VIOLET THERAPEUTICS

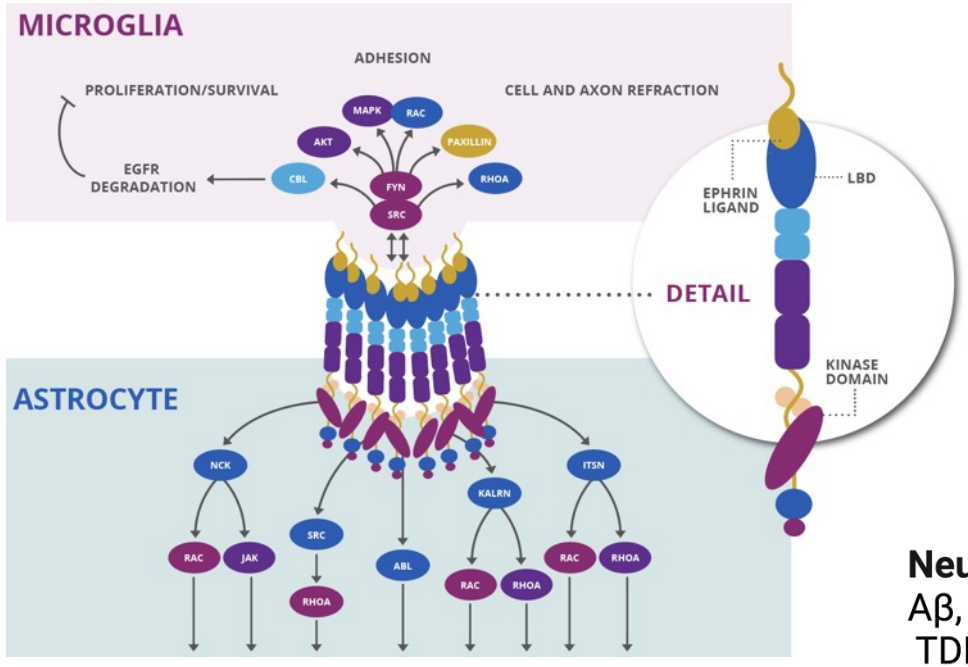
Novel Small Molecule EPHB3 Inhibitors to Treat Neurodegenerative Disease by Targeting Astrocyte-Mediated Disease Mechanisms

Evan Lebois, PhD

	No, Nothing to disclose
X	Yes, please specify

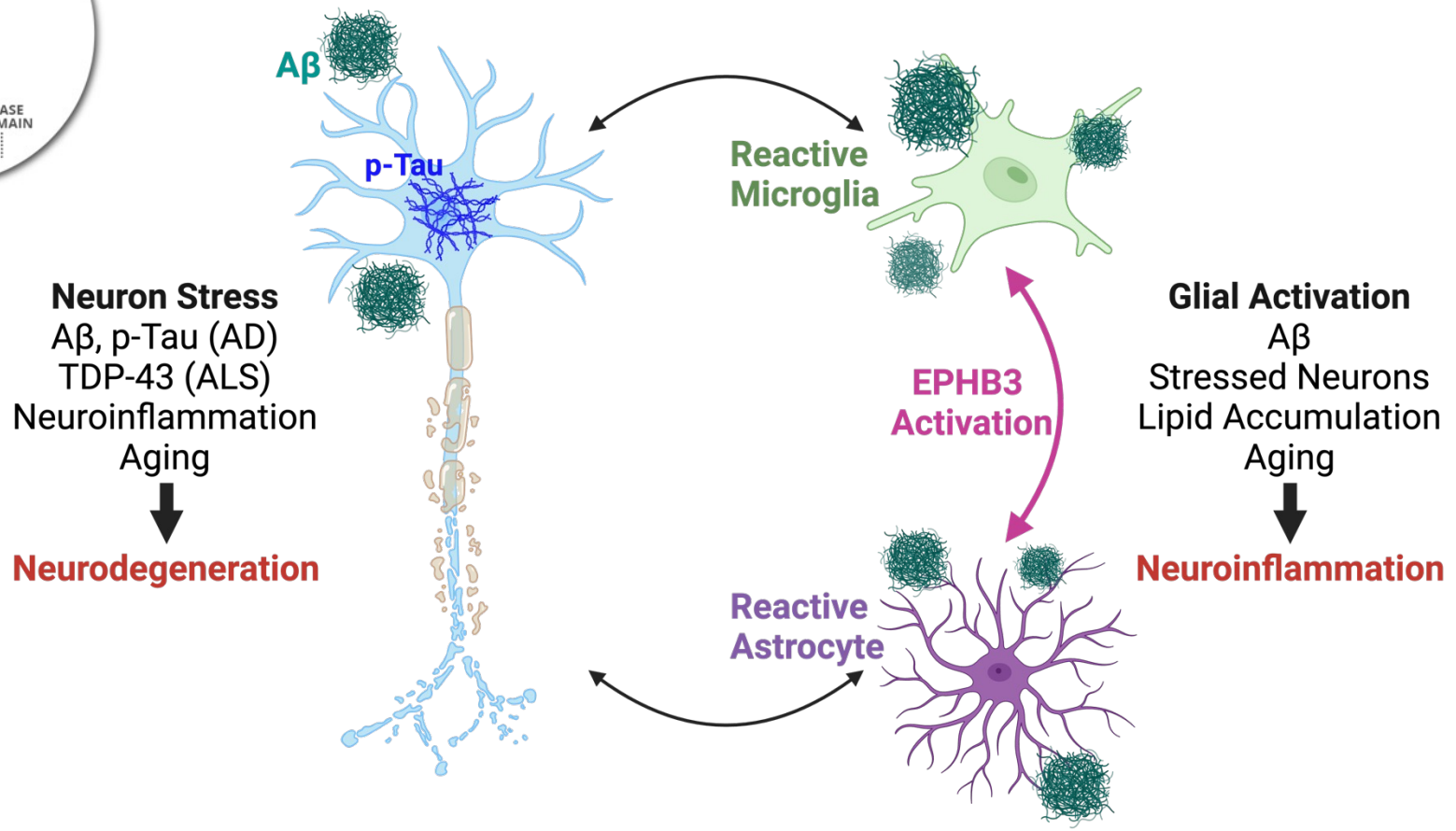
Company / Name	Honoraria / Expense	Consulting / Advisory Board	Funded Research	Royalties / Patent	Stock Options	Ownership / Equity Position	Employee	Other (Please specify)
Evan Lebois, Violet Therapeutics					X		X	

