Supplemental Materials and Methods:

Reagents: RAW264.7 macrophages and PGL3 was a gift from Dr. Michael Smith (University of Virginia). The plasmid containing the full-length murine iNOS cDNA was obtained from Dr. Lisa Palmer (University of Virginia). The Griess Reagent assay was obtained from Cayman Chemicals. The arginase activity assay was from BioAssay Systems. FBS was ordered from HyClone (Logan, UT). Thioglycollate medium was obtained from Sigma. S1P was obtained from BIOMOL Research Laboratories BAY11-7085, LPS and SEW2871 were obtained from Sigma. Cytokine ELISA kits were obtained from R&D Systems.

Conventional and Quantitative real-time PCR: Peritoneal macrophages were freshly isolated from B6 and cultured as described above for mRNA measurements. Total cellular RNA was obtained from peritoneal macrophages as previously described[1]. For measurement of S1P receptor mRNA abundance by conventional RT-PCR, 2ul cDNA from each experimental group was utilized. Conventional RT-PCR conditions for arginase-I, arginase-II, iNOS, S1P receptors, and β-actin were as follows: 95°C 3min, followed by 38 cycles of 95°C 45 sec, 59.5°C 60 sec, 72°C 60 sec, with a final extension time of 7 min at 72°C. Bands were analyzed on a 1.0% agarose gel in 1X TAE buffer. PCR for β-actin was performed as a control for normalization purposes. For quantitative real-time PCR analysis of mRNA expression (arginase-I, arginase-II, iNOS, COX-2, cytokines), cDNA was obtained as described above, and was diluted 1:8; 4uL of this dilution was used for each PCR reaction. Reagents from the BioRad real-

time PCR kit containing Sybr Green were used for quantitative PCR reactions. The PCR conditions were: 95 ^{o}C 3 min, followed by 40 cycles of 95 ^{o}C 10 secs, 60 ^{o}C 30 secs, and 72 ^{o}C 45 secs. Data were analyzed as previously described[1;2]. Primer sequences for all PCR reactions are shown in **Online Table I** and **Online Table II**.

Arginase enzymatic assay: Macrophages were plated in RPMI/10% FBS at 37 ^{o}C for 24h. Cells were then washed and treated in RPMI/1% FBS with 500nM S1P 30min prior to the stimulation with LPS for another 4h. Arginase activity was measured in cell lysates as described previously[3].

Griess reagent assay: Supernatants from macrophages were collected and assayed by the Griess assay for inducible NO production according to manufacturer's instructions (Cayman Chemicals). Briefly, 80ul aliquots of supernatant or sodium nitrate standards were combined with equal volumes of fresh Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, 2.5% H_3PO_4). Samples were incubated at room temperature for 10min, and the absorbance was measured at 540 nm. The concentration of nitrite (NO_2^-) and nitrate (NO_2^-) was determined using a sodium nitrate standard curve.

Cytokine measurements: Macrophages were cultured using RPMI containing 1% heat-inactivated FBS with 10ng/ml LPS ± 500nM S1P. Cell culture supernatant was quantitatively measured for TNFα protein levels using R&D Systems ELISA kit according to manufacturer's instructions.

Immunoblotting for NFκB and IκB: Macrophages were incubated in RPMI + 1% FBS with LPS (25ng/ml), S1P (500nM) or SEW2871 (1uM) for 1 hour.

Cytosol and nuclear extracts were collected from macrophages using the NE-PER kit (Pierce) according to the manufacturer's instructions. 50 μg of protein was analyzed by SDS-PAGE on 4-12% gels (Invitrogen). Cytosol was probed for IκBα 1:2000 (Cell Signaling) and nuclear extract for NFκB p65 1:2000 (Santa Cruz). Cytosol and nuclear extracts were normalized to tubulin 1:5000 (Sigma) or histone H1 1:2000 (Santa Cruz) respectively.

Inhibition of S1P1 gene expression by SiRNA: SiRNA for murine S1P1 was expressed in pSuper (OligoEngine, Seattle, WA). The target nucleotide sequence for S1P1 is 5'-CTATGATATCATAGTCCGG-3' Bone marrow derived macrophages from S1P2-deficient and littermate control mice were transfected with 2 μg S1P1SiRNA plasmid using a mouse macrophage nucleofector kit (Amaxa Biosystems). After 48hrs, macrophages were incubated with LPS, S1P and SEW2871 as described in the figure legends.

