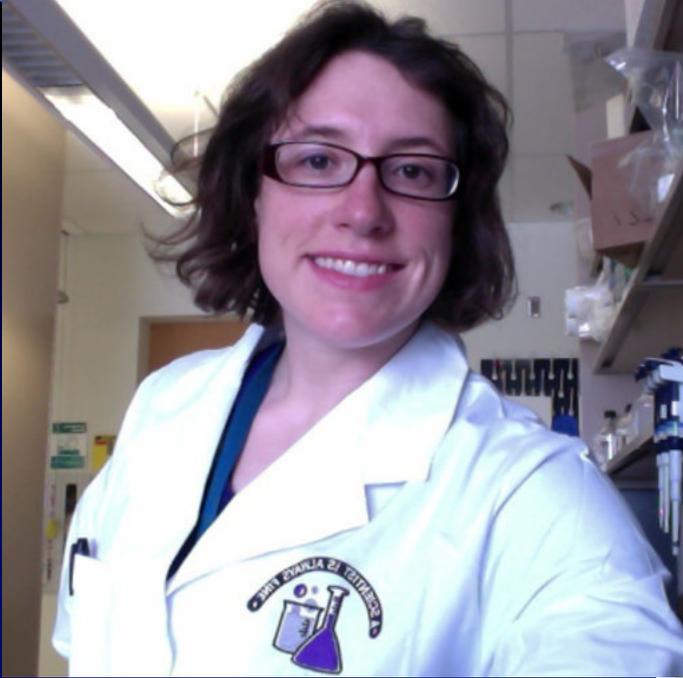


# Insights into AFM Pathogenesis & Therapy from a Mouse Model of EV-D68 AFM



Alison Hixon, (M.D.)/Ph.D.



Michael Rudy, Ph.D & "Panda"

NIAID: State of the Science  
of Acute Flaccid Myelitis & GBS  
Videocast 7 May, 2020



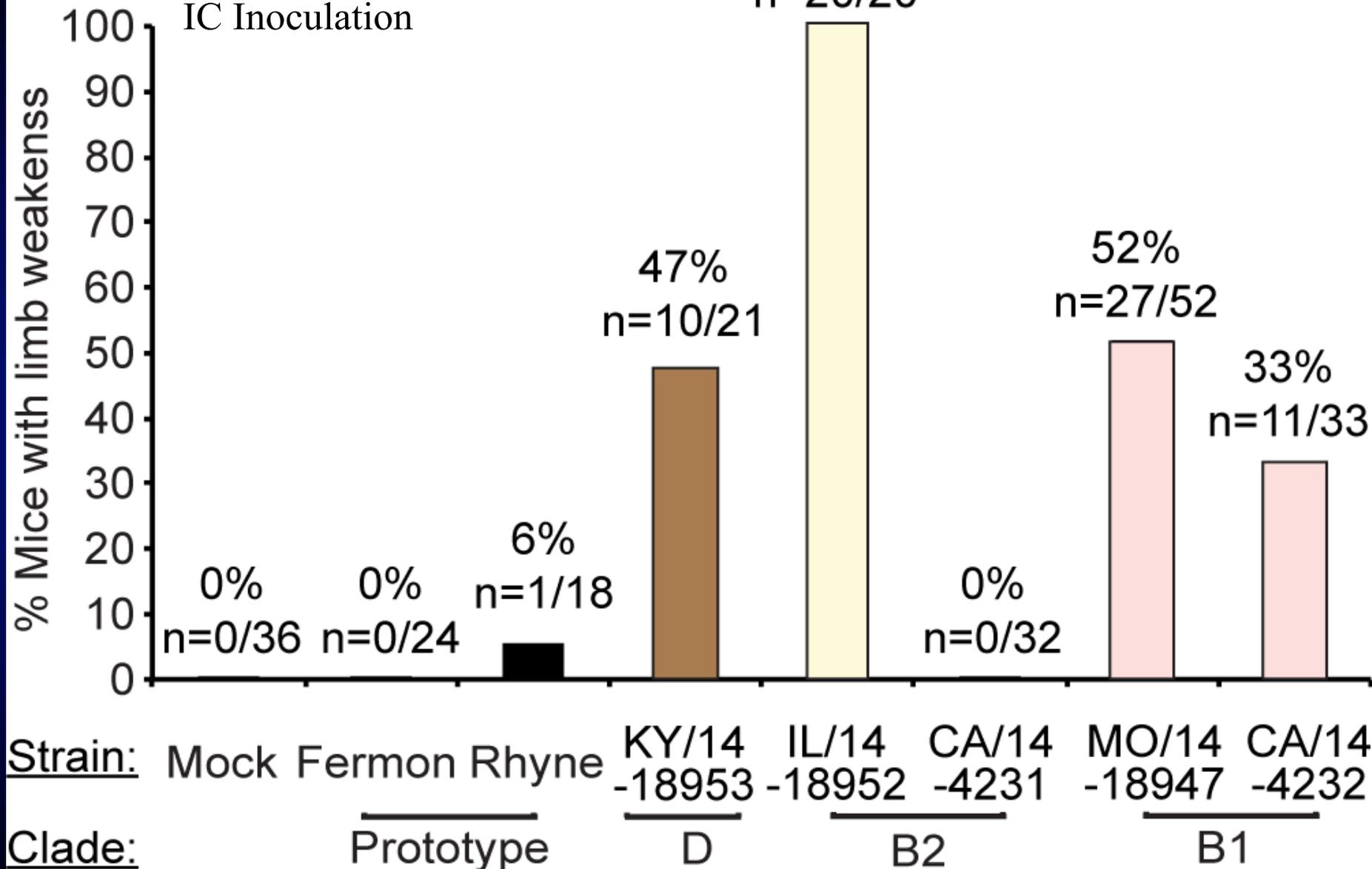
Joshua Frost, (Ph.D.)

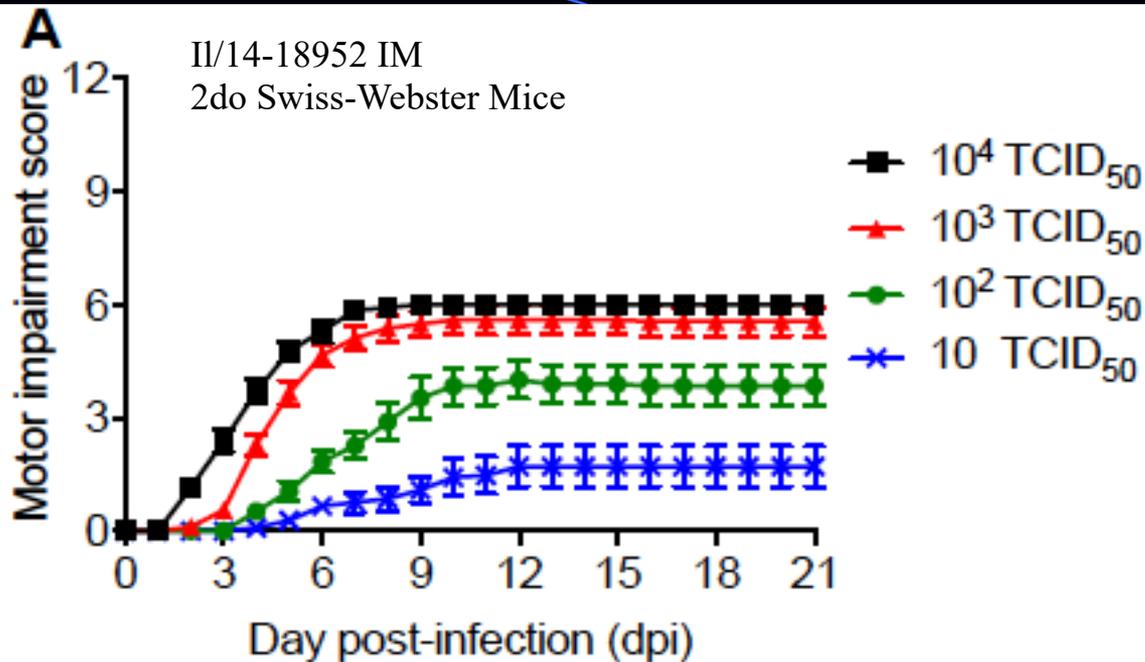
Kenneth L. Tyler, M.D.  
Louise Baum Endowed Chair of Neurology  
Professor of Medicine & Immunology-Microbiology  
University Colorado School of Medicine  
Ken.Tyler@cuanschutz.edu

R01 NS101208, F30 to Alison Hixon, (MD)/PhD, K23 to Kevin Messacar, M.D.

