Supplemental Data

Upstream transcription factor 1 influences plasma lipid and metabolic traits in mice

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Supplemental Figures

Supplemental Figure 1. (A) Schematic diagram of BAC clone CTD30030l2. Filled rectangles represent coding regions of the BAC. Arrows indicate the direction of transcription. (B) Schematic diagram of adenoviral vector (DUALGFP-CCM). AdUSF1 vector had USF1 cDNA (from pOTB7-hUSF1) inserted in the multiple cloning site (MCS) using EcoRI/XhoI restriction enzymes. The control adenoviral vector is as shown, without hUSF1 cDNA inserted.

Supplemental Figure 2. USF1 Transgenic Mice Consume More Food than Wildtypes. (A) food consumption/body weight, (B) food consumption and (C) body temperature of high expresser line female USF1 transgenic (blue, n=5) and non-tg littermates (yellow, n=7). Significance (*)=P<0.05.

Supplemental Figure 3. Human USF1 mRNA (A) and protein expression (B) in livers from Adenoviral vector transfected mice. In panel A, relative hUSF1 transcript levels are expressed relative to beta-actin, and were not detectable in the control eGFP vector mice. Panel B shows human USF1 protein bands from Western blot analyses, using the mouse monoclonal anti-human USF1 antibody AB58100 (Abcam). Each lane represents an individual sample; + indicates the cells or mouse were transfected with the AdUSF1 vector and – indicates transfection with the control eGFP vector.

Supplemental Figure 4. Apolipoprotein A-1 (ApoA1) and apolipoprotein B (ApoB) transcript levels in AdUSF1 and control eGFP transfected liver. Transcript levels were measured by real time qPCR as described in the Methods section of the paper, and expressed as a ratio to TATA box binding protein (Tbp) transcript levels. * indicates $p < 0.05$ by unpaired t-test.

Supplemental Figure 5. Trait and *Usf1* transcript levels for F2 mice homozygous for B6 (open bars) or C3H (shaded bars) alleles at the marker nearest the *Usf1* gene. Graphs depict mean +/- SEM. Significant differences between tg and wt mice by unpaired t-test are indicated by *: $p< 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

Supplemental Tables:

Supplemental Table 1. Plasma triglyceride values (mg/dL; mean ± SEM (n)) in transgenics and wild-type littermate controls for each of the transgenic lines. P-value is from unpaired t-test of transgenic vs control.

Group (line; sex)	*Controls* *Mean ±SEM (n)*	*Transgenics* *Mean ±SEM (n)*	*p-value*
High; female	170 ± 28.0 (14)	119 ± 19.4 (15)	0.137
High; male	159 ± 23.0 (10)	197 ± 40.7 (7)	0.400
Medium; female	181 ± 28.6 (11)	259 ± 45.2 (11)	0.160
Medium; male	190 ± 10.8 (24)	218 ± 17.5 (14)	0.151
Low; female	192 ± 23.6 (15)	247 ± 19.6 (18)	0.080
Low; male	167 ± 28.9 (12)	190 ± 24.9 (8)	0.575

Supplemental Table 2. (separate supplemental file). AdUSF1 signature gene sets for male and female mice. P-value determined by two-sided unpaired t-test between AdUSF1 and AdGFP control samples (n=3 each). DE fold column indicates relative fold increase or decrease of transcript levels for AdUSF1 relative to AdGFP treated mice. Transcripts of the 56 genes differentially expressed in both male and female mice are highlighted by yellow shading.

Supplemental Table 3. (separate supplemental file). Complete results for ontology enrichment analyses for the Adenoviral overexpression and F2 *Usf1* signature gene sets for each sex.

