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

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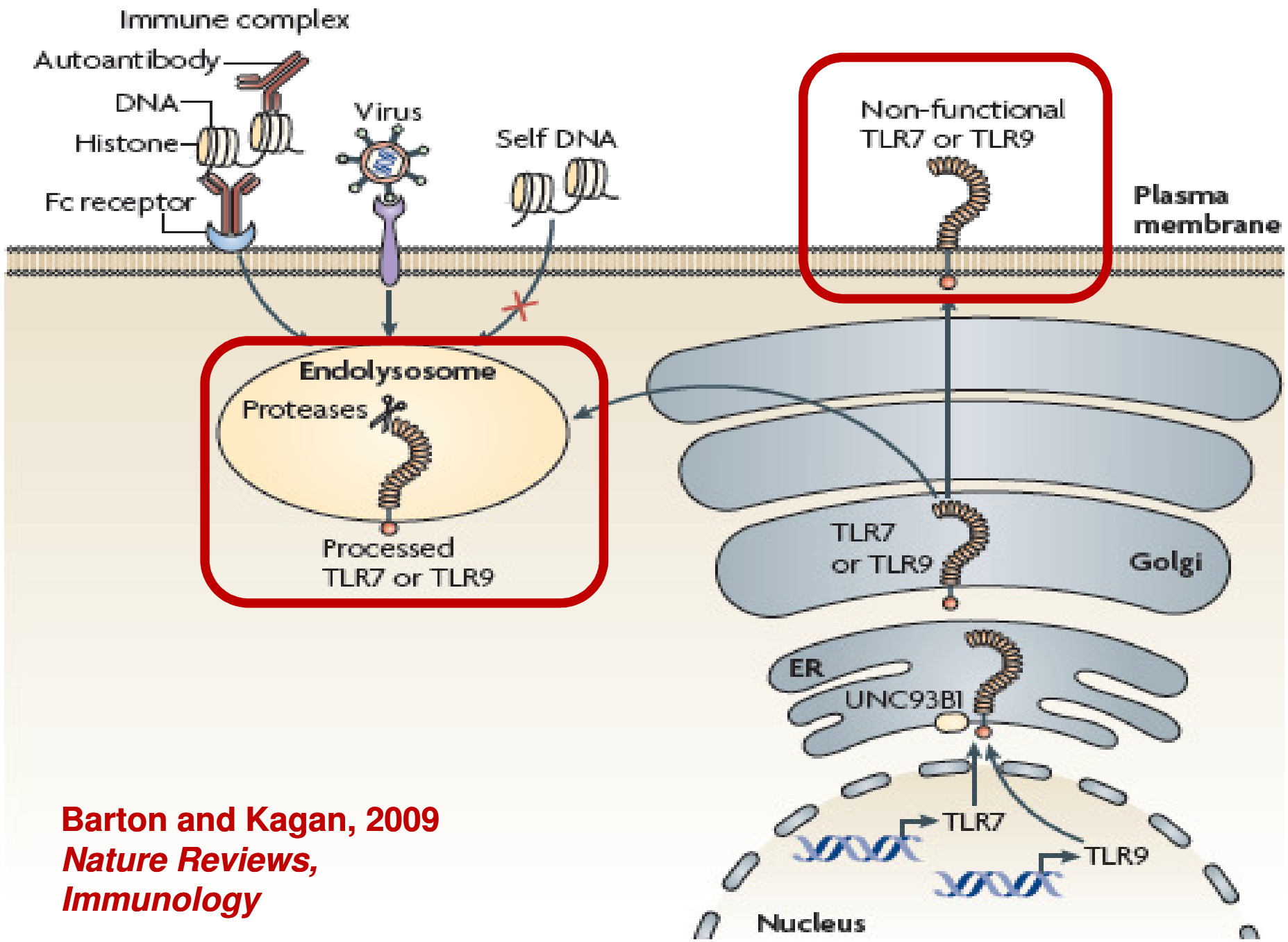
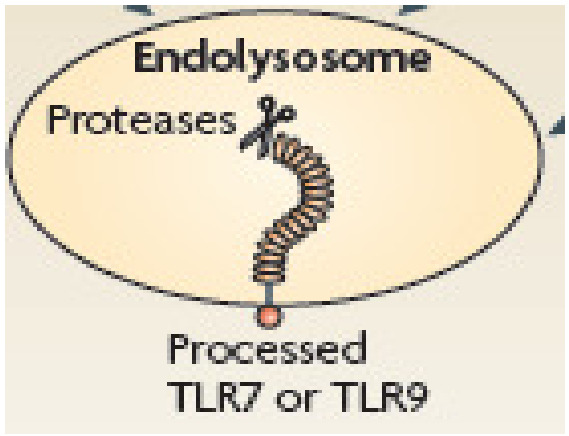
- **A novel signaling paradigm for intracellular TOLL-like receptors, TLR-7 and TLR-9**

- **Central to this process is that NMBR GPCR-Neu1-MMP9 complex is bound to TLR7 and TLR9 in naive and ligand stimulated macrophage cells.**



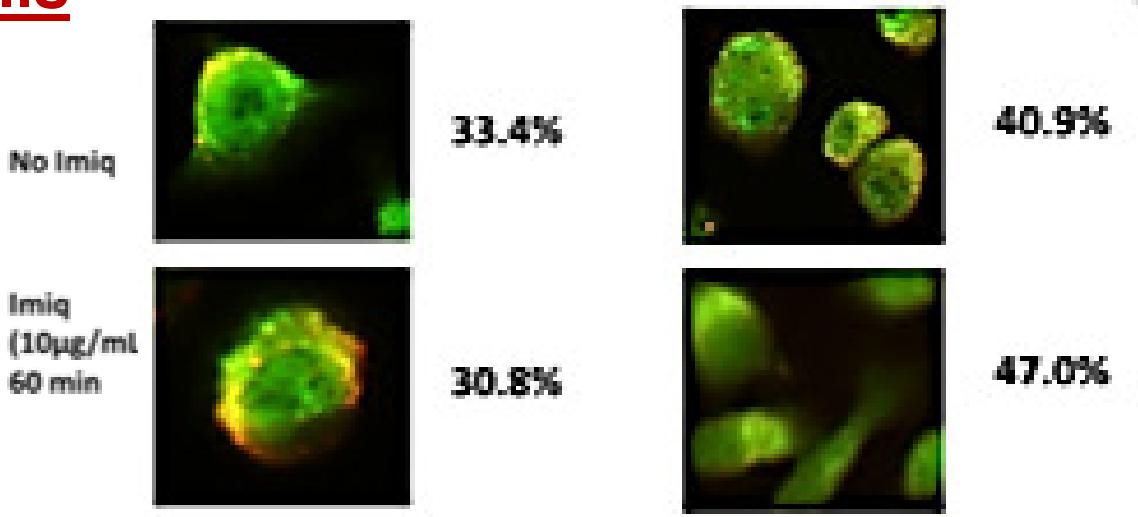
Barton and Kagan, 2009
Nature Reviews, Immunology

