = 6 for *Chrebp*$^{-/-}$).Calcium pathway induction was assessed by RT-qPCR for *Camk2d* and *Camk2g* (**A**) and by western blotting for total and Thr286 phosphorylated CaMKII (**B**). **p* < 0.05 by Student's *t* test. The data are presented as mean ± SEM.

Figure S11 (Related to Figure 3) Restoring GRP78 expression protects *Chrebp*$^{-/-}$ mice from HFrD feeding-induced liver injury.

8-wk *Chrebp*$^{-/-}$ mice were injected with either Ad-*Grp78* (n = 3) or Ad-GFP (n = 3), and then fed 70% HFrD for 2 wks. (**A**) Validation of GRP78 expression in Ad-*Grp78*-transduced primary mouse hepatocytes by western blotting. After dissection, H&E staining (**B**), TUNEL staining (**C**) and ALT assay (**D**) were conducted to assess liver injury. **p* < 0.05 by two-tailed Student's *t* test. These data are presented as mean ± SEM. Scale bar = 100 μm.

Figure S12. 34% fructose diet feeding elevates cholesterol biosynthesis in *Chrebp*$^{-/-}$ mice.

8-wk WT and *Chrebp*$^{-/-}$ mice fed 34% fructose diet for 4-wk (n = 4, both male and females). Cholesterol biosynthesis genes were assessed by RT-qPCR and western blotting (**A** and **B**). **p* < 0.05, *** p < 0.001 by two-tailed Student's *t* test. These data are presented as mean ± SEM.

Figure S13 (related to Figure 4) *Chrebp* deficiency does not affect cholesterol biosynthesis in regular chow feeding condition.
Expression levels of cholesterol biosynthesis genes in liver of *Chrebp*$^{-/-}$ mice of 8-wk old were assessed by RT-qPCR. ***p* < 0.01 by two-tailed Student's *t* test. These data are presented as mean ± SEM.

Figure S14. (Related to Figure 5) Atorvastatin administration in *Chrebp*$^{-/-}$ mice elevates cholesterol biosynthesis genes expression (**A**) and decreases serum cholesterol levels (**B**). **p* < 0.05, ** p < 0.01 by two-tailed Student's *t* test. These data are presented as mean ± SEM.

Figure S15. Quantification of protein levels in Figure 6A. **p* < 0.05 by two-tailed Student's *t* test. These data are presented as mean ± SEM.

Figure S16. (Related to Figure 6) Knocking down *Srebp2* in the liver of HFrD fed *Chrebp*$^{-/-}$ mice lowers cholesterol biosynthesis.
8-wk *Chrebp*$^{-/-}$ mice were injected with either Ad-shLacZ or Ad-sh*Srebp2* and fed 70% HFrD for 2-wk (n = 3). (**A**) mRNA levels of cholesterol biosynthesis genes were assessed with RT-qPCR. (**B**) Serum cholesterol was measured. (**C**) Quantification of hepatic free cholesterol assessed with filipin staining in **Fig6 D**. **p* < 0.05 by two-tailed Student's *t* test. These data are presented as mean ± SEM.

Figure S17. (Related to Figure 6) Expression profile of transcription factors involved in cholesterol biosynthesis in WT and *Chrebp*$^{-/-}$ mice liver.
8-wk WT and *Chrebp*$^{-/-}$ mice fed HFrD for 2-wk (n = 4 for WT, n = 6 for *Chrebp*$^{-/-}$). mRNA levels of cholesterol biosynthesis transcription factors were assessed with RT-qPCR. **p* < 0.05 by two-tailed Student's *t* test. These data are presented as mean ± SEM.

Figure S18. Longer exposure of anti-Myc IP and anti-Ubiquitin IB membrane in Fig6 I.

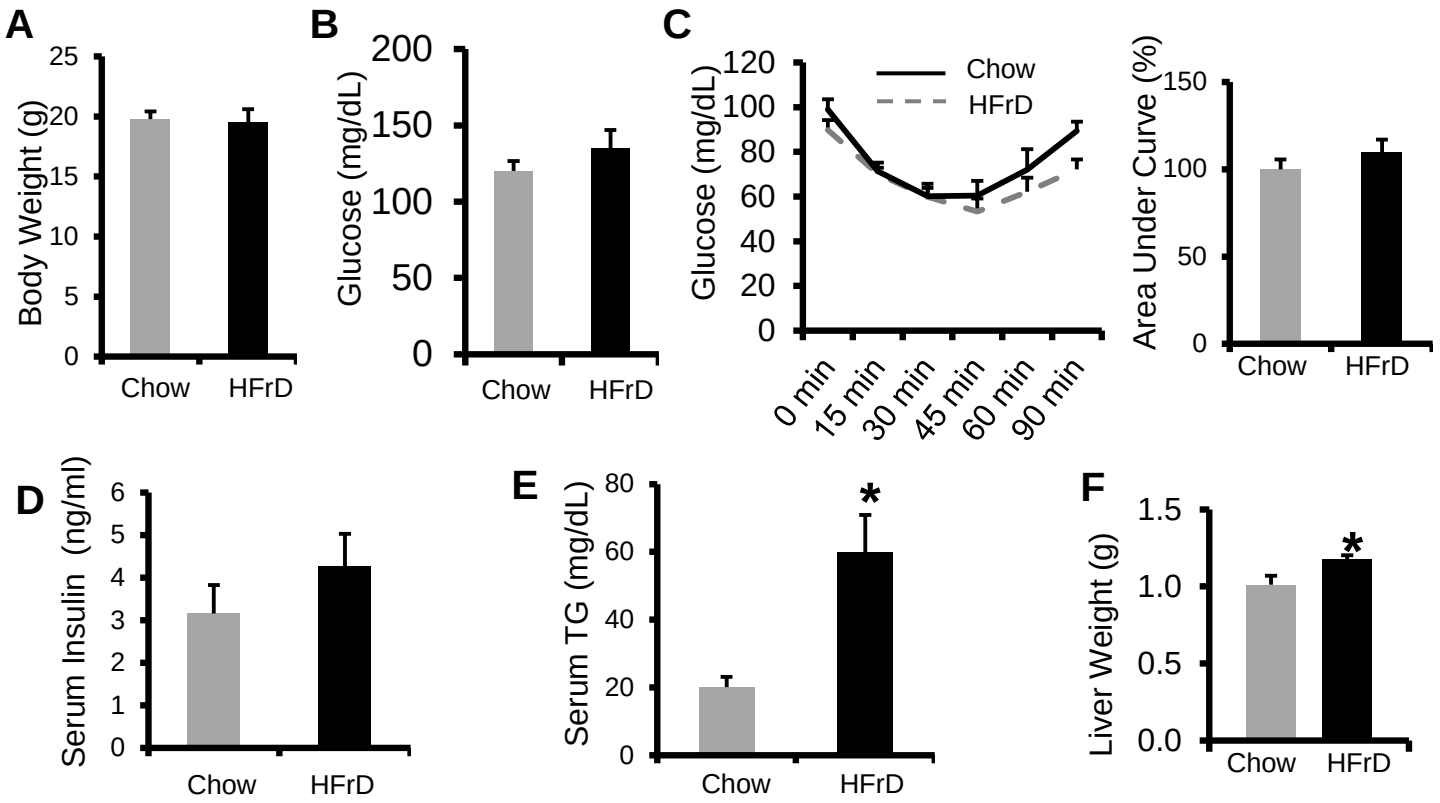
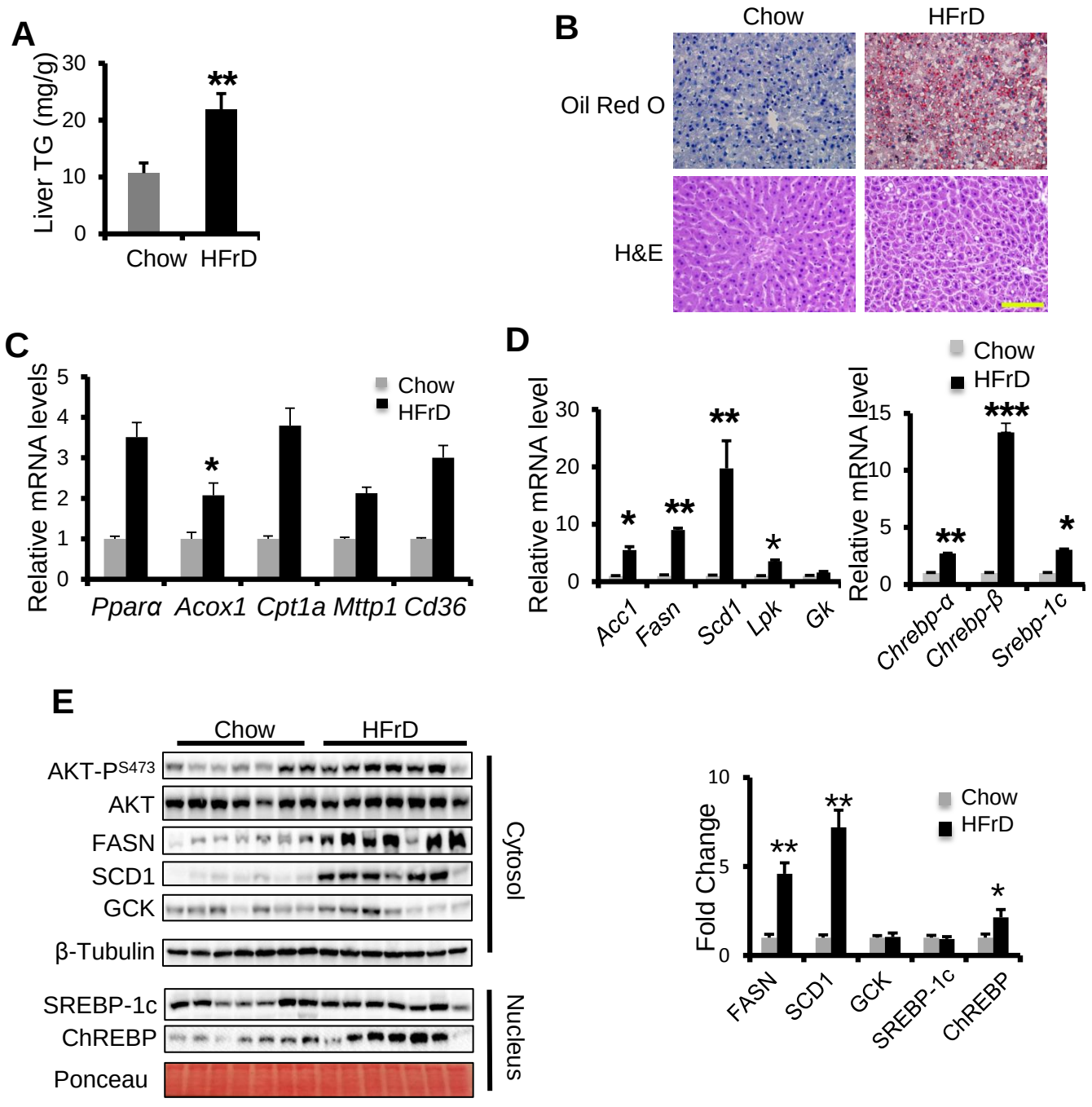


Figure S1 (Related to Figure 1) Metabolic responses of mice to regular chow (Chow) and short-term high fructose diet (HFrD) feeding.

8-wk male mice were fed chow or 70% HFrD for 3-wk before dissection (n = 7). Body weight (**A**), non-fasting blood glucose (**B**), and insulin tolerance (**C**) were measured before dissection. Serum insulin (**D**), serum triglyceride (**E**) and liver weight (**F**) were measured after dissection. *$p < 0.05$* by two-tailed Student's *t* test. These data are presented as mean ± SEM.



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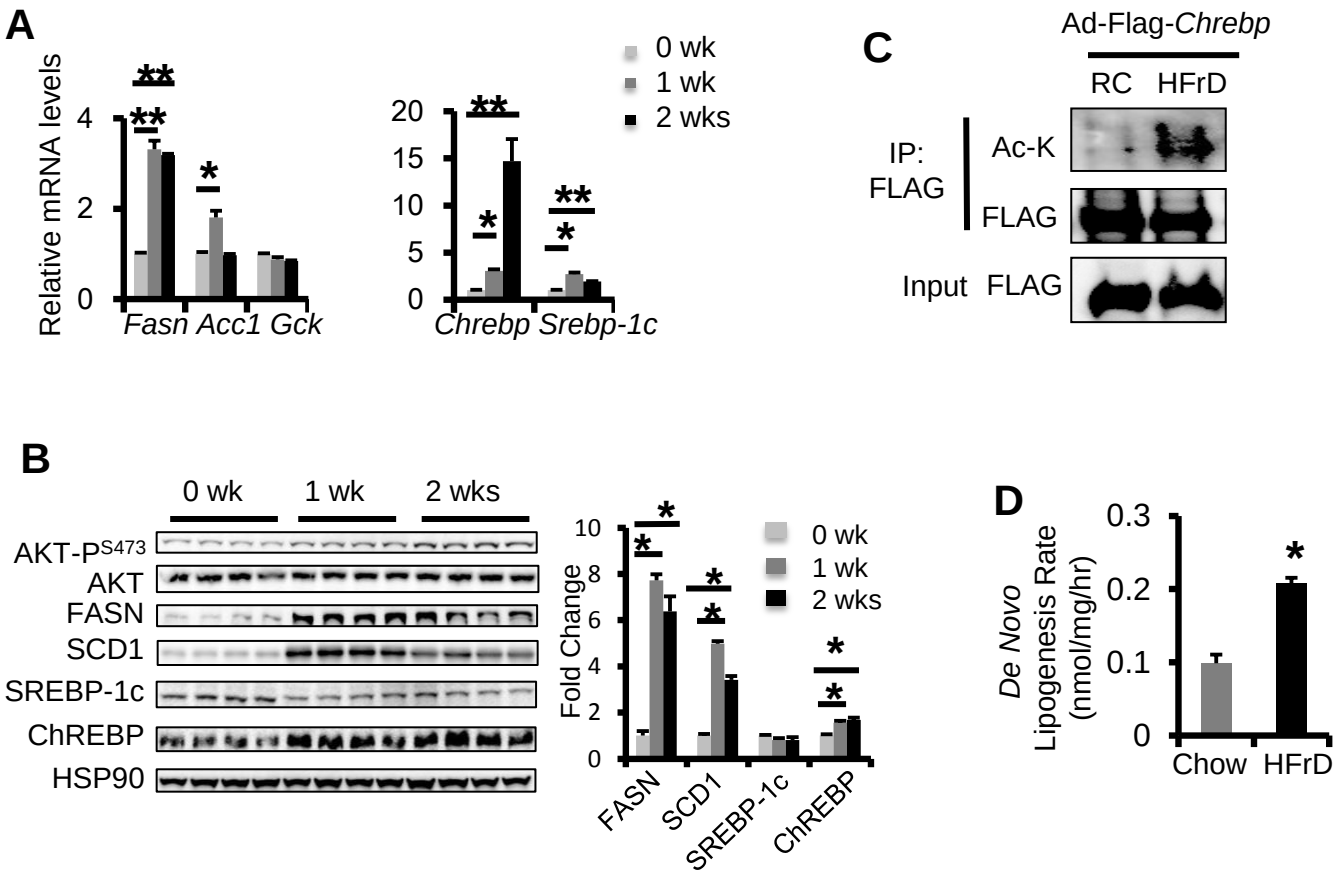

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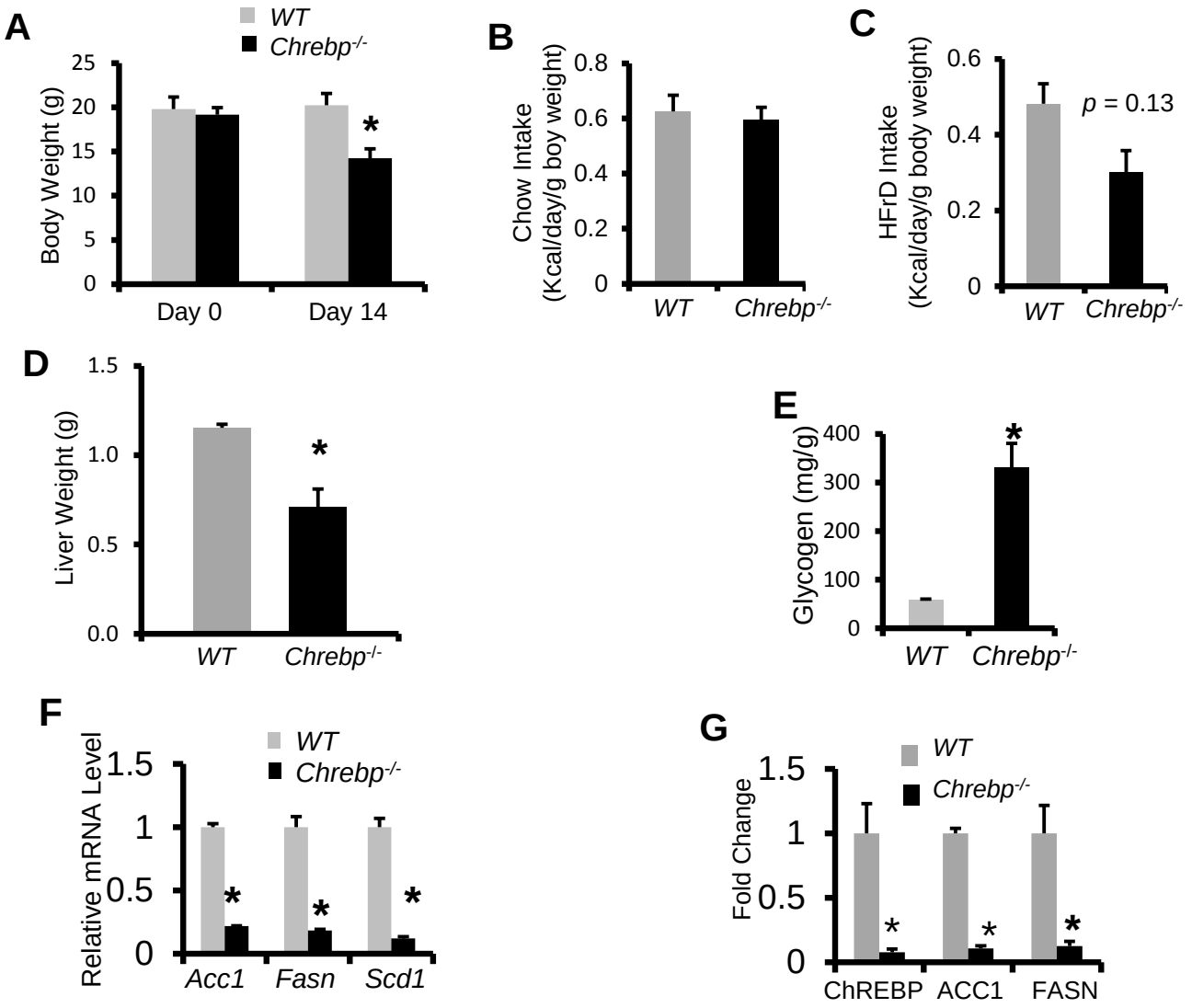
(**A-B**) HFrD feeding increases lipogenenic gene expression in hepatocytes. Lipogenic enzyme levels were assessed by RT-qPCR (**A**) and western blotting (**B**) in primary hepatocytes isolated from WT mice fed regular chow or HFrD for 1 wk and 2 wks. (**C**) HFrD feeding induces ChREBP acetylation in hepatocytes. Primary hepatocytes isolated from WT mice fed regular chow or HFrD for 1 wk were transduced with Ad-Flag-Chrebp and subjected to immunoprecipitation against FLAG and western blotting against Acetyl-Lysine (Ac-K). (**D**) *De novo* lipogenesis rate was determined in primary hepatocytes isolated from mice fed regular chow or HFrD for 1 wk (**I**). **p* < 0.05 by two-tailed Student's *t* test for (**D**). **p* < 0.05, ***p* < 0.01 by one-way ANOVA with Dunnett's test for (**A** and **B**). These data are presented as mean ± SEM.



