# Supplemental Materials for:

# The Xbp1s-GalE Axis links ER stress to Postprandial Hepatic Metabolism

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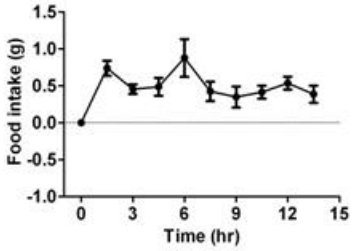
Supplemental figures 1-5

Supplemental table 1-2

# Supplemental Figure 1

**A**

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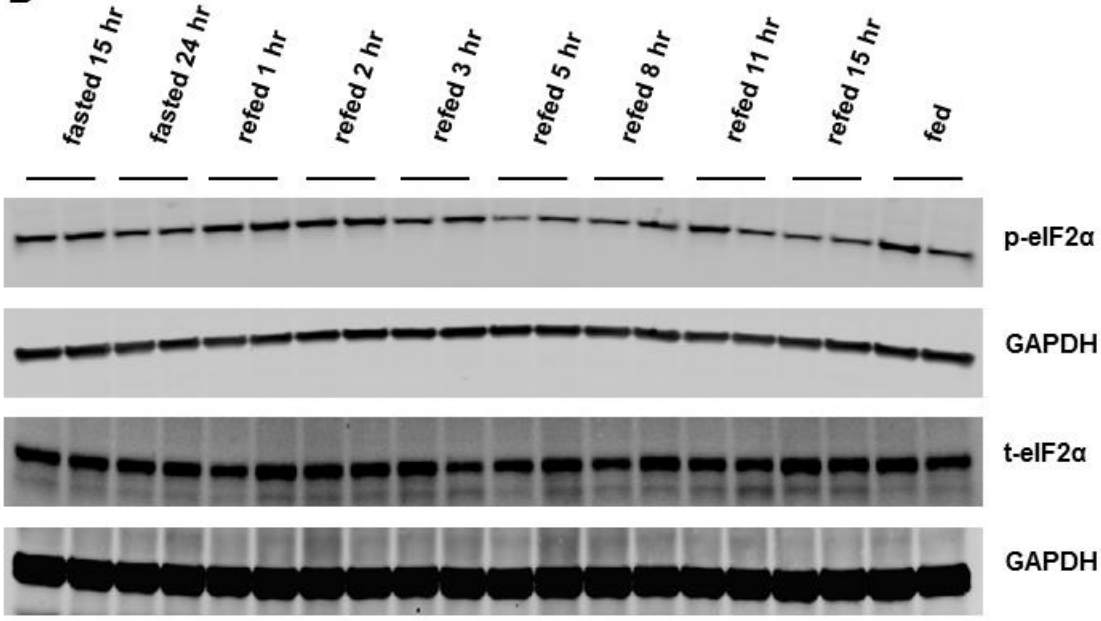


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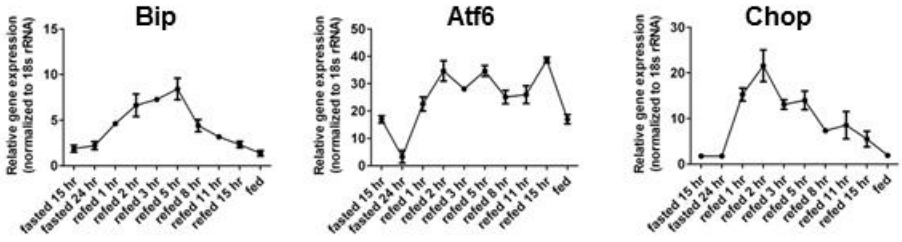

# Supplemental Figure 1 continued.

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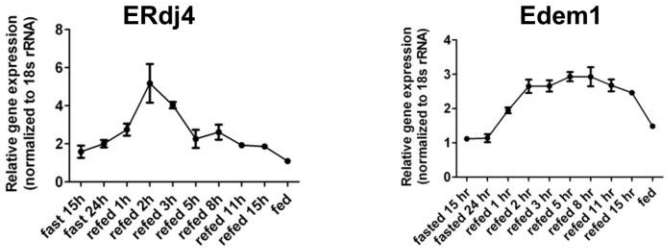


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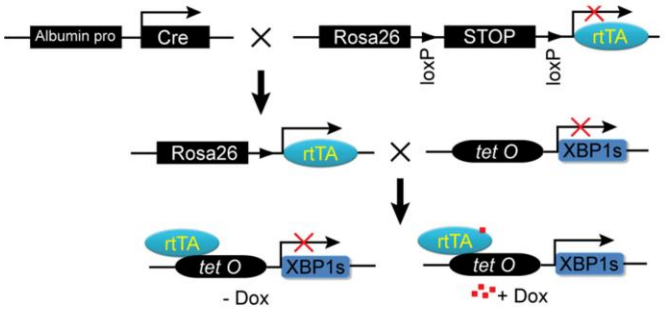


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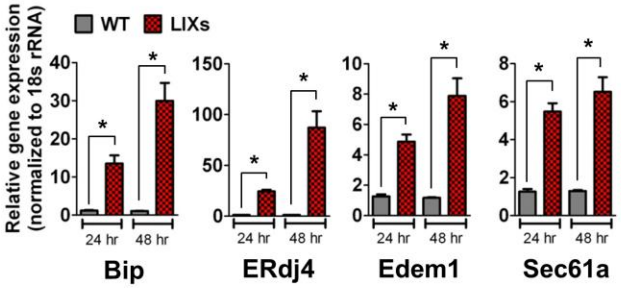


