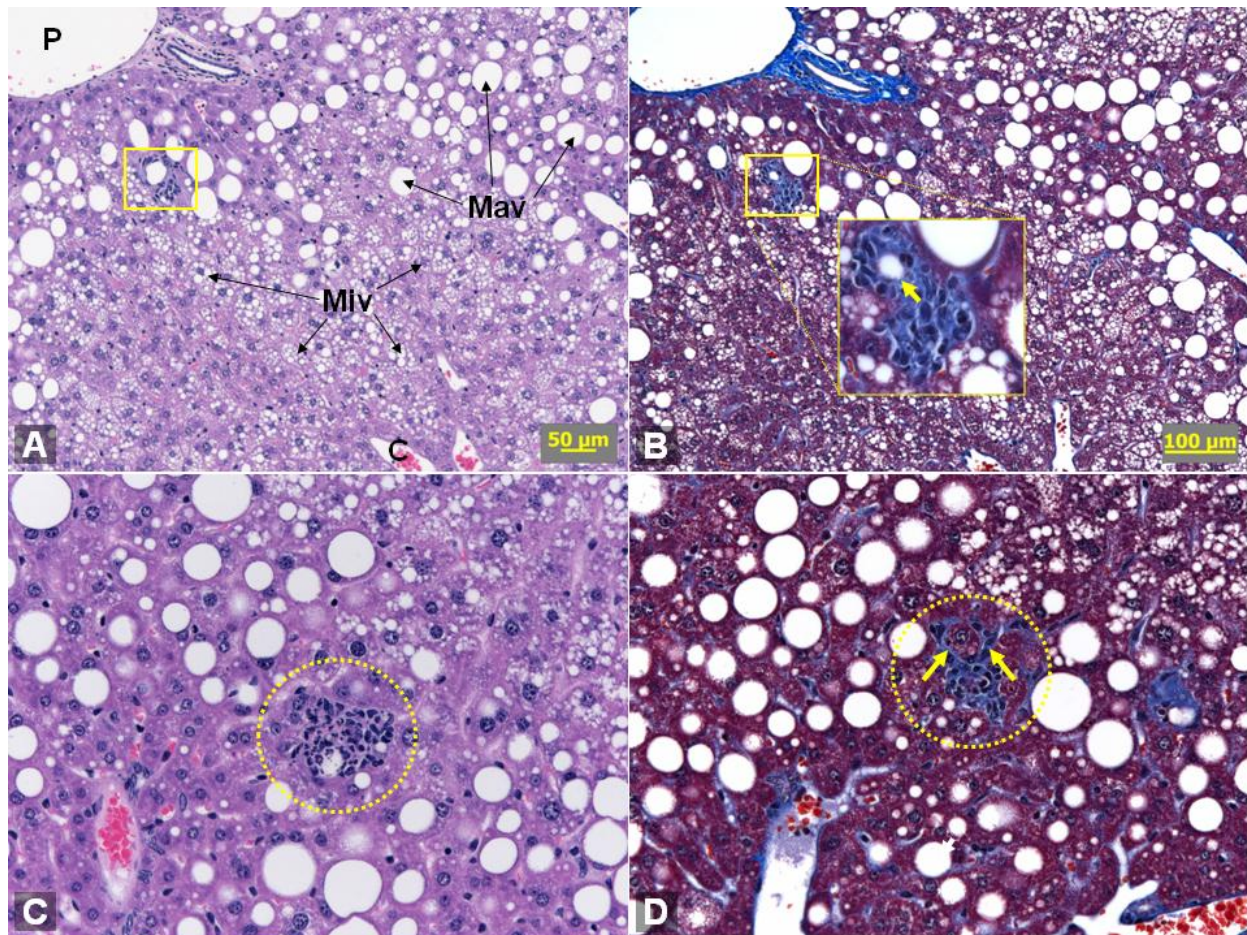


Supplemental Information, Sos, Harris et al., Profound Fatty Liver in Mice with Hepatocyte-Specific Deletion of JAK2 is Completely Rescued by Abrogation of Growth Hormone Secretion

Supplemental Figure S1

Representative Images of 20 Week Old JAK2L Mouse Livers.



