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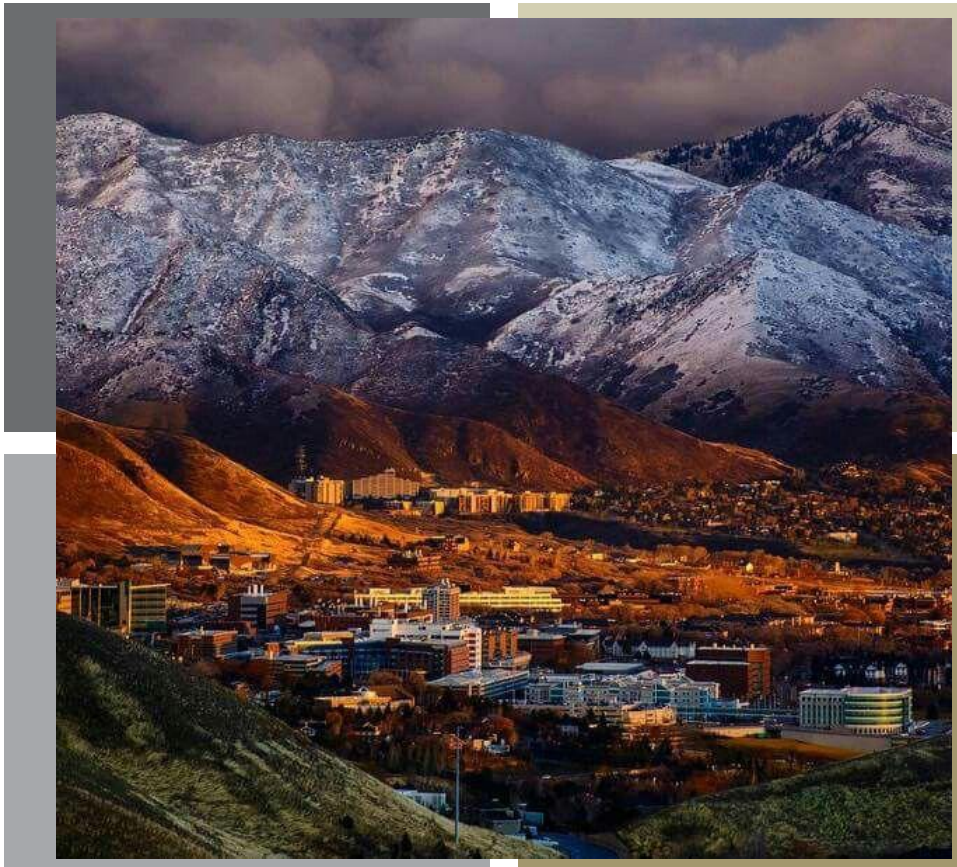
HEALTH SCIENCES

University of Utah
Department of Pathology
Grand Rounds

October 17, 2019



Innate immune responses contribute to host defense, disease, and repair in response to viral infection of the CNS

Tom Lane, Ph.D.
Division of Microbiology & Immunology
Department of Pathology
University of Utah, School of Medicine

Overview

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Laboratory Focus: Evaluate underlying molecular and cellular mechanisms contributing to neuroinflammation, neurodegeneration, and repair in pre-clinical animal models of neurologic disease.

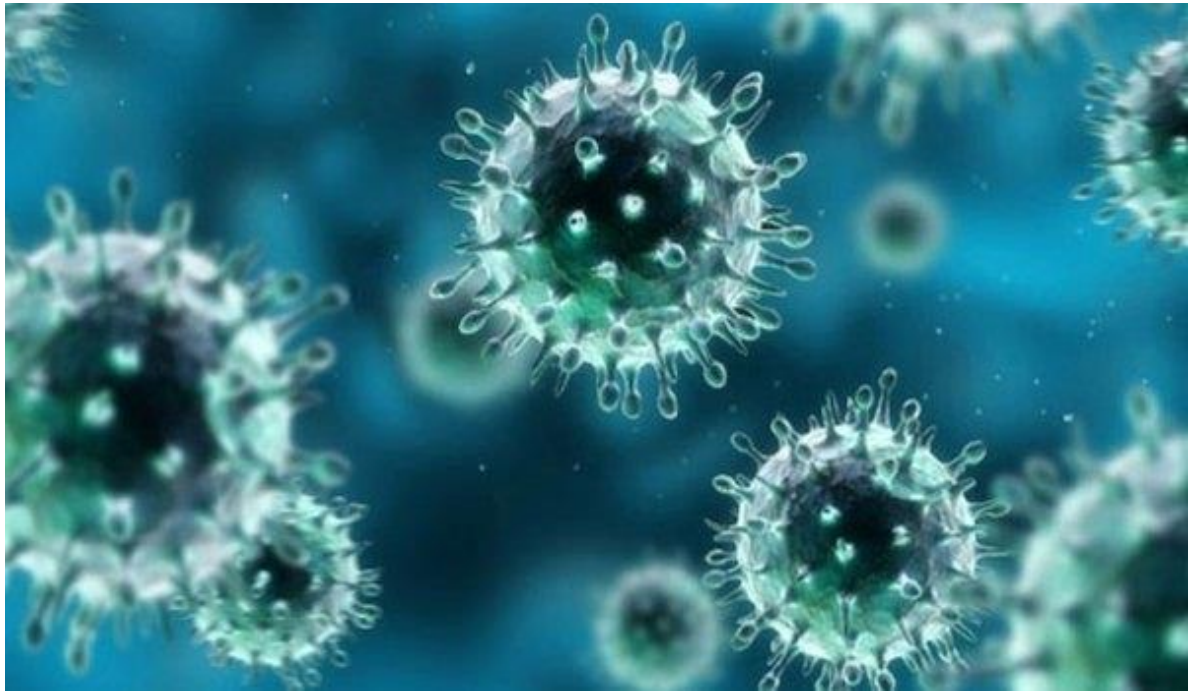
Presentation: In a viral model of neuroinflammation/ demyelination, we have demonstrated:

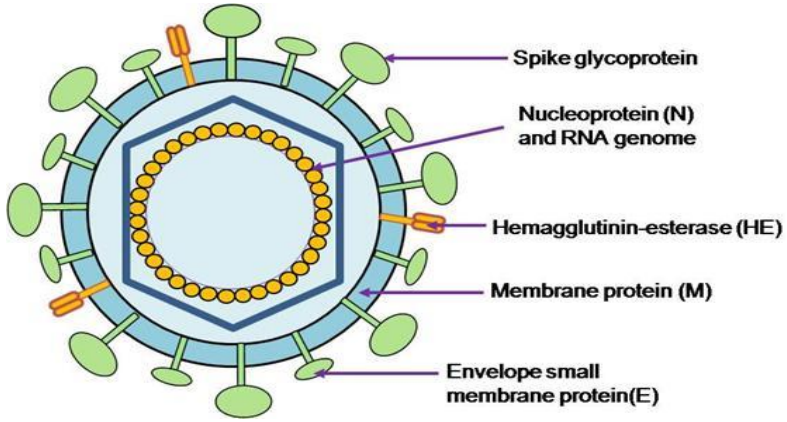
- Single cell RNA Sequencing (scRNASeq) of CD45+ cells isolated from the CNS at defined times post-infection (p.i.) with virus reveals the heterogeneity of the immune response.
- Microglia enhance host defense by influencing antigen presenting cell (APC) activation required for efficient T cell-mediated control of viral replication. Targeted ablation of microglia also results in increased demyelination associated with impaired remyelination.
- Sustained neutrophil infiltration into CNS results in increased clinical disease associated with enhanced white matter damage.

Why study viral infection of CNS?

Virus	Target cell	Geographical distribution
DNA		
Herpes Simplex Virus	Neurons	Worldwide
Human Herpesvirus 6	Oligodendrocytes	Worldwide
Cytomegalovirus	Neurons	Worldwide
JC virus	Oligodendrocytes	Worldwide
VZV	Neurons	Worldwide
RNA		
West Nile virus	Neurons	Europe, Americas, Africa
Poliovirus	Motor neurons	India, Africa
St. Louis encephalitis	Neurons	USA
LCMV	Meninges/Neurons	Worldwide
Rabies virus	Neurons	Europe, Asia, Africa, Americas
Mumps	Meninges/ependyma	Worldwide
Zika	Neural progenitors	Africa, Asia, America's
Retrovirus		
HIV	Microglia	Worldwide

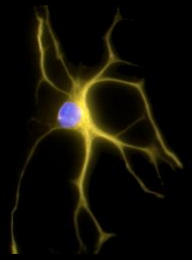
JHM strain of Mouse Hepatitis Virus (JHMV):



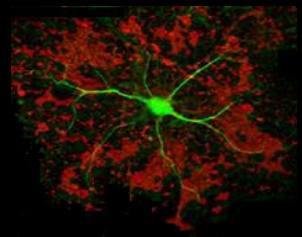


- (+) Sense, Single Stranded RNA Virus
  - *Coronaviridae*
- Intracranial Inoculation
  - Acute Encephalomyelitis
  - Glial Cell Tropism
  - Immune-Mediated Demyelination
  - Clinical Disease - mild-to-severe hind limb paralysis

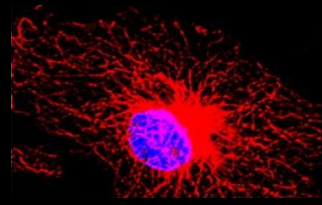
Astrocyte



Oligodendrocyte

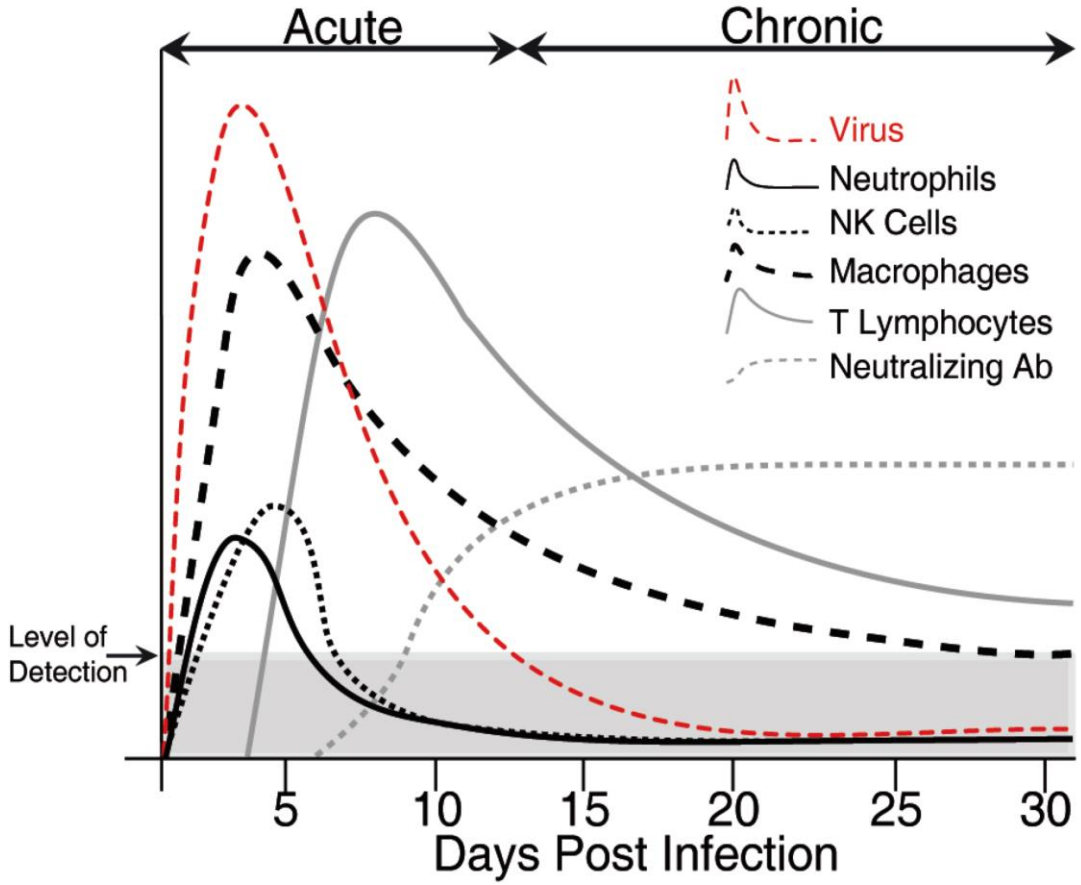


Microglia



Adams, RA et al 2007 J Exp Med

JHMV infection of the CNS evokes a rapid immune response



- Innate immune cells contribute to permeabilization of the BBB.
- CD4+ and CD8+ T cells control viral replication through IFN-γ secretion and cytolytic activity.
- Virus-specific neutralizing antibody restricts viral recrudescence.

Skinner *et al., Viral Immunol.*, 2018

Viral persistence results in immune-mediated demyelination

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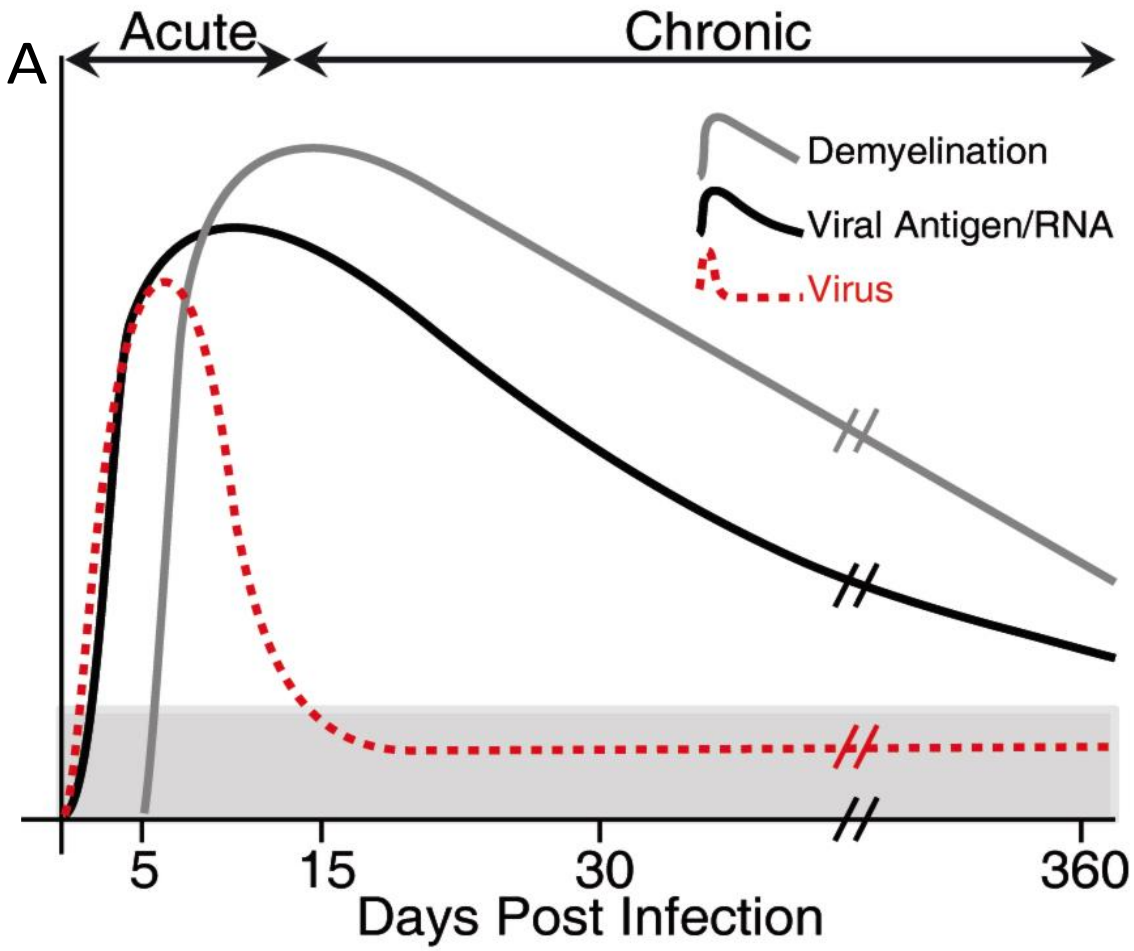
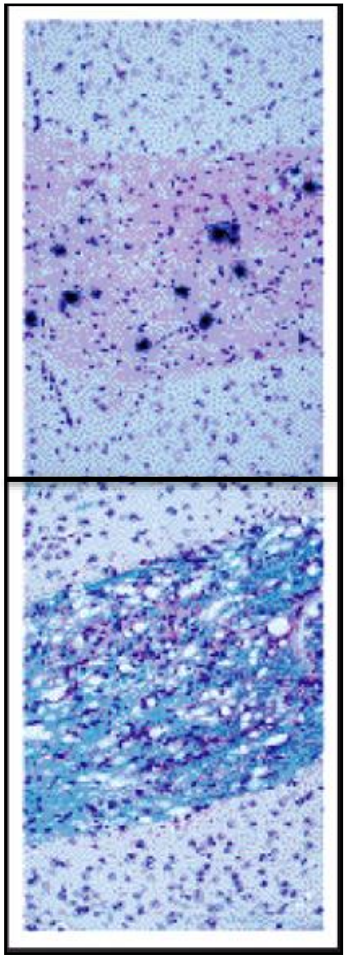


Day 28 p.i., spinal cord

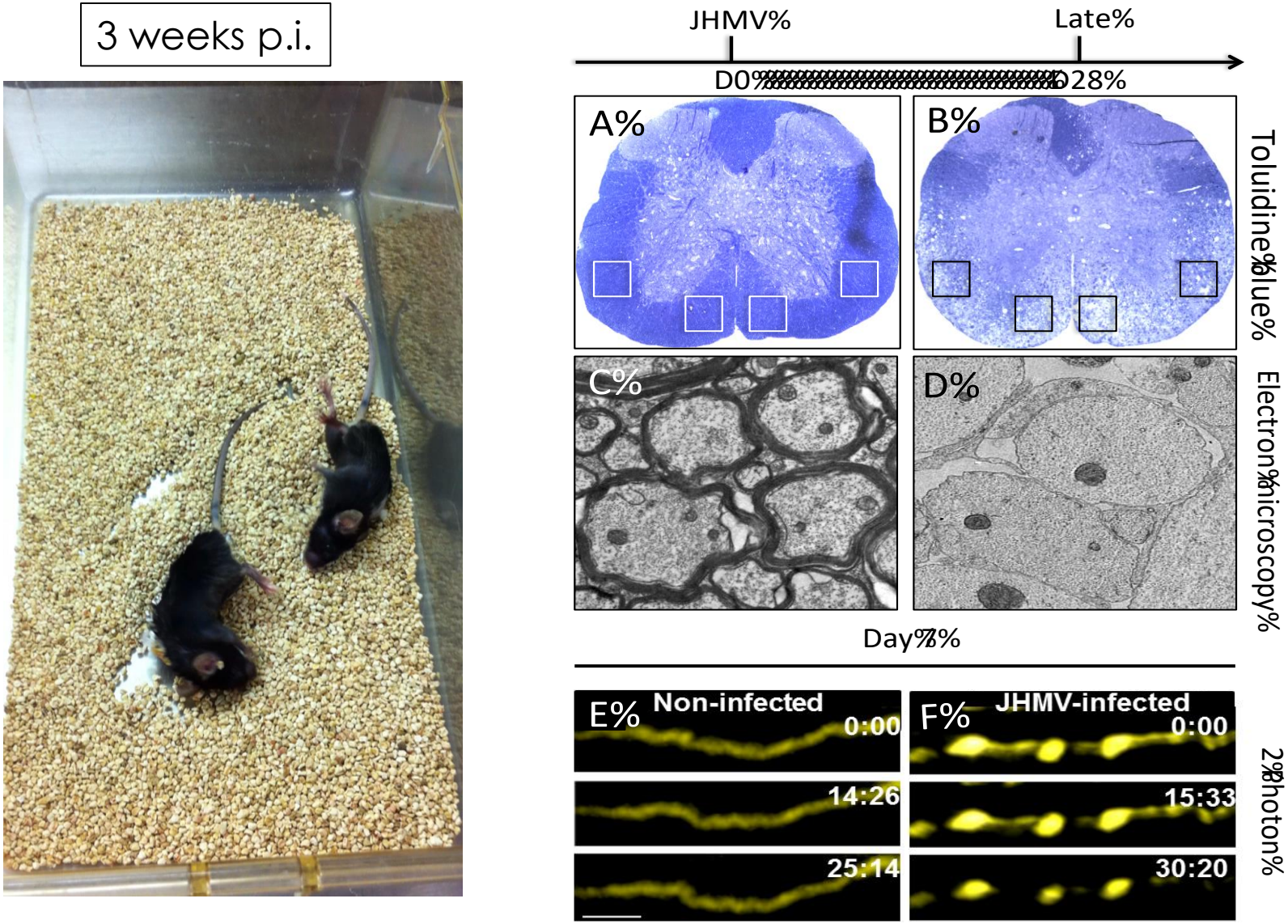


In situ viral RNA

LFB/H&E

Demyelination is mediated by inflammatory T cells and macrophages

Persistent JHMV infection in immunocompetent C57BL/6 mice results in demyelination



Greenberg *et al., PNAS*; Skinner *et al., Viral Immunol*.

