Possible Roles of Extracellular Condensates in HIV Persistence

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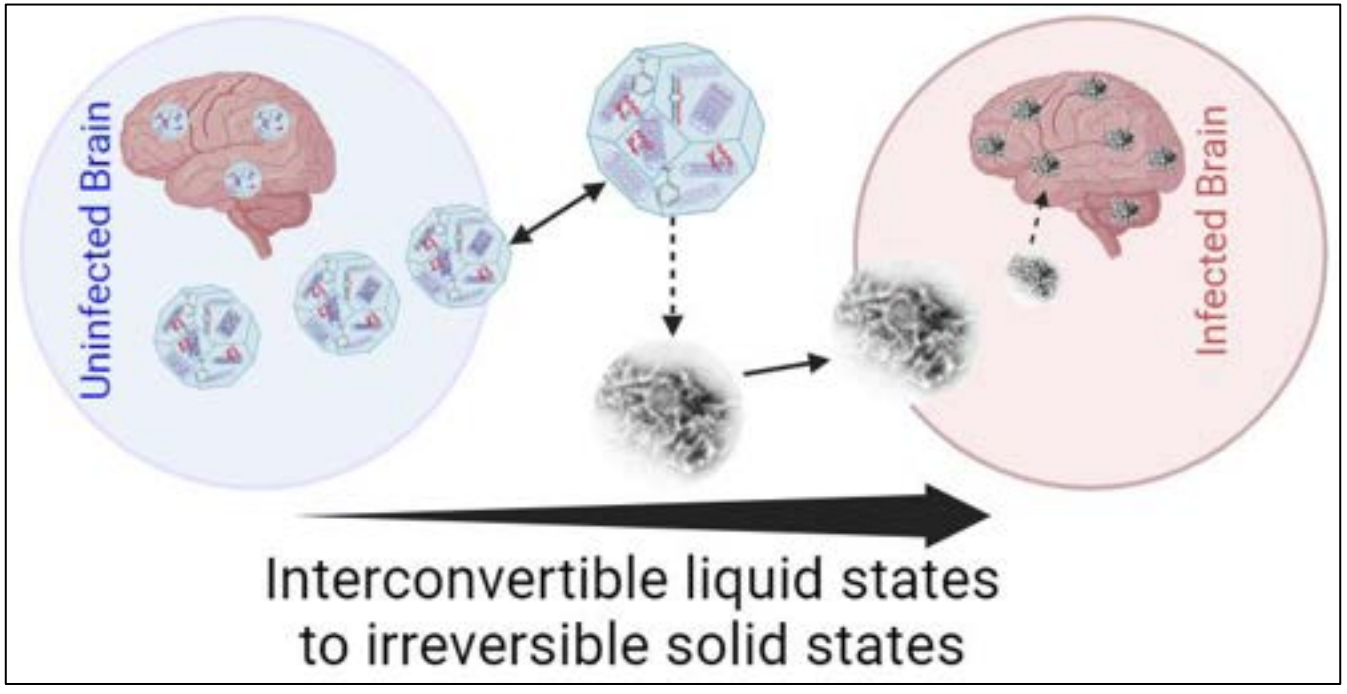
Extracellular condensates (ECs)

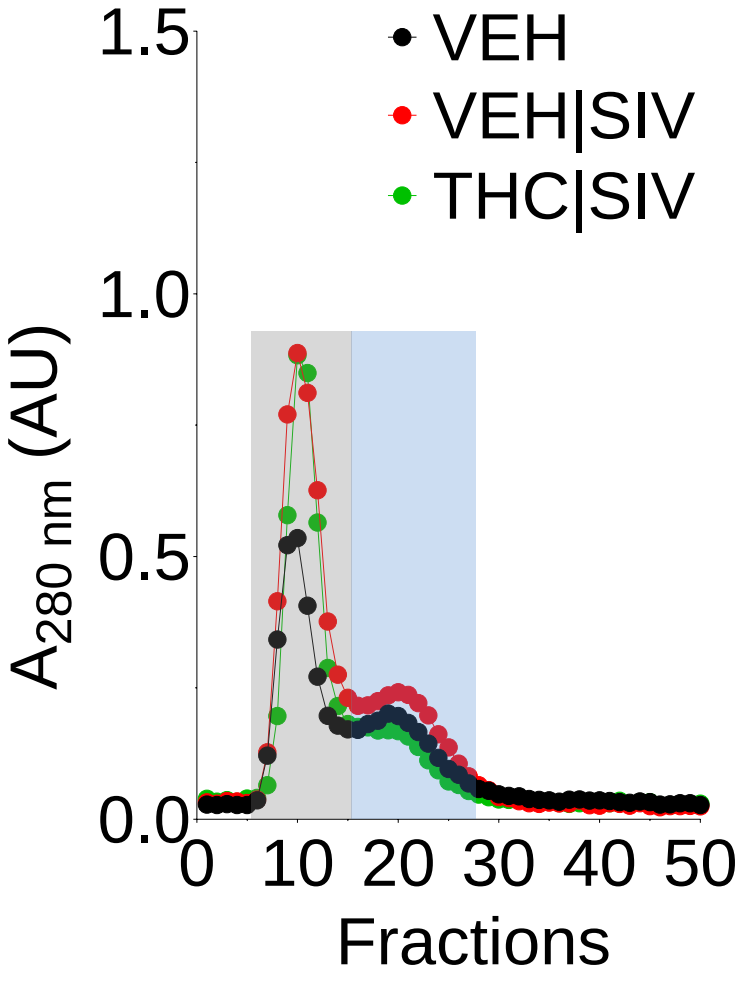
- ECs are a subset of **biomolecular condensates (BMCs) present in the extracellular milieu.**
- These condensates may form through liquid–liquid phase separation (LLPS) - de-mixing of molecules via transient interactions.