A – H+E. There is diffuse hepatocellular lipodosis affecting the entire lobule, i.e. from centrilobular (roughly corresponding to zone 3) to portal (roughly corresponding to zone 1). In contrast to typical lipid distribution in NASH (predominantly macrovesicular and zone 3), microvesicular and macrovesicular change coexist, with macrovesicular change most evident midlobular (roughly corresponding to zone 2). Small foci of inflammation occur at random within lobules (yellow square). P=portal vein. C=central vein. Miv=microvesicular change with vesicle \emptyset < hepatocellular nuclei. Mav=macrovesicular change. **B** – Trichrome. Same lobule as shown in A. Within the focus of inflammation there is deposition of sparse collagenous matrix (royal blue). Arrow: free lipid droplets are occasionally enclosed within the foci. **C** – H+E. Small intralobular focus of inflammation. **D** – Trichrome. Same focus as in C. Spindle cells and

sparse collagenous matrix extend from the focus to enclose and isolate an adjacent hepatocyte (arrows). This type of “perisinuoidal” fibrosis is typical for NASH.

Supplemental Table S1

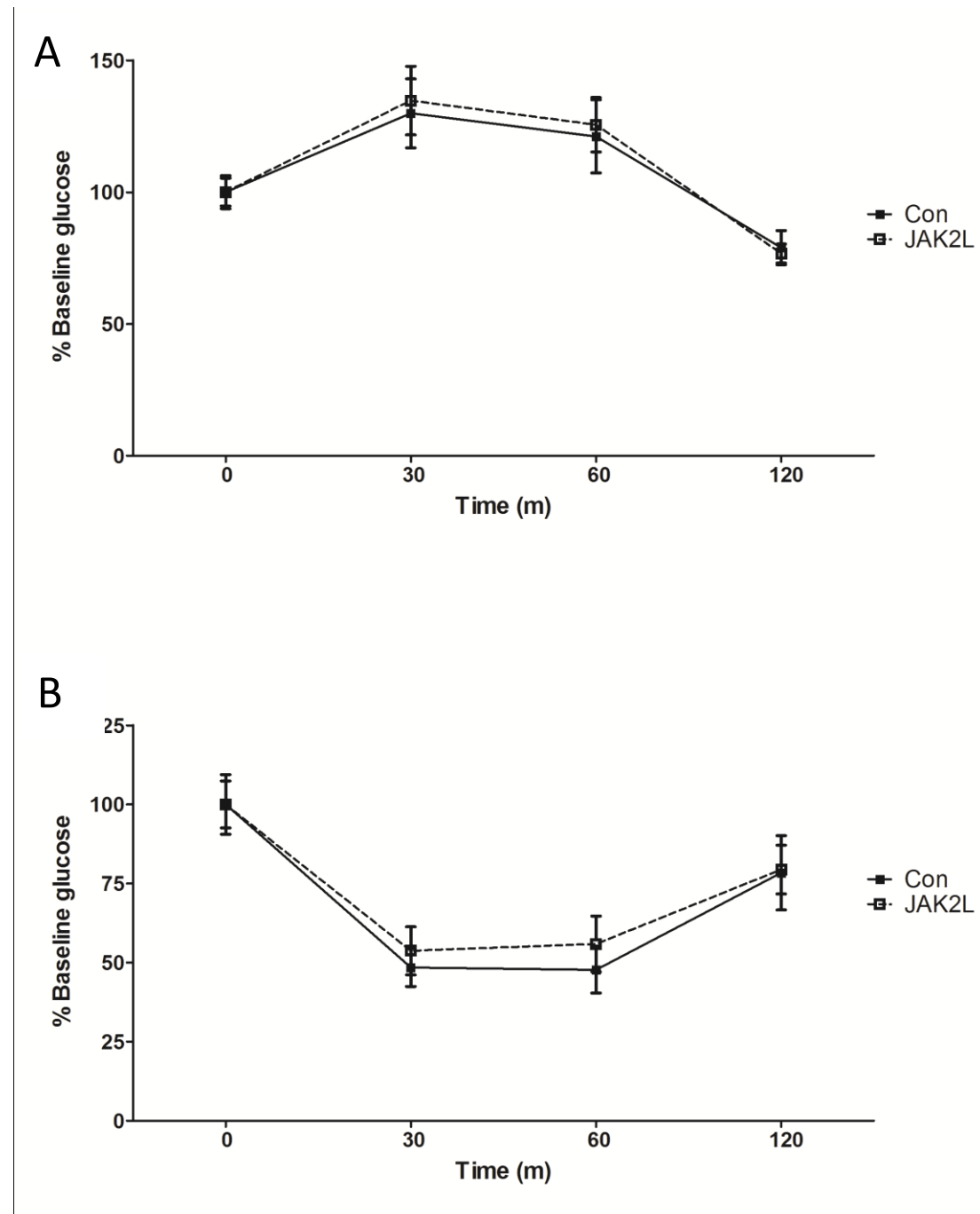
Basic Metabolic Parameters in JAK2L and Con Mice