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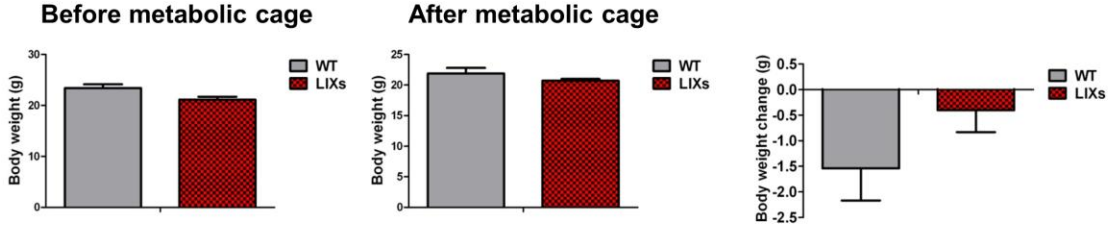
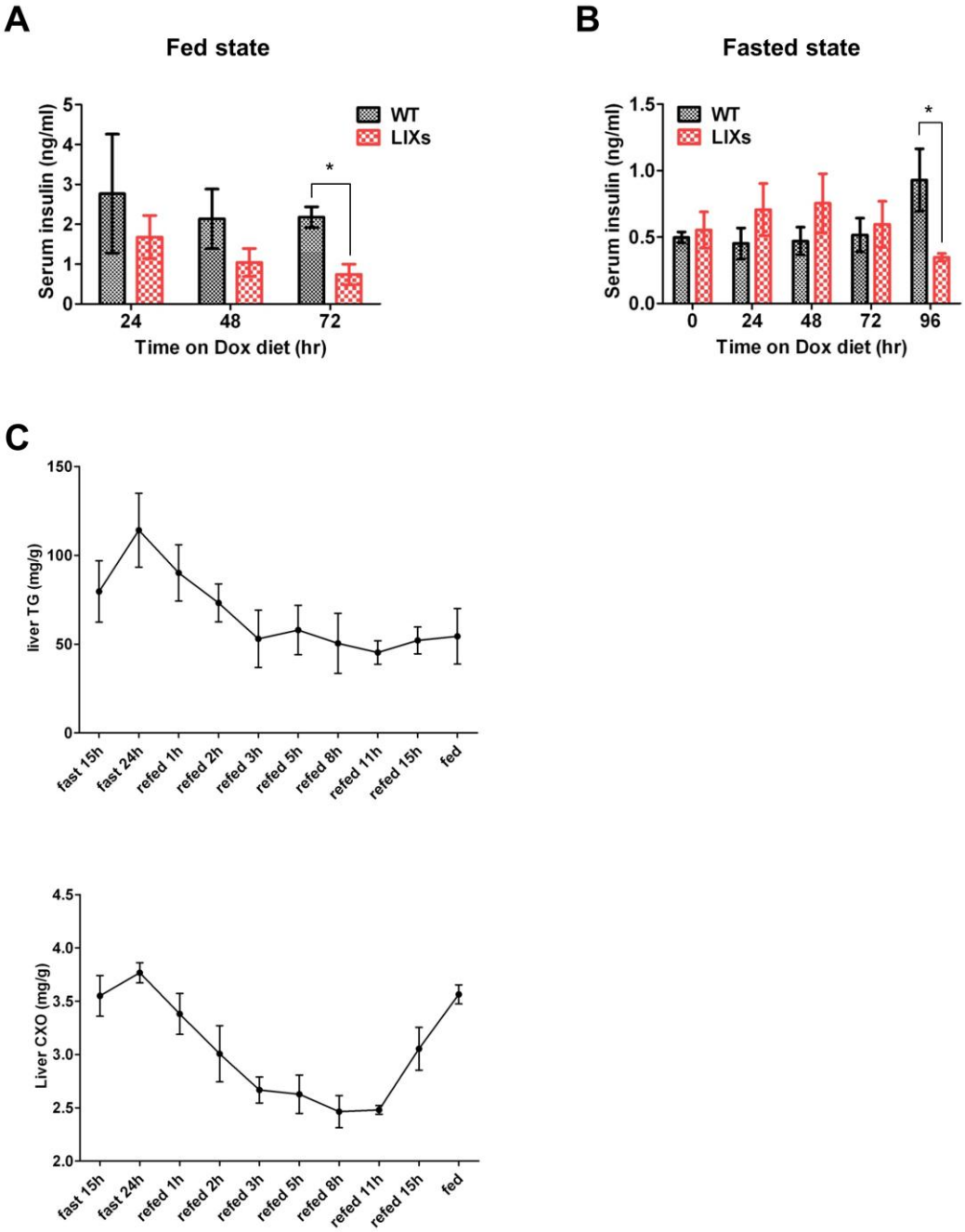


**Supplemental Figure 1. Upregulation of the UPR during fasting-refeeding in WT mice and inducible overexpression of Xbp1s in LIXs animals.**

**(A)** Food intake of WT FVB mice during fasting-refeeding in a metabolic cage study. Note that the food intake was increased in the initial phase of refeeding (1-2 hrs) and the middle of the experiment (6 hrs). This pattern is correlated with the induction Xbp1s expression. N = 3-5 per group. **(B)** WT FVB (N = 3 per group) mice were fasted up to 24 hrs and then refed up to 15 hrs before sacrifice. Immunoblotting was conducted to examine the protein levels of p-eIF2α and t-eIF2α. GAPDH serves as loading control. **(C)** Liver samples from (B) were processed for qPCR to assess the relative expression for the UPR genes, Bip, Atf6, and Chop. A representative result from two independent experiments is shown. **(D)** Liver samples from (B) were processed for qPCR to assess the relative expression for the Xbp1s target genes, ERdj4 and Edem1. A representative result from two independent experiments is shown. **(E)** Strategy of liver-specific inducible expression of Xbp1s. Liver-specific albumin-Cre mice were bred with the Rosa26-loxp-STOP-loxp-rtTA mice to achieve liver-specific expression of rtTA. This mouse was then crossed to Tre-Xbp1s transgenic mice. The resulting triple transgenic mice express Xbp1s in liver only after exposure to Dox. **(F)** Mice (N = 3 per group) were fed with Dox chow diet for 24 or 48 hrs. Before assessment, mice were fasted for 6 hrs during light cycle. qPCR was performed to examine the relative expression of Bip, ERdj4, Edem1 and Sec61a, which are known Xbp1s target genes. *, $p < 0.05$. (**G**) The body weight before and after a metabolic cage experiment was monitored. A trend to recover body weights is observed in LIXs mice compared to controls. N = 3-5 per group.

