Novel signaling paradigm regulating TOLL-like receptors in innate immune cells

Samar Abdulkhalek and **Myron R. Szewczuk***.

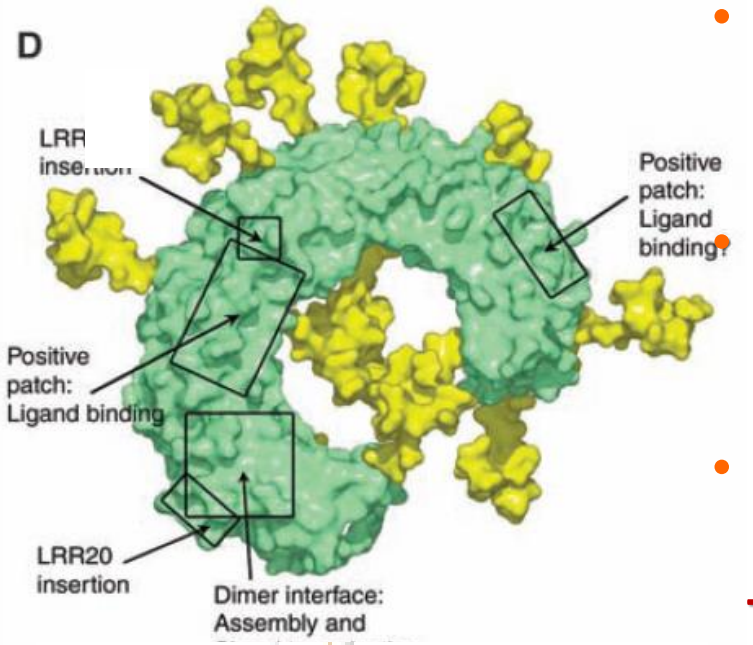
Dept. Biomedical and Molecular Sciences, Queen's University, Kingston, K7L 3N6 Ontario, Canada. ***speaker**

- **A novel signaling paradigm for cell surface TLR4 and intracellular TLR-7 and TLR-9**

- **Central to this process is that neuromedin B (NMBR) GPCR-Neu1-MMP9 complex is bound to TLRs in naive and ligand stimulated macrophage cells.**

Toll-Like Receptors

Ectodomain



TIR Domain

Common Knowledge:

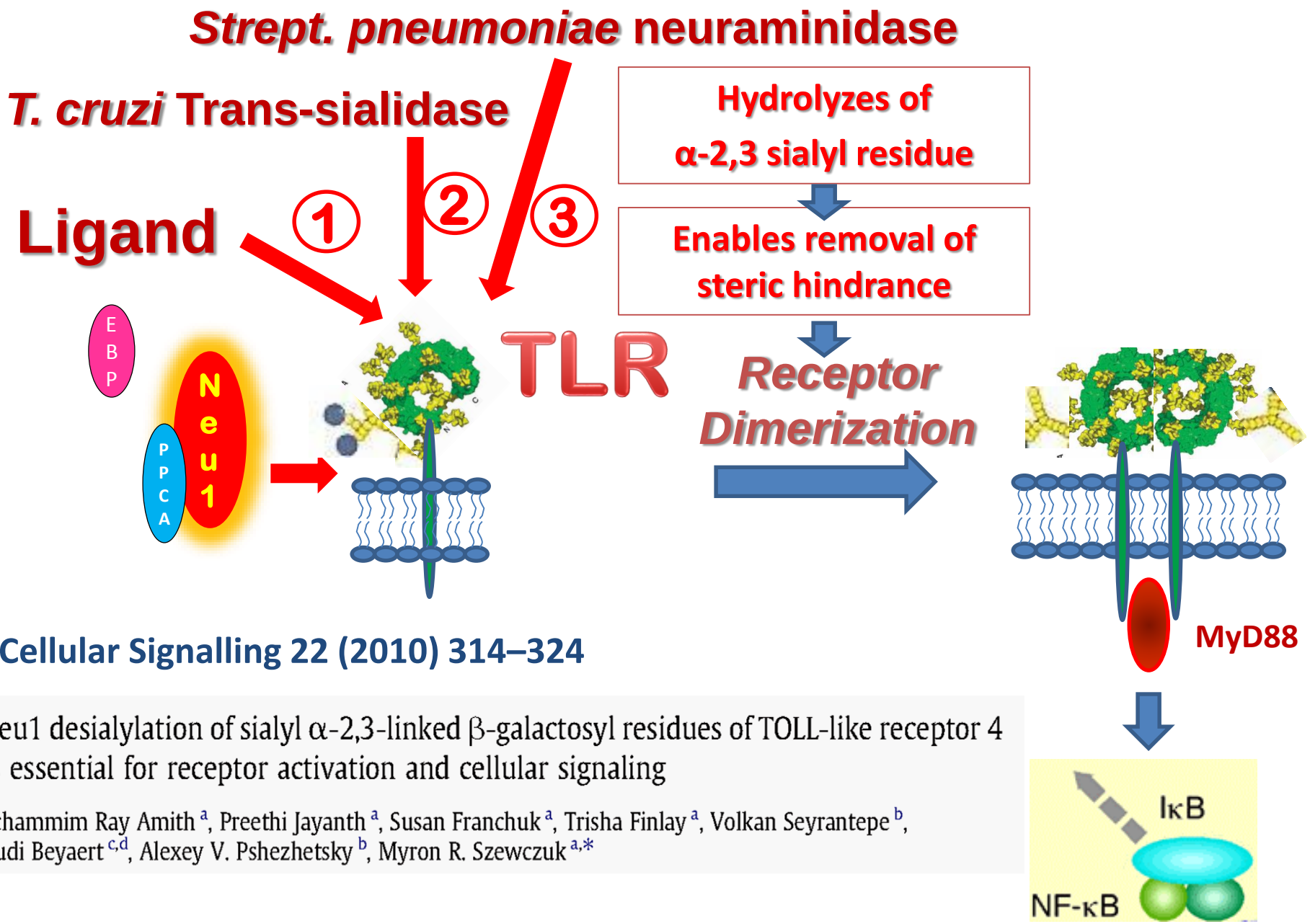
- **Front-line Pattern Recognition Receptors of the innate immune system**
- **TLRs recognize microbial pathogen-associated molecular patterns (PAMPs)**
- **Crystal structure reveals highly glycosylated ectodomain**

TLR3 **Potential 15 N-glycosylation sites depending on TLR**

Still a mystery:

- **How TLR activation is regulated?**
- **What Is the role for TLR glycosylation in this ligand–induced TLR activation?**

Crystal Structure of **TLR3** courtesy of Dr. David Segal, Centre for Cancer Research, NIH



Cellular Signalling 22 (2010) 314–324

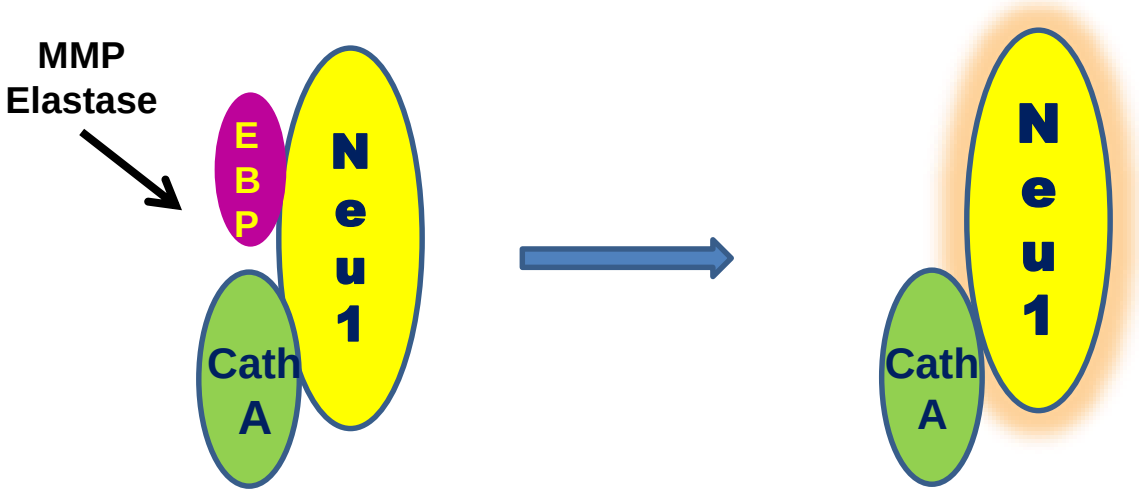
Neu1 desialylation of sialyl α-2,3-linked β-galactosyl residues of TOLL-like receptor 4 is essential for receptor activation and cellular signaling

Schammim Ray Amith [a], Preethi Jayanth [a], Susan Franchuk [a], Trisha Finlay [a], Volkan Seyrantepe [b], Rudi Beyaert [c,d], Alexey V. Pshezhetsky [b], Myron R. Szewczuk [a,*]

