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Supplementary Materials for

Ebola vaccine–induced protection in nonhuman primates correlates with antibody specificity and Fc-mediated effects

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The PDF file includes:

Figs. S1 to S12
Table S1

Other Supplementary Material for this manuscript includes the following:

Data files S1 and S2

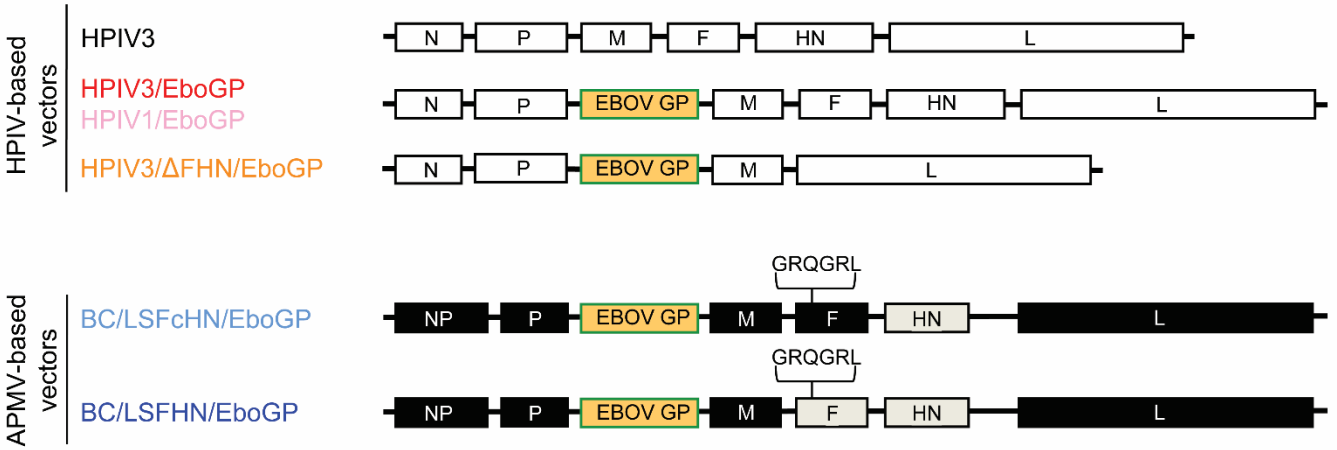

Fig. S1. Vectors utilized for the vaccine constructs. Complete maps of the paramyxovirus-vectored vaccines expressing Ebola virus (EBOV) glycoprotein (GP) transcriptional cassette inserted between the phosphoprotein (P) and matrix (M) genes and the human parainfluenza virus (HPIV) 3 vector control. The genes derived from HPIV3/HPIV1 and the Newcastle disease virus (NDV) Beaudette C (BC) strain are shown as white and black respectively. BC backbone viruses contained the fusion (F) protein cleavage sequence (GRQGRL) and the hemagglutinin-neuraminidase (HN) gene (gray) of the LaSota (LS) strain (BC/LSFcHN/EboGP) and the F and HN genes (gray) from the LS strain (BC/LSFHN/EboGP).

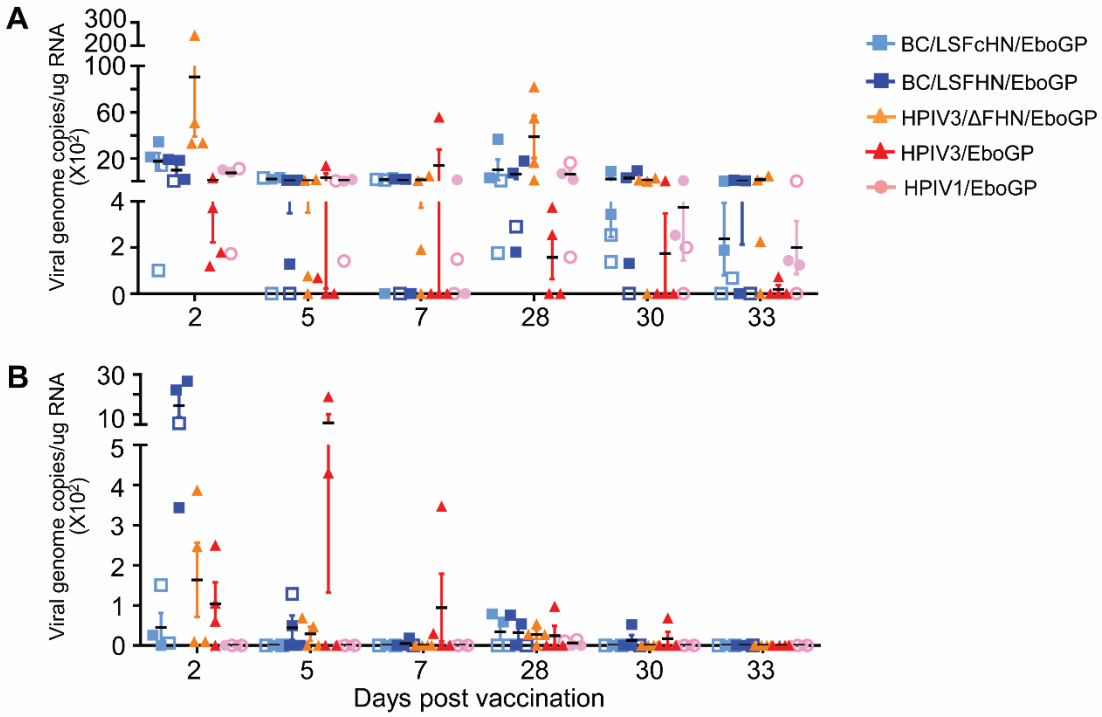

Fig. S2. Vaccine replication in the upper and lower respiratory tract. Viral genome RNA copies of candidate vaccines were measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR) in (**A**) nasal swabs and (**B**) tracheal lavages collected from vaccinated non-human primates (NHPs) at specified time points post vaccinations (n=4 per group). Open symbols indicate nonsurviving animals. Bars indicate group means ± SEM.

