

**Cell Host & Microbe, Volume 30**

**Supplemental information**

**Defining antigen targets to dissect vaccinia virus**

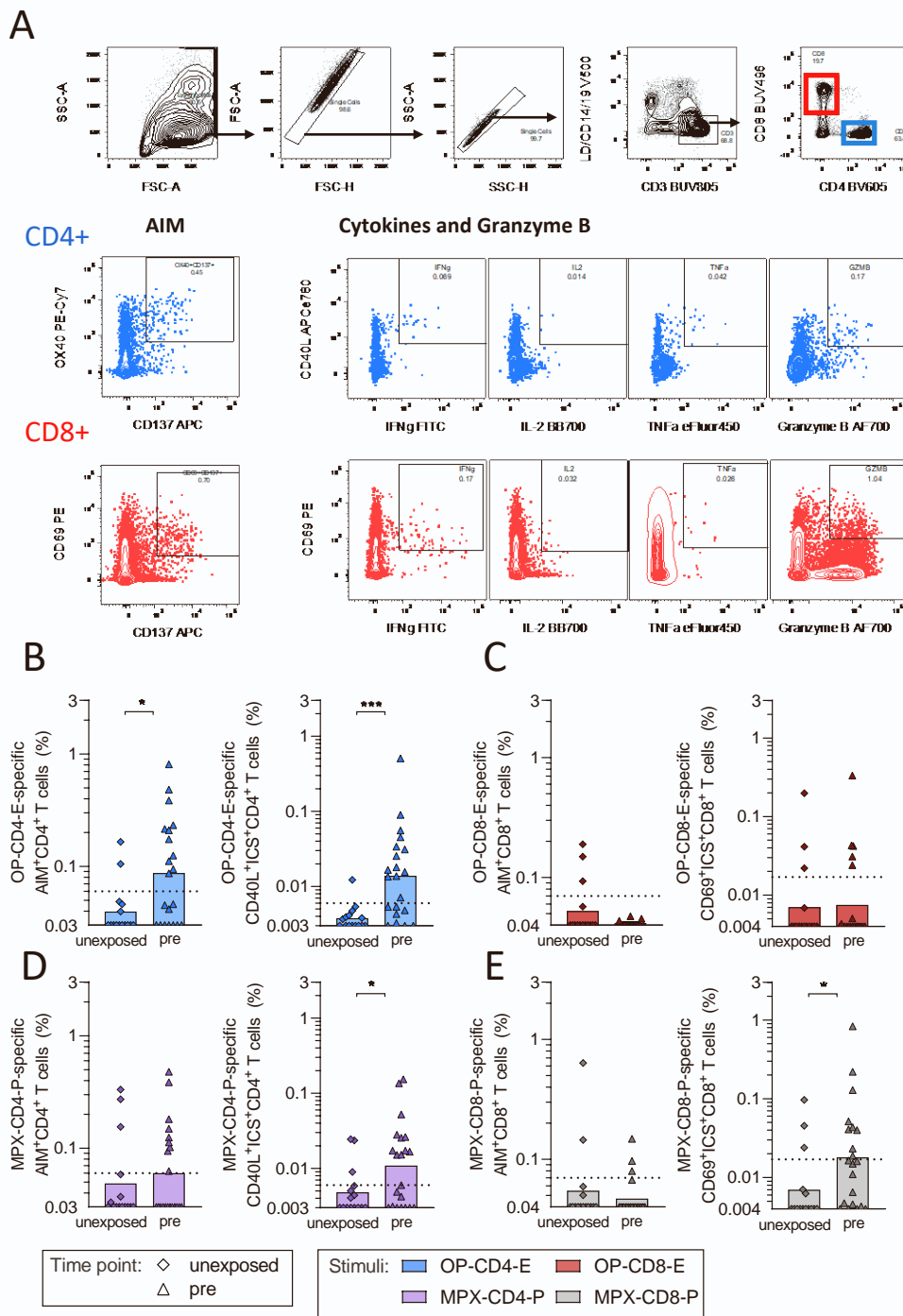
**and monkeypox virus-specific**

**T cell responses in humans**

**Alba Grifoni, Yun Zhang, Alison Tarke, John Sidney, Paul Rubiro, Maria Reina-Campos, Gilberto Filaci, Jennifer M. Dan, Richard H. Scheuermann, and Alessandro Sette**