MMP with elastase activity is required to induce NEU1

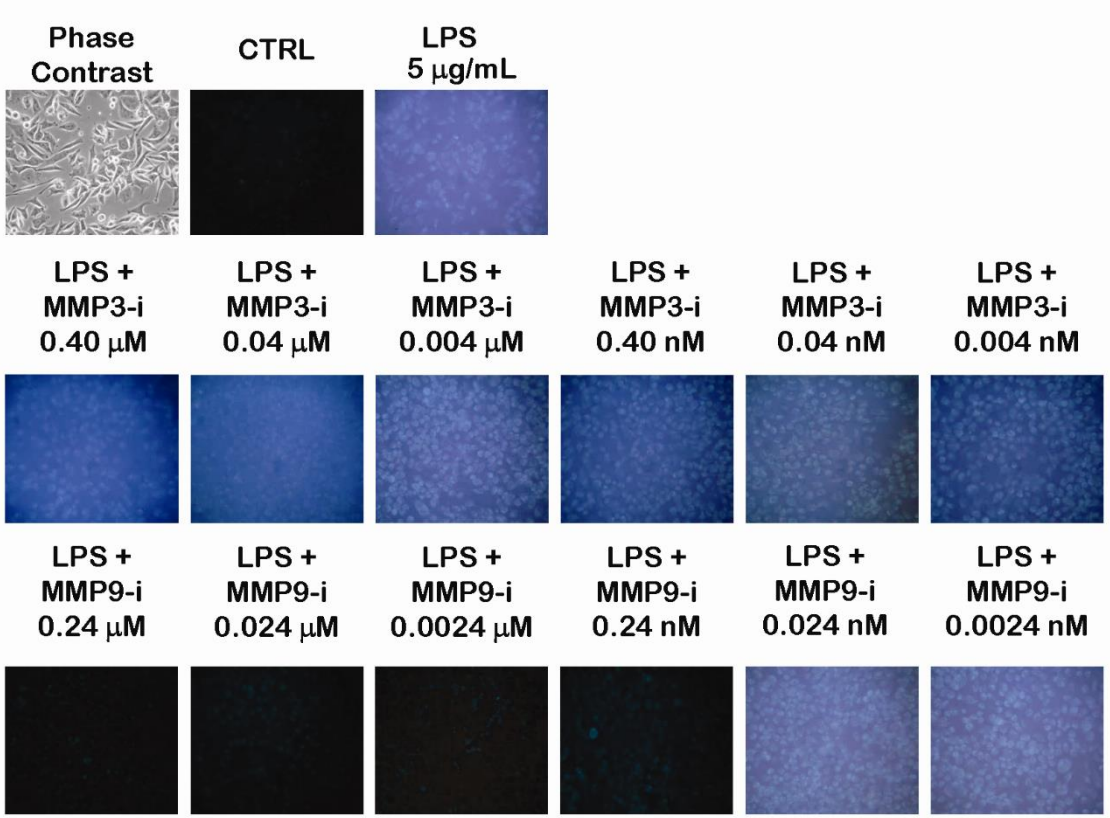
Cath A- Cathepsin A

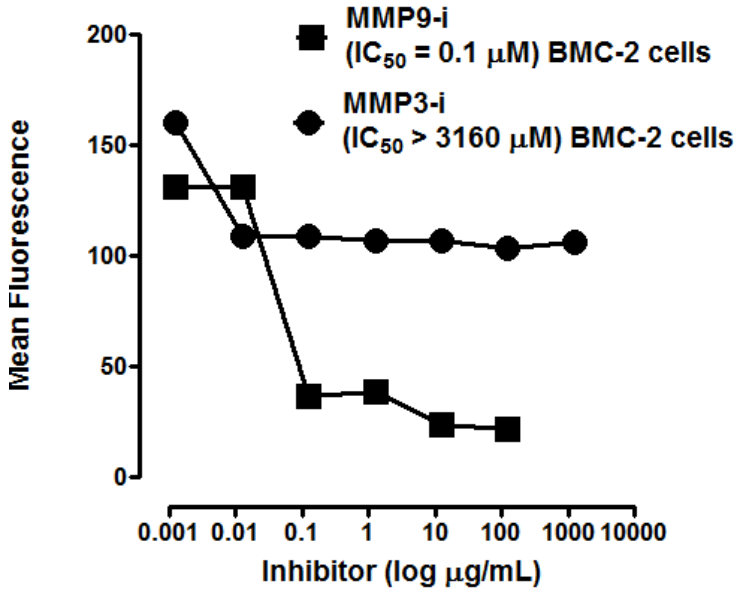
EBP- Elastin binding protein





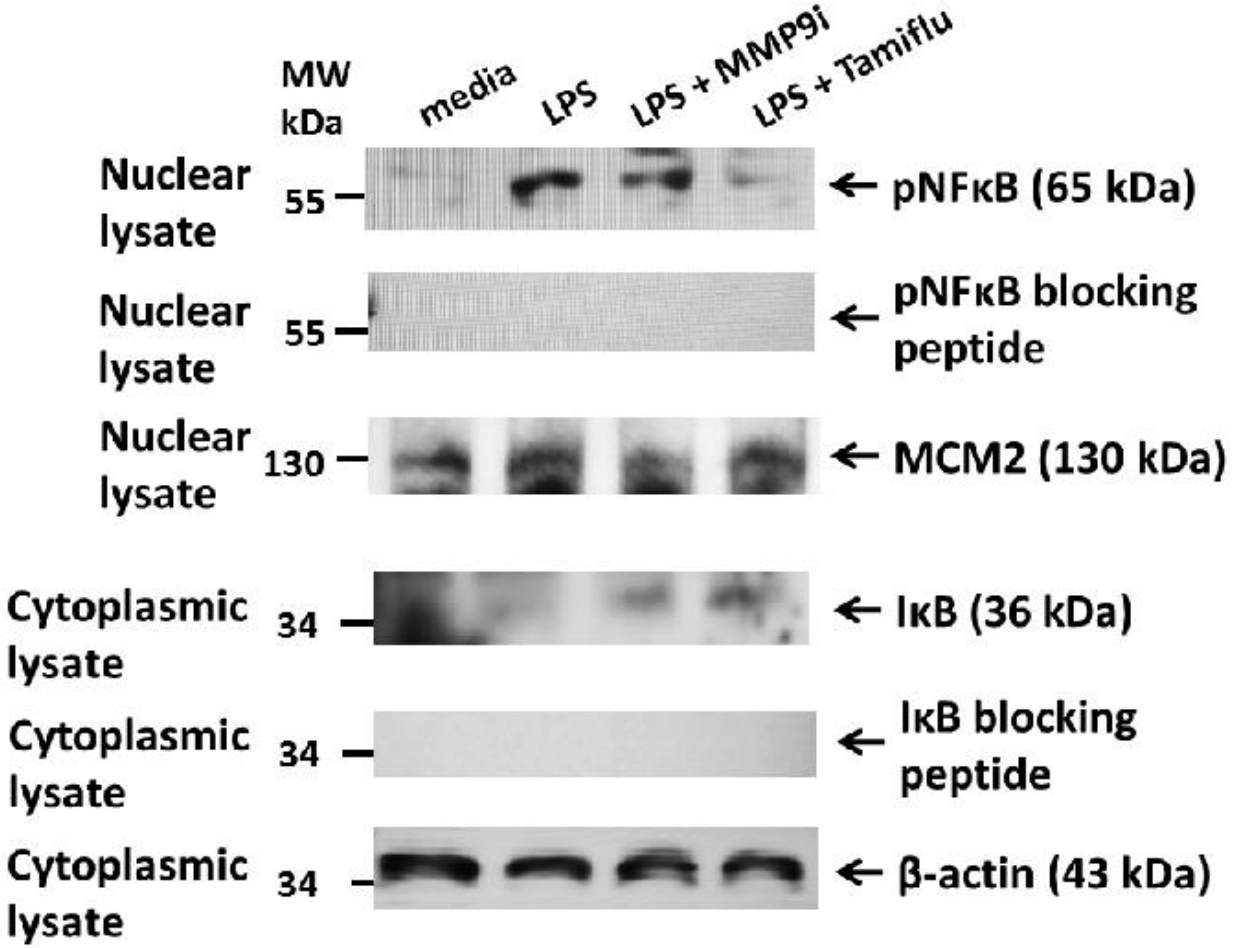
MMP9i inhibits sialidase activity – live cell sialidase assay

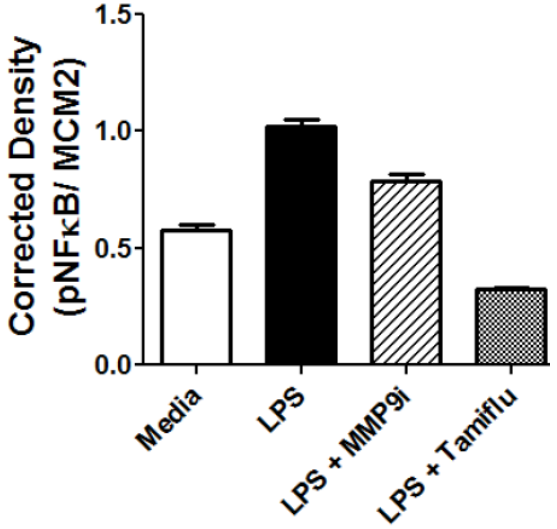


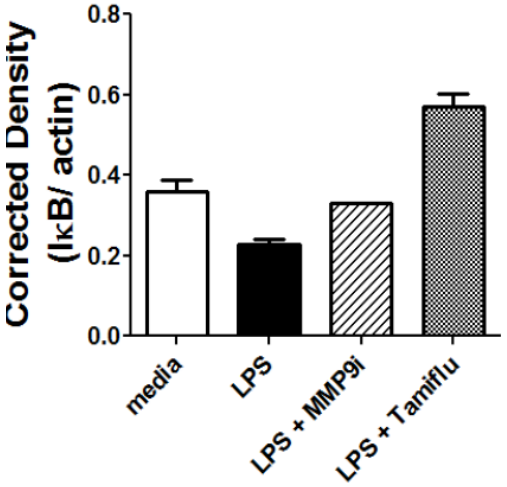


MMP9i inhibits LPS-induced pNFκB

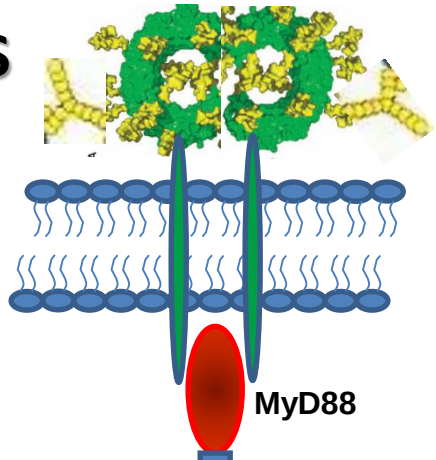
BMA cells



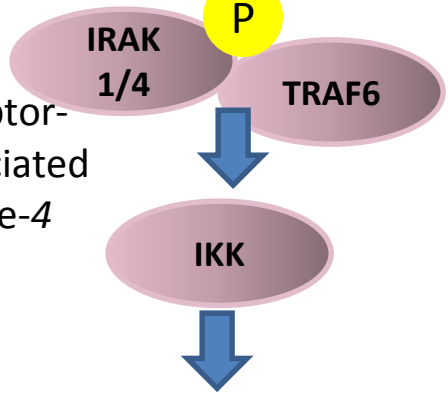


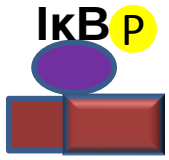


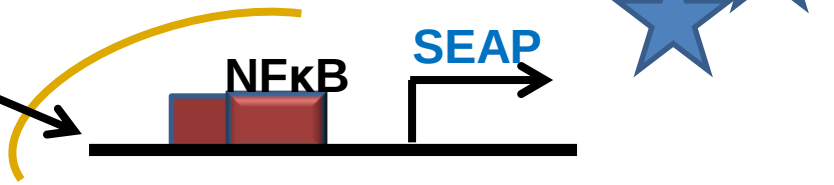
NFκB-dependent SEAP Assay – RAW-blue cells

