



The NCOR₂-Nurr₁-CoREST Transrepression Axis Impairs HIV Reactivation in Latently Infected Microglial Cells: Implication for HIV-associated Neurocognitive Disorders

David Alvarez-Carbonell, PhD

Department of Molecular Biology and Microbiology
Case Western Reserve University
Cleveland, OH

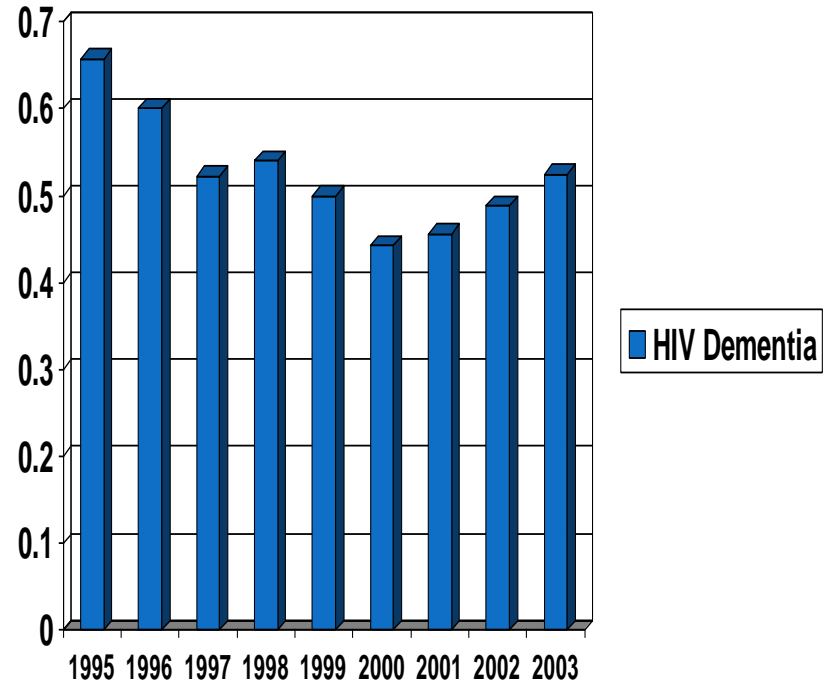
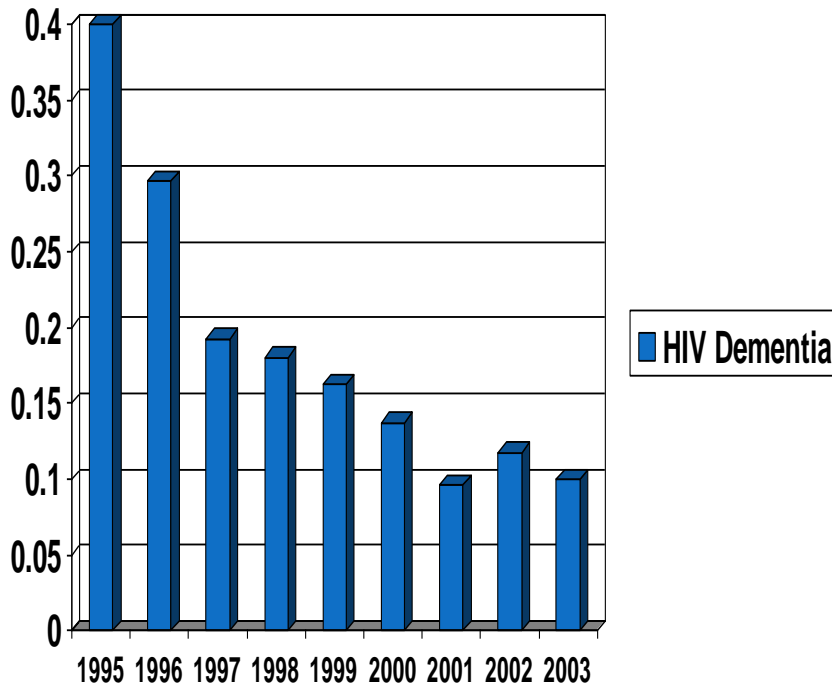


Jonathan Karn's Lab

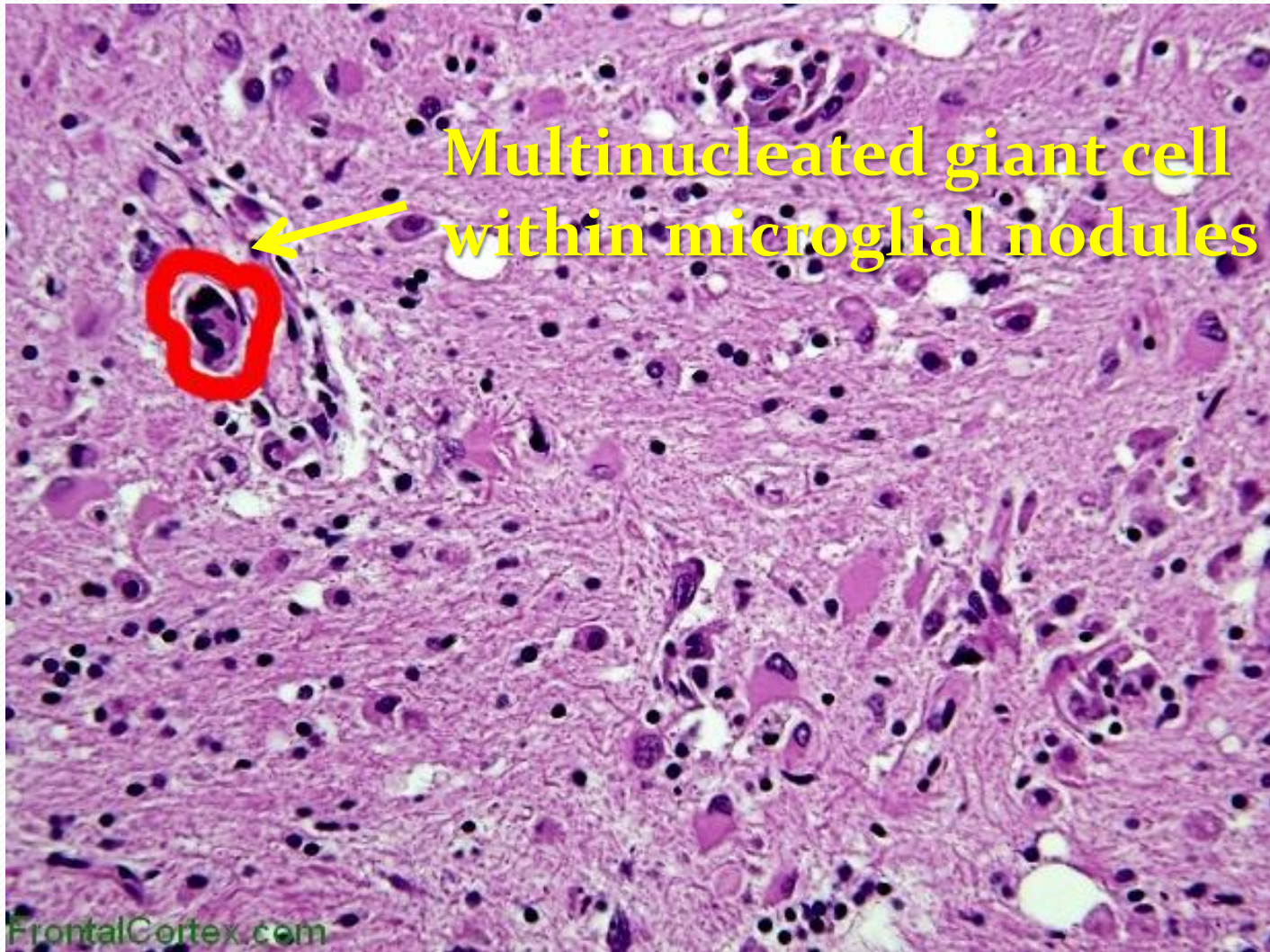
HIV Infection of the Central Nervous System



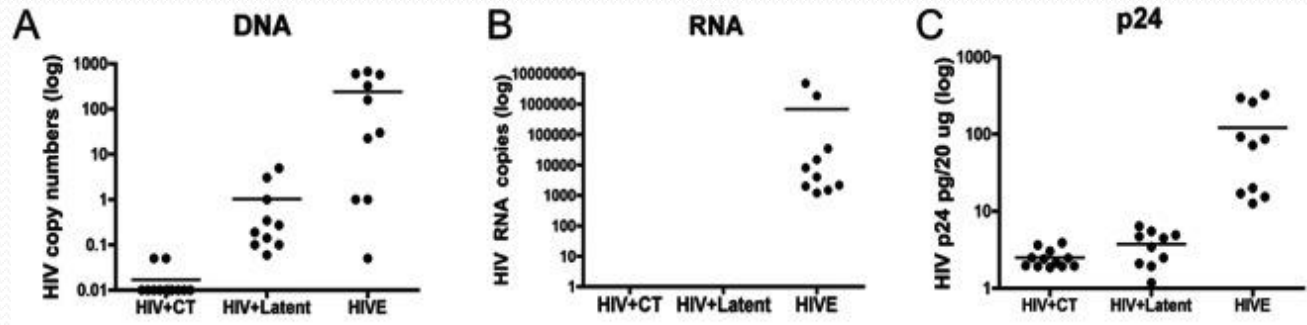
HIV-associated dementia (HAD) and conditions associated with neurocognitive disorders (HAND)



The Signature Lesion of HAD: Encephalopathy

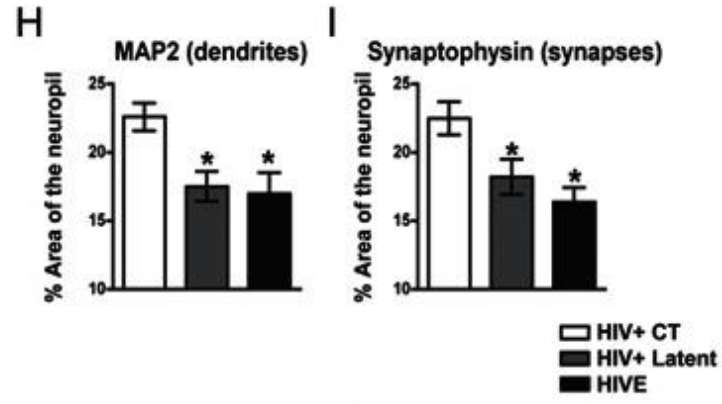
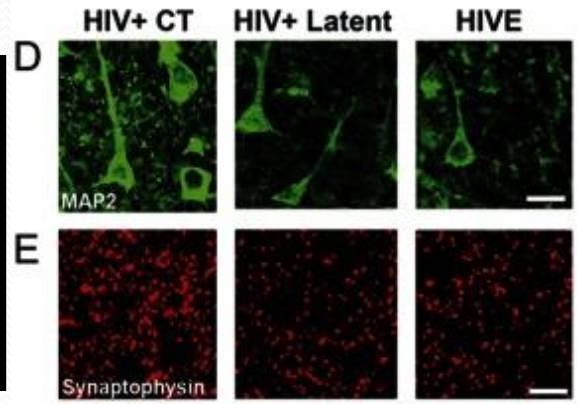


Brain as a Latent HIV Reservoir during HAART



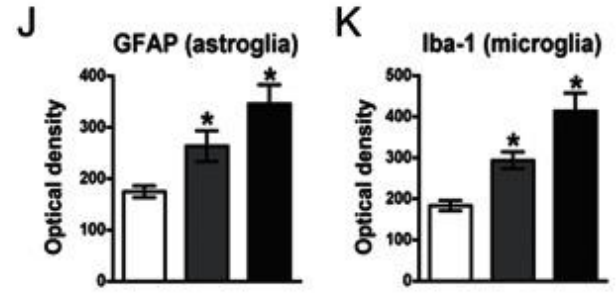
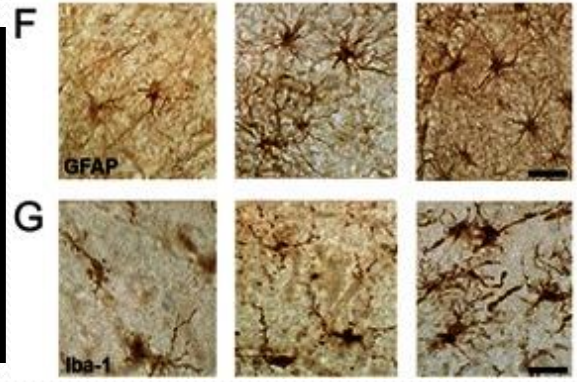
- 32 cases; 23 with antemortem neurocognitive diagnosis