JHMV-induced encephalomyelitis & demyelination

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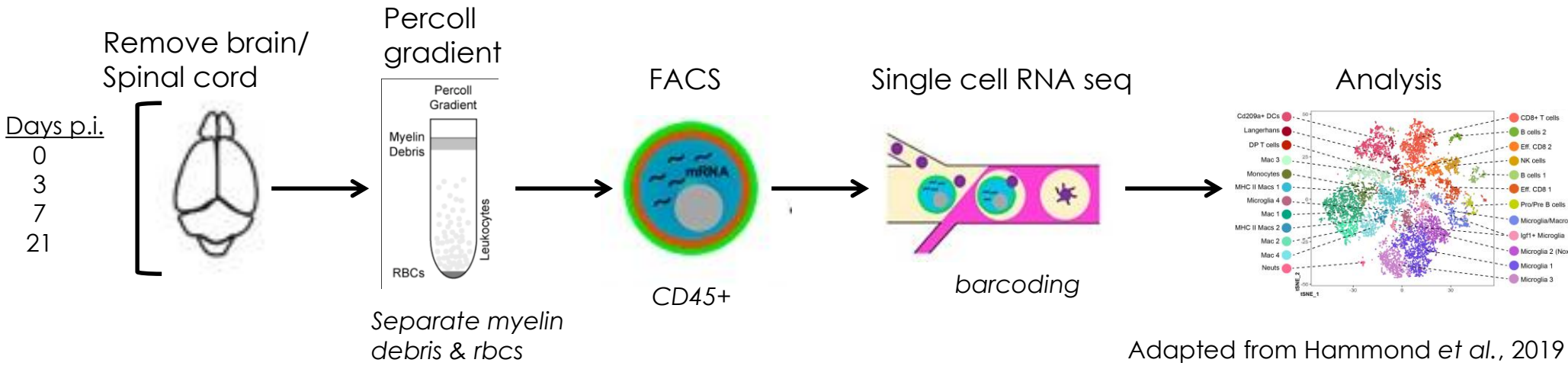
- Immunologic control of viral replication within the CNS is complex and involving controlled orchestration of innate and adaptive immune responses.
- Similarly, neuropathology *e.g.* axonal damage & demyelination in the face of persistent viral infection of the CNS is mediated by local glial responses working in combination with components of the innate & adaptive immune response.
- We employed scRNA seq on sorted CD45+ cells enriched from CNS of JHMV-infected mice at defined times p.i. to better understand these processes.

Single-cell RNA sequencing of CD45+ cells



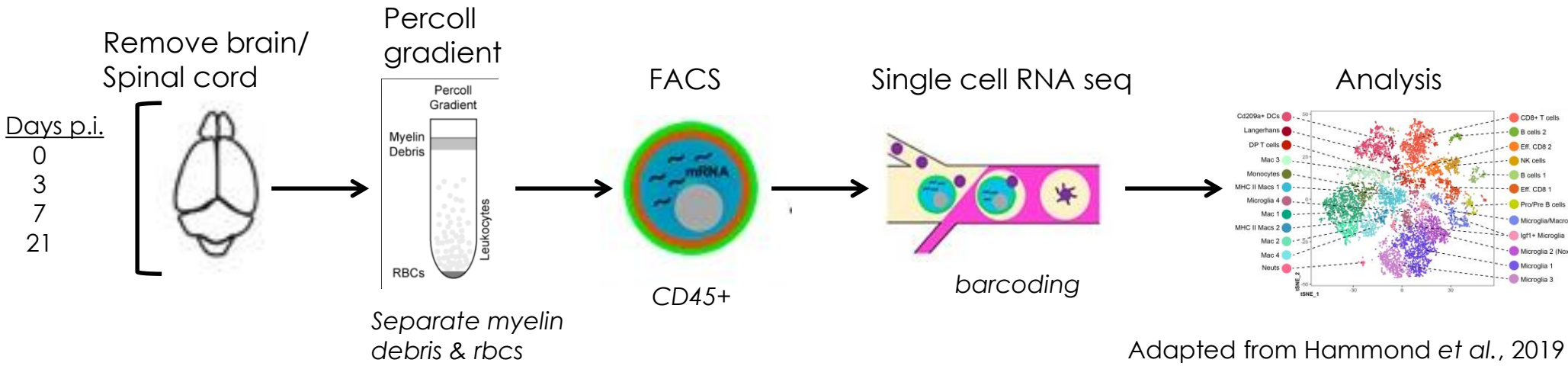
CD45+

Separate myelin debris & rbcs

Adapted from Hammond *et al.*, 2019

- Cells prepped and analyzed at HCI High-Throughput Genomics & Bioinformatics Core
- Fluidigm C1 System employed
- 3' RNA sequencing
- Version 2 Cell Ranger Pipeline to map to mouse genome
- Cell clustering analysis performed based upon similarity of gene signatures by Seurat genomics package

Single-cell RNA sequencing of CD45+ cells



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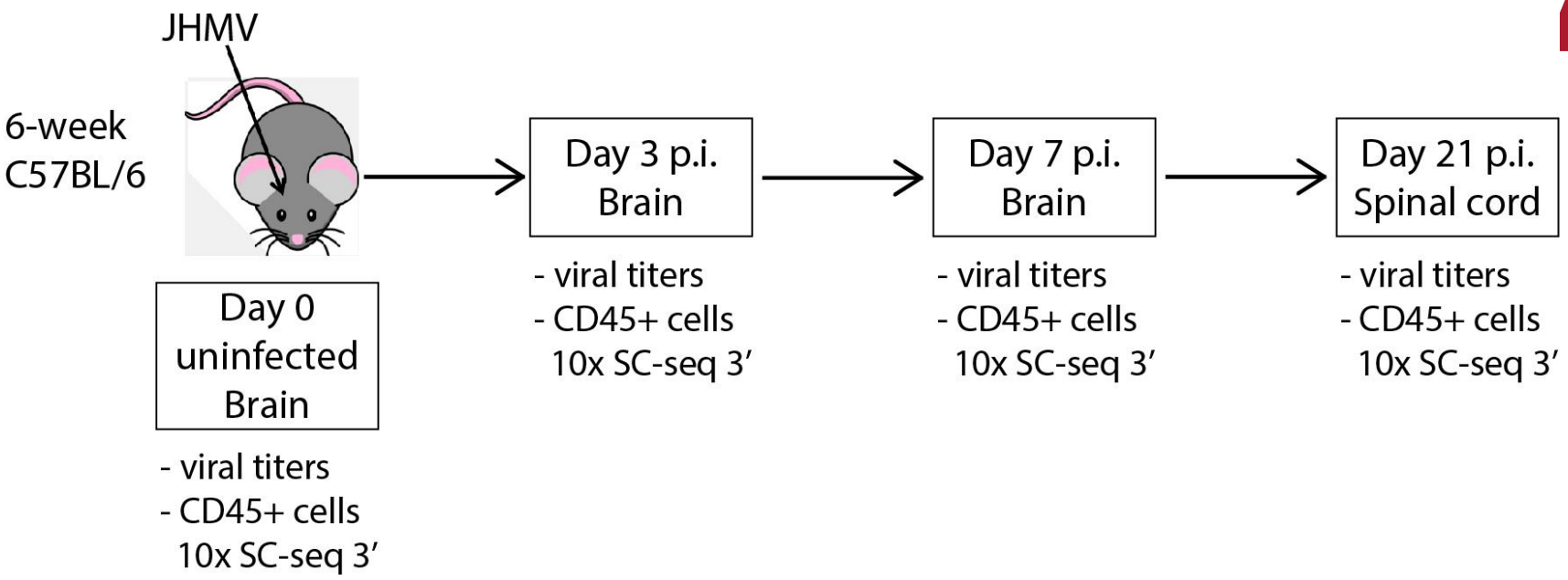
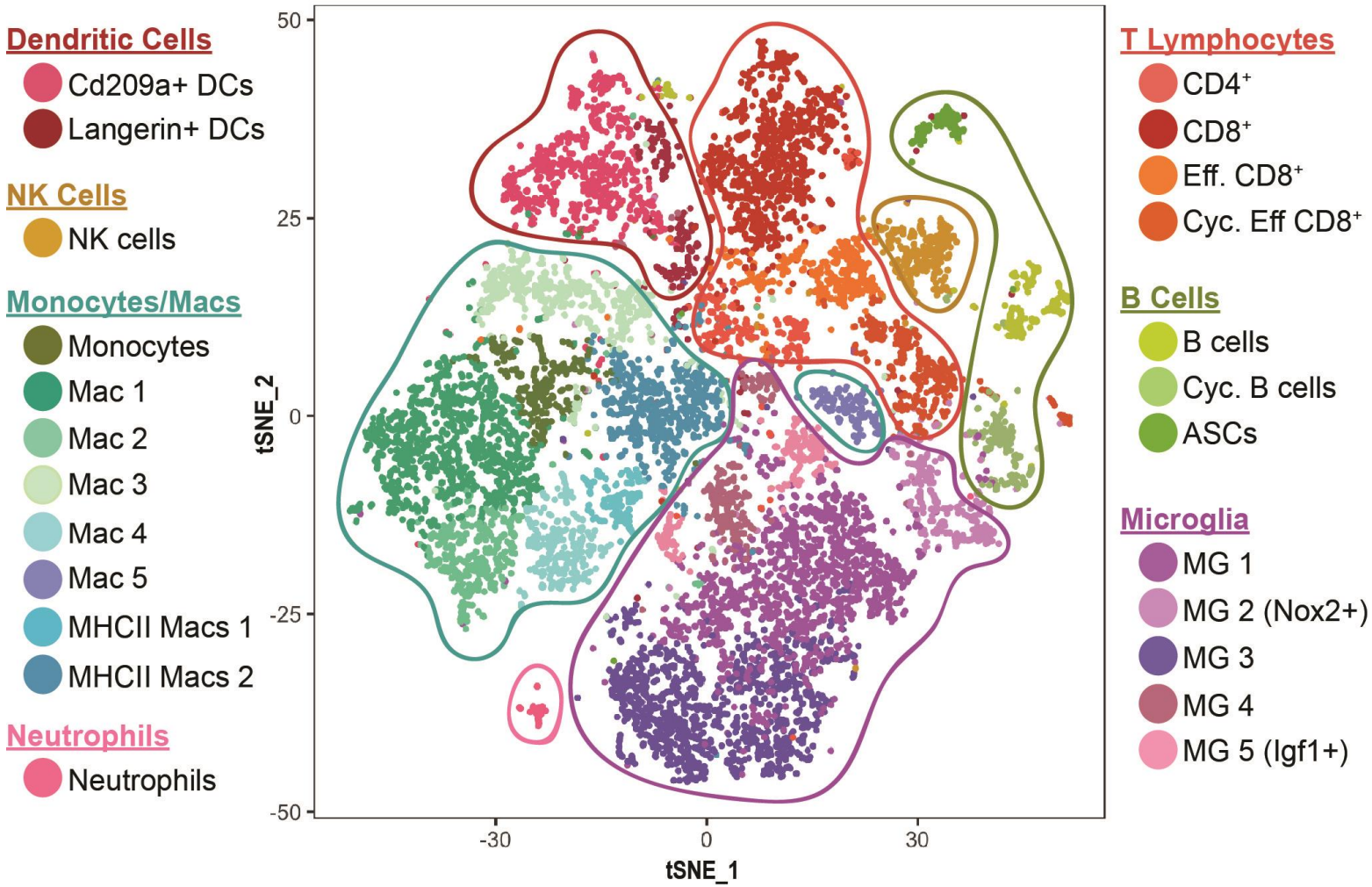
Experimental design



N	6	5	5	6
Cell #	1,769	4,397	3,880	1,920
Reads/Cell	36,399	40,055	41,717	52,751
Titer - PFU/g (Log_{10})	ND	5.1±0.4	3.5±0.2	<2.0

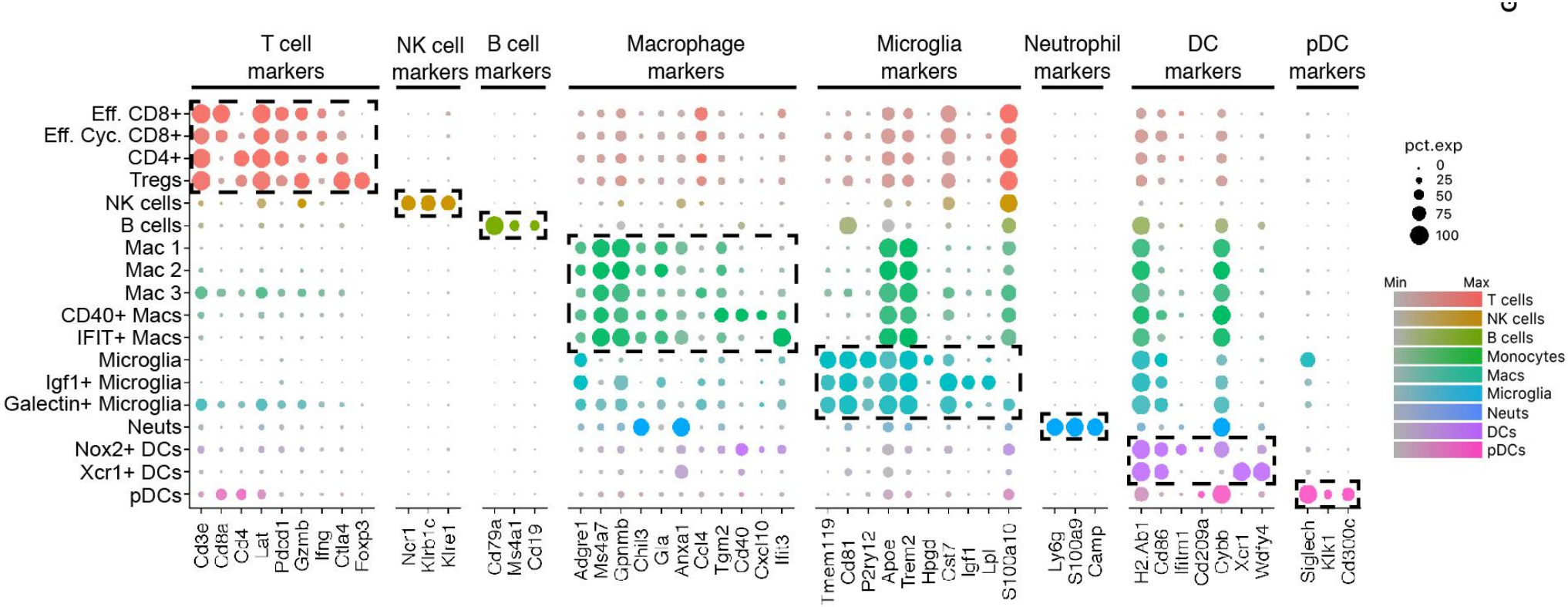
scRNA seq reveals the heterogeneity of immune response to JHMV infection of the CNS



Aggregate data from control days 0, 3, 7 and 21 days p.i. with virus

Amber Syage
Atakan Ekiz

Dot charts showing expression of selected genes in cell clusters



- Size of the dot represents the frequency of cells within cluster expressing gene of interest
- Color intensity indicates levels of expression
- Dashed boxes indicate genes that are expressed within clusters