**Supplemental Table 2.** Donor cohort vaccinated with Dryvax.

	<b>Healthcare workers</b>			
	<i>Unexposed (n=15)</i>	<i>non-vaccinated (n=21)</i>	<i>2 weeks post vaccination (n=19)</i>	<i>5-7 months post vaccination (n=18)</i>
<b>Age (years)</b>	18-26 [Median =23, IQR = 22]	23-55 [Median =34, IQR = 27]	18-62 [Median =29, IQR = 25]	23-62 [Median =43, IQR = 34]
<b>Gender</b>				
<b>Male (%)</b>	53% (8/15)	48% (10/21)	53% (10/19)	33% (6/18)
<b>Female (%)</b>	47% (7/15)	52% (11/21)	47% (9/19)	67% (12/18)
<b>Days post vaccination</b>	N/A	N/A	13-22 [Median =14, IQR = 14]	141-217 [Median =172, IQR = 142]
<b>Sample collection date</b>	Jun 2019- Dec 2021	Jun 2003-Oct 2005	Jul 2003-Sep 2007	Jun 2003-Aug 2007



**Figure S1. Gating strategy for AIM+ICS assay and T cell reactivity in unexposed and pre-vaccinated individuals.**

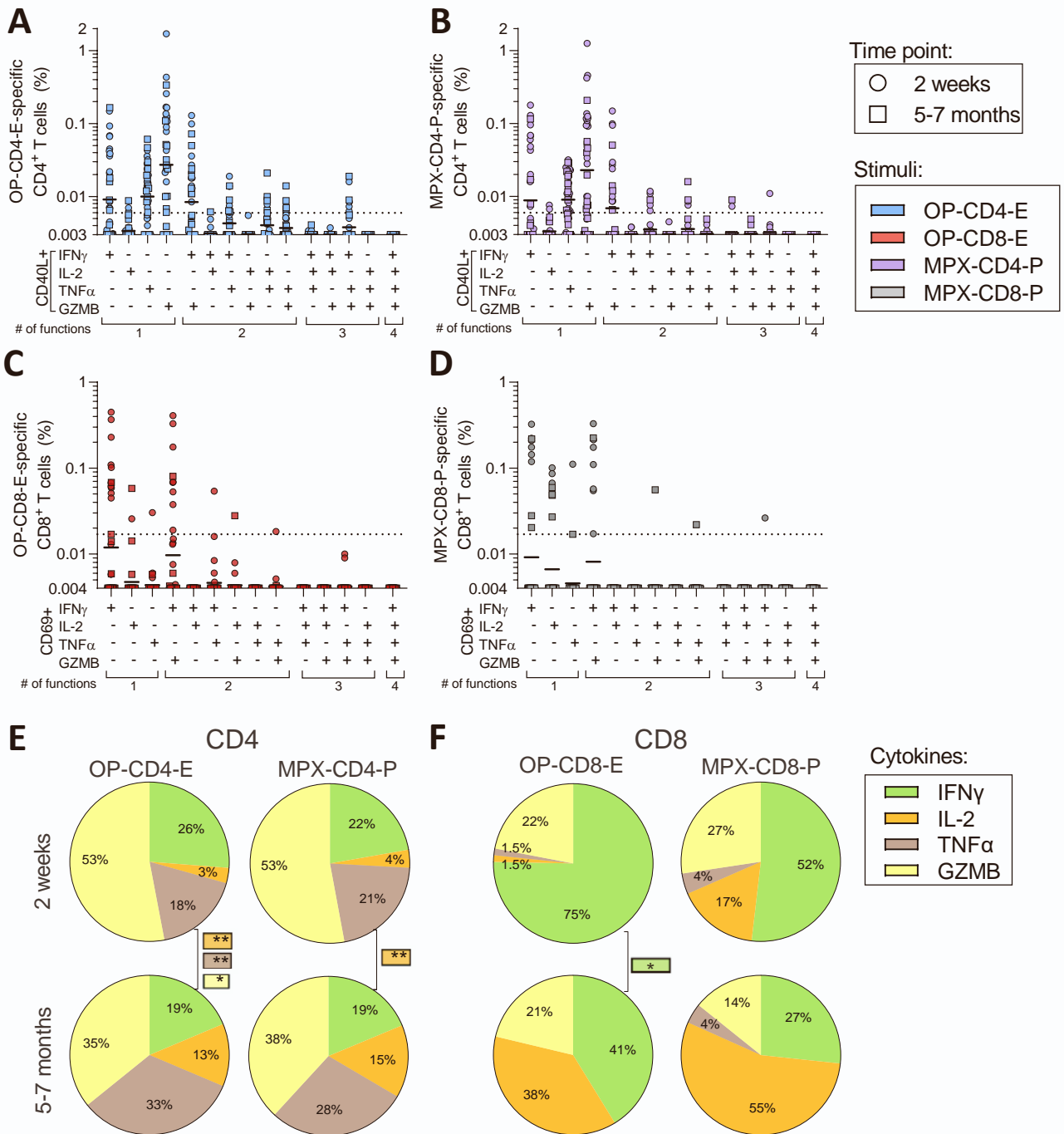
Related to Figures 1 and 3. (A) Gating strategy for the AIM/ICS assays used in the study. The indicated AIM or intracellular markers were used to assess OPXV or MPXV specific T cell reactivity. For ICS, the total reactivity was calculated from cells positive for IFN  $\gamma$ , IL-2, TNF $\alpha$  or GZMB in combination with CD40L for CD4+ or CD69 for CD8+, with the exception of single positive GZMB cells which were excluded from the CD8 sum. Unexposed individuals (n=15, diamond) and pre-vaccinated healthcare workers (n=21, triangle) were tested in AIM+ICS assays for (B) CD4+ and (C) CD8+ T cell reactivity to the OPXV megapools, OP-CD4-E (blue) and OP-CD8-E (red). The same donors were tested for (D) CD4+ and (E) CD8+ T cell reactivity for MPXV predicted peptide pools, MPX-CD4-P (purple) and MPX-CD8-P (grey) respectively. The Y axis of each bar graph starts at the LOD and the LOS is indicated with a dotted line. Mann-Whitney T test was applied to each graph and p values symbols are shown when significant. \* p<0.05, \*\*\*p<0.001.

