Online Appendix

TABLE OF CONTENTS

1. Additional Information

Antibodies and Immunoblotting. Cell and tissue lysates were prepared in Protein Extraction Reagent (Thermo Fisher) supplemented with Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher). Western blotting was performed with whole cell lysates run on 4-12% Bis-Tris NuPage gels (Life Technologies) and transferred onto Immobilon-P Transfer Membranes (Millipore) followed by antibody incubation (antibodies listed in **Supplemental Table 1**). Immunoreactive bands were visualized by chemiluminescence.

Real-time qPCR. Total RNA was extracted using the Direct-zol RNA MiniPrep kit (Zymo Research). cDNA was synthesized using SuperScript VILO Master Mix (Thermo Fisher). Relative mRNA expression was measured with Taqman Fast qPCR reagents using a QuantStudio 3 real-time PCR system (Applied Biosystems). The invariant control was *TBP*. Roche Universal Probe Gene Expression Assays are detailed in **Supplemental Table 2**.

Histology. Formalin-fixed paraffin-embedded adipose tissues sections were stained using anti-Mac3 (#550292; BD Biosciences) and hematoxylin & eosin (H&E) counterstains. Four 20x fields of view per tissue were imaged using a Nikon Ci-L Brightfield microscope and quantified using the Adiposoft ImageJ plugin for adipocyte cell size and number.

Cellular Respiration. Respiration was measured in adipocytes using an XF24 analyzer (Agilent). Preadipocytes were plated into V7-PS plates and differentiated before treatments. For the assay, media was replaced with 37˚C unbuffered DMEM containing 4.5 g/L glucose, sodium pyruvate (1 mmol/L), and L-glutamine (2 mmol/L). Basal respiration was defined before sequential addition of oligomycin, FCCP, rotenone, and antimycin A.

Fluorescence Microscopy. Mitochondria were labeled using MitoTracker CMX-ROS (ThermoFisher). Live cells were pulsed with 500 nM MitoTracker for 15 min. Mitochondrial

labeling was followed by cell fixation in 4% paraformaldehyde. Ammonium chloride was used to quench auto-fluorescence derived from residual paraformaldehyde. DAPI (Sigma) and LipidTOX were used for nuclei and lipid droplet labeling, respectively. Imaging was performed with the DeltaVision Core Image Restoration Microscope (GE Healthcare).

Metabolomics. For extraction of inguinal WAT (iWAT) metabolites, 750 µl of water/methanol (1:4) were added to snap-frozen iWAT and samples were homogenized, then mixed with 450 µl ice-cold chloroform. The resulting solution was mixed with 150 µl ice-cold water and vortexed again for 2 minutes. The solution was incubated at –20°C for 20 minutes and centrifuged at 4°C for 10 minutes to partition the aqueous and organic layers. The aqueous and organic layers were combined and dried at 37°C for 45 minutes in an automatic speed-vac system (Thermo Fisher Scientific). The extract was reconstituted in a 500-µl solution of ice-cold methanol/water (1:1) and filtered through a 3-kDa molecular filter (Amicon Ultracel 3-kDa Membrane) at 4°C for 90 minutes to remove proteins. The filtrate was dried at 37°C for 45 minutes in a speed vacuum and stored at –80°C until MS analysis. Prior to MS analysis, the dried extract was resuspended in 50-µl solution of methanol/water (1:1) containing 0.1% formic acid and then analyzed using multiple reaction monitoring (MRM).

Aqueous phase chromatographic separation was achieved using three solvents: water (solvent A), water with 5mM ammonium acetate (pH 9.9), and 100% acetonitrile (solvent B). The binary pump flow rate was 0.2 ml/min with a gradient spanning 80% B to 2% B over a 20-minute period followed by 2% B to 80% B for a 5 min period and followed by 80% B for 13-minute time period. The flow rate was gradually increased during the separation from 0.2 mL/min (0-20 mins), 0.3 mL/min (20-25 min), 0.35 mL/min (25-30 min), 0.4 mL/min (30-37.99 min), and finally set at 0.2 mL/min (5 min). Glycolytic and TCA intermediates were separated on a Luna Amino (NH2)

column (3 μm, 100A 2 x 150 mm, Phenomenex) that was maintained in a temperature-controlled chamber (37°C).

