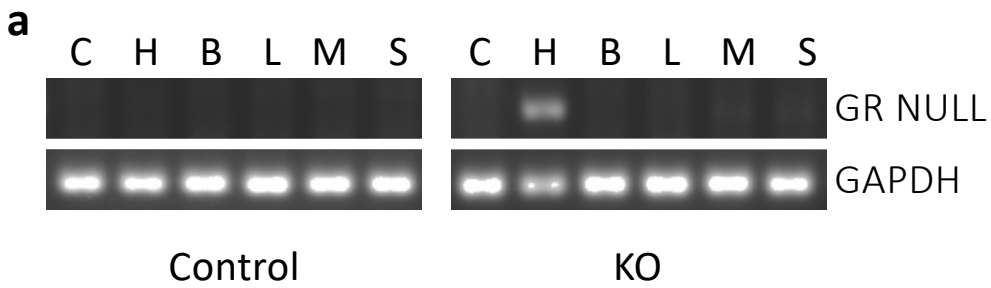
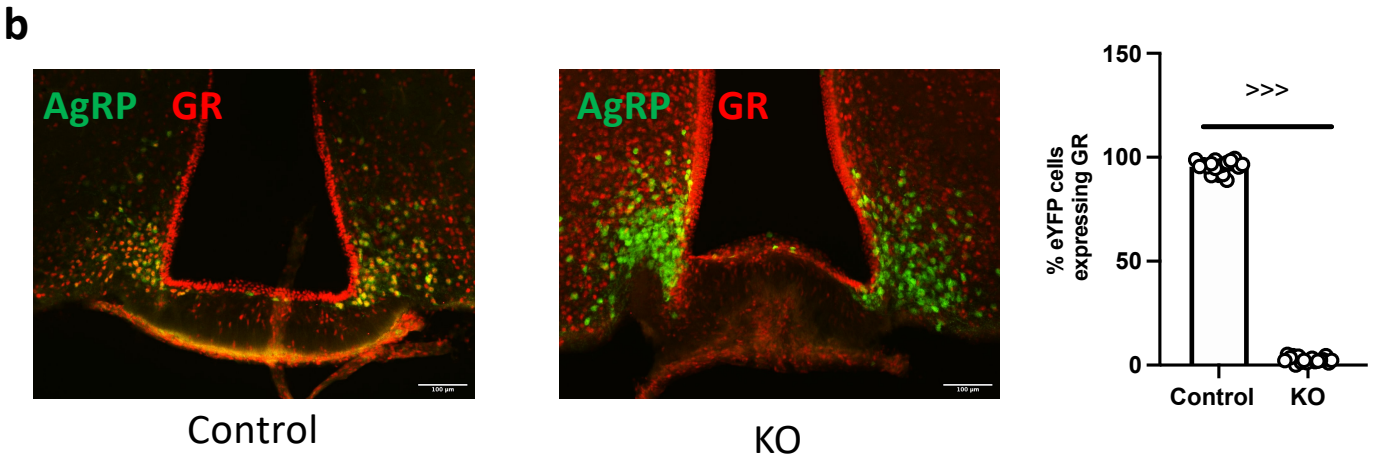
Figure 1


a
C H B L M S
C H B L M S
GR NULL
GAPDH
Control
KO


b
AgRP GR
AgRP GR
Control
KO
150
100
50
0
% eYFP cells expressing GR
>>>
Control
KO

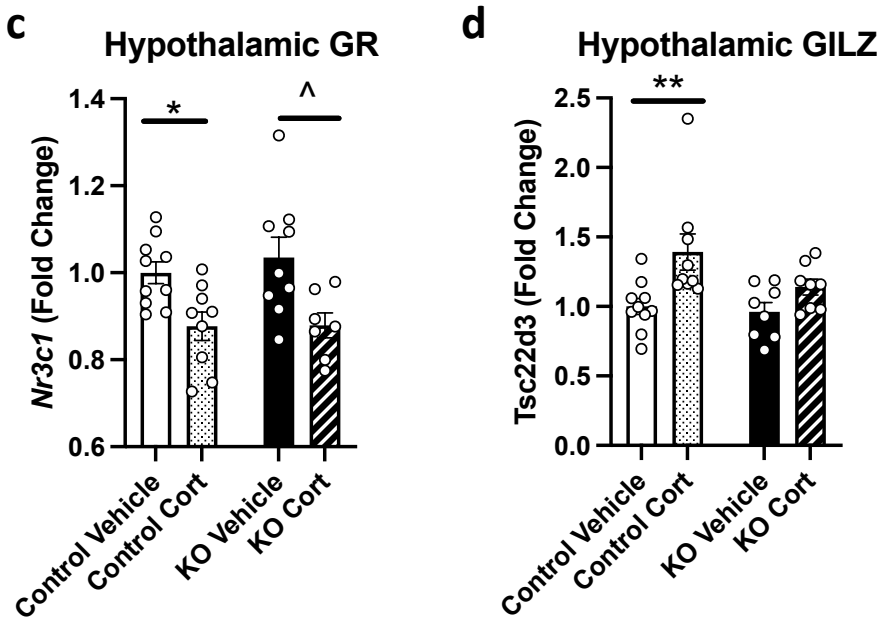

c
Hypothalamic GR
Nr3c1 (Fold Change)
1.4
1.2
1.0
0.8
0.6
*
^
Control Vehicle
Control Cort
KO Vehicle
KO Cort
d
Hypothalamic GILZ
Tsc22d3 (Fold Change)
2.5
2.0
1.5
1.0
0.5
0.0
**
Control Vehicle
Control Cort
KO Vehicle
KO Cort

