

Rationale

- ❖ Current drugs are inadequate for the treatment of visceral leishmaniasis (VL) an immunosuppressive ailment
- ❖ Regrettably, no available plant-origin antileishmanial drug present.
- ❖ Novel anti-VL therapies can rely on host immunomodulation with associated leishmanicidal action.

Theme of the Study

- Isolation of an immunostimulatory triterpenoid saponin from *G. oppositifolius*
- Identification and chemical characterization of the compound as Spergulin-A
- n-BuOH fraction and Spergulin A was screened for
- Immunostimulation and Host cell survivality
- Dose and time dependent evaluation of anti-leishmanial effect
- Evaluation of ROS, NO and different pro and anti-inflammatory cytokines
- Evaluation of molecular mechanism of anti-leishmanial effect of Spergulin-A
- Involvement of P2X7 receptors
- Involvement of p-P38 MAPK, p-JNK and p-NF-κB
- Phagolysosomal maturation promotion
- *In vivo* evaluation of anti-leishmanial activity of Spergulin-A

Materials & Methods

- ❖ Extraction, isolation and Characterization of Spergulin –A
- ❖ MΦ (RAW 264.7) Parasite (*Leishmania Donovanii*), parasite infection and treatment
- ❖ Assessment of *in vitro* leishmanicidal activity by FACS and confocal microscopy
- ❖ Measurement of extracellular NO, ROS
- ❖ Estimation of excretory cytokines
- ❖ Estimation of surface receptors and signaling intermediates by microscopy and FACS
- ❖ Measurement of phagolysosomal maturation index
- ❖ *In vivo* validation of important leishmanicidal effect of Spergulin-A