**Supplemental Table 3.** Summary of immunodominant MPXV proteins based on IEDB epitope orthologs and predicted MPXV T cell epitopes. This summary is based on epitope reassignment to MPXV orthologs and therefore encompasses differences based on different nomenclatures for the various OPXV species listed in Supplemental Table 1.

Cell type	Acc. no.	VACWR antigen	transcription classification <sup>a</sup>	Function <sup>b</sup>	Protein name	Len (OP X)	IEDB epitopes	MPx antigen	MPX protein name	Len (MP X)	# of predicted epitopes	Megapool		
CD4	P20642	A10L	Late	structural	Major core protein 4a precursor	891	26	A11L	peg.115  A11L	891	32	MPX-CD4-P		
	P21115	B5R	Early	structural	Protein B5	317	19	B6R	peg.159  EEV	317	3			
	P20643	A3L	Intermediate	structural	Major core protein 4b	644	11	A4L	peg.108  A4L	644	25			
	P21010	D5R	Early	regulation	Primase D5	785	11	E5R	peg.96  E5R	785	28			
	QKE61287.1	A26L/A30L	Late	unknown	A-type inclusion protein	725	10	A27L	peg.131  A27L	696	11			
	P20981	L4R	Intermediate	regulation	Core protein VP8	251	9	M4R	peg.0  DNA-binding virion core protein	251	6			
	P33059	H3L	Intermediate	structural	Envelope protein H3	325	9	H3L	peg.87  H3L	324	14			
	P68616	A33R	Early	structural	Protein A33	185	8	A35R	peg.139  A35R	181	3			
	P68445	B7R	Late	structural	Protein B7	182	8	B8R	peg.161	182	5			
	P20504	J6R	Early	regulation	DNA-directed RNA polymerase 147 kDa polypeptide	1286	8	L6R	peg.84  L6R	1286	38			
	P20535	A27L	Intermediate	structural	14 kDa fusion protein	110	7	A29L	peg.133  A29L	110	1			
	P68693	A48R	Early	regulation	Thymidylate kinase	204	7	A49R	peg.152  A49R	204	3			
	10AZH7/Q8JLB3	D13L	Intermediate	structural	62 kDa protein	551	7	E13L	peg.104  E13L	551	17			
	P21052	F11L	Early	unknown	Protein F11	354	7	C17L	peg.38	354	10			
	10AZK3/Q8JL90	A24R	Early	regulation	DNA-directed RNA polymerase	1164	5	A25R	peg.129  A25R	1164	29			
	10AZI2/Q8JLA8	A4L	Early	structural	Core protein	281	5	A5L	peg.109  A5L	281	1			
	10AZN3/Q8JL68	A56R	Early	structural	Hemagglutinin	319	5	B2R	peg.152  B2R	313	6			
	Q8V523	F8L	Early	unknown	DNA polymerase	1006	5	F8L	peg.52  DNA pol	1006	37			
	P20540	L1R	Late	structural	Protein L1	250	5	M1R	peg.75  M1R	250	7			
	CD8	P20979	D1R	Early	regulation	mRNA-capping enzyme catalytic subunit	844	14	E1R	peg.92  E1R	845		71	MPX-CD8-P1
		P20643	A3L	Intermediate	structural	Major core protein 4b	644	13	A4L	peg.108  A4L	644		25	
P20504		J6R	Early	regulation	DNA-directed RNA polymerase 147 kDa polypeptide	1286	12	L6R	peg.84  L6R	1286	38			
P21004		B8R	Early	virulence	Soluble interferon gamma receptor B8	272	11	B9R	peg.162  B9R	267	22			
Q8V523		F8L	Early	unknown	DNA polymerase	1006	11	F8L	peg.52  DNA pol	1006	37			
P21010		D5R	Early	regulation	Primase D5	785	11	E5R	peg.96  E5R	785	28	MPX-CD8-P2		
P20642		A10L	Late	structural	Major core protein 4a precursor	891	10	A11L	peg.115  A11L	891	32			
P33059		H3L	Intermediate	structural	Envelope protein H3	325	10	H3L	peg.87  H3L	324	14			
