

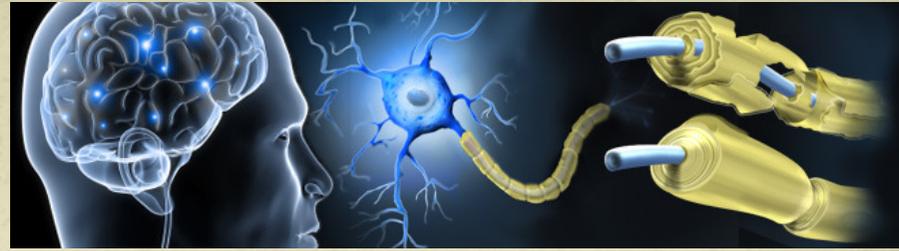
**1, 25-dihydroxyvitamin D₃ (VD₃)
enhances neural stem cell proliferation
&
oligodendrocyte differentiation**

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Thomas Jefferson University of Medical College
Neurology Department**

Investigation Objectives

- ❖ Evaluation of interaction between Vitamin D₃ and neural stem cell proliferation and differentiation in to oligodendrocyte as the myelinating cell
- ❖ Assessment of Vitamin D₃ effect on Multiple Sclerosis progression

Multiple Sclerosis



- ❖ **Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system**
- ❖ **Immunologic, infectious, genetic factors and environmental risk factors like insufficient exposure to the sunlight and naturally-produced of vitamin D3**

Vitamin D



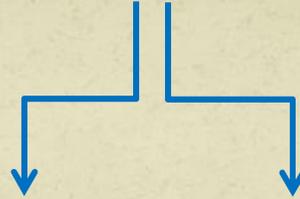
- ❖ Regulation of calcium , phosphorus , bone metabolism and neuromuscular function
- ❖ Regulation of immune responses attaching to a specific receptor (VDR) in cerebral white matter and inhibiting chronic inflammatory disease
- ❖ Regulation of cell proliferation and differentiation



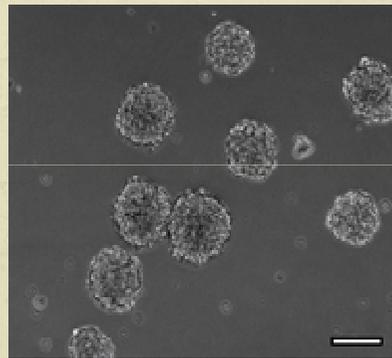
1,25 (OH)₂D₃ and Oligodendrocyte

- ★ Oligodendrocyte in brain and spinal cord expresses vitamin D receptor (VDR)**
- ★ Lack of level 1,25-(OH)₂D₃ → Decreasing of differentiation and axonal adhesion of oligodendrocyte → increasing oligodendrocyte apoptosis → immune system disorder like MS**
- ★ Oligodendrocyte apoptosis and dystrophy are important pathological features in demyelinating lesions of MS**

Analysis of proliferation and differentiation



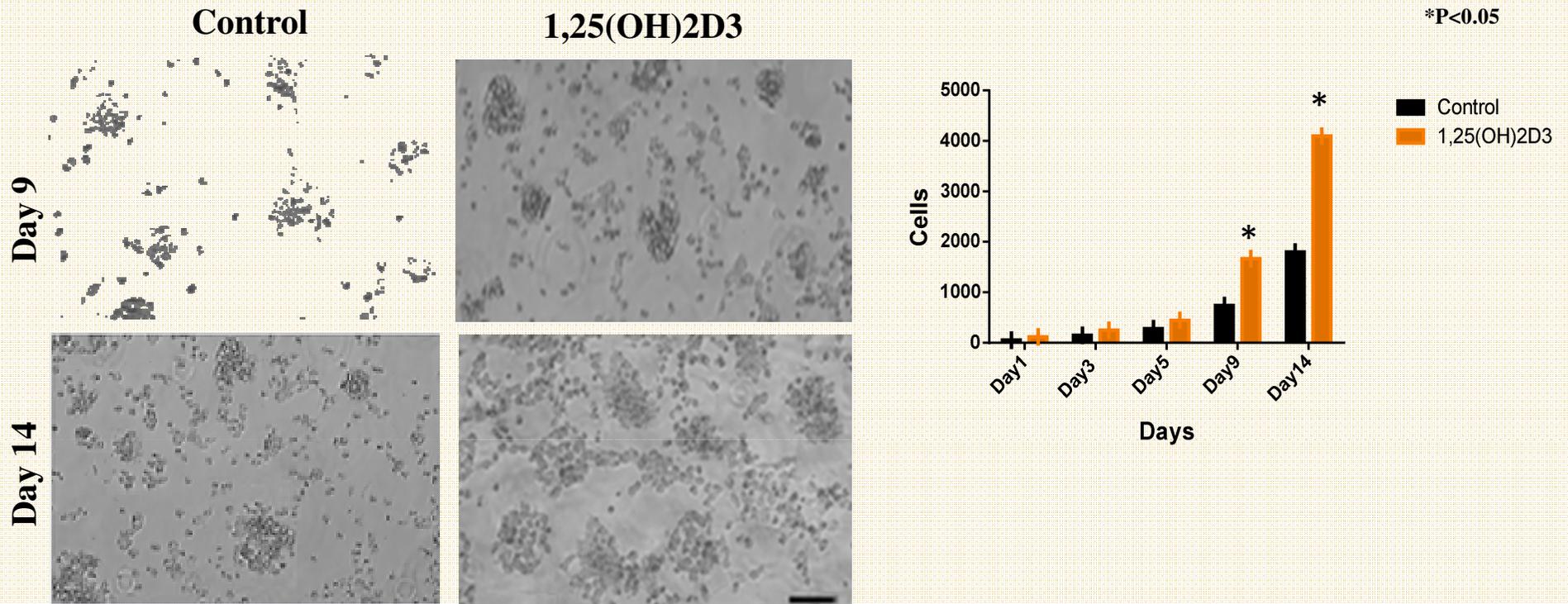
In vitro



In vivo



VD3 on NSC Proliferation on Days 1, 3, 5, 9, 14

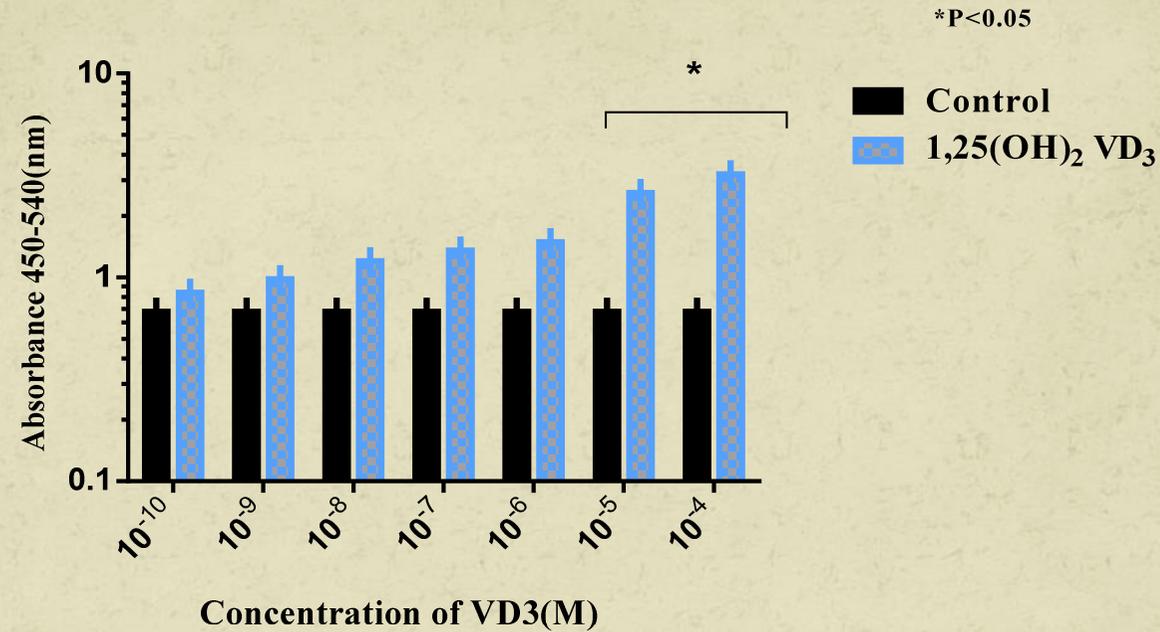


- $1,25(\text{OH})_2\text{D}_3$ $1 \times 10^{-10} - 1 \times 10^{-4}$ M
- Days 1 - 14

Result

- $1,25(\text{OH})_2\text{D}_3$ induced higher proliferation with concentration 1×10^{-4} M on Day 14
- Neurospheres were capable of secondary expansion

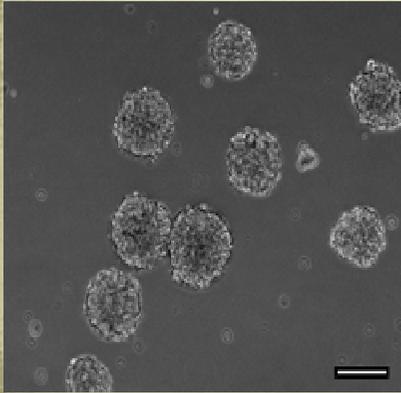
BrdU(Bromodeoxyuridine)Proliferation assay



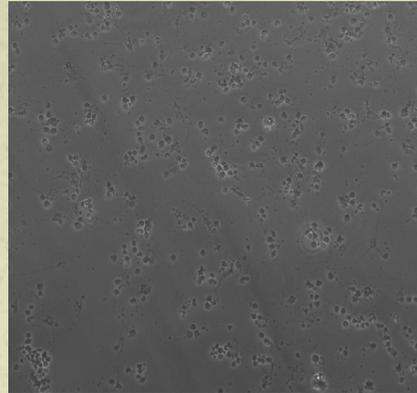
➤ **Result** : The best concentration of 1,25(OH)₂D₃ considered 1×10^{-4} M

