

Supplemental Data

***miR-210-3p* promotes obesity-induced adipose tissue inflammation and insulin resistance by targeting SOCS1 mediated NF- κ B pathway**

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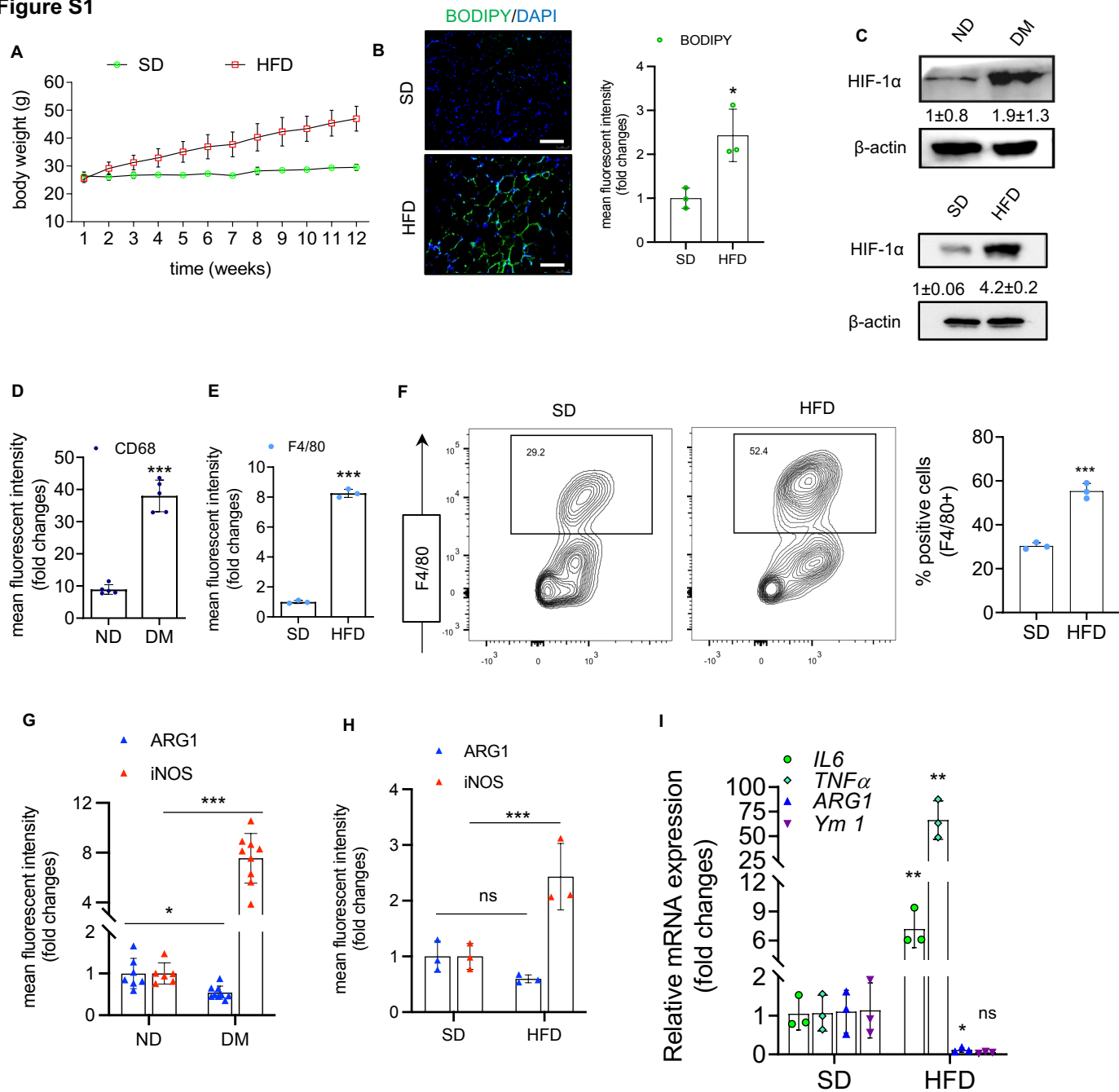
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Figure S1**Figure S1**

