## Supplementary Appendix

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Ebola virus transmission initiated by systemic Ebola virus disease relapse

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Methods

Ethics statement
The use of mAb114 during the June 2019 treatment was conducted under the Monitored Emergency Use of Unregistered and Investigational Interventions (MEURI) protocol which was review by Institut National de Recherche Biomédicale internal review board (IRB). The Ministry of Health (MoH) of the Democratic Republic of the Congo (DRC) approved this study. Oral consent was obtained at the homes of patients or in the Ebola Treatment Units prior to any sample collection by a team, including staff members of the MoH.

The sequencing and analysis of de-identified human diagnostic surveillance samples analyzed in this outbreak response project were also reviewed for the applicability of human subjects protection regulations by IRBs from Scripps and UNMC and was determined to not be human subject research.

Authors contributions
PMK, CP, MMR, MGP, TB, NJS, KGA, MRW, SAM, and JJMT designed the study, PMK, CP, MMR, MGP, FB, ANN, EKL, MF, AA, MMD, DM, BW, NB, DK, BN, MA, OT, AP, VE, ESP, YTTN, FM, FE, MM, JBB, BD, MK, MRDB, ISF, AY, MS, AWR, OF, and AS gathered the data, CP, MGP, AB, EKL, JH, KG, JM, ES, AT, MAS, IC, LH, AR, NJS, TB, KGA, and MRW analyzed the data, all authors vouch for the data and the analysis, PMK, CP, MMR, MGP, FB, ANN, AB, JH, KG, JM, IC, LH, TB, KGA, and MRW wrote the paper, and all authors listed in by-line decided to publish the paper. PMK, CP, MMR, MGP, AB, TB, KGA, and MRW wrote the first draft of our manuscript.

Viral sequencing
RNA was extracted from serum samples using the Qiagen Viral RNA Mini kit. cDNA was prepared using the ThermoFisher 1st strand synthesis system, and amplicons prepared using Q5 mastermix (New England Biolabs) and EBOV-specific amplicons generated using PrimaiSeq.1 Amplicons were quantified using a Qubit fluorometric quantification device (ThermoFisher) with dsDNA broad range kit and diluted to <500 ng for input into library preparation. Libraries were prepared using the Illumina Nextera DNA Flex kit with IDT for Illumina Unique Dual indexes, quantified, and loaded on the Illumina iSeq 100 for 2 x 150 cycles or Illumina MiSeq for 2 x 150 cycles.

Viral sequencing analysis
Short read data were analyzed with the iVar (v1.0.1)2 using reference sequence MK007330. The reads were trimmed using the ivar trim command with a quality threshold of 20 and with option -e, to prevent the removal of reads that are not directly attached to amplicon primers following Nextera Flex library prep. Consensus sequences were called using the ivar consensus command,
and a minimum coverage depth threshold of 50x, to prevent inadvertent contamination. Negative controls were included in each run to monitor potential contamination.

Multiple sequence alignment was performed with MAFFT\(^3\) after retaining only genomes that were >95% complete. The maximum likelihood analysis was performed using IQ-TREE using ModelFinder to select the best-fit model.\(^4\) The Nextstrain phylogenetics platform was used as part of the analysis.\(^5\) Bayesian phylogenetic analysis was performed using BEAST v1.10.5 to infer time-resolved phylogenies.\(^6\) We used an SDR06 nucleotide substitution model with a local clock model and a non-informative continuous time Markov chain reference prior (CTMC) on the molecular clock rate and a Skygrid coalescent prior. All the Bayesian analyses were run for 80 million Markov chain Monte Carlo steps, sampling parameters and trees every 10,000 generations.\(^7,8\) Tracer v1.7 was used to ensure run convergence (effective sample size > 200).\(^9\) The BEAST XML and log files are available at https://github.com/andersen-lab/paper_2020_drc-ebola. The final figures were created using baltic (https://github.com/evogytis/baltic) and Phylo (https://biopython.org/wiki/Phylo).