Neurodegeneration markers in frontal cortex

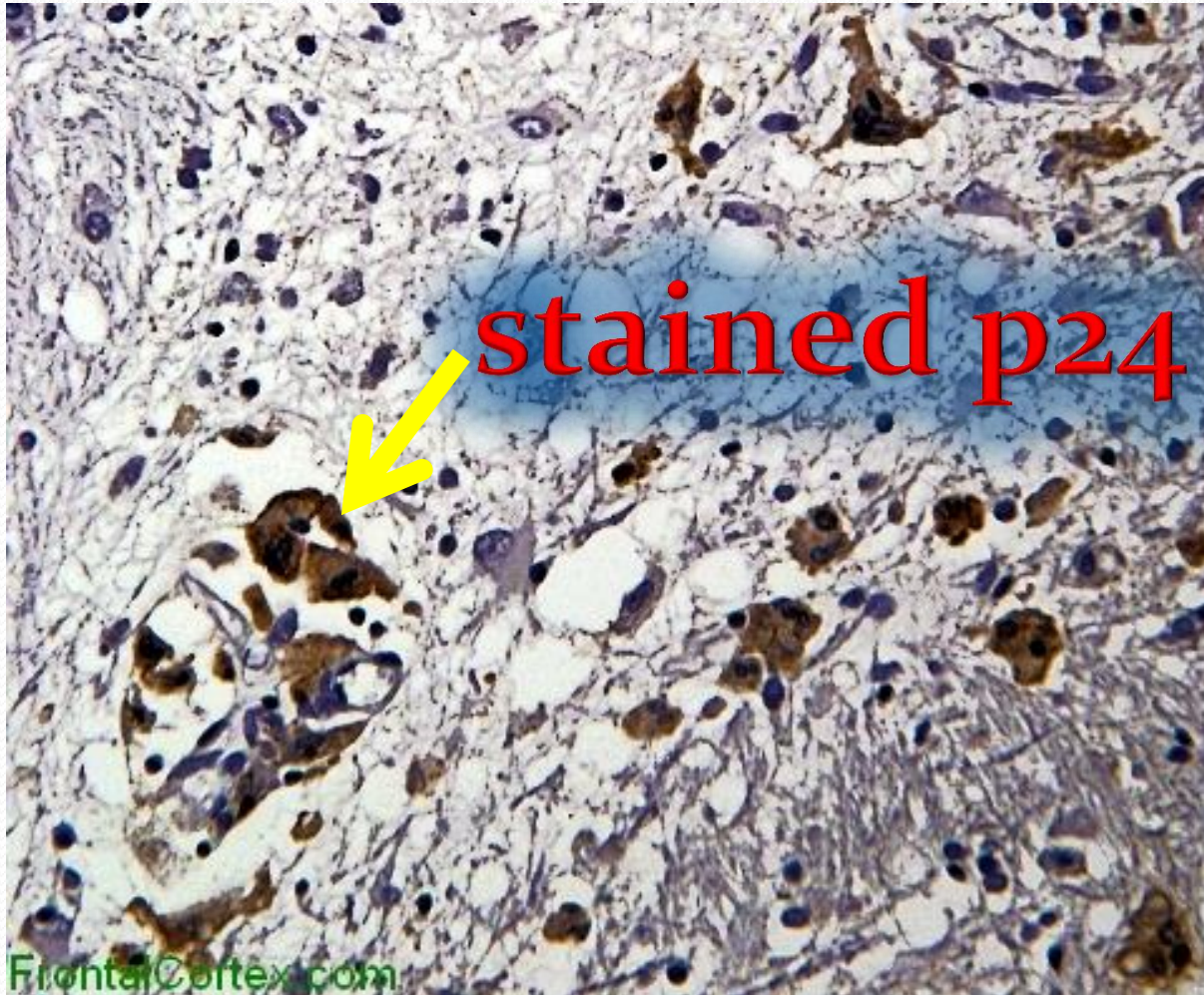


- Most "latent" cases showed mild to moderate cognitive impairment with neurodegenerative and neuroinflammatory alterations

Astroglia/microglia markers in frontal cortex



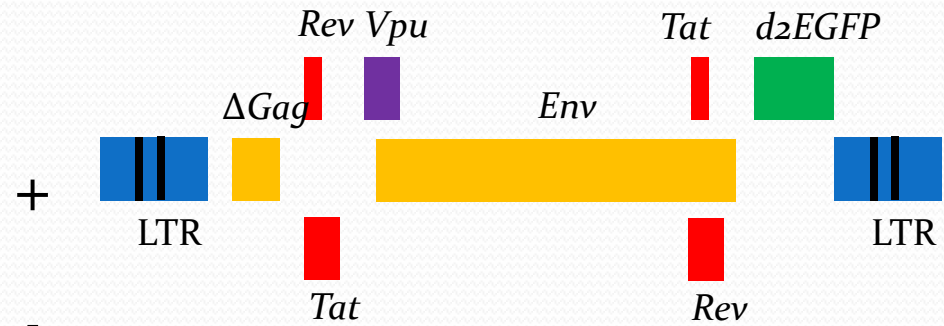
Active infection within mononuclear and microglial cells, but not within neurons



HIV Latency in Microglial Cells

CHME-5 microglial Cells
(SV40-immortalized)

hT-Hμglia microglial Cells
(hTERT-immortalized)

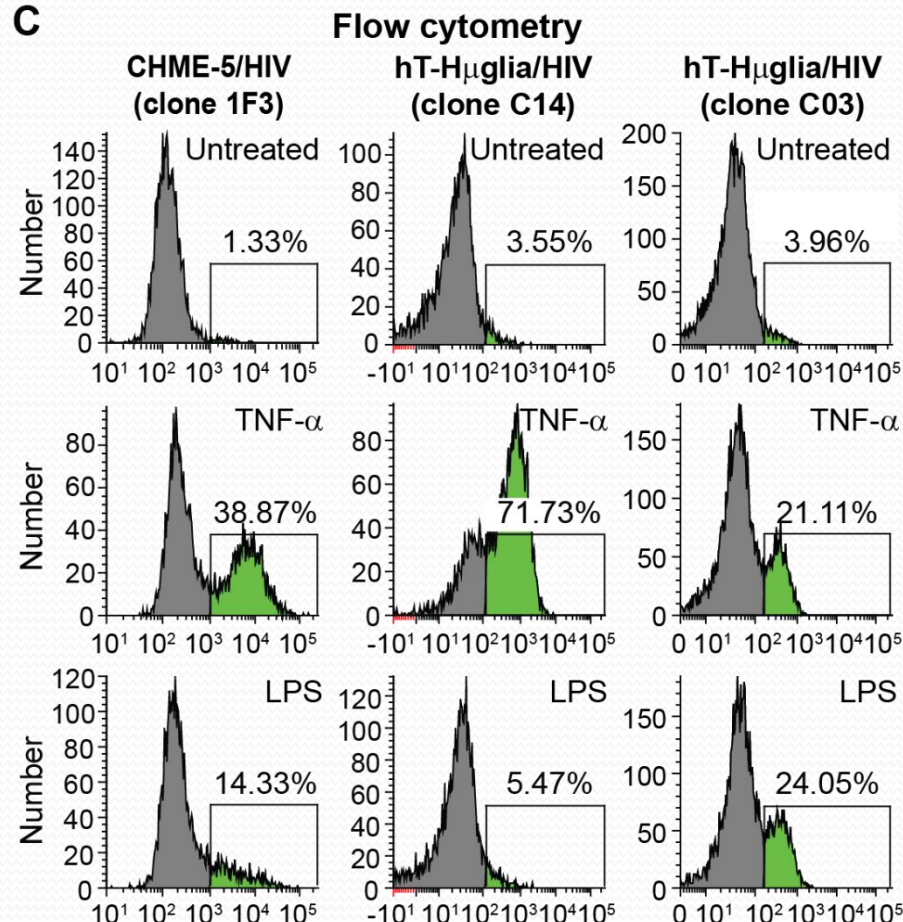
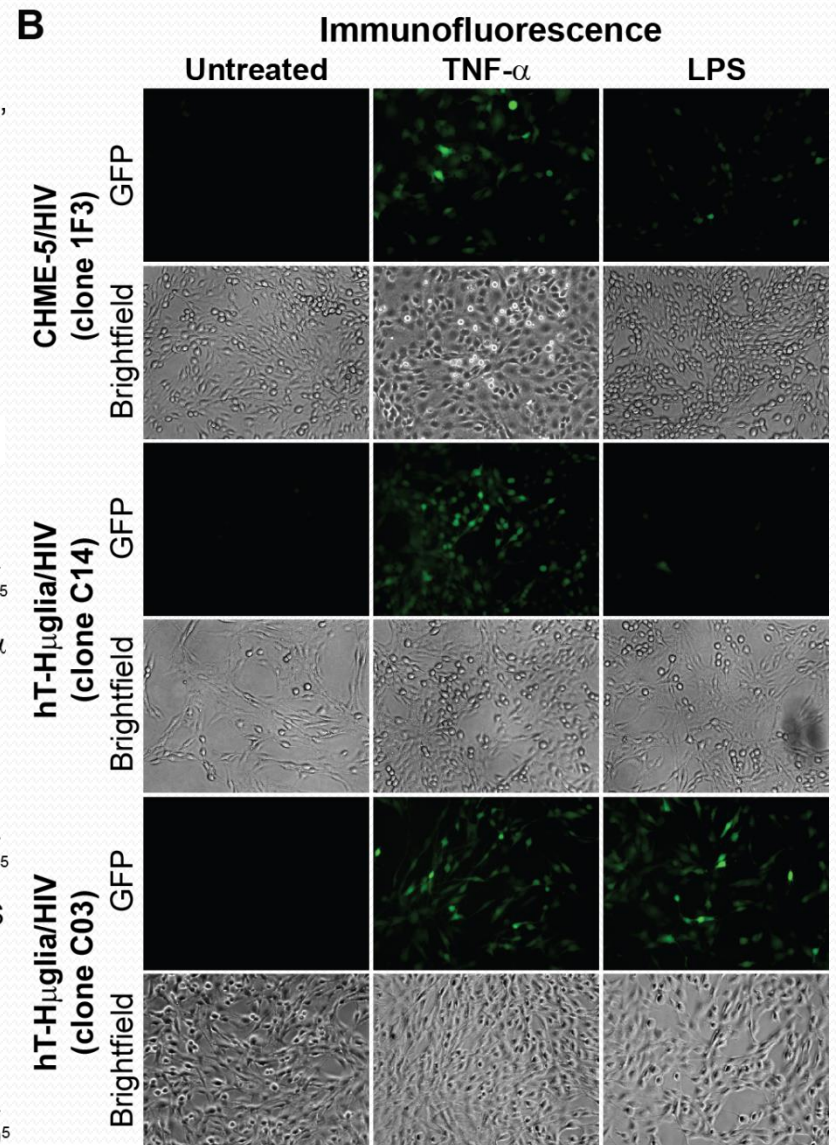
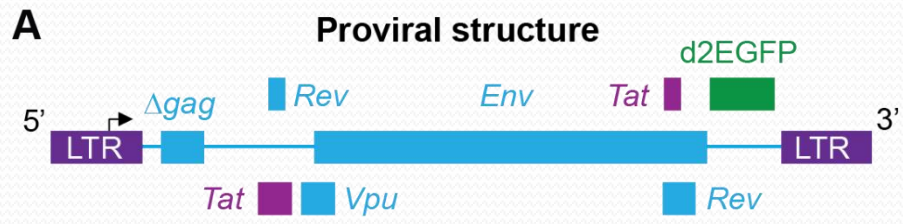