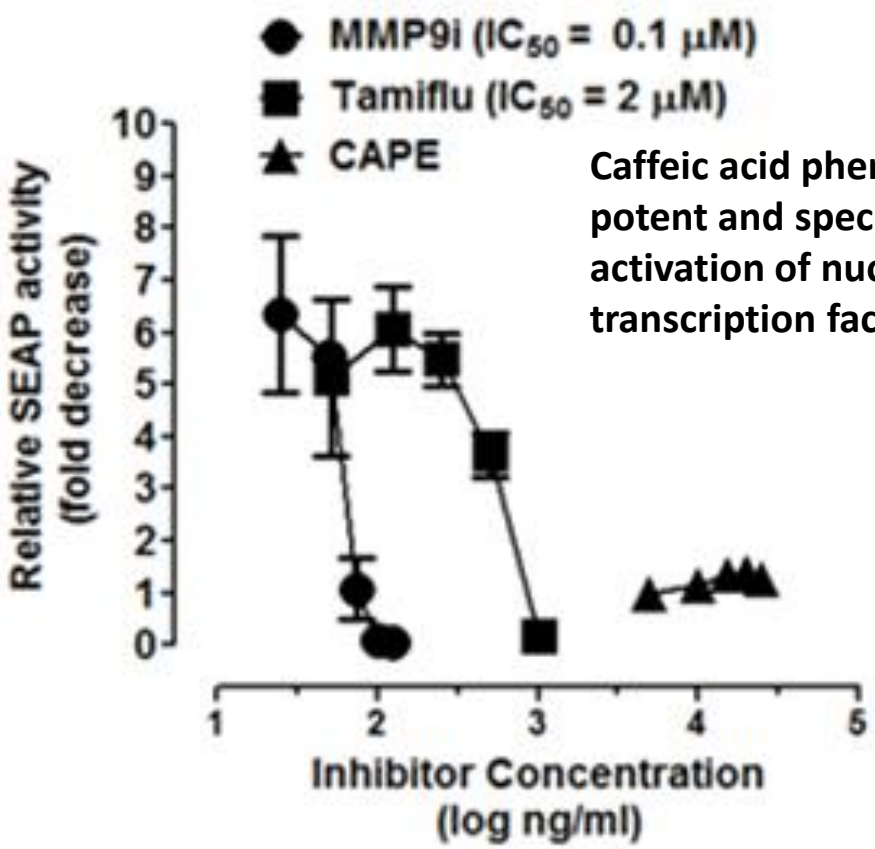


IL-1 receptor-associated kinase-*4*



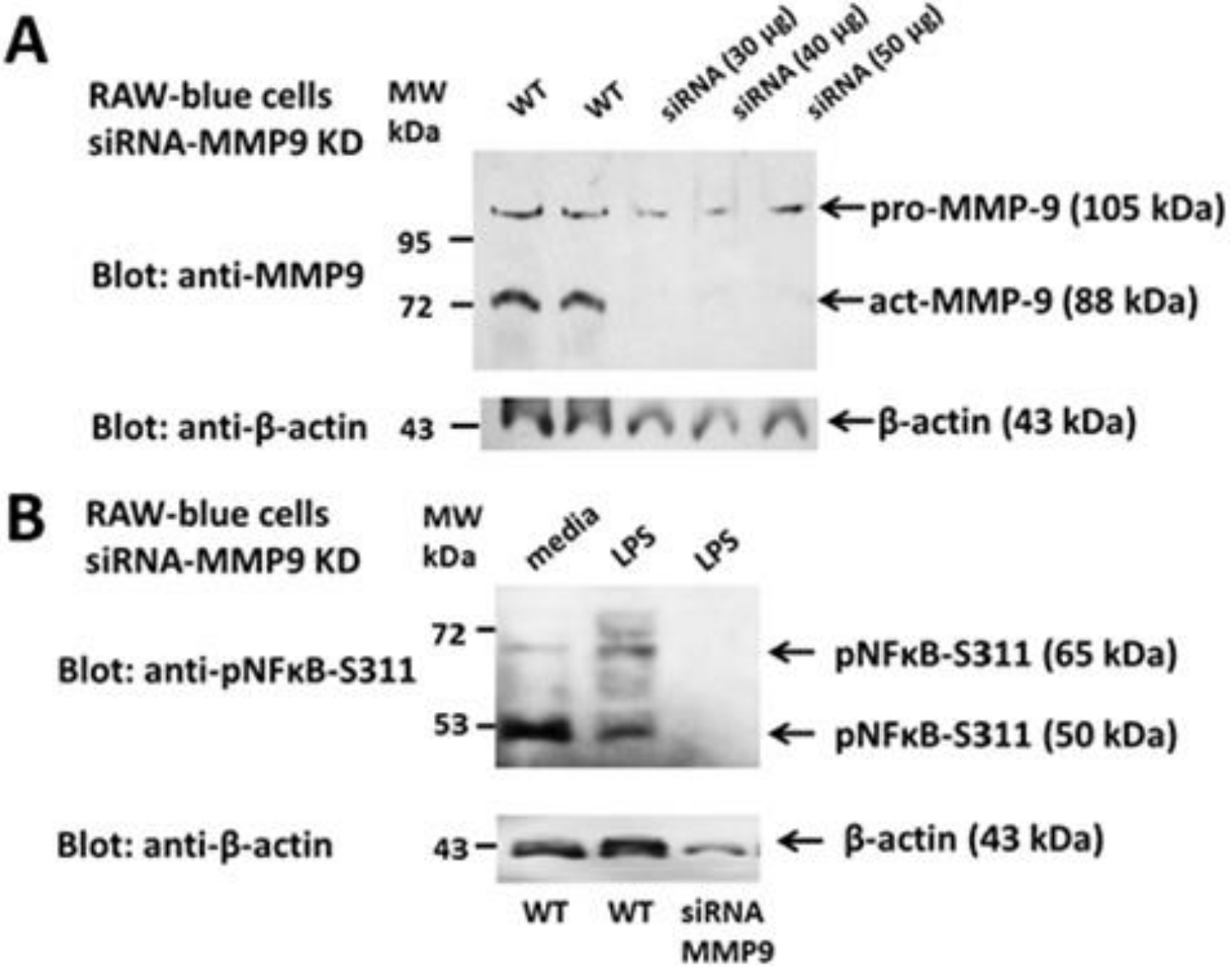




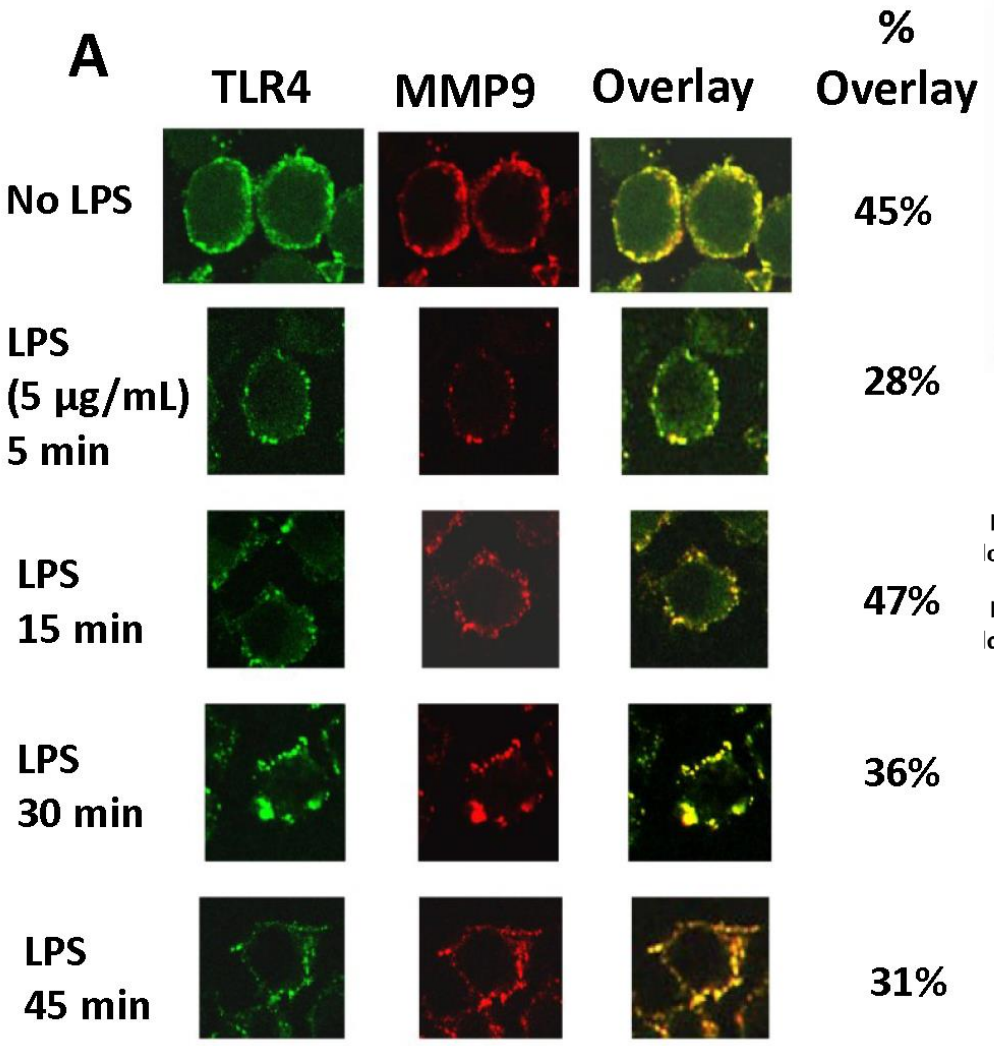


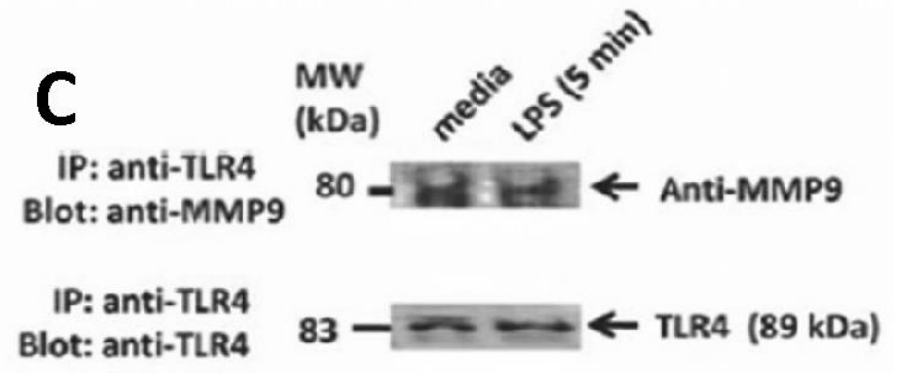
Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kappa B.

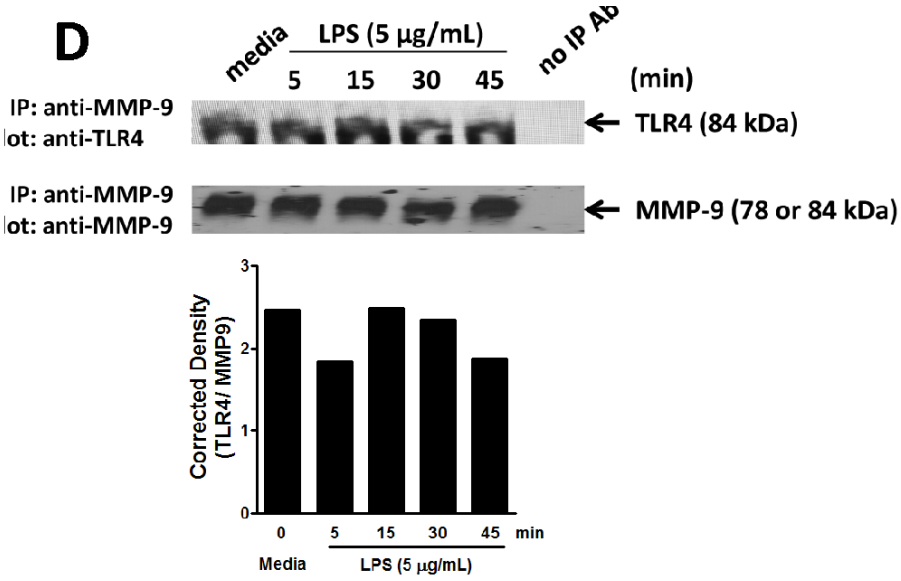
MMP9-siRNA inhibits LPS-induced NFκB in RAW-blue macrophages



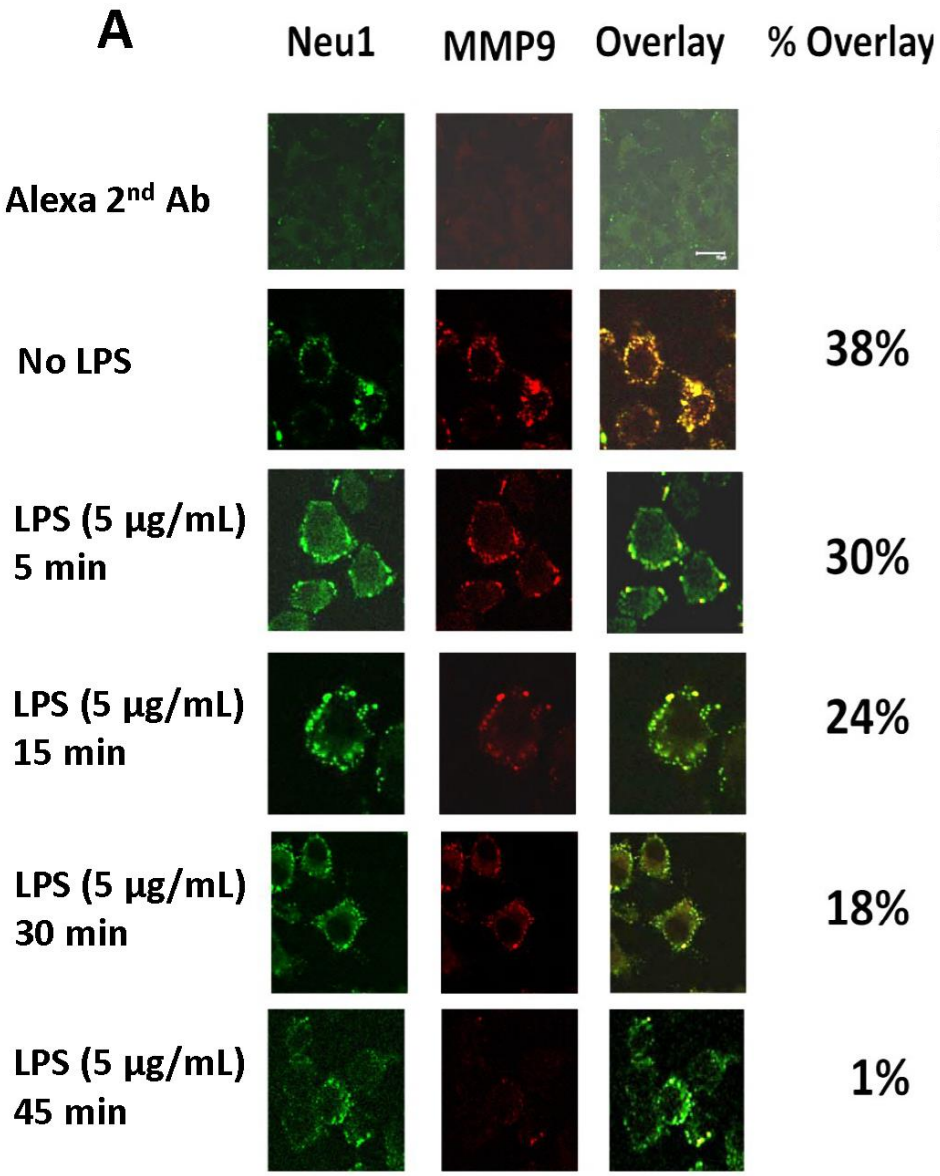
MMP-9 forms a complex with TLR4 in naïve and LPS treated BMA macrophage cells