**EBOV IgG ELISAs**

Anti-human Ebola GP IgG ELISA data was generated using the respective Alpha Diagnostic International kits, according to manufacturer’s instructions. In brief, patient serum was diluted as indicated. Final incubation with TMB substrate was carried out for 15 minutes, before stopping solution was added. ELISA Plates were read at 450nm, and optical density at 630nm was subtracted to normalize well background. To analyze the data, blank background signal was subtracted from all data. EC\(_{50}\) binding titers were determined using Graphpad Prism 8.

**Human exome sequencing and analysis**

Human genomic DNA was isolated from blood using the Qiagen DNA Blood and Tissue kit. Exome sequencing libraries were prepared using the Illumina Nextera Flex for Enrichment kit, utilizing the Illumina Exome Panel as enrichment oligos and IDT for Illumina Unique Dual indexes according to manufacturer’s instructions. Sequencing libraries were analyzed on an Agilent TapeStation using the high-sensitivity DNA reagents before dilution and loading. Two exome libraries were prepared from two independent DNA extractions of the same sample, to maximize exome coverage. Both libraries were loaded on a single Illumina MiSeq run, using 2 x 300 cycle V3 chemistry to maximize the coverage depth. Close to 20 million unique reads were obtained, resulting in an average depth of 69X across all variants found. Sequence quality control was done using the FastQC software package (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and sequences were mapped to hg19 using the BWA aligner.\(^10\) SNP and INDEL calling, annotation, classification and in-depth analyses were carried out with the Genoox platform (https://www.genoox.com/). The analysis focused on genes causing immunodeficiency disorders. Upon analysis of likely pathogenic mutations using a built-in tool in the Genoox platform we identified a single likely pathogenic variant, c.356_357insC (p.Glu119fs), in the SH2D1A gene (Supplementary Table 2). This variant
has been reported to cause Lymphoproliferative Syndrome, OMIM # 308240. However, Sanger sequencing confirmation revealed that this variant arose from a sequencing error, and was in fact not present in the patient’s genome.

Neutralization assay

mAb114 recognition of patient EBOV GP mutants was evaluated using a single-round infection and neutralization assay. Lentiviruses were produced bearing at their surface EBOV GP from either Ituri wildtype GP (first sequenced virus 18FHV089), patient’s first infection sequenced GP (d1, MAN4194) and patient’s second infection sequenced GP (d171, MAN12309). Neutralization was performed as previously described. Briefly, HEK293T cells were exposed to pseudovirus mixed with serial dilutions of mAb114 at concentrations from 0.0001–10 μg/mL. Luciferase activity measured as relative luminescence unit (RLU) was obtained after lysis of target cells by using a Luciferase Assay System Bright Glo (Promega) and an Envision Plate Reader (PerkinElmer). Assays were performed three times, each with samples in triplicate.
Supplementary Figures and Tables:

**Supplementary Table S1: Blood chemistry Data**

Blood chemistry data, as measured by the Piccolo Xpress system (Abaxis) on December 4 during the second Ebola episode. The results indicate multiple organ failure and the patient passed away the same day.

| Test   | Result  | Normal range |
|--------|---------|--------------|
| Glycemia | 126 mg/dl | 73-118       |
| BUN    | 131 mg/dl | 7-22         |
| CRE    | 7.6 mg/dl  | 0.6-1.2      |
| TBIL   | 5.4 mg/dl  | 0.2-1.6      |
| ALB    | 1.5 g/dl   | 3.3-5.5      |
| ALT    | 431 U/l    | 10-47        |
| AST    | 1221 U/l   | 11-38        |
| CK     | 1400 U/l   | 30-380       |
| AMY    | 436 U/l    | 14-97        |
| Na⁺    | 126 mmol/l | 128-145      |
| K⁺     | 4.0 mmol/l | 3.6-5.1      |
| CA     | 7.0 mg/dl  | 8.0-10.3     |
| CRP    | 169 mg/l   | 0-7.5        |
### Supplementary Table S2: Sample metadata

