Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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A Monovalent Chimpanzee Adenovirus Ebola Vaccine boosted with MVA

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Study Methods

Study Participants

The study was conducted at the Centre for Clinical Vaccinology and Tropical Medicine at the University of Oxford. Participants were healthy adults between the ages of 18 and 50 years who provided written informed consent (Supplementary Table 1).

Ethics and Regulatory Approval

The study was reviewed and approved by the UK National Research Ethics Service (NRES), Committee South Central – Oxford A (OXREC A; Ref: 14/SC/1256), the Medicines and Healthcare products Regulatory Agency (MHRA; Ref: 21584/0334/001-0001) and the Oxford University Clinical Trials and Research Governance team, who monitored GCP compliance. An independent Data Safety Monitoring Board (DSMB) provided safety oversight.

Study Design

EBL01 was a phase 1, dose-escalation, open-label study assessing the safety and immunogenicity of the experimental monovalent ChAd3 vaccine against EBOV and the first clinical trial of the MVA vectored multivalent filoviral vaccine MVA-BN® Filo in healthy adults (first-in-human study). The trial design is outlined in Supplementary Figure 1. Vaccinations with ChAd3-EBO Z began on 17th September 2014, and were completed by 18th November 2014. Vaccinations with MVA-BN® Filo began on 26th November 2014 and were completed by 9th December 2014.

The volunteers in this study all received priming vaccination with the monovalent chimpanzee adenovirus vectored ChAd3-EBO Z vaccine in the following dose groups:

Group 1;1 x 10^{10} vp (n=20), Group 2: 2.5 x 10^{10} vp (n=20), Group 3: 5 x 10^{10} vp (n=20). Thirty volunteers (n=10 per prime dose group) were then allocated to one of 2 dose groups to receive vaccination with MVA-BN® Filo 3-10 weeks after ChAd3-EBO Z prime. Four volunteers from each priming dose group received MVA-BN® Filo at 3 x 10^8 pfu (4.4 x 10^8 $TCID_{50s}$, subgroup b, n=12). The remaining six volunteers from each priming dose group received MVA-BN® Filo at 1.5 x 10^8 pfu (2.2 x 10^8 $TCID_{50s,}$ subgroup c, n=18).

An extension to the study added two further groups with a reduced prime-boost interval.

Group 4 received ChAd3 EBO Z at 2.5 x 10^{10} vp followed by MVA-BN® Filo at 1.5 x 10^8 pfu (2.2 x 10^8 $TCID_{50s}$) 1 week later (n=8). Group 5 recieved ChAd3 EBO Z at 2.5 x 10^{10} vp followed by MVA-BN® Filo at 1.5 x 10^8 pfu (2.2 x 10^8 $TCID_{50s}$) 2 weeks later (n=8). Vaccinations in this extension began on 10th Feb 2015, and were completed by 25th March 2015. All vaccines were administered intramuscularly by needle and syringe. After the administration of the first dose of MVA-BN®Filo, 48 (+/- 4) hours were allowed to elapse before the next 2 volunteers were vaccinated and only after the Chief Investigator and Local Safety Monitor had assessed and approved the safety data. Another 48 (+/- 4) hours were allowed to elapse before the next 2 volunteers were vaccinated and again, the Chief Investigator and Local Safety Monitor gave approval. After this, another 48 (+/- 4) hours elapsed before all remaining volunteers in all dose groups were vaccinated, and only when the Chief Investigator and Local Safety Monitor had assessed the available data at this point as safe to proceed. For groups 4 and 5, preliminary analysis of the safety of both vaccines was complete, therefore staggered enrolment into these groups was not required. Full details

regarding the study conduct are provided in the protocol, which is available with the full text of this article at NEJM.org.

Study Vaccines

The ChAd3 drug substance was manufactured at Advent, a subsidiary of Okairos (now GlaxoSmithKline), and the drug product was manufactured at the Vaccine Research Center Vaccine Pilot Plant, under contract with the Vaccine Clinical Materials Program, Leidos Biomedical Research. The vaccine is a sterile, aqueous, buffered solution that includes the ChAd3-vectored vaccine encoding the surface glycoprotein of EBOV in single-dose vials of 9.1×10^{14} particle units per milliliter, as determined on high-performance liquid chromatography. Different dose levels were achieved by adjusting the volume of vaccine injected from 110 μl (in group 1) to 275 μl (in group 2) and 550 μl in group 3. The MVA-BN® Filo vaccine was manufactured at IDT Biologika in Germany under contract FBS-0044-009 with NIAID and Fisher BioServices. The vaccine is a multivalent recombinant MVA (Modified Vaccinia virus Ankara, strain Bavarian Nordic [MVA-BN®]) encoding four filovirus antigens: the surface glycoproteins of Marburg marburgvirus, Ebola virus and Sudan virus as well as the nucleoprotein of Tai Forest Ebolavirus. MVA-BN® Filo was grown in primary chicken embryo fibroblast cells under serum free conditions and formulated in tris-buffered saline at 4.4×10^8 $TCID_{50}$ per 0.5 mL dose in single-dose vials.

Assessment of Safety

Participants were observed for 60 minutes after vaccination. Follow-up visits were scheduled for days 1, 7, 14, 28, 90, and 180 after vaccination, with an additional visit at day 10 for participants in group 3. All participants were given access to an

electronic diary card on which to record all solicited symptoms for 7 days after vaccination and unsolicited symptoms for 28 days after vaccination. A review of symptoms occurred at each follow-up visit, in addition to testing that included a full blood count and the measurement of urea and electrolytes, liver enzymes, activated partial-thromboplastin time, prothrombin time, and fibrinogen. Severity grading of adverse events and the assignment of a causal relationship for unsolicited adverse events were conducted according to predefined criteria.

Immunology Methods

ELISA assays

Antibody responses were measured against trimerised Zaire strain Ebola Glycoprotein (amino-acids 1-649 of GenBank protein AHX24649.1, with a C-tag). Protein was expressed in 2.5L of suspension HEK293E cells, which were transiently transfected and culture supernatant was harvested after four days when cell viability fell below 95%. Supernatant was concentrated 20-fold using a Pellicon 3 Tangential Flow Filtration system (Millipore, Herts, UK). Chromatographic purifications, performed on an ÄKTA pure system (GE Healthcare, Bucks, UK), consisted of a C-tag affinity capture step (Thermo Fisher Scientific, Leics, UK) and a polishing size exclusion chromatography (SEC) using a SepFast GF-HS-L 26/1000 column (Biotoolomics, Durham, UK). SEC peak fractions corresponding to the trimeric ebolavirus glycoprotein were pooled and the molecular weight was further confirmed by Blue native PAGE (Thermo Fisher Scientific, Leics, UK). The purified protein was quantified by Nanodrop (Thermo Fisher Scientific, Leics, UK), aliquotted and stored at -80°C until further use.

Responses were measured for MVA-boosted volunteers at day 0 (ChAd3 vaccination), day 28, MVA+14, MVA+28, MVA+90 and MVA+180 and for non-boosted volunteers at day 0, day 28, day 90 and day 180. Nunc-Immuno 96 well plates were coated with 1µg/ml of GPZ antigen in Dulbecco's PBS and left at 4°C overnight. Plates were washed 6x with PBS-Tween (PBS/T), then blocked with Casein for 1 hour at room temperature (RT). Serum was diluted in Casein to dilutions of 1:100, 1:200, 1:500, 1:1000 or 1:5000 and added to the plate in triplicate. Plates

were incubated for 2 hours at RT then washed as before. A secondary antibody (goat anti-human IgG conjugated to alkaline phosphatase, Sigma) was added at a dilution of 1:1000 in Casein for 1 hour at RT. Plates were washed a final time and developed using 4-nitrophenyl phosphate in diethanolamine buffer (Pierce). Optical density (OD) was read at 405nm using an ELx800 microplate reader.

A reference pool of GPZ-positive serum was used to form a standard curve on each plate. This pool was added at an initial dilution of 1:100 in Casein and diluted 2-fold 10 times. An arbitrary number of ELISA units were assigned to the reference pool and OD values of each dilution were fitted to a 4-parameter logistic curve using SOFTmax PRO software. Arbitrary ELISA units were calculated for each sample using the OD values of the sample and the parameters of the standard curve. An internal control made of the positive reference at a dilution of 1:800 in Casein was additionally included on each plate and used to standardise between assays.

The whole virion ELISA was performed as previously described using Zaire ebolavirus Makona strain[7], with seropositivity described as a response greater than 500AEU.

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Anti-glycoprotein IgG responses to the Sudan species of Ebolavirus were assessed using commercially available kits (AE 320620-1 and AE321600-1) from Alpha Diagnostics International, Texas, USA (ADI), according to the manufacturer's instructions with serum diluted at 1:500, as previously described [7]. The NIH ELISA to the Mayinga strain glycoprotein was performed as previously described[15].

Neutralisation assays

The whole Ebola virus neutralization assay[5] and the pseudotyped lentivirus neutralisation assay were performed as described previously [16].

The Ebola virus (EBOV) competition ELISA is based on the one-step incubation simultaneous competitive principle. If present in the sample, anti-EBOV antibodies compete with monoclonal anti-EBOV antibodies (Mab) conjugated to horseradish peroxide (HRP) for a fixed amount of recombinant EBOV GP pre-coated in the wells. When no anti-EBOV antibodies are present in the sample, the HRP labelled anti-EBOV binds to the EBOV GP antigens. Binding is reduced proportionally with increasing amounts of EBOV antibody specific for the epitope recognised by the Mab in the test serum.

