Supplementary Materials for

Nucleocapsid protein-specific monoclonal antibodies protect mice against Crimean-Congo hemorrhagic fever virus

Aura R. Garrison[1,*], Vanessa Moresco[2], Xiankun Zeng[1], Curtis R. Cline[1], Michael Ward[1], Keersten Ricks[1], Scott Olschner[1], Lisa Cazares[1], Elif Karaaslan[3], Collin Fitzpatrick[1], Éric Bergeron[3], Scott D. Pegan[2,4] and Joseph W. Golden[1,*]

*Corresponding author. E-mail: joseph.w.golden.civ@health.mil or aura.r.garrison.civ@health.mil

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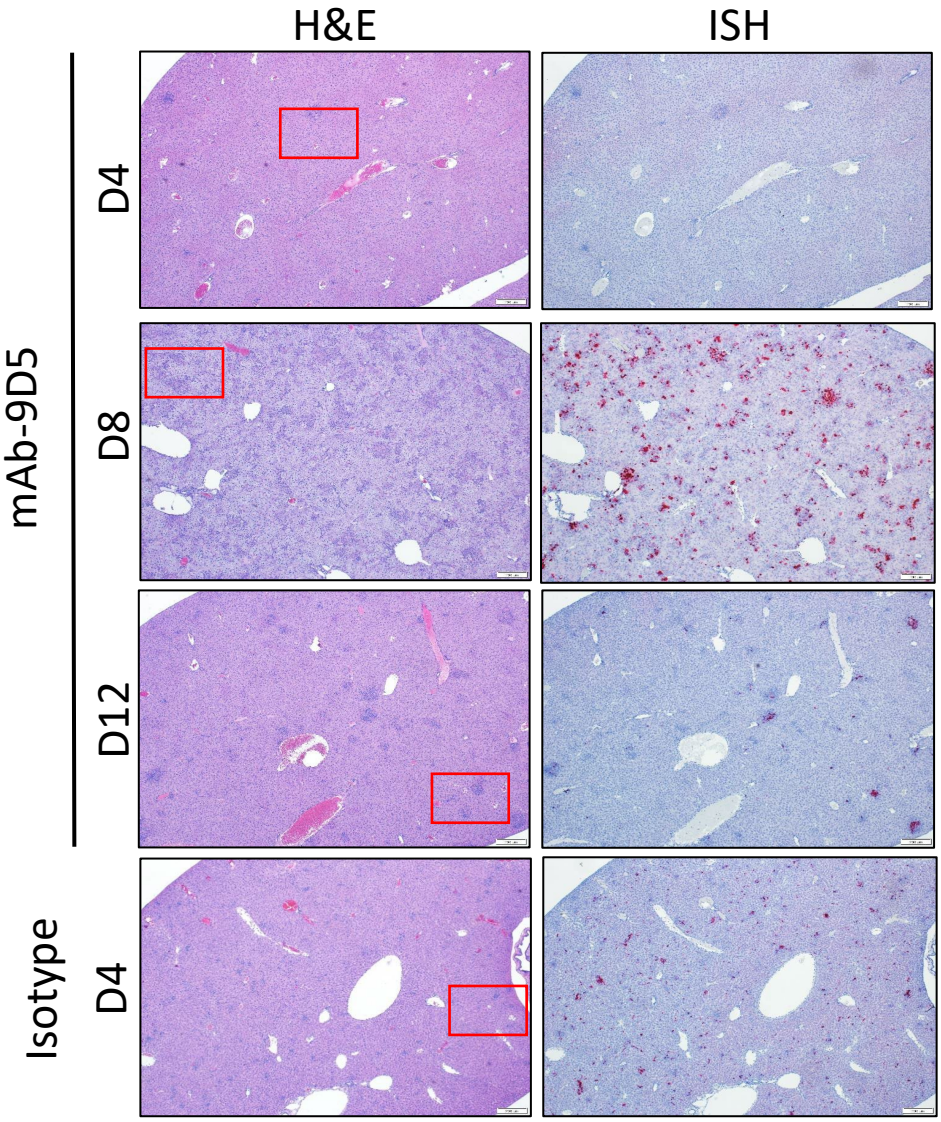

