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Supplementary Materials for

Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
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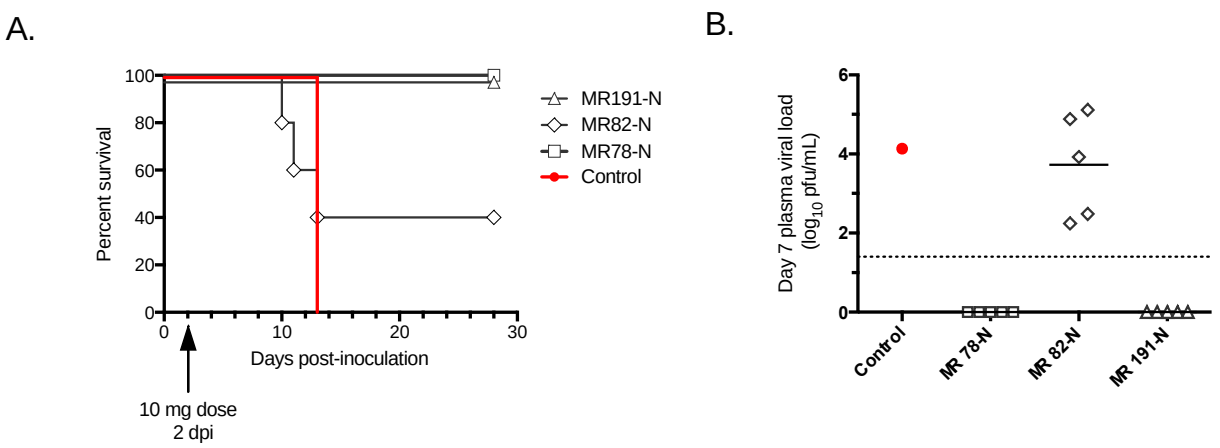

Fig. S1. Postexposure protection of guinea pigs infected with GPA MARV virus and treated 2 dpi with a single dose of mAb. Guinea pigs received 1,000 pfu of GPA MARV IP and a 10 mg dose of mAb IP 2 dpi (n=5 per treatment group; n=1 for PBS control). (**A**) Kaplan-Meier survival curves. MR78-N and MR191-N were significantly more protective than MR82-N ($P<0.05$ by Mantel-Cox test). (**B**) Plasma viral load 7 dpi, as determined by plaque assay. Limit of detection (25 pfu/mL) is indicated by a dotted line.