Atakan Ekiz
Amber Syage

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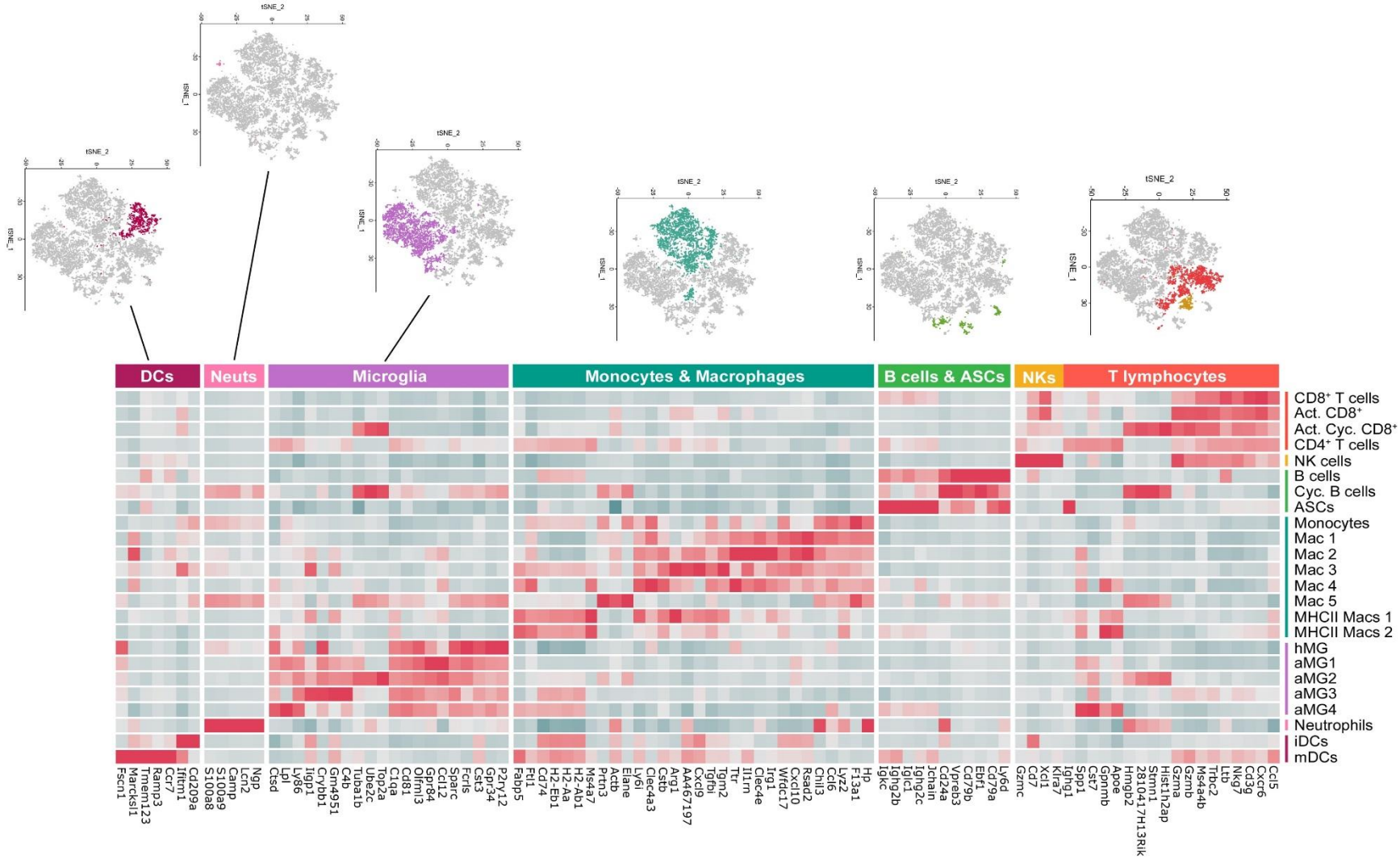
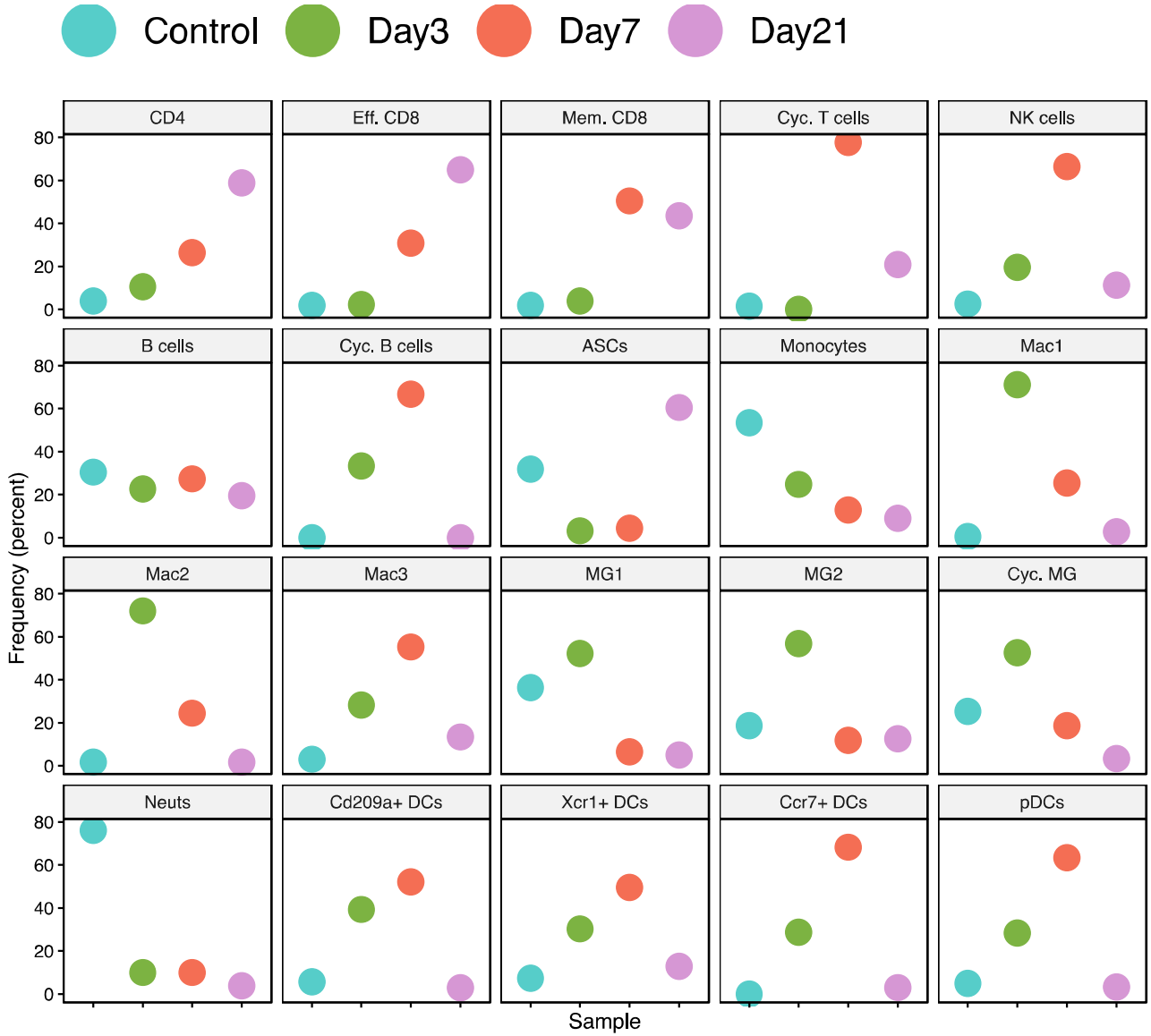
Using scRNASeq we can start to identify top genes expressed by different immune cell subsets at defined times p.i.



Kinetics of immune cell infiltration into CNS of JHMV-infected mice



Amber Syage

Microglia – development, health and disease

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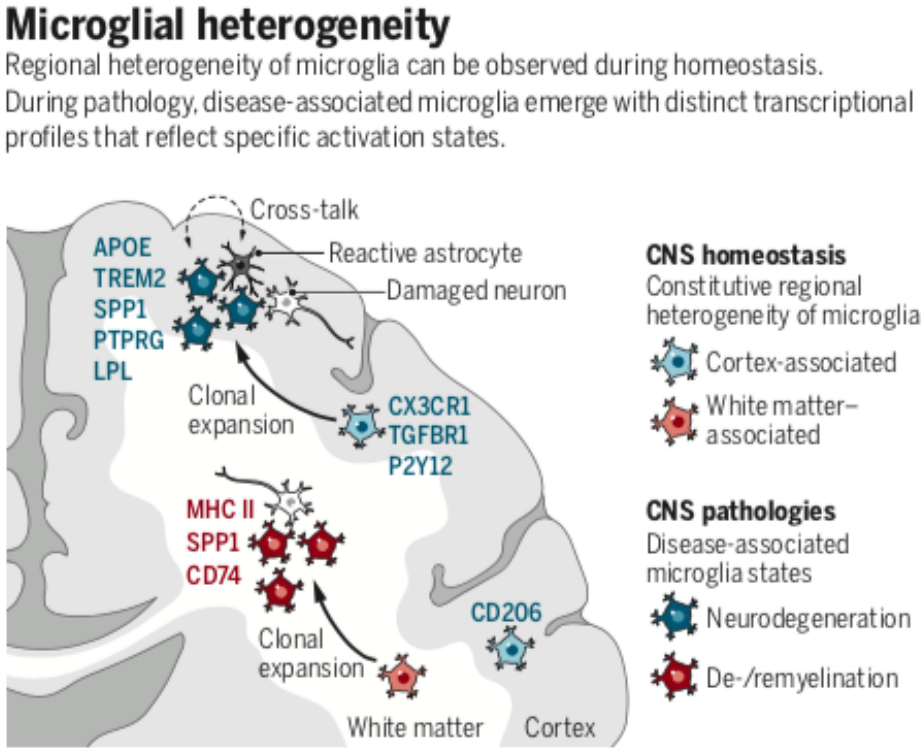
MICROGLIA

- Tissue-resident macrophages of CNS
- Originate from primitive (Kit+) erythromyeloid progenitors in yolk sac ~E8
- Colonize the embryonic CNS ~E9 (before BBB formation)
- Adult, stable CNS population (parenchymal)

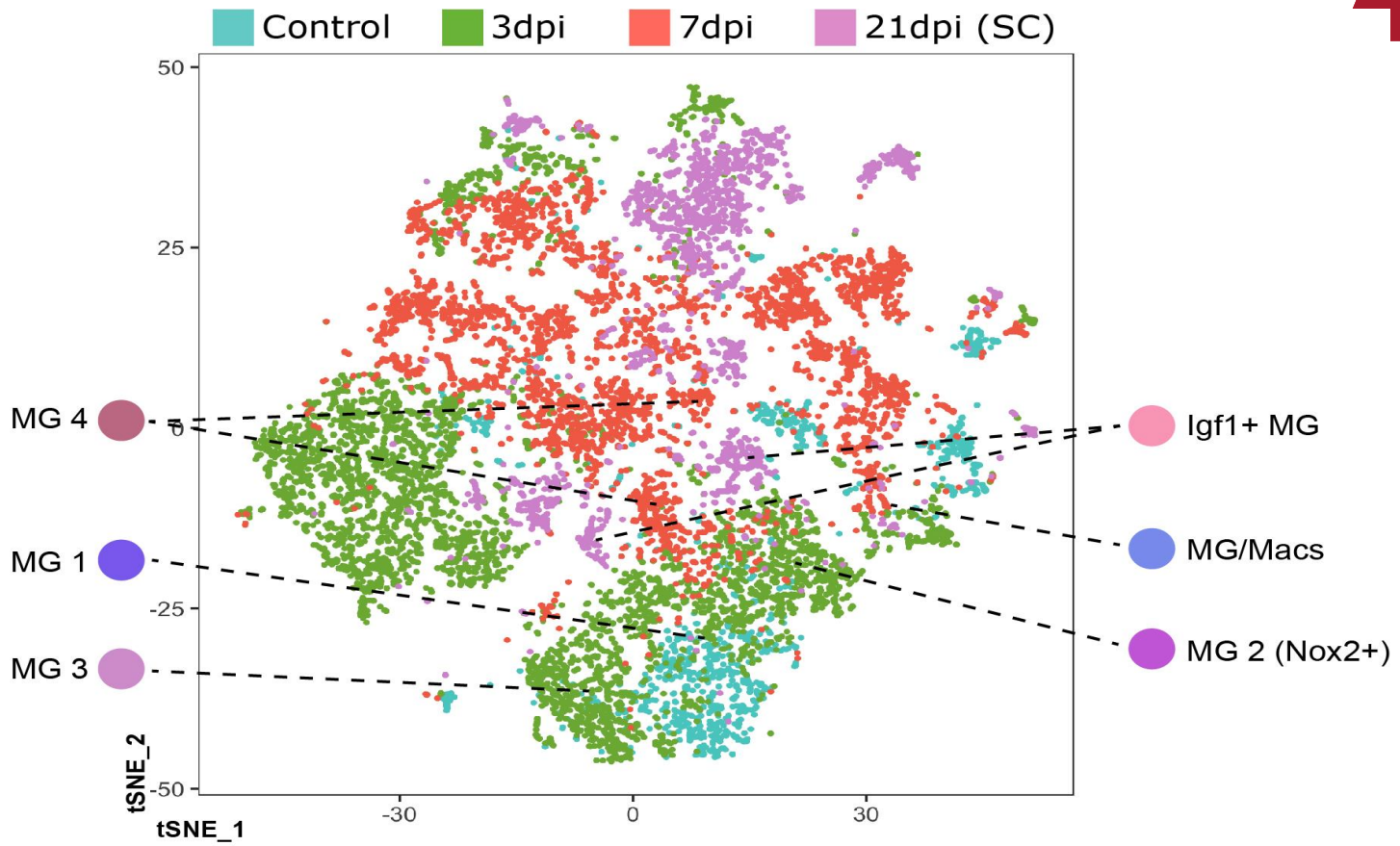
- Repopulate after experimental depletion

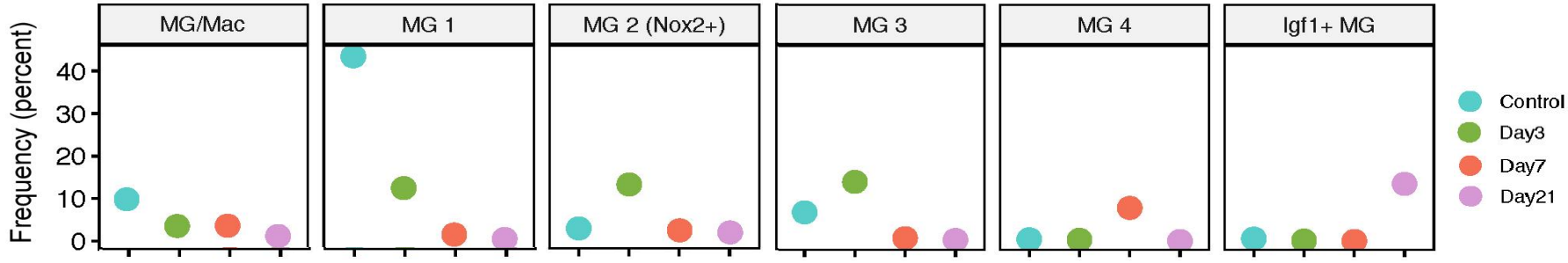


- Microglia are considered important contributors to a number of human CNS diseases including Alzheimer's disease, multiple sclerosis, CNS trauma, and psychiatric conditions.
- Targeting microglia for treatment of human CNS diseases has gained traction in recent years due to CNS permeable drugs that selectively target microglia.
- How targeting microglia impacts host defense following microbial CNS infection is an important question that is now being addressed in pre-clinical animal models.

Prinz and Priller, *Nature Neuro Rev* 2014; Priller and Prinz, *Science*, 2019

Single Cell RNA seq on CD45+ cells at days 0, 3, 7, and 21 p.i. reveals different microglia populations at defined stages of disease





Amber Syage

What is functional role of microglia in host defense and disease following JHMV infection of the CNS?

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- Do microglia contribute to host defense in response to infection with a neurotropic virus?
- What are the functional contributions of microglia to spinal cord demyelination and remyelination in mice persistently infected with a neurotropic virus?
- To address these questions, we depleted microglia using the CSF1R inhibitor PLX5622 and evaluated disease outcomes.

Microglia and host defense following viral infection of the CNS

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The Journal of Clinical Investigation

RESEARCH ARTICLE

Microglia are required for protection against lethal coronavirus encephalitis in mice

D. Lori Wheeler,[1] Alan Sariol,[1] David K. Meyerholz,[2] and Stanley Perlman[1,3]

[1]Interdisciplinary Graduate Program in Immunology, [2]Department of Pathology, and [3]Department of Microbiology and Immunology, University of Iowa, Iowa City, Iowa, USA.

RESEARCH Open Access

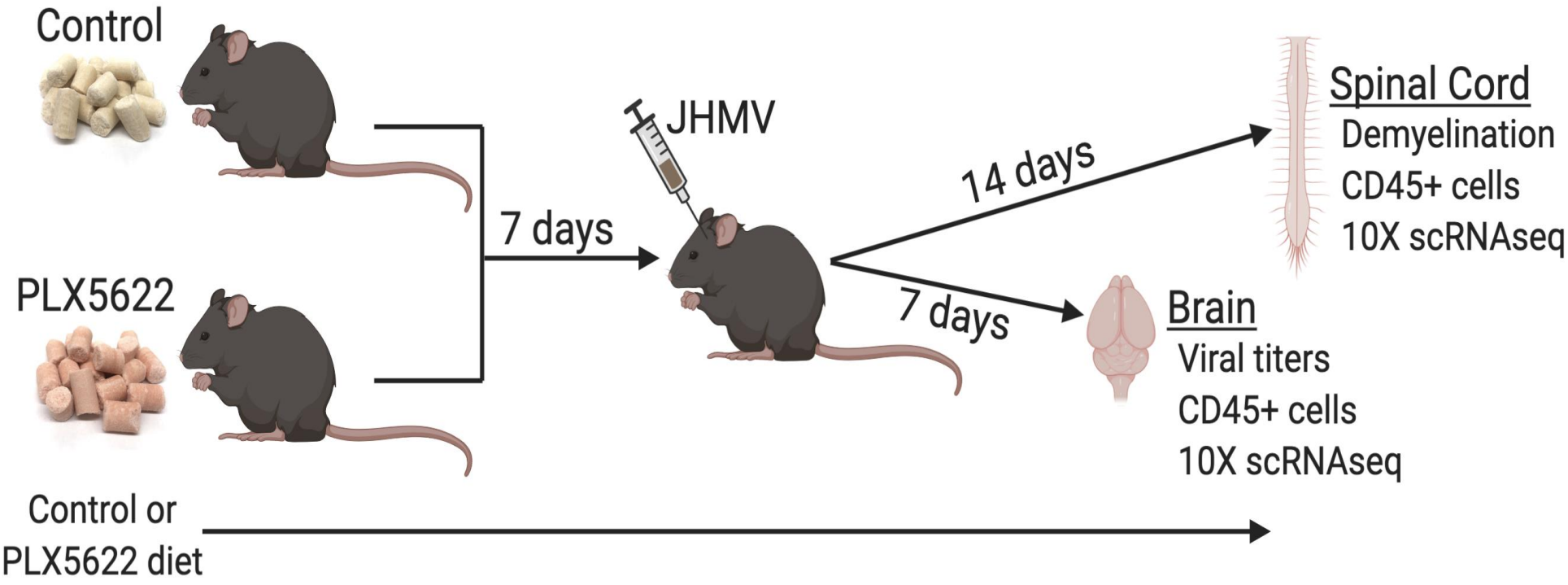
CSF1R antagonism limits local restimulation of antiviral CD8$^+$ T cells during viral encephalitis

Kristen E. Funk[1] and Robyn S. Klein[1,2,3*]

Microglial cell depletion is fatal with low level picornavirus infection of the central nervous system

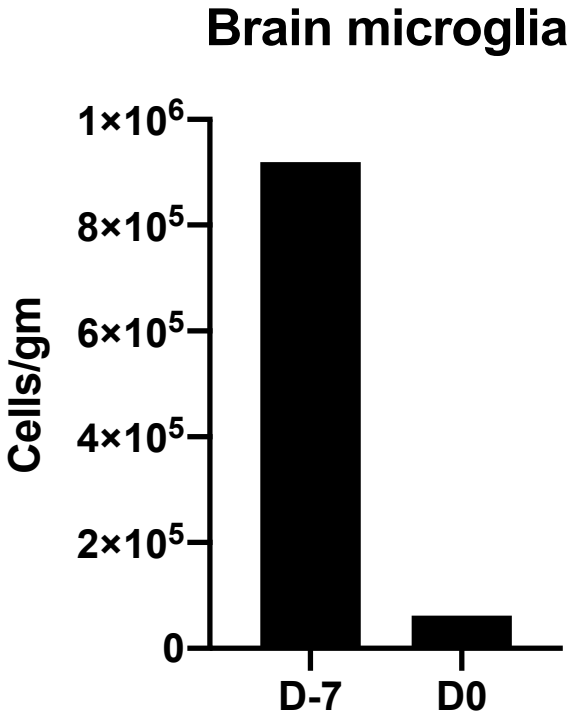
John Michael S. Sanchez[1] · Ana Beatriz DePaula-Silva[1] · Daniel J. Doty[1] · Amanda Truong[2] · Jane E. Libbey[1] · Robert S. Fujinami[1]

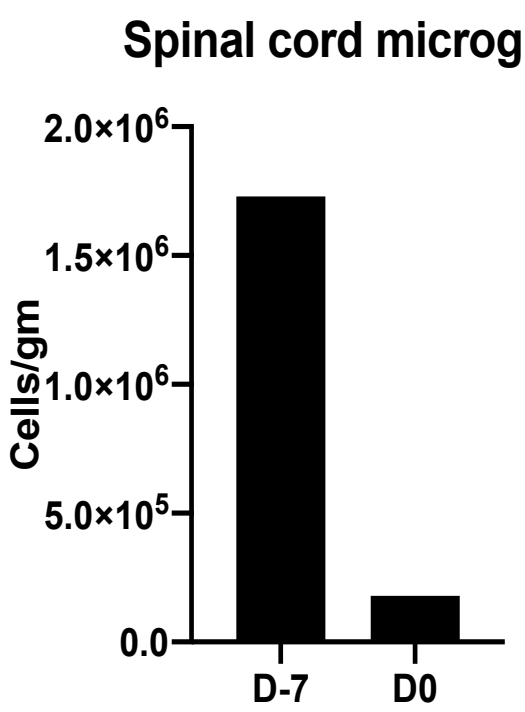
Experimental design – PLX5622 targeting of microglia

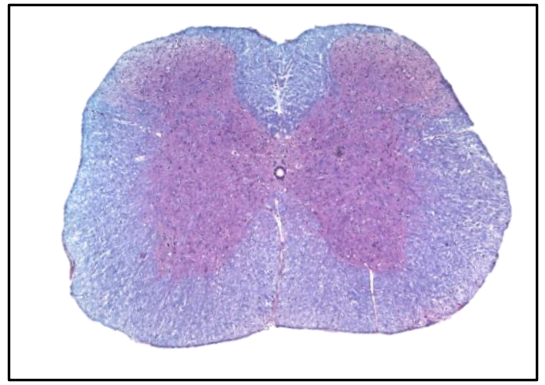


- Mice fed control chow or PLX5622 chow (1,200 mg/kg) 7 days prior to infection
- Experimental mice remain on respective chow for duration of experiment