Glycolytic and TCA intermediates were measured using negative ionization mode with an ESI voltage of −3500ev. Approximately 9–12 data points were acquired per detected metabolite. For all samples, ten microliters of sample were injected and analyzed using a 6495 QQQ triple quadrupole mass spectrometer (Agilent) coupled to a 1290 series HPLC system via SRM. The data were normalized with internal standard and log2-transformed on a per-sample basis. For every metabolite in the normalized dataset, t-tests were conducted to compare expression levels between different groups. Differential metabolites were identified by adjusting the p-values for multiple testing at an FDR threshold of <0.25.

Indirect Calorimetry. *STAT1*$^{fl/fl}$ and *STAT1*$^{a\text{-}KO}$ male mice were maintained on HFD and housed at room temperature in Comprehensive Lab Animal Monitoring System Home Cages (CLAMS-HC, Columbus Instruments). Oxygen consumption, CO_2 emission, energy expenditure, food and water intake, and activity were measured for up to 6 days (BCM Mouse Metabolic Phenotyping Core). Mouse body weight was recorded and body composition examined by MRI (Echo Medical Systems) prior to indirect calorimetry. Statistical analysis of energy balance was performed using the web-based tool CalR (https://calrapp.org/).

2. Supplemental Tables and Legends

Supplemental Table 1. Western blot antibodies

Antibody	**Vendor**	**Catalog #**
ACLY	Cell Signaling	4332
ADIPOQ	Genetex	GTX112777
β-actin	Sigma	A5441
COX IV	Genetex	GTX101499
Cytochrome C	Genetex	GTX108585
FLAG	Cell Signaling	2368
HSP60	Genetex	GTX110089
HSP90	Cell Signaling	4877
IFNGR1	Genetex	GTX54333
NDUFA12	Genetex	GTX109567
PPARg	Cell Signaling	2443
p-STAT1 Y701	Cell Signaling	9167
STAT1 total	Cell Signaling	9172
STAT2 total	Genetex	GTX103117
STAT3 total	Genetex	GTX104616
UCP1	Abcam	10983