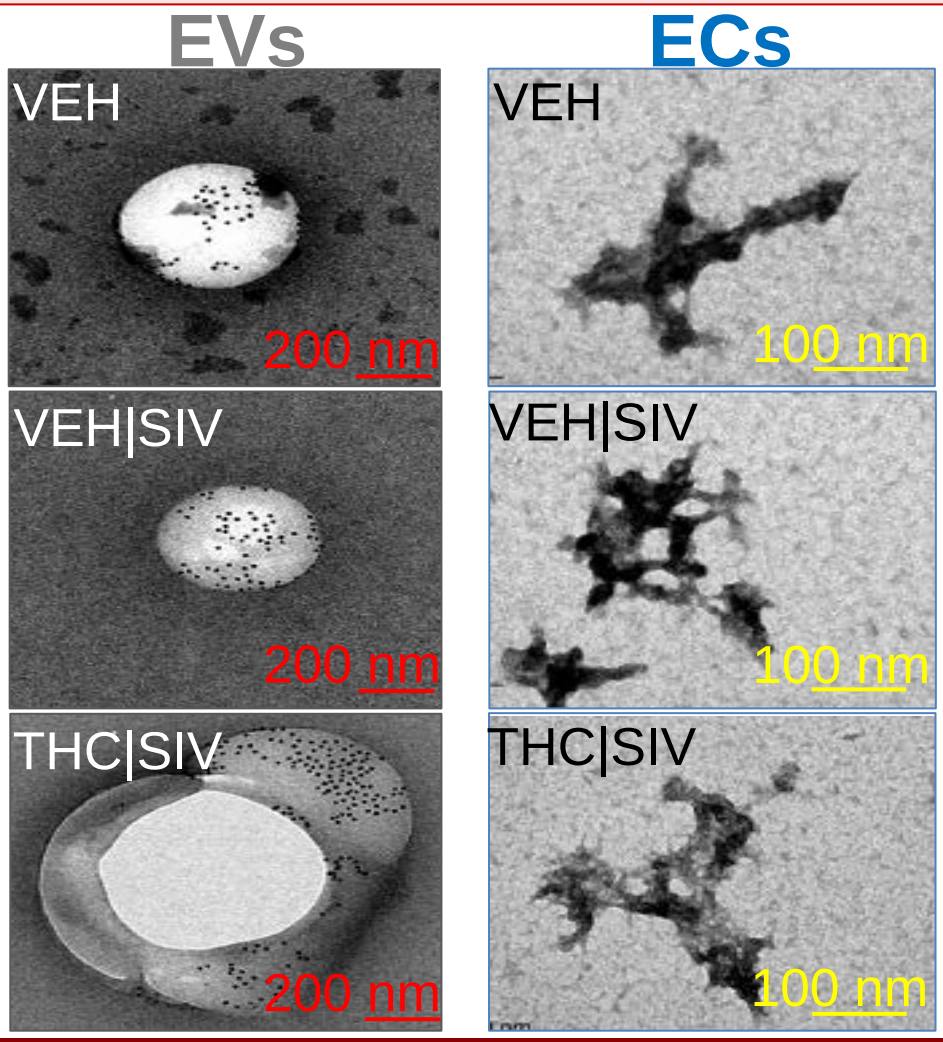
LLPS may progress from dynamic (reversible) to static (irreversible) aggregates



- ECs formation and regulation are crucial for health & disease.
- However, the significance of ECs in HIV persistence is unknown, but of great interest.

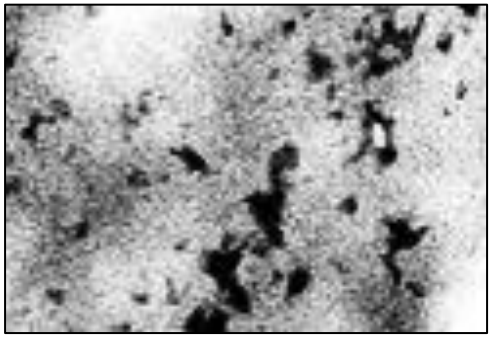
Basal ganglia contains ECs



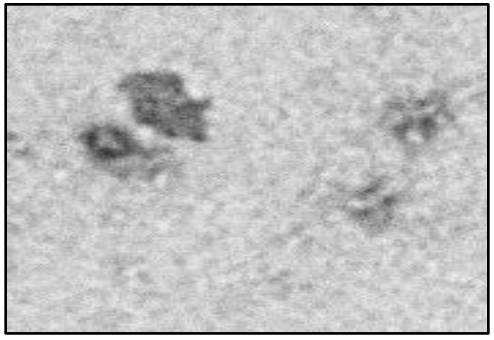


ECs from different sources and species

Human
Blood Plasma

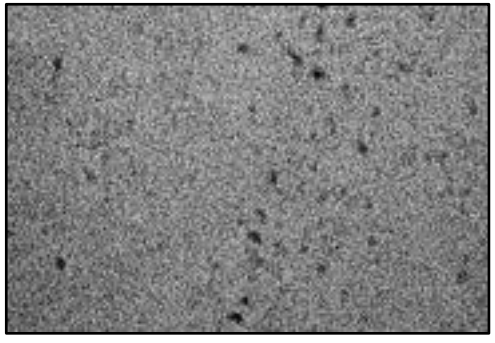


Human
Seminal Plasma

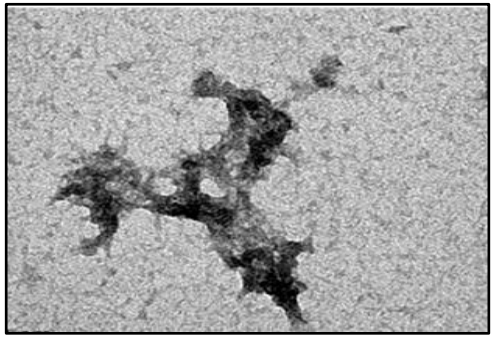


Human
Prefrontal Cortex

Macaque
Blood plasma



Macaque
basal ganglia



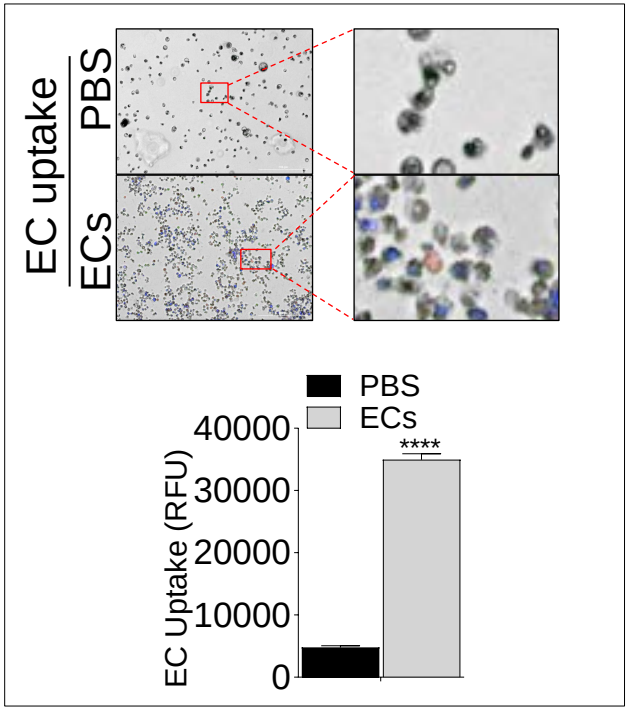
Are ECs internalized by cells latently infected with HIV?

