CHARACTERIZATION OF NANOPARTICLES FOR VACCINE DEVELOPMENT

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Introduction

Mucosal vaccines

Protective mucosal immune responses are most effectively induced by mucosal immunization

→ Elements
- Adjuvant
- Immunogen
- Delivery system

↓

New technologies

↓

Use of Nanoparticles (NPs) in vaccines

- **Carrier**: delivery and protect
- **Components**: Immunogen with or without adjuvant

Hypothesis

The use of **nanoparticles** in the formulation of mucosal vaccines or immunotherapies will allow to reduce the dose of immunogen (with or without adjuvant), protect and deliver the immunogen to the site of interest.

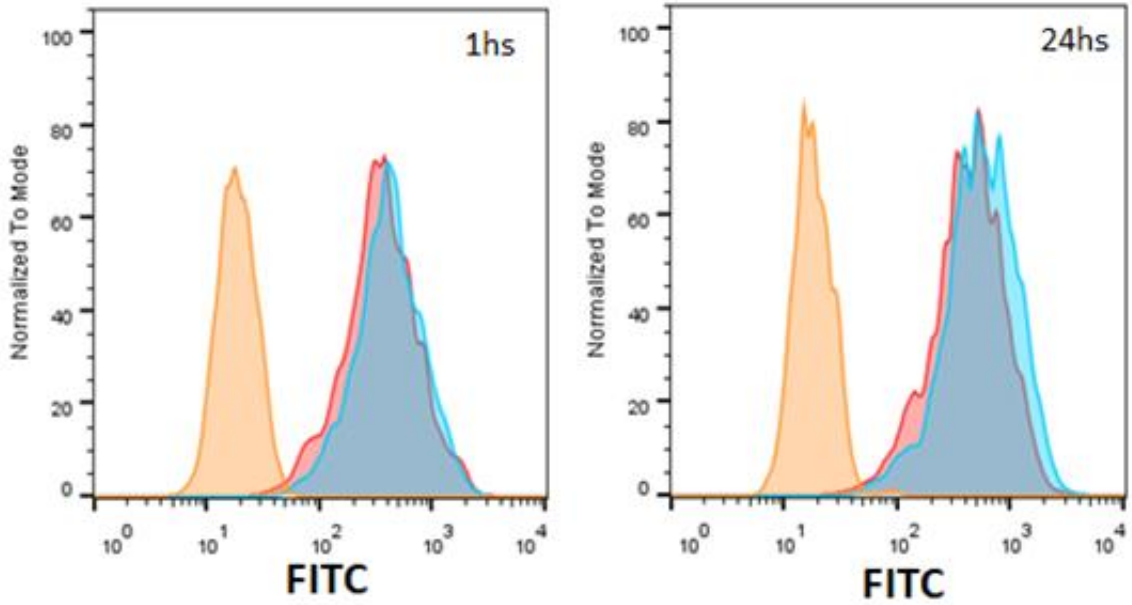
Aim

Biological **characterization** of nanoparticles in a mucosal vaccine and induction of specific immune response.

Results

In vitro characterization of NP in cell lines

Biological effect on murine macrophages RAW

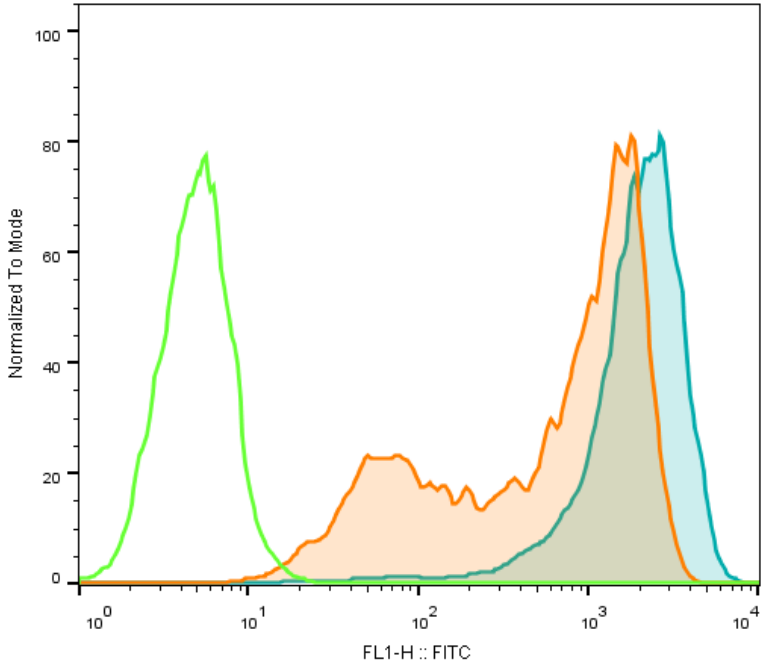


Orange: Basal
Red: LPS+NP
Blue: NP

-The interaction with NP is LPS independent
- Superficial binding or internalization of the NP-FITC?

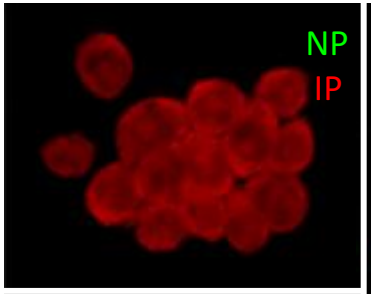
Phagocytosis of the nanoparticle

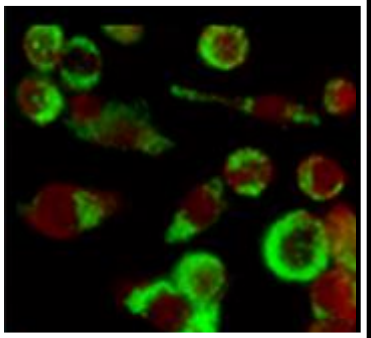
Biological effect on murine macrophages J774

