Vaccination with single plasmid DNA encoding IL-12 and antigens of severe fever with thrombocytopenia syndrome virus elicits complete protection in IFNAR knockout mice

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Running head: DNA vaccination against SFTSV in IFNAR KO mice

32 **Abstract**

33 Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne disease

34 caused by SFTS virus (SFTSV) infection. Despite a gradual increase of SFTS cases and high

35 mortality in endemic regions, no specific viral therapy nor vaccine is available. Here, we

36 developed a single recombinant plasmid DNA encoding SFTSV genes, Gn and Gc together

37 with NP-NS fusion antigen, as a vaccine candidate. The viral antigens were fused with Fms-

38 like tyrosine kinase-3 ligand (Flt3L) and IL-12 gene was incorporated into the plasmid to

39 enhance cell-mediated immunity. Vaccination with the DNA provides complete protection of

40 IFNAR KO mice upon lethal SFTSV challenge, whereas immunization with a plasmid

41 without IL-12 gene resulted in partial protection. Since we failed to detect antibodies against

42 surface glycoproteins, Gn and Gc, in the immunized mice, antigen-specific cellular immunity,

43 as confirmed by enhanced antigen-specific T cell responses, might play major role in

44 protection. Finally, we evaluated the degree of protective immunity provided by protein

45 immunization of the individual glycoprotein, Gn or Gc. Although both protein antigens

46 induced a significant level of neutralizing activity against SFTSV, Gn vaccination resulted in

47 relatively higher neutralizing activity and better protection than Gc vaccination. However,

48 both antigens failed to provide complete protection. Given that DNA vaccines have failed to

49 induce sufficient immunogenicity in human trials when compared to protein vaccines,

50 optimal combinations of DNA and protein elements, proper selection of target antigens, and

51 incorporation of efficient adjuvant, need to be further investigated for SFTSV vaccine

52 development.

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Author summary

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne infection endemic to East Asia including China, Korea, and Japan. Gradual rise of disease incidence and relatively high mortality have become a serious public health problem in the endemic countries. In this study, we developed a recombinant plasmid DNA encoding four antigens, Gn, Gc, NP, and NS, of SFTS virus (SFTSV) as a vaccine candidate. In order to enhance cell-mediated immunity, the viral antigens were fused with Flt3L and IL-2 gene was incorporated into the plasmid. Immunization with the DNA vaccine provides complete protection against lethal SFTSV infection in IFNAR KO mice. Antigen-specific T cell responses might play a major role in the protection since we observed enhanced T cell responses specific to the viral antigens but failed to detect neutralizing antibody in the immunized mice. When we immunized with either viral glycoprotein, Gn protein induced relatively higher neutralizing activity and better protection against SFTSV infection than Gc antigen, but neither generated complete protection. Therefore, an optimal combination of DNA and protein elements, as well as proper selection of target antigens, might be required to produce an effective SFTSV vaccine.

Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne infectious disease caused by SFTS virus (SFTSV), belonging to the *Phenuiviridae* family of *Bunyavirales* [1, 2]. The genome of SFTSV is composed of three segmented RNAs: large (L) segment encoding RNA-dependent RNA polymerase (RdRp), medium (M) encoding the envelop glycoproteins, Gn/Gc, and small (S) encoding the nucleocapsid and nonstructural proteins (NP and NS) [1]. Clinical manifestations include fever, gastrointestinal symptoms, leukocytopenia, and thrombocytopenia [3, 4]. Disease mortality of SFTS patients has been estimated to be $5 \sim 20\%$ [3]. Even though the majority of SFTS cases has been reported from China [3], Korea [4], and Japan [5], SFTSV infections in southern Asia, including Vietnam, have been recently reported in a retrospective survey [6]. Currently, no specific viral therapy nor vaccine is available. An effective vaccine is needed to combat its relatively high mortality, especially in elderly patients, and spread of SFTSV between humans [7, 8].

Vaccine development for SFTS is at an early discovery phase and there have only been a few studies on vaccine candidates using animal infection models [8-11]. Immunization of NS antigen with Freund's adjuvant in C57BL/6 mice, which are naturally resistant to SFTSV but partially mimic human infections [12], failed to enhance viral clearance, although it induced high titer of anti-NS antibodies and significantly elevated IFN-γ levels in sera upon viral challenge [9]. Vaccination of plasmid DNAs encoding NP and NS peptides also enhanced antigen-specific cellular immunity of T cells, such as IFN-γ and TNF-α secreting CD4$^+$ and CD8$^+$ T cells, in BALB/c mice when applied by nano-patterned microneedles [10]. However, the protective effect of cellular immunity induced by DNA vaccination was not confirmed by *in vivo* infection with SFTSV. Recently, Dong F. *et al*. reported that a single dose of live

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103 attenuated recombinant vesicular stomatitis virus (rVSV) vaccine expressing the SFTSV

104 Gn/Gc glycoproteins elicited high titers of protective neutralizing antibodies in both wild type

105 and interferon α/β receptor knockout (IFNAR KO) mice [11]. They clearly showed that a

106 single dose rVSV carrying SFTSV Gn/Gc could provide complete protection against lethal

107 challenge with SFTSV in young and old IFNAR KO mice, a promising infection model for

108 severe human infection of SFTSV [11, 13, 14]. Nevertheless, the potential role of cellular

109 immunity against the viral antigens in complete protection was not examined in this study,

110 although adoptive transfer of immune sera from mice immunized with rVSV-Gn/Gc to naïve

111 IFNAR KO mice provide partial (~ 60%) protection against lethal SFTSV challenge [11].

112 In this study, we developed a recombinant plasmid DNA (pSFTSV) encoding extracellular

113 domains of Gn and Gc, and NP-NS fusion antigen as a DNA vaccine candidate. We

114 examined whether it could provide protective immunity against lethal SFTSV infection in

115 IFNAR KO mice. In order to facilitate the processing and presentation of the SFTSV antigens

116 by dendritic cells (DCs) and enhance antigen-specific T cell responses, these recombinant

117 antigens were fused with Fms-like tyrosine kinase-3 ligand (Flt3L) [15, 16]. Moreover, we

118 generated a recombinant DNA encoding IL-12α and β in addition to the recombinant viral

119 antigens (pSFTSV-IL12) to further enhance cell-mediated immunity [17]. Vaccination of

120 pSFTSV-IL12 provided complete protection of IFNAR KO mice upon lethal SFTSV

121 challenge, whereas immunization with pSFTSV elicits only partial protection, indicating that

122 antigen-specific cellular immune responses enhanced by co-expression of IL-12 could play a

123 significant role in protection against lethal SFTSV infection. We confirmed significantly

124 higher levels of Gn and NP-specific CD4$^+$ and CD8$^+$ T cell responses in mice vaccinated with

125 pSFTSV-IL12 when compared to those in mock vector-immunized mice. Therefore, our

126 results indicate that enhanced antigen-specific T cell immunity against multiple SFTVS

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127   antigens by DNA vaccination could be a promising direction for developing an effective

128   vaccine against SFTSV infection.

129

130   **Methods**

131   **Ethics statement**

132   Animal experiments were conducted in an Animal Biosafety Level 3 facility at Seoul

133   National University Hospital and International Vaccine Institute. This study was approved by

134   the Seoul National University Hospital and International Vaccine Institute Institutional

135   Animal Care and Use Committee (SNUH IACUC No. 15-0095-C1A0 and IVI IACUC No.

136   2018-018) and conducted in strict accordance with the recommendations in the National

137   Guideline for the care and use of laboratory animals.

138

139   **Preparation of SFTSV DNA plasmid**

140   In order to generate plasmids for DNA vaccination, genes encoding ectodomains of Gn, Gc

141   (from Genebank accession no. AJO16082.1), and NP/NS fusion protein (from Genebank

142   accession no. AJO16088.1 and AKI34298.1, respectively) were synthesized (GenScript,

143   Piscataway, NJ, USA) and cloned into pGX27 vector (Genexine, Seongnam, Republic of

144   Korea). All these genes were fused with signal peptide of tissue Plasminogen Activator (tPA,

145   Uniport no. P00750) and Flt3L (Uniport no. P49771) in their N-terminus [15] (pSFTSV, Fig

146   1). In addition, murine IL-12α and β genes (Uniport no. P43432) [17] were also synthesized

147   (GenScript) and cloned into pSFTSV (pSFTSV-IL-12, Fig 1).

