

## **Supplemental Information**

### **Cross-Neutralizing and Protective Human**

### **Antibody Specificities to Poxvirus Infections**

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**SUPPLEMENTAL TABLES**

**Table S1. Generation of Human B Cell Hybridomas From PBMCs of Vaccinia-Immunized Subjects or From a Subject Who Had a History of Naturally-Acquired Monkeypox Infection, Related to Figure 1**

Subject	Vaccine or infection	Time post-exposure when blood was taken	Serum neutralizing reciprocal titer <sup>a</sup> , (fold dilution)	Number of individual hybridomas generated from sample
MVA 1	Modified Vaccinia Ankara (MVA) vaccine	Day 14 after booster vaccination	ND	16
MVA 3			ND	1
MVA 4			ND	3
MVA 12			ND	2
MVA 19			ND	1
MVA 21			ND	3
VRC-201-003-09	Dryvax vaccine	9 months	1,000	1
VRC-201-040-05		5 months	693	9
VRC-201-044-06		6 months	638	1
VRC-201-020-08		8 months	610	6
18	Acam2000 vaccine	21 days	10	1
MSK452	Monkeypox virus infection	1 year	108	45

<sup>a</sup>Based on plaque reduction neutralizing test performed using VACV strain WR.

ND indicates not determined

**Table S2. Sequence Diversity of Antibody Variable Genes Encoding Poxvirus-Specific MAbs, Related to Figure 1**

Antigen	Donor	mAb	Heavy chain variable gene sequence						Light chain variable sequence				
			V <sub>H</sub> gene	V <sub>H</sub> region nucleotide % homology to V <sub>H</sub> gene	D <sub>H</sub> gene	J <sub>H</sub> gene	HCDR3 amino acids (aa)	CDR3 length (aa)	V <sub>L</sub> gene	V <sub>L</sub> region nucleotide % homology to V <sub>L</sub> gene	J <sub>L</sub> gene	LCDR3 amino acids (aa)	CDR3 length (aa)
D8	MVA 1	VACV-8	4-34	92	1-7	6	ARVMTGITNYYYYYGM DV	18	1-9	91	2	QHLNSYPRGYT	11
		VACV-56	4-34	90	3-10	6	ARATQGSQGYKLFYFSGMDV	21	1-5	99	2	QQYNSYPYT	9
		VACV-66	4-39	87	3-10	5	ARHRRVLLWFGFQL	15	1-40	98	2	QSYDSSLSGAL	11
		VACV-77	4-59	88	6-6	4	AREDRGSPDY	10	1-51	98	3	GTWDL SLSAGV	11
		VACV-116	1-8	96	3-22	4	ARTPFDSGYYY	12	2-28 or 2D-28	99	1 or 3	MQALQTPGA	9
		VACV-117	1-8	93	3-22	4	ARTPFDDIGYYY	12	2-28 or 2D-28	81.5	1 or 3	Identical to VACV-116	9
		VACV-128	1- <sup>*</sup> 01	83	3-9	4	ARELTGYLNY	10	1-27	78	1 or 3	QKYNSAPHT	9
		VACV-136	2-26	95	1-1	4	ARMRGEYNSYYFDS	14	3-25	98	2 or 3	QSADSSGTSVV	11
	VACV-138	1-46	75	2-21	4	ASRDIVVV TATRSPFDY	17	3-20	99	2	QQYGSPPYT	10	
	MVA 3	VACV-168	4-59	96	3-22	4	ARLRGNYASSGYYNFYD	18	1-51	99	1	GTWDSLSAYV	11
	MVA 4	VACV-159	4-39	88	3-10	4	ARHLRVLLWFGELLE	15	1-40	98	1	QSHDSSLSGYV	11
	MVA 19	VACV-199	1-2	92	6-13	5	ARVPPDSSSWK	11	2-28 or 2D-28	99	ND	ND	ND
	MVA 21	VACV-228	3-21	85	6-13	4	ASRPGIAPAGPQAEQY	16	3-20	83	ND	ND	ND
		VACV-230	1-24	92	3-10	4	ARESWLRGFDY	11	3-20	74	1	QQYGSPPYT	9
		VACV-249	4-61	86	6-19	2	ARDGWYGWYLDL	12	3-15	99	4	QQYNNWPPT	9
	VRC-201-040-05	VACV-304	3-7	87	6-19	4	ATLNLELAVDAISEALK	17	3-25	82	2	QSVDNSGTYYEV	11