Selection for GFP⁺ cells

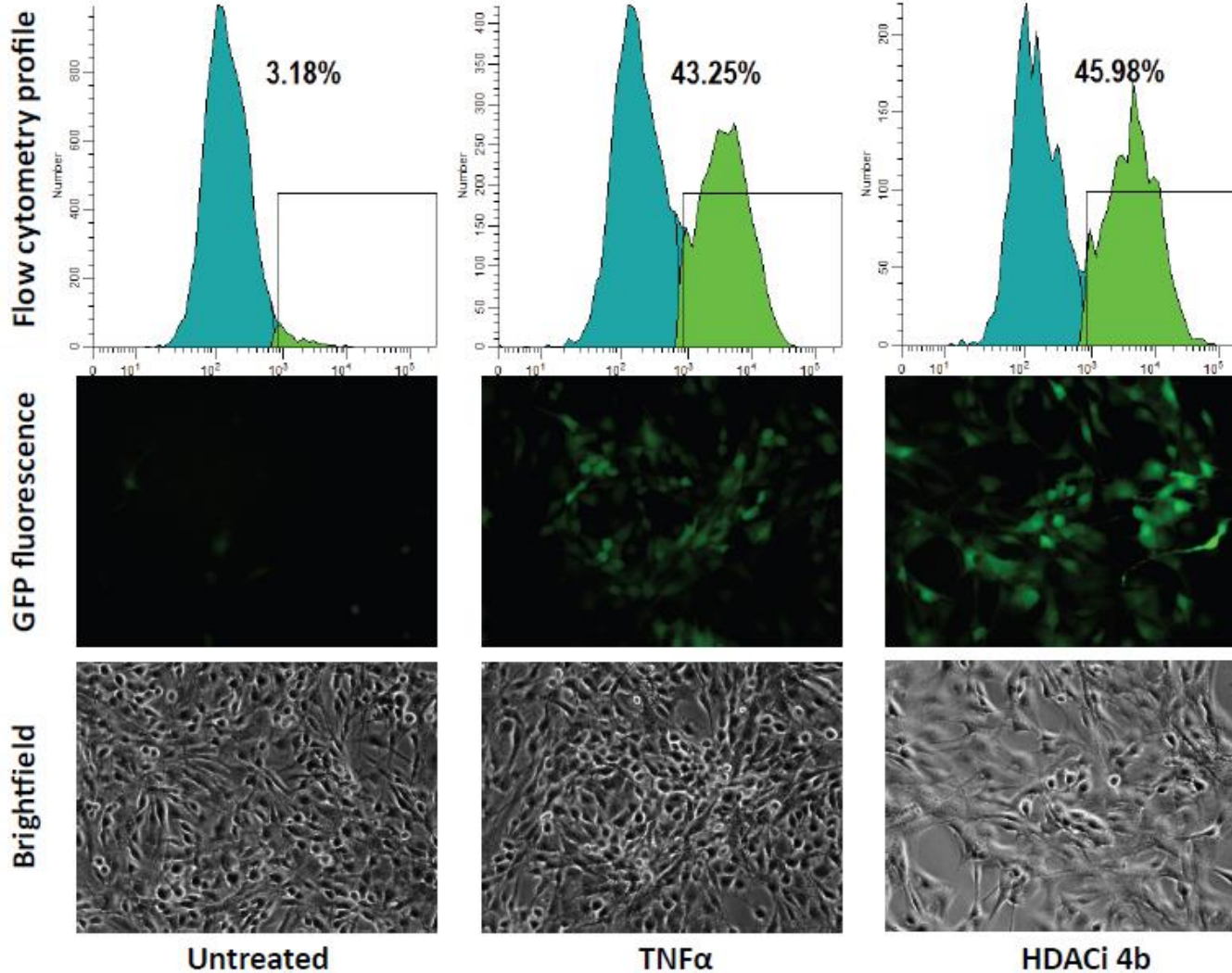


Grow infected cells until GFP signal is low (below 5%)

Re-activation of Latent HIV in Microglial/HIV Cells

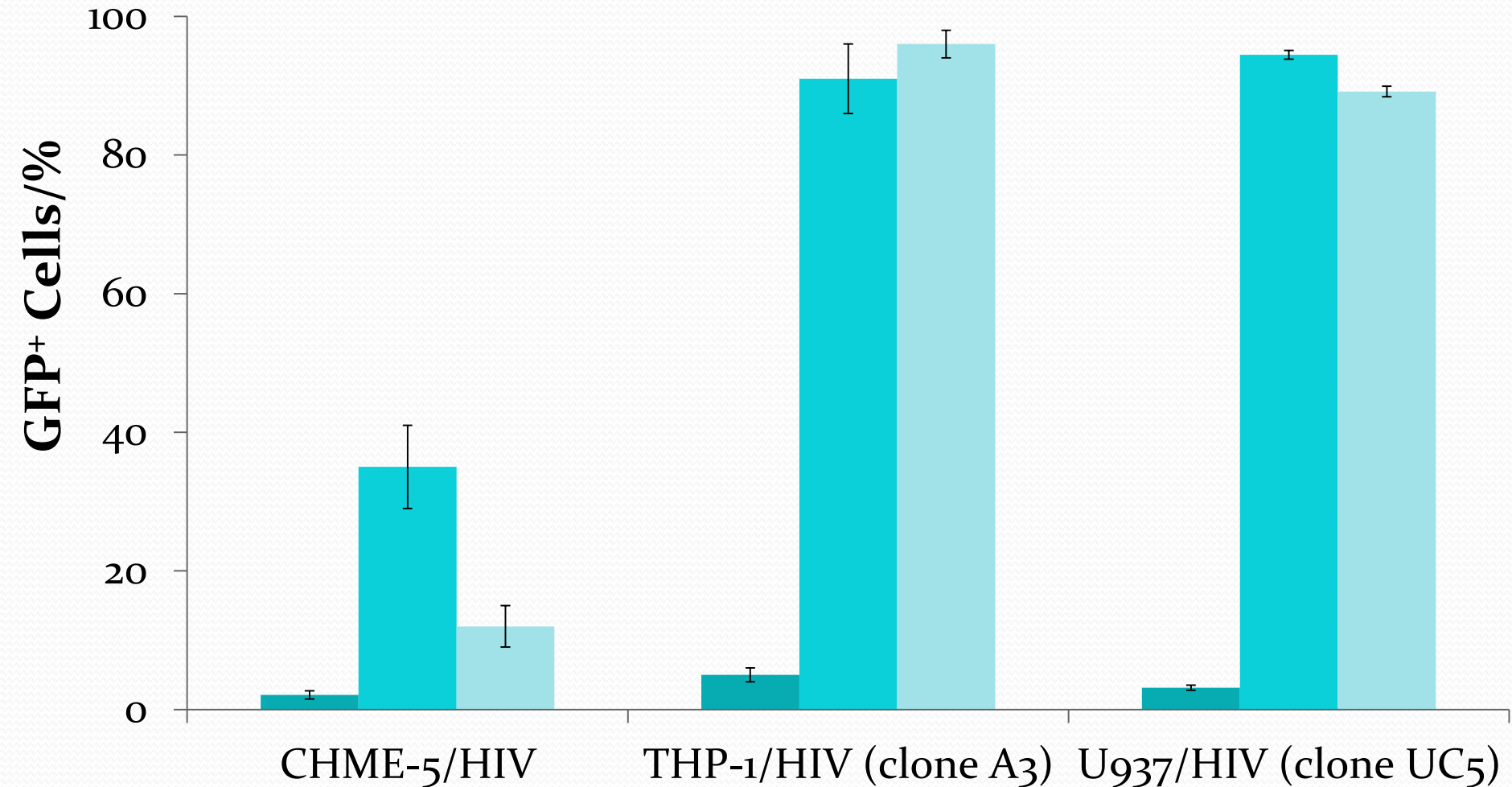


Latency is due to Chromatin Restrictions

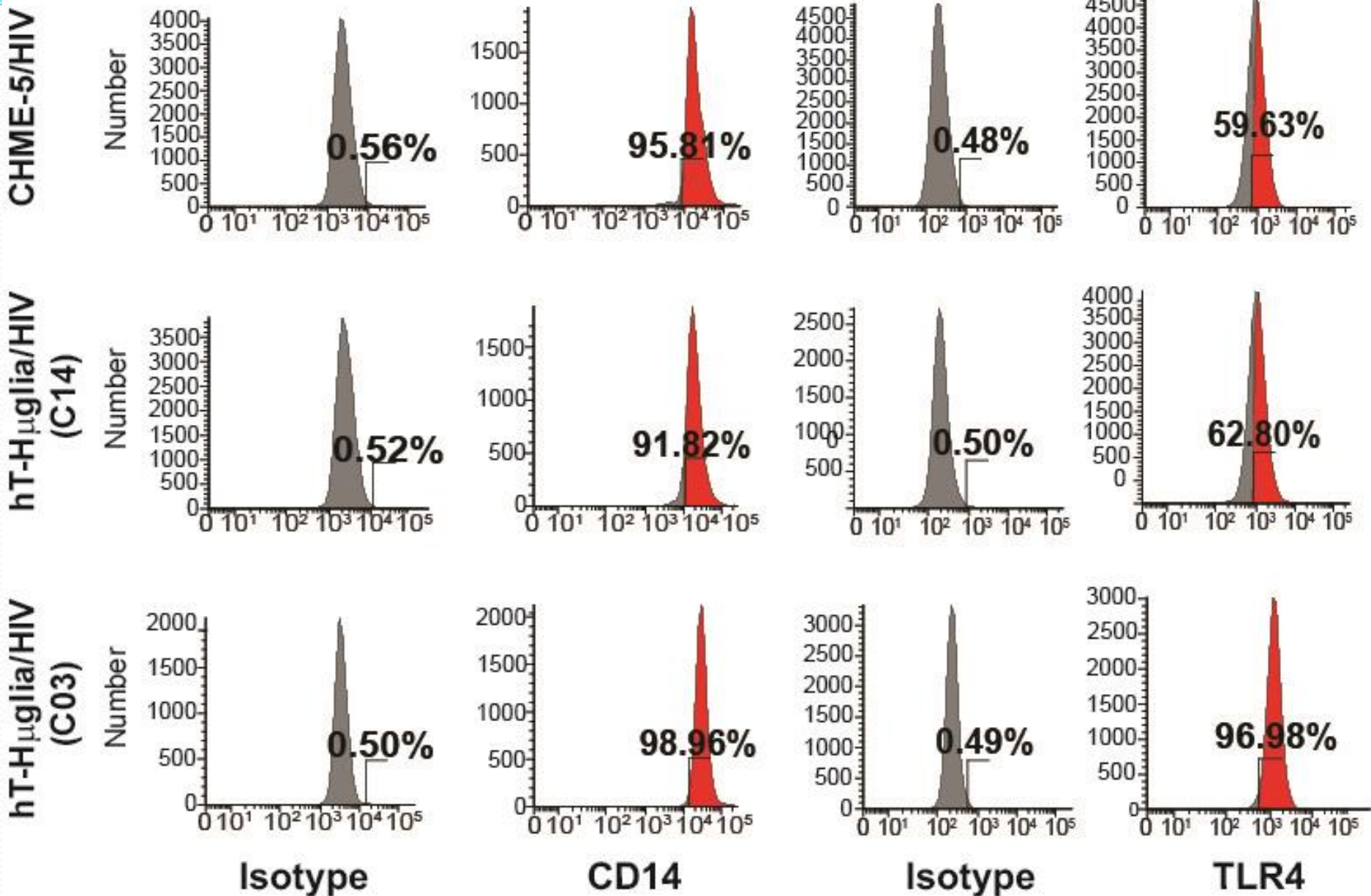


Reactivation of HIV by Potent Classical Activators is NOT Restricted in Monocytic Cells