Figure S1. Histopathology and ISH of CCHFV infected mouse liver. Representative H&E ISH staining of livers of CCHFV strain IbAr 10200 infected mice treated with mAb-9D5 (days 4, 8 and 12) or isotype control antibody (day 4). Red box denotes the magnified regions shown in Figure 1B. Day 4 N=5 mice/group, day 8 N=5 mice mAb 9D5 treated, day 12 N=2 mAb 9D5 treated were evaluated and representative images depicting the most severe pathology are shown.

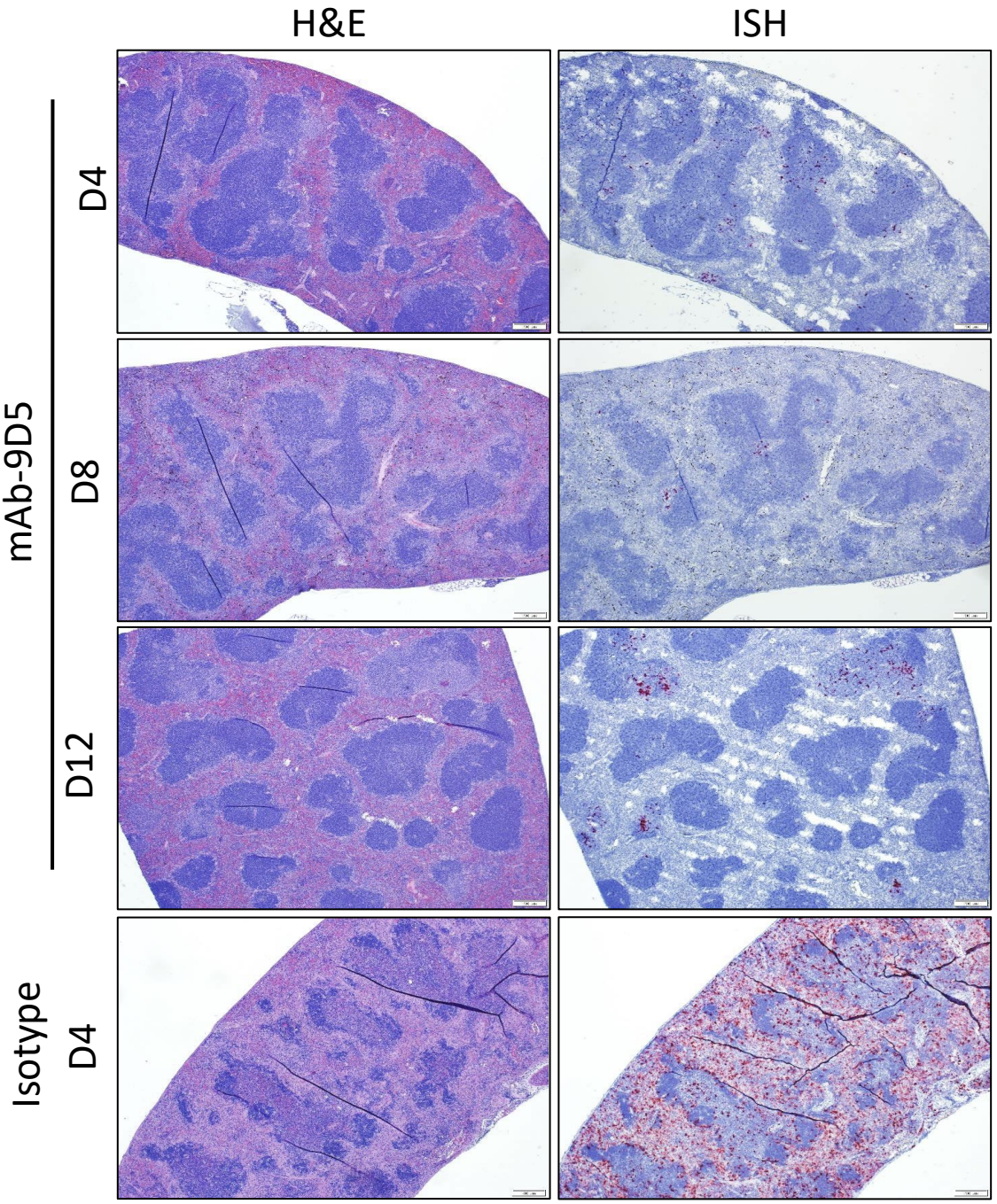

Figure S2. Histopathology and ISH of CCHFV infected mouse spleen. H&E and ISH comparison of spleen between mice treated with mAb-9D5 (days 4, 8 and 12) or isotype control animals (day 4 only) (4x magnification). Day 4, MAb-9D5 treated mice had mild ISH labeling corresponding with to areas of necrosis/inflammation. Day 8 animals had moderate lymphoid depletion and apoptosis/necrosis, indicated by areas of pallor in the white pulp, especially in the marginal zone, imparting a "moth eaten" appearance to the white pulp; ISH labeling was minimal. mAb-9D5 day 12 spleens showed mild lymphoid depletion in the white pulp with foci of inflammation and necrosis in the red pulp (and/or at the