Day 0 → Culture cells

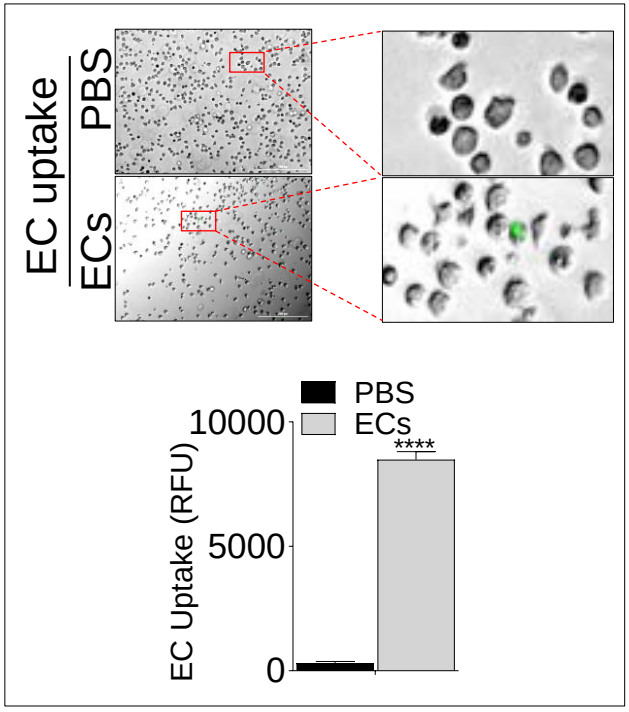
Day 1 → Add Labelled PBS or ECs

Day 2 → Image and analyze fluorescence

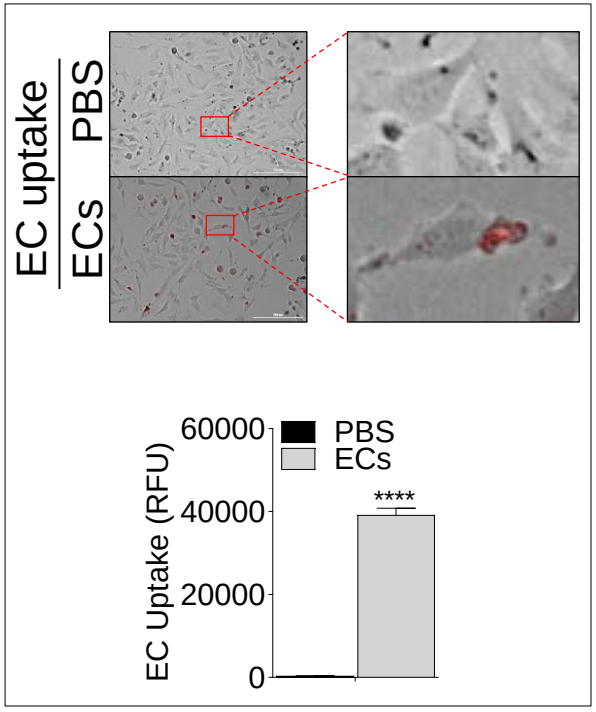
J-Lat Tat-GFP cells



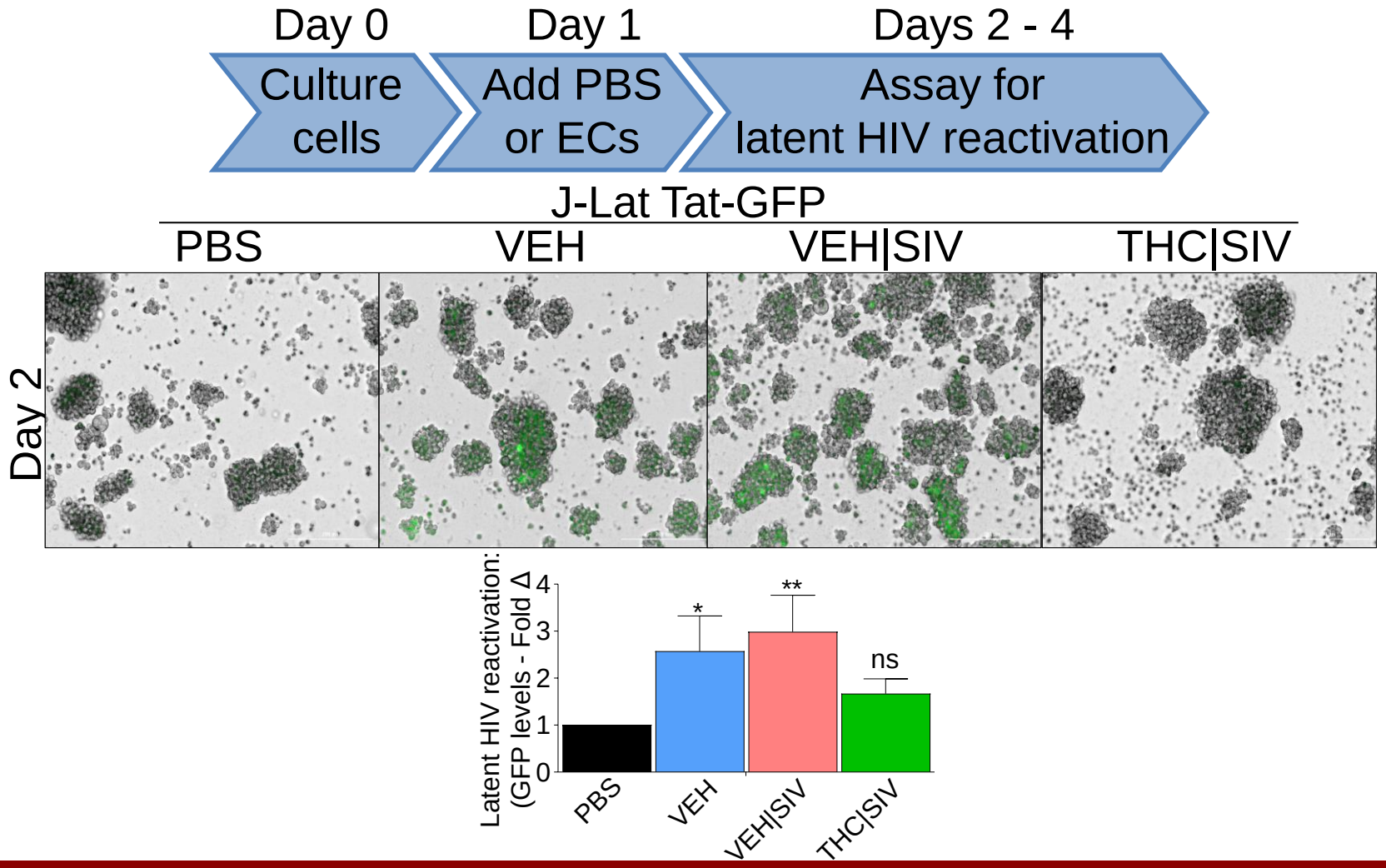
U1 cells



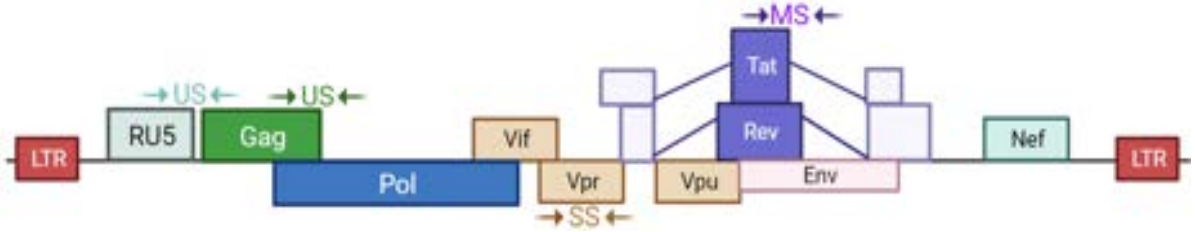
Hμglia (HC69) cells



Are basal ganglia derived ECs functional?



Do ECs alter HIV RNA expression and where in the viral genome do ECs affect?



Disrupting the balance of message ratios impairs viral replication

9 Kb

Full length HIV RNA spliced into >40 mRNAs

Unspliced (US) → Gag, Pol

Singly spliced (SS) → Vpr, Env, Vif, Vpu

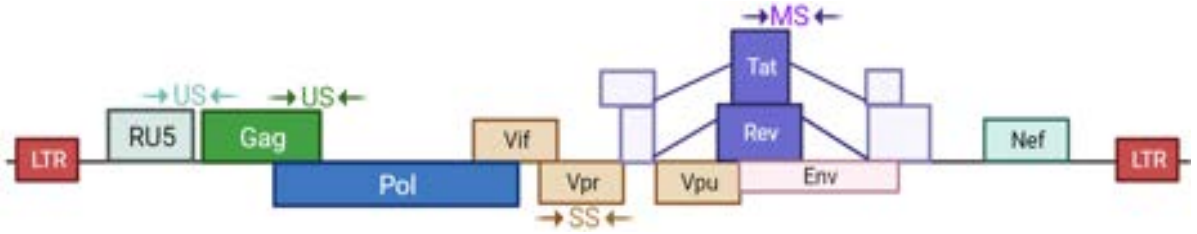
Multiply spliced (MS) → Tat, Rev, Nef

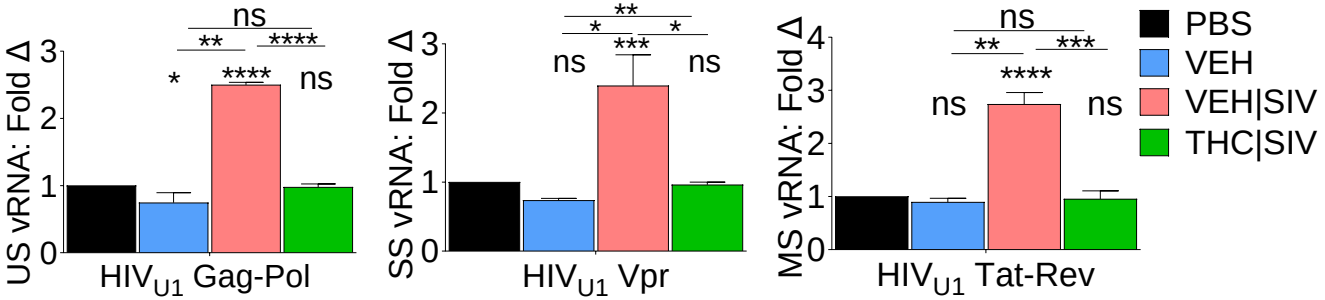
Do ECs alter HIV RNA expression and where in the viral genome do ECs affect?