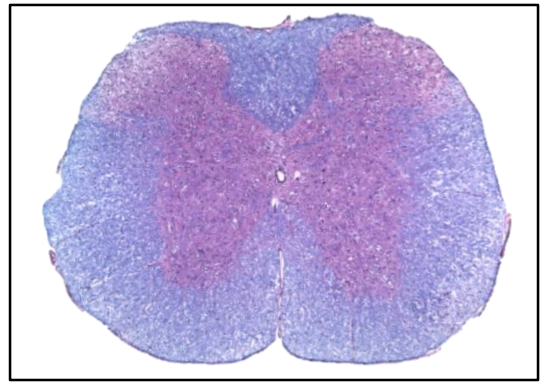
PLX5622 treatment for 7 days reduced microglia numbers within the brains and spinal cords – prior to infection







Prior to PLX5622

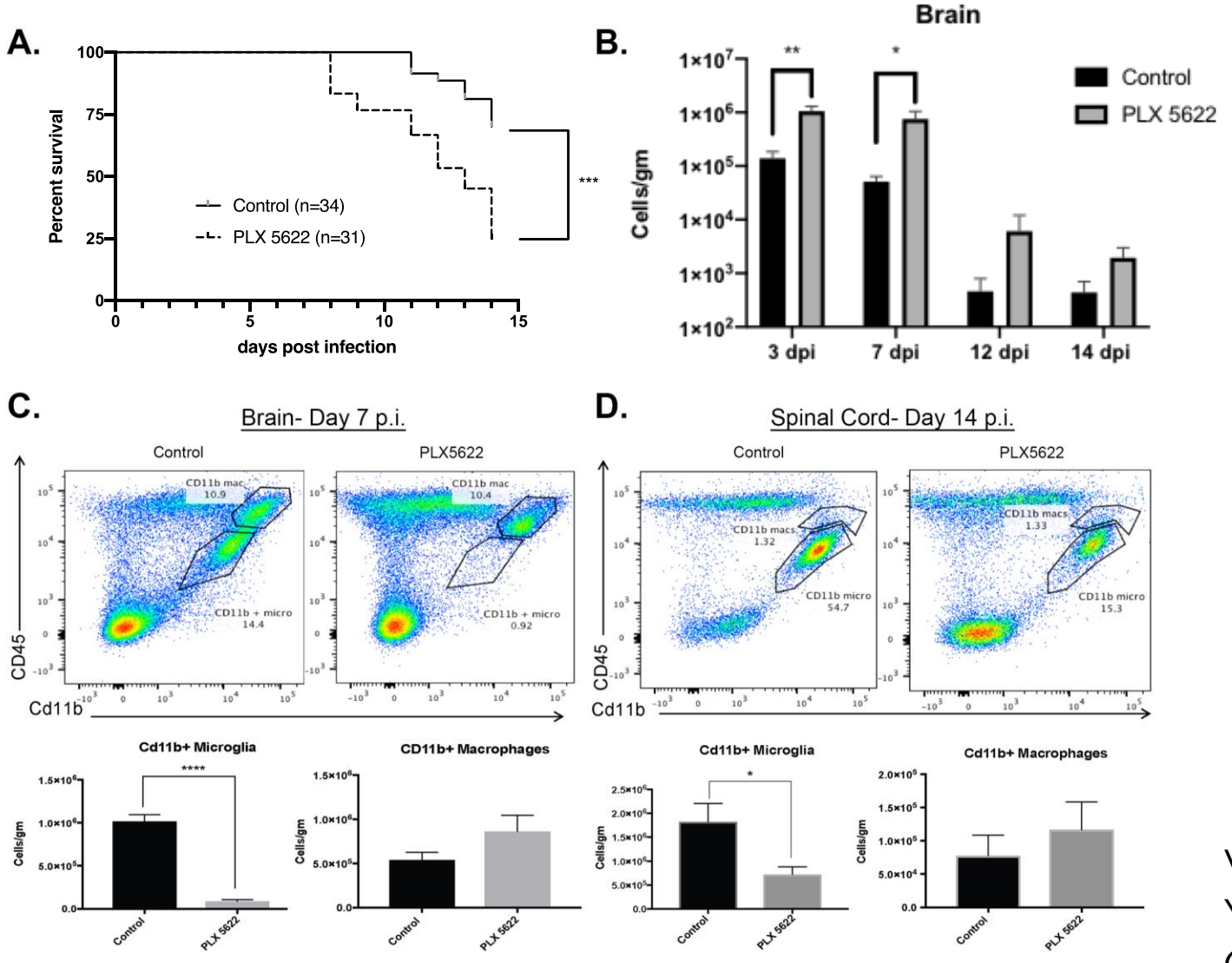


Day 7 post-PLX5622

Yuting Cheng

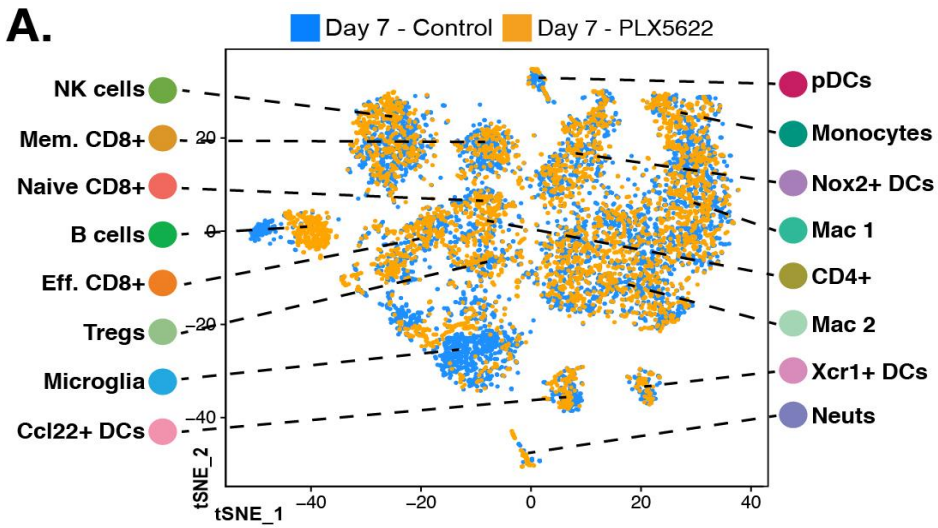
Myelin integrity not disrupted

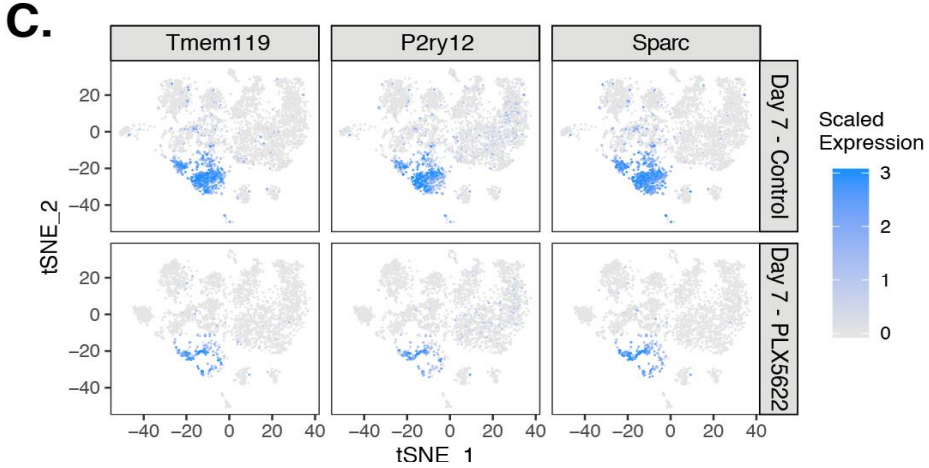
PLX5622 treatment increases mortality associated with impaired ability to control viral replication in the CNS

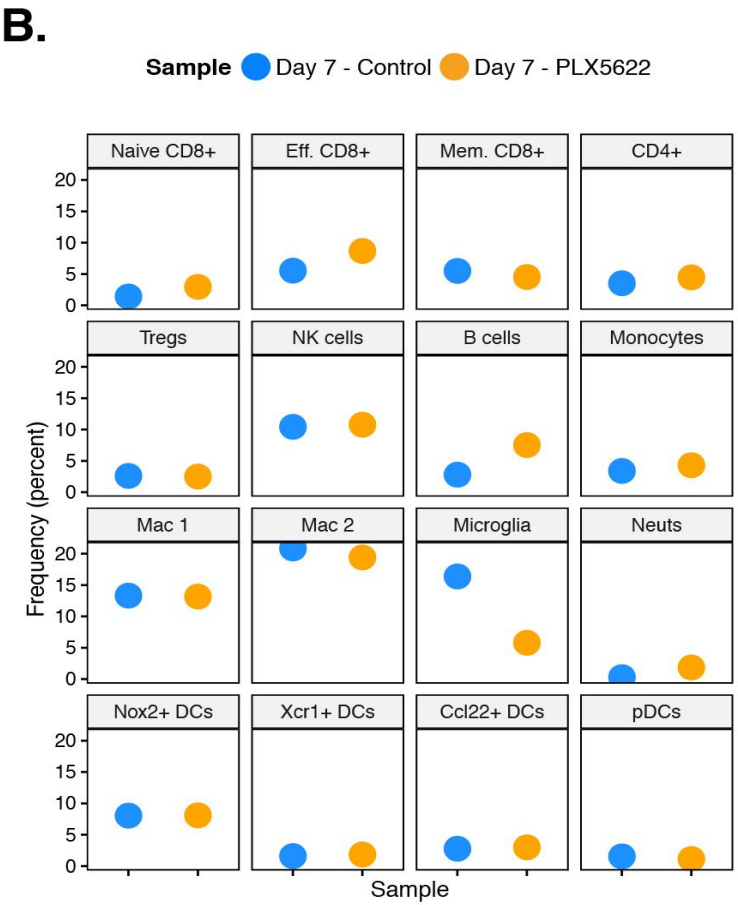


Vrushali Mangale, Ph.D.
Yuting Cheng
Colleen Stone

PLX5622 treatment does not dramatically alter immunological landscape – day 7 p.i.

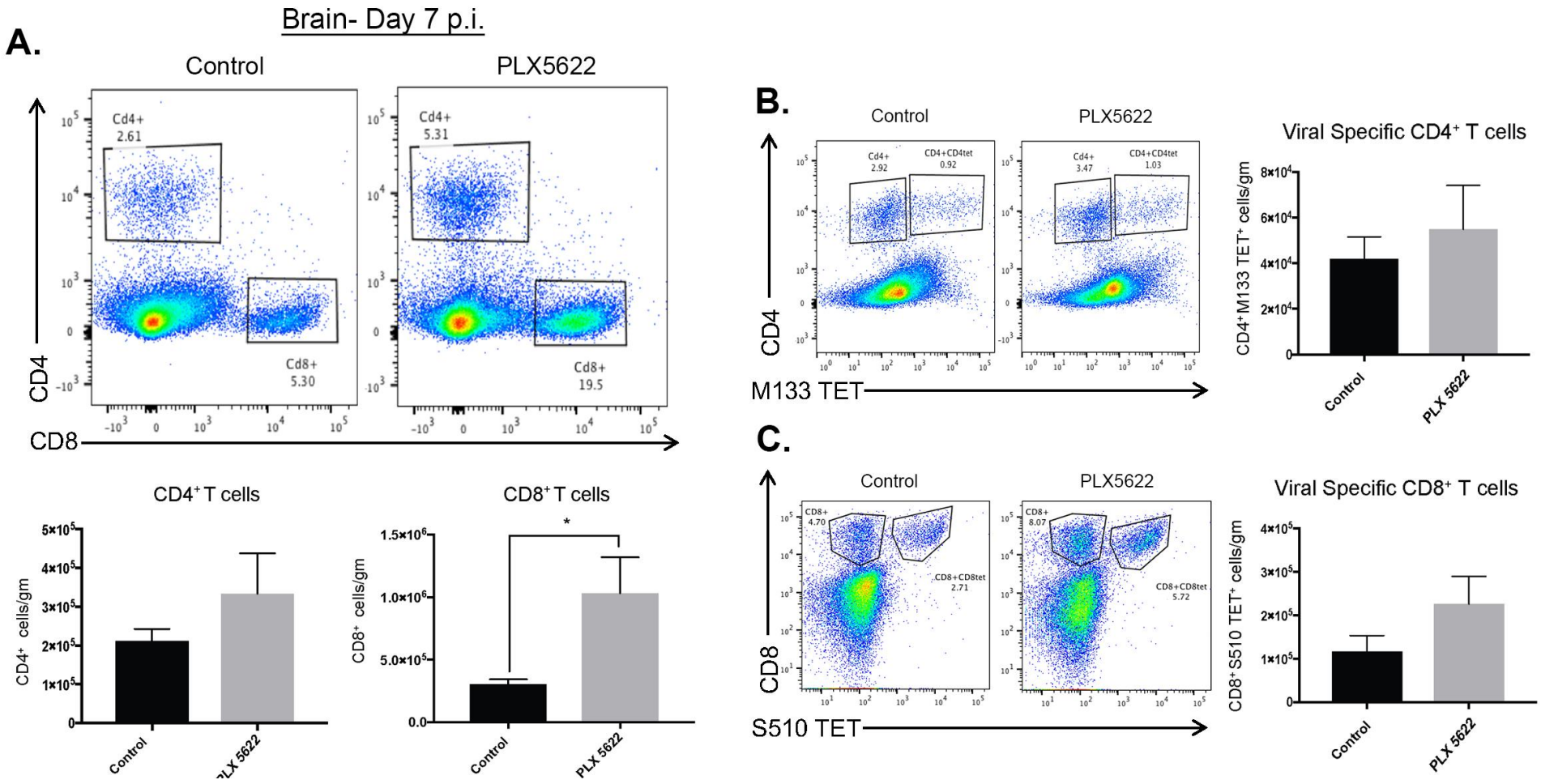






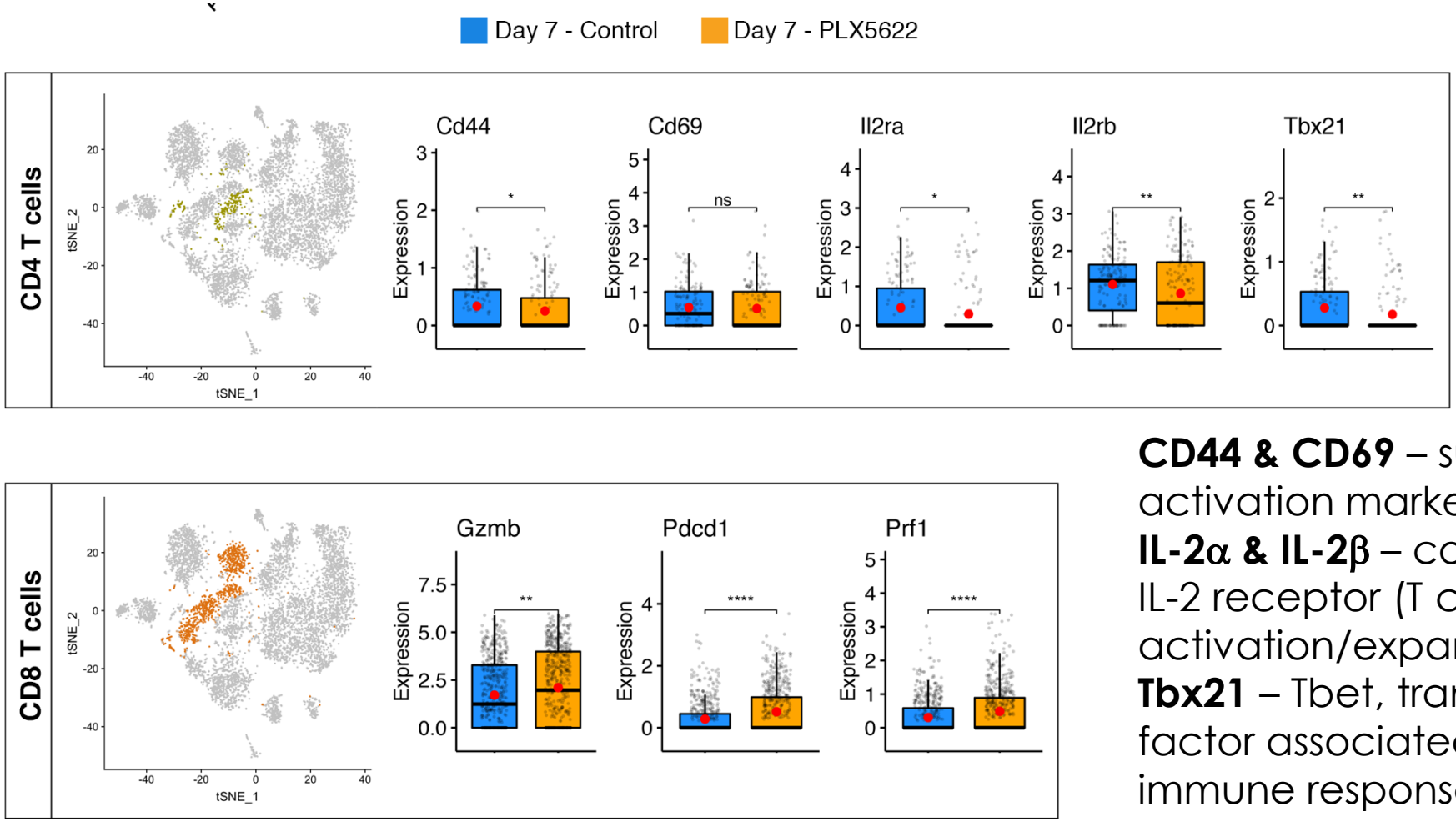
Amber Syage

T cell infiltration into the brain is increased in response to PLX5622 treatment – Day 7 p.i.



Vrushali Mangale, Ph.D.

