Journée annuelle d'échanges entre les partenaires d'Exac-t

Actions et projets soutenus en 2023

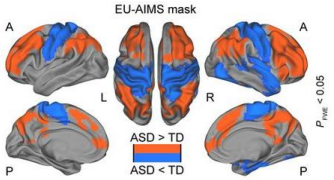
Troubles digestifs dans les TSA : rôle des vésicules extracellulaires dérivées du microbiote intestinal

Dr Martial CAILLAUD

PhD in Neuroscience

Autism Spectrum Disorder (ASD): a neurodevelopmental disease

- Behavioral symptoms: altered social interaction, communication, repetitive behavior, limited interest



- Causes are multifactorial : genetic factors, environmental factors …

- Associated with abnormal cerebral development : anomalies in neuronal connectivity and activity

Lai et al., The Lancet, 2014
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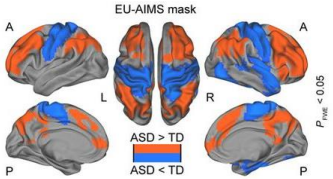

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ASD patients : strong comorbidity with gastrointestinal (GI) symptoms

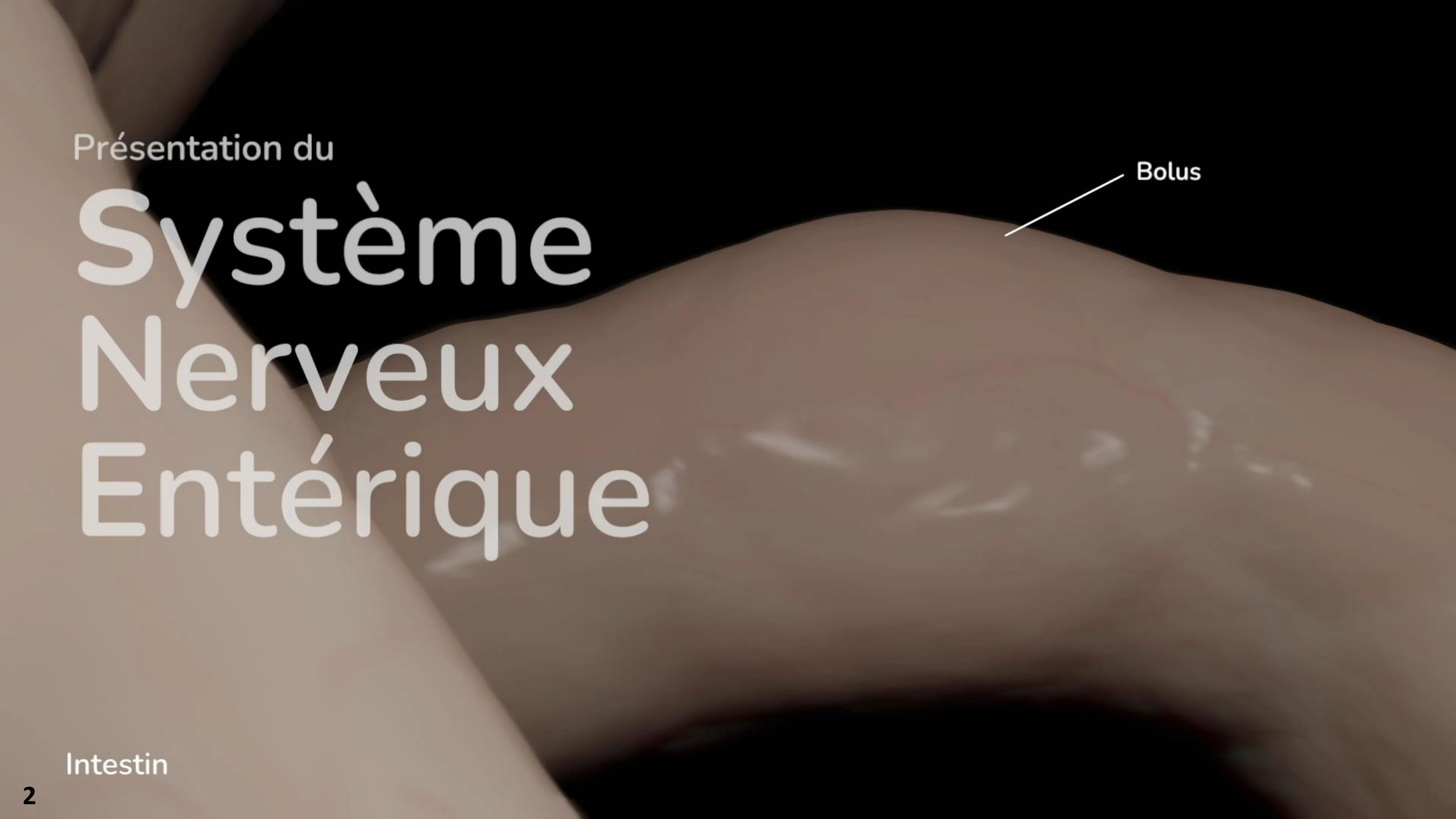
- ~70% of ASD children develop gastrointestinal (GI) symptoms (diarrhea, constipation, bloating, abdominal pain)
- Correlation between severity of behavioral symptoms and GI symptoms
- Alteration of intestinal barrier permeability and intestinal microbiota composition

- Alteration of the enteric neural network in genetic animal models of ASD

Dalton et al. 2014, Autism Res
Hughes et al., 2018 Curr Neurol Neurosci Rep

Présentation du

Système Nerveux Entérique



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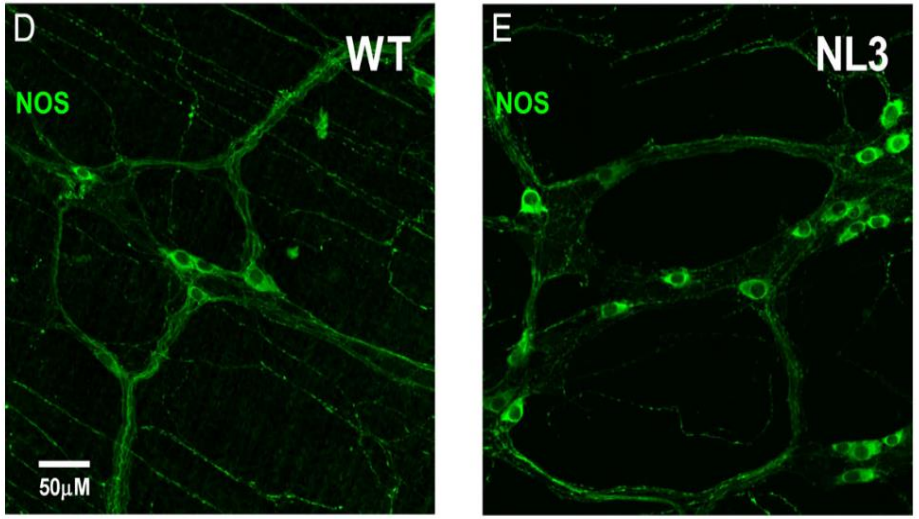
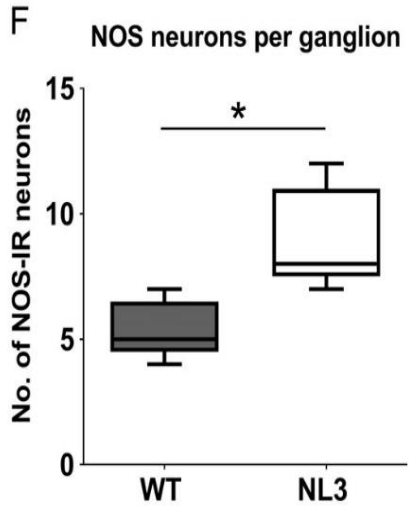
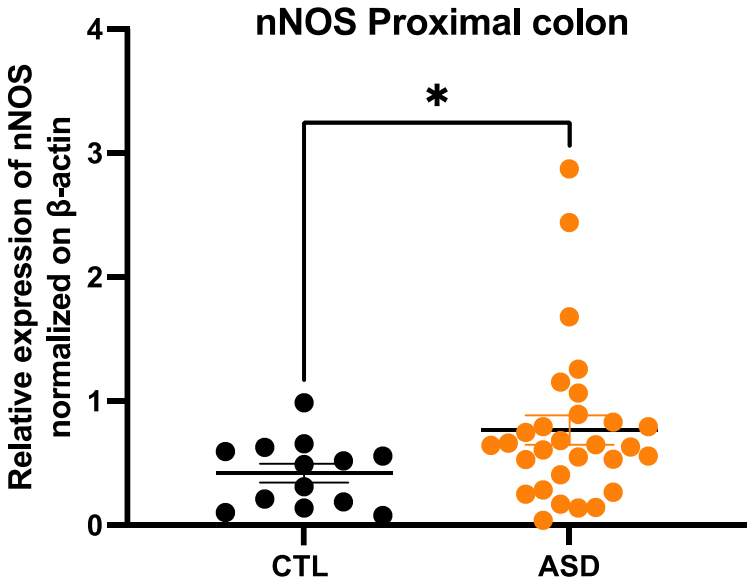
ASD mouse model (Neuroligin -/-)





Hosie et al. 2019, Autism Res.

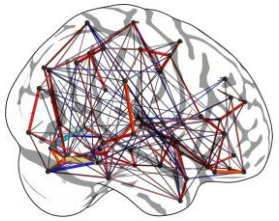


*J. Marchix et al. 2023, **in preparation***

- Fecal microbiota from ASD patient transfer into mice induced GI symptoms and ENS remodeling

Dysfunction of the “microbiota – gut – brain axis”?

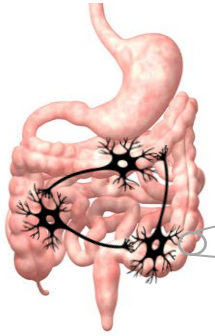
Central nervous system



Behavioral symptoms
Abnormal neuronal connectivity and activity

Mediators?

Mediators:
- Metabolites
- Membrane compounds
- Extracellular vesicles



Enteric nervous system

GI symptoms
ENS remodeling

Gut Microbiota

Alteration of gut microbiota composition

Dysfunction of the “microbiota – gut – brain axis”?

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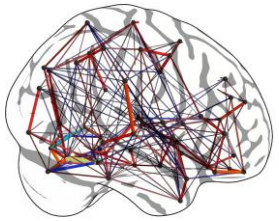

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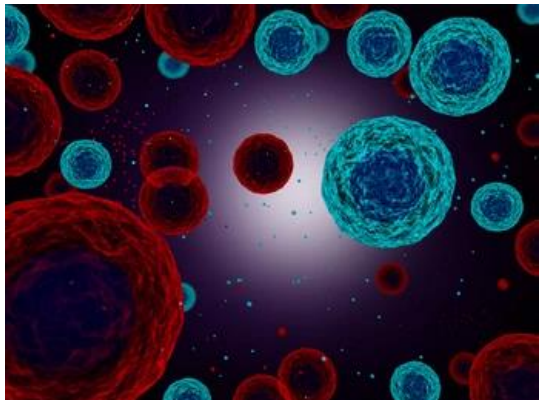
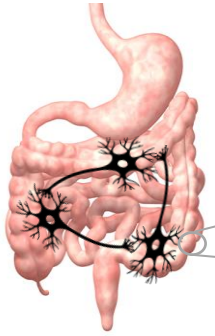
Extracellular Vesicles (EVs)





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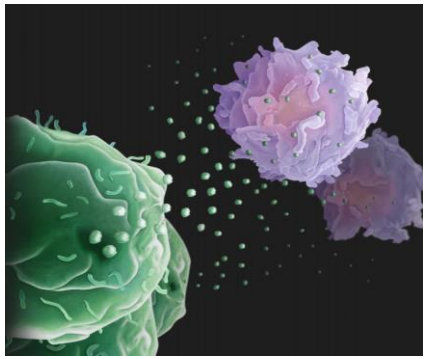
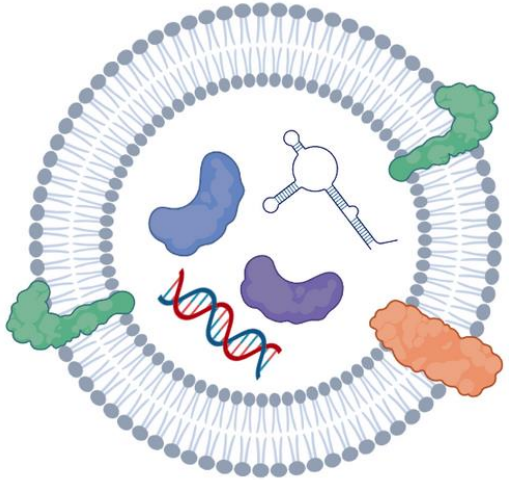
Mediators : Extracellular vesicles (EVs)

