

**Protective Monotherapy Against Lethal Ebola Virus Infection by a
Potently Neutralizing Antibody**

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46 **Abstract:**

47 Ebola virus disease in humans is highly lethal, with case fatality rates ranging from
48 25-90%. There is no licensed treatment or vaccine against the virus, underscoring
49 needs for efficacious countermeasures. Here, we demonstrate that human survivors
50 of Ebola virus disease maintain circulating antibodies against the Ebola virus
51 surface glycoprotein for more than a decade after infection, and we isolated
52 monoclonal antibodies (mAb) from one survivor of the 1995 Kikwit outbreak in the
53 Democratic Republic of Congo. These mAbs bind and neutralize recent and
54 previous outbreak strains of Ebola virus, and mediate antibody-dependent cell-
55 mediated cytotoxicity *in vitro*. Administration of a single potently neutralizing
56 antibody, mAb114, protected infected macaques from death and clinical illness
57 when administered one day after lethal challenge. Treatment with a single human
58 mAb suggests a simplified therapeutic strategy for human Ebola infection may be
59 possible.

60

61 **One Sentence Summary:**

62 A single neutralizing monoclonal antibody isolated from a human Ebola survivor
63 completely protected non-human primates from lethal Ebola infection.

64

65 **Main Text:**

66 Ebola virus disease (EVD) causes severe illness characterized by rapid onset of fever,
67 vomiting, diarrhea and bleeding diathesis (1, 2), and was first described in the
68 Democratic Republic of Congo in 1976 (2). The 2014 outbreak in West Africa has

69 affected over 27,000 and claimed at least 11,000 lives (1). The challenges of a large
70 outbreak and the failure of traditional quarantine and contact tracing measures (3, 4) to
71 control this outbreak highlights the urgency for therapies. The success in nonhuman
72 primates (NHP) of ZMapp, a cocktail of three mouse-human chimeric mAbs derived
73 from immunized mice (5, 6), illustrated the potential impact of monoclonal antibody
74 therapies against EVD, and it is currently being evaluated in human trials. To date,
75 efforts to simplify the ZMapp regimen to contain fewer mAbs have not been successful in
76 the macaque EVD model (7). We sought to isolate mAbs from human survivors of Ebola
77 virus infection, with the goal of identifying antibodies that confer clinical protection
78 either as single or dual-combination agents.

79
80 We obtained blood from two survivors of the 1995 Kikwit EVD outbreak (8) eleven
81 years after infection. These subjects were the sole survivors of a family of 15 people who
82 were infected during the outbreak. At the time of infection, subject 1 (S1) was a male 28-
83 year-old who had severe laboratory-confirmed illness and, following recovery, worked
84 for several months in the EVD ward caring for other patients. His sister (S2) was 20-
85 years old and had moderate disease severity that was clinically diagnosed based on
86 contact history and symptoms. To determine if the subjects retained circulating
87 antibodies against Ebola virus (EBOV) glycoprotein (GP), we assessed GP-specific
88 antibodies by ELISA (Fig. 1A). We observed reciprocal EC₉₀ titers of 2,326 and 275 in
89 the sera of S1 and S2, respectively. Moreover, the serum from S1, the more severely ill
90 subject, displayed potent virus neutralizing activity (Fig. 1B). The results indicated that
91 these survivors maintained serologic memory against EBOV GP more than a decade

92 following infection, and suggested the potential to clone immunoglobulins with potent
93 neutralizing activity from their memory B cells.

94
95 We focused on S1 due to high serum neutralizing activity, sorted IgG memory B-cells
96 from his peripheral blood mononuclear cells (PBMC), and immortalized individual
97 clones with Epstein-Barr virus (9). Forty immortalized clones whose supernatants
98 displayed a range of GP-binding activity by ELISA were identified (Fig. 1C). Two
99 clones, 100 and 114, expressed antibodies with markedly higher neutralizing activity than
100 all others (Fig. 1D). A second immortalization yielded 21 clones, from which two
101 additional GP-specific clones, 165 and 166, were rescued (Fig. S1).

102
103 Immunoglobulin sequences were PCR-amplified from the four clones and used to
104 produce mAb100, mAb114, mAb165 and mAb166 by transient transfection. We
105 assessed ELISA binding to EBOV GP and observed that one antibody, mAb114, stood
106 apart from the others, displaying nearly 100% higher maximal binding (Fig. 2A). The
107 remaining three antibodies, mAb100, mAb165 and mAb166, exhibited reduced levels of
108 maximal binding compared to mAb114, but were comparable to each other and to KZ52,
109 a prototype human EBOV GP-specific mAb (10). mAb114 achieved half maximal
110 binding (EC_{50}) at a concentration of 0.07 $\mu\text{g/mL}$, which was up to two orders of
111 magnitude lower EC_{50} than the other mAbs. mAb100 and mAb166 had similar binding
112 profile (0.26 $\mu\text{g/mL}$, 0.40 $\mu\text{g/mL}$) while mAb165 bound less well with an $EC_{50} > 1$
113 $\mu\text{g/mL}$.

To test potential functional properties of the mAbs we evaluated inhibition of GP-mediated entry into HEK293T cells in the absence of complement (Fig. 2B, Fig. S2). mAb165 and mAb166 both neutralized well and exhibited similar potencies for half-maximal inhibition (IC_{50}) concentration of 1.77 and 0.86 $\mu\text{g/ml}$, respectively. mAb100 and mAb114 resided in the strongest neutralizing group, with IC_{50} about one-log greater (0.06 and 0.09 $\mu\text{g/ml}$, respectively) than mAb165 and mAb166. Notably, all four of the neutralizing antibodies inhibited 100% of the input virus unlike KZ52, which consistently displayed only 80-90% maximum inhibition, and 13C6 which neutralized < 20% at 10 $\mu\text{g/mL}$. Importantly, neutralization of the 2014 West African Makona variant was achieved within similar concentration ranges seen for the Mayinga variant (Fig. S3).

Sequence analysis revealed mAb114 and mAb165 to be IgG1 isotypes, and mAb100 and mAb166 to be IgG3 isotypes. Immunoglobulins displayed between 85-95% and 89-97% germline identity for heavy and light chains, respectively (Fig. 2C). Analyses of germline gene usage and V(D)J recombination indicate that they originate from different B-cell lineages. Interestingly, mAb114 utilizes *IGHV3-13*01*, a rarely used VH gene, and *IGKV1-27*01*.

We next analyzed the role of somatic hypermutations for the two most potent antibodies, mAb100 and mAb114, using variants that were partially or completely reverted to the unmutated common ancestors (UCAs) (Fig. 2, D to G). The fully reverted version of mAb100 (UCA/UCA), as well as a variant with germline VH and a VL with a single change from germline (A89T), recognized cells expressing GP with only a 2- to 4-fold

weaker binding compared with the fully matured antibody (Fig. 2H). GP binding comparable to the fully matured mAb100 heavy and light chains (sH/sL) was observed when three HCDR3 mutations (A96V/V103Y/Y114S) were introduced in the reverted germline antibody (gH/UCA), illustrating that those mutations were sufficient to mediate binding observed with fully matured mAb100. The addition of all the other mutations did not contribute further to mAb100 binding to GP. In the case of the mAb114, the fully reverted version of mAb114 (UCA/UCA) demonstrated negligible binding to EBOV GP (Fig. 2I). Introduction of two mutations (A96V and Y108S) in the HCDR3 of mAb114 germline was sufficient to confer an increase in GP binding. It is intriguing that these mutations (A96V and Y108S) are located at the base of the HCDR3 loop which are most likely not in direct contact with GP but may have a stabilizing effect on the whole HCDR3. Indeed, restoration to the binding equivalent of the mature antibody required a fully matured light chain in addition to the two HCDR3 mutations. Inherent uncertainty in determining the germline configuration of the HCDR3 does not appear to apply to this case since the two mutations are located in the V and J regions of the junction and no polymorphisms have been described at those positions. Importantly, the fully mutated light chain gene, as shown in the case of the mAb114 UCA/sK variant, can partially compensate for the lack of somatic mutation in the heavy chain (Fig. 2I). The presence of additional mutations on either VH or VK is required to achieve the level of the fully matured mAb114 binding. These results suggest a rapid pathway of mAb114 affinity maturation through one or two somatic mutations, which became redundant as further mutations accumulated, a finding that is reminiscent of what was recently observed for the generation of broadly neutralizing influenza antibodies (11).

161

162 Since mAb100 and mAb114 were the most potently neutralizing antibodies, they were
163 considered optimal candidates for further evaluation. In order to assess the potential for
164 synergy between these antibodies in the context of combination therapy, we wished to
165 first rule out cross-competition for antigen binding or targeting of a single
166 immunodominant region of GP. We found that each antibody bound to GP in the
167 presence of the other, suggesting that they recognize distinct regions on GP (Fig. 3A) and
168 therefore could be used together in combination immunotherapy to improve efficacy and
169 diminish the likelihood of emergence viral escape mutants (12, 13). To define the regions
170 targeted by mAb100 and mAb114 we employed biolayer interferometry to assess GP
171 binding in competition with mAbs KZ52 and 13C6, which have known epitopes in the
172 GP base and glycan cap, respectively (14, 15). We found that mAb100 competes with
173 KZ52 for binding at the base of GP, while mAb114 recognizes at least in part the glycan
174 cap region, as demonstrated by the partial competition observed with 13C6 (Fig. 3, B and
175 C).

176

177 Since some EBOV GP antibodies have been suggested to mediate antibody dependent
178 cell-mediated cytotoxicity (ADCC) (16) the ADCC activity of mAb100 and mAb114
179 were determined in a flow cytometry-based assay using GP-expressing target cells (Fig.
180 3D). We found that both mAb100 and mAb114 mediated ADCC, and maximum activity
181 was observed at a mAb concentration of 0.03 $\mu\text{g/ml}$, which is similar to the IC_{50} values
182 for neutralization. Killing of target cells was demonstrated to be mediated through Fc
183 receptors since LALA mutations in the mAb Fc regions (17) of the antibodies abrogated

ADCC activity. Therefore, in addition to neutralization, these mAbs have the potential to induce direct killing of infected cells *in vivo*, a key viral clearance mechanism.

The presence of potent neutralizing and ADCC activity, and the absence of cross competition, supported testing mAb100 and mAb114 *in vivo* for protective efficacy in macaques. We challenged four rhesus macaques (*Macaca mulatta*) with a lethal dose of Ebola virus, Kikwit 1995 variant. One day post-challenge, the treatment group (n=3) was given an intravenous injection with a mixture of mAb100 and mAb114 at a total combined dose of 50 mg/kg, and the treatment was repeated twice more at 24-hour intervals (Fig. 4A). Circulating Ebola GP-specific antibody titers in the mAb recipients peaked after the second mAb injection and reciprocal ELISA titers remained above 10^5 throughout the study, suggesting minimal clearance of the mAbs during the observation period (Fig. 4B). The naive untreated macaque succumbed to EVD on day 10 with a circulating viral load exceeding 10^8 ge/ml (Fig. 4, C and D). In contrast, all three mAb-treated macaques survived challenge without detectable systemic viremia. Consistent with historic controls, the untreated animal displayed hallmark indicators of Ebola infection including hematologic, liver and renal dysfunction as indicated by thrombocytopenia and striking elevations in alanine transaminase (ALT) and creatinine from day 6 through the time of death (Fig. 4E, Figs. S4 to S7). In contrast, macaques in the treatment group remained within normal ranges for these parameters, and remained free of all EVD symptoms.

We next asked whether monotherapy is sufficient for protection of NHP, and focused on mAb114 since it showed higher maximal binding than mAb100. As in the first experiment we exposed four macaques to a lethal dose of EBOV and administered 50 mg/kg of mAb114 (n=3) to the treatment group after a one-day delay, followed by two more doses at 24-hour intervals. All treated macaques survived, whereas the control animal succumbed to EVD on day 6 with a peak viral load of 10^{10} ge/ml (Fig. 4, F to H). In contrast to the previous experiment, transient viremia was observed in the treated animals, but it remained at levels less than 0.1% of the untreated control animal, and returned to undetectable levels. Despite transient viremia, treated animals remained free of clinical and laboratory abnormalities (Fig. 4I, Figs. S8 to S11).

mAb114 has several characteristics that may contribute to protection as a monotherapy compared to KZ52 and 13C6, which were non-protective in NHPs (7, 18). Firstly, both KZ52 and mAb114 neutralize with potent IC_{50} s, however mAb114 neutralizes 100% of input virus whereas KZ52 plateaus at 80-90%. Secondly, mAb114 does not require complement for neutralizing activity in contrast to 13C6 (Figure 2B) (6). Based on these observations, one intriguing hypothesis is that protective monotherapy requires both potent binding and complete neutralization in the absence of complement. In addition, ADCC activity may contribute to the unique ability of mAb114 to protect as a monotherapy against lethal Ebola infection of macaques.

In these studies, we showed that circulating functional antibodies as well as memory B cells specific to Ebola virus are maintained in survivors for more than a decade following

infection. mAbs isolated from a survivor of the 1995 Kikwit EVD outbreak exhibited ADCC activity and showed potent neutralizing activity against two other Ebola variants, including one from the recent West Africa outbreak. Macaques who received mAb114 and mAb100 as combination therapy remained healthy with no signs of viremia after EBOV challenge. Strikingly, when a single antibody, mAb114, was therapeutically administered after lethal EBOV challenge of macaques, all treated animals were fully protected and asymptomatic, despite a low transient level of circulating virus being detected. These results contribute to understanding the mechanisms of antibody-mediated Ebola virus protection, and suggest a simplified therapeutic option for EVD may be possible.

