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Supplementary appendix

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Supplement to: Ladhani SN, Dowell AC, Jones S, et al. Early evaluation of the safety, reactogenicity, and immune response after a single dose of modified vaccinia Ankara–Bavaria Nordic vaccine against mpox in children: a national outbreak response. *Lancet Infect Dis* 2023; published online June 16. [https://doi.org/10.1016/S1473-3099\(23\)00270-0](https://doi.org/10.1016/S1473-3099(23)00270-0).

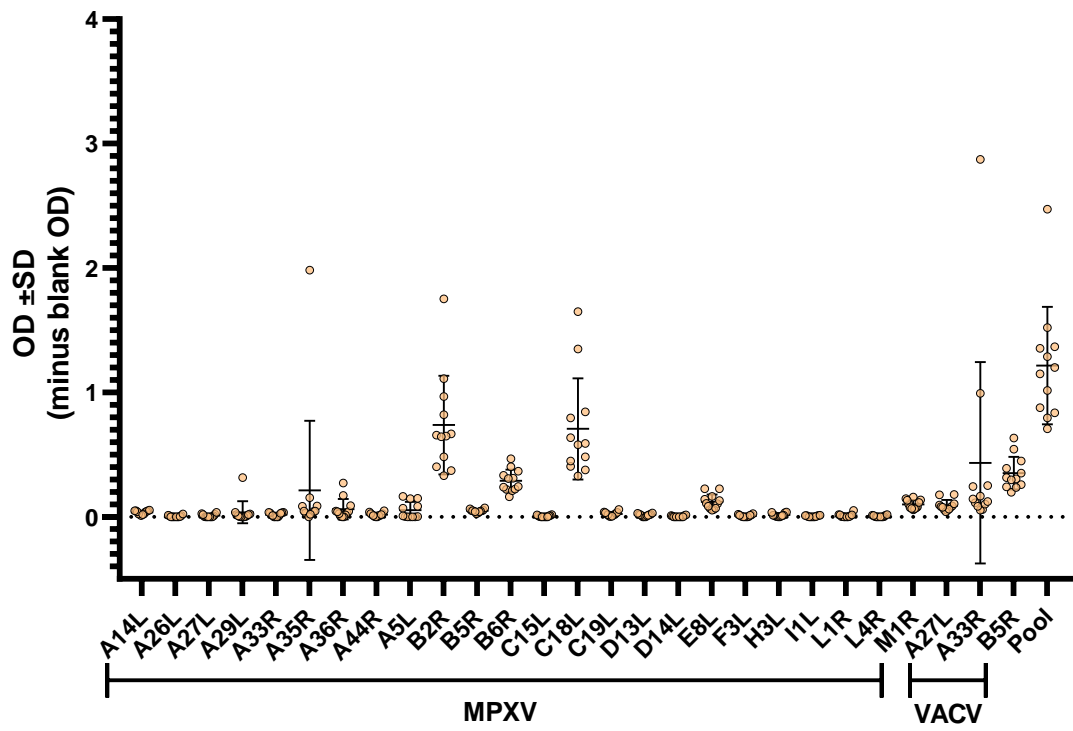
Early evaluation of the safety, reactogenicity and immune responses after a single dose of Modified Vaccinia Ankara–Bavaria Nordic (MVA-BN) vaccine against mpox in children

