Appendix Figures

Inhibition of SFTSV replication in humanized mice by a subcutaneously administered anti-PD1 nanobody

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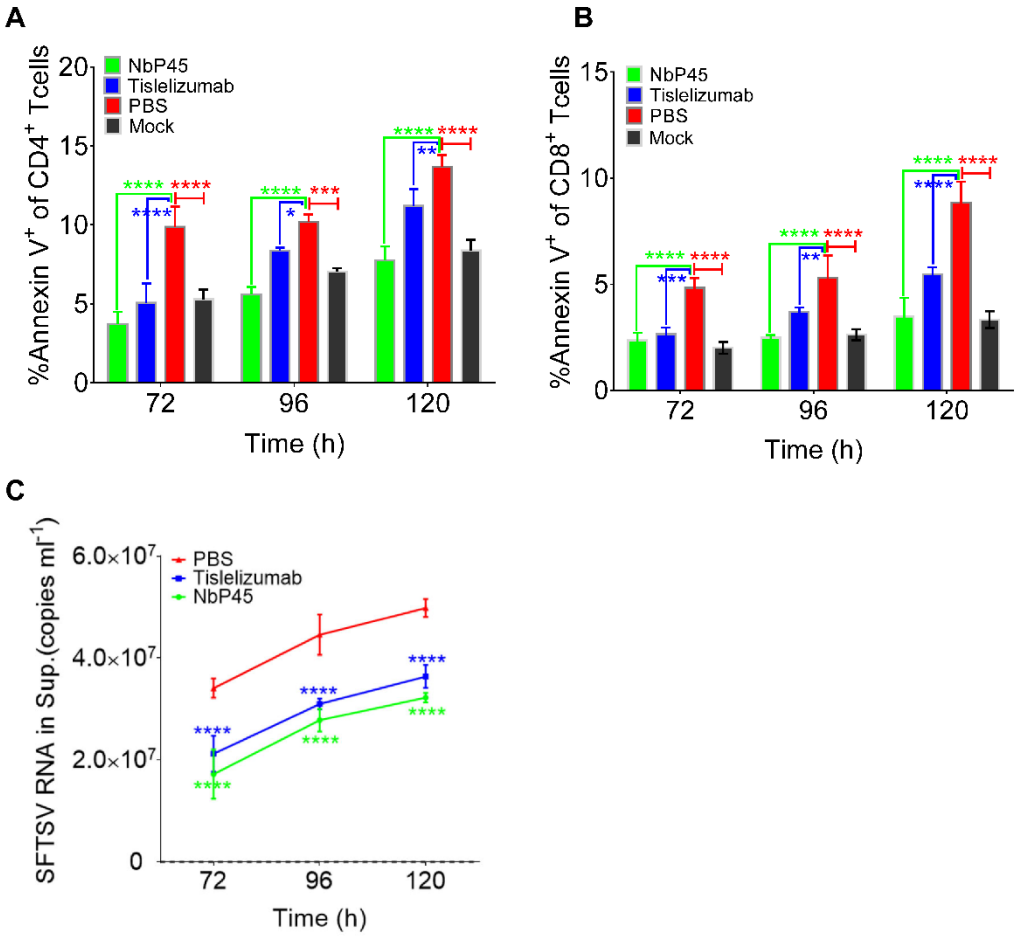
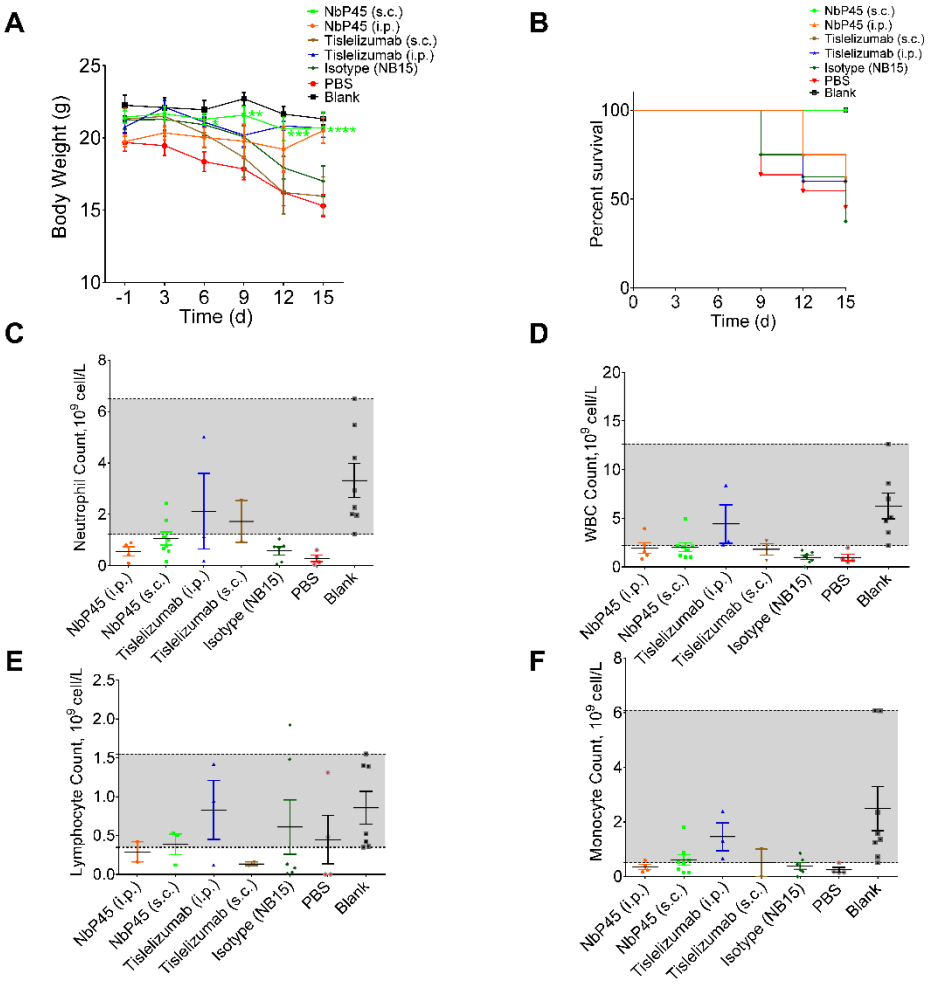