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308 the Army.

309

310 **Supplementary Materials**

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312 Materials and Methods

313 Figs. S1-S11

314 References 18-23

315

316 **Figures**

317

Figure 1

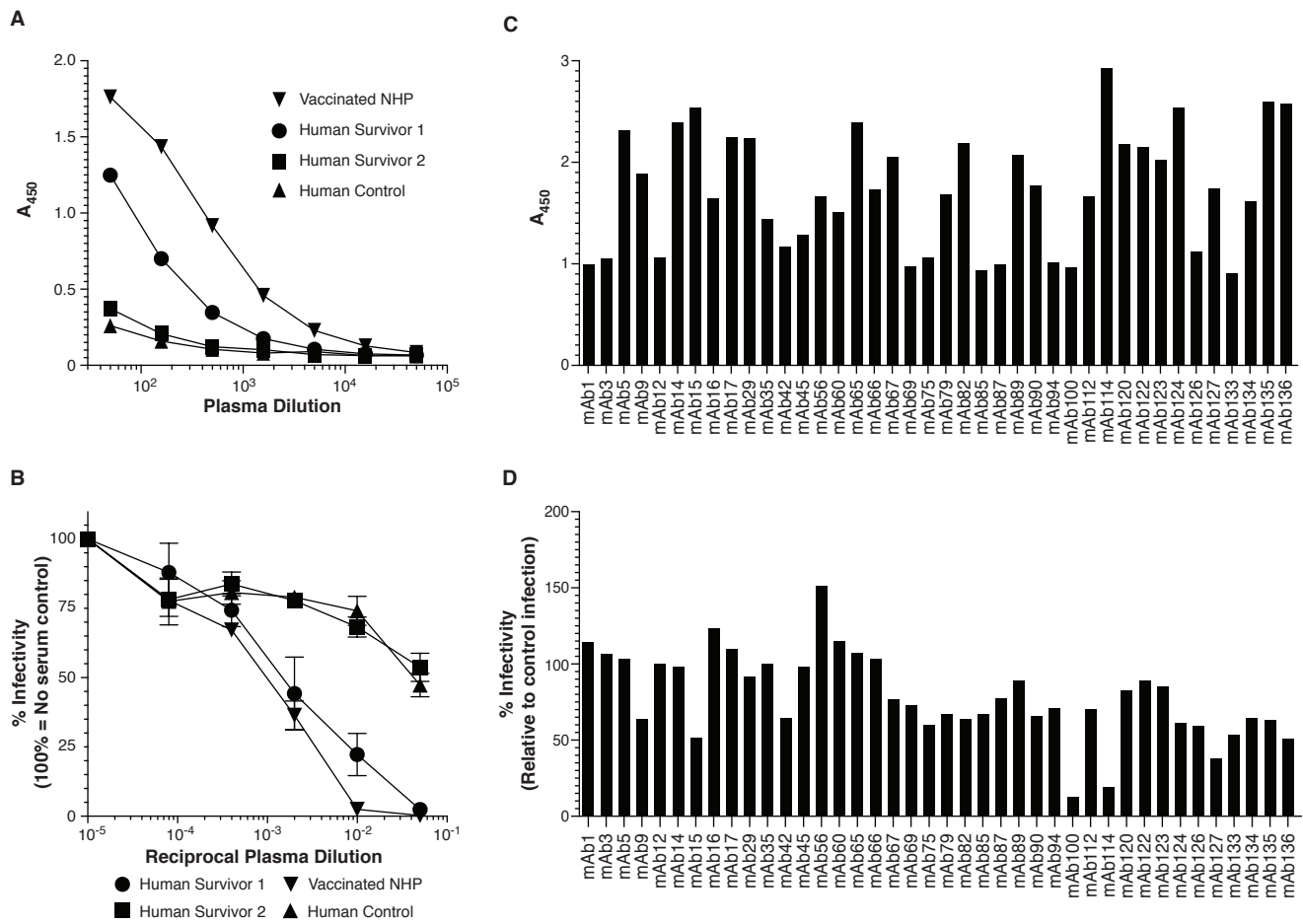
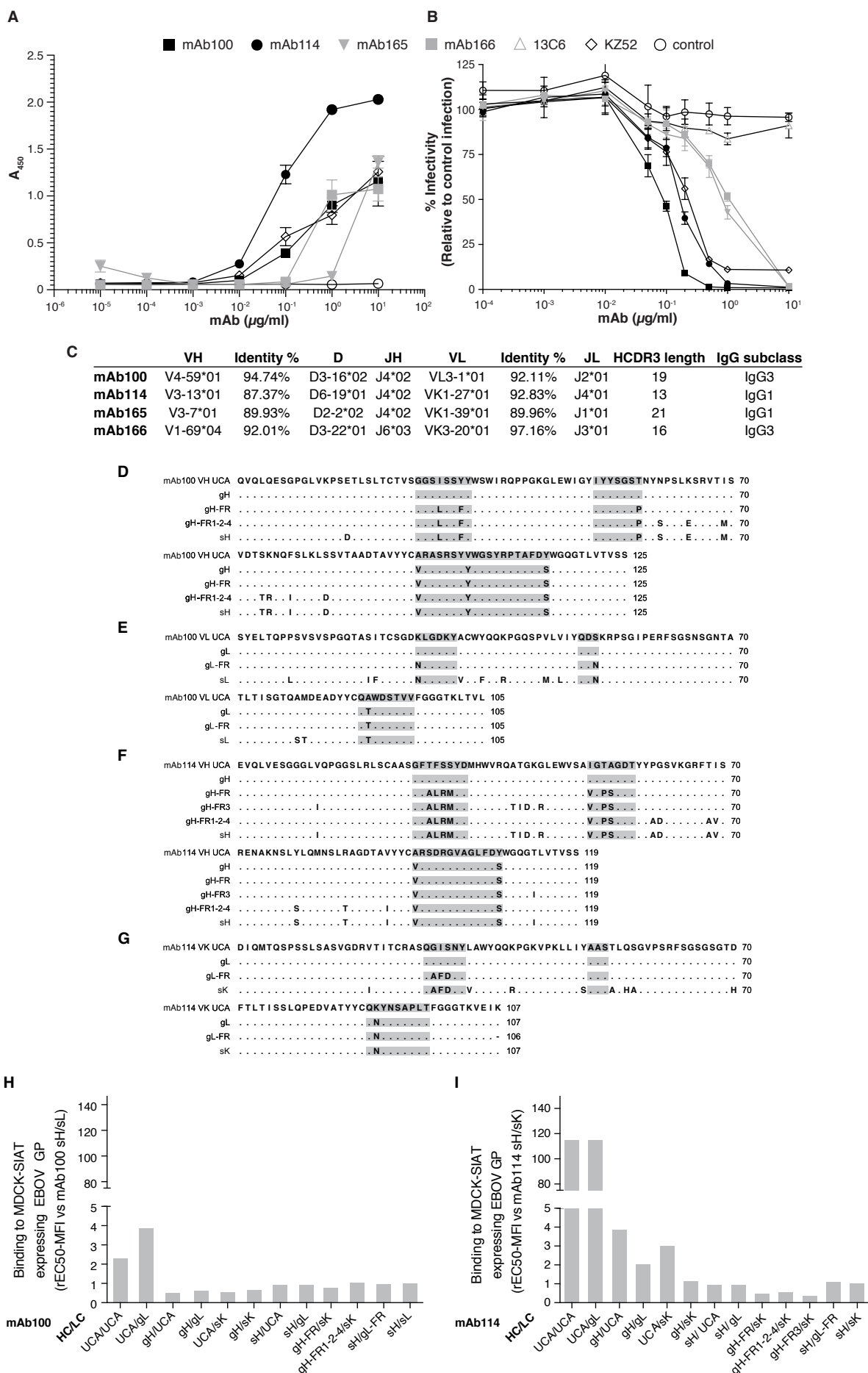


Figure 1. Isolation of antigen-specific monoclonal antibodies from Ebola virus

(A) Plasma obtained from two human survivors, an uninfected human donor and a non-human primate (NHP) vaccinated against EBOV GP were serially diluted and analyzed by GP ELISA, A_{450} (n=1). **(B)** Lentivirus particles expressing luciferase and bearing EBOV GP were incubated in the presence of heat inactivated serum for 1 hour prior to addition to HEK293T. Infection was determined by measuring relative luminescence (RLU) after 3 days. Infection % = (RLU with serum / RLU without serum) X 100% (n=3). **(C)** Immortalized B cell supernatants isolated from Survivor 1 were screened by EBOV GP ELISA A_{450} (n=1). **(D)** Immortalized B cell supernatants from **(C)** were diluted 1:50, incubated with Lentivirus particles pseudotyped with EBOV GP and infection determined as in **(B)**. Infection % = (RLU with supernatant / RLU without supernatant) X 100% (n=1).

Figure 2



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Figure 2. Characterization of purified EBOV GP monoclonal antibodies. (A) EBOV

GP ELISA in the presence of purified monoclonal antibodies as indicated, A_{450} . (B)

Lentivirus particles pseudotyped EBOV GP particles were incubated with increasing

amounts of purified monoclonal antibodies and infection measured as in Fig. 1B.

Infection % = (RLU with antibody / RLU without antibody) X 100% (n=3). (C) V gene

usage, sequence analysis and IgG subclass of antibodies from Survivor 1. (D) – (G)

Amino acid sequence of mAb100, mAb114 and variants descended from a putative

unmutated common ancestor (UCA) for heavy and light chains. Shaded regions represent

complementary determination regions 1-3. (H) and (I) Binding to EBOV GP expressed

on the surface of MDCK-SIAT cells by different mAb100 (H) and mAb114 (I) versions

in which all or subsets of somatic mutations in the wild type sH, sL (mAb100) or sK

(mAb114) chain were reverted to the germline sequence. Shown is the ratio between the

EC_{50} values of the variants and EC_{50} values of the wild-type sH/sL (mAb100) or sH/sK

(mAb114). UCA, unmutated common ancestor; gH or gL, germline V-gene revertants of

sH, sL, or sK in which the HCDR or LCDR3 are mature; gH-FR or gL-FR, germline V-

gene revertants of sH, sL or sK in which the HCDRs or LCDRs are mature; gH-FR1-2-4,

germline V-gene revertants of sH in which the HCDRs and HFR3 are mature; gH-FR3,

germline V-gene revertants of sH in which the HCDRs and HFR1, HFR2 and HFR4 are

mature; wild type, somatically mutated are sH, sL, or sK. EC_{50} ratio values above 100

indicate lack of detectable binding.

