Macrophages polarized by Toxoplasma effectors modulate hepatic stellate cells activation

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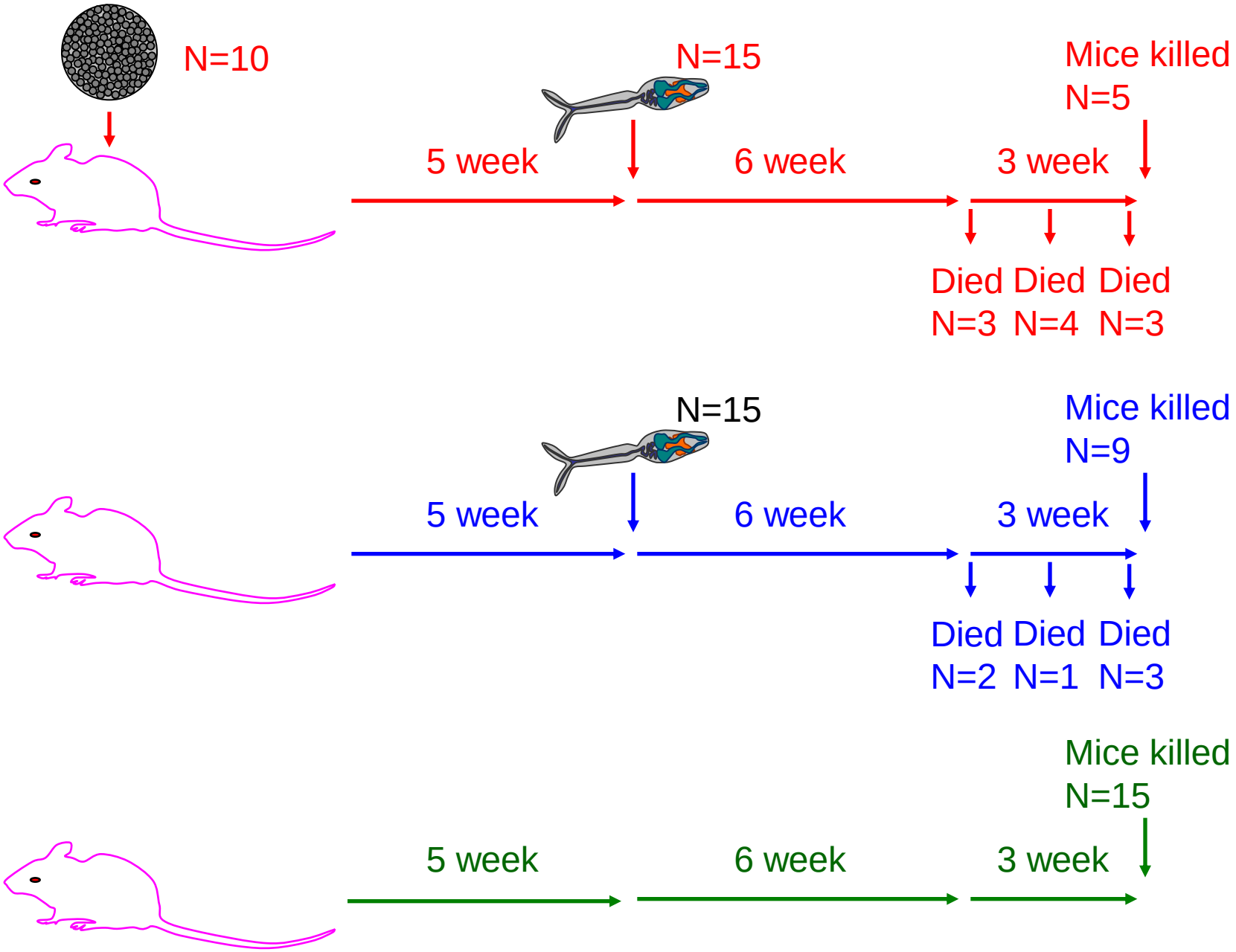
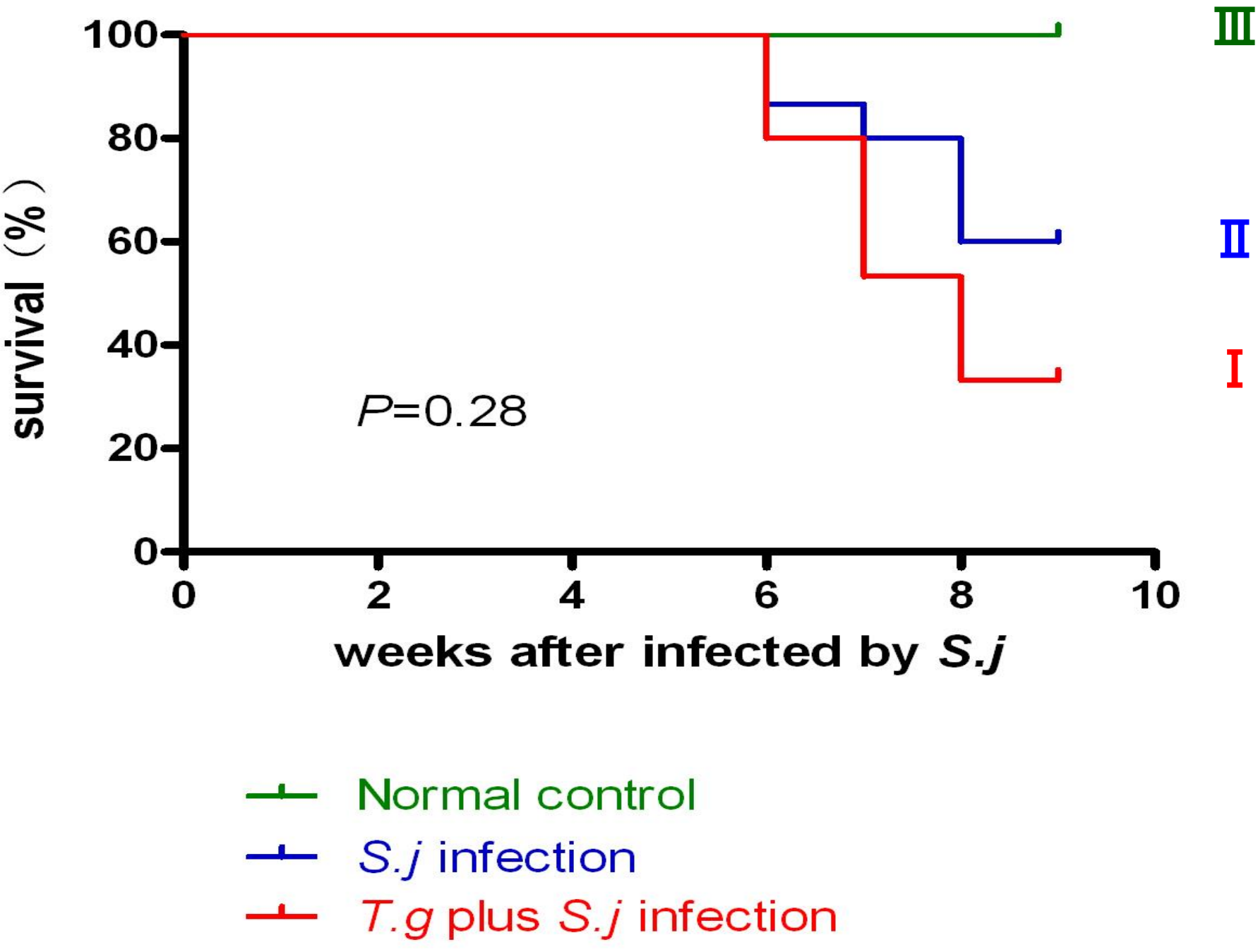
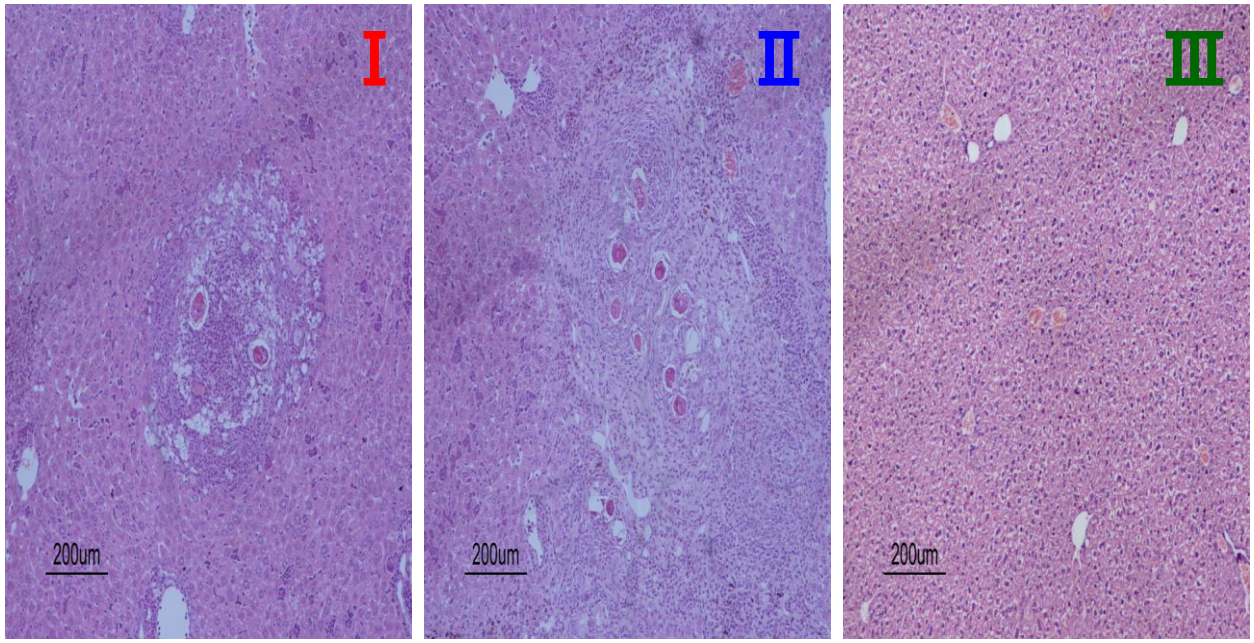
Background

