

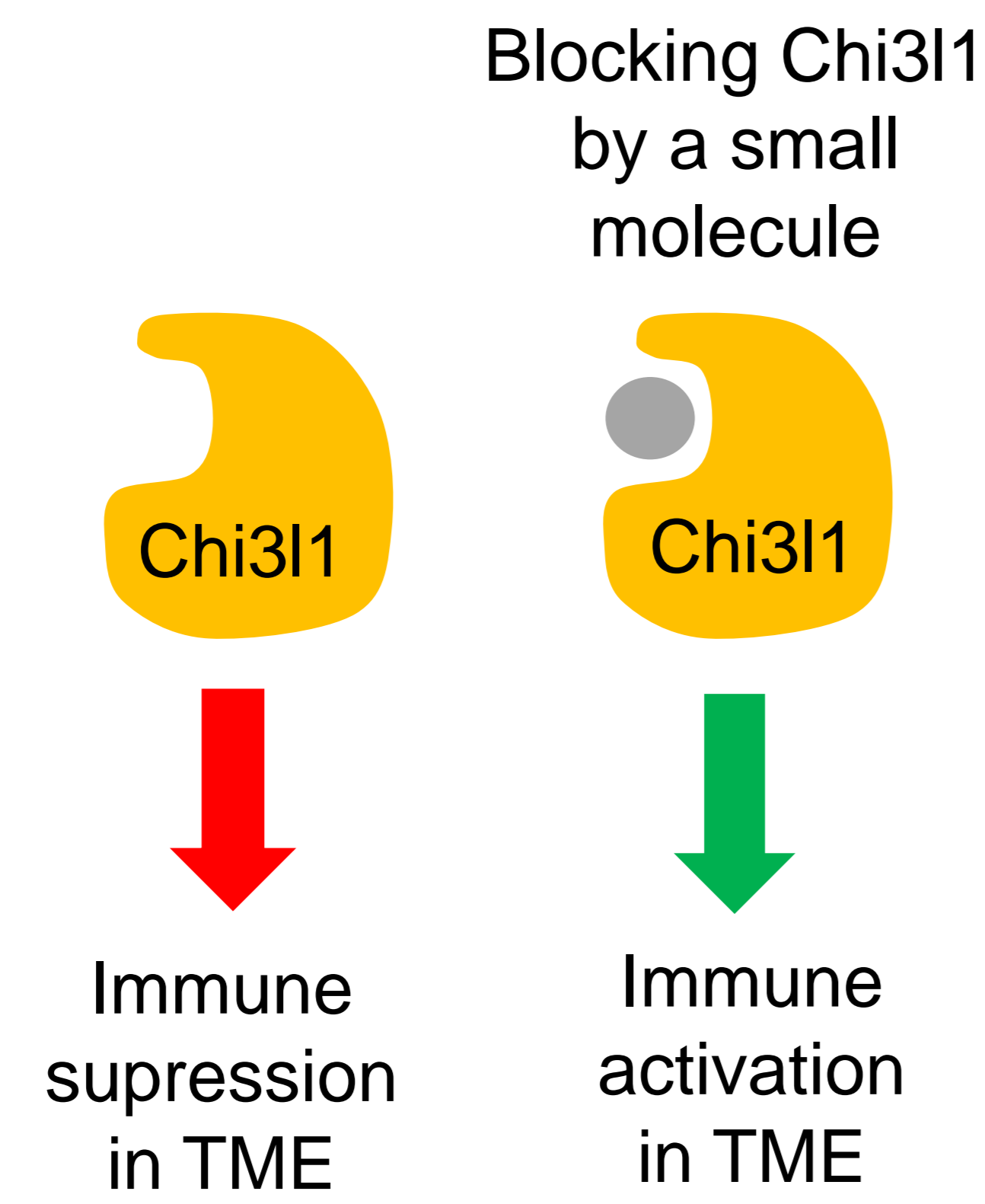
Targeting Chi311 by a small molecule activates macrophages

Katarzyna Drzewicka, Katarzyna Krysztofiak, Aleksandra Rymaszewska, Remigiusz Serwa, Anna Marusiak, Katarzyna Piwowar, Łukasz Krzemiński, Wojciech Czeszkowski, Agnieszka Bartoszewicz, Adam Gołębiowski, Zbigniew Zasłona.

