Anti-inflammatory Effects of SM07883, a Novel, Potent, and Selective Oral DYRK1A Inhibitor in Neurodegenerative Mouse Models

Ben Melchior, Ph.D., *Scott Anderson, Carolyn Lai, Karen Duong-Polk, Bora Güner, Shivan Lala, Stacy Habroun, Waseem Akhtar, and Yusuf Yazici, M.D.*

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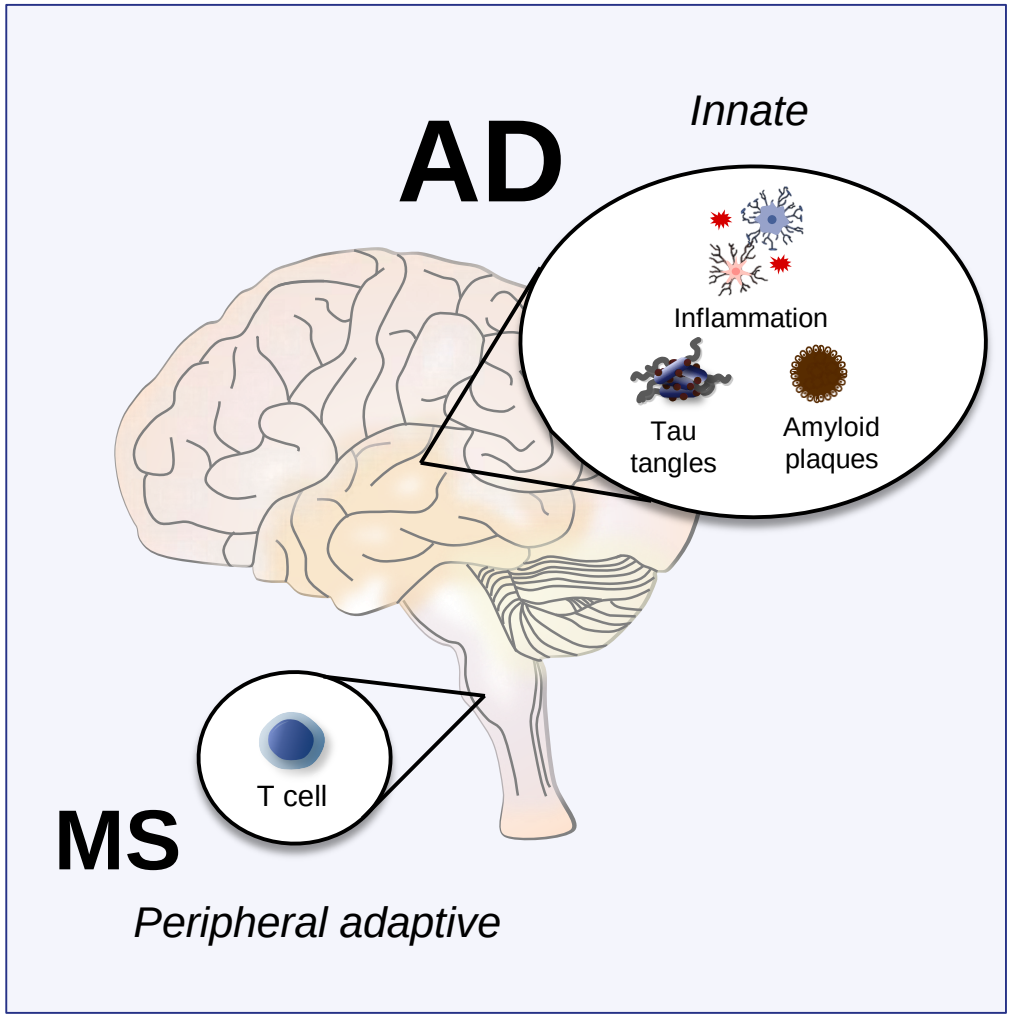
- CME/CE credits will not be awarded for this presentation
- All authors are employees and shareholders of Samumed, LLC
- This presentation is not intended to provide a comprehensive overview of all studies using SM07883
- SM07883 is an investigational compound; SM07883 has not been approved by the U.S. Food and Drug Administration (FDA) or any other pharmaceutical regulatory authority, and no conclusions can or should be drawn regarding the safety or effectiveness of the product candidate
- While the complete mechanism of action (MOA) for SM07883 is unknown, further investigation is being conducted. All of the MOA information is based on nonclinical data and the relationship to clinical benefit is unknown
- This presentation is intended as an exchange of scientific information, is provided for educational purposes only, and is not intended for any promotional purpose or to offer medical advice

AD and other neurodegenerative diseases have underlying inflammatory responses

- Alzheimer's disease (AD) pathogenesis is associated with microglia and immune function[1,2]
- Incidence of AD may be reduced in patients on immunosuppressive treatment[3,4]
- Immune response is critical in clearing misfolded proteins, but excessive activity can be deleterious[1,2]
  - **In AD, the CNS activates glial (immune) cells[2]**
    - Innate system is engaged by distressed neurons, abnormal microenvironment (plaques), and synaptic impairment sensed by glial cells
  - **In multiple sclerosis (MS), peripheral immune cells are activated[5]**
    - Adaptive immune responses against specific neuro-antigens
- Potential role for SM07883, an oral DYRK1A inhibitor, as an anti-inflammatory agent

CNS Immune Response



1. Heneka MT, et al. *Lancet Neurol.* 2015
2. Sarlus H and Heneka MT. *J Clin Invest*. 2017
3. Taglialatela G, et al. *J Alzheimers Dis*. 2015
4. Chou RC, et al. *CNS Drugs*. 2016
5. Hemmer B, et al. *Lancet Neurol.* 2015

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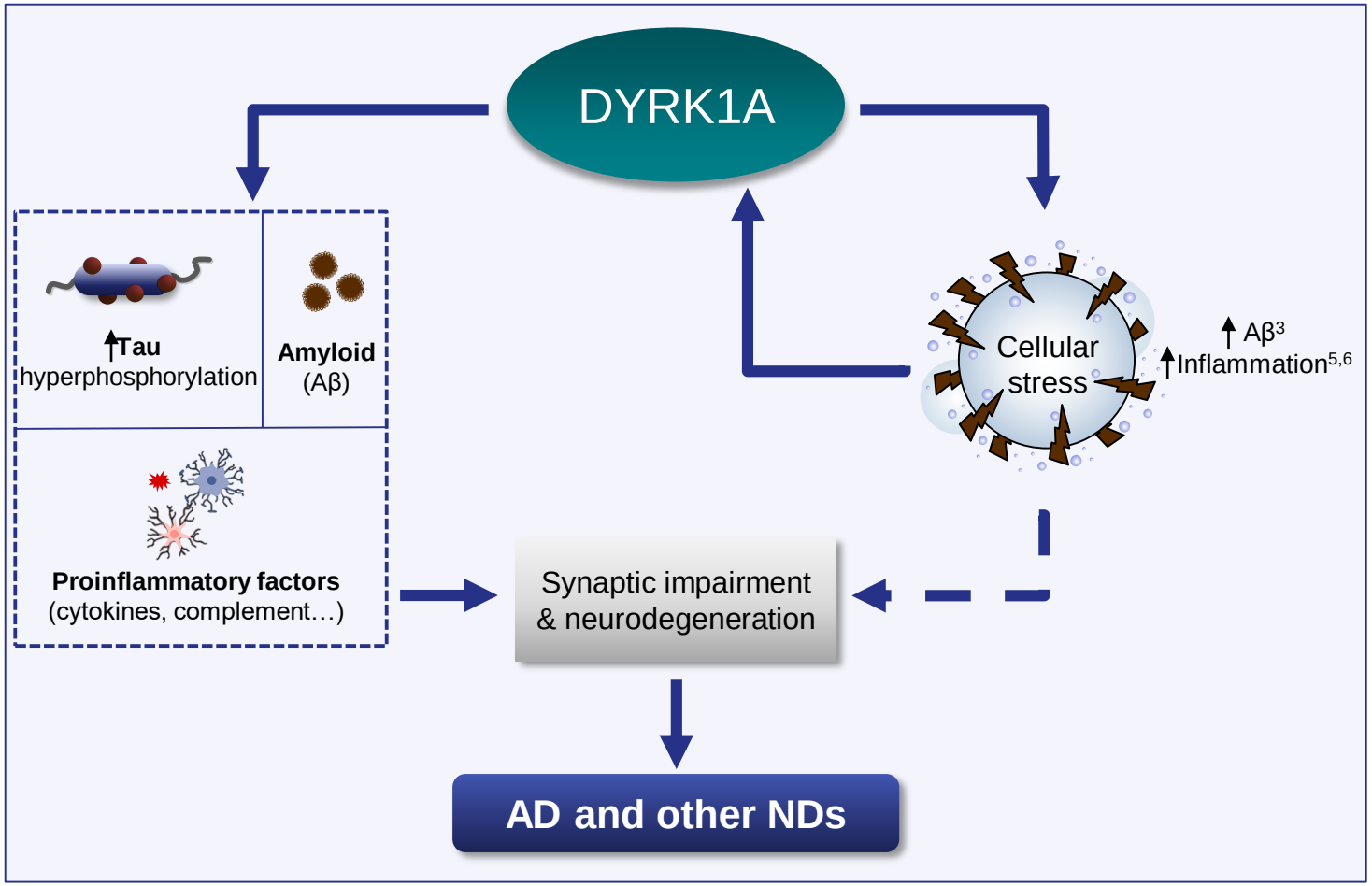
DYRK1A (Dual-Specificity Tyrosine Phosphorylation-Regulated Kinase 1A): A novel target for AD