Purified Mab 4G7, shown to neutralize EBOV[17] was conjugated to horseradish peroxidase (HRP) by standard methods[18]. Diluted test sera/plasma and the 4G7-HRP conjugate were added simultaneously to microwells coated with rGPδTM (IBT Bioservises Inc. USA) and competed for the rGP binding sites. Any serum/plasma or dilution thereof giving inhibition of 4G7-HRP binding >50% was considered to contain potential neutralising antibody. Calculation of percentage inhibition was as follows:

$$\text{Optical density 450/620nm of (NC-PC)} - \text{T/(NC-PC) x100}$$

where NC is the OD obtained for a control normal human plasma sample that does not contain EBOV antibodies, PC is the OD obtained for the a sample that inhibits the antibody-HRP conjugate and T is the OD obtained for the competing test sample.

T cell assays

Ex vivo ELISPOT and intracellular cytokine staining assays were performed as previously described[7].

Flow cytometry

Intracellular cytokine staining was performed as previously described[1]. Freshly isolated PBMC were stimulated with a pool containing all 187 peptides spanning the glycoprotein at 2.5µg/ml. Samples were analysed on a LSR II cytometer (Becton Dickinson, Oxford, UK) and a minimum of 1 million events were collected for each sample. At least 83,000 live $CD3^+$ cells were analysed per sample. Data were prepared and analysed using FlowJo v9.8.1 (Treestar Inc., Ashland, Oregon, USA), Pestle v1.6 and Spice v5.05 (Mario Roederer, Vaccine Research Centre, NIAID, NIH). A hierarchical gating strategy was used. Responses to peptide were determined after subtraction of the response in the unstimulated control for each sample. Pie-charts were created using absolute measures with a threshold of 0.004%. For analyses of multiple cytokine function, all samples had >24,000 $CD4^+$ or CD8+ T cells in the parent population. Samples where a response to the positive control of greater than 1% cytokine positive $CD4^+$ or $CD8^+$ T cells could not be detected were excluded from the analysis. The lower limit of detection for the assay was 0.004% and a positive response was greater than 2 times the medium control for the corresponding sample.

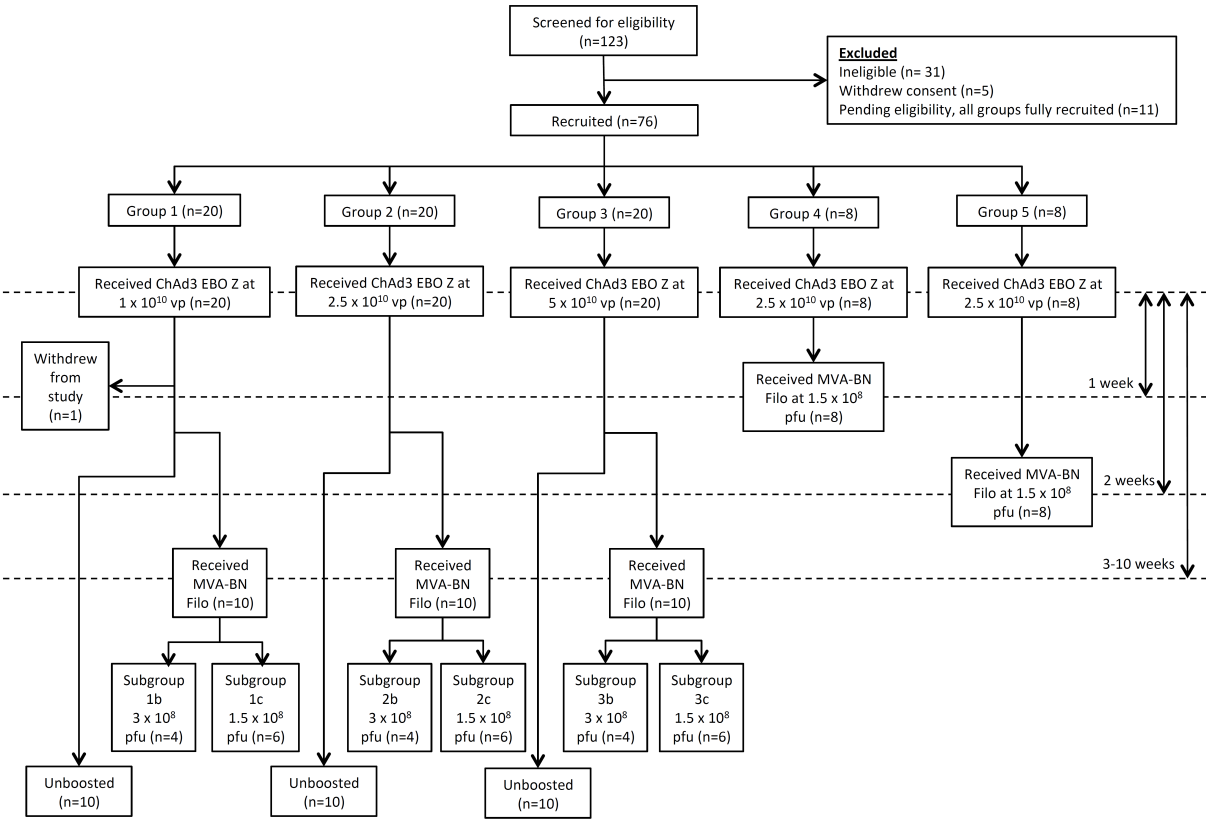
Statistical analysis

Group data are described using geometric means with 95% confidence intervals (CI) for normally distributed data or medians with inter-quartile ranges otherwise.

Matched pairs analysis excludes volunteers with missing data at any time point. Differences between groups were assessed with a Student's t test or Mann-Whitney for non-parametric data. A Kruskal-Wallis test was used to compare increases in T cell frequencies in time courses with Dunn's multiple comparisons post-test used to compare response pre- and post-vaccination. Correlations were performed using Pearson's test or Spearman's for non-parametrically distributed data. For statistical analyses, an alpha-level of 0.05 was considered significant and all p values are 2-tailed. Immunological analyses were performed in GraphPad Prism, Mac version 6. (GraphPad Software Inc., California, USA) or SPSS v23 (IBM SPSS Statistics, New York, USA).

Supplementary Figures and Data

Supplementary Figure 1. CONSORT diagram

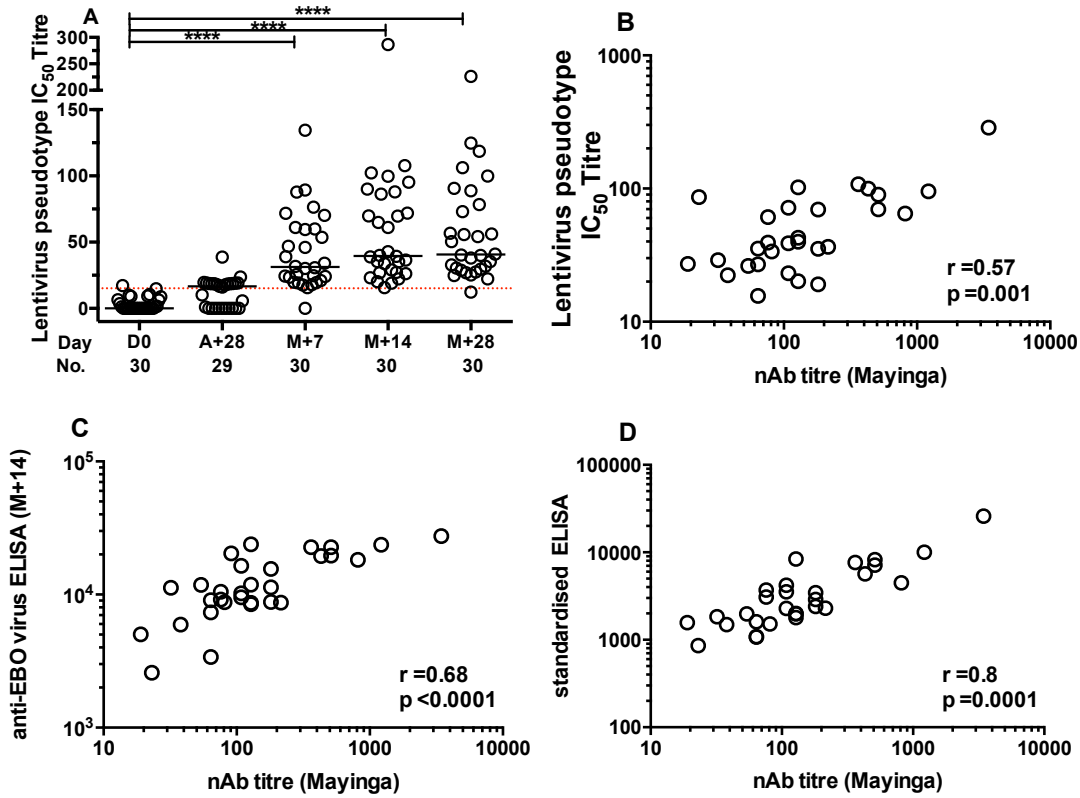


Supplementary Figure 1. CONSORT diagram of screening, enrolment, vaccination. A single group 1 subject withdrew from the study on day 1 following vaccination due to aversion to venepuncture. She had reported no adverse events at the time of withdrawal, but declined to return for further safety follow up.