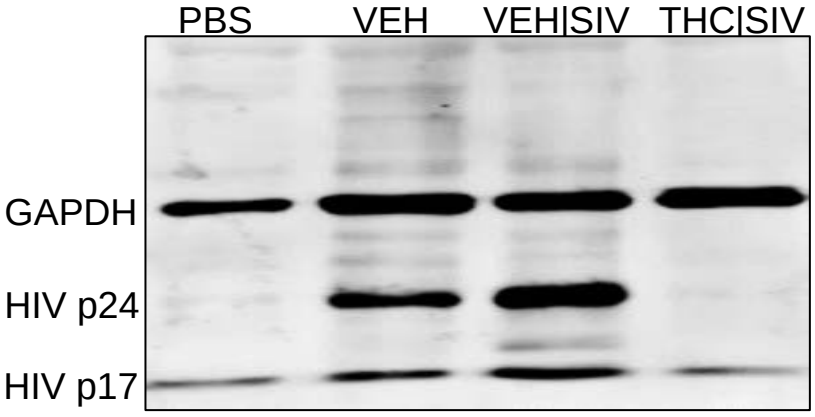
Day 0 Culture cells

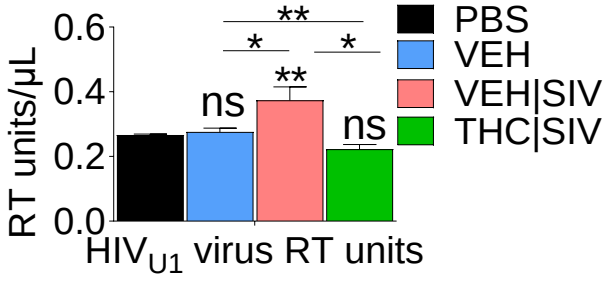
Day 1 Add PBS or ECs

Day 4 Assay for latent HIV reactivation

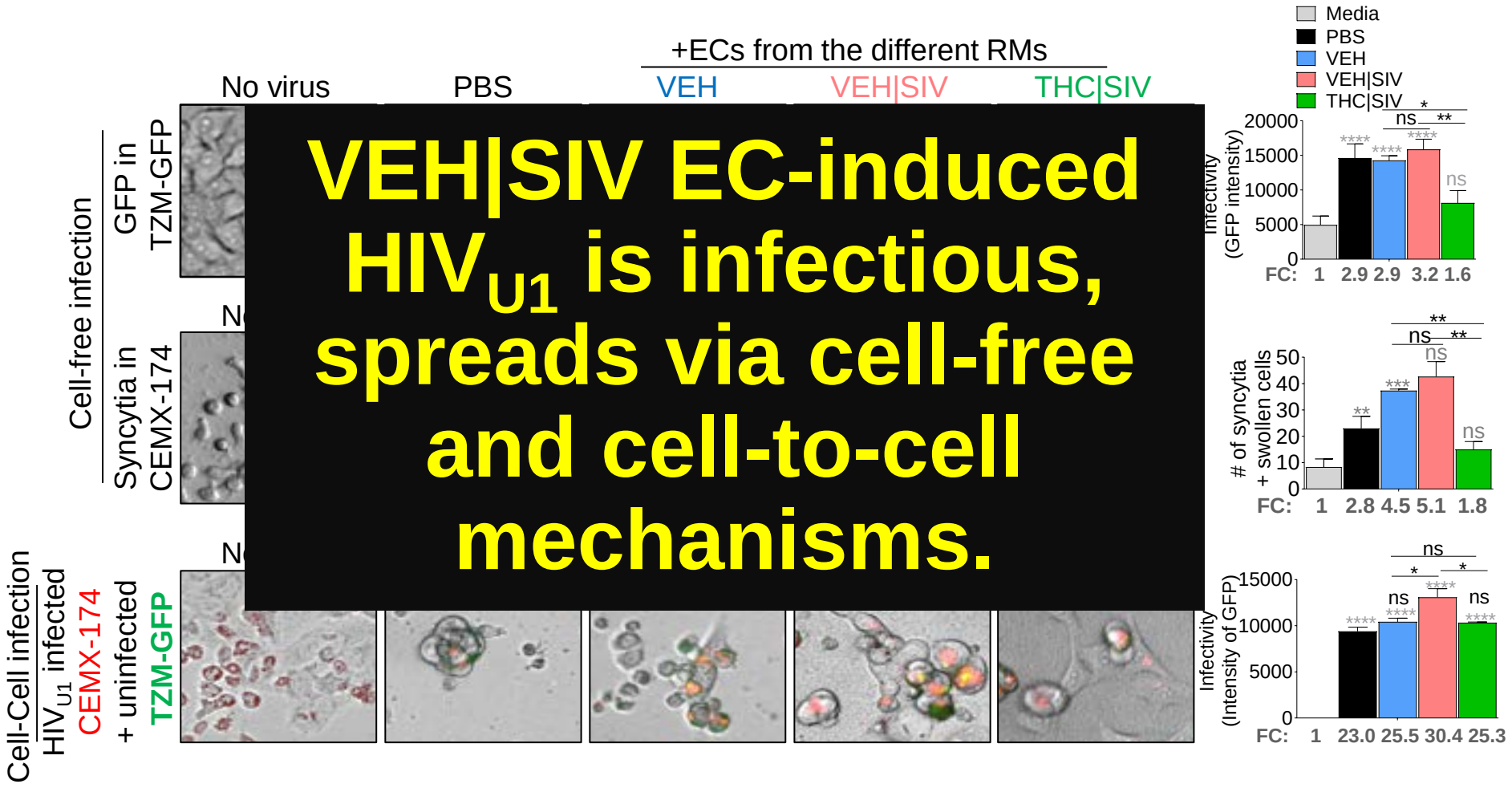






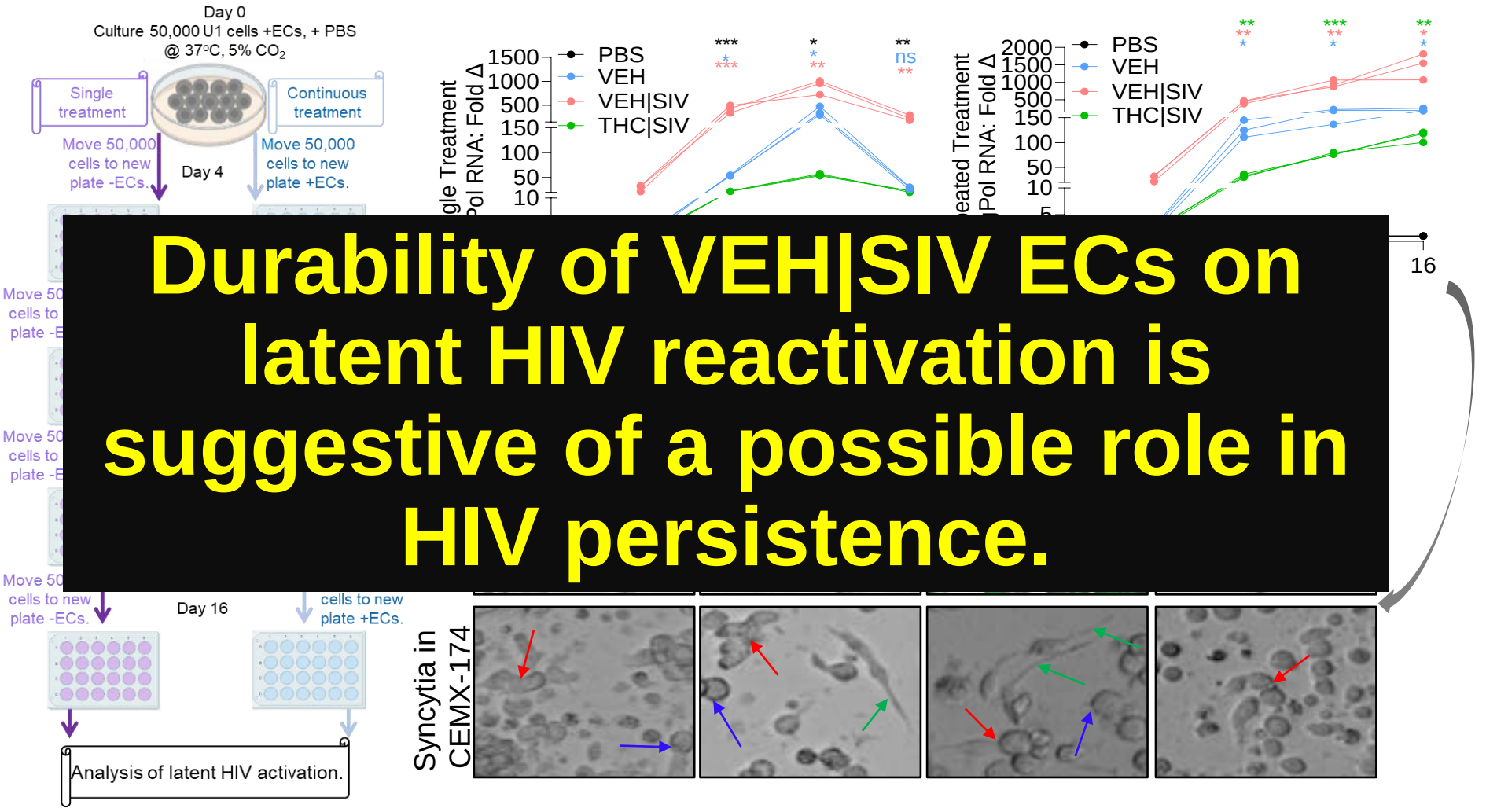


Do ECs induce the production of viral particles by HIV latently infected cells?



VEH|SIV EC-induced HIV$_{U1}$ is infectious, spreads via cell-free and cell-to-cell mechanisms.

Is the effect of ECs on HIV reactivation durable?



Durability of VEH|SIV ECs on latent HIV reactivation is suggestive of a possible role in HIV persistence.

ECs induce transcriptome & secretome changes in HIV latently infected cells

Activation of NF-kB complexes indicates that ECs may remodel the host immune system.