	MSK452	MPXV-27	4-34	93	5-12	6	ARVLSGWLPPFNYYMDV	19	1-17	95	1 or 4	LQHNSSPPT	9
		MPXV-30	1-69	82	4-23	3	ATGGNIRVHDFDI	13	2-30	98	1	MQGTHWPPRWT	11
		MPXV-40	4-34	82	2-21	6	ARISSGWIFPRYHYLDV	19	3-13	85	ND	ND	ND
		MPXV-61	1-69	92	3-10	5	ASSLPSTYYFGSGNYWGNWLDP	23	1-51	94	2 or 3	GTWDSLSSEVV	11
MPXV-96		4-34	86	5-21	4	ARGLRGNVCFD	12	3-15	98	2	QQYNNWPRT	9	
B5	MVA 1	VACV-1	3-48	86	2-15	6	ARRSVGCSGGNCAAYYGM DV	21	2-28 or 2D-28	99	5	MQALQTPIT	9
		VACV-59	3-11	86	3-10	6	ARDGDGSGSYTPPYYYGLDV	21	1-39 or 1D-39	98	4	QQSYSTPPLT	10
	MVA 4	VACV-151	3-21	98	ND	ND	ND	3-20	99	2	QQYGSPPYT	9	
	MVA 12	VACV-282	3-21	84	3-10	6	ARDRPRS RPN S GSYFWYYGM DV	23	3-21	98	ND	ND	ND
		VACV-283	1-69	84	3-16	6	ARRGGEGAAHGMDV	14	2-28 or 2D-28	99	5	LQALQTLPT	10
	MSK452	MPXV-2	1-3	87	3-9	5	ARPRASLLRYFDWLF EQ	17	3-20	85	2	QQYGNV PYS	10
		MPXV-12	4-34	85	3-10	6	ARDVYGS GTY YWFDP	15	1-39 or 1D-39	96	2	QQSYTTPYT	9
		MPXV-13	4-4	89	6-19	4	ARSGRYSSVTPFDY	14	3-11	85	3	QQRSHWPA	8
		MPXV-25	3-33	84	2-15	3	ARELGYCSGGTCY SMGAFDI	20	2-23	98.5	3	CSYVGSSTSV	10
		MPXV-38	3-15	95	ND	ND	ND	ND	3-20	95	ND	ND	ND
		MPXV-43	1-3	93	3-3	1	ARDFEDFDSWTGYYSWLH	18	3-20	97	5	QQYDSSPSIT	10
		MPXV-66	4-34	94	6-13	4	ARTARTVRYFEN	12	4-1	90	1	QQYYSTPWT	9
MPXV-70		3-11	85	2-2	3	ARGGGYCGGTTCSMGHAFDI	20	3-20	93	1 or 2	QQYGSPPY	8	
MPXV-92	4-4	88	2-21	1	ARNFYPGYLQY	11	ND	ND	ND	ND	ND		
A33	MVA 1	VACV-5	3-30	91	2-21	4	AKEACGGDCYSNYFHY	16	ND	ND	ND	ND	ND
		VACV-22	3-33	85	2-21	1	ARVPCGGDCYSGYLQH	16	3-15	98	4	QHYNWPPLLT	11
		VACV-80	3-74	84	6-13	4	ARVGAVRIAAAAPDY	15	4-1	99	1	QQYYSTPWT	9
	MSK452	MPXV-39	4-34	81	1-7	5	ASGNYR	6	2-30	97	2	MQGTHWPRT	9
		MPXV-51	4-39	87	3-16	5	GRLTPRN LFRGTLVRWVDP	19	3-20	96	4	QQYAGSLT	8
		MPXV-56	6-1	96	6-13	5	ND	ND	4-1	97	4	QQYYSSPLT	9
		MPXV-91	3-30	93	ND	ND	ND	ND	1-47	98	3	AAWDDSLSGKV	11
MPXV-99	3-30	84	3-10	4	ARADRGYFGH	10	ND	ND	ND	ND	ND		
H3	VRC-201-020-08	VACV-314	4-59	78	3-10	4	ARLAGRKP DADS	12	7-43	98	5	LLYYGGVVV	9
		VACV-315	3-23	81	4-11	4	AKGRARVNNI RYFDH	16	2-14	95	3	NSYTTSPWV	10
	MSK452	MPXV-1	3-23	87	3-22	2	ARDTYYYSRIWYFGL	16	ND	ND	ND	ND	ND
		MPXV-29	1-69	87	3-10	5	ARAVITMVRGDIPLGWFD P	19	3-20	98	1	QQYGSPPPT	9
		MPXV-72	2-5	88	6-19	3	AHRSVAGRDLAFDI	15	2-14	88	3	QLHIPSGLTWV	11
		MPXV-76	3-15	90	3-10	4	TTSFTPRRIFAY	13	3-11	90	3	QLRNSWPPT	9
MPXV-79	1-18	84	7-27	3	ARDPKLGRKGS AFDI	15	3-11	94	2	LQRS DLYT	8		
MPXV-85	3-23	84	1-26	3	AKDRVVGATYPRGV FDI	17	ND	ND	ND	ND	ND		
L1	MVA 1	VACV-33	4-39	83	6-6	4	ARQSSSTGGFHY	12	1-40	97	2	QSYDSSLSGREV	12
		VACV-34	3-9	85	3-22	4	AKETEKYYYDSSGYDY	16	3-20	99	2	QQYGSRGT	8
	MSK452	MPXV-26	3-53	92	5-12	4	AKGGGLGLDY	10	2-8	70	2 or 3	SSYAGTETVA	10
		MPXV-74	1-69	80	5-24	4	GVYNAN	6	2-28 or 2D-28	95	ND	LQARHTP	7
		MPXV-83	3-30	92	2-8	3	ND	ND	1-33 or 1D-33	94	2 or 3	QQFHSLPPT	9
MPXV-87	1-18	83	6-19	6	ARSSSGPRYYYGM DV	16	1-40	93	3	QSYDSSLSGWV	11		