Supplemental Table 2. Roche Universal Probe Library (UPL) gene expression assays

Gene	Species	Accession #	F Primer	R Primer	UPL
ADIPOQ	Human	NM_004797.2	ggtgagaagggtgagaaagga	tttcaccgatgtctcccttag	85
IRF1	Human	NM_002198.2	ggcacatcccagtggaag	cccttcctcatcctcatctg	56
STAT1	Human	NM_007315.3	tgagttgatttctgtgtctgaagtt	acacctcgtcaaactcctcag	32
UCP1	Human	NM_021833.4	ctggacacggccaaagtc	ggacacctttatacctaataacactgg	1
Acly	Mouse	NM_001199296.1	aagaaggaggggaagctgat	tcgcatgtctgggttgttta	105
Adipoq	Mouse	NM_009605.4	ggagagaaaggagatgcaggt	ctttcctgccaggggttc	17
Aspa	Mouse	NM_023113.5	ttatccaaggaatgaaagtggag	tgcaatggtttccagtcttg	46
Cidea	Mouse	NM_007702.2	aaaccatgaccgaagtagcc	aggccagttgtgatgactaagac	66
Cox8b	Mouse	NM_007751.3	ccagccaaaactcccactt	gaaccatgaagccaacgac	102
Ifngr1	Mouse	NM_010511.3	tcaaaagagttccttatgtgccta	tacgaggacggagagctgtt	69
Irf1	Mouse	NM_008390.2	gcaccactgatctgtataacctaca	cctcatcctcgtctgttgc	10
Isg15	Mouse	NM_015783.3	agtcgacccagtctctgactct	ccccagcatcttcaccttta	71
Oas1a	Mouse	NM_145211.2	gtgctgccagcctttgat	tggcatagattctgggatcag	11
Pgc1a	Mouse	NM_008904.2	gaaagggccaaacagagaga	gtaaatcacacggcgctctt	29
Pparg2	Mouse	NM_011146.3	gaaagacaacggacaaatcacc	gggggtgatatgtttgaacttg	7
Scd1	Mouse	NM_009127.4	ttccctcctgcaagctctac	cagagcgctggtcatgtagt	34
Stat1	Mouse	NM_001205313.1	aaatgtgaaggatcaagtcatgtg	catcttgtaattcttctagggtcttga	15
Stat2	Mouse	NM_019963.1	ttgaaacataaactcattgtgatctct	ggactctgaatcctgtttgagc	66
Stat3	Mouse	NM_011486.5	agtctcgcctcctccagac	gctgcttctctgtcactacgg	26
Stat4	Mouse	NM_011487.5	cggcatctgctagctcagt	tgccatagtttcattgttagaagc	48
Stat5a	Mouse	NM_011488.3	gagctggtgttccaggtga	ggtggcagtagcattgtgg	71
Stat5b	Mouse	NM_001113563.1	ctccagacactgggcgtag	gccacagcacagacaagagt	75
Stat6	Mouse	NM_009284.2	ctgcgaacccttgtgacc	ttggctgaggtccctagaaa	3
Ucp1	Mouse	NM_009463.3	ggcctctacgactcagtcca	taagccggctgagatcttgt	34

Supplemental Table 3. CRISPR guide RNA sequences to target *Stat1* and a non-mammalian targeting control (ntCR1)

Target	**Target Sequence**
ntCR1	gccacaccgttccgtataca
Stat1 g1	tgcaaaacctctcagaacag
Stat1 g2	tgtgatgttagataaacaga
Stat1 g3	gatcaccacgacaggaagag

Supplemental Table 4. GSEA was used to identify enriched, STAT1-repressed gene sets from the MSigDB C3 transcription factor target gene set collection, sorted in order by the normalized enrichment score (NES) for **(A)** iWAT and **(B)** eWAT. The NES reflects the degree to which a gene set is overrepresented at the top of the complete ranked gene list.

A

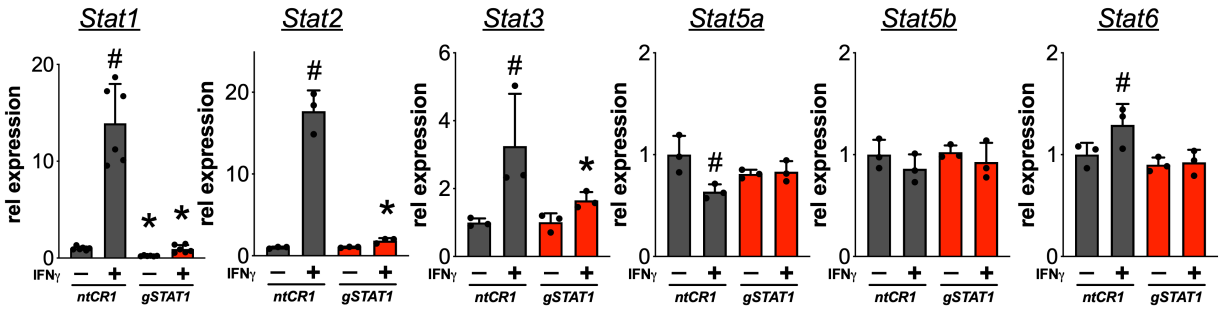
	eWAT	iWAT
GeneSet	**a-KO v fl/fl**	**a-KO v fl/fl**
SCGGAAGY_ELK1_02	-4.2408056	3.8319132
RCGCANGCGY_NRF1_Q6	-3.6902695	3.0320184
HNF1_Q6	0	2.7064352
ELK1_02	-2.1607935	2.0756822
TGCGCANK_UNKNOWN	-1.8211817	2.0353198
ACTAYRNNNCCCR_UNKNOWN	-2.339078	1.921553
GATA1_03	-1.4989849	1.9098649
GABP_B	-2.156791	1.9097301
YY1_02	-1.5859565	1.8749659

