

Supplementary Information

A broadly protective antibody targeting glycoprotein Gn inhibits severe fever with thrombocytopenia syndrome virus infection

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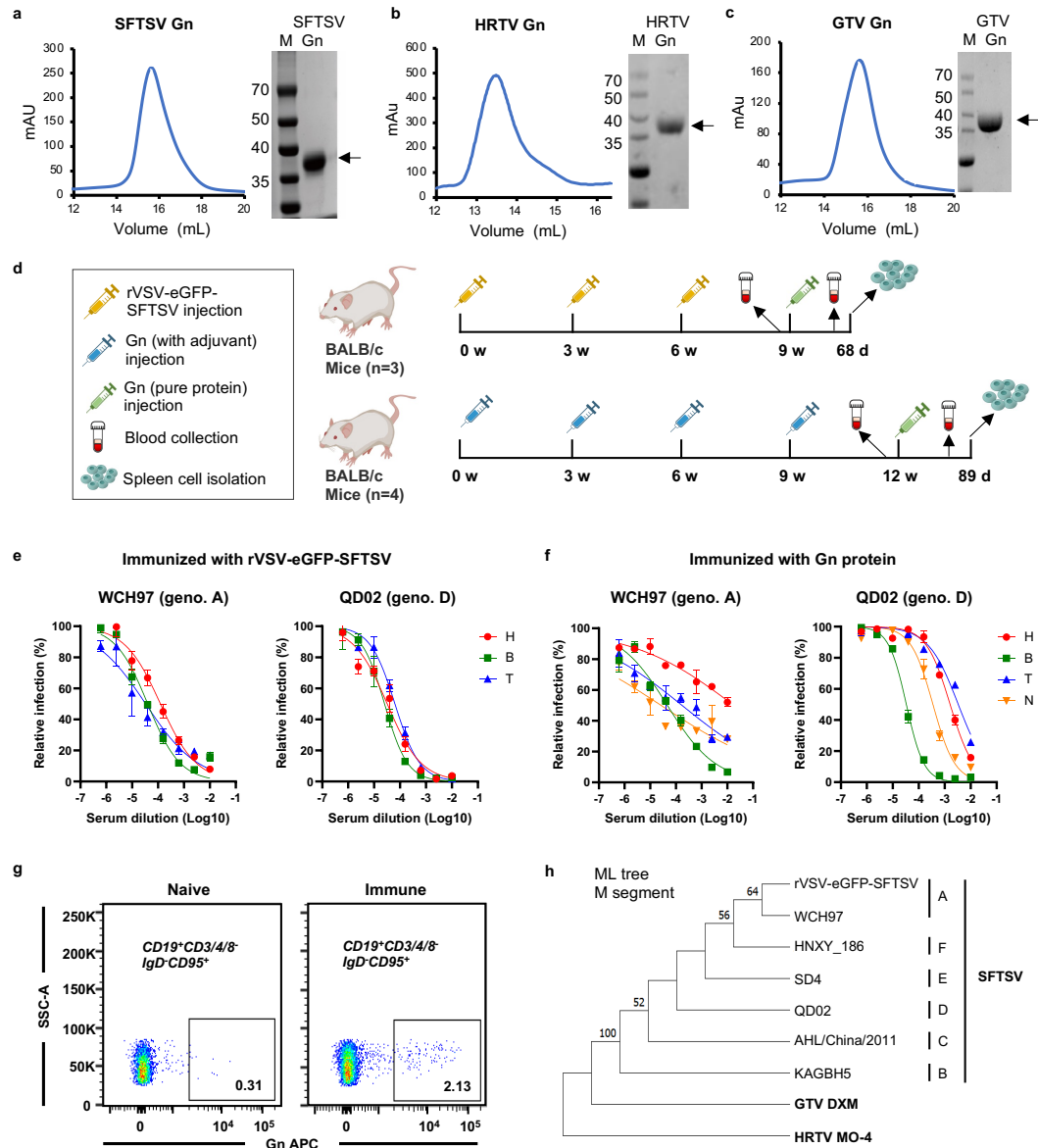
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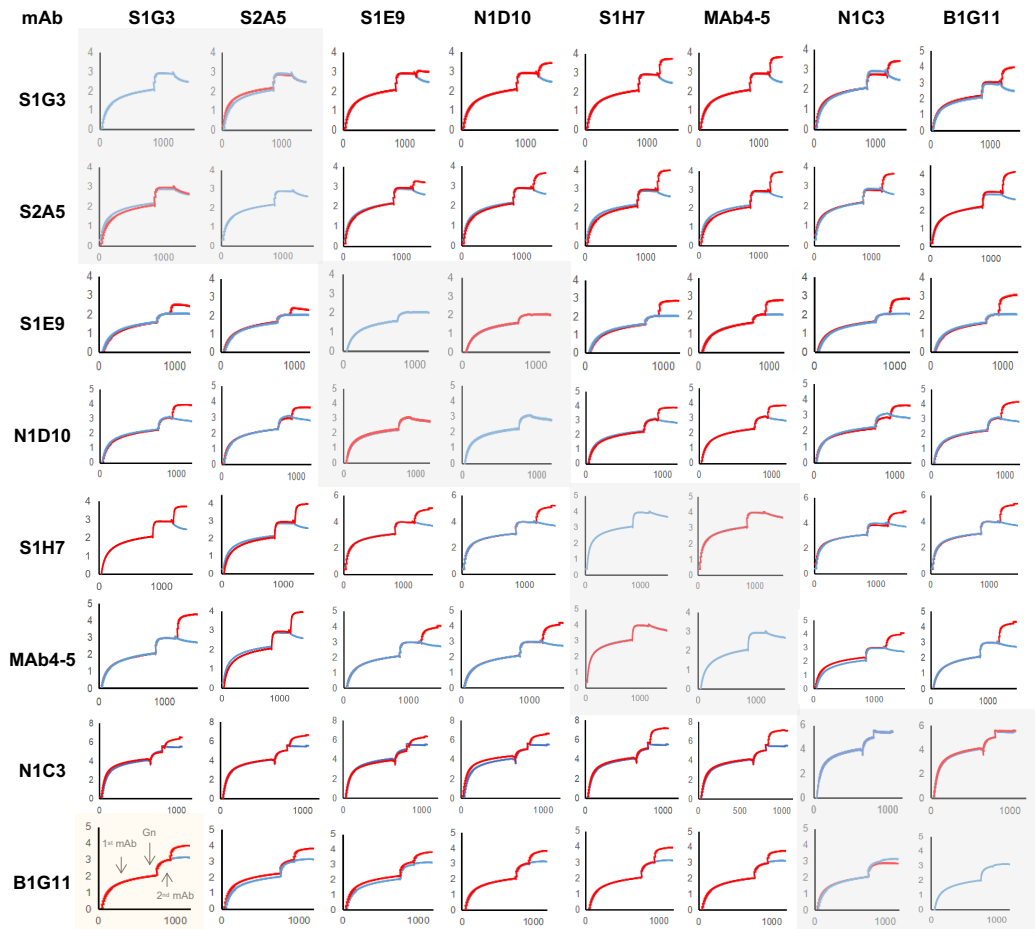
This PDF file includes:

