Supplementary Materials and Methods

Quantitative RT-PCR

A 5mm stainless steel bead was added to each tube containing tissue and tissue was homogenized using a Qiagen TissueLyser II in QIAzol Lysis Reagent. RNA extraction was carried using the RNeasy Lipid Tissue Mini Kit (Qiagen, CA, USA). Reverse transcription was performed using the Superscript VILO cDNA synthesis kit (Invitrogen, CA, USA). Gene primers for qPCR were designed to span exon-exon junctions of the gene of interest. Quantitative PCR was performed using Power SYBR Green PCR Master Mix (Invitrogen) and the PRISM 7900 instrument (Applied Biosystems, CA, USA) or Viia7 instrument (ABI). PCR conditions: 95^{o}C for 10 min, 40 cycles of 95^{o}C for 10 sec, 60^{o}C for 60 sec. Single product amplification was confirmed by disassociation curves. Each sample was amplified three times for precision and the average of these technical replicates was used to calculate expression. Gene expression was normalized to TATA-binding protein (*Tbp*) internal loading control, and analyzed using the $2^{-(\Delta\Delta CT)}$ method and was displayed as *Mecp2*/Y expression relative to wild-type.

Table S1. Primer sequences used for qRT-PCR analysis

Gene	Forward	Reverse
Acca	GGTTCCAGGCACAGTCCTTA	GGCTACCATGCCAATCTCAT
Accb	GGCCGAGGTTAAGATCAACA	ATGCAGGCTACCTTGCTTGT
Cd36	TCATGCCAGTCGGAGACATG	TGGTGCCTGTTTTAACCCAGTT
Cd68	CATCAGAGCCCGAGTACAGTCTACC	AATTCTGCGCCATGAATGTCC
Fas	TTGACGGCTCACACACCTAC	CGATCTTCCAGGCTCTTCAG
Hmgcr	TGTGGCCAGCACTAACAGAG	GGCCTCCTTTATCACTGCAA
Il6	CCAGAGATACAAAGAAATGATGG	ACTCCAGAAGACCAGAGGAAAT
Il10	GGTTGCCAAGCCTTATCGGA	AATCGATGACAGCGCCTCAG
Lss	GCGGAATGTGCAGAGTGCTA	TGTTCTCCTGAGGCCAGTCT
Mecp2	AGGAGGAGAGACTGGAGGAAAAG	CTTTCTTCGCCTTCTTAAACTTCAG
Plin2	GTGGAAAGGACCAAGTCTGTG	GACTCCAGCCGTTCATAGTTG
Plin5	AGGCTGAGCTAGCAGTCCT	CTGGGTACGGTGTTTGCTCT
Scd1	CCTGCGGATCTTCCTTATCA	CTTCTCGGCTTTCAGGTCAG
Sqle	TTGGTGGAGAGTGTGTGACC	CAGCACCGCTACTGAAAAGG
Srebp1c	CAAGGCCATCGACTACATCCG	CACCACTTCGGGTTTCATGC
Tbp	CCTTGTACCCTTCACCAATGAC	ACAGCCAAGATTCACGGTAGA
Tnfa	TGTCTACTCCCAGGTTCTCT	GGGGCAGGGGCTCTTGAC

Tolerance tests

Intraperitoneal glucose tolerance tests were performed at four and eight weeks of age in *Mecp2*/Y and wild-type male littermates, at 12 weeks and 20 weeks in *Mecp2*/+ and wild-type female littermates, and at six months of age in *Alb*-Cre, *Mecp2*^{flox}/Y, *Mecp2*^{flox}/*Y*; *Alb*-Cre, and wild-type male littermates. After a five-hour fasting period, animals were lightly anesthetized with isoflurane. A small tail amputation was made and blood was collected for baseline glucose reading. Mice were intraperitoneally injected with glucose (2g/kg lean body mass). Blood glucose was sampled from the tail incision using the AlphaTRAK Blood glucose monitoring system at 15, 30, 60, and 120 minutes after glucose injections. Intraperitoneal insulin tolerance tests were performed following a four-hour fast. Animals were lightly anesthetized with isoflurane and a small tail amputation was made. Blood glucose was measured at baseline. Insulin was then administered (0.75milliunit/g bodyweight) and glucose levels were obtained 15, 30, 60, and 120 minutes.

