1   **A highly attenuated vaccinia virus strain LC16m8-based vaccine for**

2   **severe fever with thrombocytopenia syndrome**

3

4   Short title: LC16m8-based vaccine for SFTS

5

6   Tomoki Yoshikawa[1], Satoshi Taniguchi[1], Hirofumi Kato[1], Naoko Iwata-Yoshikawa[2],

7   Hideki Tani[3], Takeshi Kurosu[1], Hikaru Fujii[4], Natsumi Omura[1], Miho Shibamura[1],

8   Shumpei Watanabe[4], Kazutaka Egawa[1], Takuya Inagaki[1], Satoko Sugimoto[1], Supranee

9   Phanthanawiboon[1], Shizuko Harada[1], Souichi Yamada[1], Shuetsu Fukushi[1], Shigeru

10   Morikawa[4], Noriyo Nagata[2], Masayuki Shimojima[1], Masayuki Saijo[1]*

11

12   [1]Department of Virology 1, National Institute of Infectious Diseases,

13   Musashimurayama-shi, Tokyo, Japan

14   [2]Department of Pathology, National Institute of Infectious Diseases,

15   Musashimurayama-shi, Tokyo, Japan

16   [3]Department of Virology, Toyama Institute of Health, Imizu-shi, Toyama, Japan

17   [4]Department of Microbiology, Faculty of Veterinary Medicine, Okayama University of

18   Science, Imabari-shi, Ehime, Japan

19

20   *Corresponding author

21   E-mail: msaijo@nih.go.jp (MS)

1

Abstract

Severe fever with thrombocytopenia syndrome (SFTS) caused by Dabie bandavirus (formerly SFTS virus [SFTSV]) is an emerging hemorrhagic infectious disease with a high case-fatality rate. One of the best strategies for preventing SFTS is to develop a vaccine, which is expected to induce both humoral and cellular immunity. We applied a highly attenuated but still immunogenic vaccinia virus strain LC16m8 (m8) as a recombinant vaccine for SFTS. Recombinant m8s expressing SFTSV nucleoprotein (m8-N), envelope glycoprotein precursor (m8-GPC), and both N and GPC (m8-N+GPC) in the infected cells were generated. Both m8-GPC- and m8-N+GPC-infected cells were confirmed to produce SFTSV-like-particles (VLP) *in vitro,* and the N was incorporated in the VLP produced by the infection of cells with m8-N+GPC. Specific antibodies to SFTSV were induced in mice inoculated with each of the recombinant m8s, and the mice were fully protected from lethal challenge with SFTSV at both 10^3 $TCID_{50}$ and 10^5 $TCID_{50}$. In mice that had been immunized with vaccinia virus strain Lister in advance of m8-based SFTSV vaccine inoculation, protective immunity against the SFTSV challenge was also conferred. The pathological analysis revealed that mice immunized with m8-GPC or m8-N+GPC did not show any histopathological changes without any viral antigen-positive cells, whereas the control mice showed focal necrosis with inflammatory infiltration with SFTSV antigen-positive cells in tissues after SFTSV challenge. The passive serum transfer experiments revealed that sera collected from mice inoculated with m8-GPC or m8-N+GPC but not with m8-N conferred protective immunity against lethal SFTSV challenge in naïve mice. On the other hand, the depletion of CD8-positive cells *in vivo* did not abrogate the

44   protective immunity conferred by m8-based SFTSV vaccines. Based on these results, the

45   recombinant m8-GPC and m8-N+GPC were considered promising vaccine candidates for

46   SFTS.

Author Summary

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging viral hemorrhagic fever with a high case-fatality rate (approximately 5% to >40%). Indigenous SFTS has been reported in China, Japan, South Korea, and Vietnam. Thus, the development of an effective vaccine for SFTS is urgently needed. Vaccinia virus (VAC) was previously used as a vaccine for smallpox. Unfortunately, after these strains, the so-called second generation of VAC used during the eradication campaign was associated with severe adverse events, and the third generation of VAC strains such as LC16m8 (m8) and modified vaccinia Ankara (MVA) was established. m8 is confirmed to be highly attenuated while still maintaining immunogenicity. m8 is licensed for use in healthy people in Japan. At the present time, approximately 100,000 people have undergone vaccination with m8 without experiencing any severe postvaccine complications. At present, third-generation VAC strains are attractive for a recombinant vaccine vector, especially for viral hemorrhagic infectious diseases, such as Ebola virus disease, Lassa fever, Crimean-Congo hemorrhagic fever, and SFTS. We investigated the practicality of an m8-based recombinant vaccine for SFTS as well as other promising recombinant VAC-based vaccines for viral hemorrhagic infectious diseases.

Introduction

64

65   Severe fever with thrombocytopenia syndrome (SFTS) is an emerging viral hemorrhagic

66   fever with a high case-fatality rate (approximately 5 to over 40%) [1-6]. The clinical

67   symptoms are in general fever, malaise, myalgia, nausea, vomiting, and diarrhea. The

68   laboratory findings include leukocytopenia and thrombocytopenia in the total blood cell

69   counts, and elevated serum levels of hepatic enzymes [1, 7-11]. The disease is caused by

70   Dabie bandavirus, which had been called SFTS virus (SFTSV), a novel tick-borne virus in

71   the order *Bunyavirales*, family *Phenuiviridae*, and genus *Bandavirus*. The viral genome

72   consists of three negative-stranded RNA segments and encodes four genes:

73   RNA-dependent RNA polymerase, envelope glycoproteins precursor (GPC), nucleoprotein

74   (N), and nonstructural protein. Indigenous SFTS had been reported in China, Japan, South

75   Korea, and Vietnam [1-3, 12-14]. Hence the development of an effective vaccine for SFTS

76   is urgently needed. Thus far, it has been reported that a live recombinant vesicular

77   stomatitis virus or a DNA vaccine expressing the SFTSV glycoproteins (GPs) originated

78   from SFTSV GPC gene-elicited protective immunity against SFTSV in lethal mouse or

79   ferret models [15, 16].

80   Vaccinia virus (VAC) was used as the smallpox vaccine, and smallpox vaccines produced

81   by using a variety of VAC strains were used during the global smallpox eradication

82   program led by the World Health Organization. Since smallpox eradication was declared in

83   1980, VAC has been used as a recombinant vaccine vector with an expectation of

84   immunogenicity [17, 18]. However, since the VAC strains used as the vaccine vector in the

85   beginning of the eradication campaign were mainly so-called second-generation smallpox

5

86   vaccines that were associated with severe side effects, such as encephalitis, encephalopathy,

87   conjunctivitis, progressive vaccinia, eczema vaccinatum and generalized fetal VAC

88   infections [17, 19, 20], the application of VAC has not been popular. The VAC strain

89   LC16m8 (m8), which is categorized as a third-generation smallpox vaccine, as well as the

90   modified vaccinia Ankara (MVA), is confirmed to have a highly attenuated phenotype but

91   to maintain immunogenicity to protect against other orthopoxvirus infections, such as

92   monkeypox [21-23]. m8 is licensed for use in healthy people in Japan, and approximately

93   100,000 people have been vaccinated with m8 thus far, with antibody response comparable

94   to the first generation vaccine [24] and without experiencing severe postvaccine

95   complications [24, 25]. This well-balanced safety and immunogenicity profile of m8

96   represents a significant advantage over the first- and second generations of VAC. In the

97   present study, recombinant m8 strains that express SFTSV N (m8-N), GPC (m8-GPC), or

98   both N and GPC (m8-N+GPC) (m8-based SFTSV vaccines) were generated as SFTS

99   vaccine candidates. It is hypothesized that N and GPC contributed to cellular immunity

100   and/or humoral immunity against SFTSV infection. The immunogenicity and protective

101   efficacy of m8-based SFTSV vaccines against SFTSV were evaluated using a mouse model

102   of lethal SFTS.

Results

Generation and characterization of recombinant m8 harboring SFTSV genes

The final recombinant m8-based SFTSV vaccines harbored the SFTSV gene expression cassette in the flanking region between the B4R and B6R genes in place of the B5R gene (Fig. 1A). The SFTSV gene expression in RK13 cells infected with m8-N, m8-GPC, or m8-N+GPC was confirmed by an indirect immunofluorescence assay (IFA) (Fig. 1B). VLP production in the m8-GPC or m8-N+GPC-infected cells was evaluated by electron microscopy (Fig. 1C). The morphological characteristics of VLP were enveloped and spherical with approximate diameters of 100 nm and seemed similar to that of SFTSV. Next, the incorporation of the N protein into the VLP, which was produced from cells infected with m8-N+GPC, was evaluated (Fig. 1D). When the VLP was pulled down in immunoprecipitation using anti-SFTSV Gc antibodies, the amount of N protein recovered from cells infected with m8-N+GPC was more extensive than that of m8-N-infected cells in comparison to when no antibodies were used. On the other hand, the addition of the anti-SFTSV Gc antibody did not affect the recovery of the N protein from the m8-N-infected-cell supernatant, which did not contain the VLP. This result indicated that the m8-GPC- or m8-N+GPC-infected cells produced SFTSV-like particles, and that the N protein was incorporated into the VLP when cells were infected with m8-N+GPC.

Immunogenicity of m8-based SFTSV vaccines in mice

Groups (5 per group) of seven- to 8-week-old naïve Type I interferon alpha receptor-deficient (IFNAR-/-) mice were subcutaneously inoculated twice at a 2-week

7

126   interval with either m8-based SFTSV vaccine or m8-EGFP as a negative control. None of

127   the mice vaccinated with any of the m8-based SFTSV vaccines, including the negative

128   control, developed observable clinical signs of illness, such as ruffled fur or pock formation

129   at the site of inoculation. To evaluate the immunogenicity of m8-based SFTSV vaccines,

130   sera were collected from IFNAR-/- mice and subjected to an IFA and N.T. assay. Five

131   naïve IFNAR-/- mice were subcutaneously infected with 10 $TCID_{50}$ of SFTSV YG-1 as a

132   positive control. Since one of the five mice died at 13 days post-SFTSV infection, sera

133   were collected from four mice at four weeks after infection. In addition, one of the sera

134   collected from four SFTSV-infected mice was omitted from the subsequent analyses, since

135   the serum did not seroconvert (i.e., both IFA and N.T. results were negative). The specific

136   IgG against SFTSV N or GPs in the sera were elicited by the infection of mice with m8-N,

137   m8-GPC, or m8-N+GPC (Fig. 2A, B). The N.T. antibodies to SFTSV were also elicited,

138   and the 50% N.T. antibody titers of the sera collected from m8-GPC-infected,

139   m8-N+GPC-infected, and even SFTSV-infected mice were approximately 1:10 dilution

140   (Fig. 2C). These results indicated that m8-based SFTSV vaccines induced humoral

141   immunity, and the induction potency was comparable to that induced by the authentic

142   SFTSV.

143

144   **Vaccine efficacy of m8-based SFTSV vaccines in mice**

145   Groups (8 to 10 per group) of six- to nine-week-old IFNAR-/- mice were subcutaneously

146   inoculated twice at a two-week interval with each m8-based SFTSV vaccine. At two weeks

147   after the second inoculation, the mice were subcutaneously challenged with 1×10^3 or $1 \times$

8

148   10^5 TCID$_{50}$ of SFTSV YG-1. While almost all of the control mice died after the

149   development of clinical signs, such as ruffled fur, hunched posture, and weight loss, all of

150   the mice inoculated with either m8-GPC or m8-N+GPC survived without developing any

151   obvious clinical signs during the two-week observation period after the SFTSV challenge

152   (Fig. 3A-D). Furthermore, all the mice that had been inoculated with m8-N survived

153   without developing any obvious clinical signs after SFTSV challenge with a dose of 1×10^3

154   TCID$_{50}$ during the two-week observation period (Fig. 3A, C). On the other hand, the mice

155   inoculated with m8-N survived but transiently developed clinical signs, when challenged

156   with 1×10^5 TCID$_{50}$ of SFTSV (Fig. 3B, D). To assess the immune response to N and GPs

157   of SFTSV, sera collected from mice that survived two weeks after the SFTSV infection

158   were subjected to an IFA to measure specific IgG against SFTSV N and GPs, which would

159   not be induced by monovalent m8-GPC and m8-N, respectively. SFTSV GPs-specific IgG

160   was not induced in the m8-N inoculated mice, while N-specific IgG was induced in five of

161   eight m8-GPC-inoculated mice when challenged with 1×10^3 TCID$_{50}$ SFTSV (Fig. 3E). On

162   the other hand, all mice inoculated with m8-N or m8-GPC seroconverted after infection

163   with 1×10^5 TCID$_{50}$ of SFTSV (Fig. 3F). These results indicated that the m8-based SFTSV

164   vaccines conferred protection against a lethal SFTSV challenge. There was a difference in

165   the degree of the efficacy between m8-N and m8-GPC including m8-N+GPC.

166

167   **Effect of preexisting immunity against VAC**

168   To evaluate the impact of preexisting immunity to VAC on the protective immunity

169   induced by m8-based SFTSV vaccines, IFNAR-/- mice were immunized with VAC and

170   then inoculated with each m8-based SFTSV vaccine and challenged with SFTSV. The

171   survival rate of the VAC-preimmunized mice infected with 1×10^3 or 1×10^5 TCID$_{50}$ of

172   SFTSV was improved by inoculation with m8-N, m8-GPC, or m8-N+GPC in comparison

173   to control mice inoculated with m8-EGFP (Fig. 4). However, the survival rates were lower

174   than those of the mice that were not immunized with VAC Lister in advance of the

175   vaccinations (Fig. 3 A, B, and Fig. 4A, B). The survival rate of m8-N+GPC-inoculated

176   mice was similar to that of m8-GPC-inoculated mice, whereas the survival rate of

177   m8-N-inoculated mice was lower in comparison to m8-N+GPC- and m8-GPC-inoculated

178   mice. The disease severity of the groups tended to show a similar trend to the survival rate.