Figure 3

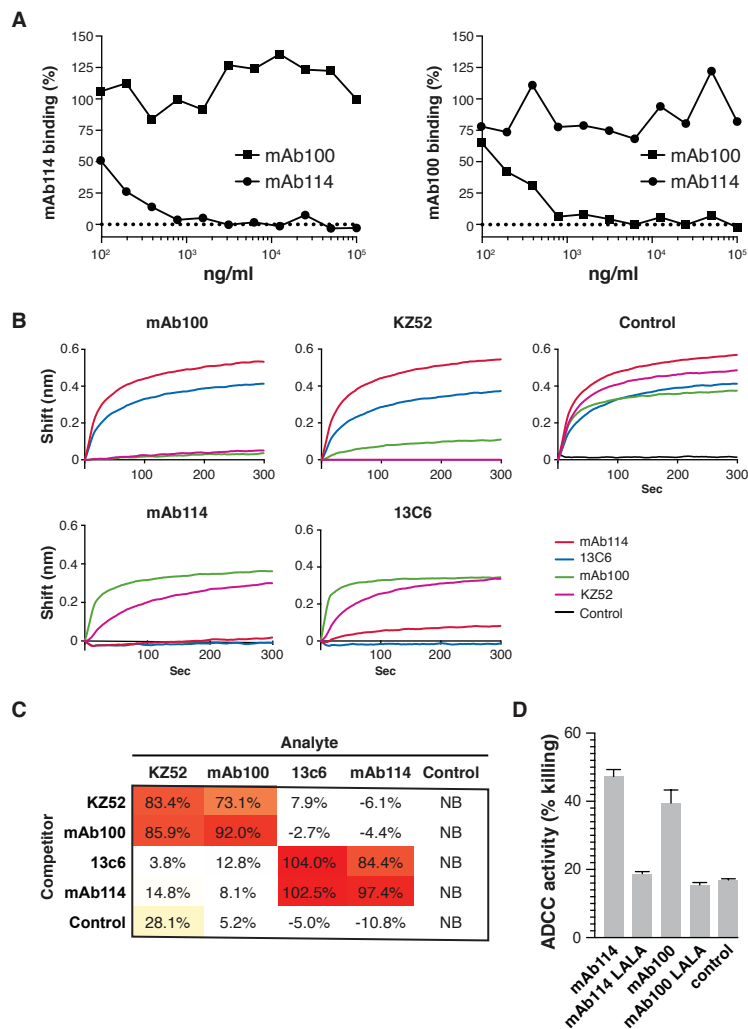
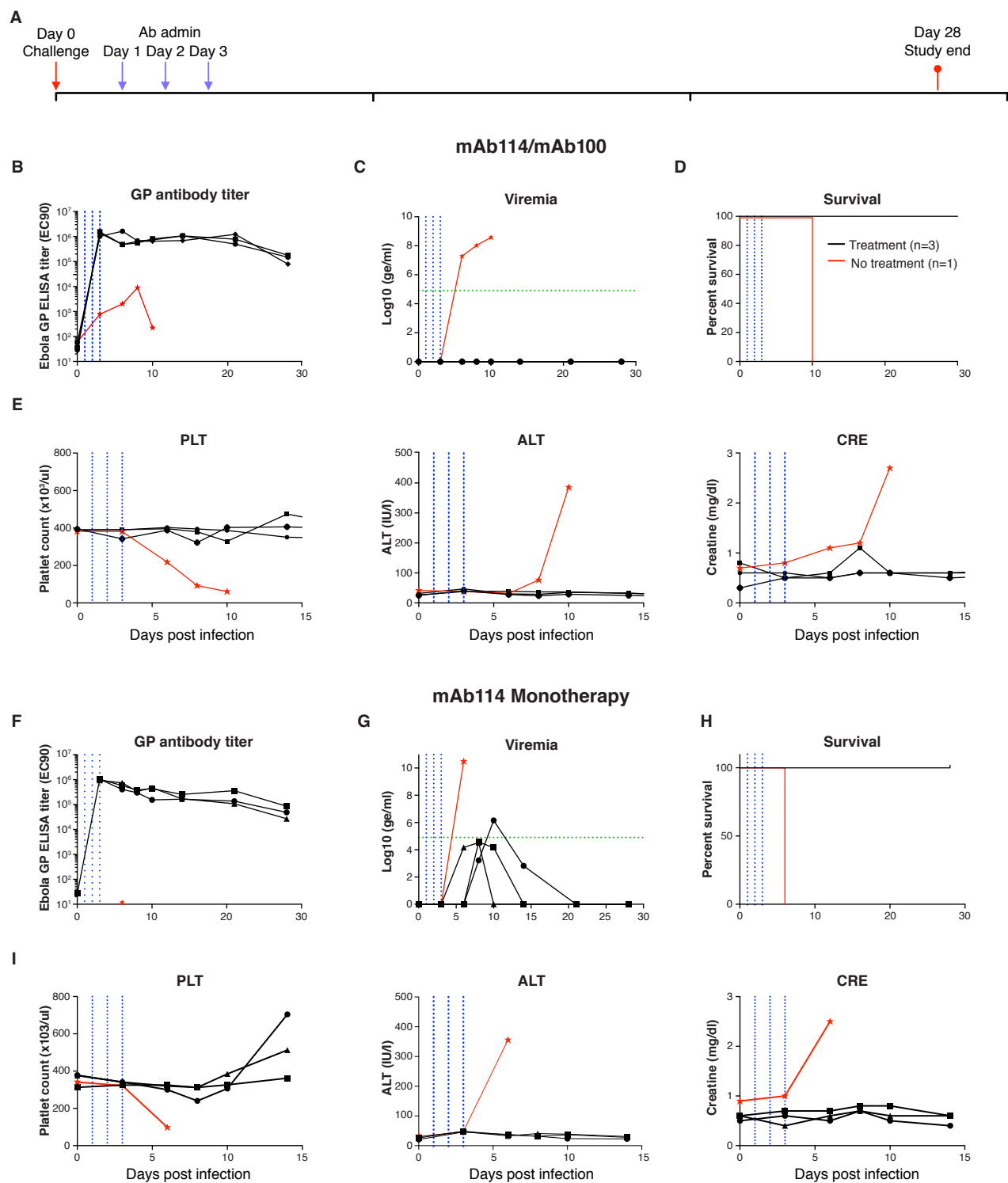


Figure 3. Binding region and effector function. (A) Inhibition of binding of biotinylated mAb114 (left) and mAb100 (right) to GP-expressing MDCK-SIAT cells by pre-incubation with increasing amounts of homologous or heterologous unlabeled antibodies. Shown is the percentage of binding of biotinylated antibodies as measured by flow cytometry using fluorophore-conjugated streptavidin. (B) and (C) Biolayer interferometry competitive binding assay to soluble EBOV GP using mAb100, mAb114, KZ52, 13C6 and isotype negative control. Biosensors were preloaded with GP followed by the competitor and analyte antibodies as indicated. Analyte binding curves (B) and quantitated % inhibition (C) are reported (n=3). (D) Antibody-dependent cell-mediated cytotoxicity (ADCC) assay was determined at 31.6 ng/mL of mAb100, mAb114 (n=3), control antibody or derivative antibodies with LALA mutations that abrogate Fc-mediated killing (n=1).

Figure 4



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368 **Figure 4. Passive transfer of antibody cocktail. (A)** Experimental challenge. Animals
369 were challenged with a lethal dose of EBOV GP on Day 0 and given injections of
370 antibody totaling 50 mg/kg at 24, 48 and 72 hours post-exposure. Surviving animals
371 were euthanized at the conclusion of the study (Day 28). Challenge data from monoclonal
372 antibody mAb114/mAb100 mixture **(B) – (E)**, or mAb114 monotherapy **(F) – (I)**.
373 Treatment animal in black, untreated control in red. **(B)** and **(F)** Ebola GP specific ELISA
374 titer (EC₉₀). **(C)** and **(G)** Viremia in blood by qRT-PCR expressed as genome equivalents
375 (ge) per mL. **(D)** and **(H)** Survival. **(E)** and **(I)** Selected hematologic and chemistry data.
376 Platelets (PLT), alanine transaminase (ALT), creatinine (CRE).
377

