



IMMUNE EPITOPE DATABASE  
AND ANALYSIS RESOURCE

# “How to use IEDB in your research”

## Examples on Orthopox

Presented by: Alba Grifoni, PhD, Instructor/Research Faculty

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# Monkeypox outbreak

## Confirmed Cases

**75,885**

Total Cases

**74,994**

in locations that have not historically reported monkeypox

**891**

in locations that have historically reported monkeypox

## Locations with cases

**109**

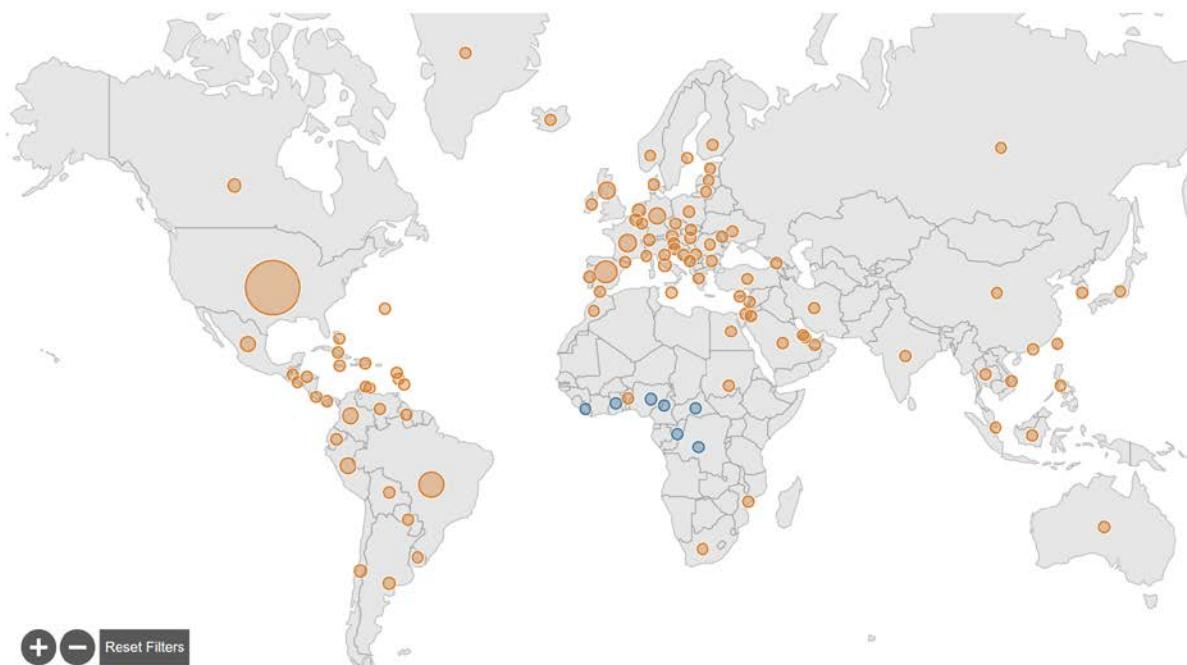
Total

**102**

Has not historically reported monkeypox

**7**

Has historically reported monkeypox

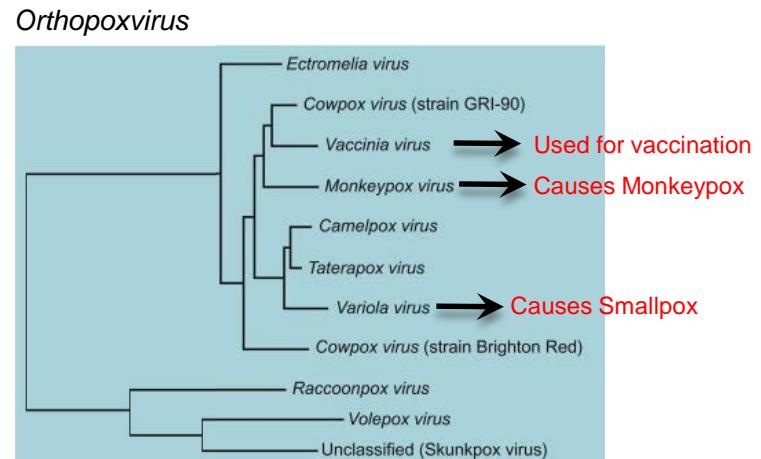
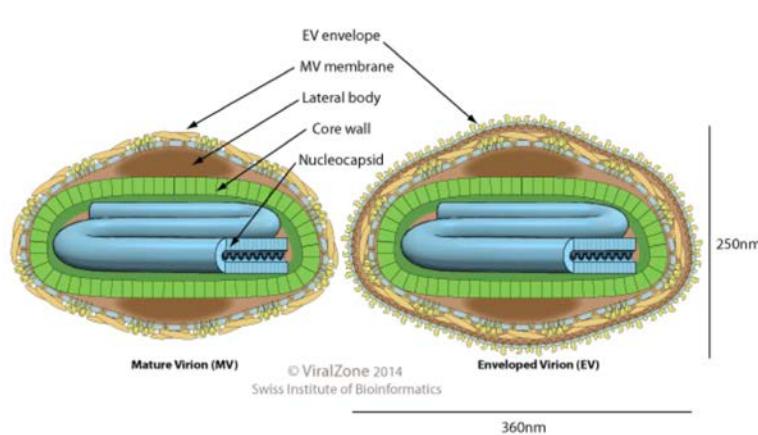


## Legend

● Has not historically reported monkeypox

● Has historically reported monkeypox

# Orthopoxviruses

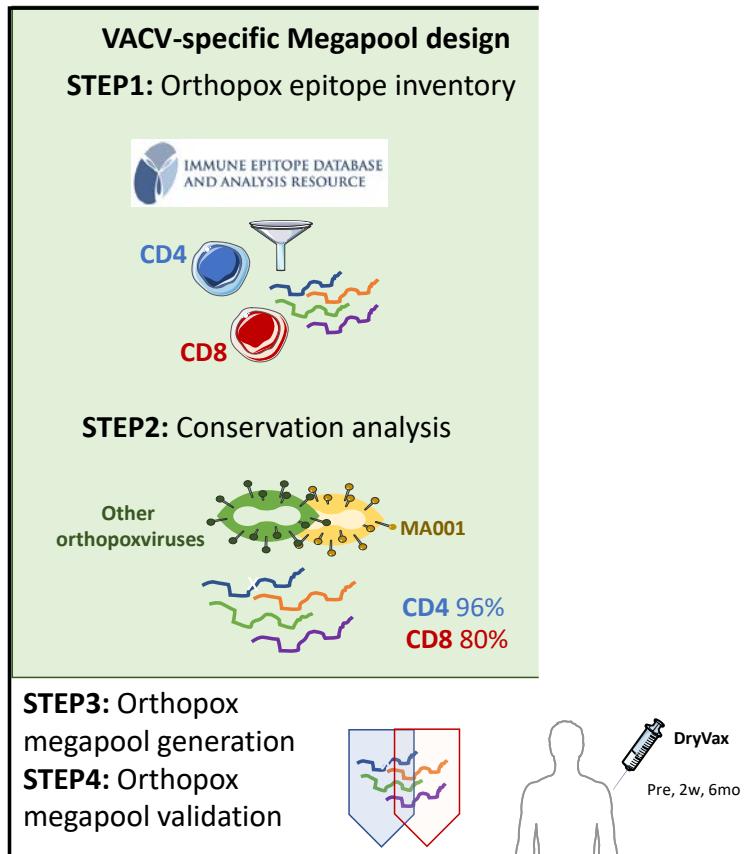


Virus Taxonomy, 2012 doi.org/10.1016/B978-0-12-384684-6.00028-8.