Results & Discussion

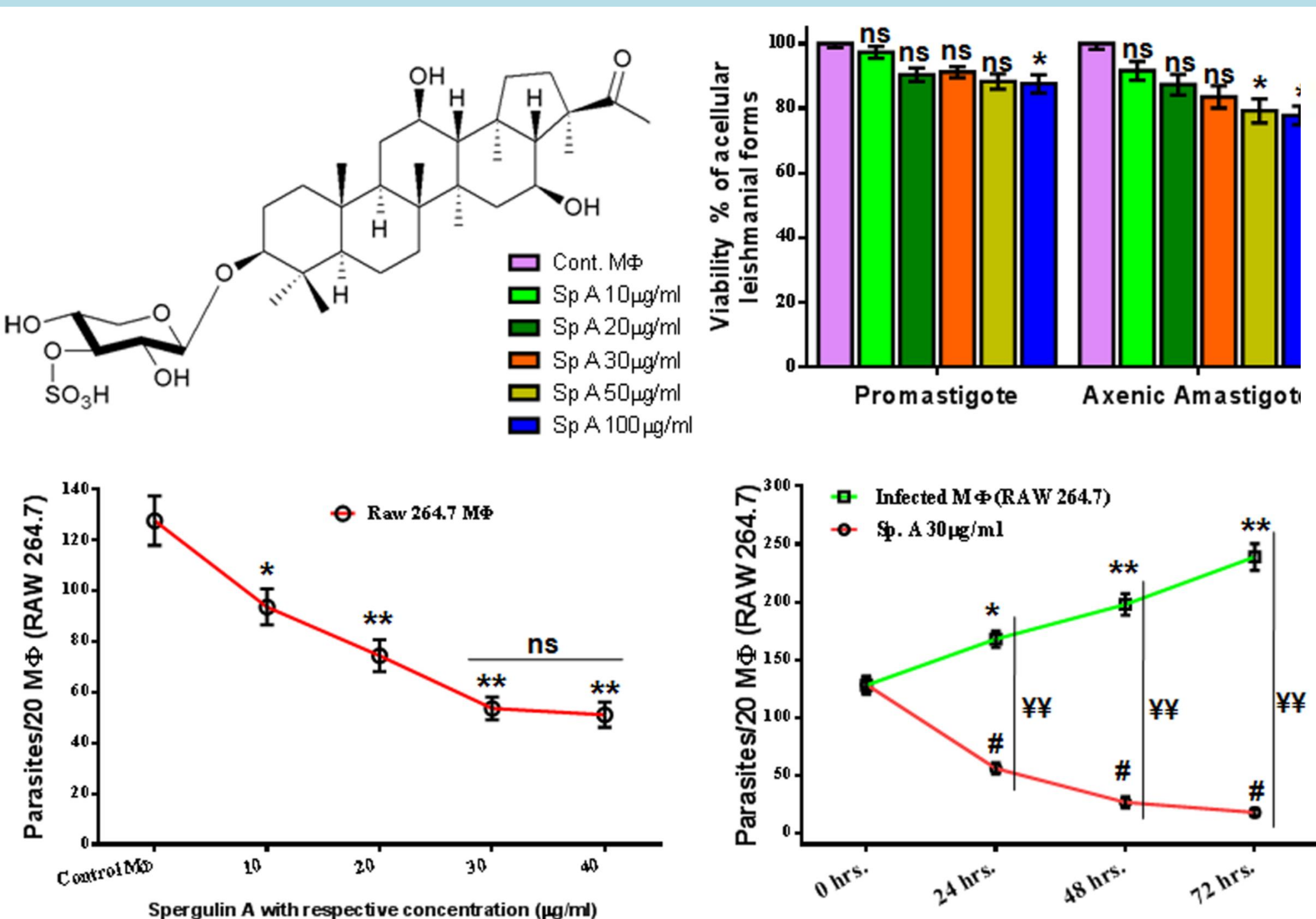


Figure 1: Spergulin-A structure. Sp-A found ineffective against promastigote and axenic amastigotes. Whereas, against amastigotes Sp-A was found significantly active within dose range of 30µg/ml.