Figure 1

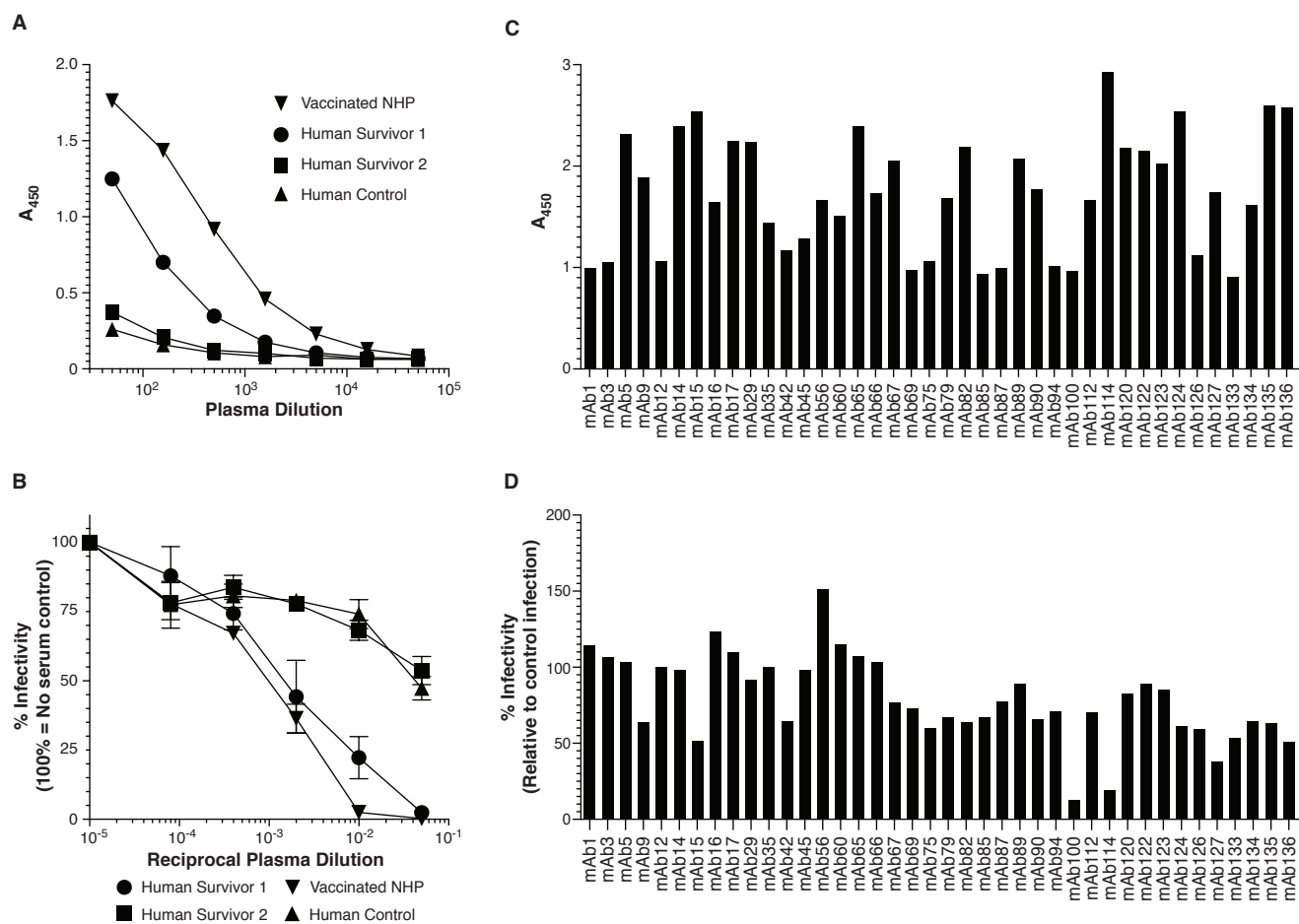


Figure 2

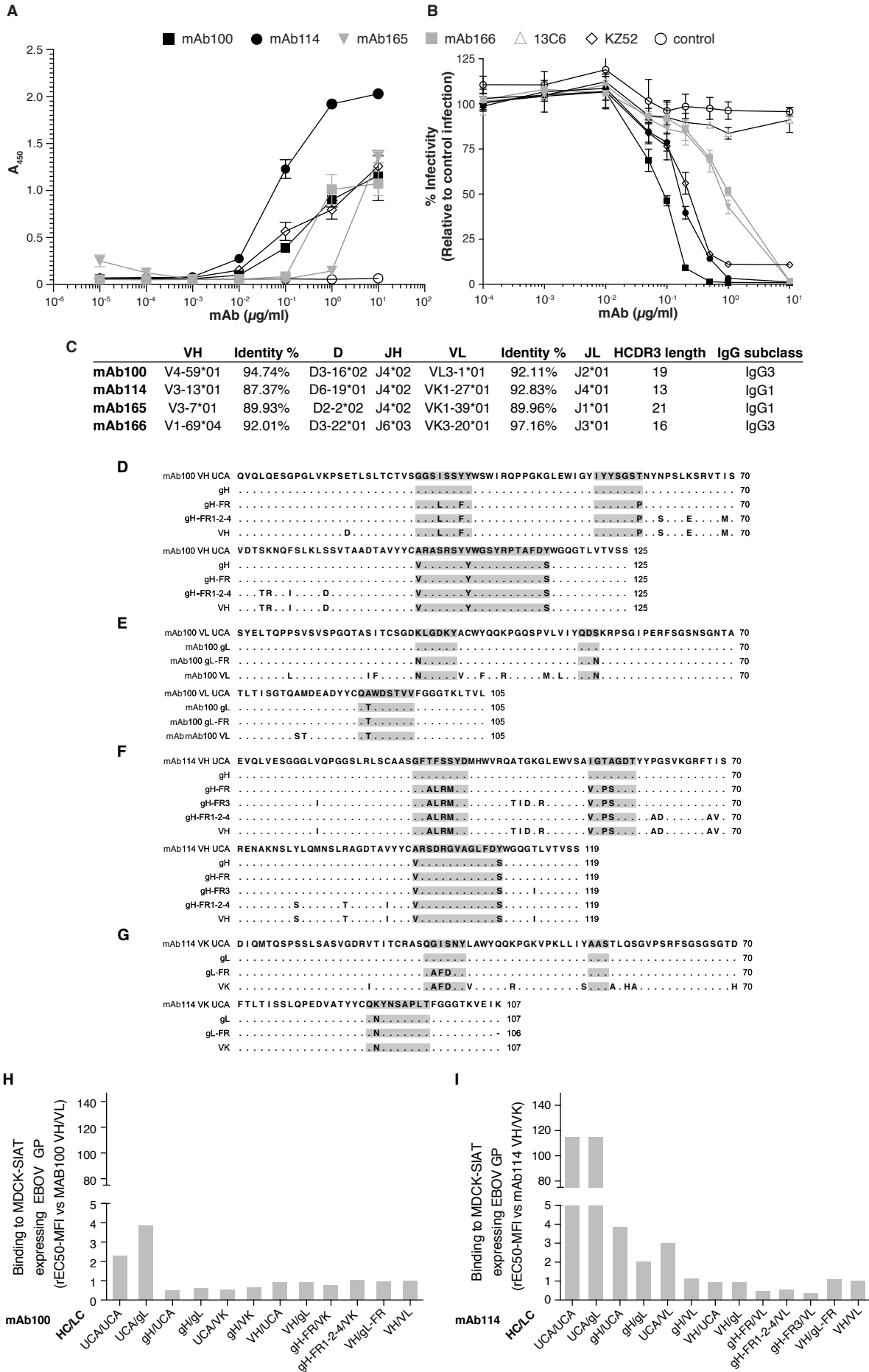


Figure 3

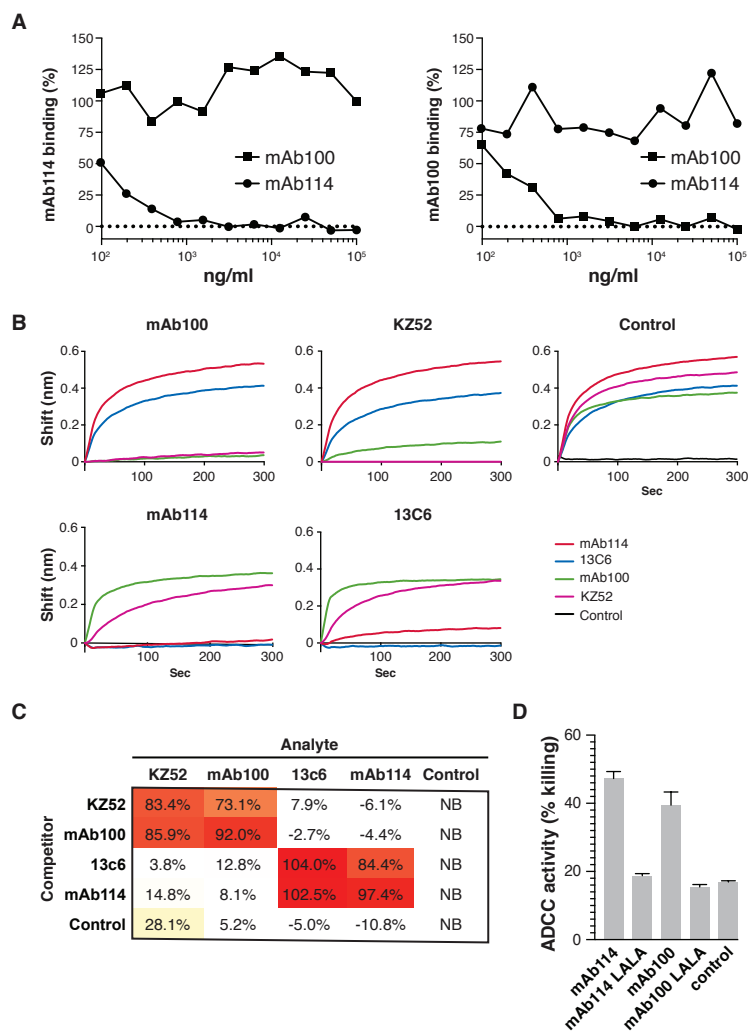
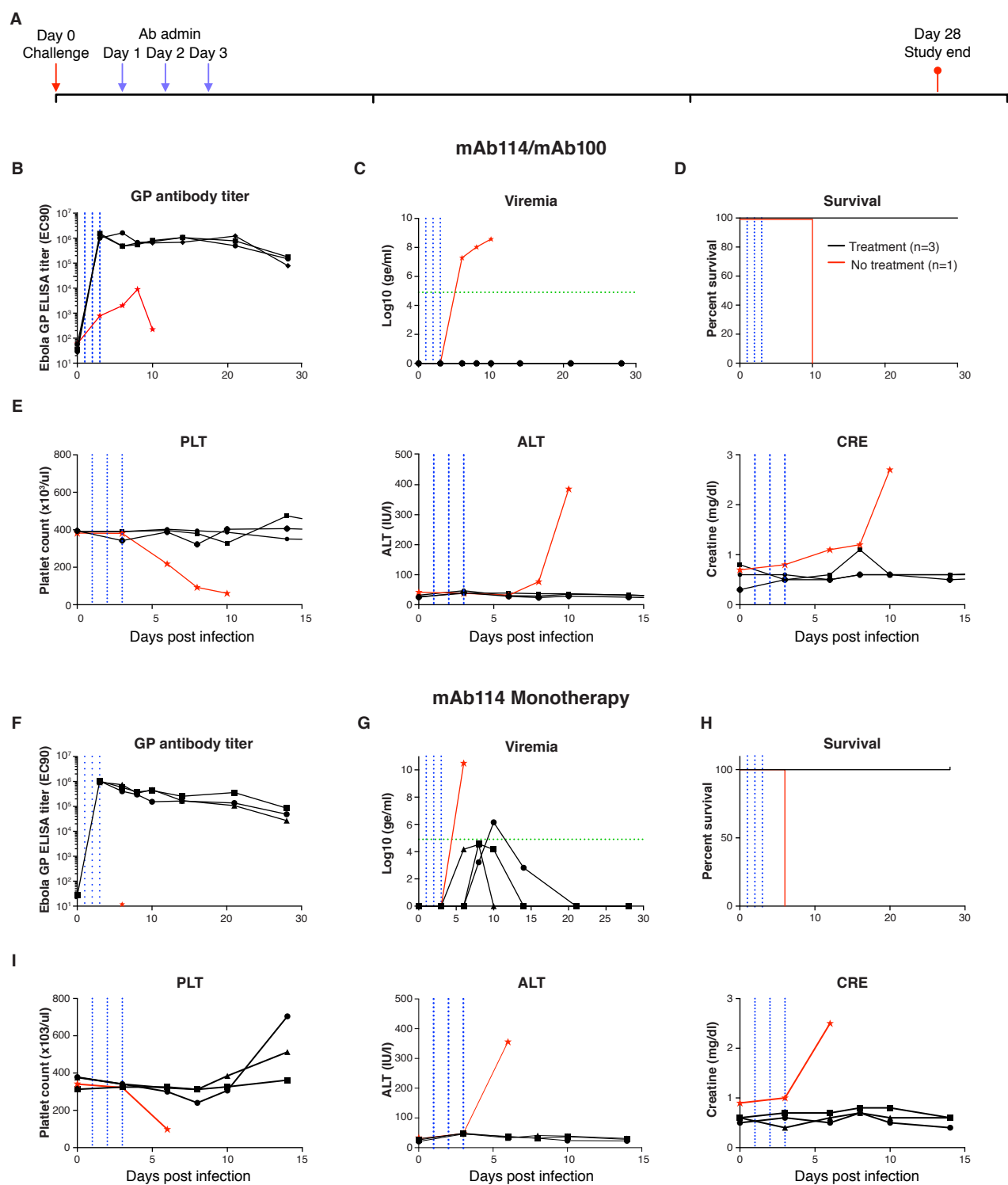


Figure 4



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Figure S1

Cell line ID	Abs 450	
	Und.	1/27
mAb151	1.119	0.162
mAb152	0.672	0.106
mAb153	0.854	0.131
mAb154	2.361	0.95
mAb155	0.115	0.08
mAb156	1.111	0.161
mAb157	2.256	0.554
mAb158	0.074	0.075
mAb159	3.298	2.529
mAb160	1.493	0.489
mAb161	0.227	0.086
mAb162	0.083	0.074
mAb163	3.099	1.805
mAb164	0.076	0.069
mAb165	3.171	1.722
mAb166	2.894	2.4
mAb167	3.368	2.974
mAb168	0.507	0.114
mAb169	0.081	0.072
mAb170	0.95	0.165
mAb171	1.998	0.895

Figure S2

mAb	IC50 (µg/mL)	95% CI	IC90 (µg/mL)	95% CI	IC99 (µg/mL)	95% CI	n
KZ52	0.06	0.02 to 0.14	17.21	8.47 to 35.00	>>1000	54,868	6
mAb 100	0.06	0.05 to 0.08	0.61	0.39 to 0.93	7.58	2.999 to 19.16	6
mAb 114	0.09	0.07 to 0.11	0.71	0.44 to 1.16	7.19	2.588 to 19.96	6
mAb 166	0.86	0.72 to 1.02	6.84	4.78 to 9.80	97.25	31.32 to 138.2	4
mAb 165	1.77	1.43 to 2.18	19.46	13.23 to 28.61	267.00	124.9 to 570.8	4

Figure S3

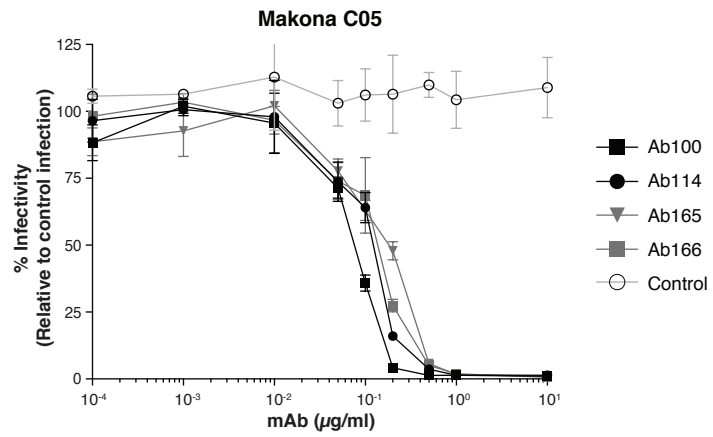


Figure S4

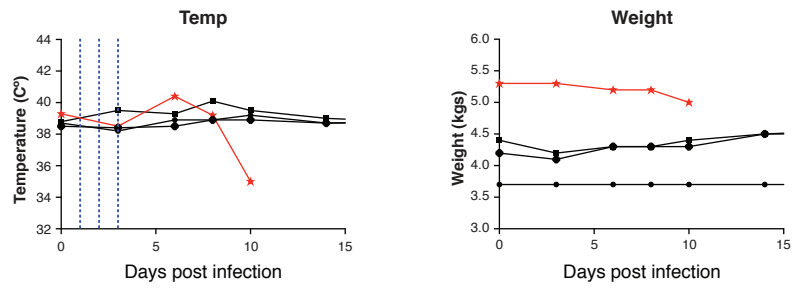
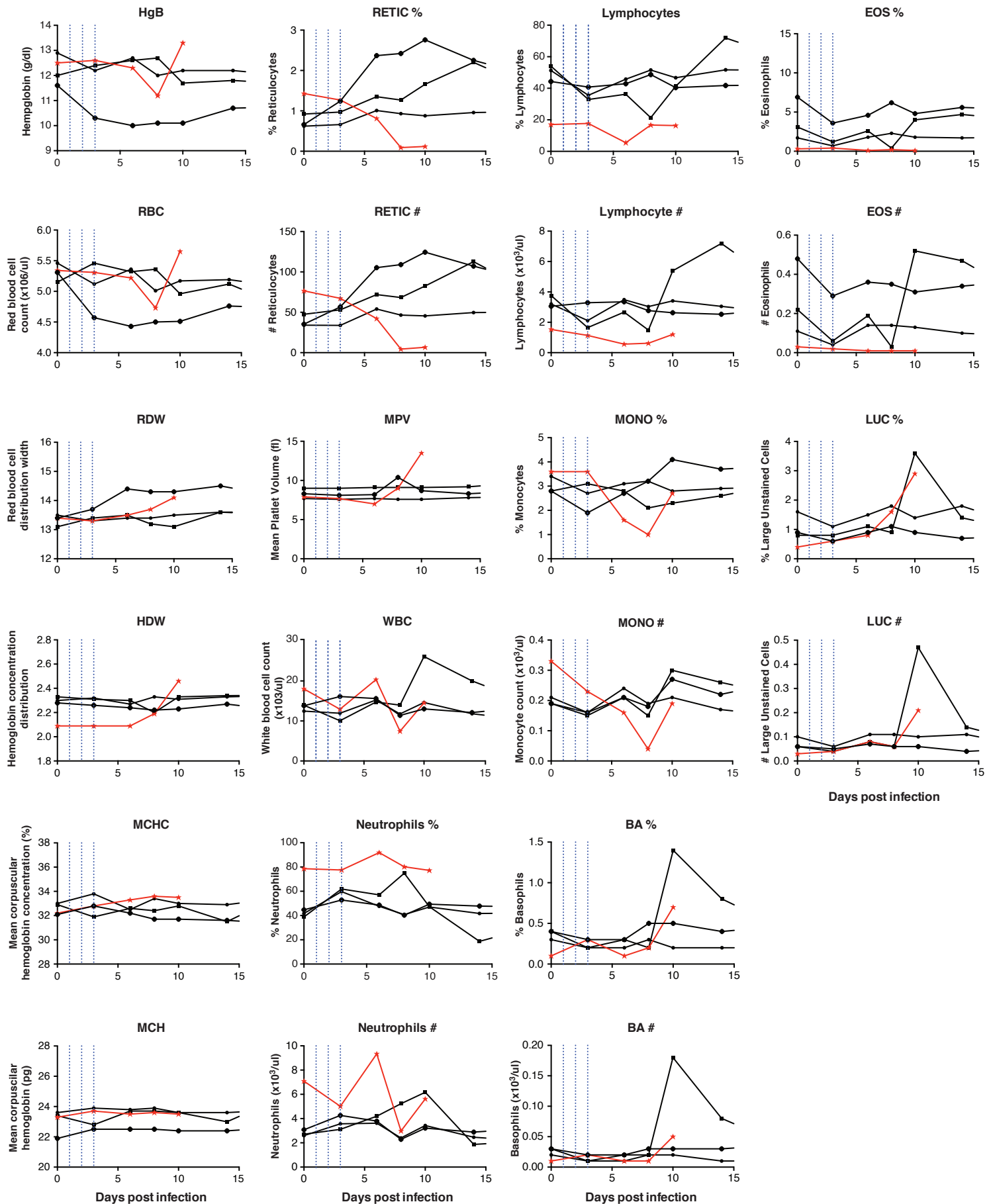
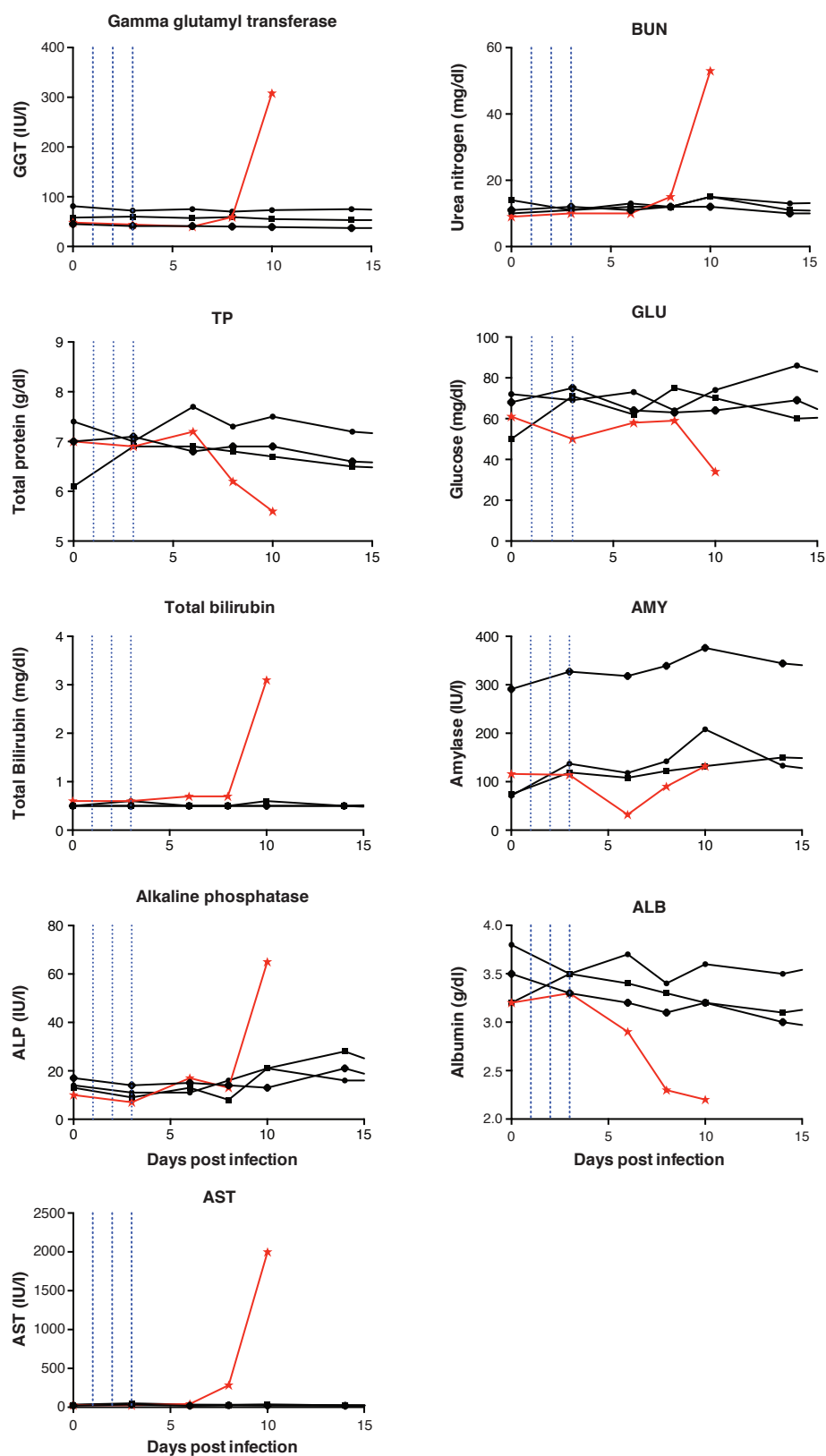


