Supplementary information for

Heterologous prime-boost regimen elicits potent humoral and cell-mediated immune responses and provides complete protection against SFTSV

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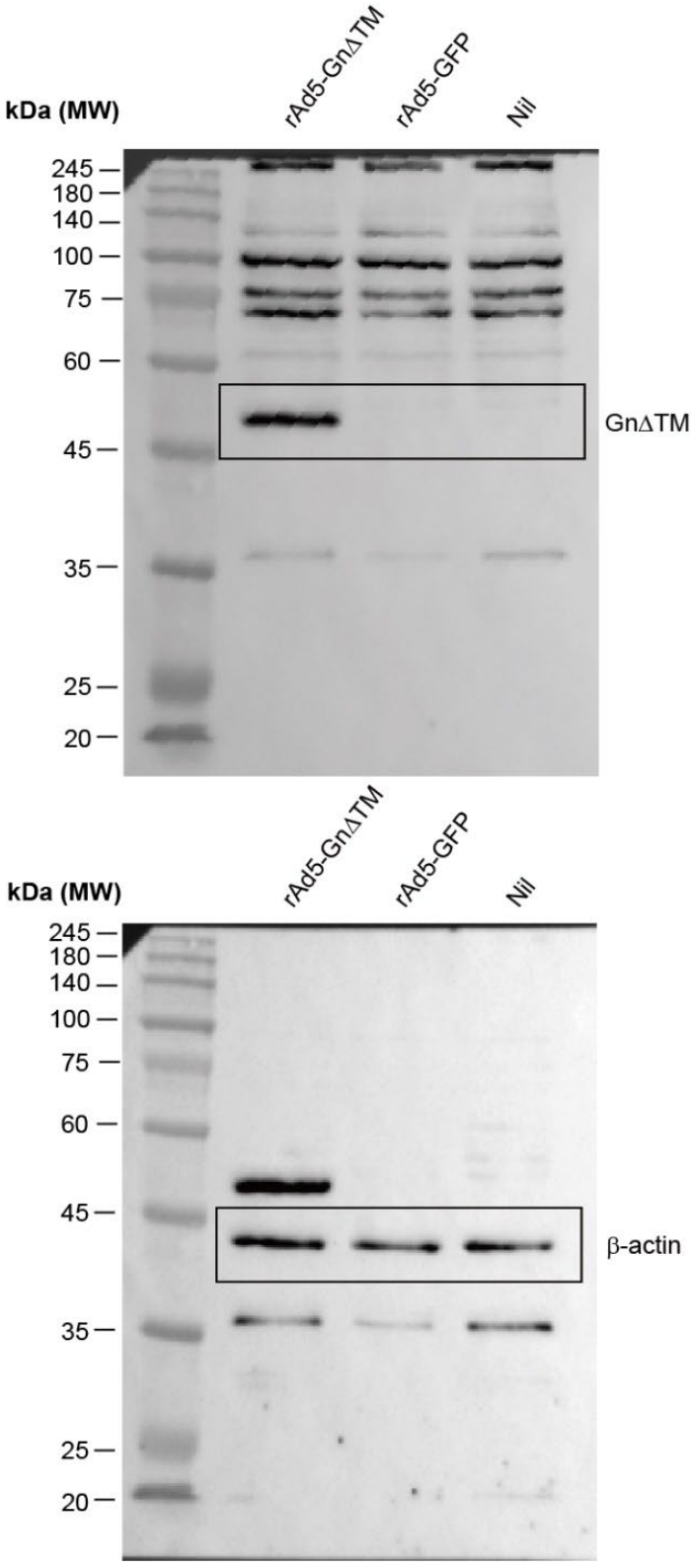
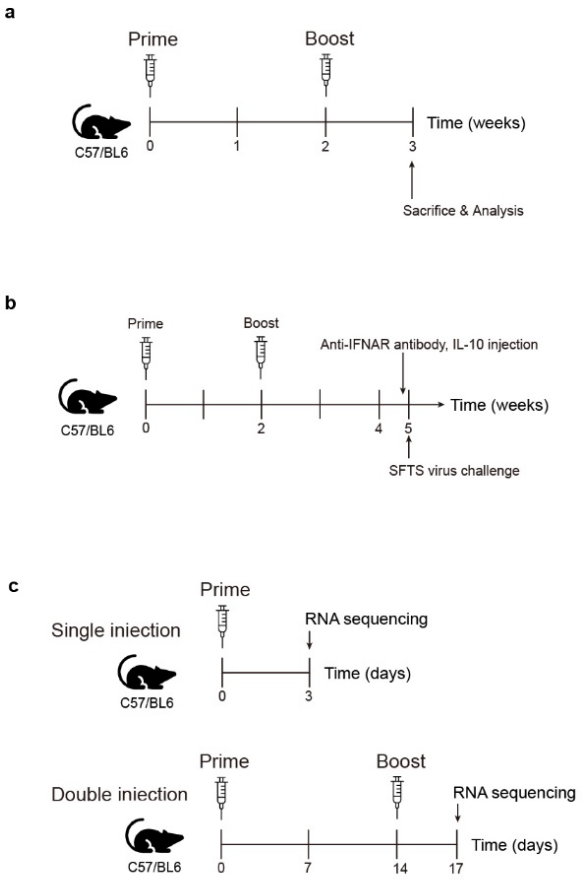
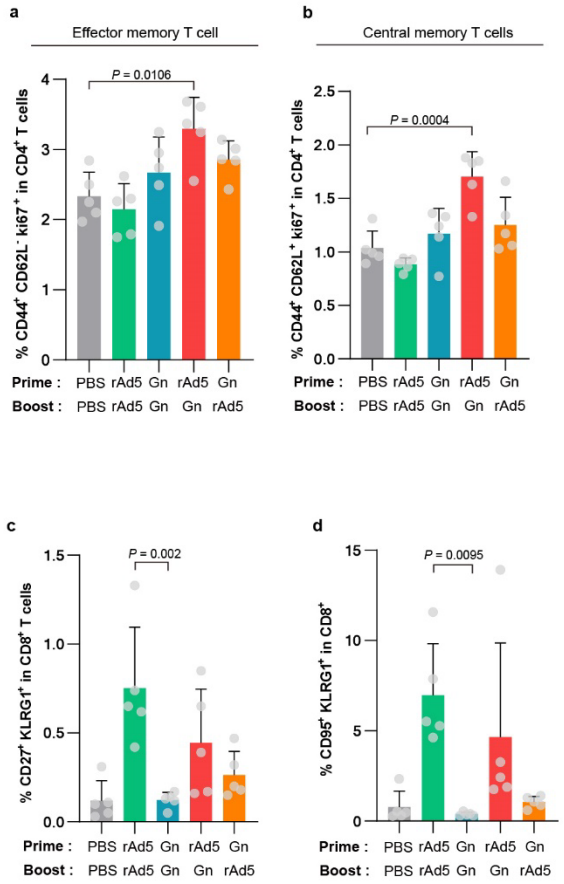
This file includes Supplementary Figures S1 to S5



