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Supplemental Information

Activation of IRF1 in Human Adipocytes Leads to Phenotypes Associated with Metabolic Disease

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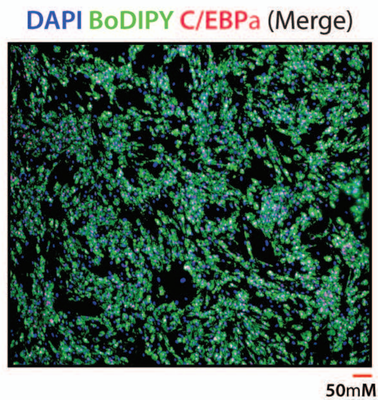
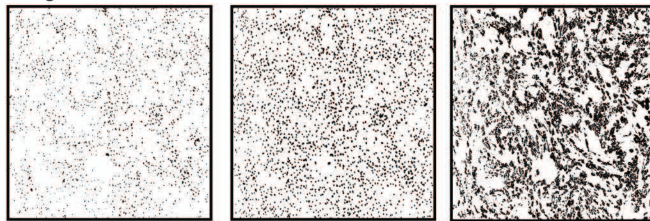
Figure S1, Adipocyte identity validation. Related to Figure 1.

A.

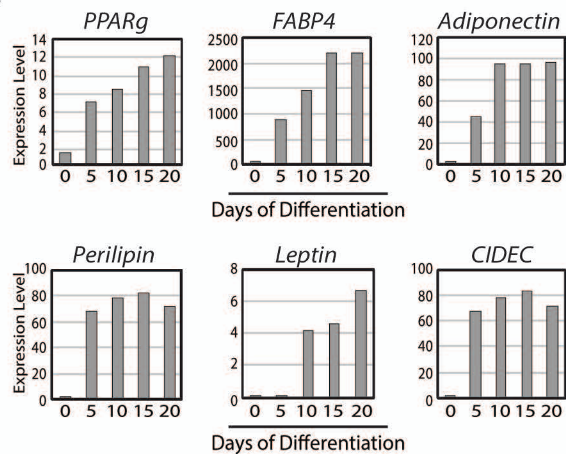




~100% BoDIPY positive cells express C/EBPa

90% (1732/1914) DAPI Positive cells express C/EBPa

B.



C.

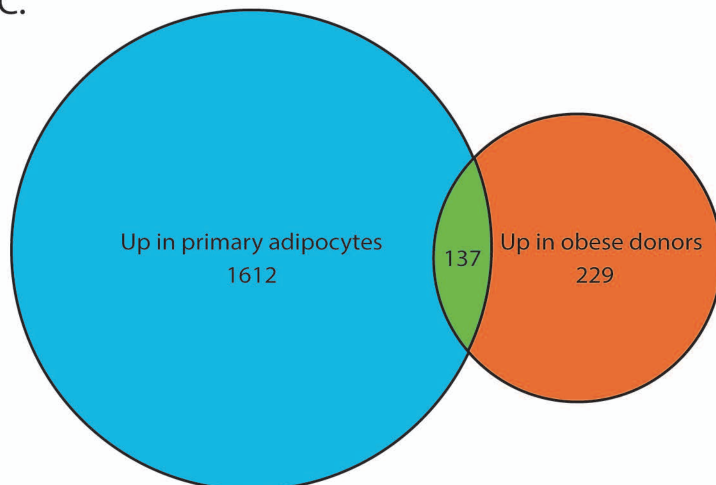


Figure S2, IRF1 overexpression. Related to Figure 2 & 3.