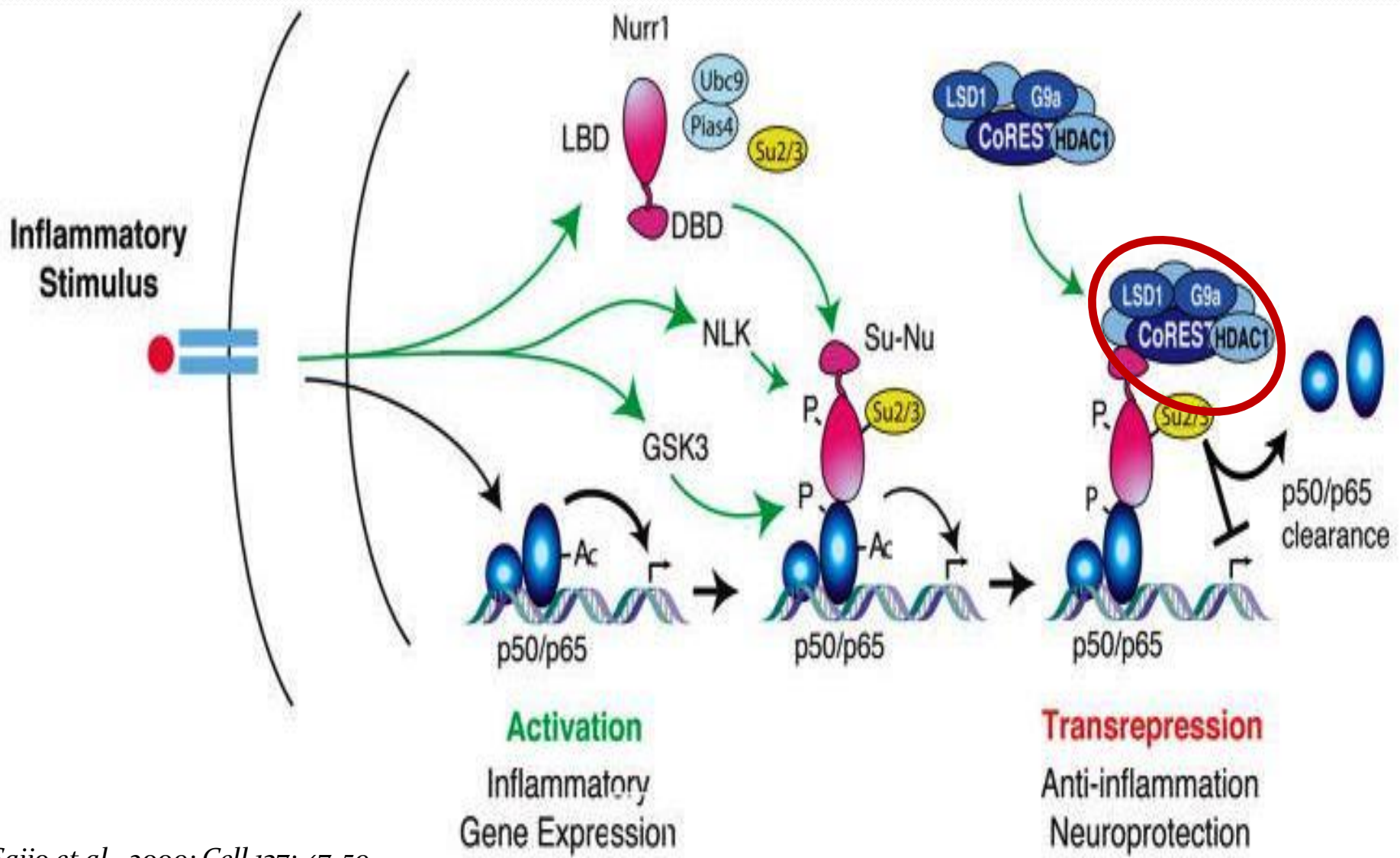
■ Untreated ■ TNF α ■ LPS



Surface Expression of CD14 and TLR4, LPS Co-Receptors



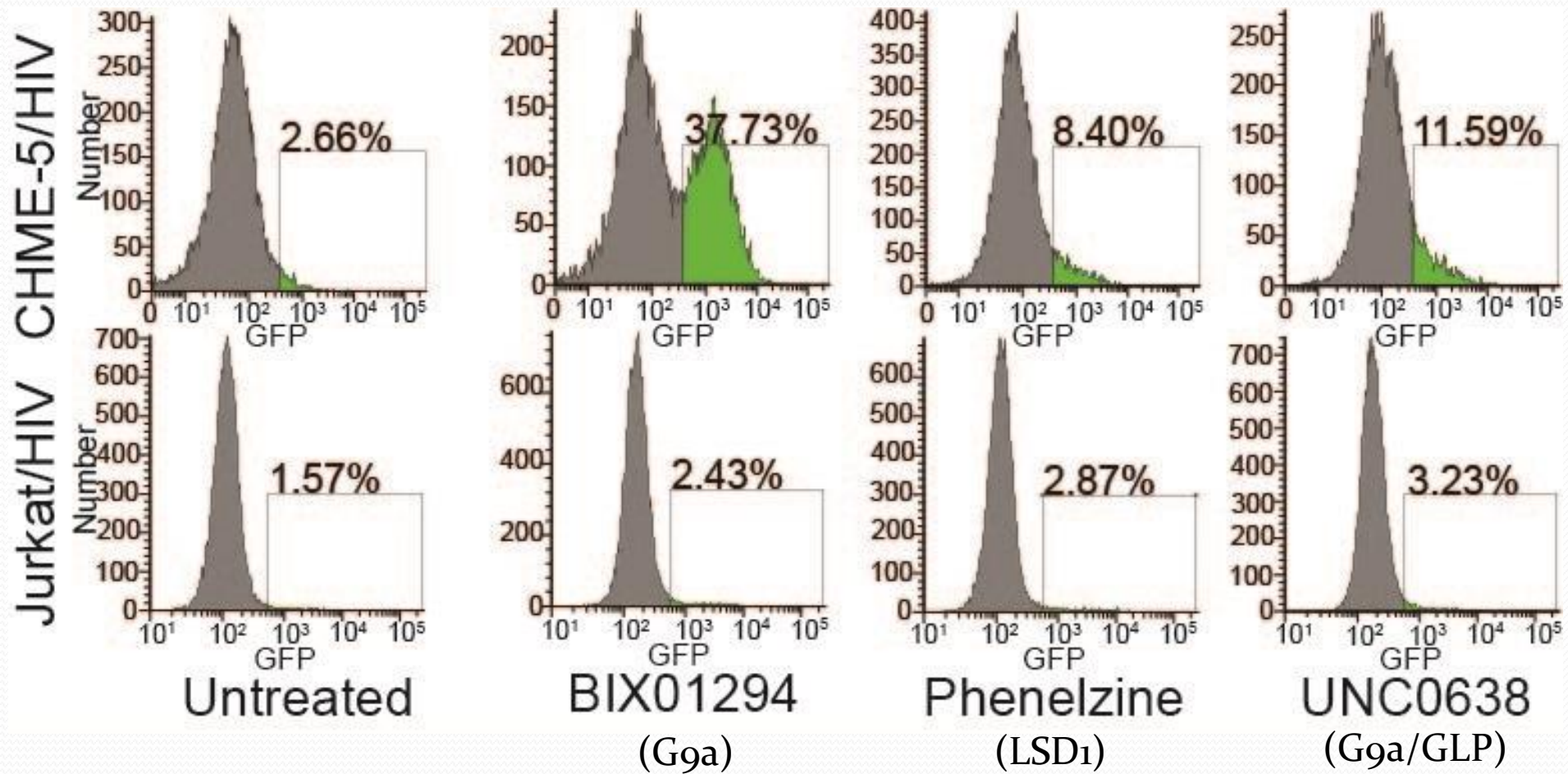
The Nurr1/CoREST Trans-Repression Pathway



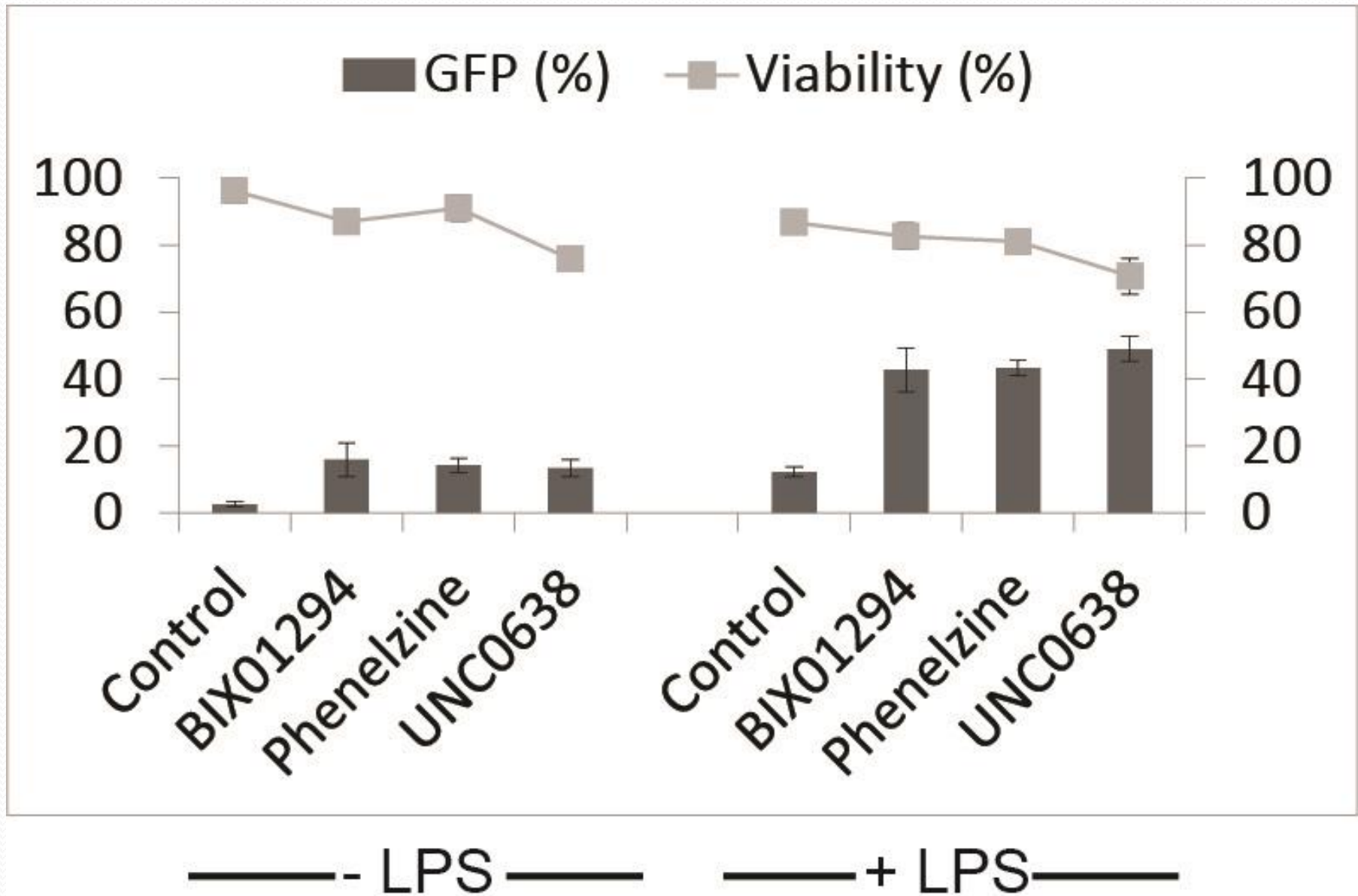
The Nurri Nuclear Receptor

- It's the NR_{4A2} of the NR₄ family of orphan nuclear receptors
- A constitutively active TF
- **Deletion of Nurri in mice results in reduction of dopaminergic neurons and perinatal lethality**
- **Human mutations in Nurri resulting in its reduced expression are associated with late-onset familial PD (protective role)**
- Expressed in non-neural cells, and induced by inflammatory stimuli including LPS in macs
- Critical role in microglia and astrocytes as a repressor, or limiting factor, preventing over-expression of pro-inflammatory neurotoxic proteins during normal inflammatory reactions in brain by recruiting CoREST complex containing G9a and LSD₁