Lipolysis analysis

In vivo lipolysis analyses were performed by the Diabetes and Endocrinology Research Center at BCM. Briefly, mice were fasted for four hours and treated with an intraperitoneal injection of CL316243 (0.1 mg per kg of body weight). Blood was collected before and 15 minutes after injection for determination of non-esterified fatty acids (NEFA) and glycerol levels in serum.

Immunohistochemistry

A small piece of subcutaneous and perigonadal white adipose tissue samples were rapidly dissected from *Mecp2*/Y (eight weeks of age) and age-matched wild-type littermates and placed in 10% neutral buffered formalin. Samples were fixed for at room temperature for 48 hours and were processed and sectioned. Sections were deparaffinized and rehydrated through graded

alcohols. Endogenous peroxide was quenched in 3% H2O2 and antigen retrieval was performed by boiling sections in 10mM Sodium Citrate buffer (pH 6.0) for 20 minutes. Sections were blocked for 30 minutes using Serum-Free Protein Block (Dako). Primary antibody against F4/F80 (ab6640) was added at 1:100 and incubated overnight at 4C. Bound antibody was detected with biotin-conjugated rabbit anti-rat IgG (Vector Labs) at 1:100, followed by avidin/biotin-HRP binding (Vector Labs), stained with DAB and counterstained with hematoxylin.

Chromatin Immunoprecipitation and ChIP-qPCR

Livers were quickly dissected, cut into ~0.2 cm^3 pieces, and incubated in Solution A (1% formaldehyde, 50mM Hepes-KOH, 100mM NaCl, 1mM EDTA, 0.5mM EGTA) for 20 minutes at room temperature. Crosslinking was quenched with 2.5M glycine for 5 minutes. Livers were quickly washed with ice-cold PBS and flash frozen. Frozen livers were dounce homogenized in PBS. Cells were strained through a 100uM filter, centrifuged, and washed with two exchanges of ice-cold PBS. Liver cells were resuspended and washed in exchanges of LB1 (50mM Hepes-KOH, pH 7.5, 140mM NaCl, 1mM EDTA, 10% glycerol, 0.5% Igepal CA-630, 0.25% Triton X-100), LB2 (10mM Tris-HCl, pH 8.0, 200mM NaCl, 1mM EDTA, 0.5mM EGTA) and LB3 (10mM Tris-HCl, pH 8.0, 100mM NaCl, 1mM EDTA, 0.5mM EGTA, 0.1% Na-Deoxycholate, 0.5% *N*-lauroylsarcosine). Chromatin in LB3 was sheared using a microtrip sonicator to a range of 200-400 base pairs. Triton X-100 was added to sheared chromatin to a final concentration of 1% and a small aliquot was removed to represent the input. Remaining sheared chromatin was incubated with anti-H3K27ac (Millipore 05-1334), anti-HDAC3 (Abcam ab7030), in the presence of 0.5% BSA-washed Protein G-Dynabeads (Invitrogen) overnight at 4C. Beads were then washed seven times with ice-cold RIPA buffer (50mM Hepes-KOH, pH 7.5, 500mM LiCl, 1mM EDTA, 1% Igepal CA-630, 0.7% Na-Deoxycholate) followed by one wash with TBS (20mM Tris-HCl, pH 7.6, 150mM NaCl). Residual TBS was removed and chromatin was eluted from the

beads in elution buffer (50mM Tris-HCl, pH 8, 10mM EDTA, 1% SDS) at 65C overnight. Input and immunoprecipitated chromatin were treated with RNAse A at 37C for 30 minutes and Proteinase K at 55C for 2 hours. DNA was purified in Phenol:Choloroform:Isoamyl Alcohol using PhaseLock Gel Light tubes (5 PRIME, Germany) and following the manufacturer's instructions and washed with ethanol. Primers used for downstream qPCR analysis of immunoprecipitated and input DNA were as follows:

Table S2. Primer sequences used for ChIP-qPCR analysis

Site	Forward	Reverse
*Sqle*_Site1	CGTAGCATGTAGAACCGCTG	GTGCAACAAAGGGAATGGGTC
*Sqle*_Site2	CTAATTATGGCAGGTTCGCCG	GCTAGAAAGGCGAGGAGGAC
Sqle Site3	ACTCTGGTTACTGTCCGCGA	CTCGGATGAGCGTCCTTCAG
*Sqle*_Site4	AAATGCGGGGATGTCACCCT	AGCGACAGGAACACCAGC
*Sqle*_Site5	GTGTGGCAGGTAAGGCTACT	CGGACTGCGCTGACTAATTG
Sqle Site6	ACAGTAGAAGGTCTCAATGCCC	GTTTCTGACAGTGGGTACGGA
Arbp (promoter)	GAGGTGGCTTTGAACCAGAG	TCTTTGTCTCTGTCTCGGAAAA
*Fasn*_Site1	GAGTGAGTGAGTGCACCCTT	CTGGAGGTTCGACCCATGAC
Fasn Site2	TAGAGGGAGCCAGAGAGACG	CAGACGACAAGCGAGGAGC
*Fasn*_Site3	TGACTGTGAGAGACCCACCA	CCGACATACCGGCTATCACC
*Cd36*_Site1	AGTATAGAAGGGGCGGCTCA	AGTCAGAGGCCAGAGAACCA
Cd36 Site2	TAGAACCGGGCCACGTAGAA	AGCACTGGTTTTCTCGCCAA
*Cd36*_Site3	AAGGGGAAGCCTACTTGCTATG	ATGGGTACTTCTACACAGGGC

Indirect Calorimetry

Mice were housed individually in calorimetry cages for a period of 24 hours beginning with a 4-hour light-phase acclimatization period (12:00-15:00), followed consecutively by a 4-hour light-phase (15:00-19:00), a 12-hour dark-phase (19:00 - 07:00), and a 4-hour light-phase (07:00-11:00). Cumulative food intake, volume of oxygen consumed and volume of carbon dioxide produced were measured. From these data the respiratory exchange ratio and energy expenditure were derived. Respiratory exchange ratio was measured by VCO_2/VO_2. Energy expenditure was adjusted for total body weight using linear regression and analysis of covariance (ANCOVA).

Supplementary Figures

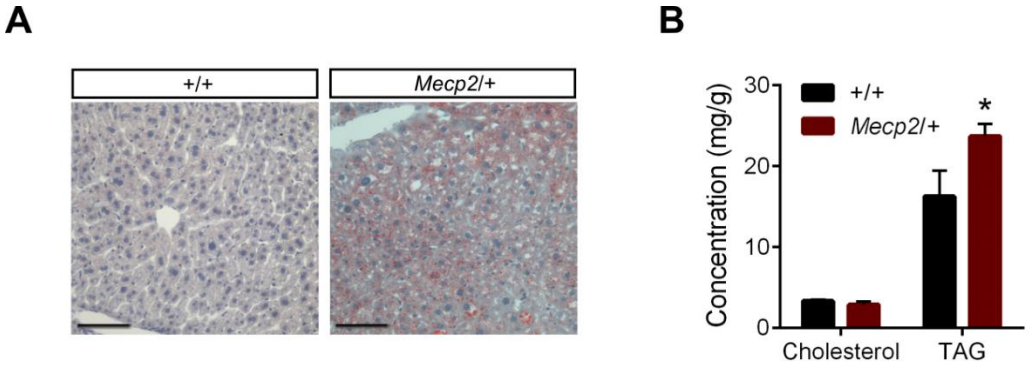

Figure S1. *Mecp2* heterozygous (*Mecp2*/+) female mice develop fatty liver.

A. Oil Red O staining of *Mecp2* heterozygous female liver and wild-type (+/+) liver at six-months of age. Scale bar indicates 100uM.

B. Quantification of lipids in livers at six-months of age (n = 7 mice per per genotype). Values are mean $\pm$ SEM. *p<0.05, **p$\leq$0.01, ***p$\leq$0.001 versus wild-type littermates.