RAW-blue MØ cells



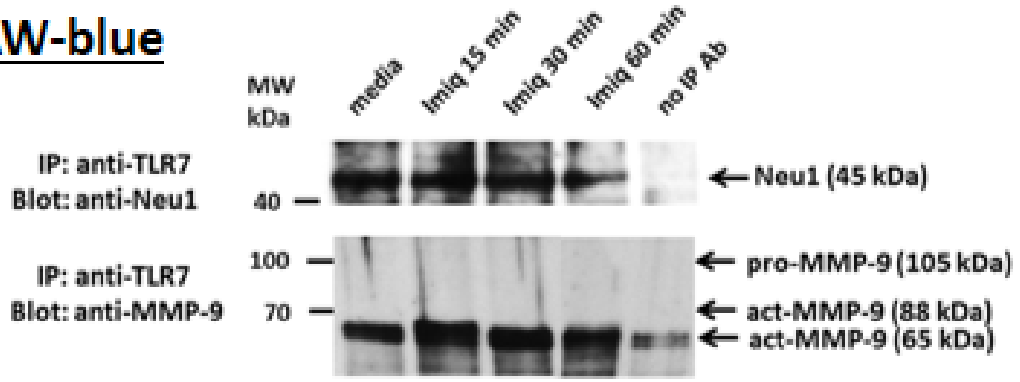
Neu1/TLR7

MMP9/TLR7

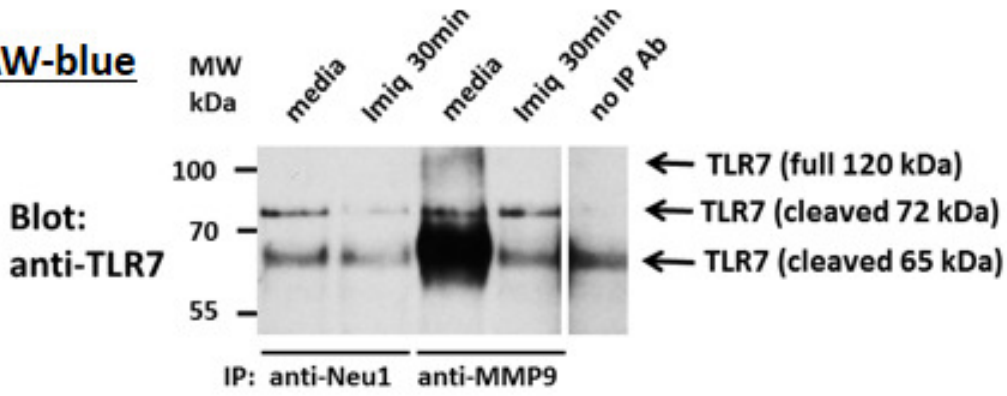


- ✓ Neu1 and MMP9 co-IP with TLR7 in RAW-blue cell lysates
- ✓ cleaved TLR7 co-IP's with Neu1 and MMP9 in RAW-blue cell lysates

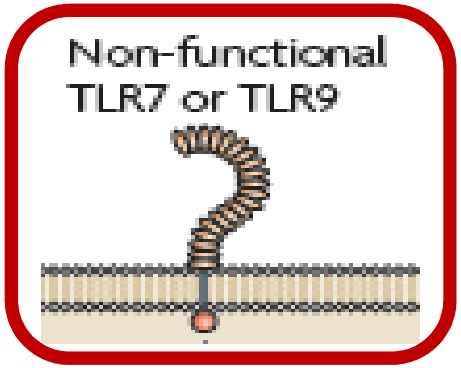
RAW-blue



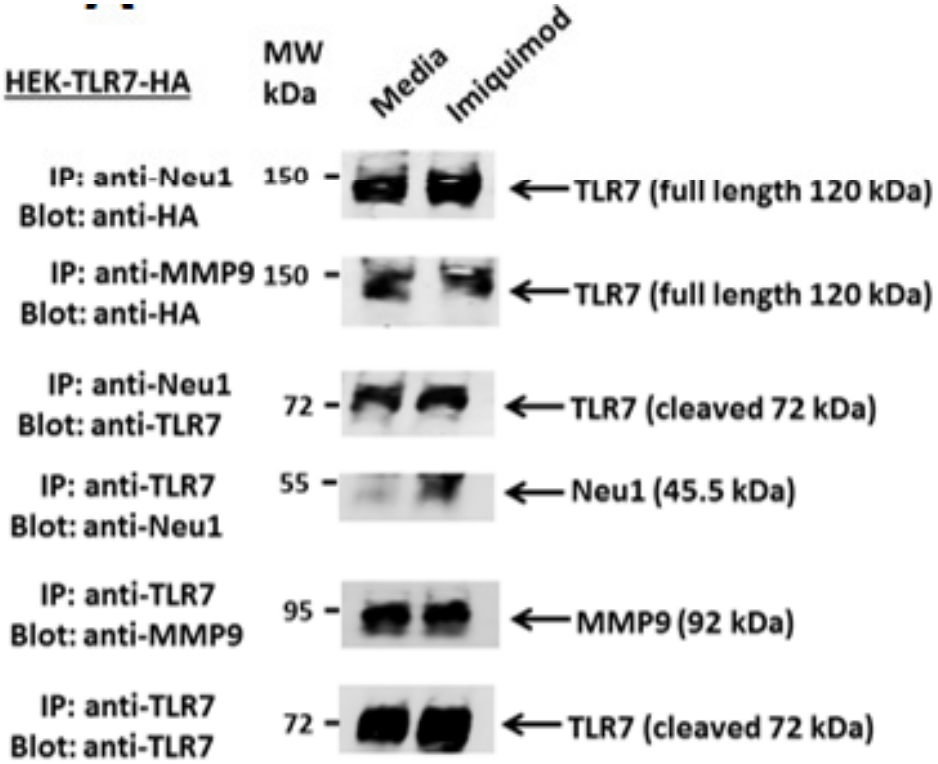
RAW-blue



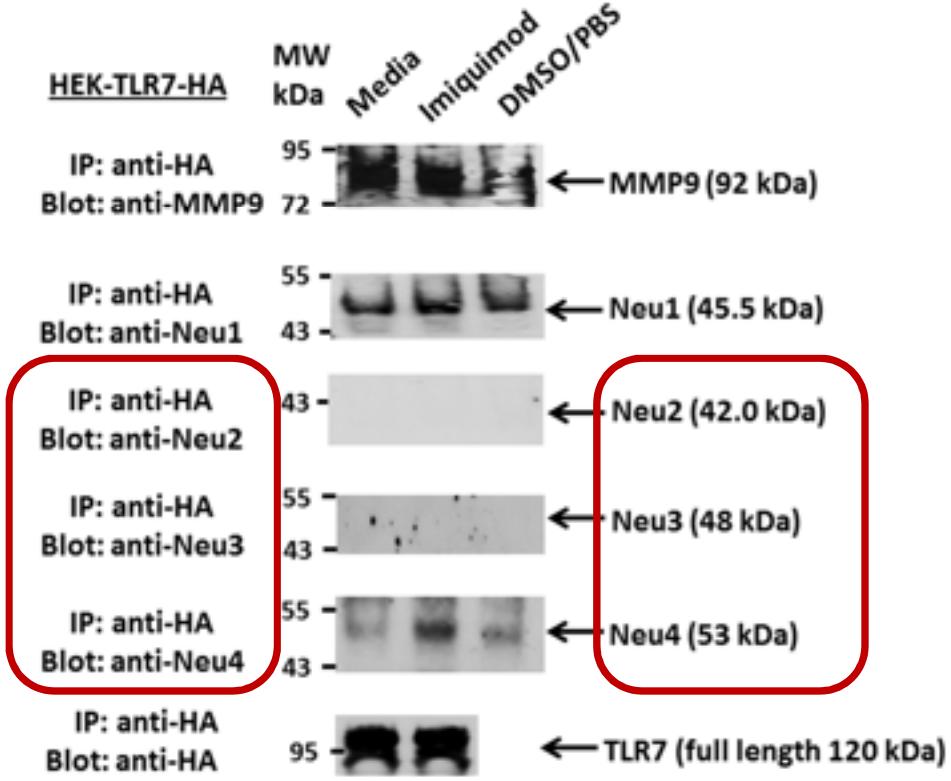
MMP9 and Neu1 form a complex with TLR7 in cell lysates of naïve and imiquimod-stimulated HEK cells expressing full length TLR7-hemaglutinine (HA) tag.