(A) Body weight of C57BL/6 mice fed with 10%kCal fat diet (SD) or 60%kCal fat diet (HFD) for 12 weeks and body weight was measured. (B) Immunofluorescence images showing BODIPY staining and quantification of mean fluorescence intensity in the adipose tissue section of SD and HFD mice. scale bar, 150μM. (C) HIF-1α protein expression was analyzed in the SVF of control (ND) and obese diabetic (DM) patients VAT and in ATMs of SD and HFD mice VAT. (D) Immunohistochemical quantification analyses of CD68 in control and obese diabetic VAT. (E) Fluorescent intensity quantification analyses of F4/80 expression in SD and HFD VAT. Scale bar, 100μM. (F) Flow cytometry analyses of F4/80+ macrophages in SVF of SD and HFD VAT. (G,H) Immunohistochemical quantification analyses of iNOS and ARG1 in non-diabetic and diabetic VAT (G); in SD and HFD mice VAT (H). (I) qRT-PCR analyses showed the expression of inflammatory cytokines (*IL6*, *TNF-α*, *ARG1*, *Ym1*) in F4/80+ ATMs from SD and HFD mice. Data are expressed as mean ± SD; human sample (n=7-9) and mice sample (n=3); *p<0.05 **p<0.01 ***p<0.001 or ns (Student's t-test).