Figure 2

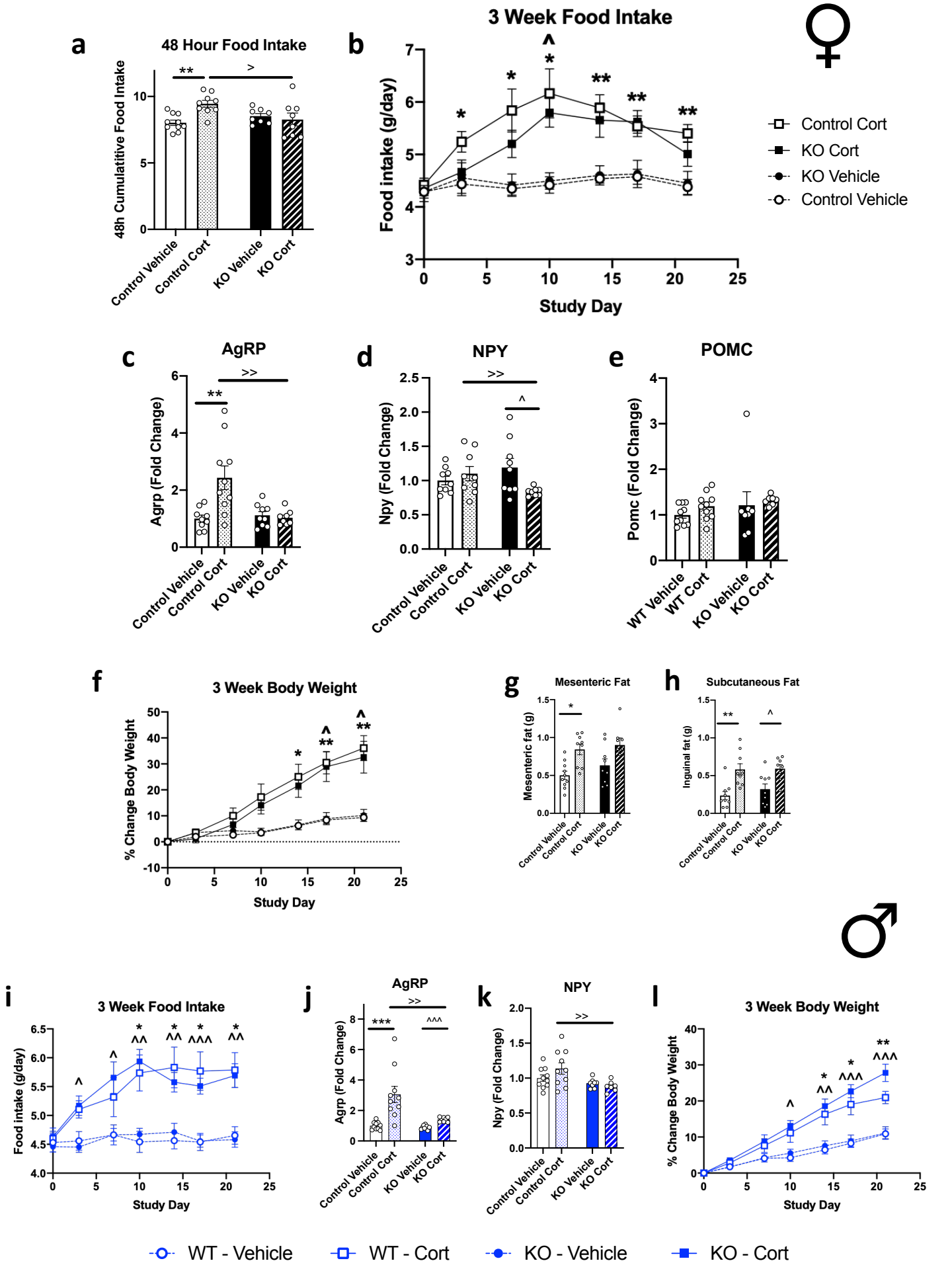

a
48 Hour Food Intake
48h Cumulatitive Food Intake
Control Vehicle
Control Cort
KO Vehicle
KO Cort
b
3 Week Food Intake
Food intake (g/day)
Study Day
Control Cort
KO Cort
KO Vehicle
Control Vehicle
c
AgRP
Agrp (Fold Change)
d
NPY
Npy (Fold Change)
e
POMC
Pomc (Fold Change)
WT Vehicle
WT Cort
f
3 Week Body Weight
% Change Body Weight
g
Mesenteric Fat
Mesenteric fat (g)
h
Subcutaneous Fat
Inguinal fat (g)
i
3 Week Food Intake
j
AgRP
k
NPY
l
3 Week Body Weight
WT - Vehicle
WT - Cort
KO - Vehicle
KO - Cort

