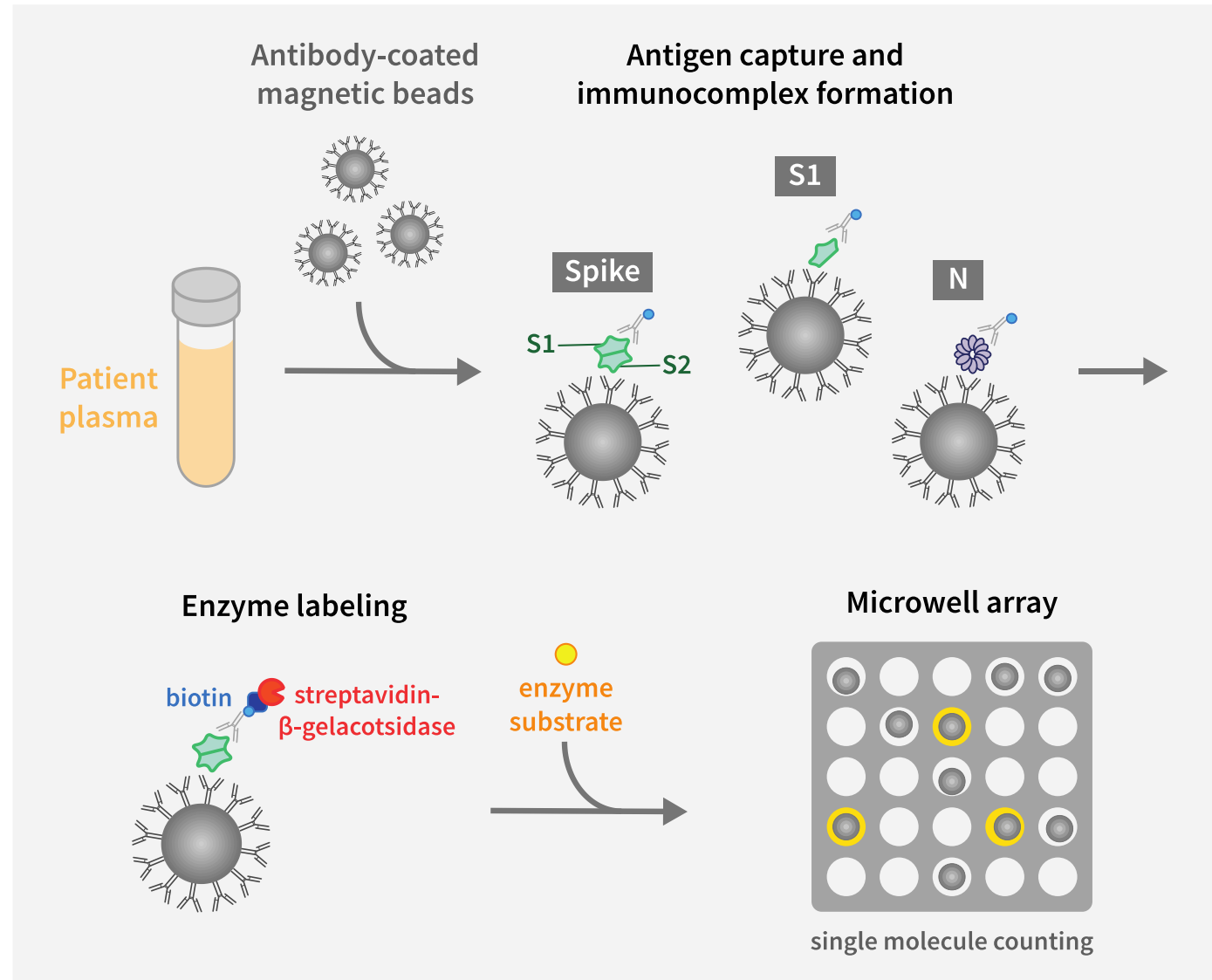
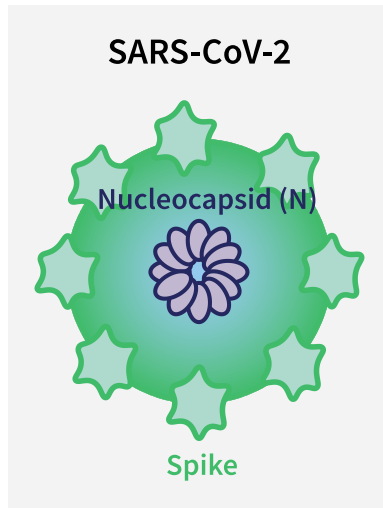


Ultrasensitive diagnostic assays: examples with SARS-CoV-2/ Long COVID

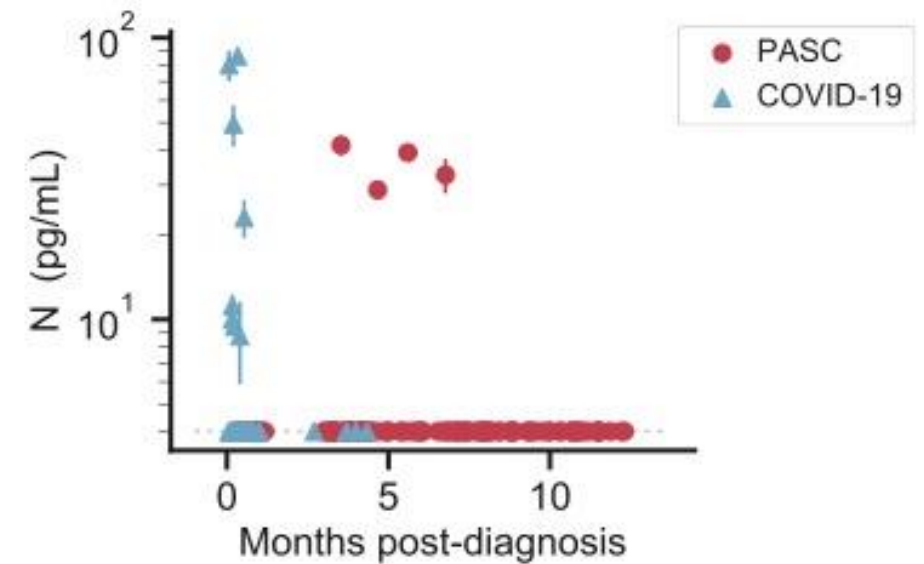
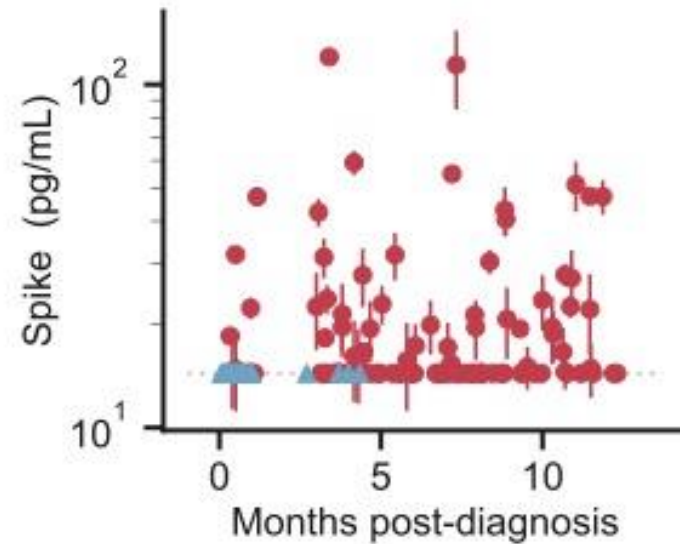
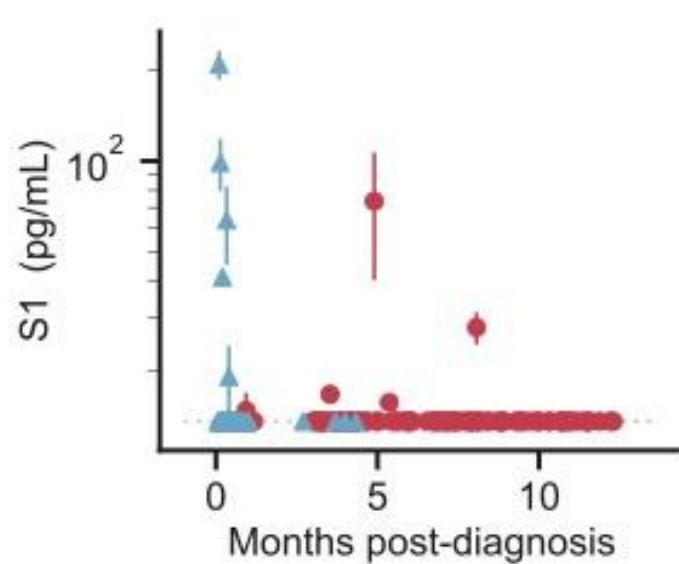
David R. Walt