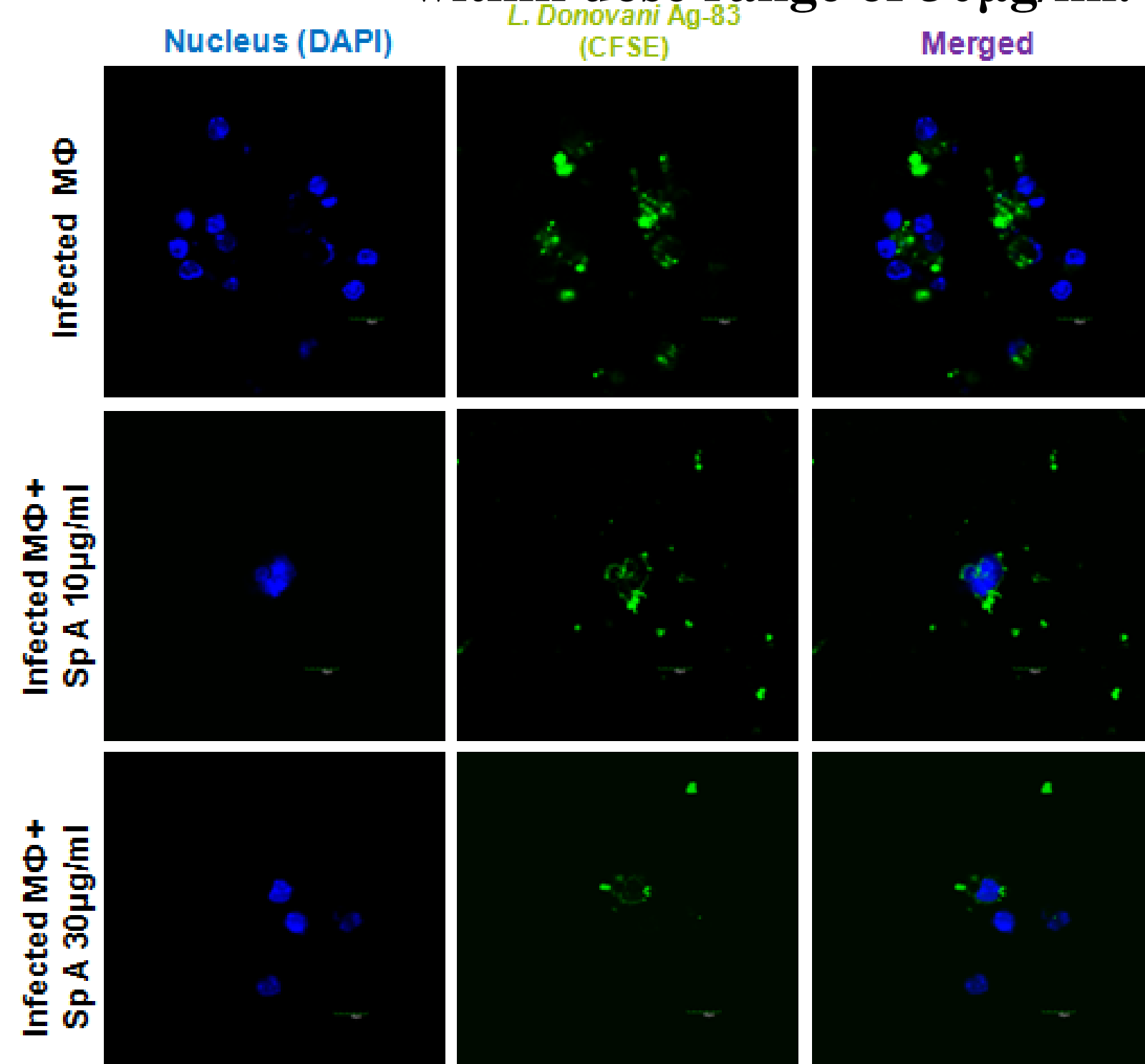


Figure 2: Dose dependent decrease of CFSE-tagged *L. donovani* after Sp-A treatment

