

Supplemental Information

Liver derived S100A6 propels β cell dysfunction in NAFLD

Surbhi Dogra^{1@}, Debajyoti Das^{2@}, Sujay K. Maity², Avishek Paul², Priya Rawat¹, P Vineeth Daniel¹,
Kausik Das³, Souveek Mitra³, Partha Chakrabarti^{2*}, Prosenjit Mondal^{1*}

¹School of Basic Sciences, IIT Mandi, Mandi, India

²Division of Cell Biology and Physiology, CSIR-Indian Institute of Chemical Biology, Kolkata, India

³Department of Hepatology, IPGMER and SSKM Hospital, Kolkata, India

@Equal contribution

*Corresponding author

Corresponding authors: pchakrabarti@iicb.res.in ; prosenjit@iitmandi.ac.in.

Supplemental Information

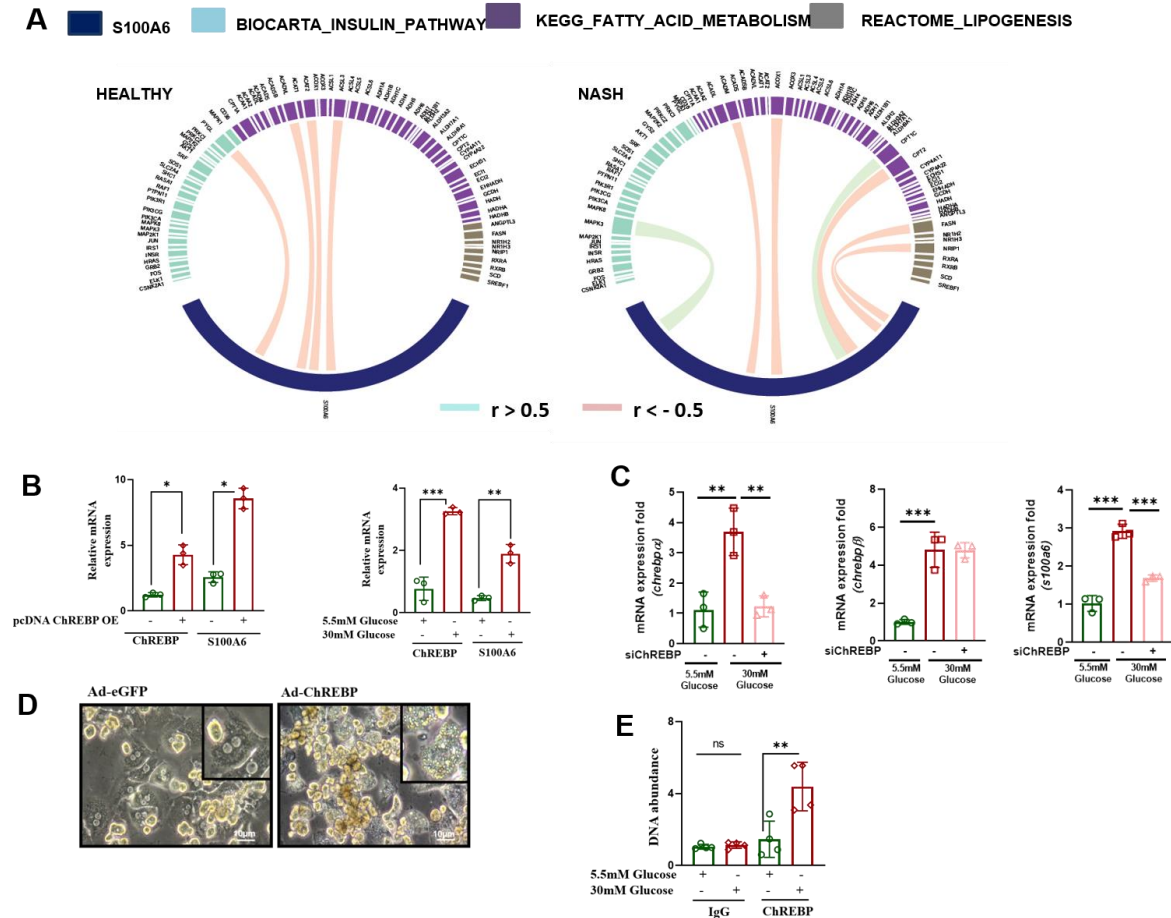


Figure S1.ChREBP stimulates both hepatic S100A6 production and intrahepatic lipid accumulation (A) Circos plot depict the correlation between S100A6 and targeted list of genes (lipid metabolism, inflammation,) for healthy and NASH groups based on the correlation coefficients among gene expressions; blue chords ($r > 0.5$), pink chords ($r < -0.5$). (B) qPCR analysis of *chrebp* and *s100a6* in HepG2 cells after ChREBP overexpression for 48hours and low (5.5mM) and high glucose (30mM) stimulation for 24hours. (C) qPCR analysis of ChREBP α and ChREBP β and S100A6 in HepG2 cells in presence of low (5.5mM) and high glucose (30mM) stimulation for 24hours and upon ChREBP knockdown in HepG2 cells. (D) Representative microscopic images depict the lipid droplet accumulation upon Ad-ChREBP treatment in primary hepatocytes. (E) *In vitro* ChREBP occupancy on ChORE site on *s100a6* promoter in HepG2 cells post high glucose stimulation. Values are expressed as mean $\pm$ sd * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (ANOVA followed by Bonferroni's Multiple Comparison).

