Exposure of CD-1 Mice to $CdCl_2$ during Gestation Induces Sex-Specific Hepatic Insulin Insensitivity, Obesity and Metabolic Syndrome in Adult Female Offspring

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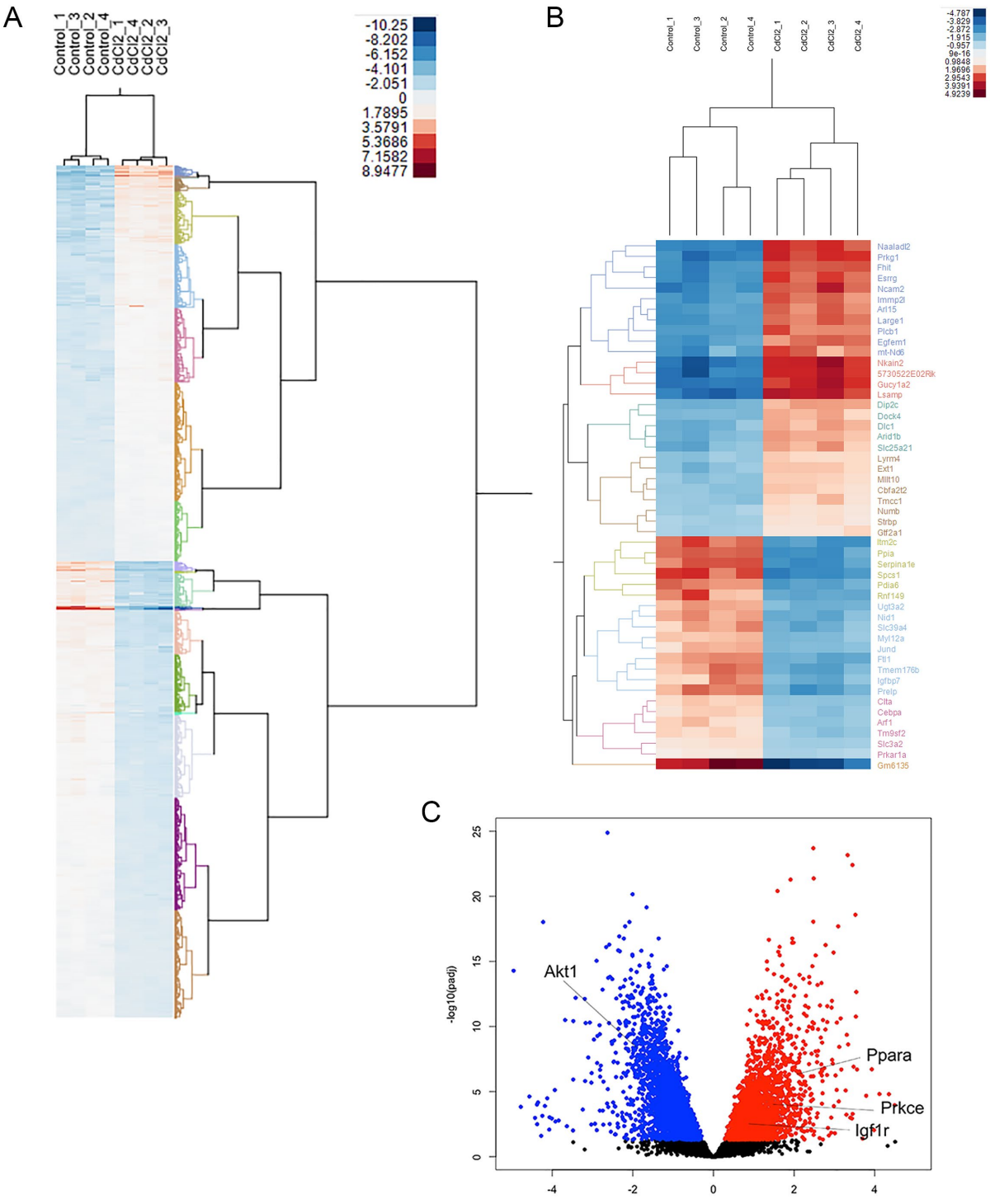
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Control_1
Control_3
Control_2
Control_4
CdCl2_1
CdCl2_4
CdCl2_2
CdCl2_3
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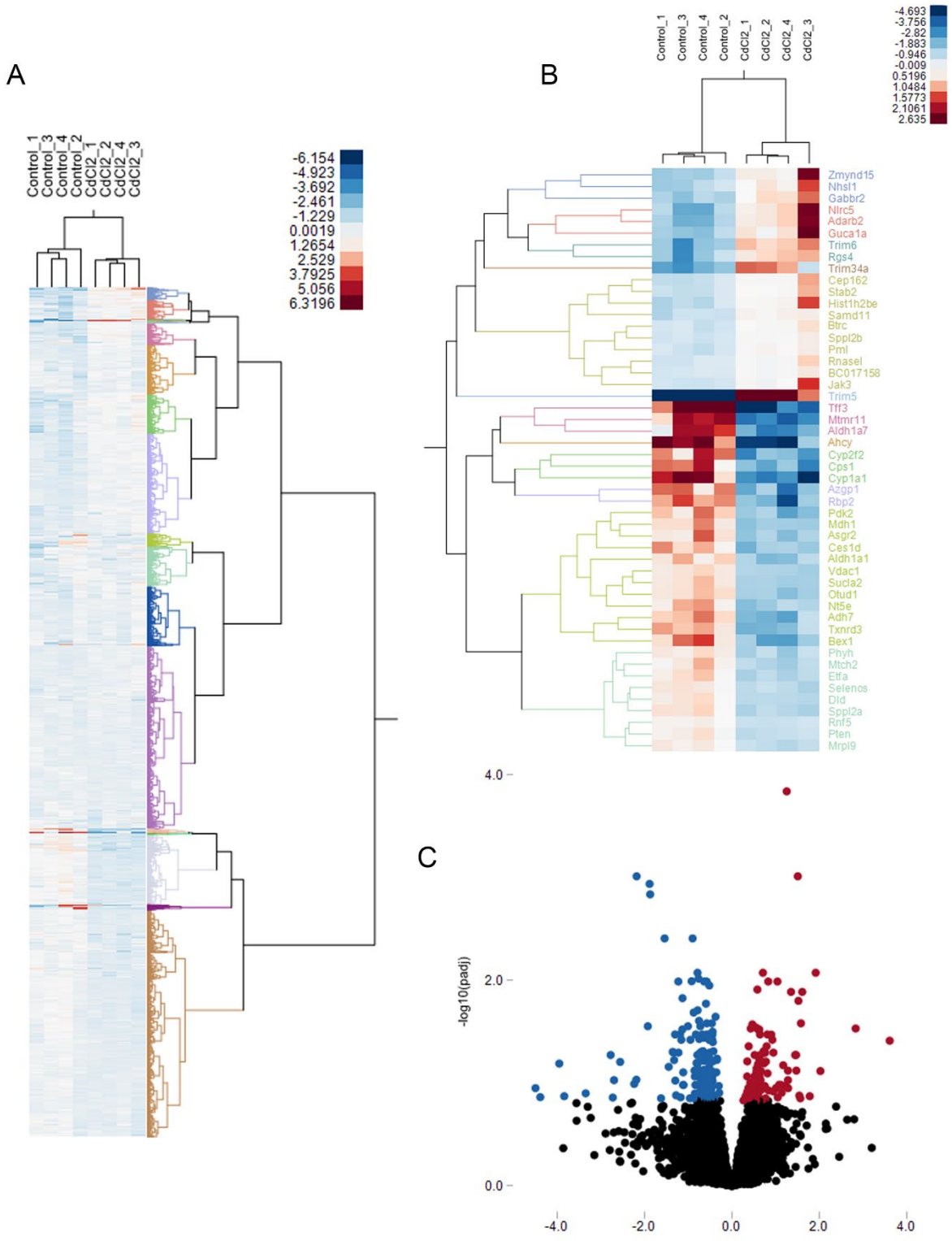
-8.202
-6.152
-4.101
-2.051
0
1.7895
3.5791
5.3686
7.1582
8.9477
B
Control_1
Control_3
Control_2
Control_4
CdCl2_1
CdCl2_2
CdCl2_3
CdCl2_4
-4.787
-3.829
-2.872
-1.915
-0.957
9e-16
0.9848
1.9696
2.9543
3.9391
4.9239
Naaladl2
Prkg1
Fhit
Esrrg
Ncam2
Immp2l
Arl15
Large1
Plcb1
Egfem1
mt-Nd6
Nkain2
5730522E02Rik
Gucy1a2
Lsamp
Dip2c
Dock4
Dlc1
Arid1b
Slc25a21
Lyrm4
Ext1
Mllt10
Cbfa2t2
Tmcc1
Numb
Strbp
Gtf2a1
Itm2c
Ppia
Serpina1e
Spcs1
Pdia6
Rnf149
Ugt3a2
Nid1
Slc39a4
Myl12a
Jund
Flt1
Tmem176b
Igfbp7
Prelp
Cita
Cebpa
Arf1
Tm9sf2
Slc3a2
Prkar1a
Gm6135
C
-log10(padj)
0
5
10
15
20
25
-4
-2
0
2
4
Akt1
Ppara
Prkce
Igf1r