A27	MVA 4	VACV-154	3-23	85	3-22	3	AKIRLDSSGYSGAFDI	16	3-25	99	2 or 3	QSADSSGTYPPVV	12
	VRC-201-040-05	VACV-300	1-69	93	6-19	5	ARASEQWLASINWFDP	16	2-23	90	3	CSYAGSSFLV	10
		VACV-301	3-23	79	3-16	4	AKWGRFESGAF	11	3-25	96	1	QSADNSGTYEV	11
		VACV-302	4-39	88	6-13	4	ARQSKAAAAGSIDY	14	2-28 or 2D-28	99	5	MQALQTPIT	9
		VACV-303	3-23	87	5-12	4	ATSLIWQLQSDY	12	1-39 or 1D-39	98	1	QQSYSTPQT	9
I1	MSK452	MPXV-10	5-51	88	4-17	4	ARAMTIVTPFDY	12	3-1	86	2 or 3	QAWDSATVV	9
		MPXV-31	5-51	81	1-26	4	ARPRQVGANRGYFDY	16	4-1	94	4	QQYYSPPAELS	11
		MPXV-53	3-33	91	6-19	4	ND	ND	3-21	95	ND	ND	ND
		MPXV-71	3-64	81	3-10	4	VRCLLRGLISPFDY	14	1-36	54	3	STWDYLSARV	11
		MPXV-97	3-30	89	6-25	2	ARLSLEAAWYFDL	13	1-40	93	1	QSYDNSLNGPWV	12
A25	VRC-201-040-05	VACV-309	3-23	86	1-1	6	AKDNNYYYYGMDV	13	1-6	100	1	LQYNYPRM	9
	VRC-201-044-06	VACV-312	3-33	88	4-11	3	ARVARDYSNIFDAFDI	16	1-39 or 1D-39	98	5	QQSYSTPIT	9
	VRC-201-020-08	VACV-313	4-39	88	6-13	4	VRIAVAAAGTDY	12	3-15	97	2	QQYNNWPPYT	10
	MSK452	MPXV-9	1-8	92	3-3	6	ARSLDSLRFLEWFHQNYFYFMDV	23	3-11	88	4	QLRST	5
F9	MSK452	MPXV-41	3-49	85	4-23	4	SATLTRGELFDY	12	3-11	96	4	QQRSNWPLT	9
A28	MSK452	MPXV-49	4-61	90	3-22	5	VREWPRHYDNRGYHTLPGT	19	1-5	92	1	QQYNTDSSRT	10
A21	VRC-201-020-08	VACV-318	3-30	93	5-12	3	QMVKVPPYF	9	3-10	84	3	YSTDSTSNQKRV	12
H5	VRC-201-003-09	VACV-308	1-69	91	5-12	4	ARPQSAYDFGPFDH	14	3-21	94	1	HVWHHTTDDHYV	11
Unknown	VRC-201-040-05	VACV-305	3-30	83	6-13	5	VRTQQVIRPFDDH	13	3-15	98	1	QQYKNWPPWT	10
		VACV-306	1-69	83	3-3	6	ARDCYGVFWSGYFSRCHFGMDV	22	3-20	97	4	QQYGGSPLLT	10
		VACV-307	3-23	85	1-26	4	AKDRGIVGTRFDS	14	3-15	99	5	QQYSNWPPIT	10
	VRC-201-020-08	VACV-311	3-73	84	2-8	6	TRRMDHARRPAREDYNNNGMDI	22	3-20	87	3	QQYSSSPT	8
		VACV-316	3-30	82	2-21	4	AKDLAMMIANPLDC	14	3-15	98	1	QQYKNWGT	8
	18	VACV-310	1-69	91	6-13	4	ARVFSAAAGH	9	1-33	94	2	QQYDNLPSGA	10
	MSK452	MPXV-8	3-30	83	6-19	6	ARGLIPSAEQWQARGGPDYYYYYG MAV	27	ND	ND	ND	ND	ND
		MPXV-28	1-69	85	6-19	4	ARGGAVTGRGYFDY	16	1-39 or 1D-39	99	2	QQSYSTPP	8
		MPXV-42	1-69	83	6-13	5	AREKLVGGGWFDP	14	3-20	94	2	QQYGHSPYT	9
		MPXV-45	1-69	95	3-3	4	ASPQVLRFLQWSPFDY	17	3-20	96	2	QQYAISPT	9
		MPXV-82	3-33	86	5-24	4	ARGVRMTTSLDY	12	2-14	98	3	SSYTTISTLGV	11
		MPXV-86	4-59	84	3-9	5	ARGVGGVYDILTGYWGNWFDP	22	1-40	97	2 or 3	QSFDSLLRGSVV	12
		MPXV-88	3-11	83	6-13	6	ARNLRAAGVNYFYFYMDV	19	3-11	96	1	QQRGKWWPPWT	10
MPXV-98	1-46	86	3-22	5	VVSSGFQQWFDP	12	3-15	96	1	Identical to MPXV-96	9		