Figure 3

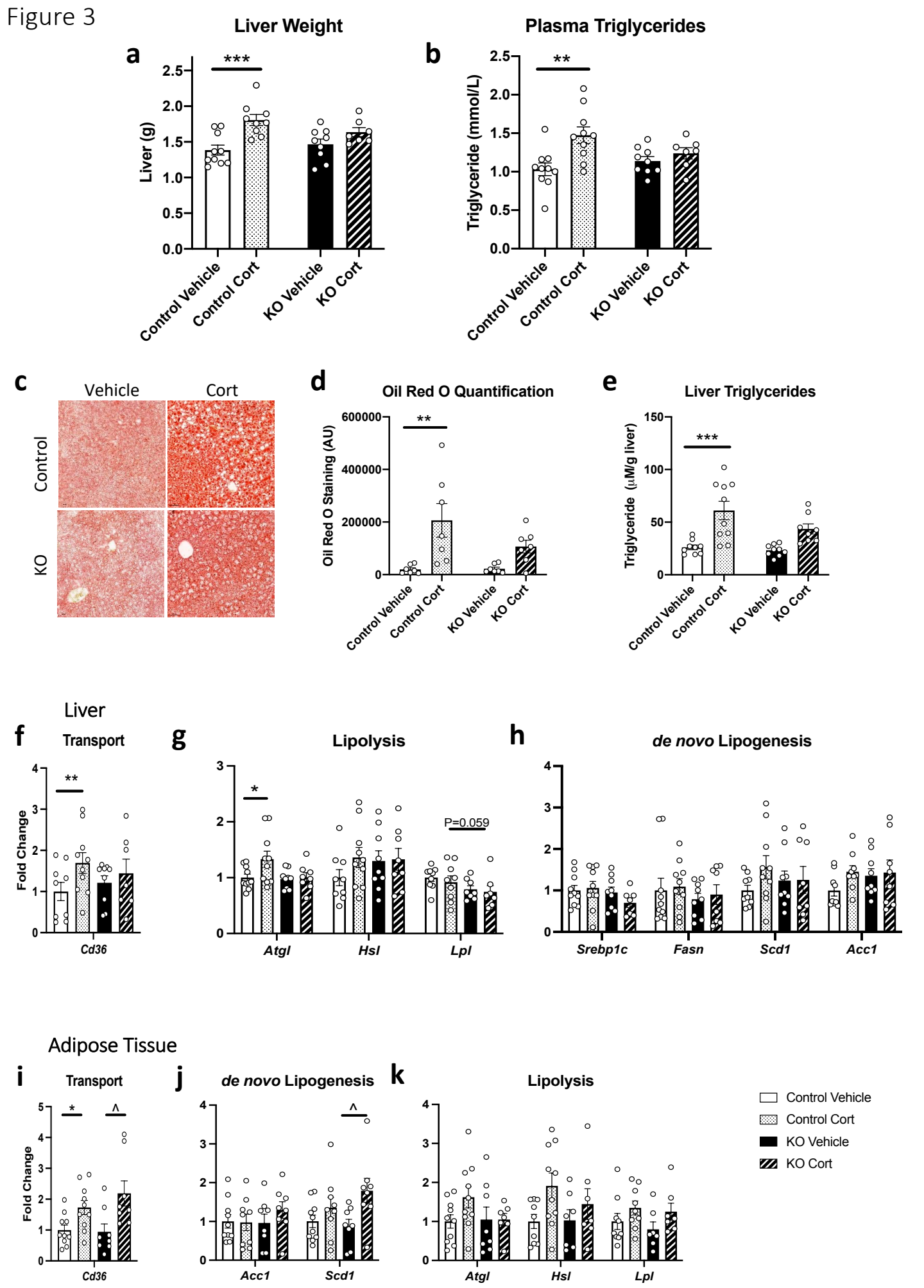
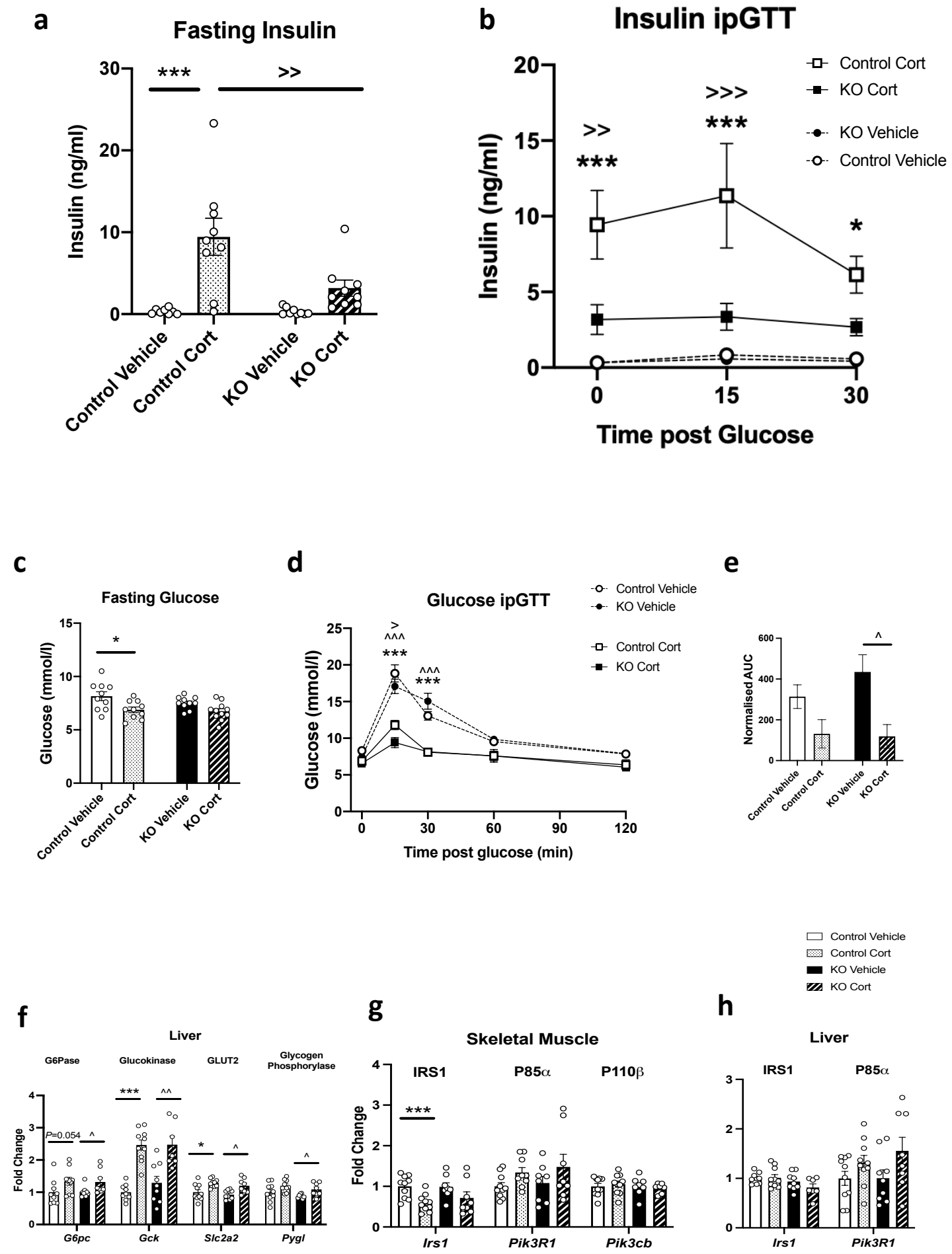

Liver Weight
a
***
Liver (g)
2.5
2.0
1.5
1.0
0.5
0.0
Control Vehicle
Control Cort
KO Vehicle
KO Cort
Plasma Triglycerides
b
**
Triglyceride (mmol/L)
c
Vehicle
Cort
Control
KO
d
Oil Red O Quantification
Oil Red O Staining (AU)
600000
400000
200000
0
e
Liver Triglycerides
Triglyceride (μM/g liver)
150
100
50
Liver
f
Transport
Fold Change
Cd36
g
Lipolysis
*
P=0.059
Atgl
Hsl
Lpl
h
de novo Lipogenesis
Srebp1c
Fasn
Scd1
Acc1
Adipose Tissue
i
Transport
^
j
de novo Lipogenesis
k
Lipolysis
Control Vehicle
Control Cort
KO Vehicle
KO Cort