\*ZabBio, San Diego, CA (SBIR, Mabs)  
Anti-plasmin technologies LLC, Dallas TX (tranexamic acid)  
Collaborations Pharmaceuticals, Inc, Raleigh NC (pleconaril derivatives)

Neonatal Swiss-Webster mice  
IC Inoculation





**C**

Titer EV-D68 injected (TCID <sub>50</sub> )	Survivors: Onset of motor deficits (dpi range)	Survivors: Avg motor score ± SEM (dpi 21)	Non-survivors: Mortality (%)	Non-survivors: Avg motor score ± SEM
10 <sup>4</sup>	2-3	6.0 ± 0.0	33	9.4 ± 0.4
10 <sup>3</sup>	2-5	5.6 ± 0.4	18	9.2 ± 0.3
10 <sup>2</sup>	3-7	3.8 ± 0.5	5	11.0 ± 0
10	4-9	1.7 ± 0.5	0	N/A

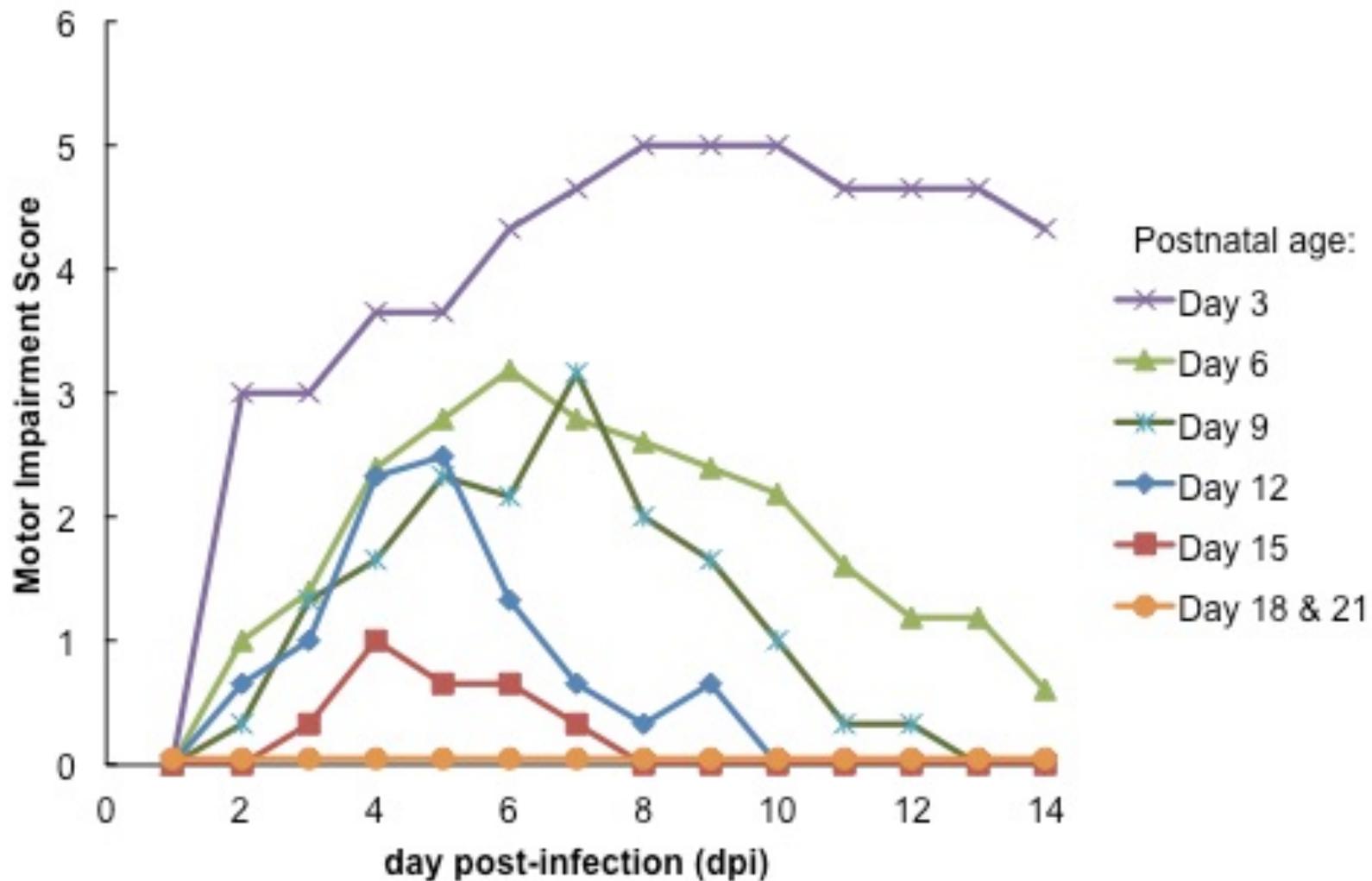
MO/14-18947  
% Paralysis  
Swiss-Webster

IM 100% (18/18)  
IC 52% (27/52)  
IP 5% (1/22)  
IN 3% (2/73)

Mouse Susceptibility:  
High: ICR, BALB/C  
Med: C57/BL6  
Low: NIH(s), KM

Sun S, et al.  
Antiviral Res.  
161:108-15, 2019

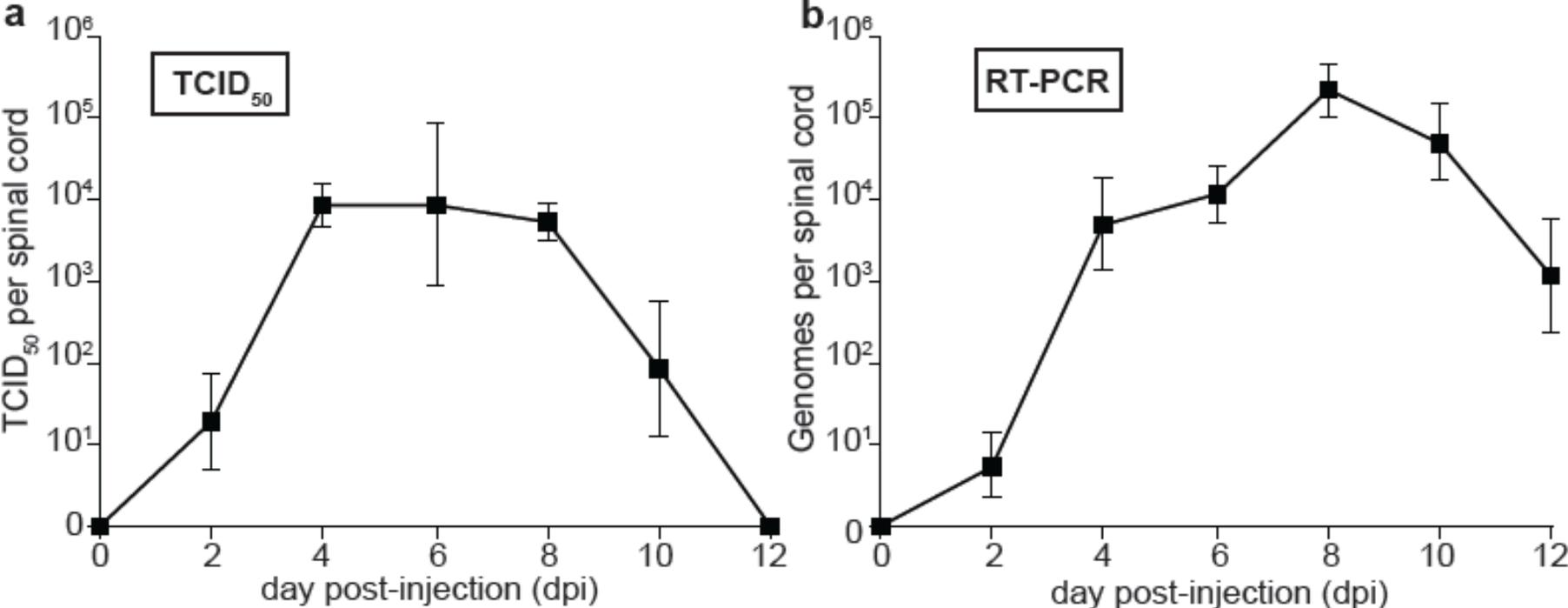
## Response to IM EV-D68 ( $10^5$ TCID<sub>50</sub>) Infection by Age





