

Supplementary Materials for

Multiple lineages of monkeypox virus detected in the United States, 2021-2022

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## **Materials and Methods**

## PCR testing

DNA was extracted from swabs collected from patient lesions using EZ-1 DNA tissue kit (Qiagen) followed by heat inactivation at 56°C for  $\geq 1$  hour. Monkeypox infection was confirmed by real-time PCR at CDC. CladeII-specific Monkeypox virus real-time PCR assay was performed as described in Li et al. (2010) (*15*). Orthopoxvirus OPX3 real-time PCR assay was performed as described (*14*), with the following changes: Each reaction (20 µL) contained 5 µL of template DNA, 0.5 µL of each primer (20 µM), 0.5 µL probe (10 µM) added to the 2X TaqMan Fast Advanced master mix (Applied Biosystems). Thermal cycling conditions for the ABI 7500 Fast Dx Real-Time PCR System (Applied Biosystems) were one cycle at 95°C for 20 seconds and 40 cycles at 95°C for 3 seconds and 60°C for 30 seconds. Primer and probe sequences for the Orthopoxvirus OPX3 real-time PCR assay (called OPX in (*14*)) are as follows:

Name	Sequence
OPX3 Forward	5'-TCA AAT ATT GAT CGT CCA ACG A-3'
OPX3 Reverse	5'-TGG ATG AAT TTC TCA ATA TTA GTT GG-3'
OPX3 Probe	5' FAM-TAA CAT CCG TCT GGA GAT ATC CCG TTA GA -BHQ1-3'

# Sequencing

ONT: Library preparation was performed on extracted DNA using Ligation Sequencing kit (Oxford Nanopore Technologies SQK-LSK-109) following the manufacturer's protocol for genomic DNA. Libraries were sequenced (one sample per flow cell) on a MinION sequencer (MPXV\_USA\_2022\_MA001, MPXV\_USA\_2021\_MD, and MPXV\_USA\_2021\_TX) or GridION sequencer (FL001, FL002, CA001, UT001, UT002, VA001) using a MIN109 R9.4.1 flow cell (Oxford Nanopore). For MA001, CA001, and VA001 data were combined from two independent swabs collected from two locations. For all other cases, data are from a single sample/swab. Basecalling was performed using guppy version 6.1.2 with high accuracy and qscore filtering (for MinION runs) or was performed on GridION using high accuracy basecalling.

Illumina: Extracted DNA (40 µl) was diluted to 130 µl in water and fragmented to 500bp on a Covaris S220 instrument using SonoLab 7 application software. Genomic DNA libraries were prepared using Swift Accel-NGS® 2S Plus DNA Library Kit (Cat# 21096) plus 2S Set A+B Indexing Kit (Cat#26396), following manufacturer's protocol using 15 cycles of PCR. Size selection was performed using Swift Accel-NGS® 2S Plus DNA Library Kit (Cat# 21096) to ensure a single peak of approximately 500bp. Libraries were prepared and normalized according to the manufacturer's recommendations to create a final DNA library with a concentration of 15 - 20nM/5ul which was then sequenced on a MiSeq instrument using MiSeq® Reagent kit v3 600 cycles (Cat# MS-102-3003, Illumina). DNA from the 2021\_MD case underwent hybridization with a custom orthopoxvirus capture probe panel prior to processing and sequencing as described in (*3*). Genome Assembly

Nanopore reads were trimmed to remove 55 bp from each end (seqtk 1.0, https://github.com/lh3/seqtk) and all reads below 50 bp were removed (trimmomatic 0.39, https://github.com/timflutre/trimmomatic) before mapping to MPXV Nigeria reference MT903344 with 6,000 bp removed from the left terminus using minimap2 2.16 (https://github.com/lh3/minimap2) to remove human and other non-MPXV reads. Illumina reads were trimmed using FaQCs 1.34 (https://github.com/LANL-Bioinformatics/FaQCs ) using parameters -q 20 --5end 10 --3end 5 -n 5 -- min\_L 30 and then mapped to MPXV Nigeria reference MT903344 with 6,000 bp removed from the left terminus using bwa mem (https://github.com/lh3/bwa). A hybrid assembly was generated from mapped Illumina and Oxford Nanopore reads using Unicyler 0.4.7 (https://github.com/rrwick/Unicycler). Assemblies were polished by mapping reads back to draft genomes containing one complete ITR and one incomplete ITR with ~6,000 bases removed using bwa mem or minimap2 and generating a consensus

sequence using samtools 1.9 and ivar 1.0 (https://github.com/andersen-lab/ivar). Inverted Terminal Repeats (ITRs) were assembled manually by copying from one end to the other.

For quality control, separate assemblies were made with either nanopore or Illumina data since DNA extracted from separate lesions for the same patient were used for Nanopore and Illumina sequencing in three cases. Illumina-only assemblies were made using SPAdes/3.13.0 and CLC Genomics Workbench 22. Oxford Nanopore-only assemblies were made using flye 2.9 (https://github.com/fenderglass/Flye).

Annotations were transferred from MPXV Clade 3 Nigeria reference MT903345, then locus\_tags were re-named with the strain ID. Alignments used to make trees were performed using MAFFT v.7.450 (37). Phylogenetic analysis (Figure 1) was performed using BEAST v. 1.8.3. All sites containing gaps were removed prior to phylogenetic analysis, and all sites containing gaps or ambiguities were removed prior to haplotype network analysis. Haplotype network analysis was generated using sequence alignments generated by whole genome alignment using MAFFT v.7.450 (37) followed by removal of alignment columns containing gaps or ambiguities with PopArt using the Median Joining method. An analysis of evolutionary rate was performed in BEAST v.1.8.3 using an alignment of 85 monkeypox virus genomes (Figure 2) after stripping all columns containing gaps. The GTR+g+i model, fixed local clock (uniform distribution 0,1), tip dates (year  $\pm$  0.5), and a coalescent Bayesian skyline tree prior with 10 groups were used. Clades I, IIa, and Lineage A were specified as taxon sets. A secondary analysis of evolutionary rate was performed for Clades I and IIa (54 monkeypox virus genomes, Figure 2) using the same alignment after removing Clade IIb sequences; GTR+g+i model, fixed local clock (uniform distribution 0,1), tip dates (year  $\pm$  0.5), and a coalescent Bayesian skyline tree prior with 10 groups were used. Lineage A sequences and KY642617 were extracted from the alignment used in Figure 2 and evolutionary rate was analyzed using both fixed local clock (for Lineage A only) and uncorrelated lognormal relaxed clock (uniform distribution 0,1). GTR+g+i model, tip dates (year  $\pm$  0.5), and a coalescent Bayesian skyline tree prior with 4 groups were used in both analyses. Sequences of all near complete MPXV genomes that were available at the time of analysis were used. Still, many genomes contained Ns and may contain errors caused by low coverage or sequencing technology bias. Availability of complete, final genomes is expected to improve resolution of future analyses.

## APOBEC3 analysis

The sequence alignment we used began with foundational sequences from Mauldin et. al., a paper that described spread of MPXV beyond the African continent (3), adding additional sequences to create our baseline dataset of 85 MPXV-related sequences. Our initial alignment was generated using MAFFTv7 (37); ends were trimmed to exclude regions that were only sporadically sequenced. The alignment further edited to resolve poorly aligned regions; generally these were in regions with differing numbers of direct repeats or that were proximal to long gaps in sequences. A maximum likelihood tree used for defining clades of interest and reconstructing ancestral states for subsequent APOBEC3 analysis in was generated using the HIV database web interface for IQ-tree

(https://www.hiv.lanl.gov/content/sequence/IQTREE/iqtree.html) (*38*), with ModelFinder (*39*); the Bayesian information criterion (BIC) best fit model was K3Pu+F+I, and a mid-point root was used. GenBank Accession numbers for these sequences are included in Tables S6 (Lineage A) and S8 (other Clade II and Clade I).

APOBEC analysis was performed by tracking all observed single nucleotide mutational events relative to the most common ancestor sequence within a given clade of interest (Figs. 2 an S2). This analysis was first performed on natural sequences (Tables S6-S8) to explore mutational patterns in subdifferent lineages within the tree. To exclude redundancy due to shared internal branches, we also compressed all SNPS observed within a clade onto a single merged sequence that included all unique mutations found within that clade (mergesnps.py, <u>https://zenodo.org/record/6970457#.YvAVPi-B1eg</u>) (*36*). This merged sequence was then compared to the most recent common ancestor of the clade generated with IQ-tree as shown in Fig. S2, and such a merged comparisons are included in Fig. 2B and Fig S3, and Table S6 – S8.

The statistical strategies for resolving if G-to-A mutations within an APOBEC3 motif context are enhanced relative other G-to-A mutations are described in the HIV-database tool HYPERMUT (https://www.hiv.lanl.gov/content/sequence/HYPERMUT/hypermut.html). HYPERMT was written for RNA viruses, so for this study we adapted the approach to a double stranded DNA scenario and built improved visualization tools. The method makes pairwise comparisons between a reference sequence and each of a set of sequences aligned to that reference. It identifies and tallies all Gs that occur in the context of APOBEC3 motifs within a reference sequence and counts both unchanged Gs and G-to-A mutations that occur within the context of that motif. Similarly, all Gs that are not embedded in an APOBEC3 motif are tallied, and the number of G-to-A mutations that occur among those G's are counted. A Fisher's exact test is then used to determine if the G-to-A substitutions events are significantly enriched in the context of APOBEC3 motifs. O-values were calculated to correct for multiple tests (40, 41). Gaps and IUPAC ambiguity codes can be present in the input sequences, but these are excluded from the comparisons. The original HYPERMUT strategy was developed for HIV-1, so we adapted it for application to MPXV by enabling simultaneously tracking G-to-A mutations in the forward and reverse complement strand using apobec.py, apocount.py, and apoplot.py (available at https://zenodo.org/record/6970457#.YvAVPi-Bleg) (36). This was critical as what simply appears as C-to-T transition on the first strand can be embedded in the APOBEC3 motif in the reverse complement. Our original HYPERMUT enables the inclusion of more complex APOBEC3 motifs, and we also explored the use of the 3-base motif pattern including a cytosine in the +2 positions (GAC-to-AAC, or GGC-to-AGC), as pattern this can partially inhibit APOBEC3 activity (42); as this strategy did not substantively change our conclusions, we present the simpler version here. Also, as a cross-check we confirmed our results with ancestral reconstruction-based analysis by using natural sequences that were most proximal to ancestral nodes as a reference strain instead; theses analyses concurred. Our method distinguishes between GA-to-AA and GG-to-AG motifs, and almost all of the substitutions we observed were in the GA-to-AA context, indicative of APOBEC3D and 3F activity, not APOBEC3G (43).