Figure 4:


a
Fasting Insulin
Insulin (ng/ml)
***
>>
Control Vehicle
Control Cort
KO Vehicle
KO Cort
b
Insulin ipGTT
Insulin (ng/ml)
Time post Glucose
Control Cort
KO Cort
KO Vehicle
Control Vehicle
>>
***
>>>
***
*
c
Fasting Glucose
Glucose (mmol/l)
d
Glucose ipGTT
Glucose (mmol/l)
Time post glucose (min)
e
Normalised AUC
Control Vehicle
Control Cort
KO Vehicle
KO Cort
f
Liver
G6Pase
Glucokinase
GLUT2
Glycogen Phosphorylase
Fold Change
P=0.054
G6pc
Gck
Slc2a2
Pygl
g
Skeletal Muscle
IRS1
P85α
P110β
Irs1
Pik3R1
Pik3cb
h
Liver
IRS1
P85α
Irs1
Pik3R1

Chronic Glucocorticoid treatment induces hepatic lipid accumulation and hyperinsulinaemia in part through actions on AgRP Neurons

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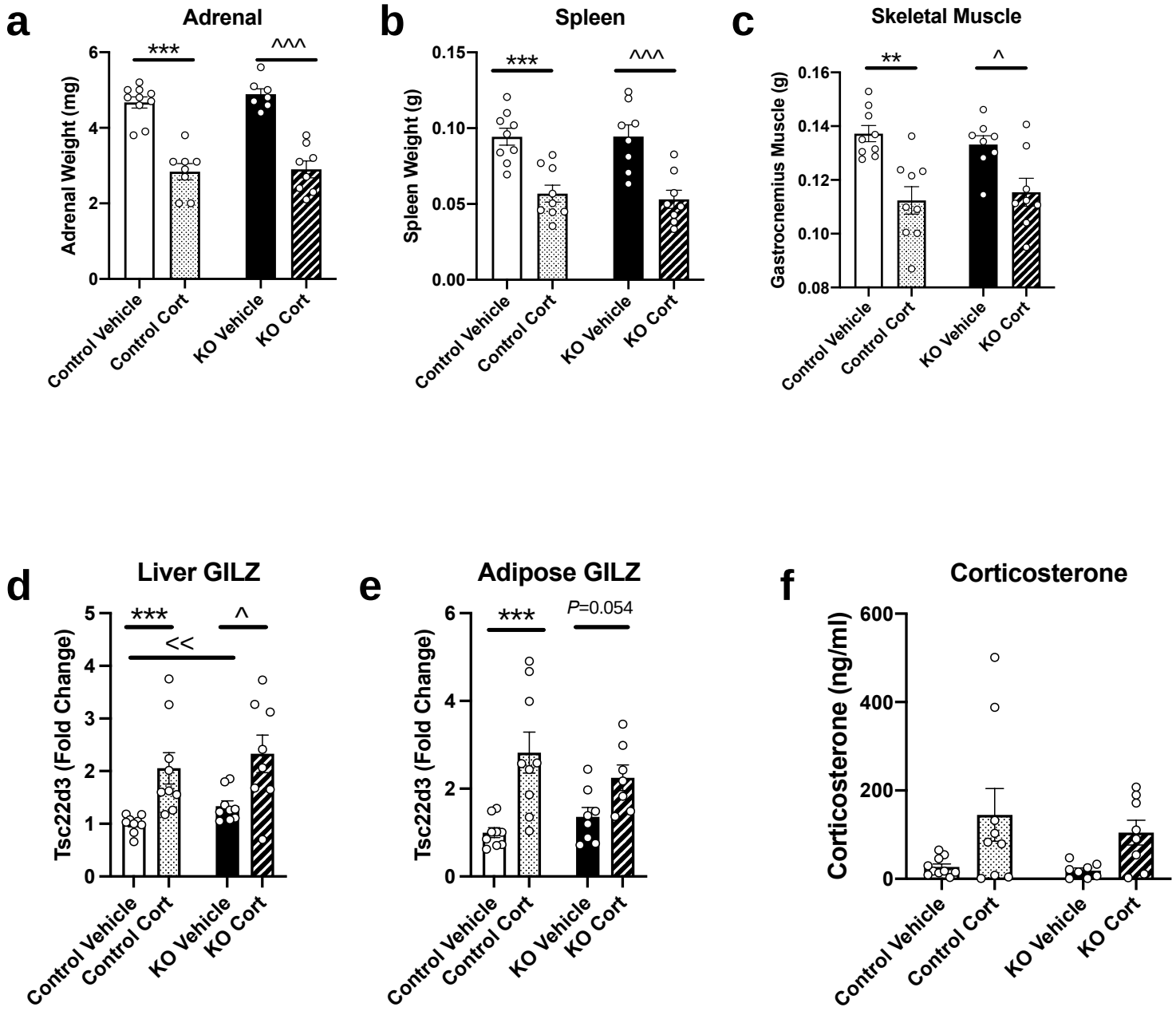
2. MRC Metabolic Diseases Unit, Wellcome-MRC Institute of Metabolic Science, University of Cambridge, Cambridge, CB2 0QQ