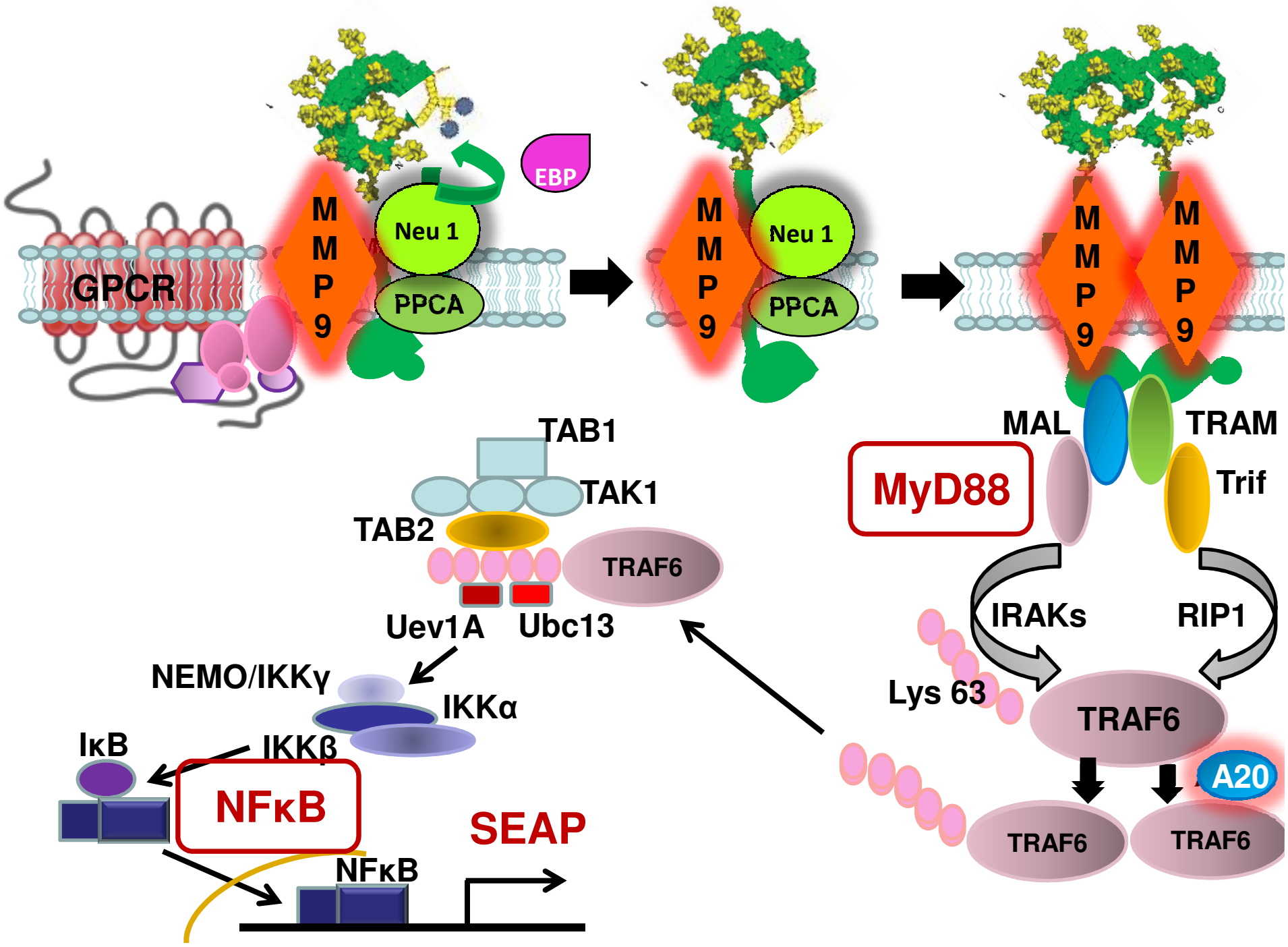


✓ **Neu1 and MMP9 co-IP with full length TLR7 in RAW-blue cell lysates**

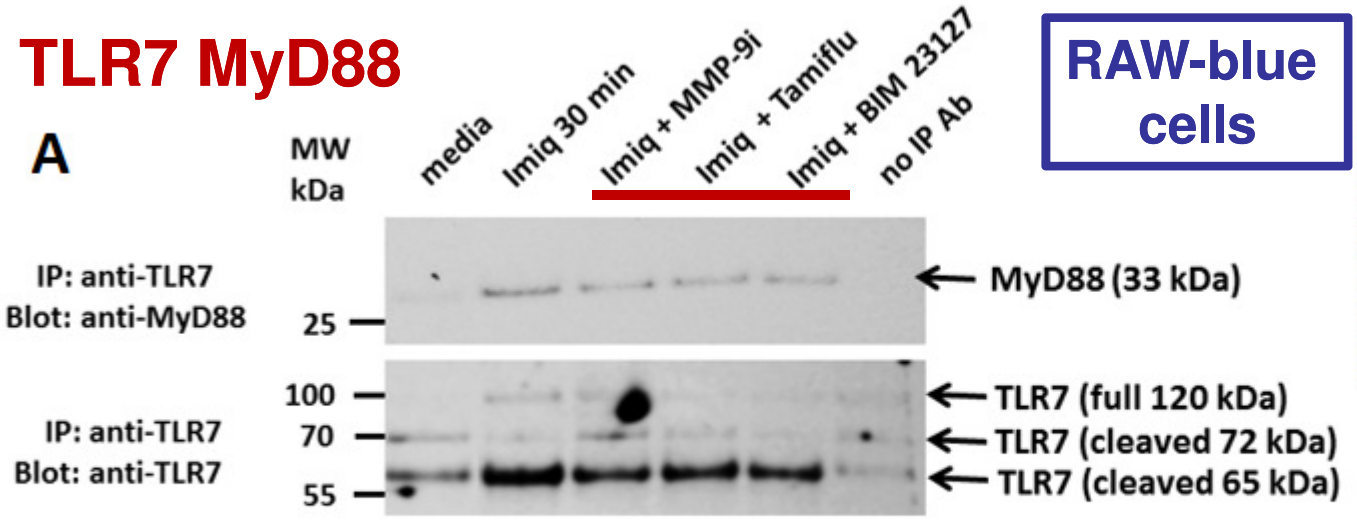


✓ **TLR7-HA co-IP's with Neu1 and MMP9 in RAW-blue cell lysates**

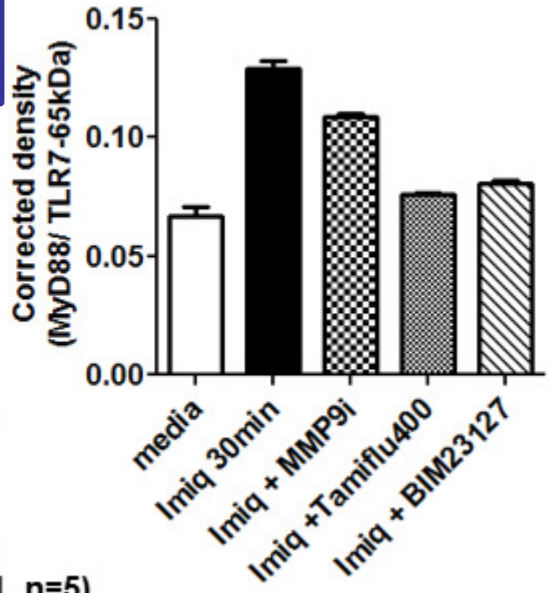



GPCR
MMP9
Neu 1
PPCA
EBP
MMP9
Neu 1
PPCA
MMP9
MMP9
MAL
TRAM
MyD88
Trif
IRAKs
RIP1
TAB1
TAK1
TAB2
TRAF6
Uev1A
Ubc13
NEMO/IKKγ
IKKα
IκB
IKKβ
NFκB
SEAP
NFκB
Lys 63
TRAF6
A20
TRAF6
TRAF6