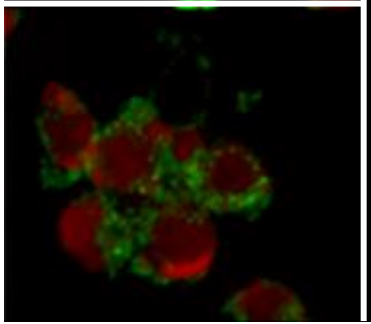


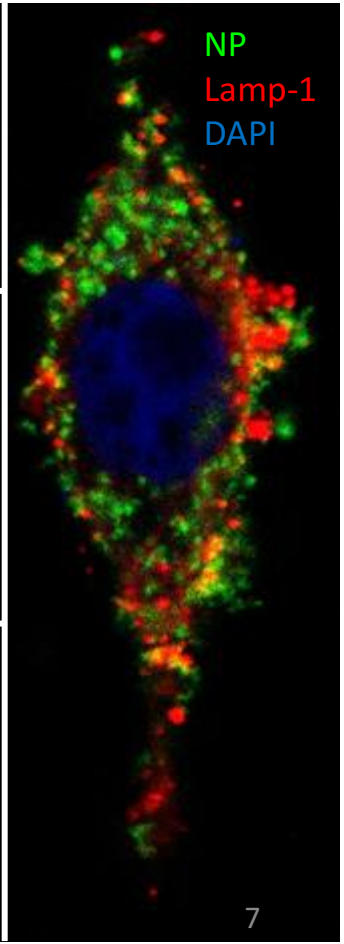
Green: Medium
Blue: NP
Orange: Cito D+NP

The Np is internalized by phagocytosis



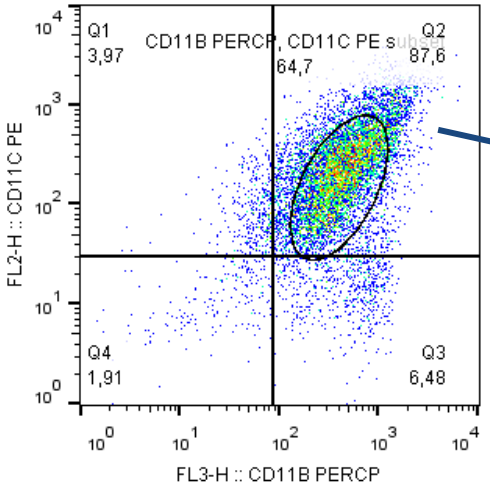


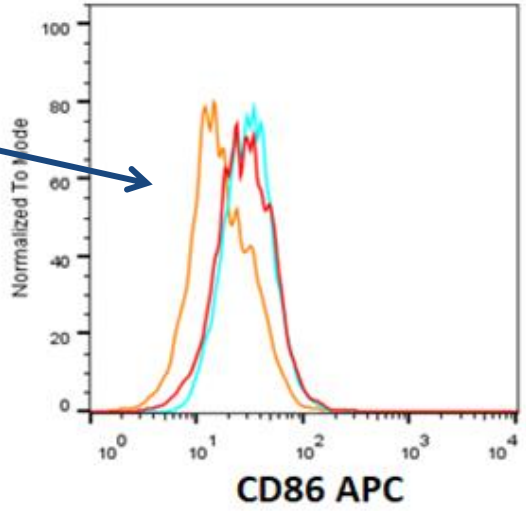




Cytochalasin D
phagocytosis inhibitor.