B

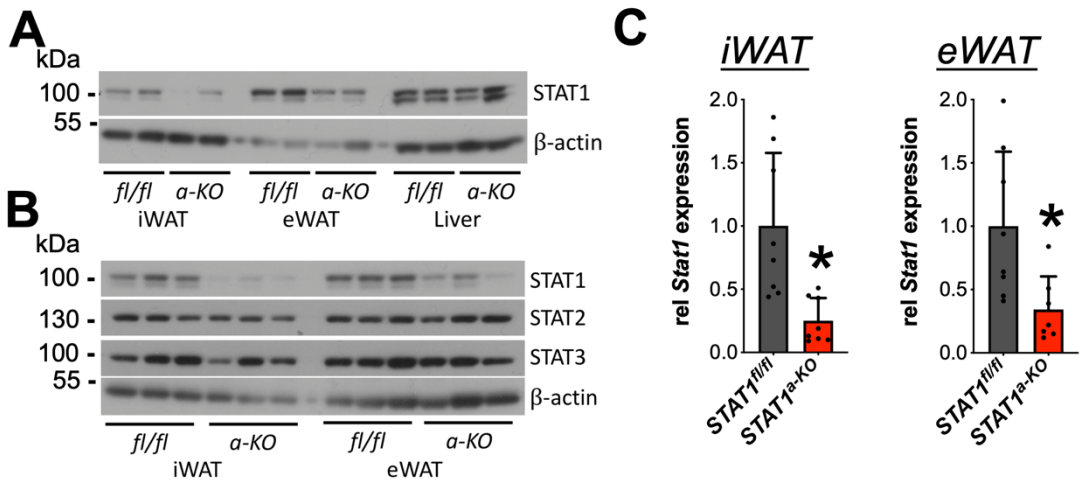
GeneSet	**a-KO v fl/fl**	**a-KO v fl/fl**
TGGNNNNNNKCCAR_UNKNOWN	2.8939285	-1.7994349
WGGAATGY_TEF1_Q6	2.438917	-2.3783414
RYCACNNRNNRNCAG_UNKNOWN	2.3840816	0
CAGCTG_AP4_Q5	2.283755	-3.2176328
SF1_Q6	2.2235904	0
HNF1_01	2.1790144	1.8113785
GCANCTGNY_MYOD_Q6	2.1511602	-2.099132
CAGGTG_E12_Q6	2.1379433	-3.9745436
RP58_01	2.1171308	0