Molecure SA, Żwirki i Wigury 101, 02-089 Warsaw, Poland;

INTRODUCTION

Chitinase 3-like 1 (Chi311) is a 40 kDa chitinase-like protein that unlike other chitinases such as Chit1 and AMCase cannot hydrolyze chitin in its chitin binding pocket. The protein has been linked to prognosis, progression and severity of numerous types of cancer, being produced and secreted by immune cells (especially macrophages) and various structural cells within cancer microenvironment. Chi311 has been demonstrated to bear anti-inflammatory effects on immune cells as well as direct pro-oncogenic effect by inducing proliferation and survival of cancer cells. Therefore, there has been lots of interest in neutralizing Chi311 by antibodies as a therapy in the immune-oncology field. In this study, we have developed first-in-class small molecule binders (OATs) that target chitin binding pocket of Chi311 with high affinity. Using macrophages as a cellular model, we propose mechanism of action of our compounds and their potential in cancer immune therapies.



RESULTS

1. Chi311 small molecule binder, OAT-3912, has low nanomolar affinity towards mouse and human Chi311

OAT-3912 activity in Alpha Screen assay

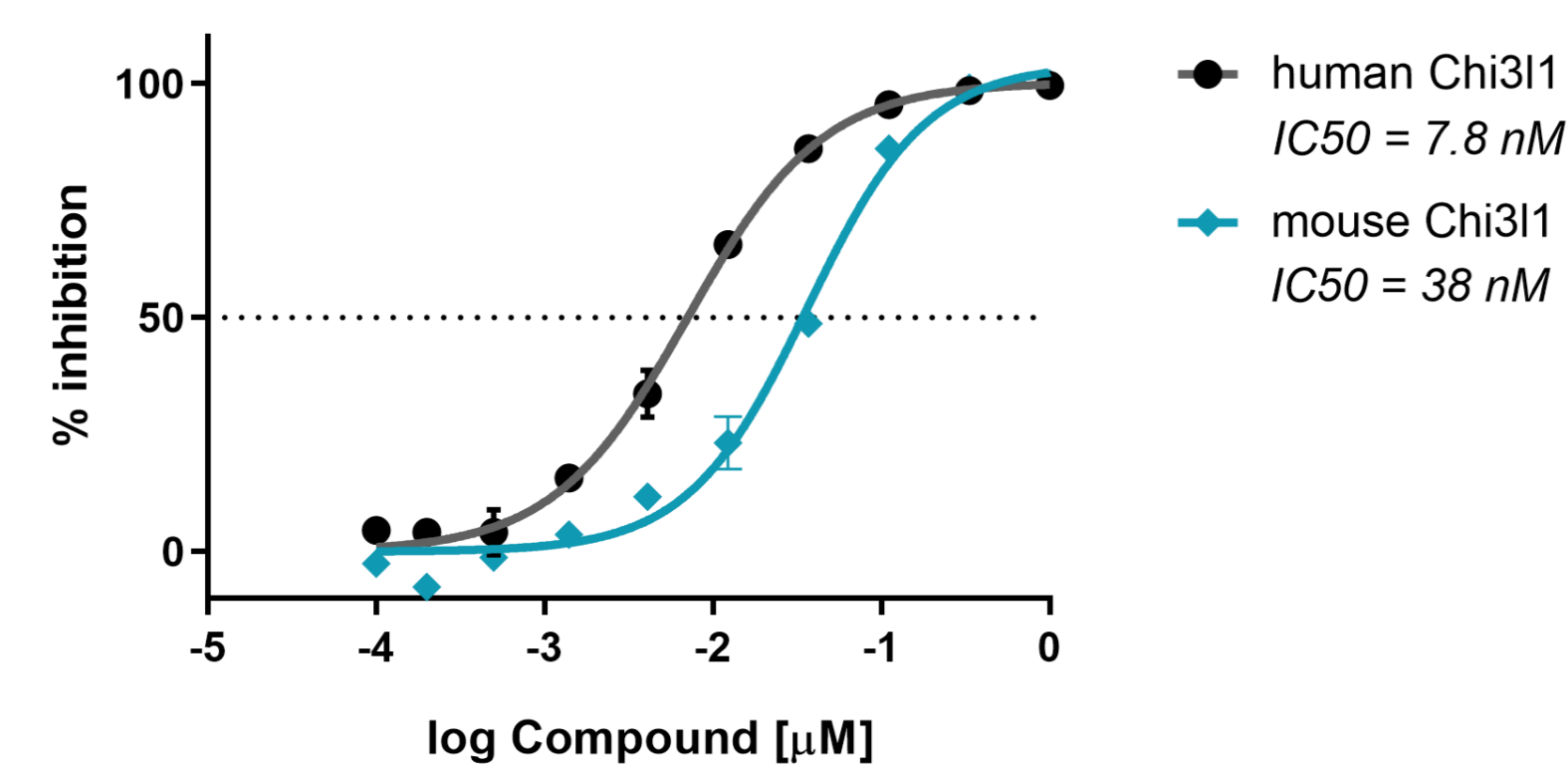


Fig.1. IC50 determination of the Chi311 small molecule binder. IC50 was measured in alpha-screen binding assay. In short, a screened compound competes with biotinylated compound immobilized on donor bead for binding to chitin binding pocket of Chi311 immobilized on the acceptor bead, which results in inhibition of alpha screen signal. Alpha screen assay is optimized to obtain IC50 values close to Kd.