179   Although all the mice, even those inoculated with m8-N, m8-GPC, m8-N+GPC in advance,

180   developed clinical symptoms after infection with 1×10^3 TCID$_{50}$ or 1×10^5 TCID$_{50}$ of

181   SFTSV, the weight loss in the mice inoculated with m8-based SFTSV vaccines, especially

182   those inoculated with m8-GPC or m8-N+GPC, was milder in comparison to mice

183   inoculated with m8-EGFP (Fig. 4C, D). These results indicated that m8-based SFTSV

184   vaccines conferred protection against lethal SFTSV challenge in mice, even when

185   preexisting immunity to VAC was present.

186

187   **SFTSV infection dynamics in mice inoculated with m8-based SFTSV vaccines**

188   To investigate the effect of m8-based SFTSV vaccines in mice on spatial and temporal

189   SFTSV infection dynamics, 6-week-old IFNAR-/- mice (three per group on each collection

190   day) were subcutaneously inoculated twice at a two-week interval with m8-based SFTSV

191   vaccines. Two weeks after the second inoculation, the mice were subcutaneously

10

192   challenged with 1×10^5 TCID$_{50}$ of SFTSV YG-1, and the sera and tissues were collected at

193   1, 3, and 5 DPI. Sera collected from mice inoculated with m8-EGFP showed a high SFTSV

194   titer (Fig. 5A). In contrast, in sera from the mice inoculated with m8-N, m8-GPC, or

195   m8-N+GPC, SFTSV was below the limit of detection or a tiny amount of the virus was

196   detected (Fig. 5A). SFTSV N gene copies were detected in the sera in one-third of the

197   m8-EGFP inoculated mice at 1 DPI, and the copy number was drastically increased at 3 and

198   5 DPI (Fig. 5B). The average N gene copies in 1 ml of serum were $1 \times 10^{6.3}$ and $1 \times 10^{7.5}$

199   copies at 3 and 5 DPI, respectively. On the other hand, the N gene copy numbers in sera of

200   mice inoculated with m8-based SFTSV vaccines contained a significantly lower in

201   comparison to mice inoculated with m8-EGFP. However, the copy number transition

202   differed among mice inoculated with m8-N, m8-GPC, and m8-N+GPC (Fig. 5B). The

203   SFTSV N genes were detected in m8-N-inoculated mice over 3-5 days, although the

204   number of copies was small. In the sera of mice inoculated with m8-GPC, the N gene had

205   been detected at 3 DPI but was below the limit of detection at 5 DPI. The N gene copies

206   were below the detection limit in the sera of the m8-N+GPC-inoculated mice not only at 3

207   DPI but also at 5 DPI.

208

209   Histopathological and immunohistochemical analysis

210   The liver, kidney, spleen, and cervical lymph nodes excised from mice inoculated with

211   m8-N, m8-GPC, m8-N+GPC, and m8-EGFP after SFTSV challenge at 1, 3, and 5 DPI

212   were subjected to histopathological and immunohistochemical analyses. The pathological

213   changes of these organs differed among the 3 m8-based SFTSV vaccination groups and the

11

214   control mice inoculated with m8-EGFP. There were no histopathological changes and any

215   cells with viral antigen in the examined organs excised from m8-GPC or

216   m8-N+GPC-inoculated mice (Table 1, 2, Fig. 5C and D). The m8-N-inoculated mice

217   showed focal infiltration of lymphocytes in the liver at 1 and 3 DPI, although viral

218   antigen-positive cells were not detected at that time. At 5 DPI, there were pathological

219   changes, including multifocal necrosis in the liver, mild interstitial lymphocytic infiltration

220   in the kidney, focal infiltration of neutrophils in the white pulp of the spleen, and depletion

221   of lymphocytes in the cervical lymph nodes (Fig. 5E top and bottom left). Furthermore, in

222   the m8-N-inoculated mice viral antigen-positive large mononuclear cells were detected

223   only spleen and cervical lymph nodes, as was also observed in m8-EGFP-inoculated mice

224   (Table 2 and Fig. 5E bottom right). In the control mice, the m8-EGFP-inoculated mice, the

225   following histopathological changes were observed. Focal necrosis with infiltration of

226   neutrophils and lymphocytes and mild infiltration of mononuclear cells were detected in the

227   liver and kidney at 1 DPI. These hepatic and renal lesions gradually spread over time

228   (Table 1). Lymphocyte depletion with apoptosis and infiltration of neutrophils were also

229   detected in some white pulps in spleen and cervical lymph nodes at 3 DPI, and the lesions

230   progressed as severe lymphocyte depletion with massive necrosis in the splenic white pulps,

231   and cervical lymph nodes caused follicular structure almost disappeared on 5 DPI (Fig. 5F

232   top and bottom left). Some viral antigen-positive cells that were large mononuclear cells

233   began to be detected from 3DPI (Table 2 and Fig. 5F bottom right), and the cells were

234   consistently localized with the lesions, including the liver, kidney, spleen, and lymph nodes.

235   These results indicated that vaccination with either of the m8-based SFTSV vaccines

12

236   conferred protective immunity that suppressed the propagation of SFTSV and inflammation

237   *in vivo*. However, it is noteworthy that the efficacy induced by the m8-based SFTSV

238   GPC vaccine is superior to that induced by vaccination with m8-N.

239   **Table 1. Temporal change of lesion-positive tissues in SFTSV-challenged mice**

Tissue	Lesion (no. of positive / no. of mice)											
	Liver			Spleen			Kidney			Cervical LN		
DPI	1	3	5	1	3	5	1	3	5	1	3	5
m8-EGFP	3/3	3/3	3/3	0/3	2/3	3/3	3/3	3/3	3/3	0/3	1/3	3/3
m8-N	3/3	3/3	3/3	0/3	0/3	1/3	0/3	0/3	1/3	0/3	0/3	3/3
m8-GPC	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
m8-G+N	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

240

241   **Table 2. Temporal change of SFTSV N antigen-positive tissues in SFTSV-challenged**

242   **mice**

Tissue	SFTSV N Antigen (no. of positive / no. of mice)											
	Liver			Spleen			Kidney			Cervical LN		
DPI	1	3	5	1	3	5	1	3	5	1	3	5
m8-EGFP	0/3	2/3	3/3	0/3	3/3	3/3	0/3	1/3	3/3	0/3	2/3	3/3
m8-N	0/3	0/3	0/3	0/3	0/3	2/3	0/3	0/3	0/3	0/3	0/3	3/3
m8-GPC	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
m8-G+N	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

13

243 **Contribution of antibodies induced by m8-based SFTSV vaccine**

244   To verify the contribution of the humoral immunity induced by m8-based SFTSV

245 vaccines, sera obtained from the mice inoculated with each vaccine was passively

246 transferred to naïve mice and challenged with SFTSV. As a control, a group of naïve mice

247 was inoculated with sera obtained from mice three weeks after the infection with 1×10^1

248 $TCID_{50}$ of SFTSV. Six- to 10-week-old naïve IFNAR-/- mice (5 or 2 per group) underwent

249 the intraperitoneal administration of sera, in which the presence of specific SFTSV

250 antibodies was confirmed in the previous experiment (Fig. 2); then, they were immediately

251 subcutaneously infected with SFTSV at a dose of 1×10^3 $TCID_{50}$. The survival rate mice

252 treated with sera collected from mice immunized with each m8-based SFTSV vaccines

253 showed no significant improvement in comparison to m8-EGFP inoculated mice (Fig. 6).

254 However, there were one or two mice that did not develop any weight loss in the groups of

255 mice treated with sera collected from m8-GPC-, m8-N+GPC-, or YG-1-inoculated mice.

256 These sera contained specific and neutralizing antibodies against SFTSV (Fig. 2). These

257 results suggested that the humoral immunity against SFTSV GPs contributed—to a certain

258 degree—to the conferred anti-SFTSV protective immunity.

259

260 **Contribution of CD8-positive cells against SFTSV infections**

261   CD8-positive cells were depleted *in vivo* during SFTSV challenge, which was performed

262 to verify the contribution of CD8-positive cells, which are known to play a significant role

263 in cellular immunity. The depletion of CD8-positive cells during the SFTSV challenge did

264 not alter the survival rate in mice inoculated with either of the m8-based SFTSV vaccines

14

265   or in the mice inoculated with the control m8-EGFP (Fig. 7). Furthermore, there was no

266   marked difference in the sequential change in weight in the groups treated with anti-CD8

267   and control monoclonal antibody (mAb) (Fig. 8). These results suggested that CD8-positive

268   cells do not contribute to conferring anti-SFTSV protective cellular immunity.

269 **Discussion**

270   An SFTS vaccine is urgently needed. One of the strategies for developing safe and

271   effective vaccines is to use recombinant vaccine vectors. VAC has been served as a

272   recombinant vaccine vector for many types of infectious diseases, including highly

273   pathogenic infectious diseases [17]. In particular, MVA-based recombinant vaccines for

274   CCHF and Ebola virus disease were reported to be effective in animal models [26-28].

275   Since there are many similarities between CCHF and SFTS (e.g., *Bunyavirales* order,

276   tick-borne infectious disease, viral hemorrhagic fever, and other clinical findings) [29, 30],

277   we hypothesized that an m8-based recombinant vaccine might be effective for SFTS.

278   Interestingly, this study elucidated both m8-N and m8-GPC conferred protection against

279   lethal SFTSV challenge in mice, although m8-N appeared less effective than m8-GPC.

280   Whereas CCHF virus (CCHFV) GPC-expressing MVA but not CCHF virus (CCHFV)

281   N-expressing MVA conferred protection against lethal CCHFV challenge [26, 27]. It is

282   hypothesized that the active acquired immunity elicited by SFTSV N and CCHFV N is

283   dominantly cellular immunity, since the specific antibody cannot access the N in the virion.

284   Therefore, cellular immunity against SFTSV infection could play a more crucial role than

285   that of CCHFV infection.

286   The expression of the GPC gene in the cells infected with the Uukuniemi virus and Rift

287   Valley fever virus in *Phenuiviridae* is necessary and sufficient for forming the VLP [31-33].

288   Since VLP presents antigens densely and repetitively in general, VLP is advantageous for

289   vaccine use as it is efficiently taken up and processed by antigen-presenting cells, and also

290   activates B cells via the physical crosslinking of B cell receptors [34, 35]. In this study, it

16

291   was confirmed that the SFTSV VLP was produced in the cells *in vitro* when infected with

292   m8-GPC or m8-N+GPC. As for the live recombinant SFTSV vaccines, m8-GPC and

293   m8-N+GPC have the advantage of VLP production, which might occur not only *in vitro* but

294   also *in vivo*, eliciting strong cellular and antibody responses. Although the immunogenicity

295   (in terms of the humoral immunity) in mice induced by authentic SFTSV infection was

296   slightly higher than that induced by recombinant m8-based SFTSV vaccines (Fig. 2), the

297   immune response induced by vaccination with m8-GPC or m8-N+GPC completely

298   protected against lethal SFTSV infection (Fig. 3). This result suggests that the mechanism

299   through which m8-N+GPC induced protective immunity to SFTSV infection by mimicked

300   that of authentic SFTSV infection.

301   The main target population for SFTS vaccination is people over 50 years of age, since age

302   is a critical risk factor for suffering from severe and lethal SFTS [4, 36-38]. All countries

303   terminated the routine smallpox vaccine program for infants by 1980. For example, the

304   routine smallpox vaccination program was terminated in Japan in 1976. Since the youngest

305   individuals who received the smallpox vaccine are now in their 40s, preexisting immunity

306   to VAC is a considerable factor that might affect the ability of m8-based vaccines to induce

307   protection against SFTSV. This study demonstrated the significant efficacy of the m8-GPC

308   and m8-N+GPC vaccine in mice pre-vaccinated with VAC, even though there was only a

309   one-month interval between the VAC (Lister) inoculation and m8-based SFTSV vaccines

310   inoculation (Fig. 4). There have been no orthopoxvirus epidemics in the more than 40 years

311   since people who received the smallpox vaccine received their last vaccination. Thus, it is

312   highly likely that m8-based SFTSV vaccines will still be effective for individuals who have

313   previously received the smallpox vaccine.

314   In general, recombinant VAC elicits both humoral (i.e., antibodies) and cellular immunity

315   (i.e., cytotoxic T lymphocytes) against a target antigen [26, 27]. The key players in the

316   humoral and cellular immunity are antibodies and cytotoxic CD8-positive T lymphocytes.

317   In the present study, it was suggested that the specific antibodies against SFTSV GPC

318   induced by m8-GPC or m8-N+GPC—but not those against N induced by

319   m8-N—contributed to the protection against the SFTSV challenge in mice (Fig. 6). Hence,

320   the major immunity that played a role in the protection of mice from the lethal SFTSV

321   challenge induced by m8-N might be cellular immunity. Furthermore, cellular immunity

322   induced by m8-GPC and m8-N+GPC may also play a role. However, SFTSV gene-specific

323   CD8-positive cells elicited by m8-based SFTSV vaccines were dispensable for the

324   protection against the SFTSV challenge. Further studies are needed to clarify the role of

325   cellular immunity in the protection of subjects, including humans, from SFTSV infection.

326   Interestingly, it was previously reported that *in vivo* depletion of CD8-positive T

327   lymphocytes in rats immunized with recombinant VAC that expresses measles genes did

328   not alter the protective effect against a measles virus challenge [39, 40]. Although the

329   authors did not test the *in vivo* depletion of CD4-positive cells, they hypothesized that the

330   CD4-positive T cell-mediated immune response specific for measles gene products was

331   sufficient to control measles virus infection. The contribution of CD4-positive T cells in

332   mice inoculated with each of the m8-based SFTSV vaccines during the SFTSV challenge

333   should be studied.

334   In summary, a novel recombinant m8 that expresses SFTSV N, GPC, or both N and GPC

335   was generated. m8-GPC-infection and m8-N+GPC-infection of the cells induced the

336   production of SFTSV-like-particles. In addition to m8-GPC and m8-N+GPC, m8-N was

337   confirmed to confer protective efficacy against lethal SFTSV infection in mice. We

338   concluded that m8-based SFTSV vaccines are promising candidates for an SFTS vaccine.

Materials and methods

Ethics statement

341   All experiments associated with animals were performed in animal biological safety level

342 2 or 3 containment laboratories at the National Institute of Infectious Diseases in Japan

343 (NIID) under strict regulations of the animal experimentation guidelines of the NIID. The

344 protocol was approved by the Institutional Animal Care and Use Committee of the NIID

345 (no. 117059, 117130, 117131, 118127, 118129 and 118173).