Simoa assays for detecting SARS-CoV-2 antigens

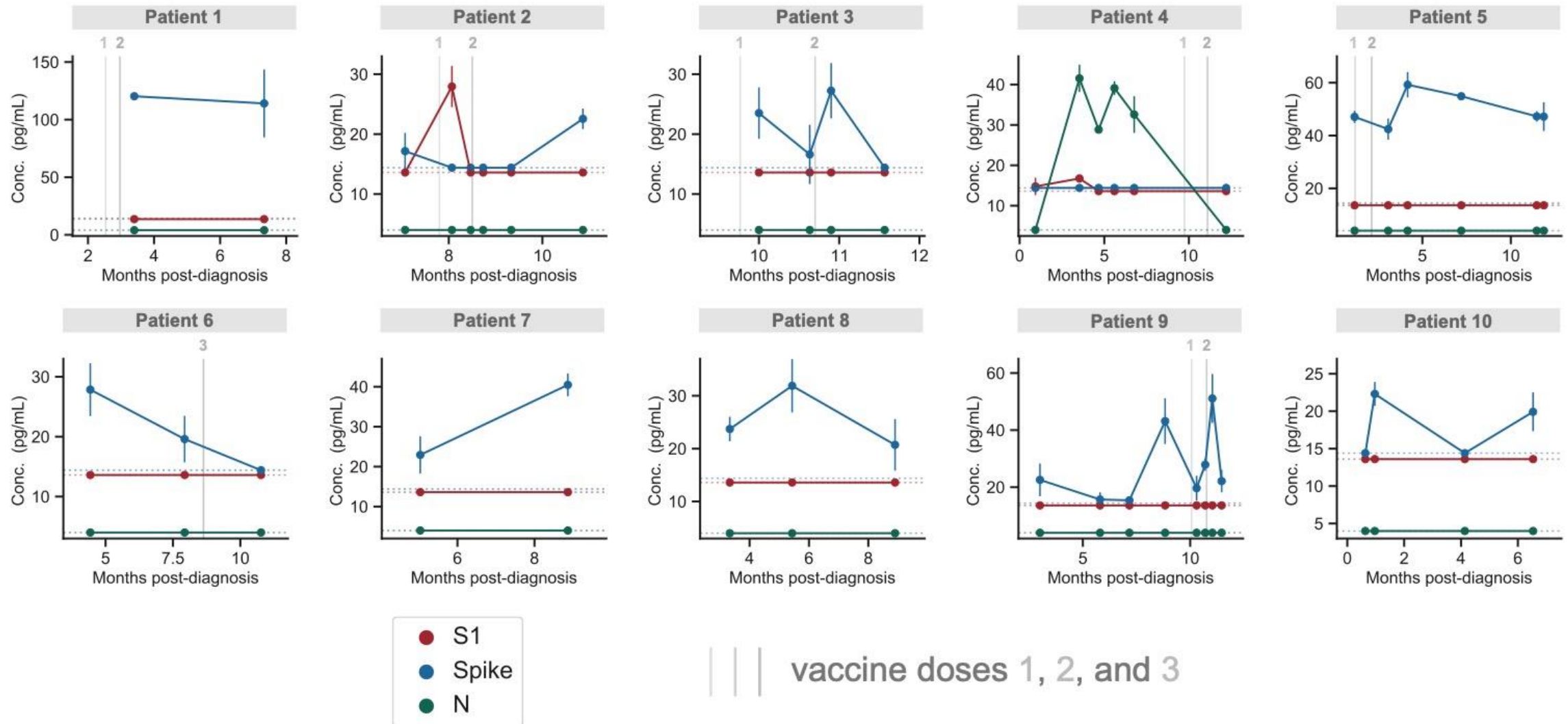


Preliminary Mass General Brigham cohort

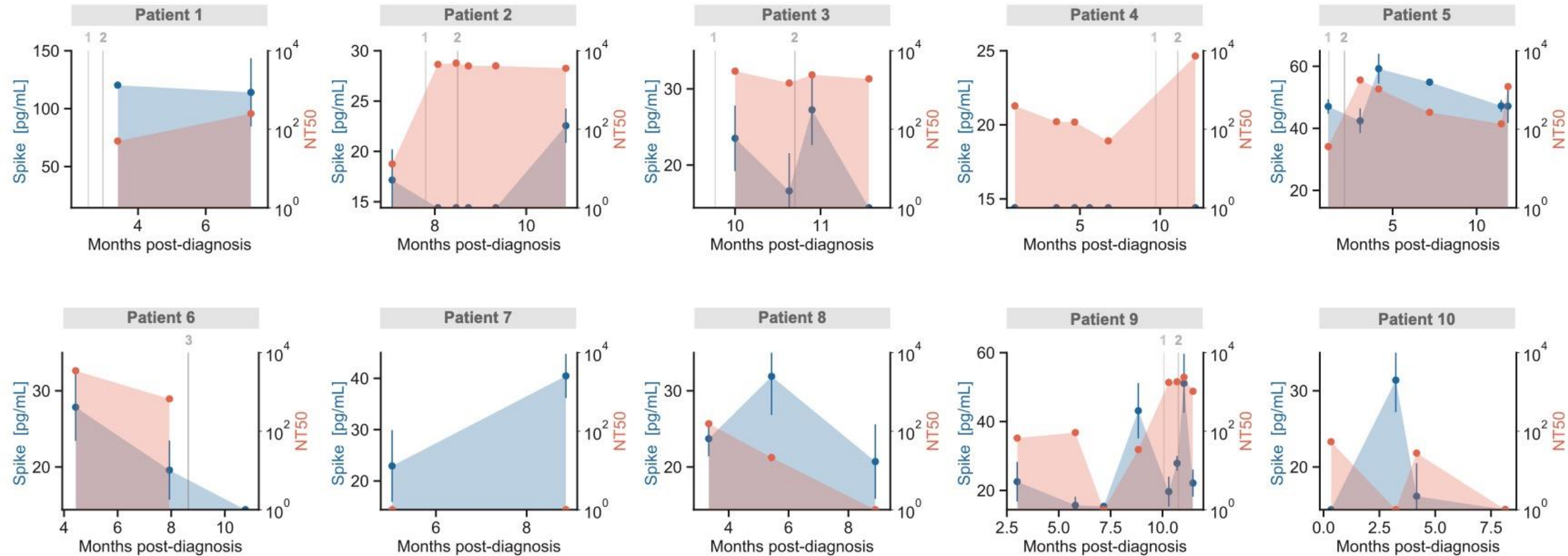


Spike detected in 60% of PASC patients

Temporal antigen profiles for individual PASC patients



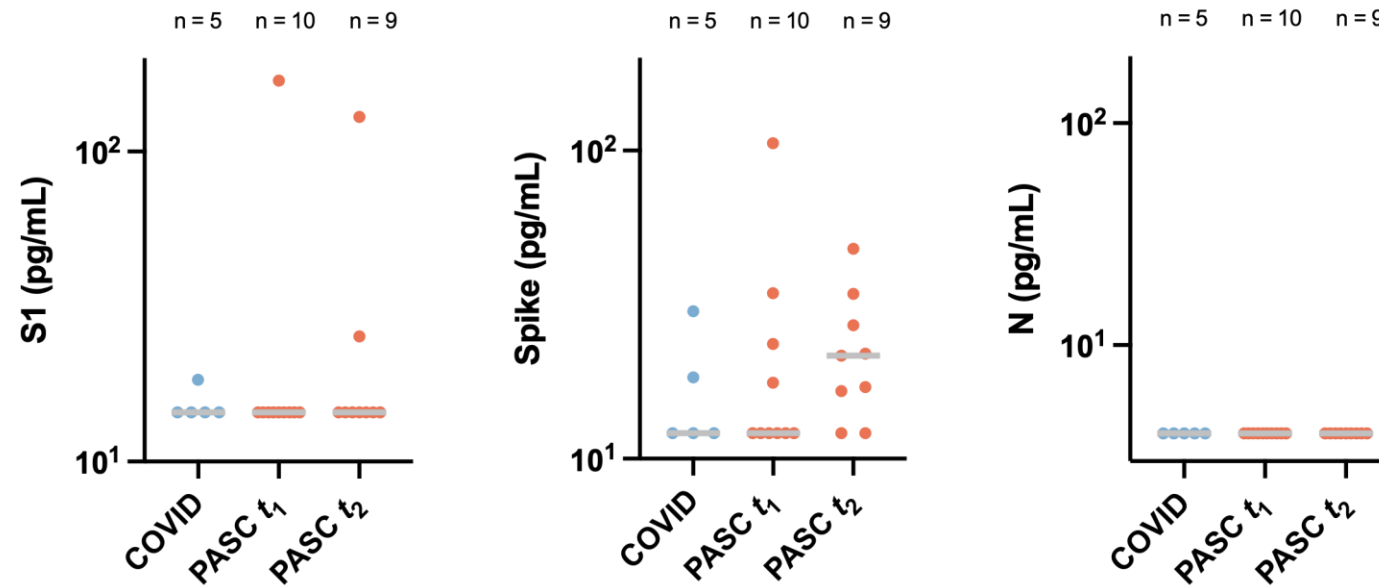
NT50s measured over time in PASC patients



||| vaccine doses 1, 2, and 3

MGH cardiology PASC/COVID-19 cohort

$t_1 \sim 3$ months post-infection
 $t_2 \sim 6$ months post-infection



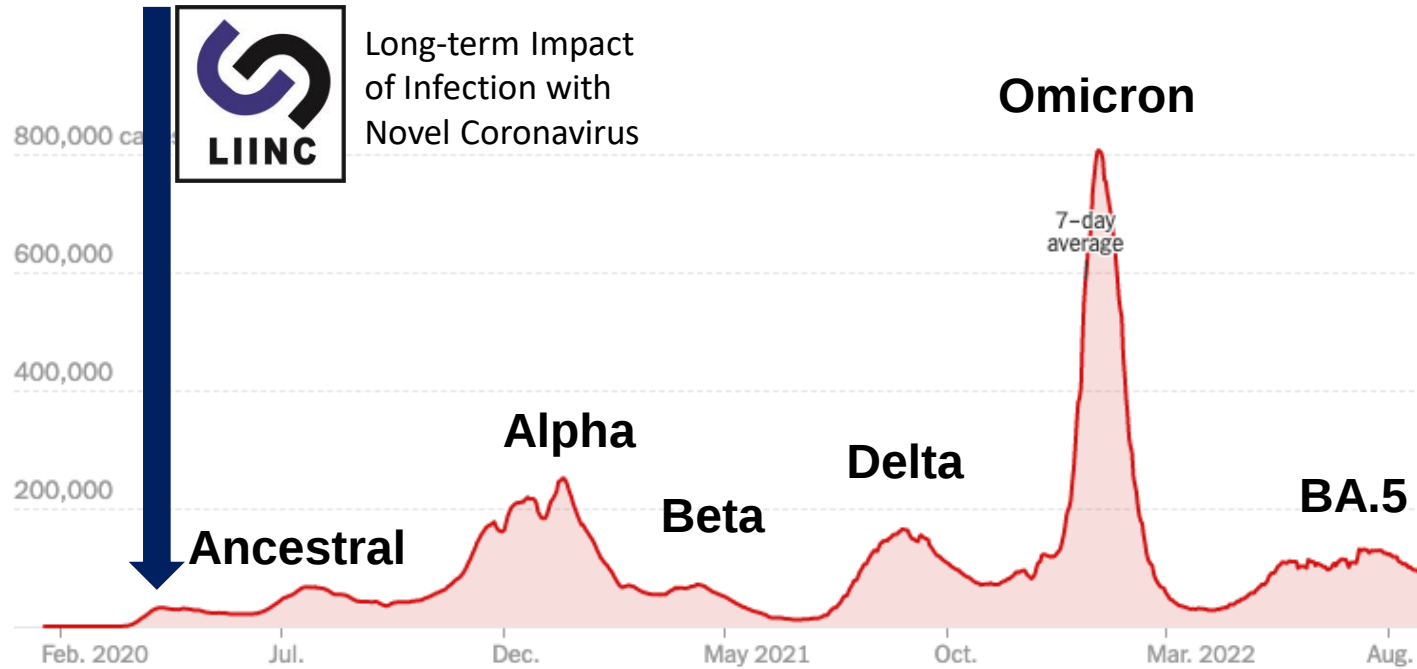
Percent of samples in which antigen was detected for each group

	COVID	PASC t ₁	PASC t ₂
S1	20%	10%	22%
Spike	40%	40%	78%
N	0%	0%	0%

RECOVER Cohort

3 Months	Analysis: Any Symptom				
		Total	Spike	S1	Nucleocapsid
PASC+		189/239 (79%)	38/189 (20%)	4/189 (2%)	7/189 (3%)
	Fatigue-Malaise	124/189 (65%)	30/124 (24%)	1/124 (0%)	7/124 (5%)
