Online Supplementary Material

Macrophage alternative activation confers protection against lipotoxicity-induced cell death

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Table S4. Top 10 DAVID functional annotation clustering analyses of genes significantly altered by GW501516 treatment as compared to vehicle

Ranking	Enrichment Score	Category	Term	PValue	Genes
1	3.1979	GOTERM_BP_FAT	GO:0016064~immunoglobulin mediated immune response	0.0003	Bcl3,CD74,Msh2,NOD2,PRKCD,C1qa,C1QB,C1QC,C1RL,C3,C4bp,Fcgr2b,H2-Ea,Igh-6,IRF7,LOC100048401,masp2,Pou2f2
2	1.8773	GOTERM_BP_FAT	GO:0042981~regulation of apoptosis	0.0105	Bcl3,CD74,Msh2,NOD2,PRKCD,1110007C09Rik,2810002N01Rik,ADRB2,ALOX12,ANGPTL4,ATM,BCL2,BCL2L11,BCL2L13,BCLAF1,birc2,BIRC3,birc6,BTG2,Camk1d,CARD9,Casp9,Cd24a,CIAPIN1,DAPK1,ddx20,Diablo,ERN1,GCH1,HIP1,HTT,Id3,IFIH1,ITSN1,KLF10,LOC100044206,LOC100048845,Lst1,LTB,MADD,MBD4,NF1,nfkbil1,NIACR1,Nlrp12,NR3C1,NR4A2,nrp,NTF3,NTRK1,PGAP2,PIK3CA,PIK3CG,PML,PRDX2,PRKDC,Prnd,Rarb,rarg,RIPK2,SERPINB9,SGK3,sgms1,Skil,Skp2,SP110,Spn,STAT5A,STK17B,tgfbr1,tgm2,TNFSF13B,Trp53inp1,TSC22D3,VEGFA,XIAP,xpa,XRCC2
3	1.5521	GOTERM_BP_FAT	GO:0043065~positive regulation of apoptosis	0.0251	Bcl3,PRKCD,2810002N01Rik,ADRB2,ATM,BCL2L11,BCLAF1,Casp9,Cd24a,DAPK1,ddx20,Diablo,ERN1,GCH1,Id3,KLF10,LOC100044206,LOC100048845,Lst1,LTB,MBD4,NF1,nfkbil1,NIACR1,NR3C1,PML,PRKDC,Rarb,rarg,RIPK2,Skp2,SP110,Spn,STK17B,tgfbr1,tgm2,Trp53inp1,xpa
4	1.4542	GOTERM_BP_FAT	GO:0046651~lymphocyte proliferation	0.0321	PRKCD,RIPK2,BCL2,PRDX2,CD86,CRIP3,CXCR4,SHB,Vpreb2,Zbtb32
5	1.2222	GOTERM_BP_FAT	GO:0045414~regulation of interleukin-8 biosynthetic process	0.0599	Apoa2,TLR7,TLR8
6	1.1397	GOTERM_BP_FAT	GO:0006779~porphyrin biosynthetic process	0.0373	ank1,FECH,HMBS,Spnb1,Urod,UROS
7	0.9784	GOTERM_BP_FAT	GO:0045625~regulation of T-helper 1 cell differentiation	0.0599	RIPK2,hlx,Il4ra
8	0.9670	GOTERM_BP_FAT	GO:0051250~negative regulation of lymphocyte activation	0.1015	hlx,Il4ra,PRDX2,Cd24a,Lst1,Spn,CD74,Fcgr2b,pAG1,TNFRSF14
9	0.9522	GOTERM_BP_FAT	GO:0045356~positive regulation of interferon-alpha biosynthetic process	0.0599	TLR7,TLR8,TLR3
10	0.8558	GOTERM_BP_FAT	GO:0046460~neutral lipid biosynthetic process	0.1311	AGPAT9,Ang,MOGAT1,PCK1

Table S5. Top 10 DAVID functional annotation clustering analyses of genes significantly altered by Il-4 treatment as compared to vehicle