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149   **Gene expression of plasmid DNAs**

150   To confirm the expression of cloned genes in pSFTSV and pSFTSV-IL12, HEK 293T cells

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151   (ATCC CRL-1573, Manassas, VA, USA) were transfected with pGX27, pSFTSV, or

152   pSFTSV-IL12 plasmid using the polyethylenimine (PEI) transfection method [18]. Briefly,

153   plasmids and PEI were diluted with Opti-MEM media (Gibco, Gaithersburg, MD, USA) and

154   incubated with HEK 293T cells. After 4 h of incubation, media were changed with

155   Dulbecco's Modified Eagle medium (DMEM) (Gibco) containing 10% fetal bovine serum

156   (FBS) and incubated in humidified CO_2 atmosphere at 37°C. After 48 h of culture, cells and

157   culture supernatants were harvested. Cells were washed with phosphate-buffered saline (PBS)

158   and lysed with NP-40 lysis buffer (1% NP-40 in 50 mM Tris-HCl, pH 8.0, 150 mM NaCl)

159   containing protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). Lysates and

160   supernatants were stored at -80°C until use.

161   For quantification of Flt3L-fused proteins and mouse IL-12 in the supernatants of transfected

162   HEK293T cells, human Flt3L and mouse IL-12p70 Quantikine ELISA kits (R&D systems,

163   Minneapolis, MN, USA) were used according to manufacturer's instructions. Expression of

164   Gc, Gn, and NP/NS fusion protein in the culture supernatants and cell lysates were examined

165   by immunoblotting using rabbit anti-Gn, Gc (NBP2-41153 and NBP2-41156, NOVUS

166   Biologicals, Centennial, Colorado, USA), and NP (produced by custom polyclonal antibody

167   production service via Abclon, Seoul, Republic of Korea) antibodies, respectively. Each

168   sample was separated on 8% polyacrylamide gels and transferred onto 0.45 μm PVDF

169   membranes (Merck, Darmstadt, Germany). PVDF membranes were blocked with PBS

170   containing 0.05% Tween 20 (PBST) and 5% skim milk at room temperature for 2 h. The

171   membranes were sequentially incubated with primary and secondary antibodies conjugated

172   with horse radish peroxidase (HRP, Invitrogen, Waltham, MA, USA). The membranes were

173   then visualized using a LAS-4000 system (Fujifilm, Tokyo, Japan).

174

Preparation of recombinant proteins

The gene encoding the NP of the KASJH strain (Genbank Accession No. KP663733) was cloned into the pET28a (+) vector (Novagen, Gibbstown, NJ, USA). NP protein was then purified from *E. coli* strain BL21 (DE3) harboring the recombinant plasmid. Following induction with 0.1 mM isopropyl β-D-thiogalactoside (IPTG) for 18 h at 16℃, the protein was purified using HisTrap HP histidine-tagged protein columns (GE healthcare, Chicago, IL, USA) according to the manufacturer's instruction. Recombinant Gn and Gc glycoproteins fused to Fc region of human immunoglobulin heavy chain were purified as previously described [19]. Briefly, vectors cloned with gene encoding Gn or Gc were transfected into HEK293F cells (Thermo Fisher Scientific, Waltham, MA, USA) using polyethylenimine. The transfected cells were cultured in FreeStyle™ 293 expression medium (Gibco) for 6 days. Overexpressed recombinant proteins in supernatants were purified by AKTA start affinity chromatography system HiTrap Mabselect (GE Healthcare) according to the manufacturer's instructions (S1 Fig).

Enzyme-linked immunosorbent assays (ELISA)

To determine the antibody titers specific to Gc, Gn, and NP in sera of immunized mice, immunoassay plates (96-well plates; Nunc, Rochester, NY, USA) were coated with 100 ng/well of purified antigens at 4°C overnight. His-tagged Gn and Gc recombinant proteins were purchased from Immune Technology Co. (New York, NY, USA). His-tagged NP recombinant protein was purified as mentioned above. After antigen coating, the immunoassay plates were blocked for 2 h at room temperature with PBST containing 5% skim milk. 100 μl of serially-diluted serum samples were incubated for 1 h at room temperature and subsequently detected using HRP-conjugated goat anti-mouse IgG (Santa

199   Cruz Biotechnology, Santa Cruz, CA, USA). 3,3',5,5'-tetramethylbenzidine peroxidase

200   substrate solution (KPL, Gaithersburg, MD, USA) was then added to develop color for 7 min,

201   and the reaction was stopped by the addition of 1 M H_3PO_4 solution. Absorbance was

202   measured at 450 nm using a microplate reader (TECAN, Mannedorf, Switzerland).

203

Flow cytometric analysis

205   Spleen cells were released into RPMI 1640 media (Gibco) by mincing the spleen through a

206   70 μm cell strainer (BD Biosciences, San Jose, CA, USA). After lysis of red blood cell with a

207   Red Blood Cell Lysing Buffer Hybri-Max™ (Sigma, St. Louis, MO, USA), splenocytes were

208   cultured for 18 h in RPMI media containing 10% FBS (Gibco) and 1%

209   penicillin/streptomycin (Gibco) in the presence of 10 μg of purified Gn or NP antigens in 96

210   well V-bottomed culture plates (Nunc, Roskilde, Denmark). For intracellular detection of

211   IFN-γ, splenocytes (2×10^6 cells/well) were treated with 1 μg of Golgiplug (BD Bioscience)

212   for the final 6 h. Cells were then blocked with ultra-block solution (10% rat sera, 10%

213   hamster sera, 10% mouse sera (Sigma), and 10 μg/ml of anti-CD16/32 (2.4G2) (BD

214   Pharmingen, Franklin Lakes, NJ, USA), followed by staining with anti-CD3 (145-2c11) (BD

215   Biosciences), CD4 (RM4-59) (BD Biosciences) and CD8 (53-6.7) (Biolegend, San Diego,

216   CA, USA) antibodies conjugated to different fluorescent dyes. After surface staining,

217   splenocytes were fixed and permeabilized with Cytofix/Cytoperm kit (BD Bioscience) and

218   incubated with anti-IFN-γ antibody (XMG1.2) (BD Pharmingen). The stained cells were

219   analyzed on a CytoFLEX S flow cytometer (Berkman Coulter Inc, Brea, CA, USA). Flow

220   cytometry data were analyzed using FlowJo software version 10.6 (Tree Star, Ashland, OR,

221   USA). Gating strategies for the flow cytometric analyses are summarized in S2 Fig.

222

Preparation of SFTSV

SFTSV (Genbank accession no. MN329148-MN329150) isolated from a Korean SFTS patient was propagated in Vero E6 cells (ATCC CRL-1586). The supernatant of infected cells was harvested at 5 d after infection and stored at - 80℃ after filtering with 0.45 μm syringe. Focus-forming unit (FFU) of SFTSV was determined by plaque assay using methylcellulose media [20]. Briefly, the filtered supernatants were serially diluted and added to a monolayer of Vero E6 cells and incubated for 1 h at 37°C. Viral supernatants were removed and cells were incubated under an overlay media (DMEM supplemented with 5% FBS and 1% methylcellulose) at 37℃ for 7 days. Cells were fixed with 4% paraformaldehyde (Intron, Seongnam, Republic of Korea) and 100% methanol (Merck, Darmstadt, Germany). The SFTSV foci were detected using rabbit anti-SFTS NP antibody (Abclon) and goat anti-rabbit IgG secondary antibody conjugated with alkaline phosphatase (Thermo Fisher Scientific). Viral plaques were visualized by incubation with NBT/BCIP solution (Roche, Mannheim, Germany).