Dendritic cell activation





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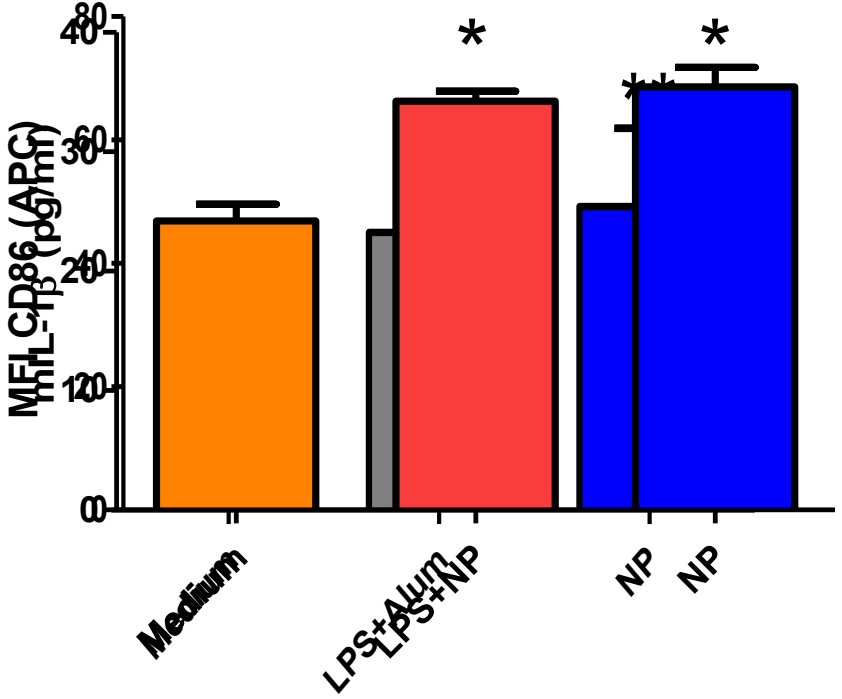
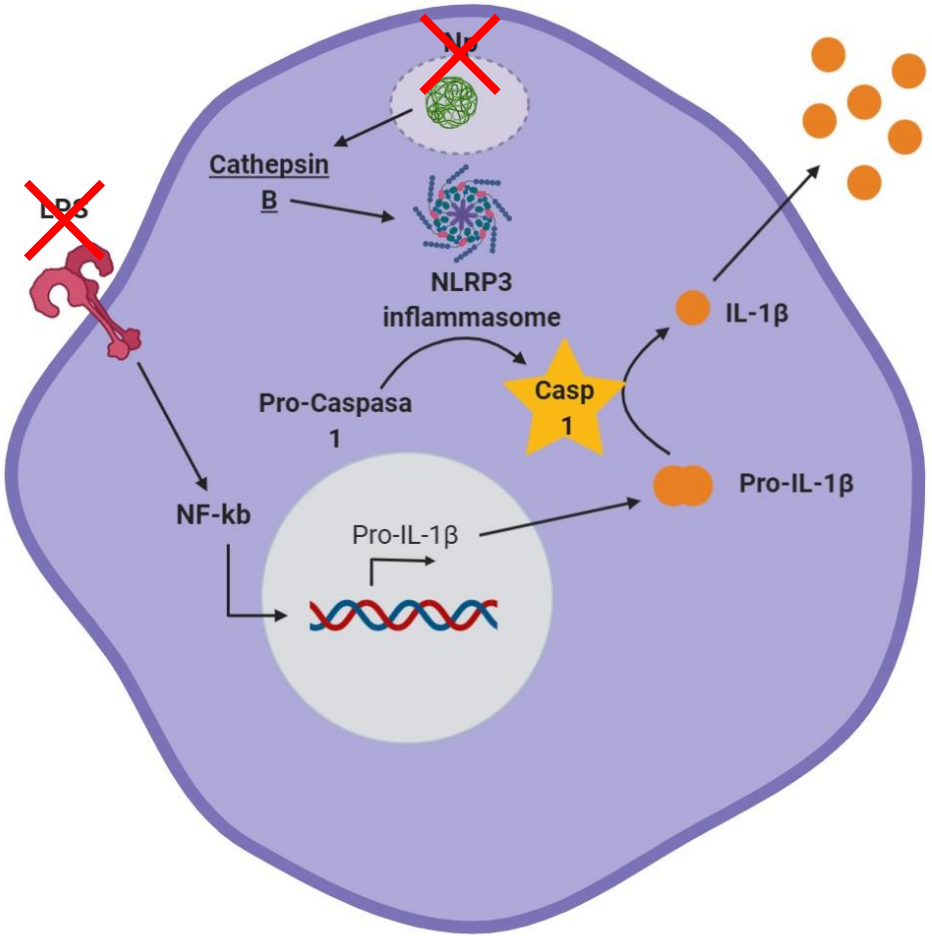
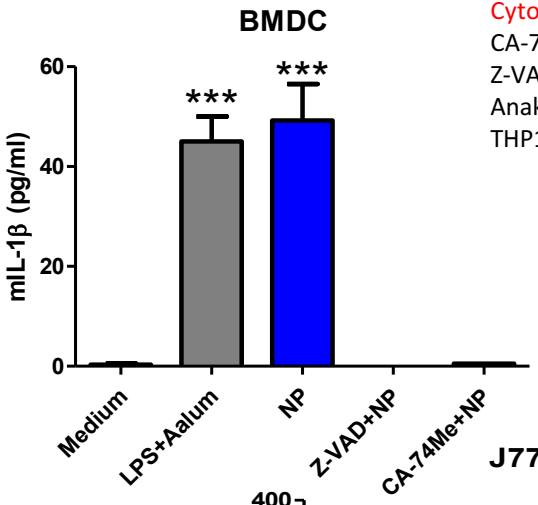
Red: LPS+NP
Blue: NPs



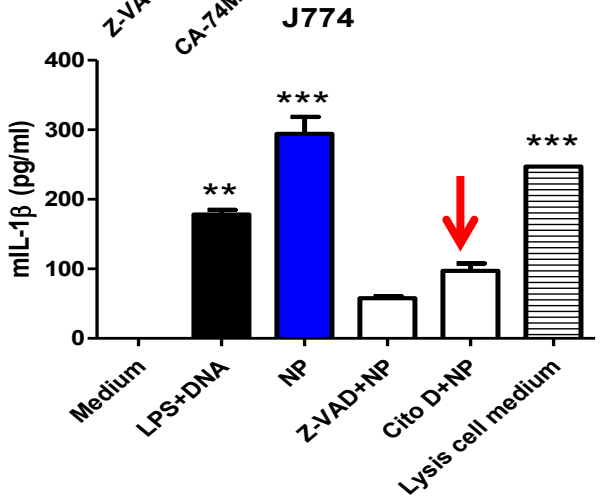
BMDC are activated with NP: induction of CD86 ($CD11c^+CD11b^+CD86^+$) and IL-1b production

Is the inflammasome pathway involved?





Cytochalasin D: phagocytosis inhibitor
CA-74Me: Catepsine B inhibitor
Z-VAD-FmKQ: Caspase-1 inhibitor
Anakinra: receptor antagonist IL-1RA
THP1-Myd88 KO



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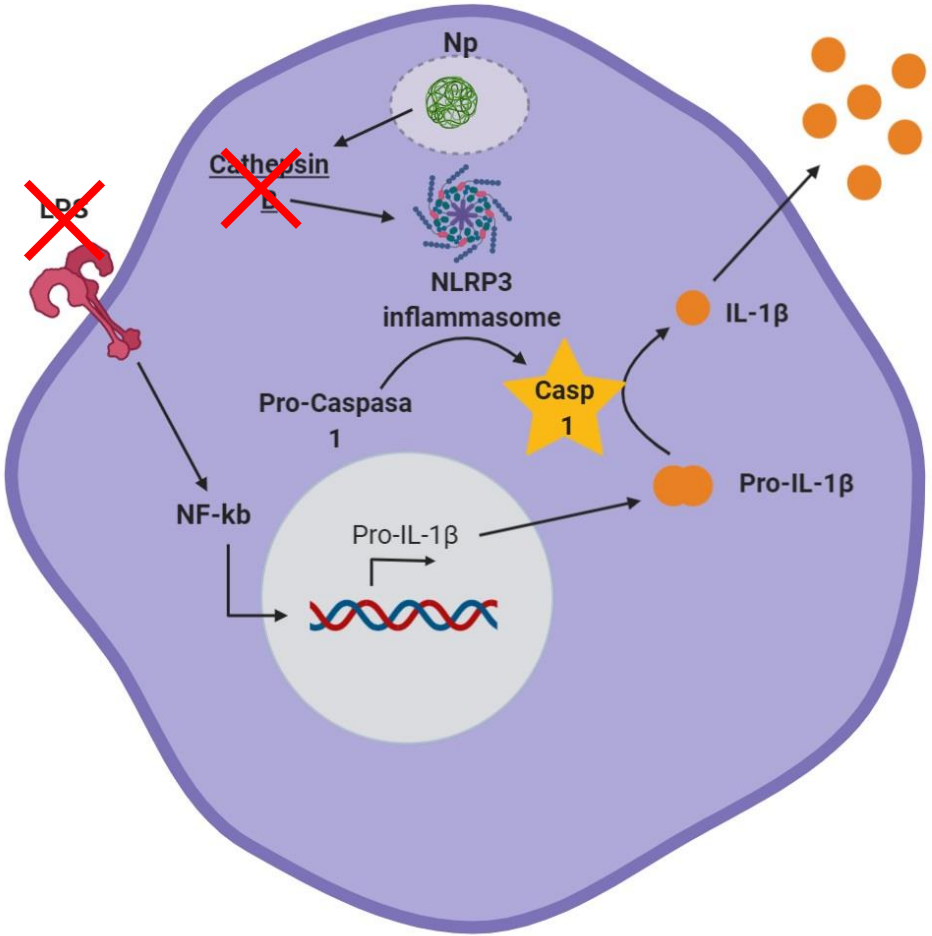