EPHB3 is an astrocyte receptor tyrosine kinase that facilitates interactions with microglia to drive neuroinflammation



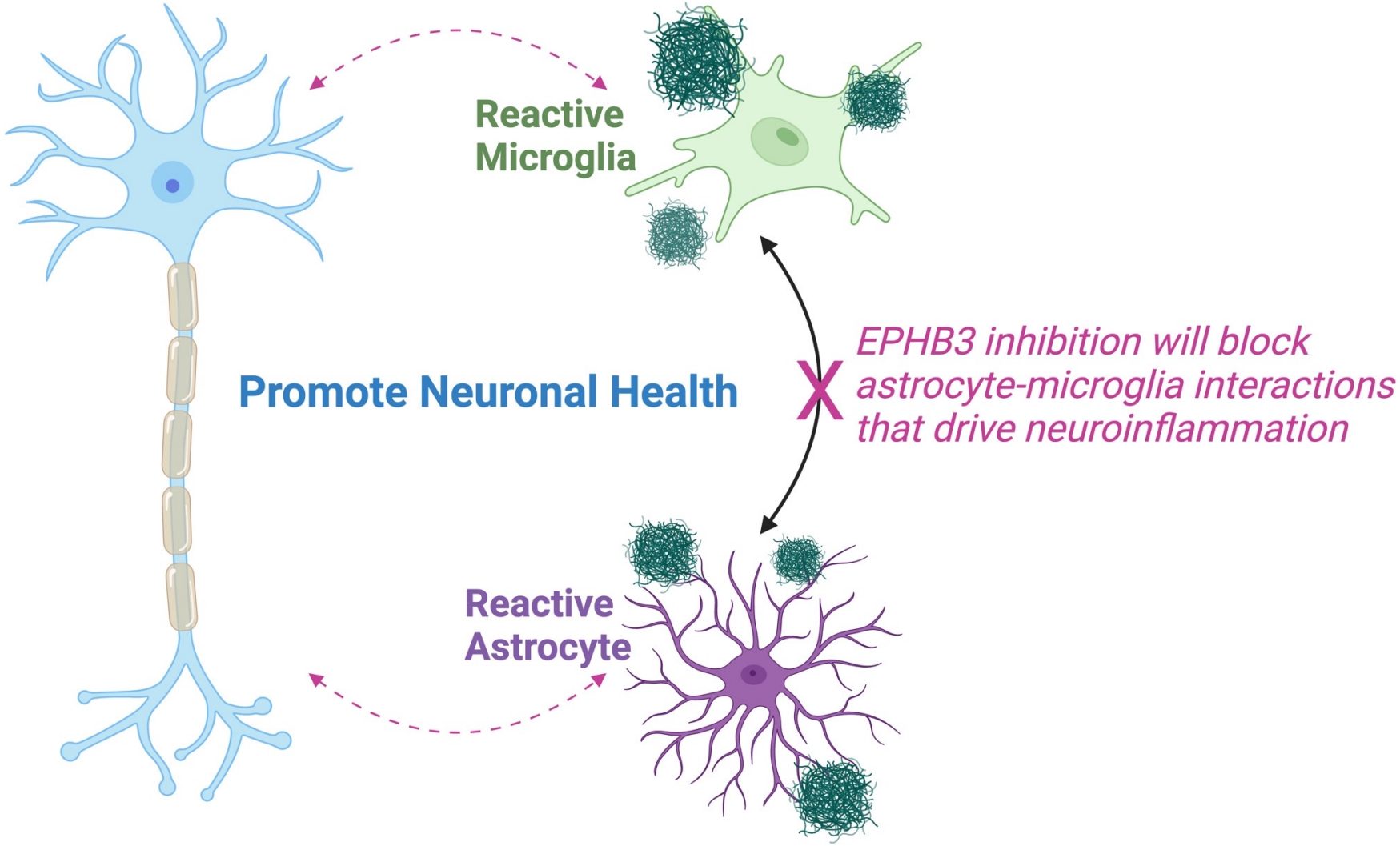
Classical role: neuronal development and axon guidance

New role: regulator of astrocyte-microglial interactions



Clark, IC, Gutiérrez-Vázquez, C, Wheeler, MA…Quintana, FJ. 2021. Science. 372(6540): eabf1230.

Therapeutic Hypothesis: EPHB3 inhibition will promote neuron health by blocking astrocyte-microglia interactions that drive neuroinflammation



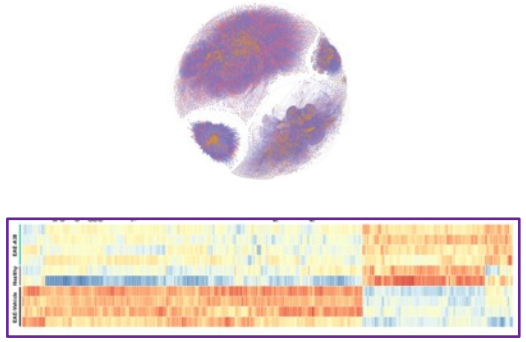
Construction of EPHB3 astrocyte activation score and in silico mapping to human disease and mouse model datasets

Target ID

RABID-seq identified EPHB3 activation in astrocytes as neuroinflammation driver in EAE