| Lab ID      | Date sample tested | Health zone | Province    | Genome coverage | Date sample sequenced |
|-------------|--------------------|-------------|-------------|-----------------|-----------------------|
| MAN4194     | 16-Jun-19          | Mabalako    | Nord-Kivu   | 99.58%          | 20-Dec-19             |
| MAN12309    | 3-Dec-19           | Mabalako    | Nord-Kivu   | 96.67%          | 20-Dec-19             |
| MAN12369    | 5-Dec-19           | Mabalako    | Nord-Kivu   | 99.65%          | 23-Jan-20             |
| MAN12448    | 7-Dec-2019         | Mabalako    | Nord-Kivu   | 99.70           | 17-Dec-19             |
| MAN12460    | 8-Dec-19           | Mabalako    | Nord-Kivu   | 99.99%          | 17-Dec-19             |
| MAN12468    | 8-Dec-19           | Mabalako    | Nord-Kivu   | 98.55%          | 17-Dec-19             |
| MAN12470    | 8-Dec-19           | Mabalako    | Nord-Kivu   | 99.97%          | 17-Dec-19             |
| MAN12472    | 8-Dec-19           | Mabalako    | Nord-Kivu   | 99.23%          | 17-Dec-19             |
| MAN12506    | 10-Dec-19          | Mabalako    | Nord-Kivu   | 99.22%          | 17-Dec-19             |
| MAN12508    | 10-Dec-19          | Mabalako    | Nord-Kivu   | 98.41%          | 17-Dec-19             |
| MAN12514    | 10-Dec-2019        | Mabalako    | Nord-Kivu   | 98.52           | 17-Dec-19             |
| MAN12535    | 10-Dec-19          | Mabalako    | Nord-Kivu   | 98.31%          | 17-Dec-19             |
| MAN12541    | 10-Dec-19          | Mabalako    | Nord-Kivu   | 99.87%          | 17-Dec-19             |
| MAN12542    | 10-Dec-19          | Mabalako    | Nord-Kivu   | 99.56%          | 17-Dec-19             |
| MAN12545    | 10-Dec-19          | Mabalako    | Nord-Kivu   | 95.52%          | 17-Dec-19             |
| MAN12546    | 10-Dec-19          | Mabalako    | Nord-Kivu   | 99.97%          | 17-Dec-19             |
| MAN12581    | 11-Dec-19          | Mabalako    | Nord-Kivu   | 99.97%          | 17-Dec-19             |
| MAN12589    | 11-Dec-19          | Biena       | Nord-Kivu   | 98.91%          | 17-Dec-19             |
| MAN12727    | 13-Dec-19          | Mabalako    | Nord-Kivu   | 99.85%          | 23-Jan-20             |
| MAN12770    | 14-Dec-19          | Mabalako    | Nord-Kivu   | 99.87%          | 23-Jan-20             |
| MAN12790    | 14-Dec-19          | Mabalako    | Nord-Kivu   | 99.85%          | 23-Jan-20             |
| BTB39991    | 17-Dec-19          | Butembo     | Nord-Kivu   | 92.10%          | 10-Jan-20             |
| MAN12952    | 17-Dec-19          | Mabalako    | Nord-Kivu   | 99.73%          | 21-Jan-20             |
| MAN12990    | 18-Dec-19          | Mabalako    | Nord-Kivu   | 99.66%          | 21-Jan-20             |
| MAN12999    | 18-Dec-19          | Mabalako    | Nord-Kivu   | 99.85%          | 27-Feb-20             |
| MAN13030    | 19-Dec-19          | Mabalako    | Nord-Kivu   | 99.27%          | 27-Feb-20             |
| MAN13175    | 21-Dec-19          | Mabalako    | Nord-Kivu   | 99.99%          | 2-Mar-20              |
| MAN13221    | 22-Dec-19          | Mabalako    | Nord-Kivu   | 96.76%          | 23-Jan-20             |
| MAN13222    | 22-Dec-19          | Mabalako    | Nord-Kivu   | 99.79%          | 21-Jan-20             |
| MAN13238    | 22-Dec-19          | Mabalako    | Nord-Kivu   | 99.91%          | 23-Jan-20             |
| MAN13273    | 23-Dec-19          | Mabalako    | Nord-Kivu   | 99.89%          | 11-Jan-20             |
| MAN13347    | 24-Dec-19          | Mabalako    | Nord-Kivu   | 99.88%          | 21-Jan-20             |
| BTB41146    | 25-Dec-19          | Butembo     | Nord-Kivu   | 99.91%          | 11-Jan-20             |
