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Ministero dell'Università e della Ricerca

Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

In vitro evaluation of synthetic peptides targeting FPR receptors for their potential anti-inflammatory effects on colon cells

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VITALITY

Università degli Studi di Urbino
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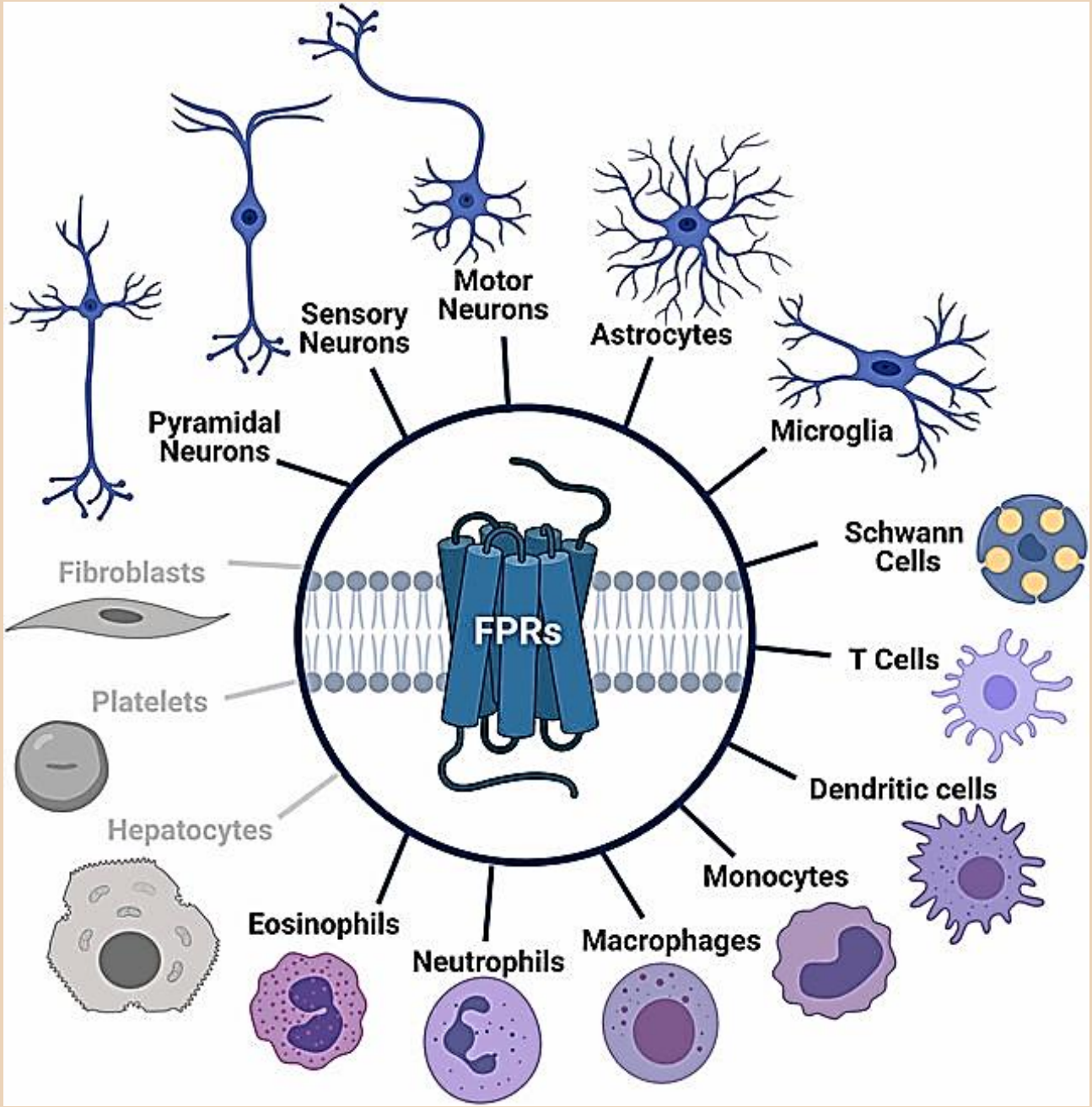
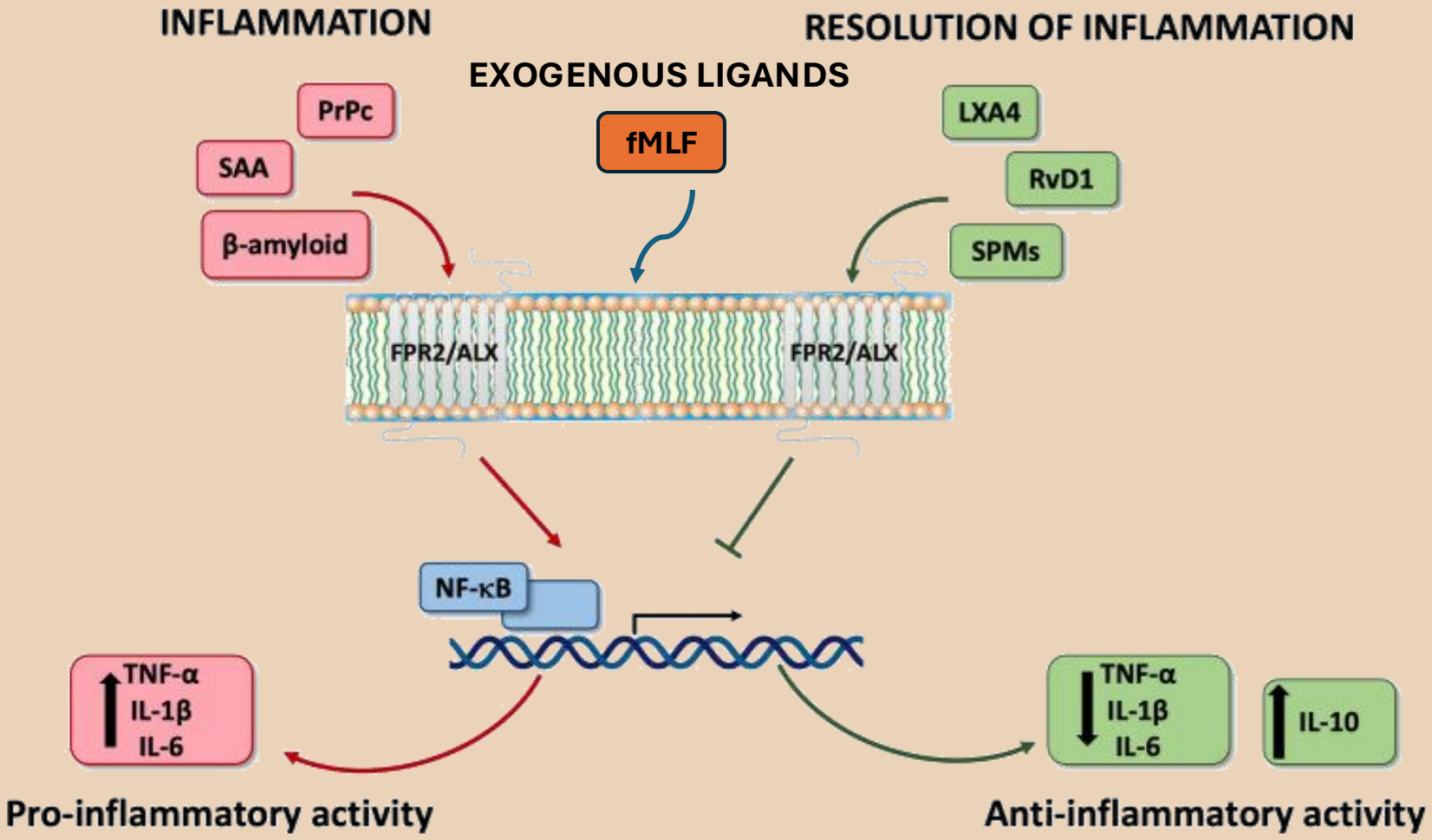
FPRs: Formyl Peptide Receptors