Activation of Replication Factor C4 (RFC4) complex indicates that ECs may remodel host cell DNA and chromatin.

Suppressed Cdk complex may have a role in cell cycle, apoptosis, and cell proliferation.

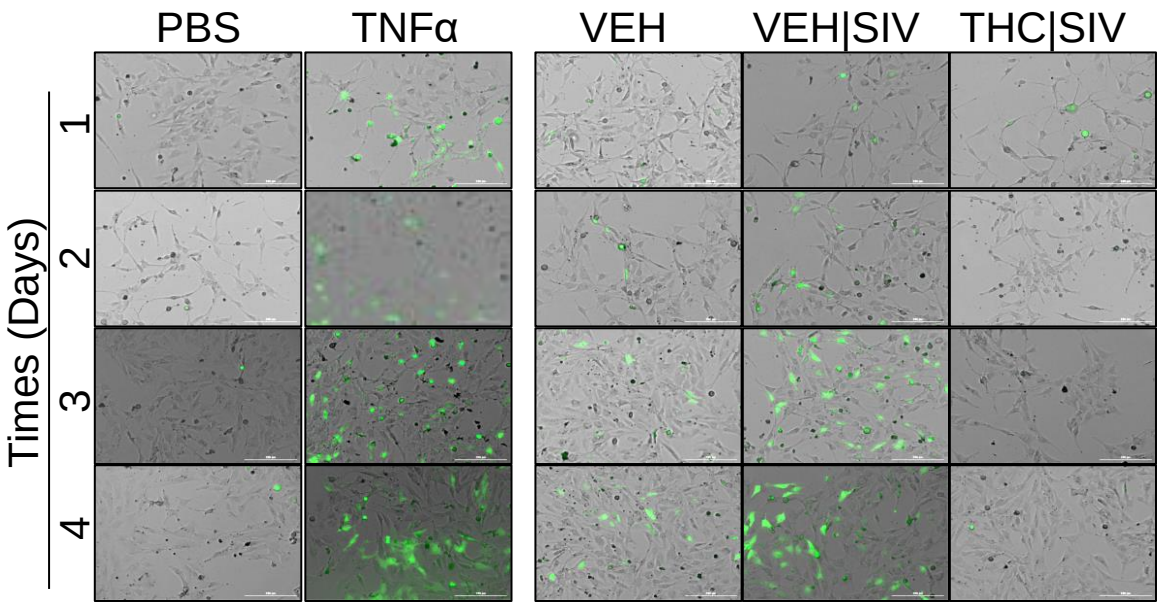
Are latently HIV infected microglia susceptible to reactivation by ECs?

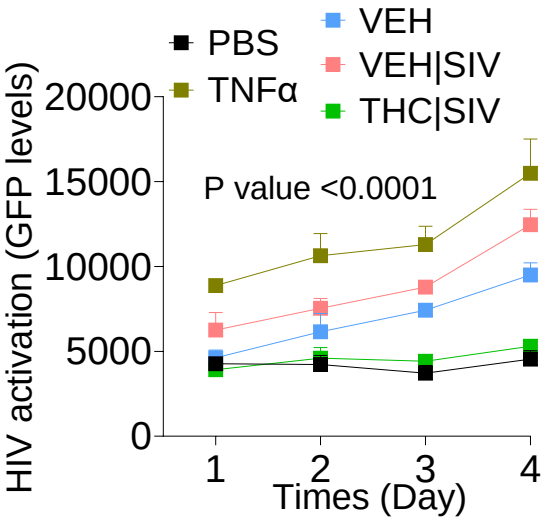
Microglia cells are resident brain cells that maintain BBB integrity

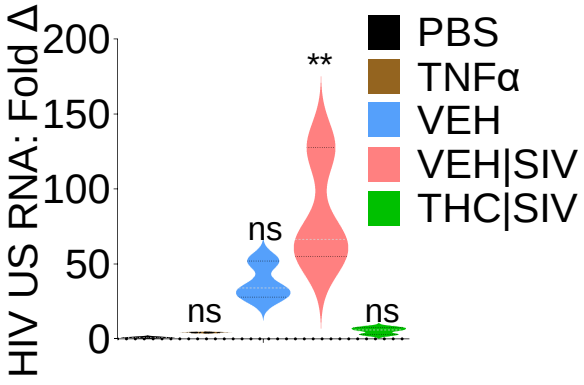
- Major reservoirs of HIV and mediators of neuroinflammation in the CNS
- Linked to HAND
- Play a role in the clearance of amyloid and Tau proteins—whose accumulation correlates with the presentation of AIDS dementia complex.