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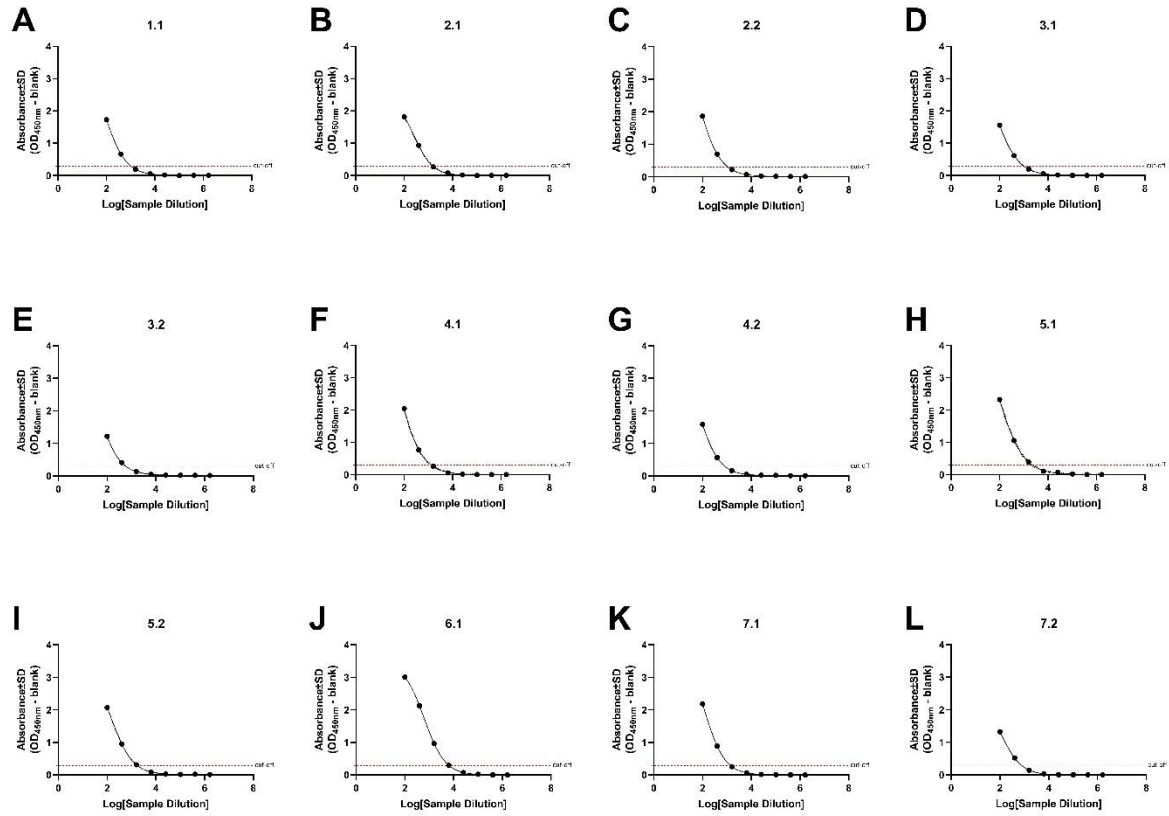
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SUPPLEMENT

