

Prior vaccination promotes early activation of memory T cells and enhances immune responses during SARS-CoV-2 breakthrough infection

In the format provided by the
authors and unedited

Supplementary Table 1: Cohort Summary

Characteristics		Longitudinal Omicron Cohort	Cross-sectional Unvaccinated	Cross-sectional Vaccinated
Total	Number	22	31	59
Age	Average (Years)	34.3	38.5	43.6
	Range (Years)	23-60	23-64	23-73
	20-30	10 (45%)	9 (29%)	12 (20%)
	30-40	7 (32%)	10 (32%)	15 (25%)
	40-50	2 (9%)	5 (16%)	8 (14%)
	50+	3 (14%)	7 (23%)	24 (41%)
Sex	Male	9 (41%)	13 (42%)	25 (42%)
	Female	13 (59%)	18 (58%)	34 (58%)
Race/Ethnicity	White - Non-Hispanic/Latino	17 (77%)	1 (3%)	25 (42%)
	White - Hispanic/Latino	1 (5%)	0	2 (3%)
	Asian	3 (14%)	0	6 (10%)
	Black	1 (5%)	30 (97%)	24 (41%)
	Native	0	0	0
	Other	0	0	2 (3%)
HLA Type	HLA-A*02:01	11 (50%)	11 (35%)	31 (53%)
	HLA-A*03:01	3 (14%)	3 (10%)	11 (19%)
Vaccine doses	2	0	0	At least 2 doses: 59 (100%)
	3	16 (73%)	0	
	4	6 (27%)	0	
Dominant Variant	Alpha	0	15 (48%)	3 (5%)
	Delta	0	14 (45%)	28 (47%)
	Omicron	22 (100%)	2 (6%)	28 (47%)
Days post-test	Mean (IQR)	NA	8.5 (6 - 10.5)	9.7 (7 - 13)

Supplementary Table 2: Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
BUV563 anti-CD3	BD Biosciences	Cat#748569
BV750 anti-CD19	Biolegend	Cat#302262
BUV805 anti-CD20	BD Biosciences	Cat#612905
BUV395 anti-CD27	BD Biosciences	Cat#563815
BUV661 anti-CD38	BD Biosciences	Cat#612969
APC-H7 anti-CD71	BD Biosciences	Cat#563671
AF700 anti-CD11c	Biolegend	Cat#337220
FITC anti-IgA	Miltenyi	Cat#130-113-475
BV480 anti-IgD	BD Biosciences	Cat#566138
PE/Dazzle 594 anti-CD21	Biolegend	Cat#354922
PE-Cy7 anti-IgG	Biolegend	Cat#410722
mouse anti-VSV Indiana G, 1E9F9	Absolute Antibody	Cat#Ab01402-2.0
goat anti-human IgG-HRP	Jackson ImmunoResearch Laboratories	RRID: AB_2337596
BUV395 CD4	BD Biosciences	Cat#563550
BUV496 CD8	BD Biosciences	Cat#612943
BUV615 CD45RA	BD Biosciences	Cat#751555
BUV737 CD27	BD Biosciences	Cat#612829
BUV805 CD3	BD Biosciences	Cat#612896
BV421 CXCR3	Biolegend	Cat#353716
BV650 CCR7	Biolegend	Cat#353234
BV605 CD69	Biolegend	Cat#310938
BV711 CD40L	Biolegend	Cat#310838
BV785 CD107a	Biolegend	Cat#328644
FITC IFN γ	Biolegend	Cat#502515
PE CD200	Biolegend	Cat#399804
PE-Cy7 OX40	Biolegend	Cat#350012
AF647 41BB	Biolegend	Cat#309810
APC-R700 CXCR5	BD Biosciences	Cat#565191
APC-Cy7 CCR6	Biolegend	Cat#353432
BB700 CD127 (anti-human)	BD	566398
AF647 TCF1 (anti-human)	CellSignaling	6709S
AF700 CCR7 (anti-human)	Biolegend	353244
APC/Fire750 KLRG1 (anti-human)	Biolegend	367718
BUV395 CD45RA (anti-human)	BD	740298
BUV496 CD8 (anti-human)	BD	612942
BUV661 CX3CR1 (anti-human)	BD	750690
BUV737 CD27 (anti-human)	BD	612830
BUV805 CD3 (anti-human)	BD	612896
BV421 PD-1 (anti-human)	Biolegend	329920
BV480 CD49d (anti-human)	BD	566134
V500 CD14 (anti-human)	BD	561391
V500 CD16 (anti-human)	BD	561394
V500 CD19 (anti-human)	BD	561121
BV510 CD41a (anti-human)	BD	563250

