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Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

Studio in vitro sugli effetti antinfiammatori di nuovi peptidi sintetici leganti i recettori FPR

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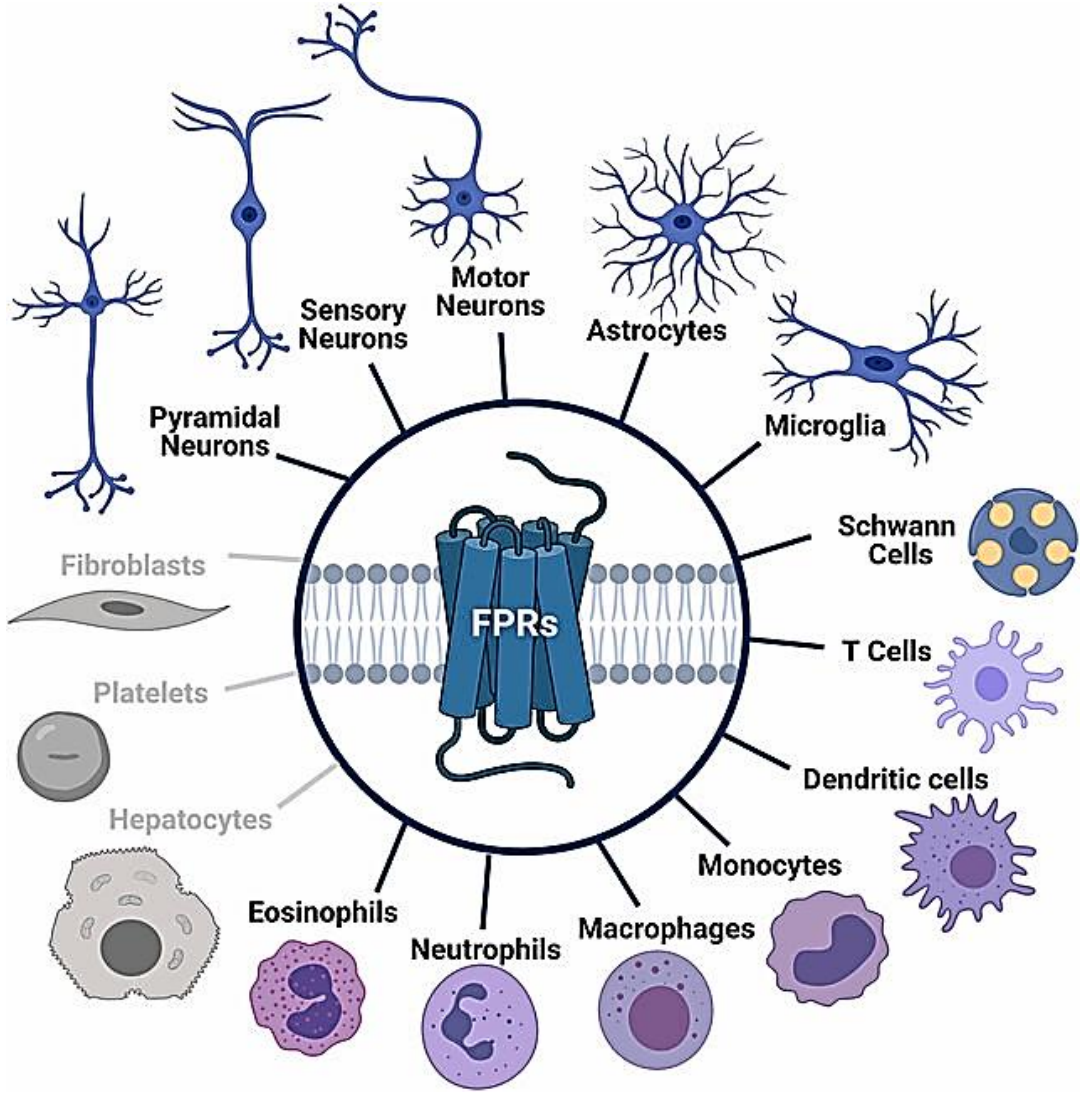
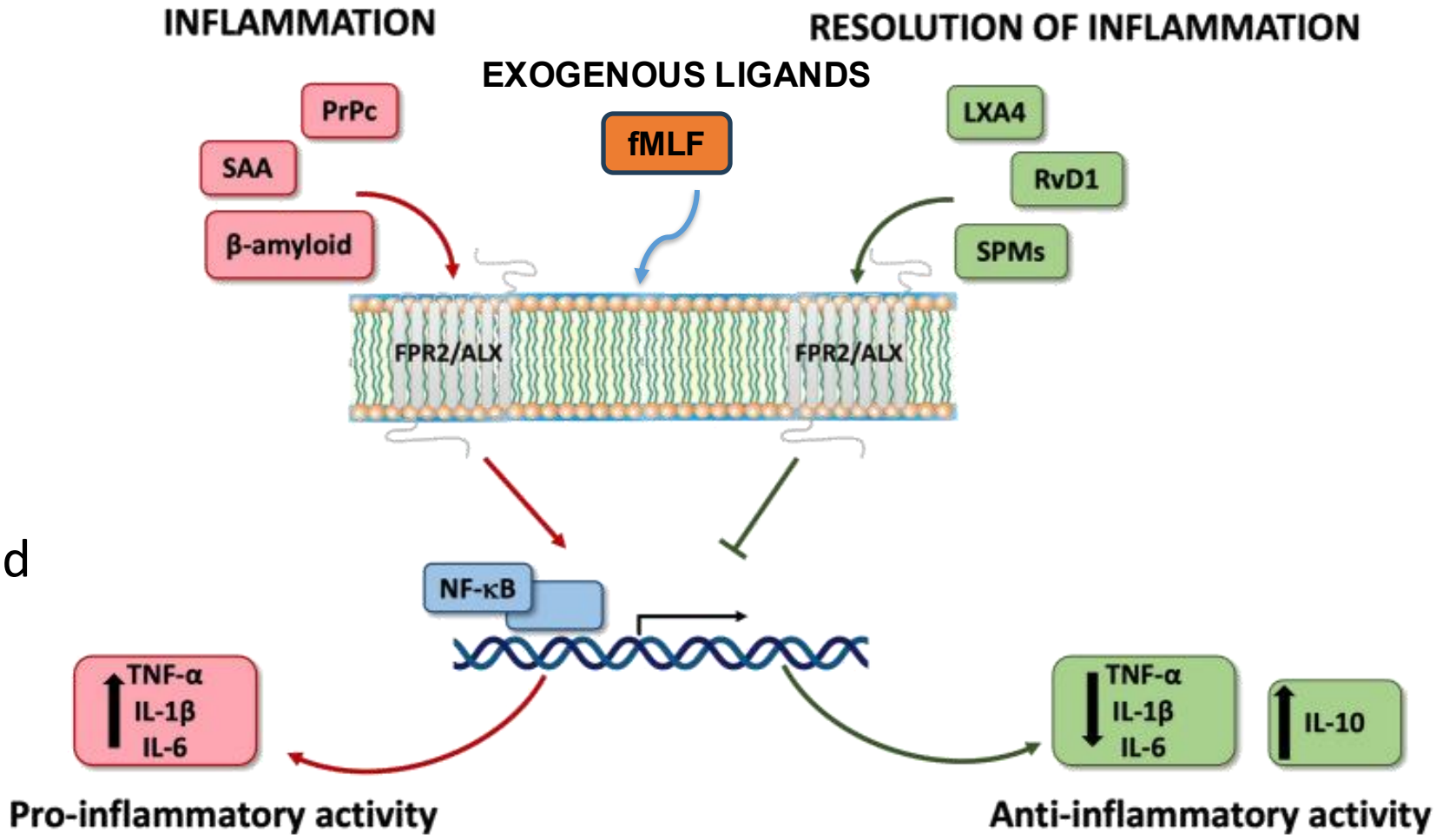
Formyl Peptide Receptors (FPRs)