Figure S2

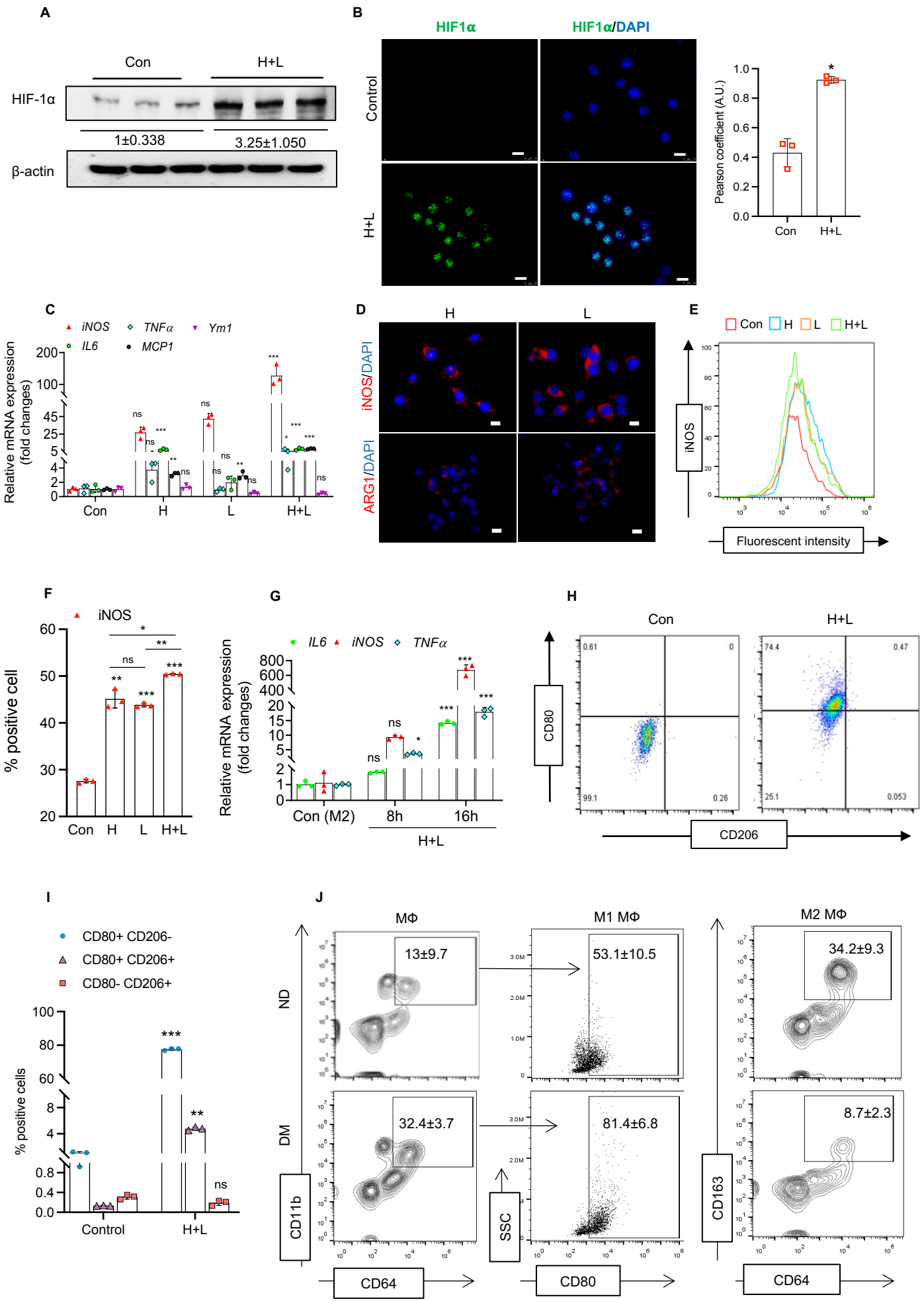


Figure S2