| MAN13348    | 25-Dec-19          | Mabalako    | Nord-Kivu   | 99.88%          | 23-Jan-20             |
| MAN13384    | 25-Dec-19          | Mabalako    | Nord-Kivu   | 99.91%          | 21-Jan-20             |
| KAT21808    | 31-Dec-19          | Butembo     | Nord-Kivu   | 88.81%          | 11-Jan-20             |
| Code       | Date       | Location 1 | Location 2  | Percentage | Date       |
|------------|------------|------------|-------------|------------|------------|
| MAN13677   | 31-Dec-19  | Mabalako   | Nord-Kivu   | 99.90%     | 27-Feb-20  |
| BTB42007   | 1-Jan-20   | Musienene  | Nord-Kivu   | 98.06%     | 11-Jan-20  |
| KAT21874   | 1-Jan-20   | Butembo    | Nord-Kivu   | 99.63%     | 10-Jan-20  |
| BTB42105   | 2-Jan-20   | Butembo    | Nord-Kivu   | 87.95%     | 11-Jan-20  |
| BTB42106   | 2-Jan-20   | Butembo    | Nord-Kivu   | 99.65%     | 11-Jan-20  |
| BTB42291   | 3-Jan-20   | Butembo    | Nord-Kivu   | 98.34%     | 11-Jan-20  |
| MAN13942   | 6-Jan-20   | Mabalako   | Nord-Kivu   | 98.09%     | 27-Jan-20  |
| BEN45861   | 7-Jan-20   | Beni       | Nord-Kivu   | 92.61%     | 30-Jan-20  |
| BEN46116   | 9-Jan-20   | Beni       | Nord-Kivu   | 95.74%     | 30-Jan-20  |
| KAT22664   | 11-Jan-20  | Butembo    | Nord-Kivu   | 93.12%     | 27-Feb-20  |
| KAT22674   | 11-Jan-20  | Mabalako   | Nord-Kivu   | 95.82%     | 27-Jan-20  |
| MAN14198   | 12-Jan-20  | Mabalako   | Nord-Kivu   | 95.48%     | 21-Jan-20  |
| BEN46488   | 13-Jan-20  | Beni       | Nord-Kivu   | 99.04%     | 2-Mar-20   |
| MAN14228   | 13-Jan-20  | Mabalako   | Nord-Kivu   | 99.73%     | 5-Feb-20   |
| MAN14231   | 13-Jan-20  | Mabalako   | Nord-Kivu   | 93.98%     | 30-Jan-20  |
| MAN14242   | 13-Jan-20  | Mabalako   | Nord-Kivu   | 99.44%     | 28-Feb-20  |
| MAN14243   | 13-Jan-20  | Mabalako   | Nord-Kivu   | 92.46%     | 30-Jan-20  |
| BEN46574   | 14-Jan-20  | Beni       | Nord-Kivu   | 86.10%     | 30-Jan-20  |
| MAN14263   | 14-Jan-20  | Mabalako   | Nord-Kivu   | 92.60%     | 30-Jan-20  |
| MAN14285   | 14-Jan-20  | Mabalako   | Nord-Kivu   | 99.89%     | 12-Feb-20  |
| BEN46697   | 15-Jan-20  | Beni       | Nord-Kivu   | 99.86%     | 3-Feb-20   |
| BEN46737   | 15-Jan-20  | Beni       | Nord-Kivu   | 99.59%     | 28-Feb-20  |
| BEN46754   | 15-Jan-20  | Beni       | Nord-Kivu   | 99.89%     | 28-Feb-20  |
| BEN46848   | 16-Jan-20  | Beni       | Nord-Kivu   | 98.98%     | 12-Feb-20  |
| BEN47083   | 18-Jan-20  | Beni       | Nord-Kivu   | 99.91%     | 27-Feb-20  |
| BEN47206   | 19-Jan-20  | Beni       | Nord-Kivu   | 99.84%     | 27-Jan-20  |
| BEN47225   | 19-Jan-20  | Beni       | Nord-Kivu   | 99.94%     | 27-Feb-20  |
| BEN47296   | 20-Jan-20  | Beni       | Nord-Kivu   | 99.91%     | 27-Feb-20  |
| BEN47371   | 20-Jan-20  | Beni       | Nord-Kivu   | 97.55%     | 27-Feb-20  |
| BEN48039   | 25-Jan-20  | Beni       | Nord-Kivu   | 99.85%     | 5-Feb-20   |
| BEN48100   | 26-Jan-20  | Beni       | Nord-Kivu   | 99.85%     | 3-Feb-20   |
| BEN48231   | 27-Jan-20  | Beni       | Nord-Kivu   | 99.89%     | 5-Feb-20   |
| BEN48404   | 28-Jan-20  | Beni       | Nord-Kivu   | 99.92%     | 5-Feb-20   |
| BEN48608   | 29-Jan-20  | Beni       | Nord-Kivu   | 99.96%     | 5-Feb-20   |
| BEN49243   | 4-Feb-20   | Beni       | Nord-Kivu   | 95.40%     | 20-Feb-20  |
| BEN49411   | 5-Feb-20   | Beni       | Nord-Kivu   | 87.39%     | 12-Feb-20  |
| BEN49660   | 7-Feb-20   | Beni       | Nord-Kivu   | 87.01%     | 12-Feb-20  |
| BEN50212   | 11-Feb-20  | Beni       | Nord-Kivu   | 97.20%     | 20-Feb-20  |
| BEN50970   | 17-Feb-20  | Beni       | Nord-Kivu   | 96.29%     | 1-Mar-20   |
### Supplementary Table S3: Exome variants potentially linked to primary immune deficiencies

