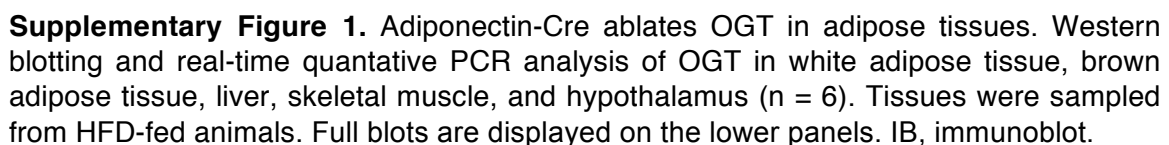


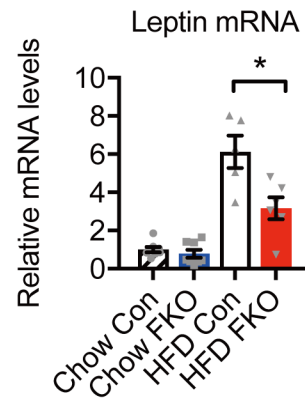
## **Supplemental Information**

Title: Adipocyte OGT governs diet-induced hyperphagia and obesity

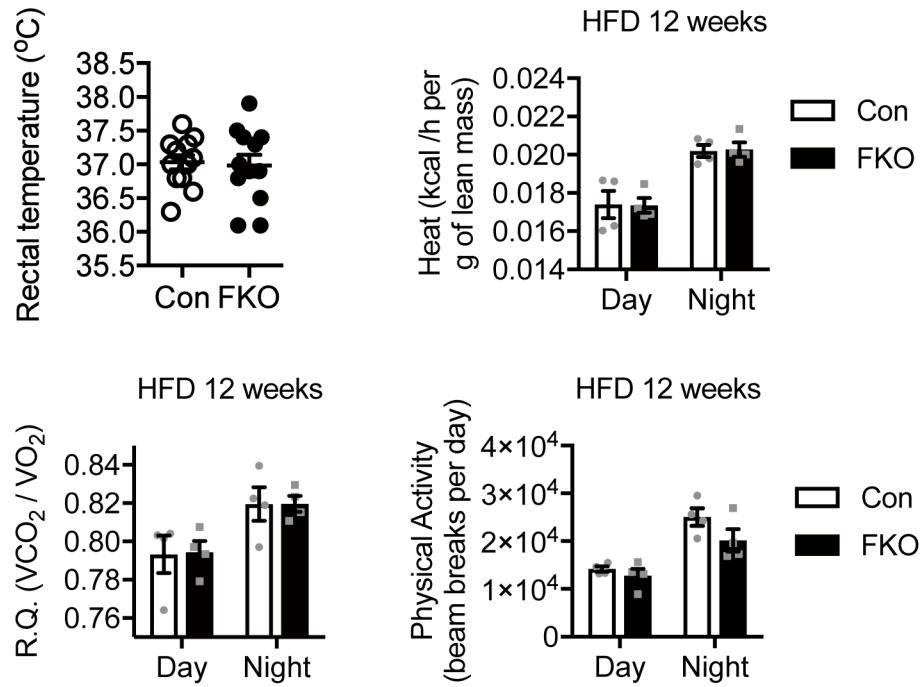
Authors: Li et al.



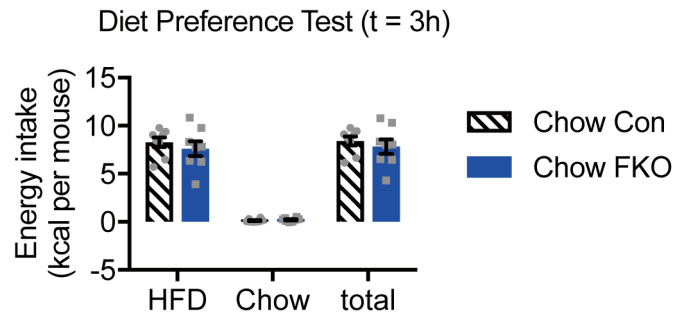
**Supplementary Figure 1.** Adiponectin-Cre ablates OGT in adipose tissues. Western blotting and real-time quantitative PCR analysis of OGT in white adipose tissue, brown adipose tissue, liver, skeletal muscle, and hypothalamus (n = 6). Tissues were sampled from HFD-fed animals. Full blots are displayed on the lower panels. IB, immunoblot.



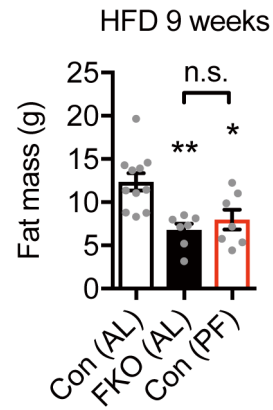
**Supplementary Figure 2.** Effects of OGT deletion on leptin expression. RT-qPCR analysis of leptin in white adipose tissue. Animal information is described in Fig. 1d. Data were presented as mean  $\pm$  s.e.m. \* $P < 0.05$ , two-tailed unpaired Student's t-test.



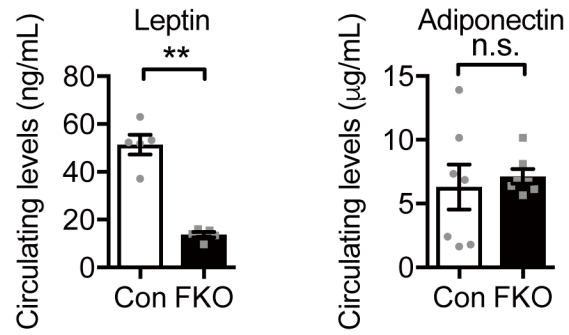
**Supplementary Figure 3.** Effects of OGT deletion on metabolic activities. Rectal temperature was recorded in Con (n = 12) and FKO (n = 12) mice that had been fed HFD for 18 weeks. Energy expenditure, respiratory quotient, and physical activity were measured in 12-week-HFD-fed Con (n = 4) and FKO (n = 4) animals. Data were presented as mean  $\pm$  s.e.m. \* $P$  < 0.05, \*\* $P$  < 0.01, two-tailed unpaired Student's t-test.



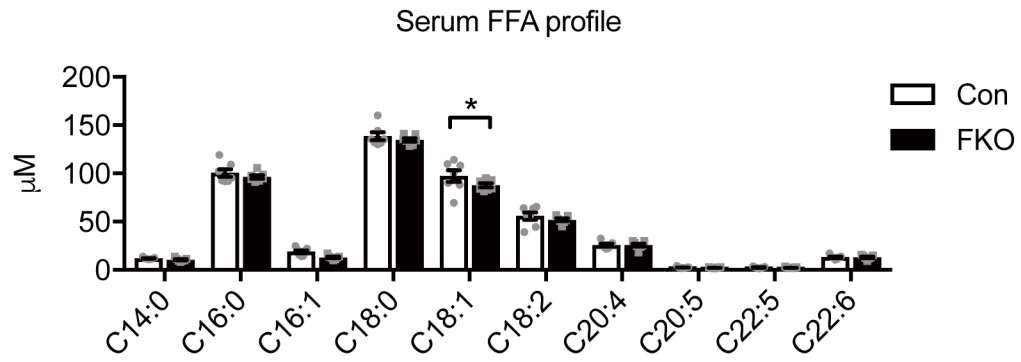
**Supplementary Figure 4.** Dietary preference is not altered in chow-fed FKO mice. 3-hour nocturnal energy intake in a two-choice test was measured in 22.5-week-old Con ( $n = 8$ ) and FKO ( $n = 8$ ) mice. Mice were acclimated by singly housing for 6 days and HFD feeding for 3 days. Data were presented as mean  $\pm$  s.e.m.  $*P < 0.05$ ,  $**P < 0.01$ , two-tailed unpaired Student's t-test.



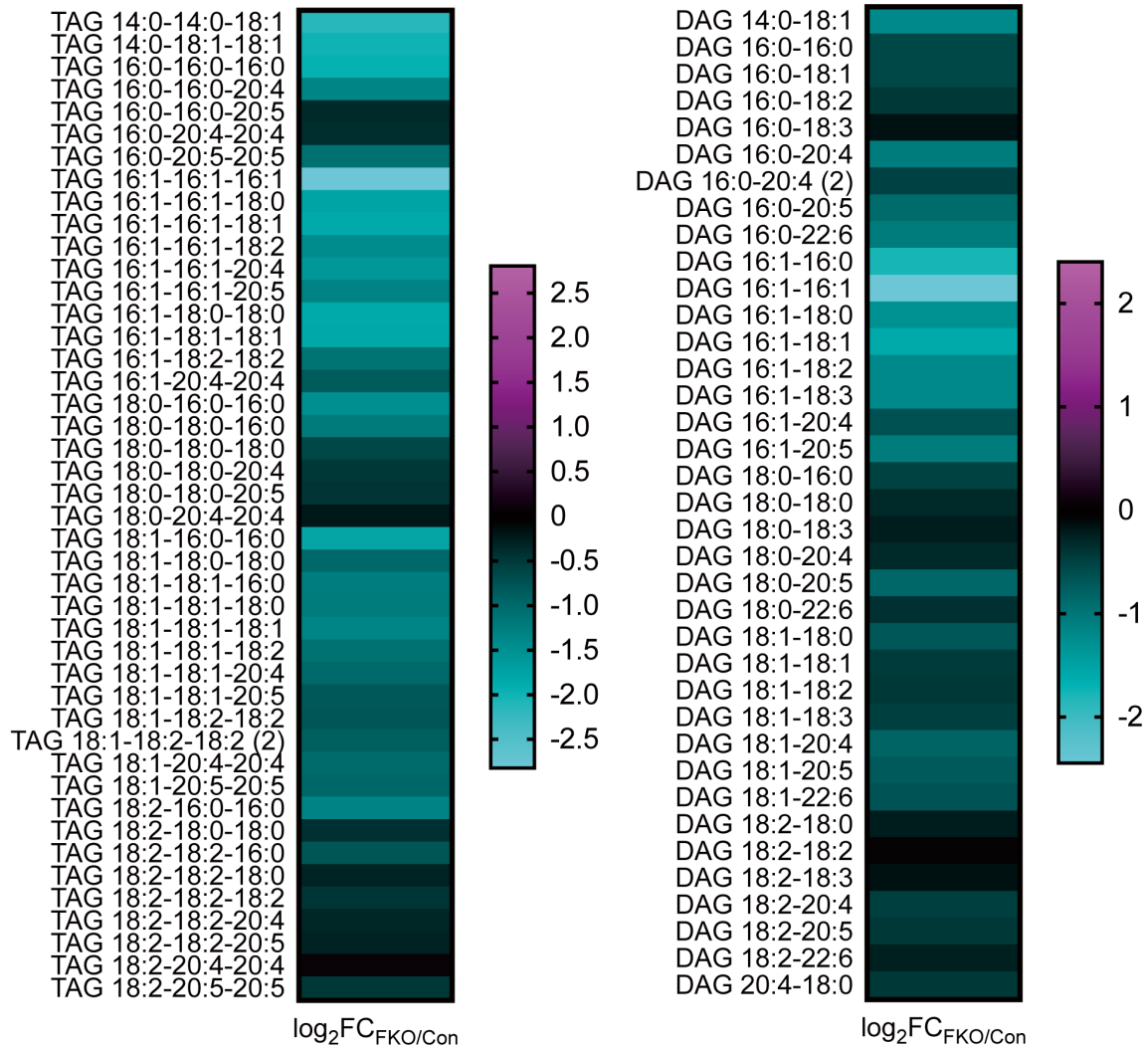
**Supplementary Figure 5.** Pair feeding normalized fat mass between FKO and Con mice. Con (AL, ad libitum,  $n = 11$ ), Con (PF, pair-fed to littermate FKO,  $n = 7$ ), FKO (AL,  $n = 7$ ). Data were presented as mean  $\pm$  s.e.m. *N.s.* not significant,  $*P < 0.05$  (compared to Con (AL)),  $**P < 0.01$  (compared to Con (AL)), *post-hoc* Tukey's test.



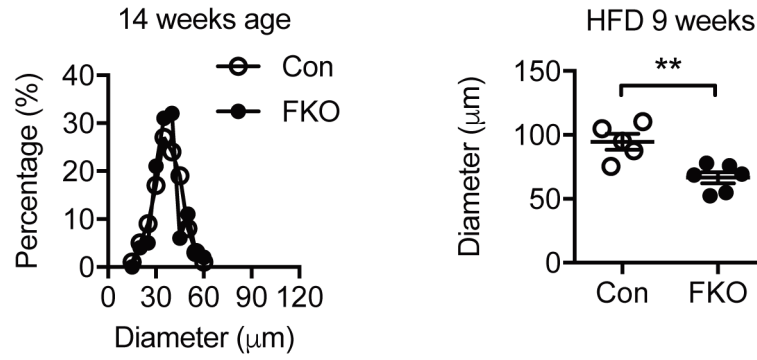
**Supplementary Figure 6.** Serum adipokines and lipid profiles in male Con (n = 7) and FKO (n = 7) mice fed HFD for 22 weeks. Data were presented as mean  $\pm$  s.e.m. *N.s.* not significant, \* $P$  < 0.05, \*\* $P$  < 0.01, two-tailed unpaired Student's t-test.



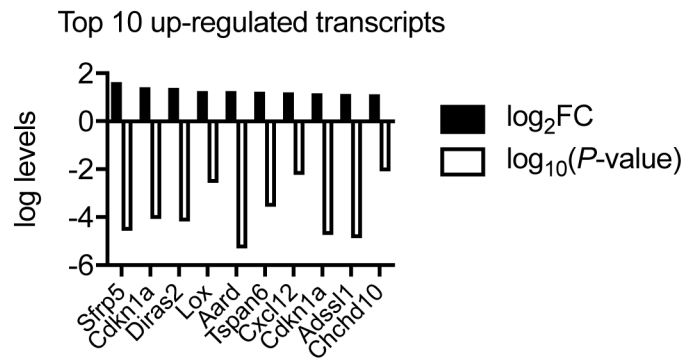
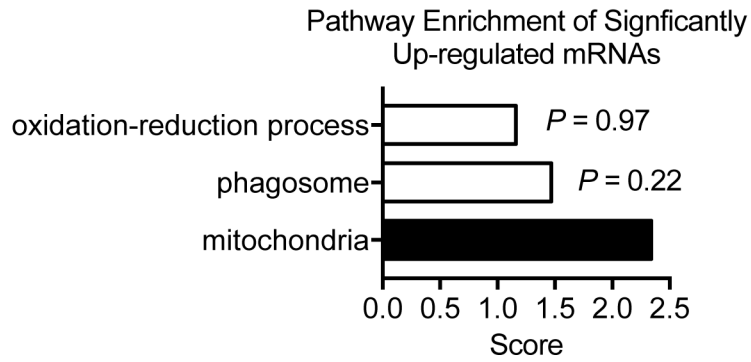
**Supplementary Figure 7.** Serum free fatty acid (FFA) profiles in male Con (n = 7) and FKO (n = 7) mice fed HFD for 22 weeks. Data were presented as mean  $\pm$  s.e.m. \* $P$  < 0.05, *post-hoc* Sidak's multiple comparisons test.



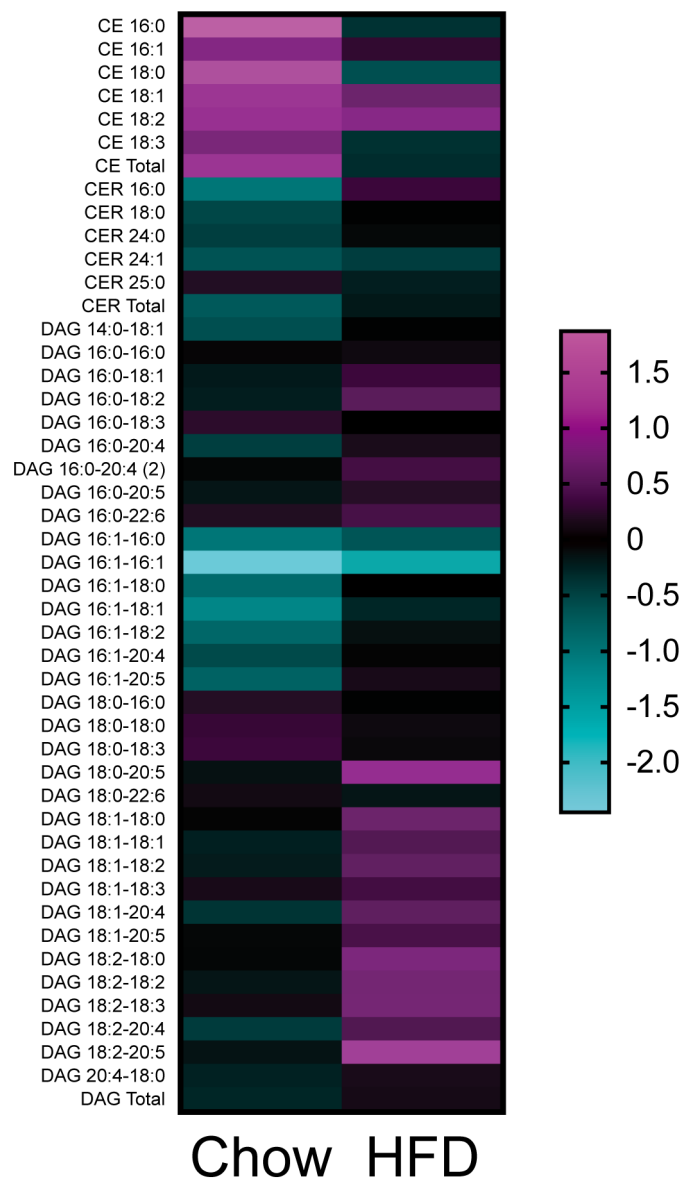
**Supplementary Figure 8.** Liver lipid profiles in male Con (n = 7) and FKO (n = 7) mice fed HFD for 22 weeks. Heatmaps show lipid profile of TAG (Left) and DAG (Right), respectively. Data were presented as the log<sub>2</sub> ratio of the fold change (FC) of lipid levels in FKO versus Con samples. A scale of log<sub>2</sub>FC<sub>FKO/Con</sub> is presented on the right of the heatmap.



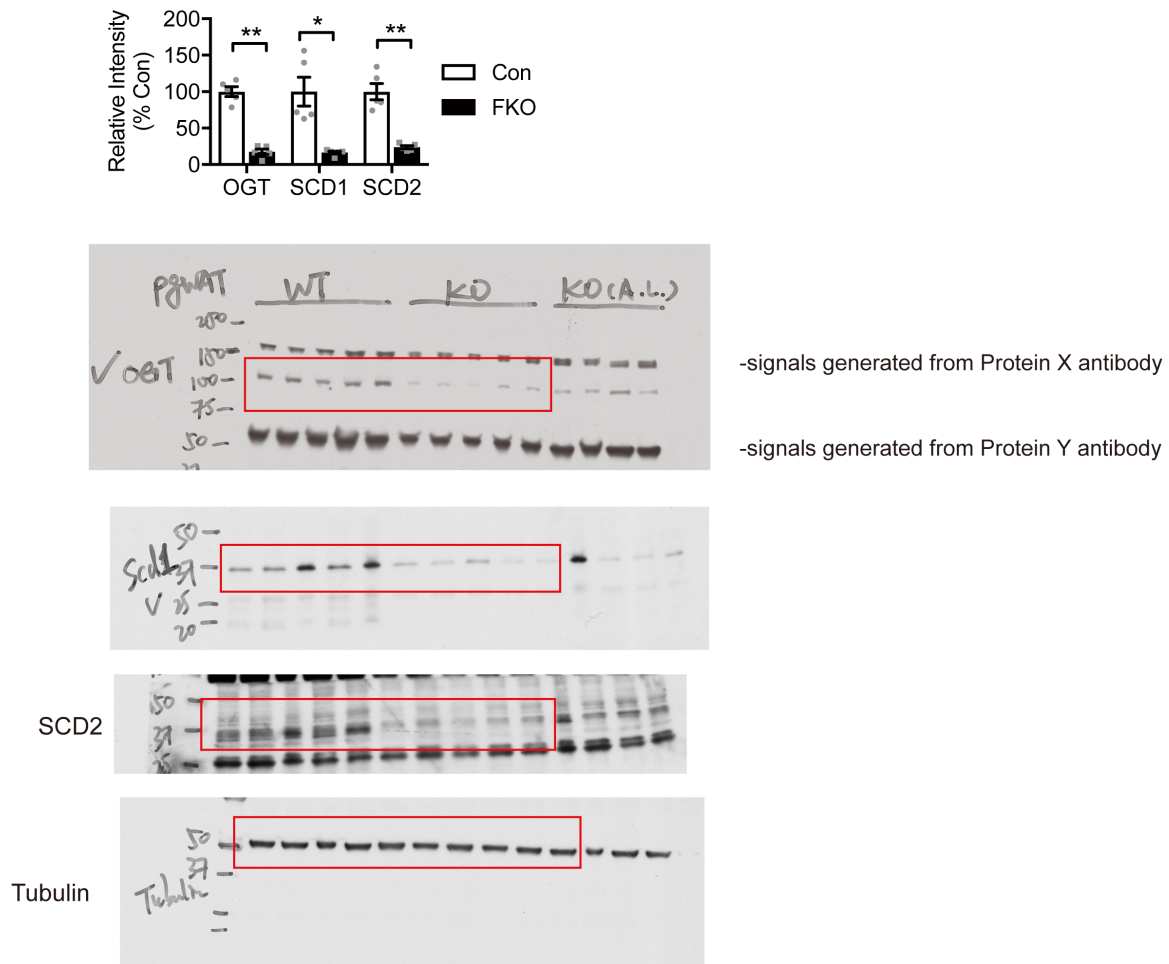
**Supplementary Figure 9.** Cell size analysis of histological sections of adipose tissue from 14-week-old chow-fed animals (Left) and 15-week-old 9-week-HFD-fed animals (Right). Data were presented as mean  $\pm$  s.e.m. \* $P$  < 0.05, \*\* $P$  < 0.01, two-tailed unpaired Student's t-test.



**Supplementary Figure 10.** Transcriptomic analysis of adipose tissue. Upper graph shows the result of pathway enrichment analysis of perigonadal white adipose tissue (pgWAT) from male Con (n = 5) and FKO (n = 3) mice fed HFD for 3 days. Genes up-regulated by OGT depletion were analyzed in the DAVID 7.8 platform. Lower graph shows the list of top 10 up-regulated genes in adipose tissue. Data were presented as the log<sub>2</sub> ratio of the fold change (FC) of signal levels in FKO and Con samples and the log<sub>10</sub> ratio of the adjusted *P* value.

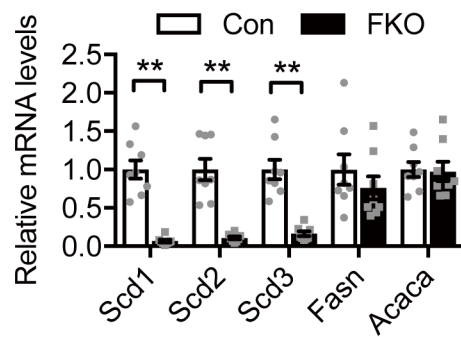


**Supplementary Figure 11.** Heatmap of lipid species in adipose tissue lipidome. CE, cholesterol ester, CER, ceramide, DAG, diacylglycerol. Data were presented as log2 ratios of FKO/Con (Chow for 14-15 weeks, Con n = 11, FKO n = 6; age-matched HFD for 9 weeks, Con n = 7, FKO n = 8).

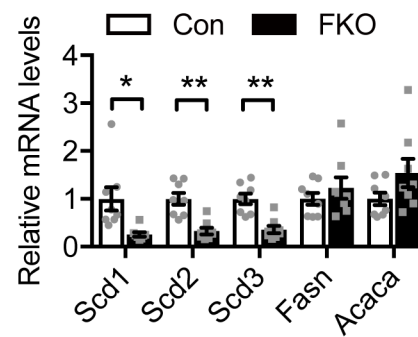


**Supplementary Figure 12.** Density graphs quantifying protein levels of SCD and OGT in adipose tissue. Data were presented as mean  $\pm$  s.e.m. \* $P$  < 0.05, \*\* $P$  < 0.01, two-tailed Student's t-test. Full blots associated with Fig. 4b are displayed. Red frames indicate cropped region displayed in the main Fig. 4b.

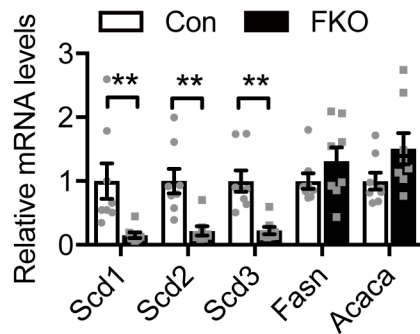
pgWAT



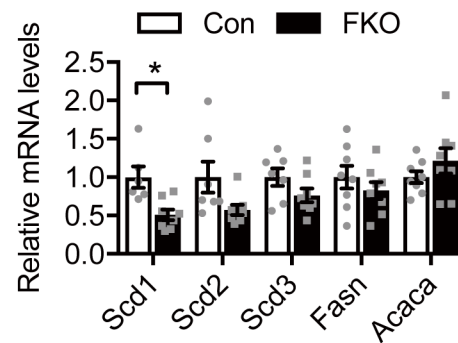
scWAT



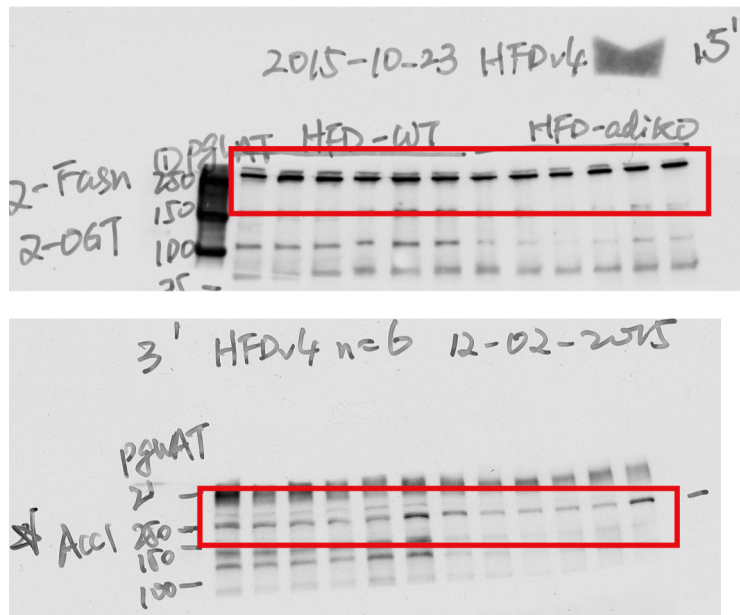
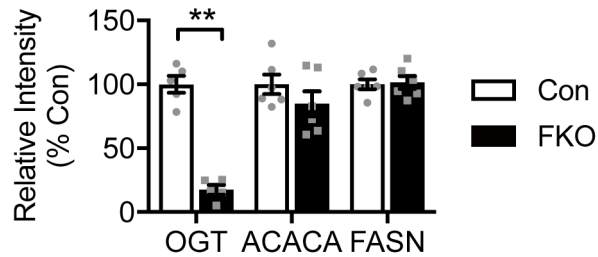
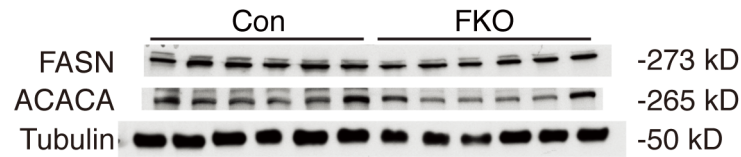
rpWAT



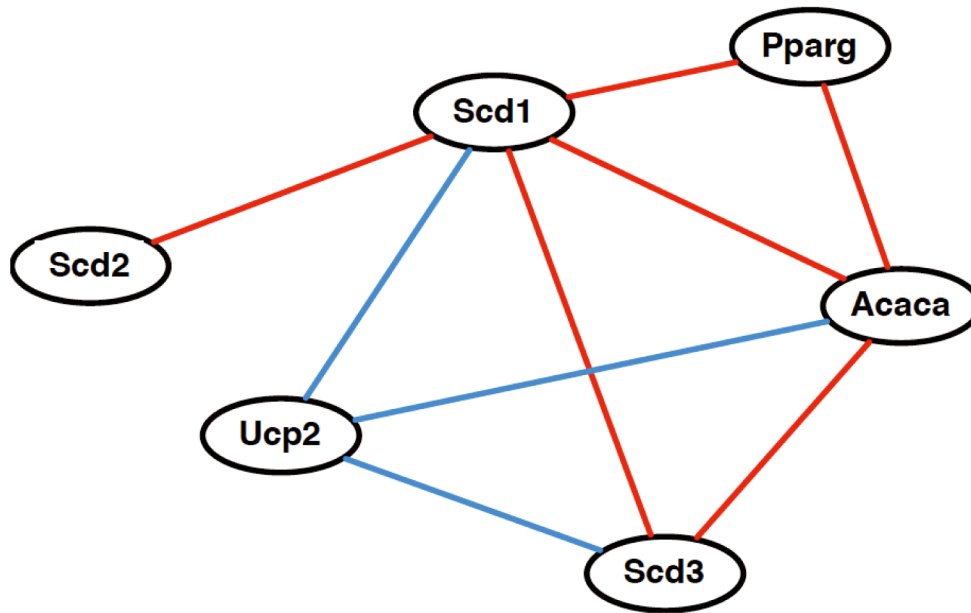
BAT



**Supplementary Figure 13.** Gene expression analysis of *de novo* lipid desaturation and synthesis in adipose depots. White adipose tissues from perigonadal (pg), subcutaneous (sc), and retroperitoneal (rp) regions, and interscapular brown adipose tissue (BAT) were collected from 15-week chow-fed Con (n = 8) and FKO (n = 8) mice. Data were presented as mean  $\pm$  s.e.m. \* $P$  < 0.05, \*\* $P$  < 0.01, two-tailed unpaired Student's t-test.

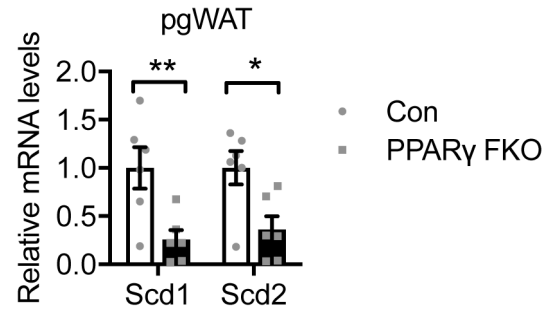


**Supplementary Figure 14.** Protein levels of Fasn and Acc1 are not altered by OGT deletion. Density graph displays the result of Western blot analysis of the pgWAT extracts from Con (n = 6) and FKO (n = 6) mice on HFD for 9 weeks. Data were presented as mean  $\pm$  s.e.m. \* $P$  < 0.05, \*\* $P$  < 0.01, two-tailed unpaired Student's t-test. Full blots are displayed. Red frames indicate cropped region displayed in the top panel.

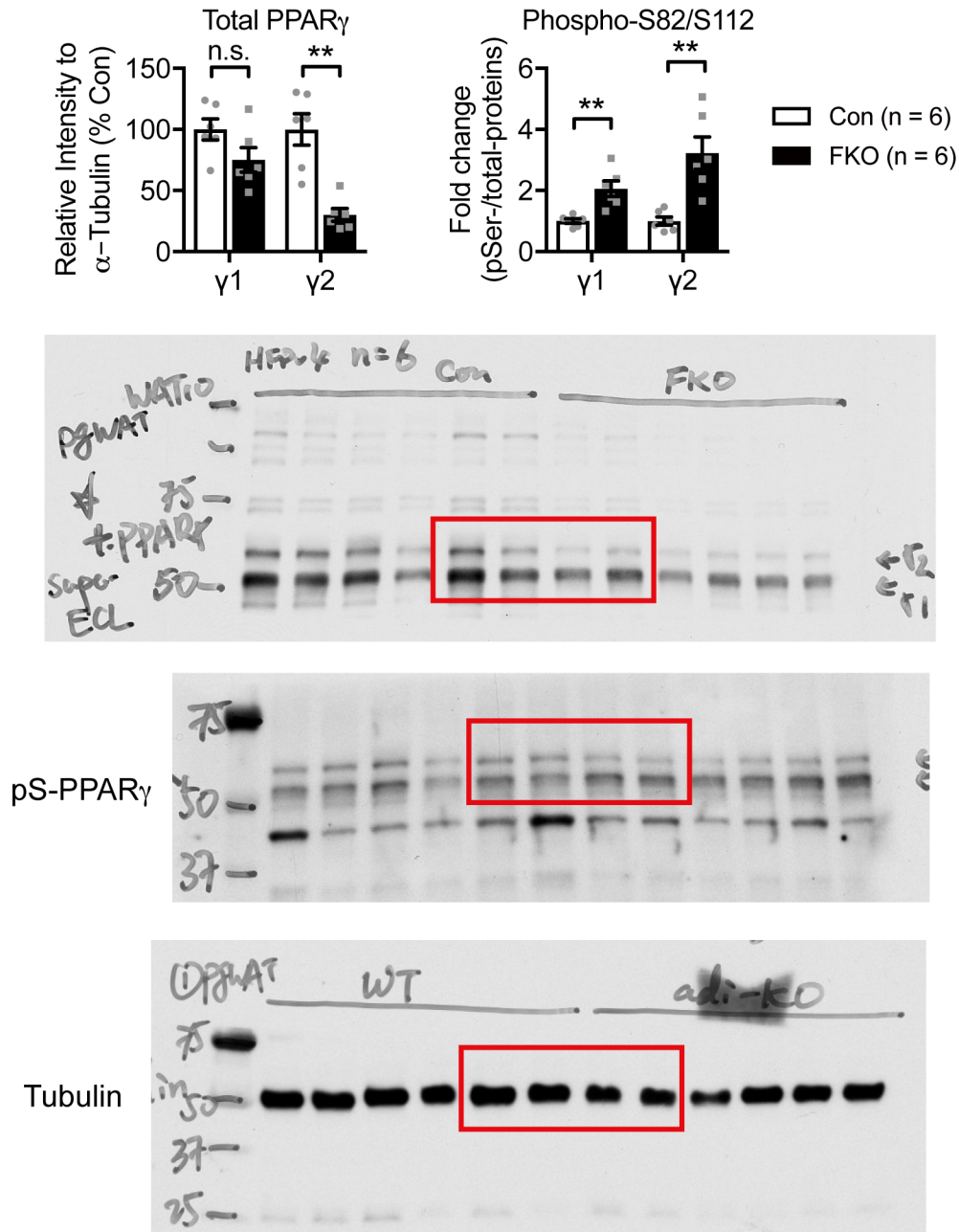


Data sources: the Hybrid Mouse Diversity Panel (HMDP)  
<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE64768>  
Red line indicates positive correlation. Blue line indicates negative correlation

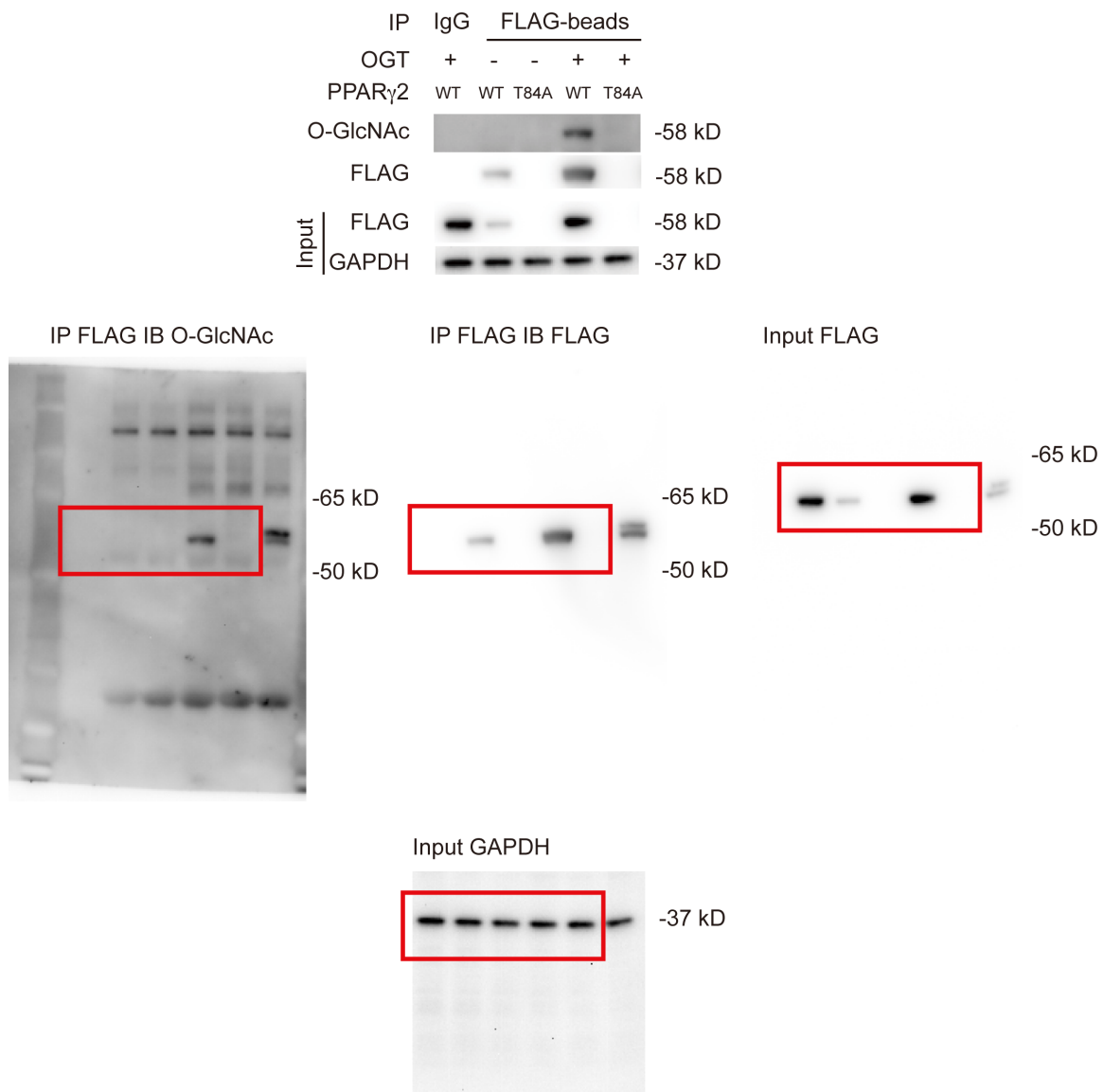
**Supplementary Figure 15.** Pparg and Scd genes are co-regulated at the transcript level. Gaussian graphical modeling was performed to estimate conditional interdependence among genes in *de novo* lipogenesis, Pparg, and Ucp2.



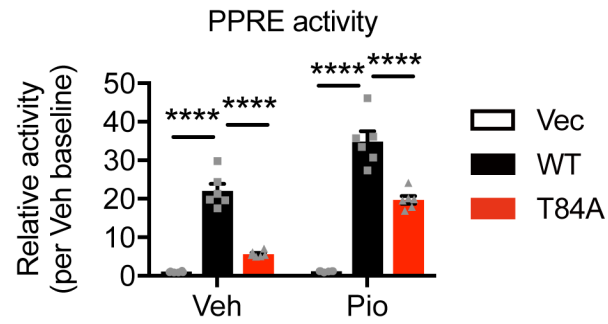
**Supplementary Figure 16.** Transcript levels of Scd1 and Scd2 in pgWAT from aP2 promoter-driven Cre PPAR $\gamma^{\text{flox/flox}}$  (PPAR $\gamma$  FKO, n = 6) mice and littermate PPAR $\gamma^{\text{flox/flox}}$  mice (Con, n = 6). Data were presented as mean  $\pm$  s.e.m. \* $P$  < 0.05, \*\* $P$  < 0.01, two-way unpaired Student's t-test.



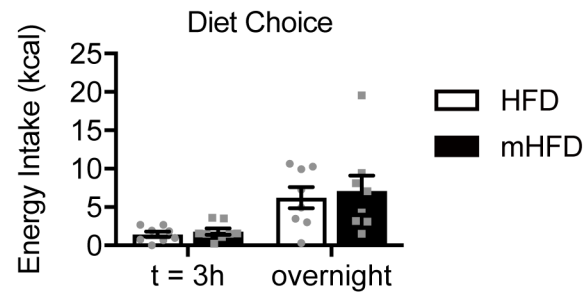
**Supplementary Figure 17.** Density graphs quantifying total or phosphorylated PPAR $\gamma$  proteins in adipose tissue from FKO and Con mice on HFD for 9 weeks (N = 6). Data were presented as mean  $\pm$  s.e.m. \* $P$  < 0.05, \*\* $P$  < 0.01, two-way unpaired Student's t-test. Full blots associated with Fig. 4c are displayed. Red frames indicate cropped region displayed in the main Fig. 4c.



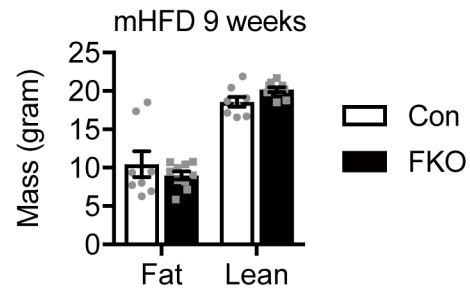
**Supplementary Figure 18.** Immunoprecipitation analysis of PPAR $\gamma$ 2 O-GlcNAcylation in 293A cells. Experiments have been repeated for three times. Full blots associated are displayed. **Red frames** indicate cropped region displayed in the upper panel.



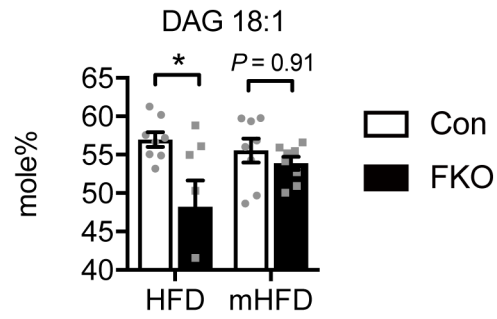
**Supplementary Figure 19.** Luciferase reporter assays of PPAR $\gamma$ 2 mutants. Expression constructs for PPAR $\gamma$ 2 and RXR $\alpha$  were co-expressed in 293A cells with a PPAR response elements (PPRE)-driven luciferase reporter (n = 6). Cells were treated with pioglitazone (Pio, 10  $\mu$ M) overnight before the assays. Luminescence was normalized to Renilla reporter activity. Experiments have been repeated for three times. Data were presented as mean  $\pm$  s.e.m. \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\*\* $P$  < 0.0001, *post-hoc* Sidak's tests.



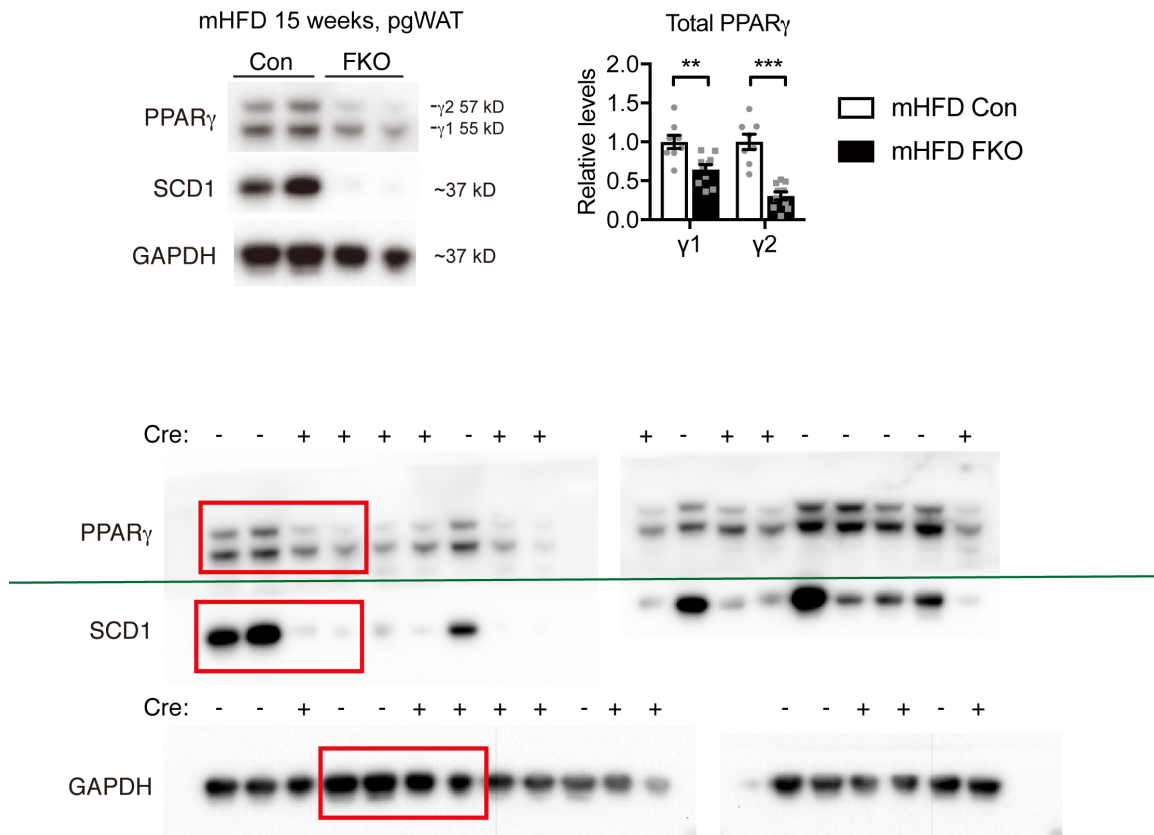
**Supplementary Figure 20.** Mice have no dietary preference between mono-unsaturated fat-fortified high fat diet (mHFD) and HFD. Energy intake (3h and 16h) in a two-choice diet preference test (n = 8). Data were presented as mean  $\pm$  s.e.m. \* $P < 0.05$ , \*\* $P < 0.01$ , two-tailed unpaired Student's t-test.



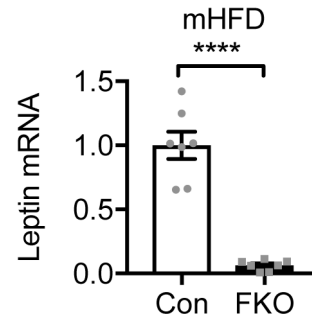
**Supplementary Figure 21.** Fat mass and lean mass in male Con (n = 8) and FKO (n = 10) mice fed mHFD for 9 weeks. Data were presented as mean  $\pm$  s.e.m. \* $P$  < 0.05, \*\* $P$  < 0.01, two-tailed unpaired Student's t-test.



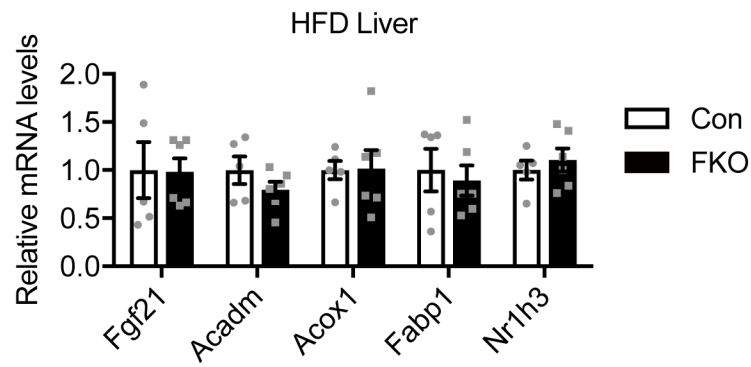
**Supplementary Figure 22.** Levels of oleoyl DAG in adipose lipidome, in mice fed HFD or mHFD for 15 weeks. Data were presented as mean  $\pm$  s.e.m. \* $P < 0.05$ , two-tailed unpaired Student's t-test.



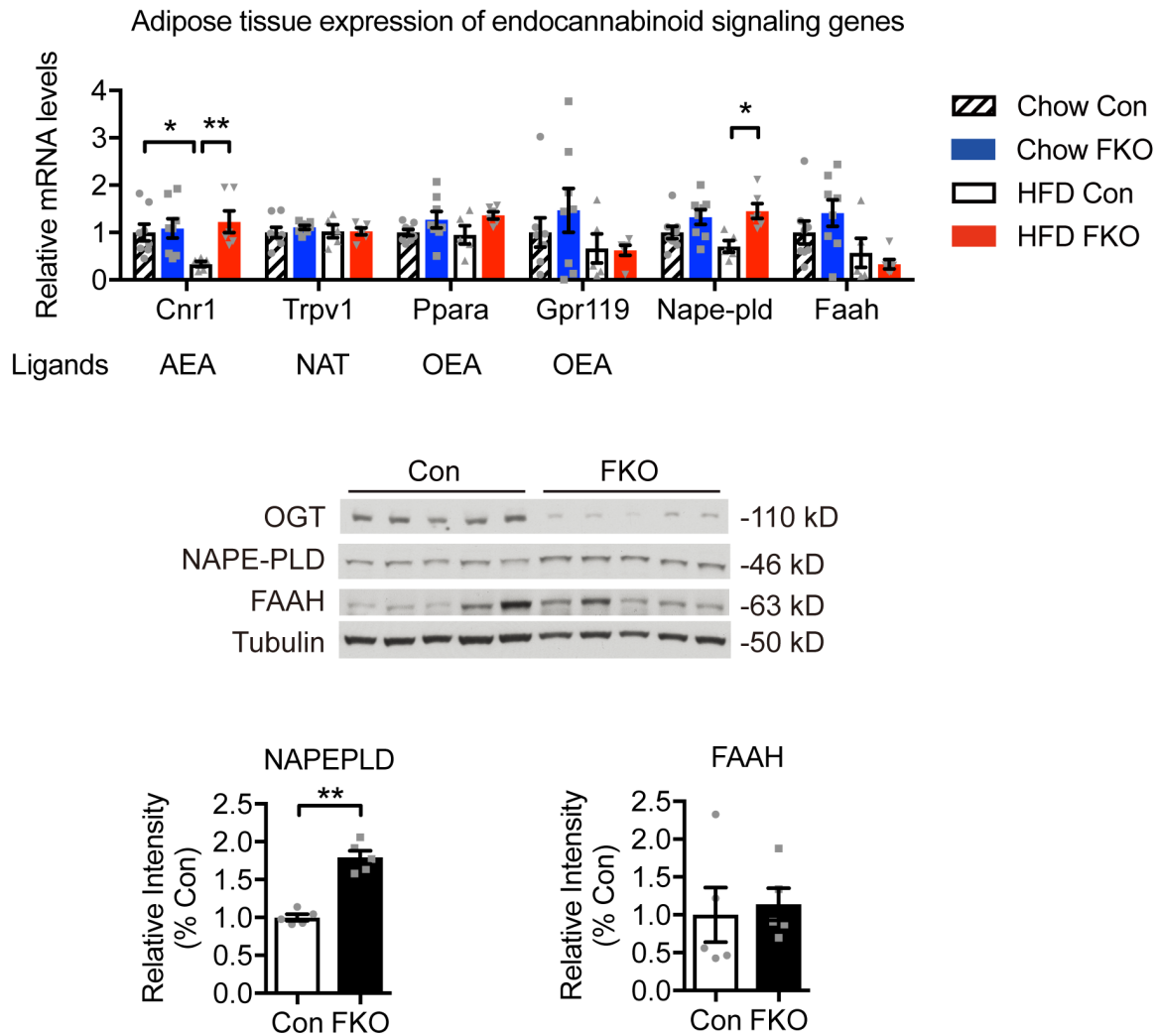
**Supplementary Figure 23.** Expression of PPAR $\gamma$  isoforms and SCD1 in mice on mHFD for 15 weeks ( $n = 8$ , Con;  $n = 10$ , FKO). Two representative mouse samples from each group were presented in Western blots. Data in the density graph were presented as mean  $\pm$  s.e.m (Density signals of PPAR $\gamma$  normalized by those of GAPDH). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , two-way ANOVA and *post-hoc* Sidak's multiple comparisons test. Full blots associated are displayed. Red frames indicate cropped region displayed in the upper panel.



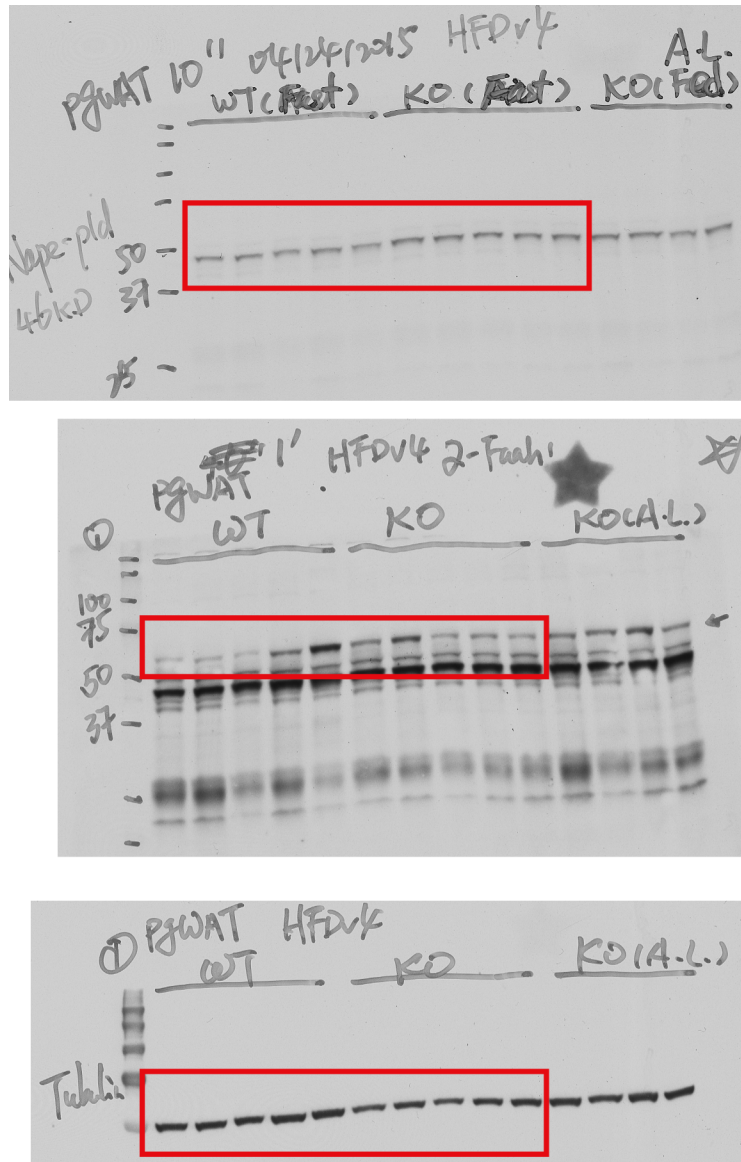
**Supplementary Figure 24.** Expression of leptin in mice on mHFD for 15 weeks (n = 7 per group). Data were presented as mean  $\pm$  s.e.m. \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\*\* $P$  < 0.0001, unpaired two-tailed Student's t-test.



**Supplementary Figure 25.** Expression of PPAR $\alpha$  target genes in liver from mice on HFD for 9 weeks. Data were presented as mean  $\pm$  s.e.m. None of these genes were differentially regulated by FKO as assessed by unpaired two-tailed Student's t-test.



**Supplementary Figure 26.** Gene expression analysis of endocannabinoid signaling and metabolism in adipose tissue. Upper graphs show the mRNA levels in adipose tissue of chow-fed mice ( $n = 8$  per group) and age-matched 9-week-HFD-fed mice ( $n = 5$  (Con) or 6 (FKO)). Lower graphs show protein levels of NAPE-PLD and FAAH in adipose tissue from 9-week-HFD-fed mice ( $n = 5$  per group). Data were presented as mean  $\pm$  s.e.m. \* $P < 0.05$ , \*\* $P < 0.01$ , unpaired two-tailed Student's t-test. NAT, N-acyl taurine. Full blots associated are displayed in Supplementary Figure 27.



**Supplementary Figure 27.** Full blots associated with Supplementary Figure 26 are displayed. Red frames indicate cropped region displayed in the Supplementary Figure 26.

**Supplementary Table 1. List of DNA Oligonucleotides**

| Oligo name  | Forward                              | Reverse                               |
|-------------|--------------------------------------|---------------------------------------|
| u36B4       | CCGATCTGCAGACACACACT                 | ACCCTGAAGTGCTCGACATC                  |
| Ogt         | AAGAGGCACGCATTTTTGAC                 | ATGGGGTTGCAGTTCGATAG                  |
| Agrp        | TGCTACTGCCGCTTCTTCAA                 | CTTTGCCCAAACAACATCCA                  |
| Npy         | TAACAAGCGAATGGGGCTGT                 | ATCTGGCCATGTCCTCTGCT                  |
| Pomc        | AGGCCTGACACGTGGAAGAT                 | AGGCACCAGCTCCACACAT                   |
| c-Fos       | CCTTCGGATTCTCCGTTTCTCT               | TGGTGAAGACCGTGTCAGGA                  |
| Scd1        | CCGGAGACCCCTTAGATCGA                 | TAGCCTGTAAAAGATTTCTGCAAACC            |
| Scd2        | TGCCTTGTATGTTCTGTGGC                 | TCCTGCAAGCTCTACACCTG                  |
| Scd3        | GCCTTGTACGTTCTGTGGCT                 | CCCTCCTGCAAGCTCTACAC                  |
| Acaca       | GAAGCCACAGTGAAATCTCG                 | GATGGTTTGGCCTTTCACAT                  |
| Acly        | AATGGCCGTCATGTGAGTTT                 | GTGGCCCCAACTATCAAGAG                  |
| Acss2       | GCTGAACTGACACACCTGGA                 | AACTTGCGCACAAAGTTGCT                  |
| Fasn        | GTTGGCCCAGAACTCCTGTA                 | GTCGTCTGCCTCCAGAGC                    |
| Elovl6      | AACTTGGCTCGCTTGTTTCAT                | CCAATGGATGCAGGAAAACCT                 |
| Srebf1      | CTGTCTCACCCCCAGCATAG                 | GATGTGCGAACTGGACACAG                  |
| Mlxipl      | CACTCAGGGAATACACGCCTAC               | ATCTTGGTCTTAGGGTCTTCAGG               |
| Nr1h3       | TGGAGAACTCAAAGATGGGG                 | TGAGAGCATCACCTTCCTCA                  |
| Pparg       | CAAGAATACCAAAGTGCGATCAA              | GAGCTGGGTCTTTTCAGAATAATAAG            |
| Lep         | GACACCAAAACCCTCAT                    | CAGTGTCTGGTCCATCT                     |
| Fgf21       | CTCCAGCAGCAGTTCTCTGA                 | CCTGGGTGTCAAAGCCTCTA                  |
| Acadm       | ATGACGGAGCAGCCAATGA                  | ATGGCCGCCACATCAGA                     |
| Acox1       | GGATGGTAGTCCGGAGAACA                 | AGTCTGGATCGTTCAGAATCAAG               |
| Fabp1       | CCAGGAGAACTTTGAGCCATTC               | TGTCCTTCCCTTTCTGGATGA                 |
| Cnr1        | TGAGAAAGAGGTGCCAGGAG                 | CTTGTAGCAGAGAGCCAGCC                  |
| Trpv1       | CATCATCAACGAGGACCCAG                 | AACCAGGGCAAAGTTCTTCC                  |
| Ppara       | AGTTCGGGAACAAGACGTTG                 | CAGTGGGGAGAGAGGACAGA                  |
| Gpr119      | TTCCAGCACTTGGTAAAGGC                 | TGGGAGCTTCACTCTGTCCT                  |
| Nape-pld    | TACTCATCCATGTCCCTCGG                 | ATATCTGCGTGGAACAGCCT                  |
| Faah        | CTGTACCAGTTGGAGCAGGG                 | GAGACCATGGACAAGGCG                    |
| Pparg2-T84A | ccattgggtcagctcttgcaatggaatgtcttcata | tatgaagacattccattcGcaagagctgacccaatgg |