P20502		18R	Intermediate	structural	RNA helicase NPH-II	676	10	18R	peg.64  RNA helicase	676	51			
10AZK3/Q8JL90		A24R	Early	regulation	DNA-directed RNA polymerase	1164	9	A25R	peg.129  A25R	1164	29			
P20640		M1L	Early	unknown	Ankyrin repeat protein M1	472	9	O1L	peg.20  ankyrin-like protein	442	24			
P68592		A17L	Late	structural	Virion membrane protein A17 precursor	203	8	A18L	peg.122  A18L	204	15			
10AZI4/Q8JLA6		A6L	Intermediate	unknown	Virion morphogenesis protein	372	8	A7L	peg.111  A7L	372	22			
P20998		A23R	Early	regulation	Intermediate transcription factor 3 large subunit	382	7	A24R	peg.128  A24R	382	25	MPX-CD8-P3		
P20535		A27L	Intermediate	structural	14 kDa fusion protein	110	7	A29L	peg.133  A29L	110	1			
10AZH7/Q8JLB3		D13L	Intermediate	structural	62 kDa protein	551	7	E13L	peg.104  E13L	551	17			
P33871		C16L	unknown	unknown	Protein F12	635	7	C18L	peg.39  EEV	635	40			
P33862		E2L	Early	unknown	Protein E2	737	7	F2L	peg.46	737	55			
P20501		I7L	Late	structural	Core protease I7	423	7	I7L	peg.63  I7L	423	26			
P21103		B25R	Late	unknown	Protein C19/B25	259	7	J1R	peg.177  J1R	588	33	MPX-CD8-P4		
P20991		A14L	Late	structural	Virion membrane protein A14	90	6	A15L	peg.119  A15L	90	5			
10AZJ7/Q8JL96		A18R	Early	regulation	DNA helicase	493	6	A19R	peg.123  A19R	492	35			
QKE61287.1		A26L/A30L	Late	unknown	A-type inclusion protein	725	6	A27L	peg.131  A27L	696	11			
10AZI2/Q8JLA8		A4L	Early	structural	Core protein	281	6	A5L	peg.109  A5L	281	1			
P20635		A7L	Late	regulation	Early transcription factor 82 kDa subunit	710	6	A8L	peg.112  A8L	710	50			
P20986		A8R	Early	regulation	Intermediate transcription factor 3 small subunit	288	6	A9R	peg.113  A9R	292	18			
P21077		B19R	Early	virulence	Surface antigen S	353	6	B16R	peg.167  B16R	351	25			
10AZH6/Q8JLB4		D12L	Early	regulation	Virus termination factor small subunit	287	6	E12L	peg.103  E12L	287	28			
10AZD2/Q8JLF2		I4L	Early	regulation	Ribonucleoside-diphosphate reductase	771	6	I4L	peg.60  I4L	771	50			
P20538		H5R	Early	regulation	Late transcription elongation factor H5	203	6	H5R	peg.89  VLTF-4	210	12			
P68467		K7R	Early	unknown	Protein K7	149	6	C6R	peg.27	149	7			
P20989		A12L	Intermediate	structural	25 kDa core protein A12L	192	5	A13L	peg.117  A13L	190	6	MPX-CD8-P5		
P20995		A20R	Early	regulation	DNA polymerase processivity factor component A20	426	5	A22R	peg.126  A22R	426	39			
P20499	I3L	Early	regulation	Protein I3	269	5	I3L	peg.59  DNA-binding phosphoprotein	269	17				
P21081	E3L	Early	virulence	RNA-binding protein E3	190	5	F3L	peg.47  dsRNA-binding protein	153	13				
P21049	E8R	Intermediate	structural	Protein E8	273	5	F7R	peg.51	273	20				
P21093	O1L	Early	unknown	Protein O1	666	5	Q1L	peg.55	665	67				
P21026	G5R	Early	unknown	Putative nuclease G5	434	5	G5R	peg.69	434	22				
P20638	F13L	Intermediate	structural	Envelope phospholipase F13	372	5	C19L	peg.40  palmytilated EEV	372	17				
P33801	C14L	unknown	unknown	Serine/threonine-protein kinase 2	439	5	C16L	peg.37  Ser Thr kinase	439	32				