Eukaryotic cell

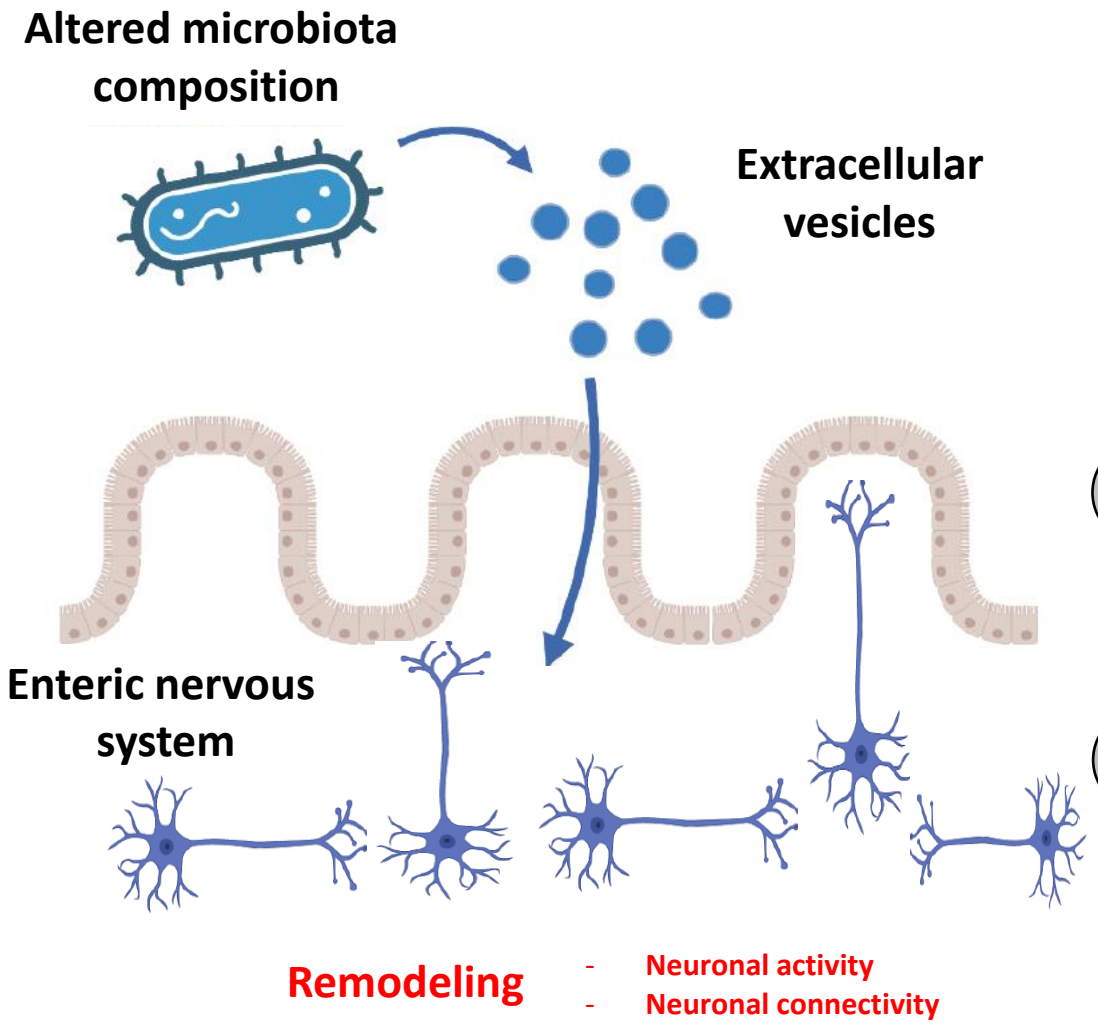


Prokaryotic cell

- **PRODUCTION**: By all cell types, including bacteria
- **SIGNALING CARGO** : Carry signaling molecules (proteins, nucleic acids, lipids)
- **PROTECTION**: Encapsulation of molecules, thus protection from enzymatic degradation
- **COMMUNICATION** : Participates in intercellular and interorgan communication
- **PATHOLOGICAL** : Release of EVs linked to neurological pathologies, including autism



Hypothesis and Objectives



Working hypothesis:

Extracellular vesicles from altered intestinal microbiota could induce structural and/or functional remodeling of the ENS in autism

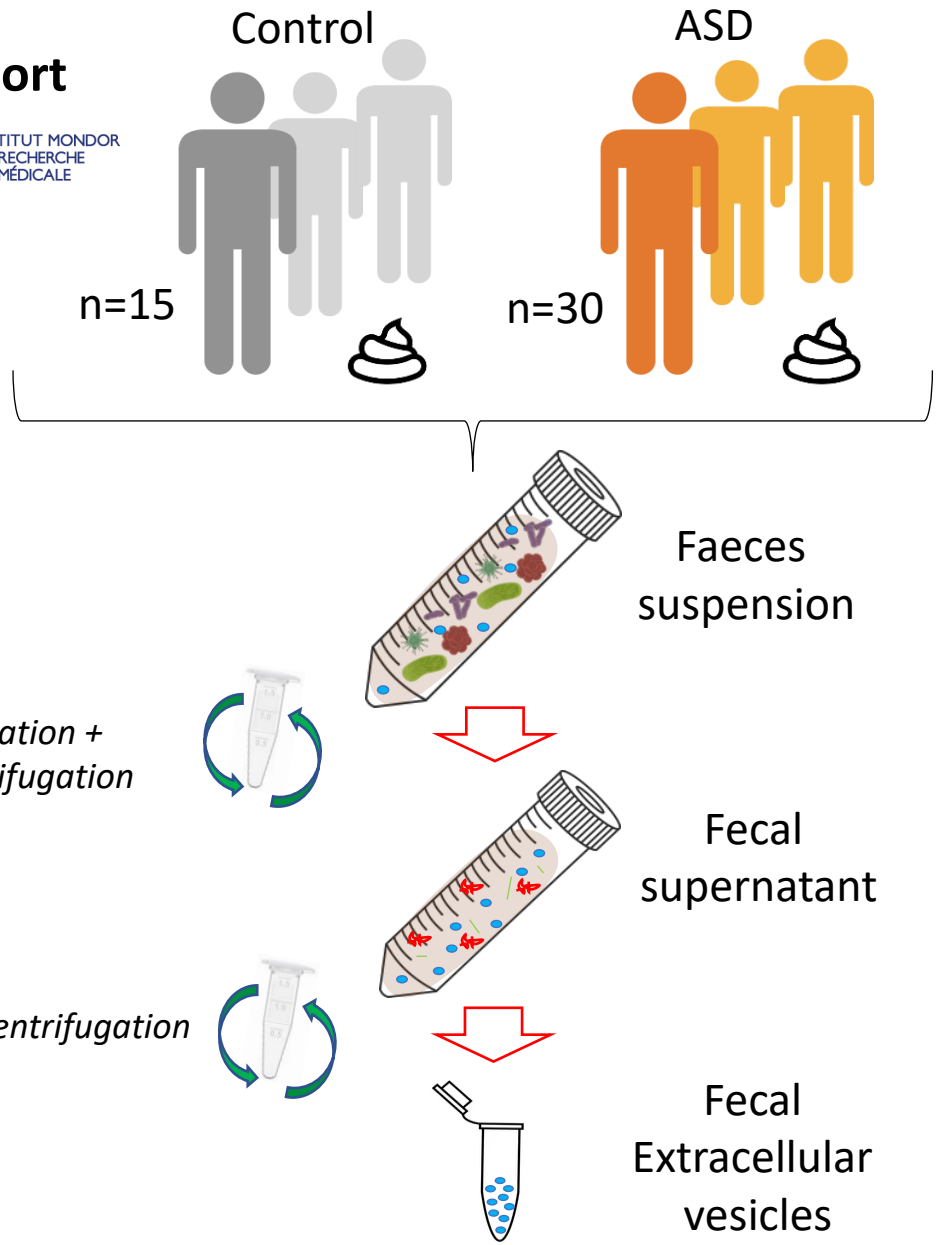
Objectives

1. **Extraction and characterization of fecal extracellular vesicles**
2. **Assessing the effect of extracellular vesicles on enteric neuronal activity and connectivity**

Isolation and characterization of fecal EVs from controls and ASD patients

Cohort

INSTITUT MONDOR DE RECHERCHE BIOMÉDICALE



Isolation and characterization of fecal EVs from controls and ASD patients

Cohort

INSTITUT MONDOR DE RECHERCHE BIOMÉDICALE

Control

ASD

n=15

n=30

Faeces suspension

Filtration + Centrifugation

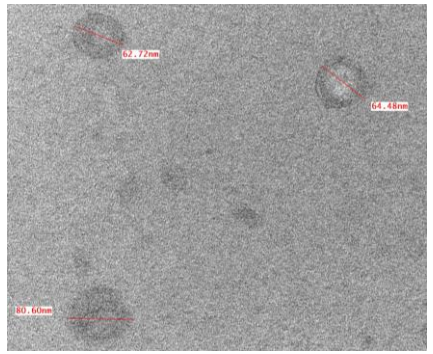
Fecal supernatant

Ultracentrifugation

Fecal Extracellular vesicles

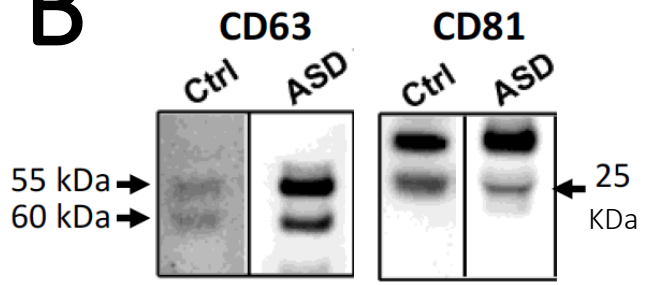
Characterization

A



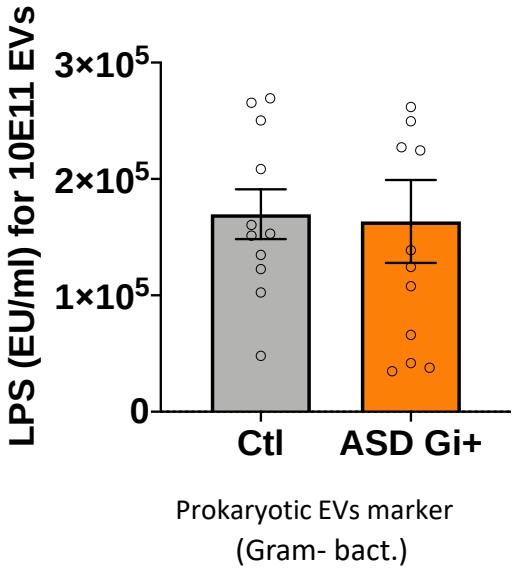
B

Eukaryotic EVs marker



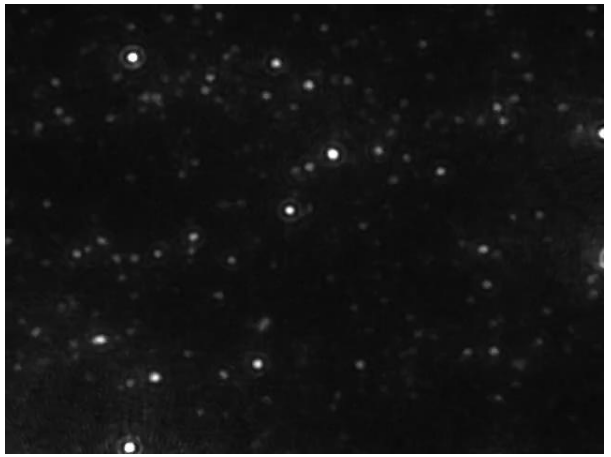
C

LPS in faecal EVs



Prokaryotic EVs marker
(Gram- bact.)

Nanoparticle Tracking Analysis



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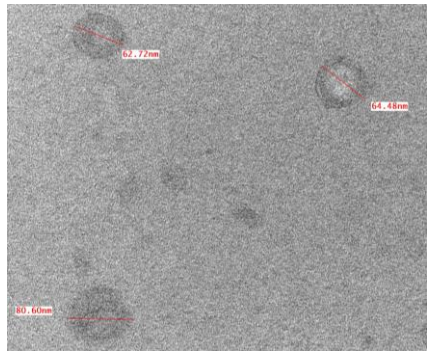

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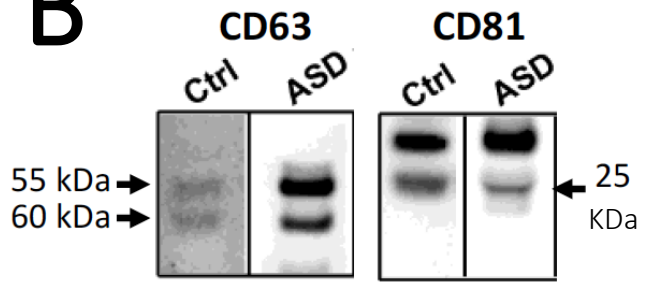

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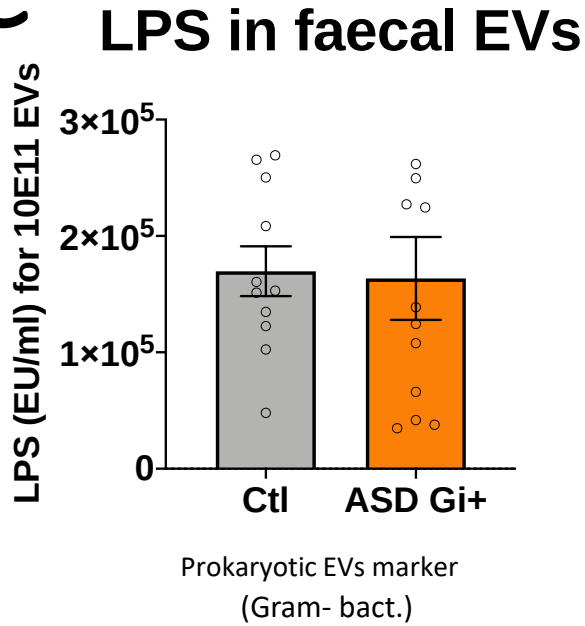

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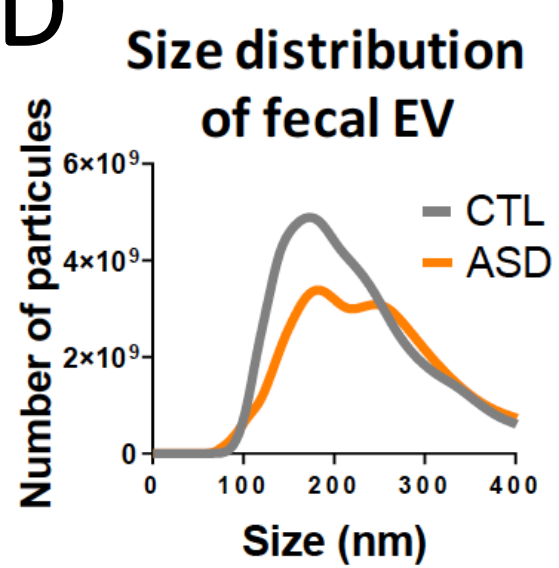
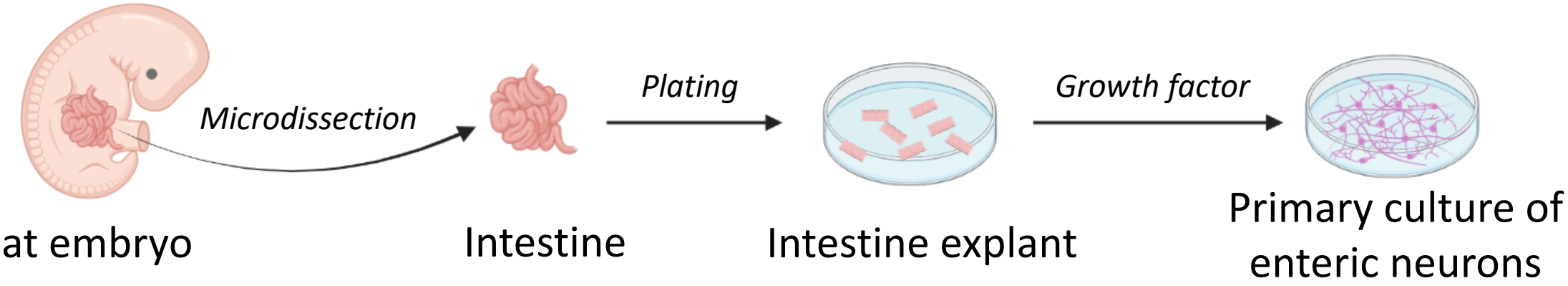