## Sensitivity of 2022 outbreak MPXV to TPOXX

A cytopathic effect (CPE) assay was used in a 96-well format.  $0.0015 - 5 \mu$ M of tecovirimat (lot: SG-14G32-M) was added to a confluent monolayer of Vero E6 cells and incubated 1 hour at 37°C and 6.0% CO<sub>2</sub>. After incubation MPXV was added (MOI = 0.01) and incubated 72 hours at 35.5°C and 6.0% CO<sub>2</sub>. To avoid edge effects, outer wells were filled with an equivalent volume of medium except for virus-only control wells which were located on the edge of the plate. Plates were inactivated, fixed, and stained using formalinized crystal violet, washed with water, and the absorbance at 570 nm was measured. Half-maximal effective concentration (EC<sub>50</sub>) was calculated using a non-linear curve fit with variable slope (four parameters) in GraphPad Prism v. 6.07 using 24 values from 4 statistical replicates from 2 biological replicates. Controls included virus-only, virus plus vehicle, cell-only, and cells plus vehicle wells as well as a drug-only plate to control for cytotoxicity.



**Fig. S1. Nucleotide changes among Clade IIb MPXV genome sequences**. The predominant 2022 MPXV outbreak variant B.1 and outbreak variant A.2 are highlighted in blue and orange, respectively. The USA\_2021\_MD sequence is highlighted in dark blue. The large node at the center of variant B.1 represents 13 identical sequences; country abbreviations are given for sake of space (sequences used can be found in Table S1). GenBank accession numbers are given for all other samples. Sequence differences between nodes are indicated by the numbers on the branches. Unlabeled nodes represent hypothetical common ancestors, lines connecting nodes do not represent direct links between cases. GBR: United Kingdom; USA: United States of America; FRA: France; BEL: Belgium; PRT: Portugal; ITA: Italy; ESP: Spain; NLD: Netherlands; CHE: Switzerland; SVN: Slovenia.



**Fig. S2. A detailed version of the ML tree shown in main text Fig. 2A.** Ancestral nodes of interest are noted here, and these are used to track the statistical exploration of G-to-A mutations in an APOBEC context through different lineages in the tree summarized in Fig. 2B in the main text and provided in detail in Tables S6 (for Lineage A) and S8 (for Clade I and II outside Lineage A). The sequence name of each of the taxa are shown, preceded by their GenBank accession number.





f R Regional distribution of monkeypox B.1 sequences available at the time of sampling, July 15, 2022

European region expanded, by country



**Figure S3. A. Details of mutational patterns among 397 variant B.1 sequences available through GISAID that were sampled between May 1, 2022, and July 15, 2022.** Each horizontal line represents a unique sequence, and tick marks indicate all mutations relative to the ancestor of the 2022 outbreak variant B.1. Sequences that are phylogenetically similar based on shared mutations are proximal to each other along the Y-axis, and so vertical columns of mutations are indicative of shared mutations between sequences. One would expect roughly the same number of red and blue mutations if G-to-A mutations were randomly occurring; the preponderance of blue ticks indicates the extreme bias favoring G-to-A mutations embedded in a 5' GA-to-AA context among these international 2022 outbreak strains. The GAto-AA pattern dominates (dark blue) over the alternative APOBEC motif 5' GG-to-AG (cyan), as we have seen throughout Lineage A. 114 sequences among the 397 variant B.1 sequences had no SNPS relative to the ancestral form of the genome and these are not shown in the figure. GY-to-AY (red) indicates a G-to-A change followed by a C or T, the G-to-A mutations that are not in an APOBEC motif. **B. Geographic distribution of sampling of the 397 sequences used for part A, based on the sequences available from GISAID sampled between May 1, 2022, and July 15, 2022.** The relative area of a given red circle reflects the number of available sequences. This map captures the level of contributions of early monkeypox genomes to GISAID the geographic distribution of the available data and is not meant to reflect the confirmed cases. The GISAID acknowledgments tables are provided in Tables S10 and S11.



A Mismatches relative to Variant B.1 ancestor

Fig. S4. Chimeric sequences within Clade IIb. A. We acquired 400 full genome MPXV sequences from GISAID sampled between May 1, 2022, and July 15, 2022, for APOBEC analysis. While confirming their lineage association, we identified 3 related sequences that were chimeric in that they carried 5

consecutive SNPs that were mirrored in each ITR that were shared with more ancestral sequences from Lineage A and Clade IIb but not found in variant B.1. Otherwise, these sequences were B.1-like throughout the remainder of the genome. These sequences originated from three different European laboratories\*. The graphic is a "Highlighter" plot (a www.hiv.lanl.gov tool) that shows every SNP relative to the USA 2022 MA001 sequence for a small set of background sequences used to explore that hypothesis that the chimeric viruses were the result of recombination. Using the Recombination Analysis Program (RAPR) (PMID: 29765018 https://www.hiv.lanl.gov/content/sequence/RAP2017/rap.html), and excluding the 5' ITR from the analysis so we did not overcount the repeated mutations in both ITRs (marked in gray). The putative recombinant sequence from Spain was incomplete and did not span the 5' ITR region, therefore we used the 3' region for analysis to enable including all three related forms. We determined that a string of 5 mutations in series that differed from the representative variant B.1 sequences and that are shared with more ancestral Clade IIb sequences is unlikely to have occurred by chance alone (p-value 3.71e-07). Thus, the RAPR analysis supports this being a recombinant lineage, although alternatively this pattern may have emerged as a systematic sequencing artifact. In either case, the analysis indicated these sequences were chimeric and not simple variant B.1 viruses, and so we removed them from the subsequent analysis of variant B.1 sequences in Fig. S3. Red sequence names indicate sequences identified by RAPR as representative of candidate parental lineages, and blue the putative recombinant lineage. **B.** The pattern of interest in the ITR was consistently found in sequences throughout Clade IIb, including samples from the 1970s through contemporary samples excluding the B.1 variant. In the 2021 MD sequence, four of the five variant B.1 mutations were apparent. The GISAID acknowledgments tables are provided in Tables S10 and S11.

\***EPI\_ISL\_13302316**: Laboratory of Clinical Microbiology, Virology and Bioemergencies. ASST-Fatebenefratelli-Sacco, L.Sacco University Hospital, Milano, Italy; **EPI\_ISL\_13331716**: Genomics Division, Instituto Tecnologico y de Energias Renovables (ITER), Poligono Industrial de Granadilla Santa Cruz de Tenerife, Spain (the 3' ITR sequence was not available); **EPI\_ISL\_13052287**: Virology, GENomique EPIdemiologique des maladies Infectieuses, Lyon, France.



F13L variant	Isolate	EC <sub>50</sub> (μM)
353E	Aggregate	0.007731 ± 0.002
353K	Aggregate	0.002860 ± 0.001
353E	MPXV Clade IIa	0.01754 ± 0.007
353E	MPXV_USA_2022_FL001	0.008412 ± 0.004
353E	MPXV_USA_2022_VA001	$0.006001 \pm 0.002$
353E	MPXV_USA_2021_MD	$0.003621 \pm 0.003$
353K	MPXV_USA_2022_MA001	0.002680 ± 0.002
353K	MPXV_USA_2022_FL002	$0.003391 \pm 0.002$
353K	MPXV_USA_2022_UT001	0.002739 ± 0.002
353K	MPXV_USA_2022_UT002	0.002658 ± 0.002

**Fig. S5. Sensitivity of 2022 outbreak MPXVs to tecovirimat (TPOXX). A.** Aggregate results of cytopathic effect (CPE) assay showing cell growth in the presence of MPXV after treatment with different doses of TPOXX. Error bars indicate 95% confidence intervals based on four statistical and two biological replicates at each dose per group. **B.** Half-maximal effective concentration EC<sub>50</sub> of TPOXX for different MPXV isolates. Average plus 95% confidence intervals were based on 24 values from four statistical and two biological replicates.

Accession numbers for Figure S1							
ON676703.1	ON676708.1						
ON563414.3	ON585037.1						
ON622713.1	ON585032.1						
ON585035.1	MT903345.1						
ON568298.1	MT903344.1						
ON614676.1	MT903343.1						
ON563414.2	MT903341.1						
ON627808.1	MT903342.1						
ON585033.1	MN648051.1						
ON622712.1	MT903340.1						
ON676705.1	MT903338.1						
ON676706.1	MT903339.1						
ON595760.1	MT903337.1						
ON676704.1	MG693723.1						
ON585031.1	ON676707.1						
ON615424.1	ON674051.1						
ON622722.1	ON675438.1						
ON609725.1	KJ642617.1						
ON622718.1	ON619835.1						
ON585038.1	ON619836.1						
ON602722.1	ON619837.1						
ON585034.1	ON619838.1						
	KJ642615.1						

 Table S1. List of GenBank accession numbers for sequences used in haplotype network analysis (Fig. S1).