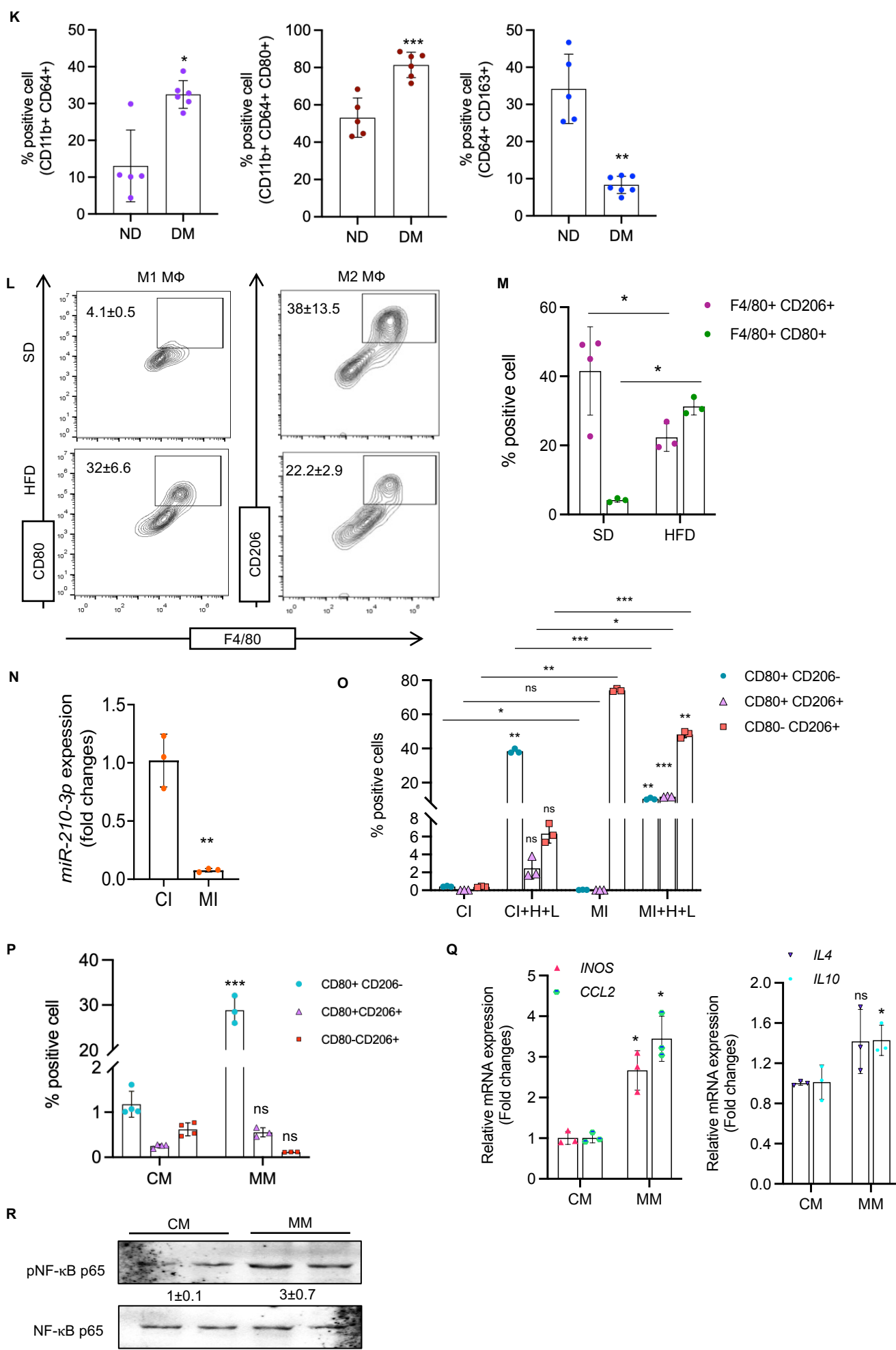
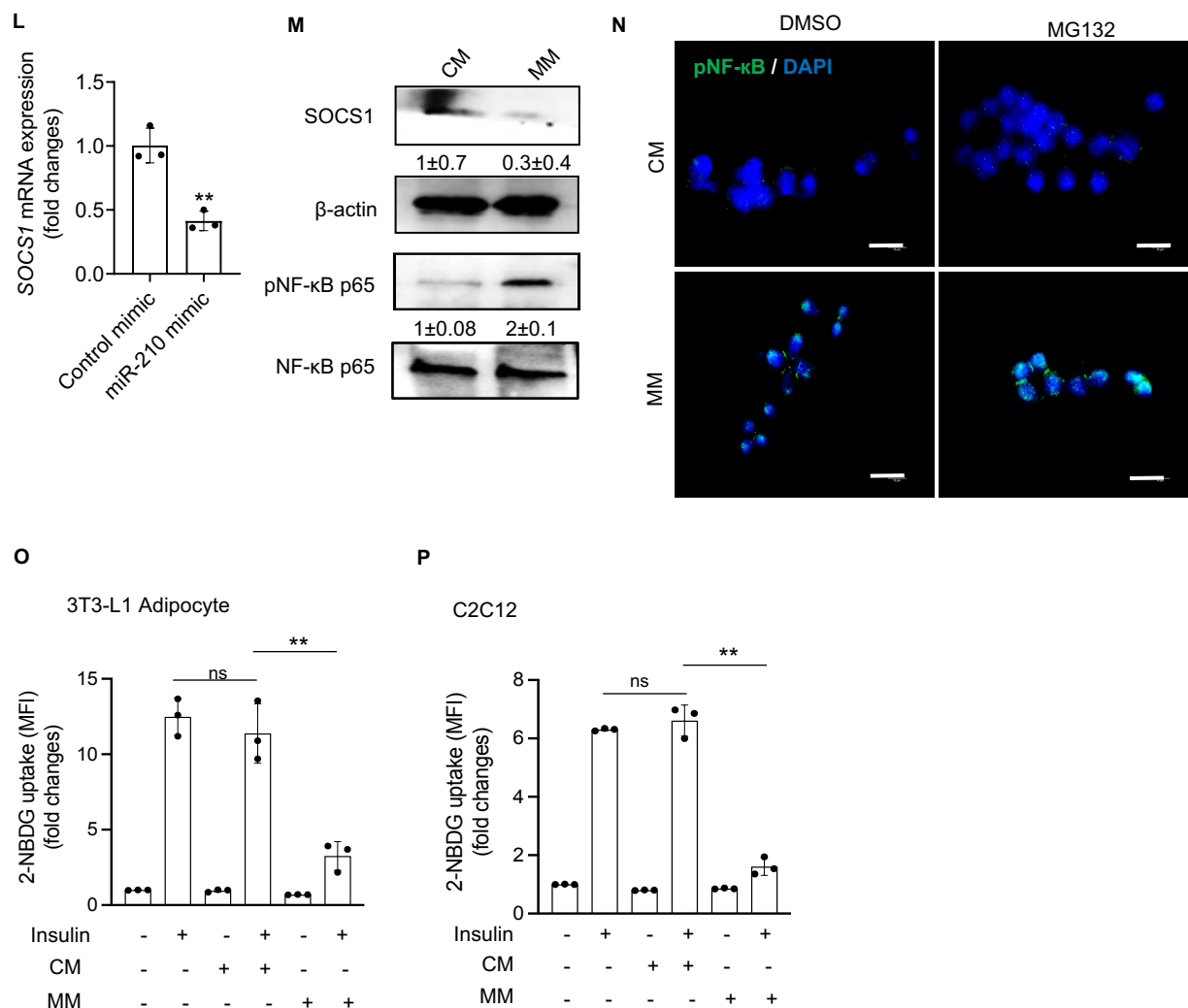