Supplemental Figure S1. RNA-seq PND42 quality control plots. RNA-sequencing of the hepatic transcriptome of females gestationally exposed to Cd revealed 5789 genes were differentially exposed between Cd and control females at PND42. Hierarchical clustering of 13092 genes in females are shown (A). The top 50 differentially expressed genes are shown, with significantly upregulated genes shown in red and significantly downregulated genes shown in blue (B). A volcano plot of the RNA-seq results shows that 3103 genes are downregulated while 2686 genes are upregulated (C).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Control_3
Control_4
Control_2
CdCl2_1
CdCl2_2
CdCl2_4
CdCl2_3
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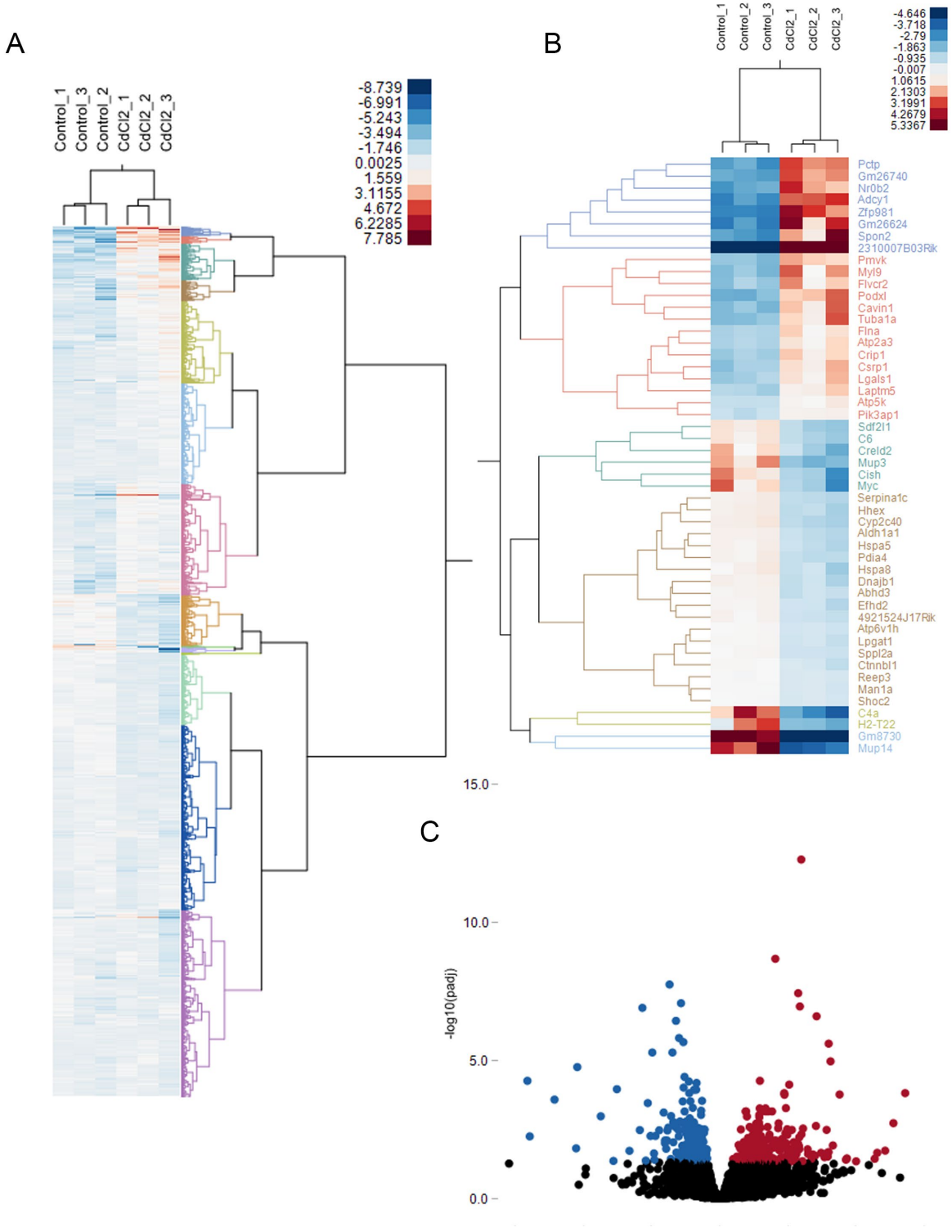
-4.923
-3.692
-2.461
-1.229
0.0019
1.2654
2.529
3.7925
5.056
6.3196
B
Control_1
Control_3
Control_4
Control_2
CdCl2_1
CdCl2_2
CdCl2_4
CdCl2_3
-4.693
-3.756
-2.82
-1.883
-0.946
-0.009
0.5196
1.0484
1.5773
2.1061
2.635
Zmynd15
Nhsl1
Gabbr2
Nlrc5
Adarb2
Guca1a
Trim6
Rgs4
Trim34a
Cep162
Stab2
Hist1h2be
Samd11
Btrc
Sppl2b
Pml
Rnasel
BC017158
Jak3
Trim5
Tff3
Mtmr11
Aldh1a7
Ahcy
Cyp2f2
Cps1
Cyp1a1
Azgp1
Rbp2
Pdk2
Mdh1
Asgr2
Ces1d
Aldh1a1
Vdac1
Sucla2
Otud1
Nt5e
Adh7
Txnrd3
Bex1
Phyh
Mtch2
Etfa
Selenos
Dld
Sppl2a
Rnf5
Pten
Mrpl9
C
4.0
2.0
0.0
-log10(padj)
-4.0
-2.0
0.0
2.0
4.0

