

Identification of a unique humoral immune signature of Sudan ebolavirus persistence in human survivors

Bronwyn Gunn

bronwyn.gunn@wsu.edu

Washington State University <https://orcid.org/0000-0001-8904-9334>

Shuangyi Bai

Washington State University School of Molecular Biosciences <https://orcid.org/0000-0001-9854-2016>

Haruna Muwonge

Makerere University Medical School

Carolyn Nasimiyu

Washington State University Global Health Kenya

Barnabas Bakamutumaho

Uganda Virus Research Institute

Cynthia Ombok

Washington State University Global Health-Kenya

Sinem Ulasan

Washington State University

Peter Elyanu

Baylor-Uganda

Moses Joloba

Makerere University Medical School

Robert Breiman

Emory University

Isaac Ssewanyana

Susan Nabadda

Uganda Central Public Health Laboratories

Julius Lutwama

Uganda Virus Research Institute

Misaki Wayengera

Makerere University

Bruce Kirenga

Makerere University Medical School

Henry Bosa

Ministry of Health Uganda

M. Njenga

Mkariuki.njenga@wsu.edu

Article

Keywords:

Posted Date: May 26th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9601480/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: There is **NO** Competing Interest.

1 **Identification of a unique humoral immune signature of Sudan ebolavirus persistence in**
2 **human survivors.**

3

4 Shuangyi Bai¹, Haruna Muwonge², Carolyn Nasimiya³, Barnabas Bakamutumaho⁴, Cynthia
5 Ombok³, Sinem Ulasan¹, Peter James Elyanu⁵, Moses L. Joloba², Robert F. Breiman⁶, Isaac
6 Ssewanyana⁷, Susan Nabadda⁷, Julius Lutwama⁴, Misaki Wayengera², Bruce Kirenga², Henry
7 Kyobe Bosa⁸, M. Kariuki Njenga^{1,3} and Bronwyn M. Gunn¹.

8

9 ¹Paul G. Allen School for Global Health, Washington State University. Pullman, WA, USA.

10 ²Makerere University Medical School. Kampala, Uganda.

11 ³Washington State University Global Health-Kenya. Nairobi, Kenya.

12 ⁴Interdisciplinary Consortium on Epidemic Research/ Uganda Virus Research Institute. Entebbe,
13 Uganda.

14 ⁵Baylor-Uganda. Kampala, Uganda

15 ⁶ Rollins School of Public Health, Emory University. Atlanta, GA, USA

16 ⁷Uganda Central Public Health Laboratories. Kampala, Uganda.

17 ⁸Ministry of Health Uganda. Kampala, Uganda.

18

19 **ABSTRACT**

20 Since finding that long-term persistence of ebolavirus RNA exists in a subset of human
21 survivors for up to 5 years post infection in several organs, viral reactivation has been implicated
22 in recurrence of acute disease among ebolavirus disease survivors and linked to small disease
23 outbreaks. Thus, identifying correlates of ebolavirus persistence are critical to the long-term care
24 of survivors and outbreak management. We analyzed the humoral immune response using a
25 comprehensive systems serology approach in 86 the 87 survivors (98.8%) of the 2022-2023
26 Sudan ebolavirus (SUDV) outbreak in Uganda. Across the survivors, 55% of eligible survivors
27 (20 of 36 survivors) were found to have persistence of viral RNA in either semen or breastmilk
28 for up to 6 months following initial infection, whereas the remaining 45% tested negative (16 of
29 36 survivors). We found an elevated, unique, and sustained humoral immune signature
30 associated with persistence of viral RNA in SUDV survivors and specifically have identified 4
31 humoral immune features that together predicted persistence: glycoprotein-specific antibody
32 dependent cellular phagocytosis [ADCP], nucleoprotein-specific IgG2, nucleoprotein-specific
33 IgA1, and VP40-specific IgM. Moreover, analysis of the 4 features in the remaining 50 SUDV
34 survivors who were ineligible for semen or breastmilk sampling, predicted an additional 17
35 survivors with humoral immune responses consistent with viral RNA persistence survivors. We
36 also find that antibodies against the VP40 (matrix protein), were associated with faster clearance
37 of persistent viral RNA. Thus, a subset of humoral immune responses could be important for
38 monitoring and clearing viral persistence in ebolavirus disease survivors.

39

40

41 INTRODUCTION

42 Orthoebolaviruses, including Zaire ebolavirus (EBOV) and Sudan ebolavirus (SUDV),
43 have caused explosive outbreaks of severe disease in multiple regions of Africa and is
44 associated with high mortality and long-term clinical sequelae for surviving individuals. Uganda
45 has experienced seven outbreaks of SUDV, with the most recent in early 2025, affecting 14
46 people with 4 deaths. A larger outbreak in 2022-2023 resulted in 142 infections with 55 deaths¹.
47 The increasing frequency of these outbreaks in the last 5 years has highlighted a persistent and
48 growing threat that orthoebolaviruses present to the region. In addition to the morbidity and
49 mortality associated with acute infection, survivors of ebolavirus disease (EVD) face a myriad of
50 long-term complications including debilitating sequelae and persistence of viral RNA in a subset
51 of survivors².

52 EBOV RNA can persist in semen, vitreous humor, breast milk, cerebrospinal fluid in EVD
53 survivors for months to years³⁻⁵. Viral RNA is estimated to persist in the semen in 90% male
54 survivors during the first 3 months in convalescence, dropping to 25% by 9 months, and is
55 estimated to be present in 1-3% of survivors at 2 years⁶. While the presence of viral RNA does
56 not necessarily prove infectious potential in a majority of survivors, virus recrudescence
57 presumably from a survivor of the 2014-2016 epidemic has been posited to be responsible for
58 the small 2021 outbreak in Guinea⁴, and transmission of the virus sexually and via breastmilk
59 have been documented^{7,8}. To date, only routine testing of semen from consenting male survivors
60 or lactating female survivors are performed, as sampling of ocular vitreous humor or cerebral
61 spinal fluid are invasive procedures and not routinely feasible. Thus, non-lactating female and
62 child survivors are rarely tested but could be at risk for persistence of viral RNA beyond the
63 convalescence period.

64 Antibodies against ebolavirus are protective against EBOV and SUDV infections in animal
65 models, providing prophylactic and therapeutic protection against challenge via both potent
66 neutralizing activity and innate immune effector functions through the antibody Fc domain⁹⁻¹¹.
67 Human efficacy trials of monoclonal antibodies led to the approval of a monotherapy as well as
68 a monoclonal antibody cocktail for treatment of acute human infection. Moreover, antibodies are
69 the correlate of protection for the WHO pre-qualified live vaccine against Ebola virus, Erevbo
70 (rVSV-ZEBOV)¹². Long-term humoral immune responses are induced in survivors of ebolavirus
71 infection¹³⁻¹⁵, and fluctuations in antibody responses observed in survivors has been posited to
72 be a reflection of recrudescence of viral antigen that stimulates a rise in ebolavirus-specific
73 antibodies¹⁶. Thus, ebolavirus-specific antibodies may be an effective biomarker of persistence
74 of viral RNA that could be measured in all survivors. However, whether a specific antibody
75 response is associated with clearance of persistent viral RNA is unknown.

76 Here, we characterize the humoral immune response of 86 survivors of the 2022 SUDV
77 outbreak in Uganda, starting at 2-3 months post infection and continued at quarterly intervals
78 through 15 months. At each follow-up, semen or breast milk from 36 eligible and consenting
79 adult survivors were tested for SUDV RNA by PCR, with 20 (55%) testing positive at least one
80 follow-up. Using a comprehensive systems serology approach that profile Fc-mediated antibody
81 effectors functions, we characterized the longitudinal humoral antibody response against SUDV
82 of survivors and a group of community controls and determined if humoral immunity was altered
83 in the context of persistent viral RNA.

84

85 **METHODS AND MATERIALS**

86 **Human serum samples.** Serum samples from 86 (98.8%) survivors of the SUDV outbreak in
87 Uganda (September-December 2022) were collected at quarterly timepoints beginning two to

88 three months after discharge from the Ebola Treatment Unit (ETU). The demographics of the
89 survivors enrolled in our study are shown in **Table 1**. In addition, 192 community controls were
90 collected in October 2023 according to approved study protocol from the School of Biomedical
91 Sciences Research Ethics Committee at Makerere University (Approval #SBS-2022-243) and
92 the Uganda National Council of Science and Technology (Approval #HS2618ES). Additional
93 administrative approvals were obtained from the Uganda Ministry of Health and participating
94 health facilities. Written informed consent was obtained from all participants prior to enrollment.
95 Serum samples were heat-inactivated at 56°C for 30 minutes prior to testing.

96 **Cell lines and reagents.** Vero E6 cells (ATCC CRL-1586) were maintained in D10 medium
97 (DMEM supplemented with 10% FBS, 1% penicillin–streptomycin and 1% L-glutamine) at 37 °C
98 in 5% CO₂. THP-1 monocytes (ATCC TIB-202) were maintained in D10 medium (DMEM
99 supplemented with 10% FBS, 1% penicillin–streptomycin, 1% L-glutamine, and 55µM beta-
100 mercaptoethanol). HL60 cells (ATCC CCL-240) were maintained in Iscove's Modified Dulbecco's
101 Medium supplemented with 10% FBS, 1% penicillin–streptomycin and 1% L-glutamine and
102 differentiated for 5 days in the presence of 1.3% DMSO. NK92 cells expressing CD16 (158V;
103 ATCC PTA-6967) were maintained in alpha-MEM (2mM L-glutamine and 1.5g/L of sodium
104 bicarbonate) supplemented with 12.5% FBS, 12.5% horse serum, 20µM folic acid, 200µM
105 inositol, 100µM beta-mercapoethanol, and 0.2mg/ml of IL-2.