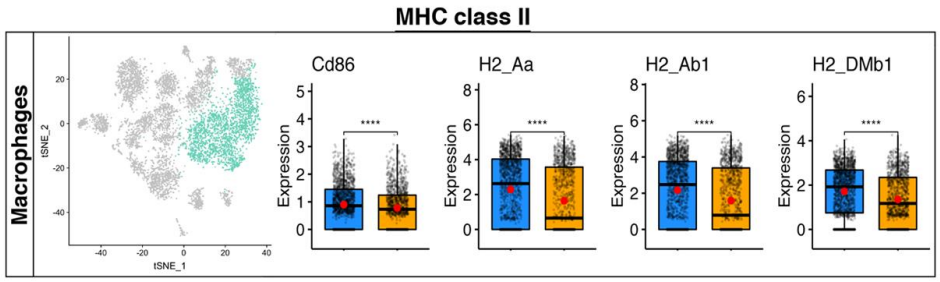
Differential activation states of CD4+ and CD8+ T cells in PLX5622-treated mice

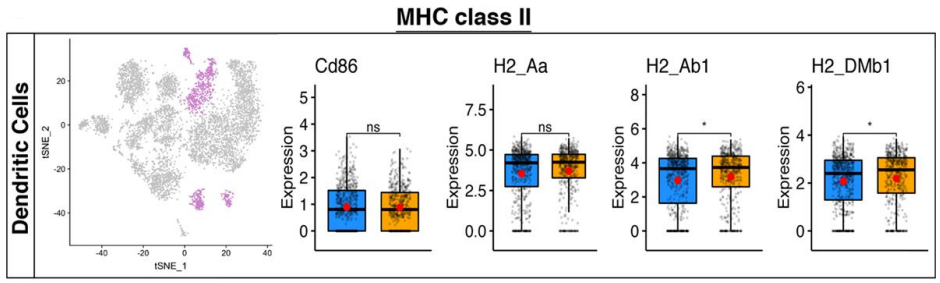


CD44 & CD69 – surface activation markers
IL-2α & IL-2β – components of IL-2 receptor (T cell activation/expansion)
Tbx21 – Tbet, transcription factor associated with Th1 immune response

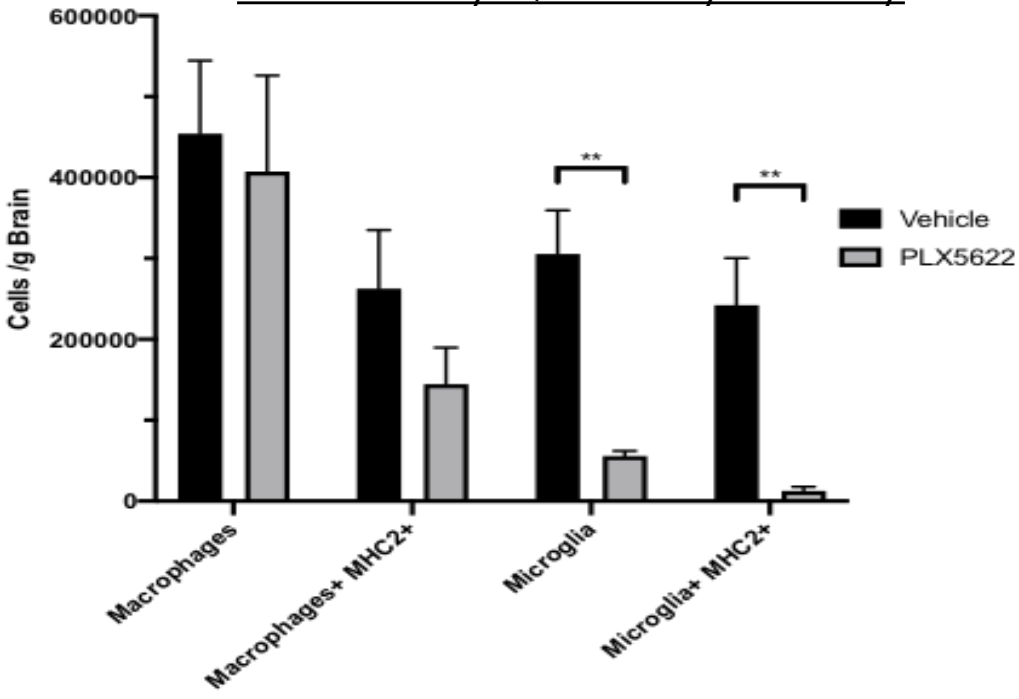
Gsmb – granzyme b; ***Pdcd1*** – PD-1; ***Prf1*** - perforin

PLX5622-treatment led to reduced expression of MHC class II transcripts and protein in macrophages – day 7 p.i.

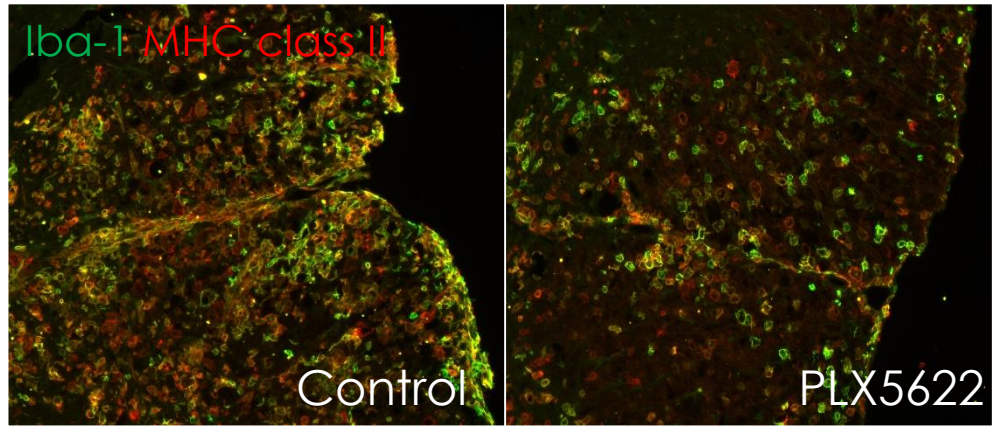




Brain – day 7, flow cytometry

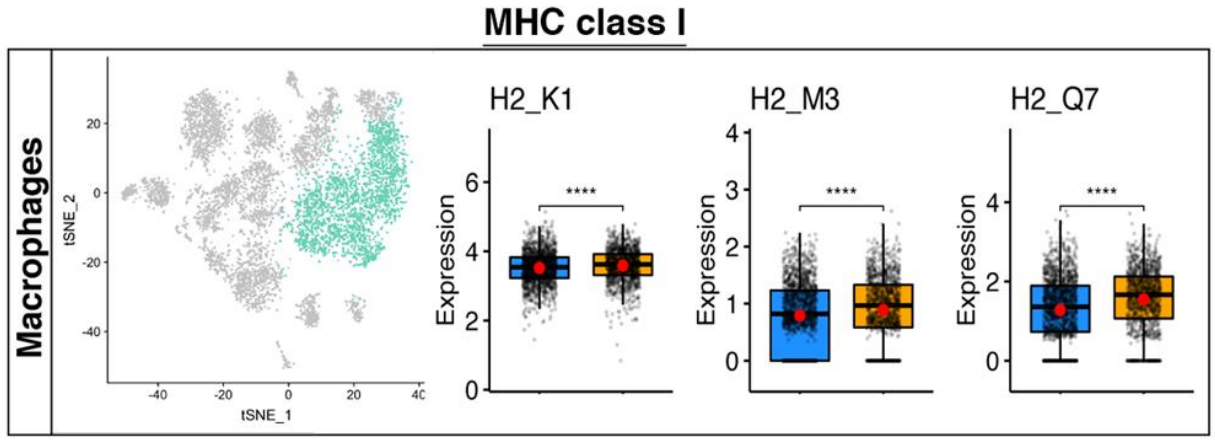


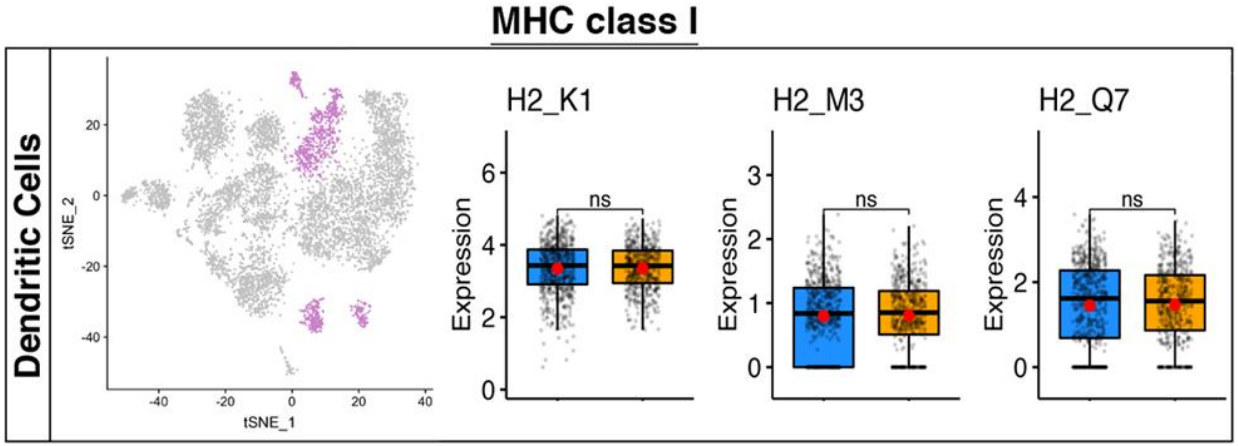
Spinal cord – day 7



Amber Syage
Atakan Ekiz, Ph.D.
Dominic Skinner

Expression of MHC class I-associated transcripts was increased in macrophages and not altered in DCs following PLX5622 treatment - day 7 p.i.

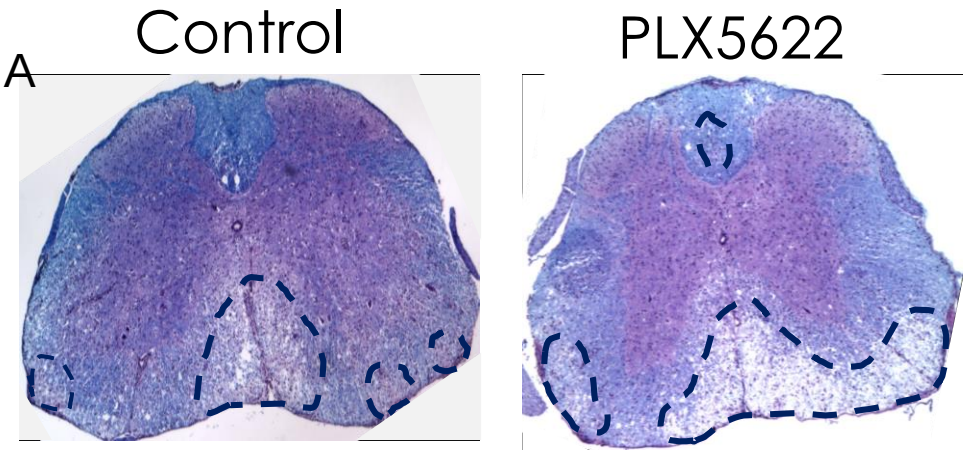


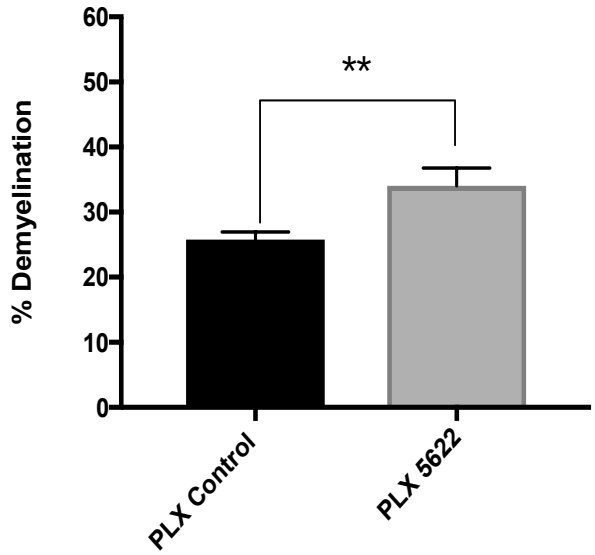


Amber Syage, Atakan Ekiz, Ph.D.

PLX5622 treatment increases the severity of demyelination in JHMV-Infected mice - day 14 p.i.

A





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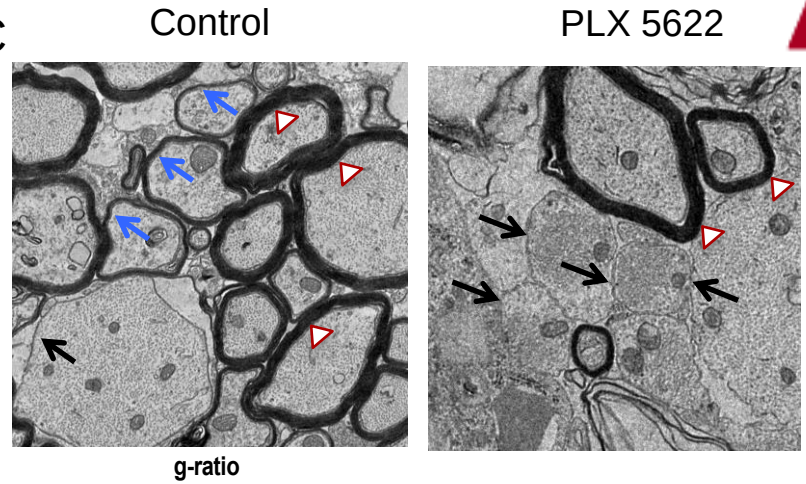

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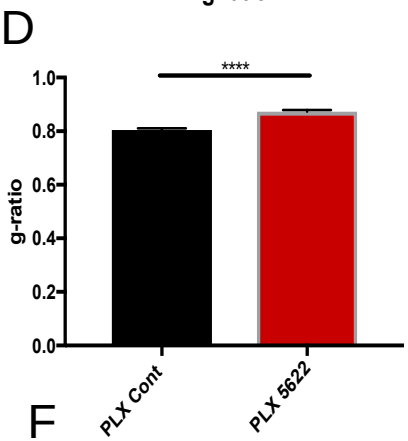

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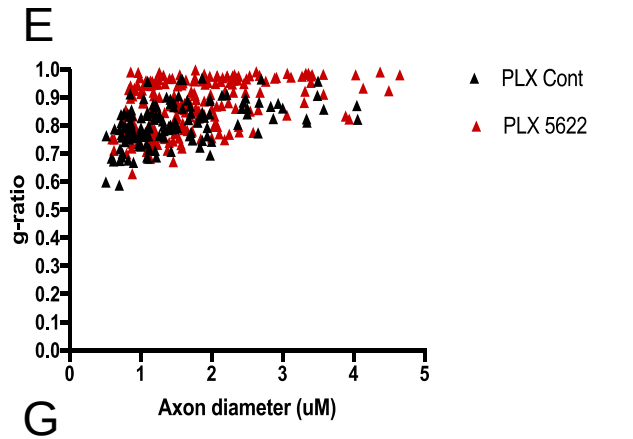

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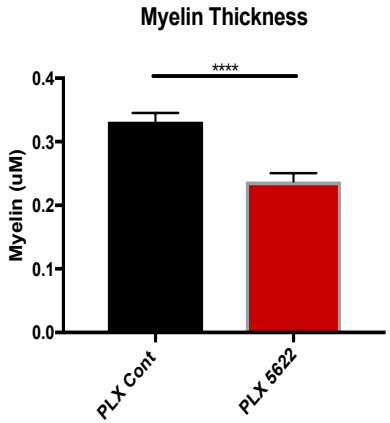

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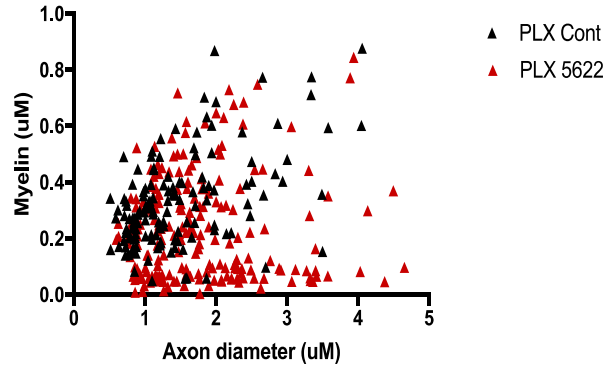

Vrushali Mangale, Ph.D., Dominic Skinner

PLX5622 reduces frequency of spinal cord oligodendrocytes

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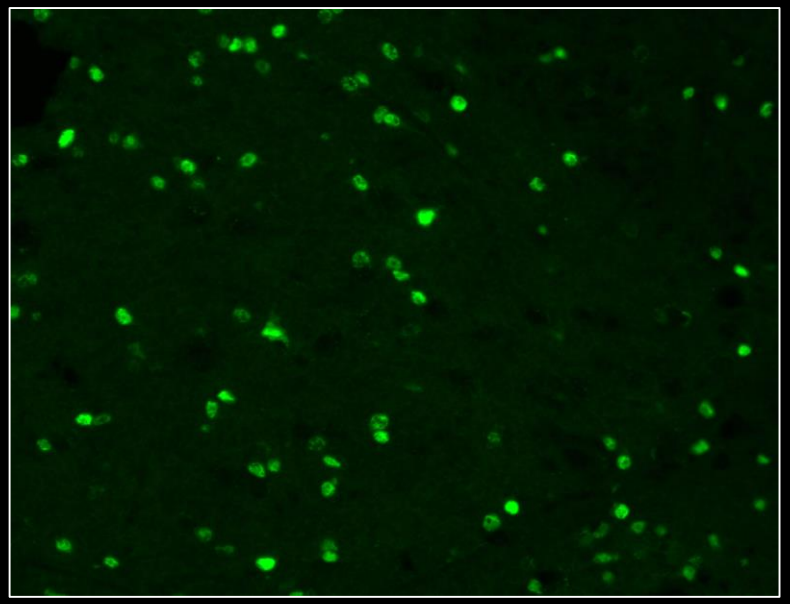
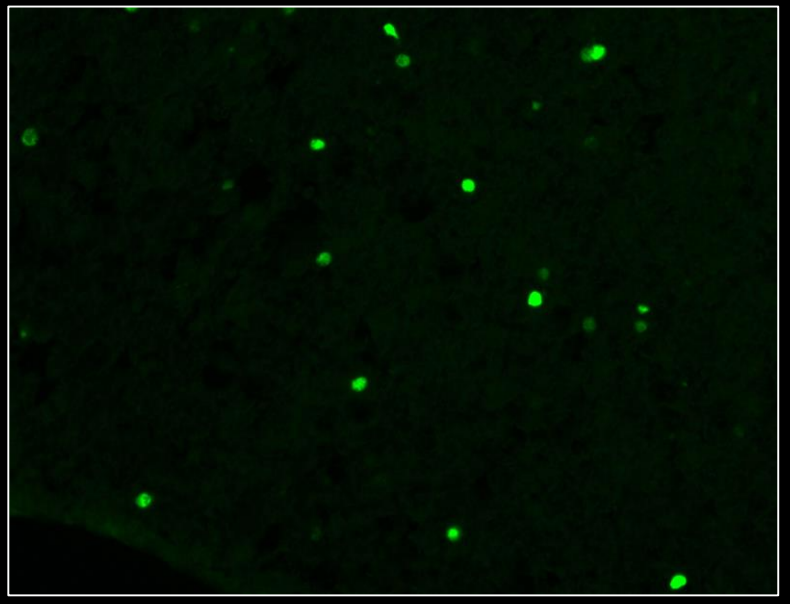
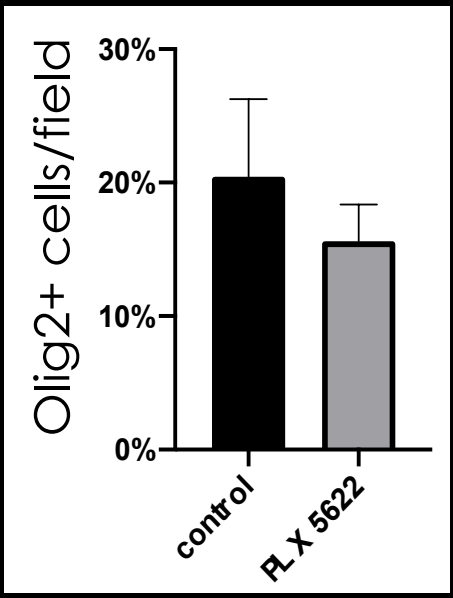
Control