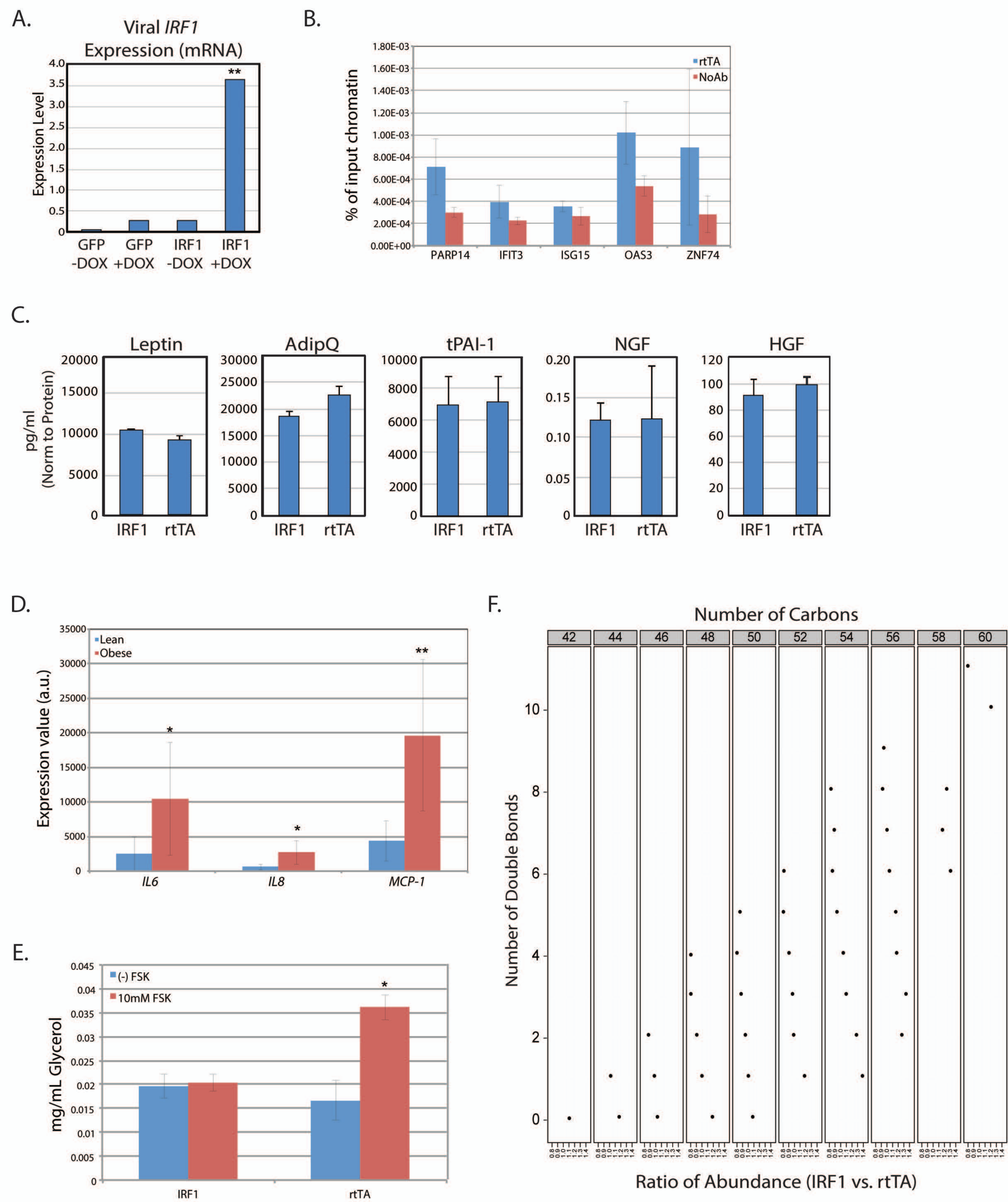
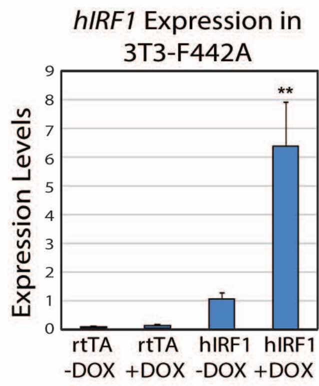
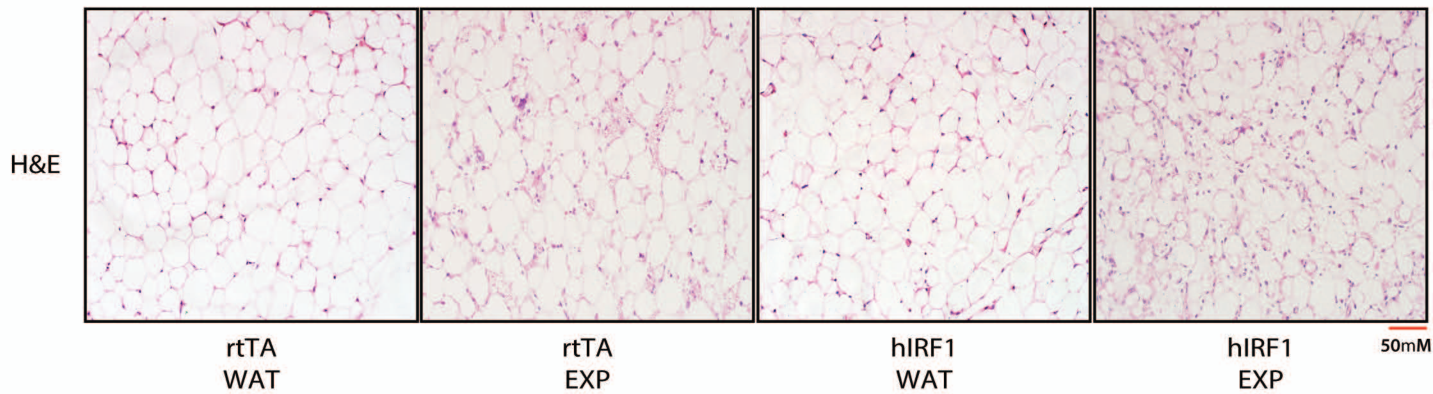


Figure S3, *In vivo* IRF1 expression. Related to Figure 4.

A.



B.



C.

