TITLE: Siah2 is expressed in adipocyte precursor cells and interacts with EBF1 and ZFP521 to promote adipogenesis

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A. SYBR Green

Gene ID	Gene Name	Accession #	Manufacturer	Sequence-Forward	Sequence-Reverse
Zfp521	Zinc finger protein 521	NM_145492	IDT	5' TGTGGCAAATCACATGATTGATGA	5' ATCCCTTCGAAGCTGTGCTC
Siah2	Seven in absentia homolog 2	NM_009174	IDT	5' ACCAGAGCATGAAGACATCTGTGA	5' TGGGCATGCATGAGATGGGA
Ebf1	Early B-cell factor 1	NM_001290709	IDT	5' ACAGCAATGGGATACGGACA	5' TGTGTGAGCAATACTCGGCA
Pparg	Peroxisome proliferator activated receptor gamma	NM_001127330	IDT	5' CACAATGCCATCAGGTTTGG	5' GCTGGTCGATATCACTGGAGATC
Adipoq	Adiponectin	NM_009605.4	IDT	5' CATGCCGAAGATGACGTTACTA	5' ACGCTGAGCGATACACATAAG
Cd11b	Cluster of differentiation molecule 11B	NM_001082960.1	IDT	5' GAAAGTAGCAAGGAGTGTGTTTG	5' CAGTGCTCTGGATATCTCCTTC
Pdgfra	Platelet derived growth factor receptor alpha	NM_001083316	IDT	5' CTGCACCAAGTCAGGTCCC	5' CTTCGGCTTCTCTGGGTGTT
Sca1	Stem cell antigen 1	NM_001271416	IDT	5' TGTGCAGAAAGAGCTCAGGG	5' AGACTCCATCAGGGTAGGGG
fapb4 (aP2)	fatty acid binding protein 4	NM_024406.2	IDT	5' GCTTTGCCACAAGGAAAGTG	5' CGACTTTCCATCCCACTTCTG

B. Taqman

Gene ID	Gene Name	Accession #	Manufacturer	Probe
Zfp423	Zinc finger protein 423	NM_033327.2	ABI	5' TGTCCTGGAGATGGTGATGACGACC
Ubb	Ubiquitin B	NM_011664.1	IDT	5' CTAGGGTGATGGTCTTGCCGGTC
Hprt	Hypoxanthine phosphoribosyl transferase	NM_013556.2	ABI	5' GGACTGATTATGGACAGGACTGAAA
Lpl	Lipoprotein lipase	NM_008509.2	ABI	5' AATAGAATTACTGGTTTGGATCCAG

C. Antibody

Antibody	Application	Concentration	Manufacturer	Catalog #
α-Flag	WB/IP	1:500	Sigma	F1804
α-HA	WB	1:1000	Covance	MMS-101R
α-EBF1	WB	1:1000	Abcam	ab108369
α-β-actin	WB	1:10,000	Bethyl	A300-491A
α-SIAH2	WB	1:1000	LsBio	LS-C112149
α-GFP	WB	1:1000	Cell Signaling	2555
α-ZFP521	WB	1:1000	Antibodies-Online	ABIN1031686
α-mouse HRP	WB	1:10,000	Jackson Immuno Research	115-035-003
α-rabbit HRP	WB	1:10,000	Jackson Immuno Research	111-035-003
Biotinylated CD11b	MACS	According to Manufacturer	Miltenyi Biotec	130-109-362
Biotinylated PDGFRα	MACS	According to Manufacturer	Miltenyi Biotec	130-101-905
Biotinylated SCA1	MACS	According to Manufacturer	Miltenyi Biotec	130-101-995
α-Goat Alexa 647	IHC	1:200	Jackson Immuno Research	705605147
α-Rabbit Alexa 488	IHC	1:200	Invitrogen	A21206
α-PDGFRα	IHC	1:100	R&D Systems	AF1062
α-PLIN1	IHC	1:500	Cell Signaling	9349S
α-CD31	IHC	1:1000	R&D Systems	AF3628-SP
α-IBA1	IHC	1:100	Wako	019-19741

Where indicated, western blots (WB) were carried out in 2% BSA in 25 mM Tris-Cl, pH 8.0 with 150 mM NaCl, 0.1% Tween 20 (TBS-T) or FLAG epitope tag in 1% BSA in TBS only. The results were visualized using HRP-conjugated secondary antibodies and chemiluminescence (Thermo Fisher/Pierce).

