

Analysis of extracellular vesicles in PLWH and their role in the immune activation: association with HIV reservoir in the brain and neurocognitive impairment

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Ramón y Cajal Research Fellow

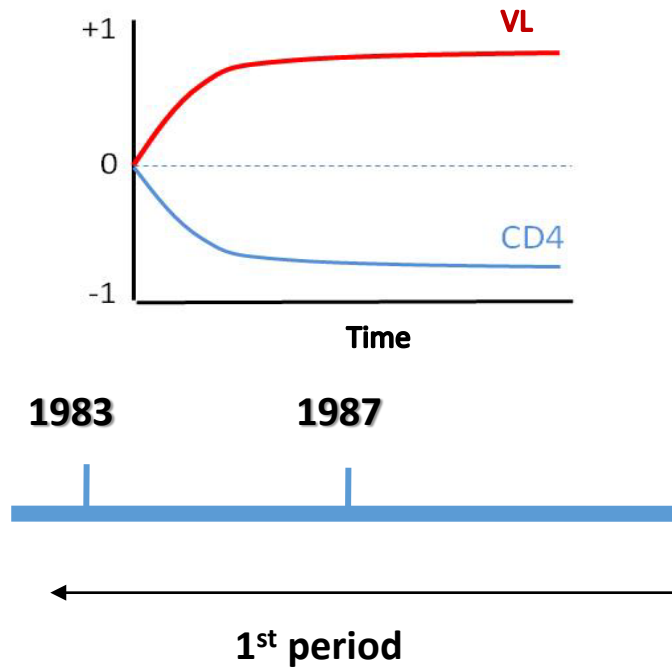
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17th International Symposium on Neuropsychiatry & HIV

Disclosures:

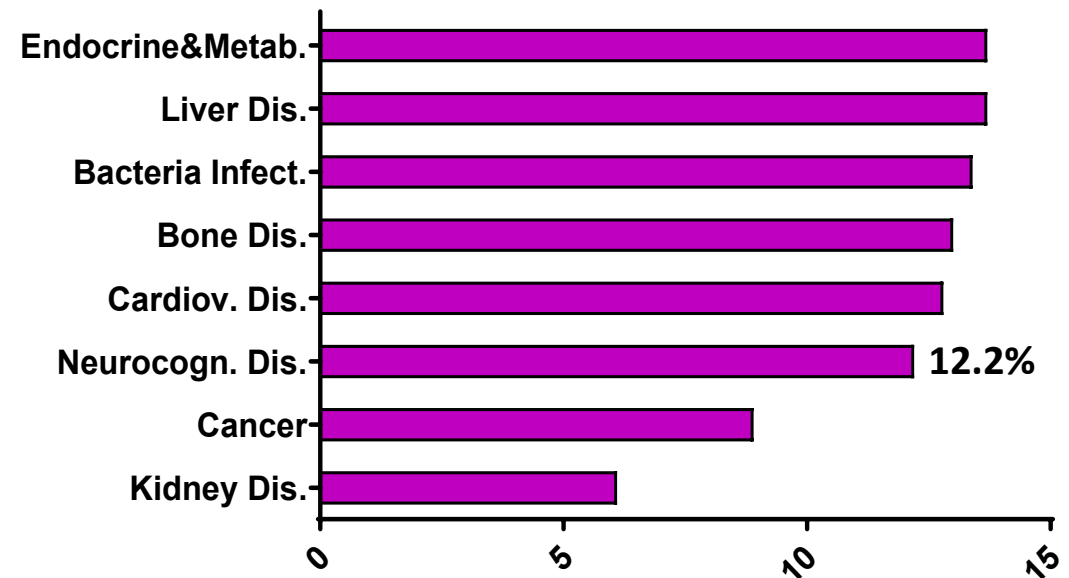
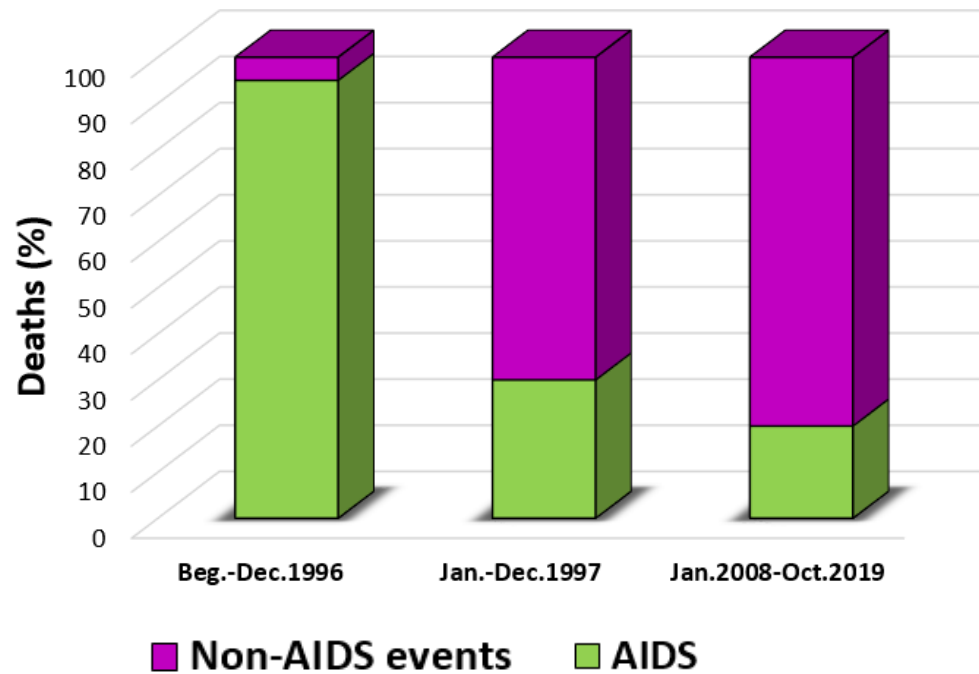
Becas Gilead a la Investigación Biomédica (GLD23/00004)

History of morbidity and mortality in people living with HIV (PLWH)



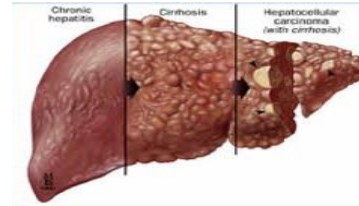
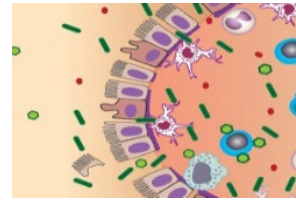
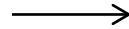
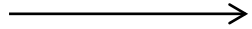
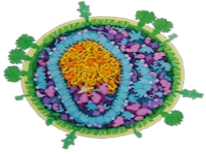
Causes of death in PLWH: Non-AIDS events

- ✓ Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection (Palella et al., N Engl J Med, 1998).
- ✓ Non-AIDs Events in the era of cART (Marin et al., AIDS, 2009; Elvstam et al., Clin Infect Dis., 2021).

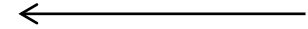
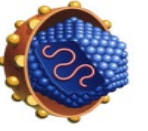


*UCEIMP Cohort, VRUH.
Ruiz-Mateos E., VIHSAEI, 2019.*

HIV



HCV



Microbial translocation
Microbiota

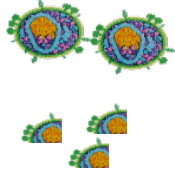
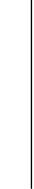
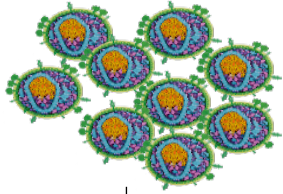


ACTIVATION/INFLAMMATION

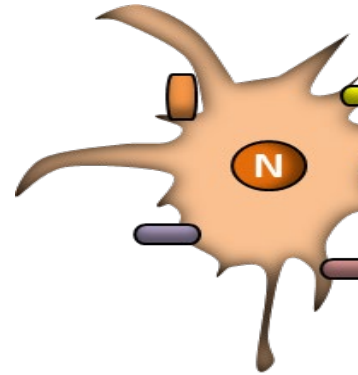
COMORBIDITIES

- Drug abuse
- Obesity
- Hypertension
- DM...