ND indicates not determined

**Table S3. Reactivity and Cross-Reactivity of Poxvirus-Specific MAb, Related to Figure 1**

Antigen	Donor	mAb	Reactivity in screen to VACV		Cross-reactivity					
			Antigen	Lysate	CPXV		MPXV		VARV <sup>a</sup>	
					Antigen	Lysate	Antigen	Lysate	Antigen	Lysate
D8	MVA 1	VACV-8	Yes	Yes	ND	Yes	ND	Yes	no	Yes (++)
		VACV-56	Yes	Yes	ND	no	ND	Yes	no	Yes (+++)
		VACV-66	Yes	Yes	ND	no	ND	Yes	no	Yes (++++)
		VACV-77 <sup>b</sup>	Yes	ND	ND	ND	ND	ND	ND	ND
		VACV-116	Yes	Yes	ND	Yes	ND	Yes	no	ND
		VACV-117	Yes	Yes	ND	Yes	ND	Yes	no	ND
		VACV-128	Yes	Yes	ND	Yes	ND	no	no	Yes (++++)
		VACV-136	Yes	Yes	ND	Yes	ND	Yes	no	Yes (++++)
		VACV-138	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (+)
	MVA 3	VACV-168	Yes	Yes	ND	Yes	ND	Yes	Yes	no
	MVA 4	VACV-159	Yes	Yes	ND	Yes	ND	Yes	no	Yes (+++)
	MVA 19	VACV-199	Yes	Yes	ND	Yes	ND	Yes	Yes	no
	MVA 21	VACV-228 <sup>b</sup>	Yes	ND	ND	ND	ND	ND	ND	ND
		VACV-230	Yes	Yes	ND	Yes	ND	Yes	no	Yes (++)
		VACV-249	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (+++)
	VRC-201-040-05	VACV-304	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (++)
	MSK452	MPXV-27	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (++)
		MPXV-30	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (+)
		MPXV-40	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (++++)
		MPXV-61	Yes	Yes	ND	Yes	ND	Yes	no	Yes (++)
MPXV-96		Yes	Yes	ND	Yes	ND	Yes	no	Yes (+)	
B5	MVA 1	VACV-1	Yes	no	ND	no	ND	no	Yes	Yes (+)
		VACV-59	Yes	no	ND	Yes	ND	no	Yes	Yes (+)
	MVA 4	VACV-151	Yes	no	ND	no	ND	no	Yes	Yes (++)
	MVA 12	VACV-282	Yes	no	ND	no	ND	no	Yes	Yes (++)
		VACV-283	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (+++)
	MSK452	MPXV-2	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (++)
		MPXV-12	Yes	Yes	ND	Yes	ND	Yes	Yes	no
		MPXV-13	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (++)
		MPXV-25	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (++)
		MPXV-38	Yes	Yes	ND	Yes	ND	Yes	Yes	no
		MPXV-43	Yes	Yes	ND	Yes	ND	no	Yes	no
		MPXV-66	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (++)
		MPXV-70	Yes	Yes	ND	Yes	ND	Yes	no	Yes (+++)
MPXV-92	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (++++)		
A33	MVA 1	VACV-5	Yes	no	ND	no	ND	no	Yes	ND
		VACV-22	Yes	Yes	ND	Yes	ND	Yes	Yes	no
		VACV-80	Yes	Yes	ND	no	ND	no	no	no
	MSK452	MPXV-39	Yes	no	ND	no	ND	Yes	no	no
		MPXV-51	Yes	Yes	ND	Yes	ND	Yes	Yes	no

