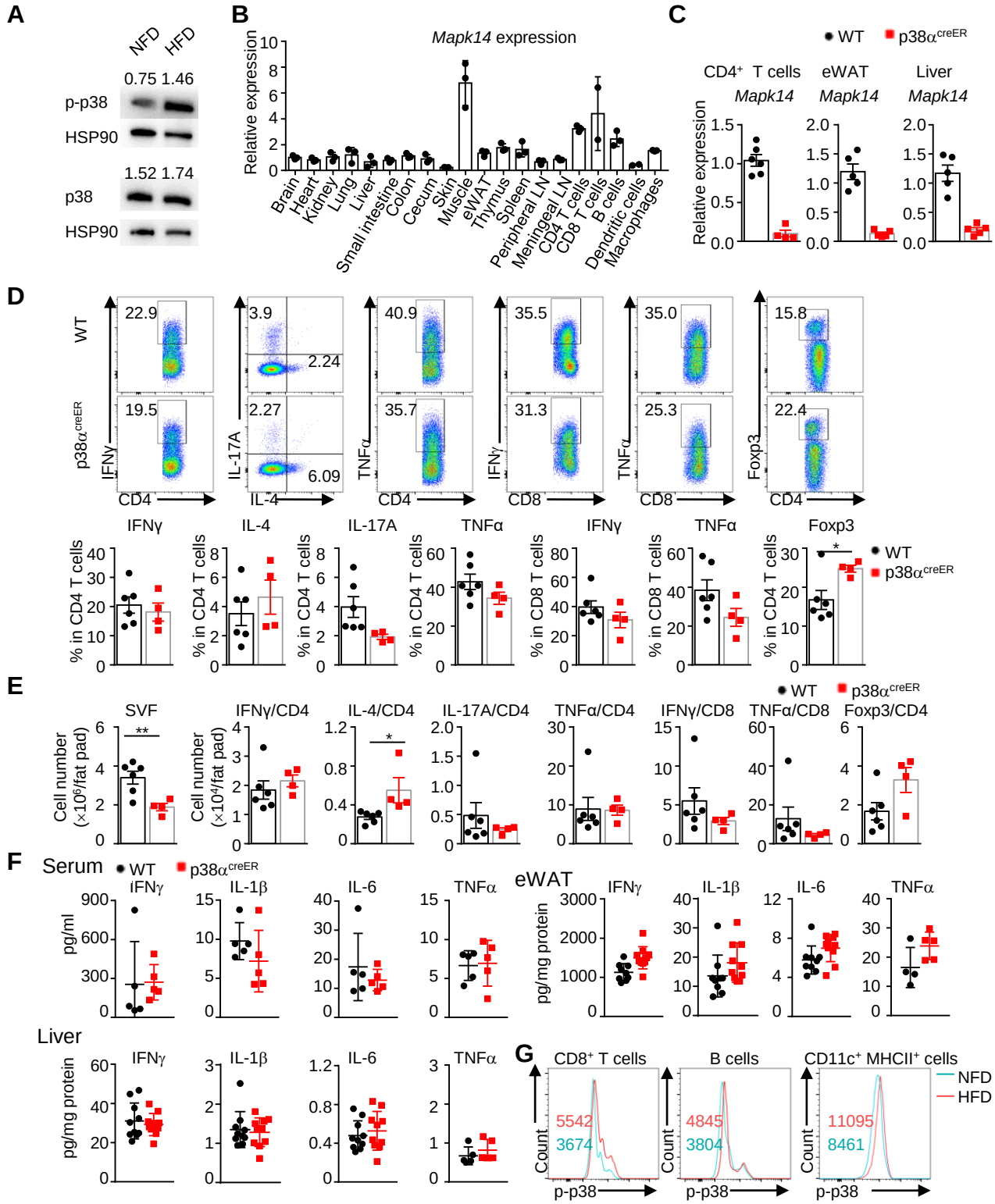
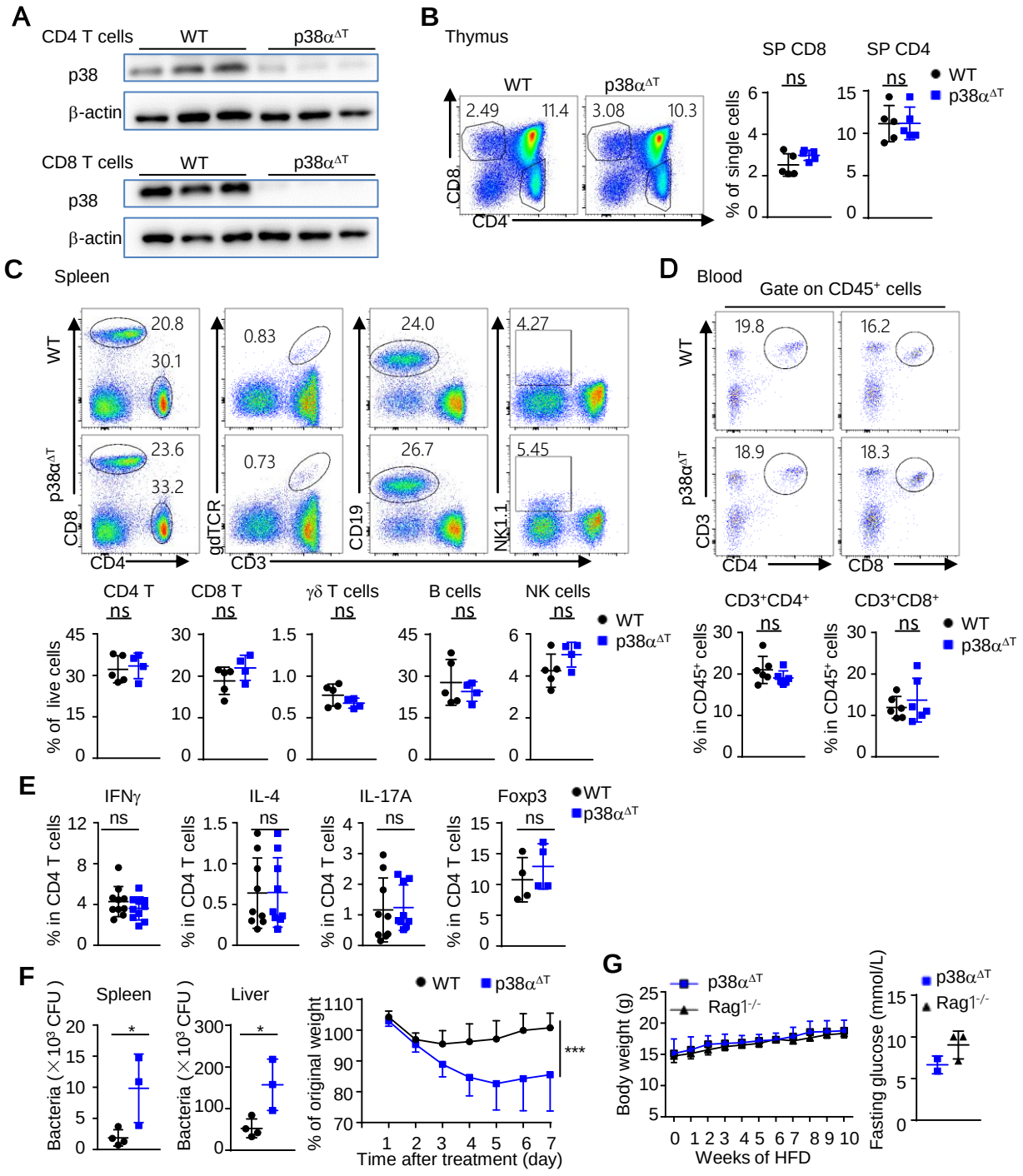
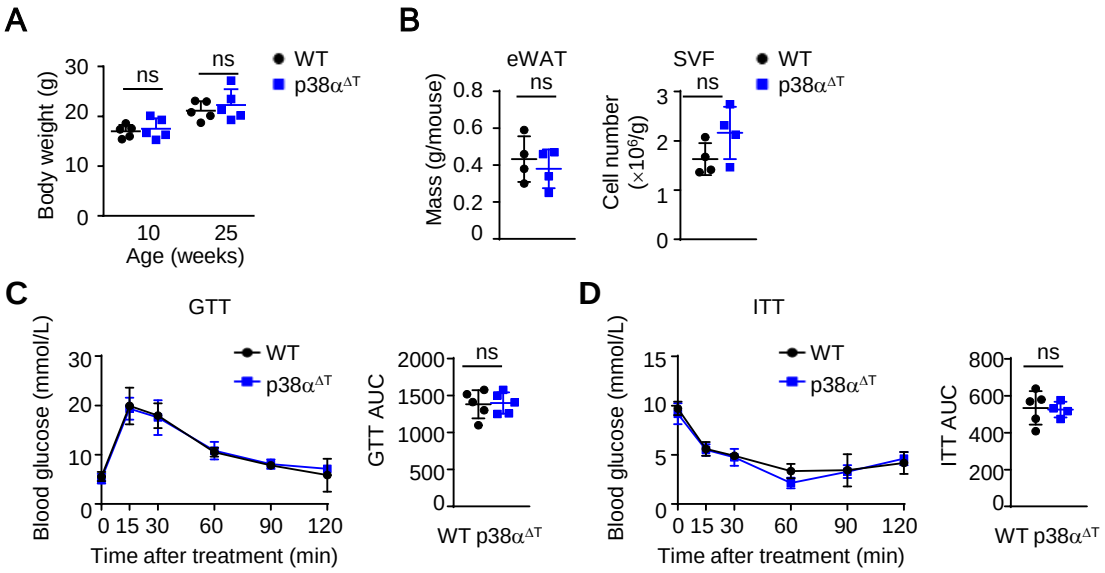


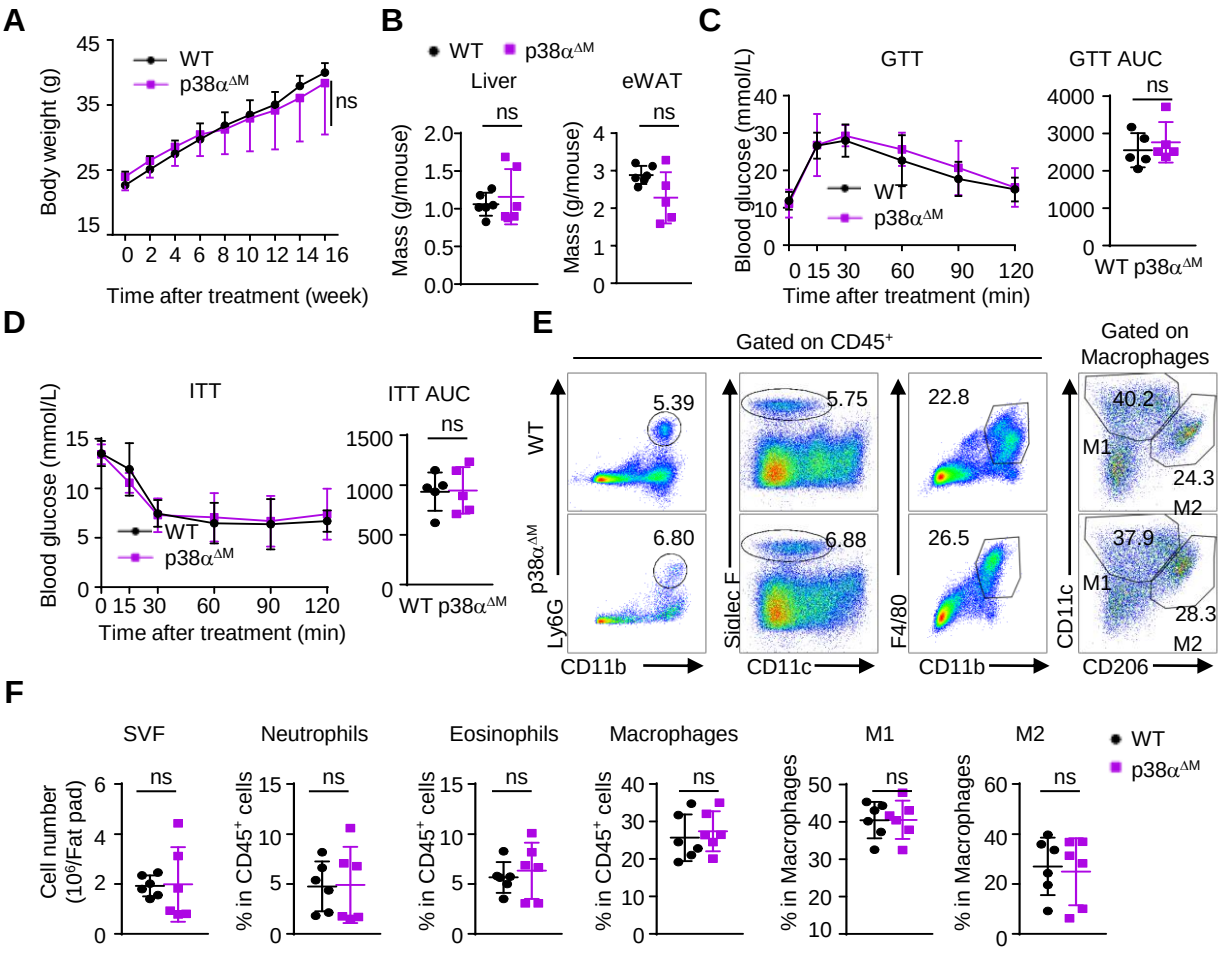
Supplementary Figure 1 – p38α expression and the effect of p38α on DIO. (A) Immunoblots of p38MAPK activity in eWAT from 16 week-NFD or HFD mice. (B) Real-time PCR analysis of *Mapk14* expression pattern in the tissues and immune cells. (C) Real-time PCR analysis of *Mapk14* expression in splenic CD4$^+$ T cells, eWAT and liver in WT and p38α^{creER} male mice treated with Tomaxifen. (D) Flow cytometry analysis of T cell subsets in the eWAT from obese WT and p38α^{creER} male mice treated with Tomaxifen. (E) Cell number analysis of infiltrating cells and immune subsets