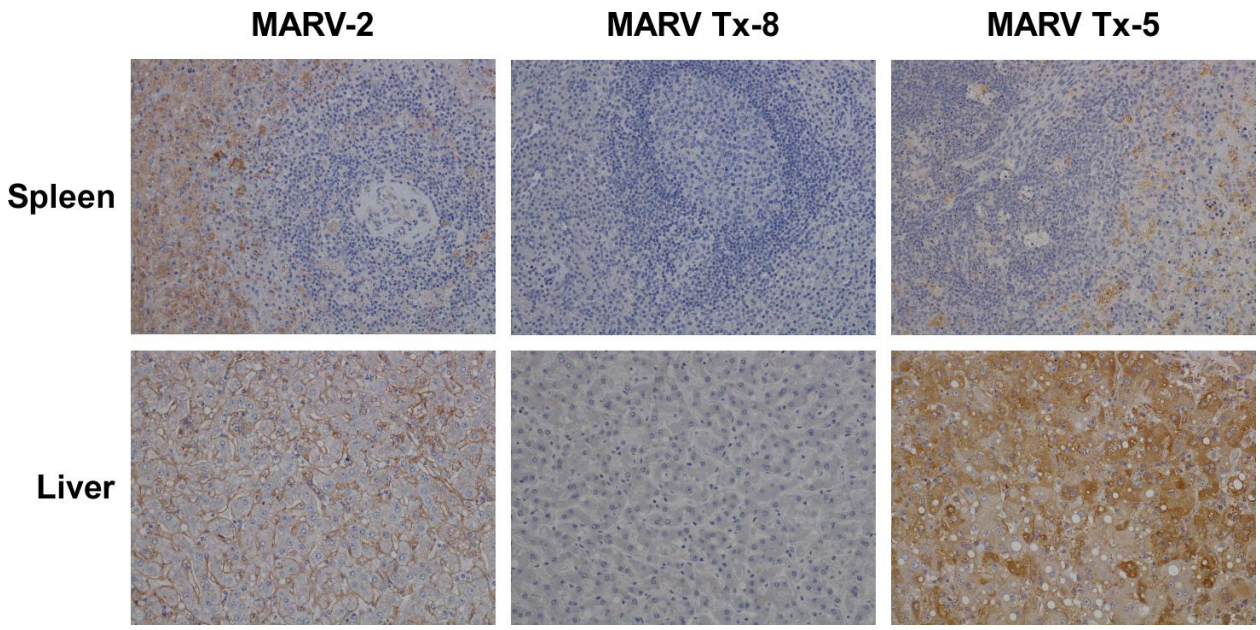

Fig. S2. MARV antigen in NHPs treated or untreated with MR191-N on 5 and 8 dpi. Top row. MARV immunolabeling of dendriform mononuclear cells in red and white pulp. Bottom row. MARV immunolabeling of sinusoidal lining and Kupffer cells. MARV-2 was not treated with mAb and succumbed to infection, MARV Tx-8 was treated with mAb and survived, and MARV Tx-5 was treated with mAb but succumbed. Labeling indicative of the presence of MARV antigen is observed for MARV-2 and MARV Tx-5 but not for MARV Tx-8.

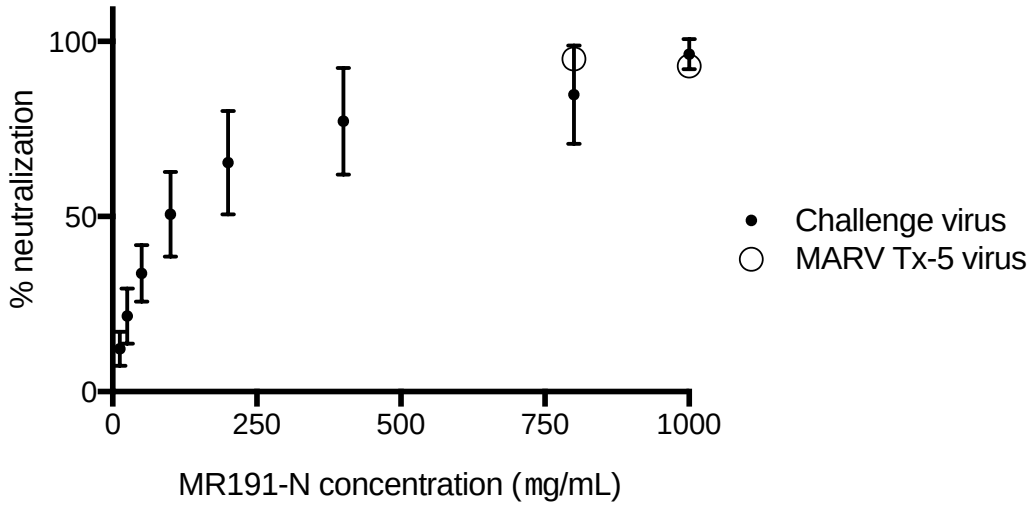

Fig. S3. Neutralization sensitivity of virus inoculum and virus isolated from MARV Tx-5. Virus isolated from a 15 dpi sample from the treated animal that succumbed to disease (MARV

Tx-5) was tested for its susceptibility to neutralization by 800 and 1,000 µg/mL of MR191-N (n=1), concentrations spanning the IC_{90} for wildtype virus. Neutralization of the original viral stock by MR191-N is plotted for comparison. Error bars denote standard deviation (n=5).

