

**A** *Ube2e2* Exon 2 (Between red dots)

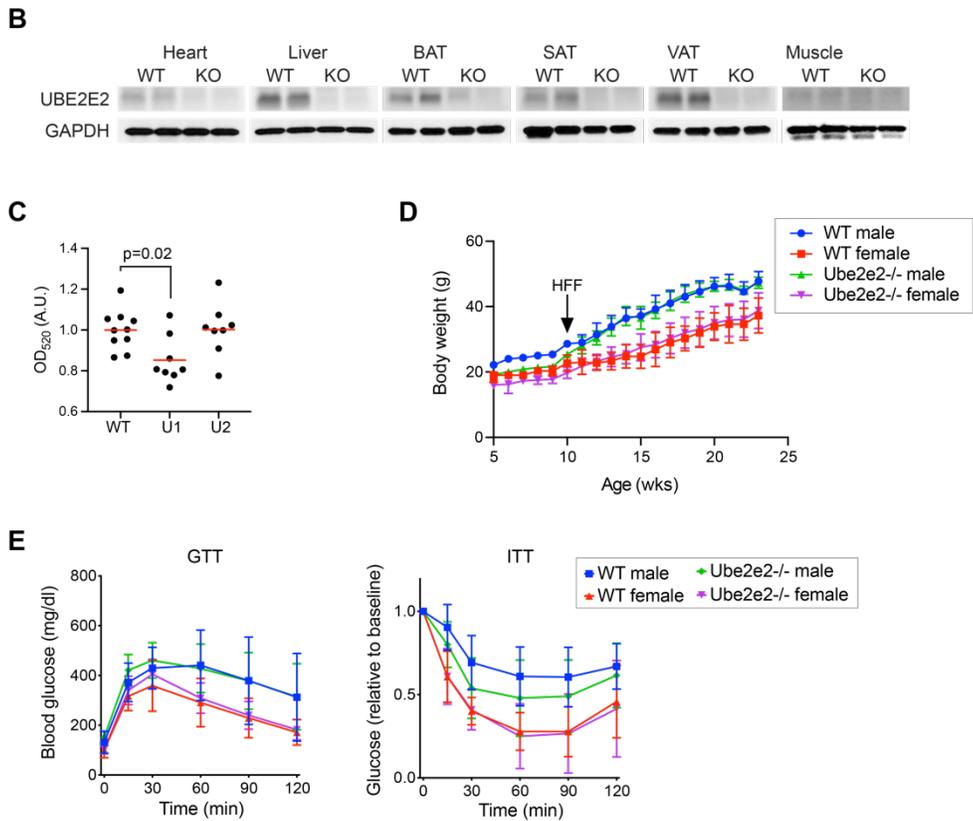
WT TGGGCTCTGTTTCTCTTCATTATGAACATACCTTGTGTAGTCCCTACCACAGCAGAATAAATGACATGCCATTTCTTAA  
 KO TGGGCTCTGTTTCTCTTCATTATGAACATACCTTGTGTAGTCCCTACCACAGCAGAATAAATGACATGCCATTTCTTAA

M S T E A Q R V D D S P S T S G G S S D G D  
 TCCAGGATCTAAGATGTCCTCACTGAGGCGCAGAGAGTTGATGACAGTCCAAGCACTAGTGGAGGAAGTTCTGATGGAGATC  
 TCCAGGATCTAAGATGTCCTCACTGAGGCGCAGAGAGTTGATGACAGTCC-----

Q R E S V Q Q E P D R E Q V Q P K K K E G K I S S K T  
 AGCGGGAGAGCGTTTCAGCAGGAACCTGATCGAGAACAAGTTACGCCTAAGAAAAAGGAGGGGAAGATATCCAGCAAGACA  
 -----

A A K L S T S A K R  
 GCCGCGAAACTGTCAACCAGTGCCAAAAGGTAGCCAGCCGTTGTTAGCACTTCATTTGAATTTCTTCTTGTACTTAGT  
 -----AACTGTCAACCAGTGCCAAAAGGTAGCCAGCCGTTGTTAGCACTTCATTTGAATTTCTTCTTGTACTTAGT

TGATTTTCTGGACTGAAGGTCATAGGGTTTATAGATTGTCTCCCTTTTATATGATGTGGTCTTTTAAAAATGTGCTTTT  
 TGATTTTCTGGACTGAAGGTCATAGGGTTTATAGATTGTCTCCCTTTTATATGATGTGGTCTTTTAAAAATGTGCTTTT



**Figure S1. Ube2e2 knockout mouse model.**

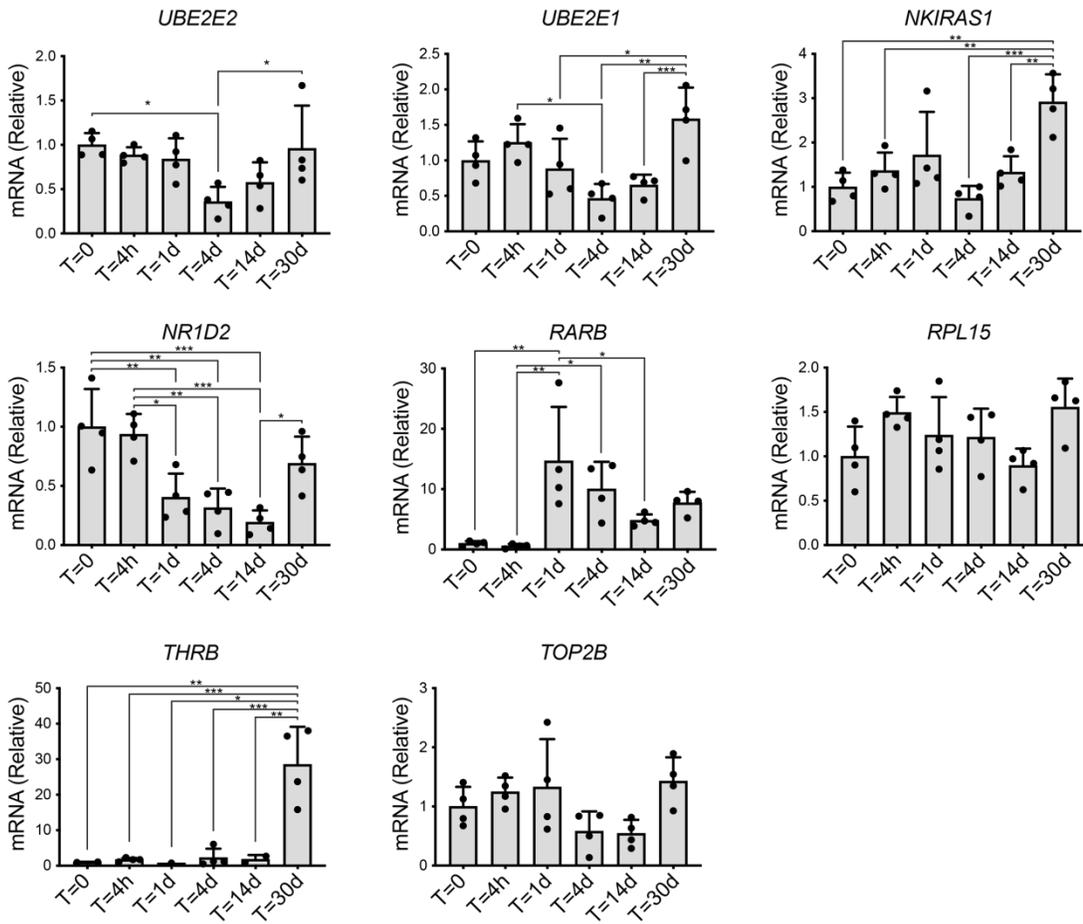
**(A)** Sequencing result demonstrating successful CRISPR targeting of exon 2 of *Ube2e2*.

**(B)** Western blot analyses of multiple tissue from *Ube2e2*<sup>-/-</sup> mice (KO) versus wildtype (WT) mice. Antibodies used were: UBE2E2 (Invitrogen, PA5-42363) and GAPDH (6C5 Santa Cruz, sc-32233).

**(C)** *Ex vivo* adipogenesis assay of primary subcutaneous adipocyte progenitors isolated from wild-type mice, the putative knockout line (U1) and a second CRISPR line (U2) that had a simple point mutation. Each dot indicates assessment of adipogenesis in progenitors isolated from a single mouse. Significance assessed by one way ANOVA, Dunnett's test, n=8-10 mice.

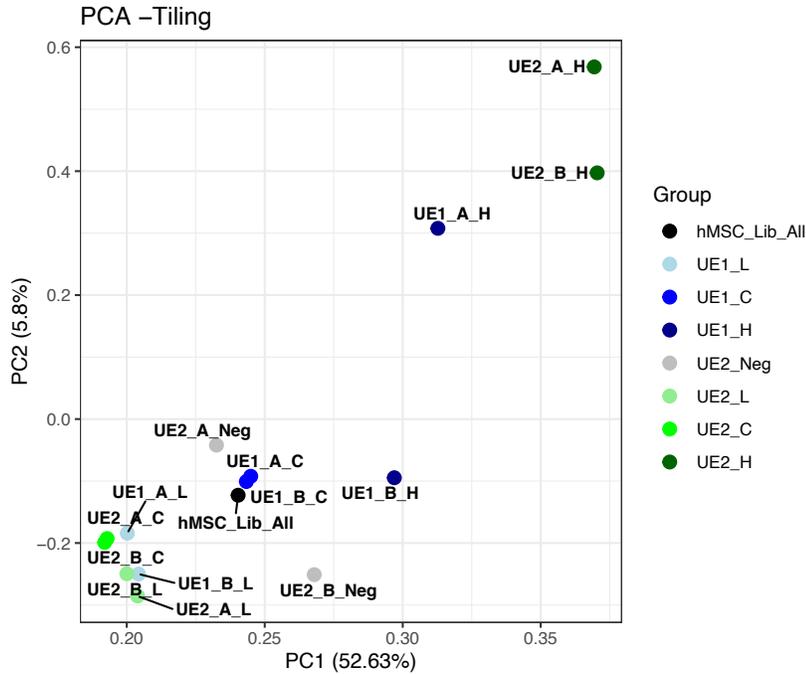
**(D)** Body weight curves for wild-type (WT) and the U1 knockout line (*Ube2e2*<sup>-/-</sup>) with high fat feeding (HFF) initiated at 10 weeks of age, n=8-9. Data shown as mean +/- s.d.m.

**(E)** Glucose tolerance testing (GTT) and insulin tolerance testing (ITT) of *Ube2e2*<sup>-/-</sup> mice and wildtype mice after 12 weeks of high fat feeding, starting at 10 weeks of age (n=13-14). Area under the curve (a.u.c.) data shown in Figure 1. No significant differences were detected (mean +/- s.d.m.).

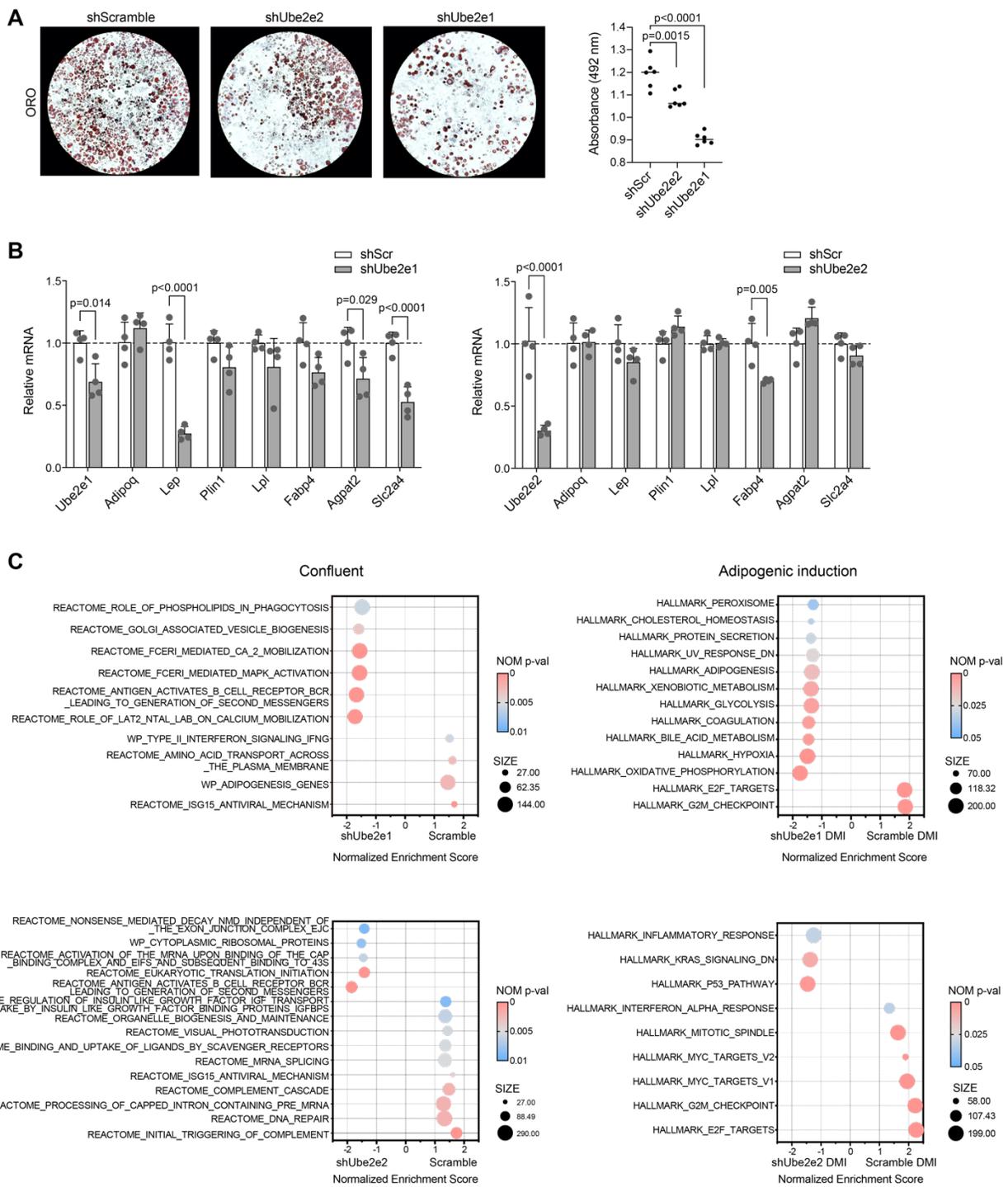


**Figure S2. Dynamic expression of genes in the topological neighborhood of *UBE2E2* during adipogenesis of mesenchymal stem cells (MSC).**

MSC were subjected to adipogenic differentiation for 30 days with harvesting of cell homogenates for qPCR at baseline, 4h, 1d, 4d, 14d, and 30d after adipogenic induction. Significance assessed by one-way ANOVA with Tukey's multiple comparison test: \*p<0.05, \*\*p<0.01, \*\*\*p<0.005. These data formed the basis for Figure 2B heatmap.



**Figure S3. gRNA sequencing discriminates MSC isolated by degree of UBE2E2/E1 expression.** gRNA library tiling across the UBE2E2 and UBE2E1 loci was introduced to MSC in culture. In situ mRNA probes were used to sort by high, low, and medium levels of *UBE2E2* and *UBE2E1* expression and then cells were sequenced to identify regions of gRNA enrichment/depletion. A sliding window approach was used to identify putative regulatory hotspots. PCA plot of these data demonstrate discrimination of high/low/medium expression MSC.



**Figure S4. Gene set enrichment analyses for Ube2e2 and Ube2e1 loss of function in 3T3L1 cells.** (A) 3T3L1 adipogenesis assay (oil-red-o) with Ube2e1 and Ube2e2 loss of function via sh-RNA and ORO staining. One way ANOVA, Dunnett's multiple comparison test, n=6 technical replicates. (B) qPCR assessment of adipogenic genes after adipogenic differentiation as in A. Two-way ANOVA with Sidak's multiple comparison test, n=4 technical replicates. (C) RNA-seq was performed on confluent 3T3L1 cells (left) and 12 hours after induction of adipogenic differentiation (right). Ube2e1 knockdown (top) or Ube2e2 knockdown (bottom) are compared to control cells transduced with sh-scramble. GSEA analyses are shown as bubble plots with nominal p-value indicated by the color scale, size indicated by bubble size, and plotted as a function of the normalized enrichment score (x-axis). These analyses complement the transcriptomics data shown in Figure 4.



- (C)** qPCR analysis of adipose tissues from the 1083 knockout line relative to wildtype and demonstrating loss of *Ube2e1* mRNA but not *Ube2e2* mRNA. Significance assessed by ANOVA and Sidak's correction, n=9-16.
- (D)** *Ex vivo* adipogenesis assay of primary subcutaneous adipocyte progenitors isolated from wild-type mice versus the 1086 *Ube2e1* knockout line (n=4 mice per group). Significance assessed by two-sided t-test. Representative Nile red images shown, scale bar = 320 $\mu$ m.
- (E)** Glucose tolerance testing (GTT) of *Ube2e1*<sup>-/-</sup> mice and wildtype mice after 12 weeks of high fat feeding. Mean +/- s.d.m. shown. Significance assessed by two-way ANOVA, Dunnett's multiple comparison test. Note: in these early cohorts the sample size was imbalanced particularly for the 1086 line: 1086 female n=2; 1086 male n=6; 1083 female n=13; 1083 male n=15; wildtype female n=6; wildtype male n=11. Two-way ANOVA p-value is displayed with color coded symbol for Dunnett's multiple comparison test \*p<0.05; \*\*p<0.01.
- (F)** Subcutaneous adipose tissues from compound heterozygous *Ube2e1*<sup>+/-</sup>; *Ube2e2*<sup>+/-</sup> mice were compared to wildtype control by qPCR and showing approximate halving of mRNA for both transcripts, n=9-12.
- (G)** Leptin and adiponectin raw values used to calculate Adiponectin:Leptin ratio shown in Figure 6H.
- (H)** Serum triglycerides from Figure 6 diet-induced obesity study, (n=11-12).