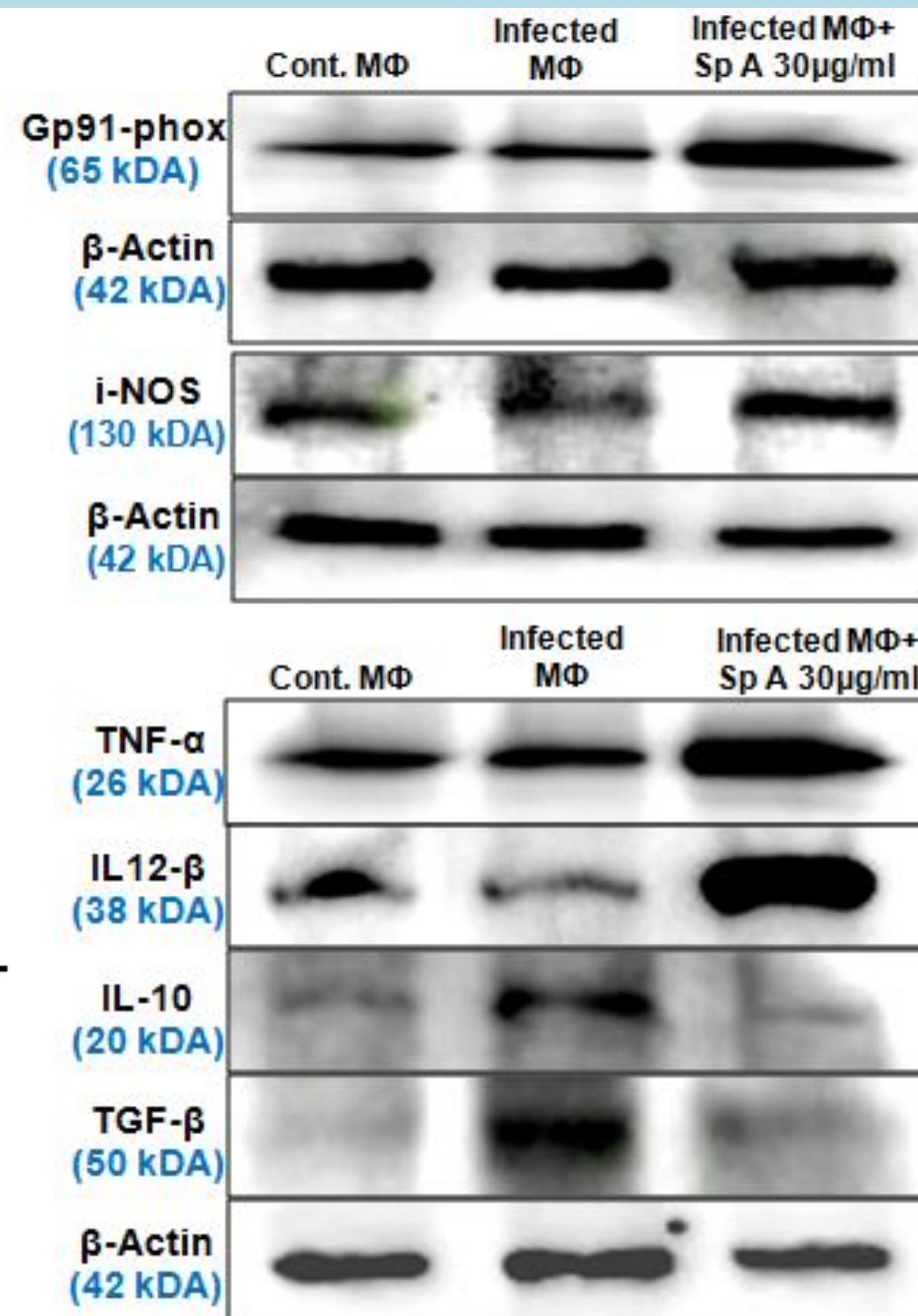


Figure 3: Western blot analyses of markers of altered redox status in the treatment panel and analyses of important pro and anti inflammatory cytokines.

Conclusions

- ❖ The inconsistency of direct leishmanicidal effect of Sp-A against acellular parasites and the proved efficacy of Sp-A against intracellular parasite was explained in the present study by demonstrating host MΦ immunostimulation.
- ❖ Sp-A mediated its action by involvement of P2X7 receptors and P38 MAPK pathway that was elaborated *in vitro* and *in vivo*.

Acknowledgements

Sincere thanks are given to Science and Engineering Research Board, Govt.of India (Grant No. PDF/2016/001437 and EMR/2015/001674) for financial assistance. And the experimental animals for their silent sacrifice.