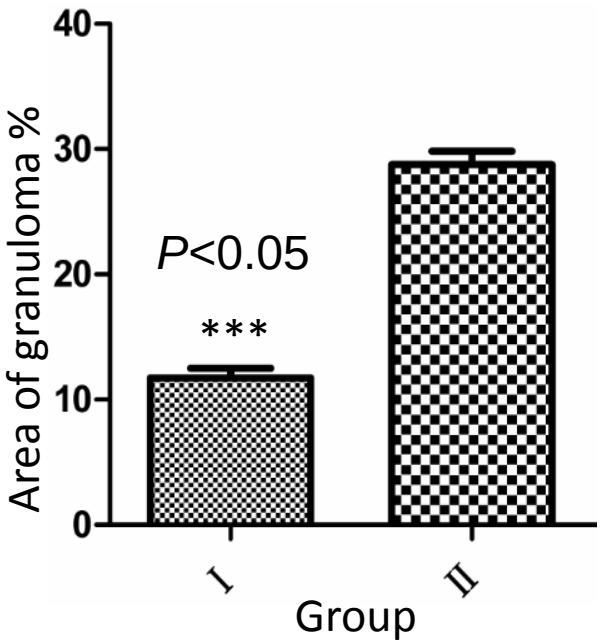
- Th1/Th2; M1(classical activated macrophage)/M2(alternatively activated macrophage)
- Our previous works
- Others previous works


N=10
N=15
Mice killed
N=5
5 week
6 week
3 week
I
Died Died Died
N=3 N=4 N=3
N=15
Mice killed
N=9
5 week
6 week
3 week
II
Died Died Died
N=2 N=1 N=3
Mice killed
N=15
5 week
6 week
3 week
III

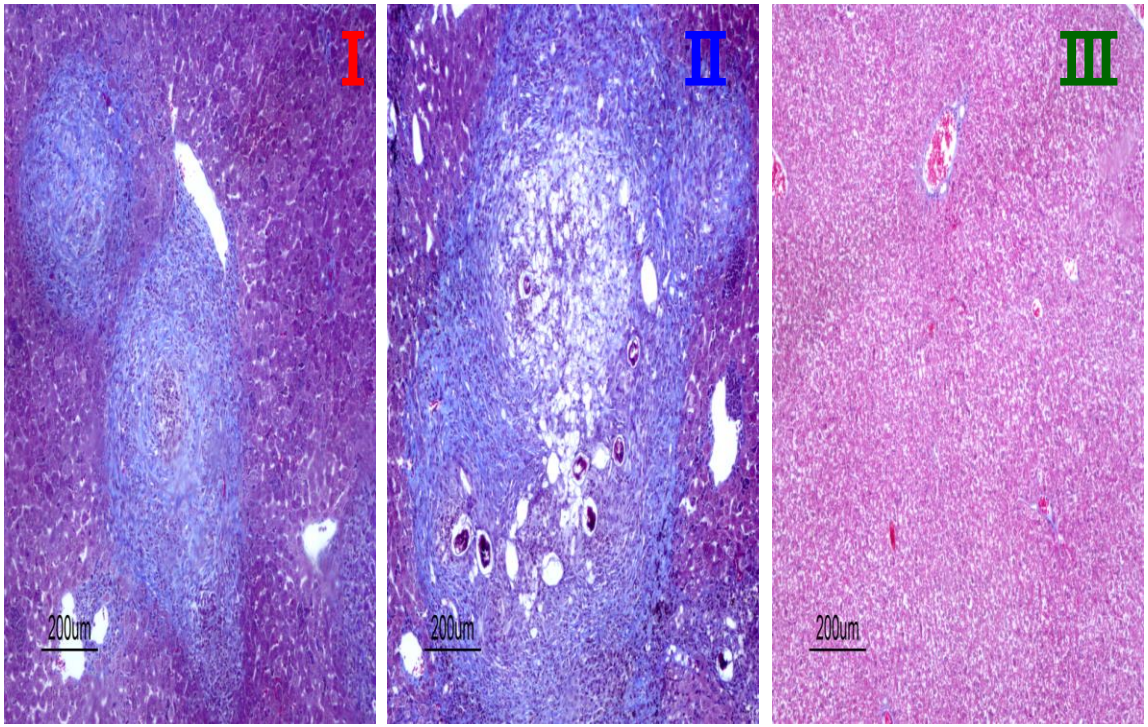

100
80
60
40
20
0
survival（%）
P=0.28
0
2
4
6
8
10
weeks after infected by S.j
Ⅲ
Ⅱ
Ⅰ
Normal control
S.j infection
T.g plus S.j infection

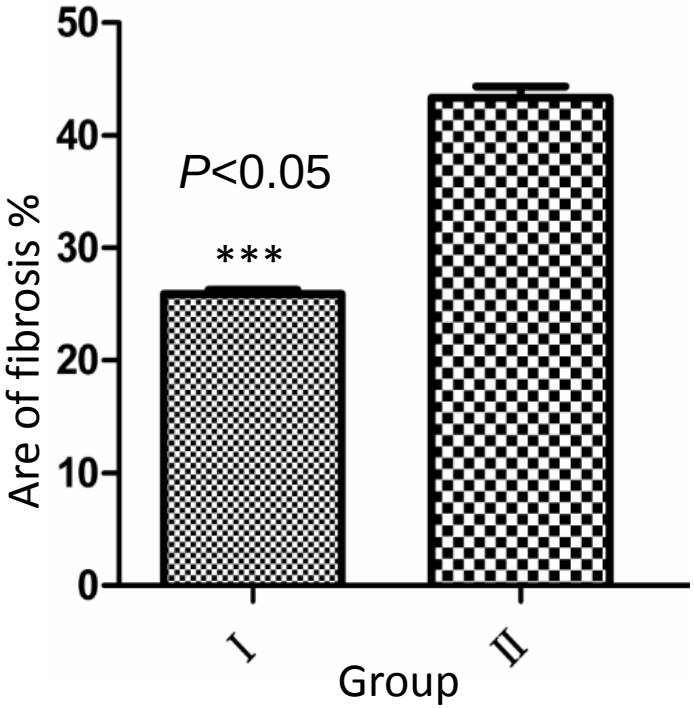
Live tissue HE staining





Live tissue Masson staining





Some results of our previous work

Liver pathology	**Egg load —**	**Worm burden —**	**granuloma ↓**	**fibrosis ↓**	**hydroxyproline ↓**
Liver tissue mRNA	**iNOS ↑**	**Arg-1 ↓**	**TGF-β1 ↓**	**IL4 ↓**	**IL13 ↓**
serum	**NO ↑**	**IFN-γ ↑**	**TGF-β1 ↓**	**IL4 ↓**	**IL13 ↓**

Suggesting that:
Th1 response increases, which drives M1 activation and inhibits Th2 response.