	Con (n = 4-6)	JAK2L (n = 4-6)
<i>Total Protein g/dL</i>	5.6 ± 0.12	5.33 ± 0.7
<i>Albumin g/dL</i>	3.63 ± 0.03	3.73 ± 0.03
<i>Glucose mg/dL</i>	199 ± 1.53	199.67 ± 2.03
<i>Total Bilirubin mg/dL</i>	0.2 ± 0	0.2 ± 0
<i>Alkaline Phosphatase U/L</i>	116.67 ± 4.98	186.67 ± 7.22 ^a
<i>Aspartate Transaminase (AST) U/L</i>	60.33 ± 1.45	101.67 ± 4.41 ^b
<i>Alanine Transaminase (ALT) U/L</i>	31.33 ± 1.86	101.33 ± 2.03 ^c
<i>Blood Urea Nitrogen (BUN) mg/dL</i>	27.5 ± 1.56	33 ± 1.50
<i>Creatinine mg/dL</i>	0.2 ± 0	0.2 ± 0
<i>Gamma glutamyl transpeptidase (GGT) U/L</i>	1.33 ± 0	1.33 ± 0.33
<i>Total Cholesterol mg/dl</i>	155 ± 21	166.25 ± 10.70
<i>Triglyceride mg/dl</i>	136.33 ± 40.40	146.36 ± 20.66
<i>High Density Lipoprotein (HDL) mg/dl</i>	105.33 ± 21.18	127.05 ± 7.39
<i>Free Fatty Acids (FFA)mmol/L</i>	0.789 ± 0.07	1.28 ± 0.11 ^a
<i>Leptin ng/ml</i>	8.30 ± 1.91	19.87 ± 2.53 ^a
<i>Insulin ng/ml</i>	0.76 ± 0.10	0.86 ± 0.11

^a $p < 0.01$; ^b $p < 0.001$; ^c $p < 0.0001$

Supplemental Figure S2

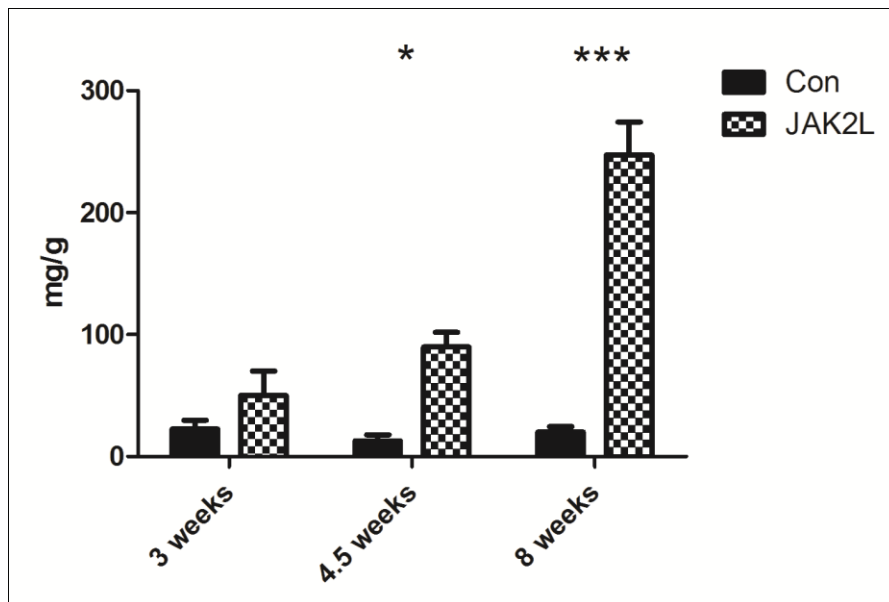
Glucose and Insulin Tolerance Tests.



