Self-assembling nanoparticles enhance brain delivery and anti-amyloid activity of NUsc1

Carraro, M. F.[1,2]; Sebollela, A. A.[3]; Santos, H. A.[2] and Lopez, R. F. V.[1]

[1] *School of Pharmaceuticals Sciences of Ribeirao Preto, University of Sao Paulo, Sao Paulo, Brazil.*

[2] *University Medical Center Groningen, University of Groningen, Groningen, the Netherlands.*

[3] *Ribeirao Preto Medical School, University of Sao Paulo, Sao Paulo, Brazil.*

Molecular Basis of Alzheimer's Disease

Dementia affects around **55 million** people globally, with projections to reach **132 million** by 2050

World Health Organization, 2023

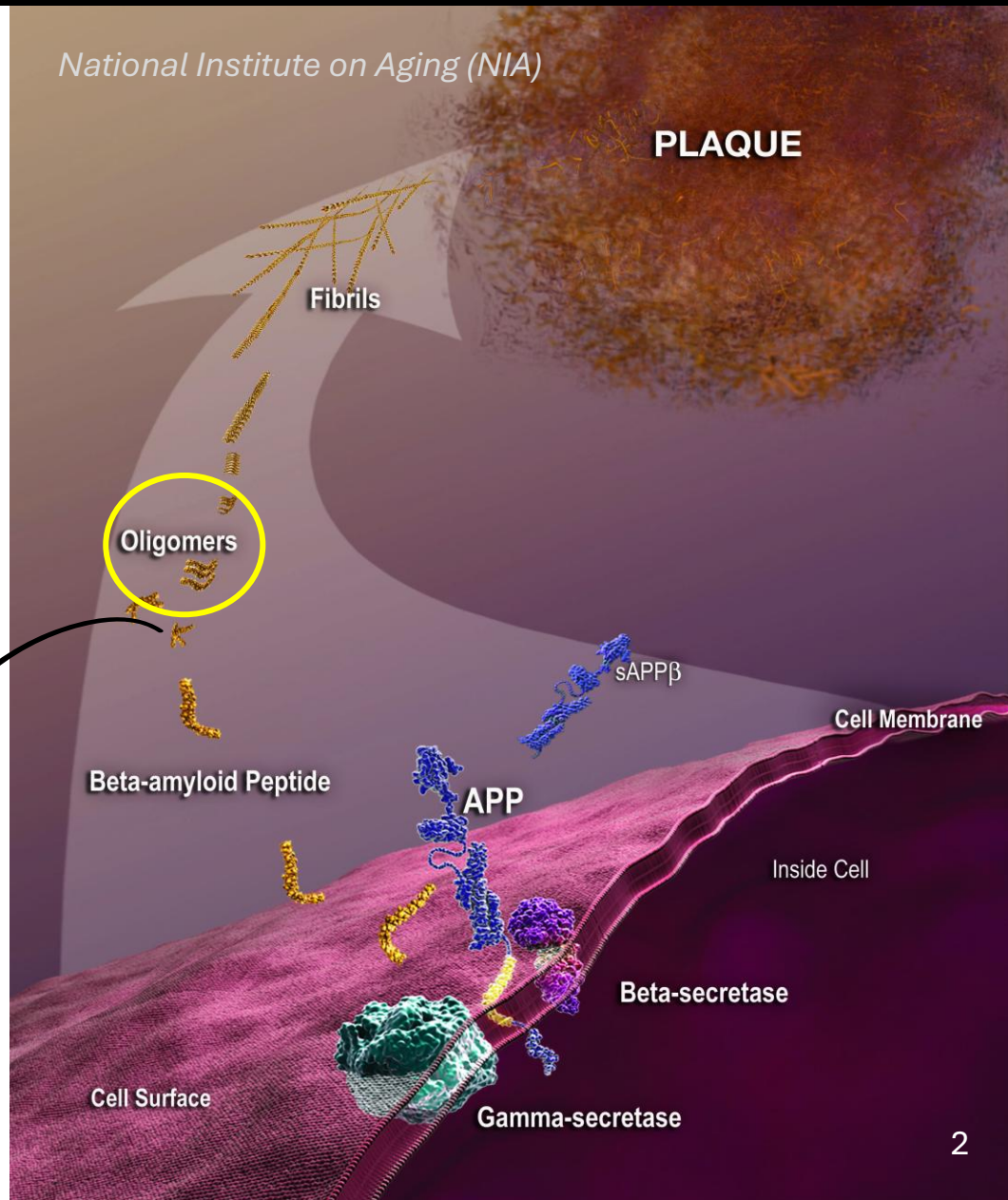
Alzheimer's disease is the **leading cause of dementia** worldwide (accounting for 60%-80% of cases)

SCHELTENS et al., 2021

Mechanisms of neurotoxicity associated with Aβ Oligomers:

- **Synapse deterioration**
- **Inhibition of axonal transport**
- **Oxidative stress**
- **Neuron death**
- **Plasticity dysfunction**

Zhao et al, 2006; Wu et al, 2010; Pigino et al, 2009
Decker et al, 2010; Lambert et al, 1998; De Felice et al, 2007; Ryan et al, 2009