Appendix Figure S1. Apoptosis of the T lymphocytes during SFTSV infected PBMC.

A, B. Annexin V^+ expression of $CD4^+$ (A) and $CD8^+$ (B) T cell was summarized for the uninfected (n = 3), SFTSV (MOI = 1) infected (n = 3) and treated controls (n = 3) at 72/96/120 h.

C. The inhibitory activity of NbP45 (n = 3) or Tislelizumab (n = 3) against SFTSV (MOI = 1) infection of PBMCs at 72/96/120 h. Each line represents data from a group with indicated treatment.

Data are shown as mean ± SEM. Two-way ANOVA with Tukey's test was performed to compare SFTSV infection, treatment group with control group. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

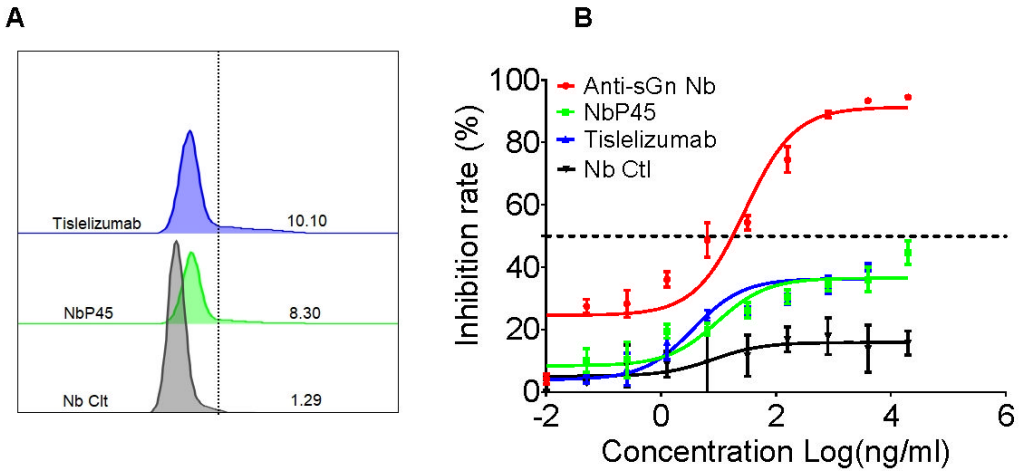


Appendix Figure S2. Evaluation of NbP45 therapeutic efficacy in SFTSV-infected NCG-HuPBL mice.

A, B. Relative weight (A) and survival (B) among 7 groups of color-coded NCG-HuPBL mice including no SFTSV challenge (n = 8), SFTSV challenge with PBS treatment as control (n = 11), SFTSV challenge with isotype (NB15) treatment as control (n = 8), NbP45 treatment by s.c. (n = 8) or i.p. (n = 8), Tislelizumab treatment by s.c. (n = 5) or i.p. (n = 5). Each line represents data from 1 group.

C-F. WBCs (C), neutrophils (D), lymphocytes (E), and monocytes (F) were analyzed in 7 groups of NCG-HuPBL mice infected with SFTSV at 15 days including no SFTSV challenge (n = 8), SFTSV challenge with PBS treatment as control (n = 5), SFTSV challenge with isotype (NB15) treatment as control (n = 7), NbP45 treatment by s.c. (n = 8) or i.p. (n = 5), Tislelizumab treatment by s.c. (n = 3) or i.p. (n = 3). The normal range is between the 2 dashed lines. Each dot represents data from 1 mouse.

Data are shown as mean ± SEM. Two-way ANOVA with Tukey's test was performed to compare treatment group with control group (PBS). *ns*, no significance; *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$.



Appendix Figure S3. Characterization of NbP45.

A. Histogram depicting the NbP45 or Tislelizumab binding to PD-1 of THP-1 cells, as evaluated using flow cytometry. Nb Ctl (one published nanobody, Nb15, specific for SARS-CoV-2 S protein) served as negative nanobody control.

B. Neutralization activity of NbP45 (n = 3) against live SFTSV infection of PBMCs. Anti-sGn Nb (one nanobody, specific for SFTSV Gn protein), Nb Ctl (one published nanobody, Nb15, specific for SARS-CoV-2 S protein) served as positive and negative nanobody control, respectively.

Data are shown as mean ± SEM.