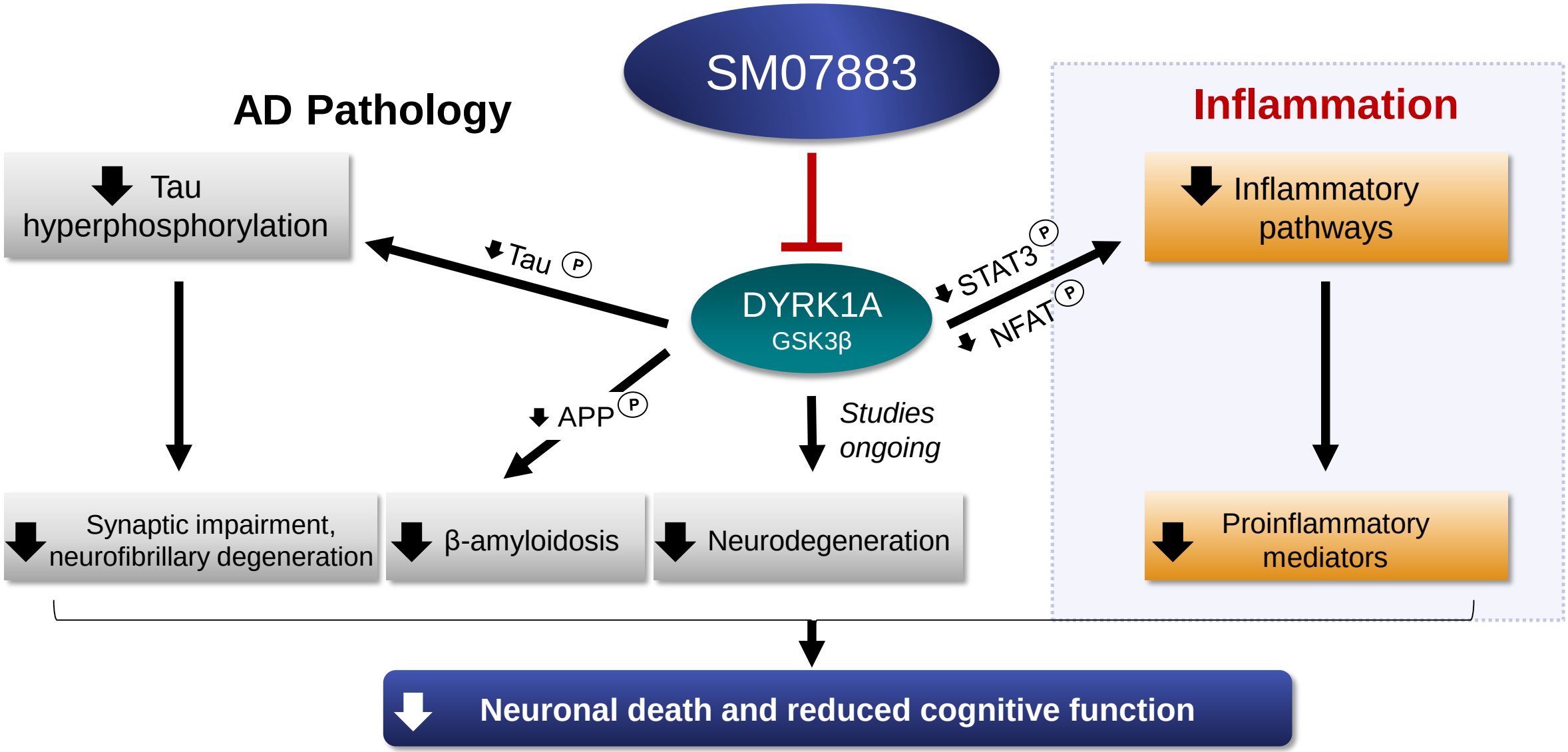
- Found to be overexpressed in AD, Pick's disease, and Down syndrome brains[1]
- Regulates phosphorylation of major AD molecular hallmarks such as tau[2], APP (Aβ)[3], and presenilin[4]
- DYRK1A regulates inflammatory signals STAT3[7], GFAP[7], and NFAT[8]

Proposed role of DYRK1A in AD



1. Ferrer I, et al. *Neurobiol. Dis.* 2005
2. Melchior B, et al. *Aging Cell.* 2019
3. Ryoo SR, et al. *J. Neurochem.* 2008
4. Ryu YS, et al. *J. Neurochem.* 2010
5. Selkoe DJ. *Nat Cell Biol.* 2004
6. Khor B, et al. *Elife.* 2015
7. Kurabayashi N, et al. *EMBO Rep.* 2015
8. Choi H and Chung K. *Exp Neurobiol.* 2011

Proposed mechanism of action of SM07883 in AD: An orally available, potent, and specific DYRK1A inhibitor

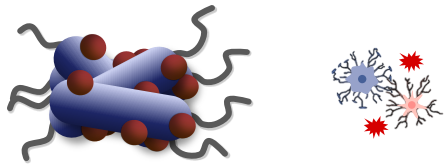


STAT3: signal transducer and activator of transcription 3, NFAT: nuclear factor of activated T cells, APP: amyloid precursor protein

Complementary preclinical mouse models

JNPL3

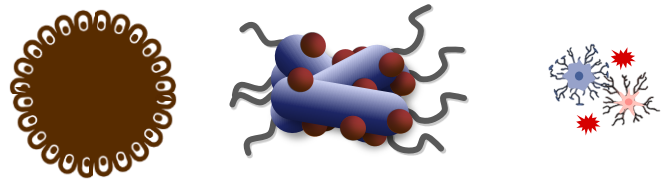
Tau transgenic model



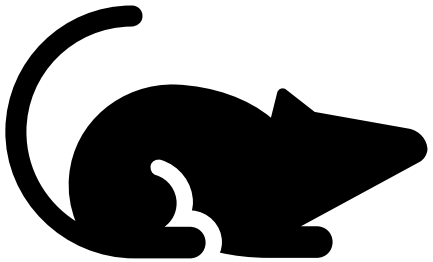
Tau P301L

3xTg-AD

Amyloid and tau transgenic model

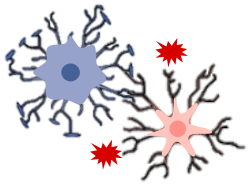


APP/PSEN/Tau P301L



LPS

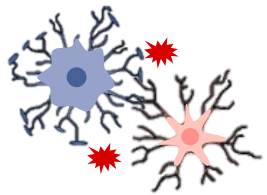
Induced inflammation model



- Acute LPS/IFNγ (IC injection)
- Chronic, low-dose LPS

EAE

Induced inflammation model



MOG immunization

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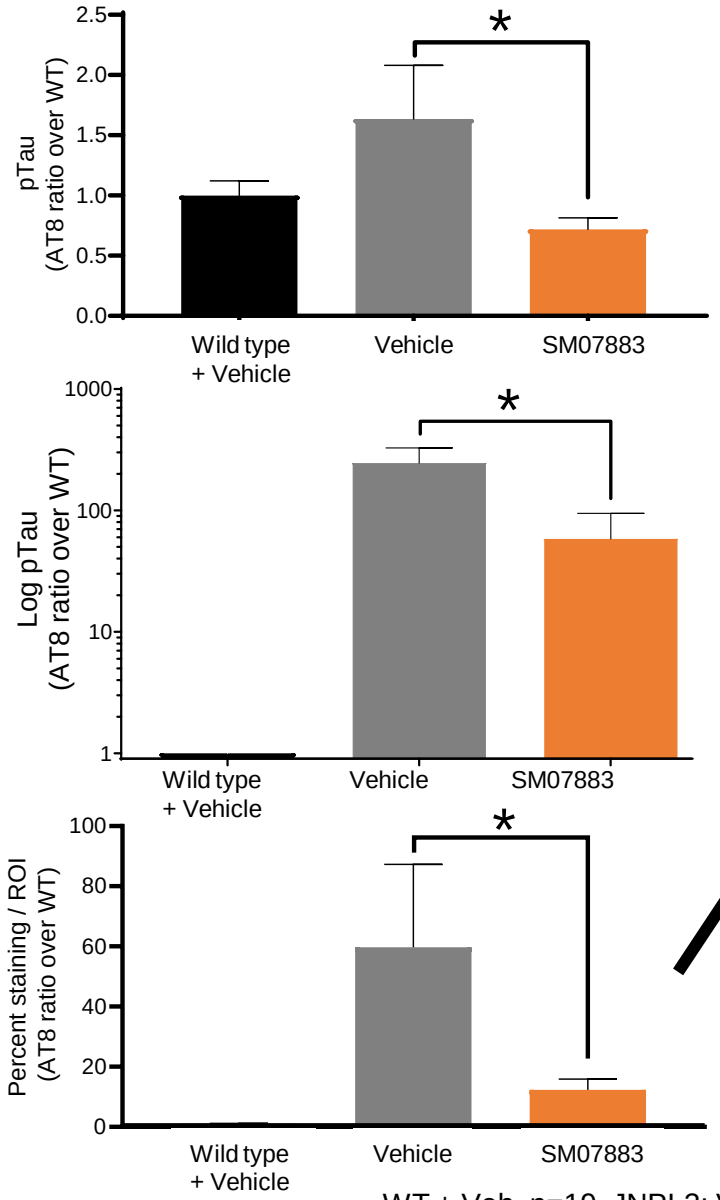
EAE: experimental autoimmune encephalomyelitis, MOG: myelin oligodendrocyte glycoprotein, IC: intracranial