Supplemental Figure S2. RNA-seq PND1 quality control plots. RNA-sequencing of the hepatic transcriptome of females gestationally exposed to Cd revealed 278 genes were differentially exposed between Cd and control females at PND1. Hierarchical clustering of 14259 genes in females are shown (A). The top 50 differentially expressed genes are shown, with significantly upregulated genes shown in red and significantly downregulated genes shown in blue (B). A volcano plot of the RNA-seq results shows that 174 genes are downregulated while 104 genes are upregulated (C).


A
Control_1
Control_3
Control_2
CdCl2_1
CdCl2_2
CdCl2_3
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-6.991
-5.243
-3.494
-1.746
0.0025
1.559
3.1155
4.672
6.2285
7.785
B
Control_1
Control_2
Control_3
CdCl2_1
CdCl2_2
CdCl2_3
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-3.718
-2.79
-1.863
-0.935
-0.007
1.0615
2.1303
3.1991
4.2679
5.3367
Pctp
Gm26740
Nr0b2
Adcy1
Zfp981
Gm26624
Spon2
2310007B03Rik
Pmvk
Myl9
Flvcr2
Podxl
Cavin1
Tuba1a
Flna
Atp2a3
Crip1
Csrp1
Lgals1
Laptm5
Atp5k
Pik3ap1
Sdf2l1
C6
Creld2
Mup3
Cish
Myc
Serpina1c
Hhex
Cyp2c40
Aldh1a1
Hspa5
Pdia4
Hspa8
Dnajb1
Abhd3
Efhd2
4921524J17Rik
Atp6v1h
Lpgat1
Sppl2a
Ctnnbl1
Reep3
Man1a
Shoc2
C4a
H2-T22
Gm8730
Mup14
C
15.0
10.0
5.0
0.0
-log10(padj)

Supplemental Figure S3. RNA-seq PND21 quality control plots. RNA-sequencing of the hepatic transcriptome of females gestationally exposed to Cd revealed 446 genes were differentially exposed between Cd and control females at PND21. Hierarchical clustering of 14259 genes in females are shown (A). The top 50 differentially expressed genes are shown, with significantly upregulated genes shown in red and significantly downregulated genes shown in blue (B). A volcano plot of the RNA-seq results shows that 336 genes are downregulated while 110 genes are upregulated (C).

The ARRIVE Guidelines Checklist

Animal Research: Reporting In Vivo Experiments

Carol Kilkenny[1], William J Browne[2], Innes C Cuthill[3], Michael Emerson[4] and Douglas G Altman[5]

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	ITEM	RECOMMENDATION	Section/ Paragraph
Title	1	Provide as accurate and concise a description of the content of the article as possible.	1-2
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	31-53
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	63-144
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	145-153
METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	156-166
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.	167-173; 331-336
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).	156-353
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	167-173

Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.	156-166
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.	167-173; 331-336
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.	167-173; 331-336
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).	175-210
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.	331-353
RESULTS			
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated).	356-372
Numbers analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%[2]). b. If any animals or data were not included in the analysis, explain why.	356-523
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).	326-523
Adverse events	17	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.	175-210
DISCUSSION			
Interpretation/ scientific implications	18	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results[2]. c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.	525-772
Generalisability/ translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.	758-772
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.	775-776

References:
1. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol* 8(6): e1000412. doi:10.1371/journal.pbio.1000412
2. Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340:c332.