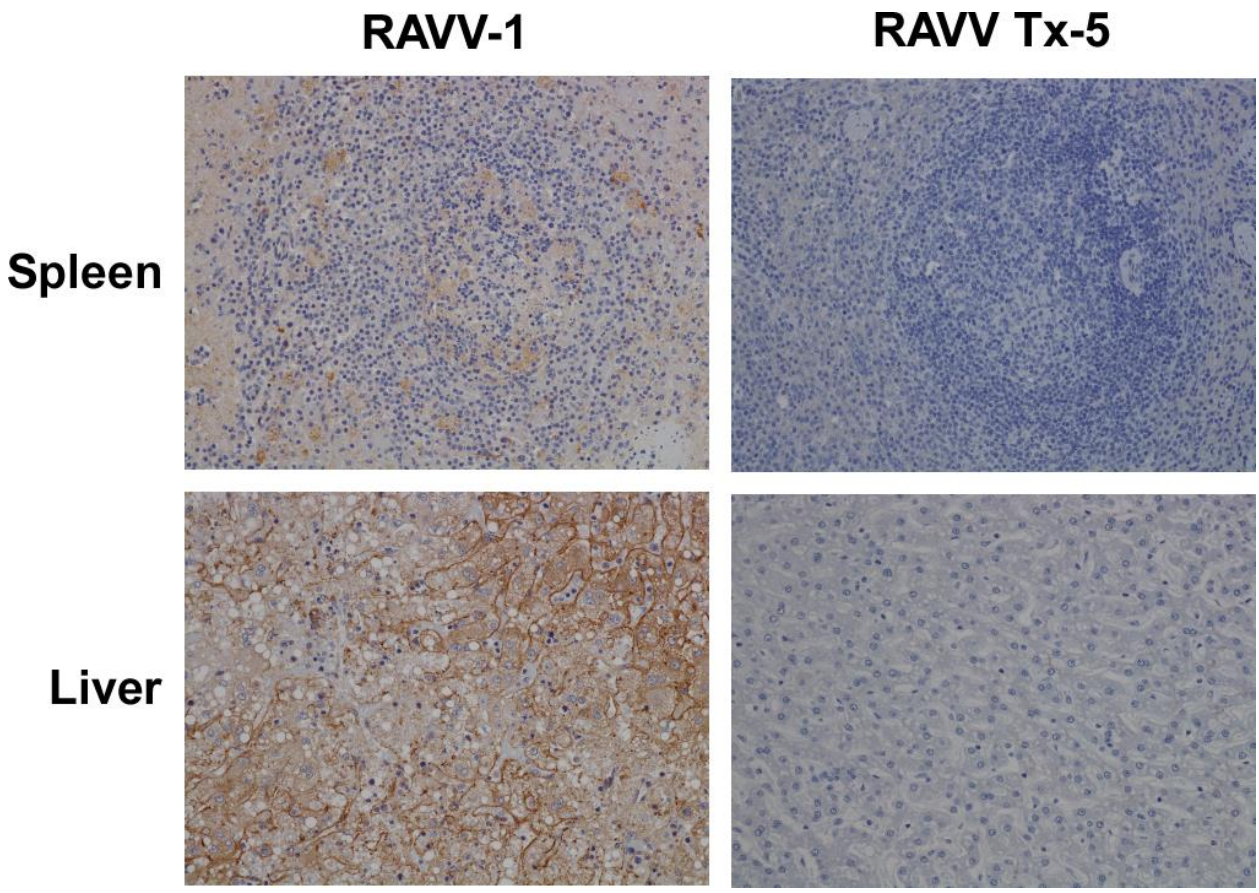

Fig. S4. RAVV antigen in NHPs treated or untreated with MR191-N on 5 and 8 dpi. Top row. RAVV immunolabeling of dendriform mononuclear cells in red and white pulp. Bottom row. RAVV immunolabeling of sinusoidal lining and Kupffer cells. RAVV-1 was not treated with mAb and succumbed to infection while RAVV Tx-5 was treated with mAb and survived. Labeling indicative of the presence of RAVV antigen is observed for RAVV-1 but not for RAVV Tx-5.