BV605 HLA-DR (anti-human)	Biolegend	307640
BV711 Ki67 (anti-human)	Biolegend	350516
BV750 CD38 (anti-human)	BD	746909
PE-Cy5 CD95 (anti-human)	BD	561977
PE-Cy5.5 CD4 (anti-human)	Invitrogen	35-0047-42
PE-Cy7 T-bet (anti-human)	Biolegend	644824
PE-TexasRed Granzyme B (anti-human)	Invitrogen	GRB17
BUV563 CD39 (anti-human)	BD	748473
Bacterial and virus strains		
SARS-CoV-2 VSV pseudotypes	Generated for this paper	
Biological samples		
Human peripheral blood samples	Collected at the University of Pennsylvania	N/A
Chemicals, peptides, and recombinant proteins		
SARS-CoV-2 Biotinylated Full Length Spike	R&D Systems	Cat#AVI10549-050
HA(Δ TM)(A/Brisbane/02/2018)(H1N1)	Immune Tech	Cat#IT-003-00110 Δ TMp
HA(Δ TM)(B/Colorado/06/2017)	Immune Tech	Cat#IT-003-B21 Δ TMp
SARS-CoV-2 Biotinylated RBD (WT)	Acro Biosystems	Cat#SPD-C82E9-25ug
SARS-CoV-2 Biotinylated RBD (Delta)	Acro Biosystems	Cat#SPD-C82E5-25ug
SARS-CoV-2 Biotinylated RBD (Omicron BA.5)	Acro Biosystems	Cat#SPD-C82Ew-25ug
SARS-CoV-2 Biotinylated RBD (Omicron BA.1)	Acro Biosystems	Cat#SPD-C82E4-25ug
SARS-CoV-2 Biotinylated N-Terminal Domain	Sino Biological	Cat#40591-V49H-B
SARS-CoV-2 Biotinylated S2	Acro Biosystems	Cat#S2N-C52E8-25ug
SARS-CoV-2 Biotinylated Nucleocapsid	R&D Systems	Cat#BT10474-050
SARS-CoV-2 (BA.1.1) Spike RBD Protein (His Tag)	Sino Biological	Cat#40592-V08H129
SARS-CoV-2 (BA.4/BA.5) Spike RBD Protein (His Tag)	Sino Biological	Cat#40592-V08H130
BV421 Streptavidin	Biolegend	Cat#405226
BV605 Streptavidin	Biolegend	Cat#405229
BV711 Streptavidin	BD Biosciences	Cat#563262
BV786 Streptavidin	BD Biosciences	Cat#563858
BUV615 Streptavidin	BD Biosciences	Cat#613013
BUV737 Streptavidin	BD Biosciences	Cat#612775
BB515 Streptavidin	BD Biosciences	Cat#564453
PE Streptavidin	Biolegend	Cat#405203
PE-Cy7 Streptavidin	Biolegend	Cat#405206
APC Streptavidin	Biolegend	Cat#405207
R848	Invivogen	Cat#tlrl-r848-5
CellTrace Violet	Thermo Fisher	Cat#C34571
Zombie NIR Viability Dye	Biolegend	Cat#423106
Ghost Viability Dye Violet 510	Tonbo	Cat#13-0870-T100
Human TruStain FcX (Fc Receptor Blocking Solution)	Biolegend	Cat#422302
EZ-Link Micro NHS-PEG4 Biotinylation Kit	Thermo Fisher	Cat#21955
Zeba Spin Desalting Columns 7K MWCO	Thermo Fisher	Cat#89894
CD4-S peptide megapool	Synthetic Biomolecules (aka A&A)	http://www.syntheticbiomolecules.com/
CD4-RE peptide megapool	Synthetic Biomolecules (aka A&A)	http://www.syntheticbiomolecules.com/

