Materials and Methods:

Chemicals and reagents were procured from the following sources:

Sigma

Giemsa stain (GS500), TRI reagent (T9424), Ethidium bromide (E7637), Ammonium persulphate (A3678), TEMED (T9281), Bromophenol blue (B8026), 2-Mercaptoethanol (M7522), BSA (B6917), Sodium azide (S2002), Saponin (S1252), Ponceau S (P3504), Phenol red (P3532), Ampicillin (A9518), and Cesium chlorides (C4036) were also provided by Sigma.

Fluka

Kanamycin sulfate (60615).

Bio-Rad

Broad range pre-stained markers for SDS-PAGE.

BD Pharmingen

Rat Anti-Mouse IFN-γ capture Ab (551216), Biotin Anti-Mouse IFN-γ detection Ab (554410), Recombinant Mouse IFN-γ (554587), Rat Anti-Mouse IL-4 capture Ab (554387), Biotin Anti-Mouse IL-4 detection Ab (554390), Recombinant Mouse IL-4 (554587), Rat Anti-Mouse IL-10 capture Ab (551215), Biotin Anti-Mouse IL-10 detection Ab (554423), Recombinant Mouse IL-10 (550070), Rat Anti-Mouse IL-12 p40/p70 capture Ab (554592), Biotin Anti-Mouse IL-12 p40/p70 detection Ab (554476), Recombinant Mouse IL-12 p70 (554592), Rat Anti-mouse TNFα capture Ab (557516), Biotin Anti-mouse TNFα detection Ab (557432), Recombinant Mouse TNFα (554589), Rat Anti-mouse TGF-β1 capture Ab (555052), Biotin Anti-mouse TGF-β1 detection Ab (555053), Recombinant Mouse TGF-β1, TMB substrate reagents Set (555214), Purified NA/LE (Clone 3/23) Rat Anti-Mouse CD40

(553787), FITC Anti-Mouse CD3 (555274), Pacific Blue Anti-Mouse CD4 (558107), APC-Cy7 Anti-Mouse CD25 (557658), PerCP-Cy5.5 Rat Anti-Mouse CD44 (560570), APC-Cy7 Rat Anti-Mouse CD62L (560514), PE-Cy7 Rat Anti-Mouse GITR (558140), Alexa Fluor 647 Rat Anti-Mouse Foxp3 (563486), FITC Anti-Mouse IL-10 (554466), PE Anti-mouse IFN-γ (554412), PE Anti-mouse IL-4 (18195A), FITC Mouse IgG1, κ Isotype control (550616), PE Rat IgG2a, κ Isotype control (554689), PerCP-Cy5.5 Mouse IgG1, κ Isotype control (550795), PE-Cy7 Hamaster IgG1, κ Isotype control (552811), APC Rat IgG2b, κ Isotype control (556924), APC-Cy7 Rat IgG1, λ Isotype control (557663), Pacific Blue Rat IgG2a, κ Isotype control (558109), Cytofix/cytoperm-Plus Kit with GolgiPlug (555028), Biotinated Anti-mouse IgG1(553441), IgG2a (553388) or IgM (553406) specific antibody, Purified NA/LE Hamster Anti-mouse CD3e (553057) and Purified NA/LE Hamster Anti-mouse CD28 (553294).

e-Bioscience

PE Anti-mouse RORγt (12-6981-82), PE Anti-mouse CCR7 (12197182).

Biolegend

APC Anti-mouse IL-17A (506916), PerCP-Cy5.5 Anti-mouse CD127 (121114), Pacific Blue Anti-mouse Foxp3 (126409), APC Anti-mouse T-BET (644804), PB Anti-mouse IFN-γ (505818), PE Anti-mouse GATA3 (653804).

MP Biomedicals LLC, France

Sodium Dodecyl Sulphate (194831), Triton X-100 (194854), DMSO (196055), Boric acid (194810), Acrylamide (193982), N,N-Methylene bis-acrylamide (195316).

USB Corporation, USA

Sodium phosphate dibasic (20232) and Sodium phosphate monobasic (20232).

R&D Systems

Recombinant mouse IL-7 (407-ML/CF).

Affymetrix, USA

Tween-20 (T1003).

Difco

Thioglycollate (225650).

Himedia

Luria Bertani broth (M1245) and Luria Bertani broth agar (M1151), Magnesium acetate (RM3921), MOPS Buffer (RM660), Potassium acetate (RM3930), Manganese chloride (RM3925), HEPES (RM380).