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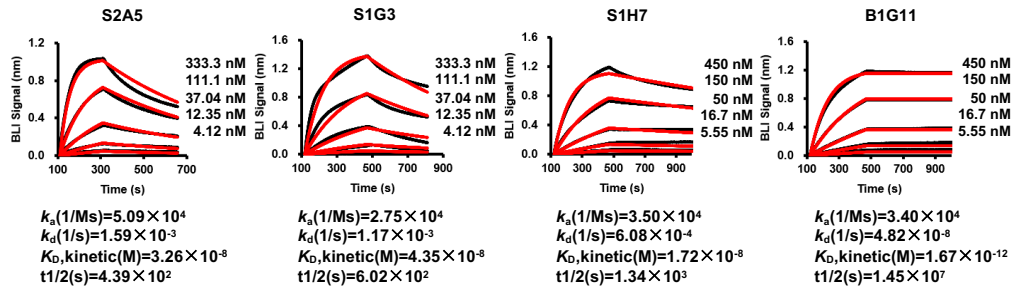
Supplementary Tables 1-6



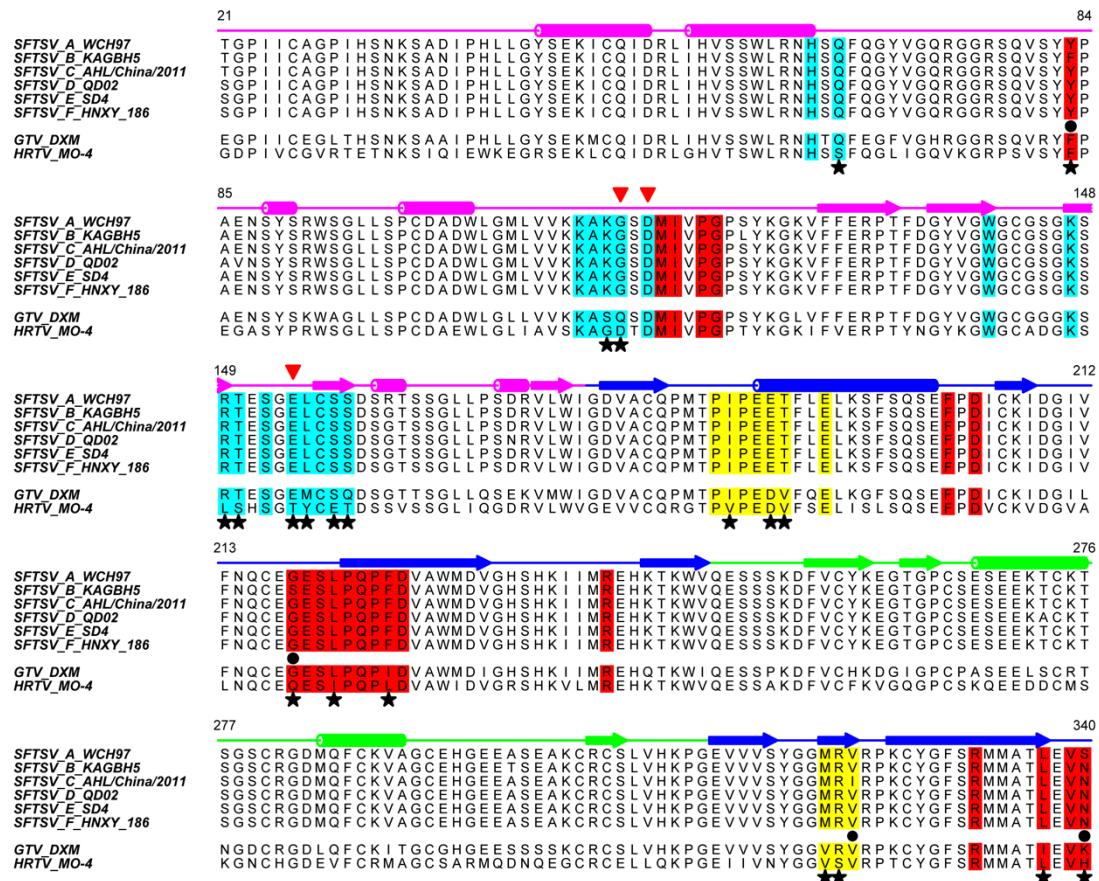
Supplementary Fig. 1 Mice immunization and single B cell sorting. **a-c** SFTSV Gn (**a**), HRTV Gn (**b**), and GTV Gn (**c**) head proteins were purified by gel filtration and analyzed by SDS-PAGE. **d** Schematic diagram of mice immunization by replication-competent SFTSV pseudovirus, rVSV-eGFP-SFTSV (upper panel), or SFTSV Gn head domain protein (lower panel). **e-f** Evaluation of the neutralizing activity of the serum collected before mice euthanization against two SFTSV pseudoviruses. Each line represents one mouse. Data represent mean $\pm$ SEM of technical duplicate and are representative of two independent experiments. **g** Sorting strategies for plasmablast. The population of CD19⁺CD3/4/8⁺IgD⁻CD95⁺APC⁺ cells from immunized mice was significantly higher than that from naïve mice. **h** The maximum likelihood (ML) tree was constructed using MEGA 11 software and inferred under Jones-Taylor-Thornton (JTT) model + Gamma distributed(G) model (also determined by MEGA). The SFTSV strains involved in this study were classified into 6 genotypes, denoted as A, B, C, D, E, and F, based on the M segment. Tree reliability was evaluated by the bootstrap method with 1000 replications. Bootstrap values (≥ 50) were labeled at nodes.