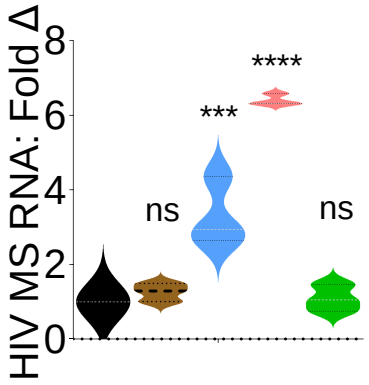
- **Hμglia - HC69 cells made by Garcia-Mesa et al., 2017**

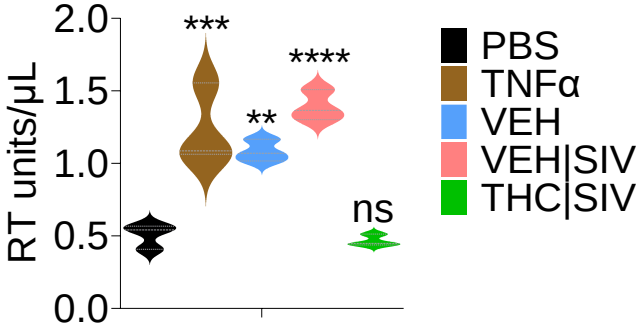
ECs potentiate latent HIV reactivation in microglia