Qualigens

Ammonium chloride (11205), Calcium chloride (22185), Sodium bicarbonate (106329), Sodium nitrite (15935), Potassium chloride (19255).

Millipore

Immobilon-PVDF membrane (IPVH00010), 0.45um (SLHV033RS) and 0.22µm (SLGP033RS) syringe filter.

Roche

Complete Mini and Streptavidin-POD Conjugate (11089153001).

Thermo Scientific

Gene jet gel extraction & PCR purification kit (K0691), Gene jet plasmid isolation kit (K0502).

Fischer Scientific

Methanol (32406), Hydrochloric acid (29505), Glacial acetic acid (11005), Chloroform (12305), Isopropyl alcohol (13825), EDTA (12635), Sodium chloride (27605) and Sodium hydroxide pellet (15895), Tris-base (15965), Glycine (24755), Glycerol (15455).

Lonza Seakem

Agarose (50004).

Invitrogen

10x PCR buffer, $MgCl_2$, 10mM dNTPs, Taq DNA polymerase, 5x RT buffer, DTT, Random primer, MMLV- RT.

NEB

Hi-fidelity DNA Polymerase Enzyme, Cutsmart buffer, Restriction Enzyme, Ligation buffer and Ligase enzyme.

Takara

SYBR-Green (RR820A).

Gibco

HBSS (14025092), FBS (16000044), Penicillin-Streptomycin (15140122), L-glutamine (25030081).

Axygen

1.7ml (MCT-175-C), 0.6ml (MCT-060-C), 0.2 ml (PCR-02-C) microtube, thin-walled real-time PCR strips and Pipette Tips.

BD Biosciences

Laboratory plastic wares used for cell and tissue culture were procured from BD Biosciences.

Corning Incorporated Costar

E.I.A./R.I.A 8 well strips (2580), cell scraper (3010).

Merck

Ethanol Absolute (1030787), Dextrose anhydrous purified (Glucose) (17809).

1.2 Buffers and Solutions

Reagents and solutions for SDS-PAGE

30% Acrylamide solution

Acrylamide	29.8 g
N, N-Methyl bis acrylamide	0.8 g

The volume was makeup to 100 ml with deionized water. The solution was stored in an amber-colored bottle at 4°C.

1.5M Tris (pH 8.8) for resolving solution

Tris base	18.17 g

Tris was dissolved in 70 ml of distilled water and the pH was adjusted to 8.8 using 1N HCl. The final volume was makeup to 100 ml with distilled water. The solution was stored at 4°C.

1.0M Tris (pH 6.8) for stacking solution

Tris base	12.11 g

Tris was dissolved in 70 ml of distilled water and the pH was adjusted to 6.8 with 1N HCl. The final volume was makeup to 100 ml with distilled water.

10% Sodium Dodecyl Sulphate (SDS) solution

SDS	10 g

SDS was dissolved in 80 ml and the volume was makeup to 100 ml with distilled water. It was stored at room temperature.

10% (w/v) Ammonium Persulphate (APS)

APS	0.1 g

0.1 g of APS was dissolved in 1 ml of distilled water. This solution was always freshly prepared before use.

10X Electrophoresis running buffer

Tris base (250mM)	30.2 g
Glycine (2.5M)	188 g
SDS (1%)	10 g

Dissolve it in distilled water and the volume was makeup to 1000 ml. Working solution (1x) was made in distilled water.

Gel composition

Resolving gel	**8%**	**10%**	**12%**
Distilled water	4.6 ml	4 ml	3.3 ml
30% Acrylamide	2.7 ml	3.3 ml	4.0 ml
1.5M Tris-HCl (pH 8.8)	2.5 ml	2.5 ml	2.5 ml
10% APS	100ul	100ul	100ul

10% SDS	100ul	100ul	100ul
TEMED	6ul	4ul	4ul

1 ml of Isopropanol or Isobutanol was added on the top of the gel.

Stacking gel	**5%**
Distilled water	6.8 ml
30% acrylamide	1.7 ml
1.0M Tris-HCl (pH 6.8)	1.25 ml
10 % APS	100ul
10% SDS	100ul
TEMED	10ul

4x Laemmli's sample buffer

Tris-HCl pH 6.8 (200mM)	2.42 g
2-mercaptoethanol (20%)	20 ml
SDS (8%)	8.0 g
Bromophenol Blue (0.4%)	0.4 g
Glycerol (40%)	40 ml

Dissolve it in 100 ml. pH was adjusted to 6.8

NP-40 Lysis Buffer

Tris-HCl (pH 7.5)	20 mM
NaCl	150 mM
EDTA	1 mM
EGTA	1 mM
Glycerol	10%
NP-40	1%
Protease Inhibitor Cocktail	1 tablet for 10 ml of lysis buffer
Phosphatase Inhibitor Cocktail	1X (10ul/ml)

#Phosphatase inhibitors were freshly used.