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



FPRs: Formyl Peptide Receptors

- Recognize a broad variety of endogenous or exogenous ligands: lipids, proteins and peptides, including N-formylayed and non- formylated peptides.
- Recognize PAMPs and DAMPs
- Mediators of inflammatory and immune responses



Synthetic ligand peptides specific to FPR receptors

Name	Formula/structure	Early phase	Late phase
Veicol	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	$65,8^{***}$	$96,3^{ns}$

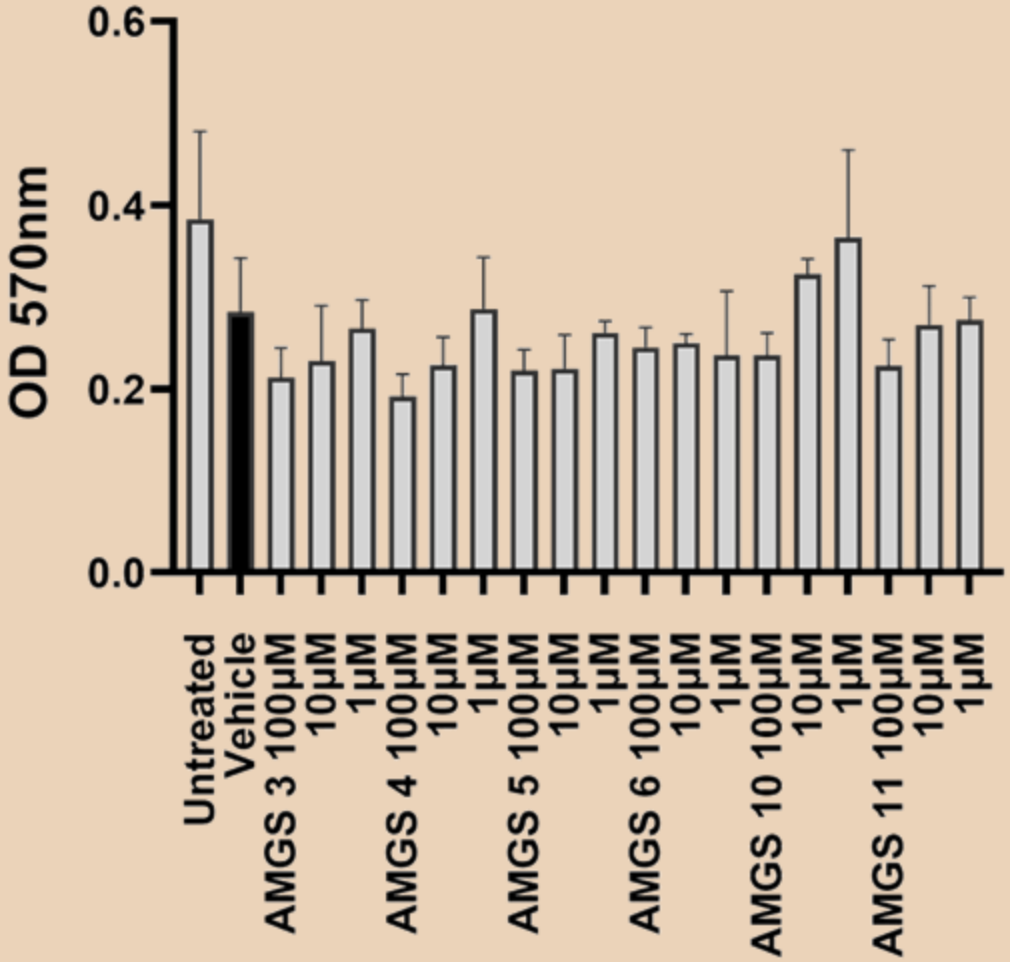
MTT citotoxicity Assay on CaCo-2 and WiDr cells