Evaluation of the effects of fEVs on enteric neuronal activity and connectivity



Caillaud et al. 2022, Frontiers in Neuroscience

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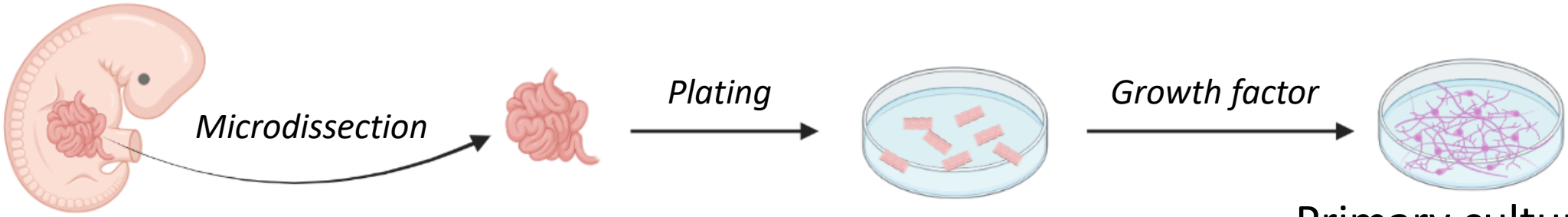

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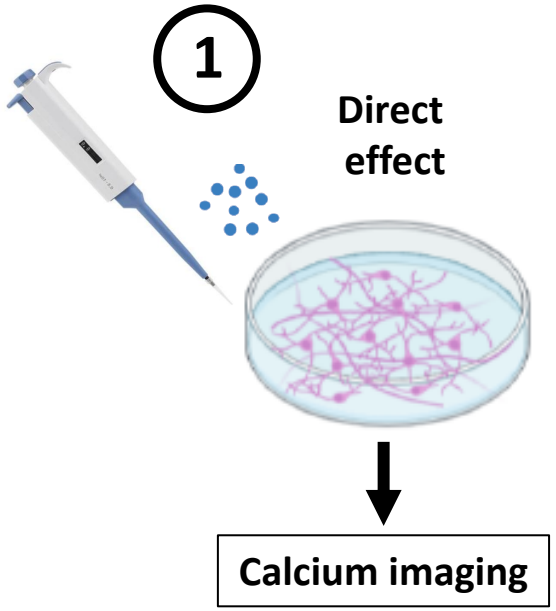
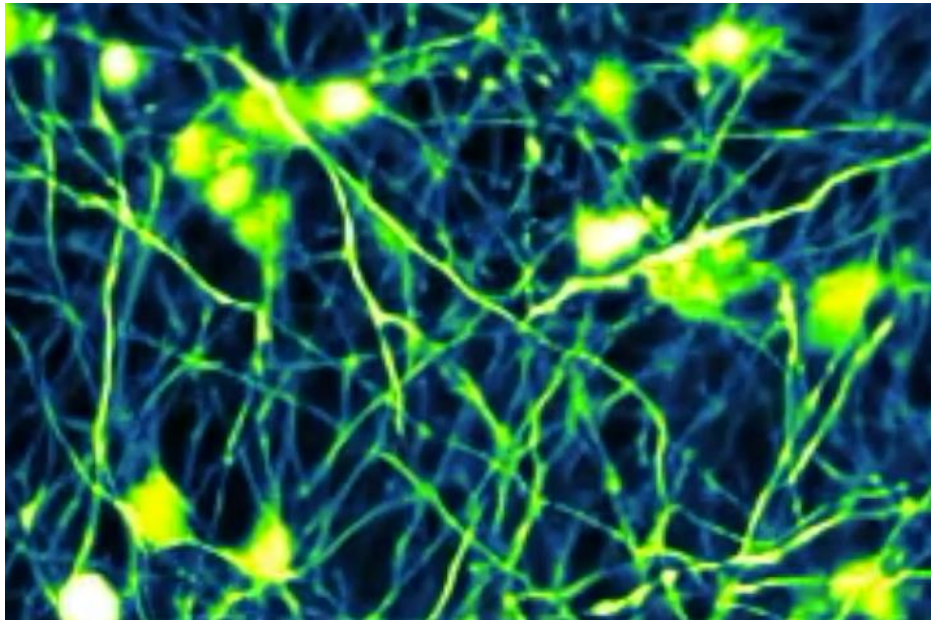


Calcium imaging



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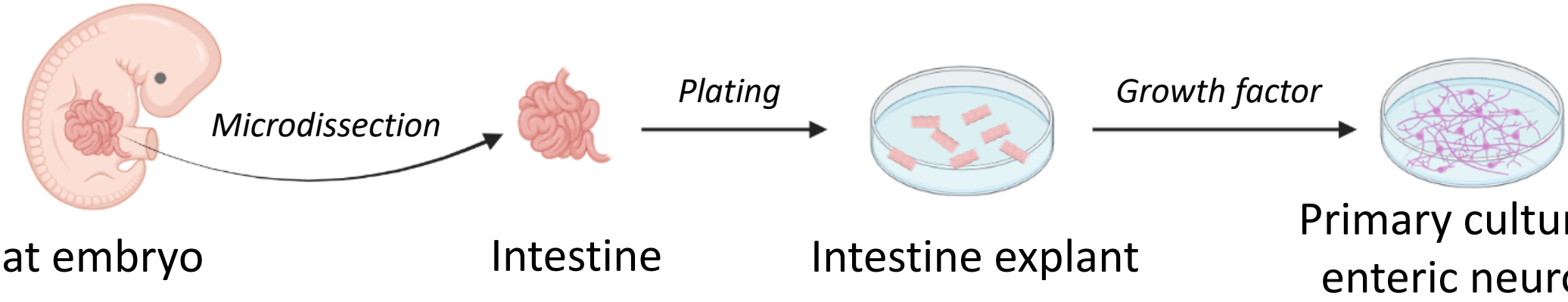

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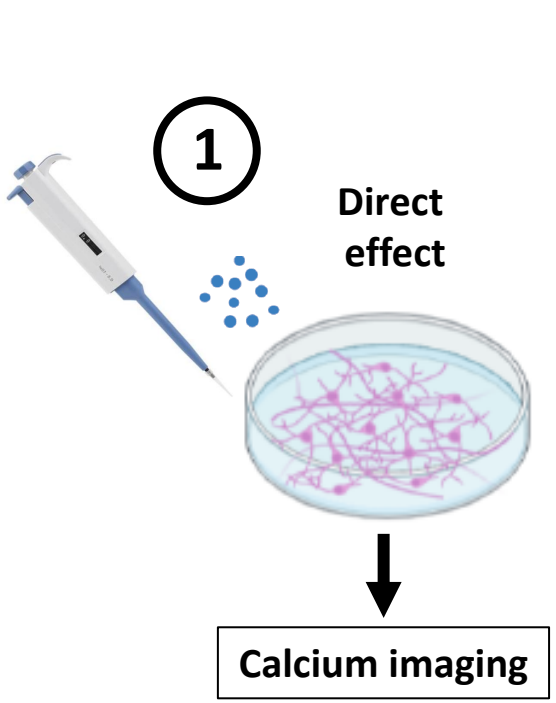

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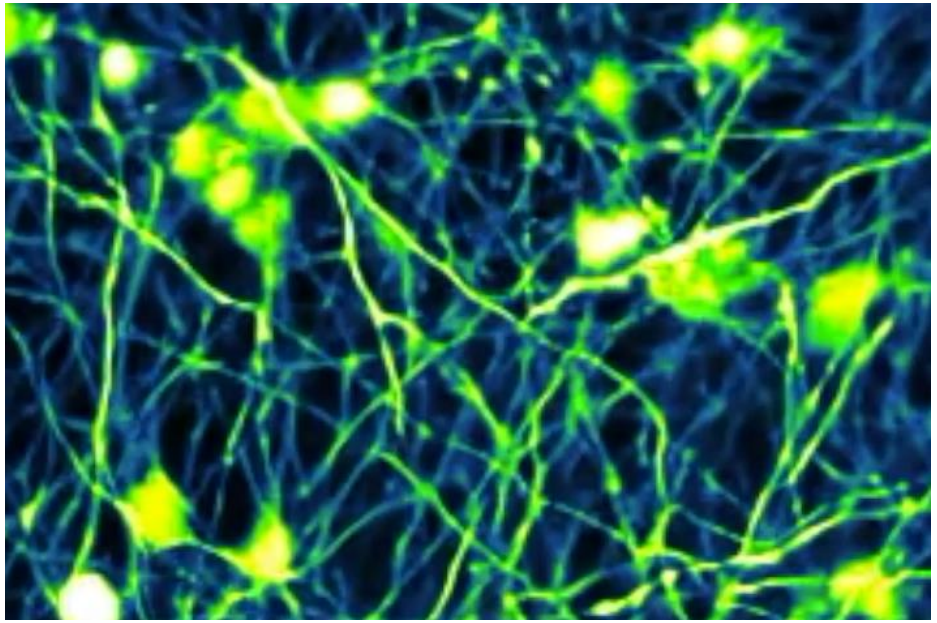
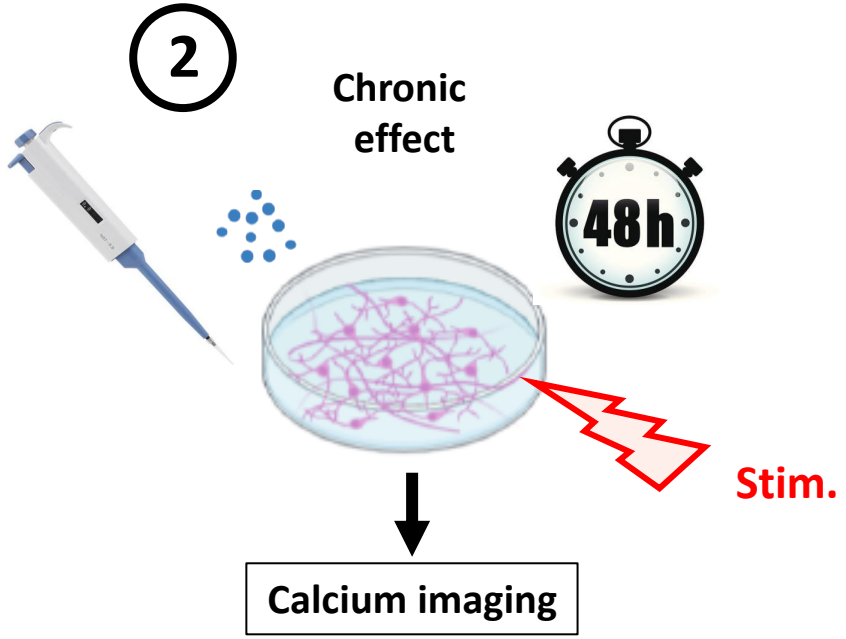
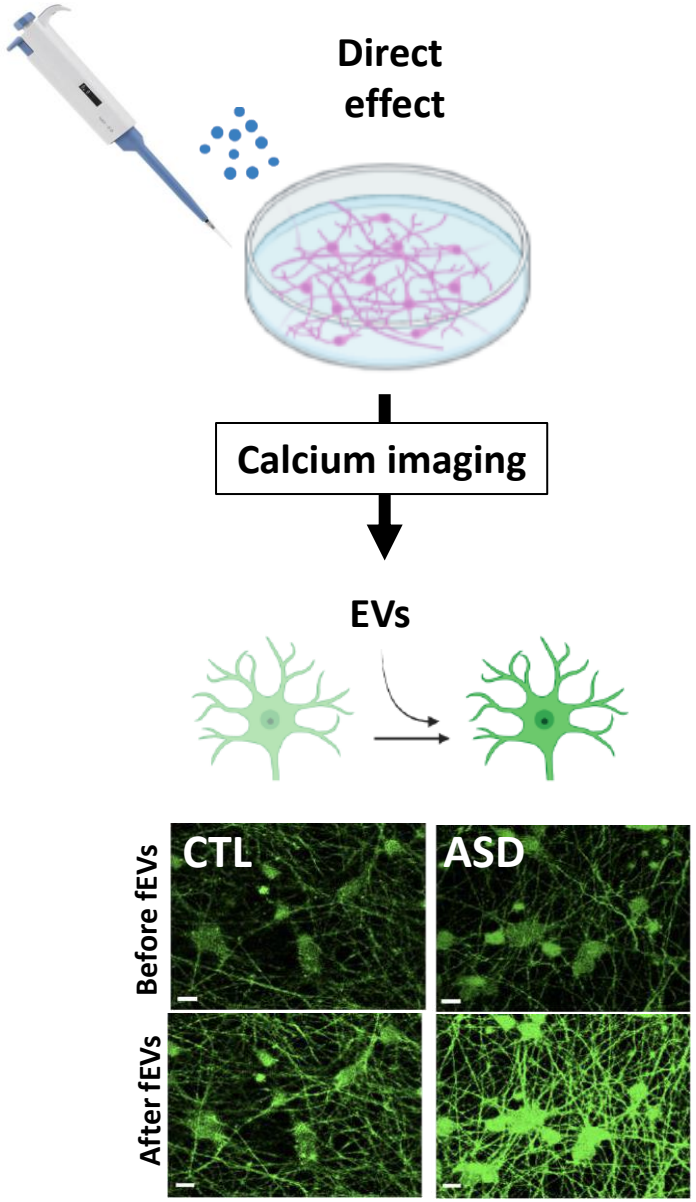




fEVs from people with ASD induce changes in neuronal activity



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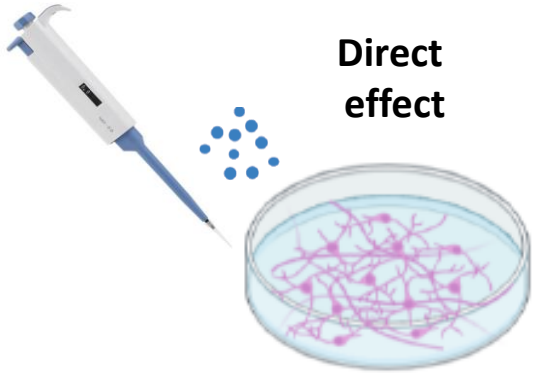