- Transmembrane chemoattractant G protein-coupled receptors
- Three isoforms: FPR1, FPR2, FPR3



FPRs

- Recognize a broad variety of endogenous or exogenous ligands
- Recognize PAMPs and DAMPs
- Mediators of inflammatory and immune responses



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Formalin Test for synthetic ligand peptides specific to FPR receptors

Name	Formula/structure	Early phase	Late phase
Vehicle	DMSO:SAL 1:3 v/v	137,3	190,3^{ns}
AMGS1	WKYMWm-NH_2	82,2*	155,3^{ns}
AMGS2	WKYMWM-NH_2	89,8^{ns}	161,0^{ns}
AMGS3	WRWWWW-NH_2	103,2^{ns}	146,3^{ns}
AMGS4	YKYMWm-NH_2	58,7****	98,5^{ns}
AMGS5	For-MLMYKW-NH_2	67,8***	124,5^{ns}
AMGS6	For-MVMYKW-NH_2	68,0***	119,8^{ns}
AMGS7	For-MLF-OH	76,5**	165,0^{ns}
AMGS8	Boc-MLF-OH	125,2^{ns}	135,5^{ns}
AMGS9	WKYMLm-NH_2	41,5****	94,2^{ns}
AMGS10	WKYMVm-NH_2	100,2^{ns}	150,7^{ns}
AMGS11	WKYFLm-NH_2	89,5^{ns}	159,2^{ns}
AMGS12	WKYFVm-NH_2	74,2**	90,0^{ns}
AMGS13	For-MLFYKW-NH_2	72,2**	110,2^{ns}
AMGS14	For-MVFYKW-NH_2	65,8***	96,3^{ns}

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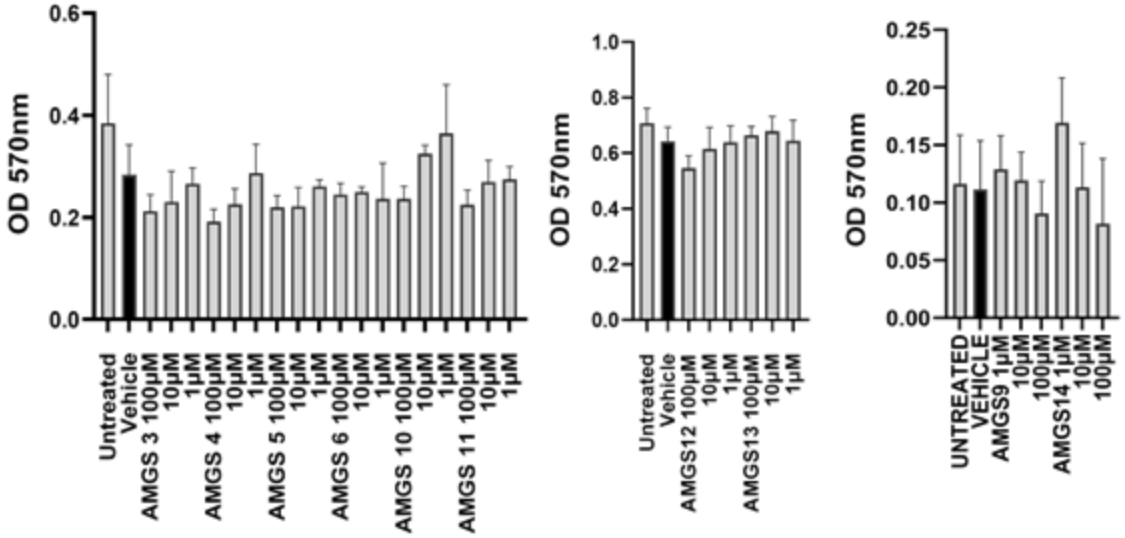
MTT cytotoxicity Assay in CaCo-2 and WiDR cells