Viral persistence
Viral proteins



cART



APC

Monocyte

S. MEDIATORS

- Cytokines
- Chemokines
- Coagulation
- Complement



Non-AIDS EVENTS

Toxicity
cART Types



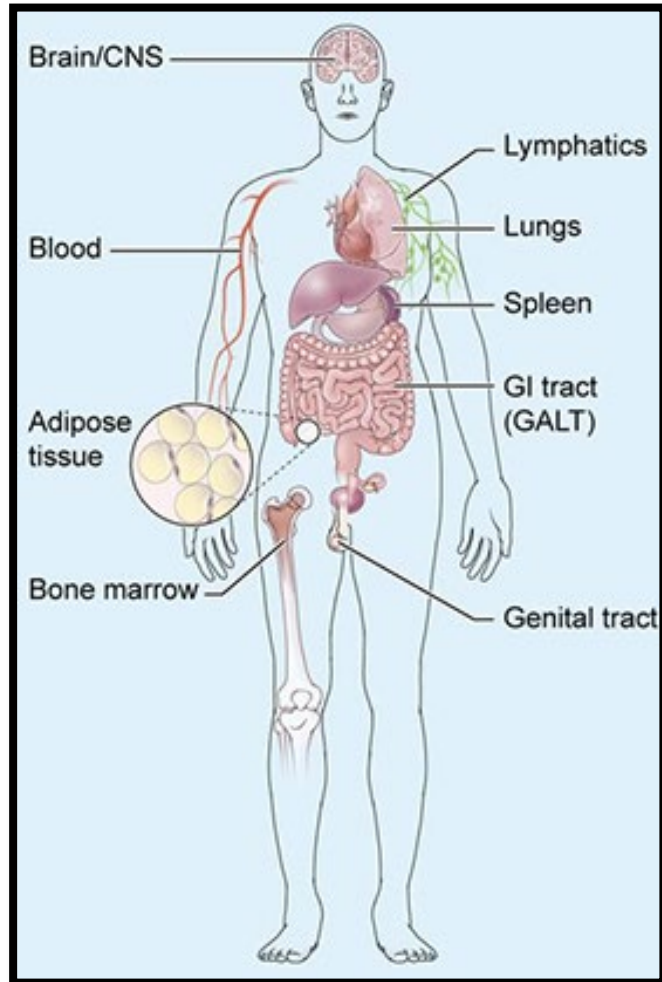
Other persistent
infections



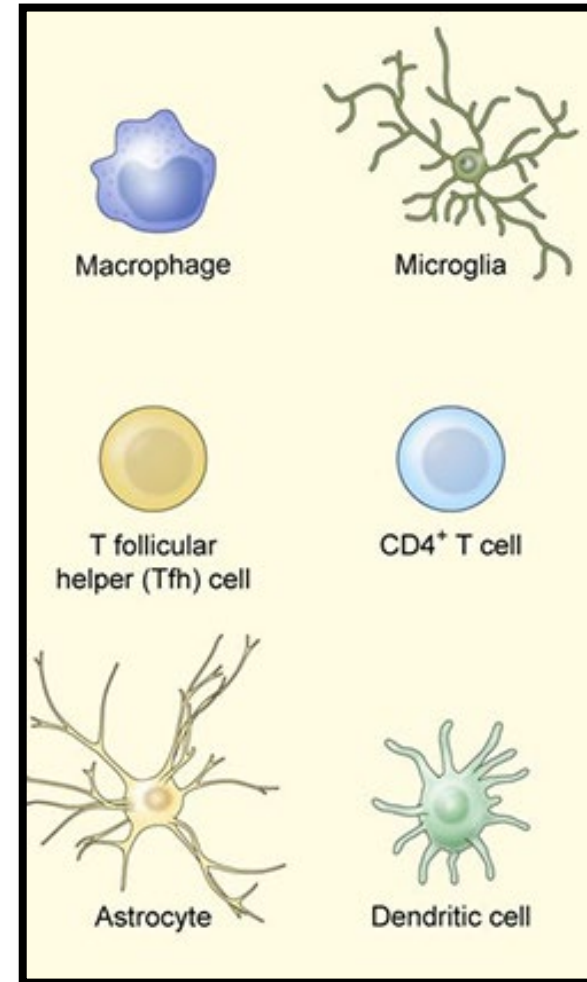
- CMV
- Other HV
- HPV
- Leishmania
- TBC
- HBV

Anatomical and cellular HIV reservoirs

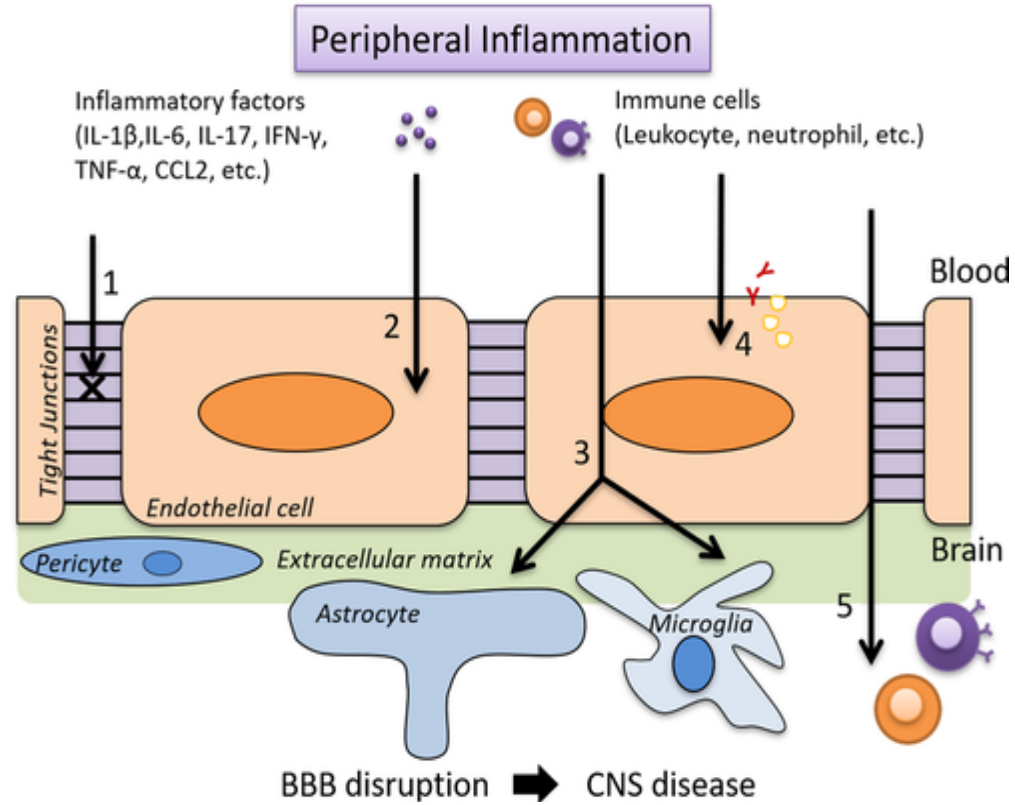
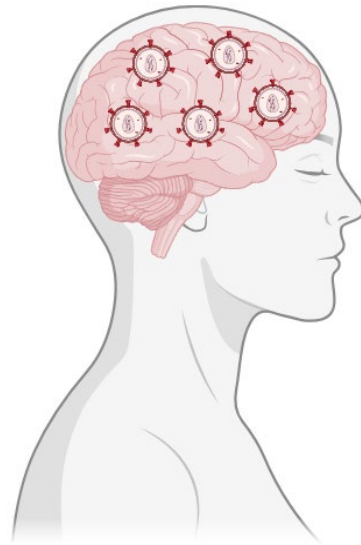
ANATOMICAL RESERVOIRS