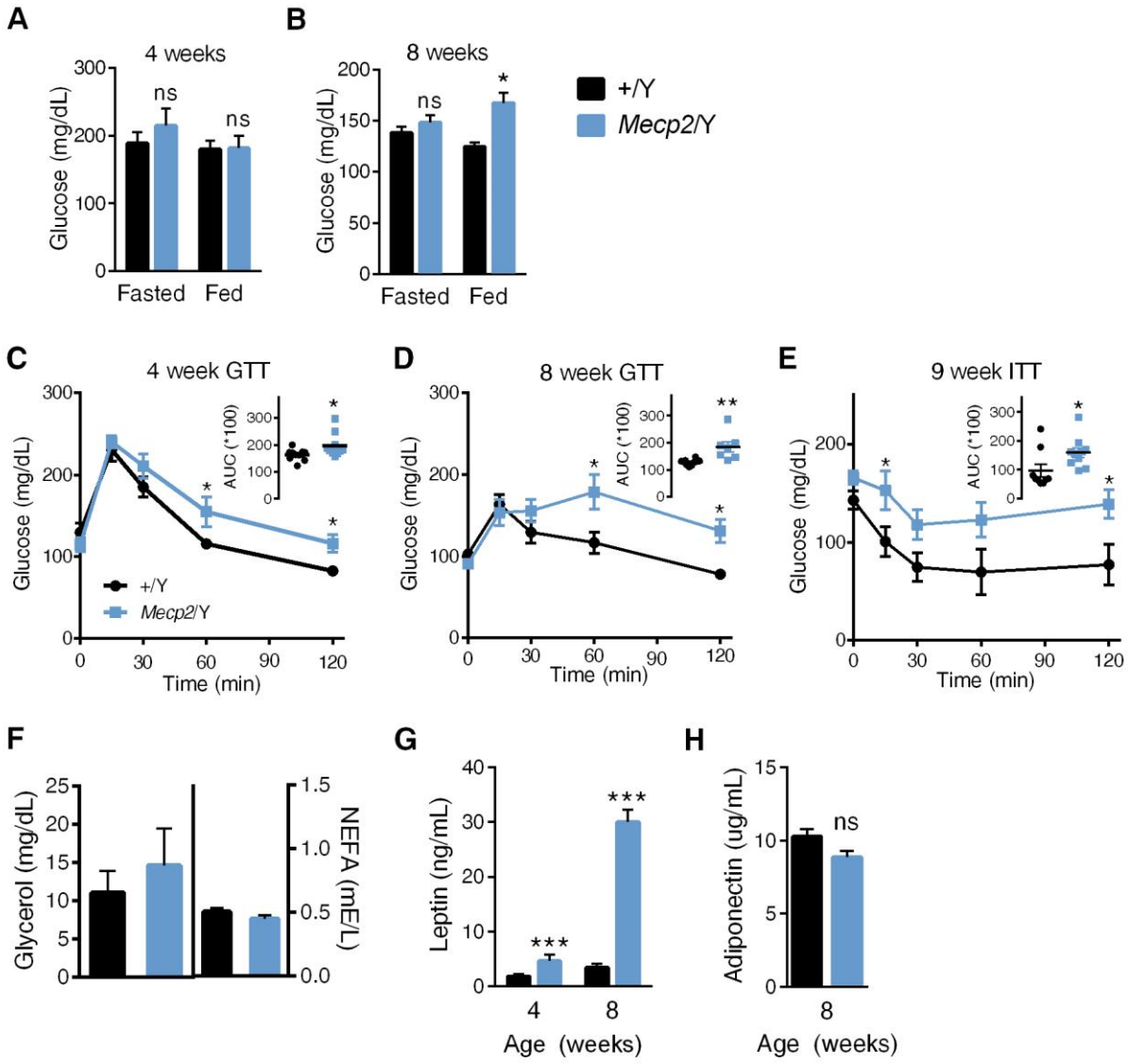

Figure S2. *Mecp2* null male mice are glucose intolerant and insulin resistant.

A. Serum glucose concentration in male null *Mecp2*/Y mice and wild-type littermates at four-weeks of age following a six-hour fast (n = 6-8 mice per genotype) or in the fed state (n = 5-7 per genotype).

B. Serum glucose concentration at eight-weeks of age following a six-hour fast or in the fed state (n = 6-8 per genotype).

C. Glucose tolerance tests (GTT) at four-weeks of age. Glucose was administered intraperitoneally after a five-hour fast, and blood glucose was measured at the indicated times (n = 7-10 per age per genotype).

D. GTT in at eight-weeks of age (n = 7-10 per age per genotype).

E. Insulin tolerance tests (ITT). Insulin was administered intraperitoneally after a four-hour fast, and blood glucose was measured at the indicated times. Mice were tested at 9 weeks of age (n = 9-10 per age per genotype).