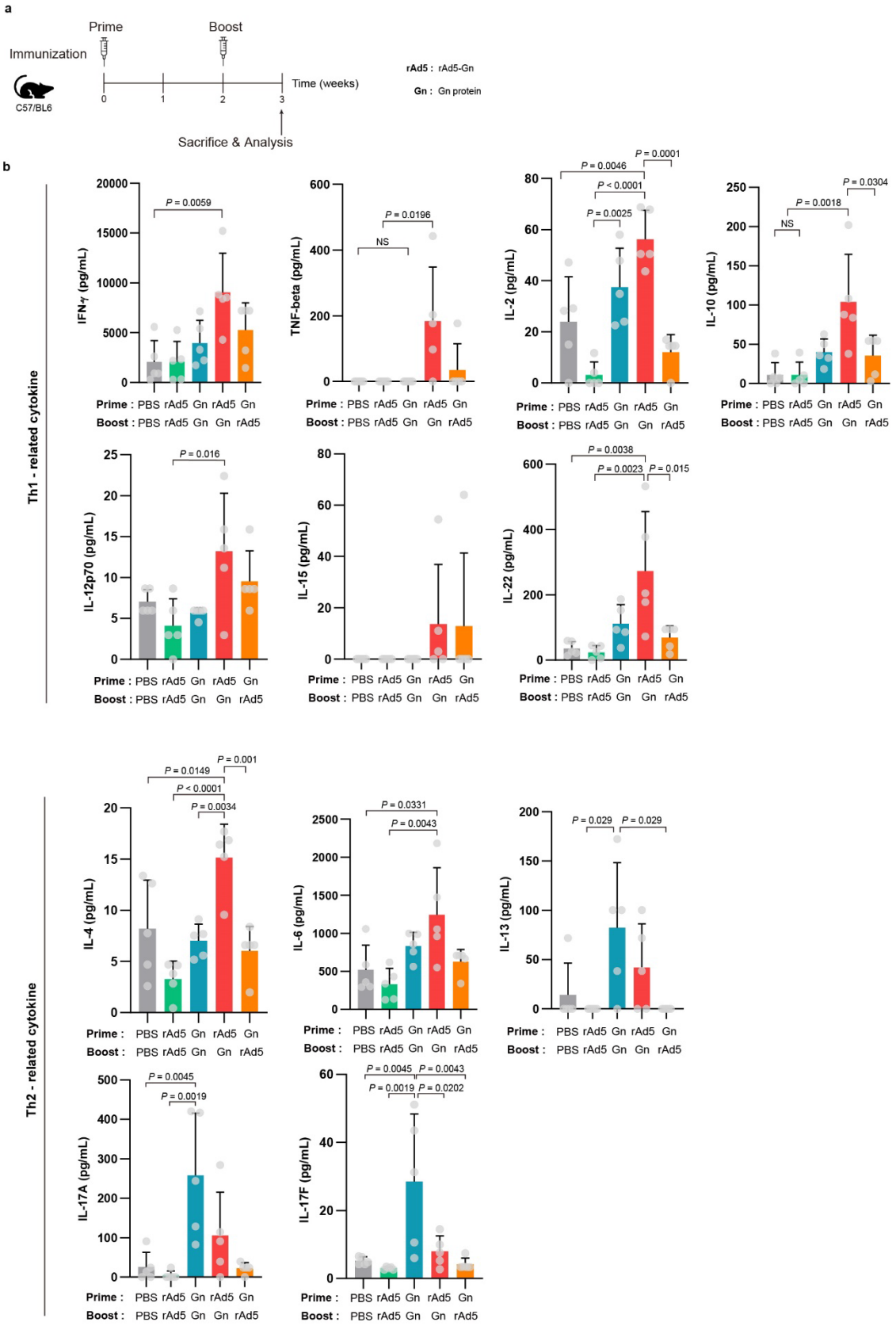
Supplementary Figure S1. Original western blot images.