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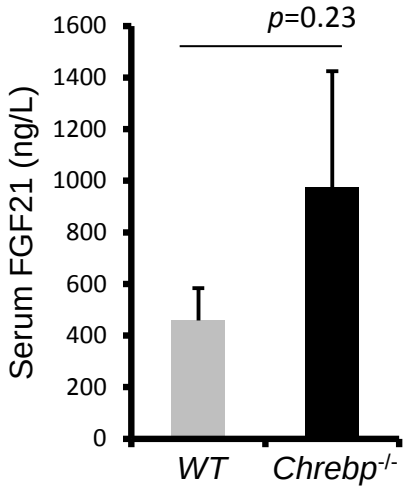


Figure S5 (Related to Figure 1) Serum FGF21 level in WT or *Chrebp*$^{-/-}$ mice following HFrD.

8-wk WT and *Chrebp*$^{-/-}$ mice fed 70% HFrD for 2-wk (n = 4 for WT, n = 6 for KO). Serum FGF21 was assessed by ELISA. The data are presented as mean ± SEM.

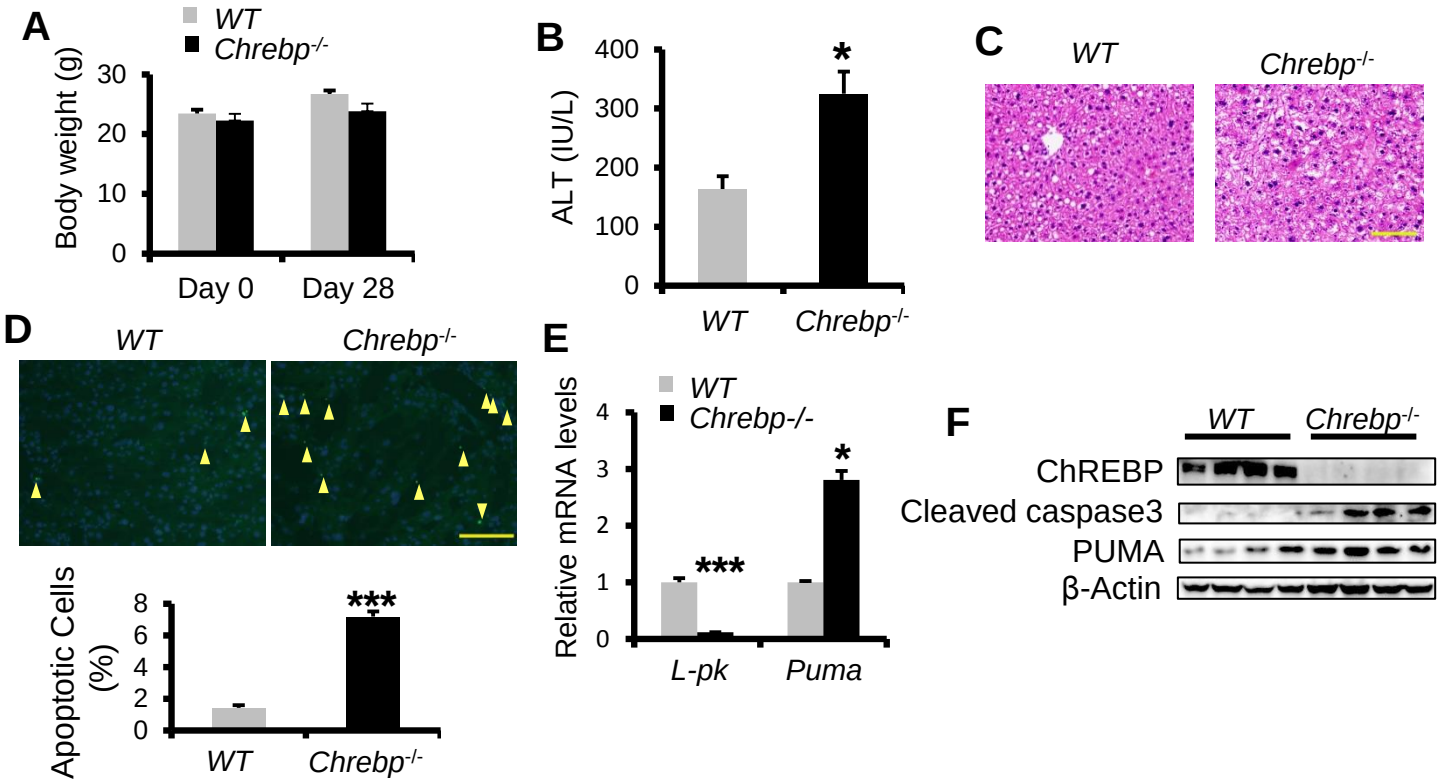


Figure S6. 34% fructose diet feeding induces liver injury in *Chrebp*$^{-/-}$ mice.

8-wk WT and *Chrebp*$^{-/-}$ mice were fed 34% fructose diet for 4 wks (n = 4, both males and females). (**A**) Body weight was measured before and after 4-wk 34% fructose diet feeding. After dissection, liver injury was assessed with ALT assay (**B**), H&E staining (**C**). Hepatocytes apoptosis was tested by TUNEL staining (**D**). Apoptotic markers were assessed by RT-qPCR and western blotting (**E** and **F**). **p* < 0.05, *** p < 0.001 by two-tailed Student's *t* test. These data are presented as mean ± SEM. Scale bar = 100 μm.

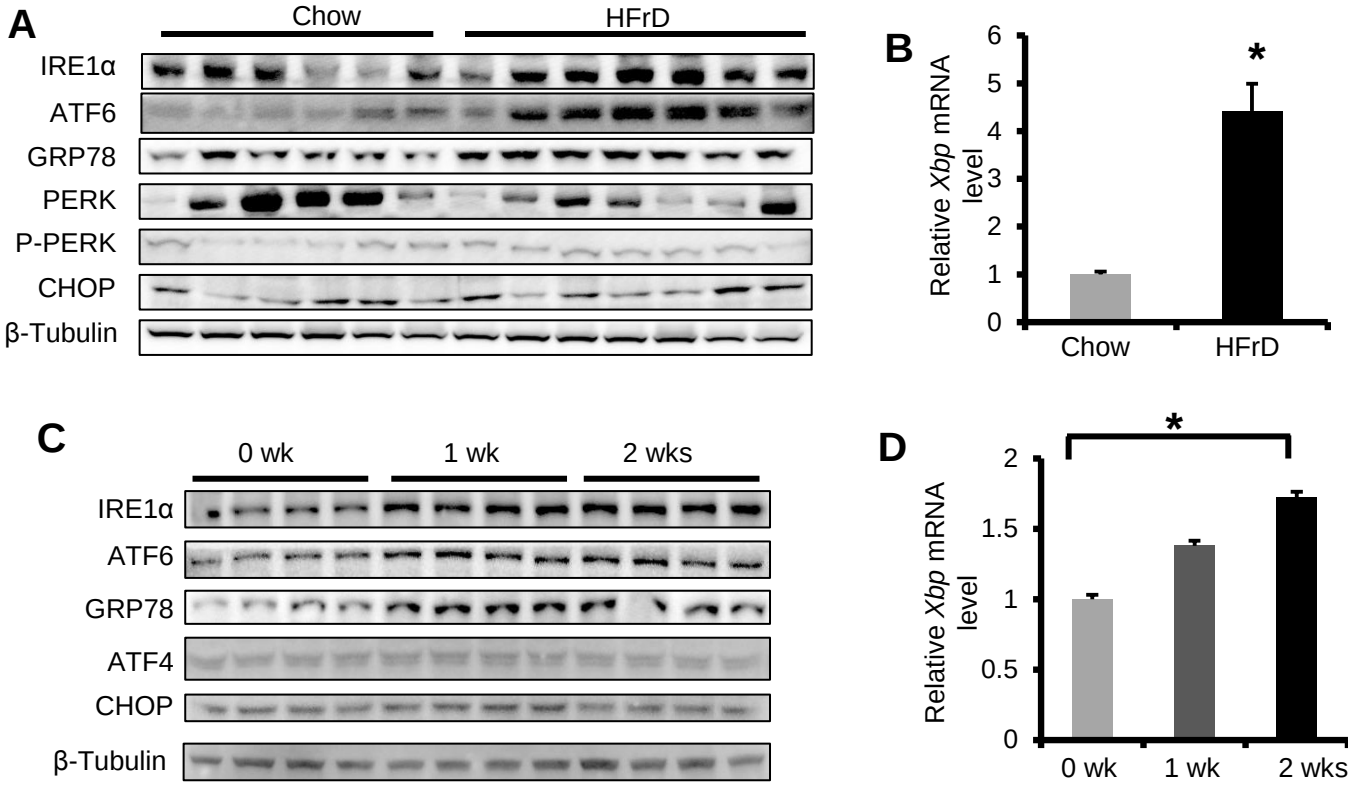


Figure S7 (related to Figure 3) HFrD induces ER stress in the liver.

(**A-B**) Mice were fed chow or 70% HFrD for 3-wk before dissection (n = 6 for chow and n = 7 for HFrD). The protein levels of ER stress markers in the liver were assessed by western blotting (**A**) and the mRNA level of spliced *Xbp1* by RT-qPCR (**B**). (**C**) Mice were fed chow or HFrD for 1-wk or 2-wk and then used for primary hepatocyte isolation. The protein levels of ER stress markers were assessed by western blotting (**C**) and the mRNA level of spliced *Xbp1* was assessed by RT-qPCR (**D**). **p* < 0.05 by two-tailed Student's *t* test for (**B)**. **p* < 0.05 by *ANOVA* with Dunnett's test for (**D**). These data are presented as mean ± SEM.

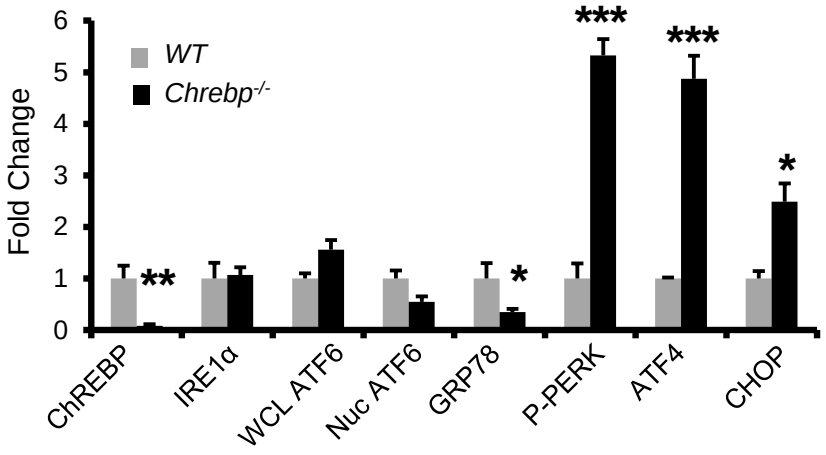


Figure S8 Quantification of protein levels in Figure 3B.
*p < 0.05; **p < 0.01; ***p < 0.001 by two-tailed Student's t test. These data are presented as mean ± SEM.

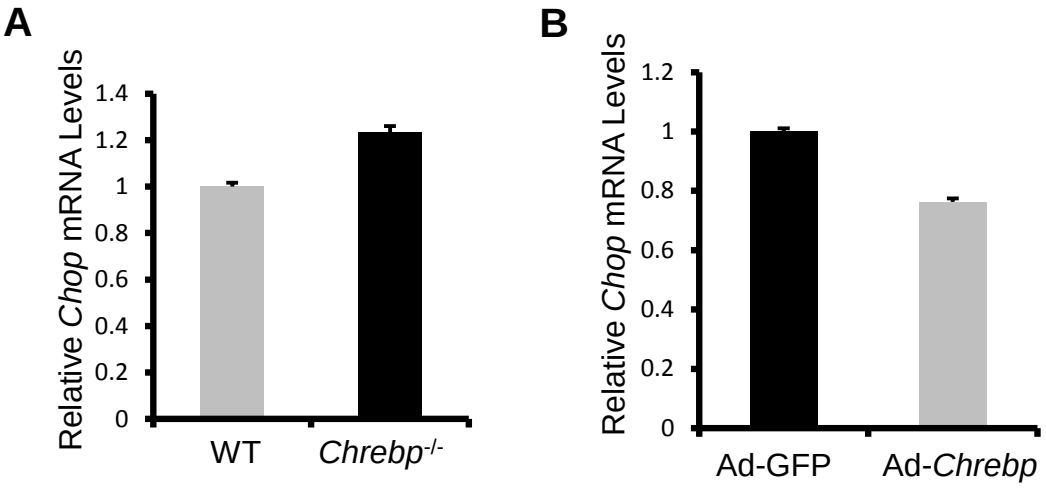


Figure S9 (related to Figure 3) ChREBP does not directly regulate *Chop* transcription.

Chop mRNA level was measured in the livers of regular chow-fed WT and *Chrebp*$^{-/-}$ mice (**A**), and in the primary hepatocytes transduced with Ad-GFP *vs.* Ad-*Chrebp* (**B**). Data are presented as mean ± SEM.

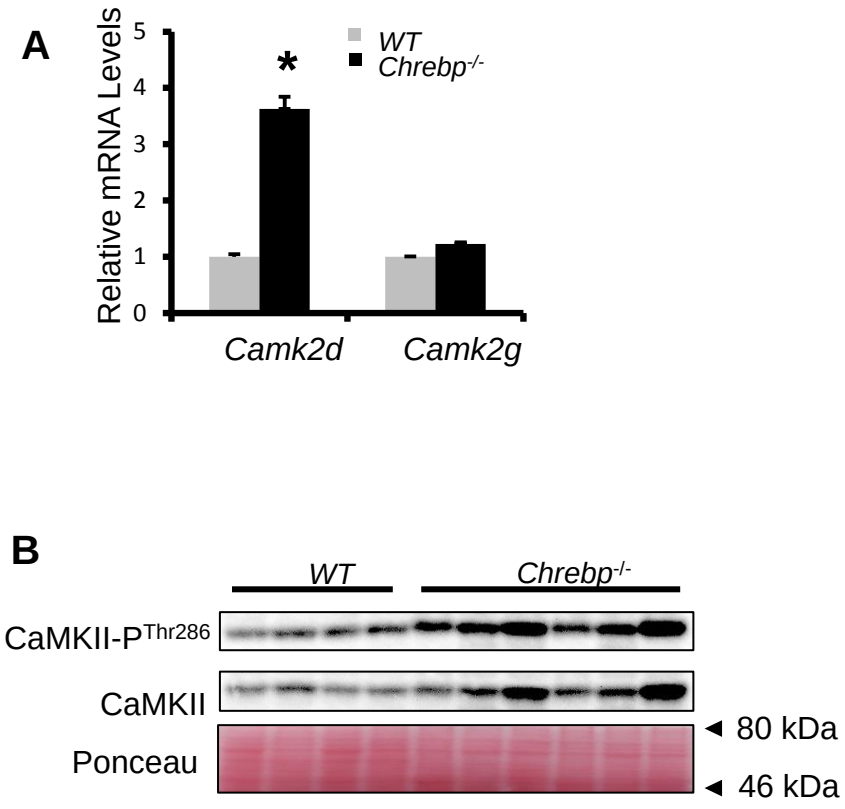


Figure S10 (related to Figure 3) HFrD feeding induces calcium signaling in *Chrebp*$^{-/-}$ mice.

8-wk WT and *Chrebp*$^{-/-}$ mice fed 70% HFrD for 2-wk (n = 4 for WT, n = 6 for *Chrebp*$^{-/-}$).Calcium pathway induction was assessed by RT-qPCR for *Camk2d* and *Camk2g* (**A**) and by western blotting for total and Thr286 phosphorylated CaMKII (**B**). *$p < 0.05$ by two-tailed Student's *t* test. The data are presented as mean ± SEM.