D12: MO/14-18947, Bilat. Hindlimb Paralysis

**Figure 2: EV-D68 spinal cord titer by TCID<sub>50</sub> and RT-PCR corresponds to paralysis onset and recovery.**



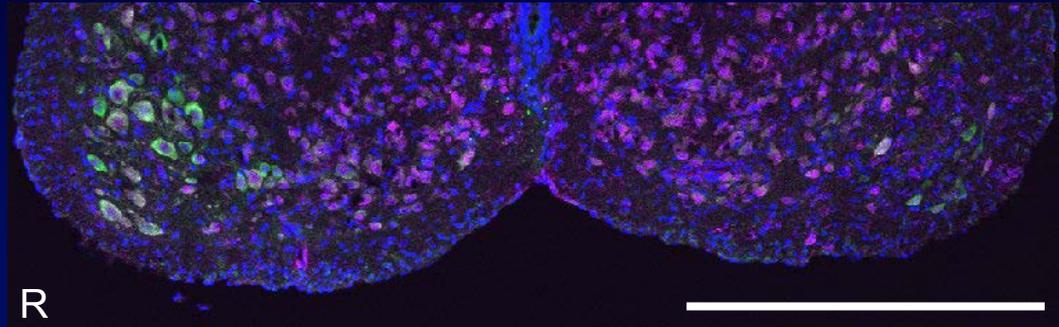
MO/14-18947, IM dpi 3



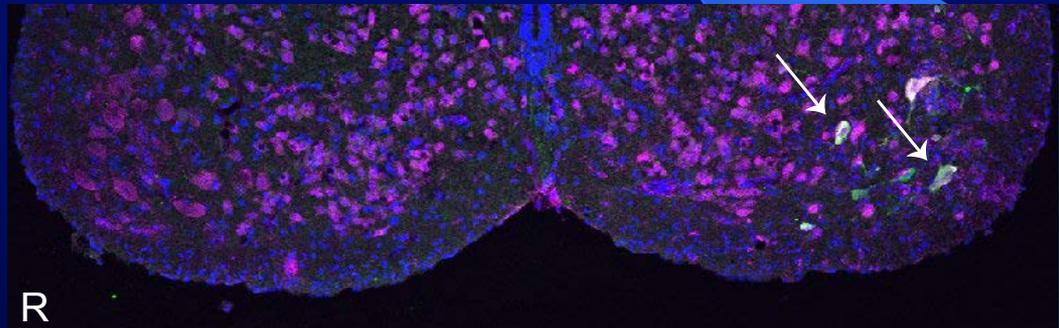
**C** intramuscular injection: dpi 3



**D** intramuscular injection: dpi 3 ChAT, NeuN, Nuclei



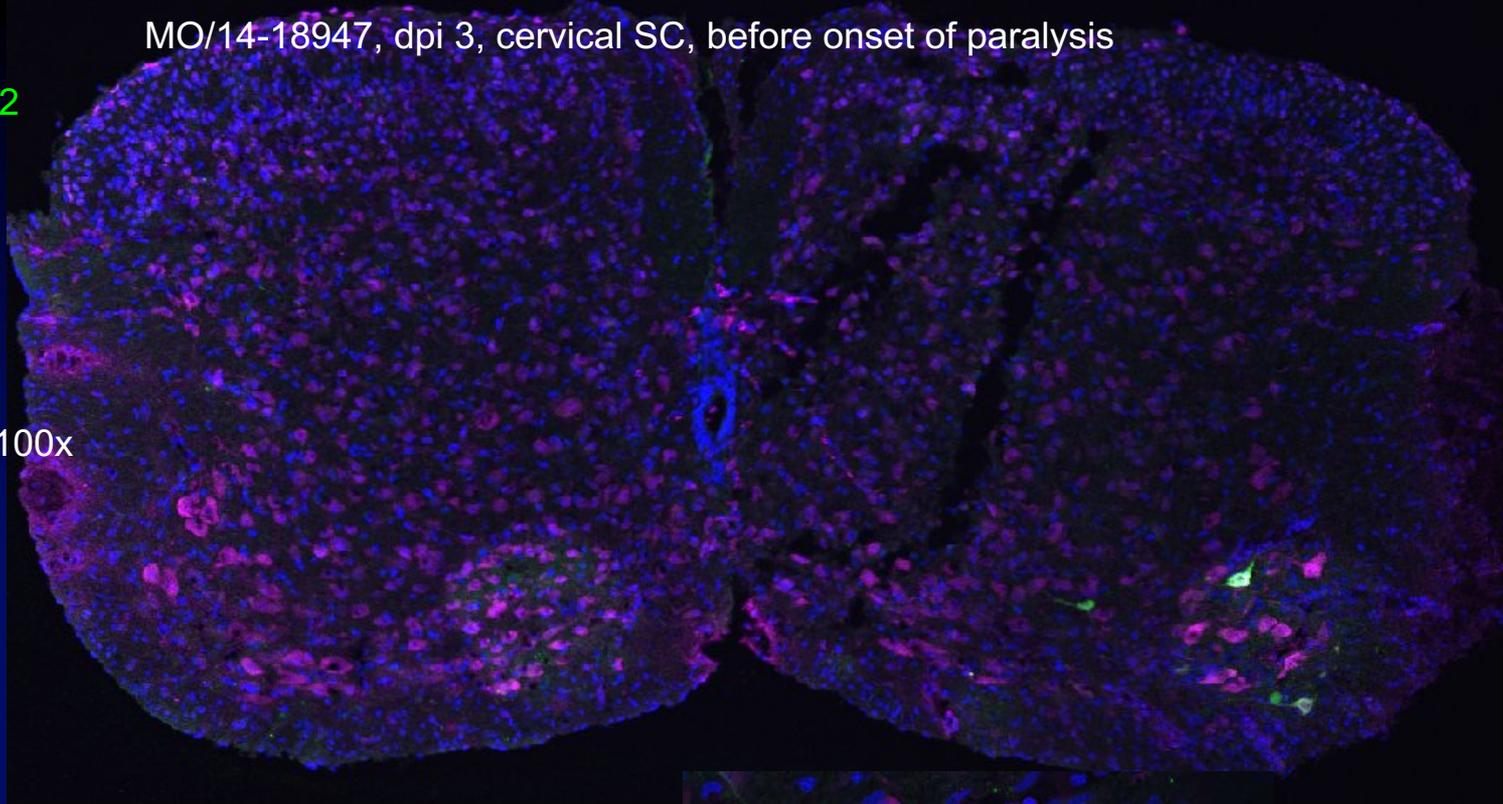
intramuscular injection: dpi 3 EV-D68 VP2, NeuN, Nuclei



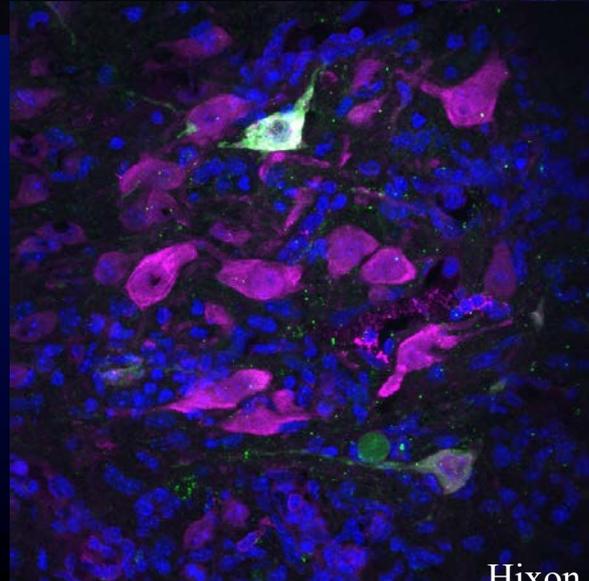
MO/14-18947, dpi 3, cervical SC, before onset of paralysis

