

# Looking at pathogenesis in AFM: The virus and the immune system

19JUN2020

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**VIPR**  
Virus Pathogen Resource

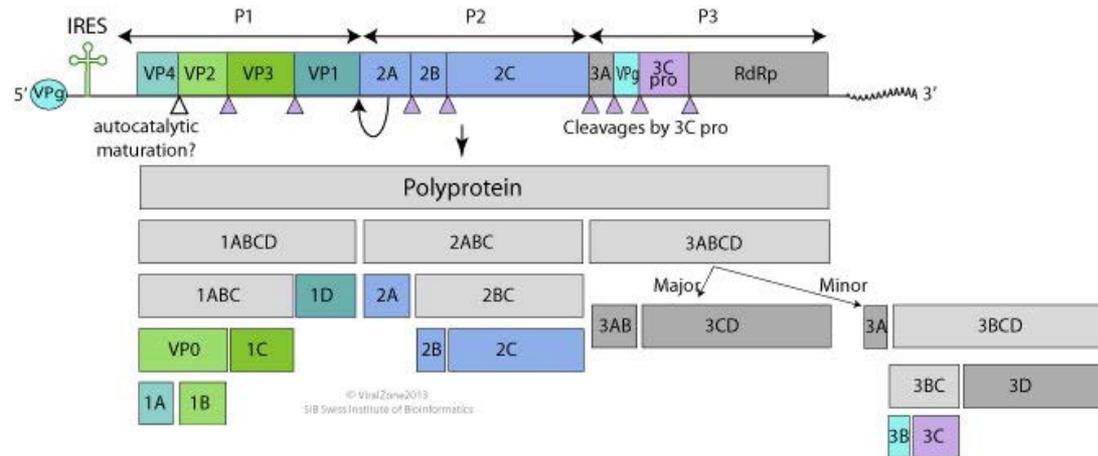
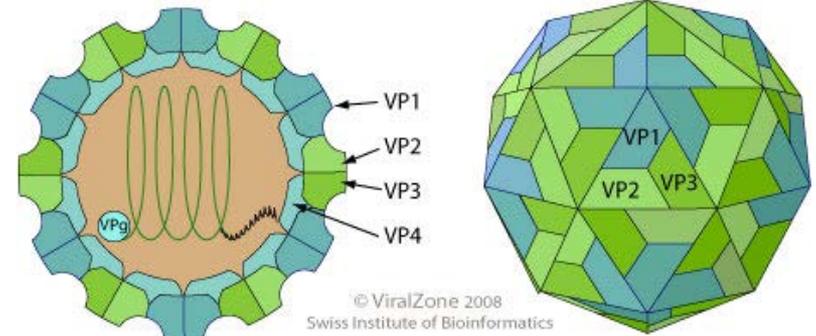


Influenza Research Database

**J. Craig Venter**<sup>®</sup>  
I N S T I T U T E

# Enterovirus D68

- Member of the *Picornaviridae* family of non-enveloped +ssRNA
- Polyprotein cleaved into mature structural and non-structural proteins by viral protease
- Typically causes mild respiratory infections
- 2014 outbreak of EV-D68 coincided with clusters of acute flaccid myelitis suggesting a causal link
- Similar outbreaks in 2016 & 2018
- Do different EV-D68 lineages exhibit different neurovirulence or neurotropism characteristics that could contribute to AFM development?



# Objectives

- Use EV-D68 genome sequence and other data to
  - determine if the virus is evolving due to diversifying (positive) selection
  - determine if the driver of diversifying selection is evasion of adaptive immunity
  - determine if there is a relationship between the drivers of virus evolution and changes in virulence characteristics

# Virus Pathogen Resource (ViPR) data summary



**Enterovirus** [www.viprbrc.org](http://www.viprbrc.org) **VI PR**

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SEARCH DATA ANALYZE & VISUALIZE WORKBENCH VIRUS FAMILIES HELP [rscheuermann@jcv.i.org](mailto:rscheuermann@jcv.i.org)

## Enterovirus

**Virion:** non-enveloped, spherical with a diameter of about 30 nm

**Genome:** positive-sense, single-stranded RNA genome of 7.2-8.5 kb, composed of a long and highly structured 5'-noncoding region and a single ORF

**Proteome:** 11 mature peptides

**Infection:** initiates by attaching to a cellular receptor, for example, CD155 as a poliovirus receptor

**RNA Transcript:** 5'-noncoding region linked to a viral protein (VPg), 3' poly-A tail

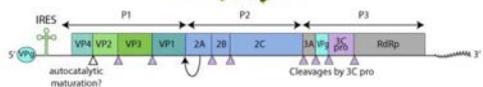
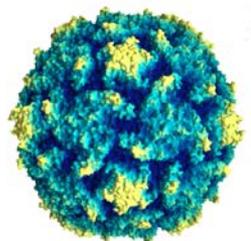
**Transmission:** fecal-oral and respiratory transmission

**Phylogeny:** 12 distinct virus species identified, including Enterovirus A-J and Rhinovirus A-C

**Epidemiology:** worldwide; 5-10 million symptomatic infections per year in the US

**Clinical:** causes a spectrum of distinct syndromes. PV: poliomyelitis; rhinovirus: common cold; CV: aseptic meningitis and encephalitis, paralysis, hand-foot-and-mouth disease (A10, A16), upper respiratory illness, hepatitis, acute hemorrhagic conjunctivitis (CVA24), cardiac disease (CVB); EV-D68: lower respiratory illness; EV-D70: acute hemorrhagic conjunctivitis, paralysis; EV-A71: hand-foot-and-mouth disease, meningoencephalitis, paralysis

**Reference:** David M. Knipe, Peter M. Howley, et al. *Fields' Virology*. 5th ed. 2007. Lippincott Williams & Wilkins, USA



**Structure:** Poliovirus 1 Mahoney, PMID 2994218, PDB 2PLV, rendered by J.Y. Sgro using GRASP (PMID 1758883)

**Schematics:** ViralZone - Enterovirus

## Data Summary Updated June 9, 2020

Enterovirus Genome Statistics	
Genera	1
Species	500
Strains	87,473
3D Protein Structures (PDB)	312
Experimentally Determined Epitopes (IEDB)	1,447
Genomes with Clinical Metadata (NIAID GSCID, manual curation)	134
Mature Peptides	7,364
Sequence Features with Variant Types	8
Proteins with Predicted Epitopes	198,352
Total Genomes	96,126
Complete Genomes	3,846
Proteins	258,819

### Search

Search our comprehensive database for:

- Sequence & Strains
- Sequence Feature Variant Types
- Immune epitopes
- 3D protein structures
- Host Factor Data
- Antiviral Drugs

[Browse All Search Types](#)

### Analyze

Analyze data online:

- Sequence Alignment
- Phylogenetic Tree
- Sequence Variation (SNP)
- Metadata-driven Sequence Analysis
- Genome Annotator
- BLAST