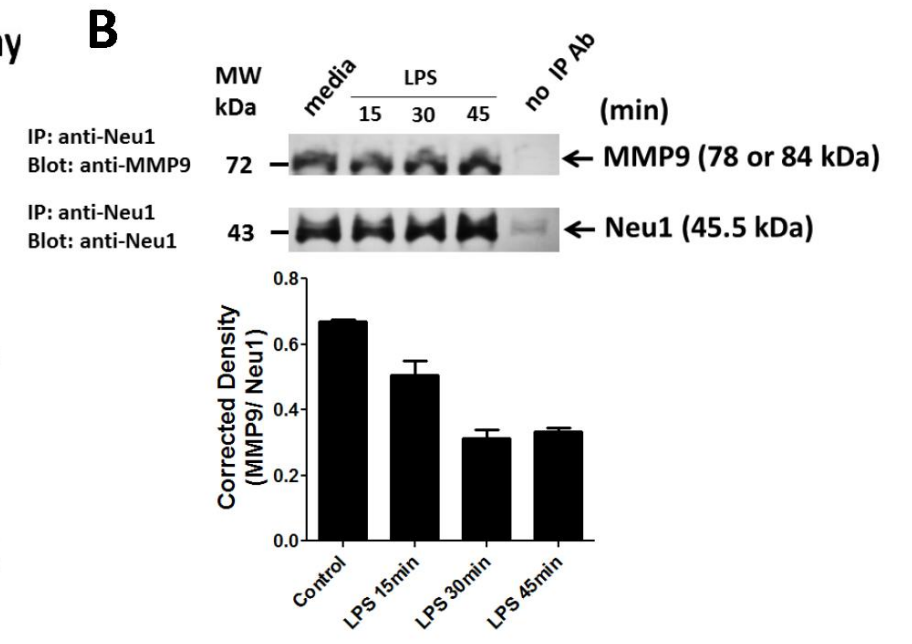


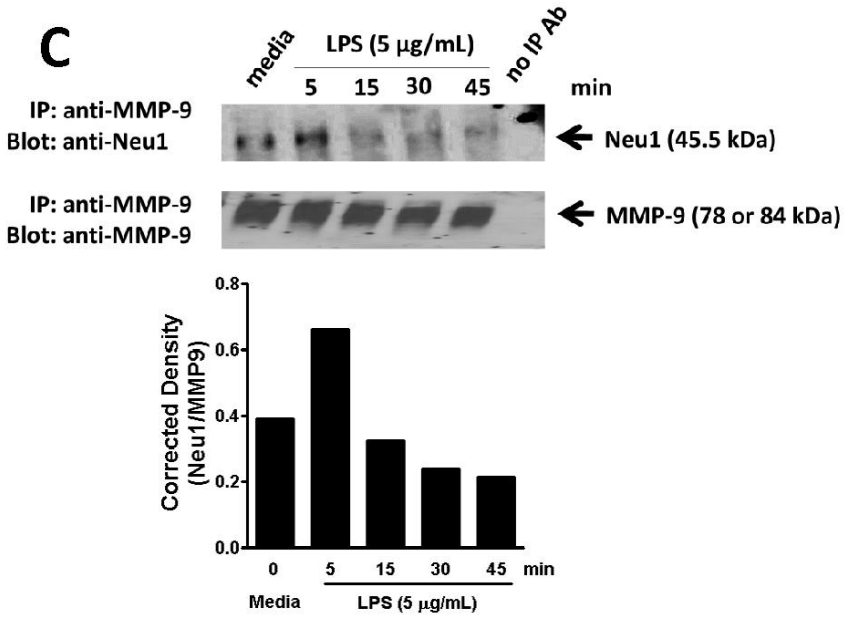




MMP9 forms a Complex with NEU1







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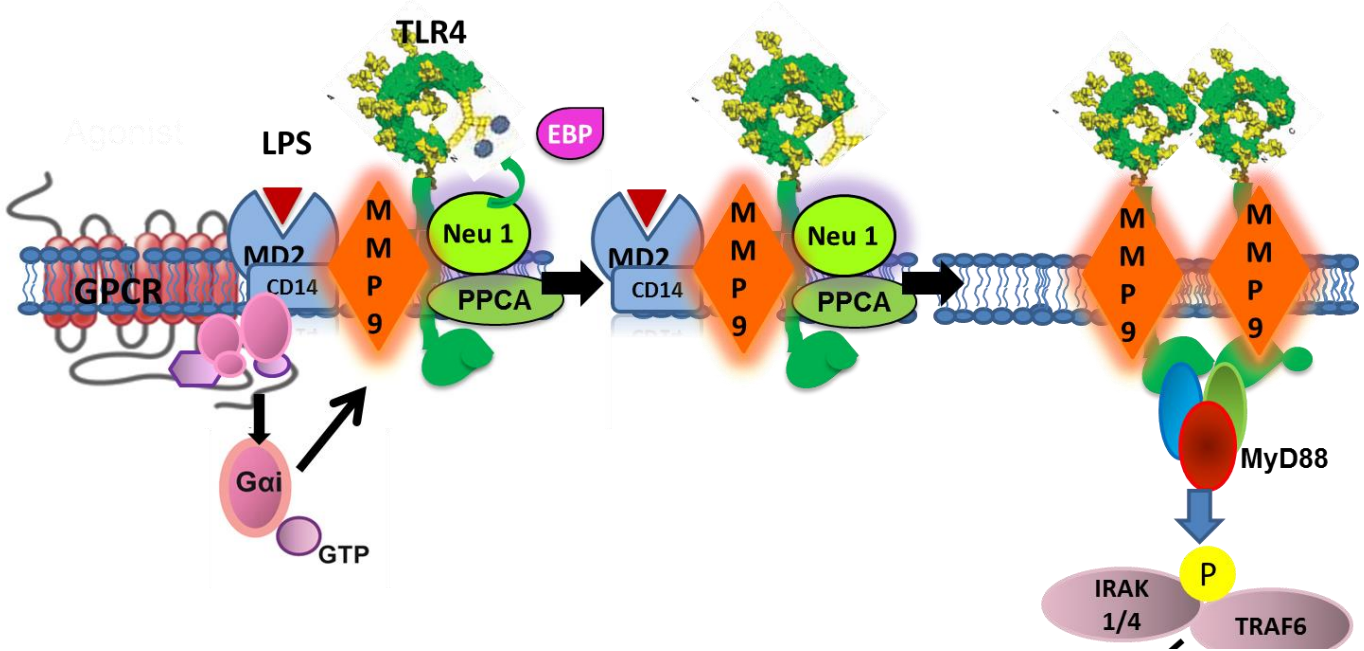
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journal homepage: www.elsevier.com/locate/cellsig

G-protein coupled receptor agonists mediate Neu1 sialidase and matrix metalloproteinase-9 cross-talk to induce transactivation of TOLL-like receptors and cellular signaling

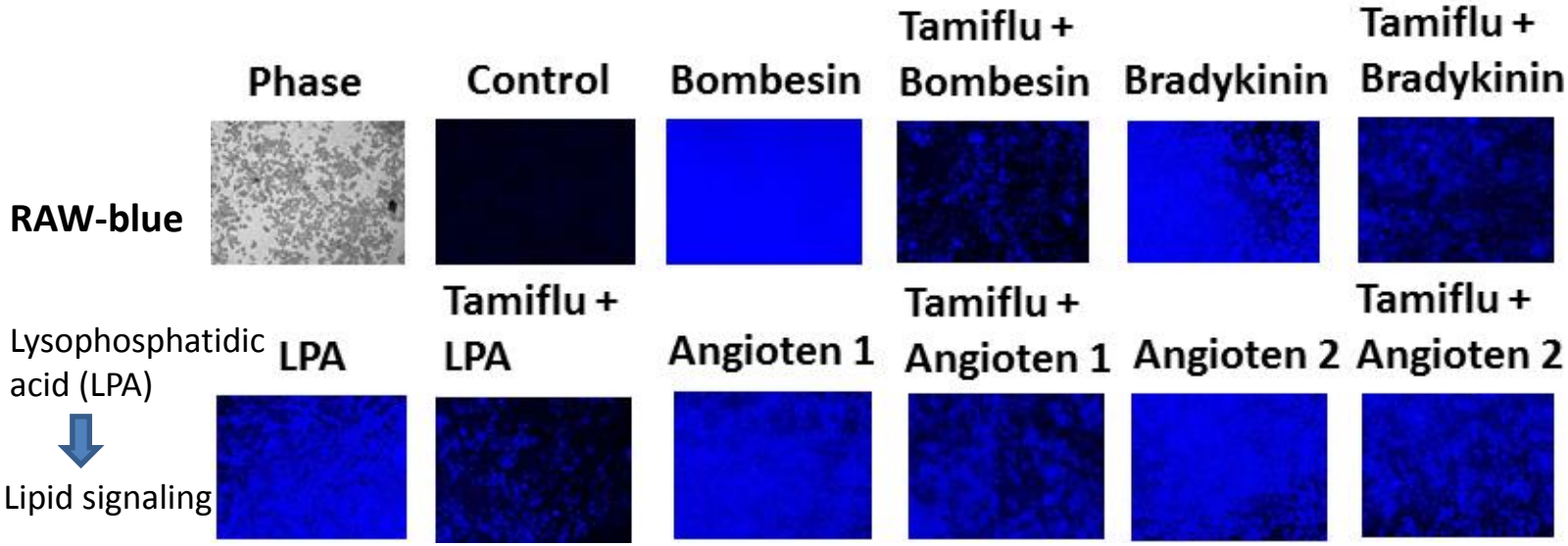
Samar Abdulkhalek, Merry Guo, Schammim Ray Amith [1], Preethi Jayanth [2], Myron R. Szewczuk *

Department of Biomedical & Molecular Sciences, Queen's University, Kingston, Ontario, Canada K7L3N6

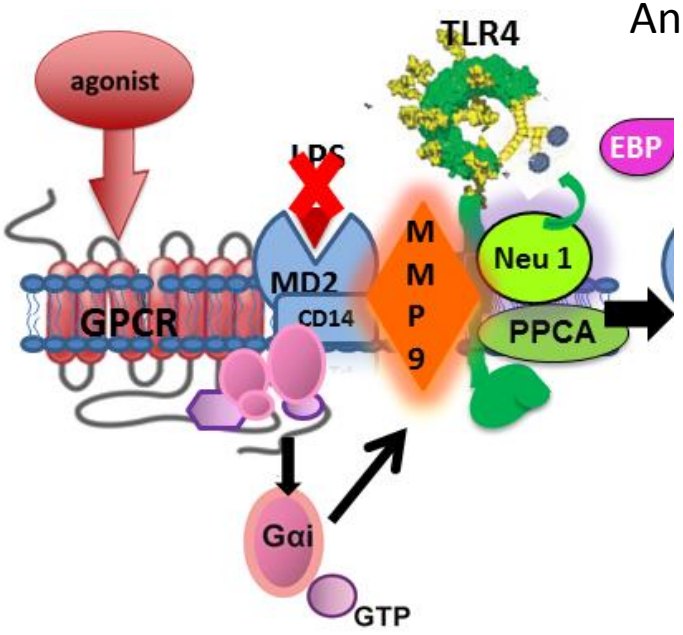


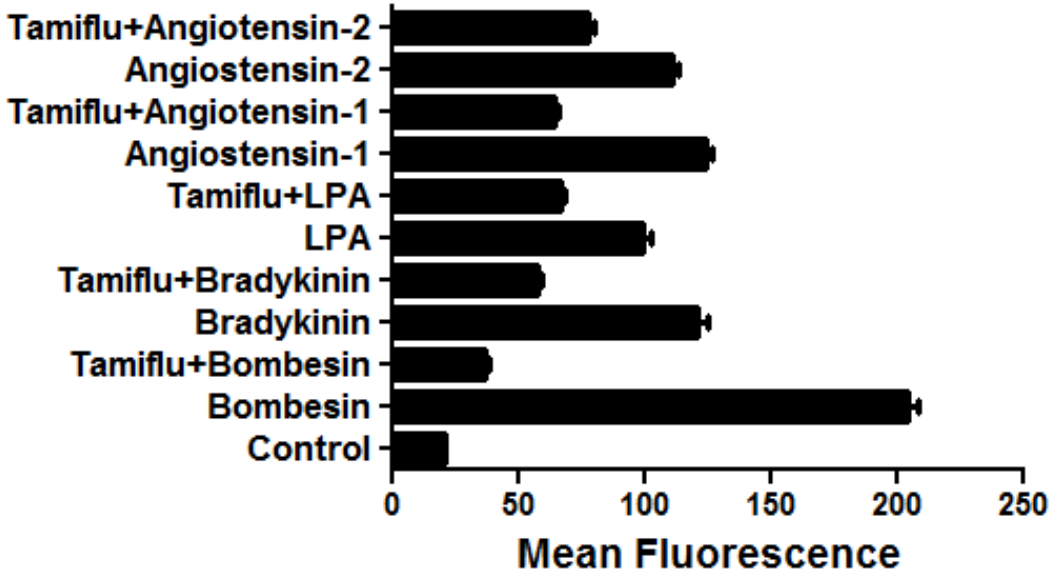
GPCR agonists induced sialidase activity is inhibited by oseltamivir phosphate