HIV Reactivation is Controlled by the CoREST Complex in Microglia

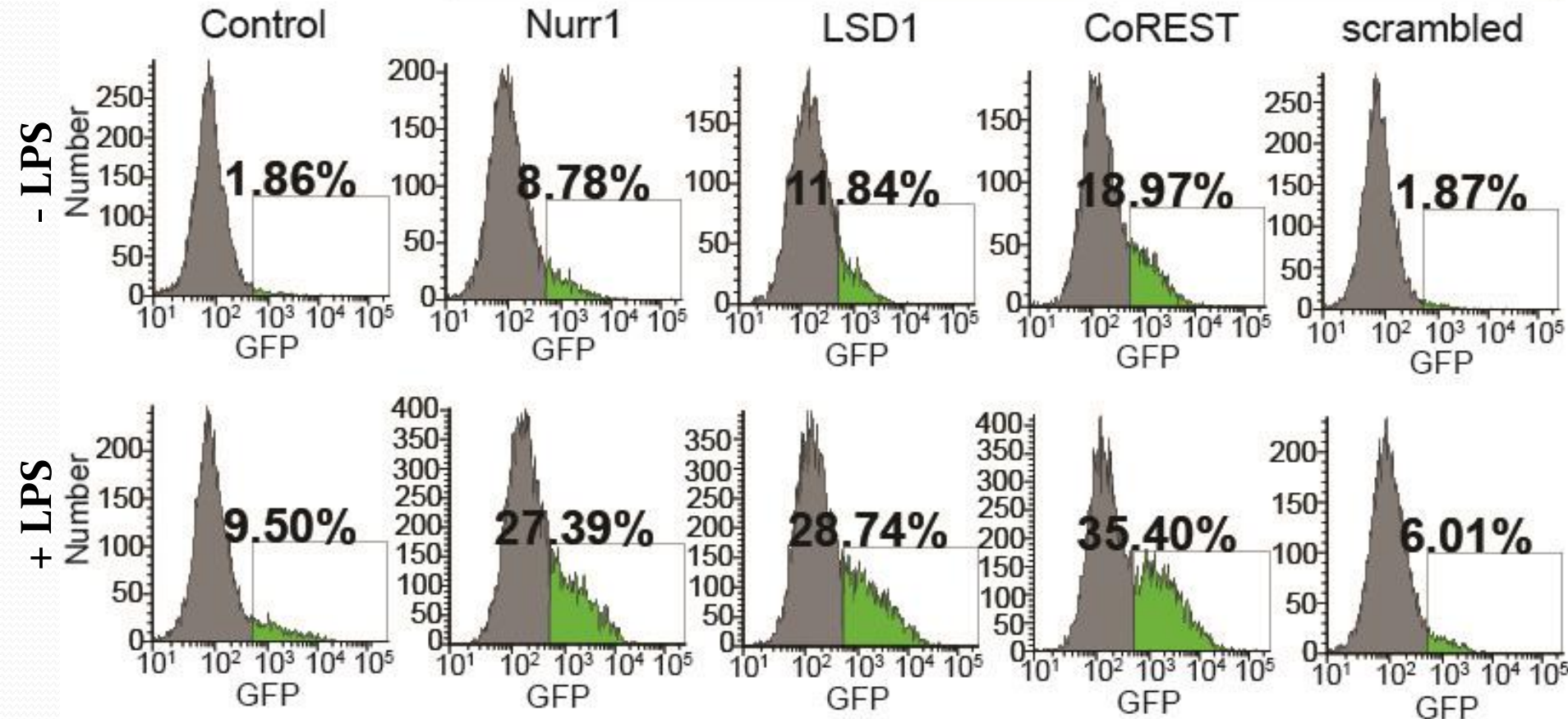


Inhibitors of the Nurrl/CoREST Complex Sensitize CHME-5/HIV Cells for LPS-Mediated HIV Reactivation



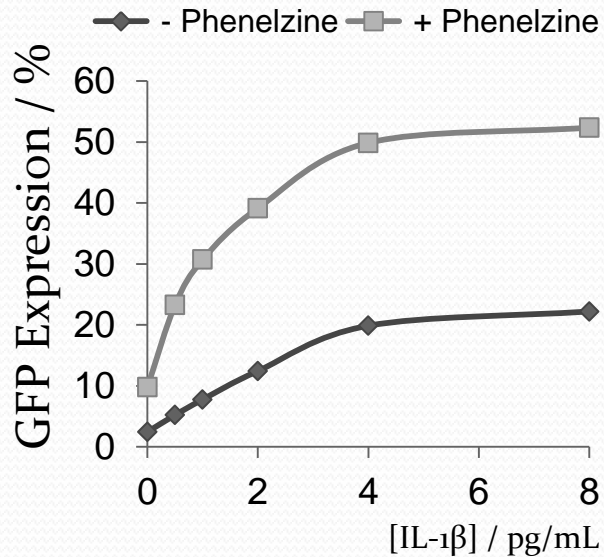
Knockdown of Nurri1/CoREST Induces and Sensitizes Cells for LPS-Mediated HIV Reactivation

shRNA

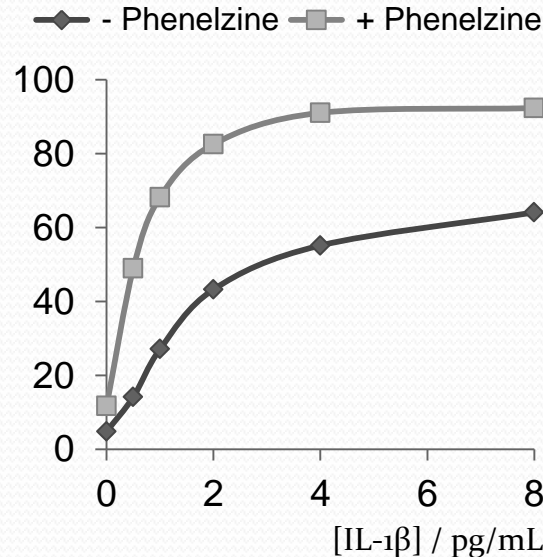


Synergy between Co-REST Complex Inhibitors and IL-1 β

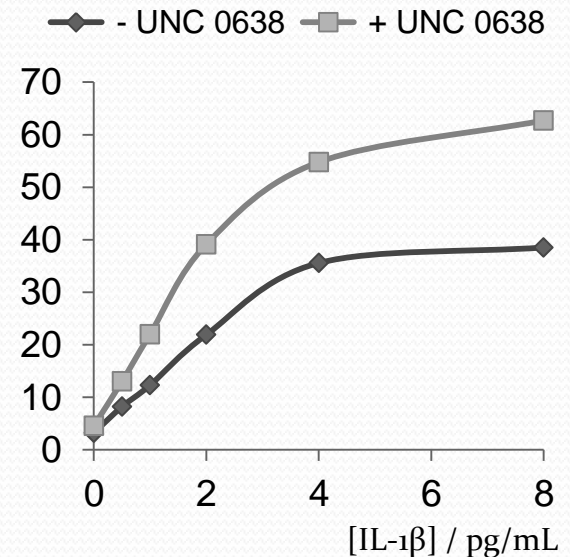
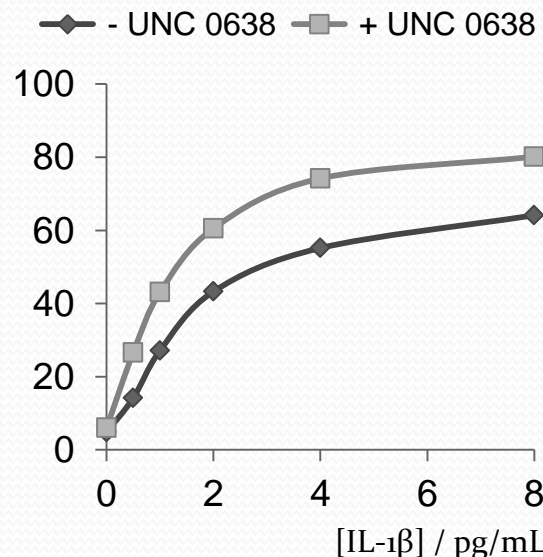
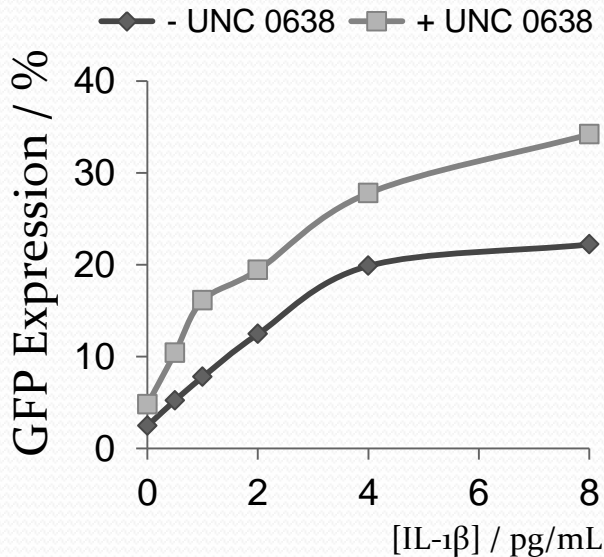
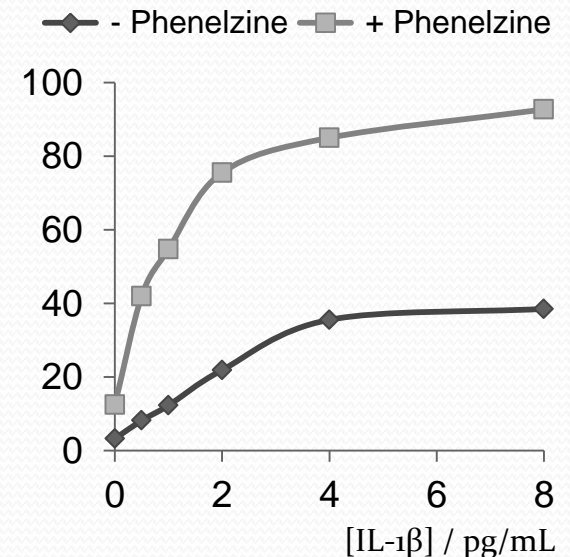
CHME-5/HIV



hT-H μ gla/HIV (C₁₄)

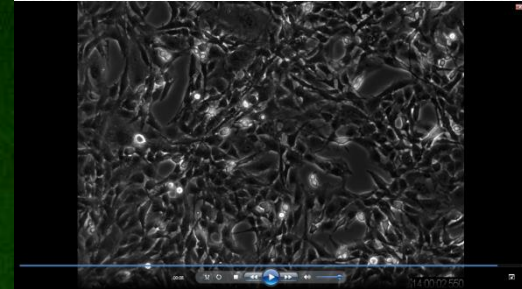


hT-H μ gla/HIV (C₀₃)



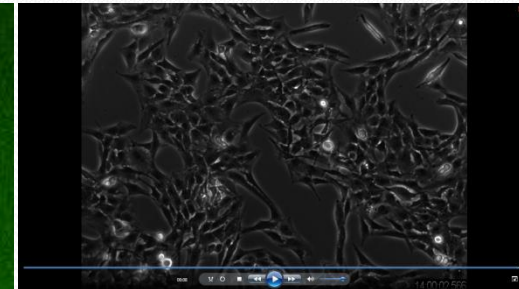
Synergistic Reactivation of Latent HIV by IL-1 β and Phenezine

Untreated



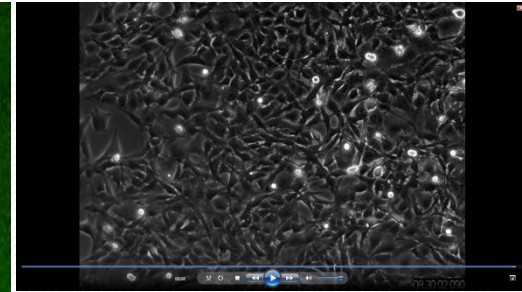
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Synergistic Reactivation of Latent HIV by IL-1 β and Phenyelzine



Synergistic Reactivation of Latent HIV by IL-1 β and Phenelzine

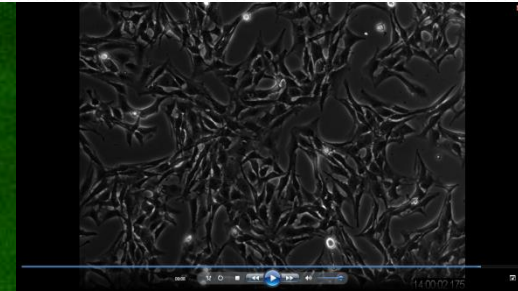
Phenelzine
(LSD1 inh)



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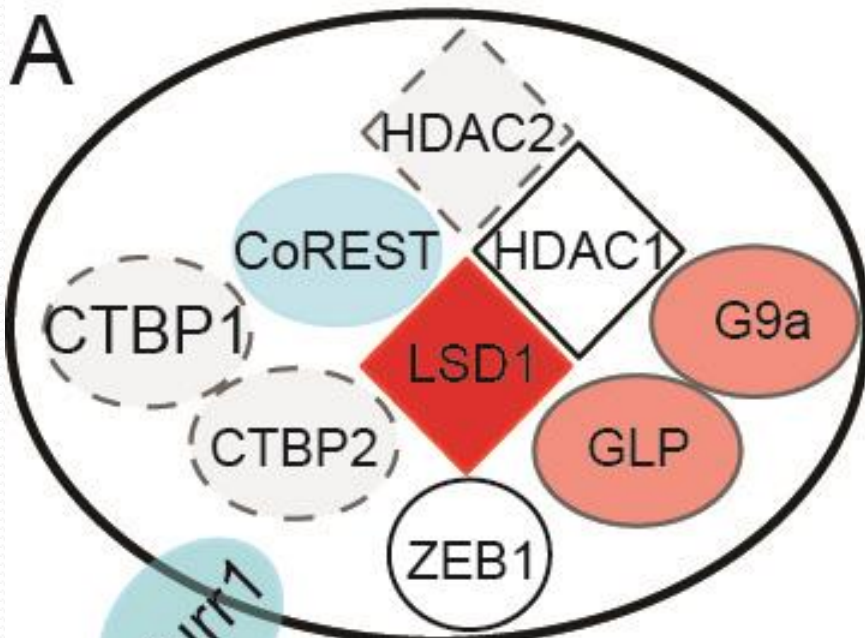
Synergistic Reactivation of Latent HIV by IL-1 β and Phenezine