Transfections: RAW264.7 macrophages were transiently transfected using the Amaxa Nucleofector II and Cell Line Nucleofector Kit V according to manufacturers protocol. The D-032 setting was determined to provide the highest transfection efficiency (>70%) with the least amount of cell death (<10%). At 24h post-transfection, cells were treated for 4h with 10ng/ml LPS ± 500nM S1P. Luciferase activities in cell extracts were measured using the Dual-Luciferase® assay system (Promega) with a Berthold Syrius Luminometer (Fisher Scientific). Luciferase activity was corrected for transfection efficiency against *Renilla* luciferase activity and expressed relative to untreated controls.

Statistical Analyses: Data for all experiments were analyzed using the StatView 6.0 software program. Comparisons between groups were performed using one-way analysis of variance (ANOVA) methods. Data are graphically represented as mean + SE, in which each mean consists of 4 experiments performed in triplicate (unless noted otherwise in the figure legends) using 6-8 mice per group. Comparisons between groups and tests of interactions were made assuming a two-factor analysis with the interaction term testing each main effect with the residual error testing the interaction. All comparisons were made using Fisher's LSD procedure, so that multiple comparisons were made at the 0.05 level only if the overall F- test from the ANOVA was significant at $p<0.05$.

References

1. Mauldin JP, Srinivasan S, Mulya A, Gebre A, Parks JS, Daugherty A, Hedrick CC. Reduction in ABCG1 in Type 2 diabetic mice increases macrophage foam cell formation. *J Biol Chem*. 2006;281:21216-21224.

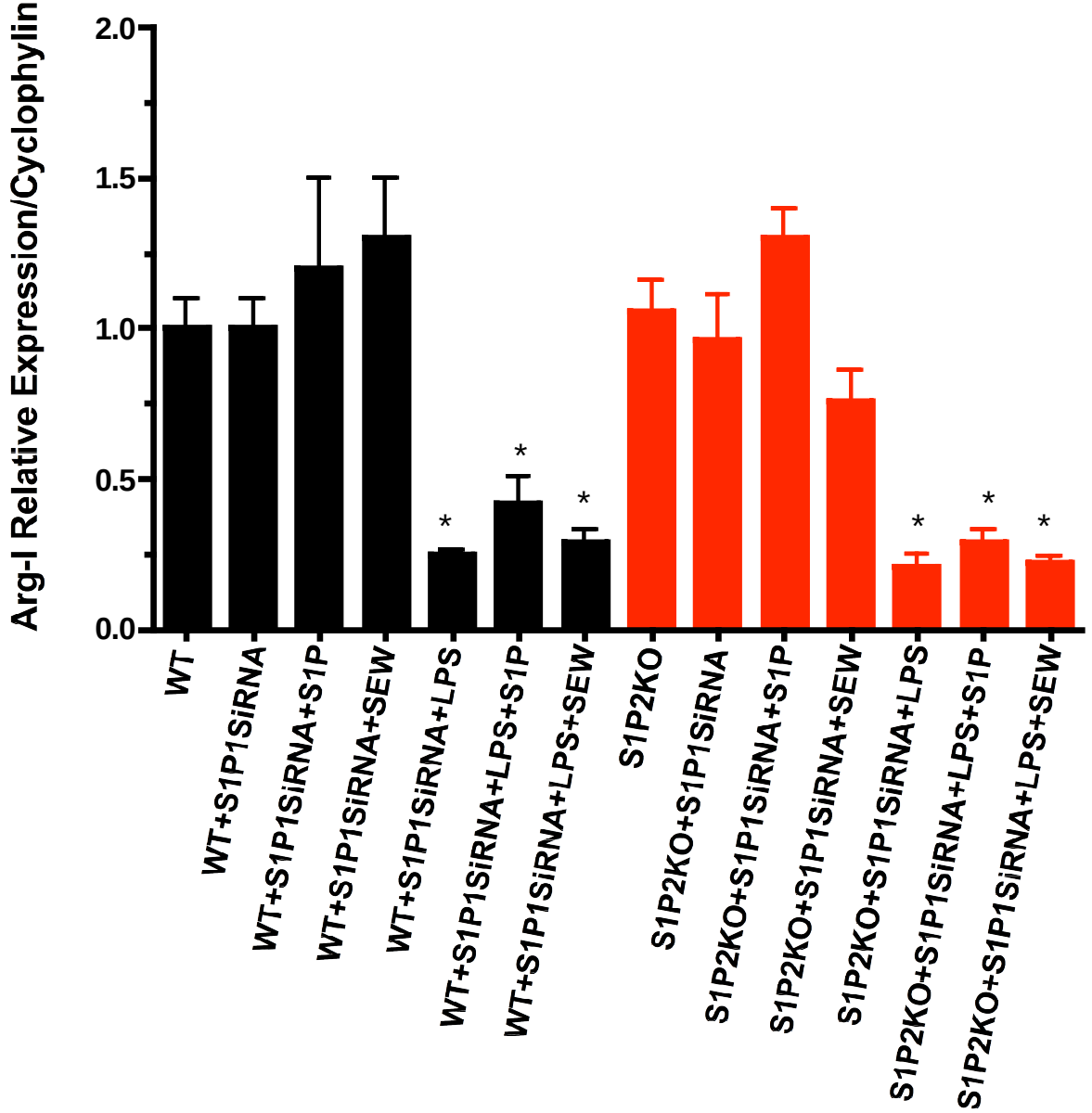
2. Bolick DT, Whetzel AM, Skaflen M, Deem TL, Lee J, Hedrick CC. Absence of the G protein-coupled receptor G2A in mice promotes monocyte/endothelial interactions in aorta. *Circ Res*. 2007;100:572-580.