	Dysautonomia	69/189 (36%)	16/69 (23%)	0/69 (0%)	3/69 (4%)
	Cardio-Pulmonary	81/189 (42%)	15/81 (18%)	1/81 (1%)	1/81 (1%)
	Musculoskeletal	130/189 (68%)	28/130 (21%)	3/130 (2%)	7/130 (5%)
	Neurologic	70/189 (37%)	18/70 (25%)	1/70 (1%)	2/70 (2%)
	Psychiatric	26/189 (13%)	10/26 (38%)	0/26 (0%)	0/26 (0%)
	Sleep	56/189 (29%)	14/56 (25%)	0/56 (0%)	3/56 (5%)
	HEENT	105/189 (55%)	20/105 (19%)	0/105 (0%)	2/105 (1%)
	Dermatologic	33/189 (17%)	10/33 (30%)	0/33 (0%)	2/33 (6%)
	Genito-urinary	39/189 (20%)	10/39 (25%)	1/39 (2%)	0/39 (0%)
PASC-		50/239 (20%)	8/50 (16%)	1/50 (2%)	2/50 (4%)
All Patients		239	46/239 (19%)	5/239 (2%)	9/239 (3%)

The UCSF LIINC study investigates COVID sequelae



- Prospective
- > 700 participants
- Detailed clinical phenotyping
- > 50K specimens collected
- > 50 collaborations
- > 25 publications