Neutralizing antibody assay

To evaluate the neutralizing activity of immune sera, a focus reduction neutralization titer (FRNT) assay was performed using immunized mice sera [21]. SFTSV (0.0001 multiplicity of infection) was pre-incubated with serially diluted sera from mice at 4℃ for 1 h. The mixture of virus and sera was added onto a monolayer of Vero E6 cells in 24-well plate. After incubation for 2 h, supernatant of cells were removed and cells were cultured under an overlay media at 37℃ for 7 days. Viral foci were visualized as described above. The percentage of focus reduction was calculated as [(No. of plaques without antibody) – (No. of

10

246   plaques with antibody)] / (No. of plaques without antibody) x 100. 50% focus reduction

247   neutralization titers ($FRNT_{50}$) were calculated by a nonlinear regression analysis

248   (log[inhibitor] versus normalized response method) embedded in GraphPad Prism Software

249   v5.01 (GraphPad Software; https://www.graphpad.com).

250

251   **Immunization of mice and SFTSV challenge**

252   Six to eight-week-old female Interferon α/β receptor knockout (IFNAR KO, B57BL/6) mice

253   [22] were used for immunization and challenge tests. They were housed and maintained in

254   the specific pathogen-free facility at Seoul National University College of Medicine. Mice

255   were intramuscularly immunized by electroporation using Orbijector EP-I model (SL

256   Vaxigen Inc., Seongnam, Republic Korea) in the hind leg three times at two-week intervals. 4

257   μg of purified pGX27, pSFTSV, or pSFTSV-IL12 in 100 μl of PBS was used for each

258   immunization. Mice were also subcutaneously immunized with 20 μg of Gn-Fc or Gc-Fc

259   protein absorbed in aluminum hydroxychloride (Alhydrogel® adjuvant 2%, InvivoGen, Hong

260   Kong). Mice sera were collected from immunized mice at one week after the third

261   immunization to determine the levels of specific antibody titers. Two weeks after the final

262   immunization, mice were subcutaneously challenged with 1 x 10^5 FFU of SFTSV. Body

263   weight and mice survival were monitored until surviving mice fully recovered. Blood and

264   tissues of mice were collected at the indicated time after viral challenge and applied for viral

265   quantitation using qRT-PCR, or hematological analysis.

266

267   **Quantitative reverse transcript – polymerase chain reaction (qRT-PCR)**

268   Total RNA was extracted from the plasma of SFTSV-infected mice using Trizol LS Reagent

269   (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instruction. Total

270 RNA was reverse transcribed into cDNA using HiSenScript™ RH (-) RT Premix kit (Intron,

271 Seongnam, Republic of Korea). cDNA was quantified using TaqMan Universal Master Mix 2

272 (Applied Biosystems, Waltham, MA, USA). qRT-PCR was performed on BioRad CFX

273 connect real-time system (Bio-Rad, Hercules, CA, USA) under following conditions: uracil-

274 N-glycosylase incubation at 50°C for 2 min, polymerase activation at 95°C for 10 min,

275 denaturation at 95°C for 15 s, annealing and extension at 53°C for 1 min. Amplification was

276 performed for 45 cycles and the fluorogenic signal was measured during annealing/extension

277 step. Primer set and detecting probe for qRT-PCR was derived from the NP gene of SFTSV:

278 NP   forward   (5'-CCTTCAGGTCATGACAGCTGG-3'),   NP   reverse   (5'-

279 ACCAGGCTCTCAATCACTCCTGT-3')   and   detecting   probe   (5'-6FAM-

280 AGCACATGTCCAAGTGGGAAGGCTCTG-BHQ1-3'). Copy numbers were calculated as

281 a ratio with respect to the standard control.

282

283 **Hematology**

284 To collect hematological data, animals were euthanized and bled by cardiac puncture. Blood

285 was prepared in tubes coated with 0.5 M EDTA (Enzynomics, Daejeon, Republic of Korea).

286 Prior to evaluation of platelet counts, 4% paraformaldehyde was added to EDTA-

287 anticoagulated whole blood samples at a 1:1 ratio to inactivate virus [23]. The platelet counts

288 were analyzed using ADVIA 2012i Hematology System (Siemens Healthimeers, Erlangen,

289 Germany).

290

291 **Statistical analysis**

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292   Data was analyzed using the Graph Pad Prism 5.01 software (GraphPad Software, La Jolla,

293   CA, USA). Statistical analysis was performed using two-tailed Student's *t*-test with 95%

294   confidence interval or one-way analysis of variance (ANOVA) followed by Newman-Keuls

295   *t*-test for comparisons of values among different groups. Data are expressed as the mean ±

296   standard deviation (S.D.). Statistical analysis on survival rates were performed using the

297   Mantel-Cox Log Rank test. A *p*-value of < 0.05 was considered statistically significant.

298

Results

300   **Characterization of SFTS DNA vaccines and their gene expression**

301   Four viral genes of SFTSV were cloned into pGX27 vector to be expressed in mammalian

302   cells (Fig. 1). Extracellular domains of viral glycoproteins, Gn and Gc, were separately

303   cloned into the plasmid vector under control of CMV promoter and IRES sequence. Full

304   lengths of viral NP and NS genes fused with a linker peptide (GSGSGSGSGSGRA) were

305   also cloned into the vector under control of RSV promoter. All the proteins were fused with

306   the extracellular domain of Flt3L and the signal sequence of tissue plasminogen activator

307   (tPA) in their N-terminus to promote antigen presentation and trafficking of the fusion

308   proteins as previously described [15]. In addition, pSFTSV-IL12 plasmid also encodes

309   murine IL-12α and β to enhance antigen-specific T cell responses [17]. Expression and

310   secretion of the viral antigens and IL-12 in HEK293 cells transfected with the plasmids were

311   confirmed by ELISA and immunoblot analysis (Fig. 2). All the viral antigens were detected

312   in cell culture supernatants, as well as in cellular lysates (Fig. 2B).

313

314   **Fig 1. Schematic diagrams of SFTSV DNA vaccines used in this study.** pSFTSV

315 was constructed by inserting genes encoding ectodomains of Gn and Gc, and NP-

316 NS fusion protein into the pGX2 vector. The viral antigens are preceded by the

317 secretory signal sequence of tPA and the extracellular domain of Flt3L. pSFTSV-IL-

318 12 includes additional genes encoding murine IL-12α and β to promote cellular

319 immunity. ColE1, ColE1-type bacterial origin of replication; gIVS, rabbit β-globin

320 intervening sequence; Kan^R, kanamycin resistance gene; IRES, internal ribosome

321 entry site.

322

323 **Fig 2. Characterization of gene expression after SFTSV DNA vaccine**

324 **transfection.** (A) Expression and secretion of the viral antigens and IL-12 in

325 HEK293 cells transfected with the DNAs was confirmed by measuring the

326 concentration of Flt3L and IL-12 in the cell culture supernatants by ELISA. Data are

327 presented as mean + S.D. from triplicated experiments. (B) Expression of the viral

328 antigens, Gn (~ 74 kDa), Gc (~ 71 kDa), and NP-NS fusion (~ 80 kDa) proteins, in

329 cell lysates (left panels) and culture supernatants (right panels) of transfected

330 HEK293 cells was assessed by immunoblot analysis using anti-Gn, Gc, or NP

331 antibodies, respectively. The specific bands of antigens corresponding to their

332 expected sizes are indicated with arrow heads. β-actin was used as loading control.