Mouse Skin

rtTA WAT

hIRF1 WAT

Brightfield

DAPI

mF4/80

mPLIN1

50mM

Supplemental Figure Legends

Figure S1. A) ASC-adipocytes were co-stained with BoDIPY (green), DAPI (blue), and anti-C/EBPα (red) to assess differentiation efficiency. An Image J binary mask was used to count stained foci. B) RT-qPCR was used to quantify expression of adipogenic genes during a timecourse of ASC-adipocyte differentiation. "Day 0" represents undifferentiated ASCs. C) Venn diagram of genes higher expressed in primary adipocytes versus ASC-adipocytes overlapping with genes higher expressed in obese versus lean individuals.

Figure S2. Error bars represent +/- standard deviation of biological triplicates and statistically significant p-values are denoted by an asterisk (*≤0.05, **≤0.005). A) RT-qPCR was used to test expression of the inducible IRF1 transgene in ASC-adipocytes, in both the presence and absence of doxycycline (+/- DOX). ASC-adipocyte transfection with a DOX-inducible GFP transgene serves as a negative control. B) ChIP/qPCR was performed for IRF1 in rtTA control cells without IRF1 expression at putative binding sites (H3K4me1 bound IRF1 motif) (*PARP14, IFIT3, ISG15*). *OAS3* and *ZNF74* represent a negative control site where IRF1 is not predicted to bind. Signals are expressed as percentage of total chromatin input. C) A multiplex ELISA assay was utilized to measure adipokines and cytokines in secreted into culture media from adipocytes expressing IRF1. D) Expression of IL6, IL8 and MCP-1 in obese and lean individuals' adipocytes from the GEO dataset, related to figure 3A. E) Glycerol release into the media in response to forskolin (FSK) activated lipolysis was measured in IRF1- and rtTA-adipocytes. Two separate biological triplicates were averaged and plotted. Replication of and related to figure 3B. F) A plot of abundance ratio against number of double bonds for each total of carbon atoms for triglycerides in IRF-adipocytes vs. control (rtTA).

Figure S3. A) RT-qPCR was used to assay for doxycycline (DOX)-inducible expression of hIRF1 in 3T3-F442A cells. B) rtTA only or IRF1-expressing 3T3-F442A cells from were injected into 6-week old nude mice. 6 weeks post-injection, exogenous fat explants (EXP) and control autologous white adipose tissue (WAT) were harvested and stained with hemotoxylin and eosin (H&E). C) Autologous WAT and skin (positive control for macrophage localization) were harvested, probed with anti-F4/80 (green) to assess macrophage infiltration, α-PLIN1 (red) to verify adipocyte identity, and stained with DAPI. Negligible numbers of macrophages are observed in control WAT.

Supplemental experimental procedures

Primer List

Expression Analysis

hMCP-1 exp For #1	CAG CCA GAT GCA ATC AAT GCC
hMCP-1 exp Rev #1	TGG AAT CCT GAA CCC ACT TCT
mf4/80 exp For #1	TTG TAC GTG CAA CTC AGG ACT
mf4/80 exp Rev #1	GAT CCC AGA GTG TTG ATG CAA
mMHCI F	GTGATCTCTGGCTGTGAAGT
mMHCI R	GTCTCCACAAGCTCCATGTC
mMHCII F	CAACCGTGACTATTCCTTCC
mMHCII R	CCACAGTCTCTGTCAGCTC
Viral IRF1 qPCR 1 For	TCT GAA GAA CAT GGA TGC CAC C
pDL38R (LentiViral 3'UTR) Rev	AGCAGCGTATCCACATAG
ADIPOQ For	GATGAAGTCCTGTCTTGGAAGG
ADIPOQ Rev	CAGCACTTAGAGATGGAGTTGG
FABP4 For	TCATGAAAGGCGTCACTTCC
FABP4 Rev	GCTTGCTAAATCAGGGAAAACA
PPARG2 For	GCAGGAGATCTACAAGGACTTG
PPARG2 Rev	CCCTCAGAATAGTGCAACTGG
Lep For	AAGGTTTGGTGTGTGGAGATG
Lep Rev	CTCCTGTCTCTTCTTTCTCTGC
PLIN1 For	CCCCCTGAAAAGATTGCTTCT
PLIN1 Rev	GGAACGCTGATGCTGTTTCTG
CIDEC For	AAGTCCCTTAGCCTTCTCTACC
CIDEC Rev	CCTTCCTCACGCTTCGATCC
HPRT For	TGACACTGGCAAAACAATGCA
HPRT Rev	GGTCCTTTTCACCAGCAAGCT

ChIP-qPCR

IFIT3 Prom ChIP-1 For	CTG AGG CAG GAG AAT CAC TT
IFIT3 Prom ChIP-1 Rev	TGA CTG TTG CTC TTT GAC CT
PARP 14 ChIP-1 For	GAT CTC TCT GCC TCC ACT CT
PARP 14 ChIP-1 Rev	GAT ATC AGG GGA TAG CCT TG
ISG15 ChIP For	CGC TTT GTG ACC AGA CCT CAC T
ISG15 ChIP Rev	ATT TTG AAG GCA TGG CCG G
IRS-1 ChIP For	TTTCTCCACCCGCCGAGATG
IRS-1 ChIP Rev	CAGCGATTCCCGAGGCAAAT