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(54) Title: ASSAYS FOR THE IDENTIFICATION OF COMPOUNDS THAT MODULATE LIPID HOMEOSTASIS

(57) Abstract: The invention describes the role of regulated IRE 1 -dependent decay (RIDD) in modulating lipid homeostasis by degradation of particular lipid metabolism genes and the role of XBPL in modulating lipid homeostasis by regulating the transcriptional activation of particular lipogenic target genes. Methods for identifying modulators of lipid homeostasis based on modulation of IRE 1 -mediated RIDD of lipid metabolism genes or based on modulation of XBPL -mediated transcriptional regulation of lipogenic target genes are provided.



ASSAYS FOR THE IDENTIFICATION OF COMPOUNDS THAT MODULATE LIPID HOMEOSTASIS

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Background of the Invention

In mammals, the liver is the principal organ that controls energy homeostasis through regulating carbohydrate and fatty acid metabolism. During starvation, the liver produces glucose to maintain circulating glucose levels by breaking down glycogen stores or by synthesizing glucose through gluconeogenesis. In contrast, ingestion of dietary carbohydrates promotes lipid synthesis in the liver to convert carbohydrates to triglyceride (TG) for long-term energy storage (see *e.g.*, Towle, H.C. *et al.* (1997) *Annu. Rev. Nutri.* 17:405-433). The incidence of metabolic syndrome, a condition characterized by the constellation of central obesity, dyslipidemia, hepatic steatosis, elevated blood glucose, and hypertension, continues to rise in industrialized nations (see *e.g.*, Mensah, G.A. *et al.* (2004) *Cardiol. Clin.* 22:485-504). Dyslipidemia, manifested by elevated levels of plasma TG and low density lipoprotein (LDL) cholesterol and low levels of high density lipoprotein (HDL) cholesterol, is a risk factor for coronary artery disease (see *e.g.*, Ginsberg, H.N. and Zhang, Y.L. (2006) *Obesity* (Silver Spring) 14 (Suppl. 1):41S-49S). Control of dyslipidemia in patients with coronary artery disease with statins and with triglyceride-lowering agents has resulted in measurable improvements in cardiovascular morbidity and mortality, suggesting clinical benefits of lowering hepatic *de novo* lipid synthesis (see *e.g.*, Brunzell, J.D. (2007) *N. Engl. J. Med.* 357:1009-1017).

The transcription factor XBP1 has been shown to be involved in the regulation of hepatic lipogenesis (for a review, see Glimcher, L.H. and Lee, A-H. (2009) *Ann. N. Y. Acad. Sci.* 1173(Suppl. 1):E2-E9). Deficiency of XBP1 in the liver in mice led to profound decreases in serum TG, cholesterol and free fatty acids without causing hepatic steatosis, and these low plasma lipid levels were primarily due to decreased *de novo* synthesis of lipids in the liver. Additionally, XBP1 protein expression is induced in the liver by a high carbohydrate diet and

directly controls the induction of critical genes involved in fatty acid synthesis (Lee, A-H. *et al.* (2008) *Science* 320:1492-1496). The mechanism by which XBP1 deficiency leads to hypolipidemia, however, remains unclear.

XBP1 is activated by a post-transcriptional modification of its mRNA by inositol requiring enzyme 1 (IRE1), a serine-threonine protein kinase and endoribonuclease (Calfon, M *et al.* (2002) *Nature* 415:92-96; Yoshida, H. *et al.* (2001) *Cell* 107:881-891). IRE1 (IRE1 α and IRE1 β) is an endoplasmic reticulum (ER) transmembrane protein that is involved in the unfolded protein response (UPR) that occurs in response to ER stress (see *e.g.*, Lee, A-H and Glimcher L.H. (2009) *Cell. Mol. Life Sci.* 66:2835-2850). It also has been shown that activated IRE1 degrades a subset of mRNAs in a process referred to as regulated IRE1-dependent decay (RIDD) (see *e.g.*, Hollier, J. *et al.* (2009) *J. Cell. Biol.* 186:323-331; Hur, K.Y. *et al.* (2012) *J. Exp. Med.* 209:307-318). The precise relationship between XBP1 and IRE1 and lipid homeostasis, however, remains unclear.

In view of the foregoing, screening assays based on elucidation of biological mechanisms involved in lipid homeostasis, in particular those involving the critical regulator protein XBP1, would be very useful and are still needed to facilitate identification of compounds that may be useful in the treatment of various disorders associated with dysregulation of lipid homeostasis.

Summary of the Invention

This invention provides screening assays based on elucidation of biological mechanisms involving XBP1 and IRE1 that are important in regulating lipid homeostasis. In particular, the invention described herein discloses the discovery that RIDD is a crucial control mechanism of lipid homeostasis. Suppression of RIDD by RNA interference or genetic ablation of IRE1 α reversed hypolipidemia in XBP1 deficient mice. Moreover, comprehensive microarray analysis of XBP1 and/or IRE1 α deficient liver identified genes involved in lipogenesis and lipoprotein metabolism as RIDD substrates, which may contribute to the suppression of plasma lipid levels by activated IRE1 α . Comprehensive microarray analysis also identified XBP1 dependent genes that represent lipogenic target genes whose transcription is directly regulated by XBP1. Additionally, ablation of XBP1 ameliorated hepatosteatosis, liver damage and hypercholesterolemia in dyslipidemic animal models, suggesting that direct targeting of either IRE1 α or XBP1 may be a feasible strategy to treat dyslipidemias.

Accordingly, in one aspect, the invention provides a method of identifying candidate modulators of lipid homeostasis comprising, a) determining the ability of a test compound to modulate regulated IRE1-dependent decay (RIDD) of a lipid metabolism gene, wherein mRNA of the lipid metabolism gene is degraded by IRE1; and b) selecting said test compound as a compound of interest when said compound modulates RIDD of the lipid metabolism gene, to thereby identify candidate modulators of lipid homeostasis.

In another aspect, the invention provides a method of identifying candidate modulators of lipid homeostasis comprising, a) determining the ability of a test compound to modulate XBP1-mediated transcription of a lipogenic target gene; and selecting said test compound as a compound of interest when said compound modulates XBP1-mediated transcription of the lipogenic target gene, to thereby identify candidate modulators of lipid homeostasis.

In one embodiment, modulation of lipid homeostasis comprises modulation of lipid metabolism. In another embodiment, modulation of lipid metabolism comprises induction of lipid clearance from blood. In another embodiment, modulation of lipid homeostasis comprises modulation of lipogenesis. In another embodiment, modulation of lipogenesis comprises induction of lipogenesis.

In one embodiment, the compound inhibits RIDD of mRNAs of lipid metabolism genes. In another embodiment, the compound stimulates RIDD of mRNAs of lipid metabolism genes. In another embodiment, the compound stimulates XBP1-mediated transcription of the lipogenic target gene. In another embodiment, the compound inhibits XBP1-mediated transcription of the lipogenic target gene.

In a preferred embodiment, the lipid metabolism gene or lipogenic target gene is a hepatic gene.

In one embodiment, the lipogenic target gene is selected from the group consisting of *Sdf2l1*, *Cflar*, *Dnajc3*, *Dnajb9*, *Gale*, *Nans*, *Edem1*, *Dnaljb11*, *Tmem39a*, *Erolb*, *Gmppa*, *Hyou1*, *Uso1*, *Sec24d*, *Slc35b1*, *Uggt1*, *Ssr3*, *Ubxn4*, *Txndc5*, *Stt3a*, *Ssr1*, *Serp1*, *Ddost*, *Sec61a1*, *Surf4*, *0610007L01Rik*, *Rrbp1*, *Ostc*, *Odc1*, *Cnpy3*, *Cdk5rap3*, *Fdps*, *Spcs3*, *P4hb*, *Spcs2*, *2810482107Rik*, *Sec61b*, *Lman2*, *Krtcap2*, *Sec11c*, *Sec61g*, *Ssr4*, *Sec63*, *H13*, *D17Wsu104e*, *Chidl1*, *Snd1*, *Ikbke*, *Tram1*, *Cbaral*, *Slc33a1*, *Pex11c*, *Mlec*, *Sqle*, *Cyp51*, *Tnfaip8l1*, *Pmvk*, *Fdtf1*, *Hsd17b7*, *Anubl1*, *Lifr*, *Sc4mol*, *Mvk*, and *Idi1*

In one embodiment, the lipid metabolism gene is selected from the group consisting of *Paqr7*, *Ptprf*, *Ces3*, *AU018778*, *Ces1*, *Ces1c*, *Ces1d*, *Ces1e*, *Ces1f*, *Ces1g*, *Lrp1*, *Bloc1s1*, *Dhcr7*, *Limd2*, *Idi1*, *2210411K11Rik*, *Es22*, *Apon*, *Glrx*, *Fads2*, *Hc*, *Oaf*, *Mvk*, *Gjbl*, *Sc4mol*, *Pla1a*, *F7*, *Cpn2*, *Pxmp2*, *Abca3*, *Gm2a*, *Kcnn2*, *Nsdh1*, *Stim2*, *Dnase2b*, *Vnn3*, *Hsd17b7*, *Hgsnat*, *Coll8a1*, *Aldoc*, *Fads1*, *Fn1*, *Smarcc2*, *Dera*, *Spp2*, *Angpt13*, *Atrnl1*, *Tpp1*, *Fam108a*, *Tmprss6*, *Sidt2*, *Es1*, *Dbt*, *Ropn1l*, *Tex264*, *Tapbp*, *Selk*, *Btbd1*, *Cyp2c68*, *Ndufa9*, *Pmvk*, *Blvra*, *Igf2r*, *Proc*, *Abca2*, *Taldo1*, *Bola3*, *Cyp1a2*, *Retsat*, *Lamp1*, *Rnf130*, *Tex2*, *Afm*, *Jkamp*, *0610007C21Rik*, *Plbd2*, *Cyp51*, *Tm7sf2*, *Clu*, *Hgfac*, *3110073H01Rik*, *Furin*, *C920025E04Rik*, *Serping1*, *Lipc*, *Ttc13*, *Coll8a1*, *Slc27a5*, *Pcsk6*, *Tmem208*, *Pm20d1*, *Sqle*, *Man2a2*, *Ppl1r15b*, *Azgp1*, *Ces6*, *Pla2g12b*, *llvbl*, *Adam9*, *Mlec*, *Pdap1*, *F2*, *Commd9*, *Itih4*, *Dgat2*, *Cyb561d2*, *Slc35f5*, *Alg1*, *Gnptg*, *Rarres1*, *Yif1a*, *Slc25a17*, *Mxd4*, *Fdps*, *Papola*, *Erp44* and *Rpn1*. In a preferred embodiment, the lipid metabolism gene is selected from the group consisting of *Ces1c*, *Ces1d*, *Ces1e*, *Ces1f*, *Ces1g* and *Angpt13*.

In one embodiment, the test compound is comprised in a combination of test compounds, such as a library of test compounds.

In one embodiment, determining the ability of a test compound to modulate RIDD of the mRNA of the lipid metabolism gene comprises contacting an indicator composition (*e.g.*, test cell, cell extract or assay composition) with the test compound and determining a level of lipid metabolism gene mRNA in the presence of the test compound. Typically, the indicator composition comprises IRE1 and mRNA of at least one lipid metabolism gene.

In another embodiment, determining the ability of a test compound to modulate RIDD of the lipid metabolism gene comprises contacting an indicator composition (*e.g.*, test cell, cell extract or assay composition) with the test compound and determining a level of lipid metabolism gene-encoded protein in the presence of the test compound. Typically, the indicator composition comprises IRE1 and mRNA of at least one lipid metabolism gene.

In another embodiment, determining the ability of a test compound to modulate XBP1-mediated transcription of the lipogenic target gene comprises contacting an indicator composition (*e.g.*, test cell, cell extract or assay composition) with the test compound and determining a level of lipogenic target gene mRNA in the presence of the test compound. Typically, the indicator composition comprises XBP1 and at least one gene encoding at least one lipogenic target gene.

In another embodiment, determining the ability of a test compound to modulate XBP1-mediated transcription of the lipogenic target gene comprises contacting an indicator composition (*e.g.*, test cell, cell extract or assay composition) with the test compound and determining a level of a reporter of lipogenic target gene transcription in the presence of the test compound. Typically, the indicator composition comprises XBP1 and at least one reporter of a lipogenic target gene.

In another embodiment, the test compound is further determined to modulate XBP1-mediated transcription of the lipogenic target gene independent of unfolded protein response (UPR) activation.

In another aspect, the invention provides a method for validating a compound as a compound useful in modulating lipid homeostasis *in vivo* comprising, selecting a candidate compound identified according to one of the screening methods described hereinbefore, and testing said candidate compound for modulation of lipid metabolism in an animal model of dyslipidemia. In various embodiments, the animal is tested for modulation of one or more of: plasma cholesterol levels, plasma and/or hepatic triglyceride levels, plasma and/or hepatic lipid levels, steatosis, and lipoprotein metabolism.

In yet another aspect, the invention provides a method for validating a compound as a compound useful in modulating lipid homeostasis *in vivo* comprising, selecting a candidate compound identified according to one of the screening methods described hereinbefore, and testing said candidate compound for modulation of lipid metabolism gene activity in an animal model deficient in XBP1 and/or IRE1 α .

Kits for carrying out the methods described herein are also encompassed by the invention.

Description of the Figures

Figures 1A-1E show results from experiments demonstrating partial restoration of plasma lipid levels by IRE1 α silencing in XBP1 deficient mice. Figure 1 A shows plasma TG levels of male mice with the indicated genotypes measured at fed state. Figure 1B shows cholesterol levels of male mice with the indicated genotypes measured at fed state. Figure 1C shows results from experiments in which Xbp1^{KLO} mice were i.v. injected with siRNAs targeting luciferase or

IRE1 α mRNA and plasma TG levels were measured at indicated time points. n = 8-9 per group. Figure 1D shows results from experiments in which Xbp1^{KLO} mice were i.v. injected with siRNAs targeting luciferase or IRE1 α mRNA and plasma cholesterol levels were measured at indicated time points, n = 8-9 per group. Figure 1E shows results from experiments in which Xbp1^{KLO} mice were sacrificed 2 or 8 days after siRNA or PBS injection and hepatic mRNA levels were measured by qRT-PCR. Values represent mean $\pm$ s.e.m. n = 2 (PBS), or 3-6 (siRNA). **P* <0.05, ***P* <0.01, ****P* <0.001.

Figures 2A-2D show results from experiments demonstrating plasma lipid levels in IRE1 α , and IRE1 α /XBP1 double deficient mice. Figure 2A shows plasma TG levels and Figure 2B shows plasma cholesterol levels that were measured before and 3 wks after poly (I:C) injection of male Ern1^{ff} and Ern1^{ff};Mx1-cre mice. Bloods were drawn at fed state. Figure 2C shows plasma TG levels and Figure 2D shows plasma cholesterol levels of Ern1^{ff};Xbp1^{ff} and Ern1^{ff};Xbp1^{ff};Mx1-cre mice before and after poly(I:C) injection.

Figures 3A-3C show results of experiments to determine the identification of direct XBP1 targets and RIDD substrates by microarray analysis. Figure 3A shows comparison of gene expression profiles between WT and Xbp1 Δ ; WT and Ire1 Δ ; untreated (NT) and tunicamycin-treated; siLuc and siIre1 injection to Xbp1^{LKO}. Shown are data for 237 genes that were decreased in Xbp1 Δ liver. Figure 3B shows mRNA levels of 64 genes that were suppressed in both Xbp1 Δ and Ire1 Δ liver in individual liver samples. Figure 3C shows mRNA levels of 112 genes that were induced by IRE1 α siRNA in Xbp1^{LKO} liver. Genes involved in protein folding processes in the ER are shown in blue. Lipid metabolism genes are highlighted in red.

Figures 4A-4I show the results of experiments to determine the identification of Ces1 and Angptl3 mRNAs as RIDD substrates. Figure 4A shows microarray signals for Ces1 genes. Figure 4B shows Western blot analysis of liver lysates of WT and Xbp1^{LKO} mice using TGH (*Ces1d*) antibody which also detects Es-X (*Ces1g*). Figure 4C shows Angptl3, -4, and -6 mRNA levels that were measured in the liver of WT and XBP1 deleted male mice. n = 4 per group. Figure 4D shows plasma Angptl3 protein levels in WT and Xbp1 Δ mice measured by ELISA. Figure 4E shows hepatic Angptl3 mRNA levels measured 8 days after siRNA injection of

Xbp1^{LKO} mice. n = 3-5 per group. Figure 4F shows plasma Angptl3 protein levels measured 4 or 8 days after siRNA injection to Xbp1^{LKO} mice. n = 3-5 per group. ****P* < 0.001 compared with the siLuc group. Figure 4G shows results from *in vitro* cleavage experiments in which *in vitro* transcribed Angptl3 mRNA was incubated with recombinant IRE1α and then resolved on an agarose gel. Angptl3mut was generated by changing the two G residues to C shown in bold face in Figure 4H. Figure 4H shows the predicted secondary structure of Angptl3 mRNA with a potential IRE1α cleavage site that is depicted by an arrow. Figure 4I shows results from *in vivo* cleavage experiments in which WT and mutant Angptl3 constructs were transfected into 293T cells together with WT or mutant IRE1α constructs. EGFP plasmid was also included in the transfection cocktail and served as a normalization control. Angptl3 mRNA levels in the transfected cells were measured by qRT-PCR.

Figures 5A-C show the results of experiments demonstrating the effects of XBP1 ablation in dyslipidemic mouse models. Figure 5A shows results of experiments in which metabolic parameters of 8-week old Xbp1^{f/f};ob/ob and Xbp1^{LKO};ob/ob mice measured after a 4-h fasting. n = 4-6 female mice per group. Figure 5b shows hepatic mRNA levels that were measured by qRT-PCR. Figure 5C shows results from experiments in which WT and Xbp1Δ mice were fed a high fat diet for 5 months, and then sacrificed to measure metabolic parameters. n = 9-10 male mice per group. ***P* < 0.01, ****P* < 0.001.

Figures 6A-6E show the results of experiments demonstrating ablation of XBP1 in apoE knock out mice reduces plasma lipids. Figures 6A-6B show results from experiments in which Apoe^{-/-};Xbp1^{f/f} and Apoe^{-/-};Xbp1^{f/f};Mx-cre mice were injected with poly(I:C) on day 0, day 4, week 12 and week 20. Bloods were drawn at fed state to measure plasma TG (Figure 6A) and plasma cholesterol levels (Figure 6B). n = 8-9 per group. ***P* < 0.01, ****P* < 0.001 compared with the control group (Apoe^{-/-};Xbp1^{f/f} mice). Figure 6C shows results from experiments in which plasma collected at weeks 8 and 17 were pooled and subjected to FPLC analysis. Figures 6D-6E show results from experiments in which descending aorta (Figure 6D) and aortic arch (Figure 6E) were stained en face with oil red O (ORO) and ORO positive areas were quantified.

Figures 7A-7I show results from experiments involving silencing of XBP1 mRNA *in vivo*. Figure 7A shows results from experiments in which female C57BL/6 mice were i.v. injected with PBS, luciferase siRNA (5 mg/kg), or varying doses of XBP1 siRNA. Mice were sacrificed 48 hrs later. Hepatic XBP1 mRNA levels were measured by qRT-PCR. $n = 4$ mice per group. *** $P < 0.001$ compared with the control groups (PBS and luciferase siRNA groups). Figure 7B shows results from a Western blot to measure XBP1s protein levels in pooled nuclear extracts. *non-specific band. Figure 7C shows results from experiments in which mice were injected with siRNAs at 7.5 mg/kg, and sacrificed after indicated days to measure hepatic XBP1s protein levels by western blot. SP1 serves as a loading control. $n = 3-4$ mice per group. Figure 7D shows results of qRT-PCR analysis of hepatic gene expression. $n = 3$ per group. Figure 7E shows results of Western blot analysis of IRE1 α in liver lysates. The panel shows IRE1 α phosphorylation measured by Phos-Tag western blot. Figures 7F-7I show the results of experiments in which Male C57BL/6 mice (Figures 7F-7G), or Apoe^{-/-} mice (Figures 7H-7I) were i.v. injected with siRNAs (7.5 mg/kg), and bled to measure plasma lipid levels. $n = 5-8$ per group. * $P < 0.05$, *** $P < 0.001$.

Figure 8 shows the expression of lipogenic genes in Xbp1^{LKO} mice determined by qRT-PCR. Values represent mean $\pm$ s.e.m. $n = 3-6$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 9A shows a schematic representation of Ces1 gene cluster.

Figure 9B shows an alignment of predicted amino acid sequences of mouse Ces1 proteins. Conserved sequences are highlighted in red.