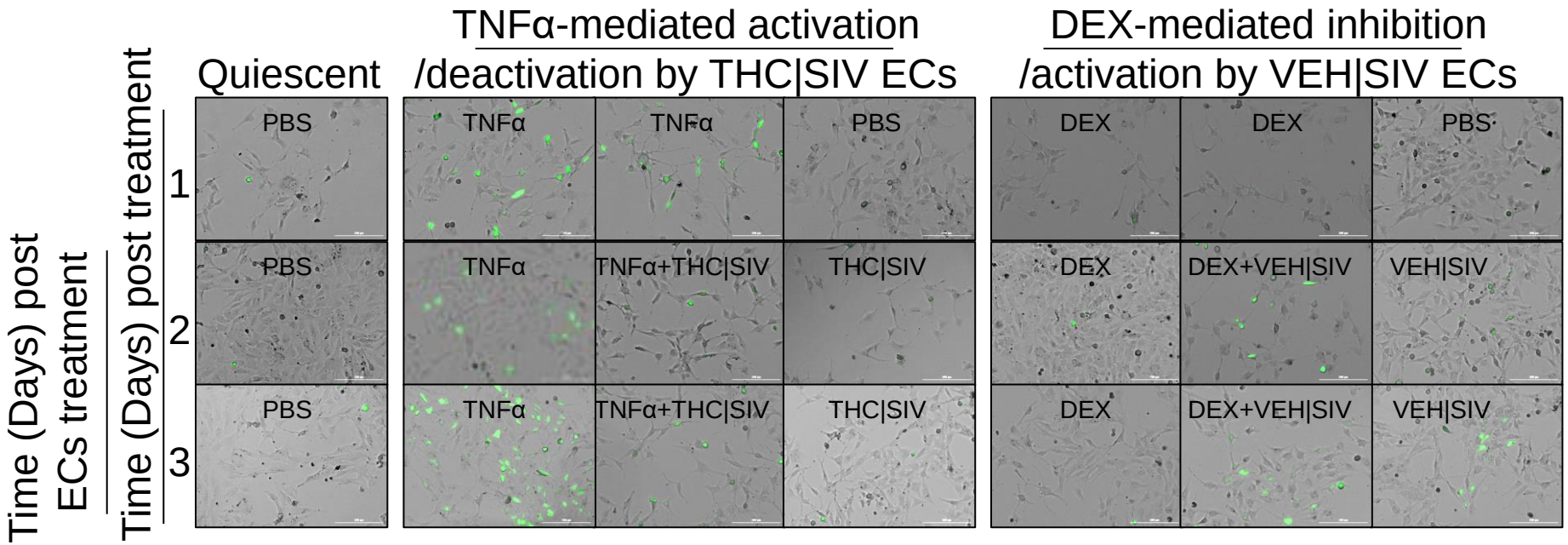


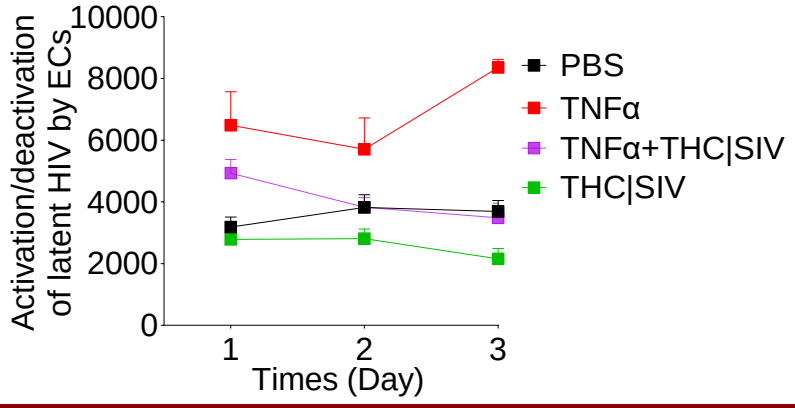






ECs are critical in the reactivation and inhbition of latent HIV in microglia





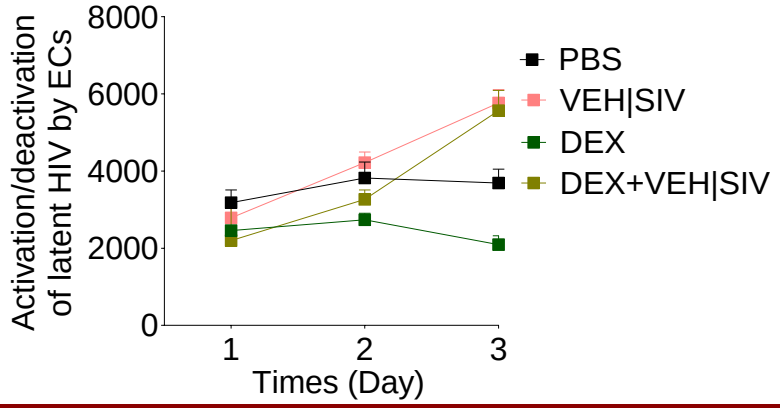
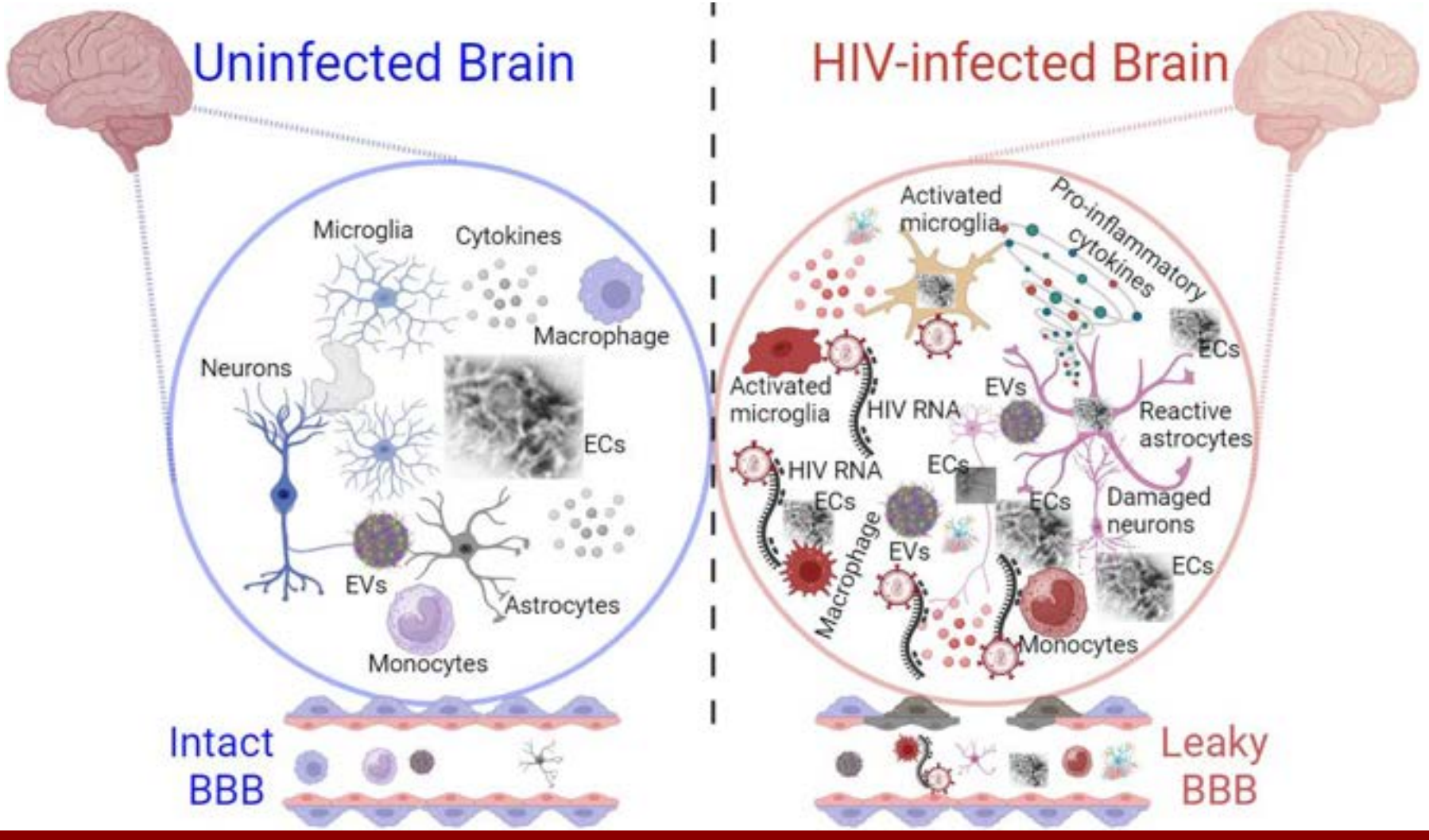


Illustration of the potential effects of pathogenic ECs in the brain of uninfected vs infected brains



Conclusions

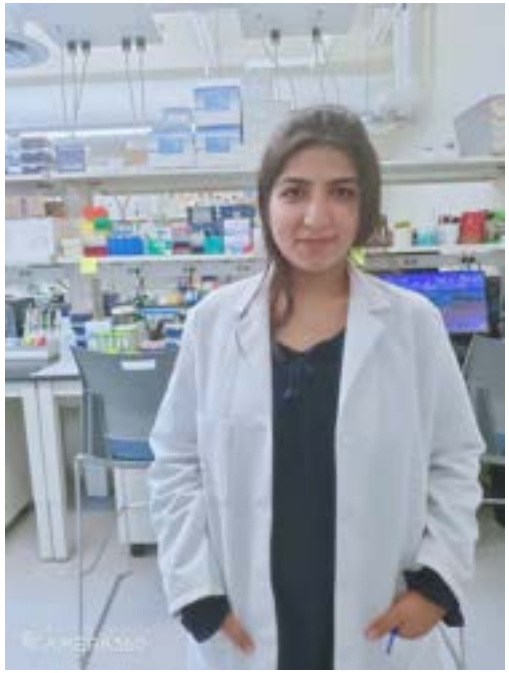
- Our work reveals new information on extracellular condensates-related effects on HIV by demonstrating that these condensates, depending on their source may promote HIV persistence.

- Our work also reveals the complexity on extracellular condensates in mediating changes in HIV latently infected cells at the level of transcriptome and secretome.