Phe/IL-1 β

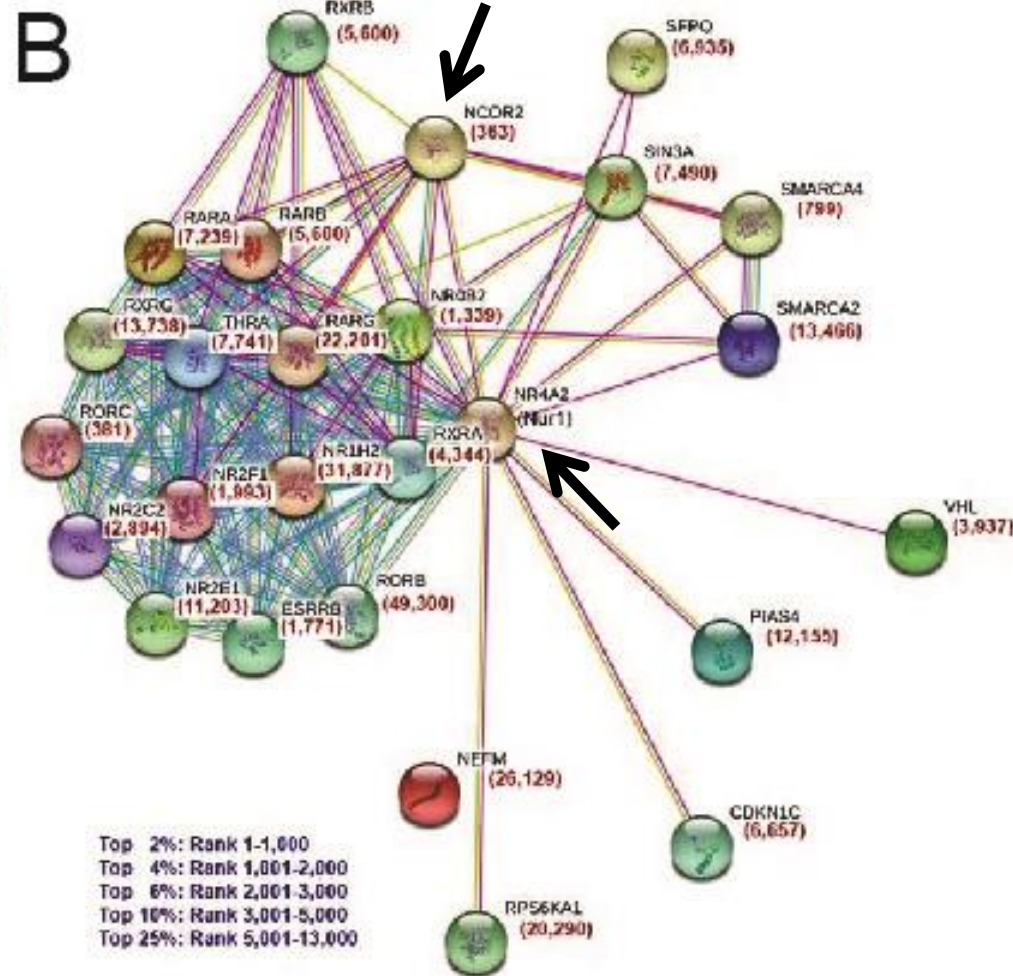


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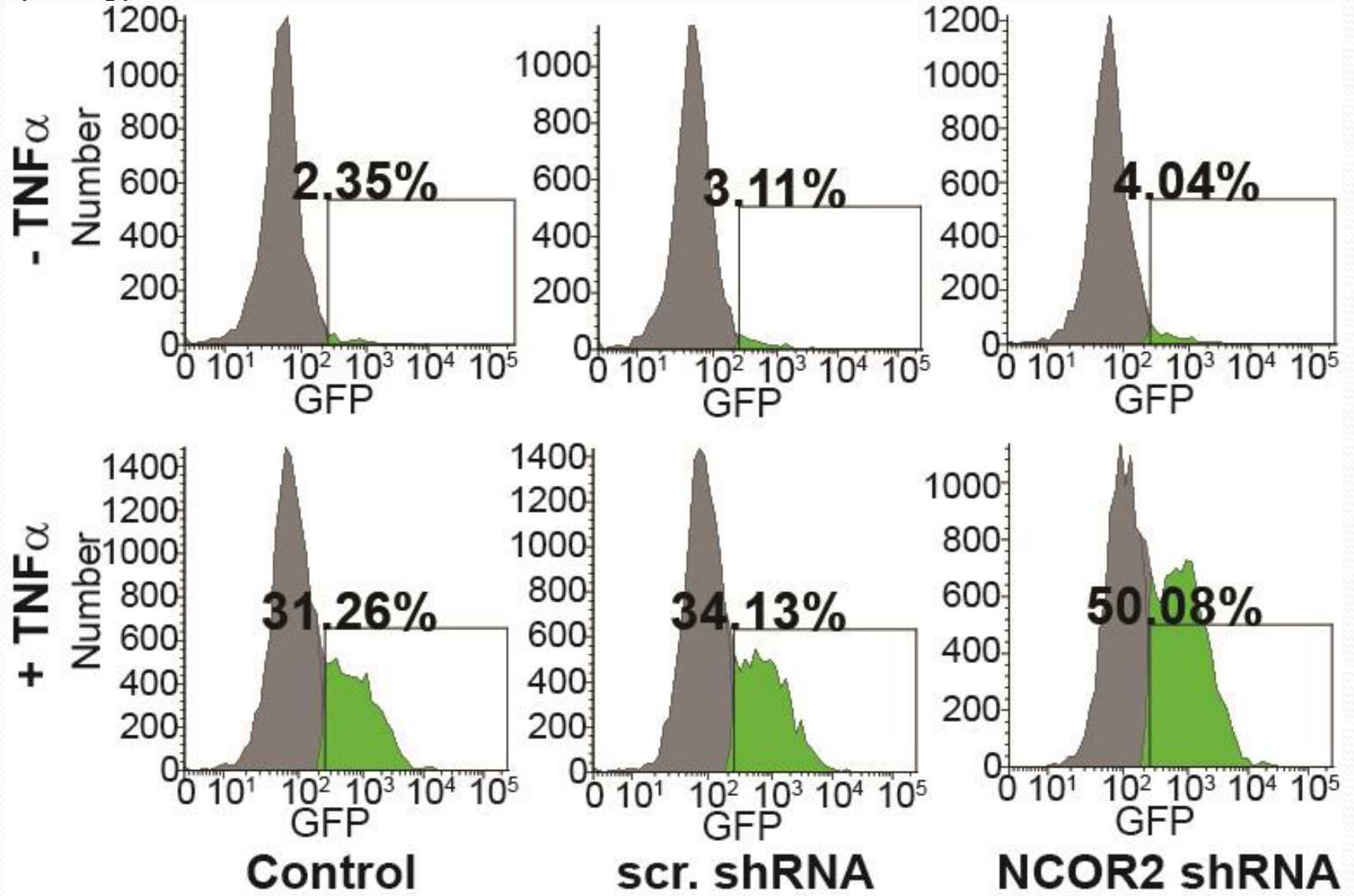
shRNA Library Screens Confirm Regulation of HIV Latency in Microglia by CoREST Complex and Other Repressors



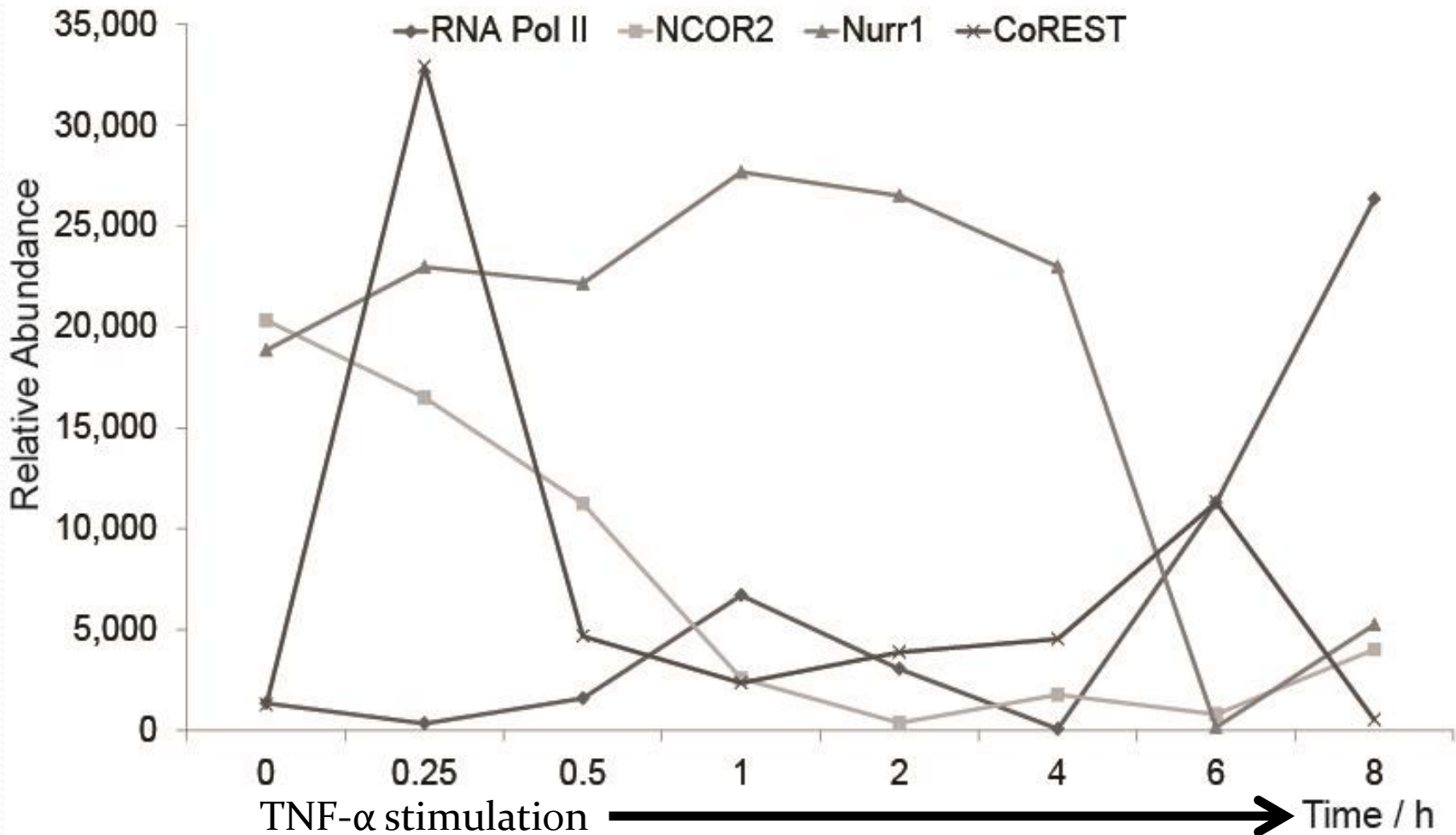
- shRNA-mediated inhibition
- pharmacological inhibition
- shRNA-mediated and pharmacological inhibition
- shRNA-mediated inhibition (shRNA library screening)



Down-regulation of NCOR2 Sensitizes hT-H μ glia/HIV (C14) Cells for TNF- α -mediated HIV Reactivation