CD8-RE peptide megapool	Synthetic Biomolecules (aka A&A)	http://www.syntheticbiomolecules.com/
GolgiStop (Containing Monensin)	BD Biosciences	Cat#51-2092K7
CD40 Antibody, anti-human, pure-functional grade	Miltenyi Biotech	Cat#130-094-133
Anti-Human CD28/CD49d Purified	BD Biosciences	Cat#347690
Foxp3 / Transcription Factor Fixation/Permeabilization Concentrate and Diluent	eBioscience	Cat#00-5521-00
ALWEIQQVV	Genscript	Custom synthesis
FLAHIQWMV	Genscript	Custom synthesis
FLLNKEMYL	Genscript	Custom synthesis
KLWAQCVQL	Genscript	Custom synthesis
SMWALIISV	Genscript	Custom synthesis
YLATALLTL	Genscript	Custom synthesis
LALLLLDRL	Genscript	Custom synthesis
GMSRIGMEV	Genscript	Custom synthesis
LLLDRLNQL	Genscript	Custom synthesis
ALSKGVHFV	Genscript	Custom synthesis
LLYDANYFL	Genscript	Custom synthesis
ALNTLVKQL	Genscript	Custom synthesis
KIADYNYKL	Genscript	Custom synthesis
RLQSLQTYV	Genscript	Custom synthesis
SIIAYTMSL	Genscript	Custom synthesis
VVFLHVTYV	Genscript	Custom synthesis
FIAGLIAIV	Genscript	Custom synthesis
NLNESLIDL	Genscript	Custom synthesis
RLNEVAKNL	Genscript	Custom synthesis
TLDSKTQSL	Genscript	Custom synthesis
VLNDILSRL	Genscript	Custom synthesis
YLQPRTFLL	Genscript	Custom synthesis
KLFAAETLK	Genscript	Custom synthesis
KLFDYFKY	Genscript	Custom synthesis
KTIQPRVEK	Genscript	Custom synthesis
VTNNTFTLK	Genscript	Custom synthesis
KTFPPTPEK	Genscript	Custom synthesis
GVYFASTEK	Genscript	Custom synthesis
KCYGVSPK	Genscript	Custom synthesis
Flex-T™ HLA-A*02:01 Monomer UVX	BioLegend	Cat#280004
Flex-T™ HLA-A*03:01 Monomer UVX	BioLegend	Cat#280005
Experimental models: Cell lines		
293T	ATCC	RRID: CVCL_0063
VeroE6/TMPRSS	Stefan Pohlman	Anderson et al., 2021
Recombinant DNA		
Plasmid: pCAGGS SARS-CoV-2 spike	Florian Krammer	Amanat et al. (2020)
Plasmid: pCAGGS SARS-CoV-2 RBD	Florian Krammer	Amanat et al. (2020)
Plasmid: pCG1 SARS-CoV-2 D614G delta18	Paul Bates Lab	N/A
Plasmid: pCG1 SARS-CoV-2 B.A.1.1 delta18	Paul Bates Lab	N/A