in the eWAT from obese WT and p38α^{creER} mice treated with Tomaxifen. (F) IFNγ, IL-1β, IL-6 and TNFα production in the serum, eWAT and Liver homogenates of obese WT and p38α^{creER} male mice treated with Tomaxifen were measured by ELISA. (G) Flow cytometric analysis of p-p38 in CD8$^+$ T cells, B cells and CD11c$^+$MHCII$^+$ cells in the eWAT of mice fed with NFD or HFD for 12 weeks. Data are mean ± SD. *$p < 0.05$, **$p < 0.01$. ns, not statistically significant.



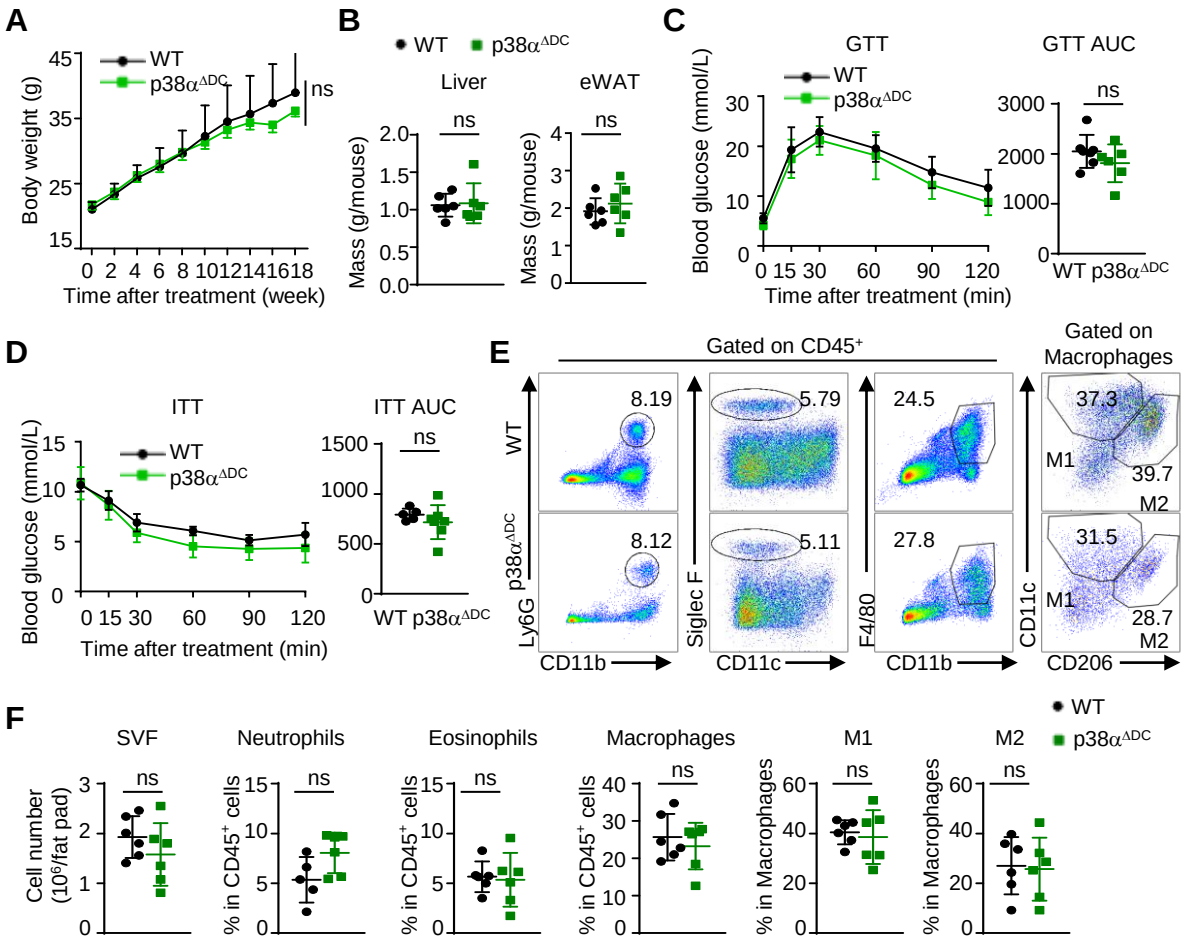
Supplementary Figure 2 – p38α in T cells is dispensable for immune homeostasis but required for defense against infection. (A) Western blot analysis of p38 in CD4$^+$ T cells and CD8$^+$ T cells isolated from the spleen of 