Figure S5



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Figure S6



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DISTRIBUTION STATEMENT A: Approved for public release; distribution is unlimited.

Figure S7

Cage 1																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NE	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	0	3	0	0	NE	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 2																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NE	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	1	2	0	0	NE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	V	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 3																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NE	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	0	0	0	0	NE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	V	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 4																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	2	2																		
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0																		
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y																		
Condition of Stool	0	0	0	0	0	0	0	3	0	3	3																		
Cough	N	N	N	N	N	N	N	N	N	N	N																		
Facial Edema	N	N	N	N	N	N	N	N	N	N	N																		
Rash	0	0	0	0	0	0	0	0	1	3	2																		
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	1	1	0	1	1	0	0																		
Bleeding	N	N	N	N	N	N	N	N	N	N	N																		
Motor Function	0	0	0	0	0	0	0	0	0	0	2																		

Observations

Biscuit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Fruit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Urine Output	Y=Yes, N=No, NE=Not Evaluated
Condition of Stool	0=normal, 1=not fully formed, 2=liquid, 3=none, NE=Not Evaluated
Cough	Y=Yes, N=No
Facial Edema	Y=Yes, N=No
Rash	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Rash/ Hemorrhage at Venipuncture Site	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Bleeding	N=None, F=Facial, V=Vaginal, O=Other, NE=Not Evaluated
Motor Function	0=normal, 1=mild coordination, dysfunction trouble manipulating items, 2=moderate dysfunction trouble grasping, moving, 3=severe dysfunction (uncoordinated actions), NE=Not Evaluated

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Figure S8

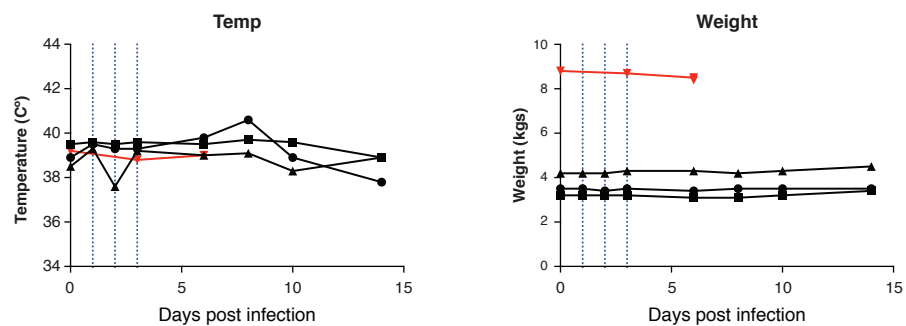
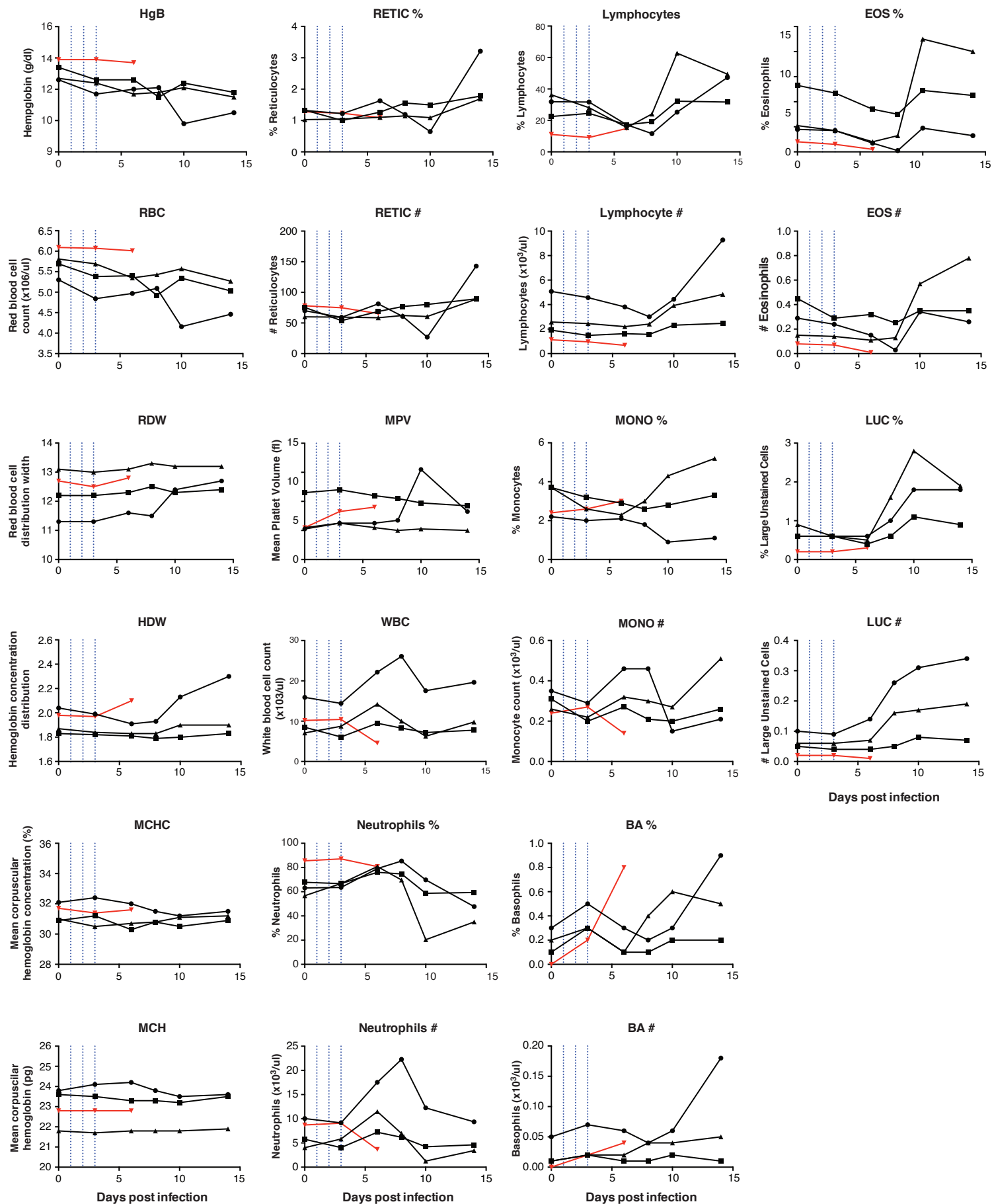
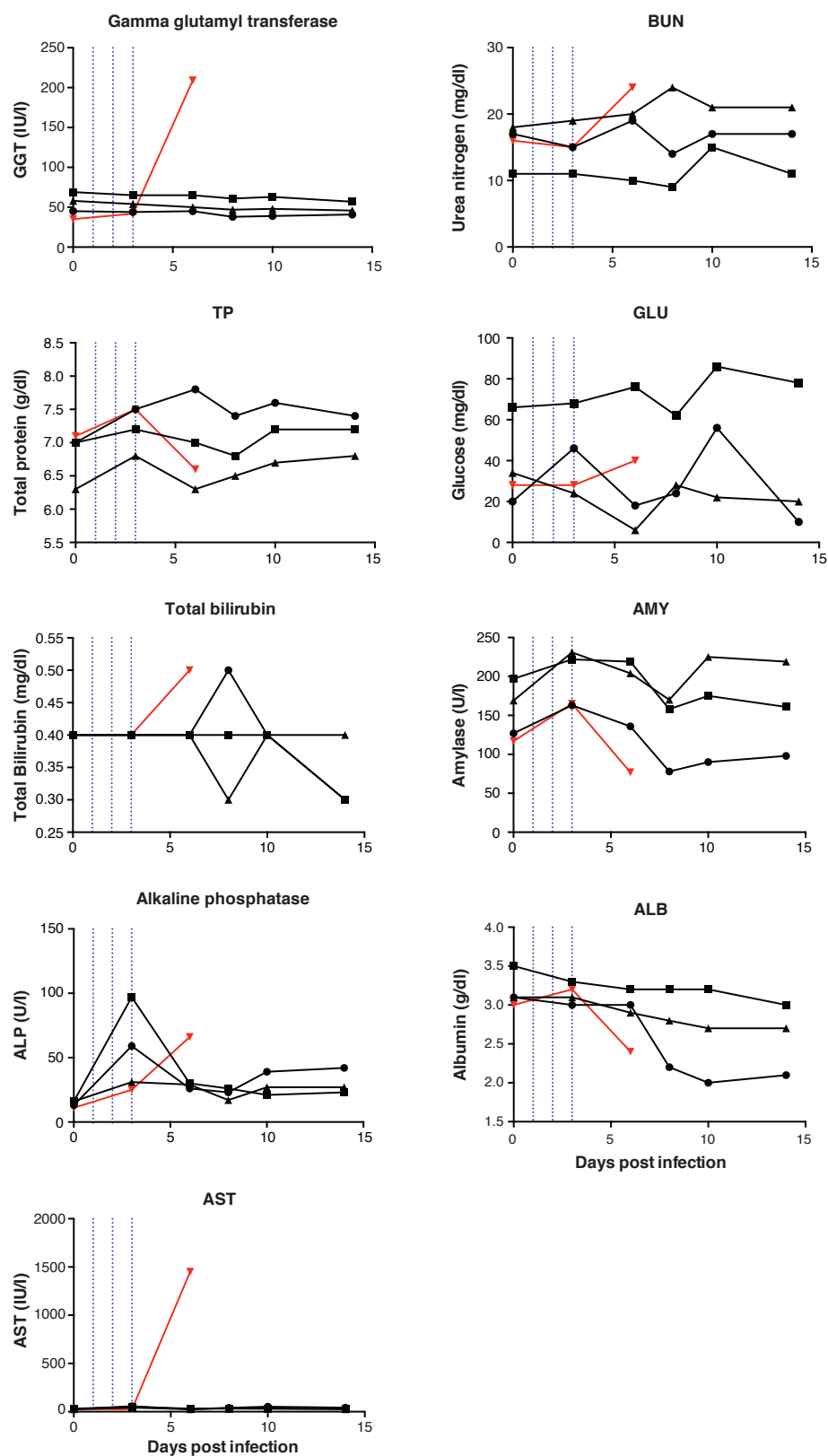