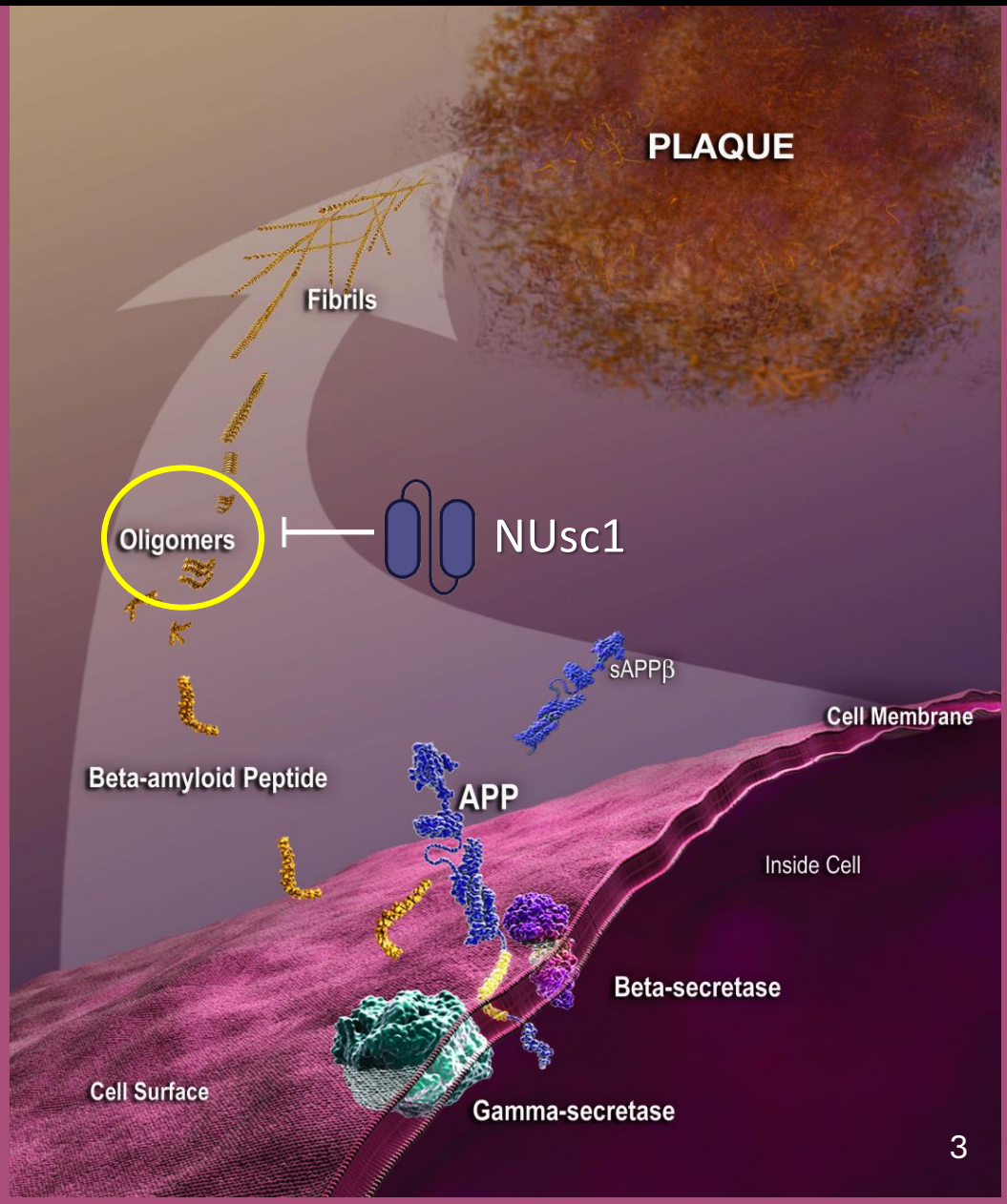
How can the neurotoxic effects of AβOs be blocked?

NUsc1 was selected against AβOs by phage display (Velasco et al., 2012);

NUsc1 shows high selectivity and specificity for AβOs (Sebollela et al., 2017);

AAV-NUsc1 treatment prevented and reversed AβO-induced memory impairment in APP/PS1 transgenic mice (Selles et al., 2022).