Supplemental Table S1. Gene list for qRT-PCR and TaqMan Assays

Gene Name	Gene Symbol	ABI Assay #	Entrez Gene ID #	$\Delta\Delta C_T$ (Mean ± SEM)	p-value
beta actin	Actb	Mm01205647_g1	11461	-	-
actin, beta	Actb	Mm02619580_g1	11461	-	-
glyceraldehyde-3-phosphate dehydrogenase	Gapdh	Mm99999915_g1	14433	-	-
glucuronidase, beta	Gusb	Mm01197698_m1	110006	-	-
hypoxanthine guanine phosphoribosyl transferase	Hprt	Mm03024075_m1	15452	-	-
18S ribosomal RNA	Rn18s	Mm03928990_g1	19791	-	-
Eukaryotic 18S rRNA	18S	Hs99999901_s1	106632259	-	-
ribosomal protein S18	Rps18	Mm02601777_g1	20084	-	-
protein kinase b	Akt1	Mm01331626_m1	11651	0.52 ± .03	.008
CD36 antigen	Cd36	Mm01135198_m1	12491	0.12 ± .04	.048
diacylglycerol O-acyltransferase 2	Dgat2	Mm00499536_m1	67800	1.50 ± .21	.047
fatty acid binding protein 4, adipocyte	Fabp4	Mm00445878_m1	11770	0.37 ± .11	.004
forkhead box protein O1	Foxo1	Mm01331626_m1	56458	0.68 ± .08	.009
histone deacetylase 3	Hdac3	Mm00515916_m1	15183	1.62 ± 09	.02
insulin-like growth factor 1 receptor	Igf1r	Mm01318459_m1	16001	1.81 ± .27	.03
insulin-like growth factor binding protein 3	Igfbp3	Mm01187817_m1	16009	0.24 ± .05	.02
insulin-like growth factor binding protein 7	Igfbp7	Mm03807886_m1	29817	0.50 ± .13	.04
insulin receptor	Insr	Mm01211875_m1	16337	1.63 ± .53	.04
perilipin 2	Plin2	Mm00475794_m1	11520	0.59 ± .11	.01
peroxisome proliferator-activated receptor alpha	PPARa	Mm00440939_m1	19013	1.46 ± .13	.04
protein kinase c epsilon type	Prkce	Mm00440894_m1	18754	1.41 ± .16	.03
solute carrier family 2, member 4	Slc2a4	Mm00436615_m1	20528	1.73 ± .79	.21
solute carrier family 39, member 8	Slc39a8	Mm00470855_m1	67547	1.9 ± 0.05	.04
solute carrier family 39, member 14	Slc39a14	Mm01317439_m1	213053	0.81 ± 0.01	.001

Supplemental Table S2: Study Animal Assignmment and Usage

ID	Wave	Litter	Sex	Exposure	Sac age	Fetal extraction	Litter size	Hemoglobin	Body weight	Adipose	Triglycerides	Thyroxine	GTT/ITT	RNAseq	Pathology
11B1F	1	11	F	Yes	42	No	12	No	Yes	No	No	Yes	No	Yes	No
11B2F	1	11	F	Yes	42	No	12	No	Yes	No	Yes	No	No	No	No
11B3F	1	11	F	Yes	21	No	12	Yes	Yes	No	No	No	No	Yes	No
11B4F	1	11	F	Yes	42	No	12	No	Yes	No	No	No	No	No	No
11B5F	1	11	F	Yes	1	No	12	Yes	Yes	No	No	No	No	No	No
11B6F	1	11	F	Yes	42	No	12	No	Yes	No	No	No	No	No	No
11B7M	1	11	M	Yes	42	No	12	No	Yes	No	No	No	No	No	No
11B8M	1	11	M	Yes	42	No	12	No	Yes	No	Yes	No	No	No	No
11B9M	1	11	M	Yes	21	No	12	Yes	Yes	No	No	No	No	No	No
11B10M	1	11	M	Yes	1	No	12	Yes	Yes	No	No	No	No	No	No
11B11M	1	11	M	Yes	42	No	12	No	Yes	No	No	No	No	No	No
11B12M	1	11	M	Yes	42	No	12	No	Yes	No	No	No	No	No	No
12B1F	1	12	F	Yes	42	No	11	No	Yes	No	No	Yes	No	No	No
12B2F	1	12	F	Yes	42	No	11	No	Yes	No	No	No	No	No	No
12B3F	1	12	F	Yes	42	No	11	No	Yes	No	Yes	No	No	Yes	No
12B4F	1	12	F	Yes	42	No	11	No	Yes	No	No	No	No	No	No
12B5F	1	12	F	Yes	42	No	11	No	Yes	No	No	No	No	No	No
12B6M	1	12	M	Yes	42	No	11	No	Yes	No	No	No	No	No	No
12B7M	1	12	M	Yes	42	No	11	No	Yes	No	No	No	No	Yes	No
12B8M	1	12	M	Yes	1	No	11	No	Yes	No	No	No	No	No	No
12B9M	1	12	M	Yes	42	No	11	No	Yes	No	No	No	No	No	No
12B10M	1	12	M	Yes	42	No	11	No	Yes	No	Yes	Yes	No	No	No
12B11M	1	12	M	Yes	42	No	11	No	Yes	No	No	No	No	No	No
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13B2F	1	13	F	Yes	21	No	15	Yes	Yes	No	No	No	No	No	No
13B3F	1	13	F	Yes	1	No	15	No	Yes	No	No	No	No	Yes	No
13B4F	1	13	F	Yes	1	No	15	Yes	Yes	No	No	No	No	No	No
13B5F	1	13	F	Yes	42	No	15	No	Yes	No	No	No	No	No	No
13B6F	1	13	F	Yes	42	No	15	No	Yes	No	No	Yes	No	No	No
13B7F	1	13	F	Yes	21	No	15	Yes	Yes	No	Yes	No	No	Yes	No
13B8M	1	13	M	Yes	42	No	15	No	Yes	No	No	No	No	No	No
13B9M	1	13	M	Yes	1	No	15	Yes	Yes	No	No	No	No	No	No
13B10F	1	13	F	Yes	1	No	15	No	Yes	No	No	No	No	No	No
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13B12M	1	13	M	Yes	42	No	15	No	Yes	No	No	Yes	No	No	No
13B13M	1	13	M	Yes	42	No	15	No	Yes	No	No	No	No	Yes	No
13B14M	1	13	M	Yes	42	No	15	No	Yes	No	No	No	No	No	No
13B15M	1	13	M	Yes	21	No	15	Yes	Yes	No	No	No	No	No	No
14B1F	1	14	F	Yes	21	No	13	No	Yes	No	No	No	No	No	No
14B2F	1	14	F	Yes	1	No	13	Yes	Yes	No	No	No	No	Yes	No
14B3F	1	14	F	Yes	42	No	13	No	Yes	No	Yes	No	No	No	No
14B4F	1	14	F	Yes	21	No	13	No	Yes	No	No	No	No	No	No
14B5F	1	14	F	Yes	42	No	13	No	Yes	No	No	Yes	No	No	No
14B6F	1	14	F	Yes	1	No	13	Yes	Yes	No	No	No	No	No	No
14B7F	1	14	F	Yes	42	No	13	No	Yes	No	No	No	No	No	No
14B8M	1	14	M	Yes	42	No	13	No	Yes	No	No	No	No	No	No
14B9M	1	14	M	Yes	42	No	13	No	Yes	No	No	Yes	No	No	No
14B10M	1	14	M	Yes	21	No	13	No	Yes	No	No	No	No	No	No