6-8-week-old WT and p38α$^{\Delta T}$ mice. (B) Flow cytometry analysis of CD4$^+$ and CD8$^+$ cells in the thymus of 6-8-week-old WT and p38α$^{\Delta T}$ mice. (C) Flow cytometry analysis of the immune subsets in the spleen of 6-8-week-old WT and p38α$^{\Delta T}$ mice. (D) Flow cytometry analysis of CD4$^+$ and CD8$^+$ T cells in blood of 6-8-week-old WT and p38α$^{\Delta T}$ mice. (E) The percentages of Th1, Th2, Th17 and Tregs in spleen of WT and p38α$^{\Delta T}$ mice fed with NFD. (F) WT and p38α$^{\Delta T}$ mice were infected with 3×10^5 *Listeria monocytogenes* for 7 days, and the bacterial loads (colony forming units, CFU, right) and body weights (left) were measured (n=4,one p38α$^{\Delta T}$ mice died at day 6). (G) 5-week old Female p38α$^{\Delta T}$ and Rag1$^{-/-}$ mice were fed with HFD for 10 weeks, body weight (right) and fasting glucose (left) were measured. Data are mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ns, not statistically significant.



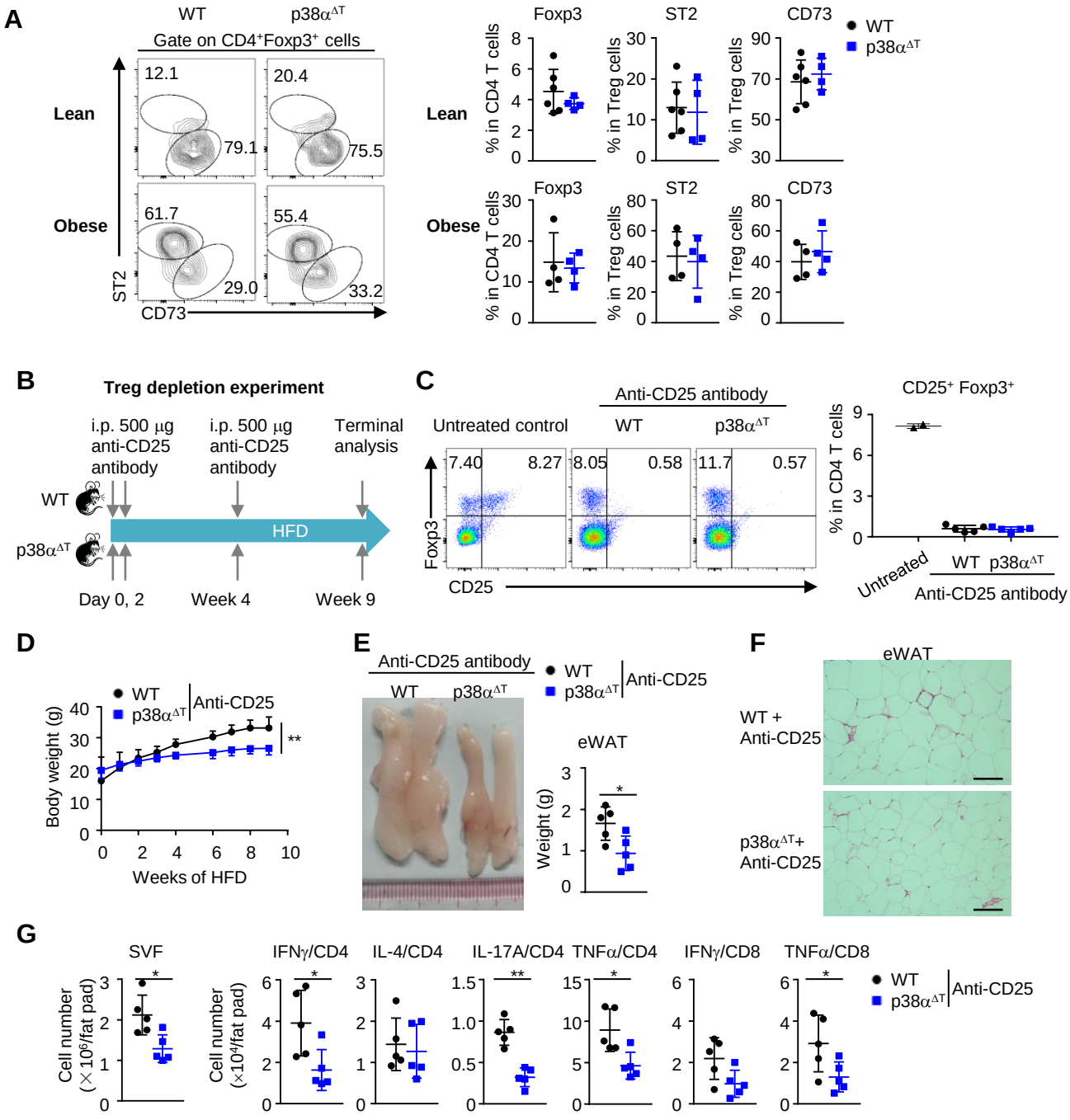
Supplementary Figure 3 – p38α deletion in T cells does not affect body weight and glucose regulation under normal diet treatment. (A) Body weight of NFD-fed female WT and p38$\alpha^{\Delta T}$ mice at 10 and 25 weeks old. (B) eWAT mass and SVF cell number in NFD-fed female WT and p38$\alpha^{\Delta T}$ mice. (C) GTT and (D) ITT were performed in NFD-fed female WT and p38$\alpha^{\Delta T}$ mice. Data are mean ± SD. ns, not statistically significant.