a potent endothelium-dependent vasodilator, leading to a drop in blood pressure

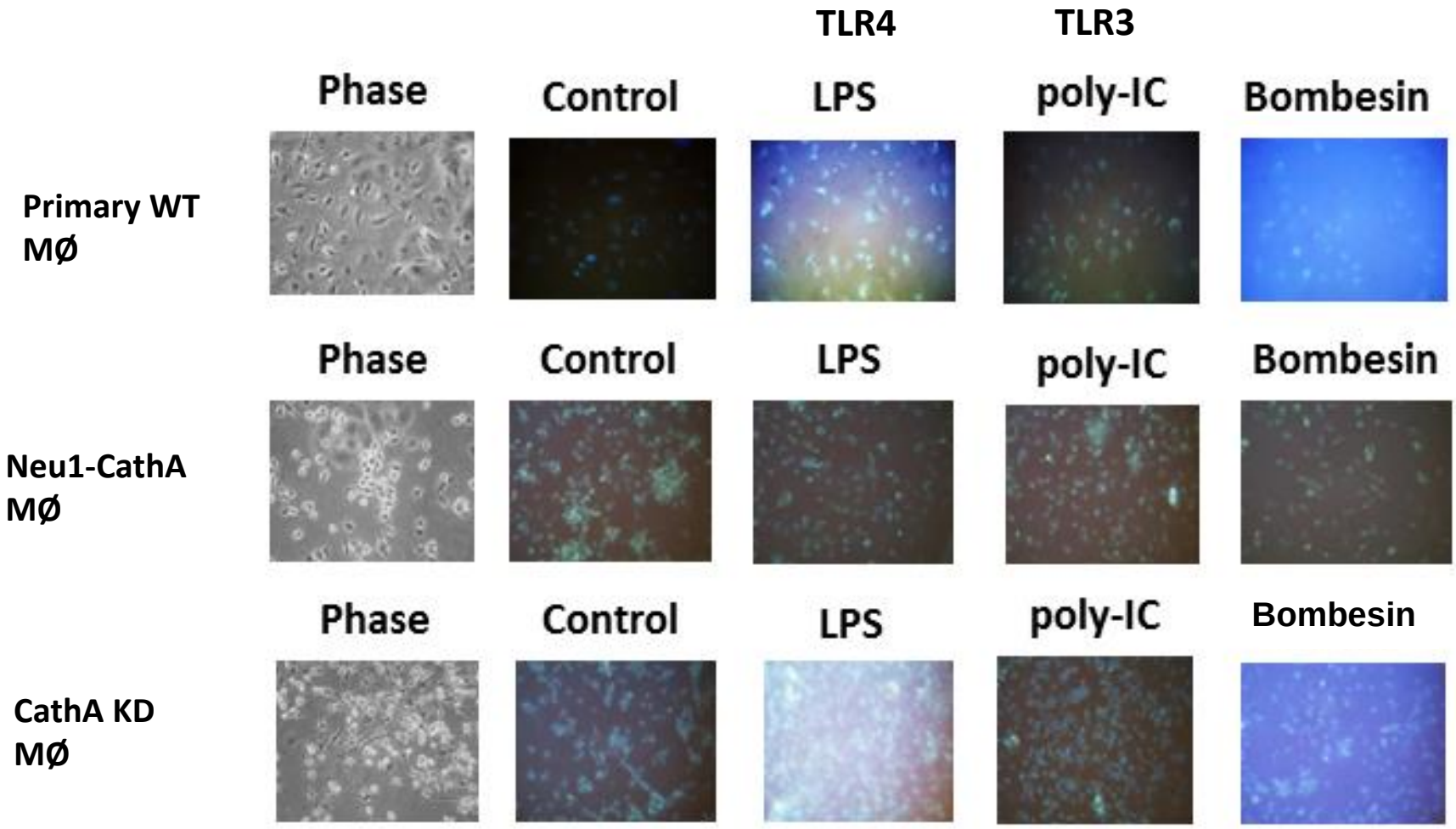


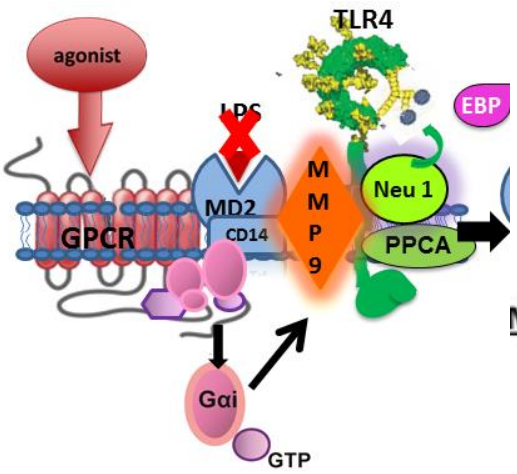
Angiotensin -vasoconstriction

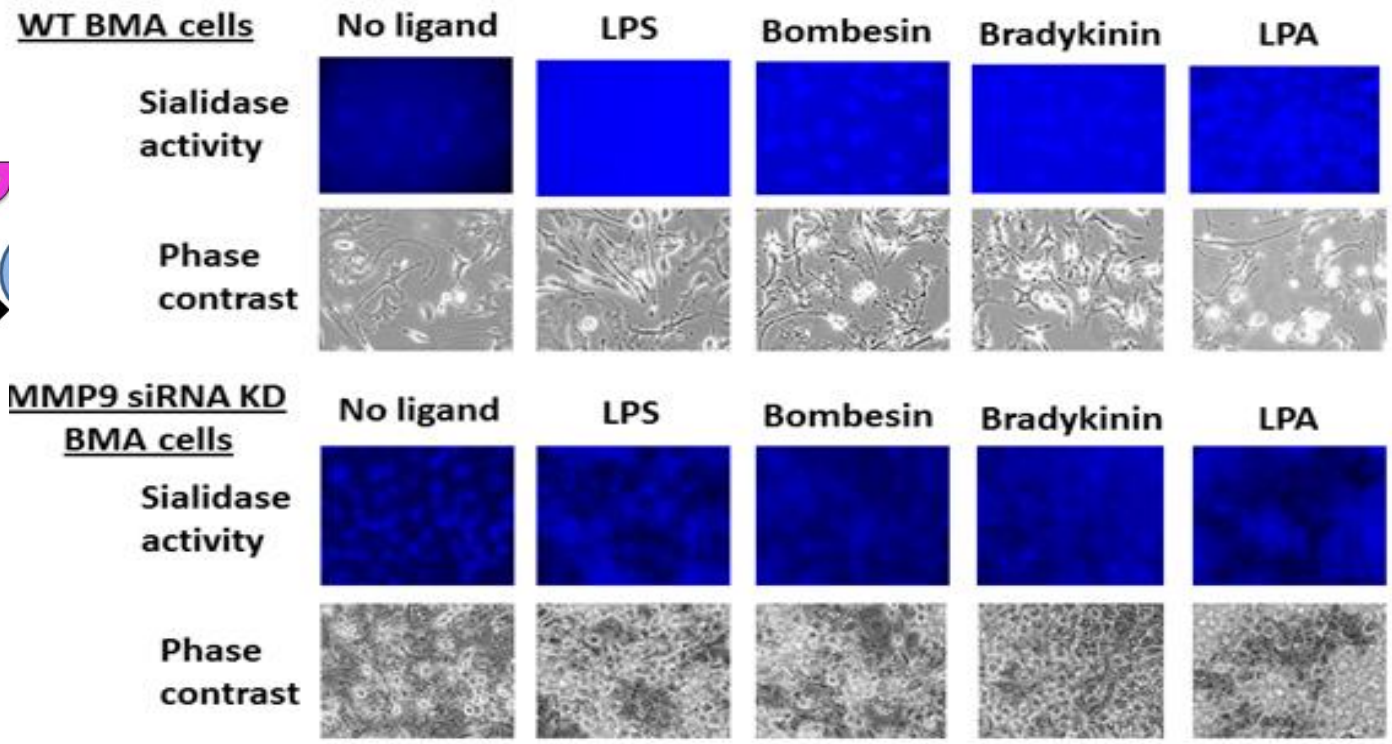




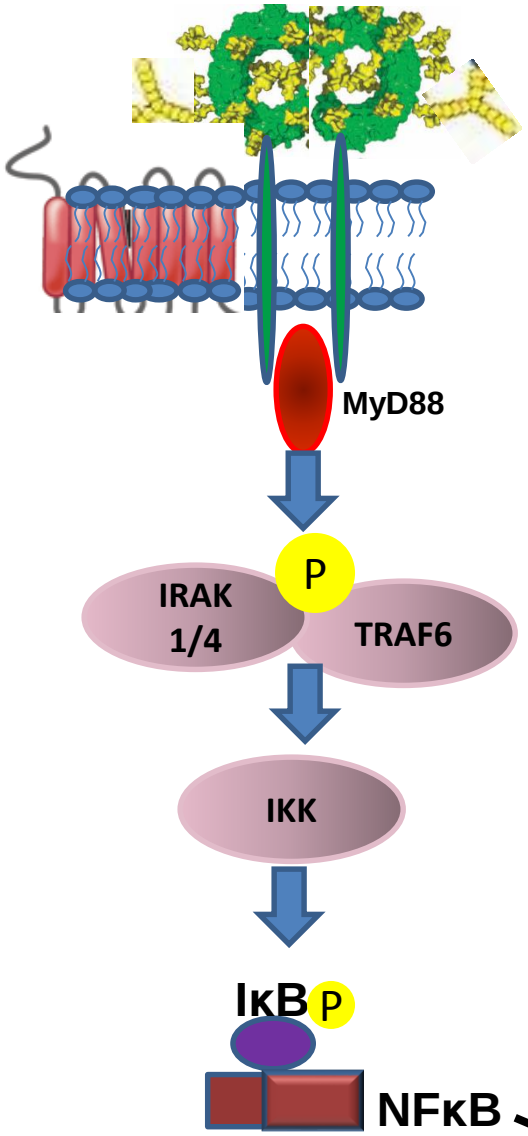
Sialidase activity is abscent in Neu1 deficient primary macrophages derived from genetically engineered mice