Figure S9



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Figure S10



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Figure S11

Cage 1																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	1	1	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	V	N	N	V	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 2																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	O	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 3																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Primary Euthanasia Criteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 4							
Day	0	1	2	3	4	5	6
Biscuit Consumption	0	1	0	0	0	0	2
Fruit Consumption	0	0	0	0	0	0	2
Urine Output	Y	Y	Y	Y	Y	Y	N
Condition of Stool	0	0	0	0	0	0	3
Cough	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0
Bleeding	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0

Observations

Biscuit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Fruit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Urine Output	Y=Yes, N=No, NE=Not Evaluated
Condition of Stool	0=normal, 1=not fully formed, 2=liquid, 3=none, NE=Not Evaluated
Cough	Y=Yes, N=No
Facial Edema	Y=Yes, N=No
Rash	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Rash/ Hemorrhage at Venipuncture Site	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Bleeding	N=None, F=Facial, V=Vaginal, O=Other, NE=Not Evaluated
Motor Function	0=normal, 1=mild coordination, dysfunction trouble manipulating items, 2=moderate dysfunction trouble grasping, moving, 3=severe dysfunction (uncoordinated actions), NE=Not Evaluated

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**Protective Monotherapy Against Lethal Ebola Virus Infection by a
Potently Neutralizing Antibody**

Davide Corti,^{1,4*} John Misasi,^{2*} Sabue Mulangu,² Daphne A. Stanley,² Masaru
Kanekiyo,² Suzanne Wollen,³ Aurélie Ploquin,² Nicole A. Doria-Rose,² Ryan P. Staupe,²
Michael Bailey,² Wei Shi,² Misook Choe,² Hadar Marcus,² Emily A. Thompson,² Alberto
Cagigi,² Chiara Silacci,¹ Blanca Fernandez-Rodriguez,¹ Laurent Perez,¹ Federica
Sallusto,¹ Fabrizia Vanzetta,⁴ Gloria Agatic,⁴ Elisabetta Cameroni,⁴ Neville Kisalu,²
Ingelise Gordon,² Julie E. Ledgerwood,² John R. Mascola,² Barney S. Graham,² Jean
Jacques Muyembe-Tamfun,⁵ John C. Trefry,^{3†} Antonio Lanzavecchia,^{1†} and Nancy J.
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44 **Supplementary Materials:**

45

46 **Materials and Methods**

47

48 **Isolation of monoclonal antibodies from EBOV survivors.** Two subjects who survived
49 the 1995 EBOV Kikwit variant outbreak in the Democratic Republic of Congo were
50 identified and enrolled in VRC200 clinical trial #NCT00067054 after giving signed
51 informed consent. Peripheral blood mononuclear cells (PBMCs) were obtained, stained
52 with directly labeled antibodies to CD22 (Pharmingen) and to immunoglobulin IgM, IgD,
53 and IgA. CD22⁺IgM⁻IgD⁻IgA⁻ B cells were isolated using FACS Aria, pulsed with
54 Epstein-Barr Virus (50% B958 supernatant) and seeded at 30 cells/well (for a total of 2.7
55 $\times 10^5$ purified cells) in replicate cultures in medium supplemented with CpG 2006 and
56 irradiated allogeneic PBMCs, as previously described (9). Culture supernatants were
57 collected after 2 weeks and tested for binding to ELISA plates coated with EBOV GP
58 (Mayinga variant), their specificity was confirmed using an unrelated antigen (tetanus
59 toxoid) and positive cultures were further tested for their ability to neutralize EBOV
60 pseudoviruses. Cultures that scored positive in the EBOV neutralization assay were sub-
61 cloned by limiting dilution.

62

63 **Antibody purification, labeling, genetic analysis, and reversion to germline.** The
64 usage of VH and VL gene segments was determined by sequencing, and analysis for
65 homology to known human V, D, and J genes was performed using the IMGT database
66 (<http://www.imgt.org/>). Human antibodies were affinity purified by protein A

67 chromatography (GE Healthcare) and dialyzed against PBS. Selected antibodies were
68 biotinylated using the EZ-Link NHS-PEO Solid Phase Biotinylation Kit (Pierce).
69 Antibodies were also produced recombinantly by cloning VH and VL genes via PCR into
70 human Igγ1, Igκ (mAb114, 165, 166), and Igλ (mAb100) expression vectors using gene-
71 specific primers (19). Antibodies used for animal studies were produced by transient
72 transfection of suspension cultured 293FreeStyle cells (Invitrogen) with PEI or Expi cells
73 with Expifectamine293 (Invitrogen). Supernatants from transfected cells were collected
74 after 6-10 days of culture and IgGs were affinity purified by Protein A chromatography
75 (GE Healthcare) and dialyzed against PBS. Purified mAbs were then concentrated with
76 Amicon Ultra centrifugal filters and sterilized by 0.22 μm filtration. The purity was
77 assessed by SEC-HPLC and SDS-PAGE. Endotoxin content was measured with the
78 Endpoint Chromogenic LAL assay (QCL-1000 TM assay, Lonza) according to
79 manufacturing instructions and shown to be below 0.25 EU/ml. Antibody concentrations
80 were determined using the BCA Protein Assay Kit (Thermo Scientific) using Rituximab
81 (Roche) as internal standard or A₂₈₀ using an Nanodrop (Thermo Scientific). Germlined
82 VH and VL nucleotide sequences were synthesized by Genscript, and their accuracy was
83 confirmed by sequencing.

84
85 **Antibodies.** KZ52 monoclonal antibody used in ELISA assay a kind gift from Dennis
86 Burton. KZ52 used elsewhere and 13C6 was purchased from IBT Bioservices. Unless
87 otherwise noted isotype control antibody was an anti-HIV gp120 IgG1.

Antibody neutralization assay. Supernatants or purified mAbs from immortalized B cell clones isolated from EVD survivor donors were assessed for neutralization potency using a single-round infection assay with EBOV GP-pseudotyped lentiviruses particles which express a luciferase reporter gene following entry (20). Unless indicated, all experiments utilized particles bearing GP from the EBOV Mayinga variant. In brief, HEK293T cells were used as infection targets and incubated in a 96-well plate 1 day before infection with pseudovirus in the presence of serially diluted supernatant or purified mAbs. Infected target cells were lysed 72 hours after infection and assayed with the Luciferase Assay System or Bright Glo (Promega), using a Victor X3 Plate Reader (PerkinElmer) to detect luciferase activity.

ELISA for serum antibody titer and GP-binding. Binding of EVD survivor's polyclonal sera, monoclonal antibodies and antibody in non-human primates to EBOV GP was evaluated by enzyme-linked immunosorbent assay (ELISA) as described previously (20). Titers for survivor and non-human primates were calculated as reciprocal EC₉₀ values (20).

Ebola virus GP vectors. Plasmid vector pVR1012 WT GP (Z) has been described previously (21). A vector expressing a soluble mucin deleted (Δ Muc) GP, GP Δ Muc Δ TM-GCN4 HisSA (Δ 309-505, Δ 657-676), was made using codon optimization and then synthesized and directly cloned in frame to a GCN4 trimerization domain-His-Strep Tactin domains (MKQIEDKIEEILSKIYHIENEIARIKKLIGEVASSSIEGRGSHHHHHHSAWSHPQFE

112 K) and sequence verified by Genscript. EBOV GP variant Makona-C05 (Acc#KJ660348)
113 was codon optimized, synthesized and sequence verified by Genscript.

114

115 **Antibody-dependent cell-mediated cytotoxicity (ADCC).** rAd5 EBOV GP-transduced
116 and non-transduced HEK293T cells were double labeled with membrane-bound and
117 intracellular stains in order to detect ADCC activity. Cells were incubated with 8 μ M
118 Plum stain (Plum cell labeling kit M.T.T.I. CellVue) followed by FBS. The cells were
119 then washed with RPMI 1640, incubated with 5 μ M Carboxyfluorescein Succinimidyl
120 ester (CFSE) (Vybrant CFDA SE cell tracer kit, Invitrogen), incubated with FBS and
121 washed again with RPMI 1640. Doubly labeled EBOV GP expressing cells were plated
122 in a V-bottomed 96-well plate at 5,000 cells/well. Antibodies were added to duplicate
123 samples at 31.6 ng/ml to the target cells for 20 minutes at room temperature. RSV
124 antibody (palivizumab) was used as a control antibody. Effector cells resuspended in
125 RPMI were then added to the target cells at the effector-to-target cell (ET) ratio 1:50
126 which was found to give the best signal to noise ratio. Each plate was incubated for 4 hr
127 at 37°C/5% CO₂. After 4 hr, plates were centrifuged at 250 x g and cells were fixed with
128 1% Paraformaldehyde (PFA) and analyzed via flow cytometry. As a control, labeled
129 non-transduced HEK293T cells were also used as targets for ADCC activity. Thirty
130 thousand non-gated events were acquired within 6 hr after the ADCC assay using an
131 LSRII cytometer (Becton Dickinson). The CFSE emission channel was read in B515
132 using a neutral density filter and Plum emission was read in R660. Following acquisition,
133 analysis was performed using FlowJo software (Tree Star). Percent killing was obtained
134 by quantifying dead cells (Plum⁺,CFSE⁻) out of the total Plum positive population. For

135 mAbs, ADCC killing was measured by subtracting percent killing of nontransduced cells
136 from percent killing of transduced cells.

137

138 **Antibody variants.** UCA sequences of the isolated antibodies were determined with
139 reference to the IMGT database (<http://www.imgt.org/>). Antibody variants in which
140 single or multiple mutations were reverted to the germline sequence were produced by
141 gene synthesis (Genscript) and used to produce a large set of mAb114 and mAb100
142 antibody variants.

143

144 **Binding of antibody variants to transfected cells.** mAb114 and mAb100 antibody
145 variants were used to stain MDCK-SIAT1 cell lines transduced to express EBOV GP as a
146 stable membrane protein (Makona variant). Binding of antibodies was analysed using a
147 Becton Dickinson FACS Canto2 (BD Biosciences) with FlowJo software (TreeStar). The
148 relative affinities of antibody binding to surface GP were determined by interpolating the
149 concentration of antibody required to achieve 50% maximal binding (EC_{50}) from the
150 plotted binding curves using the mean-fluorescence intensity (MFI) fitted with a 4-
151 parameter nonlinear regression with a variable slope.

152

153 **Inhibition of binding assay on GP-expressing cells.** mAb100 and mAb114 were
154 biotinylated using the EZ-Link NHS-PEO solid phase biotinylation kit (Pierce). Labeled
155 antibodies were tested for binding to GP-expressing MDCK-SIAT-1 cells to determine
156 the optimal concentration of each antibody to achieve 70–80% maximal binding. The
157 biotin-labelled antibodies were then used as probes to assess, by flow cytometry, whether

their binding (measured using fluorophore-conjugated streptavidin) was inhibited by pre-incubation of GP cells with homologous or heterologous unlabelled antibodies.

Production of purified GP. Expi (Invitrogen) cells were transfected with GP Δ Muc Δ TM-GCN4 HisSA and pCMV-Sport Furin (7:3 ratio) using 293Fectin (Invitrogen) at a ratio of 2 mL 293Fectin:1mg total DNA. 18-24 hours following transfection, 1/10th volume of AbBooster (ABI Scientific) was added and culture media collected 5 days later. Supernatant was filtered and protein purified as described previously (22).

Biolayer interferometry antibody cross-competition assay. Antibody cross-competition was determined based on biolayer interferometry using a fortéBio Octet HTX instrument. EBOV GP Δ Muc protein was loaded onto HIS biosensors (AR2G, fortéBio) through amine coupling for 600 s. Biosensors were equilibrated for 120 s in 1% BSA in PBS (BSA-PBS) prior to capturing competitor mAbs. GP proteins were diluted to 10 μ g/mL; mAbs KZ52, mAb100, mAb114, 13C6, and IgG1 isotype control Ab were diluted to 35 μ g/mL in BSA-PBS. Binding of competitor mAbs was assessed for 300 s followed by a brief equilibration for 60 s prior to binding assessment of probing mAbs. Binding of probing mAbs was assessed for 300 s. Percent inhibition (PI) of probing mAbs binding to GP by competitor mAbs was carried out by an equation: $PI = 100 - [(probing\ mAb\ binding\ in\ the\ presence\ competitor\ mAb) / (probing\ mAb\ binding\ in\ the\ absence\ of\ competitor\ mAb)] \times 100$. All the assays were performed in duplicate and with agitation set to 1,000 rpm at 30°C.

181

182 **Animal study and safety.** Research was conducted under an IACUC-approved protocol
183 in compliance with the Animal Welfare Act, PHS Policy, and other Federal statutes and
184 regulations relating to animals and experiments involving animals. The facilities where
185 this research was conducted are accredited by the Association for Assessment and
186 Accreditation of Laboratory Animal Care, International and adhere to principles stated in
187 the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.
188 Animal study protocols were approved by both the Vaccine Research Center and United
189 States Army Medical Research Institute of Infectious Diseases IACUCs. All animals
190 were Vietnamese-origin rhesus macaques (*Macaca mulatta*), female, approximately 2–5
191 years of age and were obtained from Covance. Animals were randomly assigned to
192 treatment groups based on sequential selection from a population inventory. Sample sizes
193 of three animals per BSL4 EBOV challenge group provide 80% power to detect a
194 difference in survival rates assuming 100% survival (3/3 treated survive) vs. 0% survival
195 in negative controls at the 95% confidence level (1-tailed Fisher exact test). Prior to
196 blood sampling or treatment, animals were anesthetized with ketamine or telazol.