PLX5622



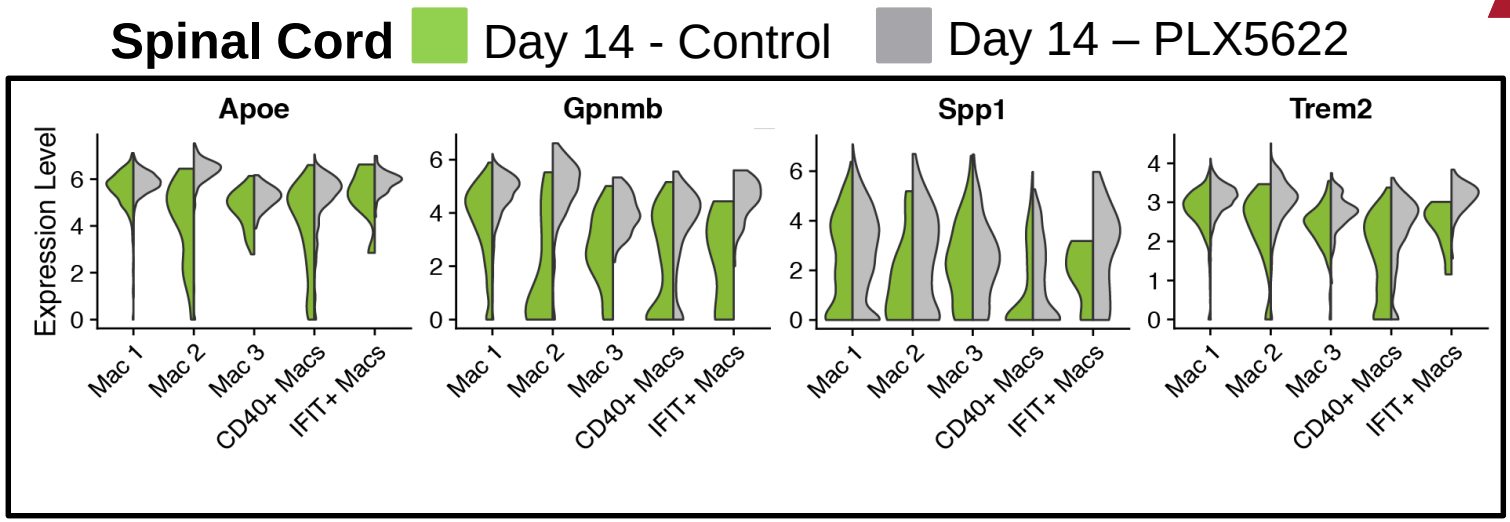


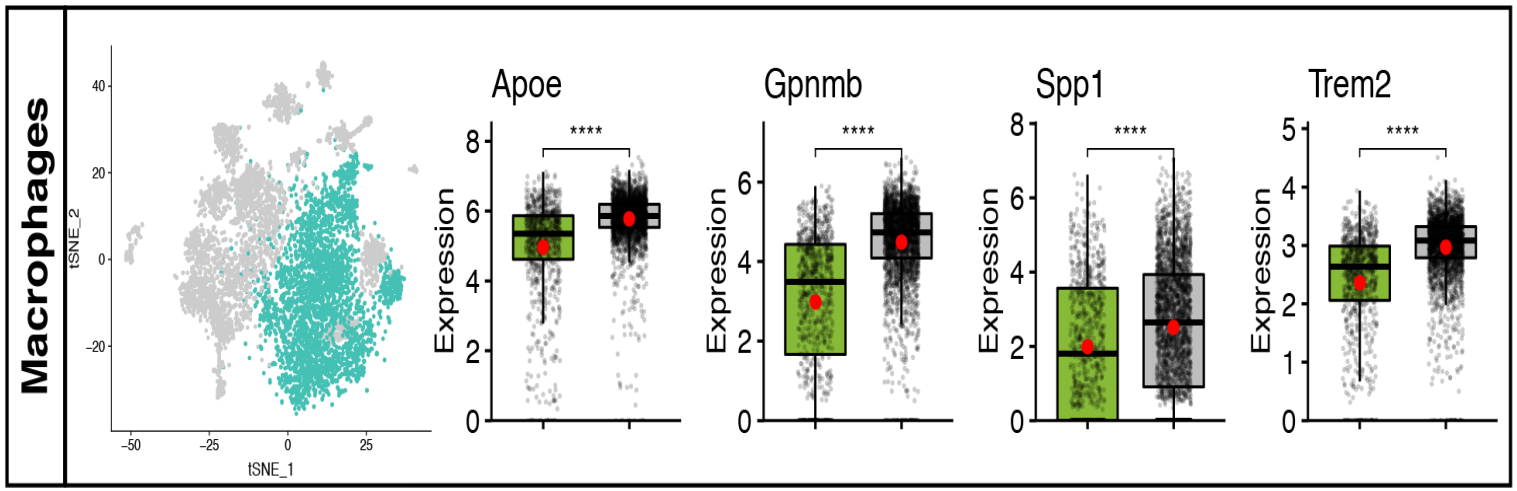
Olig2 staining – day 14 p.i.

Yuting Cheng

PLX5622 treatment results in increased expression of factors associated with demyelination

Amber Syage





Apoe, Apoliprotein E; *Gpnmb*, Transmembrane glycoprotein NMB; *Spp1*, Osteopontin; *Trem2*, Triggering receptors expressed on myeloid cells

Increased APOE expression in Iba-1+ cells within spinal cord white matter tracts – day 14 p.i.

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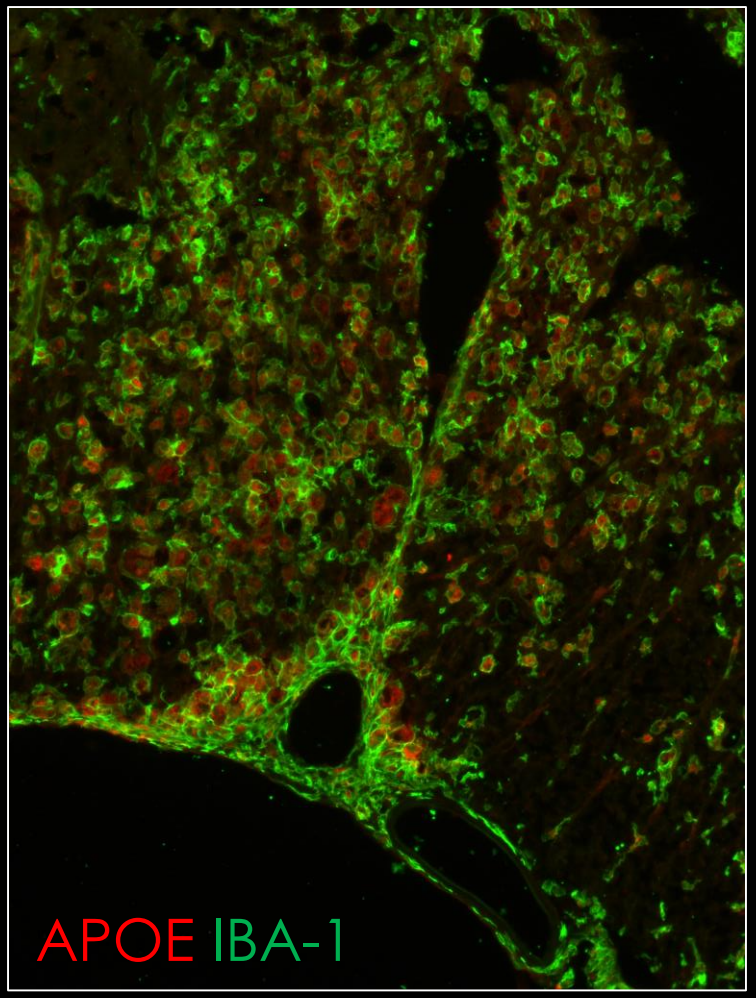
Control



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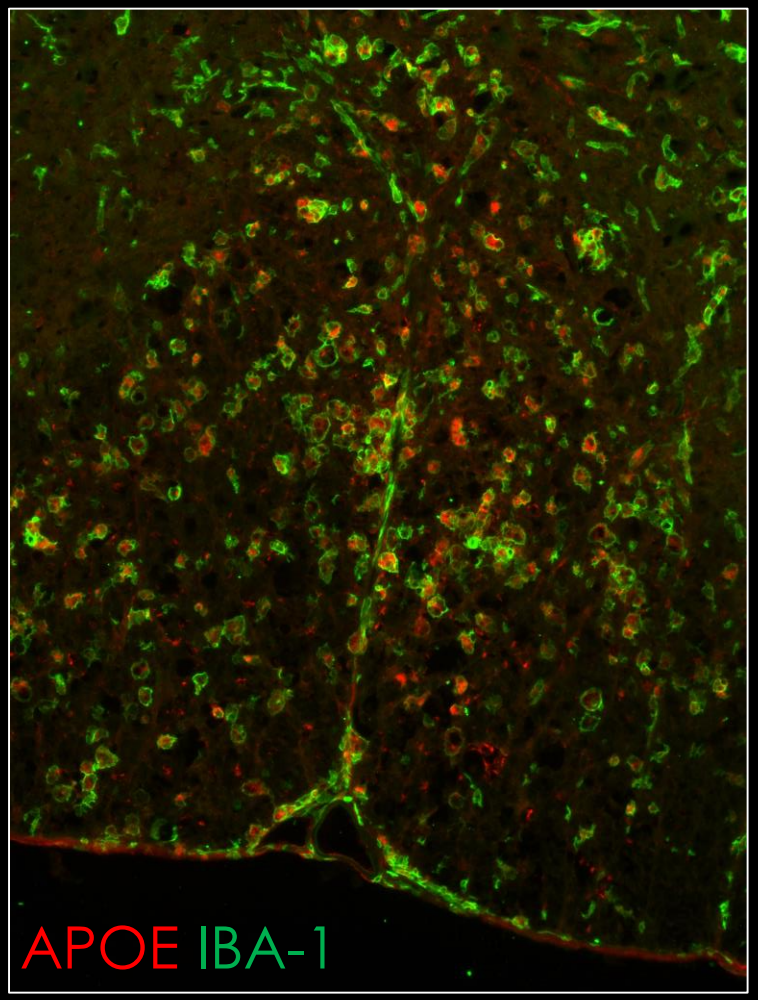
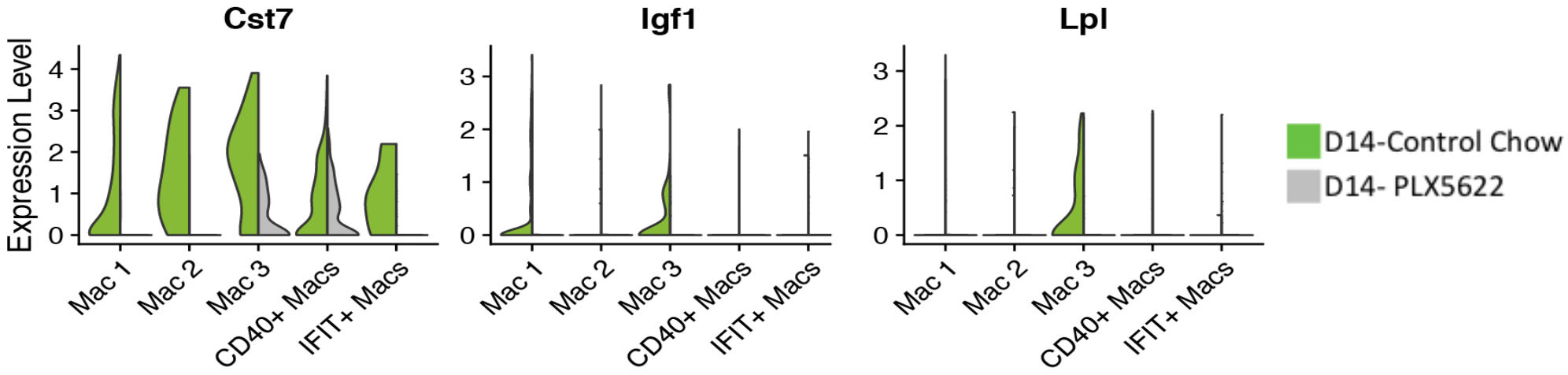
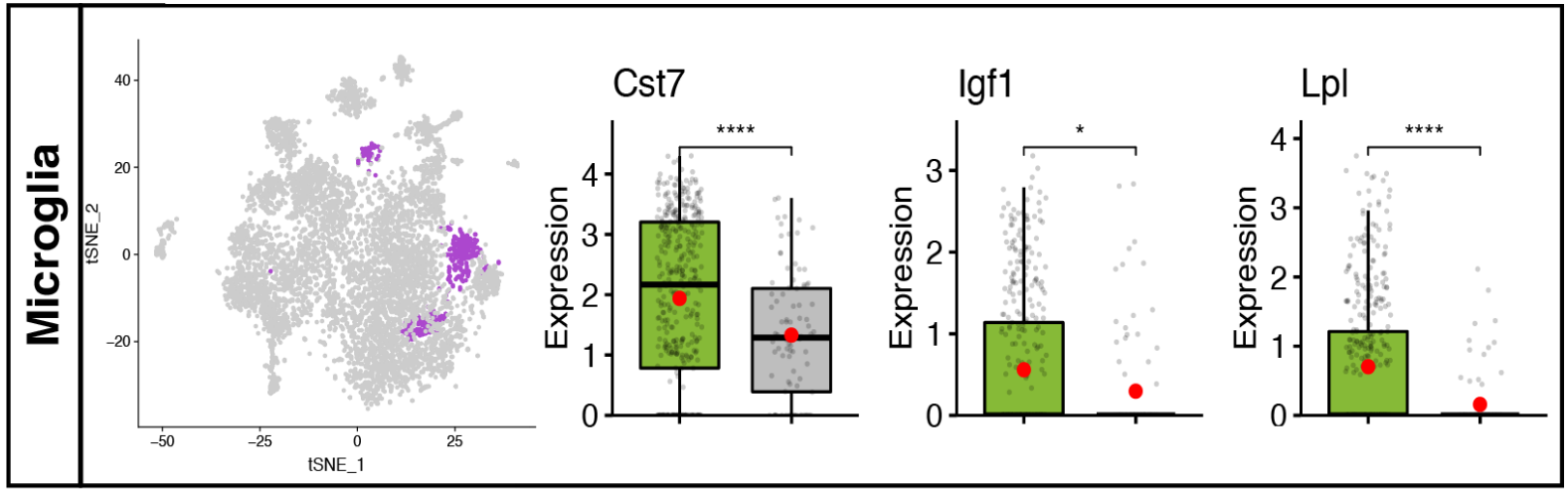


Differential expression of transcripts encoding factors associated with remyelination





Cst7, Cystatin-F; *Igf1*, Insulin growth factor 1; *Lpl,* Lipoprotein lipase

Amber Syage

Perspectives - I

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- Following JHMV infection of the CNS, microglia aid in control of viral replication , in part, through influencing antigen-presentation that aids in stimulation of CNS infiltrating virus-specific T cells.
- Administration of PLX5622 prior to infection results in increased demyelination associated with unique macrophage subpopulations characterized by distinct mRNA signatures e.g. *Apoe*, *Gpnmb*, *Trem2*, and *Spp1*.
- Remyelination efficiency was negatively impacted in PLX5622-treated mice arguing for a potential role for a population of microglia in augmenting repair possibly by regulating expression of *Igf1, Cst7*, and *Lpl* in macrophage populations
- Ongoing work will determine if i) repopulated microglia have intact immune function in face of viral infection, ii) is repopulation enforced in face of persistent CNS viral infection, and iii) does PLX5622 treatment after disease established result in increased demyelination?

Neutrophils, neuroinflammation, and demyelination

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- Emerging studies in MS patients highlight the potential importance of neutrophils in clinical disease progression and demyelination (Huber *et al., Neurology*, 2014; Rumble *et al., JEM*, 2015.; Naegele *et al., J. Neuroimmunol*. 2012).

- Preclinical mouse models of demyelination *e.g.* EAE and toxin models demonstrate neutrophils increase the severity of neuropathology and demyelination (Liu *et al., Nat. Neurosci*., 2010; Simmons *et al., J. Immunol*., 2014; Stoolman *et al., J. Immunol.*, 2014).

- A better understanding of how neutrophils influence clinical disease and demyelination in pre-clinical models of MS is necessary to determine if these cells are relevant therapeutic targets.

Question:

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Does sustained neutrophil infiltration into the CNS augment demyelination in JHMV-infected mice?

To address this question, we've generated a transgenic mouse in which the neutrophil chemoattractant chemokine CXCL1 is expressed in astrocytes upon doxycycline (DOX) injection.

Transgenic mice expressing CXCL1 from astrocytes

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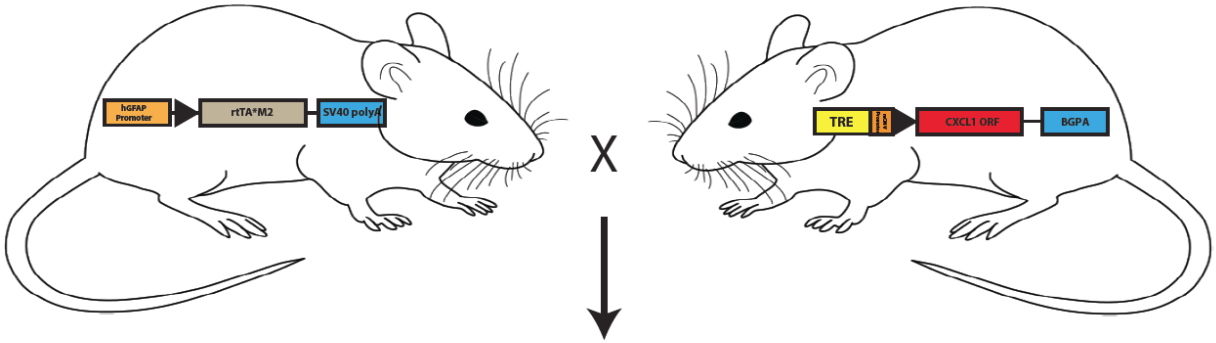
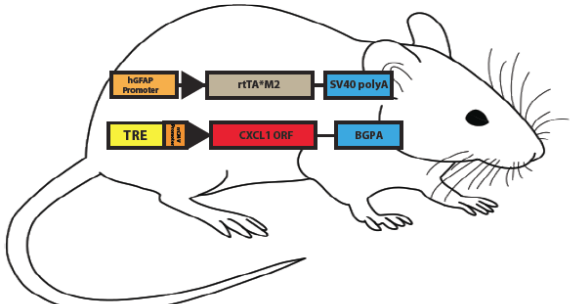
A transgenic mouse model which utilizes a doxycycline-inducible promoter to express CXCL1 in astrocytes was generated