333

334 **Antibody and T cell responses against the viral antigens in IFNAR KO mice immunized**

335 **with the DNA vaccines**

336 Next, we assessed antigen-specific adaptive immunity against the viral antigens after

337 vaccination in IFNAR KO mice. Antibody responses to NP antigen was significantly elevated

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338   (mean titer ± S.D.: 255 ± 63, n = 3) only in mice immunized with pSFTSV-IL12, and not in

339   mice vaccinated with mock vector or pSFTSV at two weeks after third immunization (Fig.

340   3A). Specific antibodies against Gn and Gc were barely detectable in all the vaccinated

341   groups (data not shown), suggesting that these DNA vaccines may not efficiently induce

342   specific antibodies against Gn or Gc, but produce NP antibody responses in the presence of

343   IL-12 expression.

344   In order to characterize the potential difference in quality of cell-mediated immunity in

345   IFNAR KO mice immunized with the DNA vaccines, we analyzed antigen-specific T cell

346   responses by assessing IFN-γ secreting T cells in an antigen-dependent manner (Fig. 3B and

347   C). Splenocytes collected from immunized mice were stimulated with Gn or NP antigens and

348   cytokine-positive T cell subsets were analyzed by flow cytometry. The frequencies of

349   cytokine-positive CD4 and CD8 T cells induced by splenocytes of pSFTSV-IL12-immunized

350   mice were significantly higher than those of vector-immunized mice. Even though the

351   cellular responses of immune splenocytes from pSFTSV-vaccinated mice were generally

352   increased, the levels were not statistically significant, suggesting a potent role of IL-12 co-

353   expression in enhancing cell-mediated immunity against the viral antigens.

354

355   **Fig 3. Generation of antigen-specific antibodies and T cell responses in IFNAR**

356   **KO mice vaccinated with SFTSV DNA vaccines.** (A) Anti-NP IgG response was

357   measured by ELISA at two weeks after the third vaccination. The antibody titers of

358   anti-NP IgG in sera of mice (n = 3) immunized with different DNA vaccines are

359   presented. Cut-off titers (dashed line, mean O.D. + 3 x S.D. at 1:100 diluents) was

360   determined using sera from vector-immunized mice. Error bar: mean ± S.D. (B and C)

361   Splenocytes were collected from mice at two weeks after the third immunization with

362 the indicated DNA vaccines. Production of IFN-γ by CD4$^+$ T or CD8$^+$ T cells were

363 analyzed by flow cytometry after stimulation with the indicated antigens.

364 Representative flow cytometric results are presented (B) and the percentile of

365 cytokine positive cells among CD4$^+$ or CD8$^+$ T cell subsets are summarized (C).

366 Data shown as mean + S.D. from duplicate assays with three mice per group. *, $p <$

367 0.05; **, $p < 0.01$.

368

369 **Vaccination with pSFTSV-IL12 provides complete protection against lethal infection of**

370 **SFTSV**

371 Since we observed significant elevation of T cell responses specific to the viral antigens in

372 IFNAR KO mice immunized with pSFTSV-IL12 DNA vaccine, we tested whether it could

373 provide protective immunity against lethal SFTSV infection. The susceptibility of IFNAR

374 KO mice to a Korean SFTSV isolate was examined by inoculating the mice with 1 x 10^1 to 1

375 x 10^5 FFU (S3 Fig A). Upon infection, all the mice gradually lost body weight and became

376 moribund from the third to fifth day after infection, depending on the infection dose. All the

377 infected mice died at 5 ~ 9 d after infection (S3 Fig A). Similar survival kinetics were

378 previously reported in IFNAR KO mice infected with other SFTSV strains [11, 14],

379 indicating that our Korean SFTSV isolate possesses an equivalent degree of virulence to prior

380 Chinese isolates. The platelet counts in mice infected with 1 x 10^5 FFU of SFTSV

381 significantly declined up to 4 d after infection, and the mean platelet volumes of the infected

382 mice were significantly higher than those of control animals (S3 Fig B), suggesting that

383 platelet destruction and the activation of platelet production simultaneously occur during

384 lethal infection [14].

385 To assess the protective efficacy of the DNA vaccine, groups of mice were immunized with

16

386   mock vector, pSFTSV, or pSFTSV-IL12 three times and then subcutaneously challenged

387   with a lethal dose (10^5 FFU/mouse) of SFTSV (Fig. 4). All the mock-immunized mice

388   expired by 5 d after SFTSV infection. In contrast, all the mice immunized with pSFTSV-IL12

389   were protected from lethal viral challenge, and 40% (2 of 5) of mice vaccinated with pSFTSV

390   survived (Fig. 4A). Consistently, pSFTSV-IL12-vaccinated animals lost weight until 4 d after

391   the viral challenge and recovered thereafter, whereas decrease in body weight of pSFTSV-

392   immunized mice was observed up to 8 d after the challenge and the surviving mice gradually

393   recovered. When we examined the progression of thrombocytopenia during the acute phase

394   of infection in the mice, platelet counts in vector-immunized mice rapidly declined until

395   expiration (Fig. 4B, left panel). However, platelet counts only marginally decreased in mice

396   vaccinated with pSFTSV or pSFTSV-IL12 and counts rebounded in pSFTSV-IL12-

397   immunized mice at 4 d after viral infection. In addition, viral loads were significantly reduced

398   in the plasma of pSFTSV or pSFTSV-IL12-vaccinated mice when compared to those of

399   vector-immunized control at 4 d after infection. Notably, despite similar initial viral loads (~

400   10^6 copies/ml of plasma) at 2 d after viral challenge among the mice groups, pSFTSV-IL12-

401   vaccinated mice (mean ± S.D. = $4.2 \times 10^6 \pm 4.6 \times 10^6$) were approximately five and fifty

402   times lower than those of pSFTSV-vaccinated mice (mean ± S.D. = $2.1 \times 10^7 \pm 1.7 \times 10^7$)

403   and vector-immunized mice (mean ± S.D. = $2.0 \times 10^8 \pm 1.9 \times 10^8$) at 4 d, respectively.

404

405   **Fig 4. Complete protection of IFNAR KO mice after vaccination with pSFTSV-**

406   **IL-12.** (A) Survival rates (left panel) and body weight changes (right panel) of mice (*n*

407   = 5/group) immunized three times with the indicated DNAs and challenged s.c. with

408   10^5 FFU of SFTSV at two weeks after vaccination. (B) Platelet counts (left panel)

409   and viral loads in plasma (right panel) of mice vaccinated with the indicated DNA

410 vaccines are presented. Blood samples were collected at 2 (D2) and 4 (D4) days

411 after viral challenge. Uninf, uninfected; *n* = 3 ~ 4 mice/group. *, *p* < 0.05; **, *p* < 0.01;

412 ***, *p* < 0.001.

413

414 **Vaccination of Gn protein provides partial protection against lethal infection of SFTSV**

415 Since antibody responses against the viral glycoproteins, Gn and Gc, were poorly induced in

416 IFNAR KO mice immunized with the DNA vaccines, we further investigate the potential

417 protective role of neutralizing antibodies against Gn or Gc protein by immunization with the

418 protein antigens fused with human Fc fragment (Fig. 5). Vaccination with Gn-Fc or Gc-Fc

419 protein with Alum adjuvant efficiently induced specific antibodies against the immunized

420 antigens in IFNAR KO mice; levels of antibody titers against both antigens were fairly

421 similar (mean titer ± S.D. = 3,584 ± 1,024, *n* = 4). In addition, levels of neutralizing

422 antibodies against SFTSV in immune sera from mice immunized with either protein antigen

423 were significantly higher than those from Fc-immunized controls, when assessed by focus

424 reduction neutralization test (FRNT, Fig. 5B). $FRNT_{50}$ titers of immune sera from mice

425 immunized with Gc-Fc (mean titer ± S.D. = 209 ± 140, *n* = 4) and Gn-Fc (mean titer ± S.D. =

426 929 ± 1,134, *n* = 4) were approximately five and twenty folds higher than those of Fc-

427 immunized mice (mean titer ± S.D. = 42 ± 29, *n* = 4). It is also notable that $FRNT_{50}$ titers of

428 Gn-Fc immune sera were generally higher than those of Gc-Fc sera, although the difference

429 was not statistically significant. Finally, we examined the protective efficacy of the protein

430 vaccines against lethal infection with SFTSV (Fig. 5C). All the control mice immunized with

431 Fc antigen died at five days after infection, whereas 50% of Gn-Fc immunized mice were

432 protected from lethal challenge and regained their lost body weight. Interestingly, weight loss

433 of Gc-Fc vaccinated mice was delayed and observed from 4 d after infection, whereas the

18

434 control mice lost weight from 2 d after infection. Even though all the mice immunized with

435 Gc-Fc succumbed to death, they survived three days more than control mice and the

436 extension of median survival time was statistically significant ($p = 0.0046$).