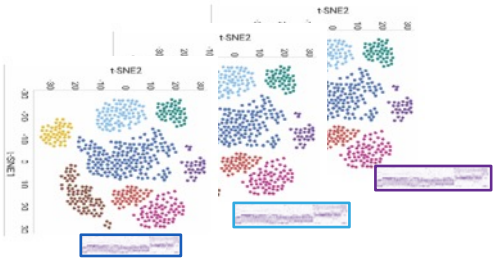
Target signature

162 transcriptional changes that occur in Astrocytes when EPHB3 is activated



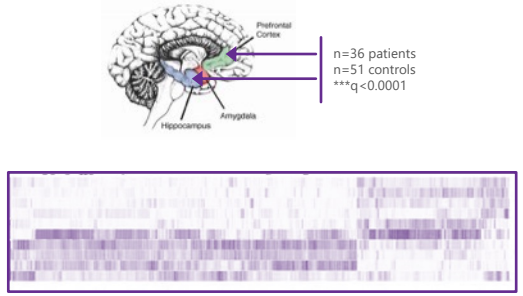
Mine human and mouse data sets

Bulk and scRNA-seq datasets are mined for target signature



Indication and mouse model selection

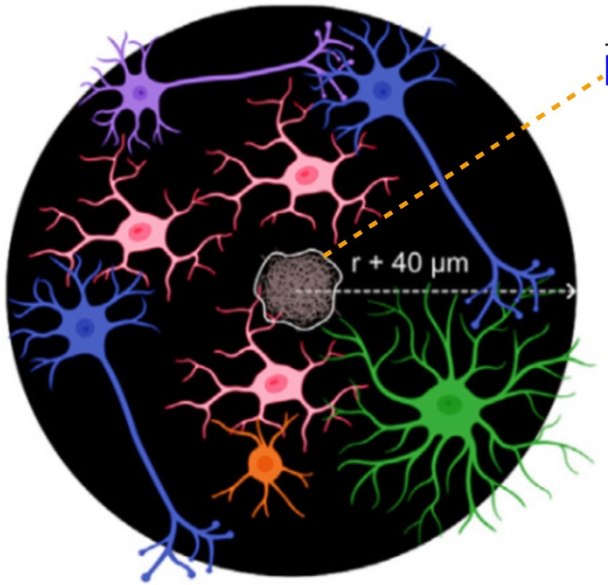
Target signature is found in human disease and mouse model data sets

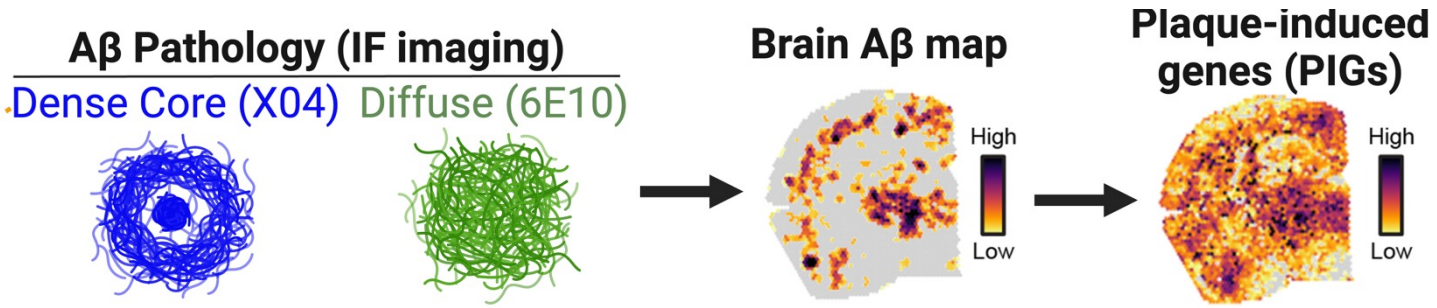


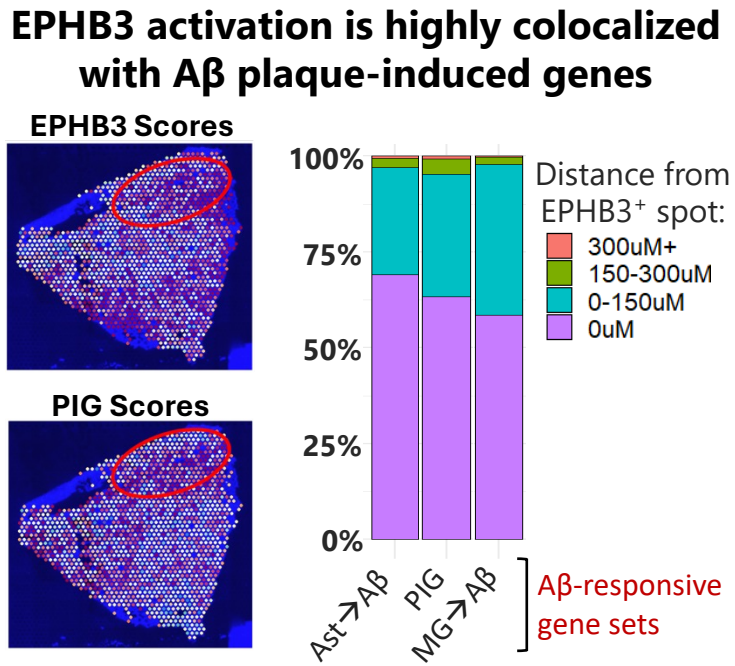
Aim = map EPHB3 activation to:

1. human disease data sets for indication relevance
2. in vivo mouse model data sets for therapeutic development

EPHB3 activation is highly co-localized and correlated with Aβ plaque-induced gene (PIG) response in AD, revealed by spatial transcriptomics