2. Chi311 is produced in BMDMs stimulated with pro-inflammatory ligands

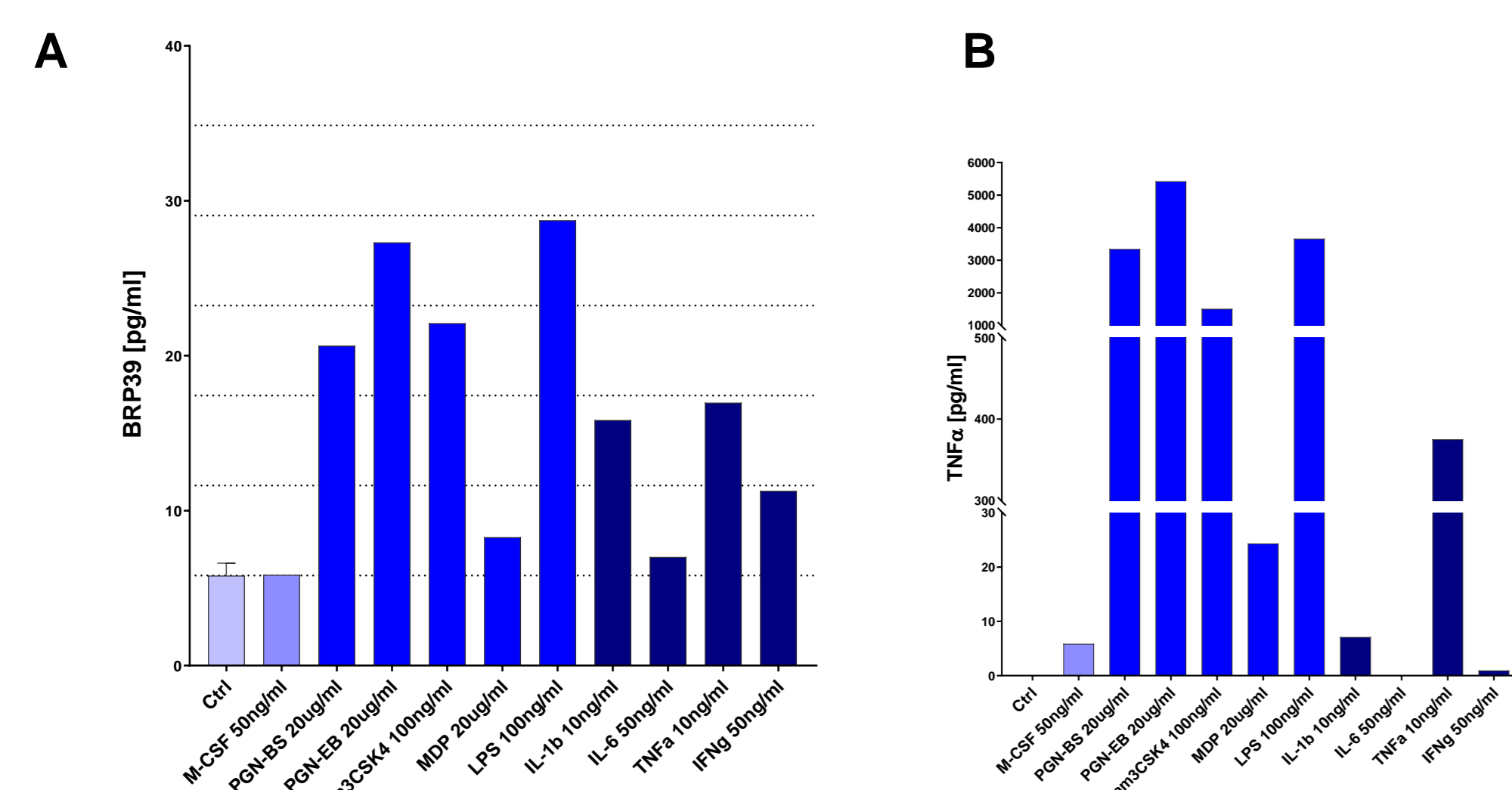


Fig.2. Chi311 is secreted after stimulation with pro-inflammatory inducers. Mouse Chi311 (A) and TNFα (B) level measured by ELISA in supernatants of mouse macrophages stimulated with pro-inflammatory ligands (PGN-BS, PGN-EB, Pam3CSK4, MDP, LPS) and cytokines (IL-1β, IL-6, TNFα, IFNγ) for 6h.