Supplemental Information

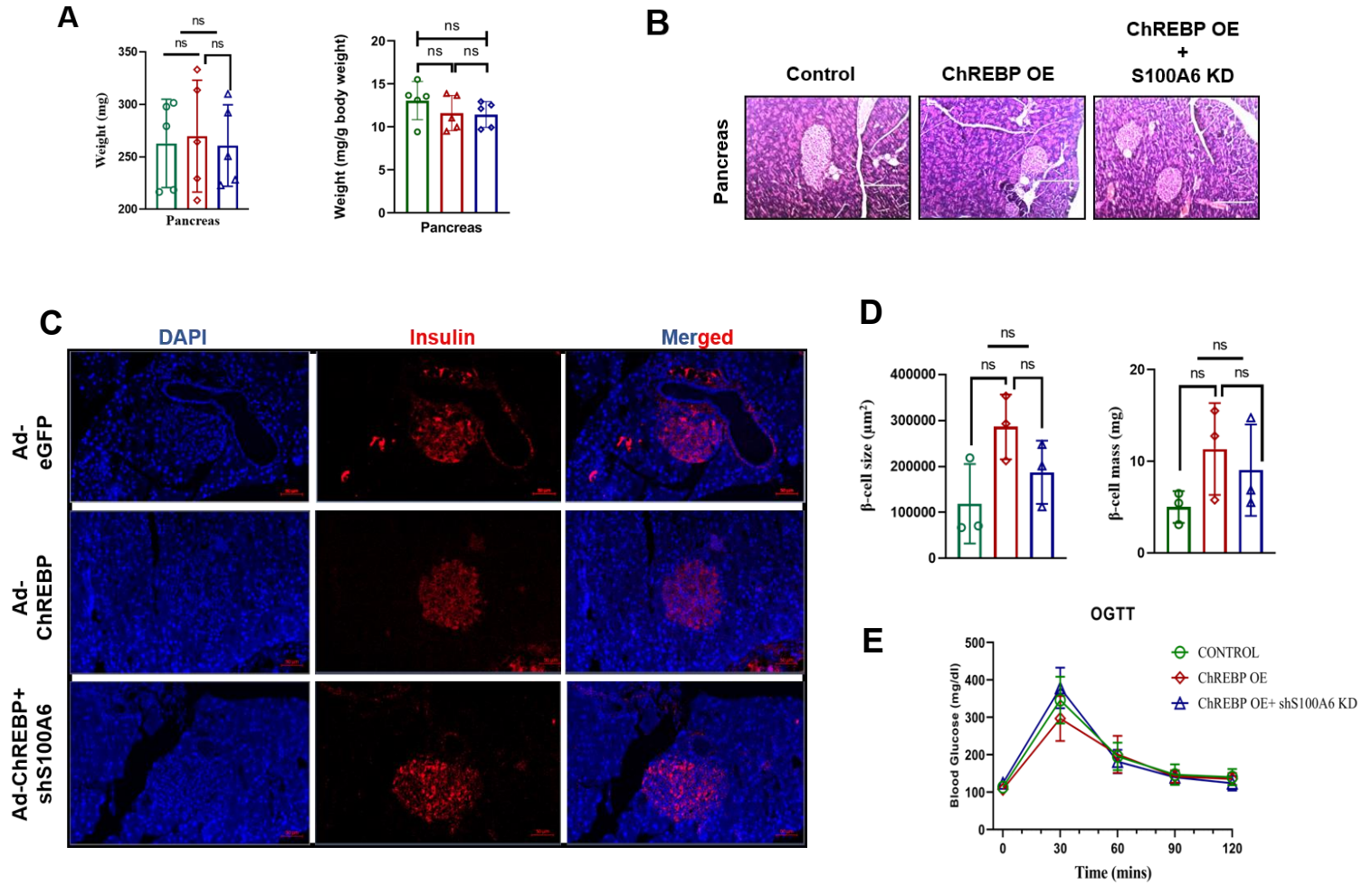
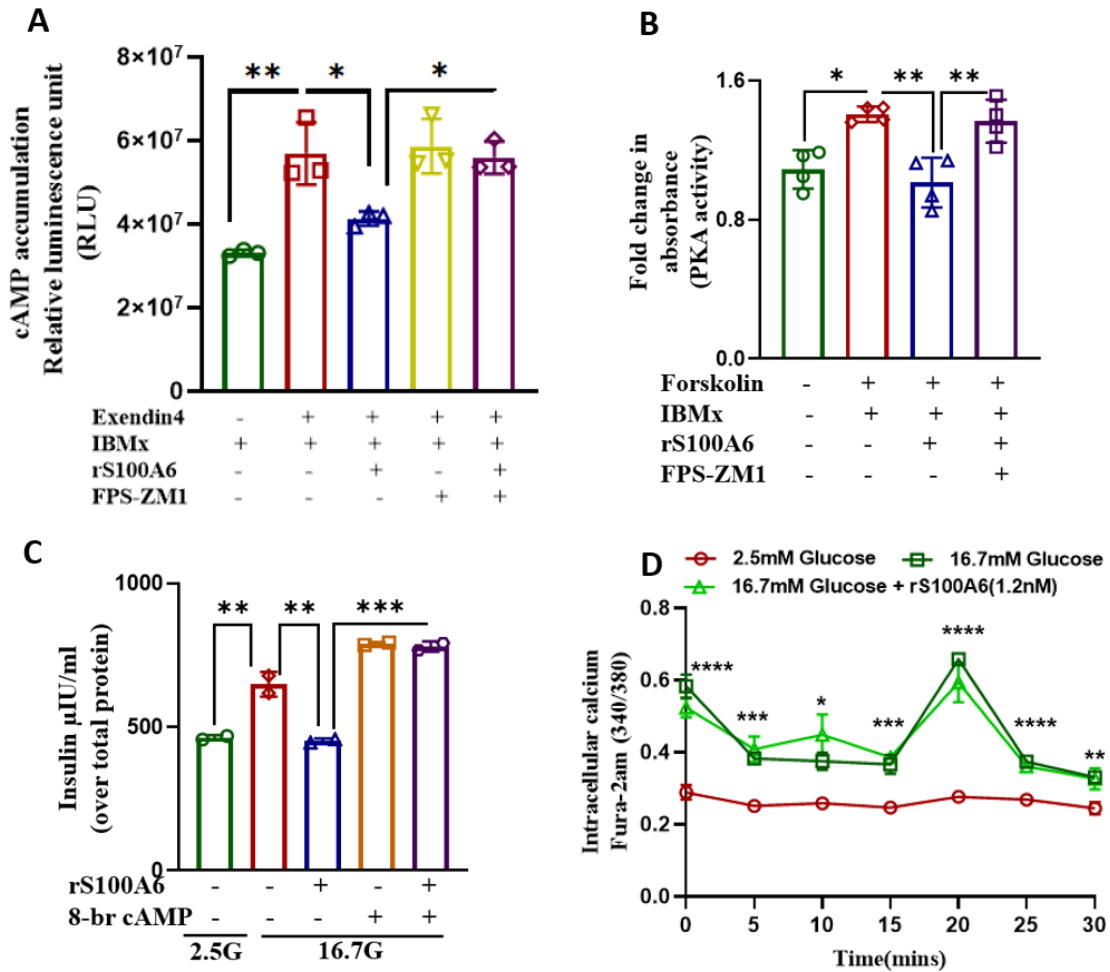