14B11M	1	14	M	Yes	1	No	13	Yes	Yes	No	No	No	No	No	No
14B12M	1	14	M	Yes	21	No	13	No	Yes	No	No	No	No	No	No
14B13M	1	14	M	Yes	42	No	13	No	Yes	No	No	No	No	No	No
15B1F	1	15	F	Yes	42	No	7	No	Yes	No	Yes	Yes	No	No	No
15B2F	1	15	F	Yes	42	No	7	No	Yes	No	No	No	No	No	No
15B3F	1	15	F	Yes	42	No	7	No	Yes	No	No	No	No	No	No
15B4M	1	15	M	Yes	42	No	7	No	Yes	No	No	No	No	No	No
15B5M	1	15	M	Yes	42	No	7	No	Yes	No	No	No	No	No	No
15B6M	1	15	M	Yes	42	No	7	No	Yes	No	No	No	No	Yes	No
15B7M	1	15	M	Yes	42	No	7	No	Yes	No	Yes	No	No	No	No
16B1F	1	16	F	Yes	42	No	14	Yes	Yes	No	No	No	No	Yes	No
16B2F	1	16	F	Yes	42	No	14	No	Yes	No	Yes	No	No	No	No
16B3F	1	16	F	Yes	42	No	14	No	Yes	No	No	Yes	No	No	No
16B4F	1	16	F	Yes	42	No	14	No	Yes	No	No	No	No	No	No
16B5F	1	16	F	Yes	1	No	14	Yes	Yes	No	No	No	No	Yes	No
16B6F	1	16	F	Yes	42	No	14	No	Yes	No	No	No	No	No	No
16B7F	1	16	F	Yes	42	No	14	No	Yes	No	No	No	No	No	No
16B8M	1	16	M	Yes	1	No	14	Yes	Yes	No	No	No	No	No	No
16B9M	1	16	M	Yes	42	No	14	Yes	Yes	No	No	No	No	No	No
16B10M	1	16	M	Yes	42	No	14	No	Yes	No	Yes	No	No	No	No
16B11M	1	16	M	Yes	42	No	14	No	Yes	No	Yes	No	No	No	No
16B12M	1	16	M	Yes	42	No	14	No	Yes	No	No	No	No	No	No
16B13M	1	16	M	Yes	42	No	14	No	Yes	No	No	No	No	No	No
16B14M	1	16	M	Yes	42	No	14	No	Yes	No	No	No	No	No	No
17B1F	1	17	F	Yes	42	No	8	No	Yes	No	No	No	No	No	No
17B2F	1	17	F	Yes	42	No	8	No	Yes	No	Yes	Yes	No	No	No
17B3F	1	17	F	Yes	42	No	8	No	Yes	No	No	No	No	No	No
17B4F	1	17	F	Yes	42	No	8	No	Yes	No	No	No	No	No	No
17B5M	1	17	M	Yes	42	No	8	No	Yes	No	No	No	No	No	No
17B6M	1	17	M	Yes	42	No	8	No	Yes	No	No	No	No	Yes	No
17B7M	1	17	M	Yes	42	No	8	No	Yes	No	Yes	No	No	No	No
17B8M	1	17	M	Yes	42	No	8	No	Yes	No	No	No	No	No	No
18B1F	1	19	F	Yes	42	No	12	Yes	Yes	No	No	No	No	No	No
18B2F	1	19	F	Yes	42	No	12	No	Yes	No	No	Yes	No	No	No
18B3F	1	19	F	Yes	42	No	12	No	Yes	No	No	No	No	No	No
18B4F	1	19	F	Yes	42	No	12	No	Yes	No	No	No	No	No	No
18B5F	1	19	F	Yes	1	No	12	Yes	Yes	No	No	No	No	No	No
18B6M	1	19	M	Yes	42	No	12	Yes	Yes	No	No	No	No	No	No
18B7M	1	19	M	Yes	42	No	12	No	Yes	No	No	Yes	No	No	No
18B8M	1	19	M	Yes	42	No	12	No	Yes	No	Yes	Yes	No	No	No
18B9M	1	19	M	Yes	1	No	12	Yes	Yes	No	No	No	No	No	No
18B10M	1	19	M	Yes	42	No	12	No	Yes	No	No	No	No	No	No
18B11M	1	19	M	Yes	42	No	12	No	Yes	No	No	No	No	No	No
18B12M	1	19	M	Yes	42	No	12	No	Yes	No	No	Yes	No	No	No
19B1F	1	19	F	Yes	42	No	12	Yes	Yes	No	No	No	No	No	No
19B2F	1	19	F	Yes	42	No	12	No	Yes	No	Yes	Yes	No	No	No
19B3F	1	19	F	Yes	42	No	12	No	Yes	No	No	No	No	No	No
19B4F	1	19	F	Yes	1	No	12	Yes	Yes	No	No	No	No	Yes	No
19B5F	1	19	F	Yes	42	No	12	No	Yes	No	No	Yes	No	Yes	No
19B6F	1	19	F	Yes	42	No	12	No	Yes	No	No	No	No	No	No
19B7M	1	19	M	Yes	42	No	12	Yes	Yes	No	No	No	No	No	No