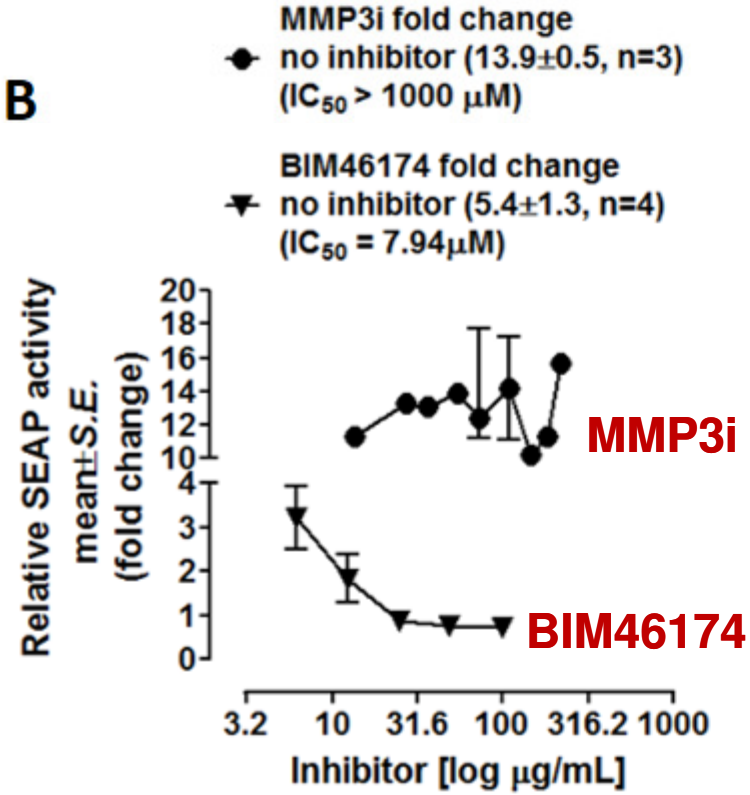
TLR7 MyD88

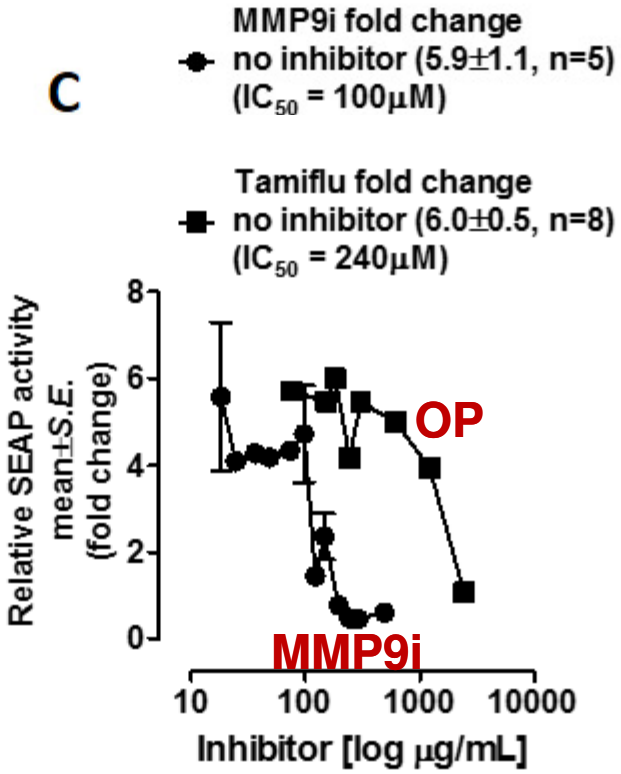


RAW-blue cells



TLR7 NFkB - SEAP





BIM-23127: neuromedin B receptor (**NMBR**) antagonist

BIM46174: selective inhibitor of heterotrimeric G-protein complex (PSEN-Institut Henri Beaufour, Les Ulis, France)

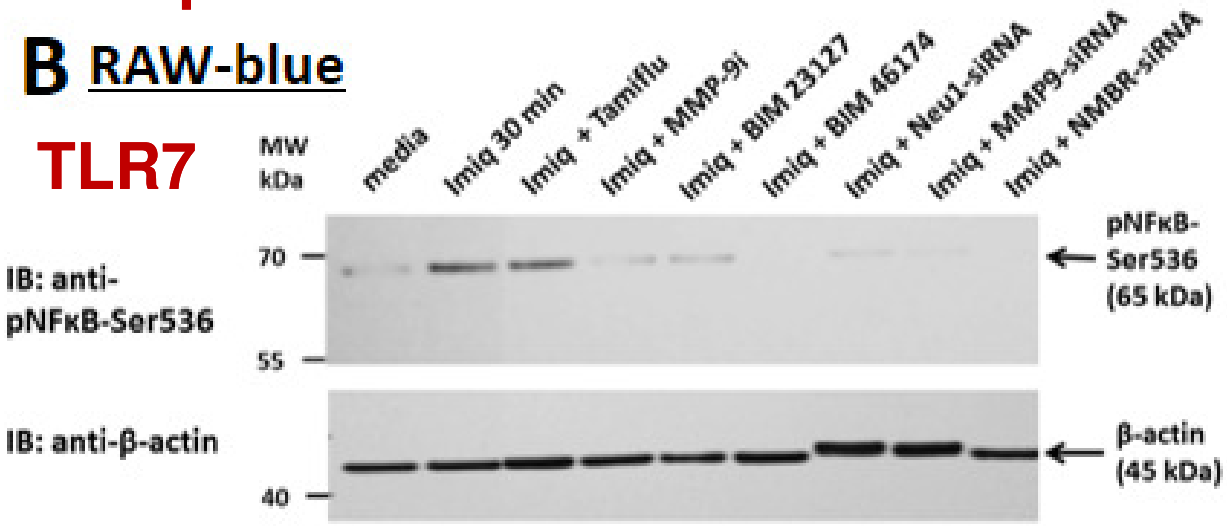
Figure 3

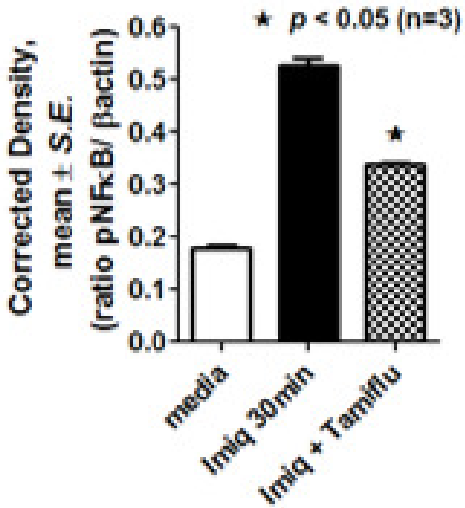
TLR7 pNFκB-ser536

siRNA

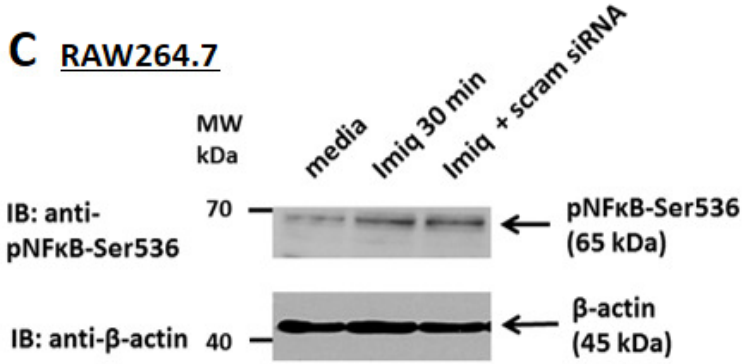
B RAW-blue

TLR7



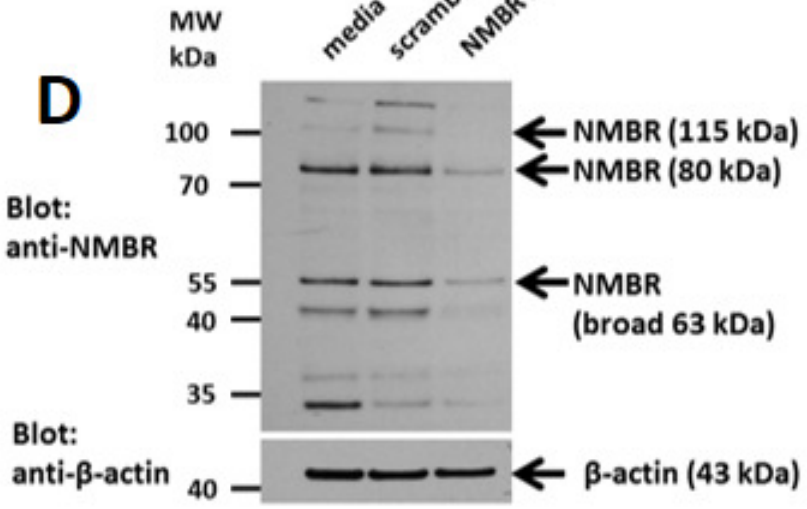


C RAW264.7



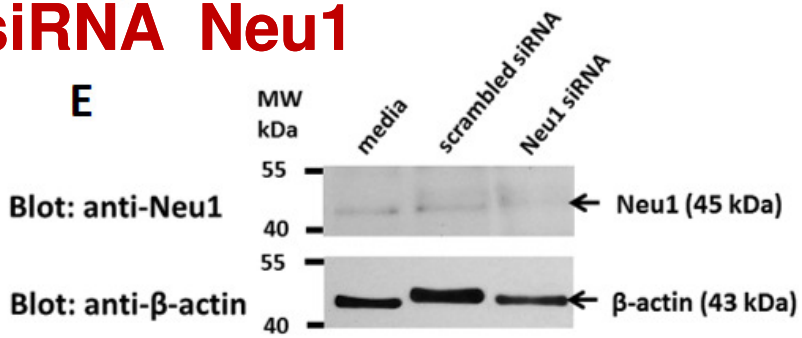
siRNA NMBR GPCR

D



siRNA Neu1

E



ODN 1826: cytosine phosphorothiolated guanine (CpG) type B dinucleotide specific for mouse TLR9