3. Pesce J, Kaviratne M, Ramalingam TR, Thompson RW, Urban JF, Jr., Cheever AW, Young DA, Collins M, Grusby MJ, Wynn TA. The IL-21 receptor augments Th2 effector function and alternative macrophage activation. *J Clin Invest*. 2006;116:2044-2055.

Online Figure I. S1P1 siRNA reduces anti-inflammatory action of S1P in macrophages. Bone marrow-derived macrophages from either wild-type littermate controls (WT; black bars) or S1P2-deficient (S1P2KO; red bars) mice (n=5 mice per group) were transfected with S1PsiRNA as described in Materials and Methods. At 48h after transfection, macrophages were treated with LPS (10 ng/ml) in the absence or presence of 500nM S1P, or 1μM SEW2871 (+SEW) for 4h. Quantitative real-time PCR for murine arginase-I (Arg-I), iNOS and TNFα were performed. Cyclophilin was measured as a housekeeping gene for normalization purposes. Data are expressed using the relative expression method using B6 control as set to 1. *Panel A, Arginase-I expression*. *p<0.0001 versus WT/S1P2KO. *Panel B iNOS expression*. $^{\#}$p<0.005 versus WT/S1P2KO; **p<0.009 versus S1P2KO by ANOVA. *Panel C, TNFα expression.* TNFα levels were measured using ELISA. $^{\#}$p<0.005 versus WT/S1P2KO; $^{\$}$p<0.05 versus WT/S1P2KO, p<0.05; $^{\&}$p<0.01 versus S1P2KO+S1P1SiRNA+LPS by ANOVA.