Additional neutralizing antibody data

In another assay, we used a pseudotyped lentivirus expressing the GP from the Mayinga strain of Zaire ebolavirus to assess the capacity of vaccine-induced antibodies to block infection, with an IC50 readout. This assay avoids the use of live Ebola virus and so may be conducted outside high containment facilities. Again, low-level inhibition of infection was observed post-prime, which increased significantly post-boost (Figure SF2A, $p<0.0001$ Kruskal-Wallis test). Neutralising antibody titres in each assay correlated positively with each other ($r=0.57$, $p=0.001$) and ELISA titres measured with both the whole virion and standardised glycoprotein ELISA (Figure SF2B, 2C and 2D).

Supplementary Figure 2. Additional antibody data I.



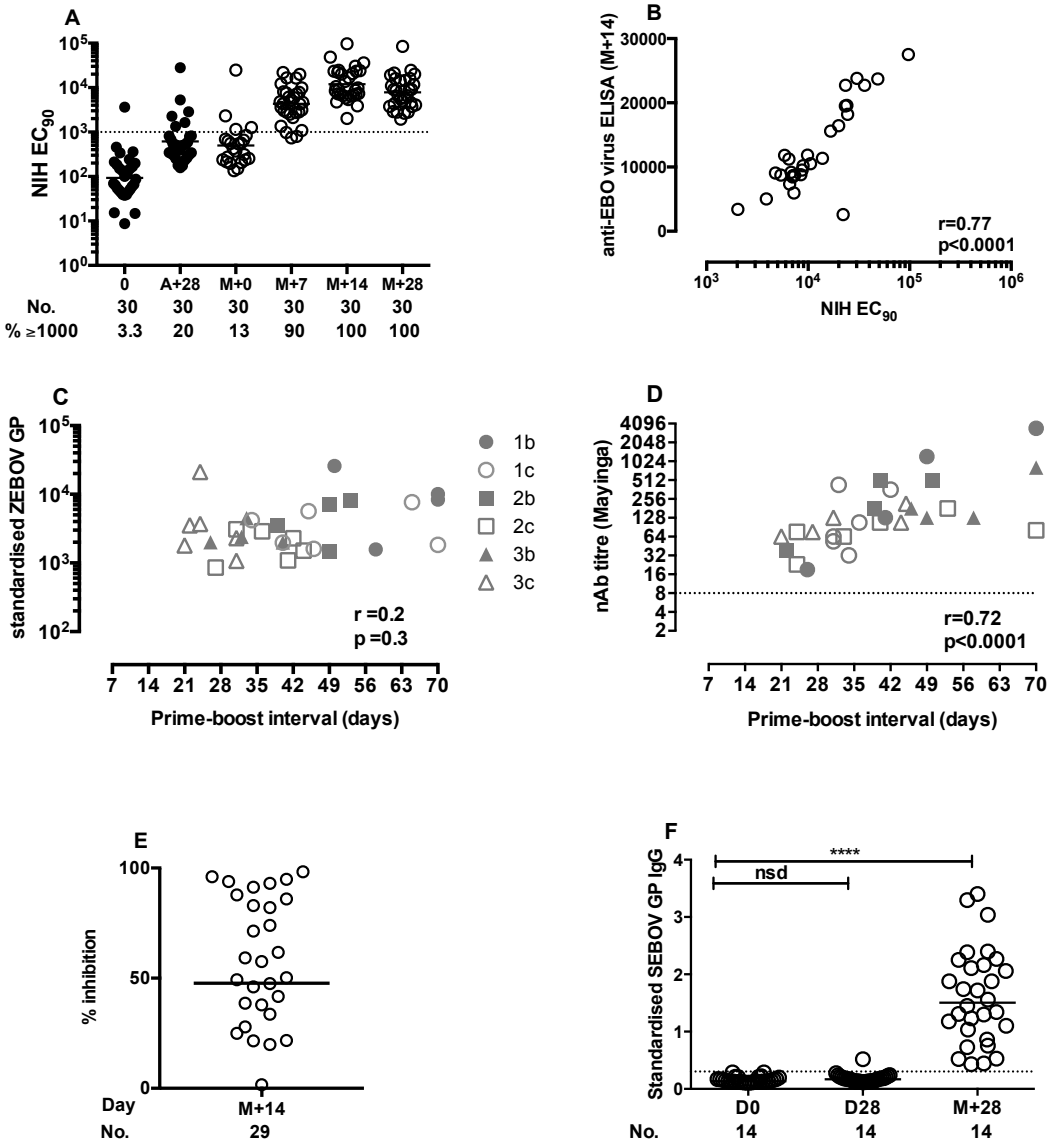
Supplementary Figure 2. Additional antibody data I. Antibody Responses to the *Zaire ebolavirus* Glycoprotein.

A Lentivirus pseudotype neutralizing activity. Dotted line represents positive threshold (IC_{50} 15.1), calculated from the mean + 3SD of the day 0 responses for all volunteers. ****, P<0.0001 Kruskal-Wallis test. B, C and D. Correlation between neutralising antibodies to Ebola virus and lentivirus pseudotype IC_{50} titre, whole virion ELISA titre and standardized ELISA titre respectively, correlations were performed with a 2-tailed Spearman test. A=ChAd3 EBO Z, M=MVA BN® Filo.

Additional IgG antibody data

Using the NIH EC90 ELISA to determine antibody titres to the Mayinga strain GP, the response peaked at fourteen days post boosting at a GM titre of 11970 (95% confidence intervals [95%CI] 8762-16353), nearly twenty times higher than the peak level detected after priming with ChAd3, and well above the level identified as a correlate of vaccine efficacy in cynomolgus macaques8 (Figure SF3A). Seven days after boosting, 90% of recipients had reciprocal titres ≥1000, a level associated with 77.1% efficacy in NHPs11, this response rate increased to 100% at day 14 and was maintained at day 28. Titres measured using this ELISA assay correlated well with the whole virion ELISA data (r=0.77, p>0.0001, Figure SF3B). Responses in the competitive ELISA assay did not correlate with neutralizing antibody titres to Ebolavirus, most likely because the competition ELISA only detects activity against a single GP epitope (Spearman's r=0.21, p=0.26).

Supplementary Figure 3. Additional antibody data II.



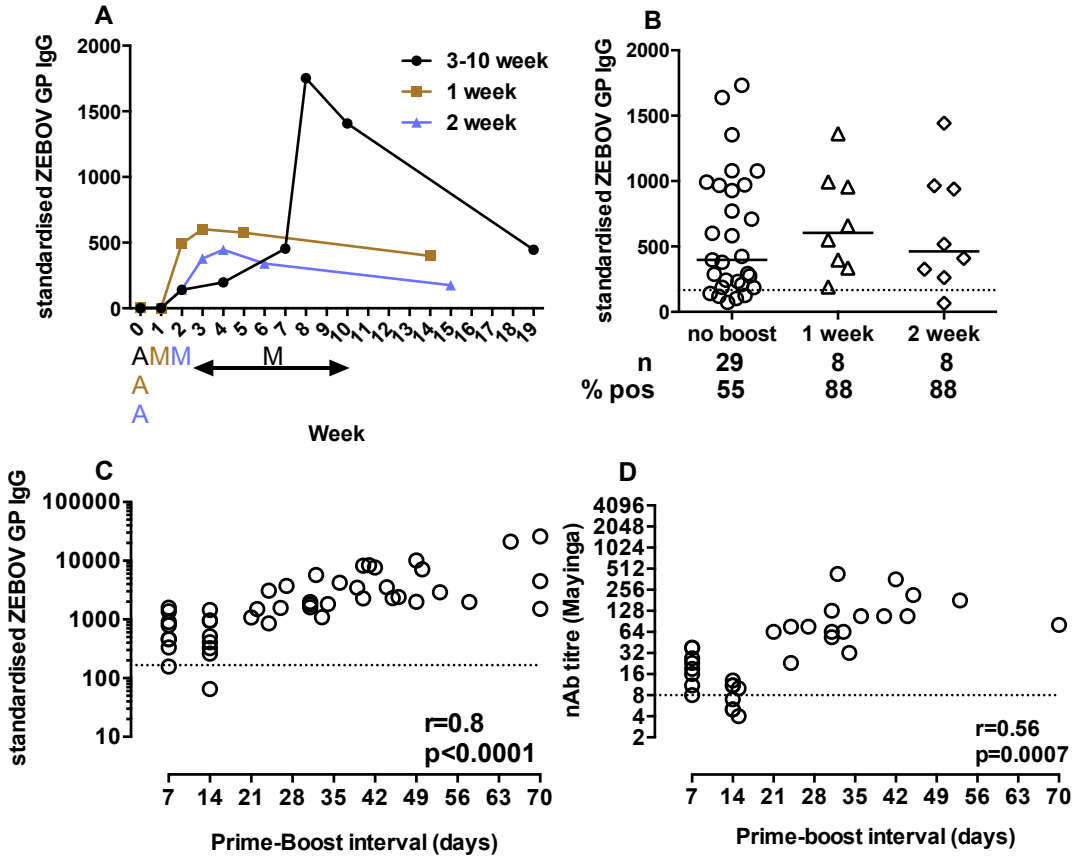
Supplementary Figure 3. Additional antibody data. A Reciprocal antibody titres measured using the NIH ELISA, bars represent GM. Percentage of vaccinees with titres ≥1000 (associated with 77% protection in NHPs) are indicated. Dotted line represents a reciprocal titre of 1000. B. Correlation between ELISA titres obtained using the NIH anti-glycoprotein ELISA and the whole-virion ELISA (n=29, 2-tailed Spearman's r). C. Effect of prime-boost interval on IgG titres to ZEBOV glycoprotein.