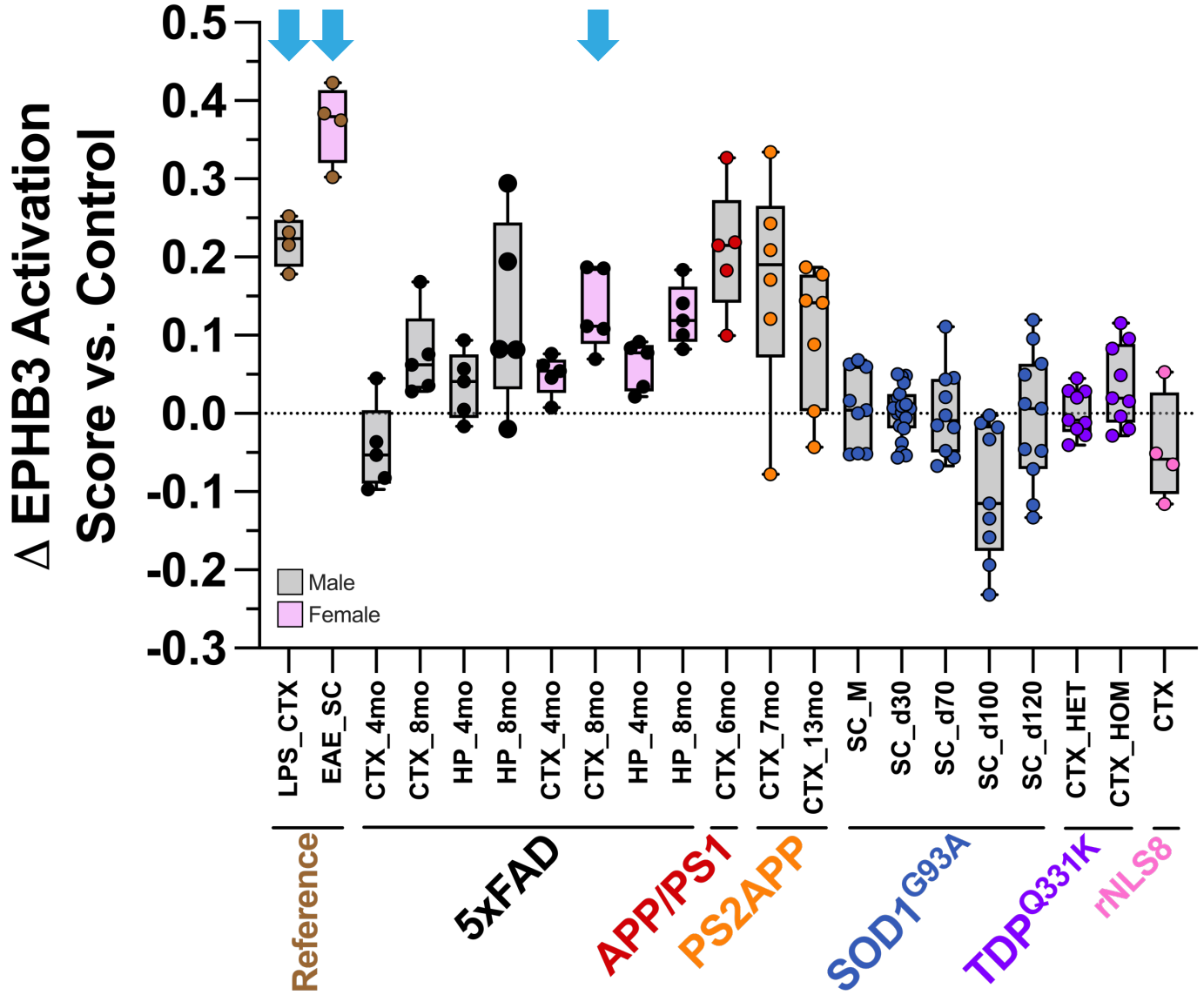


Key Takeaways:

- Astrocyte and MG gene expression altered around Aβ plaques
- This drives neuroinflammation
- EPHB3 activation happens in the same place

Miyoshi, E, … Swarup, V. 2024. Nature Genetics. 56: 2704 – 2717.
Mallach, A, … De Strooper, B. 2024. Cell Reports. 43(6): 114216.

EPHB3 activation scores in astrocytes are markedly elevated in RNA-seq data from three amyloidosis mouse models, as well as LPS and EAE models



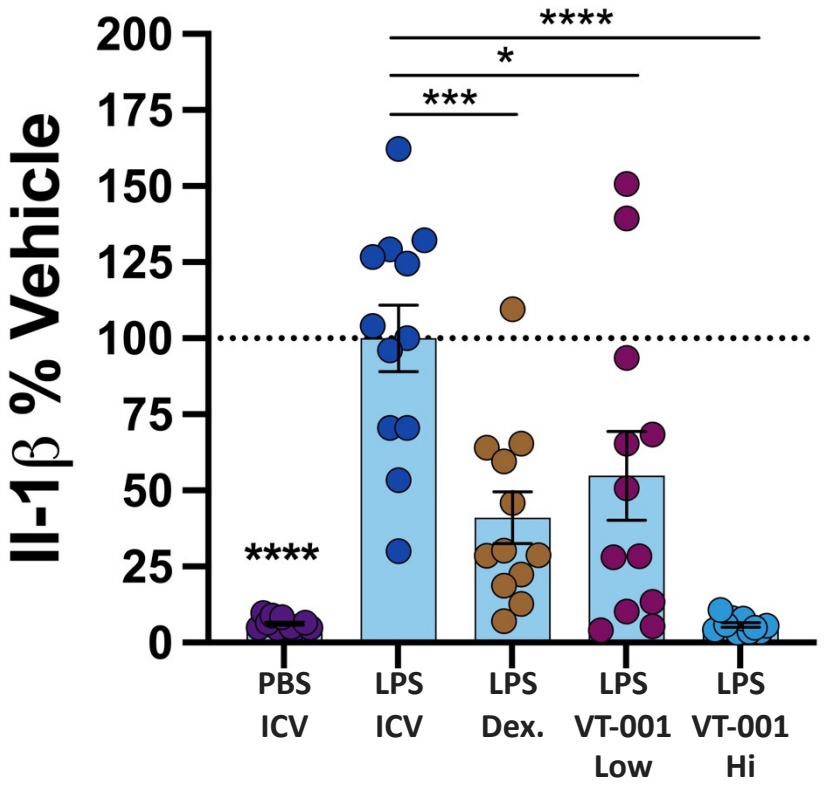
Highly selective, brain penetrant tool compound VT-001 shows robust pharmacology and in vivo efficacy in ICV LPS assay