Transfer Buffer

Methanol transfer Buffer

Glycine	14.0 g
Tris	3.0 g
Methanol	200 ml

Volume was makeup to 1000 ml with distilled water and was kept at 4°C; transfer conditions:

180 mA for 3.5 hours at 4^0C.

10X Tris Buffered Saline (TBS)

Tris base	24.2 g
NaCl	80 g

Dissolve in 900 ml of distilled water and pH was adjusted to 7.6 with 0.1N HCl and volume was makeup to 1000 ml.

Western Blot Washing buffer (TBST)

0.1 % Triton X-100 in 1X TBS solution.

Stripping solution

Glycine (100mM) pH 2.5	0.75 g
KCL (50mM)	0.372 g
Magnesium acetate (20mM)	0.428 g

Dissolve in 50 ml distilled water. pH was adjusted to 2.5 with 1N HCl and the Volume was makeup to 100 ml.

Blocking buffer (5% milk)

5% non-fat dry milk or BSA was dissolved in TBS-T.

Ponceau S

Ponceau S	0.1 g
Acetic acid	5 ml
Deionized water	95 ml

Solutions for General Use

PBS 10X (pH-7.4)

NaCl	80 g
$Na_2HPO_4.7H_2O$	14.4 g
KH_2PO_4	2.4 g
KCl	2 g
Deionized water	1000 ml

pH was adjusted to 7.4 Working solution 1X was made in distilled water.

5X TBE (Running Buffer for DNA gel)

Tris base	27 g
Boric acid	13.75 g
0.5 M EDTA	10 ml

Make up the volume with 500 ml distilled water.

ELISA REAGENTS

Binding buffer	IX PBS (pH 7.4) or 0.1M Na_2HPO_4 adjusted pH to 6.0 or pH 9.0 with 0.1M NaH_2PO_4.
Washing buffer	0.05% Tween-20 in 1X PBS.
Blocking Buffer	1% BSA in 1XPBS.
Substrate	TMB/H_2O_2.
Stop Solution	1ml H_2SO_4 + 35 ml deionized water

RBC Lysis Buffer

Ammonium chloride	4.01 gm
Sodium bicarbonate ($NAHCO_3$)	0.42 gm
EDTA	0.185 gm

Make up the volume with 50 ml distilled water. Then filter it with 0.22um.

FACS Reagent

FACS Buffer (100ml)

FBS	3 ml
1XPBS	97 ml

Perm Wash Buffer (100ml)

Saponin	0.05%
FBS	3%
1xPBS	100 ml

Perm Fix Buffer (100ml)

PFA	4%
Saponin	0.1%
1X PBS	100 ml

Fixation Buffer

1% PFA in 1xPBS

Cesium Chloride Density Gradient Ultracentrifugation

Solution1

Glucose	0.9 gm
1M Tris-cl pH 8.0	2.5 ml
EDTA pH (1M) 8.0	1 ml

Make up the volume with 100 ml Distilled water.

Solution2

10% SDS	10 ml (Final 1% SDS)
2N NAOH	10 ml (Final 0.2N NAOH)

Make up the volume with 100 ml Distilled water.

Solution3

Potassium acetate (3M)	29.4 gm
Glacial Acetic acid	11.5 ml

Make up the volume with 100 ml Distilled water.

Procedures

Mice

BALB/c mice (Jackson Laboratories, Bar Harbor, ME) were nurtured and bred in the Institute's Experimental Animal Facility (Thoren Caging Systems, PA) and all experiments were performed following the animal use protocols approved by the Institutional Animal

Care and Use Committee. The mice used in this study were 6 to 8 weeks old and sacrifice it by asphyxiation.