F. Basal serum non-esterified fatty acids (NEFA) and glycerol levels in 4-hour fasted male mice at four-weeks of age (n = 7 mice per genotype).

G. Serum leptin concentration at four- and eight-weeks of age (n = 6-8 mice per genotype).

H. Serum adiponectin concentration at eight-weeks of age (n = 6-8 mice per genotype).

A-H. Values are mean $\pm$ SEM. *$p<0.05$, **$p\leq0.01$, ***$p\leq0.001$ versus wild-type, age-matched littermates.

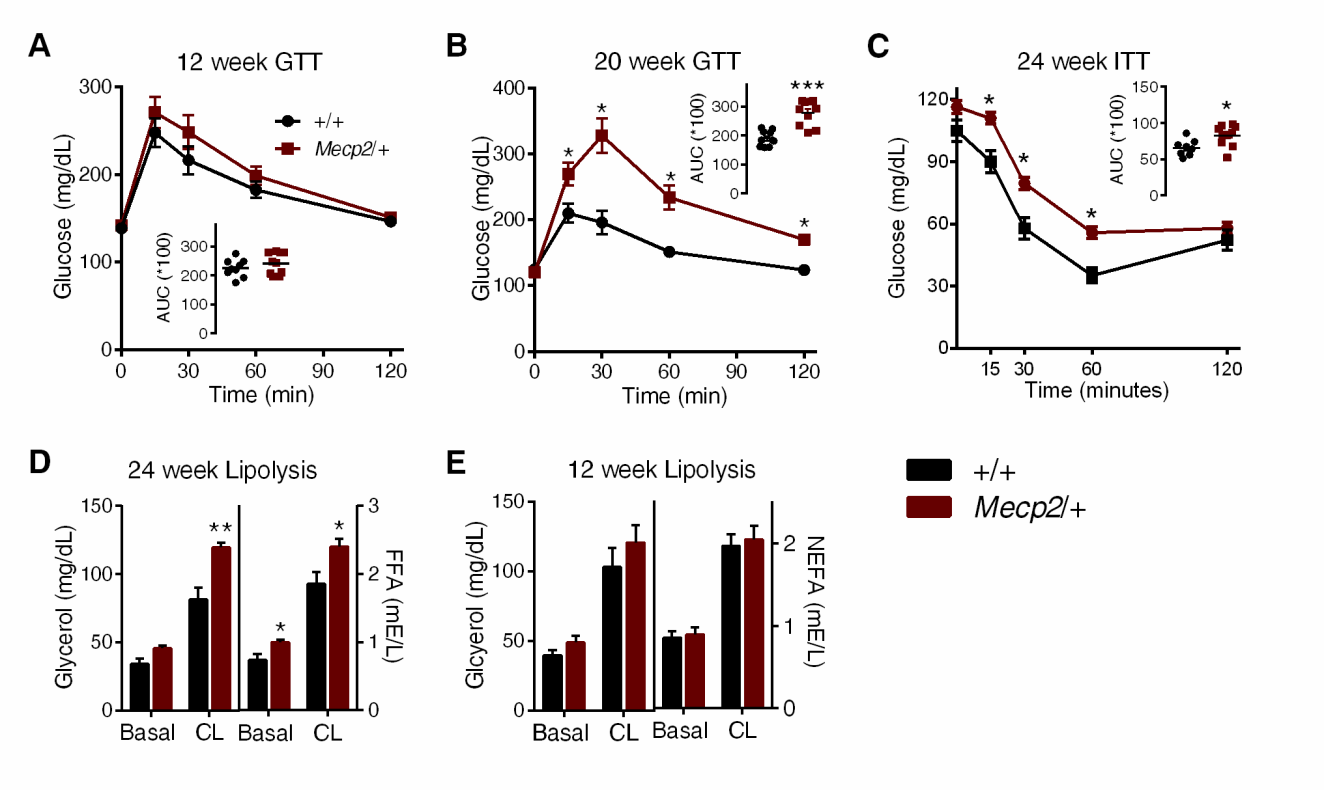

Figure S3. *Mecp2* heterozygous female mice are glucose intolerant and insulin resistant.

A. Glucose tolerance tests (GTT) in female heterozygous *Mecp2*/+ mice and wild-type littermates at 12-weeks of age (n = 8 mice per age per genotype).