Figures 10A-10F shows results from experiments demonstrating effects of XBP1 ablation in dyslipidemic mouse models. Figures 10A-10B show H&E staining of liver sections. Scale bar = 200 μ m. Figure 10C-10D shows results from experiments in which WT and Xbp1 Δ mice fed high fat diet for 4 months were subjected to glucose tolerance test (Figure 10C), and Insulin tolerance test (Figure 10D). Figure 10E shows the results of Western blot analysis and Figure 10F shows the results of qRT-PCR analysis of UPR markers in ob/ob mouse liver. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Detailed Description of the Invention

The present invention is based, at least in part, on the elucidation of biological mechanisms involving XBP1 and IRE1 that are important in regulating lipid homeostasis. In particular, the invention described herein discloses the discovery that RIDD is a crucial control mechanism of lipid homeostasis. Suppression of RIDD by RNA interference or genetic ablation of IRE1 α reversed hypolipidemia in XBP1 deficient mice. Moreover, comprehensive microarray analysis of XBP1 and/or IRE1 α deficient liver identified genes involved in lipogenesis and lipoprotein metabolism as RIDD substrates, which may contribute to the suppression of plasma lipid levels by activated IRE1 α . Comprehensive microarray analysis also identified XBP1 dependent genes that represent lipogenic target genes whose transcription is directly regulated by XBP1. Additionally, ablation of XBP1 ameliorated hepatosteatosis, liver damage and hypercholesterolemia in dyslipidemic animal models, suggesting that direct targeting of either IRE1 α or XBP1 may be a feasible strategy to treat dyslipidemias.

Various aspects of the invention are described in further detail in the following subsections:

I. Definitions

As used herein, the term "XBP1" refers to a X-box binding human protein that is a DNA binding protein and has an amino acid sequence as described in, for example, Liou, H-C. *et al.* (1990) *Science* 247:1581-1584 and Yoshimura, T. *et al.* (1990) *EMBO J.* 9:2537-2542, and other mammalian homologs thereof, such as described in Kishimoto T. *et al.*, (1996) *Biochem. Biophys. Res. Commun.* 223:746-751 (rat homologue). Exemplary proteins intended to be encompassed by the term "XBP1" include those having amino acid sequences disclosed in GenBank with accession numbers A36299 [gi:105867], NP.sub.--005071 [gi:4827058], P17861 [gi:139787], CAA39149 [gi:287645], and BAA82600 [gi:5596360] or *e.g.*, encoded by nucleic acid molecules such as those disclosed in GenBank with accession numbers AF027963 [gi:13752783]; NM.sub.--013842 [gi:13775155]; or M31627 [gi:184485]. XBP1 is also referred to

in the art as TREB5 or HTF (Yoshimura *et al.* 1990. EMBO Journal. 9:2537; Matsuzaki *et al.* 1995. J. Biochem. 117:303).

XBP1 is a basic region leucine zipper (b-zip) transcription factor isolated independently by its ability to bind to a cyclic AMP response element (CRE)-like sequence in the mouse class II MHC A α gene or the CRE-like site in the HTLV-1 21 base pair enhancer, and subsequently shown to regulate transcription of both the DR α and HTLV-1 ltr gene. Like other members of the b-zip family, XBP1 has a basic region that mediates DNA-binding and an adjacent leucine zipper structure that mediates protein dimerization. Deletional and mutational analysis identified transactivation domains in the C-terminus of XBP1 in regions rich in acidic residues, glutamine, serine/threonine and proline/glutamine.

There are two forms of XBP1 protein, unspliced and spliced, which differ markedly in their sequence and activity. Unless the form is referred to explicitly herein, the term "XBP1" as used herein includes both the spliced and unspliced forms. Spliced XBP1 protein directly controls the activation of the unfolded protein response (UPR), while unspliced XBP1 functions in this pathway only due to its ability to negatively regulate spliced XBP1.

As used herein, the term "spliced XBP1" refers to the spliced, processed form of the mammalian XBP1 mRNA or the corresponding protein. Human and murine XBP1 mRNA contain an open reading frame (ORF1) encoding bZIP proteins of 261 and 267 amino acids, respectively. Both mRNA's also contain another ORF, ORF2, partially overlapping but not in frame with ORF1. ORF2 encodes 222 amino acids in both human and murine cells. Human and murine ORF1 and ORF2 in the XBP1 mRNA share 75% and 89% identity respectively. In response to ER stress, XBP1 mRNA is processed by the ER transmembrane endoribonuclease and kinase IRE1 which excises an intron from XBP1 mRNA. In murine and human cells, a 26 nucleotide intron is excised. The boundaries of the excised introns are encompassed in an RNA structure that includes two loops of seven residues held in place by short stems. The RNA sequences 5' to 3' to the boundaries of the excised introns form extensive base-pair interactions. Splicing out of 26 nucleotides in murine and human cells results in a frame shift at amino acid 165 (the numbering of XBP1 amino acids herein is based on GenBank accession number NM.sub.--013842 [gi:13775155] and one of skill in the art can determine corresponding amino acid numbers for XBP1 from other organisms, *e.g.*, by performing a simple alignment). This causes removal of the C-terminal 97 amino acids from the first open reading frame (ORF1) and

addition of the 212 amino from ORF2 to the N-terminal 164 amino acids of ORF1 containing the b-ZIP domain. In mammalian cells, this splicing event results in the conversion of a 267 amino acid unspliced XBP1 protein to a 371 amino acid spliced XBP1 protein. The spliced XBP1 then translocates into the nucleus where it binds to its target sequences to induce their transcription. The nucleic acid and amino acid sequence of the spliced form of murine XBP1 are also shown in FIG. 8C and 8D, respectively, of US Publication No. 20040170622.

As used herein, the term "unspliced XBP1" refers to the unprocessed XBP1 mRNA or the corresponding protein. As set forth above, unspliced murine XBP1 is 267 amino acids in length and spliced murine XBP1 is 371 amino acids in length. The sequence of unspliced XBP1 is known in the art and can be found, *e.g.*, Liou, H-C. *et al.* (1990) *Science* 247:1581-1584 and Yoshimura, T. *et al.* (1990) *EMBO J.* 9:2537-2542, or at GenBank accession numbers NM.sub.--005080 [gi:14110394] or NM.sub.--013842 [gi:13775155]. The nucleic acid and amino acid sequence of the unspliced form of murine XBP1 are also shown in FIG. 8A and 8B, respectively, of US Publication No. 20040170622.

As used herein, the term "Unfolded Protein Response" (UPR) or the "Unfolded Protein Response pathway" refers to an adaptive response to the accumulation of unfolded proteins in the ER and includes the transcriptional activation of genes encoding chaperones and folding catalysts and protein degrading complexes as well as translational attenuation to limit further accumulation of unfolded proteins. Both surface and secreted proteins are synthesized in the endoplasmic reticulum (ER) where they need to fold and assemble prior to being transported.

As used herein, the term "IRE1" refers to an ER transmembrane endoribonuclease and kinase called "inositol requiring enzyme 1" which oligomerizes and is activated by autophosphorylation upon sensing the presence of unfolded proteins, see, *e.g.*, Shamu *et al.*, (1996) *EMBO J.* 15: 3028-3039. In *Saccharomyces cerevisiae*, the UPR is controlled by IREp. In the mammalian genome, there are two homologs of IRE1, IRE1 α and IRE1 β . IRE1 α is expressed in all cells and tissue whereas IRE1 β is primarily expressed in intestinal tissue. The endoribonucleases of either IRE1 α and IRE1 β are sufficient to activate the UPR. Accordingly, as used herein, the term "IRE1" includes, *e.g.*, IRE1 α , IRE1 β and IREp. In a preferred embodiment, IRE1 refers to IRE1 α .

IRE1 is a large protein having a transmembrane segment anchoring the protein to the ER membrane. A segment of the IRE1 protein has homology to protein kinases and the C-terminal

has some homology to RNases. Over-expression of the IRE1 gene leads to constitutive activation of the UPR. Phosphorylation of the IRE1 protein occurs at specific serine or threonine residues in the protein. IRE1 senses the overabundance of unfolded proteins in the lumen of the ER. The oligomerization of this kinase leads to the activation of a C-terminal endoribonuclease by trans-autophosphorylation of its cytoplasmic domains. IRE1 uses its endoribonuclease activity to excise an intron from XBP1 mRNA. Cleavage and removal of a small intron is followed by re-ligation of the 5' and 3' fragments to produce a processed mRNA that is translated more efficiently and encodes a more stable protein (Calton *et al.* (2002) *Nature* 415(3): 92-95). The nucleotide specificity of the cleavage reaction for splicing XBP1 is well documented and closely resembles that for IREp mediated cleavage of HAC1 mRNA (Yoshida *et al.* (2001) *Cell* 107:881-891). In particular, IRE1 mediated cleavage of murine XBP1 cDNA occurs at nucleotides 506 and 532 and results in the excision of a 26 base pair fragment for mouse XBP1. IRE1 mediated cleavage of XBP1 derived from other species, including humans, occurs at nucleotides corresponding to nucleotides 506 and 532 of murine XBP1 cDNA, for example, between nucleotides 502 and 503 and 528 and 529 of human XBP1.

As used herein, the term “lipid homeostasis” refers to the physiological processes and biological mechanisms and pathways involved in the maintenance of an internal metabolic equilibrium or steady-state of lipid within an organism or cell.

As used herein, the term “lipid metabolism” refers to the physiologic and metabolic processes involved in the assimilation of dietary lipids and the biosynthesis (anabolism) and degradation (catabolism) of lipids.

As used herein, the term “lipogenesis” refers to the process by which lipids (fats) are formed in the body, typically from glucose and other substrates, in particular the formation of fatty acids from acetyl coenzyme A. Lipogenesis encompasses the processes of fatty acid synthesis and subsequent triglyceride synthesis (when fatty acids are esterified with glycerol to form fats).

As used herein, the term “dyslipidemia” refers to an abnormal amount of lipids (*e.g.*, cholesterol, triglycerides, lipoproteins and/or other fats) in the blood circulation (*e.g.*, plasma levels). The term “hyperlipidemia” refers to a disorder of lipid metabolism that results in abnormally high levels of cholesterol, triglycerides, lipoproteins and/or other fats in the blood circulation (*e.g.*, plasma levels). Hyperlipidemia is a key contributor to various dyslipidemia

disorders including, but not limited, to atherosclerosis, coronary artery disease, peripheral vascular disease, obesity, metabolic syndrome, type II diabetes and pancreatitis.

As used herein, the term "lipid metabolism gene" refers to a gene, and its corresponding gene product, that is directly or indirectly involved in lipid metabolism.

As used herein, the term "lipogenic gene" refers to a gene, and its corresponding gene product, that is directly or indirectly involved in lipogenesis.

As used herein, the term "lipogenic target gene" refers to a gene, and its corresponding gene product, that is directly or indirectly involved in lipogenesis and that is a target for transcriptional regulation by XBP1.

As used herein, the term "regulated IRE1-dependent decay" or "RIDD" refers to a biological process of mRNA degradation that is mediated by IRE1, in particular the endonuclease activity of activated IRE1, as described in, for example, Hollier, J. *et al.* (2009) *J. Cell. Biol.* 186:323-331; Hur, K.Y. *et al.* (2012) *J. Exp. Med.* 209:307-318.

The various forms of the term "modulate" include stimulation (*e.g.*, increasing or upregulating a particular response or activity) and inhibition (*e.g.*, decreasing or downregulating a particular response or activity).

As used herein, the term "contacting" (*e.g.*, contacting a cell with a compound) is intended to include incubating the compound and the cell together *in vitro* (*e.g.*, adding the compound to cells in culture) or administering the compound to a subject such that the compound and cells of the subject are contacted *in vivo*. The term "contacting" is not intended to include exposure of cells to a lipid homeostasis modulator that may occur naturally in a subject (*i.e.*, exposure that may occur as a result of a natural physiological process).

As used herein, the term "compound of interest" or "test compound" or "candidate modulator" includes a compound that has not previously been identified as, or recognized to be, a modulator of lipid homeostasis.

The term "library of test compounds" is intended to refer to a panel comprising a multiplicity of test compounds.

In one embodiment, small molecules can be used as test compounds. The term "small molecule" is a term of the art and includes molecules that are less than about 7500, less than about 5000, less than about 1000 molecular weight or less than about 500 molecular weight. In one embodiment, small molecules do not exclusively comprise peptide bonds. In another

embodiment, small molecules are not oligomeric. Exemplary small molecule compounds which can be screened for activity include, but are not limited to, peptides, peptidomimetics, nucleic acids, carbohydrates, small organic molecules (*e.g.*, Cane *et al.* 1998. Science 282:63), and natural product extract libraries. In another embodiment, the compounds are small, organic non-peptidic compounds. In a further embodiment, a small molecule is not biosynthetic. For example, a small molecule is preferably not itself the product of transcription or translation.

As used herein, the term "indicator composition" refers to a composition that includes a protein of interest (*e.g.*, IRE1, such as IRE1 α , or XBP1), for example, a cell that naturally expresses the protein, a cell that has been engineered to express the protein by introducing an expression vector encoding the protein into the cell, or a cell free composition that contains the protein (*e.g.*, purified naturally-occurring protein or recombinantly-engineered protein).

As used herein, the term "cell free composition" refers to an isolated composition, which does not contain intact cells. Examples of cell free compositions include cell extracts and compositions containing isolated proteins.

As used herein, the term "reporter gene" refers to any gene that expresses a detectable gene product, *e.g.*, RNA or protein. Preferred reporter genes are those that are readily detectable. The reporter gene can also be included in a construct in the form of a fusion gene with a gene that includes desired transcriptional regulatory sequences or exhibits other desirable properties. Examples of reporter genes include, but are not limited to CAT (chloramphenicol acetyl transferase) (Alton and Vapnek (1979), Nature 282: 864-869) luciferase, and other enzyme detection systems, such as beta-galactosidase; firefly luciferase (deWet *et al.* (1987), Mol. Cell. Biol. 7:725-737); bacterial luciferase (Engbrecht and Silverman (1984), PNAS 1: 4154-4158; Baldwin *et al.* (1984), Biochemistry 23: 3663-3667); alkaline phosphatase (Toh *et al.* (1989) Eur. J. Biochem. 182: 231-238, Hall *et al.* (1983) J. Mol. Appl. Gen. 2: 101), human placental secreted alkaline phosphatase (Cullen and Malim (1992) Methods in Enzymol. 216:362-368) and green fluorescent protein (U.S. Pat. No. 5,491,084; WO 96/23898).

As used herein, the term "XBP1-responsive element" refers to a DNA sequence that is directly or indirectly regulated by the activity of the XBP1 (whereby activity of XBP1 can be monitored, for example, via transcription of a reporter gene).

As used herein, the term "cells deficient in XBP1" includes cells of a subject that are naturally deficient in XBP1, as well as cells of a non-human XBP1 deficient animal, *e.g.*, a

mouse, that have been altered such that they are deficient in XBP1. The term "cells deficient in XBP1" is also intended to include cells isolated from a non-human XBP1 deficient animal or a subject that are cultured *in vitro*.

As used herein, the term "non-human XBP1 deficient animal" refers to a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal, such that the endogenous XBP1 gene is altered, thereby leading to either no production of XBP1 or production of a mutant form of XBP1 having deficient XBP1 activity. Preferably, the activity of XBP1 is entirely blocked, although partial inhibition of XBP1 activity in the animal is also encompassed. The term "non-human XBP1 deficient animal" is also intended to encompass chimeric animals (*e.g.*, mice) produced using a blastocyst complementation system, such as the RAG-2 blastocyst complementation system, in which a particular organ or organs (*e.g.*, the lymphoid organs) arise from embryonic stem (ES) cells with homozygous mutations of the XBP1 gene.

As used herein, the term "cells deficient in IRE1" includes cells of a subject that are naturally deficient in IRE1, as well as cells of a non-human IRE1 deficient animal, *e.g.*, a mouse, that have been altered such that they are deficient in IRE1. The term "cells deficient in IRE1" is also intended to include cells isolated from a non-human IRE1 deficient animal or a subject that are cultured *in vitro*.

As used herein, the term "non-human IRE1 deficient animal" refers to a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal, such that the endogenous IRE1 gene is altered, thereby leading to either no production of IRE1 or production of a mutant form of IRE1 having deficient IRE1 activity. Preferably, the activity of IRE1 is entirely blocked, although partial inhibition of IRE1 activity in the animal is also encompassed. The term "non-human IRE1 deficient animal" is also intended to encompass chimeric animals (*e.g.*, mice) produced using a blastocyst complementation system, such as the RAG-2 blastocyst complementation system, in which a

particular organ or organs (*e.g.*, the lymphoid organs) arise from embryonic stem (ES) cells with homozygous mutations of the IRE1 gene.

II. Role of XBP1 and IRE1 α in Regulation of Lipid Homeostasis

The unfolded protein response (UPR) was originally identified as a signaling system that promotes the transcription of endoplasmic reticulum (ER) chaperone genes in response to stresses that burden the ER with increased client proteins for folding (Ron, D. and Walter, P. (2007) *Nat Rev Mol Cell Biol* 8:519-529; Schroder, M. and Kaufman, R.J. (2005) *Annu Rev Biochem* 74:739-789). In mammals, UPR is initiated by three families of unique ER transmembrane proteins, PERK, IRE1 (IRE1 α and IRE1 β), and ATF6 (ATF6 α and ATF6 β). IRE1 is evolutionarily well conserved in all eukaryotes from unicellular organisms to mammals, while the other UPR branches are present only in higher eukaryotes (Mori, K. *et al.* (1993) *Cell* 74:743-756; Ron, D. and Walter, P. (2007) *Nat Rev Mol Cell Biol* 8:519-529; Wang, X.Z. *et al.* (1998) *EMBO J* 17:5708-5717; Cox, J.S. *et al.* (1993) *Cell* 73:1197-1206).

XBP1 is the only known transcription factor downstream of IRE1 α that is activated through an unconventional mRNA splicing reaction. XBP1 activates the transcription of a variety of genes involved in protein secretory pathways (Acosta-Alvear, *et al.* (2007) *Mol Cell* 27:53-66; Shaffer, A.L. *et al.* (2004) *Immunity* 21:81-93; Lee, A.H. *et al.* (2003) *Mol Cell Biol* 23:7448-7459). In line with this, IRE1 α and XBP1 are required for the development, survival and the protein secretory function of some professional secretory cells (Lee, A.H. *et al.* (2005) *Embo J* 24:4368-4380; Reimold, A.M. *et al.* (2001) *Nature* 412:300-307; Kaser, A. *et al.* (2008) *Cell* 134:743-756; Huh, W.J. *et al.* (2010) *Gastroenterology* 139:2038-2049; Iwawaki, T. *et al.* (2010) *PLoS One* 5, e13052; Zhang, K. *et al.* (2005) *J Clin Invest* 115:268-281. In addition to activating XBP1, IRE1 α can also activate Jun N-terminal kinase (JNK) (Urano, F. *et al.* (2000) *Science* 287:664-666) and induce the degradation of certain mRNAs, a process known as regulated IRE1-dependent decay (RIDD) (Han, D. *et al.* (2009) *Cell* 138:562-575; Hollien, J. *et al.* (2009) *J Cell Biol* 186:323-331; Lipson, K.L. *et al.* (2008) *PLoS One* 3, e1648; Oikawa, D. *et al.* (2010) *Nucleic Acids Res* 38:6265-6273; Lee, A.H. *et al.* (2011) *Proc Natl Acad Sci U S A* 108:8885-8890). The physiological significance of RIDD was first explored in insect cells, where it was postulated to be a mechanism to reduce ER stress by limiting the entry of cargo

proteins to the ER, given the preferential degradation of mRNAs encoding secretory proteins by RIDD (Hollien, J. and Weissman, J.S. (2006) *Science* 313:104-107). Interestingly, in mammalian cells, IRE1 α appears to cleave mRNAs encoding not only secretory cargo proteins, but also ER resident proteins that serve in protein folding and secretory pathways. This has led to the hypothesis that IRE1 α might promote apoptosis under severe ER stress conditions by diminishing ER capacity to handle stress (Han, D. *et al.* (2009) *Cell* 138:562-575). The *in vivo* functions of RIDD are only beginning to be explored. We and others have demonstrated that IRE1 α degrades insulin mRNA as well as proinsulin-processing enzyme mRNAs, uncovering an important function of RIDD in insulin secretion from β cells (Han, D. *et al.* (2009) *Cell* 138:562-575; Lee, A.H. *et al.* (2011) *Proc Natl Acad Sci U S A* 108:8885-8890 Lipson, K.L. *et al.* (2008) *PLoS One* 3, e1648).