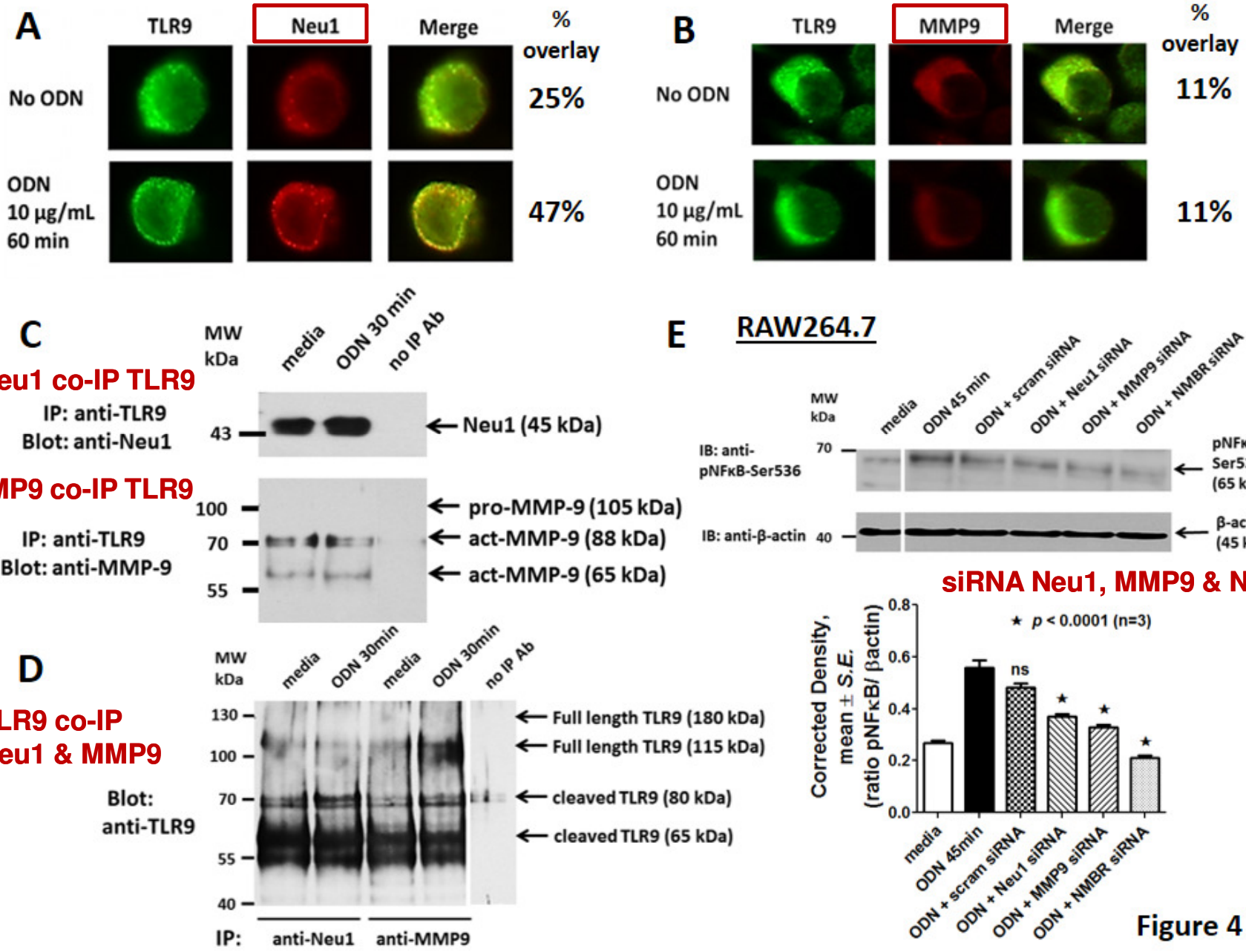
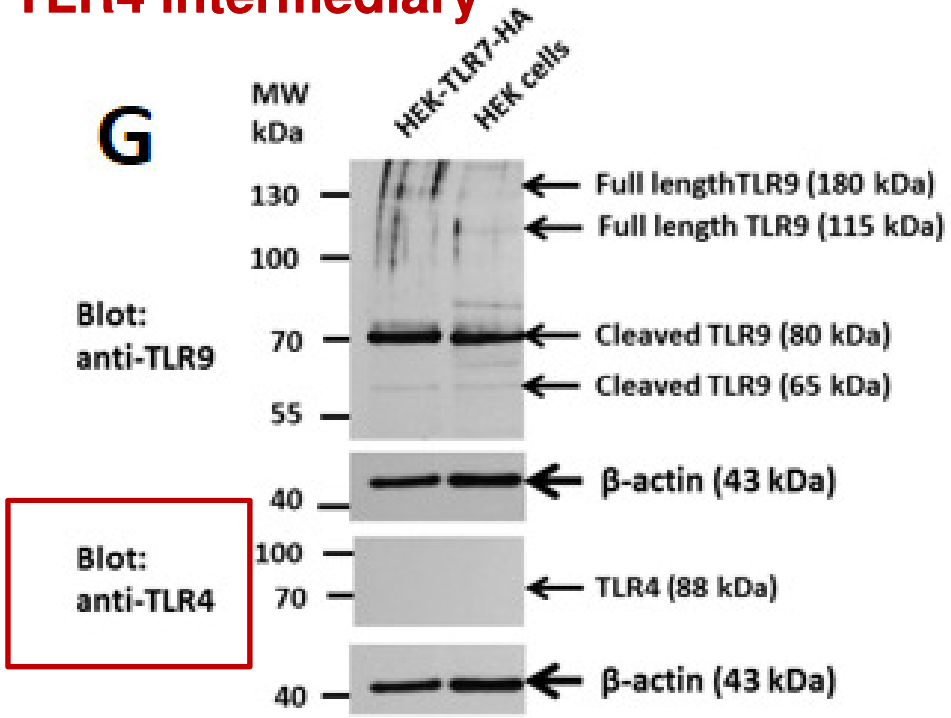


Figure 4

HMGB1 does NOT play a role in the ODN-induced NFκB activation via TLR4 intermediary



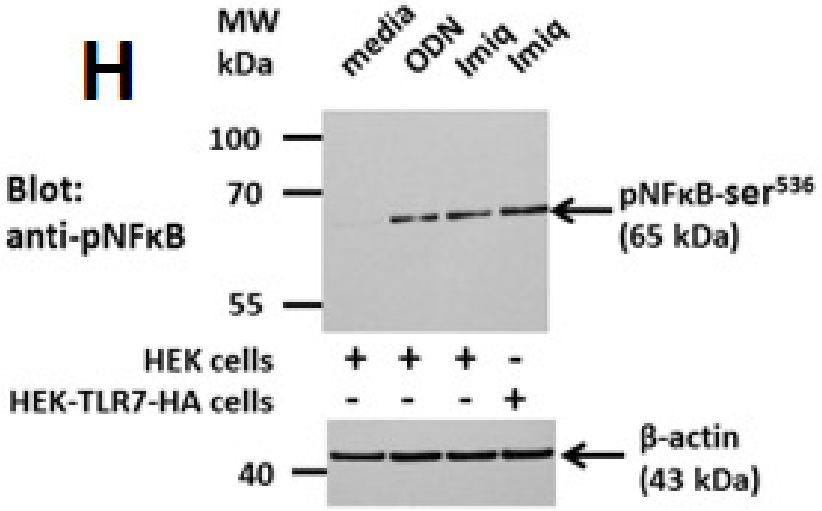
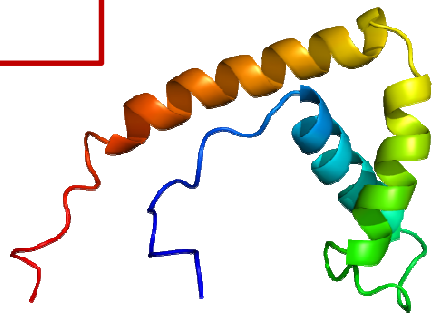