Position	reference	MA001-2022	MD-2021	TYPE	FTYPE	STRAND	EFFECT	NT CHANGE	AA CHANGE ALT	GENE	PRODUCT	Genbank Note
1271	G				009		micconco unright	2140-1	Sor105Lou	MRX// IK R2.001	MRXV/ap001	Vaccinia virus strain Copenhagen C23L; most abundant
2600	G	Â	Â	snp	CDS		missense_variant	161C>T	Ser54Phe	MPXV-UK_P2-002	MPXVgp002	TNF receptor (CrmB) (Cop-C22L); J2L
3120	G	A		snp	CDS	-	synonymous_variant	1497C>T	lle499lle	MPXV-UK_P2-003	MPXVgp003	Ankyrin (Cop-C19L); J3L
3531	G	A	А	snp	CDS		synonymous_variant	1086C>T	lle362lle	MPXV-UK_P2-003	MPXVgp003	Ankyrin (Cop-C19L); J3L
3827	С	т	т	snp	CDS	-	missense_variant	790G>A	Asp264Asn	MPXV-UK_P2-003	MPXVgp003	Ankyrin (Cop-C19L); J3L Secreted EGF-like protein (Cop-C11R); D3R; similar to
7780	с	т	т	snp	CDS	+	synonymous_variant	192C>T	lle64lle	MPXV-UK_P2-006	MPXVgp006	Vaccinia virus strain Copenhagen C11R; secreted growth factor
14009	G	т	т	snp	CDS		missense variant	1268C>A	Ala423Asp	MPXV-UK P2-012	MPXVap012	Ankyrin; Type I IFN resistance (Cop-C9L); D9L; similar to Vaccinia virus strain Copenhagen C9L; ankyrin-like
15437	G	A	А	snp			intergenic_variant				-	ANK-containing protein: apoptosis inihibitor (Cop-M1L):
21732	G	А	А	snp	CDS		synonymous variant	711C>T	Phe237Phe	MPXV-UK P2-021	MPXVap025	O1L; similar to Vaccinia virus strain Copenhagen M1L; ankvrin-like
20276	6				CDS			929C-T	Lou276Lou	MPY// IK P2.021	MRXV/ap026	Kelch-like protein (Cop-F3L); C9L; similar to Vaccinia
24062	0	Â	<u>,</u>	anp	000		missesses uniont	440C-T	Am40Cup	MPX/UK_P2-031	MDX/mp025	Kelch-like protein (Cop-F3L); C9L; similar to Vaccinia
51002	0	^	^	anp	005		masense_vanam	1420.21	Algilocys	WFX0-0K_F2-031	WP XVgp055	S-S bond formation pathway protein substrate (Cop-
34468	G	А	А	snp	CDS	-	missense_variant	232C>T	Pro78Ser	MPXV-UK_P2-037	MPXVgp041	F9L
												Vaccinia virus strain Copenhagen F12L; ct8L; similar to
3/211	G	A	A	snp	CDS		synonymous_variant	1833C>1	Phe611Phe	MPXV-UK_P2-040	MPXVgp044	tormation EEV maturation protein (Cop-F12L); C18L; similar to
38369	G	А	А	snp	CDS		synonymous_variant	675C>T	lle225lle	MPXV-UK_P2-040	MPXVgp044	Vaccinia virus strain Copenhagen F12L; actin tail formation
												EEV maturation protein (Cop-F12L); C18L; similar to Vaccinia virus strain Copenhagen F12L; actin tail
38671	С	т	Т	snp	CDS	-	missense_variant	373G>A	Glu125Lys	MPXV-UK_P2-040	MPXVgp044	formation C19L similar to Vaccinia virus strain Copenhagen F13L
39148	с	т		snp	CDS		missense_variant	1057G>A	Glu353Lys	MPXV-UK_P2-041	MPXVgp045	major envelope antigen of EEV wrapping of IMV to form IEV phospholipase D-like
												DNA polymerase (Cop-E9L); F8L; similar to Vaccinia virus strain Copenhagen E9L; DNA polymerase, catalytic
52894	G	A	А	snp	CDS	-	synonymous_variant	1554C>T	Val518Val	MPXV-UK_P2-055	MPXVgp057	subunit DNA polymerase (Cop-E9L); F8L; similar to Vaccinia
54126	G	A	А	snp	CDS		missense variant	322C>T	Leu108Phe	MPXV-UK P2-055	MPXVap057	virus strain Copenhagen E9L; DNA polymerase, catalytic subunit
							_					Sulfhydryl oxidase (FAD-linked) (Cop-E10R); F9R; similar
54644	G	А	А	snp	CDS	+	missense_variant	166G>A	Asp56Asn	MPXV-UK_P2-056	MPXVgp058	disulfide bond-forming enzyme
					0.00			1000 T			1000	Vacinia virus strain Copenhagen I7L; virion core protein;
64306	G	A	A	snp	CDS	-	synonymous_variant	4200>1	lie140lie	MPXV-UK_P2-066	мехудрова	VLTF-1 (late transcription factor 1) (Cop-G8R); G9R;
73075	с	т	т	snp	CDS	+	missense_variant	89C>T	Ser30Leu	MPXV-UK_P2-076	MPXVgp078	similar to Vaccinia virus strain Copenhagen GBR; late gene transcription factor, VLTF-1
												VLTF-1 (late transcription factor 1) (Cop-G8R); G9R; similar to Vaccinia virus strain Copenhagen G8R; late
73248	G	A		snp	CDS	+	missense_variant	262G>A	Asp88Asn	MPXV-UK_P2-076	MPXVgp078	gene transcription factor, VLTF-1 Entry/fusion complex component, myristylprotein (Cop-
74214	G	A		snp	CDS	+	missense_variant	426G>A	Met142IIe	MPXV-UK_P2-077	MPXVgp079	G9R); G10R; similar to Vaccinia virus strain Copenhagen G9R; myristylated protein
												ss/dsDNA binding protein (VP8) (Cop-L4R); M4R; similar to Vaccinia virus strain Copenhagen L4R; virion core
77392	G	А		snp	CDS	+	missense variant	484G>A	Glu162Lvs	MPXV-UK P2-081	MPXVap083	protein; ssDNA binding; stimulation of I8R helicase activity
											5,	13R similar to Vaccinia virus strain Conenhagen J3R
79357	c		т	snp	CDS	+	synonymous variant	264C>T	lle88lle	MPXV-UK P2-085	MPXVap087	poly-A polymerase stimulatory subunit simultaneously
	-			p			-,,					RNA polymerase subunit (RPO147) (Cop-J6R); L6R; similar to Vaccinia virus strain Conenhagen, I6B; RNA
81284	G	А	А	snp	CDS	+	synonymous_variant	150G>A	Lys50Lys	MPXV-UK_P2-088	MPXVgp090	polymerase, 147 kDa subunit RNA polymerase subunit (RPO147) (Cop. I6R): 1.6R
07202	c	Ŧ	т		009		concommous voriant	1249C+T	Pho/16Pho	MDY// IK D2.099	MRXV/ap000	similar to Vaccinia virus strain Copenhagen J6R; RNA
OLUGE	Ū			unp	000		oynonymous_tanam	1240021	The Forme	111 / W OK_1 2 000	nii Atgpood	RNA polymerase subunit (RPO147) (Cop-J6R); L6R; imilar to Vaccinia virus strain Conceptagen (68: RNA
82460	G	A	А	snp	CDS	+	synonymous_variant	1326G>A	Thr442Thr	MPXV-UK_P2-088	MPXVgp090	polymerase, 147 kDa subunit RNA polymerase subunit (RPO147) (Cop. I6R): 1.6P
84596	c	т		snp	CDS	+	synonymous variant	3462C>T	Phe1154Phe	MPXV-UK P2-088	MPXVap090	similar to Vaccinia virus strain Copenhagen J6R; RNA
86988	с		т	snp	CDS	-	synonymous variant	81G>A	Glu27Glu	MPXV-UK P2-091	MPXVap093	H3L similar to Vaccinia virus strain Copenhagen H3L IMV surface membrane protein
95043	G	A	А	snp	CDS	+	synonymous variant	375G>A	Val125Val	MPXV-UK P2-098	MPXVap100	Virion core (Cop-D3R); E3R; similar to Vaccinia virus strain Copenhagen D3R; virion core protein
											-	DNA helicase, transcript release factor (Cop-A1 8R); A19R: similar to Vaccinia virus strain Copenhagen A1
124139	G	А	А	snp	CDS	+	missense variant	184G>A	Glu621 vs	MPXV-UK P2-128	MPXVm129	8R; virion core associated DNA helicase; post-replicative
											51	DNA helicase, transcript release factor (Cop-A1 8R); A19R: similar to Vaccinia virus strain Copenhagen A1
124683	G	A	А	snp	CDS	+	missense variant	728G>A	Ara243GIn	MPXV-UK P2-128	MPXVap129	8R; virion core associated DNA helicase; post-replicative negative transcription elongation factor
									-		-	VITF-3 45kda subunit (Cop-A23R); A24R; similar to Vaccinia virus strain Copenhagen A23R; intermediate
128707	C GCAATCTTTCTCAA	T GCAATCTTTCT	T GCAATCTTTC	snp	CDS	+	missense_variant	920C>T	Ser307Leu	MPXV-UK_P2-133	MPXVgp134	transcription factor, VITF-3, 45 kDa
133174	TCTTTCTCAA	CAA	TCAA T	indel snp			intergenic_variant					
150480	c	т		snp	CDS	+	missense variant	661C>T	His221Tvr	MPXV-LIK P2-157	MPXVop157	IL-1/TLR signaling inhibitor (Cop-A46R); A47R; similar to Vaccinia virus strain Copenhagen A46R
	GGATATGATGGATA TGATGATATGATGG	GGATATGATG GATATGATGAT	-								or	
150564	ATATGATGATATGA	ATGATGGATAT		inde			intergenic voriant					
151472	A	C		snp			intergenic_variant					
155606	-	-		snp			intergenic_vanant					
162342		•	TCAGATACAG	snp	CDS	+	synonymous_variant	/366>1	Leuz46Leu	MPXV-UK_P2-164	MPXVgp165	Schlaten (Cop-B2R); B4R; Schlaten-like
	AGATACAGATAC		ATACAGATAC					100 1				
169732	ALACAGATACAGAT ACAGATACAGAT		ACAGATACAG ATACAGAT	indel	CDS	+	conservative_infram e_deletion	100_105delA CAGAT	Thr34_Asp35del	MPXV-UK_P2-171	Hypothetical prot	Hypothetical protein (Cop-B11R)
170273	G	А		snp	CDS	+	synonymous_variant	225G>A	Arg75Arg	MPXV-UK_P2-172	MPXVgp172	Ser/Thr Kinase (Cop-B12R); B11R; similar to Vaccinia virus strain Copenhagen B12R; protein kinase-like
178220 179163	G complex	A complex	A complex	snp indel			intergenic_variant intergenic_variant					
181995	G	А	А	snp	CDS	+	missense_variant	625G>A	Asp209Asn	MPXV-UK_P2-182	MPXVgp182	Surface glycoprotein; B21R; putative membrane- associated glycoprotein; cadherin-like domain
183534	с	т		snp	CDS	+	missense_variant	2164C>T	Pro722Ser	MPXV-UK_P2-182	MPXVgp182	Surface glycoprotein; B21R; putative membrane- associated glycoprotein; cadherin-like domain
186593	G	A	А	snp	CDS	+	missense_variant	5223G>A	Met1741lle	MPXV-UK_P2-182	MPXVgp182	Surface glycoprotein; B21R; putative membrane- associated glycoprotein; cadherin-like domain
192418 193407	complex G	complex A	complex A	indel snp	CDS	+	intergenic_variant missense_variant	790G>A	Asp264Asn	MPXV-UK_P2-188	MPXVgp189	Ankyrin (Cop-C19L); J1R; ankyrin-like
193703	с	т	т	snp	CDS	+	synonymous_variant	1086C>T	lle362lle	MPXV-UK_P2-188	MPXVgp189	Ankyrin (Cop-C19L); J1R; ankyrin-like
194114	С	т		snp	CDS	+	synonymous_variant	1497C>T	lle499lle	MPXV-UK_P2-188	MPXVgp189	Ankyrin (Cop-C19L); J1R; ankyrin-like (ITR)
194634	с	т	т	snp	CDS	+	missense_variant	161C>T	Ser54Phe	MPXV-UK_P2-189	MPXVgp190	TNF receptor (CrmB) (Cop-C22L); J2R; secreted TNF binding protein
												Chemokine binding protein (Cop-C23L); J3R; similar to Vaccinia virus strain Copenhagen B29R; CC-chemokine
195963	C		ſ	SND	CDS	+	missense variant	314C>T	Ser105Leu	MPXV-UK P2-190	MPXVap191	pinging