106 **Multiplexed-Luminex based antibody subclassing.** GP is the major surface protein on both
107 the virion and the infected cell ¹⁷; sGP is secreted from infected cells, acting as an
108 immunomodulator and antibody decoy¹⁸; NP is highly immunogenic and plays a critical role in
109 viral replication¹⁹; and VP40 is the matrix protein that is involved in viral budding and egress from
110 infected cells ²⁰. Recombinant SUDV GP, sGP, VP40 (IBT Bioservices), and NP (Sino Biologics)
111 were coupled to MagPlex beads (Luminex) according to previously published protocols²¹.

112 Samples were diluted to 1:500 (Total IgG, IgG1, IgM) or 1:25 (IgG2, IgG3, IgG4, total IgA, IgA1,
113 and IgA2) and incubated with antigen-coupled beads for 2h. Following bead washing, antibody
114 subclasses (IgG1, IgG2, IgG3, IgG4) and isotypes (IgM, IgA1, IgA2) were detected using PE-
115 labeled secondary antibodies (0.65µg/ml; Southern Biotech). The geometric mean fluorescent
116 intensity (gMFI) of 30 beads/region was recorded and analyzed on an Intelliflex instrument
117 (Luminex).

118 **Seropositivity threshold.** To establish a seropositivity threshold, we analyzed 24 randomly
119 selected serum samples from adults (without history of being exposed to ebolavirus) from
120 neighboring Kenya (n=16) to consider potential cross-reactivity from other viruses or
121 environmental factors common in East Africa, and the United States that were collected in 2020
122 (n=8), prior to the SUDV outbreak in Uganda. To limit the potential for false positives, the
123 seropositivity threshold was conservatively defined as the mean of these 24 controls + 5X the
124 standard deviation.

125 **Neutralization assay.** Vero E6 cells were seeded at 10,000 cells per well in 384-well flat-bottom
126 microplates (Thermo Scientific, cat. # 464718) in 50 µl of D10 medium and incubated 24h. On
127 Day 2, serial three-fold dilutions of serum (starting at 1:20; eight dilutions) were prepared in D10
128 medium. Recombinant vesicular stomatitis virus pseudotyped with the SUDV Gulu glycoprotein
129 (rVSV–SUDV-Gulu-GP) and encoding a luciferase reporter was obtained from IBT Bioservices
130 (cat. # 1002-002). An equal volume of pseudovirus (160 RLU µl⁻¹) was added to each dilution
131 and incubated for 1 h at 37 °C. Cell culture medium was removed by gently flicking the plate,
132 after which 25 µl of the virus–antibody mixture was added to each well. Plates were centrifuged
133 at 1,200 × g for 1 h at 4 °C and subsequently incubated for 24 h at 37 °C in 5% CO₂. Luciferase
134 activity was measured by adding 30 µl per well of Bright-Glo luciferase substrate (Promega, cat.
135 #. E2620) and reading luminescence on a Tecan Spark luminometer (Life Sciences). Percent

136 neutralization was calculated by normalizing pseudovirus-only wells to 0% neutralization and
137 cell-only wells to 100% neutralization, as described previously²². Neutralization curves were
138 fitted using nonlinear regression (“log(inhibitor) vs. normalized response—variable slope”) in
139 GraphPad Prism v10 to derive 50% inhibitory dilution (ID₅₀) values. Each assay plate included
140 a neutralizing monoclonal antibody as a positive control and either COVID-19–positive sera from
141 a US-based cohort or assay medium as a negative control.

142 **Antibody-dependent phagocytosis (ADNP and ADCP).** Biotinylated SUDV GP or VP40 was
143 conjugated to red Neutravidin beads (ThermoFisher) and incubated with samples for 2h prior to
144 adding HL-60 cells differentiated with 1.3% DMSO for 5 days blood (5x10⁴ cells/well) for 2h
145 (ADNP) or adding THP-1 cells at 2.5x10⁴ cells/well for 18h (ADCP). Uptake of antibody-bead
146 complexes by cells was determined by analysis on a Cytex Aurora spectral flow cytometer, and
147 a phagocytic score was determined: (% bead positive⁺ cells)*(gMFI red beads⁺)/10,000. The
148 gating strategy is shown in **Figure S1**.

149 **Antibody-mediated complement deposition (ADCD).** Biotinylated SUDV GP-coated red
150 Neutravidin beads (ThermoFisher) were incubated with heat-inactivated samples. Guinea pig
151 complement (Cedarlane Labs) diluted in veronal buffer containing calcium and magnesium
152 (Boston Bioproducts) was incubated with antibody-bead complexes for 20min, and C3
153 deposition onto beads was detected using an anti-guinea pig C3 antibody (MP Biomedicals) and
154 analyzed on a Cytex Aurora spectral flow cytometer. The gating strategy is shown in **Figure S1**.

155 **Antibody-dependent NK cell degranulation.** ELISA plates were coated with SUDV GP-
156 antigen (300ng/well). Wells were washed, blocked, and incubated with samples for 2h prior to
157 adding NK-92 cells expressing CD16 (5x10⁴ cells/well) for 5h with brefeldinA, GolgiStop, and
158 anti-CD107a (Biolegend). Intracellular cytokine staining to detect IFN γ and TNF α (Biolegend)

159 was performed using Fix/Perm (Biolegend), and cells were analyzed on a Cytex Aurora spectral
160 flow cytometer. The gating strategy is shown in **Figure S1**.

161 **Discriminant analysis.** A regularized discriminant analysis was performed using a stepwise
162 variable selection method using antibody measures at each timepoint (eg 2-6 months post-
163 infection) to classify survivors with and without persistent viral RNA in JMP Pro 16 (SAS).

164 **Calculation of the combined humoral immune score.** A combined score based on selected
165 features was calculated using the following formula: $((\text{Feature 1 value}) * (\text{Feature 2}$
166 $\text{value}) * (\text{Feature 3 value}) * (\text{Feature 4 value}))^{(1/4)}$.

167 **ROC analysis.** Combined humoral immune scores for survivors with and without persistent virus
168 was used to generate an ROC using Prism 10 software. The Youden's index was calculated
169 using the following formula: $(\text{sensitivity} + \text{specificity}) - 1$.

170 **Statistical analysis.** Univariate analyses were performed using Prism 10 software. Kruskal-
171 Wallis with Dunn's multiple correction test or Mann-Whitney with Bonferroni correction were used
172 to determine statistical significance between groups, and paired Wilcoxon-rank was used to
173 compare changes in immunity between timepoints in the same individual.

174

175 **RESULTS**

176 **Antibody profile of SUDV survivors 2-15 months post-infection.**

177 To characterize the humoral immune responses in SUDV survivors, Uganda community
178 controls, and confirmed ebolavirus seronegative controls from neighboring Kenya and the US,
179 we applied a systems serology approach to determine the quantity and quality of SUDV-specific
180 antibodies, including measurement of IgG, IgA, IgM, and IgG and IgA subclasses against four
181 different SUDV antigens: glycoprotein (GP), soluble glycoprotein (sGP), nucleoprotein (NP) and
182 viral protein 40 (VP40). Across the survivors, 81 (93%) were IgG seropositive against GP at

183 enrollment, which declined to 72% by 12-15 months post-infection (**Figure 1A**). This temporal
184 pattern was similar for IgG1, IgG2, and IgG3 against all the four viral antigens (**Figure S2**). In
185 contrast to the decline in IgG, levels of IgA antibodies against GP, sGP, and NP remained
186 relatively steady between enrollment and 12-15 months post-infection (**Figure 1B, Figure S4**).
187 Strikingly, we did not observe VP40-specific IgA responses, (**Figure S4**), highlighting antibody
188 profile differences against SUDV antigens. Most survivors were IgM seronegative against GP,
189 with only 15% of survivors having detectable IgM responses at enrollment (**Figure 1C**),
190 consistent with reduced detection of IgM after 12 weeks post-infection. As expected, none of the
191 seronegative controls had IgM or IgG antibodies detected. However, approximately 13-15% of
192 the Uganda community controls had detectable SUDV-specific IgG, IgA, or IgM. As samples
193 from the community controls were collected in October 2023, these responses may reflect
194 exposure to SUDV during the outbreak or asymptomatic infection.

195 As qualitative features of GP-specific antibodies are critical to protection against
196 EBOV^{9,10,23}, we next measured antibody-mediated effector function of SUDV GP-specific
197 antibodies including neutralization of a VSV-ΔG SUDV GP pseudovirus, and induction of Fc-
198 mediated phagocytosis by monocytes (ADCP), neutrophils (ADNP), activation of NK cells
199 (ADNKA), and deposition of the central complement component C3 (ADCD). Strong neutralizing
200 activity of the VSV-ΔG SUDV GP pseudovirus, defined as ID₅₀ greater than 1:200, was observed
201 in >80% of survivors 2-3 months after infection, with 45% of survivors having an ID₅₀ >1:1000.
202 At 12-15 months post-infection, 46% of tested survivors (n=41) maintained an anti-GP ID₅₀ titers
203 >1:200 (**Figure 1D**). The ability of GP-specific antibodies to activate complement (**Figure 1E**)
204 and induce monocyte-mediated phagocytosis (**Figure 1F**) was observed through 5-8 months
205 post infection but declined by 12-15 months. Similar results were observed for GP-specific
206 activation of NK cells (**Figure 1G, Figure S5**), and induction of neutrophil phagocytosis (**Figure**

207 **S5).** We found that levels of GP-specific total IgG, IgG1, IgG3, total IgA, IgA1, and IgA2
208 significantly correlated with all antibody functions across the timepoints measured (**Figure 1H**),
209 indicating a coordinated humoral immune response in SUDV survivors.