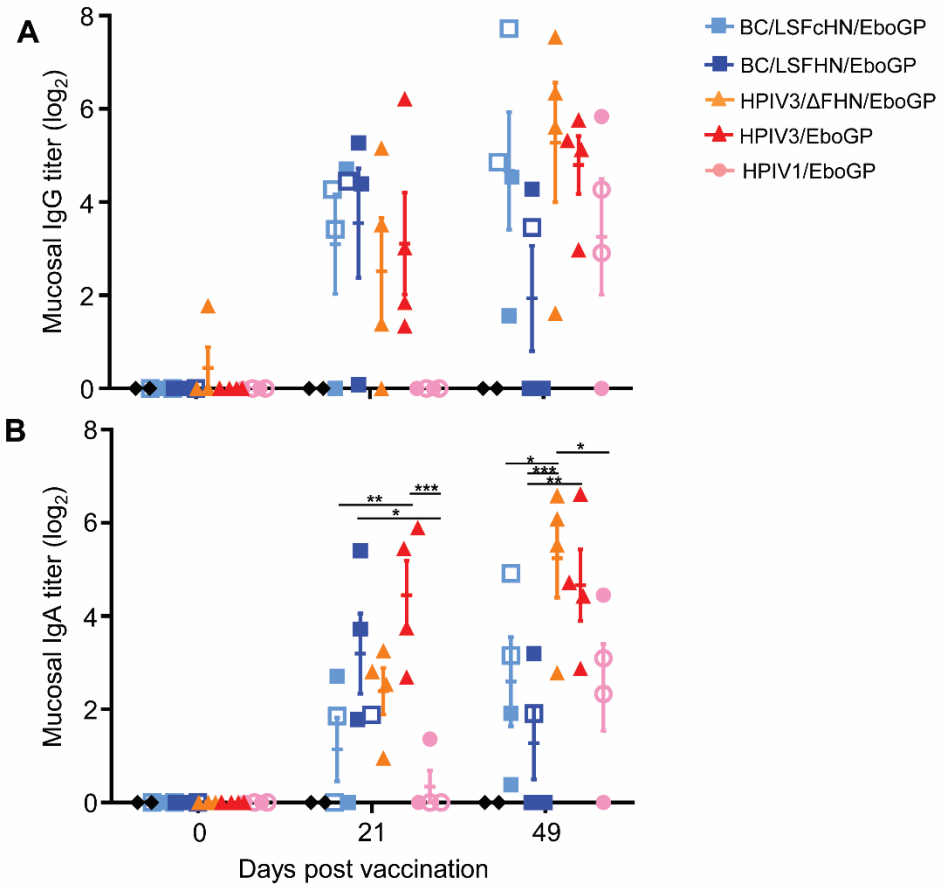

Fig. S3. GP specific mucosal IgG and IgA. GP-specific (**A**) IgG and (**B**) IgA titers were measured in bronchial lavage samples collected post vaccination by enzyme-linked immunosorbent assay (ELISA). Open symbols indicate animals that did not survive after EBOV challenge on day 55 post vaccination. Bars indicate group means ± SEM. Significance was measured by two-way analysis of variance (ANOVA) with Tukey's correction. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