We previously reported that XBP1 ablation in the liver profoundly decreased plasma triglyceride (TG) and cholesterol levels in mice, revealing an important role for this factor in hepatic lipid metabolism (Lee, A.H. *et al.* (2008) *Science* 320:1492-1496). Contrary to our speculation that XBP1 deficiency might induce ER stress in hepatocytes, leading to decreased very-low-density lipoprotein (VLDL) secretion, XBP1 deficient hepatocytes did not exhibit morphological signs of ER dysfunction, defects in apoB100 secretion, TG accumulation, increased apoptosis, or activation of XBP1 independent UPR markers, arguing against the contribution of ER stress to the hypolipidemic phenotype of the mutant mice. Instead, we found that the expression of key lipogenic enzyme genes was reduced in XBP1 deficient liver. Some but not all of these genes were directly induced by XBP1s overexpression, indicating that XBP1 acts as a pro-lipogenic transcription factor.

While XBP1 plays an important role in hepatic lipid metabolism, several studies have also reported UPR activation in alcoholic and non-alcoholic fatty liver diseases (Ji, C. and Kaplowitz, N. (2003) *Gastroenterology* 124:1488-1499; Ozcan, U. *et al.* (2004) *Science* 306:457-461; Puri, P. *et al.* (2008) *Gastroenterology* 134:568-576), suggesting the presence of ER stress in these metabolic abnormalities (Hotamisligil, G.S. (2008) *Int J Obes (Lond)* 32 Suppl 7:S52-54). Although it remains unclear how lipids activate the UPR (or cause ER stress), the idea that ER stress contributes to the pathogenesis of dyslipidemic metabolic diseases remains an interesting one. Along this line, it has been shown that XBP1 increases insulin sensitivity in type

2 diabetes animal models by resolving ER stress (Park, S.W. *et al.* (2010) *Nat Med* 16:429-437; Winnay, J.N. *et al.* (2010) *Nat Med* 16:438-445; Zhou, Y. *et al.* (2011) *Nat Med* 17:356-365).

Although XBP1 ablation did not activate PERK or ATF6, it strongly activated its upstream enzyme, IRE1 α , indicating feedback regulation of IRE1 α activity by the abundance of its substrate XBP1s (Lee, A.H. *et al.* (2008) *Science* 320:1492-1496). Hyperactivated IRE1 α possesses ribonuclease activity to induce the degradation of certain mRNAs by RIDD, such as those encoding cytochrome P450 enzymes that carry out detoxification of xenobiotics (Hur, K.Y. *et al.* (2012) *J Exp Med.*). Here, we demonstrate that RIDD also plays an important role in hepatic lipid metabolism. Suppression of RIDD by IRE1 α siRNA markedly restored plasma TG and cholesterol levels in XBP1 knock out mice, along with the induction of lipid metabolism genes. Gene expression profiling revealed a group of lipid metabolism genes regulated by RIDD, which included *Angptl3* and the carboxylesterase 1 (*Ces1*) gene family, whose suppression decreases plasma lipids (Koishi, R. *et al.* (2002) *Nat Genet* 30:151-157; Romeo, S. *et al.* (2009) *J Clin Invest* 119:70-79; Musunuru, K. *et al.* (2010) *N Engl J Med* 363:2220-2227; Quiroga, A.D. and Lehner, R. (2011) *Trends Endocrinol Metab* 22:218-225). Contrary to the notion that XBP1 mitigates ER stress induced by fat accumulation, XBP1 ablation ameliorated hepatic steatosis in ob/ob mice, and liver damage induced by long-term high fat diet feeding, consistent with the distinct role of IRE1 α /XBP1 in lipid metabolism. We also demonstrate that siRNA-mediated silencing of XBP1 mRNA in the liver effectively lowers plasma lipids in mice, providing “proof of principle” that targeting XBP1 may be a viable approach to the treatment of dyslipidemias.

We have previously demonstrated that ablation of XBP1 in the liver resulted in a profound reduction of plasma TG and cholesterol unaccompanied by hepatic steatosis (Lee, A.H. *et al.* (2008) *Science* 320:1492-1496). Here, we demonstrate that IRE1 α hyperactivation and the consequent RIDD substantially contributes to the hypolipidemia phenotype of XBP1 deficient mice. XBP1 deficiency results in a feedback activation of IRE1 α , inducing the degradation of mRNAs of a cohort of lipid metabolism genes, such as *Dgat2*, *Acacb*, *Pcsk9*, *Angptl3* and *Ces1*, which regulate TG and cholesterol metabolism at multiple levels. *Dgat2* and *Acacb* encode key lipogenic enzymes; *Pcsk9* is involved in LDL clearance; *Angptl3* suppresses LPL mediated TG clearance; *Ces1* possesses TG hydrolyzing activity and is implicated in fatty acids mobilization from lipid droplets to nascent VLDL. Hence, suppression of these genes by IRE1 α is likely to contribute to the striking hypolipidemic phenotype mediated by liver-specific loss of XBP1.

IRE1 α regulates hepatic lipid metabolism via two distinct mechanisms. First, IRE1 α promotes the degradation of mRNAs encoding lipid metabolism genes such as *Dgat2*, *Acacb*, *Ces1*, *Angptl3*, and *Pcsk9*, which play important roles in de novo lipogenesis, hydrolysis of cholesterol ester and TG, and lipoprotein catabolism. Second, IRE1 α activates its downstream transcription factor XBP1, which can directly activate certain lipid metabolism genes. Hence, the combined effects of XBP1 deficiency coupled with IRE1 α hyperactivation causes a profound reduction of plasma lipids in XBP1 deficient mice. IRE1 α deficient mice are defective in both XBP1s-mediated activation of lipogenic genes and IRE1 α -mediated mRNA degradation. Under regular chow-fed conditions, IRE1 α activity in liver is low, exerting minimal effect on the levels of RIDD substrates, but producing measurable amounts of XBP1s protein, suggesting that the modest hypolipidemia of IRE1 α deficient mice is caused mainly by the lack of XBP1s.

In that case, how does XBP1s deficiency cause hypolipidemia independently of RIDD in IRE1 α deficient mice? We previously demonstrated that XBP1 directly activated *Dgat2* and *Acacb* lipogenic genes, which might contribute to the hypolipidemia observed in the absence of XBP1. However, these genes were not suppressed in IRE1 α deficient liver, but were induced by IRE1 α siRNA in XBP1 deficient liver, indicating that these genes are primarily regulated by RIDD. In contrast, microarray analysis identified various lipid metabolism genes such as *Fdps*, *Sqle*, *Cyp51*, *Pmvk*, *Fdft1*, *Hsd17b7*, *Sc4mol*, *Mvk*, and *Idi1* that were suppressed in both XBP1 and IRE1 α deficient liver, hence representing direct XBP1 targets. We speculate that XBP1 directly regulates the expression of a subset of lipid metabolism genes including those listed above, contributing to the hypolipidemia phenotype observed in IRE1 α deficient mice. It is also possible that the alteration of ER protein folding homeostasis caused by XBP1 deficiency indirectly affected lipid metabolism. For example, VLDL assembly occurs in the ER lumen and involves the folding and lipidation of apoB-100. Although apoB turnover was not substantially altered in XBP1 or IRE1 α deficient primary hepatocytes (Lee, A.H. *et al.* (2008) *Science* 320: 1492-1496; Zhang, K. *et al.* (2011) *EMBO J* 30:1357-1375), it is possible that XBP1 deficiency impairs VLDL assembly and/or secretion *in vivo*. Supporting this scenario, IRE1 α deficient mice were reported to be more susceptible to tunicamycin-induced hepatic steatosis (Zhang, K. *et al.* (2011) *EMBO J* 30:1357-1375), which is likely to be caused by the inhibition of hepatic VLDL

secretion (Liao, W. and Chan, L. (2001) *Biochem J* 353:493-501; Lee, A.H. and Glimcher, L.H. (2009) *Cell Mol Life Sci* 66:2835-2850).

It is notable that XBP1 ablation did not cause any deleterious effect on the viability of hepatocytes. On the contrary, XBP1 ablation in the liver protected mice from hepatic steatosis and liver damage caused by prolonged HFD feeding or by leptin deficiency (ob/ob mice). XBP1 ablation in the liver of ob/ob mice markedly ameliorated hepatic steatosis, paralleled by the suppression of the expression of lipogenic genes. Feeding a high-fat diet caused liver damage in WT mice, which was ameliorated in XBP1 deficient mice. Oxidative stress has been implicated in HFD-induced liver cell death (Anstee, Q.M. and Goldin, R.D. (2006) *Int J Exp Pathol* 87:1-16). It remains to be determined if XBP1 deficiency altered the generation of ROS, or the sensitivity of hepatocytes to oxidative damage.

The ER is a crucial subcellular compartment for many physiological functions of the liver, including lipogenesis, lipoprotein production, protein secretion, and detoxification of xenobiotics. One might speculate that an increased burden of lipids, proteins, and xenobiotics on the ER would impair ER function, evoking a stress response. Supporting this hypothesis, several papers have reported UPR activation in various liver diseases as a marker of ER stress (Hotamisligil, G.S. (2008) *Int J Obes (Lond)* 32 Suppl 7:S52-54; Ozcan, U. *et al.* (2004) *Science* 306:457-461; Puri, P. *et al.* (2008) *Gastroenterology* 134:568-576). Notably, it has been shown that TG accumulation in the liver in obesity causes ER stress, and the disruption of the UPR exacerbates the consequences of metabolic abnormalities such as insulin resistance (Hotamisligil, G.S. (2008) *Int J Obes (Lond)* 32 Suppl 7:S52-54; Ozcan, U. *et al.* (2004) *Science* 306:457-461; Park, S.W. *et al.* (2010) *Nat Med* 16:429-437; Winnay, J.N. *et al.* (2010) *Nat Med* 16:438-445; Zhou, Y. *et al.* (2011) *Nat Med* 17:356-365). In stark contrast, we failed to detect any measurable UPR activation (IRE1 α and PERK phosphorylation; ATF6 α processing; CHOP and BiP mRNA induction) in the liver of ob/ob mice, arguing against the involvement of the UPR in the metabolic abnormalities in this animal model. Our data are consistent with a recent report that demonstrated increased hepatic insulin sensitivity in XBP1 deficient mice, which was associated with decreased TG accumulation in the liver and plasma (Jurczak, M.J. *et al.* (2011) *J Biol Chem*). It is important to note that our data does not disprove the involvement of ER dysfunction in metabolic stress, given that we actually did not measure "ER stress" in ob/ob mice, which is currently not feasible. Although UPR activation has been used as a marker of ER

stress, the immediate upstream stimuli activating UPR in normal and diseased liver is not defined. It is possible that metabolic stresses compromise the ER function, without UPR activation.

XBP1 deficient liver displays a qualitatively and quantitatively normal lipid profile with no hepatic steatosis. This is in contrast to ApoB siRNA treated or Mttp mutant mice where lipid accumulates in the liver due to impaired VLDL assembly/secretion (Tadin-Strapps, M. *et al.* (2011) lipid research 52:1084-1097; Raabe, M. *et al.* (1999) J Clin Invest 103:1287-1298). Dramatic reduction of plasma lipids coupled with preservation of the normal hepatic lipid profile in XBP1 deficient mice suggests that XBP1 is a promising target for drug development to treat dyslipidemias. We demonstrated that XBP1 siRNA lowered plasma lipid levels both in WT C57BL/6 and apoE deficient mice, providing proof of principle for targeting XBP1 to treat dyslipidemias. Alternatively, small molecule compounds modulating IRE1 α could be useful to lower plasma lipid levels. Since genetic ablation of IRE1 α in the liver decreased plasma lipid levels, compounds that inhibit IRE1 α activity are expected to have similar lipid-lowering effects. IRE1 α activators could also decrease plasma lipid levels by promoting the degradation of mRNAs of lipid metabolism genes. IRE1 α activation would also induce XBP1s, which can promote lipogenesis. The effects of IRE1 α activation coupled with XBP1s induction on lipid homeostasis remain to be further investigated.

III. Screening Assays

Based on the biological roles of IRE1 and XBP1 in regulating lipid homeostasis through regulation of the expression and/or activity of various lipid metabolism and lipogenic genes, as demonstrated in the Examples, screening assays are provided that are useful in identifying compounds that can modulate lipid homeostasis, *e.g.*, lipid metabolism.

Accordingly, in one aspect, the invention pertains to a method of identifying candidate modulators of lipid homeostasis comprising, a) determining the ability of a test compound to modulate regulated IRE1-dependent decay (RIDD) of a lipid metabolism gene, wherein mRNA of the lipid metabolism gene is degraded by IRE1; and b) selecting said test compound as a compound of interest when said compound modulates RIDD of the lipid metabolism gene, to thereby identify candidate modulators of lipid homeostasis.

In another aspect, the invention pertains to a method of identifying candidate modulators of lipid homeostasis comprising, a) determining the ability of a test compound to modulate XBP1-mediated transcription of a lipogenic target gene; and selecting said test compound as a compound of interest when said compound modulates XBP1-mediated transcription of the lipogenic target gene, to thereby identify candidate modulators of lipid homeostasis.

In one embodiment, modulation of lipid homeostasis comprises modulation of lipid metabolism. In one embodiment, modulation of lipid metabolism comprises induction of lipid clearance from blood (*e.g.*, clearance of cholesterol, triglyceride and/or other lipids from the blood). In one embodiment, modulation of lipid homeostasis comprises modulation of lipogenesis. In one embodiment, modulation of lipogenesis comprises induction of lipogenesis. In another embodiment, modulation of lipogenesis comprises inhibition of lipogenesis.

In one embodiment, the compound identified in a method presented herein inhibits RIDD of mRNAs of lipid metabolism genes. In another embodiment, the compound identified in a method presented herein stimulates RIDD of mRNAs of lipid metabolism genes. In one embodiment, the compound identified in a method presented herein stimulates XBP1-mediated transcription of the lipogenic target gene. In another embodiment, the compound identified in a method presented herein inhibits XBP1-mediated transcription of the lipogenic target gene.

In one embodiment, the lipid metabolism gene or lipogenic target gene is a hepatic gene.

As described in Example 4, microarray analysis has led to the identification of a panel of hepatic genes, *e.g.*, lipogenic genes and/or other genes directly or indirectly involved in lipid metabolism, whose transcription is directly regulated by XBP1, as set forth in Figure 3B.

Accordingly, in one embodiment, the lipogenic target gene regulated by XBP1 and used in a method presented herein is selected from the group consisting of *Sdf2l1*, *Cflar*, *Dnajc3*, *Dnajb9*, *Gale*, *Nans*, *Edem1*, *Dnaljb11*, *Tmem39a*, *Ero1b*, *Gmppa*, *Hyou1*, *Uso1*, *Sec24d*, *Slc35b1*, *Uggt1*, *Ssr3*, *Ubxn4*, *Txndc5*, *Stt3a*, *Ssr1*, *Serp1*, *Ddost*, *Sec61a1*, *Surf4*, *0610007L01Rik*, *Rrbp1*, *Ostc*, *Odc1*, *Cnpy3*, *Cdk5rap3*, *Fdps*, *Spcs3*, *P4hb*, *Spcs2*, *2810482107Rik*, *Sec61b*, *Lman2*, *Krtcap2*, *Sec11c*, *Sec61g*, *Ssr4*, *Sec63*, *H13*, *D17Wsu104e*, *Chid1*, *Snd1*, *Ikbke*, *Tram1*, *Cbara1*, *Slc33a1*, *Pex11c*, *Mlec*, *Sqle*, *Cyp51*, *Tnfaip8l1*, *Pmvk*, *Fdtf1*, *Hsd17b7*, *Anubl1*, *Lifr*, *Sc4mol*, *Mvk*, and *Idi1*. This subset of genes represents the 64 genes set forth in Figure 3B.

In another embodiment, the lipogenic target gene regulated by XBP1 and used in a method presented herein is selected from the group consisting of *Dnajc3*, *Edem1*, *Dnaljb11*,

Erolb, Hyoul, Sec24d, Ugg1, Ssr3, Txndc5, Stt3a, Ssr1, Ddost, Sec61a1, Rrbp1, Spcs3, P4hb, Spcs2, Sec61b, Lman2, Sec11c, Sec61g, Ssr4, Sec63 and *Tram1*. This subset of genes represents the 24 genes involved in protein processing in the ER highlighted in Figure 3B.

In yet another embodiment, the lipogenic target gene regulated by XBP1 and used in a method presented herein is selected from the group consisting of *Fdps, Sqle, Cyp51, Pmvk, Fdtfl, Hsd17b7, Sc4mol, Mvk* and *Idi1*. This subset of genes represents the 9 genes involved in lipid metabolism highlighted in Figure 3B.

Also as described in Example 4, microarray analysis has led to the identification of a panel of hepatic genes, *e.g.*, genes directly or indirectly involved in lipid metabolism, whose expression and/or activity is directly regulated by RIDD mediated by IRE1, as set forth in Figure 3C. Accordingly, in one embodiment, the lipid metabolism gene regulated by IRE1 and used in a method presented herein is selected from the group consisting of *Paqr7, Ptprf, Ces3, AU018778, Ces1, Ces1c, Ces1d, Ces1e, Ces1f, Ces1g, Lrp1, Bloc1s1, Dhcr7, Limd2, Idi1, 2210411K11Rik, Es22, Apon, Glrx, Fads2, Hc, Oaf, Mvk, Gjbl, Sc4mol, Pla1a, F7, Cpn2, Pxmp2, Abca3, Gm2a, Kcnn2, Nsdh1, Stim2, Dnase2b, Vnn3, Hsd17b7, Hgsnat, Coll8a1, Aldoc, Fads1, Fn1, Smarcc2, Dera, Spp2, Angpt13, Atrnl1, Tpp1, Fam108a, Tmprss6, Sidt2, Es1, Dbt, Ropn11, Tex264, Tapbp, Selk, Btbd1, Cyp2c68, Ndufa9, Pmvk, Blvra, Igf2r, Proc, Abca2, Taldo1, Bola3, Cyp1a2, Retsat, Lamp1, Rnf130, Tex2, Afm, Jkamp, 0610007C21Rik, Plbd2, Cyp51, Tm7sf2, Clu, Hgf, 3110073H01Rik, Furin, C920025E04Rik, Serping1, Lipc, Ttc13, Coll8a1, Slc27a5, Pcsk6, Tmem208, Pm20d1, Sqle, Man2a2, Pp1r15b, Azgp1, Ces6, Pla2g12b, llvbl, Adam9, Mlec, Pdapl, F2, Commd9, Itih4, Dgat2, Cyb561d2, Slc35f5, Alg1, Gnptg, Rarres1, Yif1a, Slc25a17, Mxd4, Fdps, Papola, Erp44* and *Rpn1*.

In another embodiment, the lipid metabolism gene regulated by XBP1 and used in a method presented herein is selected from the group consisting of *Ces3, AU018778, Ces1, Lrp1, Dhcr7, Idi1, Es22, Apon, Fads2, Mvk, Gjbl, Sc4mol, Pla1a, Abca3, Gm2a, Nsdh1, Hsd17b7, Aldoc, Fads1, Dera, Angpt13, Es1, Pmvk, Abca2, Taldo1, Retsat, Plbd2, Cyp51, Tm7sf2, Lipc, Slc27a5, Sqle, Pla2g12b, Dgat2* and *Fdps*. This subset of genes represents the 35 genes involved in lipid metabolism highlighted in Figure 3C.

In yet another embodiment, the lipid metabolism gene regulated by XBP1 and used in a method presented herein is selected from the group consisting of *Ces1c, Ces1d, Ces1e, Ces1f,*

Ces1g and *Angplt3*. This subset of genes represents a particular group of RIDD substrates described further in Examples 5 and 6.

In one embodiment, the screening methods of the invention can be carried out by testing a single test compound in the method. Alternatively, the test compound is comprised in a combination of test compounds, such as a library of test compounds. In one embodiment, the test compound is a small molecule. In another embodiment, the test compounds is comprised in a library of small molecules. Suitable test compounds for use in the methods presented herein are described further in subsection IV below.

In one embodiment of the invention, the cell based and/or cell free assays are performed in a high-throughput manner. In one embodiment, the assays are performed using a 96-well format. In another embodiment, the assays of the invention are performed using a 192-well format. In another embodiment, the assays of the invention are performed using a 384-well format. In one embodiment, the assays of the invention are semi-automated, *e.g.*, a portion of the assay is performed in an automated manner, *e.g.*, the addition of various reagents. In another embodiment, the assays of the invention are fully automated, *e.g.*, the addition of all reagents to the assay and the capture of assay results are automated.

The assays of the invention generally involve contacting an assay composition with a test compound or a compound of interest or a library of compounds for a predetermined amount of time or at a predetermined time of growth (either *in vitro* or *in vivo*) and assaying for the effect of the compound on a particular read-out. In one embodiment, an assay composition is contacted with a compound of interest or a library of compounds for the duration of the assay. In another embodiment, an assay composition is contacted with a compound of interest or a library of compounds for a period of time less than the entire assay time period. For example, cells may be cultured for a period of days or weeks and may be contacted with a compound following, for example, 14 days in culture. In one embodiment, cells are contacted with a compound of interest for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 days. In one embodiment, assay compositions of the invention are contacted with a compound for a predetermined time period, the compound is removed, and the assay composition is maintained in the absence of the compound for a predetermined period prior to assaying for a particular read-out.