CELLULAR RESERVOIRS

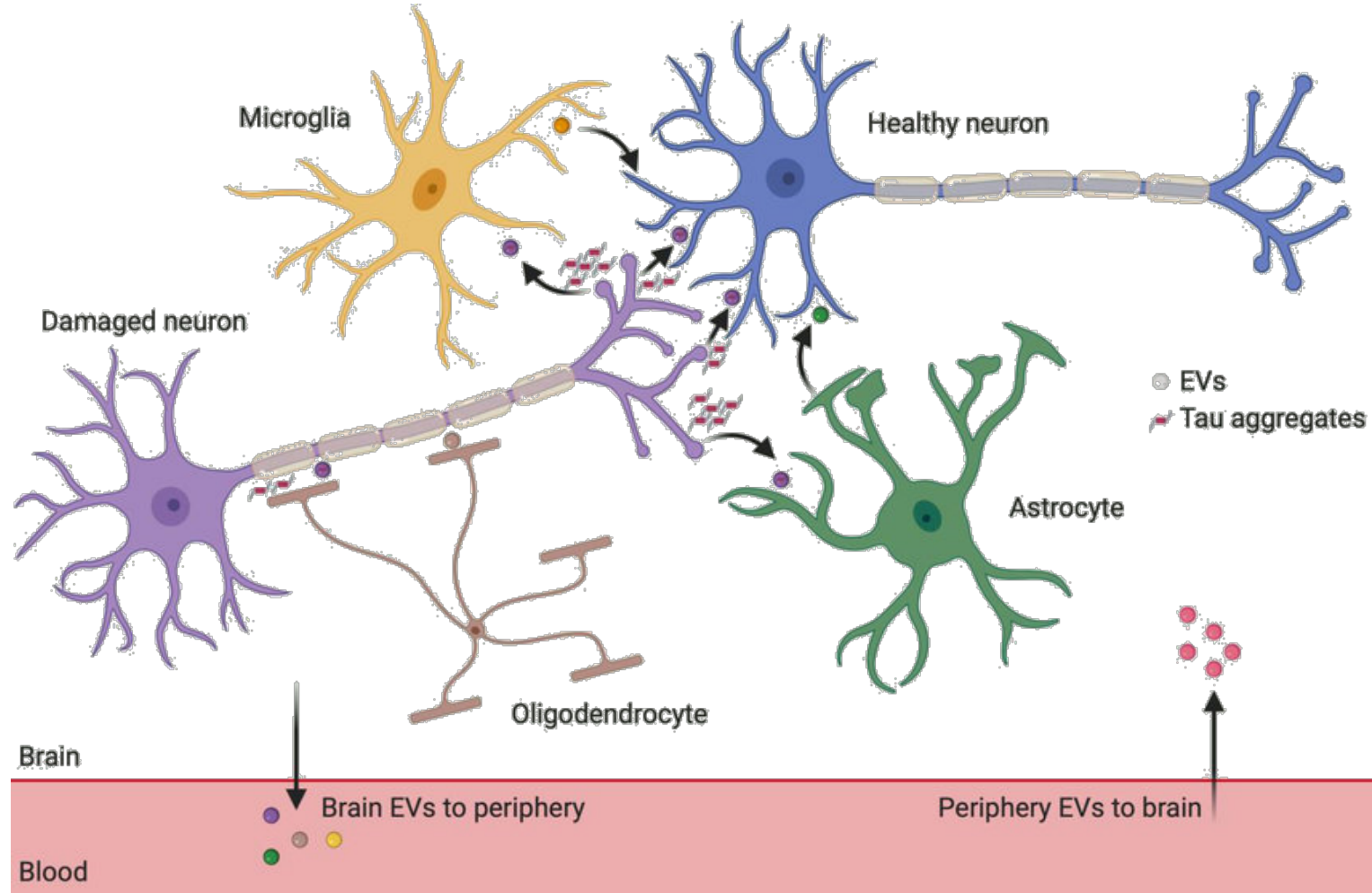


Peripheral inflammation and blood-brain barrier disruption



Huang et al., CNS Neurosci Ther., 2020.

Extracellular vesicles (EVs)



Hypothesis and Objectives

PLWH undergoes a chronic low-grade immune activation and inflammation affecting the central nervous system. Several factors may cause this inflammation, including residual HIV replication in tissues, immune dysregulation or increased inflammatory/viral cargo of extracellular vesicles (EVs).

Objectives:

1. To analyze innate and adaptive immune parameters associated with neurocognitive impairment in PLWH on cART.
2. To investigate the EVs cargo profile associated with neurocognitive impairment in PLWH on cART.
3. To analyze the HIV-reservoir in brain tissues.

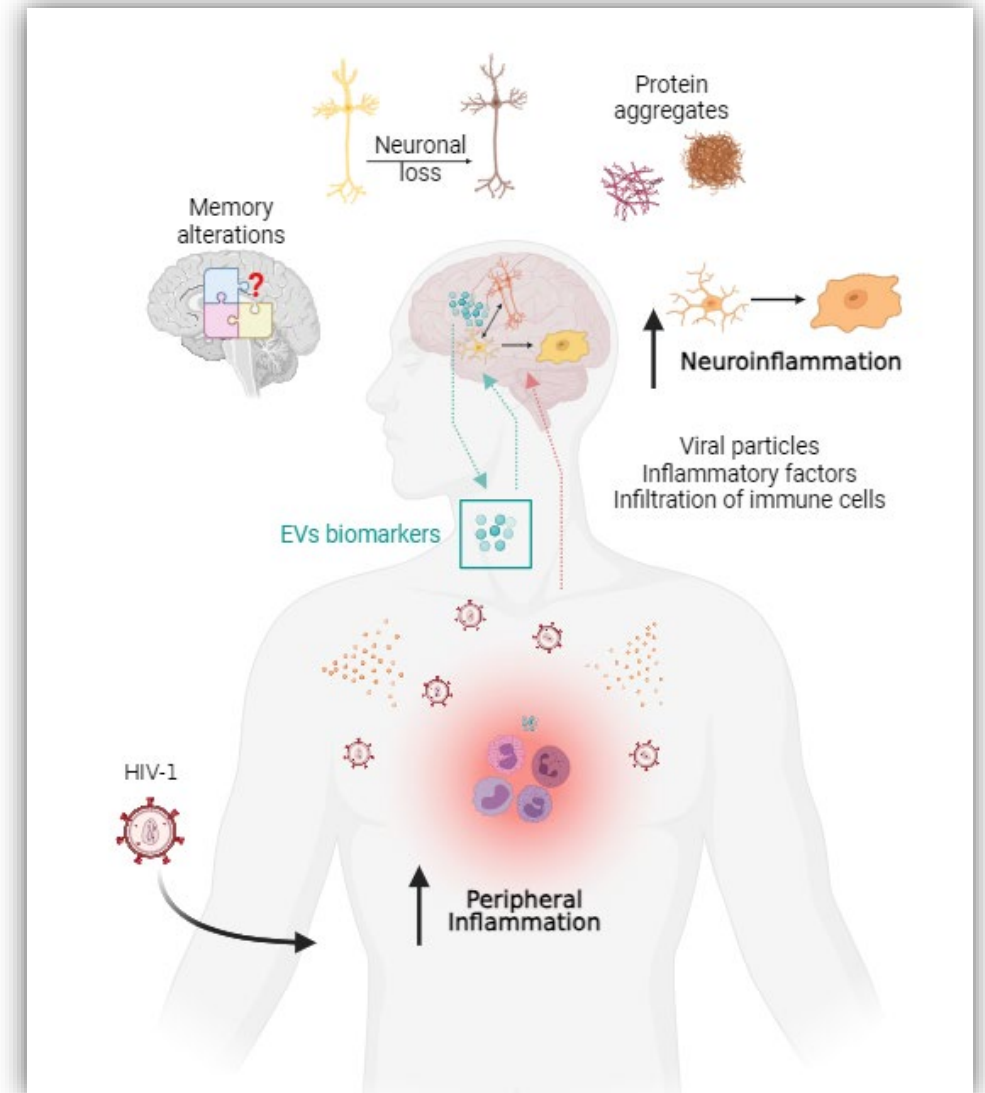
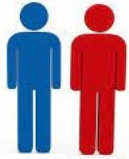


Figure created with Biorender.com.

Characteristics of participants



Inclusion criteria:

- Age: > 50 years old
- PLWH on cART > 24 months
- Indetectable Viral Load



Memory alteration test (M@T):

Encoding, temporal orientation, semantic memory, free- and cued-recall memory.