B. GTT at 20-weeks of age (n = 8 per age per genotype).

C. Insulin tolerance testing in female heterozygous *Mecp2*/+ mice at 24-weeks (n = 8 per age per genotype).

D. *In-vivo* lipolysis assay in female mice at 24-weeks of age following a four-hour fast. Serum glycerol and non-esterified fatty acids (NEFA) levels are shown at basal metabolic state (basal) and fifteen minutes after lipolysis induction by injection with CL316243 (CL), a B_3-adtrenoceptor agonist (n = 7 per genotype).

E. *In-vivo* lipolysis assay at 12-weeks of age (n = 7 per genotype).

A-E. Values are mean $\pm$ SEM. *p<0.05, **p$\leq$0.01, ***p$\leq$0.001 versus wild-type, age-matched littermates.

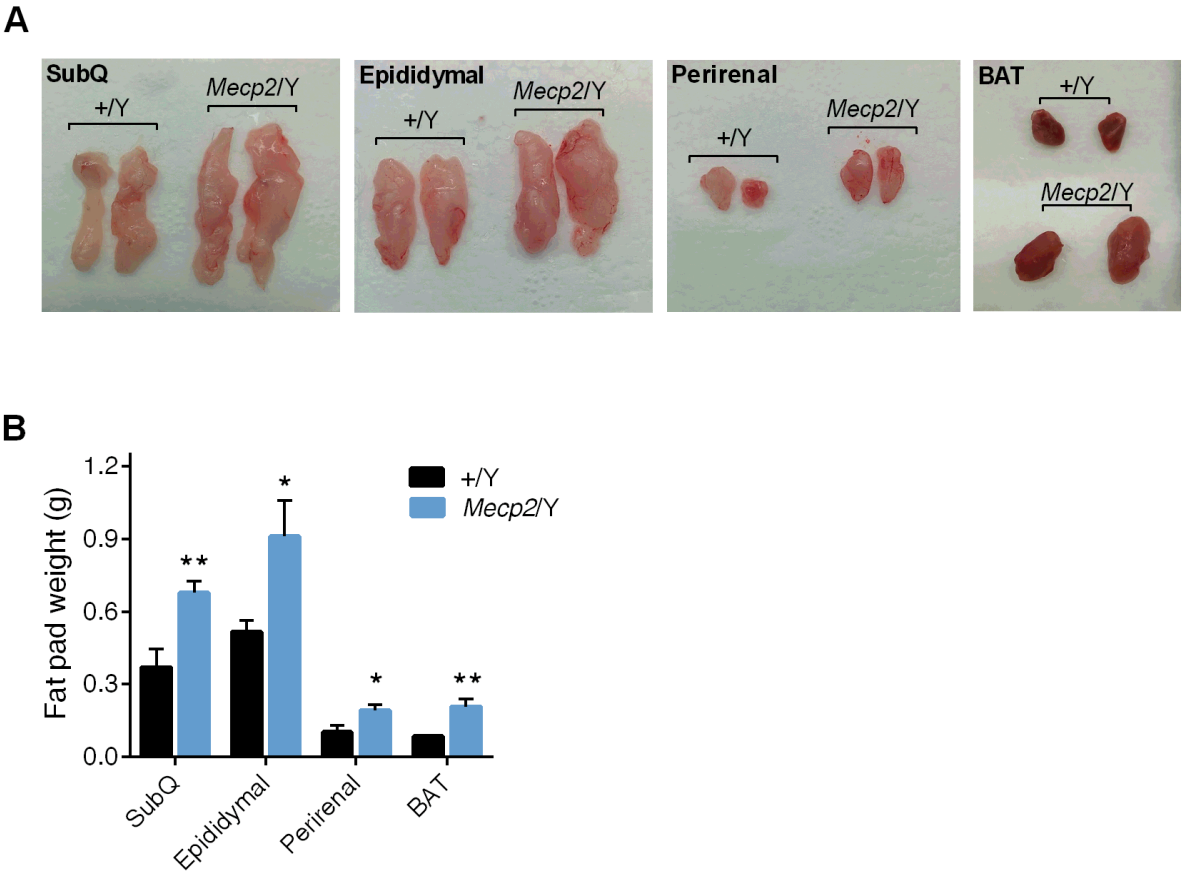

Figure S4. *Mecp2* null (*Mecp2*/Y) mice gain weight due to an expansion of white and brown adipose tissue.