210

211 **Higher viral load in acute disease associated with viral RNA persistence among survivors.**

212 Sustained detection of SUDV-specific RNA in semen or breastmilk has previously been
213 reported in a subset of survivors from the 2022 SUDV outbreak in Uganda for 3 to 7 months
214 post-infection²⁴. Of the 86 SUDV survivors, 42 were eligible for semen or breastmilk testing for
215 viral RNA persistence. Of those eligible, 36 consented and 20 (55.6%) of them tested positive
216 for SUDV RNA persistence. The number of days from admission to the Ebola Treatment Unit
217 (ETU) to the last positive PCR result after infection was calculated for each survivor and was
218 used as a metric of duration of viral RNA persistence. These survivors remained SUDV RNA
219 positive for a median of 131.5 days (range: 84 to 210 days, IQR = 83.3).

220 We investigated whether parameters of acute illness were associated with viral RNA
221 persistence, including disease severity at viral load at time of admission into the ETU. Acute
222 illness severity was classified by the non-mutually exclusive categorization as follows: Mild
223 (fever, vomiting, diarrhea, loss of appetite, abdominal pain); moderate (chest pain, muscle pain,
224 joint pain, headache, cough, sore throat); and severe (jaundice, red eye, coma, confusion,
225 bleeding). Across the 86 survivors analyzed here, 45 (52.3%) were classified as having had
226 severe illness, 39 (45.3%) were classified as having moderate illness, and 2 (2.3%) had mild
227 illness. Survivors who were tested for the presence of persistent SUDV RNA experienced either
228 severe or moderate disease during acute infection (**Table 2**), yet there was no statistically
229 significant differences in severity of disease between those with and without viral RNA
230 persistence ($p=0.3$). However, survivors with persistent viral RNA spent longer within the ETU

than survivors without persistent viral RNA (**Table 2**). The length of ETU stay was elevated in the persistent RNA survivors irrespective of acute disease severity (i.e. moderate or severe disease severity) compared to survivors without persistent viral RNA, suggesting that disease symptom severity alone is insufficient to explain increased ETU stay duration.

To investigate impact of viral load at ETU admission on survival and viral RNA persistence, we correlated admission Ct values with survival and persistence of viral RNA in semen or breast milk. Overall, survivors had higher Ct values (lower viral load) than fatal cases, and survivors with persistent SUDV RNA had lower Ct values at admission when compared to those negative for virus persistence (**Figure 2A**). These findings indicate that higher viral load at admission increased the likelihood of viral RNA persistence among survivors. However, the range of Ct values amongst those with persistent SUDV RNA varied widely (Ct values 17.21-36), perhaps reflecting differences in acute disease stage at admission. Importantly, we did not observe a correlation between admission Ct value and duration of viral RNA persistence in convalescence (**Figure 2B**).

Several humoral immune features are elevated in survivors with persistent SUDV RNA.

We performed a multiple Mann-Whitney comparison of SUDV-specific antibody levels at the enrollment timepoint (2-3 months post-infection) between survivors with and without persistent SUDV RNA (**Figure 2C, Figure S6**). The data are summarized as a volcano plot, where the adjusted q-value (after correction for multiple comparisons) for each measure is shown on the Y axis, and values that exceed $-\log_{10} q \text{ value} \geq 2$ ($q \text{ value} \leq 0.01$) were considered statistically significant. The mean difference in rank of survivors with persistent RNA vs survivors without persistent RNA is shown on the X-axis, where positive mean rank differences indicate

254 that the respective antibody response is elevated in those with RNA detected compared to those
255 without (**Figure 2C**).

256 Of the 44 antibody features included in the analysis, 28 measures were significantly
257 elevated in survivors with persistent viral RNA, indicating a significantly altered humoral immune
258 response against SUDV antigens in the context of viral RNA persistence. These measures
259 spanned all viral antigens tested, and included all IgG and IgA subclasses, except for IgG4
260 (**Table 3**). A subset of antibody responses continued to be elevated in survivors with persistent
261 RNA compared to those without, across multiple follow-up visits spanning 1-year post-infection
262 (**Table 3**). Notably, IgA (including both IgA1 and IgA2) responses against GP, sGP, and NP
263 remained elevated through to 15 months post-infection in survivors with persistent RNA.
264 Univariate plots of the top features that were elevated in survivors with persistence (GP-specific
265 total IgG [**Figure 2D**], NP-specific total IgG [**Figure 2E**], sGP-specific total IgA [**Figure 2F**], and
266 ADCP activity [**Figure 2G**]) are shown over time.

267

268 **Distinct humoral profiles can predict RNA persistence.**

269 We used these data to define the minimum number of features needed to predict viral
270 RNA persistence at enrollment using a discriminant analysis with variable selection (**Figure 3A**).
271 Subsequently, we generated a composite humoral immune score for each timepoint based on
272 the features selected to establish a cutoff above which survivors were categorized as the
273 “persistence” category and below would fall into the “no persistence” category (**Figure 3B**). At
274 the enrollment time point (2-3 months post-infection), we identified 4 immune features (GP-
275 specific antibody dependent cellular phagocytosis [ADCP], NP-specific IgG2, NP-specific IgA1,
276 and VP40-specific IgM) that produced a ROC curve with an AUC of 0.9938. We then calculated
277 the Youden’s index from the ROC curve and selected maximum sensitivity (100%) with the

highest Youden's index to determine a threshold humoral immune score that could be applied on enrolled survivors to predict prolonged viral RNA persistence. Using this approach, we estimated that 17 of the other 50 untested survivors had immune features consistent with survivors with persistent RNA (**Figure 3C**).

We further assessed the value of the composite immune score at the follow-up time points to classify persistence. The ROC curve AUC for responses measured at 5 months post-infection was 0.9868, 8 months post-infection was 0.9633, and 12 months post-infection was 0.958 (**Figure 3D**). Across these time points, 6 of the 17 untested survivors had the immune feature signature consistent with persistence across all 4 timepoints above the Youden's index with the maximum specificity (100%) to ensure no false positives (**Figure 3E**).

Level of IgG against VP40 is associated with virus clearance.

To determine if any antibody feature was associated with long term viral RNA persistence, we used the number of days from ETU admission positive PCR to last positive PCR result after infection for each patient to perform spearman correlation analysis with each humoral immune feature. Across the SUDV-specific measures collected at enrollment, we found that total IgG and IgG1 levels against VP40 were negatively correlated with days of persistent shedding (**Figure 4A, Figure S7**), indicating that higher VP40-specific IgG1 levels tracked with accelerated clearance of viral RNA from semen/breast milk. To determine if VP40-specific antibodies could mediate innate immune effector functions, we measured ADCP, ADNP, ADNKA, and ADCD against VP40-coated targets, and then determined if a specific effector response tracked with accelerated clearance of viral RNA. We found that ADCP responses were negatively associated with duration of viral RNA persistence, while ADCD and ADNP had a similar trend but not statistically significant (**Figure 4B**). Interestingly, while ADCP activity was positively correlated

302 with VP40-specific total IgG or IgG1 levels, these associations did not reach statistical
303 significance (**Figure 4C**), possibly due to the small sample size. However, ADCP activity was
304 significantly associated with VP40-specific IgG3 potentially suggesting that additional qualitative
305 features of VP40-specific IgG3 may be independently associated with accelerated viral
306 persistence.

307

308 **DISCUSSION**

309 Ebolavirus persistence and recrudescence have been demonstrated in multiple clinical,
310 epidemiological, and genomic studies, and implicated in causing acute, sometimes fatal disease
311 in survivors, and transmission that seeds new EVD clusters^{4,25-27}. Ebolavirus persistence has
312 been demonstrated in ocular fluid, semen, central nervous system including brain parenchyma
313 and cerebrospinal fluid, and breast milk^{5,6,27-30}. While persistence is well studied in survivors
314 infected with EBOV, there is a paucity of data on persistence in SUDV, and more importantly on
315 the association between host immune response and persistence of any of the ebolavirus strains.

316 Here, we used a well-characterized SUDV survivor cohort to define key antibody profiles
317 associated with viral persistence, identifying 4 immune markers that can predict viral persistence
318 among EVD survivors. As many immune-privileged sites are not easily and safely accessible,
319 surveillance of persistent viral RNA has been limited to analyses of semen and breastmilk in
320 eligible survivors. Thus, non-lactating women and children are typically not screened for
321 persistence, but other host tissue reservoirs of ebolavirus persistence may exist in these
322 survivors. When we assessed immune profiles of 50 untested survivors, over one-third (n=17)
323 had immune signatures consistent with viral RNA persistence. Importantly, the immune
324 signature was sustained in 6 of these 17 survivors (35%) through the 15 months post infection
325 timepoints that we evaluated, suggesting that these humoral immune markers can be used to

326 monitor EVD survivors for viral persistence, potentially negating the need for invasive sampling
327 and testing of tissues for the virus.

328 The underlying mechanisms for viral RNA persistence in a subset of EVD survivors are
329 not well understood. Although using a limited sample size, we observed that higher viral load
330 (lower Ct values) during acute infection had a direct association with incidence of persistence⁶,
331 but not necessarily the duration of persistence. Previous studies have posited that intermittent
332 spikes of ebolavirus-specific IgG during convalescence result from recrudescence of virus¹⁶. Our
333 finding of elevated SUDV-specific humoral immune responses in persistence survivors over 1.5
334 years post-infection supports this position. Importantly, while overall SUDV-specific IgG levels
335 decline over time, we find that IgG4 levels against GP, sGP, and NP rose over time in SUDV
336 survivors, although not statistically significant overall as a group, but GP and sGP-specific IgG4
337 significantly increased within individuals over time (**Figure S3**). While RNA persistence in
338 specific compartments was not a significant predictor of IgG4 magnitude, the longitudinal
339 increase in IgG4 across the cohort suggests that SUDV-specific B-cell maturation and class-
340 switching continue well into convalescence, potentially driven by prolonged antigen exposure in
341 a subset of individuals.