VD3 on NSC Differentiation

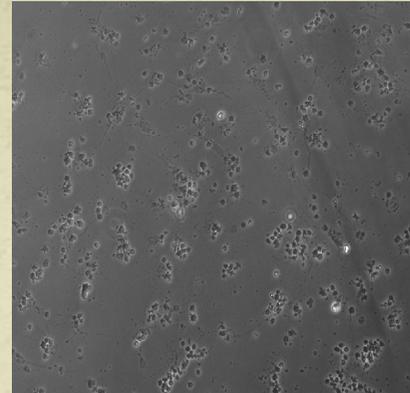
Neurosphere



Control

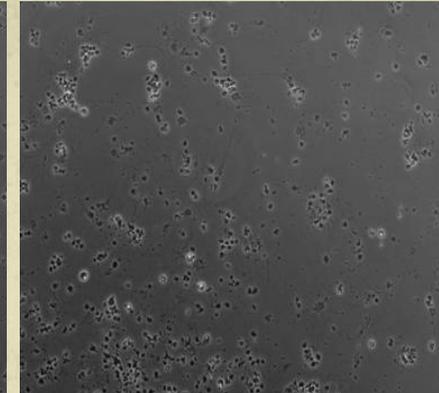


1×10^{-10} M

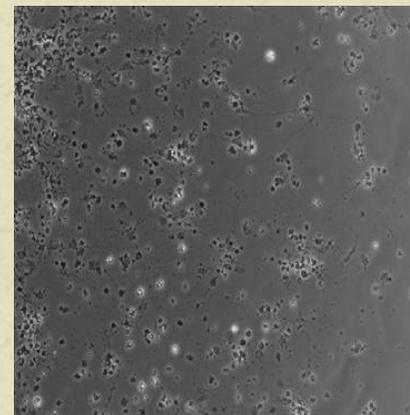


VD3

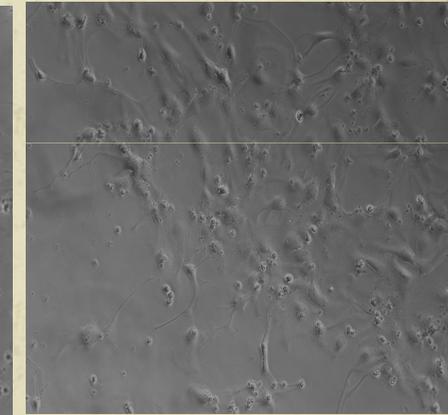
1×10^{-9} M



1×10^{-8} M

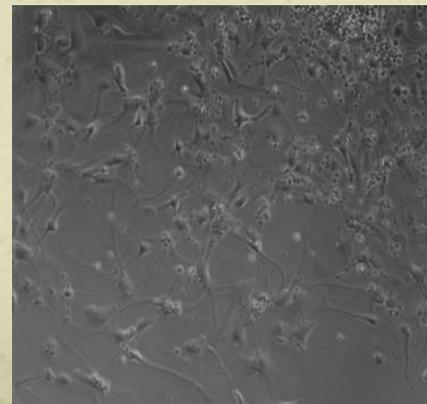


1×10^{-7} M

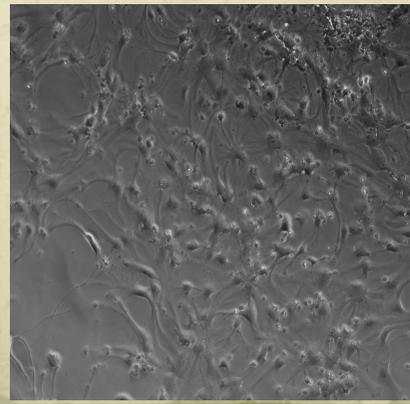


➤ **Result**

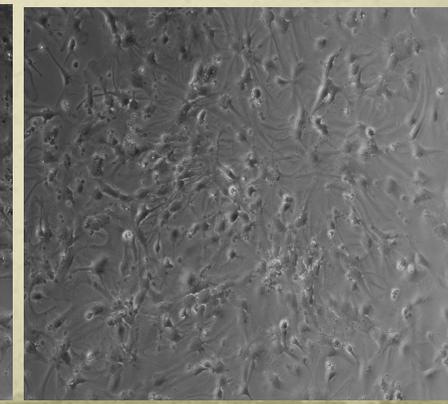
Enhanced differentiation with detected concentration 1×10^{-4} M of $1,25(\text{OH})_2\text{D}_3$



1×10^{-6} M



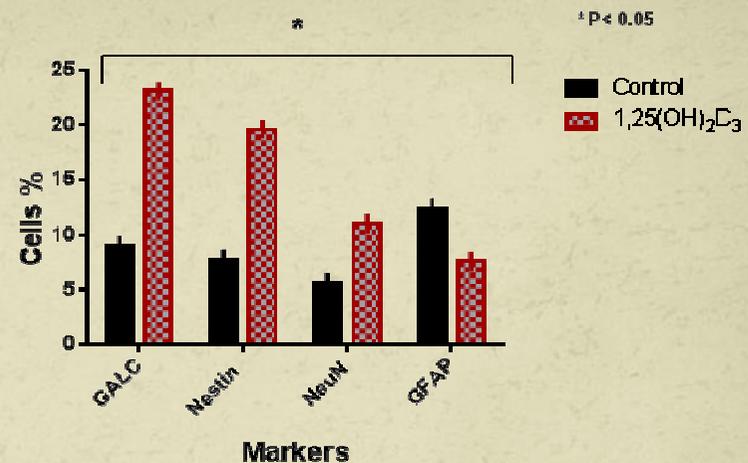
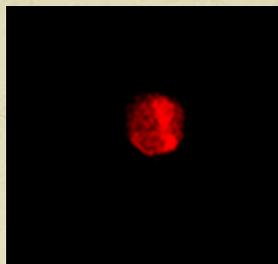
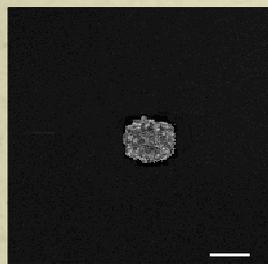
1×10^{-5} M



1×10^{-4} M

VD3 on immunoreactivity in NSC

Neurosphere

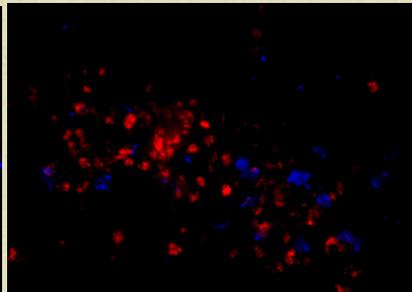
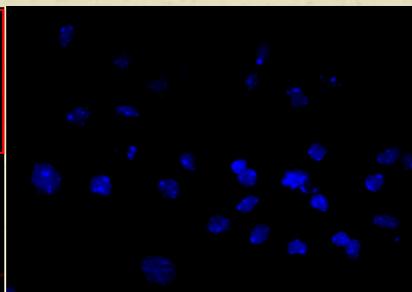
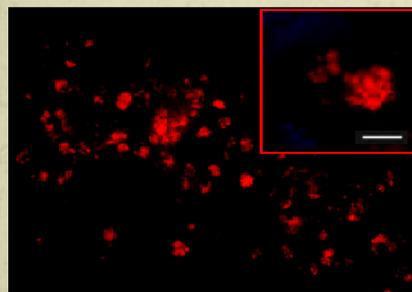


GALC

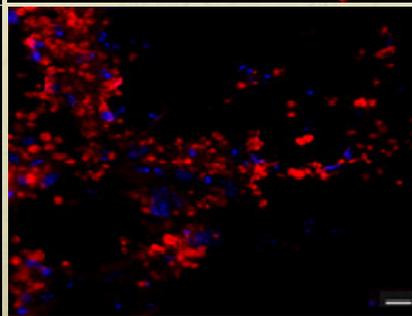
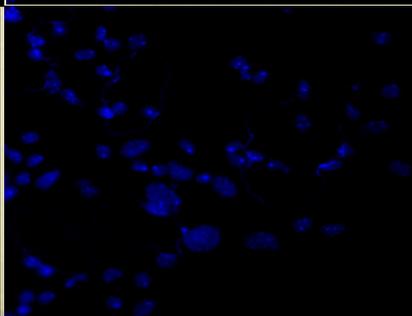
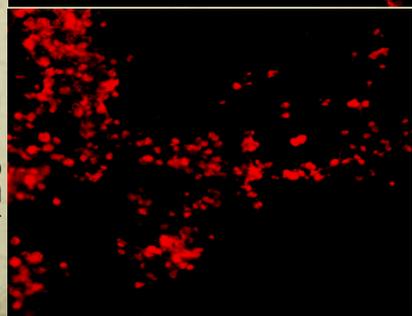
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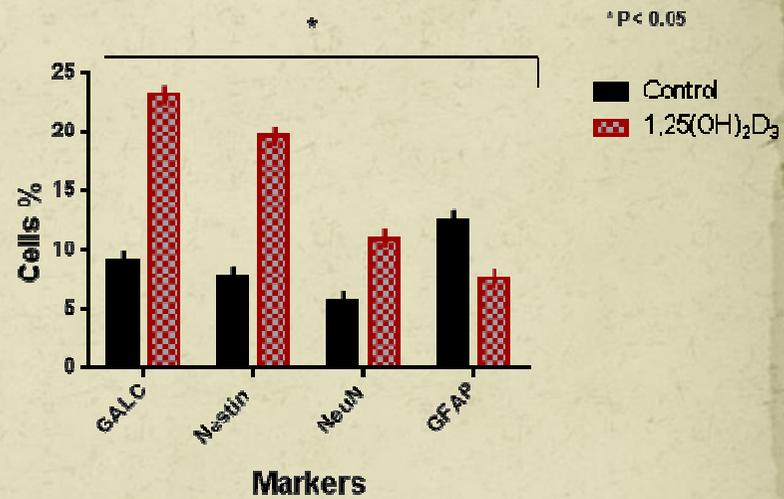
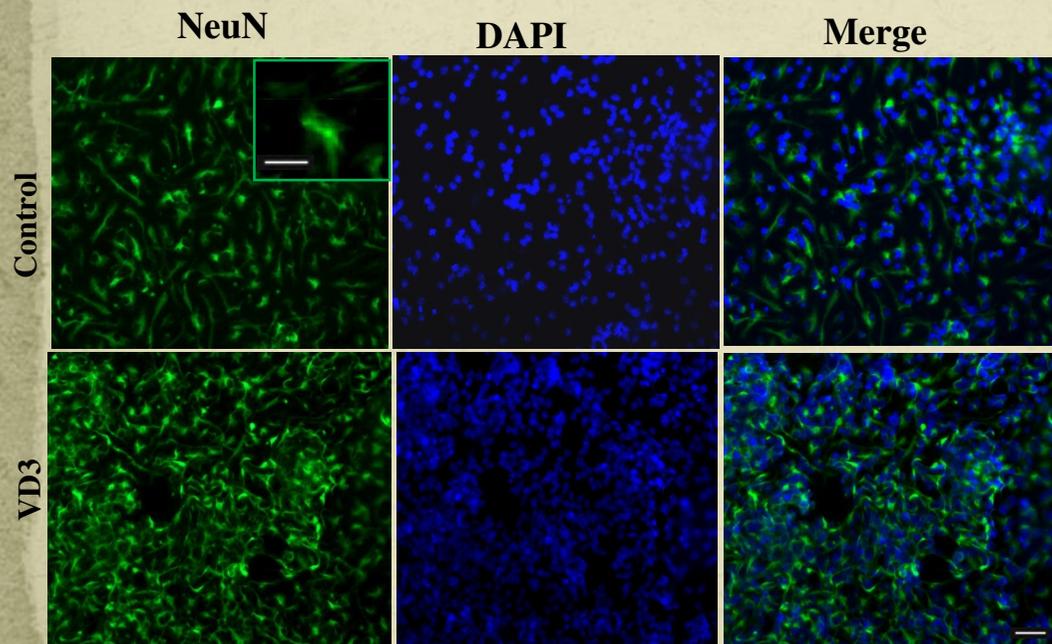
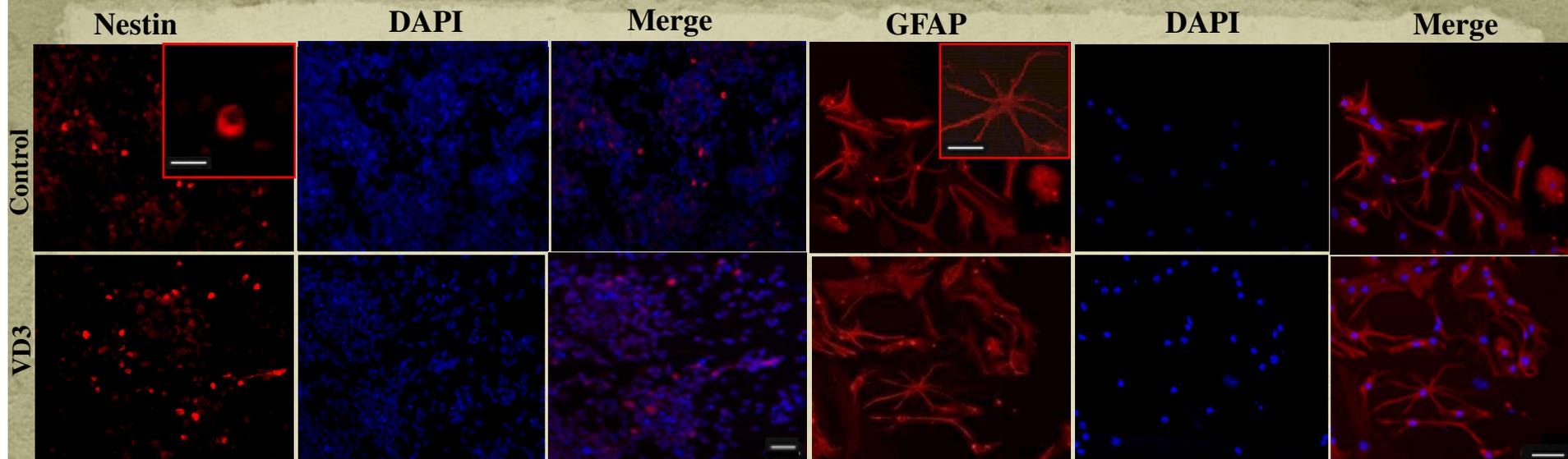
Merge