- All Orthopox viruses vaccines are based on **vaccinia virus**:
  - *Dryvax: live vaccine with combination of vaccinia strains discontinued in 1972. High efficacy, Low safety profile ORFs≈ 240*
  - *Acam2000: live vaccine, single vaccinia strains from Dryvax. Comparable to Dryvax.*
  - *MVA (Jyanneos): live vaccine non-replicating modified vaccinia Ankara Efficacy tested only in animal models, High safety profile ORFs≈ 157*

**Acam2000 and MVA are both currently licensed for Monkeypox (ORFs ≈ 200)**

# Design peptide pools able to detect vaccine responses



Grifoni, Zhang.....Scheuermann, Sette Cell Host & Microbe 2022

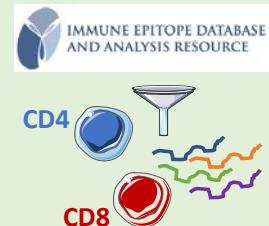
# VACV-specific Megapool design

**Table 1. IEDB inventory of orthopox virus CD4 and CD8 T cell epitopes**

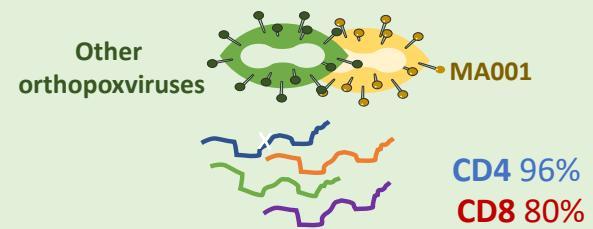
Type	Host	Orthopox virus					Total
		Cowpox	Ectromelia	Monkeypox	Vaccinia	Variola	
CD4	Humans	0	0	0	209	38	247
	Macaques	0	0	0	0	0	0
	Tg mice	0	0	0	0	0	0
	Mice	0	40	0	31	0	71
<b>Any</b>		<b>0</b>	<b>40</b>	<b>0</b>	<b>240</b>	<b>38</b>	<b>318</b>
CD8	Humans	0	0	0	207	31	238
	Macaques	0	0	2	17	0	19
	Tg mice	0	0	0	182	0	182
	Mice	1	2	0	217	0	220
<b>Any</b>		<b>1</b>	<b>2</b>	<b>2</b>	<b>623</b>	<b>31</b>	<b>659</b>

Tallies are non-redundant diminishing: macaques does not include those also recognized in humans, Tg mice does not include those recognized in humans or macaques, etc.

## STEP1: Orthopox epitope inventory



## STEP2: Conservation analysis



**OPXV CD4 MP:** 318 epitopes identified in any host

**OPXV CD8 MP:** 238 epitopes identified only in Human

# How to use IEDB to extract VACV epitopes



CD4 epitope query

The screenshot shows the IEDB search interface with a red box highlighting the search term "CD4 epitope query".

**Welcome**  
The Immune Epitope Database (IEDB) is a freely available resource funded by NIAID. It catalogs experimental data on antibody and T cell epitopes studied in humans, non-human primates, and other animal species in the context of infectious disease, allergy, autoimmunity and transplantation. The IEDB also hosts tools to assist in the prediction and analysis of epitopes.  
[Learn More](#)

**Upcoming Events & News**  
AAI Exhibitor Booth May 6-10  
FOCUS Exhibitor Booth June 21-24  
Virtual User Workshop Oct 26-28  
\* register [here](#)

[IEDB SARS-CoV-2 Epitope Analysis Videos](#)

**Summary Metrics**

Peptidic Epitopes	1,545,392
Non-Peptidic Epitopes	3,147
T Cell Assays	446,386
B Cell Assays	1,335,277
MHC Ligand Assays	4,683,598
Epitope Source Organisms	4,241
Restricting MHC Alleles	971
References	23,343

**START YOUR SEARCH HERE**

**Epitope**  
 Any  
 Linear peptide  
Exact   
 Discontinuous  
 Non-peptidic

**Assay**  
 T Cell  
 B Cell  
 MHC Ligand  
Ex: neutralization   
Outcome:  Positive  Negative

**Epitope Source**  
Organism: Orthopoxvirus (ID:10z)   
Antigen: Ex: core, capsid, myo

**MHC Restriction**  
 Any  
 Class I  
 Class II  
 Non-classical  
Ex: HLA-A\*02:01

**Host**  
 Any  
 Human  
 Mouse  
 Non-human primate  
Ex: dog, camel

**Disease**  
 Any  
 Infectious  
 Allergic  
 Autoimmune  
Ex: asthma

**Epitope Analysis Resource**

**T Cell Epitope Prediction**  
Scan an antigen sequence for amino acid patterns indicative of:  
MHC I Binding  
MHC II Binding  
MHC I Processing (Proteasome,TAP)  
MHC I Immunogenicity

**B Cell Epitope Prediction**  
Predict linear B cell epitopes using:  
Antigen Sequence Properties  
Predict discontinuous B cell epitopes using antigen structure via:  
Disotope  
ElliPro

**Epitope Analysis Tools**  
Analyze epitope sets of:  
Population Coverage  
Conservation Across Antigens  
Clusters with Similar Sequences

# How to use IEDB to extract VACV epitopes



## CD4 epitope results : Epitopes

The screenshot shows the IEDB search interface with the following filters applied:

- Pending Filters:** T Cell
- Current Filters:** Organism: Orthopoxvirus (ID:10242), Include Positive Assays, No B cell assays, No MHC assays, MHC Restriction Type: Class II

The results table displays 345 epitopes across various categories:

Epitopes (345)	Antigens (133)	Assays (720)	Receptors (0)	References (38)	
Go To Records Starting At 1200	Export Results				
345 Records Found	Page 1 of 14	25 Per Page			
Iedb ID	Epitope	Antigen	Organism	# References	# Assays
51567	QLVFNSISARALKAY	Telomere-binding protein I1	Vaccinia virus (vaccinia virus VV)	7	12
28568	ISKYAGINILNVYSP	Core protein VP8	Vaccinia virus (vaccinia virus VV)	5	11
47780	PGVMYAFTTPLISFF	Envelope protein H3	Vaccinia virus (vaccinia virus VV)	5	10
49543	PSVFINPIHTSYCY	DNA polymerase	Vaccinia virus (vaccinia virus VV)	5	13
7760	DDDYGEPIITSYQLQ	DNA-directed RNA polymerase 22 kDa subunit	Vaccinia virus (vaccinia virus VV)	4	5
36810	LKAYFTAKINEMVDE	Telomere-binding protein I1	Vaccinia virus (vaccinia virus VV)	4	6
48068	PKGFYASPVKTSVL	Transcript termination protein A18	Vaccinia virus (vaccinia virus VV)	4	5
48083	PKIIFRPTTITANVS	Scaffold protein D13	Vaccinia virus (vaccinia virus VV)	4	9
65753	TPRYIPSTSISSSNI	Protein F15	Vaccinia virus (vaccinia virus VV)	4	5
69806	VLTIKAPVNIISSKIS	Poly(A) polymerase catalytic subunit	Vaccinia virus (vaccinia virus VV)	4	5
9473	DNIFIPSVITKSGKK	DNA polymerase processivity factor component A20	Vaccinia virus (vaccinia virus VV)	3	3
17914	FTCDQGYHSSDPNAV	Protein B5	Vaccinia virus (vaccinia virus VV)	3	7
19296	GEIIRAATTSPAREN	Cell surface-binding protein	Vaccinia virus (vaccinia virus VV)	3	4
26418	IRAVTTDQVIAHESQ	DNA-directed RNA polymerase 12S subunit	Vaccinia virus (vaccinia virus VV)	2	2

# How to use IEDB to extract VACV epitopes



## CD4 epitope results : Assays

IEDB.org: View Results and Refine Search iedb.org/result\_v3.php?cookie\_id=d9f90a More IEDB

Home Specialized Searches Analysis Resource

Pending Filters Filter Options T Cell

Current Filters: Organism: Orthopoxvirus (ID:10242) Include Positive Assays No B cell assays No MHC assays MHC Restriction Type: Class II

Epitopes (345) Antigens (133) Assays (720) Receptors (0) References (38)

T Cell Assays (720) B Cell Assays (0) MHC Ligand Assays (0)

Go To Records Starting At A,b Export Results

720 Records Found Page 1 of 29 25 Per Page

IEDB ID	Reference	Epitope	Host	Immunization	Assay Antigen	Antigen Epitope Relation	MHC Restriction	Assay Description
1835642	Lichen Jing; J Immunol Methods 2009	YVLSSLHIYWGK E IMV membrane protein (61-73) Vaccinia virus (vaccinia virus VV)	Homo sapiens (human)	Prophylactic vaccination with Vaccinia virus NYCBH - Dryvax (Taxonomic Child) followed by restimulation in vitro	YVLSSLHIYWGK E IMV membrane protein (61-73) Vaccinia virus (vaccinia virus VV)	Epitope	HLA-DQA1*01:02/DQB1*06:02	3H-thymidine proliferation Positive
1835641	Lichen Jing; J Immunol Methods 2009	SAMVYSSDDIPP R double-strand RNA-binding protein (53-65) Vaccinia virus (vaccinia virus VV)	Homo sapiens (human)	Prophylactic vaccination with Vaccinia virus NYCBH - Dryvax (Taxonomic Child) followed by restimulation in vitro	SAMVYSSDDIPP R double-strand RNA-binding protein (53-65) Vaccinia virus (vaccinia virus VV)	Epitope	HLA-DQA1*01:02/DQB1*06:02	3H-thymidine proliferation Positive
2370972	David M. Koelle M.D.; IEDB Submission 2014	RKLTELNAELSD K A-type inclusion protein A25 (432-444)	Homo sapiens (human)	Administration in vivo with Vaccinia virus (vaccinia virus VV) (Source Organism)	RKLTELNAELSD K A-type inclusion protein A25 (432-444)	Epitope	HLA-DRB1*15:01	3H-thymidine proliferation Positive

# How to use IEDB to extract VACV epitopes



## CD4 epitope results : References

Screenshot of the IEDB.org interface showing CD4 epitope results for Orthopoxvirus (ID:10242).

