# Supplementary Table 1: Summary statistics for paired *ex vivo* data analysis.

Wilcoxon signed-rank test p value outcome between two vaccination visits (paired samples only) in the frequencies of CD56<sup>bright</sup>, CD56<sup>dim</sup>, CD25 and Ki67 expressing NK cells (in relation to Figure 1).

	P value			
Visit <sup>1</sup> (n)	CD56 <sup>bright</sup>	CD56 <sup>dim</sup>	CD25	Ki67
0 vs. 1 (n=7)	0.688 <sup>3</sup>	0.813 <sup>3</sup>	0.813 <sup>3</sup>	0.578 <sup>3</sup>
0 vs. 2 (n=14)	0.194 <sup>3</sup>	0.235 <sup>3</sup>	0.893 <sup>3</sup>	0.670 <sup>3</sup>
0 vs. 3 (n=10)	0.0059 <sup>2</sup>	0.0117 <sup>2</sup>	0.0137 <sup>2</sup>	0.625 <sup>3</sup>
1 vs. 2 (n=14)	0.197 <sup>3</sup>	0.122 <sup>3</sup>	0.0603 <sup>3</sup>	0.594 <sup>3</sup>
1 vs. 3 (n=13)	0.0681 <sup>3</sup>	0.0178 <sup>2</sup>	0.787 <sup>3</sup>	0.893 <sup>3</sup>
2 vs. 3 (n=24)	0.327 <sup>3</sup>	0.134 <sup>3</sup>	0.972 <sup>3</sup>	0.928 <sup>3</sup>

<sup>1</sup> Visit 0 = pre-vaccination; visit 1 = day 29 (group1), 57 (group 2) or 85 (group

3) post-dose 1; visit 2 = 14 days post-dose 2; visit 3 = 180 days post-dose 2.

<sup>2</sup> Wilcoxon signed-rank test. Significance was defined as P < 0.05.

<sup>3</sup> ns, non-significant.



### **Supplementary Figure 1:**

### Flow cytometry gating strategy for ex vivo NK cell phenotype analysis.

Plots show single, live, CD56<sup>+</sup>CD3<sup>-</sup> NK cells within whole human PBMC, and further gating into CD56<sup>bright</sup> and CD56<sup>dim</sup>CD57<sup>-/+</sup> subsets (**a**). Gating of CD56<sup>dim</sup> and CD56<sup>bight</sup> NK cell subsets, CD25<sup>+</sup> and Ki67<sup>+</sup> NK cells pre-vaccination (day 1, baseline) and day 180 post-dose 2 (visit 3) (**b**). Gating of NKG2C<sup>+</sup> and Fc $\epsilon$ R1 $\gamma$ <sup>-</sup> NK cells after gating on the CD56<sup>dim</sup> subset (**c**) at day 1, plots show one representative donor.



## Supplementary Figure 2: Analysis of NK cell *ex vivo* phenotypic data across vaccination visits.

Frequencies of CD56<sup>bright</sup> (**a**), CD56<sup>dim</sup> (**b**), CD25 (**c**) and Ki67 (**d**) expressing NK cells and of (e) CD57<sup>-</sup> (f) NKG2C<sup>+</sup> (g) Fc $\epsilon$ R1 $\gamma$ <sup>-</sup> (h) CD57/NKG2C subsets and (i) CD57/Fc $\epsilon$ R1 $\gamma$  subsets within CD56<sup>dim</sup> NK cells before vaccination (visit 0), on day 29, 57 or 85 post-dose 1 (visit 1), on day 14 post-dose 2 (visit 2) and on day 180 post-dose 2 (visit 3) (see Table 1 for n). Data points are shown with bars representing median values. Comparisons across visits were performed one-way ANOVA mixed effects analysis with Geisser-Greenhouse correction. \* p<0.05.



Supplementary Figure 3: Analysis of NK cell *ex vivo* phenotypic data between vaccine study group.

Frequencies of CD56<sup>bright</sup> (**a**), CD56<sup>dim</sup> (**b**), CD25 (**c**) and Ki67 (**d**) positive NK cells at each visit according to vaccine study group (groups 1, 2 and 3 received Ad26.ZEBOV on day 1 and MVA-BN-Filo on day 29, 57 or 85 respectively). Data points are shown with bars representing median values. Comparisons across visits were performed one-way ANOVA mixed effects analysis with Geisser-Greenhouse correction. \* p<0.05.



### **Supplementary Figure 4:**

### Flow cytometry gating strategy for *in vitro* NK cell activation analysis.

Plots show single, live, CD56<sup>+</sup>CD3<sup>-</sup> NK cell CD107a, CD16 and IFN- $\gamma$  gated following 6 hours *in vitro* culture with plate immobilised EBOV GP and pre or post-vaccination serum (serum from visit 0 and visit 2 are shown as an example). Plots shows PBMC from one non-vaccinated donor.