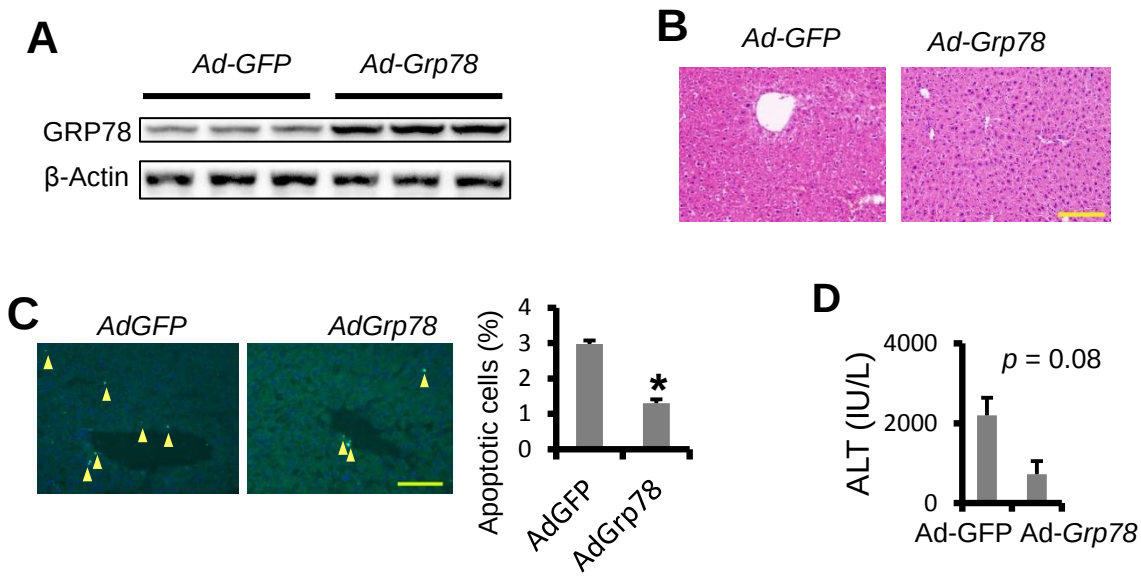


Figure S11 (Related to Figure 3) Restoring GRP78 expression protects *Chrebp*$^{-/-}$ mice from HFrD feeding-induced liver injury.

8-wk *Chrebp*$^{-/-}$ mice were injected with either Ad-*Grp78* (n = 3) or Ad-GFP (n = 3), and then fed 70% HFrD for 2 wks. (**A**) Validation of GRP78 expression in Ad-*Grp78*-transduced primary mouse hepatocytes by western blotting. After dissection, H&E staining (**B**), TUNEL staining (**C**) and ALT assay (**D**) were conducted to assess liver injury. *p < 0.05 by two-tailed Student's t test. These data are presented as mean ± SEM. Scale bar = 100 μm.

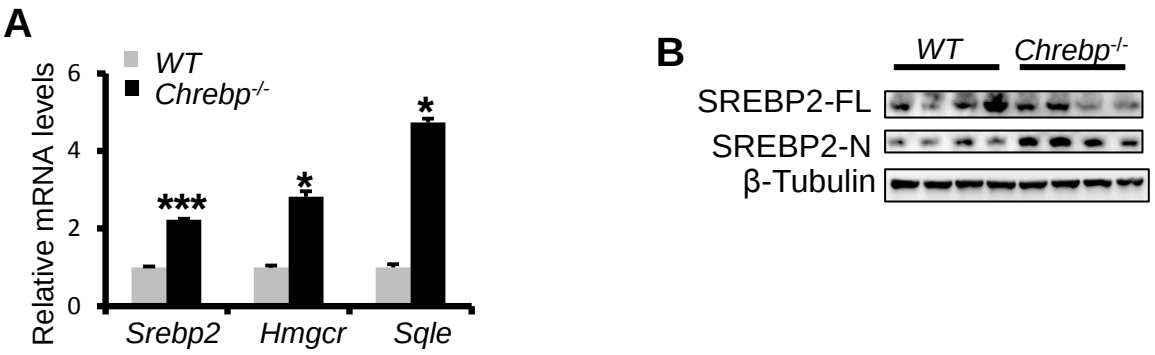


Figure S12. 34% fructose diet feeding elevates cholesterol biosynthesis in *Chrebp*$^{-/-}$ mice.

8-wk WT and *Chrebp*$^{-/-}$ mice fed 34% fructose diet for 4-wk (n = 4, both male and females). Cholesterol biosynthesis genes were assessed by RT-qPCR and western blotting (**A** and **B**). **p* < 0.05, *** p < 0.001 by two-tailed Student's *t* test. These data are presented as mean ± SEM.

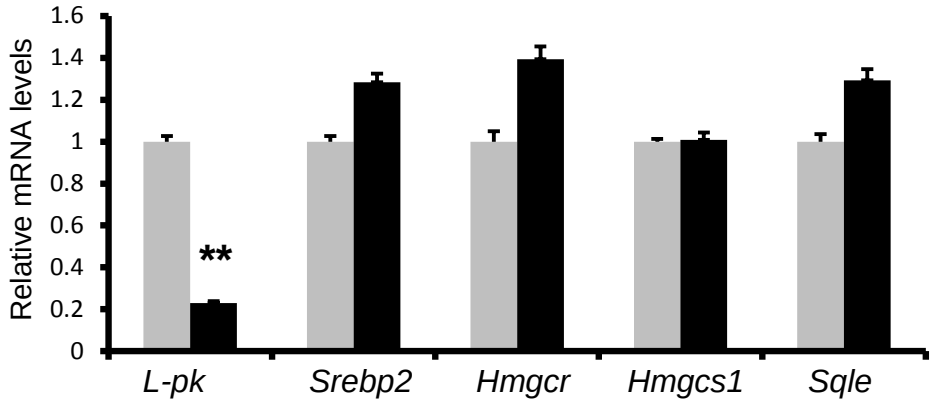


Figure S13 (related to Figure 4) *Chrebp* deficiency does not affect cholesterol biosynthesis in regular chow feeding condition.

Expression levels of cholesterol biosynthesis genes in liver of *Chrebp*$^{-/-}$ mice of 8-wk old were assessed by RT-qPCR. $^{**}p < 0.01$ by two-tailed Student's *t* test. These data are presented as mean ± SEM.

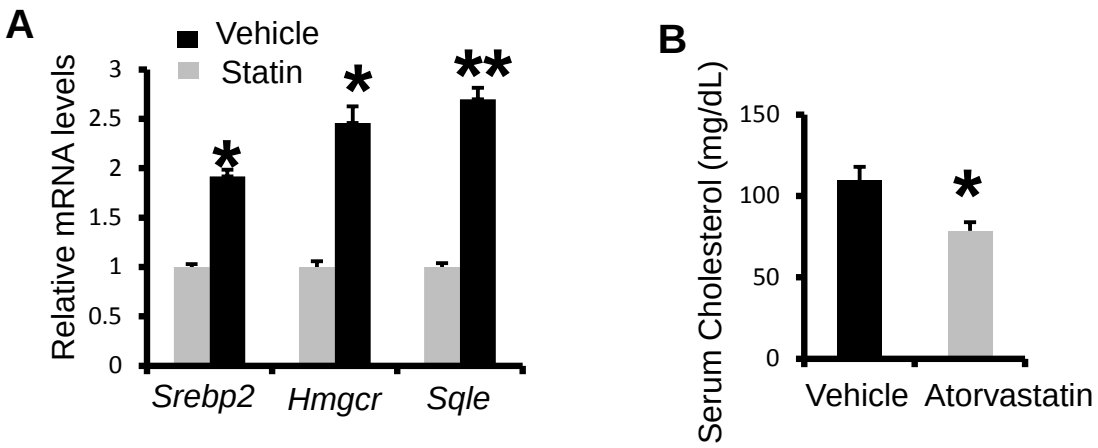


Figure S14. (Related to Figure 5) Effects of HMGCR inhibition on cholesterol biosynthesis genes in the liver and serum cholesterol in *Chrebp*$^{-/-}$ mice. Atorvastatin administration in *Chrebp*$^{-/-}$ mice elevates cholesterol biosynthesis genes expression (**A**) and decreases serum cholesterol levels (**B**). **p* < 0.05, ** p < 0.01 by two-tailed Student's *t* test. These data are presented as mean ± SEM.

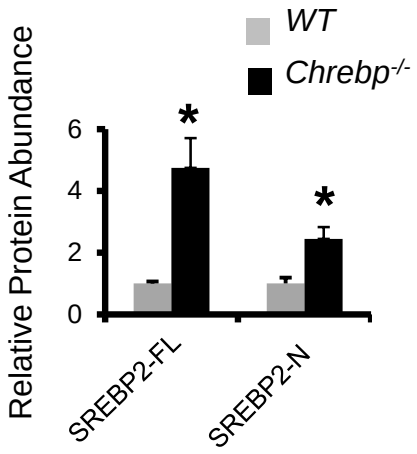


Figure S15. Quantification of protein levels in Figure 6A. *$p < 0.05$ by two-tailed Student's *t* test. These data are presented as mean ± SEM.

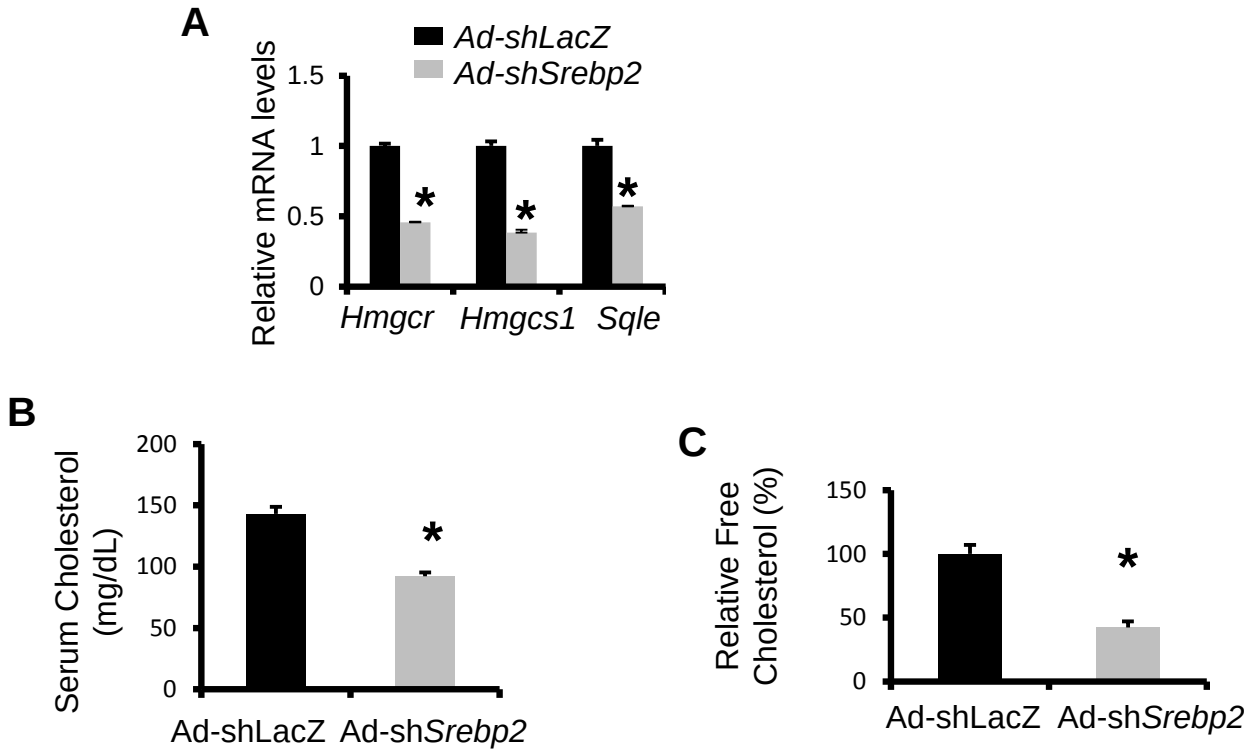


Figure S16. (Related to Figure 6) Knocking down *Srebp2* lowers cholesterol biosynthesis in the liver of HFrD-fed *Chrebp*$^{-/-}$ mice.

8-wk *Chrebp*$^{-/-}$ mice were injected with either Ad-shLacZ or Ad-sh*Srebp2* and fed 70% HFrD for 2-wk (n = 3). (**A**) mRNA levels of cholesterol biosynthesis genes were assessed with RT-qPCR. (**B**) Serum cholesterol was measured. (**C**) Quantification of hepatic free cholesterol assessed with filipin staining in **Fig6 D**. *$p < 0.05$ by two-tailed Student's *t* test. These data are presented as mean ± SEM.

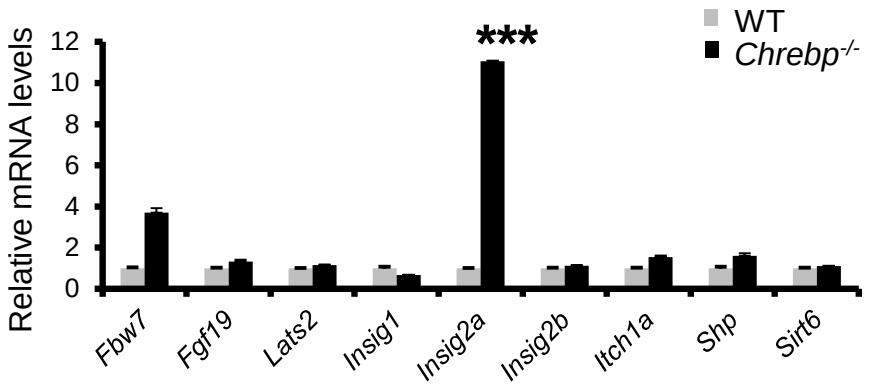


Figure S17. (Related to Figure 6) Expression profile of regulators of cholesterol biosynthesis in WT and *Chrebp*$^{-/-}$ mice liver.

8-wk WT and *Chrebp*$^{-/-}$ mice fed HFrD for 2-wk (n = 4 for WT, n = 6 for *Chrebp*$^{-/-}$). mRNA levels of cholesterol biosynthesis transcription factors were assessed with RT-qPCR. *$p < 0.05$ by two-tailed Student's *t* test. These data are presented as mean ± SEM.

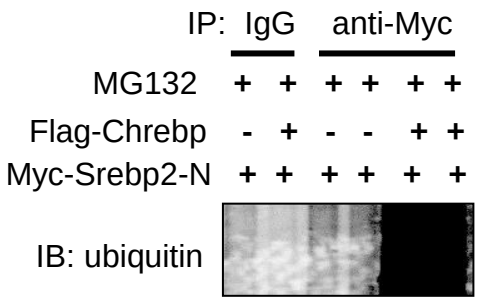
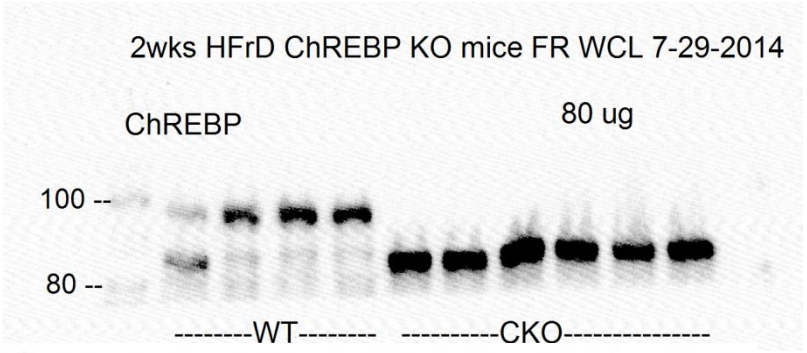
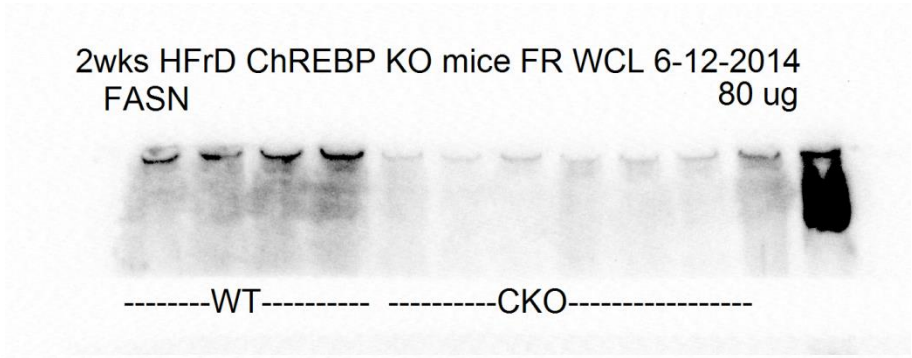
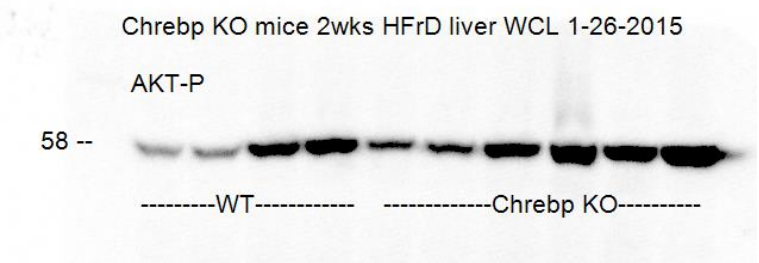


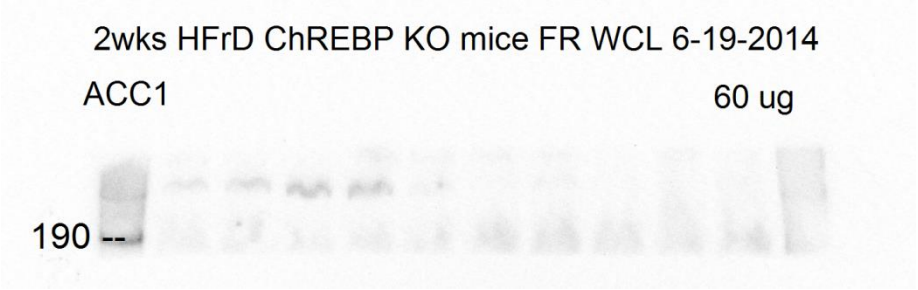
Figure S18. Longer exposure of anti-Myc IP and anti-ubiquitin IB membrane in Fig6 I.