Figure S2

(A) Immunoblot analyses showed HIF-1 α expression in RAW26.7 macrophage co-treated with or without hypoxia and lipid. (B) Immunofluorescence monograph depicts HIF-1 α (green) expression and nuclear localization in RAW 264.7 macrophages in the presence or absence of hypoxia and lipid. Colocalization quantification was performed using pearson coefficient. scale bar size, 10 μ M. (C) mRNA expression analysis of pro-inflammatory (*IL6*, *iNOS*, *TNFa*, *MCP1*) and anti-inflammatory cytokine (*Ym1*) in RAW264.7 macrophages under different conditions. (D) Immunofluorescence analyses of *iNOS* and *ARG1* in RAW 264.7 macrophages exposed to hypoxia or lipid individually. scale bar size, 10 μ M. (E-F) Flow cytometry analysis for *iNOS* in macrophage cells under different conditions and quantification analyses. (G) Quantitative RT-PCR analyses of proinflammatory cytokines (*iNOS*, *TNFa*, *IL6*) in mouse macrophage differentiated into M2 phenotype then co-incubated with hypoxia and lipid for different time periods. (H-I) Flow cytometry analysis of CD80⁺ and CD206⁺ expression in RAW264.7 macrophages under hypoxia and lipid co-exposure (H); quantification data (I). (J-K) Flow cytometry analysis for CD11b⁺/CD64⁺, CD80⁺, CD64⁺/CD163⁺ positive cells from single cell suspension of stromal vascular fraction isolated from visceral adipose tissue of control (ND) and obese diabetic patients (DM) and quantification data (K). (L-M) Flow cytometry analysis for F4/80⁺/CD80⁺, F4/80⁺/CD206⁺ positive cells from single cell suspension of stromal vascular fraction isolated from visceral adipose tissue of SD and HFD mice; Quantification data (M). (N) qRT-PCR analyses of *miR-210-3p* expression in murine macrophages transfected with control inhibitor (CI) and *miR-210-3p* inhibitor (MI). (O) FACS quantification of CD80⁺, CD206⁺ and CD80⁺CD206⁺ cells in control inhibitor (CI) and *miR-210-3p* inhibitor (MI) transfected macrophages co-incubated with or without hypoxia and lipid. (P) FACS quantification of CD80⁺, CD206⁺ and CD80⁺CD206⁺ cells in control mimic (CM) and *miR-210-3p* mimic (MM) transfected macrophages. (Q) qRT-PCR analyses showed relative mRNA expression of proinflammatory genes *iNOS*, *MCP1* and anti-inflammatory genes *IL4*, *IL10* in adipocytes transfected control mimic (CM) and *miR-210-3p* mimic MM. (R) Western blot analyses showed expression of phosphorylated NF- κ B in whole cell lysate from adipocytes transfected with control mimic (CM) and *miR-210-3p* mimic (MM). Data are expressed as means \pm SD; human sample (n=5-7), mice sample(n=3), cell culture(n=3-4) *p<0.05, **p<0.01, ***p<0.001 or ns (Student's t-test or two-way ANOVA).

Figure S3**Figure S3**

(A-B) Database (*microRNA.org*) searching result showing the interaction of *miR-210-3p* with human *SOCS1* mRNA (B) and *RNAhybrid* webserver was used to predict the minimum free energy (mfe) of different mouse *SOCS1* mRNA transcripts and *miR-210-3p* interactions (B). (C) Phosphorylated NF-κB expression was quantified by performing western blot analyses in murine macrophages co-exposed to hypoxia and lipid for different time periods. (D) Western blot analyses showed expression of phosphorylated NF-κB p65 in nuclear fraction and cytoplasmic fraction of RAW264.7 macrophages, co-incubated with hypoxia and lipid. LPS exposure counted as positive control for NF-κB activation. (E) Immunofluorescence analyses and mean fluorescence intensity quantification of phospho NF-κB p65 in hypoxia and lipid combined exposed macrophages. DAPI was used for nuclear staining. Scale bar size, 10μM. (F-G) mRNA expression analysis (F) and western blot analyses (G) showed reduced expression of *SOCS1* in *SOCS1* siRNA transfected macrophages. (H) Immunoblot analyses of phosphorylated NF-κB p65 in control siRNA or *SOCS1* siRNA transfected macrophage co-incubated with or without hypoxia and lipid. (I) Rel A (p65 subunit of NF-κB) interacting partner in EMBL INACT server. (J) Overlapping pie diagram showed selected negative regulators of NF-κB p65 and *miR-210-3p* target molecule as per the miRWALK database search. (K) qRT-PCR analyses of *MYOCD*, *CUL2*, *COMMD1*, *NF-κB1B* and *SOCS1* in control mimic and *miR-210-3p* mimic transfected macrophages. (L-M) Control mimic and *miR-210-3p* mimic transfected macrophages are considered for mRNA expression analyses of *SOCS1* (L) and protein expression of *SOCS1*, phospho NF-κB p65 (M). (N) Immunofluorescence analyses of phosphorylated NF-κB p65 in control mimic and *miR-210-3p* mimic transfected murine macrophages treated with or without MG132. DMSO was used as vehicle control. (O-P) Glucose uptake assay was performed in CM and MM transfected adipocytes (O), and C2C12 (P). Data are expressed as mean ± SD; sample (n=3); *p<0.05 **p<0.01 ***p<0.001 or ns (Student's t-test).