Control



VD3





Immunization and EAE Induction

- C57BL/6 (8-10 weeks)
- Immunization and induction of EAE (Experimental Autoimmune Encephalomyelitis)
- Myelin Oligodendrocyte Glycoprotein (MOG) 200 μ g and Complete Freund's Adjuvant(CFA) \rightarrow subcutaneous injection
- Pertussis toxin (200 ng) \rightarrow intraperitoneal injection (i.p) at the time of immunization and 48 hours later



Treatment with 1,25-(OH)₂-D₃

➤ Treated animals

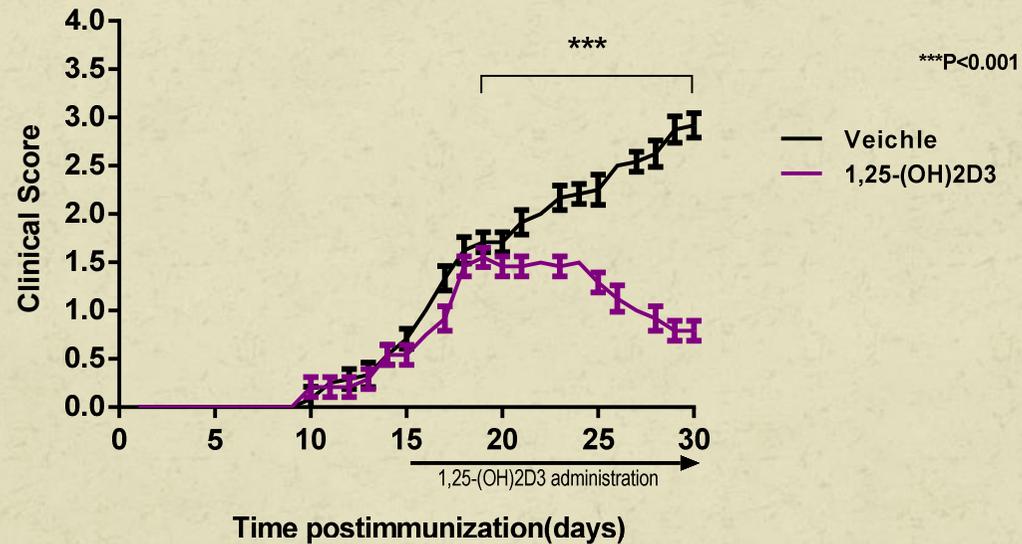
Injection 1,25-(OH)₂-D₃(0.10 μg in 0.1% ethanol) in normal saline → every other day for 15 days

➤ Control animals

Injection 0.1% ethanol in normal saline → every other day for 15 days

Disease activity assessment

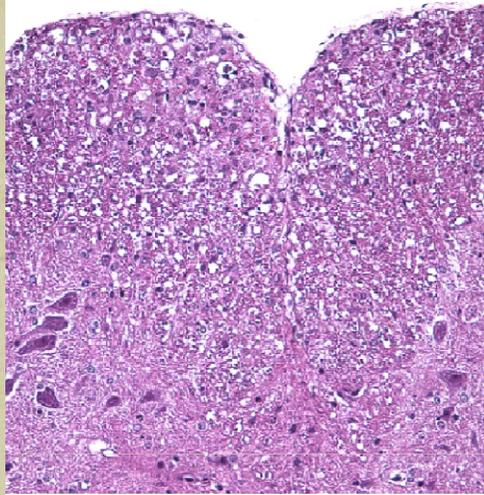
- Animals were examined daily for signs of disease and graded on a scale of 0 to 5 of increasing severity of disease



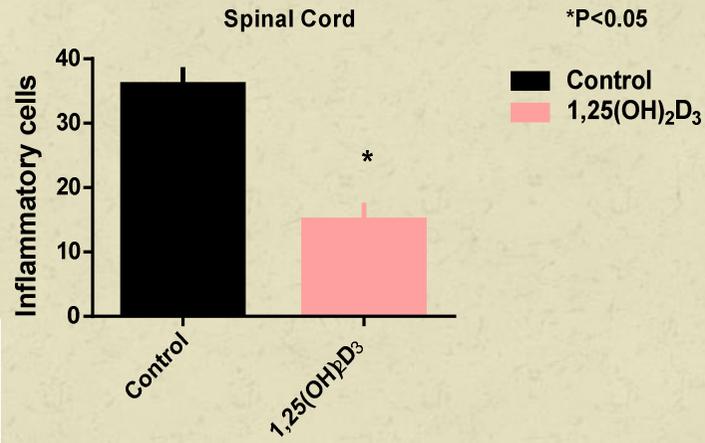
H&E

VD3 on Inflammation

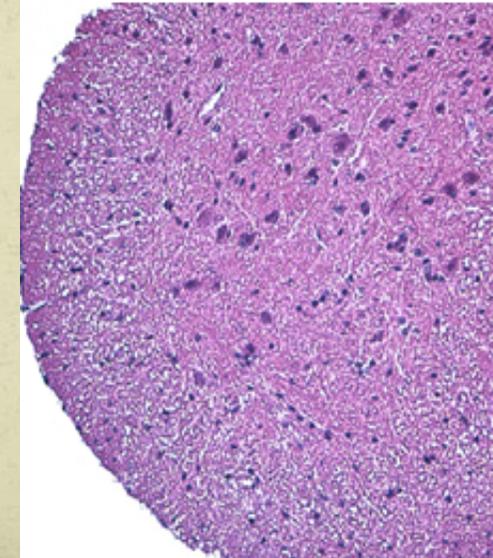
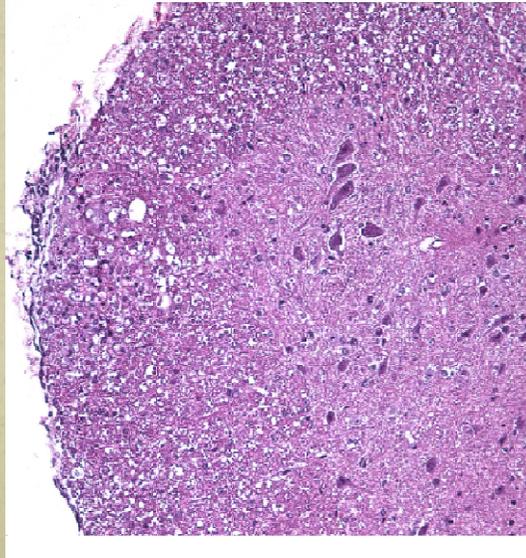
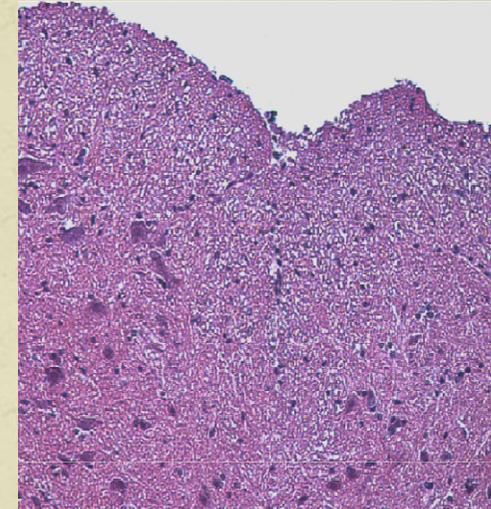
EAE