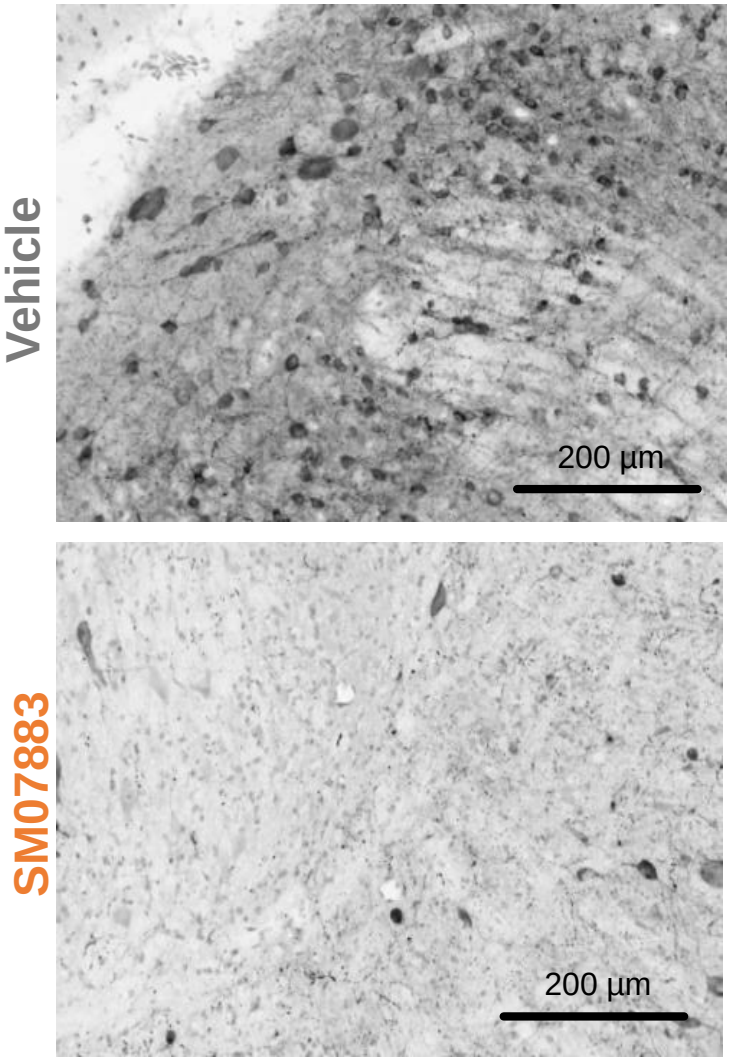
SM07883 reduced tau pathology in JNPL3 tau mice

Tau Hyperphosphorylation
(Brainstem, Ser202/Thr205 [AT8] Western blot)

Sarkosyl-insoluble Fraction
(Brainstem, AT8 Western blot)

Tau-positive Inclusions
(Brainstem, AT8 % staining of ROI)





3 mos treatment; 3 mg/kg/day

WT + Veh. n=19, JNPL3: Veh. n=19 and SM07883 n=19; Mean ± SEM; * p<0.05 vs. vehicle

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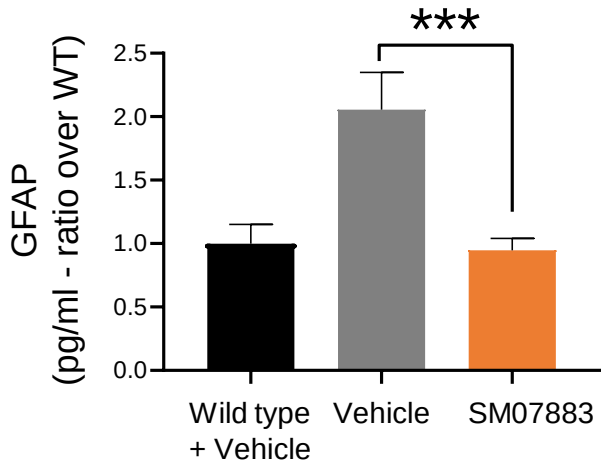
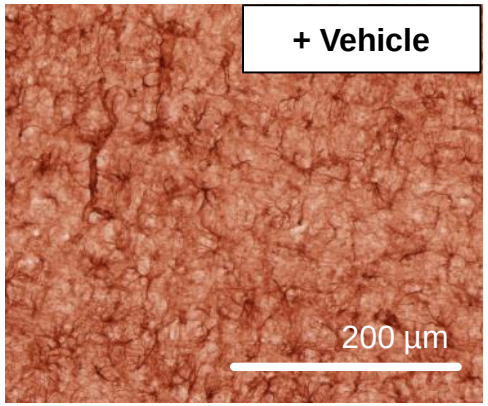
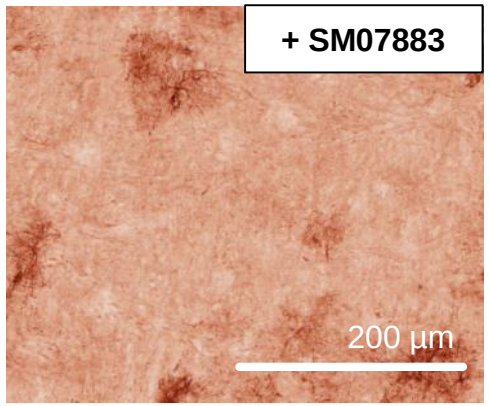
Melchior B, et al. *Aging Cell.* 2019

SM07883 reduced tau-induced glial activation in JNPL3 tau mice

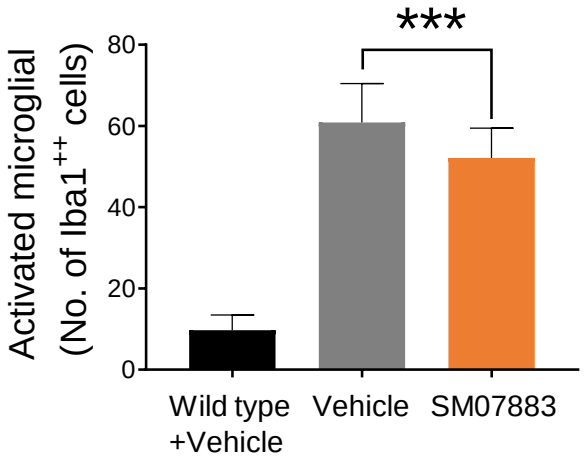
GFAP in the Spinal Cord
(ELISA)

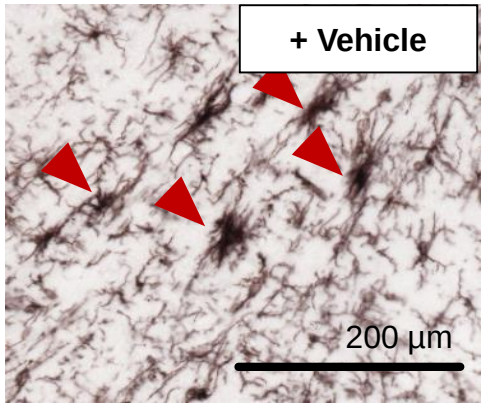


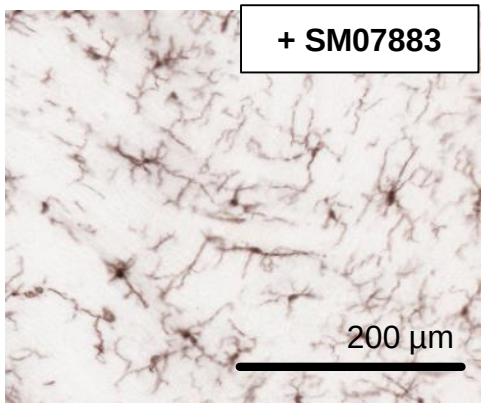




Iba1++ Activated Microglial Cells
(Hindbrain staining)







Reactive microglial cells

3 mos treatment; 3 mg/kg/day

GFAP: WT + Veh. n=9, JNPL3: Veh. n=18 and SM07883 n=19; **Iba1++**: WT + Veh. n=11, JNPL3: Veh. n=32 and SM07883 n=19
Mean ± SEM; *** p<0.001 vs. vehicle