(Rami et al., Int.J.Geriatr.Psychiatry, 2007)



Cognitive impairment (CI):

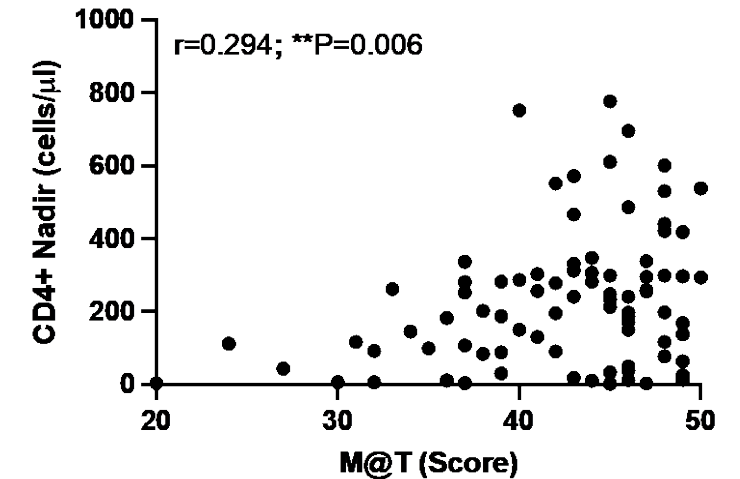
< 37 points



Non-Cognitive impairment (Non-CI):

38-50 points

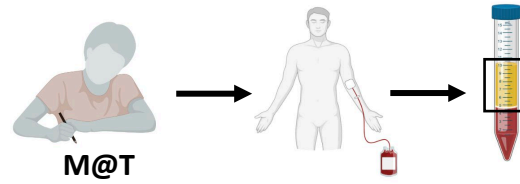
Parameter	CI (n=20) Median (IQR)	Non-CI (n=67) Median (IQR)	p-value
Age (y)	57.5 (54-65.5)	57 (53-62.75)	0.389
Sex (n, % women)	6 (30%)	10 (14.7%)	0.190
CD4+ T-Cells (cells/ μ L)	553 (298-764)	686 (534-929)	0.015
CD8+ T-Cells (cells/ μ L)	785 (479-1253)	836 (576-972)	0.800
Ratio CD4:CD8	0.81 (0.28-1.14)	0.88 (0.66-1.34)	0.225
CD4+ Nadir T-Cells	103 (9-199.5)	240 (90-331)	0.001
Indetectable VL (years)	9.2 (6.8-11.9)	6.8 (4.1-11.5)	0.217
Total M@T Score	34 (21-36.75)	45 (42.25-48)	<0.0001
Encoding Score	8 (7-9)	9 (9-10)	<0.0001
Temporal Orientation Score	4 (3-4.75)	5 (5-5)	<0.0001
Semantic Memory Score	10.5 (8-12)	14 (12-15)	<0.0001
Free-Recall Memory Score	4 (4-5.75)	8 (7-9)	<0.0001
Cued-Recall Memory Score	7 (5.25-8)	10 (9-10)	<0.0001



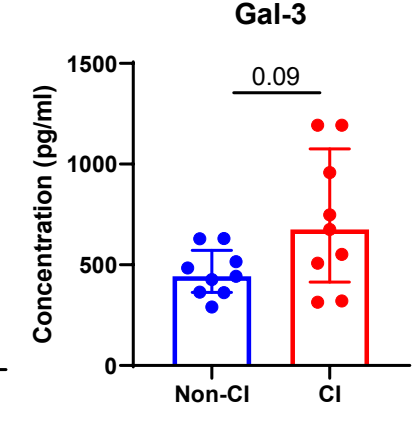
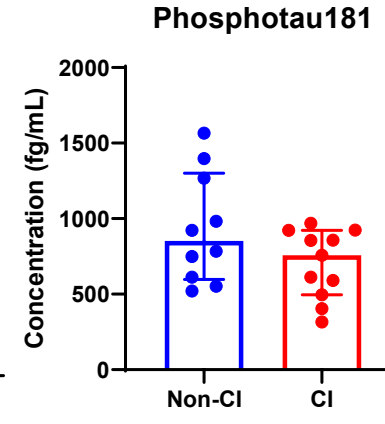
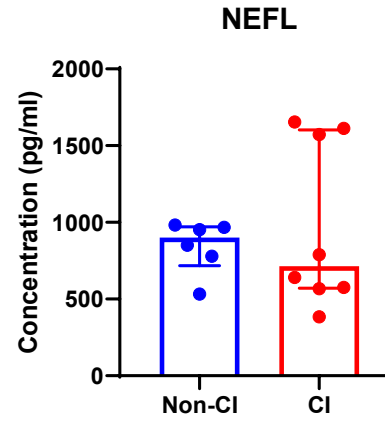
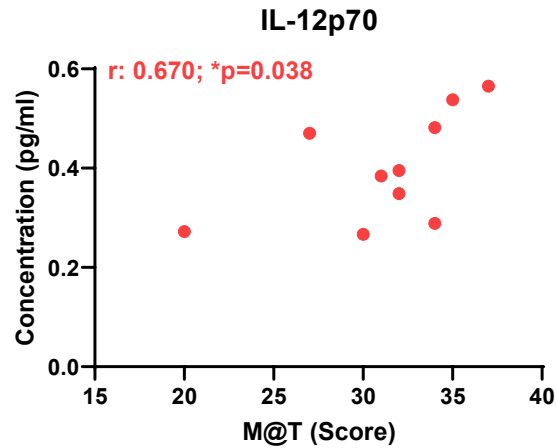
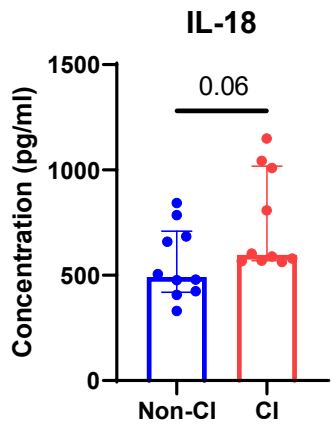
Similar results using other cognitive tests
(Ellis et al., AIDS, 2011).

Exclusion criteria: HIV-2 infected; Drug/alcohol users; Active infections (last 6 mo.); Hospital admission (last 6 mo.); Antitumour therapy; Treatments that could influence the immune system (mainly corticosteroids).

Biomarkers of inflammation and neurodegeneration in plasma



Proinflammatory cytokines: MesoScale
 Neurogeneration/neuroinflammation: ELISAs



No differences in: IFN- γ , IL-12p70, IL-1 β , IL-6, IL-8, IP-10, MIP-1 α , MIP-1 β and TNF- α .

Higher levels of plasma IL-12p70 is associated with slower cognitive decline (Yang et al., *Alzheimers Dement.*, 2021).

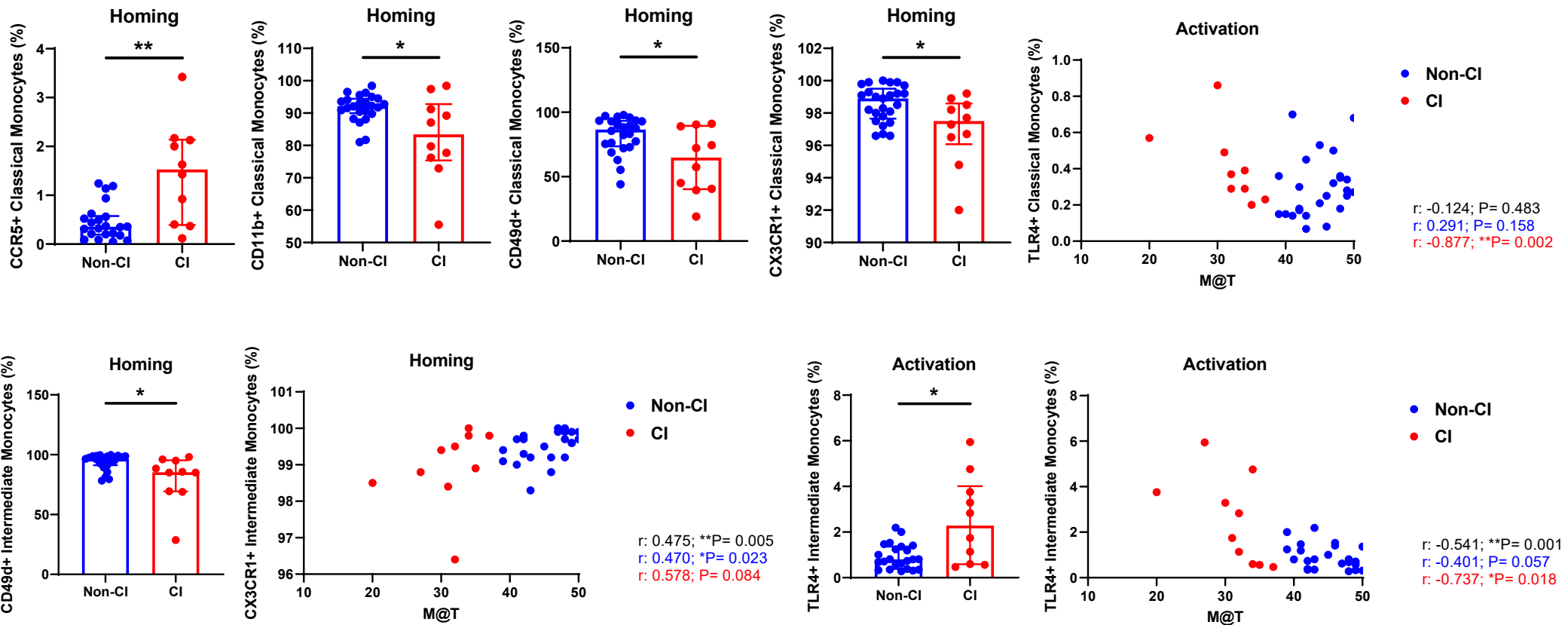
NEFL and Phosphotau181 in plasma define cognitive decline in AD (Huang et al., *CNS Neurosci Ther.*, 2022).