Parasite culture

Leishmania major (strain MHOM/Su73/5ASKH) and *Leishmania donovani* (strain MHOM/In83/Ag83) were maintained in vitro in RPMI 1640 medium (GIBCO BRL; Grand Island, NY) containing penicillin (100U/ml), streptomycin (100 ug/ml), 2-mercaptoethanol (50 uM), sodium pyruvate (1 uM), L-Glutamine (2mM), supplemented with 10% FCS (GIBCO-BRL). The parasite virulence was maintained by the passage of 2×10^7 (i.v) stationary-phase *L. donovani*, 2×10^6 (Footpad) *L. major* promastigotes through BALB/c mice. In the case of *L. major* 5 weeks infected popliteal lymph nodes were collected, whereas in *L. donovani* spleen were collected and then crushed. The supernatant was further cultured in RPMI 1640 supplemented with 10% FCS along with penicillin, streptomycin, sodium pyruvate, and L-glutamine in a 25mm culture flask (non-vented) for 5-7 days for transformation into motile promastigotes form from the amastigotes. These promastigotes were further used for in vitro macrophage infection.

***LmjPAP* cloning in pcDNA6HisA vector**

The full-length coding region of *Leishmania major* strain Friedlin PAP (LMJF_19_1350) (Phosphatidic Acid Phosphatase) was amplified by semi-quantitative PCR by using a gene-specific primer. The amplified product was cloned into a mammalian expression vector pcDNA6His/A. Different sets of primers were used for cloning in pcDNA6/HisA Forward Primer: **CGCGGATCCAG**ATGGTAGTGCTCAGCACGAAG (BamHI) Reverse Primer: **CTAGTCTAGA** TTAGATCCACGGAACGGCC (XBAI). The PCR reaction was carried out at following conditions: 95^0C for 3min (initial denaturation), 94^0C for 1minute (denaturation), 57.2^0C for 1minute (annealing), 72^0C for 1minute (extension). The reaction was carried out

for a total of 33 cycles followed by a final extension at 72^0C for 10 minutes. The 1203bp amplified product was ligated into expression vector pcDNA6/HisA and the recombinant plasmid pcDNA6/HisA-LmjPAP were transformed into *E.coli* DH5α for plasmid isolation. The recombinant plasmid pcDNA-PAP was purified by cesium chloride density gradient centrifugation.

In vivo infection to mice

$2x10^6$ *Leishmania major* promastigotes (HBSS; 50µl) in the stationary phase were used to infect the BALB/cmice subcutaneously in the hind footpad. $2×10^7$ *Leishmania donovani* promastigotes (HBSS; 50ul) in the stationary phase were used to infect the BALB/c mice intravenous in the tail vein.

Harvesting of peritoneal macrophage and *in vitro* challenge with the parasite

2 ml of 3% thioglycolate was injected intraperitoneally to Balb/c mice, 5 days later mice were sacrificed, peritoneal exudates i.e macrophages were harvested by the 18-gauge needle in a sterile HBSS (GIBCO BRL; Grand Island, NY). Peritoneal exudates were collected and centrifuged at 1200 rpm for 8minutes at 4^0C. Macrophages were in vitro cultured in advanced RPMI 1640 supplemented with 10% heat-inactivated FCS along with penicillin, streptomycin, sodium pyruvate, L-glutamine (GIBCO, BRL; Grand Island, NY) in a humidified 37^0C CO_2 incubator (Forma Scientific) for 6hr followed by washing to remove non-adherent cells. Thioglycollate-elicited peritoneal macrophages from BALB/c mice have infected with *L. major* & *L. donovani* promastigotes at a ratio of 1:10; MΦ: parasites for 6hr at 33^0C and 37^0C respectively. Extracellular parasites were then washed and the macrophages were cultured for 72hr. At 64^{th} hour stimulate the infected cells with anti-mouse CD40 NALE (3ug/ml), 8hours later lyse the cells with trizol for gene expression studies.

Co-culture of T cells with infected macrophage

BALB/c-derived thioglycolate-elicited macrophages (5×10^4/well plated in triplicates in an 8-well chamber slide; Nunc, Rochester, NY) were cultured in advanced RPMI-1640-10% FCS in a humidified 37^0C CO_2 incubator for 6hr, followed by infection with *L. donovani* promastigotes at a ratio of 1:10 for 6hr. Extracellular parasites were further washed out and the macrophages were cultured for 40hr, followed by the addition of purified $CD4^+$ T cells (1 macrophage:3 T cells) from the spleen of LmjMAPK10 immunized, *L. donovani* infected mice on day 14^{th} and 28^{th} and were cultured for another 56 hr. At the end of the incubation time, the cells were washed twice with 1X PBS to remove any traces of media and fix it with ice-cold methanol for 4min. Slides were left air-dried to overnight and later Giemsa stained. Slides were stained with Giemsa (1:10 diluted) for 45 min. Slides were washed repeatedly with deionized water and then air-dried it for overnight. The parasite load was counted through microscopically and expressed as a number of amastigotes per 100 macrophages. Culture supernatant was used for enumeration of cytokine (IL-12, IFN-γ, TNF-α, IL-10, IL-4) and NO-mediated assays.