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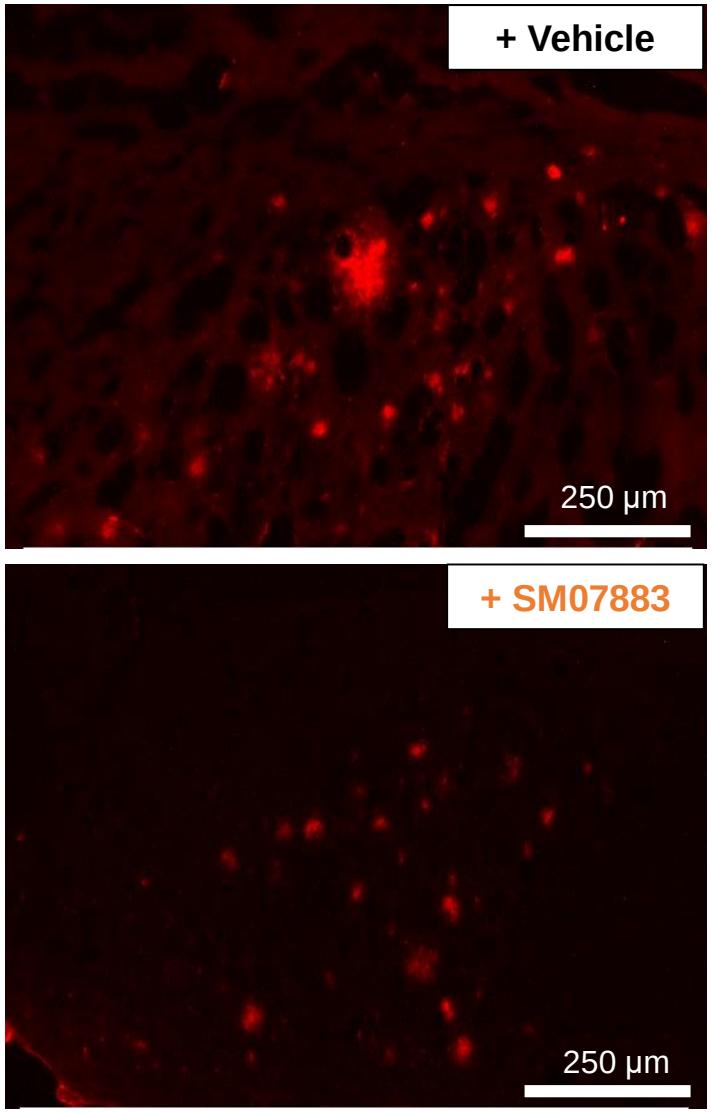
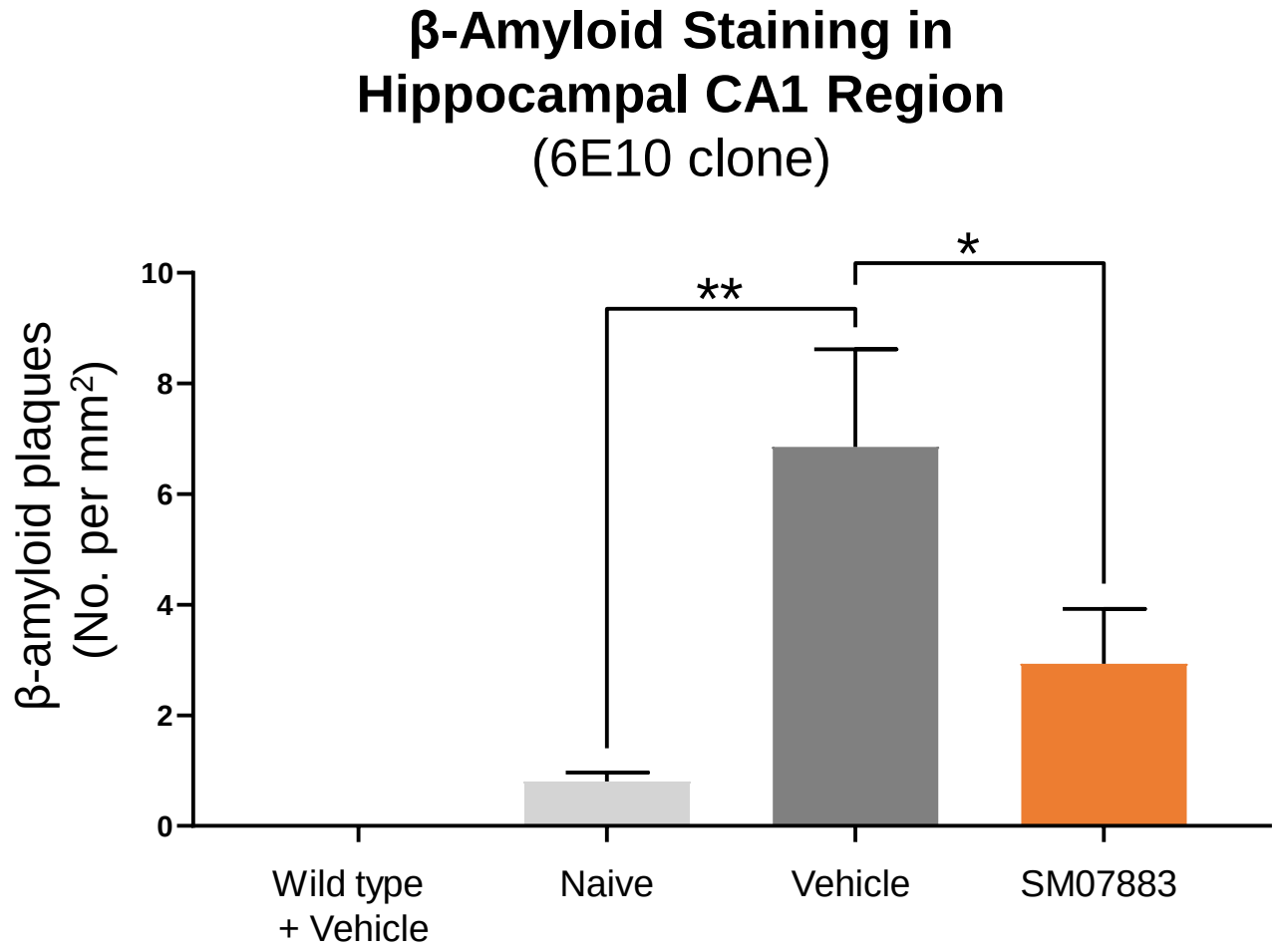
Melchior B, et al. *Aging Cell.* 2019