A33	MSK452	MPXV-56	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (+)
		MPXV-91	Yes	no	ND	Yes	ND	no	Yes	Yes (++++)
		MPXV-99	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (++)
H3	VRC-201-020-08	VACV-314	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (++++)
		VACV-315	Yes	Yes	ND	Yes	ND	Yes	no	Yes (+)
	MSK452	MPXV-1	Yes	Yes	ND	Yes	ND	Yes	no	Yes (+)
		MPXV-29	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (+)
		MPXV-72	Yes	Yes	ND	Yes	ND	Yes	no	Yes (+)
		MPXV-79	Yes	Yes	ND	Yes	ND	Yes	no	Yes (+)
		MPXV-76	Yes	Yes	ND	Yes	ND	Yes	no	no
MPXV-85	Yes	Yes	ND	Yes	ND	Yes	no	no		
L1	MVA 1	VACV-33	Yes	no	ND	Yes	ND	no	Yes	Yes (+)
		VACV-34	Yes	no	ND	no	ND	no	Yes	Yes (+)
	MSK452	MPXV-26	Yes	no	ND	Yes	ND	no	Yes	Yes (+)
		MPXV-83	Yes	no	ND	Yes	ND	no	Yes	ND
		MPXV-74	Yes	Yes	ND	Yes	ND	Yes	ND	Yes (+++)
		MPXV-87	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (++)
A27	MVA 4	VACV-154	Yes	Yes	ND	Yes	Yes	Yes	no	no
	VRC-201-040-05	VACV-300	Yes	Yes	ND	Yes	Yes	Yes	Yes	Yes (++)
		VACV-301	Yes	Yes	ND	Yes	Yes	Yes	Yes	no
		VACV-302	Yes	Yes	ND	Yes	Yes	Yes	no	Yes (++)
		VACV-303	Yes	Yes	ND	Yes	Yes	Yes	no	no
II	MSK452	MPXV-10	Yes	Yes	ND	Yes	ND	Yes	Yes	no
		MPXV-31	Yes	Yes	ND	Yes	ND	Yes	Yes	no
		MPXV-53	Yes	Yes	ND	Yes	ND	Yes	no	no
		MPXV-71	Yes	Yes	ND	Yes	ND	Yes	no	Yes (+++)
		MPXV-97	Yes	Yes	ND	Yes	ND	Yes	no	Yes (+++)
A25	VRC-201-040-05	VACV-309	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes (+)
	VRC-201-044-06	VACV-312	Yes	Yes	ND	Yes	ND	no	Yes	Yes (+)
	VRC-201-020-08	VACV-313	Yes	Yes	ND	Yes	ND	no	Yes	Yes (+)
	MSK452	MPXV-9	Yes	Yes	ND	Yes	ND	Yes	Yes	no
F9	MSK452	MPXV-41	Yes	no	ND	no	ND	no	ND	no
A28	MSK452	MPXV-49	Yes	no	ND	no	ND	no	ND	Yes (+)
A21	VRC-201-020-08	VACV-318	Yes	no	ND	no	ND	no	no	no
H5	VRC-201-003-09	VACV-308	Yes	Yes	ND	Yes	ND	Yes	Yes	no

ND indicates not determined

**Yes:** mAb reactivity was confirmed by ELISA or protein microarray or biolayer Interferometry

**No:** mAb was tested and found as not reactive

<sup>a</sup>Range of mAb binding efficiency to VARV-infected cell lysate, where numbers indicate optical density from ELISA: + (0 - 0.099 OD); ++ (0.1 - 0.299 OD);

+++ (0.3 - 0.499 OD); ++++ (0.5 - 0.799 OD); +++++ (> 0.8 OD)