342 Early (<6 months post infection) humoral immune responses in human survivors of EBOV
343 survivors are characterized by highly polyfunctional responses against the GP³¹, and similarly,
344 the humoral immune response that we have characterized here indicates that a majority of SUDV
345 survivors develop polyfunctional antibody responses against both the GP and VP40. While GP-
346 specific IgG levels declined somewhat over time, we observed consistent levels of GP-specific
347 IgA in SUDV survivors. While EBOV-specific IgA responses in EBOV survivors have been
348 described that last for at least 1-year post-infection³², the sustained IgA seroprevalence across
349 survivors is not clear. We have previously shown that GP-specific IgA can mediate antibody-

350 dependent neutrophil phagocytosis³¹, and ADNP has been linked to vaccine-mediated
351 protection in NHPs³³. SUDV GP-specific IgA levels correlated with all the Fc-mediated effector
352 functions we measured here yet may be more of a reflection of the development of a well-
353 coordinated antibody response in survivors rather than directly mediating functional activity in all
354 immune cells. The role of IgA in long-term immunity against ebolaviruses is still unclear, but the
355 longevity of the IgA response observed here may warrant further analysis of this antibody isotype
356 in the future.

357 Ultimately, clearance of infectious virus (suggested by persistent RNA) is needed to
358 ensure long-term safety and health of survivors. Interestingly, we find that the duration of
359 persistent viral RNA was inversely correlated with levels of VP40-specific IgG and with ADCP
360 activity of VP40-coated targets. These data suggest that antibodies targeting VP40 may be worth
361 pursuing as therapeutic candidates to accelerate clearance of persistent viral RNA. Therapeutic
362 targeting of VP40 has been proposed during acute infection given the role of VP40 in viral
363 budding^{34,35}, and detailed mapping of the humoral immune response in an EVD survivor showed
364 development of durable and high affinity VP40-specific IgG and IgA that preceded decline of
365 clinical symptoms³². Beyond binding to a subset of FcRs³⁶, the functions of VP40-specific
366 antibodies have not been well studied, but our data indicate that these antibodies can mediate
367 multiple effector functions and may be an important component of long-term immunity for
368 ebolaviruses.

369 Our study has several limitations that must be acknowledged. The sample size of our
370 cohort was limited to the number of survivors for the 2022-2023 outbreak. We enrolled and
371 sampled 86 of the 87 survivors (98.8%) of this outbreak, and sampling of semen and breastmilk
372 was performed for all eligible and consenting survivors. However, despite the small sample size,
373 we were able to define a four-parameter humoral immune signature of persistence of viral RNA

374 among survivors. While we cannot ascertain the predictive potential of the four-parameter
375 humoral immune signatures in survivors, our findings provide a foundation for follow-up studies
376 using future SUDV survivors such as the recent 10 SUDV survivors from a 2025 SUDV outbreak
377 in Uganda that are now part of our cohort.

378 In summary, our study highlights the potential for humoral immune responses during
379 convalescence to be used as a biomarker for viral persistence in human ebolavirus survivors.
380 Moreover, our results highlight that antibodies against non-GP viral antigens, such as VP40,
381 track with accelerated clearance of viral RNA, and thus may be an opportunity for therapeutic
382 intervention.

383

384 **ACKNOWLEDGEMENTS.**

385 We would like to thank the participants of this study for their invaluable contributions. We would
386 like to acknowledge support from the Uganda Ministry of Health, US CDC, the Central Public
387 Health Laboratory (CPHL), Makerere University's Infectious Disease Institute (IDI), the Baylor-
388 Uganda, Makerere University Lung Institute, The Inter-disciplinary Consortium for Epidemic
389 Research (ICER), Uganda Virus Research Institute's Viral Hemorrhagic Fever Laboratory,
390 Makerere University Biomedical Research Center, Uganda National Health Research
391 Organization, and the staff at the three SUDV survivor clinics for their dedication and
392 contributions to this research. This work was funded by NIH/NIAID U01AI151799 and
393 U01AI151378 (Centers for Research in Emerging Infectious Diseases-East and Central Africa),
394 and NIH/NIAID 1R01AI175698.

395

396

397 **FIGURE LEGENDS.**

398 **Figure 1. Humoral immune profiles of SUDV survivors across 2-15 months post-infection.**

399 (A-C) Levels of GP-specific total IgG (A), GP-specific total IgA (B), and GP-specific IgM (C) were
400 measured in seronegative controls (n=24), Uganda community controls (n=192), and SUDV
401 survivors across 4 timepoints. The dashed lines indicate threshold of seropositivity for each
402 measure. Kruskal-Wallis test with Dunn's correction for multiple comparisons.

403 (D-G) The inhibitory dose 50 (ID₅₀) of VSVDG-SUDV GP pseudovirus was measured for the
404 indicated groups (D) and the ability of GP-specific antibodies to induce complement activation
405 (E), phagocytosis by monocytes (F) or NK cell degranulation (G) were measured for the indicated
406 groups. Kruskal-Wallis test with Dunn's correction for multiple comparisons. The dotted lines in
407 (D) represent the ID₅₀ of 1:200 and 1:1000, as indicated to the right of the graph. The lines
408 connect survivor's ID₅₀ values at the two timepoints.

409 H. Spearman correlation analyses were performed between levels of GP-specific IgG
410 subclasses, IgA subclasses, total IgG, total IgA, or IgM with indicated antibody-mediated effector
411 functions. The spearman rho values of statistically significant associations at indicated
412 timepoints are shown (not corrected for multiple comparisons), and are colored where purple is
413 a positive correlation, and green is a negative correlation.

414

415 **Figure 2. Correlates of viral persistence in SUDV survivors.**

416 A. The Ct value of the confirmatory PCR performed at admittance to the Ebola Treatment Unit
417 during acute infection was compared between those with fatal outcomes (grey squares)
418 and all survivors (crimson circles), and between survivors with (red circles) and without
419 persistent (open circles) viral RNA detected in semen/breastmilk during convalescence. p

values were determined by Kruskal-Wallis test with Dunn's correction for multiple comparisons.

B. Spearman correlation was performed between Ct value and days persistently RNA positive in survivors with persistent viral RNA.

C. Volcano plot of a multiple Mann-Whitney tests across all SUDV-associated antibody features at the enrollment timepoint (2-6 months post-infection) between survivors with and without persistent viral RNA. The dashed line on the y-axis ($\log_{10} q \text{ value} = 2$) indicates a $q\text{-value} < 0.01$ (corrected for multiple comparisons). The statistically significant features are indicated on graph, with features to the right are elevated in survivors with persistent viral RNA compared to those without.

D-G. Univariate plots of the top four antibody features that were elevated in survivors with persistent viral RNA compared to those without across the indicated timepoints. (D) GP-specific total IgG; (E) NP-specific total IgG; (F) sGP-specific total IgA; (G) GP-specific Antibody-dependent cellular phagocytosis by monocytes (ADCP). Kruskal-Wallis test with Dunn's correction for multiple comparisons.

Figure 3. Distinct humoral profiles can predict RNA persistence.

A. A regularized discriminant analysis was performed using a stepwise variable selection method using antibody measures at the enrollment timepoint (2-6 months post-infection) to classify survivors with and without persistent viral RNA. Four features were selected by the model are indicated in the loadings biplot. A combined score using these four features was calculated using the following formula: $(\text{Feature 1 value}) * (\text{Feature 2 value}) * (\text{Feature 3 value}) * (\text{Feature 4 value})^{(1/4)}$.

- B.** A combined score using the four features identified in (A) was calculated for each survivor at the enrollment timepoint using the following formula: (Feature 1 value)*(Feature 2 value)*(Feature 3 value)*(Feature 4 value)^(1/4). The resulting combined antibody score was then used to generate a ROC curve for classification of survivors with and without persistent viral RNA. The AUC of the ROC curve is indicated on the plot.
- C.** Comparison of the combined antibody scores between survivors without persistent RNA (open circle), survivors with persistent RNA (Red circle), and survivors who were unable to be tested for the presence of persistent RNA (grey circles). The shaded region indicates the bounds of the upper and lower antibody scores from the ROC curve for either 100% sensitivity (upper) or 100% specificity (lower).
- D.** The combined antibody score for the survivors with and without persistent RNA at the later timepoints (5-8 months, 8-11 months, and 12-15 months) were used to generate ROC curves for each timepoint. The AUC for each is indicated on the plot.
- E.** The combined antibody scores between survivors without persistent RNA (open circles), survivors with persistent RNA (red circles), and survivors who were unable to be tested for the presence of persistent RNA (grey circles) for each timepoint. The shaded region indicates the bounds of the upper and lower antibody scores from the ROC curve for either 100% sensitivity (upper) or 100% specificity (lower) for the different timepoints (red shaded 5-8 months; blue shaded 8-11 months; grey shaded 12-15 months)

Figure 4. VP40-specific responses are associated with accelerated clearance of persistent viral RNA.

- A.** Spearman correlation analyses were performed between levels of total IgG specific for GP, sGP, NP, and VP40 and days persistently RNA positive in survivors with persistent viral RNA. The spearman rho and p value are indicated on each graph.