junction with the white pulp), and mild ISH labeling. Isotype controls spleens had marked lymphoid depletion with apoptosis/necrosis and hemorrhage in the white pulp, and inflammation in the red pulp. There were also marked ISH labeling. ISH stained spleens were counterstained with hematoxylin. Day 4 N=5 mice/group, day 8 N=5 mice mAb 9D5 treated, day 12 N=2 mAb 9D5 treated were evaluated and representative images depicting the most severe pathology are shown.

Table S1. Mab-9D5 does not neutralize CCHFV

	Virus strain	Antibody	PRNT50
No Complement	Afg09	9D5	<20
		11E7	>640
		6D8	<20
	IbAr 10200	9D5	<20
		11E7	>640
		6D8	<20
Complement (5%)	Afg09	9D5	<20
		11E7	>640
		6D8	<20
	IbAr 10200	9D5	<20
		11E7	>640
		6D8	<20

Table S1. Plaque reduction neutralization test (PRNT) with mAb-9D5. A dilution series from 1:20 to 1:640 of mAb-9D5, or the positive control mAb-11E7, or the negative control mAb-6D8 (anti-Ebola GP antibody) were incubated with either CCHFV strain Afg09 or IbAr 10200, with or without the addition of human complement (5% final concentration). A 50% reduction in plaques was calculated from the mAb-6D8 negative control. This experiment was conducted in duplicate.