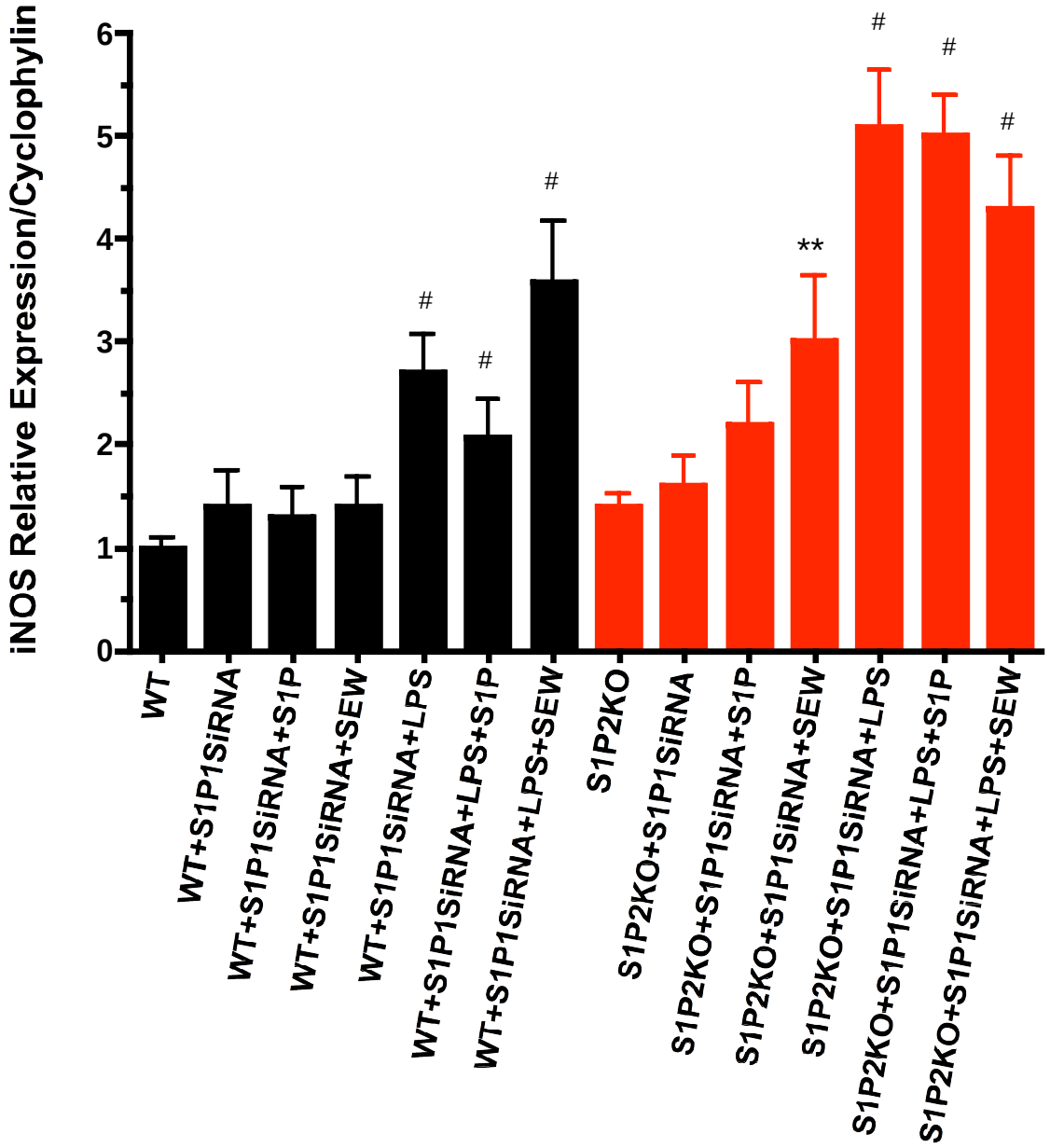
Online Figure II. NFκB regulates LPS-mediated induction of TNFα in macrophages. Peritoneal macrophages from C57BL/6J mice were incubated for 4h with 10ng/ml LPS in the absence (+LPS) or presence of a 1h-pretreatment with either 5μM or 10μM BAY11-7085 (+Bay). In some cases, cells were also incubated with 500nM S1P (+ S1P). TNFα secretion into media was measured using ELISA and normalized to total cell protein. *p<0.0001 versus Ctrl; $^{\#}$p<0.001 versus LPS; **p<0.0001 versus LPS by ANOVA.

A.