Symbols: $1x10^{10}$ vp ChAd3 prime (circles); $2.5x10^{10}$ vp ChAd3 prime, (squares); $5x10^{10}$ vp ChAd3 prime (triangles). Closed symbols, 3×10^8 pfu MVA boost; open symbols, 1.5×10^8 pfu MVA boost. D. Association between neutralising antibody titres to live Ebolavirus virus and prime-boost interval. Symbols are per C. All correlations performed at fourteen days post-boost using 2-tailed Spearman test, n=29. E. Neutralising activity determined by a competition ELISA with 4G7 monoclonal antibody, data points represent mean of two replicates. F. Antibody responses to Sudan virus glycoprotein after boosting with multivalent MVA. Lines show GM with 95% confidence intervals. Dotted line represents seroconversion threshold. ****, $p<0.0001$ Friedman test.

Effect of short interval boosting on IgG and neutralizing antibody responses

Fourteen days after reduced interval boosting, 88% of volunteers in both the one- and two-week boost intervals, were positive by ELISA compared with 55% in the group the non-boosted group (SF4B). A significant positive correlation was observed between prime-boost interval and antibody response ($r=0.8$, $p<0.0001$, 2-tailed Spearman's test, (SF 4C). Neutralising antibody titres to the live Ebola virus were positive for all volunteers in group 4 and 50% of volunteers in group 5, although the magnitude of the titres was much lower than with longer intervals, confirmed by a strong positive correlation between prime-boost interval and neutralizing antibody titre (SF4D, $r=0.56$, $p=0.0007$, 2-tailed Spearman's correlation).

Supplementary Figure 4. Effect of reduced prime-boost interval on anti-glycoprotein IgG titres.



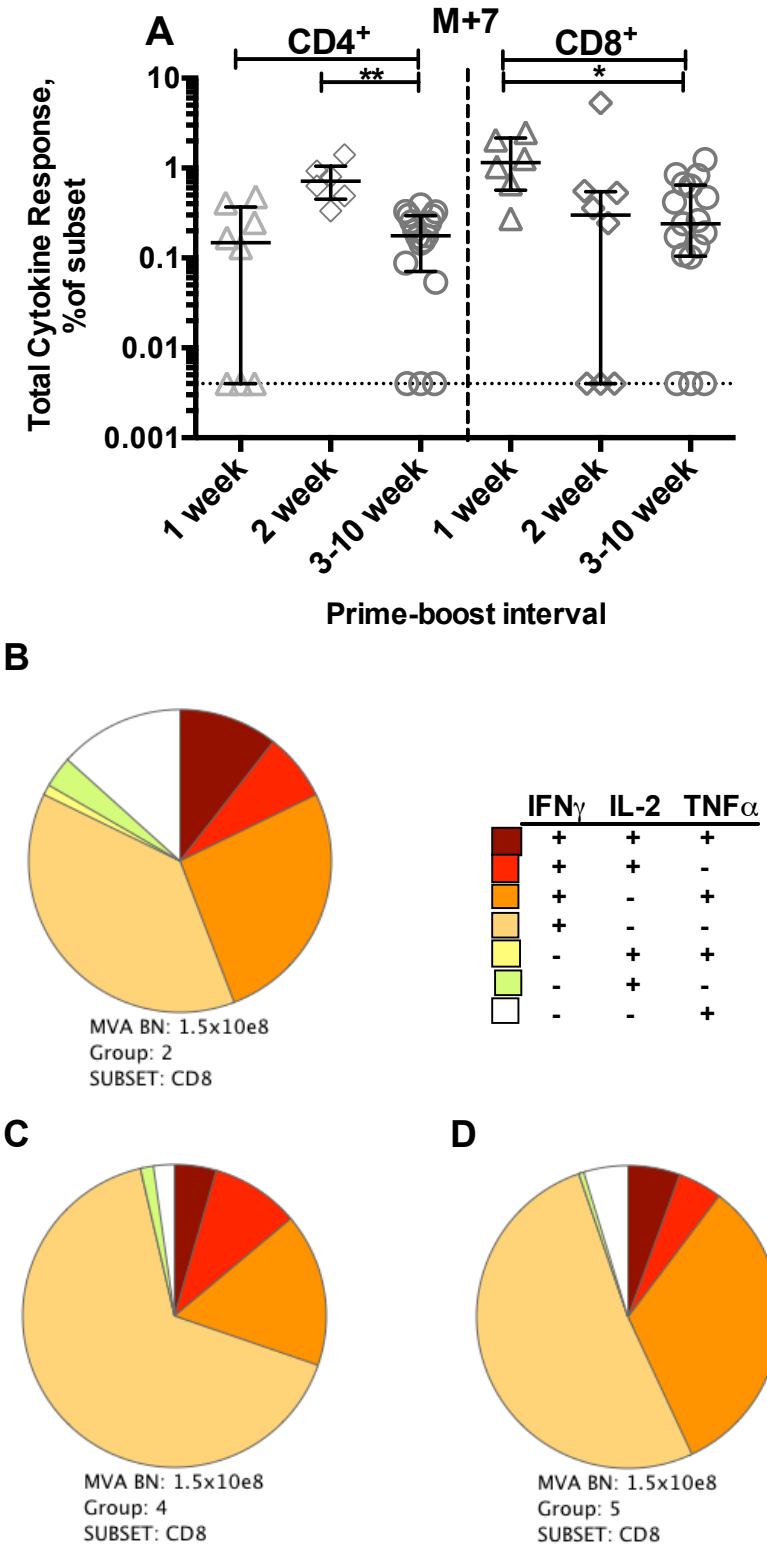
Supplementary Figure 4. Effect of reduced prime-boost interval on anti-glycoprotein IgG titres. A Geometric mean (GM) time course of anti-glycoprotein IgG levels measured with the ADI ELISA assay after vaccination with $2.5x10^{10}$vp ChAd3 EBO Z GP ("A") and boosting with $1.5x10^{8}$ pfu MVA BN® Filo ("M"). The Y-axis units are optical density (OD) without subtraction of background. B. Individual responses one month after priming in volunteers receiving either no boost, or boosted with MVA after 1 week or 2 week interval. C. Effect of prime boost interval on anti-glycoprotein IgG titres at fourteen days post boost (2-tailed Spearman test). Dotted lines represent positive threshold (OD+0.561), calculated from the mean + 3SD of

the day 0 responses for all volunteers. D. Effect of reduced prime boost interval on neutralising antibody titres at fourteen days post boost (2-tailed Spearman test).

Effect of short interval boosting on T cell responses measured by ICS

ICS analysis of T cells revealed that frequencies of cytokine-secreting CD4+ T cells were higher with two-week prime-boost interval than a 3-10 week interval ($p<0.01$, Kruskal-Wallis test, Figure S5A). For CD8+ T cells there was a slightly higher frequency of cytokine-secreting T cells after a one-week prime boost interval ($p<0.05$, Kruskal-Wallis test, Figure S5B). Subset analysis of CD8+ T cell cytokine production patterns showed cells secreting IFNγ only or IFNγ and TNFα dominated the cytokine-producing population, irrespective of prime-boost interval (Figures S5B, C and D).

Supplementary Figure 5. Effect of reduced prime-boost interval on cytokine expression.



Supplementary Figure 5. Effect of reduced prime-boost interval on cytokine expression. Flow cytometry with intracellular cytokine staining at seven days after boosting. A. IFNγ, IL2 and TNFα-secretion from $CD4^+$ and $CD8^+$ T cells was quantified for each boost dose group and expressed as the proportion expressing any one of the three cytokines. Lines and error bars show median and inter-quartile ranges. B, C and D. Proportions of $CD4^+$ and $CD8^+$ T cells producing any combination of IFNγ, IL2 and TNFα.

Supplementary Safety and Immunogenicity Tables

Supplementary Table 1: Maximum solicited local and systemic reactogenicity symptoms collected for 7 days after vaccination with ChAd3 EBO Z.