NeuN,  
EV-D68 VP2

100x

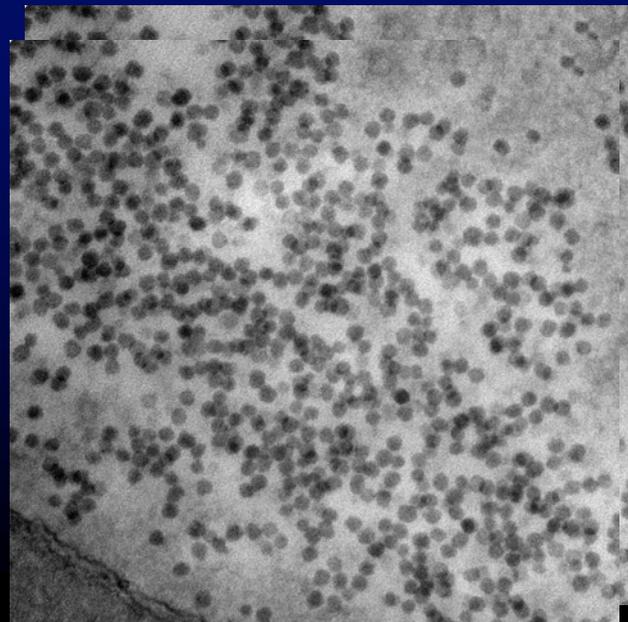
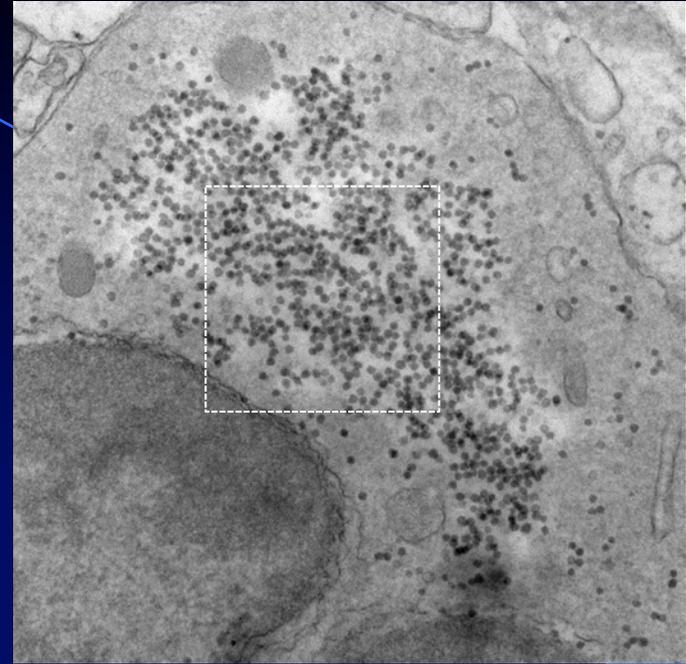
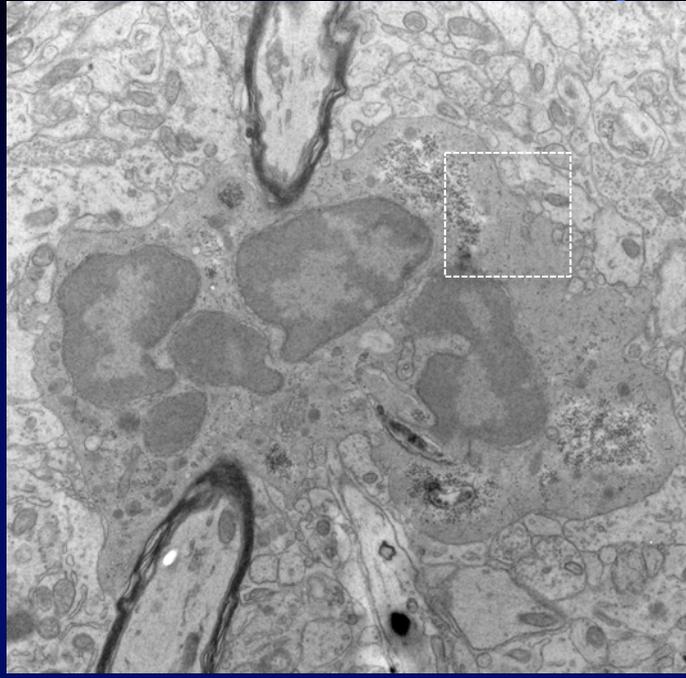