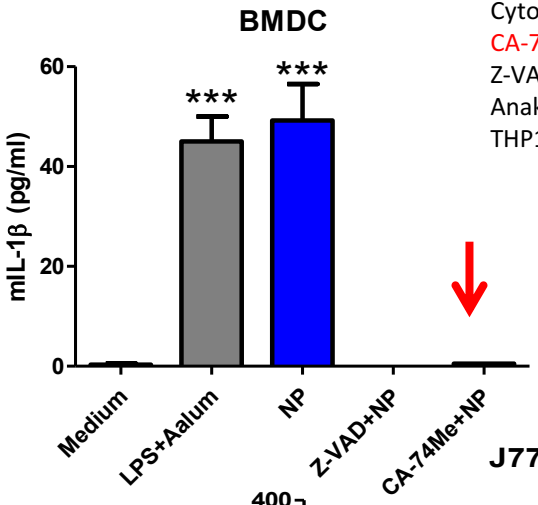

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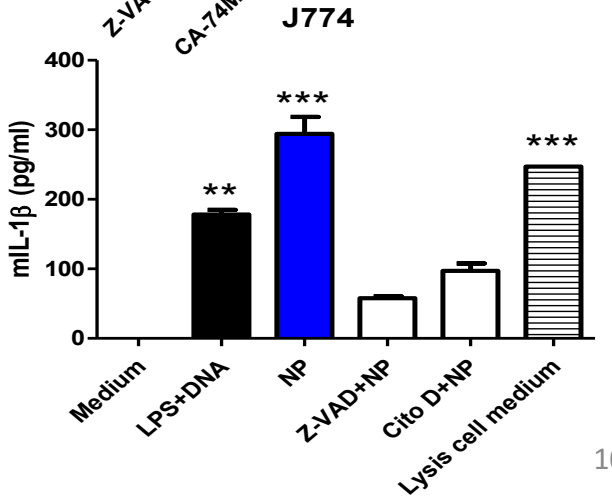

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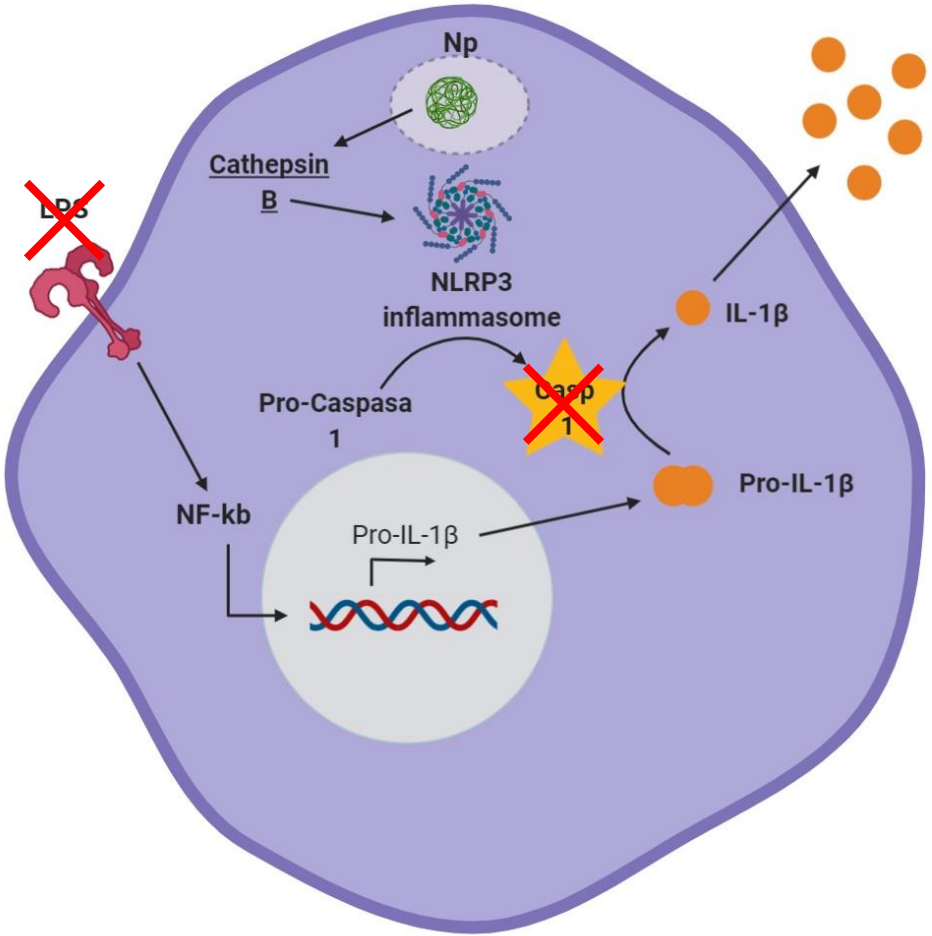