SM07883 reduced amyloid pathology in 3xTg-AD mice

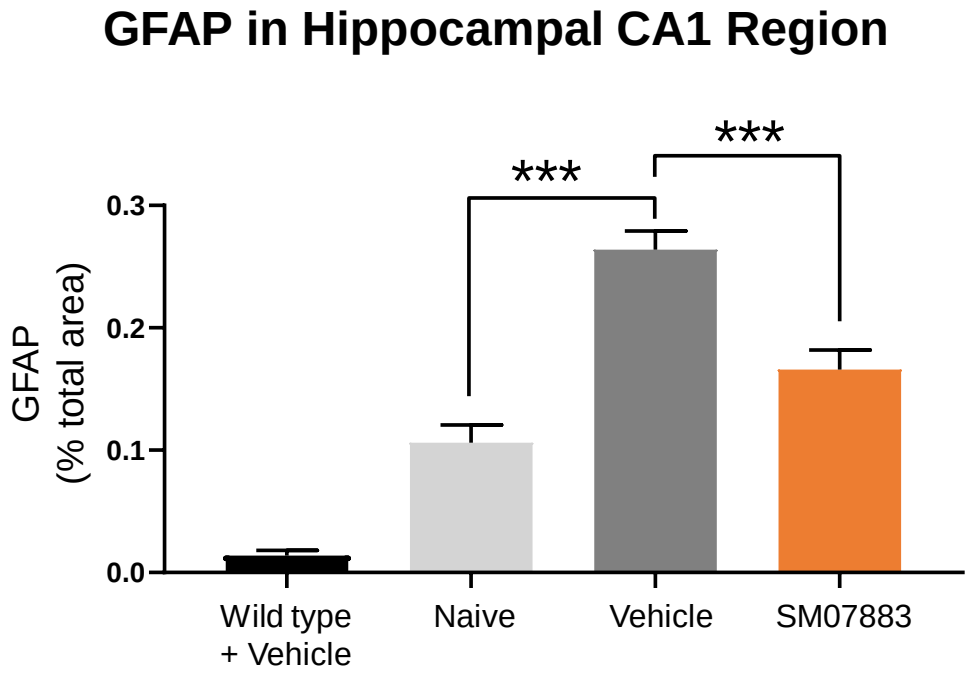


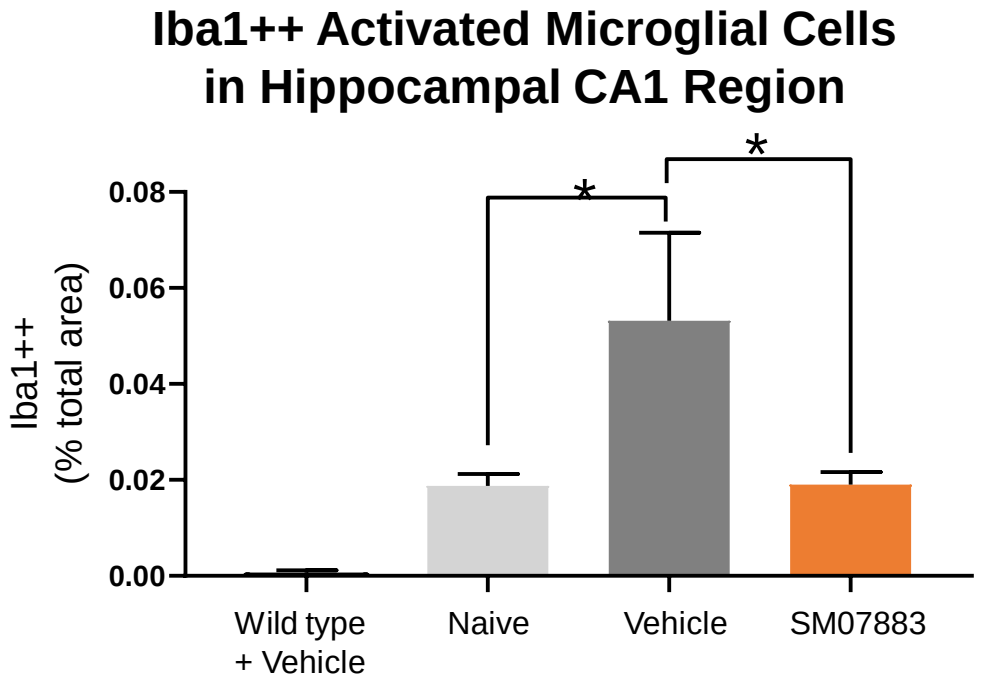


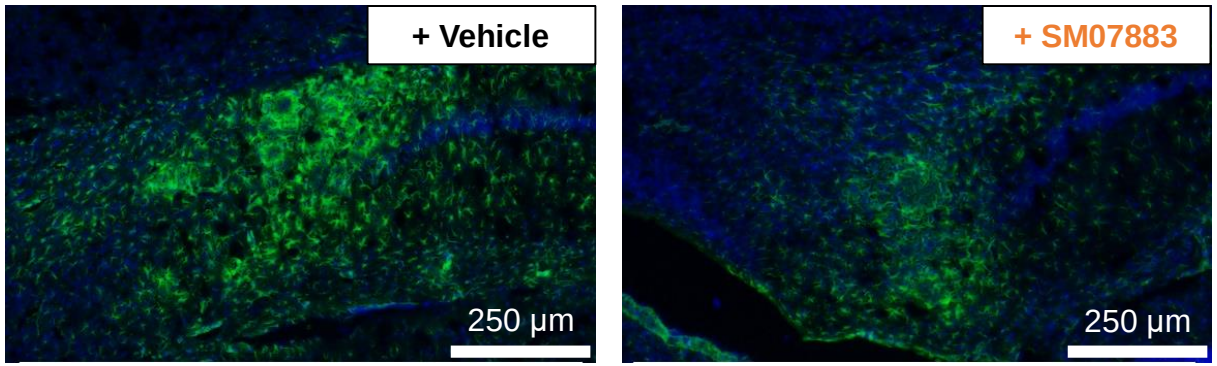
26 wks treatment; 5 mg/kg/day

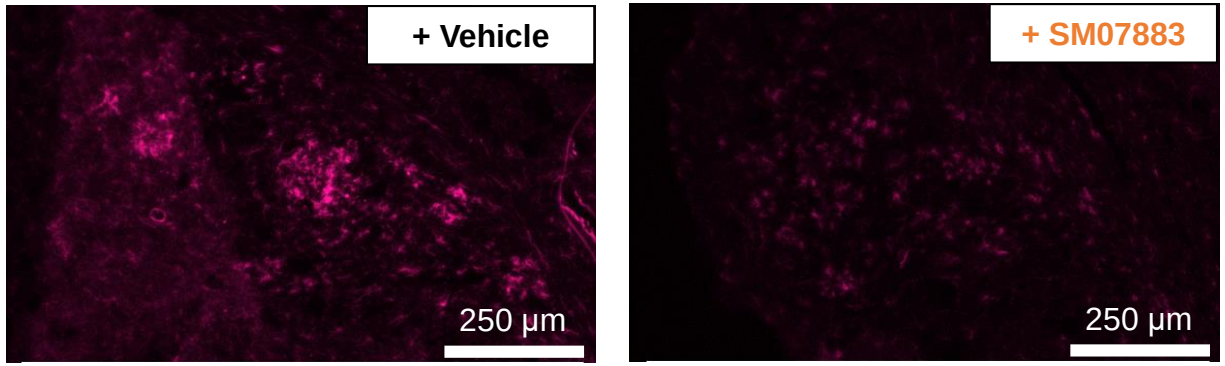
WT + Veh. n=9, 3xTg-AD: Naive n=8, Veh. n=12, and SM07883 n=13; Mean ± SEM; * p<0.05, ** p<0.01 vs. vehicle

SM07883 reduced neurodegeneration-induced glial activation in 3xTg-AD mice









26 wks treatment; 5 mg/kg/day

WT + Veh. n=9, 3xTg-AD: Naive n=8, Veh. n=9, and SM07883 n=11; Mean ± SEM; * p<0.05, *** p<0.001 vs. vehicle

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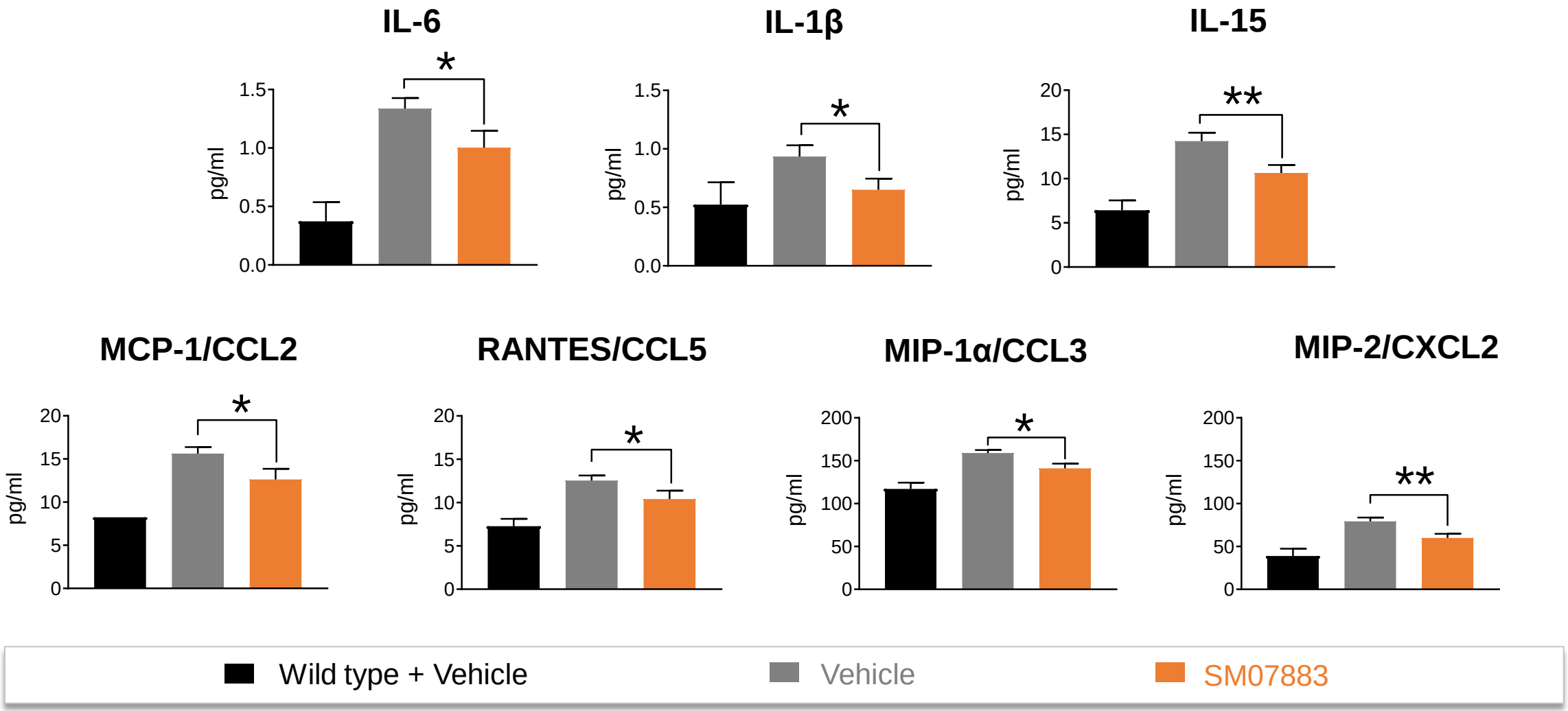
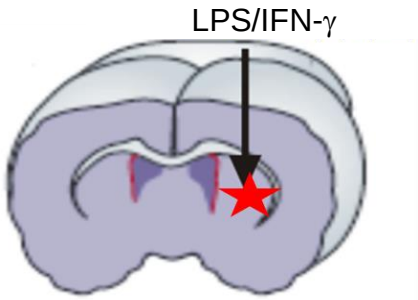
SM07883 reduced neurodegeneration-induced proinflammatory mediators in 3xTg-AD mice



26 wks treatment; 5 mg/kg/day

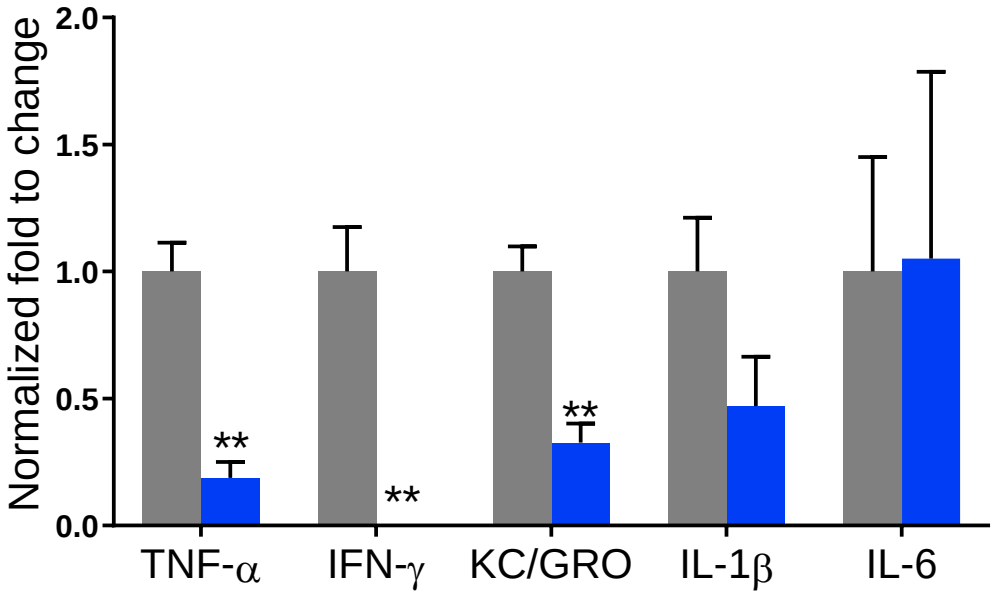
WT + Veh. n=3, 3xTg-AD: Veh. n=6 and SM07883 n=7; Mean ± SEM; * p<0.05, ** p<0.01 vs. vehicle

SM07883 reduced acute inflammation

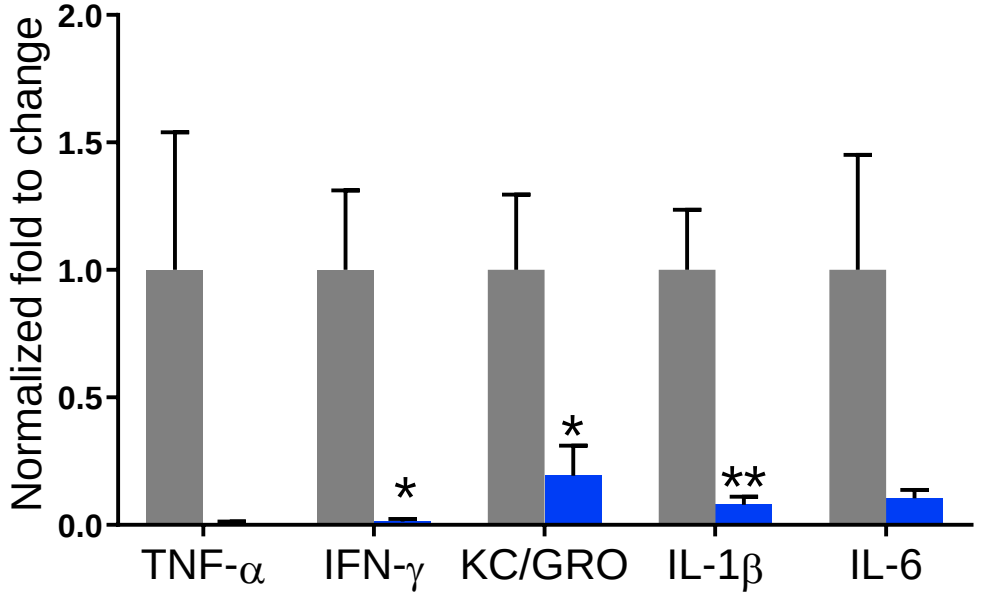


Acute intracranial LPS/IFN-γ model[1]