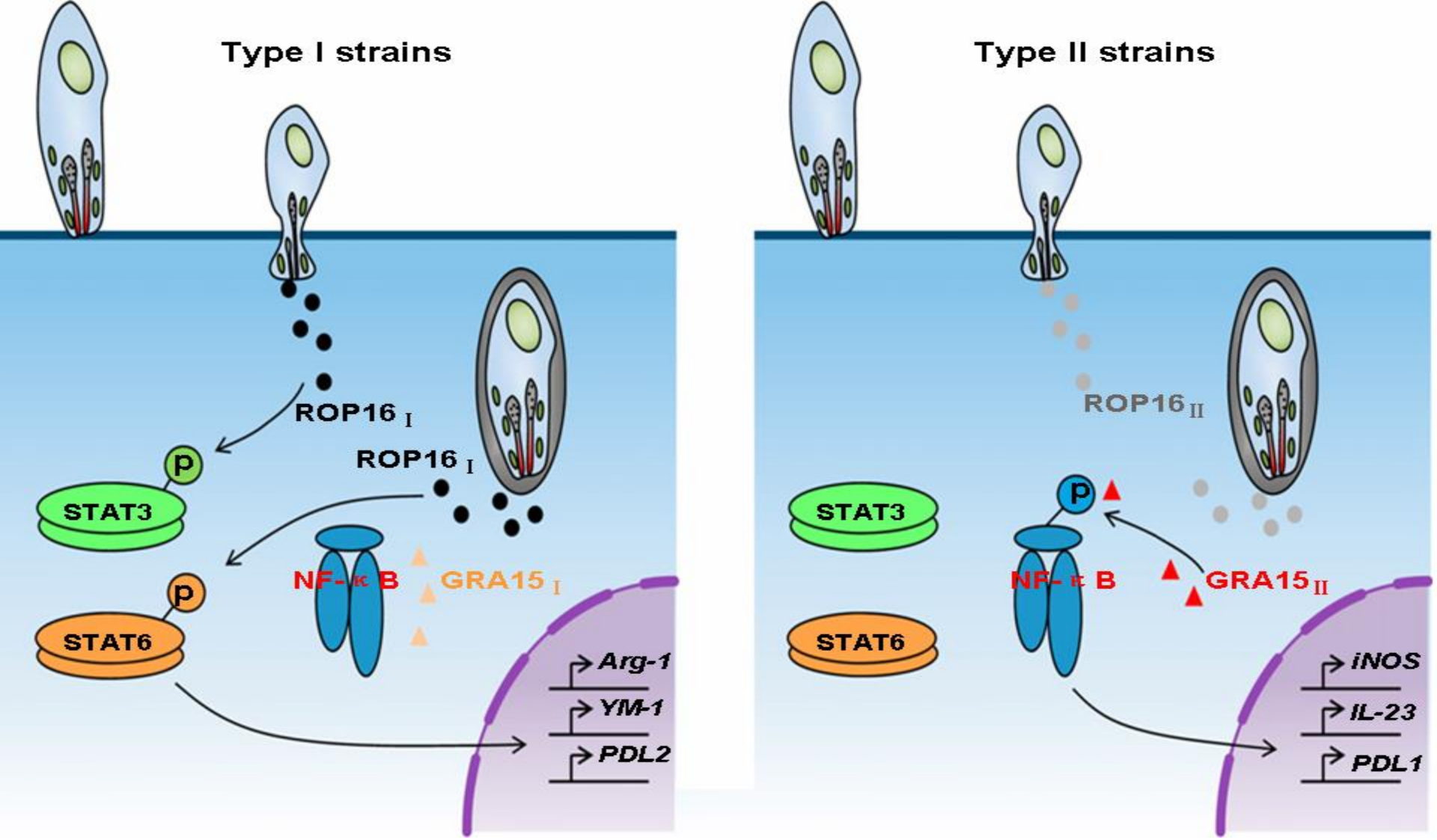
So we suppose that M1 perhaps plays an important role in decreasing the size of granuloma and the degree of fibrosis of liver in Schistosomiasis mice.

If M1 is transfered to liver of mice in a appropriate time, they maybe prevent or reverse hepatic fibrosis.

Then how to obtain the persistently activated macrophages?

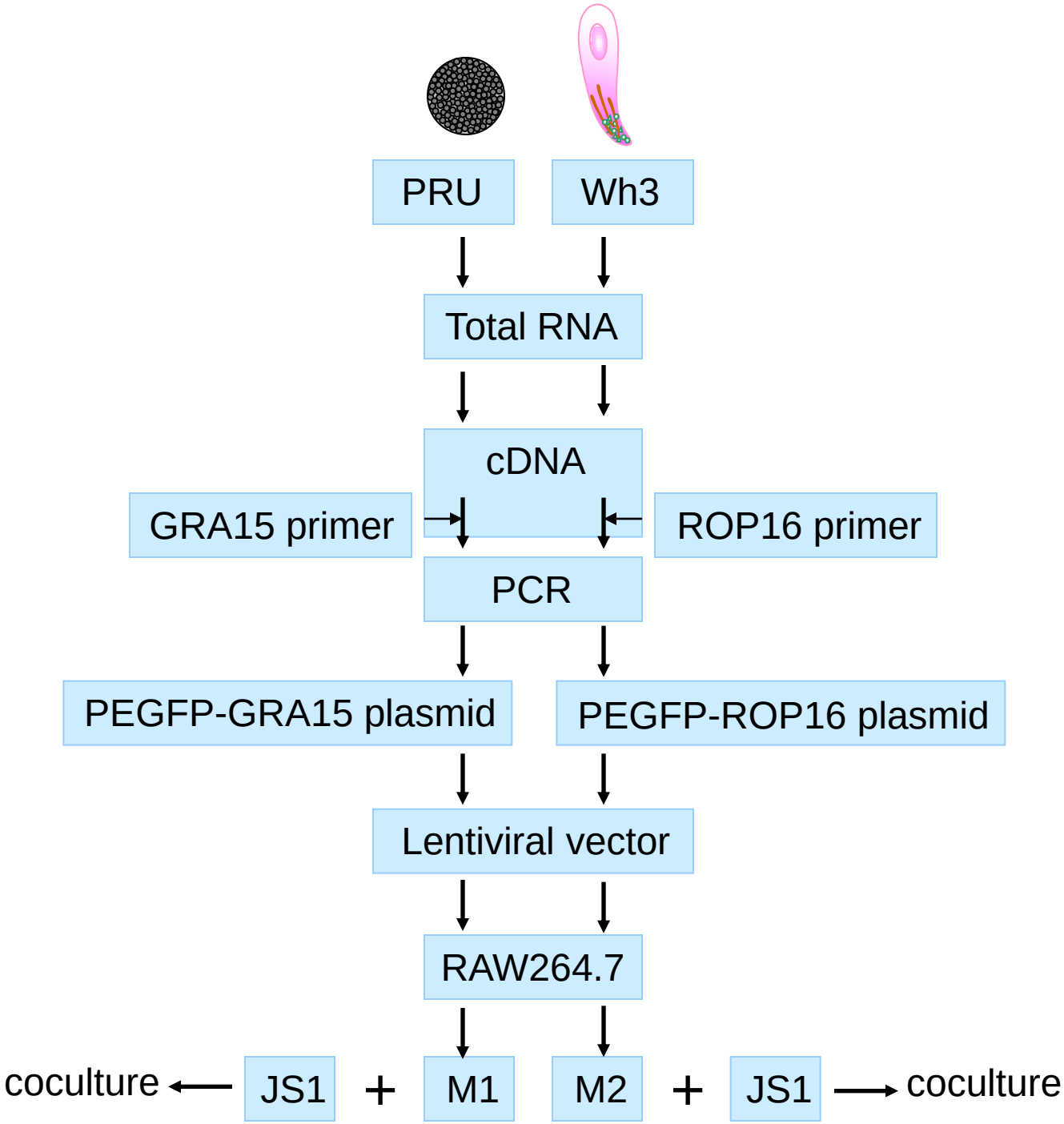
- Our previous study showed when macrophages were infected by PRU strain of *T.gondii*, *in vitro*, they could be activated to M1, when infected by Wh3 (Chinese1) strain, they could be activated to M2.