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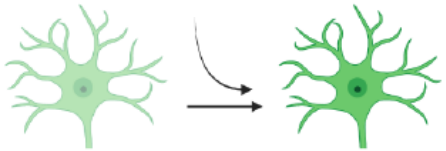
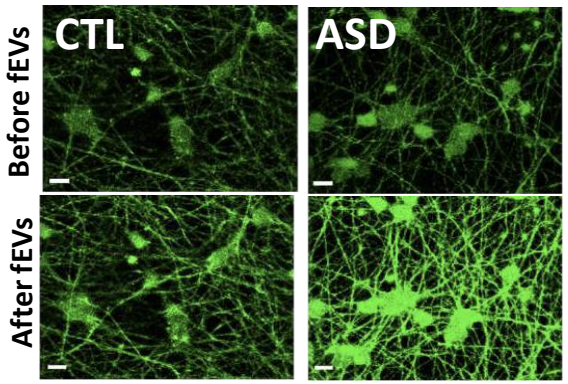
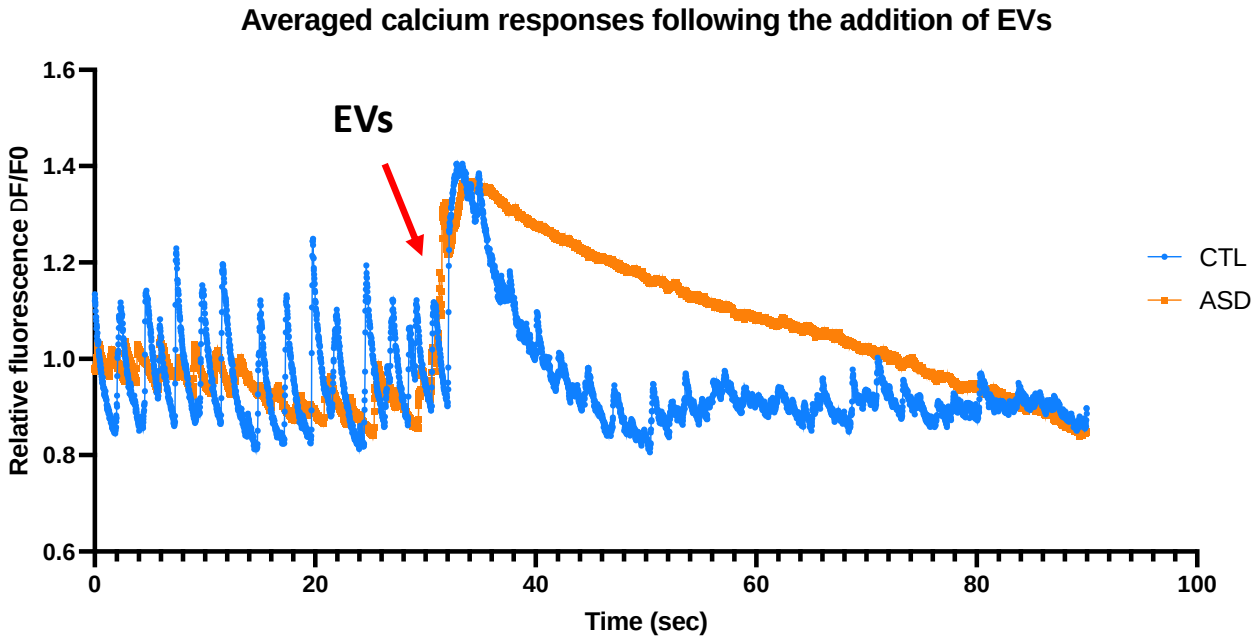
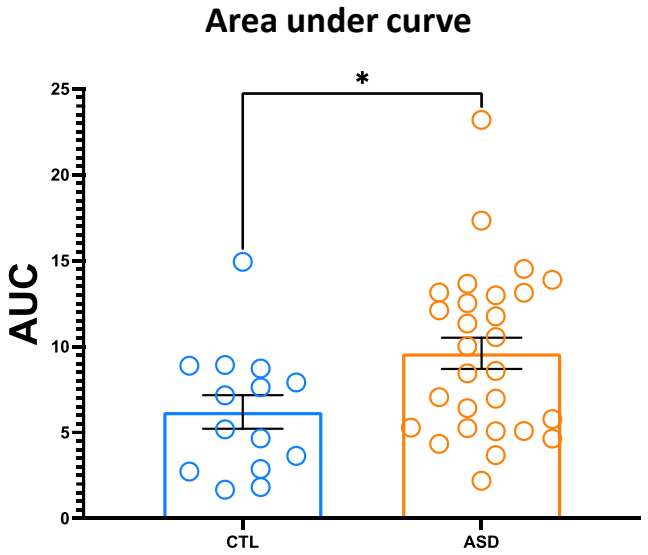
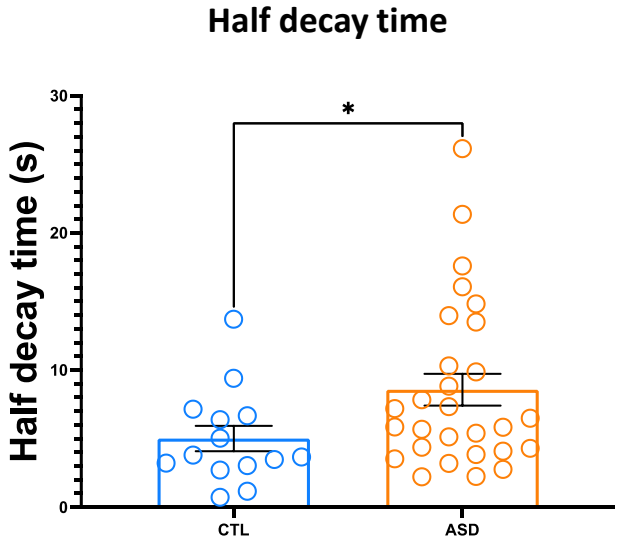


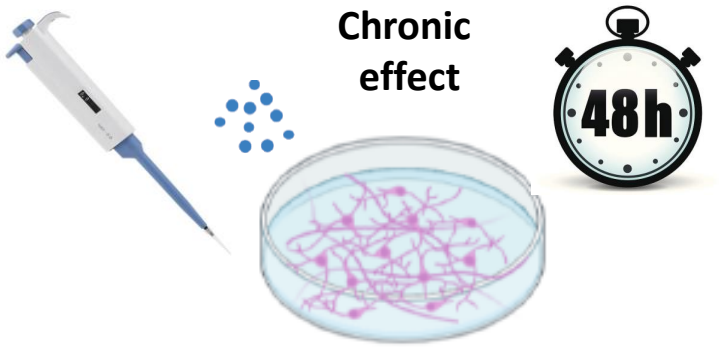






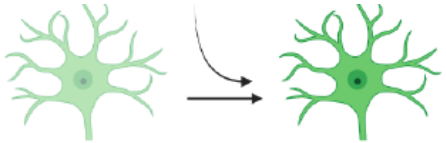


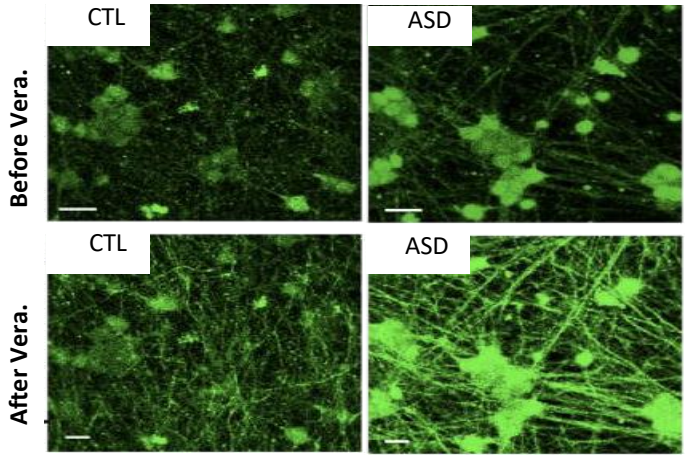
EVs from ASD patients induce a change in the Ca2+ response of enteric neurons following pharmacological stimulation



Calcium imaging

Veratridine *(opening of voltage gated Na+ channels)*





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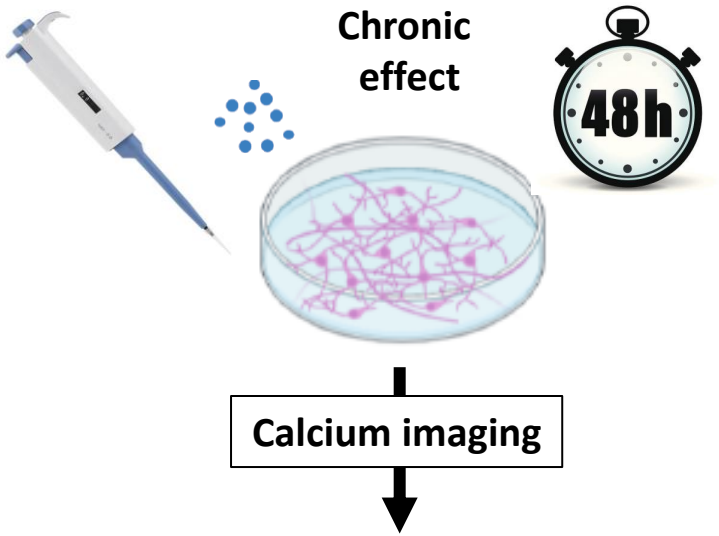

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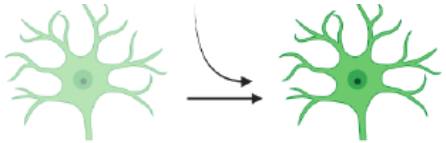
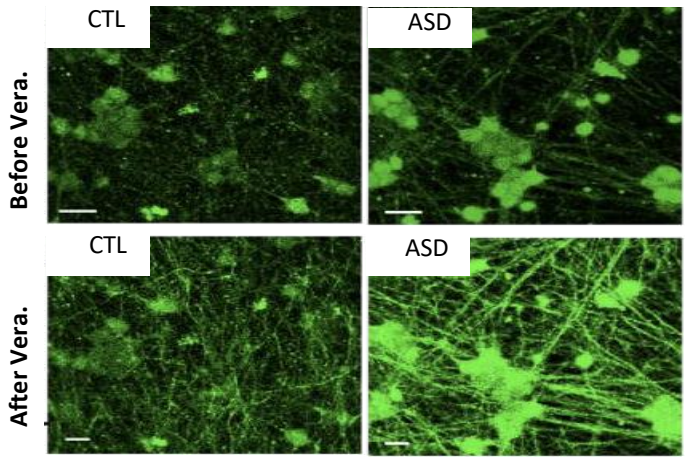
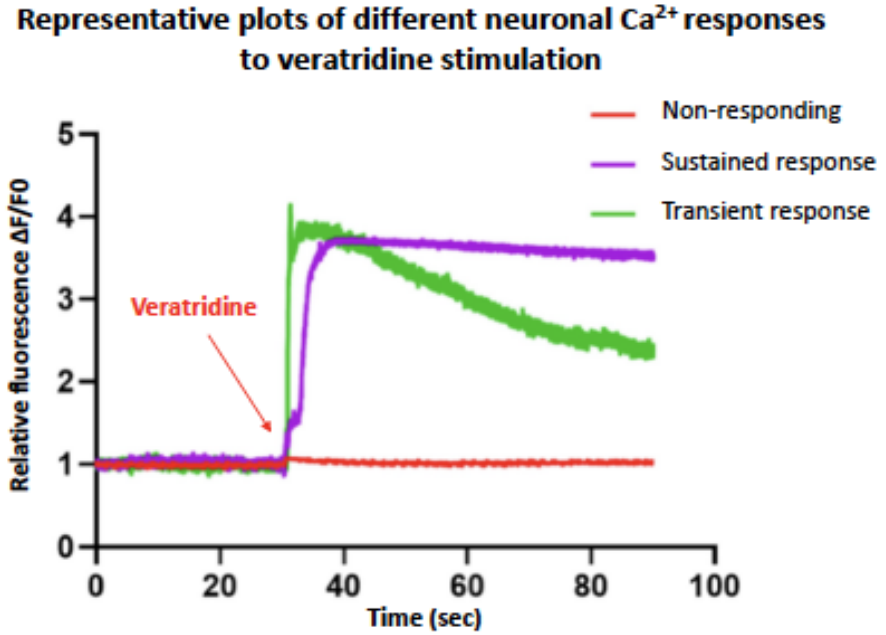
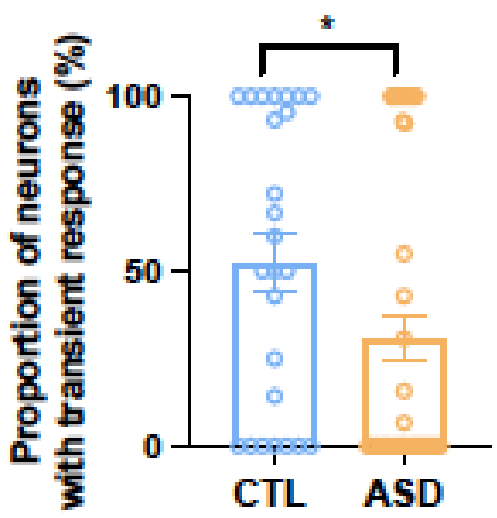
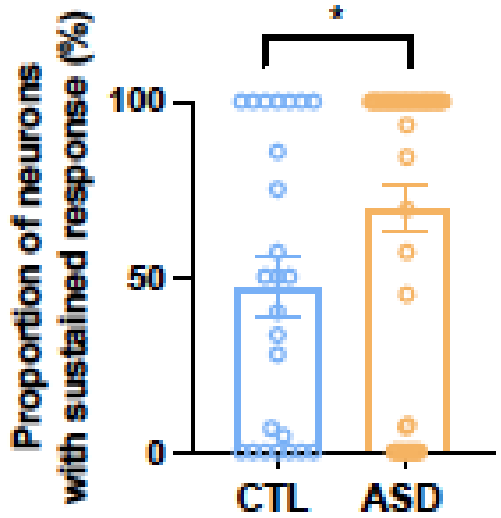