(GFAP-rtTA tg mouse) **(TRE-CXCL1 tg mouse)**



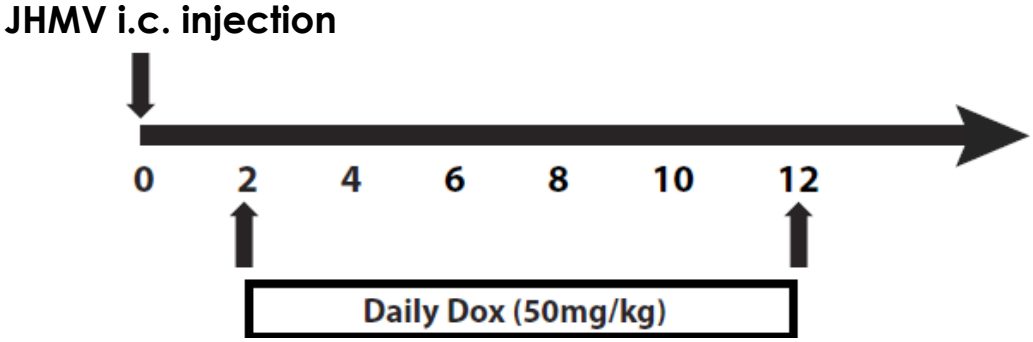
rtTA x CXCL1 Dbl tg

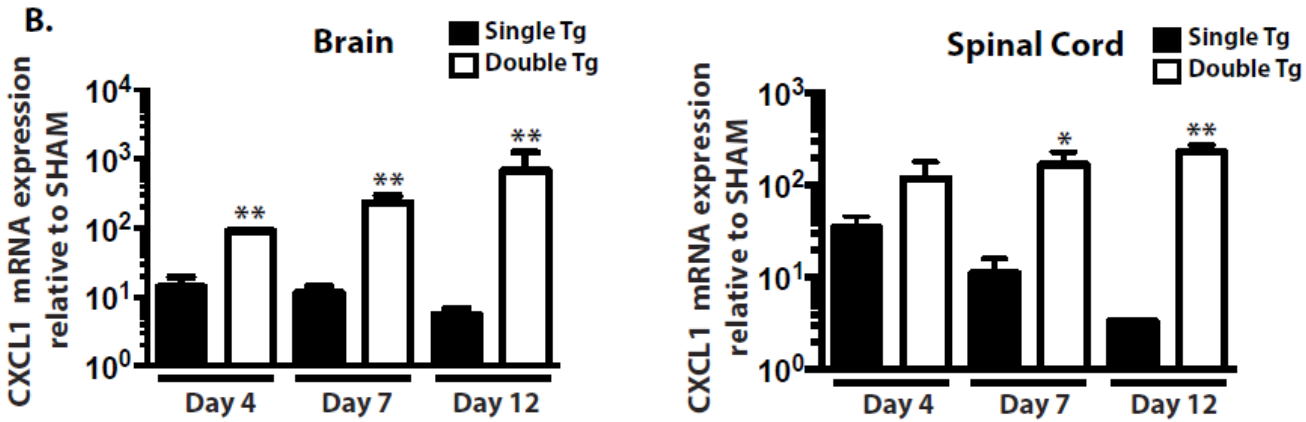


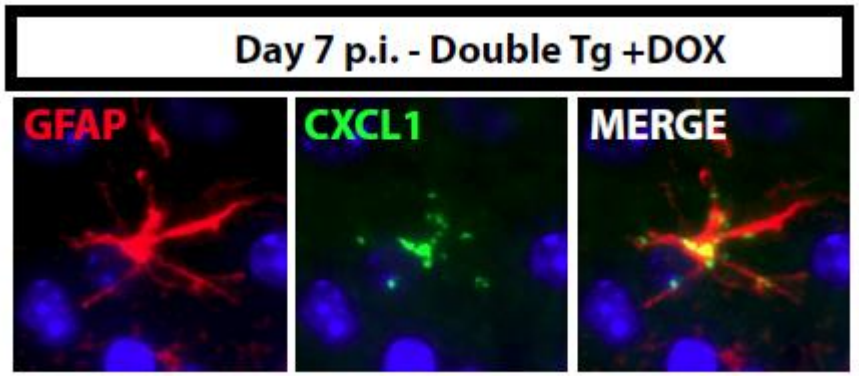
- Single Tg = CXCL1 only
- Double Tg = GFAP-rtTA and CXCL1

Marro *et al., J. Immunol., 2016*

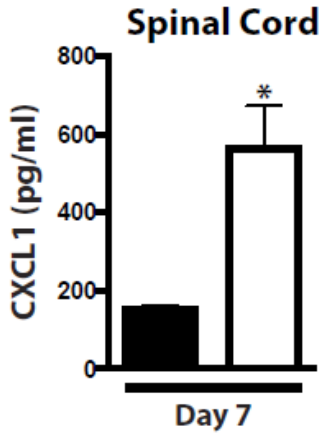
Elevated CXCL1 expression following Dox treatment of JHMV-infected mice

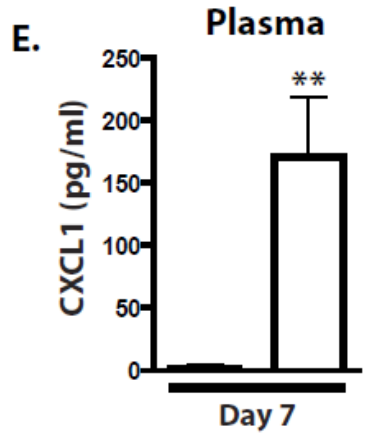




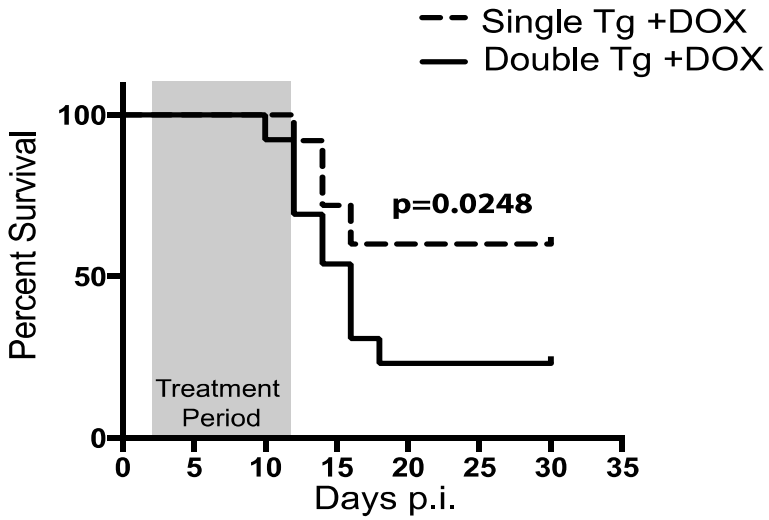


*p<0.05
**p<0.01



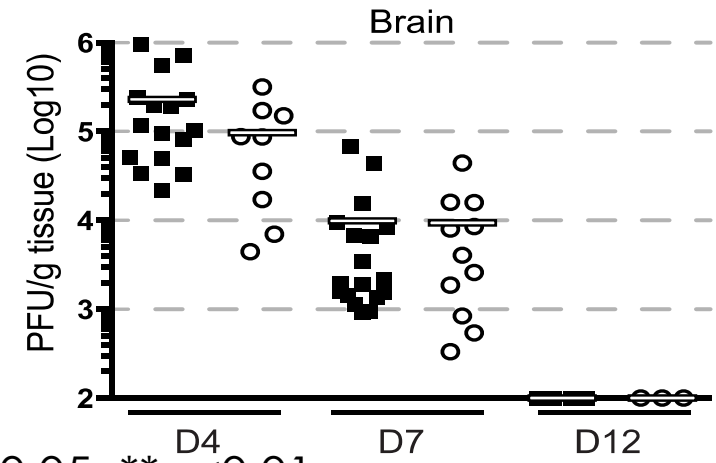


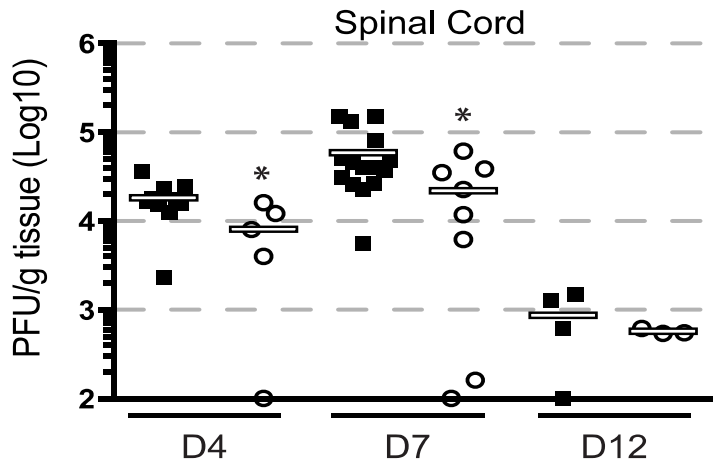
Increased mortality is independent of control of CNS viral replication



Dox-induced CXCL1 does not affect:

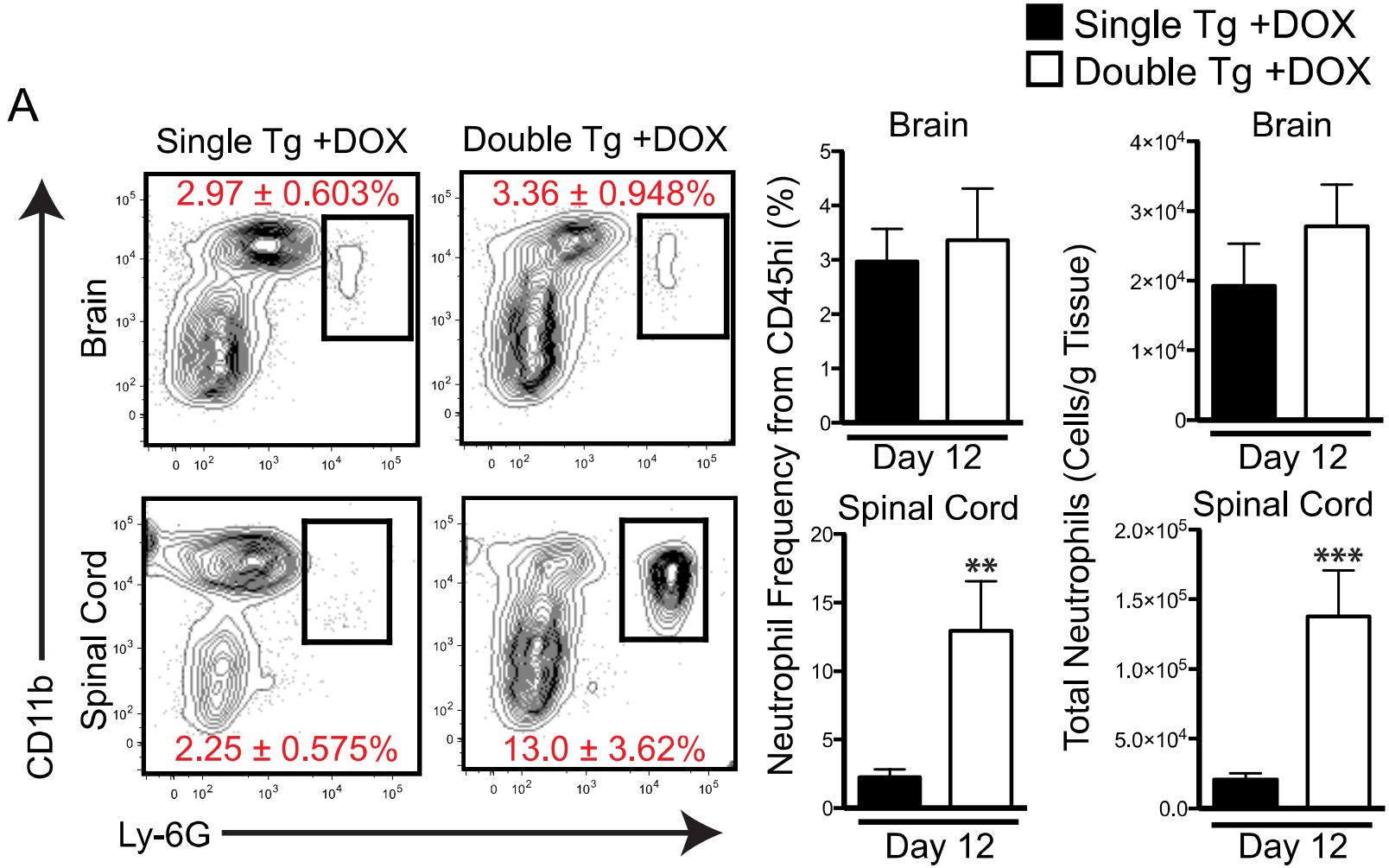
1. Infiltration of CD4+ and CD8+ T cells
2. Infiltration of virus-specific T cells
3. Infiltration/activation of activated macrophages ($CD45^{hi}F480^{+}$) or circulating monocytes (CD11b+Ly6C+Ly6G-).





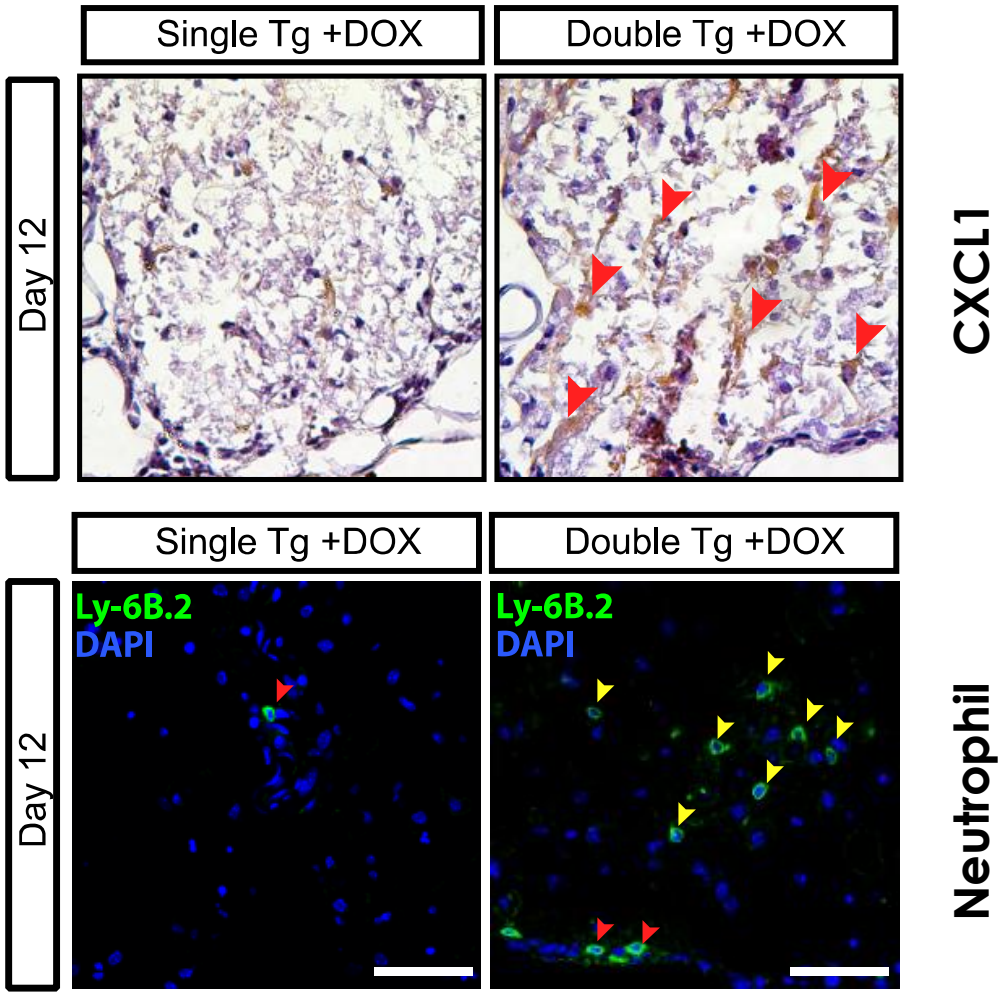
*p<0.05; **p<0.01

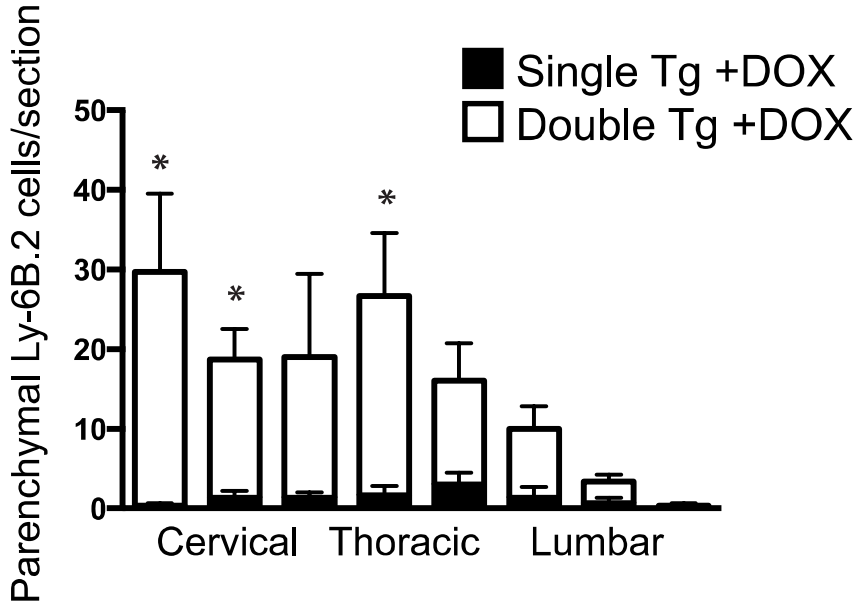
Elevated CXCL1 expression increases neutrophil CNS infiltration – day 12 p.i.



**p<0.01; ***p<0.001

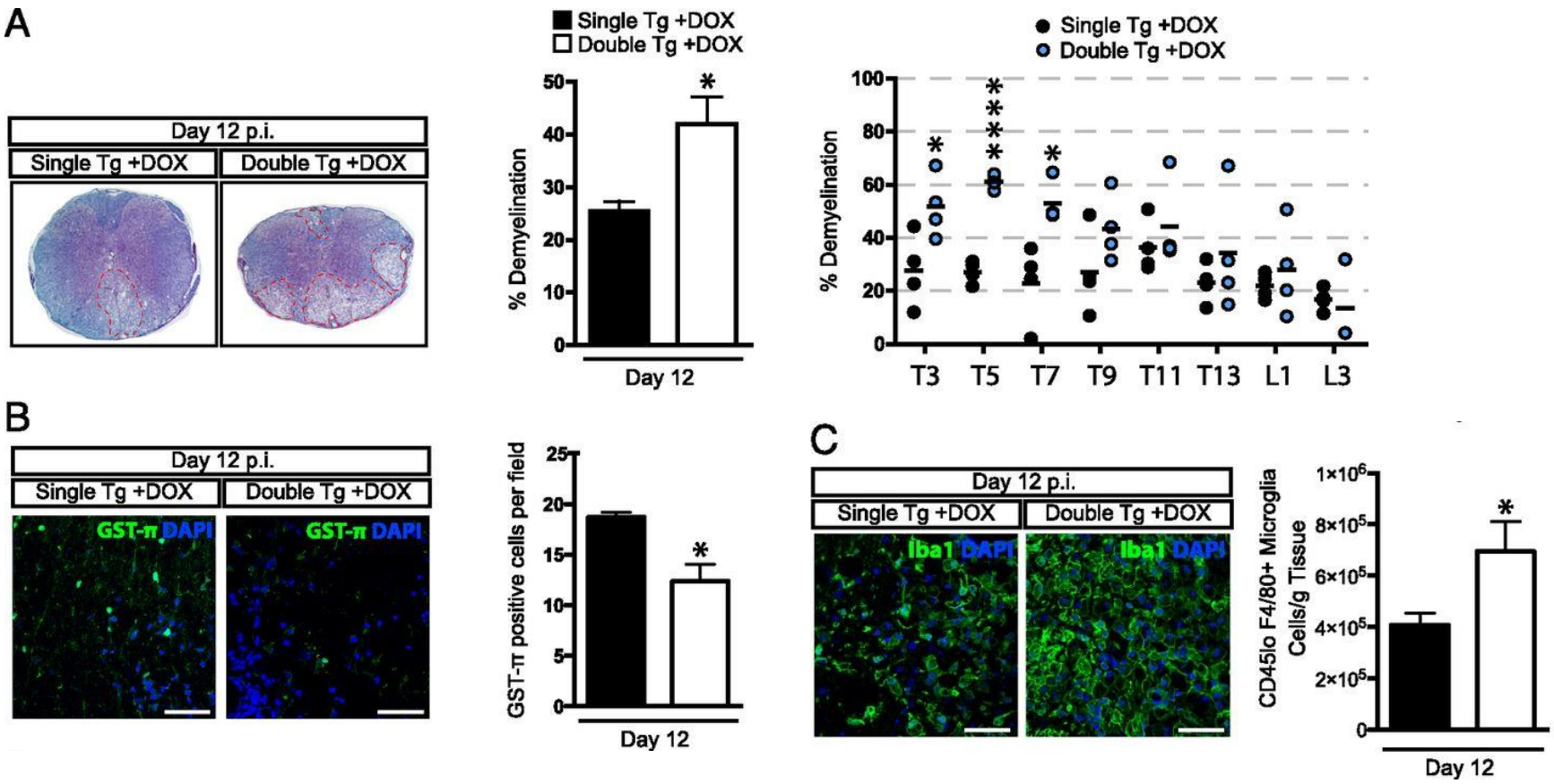
Increased neutrophil infiltration into the spinal cord of Dox-treated double tg mice