Figure S4

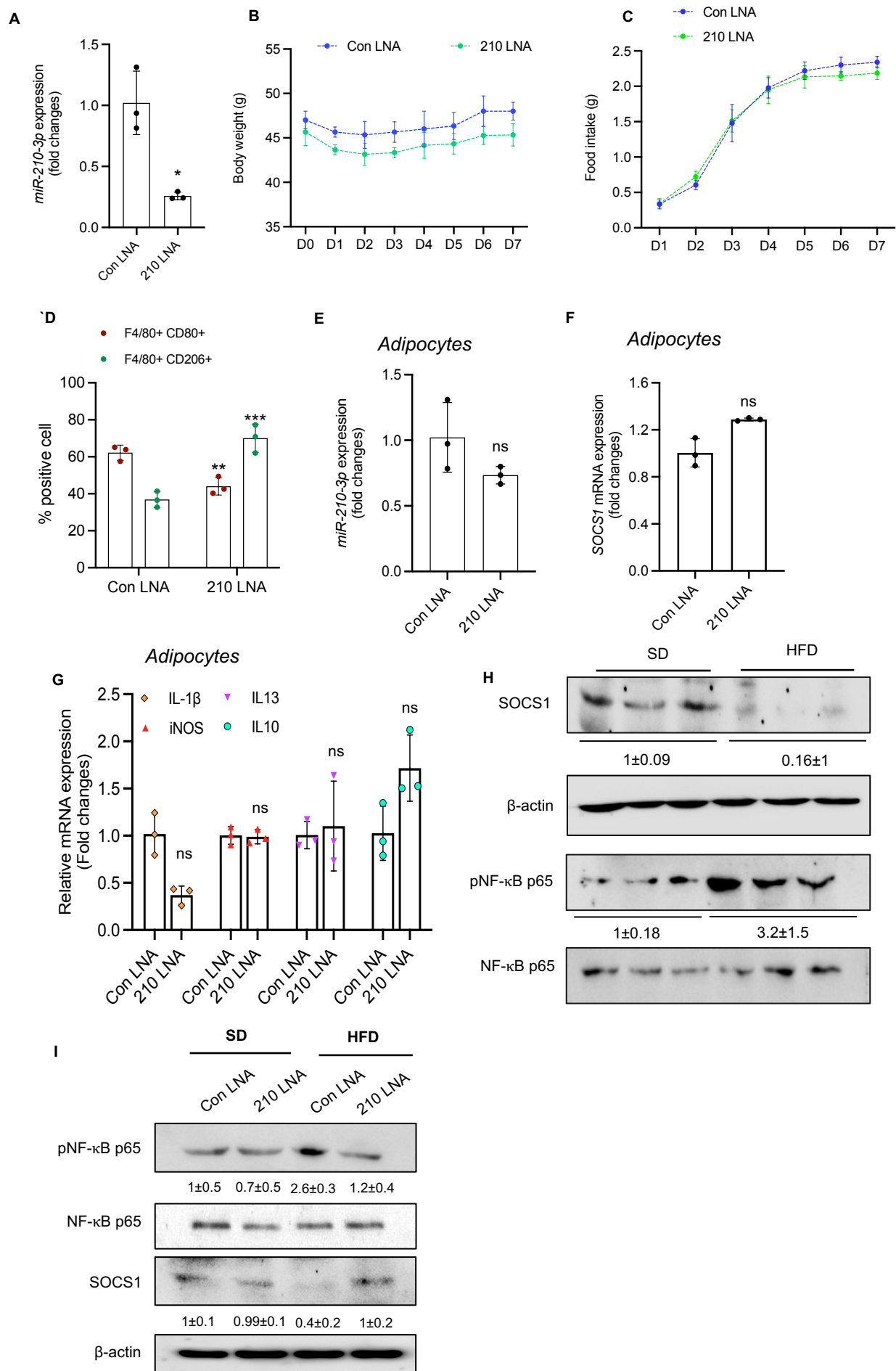


Figure S4

(A) qRT-PCR analyses showed *miR-210-3p* expression in ATMs isolated from control inhibitor LNA and *miR-210-3p* inhibitor LNA injected adipose tissue. (B-C) Body weight (B) and daily food intake data (C) in HFD mice undergo surgical procedures to administer control inhibitor LNA and anti-*miR-210-3p* LNA. (D) FACS quantification of F4/80⁺CD206⁺ and F4/80⁺CD80⁺ adipose tissue macrophage population in SVF isolated from VAT of control LNA and anti-*miR-210-3p* LNA administered HFD mice. (E-G) RT-qPCR analyses showed *miR-210-3p*(E), *SOCS1*(F), inflammatory cytokines *IL-1 β* , *iNOS*, *IL13*, and *IL10* (G) mRNA expression in adipocytes isolated from control inhibitor LNA and anti-*miR-210-3p* LNA injected adipose tissue of HFD mice. (H) Western blot analyses of *SOCS1* and phospho NF- κ B p65 in ATMs isolated from SD and HFD visceral adipose tissue. (I) Immunoblot analyses of phospho NF- κ B p65 and *SOCS1* in ATMs isolated from control inhibitor LNA and *miR-210-3p* inhibitor LNA injected adipose tissue of SD and HFD groups. Data are expressed as mean \pm SD; sample (n=3); *p<0.05 **p<0.01 ***p<0.001 or ns (Student's t-test).

Table S1: Patient demographic details:

Pathological features	
Non-obese non-diabetic patients:	
Number of patients	7
Age (median range)	42.4 ± 11.5 (19-56 years)
Gender	Male (n=2)
	Female (n=5)
BMI (median range)	24.9 ± 1.9 (22.2 - 27.4)
Fasting serum glucose (mmol/L)	3.55±0.76
Any other disease (Cancer and NAFLD)	No
Obese diabetic patients:	
Number of patients	10
Age (median range)	44.3 ± 11.9 (22-59 years)
Gender	Male (n=5)
	Female (n=5)
BMI (median range)	35 ± 3.7 (32.2 - 44)
Fasting serum glucose (mmol/L)	6.26±0.94
Any other disease (Cancer and NAFLD)	No

Table S2: List of antibodies and their details