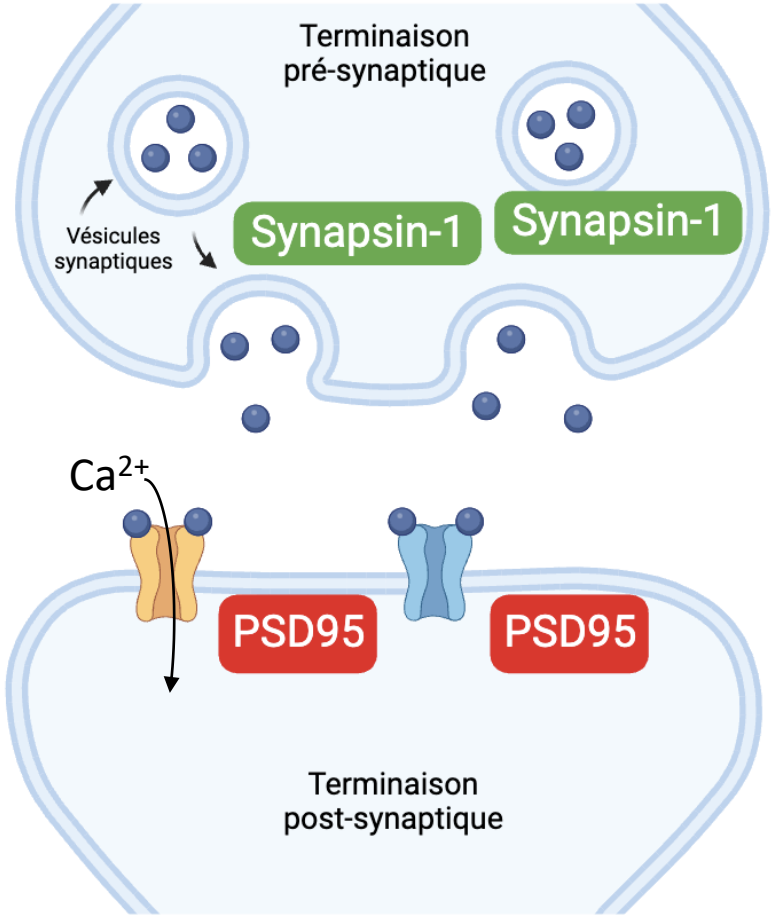








fEVs from people with ASD increase the number of synapses PSD95



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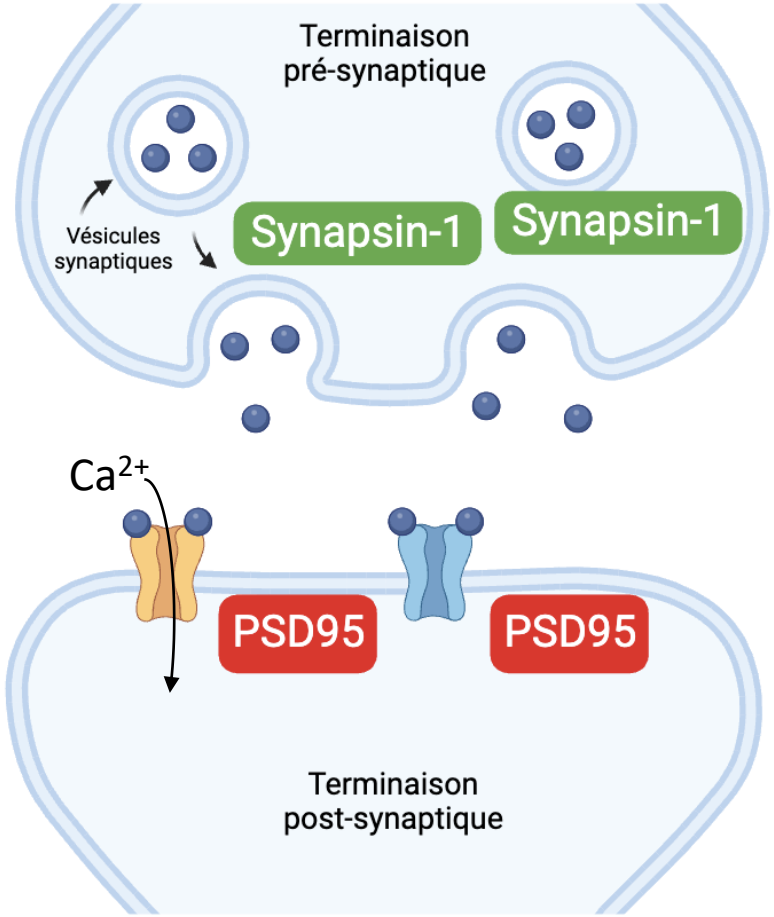
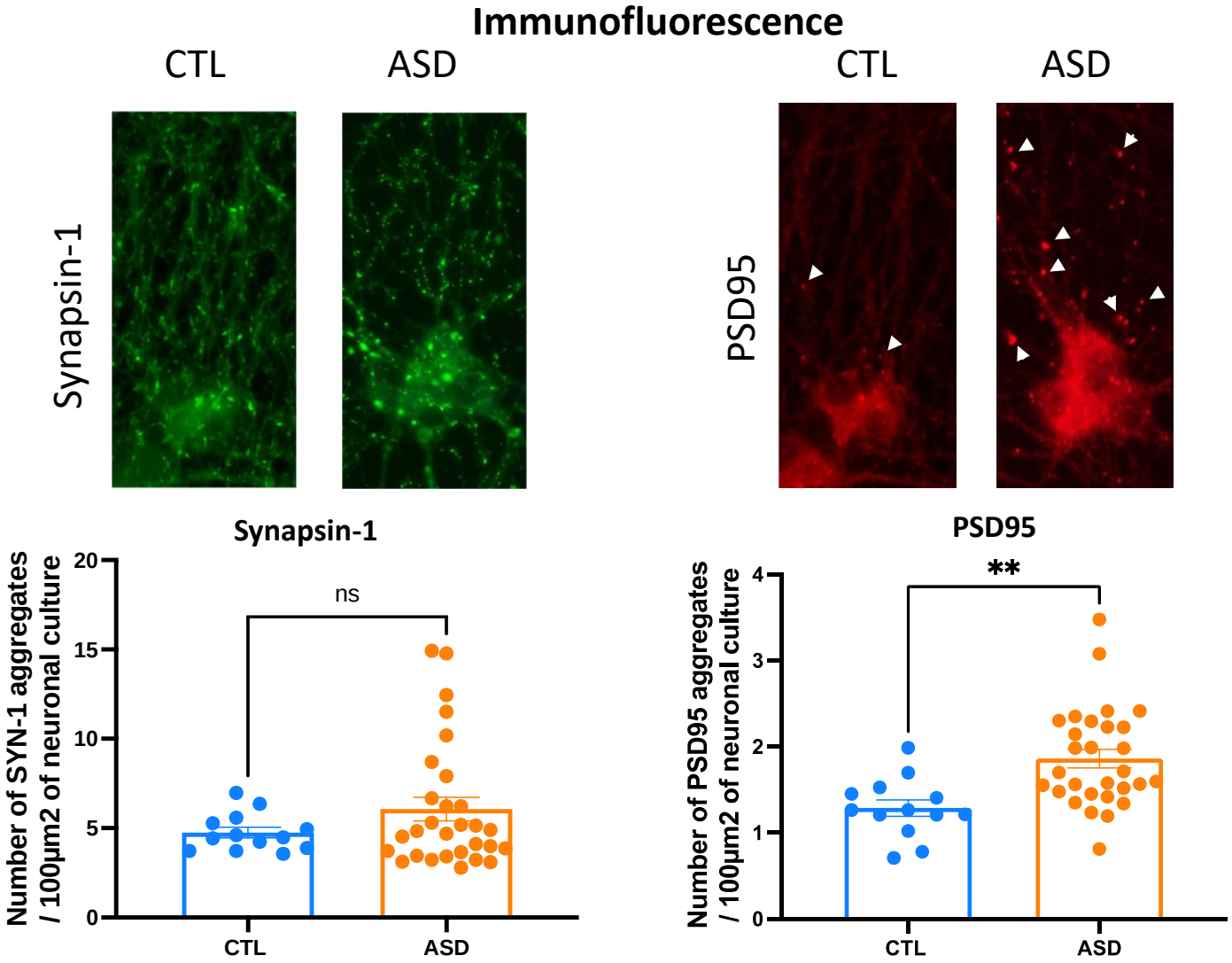
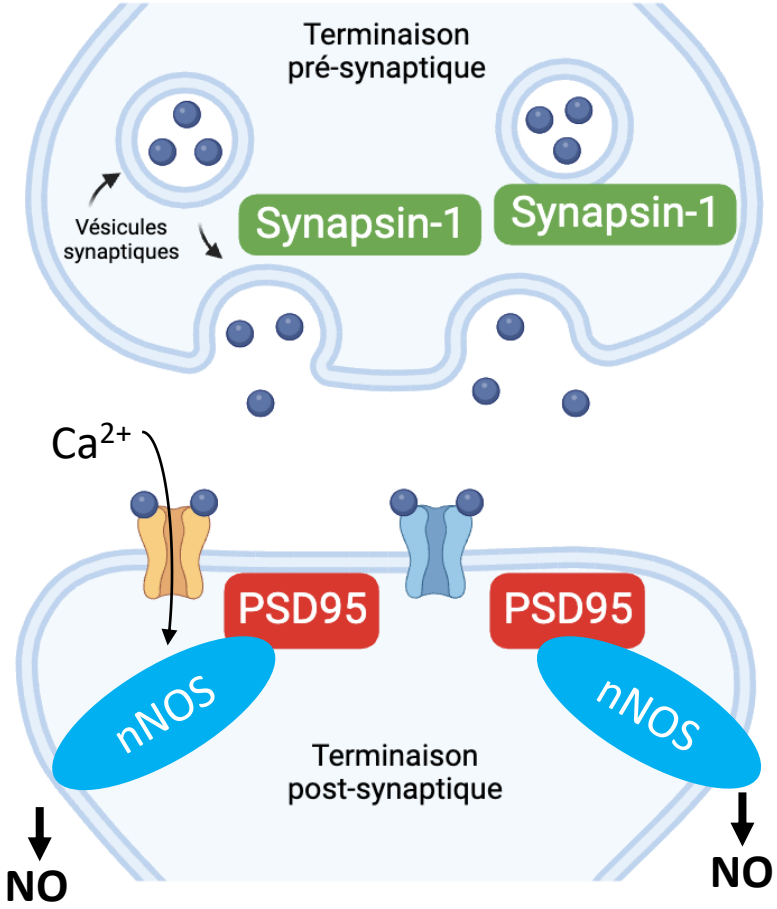


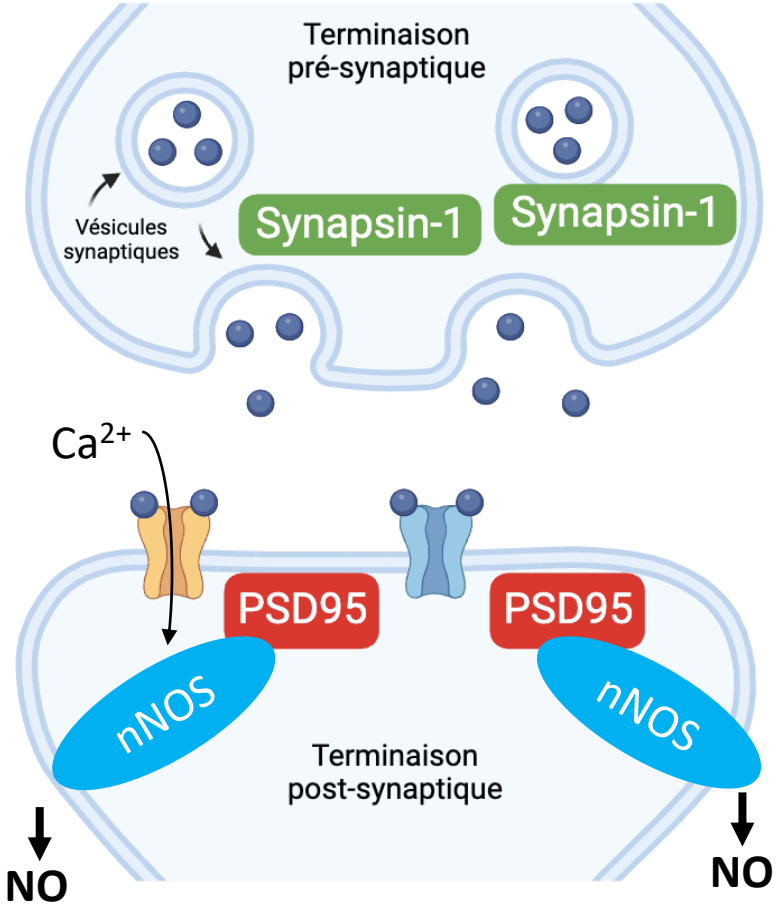


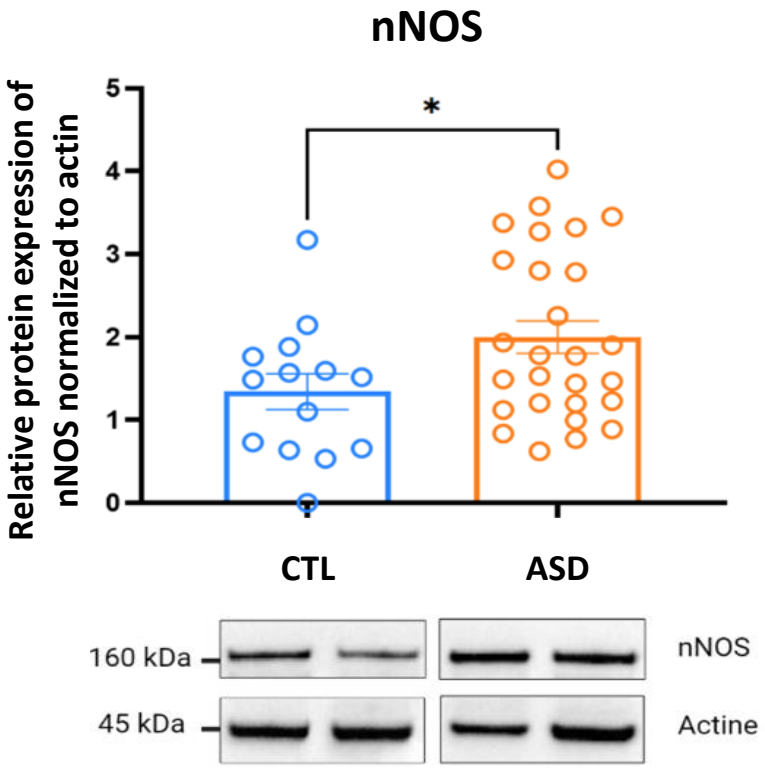
➢ **Increased PSD95 aggregates in ASD patients**