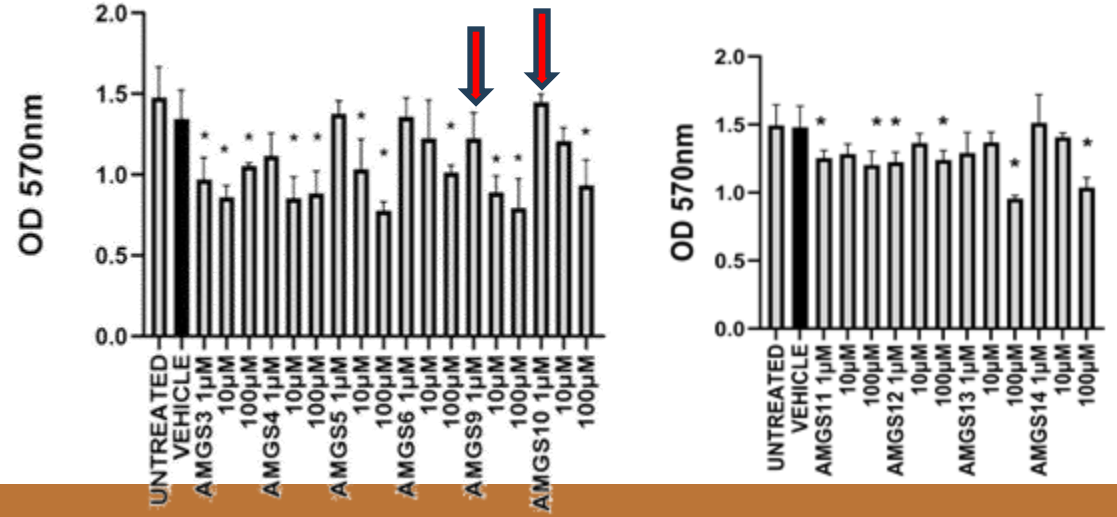
PEPTIDES
AMGS 3
AMGS 4
AMGS 5
AMGS 6
AMGS 9
AMGS 10
AMGS 11
AMGS 12
AMGS 13
AMGS 14

- **Vehicle:** 0,4% DMSO
- **C:** 1μM, 10μM, 10μM
- **Treatment:** 24h

CaCo-2



WiDR



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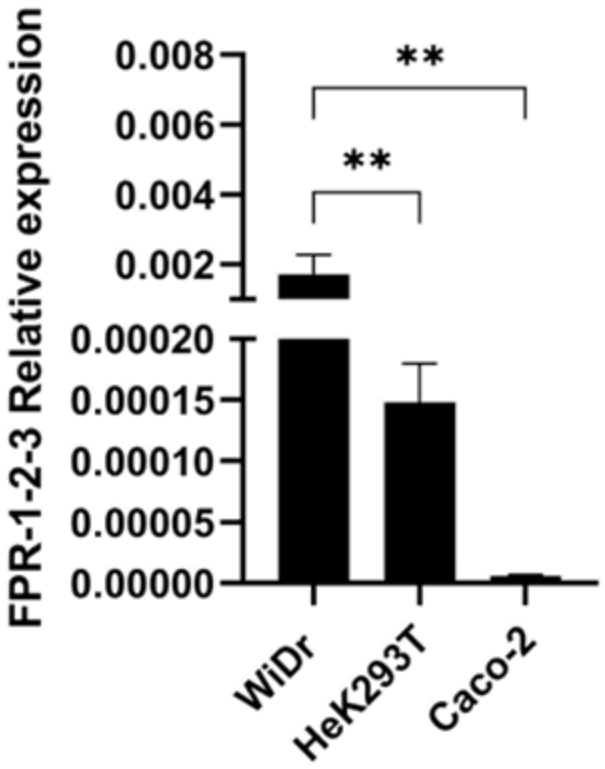
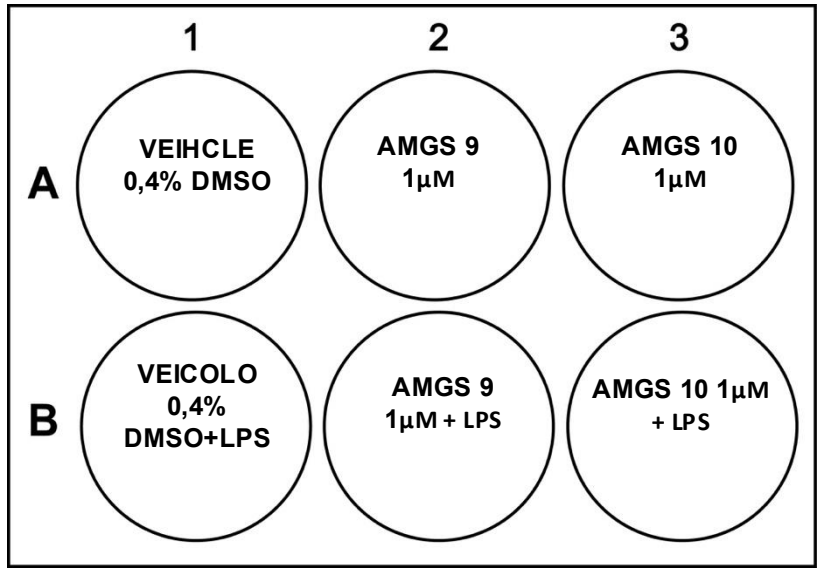
FPRs relative expression