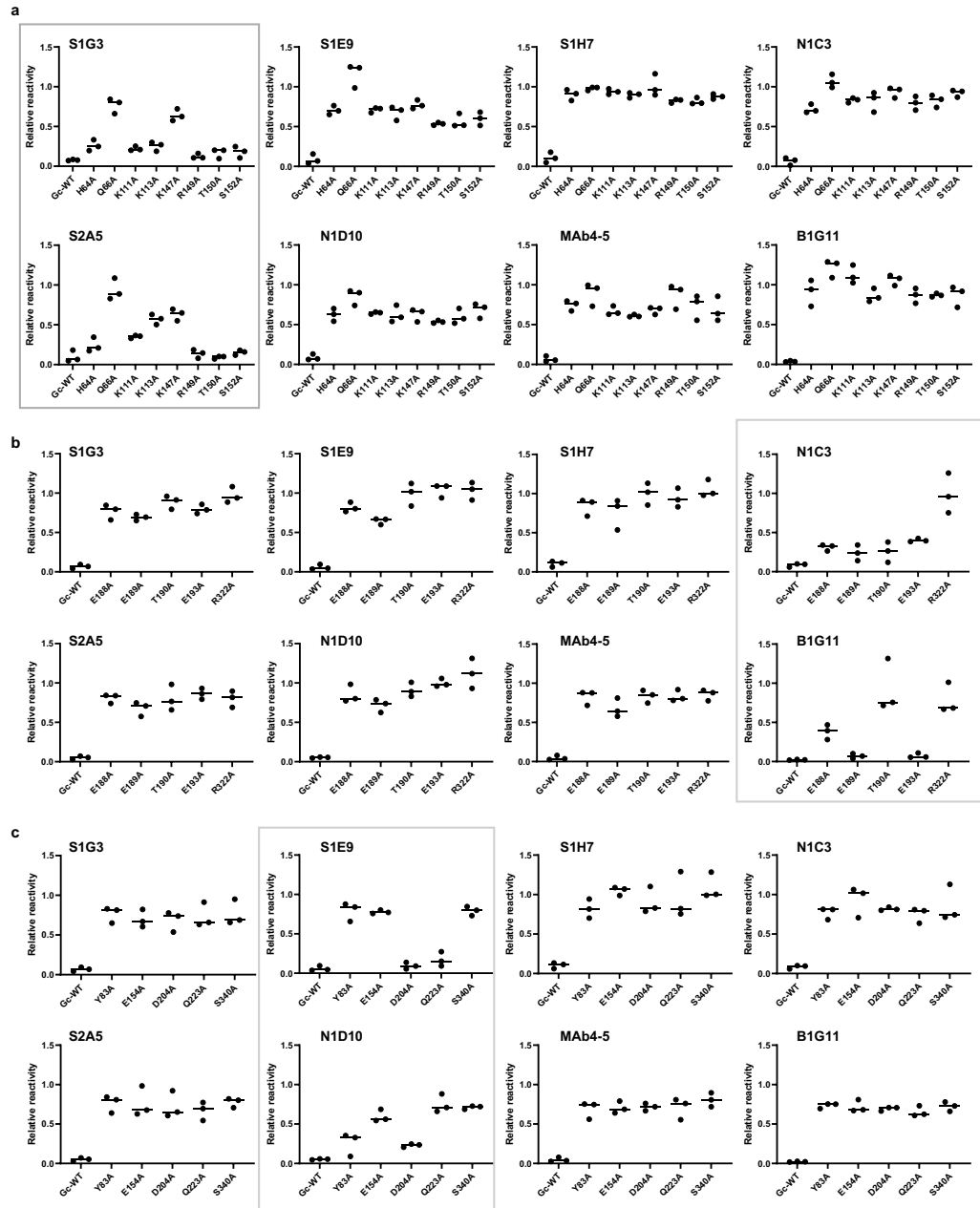
Supplementary Fig. 2 Competition-binding assay. The epitope competition relationship of mAbs was investigated by BLI assay. In this experiment, the first antibody (1st mAb) was immobilized onto the biosensor to capture the SFTSV Gn head domain (Gn), then the binding of the second antibody (2nd mAb) to captured Gn was measured (as shown in the lower left corner graph with the pale gold background). In the presented figures, each row corresponds to the same first antibody (indicated on the left), and each column corresponds to the same second antibody (indicated at the top). 1st mAb-Gn-1st mAb (in the graphs at the diagonal position, blue line) were included as controls to indicate free Fc-binding sites on the biosensor. Significantly increased BLI signal when the second mAbs (red line) are added indicates these two mAbs are non-competitors (represented by the red line giving an increased signal compared with the blue line), whereas no/weak enhanced signal indicates the first mAb competes with the second mAb for Gn binding. Antibodies with epitope competitive relationships are indicated with a soft gray background. A representative curve from two independent experiments is shown.



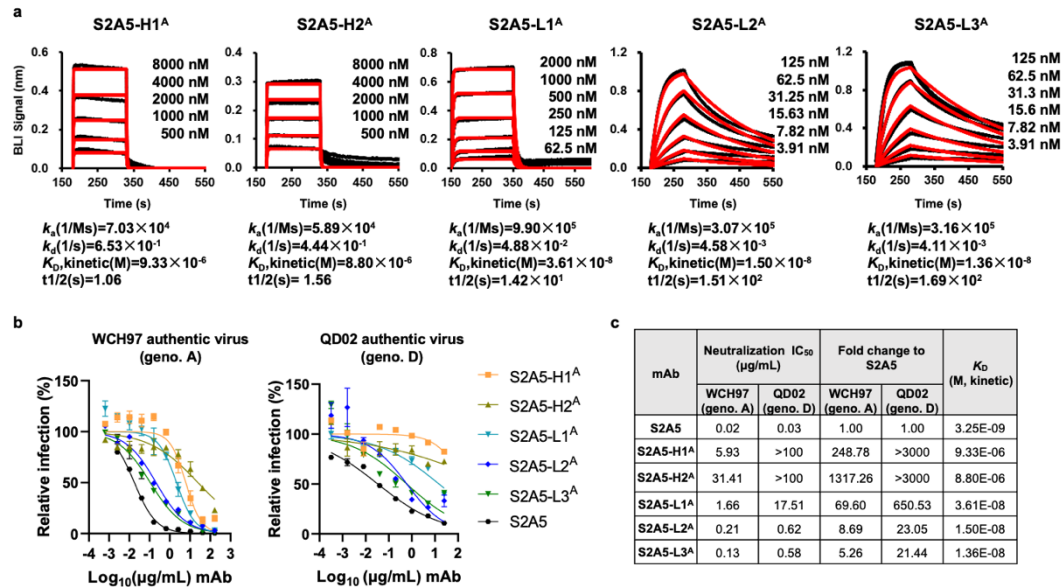
Supplementary Fig. 3 The binding affinity analysis of indicated mAbs to recombinant SFTSV Gn head protein under acid condition (pH 5.0) by BLI. Representative binding curves from two or three independent experiments are shown. The K_D and $t1/2$ values are the average of two to three independent experiments. The fitting curves are shown as red lines.