- What is the mechanisms of the genotype-associated macrophages polarization?



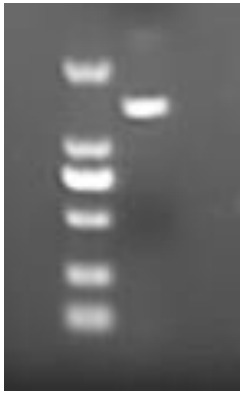
Some studies confirmed that GRA15 of Type Ⅱ strains can phosphorylate and activate NF-κB leading to an activation of M1, while the Type Ⅰ GRA15 has a negligible effect on NF-κB activation in Type Ⅰ strains. Instead, ROP16 activates STAT3 and STAT6 to drive a M2-type response. By contrast, the Type Ⅱ ROP16 has a negligible effect on STAT3 and STAT6. (Modified from Murray, 2011)

OUR PRESENT WORK


PRU
Wh3
Total RNA
cDNA
GRA15 primer
ROP16 primer
PCR
PEGFP-GRA15 plasmid
PEGFP-ROP16 plasmid
Lentiviral vector
RAW264.7
coculture
JS1
+
M1
M2
+
JS1
coculture

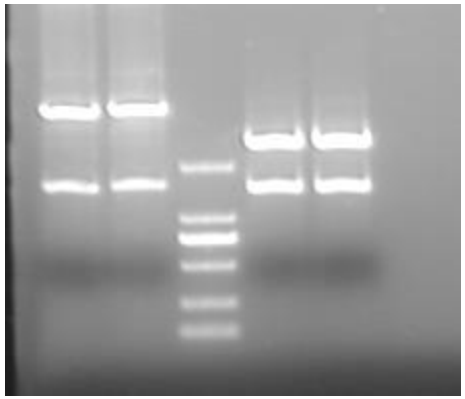