- Overall, our observations indicate that extracellular condensates regulate HIV latency reactivation or inhibition of HIV reactivation and production of viral particles from a latent state.

Acknowledgement

Dr. Naushad



Funding

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NYMC

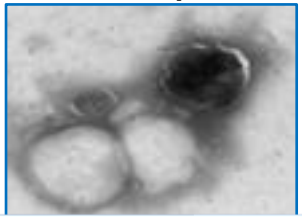
- Start-up funds

Collaborator

- Dr. Mahesh Mohan (TXBioMed Research Institute)

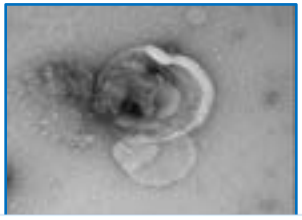
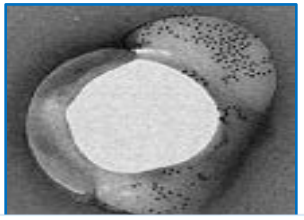
EVs & ECs are heterogenous in shape & size

Blood plasma Seminal plasma

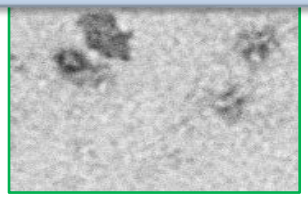
EVs

Brain tissues

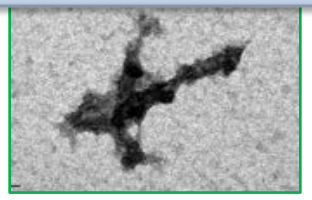



EVs

- This analysis shows that we can successfully separate EVs from ECs.

ECs

ECs

Kaddour et al., 2020, Alvarez et al., 2022, Kopcho et al., 2023, Naushad et al., unpublished