Table S1: List of primers and antibodies. A) SYBR primer sets. B) Taqman probes. C) Antibodies.
IP: immunoprecipitation; WB: Western blot; IHC: immunohistochemistry; MACS: magnetic activated cell sorting.

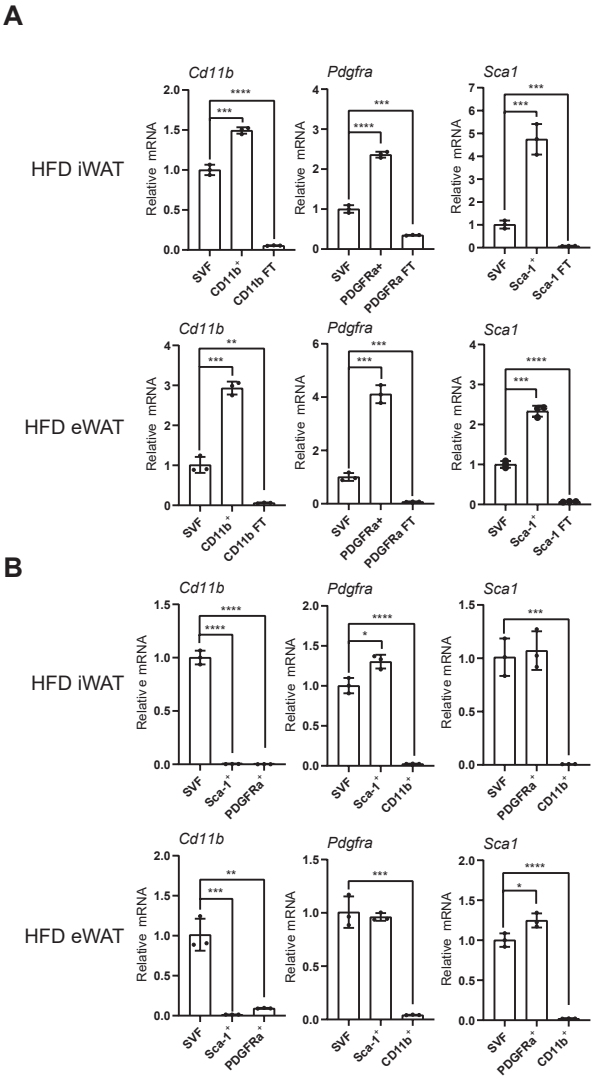

Figure S1: Magnetic immuno-purification efficiency and specificity. Macrophages ($CD11b^+$) and adipocyte precursor ($PDGFR\alpha^+$ or $Sca1^+$) cells were positively selected from stromal vascular fraction (SVF) of male C57BL/6J mice on a 60% high fat diet (HFD) via magnetic bead cell sorting. *Pdgfralpha*, *Cd11b*, and *Sca1* mRNA levels were assayed from positive-selected fraction ($PDGFR\alpha^+$, $Sca1^+$, or $CD11b^+$) and flow-through fraction (FT) to compare against total SVF cells (SVF) by qRT-PCR. **A)** Magnetic immuno-purification efficiency was assessed by comparing *Pdgfralpha*, *Cd11b*, or *Sca1* mRNA expression to total SVF within each respective cell-sorting experiment. **B)** *Pdgfralpha*, *Cd11b*, or *Sca1* mRNA expression of positively-selected cells compared against total SVF between cell-sorting experiments to assess if cross-over binding occurred. Each data point represents a technical replicate from a pooled sample with N = 8 mice/group. Figures depict mean ± SD; *, $p < 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.0001$; ****, $p \leq 0.00001$.