# Table S2. Variant table showing differences in genomes of USA\_2022\_MA001 (ON563414.3) andUSA\_2021\_MD (ON676708.1) compared to MT903344.1 UK-P2. Changes shared amongUSA\_2022\_MA001 and USA\_2021\_MD are highlighted in green. Position and annotation information isbased on reference MT903344.1.

	rererence	17-2021	FL001-2022	VA001-2022	1166	FITPE	STRAND	EFFECI	NT CHANGE	AA CHANGE A	GENE	PRODUCT	Genbank Note
2761	G	A			snp			intergenic variant					
112/1	G			^	con	CDS		synonymous variant	1560C\T	10522110	MRXV-UK R2-010	MRXV/mp010	D7I bost range antwrin-like
11541	0			A .	siip	005		synonymous_variant	1309021	ile525ile	WIPAV-UK_P2-010	INIPX V BDOTO	D/L HOST Lange ankythi-like
12108	G			A	snp	CDS	-	stop_gained	802C>T	Arg268*	MPXV-UK_P2-010	MPXVgp010	D7L host range ankyrin-like
13307	с	т	т	т	snp			intergenic_variant					
13657	c			т	snn	CDS		missense variant	1620G>A	Met540ILe	MPXV-UK P2-012	MPXVgn012	D9L similar to Vaccinia virus strain Conenhagen C9Lankyrin-like
15057	c 0			-	Ship			inissense_vanane	10200771		MINT OR_TE OIL	ten XV Spore	
15114	C			1	snp	CDS	-	missense_variant	163G>A	Asp55Asn	MPXV-UK_P2-012	MPXVgpU12	D9L similar to Vaccinia virus strain Copenhagen C9L ankyrin-like
16236	C			т	snp	CDS	-	missense_variant	151G>A	Asp51Asn	MPXV-UK_P2-013	MPXVgp013	D10L similar to Vaccinia virus strain Copenhagen C7L host range
18801	с	т	т	т	snp	CDS	-	missense_variant	37G>A	Asp13Asn	MPXV-UK_P2-016	MPXVgp016	D13L similar to Vaccinia virus strain Copenhagen C4L
23573	т	с	с	с	snp	CDS		synonymous variant	555A>G	Ser185Ser	MPXV-UK P2-023	MPXVgp027	C1L similar to Vaccinia virus strain Copenhagen K1L host range ankyrin-like
				-				-,,				0000	SDL2 C2L similar to Vaccinia virus strain Conenhagen K2L serine protease inhibitor-like
													SPI-5 CZE similar to vaccinia virus strain copernagen kZE serine protease minortor-like
25081	C		1		snp	CDS	-	missense_variant	40/G>A	Arg136GIn	MPXV-UK_P2-024	MPXVgp028	SPI-3 inhibition of the ability of infected cells to fuse
25670	A	G	G	G	snp	CDS	-	missense_variant	107T>C	Phe36Ser	MPXV-UK_P2-025	MPXVgp029	C3L
29896	с			т	snp	CDS		synonymous variant	1308G>A	Pro436Pro	MPXV-UK P2-031	MPXVgp035	C9L similar to Vaccinia virus strain Copenhagen F3L kelch-like
20645	C	т	т	т	con	CDS		missansa variant	5500>4	Acn197Acn	MDV///// D2-021	MRXV(ap025	COL similar to Vaccinia visus strain Conenhagen E21 kelch-like
50045	с -	-	<u>.</u>	-	siip	005	-	missense_vanant	55902A	ASPIO/ASI	WIPAV-UK_P2-051	INIEX V BH022	CSE similar to vacuna virus strain copernagen PSE kerch-like
34481	с	т	т	т	snp	CDS	-	synonymous_variant	219G>A	Val73Val	MPXV-UK_P2-037	MPXVgp041	C15L similar to Vaccinia virus strain Copenhagen F9L
34587	с	т	т	т	snp	CDS	-	missense_variant	113G>A	Gly38Glu	MPXV-UK_P2-037	MPXVgp041	C15L similar to Vaccinia virus strain Copenhagen F9L
36907	G		A		snp	CDS		synonymous variant	186C>T	Phe62Phe	MPXV-UK P2-039	MPXVgp043	C17L similar to Vaccinia virus strain Copenhagen F11L
												01	C19L similar to Vaccinia virus strain Copenhagen E13L major envelope antigen of EEV
20420	-	<i>c</i>	~	~		coc			10771.0	charach		1000/045	cise similar to vaccina virus strain coperinagen i ise major enverope antigen of LEV
39128	·	C	L	C	snp	CDS	-	synonymous_variant	1077A>G	GIU359GIU	IVIPXV-UK_P2-041	IMPXVgp045	wrapping or liviv to form IEV phospholipase D-like
													C19L similar to Vaccinia virus strain Copenhagen F13L major envelope antigen of EEV
39182	G	A			snp	CDS	-	synonymous_variant	1023C>T	Val341Val	MPXV-UK_P2-041	MPXVgp045	wrapping of IMV to form IEV phospholipase D-like
42110	C			т	snn	CDS	+	missense variant	155C>T	Ser52Leu	MPXV-LIK P2-046	MPXVgn049	C23R similar to Vaccinia virus strain Conenhagen F17R virion core DNA-binding
42110	c				Ship	coc		missense_variant	200001	Mark 7011 -	MID X V OK_12 040	MIN XY SPOID	C220 shulles to Vaccinia virus strain Copenhagen 12/1 virion core biot binding
42192	9		A		snp	CDS	Ŧ	missense_vanant	25/G/A	Wet/9ne	WIPAV-UK_P2-040	MINX A Shora	C25K similar to vaccinia virus strain coperinagen F1/K virion core DivA-binding
42912	C		T		snp	CDS	-	missense_variant	786G>A	Met262IIe	MPXV-UK_P2-047	MPXVgp050	F1L similar to Vaccinia virus strain Copenhagen E1L poly-A polymerase catalytic
50355	G		A		snp	CDS	+	missense_variant	361G>A	Asp121Asn	MPXV-UK_P2-053	MPXVgp055	F6R similar to Vaccinia virus strain Copenhagen E7R soluble myristylated protein
54277	с		Т		snp	CDS	-	synonymous variant	171G>A	Ala57Ala	MPXV-UK P2-055	MPXVgp057	F8L similar to Vaccinia virus strain Copenhagen E9L DNA polymerase catalytic subunit
57202	C	т			con	CDS	-	missansa variant	215654	Arg72luc	MDVV-UK D2-050	MRXV/mn061	03L similar to Vaccinia virus strain Conenhagen 03L virion-associated dutaredoxin
57255	с С			•	stip	000		missense_vananc	215028	Alg/2Lys	WIF XV-OK_F2-033	IVIP XV SPOOL	Q2E similar to vaccinia virus strain coperinagen oze vinon-associated giutaredoxin
61853	G	A			snp	CDS	-	synonymous_variant	1/5C>1	Leu59Leu	MPXV-UK_P2-063	MPXVgp065	I4L similar to Vaccinia virus strain Copenhagen I4L ribonucleotide reductase large
64225	G			A	snp	CDS	-	synonymous_variant	501C>T	Ala167Ala	MPXV-UK_P2-066	MPXVgp068	17L similar to Vaccinia virus strain Copenhagen 17L virion core protein similar to DNA
72371	т	c	с	C	snn	CDS		missense variant	586A>G	Asn196Asn	MPXV-UK P2-075	MPXVgp077	G8I similar to Vaccinia virus strain Copenhagen G7I virion protein
73635	c		т		snp	CDS	+	synonymous variant	639C>T	100213100	MPXV-IIK P2.076	MPXVan079	G9R similar to Vaccinia virus strain Copenhagen G8R late gene transcription fector VITE
7 4025	-	т			snp	CDC	-	_,vdridfit	4470-7	Lease 10Leu	MDV// UK_D2_077	MDV// 5070	C100 similar to Vaccino virus strain Copenhagen Gon atte gene transcription factor VLTF
/4235	L I	1			snp	CDS	7	synonymous_variant	44/L>1	11e14Alle	WPAV-UK_P2-077	IVIPAVgp079	GLUR SIIIIIar to vaccinia virus strain Copenhagen G9K myristylated protein
75623	G			A	snp	CDS	+	missense_variant	28G>A	Asp10Asn	MPXV-UK_P2-079	MPXVgp081	M2R similar to Vaccinia virus strain Copenhagen L2R
													L3R similar to Vaccinia virus strain Copenhagen J3R poly-A polymerase stimulatory
70/15	6		4		snn	CDS	+	missense variant	322654	Asn108Acn	MPXV-IIK P2.00F	MPXV m097	subunit simultaneously can-specific mRNA (pueleoside O2-)-methyltraneferane
/ 9415	с С				sub	CDC			0000	- OP 100ASI		WIE AV SHOOT	sadarine simultaneously cap-spectric minika (nucleoslue-02 - )-methyltransterase
80108	G	A	A	A	snp	CDS	+	synonymous_variant	99G>A	Leu33Leu	IMPXV-UK_P2-086	MPXVgp088	L4K similar to Vaccinia virus strain Copennagen J4R RNA polymerase 22 kDa subunit
83335	Т	С	С	С	snp	CDS	+	missense_variant	2201T>C	Leu734Ser	MPXV-UK_P2-088	MPXVgp090	L6R similar to Vaccinia virus strain Copenhagen J6R RNA polymerase 147 kDa subunit
87239	A	G	G	G	snp	CDS	-	missense variant	2218T>C	Tyr740His	MPXV-UK P2-092	MPXVgp094	H4L similar to Vaccinia virus strain Copenhagen H4L virion core RNA polymerase-
97206		~ ~	~	Č.		CDC		cumonumous unsignt	31517-0	Dho717Dho	MDV// UK_D2.002	MDVI/ap004	HAL similar to Vassinia visus strain Consenhagen HAL vision sere DNA polymerase
87306		0	0	0	sub	005		synonymous_variant	2131170	File/1/Phe	WIFAV-UK_P2-092	MIPA V SD094	THE SITTLE TO VACULTE VILUS STATE COPERINAGED HAL VILION COLE KINA POLYMERSE-
91737	A	G	G	G	snp			intergenic_variant					
													E1R similar to Vaccinia virus strain Copenhagen D1R mRNA capping enzyme large
93396	G	Α	Α	A	snn	CDS	+	missense variant	1657G>A	Glu553Lvs	MPXV-UK P2-096	MPXVgp098	subunit RNA 5' triphosphatase and RNA guanylyl transferase activities
05045	- -				con	CDC		missonso variant	ETCTNA	Ace 102Clu	MDXV/ UK_D2.000	MDVI/ge101	E4B similar to Vassinia visus strain Consenhagen D4B urasil DNA shusesulare
95945			A		snp	CDS	Ŧ	missense_vanant	5/01/A	Aspiszaiu	WIPAV-UK_P2-099	MIN X V SP 101	E4K similar to vaccinia virus strain coperinagen D4K uracii DivA giycosyiase
100649	c	т			snp	CDS	+	synonymous_variant	255C>T	Val85Val	MPXV-UK_P2-102	MPXVgp104	E7R similar to Vaccinia virus strain Copenhagen D7R RNA polymerase 18 kDa subunit
													E12L similar to Vaccinia virus strain Copenhagen D12L mRNA capping enzyme small
105755	6		Δ		snn	CDS		synonymous variant	223C>T	10075100	MPXV-LIK P2-107	MPXVgn109	subunit mRNA (guanine-N7-)-methyl-transferase transcription initiation factor
113553	c		т		con	CDC		missones variant	007C>A	Ace 2224 ce	MDVV UK D2 115	MDVI/ap117	A 71 similar to Vassinia visus strain Cananhagen AG
112553	L		1		snp	CDS		missense_variant	997G>A	AspasaAsn	MPXV-UK_P2-115	MPXvgp117	A /L similar to vaccinia virus strain Copennagen A6L
													A8L similar to Vaccinia virus strain Copenhagen A7L early transcription factor VETF
114668	G	A	A	A	snp	CDS	-	synonymous_variant	1038C>T	Ala346Ala	MPXV-UK_P2-116	MPXVgp118	large subunit needed for morphogenesis of the virion core
117128	C			т	snn	CDS	-	synonymous variant	2469G>A	Ala823Ala	MPXV-UK P2-119	MPXVgp121	A111 similar to Vaccinia virus strain Copenhagen A101 major virion core protein p4a
110740	c				cop	CDC		superior union	840C>T	Dhe 2020he	MDV// UK_D2 110	MDVI/ge121	A 111 similar ta Vassinia vizus straia Cononhagen A 101 maior vizion coro protein p 4a