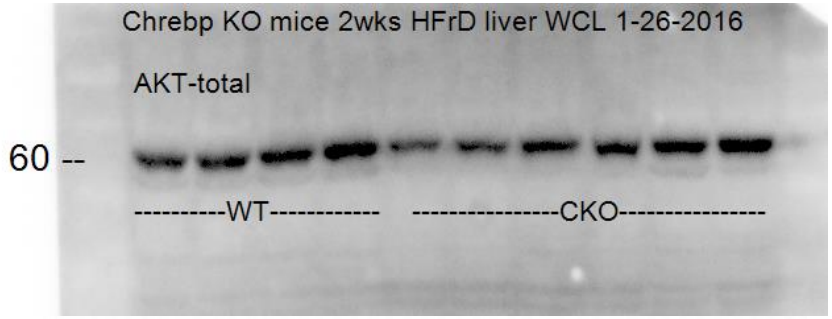
Full unedited gel for Figure1 C

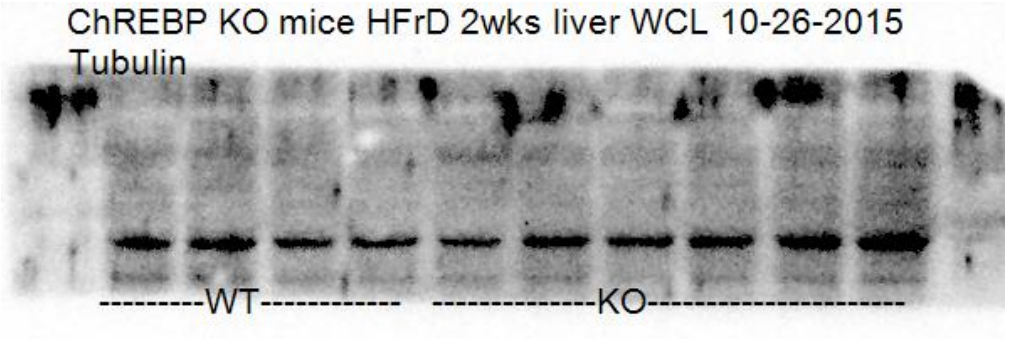


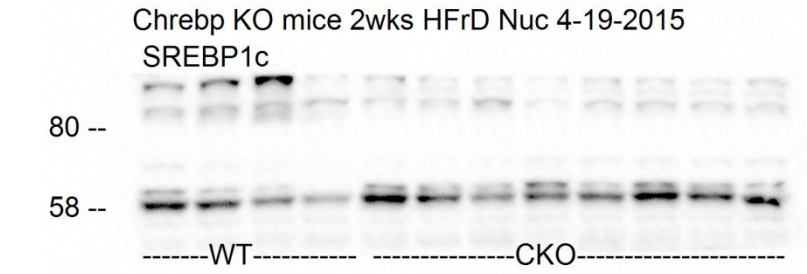












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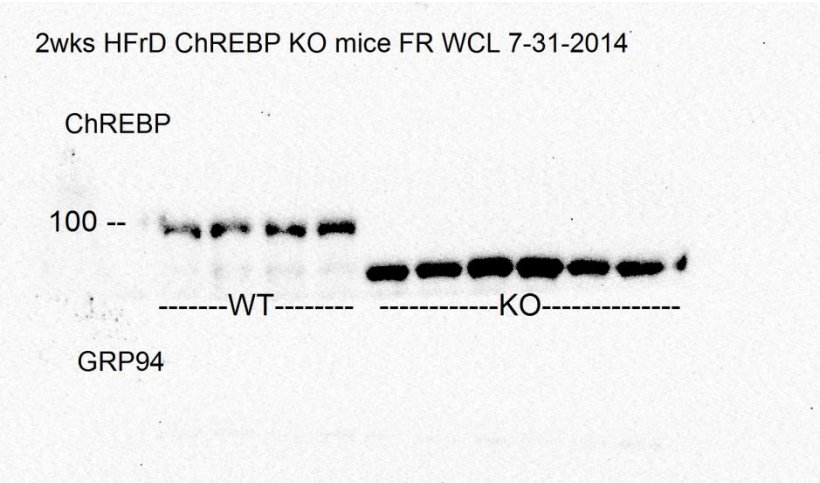

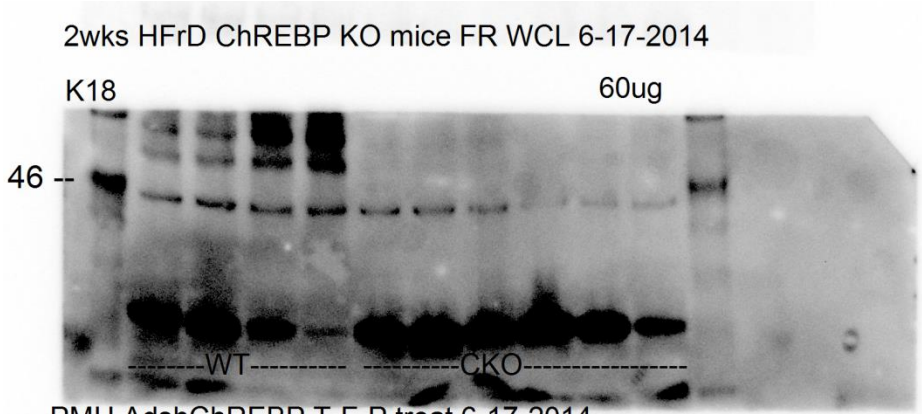

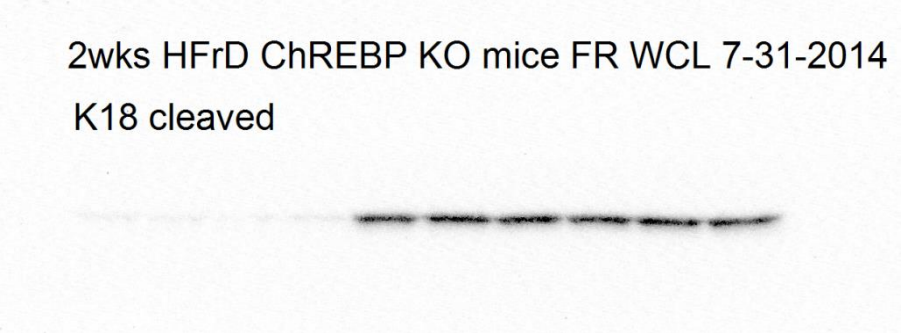

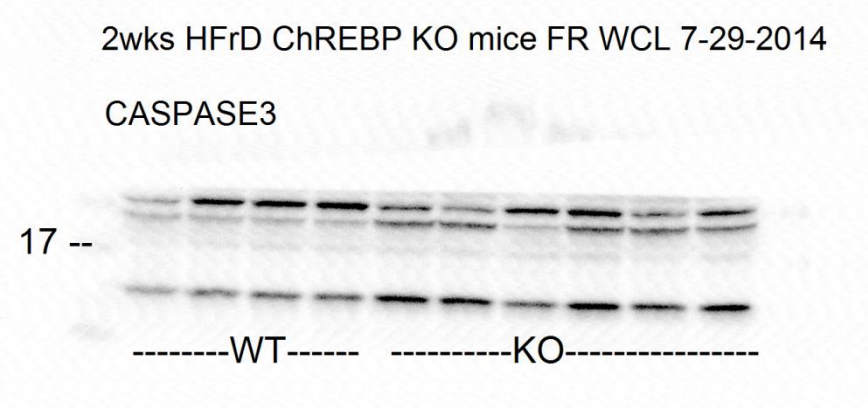

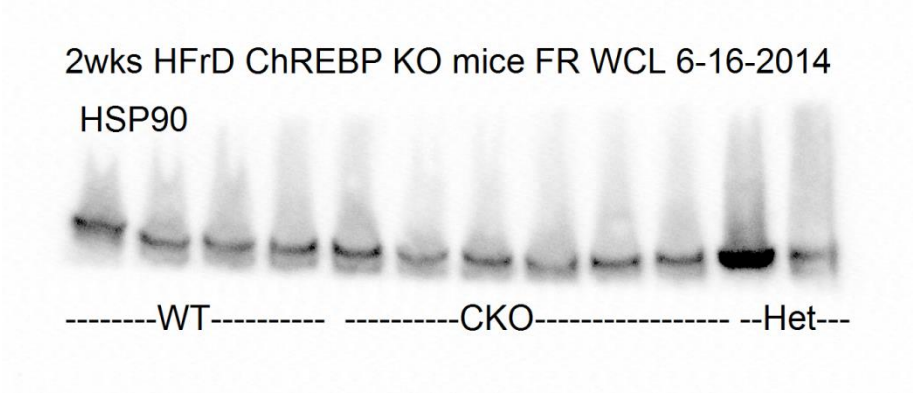

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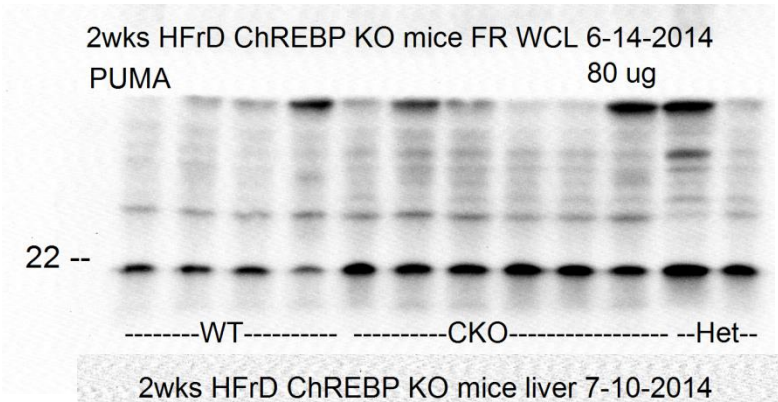

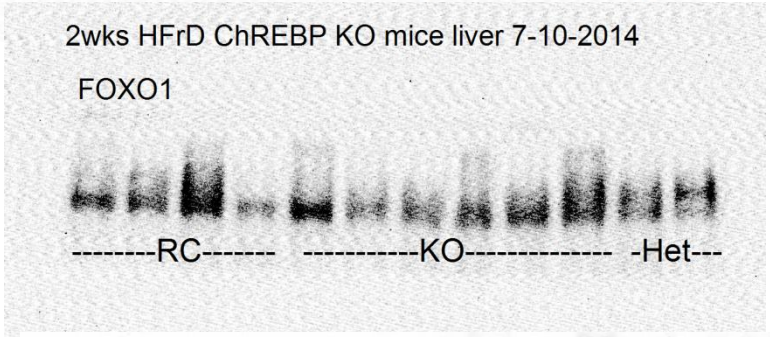

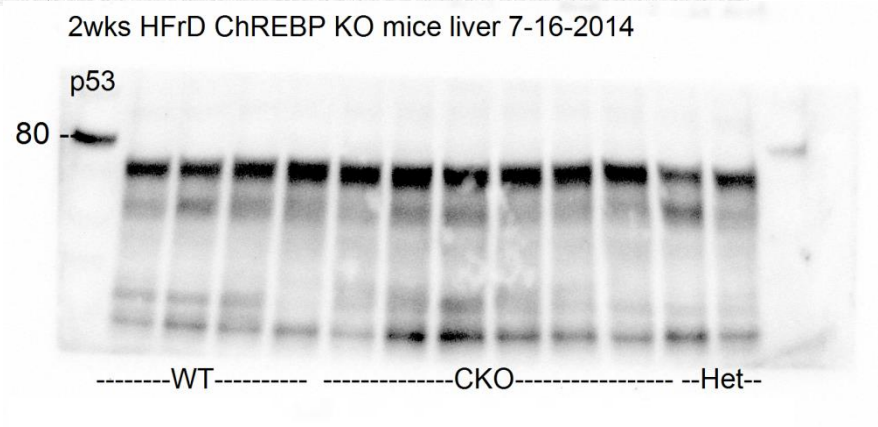

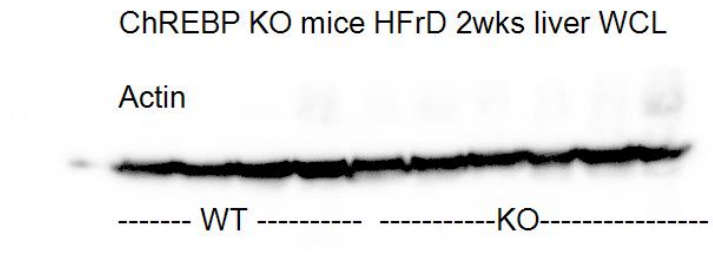

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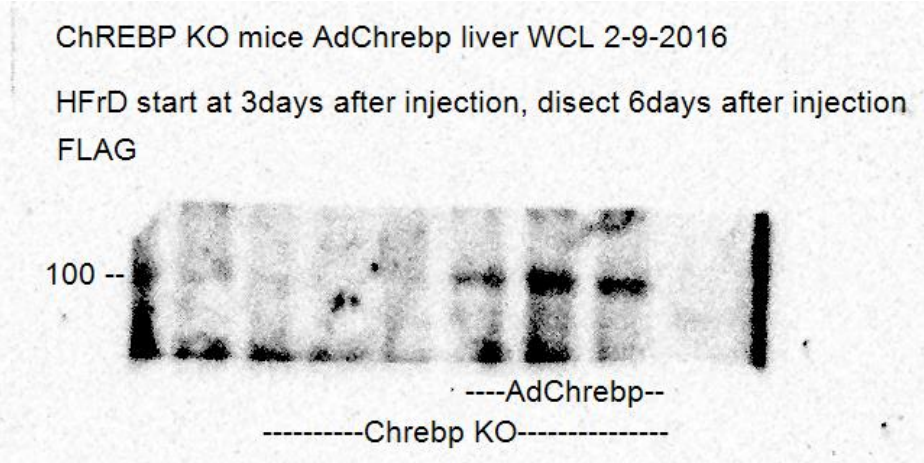

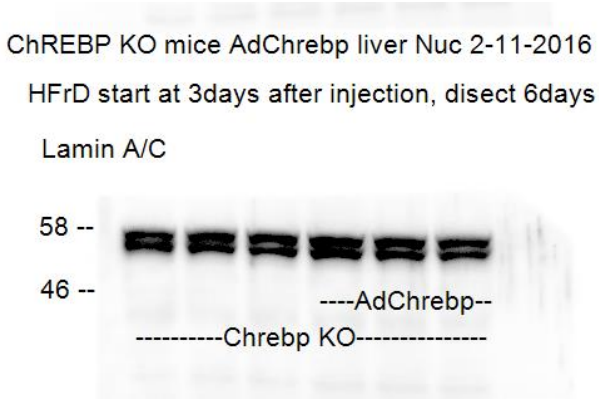

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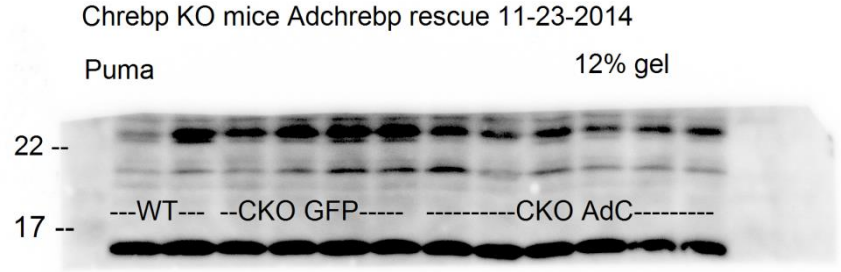

