

## Electronic Supplementary Material

### First Fatal Infection and Phylodynamic Analysis of Severe Fever with Thrombocytopenia Syndrome Virus in Jilin Province, Northeastern China

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#### Supplementary Materials and Methods

##### Indirect immunofluorescence assay

Indirect immunofluorescence assay (IFA) was developed as previously described with minor modifications (Yu *et al.* 2011). In brief, monolayers of SFTSV (strain JA1-2018) infected Vero cells were trypsinized, spotted onto the slides, and air dried, followed by fixation with acetone. The slides were then incubated with 2-fold serial dilutions of the patient's sera (starting at a dilution of 1:20) and probed with Alexa Fluor® 488 conjugated goat anti-human IgM/IgG (Invitrogen). IFA titers were expressed as the reciprocal of the highest dilution of serum resulting in specific fluorescence of SFTSV. A titer of 1:20 was considered as positive.

##### Cytokines detection

The serum sample was collected from the patient (at day 9 after the illness onset) and 7 healthy individuals that served as control. Cytokine levels were measured by a 27-plex cytokine detection kit according to the manufacturer's instructions (Bio Plex Pro<sup>TM</sup> Human Cytokine Assay Kits, Bio-Rad).

**Table S1.** Clinical blood biochemistry tests of the SFTS patient in Jilin Province, Northeastern China

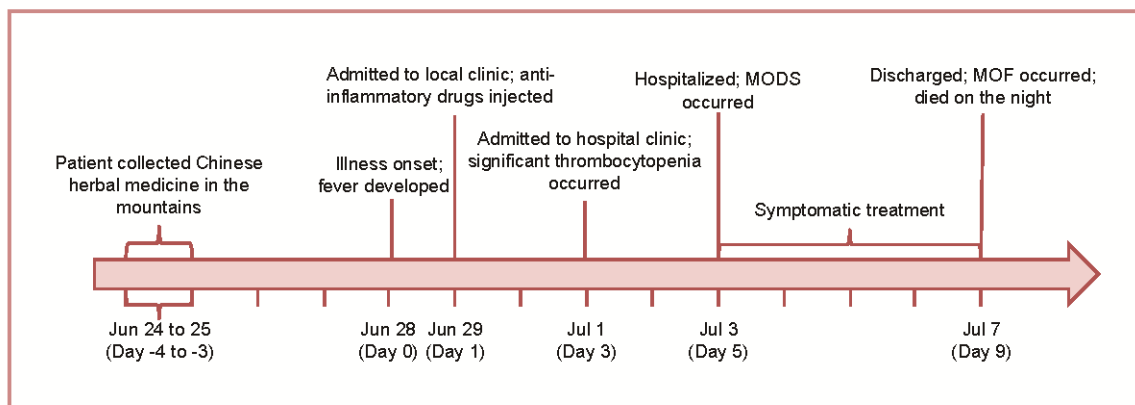
Testing items	Normal range	Days after illness onset					
		3	4	6	7	8	9
White blood cell count ( $\times 10^9$ per L)	3.50–9.50	1.65	1.16	1.70	3.95	10.94	11.70
Neutrophil count ( $\times 10^9$ per L)	1.80–6.30	1.24	0.78	0.43	1.71	6.70	8.99
Lymphocyte cell count ( $\times 10^9$ per L)	1.10–3.20	0.40	0.36	1.10	1.95	1.32	2.32
Monocyte count ( $\times 10^9$ per L)	0.10–0.60	0.01	0.01	0.06	0.09	2.78	0.32
Eosinophil count ( $\times 10^9$ per L)	0.02–0.52	0.00	0.00	0.00	0.00	0.00	0.00
Basophil count ( $\times 10^9$ per L)	0.00–0.06	0.00	0.01	0.11	0.20	0.14	0.07
Erythrocyte count ( $\times 10^{12}$ per L)	3.80–5.10	4.59	4.41	4.30	4.62	4.59	3.83
Hemoglobin (g/L)	115–150	132	126	122	132	137	109
Hematocrit (L/L)	0.35–0.45	0.41	0.39	0.37	0.41	0.41	0.34
Platelet count ( $\times 10^9$ per L)	125–350	70	44	20	18	58	60
Thrombocytocrit (%)	0.11–0.28	0.08	0.05			0.06	0.06
Hypersensitive C-reactive protein	0–8.0	0.4	2.1	3.2			
Creatine kinase (U/L)	40–200			318		780	1583
Creatine kinase isoenzymes (U/L)	0.0–25			34.0		114.0	92.0
Lactate dehydrogenase (U/L)	120–250			3158		1893	11032
$\alpha$ -hydroxybutyric dehydrogenase (U/L)	78.0–182.0			1762.0		1015	7980.0
Aspartase aminotransferase (U/L)	13.0–35.0		271.9	1262.2		754.3	3102.0
Alanine aminotransferase (U/L)	7.0–40.0		86.1	255.4		607.9	351.0
$\gamma$ -glutamyl transpeptidase (U/L)	7.0–45.0		36.1	178.9		876.1	734.0
Alkaline phosphatase (U/L)	50.0–135.0		58.5	114.7		455.6	576.0
Total protein (g/L)	65.0–85.0		67.9	51.0		77.6	50.0
Albumin (g/L)	40.0–55.0		35.4	24.8		24.7	22.0
Globulin (g/L)	20.0–40.0		32.5	26.2		52.9	28.0
A/G	1.2–2.4		1.09	0.95		0.47	0.8
Blood urea nitrogen (mmol/L)	2.6–7.5		4.95	3.51	3.82	6.56	13.8
Creatinine ( $\mu$ mol/L)	41–73		79.5	81.0	81.6	143.6	171
Uric acid ( $\mu$ mol/L)	155–357		222	247		371	590
High density lipoprotein cholesterol (mmol/L)	0.76–2.1			0.7		0.4	0.22
Low density lipoprotein cholesterol (mmol/L)	2.06–3.10			1.7		1.3	1.49
Ferritin ( $\mu$ g/L)	20–200			31371		1084	5
Glucose (mmol/L)	3.9–6.1			6.3	15.8		8.9
Calcium (mmol/L)	2.11–2.52			1.62	1.64		1.7
Sodium (mmol/L)	137–147			135	137.6		152
Chloride (mmol/L)	99–110			101	106		119
Potassium (mmol/L)	3.5–5.3			3.7	4.2		
CO <sub>2</sub> combining power (mmol/L)	23–31				17.2		15.0
D-Dimer ( $\mu$ g/L)	0–232		129			3706	>10000
Thrombin time (s)	11.0–17.8				28.2	39.6	42.0
Activated partial thromboplastin time (s)	20.0–40.0				64.7	105.5	77.9
Prothrombin time (s)	9.0–13.0				12.7	16.1	16.8
Fibrinogen	2.00–4.00				1.13	0.70	0.40