# Supplemental Figure 2



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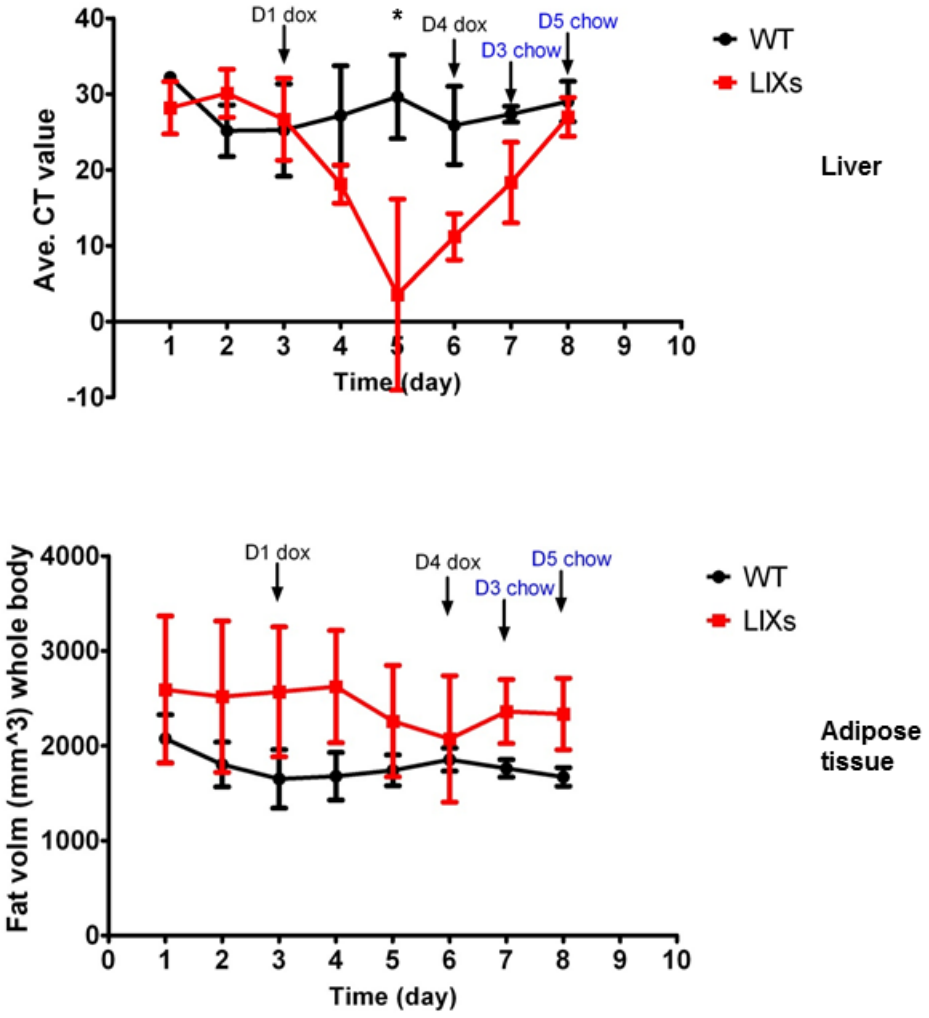

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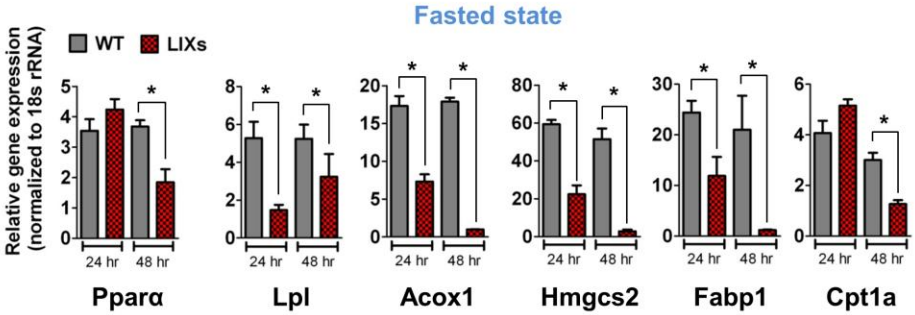


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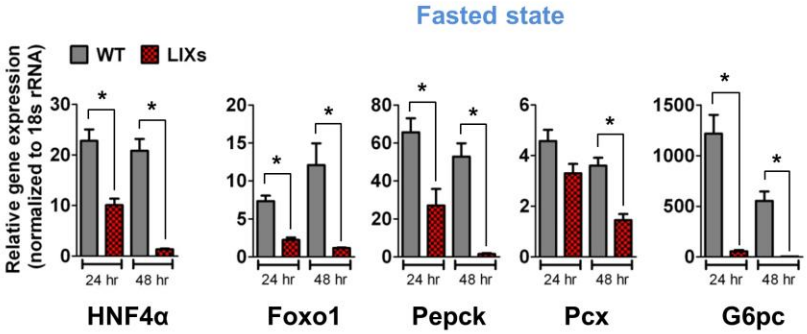


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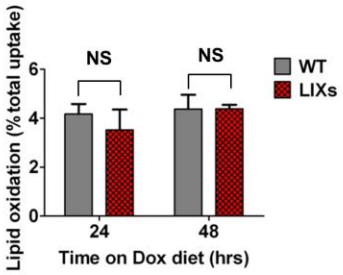