600x

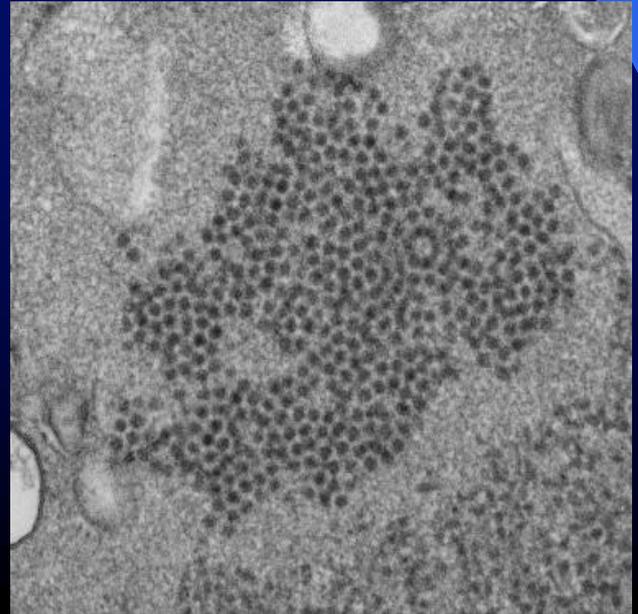


49,000x

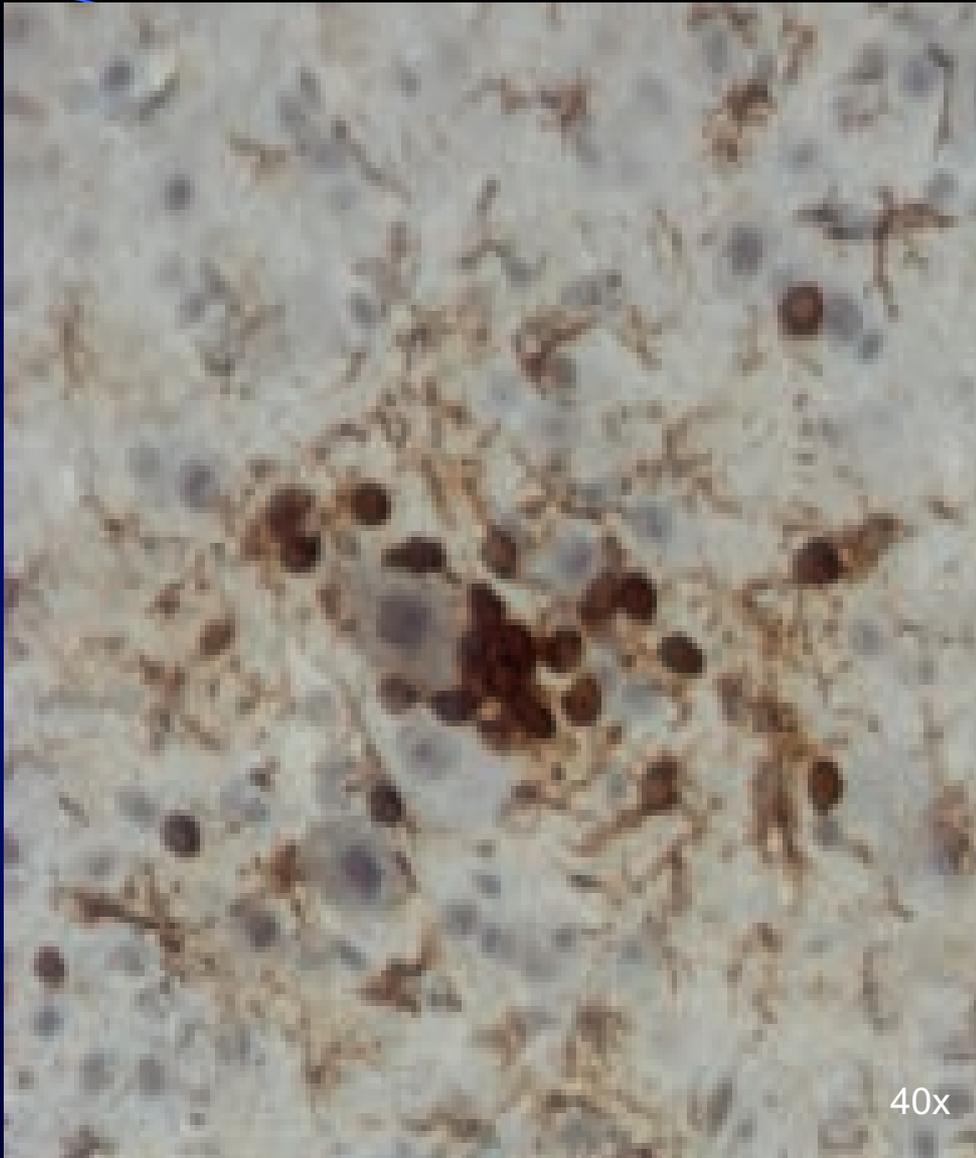
11,000x



98,000x

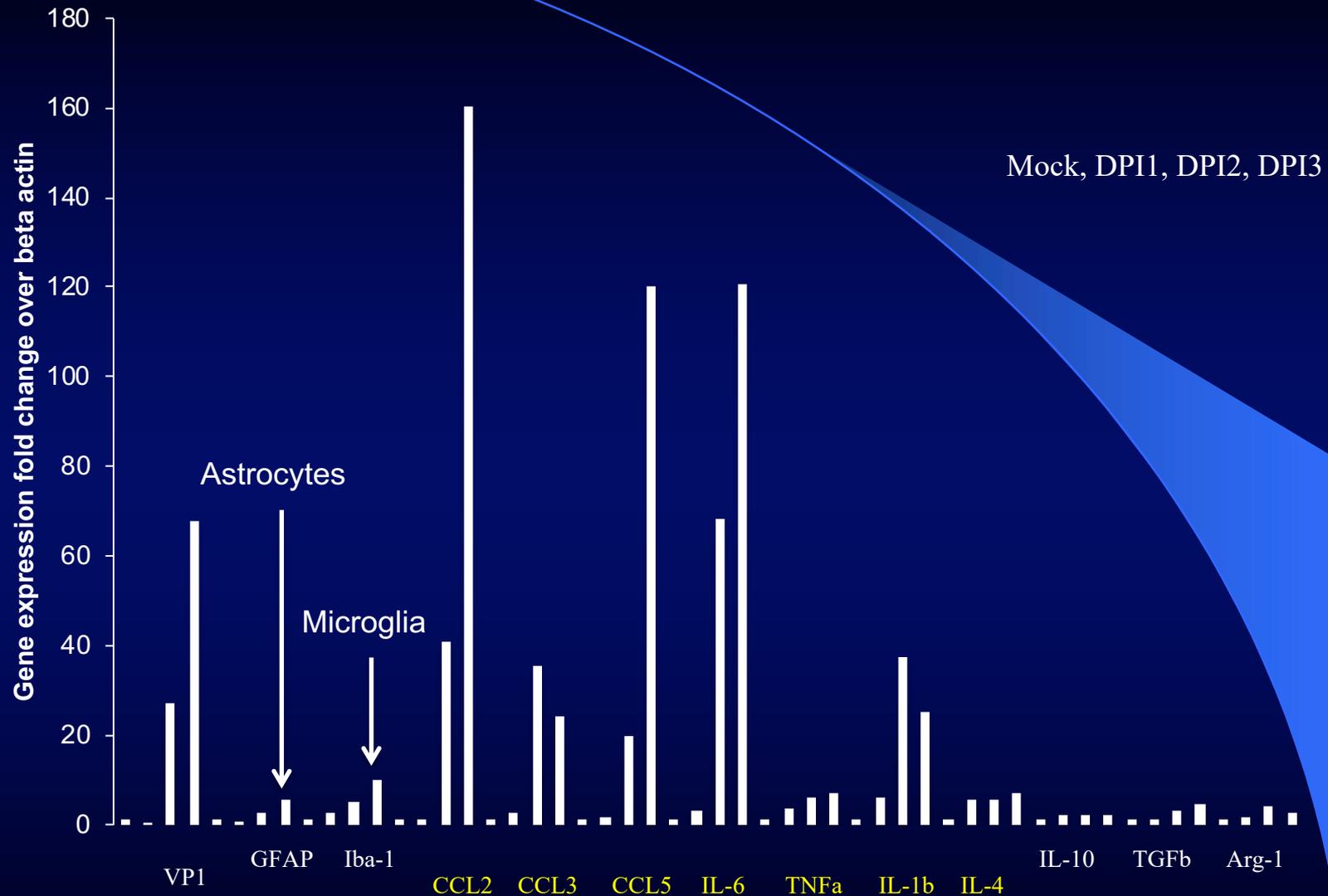


EVD68  
CDC PHIL  
ID#18258

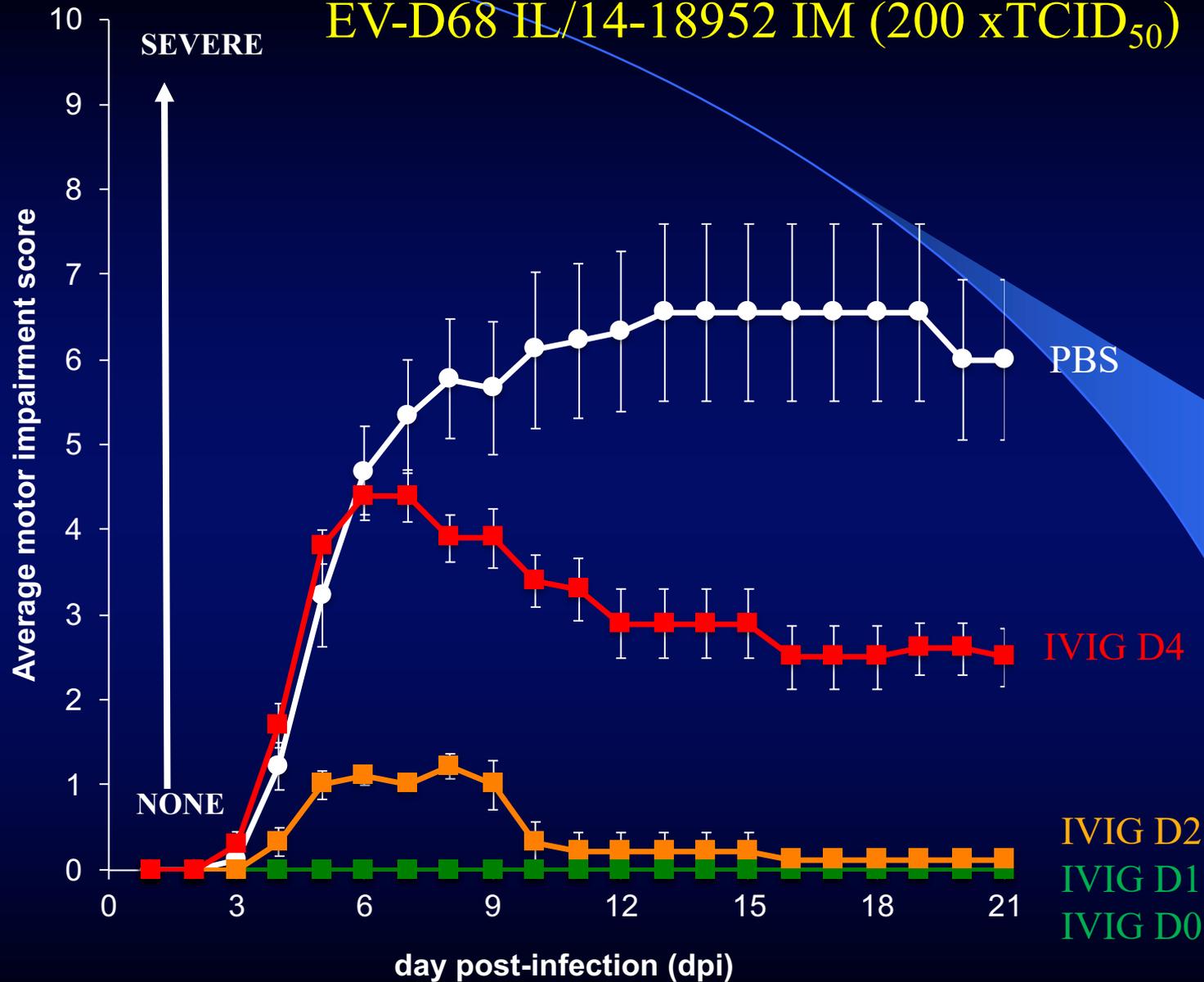


MO-14-18947, d12, CD3+ staining, anterior horn  
Hixon, Tyler (unpub.)