Table S1. Control or MR191-N–treated Marburg or Ravn virus–inoculated NHPs.

Subject Identifier	Sex	Group	Clinical illness	Clinical pathology
MARV-1	F	No Tx	Fever (D7); mild depression (D7); moderate depression (D8); lethargy (D7-8); loss of appetite (D6-8); mild rash (D7-8); Animal euthanized in pm on D8.	Lymphopenia (D7); thrombocytopenia (D7, D8); granulocytosis (D8); hypoalbuminemia (D8); >5-fold increase ALT (D7, D8); >15-fold increase in AST (D7, D8); >5-fold increase in CRP (D7, D8).
MARV-2	F	No Tx	Mild depression (D7); lethargy (D7-8); loss of appetite (D6-8); mild rash (D7-8). Animal expired in am on D8.	Lymphopenia (D5); thrombocytopenia (D5); hypoalbuminemia (D8); >10-fold increase in ALT (D8); >10-fold increase in AST (D5, D8), >4-fold increase in AST (D10); >4-fold increase in BUN (D8); >5-fold increase in CRP (D5, D8).
MARV Tx-1	F	Day 4/7	Loss of appetite (D5-12).	Lymphopenia (D7); thrombocytopenia (D7, D10); leukocytosis (D10); granulocytosis (D4, D7, D10, D14, D21, D28); >10-fold increase ALT (D7, D10), >3-fold increase in ALT (D14); >10-fold increase in AST (D7), >4-fold increase in AST (D10); >4-fold increase in CRP (D4), >2-fold increase in CRP (D7).
MARV Tx-2	F	Day 4/7	Fever (D7); loss of appetite (D9).	Lymphopenia (D7); leukocytosis (D10, D14); granulocytosis (D4); >4-fold increase ALT (D7, D10); >5-fold increase AST (D7); >2-fold increase CRP (D4).
MARV Tx-3	M	Day 4/7	Fever (D4, D7); loss of appetite (D7-D11).	Lymphopenia (D4, D7); thrombocytopenia (D7, D10); leukocytosis (D14), granulocytosis (D4, D14); >15-fold increase ALT (D7), >5-fold increase ALT (D10, D14), >2-fold increase ALT (D21); >10-fold increase AST (D7, D10); >10-fold increase CRP (D4), >5-fold increase CRP (D7)
MARV Tx-4	M	Day 5/8	Fever (D5, D8).	Lymphopenia (D5, D8); leukocytosis (D11); >10-fold increase ALT (D8, D11), >4-fold increase in ALT (D14); >15-fold increase in AST (D8); >6-fold increase in CRP (D5, D8).
MARV Tx-5	M	Day 5/8	Fever (D5, D8); mildly depressed (D10, D14); depressed (D15); lethargy (D15); loss of appetite (D6-15); severe rash (D15); facial edema (D12-D15). Animal euthanized in am on D15.	Lymphopenia (D8); thrombocytopenia (D5, D8, D11, D14, D15); leukocytosis (D10); granulocytosis (D5, D8, D11); hypoalbuminemia (D11, D14, D15); >15-fold increase ALT (D8, D11, D14, D15), >2-fold increase in ALT (D5); >10-fold increase in AST (D8, D11, D14, D15), >5-fold increase in AST (D5); >3-fold increase BUN (D15); >4-fold increase in CRP (D5, D8, D14, D15).
MARV Tx-6	M	Day 5/8	Fever (D8); mild depression (D8, D10); loss of appetite (D6-D13).	Lymphopenia (D5, D8); thrombocytopenia (D8, D11); leukocytosis (D14. D21, D28); granulocytosis (D5, D11, D14, D28); hypoalbuminemia (D11, D14); >10-fold increase ALT (D8), >5-fold increase in ALT (D11); >5-fold increase in AST (D8, D11), >2-fold increase in AST (D5); >4-fold increase in CRP (D5, D8), >2-fold increase in CRP (D28).
MARV Tx-7	F	Day 5/8	None.	Lymphopenia (D5, D8); thrombocytopenia (D8); leukocytosis (D11, D14); >10-fold increase ALT (D8, D11), >5-fold increase in ALT (D14); >10-fold increase in AST (D8), >2-fold increase in AST (D11); >4-fold increase in CRP (D8), >3-fold increase in CRP (D5).