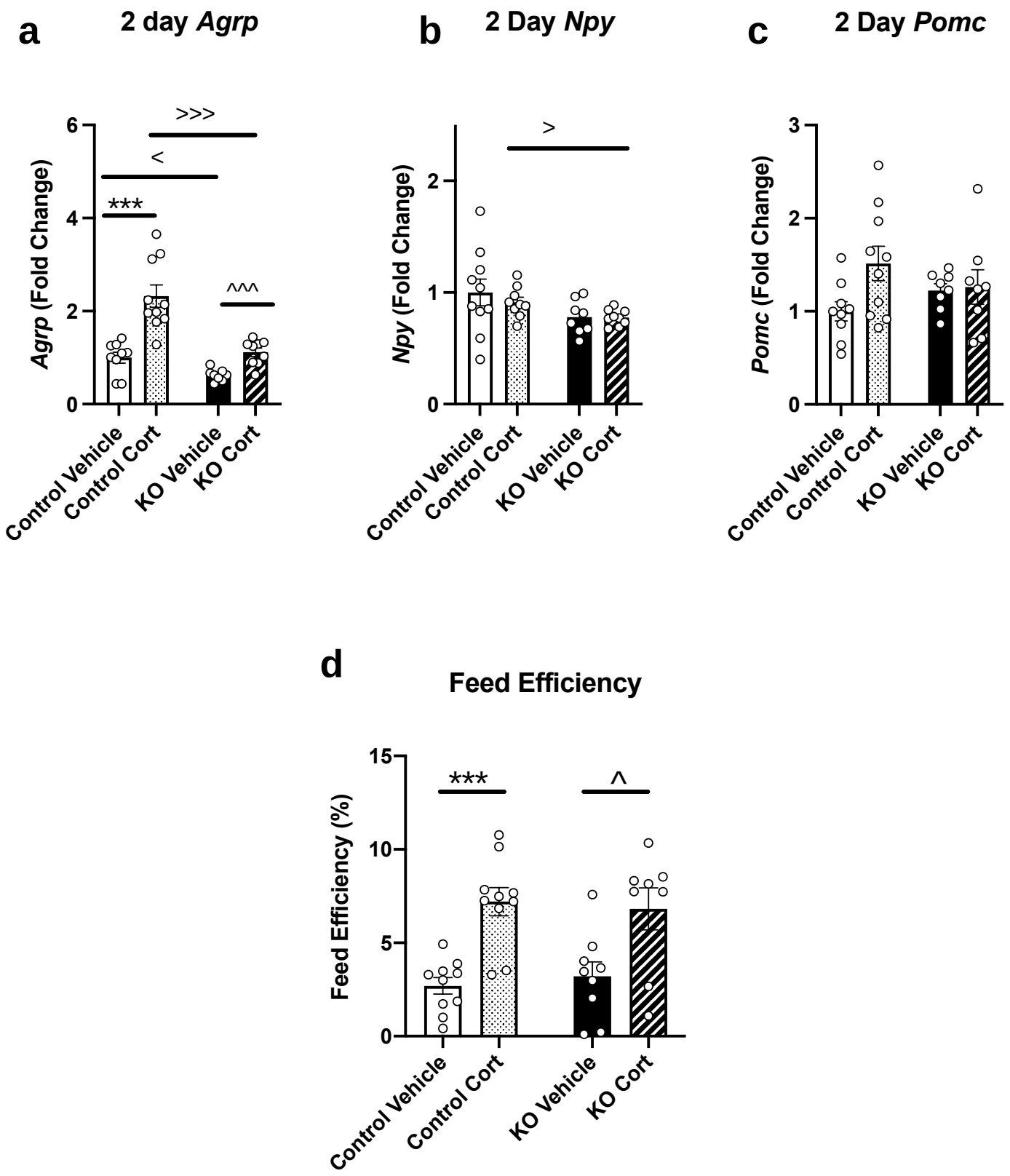
*Joint Senior Authors

Harno *et al.* Supplemental Figure S1:



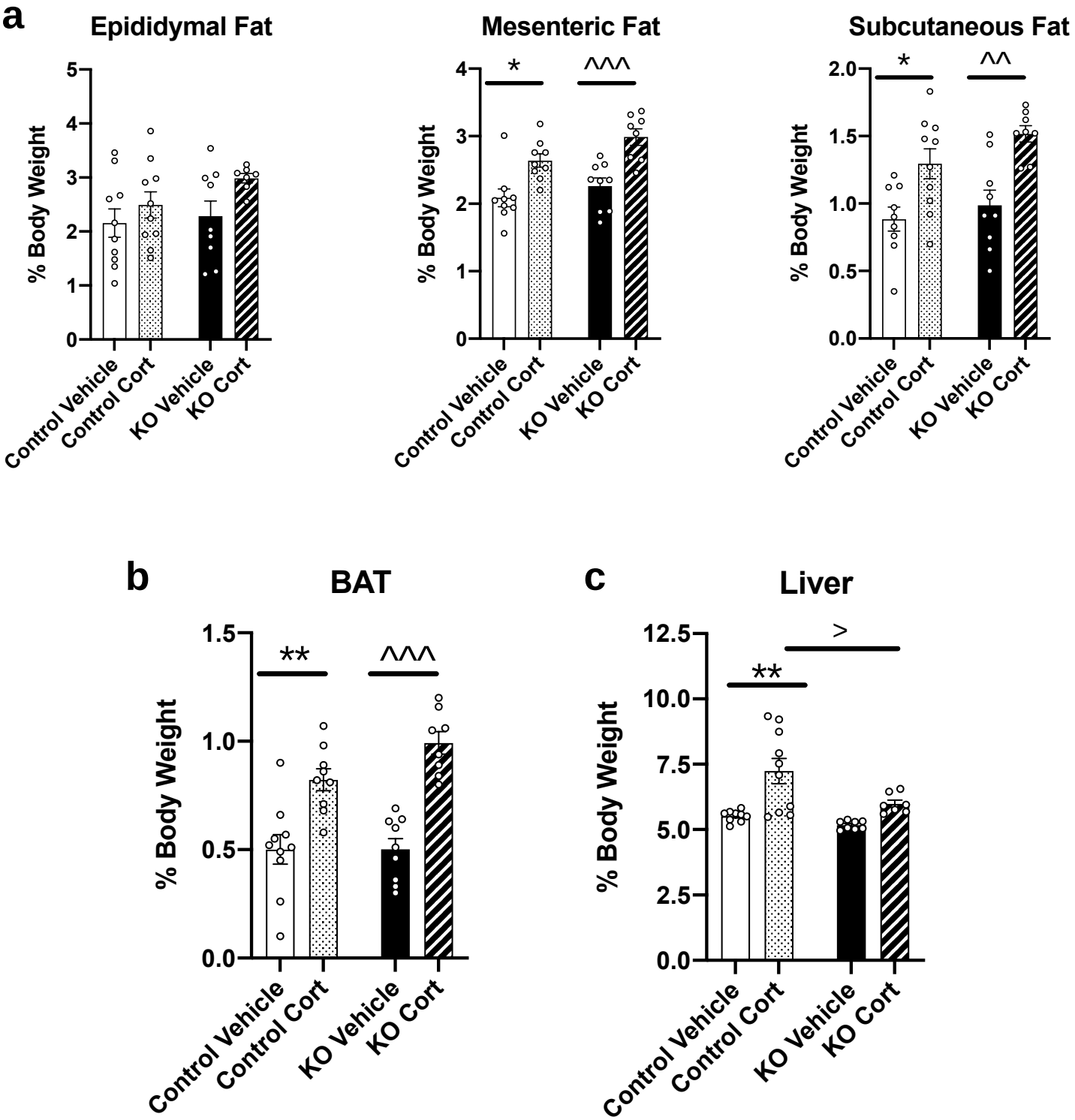
Supplemental Figure S1: Peripheral glucocorticoid target tissues are not affected by knockdown of GR on AgRP neurons in female mice. (a) adrenal, (b) spleen, and (c) skeletal muscle wet weight. (d) liver and (e) subcutaneous adipose tissue *Tsc22d3* (GILZ) mRNA expression, (f) circulating corticosterone in control and AgRP-GR KO mice treated with vehicle or 75µg/ml corticosterone (Cort) in drinking water for 3 weeks. n=7-10, (a)-(c) and (f) Two-way ANOVA with Tukey Multiple Comparison test. (d) and (e) Mann-Whitney non-parametric t-tests. ***P*<0.01 ****P*<0.001 control vehicle vs. Cort, ^*P*<0.05, ^^^*P*<0.001 KO vehicle vs. Cort, <<P<0.01 control vehicle vs. KO vehicle.