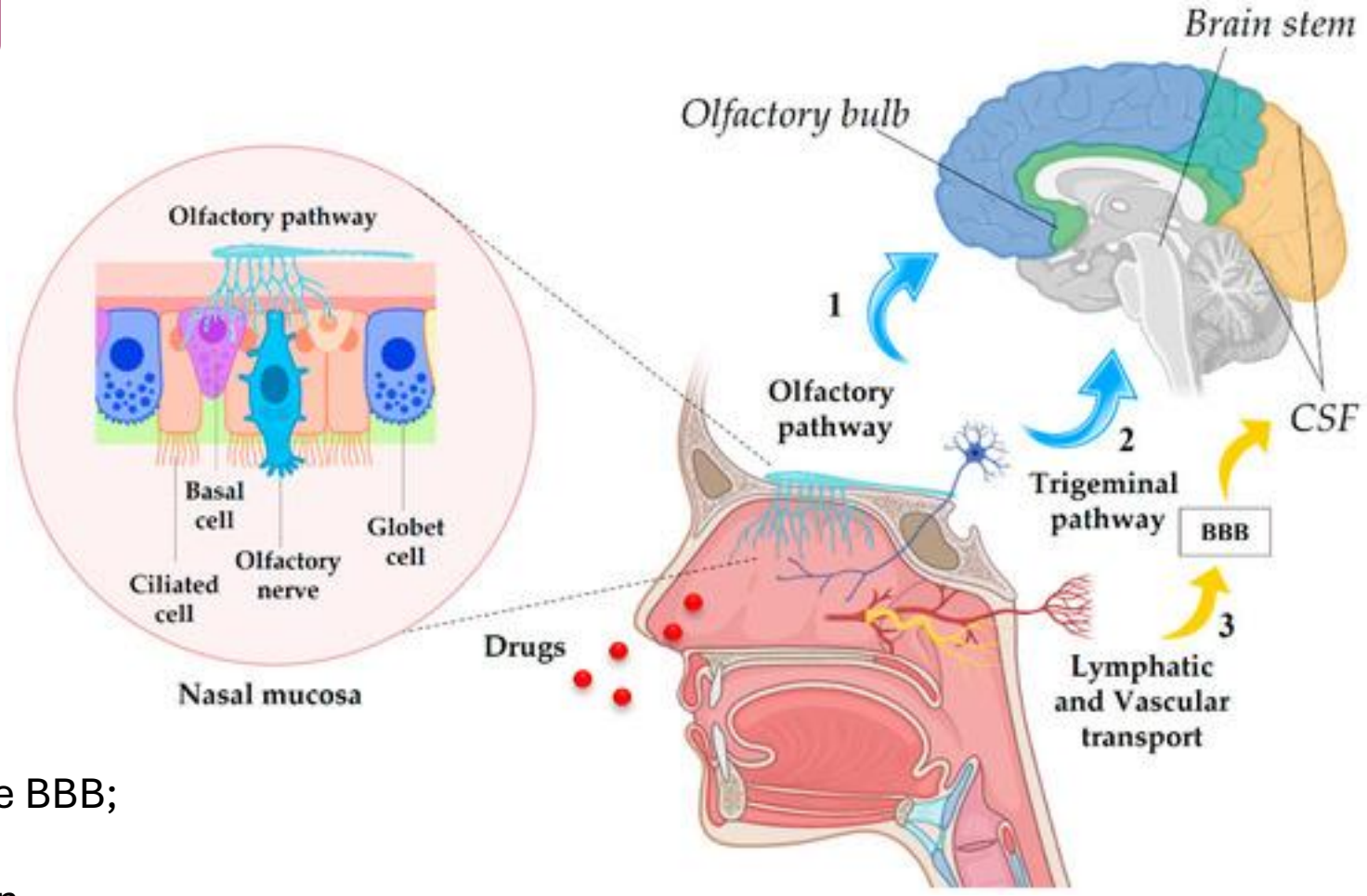
Learn more about NUsc1



How can NUsc1 be efficiently delivered to the brain?

Challenge: limited antibody transport across the Blood–Brain Barrier

Strategy: Nose-to-brain delivery



Montegiove et al, 2022

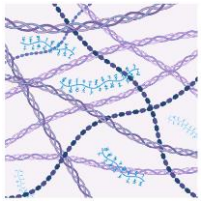
Advantages:

- Direct delivery to the brain, bypassing the BBB;
- Avoidance of first-pass metabolism ;
- Non-invasive and painless administration.

Nose-to-Brain Delivery System for NUsc1 :

Polymeric nanoparticles (NPs) loaded with NUsc1:

HA is a biopolymer and a major component of the brain extracellular matrix. Its mucoadhesive properties may enhance nasal residence time.



(Lee and Spicer, 2000)

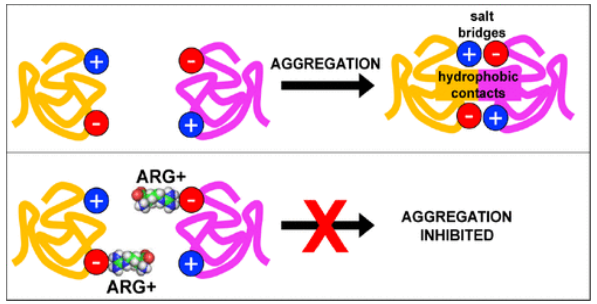
Review > Front Mol Neurosci. 2021 Oct 28:14:759729. doi: 10.3389/fnmol.2021.759729. eCollection 2021.

Arginine and Arginine-Rich Peptides as Modulators of Protein Aggregation and Cytotoxicity Associated With Alzheimer's Disease

Somayra S A Mamsa [1 2], Bruno P Meloni [2 3 4]

Affiliations + expand

PMID: 34776866 PMCID: PMC8581540 DOI: 10.3389/fnmol.2021.759729

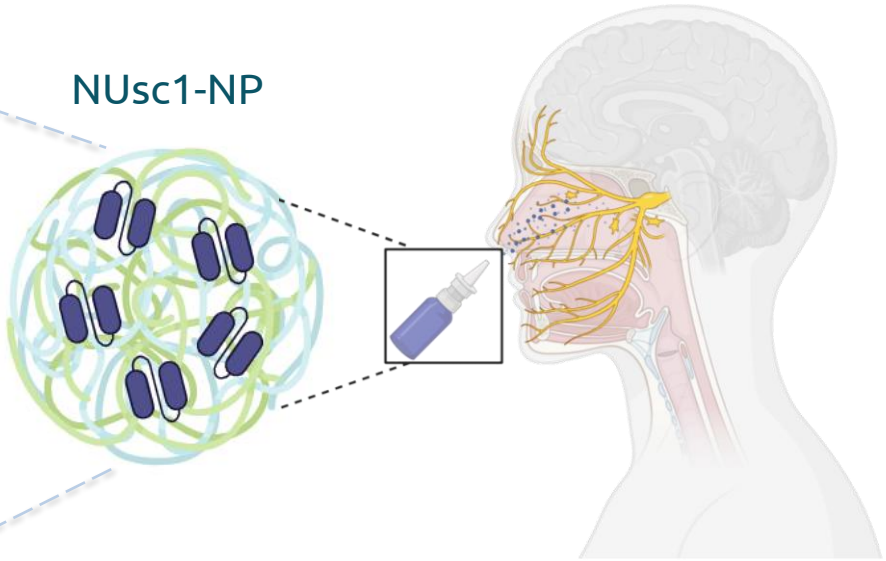


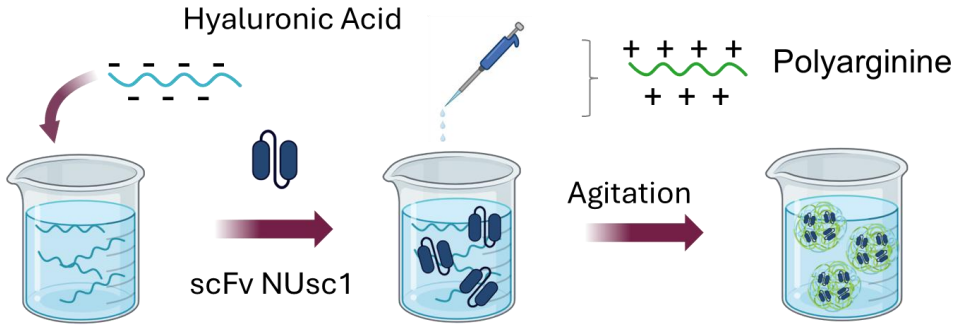
(Ng and Konermann , 2024)