Results & Discussion

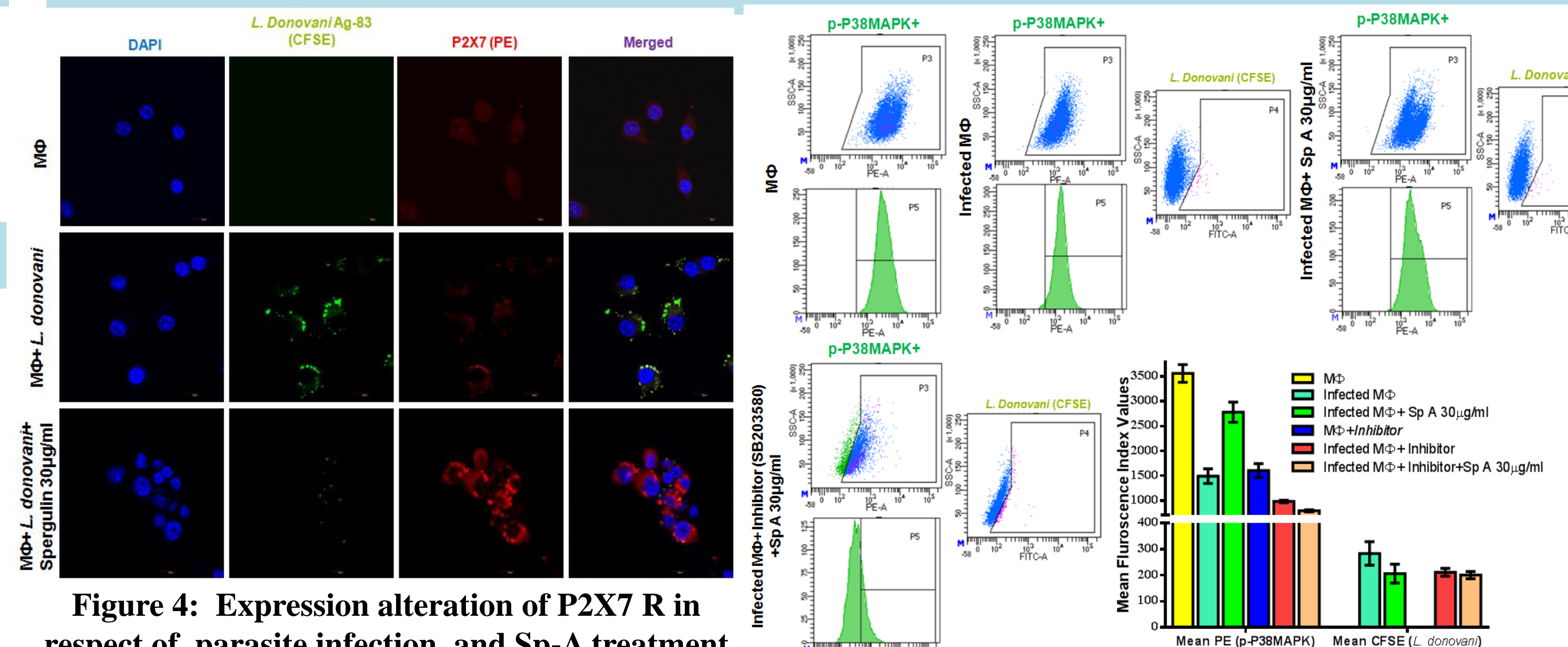


Figure 4: Expression alteration of P2X7 R in respect of parasite infection and Sp-A treatment

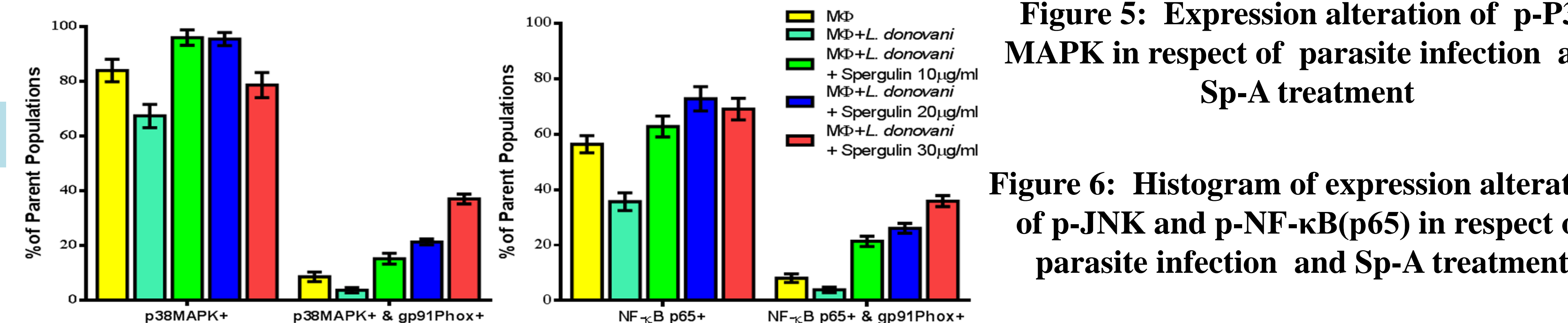


Figure 5: Expression alteration of p-P38 MAPK in respect of parasite infection and Sp-A treatment