Drug-like small molecule with excellent selectivity

	Selected assay/ Target	VT-001
Properties	CNS MPO[1]	5
	Kinetic solubility (7.4, uM)	192
	MW, cLogD	<410, <1
	PPB % unbound (m, r, d, c, h)	64, 71, 65, 61, 51
Biochemical	EphB3 (IC50, uM)	0.048
	Carna NanoBRET (196 kinases)	1/240 (EPHB3)
Kpuu	Rat	40%
In vitro tox	CYPs	< 50% @ 10uM
	hERG	37% @ 10uM
	SafetyScreen44	1/44 (3 μM AChE)

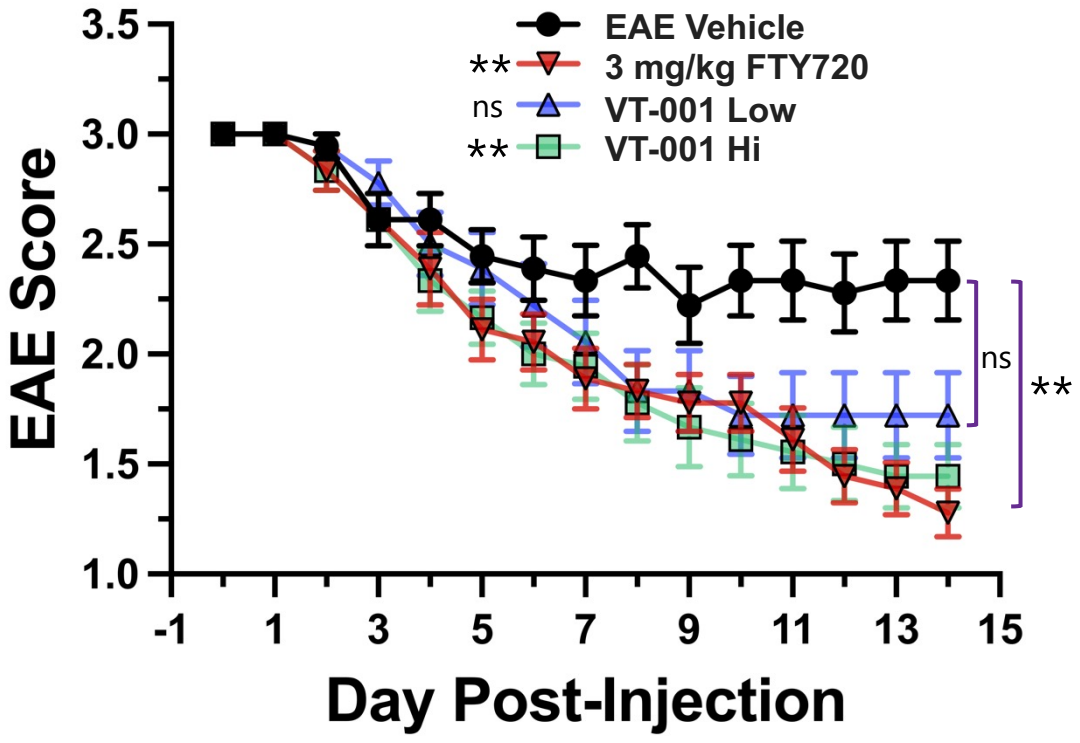
1. CNS MPO: combination of cLogp/ cLogD/ MW/ TPSA/ HBD/ pKa
2. ER – extraction ratio. Predicted Clp/hepatic blood flow; in vivo Clp/ hepatic blood flow