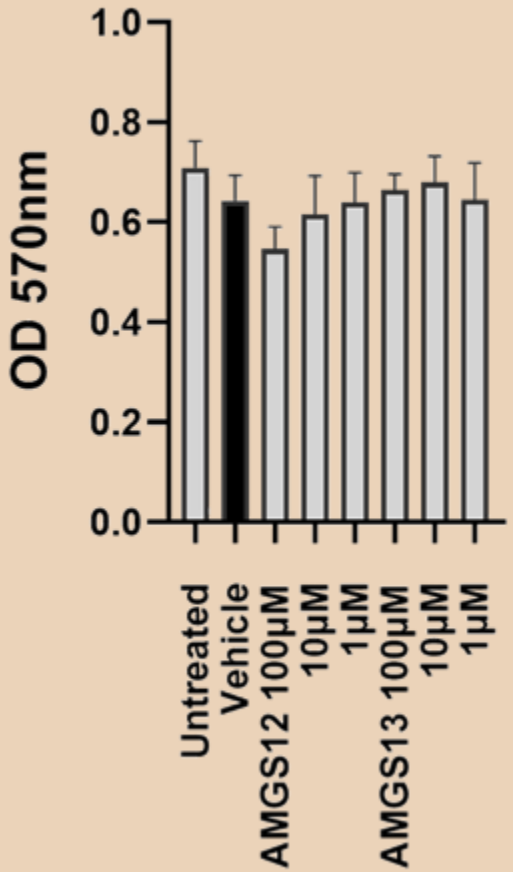
PEPTIDES
AMGS 3
AMGS 4
AMGS 5
AMGS 6
AMGS 9
AMGS 10
AMGS 11
AMGS 12
AMGS 13
AMGS 14

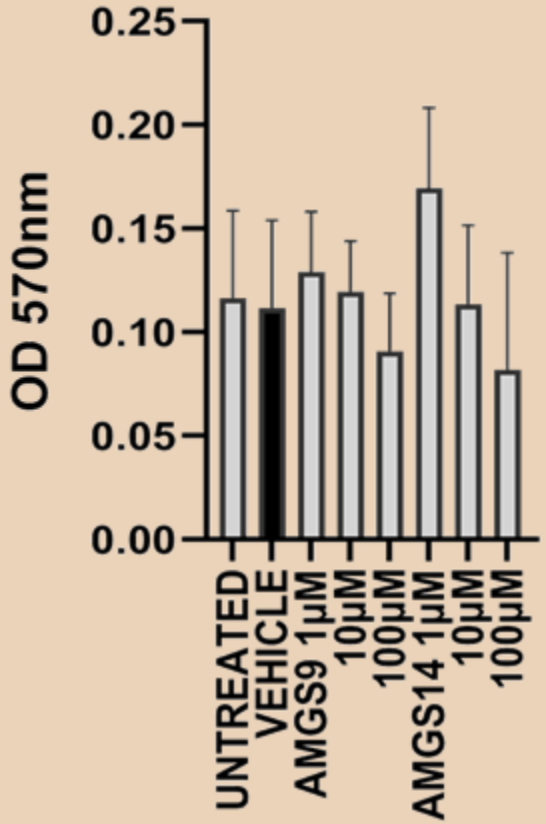
CONCENTRATION
1µM
10µM
100µM

- PEPTIDES solubilized in DMSO
- VEHICLE 0,4% DMSO
- 24h treatment

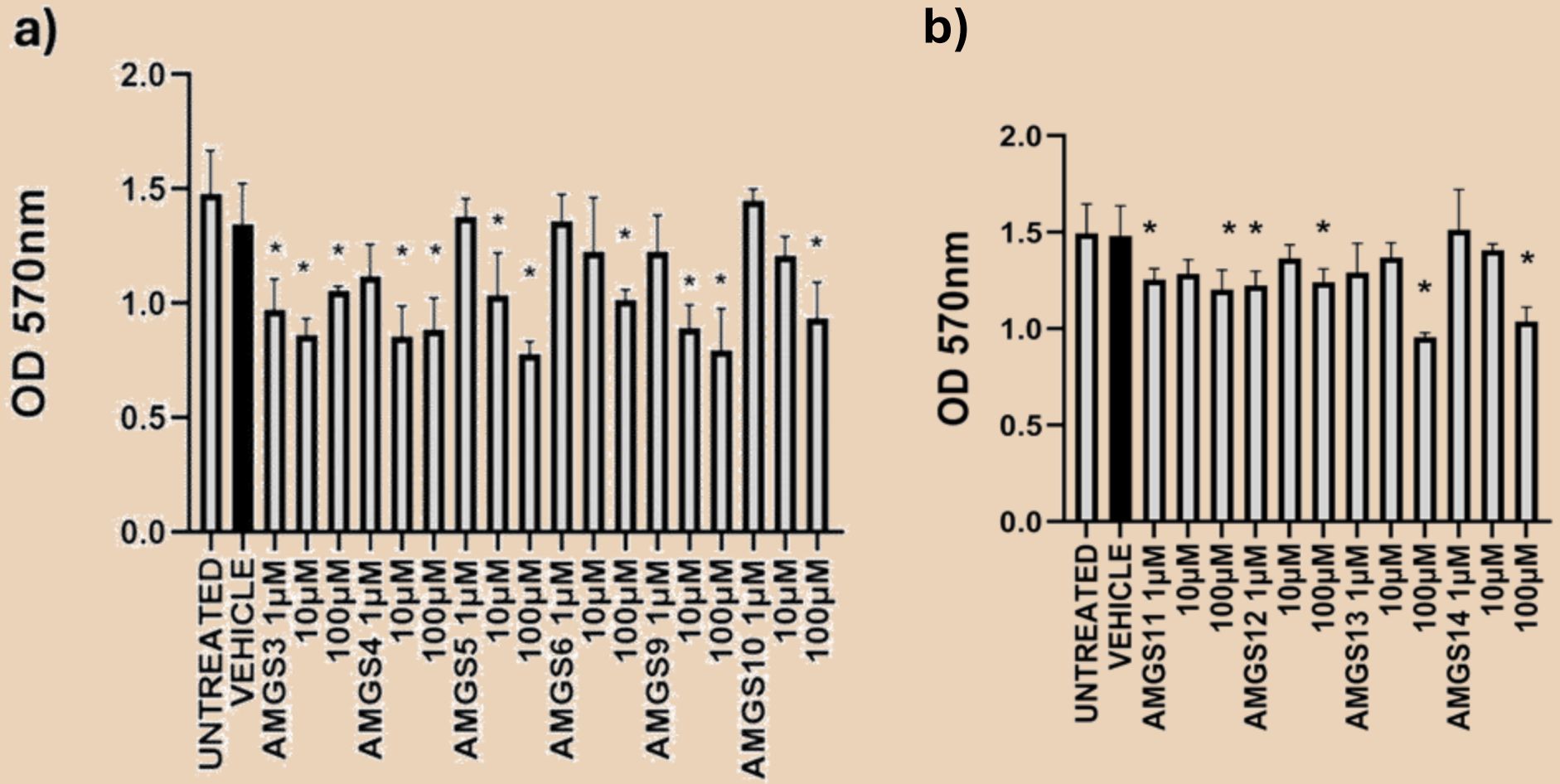
MTT citotoxicity Assay on Caco-2 cells didn’t show cytotoxic effect