Hyaluronic Acid (anionic)

scFv NUsc1 (pI= 9, cationic)

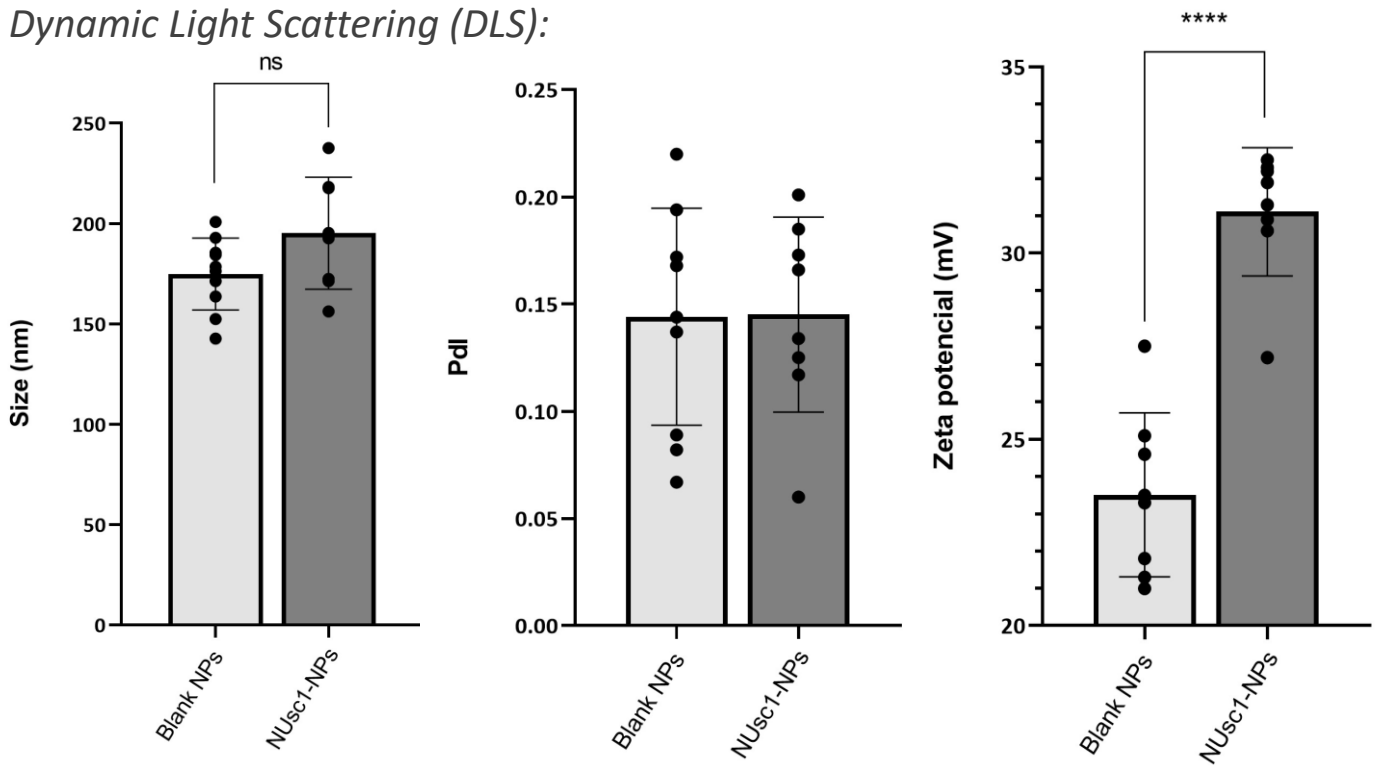
Poly-L- Arginine (cationic)





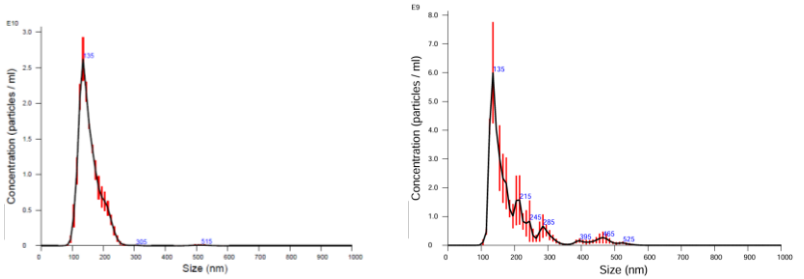
Physicochemical Characterization of NUsc1-NPs:

Dynamic Light Scattering (DLS):



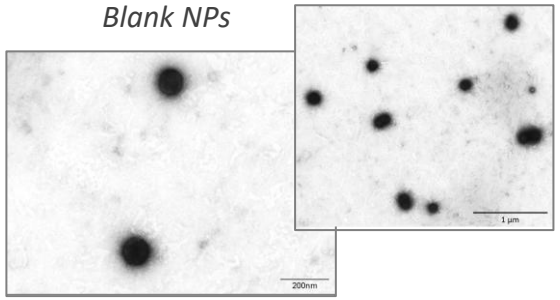
Nanoparticle Tracking Analysis:

	Mean (nm)	Span	Concentration (particles/ml) :
Blank NP	156.2 ± 1.4	0.5	$(1.72 \pm 0.02) \times 10^{11}$
NUsc1-NP	192.9 ± 3.6	0.96	$(3.68 \pm 0.23) \times 10^{10}$

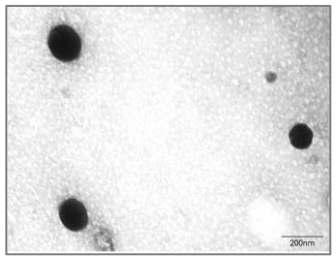


Transmission Electron Microscopy (TEM):