Ranking	Enrichment Score	Category	Term	PValue	Genes
1	4.5287	GOTERM_BP_FAT	GO:0002495~antigen processing and presentation of peptide antigen via MHC class II	0.0000	H2-EA, RMCS2, FCGR2B, H2-EB1, UNC93B1, H2-DMB1, H2-DMA, CD74, H2-DMB2
2	3.4713	GOTERM_BP_FAT	GO:0051250~negative regulation of lymphocyte activation	0.0003	LST1, NFKBID, ERBB2, IL4RA, TNFRSF14, FOXP3, CD24A, CD74, PDCD1LG2, TIGIT, IL20RB, FCGR2B, HLX, PAG1
3	2.4817	GOTERM_BP_FAT	GO:0042981~regulation of apoptosis	0.0026	IGH-1B, STAT5B, INTS1, SGMS1, ITSN1, CIAPIN1, CASP6, PAK7, NOD2, CASP9, ANK2, CUL7, RARB, LTB, RARG, MADD, ARHGEF7, PIM1, BCL2L13, NLRP3, BCL2L11, BCL2L10, XPA, CARD14, ADRB2, PSEN2, MAPK9, GHRL, ALOX12, TRAF1, ING4, CDK5R1, LST1, TNF, NFKBID, STK17B, PRKDC, SP110, CX3CL1, CD24A, CD74, BCL2, TGM2, BMF, CD27, PIK3R1, ANGPTL4, COL18A1, CARD9, NTF3, HTT, TGFBR1, NF1, NR4A2, CIDEB, IGF1, 2810002N01RIK, HGF, GAS1, ATM, NFKBIL1, FCGR3, PLEKHF1, NOTCH1, APBB3, SYNGAP1, APBB1
4	2.1631	GOTERM_BP_FAT	GO:0043065~positive regulation of apoptosis	0.0061	ING4, CDK5R1, LST1, TNF, NFKBID, IGH-1B, STK17B, PRKDC, SP110, CD24A, CASP6, CASP9, TGM2, RARB, LTB, CD27, COL18A1, RARG, ARHGEF7, TGFBR1, NF1, CIDEB, 2810002N01RIK, ATM, BCL2L11, NFKBIL1, FCGR3, PLEKHF1, XPA, ADRB2, NOTCH1, PSEN2, MAPK9, APBB1
5	1.7643	GOTERM_BP_FAT	GO:0051251~positive regulation of lymphocyte activation	0.0121	H2-EA, ICOSL, IGH-1B, IL4RA, STAT5B, TNFRSF13C, TNFSF14, SYKB, FOXP3, CD24A, PDCD1LG2, CD74, CD83, HLX, H2-DMA, CD27
6	1.7351	GOTERM_BP_FAT	GO:0030335~positive regulation of cell migration	0.0108	COL18A1, LOC100045000, PRR5, BCL2, LAMB1-1, HBEGF, ZFP640, PIK3R1, PRL2C2
7	1.6741	GOTERM_BP_FAT	GO:0051085~chaperone mediated protein folding requiring cofactor	0.0145	TOR2A, H2-DMB1, H2-DMA, CD74, H2-DMB2
8	1.6011	GOTERM_BP_FAT	GO:0032945~negative regulation of mononuclear cell proliferation	0.0251	LST1, IL20RB, FCGR2B, ERBB2, TNFRSF14, FOXP3, CD24A, PDCD1LG2
9	1.3782	GOTERM_BP_FAT	GO:0046456~icosanoid biosynthetic process	0.0228	PTGS1, SYKB, ALOX5, LTC4S, 2010111I01RIK, CD74, ALOX12
10	1.3449	GOTERM_BP_FAT	GO:0051953~negative regulation of amine transport	0.0099	TNF, LST1, LTB, NFKBIL1