[Browse All Tools](#)

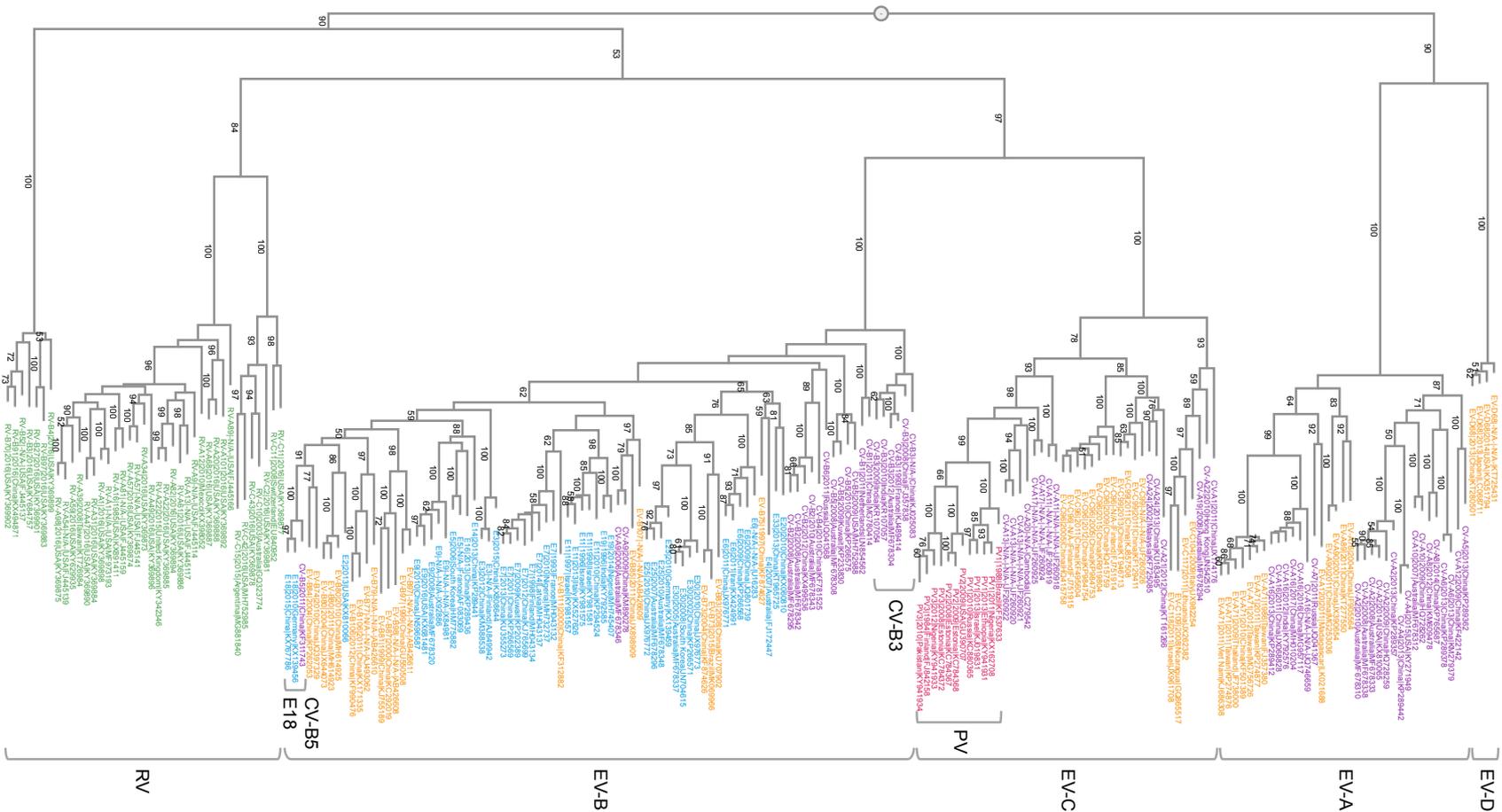
### Save to Workbench

Use your workbench to:

- Store and share data
- Combine working sets
- Integrate your data with ViPR data
- Store and share analyses
- Custom search alert

- EV-D68
  - 4673 genome sequences from 4044 strains, including 554 complete genomes
  - 13,254 protein sequences - 3,685 VP1
  - 14 protein structures – capsid and 3C protease (PDB)
  - 3 B cell/Ab epitopes (IEDB)