There were no differences in glucose **(a)** or insulin **(b)** tolerance in 8-week-old JAK2L mice versus Con.

Supplemental Figure S3

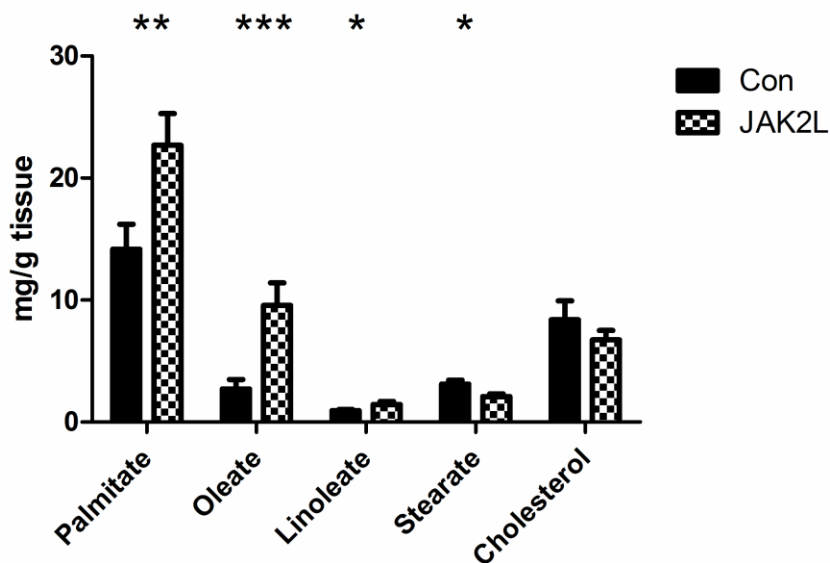
Liver Triglyceride Content in Younger JAK2L Mice.



Liver TG of JAK2L and Con mice content was measured using the colorimetric assay at 3, 4.5 and 8 weeks of age ($n = 5$ for each group). Values are expressed as mean \pm SEM. * $P < 0.05$, *** $P < 0.001$.

Supplemental Figure S4

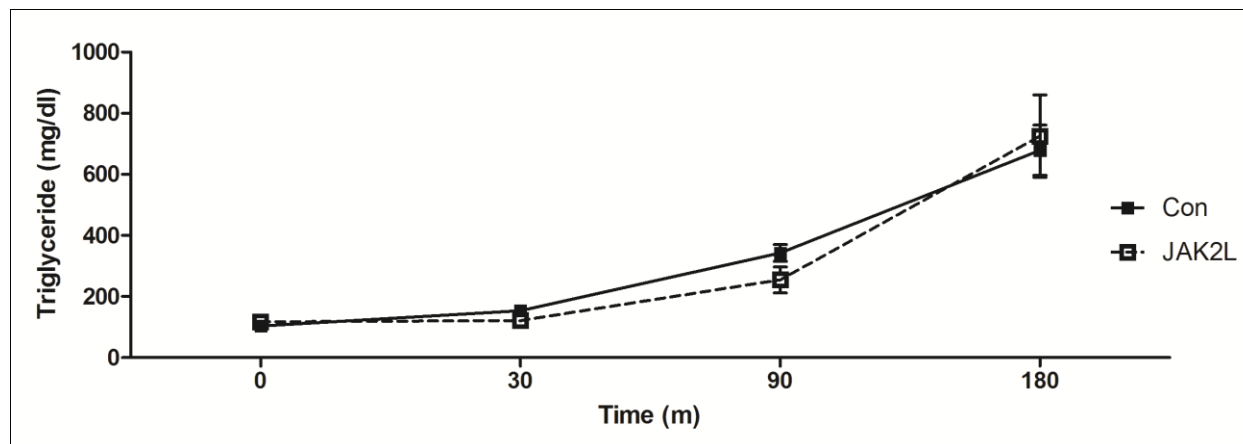
Liver Fatty Acid Content.