3. Supplemental Figures and Legends

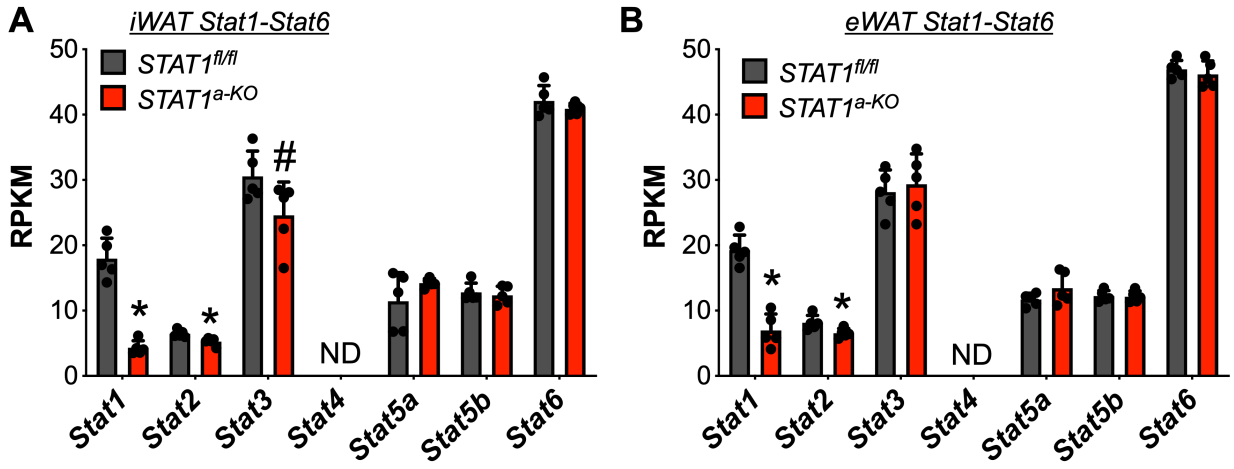
Supplemental Figure 1. Regulation of the Stat gene family in *Stat1* KO 3T3-L1 adipocytes. **(A)** Differentiated ntCR1 or *Stat1* knockout (gSTAT1) cells were treated +/- 100 ng/ml IFNγ for 24 h and then harvested for quantification of relative mRNA for *Stat* genes (n=3). *Stat4* was not detected. Gray – ntCR1; red – gSTAT1; *p<0.05 vs ntCR1, #p<0.05 vs vehicle; data are represented as mean +/- SD.



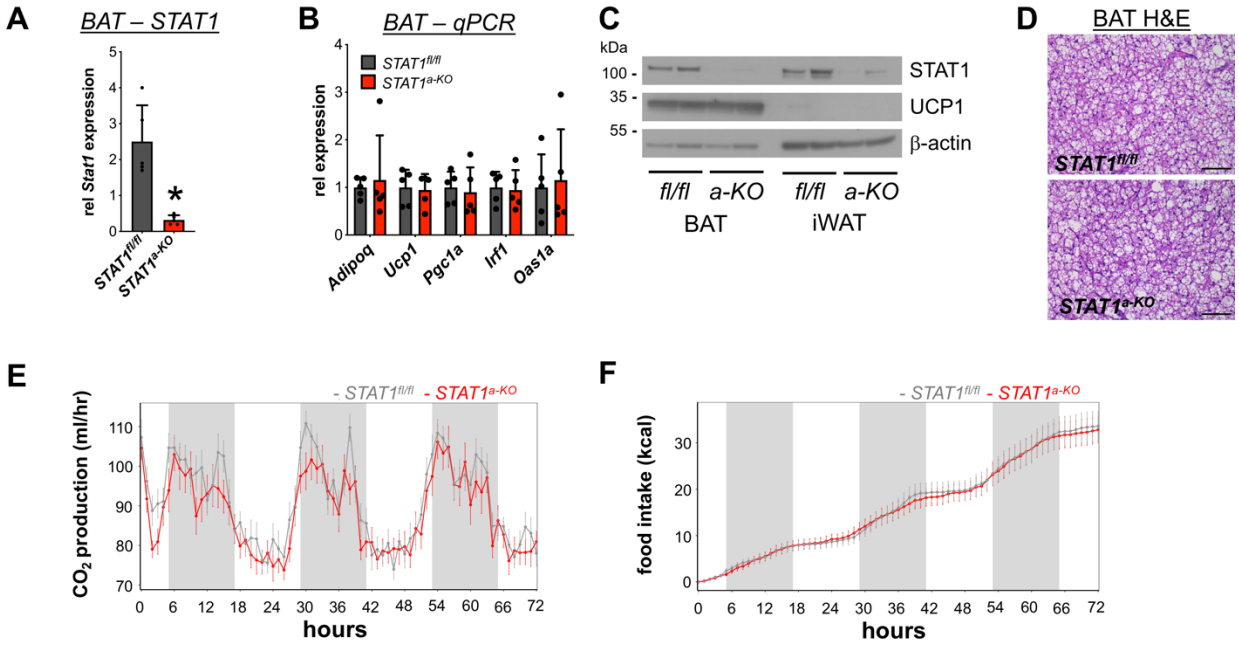
Supplemental Figure 2. Validation of adipocyte-specific knockout of *Stat1*. **(A)** Immunoblots show STAT1 knockdown in iWAT and eWAT, but not liver, of adipocyte specific (*AdipoQ-Cre*) *STAT1*$^{a\text{-}KO}$ compared to *STAT1*$^{fl/fl}$ littermate controls. **(B)** Immunoblots show unaltered STAT2 and STAT3 expression in iWAT and eWAT of *STAT1*$^{a\text{-}KO}$ mice. **(C)** Relative *Stat1* expression in the iWAT and eWAT of *STAT1*$^{fl/fl}$ and *STAT1*$^{a\text{-}KO}$ mice (n=7-8/group, *p<0.05). Data are represented as mean +/- SD.