The compounds of the invention may be assayed at concentrations suitable to the assay and readily determined by one of skill in the art. For example in one embodiment, assay compositions are contacted with millimolar concentrations of compounds. In another embodiment, assay compositions are contacted with micromolar concentrations of compounds. In another embodiment, assay compositions are contacted with nanomolar concentrations of compounds.

To determine the ability of a test compound to modulate RIDD of a lipid metabolism gene, typically the test compound is contacted with an indicator composition that comprises IRE1, or a biologically active portion thereof, and at least one lipid metabolism gene, or mRNA thereof. The indicator composition can be, for example, a cell that comprises IRE1 and the lipid metabolism gene(s)/mRNA(s), a cell extract that comprises IRE1 and the lipid metabolism gene(s)/mRNA(s), or a cell-free composition that comprises IRE1 and the lipid metabolism gene(s)/mRNA(s). Preferably, the IRE1 is IRE1 α , such as recombinant IRE1 α protein. The ability of a test compound to modulate RIDD of a lipid metabolism gene can be measured by determining the effect of the test compound on the degree of RIDD (*i.e.*, mRNA degradation), mediated by IRE1, of mRNA of the lipid metabolism gene. This can be determined by comparing the amount of RIDD of mRNA of the lipid metabolism gene in the presence of the test compound and in the absence of the test compound. The level of mRNA of the lipid metabolism gene can be directly measured or, alternatively, the amount of protein product of the lipid metabolism gene can be determined as a measure of the level of mRNA of the lipid metabolism gene.

Thus, in one embodiment, determining the ability of a test compound to modulate RIDD of the lipid metabolism gene comprises contacting an indicator composition with the test compound and determining a level of lipid metabolism gene mRNA in the presence of the test compound. In another embodiment, determining the ability of a test compound to modulate RIDD of the lipid metabolism gene comprises contacting an indicator composition with the test compound and determining a level of lipid metabolism gene-encoded protein in the presence of the test compound. In another embodiment, an *in vitro* mRNA cleavage assay, such as that described in Example 6, is used to determine the ability of the test compound to modulate RIDD of the lipid metabolism gene mediated by IRE1.

To determine the ability of a test compound to modulate XBP1-mediated transcription of the lipogenic target gene, typically the test compound is contacted with an indicator composition that comprises XBP1, or a biologically active portion thereof, and at least one lipogenic target gene, or a reporter gene thereof. The indicator composition can be, for example, a cell that comprises XBP1 and the lipogenic target gene(s)/reporter(s), a cell extract that comprises XBP1 and the lipogenic target gene(s)/reporter(s), or a cell-free composition that comprises XBP1 and the lipogenic target gene(s)/reporter(s). Preferably, the XBP1 is spliced XBP1, such as recombinant spliced XBP1 protein. The ability of a test compound to modulate XBP1-mediated transcription of the lipogenic target gene can be measured by determining the effect of the test compound on the degree of lipogenic target gene transcription (*i.e.*, as measured by mRNA or gene product levels), mediated by XBP1. This can be determined by comparing the amount of XBP1-mediated transcription of the lipogenic target gene in the presence of the test compound and in the absence of the test compound. The level of mRNA of the lipogenic target gene can be directly measured or, alternatively, the amount of protein product of the lipogenic target gene can be determined as a measure of the level of mRNA of the lipogenic target gene. Additionally or alternatively, a reporter gene construct can be used in which the transcriptional regulatory region of the lipogenic target gene is operatively linked to a reporter gene whose gene product is readily measurable (such as, for example, CAT or luciferase). Cell-based and cell-free assay systems for measuring the transcriptional activity of XBP1 for a target gene, including systems using a reporter gene, are described further in US Patent Publication No. 20040170622, the entire contents of which is expressly incorporated herein by reference. The ordinarily skilled artisan can adapt such cell-based and cell-free XBP1 assay systems for use in the methods described herein using lipogenic target genes as described herein.

Thus, in one embodiment, determining the ability of a test compound to modulate XBP1-mediated transcription of the lipogenic target gene comprises contacting an indicator composition with the test compound and determining a level of lipogenic target gene mRNA in the presence of the test compound. In another embodiment, determining the ability of a test compound to modulate XBP1-mediated transcription of the lipogenic target gene comprises contacting an indicator composition with the test compound and determining a level of a reporter of lipogenic target gene transcription in the presence of the test compound. In yet another

embodiment, the test compound is further determined to modulate XBP1-mediated transcription of the lipogenic target gene independent of unfolded protein response (UPR) activation.

In another aspect, the invention pertains to methods of validating a compound as a compound useful in modulating lipid homeostasis *in vivo* using one or more animal model systems. For example, in one embodiment, the invention provides a method for validating a compound as a compound useful in modulating lipid homeostasis *in vivo* comprising, selecting a candidate compound identified according to a screening method as disclosed herein, and testing said candidate compound for modulation of lipid metabolism in an animal model of dyslipidemia. Such animal models of dyslipidemia are well established in the art, including but not limited to, leptin deficient ob/ob mice, mice fed a high-fat diet and ApoE deficient mice. In the validation method, the animal can be tested, for example, for modulation of one or more of plasma cholesterol levels, plasma and/or hepatic triglyceride levels, plasma and/or hepatic lipid levels, steatosis, and lipoprotein metabolism. Methods for testing these parameters in animal models are well established in the art and are also described further in the Examples.

In another embodiment, the invention provides a method for validating a compound as a compound useful in modulating lipid homeostasis *in vivo* comprising, selecting a candidate compound identified according to a screening method as presented herein, and testing said candidate compound for modulation of lipid metabolism gene activity in an animal model deficient in XBP1 and/or IRE1 α . In the validation method, the animal can be tested, for example, for modulation of one or more of plasma cholesterol levels, plasma and/or hepatic triglyceride levels, plasma and/or hepatic lipid levels, steatosis, and lipoprotein metabolism. Methods for testing these parameters in animal models are well established in the art and are also described further in the Examples.

IV. Test Compounds

A variety of test compounds can be evaluated using the screening assays described herein. The term "test compound" includes any reagent or test agent which is employed in the assays of the invention and assayed for its ability to modulate RIDD of a lipid metabolism gene mediated by IRE1 and/or its ability to modulate transcriptional activation of a lipogenic target gene mediated by XBP1. More than one compound, *e.g.*, a plurality of compounds, can be tested

at the same time for their ability to modulate RIDD of a lipid metabolism gene mediated by IRE1 and/or their ability to modulate transcriptional activation of a lipogenic target gene. The term "screening assay" preferably refers to assays which test the ability of a plurality of compounds to influence the readout of choice rather than to tests which test the ability of one compound to influence a readout. Preferably, the subject assays identify compounds not previously known to have the effect that is being screened for. In one embodiment, high throughput screening can be used to assay for the activity of a compound.

In certain embodiments, the compounds to be tested can be derived from libraries (*i.e.*, are members of a library of compounds). While the use of libraries of peptides is well established in the art, new techniques have been developed which have allowed the production of mixtures of other compounds, such as benzodiazepines (Bunin *et al.* (1992). *J. Am. Chem. Soc.* 114:10987; DeWitt *et al.* (1993). *Proc. Natl. Acad. Sci. USA* 90:6909) peptoids (Zuckermann. (1994). *J. Med. Chem.* 37:2678) oligocarbamates (Cho *et al.* (1993). *Science*. 261:1303-), and hydantoins (DeWitt *et al. supra*). An approach for the synthesis of molecular libraries of small organic molecules with a diversity of 10⁴-10⁵ has been described (Carell *et al.* (1994). *Angew. Chem. Int. Ed. Engl.* 33:2059-; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061-).

The compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the 'one-bead one-compound' library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K. S. (1997) *Anticancer Drug Des.* 12:145). Other exemplary methods for the synthesis of molecular libraries can be found in the art, for example in: Erb *et al.* (1994). *Proc. Natl. Acad. Sci. USA* 91:11422-; Horwell *et al.* (1996) *Immunopharmacology* 33:68-; and in Gallop *et al.* (1994); *J. Med. Chem.* 37:1233.

Libraries of compounds can be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner U.S. Pat. No. 5,223,409), spores (Ladner U.S. Pat. No. '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.*

(1990) Proc. Natl. Acad. Sci. 87:6378-6382); (Felici (1991) J. Mol. Biol. 222:301-310); In still another embodiment, the combinatorial polypeptides are produced from a cDNA library.

Exemplary compounds which can be screened for activity include, but are not limited to, peptides, nucleic acids, carbohydrates, small organic molecules, and natural product extract libraries.

Candidate/test compounds include, for example, 1) peptides such as soluble peptides, including Ig-tailed fusion peptides and members of random peptide libraries (see, *e.g.*, Lam, K. S. *et al.* (1991) Nature 354:82-84; Houghten, R. *et al.* (1991) Nature 354:84-86) and combinatorial chemistry-derived molecular libraries made of D- and/or L-configuration amino acids; 2) phosphopeptides (*e.g.*, members of random and partially degenerate, directed phosphopeptide libraries, see, *e.g.*, Songyang, Z. *et al.* (1993) Cell 72:767-778); 3) antibodies (*e.g.*, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, and single chain antibodies as well as Fab, F(ab')₂, Fab expression library fragments, and epitope-binding fragments of antibodies); 4) small organic and inorganic molecules (*e.g.*, molecules obtained from combinatorial and natural product libraries); 5) enzymes (*e.g.*, endoribonucleases, hydrolases, nucleases, proteases, synthetases, isomerases, polymerases, kinases, phosphatases, oxidoreductases and ATPases), and 6) mutant forms of KRC (*e.g.*, dominant negative mutant forms of the molecule).

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K. S. (1997) Anticancer Drug Des. 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) Proc. Natl. Acad. Sci. U.S.A. 90:6909; Erb *et al.* (1994) Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann *et al.* (1994) J. Med. Chem. 37:2678; Cho *et al.* (1993) Science 261:1303; Carrell *et al.* (1994) Angew. Chem. Int. Ed. Engl. 33:2059; Carrell *et al.* (1994) Angew. Chem. Int. Ed. Engl. 33:2061; and Gallop *et al.* (1994) J. Med. Chem. 37:1233.

Libraries of compounds can be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner U.S. Pat. No. 5,223,409), spores (Ladner U.S. Pat. No. '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382; Felici (1991) *J. Mol. Biol.* 222:301-310; Ladner *supra.*).

Compounds identified in the subject screening assays can be used in methods of modulating lipid homeostasis, for example in therapy for disorders associated with dyslipidemia, such as atherosclerosis, obesity, metabolic syndrome and type 2 diabetes. It will be understood that it may be desirable to formulate such compound(s) as pharmaceutical compositions prior to contacting them with cells.

Once a test compound is identified that directly or indirectly modulates lipid homeostasis by one of the variety of methods described hereinbefore, the selected test compound (or "compound of interest") can then be further evaluated for its effect on cells, for example by contacting the compound of interest with cells either *in vivo* (*e.g.*, by administering the compound of interest to a subject) or *ex vivo* (*e.g.*, by isolating cells from the subject and contacting the isolated cells with the compound of interest or, alternatively, by contacting the compound of interest with a cell line) and determining the effect of the compound of interest on the cells, as compared to an appropriate control (such as untreated cells or cells treated with a control compound, or carrier, that does not modulate the biological response).

V. Kits of the Invention

Another aspect of the invention pertains to kits for carrying out the screening assays of the invention. For example, in one embodiment, a kit for carrying out a screening assay of the invention can include an indicator composition comprising IRE1 (*e.g.*, recombinant IRE1 α or a cell extract comprising IRE1), means for measuring a readout (*e.g.*, mRNA levels of one or more lipid metabolism genes regulated by RIDD) and instructions for using the kit to identify modulators of lipid homeostasis. Additionally, the kit can include mRNA or means for preparing mRNA of one or more lipid metabolism genes regulated by RIDD.

In another embodiment, a kit for carrying out a screening assay of the invention can include an indicator composition comprising XBP1 (*e.g.*, recombinant XBP1 or a cell extract comprising XBP1), means for measuring a readout (*e.g.*, mRNA levels of one or more lipogenic target genes regulated by XBP1 or a reporter gene readout of one or more lipogenic target genes regulated by XBP1) and instructions for using the kit to identify modulators of lipid homeostasis. Additionally, the kit can include one or more lipogenic target genes, or one or more reporter genes of a lipogenic target gene(s) regulated by XBP1.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, *Molecular Cloning A Laboratory Manual*, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); *DNA Cloning*, Volumes I and II (D. N. Glover ed., 1985); *Oligonucleotide Synthesis* (M. J. Gait ed., 1984); Mullis *et al.* U.S. Pat. No. 4,683,195; *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); *Transcription And Translation* (B. D. Hames & S. J. Higgins eds. 1984); *Culture Of Animal Cells* (R. I. Freshney, Alan R. Liss, Inc., 1987); *Immobilized Cells And Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); *Methods In Enzymology*, Vols. 154 and 155 (Wu *et al.* eds.), *Immunochemical Methods In Cell And Molecular Biology* (Mayer and Walker, eds., Academic Press, London, 1987); *Handbook Of Experimental Immunology*, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents, and published patent applications cited throughout this application, as well as the figures and the sequence listing, are hereby incorporated by reference.

EXAMPLES

Example 1: Materials and Methodologies Used in the Examples

The following materials and methodologies were used in Examples 2-9.

Mice. Xbp1^{flox} mice were crossed with interferon inducible B6.Cg-Tg(Mx1-cre)1Cgn/J or C57BL/6-Tg(Alb-cre)21Mgn/J strains of mice (Jackson Laboratory) that produce cre recombinase under the control of the mouse albumin promoter and efficiently delete the floxed gene in the liver as previously described (Lee, A.H. *et al.* (2008) Science 320:1492-1496) Ern1^{flox} mice have been previously described (Iwawaki, T. *et al.* (2009) Proc Natl Acad Sci U S A 106:16657-16662), and crossed with Mx1-cre mice to inducibly delete IRE1 α in the liver. Mice were housed in a specific pathogen free facility at the Harvard School of Public Health and had free access to water and standard chow diet (PicoLab Rodent diet 20, #5058, Lab diet), which consisted of 12% fat, 23.5% protein, and 64.5% carbohydrate, or a high-fat diet (45% fat, 18.6% protein, and 36.4% carbohydrate; TestDiet, #58G8). Mice heterozygous for a leptin mutation (B6.V-Lep^{ob}/J) were obtained from The Jackson Laboratory, and intercrossed to produce homozygous ob/ob mice, or bred onto Xbp1^{LKO} mice to generate Xbp1^{LKO};ob/ob mice. Xbp1^{ff};Mx1-cre mice were crossed to ApoE^{-/-} mice (Jackson laboratory, B6.129P2-Apoe^{tm1Unc}/J), and injected with poly (I:C) to generate XBP1 and ApoE double deficient mice. Animal studies and experiments were approved and carried out according to the guidelines of the Animal Care and Use Committee of Harvard University.

In vivo siRNA delivery. XBP1 siRNA (sense, 5'-CACCCUGAAUUCAUUGUCU-3' (SEQ ID NO: 1); antisense, 5'-AGACAAUGAAUUCAGGGUG-3') (SEQ ID NO: 2) was formulated with lipidoid 98N₁₂-5 as described previously (Frank-Kamenetsky, M. *et al.* (2008) Proc Natl Acad Sci U S A 105:11915-11920). XBP1 and luciferase siRNAs (Frank-Kamenetsky, M. *et al.* (2008) Proc Natl Acad Sci U S A 105:11915-11920) were diluted in PBS and injected into mice via the tail vein at 5 μ l/g body weight. IRE1 α and control luciferase siRNAs formulated with lipidoid C12-200 were injected via tail vein as described previously (Love, K.T. *et al.* (2010) Proc Natl Acad Sci U S A 107:1864-1869; Hur, K.Y. *et al.* (2012) J Exp Med.).

Blood chemistry and measurement of liver TG contents. Plasma TG and cholesterol levels were measured by using commercial Triglyceride Reagent (Sigma) and the Amplex® Red Cholesterol Assay Kit (Invitrogen), respectively, following manufacturer's instructions. Plasma Angptl3 levels were measured using a commercial ELISA kit (R&D Systems). Fast performance liquid chromatography (FPLC) analysis of plasma samples was performed as described previously (Lee, A.H. *et al.* (2011) *Proc Natl Acad Sci U S A* 108:8885-8890). Serum alanine aminotransferase (ALT) activity was measured using a commercial reagent (Bioquant). Lipids were extracted from liver tissue by Folch's method, and subjected to TG assay.

RNA isolation, qRT-PCR and microarray analysis. Total RNA was extracted from liver using Qiazol lysis reagent (Qiagen), and reverse transcribed into cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qRT-PCR was performed as described previously (Lee, A.H. *et al.* (2008) *Science* 320:1492-1496). RNA samples from individual mice were further purified using RNeasy MinElute Cleanup Kit (Qiagen), and then used for the production of biotin-labeled cRNA followed by hybridization with HT MG-430 PM Array Plate (Affymetrix). 237 XBP1-dependent genes were identified by comparing WT and Xbp1Δ liver samples with minimum fold-change criterion of 1.6 and P-value ≤ 0.005. Relaxed criteria (P-value ≤ 0.05) were used for the comparison between WT and Ire1Δ; untreated and tunicamycin treated; luciferase and IRE1α siRNA.

Cell culture and Transfection. HEK293T cells were cultured in DMEM supplemented with 10% fetal bovine serum. Transient transfection was performed using Lipofectamine 2000 (Invitrogen), as described previously (Lee, A.H. *et al.* (2011) *Proc Natl Acad Sci U S A* 108:8885-8890).

Western blot. Liver tissues were homogenized in RIPA buffer (50 mM Tris-Cl pH 8.0, 150 mM NaCl, 1% NP40, 0.5% deoxycholate, 0.1% SDS, 50 mM NaF) supplemented with protease inhibitor tablet (Roche). Homogenates were centrifuged at 12,000g for 10 min at 4°C, and the supernatants were collected. Liver nuclear extracts were prepared as described previously (Lee, A.H. *et al.* (2008) *Science* 320:1492-1496). Liver lysates were used for western blotting as described previously (Hur, K.Y. *et al.* (2012) *J Exp Med.*).

In vitro IRE1 α -mediated mRNA cleavage assays Cleavage of *in vitro* transcribed Angptl3 mRNA by recombinant IRE1 α was tested as described previously (Lee, A.H. *et al.* (2011) Proc Natl Acad Sci U S A 108:8885-8890). Briefly, pCMV-SPORT6-Angptl3 (BC019491, Open Biosystems) plasmid was linearized by BglII digestion and incubated with SP6 polymerase to produce Angptl3 RNA. Angptl3mut construct was generated using QuikChange Site-Directed Mutagenesis Kit (Stratagene), based on the predicted mRNA secondary structure (<http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>). *In vitro* transcribed RNAs were incubated with the recombinant protein including the cytosolic domain of human IRE1 α , resolved on a 1.2% denaturing agarose gel and visualized by ethidium bromide staining.

Quantification of atherosclerosis lesions. The aortas were harvested and fixed in 10% buffered formalin solution overnight. Atherosclerotic lesions were analyzed in the aortic arch, and descending aorta by oil red O staining as previously described (Gotsman, I. *et al.* (2006) Circulation 114:2047-2055; Gotsman, I. *et al.* (2007) J Clin Invest 117:2974-2982).

Glucose tolerance test. Mice were fasted for 16 h with free access to water, and then i.p. injected with glucose at 1.5 g/Kg of body weight. Blood glucose levels were measured along the time course using an Ascensia Breeze glucometer (Bayer).

Insulin tolerance test. Mice were fasted for 6 h with free access to water, and then i.p. injected with insulin (Eli Lilly) at 0.75 units/kg body weight. Blood glucose levels were measured as above.

Histological analysis. Liver tissues were fixed in 10% neutral buffered formalin solution, processed into paraffin blocks, sectioned at 5 μ m, and stained with hematoxylin and eosin (H&E).