110/40	-	A			siip	005		synonymous_variant	049021	Phezosphie	WIPAV-UK_P2-119	IVIP X V BD 121	Alle similar to vaccina virus strain coperinagen Alocinajor virion core protein p4a
119305		C	C	C	snp	CDS	-	missense_variant	292A>G	Asn98Asp	MPXV-UK_P2-119	MPXVgp121	A11L similar to Vaccinia virus strain Copenhagen A10L major virion core protein p4a
121329	т	с	с	С	snp	CDS	-	missense_variant	49A>G	Thr17Ala	MPXV-UK_P2-122	MPXVgp124	A14L similar to Vaccinia virus strain Copenhagen A13L IMV inner and outer membrane
													A 19R similar to Vaccinia virus strain Copenhagen A1 8R virion core associated DNA
124040	G		٨	^	con	CDS	-	missansa variant	95654	Acp29Acp	MDVV-11/ D2-129	MRXV/ap120	helicase post-replicative perative transcription elongation factor
124040	G	A	A	А	snp	CDS	+	missense_variant	85G>A	Asp29Asn	MPXV-UK_P2-128	MPXVgp129	helicase post-replicative negative transcription elongation factor
124040	G	А	A	A	snp	CDS	+	missense_variant	85G>A	Asp29Asn	MPXV-UK_P2-128	MPXVgp129	helicase post-replicative negative transcription elongation factor A19R similar to Vaccinia virus strain Copenhagen A1 8R virion core associated DNA
124040 125258	G A	A G	A G	A G	snp snp	CDS CDS	+	missense_variant missense_variant	85G>A 1303A>G	Asp29Asn Lys435Glu	MPXV-UK_P2-128 MPXV-UK_P2-128	MPXVgp129 MPXVgp129	helicase post-replicative negative transcription elongation factor A19R similar to Vaccinia virus strain Copenhagen A1 8R virion core associated DNA helicase post-replicative negative transcription elongation factor
124040 125258 128543	G A C	A G	A G	A G T	snp snp snp	CDS CDS CDS	+ + +	missense_variant missense_variant synonymous variant	85G>A 1303A>G 756C>T	Asp29Asn Lys435Glu Ile252lle	MPXV-UK_P2-128 MPXV-UK_P2-128 MPXV-UK_P2-133	MPXVgp129 MPXVgp129 MPXVgp134	helicase post-replicative negative transcription elongation factor A198 similar to Vaccinia virus strain Copenhagen A1.88 virion core associated DNA helicase post-replicative negative transcription elongation factor A248 similar to Vaccinia virus strain Copenhagen A28 Intermediate transcription
124040 125258 128543	G A C	A G	A G	A G T	snp snp snp	CDS CDS CDS	+ + + + + + + + + + + + + + + + + + + +	missense_variant missense_variant synonymous_variant	85G>A 1303A>G 756C>T	Asp29Asn Lys435Glu Ile252Ile Glu5111ur	MPXV-UK_P2-128 MPXV-UK_P2-128 MPXV-UK_P2-133 MPXV-UK_P2-134	MPXVgp129 MPXVgp129 MPXVgp134 MPXVgp135	helicase post-replicative negative transcription elongation factor AISR similar to Vaccinia virus strain Copenhagen A1 SR virion core associated DNA helicase post-replicative negative transcription elongation factor A2R4 similar to Vaccinia virus strain Copenhagen A23R intermediate transcription A3R4 similar to Vaccinia virus strain Copenhagen A23R intermediate transcription
124040 125258 128543 130463	G A C G	A G	A G	A G T A	snp snp snp snp	CDS CDS CDS CDS CDS	+ + + +	missense_variant missense_variant synonymous_variant missense_variant	85G>A 1303A>G 756C>T 1531G>A	Asp29Asn Lys435Glu Ile252Ile Glu511Lys	MPXV-UK_P2-128 MPXV-UK_P2-128 MPXV-UK_P2-133 MPXV-UK_P2-134	MPXVgp129 MPXVgp129 MPXVgp134 MPXVgp135	helicase post-replicative negative transcription elongation factor AISR similar to Vaccinia virus strain Copenhagen A1 SR virion core associated DNA helicase post-replicative negative transcription elongation factor A2AIs similar to Vaccinia virus strain Copenhagen A2AI intermediate transcription A25R similar to Vaccinia virus strain Copenhagen A24R RNA polymerase 132 kDa
124040 125258 128543 130463 134453	G A C G C	A G	A G	A G T A T	snp snp snp snp snp	CDS CDS CDS CDS	+ + + +	missense_variant missense_variant synonymous_variant missense_variant intergenic_variant	85G>A 1303A>G 756C>T 1531G>A	Asp29Asn Lys435Glu Ile252Ile Glu511Lys	MPXV-UK_P2-128 MPXV-UK_P2-128 MPXV-UK_P2-133 MPXV-UK_P2-134	MPXVgp129 MPXVgp129 MPXVgp134 MPXVgp135	helicase post-replicative negative transcription elongation factor AISR similar to Vaccinia virus strain Copenhagen AI SR virion core associated DNA helicase post-replicative negative transcription elongation factor A2RR similar to Vaccinia virus strain Copenhagen A23R intermediate transcription A2SR similar to Vaccinia virus strain Copenhagen A2AR RNA polymerase 132 kDa
124040 125258 128543 130463 134453 136523	G A C G C c complex	A G complex	A G complex	A G T A T complex	snp snp snp snp snp indel	CDS CDS CDS CDS CDS CDS	+ + + -	missense_variant missense_variant synonymous_variant missense_variant intergenic_variant conservative_inframe	85G>A 1303A>G 756C>T 1531G>A 21114_1122delG	Asp29Asn Lys435Glu IIe252IIe Glu511Lys Asp372_Asp37	MPXV-UK_P2-128 MPXV-UK_P2-128 MPXV-UK_P2-133 MPXV-UK_P2-134 MPXV-UK_P2-137	MPXVgp129 MPXVgp129 MPXVgp134 MPXVgp135 MPXVgp138	helicase post-replicative negative transcription elongation factor A19R similar to Vaccinia virus strain Copenhagen A18R virion core associated DNA helicase post-replicative negative transcription elongation factor A2R similar to Vaccinia virus strain Copenhagen A28R intermediate transcription A2R similar to Vaccinia virus strain Copenhagen A28R INAP on Polymerase 132 kDa A28L major component of IMV surface tubules p4c
124040 125258 128543 130463 134453 136523 140503	G A C G C complex A	A G	A G complex C	A G T A T complex	snp snp snp snp indel snp	CDS CDS CDS CDS CDS CDS CDS CDS	+ + + -	missense_variant missense_variant synonymous_variant missense_variant intergenic_variant conservative_inframe missense variant	85G>A 1303A>G 756C>T 1531G>A 1114_1122delG 5831>G	Asp29Asn Lys435Glu lle252lle Glu511Lys Asp372_Asp37 Ser195Ala	MPXV-UK_P2-128 MPXV-UK_P2-128 MPXV-UK_P2-133 MPXV-UK_P2-134 MPXV-UK_P2-137 MPXV-UK_P2-137	MPXVgp129 MPXVgp129 MPXVgp134 MPXVgp135 MPXVgp138 MPXVgp144	helicase post-replicative negative transcription elongation factor A198 similar to Vaccinia virus strain Copenhagen A1 88 virion core associated DNA helicase post-replicative negative transcription elongation factor A284 similar to Vaccinia virus strain Copenhagen A238 intermediate transcription A258 similar to Vaccinia virus strain Copenhagen A248 RNA polymerase 132 kDa A28L major component of IMV surface tubules p4c A34L similar to vaccinia virus virus Copenhagen A23L DNA packaeine into virion NTP-
124040 125258 128543 130463 134453 136523 140503	G A C G C C complex A C	A G complex	A G	A G T A T complex	snp snp snp snp indel snp	CDS CDS CDS CDS CDS CDS CDS CDS CDS	+ + + - -	missense_variant missense_variant synonymous_variant missense_variant conservative_inframe missense_variant curpopumous_variant	85G>A 1303A>G 756C>T 1531G>A 21114_1122delG 583T>G 297C-T	Asp29Asn Lys435Glu Ile252Ile Glu511Lys Asp372_Asp37 Ser195Ala	MPXV-UK_P2-128 MPXV-UK_P2-133 MPXV-UK_P2-134 MPXV-UK_P2-134 MPXV-UK_P2-137 MPXV-UK_P2-147	MPXVgp129 MPXVgp129 MPXVgp134 MPXVgp135 MPXVgp138 MPXVgp144 MPXVgp147	helicase post-replicative negative transcription elongation factor A19R similar to Vaccinia virus strain Copenhagen A18R virion core associated DNA helicase post-replicative negative transcription elongation factor A2RR similar to Vaccinia virus strain Copenhagen A28R intermediate transcription A28R similar to Vaccinia virus strain Copenhagen A24R RNA polymerase 132 kDa A28L major component of IMV surface tubules p4c A34L similar to Vaccinia virus strain Copenhagen A32. DNA packaging into virion NTP- A32R cimilar to Vaccinia virus strain Copenhagen A32. DNA packaging into virion NTP- A32R similar to Vaccinia virus strain Copenhagen A32. DNA packaging into virion NTP-
124040 125258 128543 130463 134453 136523 140503 142509	G A C G C c complex A C C	A G	A G	A G T A Complex	snp snp snp snp indel snp snp	CDS CDS CDS CDS CDS CDS CDS CDS CDS CDS	+ + + - - -	missense_variant missense_variant synonymous_variant missense_variant intergenic_variant conservative_inframe missense_variant synonymous_variant	85G>A 1303A>G 756C>T 1531G>A 1114_1122delG 5837>G 297C>T	Asp29Asn Lys435Glu Ile252lle Glu511Lys Asp372_Asp37 Ser195Ala Ile99lle	MPXV-UK_P2-128 MPXV-UK_P2-128 MPXV-UK_P2-133 MPXV-UK_P2-134 MPXV-UK_P2-137 MPXV-UK_P2-144 MPXV-UK_P2-147	MPXVgp129 MPXVgp129 MPXVgp134 MPXVgp135 MPXVgp138 MPXVgp144 MPXVgp147	helicase post-replicative negative transcription elongation factor AISR similar to Vaccinia virus strain Copenhagen AI SR virion core associated DNA helicase post-replicative negative transcription elongation factor A2R8 similar to Vaccinia virus strain Copenhagen A23R intermediate transcription A2SR similar to Vaccinia virus strain Copenhagen A24R RNA polymerase 132 kDa A28L major component of IMV surface tubules p4c A34L similar to Vaccinia virus strain Copenhagen A32L DNA packaging into virion NTP- A37R similar to Vaccinia virus strain Copenhagen A32L DNA packaging into virion NTP- A37R similar to Vaccinia virus strain Copenhagen A32R
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Table S3. Variant table showing differences in genomes of U.S. variant A.2 USA\_2022\_FL001 (ON674051.1), USA\_2022\_VA001 (ON675438.1) and USA\_2021\_TX (ON676707.1) compared to MT903344.1 UK-P2. Changes shared among all three A.2 sequences are highlighted in green. Position and annotation information is based on reference MT903344.1.