Brain (5 hrs)
(Early microglial response)



Brain (24 hrs)
(Perivascular macrophages and microglia)



Vehicle SM07883

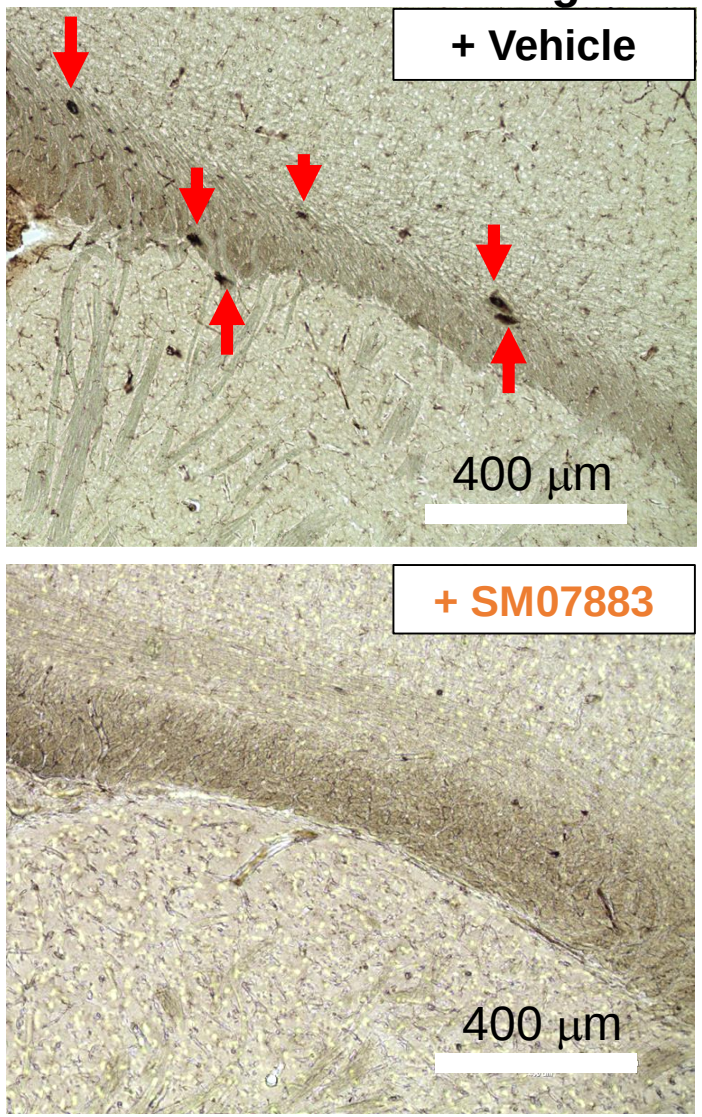
24hrs; 10 mg/kg/day

n=3/treatment group; Mean ± SEM; * p<0.05, ** p<0.01 vs. vehicle

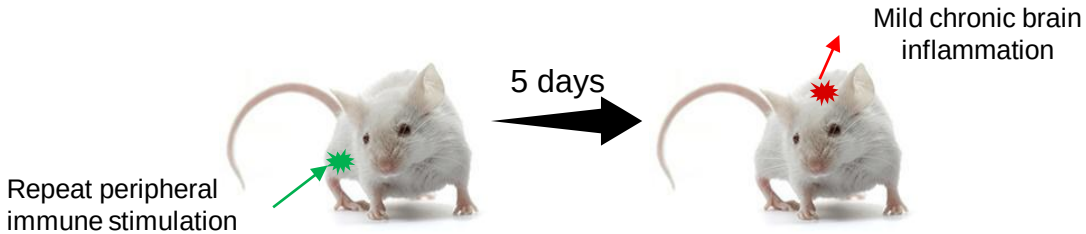
1. Schmidt CD and Melchior B. et al. *J. Neurochem.* 2006

SM07883 reduced chronic neuroinflammation

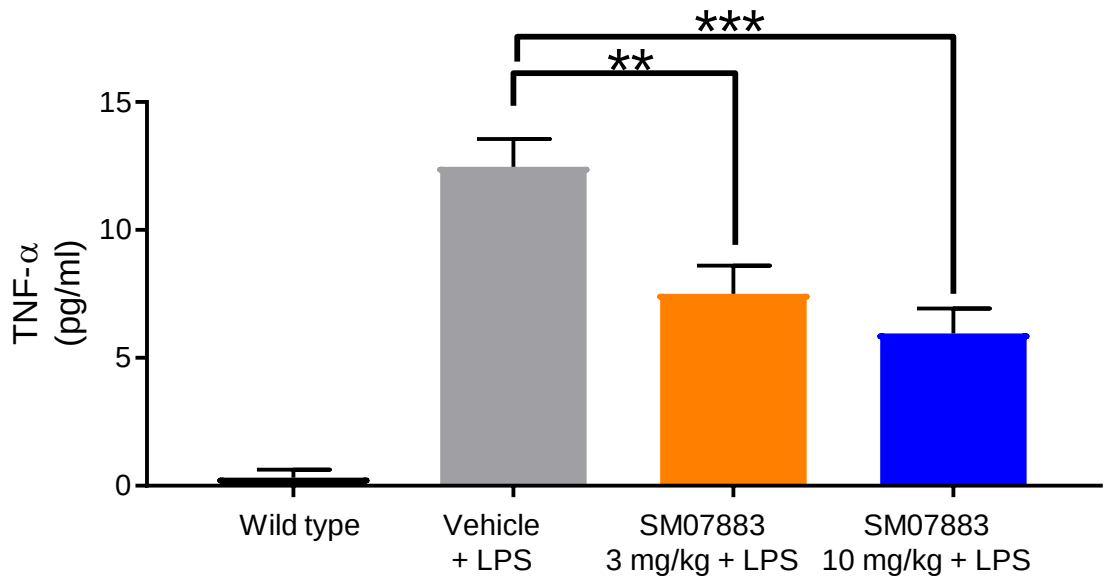
Brain CD68 Staining



Repeat, Chronic, Low-dose LPS IP Injections



TNF-α in the Brain
(ELISA)



SM07883 and LPS (0.5 mg/kg, IP) for 5 consecutive days; 3 mg/kg/day or 10 mg/kg/day

WT n=2, Veh. n=15, SM07883 + LPS: 3 mg/kg n=15 and 10 mg/kg n=15; Mean ± SEM; ** p<0.01, *** p<0.001 vs. vehicle

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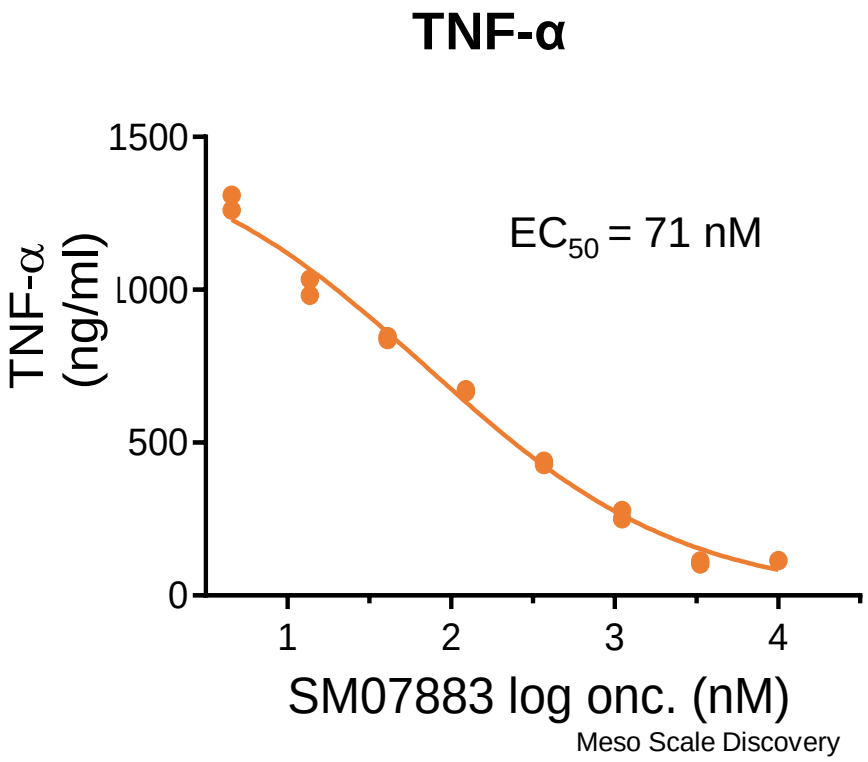
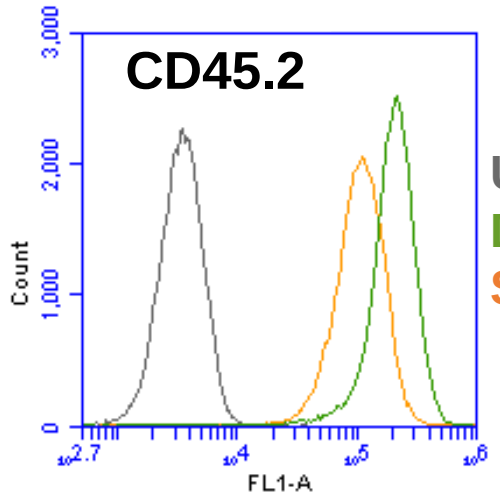
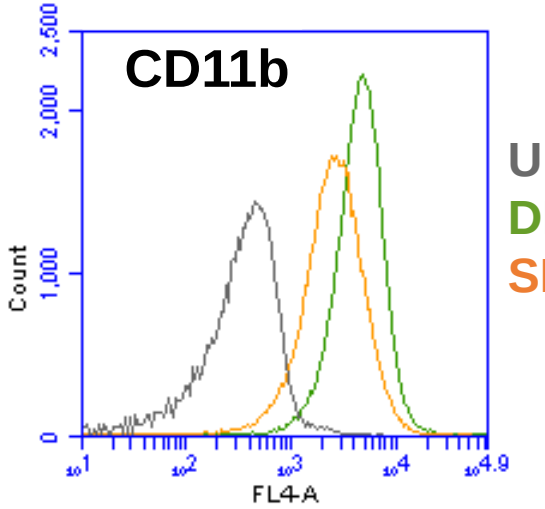
SM07883 reduced microglial cell activation *in vitro*