**Pending Filters:** T Cell

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Epitopes (345)	Antigens (133)	Assays (720)	Receptors (0)	References (38)
Go To Records Starting At 1982 <a href="#">GO</a>				
38 Records Found <a href="#">Export Results</a>				
IEDB ID	PMID	Author	Title	Journal
1037262	32747299	Jun Ando; Minhtran C Ngo; Miki Ando; Ann Leen; Cliona M Rooney	Identification of protective T-cell antigens for smallpox vaccines.	Cyotherapy
1035464	Submission	David M. Koelle M.D.; Lichen Jing Ph.D.; Kerry J. Laing Ph.D.; Lichun Dong M.S.; Victoria L. Campbell B.S.; Alessandro Sette DR. BIOL.SCI	Identification of CD4+ T cell epitopes in humans vaccinated with vaccinia	
1034653	30700590	Katherine S Forsyth; Brian DeHaven; Mark Mendonca; Sinu Paul; Alessandro Sette; Laurence C Eisenlohr	Poor Antigen Processing of Poxvirus Particles Limits CD4+ T Cell Recognition and Impacts Immunogenicity of the Inactivated Vaccine.	J Immunol
1033295	Submission	David M. Koelle M.D.; Lichen Jing Ph.D.; Kerry J. Laing Ph.D.; Lichun Dong M.S.; Victoria L. Campbell B.S.; Alessandro Sette DR. BIOL.SCI	Identification of CD4+ T cell epitopes in humans vaccinated with vaccinia	
1032285	Submission	David M. Koelle M.D.; Lichen Jing Ph.D.; Kerry J. Laing Ph.D.; Lichun Dong M.S.; Victoria L. Campbell B.S.	Identification of CD4+ T cell epitopes in humans vaccinated with Dryvax vaccine	
1029750	Submission	David M. Koelle M.D.; Lichen Jing Ph.D.; Kerry J. Laing Ph.D.; Lichun Dong M.S.; Victoria L. Campbell B.S.	Identification of CD4+ T cell epitopes in humans vaccinated with Dryvax® vaccine	
1028784	Submission	David M. Koelle M.D.; Lichen Jing Ph.D.	Identification of CD4+ T cell epitopes in	

# How to use IEDB to extract VACV epitopes



CD8 epitope query

The screenshot shows the IEDB home page with a search query for CD8 epitopes. The search parameters are:

- Epitope:** Any
- Assay:** T Cell (selected)
- Organism:** Orthopoxvirus (ID:102)
- MHC Restriction:** Class I (selected)
- Host:** Human (selected)
- Disease:** Any

The results section is currently empty, indicating no matches found for the specified criteria.

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**START YOUR SEARCH HERE**

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  - MHC I Immunogenicity
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  - Conservation Across Antigens
  - Clusters with Similar Sequences

# How to use IEDB to extract VACV epitopes

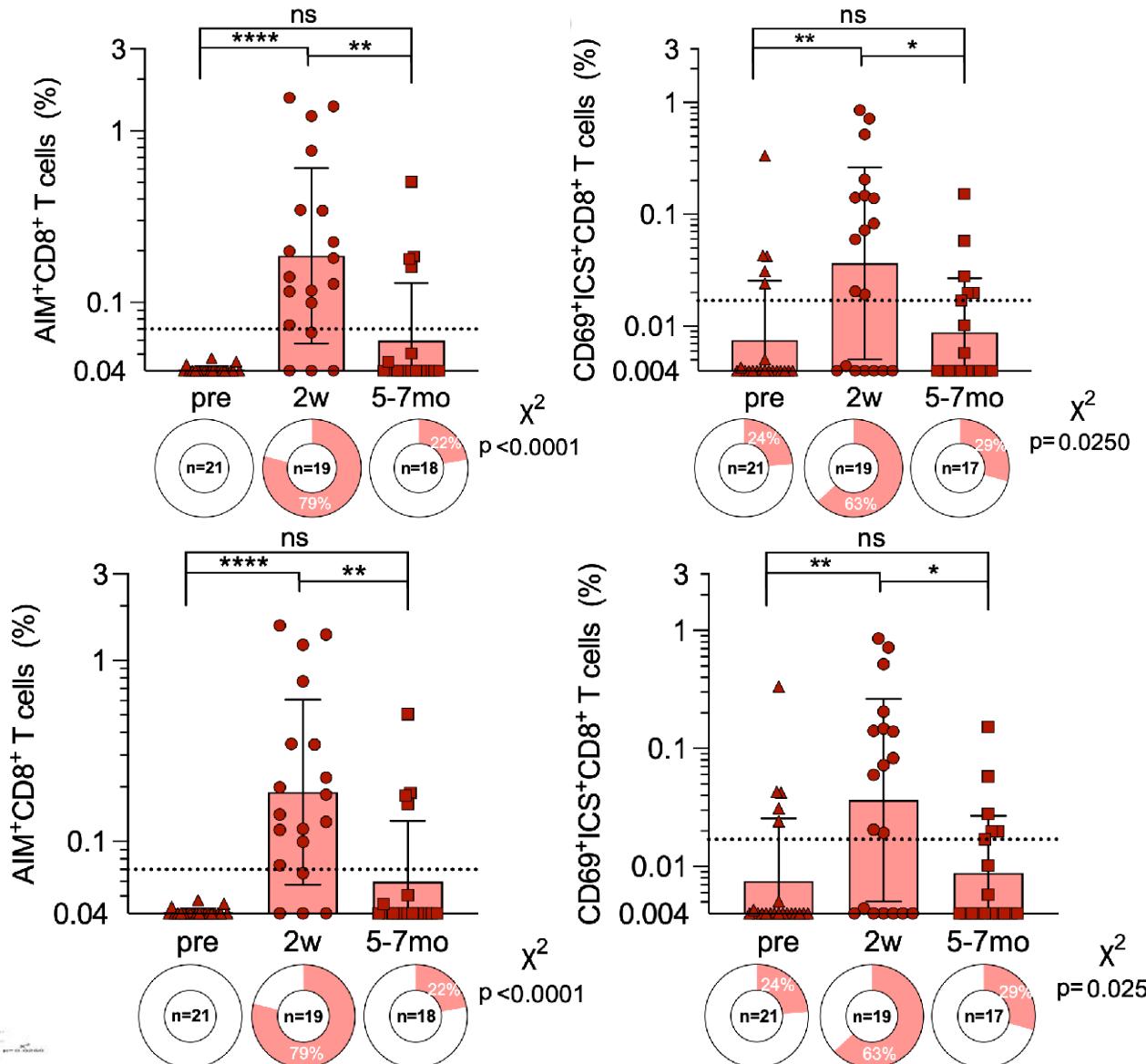


## CD8 epitope results : Epitopes

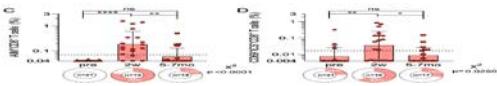
The screenshot shows the IEDB interface with the following details:

- Header:** IEDB.org: View Results and Refine Search, iedb.org/result\_v3.php?cookie\_id=25beae
- Navigation:** Home, Specialized Searches, Analysis Resource
- Pending Filters:** Reset, Search, Filter Options (T Cell selected), Epitope (Any selected), Length, Sequence, Discontinuous, Non-peptidic, 3D structure assays, Amino acid modification.
- Current Filters:** Organism: Orthopoxvirus (ID:10242), Include Positive Assays, No B cell assays, No MHC assays, MHC Restriction Type: Class I, Host: Homo sapiens (human).
- Result Summary:** Epitopes (249), Antigens (136), Assays (487), Receptors (0), References (27). Go To Records Starting At 1200, Export Results.
- Table:** 249 Records Found. Columns include: IEDB ID, Epitope, Antigen, Organism, # References, # Assays. The table lists various protein epitopes from Vaccinia virus (vaccinia virus VV) such as Interferon antagonist C7, Putative nuclease G5, Protein C16/B22, Protein E2, Protein F12, Protein O1, DNA-directed RNA polymerase 147 kDa polypeptide, Protein F12, DNA polymerase, Protein O1, Primase D5, Kelch repeat and BTB domain-containing protein 1, and Intermediate transcription factor 3 small.