# Enterovirus VP1 phylogenetic tree

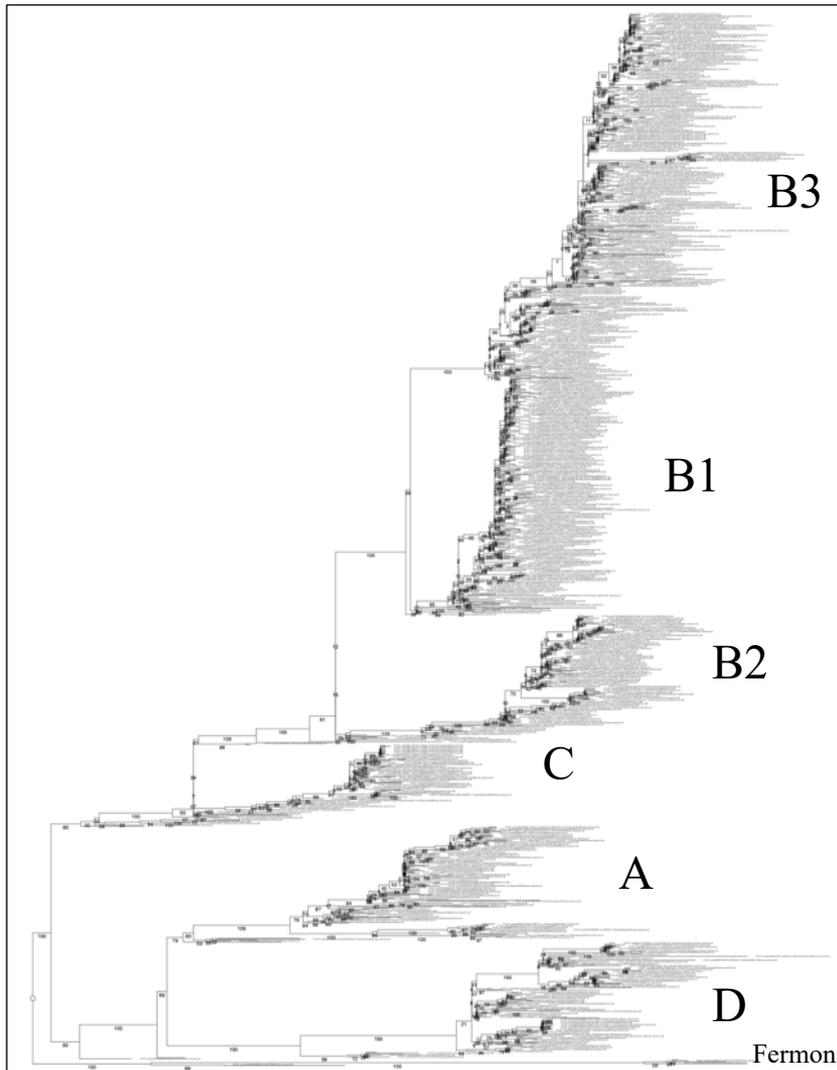


Label Color  
Species Group

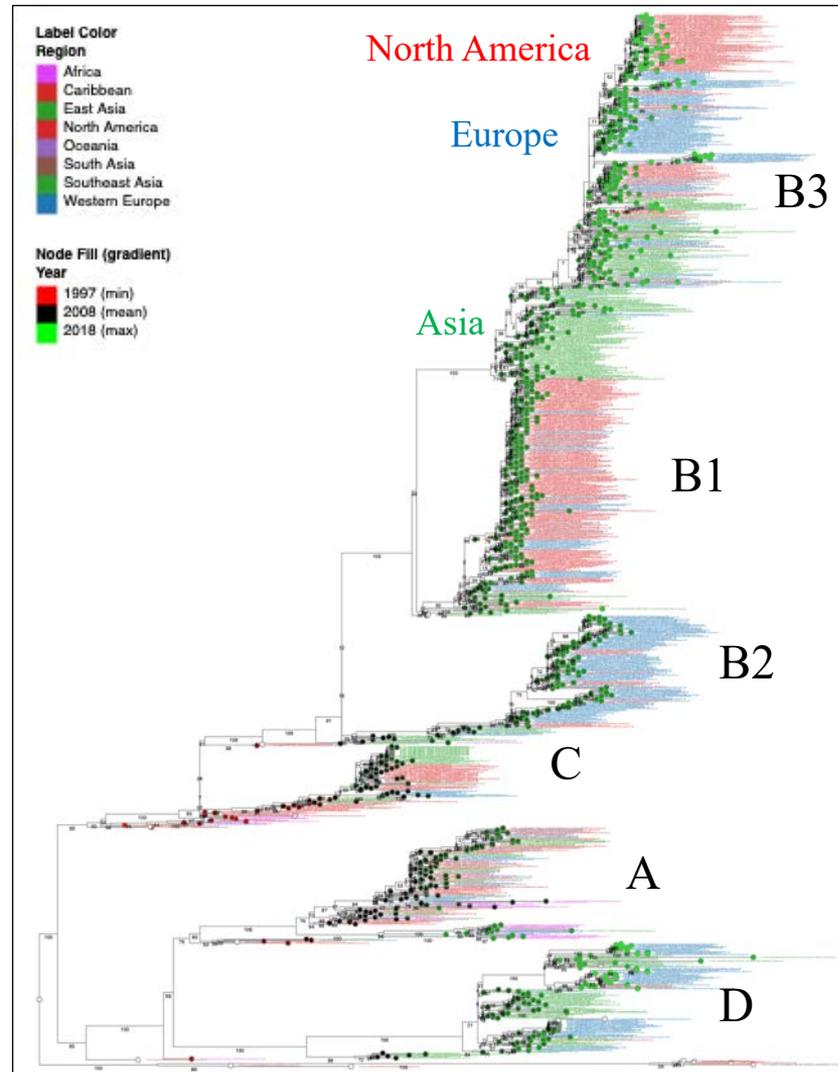
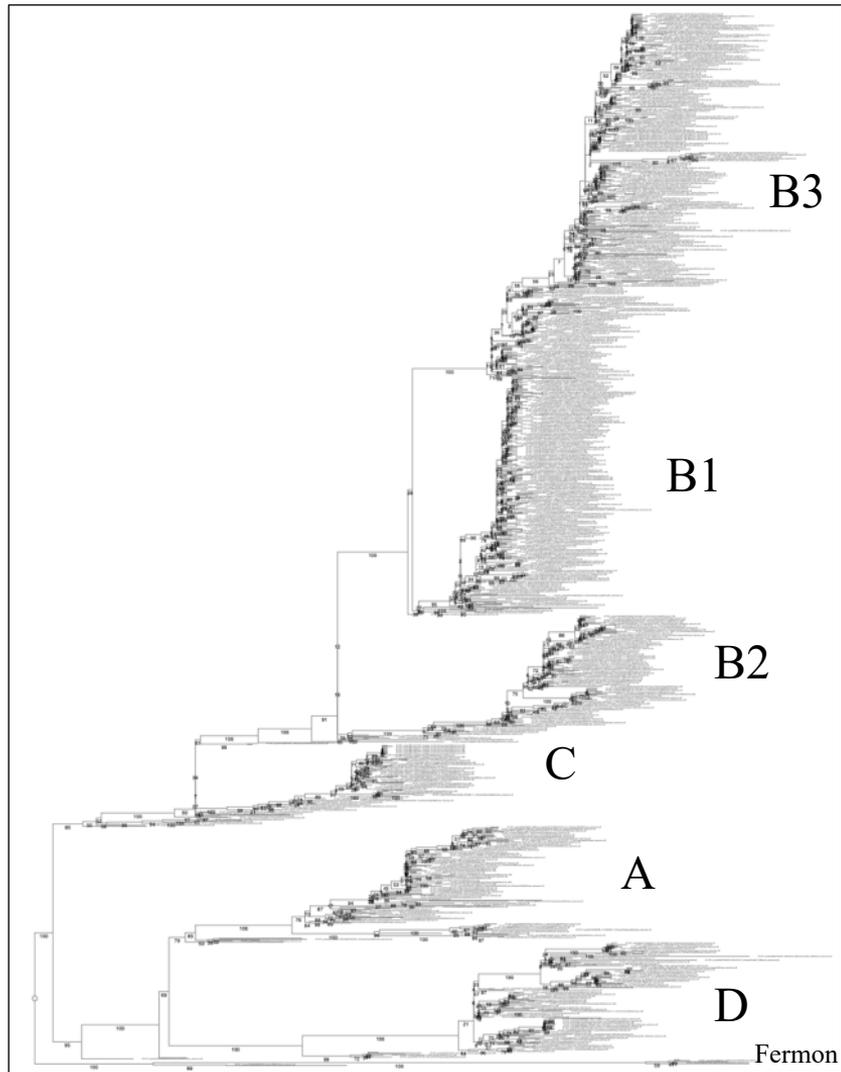
- Coxsackievirus
- Echovirus
- Enterovirus
- Poliovirus
- Rhinovirus