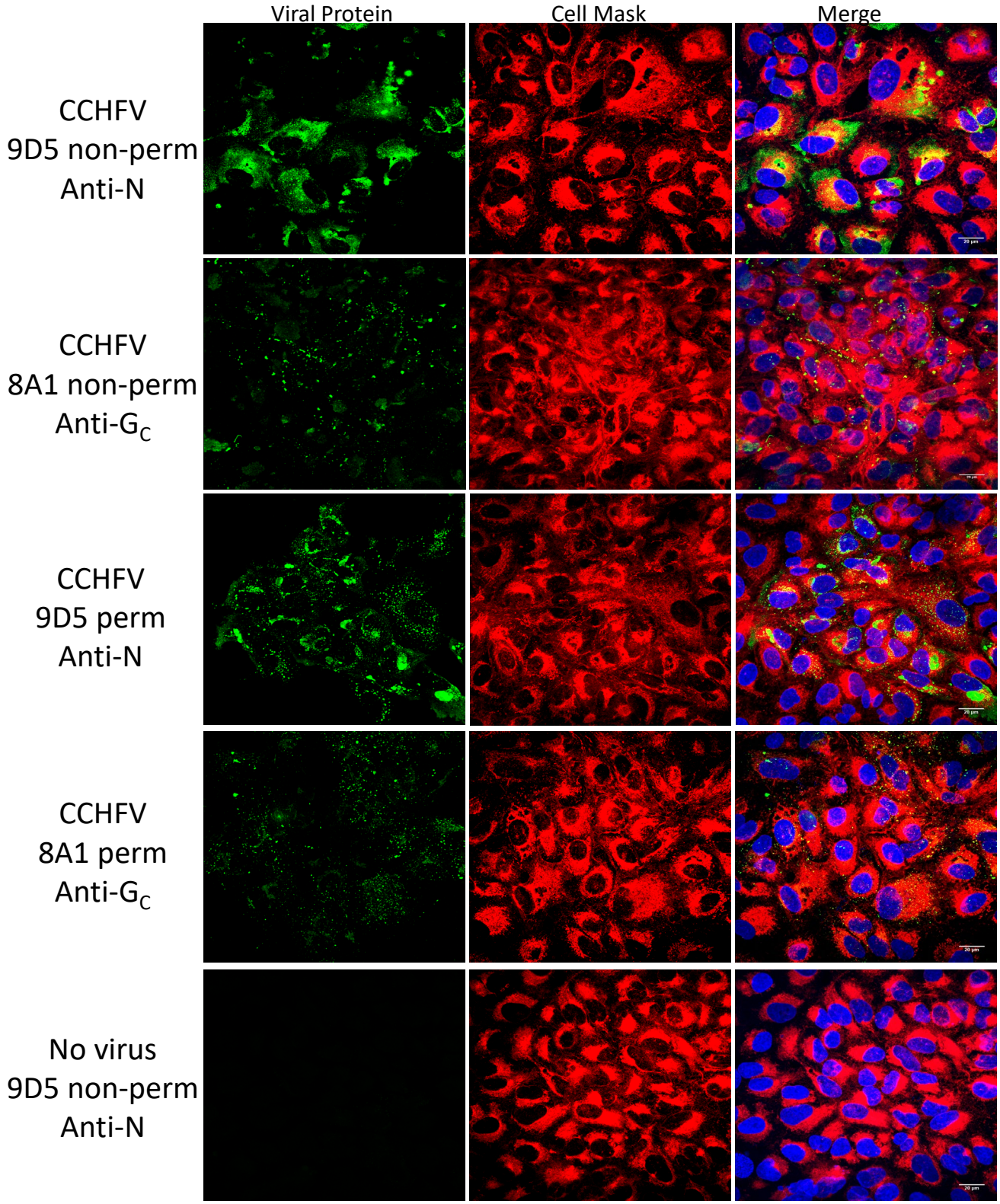

Figure S3. Surface localization of NP in CCHFV infected cells. Permeabilized and non-permeabilized A549 cells infected with CCHFV strain IbAr10200 were stained with the indicated antibodies against CCHFV viral proteins (NP;MAb-9D5 or Gc; mAb-8A1; green) and cell mask (red). Cell nuclei were stained with DAPI (blue). Two experiments were conducted with six replicates per experiment.