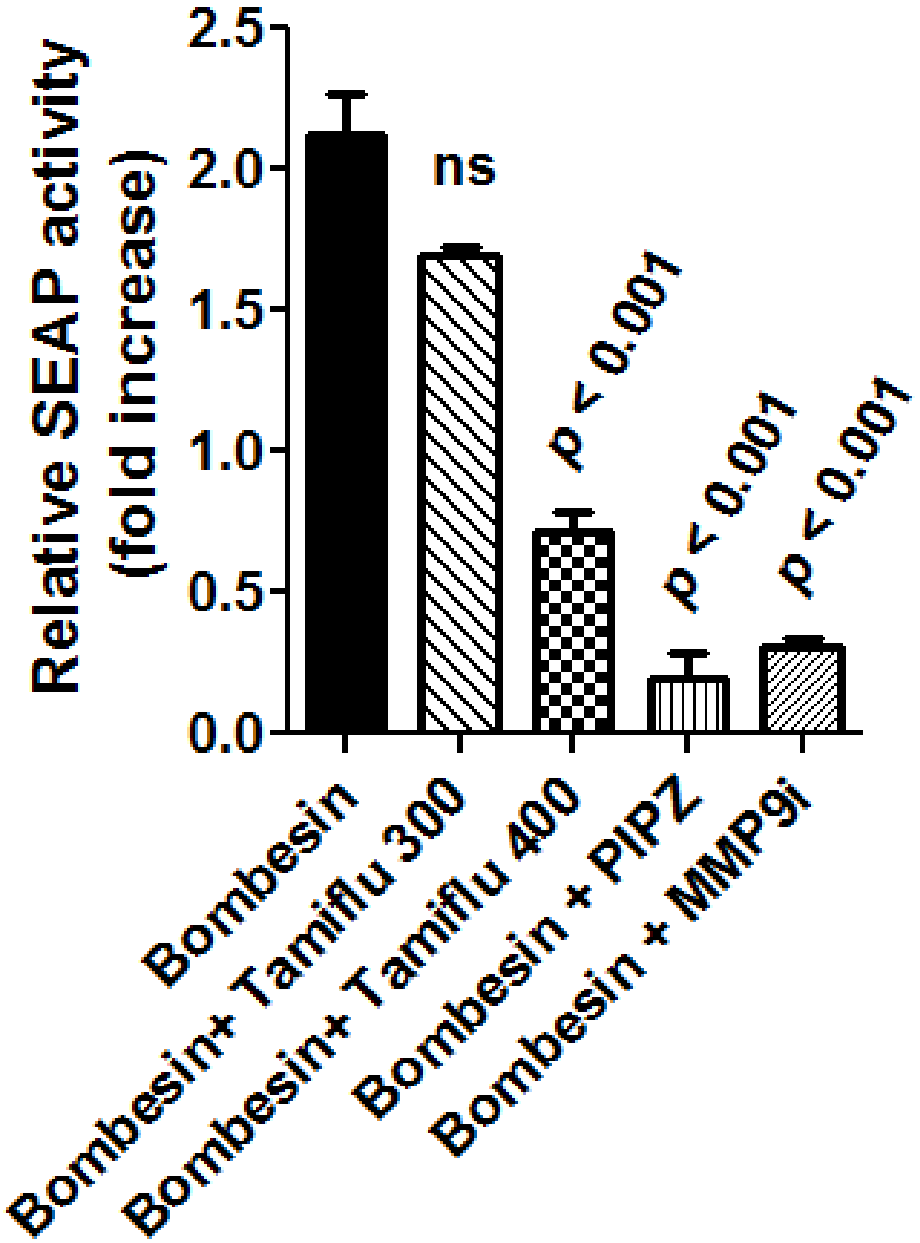



agonist
TLR4
LPS
EBP
MD2
CD14
GPCR
MMP9
Neu 1
PPCA
Gαi
GTP

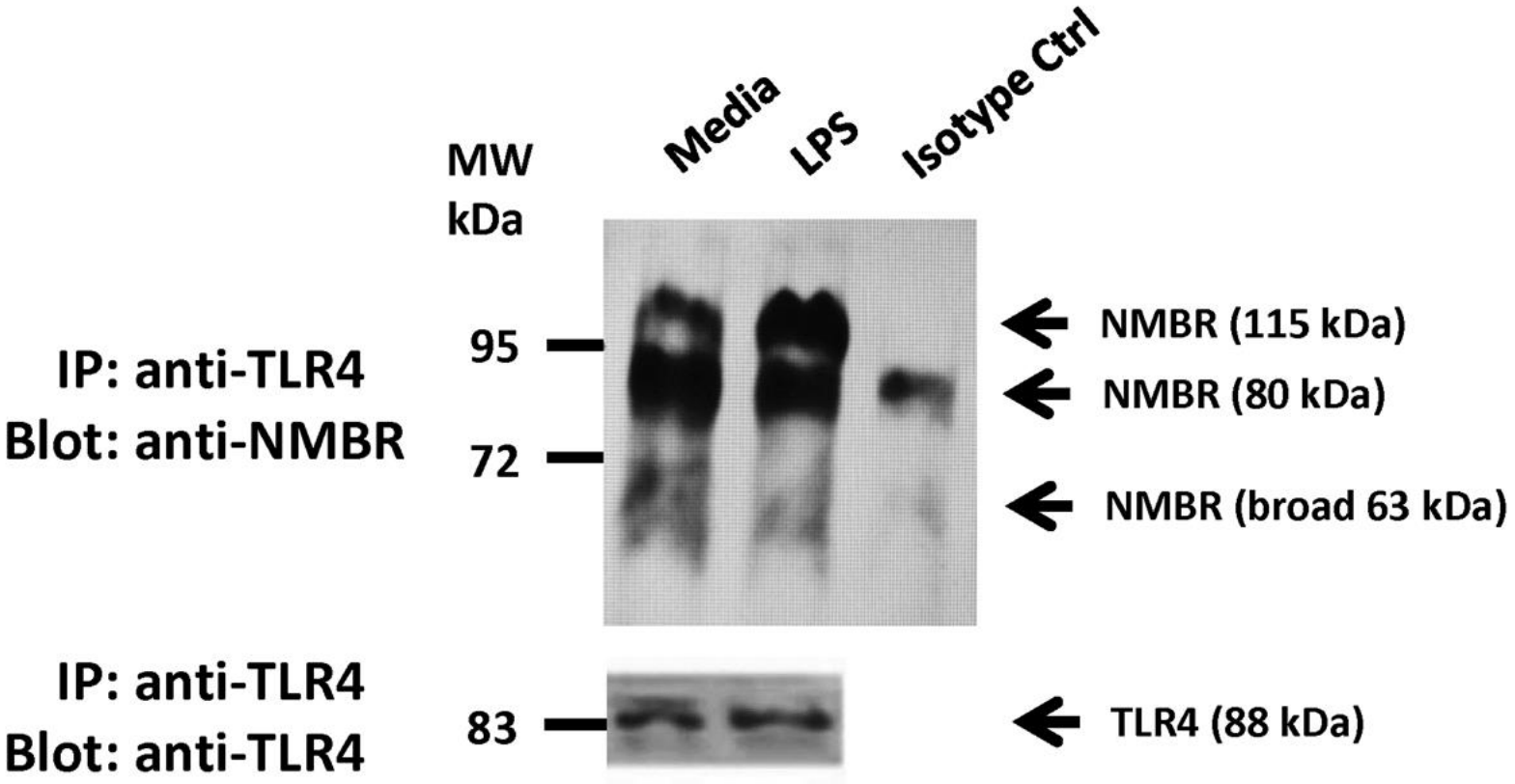

WT BMA cells
No ligand
LPS
Bombesin
Bradykinin
LPA
Sialidase activity
Phase contrast
MMP9 siRNA KD BMA cells
No ligand
LPS
Bombesin
Bradykinin
LPA
Sialidase activity
Phase contrast