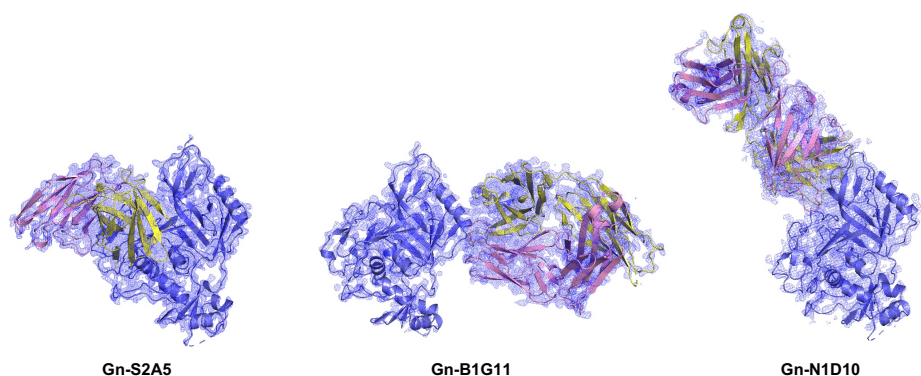
Supplementary Fig. 4 Sequence alignment of the Gn head domains among six SFTSV genotypes, HRTV and GTV. Sequences used in the alignment are listed: SFTSV genotype A, JQ341189.1; SFTSV genotype B, KP663738.1; SFTSV genotype C, JQ670930.1; SFTSV genotype D, MW526369.1; SFTSV genotype E, HM802203.1; SFTSV genotype F, KC292308.1; HRTV, LC629154.1; GTV, KT328592.1. The epitopes of S2A5, B1G11, and N1D10 are shown in cyan, yellow and red, respectively. Secondary structure annotation is displayed above the sequences in magenta, blue, and green for the domain I, II, and III, respectively. The variable positions within the epitopes across six SFTSV are marked below the alignment by the black circle. The variable positions within the epitopes between SFTSV and HRTV or GTV are marked below the alignment by the black star. The red down triangles above the sequence designate the residues contacted by both S2A5 and N1D10.



Supplementary Fig. 5 Gn mutations to identify critical residues for binding with mAbs. Gn expression plasmids with point mutations (H64A, Q66A, K111A, K113A, K147A, R149A, T150A, or S152A) within the epitope of S2A5 (**a**), Gn expression plasmids with point mutations (E188A, E189A, T190A, E193A, or R322A) within the epitope of B1G11 (**b**) and Gn expression plasmids with point mutations (Y83A, E154A, E204A, Q223A, or S340A) within the epitope of N1D10 (**c**) were individually transfected into 293T cells, and the lost-of-binding was detected by flow cytometry. Wild-type Gn and Gc expression plasmids were included as positive and negative controls, respectively. Residues were considered critical if the binding level decreased >70% compared to the wild-type Gn. Each graph represents the mean from three independent experiments. The binding of Gn point mutations corresponding to the B1G11 and N1D10 epitopes was performed simultaneously, and the same positive and negative controls were used in b and c.



Supplementary Fig. 6 Affinity and neutralization profiles of S2A5 antibody mutants. **a** The binding affinity of S2A5 mutants to Gn head domain (S2A5-H1^A [residues 31 and 32 on the heavy chain mutated to alanine], S2A5-H2^A [residues 98 and 100A on the heavy chain mutated to alanine], S2A5-L1^A [residues 26-30C on the light chain mutated to alanine], S2A5-L2^A [residues 66-69 on the light chain mutated to alanine], and S2A5-L3^A [residues 91 and 92 on the light chain mutated to alanine]) were assessed using BLI assay. Representative BLI curves from two or three independent experiments are shown. **b** The neutralization of SFTSV authentic virus was assessed using FRNT. Data represent mean $\pm$ SEM of technical duplicate and are representative of two to four independent experiments. **c** The IC₅₀ values against authentic viruses are summarized in the table, and the results are the average of two to four independent experiments.



Supplementary Fig. 7 Electron density maps for crystal structures of Gn complexed with the representative Fab. The 2Fo-Fc electron density maps are contoured at 2.0 σ for S2A5 and B1G11-Gn complex, and 1.0 σ for N1D10-Gn complex. The heavy chain and light chain of S2A5, B1G11, and N1D10 are shown in yellow and pink, respectively, and Gn is colored in slate.