Dose	**1 x10^{10} vp**	**2.5 x 10^{10} vp**	**3 x 10^{10} vp**	
Symptom and Intensity	**Group 1 (N = 19)**	**Group 2, 4 & 5 (N = 36)**	**Group 3 (N = 20)**	**All Participants (N = 59)**
Local				
Pain				
Mild	10 (52.6)	26 (72.2)	7 (35.0)	43 (57.3)
Moderate	2 (10.5)	2 (5.6)	3 (15.0)	7 (9.3)
Mild swelling	3 (15.8)	2 (5.6)	2 (10.0)	7 (9.3)
Mild redness	3 (15.8)	8 (22.20	3 (15.0)	14 (18.7)
Mild warmth	10 (52.6)	8 (22.2)	4 (20.0)	22 (29.3)
Mild itch	2 (10.5)	5 (13.9)	0	7 (9.3)
Systemic				
Moderate fever	0	1 (2.8)	1 (5.0)	2 (2.7)
Feverishness				
Mild	3 (15.8)	6 (16.7)	6 (30.0)	15 (20.0)
Moderate	0	2 (5.6)	1 (5.0)	3 (4.0)
Severe	0	1 (2.8)	0	1 (1.3)
Myalgia				
Mild	7 (36.8)	10 (27.8)	11 (55.0)	28 (37.3)

Moderate	0	3 (8.3)	0	3 (4.0)
Arthralgia				
Mild	1 (5.3)	6 (16.7)	3 (15.0)	10 (13.3)
Moderate	0	1 (2.8)	1 (5.0)	2 (2.7)
Headache				
Mild	7 (36.8)	18 (50.0)	8 (40.0)	33 (44.0)
Moderate	2 (10.5)	3 (8.3)	1 (5.0)	6 (8.0)
Fatigue				
Mild	11 (58)	10 (27.8)	10 (50.0)	31 (41.3)
Moderate	2 (10.5)	3 (8.3)	3 (15.0)	8 (10.7)
Nausea				
Mild	3 (15.8)	4 (11.1)	4 (20.0)	11 (14.7)
Moderate	1 (5.3)	2 (5.6)	1 (5.0)	4 (5.3)
Severe	0	1 (2.8)	0	1 (1.3)
Malaise				
Mild	6 (31.6)	9 (25.0)	7 (35.0)	22 (29.3)
Moderate	2 (10.5)	1 (2.8)	2 (10.0)	5 (6.7)
Severe	0	1 (2.8)	0	1 (1.3)
Use of acetaminophen, NSAID, or aspirin for symptoms	8 (42.1)	9 (25.0)	7 (35.0)	24 (32.0)

Supplementary Table 1: Maximum solicited local and systemic reactogenicity symptoms collected for 7 days after vaccination with ChAd3 EBO Z. Frequency is calculated as the number of participants counted once at the worst severity of each symptom. Severity categories returning all zero values have not been shown. NSAID denotes non-steroidal anti-inflammatory drug. The case definition and severity grading for adverse events can be found in the protocol.

Supplementary Table 2: Maximum unsolicited reactogenicity summary after vaccination with ChAd3 EBO Z.

Dose	**1 x 10^{10}vp**	**2.5 x 10^{10}vp**	**5 x 10^{10}vp**	
	Group 1	**Groups 2, 4 & 5**	**Group 3**	**All Groups**
All unsolicited AEs	**(n=19)**	**(n=36)**	**(n=20)**	**(n=75)**
Mild	14 (73.7%)	13 (36.1%)	9 (45.0%)	36 (48.0%)
Moderate	2 (10.5%)	7 (19.4%)	8 (40.0%)	17 (22.7%)
Severe	0	2 (5.6%)	0	2 (2.7%)
Unsolicited AEs deemed possibly, probably or definitely related to vaccination				
Mild	7 (36.8%)	12 (33.3%)	9 (45.0%)	28 (37.3%)
Moderate	2 (10.5%)	3 (8.3%)	3 (15.0%)	8 (10.7%)
Severe	0	1 (2.8%)	0	1 (1.3%)

Supplementary Table 2: Maximum unsolicited reactogenicity summary after vaccination with ChAd3 EBO Z. Unsolicited reactogenicity is collected to day 28, or until boosting vaccination with MVA BN®-Filo, whichever comes first. Causality has been assigned to all unsolicited AEs based on type of event and temporal

relationship to vaccination. Causality gradings are: unrelated; unlikely relationship to vaccination; possible relationship to vaccination; probable relationship to vaccination; definite relationship to vaccination.

Supplementary Table 3: All laboratory adverse events following ChAd3 EBO Z.

Dose	**1x10^{10} vp**	**2.5 x 10^{10} vp**	**5 x 10^{10} vp**	
	Group 1	**Groups 2, 4 & 5**	**Group 3**	**Total**
Lab AE/Severity	**(n=19)**	**(n=36)**	**(n=20)**	**(n=75)**
Anaemia				
Mild	6 (31.6%)	0	0	6 (8.0%)
Decreased fibrinogen				
Moderate	0	0	1 (5.0%)	1 (1.3%)
Thrombocytopenia				
Moderate	0	0	1 (5.0%)	1 (1.3%)
Elevated ALT				
Mild	1 (5.3%)	0	0	1 (1.3%)
Elevated aPTT				
Mild	0	2 (5.7%)	1 (5.0%)	3 (4.0%)
Moderate	0	1 (2.7%)	0	1 (1.3%)
Eosinophilia				
Mild	0	0	2 (10.0%)	2 (2.7%)
Moderate	0	0	1 (5.0%)	1 (1.3%)
Hyperbilirubinaemia				
Mild	0	2 (5.7%)	0	2 (2.7%)
Moderate	0	1 (2.7%)	3 (15.0%)	4 (5.3%)
Severe	0	1[‡] (2.7%)	1 (5.0%)	2 (2.7%)
Hypernatraemia				
Mild	1 (5.3%)	1 (2.7%)	0	2 (2.7%)
Hypokalaemia				

Mild	0	1 (2.7%)	1 (5.0%)	2 (2.7%)
Hyponatraemia				
Mild	1 (5.3%)	1 (2.7%)	0	2 (2.7%)
Moderate	1 (5.3%)	0	0	1 (1.3%)
Lymphopenia				
Mild	5 (26.3%)	5 (13.9%)	8 (40.0%)	18 (24.0
Moderate	0	2 (5.7%)	2 (10.0%)	4 (5.3%)
Neutropenia				
Mild	1 (5.3%)	1 (2.7%)	2 (10.0%)	4 (5.3%)

Supplementary Table 3: All laboratory adverse events following ChAd3 EBO Z.

Adverse events are defined as a deviation from the baseline result collected pre-vaccination on Day 0. This table shows results for 75 volunteers followed to trial end, or until boosting vaccination with MVA BN®-Filo vaccination, whichever came first.

‡Severe hyperbilirubinaemia detected in a single subject with a pre-vaccination diagnosis of Gilbert's disease. Serum bilirubin was in the moderate AE range before vaccination, in this subject.

Supplementary Table 4: Maximum solicited local and systemic reactogenicity symptoms collected for 7 days after vaccination with MVA-BN® Filo.

	Subgroup b (3×10^8 pfu)	**Subgroup c (1.5×10^8 pfu)**	**Groups 4 & 5 (1.5×10^8 pfu)**	**All Groups**
Symptom/Intensity	**n=12**	**n=18**	**n=16**	**n=46**
Pain				
Mild	5 (41.7%)	13 (72.2%)	13 (81.3%)	31 (67.4%)
Moderate	6 (50.0%)	3 (16.7%)	2 (12.5%)	11 (23.9%)
Severe	0	1 (5.6%)	0	1 (2.2%)
Swelling				

Mild	3 (25.0%)	8 (44.4%)	2 (12.5%)	13 (28.3%)
Severe	0	1 (5.6%)	0	0
Redness				
Mild	7 (58.3%)	4 (22.2%)	4 (25.0%)	15 (32.6%)
Warmth				
Mild	4 (33.3%)	6 (33.3%)	4 (25.0%)	14 (30.4%)
Moderate	1 (8.3%)	2 (11.1%)	0	3 (6.5%)
Itch				
Mild	2 (16.7%)	1 (5.6%)	0	3 (6.5%)
Fever				
Mild	3 (25.0%)	1 (5.6%)	0	4 (8.7%)
Feverishness				
Mild	1 (8.3%)	0	1 (6.3%)	2 (4.3%)
Moderate	2 (16.7%)	2 (11.1%)	0	4 (8.7%)
Myalgia				
Mild	5 (41.7%)	2 (11.1%)	7 (43.8%)	14 (30.4%)
Moderate	3 (25.0%)	2 (11.1%)	1 (6.3%)	6 (13.0%)
Arthralgia				
Mild	1 (8.3%)	1 (5.6%)	1 (6.3%)	3 (6.5%)
Moderate	1 (8.3%)	0	1 (6.3%)	2 (4.3%)
Headache				
Mild	5 (41.7%)	4 (22.2%)	8 (50.0%)	17 (37.0%)
Moderate	2 (16.7%)	2 (11.1%)	0	4 (8.7%)

Fatigue				
Mild	7 (58.3%)	6 (33.3%)	5 (31.3%)	18 (39.1%)
Moderate	1 (8.3%)	2 (11.1%)	1 (6.3%)	4 (8.7%)
Nausea				
Mild	1 (8.3%)	1 (5.6%)	1 (6.3%)	3 (6.5%)
Moderate	1 (8.3%)	0	0	1 (2.2%)
Malaise				
Mild	4 (33.3%)	2 (11.1%)	4 (25.0%)	10 (21.7%)
Moderate	2 (16.7%)	2 (11.1%)	0	4 (8.7%)

Supplementary Table 4: Maximum solicited local and systemic reactogenicity symptoms collected for 7 days after vaccination with MVA-BN® Filo. Frequency is calculated as the number of participants counted once at the worst severity of each symptom. Severity categories returning all zero values have not been shown. NSAID denotes non-steroidal anti-inflammatory drug. The case definition and severity grading for adverse events can be found in the protocol.

Supplementary Table 5: Maximum unsolicited reactogenicity summary after vaccination with MVA-BN® Filo.