FC = 1000, WiDR *vs* Caco2

Treatment with synthetic peptides



3 X

Peptide treatment

LPS (5 µg/ml) stimulation

End of treatment

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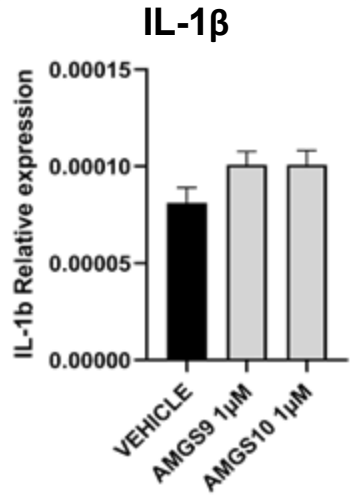
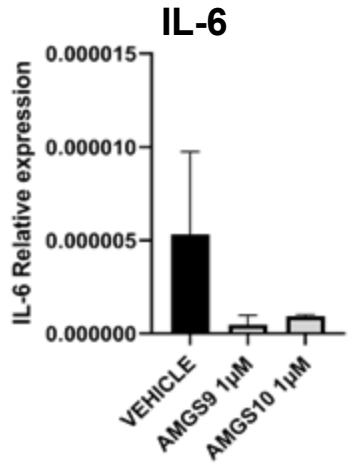
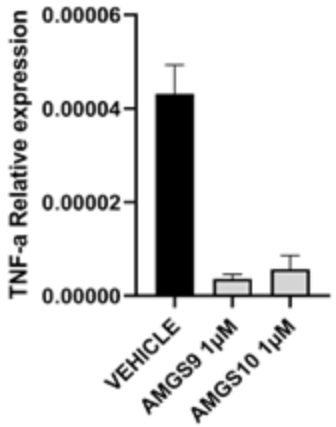
Analysis of transcriptional effects on pro-inflammatory cytokines