Sl. No.	Antibody	Dilution	Company	Catalog No.
1	Phospho-NF-κBp65 (S-536)	1:1000 for ICC 1:2000 for WB	Abcam	#ab86299
2	SOCS1	1:100 for IHC 1:1000 for WB	Invitrogen	#38-5200
3	iNOS	1:400 for ICC 1:400 for IHC 1:1500 for FC	Cell Signaling Technology	#13120
4	Arginase 1	1:50 for ICC 1:400 for IHC	Cell Signaling Technology	#93668
5	Anti-Mouse IgG (Alexa Fluor 488 conjugated)	1:1000 for ICC 1:1000 for IHC	Cell Signaling Technology	#4408
6	Anti-Mouse IgG (Alexa Fluor 594 conjugated)	2µg/ml for ICC	Invitrogen	#A-11032
7	Anti-Rabbit IgG (Alexa Fluor 488 conjugated)	5µg/ml for ICC 1:500 for IHC	Invitrogen	#A-11034
8	Anti-Rabbit IgG (Alexa Fluor 568 conjugated)	2µg/ml for ICC 2µg/ml for IHC	Invitrogen	#A-1101
9	HIF1α	10µg/ml for IHC	Abcam	#ab16066
10	F4/80	1:50 for IHC	Santa Cruz Biotechnology	#sc-377009
11	CD68	1µg/ml for IHC	Abcam	#ab125212
12	PE anti-mouse CD80	0.5µg/million cells for FC	BioLegend	#104707
13	FITC anti-mouse CD80	1µg/million cells for FC	BioLegend	#104705
14	APC anti-mouse CD206 (MMR)	0.5µg/million cells for FC	BioLegend	#141707
15	PE anti-mouse F4/80	1µg/million cells for FC	BioLegend	#123110
16	PE anti-human CD80	5µl/million cells for FC	BioLegend	#305207
17	APC anti-human CD163	5µl/million cells for FC	BioLegend	#326510
18	FITC anti-human CD64	5µl/million cells for FC	BioLegend	#305006
19	APC anti-mouse/human CD11b	0.25µg/million cells for FC	BioLegend	#101212
20	PE anti-human CD68 Antibody	5µl/million cells for FC	BioLegend	#333807
21	TruStainFcX™ (anti-mouse CD16/32)	0.1µg/million cells for FC	BioLegend	#101319
22	NF-κB p65	2µg/ml for WB	Invitrogen	#PA1-186
23	HRP conjugated Anti-Mouse IgG antibody	1:20000 for WB	Sigma-Aldrich	#A9044
24	HRP conjugated Anti-Rabbit IgG antibody	1:20000 for WB	Sigma-Aldrich	#A9169
25	β-actin	1:1000 for WB	Invitrogen	#AM4302