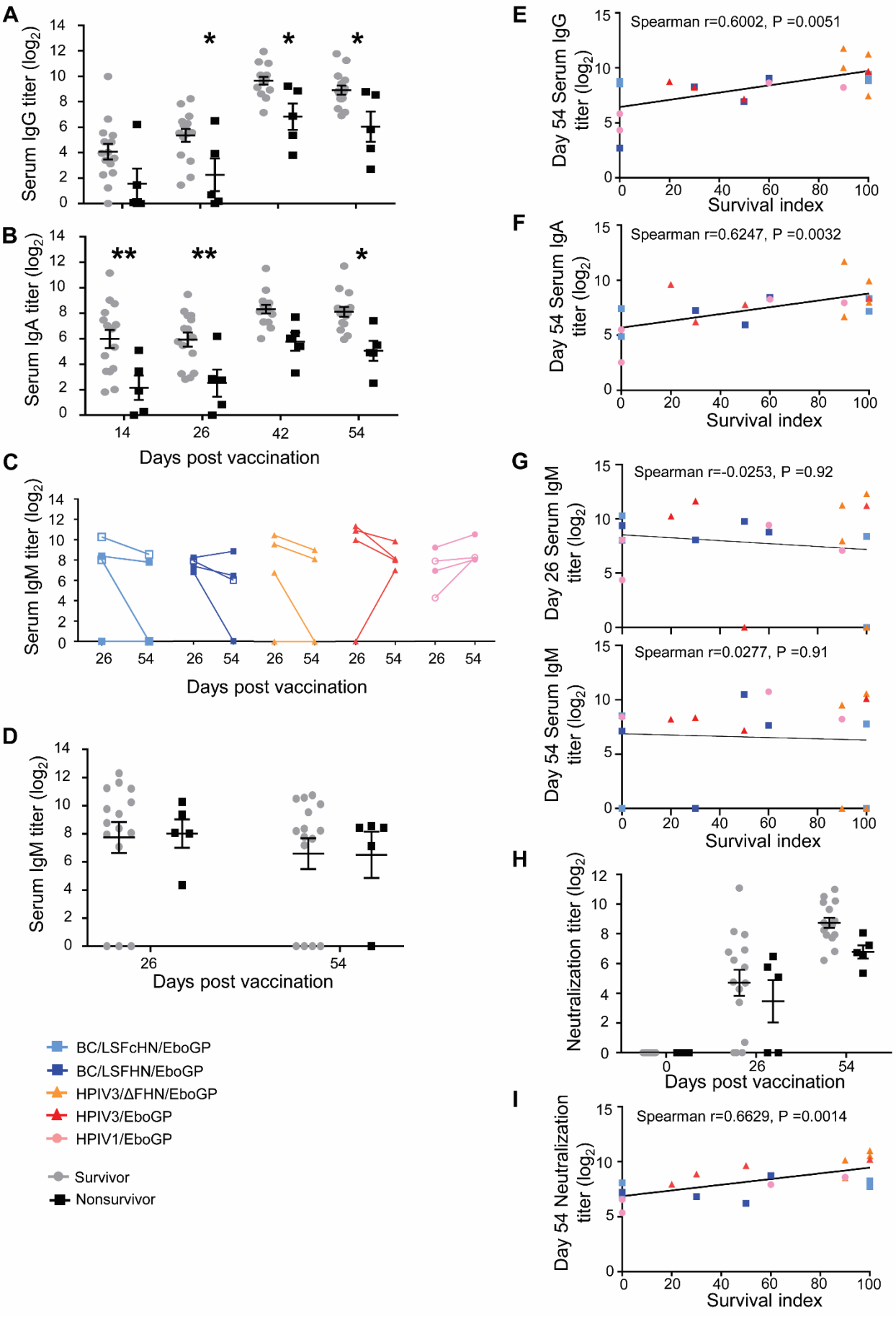
A
Serum IgG titer (log2)
B
Serum IgA titer (log2)
Days post vaccination
C
Serum IgM titer (log2)
Days post vaccination
D
Serum IgM titer (log2)
Days post vaccination
BC/LSFcHN/EboGP
BC/LSFHN/EboGP
HPIV3/ΔFHN/EboGP
HPIV3/EboGP
HPIV1/EboGP
Survivor
Nonsurvivor
E
Day 54 Serum IgG titer (log2)
Spearman r=0.6002, P =0.0051
Survival index
F
Day 54 Serum IgA titer (log2)
Spearman r=0.6247, P =0.0032
Survival index
G
Day 26 Serum IgM titer (log2)
Spearman r=-0.0253, P =0.92
Day 54 Serum IgM titer (log2)
Spearman r=0.0277, P =0.91
Survival index
H
Neutralization titer (log2)
Days post vaccination
I
Day 54 Neutralization titer (log2)
Spearman r=0.6629, P =0.0014
Survival index

Fig. S4. Serum antibody responses in vaccinated NHPs. GP-specific (**A**) IgG and (**B**) IgA antibody titers were measured by ELISA in serum of vaccinated NHP and differentiated according to NHPs that went on to be survivors (gray circles, n=15) or nonsurvivors (black squares, n=5). (**C and D**) GP-specific IgM titers in NHPs grouped according (**C**) vaccine or (**D**) survivors (gray circles) and nonsurvivors (black squares). (**E to G**) Correlations between survival index and IgG (E), IgA (F), and IgM (G) titers measured at day 26 or 54 post vaccination for all vaccinated NHPs (n=20 total, n=4 per vaccine group). Line denote linear regression fit. P and r values were determined by two-sided Spearman rank test. (**H**) EBOV neutralizing titers in serum of vaccinated NHP survivors (gray circles) and nonsurvivors (black squares). (**I**) Correlations between survival index and neutralizing titers measured at day 54 for all vaccinated NHPs (n=20, n=4 per vaccine group). Line denote linear regression fit. P and r values were determined by two-sided Spearman rank test. Significance was measured by two-way ANOVA with Sidak's correction for all comparisons between survivors and nonsurvivors (A, B, D, H) or Tukey's correction for all comparisons (C). *P ≤ 0.05 and **P ≤ 0.01.