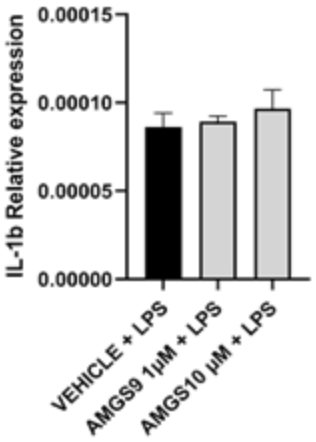
NO LPS

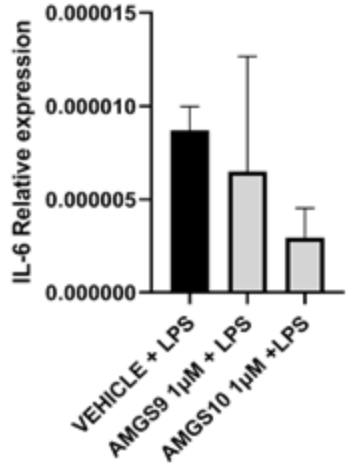
LPS

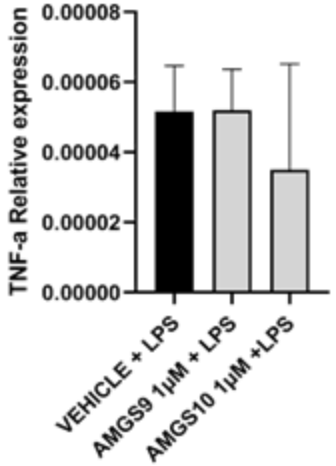








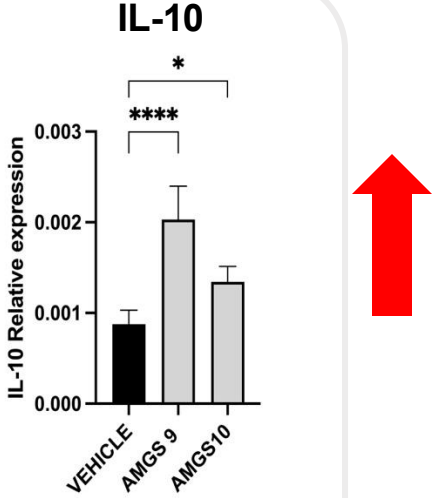


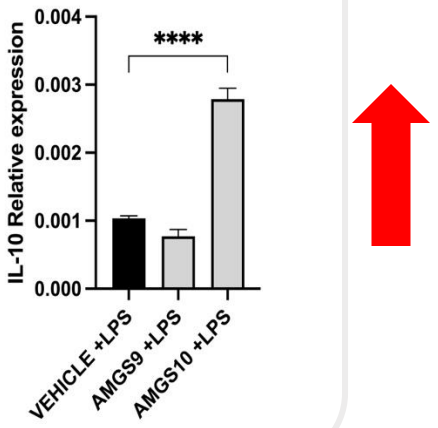


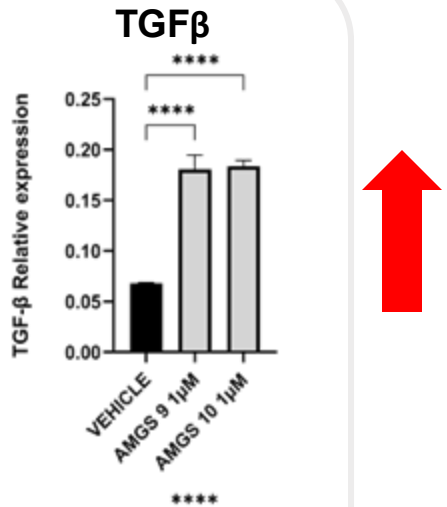
Analysis of transcriptional effects