Bombesin induces NFκB activation in Raw-blue cells

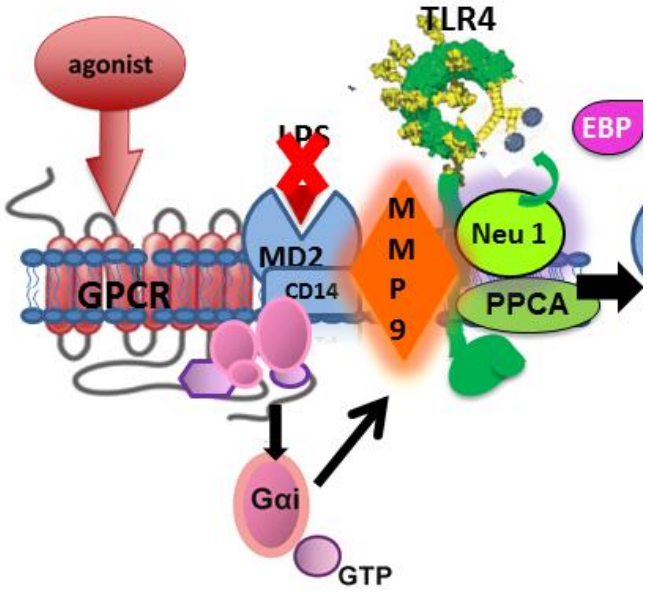


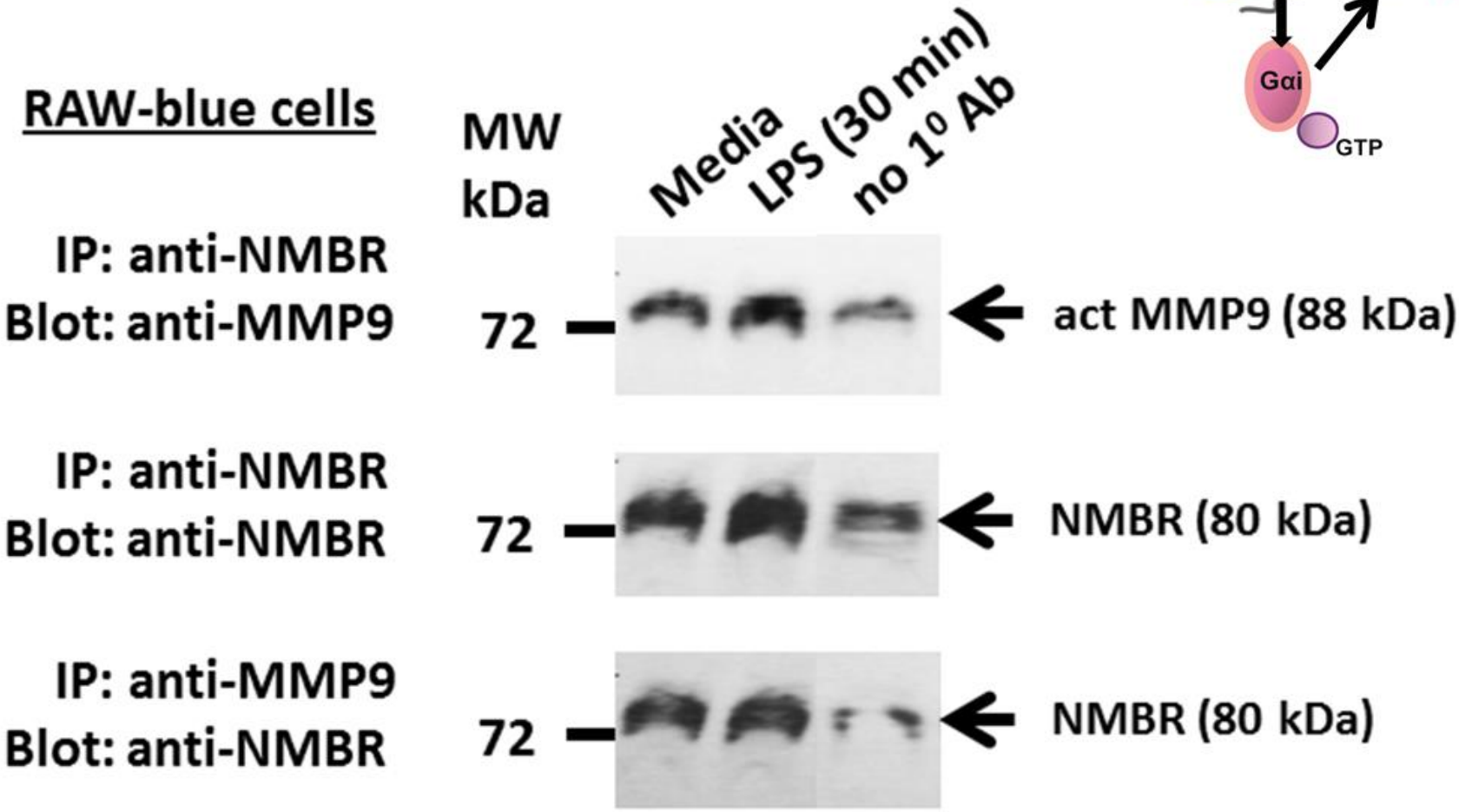


Bombesin receptor neuromedin B (NMBR) co-IP's with TLR4 in BMA MØ cell lysates

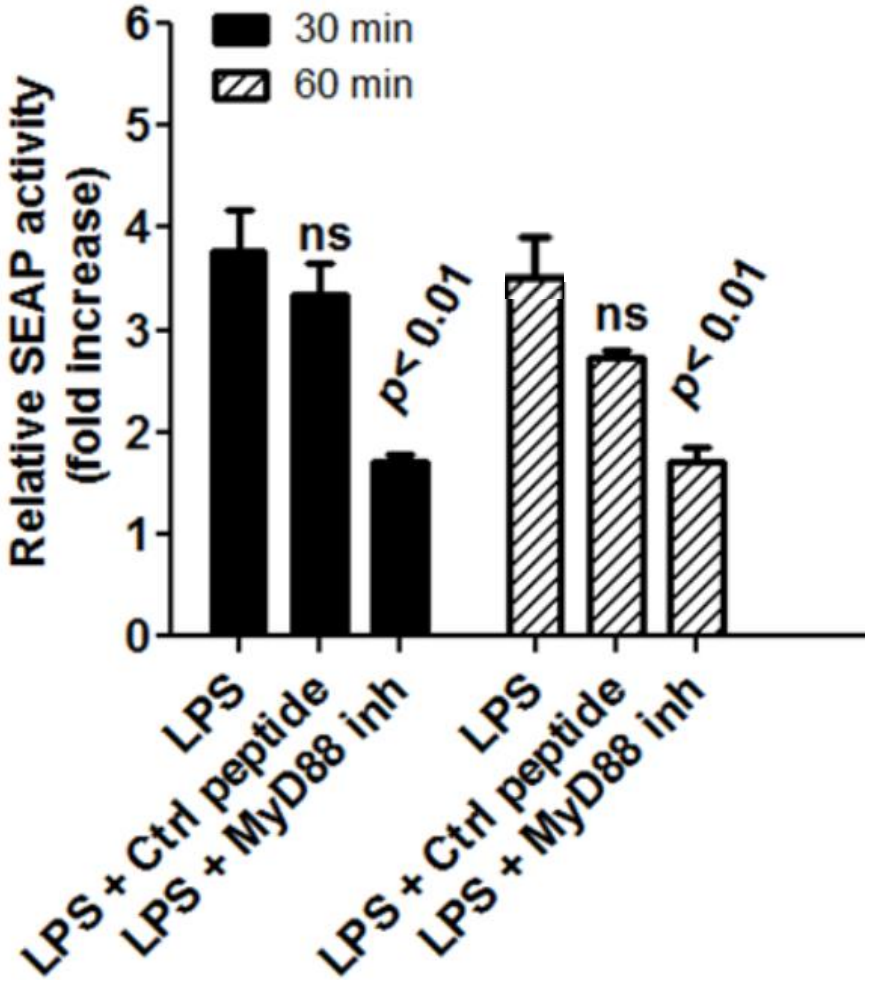


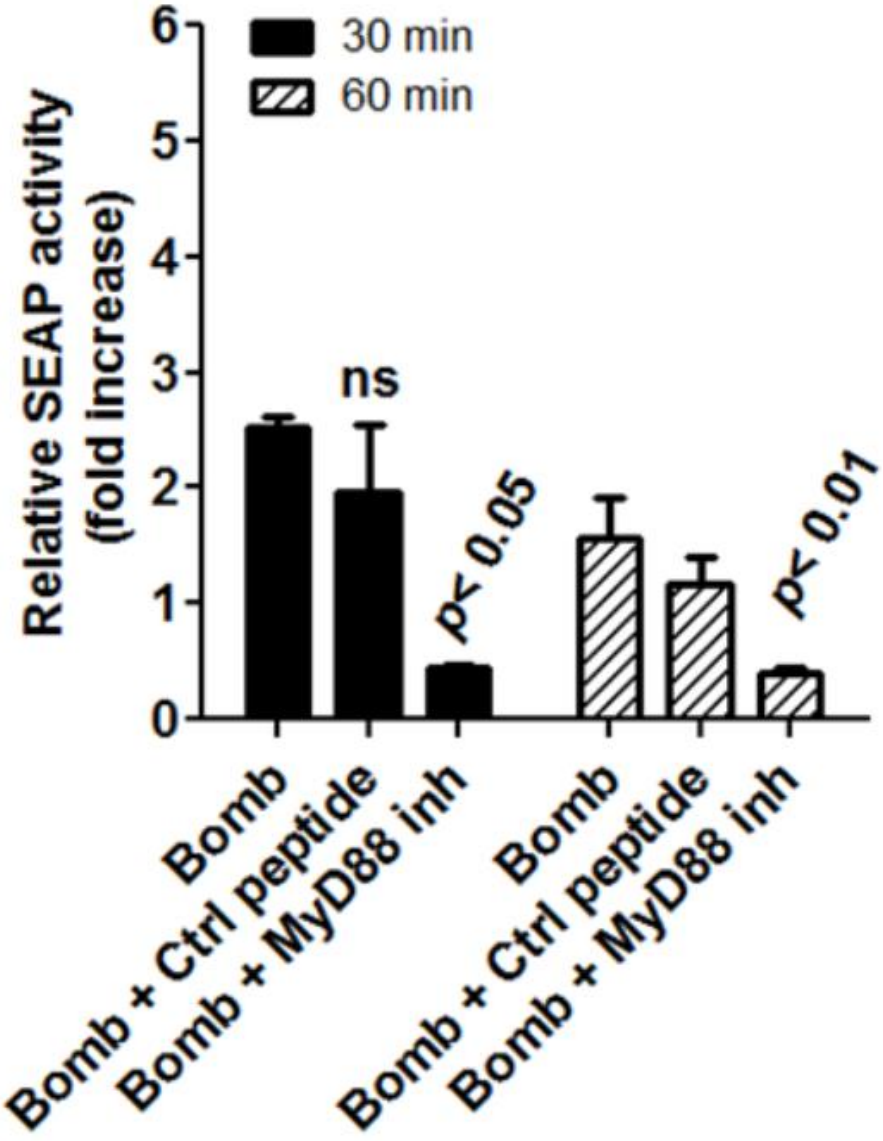
Bombesin receptor NMBR co-IP's with MMP-9 in RAW-blue MØ cell lysates





LPS and Bombesin agonists induced SEAP activity in RAW-blue cells is blocked by MyD88 specific inhibitor





Cellular Signalling 25 (2013) 2093–2105

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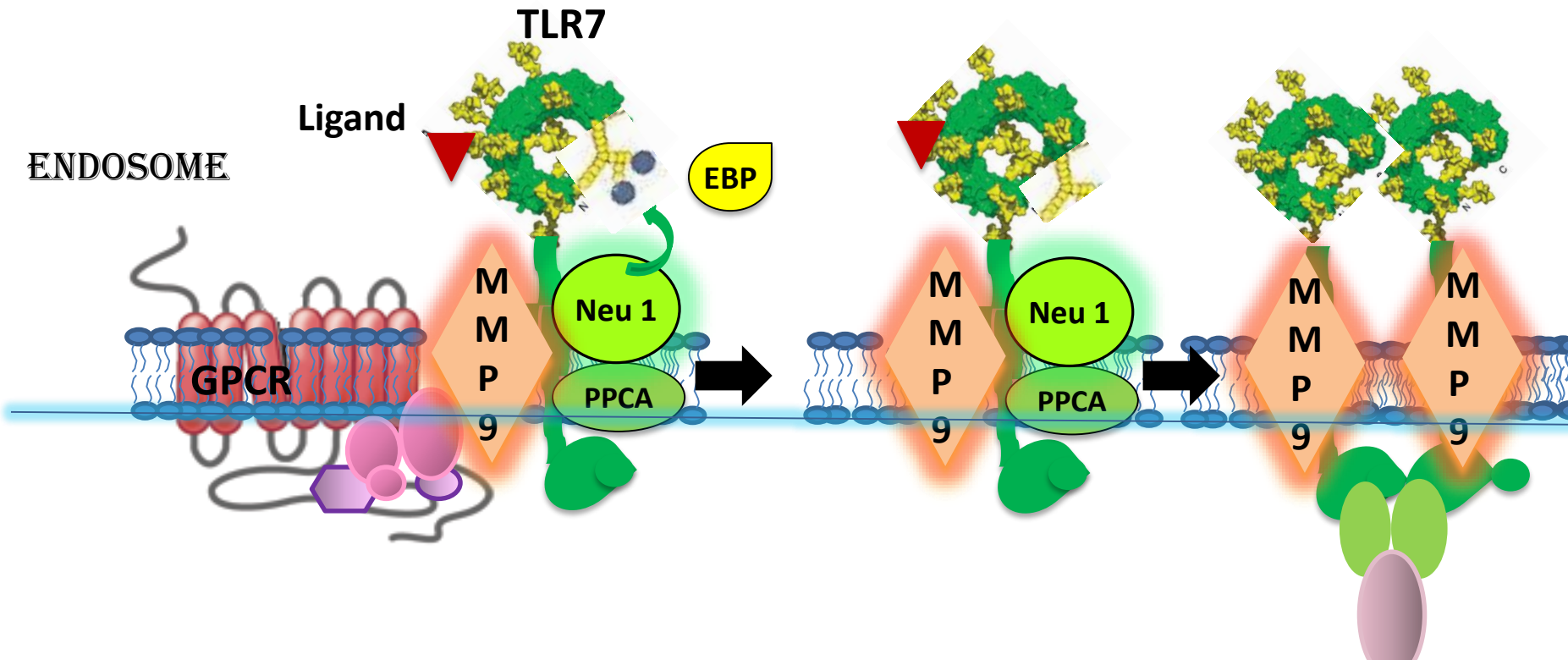
Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

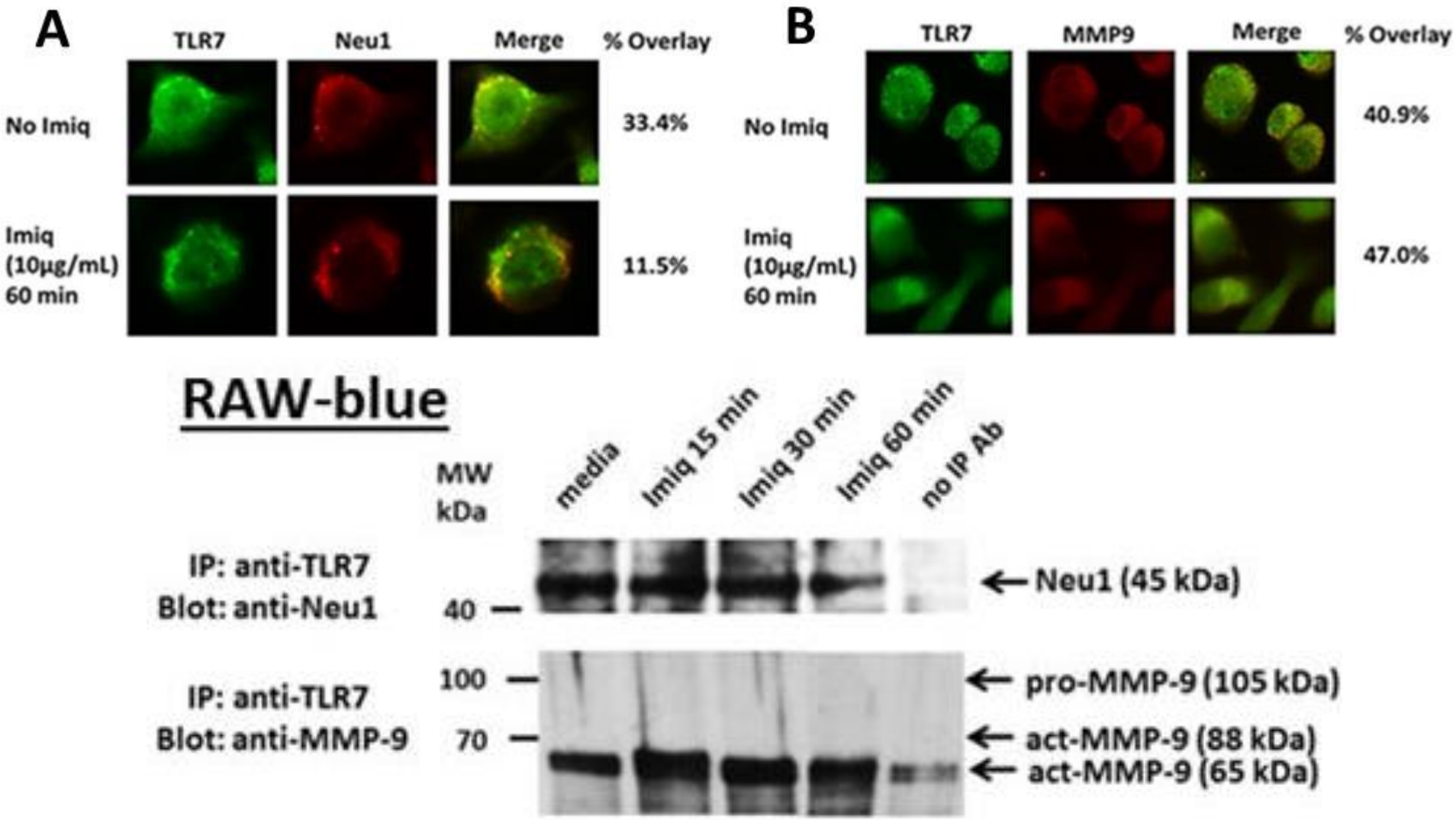
Neu1 sialidase and matrix metalloproteinase-9 cross-talk regulates nucleic acid-induced endosomal TOLL-like receptor-7 and -9 activation, cellular signaling and pro-inflammatory responses ☆