Figure S2. Effect S100A6 knockdown in Ad-ChREBP overexpression mice model on beta-cell morphometry (A) The graphs represent the pancreatic weight and normalized pancreas weight upon S100A6 knockdown in adenoviral mediated ChREBP overexpressed mice model. (B) H&E staining images of pancreas from control, Ad-ChREBP overexpression and S100A6 knockdown mice. (C) The microscopic images reveal insulin immunostaining in pancreatic beta cells (scale bar-50μm). (D) The graphs represent the beta-cell size and beta-cell mass upon adenoviral mediated ChREBP overexpression and S100A6 knockdown. (E) Plasma glucose levels during oGTT upon ChREBP overexpression and S100A6 knockdown. Values are expressed as mean±sd * $P < 0.05$, ** $P < 0.01$, ns-not significant, ** $P < 0.01$. (ANOVA followed by Bonferroni's Multiple Comparison).

Supplemental Information



FigS3- S100A6 suppresses cAMP synthesis and inhibits PKA activity

(A) cAMP synthesis in response to S100A6 and incretin analog exendin-4 in INS1 cells in the presence and absence of RAGE antagonist (FPS-ZM1) (B) PKA activity in response to S100A6 in the presence and absence of RAGE antagonist (FPS-ZM1) (C) GSIS analysis from INS1 cells post 8-bromo cAMP(100nM) and recombinant S100A6 protein treatment. (D) Intracellular calcium dynamics in high glucose induced INS1 cells upon recombinant S100A6 protein treatment using Fura-2AM. Values are expressed as mean $\pm$ sd, * P <0.05, ** P <0.01, **** P <0.0001. (ANOVA followed by Bonferroni's Multiple Comparison).