Figure 6: Histogram of expression alteration of p-JNK and p-NF-κB(p65) in respect of parasite infection and Sp-A treatment

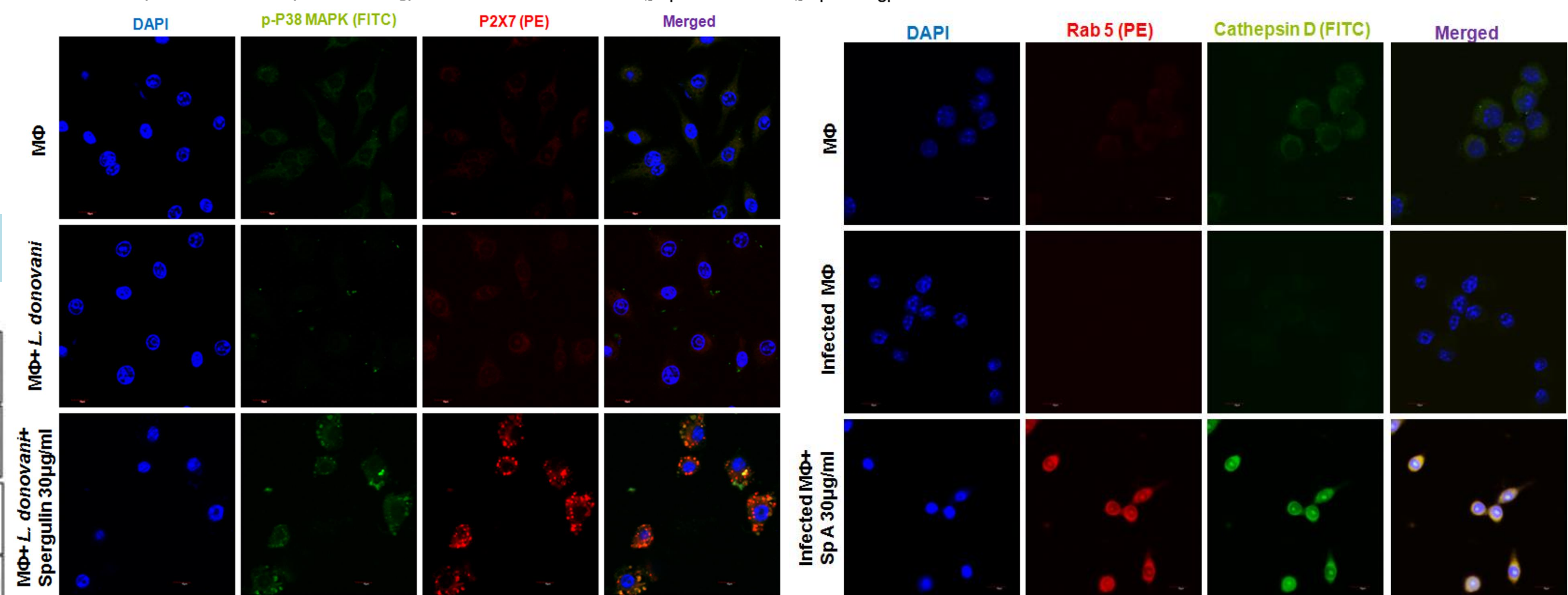
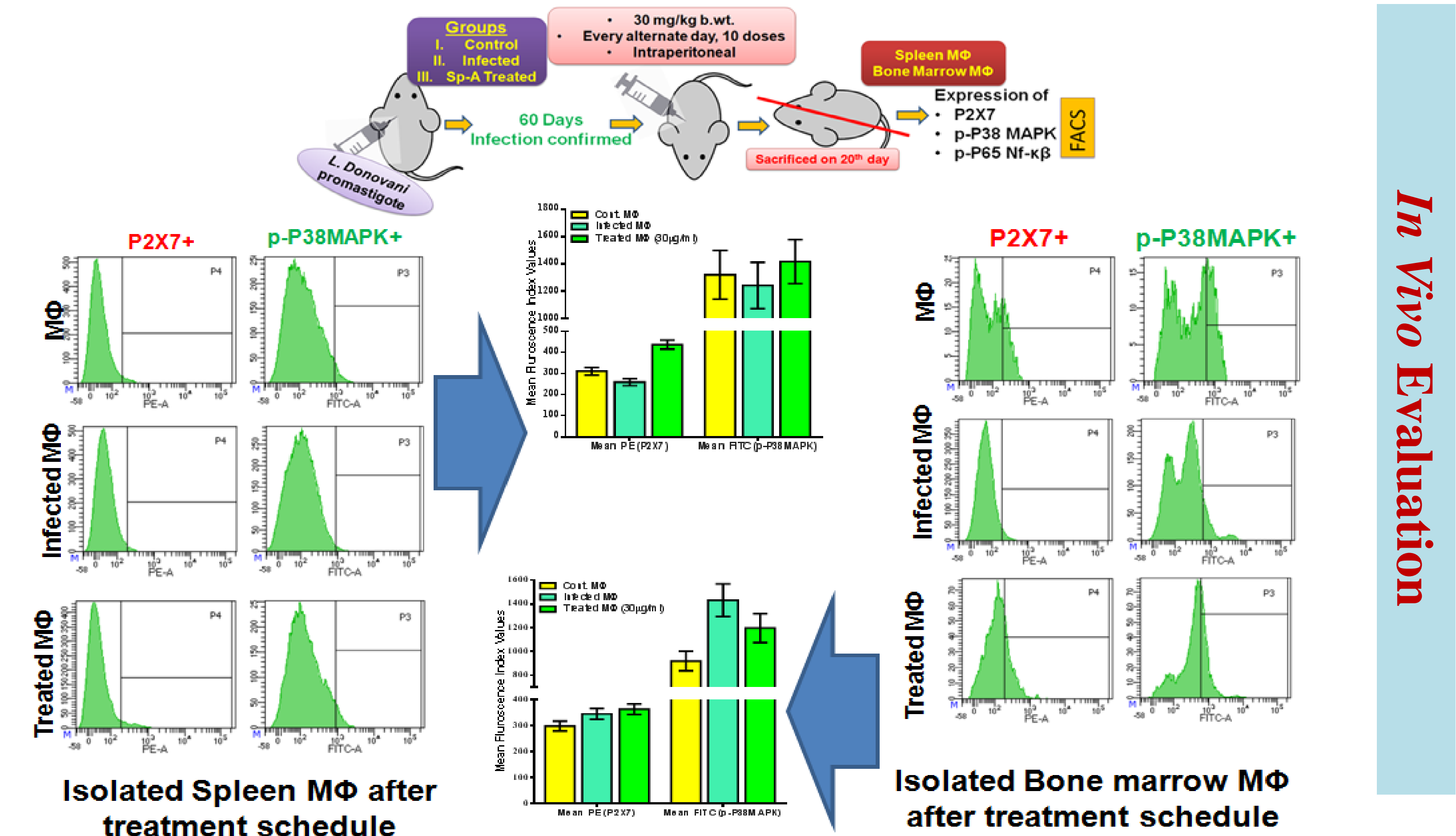


Figure 7: Confocal micrograph of co-dependent expression alteration of p-P38 MAPK and P2X7 R in respect of parasite infection and Sp-A treatment

Figure 8: Confocal micrograph of phagolysosomal maturation index in respect of parasite infection and Sp-A treatment



In Vivo Evaluation

References
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