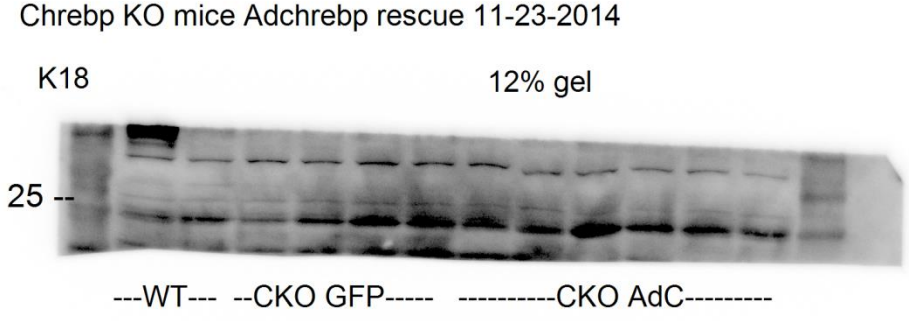

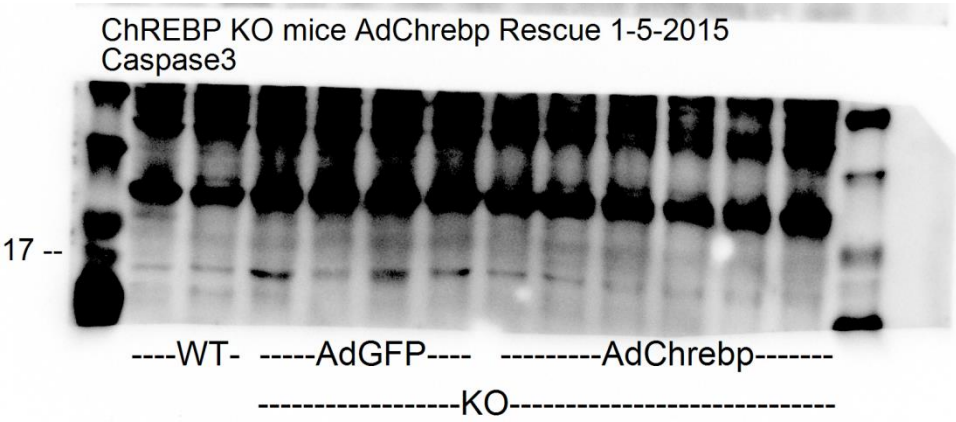

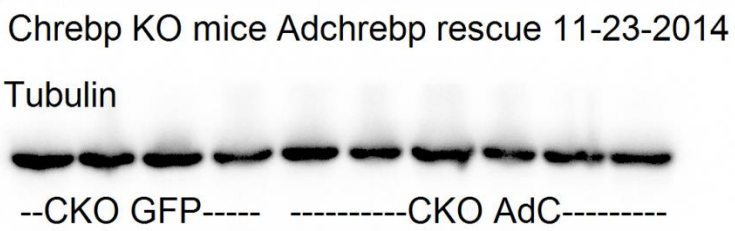

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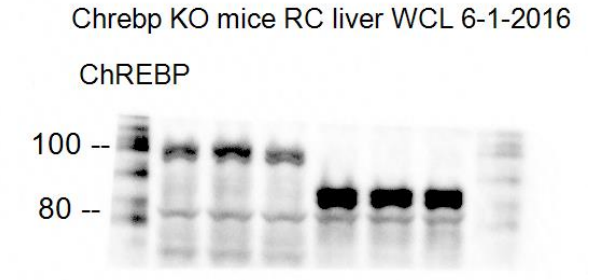

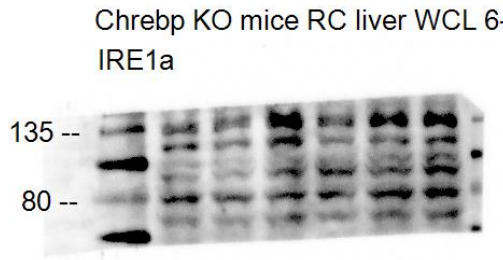

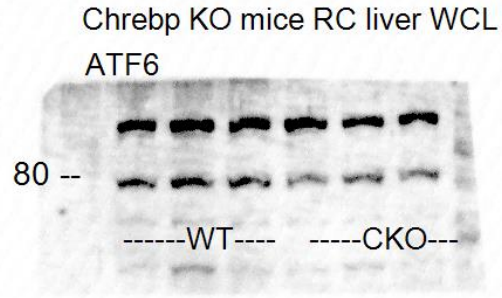

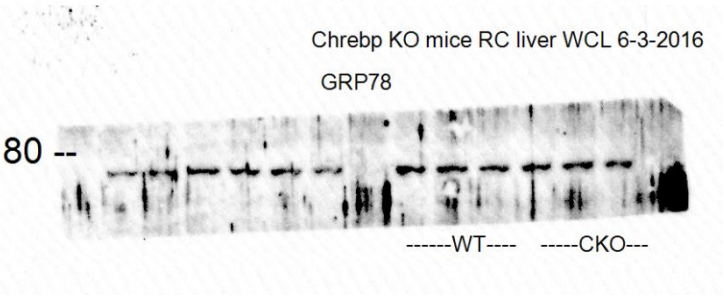

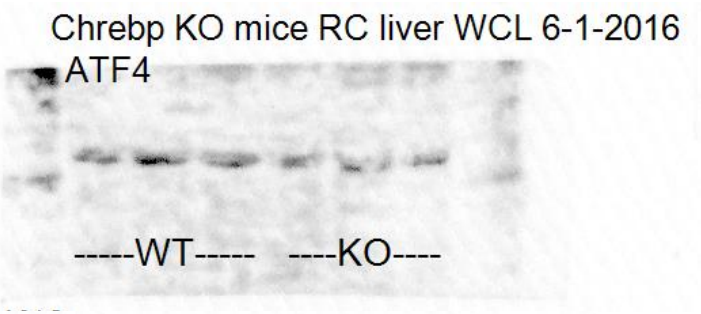

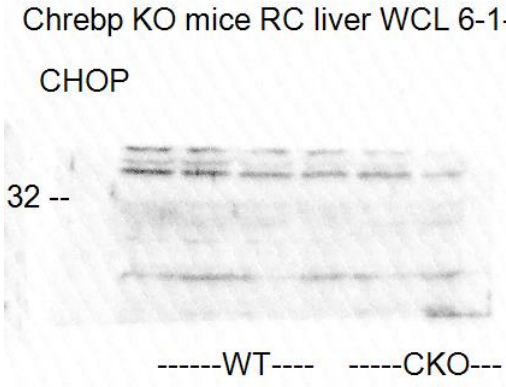

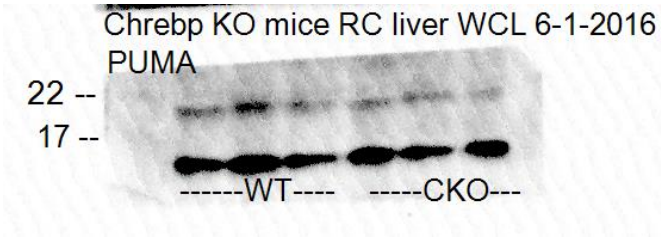

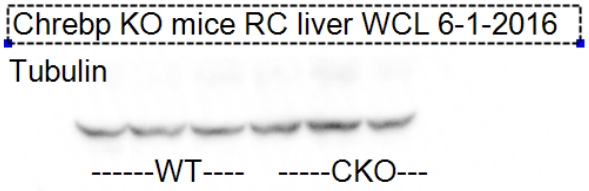

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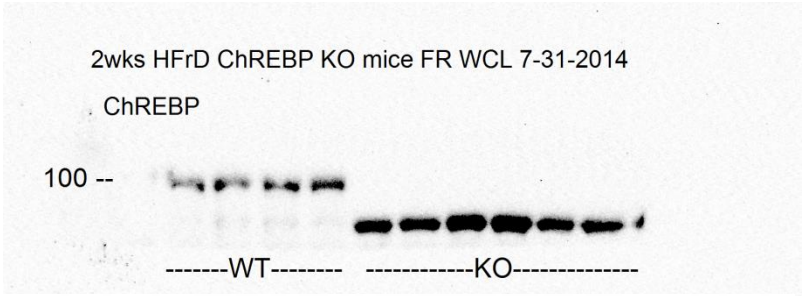
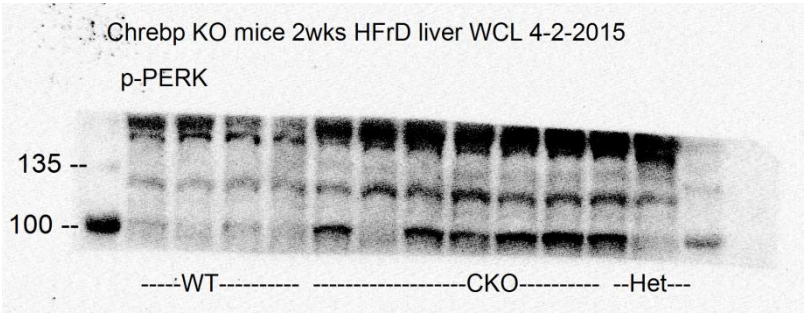
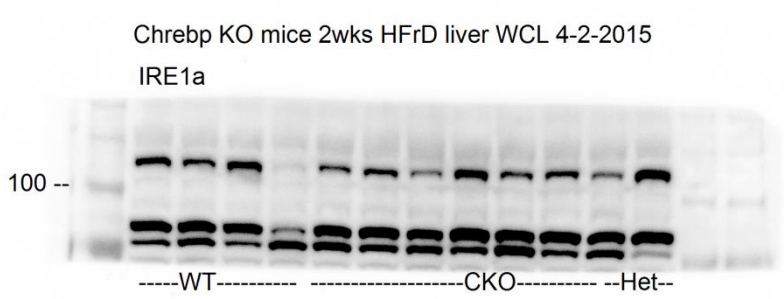
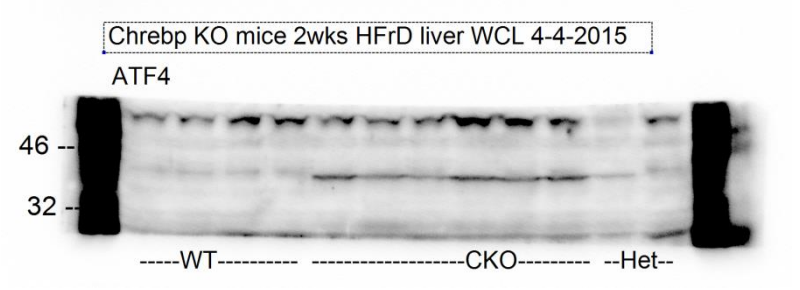
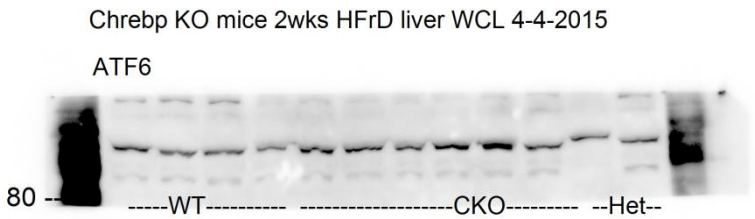
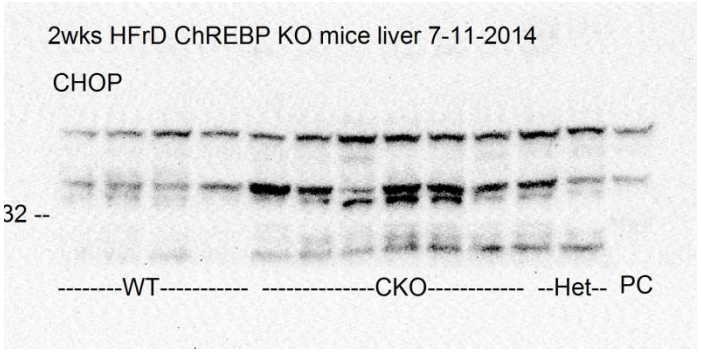