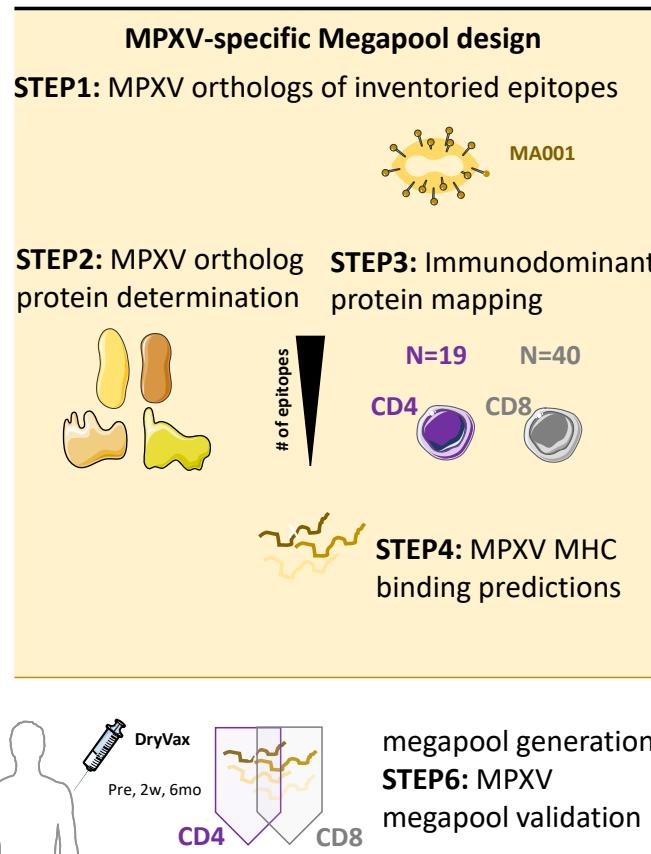
# Testing VACV pools on a Dryvax vaccinated cohort



Grifoni, Zhang.....  
Scheuermann, Sette Cell  
Host & Microbe 2022



# Design peptide pools able to detect MPXV responses



Grifoni, Zhang.....Scheuermann, Sette Cell Host & Microbe 2022

# MPXV-specific Megapool design

**STEP1:** MPXV orthologs of inventoried epitopes



MA001

**STEP2:** MPXV ortholog protein determination



# epitopes

N=19      N=40  
CD4      CD8

**STEP3:** Immunodominant protein mapping

Selected proteins with 5 or more epitopes

CD4

67%

# protein
28 A10L
20 B5R
11 D5R
11 A3L
11 J6R
10 L4R
9 A33R
9 D13L
9 H3L
8 B7R
7 A27L
7 A48R
7 F11L
6 A24R
5 L1R
5 F8L
5 A20R
5 A4L
5 E3L
5 A56R
5 D8L
92 Others

CD8

61%

# protein	# protein
14 D1R	7 B25R
13 A3L	6 A7L
12 J6R	6 D12L
11 D5R	6 G2R
11 B5R	6 B19R
11 F8L	6 I4L
10 A10L	6 A8R
10 I8R	6 A18R
10 H3L	6 A14L
10 H3L	6 H5R
9 A24R	6 A14L
9 A24R	6 K7R
9 A47L	5 G5R
9 A47L	5 I3L
8 A6L	5 F13L
8 A17L	5 C14L
7 I7L	5 O1L
7 A23R	5 C16L
7 E2L	5 E8R
7 C16L	5 E3L
7 A29L	5 A20R
7 D13L	198 Others
7 A27L	