Spinal cord



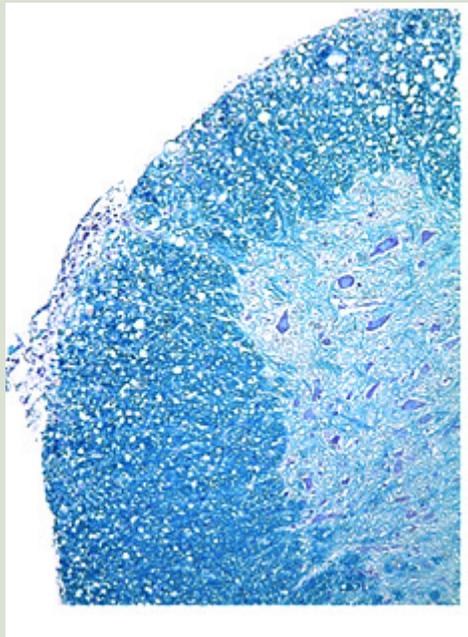
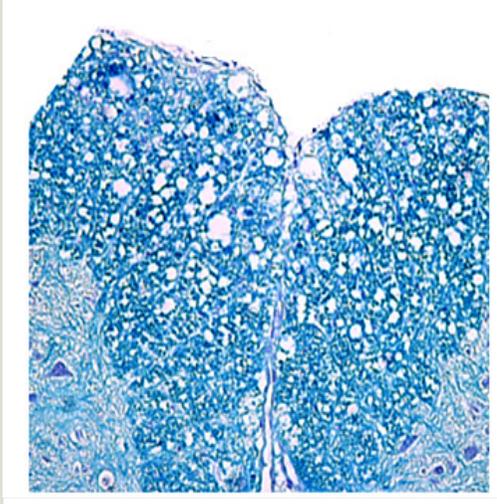
VD3 treated



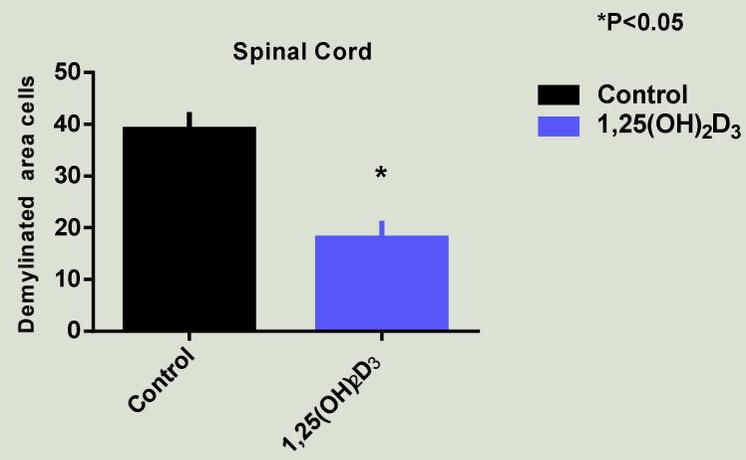
LFB

VD3 on Demyelination

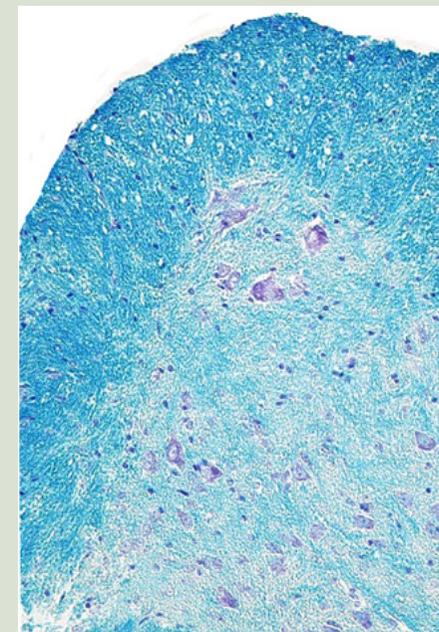
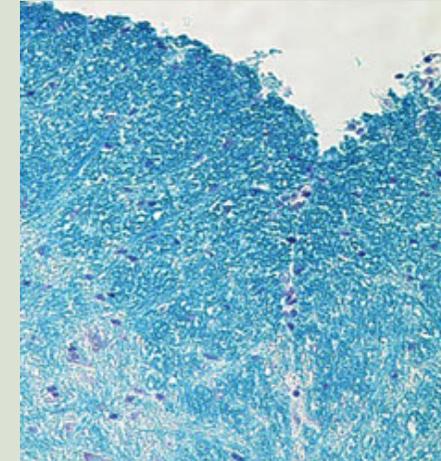
EAE



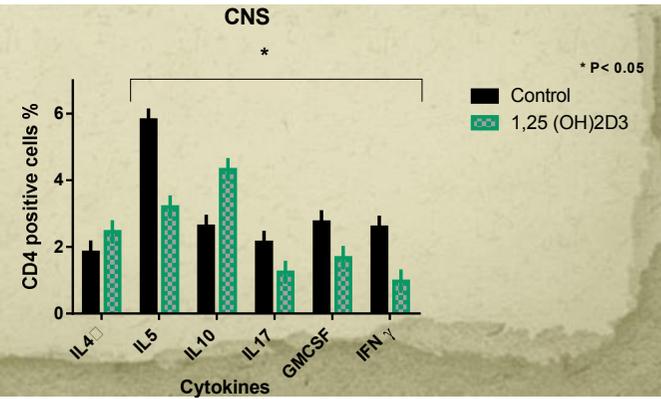
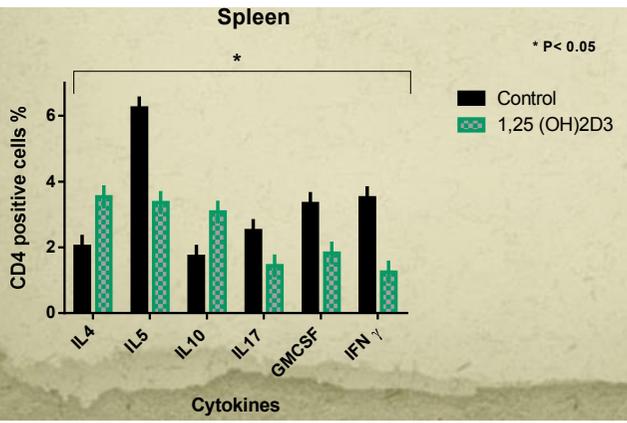
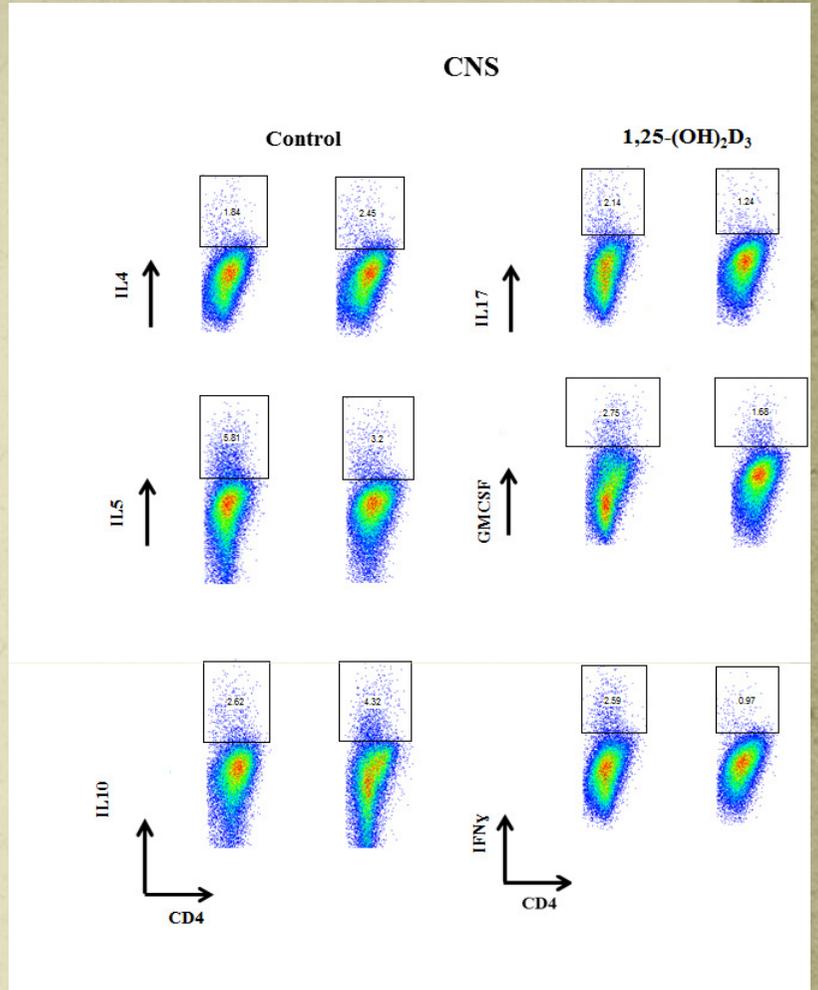
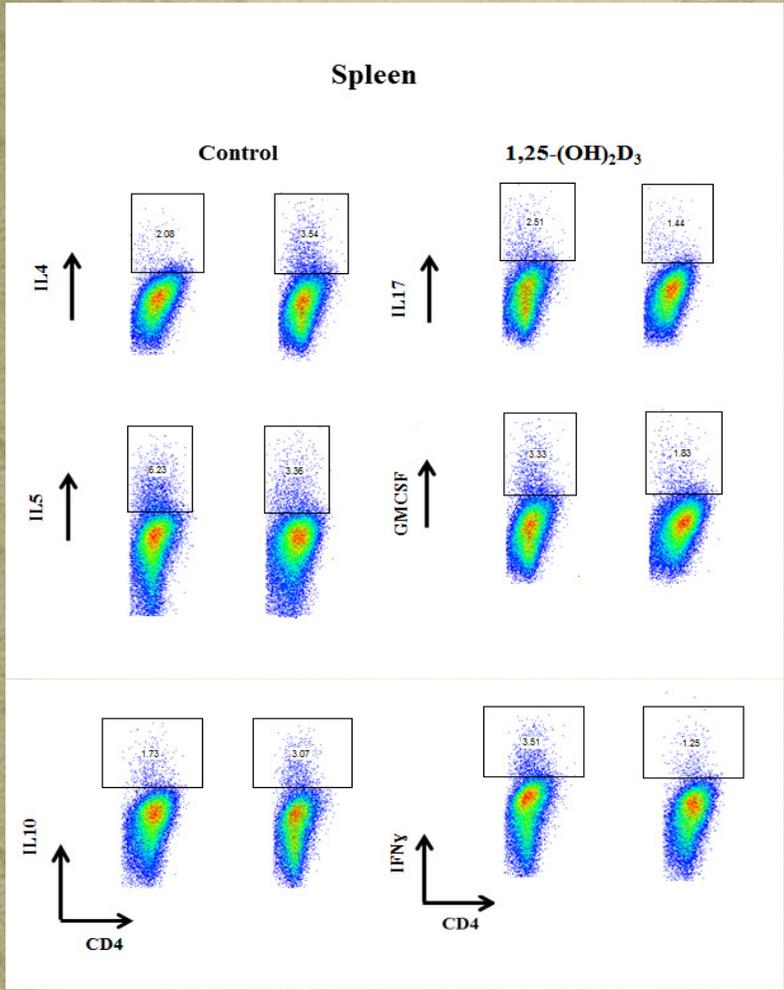
Spinal cord