Neurodegeneration

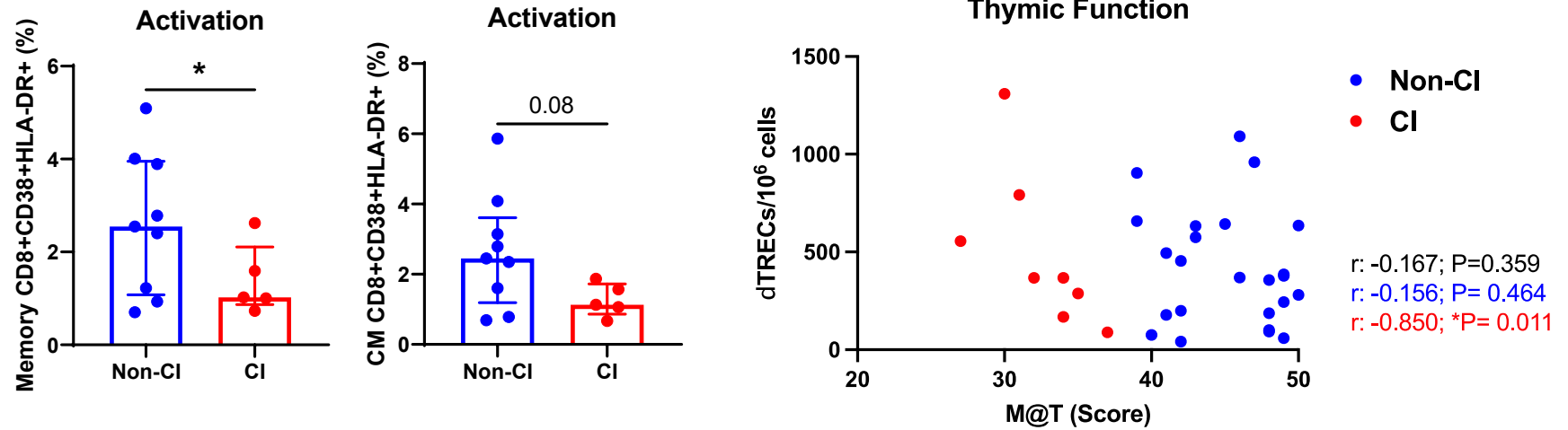
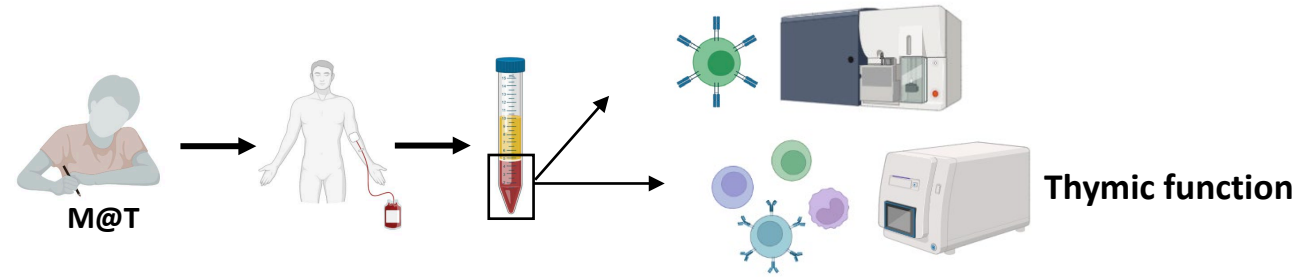
Cognitive decline

Inflammation

Monocytes are altered in PLWH with memory impairment

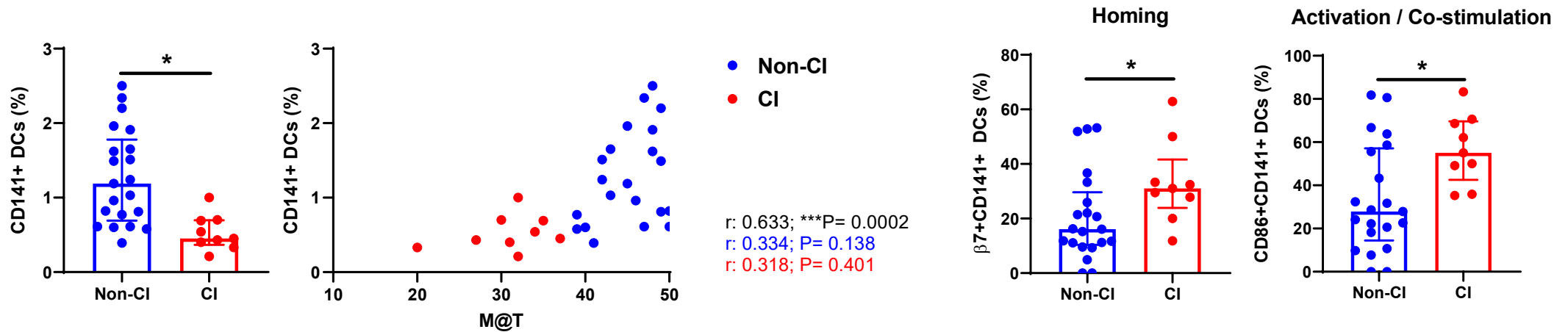
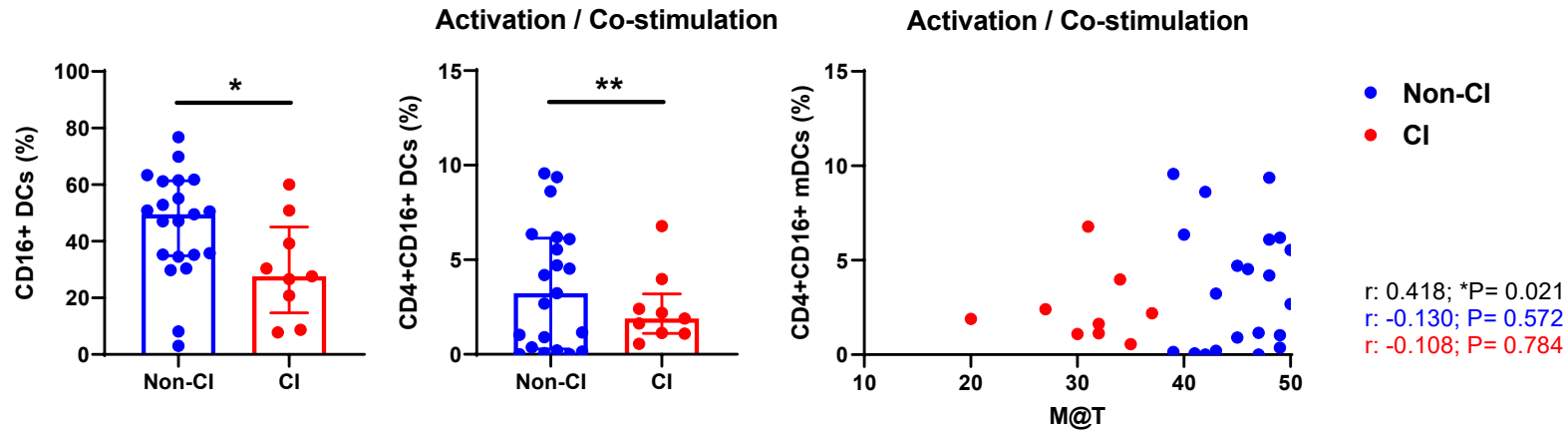
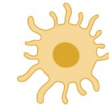