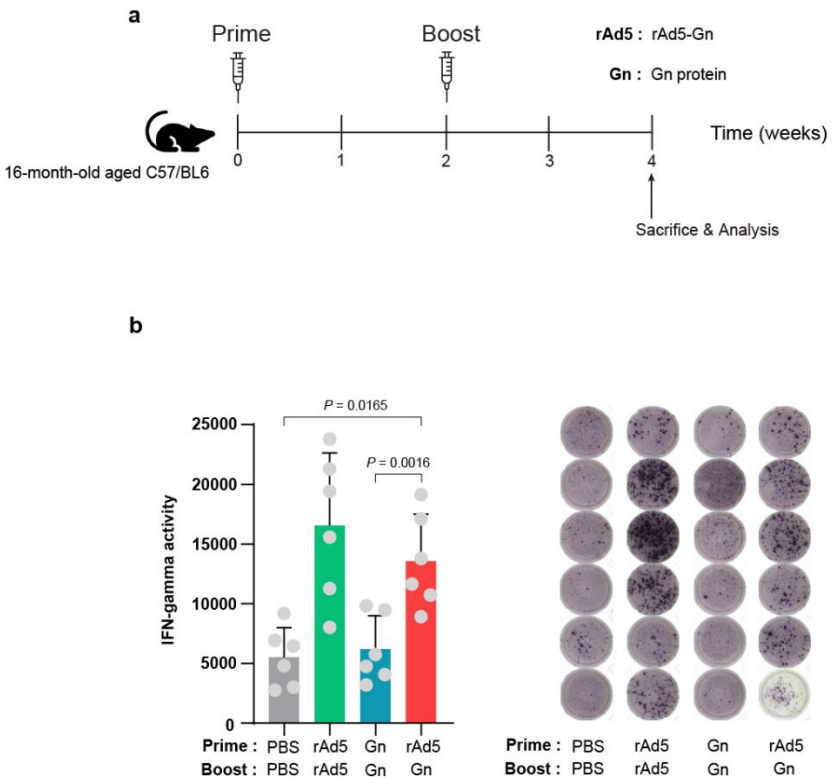
Supplementary Figure S2. Immunization and virus challenge schedule of mice. (a) C57/BL6 wildtype mice (*n* = 5/group) are immunized with rAd5-Gn (1×10^8 IU) and/or Gn protein intramuscularly at 2-week intervals. One week after the boosting mice are sacrificed and analyzed. (b) C57/Bl6 wildtype mice (*n* = 5/group) are immunized with rAd5-Gn (1×10^8 IU) and/or Gn protein intramuscularly at 2-week intervals. Mice are virus challenged 3 weeks after boosting. Mice receive the anti-interferon receptor antibody and IL-10 4 days and 1 day before the virus challenge. (c) C57/BL6 wildtype mice (*n* = 4 or 5/group) are immunized with rAd5-Gn (1×10^8 IU) and/or Gn protein. Single-injection or double-injection groups are sacrificed 3 days after priming or boosting.