- B.** Spearman correlation analyses were performed between levels of VP40-specific antibody-mediated effector functions (antibody-dependent cellular phagocytosis by monocytes [ADCP], antibody-dependent complement deposition [ADCD], antibody-dependent neutrophil phagocytosis [ADNP], or antibody-dependent NK cell degranulation [ADNKA] and days persistently RNA positive in survivors with persistent viral RNA. The spearman rho and p value are indicated on each graph.
- C.** Spearman correlation heatmap of VP40-specific IgG subclasses, IgA subclasses, and IgM with VP40-specific antibody-mediated effector functions. The spearman rho values of statistically significant associations at indicated timepoints are shown (not corrected for multiple comparisons), and are colored where purple is a positive correlation, and green is a negative correlation.

Table 1. Demographic characteristics of the SUDV survivors included in this study.

The age, sex, and occupation of all SUDV survivors (n=87), or survivors with (n=20) and without persistent viral RNA (n=16) are shown. P values compare means between survivors with and without persistent viral RNA by Mann-Whitney test for age; Fisher's exact test was used to determine statistical significance between male/female distribution in survivors with and without persistent viral RNA.

	All	Persistent	Non-persistent	p value
n	87	20	16	
Age				
Mean (SD)	29.8 (13.05)	29.32 (9.061)	32.62 (7.375)	0.336
Median (IQR)	29.65 (15.96)	29.18 (15.91)	30.31 (10.31)	
<10	6 (6.9%)	0 (0%)	0 (0%)	N/A
10-19	11 (12.6%)	3 (15%)	1 (6.25%)	
20-39	55 (63.2%)	15 (75%)	12 (75%)	
40-49	10 (11.5%)	2 (10%)	3 (18.75%)	
>50	5 (5.7%)	0 (0%)	0 (0%)	
Sex				
Male (%)	56 (64.4%)	15 (75%)	16 (100%)	0.0527
Female (%)	31 (35.6%)	5 (25%)	0 (0%)	
Occupation				
HCW (%)	8 (9.2%)	3 (15%)	4 (25%)	N/A
Professional/skilled labour (%)	7 (8%)	2 (10%)	1 (6.25%)	
Unskilled labour (%)	8 (9.2%)	2 (10%)	0 (0%)	
Farmer (%)	32 (36.8%)	10 (50%)	4 (25%)	
Student (%)	10 (11.5%)	1 (5%)	2 (12.5%)	
Other/NA (%)	22 (25.3%)	2 (10%)	5 (31.25%)	

Table 2. Acute EVD parameters of the SUDV survivors included in this study.

Acute EVD parameters of SUDV survivors (n=87), or survivors with and without persistent viral RNA are shown, including the date range of illness confirmation, disease symptom severity, and the number of days spent in the ETU. Fisher's exact test was used to determine statistical significance in disease symptom severity (moderate vs severe) in survivors with and without persistent viral RNA. P values of mean of days spent in ETU between survivors with and without persistent viral RNA that experienced moderate or severe symptoms was determined by Kruskal-Wallis test with Dunn's correction for multiple comparisons.

		All	Persistent	Non-persistent	p value
n		87	20	16	N/A
Date range of illness confirmation					
		9/20/22-11/15/2022	9/22/22-11/10/2022	9/23/22-10/28/22	N/A
Severity of acute disease					
Mild		3	0	0	0.3145
Moderate		39	8	10	
Severe		45	12	6	
Number of days in ETU					
Mean (SD)		12.39 (7.95)	17.42(7.94)	9.69 (9.53)	<.001
Median (IQR)		10 (9)	18 (9)	7 (4)	
Moderate	Mean (SD)	12.27 (9.92)	18.38 (11.13)	9.7 (11.66)	p<0.05
	Median (IQR)	9 (10)	17.5 (9.25)	5.5 (5.75)	
Severe	Mean (SD)	12.85 (5.77)	16.73 (5.04)	9.67 (5.28)	p=0.08
	Median (IQR)	12 (8.5)	18 (10)	8.5 (5.25)	

498 **Table 3.** q values of antibody measures between shedding and non-shedding SUDV survivors.
 499 All measures with significant q values ($q < 0.05$) were elevated in survivors with persistent viral
 500 RNA compared to survivors without viral RNA.

Isotype	Antigen	2-6 mo.	5-8 mo.	8-11 mo.	12-15 mo.
Total IgA	GP	0.00049	0.00033	0.00094	0.00106
IgA1	GP	0.00036	0.00016	0.00094	0.00106
IgA2	GP	0.00057	0.00016	0.00186	0.00064
Total IgG	GP	0.00008	0.00007	0.00068	0.00135
IgG1	GP	0.00061	0.00106	ns	ns
IgG2	GP	0.00621	0.00124	0.00588	ns
IgG3	GP	ns	0.00501	ns	ns
IgG4	GP	ns	ns	ns	ns
IgM	GP	0.00040	0.00164	ns	0.00396
Total IgA	sGP	0.00005	0.00002	0.00002	0.00054
IgA1	sGP	0.00005	0.00002	0.00002	0.00064
IgA2	sGP	0.00294	0.00164	0.00186	0.00318
Total IgG	sGP	0.00021	0.00002	0.00000	0.00002
IgG1	sGP	0.00021	0.00002	0.00000	0.00003
IgG2	sGP	0.00246	0.00124	0.00189	0.00949
IgG3	sGP	0.00038	0.00002	0.00002	0.00064
IgG4	sGP	ns	ns	ns	ns
IgM	sGP	ns	ns	ns	ns
Total IgA	NP	0.00066	0.00026	0.00094	0.00294
IgA1	NP	0.00012	0.00016	0.00047	0.00172
IgA2	NP	ns	ns	ns	ns
Total IgG	NP	0.00005	0.00001	0.00002	0.00029
IgG1	NP	0.00008	0.00001	0.00004	0.00029
IgG2	NP	0.00005	0.00001	0.00000	0.00029
IgG3	NP	0.00127	0.00036	0.00186	ns
IgG4	NP	ns	ns	ns	ns
IgM	NP	0.00349	ns	ns	ns
Total IgA	VP40	ns	ns	ns	ns
IgA1	VP40	ns	ns	ns	ns
IgA2	VP40	ns	ns	ns	ns
Total IgG	VP40	ns	ns	ns	ns
IgG1	VP40	ns	ns	ns	ns
IgG2	VP40	ns	ns	0.00189	ns
IgG3	VP40	0.00053	0.00020	0.00186	0.00688
IgG4	VP40	ns	ns	ns	ns
IgM	VP40	0.00081	ns	ns	0.00640
Function	Antigen	2-6 mo.	5-8 mo.	8-11 mo.	12-15 mo.
Neutralization	GP	0.0032	n.d.	n.d.	n.d.
ADCP	GP	0.0001	0.00002	0.0251	0.0009
ADNP	GP	0.0001	0.0011	ns	ns
ADCD	GP	ns	0.0006	0.0021	0.0160
ADNKA: CD017a	GP	0.0087	0.0003	0.0061	0.0361
ADNKA: TNFa	GP	0.0057	0.0002	0.0330	0.0568

ns = not significant, $q < 0.05$

n.d. = not determined

501

502 SUPPLEMENTAL FIGURE LEGENDS

503 **Figure S1.** The gating strategies for ADNP (A), ADCP (B), ADCD (C) and ADNKA (D) are shown
504 for representative SUDV GP seronegative and seropositive samples as indicated.

505

506 **Figure S2.** Levels of GP-specific IgG1, IgG2, IgG3, IgG4 (A), sGP-specific IgG1, IgG2, IgG3,
507 IgG4 (B), NP-specific IgG1, IgG2, IgG3, IgG4 (C) and VP40-specific IgG1, IgG2, IgG3, IgG4 (D)
508 were measured in seronegative controls (n=24), Uganda community controls (n=192), and
509 SUDV survivors across 4 timepoints. Kruskal-Wallis test with Dunn's correction for multiple
510 comparisons.

511

512 **Figure S3.** The GP- and sGP-specific IgG4 responses for each survivor is plotted longitudinally
513 across the four timepoints (2-15 months). Mixed-effects analysis with Holm-Sidak's multiple
514 comparison test was used to determine if later timepoints were significantly different from the
515 enrollment (2-6 month) timepoint.

516

517 **Figure S4.** Levels of total IgG (A), total IgA (B), and IgM (C) were measured in seronegative
518 controls (n=24), Uganda community controls (n=192), and SUDV survivors across 4 timepoints.
519 Kruskal-Wallis test with Dunn's correction for multiple comparisons.

520

521 **Figure S5.** The ability of GP-specific antibodies to induce antibody-mediated neutrophil
522 phagocytosis and antibody-mediated NK cell secretion of $\text{TNF}\alpha$ were measured for the indicated
523 groups. Kruskal-Wallis test with Dunn's correction for multiple comparisons.

524

525 **Figure S6.** Volcano plot of a multiple Mann-Whitney tests across all SUDV-associated antibody
526 features at the 5-8 months post-infection (A), 8-11 months post-infection (B), and 12-15 months
527 post-infection (C) between survivors with and without persistent viral RNA. The dashed line on
528 the y-axis ($\log_{10} q \text{ value} = 1.3$) indicates a $q\text{-value} < 0.05$ (corrected for multiple comparisons).
529 The statistically significant features are indicated on graph, with features to the right are elevated
530 in survivors with persistent viral RNA compared to those without.

531

532 **Figure S7.** Spearman correlation analyses were performed between levels of VP40-specific
533 IgG1, IgG2, IgG3, IgG4 at 2-6 months post-infection and days persistently RNA positive in
534 survivors with persistent viral RNA. The spearman rho and p value are indicated on each graph.