197

198 **Antibody administration.** In the mAb100/mAb114 cocktail challenge, antibodies were
199 mixed in PBS at 4 mg/mL of mAb100 and 46 mg/mL of mAb114 for a total antibody
200 concentration of 50 mg/mL. In the second challenge, animals received 50 mg/mL of
201 mAb114 in PBS. Antibodies were administered via intravenous injection in peripheral
202 veins using ≤ 20 gauge butterfly needles over a period ≥ 15 minutes in a single bolus via
203 syringe pump.

204

205 **EBOV challenge.** Animal studies conducted at USAMRIID were approved by the
206 IACUC. Animals were transferred one week prior to challenge to the Bio-Safety Level-4
207 (BSL-4) facility for exposure to a lethal (1000 PFU) i.m. EBOV Kikwit variant
208 challenge. Challenge studies included a single unvaccinated animal (control); the use of
209 historical control (n>50) allows for one untreated control to be used in each challenge
210 experiment. While at USAMRIID the monkeys were fed and checked daily. During the
211 EBOV challenge study, blood was collected from the NHP for hematological,
212 biochemical and virological analyses. Following the development of clinical signs,
213 animals were checked multiple times daily. Institute scoring criteria were used to
214 determine timing of humane euthanasia under anesthesia.

215

216 **Detection of EBOV.** RNA was isolated from plasma of EBOV-exposed NHP by real
217 time qPCR as described previously (23). EDTA plasma was added to TriReagent LS
218 (Sigma), 1 part to 3 parts, in preparation for qRT-PCR. Inactivated samples were
219 extracted and eluted with AVE Buffer (QIAGEN, Valencia, CA) using a QIAamp Viral
220 RNA Mini Kit (Qiagen, Valencia, CA). All samples were run on an Applied Biosystems
221 7500 Fast Dx Real-Time PCR instrument (Life Technologies, Grand Island, NY).
222 Reactions were performed with SuperScript II One-Step RT-PCR System (Life
223 Technologies, Grand Island, NY) with additional MgSO₄ added to a final
224 concentration of 3.0 mM. All samples were run in triplicate 5 µL each. The average of
225 the triplicates was multiplied by 200 to obtain genomes equivalents per mL, then
226 multiplied by a dilution factor of 4 for the final reported value. The sequence of the

227 primer and probes for the EBOV glycoprotein are described below. The genomic
228 equivalents were determined using a synthetic RNA standard curve of known
229 concentration. Forward primer: 5' - TTT TCA ATC CTC AAC CGT AAG GC - 3' ;
230 REVERSE PRIMER : 5' - CAG TCC GGT CCC AGA ATG TG - 3' ; PROBE: 6FAM - CAT
231 GTG CCG CCC CAT CGC TGC - TAMRA.

232

233 **Supplementary Figures**

234

235 **Figure S1-S11**

236

Figure S1

Cell line ID	Abs 450	
	Und.	1/27
mAb151	1.119	0.162
mAb152	0.672	0.106
mAb153	0.854	0.131
mAb154	2.361	0.95
mAb155	0.115	0.08
mAb156	1.111	0.161
mAb157	2.256	0.554
mAb158	0.074	0.075
mAb159	3.298	2.529
mAb160	1.493	0.489
mAb161	0.227	0.086
mAb162	0.083	0.074
mAb163	3.099	1.805
mAb164	0.076	0.069
mAb165	3.171	1.722
mAb166	2.894	2.4
mAb167	3.368	2.974
mAb168	0.507	0.114
mAb169	0.081	0.072
mAb170	0.95	0.165
mAb171	1.998	0.895

Figure S1. Second screening of immortalized memory B-cells from Survivor. 14

million PBMCs were used to isolate 59,500 IgG memory B cells which were immortalized as in Fig. 1A-D. After removal of non-specific binding, 21 culture supernatants were found to specifically bind Ebola GP as measured by ELISA. Shown are ELISA A_{450} values for undiluted and 1:27 dilutions of the supernatants. Amongst the 21 supernatants, only 2 B cell clones (mAb165, mAb166) were rescued for further analysis.

Figure S2

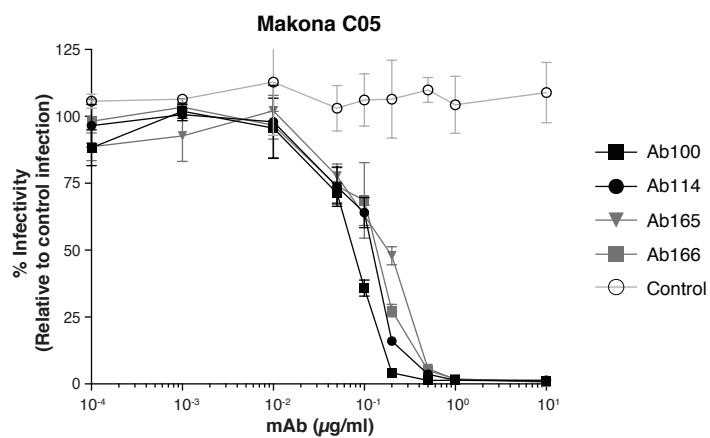
mAb	IC50 (µg/mL)	95% CI	IC90 (µg/mL)	95% CI	IC99 (µg/mL)	95% CI	n
KZ52	0.06	0.02 to 0.14	17.21	8.47 to 35.00	>>1000	54,868	6
mAb 100	0.06	0.05 to 0.08	0.61	0.39 to 0.93	7.58	2.999 to 19.16	6
mAb 114	0.09	0.07 to 0.11	0.71	0.44 to 1.16	7.19	2.588 to 19.96	6
mAb 166	0.86	0.72 to 1.02	6.84	4.78 to 9.80	97.25	31.32 to 138.2	4
mAb 165	1.77	1.43 to 2.18	19.46	13.23 to 28.61	267.00	124.9 to 570.8	4

246 **Figure S2. Neutralization of isolated monoclonal antibodies.** Neutralization assays
247 were performed as in Fig. 2B. IC₅₀, IC₉₀, and IC₉₉ were determined using non-linear
248 regression-variable slope (Graph Pad).

249

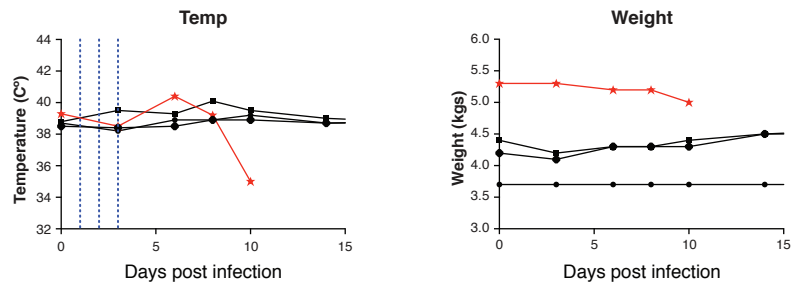
250

Figure S3



251 **Figure S3. Inhibition of EBOV Makona variant by mAb100 and mAb114.** Lentivirus
252 particles bearing GPs from EBOV Makona variant were incubated with serially diluted
253 mAb100, mAb114 or isotype control. Infection measured as in Fig. 2B (n=3).
254
255

Figure S4

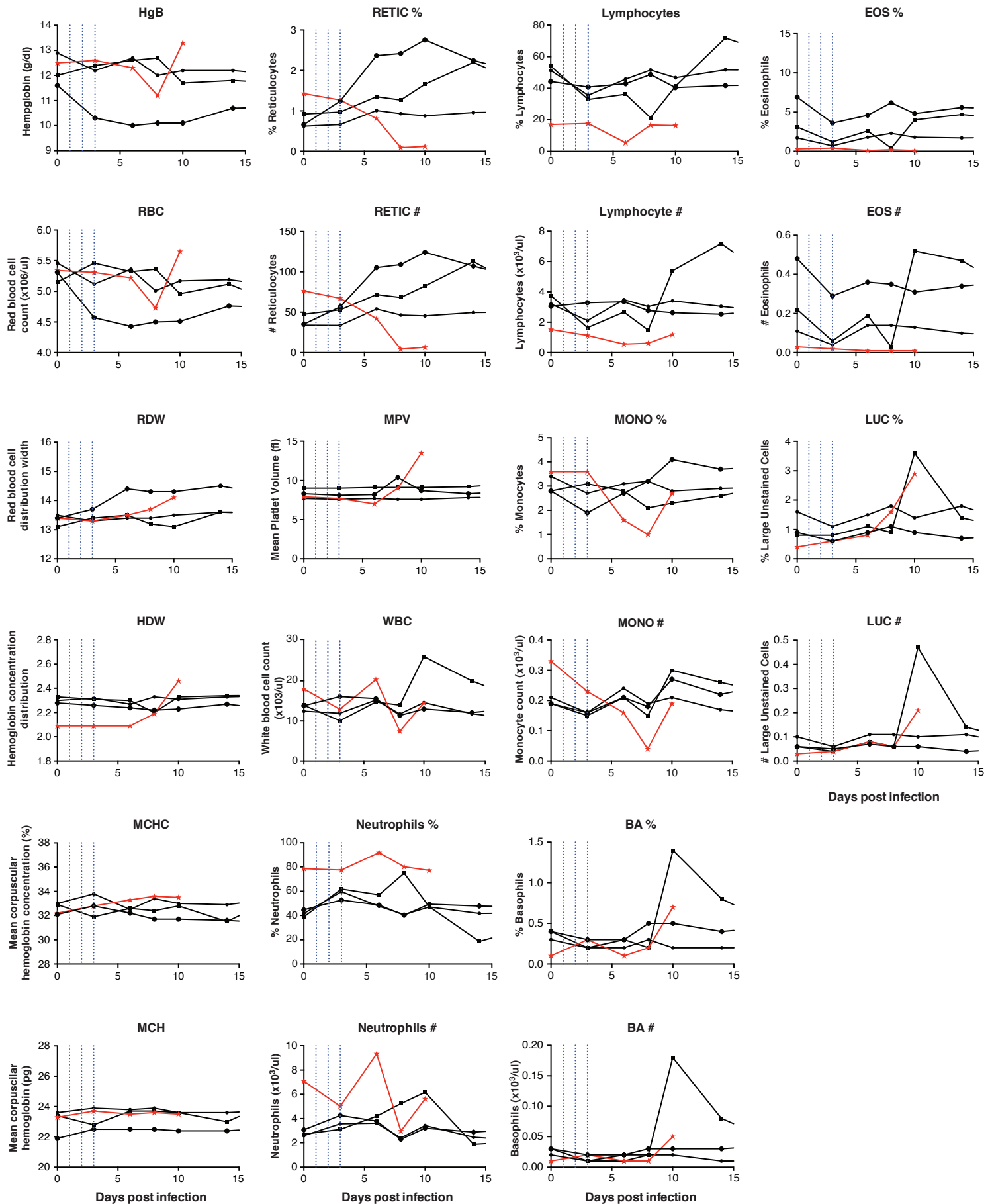


256 **Figure S4. Additional clinical data from passive transfer of mAb114/mAb100.**

257

258

Figure S5



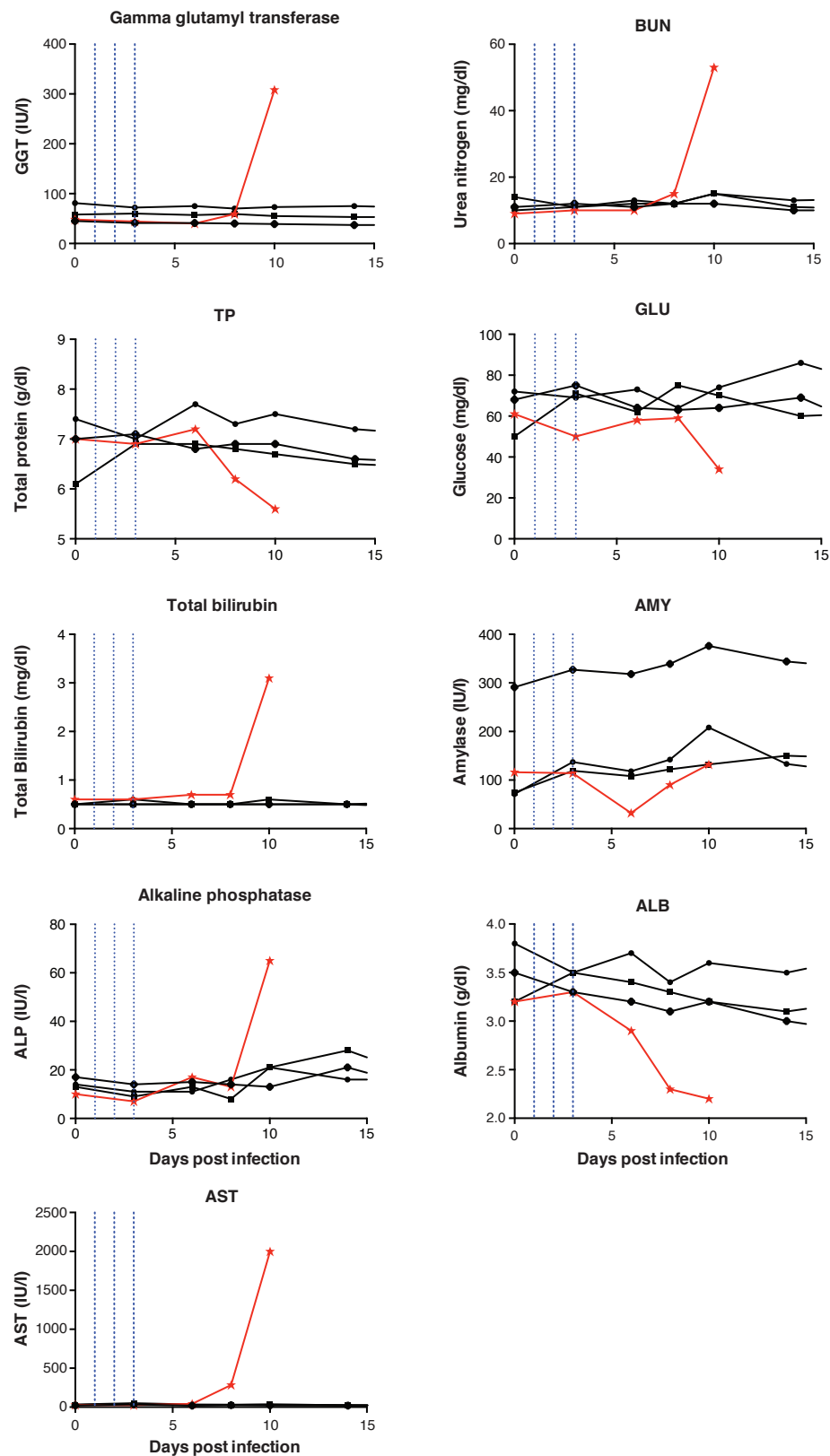
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259 **Figure S5. Additional hematology data from passive transfer of mAb114/mAb100.**