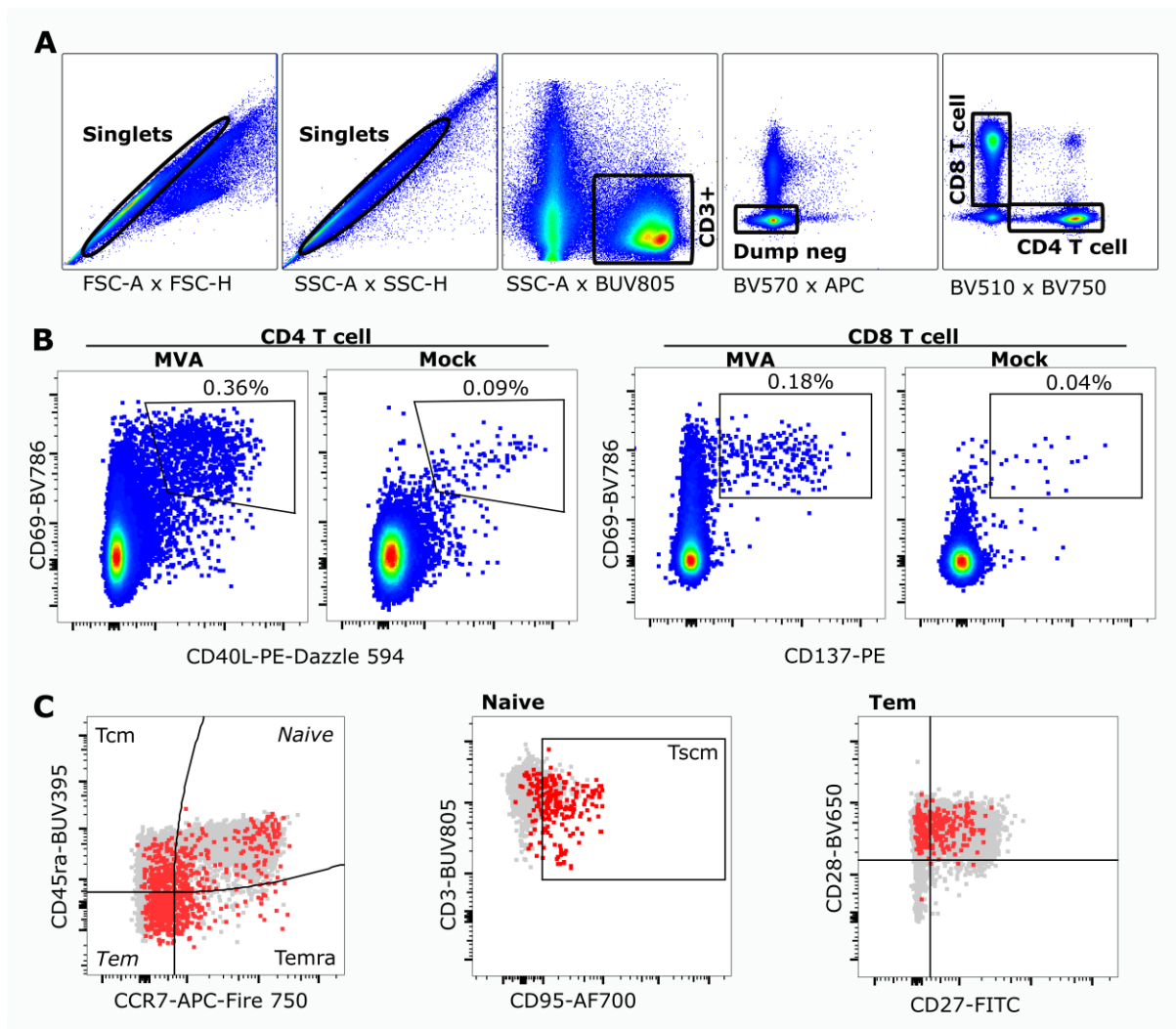
- **Supplementary Figure S1. Antibody absorbance of samples to panels of MPXV and VACV recombinant antigens**
- **Supplement Figure S2: Endpoint titres using the pooled antigen ELISA for each individual at each time-point**
- **Supplement Figure S3. Cellular response in un-vaccinated children (Control Group).** Frequency of cellular response to MVA infection (MVA) or a pool of conserved *Poxviridae* immunogenic peptide epitopes (Pox-Pep), by IFN γ ELISpot, in 9 healthy children without a history of known MPX exposure or vaccination
- **Supplement Figure S4. Activation Induced Marker assay gating strategy.** A) A representation of the gating strategy used to identify CD4+ and CD8+ T cells, firstly doublets were excluded and CD3 expressing cells gated, followed by exclusion of CD14/19 and viability dye positive cells. B) A representation of AIM+ CD4 and CD8 staining, showing CD69+CD40L+ CD4+ T cells and CD69+CD137+ CD8+ T cells in response to MVA or mock infection. C) Representation of further phenotype characterisation of AIM+ cells, shown are AIM+ CD4+ T cells (red) or bulk CD4+ T cells (grey)
- **Supplement Figure S5. Comparison of Cellular and Serology responses.** Frequency of cellular response to MVA infection (yellow circles) or a pool of conserved *Poxviridae* immunogenic peptide epitopes (orange squares), determined by IFN γ ELISpot, in MVA-BN vaccinated children, plotted against measurement of serology responses against pooled antigen (A) or mpox antigen B2R (B) determined by ELISA.
- **Supplement Table S1. Flow Cytometry antibody suppliers and dilutions**
- **Supplementary Table S2. Summary table of Cellular and Serology responses.** Timing of first sample and second sample (if taken) are given as days relative to the vaccine dose. ELISpot responses are shown for response to MVA infection (or Pan-Poxviridae peptide pool) as sfc/10⁶ PBMC. Frequency of CD4+ and CD8+ T cells defined by AIM assay in response to MVA virus are also shown. Serology results against pooled antigen determined by ELISA are given as Absorbance (O.D. 450nm) values.



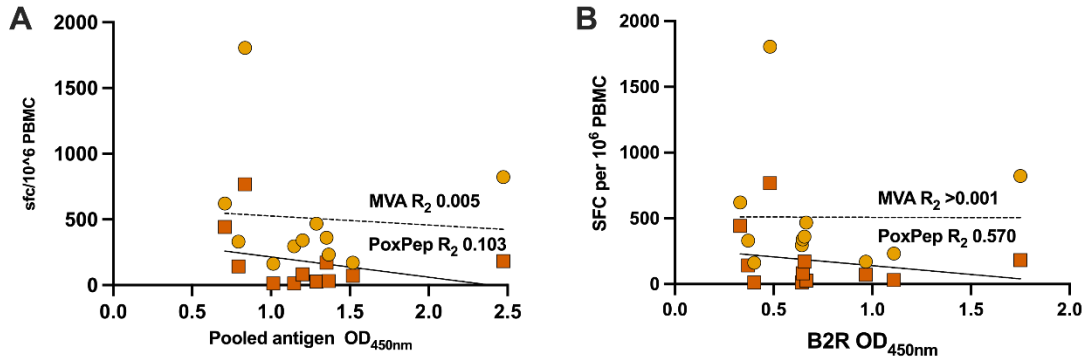
Supplementary Figure S1. Antibody absorbance of samples to panels of MPXV and VACV recombinant antigens