19B8M	1	19	M	Yes	42	No	12	No	Yes	No	No	Yes	No	No	No
19B9M	1	19	M	Yes	1	No	12	Yes	Yes	No	No	No	No	No	No
19B10M	1	19	M	Yes	42	No	12	No	Yes	No	No	No	No	No	No
19B11M	1	19	M	Yes	42	No	12	No	Yes	No	No	No	No	No	No
19B12M	1	19	M	Yes	42	No	12	No	Yes	No	No	No	No	No	No
20B1F	1	20	F	Yes	42	No	12	No	Yes	No	No	Yes	No	No	No
20B2F	1	20	F	Yes	42	No	12	No	Yes	No	Yes	No	No	Yes	No
20B3F	1	20	F	Yes	42	No	12	No	Yes	No	No	No	No	No	No
20B4F	1	20	F	Yes	42	No	12	No	Yes	No	No	No	No	No	No
20B5M	1	20	M	Yes	42	No	12	No	Yes	No	No	No	No	No	No
20B6M	1	20	M	Yes	42	No	12	No	Yes	No	No	No	No	No	No
20B7M	1	20	M	Yes	1	No	12	Yes	Yes	No	No	No	No	No	No
20B8M	1	20	M	Yes	42	No	12	No	Yes	No	No	Yes	No	No	No
20B9M	1	20	M	Yes	1	No	12	Yes	Yes	No	No	No	No	No	No
20B10M	1	20	M	Yes	42	No	12	No	Yes	No	Yes	No	No	No	No
20B11M	1	20	M	Yes	42	No	12	No	Yes	No	No	No	No	No	No
20B12M	1	20	M	Yes	42	No	12	No	Yes	No	No	No	No	No	No
31D1F	1	31	F	No	42	No	13	No	Yes	No	Yes	Yes	No	No	No
31D2F	1	31	F	No	21	No	13	No	Yes	No	No	No	No	No	No
31D3F	1	31	F	No	1	No	13	Yes	Yes	No	No	No	No	Yes	No
31D4F	1	31	F	No	42	No	13	No	Yes	No	No	No	No	No	No
31D5F	1	31	F	No	42	No	13	No	Yes	No	No	No	No	No	No
31D6M	1	31	M	No	21	No	13	No	Yes	No	No	No	No	No	No
31D7M	1	31	M	No	1	No	13	Yes	Yes	No	No	No	No	No	No
31D8M	1	31	M	No	42	No	13	No	Yes	No	Yes	Yes	No	Yes	No
31D9M	1	31	M	No	42	No	13	No	Yes	No	Yes	Yes	No	No	No
31D10M	1	31	M	No	1	No	13	No	Yes	No	No	No	No	No	No
31D11M	1	31	M	No	1	No	13	Yes	Yes	No	No	No	No	No	No
31D12M	1	31	M	No	42	No	13	No	Yes	No	No	No	No	No	No
31D13M	1	31	M	No	42	No	13	No	Yes	No	No	No	No	No	No
32D1F	1	32	F	No	21	No	10	Yes	Yes	No	No	No	No	Yes	No
32D2F	1	32	F	No	42	No	10	No	Yes	No	Yes	Yes	No	No	No
32D3F	1	32	F	No	1	No	10	Yes	Yes	No	No	No	No	Yes	No
32D4F	1	32	F	No	42	No	10	No	Yes	No	Yes	Yes	No	Yes	No
32D5F	1	32	F	No	42	No	10	No	Yes	No	No	No	No	No	No
32D6M	1	32	M	No	21	No	10	Yes	Yes	No	No	No	No	No	No
32D7M	1	32	M	No	1	No	10	Yes	Yes	No	No	No	No	No	No
32D8M	1	32	M	No	42	No	10	No	Yes	No	Yes	No	No	No	No
32D9M	1	32	M	No	42	No	10	No	Yes	No	Yes	Yes	No	No	No
32D10M	1	32	M	No	42	No	10	No	Yes	No	No	No	No	Yes	No
33D1F	1	33	F	No	21	No	11	No	Yes	No	No	No	No	Yes	No
33D2F	1	33	F	No	42	No	11	No	Yes	No	Yes	Yes	No	No	No
33D3F	1	33	F	No	42	No	11	No	Yes	No	Yes	No	No	No	No
33D4F	1	33	F	No	42	No	11	No	Yes	No	No	No	No	No	No
33D5F	1	33	F	No	42	No	11	No	Yes	No	No	No	No	No	No
33D6M	1	33	M	No	1	No	11	Yes	Yes	No	No	No	No	No	No
33D7M	1	33	M	No	42	No	11	No	Yes	No	Yes	No	No	No	No
33D8M	1	33	M	No	21	No	11	No	Yes	No	No	No	No	No	No
33D9M	1	33	M	No	42	No	11	No	Yes	No	No	Yes	No	No	No
33D10M	1	33	M	No	42	No	11	No	Yes	No	No	No	No	No	No
33D11M	1	33	M	No	42	No	11	No	Yes	No	No	No	No	No	No