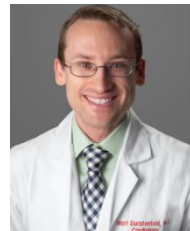
Michael
Peluso



Tim
Henrich



Priscilla
Hsue



Matt
Durstenfeld



Kim
Rhoads



Carina
Marquez



Dan
Kelly



Lekshmi
Santhosh



Jeff
Martin



Steven
Deeks

LIINC Persistent Antigen: Design and Cohort

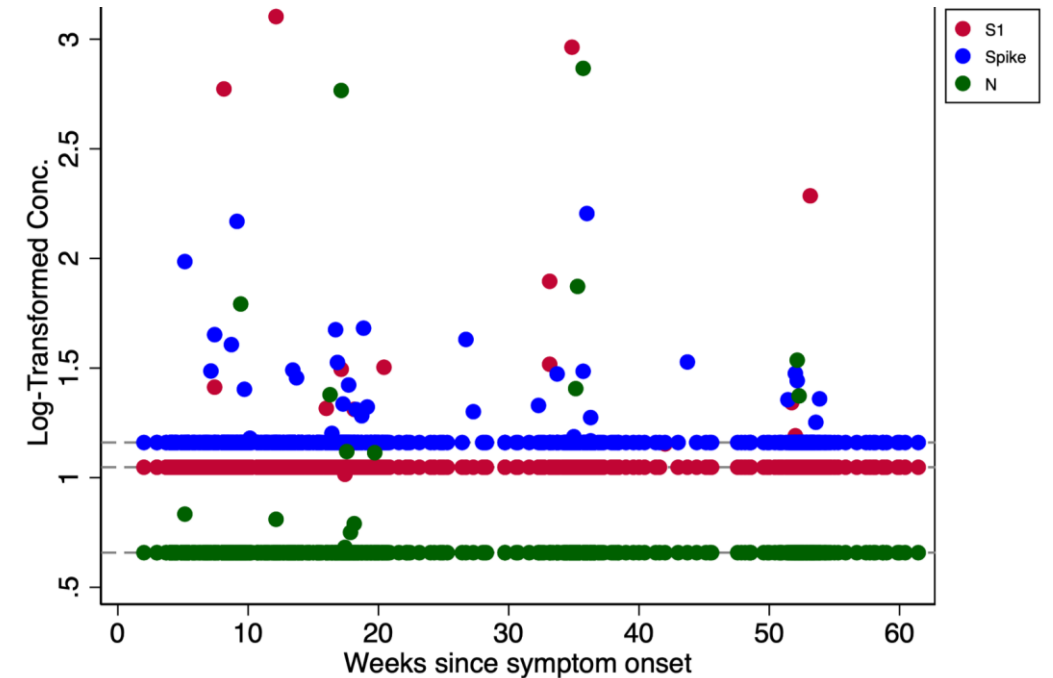


- 172 individuals across 661 timepoints using banked plasma specimens from 2020-2021
- All participants with prior confirmed SARS-CoV-2 infection
- Up to 5 samples per person, 30-450 days post-infection
- 77% of samples before any SARS-CoV-2 vaccine ever received
- Each biospecimen annotated with presence/absence of 32 individual symptoms around time of visit

Characteristic	
Age	46 (37-57)
Female	50%
Race/Ethnicity	
White	50%
Hispanic/Latino	27%
Asian	10%
Black/African American	5%
Pacific Islander	2%
Prior COVID Hospitalization	19%
Max Symptoms Across Visits	5 (1-8)
Median Symptoms Across Visits	2 (0-5)

LIINC Persistent Antigen: Results 1

- 61/661 (9.2%) samples positive for at least 1 antigen
- These 61 samples came from 42 unique people (26% of the cohort)
 - 29/42 individuals had antigen positive at 1 time point
 - 13/42 individuals had antigen positive at >1 time point
- Prevalence of antigen positivity
 - In visits with persistent symptoms = 9.8%
 - In visits without persistent symptoms = 7.8%



LIINC Persistent Antigen: Results 2

Prevalence of antigen by median symptom count in the cohort (by individuals)

Median Symptom Count	Ever S1 Positive	Ever Spike Positive	Ever N Positive	Ever Antigen Positive
0	3 (27%)	6 (23%)	2 (20%)	10 (24%)
0.5-3	5 (45%)	11 (42%)	2 (20%)	16 (38%)
3.5-7.5	1 (9.1%)	4 (15%)	3 (30%)	8 (19%)
≥8	2 (18%)	4 (19%)	3 (30%)	8 (19%)
Total	11	26	10	42

Interpretation: Highest concentration of positivity in those with relatively low number of symptoms (not necessarily the most extreme cases).

Prevalence of antigen by median symptom count (running median) in the cohort (by visit)

Median Symptom Count	S1 Positive at Timepoint	Spike Positive at Timepoint	N Positive at Timepoint	Antigen Positive
0	5 (33%)	10 (30%)	3 (20%)	18 (30%)
0.5-3	4 (27%)	9 (27%)	1 (6.7%)	14 (23%)
3.5-7.5	3 (20%)	8 (24%)	7 (47%)	17 (28%)
≥8	3 (20%)	6 (18%)	4 (27%)	12 (20%)
Total	15	33	15	61

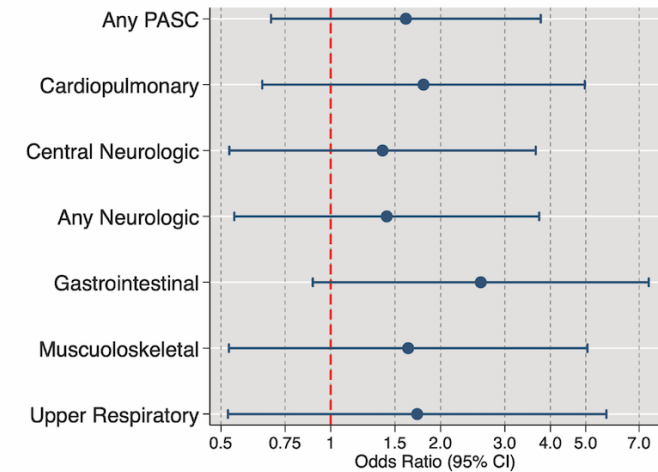
Interpretation: Among those with PASC, antigen detected among those with moderate number of symptoms up to that point. But a higher proportion without symptoms to that point also have antigen.

Ongoing analyses (Feb 2023) to look at overall health scores and severity of symptoms

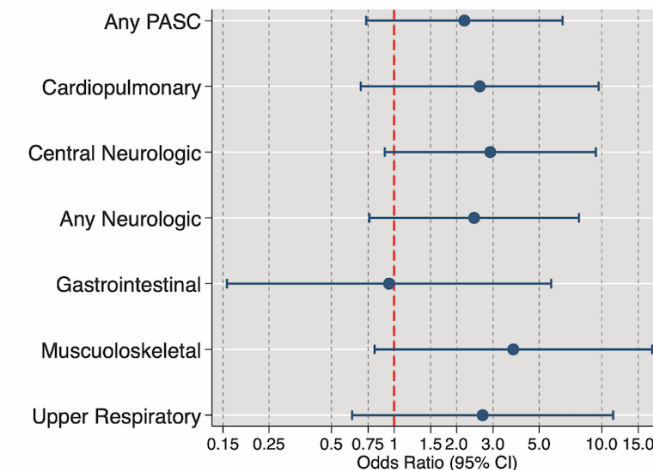
LIINC Persistent Antigen: Results 3

- Spike associated with GI symptoms (OR 2.2, $p=0.036$)
- N associated with CNS symptoms (OR 2.83, $p=0.06$) and musculoskeletal symptoms (OR 2.98, $p=0.038$)
- In regression models, persistent antigen associated with prior hospitalization (more severe illness), (OR 2.54, $p=0.004$)

Spike



Nucleocapsid





ALLEN INSTITUTE *for*
IMMUNOLOGY

Assessing viral persistence in an inflammatory sub-category of long COVID

Collaborative study with Fred Hutch to study the immune responses underlying long COVID