Harno *et al.* Supplemental Figure S2:



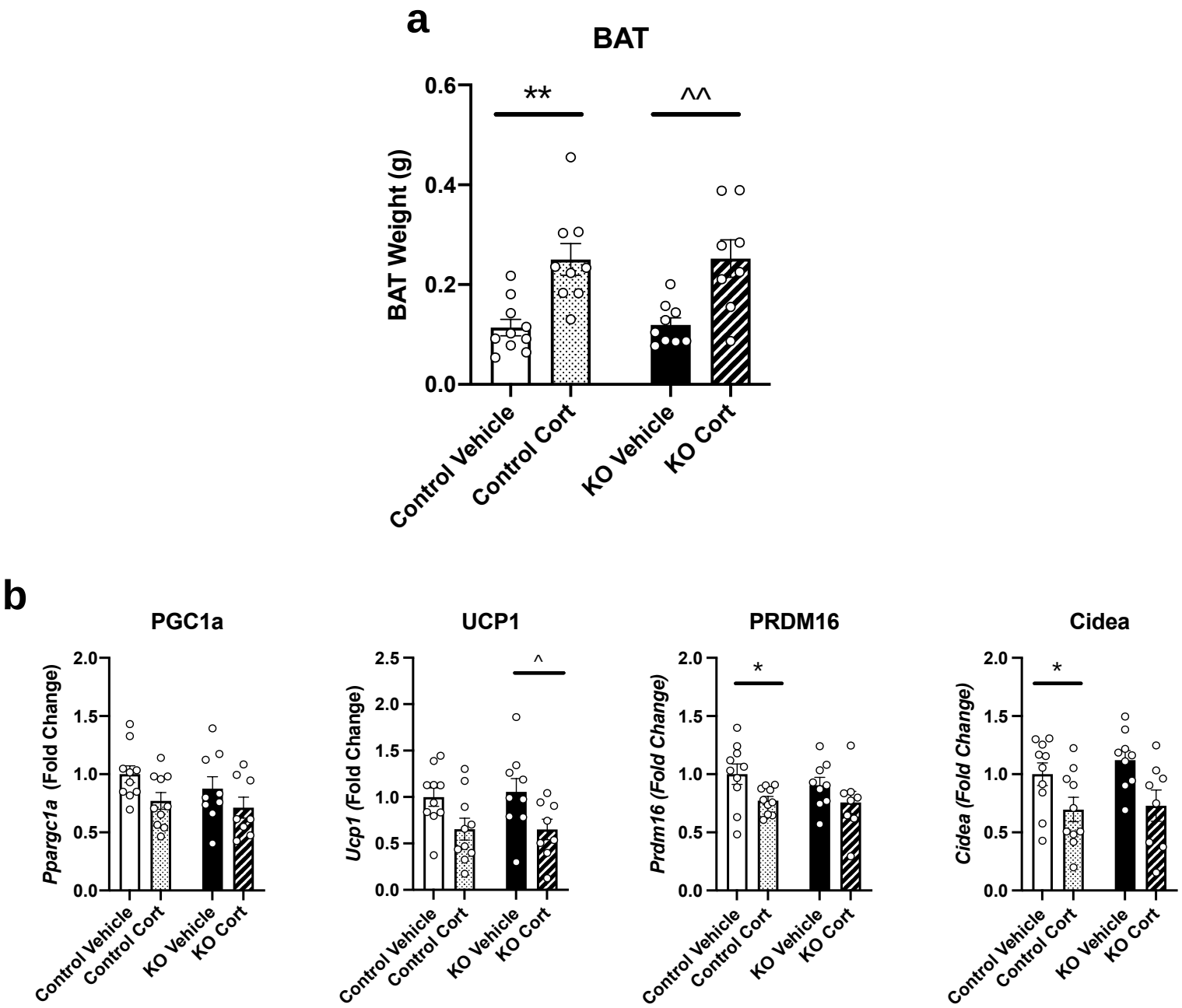
Supplemental Figure S2: Neuropeptide expression in 2 day Cort treated female control and AgRP-GR KO mice. mRNA expression of (a) *Agrp*, (b) *Npy* and (c) *Pomc* in control and AgRP-GR KO mice treated with vehicle or 75µg/ml corticosterone (Cort) in drinking water for 2 days. (d) Feed efficiency in control and AgRP-GR KO mice treated with vehicle or 75µg/ml corticosterone (Cort) in drinking water for 3 weeks. (a) – (c) n=8-10, Mann-Whitney non-parametric t-tests. (d) n=8-10, Two-way ANOVA with Tukey Multiple Comparison test. ***$P<0.001$ control vehicle vs. Cort, ^$P<0.05$ KO vehicle vs. Cort, $^{>}P<0.05$, $^{>>>}P>0.001$ control Cort vs. KO Cort, $^{<}P<0.05$ control vehicle vs. KO vehicle.