Supplemental Figure 3. Expression of the Stat gene family in iWAT and eWAT of *STAT1$^{a\text{-}KO}$* mice. RNA-Seq analysis was performed on total mRNA from **(A)** iWAT and **(B)** eWAT of obese *STAT1$^{a\text{-}KO}$* (red) and *STAT1$^{fl/fl}$* littermate controls (gray). RPKM values for *Stat* genes (n=5/group). *Stat4* was not detected (ND). *p<0.05, #p<0.1; data are represented as mean +/- SD.



Supplemental Figure 4. Impacts of *Stat1* deletion on brown adipose tissue (BAT) features and energy expenditure. Relative expression of **(A)** *Stat1*, **(B)** *Adipoq*, *Pgc1a*, *Ucp1*, and inflammatory marker (*Irf1*, *Oas1a*) genes in the BAT of $STAT1^{fl/fl}$ (gray) and $STAT1^{a\text{-}KO}$ (red) mice (n=5/group). *p<0.05, data are represented as mean +/- SD. **(C)** Immunoblots show STAT1 depletion in BAT and inguinal WAT (iWAT), with UCP1 expression predominantly expressed in the BAT compared to iWAT of $STAT1^{a\text{-}KO}$ and $STAT1^{fl/fl}$ littermate controls. **(D)** H&E staining of BAT tissue sections from $STAT1^{fl/fl}$ and $STAT1^{a\text{-}KO}$ mice. Scale bar, 100 µm Mice were individually housed and monitored in metabolic cages (Columbus CLAMS-HC) for 3 days (n=7-8/group). Recorded traces of **(E)** carbon dioxide (CO_2) production (ml/hour) and **(F)** food intake (kcal) during the dark (gray) and light (white) periods in obese $STAT1^{fl/fl}$ (gray lines) and $STAT1^{a\text{-}KO}$ (red lines) mice. Data are represented as mean +/- SD.



Supplemental Figure 5. Gene expression of *Stat* family members in iWAT and adipocytes from *IFNGR1* knockout mice. (A) qPCR was used to measure relative mRNA expression of *Ifngr1, Stat1, Stat2*, *Stat3*, *Stat4*, *Stat5a*, *Stat5b*, and *Stat6* from iWAT of *IFNGR1*$^{+/+}$ (gray) and *IFNGR1*$^{-/-}$ (red) mice fed high fat diet (n=9-13 mice/group). *p<0.05; data are represented as mean +/- SD. **(B)** qPCR was used to measure relative mRNA levels of *Stat1, Stat2*, *Stat3*, *Stat4*, *Stat5a*, *Stat5b*, and *Stat6* from *IFNGR1*$^{+/+}$ (gray) and *IFNGR1*$^{-/-}$ (red) SVF-derived adipocytes treated +/- 100 ng/ml IFNγ for 24 hours (n=3). *p<0.05 vs *IFNGR1*$^{+/+}$, #p<0.05 vs vehicle. Data are represented as mean +/- SD.