Sample	Year	Month	Country	State	Specimen	Travel	WA_MPXV	PCR	Clade	Variant
MD	2021	November	USA	MD	swab	Nigeria	19.2	positive	llb	A.1.1
TX	2021	July	USA	TX	swab	Nigeria	18.6	positive	llb	A.2
MA001	2022	May	USA	MA	swab	Canada	22.7	positive	llb	B.1
UT001	2022	May	USA	UT	swab	Europe	24.1	positive	llb	B.1
UT002	2022	May	USA	UT	swab	Europe	24.9	positive	llb	B.1
FL001	2022	May	USA	FL	swab	Middle East	21.5	positive	llb	A.2
FL002	2022	May	USA	FL	swab	Domestic USA	23.4	positive	llb	B.1
VA001	2022	May	USA	VA	swab	Western Africa	18.8	positive	llb	A.2
CA001	2022	May	USA	CA	swab	Europe	21.7	positive	llb	B.1
		Oxfo	ord Nanopore	e				umina		
Sample	platform	reads	mapped	perc_map	avg_cov	platform	reads	mapped	perc_map	avg_cov
MD	MinION	9,555,644	20,924	0 220/	01	110	4075400			1705
TX				0.22 /0	91	MiSeq	4075122	2009295	49.31	1100
	MinION	9,806,213	57,432	0.22%	64	MiSeq	4075122 3,392,364	2009295 181,357	49.31 5.35	59
MA001	MinION	9,806,213 1,843,299	57,432 10,548	0.59% 0.57%	64 35	MiSeq MiSeq MiSeq	4075122 3,392,364 59,677,436	2009295 181,357 17501	49.31 5.35 0.03	59 1.5
MA001 UT001	MinION MinION GridION	9,806,213 1,843,299 2,979,999	57,432 10,548 8,855	0.59% 0.57% 0.30%	91 64 35 42	MiSeq MiSeq MiSeq MiSeq	4075122 3,392,364 59,677,436 18896008	2009295 181,357 17501 87807	49.31 5.35 0.03 0.46	59 1.5 92
MA001 UT001 UT002	MinION MinION GridION GridION	9,806,213 1,843,299 2,979,999 1,132,000	57,432 10,548 8,855 4,728	0.22% 0.59% 0.57% 0.30% 0.42%	91 64 35 42 23	MiSeq MiSeq MiSeq MiSeq MiSeq	4075122 3,392,364 59,677,436 18896008 12570222	2009295 181,357 17501 87807 63578	49.31 5.35 0.03 0.46 0.51	59 1.5 92 61
MA001 UT001 UT002 FL001	MinION MinION GridION GridION GridION	9,806,213 1,843,299 2,979,999 1,132,000 2,555,998	57,432 10,548 8,855 4,728 24,451	0.22% 0.59% 0.57% 0.30% 0.42% 0.96%	91 64 35 42 23 150	MiSeq MiSeq MiSeq MiSeq MiSeq MiSeq	4075122 3,392,364 59,677,436 18896008 12570222 18434660	2009295 181,357 17501 87807 63578 253654	49.31 5.35 0.03 0.46 0.51 1.38	59 1.5 92 61 258
MA001 UT001 UT002 FL001 FL002	MinION MinION GridION GridION GridION	9,806,213 1,843,299 2,979,999 1,132,000 2,555,998 1,456,000	57,432 10,548 8,855 4,728 24,451 17,786	0.22% 0.59% 0.57% 0.30% 0.42% 0.96% 1.22%	91 64 35 42 23 150 61	MiSeq MiSeq MiSeq MiSeq MiSeq MiSeq MiSeq	4075122 3,392,364 59,677,436 18896008 12570222 18434660 11192710	2009295 181,357 17501 87807 63578 253654 90699	49.31 5.35 0.03 0.46 0.51 1.38 0.81	59 1.5 92 61 258 97
MA001 UT001 UT002 FL001 FL002 VA001	MinION MinION GridION GridION GridION GridION	9,806,213 1,843,299 2,979,999 1,132,000 2,555,998 1,456,000 5,119,998	57,432 10,548 8,855 4,728 24,451 17,786 11,446	0.22% 0.59% 0.57% 0.30% 0.42% 0.96% 1.22% 0.22%	91 64 35 42 23 150 61 28	MiSeq MiSeq MiSeq MiSeq MiSeq MiSeq MiSeq MiSeq	4075122 3,392,364 59,677,436 18896008 12570222 18434660 11192710 17421364	2009295 181,357 17501 87807 63578 253654 90699 39206	49.31 5.35 0.03 0.46 0.51 1.38 0.81 0.23	59 1.5 92 61 258 97 37

Table S4. Details for U.S. MPXV genomes from nine 2021 – 2022 cases.

Sample	Clade	Variant	OPX3	MPXV-Cladell	diff
ON563414_MPXV_USA_2022_MA001	Clade Ilb	B.1	31.0	22.7	8.3
ON676704_MPXV_USA_2022_FL002	Clade Ilb	B.1	30.6	23.4	7.2
ON676705_MPXV_USA_2022_UT001	Clade Ilb	B.1	29.4	24.1	5.3
ON676706_MPXV_USA_2022_UT002	Clade Ilb	B.1	32.1	24.6	7.5
ON676703_MPXV_USA_2022_CA001	Clade Ilb	B.1	29.1	21.7	7.4
ON676708_MPXV_USA_2021_MD	Clade Ilb	A.1.1	24.8	19.2	5.6
ON674051_MPXV_USA_2022_FL001	Clade Ilb	A.2	21.7	21.2	0.5
ON675438_MPXV_USA_2022_VA001	Clade Ilb	A.2	17.8	18.8	-1.0
ON676707_MPXV_USA_2021_TX	Clade Ilb	A.2	17.2	18.6	-1.4
MT903337_MPXV_Nigeria_2017_FCT	Clade Ilb	А	18.0	19.2	-1.2
Positive control			23.4	23.2	0.2

**Table S5. Summary PCR results for OPX3** (*15*) **and Clade II-specific** (*14*) **real-time PCR assay.** Average Ct value is based on triplicate testing for each assay. Difference (diff) was calculated by subtracting Clade II-specific average Ct from OPX3 average Ct value.