Harno *et al.* Supplemental Figure S3:



Supplemental Figure S3: Male AgRP-GR KO mice have similar increases in relative adiposity and BAT tissue weight, but not liver weight with corticosterone (Cort) treatment. (a) Epididymal, mesenteric and subcutaneous adipose tissue weights, (b) brown adipose tissue (BAT) weight and (c) liver weight in male control and AgRP-GR KO mice treated with Cort in drinking water for 3 weeks. (a) – (c) n=7-10, Two-ANOVA with Tukey Multiple Comparison test. *P<0.05, **P<0.01 control vehicle vs. Cort, ^^P<0.01, ^^^P<0.001 KO vehicle vs Cort, $^{>}P$<0.05 Control Cort vs. KO Cort. Data expressed as % body weight.

Harno *et al.* Supplemental Figure S4:



Supplemental Figure S4: Knockout of GR on AgRP neurons does not affect corticosterone (Cort)-induced changes in brown adipose tissue (BAT) of female mice. (a) brown adipose tissue wet weight (b) mRNA expression of genes associated with thermogenesis in BAT in female control and AgRP-GR KO mice treated with Cort in drinking water for 3 weeks. n=7-10, (a) Two-way ANOVA with Tukey Multiple Comparison test. (b) Mann-Whitney non-parametric t-tests. *P<0.05, **P<0.01, control vehicle vs. Cort, ^P<0.05, ^^P<0.01, KO vehicle vs. Cort.

Harno *et al.* Supplemental Table 1

Gene Name	Taqman Probe
Agrp	Mm00475829_g1
Cidea	Mm00432554_m1
Hprt	Mm03024075_m1

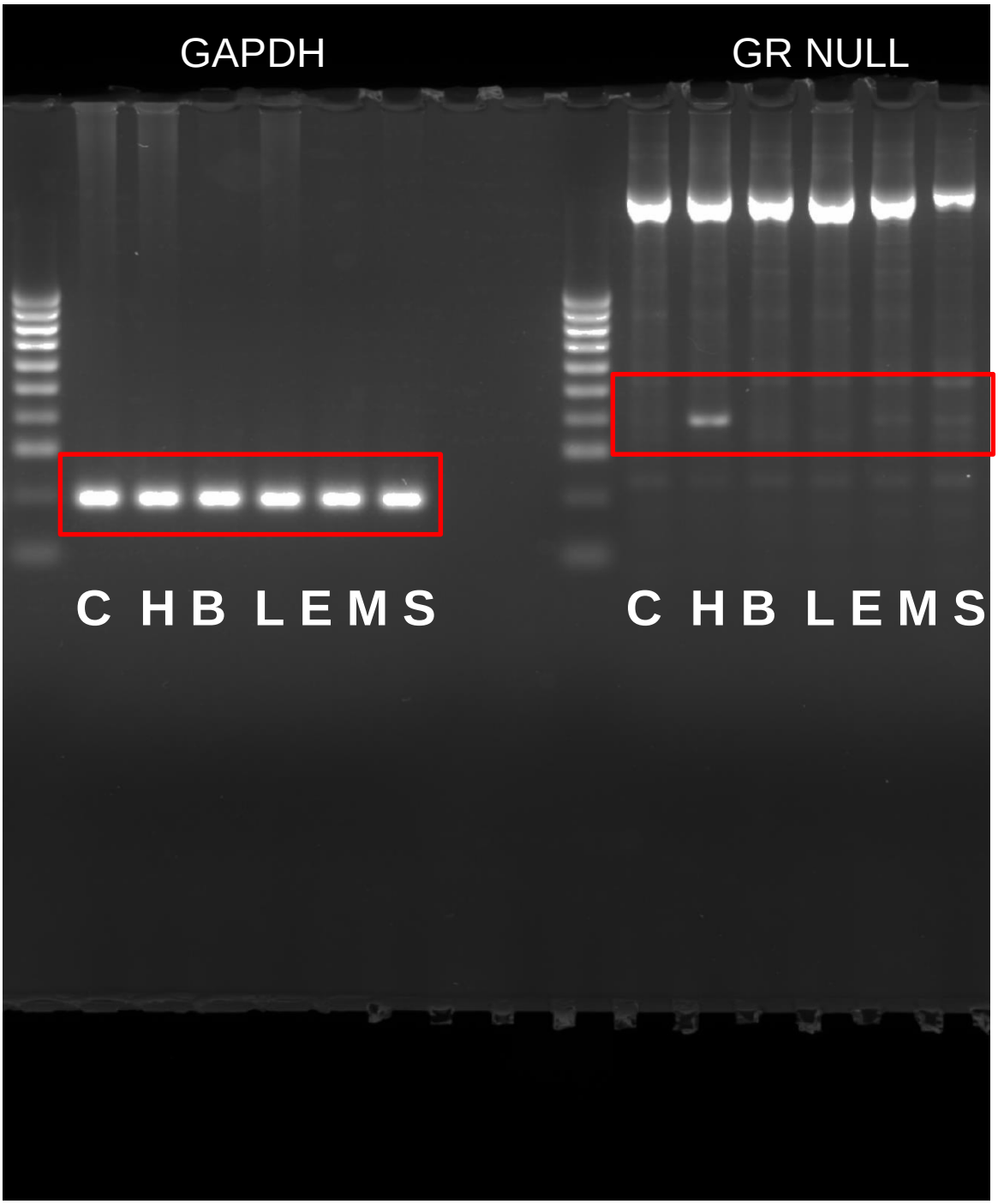
Supplemental Table 1: Taqman probe catalogue numbers used in the real-time quantitative PCR experiments.