535

536 REFERENCES

- 537 1. Kabami, Z., *et al.* Ebola disease outbreak caused by the Sudan virus in Uganda, 2022:
538 a descriptive epidemiological study. *The Lancet. Global health* **12**, e1684-e1692 (2024).
- 539 2. Group, P.I.S., *et al.* A Longitudinal Study of Ebola Sequelae in Liberia. *The New*
540 *England journal of medicine* **380**, 924-934 (2019).
- 541 3. Keita, A.K., *et al.* A 40-Month Follow-Up of Ebola Virus Disease Survivors in Guinea
542 (PostEbogui) Reveals Long-Term Detection of Ebola Viral Ribonucleic Acid in Semen
543 and Breast Milk. *Open Forum Infect Dis* **6**, ofz482 (2019).
- 544 4. Keita, A.K., *et al.* Resurgence of Ebola virus in 2021 in Guinea suggests a new
545 paradigm for outbreaks. *Nature* **597**, 539-543 (2021).
- 546 5. Varkey, J.B., *et al.* Persistence of Ebola Virus in Ocular Fluid during Convalescence.
547 *The New England journal of medicine* **372**, 2423-2427 (2015).
- 548 6. Thorson, A.E., *et al.* Persistence of Ebola virus in semen among Ebola virus disease
549 survivors in Sierra Leone: A cohort study of frequency, duration, and risk factors. *PLoS*
550 *medicine* **18**, e1003273 (2021).
- 551 7. Mate, S.E., *et al.* Molecular Evidence of Sexual Transmission of Ebola Virus. *The New*
552 *England journal of medicine* **373**, 2448-2454 (2015).
- 553 8. Sissoko, D., *et al.* Ebola Virus Persistence in Breast Milk After No Reported Illness: A
554 Likely Source of Virus Transmission From Mother to Child. *Clinical infectious diseases :
555 an official publication of the Infectious Diseases Society of America* **64**, 513-516 (2017).

- 556 9. Gunn, B.M., *et al.* A Role for Fc Function in Therapeutic Monoclonal Antibody-Mediated
557 Protection against Ebola Virus. *Cell host & microbe* **24**, 221-233 e225 (2018).
- 558 10. Saphire, E.O., *et al.* Systematic Analysis of Monoclonal Antibodies against Ebola Virus
559 GP Defines Features that Contribute to Protection. *Cell* **174**, 938-952 e913 (2018).
- 560 11. Bornholdt, Z.A., *et al.* A Two-Antibody Pan-Ebolavirus Cocktail Confers Broad
561 Therapeutic Protection in Ferrets and Nonhuman Primates. *Cell host & microbe* **25**, 49-
562 58 e45 (2019).
- 563 12. Grais, R.F., *et al.* Estimation of the correlates of protection of the rVSVDeltaG-ZEBOV-
564 GP Zaire ebolavirus vaccine: a post-hoc analysis of data from phase 2/3 clinical trials.
565 *Lancet Microbe* **2**, e70-e78 (2021).
- 566 13. Rimoin, A.W., *et al.* Ebola Virus Neutralizing Antibodies Detectable in Survivors of
567 the Yambuku, Zaire Outbreak 40 Years after Infection. *The Journal of infectious diseases*
568 **217**, 223-231 (2018).
- 569 14. Sobarzo, A., *et al.* Persistent immune responses after Ebola virus infection. *The New*
570 *England journal of medicine* **369**, 492-493 (2013).
- 571 15. Natesan, M., *et al.* Human Survivors of Disease Outbreaks Caused by Ebola or
572 Marburg Virus Exhibit Cross-Reactive and Long-Lived Antibody Responses. *Clinical and*
573 *vaccine immunology : CVI* **23**, 717-724 (2016).
- 574 16. Adaken, C., *et al.* Ebola virus antibody decay-stimulation in a high proportion of
575 survivors. *Nature* **590**, 468-472 (2021).
- 576 17. Lee, J.E. & Saphire, E.O. Ebolavirus glycoprotein structure and mechanism of entry.
577 *Future virology* **4**, 621-635 (2009).
- 578 18. de La Vega, M.A., Wong, G., Kobinger, G.P. & Qiu, X. The multiple roles of sGP in
579 Ebola pathogenesis. *Viral immunology* **28**, 3-9 (2015).
- 580 19. Emanuel, J., Marzi, A. & Feldmann, H. Filoviruses: Ecology, Molecular Biology, and
581 Evolution. *Advances in virus research* **100**, 189-221 (2018).
- 582 20. Jasenosky, L.D., Neumann, G., Lukashevich, I. & Kawaoka, Y. Ebola virus VP40-
583 induced particle formation and association with the lipid bilayer. *Journal of virology* **75**,
584 5205-5214 (2001).
- 585 21. Brown, E.P., *et al.* High-throughput, multiplexed IgG subclassing of antigen-specific
586 antibodies from clinical samples. *Journal of immunological methods* **386**, 117-123
587 (2012).
- 588 22. Ferrara, F. & Temperton, N. Pseudotype Neutralization Assays: From Laboratory Bench
589 to Data Analysis. *Methods Protoc* **1**(2018).
- 590 23. Saphire, E.O., Schendel, S.L., Gunn, B.M., Milligan, J.C. & Alter, G. Antibody-mediated
591 protection against Ebola virus. *Nature immunology* **19**, 1169-1178 (2018).
- 592 24. Muwonge, H., *et al.* Long-term clinical sequelae among Sudan ebolavirus disease
593 survivors 2 years post-infection: a matched cohort study. *BMC Med* **23**, 432 (2025).
- 594 25. Jacobs, M., *et al.* Late Ebola virus relapse causing meningoencephalitis: a case report.
595 *Lancet* **388**, 498-503 (2016).
- 596 26. Mbala-Kingebeni, P., *et al.* Ebola Virus Transmission Initiated by Relapse of Systemic
597 Ebola Virus Disease. *The New England journal of medicine* **384**, 1240-1247 (2021).
- 598 27. Mukadi-Bamuleka, D., *et al.* Fatal meningoencephalitis associated with Ebola virus
599 persistence in two survivors of Ebola virus disease in the Democratic Republic of the
600 Congo: a case report study. *Lancet Microbe* **5**, 100905 (2024).
- 601 28. Deen, G.F., *et al.* Ebola RNA Persistence in Semen of Ebola Virus Disease Survivors -
602 Final Report. *The New England journal of medicine* **377**, 1428-1437 (2017).

- 603 29. Sissoko, D., *et al.* Persistence and clearance of Ebola virus RNA from seminal fluid of
604 Ebola virus disease survivors: a longitudinal analysis and modelling study. *The Lancet.*
605 *Global health* **5**, e80-e88 (2017).
- 606 30. Fischer, W.A., *et al.* Ebola Virus Ribonucleic Acid Detection in Semen More Than Two
607 Years After Resolution of Acute Ebola Virus Infection. *Open Forum Infect Dis* **4**, ofx155
608 (2017).
- 609 31. Gunn, B.M., *et al.* Survivors of Ebola Virus Disease Develop Polyfunctional Antibody
610 Responses. *The Journal of infectious diseases* **221**, 156-161 (2020).
- 611 32. Khurana, S., *et al.* Longitudinal Human Antibody Repertoire against Complete Viral
612 Proteome from Ebola Virus Survivor Reveals Protective Sites for Vaccine Design. *Cell*
613 *host & microbe* **27**, 262-276 e264 (2020).
- 614 33. Gunn, B.M., *et al.* Antibodies against the Ebola virus soluble glycoprotein are associated
615 with long-term vaccine-mediated protection of non-human primates. *Cell reports* **42**,
616 112402 (2023).
- 617 34. Madara, J.J., Han, Z., Ruthel, G., Freedman, B.D. & Harty, R.N. The multifunctional
618 Ebola virus VP40 matrix protein is a promising therapeutic target. *Future virology* **10**,
619 537-546 (2015).
- 620 35. Stahelin, R.V. Could the Ebola virus matrix protein VP40 be a drug target? *Expert Opin*
621 *Ther Targets* **18**, 115-120 (2014).
- 622 36. Sobarzo, A., *et al.* Multiple viral proteins and immune response pathways act to
623 generate robust long-term immunity in Sudan virus survivors. *EBioMedicine* **46**, 215-226
624 (2019).
- 625