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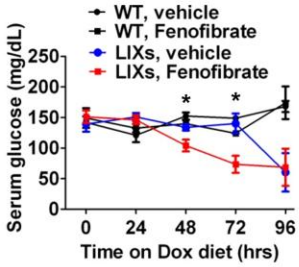

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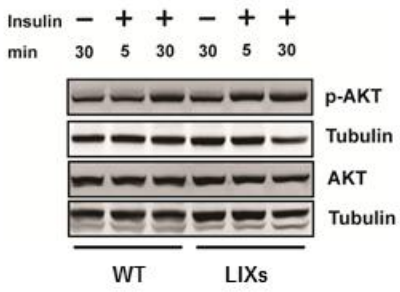


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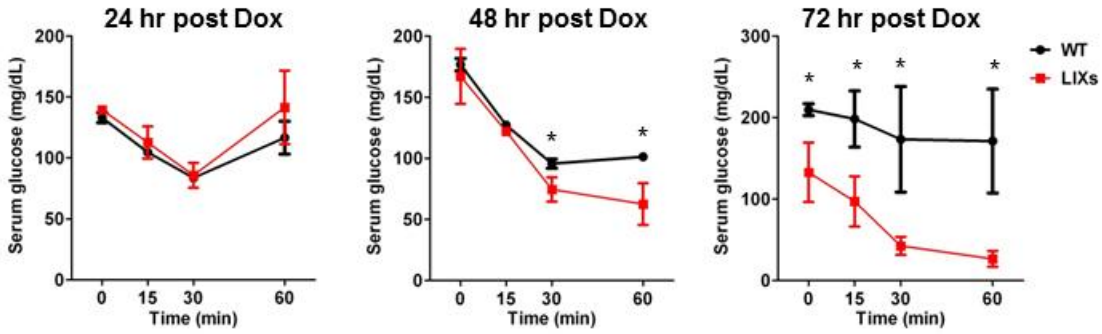


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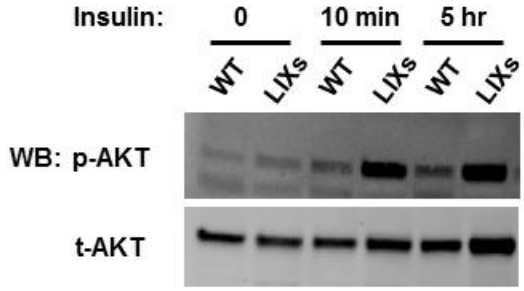
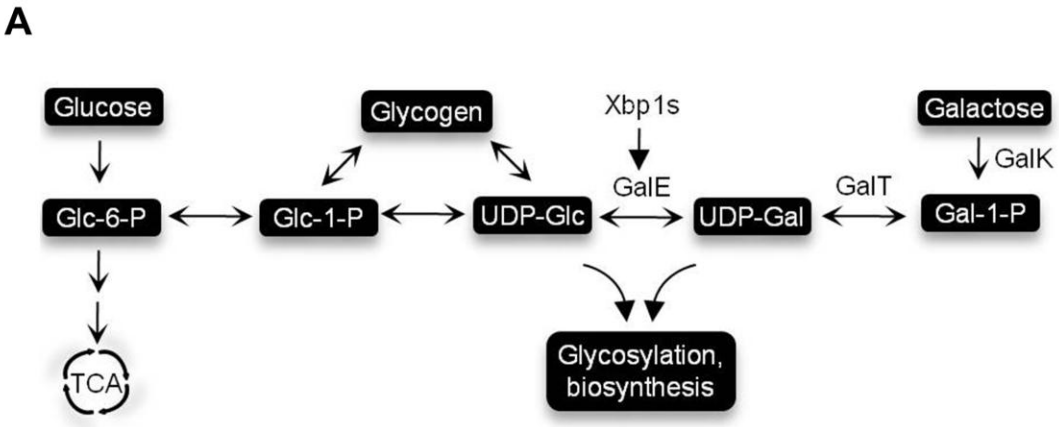


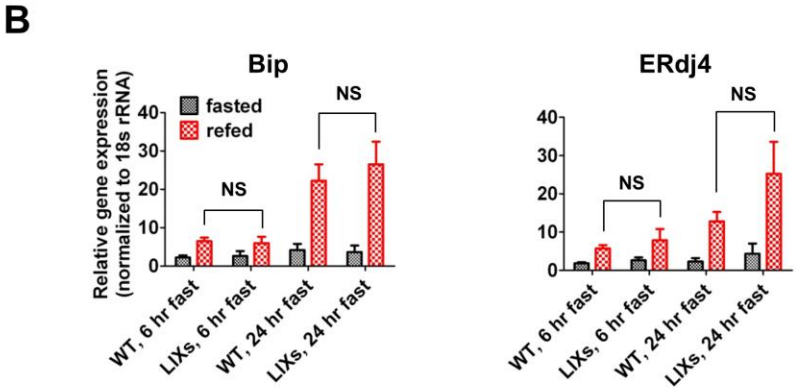
**Supplemental Figure 2. Acute induction of Xbp1s in liver caused profound metabolic changes.**