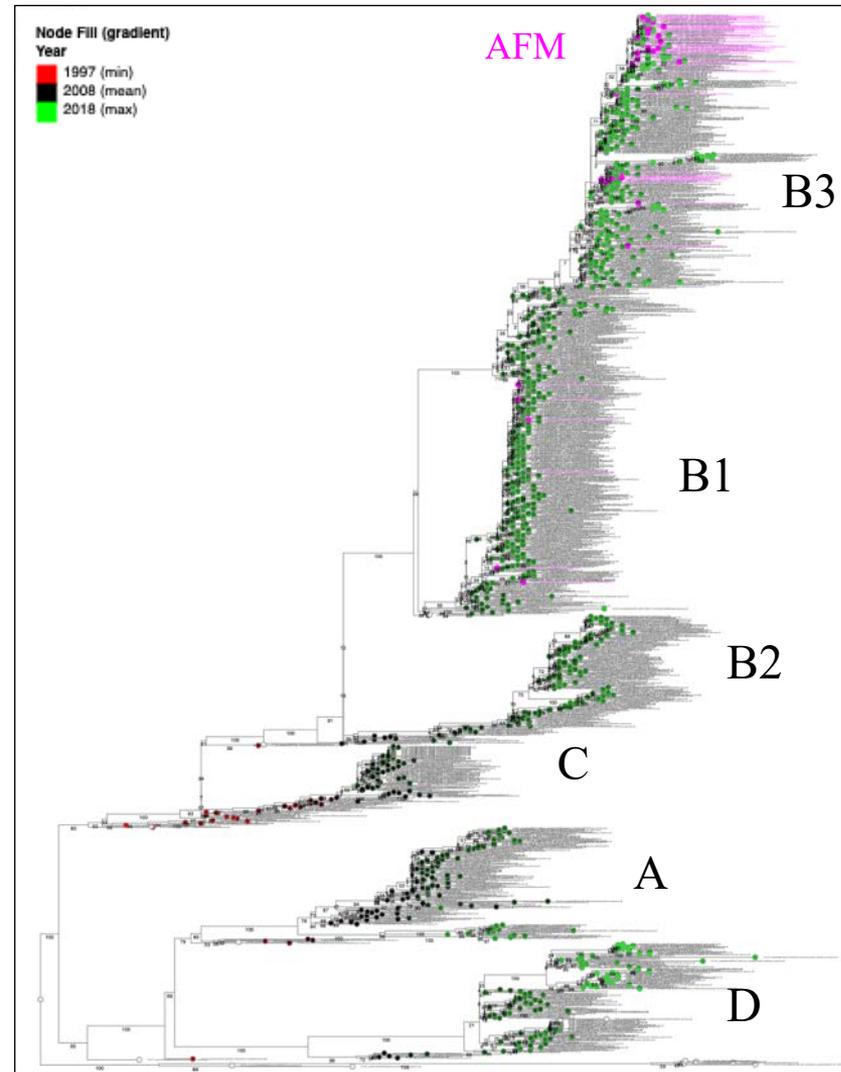
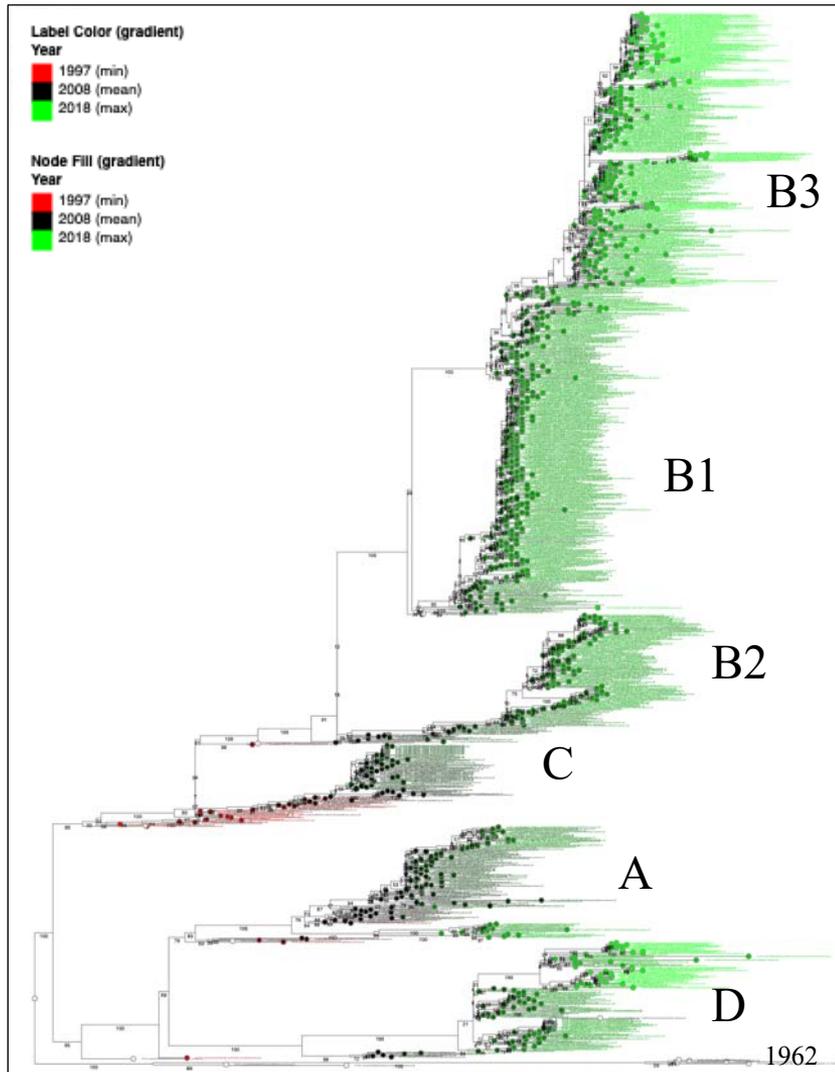
## EV-D68 phylogenetic analysis using the hypervariable VP1 capsid gene

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- 1608 DNA sequences for the EV-D68 VP1 protein with a minimal length of 800 nucleotides from ViPR
- The CD-HIT program was used to remove highly similar and identical sequences
- Multiple sequence alignments were inferred using MAFFT
- Maximum likelihood trees inferred using the RAxML program with the GTR model and rapid bootstrapping algorithm
- *Representation in sequence record should not be interpreted as prevalence!*