Dr. Julie McElrath



Julie Czartoski

Greater Seattle area, Washington, USA

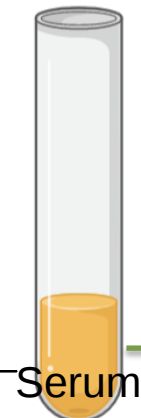
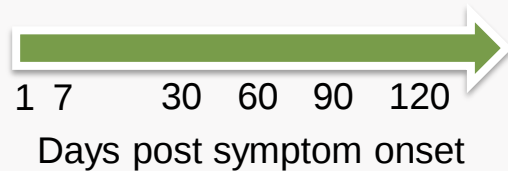
Uninfected
22



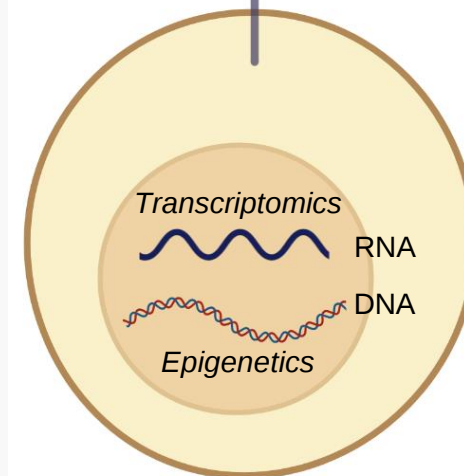
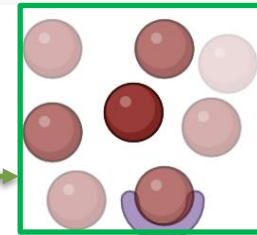
Infected
Recovered
24