BALB/c mice immunization with M10 DNA

BALB/c mice (n=8 per group) were immunized (i.m) using a 26-gauge needle with three doses of 100µg (in 100µl 1x HBSS) M10 DNA or pcDNA6/HisA empty vector (i.e Control DNA) on days 0, 15 and 30 following the previous immunization protocol. Four weeks post-immunization, the primed mice were infected (i.v) with 2×10^7 (in 100µl 1x HBSS) infective stationary-phase promastigotes as follows: Control DNA, M10 DNA, and only infection without immunization (but they remain naïve before infection) except naïve mice group. Sera were collected before and 14 and 28 days after *L. donovani* infection.

Giemsa-staining and Leishman Donovan Unit (LDU)

Splenic and Liver tissue weight were measured later on splenic and liver tissue pieces were cut and exactly imprinted on the slides. Slides were fixed in chilled methanol for 4min. Slides were air-dried overnight and later stained with Giemsa. Slides were stained with Giemsa (1:10 diluted) for 45 min. Slides were washed repeatedly with deionized water and then air-dried it for overnight. Giemsa-stained splenic and liver stamp-smears were enumerated under a Nikon microscope and expressed by the formula- (amastigotes number/1000 nucleated cells) × organ weight in mg.

Determination of antibody titers

96-well ELISA plates were coated with CSA (Crude soluble antigen of *L. donovani*) 15µg/ml in 50µl 1xPBS (overnight at 4^0C). CSA was prepared by rapid-freeze thaw up to (7 cycles), later on, sonicate it and wait for clear the suspension followed by centrifugation. The protein content was analyzed by the Bradford method. The plates were blocked with 1% BSA (2hr, RT) and washed thrice with PBS-0.05% Tween-20. Serially diluted serum samples were added from all the groups of mice and kept overnight at 4^0C. Later on, the plates were washed thrice with wash buffer and anti-LdMAPK10 antibodies were probed sequentially with biotinylated anti-mouse IgG1 (0.25µg/ml), IgG2a (0.25µg/ml) or IgM (1µg/ml) (BD Pharminogen, San Diego, CA), HRP-streptavidin and a TMB substrate. Finally, the enzymatic reaction was stopped using 1N H_2SO_4 and absorbance was read at 450nm.

Cytokine ELISA

1×10^6 splenocytes from the immunized, *L. donovani*-infected mice were collected and stimulated with CSA (25µg/ml) and kept at 37^0C. After 56h, supernatants were collected and estimated for IL-12, IFN-γ, IL-4 and IL-10 secretion by sandwich ELISA from all groups of mice. Briefly, 96 well ELISA plates were coated with anti-cytokine capture antibody and incubate it overnight at 4^0C. Wells were washed twice with washing buffer and then blocked

it for 4 hours with 200 ul of 1% BSA at 4^0c. Then wash thrice with wash buffer followed by the addition of samples and standards (50ul each) and plates were then kept overnight at 4^0C. After 3 washes biotin-conjugated antibody (biotin-labelled anti-cytokine antibody) was added for 4 hours and incubate it at 4^0C. Wells were then washed thrice and streptavidin POD was added for 45 minutes and plates were kept for incubation at 4^0C. Then wells were washed twice and 100ul substrate was added. Wait for colour development for 20 minutes, the reaction was stopped by the addition of H_2SO_4 stop solution (50ul) and read at 450 nm in an ELISA reader (VERSA max microplate reader, Molecular Devices). The values of standards were plotted and the quantity of the cytokines was assessed in the samples and expressed in pg/mL.

Quantification of NO

NO production in supernatant from the co-culture experiment was measured by Griess reagent (Sigma-Aldrich, USA). Standard and samples were incubated with an equal amount of Griess reagent for 20 min at RT to develop colour and absorbance were read at 540nm in an ELISA reader.