Harno *et al.* Supplemental Table 2

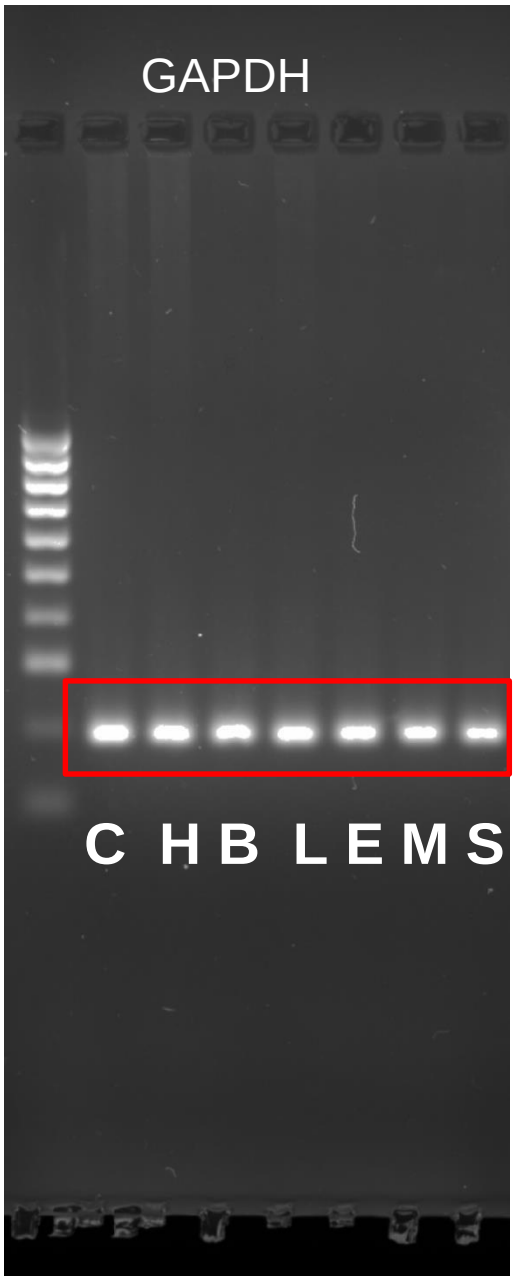
Gene Name	Forward 5' - 3'	Reverse 5' - 3'	Genbank Accession Number
Acc1	CCTCCGTCAGCTCAGATACA	TTTACTAGGTGCAAGCCAGACA	NM_133360.2
Atgl	AAAGACCCTGGCTGCTGATT	TGCAGACATTGGCCTGGATG	NM_01163689.1
Cd36	CAAAACGACTGCAGGTCAACA	CACCAATGGTCCCAGTCTCAT	XM_006535625.2
Fasn	GCTGCTGTTGGAAGTCAGC	AGTGTTCGTTCCTCGGAGTG	NM_007988.3
G6pc	GTGAGACCGGACCAGGAAGTC	ATCCCAACCACAAGATGACGTT	NM_008061.4
Gck	AAGCTGCACCCGAGCTTCAA	GCTGCCCTCCTCTGATTCAA	NM_010292.5
Hsl	ACTGTGACCTGCTTGGTTCAA	TGTCCCCTGCAAGGCATATC	NM_001039507.2
Irs1	CAAGACGCTCCAGTGAGGATT	TTTAGGTCTTCATTCTGCTGTGA	NM_010570.4
Lpl	GCTGGTGGGAAATGATGTG	TGGACGTTGTCTAGGGGGTA	NM_008509.2
Npy	ATGCTAGGTAACAAGCGAATGG	TGTCGCAGAGCGGAGTAGTAT	NM_023456.3
Nr3c1	AGCTCCCCCTGGTAGAGAC	GGTGAAGACGCAGAAACCTTG	NM_008173.4
Pik3cb	GCGCGGGGCAGTTCATCTTCTAA	GAGGCATGATAGGGCGGAAGCA	NM_029094.3
Pik3r1	GCCAAGGAAACTGTCGCACACA	GGGGCAGTGCTGGTGGATCCAT	NM_001024955.2
Pomc	ATGCCGAGATTCTGCTACAGT	TCCAGCGAGAGGTCGAGTTT	NM_001278581.1
Ppargc1a	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG	NM_008904.2
Prdm16	GACATTCCAATCCCACCAGA	CACCTCTGTATCCGTCAGCA	NM_001177995.1
Pygl	CCACTCGGACATCGTGAAGA	CCAATTTTCTCCGCTATCAAGTC	NM_133198.2
Scd1	CTGTACGGGATCATACTGGTTC	GCCGTCCCTTGTAAGTTCTG	NM_009127.4
Slc2a2	GATCGCTCCAACCACACTCA	CTGAGGCCAGCAATCTGACTA	NM_031197.2
Srebp1c	GGAGCCATGCATTGCACATT	GGCCCGGGAAGTCACTGTACATT	NM_001358314.1
Tbp	GGGAGAATCATGGACCAGAA	GATGGGAATTCCAGGAGTCA	NM_013684.3
Tsc22d3	GCAGGCCATGGACCTCGTGAAG	TCAGGAGGGTGTTCTCGCGCT	NM_001077364.1
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG	NM_009463.3

Supplemental Table 1: Primer sequences and GenBank accession numbers used in the real-time quantitative PCR experiments.

Unedited blot in Figure 1a: GR-AgRP KO



Unedited blot in Figure 1a: Control



The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Throughout the results section Animals are singly housed throughout (line 440)
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Line 449 and figure legends Lines 449-453
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	Lines 556-557 Lines 556-557 In figure legends and individual data points are represented in graphs
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Line 441-442 Lines 445-447
Blinding	5	'HVFULEHZKRZDVDZDUHRIWKHJURXSDOORFDWLRQDWWKHGLNjHUHQWVWDJHVRIWKH experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Lines 456-458
Outcome measures	6	a. &OHDUO\GHnjQHDOORXWFRPHPHDVXUHVDVVHVVHGHJFHOOGHDWKPROHFXODUPDUNHUV or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Throughout the results
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Lines 552-556

Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune VWDWXVXVHOHFWLRQ&LUFXPVWDQFHVXVHGWRDVVLJQDQLPDOVWRJURXSV\SHDQGDQ\SUHYLRXVSURFHGXUHV	Lines 406-412, 432-434 and 440
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	In the methods section and then repeated where appropriate in the results
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. IDSSOLEDSOUWKUINIUEWVL]H7UWKDERQ&IGHOHUOWHUYDO	Statistical tests used are listed in each figure legend. Variability can be seen through SEM error bars and the individual data points.