The content of each indicated lipid was measured in 4-week-old male Con ($n=9$) and JAK2L ($n=6$) mice. Values are expressed as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplemental Figure S5

Triglyceride Secretion.



There were no differences in TG secretion rates after injection with Triton WR-1339.

Supplemental Table S2

Liver Expression of Genes Related to Lipid Metabolism

MGI Name	MGI Name Full	Male JAK2L:Con			Female JAK2L:Con		
		Fold Change	SEM	P-Value	Fold Change	SEM	P-Value
<u>Peroxisome Proliferator Activated Receptor Related</u>							
Ppara	peroxisome proliferator activated receptor alpha	1.29	0.06	0.00450	1.24	0.10	0.04774
Ppard	peroxisome proliferator activator receptor delta	1.01	0.08	0.92385	1.07	0.06	0.31174
Pparg	peroxisome proliferator activated receptor gamma	2.95	0.84	0.00363	2.47	0.34	0.00347
Ppargc1a	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	1.42	0.20	0.03932	1.58	0.21	0.01726
Ppargc1b	peroxisome proliferative activated receptor, gamma, coactivator 1 beta	1.06	0.06	0.33986	1.19	0.08	0.06864
<u>Lipid Metabolism Related</u>							
Acaca	acetyl-Coenzyme A carboxylase alpha	2.47	0.68	0.00692	0.81	0.27	0.53245
Acacb	acetyl-Coenzyme A carboxylase beta	1.21	0.57	0.67643	0.25	0.09	0.09487
Acly	ATP citrate lyase	1.54	0.57	0.22308	0.46	0.13	0.08967
Fasn	fatty acid synthase	2.86	1.58	0.02808	0.86	0.35	0.71551
Insig1	insulin induced gene 1	1.03	0.29	0.92557	0.84	0.15	0.39821
Insig2	insulin induced gene 2	1.58	0.24	0.02414	1.55	0.26	0.04697
Scd1	stearoyl-Coenzyme A desaturase 1	2.25	1.02	0.05064	0.94	0.20	0.77399
Scd2	stearoyl-Coenzyme A desaturase 2	10.99	4.09	0.05590	3.96	0.50	0.00318
Scd4	stearoyl-Coenzyme A desaturase 4	1.26	0.09	0.04018	1.11	0.06	0.11260
Agpat1	1-acylglycerol-3-phosphate O-acyltransferase 1 (lysophosphatidic acid acyltransferase, alpha)	1.04	0.09	0.67748	1.16	0.09	0.14703
Agpat2	1-acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta)	1.50	0.14	0.01135	1.67	0.31	0.02449
Agpat3	1-acylglycerol-3-phosphate O-acyltransferase 3	1.26	0.03	0.00069	1.28	0.06	0.00815
Agpat4	1-acylglycerol-3-phosphate O-acyltransferase 4 (lysophosphatidic acid acyltransferase, delta)	1.06	0.05	0.29438	0.88	0.09	0.26311
Agpat5	1-acylglycerol-3-phosphate O-acyltransferase 5 (lysophosphatidic acid acyltransferase, epsilon)	1.17	0.06	0.03478	1.18	0.07	0.06007
Agpat6	1-acylglycerol-3-phosphate O-acyltransferase 6 (lysophosphatidic acid acyltransferase, zeta)	0.82	0.03	0.00849	1.08	0.03	0.03854
Agpat9	1-acylglycerol-3-phosphate O-acyltransferase 9	1.48	0.12	0.01489	1.40	0.18	0.04225
Dgat1	diacylglycerol O-acyltransferase 1	1.13	0.08	0.19599	1.36	0.13	0.03022
Dgat2	diacylglycerol O-acyltransferase 2	1.30	0.09	0.01789	1.20	0.14	0.22365
Dgat2l3	diacylglycerol O-acyltransferase 2-like 3	1.06	0.06	0.41006	1.08	0.04	0.11499