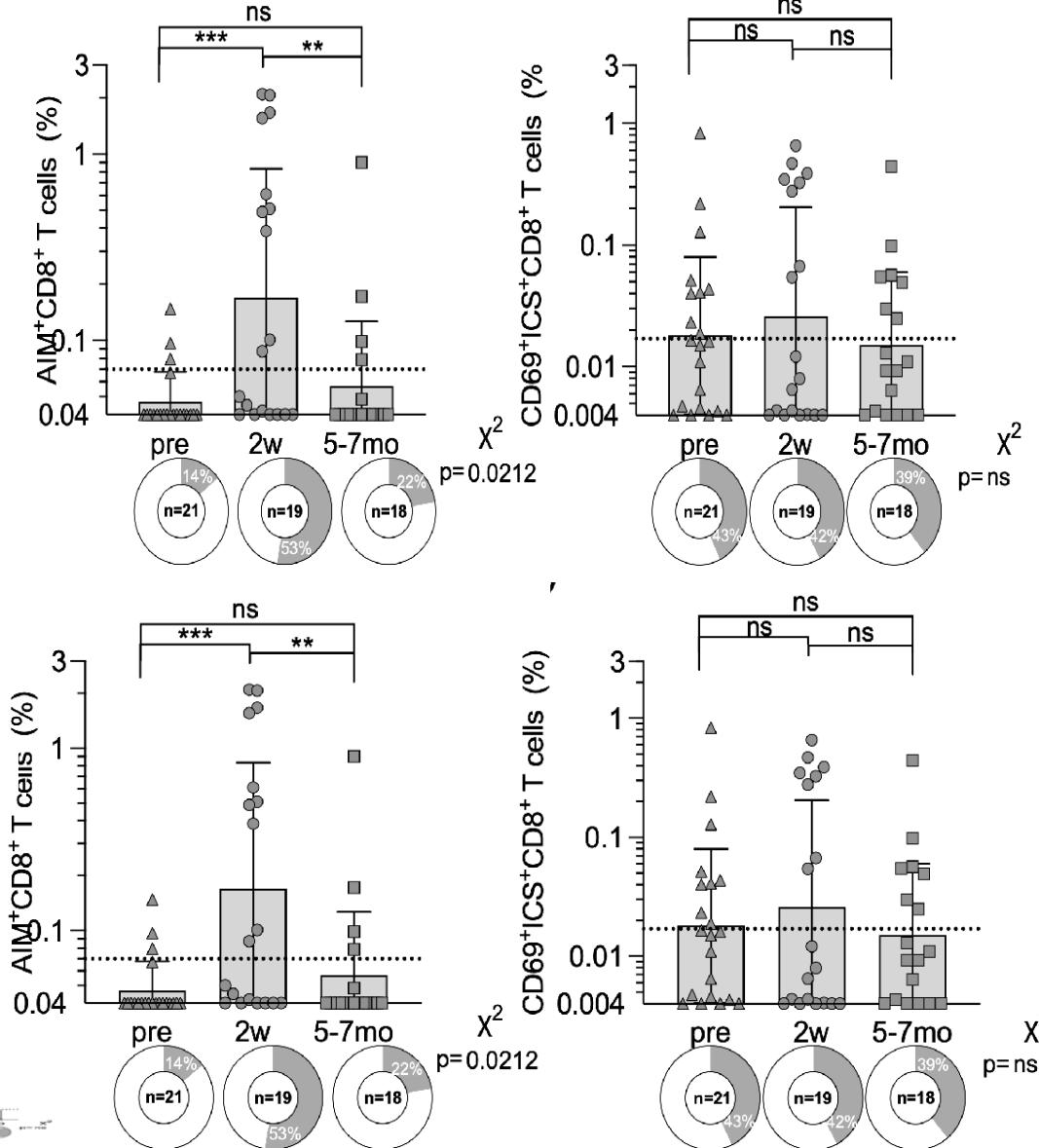


**STEP4:** MPXV MHC binding predictions

MPXV CD4 MP: 276

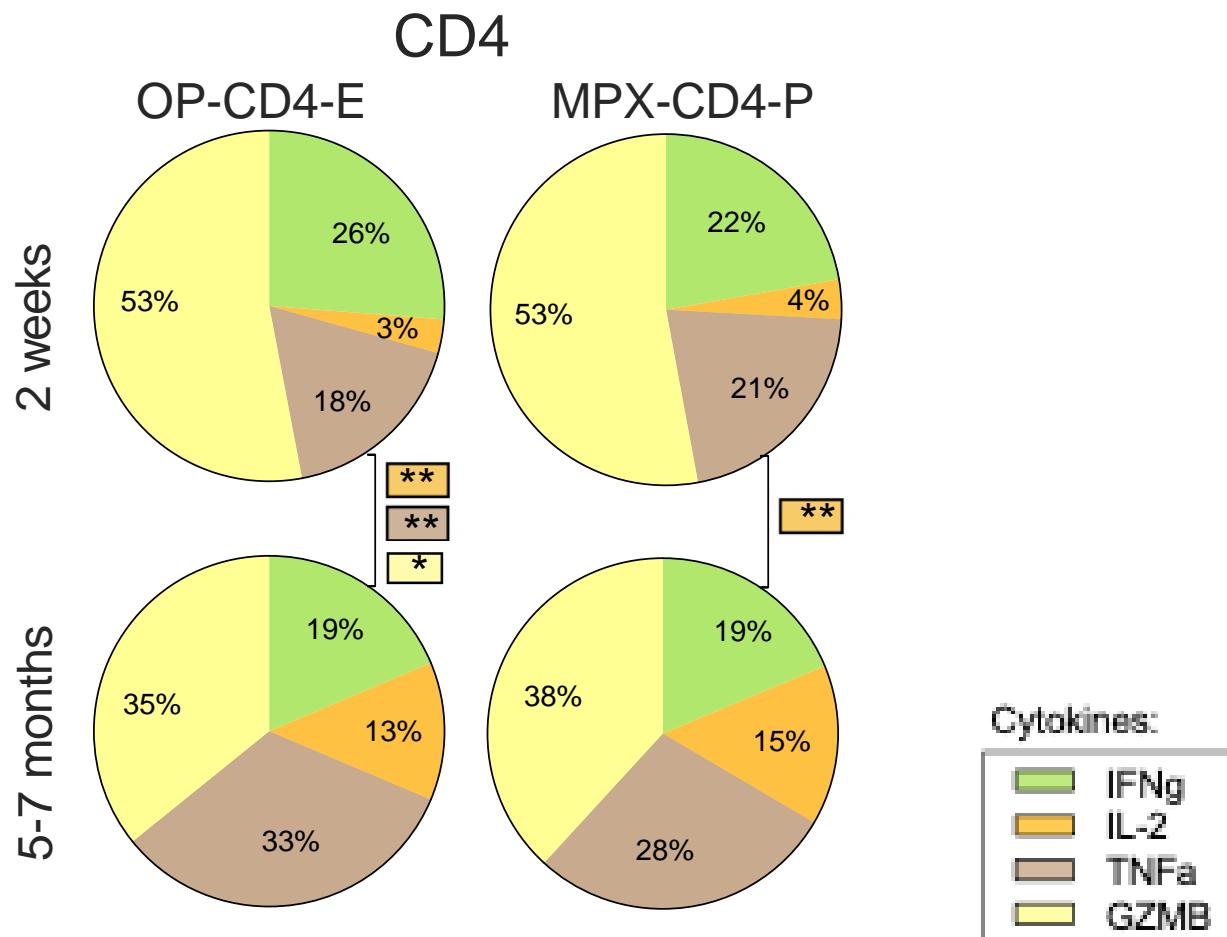
MPXV CD8 MP P1-P5: 1647- divided in 5 pools

# Testing MPXV pools to assess T cell cross-reactivity on a Dryvax vaccinated cohort

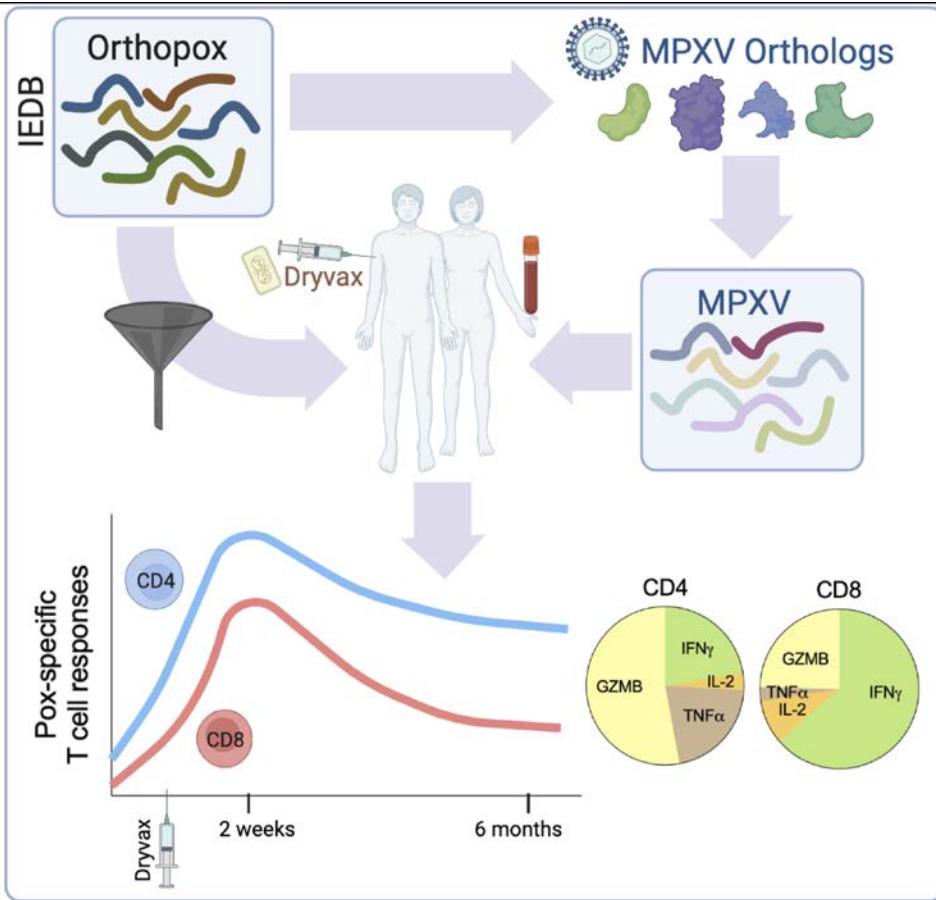


Grifoni, Zhang.....  
Scheuermann, Sette Cell Host  
& Microbe 2022

# Pox-specific T cells produce multiple cytokines



# Conclusions



- We defined dominant cross-reactive ORFs to generate peptide MPs to measure T cell responses
- Wide breadth of both CD4+ and CD8+ T cell immune responses
- T cell epitopes are largely conserved between VACV and MPXV
- CD4 responses are more durable over time than CD8
- There is a large population of Pox-specific Granzyme B+ CD40L+ CD4 T cells