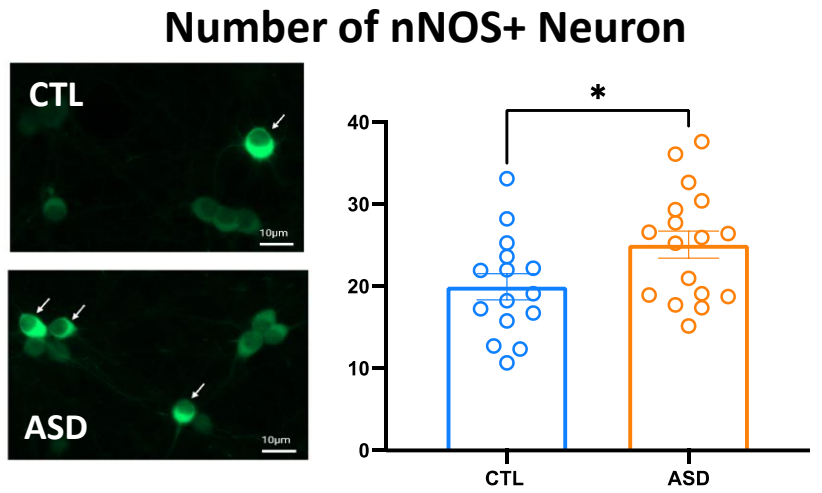
fEVs from people with ASD induce a remodeling of the nitregic pathway

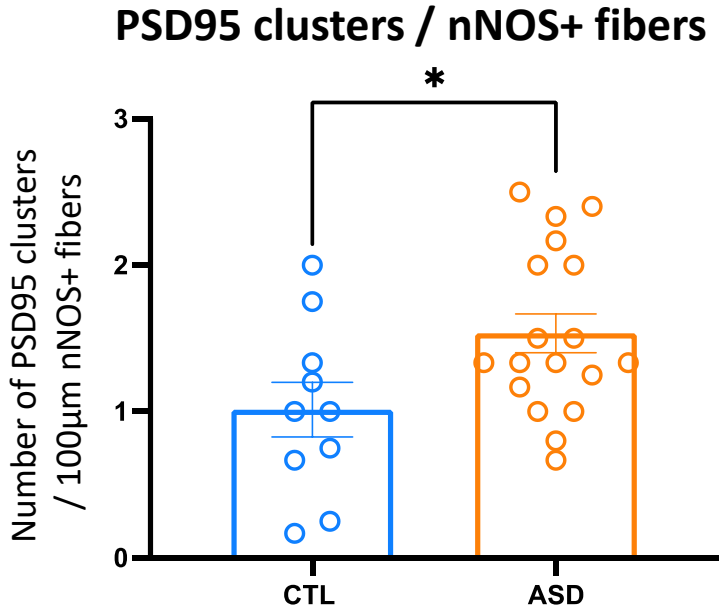


fEVs from people with ASD induce a remodeling of the nitregic pathway

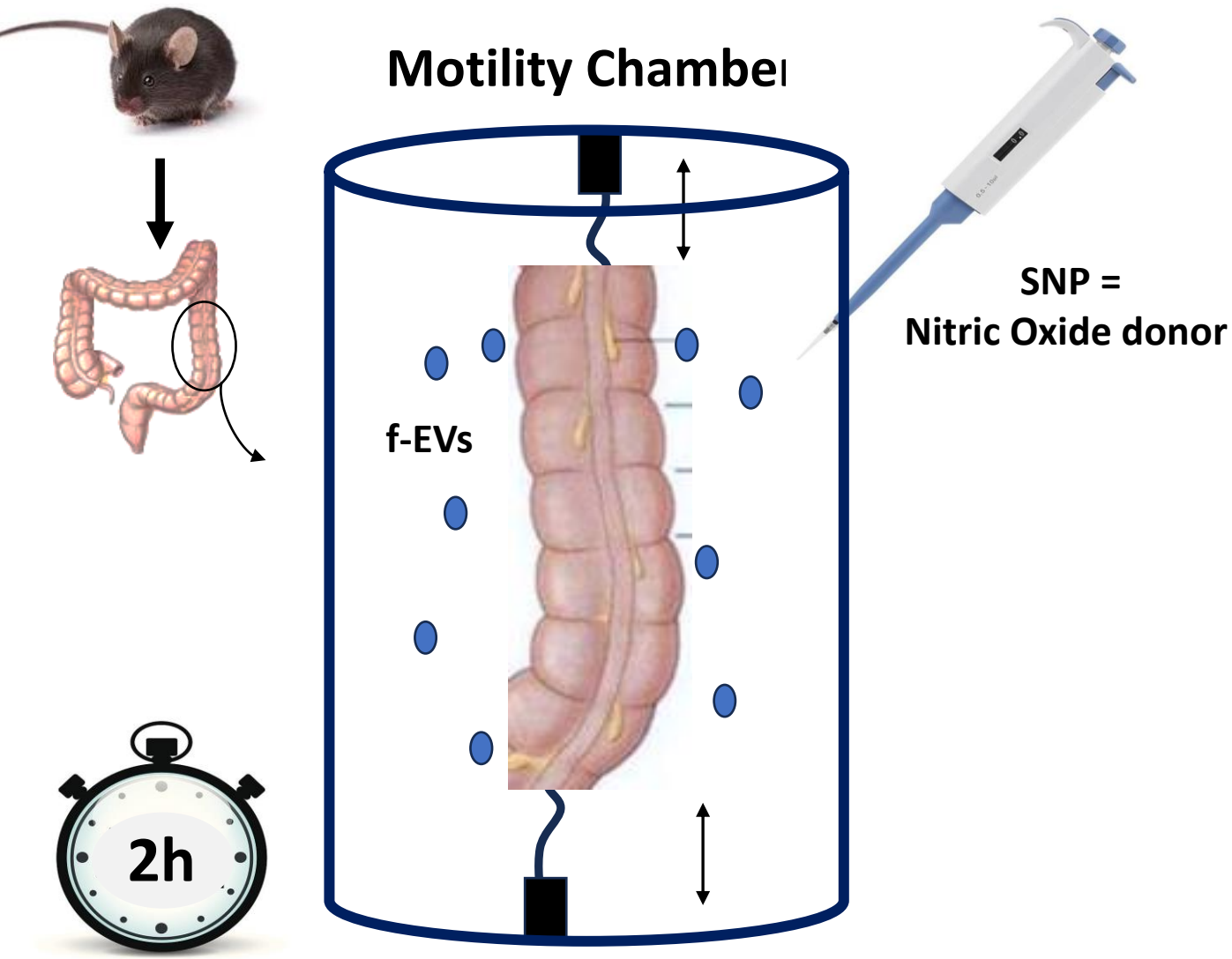




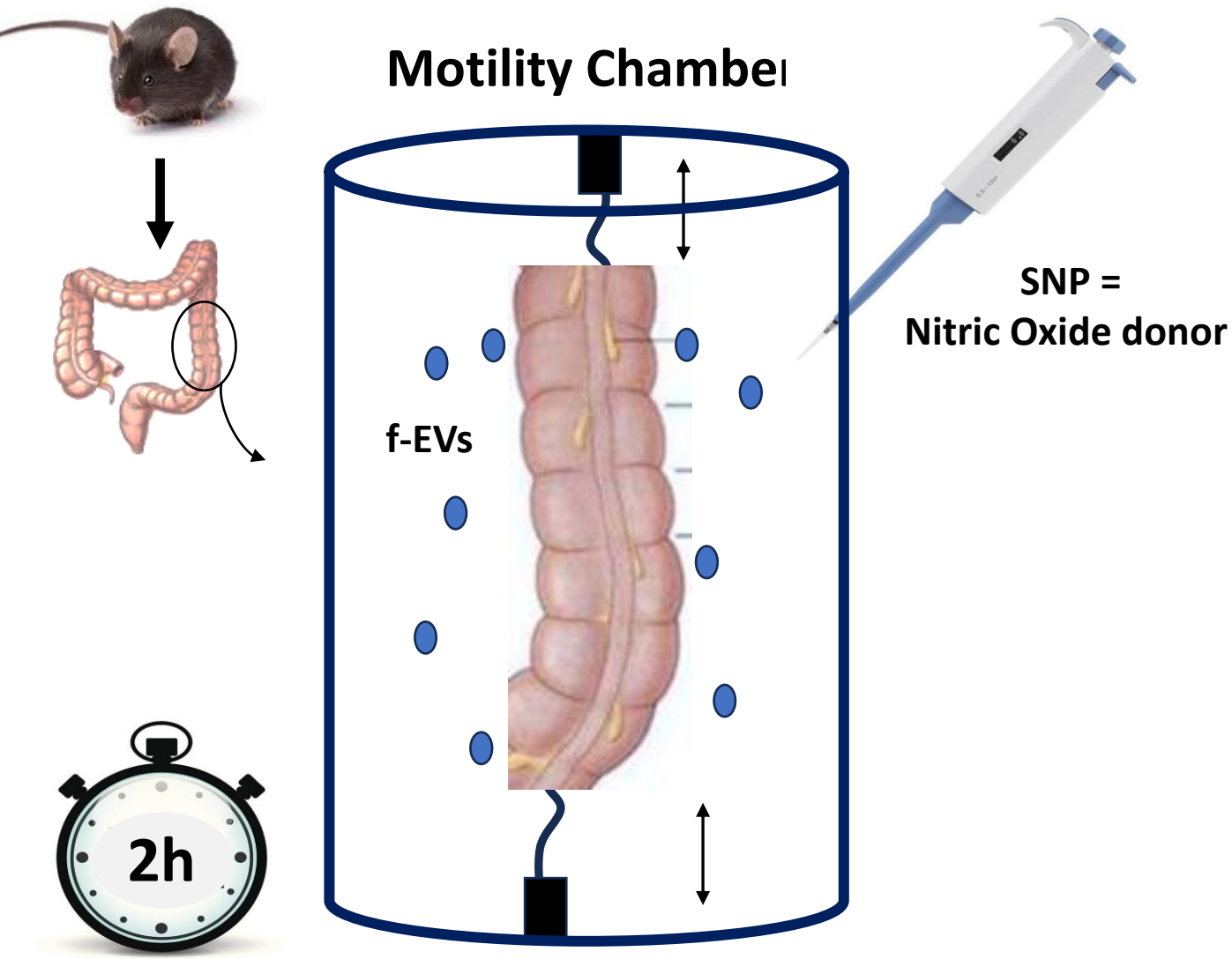


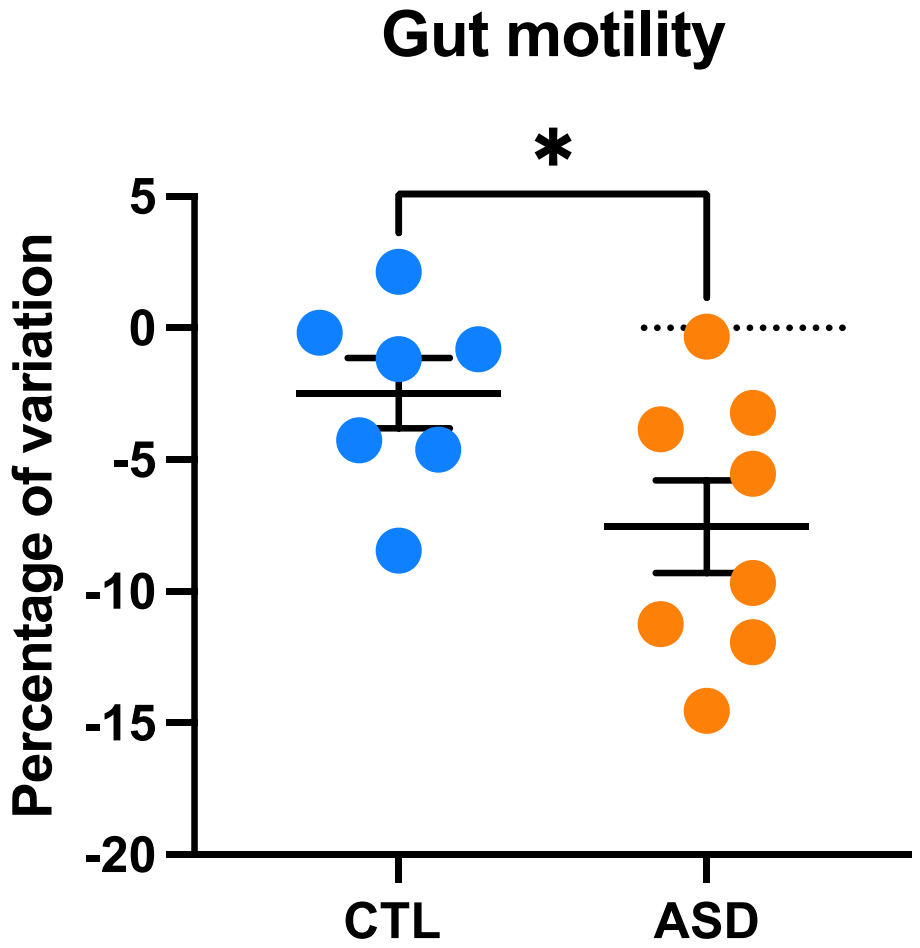


fEVs from people with ASD induce a change in gut motility



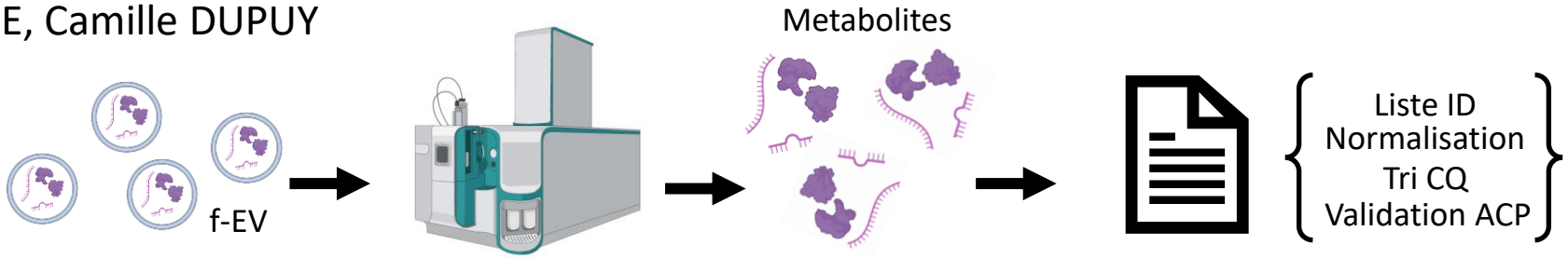
fEVs from people with ASD induce a change in gut motility