Supplementary Figure S3. Analysis of T cell activation after heterologous or homologous immunization with the Ad5-Gn and Gn protein. (a) C57/BL6 wildtype mice are immunized with rAd5-Gn (1×10^8 IU) and/or Gn protein intramuscularly at 2-week intervals and sacrificed 1 week after boosting. Splenocytes (5×10^5) from each mouse are cultured in the presence of Gn protein for 12 h. (b) Percentages of Ki-67$^+$Tcm and Ki-67$^+$Tem are assessed by flow cytometry. (c) The CD27+KLRG1+ population in CD8$^+$ T cells in the spleen is assessed using flow cytometry. (d) The CD95+KLRG1+ population in CD8$^+$ T cells in the spleen is assessed using flow cytometry. Data are presented as mean values ± SD. *P*-values are calculated using one-way ANOVA with Bonferroni multiple comparison test.


a
Prime
Boost
Immunization
C57/BL6
0
1
2
3
Time (weeks)
Sacrifice & Analysis
rAd5 : rAd5-Gn
Gn : Gn protein
b
Th1 - related cytokine
IFN-γ (pg/mL)
TNF-beta (pg/mL)
IL-2 (pg/mL)
IL-10 (pg/mL)
IL-12p70 (pg/mL)
IL-15 (pg/mL)
IL-22 (pg/mL)
Th2 - related cytokine
IL-4 (pg/mL)
IL-6 (pg/mL)
IL-13 (pg/mL)
IL-17A (pg/mL)
IL-17F (pg/mL)
Prime : PBS rAd5 Gn rAd5 Gn
Boost : PBS rAd5 Gn Gn rAd5
P = 0.0059
P = 0.0196
NS
P = 0.0046
P = 0.0001
P < 0.0001
P = 0.0025
P = 0.0304
P = 0.0018
P = 0.016
P = 0.0038
P = 0.0023
P = 0.015
P = 0.001
P = 0.0149
P < 0.0001
P = 0.0034
P = 0.0331
P = 0.0043
P = 0.029
P = 0.0045
P = 0.0019
P = 0.0202

Supplementary Figure S4. Multiplex cytokine profiling of antigen-stimulated splenocytes from homologous or heterologous prime-boosted mice. (a) C57/BL6 wildtype mice are immunized with rAd5-Gn (1×10^8 IU) and/or Gn protein intramuscularly at 2-week intervals and sacrificed 1 week after boosting. Splenocytes (5×10^5) from each mouse are cultured in the presence of Gn protein for 3 days. (b) Cytokine concentrations in the cell culture supernatants are measured using multiplex analysis. Data are presented as mean values ± SDW. *P*-values are calculated using one-way ANOVA with Bonferroni multiple comparison test.



Supplementary Figure S5. Analysis of IFN-γ-producing T cells after stimulation Gn peptide mix. (a) 16-month-old C57/BL6 wildtype mice are immunized with rAd5-Gn (1×10^8 IU) and/or Gn protein intramuscularly at 2-week intervals. 1 week after boosting mice are sacrificed and analyzed. (b) Gn peptide mix-specific IFN-γ-producing T cells in the spleen were quantified using ELISPOT. Data are presented as mean values ± SD. *P*-values are calculated using one-way ANOVA with Bonferroni multiple comparison test.