Blank NPs



NUsc1-NPs

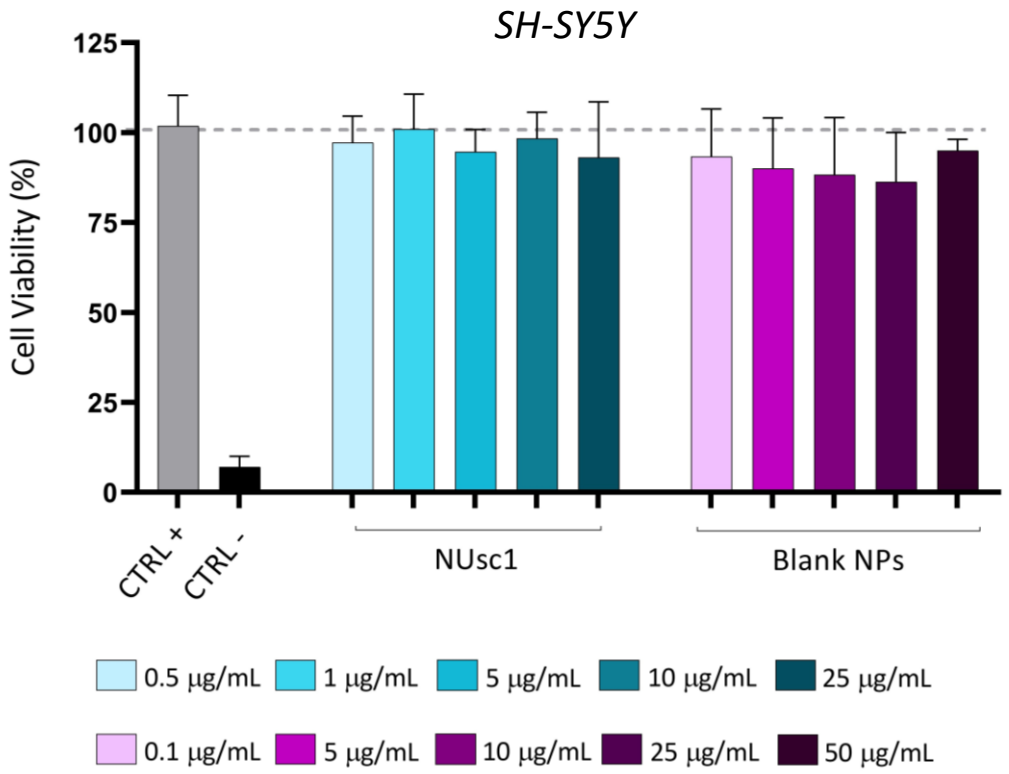


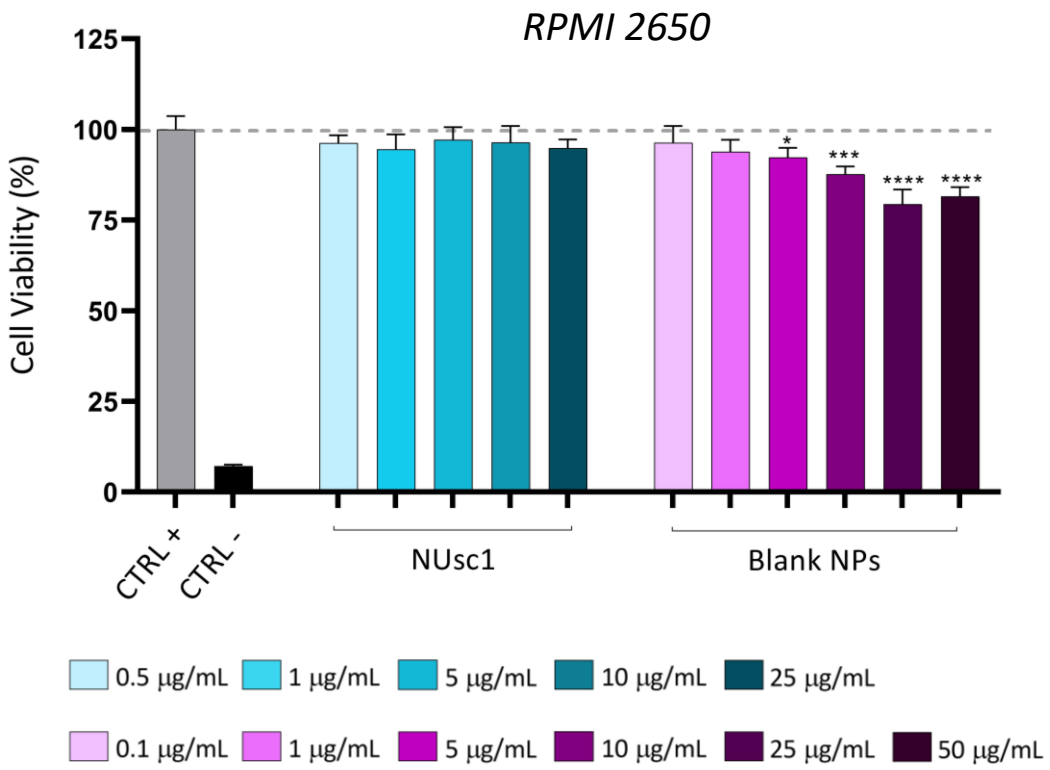
NUsc1 Encapsulation Efficiency (%EE):

NUsc1-NPs	Free NUsc1 (μg/mL)	Encapsulated NUsc1 (μg/mL)	%EE
Average ± SD	6.9 ± 0.5	13.07 ± 0.5	65.3 ± 2.5