	Subgroup b	**Subgroup c**	**Groups 4 & 5**	**All Groups**
All unsolicited AEs	**(n=12)**	**(n=18)**	**(n=16)**	**(n=46)**
Mild	3 (25.0%)	4 (22.2%)	8 (50.0%)	15 (32.6%)
Moderate	5 (41.7%)	6 (33.3%)	3 (18.8%)	14 (30.4%)
Severe	0	2 (11.1%)	0	2 (4.3%)
Unsolicited AEs deemed possibly, probably or definitely related to vaccination				
Mild	5 (41.7%)	6 (33.3%)	8 (50.0%)	19 (41.3%)
Moderate	2 (16.7%)	3 (16.7%)	1 (6.3%)	6 (13.0%)
Severe	0	1 (5.6%)	0	1 (2.2%)

Supplementary Table 5: Maximum unsolicited reactogenicity summary after vaccination with MVA-BN® Filo. Unsolicited reactogenicity is collected to day 28 after vaccination. Causality has been assigned to all unsolicited AEs based on type of event and temporal relationship to vaccination. This process is further described in the study protocol. Causality gradings are: unrelated; unlikely relationship to vaccination; possible relationship to vaccination; probable relationship to vaccination; definite relationship to vaccination.

Supplementary Table 6: All laboratory adverse events.

	Subgroup b	Subgroup c	Groups 4 & 5	Total
Lab AE/Severity	**(n=12)**	**(n=18)**	**(n=16)**	**(n=46)**
Anemia				
Mild	1 (8.3%)	0	0	1 (2.2%)
Moderate	0	1 (8.3%	0	1 (2.2%)
Hyperbilirubinaemia				
Mild	1 (8.3%)	1 (5.6%)	0	2 (4.3%)
Moderate	0	2 (11.1%)	0	2 (4.3%)
Severe	0	1	0	1 (2.2%)[‡]
Hypokalemia				
Mild	4 (33.3%)	0	1 (6.3%)	5 (10.9%)
Moderate	0	1 (5.6%)	0	1 (2.2%)
Hypernatremia				
Mild	0	0	2 (12.5%)	2 (4.3%)
Lymphopenia				
Mild	4 (33.3%)	5 (27.8%)	1 (6.3%)	10 (21.7%)
Moderate	2 (16.7%)	1 (5.6%)	1 (6.3%)	4 (8.7%)
Neutropenia				
Mild	0	1 (5.6%)	0	1 (2.2%)
Raised ALT				
Mild	1 (8.3%)	0	0	1 (2.2%)

Supplementary Table 6: All laboratory adverse events. Adverse events are defined as a deviation from the baseline result collected pre-vaccination on Day 0. This table shows results for 59 volunteers followed to at least day 28. [‡]Severe hyperbilirubinaemia detected in a single subject with a pre-vaccination diagnosis of Gilbert's disease. Serum bilirubin was in the moderate AE range before vaccination, in this subject.

Supplementary Table 7: All unsolicited AEs reported

Symptom	Severity/Intensity	Subgroup b (n=12)	Subgroup c (n=18)	Groups 4 & 5 (n=16)	All groups (n=46)
Abdominal discomfort	Mild	1 (8.3%)	0	0	1 (2.2%)
Abdominal pain	Moderate	0	1 (5.6%)	0	1 (2.2%)
Abdominal pain upper	Moderate	0	0	1 (6.3%)	1 (2.2%)
Abdominal pain upper	Mild	0	0	2 (12.5%)	2 (4.3%)
Apthous stomatitis	Mild	0	2 (11.1%)	0	2 (4.3%)
Arthralgia	Moderate	0	1 (5.6%)	0	1 (2.2%)
Back pain	Mild	0	1 (5.6%)	0	1 (2.2%)
Back pain	Moderate	0	0	1 (6.3%)	1 (2.2%)
Chest discomfort	Mild	2 (16.7%)	0	0	2 (4.3%)
Cough	Moderate	2 (16.7%)	1 (5.6%)	0	3 (6.5%)
Cough	Mild	0	2 (11.1%)	1 (6.3%)	3 (6.5%)
Depressed mood	Moderate	0	1 (5.6%)	0	1 (2.2%)
Diarrhoea	Moderate	0	1 (5.6%)	1 (6.3%)	2 (4.3%)
Diarrhoea	Mild	0	1 (5.6%)	0	1 (2.2%)
Diastolic hypertension	Moderate	1 (8.3%)	0	0	1 (2.2%)
Diastolic	Mild	0	2 (11.1%)	0	2 (4.3%)

hypertension					
Dizziness	Mild	0	0	1 (6.3%)	1 (2.2%)
Dry skin	Mild	0	1 (5.6%)	0	1 (2.2%)
Dyspepsia	Mild	1 (8.3%)	0	1 (6.3%)	2 (4.3%)
Epistaxis	Mild	0	0	1 (6.3%)	1 (2.2%)
Fatigue	Mild	1 (8.3%)	0	1 (6.3%)	2 (4.3%)
Headache	Mild	2 (16.7%)	3 (16.7%)	4 (25.0%)	9 (19.6%)
Headache	Moderate	1 (8.3%)	2 (11.1%)	0	3 (6.5%)
Injection site bruising	Mild	0	1 (5.6%)	0	1 (2.2%)
Injection site erythema	Mild	0	1 (5.6%)	0	1 (2.2%)
Injection site pain	Moderate	1 (8.3%)	0	0	1 (2.2%)
Insomnia	Moderate	0	0	1 (6.3%)	1 (2.2%)
Joint dislocation	Severe	0	1 (5.6%)	0	1 (2.2%)
Joint stiffness	Mild	1 (8.3%)	0	0	1 (2.2%)
Lymphadenopathy	Mild	0	0	1 (6.3%)	1 (2.2%)
Malaise	Moderate	0	1 (5.6%)	0	1 (2.2%)
Migraine	Moderate	1 (8.3%)	0	0	1 (2.2%)
Muscle strain	Moderate	0	1 (5.6%)	0	1 (2.2%)
Myalgia	Mild	1 (8.3%)	0	0	1 (2.2%)
Nasal congestion	Mild	2 (16.7%)	2 (11.1%)	0	4 (8.7%)
Nasal congestion	Moderate	0	1 (5.6%)	0	1 (2.2%)
Nasopharyngitis	Moderate	1 (8.3%)	1 (5.6%)	0	2 (4.3%)

Nasopharyngitis	Mild	1 (8.3%)	2 (11.1%)	0	3 (6.5%)
Nausea	Moderate	0	1 (5.6%)	0	1 (2.2%)
Nausea	Mild	0	0	2 (12.5%)	2 (4.3%)
Oral herpes	Mild	0	1 (5.6%)	0	1 (2.2%)
Oropharyngeal pain	Mild	2 (16.7%)	2 (11.1%)	0	4 (8.7%)
Oropharyngeal pain	Moderate	1 (8.3%)	1 (5.6%)	0	2 (4.3%)
Pain in extremity	Mild	0	1 (5.6%)	0	1 (2.2%)
Procedural pain	Moderate	0	1 (5.6%)	0	1 (2.2%)
Productive cough	Mild	0	0	1 (6.3%)	1 (2.2%)
Renal pain	Mild	1 (8.3%)	0	0	1 (2.2%)
Rhinorrhoea	Mild	0	0	2 (12.5%)	2 (4.3%)
Sneezing	Mild	1 (8.3%)	1 (5.6%)	0	2 (4.3%)
Systolic hypertension	Mild	2 (16.7%)	1 (5.6%)	1 (6.3%)	4 (8.7%)
Tachycardia	Mild	0	1 (5.6%)	1 (6.3%)	2 (4.3%)
Thirst	Mild	0	1 (5.6%)	0	1 (2.2%)
Throat irritation	Mild	0	0	1 (6.3%)	1 (2.2%)
Toothache	Mild	0	1 (5.6%)	0	1 (2.2%)
Vaccination site bruising	Mild	0	0	1 (6.3%)	1 (2.2%)
Vaccination site erythema	Mild	0	0	1 (6.3%)	1 (2.2%)
Vaccination site swelling	Mild	0	0	2 (12.5%)	2 (4.3%)

Vomiting	Moderate	0	1 (5.6%)	0	1 (2.2%)
Vomiting	Severe	0	1 (5.6%)	0	1 (2.2%)

Supplementary Table 7: All unsolicited AEs reported. Frequency of unsolicited AEs reported in the 28 days following vaccination. Frequency is calculated as the number of volunteers counted once at worst severity. Rows with all zero values are not shown. AEs have been classified according to the Medical Dictionary for Regulatory Activities (MedDRA) at the Preferred Term level.

Supplementary Table 8: Related unsolicited AEs.