260

261

Figure S6



262 **Figure S6. Additional serum chemistries from passive transfer of mAb114/mAb100.**

263

264

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Figure S7

Cage 1																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NE	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	0	3	0	0	NE	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 2																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NE	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	1	2	0	0	NE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	V	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 3																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NE	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	0	0	0	0	NE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	V	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 4																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	2	2																		
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0																		
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y																		
Condition of Stool	0	0	0	0	0	0	0	3	0	3	3																		
Cough	N	N	N	N	N	N	N	N	N	N	N																		
Facial Edema	N	N	N	N	N	N	N	N	N	N	N																		
Rash	0	0	0	0	0	0	0	0	1	3	2																		
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	1	1	0	1	1	0	0																		
Bleeding	N	N	N	N	N	N	N	N	N	N	N																		
Motor Function	0	0	0	0	0	0	0	0	0	0	2																		

Observations

Biscuit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Fruit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Urine Output	Y=Yes, N=No, NE=Not Evaluated
Condition of Stool	0=normal, 1=not fully formed, 2=liquid, 3=none, NE=Not Evaluated
Cough	Y=Yes, N=No
Facial Edema	Y=Yes, N=No
Rash	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Rash/ Hemorrhage at Venipuncture Site	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Bleeding	N=None, F=Facial, V=Vaginal, O=Other, NE=Not Evaluated
Motor Function	0=normal, 1=mild coordination, dysfunction trouble manipulating items, 2=moderate dysfunction trouble grasping, moving, 3=severe dysfunction (uncoordinated actions), NE=Not Evaluated

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265 **Figure S7. Clinical observation scoring from passive transfer of mAb114/mAb100.**

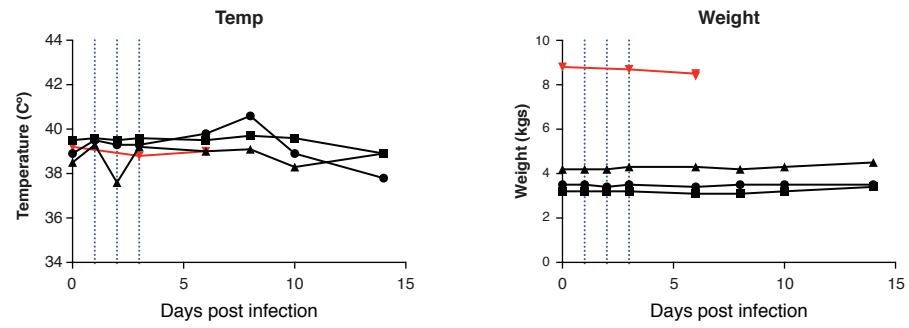
266 Data from multiple observations each day post-exposure are presented as the maximal

267 level observed for that day.

268

269

Figure S8

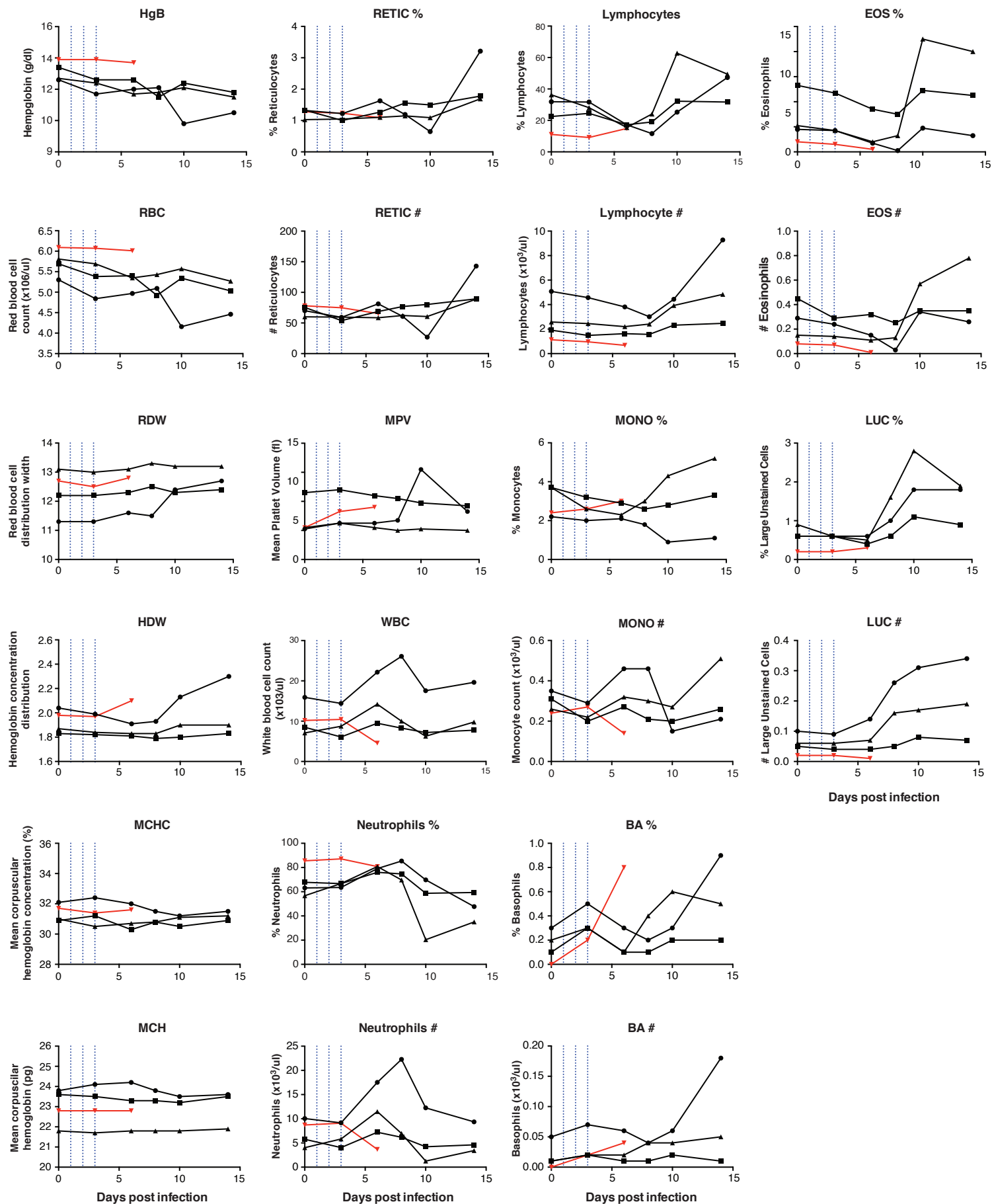


270 **Figure S8. Additional clinical data from passive transfer of mAb114.**

271

272

Figure S9



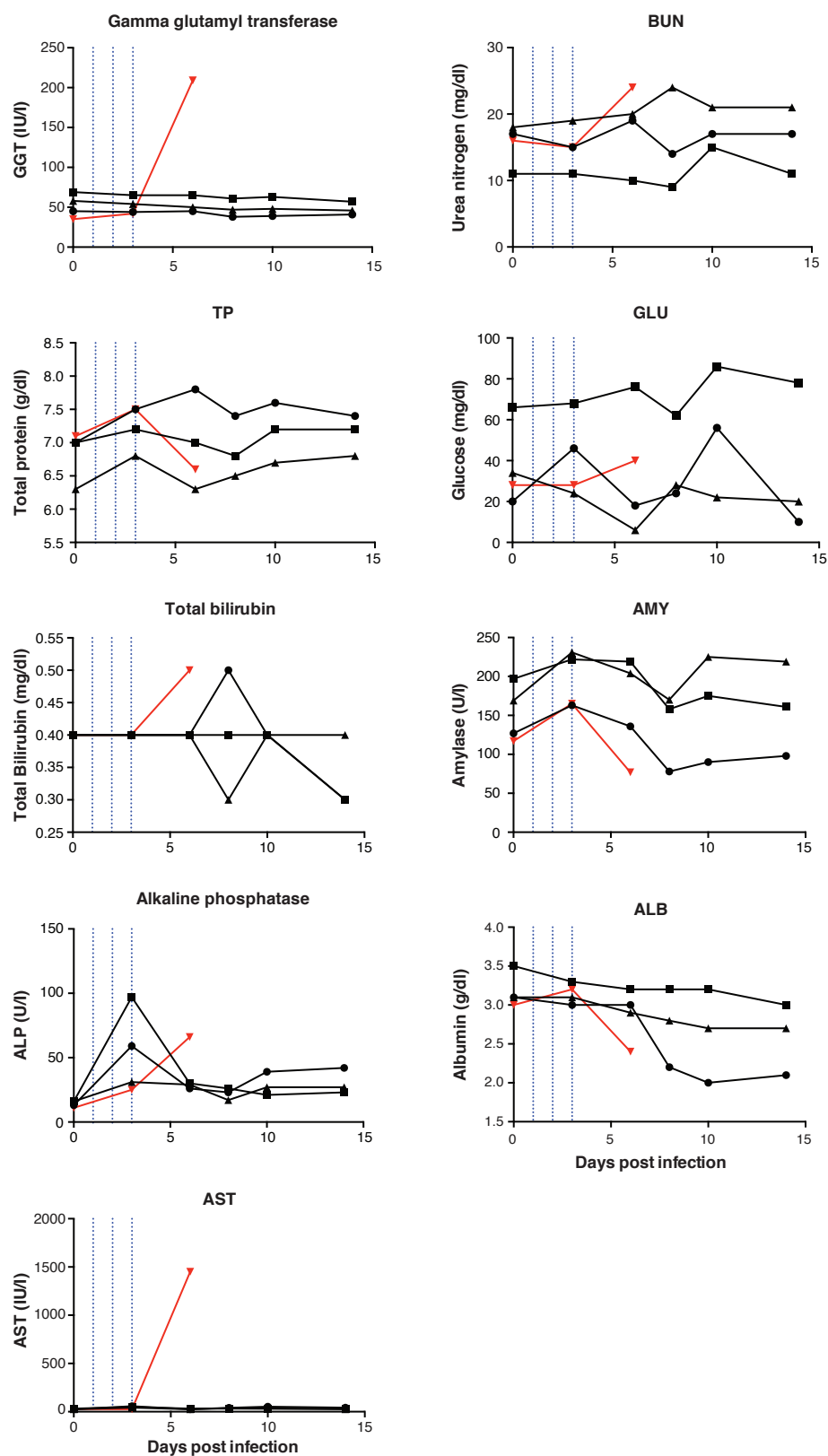
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273 **Figure S9. Additional hematology data from passive transfer of mAb114.**

274

275

Figure S10



276 **Figure S10. Additional serum chemistries from passive transfer of mAb114.**

277

278

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Figure S11

Cage 1																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	1	1	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	V	N	N	V	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 2																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	O	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 3																													
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Primary Euthanasia Criteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 4							
Day	0	1	2	3	4	5	6
Biscuit Consumption	0	1	0	0	0	0	2
Fruit Consumption	0	0	0	0	0	0	2
Urine Output	Y	Y	Y	Y	Y	Y	N
Condition of Stool	0	0	0	0	0	0	3
Cough	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipuncture Site	0	0	0	0	0	0	0
Bleeding	N	N	N	N	N	N	N
Motor Function	0	0	0	0	0	0	0

Observations

Biscuit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Fruit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Urine Output	Y=Yes, N=No, NE=Not Evaluated
Condition of Stool	0=normal, 1=not fully formed, 2=liquid, 3=none, NE=Not Evaluated
Cough	Y=Yes, N=No
Facial Edema	Y=Yes, N=No
Rash	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Rash/ Hemorrhage at Venipuncture Site	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Bleeding	N=None, F=Facial, V=Vaginal, O=Other, NE=Not Evaluated
Motor Function	0=normal, 1=mild coordination, dysfunction trouble manipulating items, 2=moderate dysfunction trouble grasping, moving, 3=severe dysfunction (uncoordinated actions), NE=Not Evaluated

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279 **Figure S11. Clinical observation scoring from passive transfer of mAb114.**

280 Data from multiple observations each day post-exposure are presented as the maximal

281 level observed for that day.