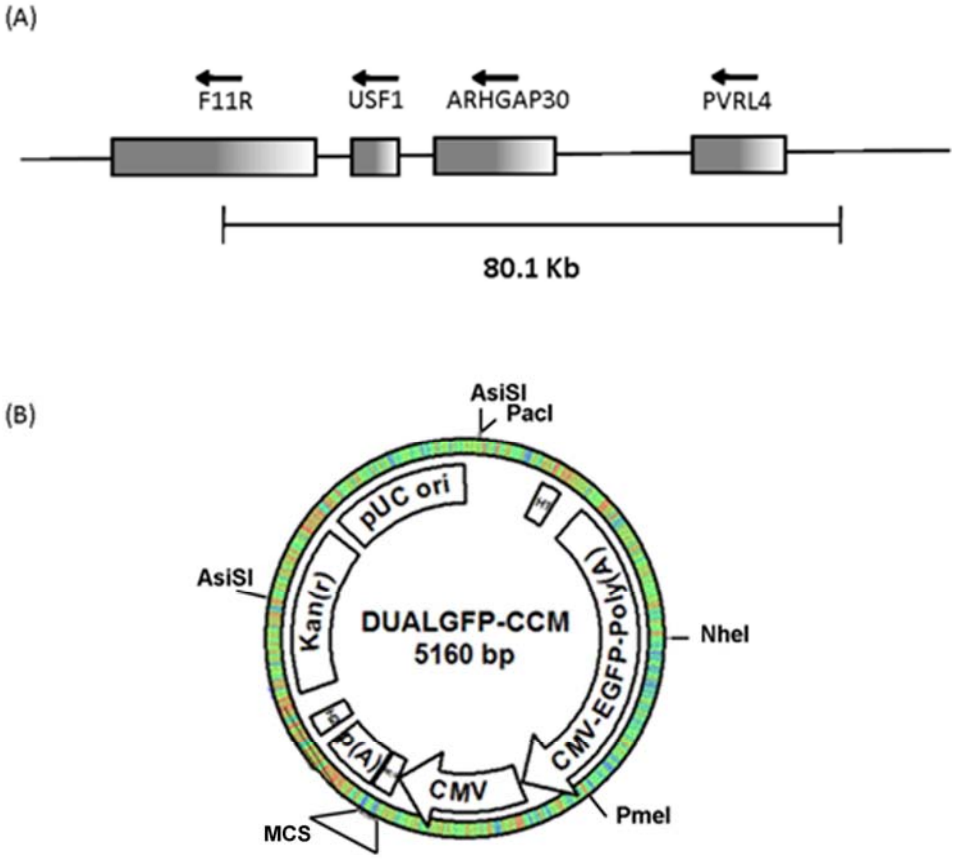
Supplemental Table 4. Pearson correlation coefficients between hepatic *Usf1* mRNA levels and phenotypic traits from BXH ApoE null F2 mice. *: P<0.05, **: P<0.001. Superscript C indicates causative role of *Usf1* to the corresponding trait listed in each column, determined by likelihood-based causality model selection procedure (references 32 and 34 in manuscript). Adiposity is calculated as the total fat mass divided by body mass, expressed as a %.

Trait	**Male**	**Female**
Body weight	-0.01	0.17*
Total Fat	-0.17*	0.17*,C
Adiposity	-0.19*,C	0.15
Plasma Glucose	0.32**,C	0.29**
Plasma Insulin	0.20*,C	0.20*,C
Plasma Glucose/Insulin ratio	-0.11	-0.08
Plasma Total Chol	0.30**,C	0.10
Plasma HDL Chol	0.43**,C	0.40**,C
Plasma LDL/VLDL Chol	0.30**	0.08
Plasma Unesterified Chol	0.30**,C	0.12
Plasma Triglycerides	0.34**	0.29*,C
Plasma Free Fatty Acid	0.36**,C	0.26*,C