Cells and viruses

347   293FT cells (Thermo Fisher Scientific, Waltham, MA), Vero cells, and RK13 cells were

348 grown in Dulbecco's Modified Eagle's Medium (DMEM; Wako, Osaka, Japan)

349 supplemented with 5% of heat-inactivated fetal bovine serum (FBS) and antibiotics

350 (Sigma-Aldrich Japan, Tokyo). VAC strain Lister, which is the grandparental strain of m8

351 (accession No. AY678276) [41], m8 (accession No. AY678275), and recombinant m8

352 expressing an enhanced green fluorescent protein (m8-EGFP), which was generated

353 previously [42] were propagated in RK13 cells. The infectious titer in RK13 cells was

354 determined by a standard plaque-forming unit (PFU) assay [41]. SFTSV strains YG-1

355 (accession No. AB817979, AB817987, AB817995), SPL005 (AB817982, AB817990,

356 AB817998), and SPL010 (AB817983, AB817991, AB817999) were propagated in Vero

357 cells. The infectious dose in Vero cells was determined with the 50% standard tissue

358 culture infectious dose ($TCID_{50}$) assay, with visualization of infection by an indirect

359 immunofluorescence assay, as described previously [13]. Briefly, cells infected with

360 SFTSV were reacted with an in-house-made rabbit anti-SFTSV N polyclonal antibody,

20

361 then stained with Alexa Fluor 488-conjugated goat anti-rabbit IgG H+L antibody (Thermo

362 Fisher Scientific).

363

364 **Plasmids**

365 The open reading frames (ORFs) of N and GPC of SFTSV strain SPL005 were amplified

366 using RNA purified from cells infected with SFTSV by two-step conventional RT-PCR.

367 These ORFs containing a synthetic VAC early/late promoter [43] were also cloned into the

368 NotI (N) or an XhoI (GPC) site of precB5R, a plasmid for generating recombinant m8s by

369 homologous recombination [42]. The plasmids inserted with N and GPC genes were named

370 precB5R-N and precB5R-GPC, respectively. The ORF of N with the vaccinia early/late

371 promoter was inserted into the NotI site of precB5R-GPC to construct a plasmid named

372 precB5R-N+GPC that contained both ORFs: N and GPC. The mammalian expression

373 plasmid pHEK293 Ultra Expression Vector II (Takara Bio Inc., Shiga, Japan) was also

374 inserted with the N or GPC ORF at the BamHI site of multicloning sites. Plasmids with the

375 insertion of N and GPC were named pHEK293-N and -GPC, respectively. The N protein

376 expression plasmid was inserted with an N gene with a FLAG-tag at the C-terminus of the

377 gene.

378

379 **Generation of recombinant m8 harboring SFTSV genes**

380 Recombinant m8, m8-N, m8-GPC and m8-N+GPC, with the insertion of only the N gene,

381 only the GPC gene, and both the N and GPC genes, respectively, in the flanking region

382 between B4R and B6R genes, were generated with a method employing homologous

21

383   recombination for foreign gene insertion, as described previously [42]. Briefly, 293FT cells

384   were transfected with each of the following plasmids: precB5R-N, precB5R-GPC, and

385   precB5R-N+GPC by using X-tremeGENE 9 (Roche Diagnostics K.K., Tokyo, Japan).

386   Cells transfected with each plasmid were then infected with m8 at a multiplicity of

387   infection (MOI) of 0.05 per cell. The cells were cultured in DMEM supplemented with 5%

388   FBS for 3 days. The culture medium, along with the cells, was then collected and was

389   freeze-thawed three times to prepare crude intermediate recombinant m8 stocks. RK13 cell

390   monolayers were inoculated with each of the crude intermediate recombinant m8 stocks.

391   Then, the inoculated stock was removed, and the cells were overlaid with Eagle's Minimal

392   Essential Medium (EMEM, Wako) containing 2% of FBS, 20 μg/ml mycophenolic acid

393   (MPA, Sigma-Aldrich), 250 μg/ml xanthine, 15 μg/ml hypoxanthine, and 1% agarose M.E.

394   (Iwai Chemicals, Tokyo, Japan). After three days, the mCherry fluorescence-positive

395   plaques were identified by either fluorescence microscopy or an LED transilluminator

396   (GELmieru, WAKO) at the wavelength of 500 nm. One fluorescence-positive plaque in the

397   agarose plug was collected and mixed with DMEM supplemented with 5% FBS to prepare

398   stocks of the intermediate recombinant m8 clones. RK13 cells in a monolayer in a 96-well

399   plate were inoculated with the intermediate clones to confirm the expression capability of

400   SFTSV N and/or GPC. The cells inoculated were fixed with a 1:1 methanol: acetone

401   mixture at two days post-infection (DPI). The cells were then reacted with either an

402   in-house-made rabbit anti-SFTSV N polyclonal antibody or an in-house mouse anti-SFTSV

403   Gc mAb clone C6C1, followed by reaction with DyLight 594-conjugated goat anti-rabbit

404   IgG H+L antibody (Abcam, Cambridge, UK) and DyLight 488-conjugated goat anti-mouse

405   IgG H+L antibody (Abcam), respectively. The fluorescence-positive expression clones

406   were then further purified by plaque isolation at least three times. RK13 cells were infected

407   with the purified intermediate clones without supplementation with MPA, xanthine, and

408   hypoxanthine to prepare the crude final recombinant m8 stocks. Final recombinant

409   m8-based SFTSV vaccines, from which the selection marker genes were self-excised,

410   resulting in their deletion, were cloned by selecting fluorescence-negative plaques. Hence,

411   monolayered RK13 cells were infected with one of the final crude recombinant m8 stocks,

412   and the cells were overlaid with agarose containing MEM supplemented with 2% FBS

413   without supplementation with MPA, xanthine, and hypoxanthine. After three days, the

414   mCherry fluorescence-negative plaques were collected under an LED transilluminator. The

415   N and/or GPC expression-positive and the mCherry fluorescence-negative clones were next

416   purified by plaque isolation at least three times to establish the final recombinants. The

417   m8-N, m8-GPC, m8-N+GPC, and m8-EGFP were propagated in RK13 cells, and their

418   infectious doses were determined with the standard plaque assay.

419

420   **Confirmation of SFTS virus-like-particles (VLP)**

421   RK13 cells were infected with m8-GPC or m8-N+GPC at an MOI of 0.1. The culture

422   supernatant was collected at 3 DPI and mixed with polyethylene glycol (PEG) 6000 at a

423   final concentration of 8 w/v % for pelleting the VLP, and the mixture was incubated at 4°C

424   overnight. The PEG precipitates were pelleted by centrifugation for 30 min at 1,500 ×g at

425   4 °C in a swing-out rotor. The pellets were resuspended with DMEM supplemented with

426   5% FBS. The VLP was purified through a double-cushion of 20% and 60% sucrose in H_2O

427   in an SW-41 swing-out rotor by ultracentrifugation (Beckman Coulter, Brea, CA) for 2

428   hours at 25,000 rpm at 4 °C in a swing-out rotor. The accumulated VLP that was banded at

429   the 20/60% interface was negatively stained with 2% phosphotungstic acid and then

430   observed using a JEM-1400 transmission electron microscope (JEOL, Tokyo, Japan).

431

432   **Immunoprecipitation**

433   The incorporation of SFTSV N into the VLP was confirmed by immunoprecipitation.

434   Briefly, RK13 cells were infected with m8-EGFP, m8-N, or m8-N+GPC at an MOI of 0.05.

435   The culture supernatants were collected at 3 DPI. The remaining cells were lysed with

436   sodium dodecyl sulfate (SDS) sample buffer and heated at 95 °C for 5 min. One milliliter

437   of each supernatant was incubated with or without 3 μg of mouse anti-SFTSV Gc mAb

438   (clone C6C1) with rotation for 1 hour at room temperature followed by further incubation

439   for 1 hour with the addition of 15 µl of goat anti-mouse IgG magnetic beads (New England

440   Biolabs, Ipswich, MA, USA). The magnetic beads in the supernatant were collected on a

441   magnetic stand and washed 2 times with PBS. Twenty microliters of SDS sample buffer

442   was added to the beads and heated at 95 °C for 5 min. The infected cell lysates and

443   immunoprecipitated samples were fractionated by SDS polyacrylamide gel electrophoresis

444   (SDS-PAGE) and subjected to Western blotting. The membrane was reacted with rabbit

445   anti-SFTSV N polyclonal antibody, followed by reaction with horseradish peroxidase

446   (HRP)-conjugated goat anti-rabbit IgG (H+L) antibody (KPL, Gaithersburg, MD). The

447   immunoreactive proteins were visualized by LAS-4000mini (Fujifilm, Tokyo, Japan) with

448   ECL Prime Western blotting detection reagents (G.E. Healthcare, Chicago, IL, USA).

449

Animals

451 IFNAR-/- mice on the C57BL/6 background were used for all of the animal experiments.

452 The IFNAR-/- mice were previously produced by mating DNase II/type I interferon

453 receptor double-knockout mice (strain B6.129-Dnase2a<tm1Osa> Ifnar1<tm1Agt>)

454 [44-46] and C57BL/6 mice (age, 4–10 weeks; sex, male or female) and bred under specific

455 pathogen-free (SPF) conditions at the NIID [47].

456

Evaluation of vaccine efficacy in VAC-immunonegative mice

458 The mice were subcutaneously inoculated twice at a two-week interval with 1×10^6 PFU

459 of m8-EGFP, m8-N, m8-GPC, or m8-N+GPC in a volume of 100 µl per mouse under

460 anesthesia with midazolam, medetomidine and butorphanol tartrate combination. At two

461 weeks after the second inoculation, the mice were euthanized with Isoflurane and sacrificed

462 to collect blood to evaluate immunogenicity or subcutaneously challenged with 1×10^3 or 1

463 $\times 10^5$ TCID$_{50}$ of SFTSV strain YG-1 in a volume of 100 µl per mouse under anesthesia

464 with midazolam, medetomidine and butorphanol tartrate combination to evaluate the

465 protective efficacy of each m8-based SFTSV vaccine candidate. The body weight and

466 clinical signs of the mice challenged with SFTSV was monitored daily for two weeks. In

467 the experiments challenged with SFTSV, the mice were euthanized with Isoflurane when

468 the mice became moribund or expected to be die since of the difficulty in eating and/or

469 drinking.

470

25

471 **Evaluation of vaccine efficacy in VAC-immunopositive mice**

472 Four- to five-week-old IFNAR-/- mice (10 per group) were subcutaneously inoculated

473 with 1×10^6 PFU of a VAC strain Lister, a second-generation smallpox vaccine strain, in a

474 volume of 100 µl per mouse at 4 weeks prior to the first inoculation with the m8-based

475 SFTSV vaccine. The VAC pre-inoculated mice were then subcutaneously inoculated twice

476 at a two-week interval with each m8-based SFTSV vaccine followed by subcutaneous

477 challenge with 1×10^3 or 1×10^5 TCID$_{50}$ of SFTSV YG-1, as described above. The body

478 weight and clinical signs of the mice challenged with SFTSV was monitored daily for two

479 weeks.

480

481 **Evaluation of the anti-SFTSV antibody transfer efficacy and cellular immunity in**

482 **mice**

483 The following experiment was designed to evaluate the humoral immunity induced by

484 vaccination with m8- based SFTSV vaccines. Sera were collected from mice

485 subcutaneously inoculated twice with 1×10^6 PFU of m8-EGFP, m8-N, m8-GPC, or

486 m8-N+GPC at a two-week interval, as described above. Naïve mice were passively

487 transferred twice with 100 µl of sera at 1 day and immediately before the subcutaneous

488 SFTSV challenge at 1×10^3 TCID$_{50}$. to evaluate the cellular immunity induced by based

489 SFTSV vaccines, CD8-positive cells were depleted *in vivo* by the administration of

490 anti-CD8 mAb, as described previously [48]. It was confirmed that 96.1 and 99.2% of

491 CD8-positive cells were depleted from the spleen in naïve IFNAR-/- mice at 1 and 3 days

492 post-mAb inoculation, respectively, when 250 µg of rat anti-mouse CD8 mAb clone 2.43

26

493   (BioXCell, West Lebanon, NH) intraperitoneally administered. Rat anti-keyhole limpet

494   hemocyanin mAb clone LTF-2 (BioXCell) was used as the control antibody. The 6- to

495   9-week-old IFNAR-/- mice (4 to 5 per group) were subcutaneously inoculated twice with 1

496    $\times$ 10^6 PFU of m8-EGFP, m8-N, m8-GPC, or m8-N+GPC at a 2-week interval, as described

497   above. Two weeks later, the mice were subcutaneously challenged with 1×10^3 or 1×10^5

498    $TCID_{50}$ of SFTSV YG-1 and were intraperitoneally inoculated with 250 μg of anti-CD8

499   mAb clone 2.43 on day -1, 2, 5, and 8 after SFTSV challenge, taking the day on which the

500   mice were challenged with SFTSV as day 0. The body weight and clinical signs of the mice

501   challenged with SFTSV was monitored daily for two weeks.

502

503   **Indirect immunofluorescence assay**

504   IFA antigen-spotted slides, 293FT cells were transfected with either pHEK293-N or

505   pHEK293-GPC with an expression enhancer plasmid, pHEK293 (Takara Bio Inc.). A

506   mixture of the 293FT cells transfected with expression vectors in combination and

507   untransfected 293FT cells at a ratio of 1:3 were washed with PBS, spotted on glass slides

508   (Matsunami glass IND., Ltd., Osaka, Japan), dry-fixed, and treated with acetone. To

509   measure the titer of the SFTSV N- or GPC-specific IgG in mice, serum samples

510   immobilized at 56 °C for 30 min were serially diluted two-fold and added onto the antigens

511   on the glass slides. The antigens were then reacted with an Alexa Fluor 488-conjugated

512   goat anti-mouse IgG H+L antibody (Thermo Fisher Scientific). The antibody titer was

513   defined as the reciprocal of the highest dilution level at which a specific fluorescent signal

514   was detected.