Table S6. List of primers used for qPCR

Genes	Forward Sequence	Reverse Sequence	Accession Number
Plin2	GACCTTGTGTCCTCCGCTTAT	CAACCGCAATTTGTGGCTC	NM_007408
Angptl4	CATCCTGGGACGAGATGAACT	TGACAAGCGTTACCACAGGC	NM_020581
Sgms1	CCAGGACTTAATCAACCTAACCC	GTCAGAGGAGACTCGGTACAG	NM_001168525
Nlrp3	TCCTGCAGAGCCTACAGTTGGGT	GGTGGTCCTGCTTCCACGCC	NM_145827
Mgl1	TGAGAAAGGCTTTAAGAACTGGG	GACCACCTGTAGTGATGTGGG	NM_001204252
Igf1	TGCTGTGTAAACGACCCGGACC	GGTGAGCAAGCAGAGCGCCA	NM_001111274
Bcl2	ATGCCTTTGTGGAACTATATGGC	GGTATGCACCCAGAGTGATGC	NM_177410
Tnf-α	AAATGGCCTCCCTCTCATCAG	GTCACTCGAATTTTGAGAAGATGATC	NM_013693
Il-1β	AATCTATACCTGTCCTGTGTAATGAAAGAC	GGTATTGCTTGGGATCCACACT	NM_008361
Arg1	CACAGTCTGGCAGTTGGAAG	GGGAGTGTTGATGTCAGTGTG	NM_007482
Ym1	AGAAGGGAGTTTCAAACCTGGT	GTCTTGCTCATGTGTGTAAGTGA	NM_009892
Acadvl	TCGGAGGCGGCCTGGAAAGT	TGCCCAGCCCAGTGAGTTCCT	NM_017366
Cd36	GATGTGGAACCCATAACTGGATTCAC	GGTCCCAGTCTCATTTAGCCACAGTA	NM_001159556
Slc25a20	ACTGCACCTCCTGGAAAATATCC	TCTTCTCGGATCAGCTCTCTCAAC	NM_020520
Mogat1	TCCCGTTGTTCCGAGAATATCT	TGCTCAGCACATGAGACAAAC	NM_026713
H2-Eb1	CCTGCAGCATGGTGTGGCTCC	CGCGCTGCGTCCCGTTGTAG	NM_010382