RESULTS

3. OAT-3912 reduced Chi311 levels in BMDMs stimulated with TLR triggering ligands

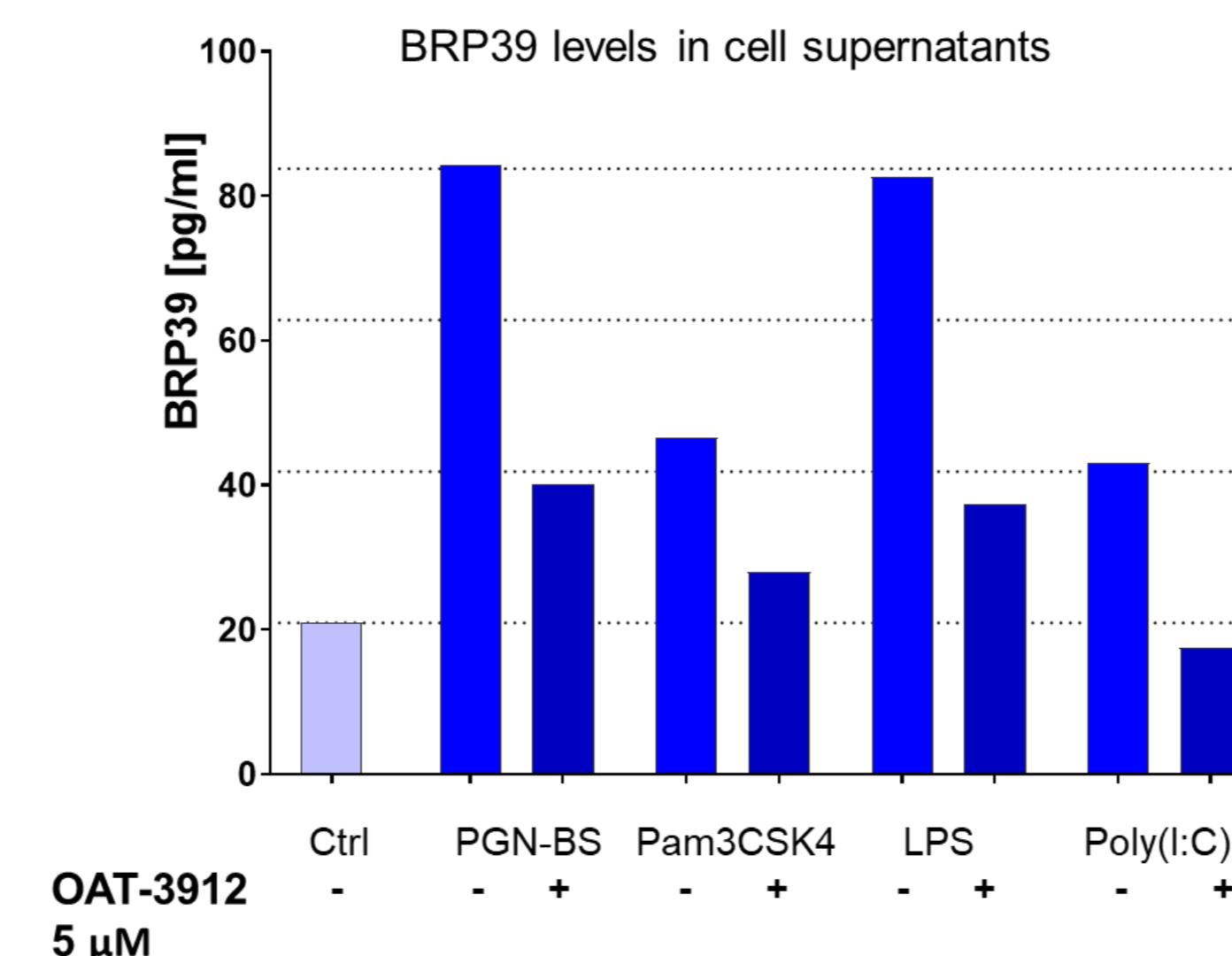


Fig.3. OAT-3912 reduce Chi311 levels in supernatants of mouse macrophages stimulated with various bacterial ligands and Poly(I:C) after 6h of treatment. OAT-3912 at 5 µM was added to macrophage medium 0.5h before addition of pro-inflammatory stimulants. Then, 6h later Chi311 levels were measured in cell supernatants by ELISA.