CD8+ T-cells are decreased in PLWH with memory impairment

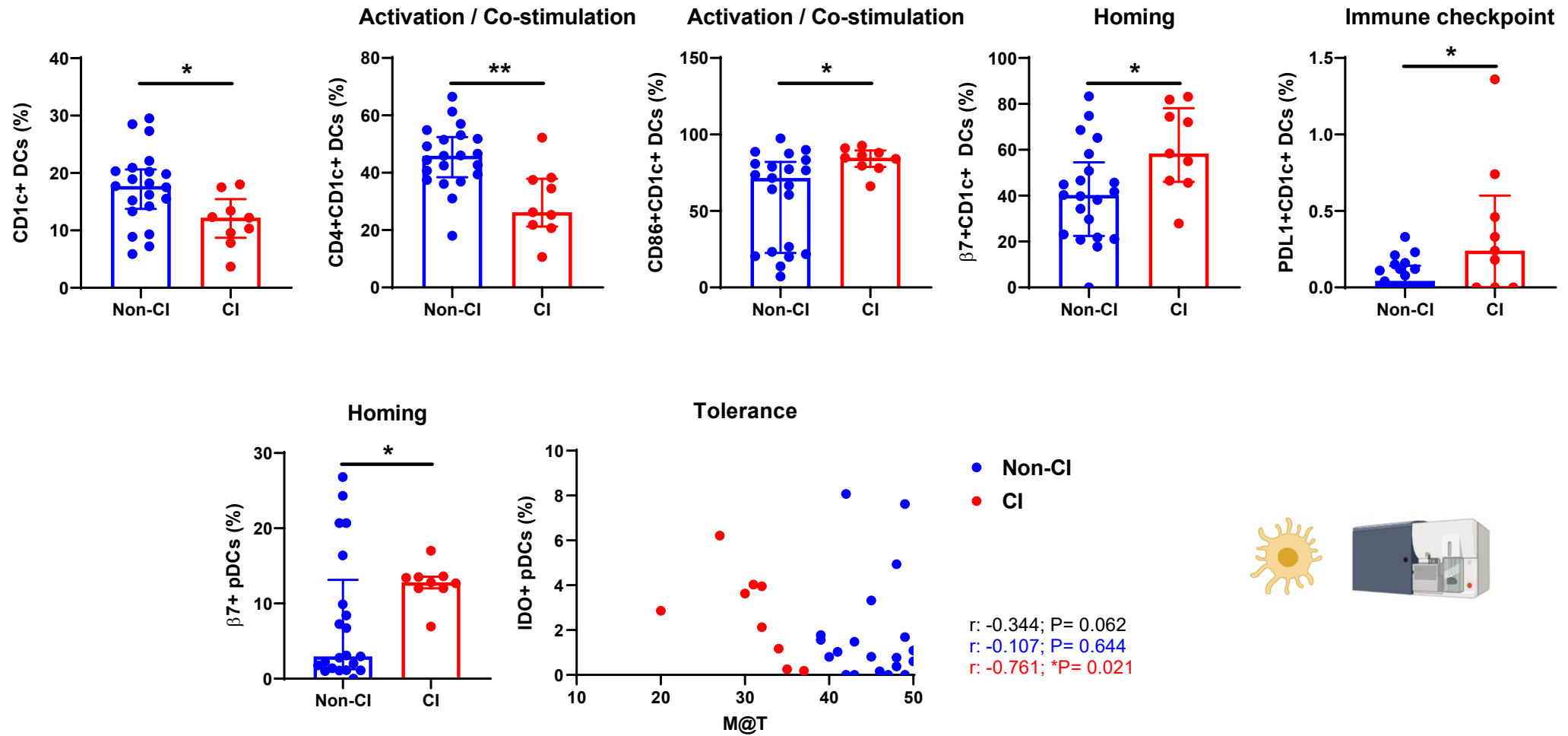


The role of antiviral CD8+ T-cells in cognitive impairment
(Reagin and Funk, Curr Opin in Neurobiol., 2022).

Dendritic Cells are decreased in PLWH with memory impairment

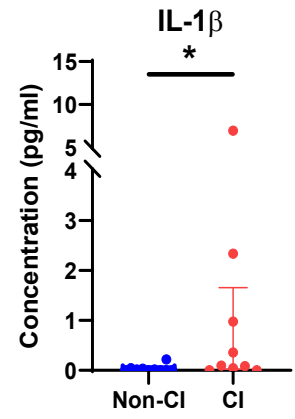
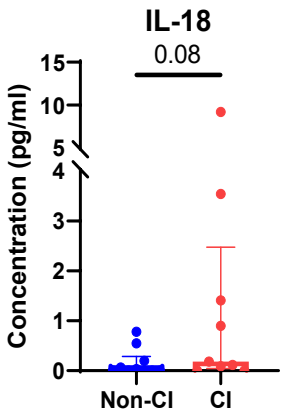
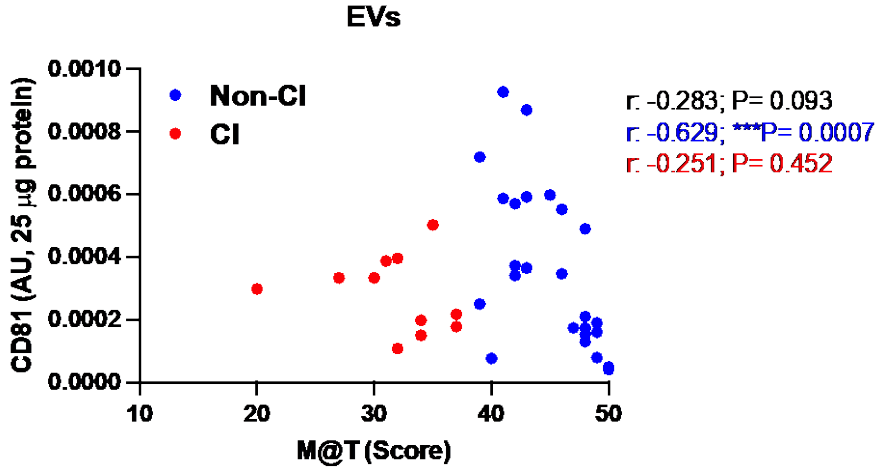
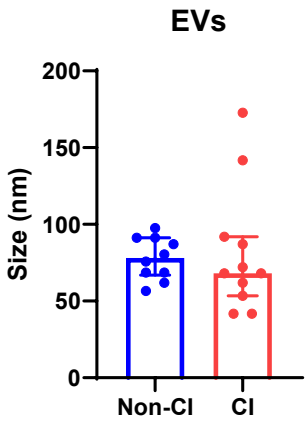
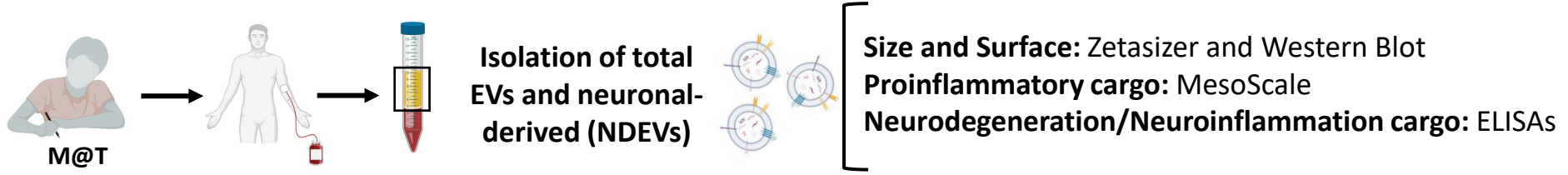