Example 2: Partial restoration of plasma lipid levels by suppression of IRE1 α in Xbp1 Δ mice

We previously demonstrated that an inducible deletion of Xbp1 in the liver decreased plasma cholesterol and TG levels in mice, while preserving normal hepatic lipid contents and composition (Lee, A.H. *et al.* (2008) Science 320:1492-1496). As XBP1 deficiency also resulted in constitutive activation of IRE1 α , which could potentially mediate XBP1-independent functions, we sought to determine the contribution of hyperactivated IRE1 α to the hypolipidemic phenotype of XBP1 deficient mice. Xbp1^{fl/fl};Alb-cre (Xbp1^{LKO}) mice which expressed Cre recombinase in postnatal liver under the control of the mouse albumin enhancer/promoter displayed markedly lower plasma TG and cholesterol levels compared to their Xbp1^{fl/fl} littermate controls (Figure 1A and 1B). Plasma lipid levels were not altered in heterozygous Xbp1^{fl/+};Alb-cre mice. Lipogenic enzyme genes such as *Dgat2*, *Acacb*, and *Scd1*, were suppressed in the liver of Xbp1^{LKO} mice (Figure 8), similar to what we observed in the inducible XBP1 deficient mouse strain (Lee, A.H. *et al.* (2008) Science 320:1492-1496).

To investigate the function of hyperactivated IRE1 α in hepatic lipid metabolism, we silenced IRE1 α expression in the liver of Xbp1^{LKO} mice using siRNA, as described elsewhere (Hur, K.Y. *et al.* (2012) J Exp Med.). IRE1 α siRNA markedly increased plasma TG and cholesterol levels in Xbp1^{LKO} mice, indicating that hyperactivated IRE1 α contributed to the reduction of plasma lipids in the setting of XBP1 deficiency (Figure 1C and 1D). Injection of IRE1 α siRNA into Xbp1^{LKO} mice significantly increased *Dgat2* and *Acacb* mRNAs, which play crucial roles in fatty acid metabolism and were decreased in Xbp1 deficient liver (Figure 1E and 1F). *Pcsk9*, which promotes the degradation of LDL receptor (Horton, J.D. *et al.* (2007) Trends Biochem Sci 32:71-77), was also induced by IRE1 α siRNA, suggesting that these mRNAs are degraded after cleavage by hyperactivated IRE1 α in XBP1 deficient liver.

Example 3: Modest hypolipidemia in IRE1 α deficient mice

Since IRE1 α silencing restored the plasma lipid concentrations to near normal levels in Xbp1 knock-out mice, we asked whether XBP1 regulates plasma lipid levels solely by

modulating IRE1 α activity or has a separate function in hepatic lipid metabolism. We sought to determine the relative contributions of XBP1 deficiency and IRE1 α hyperactivation to the dyslipidemia phenotype of Xbp1^{LKO} mice. It is notable that IRE1 α siRNA only partially restored plasma lipid levels in Xbp1^{LKO} mice (Figure 1), suggesting that XBP1 itself may also play a role in lipid metabolism independent of RIDD. XBP1 deficient mice with normal IRE1 α activity would reveal the direct role of XBP1 in hepatic lipid metabolism, but this was not a feasible approach. As an alternative, we examined IRE1 α deficient mice that do not produce the active XBP1s protein. IRE1 α deficient mice were generated by crossing Ern1^{flox} mice with Mx1-cre mice, which allowed inducible deletion of IRE1 α in the liver upon poly (I:C) administration. Without poly (I:C) administration, plasma TG and cholesterol levels were comparable between Ern1^{ff} and Ern1^{ff};Mx1-cre mice (Figure 2A and 2B). Notably, poly (I:C) administration significantly decreased both plasma TG and cholesterol levels in Ern1^{ff};Mx1-cre mice (Ern1 Δ), but not in the control Ern1^{flox/flox} mice, indicating that XBP1s is required to maintain optimal plasma lipid levels. Moreover, IRE1 α and XBP1 double-deficient mice exhibited modestly decreased plasma TG and cholesterol levels compared with WT littermates (Figure 2C and 2D), consistent with the results from IRE1 α siRNA-injected Xbp1^{LKO} mice (Figure 1C and 1D). These data suggest that the lack of XBP1s and the hyperactivation of IRE1 α both contribute to the dramatic decrease of plasma lipids observed in the setting of XBP1 deficiency and do so via separate mechanisms.

Example 4: Identification of direct XBP1 targets and RIDD substrates in liver

We next sought to identify lipid metabolism genes that are directly regulated by XBP1 and those regulated by RIDD. We reasoned that genes down-regulated in XBP1 deficient liver would fall into two groups: RIDD substrates and direct XBP1 targets. To identify RIDD substrate mRNAs and direct XBP1 targets in the liver, we performed a comprehensive microarray analysis of three groups of RNA samples: WT and XBP1 deficient mice, WT and IRE1 α deficient mice untreated or injected with tunicamycin, and XBP1 deficient mice injected with luciferase or IRE1 α siRNA. We identified 237 genes whose mRNA levels were significantly lower in XBP1 knockout than in the WT littermate controls. Among these XBP1-

dependent genes, 64 genes were also down regulated in IRE1 α deficient liver, hence representing likely direct XBP1 target genes (Figure 3B). A majority of these genes were induced by tunicamycin treatment in WT, but not in *Ern1* Δ mice, indicating that XBP1s directly activates their transcription (Figure 3A and 3B). Gene ontology analysis revealed that genes involved in “Protein processing in endoplasmic reticulum (KEGG mmu04141)” were highly enriched in this group, consistent with the critical function of XBP1 in protein secretory pathways (Shaffer, A.L. *et al.* (2004) *Immunity* 21:81-93; Lee, A.H. *et al.* (2003) *Mol Cell Biol* 23:7448-7459). Notably, the expression of a number of sterol biosynthesis-related genes such as *Fdps*, *Sqle*, *Cyp51*, *Pmvk*, *Fdft1*, *Hsd17b7*, *Sc4mol*, *Mvk*, and *Idi1* was also reduced in both XBP1 and IRE1 α deficient mice, and hence might contribute to the decreased lipid levels in these mutant mice.

Among the 237 genes that were suppressed in XBP1 deficient liver, 112 genes were induced by IRE1 α siRNA treatment in XBP1 deficient mice- these genes represent RIDD substrates (Figure 3C). Notably, several genes involved in lipid metabolism in addition to *Dgat2*, *Acacb* and *Pcsk9* were identified as RIDD substrates, likely contributing to the suppression of plasma lipid levels by activated IRE1 α . *Acacb* and *Pcsk9* were not included in the microarray analysis because of weak signals. Tunicamycin activates IRE1 α and hence would decrease the expression of RIDD substrates. Indeed, most of the genes that were suppressed by tunicamycin were induced by IRE1 α siRNA in XBP1 deficient liver. (Figure 3A). Among RIDD substrates, *Hsd17b7*, *Idi1*, *Mvk*, *Sc4mol* and *Pmvk*, which participate in sterol biosynthesis were suppressed in IRE1 α deficient mice, suggesting that XBP1 regulates these genes in both RIDD-dependent and -independent manners.

Example 5: Identification of *Ces1* and *Angptl3* mRNAs as RIDD substrates

A notable group of RIDD substrates was the carboxylesterase 1 gene family. The mouse genome contains eight highly homologous CES1-like genes in tandem, generated by gene duplication events during evolution (Figures 9A and 9B) (Holmes, R.S. *et al.* (2010) *Mamm Genome* 21:427-441). *Ces1* genes encode enzymes that possess TG and cholesterol-ester hydrolase activities, and have been implicated in the hydrolysis of neutral lipids stored in lipid droplet and VLDL formation (Parathath, S. *et al.* (2011) *J Biol Chem* 286:39683-39692;

Quiroga, A.D. and Lehner, R. (2011) Trends Endocrinol Metab 22:218-225; Wei, E. *et al.* (2010) Cell Metab 11:183-193). Notably, ablation of Ces3/TGH encoded by the *Ces1d* gene decreased plasma TG and cholesterol levels without causing steatosis (Wei, E. *et al.* (2010) Cell Metab 11:183-193). *Ces1c*, *Ces1d*, *Ces1e*, *Ces1f* and *Ces1g* mRNA levels were strikingly lower in XBP1 deficient, but not in IRE1 α deficient liver compared with WT controls (Figure 4A). Further, these mRNAs were induced by IRE1 α siRNA in Xbp1^{LKO} liver, and suppressed by tunicamycin treatment in WT mice. Western blot analysis of liver lysates revealed marked reduction of TGH (*Ces1d*) and Es-X (*Ces1g*) proteins in XBP1 deficient liver (Figure 4B). IRE1 α -induced degradation of Ces1 mRNAs may contribute to the hypolipidemic phenotype of XBP1 deficient mice.

Interestingly, angiopoietin-like protein 3 (*Angptl3*) mRNA was also identified as a RIDD substrate in the microarray analysis. ANGPTL3 protein is produced and secreted mainly by liver both in humans and mice, and possesses inhibitory activity toward lipoprotein lipase and endothelial lipase (Oike, Y. *et al.* (2005) Trends Mol Med 11:473-479). Accordingly, genetic loss of ANGPTL3 resulted in decreased plasma TG and cholesterol levels in both species (Koishi, R. *et al.* (2002) Nat Genet 30:151-157; Fujimoto, K. *et al.* (2006) Exp Anim 55:27-34; Shimamura, M. *et al.* (2007) Arterioscler Thromb Vasc Biol 27:366-372; Musunuru, K. *et al.* (2010) Engl J Med 363:2220-2227. qRT-PCR confirmed that *Angptl3* mRNA levels were markedly lower in XBP1 deficient liver compared with WT (Figure 4C). *Angptl4* and *Angptl6* mRNAs that are also abundantly produced in liver were not altered by XBP1 deficiency (Figure 4C). Consistent with the reduction of *Angptl3* mRNA by XBP1 deficiency, plasma *Angptl3* protein concentration was also markedly decreased in Xbp1 Δ mice (Figure 4D). Administration of IRE1 α siRNA into Xbp1^{LKO} mice significantly induced hepatic *Angptl3* mRNA and plasma *Angptl3* protein levels, indicating that hyperactivated IRE1 α reduces *Angptl3* expression in the liver (Figure 4E and 4F).

Example 6: Cleavage of *Angptl3* mRNA by IRE1 α

To directly demonstrate that *Angptl3* mRNA is cleaved by IRE1 α , we performed *in vitro* cleavage assays using recombinant IRE1 α . *Angptl3* RNA synthesized *in vitro* was efficiently cleaved by IRE1 α into two smaller fragments (Figure 4G). Analysis of the mRNA secondary

structure predicted the presence of a hairpin structure in Angptl3 mRNA, which was similar to the IRE1 α cleavage site in XBP1 mRNA (Figure 4H). Mutation of the potential cleavage site abolished the cleavage of Angptl3 mRNA by IRE1 α , demonstrating that IRE1 α recognizes the hairpin structure and cleaves Angptl3 mRNA (Figure 4G). To further validate the IRE1 α cleavage site in Angptl3 mRNA, we transfected 293T cells with WT or cleavage site mutant Angptl3 expression plasmids together with IRE1 α , and measured the mRNA levels. IRE1 α is activated by overexpression in 293T cells and reduced the expression of WT, but not the mutant Angptl3 mRNA (Figure 4I). Given the critical role of Angptl3 in the catabolism of lipoprotein particles (Lichtenstein, L. and Kersten, S. (2010) *Biochim Biophys Acta* 1801:415-420), IRE1 α -induced degradation of Angptl3 mRNA may contribute to the hypolipidemia phenotype of XBP1 deficient mice.

Example 7: Ablation of XBP1 ameliorates hepatic steatosis and liver damage in dietary and genetic animal disease models

IRE1 α and XBP1 activate hepatic lipid metabolism via transcriptional and post-transcriptional regulation of genes in lipid metabolic pathways. However, the IRE1 α /XBP1 signaling pathway is also considered to have a cytoprotective role against ER stress, which has been implicated in metabolic abnormalities in various organs (Cnop, M. *et al.* (2011) *Trends Mol Med.*; Ron, D. and Walter, P. (2007) *Nat Rev Mol Cell Biol* 8:519-529). Several reports have demonstrated that fat accumulation causes ER stress in liver (Sha, H. *et al.* (2011) *Trends Endocrinol Metab* 22:374-381; Ozcan, U. *et al.* (2004) *Science* 306:457-461; Puri, P. *et al.* (2008) *Gastroenterology* 134:568-576; Nakatani, Y. *et al.* (2005) *J Biol Chem* 280:847-851). We asked whether XBP1 deficiency would ameliorate fat accumulation in liver and blood under metabolic stress conditions, given its lipid-lowering effect, or would instead heighten ER stress and lipotoxicity, leading to increased cell death. We investigated the effects of XBP1 deficiency in two dyslipidemic animal models: leptin deficient ob/ob mice, and mice fed a high-fat diet.

Ablation of Xbp1 in the liver of ob/ob mice was achieved by generating compound mutant mice (Xbp1^{LKO};ob/ob) harboring a homozygous Xbp1 flox allele, Alb-cre transgene, and ob/ob mutation. Compared with the Xbp1^{fl/fl};ob/ob mice littermate control, Xbp1^{LKO};ob/ob mice displayed markedly lower hepatic TG and plasma cholesterol levels (Figure 5A and Figure 10),

associated with decreased lipogenic gene mRNA levels (Figure 5B), similar to $Xbp1^{LKO}$ mice. Plasma ALT levels were comparable between $Xbp1^{ff};ob/ob$ and $Xbp1^{LKO};ob/ob$ mice, indicating that the ablation of $Xbp1$ did not augment liver damage in ob/ob mice (Figure 5A). CHOP, GADD34, *Herpud1* and BiP mRNAs which are strongly induced under ER stress conditions by the PERK and ATF6 pathways were not induced in $Xbp1^{LKO};ob/ob$ mice (Figure 5B), suggesting that XBP1 ablation did not induce measurable ER stress in ob/ob mouse liver. Furthermore, contrary to the notion that fat accumulation induces ER stress in liver, we failed to detect any measurable induction of UPR markers such as phospho-IRE1 α , phospho-PERK, phospho-eIF2 α , processed nuclear ATF6 α and XBP1s proteins, and BiP, CHOP and ERdj4 mRNAs in ob/ob mouse liver (Figure 10), suggesting that the causal relationship between fat accumulation and ER stress induction in liver should be reassessed.

Feeding a high-fat diet increased plasma TG in $Xbp1\Delta$ mice to a level similar to WT mice (Figure 5C). However, plasma cholesterol levels were lower in HFD fed $Xbp1\Delta$ mice than in WT mice. Interestingly, the plasma ALT level was significantly lower in $Xbp1\Delta$ compared to WT mice (Figure 5C), indicating decreased hepatotoxicity in the absence of XBP1. We did not observe any differences in glucose tolerance and insulin tolerance tests between WT and $Xbp1\Delta$ mice under HFD conditions (Figure 10).

Example 8: Ablation of XBP1 in ApoE deficient mice can significantly decrease plasma cholesterol

ApoE deficient mice display high levels of plasma cholesterol and are widely used as a model for atherosclerosis (Zadelaar, S. *et al.* (2007) *Arterioscler Thromb Vasc Biol* 27:1706-1721). To test whether XBP1 ablation could lower cholesterol levels and reduce atherosclerotic lesion development under such extreme conditions, we generated $ApoE^{-/-};Xbp1^{ff};Mxcre$ mice that harbored a poly (I:C)-inducible cre recombinase transgene. Poly (I:C) administration activated the Cre and ablated XBP1 to generate ApoE and XBP1 double knock out ($ApoE^{-/-};Xbp1\Delta$) mice. Inducible XBP1 deletion in $ApoE^{-/-}$ mice significantly decreased plasma TG and cholesterol levels, although the latter still remained substantially elevated at >200 mg/dL (Figure 6A and 6B). FPLC analysis of the lipoprotein profile demonstrated that the cholesterol distribution was not altered by XBP1 ablation in $ApoE^{-/-}$ mice (Figure 6C). Not surprisingly,

despite reductions in plasma cholesterol and TG levels in ApoE^{-/-};Xbp1Δ compared with ApoE^{-/-} mice, atherosclerotic lesion formation was not significantly different between these two groups, as measured by the oil red O stained areas in the descending aorta (Figure 6D) and aortic arch (Figure 6E). Thus the 30-40% reduction in plasma cholesterol levels achieved by XBP1 ablation, while very significant, was not sufficient to prevent lesion formation in the setting of ApoE deficiency.

Example 9: Effect of XBP1 siRNA on plasma lipids

In recent years, RNA interference has received considerable attention as a novel therapeutic strategy, as sequence specific siRNA molecules can target virtually any disease-associated gene with high specificity (Vaishnaw, A.K. *et al.* (2010) *Silence* 1, 14). XBP1 is an attractive target for RNA therapeutics, given the profound reduction of plasma TG and cholesterol by XBP1 ablation. We investigated if the targeted delivery of XBP1 siRNA to the liver would decrease plasma lipid levels. Intravenous injection of lipidoid formulated XBP1 siRNA decreased XBP1 mRNA and protein levels in the liver in a dose-dependent manner, resulting in near complete suppression of XBP1s protein at doses > 5 mg/kg (Figure 7A and 7B). It is notable that a intermediate dose of 2.5 mg/kg siRNA had no effect on XBP1s protein level, despite ~50% reduction of XBP1 mRNA, indicating precise regulation of IRE1α activation to maintain constant XBP1s protein levels in the liver. Silencing of XBP1s expression was stably maintained for more than a week after a single injection of XBP1 siRNA at 7.5 mg/kg (Figure 7C). Similar to the effect of genetic ablation of XBP1, XBP1 siRNA induced IRE1α hyperactivation and decreased the expression of genes involved in lipid metabolism, similar to what we observed in XBP1 knock-out mice (Figure 7D and 7E). XBP1 siRNA transiently decreased plasma TG and cholesterol levels not only in C57BL/6 mice (Figure 7F and 7G), but also in apoE deficient mice (Figure 7H and 7I), indicating that XBP1 siRNA can efficiently reduce even very elevated plasma lipid levels.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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Table S1. Genes that are suppressed in XBP1 deficient liver.