IMMUNE EPITOPE DATABASE  
AND ANALYSIS RESOURCE

# “How to use IEDB in your research”

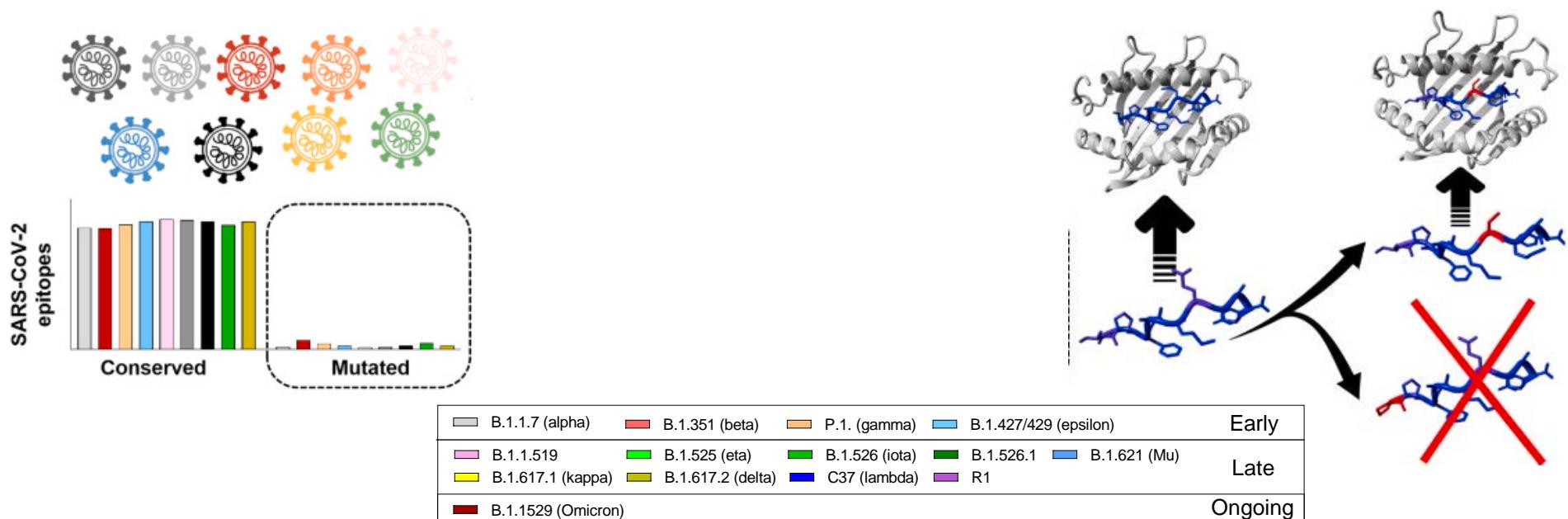
## Examples on SARS-CoV-2

Presented by: Alba Grifoni, PhD, Instructor/Research Faculty

La Jolla  
Institute  
FOR IMMUNOLOGY

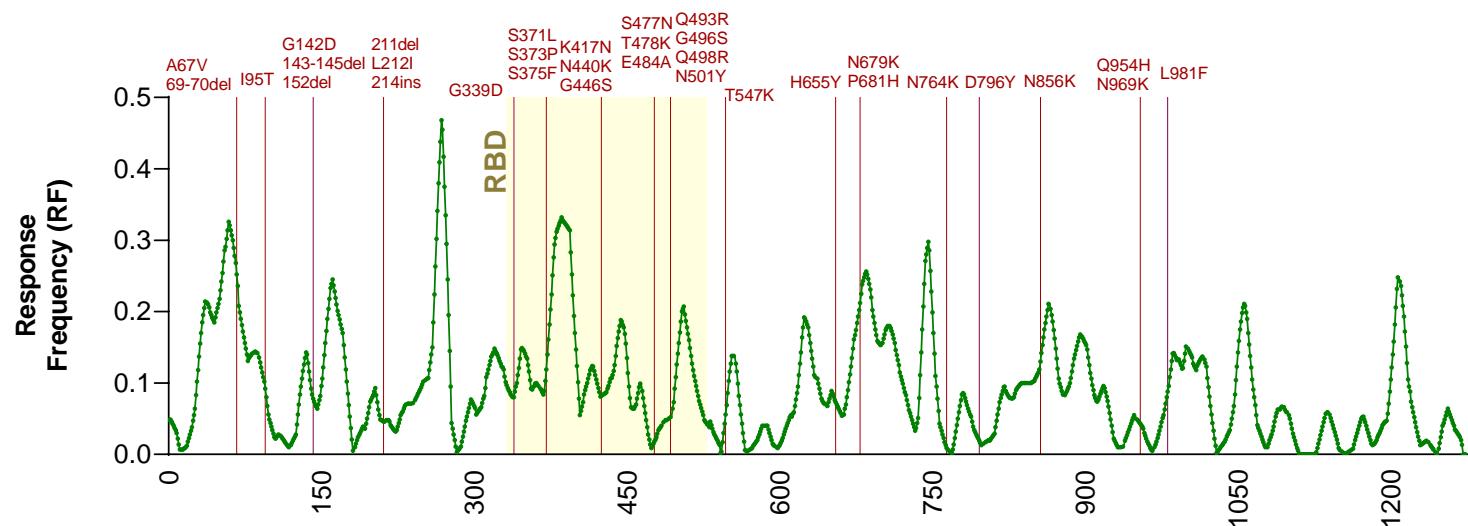
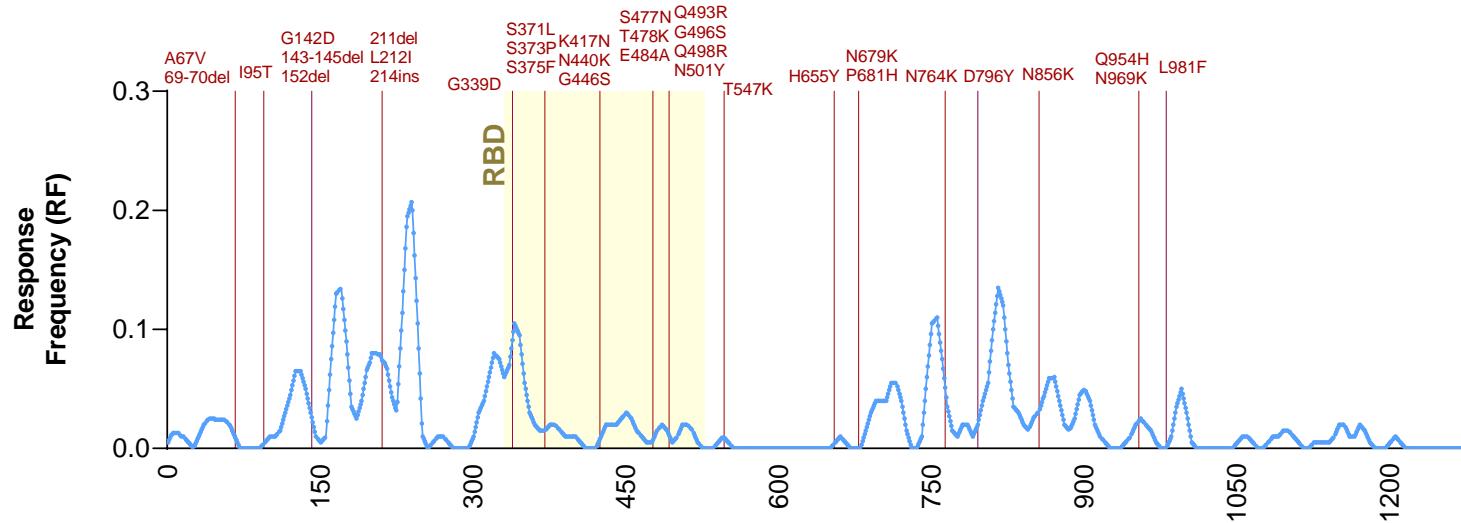
Life  
Without  
Disease.®

# Predicted impact of mutated epitopes



Tarke..... Grifoni and Sette Cell 2022  
Grifoni and Sette CRI 2022

# Impact of spike Omicron mutations on T cell epitopes



# How to use ImmunomeBrowser to define spike T cell immunodominant regions



CD4 epitope query

The screenshot shows the IEDB homepage with a search query for "CD4 epitope query".

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Epitope Source Organisms	4,241
Restricting MHC Alleles	971
References	23,343

**START YOUR SEARCH HERE:**

**Epitope:** Any, Linear peptide, Discontinuous, Non-peptidic. Exact: SIINFEKL.

**Assay:** T Cell (checked), B Cell, MHC Ligand. Ex: neutralization. Outcome: Positive (checked).

**Epitope Source:** Organism: SARS-CoV2 (ID:2697). Antigen: core, capsid, myo. Find.

**MHC Restriction:** Any, Class I, Class II (checked), Non-classical. Ex: HLA-A\*02:01. Find.

**Host:** Any, Human (checked), Mouse, Non-human primate. Ex: dog, camel. Find.

**Disease:** Any, Infectious, Allergic, Autoimmune. Ex: asthma. Find.

**Epitope Analysis Resource:**

**T Cell Epitope Prediction:** Scan an antigen sequence for amino acid patterns indicative of: MHC I Binding, MHC II Binding, MHC I Processing (Proteasome,TAP), MHC I Immunogenicity.

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**Epitope Analysis Tools:** Analyze epitope sets of: Population Coverage, Conservation Across Antigens, Clusters with Similar Sequences.