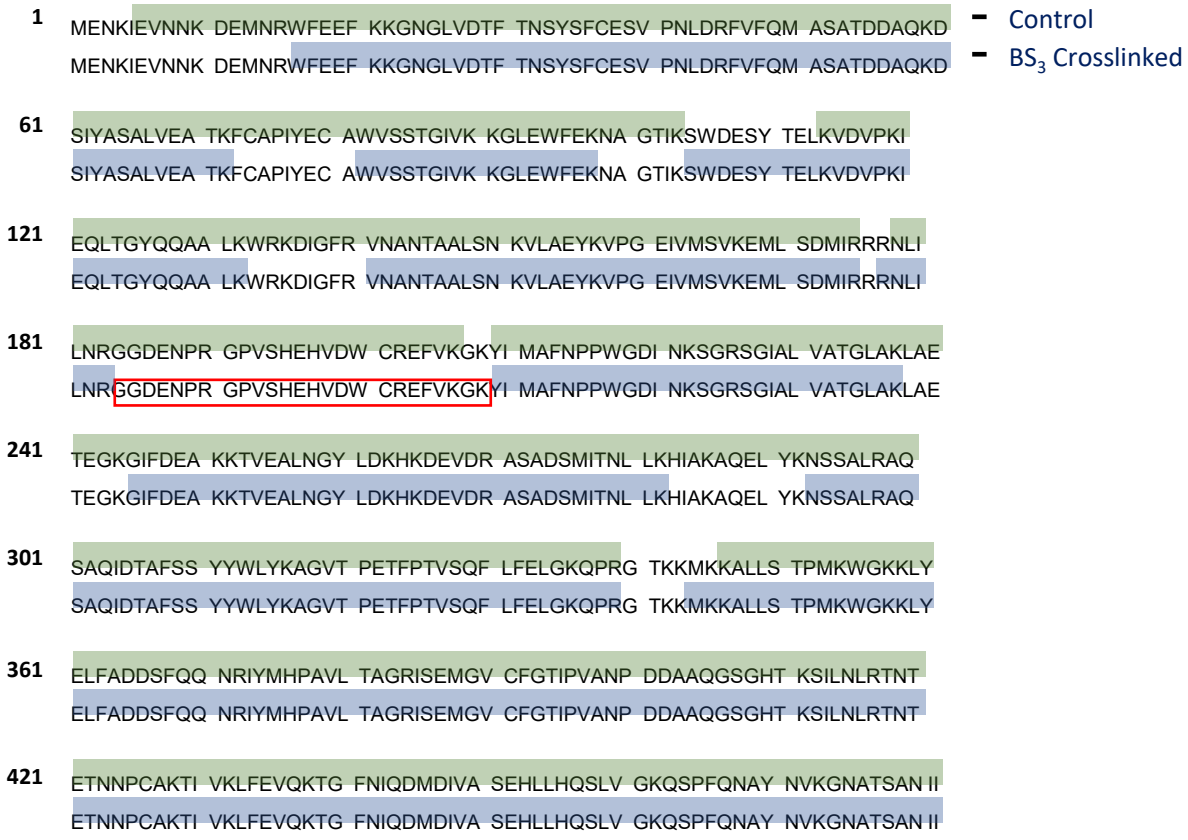

Figure S4. LC-MS epitope mapping. mAb-9D5 was bound to magnetic beads and the recombinant NP was bound to the antibody, cross-linked, and then digested with trypsin/Lyc C. As a control, a non-cross-linked sample was also analyzed. This analysis identified the mAb-9D5 protected region between amino acids 184 to 208 of IbAr 10200 N protein (Accession # MH483987.1). Red box indicates the mAb-95D binding domain. Two biological replicates were analyzed in triplicate to identify the mAb-9D5 epitope.

Percent Identical

Strain	10200	Afg09	Aigai	Erve	Hazara	Hoti	Oman	Semnya	Senegal
10200		92.0	84.0	46.2	65.4	92.0	88.0	92.0	88.0
Afg09	92.0		84.0	46.2	65.4	100.0	96.0	100.0	92.0
Aigai	84.0	84.0		38.5	65.4	84.0	88.0	84.0	88.0
Erve	46.2	46.2	38.5		38.5	46.2	46.2	46.2	42.3
Hazara	65.4	65.4	65.4	38.5		65.4	65.4	65.4	61.5
Hoti	92.0	100.0	84.0	46.2	65.4		96.0	100.0	92.0
Oman	88.0	96.0	88.0	46.2	65.4	96.0		96.0	88.0
Semnya	92.0	100.0	84.0	46.2	65.4	100.0	96.0		92.0
Senegal	88.0	92.0	88.0	42.3	61.5	92.0	88.0	92.0	

Percent Identical

Table S2. Percent similarity of the MAb-9D5 epitope. Percent amino acid identify and dissimilarity of the MAb-9D5 epitope was calculated by Geneious Prime 2023.2.