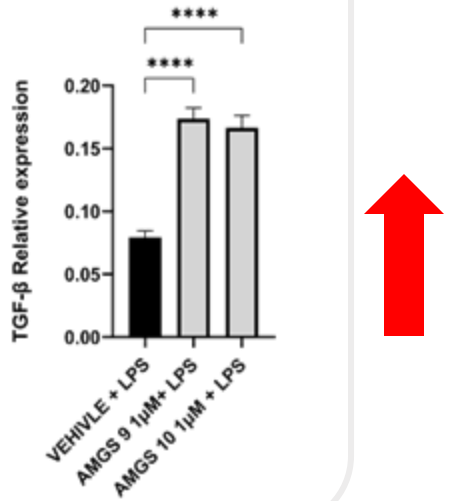
NO LPS

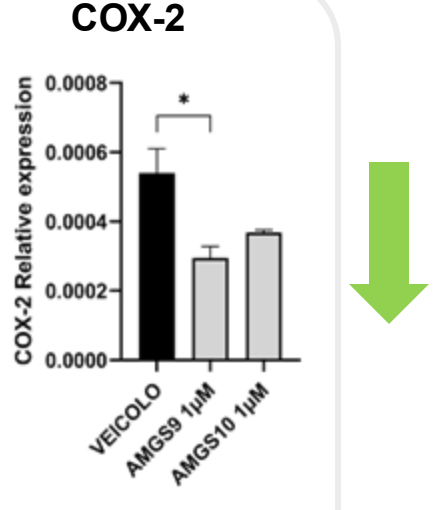
LPS

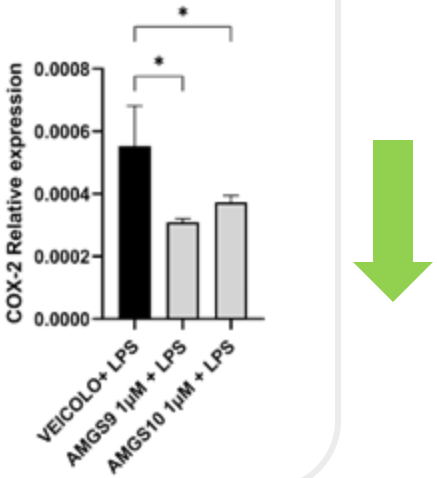












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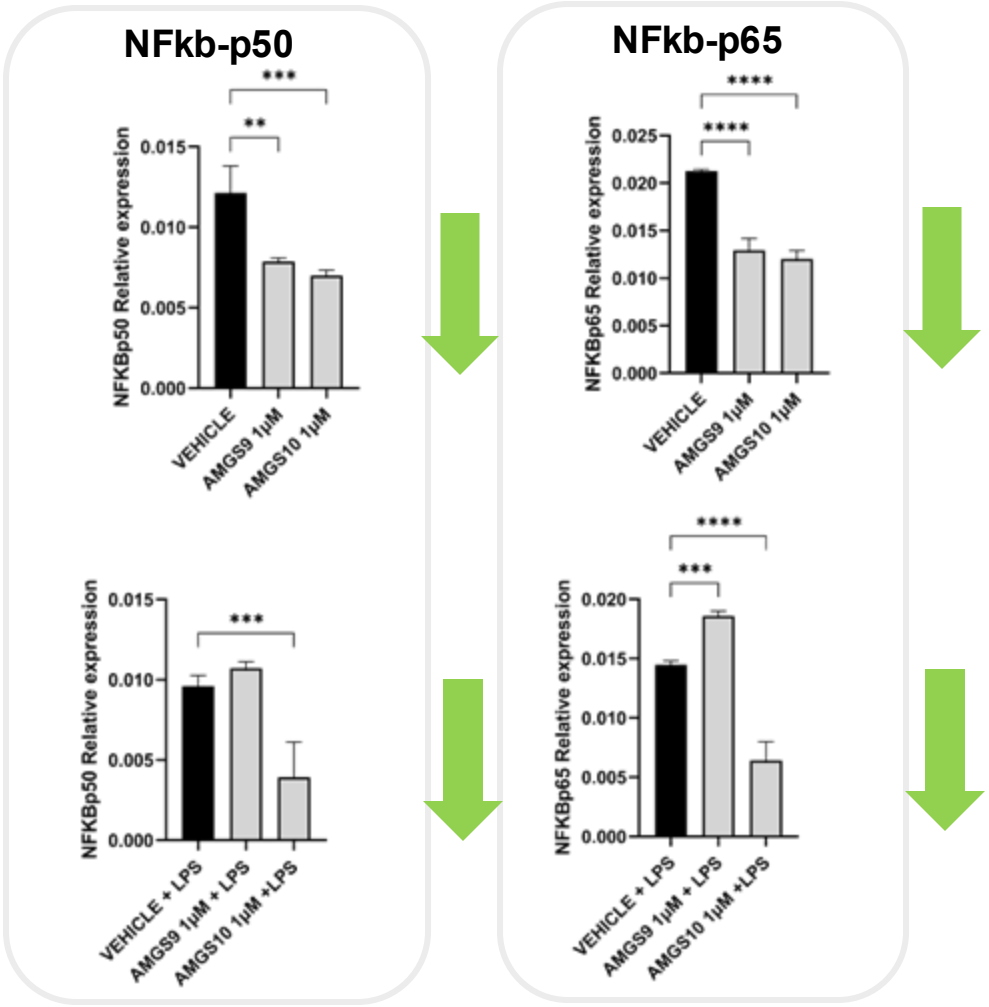
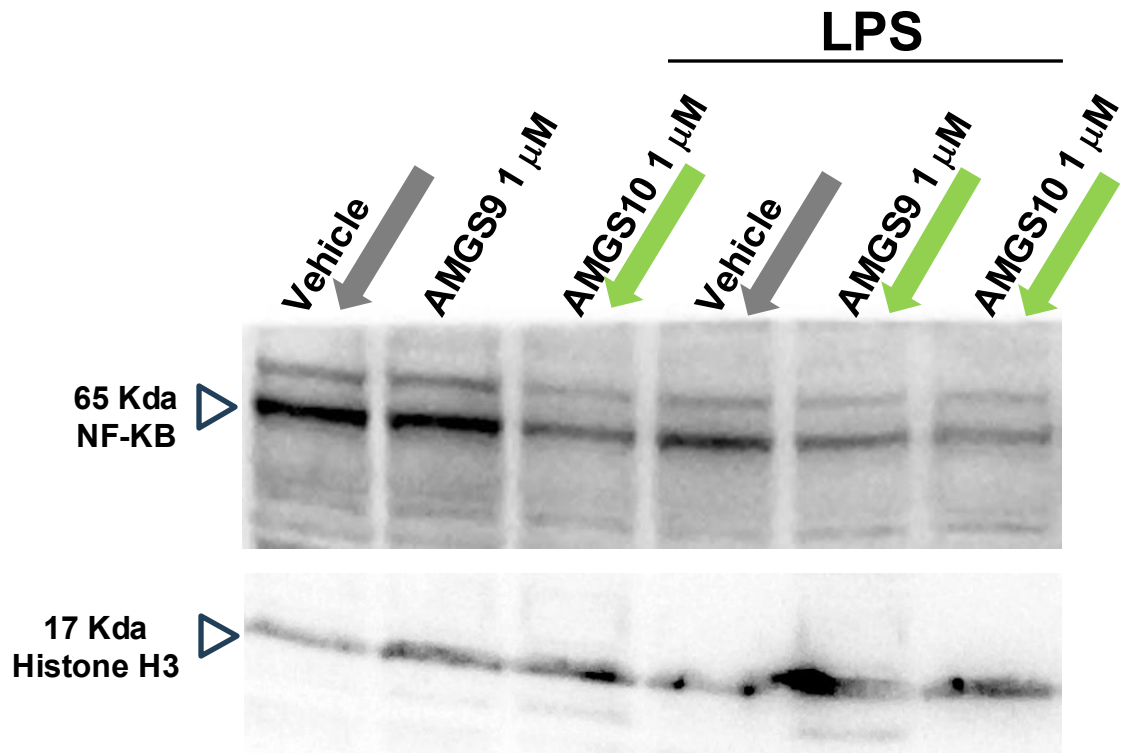
Analysis of transcriptional effects on NF-KB