437

438 **Fig 5. Partial protection of IFNAR KO mice after vaccination with Gn-Fc antigen.**

439 (A) Anti-Gc (blue dots) or Gn (red dots) IgG response was measured by ELISA at

440 two weeks after the third immunization with Gn-Fc or Gc-Fc in IFNAR KO mice (n =

441 4/group). Cut-off titers (dashed line, mean O.D. + 3 x S.D. at 1:100 diluents) was

442 determined using Fc-immune sera (black dots). The antibody titers of anti-Gc or Gn

443 IgG in sera of immunized mice are summarized in the right panel. Error bar, mean +

444 S.D.; black line, mean. (B) Neutralizing antibody response to SFTSV generated by

445 SFTSV DNA vaccines in IFNAR KO mice. The amount of neutralizing antibody

446 against SFTSV was determined based on $FRNT_{50}$ (left panel) and summarized (right

447 panel). (C) Survival rates (left panel) and body weight changes (right panel) of mice

448 immunized three times with Fc (black dots, n = 6), Gc-Fc (blue dots, n = 4), or Gn-Fc

449 (red dots, n = 6) antigen and challenged s.c. with 10^5 FFU of SFTSV at two weeks

450 after vaccination. *, $p < 0.05$; **, $p < 0.01$.

451

Discussion

453 DNA vaccination has been widely investigated for various infectious diseases, especially

454 targeting viral infections, during the last two decades [24, 25]. Although, human clinical trials

455 of DNA vaccines have yielded poor immunogenicity and less than optimal results, the

456 approval of a few veterinary vaccines is a testimony of proof-of-concept and the hope that

457 licensed DNA vaccines for human use may not be too far away [24]. While we were

19

458 preparing this manuscript, Kwak J.E. *et al*. reported a promising demonstration of DNA

459 vaccination against lethal SFTSV infection in old-aged (> 4-years-old) ferret model [26].

460 They used five plasmid DNAs encoding individual viral gene, Gn, Gc, NP, NS, or RdRp, and

461 found that vaccination of old ferrets with a mixture of the five plasmids completely protected

462 ferrets from lethal SFTSV challenge without developing any clinical symptoms and systemic

463 viremia [26]. In addition, adoptive transfer of immune sera from ferrets immunized with two

464 plasmids encoding Gn and Gc transiently induced systemic viremia but provided complete

465 protection against lethal challenge, suggesting that Gn/Gc may be the most effective antigens

466 for inducing protective immunity. They also found that non-envelop (NP, NS, and RdRp)-

467 specific T cell responses also contribute to protection against SFTSV infection although it

468 failed to induce neutralizing activity [26]. Here we also observed that vaccination with a

469 plasmid encoding Gn, Gc, NP, and NS, together with murine IL-12, could provide protective

470 immunity in IFNAR KO mice against lethal SFTSV challenge. However, our current DNA

471 vaccine failed to suppress initial systemic viremia and weight loss (Fig. 4). This might be due

472 to the lack of type I interferon signaling and/or antibody responses against Gn and Gc

473 glycoproteins, which are required for viral neutralization during the early stage of viral

474 infection. Inefficient generation of antibodies against Gn and Gc might be due to the absence

475 of type I interferon signaling in IFNAR KO mice or fusion of Flt3L with the glycoproteins,

476 resulting in conformation defect of the antigens and/or dysregulation of B cell responses.

477 Given that immunization with Gn-Fc and Gc-Fc proteins can induce specific antibodies in

478 IFNAR KO mice (Fig. 5), the absence of type I IFN signaling might not be the cause of

479 inefficient antibody generation. Instead, Flt3L may suppress specific antibody responses as

480 observed in other studies [16, 27-29]. Flt3L may exert tolerogenic effect on CD4 T cells via

481 dendritic cells, thereby promoting B cell hypo-responsiveness *in vivo*; however, the

482   underlying mechanisms of CD4 T cell dysregulation are still unclear [28]. Nevertheless,

483   Flt3L can significantly enhance CD8 T cell responses when fused with a target antigen and

484   promote persistent maintenance of antigen-specific CD8 T cells *in vivo* [16, 29]. In this study,

485   we also observed significant enhancement of Gn and NP-specific CD8 T cell responses, as

486   well as CD4 T cells, in the presence of IL-12 expression (Fig. 3B and C), which correlated

487   well with vaccine protective efficacy in mice infected with lethal doses of SFTSV. The potent

488   effect of antigen-specific T cell responses in protection against SFTSV infection is consistent

489   with the results of the aforementioned ferret infection model [26].

490   Finally, we evaluated the degree of protective immunity provided by the individual

491   glycoprotein, Gn or Gc, after protein antigen immunization for the first time (Fig. 5), since

492   previous studies showed that vaccination with both antigens retained in a live viral vector [11]

493   or DNA vaccine [26] can provide complete protection against lethal SFTSV challenge in

494   IFNAR KO mice or old ferrets, respectively. Both protein antigens are immunogenic and

495   induce significant levels of neutralizing activity against SFTSV, although anti-Gn antibodies

496   retain relatively higher neutralizing capacity than anti-Gc antibodies. Consistently, the

497   protective efficacy of Gn protein vaccination is relatively higher than Gc protein

498   immunization. While vaccination with either protein antigen failed to provide complete

499   protection against lethal SFTSV challenge, Gc vaccine prolonged the survival time of

500   infected mice and Gn antigen can provide partial protection. Considering that Gn and Gc

501   glycoproteins function as viral ligands for cellular receptor binding and viral membrane

502   fusion in host endosome, respectively [30-32], neutralization of both antigens might be

503   required to completely block viral attachment and entry into host cells.

504   Taken together, we confirmed that antigen-specific T cell responses against SFTSV induced

505   by DNA vaccination can provide complete protection against lethal viral challenge and

21

506   immunization with each individual glycoprotein of SFTSV can also confer partial protective

507   immunity. Given that the Achilles heel of DNA vaccines remains their poor immunogenicity

508   in human trials when compared to protein vaccines [25], optimal combinations of DNA

509   and/or protein vaccines, proper selection of target antigens, and incorporation of efficient

510   vaccine adjuvant, need to be further investigated for development of the best SFTSV vaccine

511   formulation for human application.

512

Supporting information

514   **S1 Fig.** Purified Gn-Fc and Gc-Fc proteins were resolved by SDS-PAGE and stained with

515   Coomassie blue.

516   **S2 Fig.** Gating strategies for T cell analysis by flow cytometry.

517   **S3 Fig.** Clinical manifestations in IFNAR KO mice infected with SFTSV. (A) IFNAR KO

518   mice were subcutaneously infected with different doses (0, 10^1, 10^3, or 10^5 FFU/mouse) of

519   SFTSV. The mice were monitored daily to assess survival rate (left) and body weight change

520   (right) up to 10 d after infection when all the infected mice died. (B) The blood from the

521   infected mice were collected and platelet counts and their volumes were examined by

522   hematological analyzer. Red line: mean value, *: $p < 0.05$, **: $p < 0.01$, compared with

523   mock-infected controls.