**Search Buttons:** Reset, Search.

# How to use ImmunomeBrowser to define spike T cell immunodominant regions



CD4 epitope results : Antigen

The screenshot shows the IEDB interface with the following details:

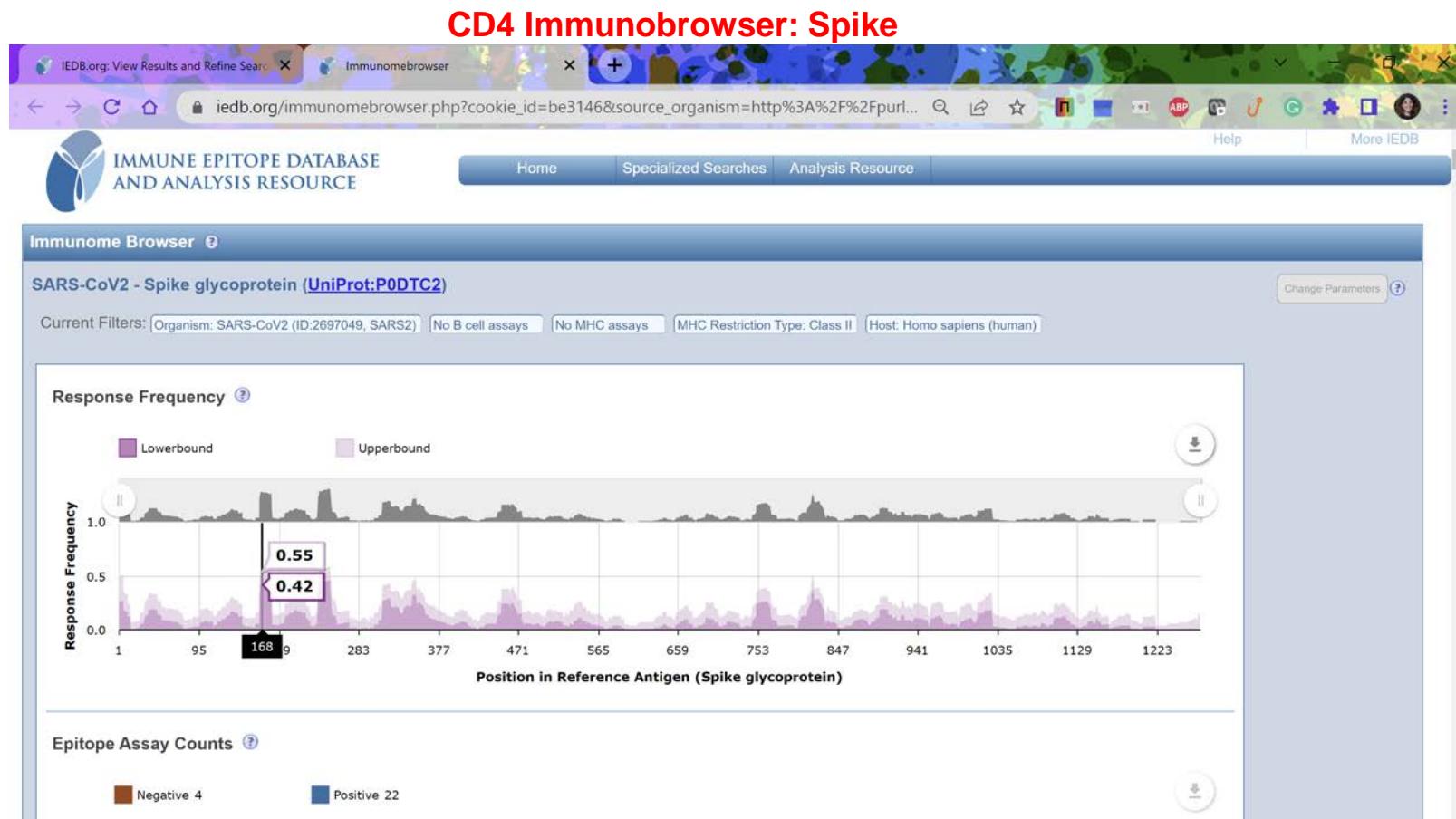
- Pending Filters:** T Cell
- Current Filters:** Organism: SARS-CoV2 (ID:2697049, SARS2), Host: Homo sapiens (human)
- Epitopes:** 1039
- Antigens:** 12
- Assays:** 3309
- Receptors:** 800
- References:** 50

Below the filters, there is a table of antigens:

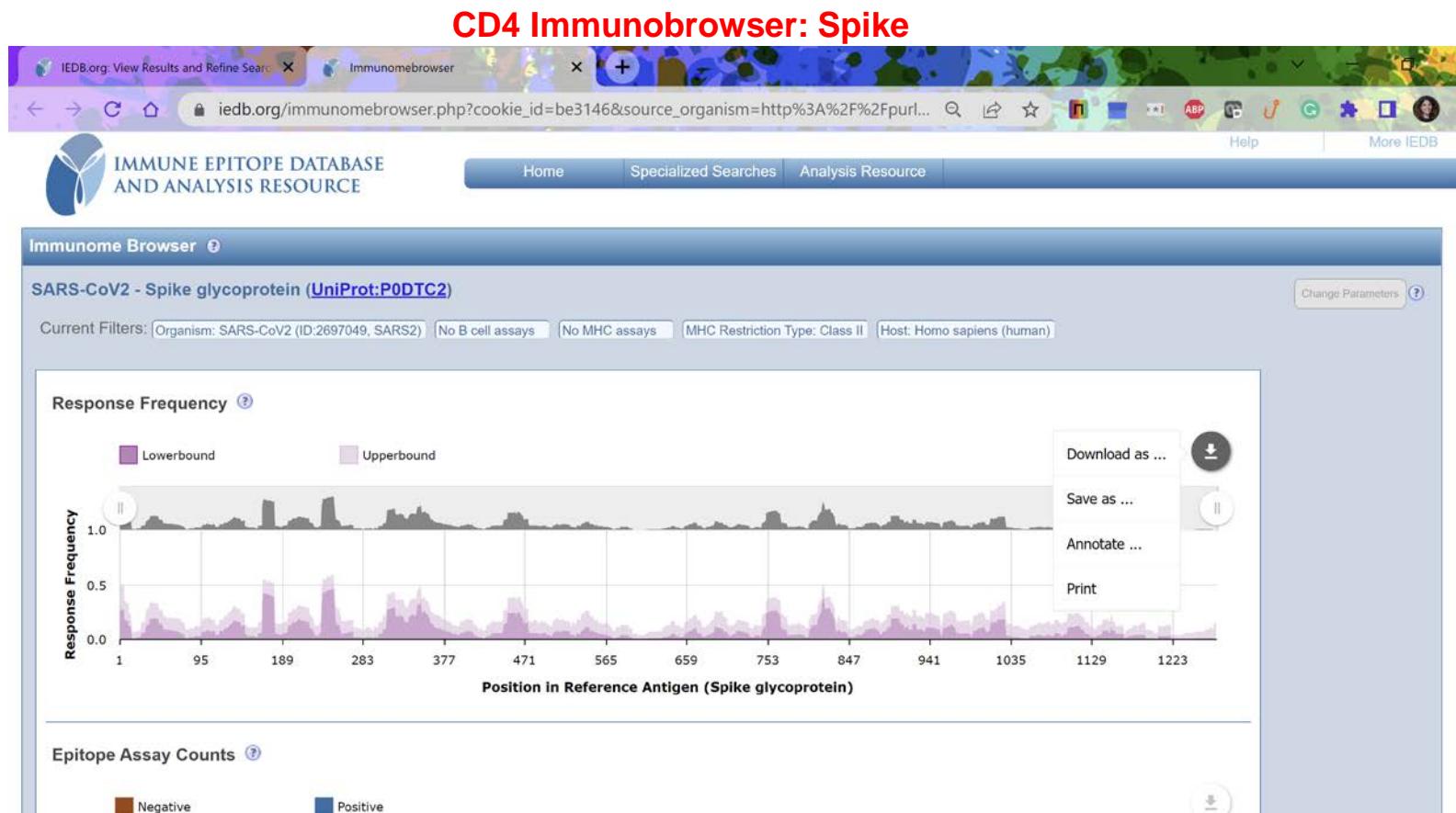
Antigen	Organism	# Epitopes	# Assays	# References
Spike glycoprotein	Influenza A Hemagglutinin	42	60	42
Nucleoprotein	Host: Homo sapiens	27	27	27
Membrane protein		23	23	23
Replicase polyprotein 1ab	Assay: B cell assays	12	12	12
Envelope small membrane protein		11	11	11
ORF8 protein		8	8	8
ORF7a protein		8	8	8
ORF6 protein		5	5	5
ORF3a protein		5	5	5
ORF10 protein		3	3	3
Replicase polyprotein 1a		2	2	2
ORF7b protein		1	1	1

A modal window titled "Click icon to view Immunome Browser" is open, showing a "Response Frequency" plot for the Influenza A Hemagglutinin antigen. The plot shows two shaded areas representing the Lowerbound and Upperbound response frequencies across positions 1 to 183. Below the plot, text explains the function of the Immunome Browser.