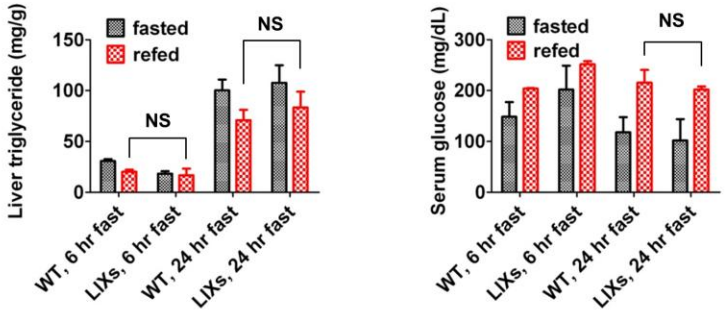
**(A)** WT and LIXs mice were exposed to Dox diet for different time. Serum insulin levels were measured in the fed state. N = 3 for each group. *, $p < 0.05$. **(B)** WT and LIXs mice were exposed to Dox diet for different time. Serum insulin levels were measured in the fasted state. N = 3 for each group. *, $p < 0.05$. **(C)** WT animals (8 weeks of age) were subjected to fasting and refeeding. Livers were harvested for biochemical measurements of TG and cholesterol. N = 3 for each group. Note that this is a representative result of two independent repeats. **(D)** WT and LIXs animals were subjected to whole body CT scan before, during and after Xbp1s induction. The values for liver and adipose tissue are shown. N = 3 for each group. *, $p < 0.05$. Note that a low numerical value indicates high TG content. **(E)** Mice (N = 3 per group) were fed with Dox chow diet for 24 or 48 hrs. Before assessment, mice were fasted for 6 hrs during light cycle. qPCR was performed to examine the relative expression of β-oxidation genes. *, $p < 0.05$. **(F)** Mice (N = 3 per group) were fed with Dox chow diet for 24 or 48 hrs. Before assessment, mice were fasted for 6 hrs during light cycle. qPCR was performed to examine the relative expression of gluconeogenic genes. *, $p < 0.05$. **(G)** β-oxidation assay was performed in livers from mice fed 24 hrs or 48 hrs of Dox food (N = 3 per group). NS, not significant. **(H)** Mice (N = 3-4 per group) were switched to Dox food and gavaged with fenofibrate daily for 5 days. Serum glucose levels were recorded daily. *, $p < 0.05$. **(I)** Hepatic insulin action was assayed by p-AKT immunoblotting in WT and LIXs animals after intraperitoneal insulin injection. **(J)** Insulin sensitivity was examined by insulin tolerance tests at 24 hrs, 48 hrs and 72 hrs post initiation of Dox food exposure. N = 3 for each

group. *, $p < 0.05$. LIXs mice show enhanced insulin sensitivity at later time points. **(K)** WT and LIXs primary hepatocytes were isolated and Dox was used to induce Xbp1s overexpression. Insulin signaling was surveyed by p-AKT levels.