VD3 treated

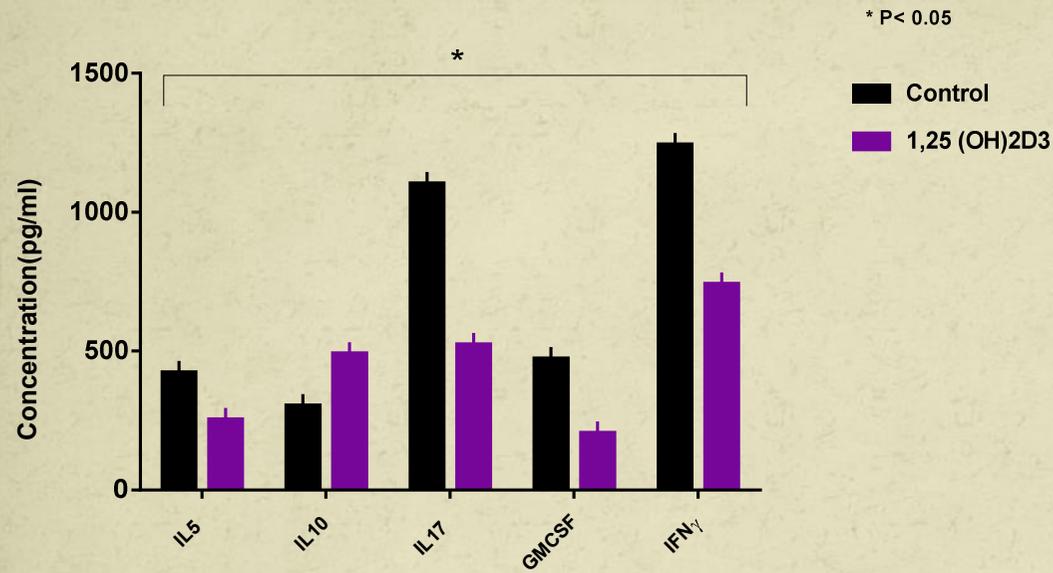


VD3 on Immunoregulation

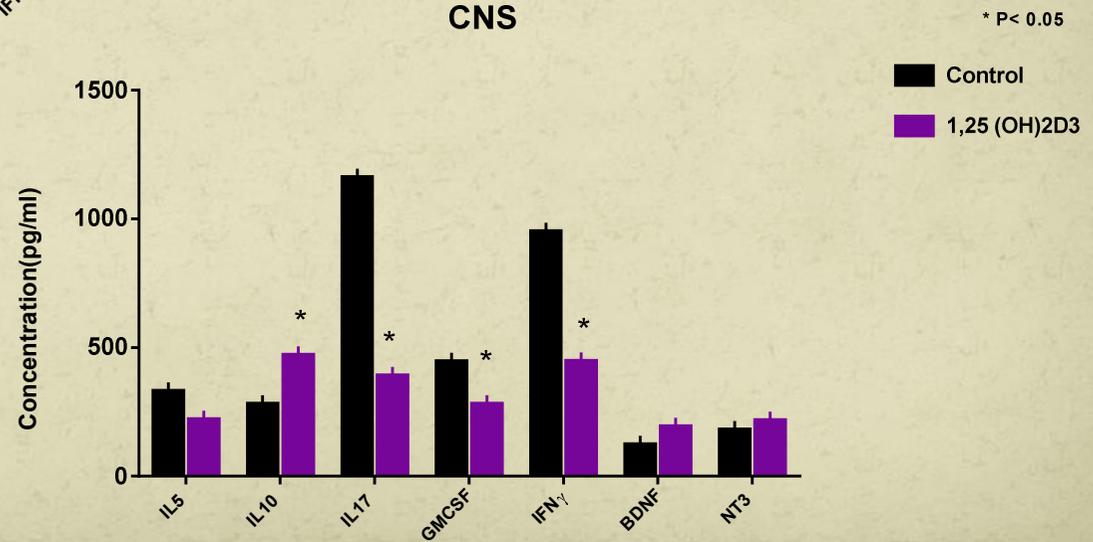


VD3 on Cytokine levels & Immunoassay

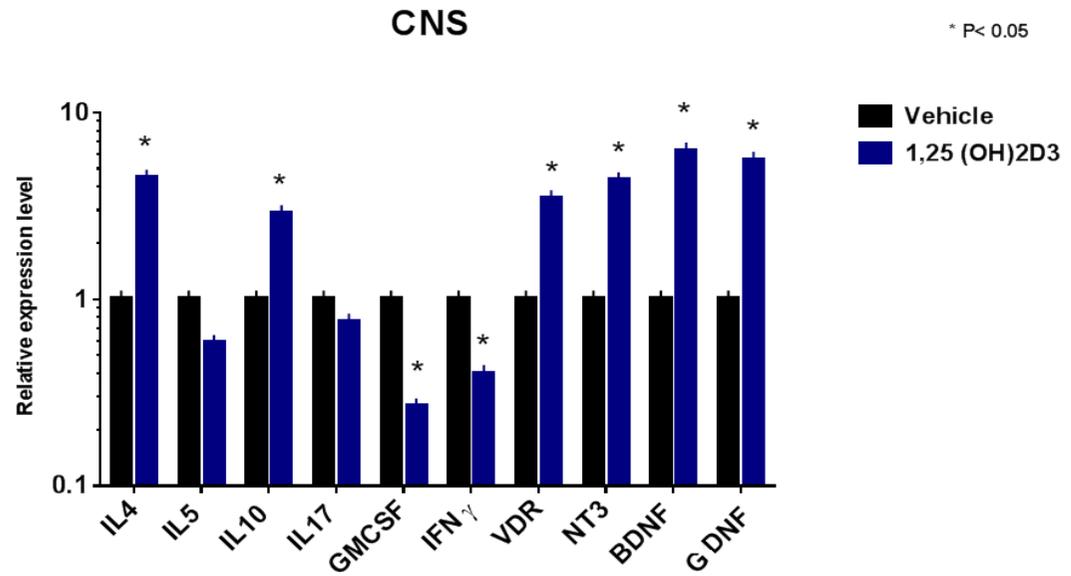
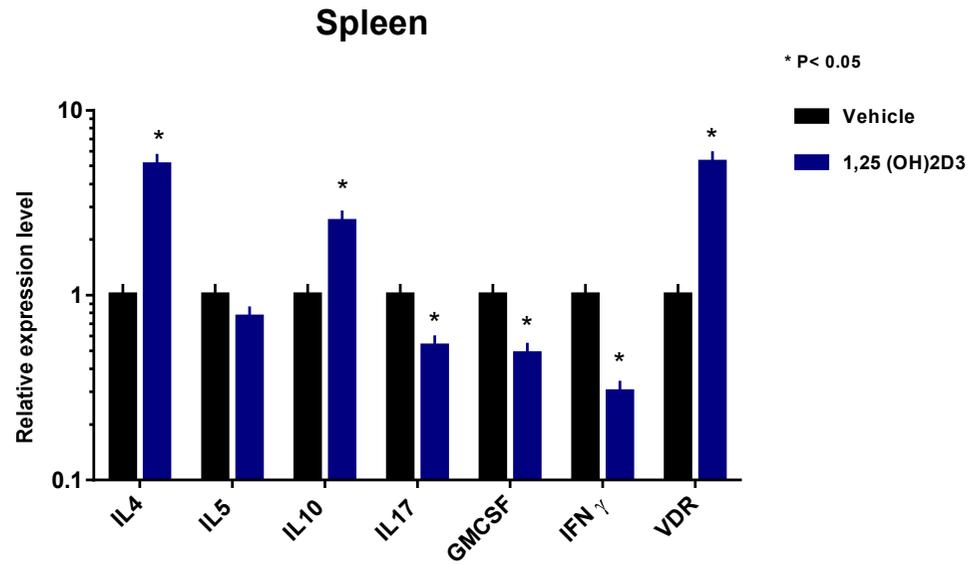
Spleen