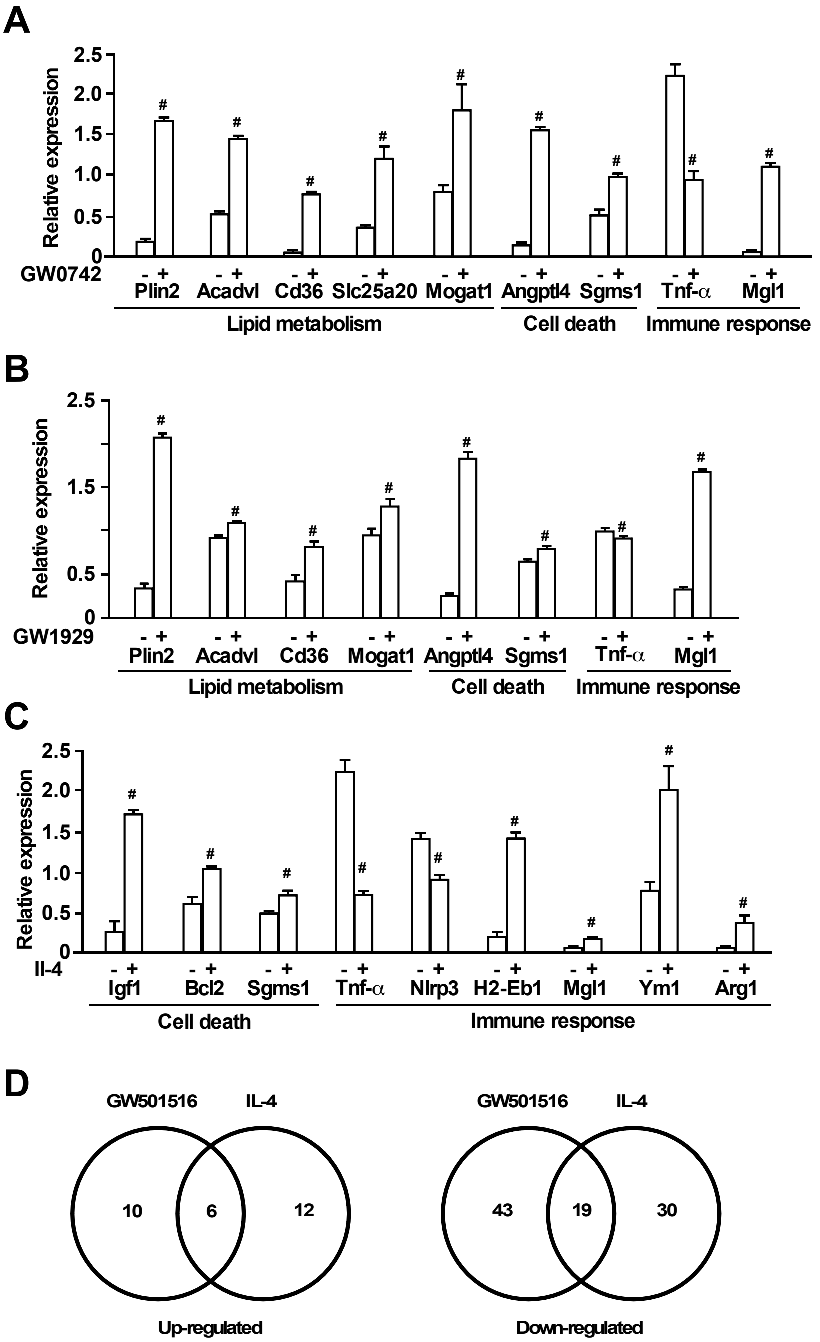

Figure S1. Validation of Pparδ/Pparγ and Stat6 target genes in RAW264.7 macrophages. (A) Validation of Ppar target genes identified by microarray in RAW-Pparδ cells cultured with GW0742 (0.1μM) overnight. Vehicle (DMSO) was included as a control. Gene expression was determined by qPCR. (B) Gene expression in Pparγ-overexpressing RAW264.7 cells treated with Pparγ agonist GW1929 (1 μM) overnight. (C) Validation of Il4-regulated genes identified by microarray in RAW-Pparδ cells treated with rIl-4 (10 ng/ml) overnight. (D) Venn diagrams of cell-death related genes significantly up-regulated or down-regulated by GW501516 or Il-4 treatment, compared to vehicle control. Data were presented as means ± SEM. #$p < 0.05$ between vehicle and GW0742, GW1929, or rIl-4.

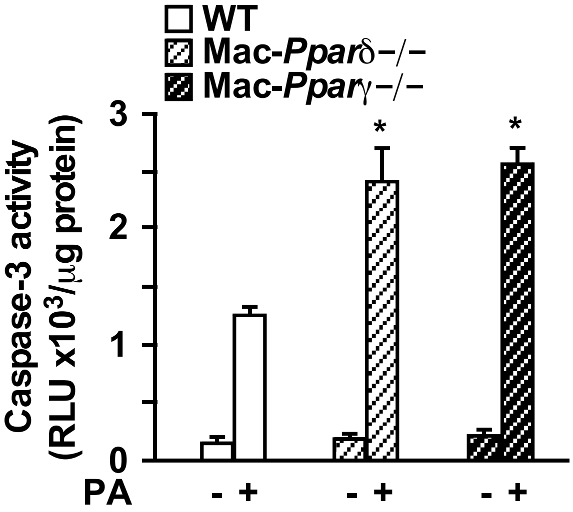

Figure S2. Macrophages lacking *Pparδ* or *Pparγ* are prone to lipotoxicity-triggered cell death.

WT, Mac-*Pparδ*-/- and Mac-*Pparγ*-/- BMDMs were treated with vehicle or PA (300 μM) for 16 hr and caspase-3 activity was measured by Caspase-Glo® 3/7 Assay kits. Values were expressed as means ± SEM. $^*p < 0.05$ between WT and Mac-*Pparδ*-/- or Mac-*Pparγ*-/-.