Infected PASC
55

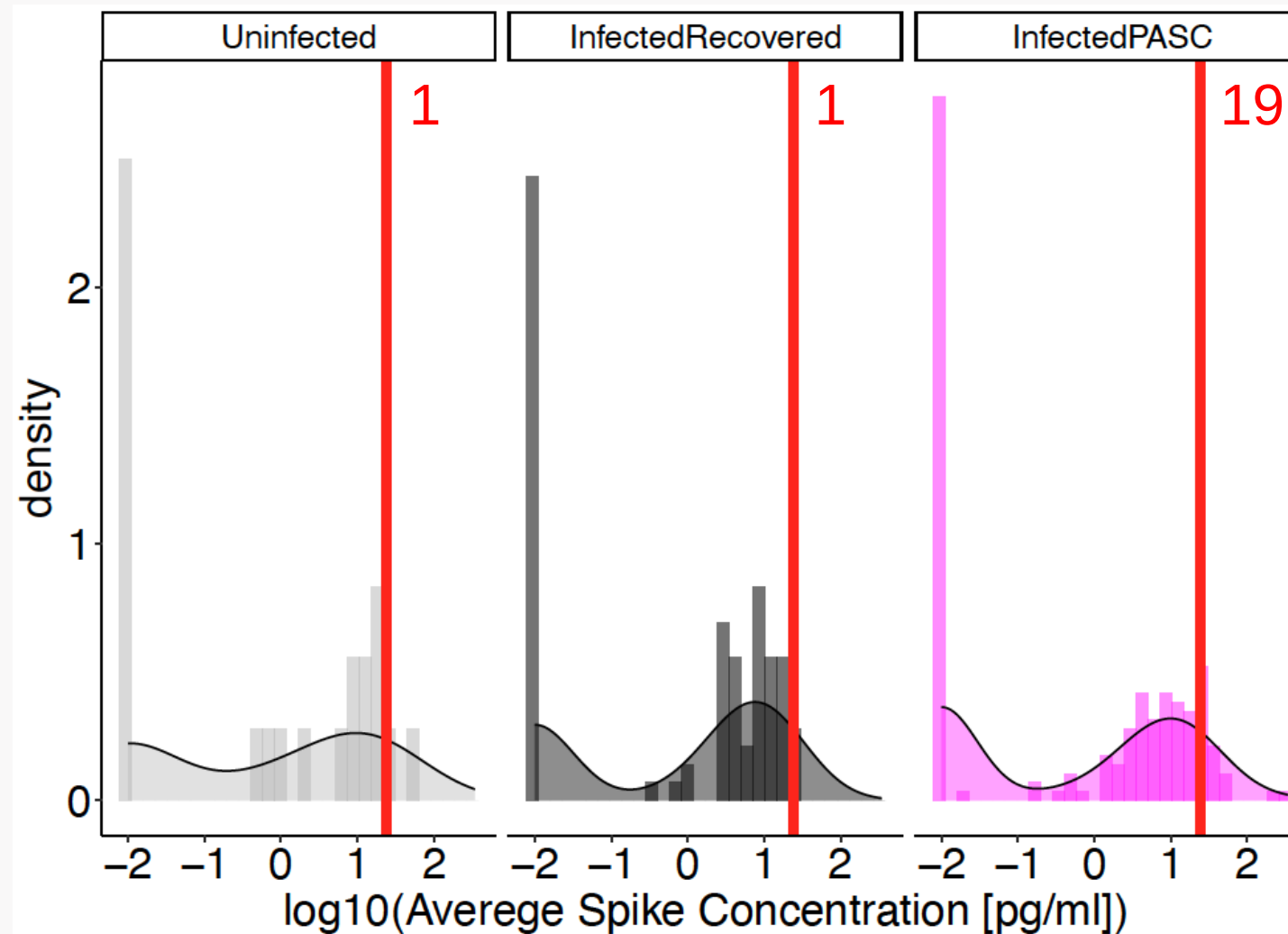


Serum proteins
indicative of an
active immune
system

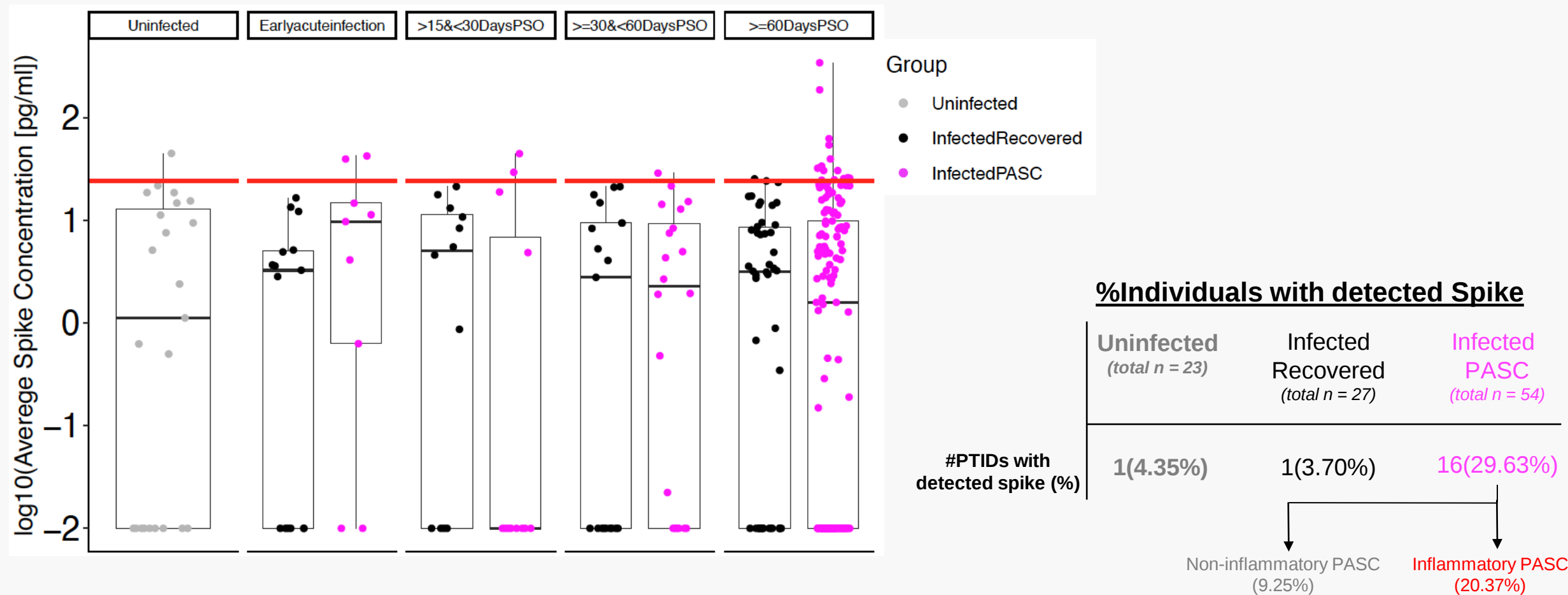


Spike concentration distribution in participant groups in samples CV < 50%

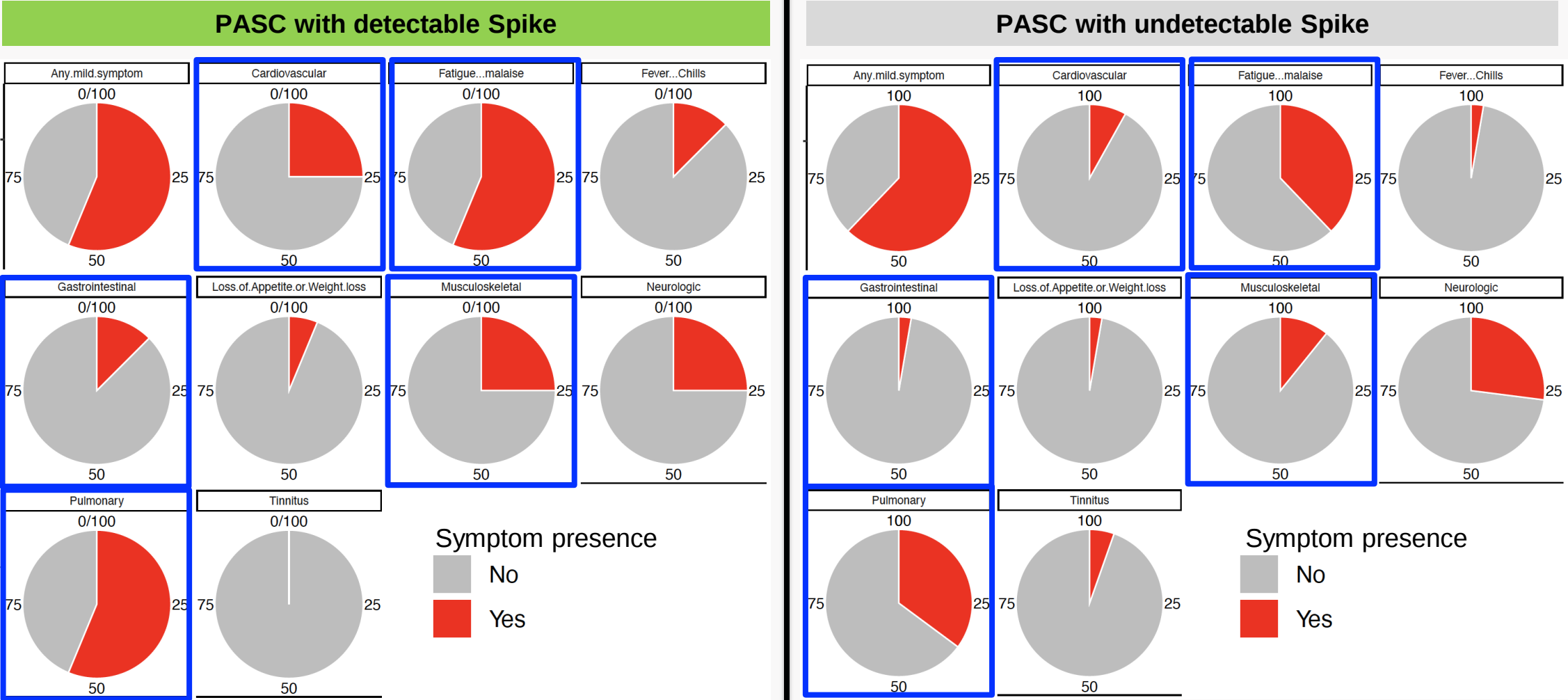
1 uninfected, 1 Recovered and 19 PASC samples had spike detected (>LOD)



Spike concentration distribution across timepoint bins in samples CV < 50%

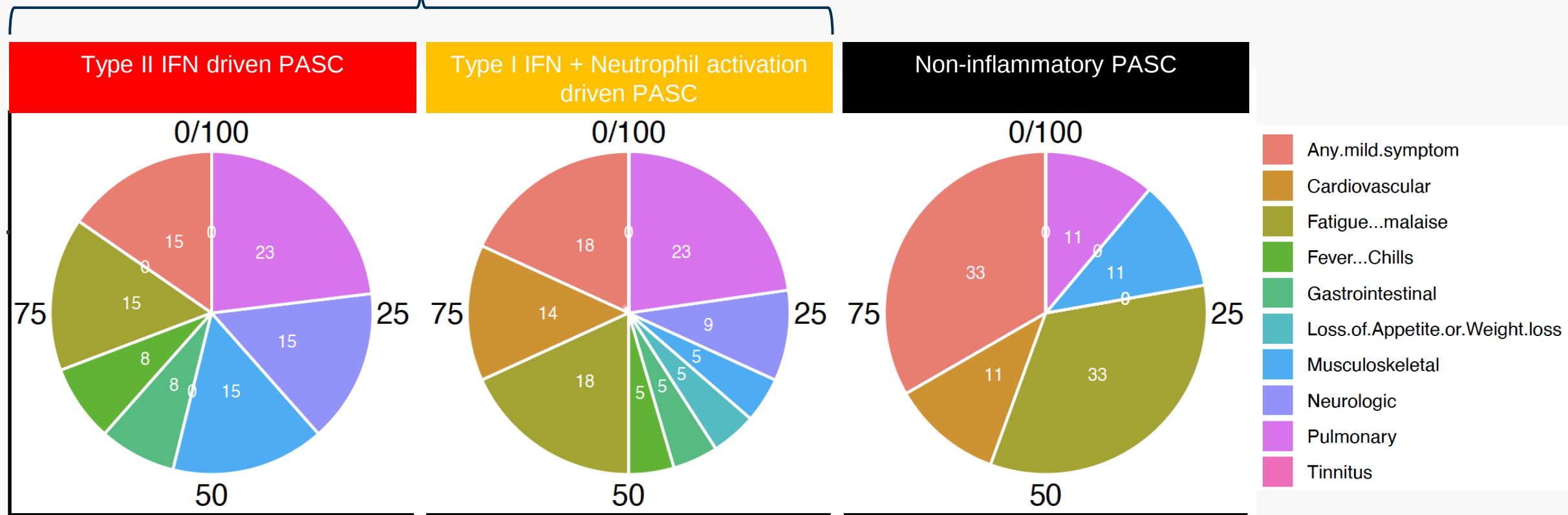


Cardiovascular, Gastrointestinal, Musculoskeletal, Pulmonary symptoms and Fatigue were more prevalent among PASC individuals with detectable Spike compared to PASC individuals with undetectable Spike



Among PASC with detected Spike, Pulmonary, Neurologic and gastrointestinal symptoms were more prevalent among the “inflammatory PASC” individuals

Inflammatory PASC groups



Acknowledgements



- Troy Torgerson
- Xiaojun Li
- Peter Skene
- Suhas Vasaikar

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- Lynne Becker



- Julie McElrath
- Julie Czartoski
- Zoe Moodie
- Maria Lemos
- Evan Newall
- Hugh MacMillan