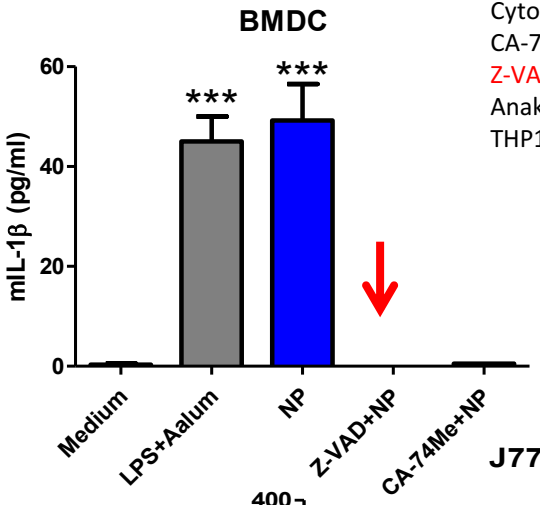

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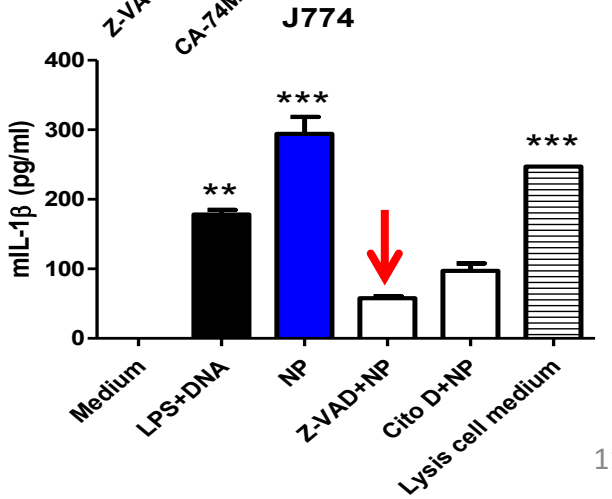

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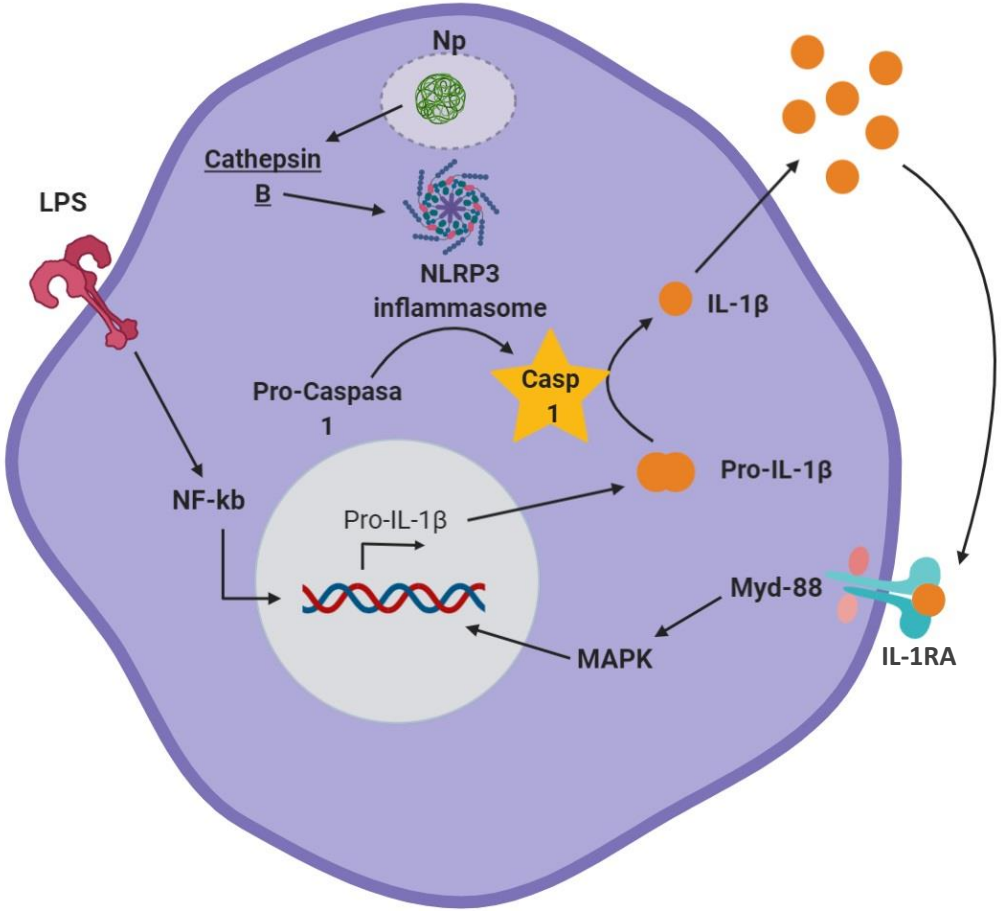