Probe	Gene	Symbol	Signal intensity																				Fold change										P value									
			wt-1	wt-2	wt-3	wt-4	wt-5	wt-6	wt-7	wt-8	wt-9	wt-10	wt-11	wt-12	wt-13	wt-14	wt-15	wt-16	wt-17	wt-18	wt-19	wt-20	wt-21	wt-22	wt-23	wt-24	wt-25	wt-26	wt-27	wt-28	wt-29	wt-30										
140806	Ubat		192	223	270	118	50	49	68	51	56	57	52	43	41	33	49	47	238	313	515	230	2450	5567	5004	4871	69	275	73	2233	1923	2393	2685	0.27	0.92	16.11	16.62	0.43	0.0039	0.3350	0.0000	0.1141
141870	Sdf2l1		179	167	169	156	54	34	70	45	32	28	34	19	34	37	32	35	151	196	254	94	2531	2271	2318	1961	63	62	61	1381	1321	1607	1401	0.30	1.23	13.07	13.01	0.36	0.0000	0.1177	0.0000	0.0399
144931	Ctfr	Wt	28	30	36	28	21	13	18	19	28	21	22	26	30	32	25	27	42	42	54	47	395	408	366	347	26	27	23	72	55	59	86	0.59	1.18	8.38	2.52	0.56	0.0013	0.1148	0.0000	0.0032
141719	Dnao9		241	288	223	157	63	88	70	77	57	62	58	68	58	58	52	74	196	131	192	266	1332	1611	1641	1472	52	111	51	250	272	243	316	0.35	0.99	7.97	1.80	0.36	0.0017	0.8899	0.0000	0.0191
142416	Gale		17	17	18	15	12	9	10	7	9	8	8	7	6	8	17	17	12	10	125	112	63	93	9	8	9	6	6	6	6	9	0.60	0.85	6.92	0.74	0.61	0.0002	0.1337	0.0009	0.0482	
143369	Hsl		20	19	22	16	11	10	12	11	12	12	12	8	10	9	8	10	12	14	18	10	79	104	89	98	9	8	10	14	15	15	0.57	0.86	6.54	1.63	0.63	0.0006	0.2551	0.0000	0.0699	
141777	Nam		44	38	47	41	20	18	28	19	28	30	30	26	22	21	21	28	58	64	76	64	444	412	398	421	26	36	17	39	52	59	83	0.50	0.81	6.36	2.21	0.40	0.0005	0.0540	0.0000	0.0015
144932	Dnao3		911	918	958	827	501	463	536	428	285	274	302	282	286	324	244	250	529	461	724	581	8648	8836	9789	3697	446	468	377	1687	1851	1751	1950	0.51	0.97	6.00	4.30	0.69	0.0000	0.3367	0.0000	0.0178
145311	Ecm1		133	173	167	91	68	69	63	68	64	57	48	53	63	75	54	65	165	112	135	101	763	727	757	663	71	88	69	57	274	286	341	0.54	1.17	5.64	3.82	0.59	0.0030	0.1539	0.0000	0.0270
142434	Erla		102	99	94	98	70	53	62	50	80	57	70	55	61	55	57	64	120	73	101	105	504	625	481	523	78	67	73	365	377	415	416	0.40	0.90	5.34	5.40	0.73	0.0002	0.3458	0.0000	0.0736
142315	Dnao11		358	362	398	434	214	158	203	253	126	103	126	106	152	147	147	155	395	396	414	296	1959	1963	1867	2063	237	206	187	1215	1135	1282	1231	0.53	1.31	5.23	5.79	0.56	0.0005	0.0019	0.0000	0.0046
145209	Haf		38	38	35	34	20	17	23	22	21	19	18	18	21	22	23	19	30	38	35	35	180	201	195	135	22	22	28	59	55	69	64	0.57	1.11	5.15	2.55	0.70	0.0001	0.0941	0.0001	0.0080
142316	Trim39a		21	23	23	20	13	12	16	8	15	18	16	20	14	14	15	14	38	27	43	42	172	177	147	141	23	16	18	15	18	17	19	0.55	0.83	4.39	0.53	0.52	0.0013	0.0783	0.0000	0.0147
141613	Sec11a		67	73	79	74	20	17	29	36	13	14	11	10	9	15	10	15	31	30	37	32	144	141	157	122	12	11	12	18	15	19	20	0.35	1.01	4.21	1.51	0.36	0.0001	0.0543	0.0000	0.0001
142570	Emlb		38	31	35	34	17	16	17	16	10	12	13	11	12	13	11	14	13	17	25	24	86	89	88	73	12	12	9	33	38	43	49	0.51	1.10	4.00	3.77	0.55	0.0021	0.2941	0.0000	0.0467
141757	Gmpa		26	25	28	32	13	16	15	11	22	18	15	22	17	19	18	20	31	28	38	37	136	129	120	128	15	13	17	18	17	18	17	0.50	0.96	3.82	1.14	0.45	0.0003	0.7002	0.0000	0.0013
142339	Ppyat		253	287	296	256	110	91	114	97	37	52	35	33	59	45	51	49	225	317	318	175	971	1037	1061	781	105	101	83	737	751	976	804	0.34	1.34	3.73	7.43	0.37	0.0000	0.0523	0.0001	0.0117
142864	Ptmc		739	737	756	653	460	416	565	475	270	243	258	276	257	256	257	288	650	695	713	544	2375	2330	2336	2567	521	392	466	1946	1831	1971	1889	0.61	1.07	3.60	4.12	0.70	0.0002	0.0244	0.0000	0.0130
142427	Ubat		177	200	218	155	95	103	122	118	118	102	105	117	95	80	101	114	198	193	190	190	581	621	725	665	109	117	91	214	199	219	228	0.59	0.93	3.49	2.03	0.55	0.0021	0.2417	0.0000	0.0001
142897	Sec24d		203	220	244	169	139	137	136	120	82	74	67	66	68	79	76	84	198	155	191	167	648	651	599	554	100	76	75	121	112	121	145	0.64	1.07	3.45	1.49	0.47	0.0036	0.3637	0.0000	0.0001
144876	Sec15b		293	285	338	254	133	131	173	168	106	98	102	81	64	80	86	105	324	263	293	246	908	947	958	912	106	109	104	510	510	547	532	0.52	0.87	3.34	4.96	0.38	0.0005	0.2392	0.0000	0.0003
141776	LOC36353		193	146	195	197	92	81	88	81	85	84	53	112	98	46	57	94	108	139	124	333	413	394	312	66	77	74	59	62	64	69	0.49	1.01	3.12	1.06	0.54	0.0003	0.0633	0.0001	0.0064	
141570	Grf		512	540	548	573	309	292	320	312	244	249	271	266	238	247	259	255	451	517	513	434	1462	1465	1672	1700	290	276	220	323	316	379	424	0.57	0.82	3.07	1.59	0.54	0.0029	0.1167	0.0000	0.0001
142086	Ime2		186	212	196	175	115	113	146	124	105	101	90	82	107	79	85	137	131	133	145	380	374	420	367	119	105	93	145	115	135	155	0.65	0.93	2.92	1.30	0.80	0.0009	0.4304	0.0000	0.0389	
142607	Gpr108		14	21	24	15	7	8	7	7	6	7	7	7	9	8	10	10	9	13	11	29	32	28	36	9	10	9	11	12	11	11	11	0.40	1.29	2.90	1.15	0.88	0.0029	0.2951	0.0000	0.0722
142648	Ubat		192	185	203	141	110	114	112	122	99	99	103	104	79	91	96	111	167	138	187	162	395	427	457	458	91	110	80	214	186	215	252	0.65	0.92	2.67	2.32	0.58	0.0009	0.2746	0.0000	0.0001
145109	Trim5c		493	472	535	423	188	195	188	200	245	272	266	268	281	376	274	264	555	552	557	570	1286	1548	1536	1349	295	312	321	325	298	349	375	0.40	1.13	2.59	1.09	0.56	0.0000	0.0000	0.0000	0.0000
145842	Sec3		219	200	197	233	101	98	214	104	87	81	104	87	105	96	95	164	192	192	144	455	433	512	395	112	74	103	108	107	111	132	0.48	1.08	2.59	1.18	0.56	0.0000	0.2863	0.0000	0.0061	
143390	MD00776		101	90	96	81	59	66	52	60	70	71	65	75	57	70	66	139	94	139	126	350	304	313	66	73	61	78	75	86	102	0.64	0.93	2.58	1.27	0.54	0.0007	0.3407	0.0000	0.0066		
141955	Maj1		153	154	140	85	58	48	20	59	33	26	28	26	28	25	21	51	60	71	60	165	144	170	162	41	35	50	60	84	72	85	0.46	0.87	2.56	1.80	0.67	0.0042	0.1214	0.0000	0.0093	
141612	Dnpep		123	123	151	109	65	70	84	70	101	104	86	87	119	131	106	130	166	181	186	172	435	367	361	445	142	139	111	203	206	278	213	0.57	1.29	2.56	1.73	0.74	0.0013	0.0113	0.0000	0.0054
141582	Secp1		991	1013	1013	1039	488	493	496	567	290	333	288	281	249	299	287	275	639																							

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143437	Abd4	97	105	116	93	49	63	64	62	61	60	69	60	81	100	116	104	129	120	140	110	181	164	215	212	125	114	96	74	64	80	84	1054	1053	1057	0.48	0.89	0.0007	0.0004	0.0019	0.2526	
142493	St25a17	124	136	167	158	63	49	70	58	53	73	62	79	144	178	142	125	151	138	167	176	251	254	232	219	140	197	158	676	434	361	503	0.41	1.20	1.51	2.99	1.04	0.0002	0.0007	0.0004	0.7085	
142346	UfPa	288	248	313	328	157	153	191	185	204	193	185	198	305	296	297	316	361	346	373	388	559	543	563	528	313	271	349	285	280	375	362	0.57	1.56	1.49	1.05	0.85	0.0006	0.0000	0.0000	0.4997	
144852	Ser4	789	831	780	856	299	425	478	401	467	438	433	450	364	411	412	427	487	427	487	796	1131	1230	1292	1078	422	425	310	430	478	433	455	0.52	0.90	1.48	0.99	0.57	0.0000	0.0308	0.0009	0.0015	
143469	Me13	155	178	159	148	84	104	106	123	104	105	111	93	125	113	130	120	100	107	147	127	185	170	157	108	96	95	108	124	113	91	0.66	1.18	1.47	1.09	0.85	0.0019	0.0106	0.0156	0.2160		
141728	H12	451	469	457	478	248	330	213	298	218	295	321	319	248	276	283	285	367	419	400	425	589	655	535	275	270	252	767	241	229	248	0.66	0.86	1.46	0.90	0.65	0.0002	0.0401	0.0006	0.0020		
143895	Harvey1	255	274	335	263	92	103	188	132	142	165	183	133	218	185	232	293	248	202	175	194	371	155	342	315	171	256	274	533	473	545	0.42	1.42	1.52	1.44	1.14	0.0017	0.0124	0.1230	0.4147		
141669	D17Wu10	110	132	141	122	67	67	91	62	99	110	99	81	79	99	88	107	173	157	171	154	245	232	233	235	120	98	85	117	135	127	140	0.57	0.93	1.44	1.29	0.62	0.0012	0.1434	0.0000	0.0016	
144926	Chd1	49	37	48	38	8	8	14	10	12	14	15	11	15	17	13	21	49	53	61	55	84	82	75	73	23	18	18	19	17	22	74	0.24	1.26	1.43	1.03	0.37	0.0001	0.1018	0.0006	0.0001	
145453	Opp191	28	43	27	46	33	24	22	27	20	24	18	21	20	25	22	24	31	34	42	25	42	39	56	31	37	30	27	40	42	38	47	0.65	1.09	1.43	1.12	0.96	0.0036	0.3003	0.0339	0.2719	
141834	H12-07	60	66	64	70	28	18	43	16	36	49	38	31	22	32	32	42	89	53	84	44	111	136	74	68	72	38	39	106	80	75	61	0.40	0.95	1.39	1.67	0.71	0.0007	0.2763	0.2242	0.2997	
141894	Rgn2	235	239	247	212	136	105	137	117	119	121	128	126	146	169	154	161	216	232	265	258	352	328	352	308	179	154	169	182	181	204	171	0.53	1.28	1.38	1.10	0.69	0.0001	0.0007	0.0010	0.0037	
141981	Sec63	128	139	134	100	69	82	90	76	95	101	83	81	77	95	78	81	136	136	138	128	170	181	211	179	101	94	91	132	109	124	122	0.63	0.92	1.38	1.18	0.71	0.0035	0.2888	0.0013	0.0001	
145409	Prp12p	22	29	25	28	17	13	19	17	21	19	14	15	19	18	21	20	28	25	19	25	32	29	46	29	18	19	12	18	15	19	29	0.63	1.16	1.37	1.24	0.66	0.0074	0.1705	0.0813	0.0344	
141603	Enr1	225	218	231	120	114	100	128	124	95	102	108	108	69	190	85	105	127	152	172	120	234	277	254	282	83	72	82	119	110	106	133	0.52	0.89	1.37	1.45	0.42	0.0000	0.2318	0.0062	0.0007	
141781	Boke	40	37	52	32	16	17	23	16	22	21	19	17	17	26	21	23	84	51	50	45	71	95	68	79	24	14	19	22	20	26	24	0.44	1.09	1.33	1.34	0.33	0.0214	0.4614	0.1068	0.1237	
145126	Sharn	101	88	106	87	60	60	62	62	76	74	62	84	91	109	107	113	126	115	105	138	157	149	160	178	108	131	106	89	75	108	93	0.60	1.42	1.33	0.79	0.95	0.0010	0.0033	0.0057	0.5781	
141813	hsc2b	73	65	55	54	38	33	40	45	44	32	38	42	29	34	48	89	53	46	43	60	86	44	81	71	28	47	32	23	20	29	82	0.63	1.16	1.28	0.73	0.70	0.0047	0.5437	0.1846	0.0697	
145468	hsc10	44	47	46	42	23	27	35	32	38	31	36	34	39	37	38	28	35	54	47	47	72	54	61	41	31	27	38	39	40	31	35	0.66	1.00	1.28	1.15	0.71	0.0019	0.9630	0.1479	0.0651	
142223	hsc1	658	806	758	711	354	373	382	384	307	300	314	276	292	285	273	300	672	690	819	650	863	671	670	678	401	341	351	269	281	247	273	0.51	0.96	1.27	0.73	0.51	0.0000	0.2994	0.0061	0.0008	
143632	hsc2	11	12	10	7	8	6	6	8	8	8	8	8	10	13	10	14	10	11	13	10	15	13	13	14	11	10	15	13	11	14	10	0.62	1.33	1.25	0.93	1.07	0.0016	0.0154	0.0707	0.6173	
141637	hsc2	176	133	132	145	69	87	96	90	136	147	145	114	163	159	205	211	219	222	189	221	238	337	245	236	197	189	224	300	267	249	261	0.64	1.36	1.24	1.33	0.95	0.0005	0.0178	0.0911	0.4848	
142874	hsc2	153	137	146	178	85	98	67	65	95	108	89	105	128	140	142	138	156	151	154	182	238	183	199	174	154	156	147	133	159	159	127	0.51	1.38	1.23	0.95	0.95	0.0000	0.0009	0.0295	0.3634	
145120	hsc2	60	55	59	49	33	33	32	32	39	74	75	83	73	58	59	65	80	34	55	50	60	60	64	70	49	29	33	33	39	38	28	37	0.57	0.86	1.23	1.11	0.64	0.0001	0.0948	0.1504	0.0423
143734	hsc1	133	128	129	116	74	72	76	72	73	109	103	94	149	150	156	153	158	189	182	161	115	106	109	158	148	171	158	47	131	131	131	0.63	1.19	0.71	0.94	0.53	0.0000	0.0000	0.0076	0.0020	
142362	hsc3	61	80	87	76	35	36	47	41	32	34	34	31	37	32	32	31	84	75	85	84	104	91	100	144	41	33	28	40	45	38	37	0.51	1.00	1.21	1.17	0.41	0.0017	0.9848	0.0300	0.0003	
145136	hsc3	20	16	19	18	11	11	12	10	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	0.66	1.47	1.10	1.05	0.86	0.0010	0.0000	0.4360	0.3115
144869	hsc3	1407	1428	1484	1256	909	909	973	834	949	966	916	900	911	962	957	952	1324	1466	1556	1559	1722	1882	1894	1817	1019	921	1016	1172	1049	1059	1056	0.66	1.01	1.18	1.10	0.69	0.0001	0.5323	0.0195	0.0013	
145205	hsc3	43	51	38	46	28	23	32	30	23	16	26	26	44	53	51	38	50	47	48	58	46	53	57	56	42	42	50	63	51	57	0.64	1.12	1.15	1.19	1.02	0.0038	0.0003	0.0818	0.3794		
143556	hsc3	45	47	59	58	33	32	34	32	21	23	20	23	15	23	17	20	24	22	23	21	28	35	31	29	20	20	28	28	24	24	27	0.63	1.19	0.71	0.94	0.53	0.0000	0.0000	0.0000	0.0000	
143644	hsc3	16	18	15	16	11	8	10	9	15	13	11	15	19	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	0.66	1.47	1.10	1.05	0.86	0.0010	0.0000	0.4360	0.3115
141826	hsc1	308	301	339	276	206	167	217	214	250	237	239	256	346	358	361	369	437	379	573	432	492	417	574	514	415	378	380	415	421	397	415	0.66	1.47	1.10	1.05	0.86	0.0010	0.0000	0.4360	0.3115	
143085	hsc1	141	100	128	131	64	72	77	82	80	60	68	60	77	57	72	82	73	67	67	67	67	67	67	67	67	67	67	67	67	67	67	67	0.66	1.47	1.10	1.05	0.86	0.0010	0.0000	0.4360	0.3115
145145	hsc1	62	63	73	58	31	33	37	30	51	54	46	52	66	80	84	72	62	67	64	90	81	83	75	79	67	72	42	46	45	58	59	0.52	1.29	1.04	0.87	0.44	0.0002	0.0000	0.0000	0.0000	
142084	hsc1	977	705	1155	920	434	365	468	295	364	328	366	442	314	316	316	316	316	316	316	316	316	316	316	316	316	316	316	316	316	316	316	316	316	316	316	316	316				
142767	hsc2	1069	1097	1106	1038	203	282	278	318	230	203	205	286	791	1055	963	828	1179	1013	1330	1216	1243	1046	1054	1106	1062	1317															

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1451232 Jamp	76	78	83	88	61	45	45	48	40	41	31	43	47	72	73	70	64	55	88	75	34	51	52	48	72	82	82	84	81	69	81	0.63	1.39	0.65	0.95	1.10	0.0006	0.0000	0.0239	0.4547	
142726 JAm	726	554	633	492	201	239	221	216	207	175	173	267	489	594	606	463	584	586	704	764	361	391	488	473	618	666	806	431	524	550	543	0.36	2.62	0.65	0.73	1.06	0.0003	0.0002	0.0054	0.6247	
144884 Torib	50	49	55	55	27	39	35	37	48	63	64	58	74	85	73	94	108	84	101	86	54	67	54	69	87	80	68	71	78	82	85	0.64	1.36	0.65	1.01	0.83	0.0010	0.0004	0.0106	0.1006	
142638 Camkid	24	35	32	32	15	14	15	13	7	11	8	10	9	9	9	11	13	8	6	7	5	7	7	10	6	6	5	7	7	7	0.46	1.09	0.63	0.80	0.79	0.0004	0.0004	0.0385	0.0188		
145221 Tm2	275	288	279	236	77	73	59	66	55	46	49	70	145	189	183	170	247	215	245	269	138	142	167	163	207	229	169	191	187	141	177	0.26	1.32	0.83	0.86	0.82	0.0000	0.0000	0.0005	0.0488	
142926 Tm3	284	347	285	270	68	75	85	87	73	76	75	88	209	289	284	221	307	303	335	337	131	187	230	191	244	355	309	287	290	265	289	0.26	3.16	0.51	0.81	1.05	0.0000	0.0001	0.0001	0.3474	
141585 Lamg1	1306	1295	1279	1216	416	432	513	535	421	464	410	488	1059	1215	1050	1043	1321	1380	1300	1293	808	692	841	875	1418	1318	1324	1079	946	1029	1023	0.38	2.45	0.61	0.75	1.02	0.0000	0.0000	0.0000	0.4440	
144912 Tm1g81	93	114	123	134	53	61	46	72	126	85	93	66	72	111	122	108	168	167	241	105	98	87	118	113	95	69	63	37	33	44	101	0.30	1.12	0.61	0.78	0.44	0.0014	0.0014	0.0519	0.0289	
142471 Retat	1091	1088	807	970	152	250	91	168	143	114	120	243	471	1047	682	591	729	820	1470	1247	594	489	479	596	662	966	700	561	771	628	375	0.17	1.450	0.60	0.75	0.87	0.0000	0.0004	0.0070	0.4890	
145075 Cym12	1871	1778	2322	1659	859	718	727	649	778	954	718	1135	1598	1578	1764	1714	1596	1446	1854	1085	1489	1054	1059	1592	1590	1574	1580	1649	1420	1500	1708	0.38	1.83	0.39	0.52	1.04	0.0002	0.0004	0.0002	0.8958	
143370 Bm3	542	496	517	454	272	224	289	301	347	310	298	360	341	380	359	345	629	535	587	618	363	303	359	371	607	649	667	931	443	559	565	0.54	1.73	0.99	0.80	1.09	0.0001	0.0000	0.0001	0.1388	
142612 Tm1g1	710	725	722	710	159	132	159	121	178	187	151	178	259	290	268	277	245	297	264	272	163	138	153	162	322	296	254	204	209	225	202	0.66	1.52	0.57	0.72	1.08	0.0004	0.0001	0.0001	0.3123	
144930 Abca2	7	7	9	7	4	6	4	6	5	4	6	7	10	8	9	6	10	10	8	5	5	5	5	6	7	7	5	5	4	5	0.60	1.63	0.57	0.73	0.78	0.0013	0.0063	0.0064	0.1569		
141884 Proc	1719	1665	1790	1674	504	553	632	606	682	616	559	753	1617	1866	1703	1646	1947	1937	1773	2050	1121	966	1107	1199	1742	2089	2121	1428	1618	1524	1460	0.33	2.62	0.57	0.76	1.03	0.0000	0.0000	0.0000	0.6379	
142411 Tm1g	20	13	24	17	8	7	7	7	8	4	5	5	6	11	13	15	10	12	10	12	12	7	7	7	6	6	11	13	12	10	8	9	0.40	2.44	0.87	0.89	1.05	0.0000	0.0000	0.0000	0.5958
145468 BC999997	287	291	272	289	193	177	164	195	264	286	260	353	303	484	553	441	523	421	356	516	224	161	286	363	267	448	477	428	399	513	530	0.66	1.15	0.57	1.18	0.87	0.0000	0.0000	0.0000	0.4551	
145301 Bm2	55	42	54	48	16	26	25	23	46	44	29	40	37	65	43	57	46	35	53	62	25	29	24	37	45	37	38	15	19	46	31	0.43	1.26	0.96	0.52	1.09	0.0004	0.0000	0.0000	0.7321	
142858 Bm9	27	24	26	30	15	11	13	15	17	14	15	20	29	39	31	43	34	35	31	37	19	17	24	17	33	35	30	26	25	31	33	0.50	2.19	0.56	0.89	0.95	0.0001	0.0016	0.0004	0.3975	
142785 Pm1k	55	70	42	44	20	20	26	19	27	25	31	20	38	42	58	55	134	99	79	100	55	60	48	59	68	50	41	38	34	57	44	0.40	1.89	0.56	0.82	0.51	0.0079	0.0063	0.0095	0.0200	
142054 Masp2	287	288	281	263	190	176	194	181	159	155	176	225	256	235	242	219	273	216	280	140	129	135	147	210	231	247	155	144	139	178	0.66	1.43	0.55	0.70	0.94	0.0000	0.0002	0.0008	0.5294		
141666 Mm1g	474	487	444	441	177	145	173	197	181	167	142	194	413	445	404	433	460	438	420	498	224	236	259	276	494	550	490	388	443	392	415	0.38	1.47	0.55	0.80	1.13	0.0000	0.0000	0.0000	0.0001	
144897 Sml161	276	287	276	247	165	151	123	169	137	139	141	207	221	218	144	160	136	152	210	173	77	68	128	93	200	272	162	95	133	125	129	0.59	1.19	0.55	0.57	1.26	0.0008	0.0000	0.0000	0.2414	
142324 Cym2B	1025	996	1228	923	414	441	518	384	409	372	412	643	920	886	975	1057	986	907	1086	1111	711	332	681	493	604	661	956	417	281	352	636	0.42	2.13	0.34	0.67	0.72	0.0002	0.0000	0.0000	0.4063	
145324 Bm1g1	114	95	88	95	48	39	37	48	38	38	42	47	70	78	82	71	83	77	88	47	42	46	48	45	86	83	68	81	111	94	78	0.44	1.43	0.54	0.51	0.94	0.0001	0.0000	0.0000	0.3937	
142791 BC952889	93	57	52	59	19	17	25	27	19	14	18	14	33	29	30	18	72	132	126	166	89	48	53	78	124	130	144	98	77	78	126	0.33	1.67	0.64	0.63	1.10	0.0000	0.0000	0.0000	0.4112	
145327 Tm1g	38	29	27	34	14	11	14	15	9	13	15	10	14	23	16	24	19	21	24	23	9	12	13	12	34	23	19	17	16	15	17	0.44	1.63	0.54	0.63	1.18	0.0004	0.0000	0.0000	0.3775	
142366 Tm2B	87	84	72	57	13	13	18	20	20	23	20	27	66	94	80	68	101	91	106	120	63	47	61	53	116	98	81	83	85	88	81	0.21	0.94	0.54	0.86	0.94	0.0002	0.0000	0.0000	0.4666	
142595 Bm1g1	35	34	31	24	14	18	17	15	15	16	15	20	23	35	32	30	34	35	39	34	15	19	15	20	26	63	23	18	17	27	21	0.52	1.77	0.53	0.36	1.14	0.0015	0.0183	0.0005	0.6889	
144911 Dm1	153	133	139	127	92	78	86	69	58	53	87	95	119	109	116	122	80	118	111	44	61	57	65	85	146	101	139	138	126	135	0.55	1.48	0.53	0.42	1.03	0.0029	0.0004	0.0000	0.4864		
145326 Tm1g	155	172	143	165	97	103	99	100	125	139	123	146	391	394	185	188	229	221	227	106	111	127	129	232	265	199	446	350	171	141	0.62	1.42	0.51	0.68	1.00	0.0001	0.0000	0.0000	0.4112		
142366 Sml161	82	73	82	78	13	29	27	20	30	26	33	39	71	82	75	84	141	139	120	125	66	64	75	57	185	132	100	215	216	129	161	0.29	2.44	0.51	1.30	1.08	0.0000	0.0000	0.0000	0.6588	
142786 Tm1g	519	550	547	451	170	196	151	220	184	182	180	220	475	566	531	526	685	592	634	688	332	285	338	365	647	630	536	439	454	464	434	0.36	2.74	0.51	0.74	0.93	0.0000	0.0000	0.0000	0.3070	
144284 Tm1g	50	55	40	49	31	33	22	30	29	29	32	48	58	48	47	62	47	62	60	28	26	34	27	58	63	58	32	34	36	36	0.60	1.66	0.50	0.58	1.02	0.0000	0.0000	0.0000	0.4005		
145466 Cym3	97	88	107	98	60	62	57	42	73	80	71	87	75	89	99	75	92	113	96	48	53	78	124	130	144	98	77	78	78	78	0.54	1.24	0.49	0.52	0.88	0.0002	0.0000	0.0000	0.4005		
141666 BC999997	243	259	247	209	119	150	145	166	177	181	148	186	228	222	265	207	271	270	126	114	123	118	200	213	220	150	126	121	124	60	1.33	0.49	0.59	0.86	0.0008	0.0004	0.0000	0.3136			
143474 Tm1g	33	36	36	31	15	14	17	17	16	14	18	19	38	52	43	43	38	42	45	41	20	16	25	18	72	49	45	44	48	33	39	0.47	2.63	0.48	0.74	1.33	0.0001	0.0000	0.0000	0.1173	
141992 Tm1g	50	45	4																																						