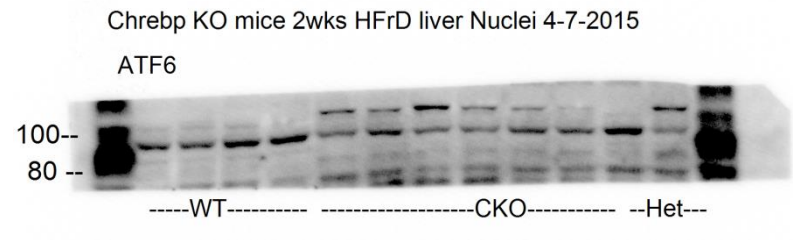


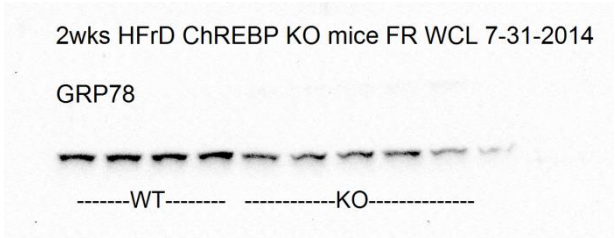


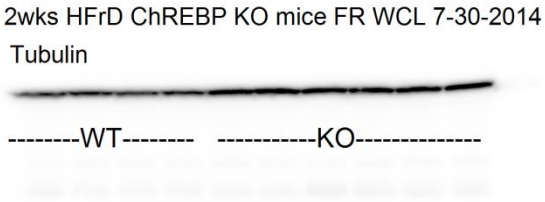




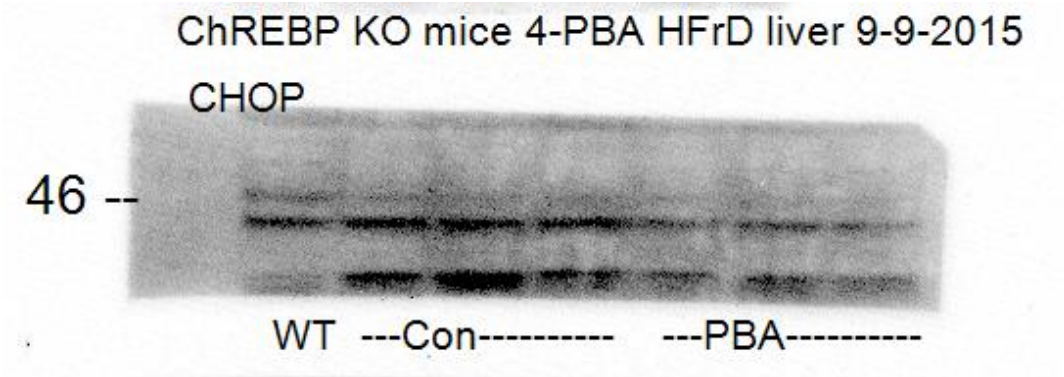


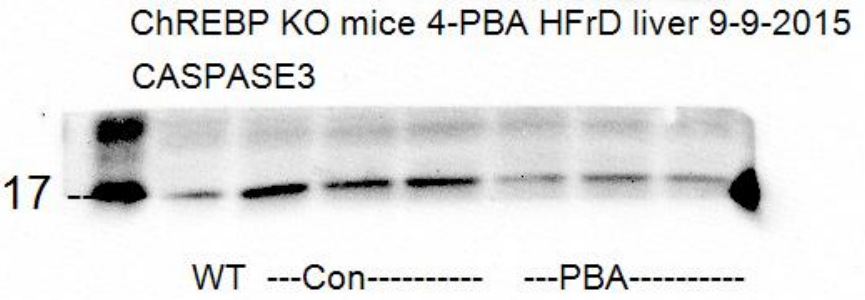


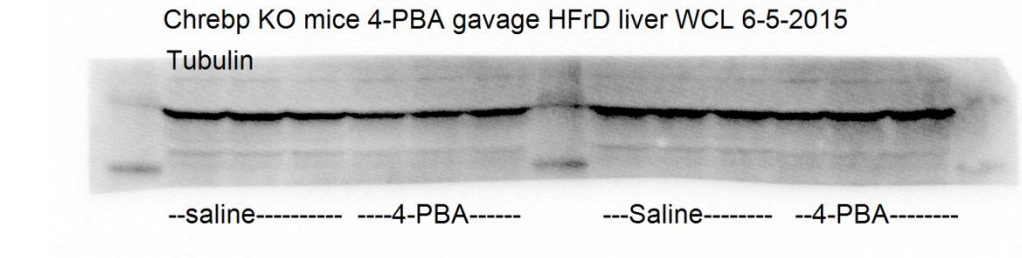




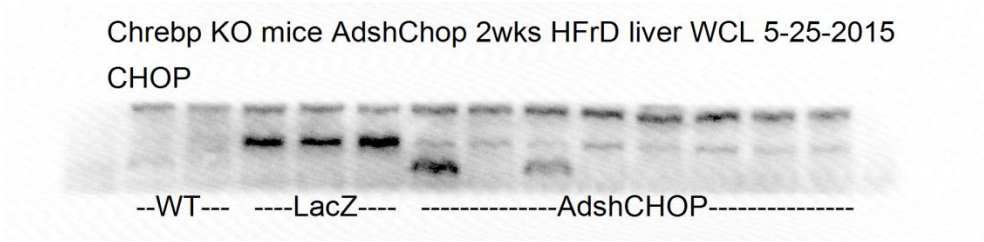
Full unedited gel for Figure3 G

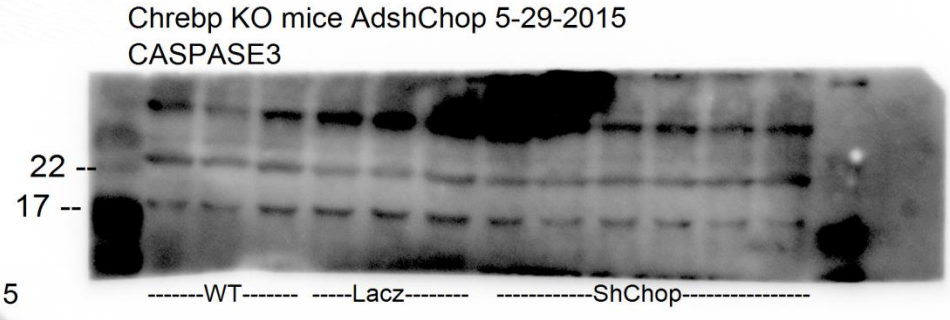


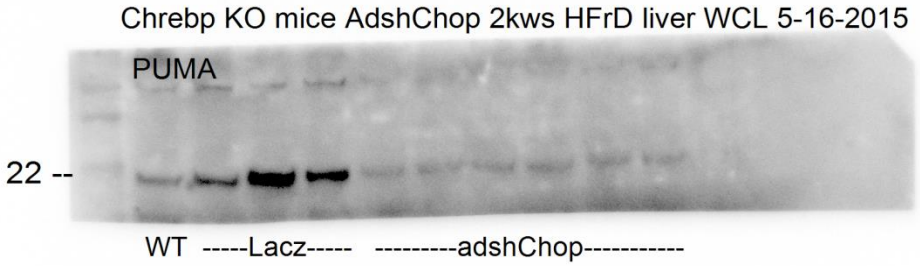


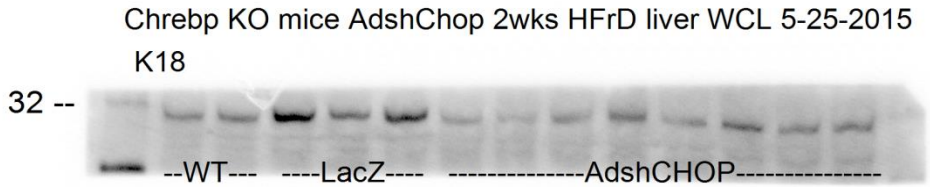


Full unedited gel for Figure3 L

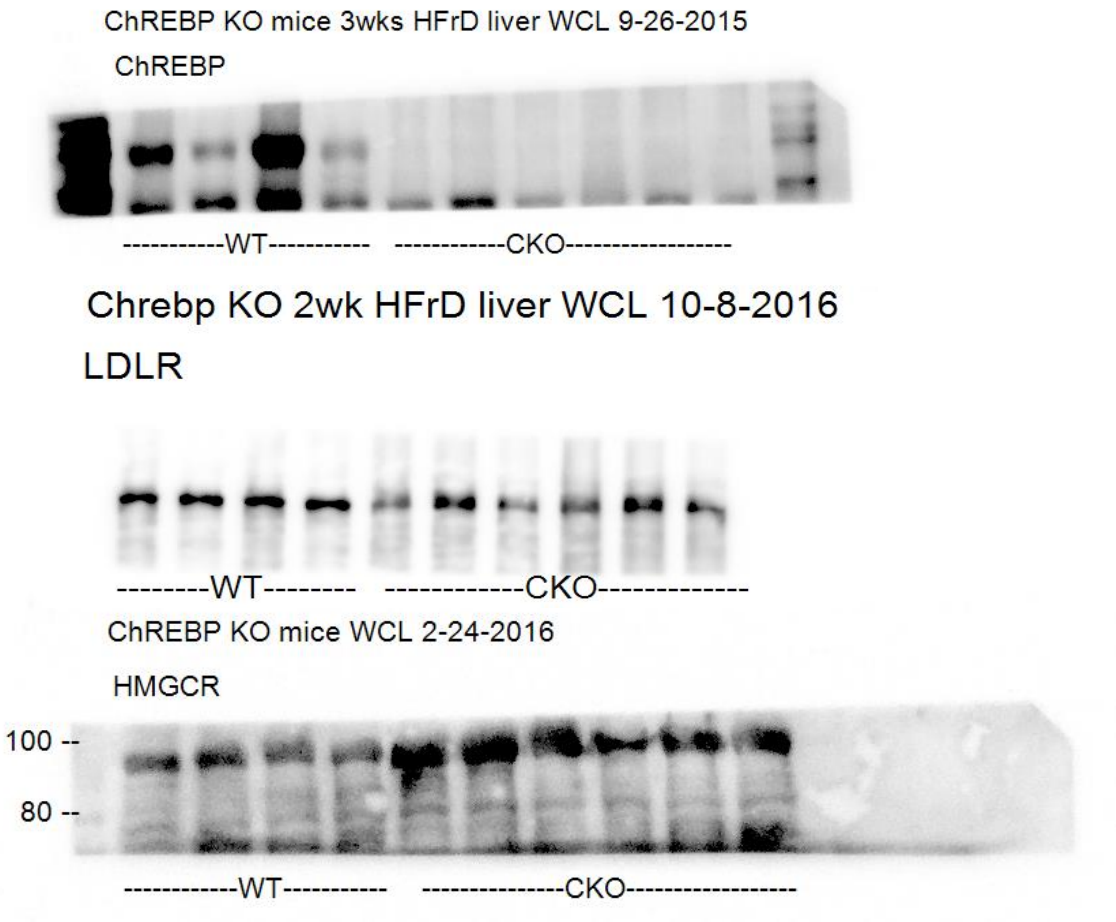




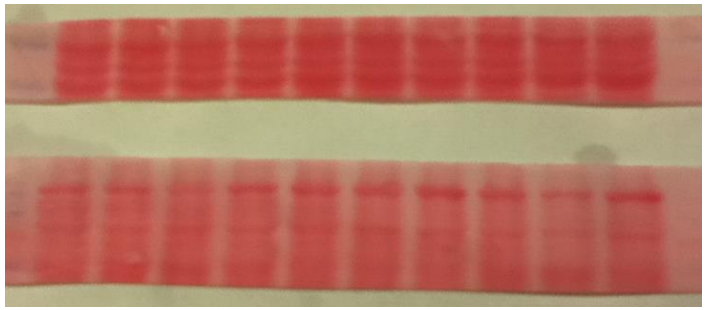




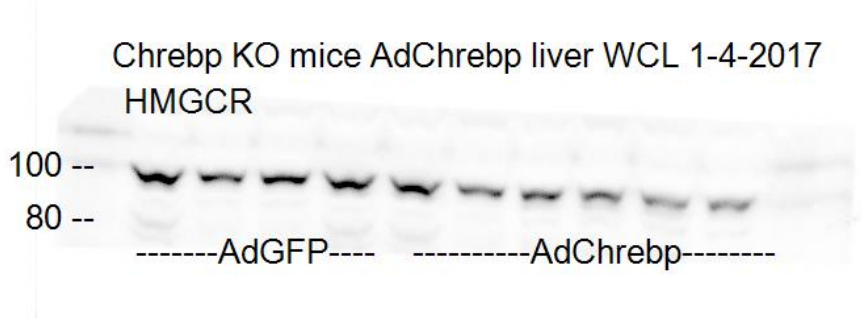
Full unedited gel for Figure4 D

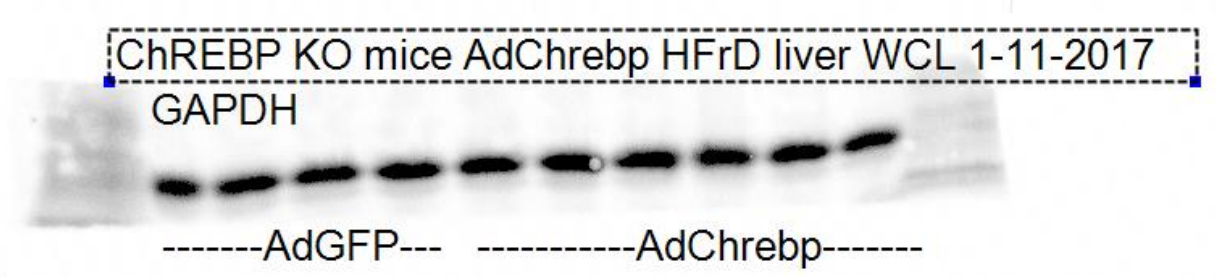


Pauseau Staining



Full unedited gel for Figure4 F

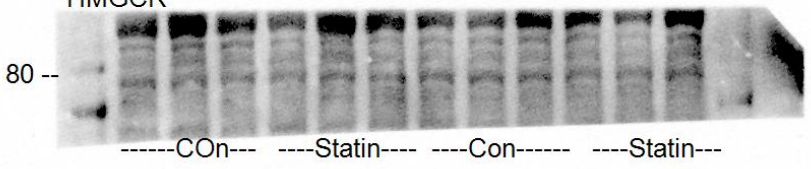




Full unedited gel for Figure5 H

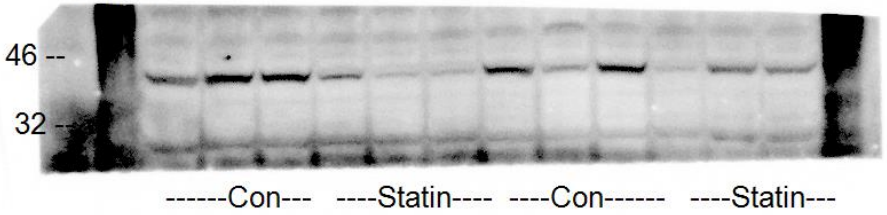
ChREBP KO mice Atorvastatin treated liver WCL 3-3-2016

HMGCR



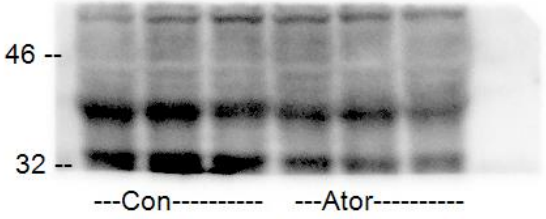
ChREBP KO mice Atorvastatin treated liver WCL 3-3-2016

ATF4



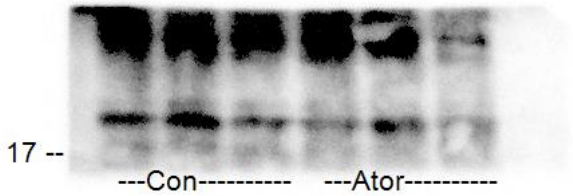
ChREBP KO mice Atorvastatin HFrD liver 9-29-2015

CHOP



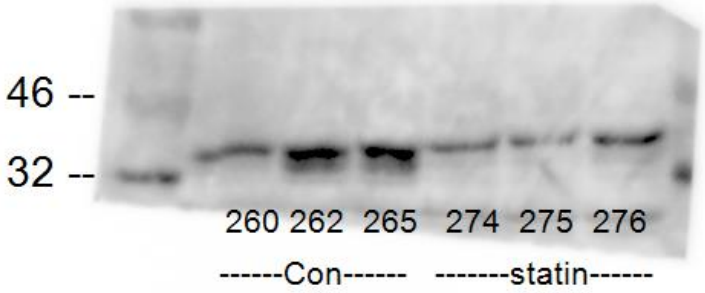
ChREBP KO mice Atorvastatin HFrD liver 9-29-2015

PUMA

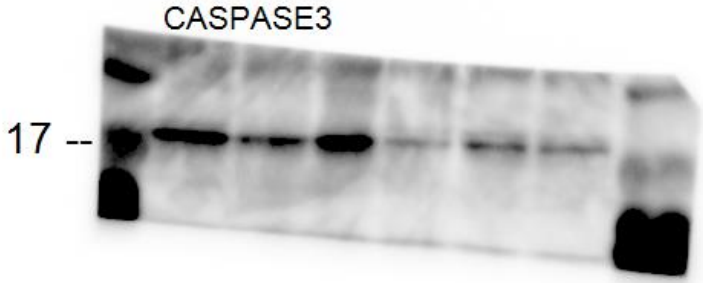


Chrebp KO mice Atovastatin 10days liver WCL 1-8-2016

K18

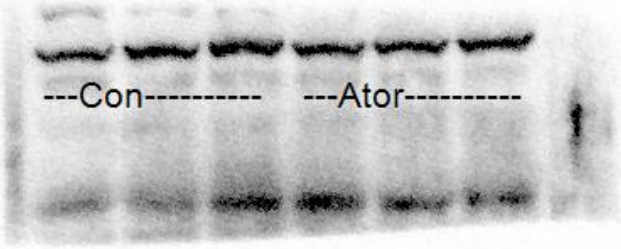


CASPASE3

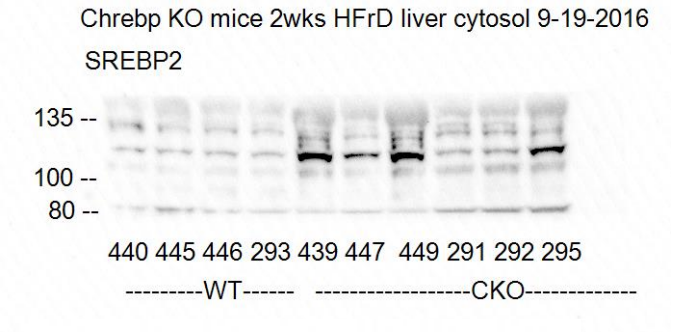


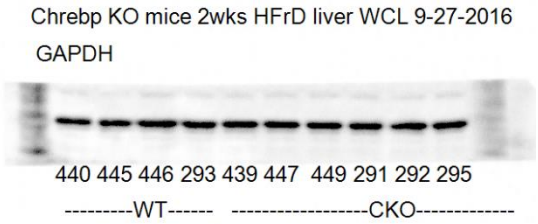
ChREBP KO mice Atorvastatin HFrD liver 9-29-2015

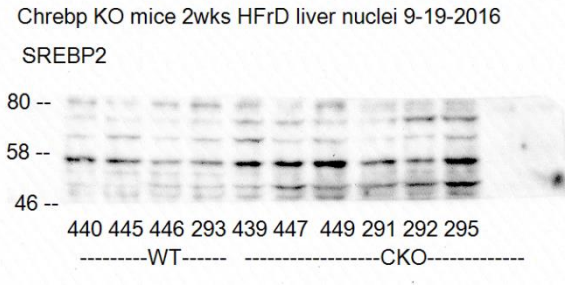
TUBULIN

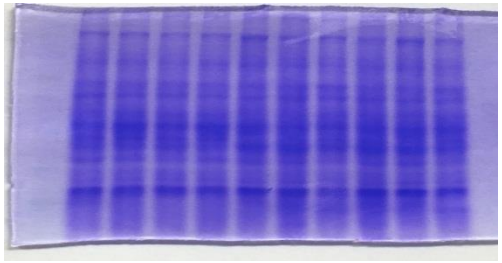


Full unedited gel for Figure6 A

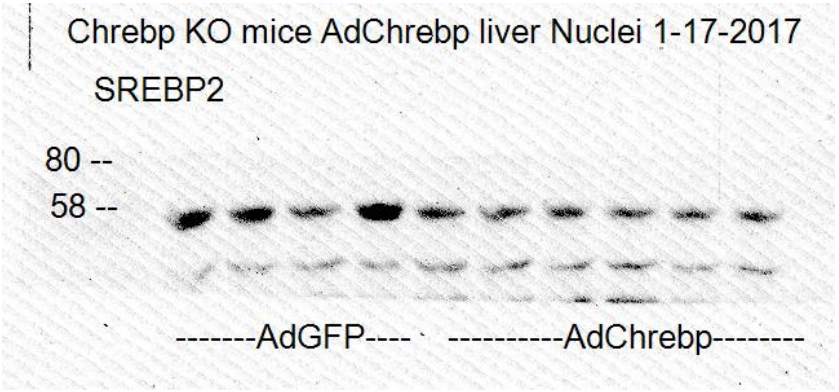


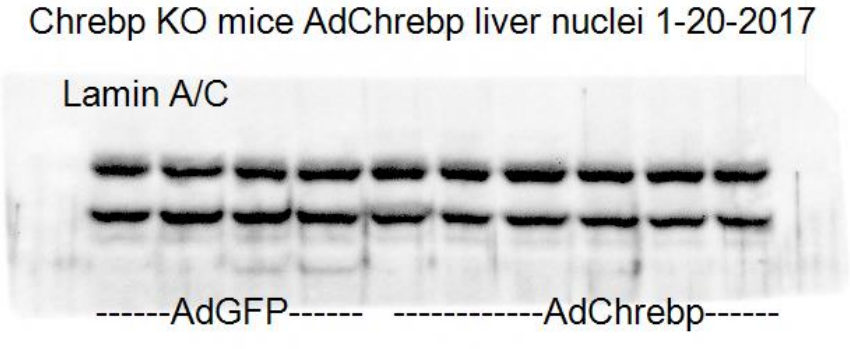






Full unedited gel for Figure6 B

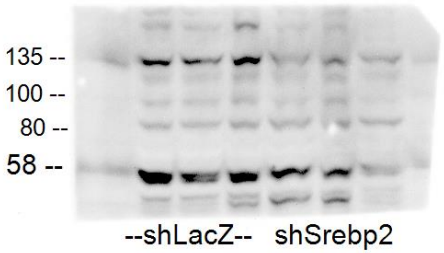




Full unedited gel for Figure6 C

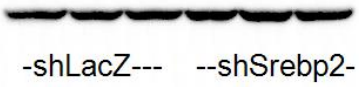
Chrebp KO mice AdshSrebp2 HFrD 10 days liver WCL 12-20-2016

SREBP2



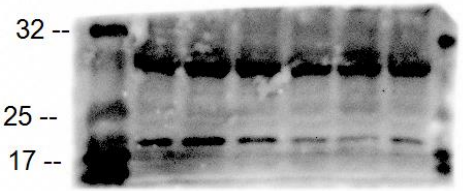
Chrebp KO mice AdshSrebp2 liver WCL 1-12-2017

Actin



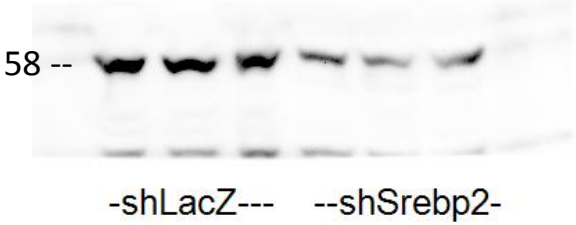
Chrebp KO mice AdshSrebp2 HFrD 10 days liver WCL 12-14-2016

Caspase3



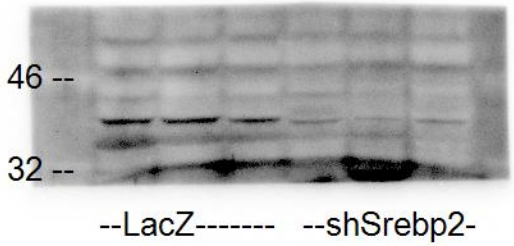
CKO mice AdshSrebp2 liver Nuc 1-4-2017

SREBP2



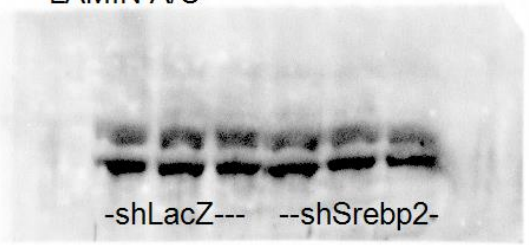
Chrebp KO mice AdshSrebp2 10 days HFrD 2-4-2017