Supplemental Information

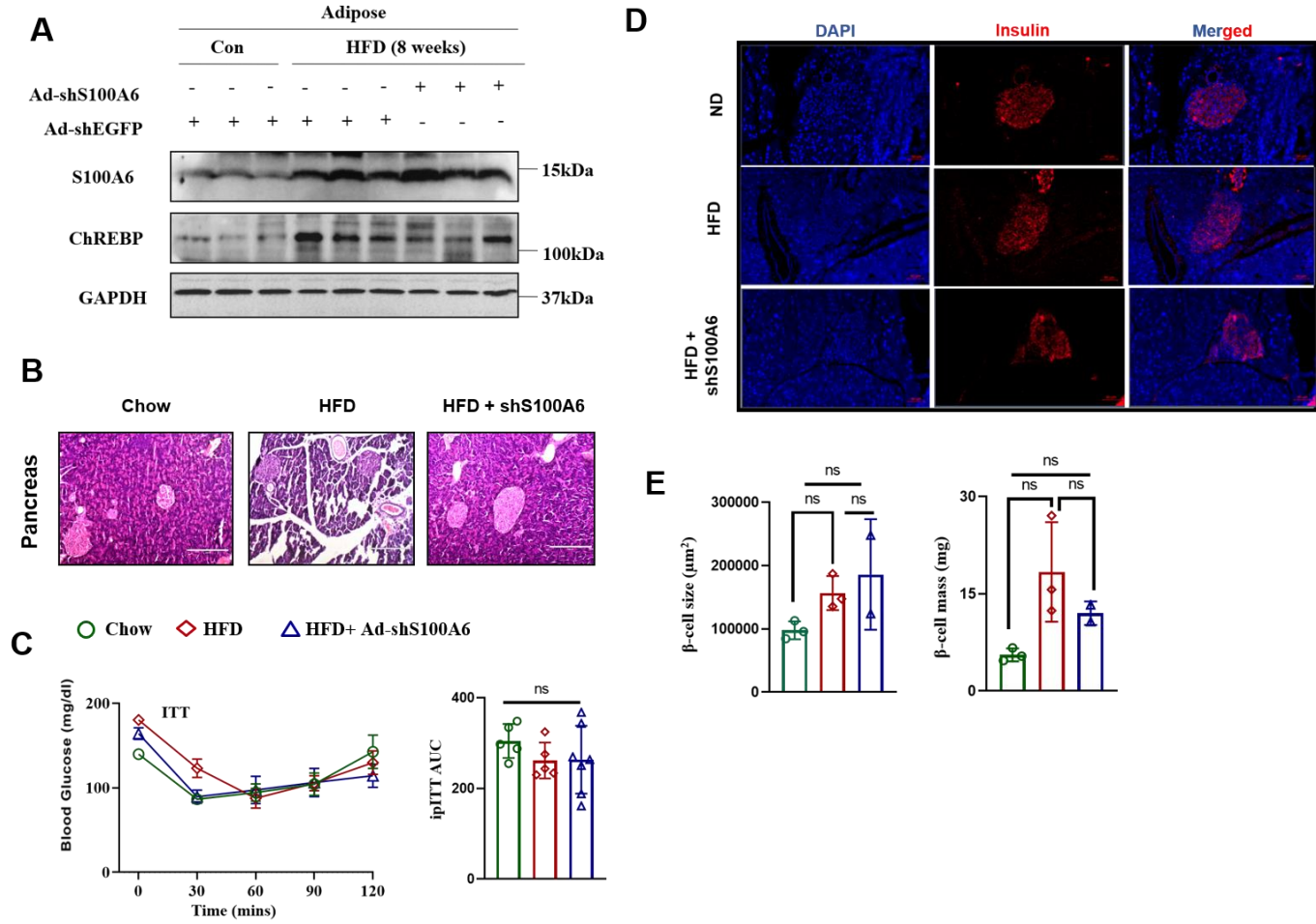


Figure S4. Effect of Hepatic S100A6 deficiency on beta-cell morphometry in HFD included NAFLD mice (A) Immunoblot analysis of ChREBP and S100A6 in adipose tissue from ND, HFD and HFD and HFD+shS100A6 treated groups. (B) H&E staining images of pancreas from chow diet mice, high fat diet fed mice and S100A6 knockdown in high fat diet mice. (C) The blood glucose levels during an ipITT in ND, HFD, HFD+shS100A6. AUCs for blood glucose levels as determined during ipITT. (D) The microscopic images reveal insulin immunostaining in pancreatic beta-cells (scale bar-50μm). (E) The graphs represent beta-cell size and beta-cell mass upon chow diet, HFD and S100A6 knockdown in HFD group. Values are expressed as mean±sd ns- not significant. (ANOVA followed by Bonferroni's Multiple Comparison).

Supplemental Information

S.No.	Accession	Description
1	P83916	Chromobox protein homolog 1 OS=Homo sapiens OX=9606 GN=CBX1 PE=1 SV=1
2	Q14011	Cold-inducible RNA-binding protein OS=Homo sapiens OX=9606 GN=CIRBP PE=1 SV=1
3	P06703	Protein S100-A6 OS=Homo sapiens OX=9606 GN=S100A6 PE=1 SV=1
4	P09038	Fibroblast growth factor 2 OS=Homo sapiens OX=9606 GN=FGF2 PE=1 SV=3
5	Q5IS69	Fibroblast growth factor 2 OS=Pan troglodytes OX=9598 GN=FGF2 PE=2 SV=2
6	Q15435	Protein phosphatase 1 regulatory subunit 7 OS=Homo sapiens OX=9606 GN=PPP1R7 PE=1 SV=1
7	P51649	Succinate-semialdehyde dehydrogenase, mitochondrial OS=Homo sapiens OX=9606 GN=ALDH5A1 PE=1 SV=2