[illegible]

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We claim:

1. A method of identifying candidate modulators of lipid homeostasis comprising, a) determining the ability of a test compound to modulate regulated IRE1-dependent decay (RIDD) of a lipid metabolism gene, wherein mRNA of the lipid metabolism gene is degraded by IRE1; and b) selecting said test compound as a compound of interest when said compound modulates RIDD of the lipid metabolism gene, to thereby identify candidate modulators of lipid homeostasis.
2. A method of identifying candidate modulators of lipid homeostasis comprising, a) determining the ability of a test compound to modulate XBP1-mediated transcription of a lipogenic target gene; and selecting said test compound as a compound of interest when said compound modulates XBP1-mediated transcription of the lipogenic target gene, to thereby identify candidate modulators of lipid homeostasis.
3. The method of claim 1 or 2, wherein said modulation of lipid homeostasis comprises modulation of lipid metabolism.
4. The method of claim 3, wherein said modulation of lipid metabolism comprises induction of lipid clearance from blood.
5. The method of claim 1 or 2, wherein said modulation of lipid homeostasis comprises modulation of lipogenesis.
6. The method of claim 5, wherein said modulation of lipogenesis comprises induction of lipogenesis.
7. The method of any one of claims 1, 5 and 6, wherein said compound inhibits RIDD of mRNAs of lipid metabolism genes.

8. The method of any one of claims 1, 5 and 6, wherein said compound stimulates XBP1-mediated transcription of the lipogenic target gene.

9. The method of any one of claims 1 to 8, wherein said lipid metabolism gene or lipogenic target gene is a hepatic gene.

10 The method of any one of claims 2 to 5, wherein the lipogenic target gene is selected from the group consisting of *Sdf2l1*, *Cflar*, *Dnajc3*, *Dnajb9*, *Gale*, *Nans*, *Edem1*, *Dnaljb11*, *Tmem39a*, *Ero1b*, *Gmppa*, *Hyou1*, *Uso1*, *Sec24d*, *Slc35b1*, *Uggt1*, *Ssr3*, *Ubxn4*, *Txndc5*, *Stt3a*, *Ssr1*, *Serp1*, *Ddost*, *Sec61a1*, *Surf4*, *0610007L01Rik*, *Rrbp1*, *Ostc*, *Odc1*, *Cnpy3*, *Cdk5rap3*, *Fdps*, *Spcs3*, *P4hb*, *Spcs2*, *2810482107Rik*, *Sec61b*, *Lman2*, *Krtcap2*, *Sec11c*, *Sec61g*, *Ssr4*, *Sec63*, *H13*, *D17Wsu104e*, *Chid1*, *Snd1*, *Ikbke*, *Tram1*, *Cbara1*, *Slc33a1*, *Pex11c*, *Mlec*, *Sqle*, *Cyp51*, *Tnfaip8l1*, *Pmvk*, *Fdtf1*, *Hsd17b7*, *Anubl1*, *Lifr*, *Sc4mol*, *Mvk*, and *Idi1*

11. The method of any one of claims 1 and 3 to 5, wherein the lipid metabolism gene is selected from the group consisting of *Paqr7*, *Ptprf*, *Ces3*, *AU018778*, *Ces1*, *Ces1c*, *Ces1d*, *Ces1e*, *Ces1f*, *Ces1g*, *Lrp1*, *Bloc1s1*, *Dhcr7*, *Limd2*, *Idi1*, *2210411K11Rik*, *Es22*, *Apon*, *Glrx*, *Fads2*, *Hc*, *Oaf*, *Mvk*, *Gjbl*, *Sc4mol*, *Pla1a*, *F7*, *Cpn2*, *Pxmp2*, *Abca3*, *Gm2a*, *Kcnn2*, *Nsdh1*, *Stim2*, *Dnase2b*, *Vnn3*, *Hsd17b7*, *Hgsnat*, *Coll8a1*, *Aldoc*, *Fads1*, *Fnl*, *Smarcc2*, *Dera*, *Spp2*, *Angpt13*, *Atrnl1*, *Tpp1*, *Fam108a*, *Tmprss6*, *Sidt2*, *Es1*, *Dbt*, *Ropn1l*, *Tex264*, *Tapbp*, *Selk*, *Btbd1*, *Cyp2c68*, *Ndufa9*, *Pmvk*, *Blvra*, *Igf2r*, *Proc*, *Abca2*, *Taldo1*, *Bola3*, *Cyp1a2*, *Retsat*, *Lamp1*, *Rnf130*, *Tex2*, *Afm*, *Jkamp*, *0610007C21Rik*, *Plbd2*, *Cyp51*, *Tm7sf2*, *Clu*, *Hgfac*, *3110073H01Rik*, *Furin*, *C920025E04Rik*, *Serping1*, *Lipc*, *Ttc13*, *Coll8a1*, *Slc27a5*, *Pcsk6*, *Tmem208*, *Pm20d1*, *Sqle*, *Man2a2*, *Pp1r15b*, *Azgp1*, *Ces6*, *Pla2g12b*, *llvbl*, *Adam9*, *Mlec*, *Pdap1*, *F2*, *Commd9*, *Itih4*, *Dgat2*, *Cyb561d2*, *Slc35f5*, *Alg1*, *Gnptg*, *Rarres1*, *Yif1a*, *Slc25a17*, *Mxd4*, *Fdps*, *Papola*, *Erp44* and *Rpn1*.

12. The method of claim 11, wherein the lipid metabolism gene is selected from the group consisting of *Ces1c*, *Ces1d*, *Ces1e*, *Ces1f*, *Ces1g* and *Angptl3*.

13. The method of any one of the preceding claims, wherein said test compound is comprised in a combination of test compounds.

14. The method of claim 1, wherein determining the ability of a test compound to modulate RIDD of the lipid metabolism gene comprises contacting an indicator composition with the test compound and determining a level of lipid metabolism gene mRNA in the presence of the test compound.

15. The method of claim 1, wherein determining the ability of a test compound to modulate RIDD of the lipid metabolism gene comprises contacting an indicator composition with the test compound and determining a level of lipid metabolism gene-encoded protein in the presence of the test compound.

16. The method of claim 2, wherein determining the ability of a test compound to modulate XBP1-mediated transcription of the lipogenic target gene comprises contacting an indicator composition with the test compound and determining a level of lipogenic target gene mRNA in the presence of the test compound.

17. The method of claim 2, wherein determining the ability of a test compound to modulate XBP1-mediated transcription of the lipogenic target gene comprises contacting an indicator composition with the test compound and determining a level of a reporter of lipogenic target gene transcription in the presence of the test compound.

18. The method of any one of claims 2, 16 and 17, wherein the test compound is further determined to modulate XBP1-mediated transcription of the lipogenic target gene independent of unfolded protein response (UPR) activation.

19. A method for validating a compound as a compound useful in modulating lipid homeostasis *in vivo* comprising, selecting a candidate compound identified according to the method of any one of the preceding claims, and testing said candidate compound for modulation of lipid metabolism in an animal model of dyslipidemia.

20. The method of claim 19, wherein said animal is tested for modulation of one or more of plasma cholesterol levels, plasma and/or hepatic triglyceride levels, plasma and/or hepatic lipid levels, steatosis, and lipoprotein metabolism.

21. A method for validating a compound as a compound useful in modulating lipid homeostasis *in vivo* comprising, selecting a candidate compound identified according to the method of any one of the claims 1 to 18, and testing said candidate compound for modulation of lipid metabolism gene activity in an animal model deficient in XBP1 and/or IRE1 α .