BV2 Microglial Cells + LPS





Unstained
DMSO
SM07883 (0.370 µM, 24 hrs)



Unstained
DMSO
SM07883 (0.370 µM, 96 hrs)

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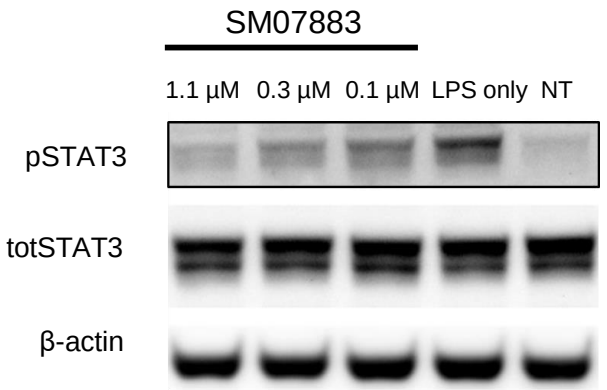
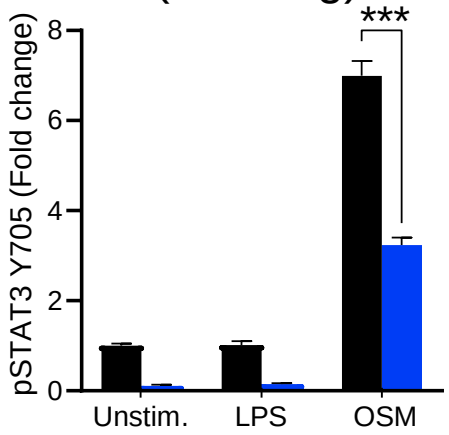
SM07883 reduced STAT3 phosphorylation and translocation

STAT3 Phosphorylation

THP-1 Monocytes + LPS
(Western blot)

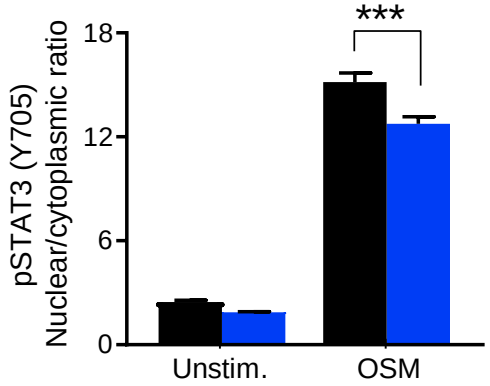


Primary Mouse Astrocytes + OSM
(Staining)

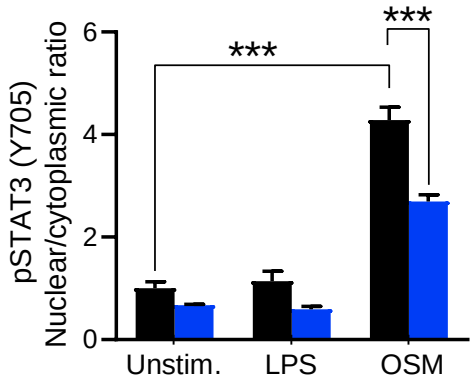


STAT3 Translocation

Human Microglia (HMC-3) + OSM
(Staining)



Primary Mouse Astrocytes + OSM
(Staining)



DMSO SM07883 1.1 µM

OSM: Oncostatin M
Mean ± SEM
*** p<0.001 vs. vehicle

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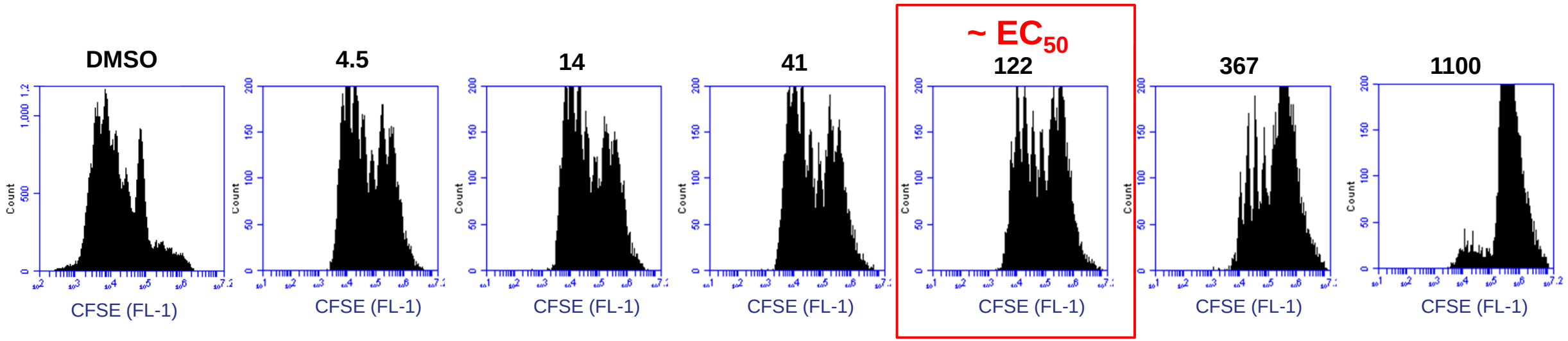
SM07883 prevented T cell proliferation and proinflammatory cytokines secretion

CD3/CD28 Stimulated Mouse Splenocytes for 5 Days +/- SM07883

Quiescence

SM07883 (nM)

Dose



CFSE (carboxyfluorescein diacetate succinimidyl ester) staining analyzed by flow cytometry

Reduction at 5 days of: **IL-17a**: EC_{50} = 15 nM **IFN-γ**: EC_{50} = 42 nM **TNF-α**: EC_{50} = 46 nM Meso Scale Discovery

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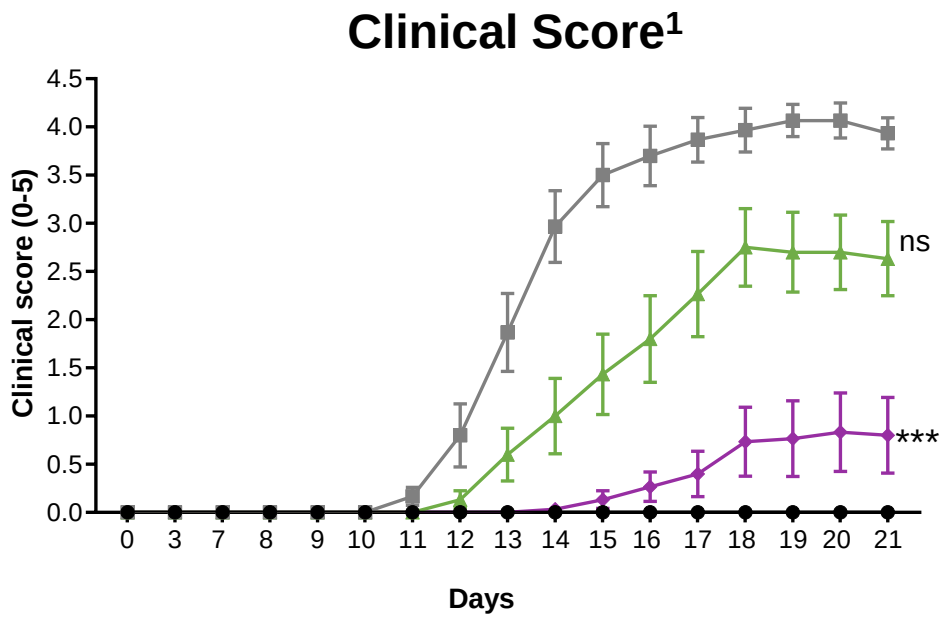
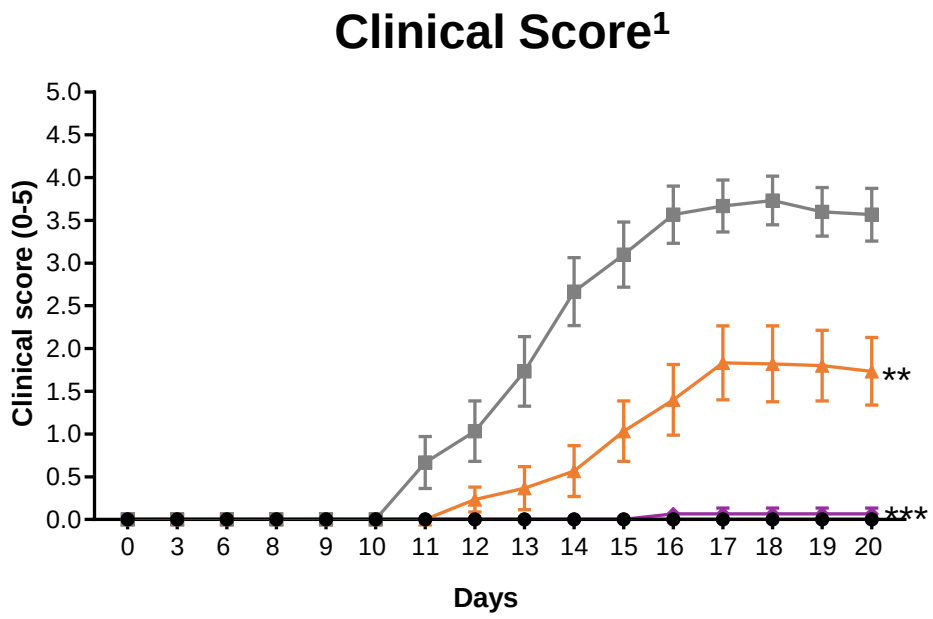
MOG-induced EAE acute symptoms were reduced with SM07883