524

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530

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532

533   **Author Contributions**

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539   Writing - original draft: JGK KJ NHC.

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541

542   **References**

543   1.   Silvas JA, Aguilar PV. The Emergence of Severe Fever with Thrombocytopenia

544   Syndrome Virus. Am J Trop Med Hyg. 2017;97(4):992-6. Epub 2017/08/19. doi:

545   10.4269/ajtmh.16-0967.   PubMed   PMID: 28820686;   PubMed   Central   PMCID:

546   PMCPMC5637595.

547   2.   Abudurexiti A, Adkins S, Alioto D, Alkhovsky SV, Avsic-Zupanc T, Ballinger MJ,

548   et al. Taxonomy of the order Bunyavirales: update 2019. Arch Virol. 2019;164(7):1949-65.

549   Epub 2019/05/09. doi: 10.1007/s00705-019-04253-6. PubMed PMID: 31065850; PubMed

550   Central PMCID: PMCPMC6641860.

551   3.   Li H, Lu QB, Xing B, Zhang SF, Liu K, Du J, et al. Epidemiological and clinical

552   features of laboratory-diagnosed severe fever with thrombocytopenia syndrome in China,

553 2011-17: a prospective observational study. Lancet Infect Dis. 2018;18(10):1127-37. Epub

554 2018/07/29. doi: 10.1016/S1473-3099(18)30293-7. PubMed PMID: 30054190.

555 4. Park SW, Lee CS, Kim JH, Bae IG, Moon C, Kwak YG, et al. Severe fever with

556 thrombocytopenia syndrome: comparison with scrub typhus and clinical diagnostic prediction.

557 BMC infectious diseases. 2019;19(1):174. Epub 2019/02/21. doi: 10.1186/s12879-019-3773-

558 1. PubMed PMID: 30782137; PubMed Central PMCID: PMCPMC6381645.

559 5. Nakamura S, Iwanaga N, Hara S, Shimada S, Kashima Y, Hayasaka D, et al. Viral

560 load and inflammatory cytokine dynamics associated with the prognosis of severe fever with

561 thrombocytopenia syndrome virus infection: An autopsy case. J Infect Chemother.

562 2019;25(6):480-4. Epub 2019/03/03. doi: 10.1016/j.jiac.2019.01.013. PubMed PMID:

563 30824300.

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

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

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

567 PMCID: PMCPMC6478219.

568 7. Jung IY, Choi W, Kim J, Wang E, Park SW, Lee WJ, et al. Nosocomial person-to-

569 person transmission of severe fever with thrombocytopenia syndrome. Clin Microbiol Infect.

570 2019;25(5):633 e1- e4. Epub 2019/01/25. doi: 10.1016/j.cmi.2019.01.006. PubMed PMID:

571 30677496.

572 8. Reece LM, Beasley DW, Milligan GN, Sarathy VV, Barrett AD. Current status of

573 Severe Fever with Thrombocytopenia Syndrome vaccine development. Current opinion in

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

575 29642053.

576 9. Liu R, Huang DD, Bai JY, Zhuang L, Lu QB, Zhang XA, et al. Immunization with

577   recombinant SFTSV/NSs protein does not promote virus clearance in SFTSV-infected

578   C57BL/6J   mice.   Viral   Immunol.   2015;28(2):113-22.   Epub   2015/01/17.   doi:

579   10.1089/vim.2014.0100.   PubMed   PMID: 25594805;   PubMed   Central   PMCID:

580   PMCPMC4334103.

581   10.   Jung D, Rejinold NS, Kwak JE, Park SH, Kim YC. Nano-patterning of a stainless

582   steel microneedle surface to improve the dip-coating efficiency of a DNA vaccine and its

583   immune response. Colloids Surf B Biointerfaces. 2017;159:54-61. Epub 2017/08/07. doi:

584   10.1016/j.colsurfb.2017.07.059. PubMed PMID: 28780461.

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

586   vaccine elicits complete protection against severe fever with thrombocytopenia syndrome

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

588   PMID: 30701094; PubMed Central PMCID: PMCPMC6347601 All the remaining authors

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590   12.   Jin C, Liang M, Ning J, Gu W, Jiang H, Wu W, et al. Pathogenesis of emerging

591   severe fever with thrombocytopenia syndrome virus in C57/BL6 mouse model. Proc Natl

592   Acad Sci U S A. 2012;109(25):10053-8. Epub 2012/06/06. doi: 10.1073/pnas.1120246109.

593   PubMed PMID: 22665769; PubMed Central PMCID: PMCPMC3382536.

594   13.   Liu Y, Wu B, Paessler S, Walker DH, Tesh RB, Yu XJ. The pathogenesis of severe

595   fever with thrombocytopenia syndrome virus infection in alpha/beta interferon knockout mice:

596   insights into the pathologic mechanisms of a new viral hemorrhagic fever. J Virol.

597   2014;88(3):1781-6. Epub 2013/11/22. doi: 10.1128/JVI.02277-13. PubMed PMID: 24257618;

598   PubMed Central PMCID: PMCPMC3911604.

599   14.   Matsuno K, Orba Y, Maede-White K, Scott D, Feldmann F, Liang M, et al. Animal

600   Models of Emerging Tick-Borne Phleboviruses: Determining Target Cells in a Lethal Model

25

601   of SFTSV Infection. Front Microbiol. 2017;8:104. Epub 2017/02/15. doi:

602   10.3389/fmicb.2017.00104. PubMed PMID: 28194148; PubMed Central PMCID:

603   PMCPMC5276813.

604   15.   Kim TJ, Jin HT, Hur SY, Yang HG, Seo YB, Hong SR, et al. Clearance of persistent

605   HPV infection and cervical lesion by therapeutic DNA vaccine in CIN3 patients. Nat

606   Commun. 2014;5:5317. Epub 2014/10/31. doi: 10.1038/ncomms6317. PubMed PMID:

607   25354725; PubMed Central PMCID: PMCPMC4220493.

608   16.   Sailaja G, Husain S, Nayak BP, Jabbar AM. Long-term maintenance of gp120-

609   specific immune responses by genetic vaccination with the HIV-1 envelope genes linked to

610   the gene encoding Flt-3 ligand. J Immunol. 2003;170(5):2496-507. Epub 2003/02/21. doi:

611   10.4049/jimmunol.170.5.2496. PubMed PMID: 12594275.

612   17.   Ha SJ, Chang J, Song MK, Suh YS, Jin HT, Lee CH, et al. Engineering N-

613   glycosylation mutations in IL-12 enhances sustained cytotoxic T lymphocyte responses for

614   DNA immunization. Nat Biotechnol. 2002;20(4):381-6. Epub 2002/03/30. doi:

615   10.1038/nbt0402-381. PubMed PMID: 11923845.

616   18.   Longo PA, Kavran JM, Kim MS, Leahy DJ. Transient mammalian cell transfection

617   with polyethylenimine (PEI). Methods Enzymol. 2013;529:227-40. Epub 2013/09/10. doi:

618   10.1016/B978-0-12-418687-3.00018-5. PubMed PMID: 24011049; PubMed Central PMCID:

619   PMCPMC4012321.

620   19.   Kim KH, Kim J, Ko M, Chun JY, Kim H, Kim S, et al. An anti-Gn glycoprotein

621   antibody from a convalescent patient potently inhibits the infection of severe fever with

622   thrombocytopenia syndrome virus. PLoS Pathog. 2019;15(2):e1007375. Epub 2019/02/02.