<sup>a</sup>based on Yang et al., 2011 JVI

<sup>b</sup>based on Moutfias et al., 2010 Future Microb



**Figure S2. Quality of OPXV and MPXV-specific- T cell responses.**

Related to Figures 1 and 3. PBMCs were collected from donors two weeks after vaccination with Dryvax (n=19, circle) and five to seven months after vaccination (n=18, squares) and tested in AIM+ICS assays for T cell reactivity to OPXV and MPXV. (A – B) Boolean gating of (A) OP-CD4-E- or (B) MPX-CD4-P-specific CD4<sup>+</sup> ICS reactivity is shown for CD40L<sup>+</sup> CD4<sup>+</sup> T cells in combination with IFN $\gamma$ , IL-2, TNF $\alpha$  and GZMB. (C - D) Boolean gating of (C) OP-CD8-E- or (D) MPX-CD8-P-specific CD8 ICS reactivity is shown for CD69<sup>+</sup> CD8 T cells in combination with IFN $\gamma$ , IL-2, TNF $\alpha$  or GZMB. The Y axis of each bar graph starts at the LOD and the LOS is indicated with a dotted line. Contribution of IFN $\gamma$ , IL-2, TNF $\alpha$  and GZMB to the total of (E) CD4 or (F) CD8<sup>+</sup> ICS responses to OP and MPX megapools at two weeks and 5-7 months post vaccination. Mann-Whitney T test was applied to each pie chart and p values symbols are shown when significant. \* p<0.05, \*\*p<0.01.