Supplement Figure S2: Endpoint titres using the pooled antigen ELISA for each individual at each time-point. A four-parameter logistic (4PL) regression model was used to fit the curves



Supplement Figure S3. Activation Induced Marker (AIM) assay gating strategy. A) A representation of the gating strategy used to identify CD4+ and CD8+ T cells, firstly doublets were excluded and CD3 expressing cells gated, followed by exclusion of CD14/19 and viability dye positive cells. B) A representation of AIM+ CD4 and CD8 staining, showing CD69+CD40L+ CD4+ T cells and CD69+CD137+ CD8+ T cells in response to MVA or mock infection. C) Representation of further phenotype characterisation of AIM+ cells, shown are AIM+ CD4+ T cells (red) or bulk CD4+ T cells (grey).



Supplement Figure S4. Comparison of Cellular and Serology responses. Frequency of cellular response to MVA infection (yellow circles) or a pool of conserved *Poxviridae* immunogenic peptide epitopes (orange squares), determined by IFN γ ELISpot, in MVA-BN vaccinated children, plotted against measurement of serology responses against pooled antigen (A) or mpox antigen B2R (B) determined by ELISA. show linear regression and R₂ values added to assess any significant correlation between antibody response (pooled antigen (A), or B2R-specific (B), x-axis) and cellular response (y-axis) to MVA infection (MVA, dashed line) or a pool of conserved *Poxviridae* immunogenic peptide epitopes (PoxPep, solid line), no correlation was evident.

Antigen	Fluorochrome	Supplier	Final Dilution
CD3	BUV805	BD Bioscience	1/100
CD4	BV750	Biolegend	1/100
CD8	BV510	Biolegend	1/100
CD45RA	BUV395	BD Bioscience	1/200
CCR7	APC-Fire-750	Biolegend	1/20
CD27	Fitc	Biolegend	1/50
CD28	BV650	Biolegend	1/20
CD95	AF700	Biolegend	1/50
CD69	BV786	Biolegend	1/25
CD154 (CD40L)	PE-Dazzle-594	Biolegend	1/25
CD137 (4-1BB)	PE	Biolegend	1/25
CD14/CD19	BV570	Biolegend	1/100
Far Red Fixable Viability stain	APC	Thermo-Fisher Scientific	1/1000

Supplement Table S1. Flow Cytometry antibody suppliers and dilutions

Donor	1	2	3	4	5	6	7
<i>Sample 1 days post vaccine</i>	34	44	44	44	44	47	64
<i>Sample 2 days post vaccine</i>	-	108	108	108	108	-	91
<i>Sample 1 ELISpot sfc/10⁶PBMC against MVA (Pox-Pep)</i>	295 (13)	467 (28)	1805 (768)	340 (83)	233 (30)	822 (182)	360 (170)
<i>Sample 2 ELISpot sfc/10⁶PBMC against MVA (Pox-Pep)</i>	-	162 (12)	620 (443)	330 (143)	170 (70)	-	85 (40)
<i>Sample 1 MVA CD4/CD8 AIM+ T cells</i>	0.17/ 0.06%	0.12/ 0.04%	1.04/ 0.32%	0.27/ 0.18%	0.23/ 0.01%	0.27/ 0.14%	0.31/ 0.21%
<i>Sample 2 MVA CD4/CD8 AIM+ T cells</i>	-	0.05/ 0.06%	0.30/ 0.19%	0.23/ 0.26%	0.27/ 0.11%	-	0.34/ 0.24%
<i>Sample 1 Serology ELISAPool Absorbance</i>	1.1471	1.288	0.8357	1.2007	1.3665	2.4724	1.3525
<i>Sample 2 Serology ELISA Pool Absorbance</i>	-	1.0143	0.7081	0.7940	1.5194	-	0.877

Supplement Table S2. Summary table of Cellular and Serology responses. Timing of first sample and second sample (if taken) are given as days relative to the vaccine dose. ELISpot responses are shown for response to MVA infection (or Pan-Poxviridae peptide pool) as sfc/10⁶ PBMC. Frequency of CD4+ and CD8+ T cells defined by AIM assay in response to MVA virus are also shown. Serology results against pooled antigen determined by ELISA are given as Absorbance (O.D. 450nm) values.