| Gene | Variation Type | Chr | Start Position | Stop Position | Ref | Alt | Transcript | AA Change | Nucleotide | Exon | Zygosity | Region | Effect | Confidence Quality | Genotype C | Genotype Depth | Ref Depth | Obs Depth | Genomic Classification |
|------|---------------|-----|----------------|---------------|-----|-----|------------|-----------|------------|-----|----------|--------|--------|-------------------|------------|---------------------|----------|-----------|---------------------|
| CFTR | SNP           | chr7| 117306984       | 117306984     | G   | A   | NM_000492.3 | p.Arg1422Gln | c.4265G>A  | 27  | het      | Exonic | Missense | High              | 927.77     | 99:956:0:1614 | 135:80  | 55:55     | Possibly Pathogenic (Low) |
| CXTA | SNP           | chr16| 10692835        | 10692835      | T   | C   | NM_000246.3 | p.Val138Ala  | c.413T>C   | 5   | het      | Exonic | Missense | High              | 876.77     | 99:306:0:2275 | 172:114 | 58:58     | Possibly Pathogenic (Low)   |
| SH2D1A| InDel       | chrX| 123060210        | 123060210     | A   | AC  | NM_002351.4 | p.Glu119Asp | c.336_337insG| 4   | het      | Exonic | Frameshift | Low               | 13.9273   | 14:13       | 10:10   | 3:3       | Likely Pathogenic          |
| GHR  | SNP           | chr5 | 42713631         | 42713631      | G   | T   | NM_000163.5 | c.875+10G>T  |            | 8   | het      | Splice Region | Medium     | 137.77    | 99:166:0:380 | 27:17  | 10:10     | Uncertain Significance     |
| GHR  | SNP           | chr5 | 42718592         | 42718592      | T   | G   | NM_000163.5 | p.Thr328Thr | c.953T>C   | 10  | het      | Exonic | Missense | High              | 502.77     | 99:531:0:466 | 54:26  | 28:28     | Uncertain Significance     |
| XIAP | SNP           | chrX| 1230625117       | 1230625117    | A   | T   | NM_001167.3 | p.Glu368Leu | c.1007A>T  | 4   | het      | Exonic | Missense | Low               | 10.6382    | 11:10       | 8:8     | 2:2       | Uncertain Significance     |
| SCDH | SNP           | chr1 | 17354329         | 17354329      | G   | A   | NM_003002.2 | p.Ser152Phe  | c.450C>T   | 5   | het      | Exonic | Missense | High              | 902.77     | 99:931:0:1583 | 135:81 | 54:54     | Possibly Pathogenic (Low)   |
| C5   | SNP           | chr9 | 123737151        | 123737151     | C   | T   | NM_001736.2 | p.Arg130His  | c.392G>A   | 30  | het      | Exonic | Missense | High              | 620.77     | 99:649:0:550 | 64:30  | 34:34     | Uncertain Significance     |
| OFD1 | SNP           | chrX| 13774707         | 13774707      | A   | T   | NM_003611.3 | p.Glu411Val  | c.1232A>T  | 13  | het      | Exonic | Missense | Low               | 23.842     | 21:4        | 2:2     | 2:2       | Possibly Pathogenic (Low)   |
Figure S1: Maximum Likelihood tree of 297 genomes from the current Nord-Kivu EBOV outbreak in DRCA