Supplementary Table 1. X-ray diffraction data collection and structure refinement statistics

PDB Code	Gn-SNB1G11 Fab complex (8XK5)	Gn-S2A5 Fab complex (8XK6)	Gn-N1D10 Fab complex (8XK8)
Data collection			
Space group	$P2_12_12_1$	$P6_322$	$P2$
Cell dimensions			
a, b, c (Å)	113.7, 181.0, 264.0	158.0, 158.0, 190.5	84.1, 111.9, 87.9
α , β , γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 120.0	90.0, 94.3, 90.0
Resolution (Å)	30.00–3.05 (3.10–3.05) ^a	50.00–2.45 (2.53–2.45)	47.00–3.52 (3.85–3.52)
No. reflections	104166	50670	20028
R_{merge}	0.108 (1.269)	0.102 (1.054)	0.246 (0.358)
R_{meas}	0.129 (1.589)	0.110 (1.135)	0.347 (0.505)
$CC_{1/2}$	0.997 (0.543)	0.999 (0.845)	0.758 (0.626)
$I/\sigma I$	8.5 (1.2)	15.6 (2.9)	3.4 (2.5)
Completeness (%)	99.8 (100.0)	98.0 (98.9)	98.5 (96.4)
Redundancy	6.3 (5.1)	13.6 (13.7)	2.7 (2.6)
Refinement			
Resolution (Å)	3.05	2.45	3.52
No. reflections	79582	50657	19778
$R_{\text{work}}/R_{\text{free}}$ (%)	21.5/28.1	23.1/25.0	25.0/32.4
No. atoms			
Protein	27019	4173	11394
Water		160	
B -factors (Å ²)			
Protein	45.8	48.7	34.8
Water		50.6	
R.m.s. deviations			
Bond lengths, (Å)	0.012	0.008	0.004
Bond angles, (°)	1.458	0.995	0.799
Ramachandran	94.3/5.2/0.5	95.6/4.4/0.0	94.2/5.5/0.3
statistics ^b			

^aValues in parentheses are for the highest-resolution shell.

^bValues are in percentage for favored, allowed, and disallowed regions in Ramachandran plots, respectively.

Supplementary Table 2. Contacts for S2A5 Fab and Gn head domain complex

Hydrogen bond contacts		
SFTSV-Gn	Heavy chain	Light chain
His ⁶⁴ (ND1)	Asp ³¹ (OD2)	
Gln ⁶⁶ (OE1)	Arg ⁹⁴ (NH2)	
Lys ¹¹¹ (NZ)	Asp ³² (OD1)	
Lys ¹¹³ (NZ)	Asp ⁵² (OD1), Asp ³¹ (O), Ser ³⁰ (O)	
Lys ¹¹³ (O)	Tyr ⁹⁸ (OH)	
Asp ¹¹⁶ (O)	Tyr ⁹⁸ (OH)	
Lys ¹⁴⁷ (NZ)		Glu ⁵⁵ (OE2)
Arg ¹⁴⁹ (NH2)	Tyr ⁹⁸ (O)	
Arg ¹⁴⁹ (NH1)	Tyr ⁹⁸ (O)	
Thr ¹⁵⁰ (OG1)		Asp ^{30D} (OD2)
Ser ¹⁵² (OG)		Asp ^{30D} (OD2)
Glu ¹⁵⁴ (OE2)	Arg ^{100A} (NH2)	
Glu ¹⁵⁴ (O)	Arg ^{100A} (NH2)	
Cys ¹⁵⁶ (O)		Tyr ⁴⁹ (OH)
Cys ¹⁵⁶ (N)	Gly ⁹⁹ (O)	
Ser ¹⁵⁸ (N)		Asn ⁵³ (OD1)
Van der Waals contacts		
SFTSV-Gn	Heavy chain	Light chain
His ⁶⁴ (3)	Asp ³¹ (3)	
Gln ⁶⁶ (3)	Tyr ²⁷ (1), Arg ⁹⁴ (2)	
Lys ¹¹¹ (4)	Asp ³¹ (3), Asp ³² (1)	
Ala ¹¹² (3)	Tyr ⁹⁸ (3)	
Lys ¹¹³ (9)	Asp ⁵² (3), Asp ³¹ (1), Asp ⁵³ (1) Ser ³⁰ (1), Tyr ⁹⁸ (3)	
Gly ¹¹⁴ (5)	Tyr ⁹⁸ (2), Tyr ⁹⁷ (3)	
Trp ¹⁴¹ (9)	Tyr ⁹⁸ (9)	
Lys ¹⁴⁷ (3)		Glu ⁵⁵ (1), Tyr ⁴⁹ (2)
Arg ¹⁴⁹ (3)	Tyr ⁹⁸ (3)	
Thr ¹⁵⁰ (5)		Asp ^{30D} (5)
Ser ¹⁵² (1)		Ala ^{30B} (1)
Glu ¹⁵⁴ (9)	Arg ^{100A} (4), Tyr ⁹⁸ (3),	Tyr ³² (2)
Leu ¹⁵⁵ (10)	Gly ⁹⁹ (4), Tyr ⁹⁸ (1)	Ala ⁵⁰ (1), Tyr ³² (2), Asp ³⁰ (2)
Cys ¹⁵⁶ (7)	Tyr ⁹⁸ (1), Gly ⁹⁹ (3)	Tyr ⁴⁹ (3)
Ser ¹⁵⁷ (5)		Asn ⁵³ (4), Tyr ⁴⁹ (1)

The number in superscript indicates the residue positions in the Gn or mAb S2A5. The numbers in parentheses show the interacting numbers contributed by the indicated residues. Interactions were assessed using LigPlot+ with a cutoff distance of 3.9 Å.