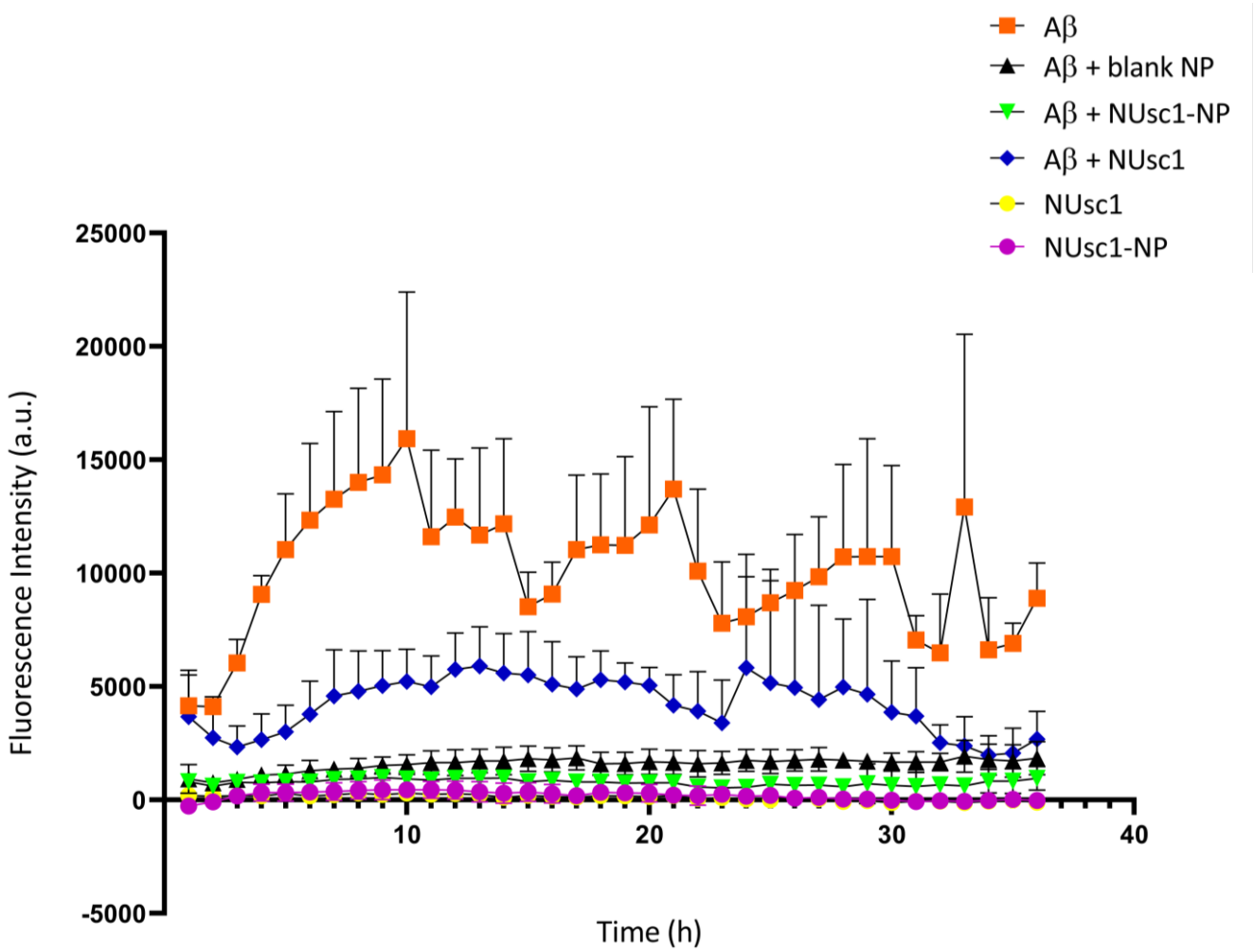
Evaluation of NUsc1-NPs Cytotoxicity:

- Resazurin-Based Viability Assay in Human Neuroblastoma and Human Nasal Epithelial Cells
- 24-hour treatment

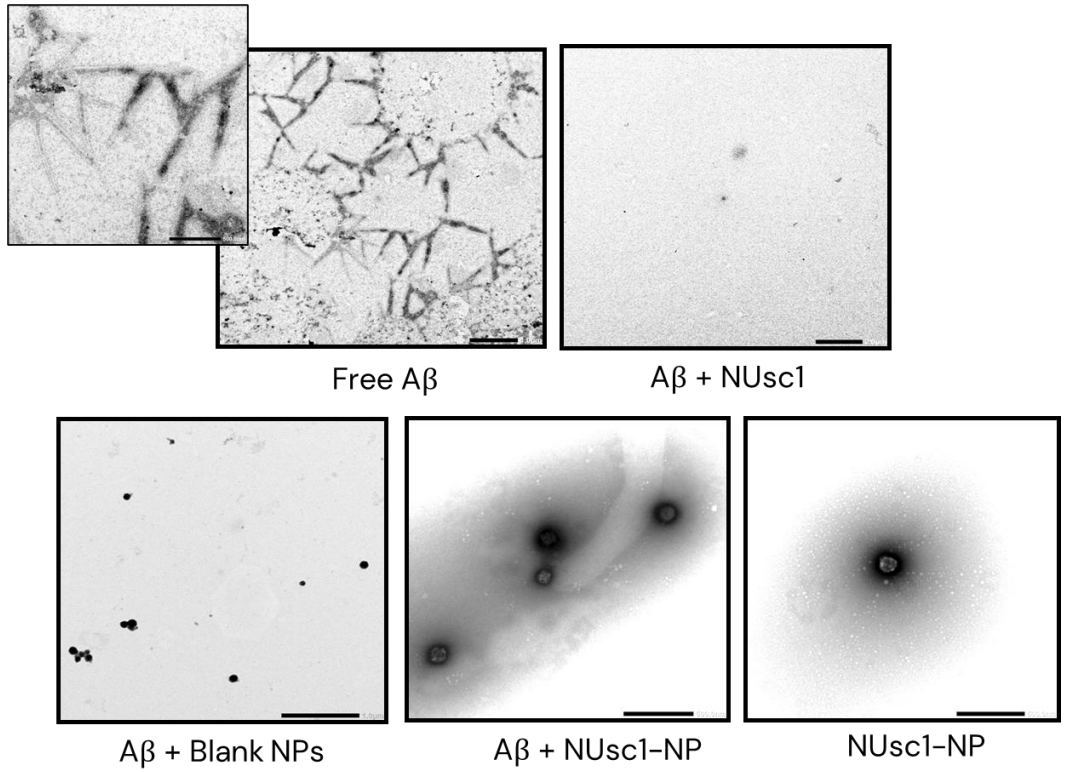




Monitoring of the Aβ aggregation pathway by thioflavin T:



Transmission Electron Microscopy (TEM):



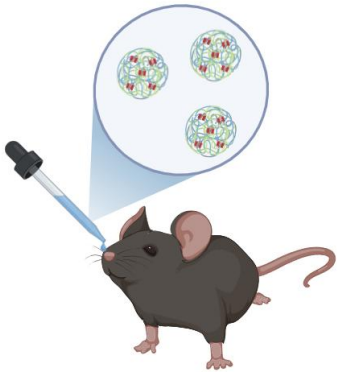
A 10 µM solution of Aβ peptide was incubated at 37 °C for 36 h in the presence of free NUsc1 (10 µg/mL), blank NPs, or NUsc1-NPs (33% v/v per well) (n = 4). Amyloid fibril formation was monitored by the increase in Thioflavin T (ThT) fluorescence intensity.

Intranasal administration of NUsc1-NPs:

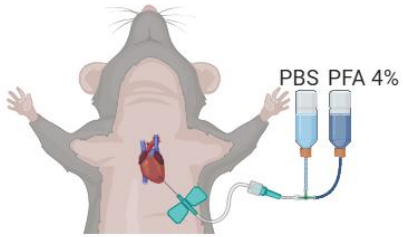
Preliminary *in vivo* study of brain delivery of NUsc1-NPs (WT mice):

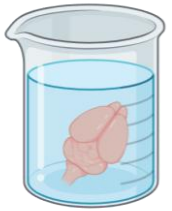
Intranasal administration
Total vol.: 20 µL (10 µL per nostril)
NUsc1 dose: 20 µg

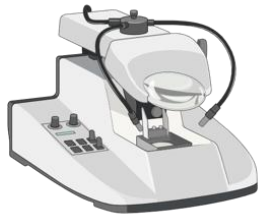
→ Euthanasia/ Perfusion → Brain Extraction → Slicing → Immunofluorescence

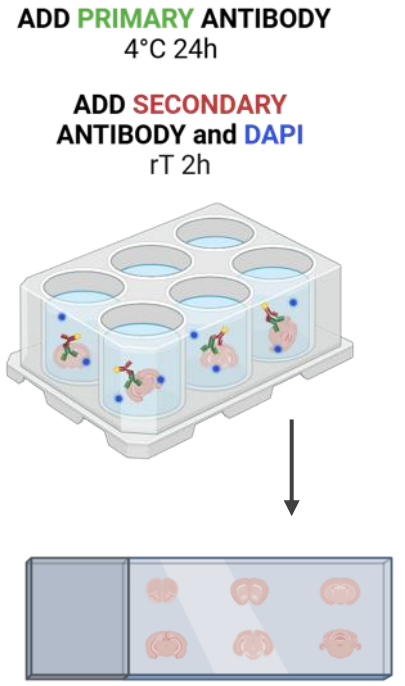


CEUA- FCFRP (nº 25.1.54.60.3)