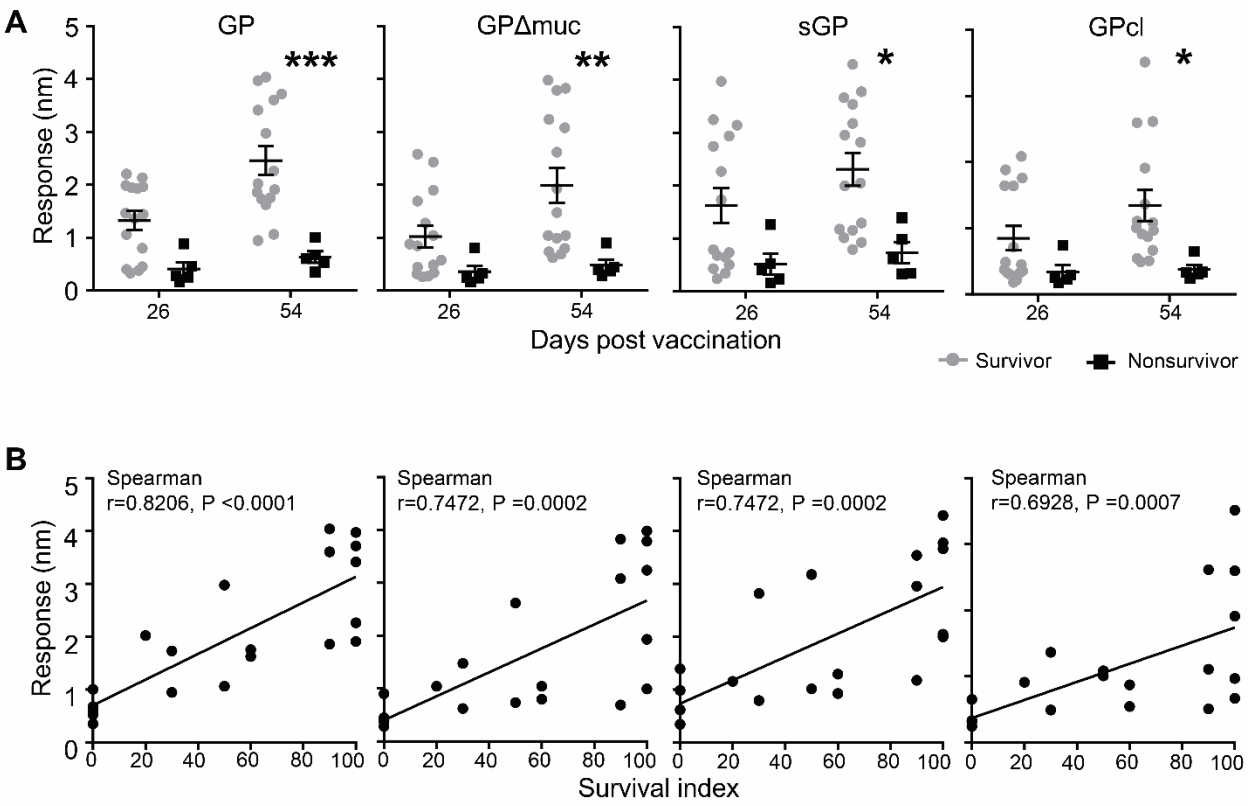

Fig. S5. Serum antibody responses in vaccinated NHPs measured by BLI. (**A**) Maximum response units of total post-vaccination sera binding to the different truncated GP forms using biolayer interferometry (BLI). NHPs were organized according to survivors (gray circles) and nonsurvivors (black squares). Lines denote group means ± SEM. Significance measured by two-way ANOVA with Sidak's correction for all comparisons between survivors and nonsurvivors. *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.001. (**B**) Correlations between the survival index and the BLI maximum response units for day 54 total sera binding to the different truncated GP forms (n=20). Line denotes linear regression fit. P and r values were determined by two-sided Spearman rank test.

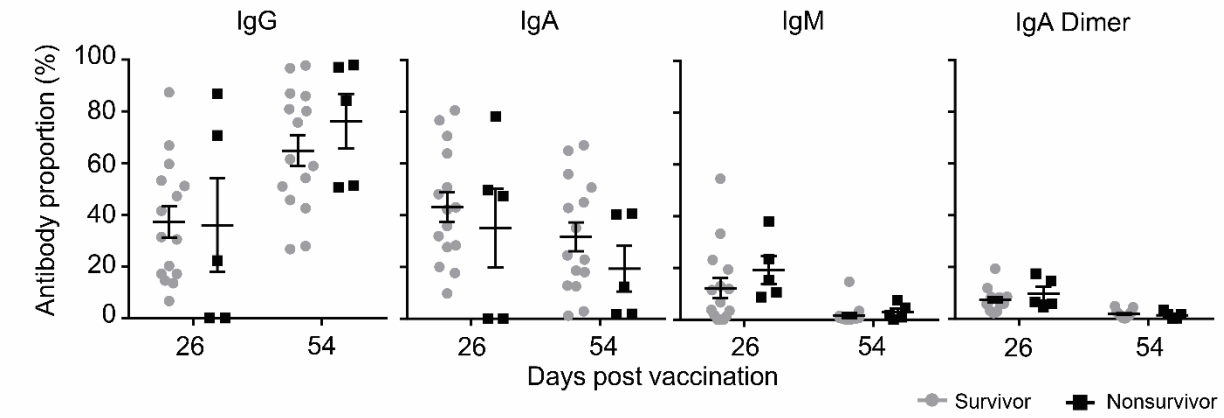

Fig. S6. Serum antibody isotype frequencies in vaccinated surviving and nonsurviving NHPs. NHPs were organized according to survivors (gray circles) and nonsurvivors (black squares). Lines denote group means ± SEM. Significance was measured by two-way ANOVA with Sidak's correction for multiple comparisons. No significant differences in antibody isotype frequencies were observed between survivors and nonsurvivors.

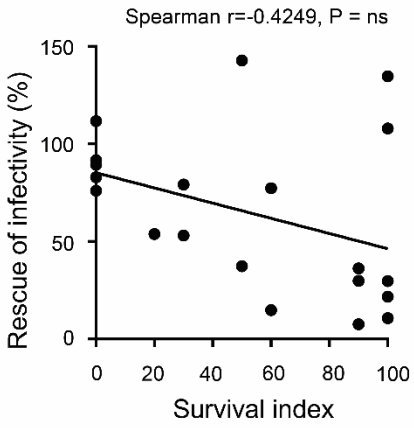

Fig. S7. Correlation between rescue of infectivity in the presence of GPΔmuc and the survival index for each vaccinated NHP. Correlations between the rescue of infectivity levels achieved following preadsorption of day 54 serum from vaccinated NHPs (n=20) with 2 µg GPΔmuc and the survival index, represented by a linear regression with a two-sided spearman correlation coefficient r and probability P. ns, not significant.