Dendritic Cells are decreased in PLWH with memory impairment



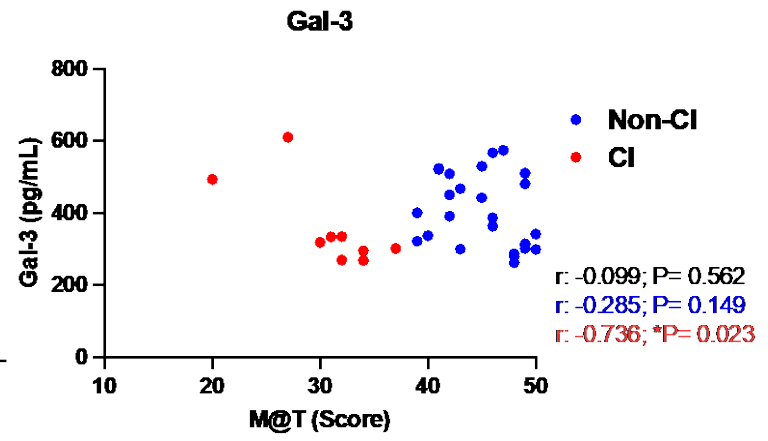
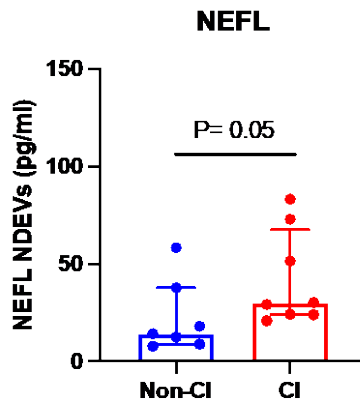
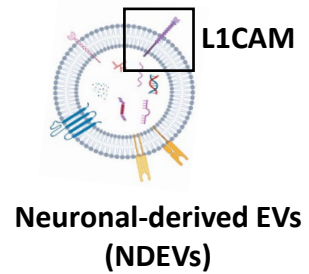
Decreased DCs are associated with AD progression (Ciaramella et al., J Neuroinflammation, 2016).

Extracellular vesicles cargo is associated with memory impairment

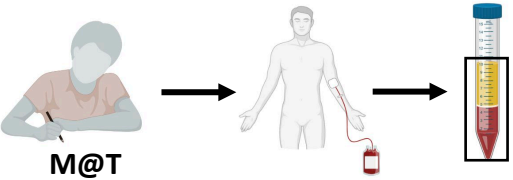


IL-18 in EVs from different phenotypes of PLWH (Poveda et al., J Infect Dis., 2023).

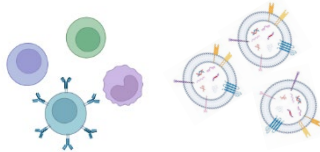
EVs proinflammatory cargo is associated to cognitive progression in AD (Aharon et al., Mol Neurobiol., 2020).



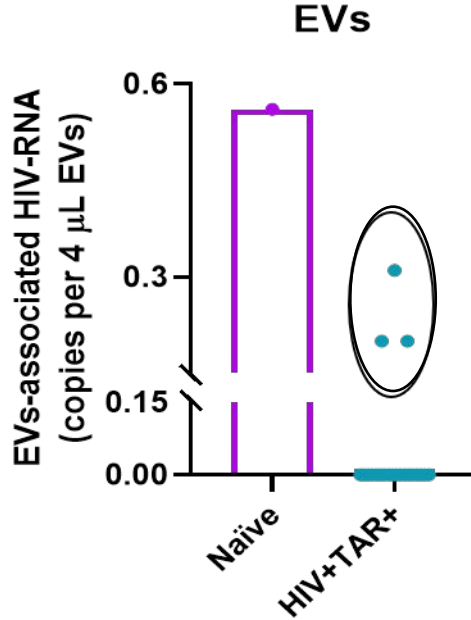
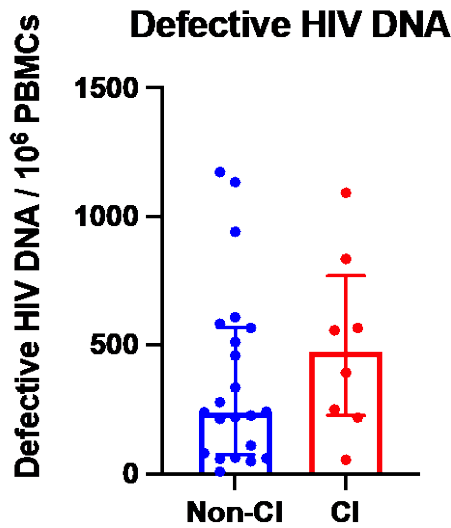
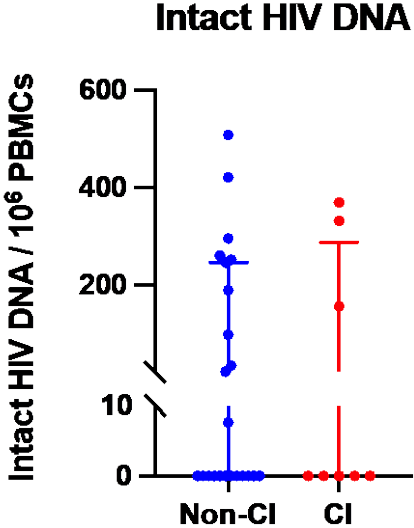
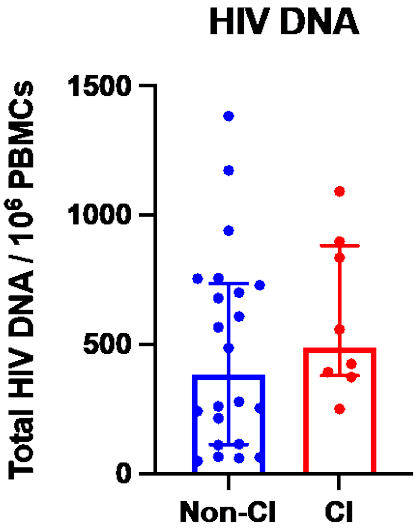
HIV reservoir in PBMCs and EVs