Neuroinflammatory reduction in mouse LPS model greater than positive control

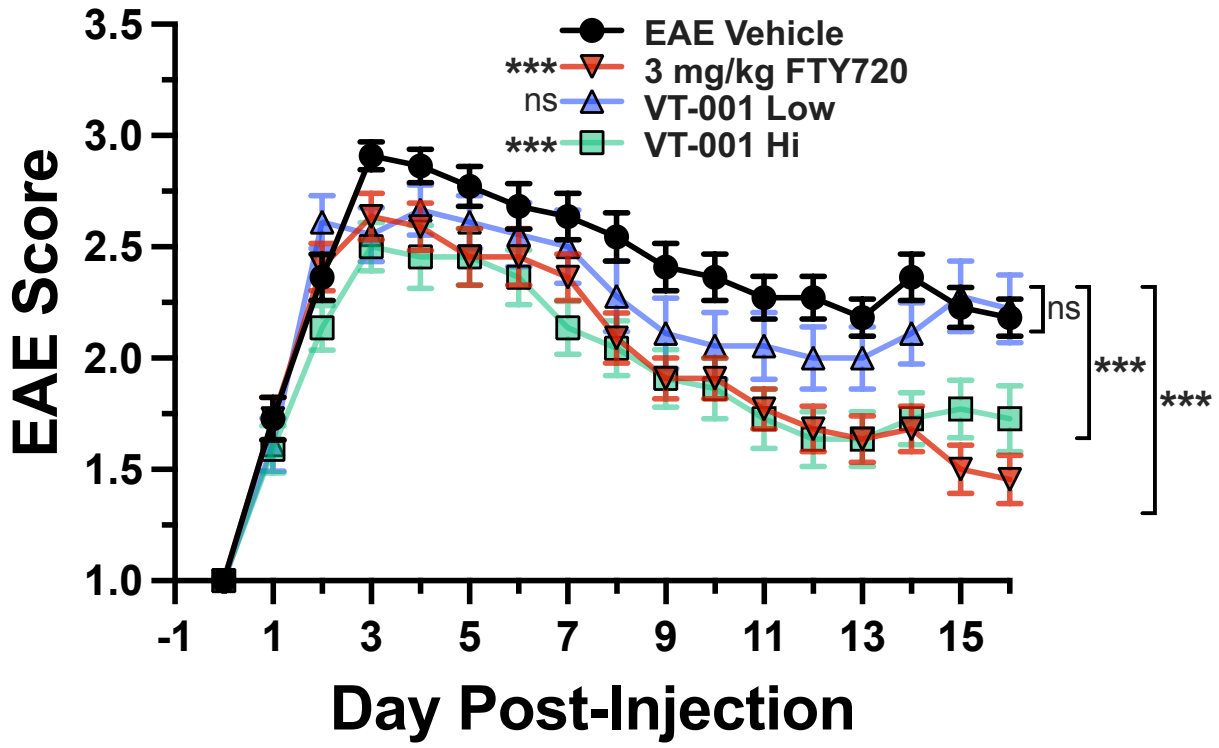


VT-001 significantly rescues clinical EAE score deficits in mice

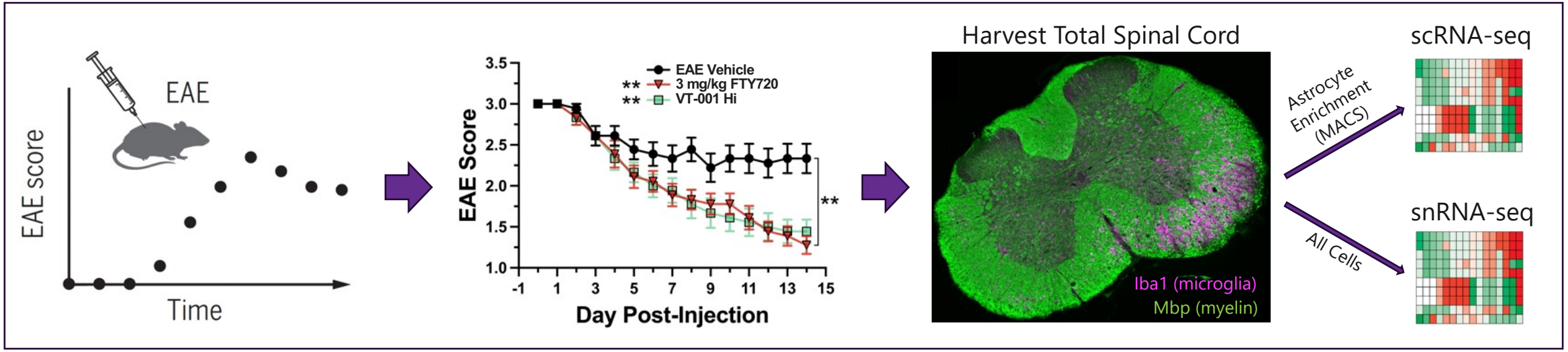
Dosing initiated at peak EAE



Dosing initiated at first detectable EAE symptoms (EAE score = 1)



EAE scRNA-seq studies to reveal VT-001 mechanism of action (MOA)



Are experimental groups different from one another?

Identify clusters that differentiate EAE and VT-001 groups:

- Differentially expressed gene (DEG) analysis and clustering of **astrocyte scRNA-seq data**

 # Clusters

32

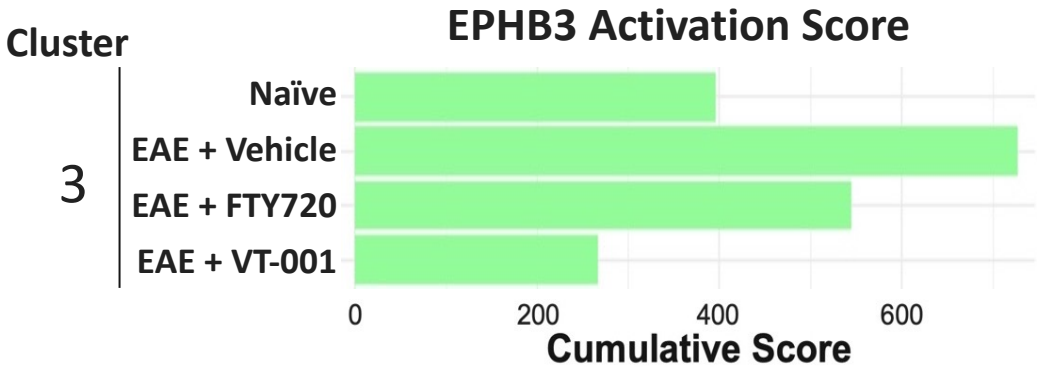
How are experimental groups different?

Attach biological function to these clusters:

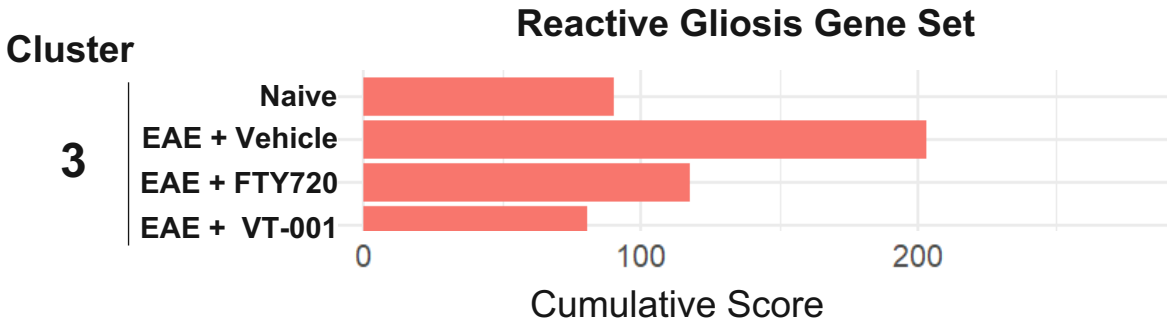
1. What clusters are associated with VT-001 treatment effects?
2. Of these, which clusters are associated with: EAE induction, EPHB3 activation, and neuroinflammatory gene expression?