515

Focus reduction neutralization test

517   To measure the titer of neutralizing antibody to SFTSV in mouse serum, 100 TCID$_{50}$

518 aliquots of SFTSV were mixed with 4-fold serially diluted immobilized sera, and the

519 mixtures were incubated for 1 hour. Confluent Vero cell cultures in 12-well microtiter

520 plates were inoculated with each virus and serum mixture for 1 hour, overlaid with DMEM

521 containing 2% FBS and 1% methylcellulose, and then cultured for 4 to 5 days. The cells

522 were fixed with PBS containing 10% formalin and were reacted with rabbit anti-SFTSV N

523 polyclonal antibody. The foci of SFTSV infection were then visualized using a standard

524 immunoperoxidase method. The percent N.T. antibody titer was determined to divide the

525 number of foci in the well inoculated with the virus and serum from m8-SFTSV gene or

526 SFTSV inoculated mouse by that of naïve mice.

527

Quantitative one-step RT-PCR

529   Specific quantitative one-step reverse transcription-PCR (qPCR) was performed as

530 reported previously [49]. Specific qPCR primer and probe sets targeted to the N gene

531 (forward; TGTCAGAGTGGTCCAGGATT, reverse; ACCTGTCTCCTTCAGCTTCT,

532 probe; FAM-TGGAGTTTGGTGAGCAGCAGC-BHQ1) were used. Total RNAs were

533 extracted from 200 μl of mice sera using a High Pure viral RNA kit (Roche Applied

534 Science) according to the manufacturer's protocol. The elution volume for RNA extraction

535 was 50 μl. For the qPCR assay, an aliquot of the extracted RNA solution was added to the

536 reaction mixture for the QuantiTect probe RT-PCR kit (Qiagen, Hilden, Germany), which

28

537   contained 2x QuantiTect probe RT-PCR master mix, QuantiTect RT mix, H2O, and 10x

538   primer-probe mix, which contained 4 μM of each specific primer, 2 μM TaqMan probe(s),

539   and 2 μl of contamplicon probe. After PCR activation at 95°C for 15 min, the reverse

540   transcription reaction was carried out at 50°C for 30 min, followed by 45 cycles of

541   amplification under the following conditions: 94°C for 15 s and 60°C for 60 s in a

542   LightCycler 96 (Roche).

543

544   **Histopathology and immunohistochemistry**

545   As described above, IFNAR-/- mice were subcutaneously inoculated with 1×10^6 PFU of

546   the m8-EGFP or each m8-based SFTSV vaccine twice at a 2-week interval and were

547   intraperitoneally challenged with 1×10^5 $TCID_{50}$ of SFTSV YG-1, 2 weeks after the second

548   inoculation. The SFTSV-infected mice were euthanized with Isoflurane and sacrificed at 1,

549   3, and 5 DPI, and the blood, cervical lymph nodes, spleen, liver, and kidneys were collected.

550   The tissues were fixed with PBS containing 10% formalin and embedded with paraffin to

551   prepare blocks. Sections were prepared from the tissue block and were stained with

552   hematoxylin-eosin for a histopathological examination. An immunohistochemical analysis

553   to detect SFTSV N protein was performed as previously described [13]. The sections were

554   first reacted with a rabbit anti-SFTSV N polyclonal antibody. Antigens were retrieved by

555   hydrolytic autoclaving in citrate buffer (pH 6.0) for 10 min at 121°C. IHC staining was then

556   performed using a standard immunoperoxidase method.

557

Statistical analysis

558

559   All statistical analyses were performed using GraphPad Prism 7 (GraphPad Software, La

560   Jolla, CA). Survival curves were plotted according to a Kaplan-Meier analysis, and the

561   protective efficacy of the m8-based SFTSV vaccines against SFTSV challenge in mice was

562   evaluated using a log-rank test with Bonferroni correction to adjust the significance level

563   for multiple comparisons. The equality of the means of the virulent virus titer or the viral

564   RNA levels in mouse sera was evaluated using a two-way analysis of variance (ANOVA)

565   with Sidak's multiple-comparison test. P values of <0.05 were considered to indicate

566   statistical significance.

References

570

571 1.   Yu XJ, Liang MF, Zhang SY, Liu Y, Li JD, Sun YL, et al. Fever with

572 thrombocytopenia associated with a novel bunyavirus in China. N Engl J Med.

573 2011;364(16):1523-32. Epub 2011/03/18. doi: 10.1056/NEJMoa1010095. PubMed PMID:

574 21410387; PubMed Central PMCID: PMC3113718.

575 2.   Xu B, Liu L, Huang X, Ma H, Zhang Y, Du Y, et al. Metagenomic analysis of

576 fever, thrombocytopenia and leukopenia syndrome (FTLS) in Henan Province, China:

577 discovery of a new bunyavirus. PLoS Pathog. 2011;7(11):e1002369. Epub 2011/11/25. doi:

578 10.1371/journal.ppat.1002369. PubMed PMID: 22114553; PubMed Central PMCID:

579 PMC3219706.

580 3.   Zhang YZ, Zhou DJ, Xiong Y, Chen XP, He YW, Sun Q, et al. Hemorrhagic fever

581 caused by a novel tick-borne Bunyavirus in Huaiyangshan, China. Zhonghua Liu Xing

582 Bing Xue Za Zhi. 2011;32(3):209-20. Epub 2011/04/05. PubMed PMID: 21457654.

583 4.   Kato H, Yamagishi T, Shimada T, Matsui T, Shimojima M, Saijo M, et al.

584 Epidemiological and Clinical Features of Severe Fever with Thrombocytopenia Syndrome

585 in Japan, 2013-2014. PLoS One. 2016;11(10):e0165207. Epub 2016/10/25. doi:

586 10.1371/journal.pone.0165207. PubMed PMID: 27776187; PubMed Central PMCID:

587 PMCPMC5077122.

588 5.   Reece LM, Beasley DW, Milligan GN, Sarathy VV, Barrett AD. Current status of

589 Severe Fever with Thrombocytopenia Syndrome vaccine development. Curr Opin Virol.

590 2018;29:72-8. Epub 2018/04/12. doi: 10.1016/j.coviro.2018.03.005. PubMed PMID:

591 29642053.

592   6.   Choi SJ, Park SW, Bae IG, Kim SH, Ryu SY, Kim HA, et al. Severe Fever with

593   Thrombocytopenia Syndrome in South Korea, 2013-2015. PLoS neglected tropical

594   diseases. 2016;10(12):e0005264. doi: 10.1371/journal.pntd.0005264. PubMed PMID:

595   28033338; PubMed Central PMCID: PMCPMC5226827.

596   7.   Hu J, Li S, Zhang X, Zhao H, Yang M, Xu L, et al. Correlations between clinical

597   features and death in patients with severe fever with thrombocytopenia syndrome. Medicine

598   (Baltimore). 2018;97(22):e10848. Epub 2018/06/01. doi: 10.1097/MD.0000000000010848.

599   PubMed PMID: 29851797; PubMed Central PMCID: PMCPMC6392624.

600   8.   Shin J, Kwon D, Youn SK, Park JH. Characteristics and Factors Associated with

601   Death among Patients Hospitalized for Severe Fever with Thrombocytopenia Syndrome,

602   South Korea, 2013. Emerg Infect Dis. 2015;21(10):1704-10. Epub 2015/09/25. doi:

603   10.3201/eid2110.141928. PubMed PMID: 26402575; PubMed Central PMCID:

604   PMCPMC4593431.

605   9.   Ding YP, Liang MF, Ye JB, Liu QH, Xiong CH, Long B, et al. Prognostic value of

606   clinical and immunological markers in acute phase of SFTS virus infection. Clin Microbiol

607   Infect. 2014;20(11):O870-8. Epub 2014/04/02. doi: 10.1111/1469-0691.12636. PubMed

608   PMID: 24684627.

609   10.   Liu S, Chai C, Wang C, Amer S, Lv H, He H, et al. Systematic review of severe

610   fever with thrombocytopenia syndrome: virology, epidemiology, and clinical

611   characteristics. Reviews in medical virology. 2014;24(2):90-102. doi: 10.1002/rmv.1776.

612   PubMed PMID: 24310908; PubMed Central PMCID: PMC4237196.

613   11.   Deng B, Zhang S, Geng Y, Zhang Y, Wang Y, Yao W, et al. Cytokine and

614   chemokine levels in patients with severe fever with thrombocytopenia syndrome virus.

615   PLoS One. 2012;7(7):e41365. Epub 2012/08/23. doi: 10.1371/journal.pone.0041365.

616   PubMed PMID: 22911786; PubMed Central PMCID: PMCPMC3404083.

617   12.   Kim KH, Yi J, Kim G, Choi SJ, Jun KI, Kim NH, et al. Severe fever with

618   thrombocytopenia syndrome, South Korea, 2012. Emerg Infect Dis. 2013;19(11):1892-4.

619   doi: 10.3201/eid1911.130792. PubMed PMID: 24206586; PubMed Central PMCID:

620   PMC3837670.

621   13.   Takahashi T, Maeda K, Suzuki T, Ishido A, Shigeoka T, Tominaga T, et al. The

622   first identification and retrospective study of Severe Fever with Thrombocytopenia

623   Syndrome in Japan. J Infect Dis. 2014;209(6):816-27. doi: 10.1093/infdis/jit603. PubMed

624   PMID: 24231186.

625   14.   Tran XC, Yun Y, Van An L, Kim SH, Thao NTP, Man PKC, et al. Endemic Severe

626   Fever with Thrombocytopenia Syndrome, Vietnam. Emerg Infect Dis. 2019;25(5):1029-31.

627   Epub 2019/04/20. doi: 10.3201/eid2505.181463. PubMed PMID: 31002059; PubMed

628   Central PMCID: PMCPMC6478219.

629   15.   Dong F, Li D, Wen D, Li S, Zhao C, Qi Y, et al. Single dose of a rVSV-based

630   vaccine elicits complete protection against severe fever with thrombocytopenia syndrome

631   virus. NPJ Vaccines. 2019;4:5. Epub 2019/02/01. doi: 10.1038/s41541-018-0096-y.

632   PubMed PMID: 30701094; PubMed Central PMCID: PMCPMC6347601 All the remaining

633   authors declare no competing interests.

634   16.   Kwak JE, Kim YI, Park SJ, Yu MA, Kwon HI, Eo S, et al. Development of a

635   SFTSV DNA vaccine that confers complete protection against lethal infection in ferrets.

34

636   Nat Commun. 2019;10(1):3836. Epub 2019/08/25. doi: 10.1038/s41467-019-11815-4.

637   PubMed PMID: 31444366; PubMed Central PMCID: PMCPMC6707330.

638   17.   Walsh SR, Dolin R. Vaccinia viruses: vaccines against smallpox and vectors against

639   infectious diseases and tumors. Expert Rev Vaccines. 2011;10(8):1221-40. doi:

640   10.1586/erv.11.79.   PubMed   PMID: 21854314;   PubMed   Central   PMCID:

641   PMCPMC3223417.

642   18.   Ramezanpour B, Haan I, Osterhaus A, Claassen E. Vector-based genetically

643   modified vaccines: Exploiting Jenner's legacy. Vaccine. 2016;34(50):6436-48. Epub

644   2016/12/29. doi: 10.1016/j.vaccine.2016.06.059. PubMed PMID: 28029542; PubMed

645   Central PMCID: PMCPMC7115478.

646   19.   Lane JM, Ruben FL, Neff JM, Millar JD. Complications of smallpox vaccination,

647   1968: results of ten statewide surveys. J Infect Dis. 1970;122(4):303-9. Epub 1970/10/01.

648   doi: 10.1093/infdis/122.4.303. PubMed PMID: 4396189.

649   20.   Kenner J, Cameron F, Empig C, Jobes DV, Gurwith M. LC16m8: an attenuated

650   smallpox vaccine. Vaccine. 2006;24(47-48):7009-22. doi: 10.1016/j.vaccine.2006.03.087.

651   PubMed PMID: 17052815.

652   21.   Belyakov IM, Earl P, Dzutsev A, Kuznetsov VA, Lemon M, Wyatt LS, et al. Shared

653   modes of protection against poxvirus infection by attenuated and conventional smallpox

654   vaccine viruses. Proc Natl Acad Sci U S A. 2003;100(16):9458-63. doi:

655   10.1073/pnas.1233578100.   PubMed   PMID: 12869693;   PubMed   Central   PMCID:

656   PMCPMC170940.

657   22.   Earl PL, Americo JL, Wyatt LS, Eller LA, Whitbeck JC, Cohen GH, et al.

658   Immunogenicity of a highly attenuated MVA smallpox vaccine and protection against

659   monkeypox. Nature. 2004;428(6979):182-5. doi: 10.1038/nature02331. PubMed PMID:

660   15014500.

661   23.   Wyatt LS, Earl PL, Eller LA, Moss B. Highly attenuated smallpox vaccine protects

662   mice with and without immune deficiencies against pathogenic vaccinia virus challenge.

663   Proc Natl Acad Sci U S A. 2004;101(13):4590-5. doi: 10.1073/pnas.0401165101. PubMed

664   PMID: 15070762; PubMed Central PMCID: PMCPMC384791.

665   24.   Kennedy JS, Gurwith M, Dekker CL, Frey SE, Edwards KM, Kenner J, et al. Safety

666   and immunogenicity of LC16m8, an attenuated smallpox vaccine in vaccinia-naive adults. J

667   Infect Dis. 2011;204(9):1395-402. Epub 2011/09/17. doi: 10.1093/infdis/jir527. PubMed

668   PMID: 21921208; PubMed Central PMCID: PMCPMC3218648.

669   25.   Saito T, Fujii T, Kanatani Y, Saijo M, Morikawa S, Yokote H, et al. Clinical and

670   immunological response to attenuated tissue-cultured smallpox vaccine LC16m8. JAMA.

671   2009;301(10):1025-33. Epub 2009/03/13. doi: 10.1001/jama.2009.289. PubMed PMID:

672   19278946.