A. Subcutaneous, epididymal, perirenal white adipose tissue fat, and subscapular brown adipose tissue at eight-weeks of age.

B. Weights of fat pads (n = 4 mice per age per genotype). Values are mean ± SEM. *$p<0.05$, **$p\leq0.01$, ***$p\leq0.001$ versus wild-type (+/Y) littermates.

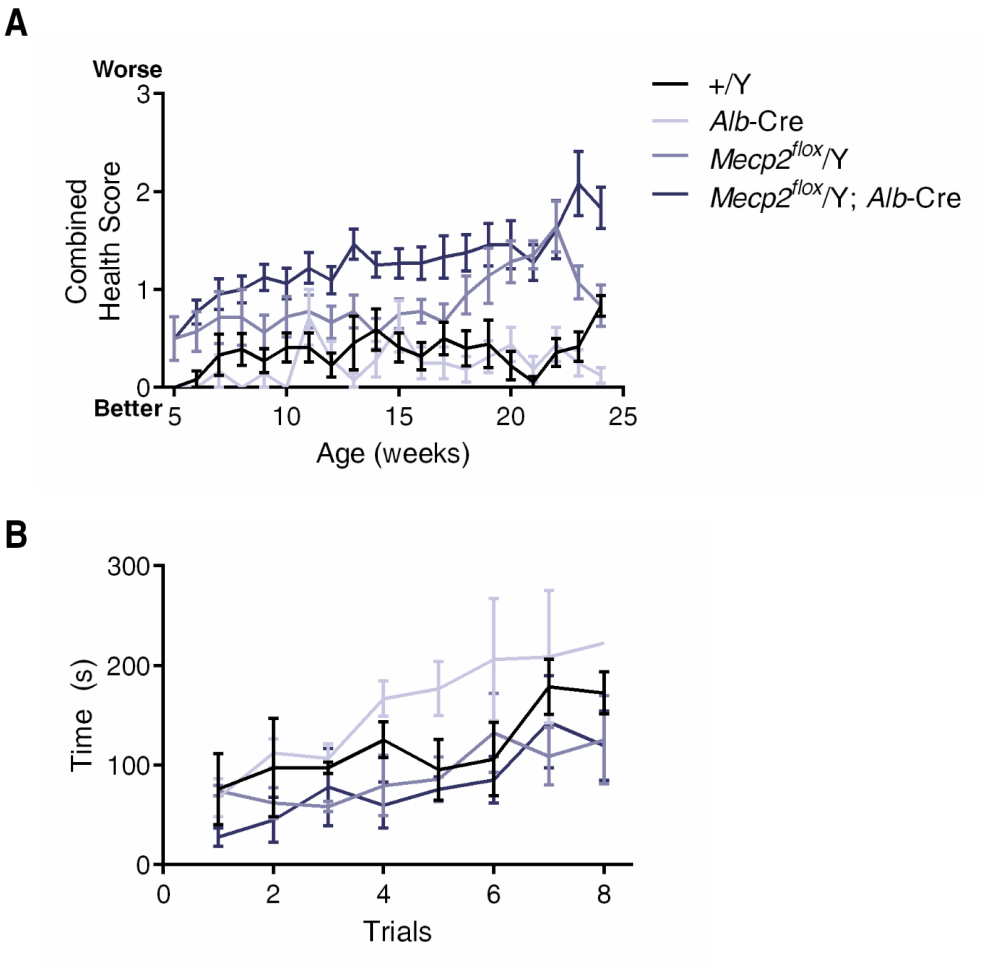

Figure S5. *Mecp2* liver-specific deletion mice do not develop neurological phenotypes.

A. Average subjective health scores for *Mecp2*^{flox}/Y; *Alb*-Cre mice and control littermates (n = 9-11 mice per genotype) from five to 24-weeks of age. Maximum subjective health score is 8, a combined score of 0 (better) – 2 (worse) for limbclasping, tremors, activity, and grooming.

B. Rotarod trials *Mecp2*^{flox}/Y; *Alb*-Cre mice and control littermates (n = 3 mice per genotype). Mice were assayed for four trials per day for two consecutive days.

A-B. Values are mean ± SEM. ns = no significance, *p≤0.05, **p≤0.01, ***p≤0.001. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis.