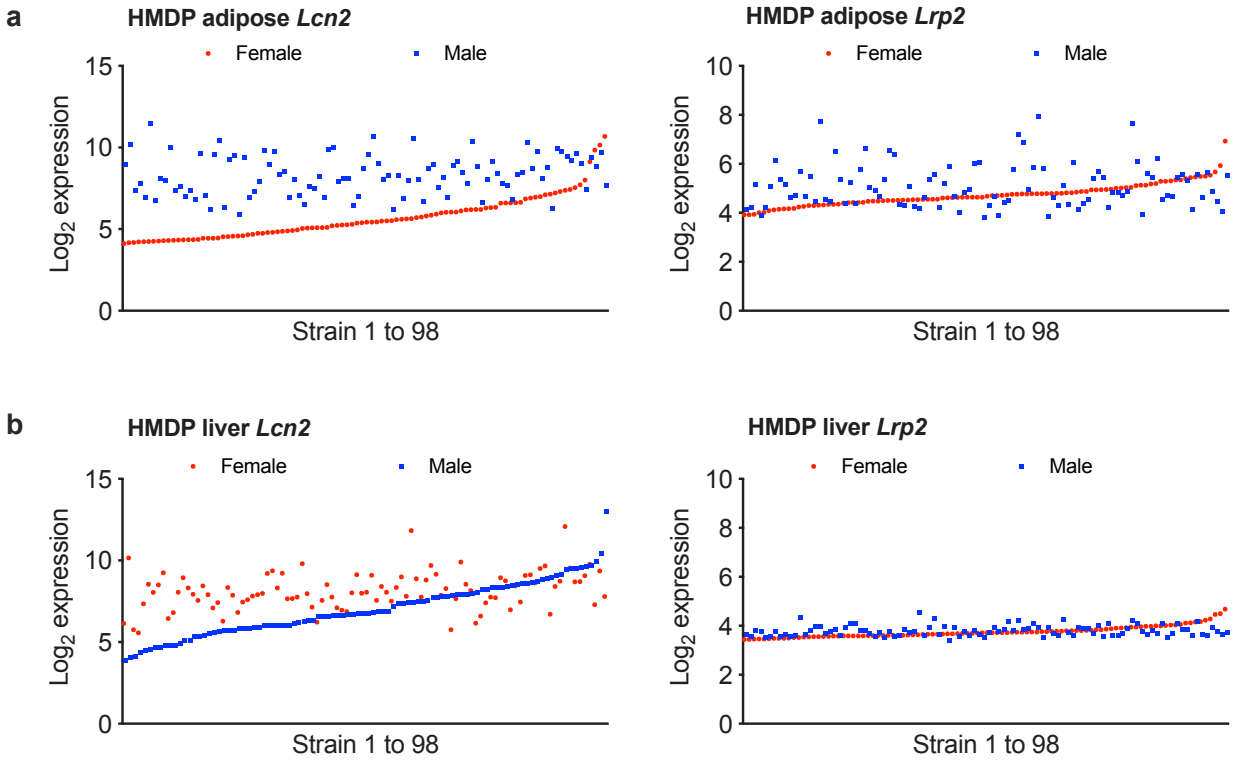

Figure S1. Gene-by-sex control of *Lcn2* and *Lrp2* expression in adipose and liver tissues. Individual matched-strain average expression profiles of **a,** adipose *Lcn2* or *Lrp2* and **b,** liver *Lcn2* or *Lrp2* in female (red) and male (blue) strains form the HF/HS fed HMDP cohort (n = 2-4 per sex/strain).

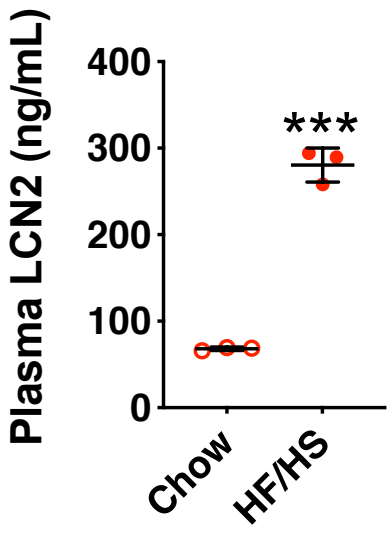

Figure S2. Diet-induced LCN2 upregulation in female C57BL/6J mice.
Plasma levels of LCN2 in female C57BL/6J mice fed either a chow (open circles) or HF/HS diet (closed circles), respectively. (n = 3 mice per diet). *P* values were calculated by Unpaired Student's t test. ***P < 0.001.

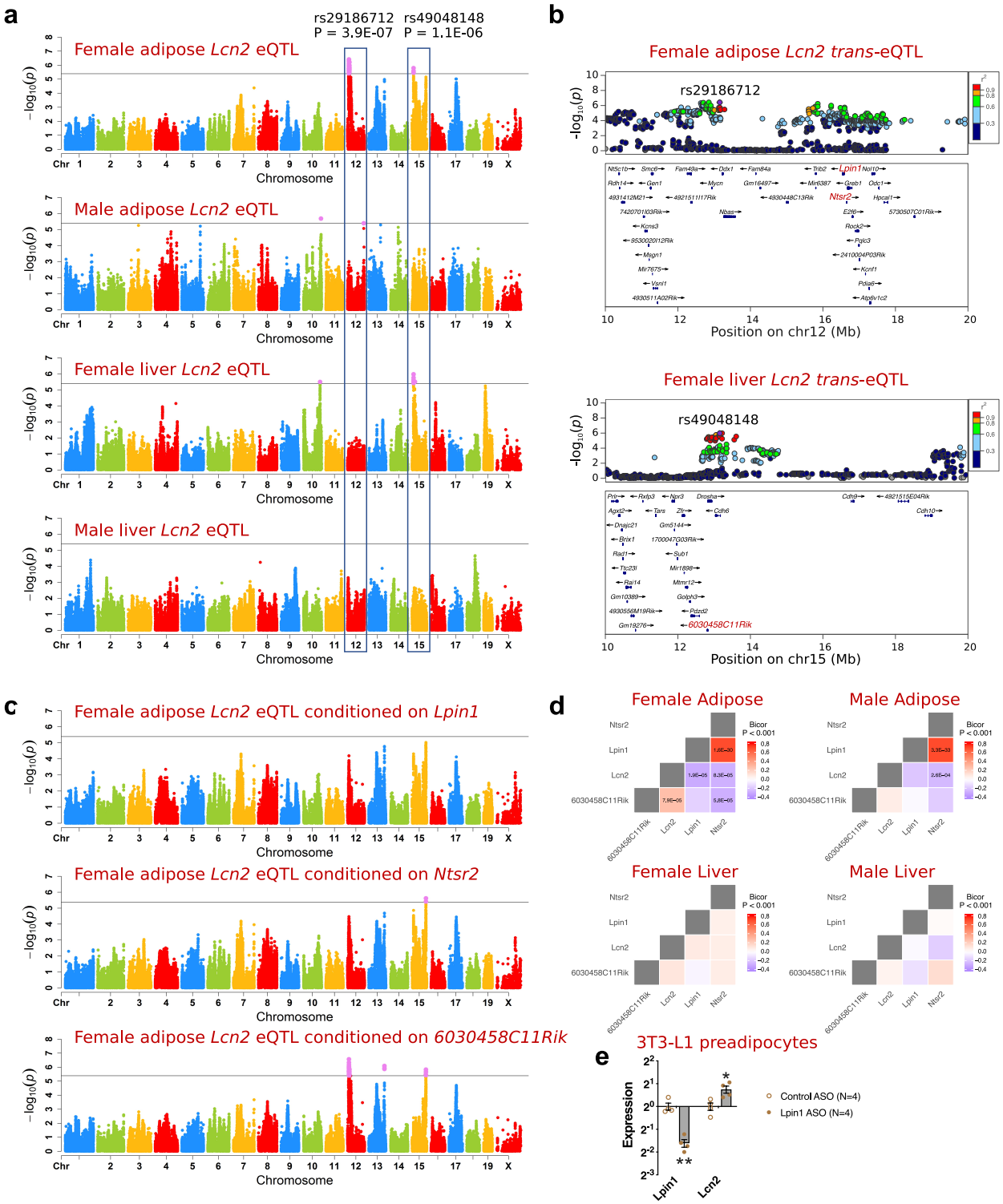

Figure S3. Genetic mapping *Lcn2* expression identified *Lpin1* as a potential trans-regulator. **a,** Association mapping of female adipose, male adipose, female liver and male liver *Lcn2* expression. **b,** Regional plots focusing on female adipose and liver *Lcn2* *trans*-eQTLs on chromosomes 12 and 15, respectively. Candidate genes are highlighted in red. **c,** Association

mapping of female adipose *Lcn2* expression by conditioning on *Lpin1*, *Ntsr2* and *6030458C11Rik* expression. Association significance threshold was set at $P < 4.1E-06$. **d,** Gene-by-gene bicorrelation matrix between *Lcn2* and candidate gene expression in their respective tissues. Positive bicorrelation was shown in red and negative bicorrelation in purple. Significance threshold was set at $P < 0.001$. Individual P values are indicated. **e,** Relative normalized expression values of *Lpin1* and *Lcn2* in 3T3-L1 preadipocytes treated with antisense oligonucleotide (ASO) against *Lpin1*. Data are presented as mean ± SEM (n = 4 per treatment). P values were calculated by Unpaired Student's t test. $*P < 0.05$; $**P < 0.01$.

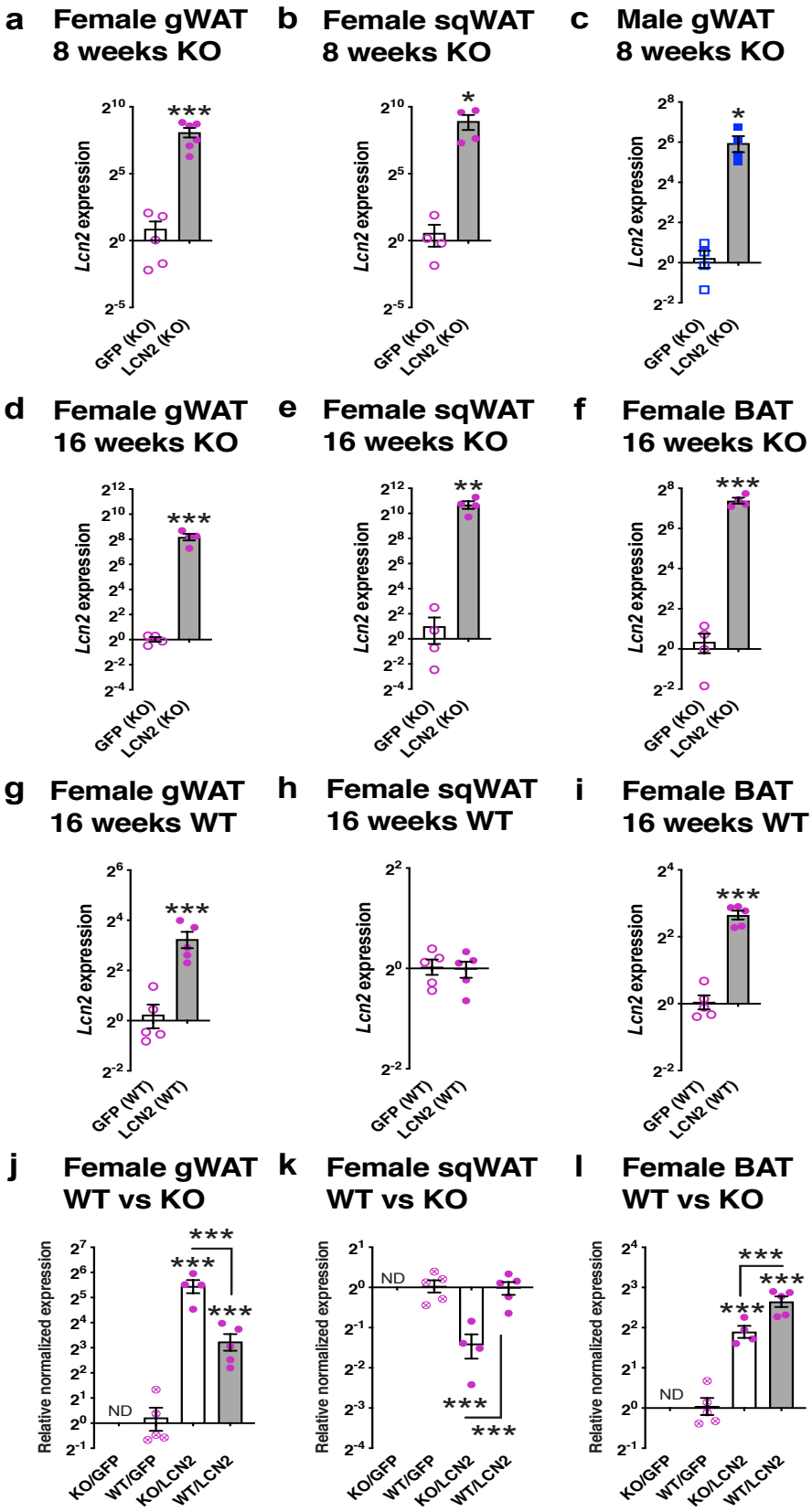
a
Female gWAT
8 weeks KO
b
Female sqWAT
8 weeks KO
c
Male gWAT
8 weeks KO
d
Female gWAT
16 weeks KO
e
Female sqWAT
16 weeks KO
f
Female BAT
16 weeks KO
g
Female gWAT
16 weeks WT
h
Female sqWAT
16 weeks WT
i
Female BAT
16 weeks WT
j
Female gWAT
WT vs KO
k
Female sqWAT
WT vs KO
l
Female BAT
WT vs KO
Lcn2 expression
Relative normalized expression
GFP (KO)
LCN2 (KO)
GFP (WT)
LCN2 (WT)
KO/GFP
WT/GFP
KO/LCN2
WT/LCN2
ND
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Figure S4. Adipose-specific expression of *Lcn2 via* AAV delivery.
Quantitative measurements of *Lcn2* expression in multiple adipose depots isolated from **a – c,** 8-week and **d – f,** 16-week HF/HS fed *Lcn2*-null mice or **g – i,** 16-week HF/HS fed C57BL/6J wildtype mice. **j – l,** Comparisons of *Lcn2* expression between *Lcn2*-null and C57BL/6J wildtype female mice in multiple adipose depots. Data are presented as mean ± SEM (n = 4-6 mouse/group for qPCR). *P* values were calculated by Unpaired Student's test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

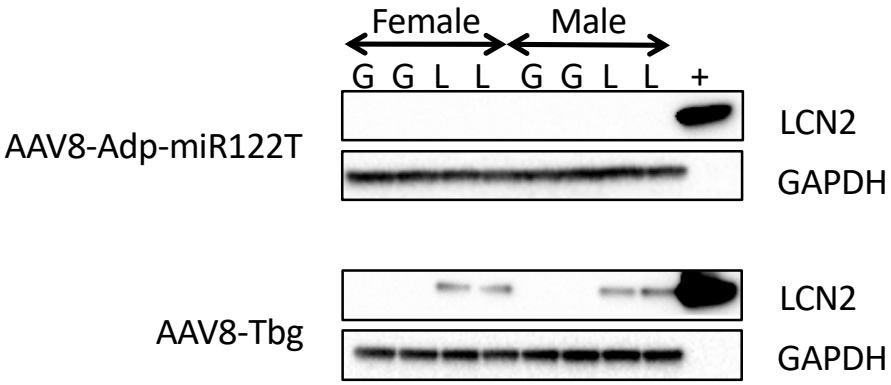

Figure S5. miR122 target sequence eliminates liver expression.
Immunoblot analyses of LCN2 expression in liver tissues extracted from female or male animals receiving either AAV8-Adp-miR122T (top) or AAV8-Tbg vectors (bottom). Recombinant LCN2 was used as positive control and GAPDH was used as loading control. G, GFP; L, LCN2 and +, positive control.

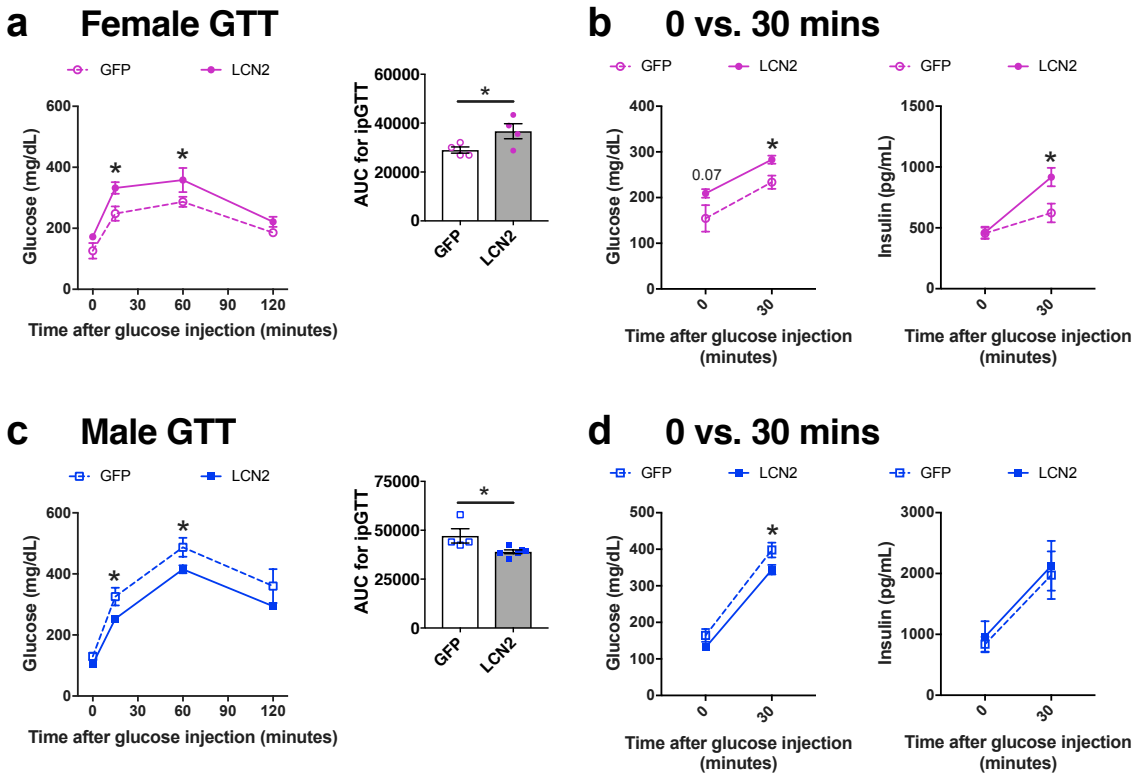

Figure S6. Adipose LCN2 alters glucose metabolism and liver mitochondria in females. Glucose-tolerance tests and their respective AUC, plasma glucose and insulin levels measured independently at 0 and 30 minutes after glucose injection in adipose-specific GFP or LCN2 overexpressing **a and b**, females and **c and d**, males, respectively after 6-weeks of HF/HS diet challenge. State 3 oxygen consumption rate (OCR) measured with CI (pyruvate/malate) or CII (succinate/rotenone) or fatty acid oxidation (palmitoyl-carnitine/malate) substrates for isolated liver mitochondria from **e**, female and **f** male animals, respectively. Data are presented as mean ± SEM (n = 4-6 animals per group for GTT; n = 5 mitochondria per group for OCR). *P* values were calculated by unpaired Student's t test for AUC and plasma analytes; Repeated measures 2-factor ANOVA corrected by post-hoc "Holm-Sidak's" multiple comparisons test for GTT and OCR measures; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

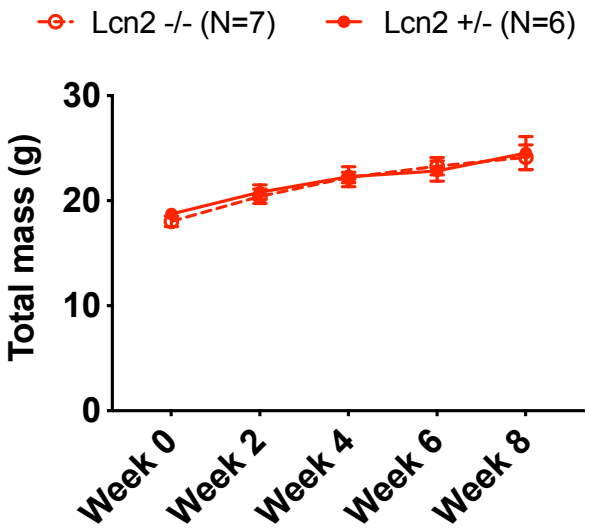

Figure S7. No bodyweight changes between *Lcn2*-null and heterozygous female mice.
Eight-week old female littermates of *Lcn2*-null and heterozygous mice fed a HF/HS diet for eight additional weeks. Total mass was monitored every two weeks. Data are presented as mean ± SEM (n = 6-7 animals).

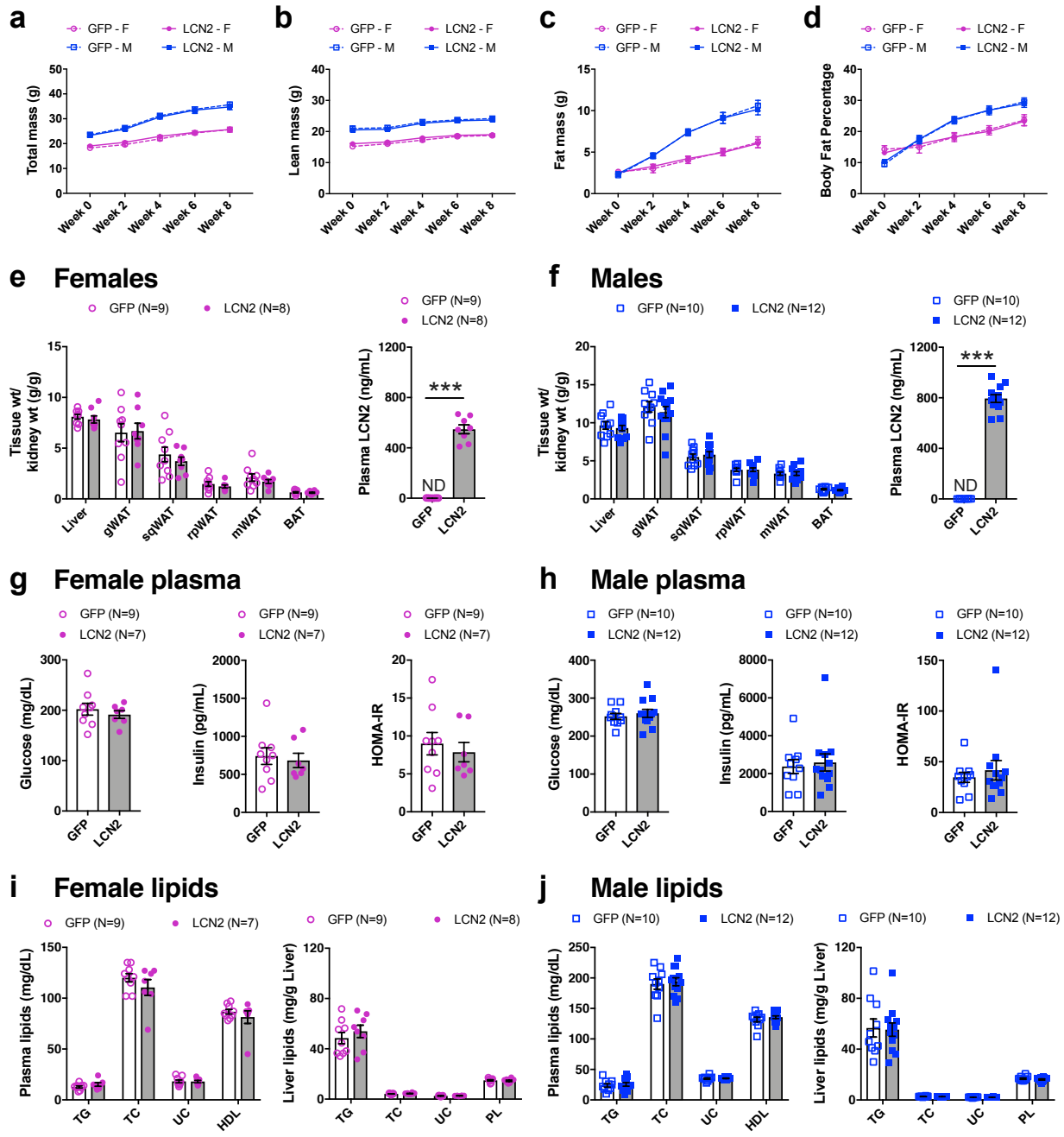

Figure S8. No phenotypic changes observed in animals overexpressing liver LCN2.
Eight-week old females and males of C57BL/6J mice were injected with AAV vectors carrying either GFP or LCN2 cDNA under the control of Tbg promoter and fed with HF/HS diet for eight additional weeks. Body weight composition such as **a**, total mass **b**, lean mass **c**, fat mass and **d**, body fat percentage were monitored every two weeks. Comparisons of kidney-normalized tissue weights and plasma levels of LCN2 between GFP and LCN2 groups in **e**, females and **f**, males, respectively. Comparisons of plasma levels of glucose, insulin and HOMA-IR from **g**, female and **h**, male animals, respectively. Similarly, comparisons of plasma levels of triglycerides (TG), total cholesterol (TC), unesterified cholesterol (UC) and HDL as well as hepatic TG, TC, UC and

phospholipid (PL) levels from i, female and j, male animals, respectively. Data are presented as mean ± SEM (n = 7-12 animals). *P* values were calculated by **a – d**, Repeated-measures 2-factor ANOVA corrected by post-hoc "Holm-Sidak's" multiple comparisons test; **e – j**, Unpaired Student's t test for tissue weights, plasma and liver analytes. ***$P < 0.001$.

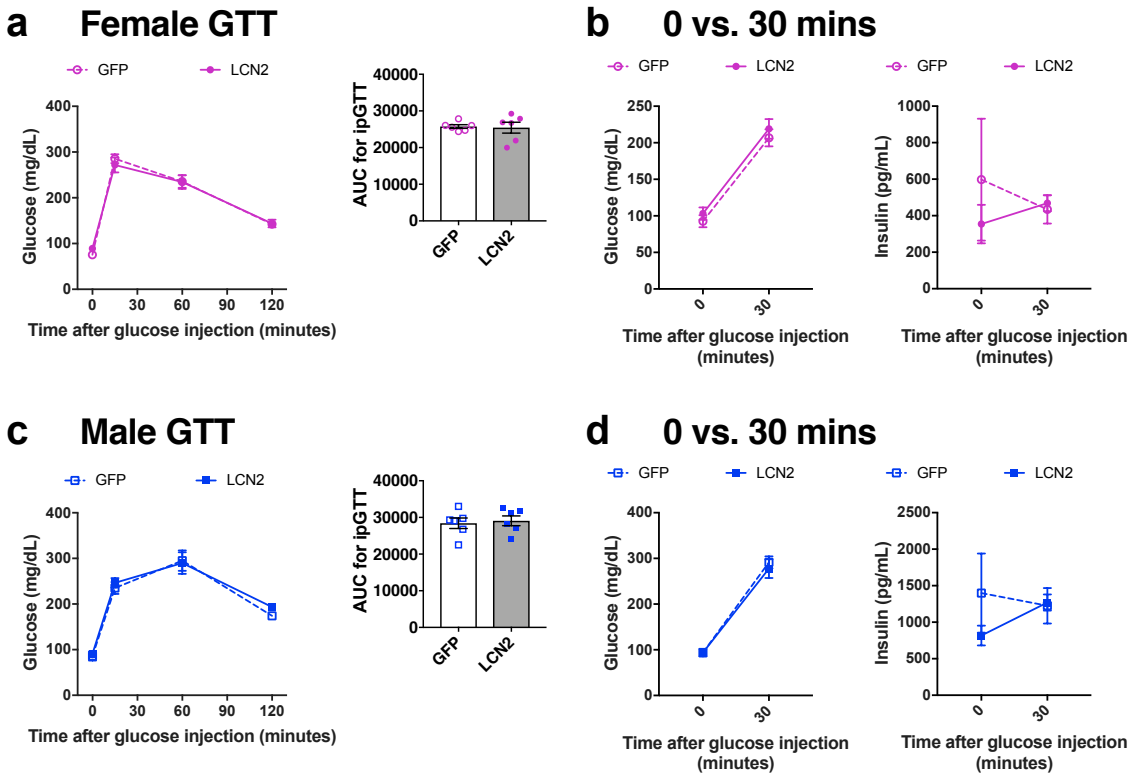

Figure S9. Liver LCN2 does not affect glucose metabolism or liver mitochondria.
Glucose-tolerance tests and their respective AUC, plasma glucose and insulin levels measured independently at 0 and 30 minutes after glucose injection in liver-specific GFP or LCN2 overexpressing **a and b**, females and **c and d**, males, respectively after 6-weeks of HF/HS diet challenge. State 3 oxygen consumption rate (OCR) measured with CI (pyruvate/malate) or CII (succinate/rotenone) or fatty acid oxidation (palmitoyl-carnitine/malate) substrates for isolated liver mitochondria from **e**, female and **f** male animals, respectively. Data are presented as mean ± SEM (n = 6 animals per group for GTT; n = 3-5 mitochondria per group for OCR). *P* values were calculated by unpaired Student's t test for AUC and plasma analytes; Repeated measures 2-factor ANOVA corrected by post-hoc "Holm-Sidak's" multiple comparisons test for GTT and OCR measures.

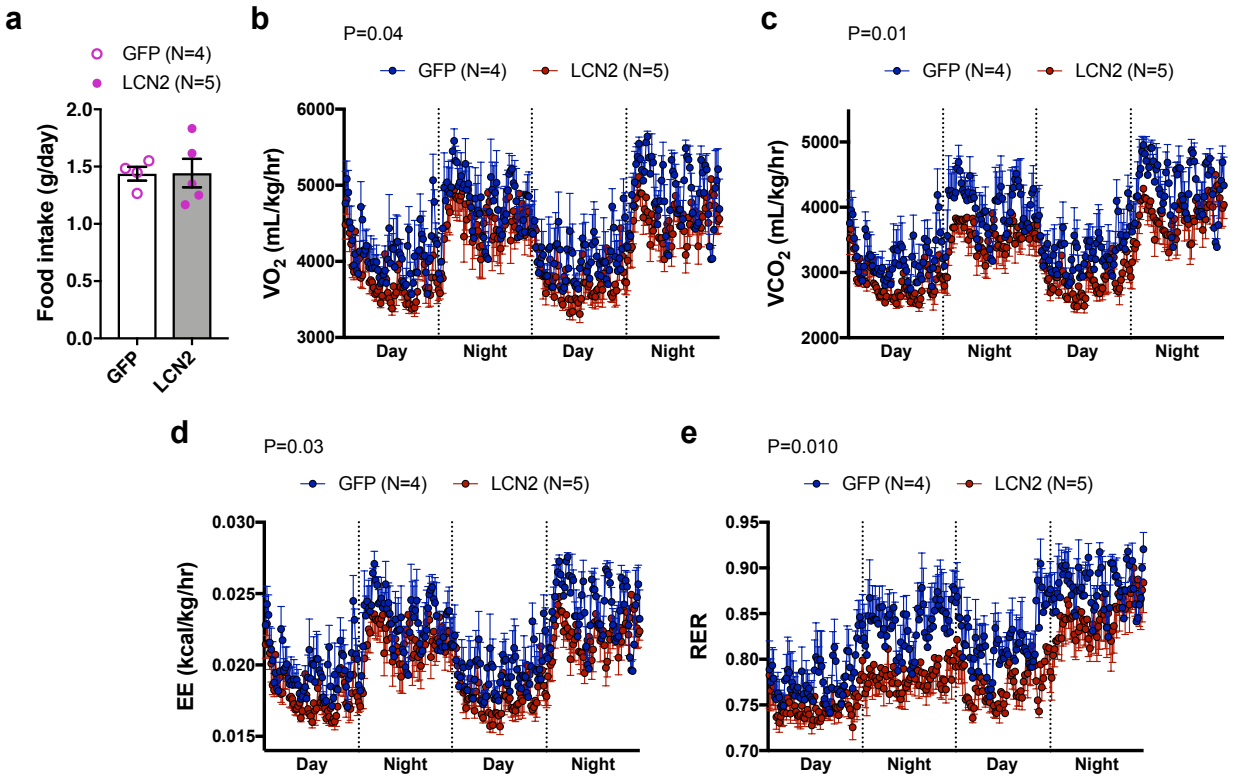

Figure S10. Metabolic chamber measurements as time-series data.
Metabolic chamber measurements such as **a**, food intake, **b**, oxygen consumption rate (VO_2), **c**, carbon dioxide production rate (VCO_2), **d**, energy expenditure (EE) and **e**, respiratory exchange rate (RER) between GFP and LCN2 overexpressing females presented as time-series data. Data are presented as mean ± SEM (n = 4-5 animals per group). *P* values were calculated by repeated measures 2-factor ANOVA.

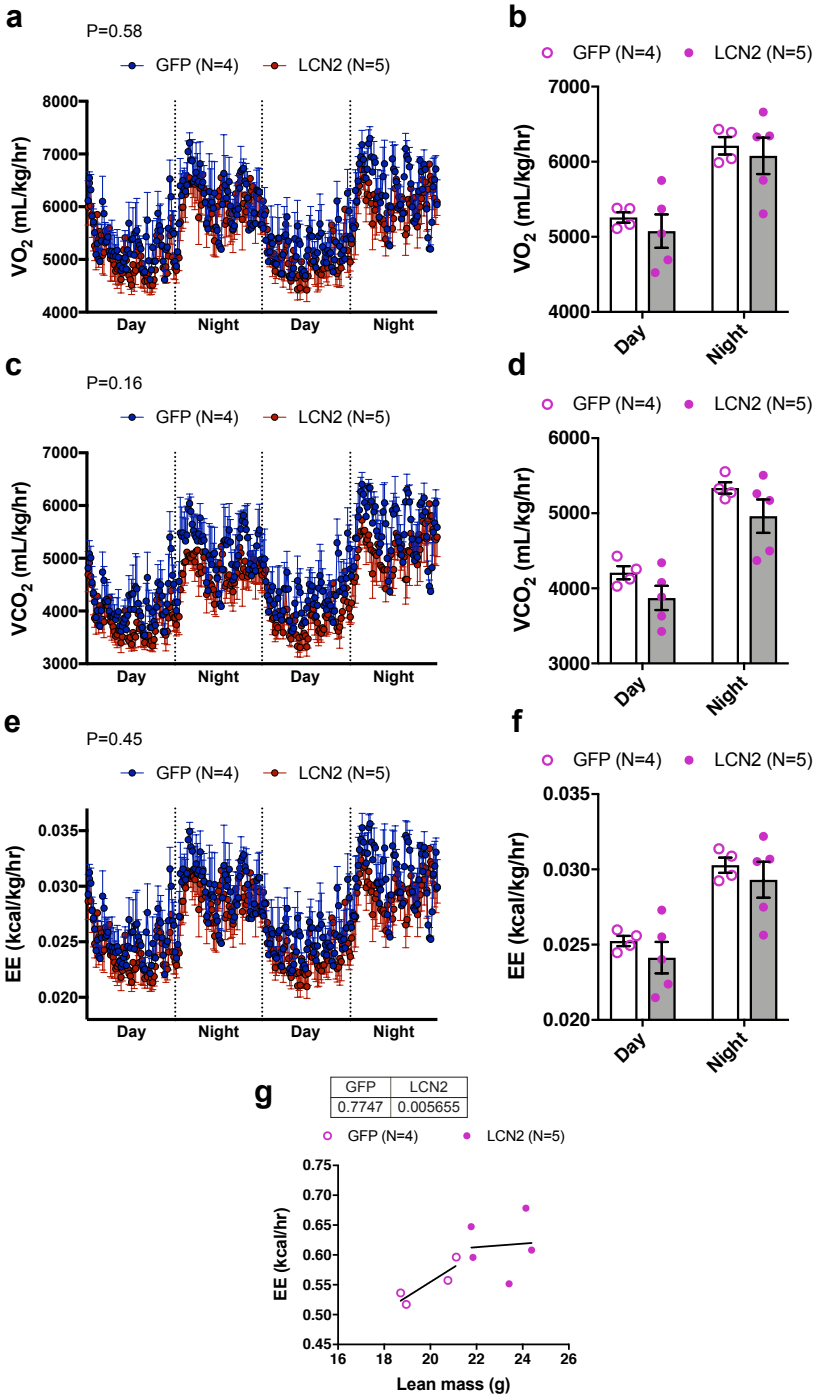

Figure S11. Metabolic chamber measurements normalized to lean body mass.
Metabolic chamber measurements such as **a and b**, oxygen consumption rate (VO_2), **c and d**, carbon dioxide production rate (VCO_2), **e and f**, energy expenditure (EE) normalized to lean body mass, between GFP and LCN2 overexpressing females, presented as time-series data or aggregated. **g,** Unnormalized EE is plotted against lean body mass, regression coefficients are listed in the table inset. Data are presented as mean ± SEM (n = 4-5 animals per group). *P* values were calculated by repeated measures 2-factor ANOVA.

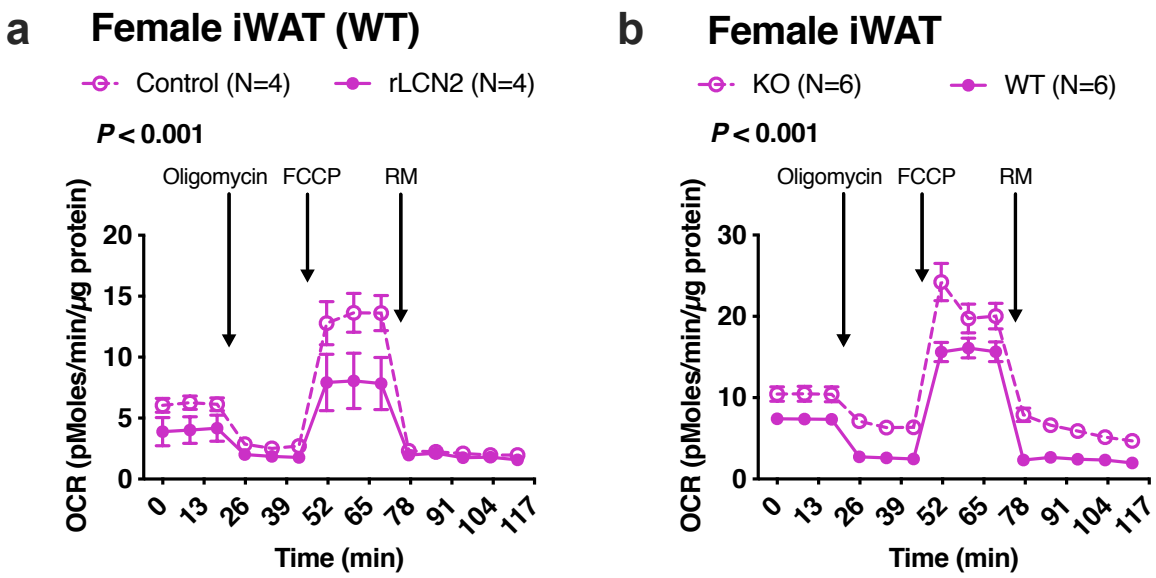

Figure S12. Adipose LCN2 reduced mitochondrial respiration.
Comparisons of oxygen consumption rate (OCR) profiles of *ex vivo* differentiated inguinal adipocytes (iWAT) between **a,** control and recombinant LCN2-treated groups from WT females, **b,** untreated KO and WT females. Data are presented as mean ± SEM (n = 4-6 per group). *P* values were calculated by Repeated measures 2-factor ANOVA.

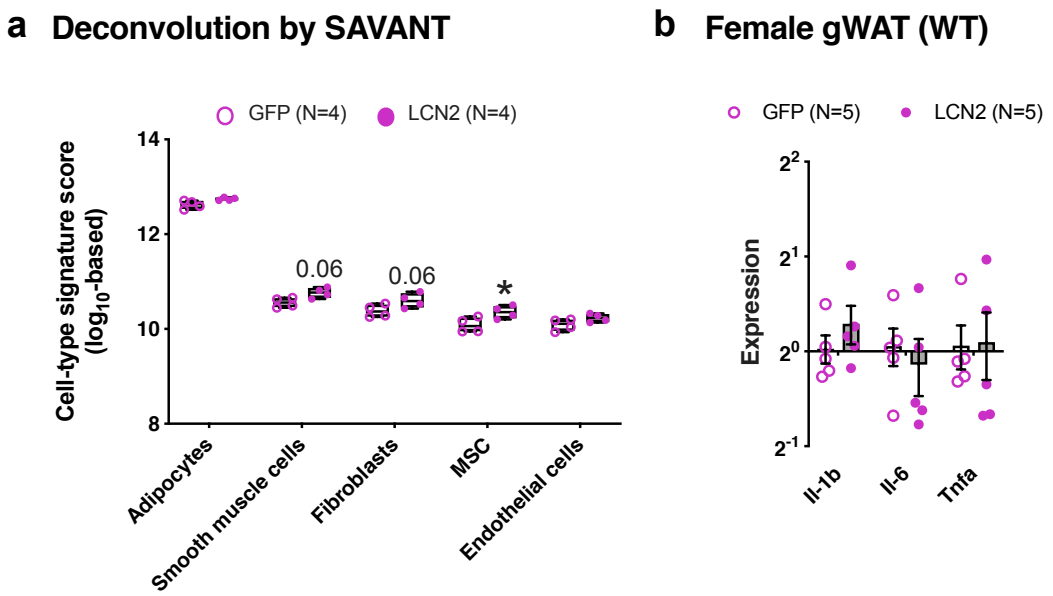

Figure S13. Changes in other cell populations and inflammation markers by LCN2 overexpression.

a, Adipose deconvolution focusing on all cell populations shown in Figure 5e. **b,** Quantitative measurements of adipose inflammation markers such as *Il-1b*, *Il-6* and *Tnfa* in female gonadal adipose depots isolated from GFP or LCN2 overexpressing C57BL/6J WT mice. Data are presented as mean ± SEM (n = 4 mouse/group for global RNA sequencing; n = 5 mouse/group for qPCR). *P* values were calculated by 2-factor ANOVA corrected by post-hoc "Holm-Sidak's" multiple comparisons test. $*P < 0.05$.

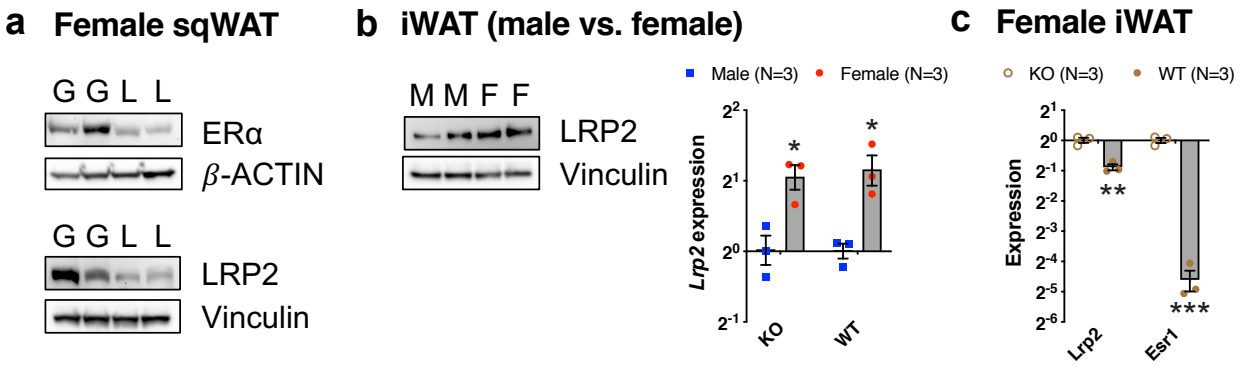

Figure S14. Adipose LCN2 downregulated both LRP2 and ERα expression.
a) Immunoblot analyses of ERα and LRP2 protein levels in female subcutaneous adipose depots extracted from GFP or LCN2 overexpressing *Lcn2*-null mice. *β*-ACTIN and Vinculin were used as loading control. G, GFP; L, LCN2. **b)** Immunoblot analyses and quantitative measurements of LRP2 expression in *ex vivo* differentiated primary inguinal adipocytes isolated from both sexes of *Lcn2*-null or C57BL/6J wildtype mice. Vinculin was used as loading control. M, Male; F, Female. **c)** Quantitative measurements of *Lrp2* and *Esr1* expression in *ex vivo* differentiated primary inguinal adipocytes isolated from both *Lcn2*-null and C57BL/6J wildtype mice. Data are presented as mean ± SEM (n = 2 mouse/group for WB; 3 mouse/iWAT/group for qPCR). *P* values were calculated by Unpaired Student's t test. *P < 0.05; **P < 0.01; ***P < 0.001.

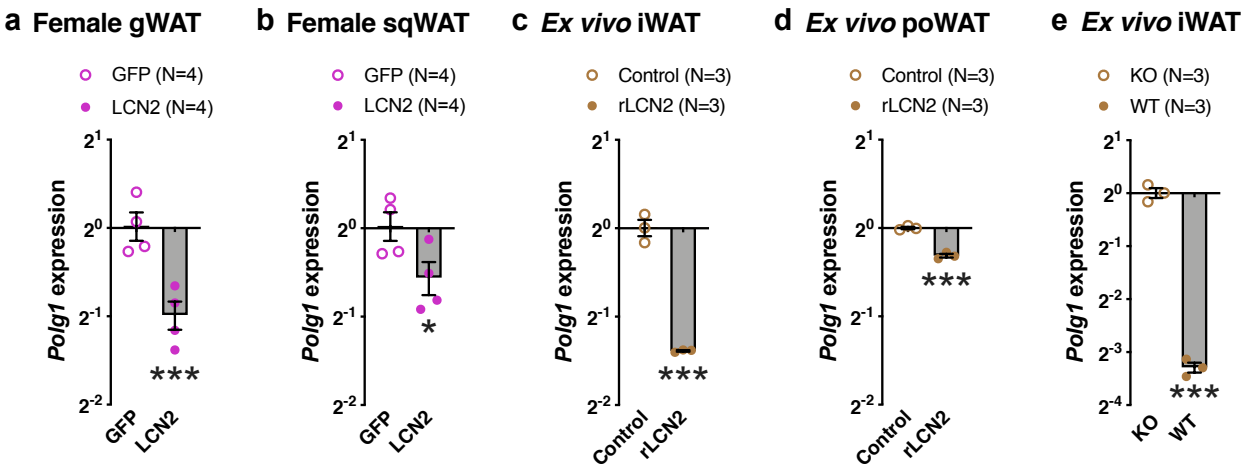

Figure S15. LCN2 overexpression downregulated *Polg1* expression in adipose tissues. Quantitative measurements of mitochondrial polymerase *Polg1* in **a,** female gonadal WAT and **b,** female subcutaneous WAT, **c and d,** *ex vivo* differentiated primary inguinal or periovarian adipocytes isolated from female *Lcn2*-null mice treated with recombinant LCN2, **e,** *ex vivo* differentiated primary inguinal adipocytes isolated from female *Lcn2*-null or WT mice. Data are presented as mean ± SEM (n = 4 mouse/group for qPCR; n = 6 female mouse/iWAT or poWAT divided into two groups for *ex vivo* treatment). *P* values were calculated by Unpaired Student's test. **P* < 0.05; ****P* < 0.001.