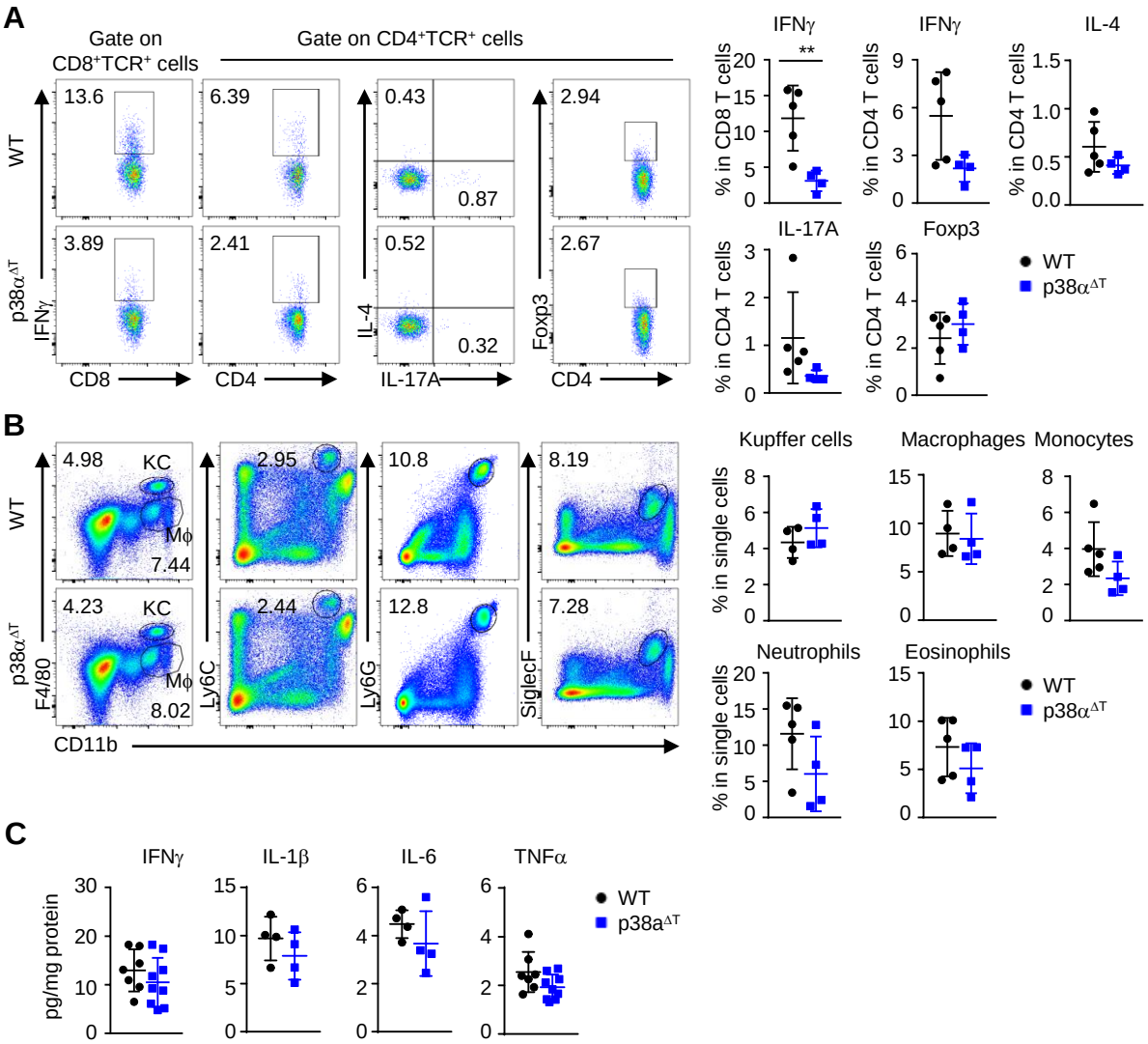
Supplementary Figure 4 – p38α in macrophages is dispensable for DIO. WT and p38α$^{\Delta M}$ male mice were fed with HFD for 16 weeks. Body weight (A) Liver and eWAT mass were measured (B). GTT (C) and ITT (D) were performed at 15 weeks. Flow cytometric analysis of infiltrated myeloid cells in the eWAT (E). Quantification of SVF cell number and myeloid cell percentages (F). Data are representative of two independent experiments with five to six mice per group. Data are mean ± SD. ns, not statistically significant.