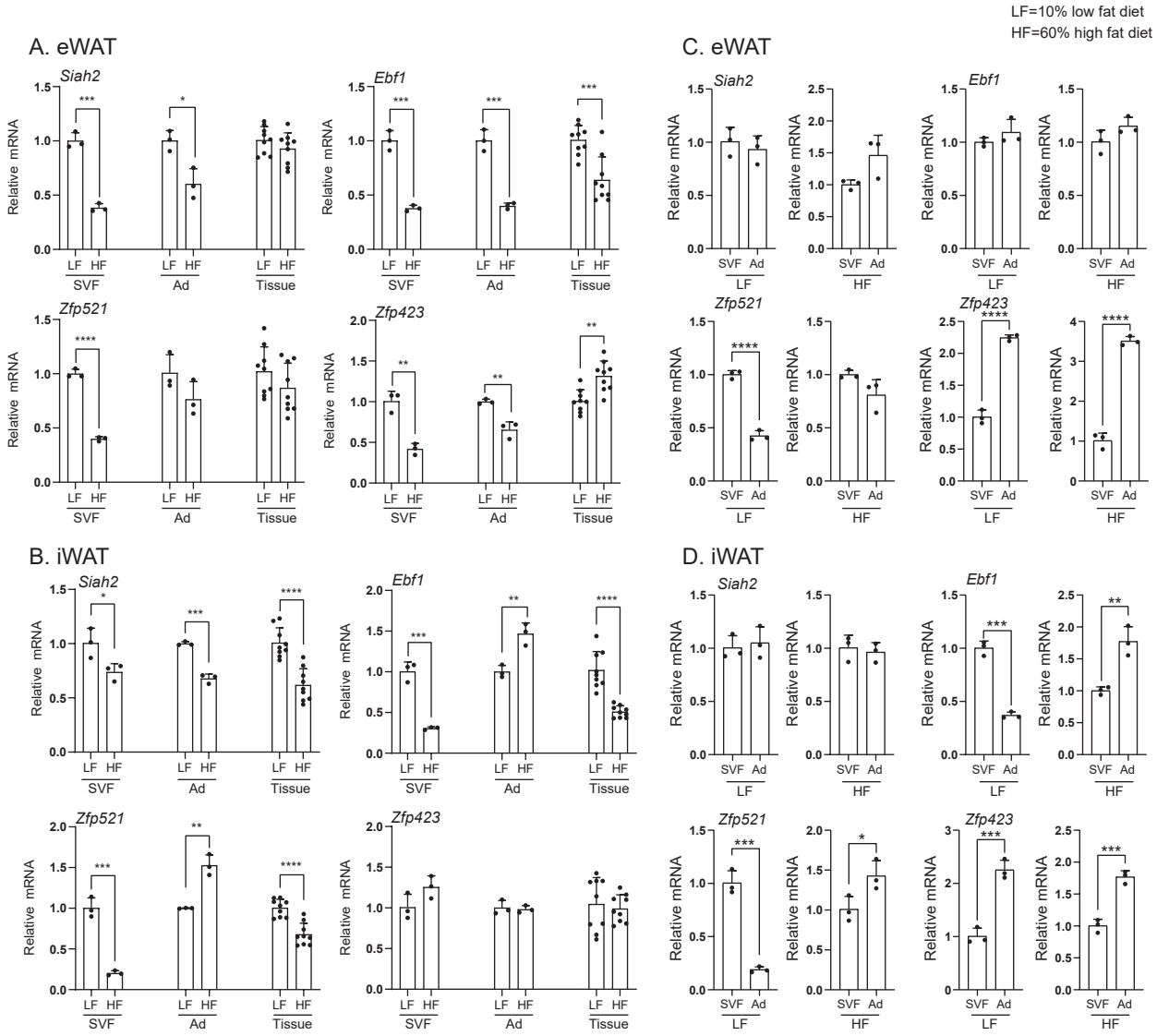

Figure S2: Diet-induced obesity regulates *Siah2*, *Ebf1*, *Zfp521*, and *Zfp423* gene expression. Ten-week old wild-type C57BL/6J male mice fed either a 10% low-fat diet (LF) or 60% high-fat diet (HF) for four-week were purchased from Jackson Laboratory (N=3 mice/group). Stromal vascular fraction (SVF), adipocyte fraction (Ad), and whole tissue (Tissue) from epididymal (eWAT and inguinal (iWAT) adipose tissues were collected. *Siah2*, *Ebf1*, *Zfp521*, and *Zfp423* gene expression were analyzed by qRT-PCR. Gene expression was normalized to *UbB* gene expression and compared to LF within each respective sample group for eWAT (**A)** or iWAT (**B**). Gene expression from eWAT (**C)** or iWAT (**D**) compared between the adipose tissue fractions. Stromal vascular fraction and adipocyte fraction were pooled into a single sample from 3 mice while tissues were analyzed from each mouse separately. Points on the bar graph represent technical replicates. Figures depict mean ± SD; *, $p < 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.0001$; ****, $p \leq 0.00001$.