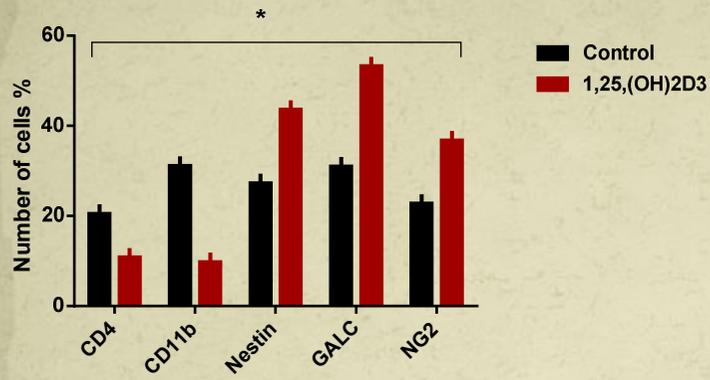
CNS



VD3 on mRNA expression

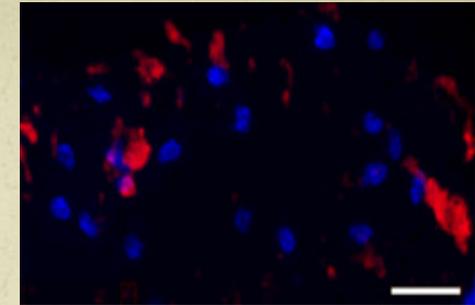


VD3 on immunoreactivity



Spinal cord

Nestin

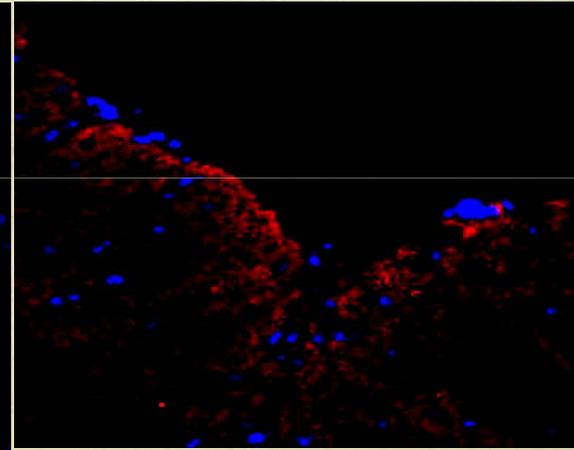
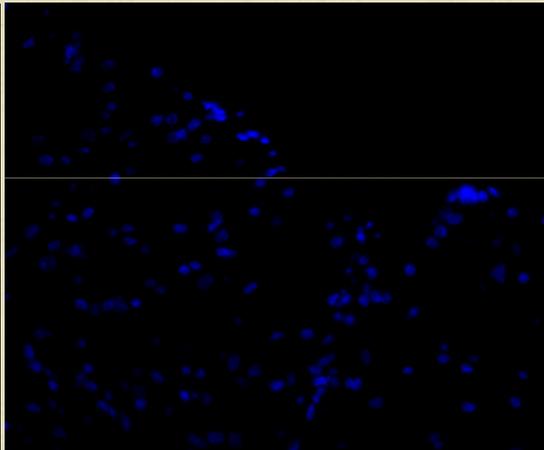
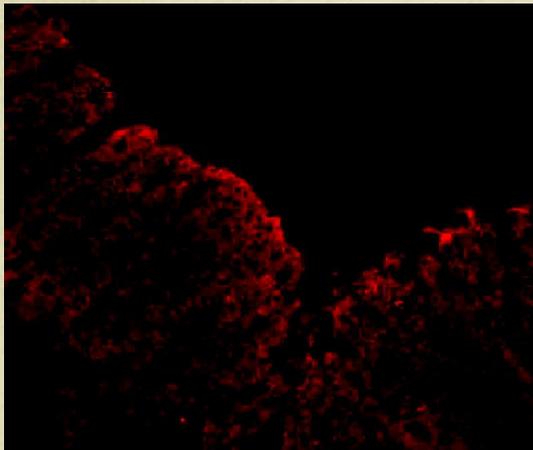


Nestin

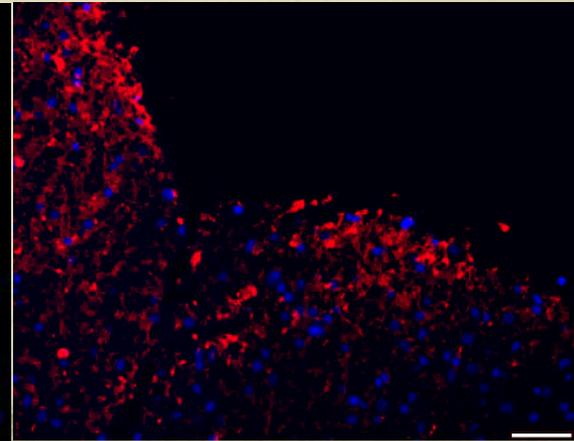
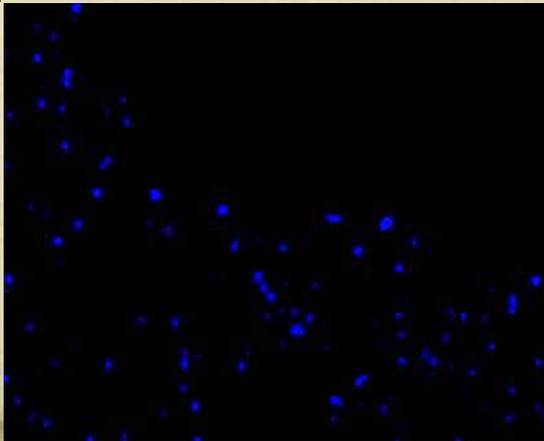
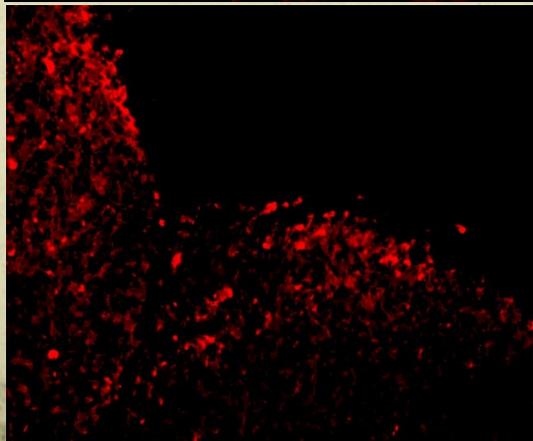
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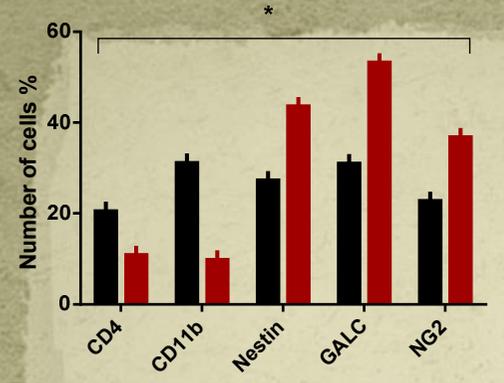
Merge

Control



VD3

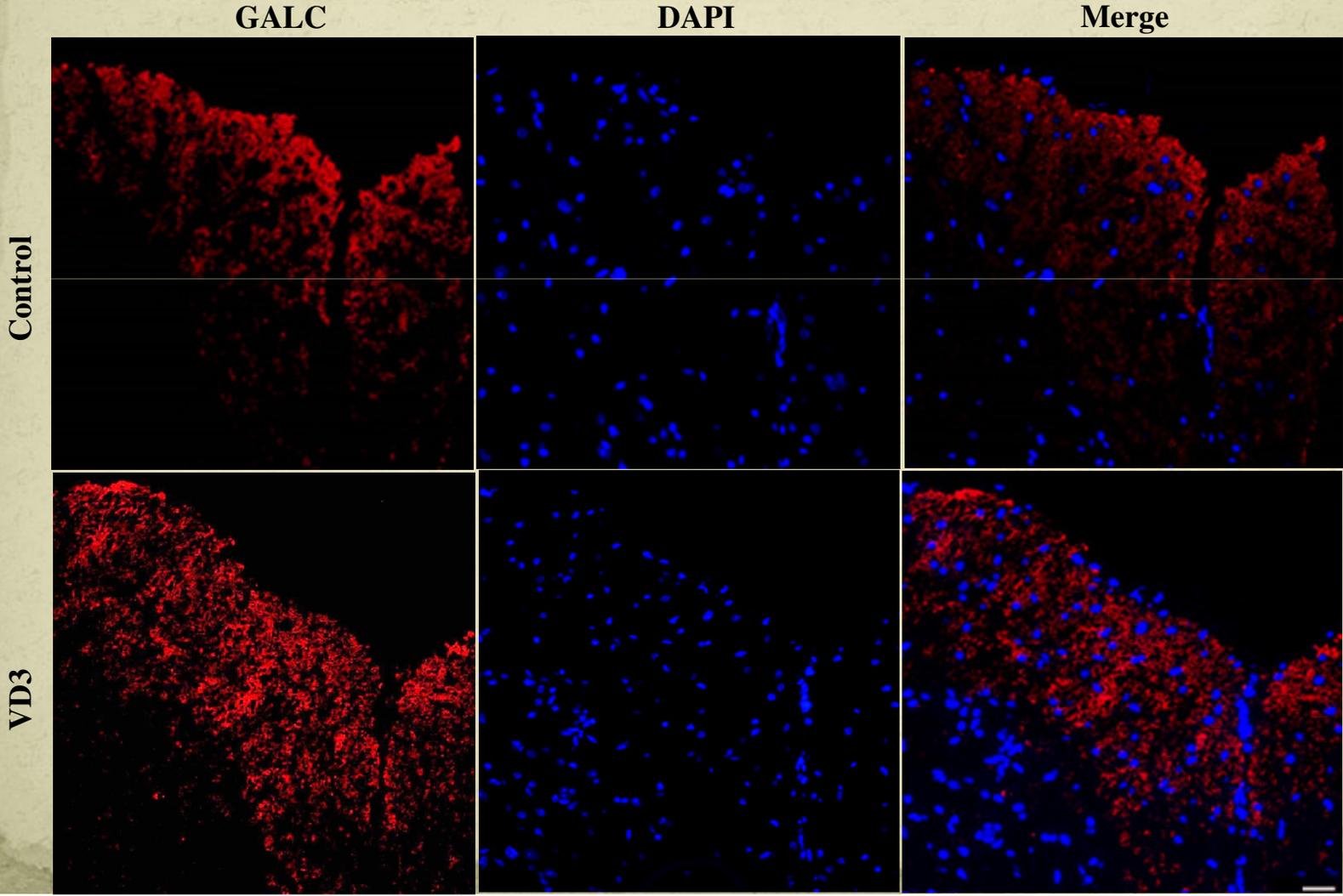
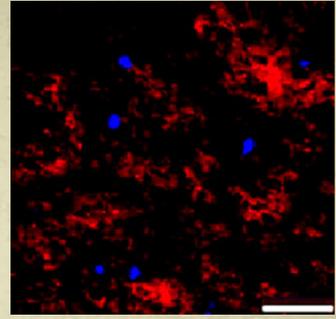




VD3 on immunoreactivity

Spinal cord

Oligodendrocyte



Control

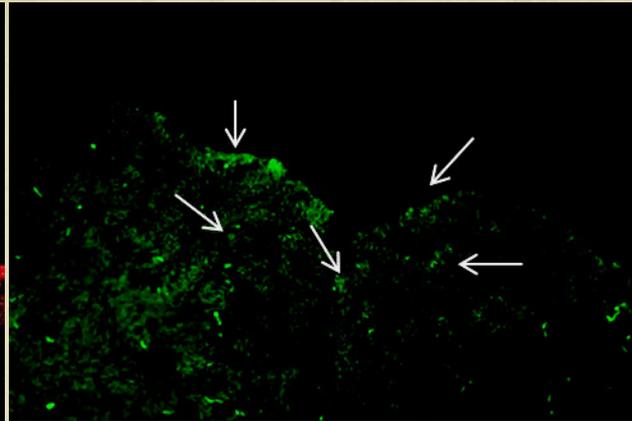
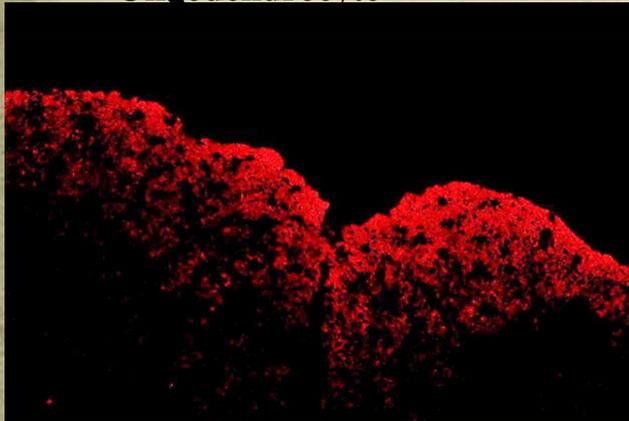
VD3