34D1F	1	34	F	No	21	No	11	Yes	Yes	No	No	No	No	No	No
34D2F	1	34	F	No	42	No	11	No	Yes	No	Yes	No	No	No	No
34D3F	1	34	F	No	42	No	11	No	Yes	No	No	No	No	No	No
34D4F	1	34	F	No	42	No	11	No	Yes	No	No	No	No	No	No
34D5F	1	34	F	No	1	No	11	Yes	Yes	No	No	No	No	Yes	No
34D6F	1	34	F	No	42	No	11	No	Yes	No	No	No	No	No	No
34D7F	1	34	F	No	42	No	11	No	Yes	No	No	No	No	No	No
34D8F	1	34	F	No	42	No	11	No	Yes	No	No	No	No	No	No
34D9M	1	34	M	No	1	No	11	Yes	Yes	No	No	No	No	No	No
34D10M	1	34	M	No	21	No	11	Yes	Yes	No	No	No	No	No	No
34D11M	1	34	M	No	42	No	11	No	Yes	No	No	Yes	No	No	No
35D1F	1	35	F	No	21	No	10	Yes	Yes	No	No	No	No	Yes	No
35D2F	1	35	F	No	21	No	10	Yes	Yes	No	No	No	No	Yes	No
35D3F	1	35	F	No	42	No	10	No	Yes	No	No	Yes	No	No	No
35D4F	1	35	F	No	42	No	10	No	Yes	No	Yes	Yes	No	No	No
35D5F	1	35	F	No	42	No	10	No	Yes	No	No	No	No	No	No
35D6F	1	35	F	No	42	No	10	No	Yes	No	No	No	No	No	No
35D7F	1	35	F	No	42	No	10	Yes	Yes	No	No	No	No	No	No
35D8F	1	35	F	No	1	No	10	No	Yes	No	No	No	No	No	No
35D9F	1	35	F	No	42	No	10	No	Yes	No	No	No	No	No	No
35D10M	1	35	M	No	1	No	10	Yes	Yes	No	No	No	No	No	No
36D1F	1	36	F	No	1	No	9	Yes	Yes	No	No	No	No	No	No
36D2F	1	36	F	No	21	No	9	Yes	Yes	No	No	No	No	Yes	No
36D3F	1	36	F	No	42	No	9	No	Yes	No	Yes	Yes	No	No	No
36D4F	1	36	F	No	42	No	9	No	Yes	No	No	Yes	No	No	No
36D5F	1	36	F	No	42	No	9	No	Yes	No	No	No	No	No	No
36D6F	1	36	F	No	42	No	9	No	Yes	No	No	No	No	No	No
36D7F	1	36	F	No	42	No	9	No	Yes	No	No	No	No	No	No
36D8M	1	36	M	No	21	No	9	Yes	Yes	No	No	No	No	No	No
36D9M	1	36	M	No	1	No	9	Yes	Yes	No	No	No	No	No	No
37D1F	1	37	F	No	42	No	8	No	Yes	No	No	No	No	No	No
37D2F	1	37	F	No	42	No	8	No	Yes	No	Yes	Yes	No	No	No
37D3F	1	37	F	No	42	No	8	No	Yes	No	No	No	No	No	No
37D4M	1	37	M	No	21	No	8	No	Yes	No	No	No	No	No	No
37D5M	1	37	M	No	21	No	8	No	Yes	No	No	No	No	No	No
37D6M	1	37	M	No	42	No	8	No	Yes	No	Yes	No	No	No	No
37D7M	1	37	M	No	42	No	8	No	Yes	No	No	No	No	No	No
37D8M	1	37	M	No	42	No	8	No	Yes	No	No	No	No	No	No
38D1F	1	38	F	No	42	No	12	No	Yes	No	No	Yes	No	Yes	No
38D2F	1	38	F	No	42	No	12	No	Yes	No	Yes	No	No	No	No
38D3F	1	38	F	No	21	No	12	No	Yes	No	No	No	No	No	No
38D4F	1	38	F	No	42	No	12	No	Yes	No	No	No	No	No	No
38D5F	1	38	F	No	21	No	12	No	Yes	No	No	No	No	No	No
38D6M	1	38	M	No	42	No	12	No	Yes	No	No	Yes	No	Yes	No
38D7M	1	38	M	No	42	No	12	No	Yes	No	Yes	No	No	No	No
38D8M	1	38	M	No	21	No	12	No	Yes	No	No	No	No	No	No
38D9M	1	38	M	No	21	No	12	No	Yes	No	No	No	No	No	No
38D10M	1	38	M	No	21	No	12	No	Yes	No	No	No	No	No	No
38D11M	1	38	M	No	42	No	12	No	Yes	No	No	No	No	No	No
38D12M	1	38	M	No	21	No	12	No	Yes	No	No	No	No	No	No
39D1F	1	39	F	No	1	No	14	Yes	Yes	No	No	No	No	Yes	No

39D2F	1	39	F	No	21	No	14	Yes	Yes	No	No	No	No	No	No
39D3F	1	39	F	No	42	No	14	No	Yes	No	No	No	No	No	No
39D4F	1	39	F	No	42	No	14	No	Yes	No	No	No	No	No	No
39D5F	1	39	F	No	42	No	14	No	Yes	No	No	No	No	No	No
39D6F	1	39	F	No	42	No	14	No	Yes	No	No	No	No	No	No
39D7M	1	39	M	No	21	No	14	Yes	Yes	No	No	No	No	No	No
39D8M	1	39	M	No	42	No	14	No	Yes	No	No	No	No	No	No
39D9M	1	39	M	No	42	No	14	No	Yes	No	No	No	No	No	No
39D10M	1	39	M	No	42	No	14	No	Yes	No	No	No	No	No	No
39D11M	1	39	M	No	42	No	14	No	Yes	No	No	No	No	No	No
39D12M	1	39	M	No	42	No	14	No	Yes	No	No	No	No	No	No
39D13M	1	39	M	No	1	No	14	Yes	Yes	No	No	No	No	No	No
39D14M	1	39	M	No	42	No	14	No	Yes	No	No	No	No	No	No
40D1F	1	40	F	No	42	No	9	No	Yes	No	Yes	Yes	No	No	No
40D2F	1	40	F	No	42	No	9	No	Yes	No	No	No	No	Yes	No
40D3F	1	40	F	No	1	No	9	No	Yes	No	No	No	No	No	No
40D4F	1	40	F	No	42	No	9	No	Yes	No	No	No	No	No	No
40D5F	1	40	F	No	21	No	9	No	Yes	No	No	No	No	No	No
40D6M	1	40	M	No	21	No	9	No	Yes	No	No	No	No	No	No
40D7M	1	40	M	No	42	No	9	No	Yes	No	Yes	No	No	No	No
17	2	Dam	F	No	N/A	No	15	No	N/A	N/A	N/A	N/A	N/A	N/A	N/A
18	2	Dam	F	No	N/A	No	N/A	No	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Supplemental Table S3. Blood Hemoglobin Levels (g/dL)

	Control (0 Cd)	Gestational $CdCl_2$
Dams at study onset	15.45 ± 0.71 (10)	15.36 ± 0.70 (10)
Dams at breeding (GD0)	15.94 ± 0.82 (10)	16.25 ± 0.89 (10)
PND1 Female	13.17 ± 0.86 (6)	**11.49 ± 1.15 (7)***
PND1 Male	13.31 ± 1.00 (7)	**11.59 ± 0.49 (5)***
PND21 Female	16.36 ± 1.11 (5)	16.00 ± 0.49 (5)
PND21 Male	16.20 ± 1.30 (4)	15.38 ± 0.89 (5)

Values represent group mean ± SD; the number of samples analyzed is shown in parenthesis. * $p < .05$