Figure 1

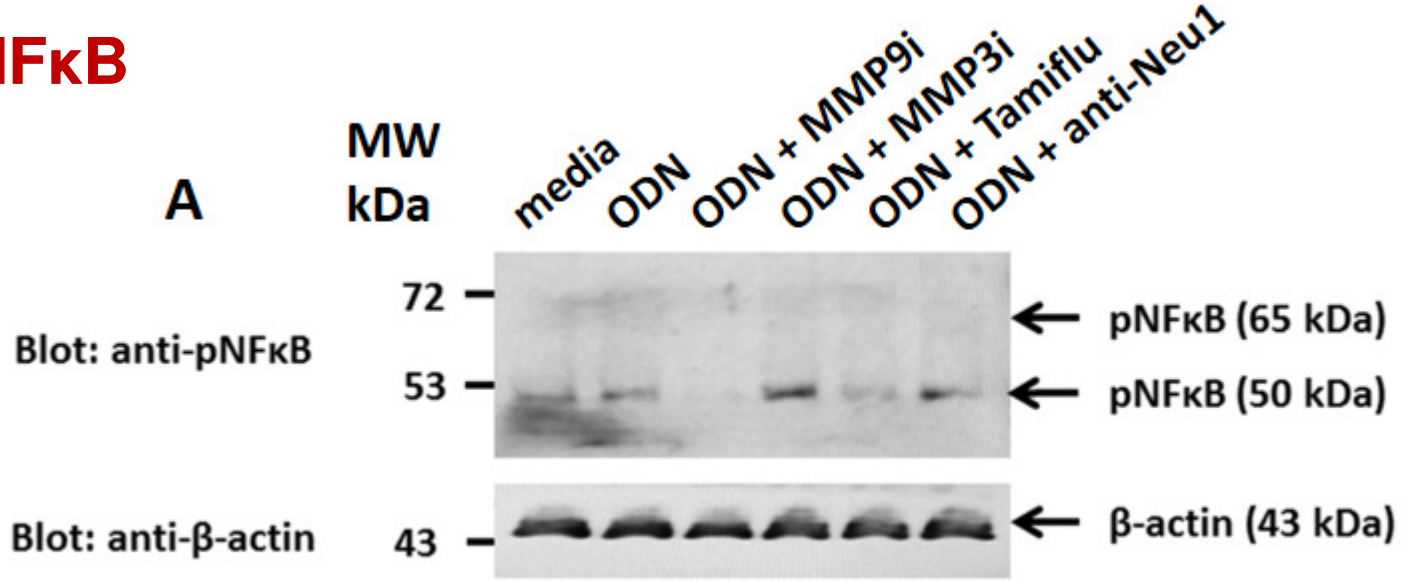
High-mobility group protein B1, also known as **high-mobility group protein 1** (HMG-1) or **amphoterin**

HMGB1

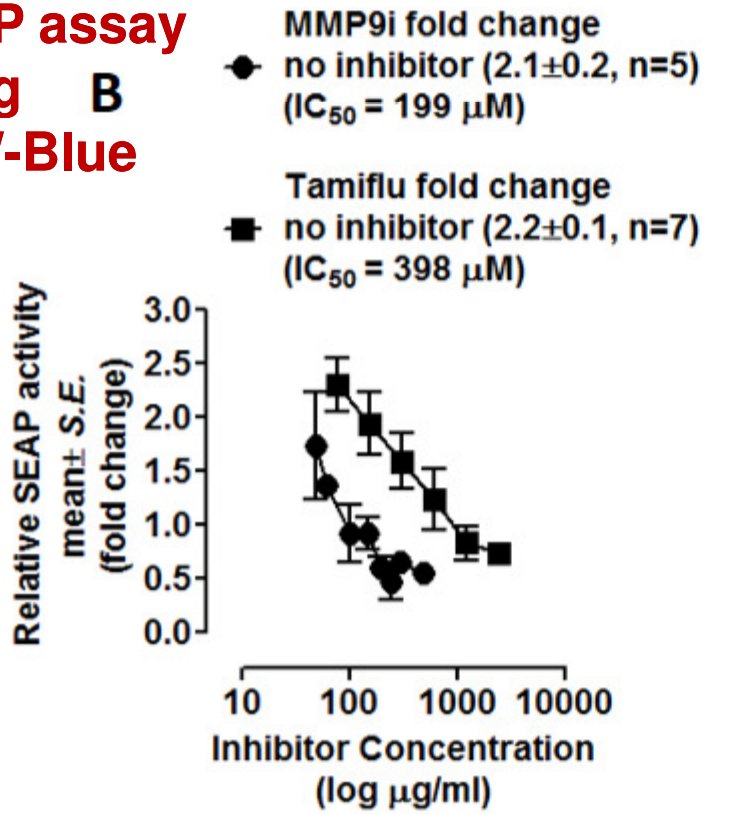


ODN can induce **HMGB1** secretion which binds to **TLR4,** and mediates NFkB activation and macrophage cytokine release during cell damage and inflammation.

pNFκB



SEAP assay using RAW-Blue cells



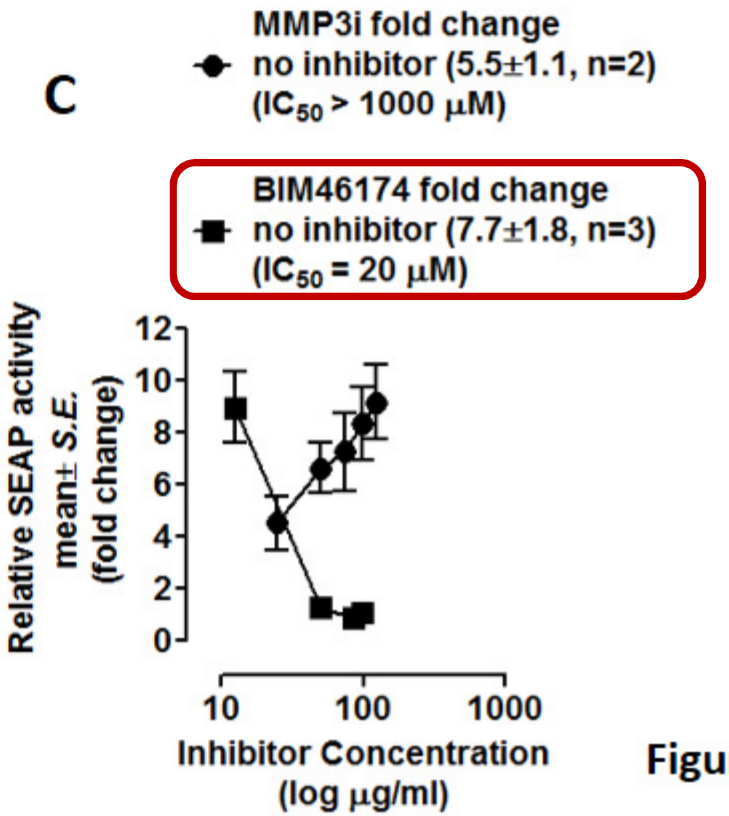
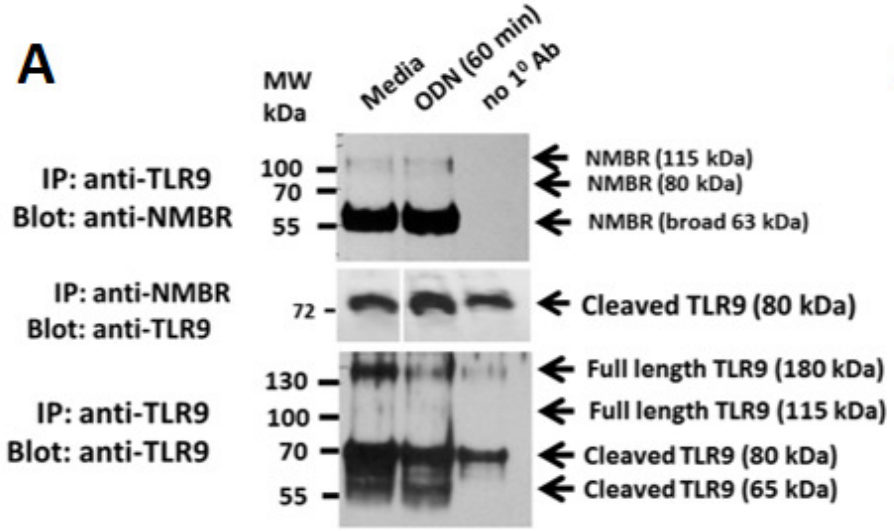
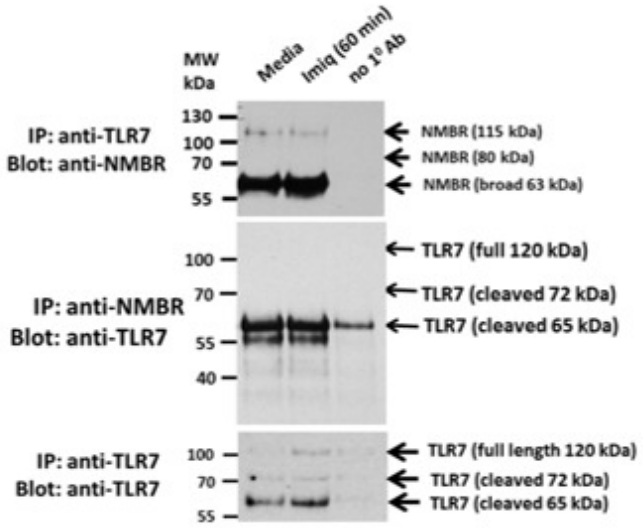
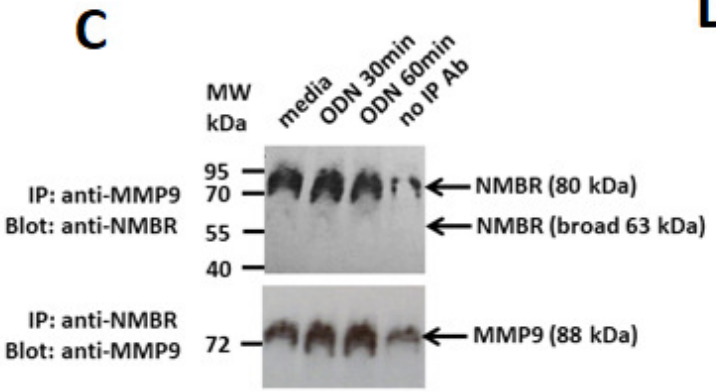