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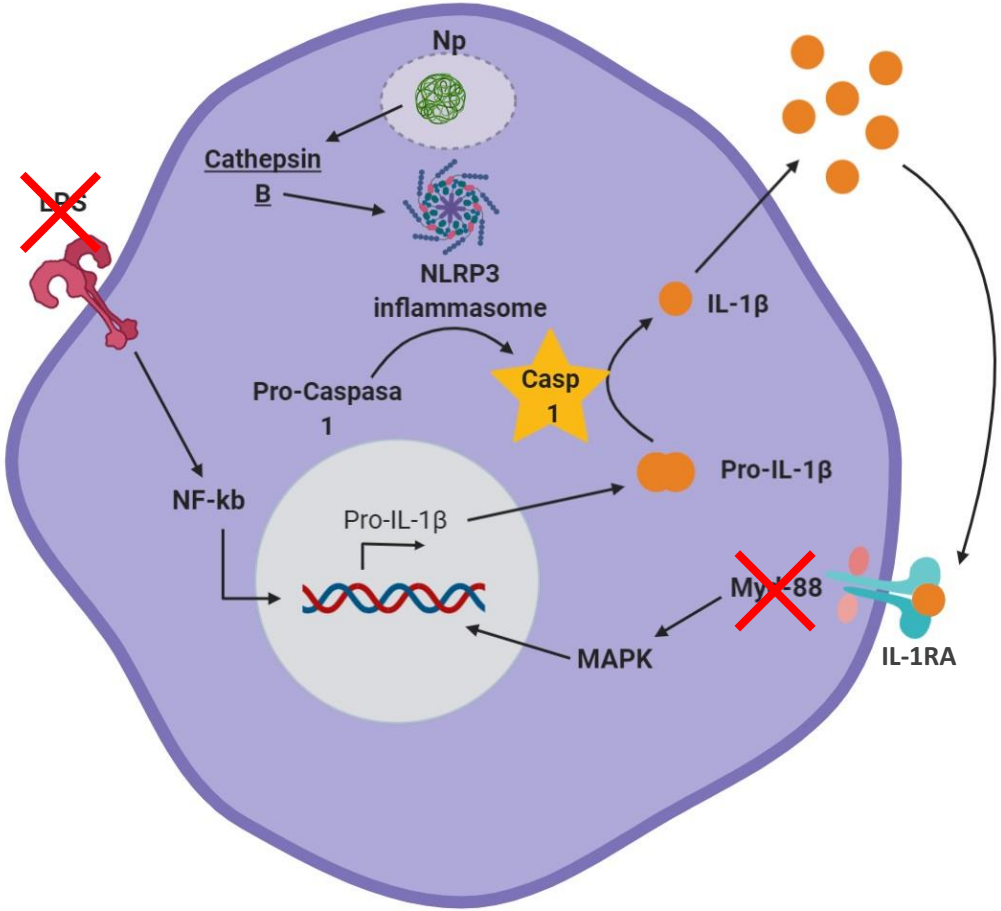

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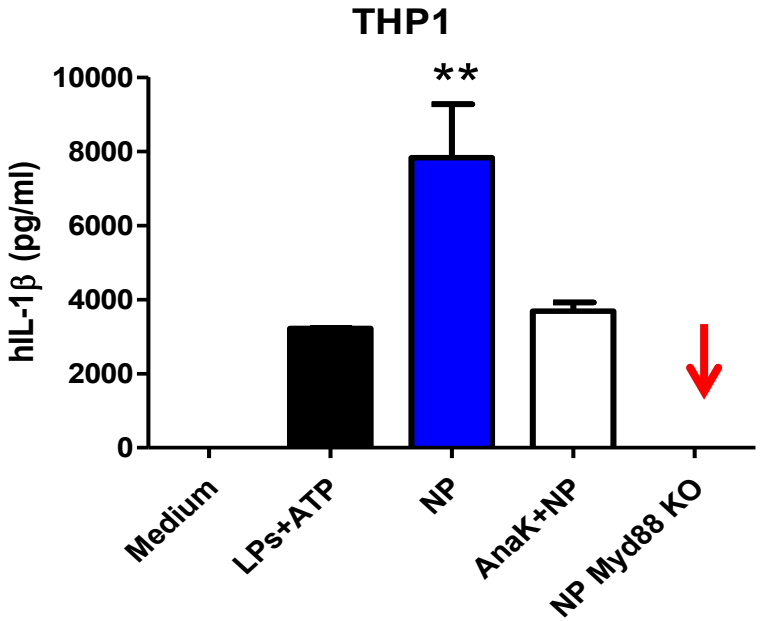

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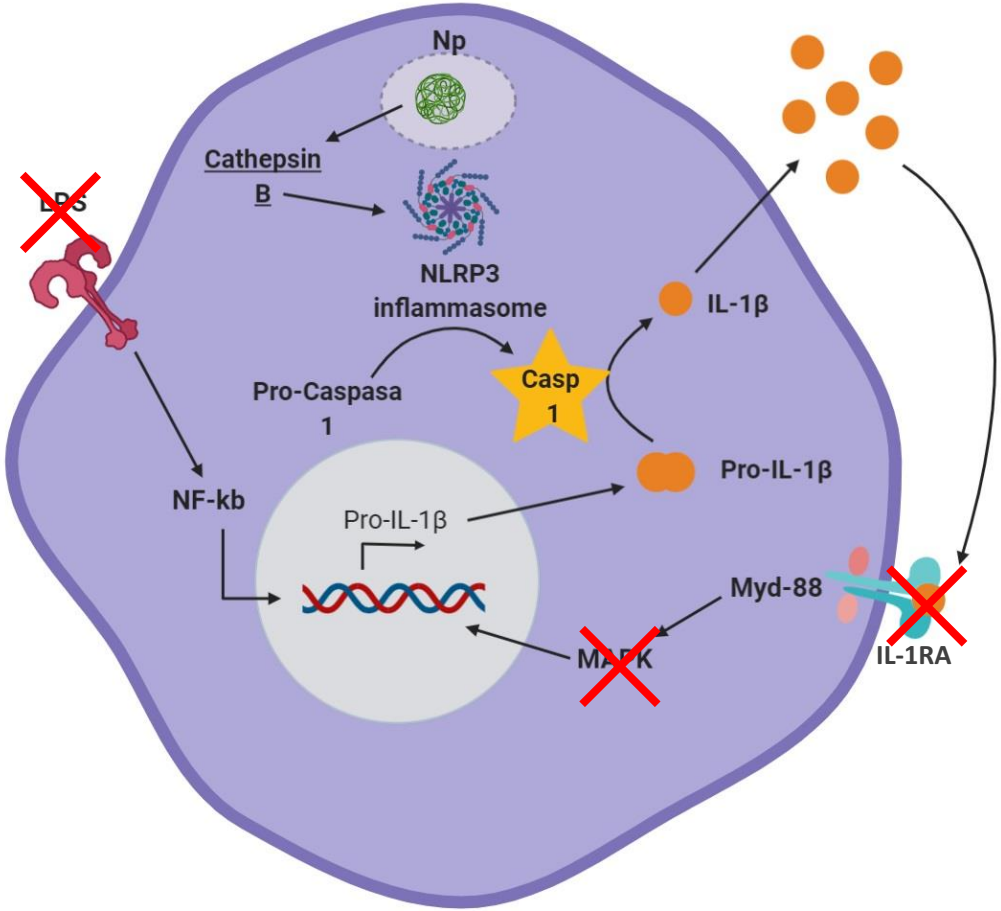