673   26.   Dowall SD, Buttigieg KR, Findlay-Wilson SJ, Rayner E, Pearson G, Miloszewska

674   A, et al. A Crimean-Congo hemorrhagic fever (CCHF) viral vaccine expressing

675   nucleoprotein is immunogenic but fails to confer protection against lethal disease. Human

676   vaccines   &   immunotherapeutics.   2016;12(2):519-27.   Epub   2015/08/27.   doi:

677   10.1080/21645515.2015.1078045. PubMed PMID: 26309231; PubMed Central PMCID:

678   PMCPMC5049717.

679   27.   Buttigieg KR, Dowall SD, Findlay-Wilson S, Miloszewska A, Rayner E, Hewson

680   R, et al. A novel vaccine against Crimean-Congo Haemorrhagic Fever protects 100% of

681   animals against lethal challenge in a mouse model. PLoS One. 2014;9(3):e91516. Epub

682   2014/03/14. doi: 10.1371/journal.pone.0091516. PubMed PMID: 24621656; PubMed

683   Central PMCID: PMCPMC3951450.

684   28.   Domi A, Feldmann F, Basu R, McCurley N, Shifflett K, Emanuel J, et al. A Single

685   Dose of Modified Vaccinia Ankara expressing Ebola Virus Like Particles Protects

686   Nonhuman Primates from Lethal Ebola Virus Challenge. Sci Rep. 2018;8(1):864. Epub

687   2018/01/18. doi: 10.1038/s41598-017-19041-y. PubMed PMID: 29339750; PubMed

688   Central PMCID: PMCPMC5770434.

689   29.   Shayan S, Bokaean M, Shahrivar MR, Chinikar S. Crimean-Congo Hemorrhagic

690   Fever.   Lab   Med.   2015;46(3):180-9.   Epub   2015/07/23.   doi:

691   10.1309/LMN1P2FRZ7BKZSCO. PubMed PMID: 26199256.

692   30.   Miyamoto S, Ito T, Terada S, Eguchi T, Furubeppu H, Kawamura H, et al.

693   Fulminant myocarditis associated with severe fever with thrombocytopenia syndrome: a

694   case   report.   BMC   Infect   Dis.   2019;19(1):266.   Epub   2019/03/20.   doi:

695   10.1186/s12879-019-3904-8. PubMed PMID: 30885147; PubMed Central PMCID:

696   PMCPMC6423866.

697   31.   Strandin T, Hepojoki J, Vaheri A. Cytoplasmic tails of bunyavirus Gn

698   glycoproteins-Could they act as matrix protein surrogates? Virology. 2013;437(2):73-80.

699   Epub 2013/01/30. doi: 10.1016/j.virol.2013.01.001. PubMed PMID: 23357734.

700   32.   Piper ME, Sorenson DR, Gerrard SR. Efficient cellular release of Rift Valley fever

701   virus requires genomic RNA. PLoS One. 2011;6(3):e18070. Epub 2011/03/30. doi:

37

702   10.1371/journal.pone.0018070. PubMed PMID: 21445316; PubMed Central PMCID:

703   PMCPMC3061922.

704   33.   Overby AK, Popov V, Neve EP, Pettersson RF. Generation and analysis of

705   infectious virus-like particles of uukuniemi virus (bunyaviridae): a useful system for

706   studying bunyaviral packaging and budding. J Virol. 2006;80(21):10428-35. Epub

707   2006/08/25. doi: 10.1128/JVI.01362-06. PubMed PMID: 16928751; PubMed Central

708   PMCID: PMCPMC1641803.

709   34.   Zabel F, Mohanan D, Bessa J, Link A, Fettelschoss A, Saudan P, et al. Viral

710   particles drive rapid differentiation of memory B cells into secondary plasma cells

711   producing increased levels of antibodies. J Immunol. 2014;192(12):5499-508. Epub

712   2014/05/14. doi: 10.4049/jimmunol.1400065. PubMed PMID: 24821969.

713   35.   Li C, Liu F, Liang M, Zhang Q, Wang X, Wang T, et al. Hantavirus-like particles

714   generated in CHO cells induce specific immune responses in C57BL/6 mice. Vaccine.

715   2010;28(26):4294-300. Epub 2010/05/04. doi: 10.1016/j.vaccine.2010.04.025. PubMed

716   PMID: 20433802.

717   36.   Fu Y, Li S, Zhang Z, Man S, Li X, Zhang W, et al. Phylogeographic analysis of

718   severe fever with thrombocytopenia syndrome virus from Zhoushan Islands, China:

719   implication for transmission across the ocean. Sci Rep. 2016;6:19563. doi:

720   10.1038/srep19563. PubMed PMID: 26806841; PubMed Central PMCID:

721   PMCPMC4726339.

722   37.   Ding S, Niu G, Xu X, Li J, Zhang X, Yin H, et al. Age is a critical risk factor for

723   severe fever with thrombocytopenia syndrome. PLoS One. 2014;9(11):e111736. Epub

38

724   2014/11/05. doi: 10.1371/journal.pone.0111736. PubMed PMID: 25369237; PubMed

725   Central PMCID: PMCPMC4219771.

726   38.   Guo CT, Lu QB, Ding SJ, Hu CY, Hu JG, Wo Y, et al. Epidemiological and clinical

727   characteristics of severe fever with thrombocytopenia syndrome (SFTS) in China: an

728   integrated data analysis. Epidemiol Infect. 2016;144(6):1345-54. Epub 2015/11/07. doi:

729   10.1017/S0950268815002678. PubMed PMID: 26542444.

730   39.   Brinckmann UG, Bankamp B, Reich A, ter Meulen V, Liebert UG. Efficacy of

731   individual measles virus structural proteins in the protection of rats from measles

732   encephalitis. J Gen Virol. 1991;72 (Pt 10):2491-500. Epub 1991/10/01. doi:

733   10.1099/0022-1317-72-10-2491. PubMed PMID: 1833505.

734   40.   Bankamp B, Brinckmann UG, Reich A, Niewiesk S, ter Meulen V, Liebert UG.

735   Measles virus nucleocapsid protein protects rats from encephalitis. J Virol.

736   1991;65(4):1695-700. Epub 1991/04/01. PubMed PMID: 1825854; PubMed Central

737   PMCID: PMCPMC239973.

738   41.   Morikawa S, Sakiyama T, Hasegawa H, Saijo M, Maeda A, Kurane I, et al. An

739   attenuated LC16m8 smallpox vaccine: analysis of full-genome sequence and induction of

740   immune   protection.   J   Virol.   2005;79(18):11873-91.   doi:

741   10.1128/JVI.79.18.11873-11891.2005. PubMed PMID: 16140764; PubMed Central

742   PMCID: PMCPMC1212643.

743   42.   Omura N, Yoshikawa T, Fujii H, Shibamura M, Inagaki T, Kato H, et al. A Novel

744   System for Constructing a Recombinant Highly-Attenuated Vaccinia Virus Strain

745   (LC16m8) Expressing Foreign Genes and Its Application for the Generation of

746 LC16m8-Based Vaccines against Herpes Simplex Virus 2. Japanese journal of infectious

747 diseases. 2018;71(3):229-33. doi: 10.7883/yoken.JJID.2017.458. PubMed PMID:

748 29709968.

749 43.   Chakrabarti S, Sisler JR, Moss B. Compact, synthetic, vaccinia virus early/late

750 promoter for protein expression. BioTechniques. 1997;23(6):1094-7. PubMed PMID:

751 9421642.

752 44.   Yoshida H, Okabe Y, Kawane K, Fukuyama H, Nagata S. Lethal anemia caused by

753 interferon-beta produced in mouse embryos carrying undigested DNA. Nat Immunol.

754 2005;6(1):49-56. Epub 2004/11/30. doi: 10.1038/ni1146. PubMed PMID: 15568025.

755 45.   Kawane K, Ohtani M, Miwa K, Kizawa T, Kanbara Y, Yoshioka Y, et al. Chronic

756 polyarthritis caused by mammalian DNA that escapes from degradation in macrophages.

757 Nature. 2006;443(7114):998-1002. Epub 2006/10/27. doi: 10.1038/nature05245. PubMed

758 PMID: 17066036.

759 46.   Muller U, Steinhoff U, Reis LF, Hemmi S, Pavlovic J, Zinkernagel RM, et al.

760 Functional role of type I and type II interferons in antiviral defense. Science.

761 1994;264(5167):1918-21. Epub 1994/06/24. doi: 10.1126/science.8009221. PubMed

762 PMID: 8009221.

763 47.   Tani H, Fukuma A, Fukushi S, Taniguchi S, Yoshikawa T, Iwata-Yoshikawa N, et

764 al. Efficacy of T-705 (Favipiravir) in the Treatment of Infections with Lethal Severe Fever

765 with   Thrombocytopenia   Syndrome   Virus.   mSphere.   2016;1(1).   doi:

766 10.1128/mSphere.00061-15. PubMed PMID: 27303697; PubMed Central PMCID:

767 PMCPMC4863605.

768   48.   Jing W, Gershan JA, Johnson BD. Depletion of CD4 T cells enhances

769   immunotherapy for neuroblastoma after syngeneic HSCT but compromises development of

770   antitumor immune memory. Blood. 2009;113(18):4449-57. Epub 2009/02/03. doi:

771   10.1182/blood-2008-11-190827. PubMed PMID: 19182203; PubMed Central PMCID:

772   PMCPMC2676098.

773   49.   Yoshikawa T, Fukushi S, Tani H, Fukuma A, Taniguchi S, Toda S, et al. Sensitive

774   and specific PCR systems for detection of both Chinese and Japanese severe fever with

775   thrombocytopenia syndrome virus strains and prediction of patient survival based on viral

776   load. Journal of clinical microbiology. 2014;52(9):3325-33. doi: 10.1128/JCM.00742-14.

777   PubMed PMID: 24989600; PubMed Central PMCID: PMCPMC4313158.

778

Figure Captions

Fig 1. The construction, generation, and characterization of recombinant LC16m8 harboring SFTSV N, GPC, and both N and GPC, and EGFP genes.

A schematic illustration of the site harboring EGFP, SFTSV genes in the genome in m8-EGFP, or m8-based SFTSV vaccines (A). The VAC B5R gene, flanked by the B4R and B6R genes, was substituted for the SFTSV gene. Synthetic vaccinia early/late promoter is indicated as P in the arrow-shaped box. The expression of SFTSV N protein and GPs was confirmed in the cells infected with m8-based SFTSV vaccines (B). Purified VLP from the supernatant of the m8-N+GPC-infected cell was negatively stained and observed under a transmission electron microscope (C). The supernatant was precipitated using PEG 6000, and the VLP was banded by ultracentrifugation at the interface of 20% and 60% (w/v) double-cushion sucrose. RK13 cells were infected with m8-N+GPC at an MOI of 0.1 per cell, and the culture supernatant was collected at 3 DPI. The collected VLP in the culture supernatant was banded at the 20/60% interface and then negatively stained with 2% phosphotungstic acid. The bar in the image indicates the length of 100 nm. The picture of the VLP was derived from the supernatant of the m8-N+GPC-infected cells. The morphology of SFTS VLP from the m8-GPC-infected cells was indistinguishable from that of m8-N+GPC. SFTS N protein incorporation into the VLP was evaluated (D). The VLP in the supernatant from the RK13 cells infected with m8-EGFP, m8-N, or m8-N+GPC was reacted with or without anti-SFTSV GPC mAb (clone C6C1) and then precipitated with anti-mouse IgG magnetic beads. The precipitated magnetic beads were then resolved on SDS-PAGE and analyzed by Western blotting using rabbit anti-SFTSV N polyclonal

42

802   antibody. RK13 cell lysates infected with m8-EGFP, m8-N, or m8-N+GPC were shown as

803   a positive control.

804

805   **Fig 2. Immunogenicity of the m8-based SFTSV vaccines in mice.**

806   Naïve IFNAR-/- mice were subcutaneously inoculated twice at a 2-week interval with a

807   dose of 1×10^6 PFU of m8-EGFP, m8-N, m8-GPC or m8-N+GPC or subcutaneously

808   infected with 10 $TCID_{50}$ of SFTSV YG-1 (SFTSV infection). Two weeks after the second

809   inoculation of mice with the recombinant m8s or 4 weeks after SFTSV infection, the mice

810   were euthanized and sacrificed. Blood was collected to evaluate Anti-SFTSV N-specific

811   (A) or GPs-specific (B) IgG production. Each dot represents the antibody titer. Horizontal

812   lines represent the mean value. The horizontal dotted line at "2^3" indicates the detection

813   limit. The neutralizing antibodies to SFTSV were also measured (C). SFTSV YG-1 was

814   mixed and incubated with 3 serial 4-fold dilutions (1/10, 1/40, or 1/160) of serum. The

815   percent neutralization titer was determined to divide the number of foci by that of naïve

816   mice.

817

818   **Fig 3. Survival, weight change, and seropositivity in m8-based SFTSV**

819   **vaccine-inoculated mice followed by lethal SFTSV challenge.**

820   IFNAR-/- mice were subcutaneously inoculated twice at a 2-week interval with a dose of

821   1×10^6 PFU of m8-EGFP, m8-N, m8-GPC or m8-N+GPC. Two weeks later, from the

822   second inoculation of mice with m8-EGFP or m8-based SFTSV vaccines, the mice were

823   subcutaneously challenged with 1×10^3 (A, C) or 1×10^5 (B, D) $TCID_{50}$ of SFTSV YG-1.

43

824   Survival (A, B) and the sequential change in percent weight from the initial (C, D) were

825   followed daily for 2 weeks. The number of mice in a group is shown as "n" in the legend.

826   The log-rank test was used to determine the level of statistical significance. The calculated

827   p-values are shown beside the groups that were compared. Seropositivity in mice

828   inoculated with m8-N or m8-GPC then infected with SFTSV was evaluated. Serum was

829   collected from the m8-N and m8-GPC-inoculated mice at 2 weeks after 1×10^3 (E) or $1 \times$

830   10^5 (F) $TCID_{50}$ of SFTSV infection. Specific IgG against SFTSV GPs or N, which should

831   be induced by SFTSV infect, and which should never be induced by monovalent m8-N or

832   m8-GPC, was measured. Each dot represents the antibody titer. The horizontal dotted line

833   at 2^3 indicates the detection limit.