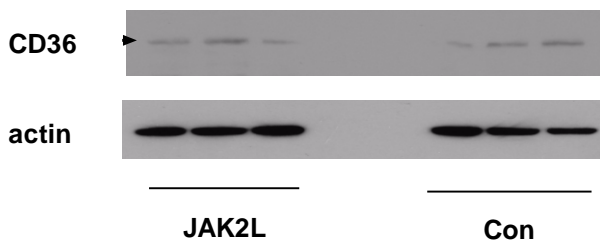
Dgat2l4	diacylglycerol O-acyltransferase 2-like 4	0.95	0.06	0.41574	1.02	0.05	0.65053
Dgat2l6	diacylglycerol O-acyltransferase 2-like 6	1.09	0.06	0.16311	0.99	0.12	0.94058
Gpam	glycerol-3-phosphate acyltransferase, mitochondrial	2.31	0.79	0.02047	1.24	0.26	0.34902
Mogat1	monoacylglycerol O-acyltransferase 1	7.63	1.74	0.01515	4.58	1.64	0.09352
Mogat2	monoacylglycerol O-acyltransferase 2	0.86	0.08	0.15933	0.87	0.17	0.51045
<u>Lipid Oxidation Related</u>							
Acaa1a	acetyl-Coenzyme A acyltransferase 1A	1.65	0.15	0.00458	1.56	0.14	0.00423
Acaa1b	acetyl-Coenzyme A acyltransferase 1B	2.40	0.35	0.00102	1.90	0.26	0.01093
Acaa2	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	1.16	0.04	0.00683	1.21	0.09	0.06057
Acox1	acyl-Coenzyme A oxidase 1, palmitoyl	1.53	0.10	0.00110	1.56	0.13	0.00551
Acox2	acyl-Coenzyme A oxidase 2, branched chain	1.16	0.04	0.01404	1.68	0.21	0.01215
Acox3	acyl-Coenzyme A oxidase 3, pristanoyl	0.85	0.08	0.16287	0.88	0.03	0.03240
Acox1	acyl-Coenzyme A oxidase-like	1.09	0.06	0.14479	0.99	0.07	0.90385
Cpt1a	carnitine palmitoyltransferase 1a, liver	1.29	0.07	0.00597	1.27	0.13	0.09155
Cpt1b	carnitine palmitoyltransferase 1b, muscle	1.39	0.07	0.00483	1.73	0.18	0.00671
Cpt1c	carnitine palmitoyltransferase 1c	1.05	0.05	0.38268	0.95	0.11	0.70005
Cpt2	carnitine palmitoyltransferase 2	1.26	0.08	0.01621	1.60	0.10	0.00273
Ehhadh	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	2.91	0.35	0.00011	1.74	0.36	0.08910
<u>Lipid Transport Related</u>							
Cd36	CD36 antigen	16.83	3.78	0.00004	6.49	1.00	0.00125
Slc27a1	solute carrier family 27 (fatty acid transporter), member 1	1.01	0.06	0.87355	1.02	0.03	0.64989
Slc27a2	solute carrier family 27 (fatty acid transporter), member 2	1.21	0.04	0.00575	1.41	0.20	0.07626
Slc27a3	solute carrier family 27 (fatty acid transporter), member 3	1.04	0.06	0.56106	0.90	0.05	0.14043
Slc27a4	solute carrier family 27 (fatty acid transporter), member 4	1.08	0.01	0.00193	1.11	0.04	0.06044
Slc27a5	solute carrier family 27 (fatty acid transporter), member 5	1.05	0.07	0.56940	1.06	0.10	0.56584
Slc27a6	solute carrier family 27 (fatty acid transporter), member 6	0.85	0.05	0.08302	0.98	0.09	0.82817