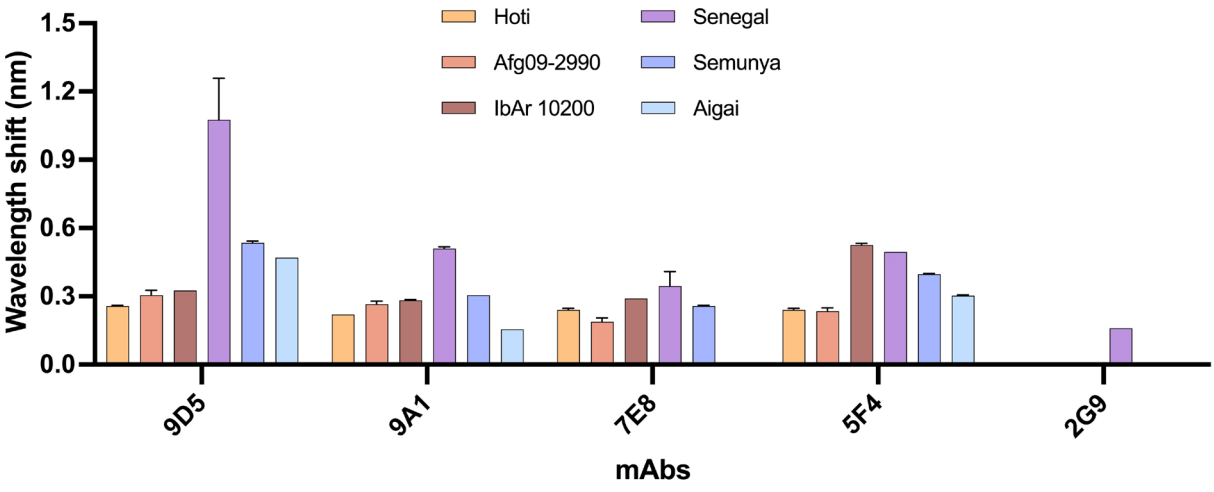

Figure S5. Mouse ascite-mAbs binding affinity screening to NP. BLI SA biosensors were loaded with NP (antigen) at concentrations of 500 nM and a single dilution (1:64) of the ascite-mAbs was employed in triplicate. Wavelength shift (nm) correspond to a semi-quantitative measurement of the binding affinities between the mAbs and NP. Erve-virus did not bind to any of the tested mAbs.

	Afg09		Hoti		IbAr 10200		Senegal		Semunya		Aigai	
mAb-ascites	R^2	nm shift ± SD	R^2	nm shift ± SD	R^2	nm shift ± SD	R^2	nm shift ± SD	R^2	nm shift ± SD	R^2	nm shift ± SD
9D5	0.99	0.305 ± 0.02	0.974	0.257 ± 0.03	0.984	0.325	0.981	1.075 ±0.183	0.985	0.535 ± 0.007	0.972	0.47
9A1	0.982	0.265 ± 0.014	0.99	0.22	0.982	0.2825 ± 0.003	0.98	0.51 ± 0.007	0.984	0.305	0.981	0.155
7E8	0.967	0.18 ± 0.017	0.973	0.24 ± 0.007	0.975	0.29	0.977	0.35 ± 0.063	0.972	0.257 ± 0.003		NB
5F4	0.961	0.235 ± 0.014	0.955	0.24 ± 0.007	0.968	0.525 ± 0.007	0.975	0.495	0.931	0.397 ± 0.003	0.971	0.305 ± 0.003
2G9		NB		NB		NB	0.961	0.16		NB		NB

NP 500nM; mouse-ascites mAbs 1:64 dilution
NB: no binding
Erve virus: no binding for all tested mAbs

Table S3. Mouse ascites-mAbs to NP BLI. Wavelength shift (nm) (mean ± SD) correspond to a semi-quantitative measurement of the binding affinities between the mAbs and NP. Erve-virus did not bind to any of the tested mAbs. Samples were evaluated in triplicate.