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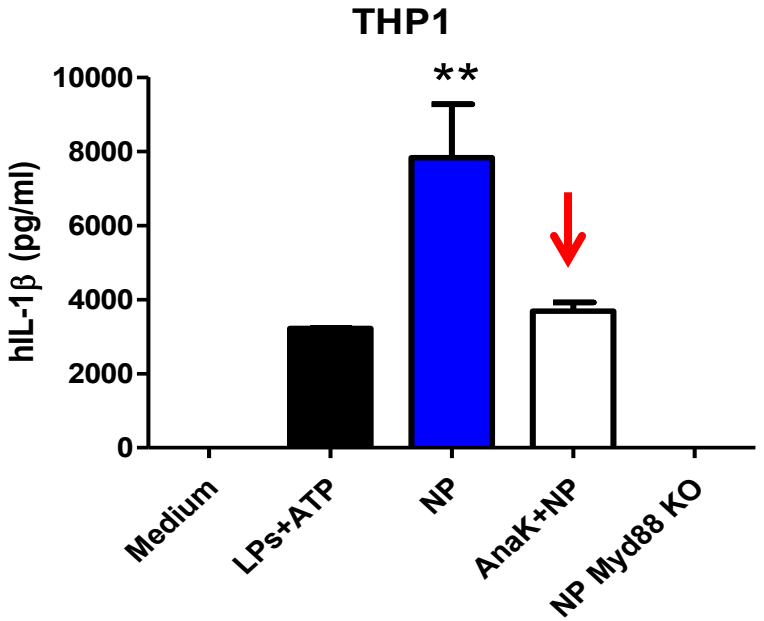
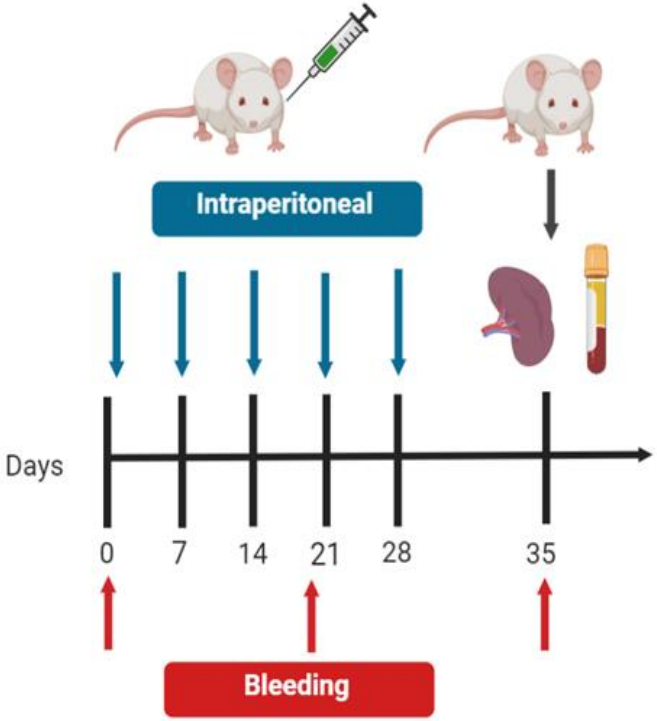


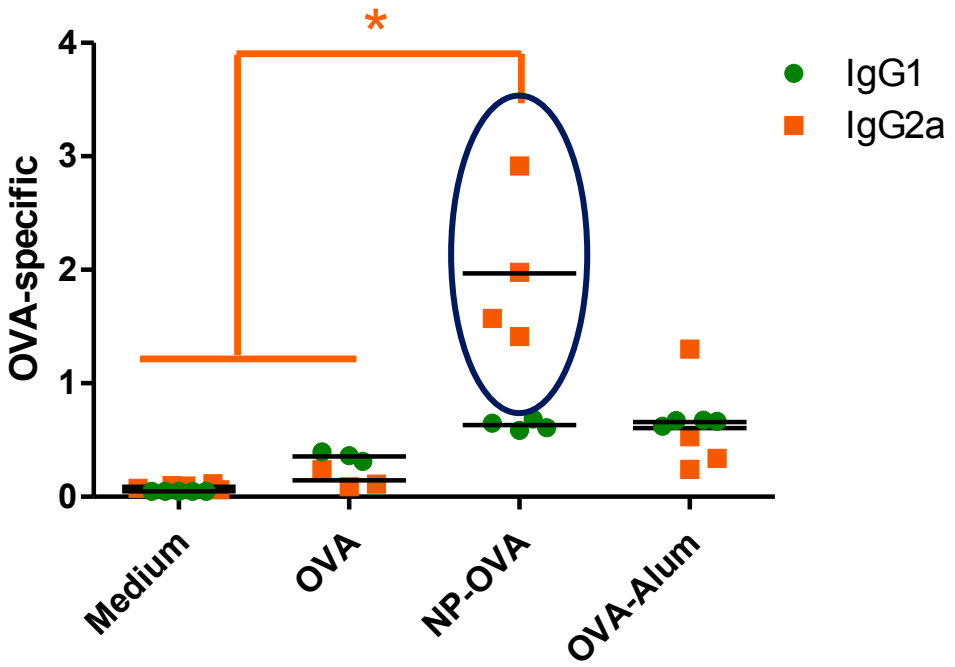
First conclusions

- Inflammasome was activated with IL-1β-production
  - IL-1β-production was TLR-independent
  - Cell activation was Myd88-dependent

Does the nanoparticle have adjuvant properties?