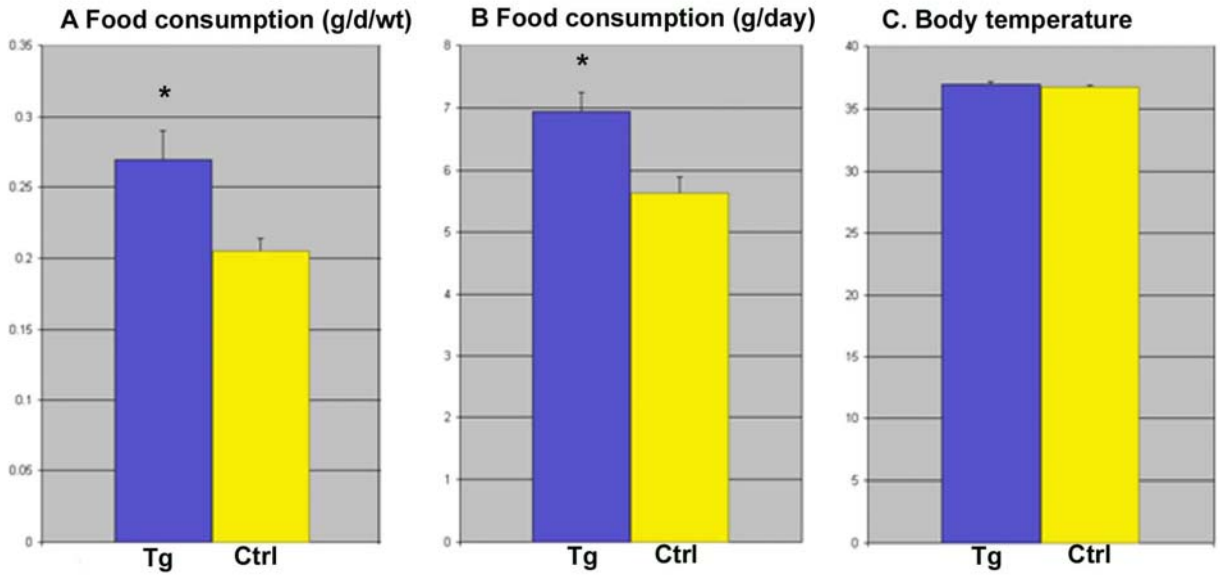
Supplemental Table 5. (separate supplemental file). BXH ApoE null F2 *Usf1* signature gene sets. Column headings are: transcript_id, gene_symbol, Entrez geneID, corrPm (r from Pearson's correlation with Usf1 transcript levels), pvaluePm (p-value for Pearson's correlation), q.value (q-value from Storey false discovery rate analysis as described in article), module (module assignment from co-expression analysis), k.in.normed (normalized Kin value for transcript from co-expression analysis as described in article). (There is no implied relationship between modules from different sexes that have the same assigned color designation.) For the Female lightcyan module, the genes depicted in Figure 5 of the paper are correspondingly color coded here with yellow shading indicating shared with AdUSF1 signature; pink: depicted in graph but not shared.

Supplemental Table 6: (separate supplemental file). Module significance results for all modules and traits (Pearson correlation coefficient and corresponding nominal p-value for module eigengene vs each phenotypic trait, as described in article). Genes in each module are given in Supplemental Table 5. (There is no implied relationship between modules from different sexes that have the same assigned color designation.) Column headings are: Module, Trigly (plasma triglyceride), Total_Chol_log (log total plasma cholesterol), LDL_plus_VLDL (LDL plus VLDL fractions of total cholesterol in plasma), HDL_Chol_log (log plasma HDL cholesterol), UC (plasma unesterified cholesterol), FFA (plasma free fatty acids), Glucose (plasma glucose), Insulin_log (log plasma insulin), Glucose_Insulin (plasma glucose insulin ratio), weight_g (body weight in grams), ab_fat (abdominal fat mass), other_fat (sum of subcutaneous, retroperitoneal, and gonadal fat mass), total_fat (sum of all fat pad mass), X_00xfat_weight (adiposity as % sum of fat mass divided by body mass), Leptin_sqrt (square root of plasma leptin).