Supplemental Table S5. Shared DEGs: PND1, PND21, PND43

Symbol	Ensembl ID	MGI Gene/Marker ID	Feature Type	Chr	Strand	Start	End	log2FC_1	padj_1	log2FC_21	padj_21	log2FC_42	padj_42
Aldh1a1	ENSMUSG00000053279	MGI:1353450	protein coding gene	19	+	20492715	20643465	-0.75497	8.19E-06	-1.05358	2.23E-06	-1.31199	0.006123
Ccdc117	ENSMUSG00000020482	MGI:2144383	protein coding gene	11	-	5528887	5542187	-0.65193	0.000242	-0.85778	0.003579	-0.92357	0.001628
Cebpa	ENSMUSG00000034957	MGI:99480	protein coding gene	7	+	35119293	35121928	-0.62226	0.002649	-0.91721	0.010818	-1.60089	2.57E-15
Cyp4a31	ENSMUSG00000028712	MGI:3028580	protein coding gene	4	+	115563649	115579015	-0.83974	0.000822	-0.95869	0.038189	-2.07547	0.000732
Dnttip2	ENSMUSG00000039756	MGI:1923173	protein coding gene	3	+	122274414	122285271	-0.41532	0.002539	-0.60152	0.014212	-0.98805	1.89E-05
Gm16332	ENSMUSG00000089991	MGI:3840155	pseudogene	1	-	139878094	139936477	-0.84712	0.000604	-1.05943	0.012303	-0.95554	0.001148
Gm26740	ENSMUSG00000097705	MGI:5477234	lncRNA gene	10	-	22187013	22302835	0.934274	0.000164	2.322579	3.79E-08	2.247914	0.011753
Phyh	ENSMUSG00000026664	MGI:891978	protein coding gene	2	+	4919019	4938730	-0.57014	1.25E-05	-0.64074	0.003236	-0.8117	0.000796
Rnf5	ENSMUSG00000015478	MGI:1860076	protein coding gene	17	-	34601099	34603561	-0.36618	4.48E-05	-0.37508	0.016767	-0.73563	0.001183
Slc25a16	ENSMUSG00000071253	MGI:1920382	protein coding gene	10	+	62920632	62946494	-0.51992	0.001486	-0.82351	0.001654	-0.44834	0.018766
Slc39a4	ENSMUSG00000063354	MGI:1919277	protein coding gene	15	-	76612383	76617384	-0.55081	0.002165	-0.64939	0.049778	-2.01368	3.03E-16
Sppl2a	ENSMUSG00000027366	MGI:1913802	protein coding gene	2	-	126890391	126933235	-0.58441	2.99E-05	-0.77593	0.000534	-0.61585	0.00198
St8sia3os	ENSMUSG00000086128	MGI:1920838	antisense lncRNA gene	18	-	64157724	64277180	1.09081	0.001181	0.922975	0.049263	1.244141	0.039759

Supplemental Table S6. Shared DEGs: PND1, PND21

Symbol	Ensembl ID	MGI Gene/Marker ID	Feature Type	Chr	Strand	Start	End	log2FC_1	padj_1	log2FC_21	padj_21
Aldh1a1	ENSMUSG00000053279	MGI:1353450	protein coding gene	19	+	20492715	20643465	-1.05358	2.23E-06	-1.31199	0.006123
Ccdc117	ENSMUSG00000020482	MGI:2144383	protein coding gene	11	-	5528887	5542187	-0.85778	0.003579	-0.92357	0.001628
Cebpa	ENSMUSG00000034957	MGI:99480	protein coding gene	7	+	35119293	35121928	-0.91721	0.010818	-1.60089	2.57E-15
Cyp2c68	ENSMUSG00000074882	MGI:3612287	protein coding gene	19	-	39688834	39741054	-0.66988	0.053779	-0.9681	0.001654
Cyp4a31	ENSMUSG00000028712	MGI:3028580	protein coding gene	4	+	115563649	115579015	-0.95869	0.038189	-2.07547	0.000732
Dnttip2	ENSMUSG00000039756	MGI:1923173	protein coding gene	3	+	122274414	122285271	-0.60152	0.014212	-0.98805	1.89E-05
Fxr1	ENSMUSG00000027680	MGI:104860	protein coding gene	3	+	34020058	34069980	-0.28147	0.001865	-0.3547	0.035803
G530011O06Rik	ENSMUSG00000072844	MGI:3603513	lncRNA gene	X	-	169975043	169978917	1.78973	0.002256	2.610377	0.024758
Gm16332	ENSMUSG00000089991	MGI:3840155	pseudogene	1	-	139878094	139936477	-1.05943	0.012303	-0.95554	0.001148
Gm26740	ENSMUSG00000097705	MGI:5477234	lncRNA gene	10	-	22187013	22302835	2.322579	3.79E-08	2.247914	0.011753
Hhex	ENSMUSG00000024986	MGI:96086	protein coding gene	19	+	37434841	37440731	-0.63913	0.036475	-1.17918	1.59E-06
Ndufa10	ENSMUSG00000026260	MGI:1914523	protein coding gene	1	-	92439486	92473825	-0.42935	0.000181	-0.56529	0.003017
Pdk2	ENSMUSG00000038967	MGI:1343087	protein coding gene	11	-	95026258	95041354	-0.89152	0.003952	-0.7312	0.028479
Phyh	ENSMUSG00000026664	MGI:891978	protein coding gene	2	+	4919019	4938730	-0.64074	0.003236	-0.8117	0.000796
Rnf5	ENSMUSG00000015478	MGI:1860076	protein coding gene	17	-	34601099	34603561	-0.37508	0.016767	-0.73563	0.001183
Sdhb	ENSMUSG00000009863	MGI:1914930	protein coding gene	4	+	140961271	140979198	-0.47495	0.001406	-0.5576	0.041193
Slc25a16	ENSMUSG00000071253	MGI:1920382	protein coding gene	10	+	62920632	62946494	-0.82351	0.001654	-0.44834	0.018766
Slc39a4	ENSMUSG00000063354	MGI:1919277	protein coding gene	15	-	76612383	76617384	-0.64939	0.049778	-2.01368	3.03E-16
Sppl2a	ENSMUSG00000027366	MGI:1913802	protein coding gene	2	-	126890391	126933235	-0.77593	0.000534	-0.61585	0.00198
St8sia3os	ENSMUSG00000086128	MGI:1920838	antisense lncRNA gene	18	-	64157724	64277180	0.922975	0.049263	1.244141	0.039759