Supplementary Table 3. Contacts for B1G11 Fab and Gn head domain complex

Hydrogen bond contacts		
SFTSV-Gn	Heavy chain	Light chain
Pro ¹⁸⁵ (O)		Ser ³⁰ (OG)
Pro ¹⁸⁵ (O)		Tyr ³² (OH)
Glu ¹⁸⁸ (N)		Ser ⁹² (O)
Glu ¹⁸⁸ (OE1)		Phe ⁹⁴ (N)
Glu ¹⁸⁹ (OE1)		Arg ⁹⁶ (NH2)
Glu ¹⁸⁹ (OE1)	Arg ⁹⁷ (NH2)	
Thr ¹⁹⁰ (OG1)	Tyr ^{100D} (OH)	
Glu ¹⁹³ (OE1)	Arg ⁵² (NH1)	
Arg ³²² (O)	Tyr ^{100B} (OH)	
Van der Waals contacts		
SFTSV-Gn	Heavy chain	Light chain
Pro ¹⁸⁵ (5)		Ser ³⁰ (2), Tyr ³² (3)
Ile ¹⁸⁶ (6)	Tyr ^{100B} (5)	Tyr ³² (1)
Pro ¹⁸⁷ (12)	Tyr ^{100B} (3), Tyr ^{100D} (1)	Tyr ³² (4), Tyr ⁹¹ (2), Ser ⁹² (2)
Glu ¹⁸⁸ (10)		Ser ⁹² (3), Lys ⁹³ (2), Phe ⁹⁴ (5)
Glu ¹⁸⁹ (7)	Arg ⁹⁷ (4)	Arg ⁹⁶ (3)
Thr ¹⁹⁰ (2)	Tyr ^{100D} (2)	
Glu ¹⁹³ (8)	Tyr ³³ (2), Arg ⁵² (1), Lys ⁵³ (5)	
Met ³²¹ (2)	Asp ⁹⁹ (2)	
Arg ³²² (3)	Asp ¹⁰⁰ (1), Tyr ^{100B} (2)	
Val ³²³ (2)	Tyr ^{100B} (2)	

The number in superscript indicates the residue positions in the Gn or mAb B1G11. The numbers in parentheses show the interacting numbers contributed by the indicated residues. Interactions were assessed using LigPlot+ with a cutoff distance of 3.9 Å.

Supplementary Table 4. Contacts for N1D10 Fab and Gn head domain complex

Hydrogen bond contacts		
SFTSV-Gn	Heavy chain	Light chain
Tyr ⁸³ (OH)	Ser ⁵⁶ (OG)	
Asp ¹¹⁶ (O)		Tyr ^{30C} (OH)
Ile ¹¹⁸ (N)		Tyr ^{30C} (OH)
Glu ¹⁵⁴ (OE1)		Ser ^{30B} (OG)
Asp ²⁰⁴ (OD2)	Thr ²⁸ (OG1)	
Asp ²⁰⁴ (OD1)	Tyr ³² (OH)	
Glu ²¹⁹ (O)		Tyr ⁴⁹ (OH)
Pro ²²² (O)		Tyr ³² (OH)
Gln ²²³ (NE2)	Ser ⁹⁵ (O)	
Asp ²²⁶ (O)	Asn ^{52A} (ND2)	
Asp ²²⁶ (N)	Asn ^{52A} (OD1)	
Ser ³⁴⁰ (OG)		Ser ⁵⁶ (OG)
Van der Waals contacts		
SFTSV-Gn	Heavy chain	Light chain
Tyr ⁸³ (2)	Ser ⁵⁶ (2)	
Gly ¹¹⁴ (2)		Tyr ^{30C} (2)
Asp ¹¹⁶ (3)		Tyr ^{30C} (3)
MET ¹¹⁷ (9)		Tyr ^{30C} (9)
Ile ¹¹⁸ (6)		Tyr ^{30C} (6)
Pro ¹²⁰ (3)	Tyr ⁹⁶ (1), Tyr ⁵⁸ (2)	
Gly ¹²¹ (1)	Tyr ⁵⁸ (1)	
Glu ¹⁵⁴ (13)		Tyr ^{30C} (7), Ser ^{30B} (6)
Phe ²⁰² (2)	Thr ³¹ (2)	
Asp ²⁰⁴ (10)	Thr ²⁸ (1), Tyr ³² (9)	
Gly ²¹⁸ (1)		Tyr ⁴⁹ (1)
Glu ²¹⁹ (4)		Tyr ⁴⁹ (4)
Ser ²²⁰ (3)	Asn ⁹⁸ (2)	Tyr ⁴⁹ (1)
Leu ²²¹ (6)		Thr ³¹ (1), Asn ^{30E} (1), Gly ⁵⁰ (2), Tyr ³² (2)
Pro ²²² (8)	Tyr ⁹⁶ (2)	Asn ^{30E} (2), Tyr ³² (4)
Gln ²²³ (6)	Tyr ⁹⁶ (4), Ser ⁹⁵ (2)	
Pro ²²⁴ (12)	Thr ³³ (1), Tyr ⁵⁰ (3), Tyr ⁹⁶ (8)	
Phe ²²⁵ (5)	Asn ^{52A} (5)	
Asp ²²⁶ (10)	Ser ⁵² (1), Asn ^{52A} (9)	
Arg ²⁴¹ (3)	Thr ²⁸ (2), Ser ³⁰ (1)	
Arg ³³² (5)	Ser ⁵² (1), Ser ⁵⁶ (4)	
Leu ³³⁷ (6)	Thr ³¹ (1), Tyr ³² (5)	
Val ³³⁹ (11)		Tyr ⁴⁹ (3), Phe ⁵⁵ (5), Ser ⁵⁶ (3)
Ser ³⁴⁰ (4)		Ser ⁵⁶ (4)