834

835   **Fig 4. Survival and weight change in mice immunized with VAC before m8-based**

836   **SFTSV vaccine inoculation.**

837   IFNAR-/- mice were inoculated twice at a 2-week interval with a dose of 1×10^6 PFU of

838   m8-EGFP, m8-N, m8-GPC or m8-N+GPC, 1 month after inoculation with 1×10^6 PFU

839   VAC strain Lister. Two weeks after the second inoculation of m8-EGFP, m8-N, m8-GPC,

840   or m8-N+GPC, the mice were subcutaneously challenged with 1×10^3 (A, C) or 1×10^5 (B,

841   D) $TCID_{50}$ of SFTSV YG-1. Survival (A, B) and percent weight sequential change from the

842   initial (C, D) were followed daily for 2 weeks. The number of mice used in a group is

843   shown as "n" in the legend. The log-rank test with Bonferroni correction was used to

844   determine the level of statistical significance. To adjust the significance level, the basic

845   significance level was divided by the number of null hypotheses tested (i.e., 0.05/3=0.017).

44

846   The calculated p-values are shown beside the groups that were compared.

847

848   **Fig 5. Infectious SFTSV and SFTSV genome levels and the histopathological changes**

849   **in the tissue of m8-based vaccine-inoculated mice.**

850   IFNAR-/- mice were inoculated with m8-N, m8-GPC, m8-N+GPC, or m8-EGFP. At two

851   weeks after the second inoculation, the mice were subcutaneously challenged with 1×10^5

852    $TCID_{50}$ of SFTSV YG-1. Three infected mice were sacrificed, and blood was collected at 1,

853   3, and 5 DPI. The sequential change in the infectious SFTSV titers (A) and SFTSV N gene

854   copies (B) in the sera were measured by a standard $TCID_{50}$ assay and qPCR. A two-way

855   ANOVA with Sidak's multiple-comparison test was used to determine the level of

856   statistical significance. The calculated p-values are shown above the groups that were

857   compared. U.D., under the detection limit, which is 32 $TCID_{50}$ per ml or 125 copies per ml

858   of serum. Histopathological changes in the spleen at 5 days of mice inoculated with

859   m8-N+GPC (C), m8-GPC (D), m8-N (E), and m8-EGFP (F) after SFTSV challenge.

860   High-magnification views of hematoxylin and eosin-stained tissue sections (left) and

861   immunohistochemistry (right) of the areas of interest are shown at the bottom of the low

862   magnification views of each group.

863

864   **Fig 6. Weight change in mice to which sera collected from mice inoculated with each**

865   **m8-based SFTSV vaccine was passively administered.**

866   IFNAR-/- mice were passively transferred with 100 μl of sera collected from mice

867   inoculated m8-EGFP (A), m8-N (B), m8-GPC (C) or m8-N+GPC (D) at 1 day before and

45

868   immediately before the subcutaneous challenge with SFTSV YG-1 at a dose of 1×10^3

869    $TCID_{50}$. As a positive control, sera obtained from mice 3 weeks after the infection with 10

870    $TCID_{50}$ of SFTSV YG-1 was administered to a group of naïve mice (E). The percent weight

871   change of each individual in the groups from the initial weight was measured daily for 2

872   weeks. The number of mice used in a group is shown as "n" in the legend. The asterisk

873   indicates the endpoint of the individuals.

874

875   **Fig 7. Survival in m8-SFTSV gene-inoculated mice following lethal SFTSV challenge**

876   **with CD8-positive cell depletion.**

877   IFNAR-/- mice were subcutaneously inoculated twice at a 2-week interval with a dose of

878   1×10^6 PFU of the m8-based SFTSV vaccines. Two weeks after the second inoculation of

879   mice with m8-N+GPC (A), m8-GPC (B), m8-N (C) or m8-EGFP (D), the mice were

880   challenged with 1×10^3 (blue circle) or 1×10^5 (red square) $TCID_{50}$ of SFTSV YG-1 and

881   were inoculated with anti-CD8 (strait line with filled symbol) mAb, to deplete the

882   CD8-positive cells, or control (dotted line with open symbol) mAb on -1, 2, 5, and 8 DPI

883   before and after SFTSV challenge. Survival was evaluated daily for 2 weeks. The number

884   of mice in a group is shown as "n" in the legend.

885

886   **Fig 8. Weight change in m8-SFTSV gene-inoculated mice following a lethal SFTSV**

887   **challenge with CD8-positive cell depletion.**

888   The inoculation schedule and figure legend are described in Fig. 7. The percent weight

889   change from the initial weight was measured daily for 2 weeks.