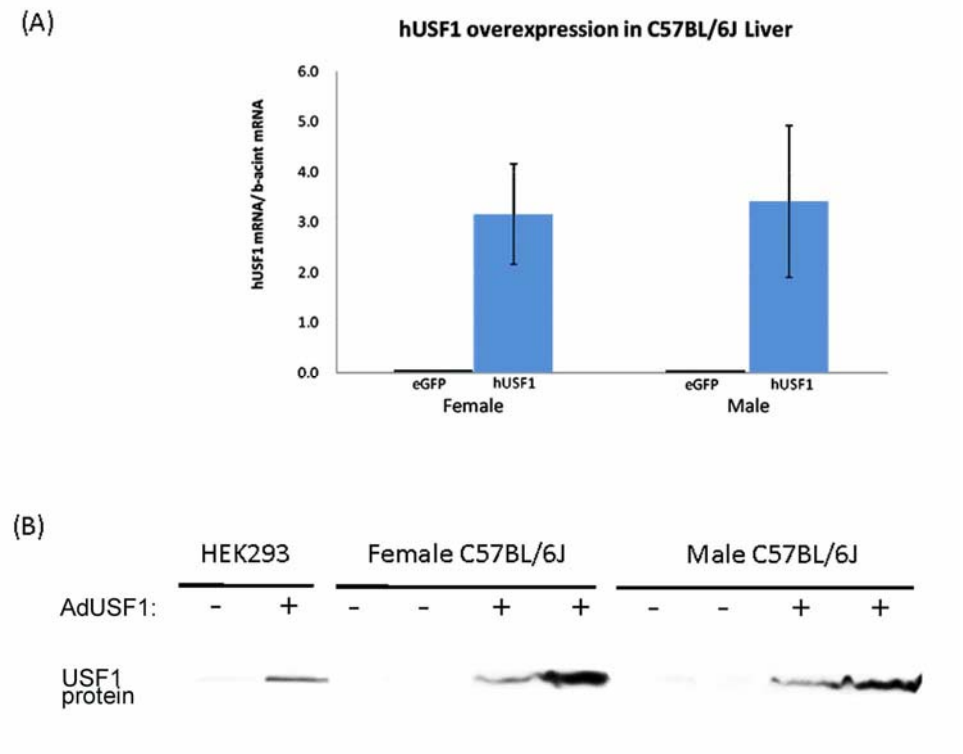
Supplemental Figure 1.



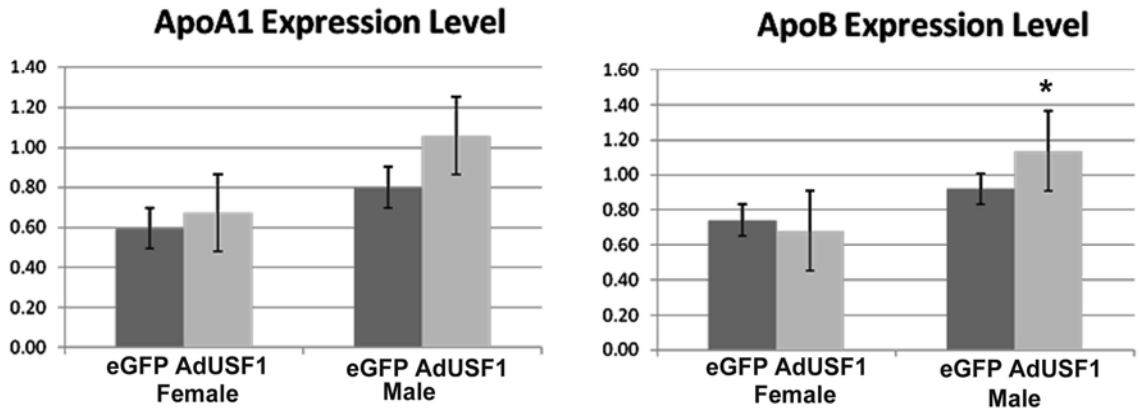
Supplemental Figure 2.



Supplemental Figure 3.



Supplemental Figure 4.



Supplemental Figure 5.