NO LPS

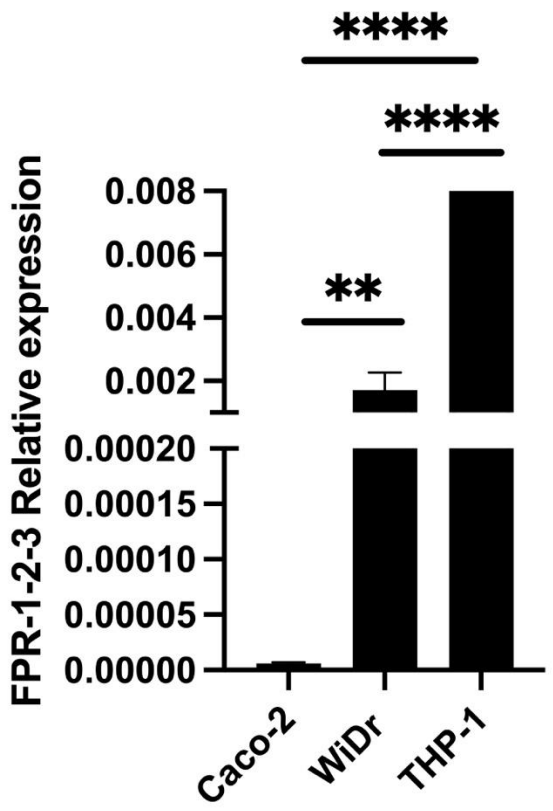
LPS



NF-KB p65 immunoblot from nulear lysate of WiDR cells

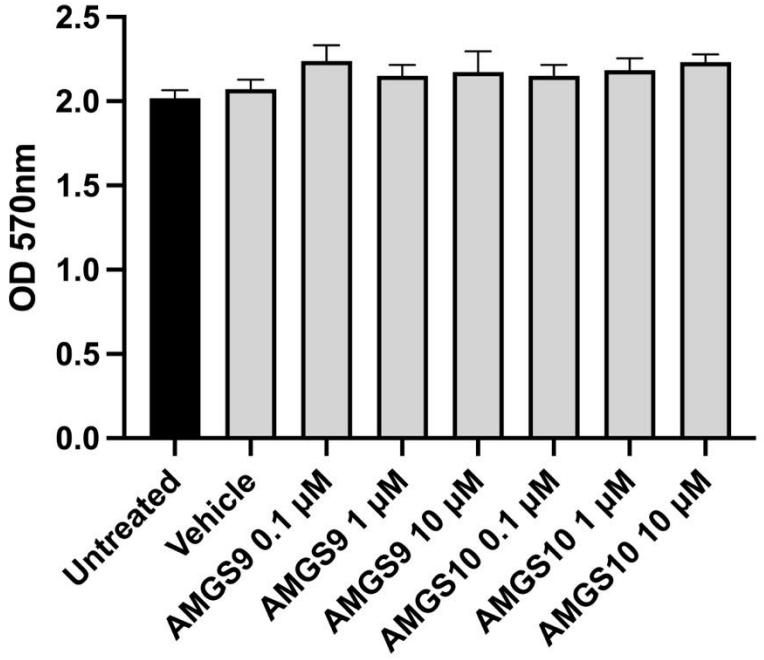


FPRs relative expression

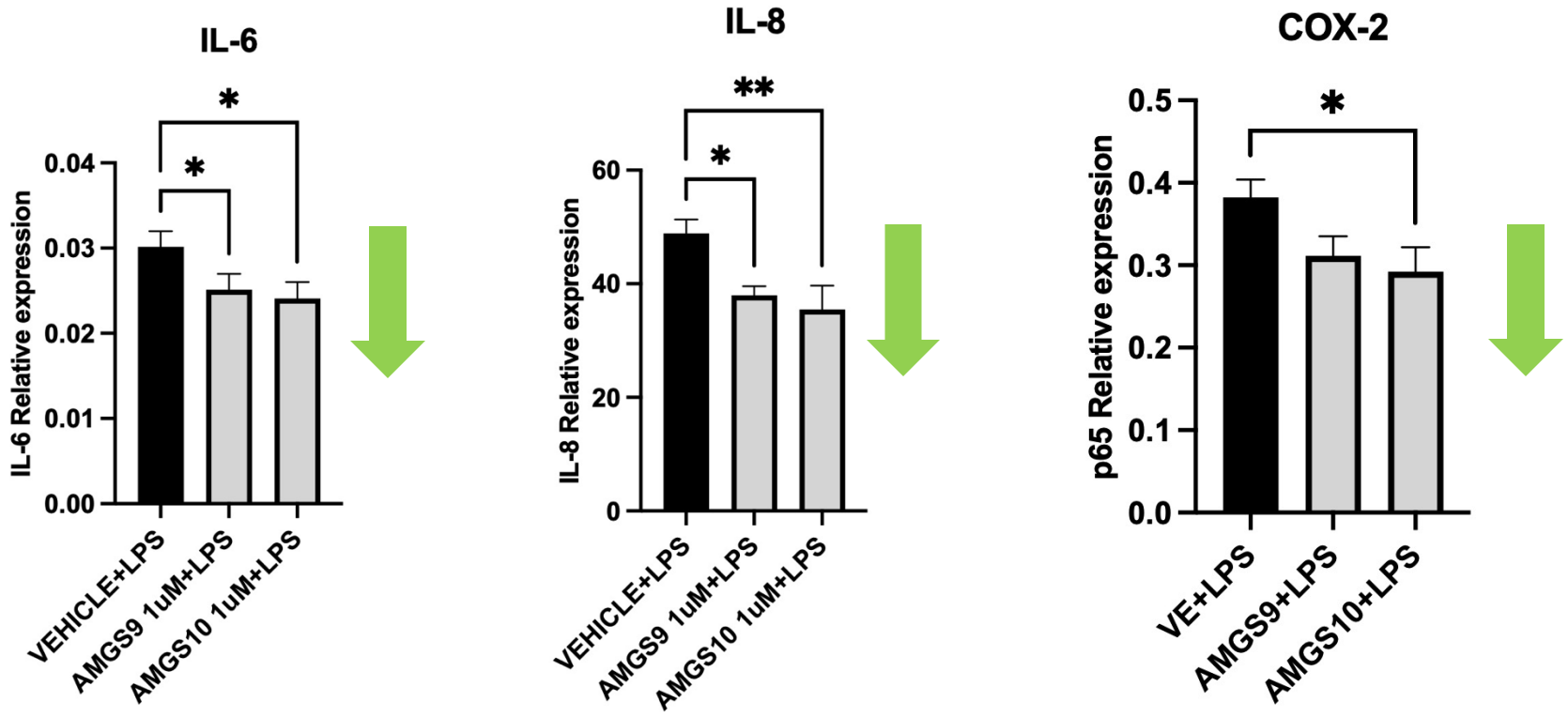


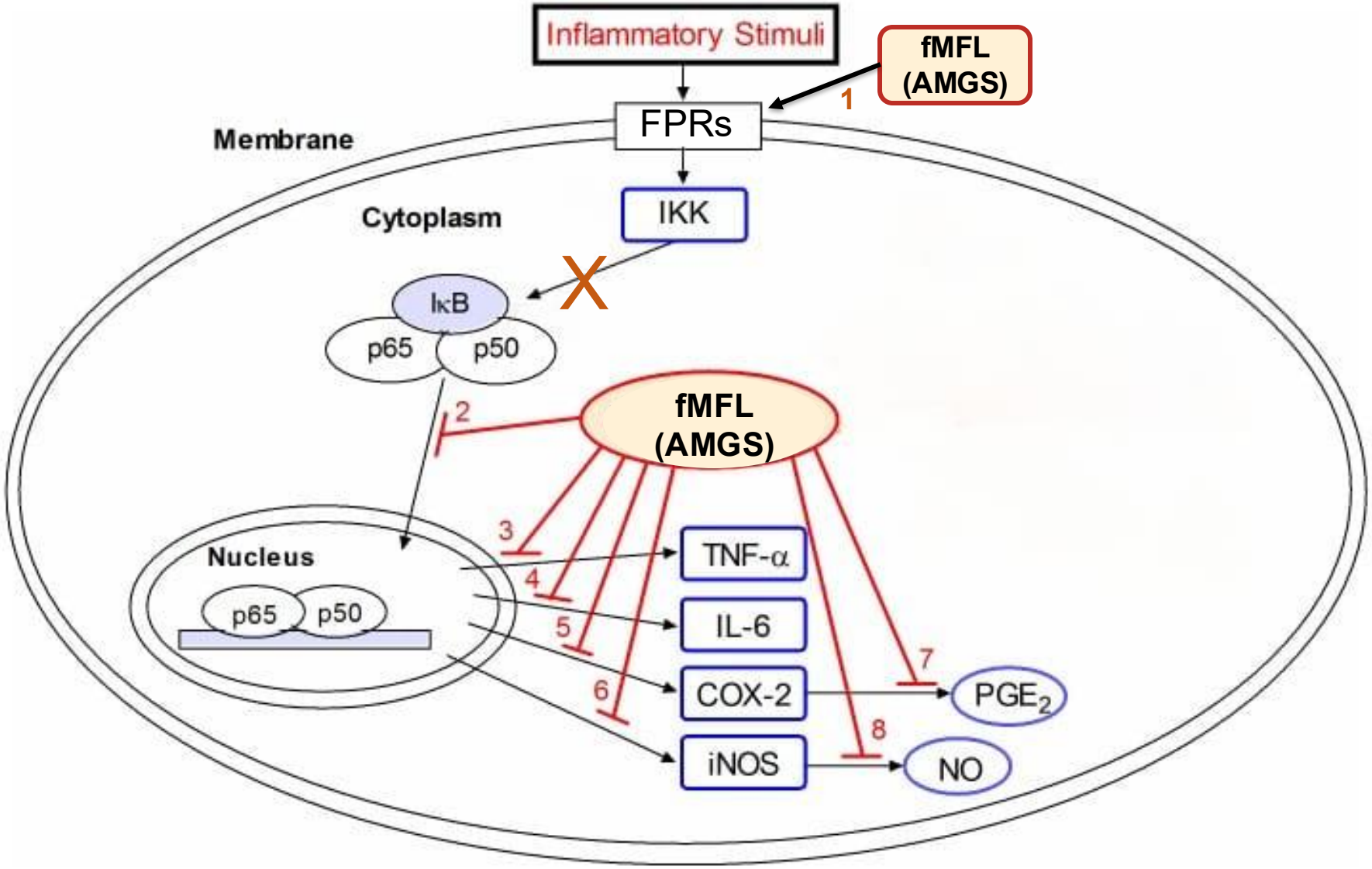
MTT cytotoxicity assay in THP-1 cells

AMGS9 and AMGS10 CONCENTRATION
1µM
10µM
100µM



Analysis of transcriptional effects on THP-1 macrophages after LPS stimulation




Inflammatory Stimuli
fMFL (AMGS)
1
FPRs
Membrane
Cytoplasm
IKK
X
IκB
p65
p50
2
fMFL (AMGS)
3
4
5
6
7
8
Nucleus
p65
p50
TNF-α
IL-6
COX-2
iNOS
PGE2
NO

Conclusions

- Synthetic formylated peptides could be considered a promising new therapeutic approach for treating intestinal diseases.

- Developing a nanoparticle-based delivery system could enhance their anti-inflammatory effects.

This step is ongoing in collaboration with laboratories of University of Urbino affiliated with the Spoke 8.

UdA teams involved in the project

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