Results

- **Construct plasmid**



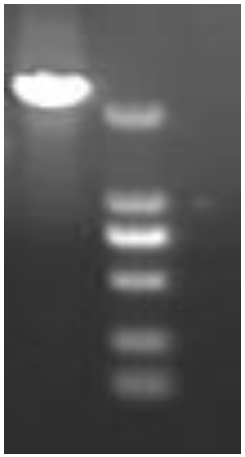
GRA15 PCR

GRA15-PEGFP plasmid

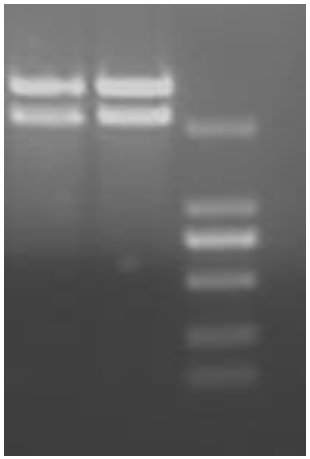


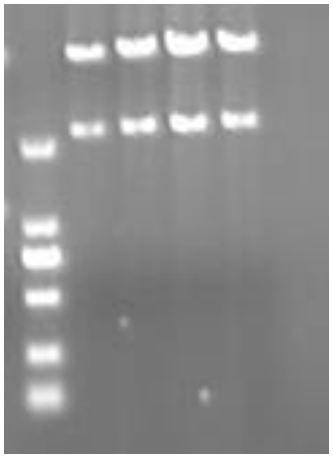
GRA15-T plasmid

ROP16 PCR



ROP16-T

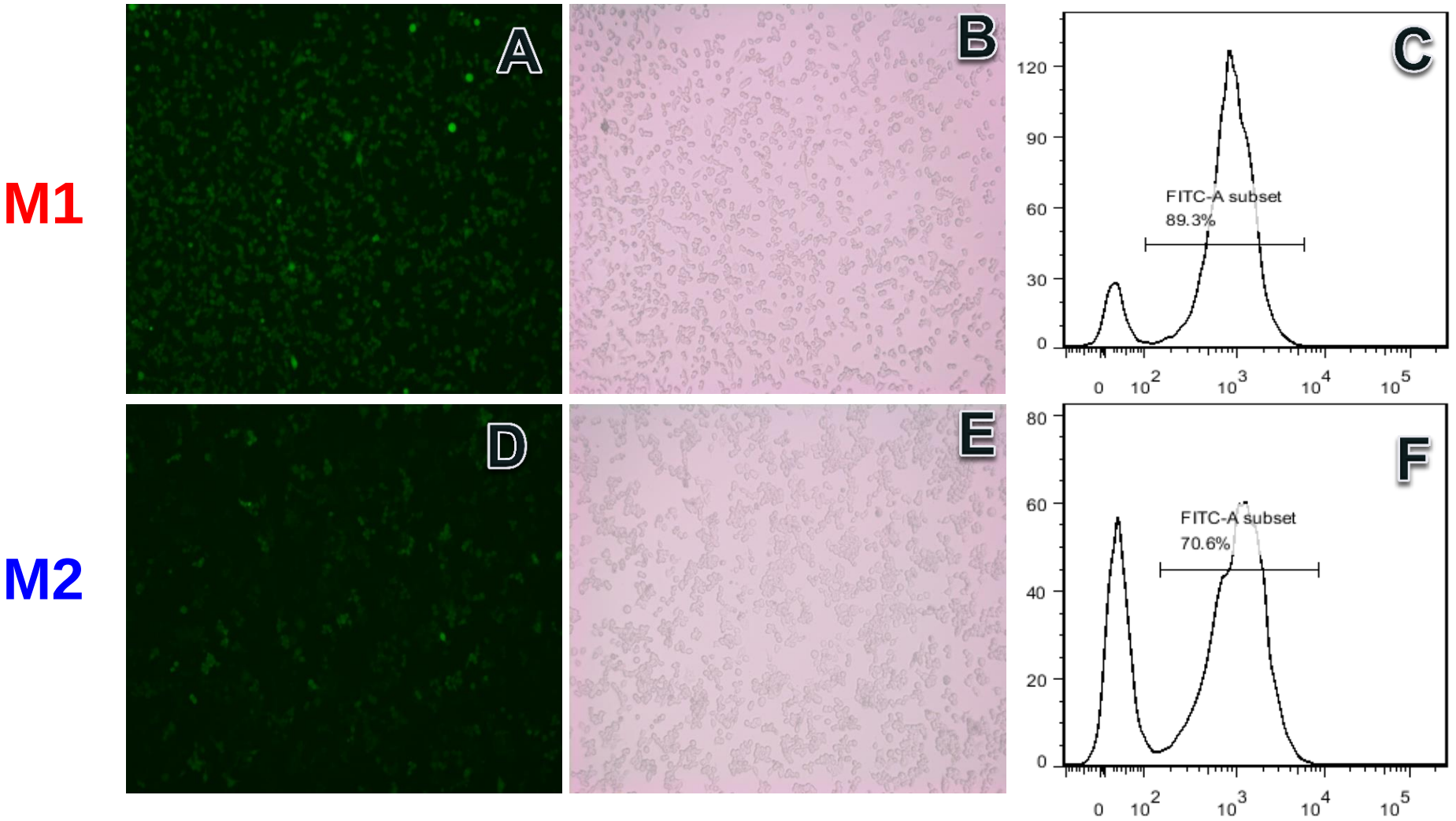




ROP16-PEGFP plasmid

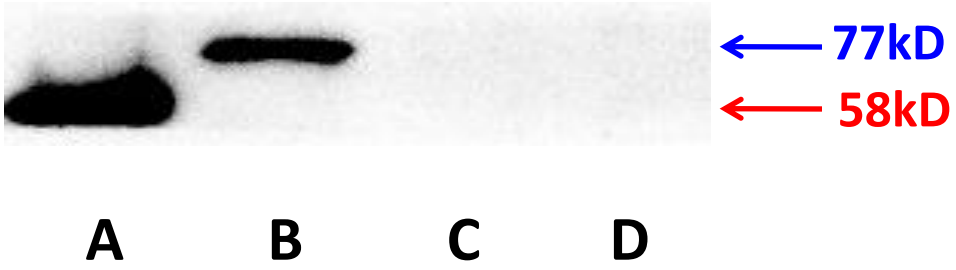
Results

- **Lentiviral vectors infect RAW264.7 (transfection efficiency)**



Results

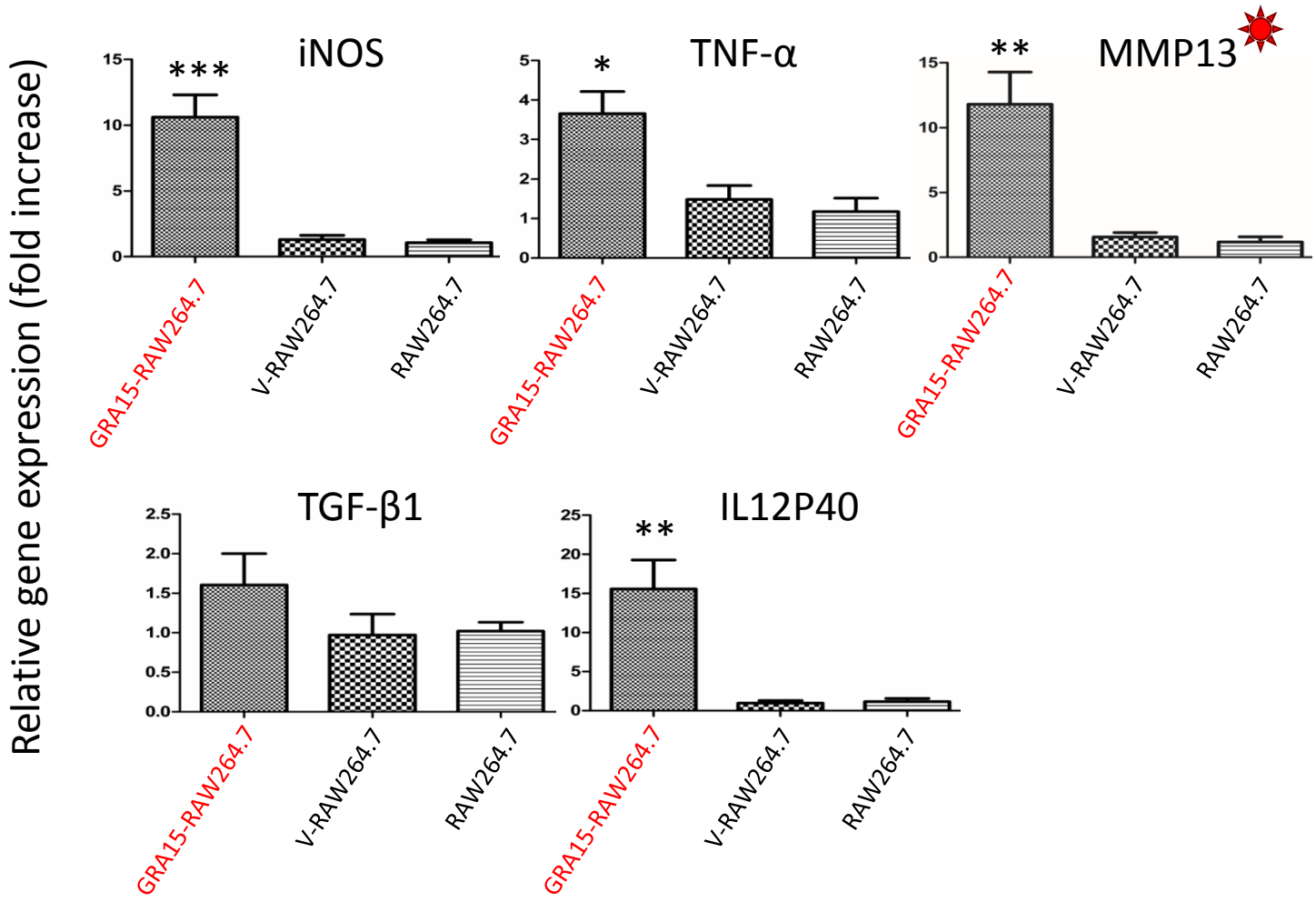
- **Western blotting verifies Flag tag protein in M1 and M2**



A: GRA15-RAW264.7;
B: ROP16-RAW264.7;
C: v-RAW264.7;
D: RAW264.7

Results

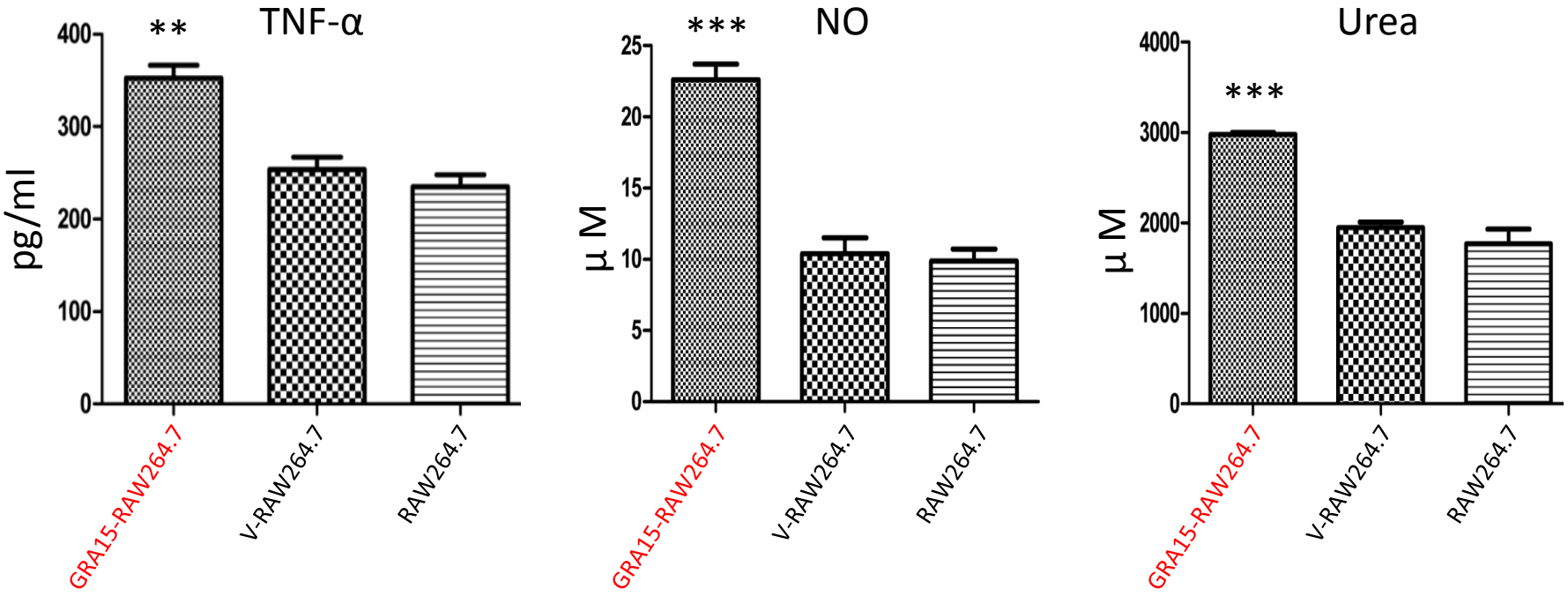
- **GRA15 protein drives RAW264.7 to M1**