# RT-PCR for inflammatory markers, 7 pooled lumbar SC sections Mo/14-18947 IM



# IVIG Rx Reduces Motor Impairment : EV-D68 IL/14-18952 IM (200 xTCID<sub>50</sub>)

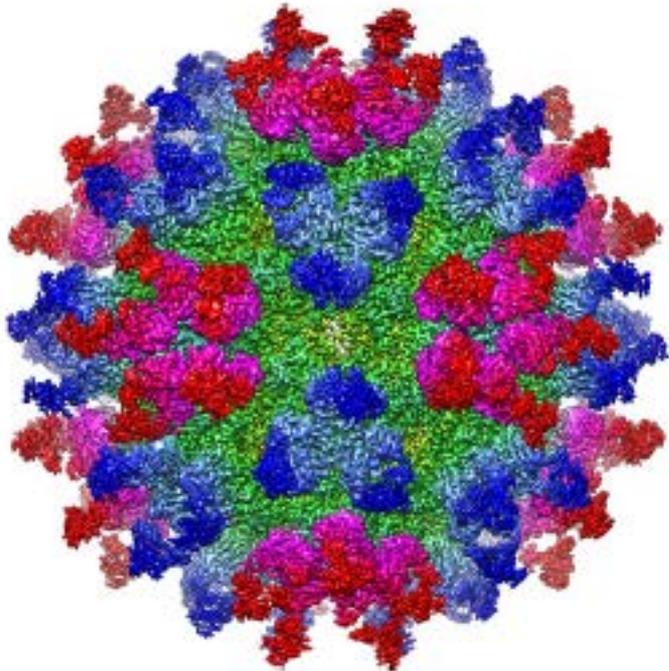
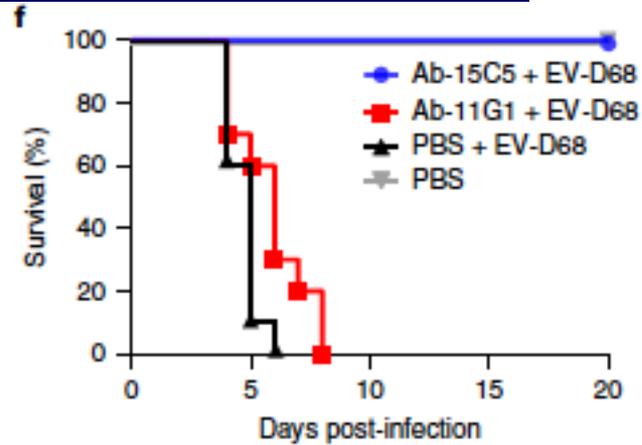
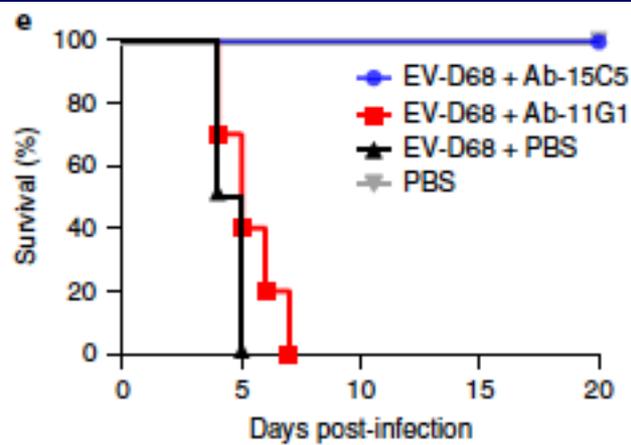
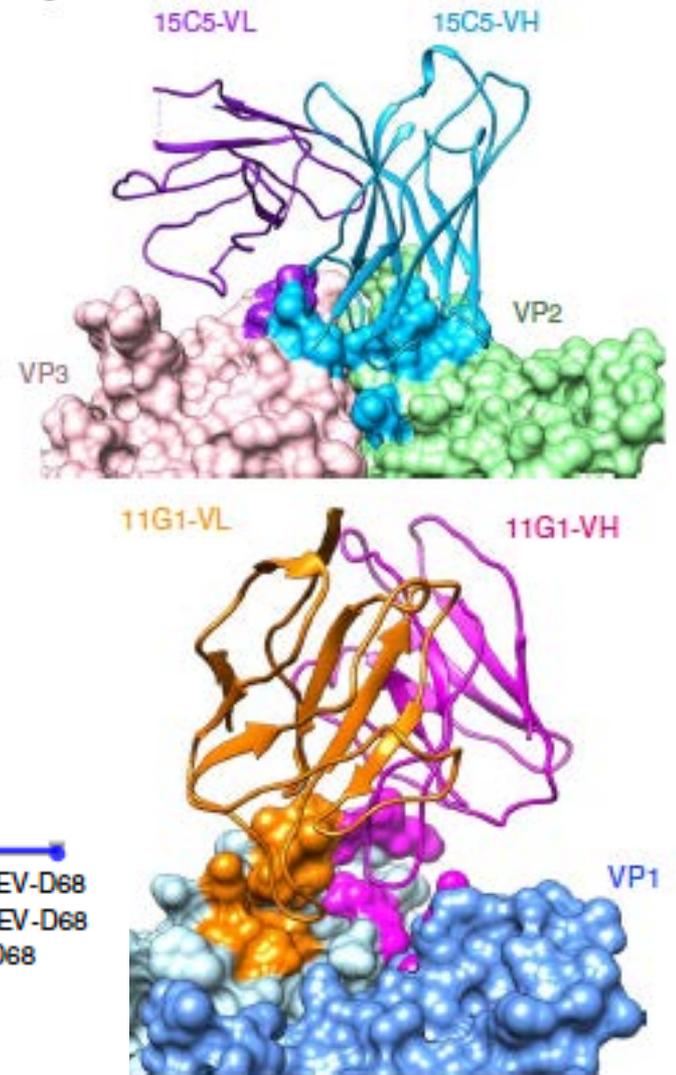