MARV Tx-8	F	Day 5/8	Fever (D5); loss of appetite (D6-12).	Lymphopenia (D5, D8); thrombocytopenia (D8, D11); leukocytosis (D11, D14); hypoalbuminemia (D11); >15-fold increase ALT (D8, D11), >2-fold increase in ALT (D14, D21); >10-fold increase in AST (D8), >3-fold increase in AST (D5, D11); >4-fold increase in CRP (D5, D8), >2-fold increase in CRP (D11).
RAVV-1	F	No Tx	Fever (D8); moderate depression (D8-10); lethargy (D8-10); loss of appetite (D7-10); mild rash (D8); moderate rash (D9-10). Animal expired in am on D10.	Lymphopenia (D5); thrombocytopenia (D8); >15-fold increase ALT (D8); >15-fold increase in AST (D8), >2-fold increase in AST (D5); >2-fold increase in CRP (D5, D8).
RAVV Tx-1	M	Day 5/8	None.	Lymphopenia (D5, D8); leukocytosis (D11); granulocytosis (D5); >6-fold increase in CRP (D5), >2-fold increase in CRP (D8).
RAVV Tx-2	M	Day 5/8	Fever (D5).	Lymphopenia (D5, D8); thrombocytopenia (D8); leukocytosis (D11); granulocytosis (D5, D11); >10-fold increase ALT (D8), >3-fold increase in ALT (D11, D14); >5-fold increase in AST (D8), >2-fold increase in AST (D11); >6-fold increase in CRP (D5), >2-fold increase in CRP (D8).
RAVV Tx-3	M	Day 5/8	Fever (D5).	Lymphopenia (D5, D8); thrombocytopenia (D8); leukocytosis (D11); granulocytosis (D5); >5-fold increase ALT (D8), >2-fold increase in ALT (D11, D14); >4-fold increase in AST (D8), >2-fold increase in AST (D11); >5-fold increase in CRP (D5).
RAVV Tx-4	F	Day 5/8	Fever (D5).	Leukocytosis (D11); >3-fold increase ALT (D8); >6-fold increase in CRP (D5).
RAVV Tx-5	F	Day 5/8	None.	Lymphopenia (D5); leukocytosis (D11, D14); >5-fold increase ALT (D8), >2-fold increase in ALT (D11); >3-fold increase in AST (D8); >4-fold increase in CRP (D5), >2-fold increase in CRP (D8).

[a] Days after MARV or RAVV inoculation are in parentheses. Fever is defined as a body temperature more than 2.5 ºF over baseline or at least 1.5 ºF over baseline and ≥103.5 ºF. Mild rash: focal areas of petechiae covering less than 10% of the skin; Moderate rash: areas of petechiae covering between 10% and 40% of the skin; severe rash: areas of petechiae and/or echymosis covering more than 40% of the skin. Lymphopenia and thrombocytopenia are defined by a ≥35% drop in numbers of lymphocytes and platelets, respectively. Leukocytosis and granulocytosis are defined by ≥35% increase in numbers of white blood cells. Hypoalbuminemia is defined by a ≥35% decrease in levels of albumin. (ALP) alkaline phosphatase, (ALT) Alanine aminotransferase, (AST) aspartate aminotransferase, (GGT) gamma glutamyltransferase, (BUN) blood urea nitrogen, (CRE) creatinine, (CRP) C-reactive protein

Table S2. Single-nucleotide polymorphism changes from MARV deep sequencing data.