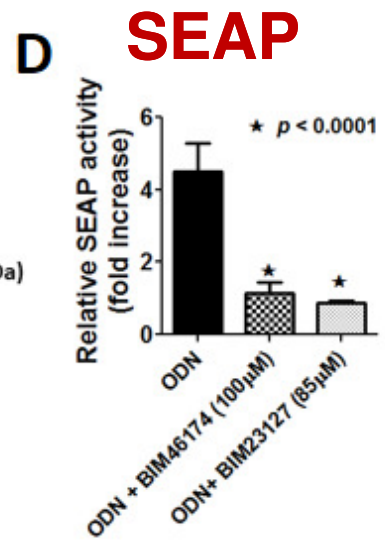
Figure 5

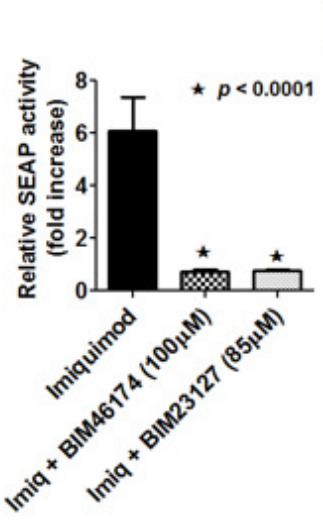
NMBR GPCR receptor co-IP's with cleaved TLR-7, -9 and MMP9

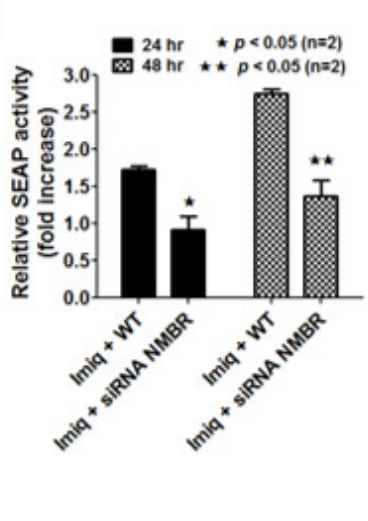






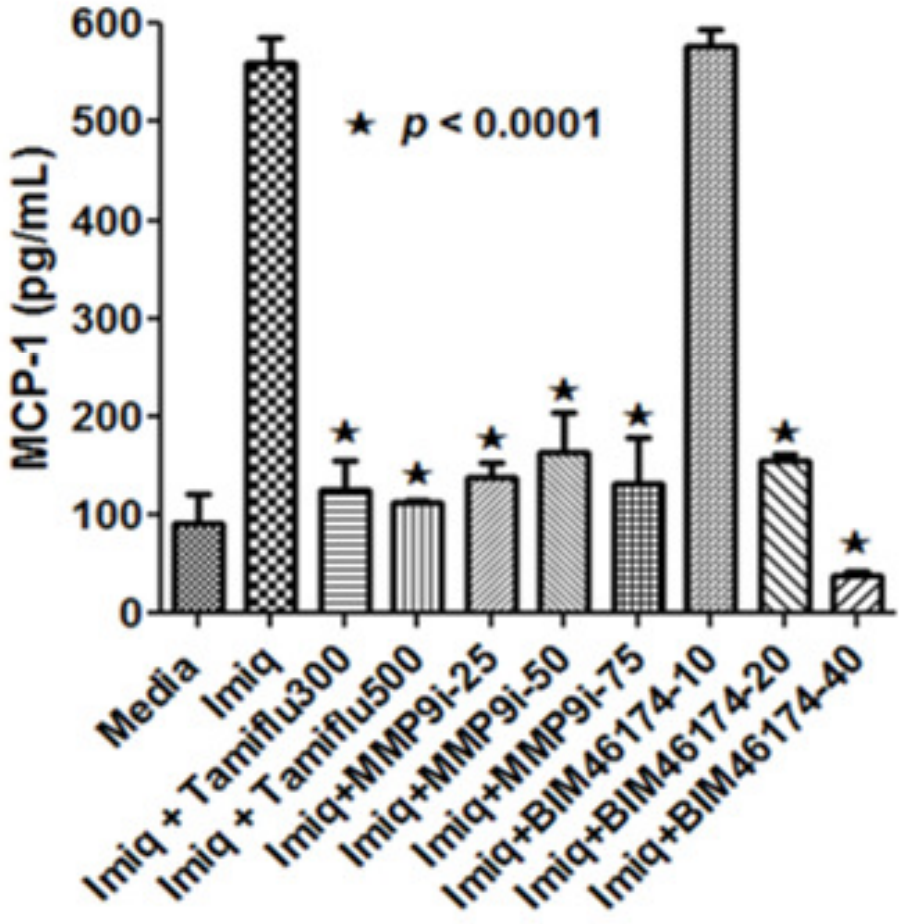




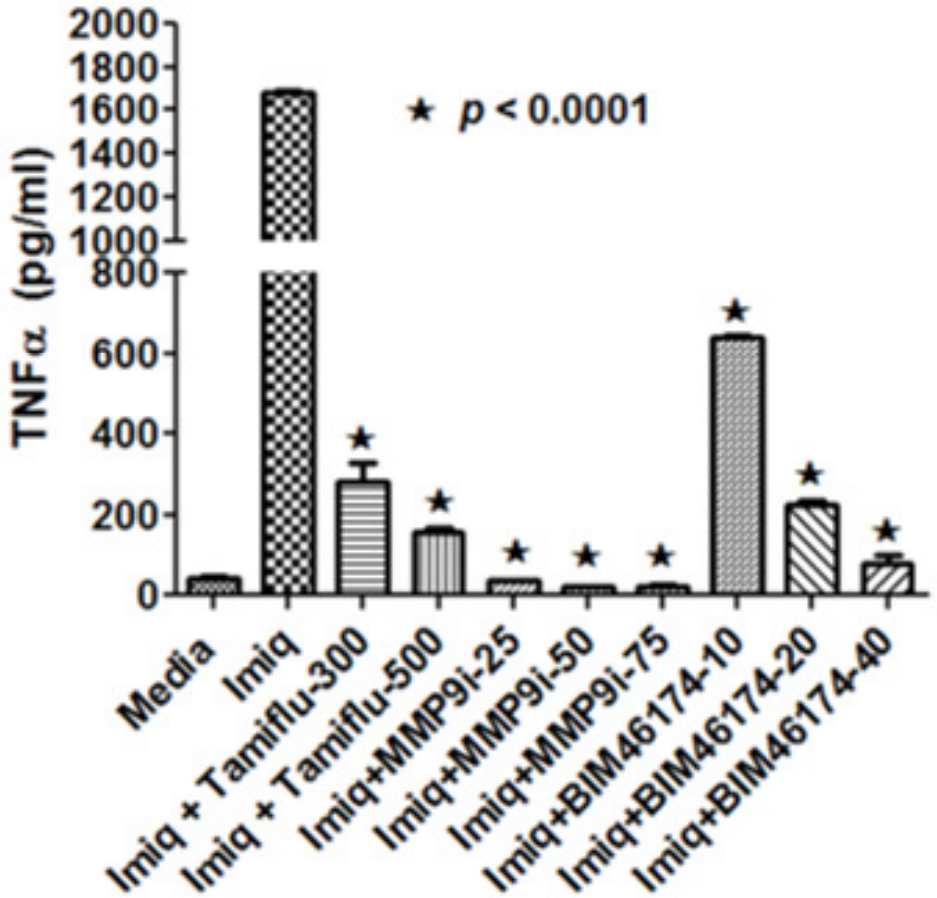


RAW-blue cells stimulated for 24h with 20µg/mL imiquimod (TLR7)