2011

B3

B1

- - AFM
- - Year
- - AFM/Year

2012

B3

B1

2013

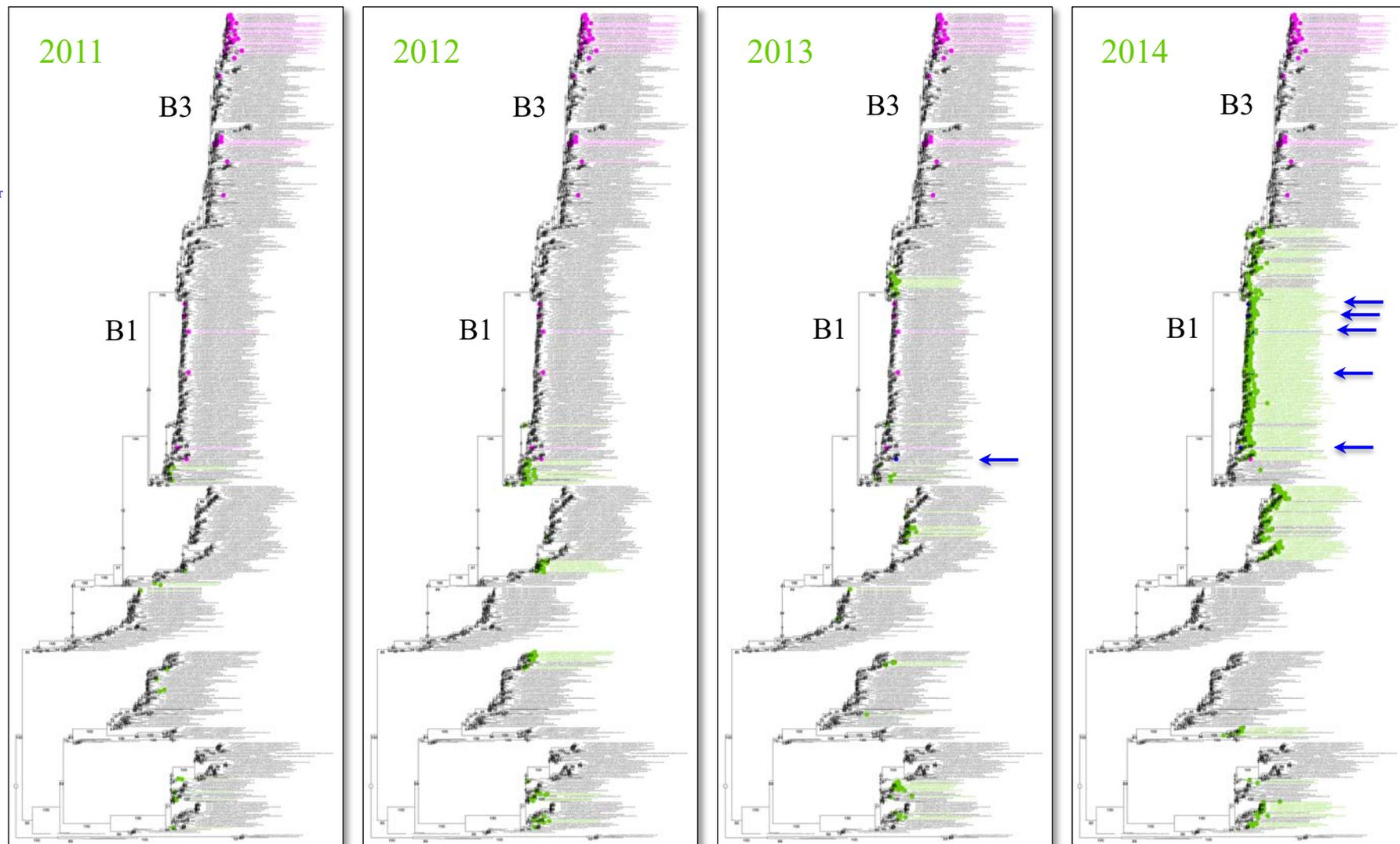
B3

B1

2014

B3

B1



2015

B3

B1

● - AFM  
● - Year  
● - AFM/Year

2016

B3

B1

2017

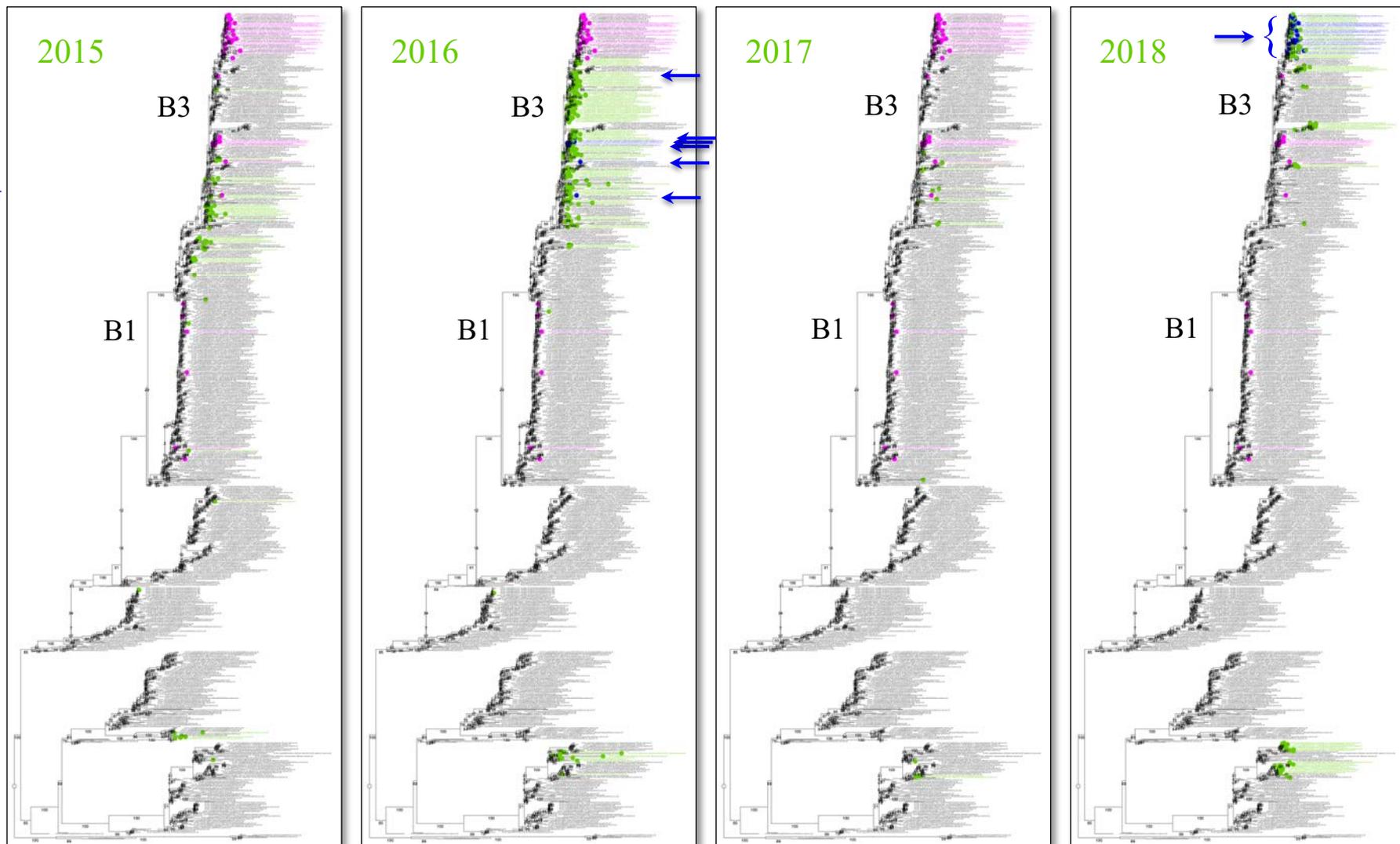
B3

B1

2018

B3

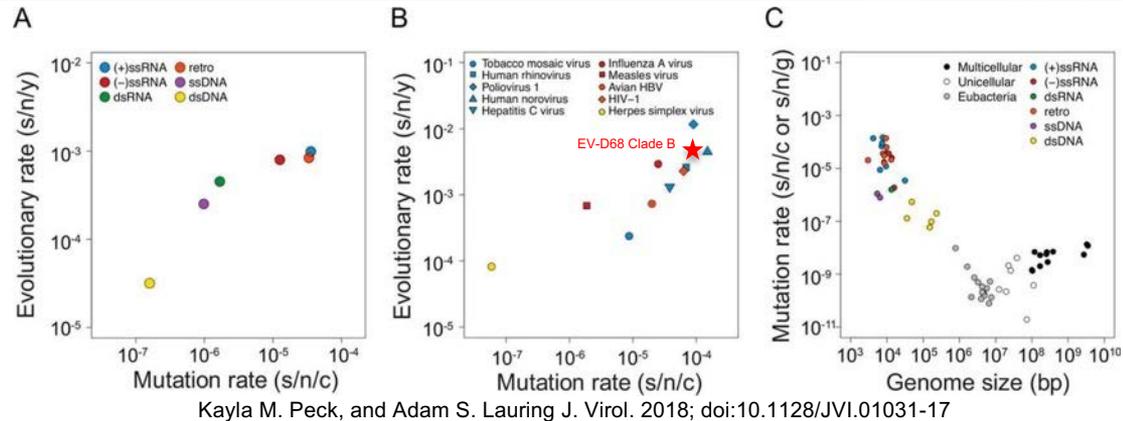
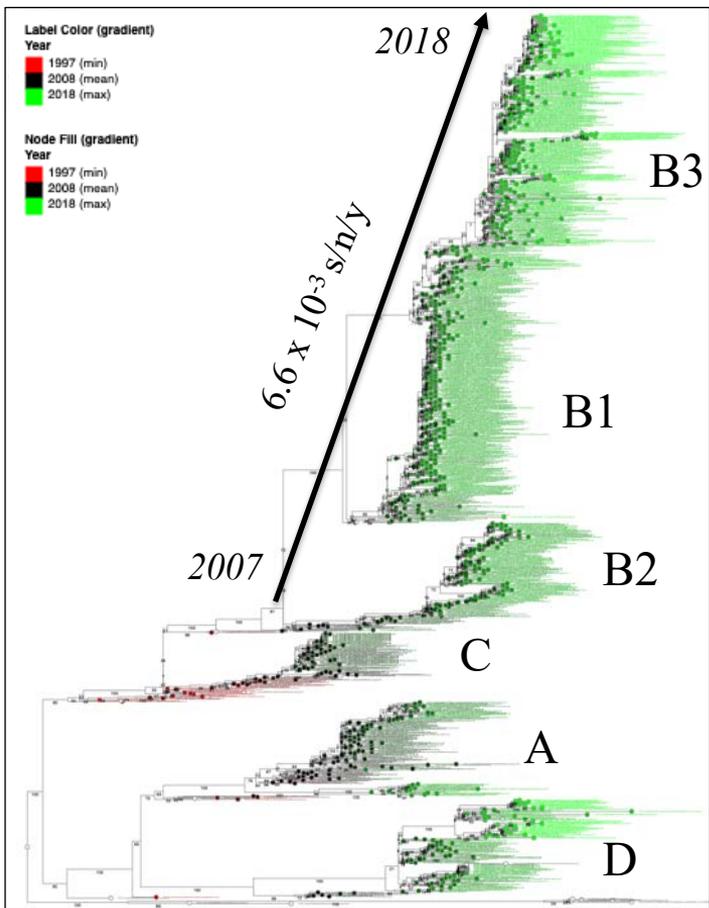
B1



# Evolutionary Drivers $\Leftrightarrow$ Virulence

- Evolution as mutation + selection
  - Evolutionary rate  $\neq$  mutation rate
- Drivers of diversifying (positive) selection
  - Enhance transmissibility (increasing  $R_0$ )
  - Escape from immunity
- Drivers of purifying (negative) selection
  - Important structural amino acid
  - Important functional amino acid
- Increased virulence?

# Evolution of EV-D68 Clade B: 2007-2018



	Substitutions	Aligned length	Percent change
genome (nucs)	530	7285	7.28%
5'UTR (nucs)	25	698	3.58%
proteome (aa)	43	2188	1.97%
2A	3	147	2.04%
2B	0	99	0.00%
2C	6	330	1.82%
3A	0	89	0.00%
3B	0	22	0.00%
3C	4	183	2.19%
3D	8	457	1.75%
VP1	14	309	4.53%
VP2	6	248	2.42%
VP3	1	235	0.43%
VP4	1	69	1.45%

Characteristic	VP1 position	1	2	5	6	90	91	92	93	94	95	96	98	99	143	144	148	168	169	171	207	218	239	240	241	243	280	282	283	290	297	