Samar Abdulkhalek, Myron R. Szewczuk *

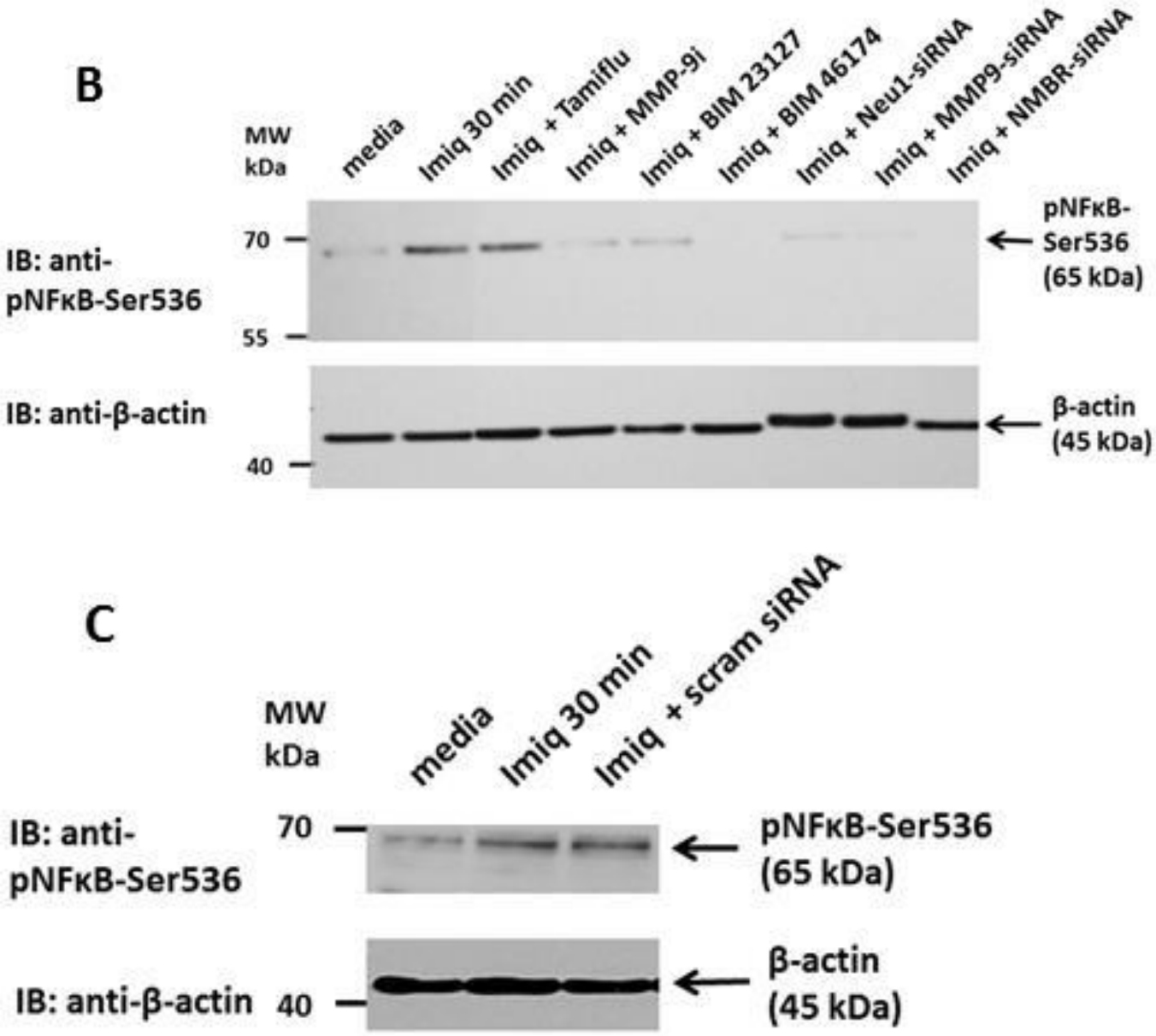
Department of Biomedical & Molecular Sciences, Queen's University, Kingston, Ontario K7L3N6, Canada



NEU1 and MMP-9 form a complex with TLR7 in naïve and ligand-stimulated macrophage cells



NEU1, MMP-9 and NMBR are essential for TLR7 activation



NMBR is involved in intracellular TLRs signaling

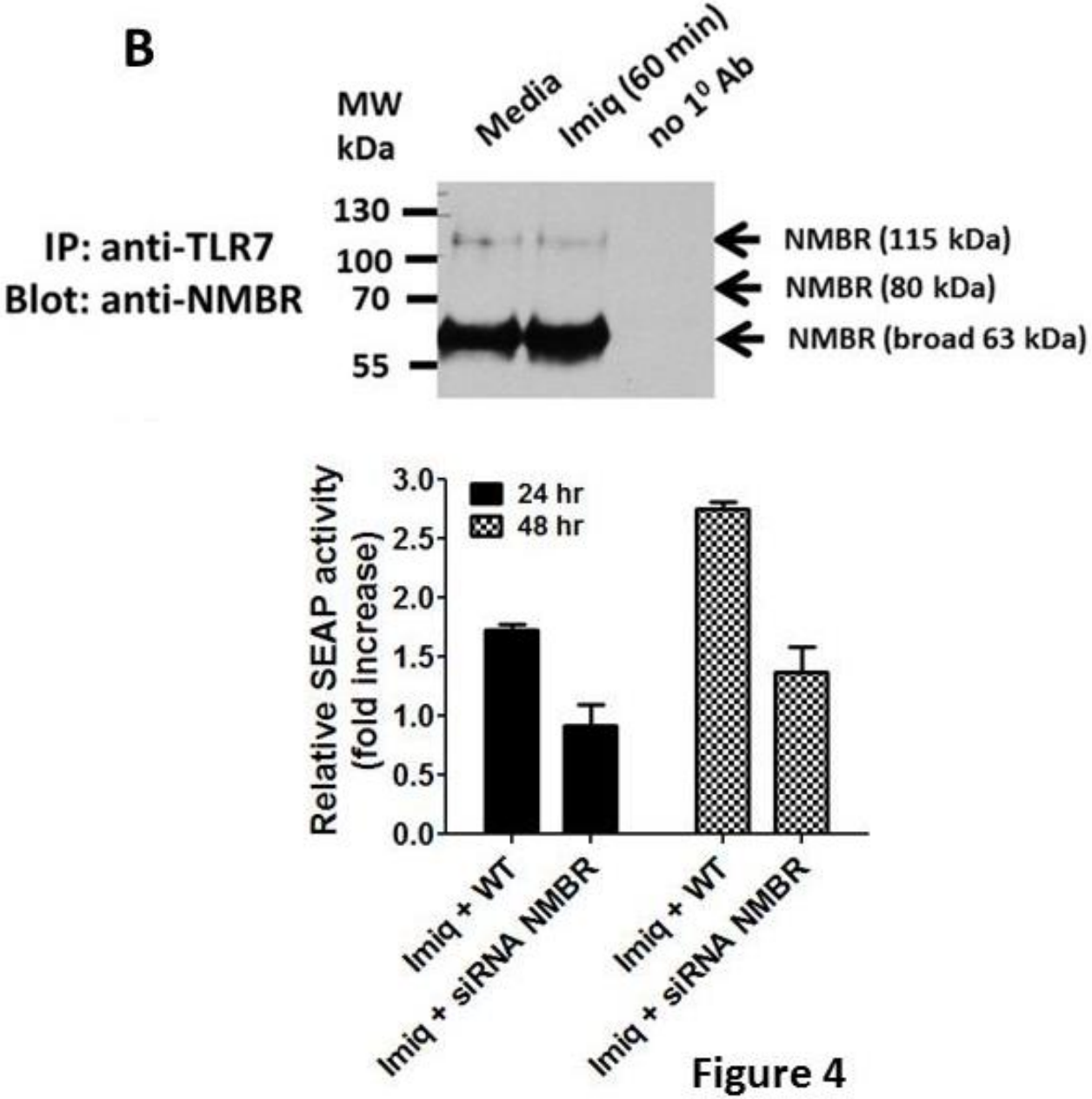
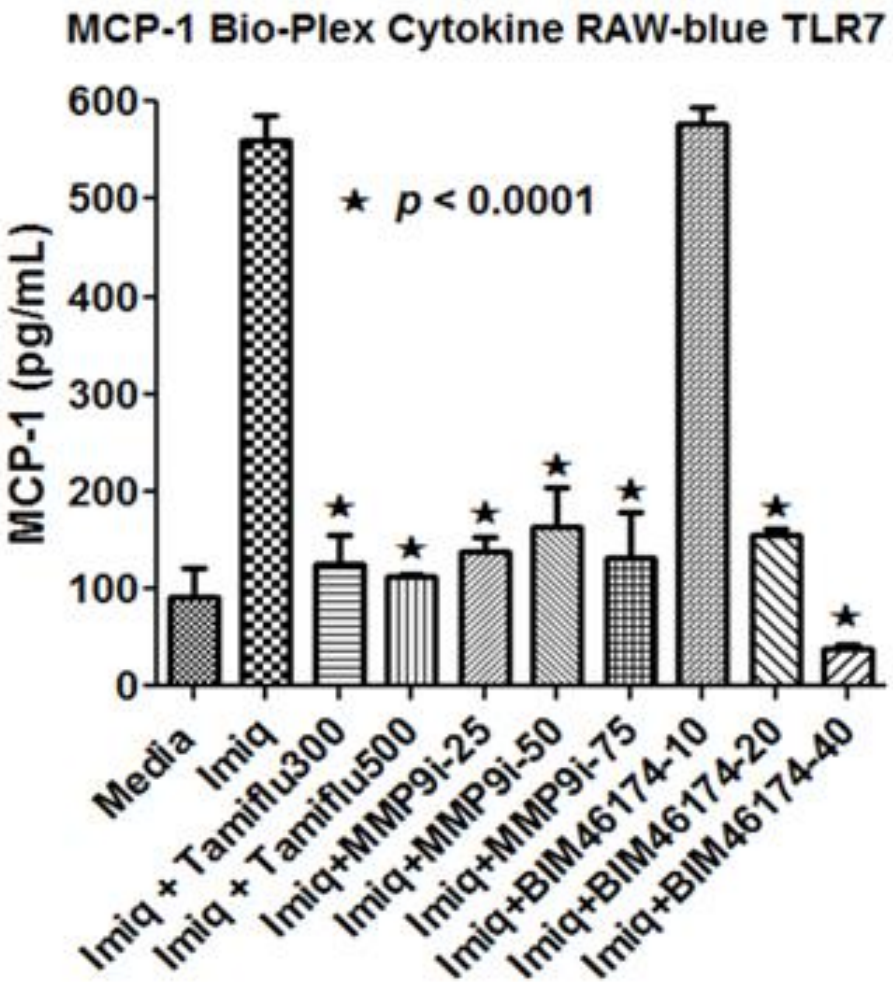
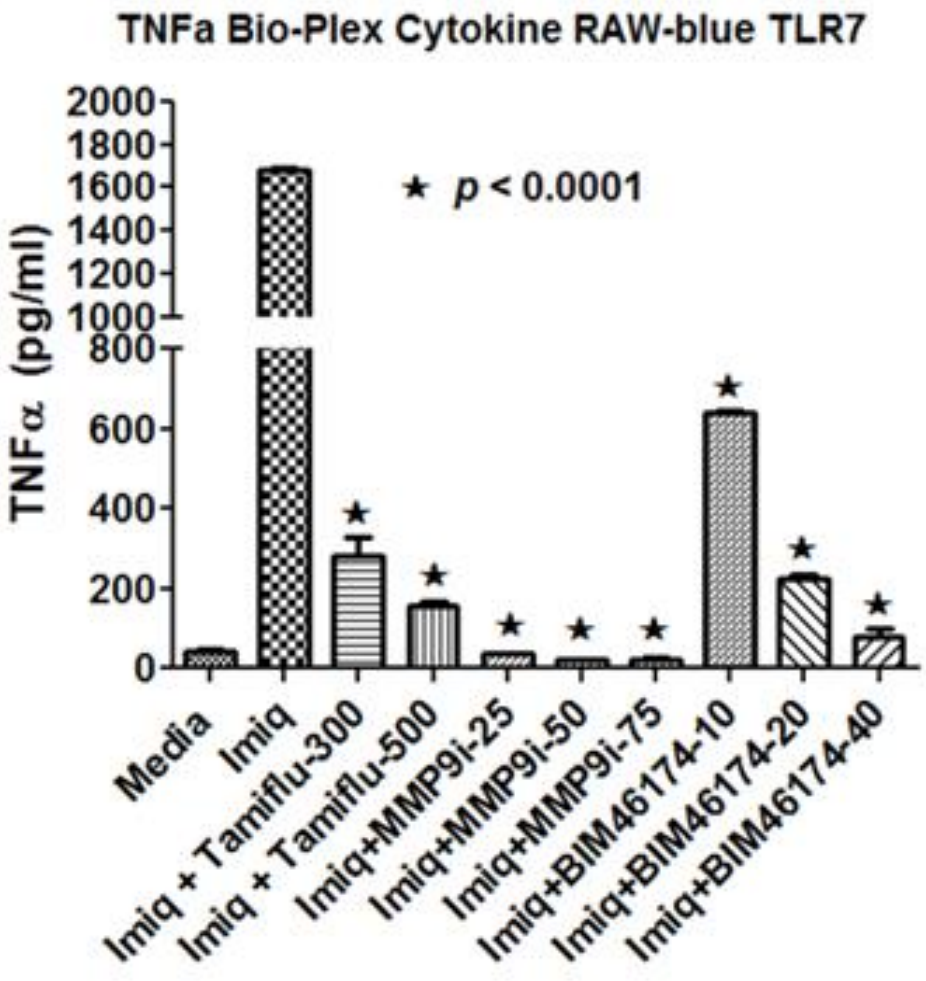


Figure 4

Tamiflu, MMP9i and BIM46174 abrogates TLR7 ligand-induced inflammatory cytokines production





CONCLUSIONS

- Neu1 plays a central role in mediating nucleic acid-induced intracellular TLR activation,
- GPCR NMBR–MMP9–Neu1 cross-talk constitutes a novel intracellular TLR signaling platform that is essential for NF-κB activation and pro-inflammatory responses.