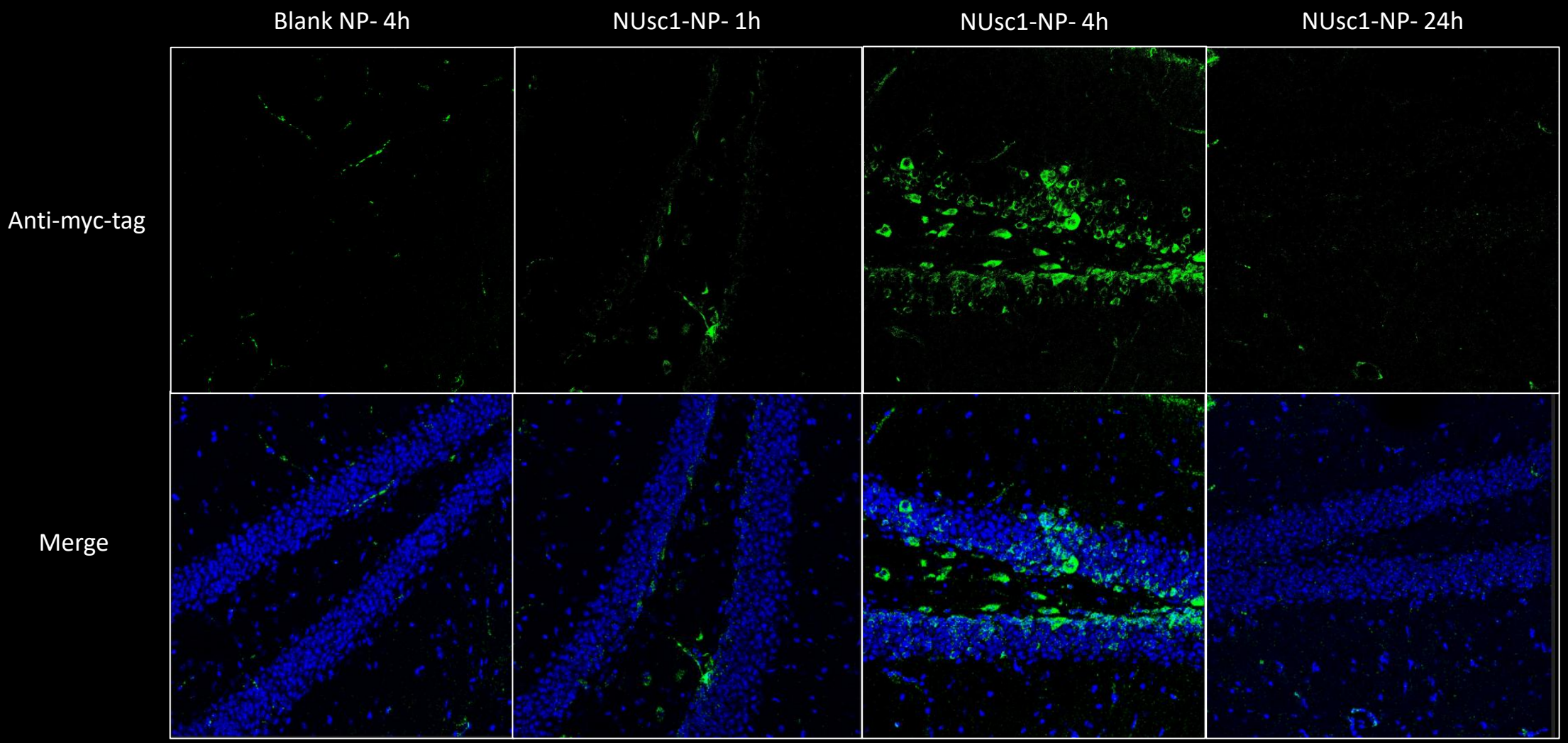




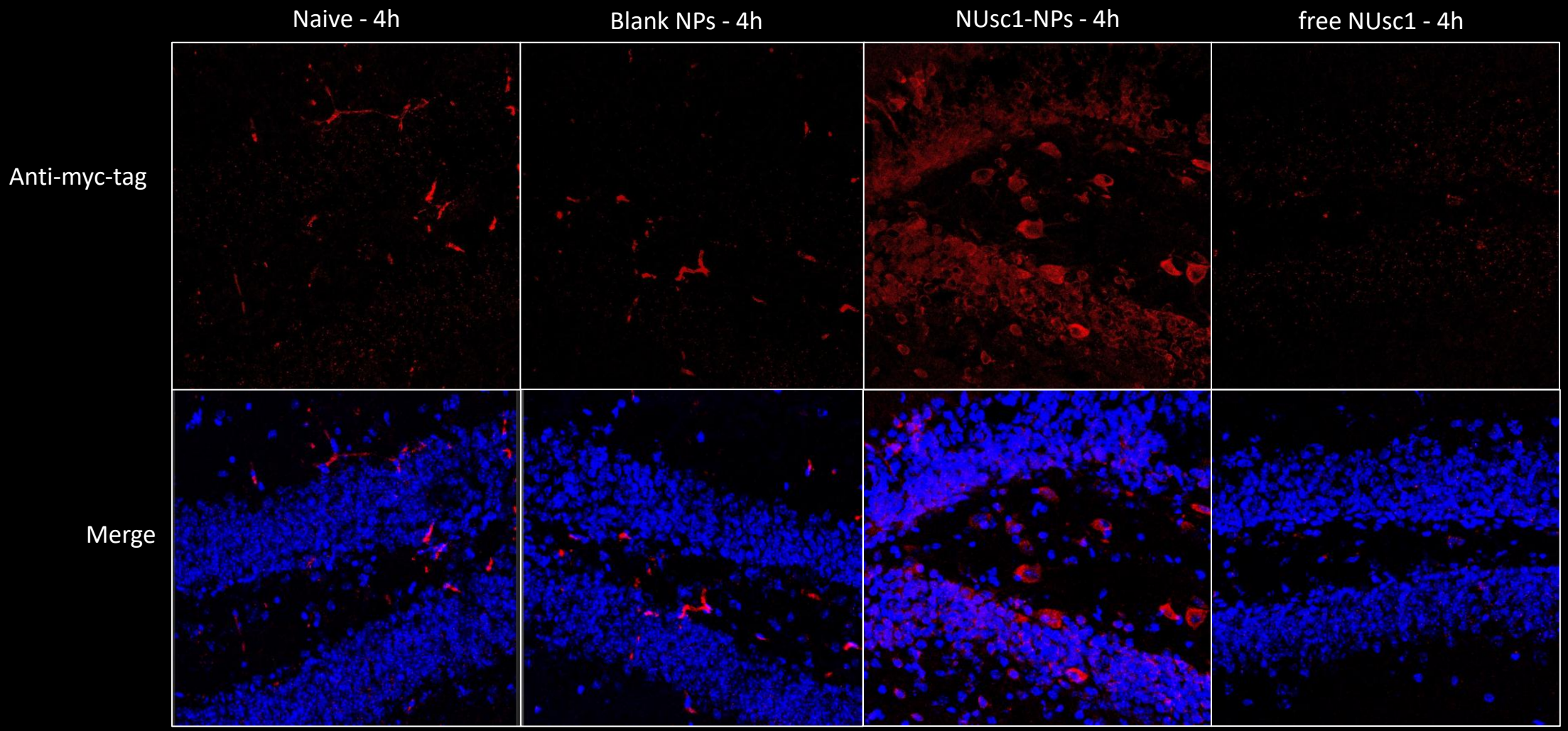


Groups:
Naïve
NUsc1-NPs (1 h, 4 h and 24 h)
Blank NPs (4 h)
free NUsc1 (4 h)

Intranasal Delivery of NUsc1 to the Hippocampus



Intranasal Delivery of NUsc1 to the Hippocampus



Acknowledgments

Prof. Dr. Renata Lopez (Supervisor - FCFRP/USP)
Prof. Dr. Hélder Santos (Supervisor - UMCG/UG)
Prof. Dr. Adriano Sebollela (Collaborator – FMRP/USP)
Prof. Dr. Luciane Alberici (Collaborator – FMRP/USP)
Eduarda Rios (FCFRP/USP)
Dr. Jonathas Santos (FCFRP/USP)
Silvana Chrodrau (FMRP/USP)

Funding Agencies : FAPESP CAPES CNPq

Nanotop

Santos' Lab

SebolleLab

Thank you for your attention.

mariane.carraro@usp.br