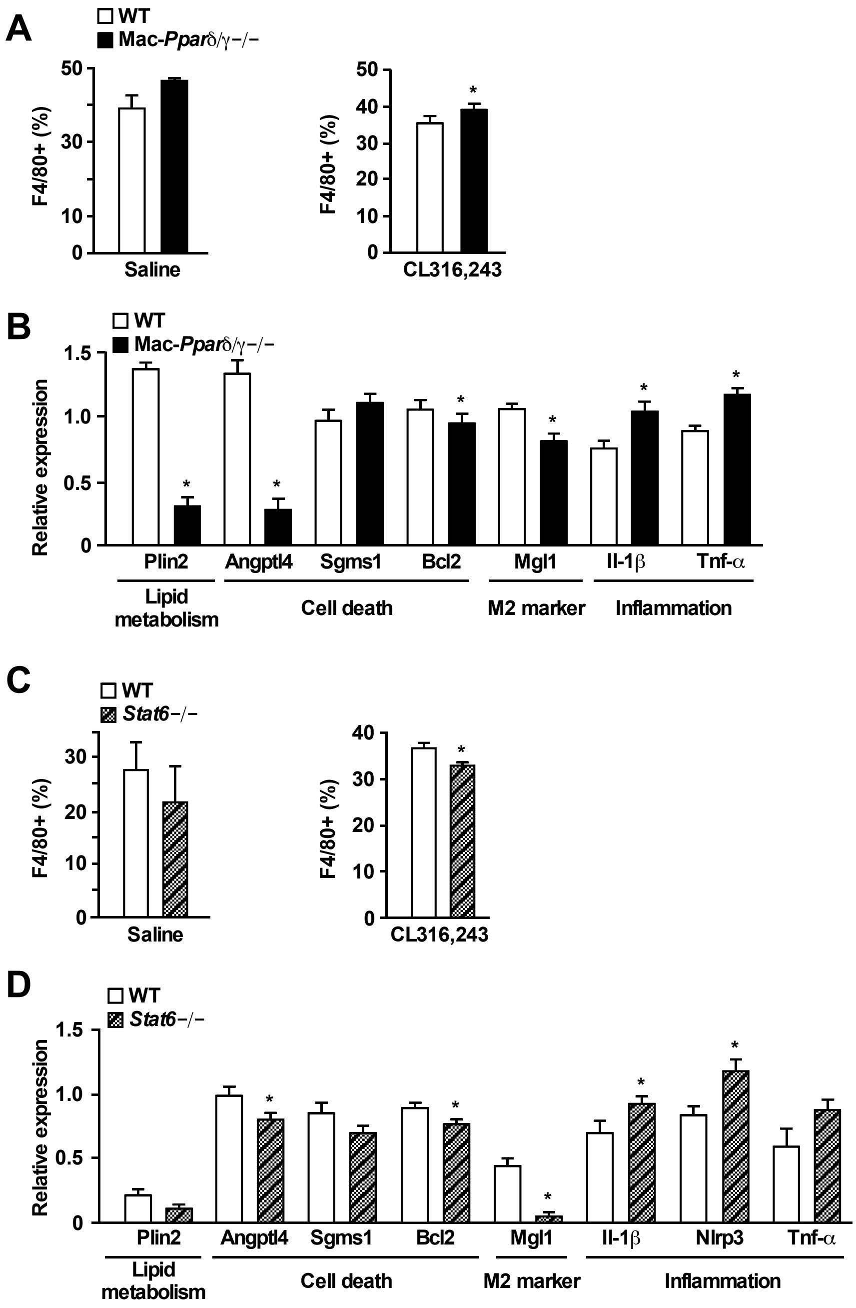

Figure S3. Assessment of ATMs from WT, Mac-*Pparδ/γ-/-* and *Stat6-/-* mice.

Chow-fed WT, Mac-*Pparδ/γ*-/- and *Stat6*-/- mice (3-month-old males) were fasted for 14 hr, followed by intraperitoneal injection of saline or 1 mg/kg CL316,243. Animals were sacrificed 2.5 hr after injection. SVF and ATMs were isolated from gonadal WAT. (A) and (C) Flow cytometry quantification of the percentage of F4/80$^+$ cells in SVF of WAT from Mac-*Pparδ/γ*-/- and *Stat6*-/- mice, respectively. Left and right panels were from saline and CL316,243 injected mice respectively. (B) and (D) Gene expression analyses in ATMs from saline injected Mac-*Pparδ/γ*-/- and *Stat6*-/- mice. Values were expressed as means ± SEM. *p < 0.05 between WT and Mac-*Pparδ/γ*-/- or *Stat6*-/- mice.