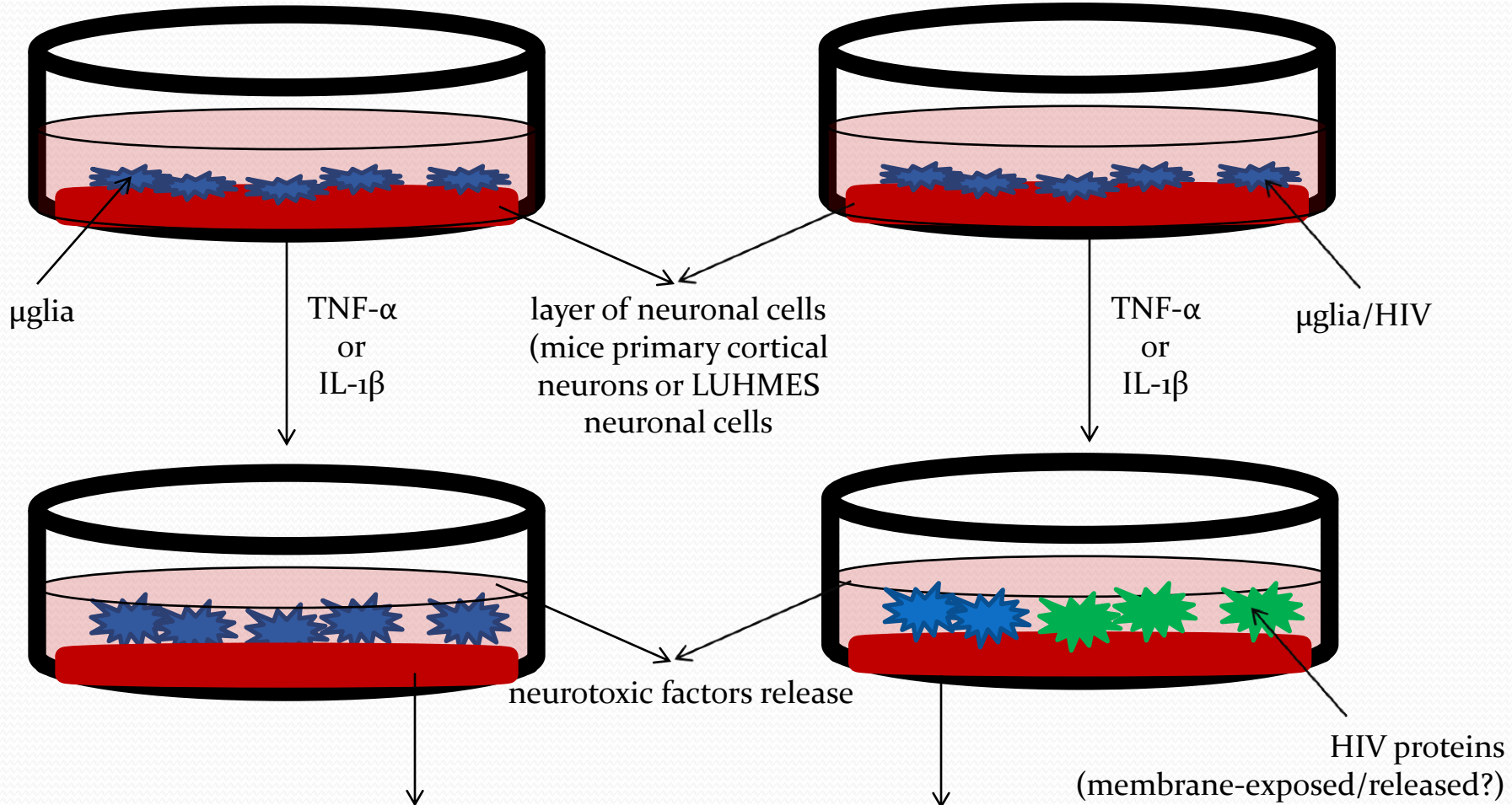
Occupancy Kinetics of NCOR2, Nurri1, and CoREST onto HIV Promoter



Questions:

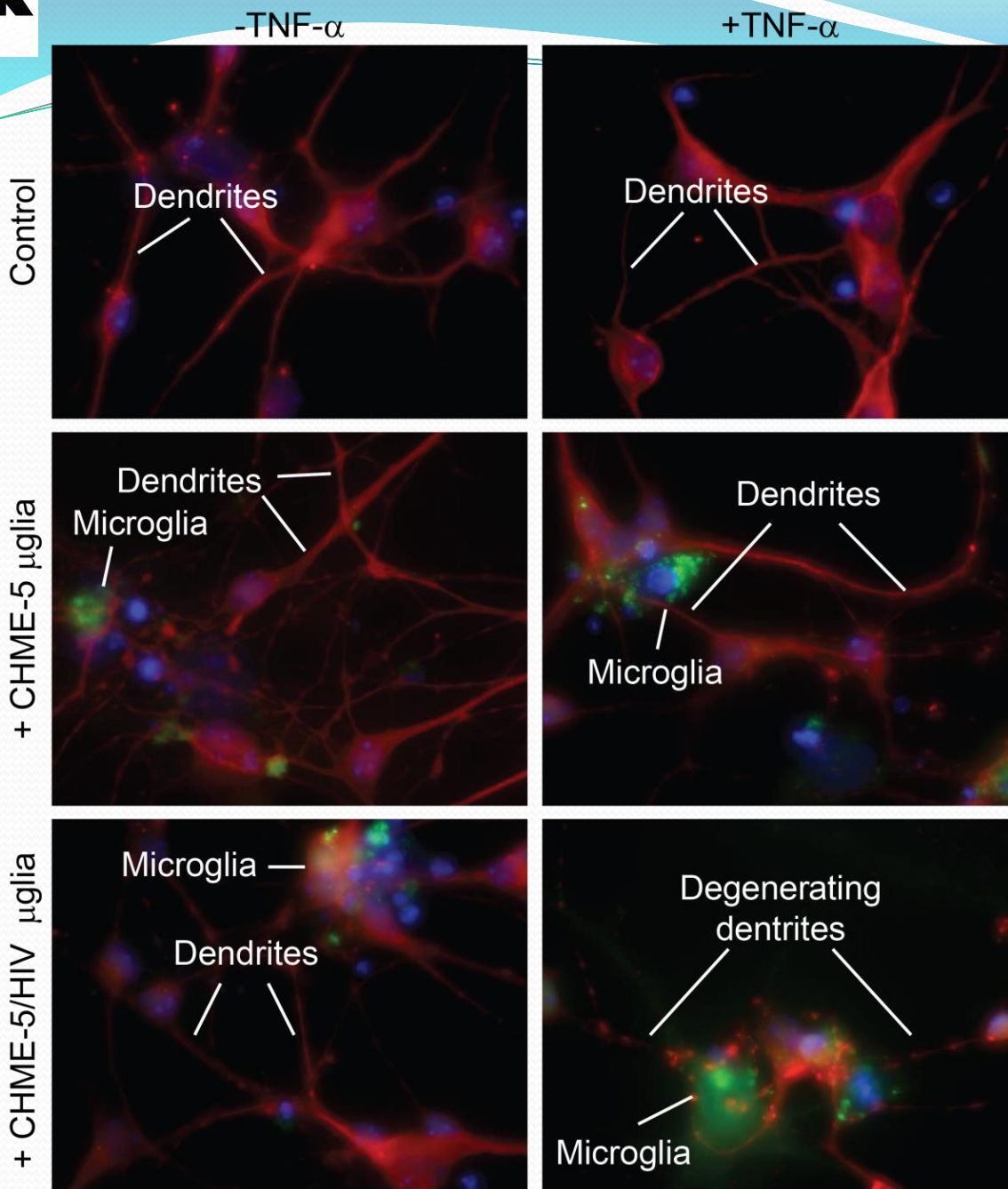
1. Can disruption of protective/repressor systems in μ glial/HIV cells exacerbate neurodegeneration?
2. Interplay between HIV repressors in μ glia and drugs of abuse (METH)
3. Can we prevent these HIV-induced neurodegenerative processes?

A Glial-Neuron Co-Culture System



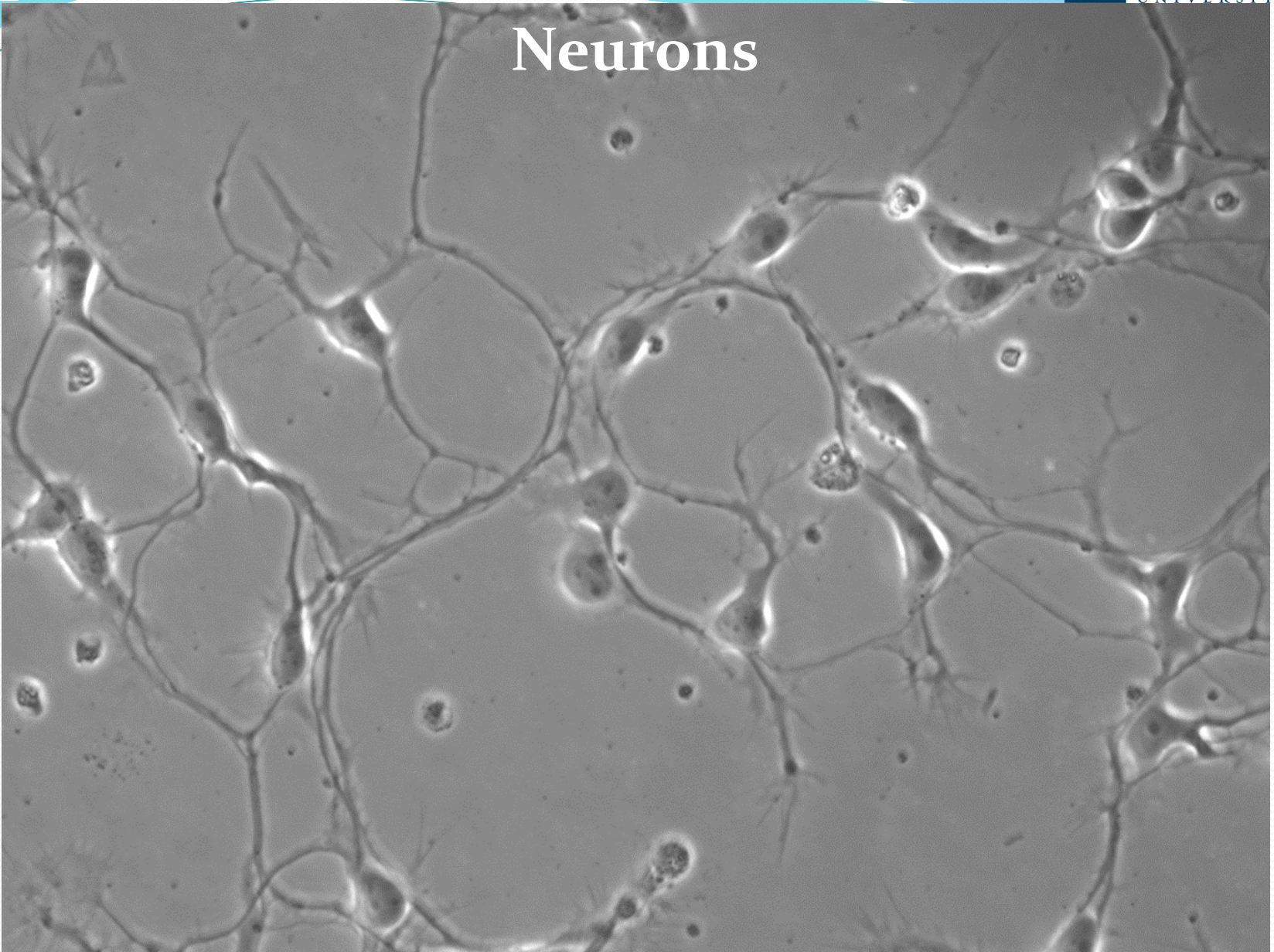
Interrogate neuronal cells for signs of neurodegeneration