9-10 mice per group

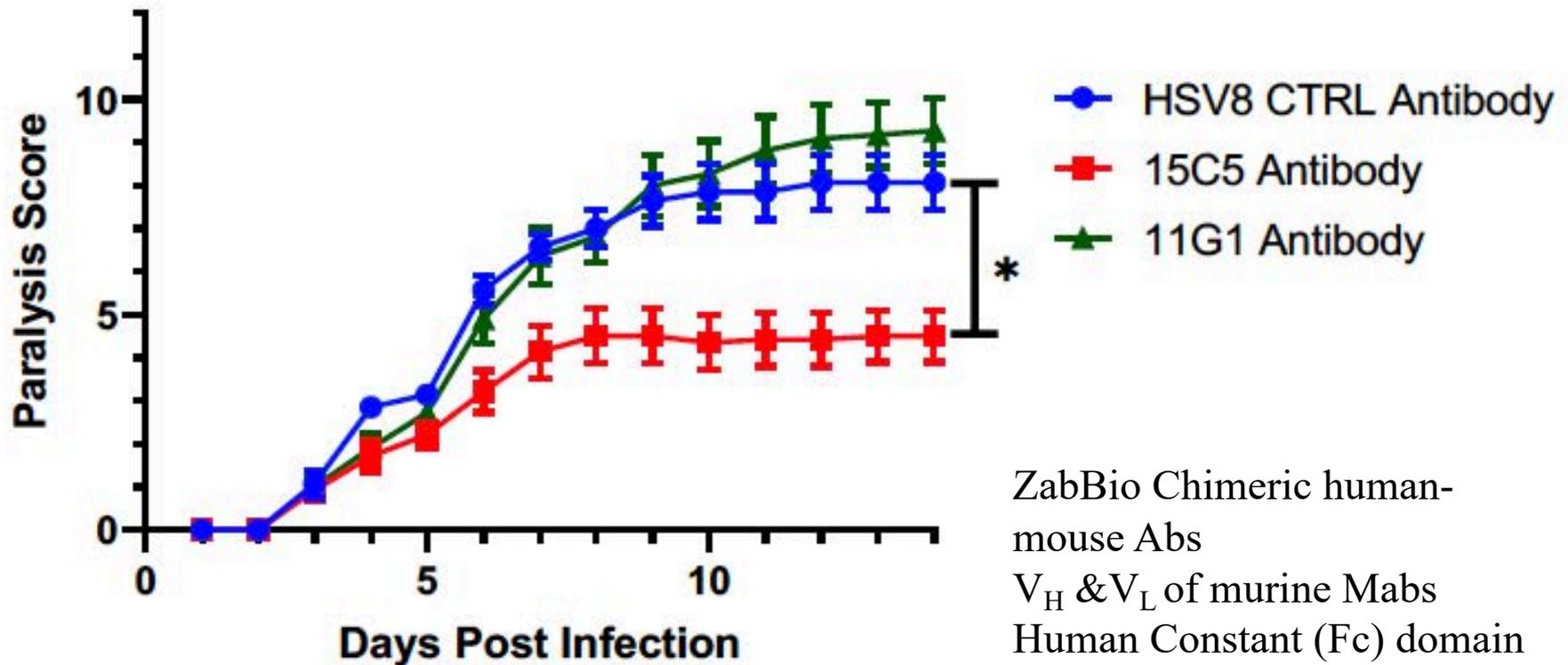
Hixon et al. JID 216:1245, 2017

Error bars are s.e.m



**a****c**

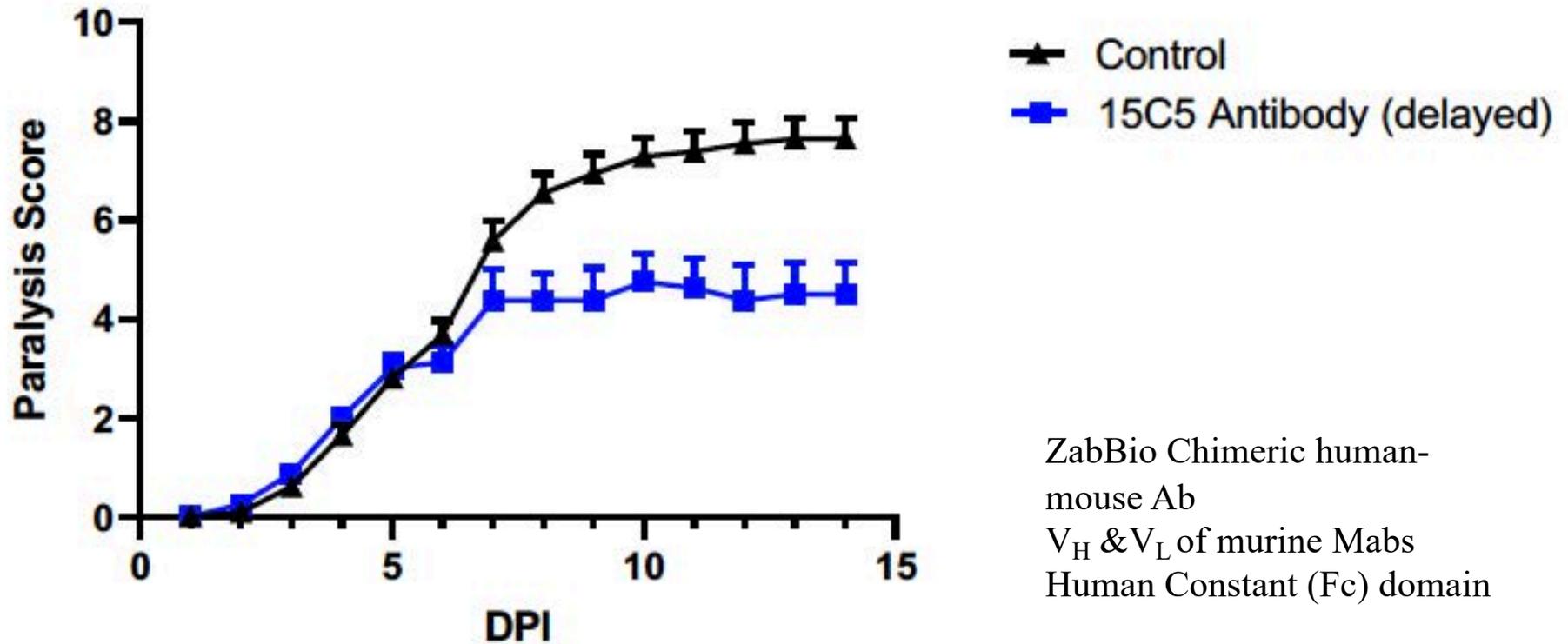
## In Vivo Antibody Treatment



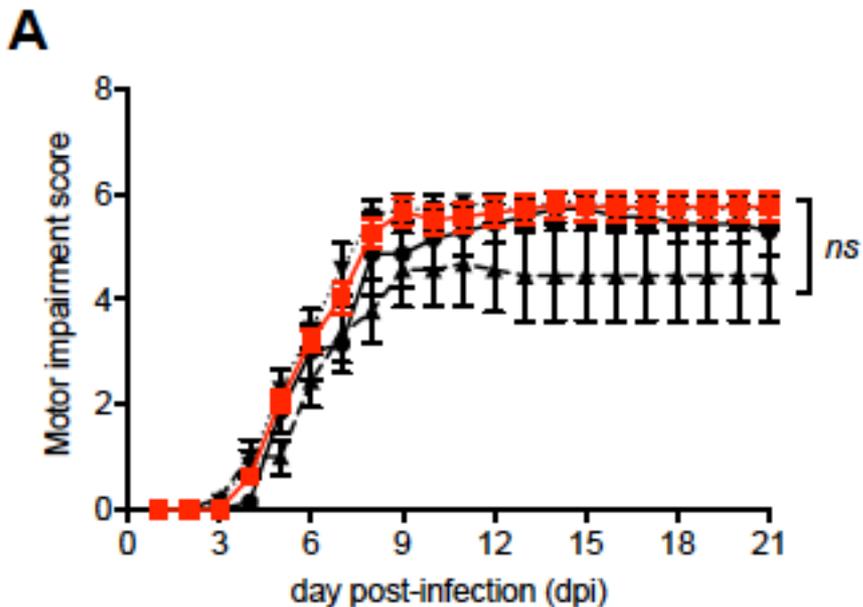
EV-D68 IL/14-18952, 1000 TCID<sub>50</sub> IM, ~100 ug MAb IP on dpi +1

Frost & Rudy, 2020, unpub.,

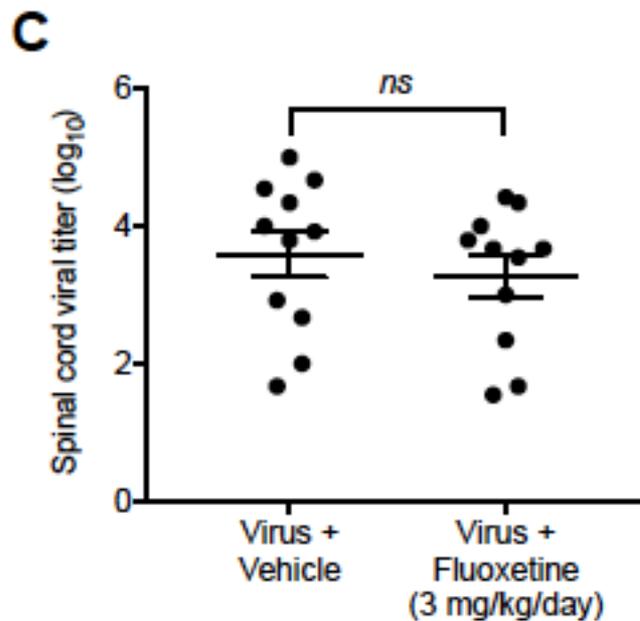
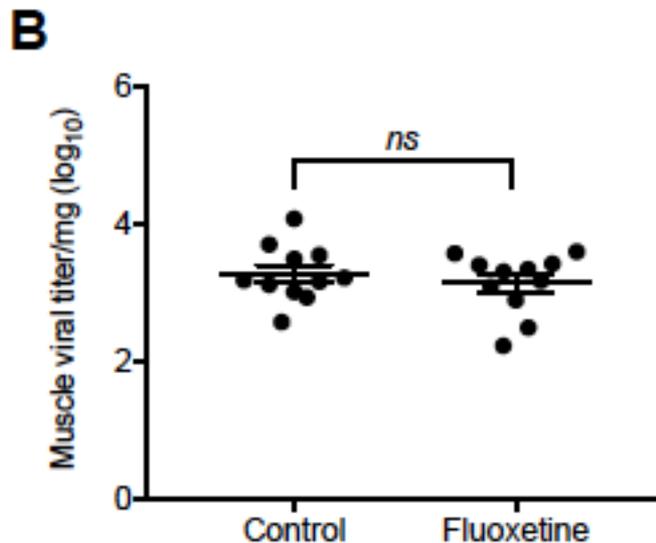
## Antibody Paralysis Scores



EV-D68 IL/14-18952, 1000 TCID<sub>50</sub> IM, ~100 ug MAb IP on day paralysis score  $\geq 2$



■ Virus + Vehicle      ▲ Virus + Fluoxetine - 1.5 mg/kg  
 ● Virus + Fluoxetine - 0.75 mg/kg      ▼ Virus + Fluoxetine - 3.0 mg/kg



IL/14-18952 IM ( $10^3$  TCID<sub>50</sub>)  
 100 ul of 0.1 mg/ml stock  
 QD x6D IP, tissue coll. D6 pi

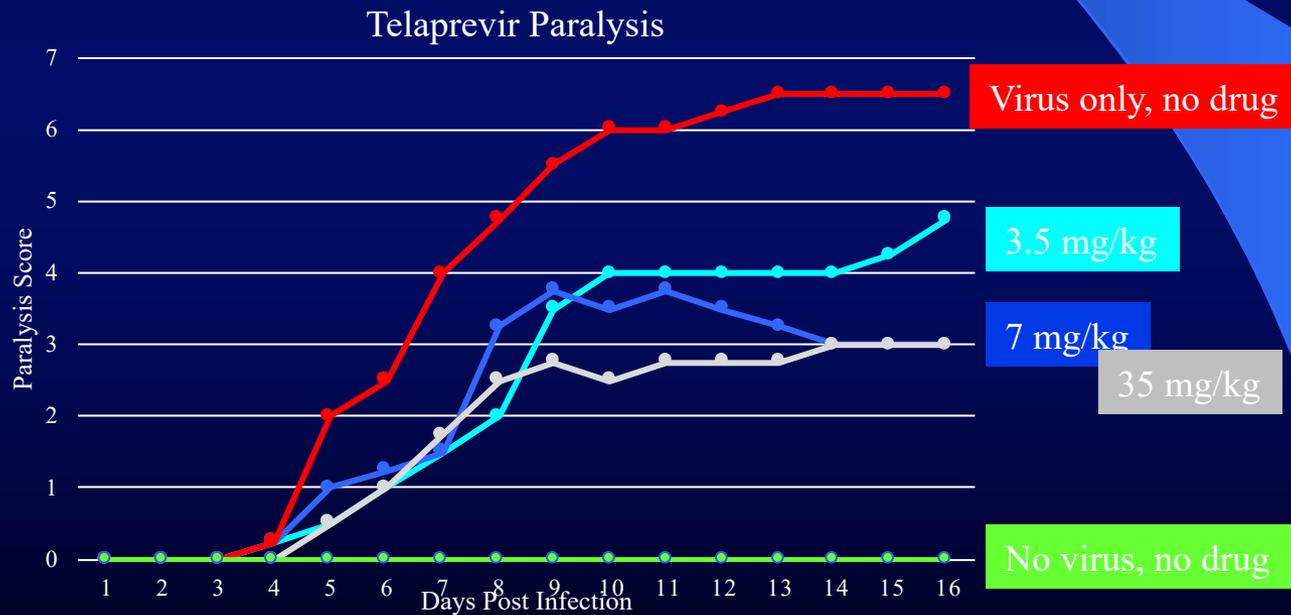


## Validating Enterovirus D68-2A<sup>pro</sup> as an Antiviral Drug Target and the Discovery of Telaprevir as a Potent D68-2A<sup>pro</sup> Inhibitor

Rami Musharrafieh,<sup>a,b</sup> Chunlong Ma,<sup>a</sup> Jiantao Zhang,<sup>a</sup> Yanmei Hu,<sup>a</sup> Jessica M. Diesing,<sup>b</sup> Michael T. Marty,<sup>b</sup> Jun Wang<sup>a</sup>