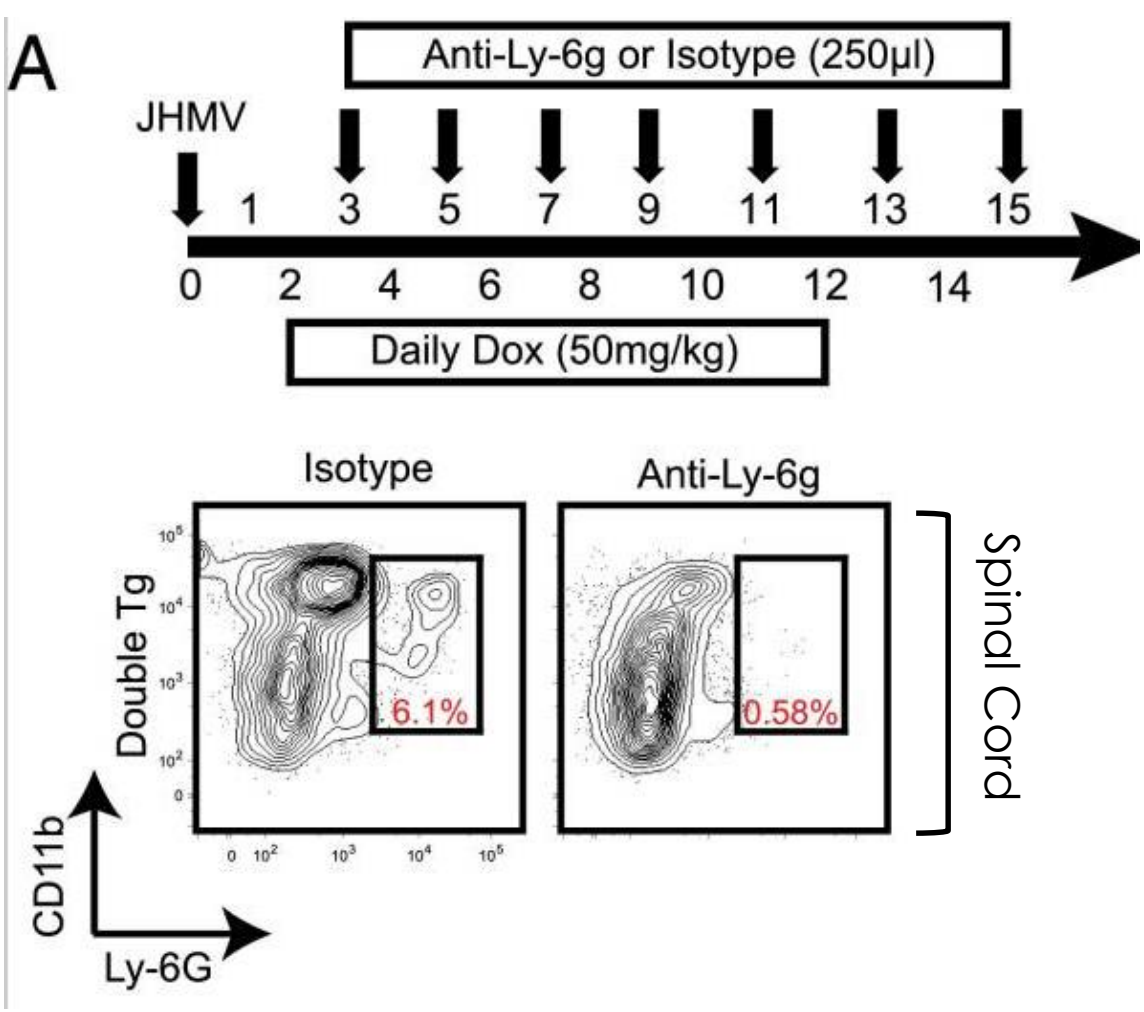


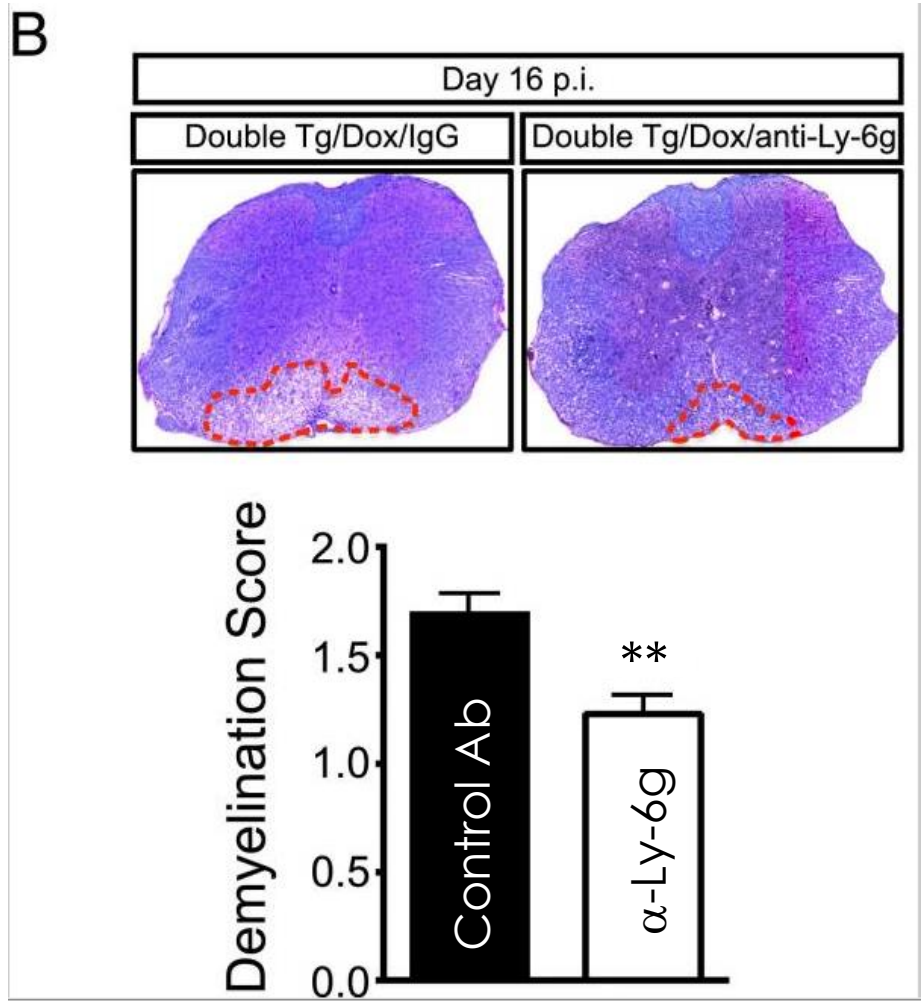
*p<0.05

Demyelination is increased in response to elevated expression of CXCL1



Ablation of neutrophils diminishes the severity of demyelination





**p<0.01

Sustained neutrophil infiltration into the CNS contributes to increased demyelination in experimental autoimmune encephalomyelitis - EAE (prototypic model of MS)

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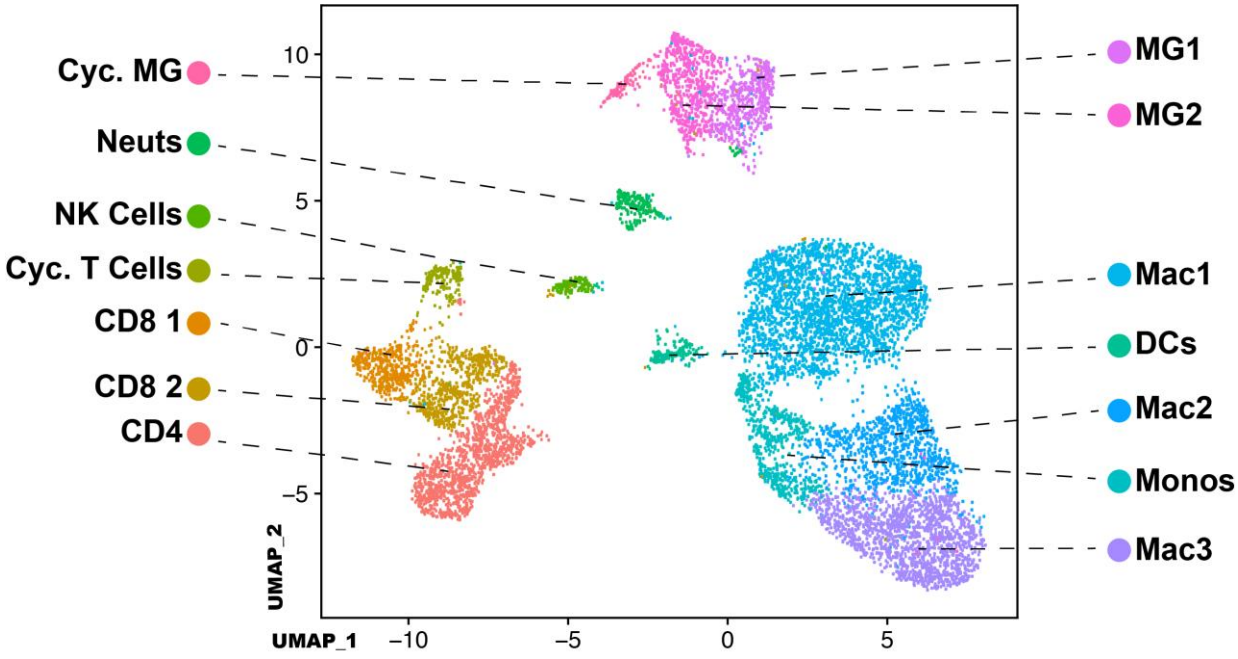
Research Article | Basic | Open Access

Induced CNS expression of CXCL1 augments neurologic disease in a murine model of multiple sclerosis via enhanced neutrophil recruitment

Jonathan J. Grist, Brett S. Marro, Dominic D. Skinner, Amber R. Syage, Colleen Worne, Daniel J. Doty, Robert S. Fujinami, Thomas E. Lane

Grist *et al.*, EJI 2018

What are mechanisms by which neutrophils augment spinal cord demyelination in JHMV-infected mice?

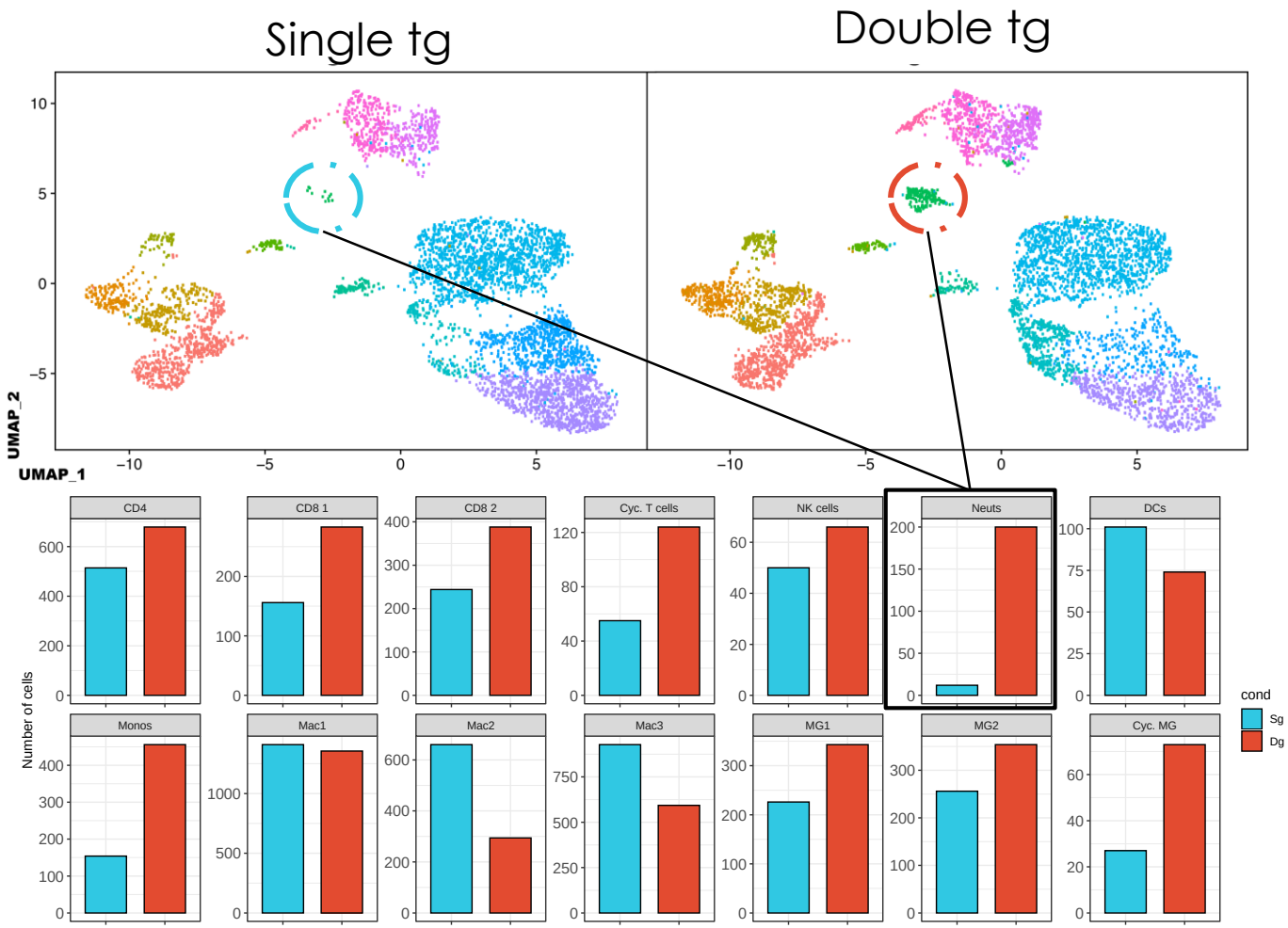


	SG Mice	DG Mice
Tissue	Spinal Cord	Spinal Cord
N	4	4
Cell #	5,381	6,003
Reads /Cell	38,609	32,124

We employed scRNAseq on spinal cords isolated from JHMV-infected Sg and Dg mice treated with Dox at day 12 p.i.

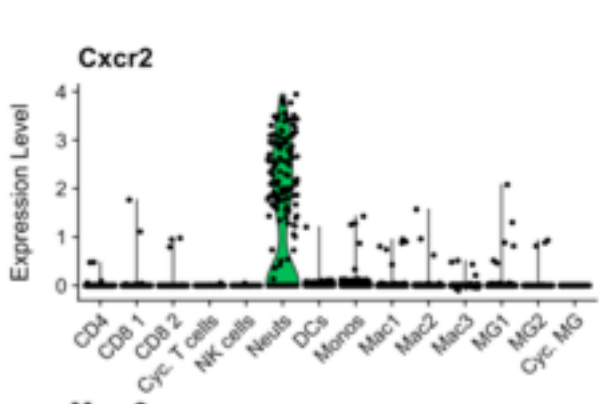
Dominic Skinner

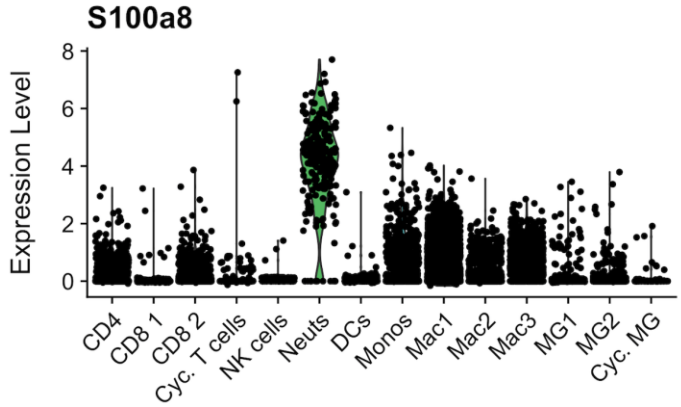
Neutrophils numbers are increased in spinal cords of Dg mice

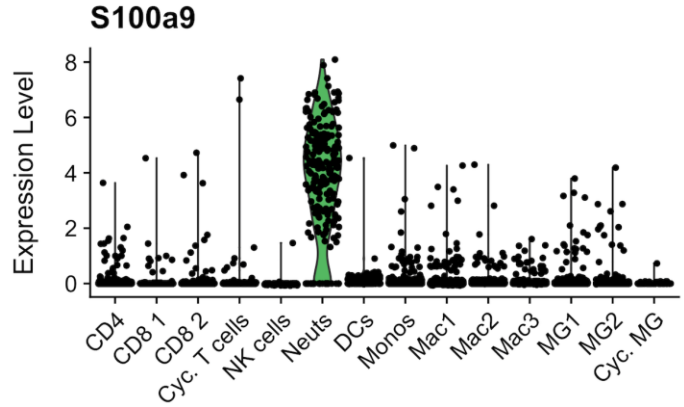


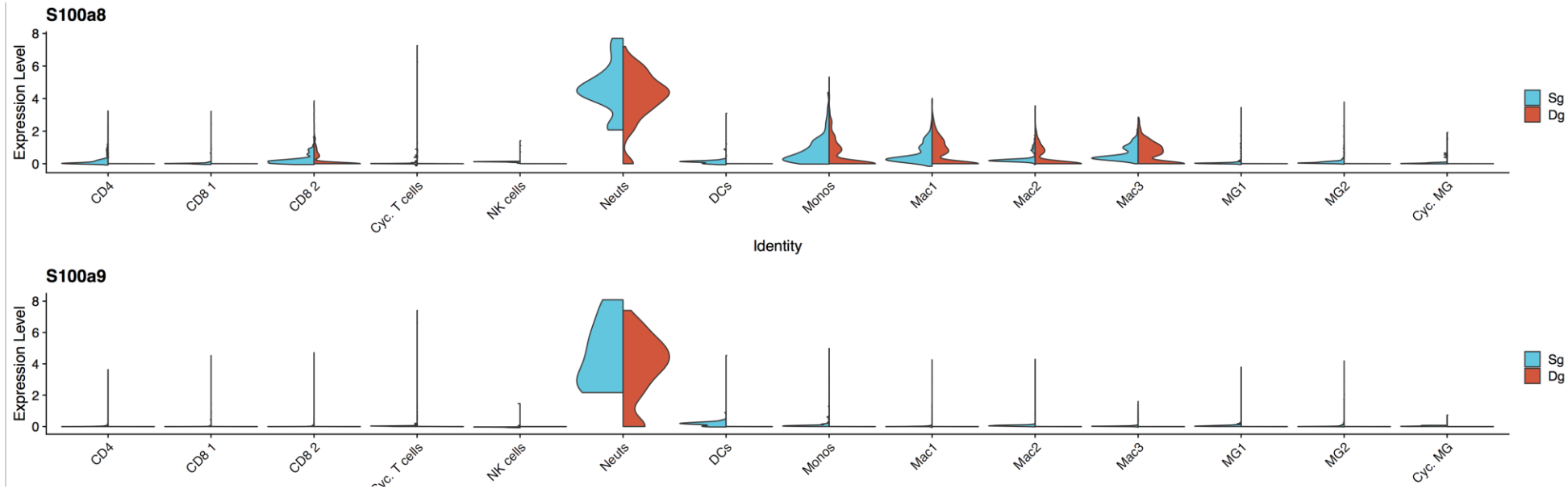
Day 12 p.i.

Preliminary characterization of neutrophils isolated from spinal cords of experimental mice – day 12 p.i.









Dominic Skinner

Perspectives - II

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- Overexpression of astrocyte-derived CXCL1 results in increased mortality in JHMV-infected mice that is not the result of impaired control of viral replication in the CNS.
- Dox-induced CXCL1 from the CNS selectively increased neutrophil mobilization from bone-marrow that subsequently resulted in increased neutrophil infiltration into the CNS.
- Increased neutrophil accumulation within the CNS correlated with enhanced demyelination. Targeting neutrophils attenuated the severity of white-matter damage.
- Ongoing studies are focused on determining the mechanism(s) of action by which neutrophils contribute to demyelination in JHMV-infected mice.

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