Clade B 2007-2018 evolved positions	1,5,6,90,95,143,144,148,168,169,207,218,243,280	x		x	x	x					x				x	x	x	x	x		x	x				x	x					
Clade B diverifying sites (FUBAR)	1,2,148,297	x	x														x														x	
Sia-independent differences	169,282,283																		x								x	x				
B cell epitope - 11G1 (IEDB)	91,92,93,94,95,96,98,99,169,171,239,240,241						x	x	x	x	x	x	x	x					x	x			x	x	x							
D68-specific CSF Ab binding	282-303	.																										x	x	x	x	

## Enterovirus D68 receptor requirements unveiled by haploid genetics

Jim Baaggen<sup>a,1</sup>, Hendrik Jan Thibaut<sup>a,1</sup>, Jacqueline Staring<sup>b</sup>, Lucas T. Jae<sup>b</sup>, Yue Liu<sup>c</sup>, Hongbo Guo<sup>d</sup>, Jasper J. Slager<sup>e</sup>, Jost W. de Bruin<sup>f</sup>, Arno L. W. van Vliet<sup>g</sup>, Vincent A. Blomen<sup>h</sup>, Pieter Overduin<sup>h</sup>, Ju Sheng<sup>i</sup>, Cornelis A. M. de Haan<sup>a</sup>, Erik de Vries<sup>a</sup>, Adam Meijer<sup>a</sup>, Michael G. Rossmann<sup>h,2</sup>, Thijn R. Brummelkamp<sup>b,h,\*</sup>, and Frank J. M. van Kuppeveld<sup>a,2</sup>