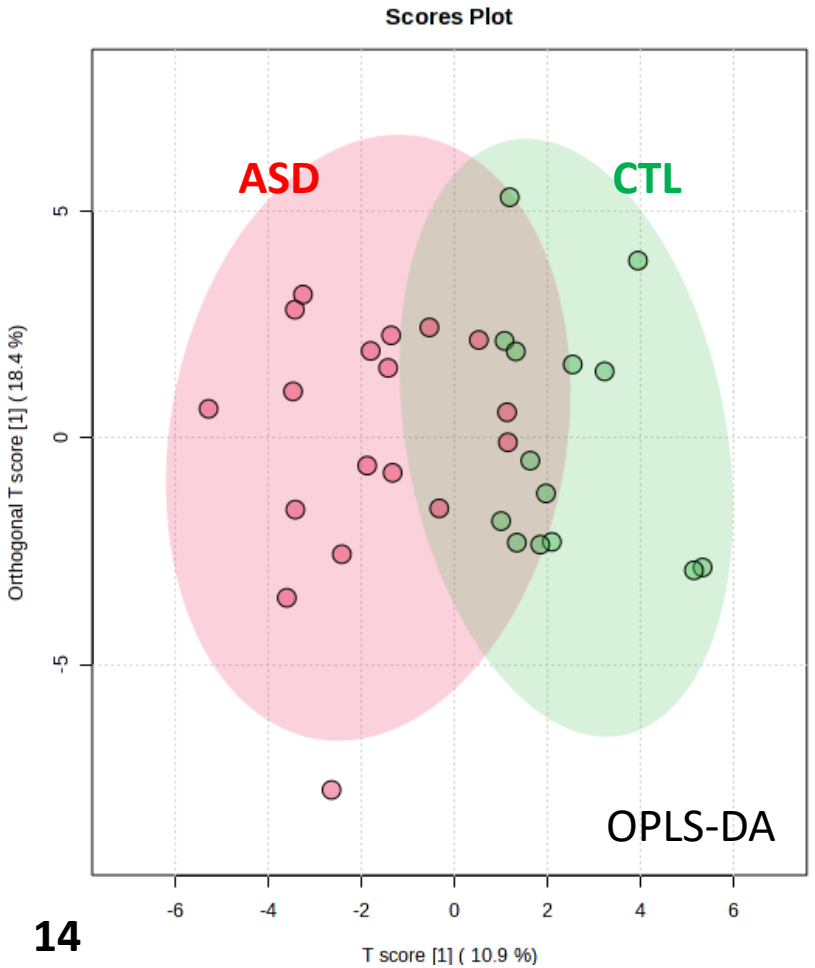
Metabolomic analysis of f-EV

Patrick EMOND, Antoine LEFEVRE, Camille DUPUY



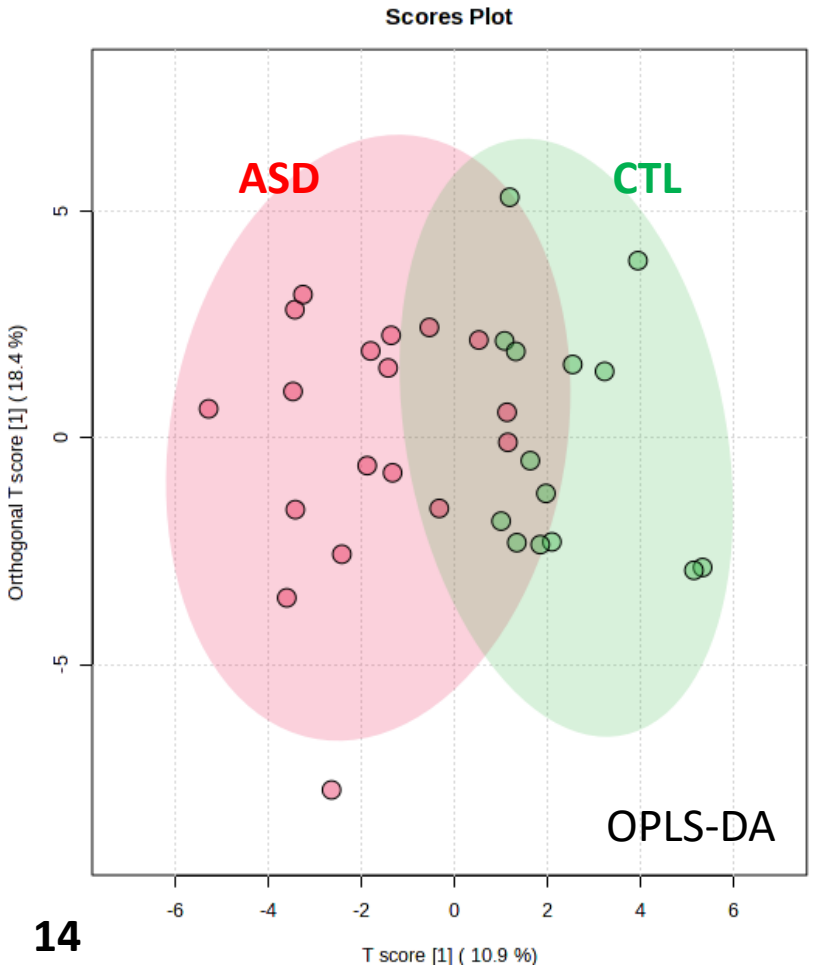
Metabolomic analysis of f-EV

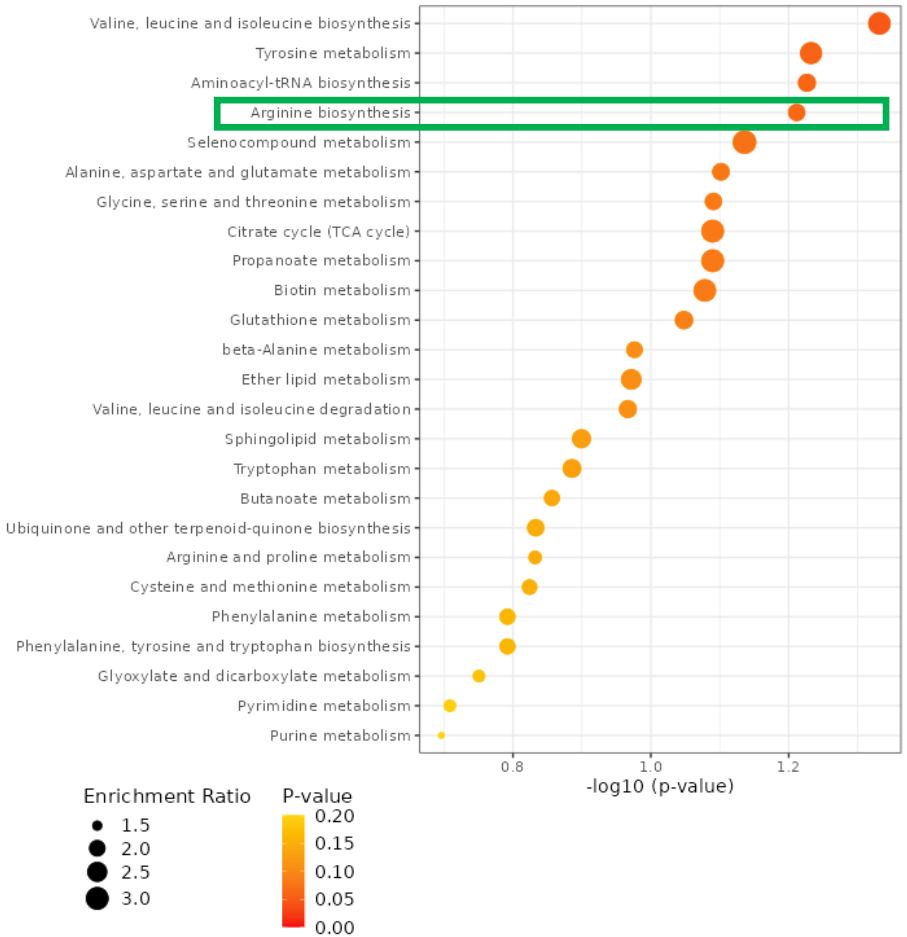
Patrick EMOND, Antoine LEFEVRE, Camille DUPUY



Metabolomic analysis of f-EV

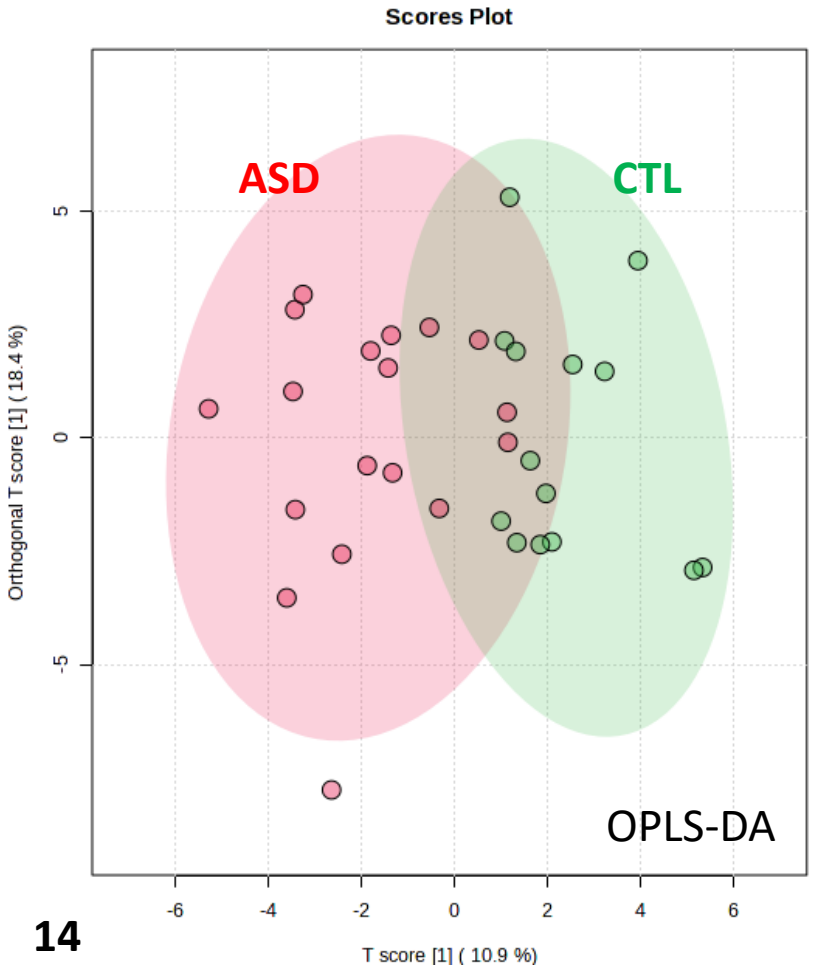
Patrick EMOND, Antoine LEFEVRE, Camille DUPUY