VDR detection in Oligodendrocyte

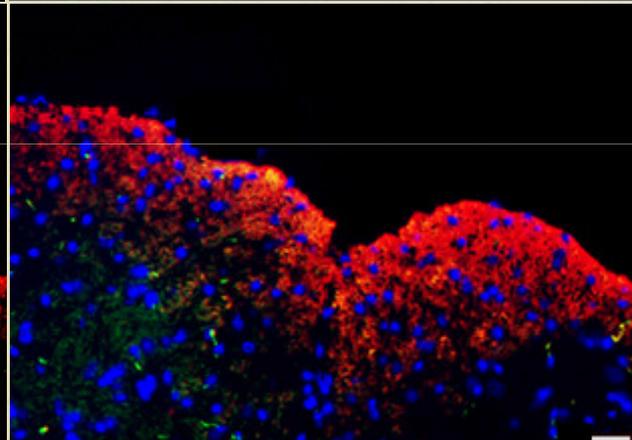
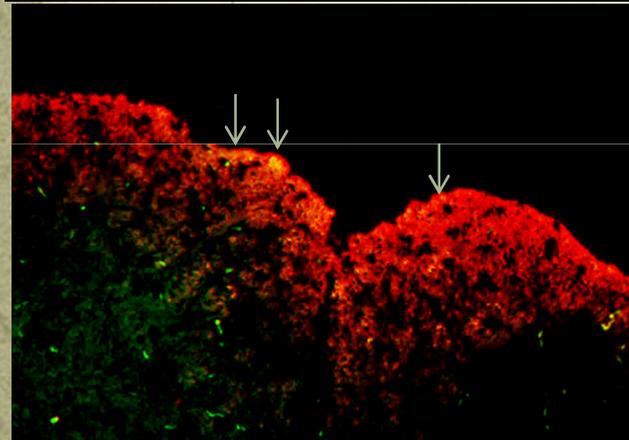
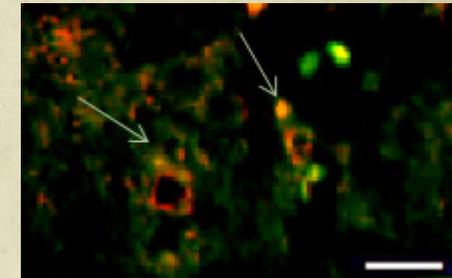
Spinal cord

Oligodendrocyte

VDR



Oligodendrocyte & VDR



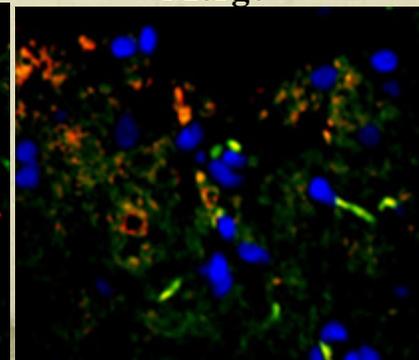
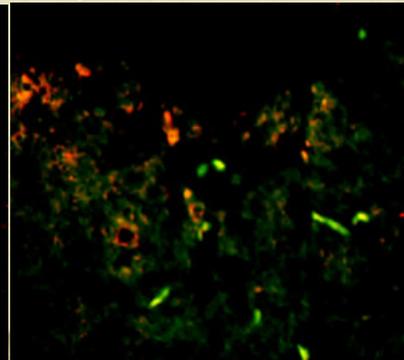
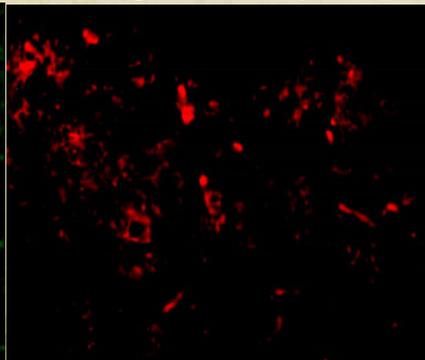
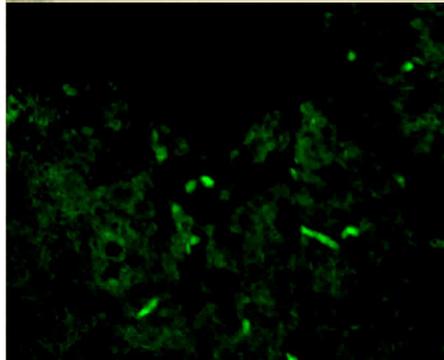
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VDR

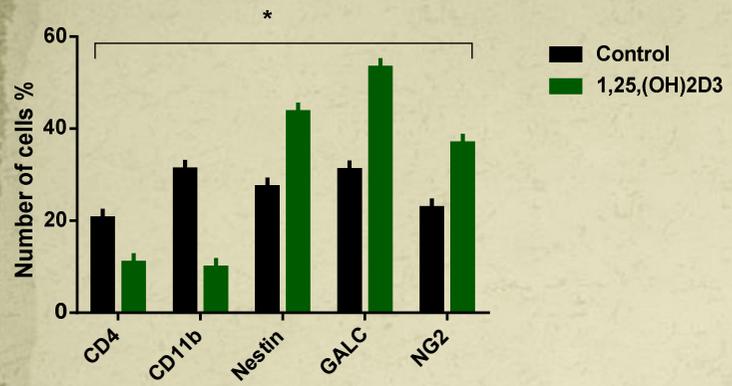
Oligodendrocyte

Oligodendrocyte & VDR

Merge

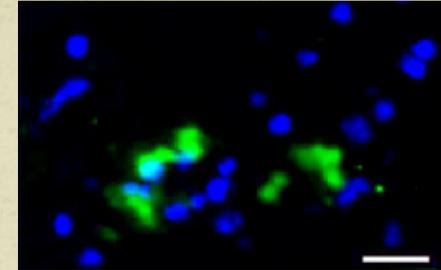


VD3 on immunoreactivity



Spinal cord

CD4



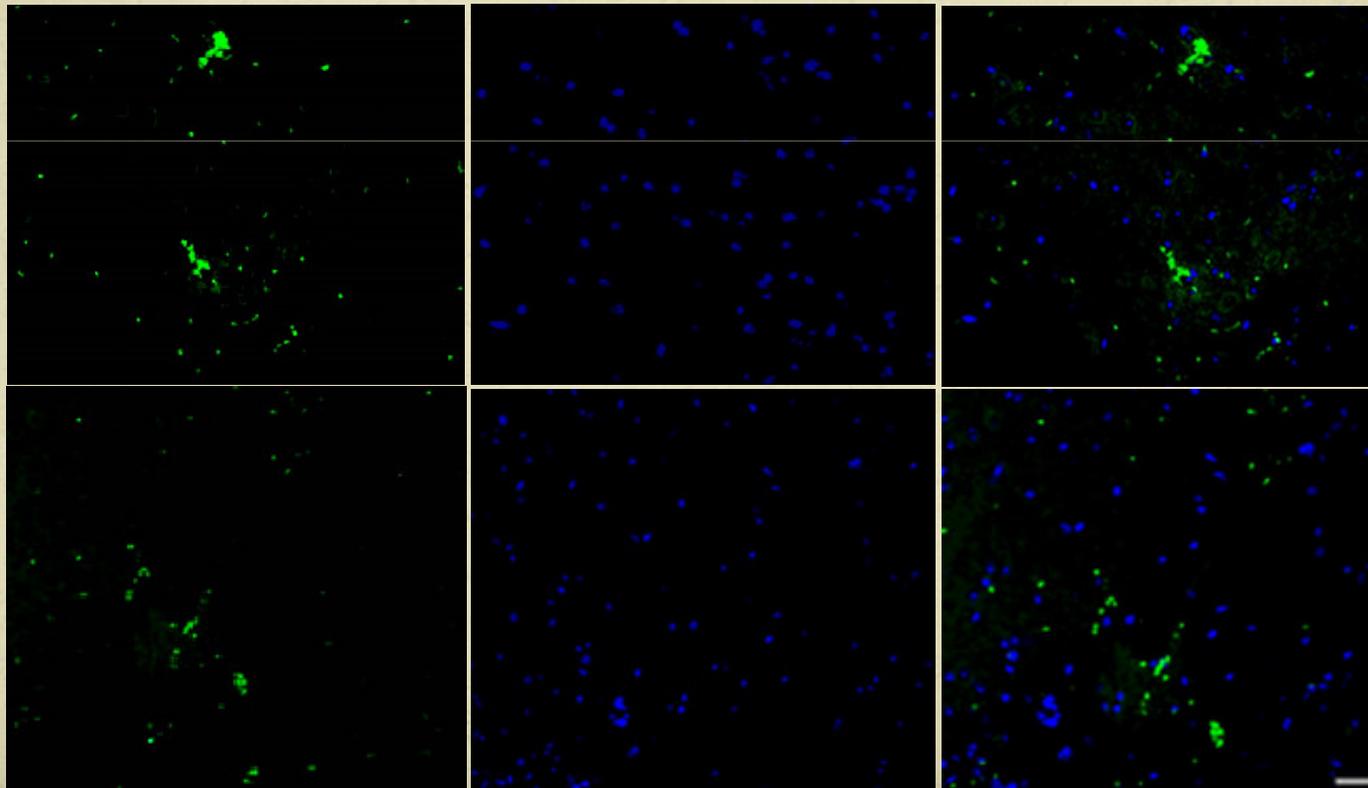
CD4

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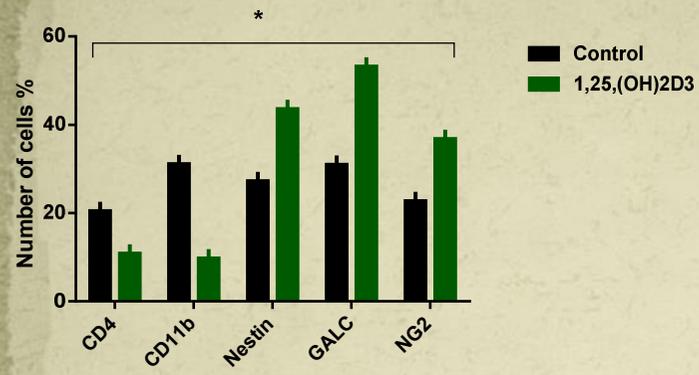
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Control