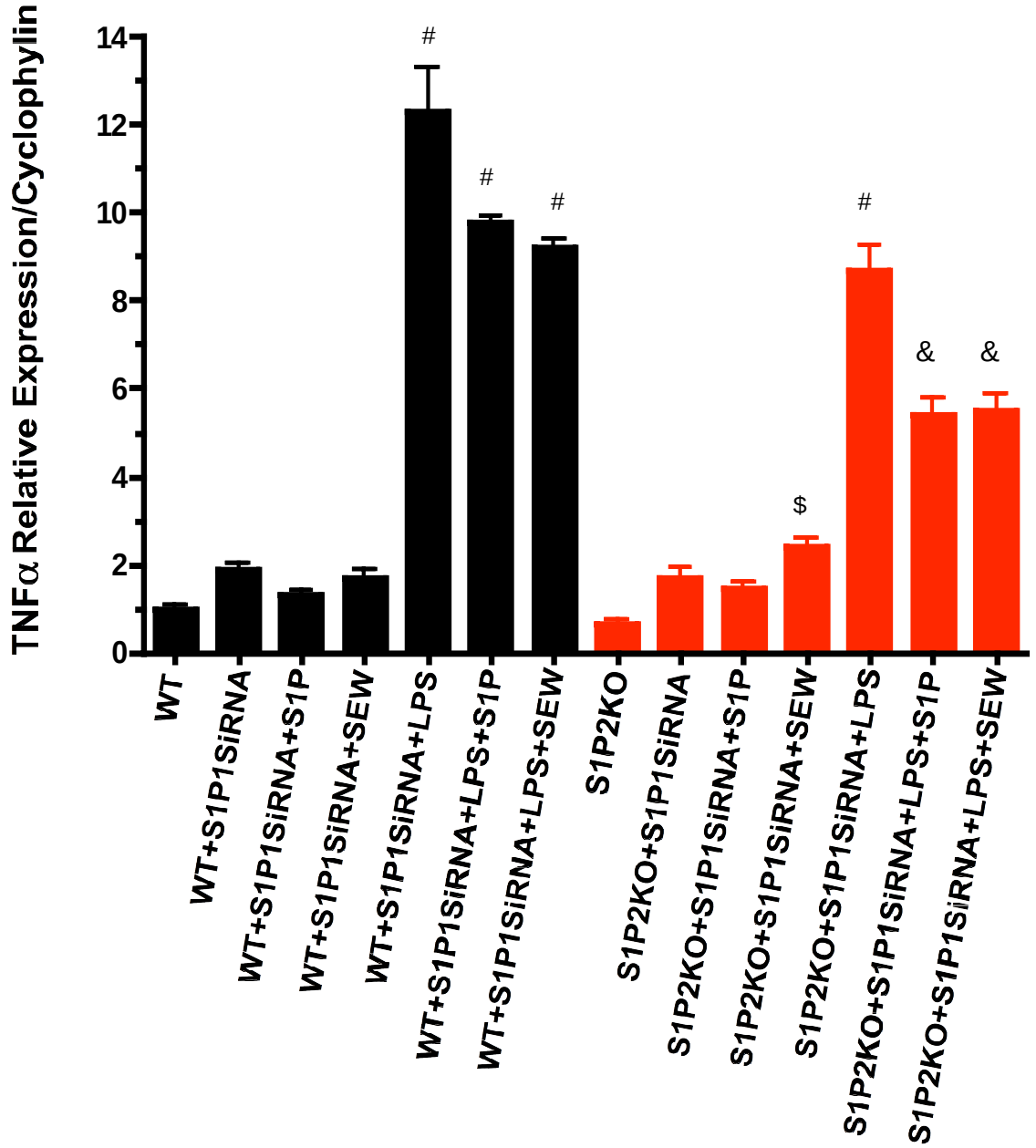
Online Figure IA

B.