Some mRNA of RAW264.7 detected by rq-PCR

Results

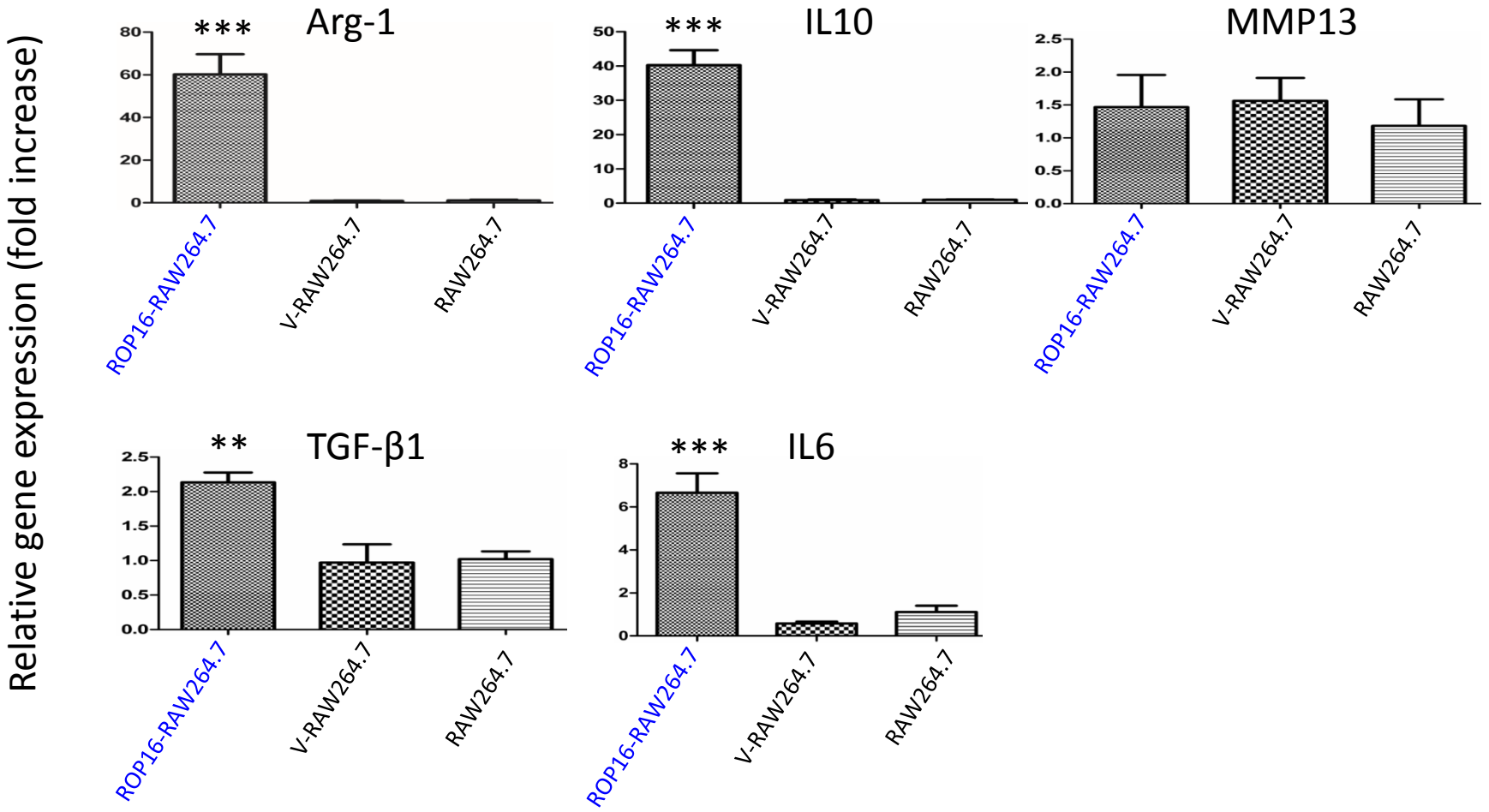
- **GRA15 protein drives RAW264.7 to M1**



Some cytokines of RAW264.7 detected in supernatant by ELISA

Results

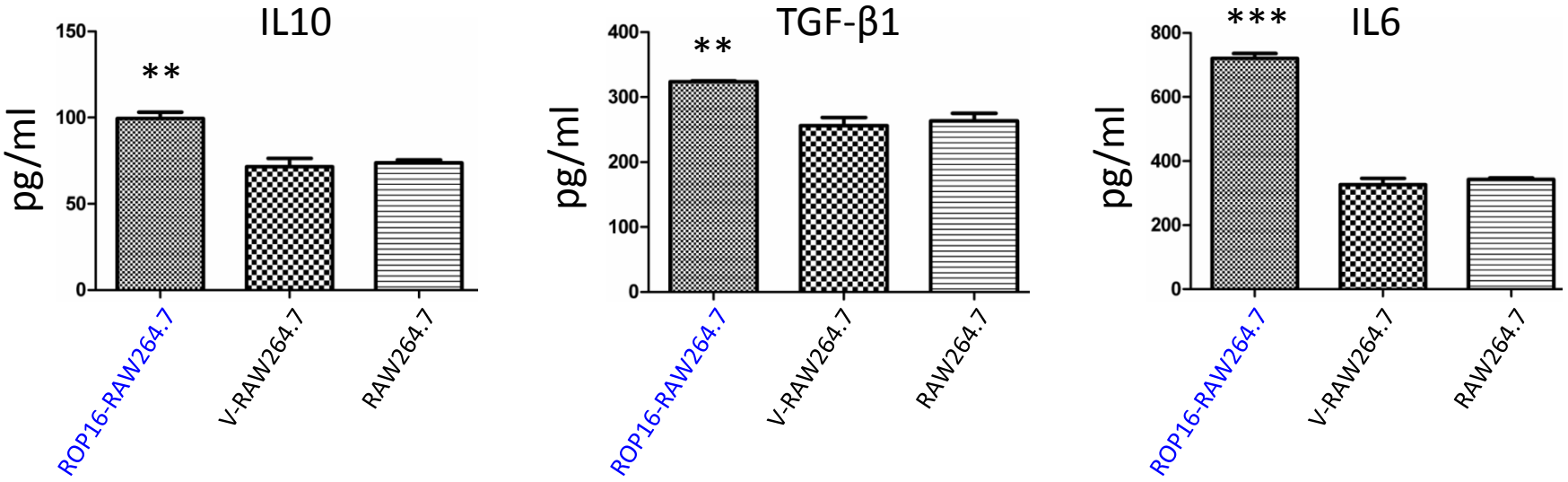
- **ROP16 protein drives RAW264.7 to M2**



Some mRNA of RAW264.7 detected by rq-PCR

Results

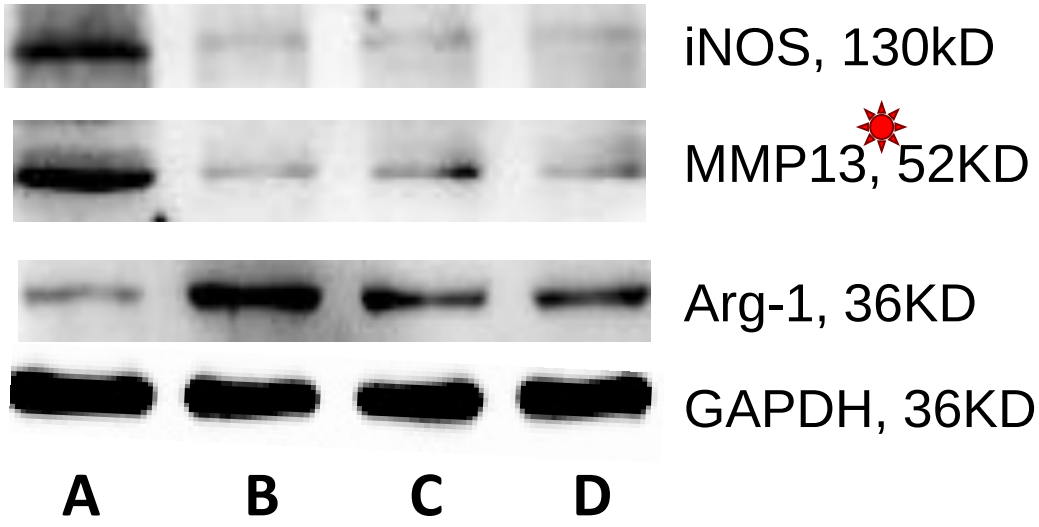
- **ROP16 protein drives RAW264.7 to M2**



Some cytokines of RAW264.7 detected in supernatant by ELISA

Results

- **GRA15 protein drives RAW264.7 to M1**
- **ROP16 protein drives RAW264.7 to M2**