<sup>b</sup>MAbs with low expression that were excluded from the analysis

**Table S4. Binding of Poxvirus-Specific MAbs to Purified Antigens or Infected Cell Lysates, Related to Figure 1**

Antigen	Donor	mAb	EC <sub>50</sub> (µg/mL)							
			Purified antigen				Virus-infected cell lysate			
			VACV	CPXV	MPXV	VARV	VACV	CPXV	MPXV	VARV
D8	MVA 1	VACV-8	ND	ND	ND	ND	0.04	0.1	0.06	ND
		VACV-56	ND	ND	ND	ND	0.01	>	0.008	ND
		VACV-66	ND	ND	ND	ND	0.01	>	>0.3	ND
		VACV-77	ND	ND	ND	ND	ND	ND	ND	ND
		VACV-116	1.8	ND	ND	ND	0.01	0.01	>26	ND
		VACV-117	1.8	ND	ND	ND	0.01	0.01	>26	ND
		VACV-128	1.1	ND	ND	ND	0.3	0.25	>	ND
		VACV-136	ND	ND	ND	ND	0.06	0.05	0.04	ND
	VACV-138	0.5	ND	ND	ND	0.08	0.04	0.1	ND	
	MVA 3	VACV-168	ND	ND	ND	ND	0.4	0.8	0.5	ND
	MVA 4	VACV-159	ND	ND	ND	ND	0.009	>28	>14	ND
	MVA 19	VACV-199	ND	ND	ND	ND	0.008	0.008	>19	ND
	MVA 21	VACV-228	ND	ND	ND	ND	ND	ND	ND	ND
		VACV-230	ND	ND	ND	ND	0.6	3.3	1	ND
		VACV-249	2.8	ND	ND	ND	0.3	0.4	0.1	ND
	VRC-201-040-05	VACV-304	0.05	ND	ND	ND	0.04	0.05	0.1	ND
	MSK452	MPXV-27	0.09	ND	ND	ND	0.02	0.02	0.02	ND
		MPXV-30	ND	ND	ND	ND	0.004	0.004	0.003	ND
		MPXV-40	0.09	ND	ND	ND	0.01	0.02	0.01	ND
		MPXV-61	ND	ND	ND	ND	0.007	0.009	0.004	ND
MPXV-96		ND	ND	ND	ND	0.001	0.003	0.001	ND	
B5	MVA 1	VACV-1	0.02	ND	ND	0.02	>	>	>	ND
		VACV-59	0.14	ND	ND	0.18	>	0.78	>	ND
	MVA 4	VACV-151	0.04	ND	ND	0.04	>	>	>	ND
		VACV-282	0.155	ND	ND	3.15	>	>	>	ND
	MVA 12	VACV-283	0.06	ND	ND	0.08	>48	>41	>50	ND
		MPXV-2	0.03	ND	ND	0.06	>6	>13	>6	ND
	MSK452	MPXV-12	0.1	ND	ND	0.1	5.85	12.62	5.62	ND
		MPXV-13	>10	ND	ND	0.03	>0.1	0.004	>0.1	ND
		MPXV-25	0.19	ND	ND	0.19	0.066	0.05	>0.24	ND
		MPXV-38	0.08	ND	ND	0.08	>50	>50	>50	ND
		MPXV-43	10.1	ND	ND	10.8	0.011	>62.8	>	ND
		MPXV-66	0.1	ND	ND	0.16	>33	>29	>16	ND
		MPXV-70	0.1	ND	ND	>	>9	>24	>13	ND
MPXV-92		0.7	ND	ND	1.2	0.064	0.014	0.046	ND	
A33	MVA 1	VACV-5	0.03	ND	ND	0.01	>	>	>	ND
		VACV-22	0.03	ND	ND	0.02	>0.008	>0.009	>0.006	ND
		VACV-80	0.065	ND	ND	>	> 69	>	>	ND
	MSK452	MPXV-39	0.07	ND	ND	>	>	>	>47	ND
		MPXV-51	0.04	ND	ND	0.04	0.05	0.024	0.025	ND
		MPXV-56	0.06	ND	ND	0.05	>0.07	>0.07	>26	ND
		MPXV-91	0.03	ND	ND	0.03	>	0.26	>	ND
		MPXV-99	0.01	ND	ND	0.04	>49	>18	>0.008	ND
H3	VRC-201-020-08	VACV-314	0.1	ND	ND	ND	0.003	0.004	0.003	ND
	VRC-201-020-08	VACV-315	0.2	ND	ND	ND	0.002	0.002	0.001	ND

H3	MSK452	MPXV-1	0.4	ND	ND	ND	0.009	0.011	0.005	ND
		MPXV-29	ND	ND	ND	ND	0.001	0.002	0.001	ND
		MPXV-72	0.04	ND	ND	ND	0.005	0.003	0.003	ND
		MPXV-79	0.06	ND	ND	ND	0.004	0.004	0.003	ND
		MPXV-76	ND	ND	ND	ND	0.008	0.01	0.006	ND
		MPXV-85	0.04	ND	ND	ND	0.003	0.002	0.002	ND
L1	MVA 1	VACV-33	0.02	ND	ND	0.02	>	0.06	>	ND
		VACV-34	0.04	ND	ND	0.02	>	>	>	ND
	MSK452	MPXV-26	0.05	ND	ND	0.02	>	0.05	>	ND
		MPXV-83	0.065	ND	ND	0.065	>	0.03	>	ND
		MPXV-74	ND	ND	ND	ND	0.02	0.02	0.02	ND
		MPXV-87	0.3	ND	ND	0.3	0.01	0.003	0.001	ND
A27	MVA 4	VACV-154	0.06	ND	0.06	0.3	>0.1	0.02	0.02	ND
	VRC-201-040-05	VACV-300	0.22	ND	0.14	0.79	>0.37	0.06	>0.68	ND
		VACV-301	0.03	ND	0.04	0.11	>21	>1.4	>5.6	ND
		VACV-302	0.08	ND	0.04	0.55	0.01	0.01	0.01	ND
		VACV-303	0.02	ND	0.02	0.16	0.05	0.004	0.004	ND
I1	MSK452	MPXV-10	ND	ND	ND	ND	0.003	0.006	>0.02	ND
		MPXV-31	ND	ND	ND	ND	0.01	0.01	0.04	ND
		MPXV-53	ND	ND	ND	ND	0.008	0.011	>0.08	ND
		MPXV-71	ND	ND	ND	ND	0.022	0.014	0.006	ND
		MPXV-97	ND	ND	ND	ND	0.008	>50	0.001	ND
A25	VRC-201-040-05	VACV-309	ND	ND	ND	ND	0.005	0.005	0.001	ND
	VRC-201-044-06	VACV-312	ND	ND	ND	ND	0.002	>50	>	ND
	VRC-201-020-08	VACV-313	ND	ND	ND	ND	0.001	1.172	>	ND
	MSK452	MPXV-9	ND	ND	ND	ND	0.022	0.876	0.107	ND
F9	MSK452	MPXV-41	0.008	ND	ND	ND	>	>	>	ND
A28	MSK452	MPXV-49	0.02	ND	ND	ND	>	>	>	ND
A21	VRC-201-020-08	VACV-318	1.3	ND	ND	ND	>	>	>	ND
H5	VRC-201-003-09	VACV-308	ND	ND	ND	ND	0.006	0.002	0.003	ND
Unknown	VRC-201-040-05	VACV-305	ND	ND	ND	ND	0.003	0.001	0.002	ND
		VACV-306	ND	ND	ND	ND	0.003	0.001	0.001	ND
		VACV-307	ND	ND	ND	ND	0.004	0.003	0.002	ND
	VRC-201-020-08	VACV-311	ND	ND	ND	ND	0.517	>	>	ND
		VACV-316	ND	ND	ND	ND	3.521	>	>	ND
	18	VACV-310	ND	ND	ND	ND	0.027	0.004	>	ND
	MSK452	MPXV-8	ND	ND	ND	ND	0.118	0.058	0.039	ND
		MPXV-28	ND	ND	ND	ND	0.001	>16	0.001	ND
		MPXV-42	ND	ND	ND	ND	0.017	0.036	0.018	ND
		MPXV-45	ND	ND	ND	ND	0.0002	0.0004	0.0001	ND
		MPXV-82	ND	ND	ND	ND	0.003	0.004	0.002	ND
		MPXV-86	ND	ND	ND	ND	ND	ND	ND	ND
		MPXV-88	ND	ND	ND	ND	>62	0.03	0.05	ND
MPXV-98	ND	ND	ND	ND	0.014	0.006	0.004	ND		