- Immunocytochemistry staining
- Western blot
- Flow cytometry

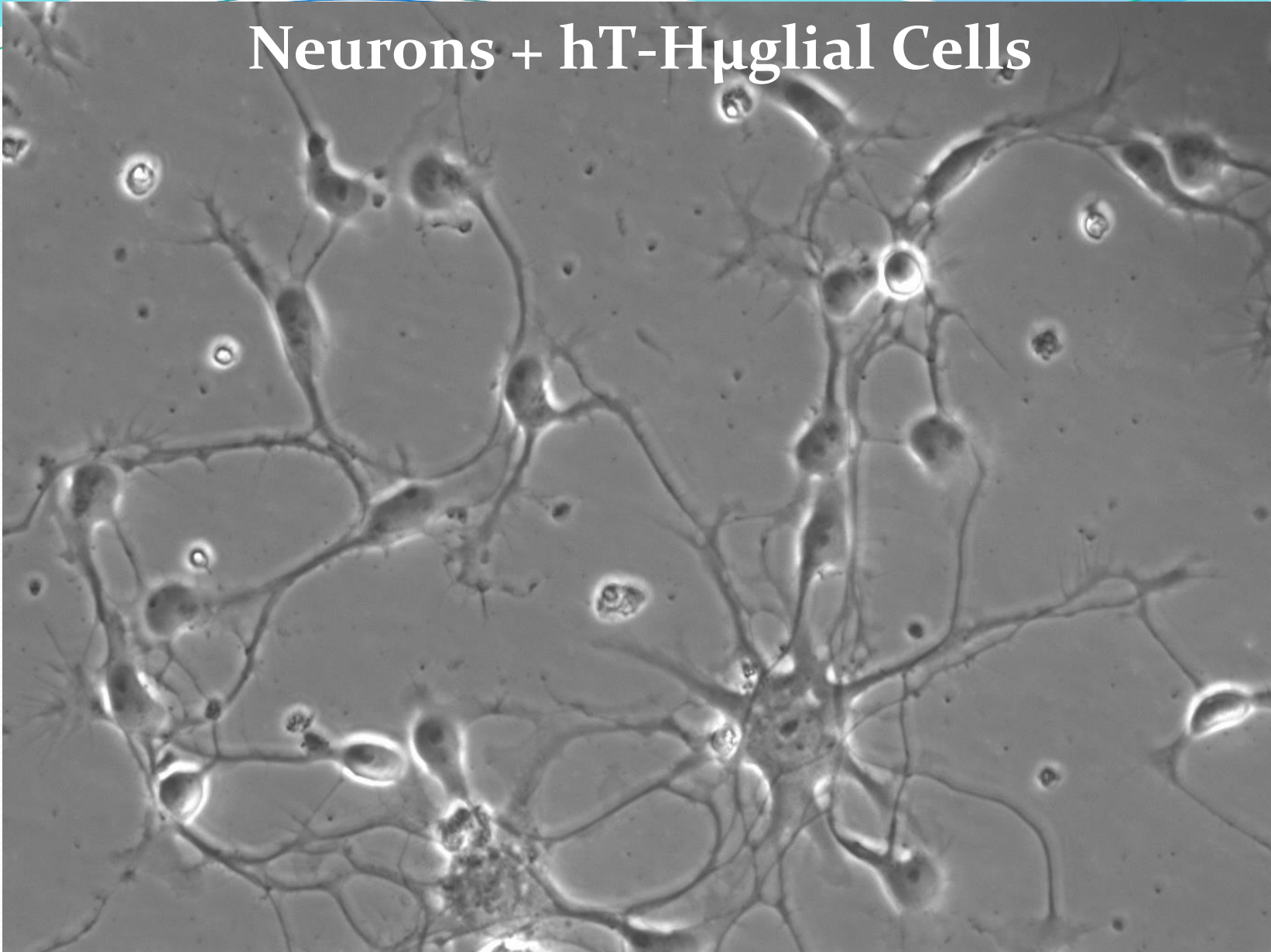


HIV- induced Neuro- damage

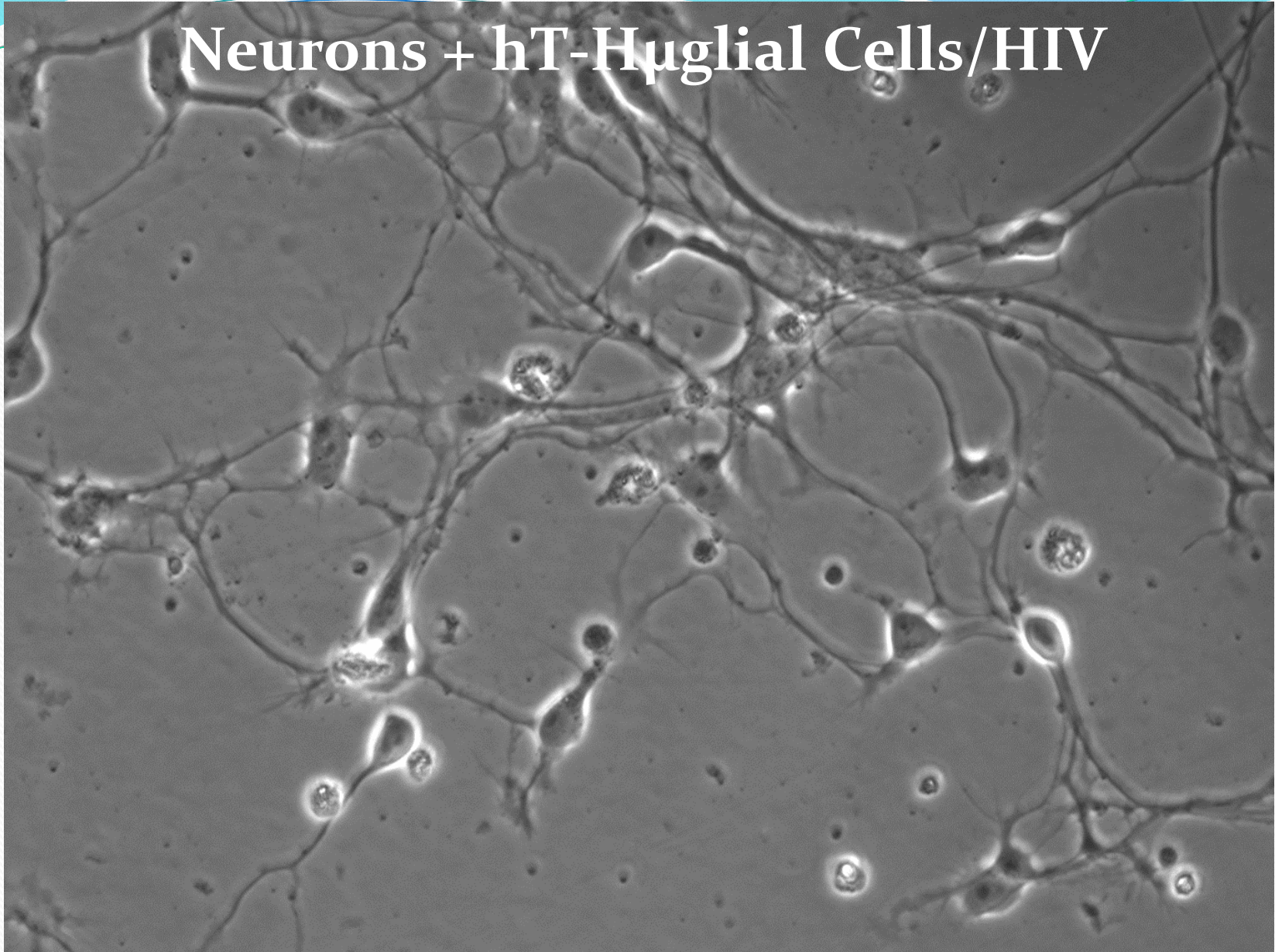
Neurons



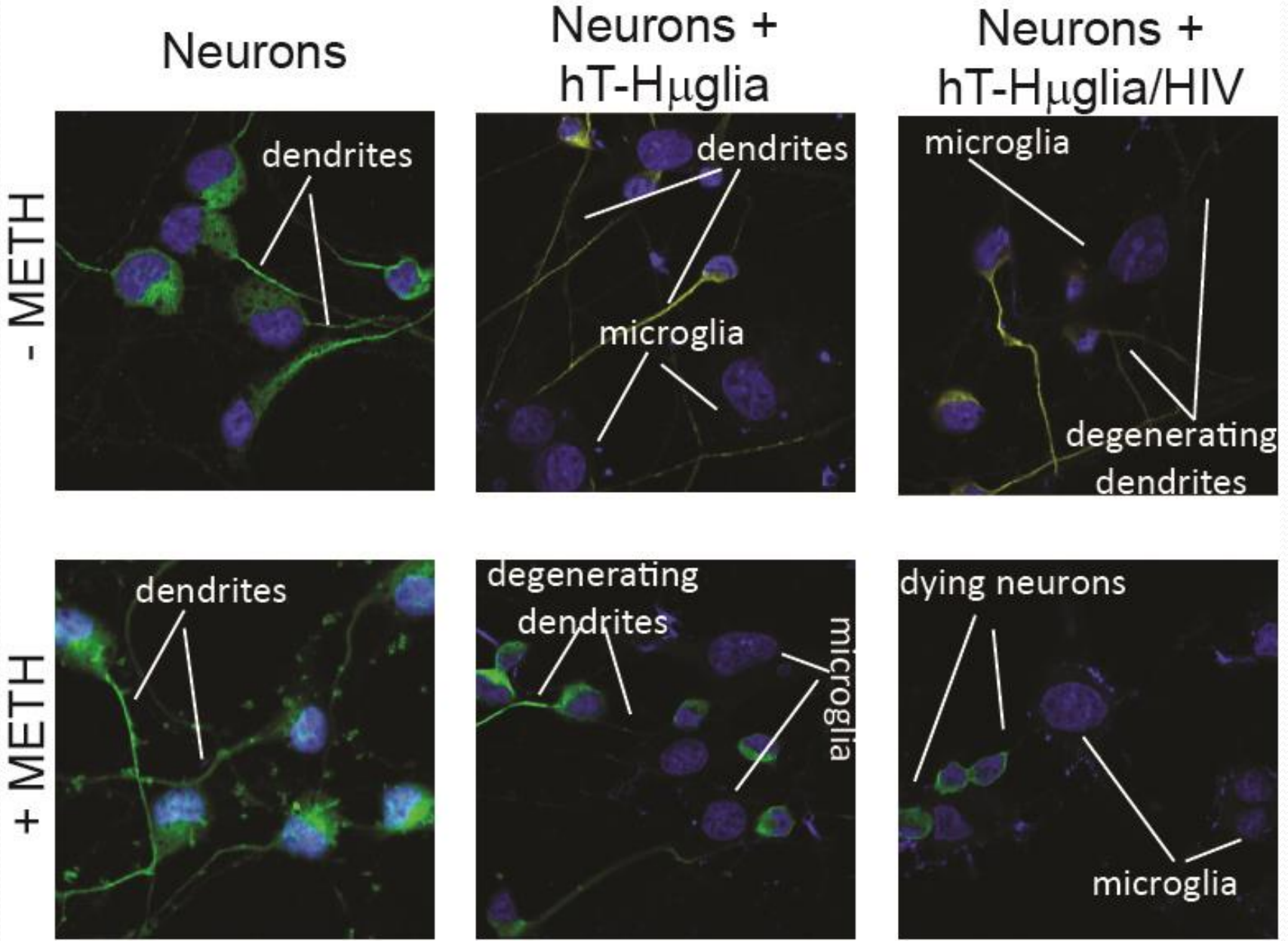
Neurons + hT-H μ glial Cells



Neurons + hT-Hu glial Cells/HIV



METH Exacerbates HIV-induced Neurodamage



Conclusions

- 1. Latently infected CHME-5 and hT-H μ glial cells serve as in vitro models for studying molecular events associated with emergence from latency of proviral HIV in microglial cells**
- 2. Reactivation of proviral HIV in different lines of latently-infected microglial cells is severely impaired**
- 3. Pharmacological inhibition of chromatin-modifying enzymes of the CoREST complex, partially re-activates HIV and permits proviral over-activation upon inflammatory stimuli(LPS, TNF- α , IL-1 β) in latently-infected microglia**
- 4. Partial induction of HIV and/or cell sensitization are observed when NCOR2, Nurr1, LSD1, or CoREST are knocked-down by shRNA silencing**
- 5. Our ex-vivo co-culture system between neurons and microglial/HIV cells serves as an in vitro model for studying HIV-induced neurodegenerative processes , and drugs of abuse exacerbation of neurodegeneration**

Acknowledgement

Jonathan Karn Lab

Present Members:

Yoelvis Garcia

Curtis Dobrowolsky

Biswajit Das

Mary Ann Checkley

Michael Greenberg

Hongxia Mao

Uri Mbonye

Kien Nguyen

Meenakshi Shukla

Past Members:

Amy Graham

Julia Freedman

Julian Wong

Brandon Harvey Lab

At NIDA-NIH

Kurt Hauser Lab

At VCU