CHOP

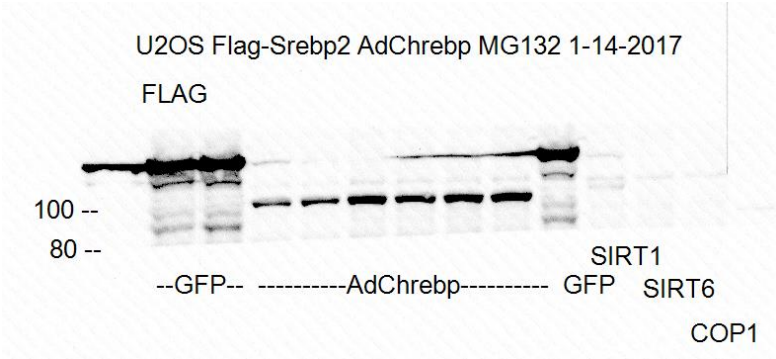


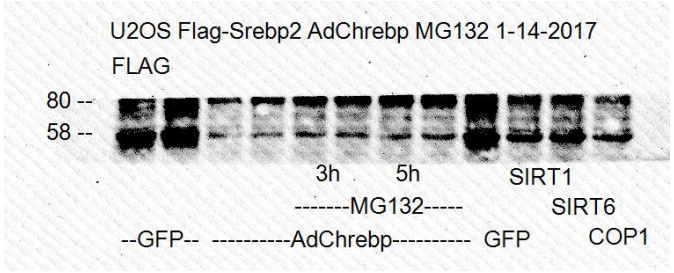
CKO mice AdshSrebp2 liver Nuc 1-5-2017

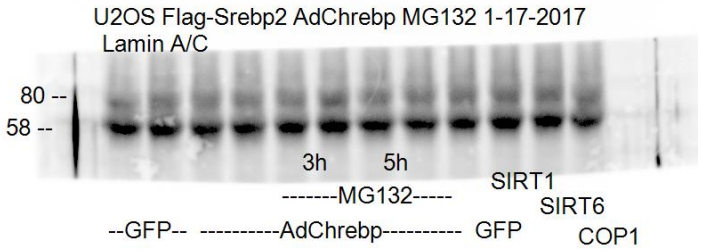
LAMIN A/C



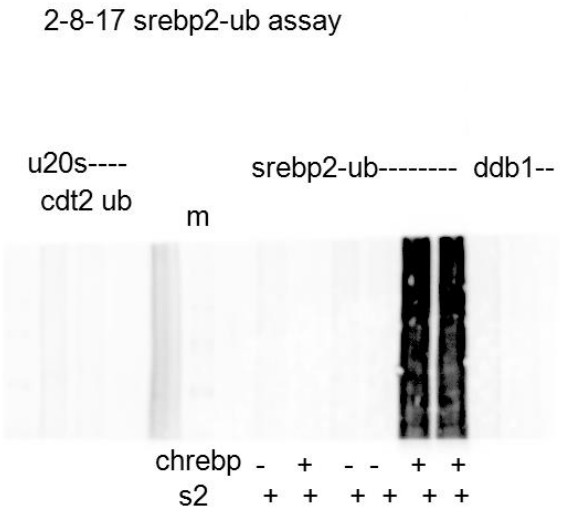
Full unedited gel for Figure6 H

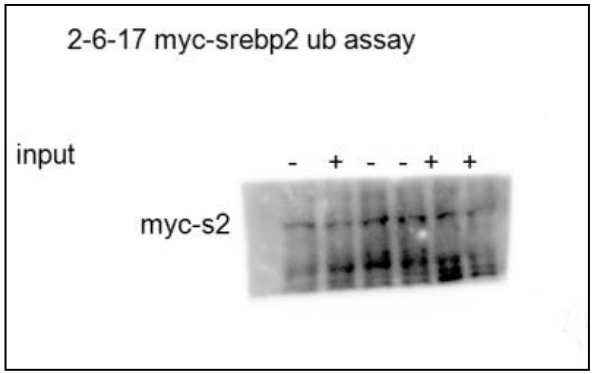




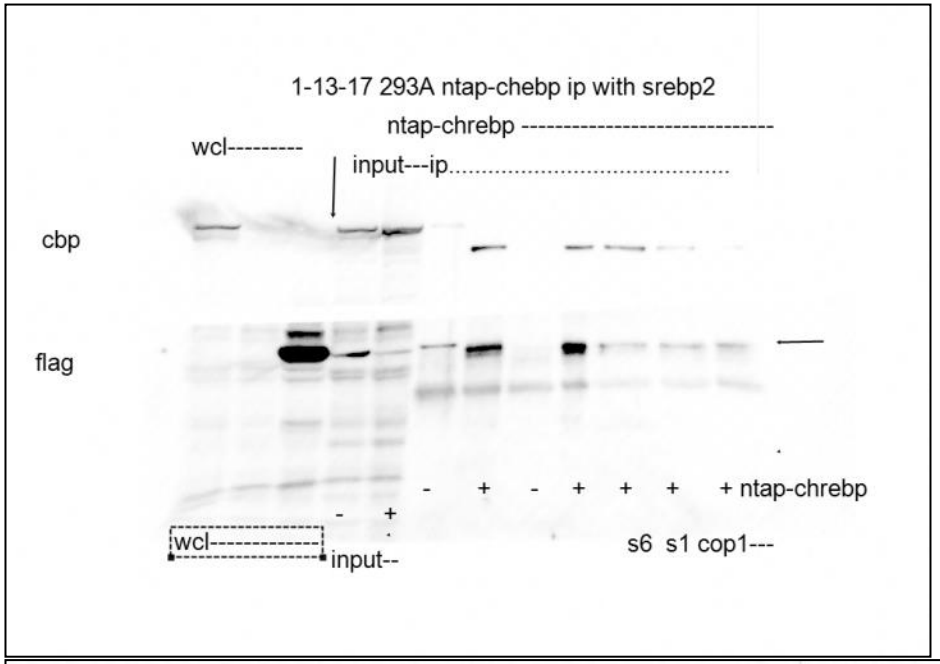


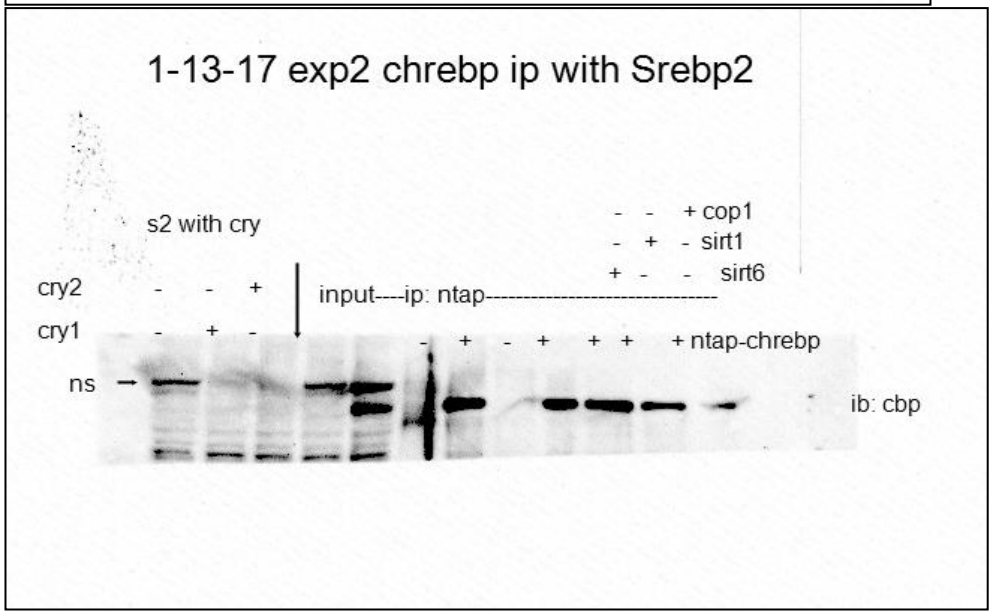
Full unedited gel for Figure6 I



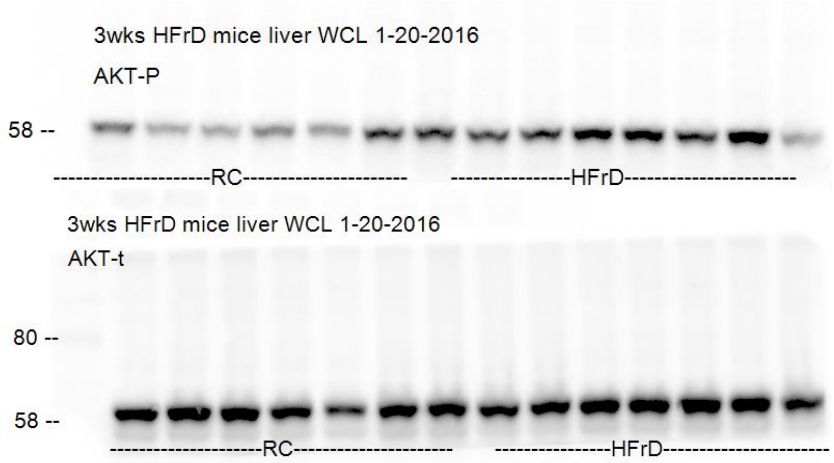


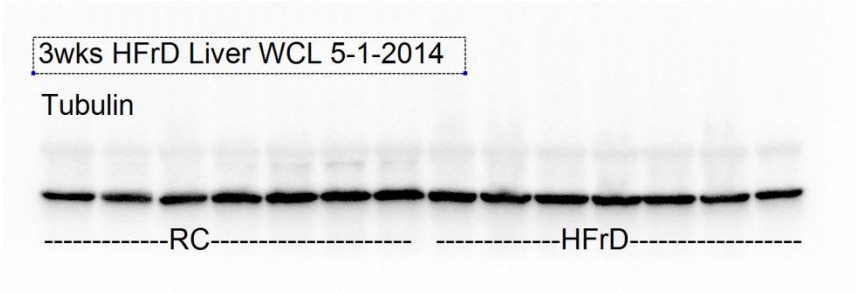
Full unedited gel for Figure6 J

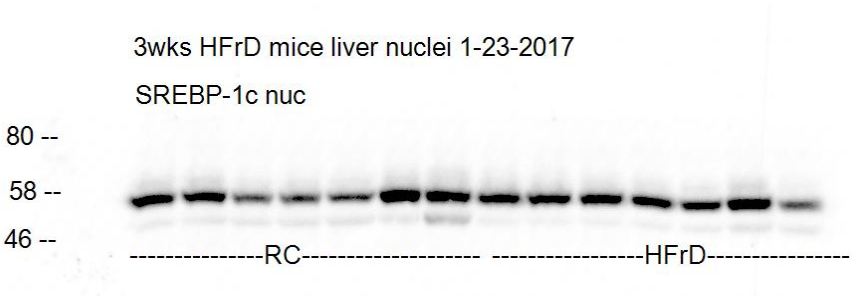


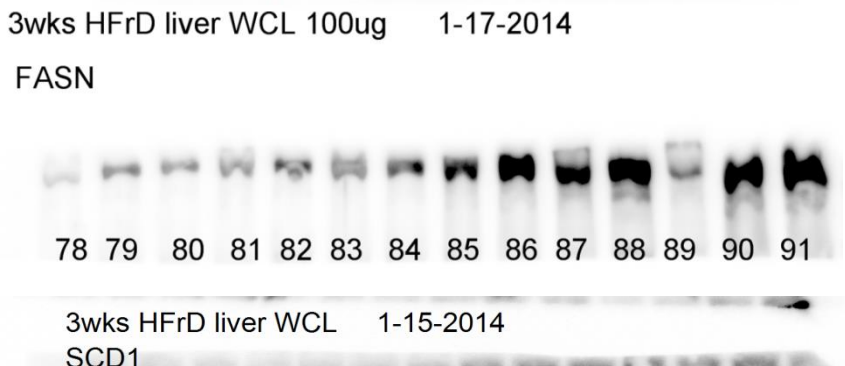


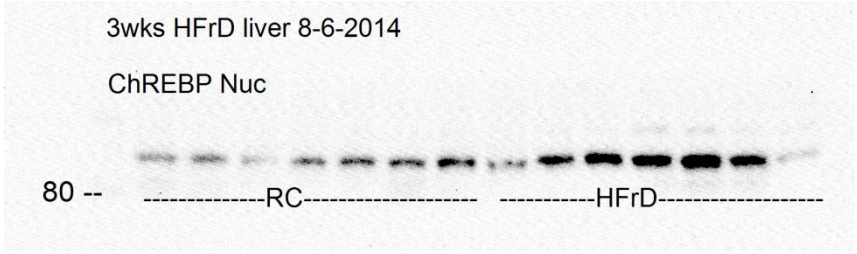
Full unedited gel for Figure S2 E

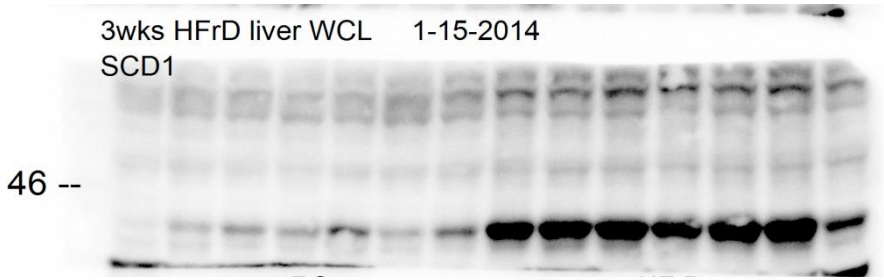




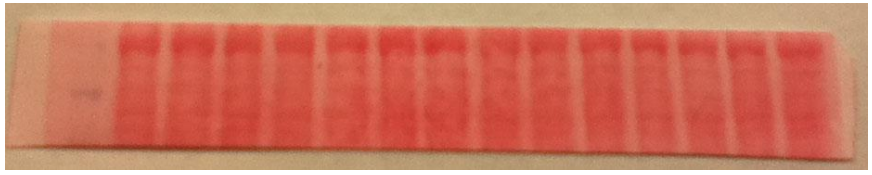


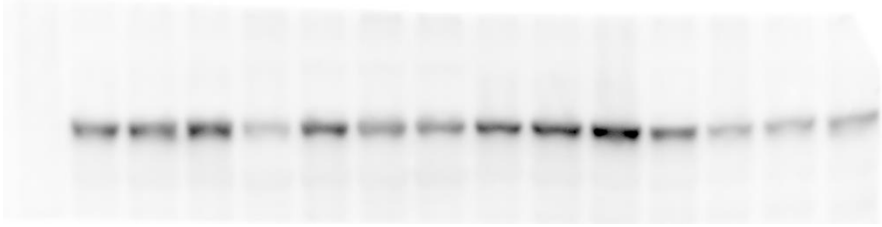




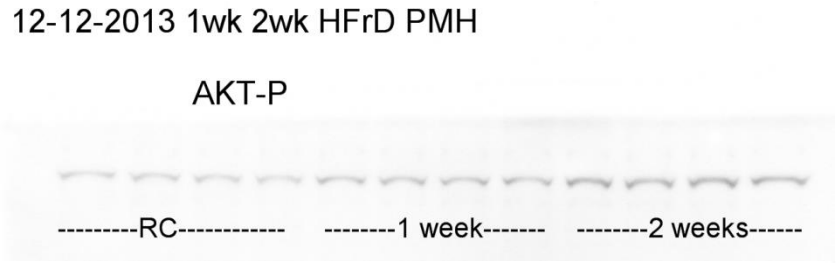


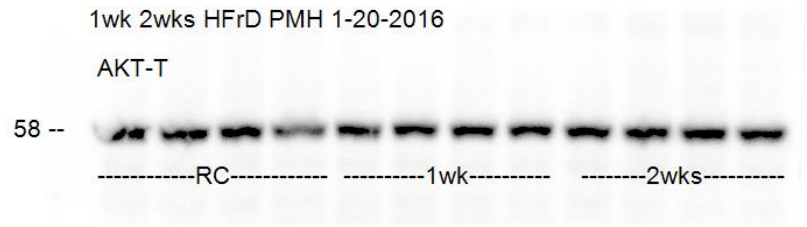
Ponceau

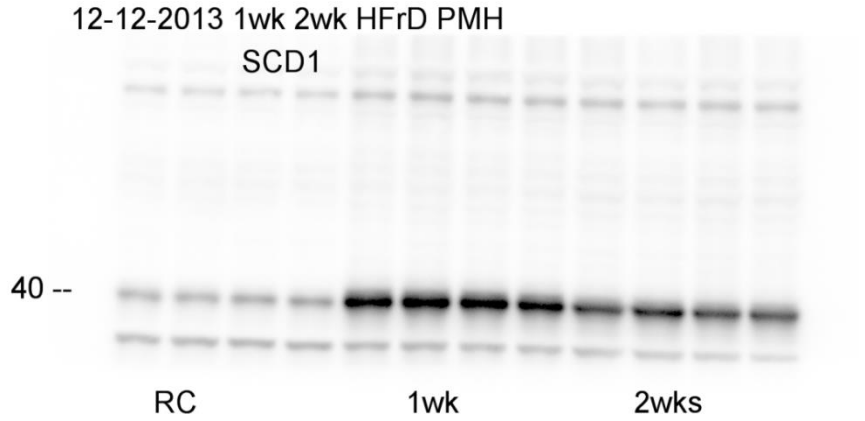


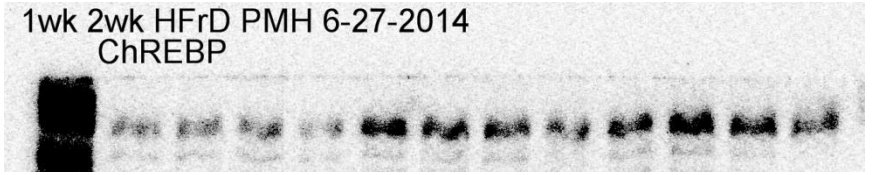


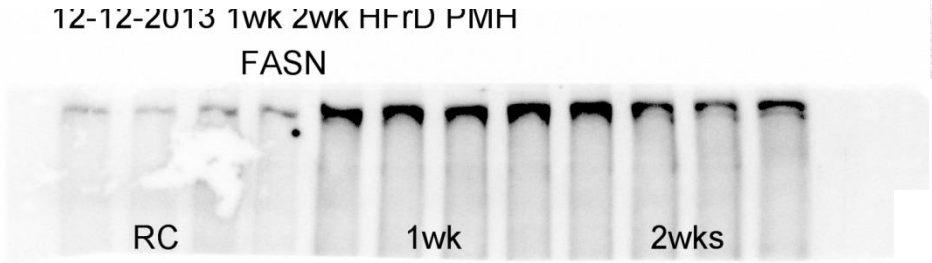
Full unedited gel for Figure S3 B

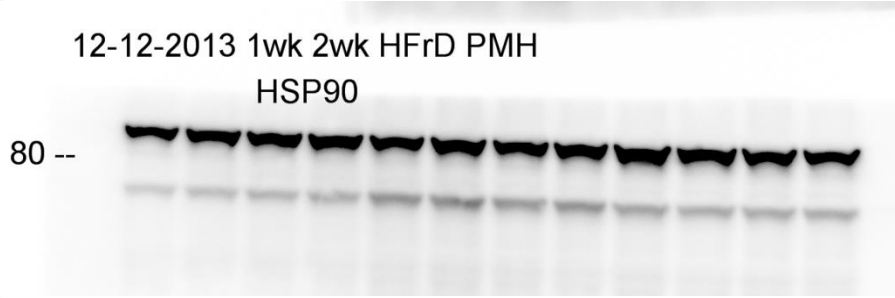








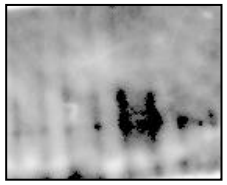