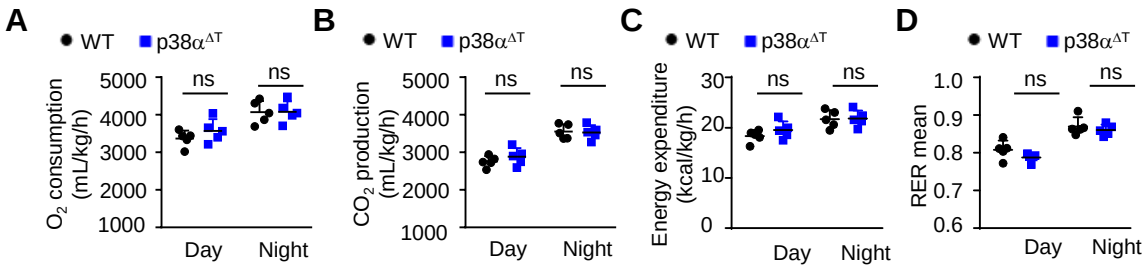
Supplementary Figure 5 – p38α in DCs is dispensable for DIO. WT and p38α$^{\Delta DC}$ male mice were fed with HFD for 18 weeks. Body weight (A), Liver and eWAT mass were measured (B). GTT (C) and ITT (D) were performed. Flow cytometric analysis of infiltrated myeloid cells in the eWAT (E). Quantification of SVF cell number and myeloid cell percentages (F). Data are representative of two independent experiments with six mice per group. Data are mean ± SD. ns, not statistically significant.



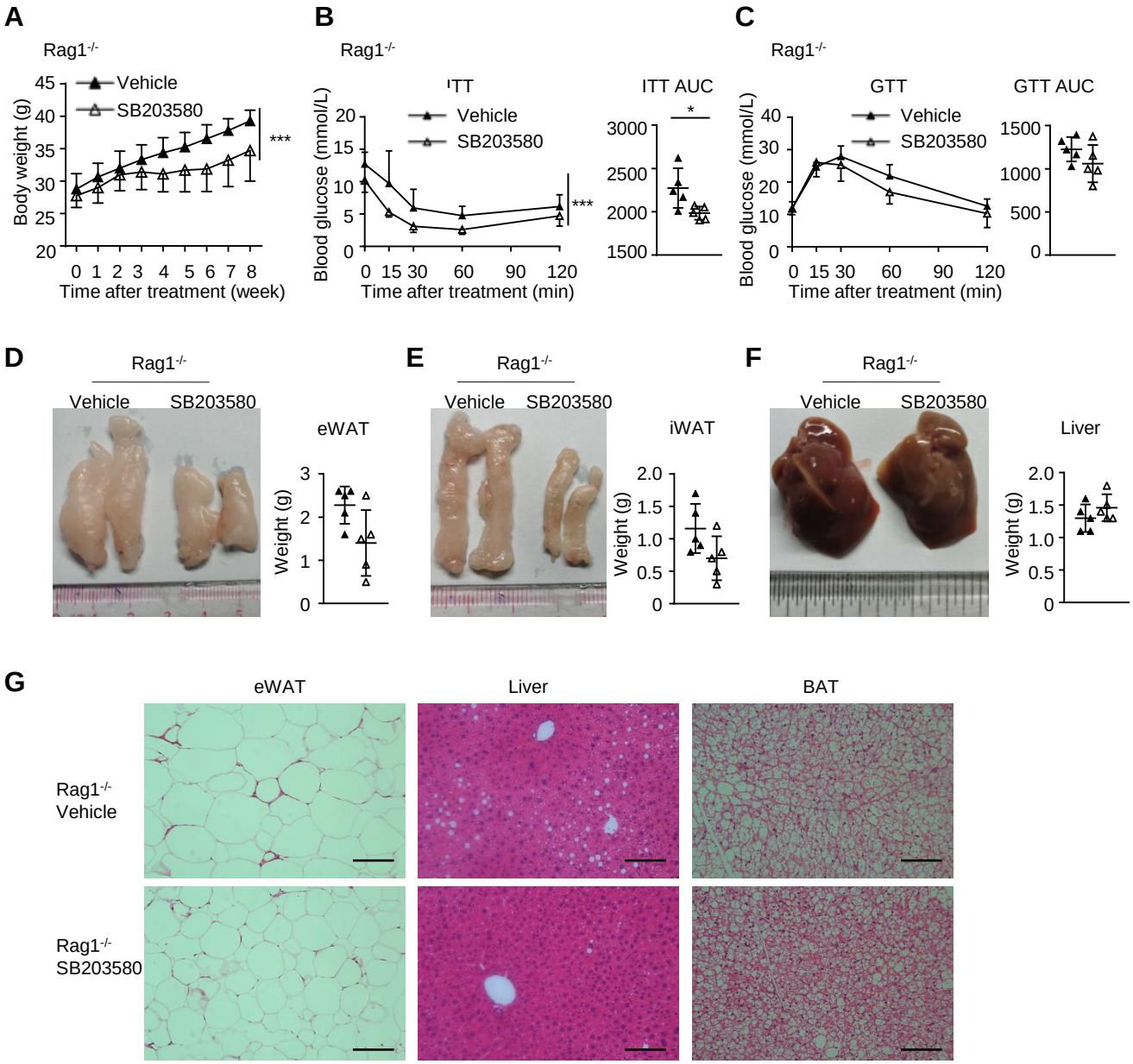
Supplementary Figure 6 – Effect of p38α in Treg cells on DIO. (A) Adipose tissue Treg subsets in 6 week-old lean and 8 week-HFD obese WT and p38a$^{\Delta T}$ mice. (B-G) WT and p38a$^{\Delta T}$ mice were treated with Anti-CD25 for DIO analysis. Experimental design, i.p., intraperitioneal (B) .Flow cytometry analysis of CD25^{+} Foxp3^{+} cell in CD4^{+} T cells in the spleen(C). Body weight of WT and p38a$^{\Delta T}$ mice (D). Image of eWAT and eWAT mass from HFD-fed WT and p38a$^{\Delta T}$ mice (E). Representative H&E staining of eWAT sections (scale bars: 100μm) (F). Cell numbers of the infiltrated SVF and Th1, Th2, Th17 and IFNγ^{+}CD8^{+}, TNFα^{+}CD8^{+} T cells in the eWAT(G). Data are mean ± SD. *p < 0.01, **p < 0.01, ***p < 0.001. ns, not statistically significant.