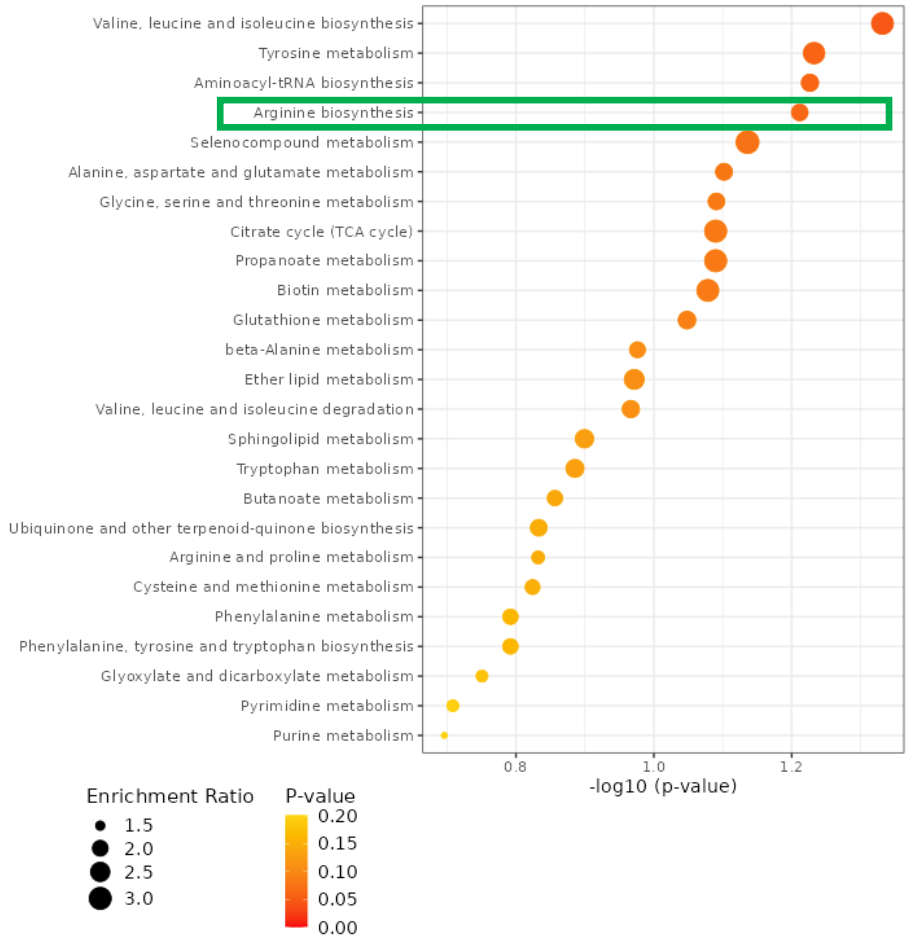


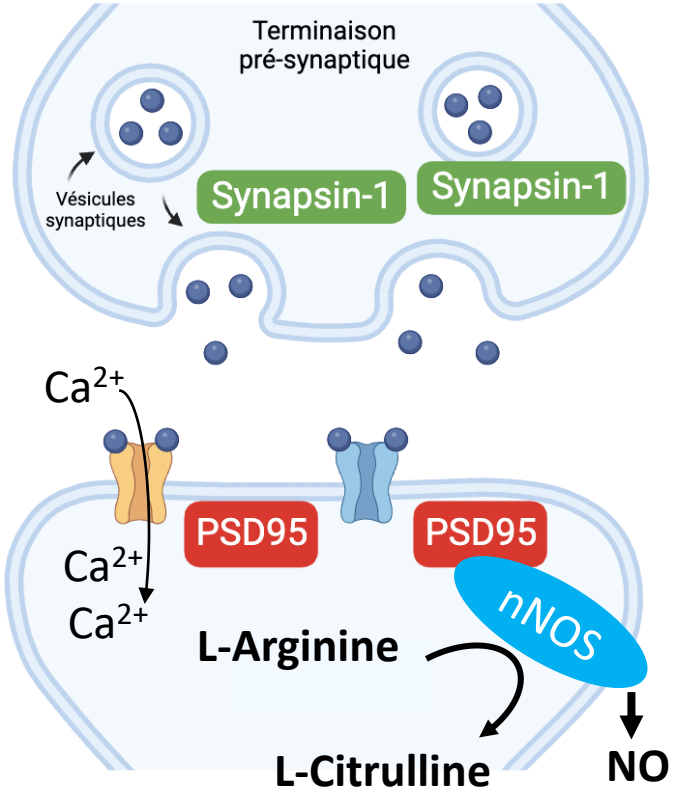


Metabolomic analysis of f-EV

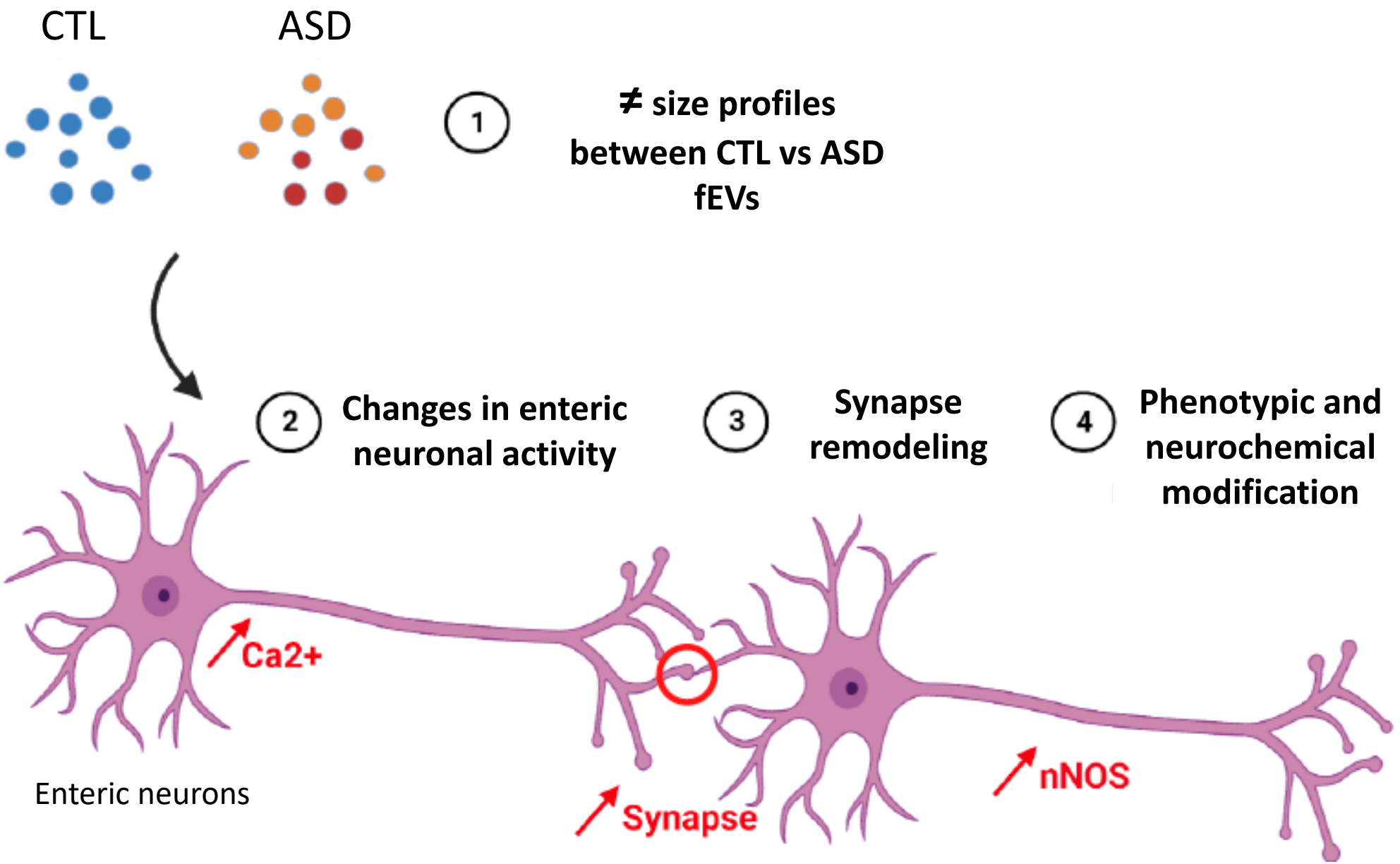
Patrick EMOND, Antoine LEFEVRE, Camille DUPUY





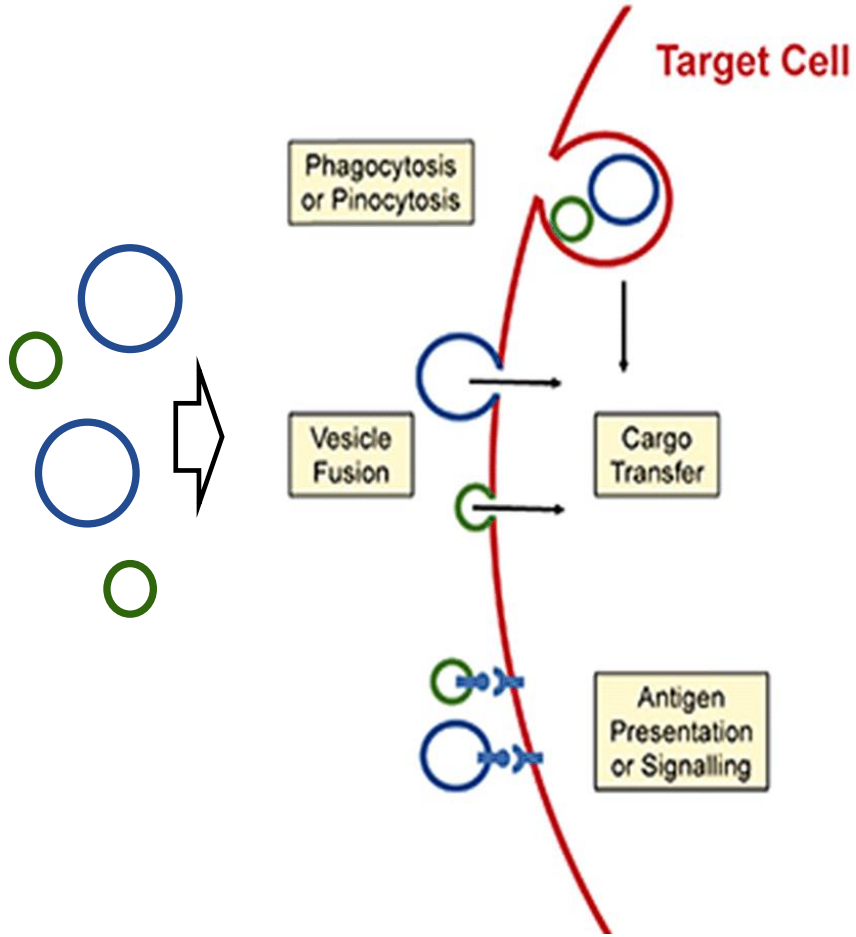


CONCLUSION


CTL
ASD
1
≠ size profiles between CTL vs ASD fEVs
2
Changes in enteric neuronal activity
3
Synapse remodeling
4
Phenotypic and neurochemical modification
Ca2+
Synapse
nNOS
Enteric neurons

PERSPECTIVES

Mode of action



In vivo study

f-EVs Gavage (CTL / ASD)

- Behavioural tests
- Gastrointestinal functional tests
- Histological evaluation

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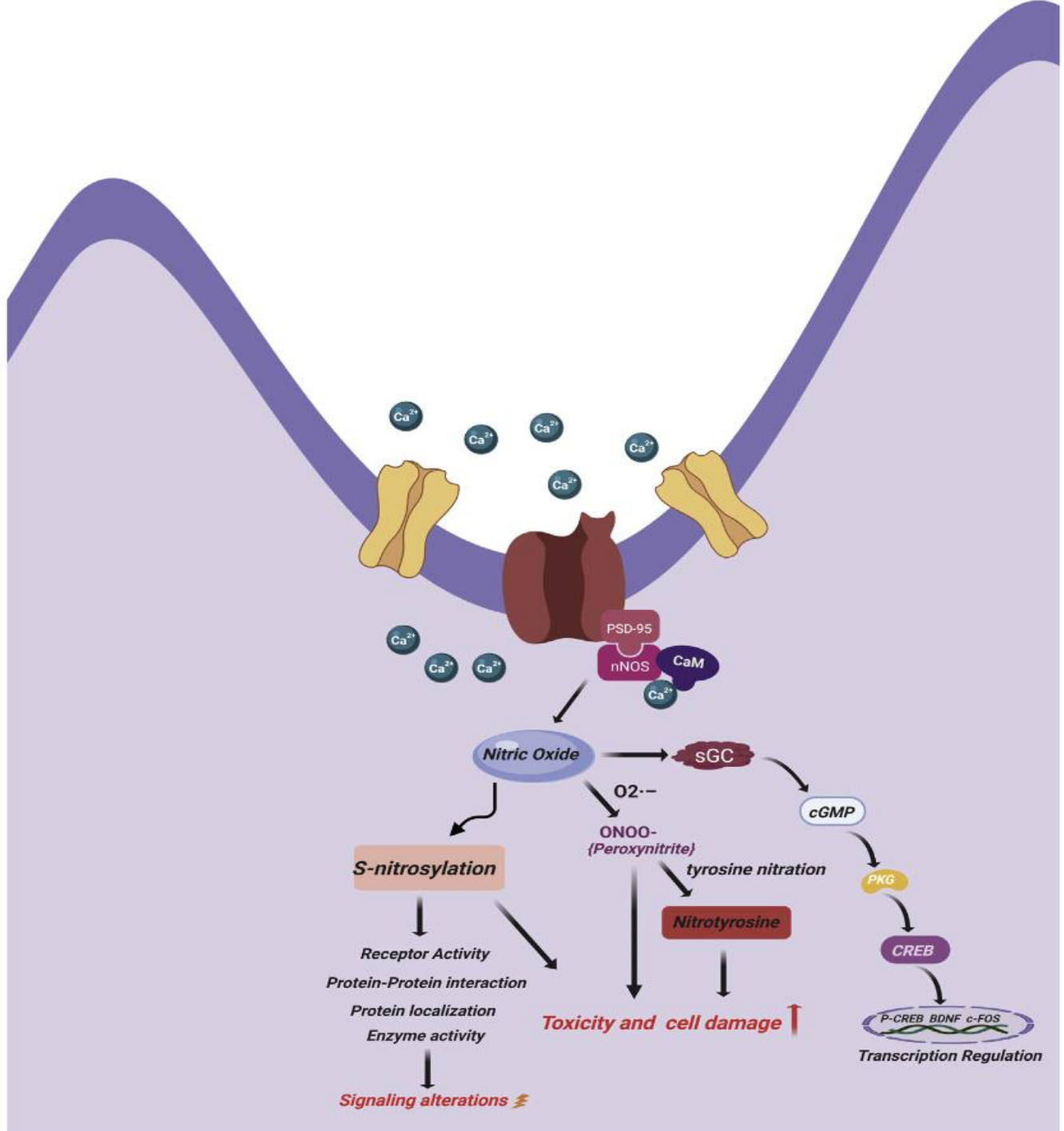
Ca2+
PSD-95
nNOS
CaM
Nitric Oxide
sGC
O2·−
ONOO-
{Peroxynitrite}
tyrosine nitration
cGMP
PKG
CREB
P-CREB BDNF c-FOS
Transcription Regulation
S-nitrosylation
Receptor Activity
Protein-Protein interaction
Protein localization
Enzyme activity
Signaling alterations
Nitrotyrosine
Toxicity and cell damage