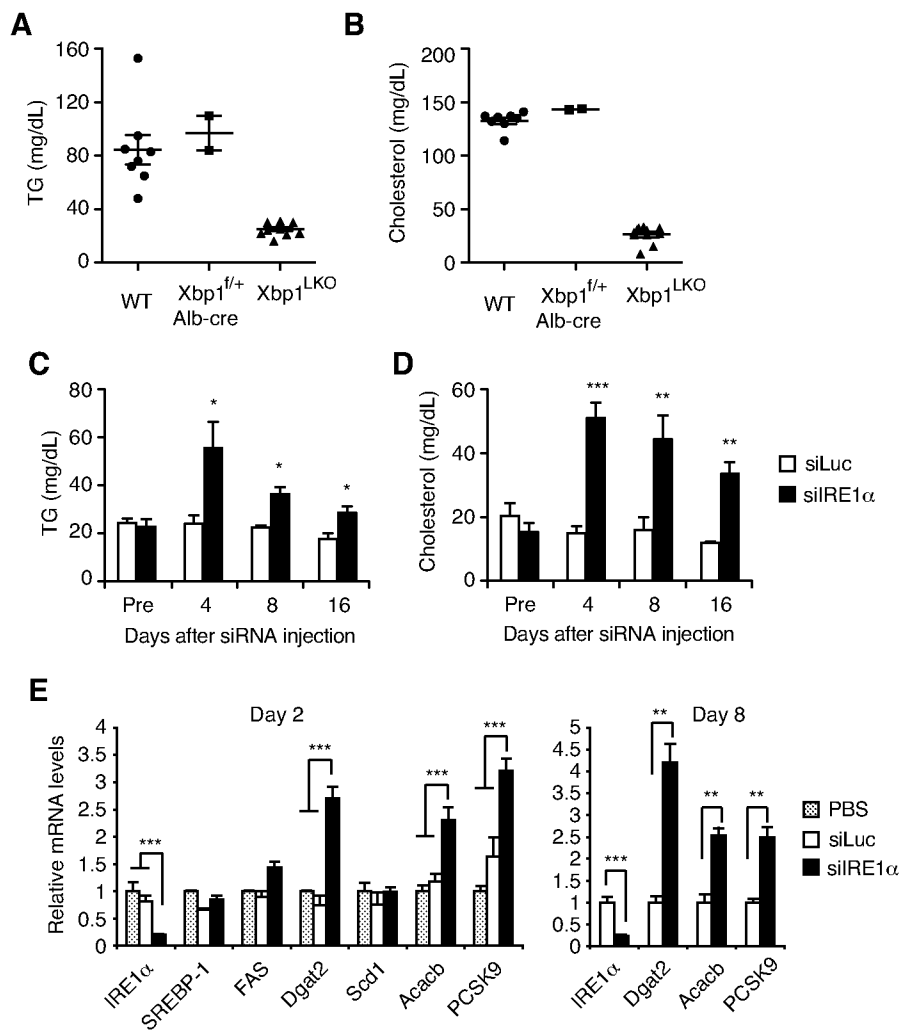


Fig 1

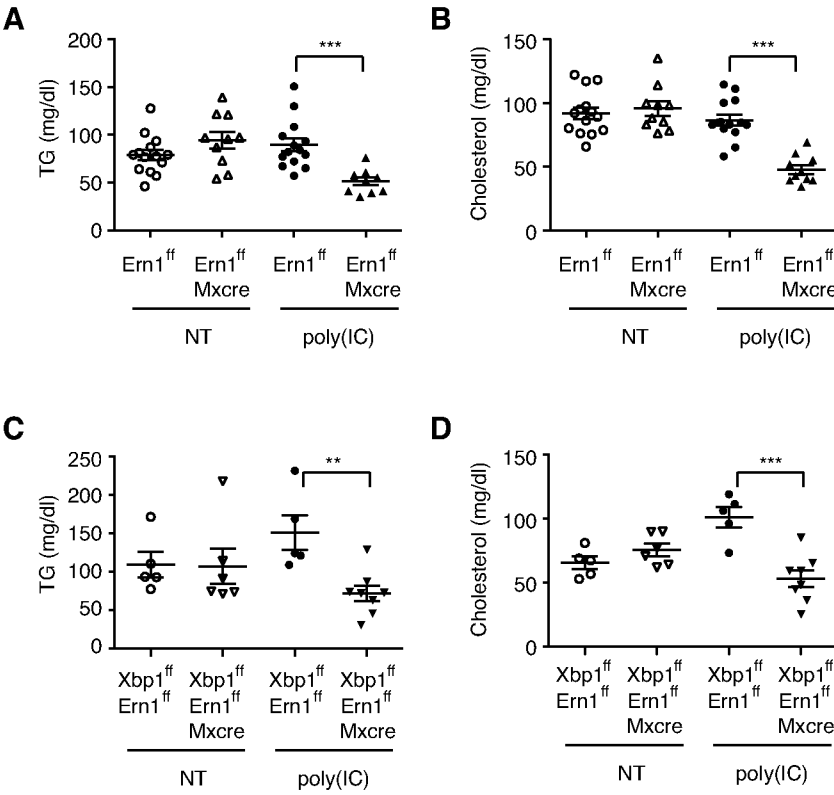


Fig 2

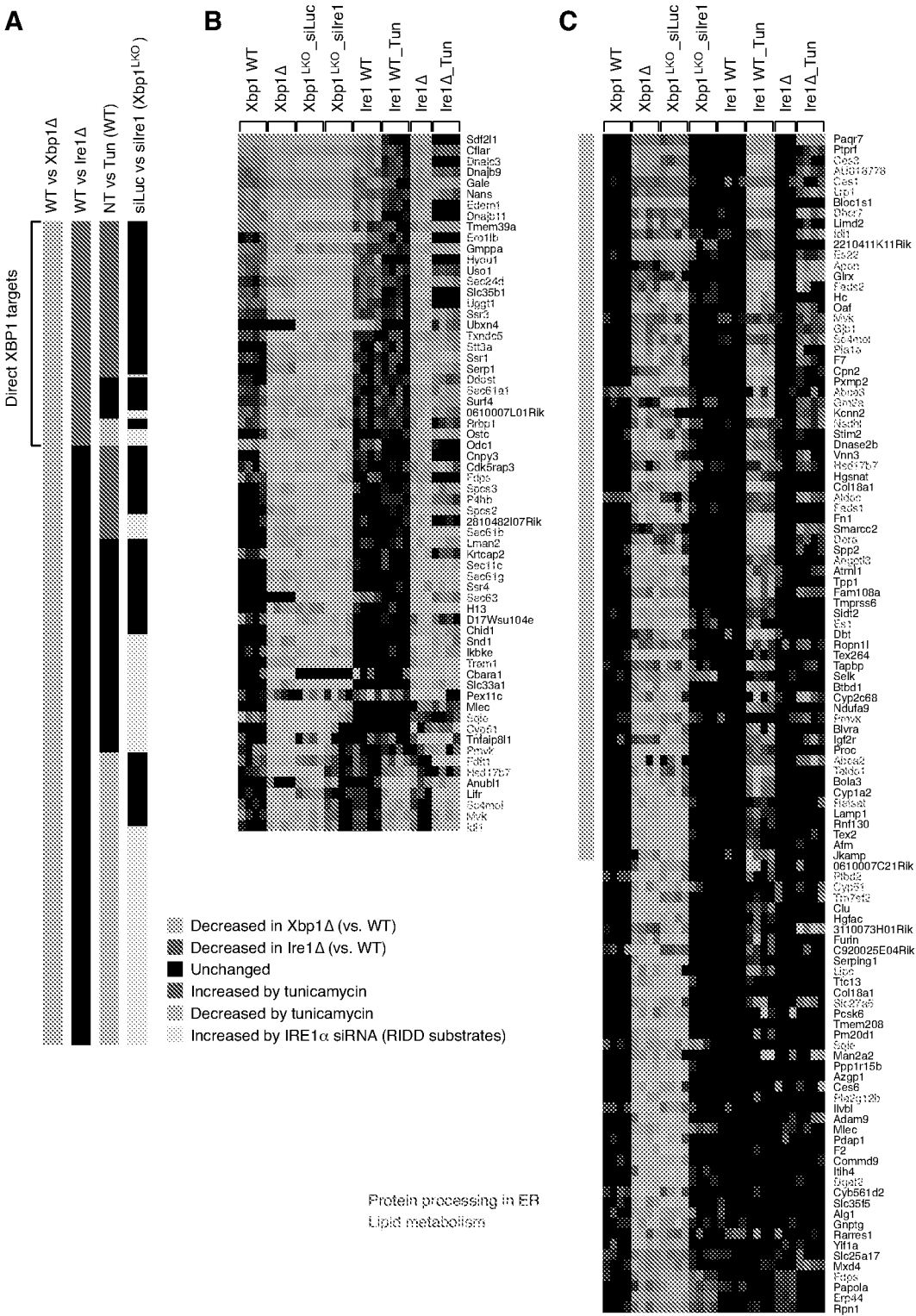


Fig 3

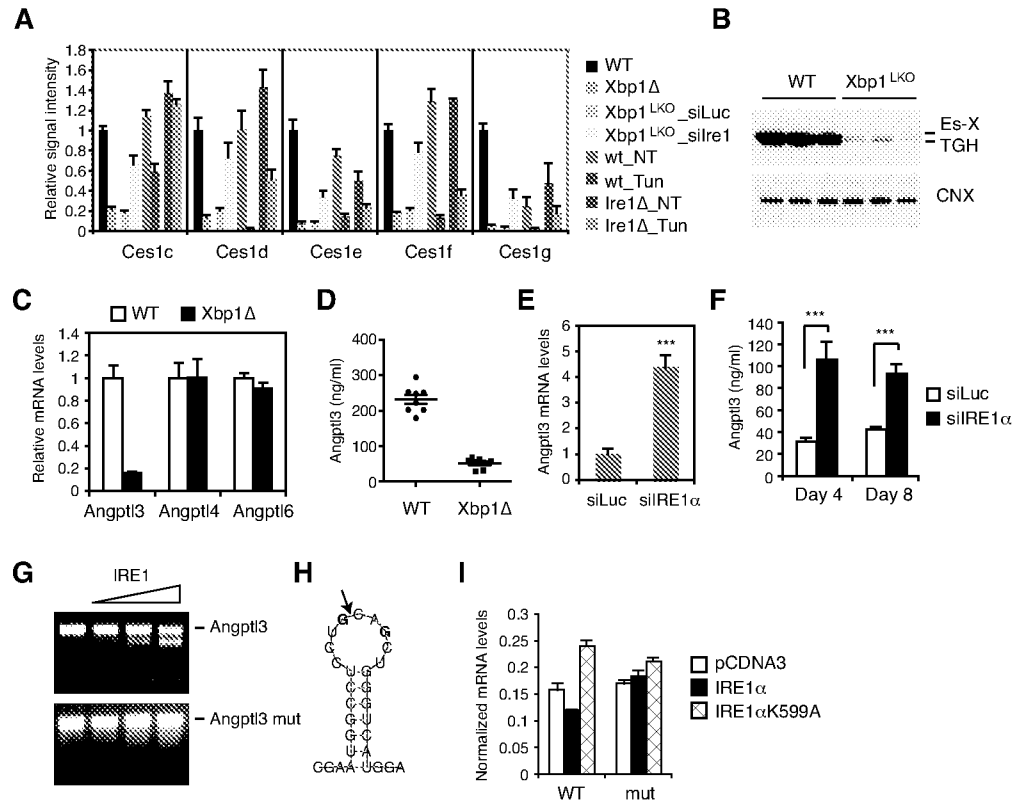


Fig 4

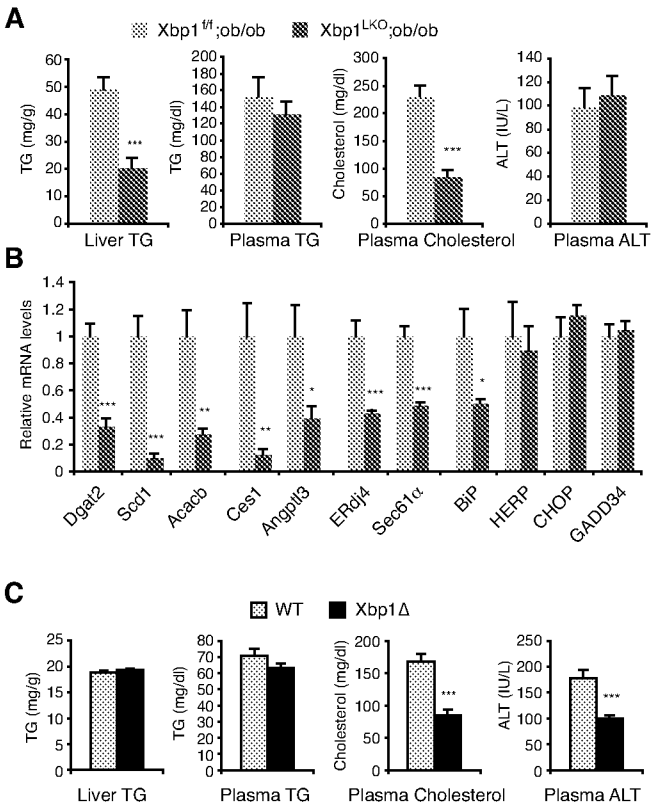


Fig 5

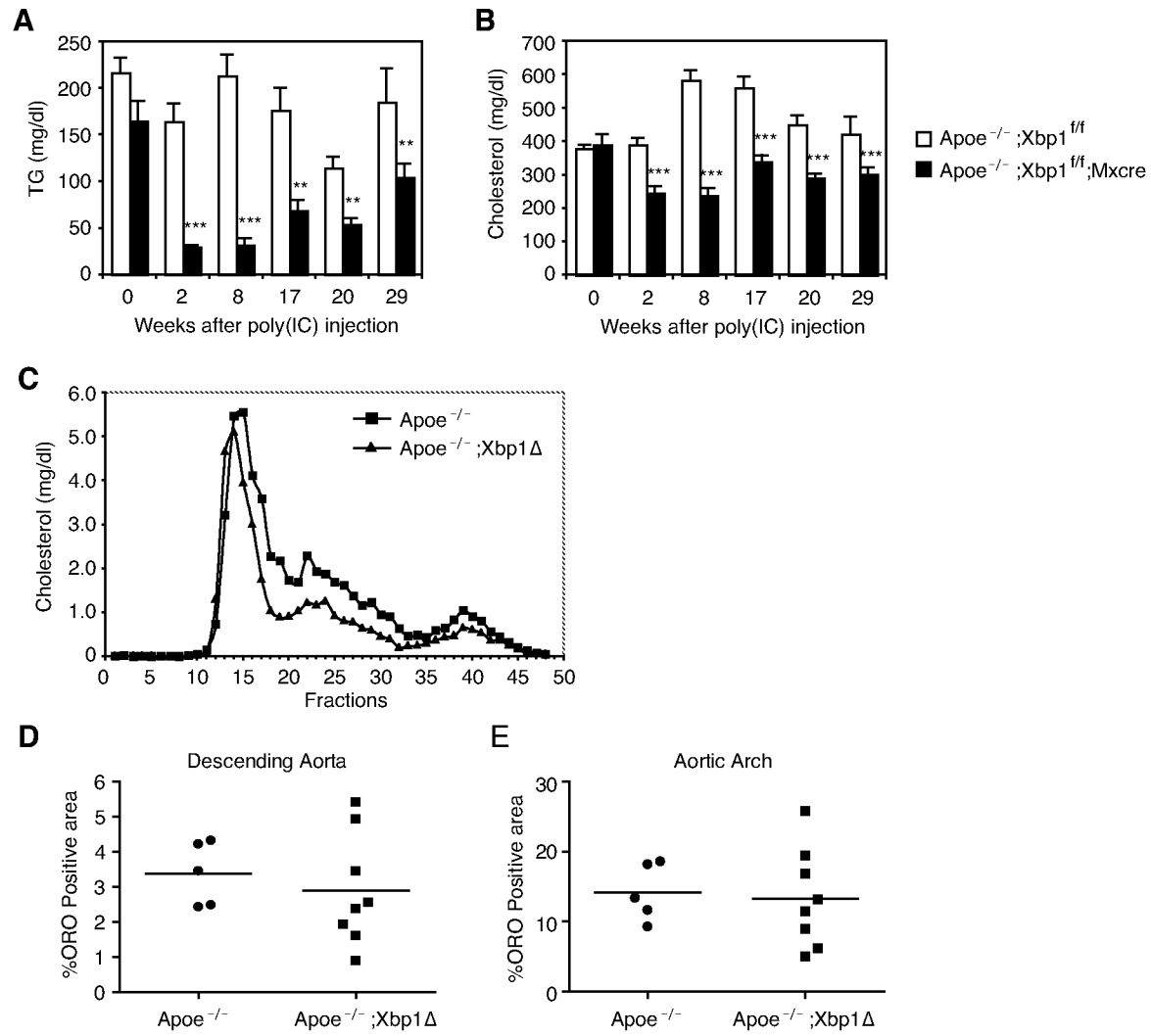


Fig 6

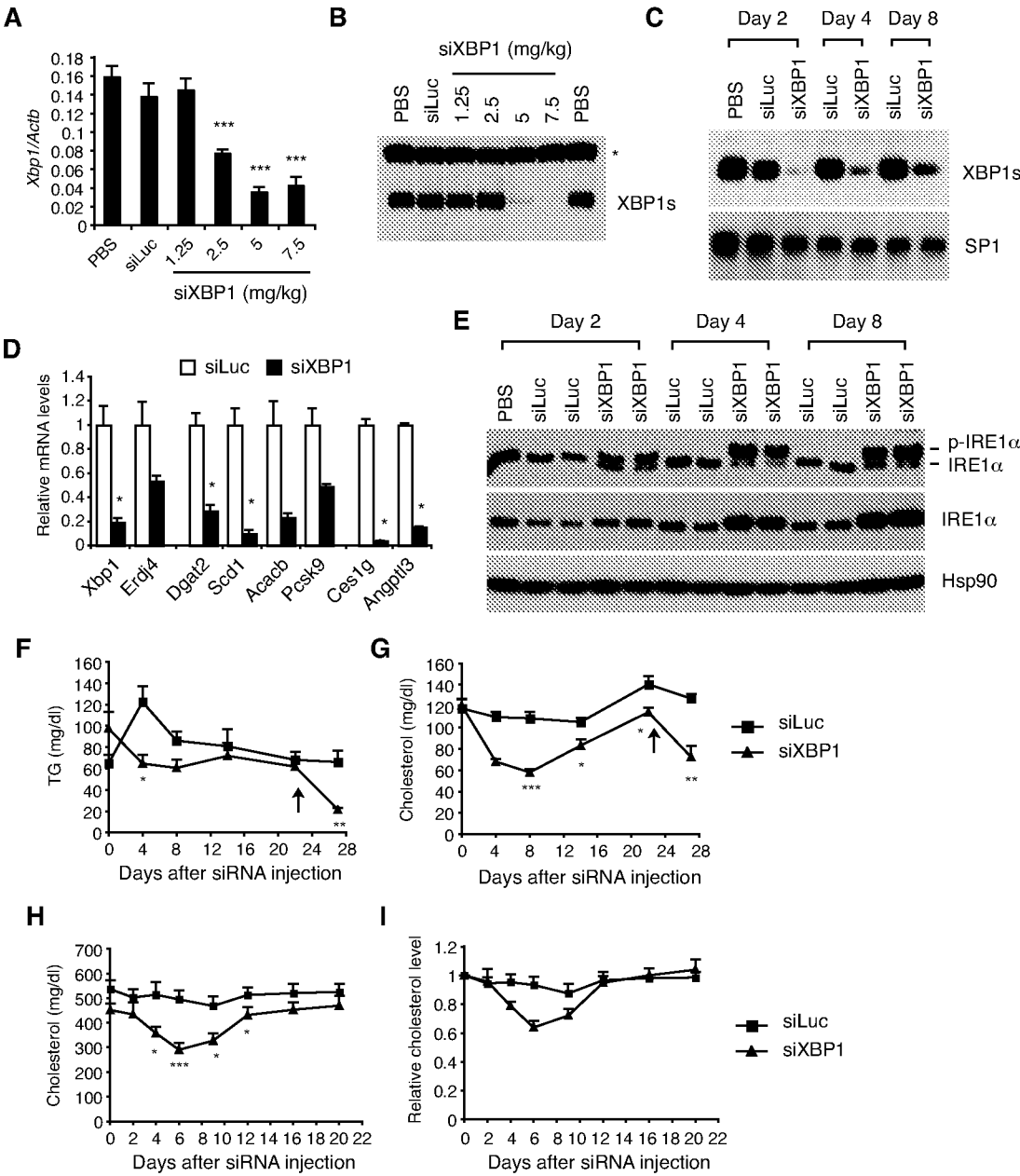


Fig 7

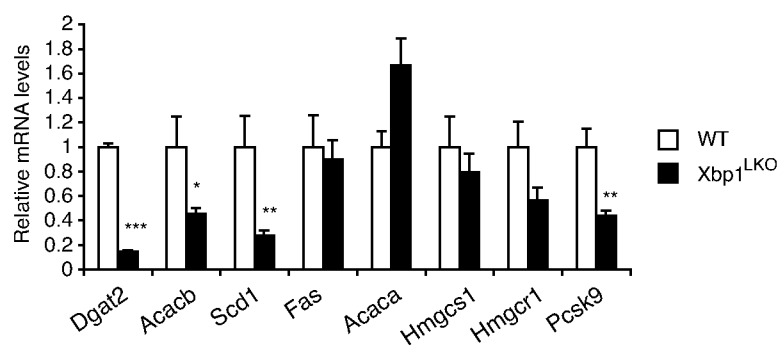


Figure S1. Expression of lipogenic genes in Xbp1^{LKO} mice determined by qRT-PCR.

Values represent mean $\pm$ s.e.m. n = 3-6. * P < 0.05, ** P < 0.01, *** P < 0.001.

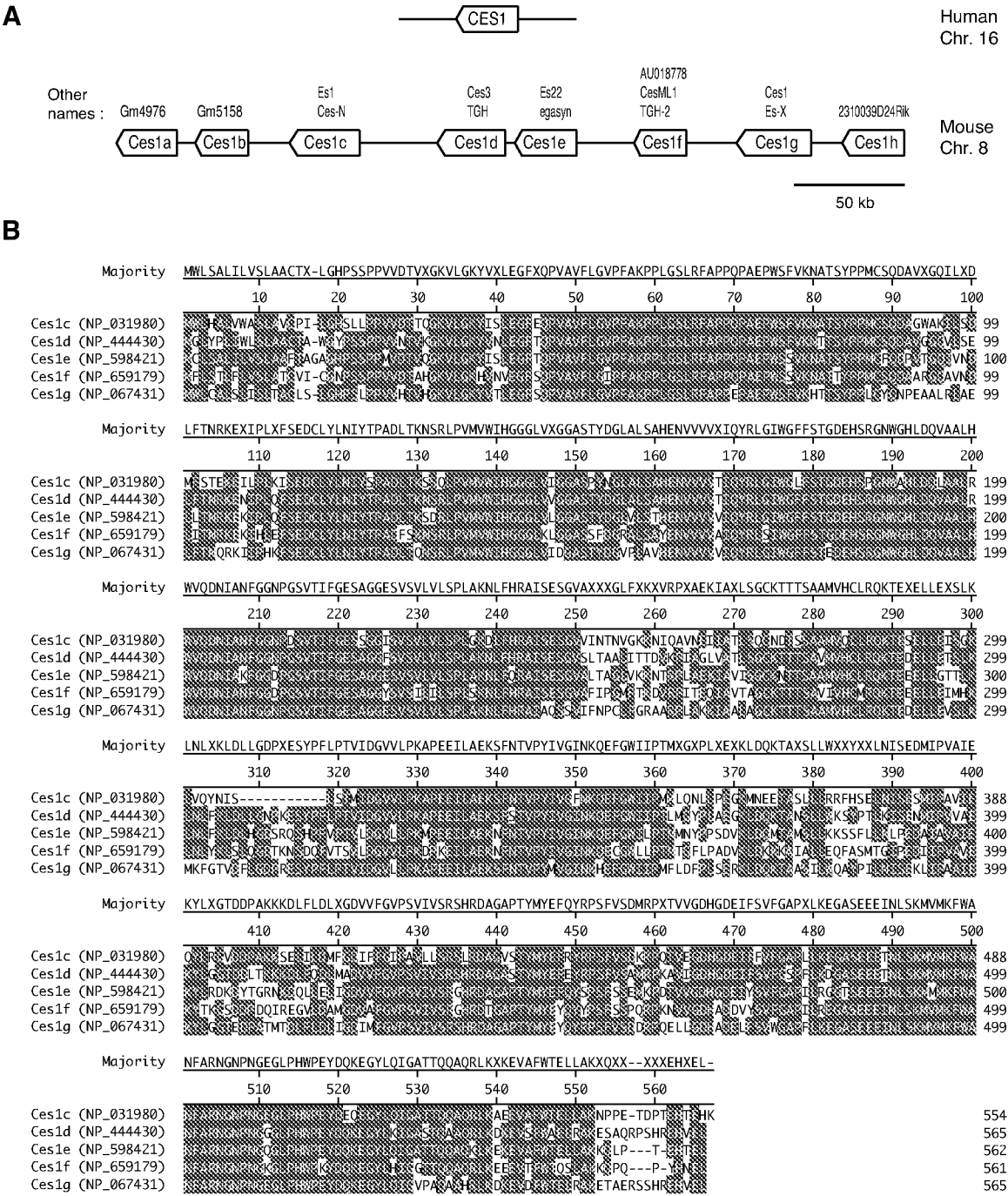


Figure S2. (A) Schematic representation of Ces1 gene cluster. (B) Alignment of predicted amino acid sequences of mouse Ces1 proteins. Conserved sequences are highlighted in red.

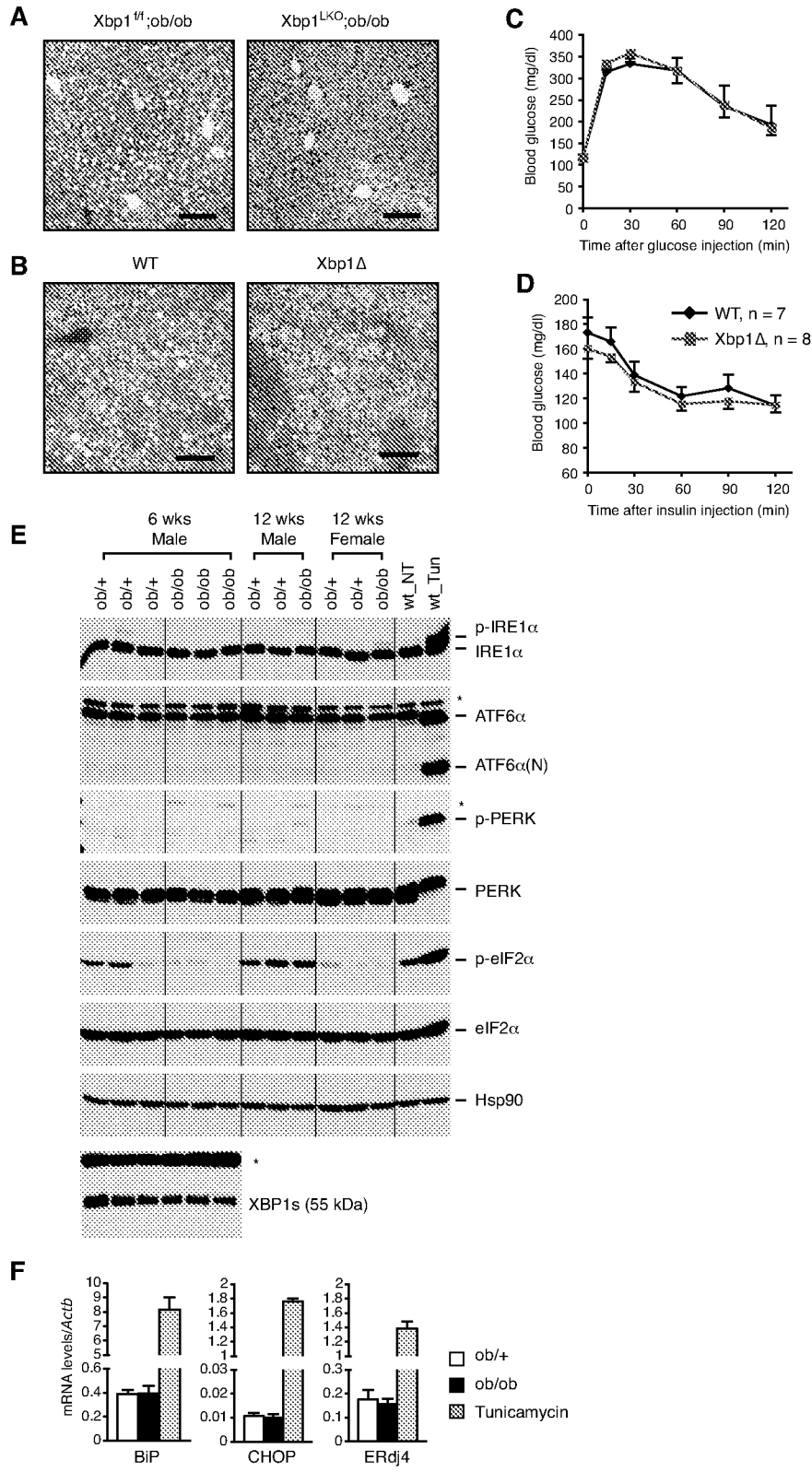


Fig S3