Supplementary Figure 7 – Immune profile analysis of livers in obese WT and p38α$^{\Delta T}$ mice. (A) Flow cytometry analysis of T cell subsets in the liver of obese WT and p38α$^{\Delta T}$ male mice. (B) Flow cytometry analysis of myeloid cell infiltration in the liver of obese WT and p38α$^{\Delta T}$ male mice. (C) ELISA analysis of IFNγ, IL-1β. IL-6 and TNFα production in the Liver homogenates of obese WT and p38α$^{\Delta T}$ male mice. N/A, Not applicable. Data are mean ± SD. *$p < 0.05$, **$p < 0.01$. ns, not statistically significant.



Supplementary Figure 8 – p38α in T cells is dispensable for energy expenditure in mice with NFD. Metabolic cage analyses were performed to measure O_2 consumption (A), CO_2 production (B), energy expenditure (C) and respiratory exchange ratio (D) in WT and p38$\alpha^{\Delta T}$ mice fed with NFD (n = 5). Data are mean ± SD. ns, not statistically significant.



Supplementary Figure 9 – Inhibition of p38α in Rag1$^{-/-}$ mice ameliorates DIO. (A-G) 3 month-old male Rag1$^{-/-}$mice fed with HFD were treated weekly with SB203580 or Vehicle for 8 weeks. Body weight analysis of Vehicle- and SB203580-treated Rag1$^{-/-}$ mice (A) . ITT (B) and GTT (C) were performed at 8 weeks. Images of eWAT(D), iWAT (E), and liver (F). Representative H&E staining of eWAT, liver and BAT (scale bars: 100μm) (G). Data are mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ns, not statistically significant.

Supplementary Table 1. List of Main Materials

	Name	Concentration	Source	Product Code
Antibody	Phospho-p38 MAPK (Thr180/Tyr182)	1:1000	Cell Signaling Technology	4511
	p38 MAPK	1:1000	Cell Signaling Technology	8690
	Phospho-MK-2 (Thr334)	1:1000	Cell Signaling Technology	3007
	Phospho-Akt (Thr308)	1:1000	Cell Signaling Technology	2965
	Phospho-Akt (Ser473)	1:1000	Cell Signaling Technology	4060
	Phospho-S6 (Ser235/236)	1:1000	Cell Signaling Technology	4858
	Phospho-AMPKα (Thr172)	1:1000	Cell Signaling Technology	2535
	c-Myc	1:1000	Cell Signaling Technology	5605
	Phospho-p70 S6 Kinase (Thr389)	1:1000	Cell Signaling Technology	9234
	Phospho-FoxO1 (Ser256)	1:1000	Cell Signaling Technology	9461
	PTEN	1:1000	Cell Signaling Technology	9559
	β-Actin	1:1000	Cell Signaling Technology	4970
	GAPDH	1:10000	Proteintech	10494
	HSP90	1:1000	Cell Signaling Technology	4877
	Goat Anti-Rabbit IgG	1:5000	Cell Signaling Technology	98164
	Goat anti-Rabbit IgG-AF555	1:200	invitrogen	A27039
	Fixable Viability Dye eFluor780	1:1000	Invitrogen	65-0865-18
	Anti-CD4- eFluor450	1:200	Invitrogen	48-0042-82
	Anti CD8a-BV605	1:200	BD	563152
	Anti-TCR-Percp-cy5.5	1:200	Invitrogen	
	Anti-CD25-FITC	1:200	Invitrogen	53-0251-82
	Anti-Foxp3-PE	1:100	Invitrogen	12-5773-82
	Anti-IL-4-PE	1:200	Invitrogen	12-7041-82
	Anti-IL-17A-PE-cy7	1:200	Invitrogen	25-7177-82
	Anti-IFNγ-FITC	1:200	Invitrogen	11-7311-82
	Anti-TNFα-APC	1:200	Invitrogen	17-7321-82
	Anti-CD3-FITC	1:400	Invitrogen	11-0032-82
	Anti- NK1.1-APC	1:400	Invitrogen	17-5941-82
	Anti-B220- eFluor 450	1:400	Invitrogen	48-0452-82
	Anti-CD16/32	1:100	Bio-X-Cell	CUSTOM24 G2
	Anti-F4/80-FITC	1:400	Invitrogen	11-4801-82
	Anti-SiglecF-APC	1:400	BD	562680
	Anti-CD11c-PE-cy7	1:400	Invitrogen	25-0114-82
	Anti-CD11b-BV605	1:400	BD	563015
	Anti-Ly6G-Percp-cy5.5	1:400	BD	560602
	Anti-CD45-eF450	1:400	Invitrogen	48-0451-82
	Anti-ST2-APC	1:100	Invitrogen	17-9335-82
	Anti-CD73-Percp-cy5.5	1:100	Invitrogen	127214
	Phospho-p38 MAPK (Thr180/Tyr182) -AF488	1:50	Cell Signaling Technology	4551
Mitochondrial Related reagents	CM-H2DCFDA (ROS-FITC)	10 μM	Invitrogen	C6827