A
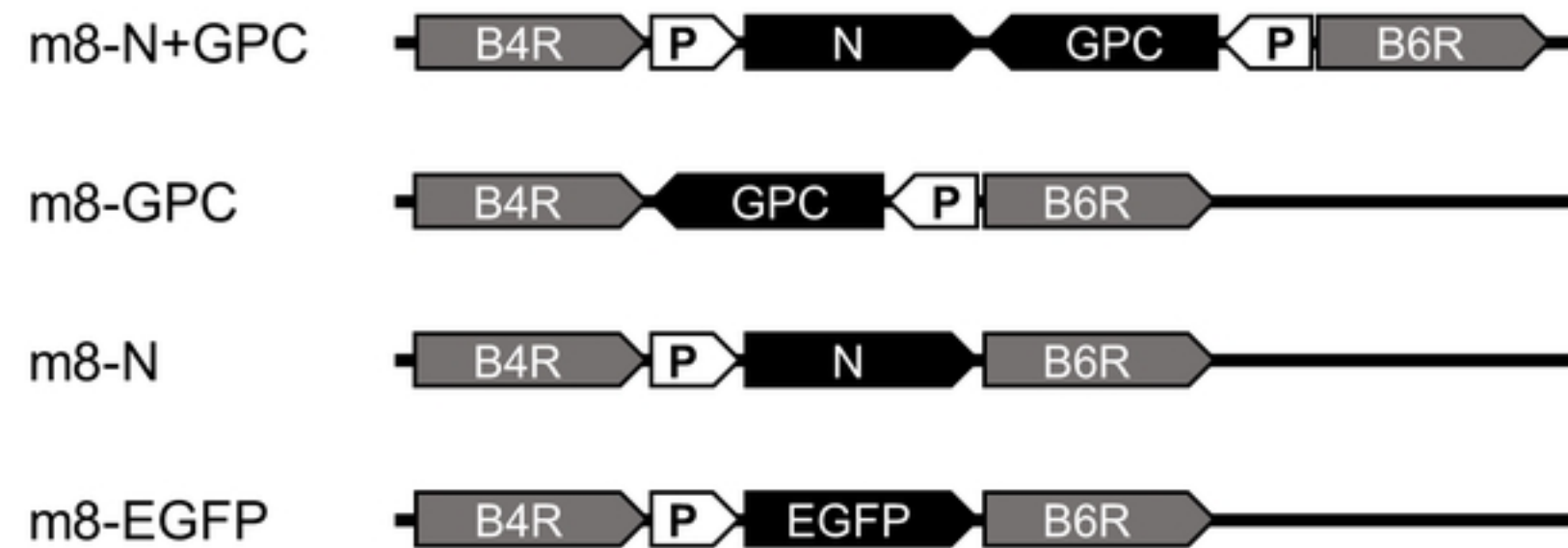
m8-N+GPC

m8-GPC

m8-N

m8-EGFP

B

m8-N+GPC   m8-GPC   m8-N   m8-EGFP

Anti-N

Anti-GP

C

100nm

D

Supernatant

Cell lysate

without Anti-Gc

with Anti-Gc

m8-EGFP
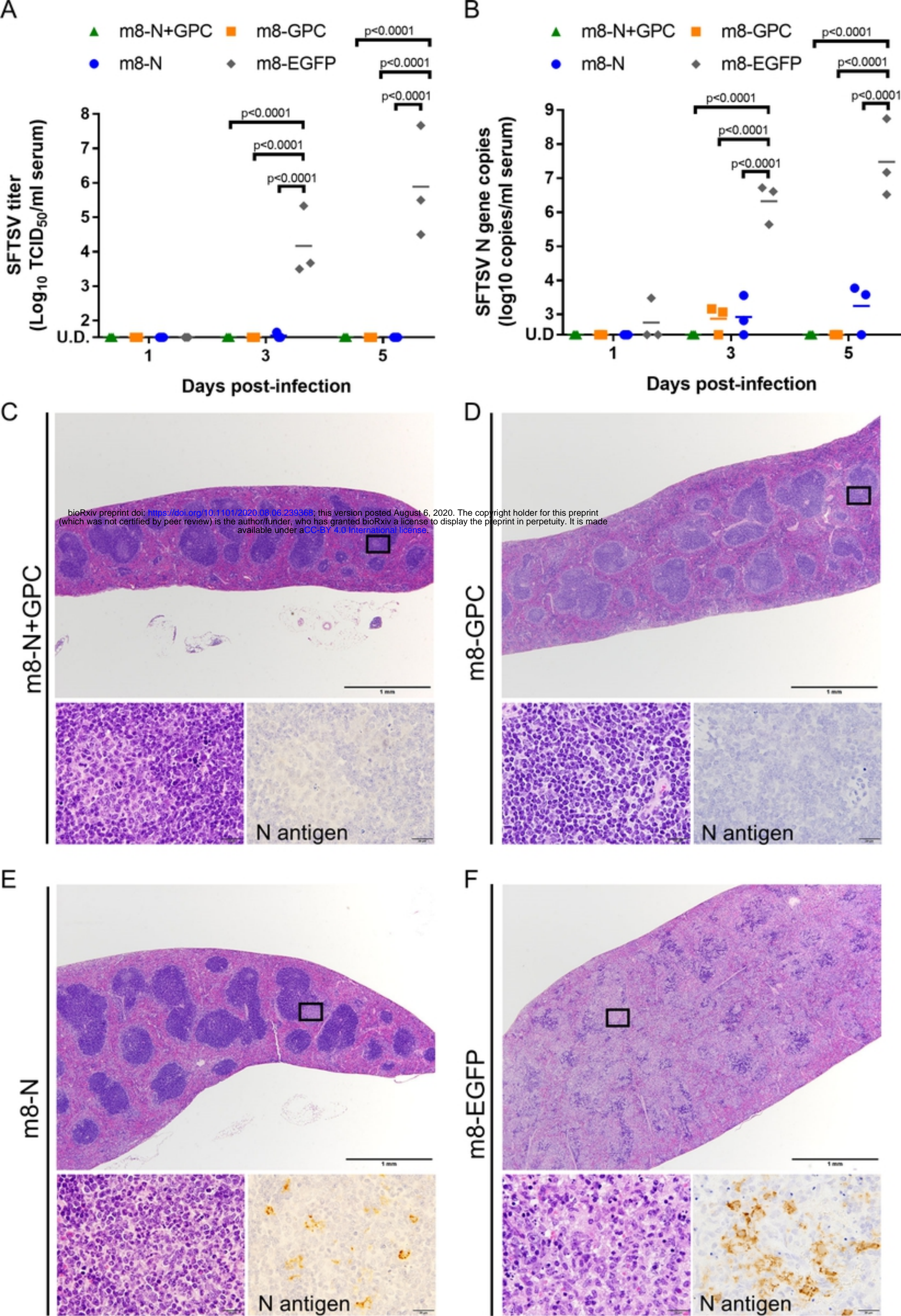
m8-N

m8-N+GPC

m8-EGFP

m8-N

m8-N+GPC

m8-EGFP

m8-N
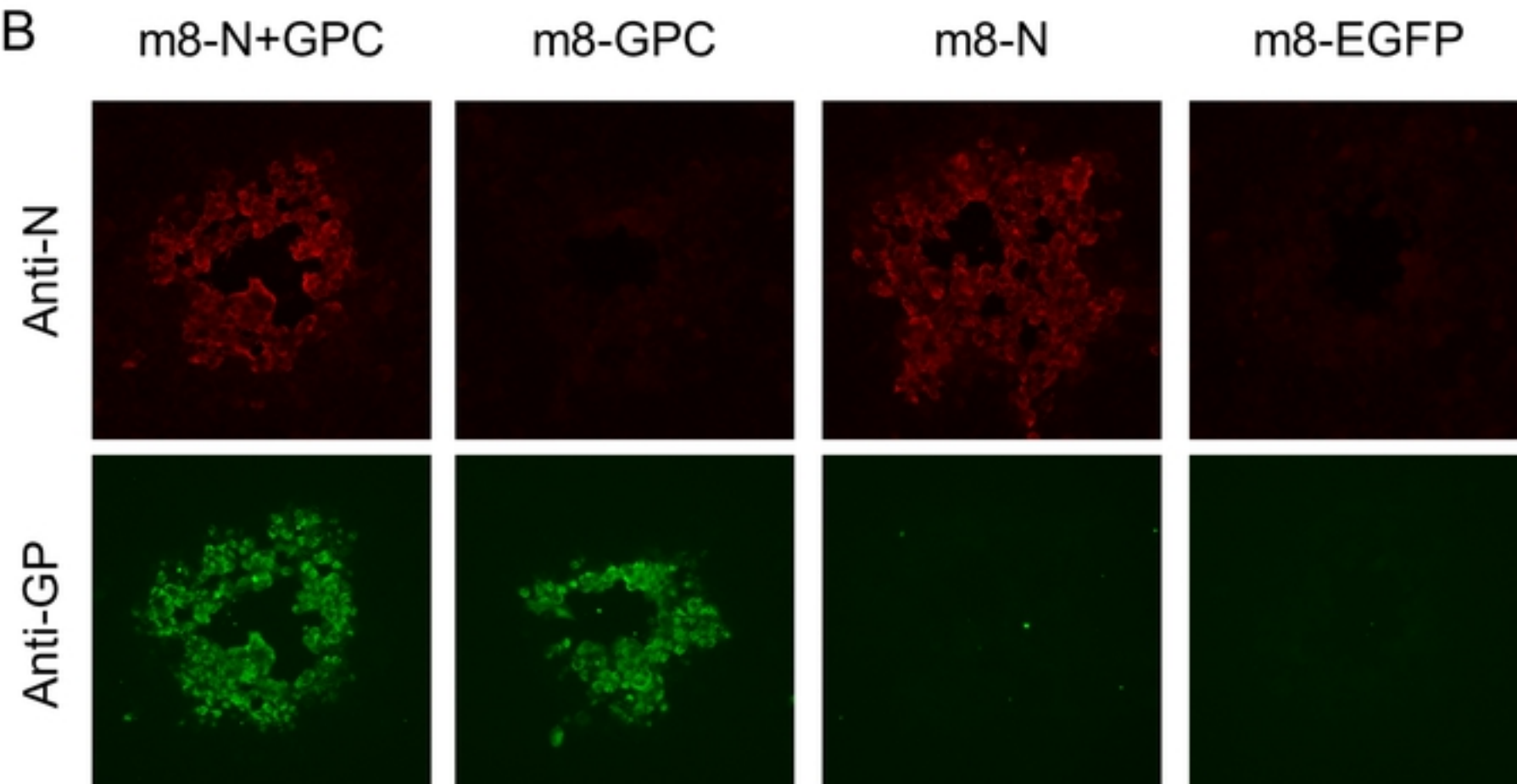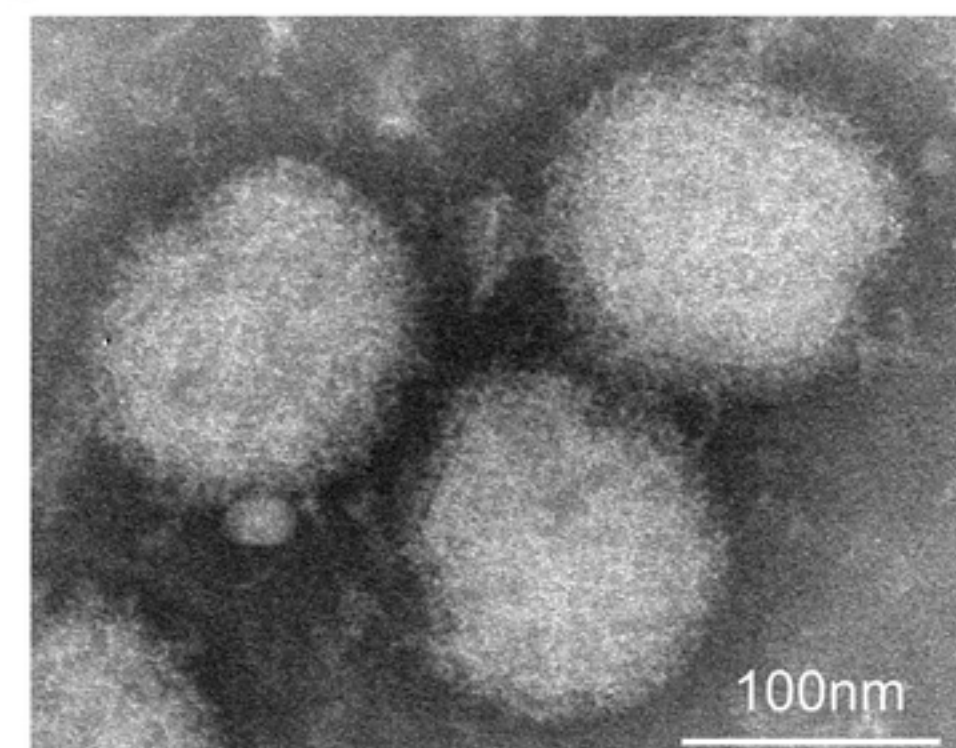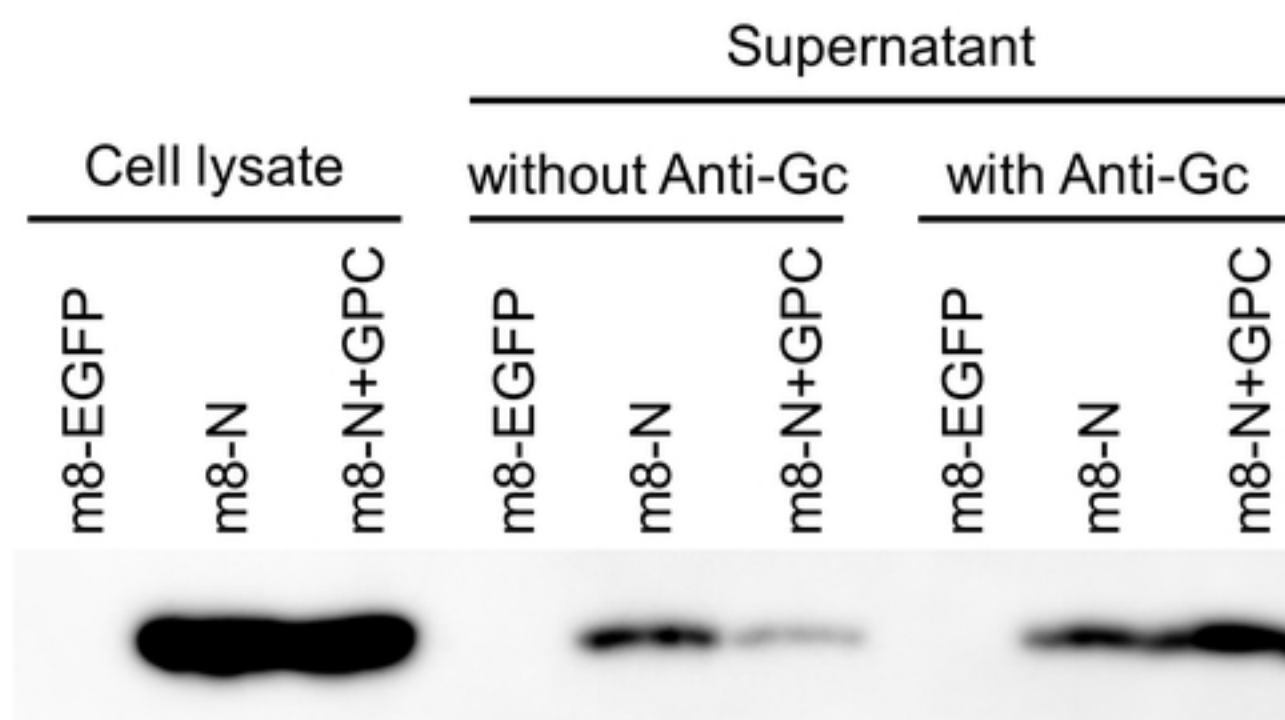
m8-N+GPC
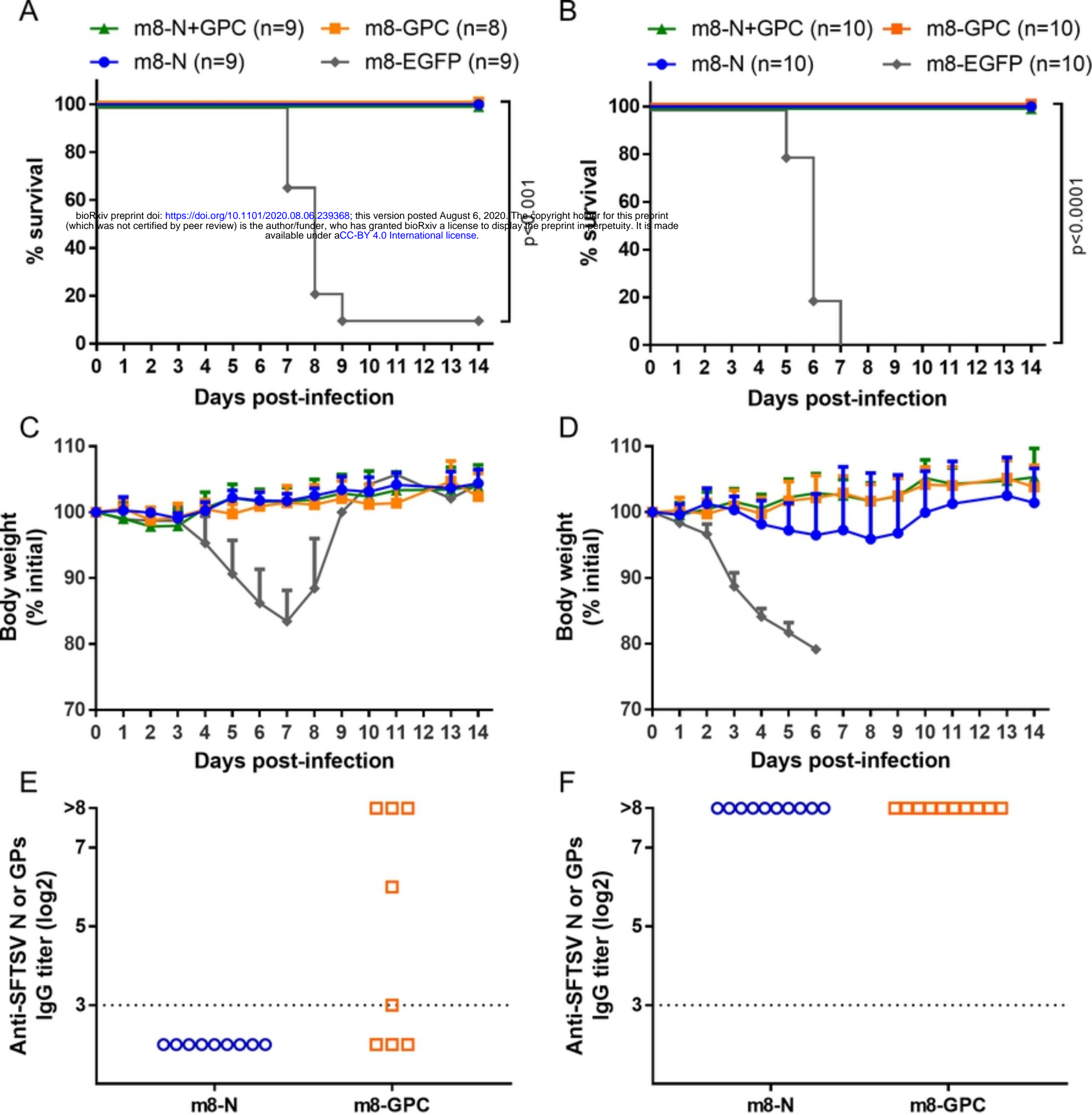
Figure 1
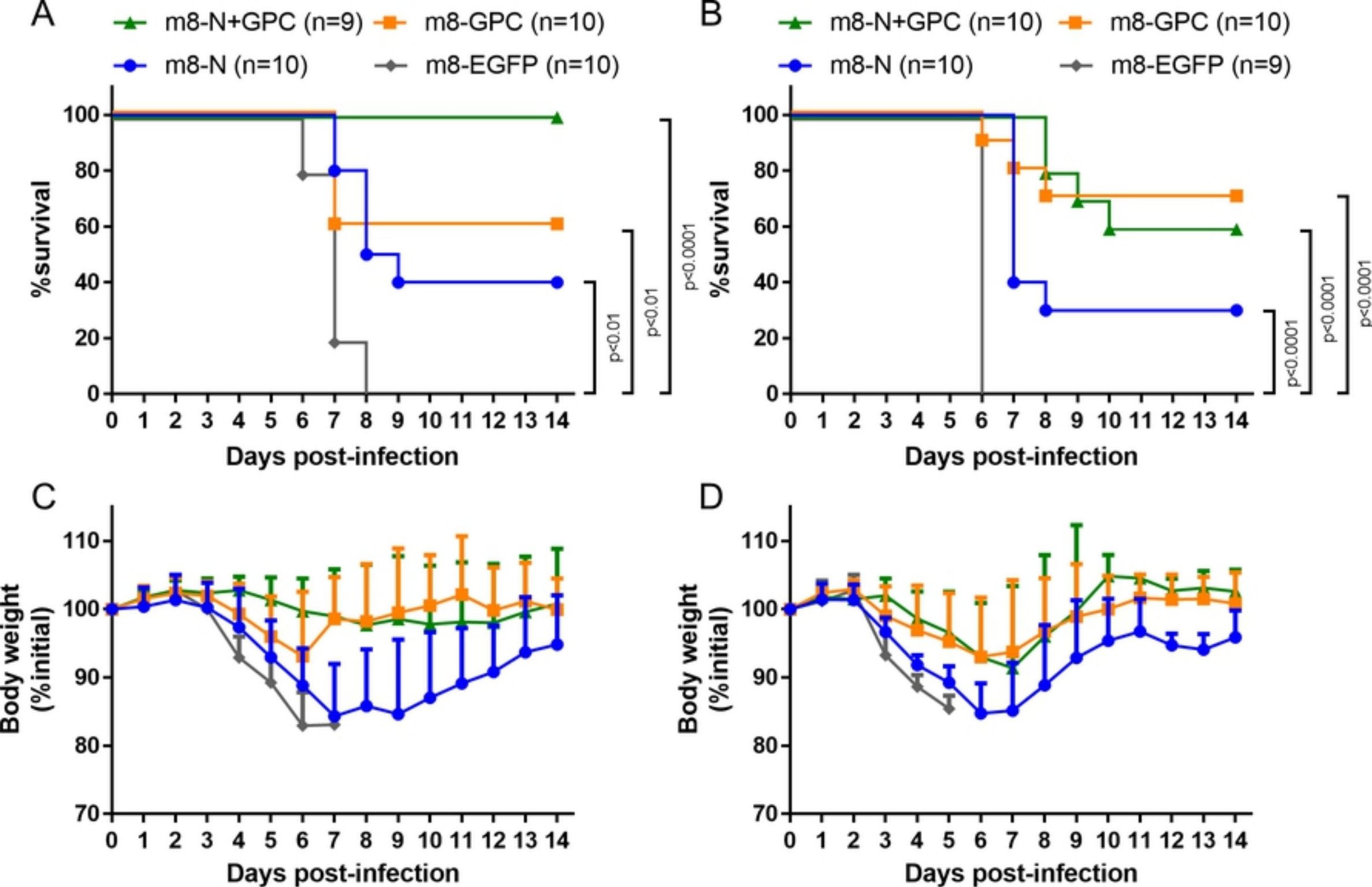
Figure 2