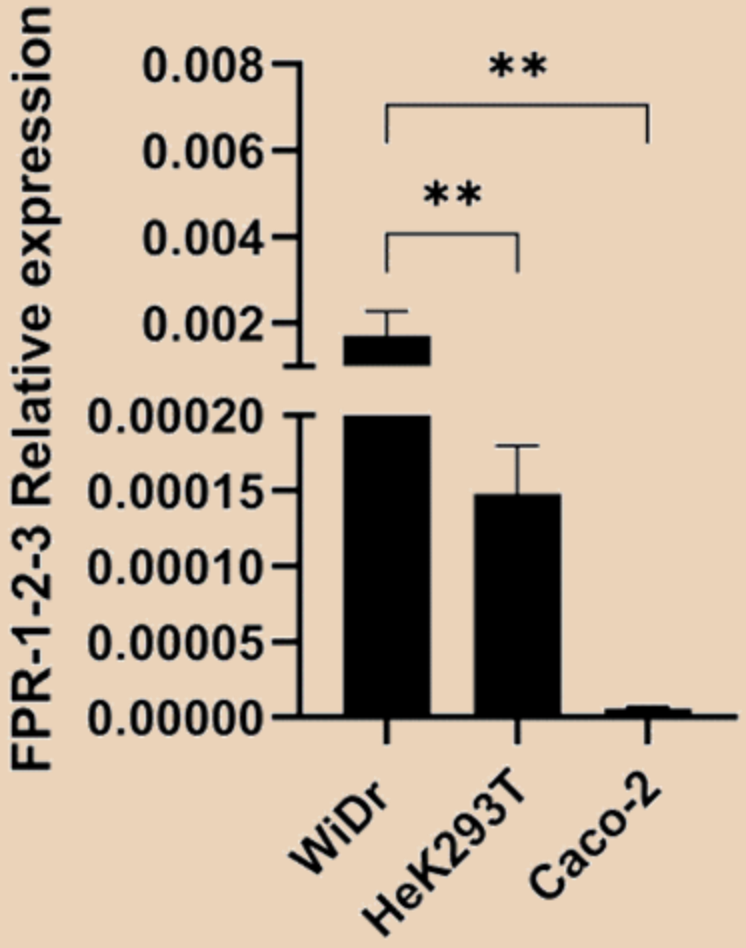




MTT cytotoxicity Assay on WiDr cells showed variable concentration-dependent toxic effect