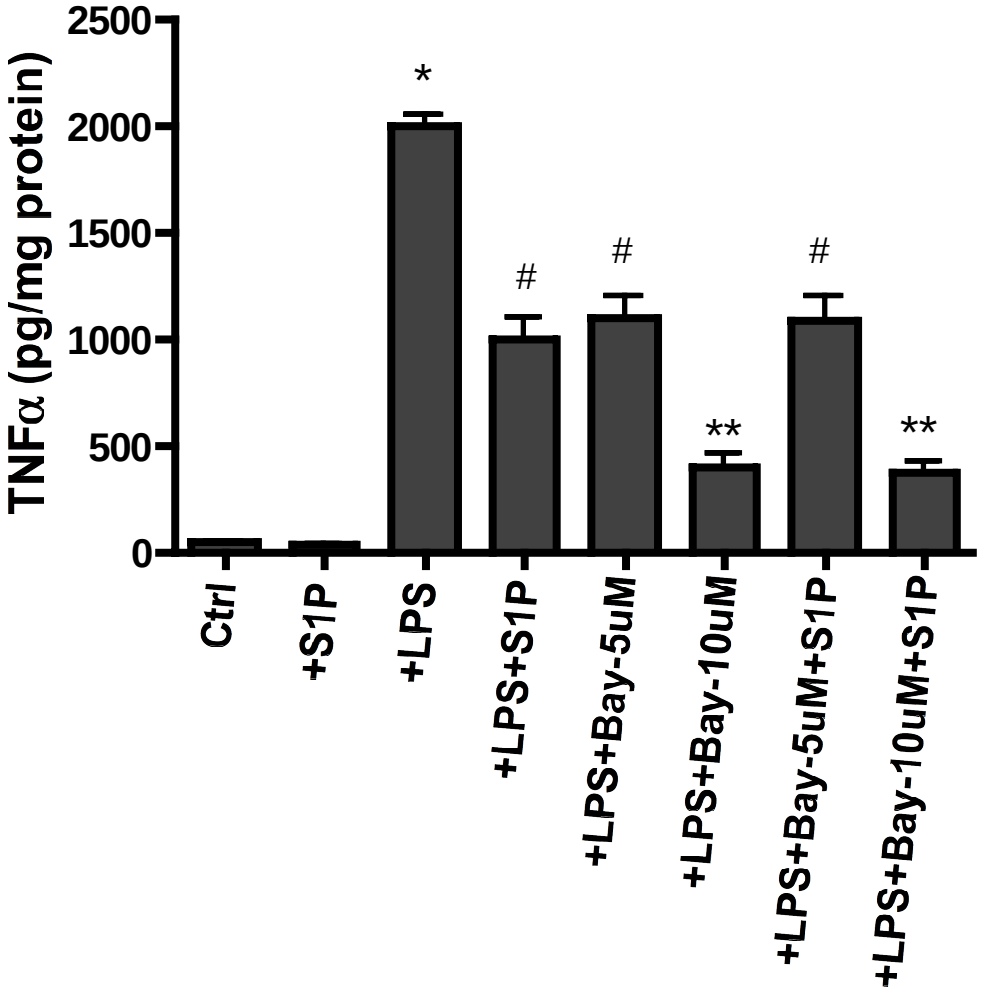


Online Figure IB

C.



Online Figure IC



Online Figure II

Online Table I

Quantitative Real-time PCR primers used in this study:

Name		Sequence
TNFα	Forward	5'- TGG AAC TGG CAG AAG AG -3'
	Reverse	5'- CCA TAG AAC TGA TGA GAG G-3'
MCP-1	Forward	5'- ACT CAT TCA CCA GCA AGA TG-3'
	Reverse	5'- TGT AGG TTC TGA TCT CAT TTG G-3'
Arginase-1	Forward	5'- AAG ACA GCA GAG GAG GTG AAG AG-3'
	Reverse	5'- TGG GAG GAG AAG GCG TTT GC-3'
iNOS	Forward	5'-CTG CAG CAC TTG GAT CAG GAA CCT G-3'
	Reverse	5'- GGG AGT AGC CTG TGT GCA CCT GGA A-3'
IL12p40	Forward	5'- GGA AGC ACG GCA GCA GAA TA-3'
	Reverse	5'- AAC TTG AGG GAG AAG TAG GAA TGG-3'
Cyclophilin	Forward	5'- TGG AGA GCA CCA AGA CAG ACA-3'
	Reverse	5'- TGC CGG AGT CGA CAA TGA T-3'
KC	Forward	5'- CAA GCA GAA CTG AAC TAC CAT C-3'
	Reverse	5'- AAC CGA AGT CAT AGC CAC AC -3'
COX-2	Forward	5'- ACC TCT CCA CCA ATG ACG TG -3'
	Reverse	5'- GGG AGA GAG TTC ATC CCT GA -3'
MIP-2	Forward	5'-CCC AAC TCA CCC TCT CC-3'
	Reverse	5'-TGA TGT GCC TCG CTG TCT G-3'

Online Table II
Conventional PCR primers used in this study:

Name		Sequence
S1P1	Forward	5'- CAC CGG CCC ATG TAC TAT TT-3'
	Reverse	5'- GAC TGC CCT TGG AGA TGT TC-3'
S1P2	Forward	5'- GGG CAT GTC ACT CTG TCC TT -3'
	Reverse	5'- GAA CGG GAC AGG TGG AGT CTA-3'
S1P3	Forward	5'- GAG CAA CCT TGG CTA CTT GC-3'
	Reverse	5'- AGC TTC GGG TTC AGT GAG AA-3'
S1P4	Forward	5'-GGC TAC TGG CAG CTA TCC TG-3'
	Reverse	5'- GCT GAG TGA CCG AGA AGT CC-3'
S1P5	Forward	5'- GCC GGT GAG TGA GGT TAT TG-3'
	Reverse	5'- CGC GAC ATC CAG TAA TAG CA-3'
Arginase-1	Forward	5'- CAG AAG AAT GGA AGA GTC AG -3'
	Reverse	5'- CAG ATA TGC AGG GAG TCA CC-3'
Arginase-2	Forward	5'- TGA TTG GCA AAA GGC AGA GG-3'
	Reverse	5'- CTA GGA GTA GGA AGG TGG TC-3'
iNOS	Forward	5'- CGC TTG GGT CTT GTT CAC TCC-3'
	Reverse	5'- AGG GGC AAG CCA TGT CTG AG-3'
β-actin	Forward	5'-CCA TGT TTG AGA CCT TCA ACA C-3'
	Reverse	5'-CTG CTT GCT GAT CCA CAT CT-3'