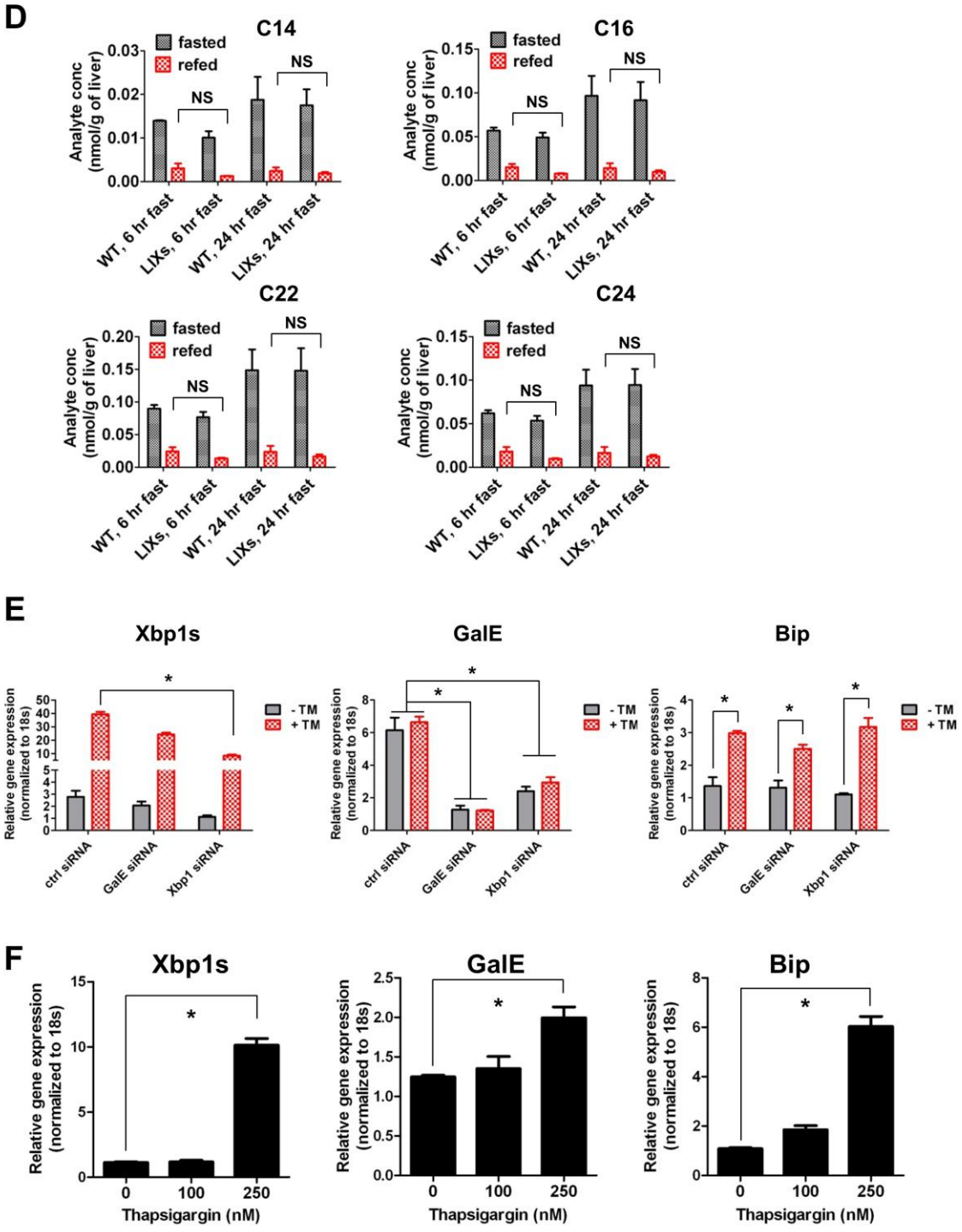
# Supplemental Figure 3



# Supplemental Figure 3 continued.

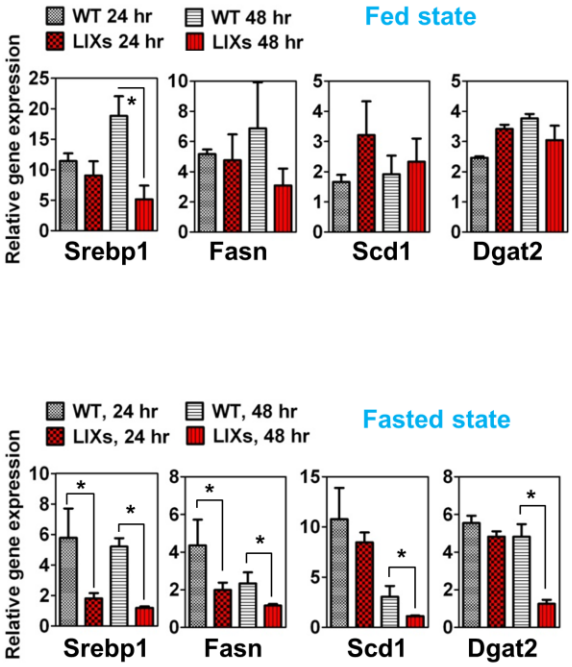


**Supplemental Figure 3. Metabolic parameters in WT and LIXs animals under fasting and refeeding conditions.**

**(A)** GalE is responsive for the interconversion of UDP-Gal and UDP-Glc, UDP-GalNAc and UDP-GlcNAc, which are critical for protein/lipid glycosylation in the biosynthetic pathway. **(B)** Mice (N = 3 per group) were fasted for 6 hrs or 24 hrs followed by 2 hr refeeding with regular chow. One dose gavage of Dox water was performed 3 hrs before refeeding or sacrifice of fasted groups. Gene expression of Bip and ERdj4 was determined by qPCR. NS, not significant. **(C)** Mice (N = 3 per group) were fasted for 6 hrs or 24 hrs followed by 2 hr refeeding with regular chow. One dose gavage of Dox water was performed 3 hrs before refeeding or sacrifice of fasted groups. No changes in liver triglyceride content and serum glucose levels were detected in WT and LIXs mice. NS, not significant. **(D)** Liver acyl-carnitine composition was shown from the same mice as (**B**). NS, not significant. **(E)** WT primary hepatocytes were treated with tunicamycin. Gene expression of Xbp1s, GalE and Bip were examined. siRNA for Xbp1s and GalE were used to test the relationship between tunicamycin and GalE expression. Note that while tunicamycin induces Xbp1s expression, no induction of GalE was observed. Bip expression, on the other hand, was stimulated by tunicamycin. N = 3 for each group. *, $p < 0.05$. **(F)** WT primary hepatocytes were treated with thapsigargin. Gene expression of Xbp1s, GalE and Bip were examined. Note that thapsigargin, in contrast to tunicamycin, can effectively stimulate Xbp1s, Bip and GalE. N = 3 for each group. *, $p < 0.05$.

# Supplemental Figure 4

## A

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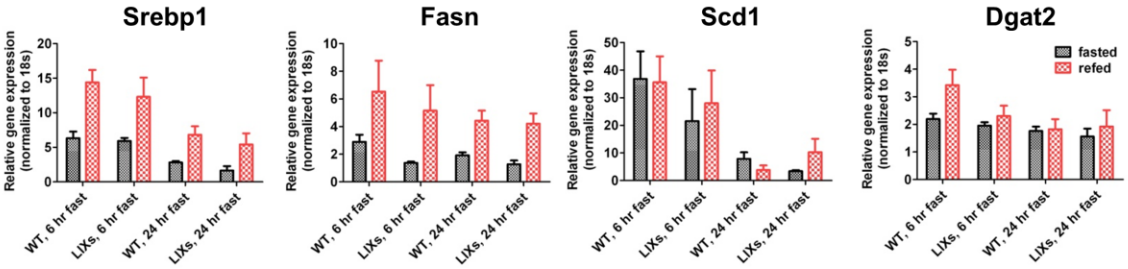

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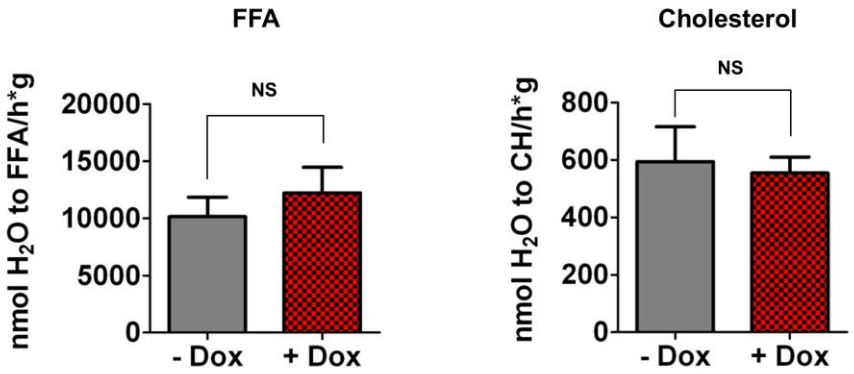
**C**