Supplemental Figure S6

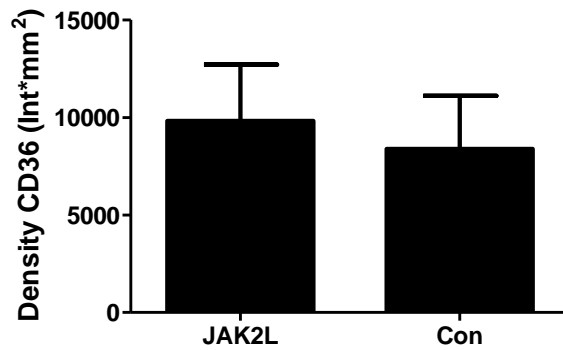
CD36 Protein Content in Heart

(a) Western blots of heart tissue from JAK2L and Con mice. In the upper panel, individual mouse samples were incubated with a commercially available polyclonal antibody against CD36. In the bottom panels, the blots were stripped and incubated with an antibody against actin. There is no difference in the content of CD36 in the JAK2L samples versus Con. (b) Quantified density (Intensity*mm²) of the CD36 band from the blot in (a).

A



B



Supplemental Table S3

Taqman® Primer and Probe Sequences

MGI Name	MGI Name Full	Probe	Sequence
<i>Cd36</i>	CD36 antigen	Forward	CCTGGGAGTTGGCGAGAAA
		Reverse	CGATCACAGCCCATTCTCCT
		Probe	CCAGTGCTCTCCCTTGATTCTGCTGC
<i>Pparg</i>	peroxisome proliferator activated receptor gamma	Forward	CCCCTGCTCCAGGAGATCTAC
		Reverse Probe	GCAATCAATAGAAGGAACACGTTGT ACTTGTATTAGCAGGAAAGTCCCACCCGC
<i>Jak2</i>	Janus kinase 2	Forward	ATGGCCTGCCTTACAATGACAG
		Reverse	ATTAGCACTTCCAGGAATATCACCA
		Probe	AGGCAACCTCCACATCTCCTGTACATCAGA
<i>Igf1</i>	insulin-like growth factor 1	Forward	TCGGCCTCATAGTACCCACTCT
		Reverse	TTATTGCAGGTGCGGTCATTT
		Probe	CTGCTGTGTAAACGACCCGGACCTACC
<i>Igf1r</i>	insulin-like growth factor 1 receptor	Forward	GCGATTTAGAGAAACGAACATTCC
		Reverse Probe	CAAGTCCAAATATGGTCCATGCT CACCTTGGTAACCGGCATCGTGA
<i>Igfals</i>	insulin-like growth factor binding protein, acid labile subunit	Forward	GACAATCAGATCCATGAGGTCAAG
		Reverse Probe	AGTTGCCGGAGAGATTCATAACA CGCCTTCTTTGGCCTCTTCAACGTG
<i>Igfbp3</i>	insulin-like growth factor binding protein 3	Forward	GCTGGTGTGTGGACAAGTATGG
		Reverse Probe	GGCAATGTACGTCGTCTTTCC AGCCCTTGCCAGGCTACGACACC
<i>Gh</i>	growth hormone	Forward	GCTGCTCTCCTGCTTCAAGAA
		Reverse	AGCGGCGACACTTCATGAC
		Probe	ACCTGCACAAAGCGGAGACCTACCTG
<i>Ghrh</i>	growth hormone releasing hormone	Forward	TCAGGATGCAGCGACACGTA
		Reverse Probe	GATCACTTTCCGGGCATACAG ATGCCATCTTCACCACCAACTACAGGAAAC
<i>Ghrhr</i>	growth hormone releasing hormone receptor	Forward	CACTGCCCCAGGAACTACATC
		Reverse Probe	AGAACACAGCACTGGCCTTGA ACACGCAGCTGTTTGCCACCTTCA