HIV reservoir:
PBMCs
RNA-EVs

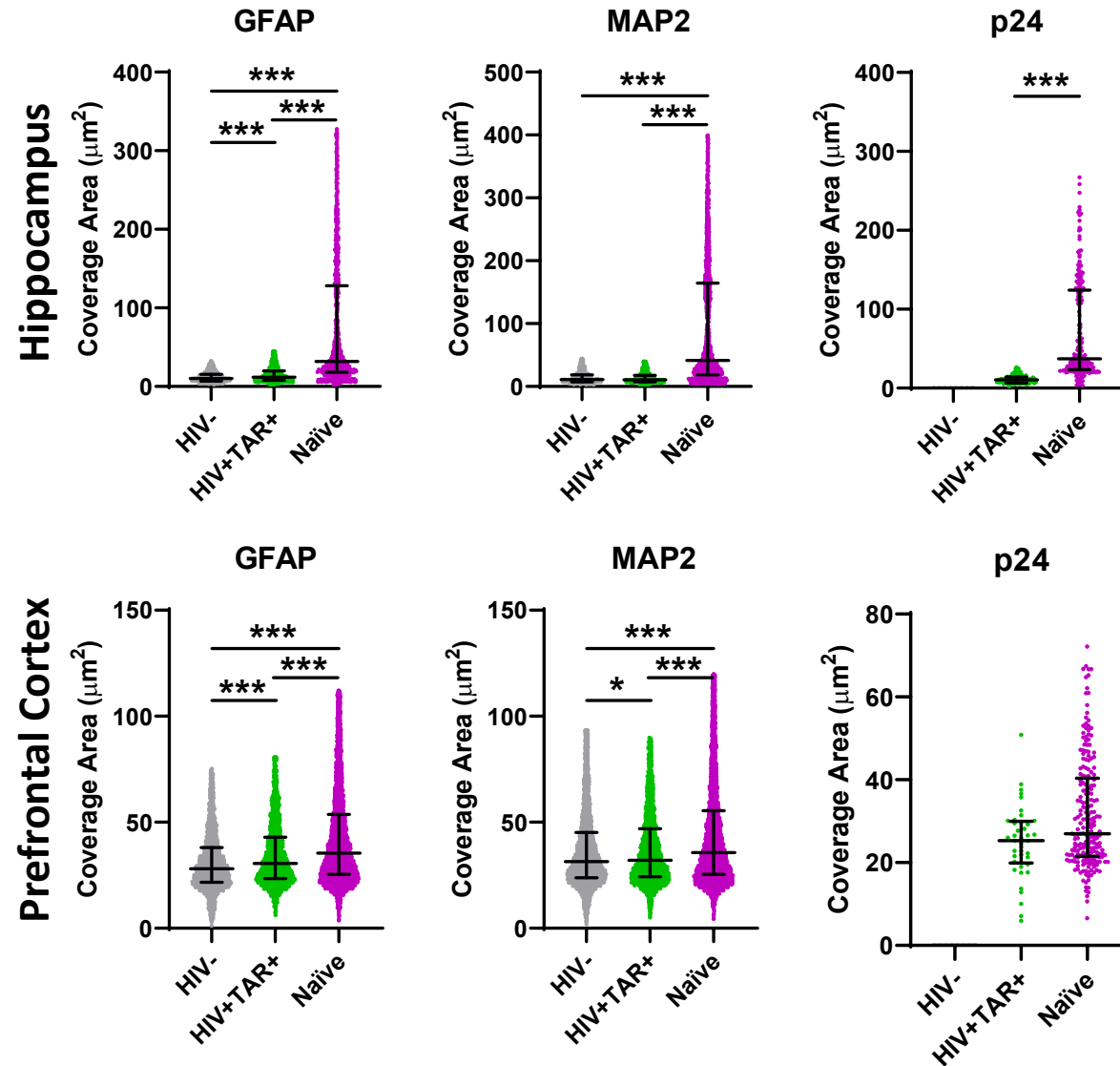
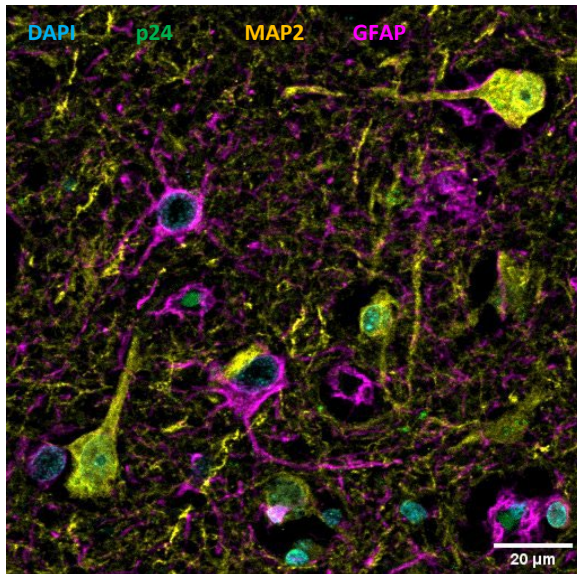


Intact Proviral DNA Assay (IPDA)
ddPCR: EVs-associated HIV-RNA



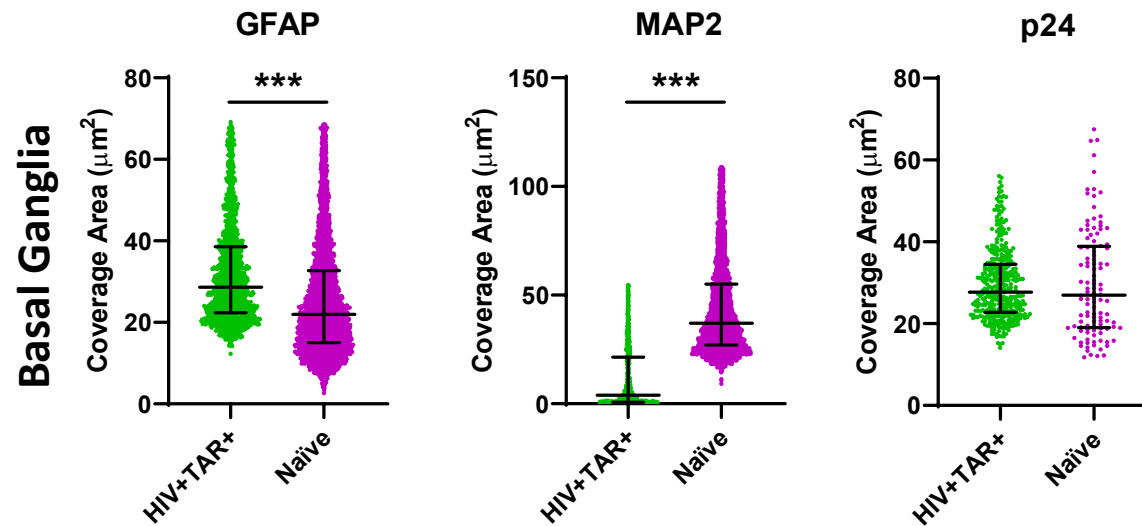
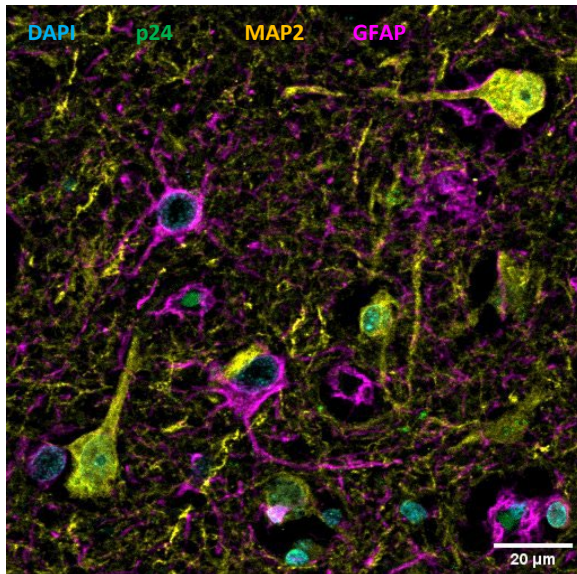
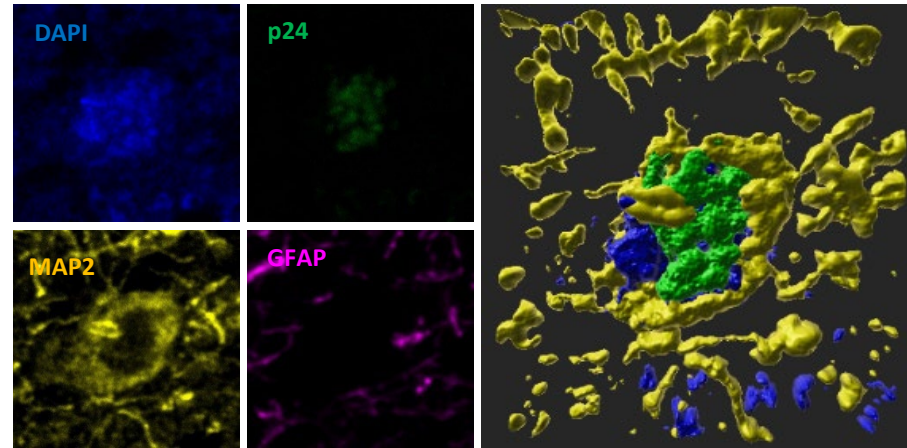
Neurodegenerative-neuroinflammatory markers and HIV-1 reservoir distribution followed a brain region-dependent manner

- 4 non-HIV
- 2 PLWH on cART
- 4 PLWH off cART



Neurodegenerative-neuroinflammatory markers and HIV-1 reservoir distribution followed a brain region-dependent manner

- 4 non-HIV
- 2 PLWH on cART
- 4 PLWH off cART



CONCLUSIONS

1. Immunological alterations were found in PLWH with memory alterations.
2. The size, surface composition, and EVs proinflammatory cargo were associated with memory impairment.
3. NEFL and Gal-3 levels in NDEVs were related with memory alterations.
4. The expression of p24, MAP-2 and GFAP followed a brain region-dependent distribution in PLWH on cART compared to PLWH off cART.

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