The number in superscript indicates the residue positions in the Gn or mAb N1D10. The numbers in parentheses show the interacting numbers contributed by the indicated residues. Interactions were assessed using LigPlot+ with a cutoff distance of 3.9 Å.

Supplementary Table 5. Antibodies germline and corresponding immunogens

Group ^a	Chain	H ^b			K ^b		Immunogen ^c
	mAb	Top V gene match	Top D gene match	Top J gene match	Top V gene match	Top J gene match	
A	S1G3	IGHV1S135*01	IGHD1-1*01	IGHJ4*01	IGKV3-4*01	IGKJ1*01	rVSV-eGFP-SFTSV
	S2A5	IGHV1S135*01	IGHD1-1*02	IGHJ4*01	IGKV3-4*01	IGKJ1*01	
B	S1E9	IGHV5-6*01	IGHD2-4*01	IGHJ3*01	IGKV4-72*01	IGKJ1*01	Gn
	N1D10	IGHV5-12-2*01	IGHD1-1*01	IGHJ1*01	IGKV1-88*01	IGKJ1*01	
C	S1H7	IGHV1-87*01	IGHD2-1*01	IGHJ3*01	IGKV6-23*01	IGKJ4*01	rVSV-eGFP-SFTSV
D	N1C3	IGHV5-6-5*01	IGHD1-2*01	IGHJ1*01	IGKV10-94*01	IGKJ1*01	Gn
	B1G11	IGHV7-3*02	IGHD2-14*01	IGHJ4*01	IGKV10-94*01	IGKJ1*01	

^aBased on the competition binding assay, Gn mAbs were categorized into groups A, B, C, and D.

^bThe V(D)J composition of the variable antibody domains was analyzed using the IgBlast website (IgBLAST, [http:// www.ncbi.nlm.nih.gov/igblast](http://www.ncbi.nlm.nih.gov/igblast)).

^cTo generate mAbs, mice were immunized with SFTSV Gn head protein (Gn, i.m.) or VSV-based replication-competent SFTSV (rVSV-eGFP-SFTSV, i.p.) four or three times and then boosted with 25 µg of SFTSV Gn head protein.

Supplementary Table 6. Antibody affinity summary^a

^b pH	^c mAb		^d Affinity for SFTSV Gn			
			<i>K_D</i> (M)	<i>k_a</i> (1/Ms)	<i>k_d</i> (1/s)	t1/2(min)
7.4	Gn mAbs	S1G3	1.33E-08	6.28E+05	7.93E-03	1.462
		S2A5	3.25E-09	3.17E+05	9.96E-04	11.59
		S1E9	1.67E-08	1.23E+05	2.09E-03	5.52
		N1D10	1.36E-08	2.10E+05	2.82E-03	4.10
		S1H7	2.03E-09	2.86E+05	5.72E-04	20.20
		N1C3	1.90E-08	5.12E+05	9.79E-03	1.18
		B1G11	1.90E-10	2.79E+05	5.10E-05	226.28
	S2A5 mutants	S2A5-H1 ^A	9.33E-06	7.03E+04	6.53E-01	0.02
		S2A5-H2 ^A	8.80E-06	5.89E+04	4.44E-01	0.03
		S2A5-L1 ^A	3.61E-08	9.90E+05	4.88E-02	0.24
		S2A5-FR3 ^A	1.50E-08	3.07E+05	4.58E-03	2.52
		S2A5-L3 ^A	1.36E-08	3.16E+05	4.11E-03	2.81
5.0	Gn mAbs	S1G3	4.35E-08	2.75E+04	1.17E-03	10.03
		S2A5	3.26E-08	5.09E+04	1.59E-03	7.31
		S1H7	1.72E-08	3.50E+04	6.08E-04	22.38
		B1G11	1.67E-12	3.40E+04	4.82E-08	239502.40

^aThe affinity of seven Gn mAbs and five S2A5 mutations to Gn head domain protein.

^bThe affinity of Gn mAbs to the Gn head domain was assessed using BLI assay under neutral or acidic conditions.

^cGn mAbs were arranged according to epitope classification.

^dThe *k_a*, *k_d*, *K_D* and t1/2 values of mAbs affinity to recombinant Gn head protein are the average of two to three independent experiments.