Do nanoparticle have adjuvant properties?

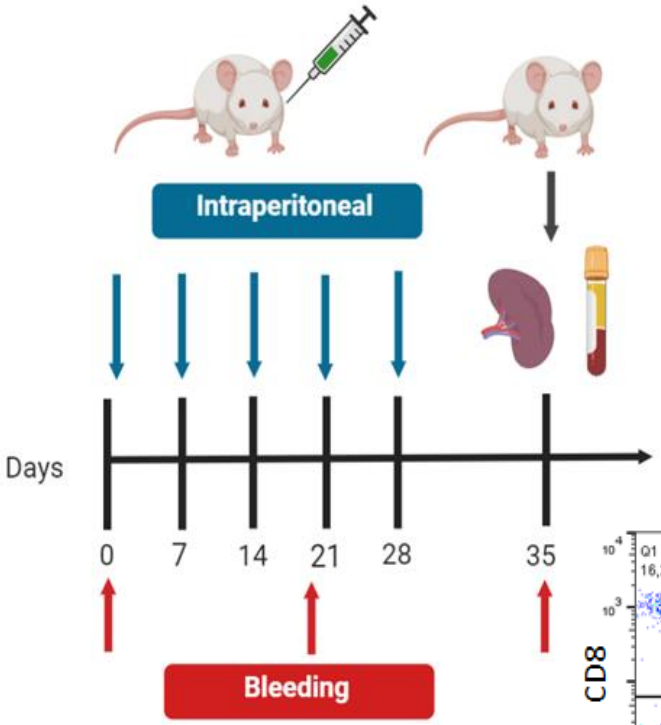




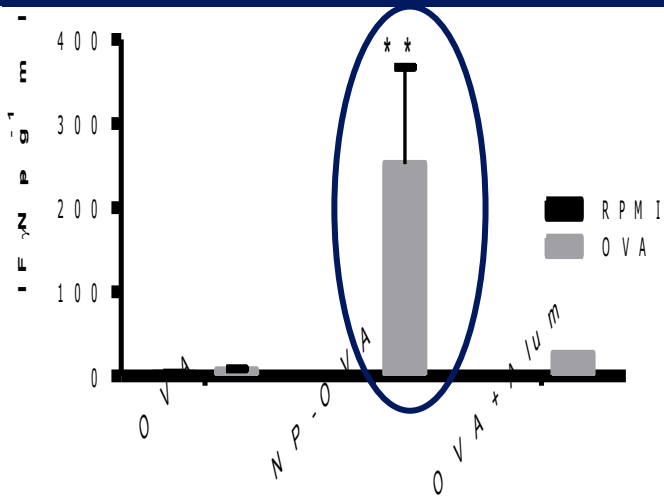
n=4	NP-OVA
n=4	OVA+Alum
n=3	OVA

Np-OVA induces a Th1 profile

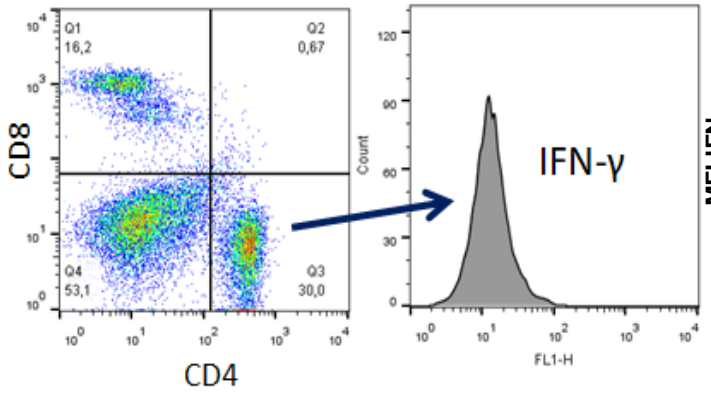
Do nanoparticle have adjuvant properties?

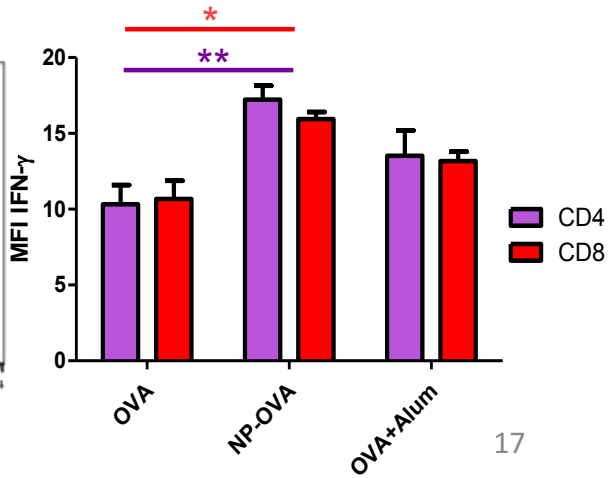


n=4 NP-OVA
n=4 OVA+Alum
n=3 OVA



Splenocyte IFN-γ secretion
NP induced cellular immunity





Final conclusions

- ✓ The nanoparticles were internalized by phagocytosis and cells were activated:
- ✓ Induction of $CD86^{+}$
- ✓ Production of IL-1β
- ✓ Inflammasome activation in macrophages: non-cannonical activation (without the signal 1)

In vivo adjuvant effect

- ✓ Production of OVA-specific Th1 antibodies
- ✓ Cellular immune response with induction of IFN-γ-producing CD4 and CD8 T cells

Potential applications:

- ✓ Characterization as a mucosal carrier/adjuvant (IgA?)
- ✓ Use in mucosal immunotherapies for food allergy (OIT and SLIT) (CD4 and CD8 IFN-γ)
- ✓ Use as a mucosal vaccine for infectious diseases (CD4 and CD8 IFN-γ)

Thanks for your attention!!

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