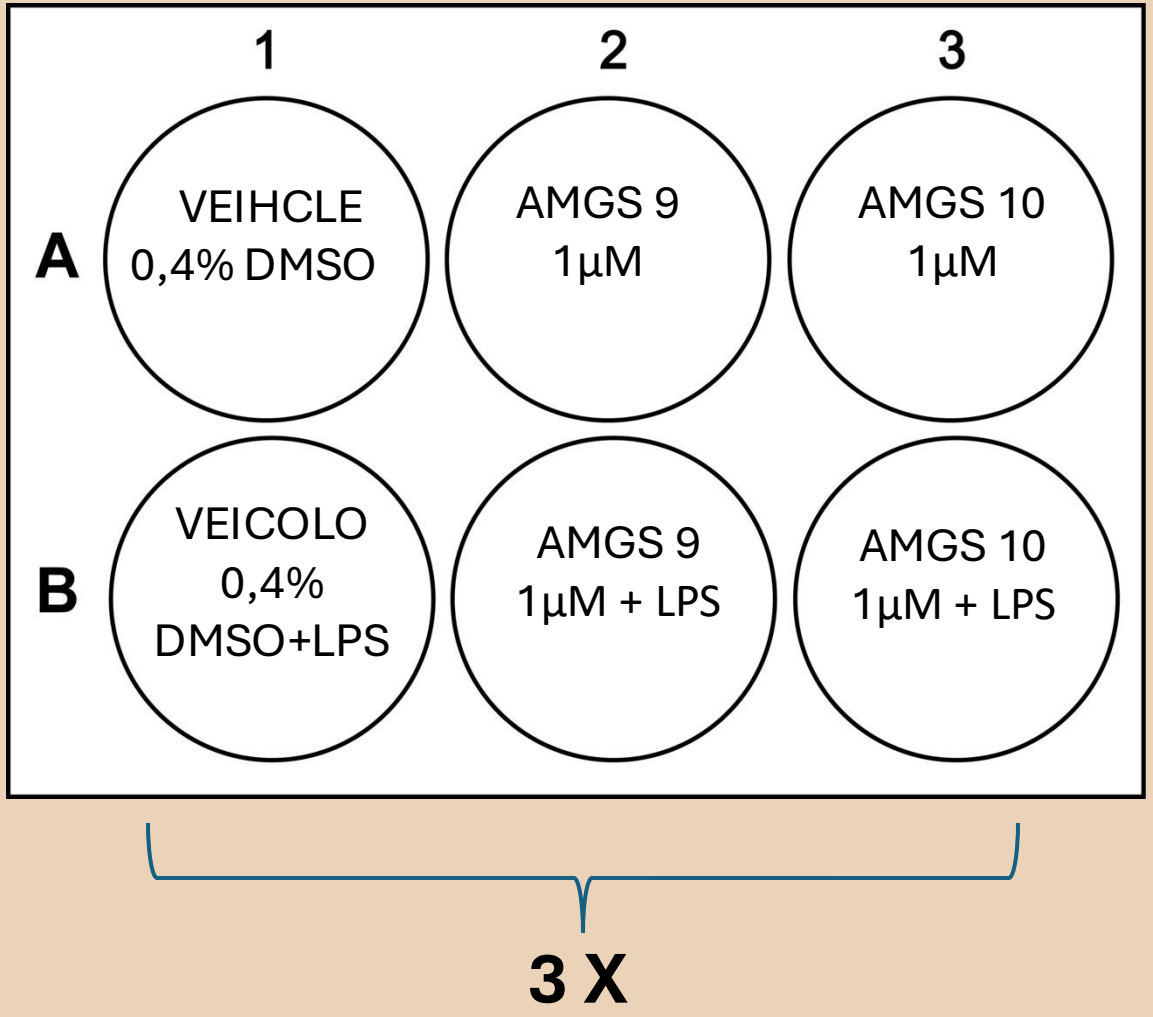


FPRs Relative expression



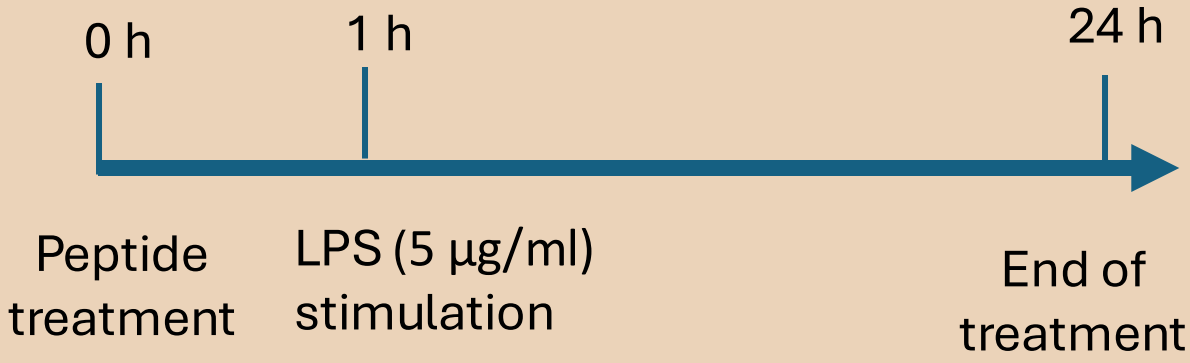
- WiDR: colon cancer cells derived from HT29
- Approximately 1000-fold higher expression of FPR genes in WiDr compared to Caco-2.
- HEK293T (Human Embryonic Kidney cell line) served as a control due to its documented low FPR expression in literature.

SYNTHETIC LIGAND PEPTIDES TREATMENT



- WiDr cells were plated in triplicate in 6-well plates and treated with AMGS9 and AMGS10 peptides at the concentration of 1µM for 24 hours, with or without stimulation with LPS from E. coli