Supplementary Table 3: Antibody Dilutions for Flow Cytometry

REAGENT or RESOURCE	SOURCE	Dilution Factor	Clone	IDENTIFIER
BUV563 anti-CD3	BD Biosciences	200	UCHT1	Cat#748569
BV750 anti-CD19	Biolegend	100	HIB19	Cat#302262
BUV395 anti-CD27	BD Biosciences	200	L128	Cat#563815
BUV661 anti-CD38	BD Biosciences	1000	HIT2	Cat#612969
APC-H7 anti-CD71	BD Biosciences	50	M-A712	Cat#563671
FITC anti-IgA	Miltenyi	400	IS11-8E10	Cat#130-113-475
BV480 anti-IgD	BD Biosciences	50	IA6-2	Cat#566138
PE/Dazzle 594 anti-CD21	Biolegend	500	Bu32	Cat#354922
AF700 anti-IgG	BD	200	G18-145	Cat#561296
BUV395 CD4	BD Biosciences	400	SK3	Cat#563550
BUV496 CD8	BD Biosciences	400	RPA-T8	Cat#612943
BUV615 CD45RA	BD Biosciences	2000	HI100	Cat#751555
BUV737 CD27	BD Biosciences	400	L128	Cat#612829
BUV805 CD3	BD Biosciences	800	UCHT1	Cat#612896
BV421 CXCR3	Biolegend	800	G02587	Cat#353716
BV650 CCR7	Biolegend	400	G043H7	Cat#353234
BV605 CD69	Biolegend	400	FN50	Cat#310938
BV711 CD40L	Biolegend	50	24-31	Cat#310838
BV785 CD107a	Biolegend	100	H4A3	Cat#328644
FITC IFN γ	Biolegend	400	4S.B3	Cat#502515
PE CD200	Biolegend	100	A18042B (OX2)	Cat#399804
PE-Cy7 OX40	Biolegend	1600	Ber-ACT35	Cat#350012
AF647 41BB	Biolegend	400	4B4-1	Cat#309810
APC-R700 CXCR5	BD Biosciences	100	RF8B2	Cat#565191
APC-Cy7 CCR6	Biolegend	800	G034E3	Cat#353432
BB700 CD127 (anti-human)	BD	50	HIL-7R-M21	566398
AF647 TCF1 (anti-human)	CellSignaling	400	C63D9	6709S
AF700 CCR7 (anti-human)	Biolegend	100	G043H7	353244
APC/Fire750 KLRG1 (anti-human)	Biolegend	100	SA231A2	367718
BUV395 CD45RA (anti-human)	BD	800	HI100	740298
BUV496 CD8 (anti-human)	BD	400	RPA-T8	612942
BUV661 CX3CR1 (anti-human)	BD	100	2A9-1	750690
BUV737 CD27 (anti-human)	BD	200	L128	612830
BUV805 CD3 (anti-human)	BD	400	UCHT1	612896
BV421 PD-1 (anti-human)	Biolegend	50	EH12.2H7	329920
BV480 CD49d (anti-human)	BD	50	9F10	566134
V500 CD14 (anti-human)	BD	400	M5E2	561391
V500 CD16 (anti-human)	BD	100	3G8	561394
V500 CD19 (anti-human)	BD	800	HIB19	561121
BV510 CD41a (anti-human)	BD	800	HiP8	563250
BV605 HLA-DR (anti-human)	Biolegend	200	L243	307640
BV711 Ki67 (anti-human)	Biolegend	2500	Ki-67	350516
BV750 CD38 (anti-human)	BD	100	HIT2	746909
PE-Cy5 CD95 (anti-human)	BD	50	DX2	561977
PE-Cy5.5 CD4 (anti-human)	Invitrogen	1600	SK3	35-0047-42

PE-Cy7 T-bet (anti-human)	Biolegend	1250	4B10	644824
PE-TexasRed Granzyme B (anti-human)	Invitrogen	5000	GB11	GRB17
BUV563 CD39 (anti-human)	BD	50	TU66	748473

Penn Medicine BioBank Banner Author List and Contribution Statements

PMBB Leadership Team

Daniel J. Rader, M.D., Marylyn D. Ritchie, Ph.D., Michael D. Feldman M.D.

Contribution: All authors contributed to securing funding, study design and oversight. All authors reviewed the final version of the manuscript.

Patient Recruitment and Regulatory Oversight

JoEllen Weaver, Nawar Naseer, Ph.D., M.P.H., Afiya Poindexter, Ashlei Brock, Khadijah Hu-Sain, Yi-An Ko

Contributions: JW manages patient recruitment and regulatory oversight of study. NN manages participant engagement, assists with regulatory oversight, and researcher access. AP, AB, KH, YK perform recruitment and enrollment of study participants.

Lab Operations

JoEllen Weaver, Meghan Livingstone, Fred Vadivieso, Ashley Kloter, Stephanie DerOhannessian, Teo Tran, Linda Morrel, Ned Haubein, Joseph Dunn

Contribution: JW, ML, FV, SD conduct oversight of lab operations. ML, FV, AK, SD, TT, LM perform sample processing. NH, JD are responsible for sample tracking and the laboratory information management system.

Clinical Informatics

Anurag Verma, Ph.D., Colleen Morse, M.S., Marjorie Risman, M.S., Renae Judy, B.S.

Contribution: All authors contributed to the development and validation of clinical phenotypes used to identify study subjects and (when applicable) controls.

Genome Informatics

Anurag Verma Ph.D., Shefali S. Verma, Ph.D., Yuki Bradford, M.S., Scott Dudek, M.S., Theodore Drivas, M.D., PH.D.

Contribution: A.V., S.S.V. are responsible for the analysis, design, and infrastructure needed to quality control genotype and exome data. Y.B. performs the analysis. T.D. and A.V. provides variant and gene annotations and their functional interpretation of variants.