4. OAT-3912 inhibits secretion of Chi311 in BMDMs in dose dependent manner

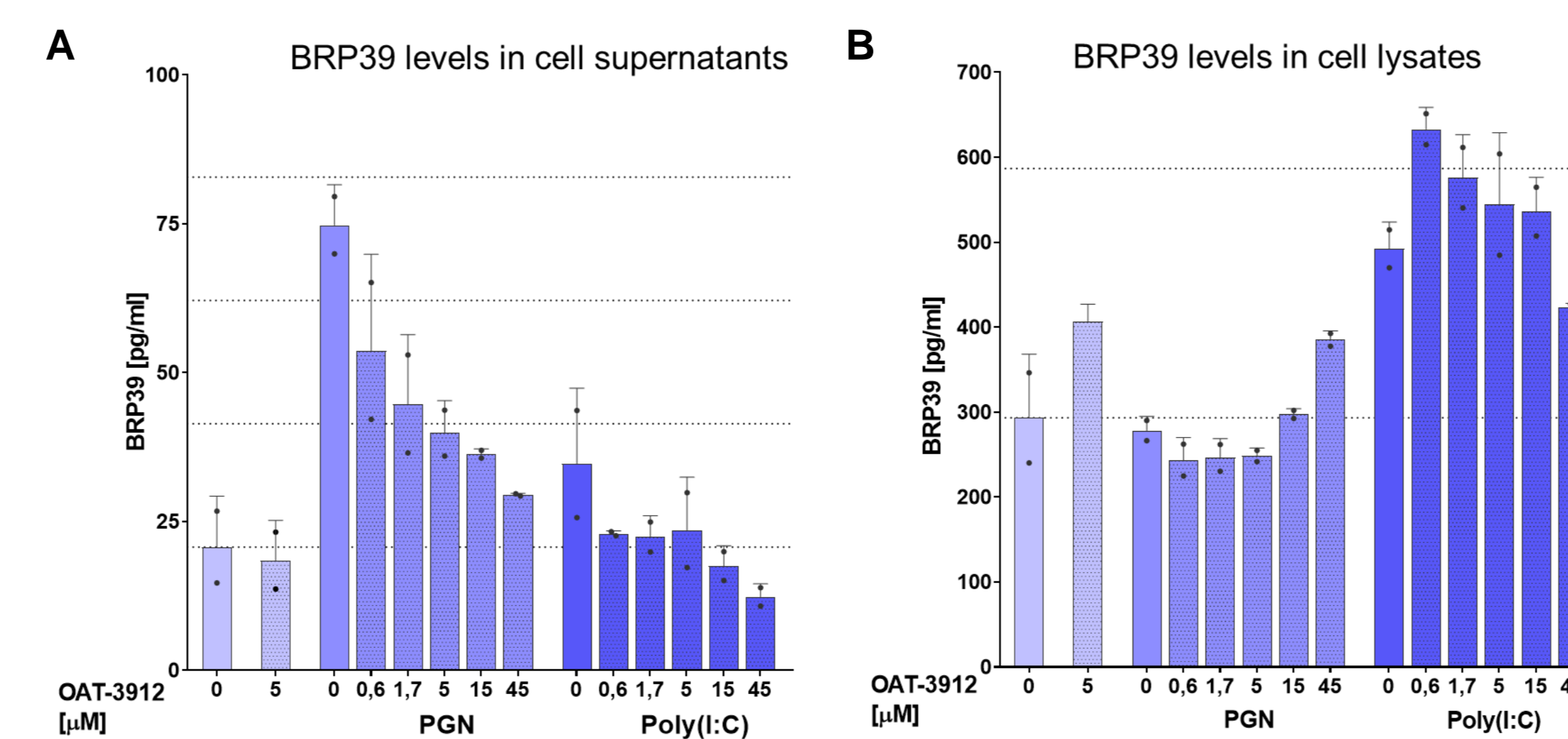


Fig.4. OAT-3912, reduce extracellular Brp39 (murine Chi311) levels in dose dependent manner in mouse macrophages stimulated with peptidoglycan and Poly(I:C). BMDMs were treated with OAT-3912 at different concentration 0.5 h before adding peptidoglycan (20 µg/ml) and Poly(I:C) (5 µg/ml). 6 h later, cell supernatants were collected and cells were lysed in RIPA buffer for ELISA test to evaluate Brp39 levels (A and B).