Symptom	Severity/Intensity	Subgroup b (n=12)	Subgroup c (n=18)	Group 4 & 5 (n=16)	All groups (n=46)
Abdominal pain upper	Mild	0	0	1 (6.3%)	1 (2.2%)
Arthralgia	Moderate	0	1 (5.6%)	0	1 (2.2%)
Chest discomfort	Mild	2 (16.7%)	0	0	2 (4.3%)
Cough	Moderate	1 (8.3%)	0	0	1 (2.2%)
Cough	Mild	0	2 (11.1%)	1 (6.3%)	3 (6.5%)
Depressed mood	Moderate	0	1 (5.6%)	0	1 (2.2%)
Diarrhoea	Moderate	0	1 (5.6%)	0	1 (2.2%)
Diarrhoea	Mild	0	1 (5.6%)	0	1 (2.2%)
Diastolic hypertension	Mild	0	1 (5.6%)	0	1 (2.2%)
Dizziness	Mild	0	0	1 (6.3%)	1 (2.2%)
Dry skin	Mild	0	1 (5.6%)	0	1 (2.2%)
Dyspepsia	Mild	1 (8.3%)	0	0	1 (2.2%)
Fatigue	Mild	1 (8.3%)	0	0	1 (2.2%)
Headache	Mild	0	1 (5.6%)	0	1 (2.2%)
Injection site bruising	Mild	0	1 (5.6%)	0	1 (2.2%)
Injection site erythema	Mild	0	1 (5.6%)	0	1 (2.2%)

Injection site pain	Moderate	1 (8.3%)	0	0	1 (2.2%)
Insomnia	Moderate	0	0	1 (6.3%)	1 (2.2%)
Joint stiffness	Mild	1 (8.3%)	0	0	1 (2.2%)
Lymphadenopathy	Mild	0	0	1 (6.3%)	1 (2.2%)
Nasal congestion	Mild	1 (8.3%)	2 (11.1%)	0	3 (6.5%)
Nasopharyngitis	Moderate	0	1 (5.6%)	0	1 (2.2%)
Nausea	Moderate	0	1 (5.6%)	0	1 (2.2%)
Oropharyngeal pain	Mild	1 (8.3%)	1 (5.6%)	0	2 (4.3%)
Oropharyngeal pain	Moderate	1 (8.3%)	0	0	1 (2.2%)
Pain in extremity	Mild	0	1 (5.6%)	0	1 (2.2%)
Productive cough	Mild	0	0	1 (6.3%)	1 (2.2%)
Renal pain	Mild	1 (8.3%)	0	0	1 (2.2%)
Rhinorrhoea	Mild	0	0	2 (12.5%)	2 (4.3%)
Sneezing	Mild	1 (8.3%)	0	0	1 (2.2%)
Systolic hypertension	Mild	2 (16.7%)	0	0	2 (4.3%)
Tachycardia	Mild	0	1 (5.6%)	1 (6.3%)	2 (4.3%)
Thirst	Mild	0	1 (5.6%)	0	1 (2.2%)
Vaccination site bruising	Mild	0	0	1 (6.3%)	1 (2.2%)
Vaccination site erythema	Mild	0	0	1 (6.3%)	1 (2.2%)
Vaccination site swelling	Mild	0	0	2 (12.5%)	2 (4.3%)

Vomiting	Severe	0	1 (5.6%)	0	1 (2.2%)

Supplementary Table 8: Related unsolicited AEs. Frequency of unsolicited AEs reported in the 28 days following vaccination assessed as being possibly, probably or definitely related to vaccination. Causality has been assigned to all unsolicited AEs based on type of event and temporal relationship to vaccination. Causality gradings are: unrelated; unlikely relationship to vaccination; possible relationship to vaccination; probable relationship to vaccination; definite relationship to vaccination. Frequency is calculated as the number of volunteers counted once at worst severity. Rows with all zero values are not shown. AEs have been classified according to the Medical Dictionary for Regulatory Activities (MedDRA) at the Preferred Term level.

Supplementary Table 9: Geometric mean titers (GMT) and seropositivity rates to ChAd3 MVA measured by Oxford Standardised GP ELISA.

				Oxford Standardised GP ELISA					
Priming dose ChAd3 (vp)	Boosting dose MVA (pfu)	Prime-boost interval (w)	Group	Time point	N	GMT	Lower 95% CI	Upper 95% CI	Seropositivity (>166 AEU, %)
$1x10^{10}$	n/a	n/a	1a	0	9	4.027	1.271	12.76	0.0
				7	9	4.576	1.077	19.43	0.0
				14	9	141.1	48.08	414.2	44.4
				28	9	328.2	163.3	659.8	77.8
				90	9	124.1	51.35	299.9	33.3
				180	9	84.34	32.62	218	22.2
$2.5x10^{10}$	n/a	n/a	2a	0	10	4.009	1.527	10.52	0
				7	10	4.655	1.889	11.47	0
				14	10	182	90.59	365.5	50
				28	10	359	199.9	644.8	80
				90	10	226.2	113.1	452.4	60
				180	10	56.34	10.75	295.3	40
$5x10^{10}$	n/a	n/a	3a	0	10	3.612	1.205	10.83	0
				7	10	6.54	2.104	20.33	0
				14	10	315.6	147.3	676.5	70
				28	10	580.7	290.2	1162	90
				90	10	274.1	136.8	549.1	70
				180	10	158.6	67.43	373.3	50
$1x10^{10}$	$3x10^{8}$	3-10	1b	0	4	1.965	0.229	16.85	0
				7	4	1.707	0.3111	9.372	0
				14	4	240.5	71.28	811.2	75

				28	4	342.2	195.8	598.2	100
				M+7	4	3815	907.3	16038	100
				M+14	4	7650	1196	48939	100
				M+28	4	5189	873.4	30824	100
				M+90	4	1244	424.8	3645	100
				M+180	4	1014	241.1	4261	100
				0	4	3.15	0.3698	26.83	0
				7	4	3.677	0.3119	43.34	0
				14	4	184.1	124.6	271.8	50
				28	4	527.7	304.4	914.5	100
$2.5x10^{10}$	$3x10^{8}$	3-10	2b	M+7	4	2044	545.1	7667	100
				M+14	4	4177	1200	14536	100
				M+28	4	3394	1229	9371	100
				M+90	4	1083	354.7	3305	100
				M+180	4	588.6	222	1561	100
				0	4	12.81	0.5922	277	0
				7	4	10.12	0.2237	458.2	25
				14	4	220.8	75.28	647.5	50
				28	4	180.5	70.94	459.2	50
$5x10^{10}$	$3x10^{8}$	3-10	3b	M+7	4	1281	1026	1601	100
				M+14	4	2559	1385	4726	100
				M+28	4	2050	1014	4143	100
				M+90	4	735.8	345.3	1568	100
				M+180	4	407.8	202.8	820	100
				0	6	8.599	1.444	51.22	0
				7	6	11	1.301	92.95	0.0
$1x10^{10}$	$1.5x10^{8}$	3-10	1c	14	6	105.6	9.23	1208	66.7
				28	6	418.9	219	801.5	100.0

				M+7	6	1550	743.9	3228	100.0
				M+14	6	3197	1599	6392	100.0
				M+28	6	2246	1020	4943	100.0
				M+90	6	655.9	339.2	1268	100.0
				M+180	6	269	128.3	563.8	83.3
				0	6	2.059	0.3218	13.17	0.0
				7	6	2.2	0.4359	11.1	0.0
				14	6	141	35.41	561.2	50.0
				28	6	197.4	70.25	554.5	50.0
$2.5x10^{10}$	$1.5x10^{8}$	3-10	2c	M+7	6	454.2	121.1	1704	83.3
				M+14	6	1752	1005	3053	100.0
				M+28	6	1407	642.4	3081	100.0
				M+90	6	445.8	180.8	1099	83.3
				M+180	6	237.8	112	505.2	66.7
				0	6	4.333	0.3279	57.26	16.7
				7	6	6.499	0.3427	123.3	16.7
				14	5	324.3	136.7	769.3	83.3
				28	6	916.3	380.7	2206	100.0
$5x10^{10}$	$1.5x10^{8}$	3-10	3c	M+7	6	2557	642.4	10182	100
				M+14	6	3273	1119	9578	100
				M+28	6	2353	843.4	6566	100
				M+90	6	1037	352.7	3051	100
				M+180	6	562.8	172.4	1837	100
				D0	8	4.453	1.263	15.7	0
				D7/M+0	8	2.108	0.6571	6.765	0
$2.5x10^{10}$	$1.5x10^{8}$	1	4	M+7	8	492.3	198.2	1223	75
				M+14	8	602.1	316	1147	87.5
				M+21	8	575.4	334.1	991.1	100

				M+90	8	399.5	269.7	591.6	100
				D0	8	3.007	0.847	10.67	0
				D7	8	2.042	0.6587	6.329	0
				D14/M+0	8	144.3	62.75	331.8	50
$2.5x10^{10}$	$1.5x10^{8}$	2	5	M+7	8	378.9	164.8	871.1	87.5
				M+14	8	445	197.3	1004	87.5
				M+28	8	340.3	178.2	650	87.5
				M+90	8	176.4	85.14	365.5	50

Supplementary Table 9: Geometric mean titers (GMT) and seropositivity rates to ChAd3 MVA measured by Oxford Standardised GP ELISA. Results are expressed in arbitrary ELISA units (AEU)/ml with 95% confidence intervals. Seropositivity is defined by a GMT > 166 AEU.