8	O94979	Protein transport protein Sec31A OS=Homo sapiens OX=9606 GN=SEC31A PE=1 SV=3
9	Q13310	Polyadenylate-binding protein 4 OS=Homo sapiens OX=9606 GN=PABPC4 PE=1 SV=1
10	P02787	Serotransferrin OS=Homo sapiens OX=9606 GN=TF PE=1 SV=3
11	P35555	Fibrillin-1 OS=Homo sapiens OX=9606 GN=FBN1 PE=1 SV=4
12	O00232	26S proteasome non-ATPase regulatory subunit 12 OS=Homo sapiens OX=9606 GN=PSMD12 PE=1 SV=3
13	Q16531	DNA damage-binding protein 1 OS=Homo sapiens OX=9606 GN=DDB1 PE=1 SV=1
14	Q00169	Phosphatidylinositol transfer protein alpha isoform OS=Homo sapiens OX=9606 GN=PITPNA PE=1 SV=2
15	P01042	Kininogen-1 OS=Homo sapiens OX=9606 GN=KNG1 PE=1 SV=2
16	Q6FI81	Anamorsin OS=Homo sapiens OX=9606 GN=CIAPIN1 PE=1 SV=2
17	P49748	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial OS=Homo sapiens OX=9606 GN=ACADVL PE=1 SV=1
18	Q8IZP0	Abl interactor 1 OS=Homo sapiens OX=9606 GN=ABI1 PE=1 SV=4
19	O95361	Tripartite motif-containing protein 16 OS=Homo sapiens OX=9606 GN=TRIM16 PE=1 SV=3
20	Q9BUL8	Programmed cell death protein 10 OS=Homo sapiens OX=9606 GN=PDCD10 PE=1 SV=1
21	P50897	Palmitoyl-protein thioesterase 1 OS=Homo sapiens OX=9606 GN=PPT1 PE=1 SV=1
22	P35443	Thrombospondin-4 OS=Homo sapiens OX=9606 GN=THBS4 PE=1 SV=2
23	Q16186	Proteasomal ubiquitin receptor ADRM1 OS=Homo sapiens OX=9606 GN=ADRM1 PE=1 SV=2
24	Q9BYE2	Transmembrane protease serine 13 OS=Homo sapiens OX=9606 GN=TMPRSS13 PE=2 SV=5
25	P09497	Clathrin light chain B OS=Homo sapiens OX=9606 GN=CLTB PE=1 SV=1
26	P55265	Double-stranded RNA-specific adenosine deaminase OS=Homo sapiens OX=9606 GN=ADAR PE=1 SV=4