MCP-1 (monocyte chemotactic protein-1)

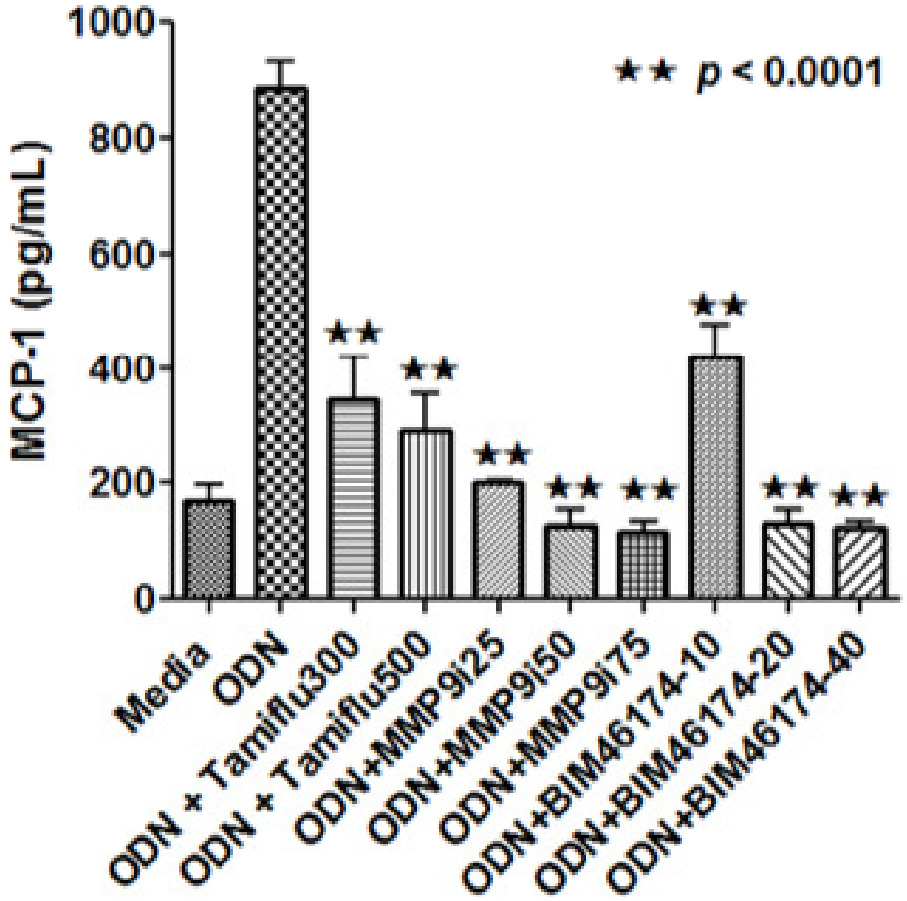


TNF-α

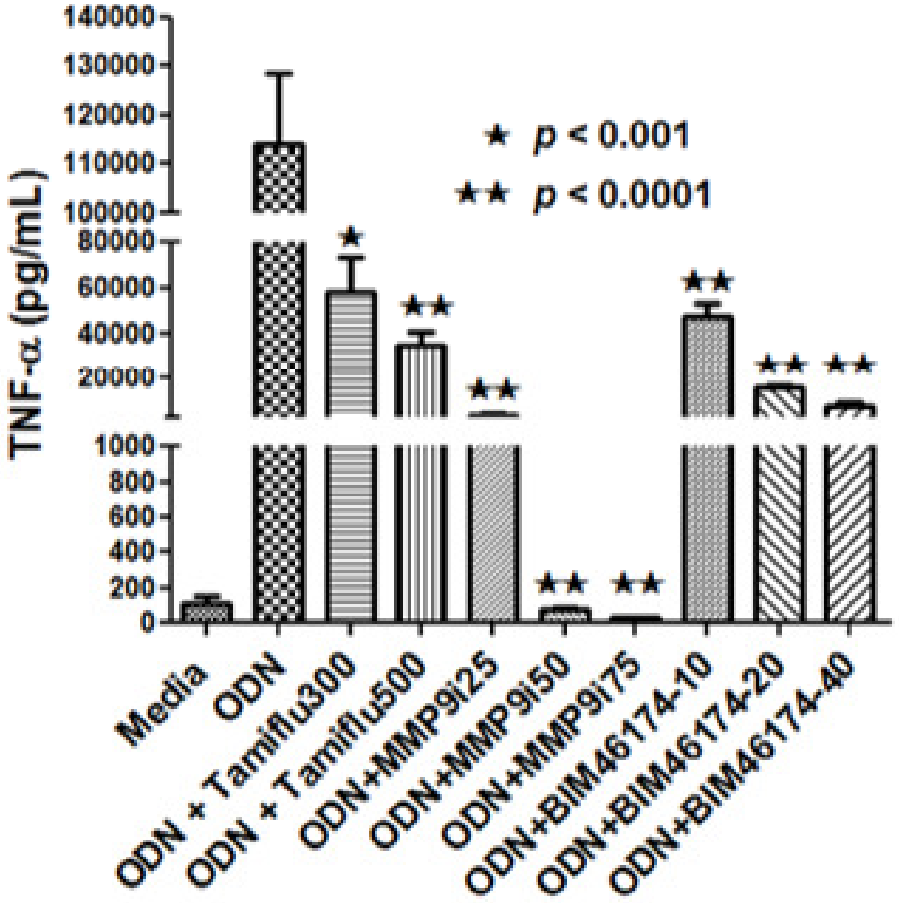


RAW-blue cells stimulated for 24h with 20µg/mL ODN (TLR9)

MCP-1

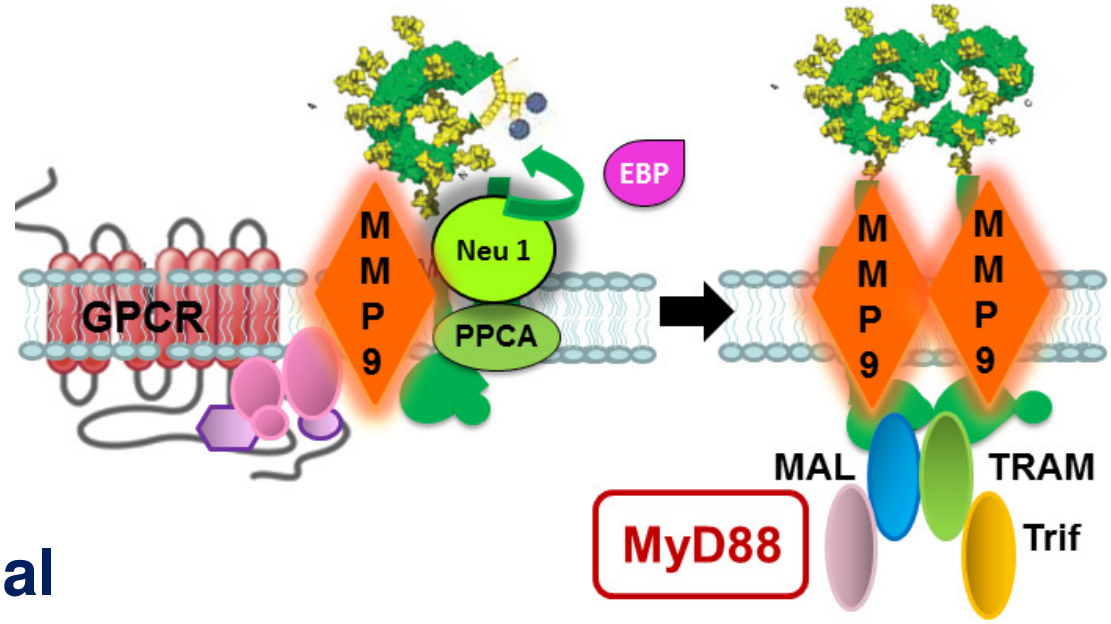


TNF-α



Conclusions

- A novel molecular GPCR signaling platform of a Neu1-MMP9 cross-talk in alliance with NMBR tethered to endosomal TLR-7 & -9.



- Essential for nucleic acid activation of TLR receptors, cellular signaling and pro-inflammatory responses.