Supplementary Table 10: Geometric mean titers (GMT) and seropositivity rates to ChAd3 MVA measured by ADI GP ELISA

				ADI GP ELISA					
Priming dose ChAd3 (vp)	Boosting dose MVA (pfu)	Prime-boost interval (w)	Group	Time point	N	GMT	Lower 95% CI	Upper 95% CI	Seropositivity (>166 AEU, %)
$1x10^{10}$	n/a	n/a	1a	0	9	0.13	0.10	0.17	0.0
				28	9	0.46	0.24	0.87	33.3
				90	9	0.27	0.15	0.51	11.1
				180	9	0.29	0.17	0.47	11.1
$2.5x10^{10}$	n/a	n/a	2a	0	10	0.11	0.07	0.18	0
				28	10	0.39	0.25	0.62	20
				90	10	0.36	0.19	0.67	20
				180	9	0.47	0.26	0.82	20
$5x10^{10}$	n/a	n/a	3a	0	10	0.11	0.08	0.17	0
				28	10	0.62	0.35	1.10	50
				90	10	0.52	0.26	1.04	60
				180	10	0.50	0.25	1.02	50
$1x10^{10}$	$3x10^{8}$	3-10	1b	0	4	0.17	0.02	1.36	25
				28	4	0.42	0.19	0.91	25
				M+7	4	2.84	1.82	4.44	100
				M+14	4	3.17	2.50	4.02	100
				M+28	4	3.01	2.08	4.37	100
				M+90	4	1.74	0.72	4.25	100
				M+180	4	1.19	0.51	2.81	100
$2.5x10^{10}$	$3x10^{8}$	3-10	2b	0	3	0.12	0.01	1.37	0
				28	4	0.61	0.24	1.51	75

				M+7	4	1.98	0.78	5.03	100
				M+14	4	3.01	2.24	4.03	100
				M+28	4	2.54	1.71	3.76	100
				M+90	4	1.43	0.51	4.03	100
				M+180	3	1.18	0.30	4.62	75
				0	4	0.18	0.06	0.57	0
				28	4	0.27	0.10	0.77	25
				M+7	4	1.19	0.88	1.60	100
$5x10^{10}$	$3x10^{8}$	3-10	3b	M+14	4	2.30	1.30	4.05	100
				M+28	4	2.09	1.27	3.45	100
				M+90	4	1.31	0.54	3.19	100
				M+180	4	0.79	0.43	1.48	75
				0	6	0.10	0.07	0.15	0
				28	6	0.31	0.13	0.72	33.3
				M+7	6	1.93	1.03	3.61	100.0
$1x10^{10}$	$1.5x10^{8}$	3-10	1c	M+14	6	2.76	2.08	3.65	100.0
				M+28	6	2.39	1.49	3.84	100.0
				M+90	6	0.99	0.45	2.18	83.3
				M+180	5	0.47	0.19	1.12	33.3
				0	6	0.10	0.06	0.17	0.0
				28	6	0.37	0.16	0.86	33.3
				M+7	6	0.94	0.71	1.24	100.0
$2.5x10^{10}$	$1.5x10^{8}$	3-10	2c	M+14	6	2.71	2.21	3.32	100.0
				M+28	6	2.01	1.24	3.25	100.0
				M+90	6	0.95	0.44	2.06	66.7
				M+180	6	0.56	0.24	1.31	66.7
				0	6	0.18	0.09	0.35	16.7
$5x10^{10}$	$1.5x10^{8}$	3-10	3c	28	6	0.78	0.31	1.95	66.7

				M+7	6	2.14	1.50	3.05	100.0
				M+14	6	2.52	1.93	3.29	100.0
				M+28	6	2.34	1.33	4.11	100.0
				M+90	6	1.17	0.64	2.12	100.0
				M+180	6	0.77	0.36	1.63	83.3
$2.5x10^{10}$	$1.5x10^{8}$	1	4	D0	8	0.07	0.04	0.11	0
				D7/M+0	8	0.06	0.05	0.08	0
				M+7	8	0.60	0.33	1.08	38
				M+14	8	1.09	0.69	1.73	88
				M+21	8	1.10	0.69	1.74	88
				M+90	8	0.89	0.54	1.45	75
$2.5x10^{10}$	$1.5x10^{8}$	2	5	D0	8	0.07	0.05	0.12	0
				D14/M+0	8	0.29	0.11	0.79	0
				M+7	8	0.83	0.44	1.59	38
				M+14	8	0.99	0.50	1.94	63
				M+28	8	0.90	0.51	1.59	75
				M+90	6	0.43	0.20	0.94	88

Supplementary Table 10: Geometric mean titers (GMT) and seropositivity rates to ChAd3 MVA measured by ADI GP ELISA. Results are expressed in arbitrary ELISA units (AEU)/ml with 95% confidence intervals. Seropositivity is defined by a GMT > 0.561 EU.

Supplementary Table 11: Geometric mean titers (GMT) and seropositivity rates to ChAd3 MVA measured by Marburg whole ZEBOV virion ELISA

				Marburg whole ZEBOV virion ELISA					
Priming dose ChAd3 (vp)	Boosting dose MVA (pfu)	Prime-boost interval (w)	Group	Time point	N	GMT	Lower 95% CI	Upper 95% CI	Seropositivity (>500 AEU, %)
$1x10^{10}$	n/a	n/a	1a	180	9	654.6	428.4	1000	22
$2.5x10^{10}$	n/a	n/a	2a	180	9	814	381.8	1735	22
$5x10^{10}$	n/a	n/a	3a	180	10	810.3	457.7	1435	30
				28	4	612.6	320.9	1170	25
				M+7	4	13942	5569	34906	100
$1x10^{10}$	$3x10^{8}$	3-10	1b	M+14	4	16719	4653	60079	100
				M+28	4	15095	5045	45171	100
				M+180	4	3565	744.1	17077	100
				28	4	764.6	346.9	1685	50
				M+7	4	8485	2007	35873	100
$2.5x10^{10}$	$3x10^{8}$	3-10	2b	M+14	4	13166	5003	34651	100
				M+28	4	10914	4914	24241	100
				M+180	4	3020	1027	8885	100
				28	4	500	500	500	0
				M+7	3	4855	2312	10194	75
$5x10^{10}$	$3x10^{8}$	3-10	3b	M+14	4	11256	6407	19775	100
				M+28	4	10575	6500	17203	100
				M+180	4	1383	421.9	4531	75
				28	6	570.6	406.3	801.5	16.7
$1x10^{10}$	$1.5x10^{8}$	3-10	1c	M+7	6	7800	3540	17186	100.0
				M+14	6	13868	8971	21436	100.0

				M+28	6	10516	7126	15518	100.0
				M+180	6	1044	427.1	2554	50.0
				28	6	661.3	322.3	1357	16.7
				M+7	6	2723	1016	7292	83.3
$2.5x10^{10}$	$1.5x10^{8}$	3-10	2c	M+14	6	8270	4373	15638	100.0
				M+28	6	4812	1450	15961	83.3
				M+180	6	1228	522.4	2886	66.7
				28	6	1682	304.3	9295	50.0
				M+7	6	8261	3843	17757	100.0
$5x10^{10}$	$1.5x10^{8}$	3-10	3c	M+14	6	8876	4874	16162	100.0
				M+28	6	8090	4992	13110	100.0
				M+180	6	2115	743.4	6018	83.3
$2.5x10^{10}$	$1.5x10^{8}$	1	4	M+21	8	1617	617.2	4238	62.5
$2.5x10^{10}$	$1.5x10^{8}$	2	5	M+28	8	1527	660	3533	62.5

Supplementary Table 11: Geometric mean titers (GMT) and seropositivity rates to ChAd3 MVA measured by Marburg whole ZEBOV virion ELISA.

Results are expressed in arbitrary ELISA units (AEU)/ml with 95% confidence intervals. Seropositivity is defined by a GMT > 500 AEU/ml.

Supplementary Table 12: Geometric mean titers (GMT) and seropositivity rates to ChAd3 MVA measured by Marburg neutralising antibody assay

			Marburg neutralising antibody assay						
Priming dose ChAd3 (vp)	Boosting dose MVA (pfu)	Prime-boost interval (w)	Group	Time point	N	GMT	Lower 95% CI	Upper 95% CI	Seropositivity (>8 titre, %)

$1x10^{10}$	$3x10^{8}$	3-10	1b	28	4	13.52	8.334	21.94	50
				M+14	4	317.8	7.835	12892	100
$2.5x10^{10}$	$3x10^{8}$	3-10	2b	28	4	19.8	9.72	40.35	75
				M+14	4	206.1	29.15	1457	100
$5x10^{10}$	$3x10^{8}$	3-10	3b	28	4	13.89	9.245	20.86	25
				M+14	4	221.6	54.47	901.5	100
$1x10^{10}$	$1.5x10^{8}$	3-10	1c	28	6	11.48	7.349	17.93	17
				M+14	6	110.9	36.51	337.1	100
$2.5x10^{10}$	$1.5x10^{8}$	3-10	2c	28	6	18.09	9.031	36.23	67
				M+14	6	74.94	36.57	153.6	100
$5x10^{10}$	$1.5x10^{8}$	3-10	3c	28	6	14.83	5.436	40.48	33
				M+14	6	104.7	66.67	164.3	100
$2.5x10^{10}$	$1.5x10^{8}$	1	4	M+14	8	19.84	12.44	31.64	88
$2.5x10^{10}$	$1.5x10^{8}$	2	5	M+14	8	7.59	5.218	11.04	50

Supplementary Table 12: Geometric mean titers (GMT) and seropositivity rates to ChAd3 MVA measured by Marburg neutralising antibody assay.

Results are expressed as neutralization titers with 95% confidence intervals.

Seropositivity is defined by a GMT > 8.

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