- Ken Stuart
- Paurvi Shinde

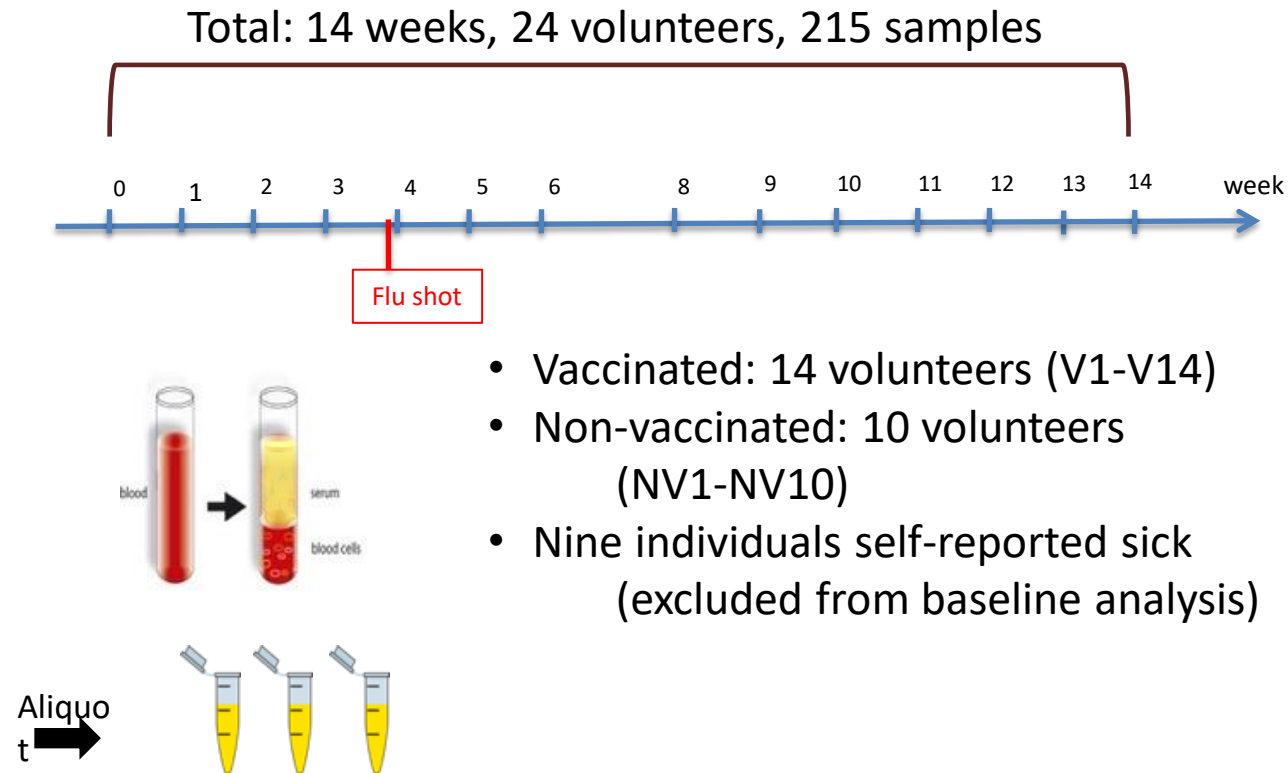
Long-term monitoring of baseline serum cytokines in healthy subjects at sub-femtomolar concentrations



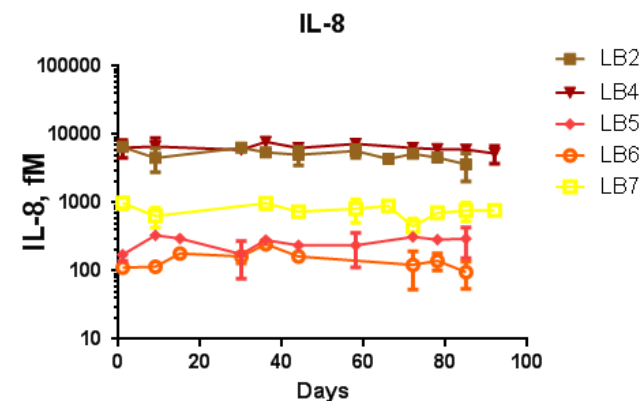
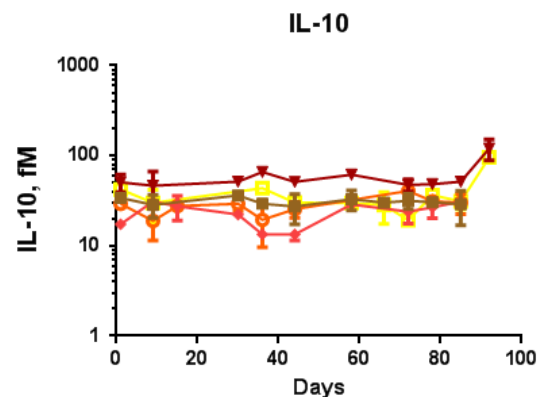
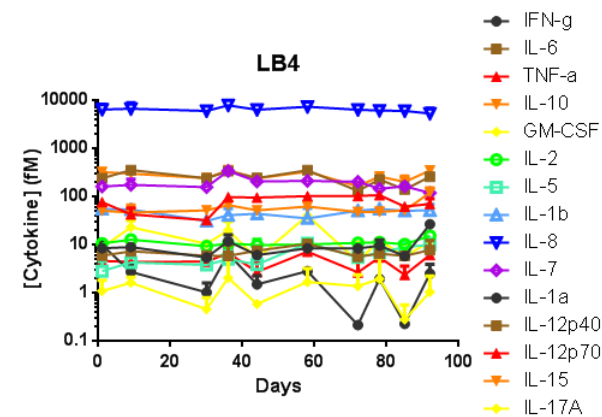
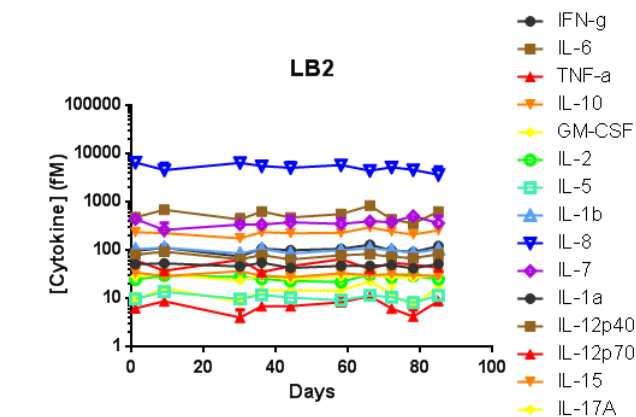
Bruce Bausk



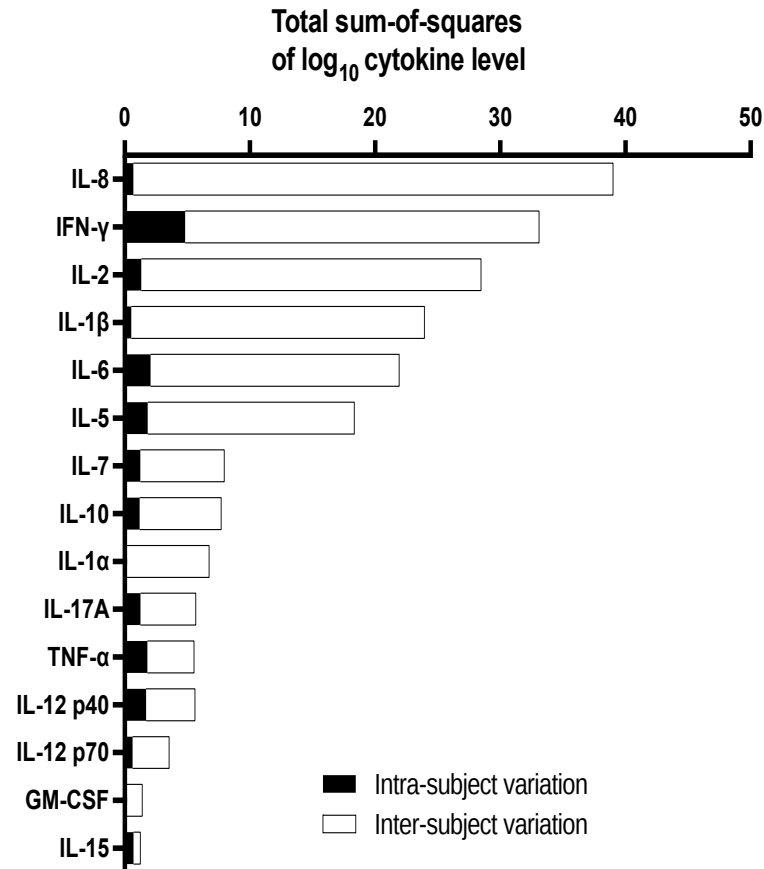
Study design



Baseline Study (Control Healthy Samples)