# How to use ImmunomeBrowser to define spike T cell immunodominant regions



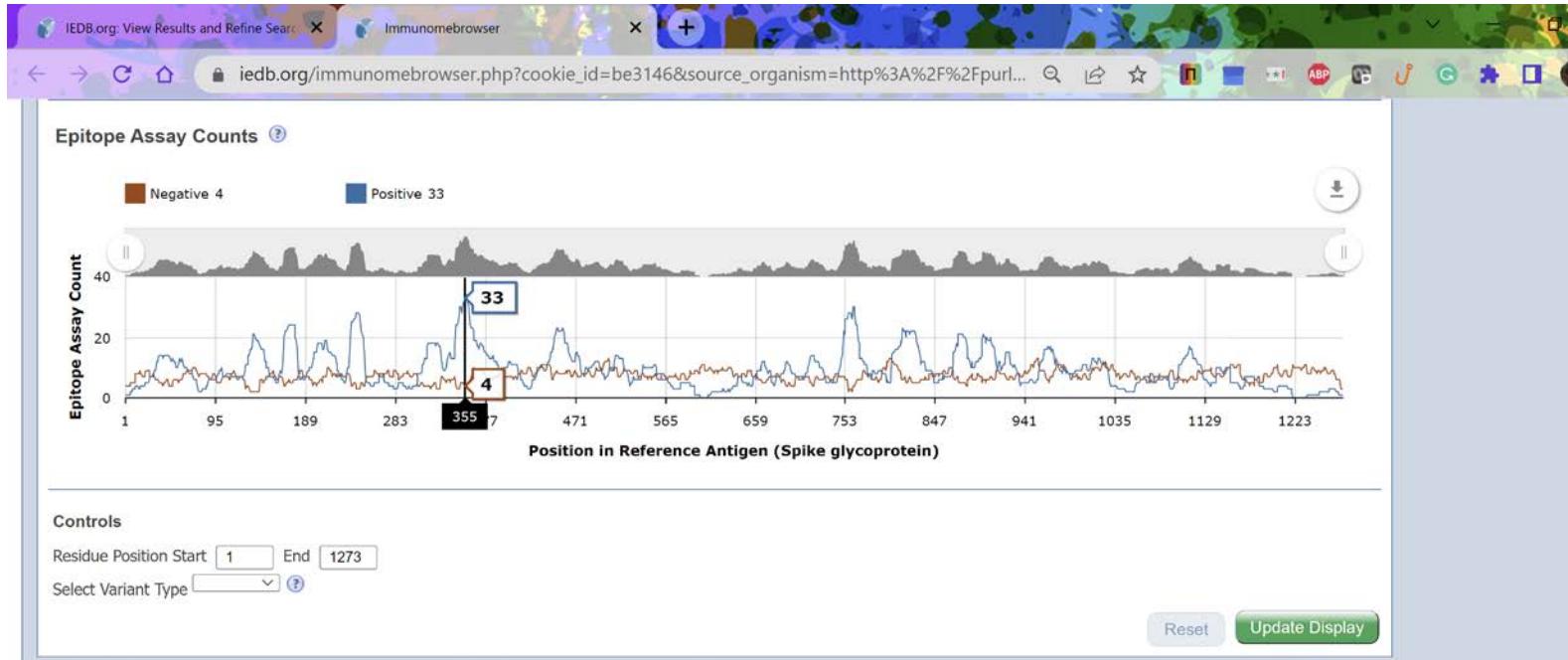
# How to use ImmunomeBrowser to define spike T cell immunodominant regions



# How to use ImmunomeBrowser to define spike T cell immunodominant regions



## CD4 Immunobrowser: Spike



# How to use ImmunomeBrowser to define spike T cell immunodominant regions

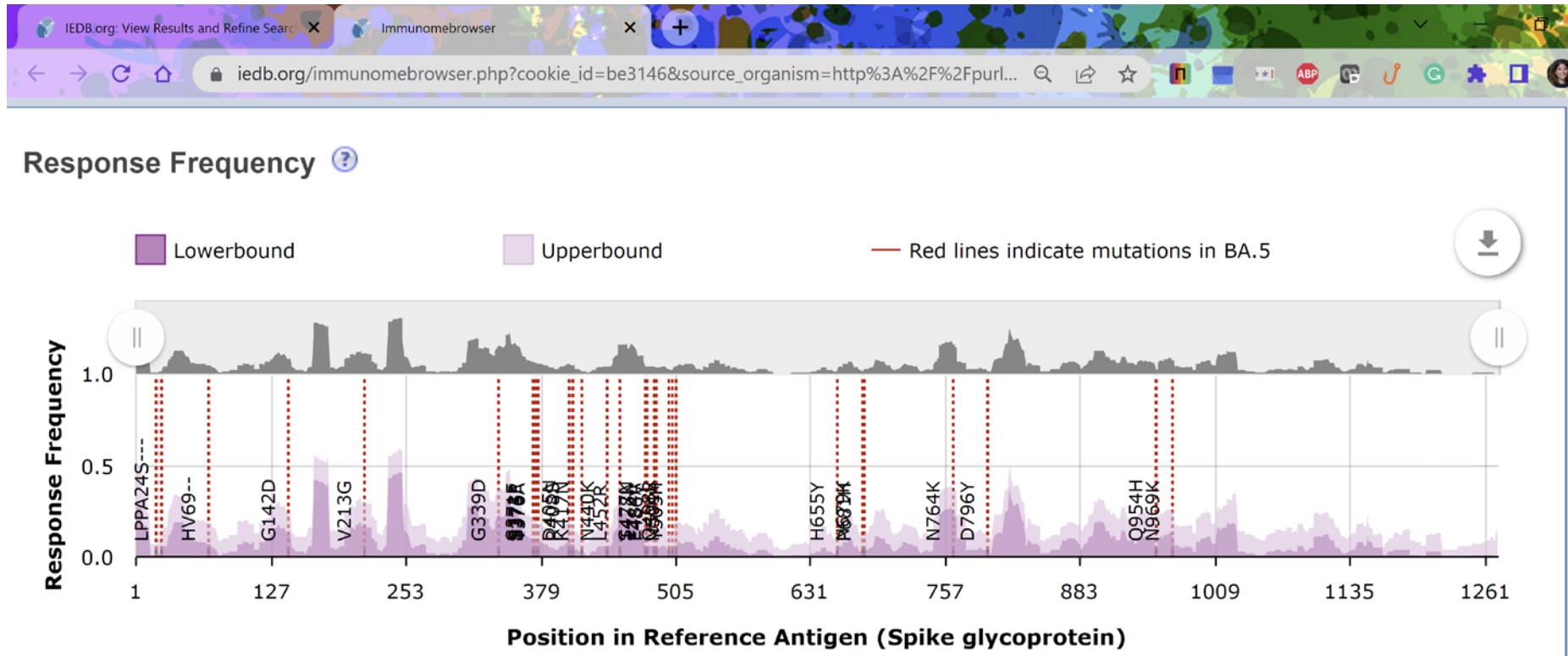


CD4 Immunobrowser: Spike

# How to use ImmunomeBrowser to define spike T cell immunodominant regions



## CD4 Immunobrowser: Spike



# ....Science is teamwork



Prof. Alex Sette



Alba Grifoni, PhD



Alison Tarke



John Sidney



Ricardo Da Silva  
Antunes, PhD



Esther Yu, MD



Prof. Daniela Weiskopf



Prof. Shane Crotty



Prof. Camila Coelho



Zeli Zhang, PhD



Prof. Jen Dan MD/PhD

Other members of  
Crotty Lab  
Nate Bloom  
Ben Goodwin

Other Members of  
Sette Lab  
Nils Methot  
Maria Reina-  
Campos  
April Frazier

Clinical Studies Core  
Gina Levi  
Shariza Bautista  
Quinn Bui  
Jasmine Cardenas



Prof. Richard  
Scheuermann



Yun Zhang,  
PhD

J. Craig Venter®  
INSTITUTE

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