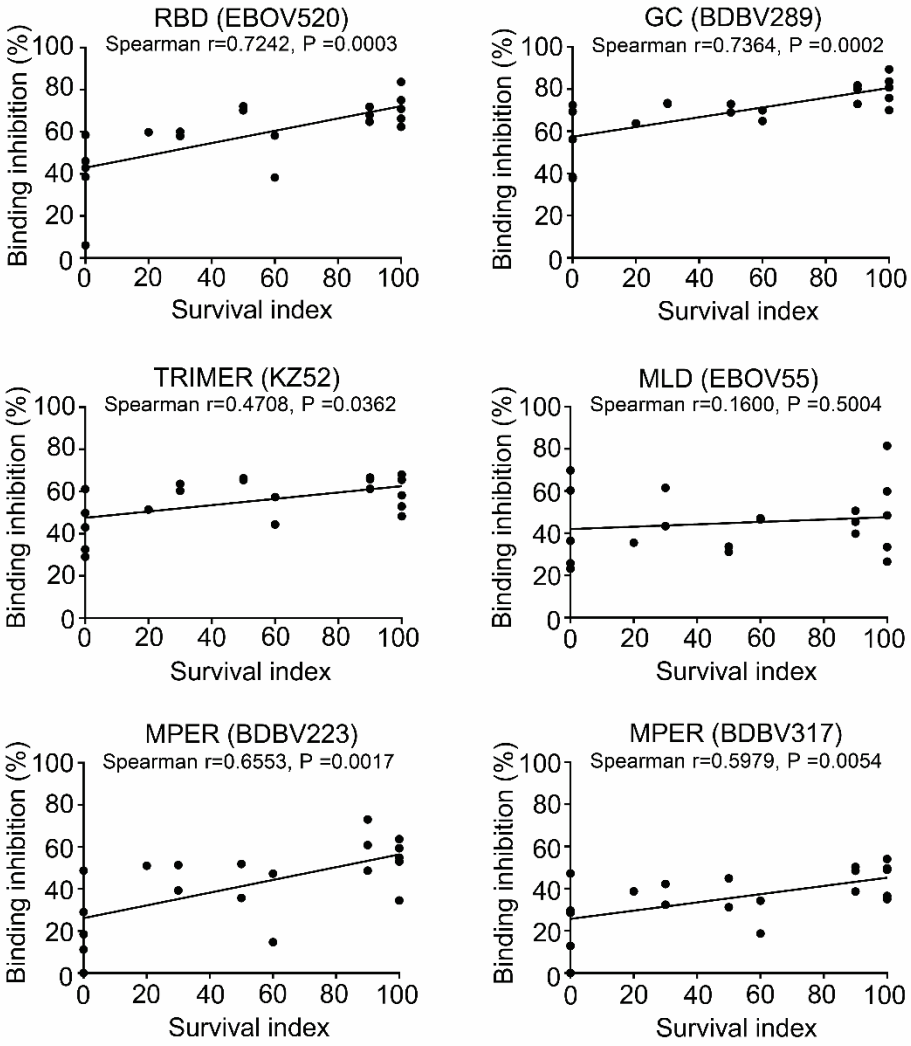

Fig. S8. Correlations between NHP survival index and serum competition for known mAb epitopes. Correlations between serum binding inhibition for each indicated epitope was measured at day 54 and correlated with survival index for all vaccinated NHPs (n=20), represented by a linear regression with a two-sided spearman correlation coefficient r and probability P.