Variation in baseline cytokine levels in healthy subjects



Am. J. Pathology 2017



Conclusions and outlook

- Determining the link between antigen persistence and PASC may depend on:
 - Definition of PASC
 - Pandemic wave
 - Vaccination status and comorbidities
 - Sample timing
 - Source of circulating antigen
- Later time points have higher prevalence.
- Original strain is different and had higher spike than post omicron
- “Uninfected”—not really
- Should be thought of as qualitative not quantitative.
- Oscillatory—mobilization and immune response
- Viral persistence does not necessarily cause PASC



RECOVER-VITAL

(Viral persIstence and reacTivation, And immune dysreguLation)

Key Question: Does a study intervention improve outcomes for people with ongoing symptoms from PASC?



Population

~900 adults with
3 symptom clusters



Intervention(s)

Antiviral drug



Health Measures

- Patient-reported outcomes
- Performance-based outcomes
- Safety and tolerability



Sites

Up to 100



Launch

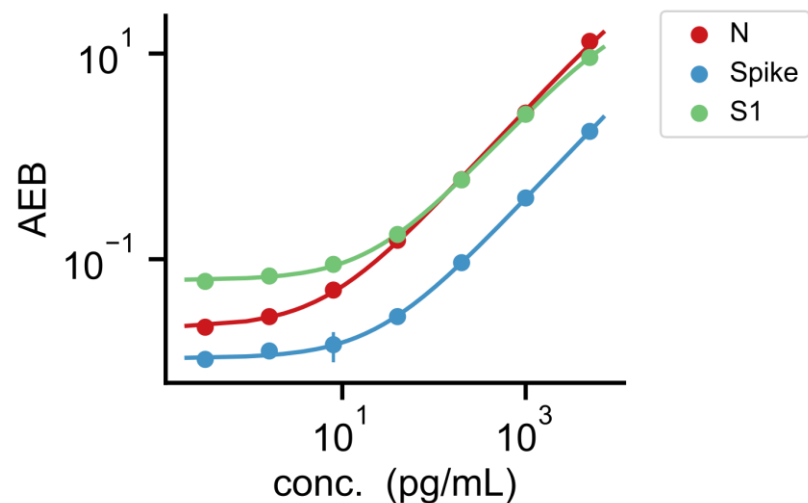
Summer
2023



RECOVER Antigen Assay

CLIA Validation Update

Linearity and Dynamic Range

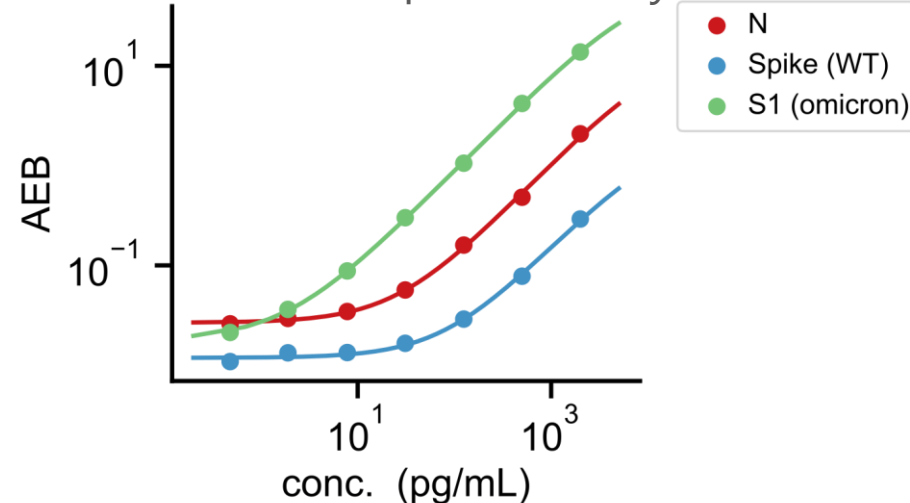


N

Spike

S1

Omicron specific assay



	Sample 1	Sample 2
Conc.	Recovery	Recovery
100 pg/mL	60%	70%
500 pg/mL	75%	69%

0.5% tween

	Sample 1	Sample 2
Conc.	Recovery	Recovery
100 pg/mL	58%	60%
500 pg/mL	70%	54%

0.5% triton

	Sample 1	Sample 2
Conc.	Recovery	Recovery
100 pg/mL	136%	149%
500 pg/mL	116%	134%

0.5% triton

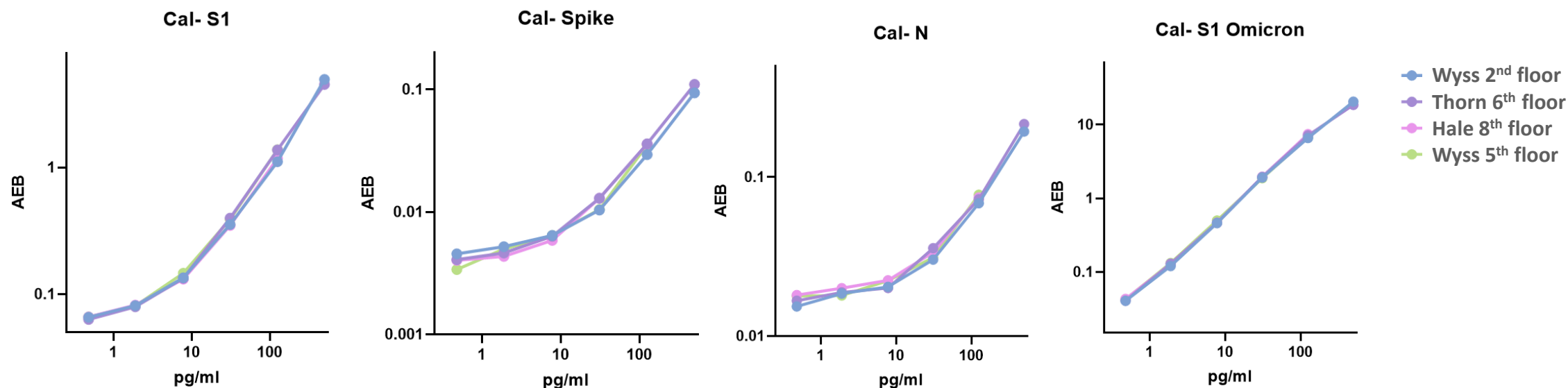
Conc.	Recovery	Recovery
100 pg/mL	95%	100%
500 pg/mL	124%	115%

Conc.	Recovery	Recovery
100 pg/mL	83%	82%
500 pg/mL	94%	81%

Conc.	Recovery	Recovery
100 pg/mL	103%	117%
500 pg/mL	117%	109%

Equipment equivalency

Calibration curves and spike and recovery measurements were performed on four HDx instruments: Wyss 2nd floor, Thorn 6th floor, Hale 8th floor, and Wyss 5th floor



	S1	Spike	N	S1 Omicron	Average Calibration Curve CV%	6
LoD AVERAGE (fg/ml)	66.94	6.09	19.39	12.66	Inter-Assay Calibration Curve CV%	7
LoD CV%	6	16	8	13		

Thank you!

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