<sup>a</sup>Virology Division, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, 3584 CL Utrecht, The Netherlands; <sup>b</sup>Biochemistry Division, Netherlands Cancer Institute, 1006 BE, Amsterdam, The Netherlands; <sup>c</sup>Department of Biological Sciences, Purdue University, West Lafayette, IN 47907; <sup>d</sup>Virology Division, Centre for Infectious Diseases Research, Diagnostics and Screening, National Institute for Public Health and the Environment, 3720 BA, Bilthoven, The Netherlands; and <sup>e</sup>Cancer Genomics Centre, 3584 CG, Utrecht, The Netherlands

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- Defined sialic acid dependent and independent infection
- Some D68 isolates capable of infection without sialic acid
- Mapped amino acid differences to VPs

## Atomic structures of enterovirus D68 in complex with two monoclonal antibodies define distinct mechanisms of viral neutralization

Qingbing Zheng<sup>1,6</sup>, Rui Zhu<sup>1,6</sup>, Longfa Xu<sup>1,6</sup>, Maozhou He<sup>1,6</sup>, Xiaodong Yan<sup>1,2,6</sup>, Dongxiao Liu<sup>1</sup>, Zhichao Yin<sup>1</sup>, Yangtao Wu<sup>1</sup>, Yongchao Li<sup>1</sup>, Lisheng Yang<sup>1</sup>, Wangheng Hou<sup>1</sup>, Shuxuan Li<sup>1</sup>, Zizhen Li<sup>1</sup>, Zhenqin Chen<sup>1</sup>, Zhihai Li<sup>1</sup>, Hai Yu<sup>1</sup>, Ying Gu<sup>1</sup>, Jun Zhang<sup>1</sup>, Timothy S. Baker<sup>2,7</sup>, Z. Hong Zhou<sup>3,4,7</sup>, Barney S. Graham<sup>5,\*</sup>, Tong Cheng<sup>1\*</sup>, Shaowei Li<sup>1\*</sup> and Ningshao Xia<sup>1\*</sup>

NATURE MICROBIOLOGY | VOL 4 | JANUARY 2019 | 124-133 | www.nature.com/naturemicrobiology

- Two neutralizing mouse monoclonal Abs
- Binding sites determined using cryoEM

## Antibodies to Enteroviruses in Cerebrospinal Fluid of Patients with Acute Flaccid Myelitis

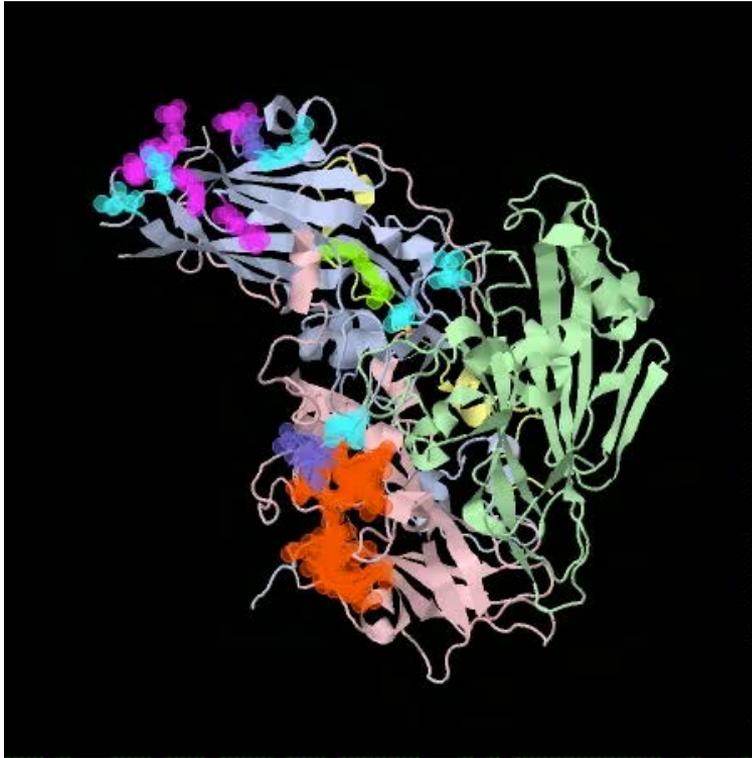
Nischay Mishra,<sup>a</sup> Terry Fei Fan Ng,<sup>b</sup> Rachel L. Marine,<sup>b</sup> Komal Jain,<sup>a</sup> James Ng,<sup>a</sup> Riddhi Thakkar,<sup>a</sup> Adrian Caciula,<sup>a</sup> Adam Price,<sup>a</sup> Joel A. Garcia,<sup>a</sup> Jane C. Burns,<sup>a</sup> Kiran T. Thakur,<sup>a</sup> Kimbell L. Hetzler,<sup>a</sup> Janell A. Routh,<sup>b</sup> Jennifer L. Konopka-Anstadt,<sup>b</sup> W. Allan Nix,<sup>b</sup> Rafal Tokarz,<sup>a</sup> Thomas Briese,<sup>a</sup> M. Steven Oberste,<sup>b</sup> W. Ian Lipkin<sup>a</sup>

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- Binding of CSF antibodies from AFM patients to EV-specific peptide arrays
- One D68-specific region with overlapping peptides