	All other	G-to-A in an	APOBEC	G-to-A	Non-APOBEC			Odds	Clade or
Data subsets description and sequence names	mutatio	APOBEC	context G, no	non-APOBEC	context G, no	p-value	q-value	Ratio	Lineage
	ns	context	change	context	change				8
All mutations in Lineage A, from Node 48									
All unique SNPs in lineage A merged	14	167	36415	9	30669	< 0.000001	0.000006	15.63	Lineage A
ON627808.1.MPX.USA.UT-UPHL-82200022.197114.2022-05-20	2	58	36524	2	30676	<0.000001	0.000006	24.36	A B.1
ON568298.1.MPX-BY-IMB25241.197378.Germany.2022-05-19	2	59	36523	2	30676	<0.000001	0.000006	24.78	A B.1
ON585033.1.MPX.PT0006.197220.Portugal.2022-05-15	2	59	36523	2	30676	<0.000001	0.000006	24.78	A B.1
ON585035.1.MPX.P10009.197220.P0rtugal.2022-05-15	2	58	36524	2	30676	<0.000001	0.000006	24.30	A B.1
	2	50	30524	2	30676	<0.000001	0.000006	24.50	A B.1
	2	50	30524	2	30676	<0.000001	0.000006	24.50	A B.1
ON676705.IVIPXV_USA_2022_01001	2	59	30523	2	30676	<0.000001	0.000006	24.70	A B.1
ON676706 MPXV LISA 2022_FL002	2	58	36524	2	30676	<0.000001	0.000000	23.2	A B.1
ON670700.WFXV_03A_2022_01002	2	59	36523	2	30676	<0.000001	0.000000	24.30	A B.1
ON622713.1 MPX LIZ Belgium BEGA 2 198016 2022-05-22	2	60	36522	2	30676	<0.000001	0.000000	25.2	Δ B 1
ON614676 1 MPX INML-Pt1 190289 Italy 2022-05-18	2	53	36529	2	30676	<0.000001	0.000000	22.2	A B 1
ON676708 MPXV LISA 2021 MD	2	49	36533	3	30675	<0.000001	0.000000	13 71	ΔΔ11
MT903344.1 MPXV UK P2	0	15	36567	1	30677	0.001513	0.004144	12.58	A A.1
MT903345.1 MPXV UK P3	0	15	36567	1	30677	0.001513	0.004144	12.58	A A.1
MT903341.1 MPXV M5320 M15 Bavelsa	0	13	36569	1	30677	0.005002	0.011892	10.91	A A.1
MT903343.1 MPXV UK P1	0	14	36568	2	30676	0.009786	0.022019	5.872	A A.1
MN648051.1 MPXV Israel 2018	1	16	36566	1	30677	0.000829	0.002678	13.42	A A.1
MT903342.1 MPXV Singapore	0	16	36566	1	30677	0.000829	0.002678	13.42	A A.1
MT250197.1 MPXV Singapore 2019	2	16	36566	1	30677	0.000829	0.002678	13.42	A A.1
ON674051 MPXV USA 2022 FL001	2	28	36554	2	30676	0.000008	0.000042	11.75	A A.2
ON676707_MPXV_USA_2021_TX	0	32	36550	2	30676	0.000001	0.000006	13.43	A A.2
ON675438_MPXV_USA_2022_VA001	1	34	36548	2	30676	< 0.000001	0.000006	14.27	A A.2
MT903338.1_MPXV_M2957_Lagos	0	4	36578	0	30678	0.130775	0.189398	inf	A
MT903340.1_MPXV_M5312_HM12_Rivers	0	5	36577	0	30678	0.067325	0.114635	inf	A
MG693724.1_MPXV_Nigeria_2017	3	5	36577	0	30678	0.067325	0.114635	inf	A
MT903339.1_MPXV_M3021_Delta	0	4	36578	0	30678	0.130775	0.189398	inf	A
MT903337.1_MPXV_M2940_FCT	1	3	36579	1	30677	0.630746	0.749755	2.516	A
MG693723.1_MPXV_Nig_2017_297957	1	11	36571	0	30678	0.001409	0.004144	inf	A
Internal Branches within Lineage A									
Node 52-56	1	35	36535	1	30676	< 0.000001	0.00006	29.39	A
Node 56-57	1	11	36524	0	30675	0.001403	0.004144	inf	A
Node 48-52	0	12	36570	1	30677	0.004944	0.011892	10.07	A
Node 48-71	0	14	36568	1	30677	0.002755	0.007232	11.74	A
All mutations in Variant B.1, from Node 57									
All unique SNPs in variant B.1 merged	0	8	36517	0	30675	0.009498	0.021759	inf	Lineage A B.1
ON627808.1.MPX.USA.UT-UPHL-82200022.197114.2022-05-20	0	0	36525	0	30675	1	1.000000		A B.1
ON568298.1.MPX-BY-IMB25241.197378.Germany.2022-05-19	0	1	36524	0	30675	1	1.000000	inf	A B.1
ON585033.1.MPX.PT0006.197220.Portugal.2022-05-15	0	1	36524	0	30675	1	1.000000	inf	A B.1
ON585035.1.MPX.PT0009.197220.Portugal.2022-05-15	0	0	36525	0	30675	1	1.000000		A B.1
ON563414.MPXV_USA_2022_MA001	0	0	36525	0	30675	1	1.000000		A B.1
ON676703.MPXV_USA_2022_CA001	0	0	36525	0	30675	1	1.000000		A B.1
ON676705.MPXV_USA_2022_UT001	0	1	36524	0	30675	1	1.000000	inf	A B.1
ON676704.MPXV_USA_2022_FL002	0	2	36523	0	30675	0.503782	0.616338	Inf	A B.1
ON676706.MPXV_USA_2022_UT002	0	0	36525	0	30675	1	1.000000		A B.1
ON622712.1.MPX.Belgium.UZ_REGA_1.198010.2022-05-19	0	1	36524	0	30675	1	1.000000	inf	A B.1
ON622/13.1.MPX.U2_Belgium.REGA_2.198016.2022-05-22	0	2	36523	0	30675	0.503782	0.616338	INT	A B.1
ON614676.1.MPX.INMI-Pt1.190289.Italy2022-05-18	0	0	36525	0	30675	1	1.000000		A B.1
All unique mutations in extended variant B1 data, including 397									
International GISAID sequences sampled between May 1 - July 15,									
2022, details in Sup. Fig. 52 and 55.	145	275	25064	22	20702	-0.000001	0.000000	7.00	1.5.4
All unique SNPs in extended lineage B.1 merged	115	2/5	35064	33	29703	<0.000001	0.000006	7.06	A B.1
All mutations in variant A.2, from Node 71	-	50	0.051.0	-	200774		0.000000	44.50	
All unique SNPs in variant A.2 merged	3	52	36516	3	30674	<0.000001	0.000006	14.56	Lineage A A.2
ON674051_MPXV_USA_2022_FL001	2	14	36554	1	30676	0.002755	0.007232	11.75	A A.2
ON676707_MPXV_USA_2021_1X	1	18	36550	1	30676	0.000248	0.000977	16.70	A A.2
ON6/5438_NPXV_USA_2022_VA001	1	20	30348	1	30676	0.000074	0.000322	10.79	A A.Z
All mutations in the 2018/2019 imports set, from Node 52	2	10	26554	1	20676	0.000920	0.002678	12 42	
All unique sives in the 2018/2019 import set merged	5	10	30334	1	20677	0.000629	0.002078	15.45	Lineage A A.1
MT003245 1 MDYV UK D2	0	<u> </u>	36567	0	30677	0.233748	0.334113	int	A A 1 1
MT002241 1 MDXV ME220 M1E Pavalca	0	2	36568	0	30677	0.200748	0.534115	inf	A A 1 1
MT903343 1 MPXV 1K P1	0	2	36568	1	30676	1	1 000000	1 679	ΔΔ11
MN648051 1 MPXV Israel 2018	1	4	36566	0	30677	0.130756	0 189309	inf	ΔΔ11
MT903342 1 MPXV Singapore		4	36566	0	30677	0.130756	0 189309	inf	ΔΔ11
MT250197 1 MPXV Singapore 2019	2	4	36566	0	30677	0 130756	0 180300	inf	ΔΔ11
All mutations in the Lineage & Nigerian set from Node 49	2	-	30300	0	50077	0.100700	3.133330		A A.1.1
All unique SNPs in the Lineage A Nigerian set merged	5	24	36558	1	30677	0.000007	0.000038	20 14	Lineage A
MT903338.1 MPXV M2957 Lagos	0	4	36578	0	30678	0.130775	0.189398	inf	A
MT903340.1 MPXV M5312 HM12 Rivers	0	5	36577	n	30678	0.067325	0.114635	inf	A
MG693724.1 MPXV Nigeria 2017	3	5	36577	n	30678	0.067325	0.114635	inf	A
MT903339.1 MPXV M3021 Delta	0	4	36578	0	30678	0.130775	0.189398	inf	A
MT903337.1 MPXV M2940 FCT	1	3	36579	1	30677	0.630746	0.749755	2.516	A
	-								
MG693723.1 MPXV Nig 2017 297957	1	11	36571	0	30678	0.001409	0.004144	inf	A

Table S6. Details and statistical analysis of mutational patterns within Lineage A, based on the trees shown in Fig. 2 and Fig. S2. Here we compare the frequencies of G-to-A substitutions in APOBEC3 and non-APOBEC3 contexts in Lineage A and each relevant sub-lineage within Lineage A. This table details the mutational patterns throughout the entire Lineage A, and G-to-A mutations in an APOBEC3 context are highly enriched relative to other mutations. First, to determine the overall level of enrichment for these mutations in Lineage A, we compressed every unique SNP mutation that arose within Lineage A onto a single sequence we call "All unique SNPs in Lineage A merged"; the analysis of the merged data is highlighted in gray at the top of the table, and this summary was also used in Figure 2 in the main text. Within Lineage A, 167 G-to-A mutations arose in an APOBEC3 context (5' GA-to-AA or GG-to-AG, in blue); 36,415 Gs in a APOBEC context, GA or GG in the ancestral sequence, did not change. In contrast, only 9 G-to-A mutations arose out of outside of an APOBEC3 context (GY-to-AY, where Y is C or T, in red), while 30,669 GY's remained unchanged. A simple two-sided Fisher's exact text was used to test the null hypothesis that the distributions between the two mutational patterns was random; q-values (37, 38) were calculated based on all p-values included Tables S6 and S8 to correct for multiple tests. The G-to-A mutations are obviously highly enriched. When the odds ratio is greater than one (highlighted in light blue) it is indicative more G-to-A mutations in an APOBEC3 context. We also tallied all other SNPs that were not G-to-A (in gray); these were rare, there were only 14 of these in all of Lineage A. To resolve if the enrichment pattern for G-to-A mutations in an APOBEC context was observed throughout Lineage A, we compared the mutational patterns on the internal branches within Lineage A, and we found all partitions of the data throughout Lineage A were enriched for G-to-A in an APOBEC3 context. While variant B.1, the predominant 2022 outbreak, had too few changes between the ancestor and any one leaf for a single sequence to be significant, all of the 8 mutations observed in the 12 earliest sequences from variant B.1 available for our initial analysis were G-to-A in an APOBEC3 context, and this enrichment was significant when considered together as a merged sequence. There were enough mutations in the longer branches in variant A.2 for statistical significance of the pattern to be evident for each of the 3 taxa independently. G-to-A in an APOBEC3 context was also enriched among the Nigerian sequences, in 2018/2019 export set, and on each of 4 major internal branches within Lineage A.