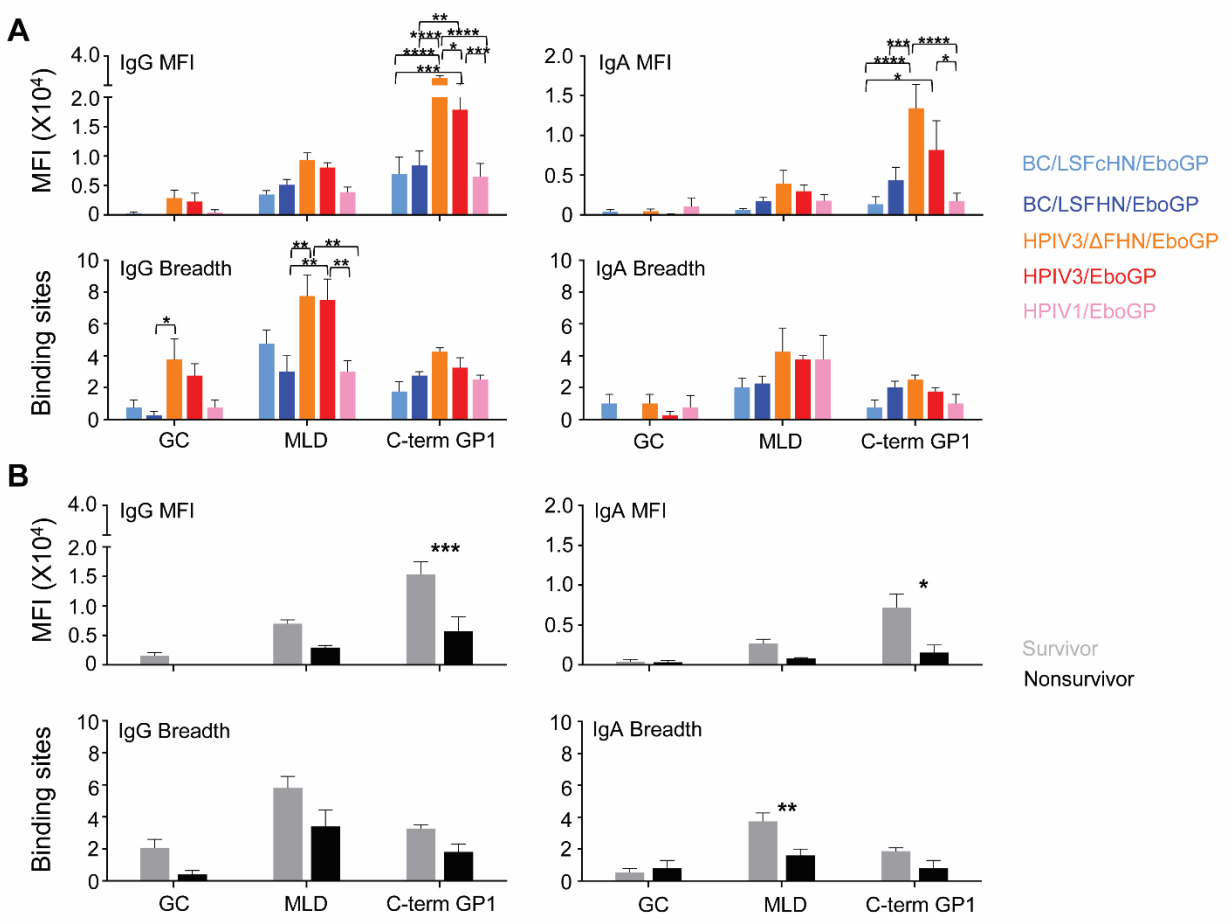

Fig. S9. Magnitude and breadth of GP specific IgG and IgA antibody binding to GP regions measured by peptide microarray. Antibody responses for (**A**) vaccinated groups or (**B**) survivors (gray) versus nonsurvivors (black) are plotted by GP regions, glycan cap (GC), MLD and C-terminus of GP1. Bar represent the mean fluorescence intensity (MFI) of positive peptides (magnitude) and the number of binding sites (breadth) ± SEM. Significance measured by two-way ANOVA with (A) Tukey's or (B) Sidak's post-hoc tests. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 and ****P ≤ 0.0001.

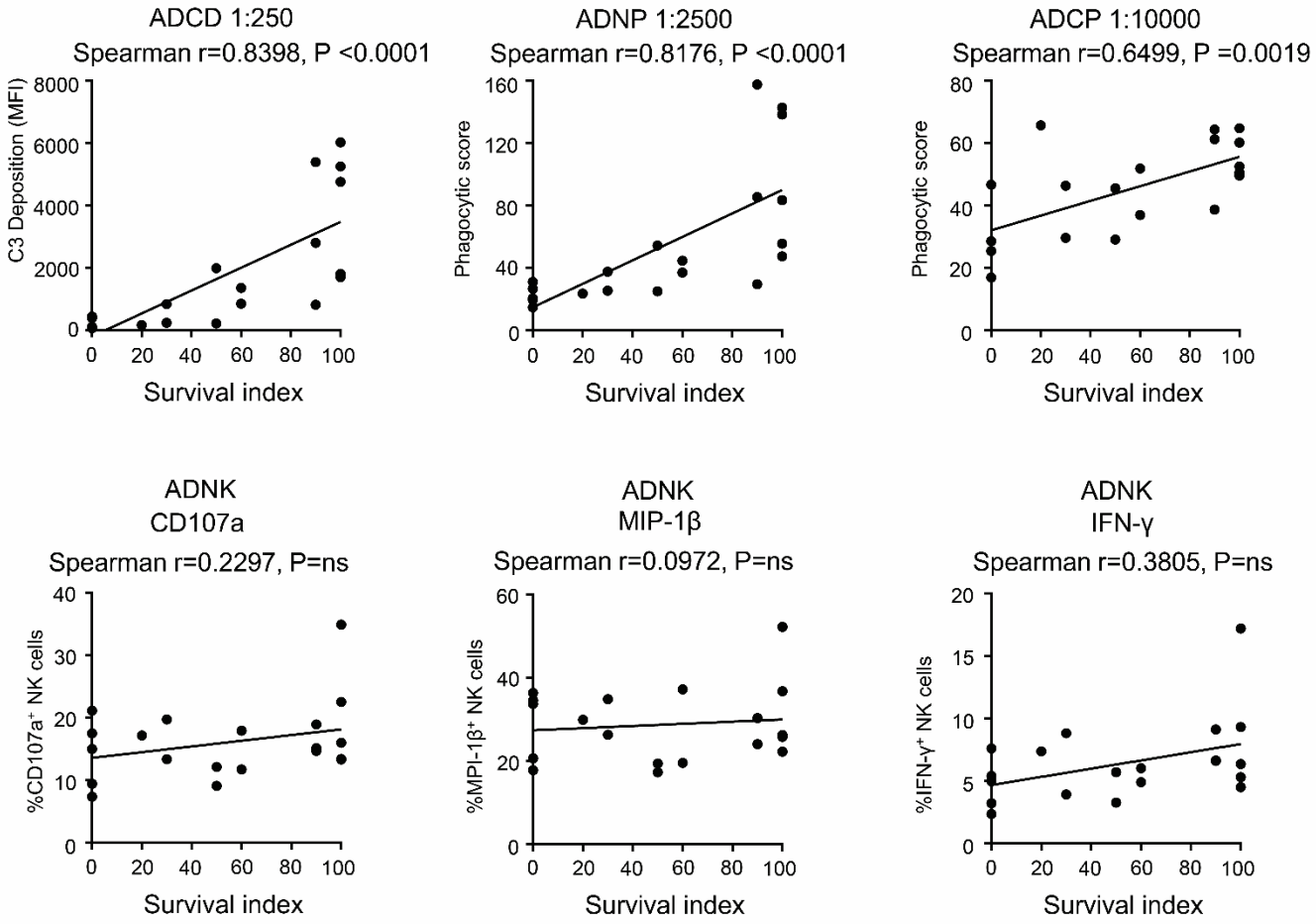

Fig. S10. Correlations between NHP survival index and Fc effector functions. Correlations between the survival index and day 54 serum-mediated Fc effector functions (ADCD, ADNP, ADCP and NK cells activity (CD107a, MIP-1β and IFN-γ production) of all vaccinated NHPs (n=20) represented by a linear regression with a two-sided spearman correlation coefficient r and probability P. ns, not significant. ADCD, antibody-dependent complement deposition; ADNP, antibody-dependent cellular phagocytosis mediated by neutrophils; ADCP, antibody-dependent cellular phagocytosis mediated by monocytes; NK, natural killer; MIP, macrophage inflammatory protein; IFN, interferon.