Table S3: List of primers, antisense oligos (ASOs) and their sequences

	Mouse Primers		
	Gene	F (5'-3')	R (5'-3')
1	SOCS1	CAACGGAACTGCTTCTTCGC	AGCTCGAAAAGGCAGTCGAA
2	IL1 β	TGCCACCTTTTGACAGTGATG	GAAGGTCCACGGGAAAGACA
3	CD163	TGCTCAGGAAACCAATCCCA	ACCTCCACTCTTCCAGCG
4	CD206	TTCAGCTATTGGACGCGAGG	GAATCTGACACCCAGCGGAA
5	CD86	CTGTAGGCAGCACGGACTTG	CATGGTGCATCTGGGGTCCAT
6	CD200R1	TCAGTGGCTTCAGAAAATGCAA	GTGCCATTGACTTCGCCTTG
7	FPR2	GAGACCTCAGCTGGTTGTGC	CATCCGGAATCCAGCTACCC
8	MMP9	CCGACTTTTGTGGTCTTCCC	TTTGAATCGACCCACGTCT
9	CCR2	AGGAGCCATACCTGTAAATGC	GGCAGGATCCAAGCTCCAAT
10	Ym1	GTTTGGACCTGCCCCGTTT	CCTTGAATGTCTTTCTCCACAG
11	MHC-IIa	GAAGACGACATTGAGGCCGA	GGAACACAGTCGCTTGAGGA
12	IL13	GTATGGAGTGTGGACCTGGC	CTCTGGGTCCTGTAGATGGC
13	IL10	CTCGAATGTACCAGGAGCCA	AGGACGTTTGGCACATCCAT
14	IL4	GCATGGCCCAGAAATCAAGG	GAGAAATCGATGACAGCGCC
15	iNOS	CTTGGTGAAGGGACTGAGCTG	CGTTCTCCGTTCTCTTGCAGT
16	CCL2	GATGCAGTTAACGCCCCACT	AGCTTCTTTGGGACACCTGC
17	Arg1	ACATTGGCTTGCGAGACGTA	ATCACCTTGCCAATCCCCAG
18	TNF α	CGCTGAGGTCAATCTGCCCAAG	GGTCAGAGTAAAGGGGTCAGAGTGG
19	β -Actin	GTA CTCTGTGTGGATCGGTGG	AGGGTGTA AACGCAGCTCAG
20	IL6	GGGACTGATGCTGGTGACAA	ACAGGTCTGTTGGGAGTGGT
21	CCL5	CTGCCTCCCCATATTCCTCG	TCGGGTGACAAAGACGACTG
22	CXCL5	TAAAAGGGGTGCAGTGGGTT	GAGCACCAGCTCGGGATATG
23	CD64	TCTGCTACTTTGGGTTCCAGTC	ATGTGTAGCGGTGTCTTCCC
24	MYOCD	ATGACATCAGCCAGGAACGC	GGACCTTTCAGTGGCGGTATT
25	CUL2	CCTGGACTGGACCATTGCATC	AAGTTTAGTGTTGAAACCTGCTCC
26	NF-kBIB	GACCTCAATAAACCGGAGCCT	ACCAAGCCTGAGAGAAGCCT
27	COMMD1	CTGCACAGCCAACTCTATCC	ACACAAAAATTGAGATTCTGTCCA
28	GLUT1	GCTGTGCTTATGGGCTTCTC	CACAAGTCTGCATTGCCCAT

(F: Forward primer; R: Reverse primer)

Human Primers			
	Gene	F (5'-3')	R (5'-3')
1	SOCS1	CACTTCCGCACATTCCGTTC	AGGCCATCTTCACGCTAAGG
2	β-actin	GAGCACAGAGCCTCGCCTTT	ACATGCCGGAGCCGTTGTC

SOCS1 3' UTR primer details			
	Gene	F (5'-3')	R (5'-3')
1	SOCS1 (mut-1)	TAATGCTGCGTGCGGGCGCTGCCG	CGGCAGCGCCCGCACGCAGCATTA
2	SOCS1 (mut-2)	GCCCGCCGTGCACGATTA ACTGGGAT GC	CATCCCAGTTAATCGTGACAGGCGGGC

microRNA Primers				
		Sequences	Assay ID	Accession No.
1	U6 snRNA	GTGCTCGCTTCGGCAGCACATATACTAA ATTGGAACGATACAGAGAAGATTAGCAT GGCCCCTGCGCAAGGATGACACGCAAAT TCGTGAAGCGTTCCATATTTT	001973	NR_004394
2	miR-210-3p	CUGUGCGUGUGACAGCGGCUGA	000512	MI0000286

(F: Forward primer; R: Reverse primer)