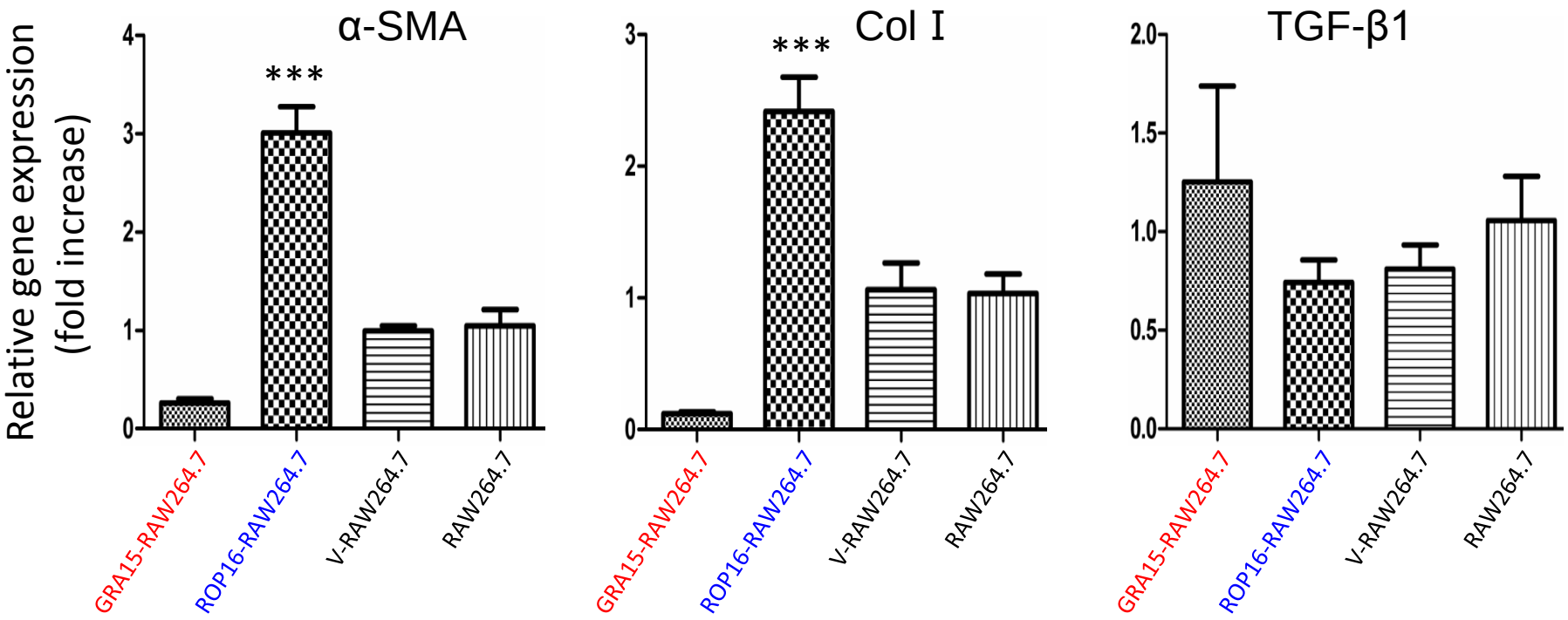


A: GRA15-RAW264.7; B: ROP16-RAW264.7;
C: V-RAW264.7; D: RAW264.7

Some proteins of RAW264.7 detected by western blotting

Results

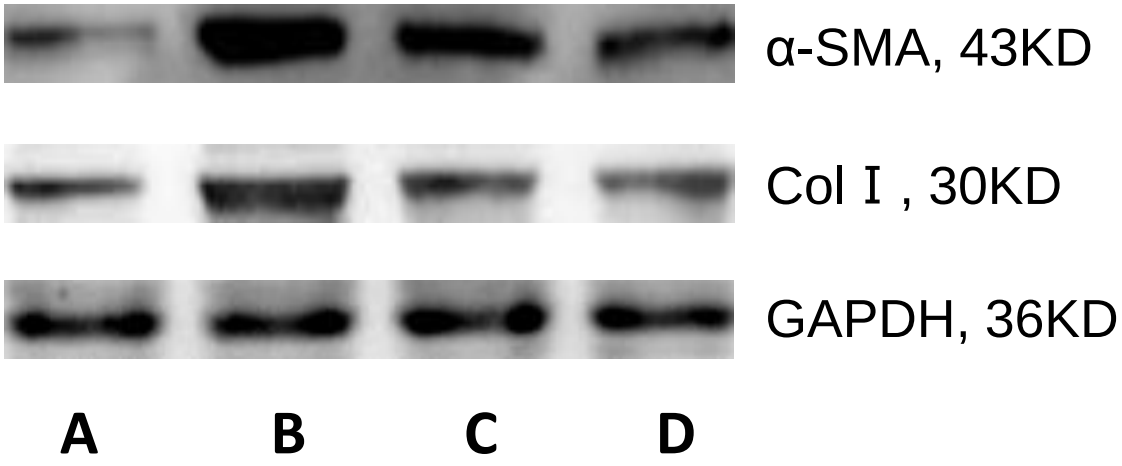
- **Effect of M1 or M2 on JS1**



Some mRNA of JS1 detected by rq-PCR

Results

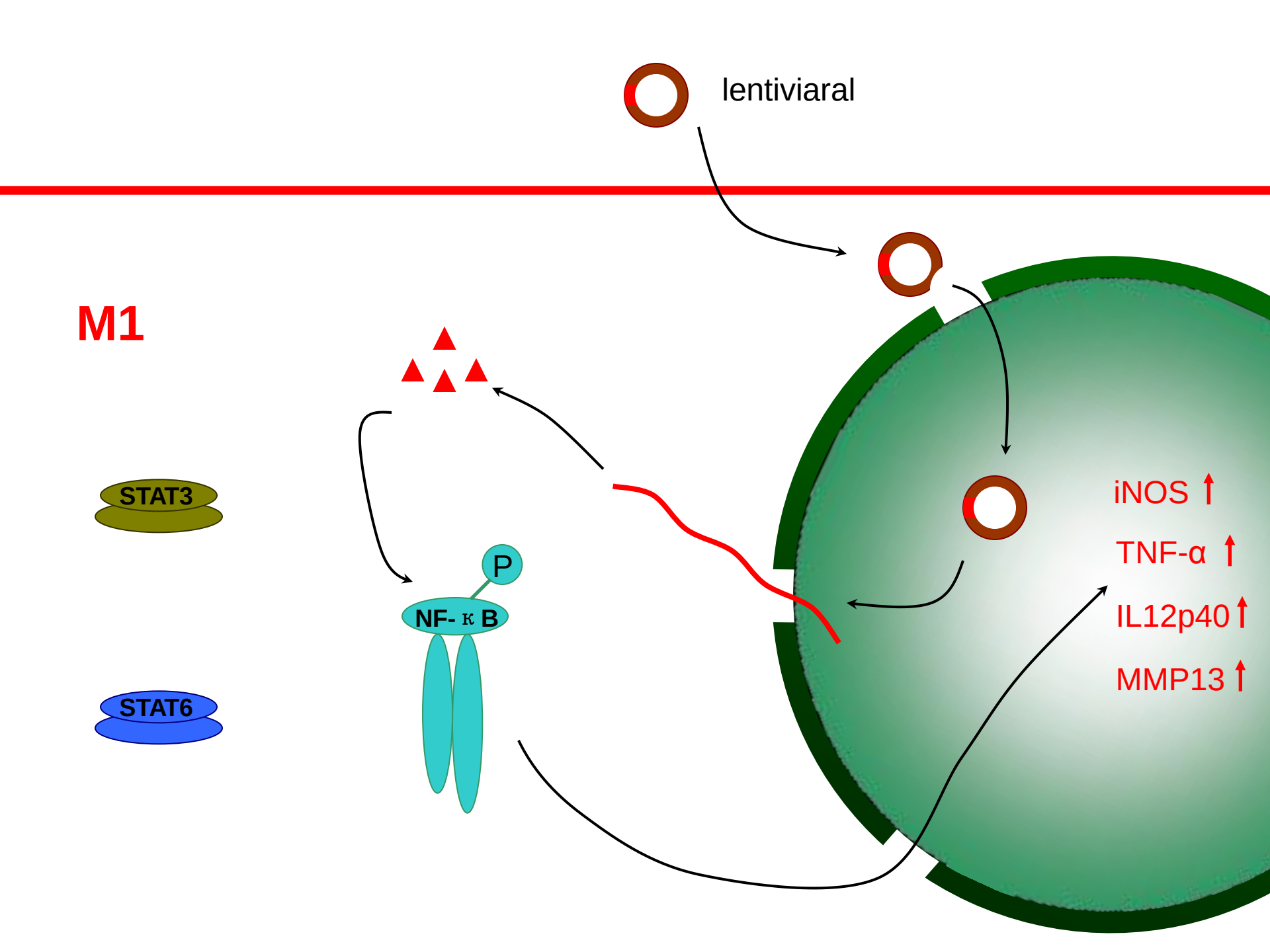
- **Effect of M1 or M2 on JS1**

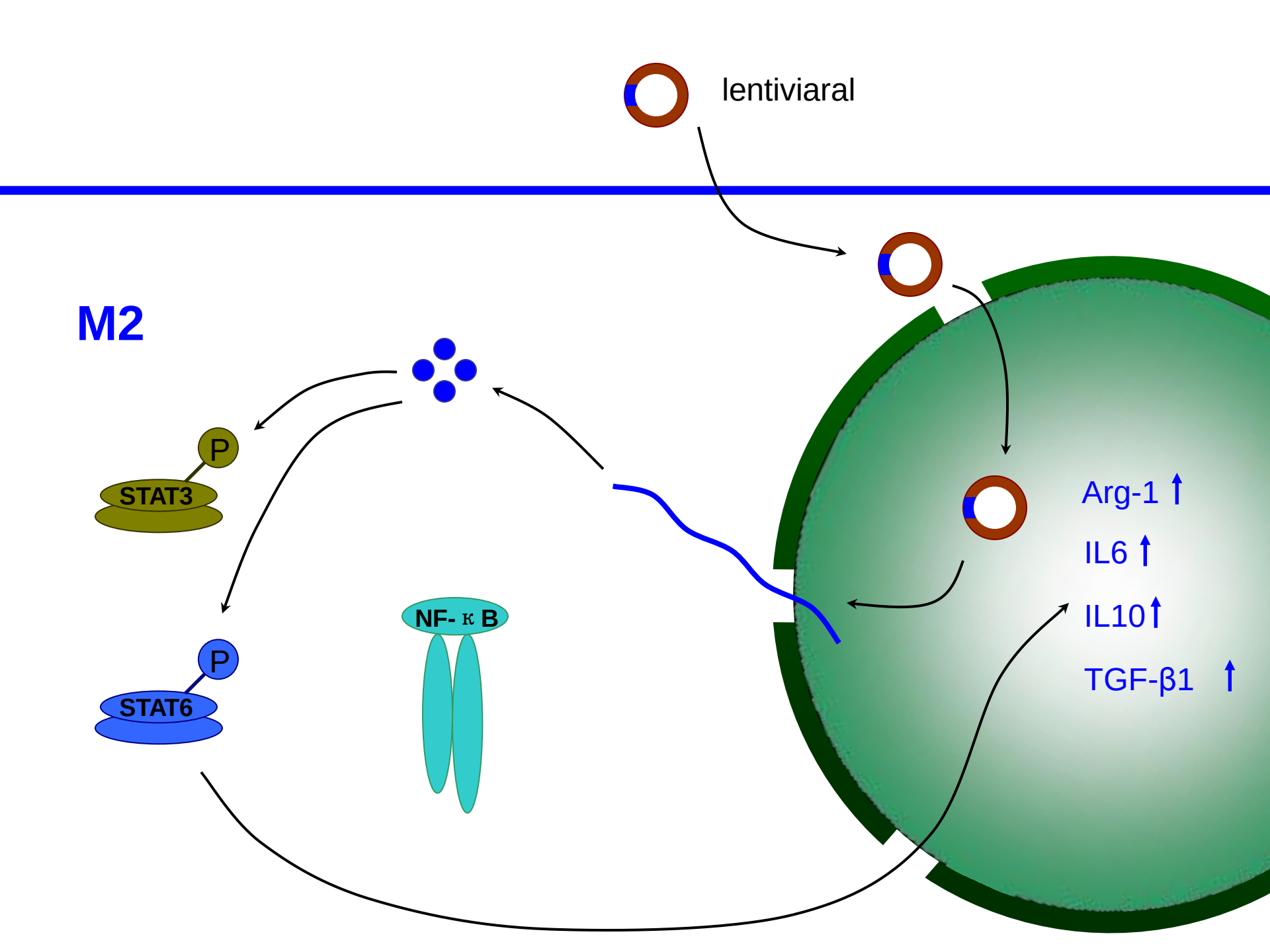


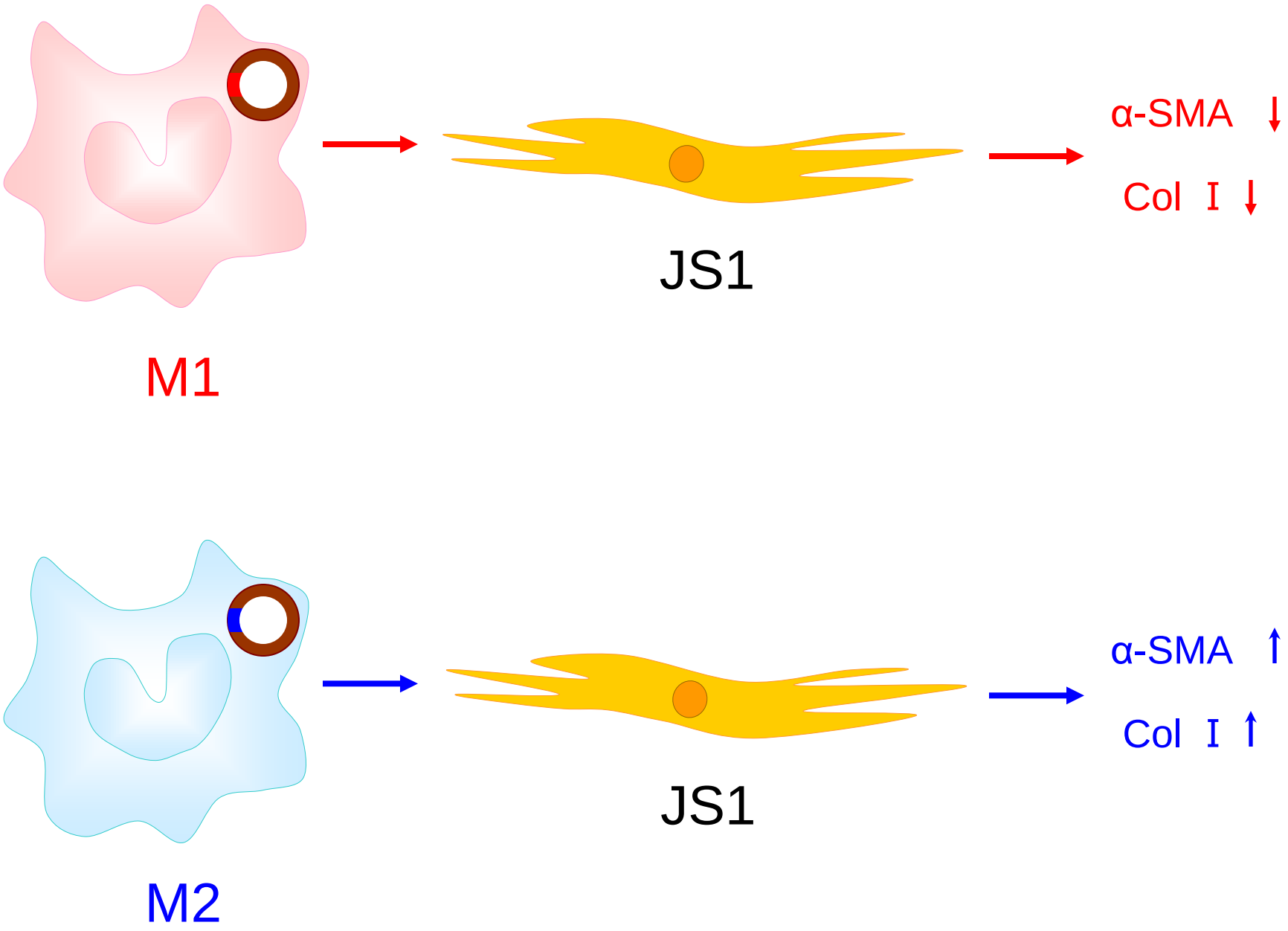
A: GRA15-RAW264.7; B: ROP16-RAW264.7;
C: V-RAW264.7; D: RAW264.7

Some proteins of JS1 detected by western blotting

CONCLUSION


lentiviaral
M1
STAT3
STAT6
P
NF- κ B
iNOS
TNF-α
IL12p40
MMP13


lentiviaral
M2
P
STAT3
P
STAT6
NF- κ B
Arg-1
IL6
IL10
TGF-β1


α-SMA
Col I
JS1
M1
α-SMA
Col I
JS1
M2

Acknowledgments

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- Appreciation is given to the colleagues and collaborators who are dedicated to this work.

Thank you for attention