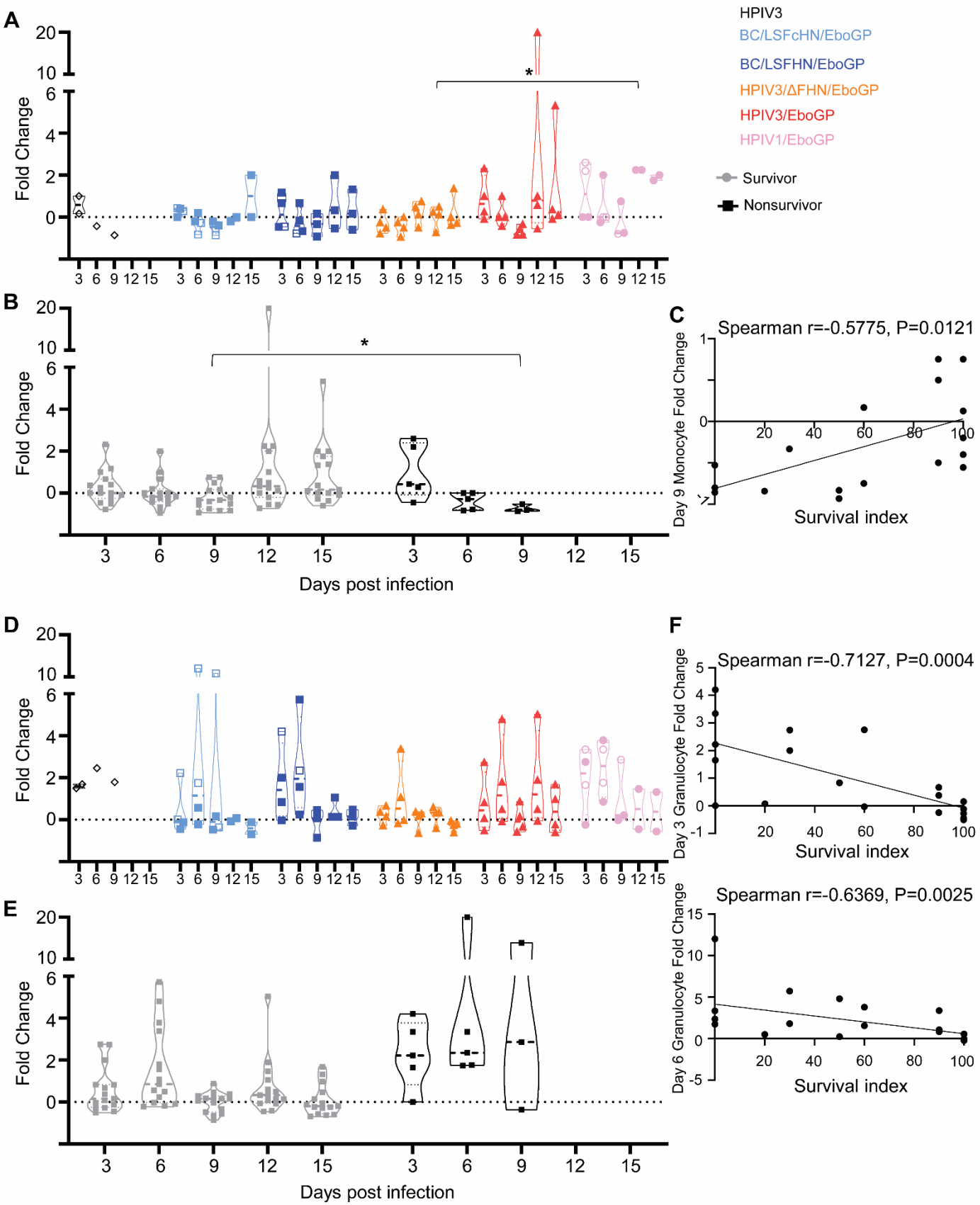
A
B
C
D
E
F
Fold Change
Days post infection
HPIV3
BC/LSFcHN/EboGP
BC/LSFHN/EboGP
HPIV3/ΔFHN/EboGP
HPIV3/EboGP
HPIV1/EboGP
Survivor
Nonsurvivor
Spearman r=-0.5775, P=0.0121
Day 9 Monocyte Fold Change
Survival index
Spearman r=-0.7127, P=0.0004
Day 3 Granulocyte Fold Change
Survival index
Spearman r=-0.6369, P=0.0025
Day 6 Granulocyte Fold Change
Survival index

Fig. S11. Circulating monocytes and granulocytes frequencies during infection. (**A to C**) monocytes and (**D to F**) granulocytes frequencies were evaluated over the course of infection. (**A, D**) numbers in blood collected periodically from vaccine groups during infection, displayed as the fold change from baseline numbers measured just prior to infection. Open symbols indicate nonsurviving animals. (**B, E**) fold change in monocyte and granulocyte numbers in vaccinated NHPs grouped according to survivors (gray circles) and nonsurvivors (black squares). Significance measured by two-way ANOVA with (A, D) Tukey's posthoc test for multiple comparisons between vaccine groups or (B, E) Sidak's post-hoc test for multiple comparisons between survivors and nonsurvivors, *P ≤ 0.05. Correlations between fold changes in the frequency of (**C**) monocytes (n=18) or (**F**) granulocytes (n=20) at specified days post infection was correlated with the survival index for all vaccinated NHPs, represented by a linear regression with a two-sided spearman correlation coefficient r and probability P.