- Vehicle: 0,4% DMSO

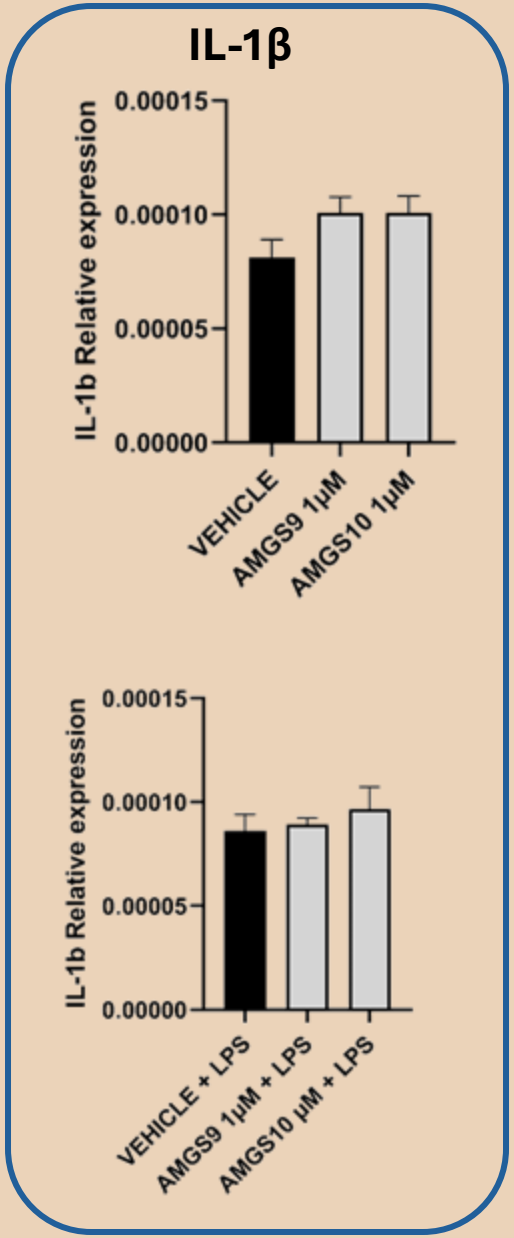


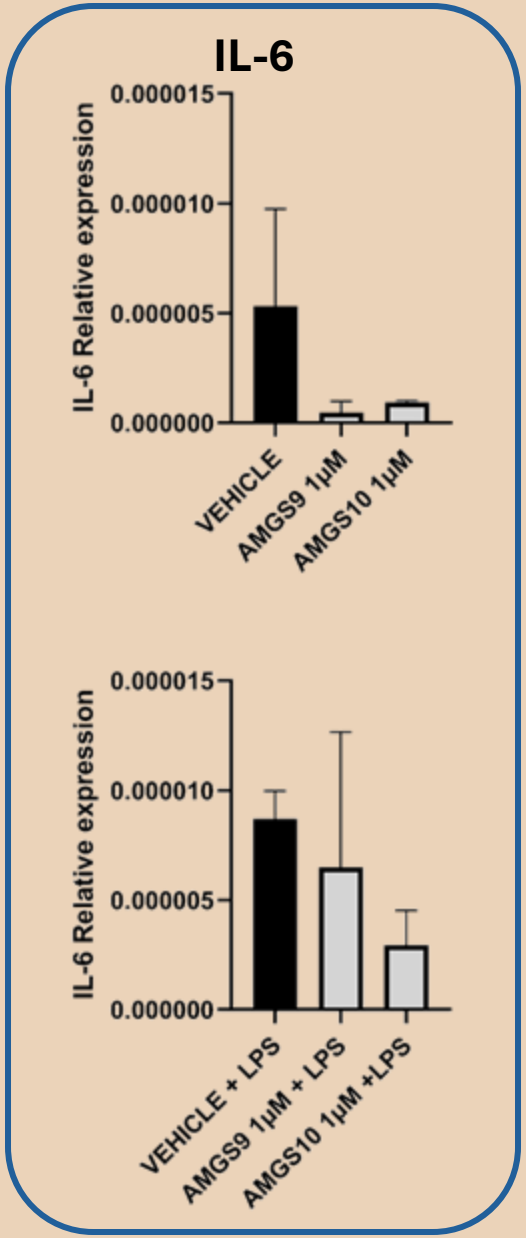
Cells were recovered for gene expression analysis.

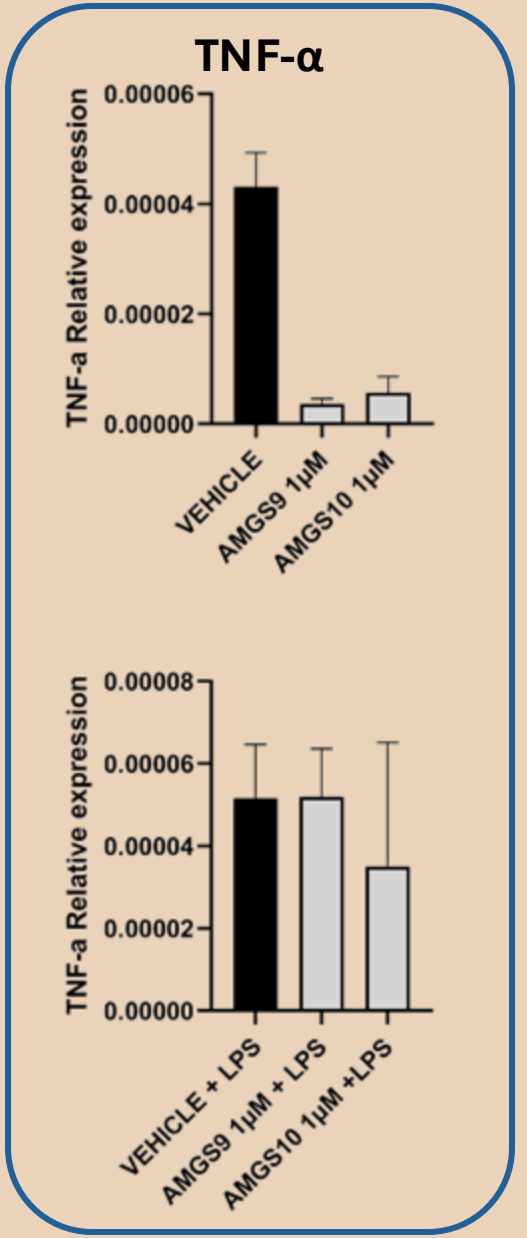
ANALYSIS OF TRANSCRIPTIONAL EFFECTS

W/O LPS

LPS







- No significant effects were observed in the relative expression of pro-inflammatory cytochines IL-1b, IL-6, TNF-a

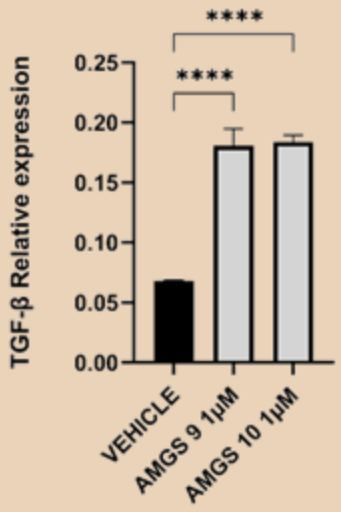
- Data confirmed by ELISA analisys for TNF-α protein expression.