EC<sub>50</sub> values (µg/mL) indicated in colors:

<0.001	0.0011-0.01	0.011-0.1	0.11-1.0	1.1-10	10.1-100	>100
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> indicates that binding was not detected even when mAb was tested at the highest concentration of 100 µg/mL  
 ND indicates not determined





H3	VRC-201-020-08	VACV-314	IgG3	<	0.1 (74)	ND	ND	<	0.7 (85)	ND	ND	<	0.8 (84)	ND	ND	
		VACV-315	IgG1	<	2.2 (72)	ND	ND	<	2.8 (70)	ND	ND	<	<	ND	ND	
	MSK452	MPXV-1	IgG1	<	<	ND	ND	<	2.4 (53)	ND	ND	25 (77)	3 (85)	ND	ND	
		MPXV-29	IgG1	<	<	<	<	<	<	ND	ND	<	<	<	<	
		MPXV-72	IgG1	<	11.4 (66)	ND	ND	<	1.7 (84)	ND	ND	<	6.2 (64)	ND	ND	
		MPXV-76	IgG1	<	<	<	<	<	<	ND	ND	<	<	<	<	
		MPXV-79	IgG1	<	4.7 (62)	ND	ND	<	0.2 (81)	ND	ND	12.5 (67)	<	ND	ND	
MPXV-85	IgG1	<	3.4 (63)	ND	ND	<	0.6 (82)	ND	ND	12.5 (77)	100 (51)	ND	ND			
L1	MVA 1	VACV-33	IgG1	0.3 (56)	0.7 (71)	ND	ND	0.5 (74)	0.2 (75)	ND	ND	70 (50)	<	ND	ND	
		VACV-34	IgG1	3.3 (76)	0.96 (78)	ND	ND	2.7 (88)	7 (88)	ND	ND	63 (64)	<	ND	ND	
	MSK452	MPXV-26	IgG1	0.3 (95)	0.7 (80)	ND	ND	0.07 (99)	0.2 (93)	ND	ND	3 (96)	6.2 (97)	ND	ND	
		MPXV-83	IgG1	0.3 (56)	0.2 (75)	ND	ND	0.2 (69)	0.1 (78)	ND	ND	<	12.5 (67)	ND	ND	
		MPXV-74	IgG1	<	0.09 (79)	ND	ND	<	0.08 (71)	ND	ND	<	<	ND	ND	
MPXV-87	IgG1	5 (61)	0.8 (72)	ND	ND	0.8 (63)	0.6 (82)	ND	ND	50 (57)	50 (65)	ND	ND			
A27	MVA 4	VACV-154	IgG1	<	<	ND	ND	<	<	ND	ND	15 (66)	14 (69)	ND	ND	
	VRC-201-040-05	VACV-300	IgG1	<	<	ND	ND	<	<	ND	ND	<	<	ND	ND	
		VACV-301	IgG3	0.5 (61)	0.1 (77)	ND	ND	<	4 (86)	ND	ND	1.6 (84)	0.8 (92)	ND	ND	
		VACV-302	IgG1	12.3 (81)	0.1 (53)	ND	ND	11 (93)	0.2 (81)	ND	ND	0.1 (88)	6.3 (82)	ND	ND	
VACV-303	IgG1	<	<	ND	ND	<	<	ND	ND	25 (51)	15 (64)	ND	ND			
I1	MSK452	MPXV-10	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
		MPXV-31	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
		MPXV-53	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
		MPXV-71	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
		MPXV-97	IgG1	<	<	<	<	<	<	ND	ND	0.02 (96)	0.02 (76)	<	<	
A25	VRC-201-040-05	VACV-309	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
	VRC-201-044-06	VACV-312	IgG1	<	<	<	<	ND	ND	ND	ND	ND	ND	<	<	
	VRC-201-020-08	VACV-313	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
	MSK452	MPXV-9	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
F9	MSK452	MPXV-41	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
A28	MSK452	MPXV-49	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
A21	VRC-201-020-08	VACV-318	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
H5	VRC-201-003-09	VACV-308	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
Unknown	VRC-201-040-05	VACV-305	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
		VACV-306	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
		VACV-307	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
	VRC-201-020-08	VACV-311	IgG1	<	<	<	<	<	<	ND	ND	ND	<	<	<	<
		VACV-316	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
	18	VACV-310	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
	MSK452	MPXV-8	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
		MPXV-28	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
		MPXV-42	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
		MPXV-45	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<	
MPXV-82		IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<		
MPXV-86		ND	<	<	<	<	ND	ND	ND	ND	<	<	<	<		
MPXV-88		ND	<	<	<	<	ND	ND	ND	ND	<	<	<	3.1 (78)		
MPXV-98	IgG1	<	<	<	<	ND	ND	ND	ND	<	<	<	<			