Vehicle

SM07883





Naive | Vehicle | SM07883 3 mg/kg BID | SM07883 5 mg/kg BID | SM07883 5 mg/kg QD

[1]Per Hooke Lab Mouse EAE Scoring Guide (hookelabs.com)

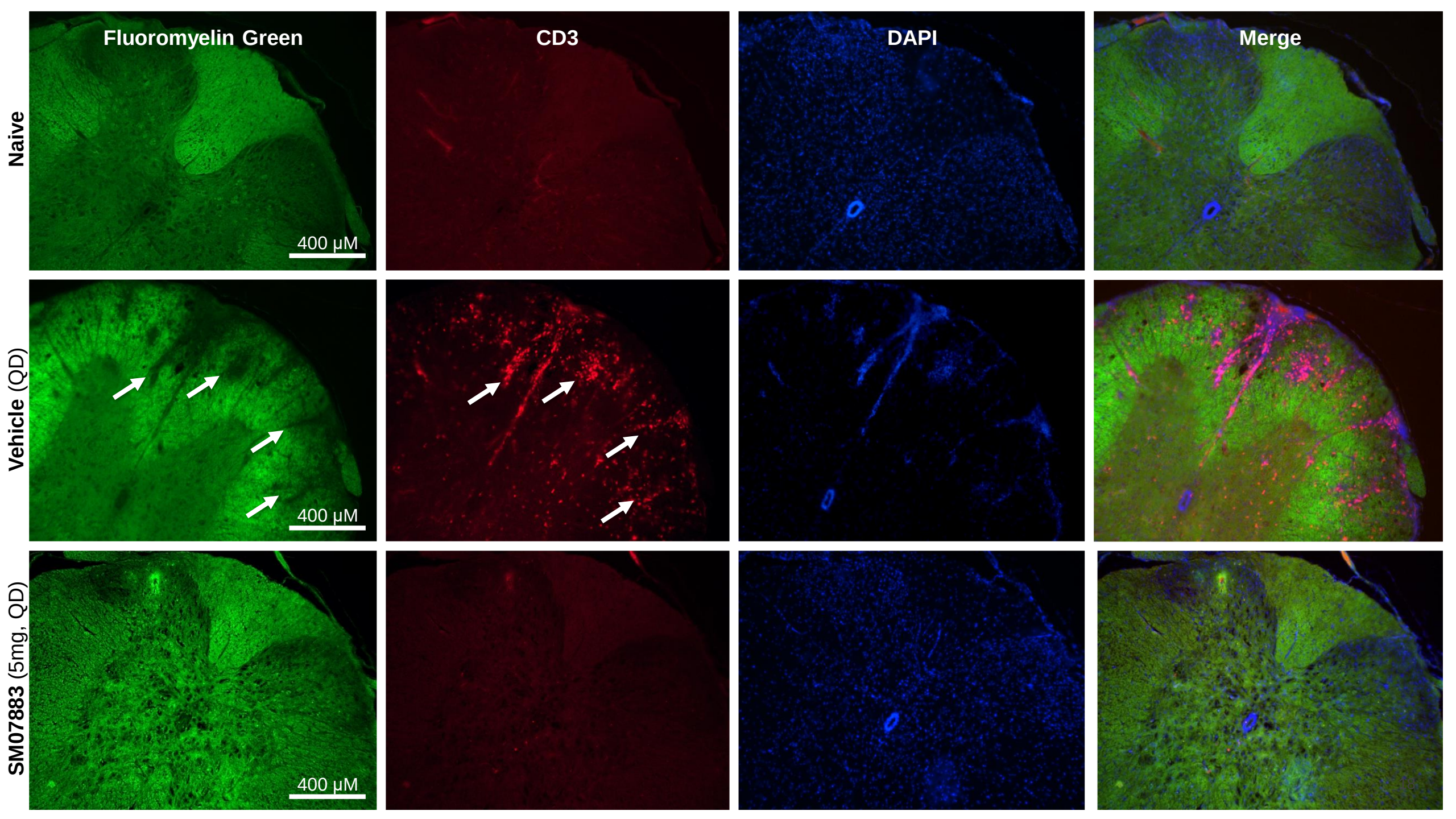
Dosed full length of study; 3 mg/kg/day BID, 5 mg/kg/BID, and 5 mg/kg/day QD

Left: Naive n=2, EAE: Veh. n=15, SM07883 3 mg/kg BID n=15, SM07883 5 mg/kg BID n=15

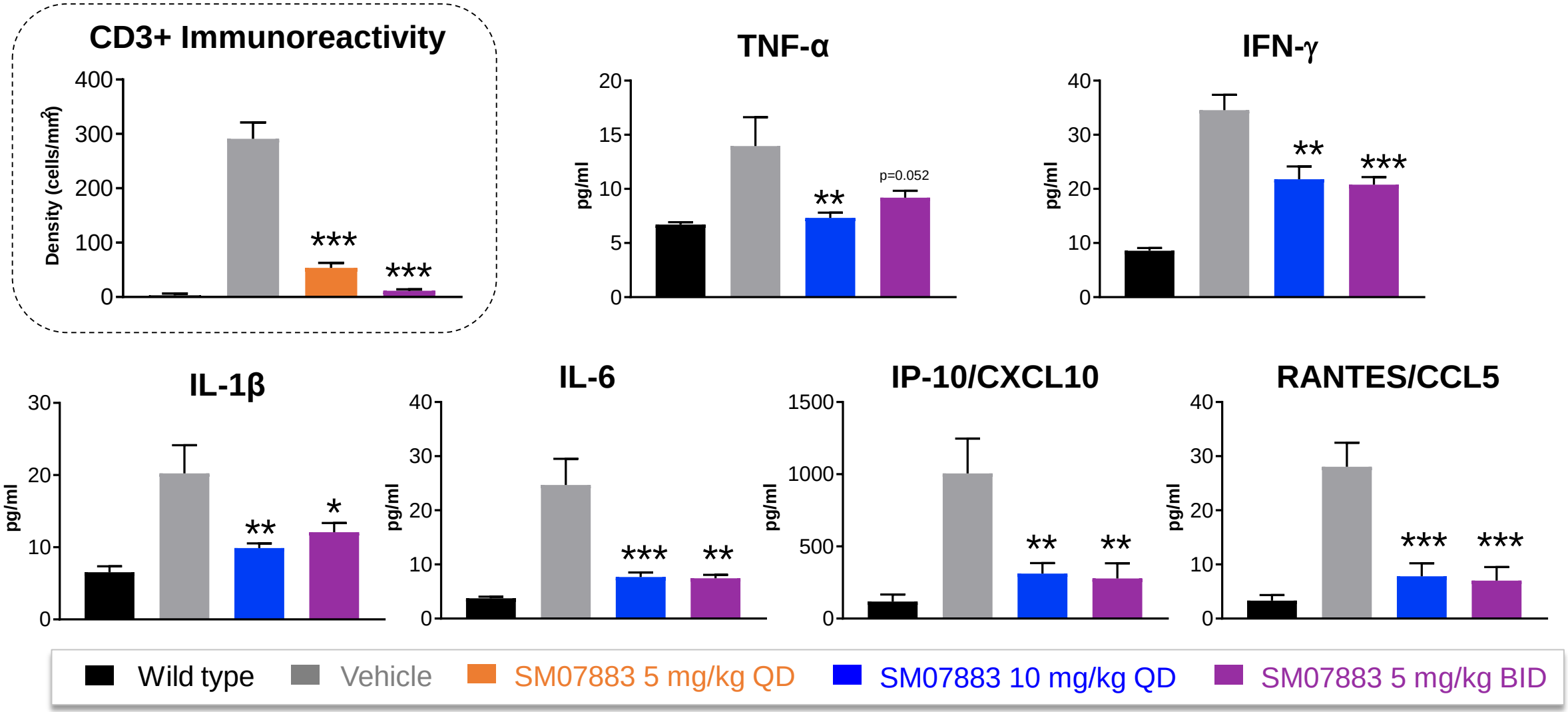
Right: Naive n=2, EAE: Veh. n=12, SM07883 5 mg/kg BID n=10, SM07883 10 mg/kg QD n=12

Mean ± SEM; ** p<0.01, *** p<0.001 vs. vehicle

samumed


Fluoromyelin Green
CD3
DAPI
Merge
Naive
Vehicle (QD)
SM07883 (5mg, QD)
400 µM
400 µM
400 µM

SM07883 reduced EAE-induced proinflammatory mediators in the spinal cord



Also reduced IL-4, MIP-1α, MIP-1β, and GM-CSF compared to vehicle

samumed

Mean ± SEM; * p<0.05, ** p<0.01, *** p<0.001 vs. vehicle

Conclusion

- SM07883 ameliorated neuroinflammatory responses in preclinical models compared to vehicle
  - Reduced AD-associated neuroinflammation
  - Reduced acute and chronic neuroinflammation in absence of neurodegeneration
  - Not restricted to innate immunity with a potent effect on CNS-related adaptive immune responses
- Potential role of DYRK1A inhibition in both innate and adaptive immunity
- Immune mediators may be useful biomarkers for clinical trials in DYRK1A systemic intervention

samumed

Thank you