Full unedited gel for Figure S3 C

RC 1wk HFrD PMH AdF-Chrebp FLAG IP

IB: Ac-K



RC HFrD

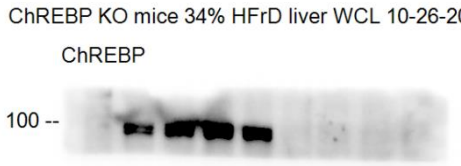
IB: FLAG

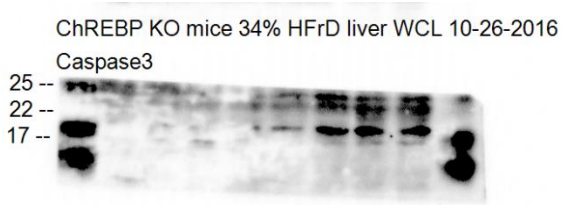
RC HFrD

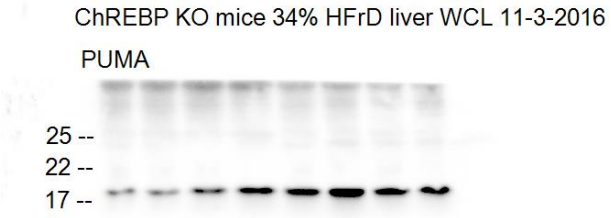
Input IB: FLAG

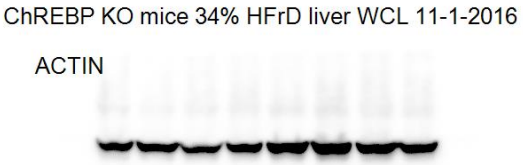
RC HFrD

Full unedited gel for Figure S6 F

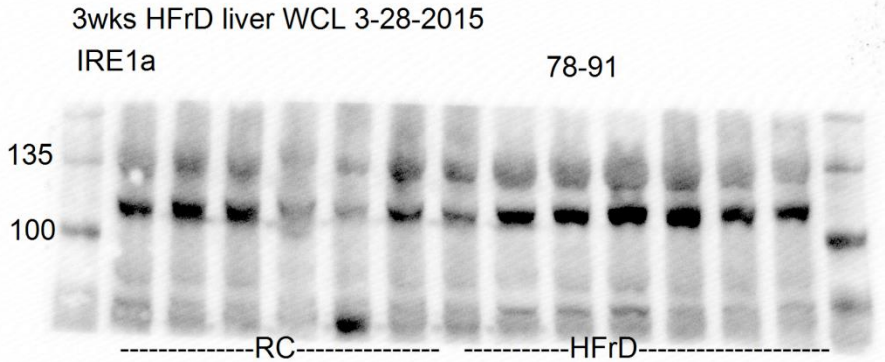


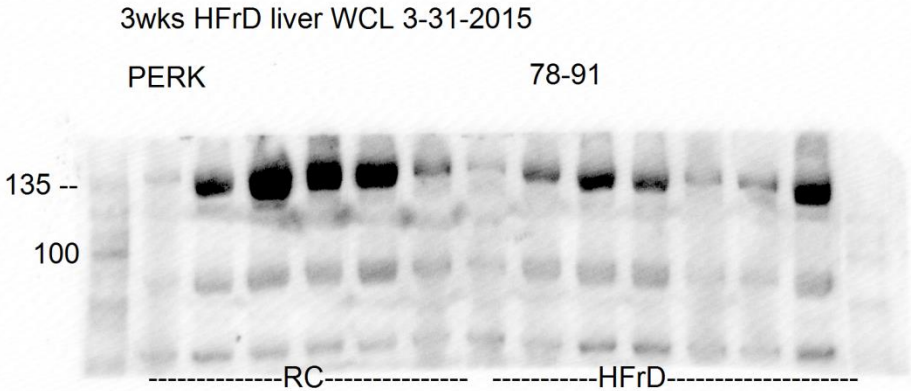


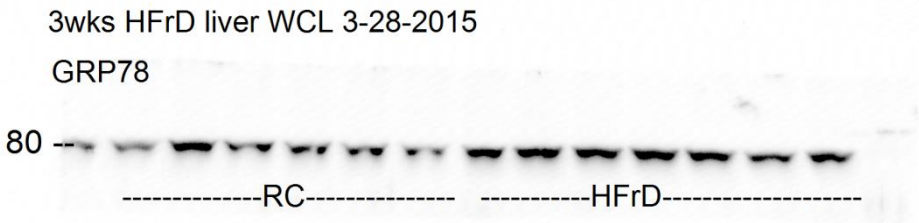


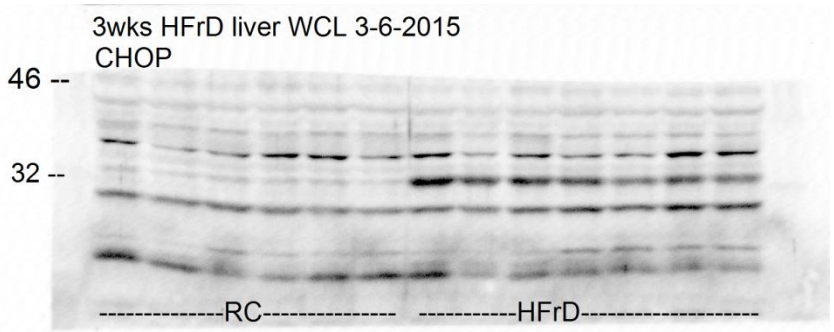


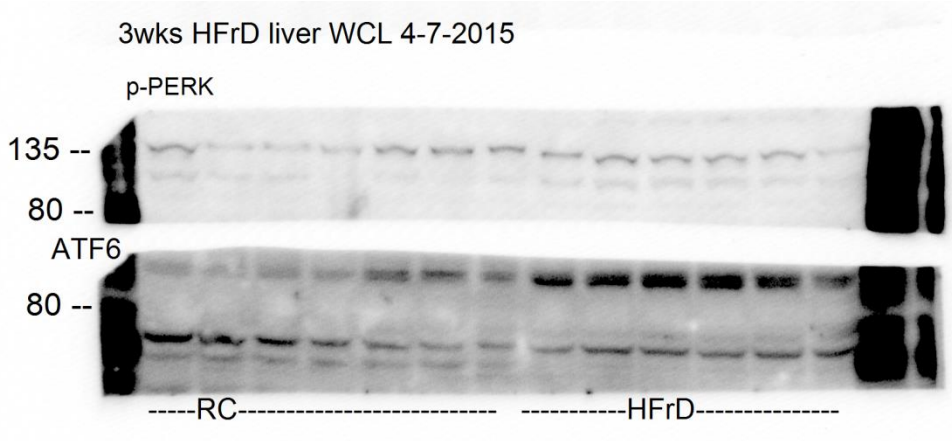
Full unedited gel for Figure S7A

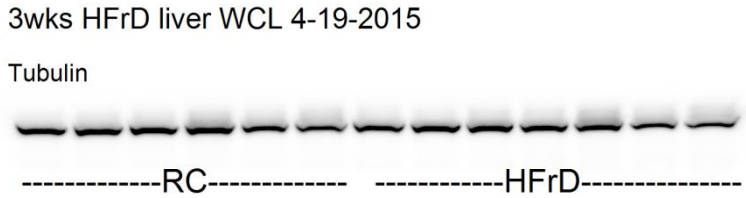




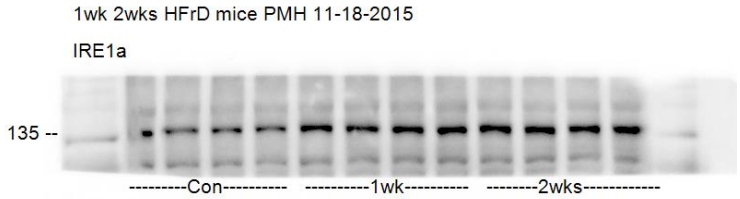


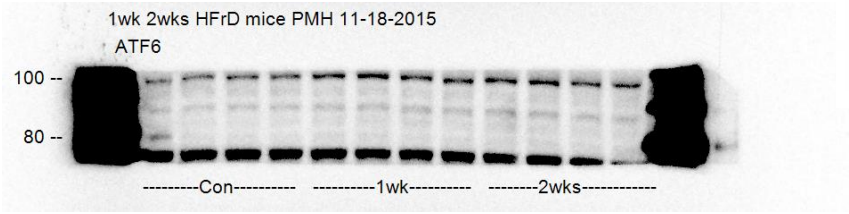


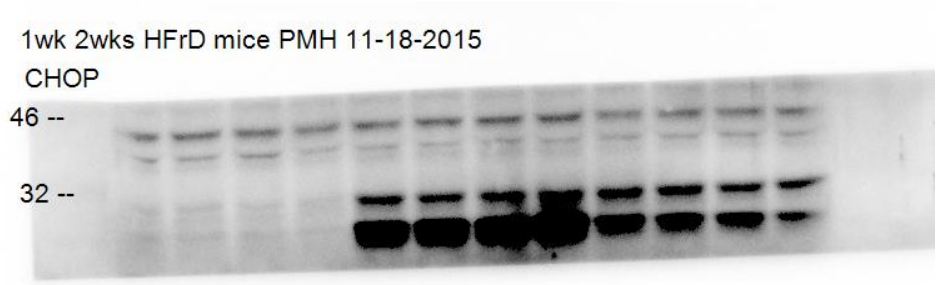


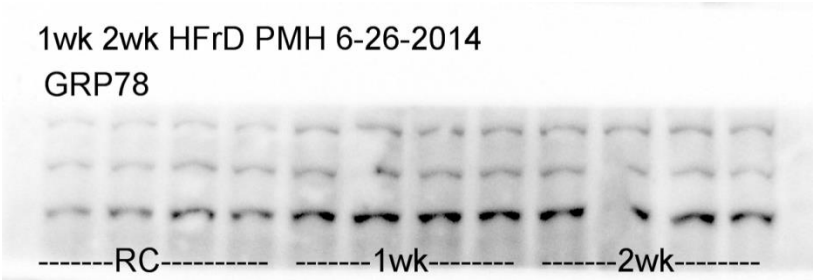


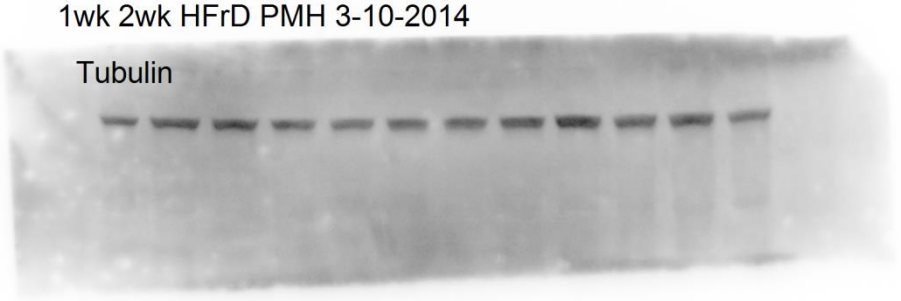
Full unedited gel for Figure S7 C



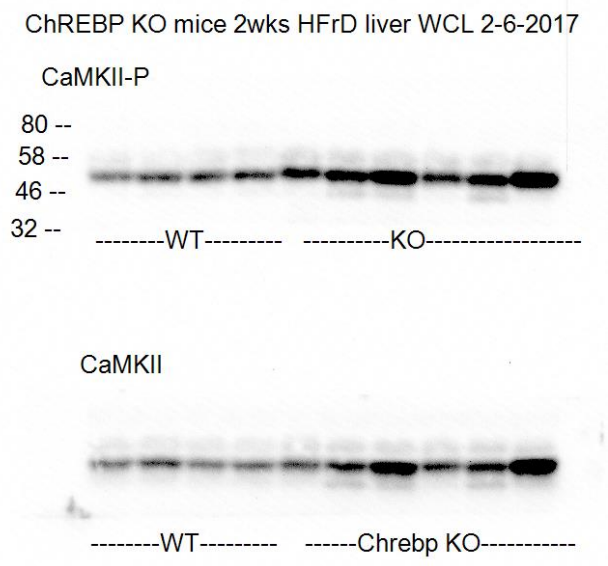








Full unedited gel for Figure S10 B



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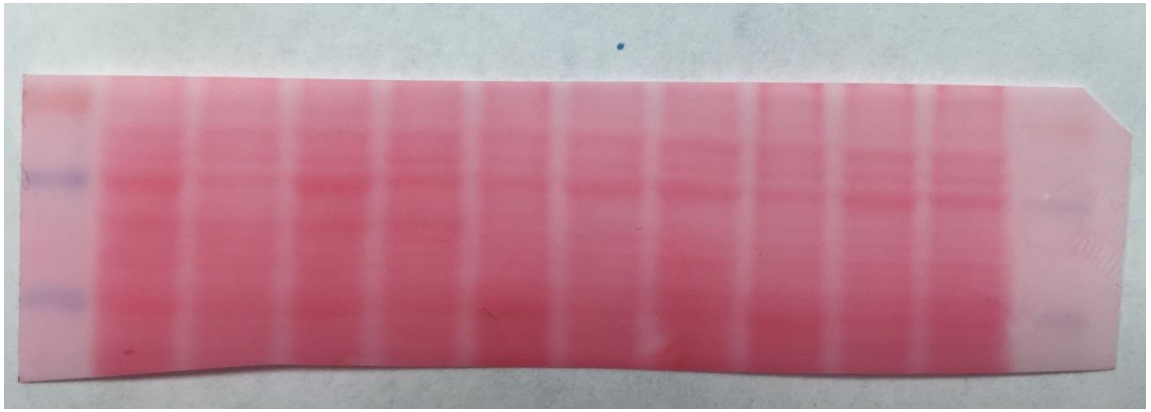
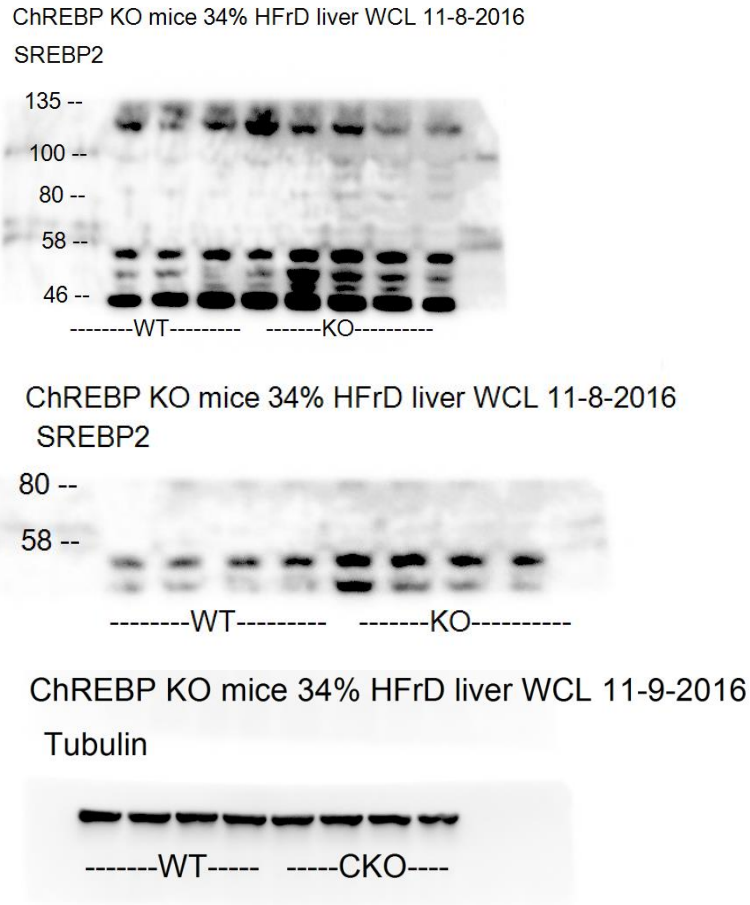


Full unedited gel for Figure S11 A

Full unedited gel for Figure S12 B



Full unedited gel for Figure S18