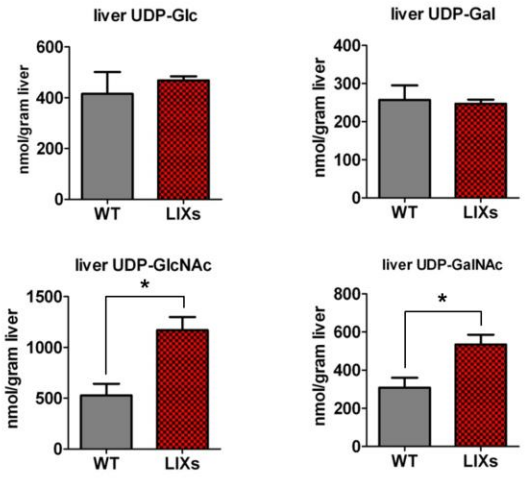



**Supplemental Figure 4. The effects of Xbp1s overexpression on lipogenesis.**

**(A)** Mice (N = 3 per group) were fed with Dox chow diet for 24 or 48 hrs. qPCR was performed to examine the relative expression of lipogenic genes under fed and fasted (6 hrs) conditions. *, $p < 0.05$. **(B)** Mice (N = 3 per group) were fasted for 6 hrs or 24 hrs followed by 2 hr refeeding with regular chow. One dose gavage of Dox water was performed 3 hrs before refeeding or sacrifice of fasted groups. Lipogenic gene expression was determined by qPCR. **(C)** LIXs mice were maintained on either control or Dox chow diet for 3 days. An *in vivo* for lipogenesis and cholesterol synthesis was performed (N = 5 per group). NS: not significant.

# Supplemental Figure 5

**A**

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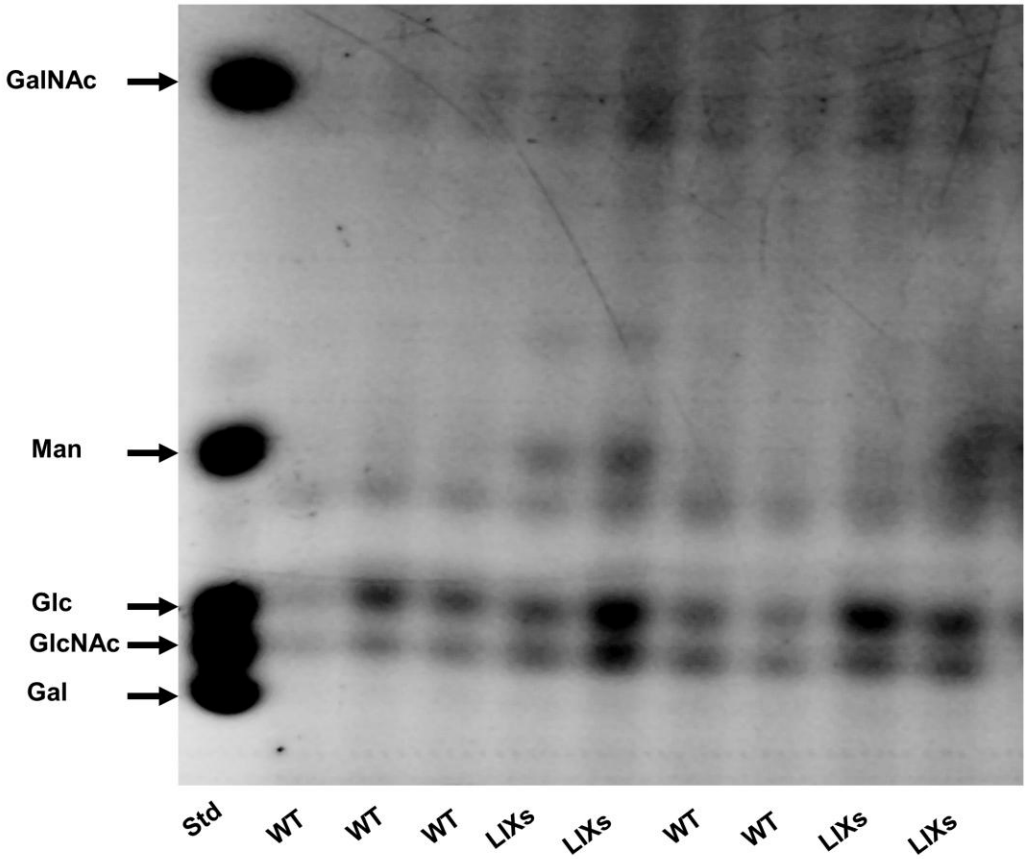

**Supplemental Figure 5. Liver nucleotide sugar and single-sugar O-glycosylation analysis. (A)** Mice (N = 3 per group) were fed with Dox chow died for 48 hrs and fasted for 6 hrs during light cycle before sacrifice for analysis of liver nucleotide sugars. *, $p < 0.05$. **(B)** Mice (N = 4-5 per group) were maintained on Dox food for 48 hrs and sacrificed under fed status. A representative FACE gel image was shown for AMAC labeled neutral O-glycan. Only mono- or disaccharides got into the gel.