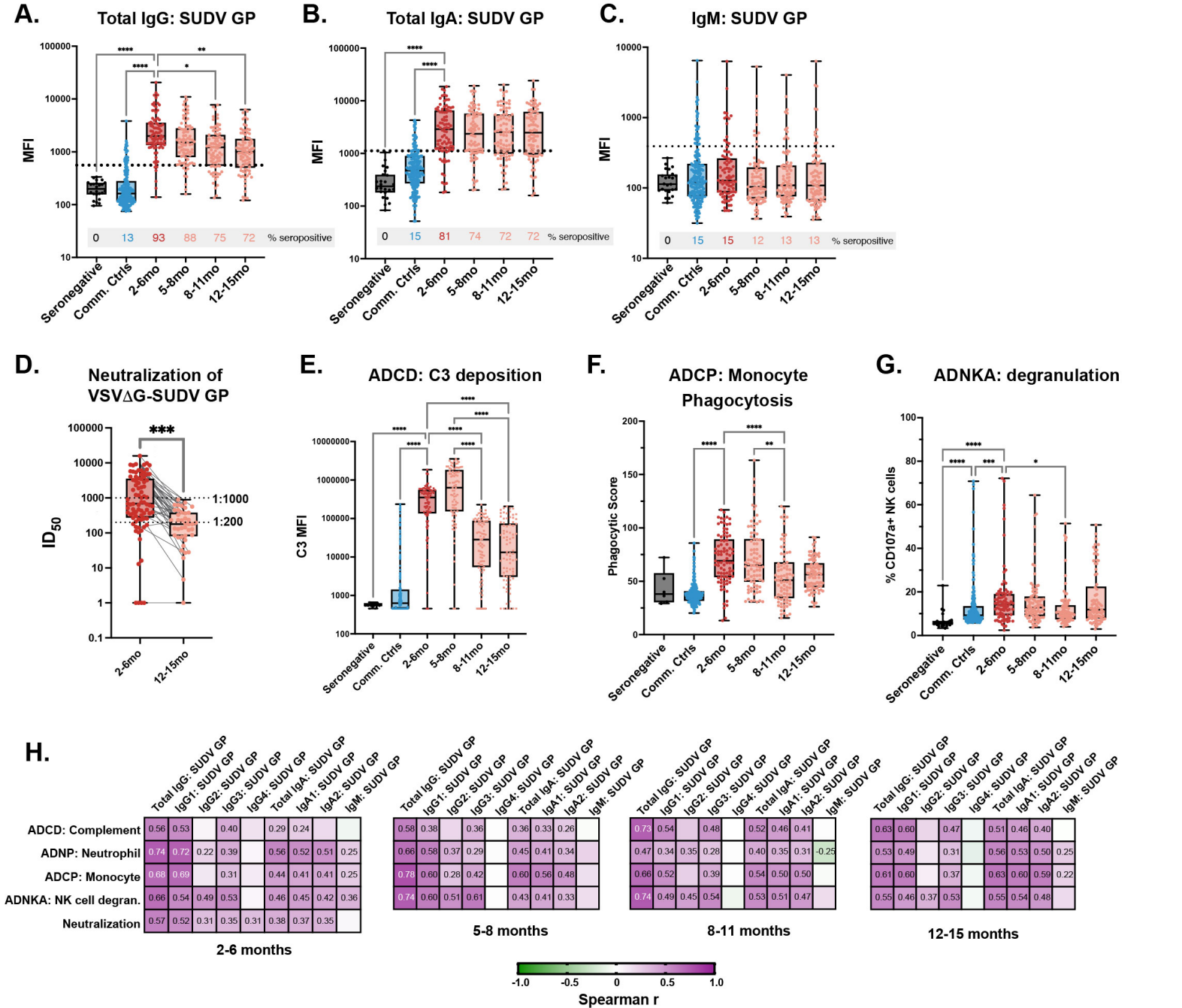


Figure 1. Humoral immune profiles of SUDV survivors across 2-15 months post-infection.

(A-C) Levels of GP-specific total IgG (A), GP-specific total IgA (B), and GP-specific IgM (C) were measured in seronegative controls (n=24), Uganda community controls (n=192), and SUDV survivors across 4 timepoints. The dashed lines indicate threshold of seropositivity for each measure. Kruskal-Wallis test with Dunn's correction for multiple comparisons.

(D-G) The inhibitory dose 50 (ID_{50}) of VSVΔG-SUDV GP pseudovirus was measured for the indicated groups (D) and the ability of GP-specific antibodies to induce complement activation (E), phagocytosis by monocytes (F) or NK cell degranulation (G) were measured for the indicated groups. Kruskal-Wallis test with Dunn's correction for multiple comparisons. The dotted lines in (D) represent the ID_{50} of 1:200 and 1:1000, as indicated to the right of the graph. The lines connect survivor's ID_{50} values at the two timepoints.

H) Spearman correlation analyses were performed between levels of GP-specific IgG subclasses, IgA subclasses, total IgG, total IgA, or IgM with indicated antibody-mediated effector functions. The spearman rho values of statistically significant associations at indicated timepoints are shown (not corrected for multiple comparisons), and are colored where purple is a positive correlation, and green is a negative correlation.

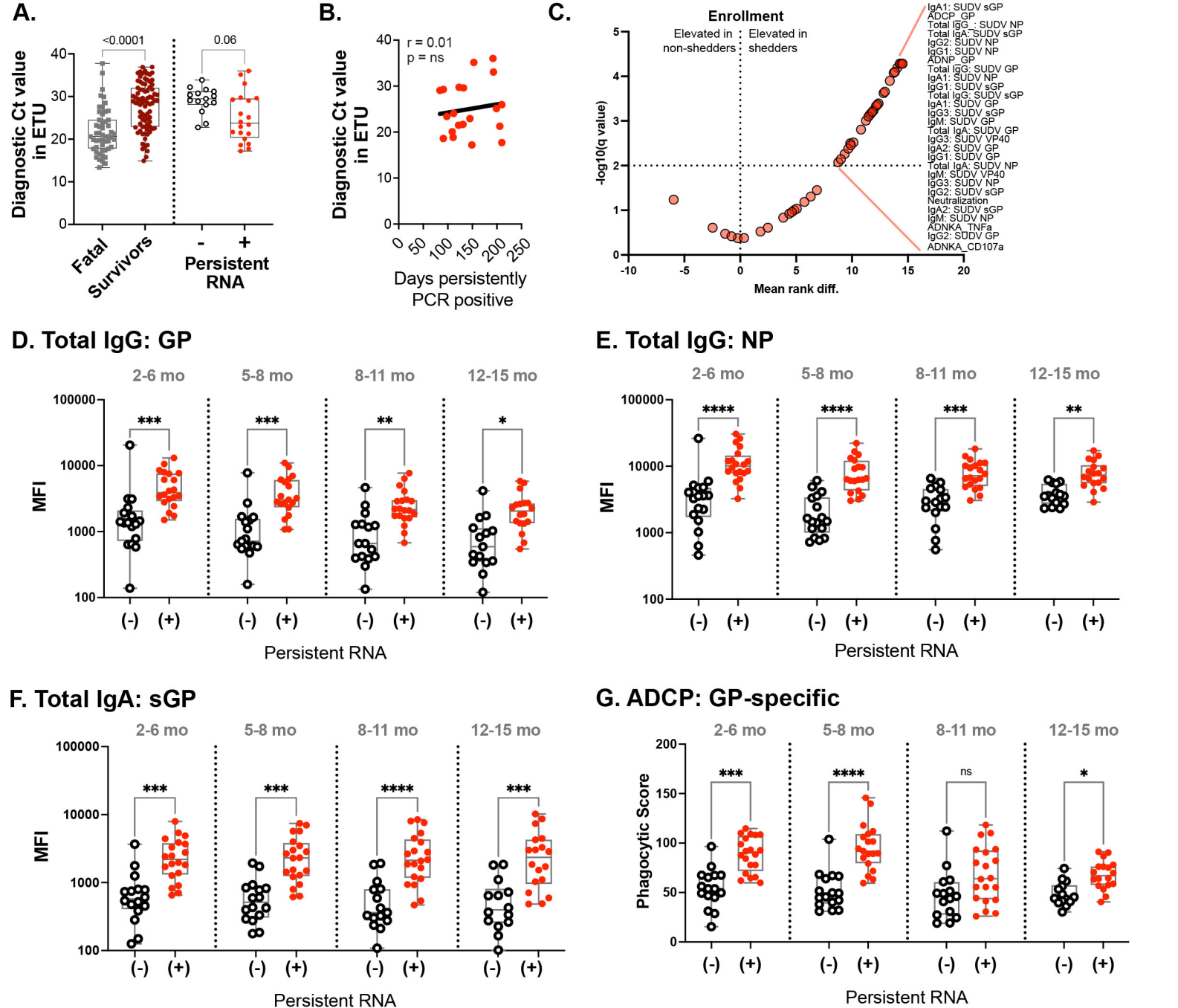


Figure 2. Correlates of viral persistence in SUDV survivors.

A) The Ct value of the confirmatory PCR performed at admittance to the Ebola Treatment Unit during acute infection was compared between those with fatal outcomes (grey squares) and all survivors (crimson circles), and between survivors with (red circles) and without persistent (open circles) viral RNA detected in semen/breastmilk during convalescence. p values were determined by Kruskal-Wallis test with Dunn's correction for multiple comparisons.

B) Spearman correlation was performed between Ct value and days persistently RNA positive in survivors with persistent viral RNA.

C) Volcano plot of a multiple Mann-Whitney tests across all SUDV-associated antibody features at the enrollment timepoint (2-6 months post-infection) between survivors with and without persistent viral RNA. The dashed line on the y-axis ($\log_{10} q \text{ value} = 2$) indicates a q-value < 0.01 (corrected for multiple comparisons). The statistically significant features are indicated on graph, with features to the right are elevated in survivors with persistent viral RNA compared to those without.

(D-G) Univariate plots of the top four antibody features that were elevated in survivors with persistent viral RNA compared to those without across the indicated timepoints. (D) GP-specific total IgG; (E) NP-specific total IgG; (F) sGP-specific total IgA; (G) GP-specific Antibody-dependent cellular phagocytosis by monocytes (ADCP). Kruskal-Wallis test with Dunn's correction for multiple comparisons.