	MitoTracker Deep Red FM	50 nM	Invitrogen	M22426
	Image-iT TMRM Regant	1:1000	Invitrogen	I34361
ELISA kit	Mouse IFNg gamma ELISA		Invitrogen	88-7314-86

	Mouse IL-6 ELISA		Invitrogen	88-7064-86
	Mouse IL-1 beta ELISA		Invitrogen	88-7013-86
	TNF alpha Mouse ELISA Kit		Invitrogen	88-7324-77
	Ultra Sensitive Mouse Insulin ELISA Kit		BioTNT	90080
Cytokines for differentiation	mIL-2		Bio-X-Cell	BE0043
	mIL-12		BD	419-ML-010
	mIL-4		BD	404-ML-010
	hTGFβ		BD	240-B-010
	mIL-6		BD	554582
	mIL-23		R&D	1887-ML-010
Antibody for differentiation	Anti-CD3 (145-2C11)		Bio-X-Cell	BE0001-1
	Anti-CD28 (37.51)		Bio-X-Cell	BE0015-1
	Anti-IFNγ (XMG1.2)		Bio-X-Cell	BE0055
	Anti-IL-4 (11B11)		Bio-X-Cell	BE0045
Reagent kit	Cytofix/Cytoperm™ fixation/permeabilization solution kit		eBioscience	00-8222-49
	Foxp3/Transcription Factor Staining Buffer Set Kit		eBioscience	00-5523-00
	Seahorse XF Glycolysis Stress Test Kit		Agilent Seahorse	103020-100
	Seahorse XF Cell Mito Stress Test Kit		Agilent Seahorse	103015-100
Others	D-glucose		Sigma	G8270
	Human insulin (Humulin R)		Eli Lilly	HI-213
	RIPA Buffer (10X)		Cell Signaling Technology	9806S
	Protease/Phosphatase Inhibitor Cocktail (100X)		Cell Signaling Technology	5872S
	PMSF		Cell Signaling Technology	8553
	Trizol		Invitrogen	15596-026
	PrimeScript RT reagent Kit		TAKARA	RR037A
	Power SYBR Green PCR Master Mix		ABI	4367659
	Type II collagenase		Sigma	C6885
	SB203580		MedChemExpress	HY-10256
	PEG300		Selleck	S6704
	Anti-CD25 antibody (PC-61.5.3)		Bio-X-Cell	BE0012
	Phorbol-12-myristate-13-acetate	50ng/mL	Sigma	P8139
	Ionomycin	1μM	Sigma	I3909
	GolgiStop	1:1000	BD	554724
	Senescence-Associated β-Galactosidase Staining		Servicebio	G1073
	Triglyceride test kit		Nanjing Jiancheng Bioengineering Institute	A110-1-1

Supplementary table 2. Mouse primer sequence for qRT-PCR

Name	Forward	Reverse
Hprt	TCAGTCAACGGGGGACATAAA	GGGGCTGTACTGCTTAACCAG
Mapk14	GAGGTGCCCGAACGATAC	TGGCGTGAATGATGGACT
Ifng	GCCACGGCACAGTCATTGA	TGCTGATGGCCTGATTGTCTT
Tnfa	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG
Ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Ccl5	GCTGCTTTGCCTACCTCTCC	TCGAGTGACAAACACGACTGC
Adipoq	TGTTCCTCTTAATCCTGCCCA	CCAACCTGCACAAGTTCCCTT
Pgc1a	TGATGTGAATGACTTGGATACAGACA	GCTCATTGTTGTACTGGTTGGATATG
G6pase	CGACTCGCTATCTCCAAGTGA	GTTGAACCAGTCTCCGACCA
Pepck	CTGGTTCCGGAAAGACAAAA	GCTCGGAAGCTCCCTCTCTAT
Foxo1	GCGGGCTGGAAGAATTCAAT	TCCAGTTCCTTCATTCTGCA
Fbp1	CAGCTGCTGAATTCGCTCTG	ACATTGGTTGAGCCAGCGATA
Pcx	CTGAAGTTCCAAACAGTTCGAAGG	CGCACGAAACACTCGGATG
Hk2	TGATCGCCTGCTTATTCACGG	AACCGCCTAGAAATCTCCAGA
Tpi	CCAGGAAGTTCTTCGTTGGGG	CAAAGTCGATGTAAGCGGTGG
Eno1	TGCGTCCACTGGCATCTAC	CAGAGCAGGCGCAATAGTTTTA
Pkm	GCCGCCTGGACATTGACTC	CCATGAGAGAAATTCAGCCGAG
Ldha	CATTGTCAAGTACAGTCCACACT	TTCCAATTACTCGGTTTTTGGGA
Fasn	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
Acc1	GATGAACCATCTCCGTTGGC	GACCCAATTATGAATCGGGAGTG
Scd1	TTCTTGCGATACACTCTCGTGC	CGGGATTGAATGTTCTTGTCGT
Scd2	GCATTTGGGAGCCTTGTACG	AGCCGTGCCTTGTATGTTCTG
Cpt1a	CTATGCGCTACTCGCTGAAGG	GGCTTTCGACCCGAGAAGA
Cpt2	CAAAAGACTCATCCGCTTTGTTC	CATCACGACTGGGTTTGGGTA
Acsl1	ACCAGCCCTATGAGTGGATTT	CAAGGCTTGAACCCCTTCTG
Acsl3	TGTCTTTCTCATGGATGCCGA	CAGCACGGATGTGTCTCCTT
Cd28	CTATCAGCCCCAGTTTCGCTC	CGGAACGTCACTGTTTCGTTG
Il2	TGAGCAGGATGGAGAATTACAGG	GTCCAAGTTCATCTTCTAGGCAC
Il13	TGACCAACATCTCCAATTGCA	TTGTTATAAAGTGGGCTACTTCGAT
Il17a	TCAGCGTGTCCAAACACTGAG	CGCCAAGGGAGTTAAAGACTT
p16ink4a	ATGGAGTCCGCTGCAGACAGACTG	CGTTGCCCATCATCATCACCTGAATCGG
p19arf	GGTTCTTGGTCACTGTGAGGATTC	TTGCCCATCATCATCACCTGGTC
p21	CCTGGTGATGTCCGACCTG	CCATGAGCGCATCGCAATC
Igfbp5	CCAAGCACACTCGCATTTCC	CCTTGTTCGGATTCCTGTCTCAT