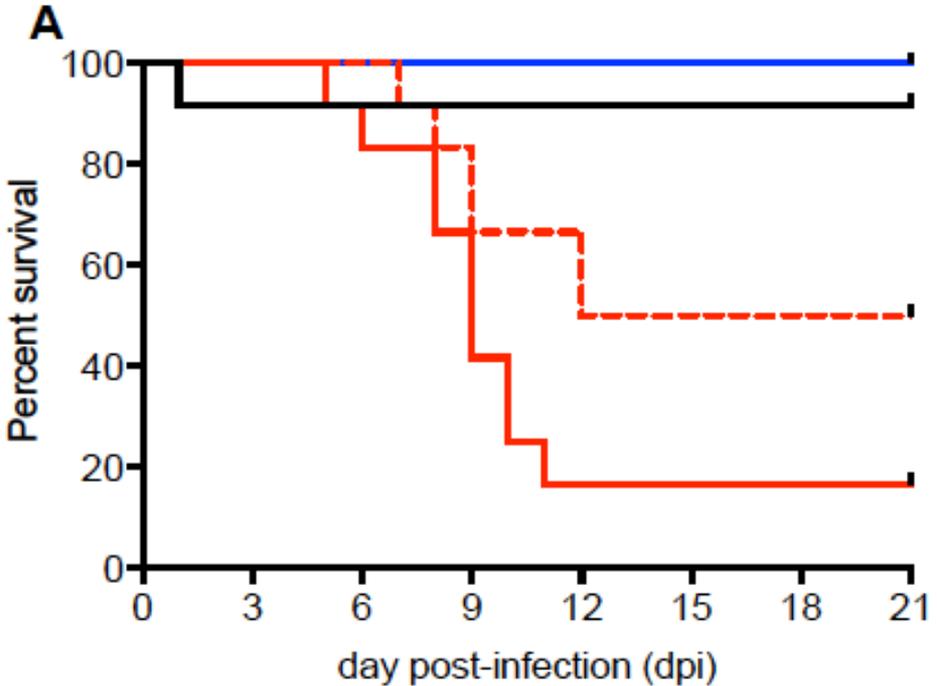
<sup>a</sup>Department of Pharmacology and Toxicology, College of Pharmacy, The University of Arizona, Tucson, Arizona, USA

<sup>b</sup>Department of Chemistry and Biochemistry, The University of Arizona, Tucson, Arizona, USA

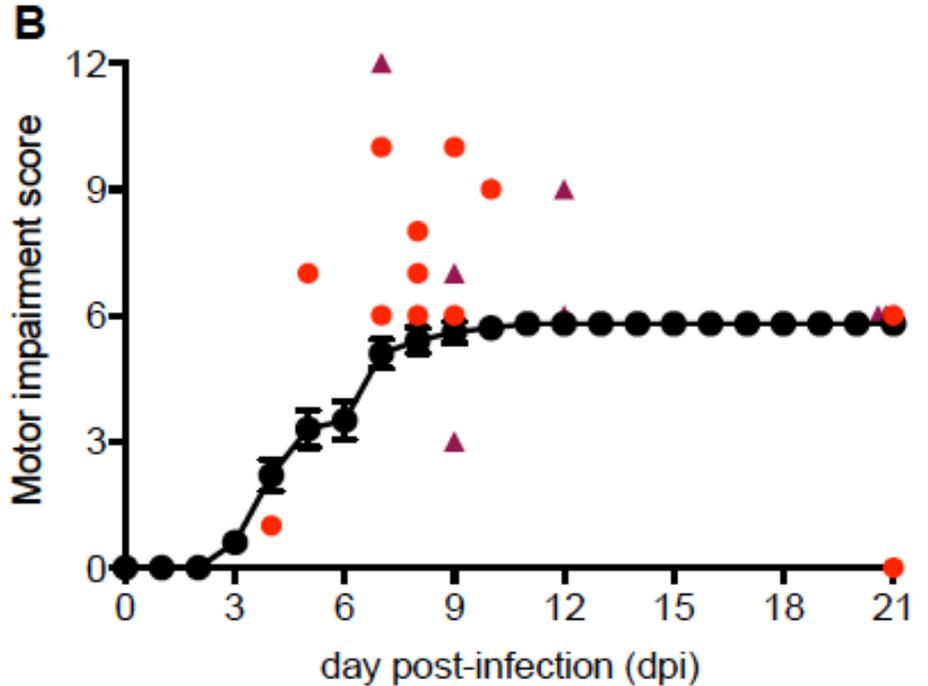


IL52 IM, 1000 TCID<sub>50</sub> SW Mice, IP drug d=0 then daily, 4-5 per group for paralysis

Frost J, & Ruddy M, unpub.

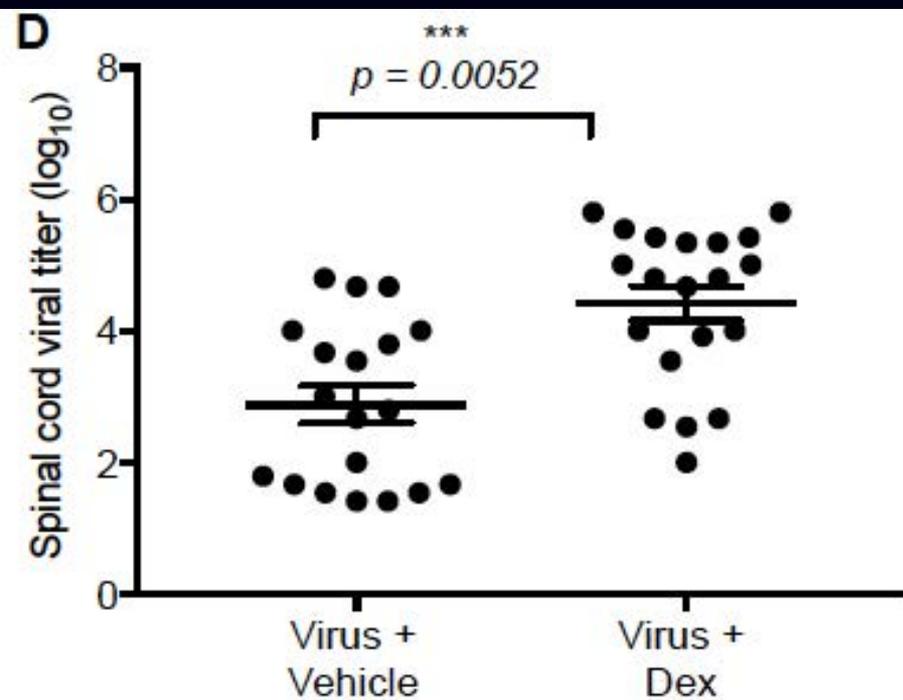
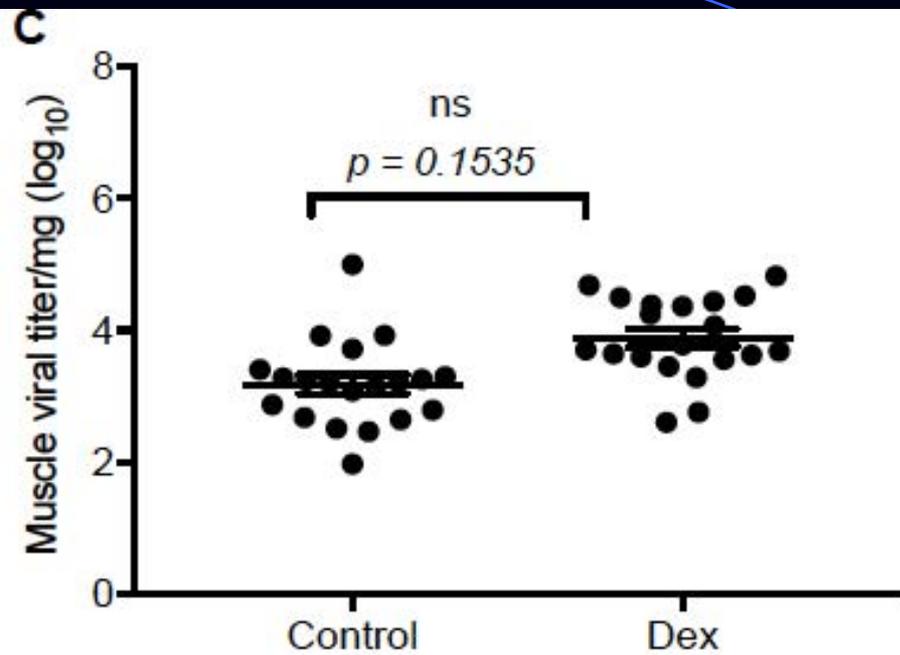


- Virus + Vehicle
- Virus + Dex (early) D3 + D4 pi
- - - Virus + Dex (late) D6 +D7 pi
- Dex only



- Virus + Vehicle
- Virus + Dex (early)
- ▲ Virus + Dex (late)

IL/14-18952 IM ( $10^3$  TCID<sub>50</sub>)  
 Dexamethasone ~1.8-3.7mg/kg  
 100ul of 0.1 mg/ml stock IP



IL/14-18952 IM,  $10^3$  TCID<sub>50</sub>  
 Dexamathasone ~1.8-3.7 mg/kg  
 (100 ul of 0.1 mg/ml stock IP)