5. OAT-3912 treatment inhibits secretome in BMDMs

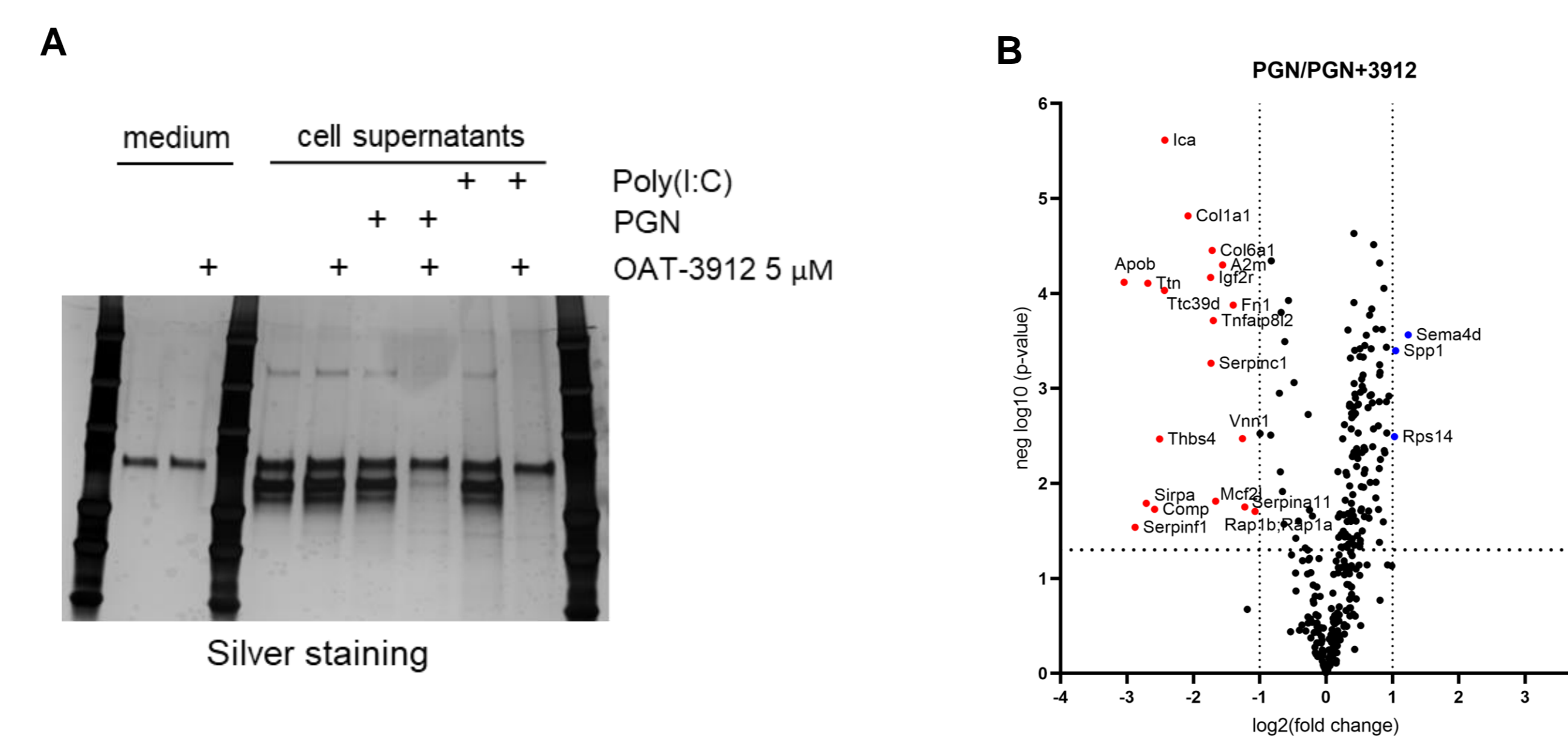
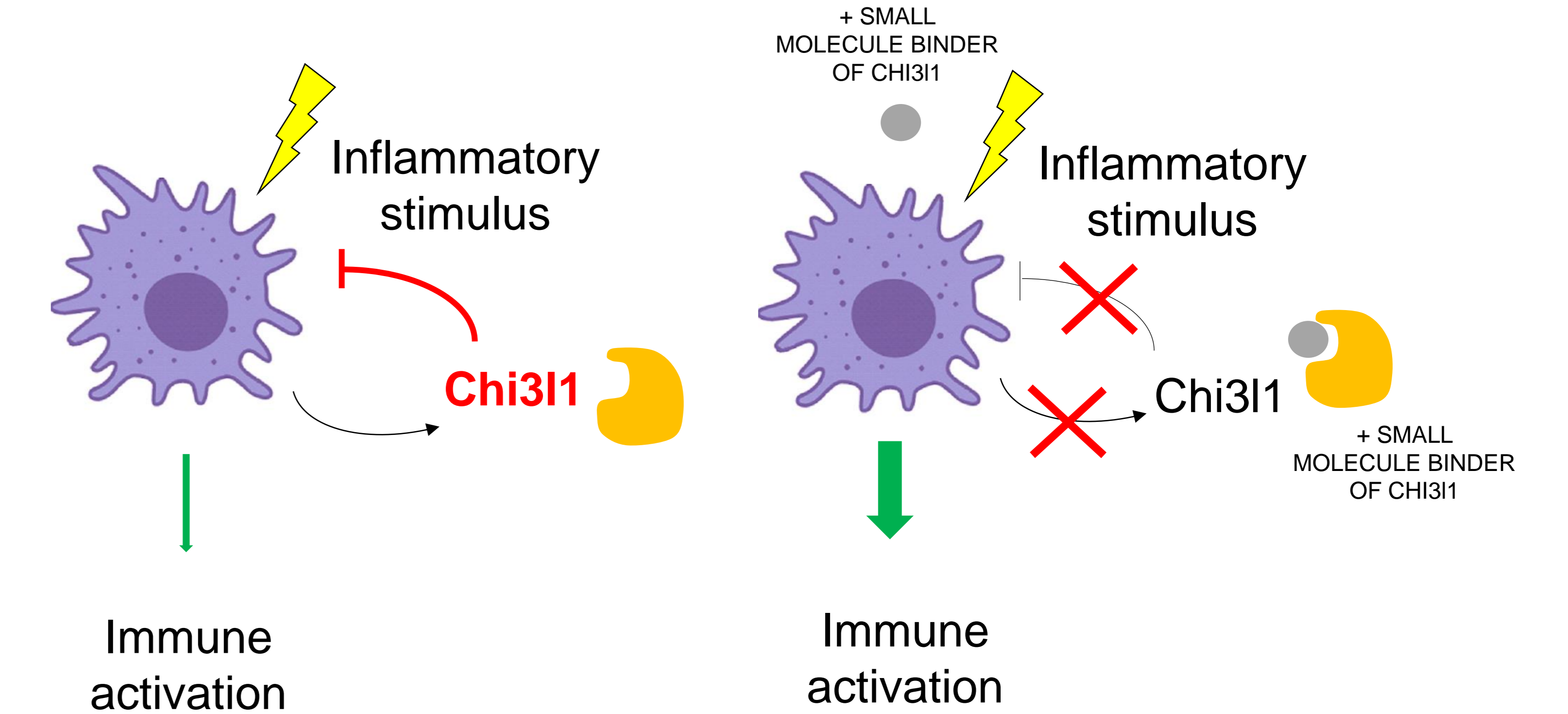