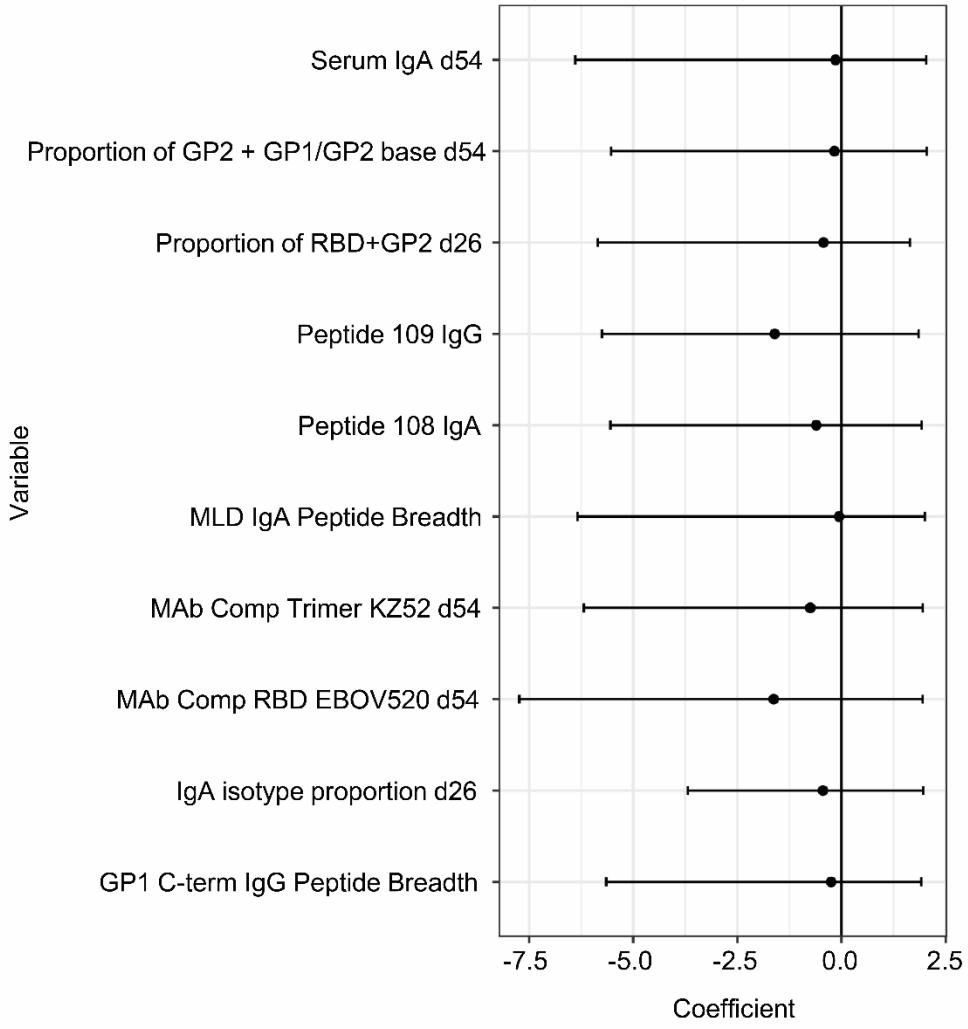

Fig. S12. Cox regression coefficients using measured features of the humoral response to model vaccine-mediated survival. A variable with a positive coefficient indicates it is associated with nonsurvival and a negative coefficient indicates a protective effect by the variable with which it is associated. RBD, receptor binding domain; MAb Comp, monoclonal antibody competition; GP1 C-term, C-terminus of GP1.

Table S1. Survival index scores.

	Clinical and disease parameters					
Vaccine group	Clinical score [a]	Liver function score [b]	Kidney function score [b]	Viremia plaque assay score [c]	Viremia qRT-PCR score [d]	**Survival index [e]**
BC/LSFcHN/EboGP	0	0	0	0	0	**100**
BC/LSFcHN/EboGP	0	0	0	0	0	**100**
BC/LSFcHN/EboGP	20	20	20	20	20	**0**
BC/LSFcHN/EboGP	20	20	20	20	20	**0**
BC/LSFHN/EboGP	10	20	20	0	20	**30**
BC/LSFHN/EboGP	10	20	0	0	20	**50**
BC/LSFHN/EboGP	20	20	0	20	20	**20**
BC/LSFHN/EboGP	0	20	0	0	20	**60**
HPIV3/ΔFHN/EboGP	0	0	0	0	0	**100**
HPIV3/ΔFHN/EboGP	0	0	0	0	10	**90**
HPIV3/ΔFHN/EboGP	0	0	0	0	10	**90**
HPIV3/ΔFHN/EboGP	0	0	0	0	0	**100**
HPIV3/EboGP	0	0	0	0	0	**100**
HPIV3/EboGP	10	20	0	0	20	**50**
HPIV3/EboGP	10	20	20	0	20	**30**
HPIV3/EboGP	20	20	20	0	20	**20**
HPIV1/EboGP	0	20	0	0	20	**60**
HPIV1/EboGP	20	20	20	20	20	**0**
HPIV1/EboGP	20	20	20	20	20	**0**
HPIV1/EboGP	0	0	0	0	10	**90**
HPIV3	20	20	20	20	20	**0**
HPIV3	20	20	20	20	20	**0**

[a] Clinical score of 2 = 0; ≤5 = 10; >5 = 20
[b] Normal function = 0; abnormal function = 20
[c] not detected = 0; detected = 20
[d] not detected = 0; detected on 1 day = 10; detected > 1 day = 20)
[e] Survival index = 100 - total score