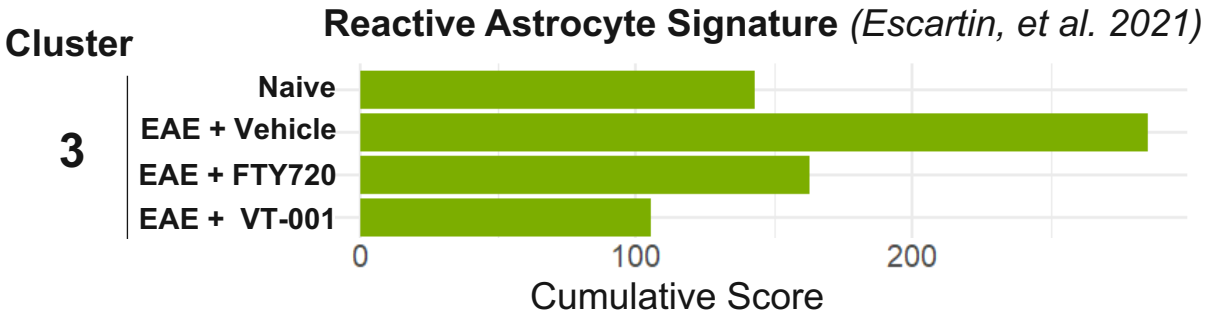
8→1

VT-001 rescues expression of pro-inflammatory and reactive astrocyte signature gene sets in astrocytes



~30-40% of total astrocytes in sample



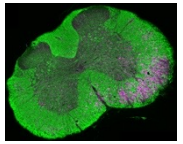


scRNA-seq in EAE spinal cord shows, in astrocytes, VT-001 decreases EPHB3 activation scores, pro-inflammatory, and reactive astrocyte gene expression signatures

Global proteomic analysis of spinal cord reveals VT-001 treatment reduces inflammation and restores neuronal signaling pathways

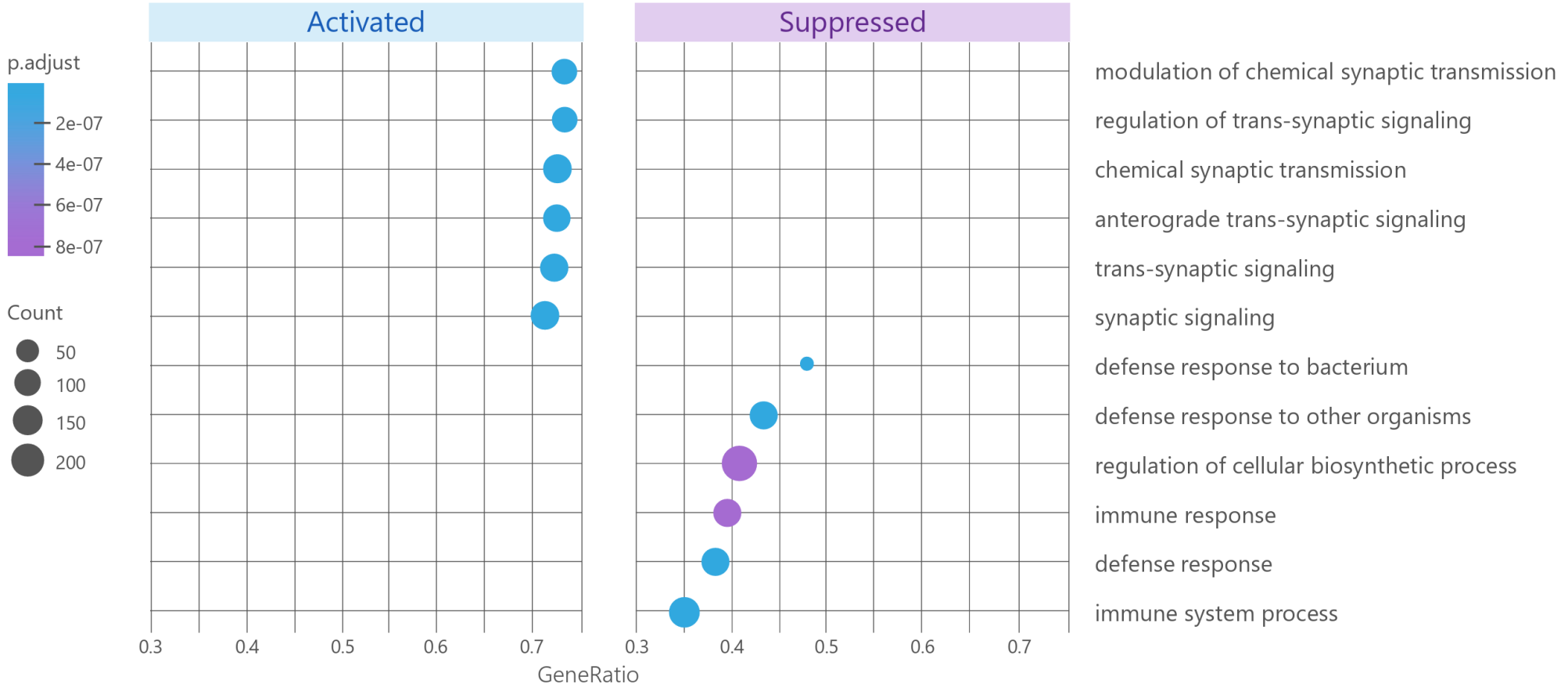
VT-001 dosing (2 weeks)