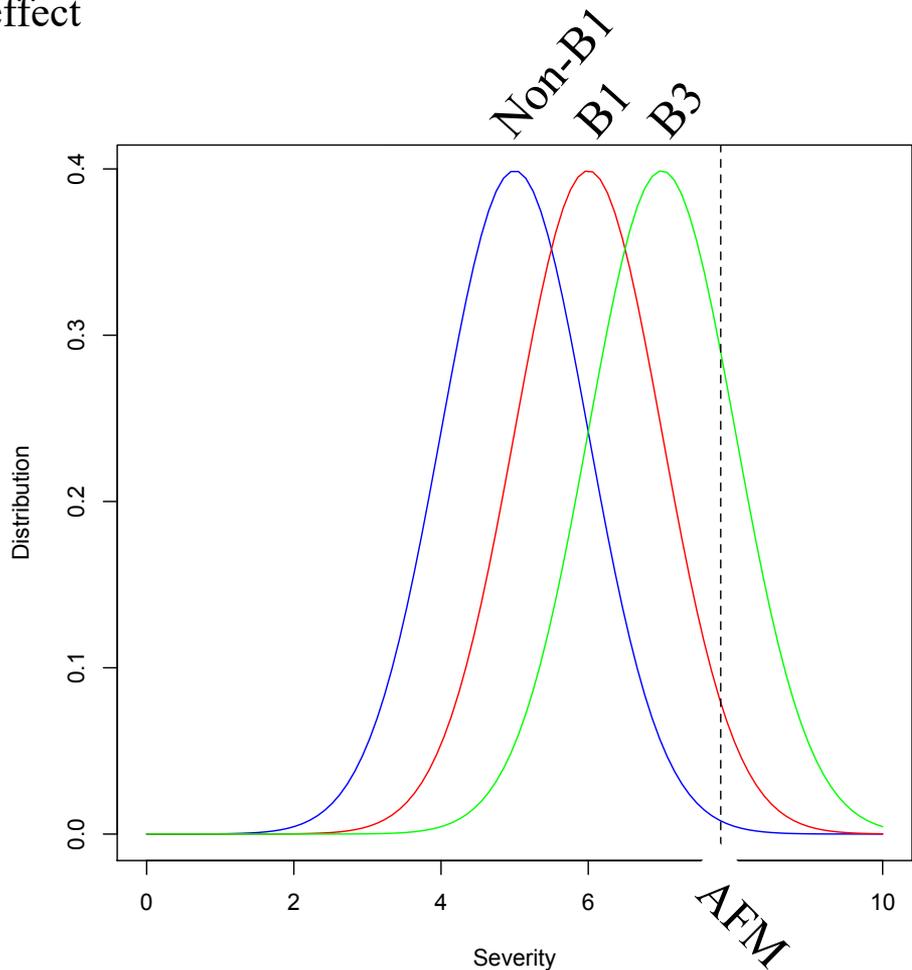
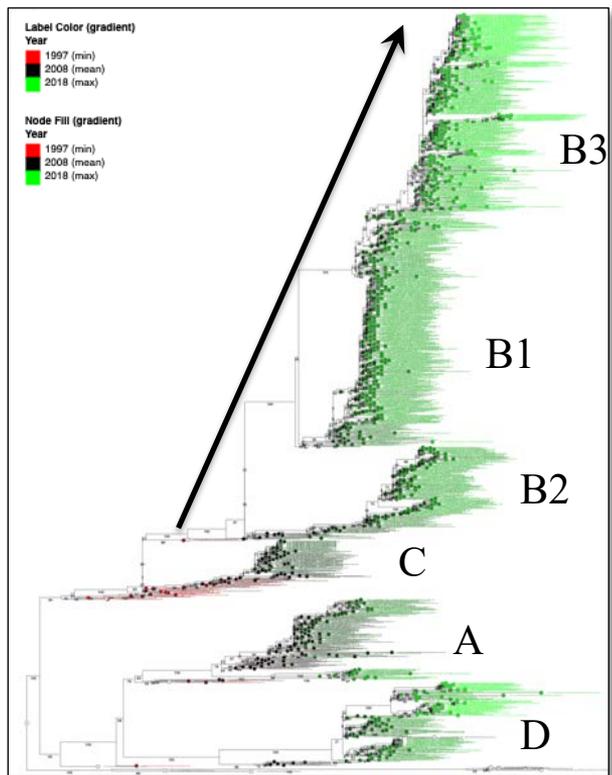
# Co-localization of evolution drivers and receptor specificity determinants



## Capsid canyon

- - B3 evolved sites
- - Neutralizing Ab epitope
- - CSF antibody peptide
- - Sia-independent infection
- - Hydrophobic pocket factor

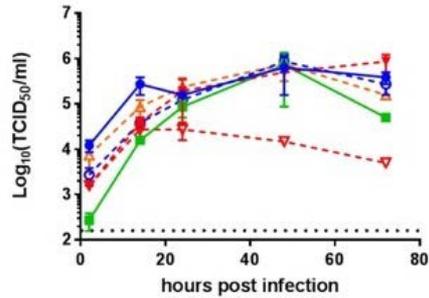
- Evasion of herd immunity => substitution in Ab epitopes
- Affected receptor binding/severity as a side effect
- Severity setpoint based on viral genome
- Severity spread by non-viral factors



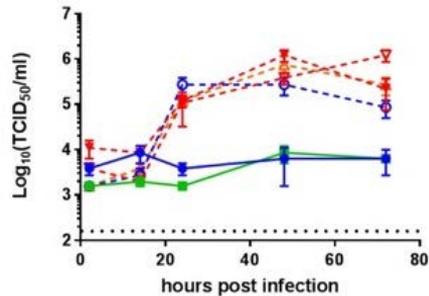
# Virion binding and entry in neurons

## Infection

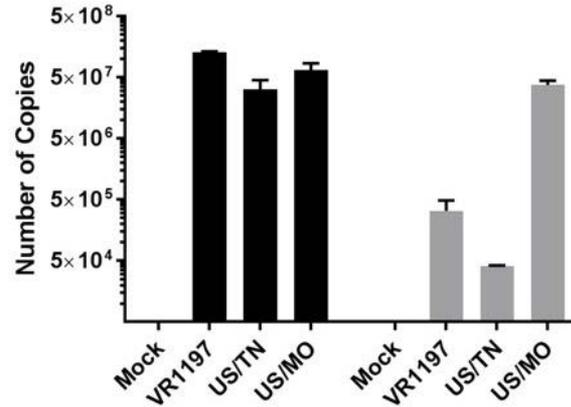
HeLa



SH-SY5Y



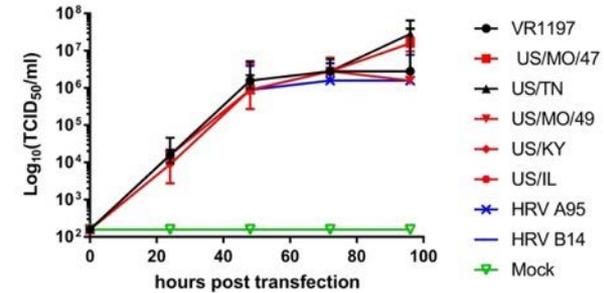
## Binding



## Transfection

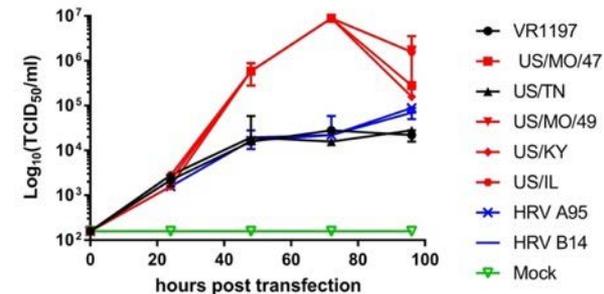
A

HeLa



B

SH-SY5Y



Brown DM, et al. (2018) mBio 9(5)

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**Alison Hixon**

**Ken Tyler**

Data providers!

NIAID HHSN272201200005C  
and HHSN272201400028C



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I N S T I T U T E

# Looking at pathogenesis in AFM: the virus and the cells



William T. Jackson, PhD.

*Associate Professor of Microbiology and  
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