VD3

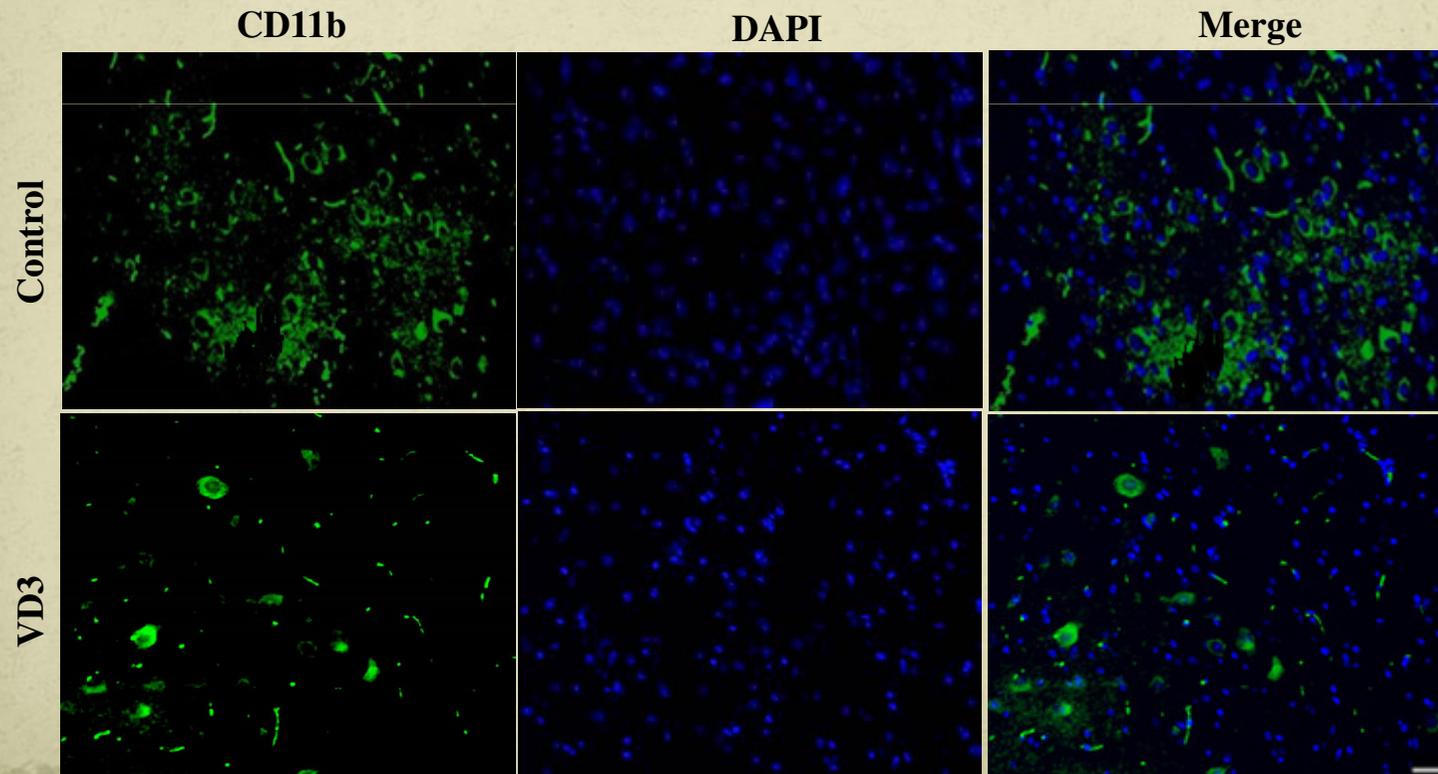
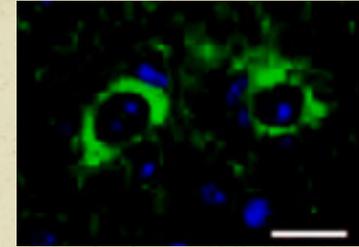


VD3 on immunoreactivity



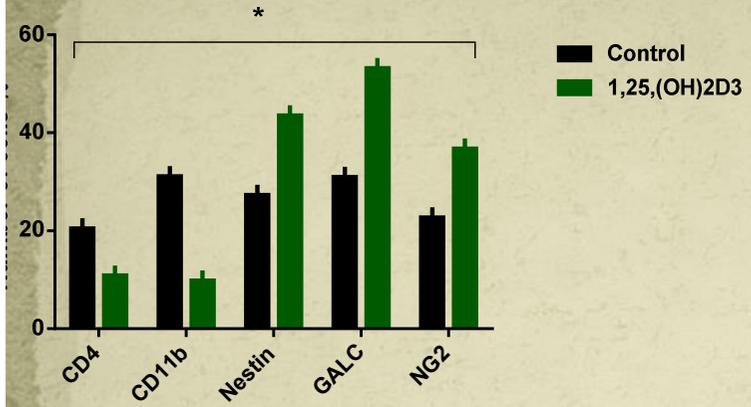
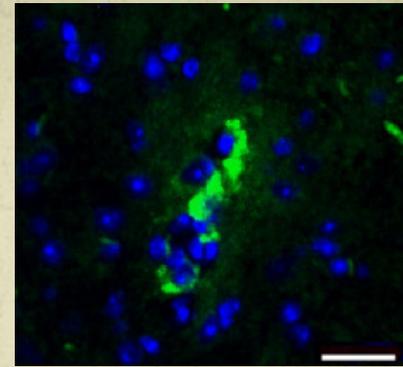
Spinal cord

CD11b

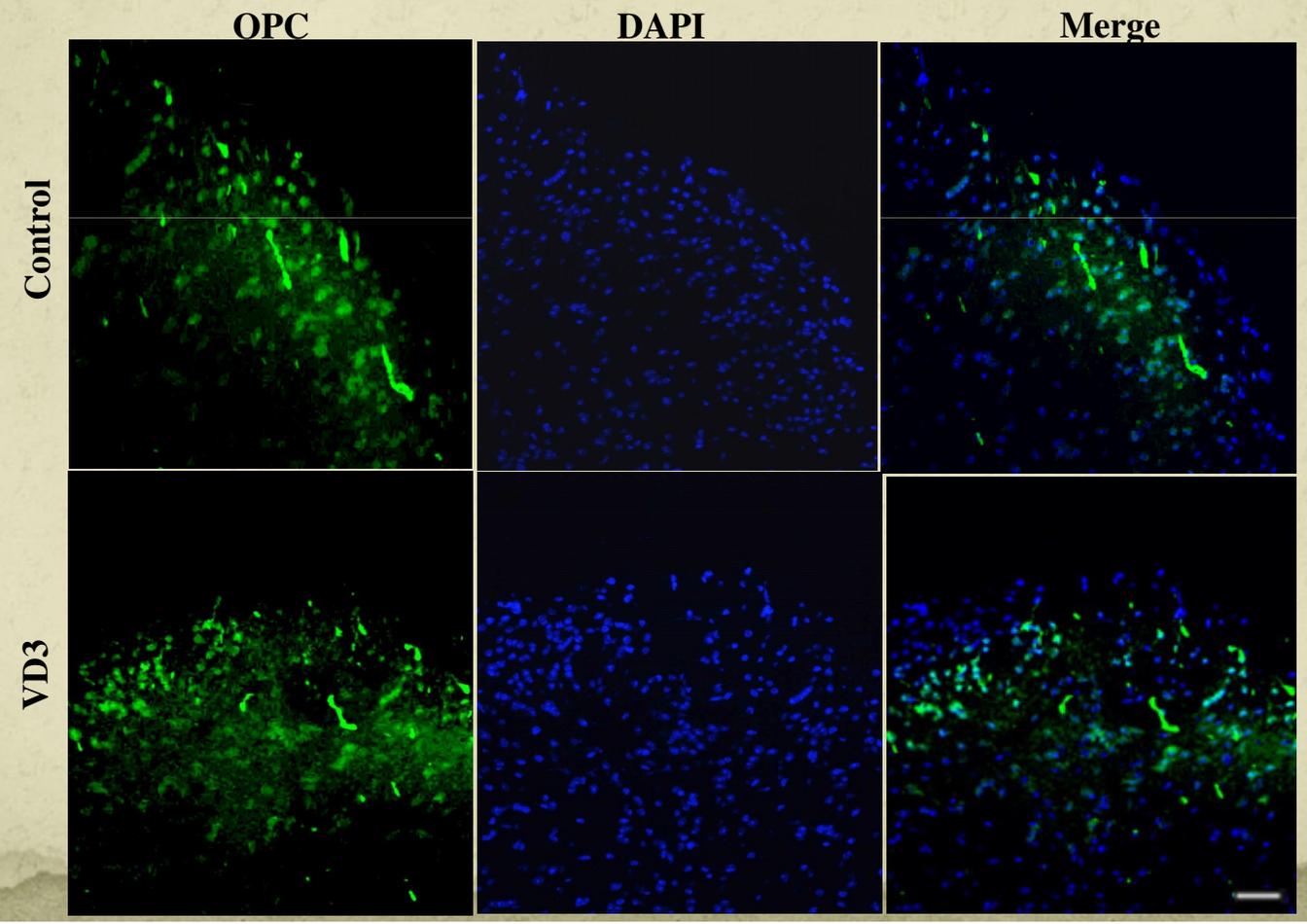


VD3 on immunoreactivity

OPC



Spinal cord



Summary

- ❖ This study provides evidence for strong association of vitamin D₃ with development of MS and EAE progression suggesting as a neuroprotective factor and possessing of potential effects on NSC proliferation and oligodendrocyte differentiation.
- ❖ Vitamin D₃ advantages are selective immunosuppression of autoimmune cells, the positive regulation of other immune cells and ease of delivery that might decrease the risk of infection, so can be suggested as a novel and effective treatment promoting endogenous remyelination .
- ❖ Moderate sun exposure and vitamin D supplement can be effective complements for MS prevention and management.

Acknowledgment

- ❖ *Professor Guang –Xian Zhang (MD, PhD) ,Thomas Jefferson University of Medical College*
- ❖ *Professor Abdolmohamad Rostami (MD, PhD), Thomas Jefferson University of Medical College*
- ❖ *National Institutes of Health (NIH) and Multiple Sclerosis International Federation (MSIF)*