Figure 3

Figure 4

Figure 5

A. m8-N+GPC (n=5)
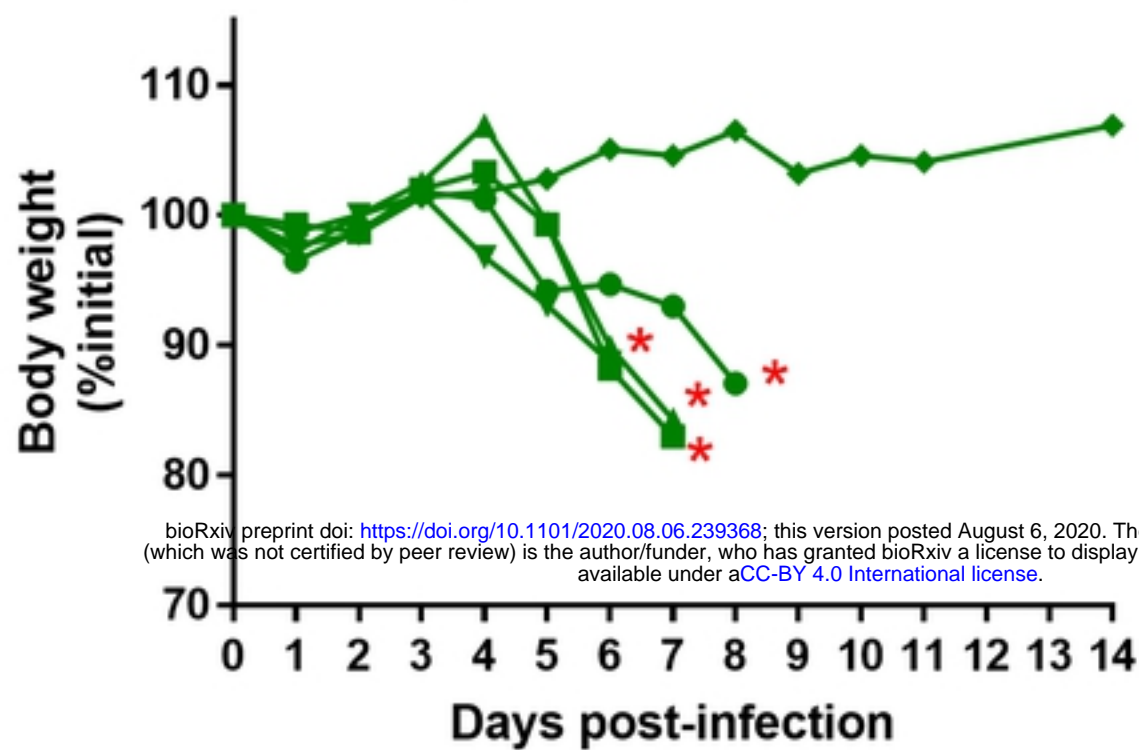
B. m8-GPC (n=5)
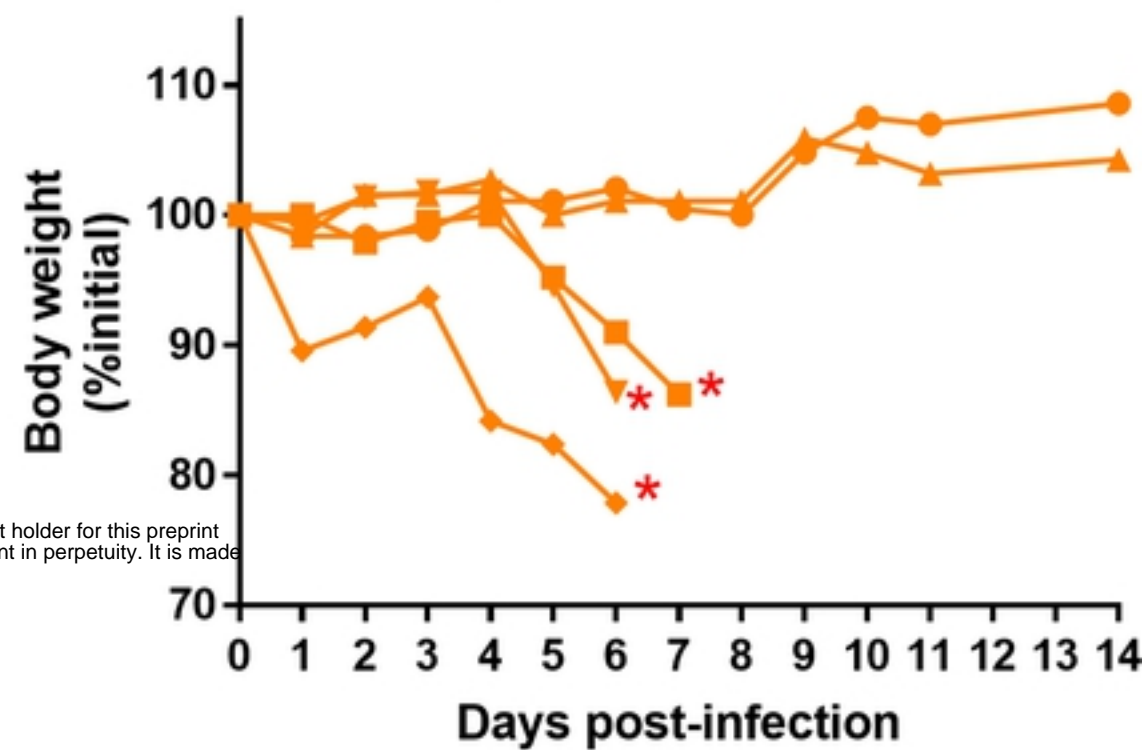
C. m8-N (n=5)
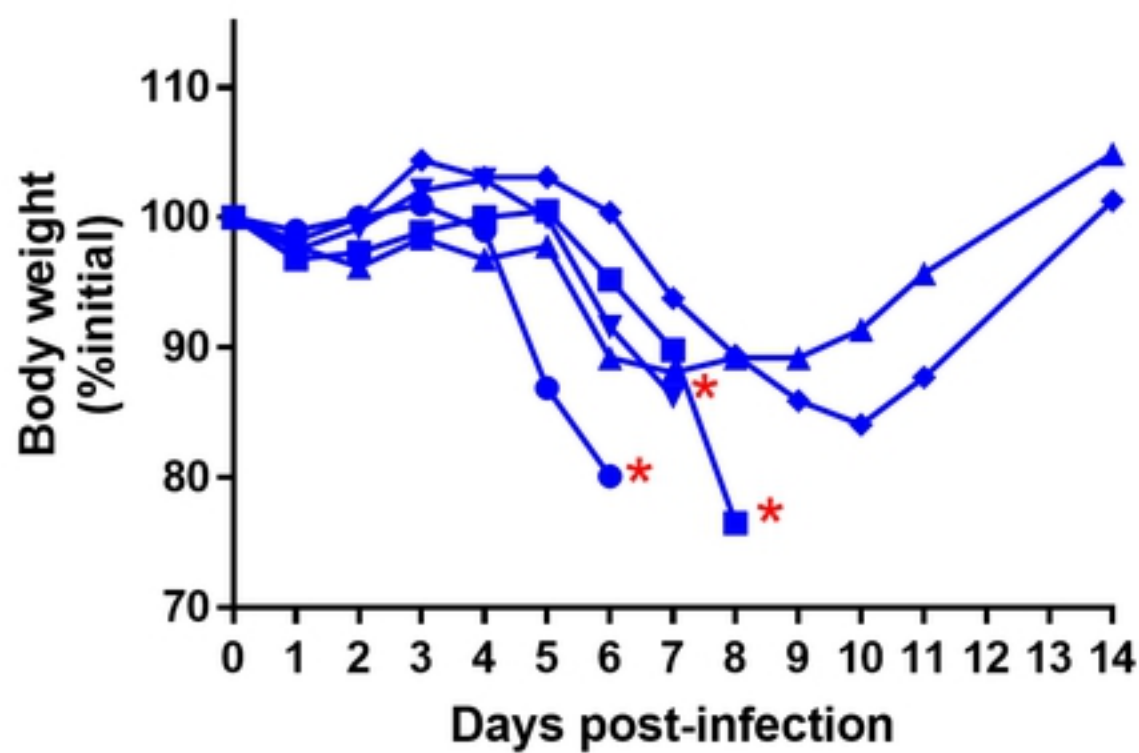
D. m8-EGFP (n=5)
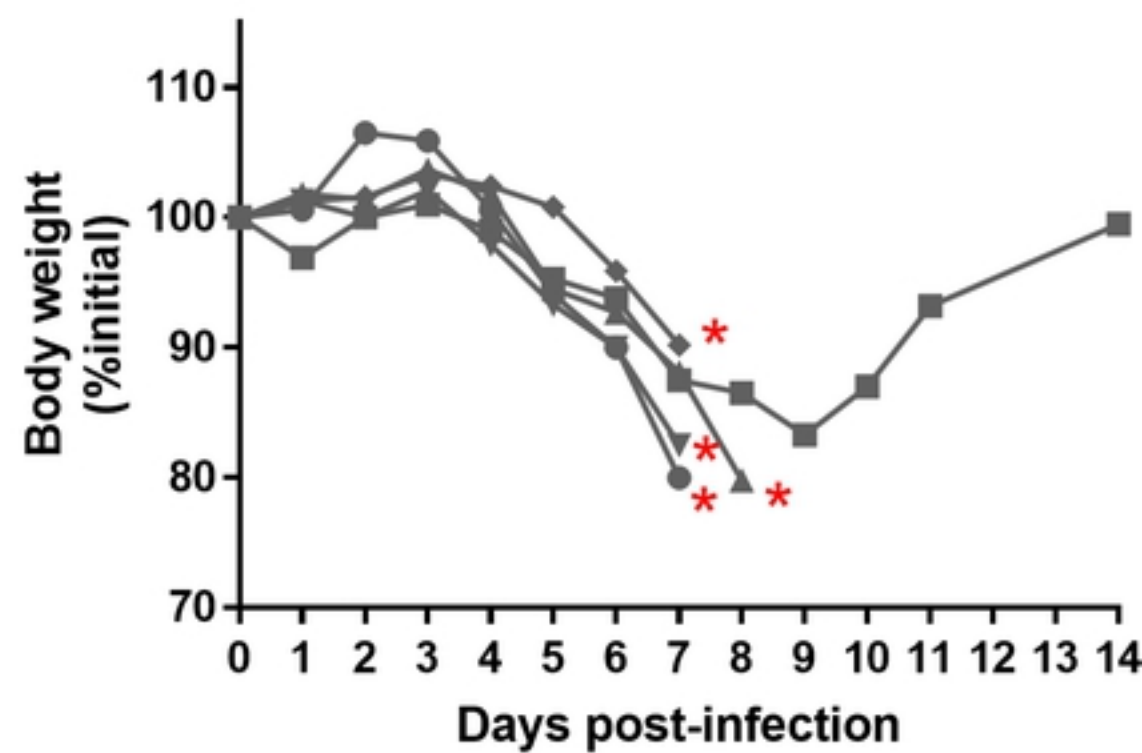
E. SFTSV (n=2)
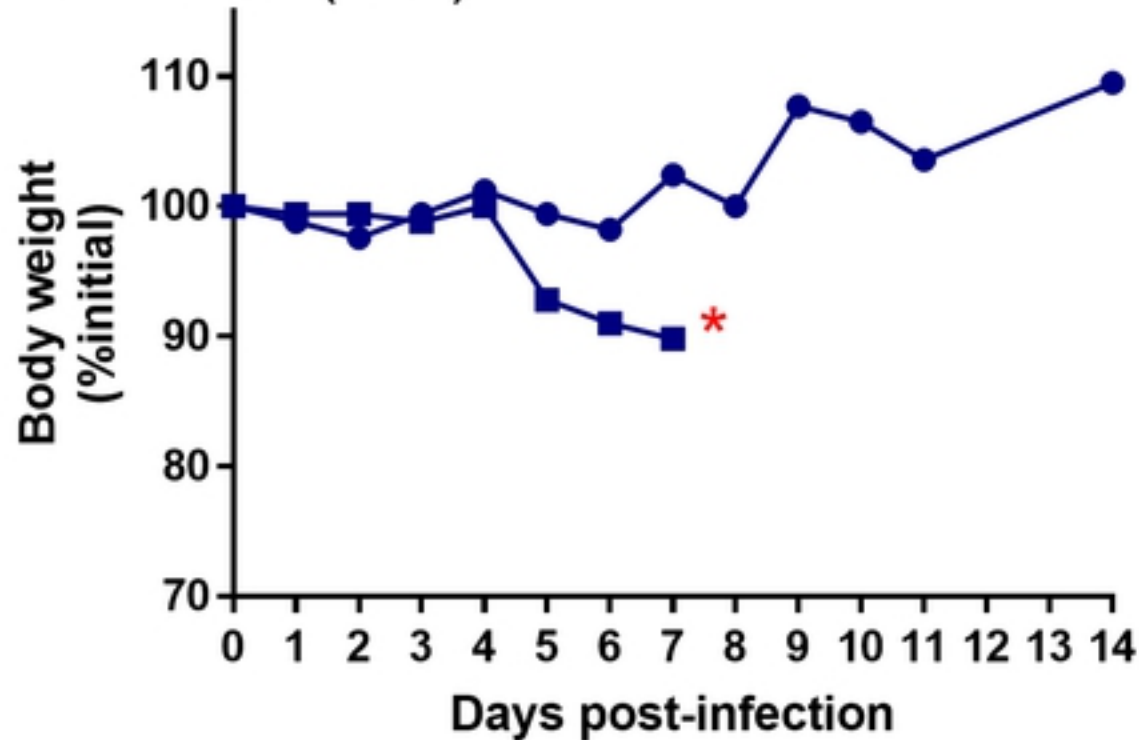
Figure 6

A. m8-N+GPC

B. m8-GPC

- -○- Ctl. Ab 10^3 (n=5)
- -●- Anti-CD8 10^3 (n=5)

C. m8-N

D. m8-EGFP

Figure 7

A. m8-GPC

B. m8-GPC

C. m8-N

D. m8-EGFP

Figure 8