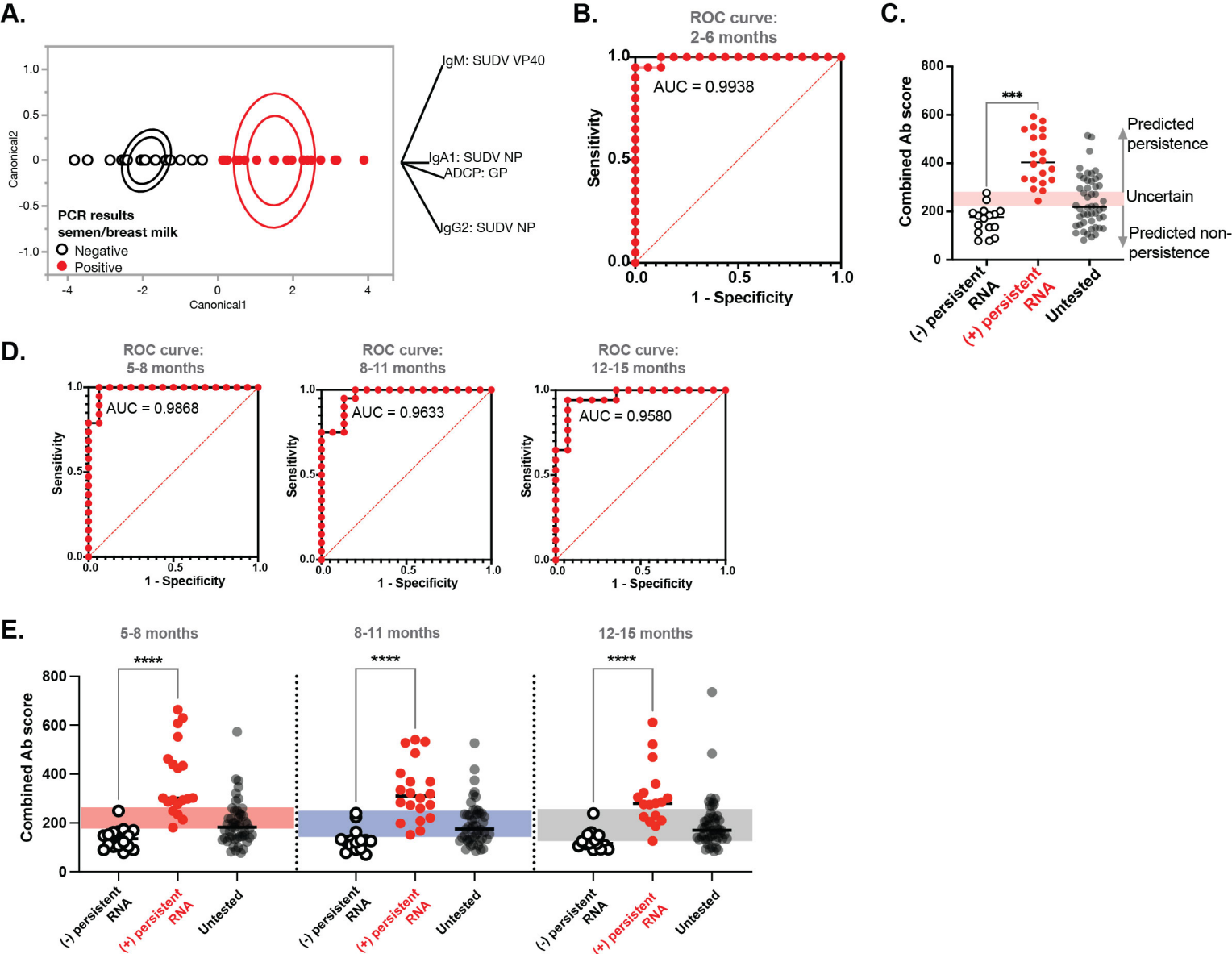


Figure 3. Distinct humoral profiles can predict RNA persistence.

A) A regularized discriminant analysis was performed using a stepwise variable selection method using antibody measures at the enrollment timepoint (2-6 months post-infection) to classify survivors with and without persistent viral RNA. Four features were selected by the model are indicated in the loadings biplot.

B) A combined score using the four features identified in (A) was calculated for each survivor at the enrollment timepoint using the following formula: $(\text{Feature 1 value}) \times (\text{Feature 2 value}) \times (\text{Feature 3 value}) \times (\text{Feature 4 value})^{1/4}$. The resulting combined antibody score was then used to generate a ROC curve for classification of survivors with and without persistent viral RNA. The AUC of the ROC curve is indicated on the plot.

C) Comparison of the combined antibody scores between survivors without persistent RNA (open circle), survivors with persistent RNA (Red circle), and survivors who were unable to be tested for the presence of persistent RNA (grey circles). The shaded region indicates the bounds of the upper and lower antibody scores from the ROC curve for either 100% sensitivity (upper) or 100% specificity (lower).

D) The combined antibody score for the survivors with and without persistent RNA at the later timepoints (5-8 months, 8-11 months, and 12-15 months) were used to generate ROC curves for each timepoint. The AUC for each is indicated on the plot.

E) The combined antibody scores between survivors without persistent RNA (open circles), survivors with persistent RNA (red circles), and survivors who were unable to be tested for the presence of persistent RNA (grey circles) for each timepoint. The shaded region indicates the bounds of the upper and lower antibody scores from the ROC curve for either 100% sensitivity (upper) or 100% specificity (lower) for the different timepoints (red shaded 5-8 months; blue shaded 8-11 months; grey shaded 12-15 months)

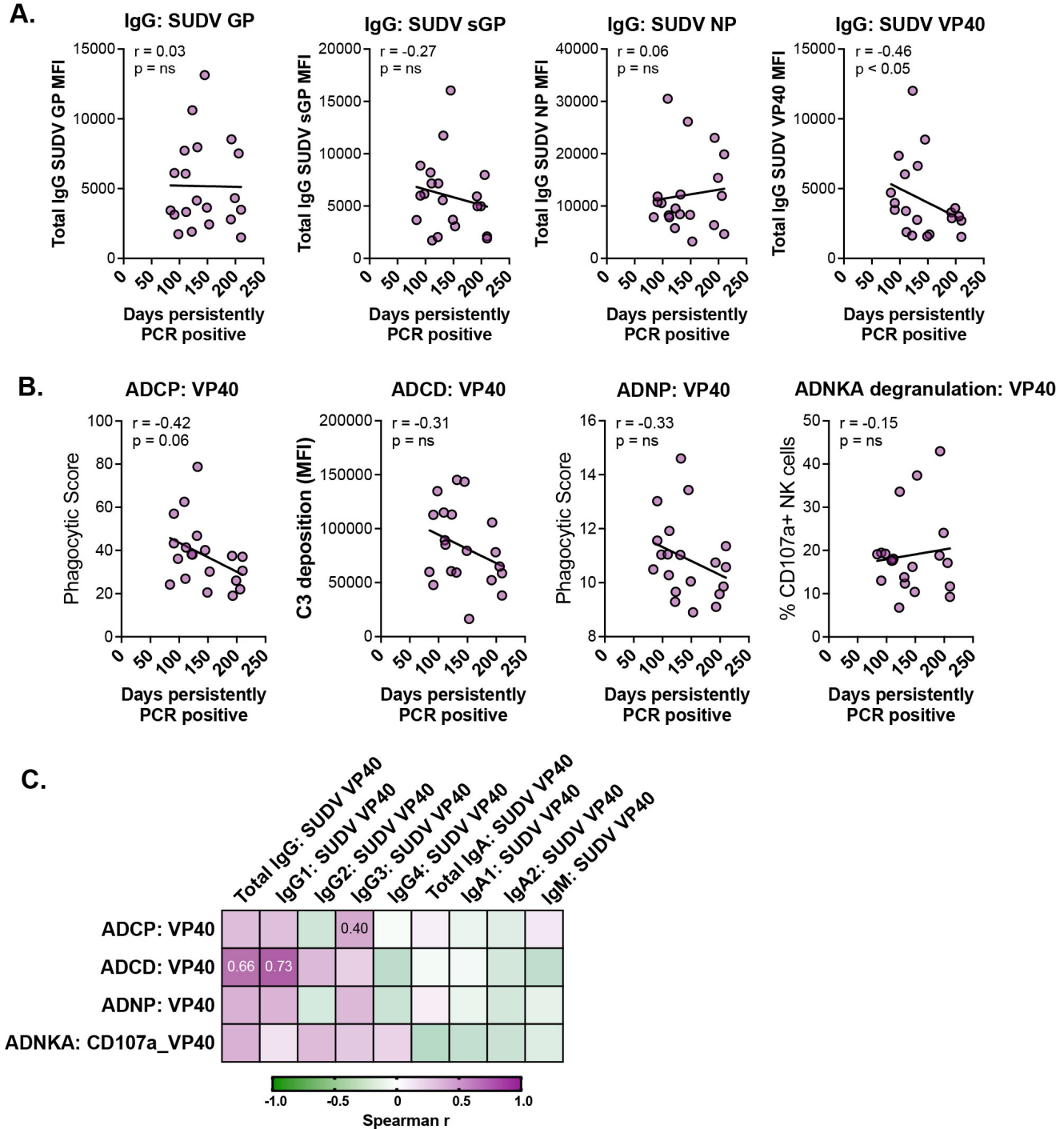


Figure 4. VP40-specific responses are associated with accelerated clearance of persistent viral RNA.

A) Spearman correlation analyses were performed between levels of total IgG specific for GP, sGP, NP, and VP40 and days persistently RNA positive in survivors with persistent viral RNA. The spearman rho and p value are indicated on each graph.

B) Spearman correlation analyses were performed between levels of VP40-specific antibody-mediated effector functions (antibody-dependent cellular phagocytosis by monocytes [ADCP], antibody-dependent complement deposition [ADCD], antibody-dependent neutrophil phagocytosis [ADNP], or antibody-dependent NK cell degranulation [ADNKA] and days persistently RNA positive in survivors with persistent viral RNA. The spearman rho and p value are indicated on each graph.

C) Spearman correlation heatmap of VP40-specific IgG subclasses, IgA subclasses, and IgM with VP40-specific antibody-mediated effector functions. The spearman rho values of statistically significant associations at indicated timepoints are shown (not corrected for multiple comparisons), and are colored where purple is a positive correlation, and green is a negative correlation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SUDVsupplementaryfigurescombined.pdf](#)