623   doi: 10.1371/journal.ppat.1007375. PubMed PMID: 30707748; PubMed Central PMCID:

624   PMCPMC6380599 based on these findings (PCT/KR2017/003156).

625   20.   Watanabe T, Horikawa Y, Sato K, Saito H. Methylcellulose media for plaque assay

626   of murine leukemia virus. J Clin Microbiol. 1982;16(3):542-4. Epub 1982/09/01. PubMed

627   PMID: 6290531; PubMed Central PMCID: PMCPMC272405.

628   21.   Kim YS, Aigerim A, Park U, Kim Y, Rhee JY, Choi JP, et al. Sequential Emergence

629   and Wide Spread of Neutralization Escape Middle East Respiratory Syndrome Coronavirus

630   Mutants, South Korea, 2015. Emerg Infect Dis. 2019;25(6):1161-8. Epub 2019/03/23. doi:

631   10.3201/eid2506.181722.   PubMed   PMID: 30900977;   PubMed   Central   PMCID:

632   PMCPMC6537729.

633   22.   Min CK, Kim HI, Ha NY, Kim Y, Kwon EK, Yen NTH, et al. A Type I Interferon

634   and IL-10 Induced by Orientia tsutsugamushi Infection Suppresses Antigen-Specific T Cells

635   and Their Memory Responses. Frontiers in immunology. 2018;9:2022. Epub 2018/09/21. doi:

636   10.3389/fimmu.2018.02022.   PubMed   PMID: 30233599;   PubMed   Central   PMCID:

637   PMCPMC6131522.

638   23.   Gowen BB, Westover JB, Miao J, Van Wettere AJ, Rigas JD, Hickerson BT, et al.

639   Modeling Severe Fever with Thrombocytopenia Syndrome Virus Infection in Golden Syrian

640   Hamsters: Importance of STAT2 in Preventing Disease and Effective Treatment with

641   Favipiravir. J Virol. 2017;91(3). Epub 2016/11/25. doi: 10.1128/JVI.01942-16. PubMed

642   PMID: 27881648; PubMed Central PMCID: PMCPMC5244333.

643   24.   Porter KR, Raviprakash K. DNA Vaccine Delivery and Improved Immunogenicity.

644   Current Issues in Molecular Biology. 2017;22:129-38. doi: 10.21775/cimb.022.129. PubMed

645   PMID: WOS:000395963200008.

646   25.   Li L, Saade F, Petrovsky N. The future of human DNA vaccines. Journal of

647   Biotechnology. 2012;162(2-3):171-82. doi: 10.1016/j.jbiotec.2012.08.012. PubMed PMID:

648   WOS:000311642000001.

649   26.   Kwak JE, Kim YI, Park SJ, Yu MA, Kwon HI, Eo S, et al. Development of a SFTSV

650   DNA vaccine that confers complete protection against lethal infection in ferrets. Nat

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

652   PMID: 31444366; PubMed Central PMCID: PMCPMC6707330.

653   27.   Kwon TK, Park JW. Intramuscular co-injection of naked DNA encoding HBV core

654   antigen and Flt3 ligand suppresses anti-HBc antibody response. Immunol Lett.

655   2002;81(3):229-34. Epub 2002/04/12. doi: 10.1016/s0165-2478(02)00039-1. PubMed PMID:

656   11947930.

657   28.   Venanzi FM, Barucca A, Havas K, Capitani M, Provinciali M, Scotti S, et al. Co-

658   expression of Flt-3 ligand gene ablates tumor immunity elicited by HER-2/neu DNA vaccine

659   in   transgenic   mice.   Vaccine.   2010;28(22):3841-7.   Epub   2010/03/31.   doi:

660   10.1016/j.vaccine.2010.03.010. PubMed PMID: 20350624.

661   29.   Hung CF, Hsu KF, Cheng WF, Chai CY, He L, Ling M, et al. Enhancement of DNA

662   vaccine potency by linkage of antigen gene to a gene encoding the extracellular domain of

663   Fms-like tyrosine kinase 3-ligand. Cancer research. 2001;61(3):1080-8. Epub 2001/02/28.

664   PubMed PMID: 11221836.

665   30.   Spiegel M, Plegge T, Pohlmann S. The Role of Phlebovirus Glycoproteins in Viral

666   Entry, Assembly and Release. Viruses-Basel. 2016;8(7). doi: ARTN 202

667   10.3390/v8070202. PubMed PMID: WOS:000380766700024.

668   31.   Halldorsson S, Behrens AJ, Harlos K, Huiskonen JT, Elliott RM, Crispin M, et al.

669   Structure of a phleboviral envelope glycoprotein reveals a consolidated model of membrane

670   fusion. P Natl Acad Sci USA. 2016;113(26):7154-9. doi: 10.1073/pnas.1603827113. PubMed

671   PMID: WOS:000379033400057.

672   32.   Wu Y, Zhu YH, Gao F, Jiao YJ, Oladejo BO, Chai Y, et al. Structures of phlebovirus

673   glycoprotein Gn and identification of a neutralizing antibody epitope. P Natl Acad Sci USA.