**Supplemental Table 1. The top 10 genes upregulated in LIXs animals from the microarray study.**

| Top 10 | Symbol | Entrez Gene Name | Fold Change (24 hr LIXs *vs* WT) | Fold change (48 hr LIXs *vs* WT) | Fold change (2 hr refed *vs* 24 hr fast) | Type | Entrez Gene ID |
|---|---|---|---|---|---|---|---|
| 1 | RDBP | RD RNA binding protein | 4.003 | 1.442 | -1.228 | other | 27632 |
| 2 | CAMK2N2 | calcium/calmodulin-dependent protein kinase II inhibitor 2 | 3.683 | 1.558 | -1.081 | other | 73047 |
| 3 | SCARF2 | scavenger receptor class F, member 2 | 3.599 | 1.572 | 1.841 | receptor | 224024 |
| 4 | FKBP11 | FK506 binding protein 11, 19 kDa | 3.565 | 1.362 | -1.26 | enzyme | 66120 |
| 5 | ICA1 | islet cell autoantigen 1, 69kDa | 3.455 | 1.886 | 1.301 | other | 15893 |
| 6 | STX5 | syntaxin 5 | 3.378 | 1.477 | 1.359 | transporter | 56389 |
| 7 | GALE | UDP-galactose-4-epimerase | 3.328 | 1.831 | 1.193 | enzyme | 74246 |
| 8 | ALDH18A1 | aldehyde dehydrogenase 18 family, member A1 | 3.202 | 1.591 | 1.914 | kinase | 56454 |
| 9 | DYNLL1 | dynein, light chain, LC8-type 1 | 3.168 | 1.425 | 1.119 | other | 56455 |
| 10 | IGSF5 | immunoglobulin superfamily, member 5 | 3.113 | 1.999 | 1.616 | other | 72058 |

**Supplemental Table 2**

| Gene | Primers | Use |
|---|---|---|
| Xbp1 (mouse) | ACACGCTTGGGAATGGACAC | PCR |
| | CCATGGGAAGATGTTCTGGG | |
| GAPDH (mouse) | AACTTTGGCATTGTGGAAGG | PCR |
| | ACACATTGGGGGTAGGAACA | |
| Actin (mouse) | TACCACAGGCATTGTGATGG | PCR |
| | TTTGATGTCACGCACGATTT | |
| Xbp1s (mouse/human) | GGTCTGCTGAGTCCGCAGCAGG | qPCR |
| | GAAAGGGAGGCTGGTAAGGAAC | |
| ERdj4 (mouse) | CAGAATTAATCCTGGCCTCC | qPCR |
| | ACTATTGGCATCCGAGAGTG | |
| Scd1 (mouse) | GCCGAGAAGCTGGTGATGTT | qPCR |
| | ATAGAGATGCGCGGCACTGT | |
| Fasn (mouse) | AGGAGGTGGTGATAGCCGGT | qPCR |
| | GGTCCATTGTGTGTGCCTGC | |
| Bip (mouse) | CCTCTCTGGTGATCAGGATA | qPCR |
| | CGTGGAGAAGATCTGAGACT | |
| Edem1 (mouse) | CTGCAATGAAGGAGAAGGAG | qPCR |
| | TAGAAGGCGTGTAGGCAGAT | |
| Sec61 (mouse) | CTCATGAACCTGATTGCCAC | qPCR |
| | ACCAGAGCAGACTGCAAGAT | |
| Pparα (mouse) | TCACAAGTGCCT GTCTGTCG | qPCR |
| | CAGGTAGGCTTCGTGGATTC | |
| Lpl (mouse) | GTGTGATTGCAGAGAGAGGA | qPCR |
| | TTCTACAACTCAGGCAGAGC | |
| Acox1 (mouse) | CTCACTCGAAGCCAGCGTTA | qPCR |
| | CGTGATCTCCAGATTCCAGG | |
| Fabp1 (mouse) | ACCAATTGCAGAGCCAGGAG | qPCR |
| | TCACCTTCCAGCTTGACGAC | |
| Cpt1a (mouse) | GTGACGTTGGACGAATCGGA | qPCR |
| | TCGGTGGCCATGACATACTC | |
| Hmgcs2 (mouse) | CATGGAGAACGCGTACGACT | qPCR |
| | TGAACATCAACCGAGCCAGG | |
| HNF4α (mouse) | GCATGGATATGGCCGACTAC | qPCR |
| | TGTGGTTCTTCCTCACGCTC | |
| Pepck (mouse) | TGA CAA CTG TTG GCT GGCTC | qPCR |
| | GAC ATA CAT GGTG CGG CCTT | |
| Pcx (mouse) | GTG AGA T TGC CAT CCGA GTG | qPCR |
| | TCTG CTC GCT CTGA GAG GAA | |
| G6pc (mouse) | GGCGCAGCAGGTGTATACTA | qPCR |
| | ATGCCTGACAAGACTCCAGC | |
| GalE (mouse) | CCATAACGCCATTCGTGGAG | qPCR |
| | TCCAGAGGCTTCTGCACTGA | |
| GalK (mouse) | TGGCCACGTACACCTTCATC | qPCR |
| | ACAGTGGCACCAGGCTTGTT | |

| | | |
|---|---|---|
| GalE (human) | CCATAATGCCTTCCGTGGAG | qPCR |
| | AATCCAGAGGCTTCTGCACC | |
| 18s (mouse) | AGGGTTCGATTCCGGAGAGG | qPCR |
| | CAACTTTAATATACGCTATTGG | |
| 18s (human) | AAACGGCTACCACATCCAAG | qPCR |
| | CCTCCAATGGATCCTCGTTA | |
| GalE promoter | GCTAGCCGAAGTCCAGAGCTAAGCTCC | cloning |
| | CTCGAGGGACATCAGACACACCATGC | |
| GalE promoter (mouse) | TGTGTGGACTGCCTCTCTGT | CHIP assay |
| | GGAAGCAGTAAGAACGCCAA | |
| Atf6 (mouse) | GCCGACTGTGGTTCAACTTC | qPCR |
| | TCCTCAGCACAGCGATATCC | |
| CHOP (mouse) | GTATGAGGATCTGCAGGAGG | qPCR |
| | CTGACTGGAATCTGGAGAGC | |
| FoxO1 (mouse) | GCAGCCAGGCATCTCATAA | qPCR |
| | CCTACCATAGCCATTGCAGC | |
| Srebp1 (mouse) | TGCCATTGAGAAGCGCTACC | qPCR |
| | TCCACTGCCACAAGCTGACA | |
| Dgat2 (mouse) | GCCTGCAGTGTCATCCTCAT | qPCR |
| | TGGTGGTCAGCAGGTTGTGT | |