FACS analyses

1×10^6 Splenic T cells from immunized mice were studied for Treg, T_H17, T_H1, T_H2, T_m by multicolour FACS analyses. After blocking with 30% FCS for 30 minutes at 4ºC, the cells were washed twice with FACS buffer (1x PBS with 3% FCS) and stained with fluorescently labelled Abs anti-CD3-FITC, anti-CD4-Pacific Blue, anti-CD25-APC-Cy7, anti-GITR-PE-Cy7, and anti-CD127-PerCP-Cy5.5, anti-CCR7 PE for 45 minutes at 4ºC in dark and washed twice with FACS buffer. For intracellular staining, the cells were permeabilized by using Cytofix/Cytoperm-Plus Kit with Golgi Plus and washed twice with Perm/Wash buffer. The cells were then stained with anti-Foxp3-Alexa Fluor 647, anti-IL-10-PE, anti-RORγt-PE and

anti-IL-17-APC, anti-IFN-γ PE, anti-T-BET APC, anti-IL-4 PE, anti-GATA3 APC antibodies for 60 minutes at 4ºC in the dark. Cells were acquired in the $CD3^+CD4^+CD25^+CD127dim$-gate for T-reg cells and $CD3^+CD4^+CD44^+CD62L$- gate for T_H17 cells, $CD3^+CD4^+$ gate for T_H1 and T_H2 cells, $CD3^+CD4^+CD44^+CD62L$ (Low) -gate for T_{EM} and (High) for T_{CM} cells by FACS Canto II flow cytometer (BD Biosciences) and analyzed for expression of GITR and Foxp3 or Foxp3 and IL-10, GITR and IL-10, IFN-γ and T-bet, IL-4 and GATA-3, and RORγt and IL-17A, and CCR7 +ve and -ve using BD FACS Diva software (version 5.2; BD Biosciences). Cells stained with specific Isotype or single +ve antibodies were used as controls.

RNA isolation and Real Time-PCR

Using TRI-reagent (Sigma-Aldrich, St. Louis, MO), total RNA was isolated from the splenocytes (3×10^6) of uninfected or *L. donovani*-infected BALB/c mice. For first-strand cDNA synthesis (Life Technologies, Carlsbad, CA), total RNA (2µg) of each sample was incubated with 1X RT buffer, 0.6 µg Random Primer and heat inactivation at 65^0C (5min), followed by incubation with 0.1 mM DTT, 500µm dNTPs, 40U RNase inhibitor, 200U MMLV-reverse transcriptase at 37^0C (1h) followed by heat inactivation at 65^0C (10min). Real-time PCR was performed using gene-specific primers (Table-3). Quantitative Real-time PCR was performed in duplicates in 0.1 ml Micro Amp Fast Reaction strip tubes (Applied Biosystems; Foster City, CA) in a 10 µl reaction mixture containing 10 ng cDNA, 2 ng forward primer, 2 ng reverse primer, and 2X SYBR Premix Ex Taq II (5µl; Takara; Kusatsu, Shiga). Real-time PCR was performed on Step One Plus Real-Time PCR Systems (Applied Biosystems; Foster City, CA) under the following conditions: 95°C for the 30s, 45 cycles of 95°C for 5s, 60°C for 34s. Relative quantitation was done using the comparative threshold (ΔΔCt) method. mRNA expression levels of the target genes were normalized against those of GAPDH and expressed as relative fold change compared with untreated controls.

Statistical analysis

All the sets of in vivo experiments were repeated thrice with 8 mice per group. The results from one of the representative experiments are presented as mean ± SEM. Statistical analysis was performed using the Sigma-plot 10 and STAT3.0. The significance of the difference between the mean was determined by one-way ANOVA (Student-Newman-Keuls test). Differences were considered statistically significant when $p<0.05$(*), $p<0.01$(**) or $p<0.001$(***). Compared groups are indicated.

Table -3: Sequences of the real-time PCR primers used

No.	Gene name	Forward primer	Reverse Primer
1	GAPDH	CTCATGACCACAGTCCATGC	CACATTGGGGGTAGGAACAC
2	IL-12	CCTGAAGTGTGAAGCACCAA	AGACAGAGACGCCAT TCCA
3	IFN-γ	TGGATATCTGGAGGAACTGG	TCGGCTTGCTGTTGCTGA
4	IL-4	GTGTTCTTCGTTGCTGTGA	TCT CGAATGTACCACGAGCC
5	IL-10	AACATACTGCTAACCGACTCC	TCCTTGATTTCTGGGCCATG
6	T-Bet	GTTTCTACCCCGACCTTCCA	ACTGTGTTCCCGAGGTGTC
7	FoxP3	GGCCCTTCTCCAGGACAGA	CAGCAGAAGGTGGTGGGAG
8	Rorγt	CTTTTCACGGGAGGAGGT	ATGAAGCCTGAAAGCCGC
9	IL-17	CTCCACCGCAATGAAGACC	TTTCCCTCCGCATTGACACA