674   2017;114(36):E7564-E73.   doi: 10.1073/pnas.1705176114.   PubMed   PMID:
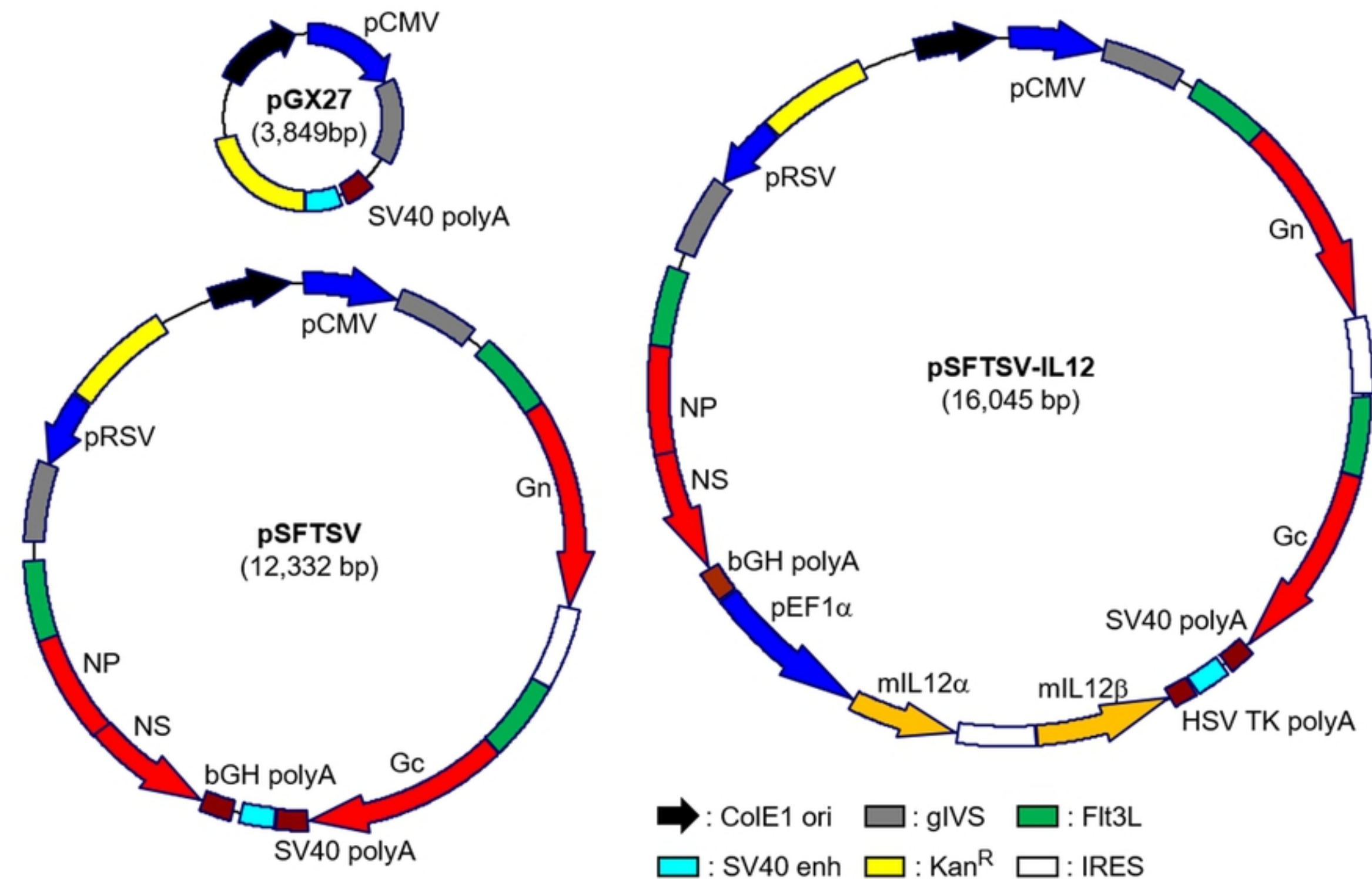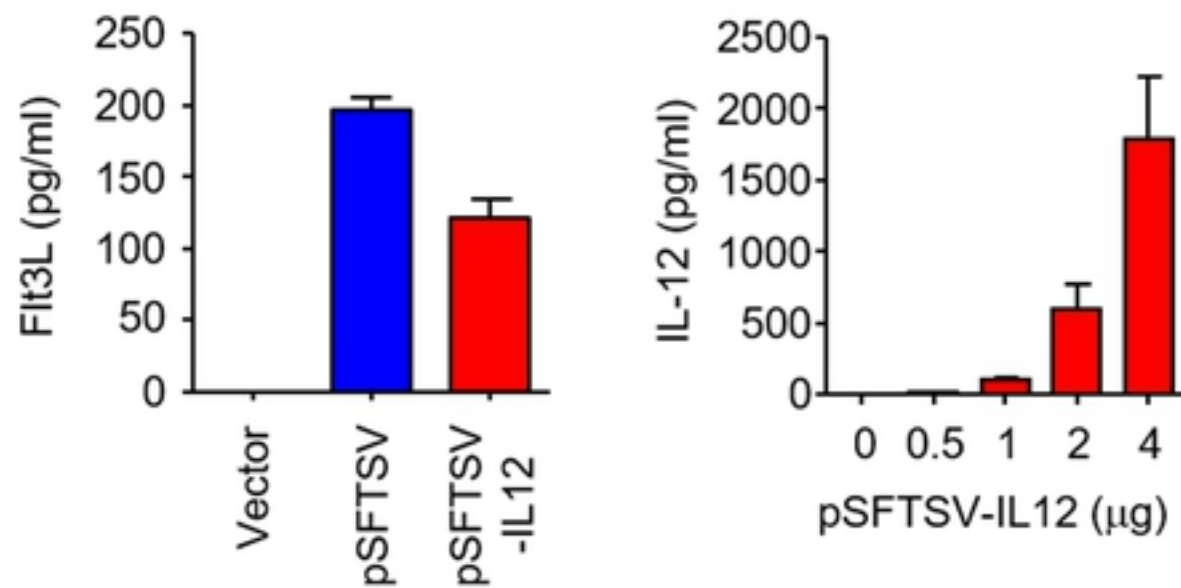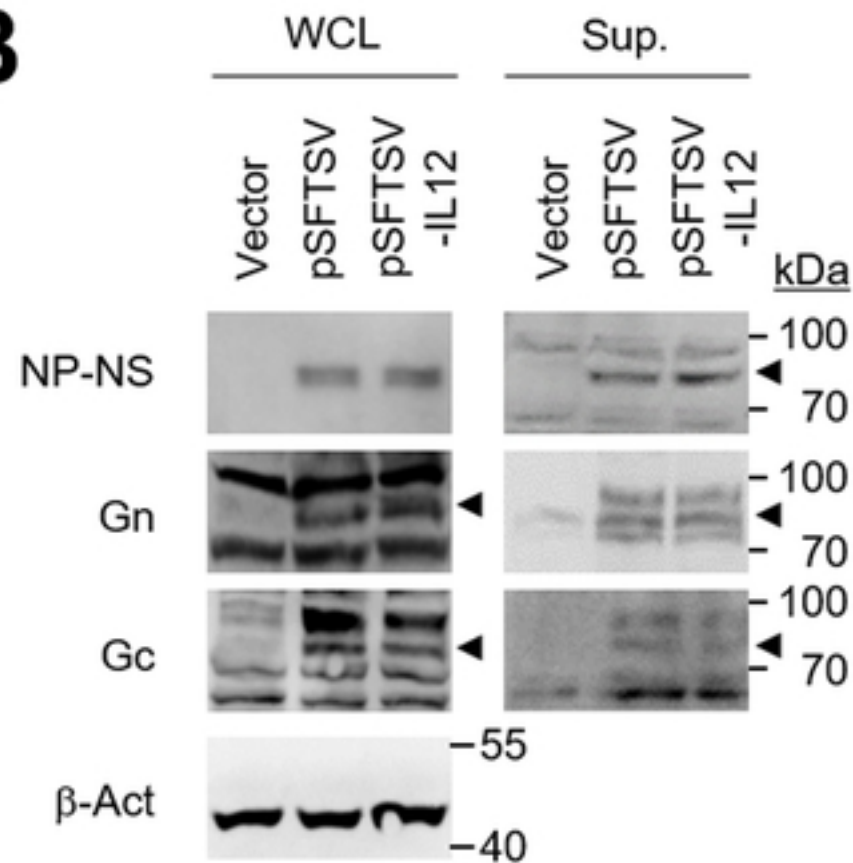
675   WOS:000409182200018.
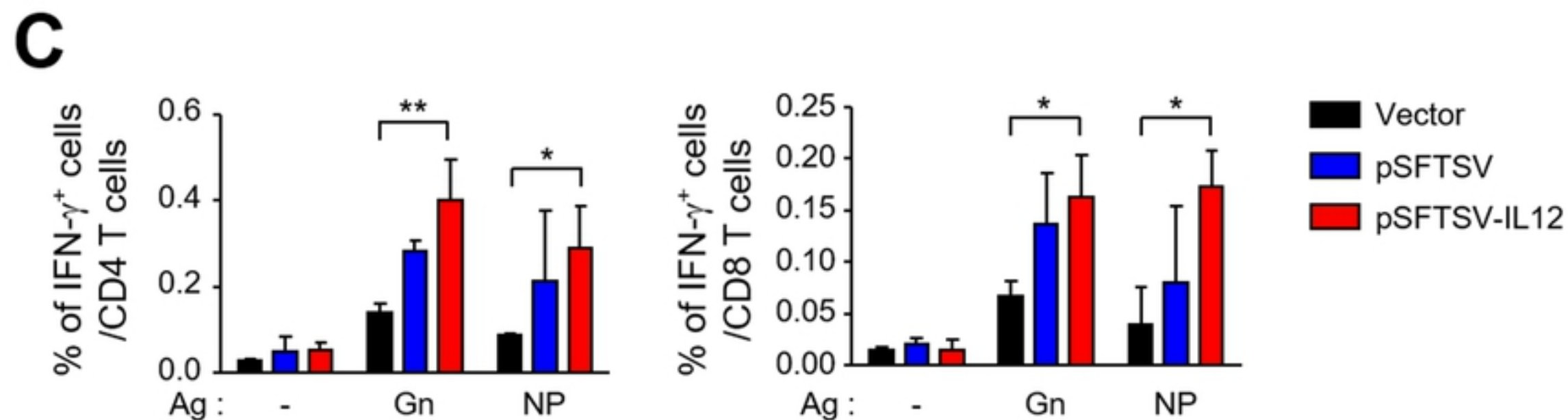

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Figure 1

Figure 2

Figure 3

Figure 4

Figure 5