Table S5: Lis of upregulated proteins from ChREBP overexpressed HepG2 cells conditioned media

Supplemental Information

Chemicals:

S.No.	Chemical	Company	Cat. No.
1.	RNA Xpress Reagent	Himedia	MB601
2.	iScript cDNA synthesis kit	Biorad	170881
3.	iTaq Universal SYBR Green Supermix	Biorad	1725121
4.	Recombinant S100A6 protein	Elabscience	PKSH031347
5.	FPS-ZM1	Merck	553030
6.	Forskolin	Sigma	F3917
7.	IBMX	Sigma	I5879
8.	8-bromo cAMP	Sigma	B7880
9.	JC-1 dye	Thermo	T3168
10.	Cell Mito stress kit	Agilent Technologies	103010
11	Exendin-4	Sigma	E7144

Antibodies:

S.No.	Antibody	Company	Cat. No.
1.	ChREBP	CST	58069
2.	S100A6	CST & Abclonal	13162& A3461
3.	B-actin	Santa Cruz	SC-47778
4.	insulin	abcam	ab7842
5.	Alexa-647-anti-mouse IgG	Jackson ImmunoResearch	115-605-006

Primers:

Gene	Species	Sequence
ChREBP FP	Human	AGAGACAAGATCCGCCTGAA
ChREBP RP	Human	CTTCCAGTAGTTCCCTCCA
S100A6 FP	Human	AAGCTGCAGGATGCTGAAAT
S100A6 RP	Human	CCCTTGAGGGCTTCATTGTA
β -actin FP	Human	AGGCACCAGGGCGTGAT
β -actin RP	Human	GCCACATAGGAATCCTTCTGAC
S100A6 FP	Mouse	CAAGGAAGGTGACAAGCACA
S100A6 RP	Mouse	AAGGCGACATACTCCTGGAA
ChREBP FP	Mouse	CTGGGGACCTAAACAGGAGC
ChREBP RP	Mouse	GAAGCCACCCTATAGCTCCC
18S FP	Mouse	GCAATTATTCCCCATGAACG
18S RP	Mouse	GGCCTCACTAAACCATCCAA
S100A6 promoter FP	Human	CCCAAGCTTGGGGGGTGACCCTGAGTATCCTC
S100A6 promoter RP	Human	CCCAAGCTTGGGGTAGGGAGGCGGCCAAATGC
S100A6 promoter FP	Mouse	AGGATGTGGTAGACAGACTGCTT
S100A6 promoter RP	Mouse	GAACATCCCAGCCCCCTCAACTT

Supplemental Information

siRNA & shRNA

Gene	Species	Sequence
siRNA S100A6	Human	Sense- GCAGGAUGCCUGAAAUUGCAtt
		Antisense- UGCAAUUUCAGCAUCCUGCag
shRNA S100A6	Mouse	GGACCGTAACAAGGATCAGGA

Plasmids and Adenovirus

S.No.	Gene	Company & Cat. No.
1.	pcDNA-eGFP	Addgene-21073
2.	pcDNA-ChREBP	Addgene-39235
3.	pAd-eGFP	abm-000541A
4.	pAd-MLXIPL	abm-198553A