MARV[a]											
Ref Pos	**Type**	**Ref Base[b]**	**Called Base**	**SNP %**	**Depth**	**A Cnt**	**C Cnt**	**G Cnt**	**U Cnt**	**Gene**	**Change**
124	SNP	A	U	26.70%	285	-	0	0	76	NP	L7F
696	SNP	A	G	6.10%	3928	-	49	238	69	NP	H198R
2834	SNP	A	G	11.50%	287	-	0	33	0		Non-coding
7329	SNP	U	C	7.30%	123	0	9	0	-	GP	Silent
8433	SNP	A	G	9.00%	4638	-	0	419	0		Non-coding
14547	SNP	C	U	6.50%	1428	0	-	17	93	L	Silent
18679	SNP	A	U	5.20%	2240	-	1	6	117		Non-coding
MARV Tx-5 15 dpi[c]											
35	SNP	A	G	35.50%	124	-	1	44	1		Non-coding
38	SNP	A	U	25.60%	152	-	0	0	32		Non-coding
124	SNP	A	U	28.70%	286	-	0	0	82	NP	L7F
127	SNP	A	G	5.80%	346	-	0	20	6	NP	Silent
696	SNP	A	G	6.50%	3984	-	28	257	39	NP	H198R
2590	SNP	U	C	9.50%	1847	0	176	8	-		Non-coding
2598	SNP	U	C	9.20%	1985	0	182	0	-		Non-coding
2640	SNP	U	C	9.20%	1869	0	172	0	-		Non-coding
2643	SNP	U	C	9.40%	1838	0	172	0	-		Non-coding
2663	SNP	U	C	10.40%	1780	1	186	1	-		Non-coding
2724	SNP	U	C	10.90%	2377	0	259	0	-		Non-coding
2834	**SNP**	**A**	**G**	**46.90%**	**3631**	**-**	**0**	**1702**	**0**		**Non-coding**
2836	SNP	U	C	6.50%	3672	10	238	0	-		Non-coding
3400	SNP	A	U	5.40%	3543	-	0	0	192	VP35	Silent
4522	SNP	G	U	5.90%	778	0	0	-	46		Non-coding
6891	SNP	U	C	6.80%	1770	2	121	0	-	GP	Silent
7329	SNP	U	C	6.60%	500	0	33	0	-	GP	Silent

8860	SNP	A	G	5.00%	2225	-	24	112	22		Non-coding
10203	SNP	A	G	7.70%	3174	-	26	245	48		Non-coding
14539	SNP	G	U	17.80%	1700	0	0	-	303	L	C1026F
14547	SNP	C	U	7.10%	2525	4	-	22	180	L	Silent
14833	SNP	C	U	7.30%	6280	20	-	139	460	L	A1124V
14834	SNP	A	G	9.30%	6260	-	279	585	176	L	Silent
15768	SNP	A	U	16.30%	5248	-	0	5	856	L	S1436C
15775	SNP	A	G	15.30%	7049	-	59	1082	95	L	N1438S
15780	SNP	A	G	9.90%	7959	-	164	786	232	L	S1440G
16206	SNP	C	U	5.70%	1431	0	-	0	82	L	L1582F
18679	SNP	A	U	13.30%	2884	-	0	1	385		Non-coding
18684	SNP	A	G	9.70%	3366	-	5	328	51		Non-coding
18961	SNP	C	A	5.90%	4477	264	-	0	3		Non-coding
19084	SNP	C	A	5.30%	225	12	-	0	0		Non-coding

SNP, single nucleotide polymorphism; SNP%, percentage of reads containing SNP, Ref Pos; Nucleotide position in reference genome; Depth, number of reads over base; Cnt, number counted.

[a] MARV passage 2 seed stock used for inoculations presented in this study.

[b]The MARV reference strain used is GenBank Accession number DQ447653.

[c] Virus isolate from MARV Tx-5 15 dpi serum.

Bold face type highlights a notable change in population of genomes.

Underlined type highlights the only change in GP sequence compared to the MARV passage 2 seed stock.