Range of IC<sub>50</sub> values (µg/mL) indicated by the heat map as below:

0.01-0.1	0.11-1.0	1.1-10	10.1-100	>100
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< indicates that the E<sub>max</sub> was below 50% even at the highest concentration of 100 µg/mL

ND indicates not determined

C' indicates neutralization assay was performed in the presence of complement

**Table S6. Composition of MAb Mixtures, Related to Figure 3 and Figure 5**

Mixture	Human mAb specificity						Ratio	Total amount of mAbs per mouse, mg	MAb clones included
	Anti-A27	Anti-D8	Anti-H3	Anti-L1	Anti-A33	Anti-B5			
Mix6	1	1	1	1	1	1	1:1:1:1:1:1	1.2	VACV-301, VACV-249, MPXV-72, MPXV-26, VACV-22, VACV-283
Mix6 ( $\Delta$ D8)	1	-	1	1	1	1	1:1:1:1:1	1	VACV-301, MPXV-72, MPXV-26, VACV-22, VACV-283
Mix6 ( $\Delta$ L1)	1	1	1	-	1	1	1:1:1:1:1	1	VACV-301, VACV-249, MPXV-72, VACV-22, VACV-283
Mix6 ( $\Delta$ A27)	-	1	1	1	1	1	1:1:1:1:1	1	VACV-249, MPXV-72, MPXV-26, VACV-22, VACV-283
Mix6 ( $\Delta$ H3)	1	1	-	1	1	1	1:1:1:1:1	1	VACV-301, VACV-249, MPXV-26, VACV-22, VACV-283
Mix6 ( $\Delta$ A33)	1	1	1	1	-	1	1:1:1:1:1	1	VACV-301, VACV-249, MPXV-72, MPXV-26, VACV-283
Mix6 ( $\Delta$ B5)	1	1	1	1	1	-	1:1:1:1:1	1	VACV-301, VACV-249, MPXV-72, MPXV-26, VACV-22
Anti-D8/mix	-	5	-	-	-	-	1:1:1:1:1	1	VACV-249, VACV-8, VACV-304, VACV-66, MPXV-40
Anti-H3/mix	-	-	3	-	-	-	1:1:1	0.6	VACV-314, VACV-315, MPXV-72
Mix4	1	-	-	1	1	1	1:1:1:1	0.8	VACV-301, MPXV-26, VACV-22, VACV-283
Mix4 ( $\Delta$ L1)	1	-	-	-	1	1	2:1:1	0.8	VACV-301, VACV-22, VACV-283
Mix4 ( $\Delta$ B5)	1	-	-	1	1	-	1:1:2	0.8	VACV-301, MPXV-26, VACV-22
Mix6 ( $\Delta$ EV)	1	1	1	1	-	-	1:1:1:1:1	0.8	VACV-301, VACV-249, MPXV-72, MPXV-26
Mix6 ( $\Delta$ MV)	-	-	-	-	1	1	1:1	0.4	VACV-22, VACV-283

An entry using the “-“ symbol above indicates that the mAb listed in the column header was not included in the mix listed to the left.