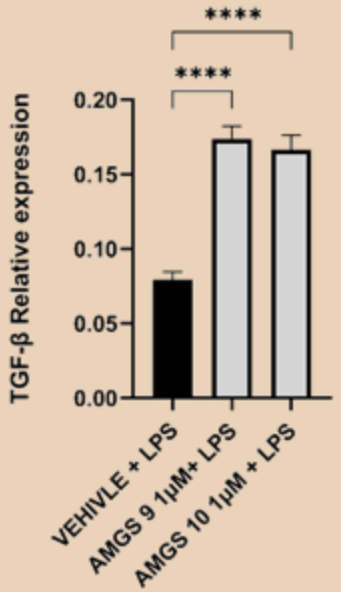
ANALYSIS OF TRANSCRIPTIONAL EFFECTS

W/O LPS

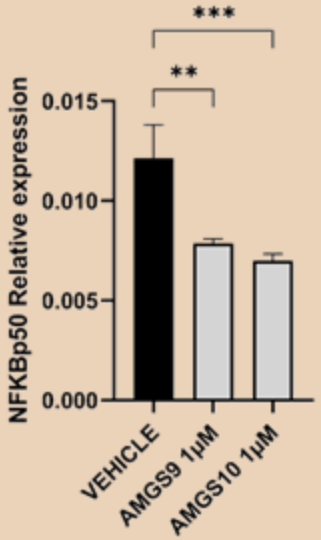
LPS

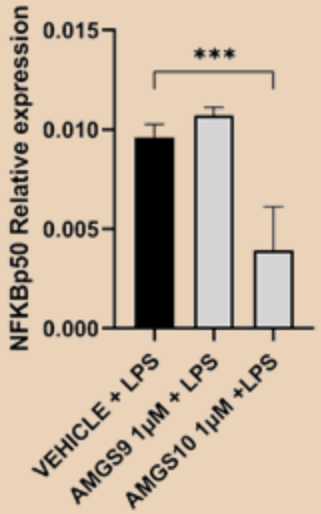
TGFβ



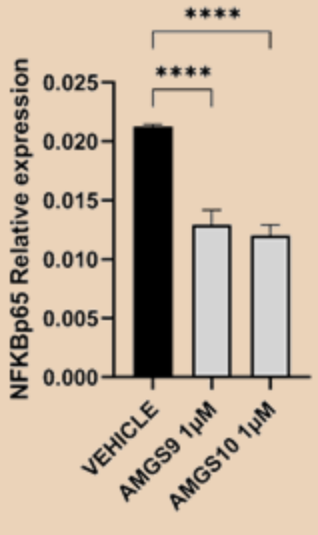


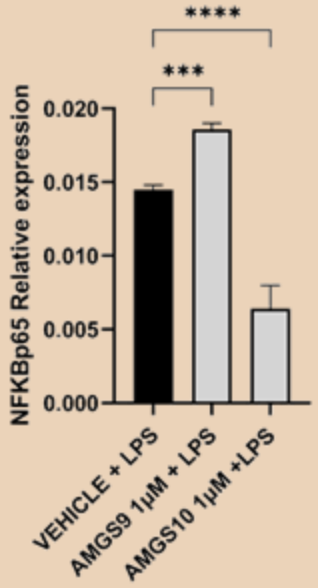
NFkb-p50



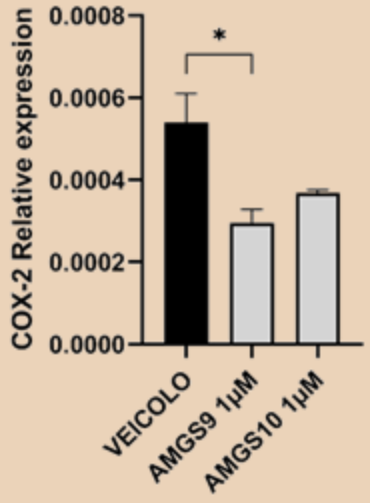


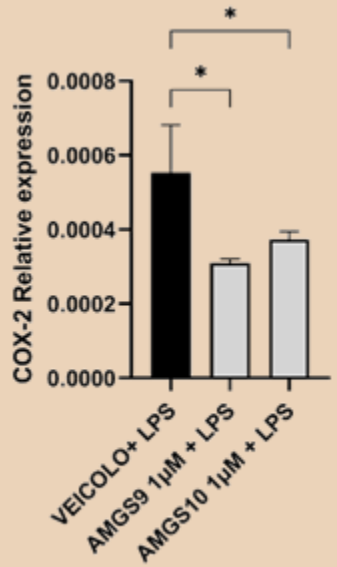
NFkb-p65

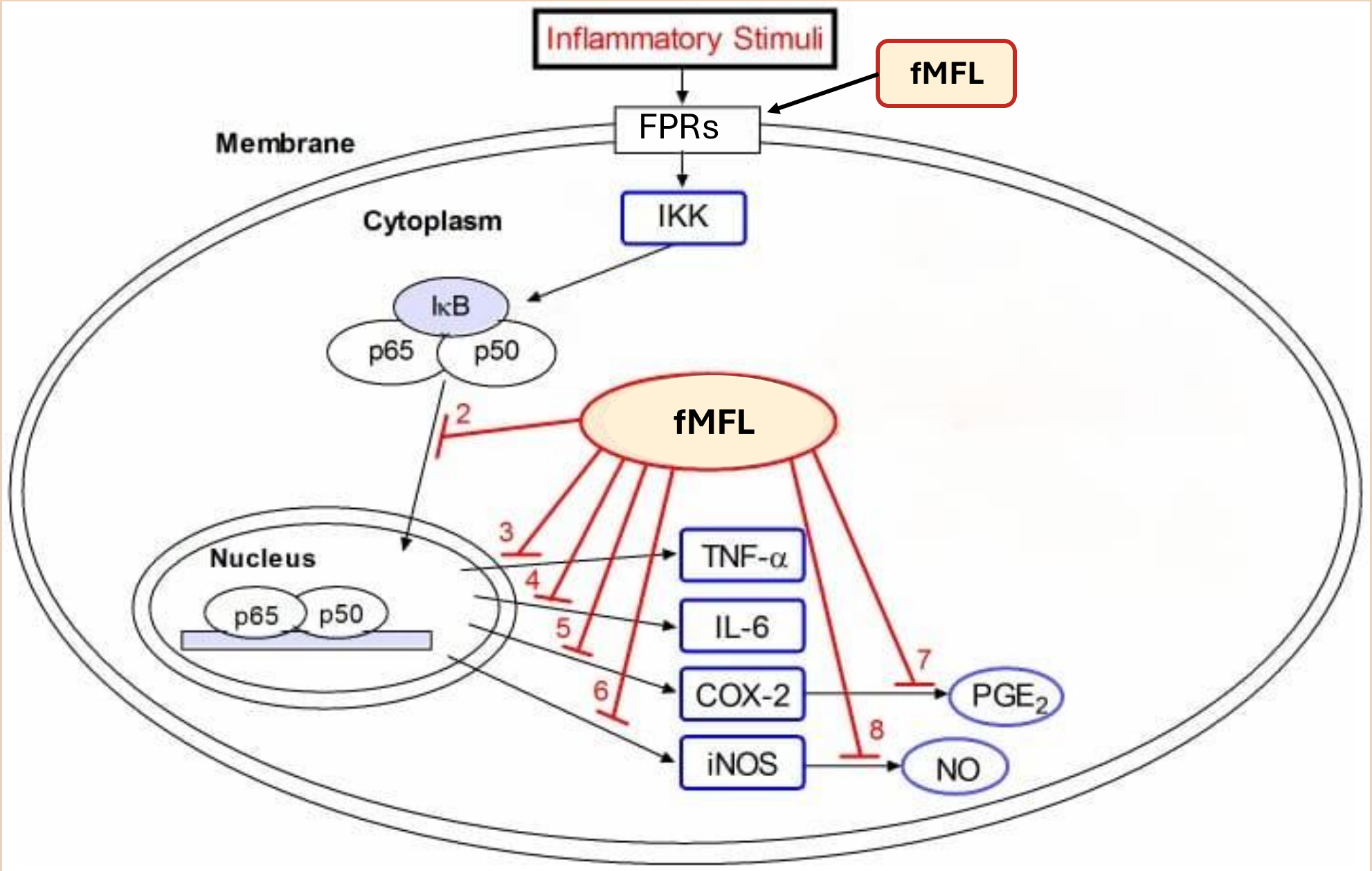


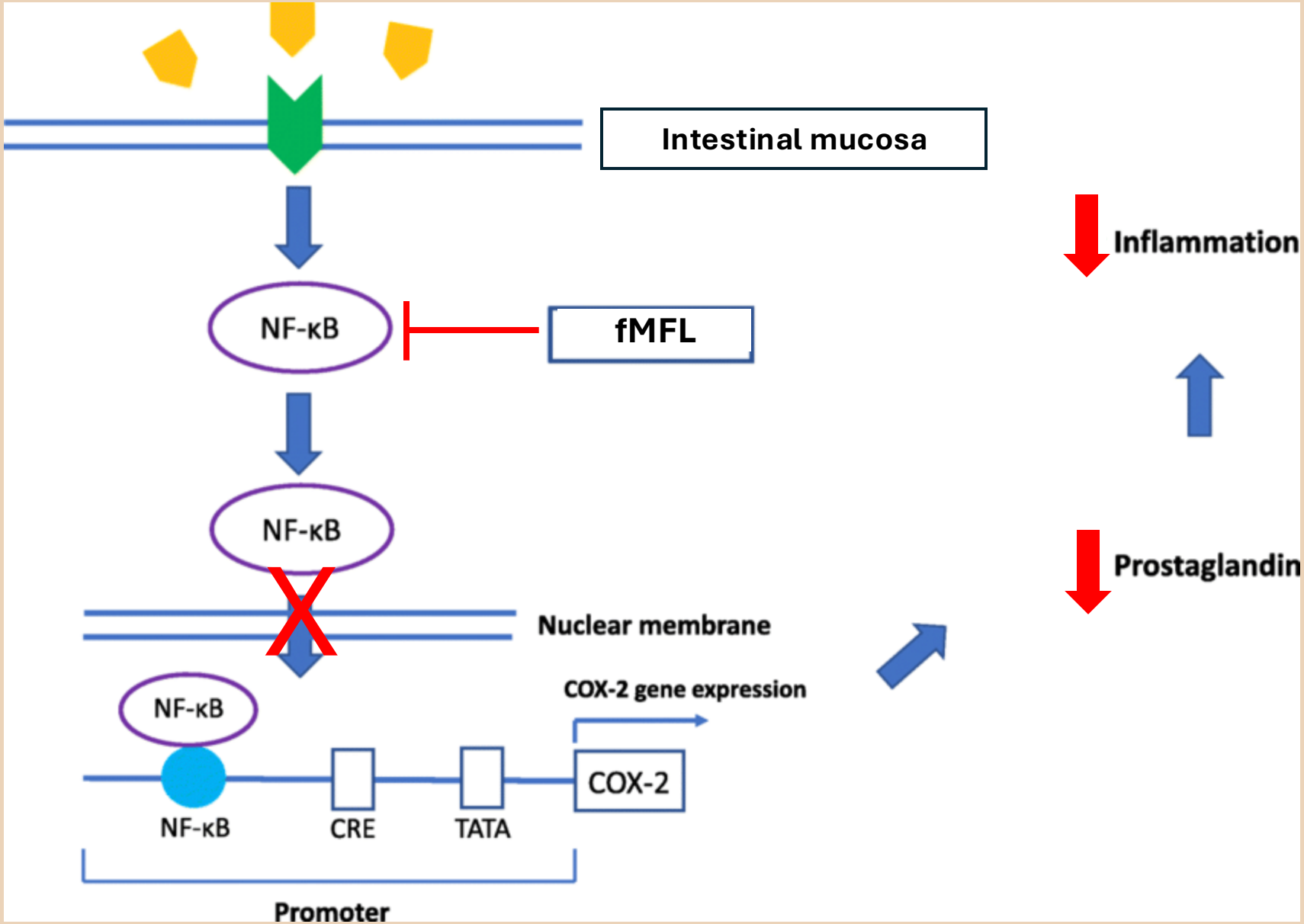


COX-2






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


Intestinal mucosa
NF-κB
fMFL
NF-κB
Nuclear membrane
NF-κB
COX-2 gene expression
COX-2
NF-κB
CRE
TATA
Promoter
Inflammation
Prostaglandin

CONCLUSIONS:

↑ TGFβ: antinflammatory and immunosuppressive

↓ NFkb: - inhibition of pro-inflammatory cytochine production
- no activation of COX-2, which is its target gene

↓ COX-2: inhibition of prostanoid production

PERSPECTIVES:

- Synthetic formylated peptides could be considered as a new therapeutic approach in treating colorectal and intestinal deseases.

- The development of a nanoparticles-based delivery system could improve their antinflammatory effect.

This step could be elaborated in collaboration with the laboratories affiliated to this WP3, of the Spoke 8.

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PIANO NAZIONALE DI RIPRESA E RESILIENZA

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VITALITY