Fig. 5. OAT-3912 reduces protein secretion in activated BMDMs. A. BMDMs were treated or not with peptidoglycan (20 µg/ml), Poly(I:C) (5 µg/ml) and OAT-3912 (5 µM) for 6h prior to collection of cell supernatants. 10 µl of cell supernatants was loaded on SDS-PAGE gel and analysed by silver staining. For a control, pure culture media (Optimem I reduced serum media) with or without of OAT-3912 was loaded to test for unspecific protein precipitation. B. Proteomic analysis of extracellular proteins regulated by 3912 in stimulated BMDMs.

WORKING HYPOTHESIS



We believe that Chi311 is induced by pro-inflammatory ligands to act as a negative feedback loop. Targeting Chi311 by a small molecule binder blocks Chi311 secretion and enhances activation of macrophages, suggesting potential of the compound in cancer immune therapies

FUTURE PLANS

- Experiments on BMDMs Chi311 KO and THP1 Chi311 KO to test specificity of OAT
- Comparing the effect of OAT to Chi311 neutralizing antibodies in macrophages
- Co-culture experiments with macrophages stimulated with OAT and cancer cells
- Syngeneic mouse model to study efficacy of OAT-3912 is ongoing

ACKNOWLEDGEMENTS

"Development of a first-in-class small molecule drug candidate for cancer treatment through YKL 40 inhibition"



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