Maximum Likelihood (ML) tree of sequenced isolates from the current EBOV outbreak in DRC (n=297), colored by health zones relevant to this study as indicated. B) Zoomed in view of the ML tree showing the first (d1) and second EVD episodes (d171) of the relapse patient, as well as 61 viral genomes sampled from epidemiologically linked cases. Two mutations (T5578C - non coding, A6867G - GP E280G) developed during the persistent infection of the relapse patient and are unique to Sample d171 and the 61 samples from the relapse cluster, showing human-to-human transmission originating from the relapse patient. The horizontal axes show the number of nucleotide substitutions per site. Data taken from https://nextstrain.org/community/inrb-drc/ebola-nord-kivu and released on NCBI GenBank database.
Figure S2: Comparison of evolutionary rates between persistent infection branches and the overall outbreak.

Based on preliminary Bayesian phylogenetic analysis using Beast with a relaxed clock model, we allowed three branches originating from samples d1, KAT21596 and MAN14985, which are strongly presumed to represent persistent infections, to have a different evolutionary rate from the rest of the tree, under a local clock model. This allows for the comparison of persistent infection and overall outbreak evolutionary clock rates. The median evolutionary rate for the overall outbreak was 0.00077 substitutions/site/year (clock rate; 95% HPD: 0.00066 - 0.00088), while the median persistent evolutionary rate was approximately 4-fold lower at 0.000207 substitutions/site/year (persistent clock rate; 95% HPD: 0.00007, 0.00038). Shown are posterior density distributions with median and the range indicated by black lines.
Supplementary Figure S3: Anti-Ebola GP IgG ELISA data.

Anti- Ebola GP IgG ELISA results of serial serum dilutions from the first and second EVD episodes. Titers were calculated using Graphpad Prism 8 and are listed in Table 1.
Supplementary Figure S4: Exome pathogenic variant analysis summary.

Over 20 million exome reads were aligned to the human reference genome, yielding an average variant coverage of 69-fold across all 221,821 screened variants. The analysis was focused on variants that are potentially causing primary immune deficiencies, of which we found nine candidates in the filtered data set. Only one variant was found to be likely pathogenic, which we attempted to confirm with Sanger sequencing confirmation. However, the detected variant could be traced to a sequencing error. MAF: Minor Allele Frequency, ACMG: American College of Medical Genetics, VUS: Variant of Uncertain Significance, LP: Likely Pathogenic, P: Pathogenic.
Supplementary Figure S5: mAb114 neutralization capacity against patient’s GP mutants.

Neutralization capacity of mAb114 was evaluated against pseudoparticles harboring either Ituri wildtype GP (18FHV089), patient’s first infection GP (d1, MAN4194) and patient’s second infection GP (d171, MAN12309).
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