Total spinal cord



Global proteomics

Enriched Pathways VT-001 vs. Vehicle



Using global proteomics, the top upregulated protein pathways by VT-001 treatment were synaptic signaling pathways and top suppressed pathways are immune pathways

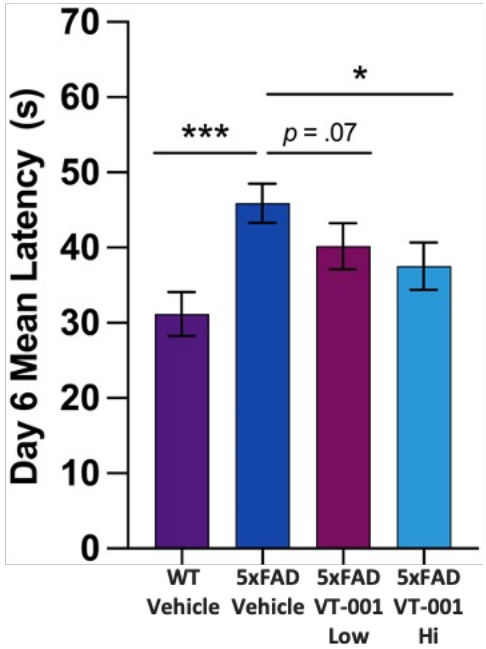
VT-001 rescues cognitive deficits and Aβ plaque-induced gene (PIG) expression signature in cortex of 5xFAD mice

5xFAD

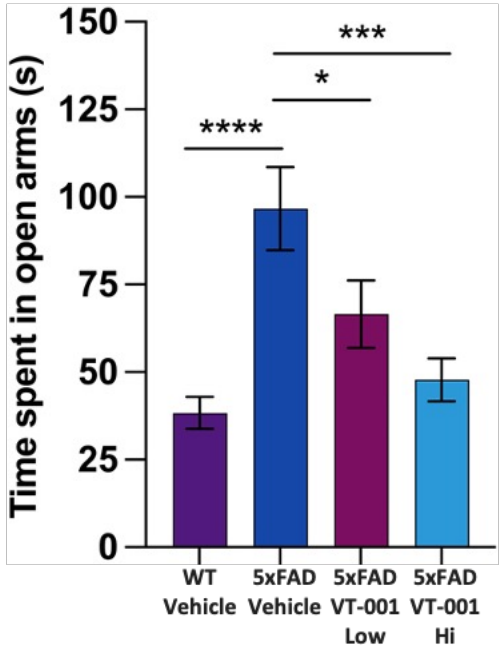
2-month treatment

Behavioral efficacy

Morris water maze

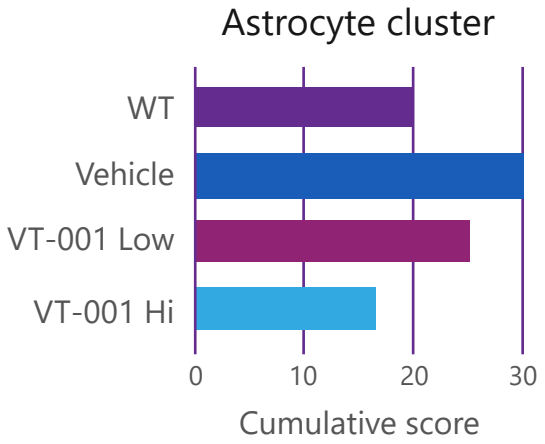


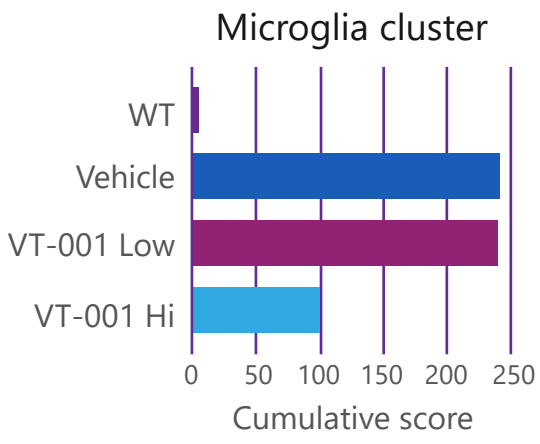
Elevated Plus Maze



Reduced astrocyte and microglia Aβ plaque-induced gene expression in cortex (scRNA-seq)

Aβ plaque induced gene (PIG) response





Study Timeline

Study Time: t_0 — 2 mos
Mouse Age: 6.5 m/o — 8.5 m/o

MWM
EPM
Tissue

Astrocytes in 5xFAD mice have **increased plaque inflammatory response**

- Inflammation local to Aβ plaques damaging to neurons that represents pathogenic astrocyte-microglia crosstalk

VT-001 attenuates astrocyte and microglia inflammatory response

Mallach, A, … De Strooper, B. 2024. Cell Reports. 43(6): 114216.

Conclusions and next steps

Overall Conclusion: VT-001 is highly efficacious in vivo in LPS, EAE, and 5xFAD models

1. LPS
   - ✓ VT-001 robustly attenuates LPS-evoked IL-1β release to WT levels (MSD)

2. EAE
   - ✓ Spinal cord gliosis and reactive astrocyte gene signatures attenuated by VT-001 (scRNA-seq)
   - ✓ Rescue of neuronal signaling pathways and inhibition of immune response pathways (proteomics)

3. 5xFAD
   - ✓ Significant Aβ PIG expression in cortex astrocytes and microglia attenuated by VT-001 (scRNA-seq)

Next Steps

1. 5xFAD follow-up studies
   - Neurohistology to characterize VT-001 efficacy at molecular level
   - Spatial transcriptomics to establish VT-001 MOA local to Aβ plaque pathology
   - Fluid and tissue proteomics to identify VT-001 biomarkers

2. PS19 study to test VT-001 efficacy in setting of tauopathy

Thank you

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