Table S7. G-A substitutions are enriched in an APOBEC3 context among 397 variant B.1 sequences available in GISAID through mid-July and among 4 additional variant A.2 sequences\*. To confirm the enrichment for G-to-A substitutions was retained in the rapidly expanding available outbreak sequence data, we extended our original analysis to include an additional 397 variant B.1 sequences that were available in GISAID as of July 15, 2022; these data supported our initial findings. A summary of all merged unique mutations in this set of 397 sequences is provided in this table, and the merged data is presented in Fig. 2B in the main text; a detailed graphic displaying the details of the analyses is provided in the supplemental Fig. S3. We also identified four additional variant A.2 sequences, two from Thailand and two from India, that became available through GISAID later in July. These too were highly enriched for G-to-A substitutions, and the statistics are provided in this table. The column headers are described in Table S6. \*EPI\_ISL\_14011193: Bangkok Hospital Phuket, Thai Red Cross Emerging Infectious Diseases Clinical Center and Faculty of Medicine, Chulalongkorn University; EPI ISL 13953611: Indian Council of Medical Research-National Institute of Virology; EPI ISL 14049245, Indian Council of Medical Research-National Institute of Virology; EPI ISL 13983888: Bangkok Hospital Phuket, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand. The GISAID acknowledgments tables for these two data sets are provided in Tables S10 and S11.

Data subset descriptions and sequence names	All other mutations	G-to-A in an APOBEC context	APOBEC context G, no change	G-to-A non- APOBEC context	Non-APOBEC context G, no change	p-value	q-value	Odds Ratio	Clade or Lineage
All mutations in Clade IIa, from Node 76									
Merged-SNPs	225	65	36548	132	30578	< 0.000001	0.000006	0.412	Clade IIa
KJ642615.1_MPXV_W-Nigeria_1978	112	17	36596	48	30662	0.000005	0.000029	0.2967	lla
DQ011156.1_MPXV_Liberia_1970	33	14	36599	21	30689	0.092181	0.150842	0.559	lla
AY741551.1_MPXV_Sierra_Leone_1970	28	20	36593	24	30686	0.289194	0.391811	0.6988	lla
AY753185.1_MPXV_COP-58_cynomolgus_monkey_West_African_origin_1958	26	12	36601	15	30695	0.336987	0.442295	0.6709	lla
AY603973.1_MPXV_MPXV-WRAIR7-61-cynomolgus-monkey-1962	27	10	36603	17	30693	0.082367	0.136556	0.4933	lla
KJ642616.1_MPXV_PCH_Paris_1962	39	8	36605	22	30688	0.002826	0.007267	0.3049	lla
MT903346.1_MPXV_USA_2003_099	40	9	36604	32	30678	0.000028	0.000141	0.2357	lla
DQ011157.1 MPXV USA 2003 039	40	10	36603	32	30678	0.000071	0.000320	0.2619	lla
DQ011153.1 MPXV USA 2003 044	40	10	36603	32	30678	0.000071	0.000320	0.2619	lla
Mutations in Clade IIb. prior to the emergence of Lineage A									
Node 60 to KJ642615.1 MPXV W-Nigeria 1978	29	4	36604	19	30693	0.00047	0.001745	0.1765	Clade IIb
Node 46-47	60	25	36583	52	30660	0.00013	0.000546	0.4029	IIb
Node 47-48	11	11	36584	8	30675	0.821212	0.967035	1.153	lib
Mutations in Clade I. from Node 5: Clade I				-					
Merged-SNPs	277	72	36552	176	30580	<0.000001	0.000006	0.3423	Clade I
1X878428 1 MPXV DRC 07-0514	1	0	36624	1	30755	0.456456	0.575264	0	I
1X878422.1 MPXV_DRC_07-0287	3	1	36623	1	30755	1	1.000000	0,8398	
IX878413.1 MPXV_DRC_07-0045	0	0	36624	0	30756	1	1.000000	0.0000	1
1X979427 1 MRXV DRC 07 0490	1	0	26624	0	20756	1	1.000000		
JX878427.1_WFXV_DRC_07-0480	1	0	36624	0	30750	1	1.000000		-
JX878421.1_MPXV_DRC_07-0280	1	0	36624	0	30756	1	1.000000		1
JX878414.1_MPXV_DRC_07-0046	0	0	30024	0	30756	1	1.000000		
JX878415.1_MPXV_DRC_07-0092	0	1	36624	0	30756	1	1.000000	t a f	
JX878410.1_MPXV_DRC_07-0095	0	1	30023	0	30756	1	1.000000		
JX878410.1_MPXV_DRC_06-1070	9	0	36624		30756	1	1.000000	0.4700	
JX8/8411.1_MPXV_DRC_06-10/5	17	4	36620	-	30749	0.245298	0.347276	0.4798	
JX8/8409.1_MPXV_DRC_06-0999	1/	4	36620	/	30749	0.245298	0.347276	0.4798	
JX878412.1_MPXV_DRC_06-1076	18	3	36621	9	30747	0.046645	0.087720	0.2799	
KP849471.1_MPXV_Yambuku_DRC_1985	1/	6	36618	8	30748	0.430146	0.553045	0.6298	I
MN702448.1_MPXV_015c	36	7	36617	15	30741	0.051532	0.094102	0.3918	1
MN702447.1_MPXV_18	36	6	36618	15	30741	0.026145	0.051473	0.3358	1
MN702450.1_MPXV_B1	43	7	36617	17	30739	0.022071	0.044854	0.3457	I
MN702453.1_MPXV_A1	34	5	36619	14	30742	0.019292	0.041200	0.2998	1
MN702452.1_MPXV_A2	35	6	36618	14	30742	0.040971	0.078217	0.3598	I
DQ011154.1_MPXV_Congo_2003_358	38	5	36619	16	30740	0.007076	0.016511	0.2623	1
JX878429.1_MPXV_DRC_07-0662	39	7	36617	17	30739	0.022071	0.044854	0.3457	1
JX878425.1_MPXV_DRC_07-0354	39	7	36617	17	30739	0.022071	0.044854	0.3457	1
JX878424.1_MPXV_DRC_07-0338	41	5	36619	15	30741	0.011744	0.025513	0.2798	1
JX878423.1_MPXV_DRC_07-0337	43	6	36618	15	30741	0.026145	0.051473	0.3358	1
JX878407.1_MPXV_DRC_06-0950	42	5	36619	17	30739	0.004225	0.010647	0.2469	1
KP849469.1_MPXV_Boende_DRC_2008	41	7	36617	14	30742	0.077307	0.129876	0.4198	1
MN702451.1_MPXV_A6	37	8	36616	17	30739	0.027567	0.053438	0.3951	1
KJ642613.1_MPXV_Congo_8	33	5	36619	12	30744	0.050168	0.092958	0.3498	I
MN702449.1_MPXV_B2	34	10	36614	13	30743	0.304321	0.403626	0.6459	1
MN702446.1_MPXV_38c	37	11	36613	13	30743	0.41972	0.545203	0.7105	I
MN702445.1 MPXV_A4	36	10	36614	16	30740	0.117126	0.184473	0.5247	I
 MN702444.1 MPXV A5	36	10	36614	16	30740	0.117126	0.184473	0.5247	I
D0011155.1 MPXV Zaire 1979-005	37	6	36618	13	30743	0.063366	0.114059	0.3875	1
H0857562.1 MPXV V79-I-005	45	10	36614	13	30743	0.304321	0.403626	0.6459	1
H0857563.1 MPXV D14L knockout	51	10	36614	12	30744	0 52176	0.632132	0.6997	1
HM172544.1 MPXV Zaire 1979-005	54	11	36613	35	30721	0.000039	0.000189	0.2637	
KC257460.1 MPXV_DBC_Vandongi 1985	39	7	36617	9	30747	0.456559	0.575264	0.6531	· ·
KI642619.1 MPXV Gabon-1988	40	7	36617	13	30743	0.114327	0.184473	0.4521	
KI642612.1 MRXV Jkubi	40	,	26616	12	20743	0.261606	0.259410	0.5598	
19972012.1_IVIFAV_INUU	42	0	30010	21	20725	0.201090	0.000507	0.3556	
1X070420.1_WEXV_DEC_06.0070	43	4	26620	15	20741	0.000147	0.000597	0.1333	1
1/070400.1_1VIF.AV_DRC_07.0393	40	4	36620	17	30720	0.004551	0.011244	0.2233	
JA0/0420.1_VIFAV_UKL_U/-U283	43	4	30020	17	30/39	0.001484	0.004144	0.1975	1
1X070419.1_VIFAV_DRC_07.0120	45	4	30020	21	30739	0.001484	0.004144	0.1975	
JX8/8418.1_IVIPAV_DRC_0/-0120	45	5	36619	21	30/35	0.000501	0.001804	0.1998	
AF380138.1_MPXV_2aire-96-1-16	109	16	36608	30	30726	0.011006	0.024329	0.44/6	
JX878417.1_MPXV_DRC_07-0104	48	4	36620	19	30737	0.000471	0.001745	0.1767	

**Table S8. Details and statistical analysis of mutational patterns outside of Lineage A, based on the trees shown in Fig. 2 and Fig. S2.** Here we compare Clade I, Clade IIa and Clade IIb data prior to the emergence of Lineage A. In these clades, there was no evident enrichment for G-to-A changes in APOBEC3 context. Instead, there was a modest, but overall significant, enrichment for G-to-A substitutions to occur *not* in a APOBEC3 context (the two-sided p-values were for the merged data for Clades I and IIa were significant) and the Odds Ratios were less than one throughout these clades indicating G-to-A changes in APOBEC3 context were relatively diminished compared to other G-to-A mutations.

Alignment	Full	Clades I and IIa	LineageA + Nigerian-SE-1971	LineageA + Nigerian-SE-1971
taxa	Clade I, Clade IIa, Lineage A	Clade I, Clade IIa	LineageA	LineageA
model	GTR+g+i	GTR+g+i	GTR+g+i	GTR+g+i
clock	fixed local	fixed local	fixed local	Uncorrelated lognormal
tree	Bayesian skyline	Bayesian skyline	Bayesian skyline	Bayesian skyline
LineageA rate	7.1817E-6 (5.5355E-6, 8.9951E-6)	NA	8.7045E-6 (6.4756E-6, 1.108E-5)	2.8395E-5 (1.4592E-5, 4.604E-5)
Cladella rate	3.8539E-6 (2.1937E-6, 5.5976E-6)	3.4138E-6 (1.4092E-6, 5.5292E-6)	NA	NA
Cladel rate	1.8768E-6 (2.1937E-6, 5.5976E-6)	1.8471E-6 (1.209E-6, 2.4765E-6)	NA	NA
meanrate	3.2883E-6 (2.339E-6, 4.217E-6)	2.3403E-6 (1.5014E-6, 3.1729E-6)	8.026E-6 (6.6458E-6, 1.1203E-5)	1.4876E-5 (1.2073E-5, 1.7897E-5)

**Table S9. Rate estimates for Lineage A, Clade I and Clade IIa.** Analysis was performed in BEAST v1.8.3 using the alignment from Figure 2. Mean rates are shown with 95% highest posterior density intervals in parenthesis.

Table S10. GISAID acknowledgement table for variant B.1 sequences

Table S11. GISAID acknowledgement table for variant A.2 sequences