**Table S2.** Complications, treatment, and clinical outcome of the patient

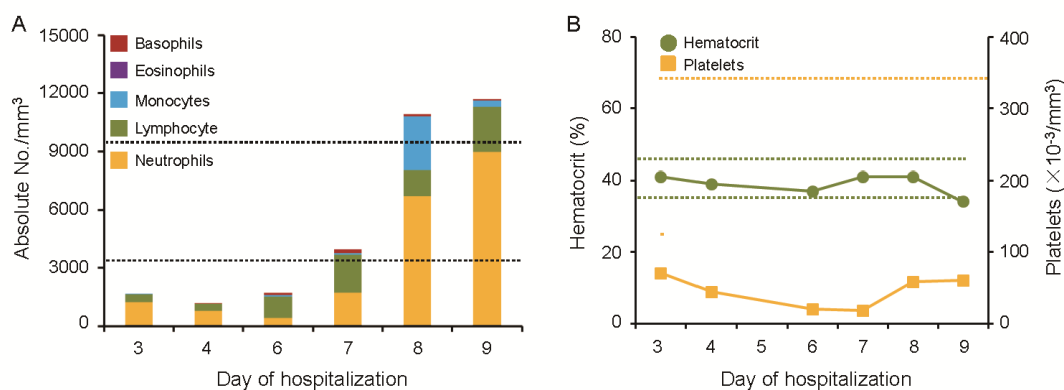
<b>Complications/Treatment</b>	
Fever	Yes
Temperature on admission (°C)	37.5
Highest temperature (°C)	39.4
<b>Complications</b>	
Multiple organ failure	Yes
Unconsciousness	Yes
Bronchitis; pneumonia	Yes
Hypoproteinemia	Yes
Bilateral pleural effusion	Yes
Urinary tract infection	Yes
Hepatomegaly	Yes
Bacterial co-infection	No (negative sputum and blood cultures)
Viral co-infection	No
Oxygen treatment	Yes (started on day 6)
<b>Hemostatic treatment</b>	
Day 5–9	Hemocoagulase bothrops atrox (1 U per day? given intravenously)
Day 6–9	Pantoprazole sodium (42.3 mg per day? given intravenously)
<b>Thrombocytopenia treatment</b>	
Day 5–7	Recombinant human interleukin-11 (3.0 mg given subcutaneously) Recombinant human thrombopoietin (15000 U given subcutaneously)
<b>Antibiotic treatment</b>	
Day 5–9	Cefotaxime sodium (two 3 g doses given intravenously)
Day 6–8	Vancomycin (500 mg given intravenously every 8 h), meropenem (1 g given intravenously every 8 h), moxifloxacin (one 0.4 g dose given intravenously)
Day 7–9	Moxifloxacin hydrochloride (0.4 g given intravenously)
Glucocorticoids	Dexamethasone (day 6–9, one 10 mg dose given intravenously)
Antiviral treatment	Ganciclovir sodium (day 5–9, 0.25 g given intravenously)
Plasma transfusion	400 mL (day 7–9)
Platelet transfusion	One dose (day 7)
Leukopenia treatment	Recombinant human granulocyte colony-stimulating factor injection (day 6, 300 µg given subcutaneously)
Intravenous immunoglobulin therapy	20 g (day 7–8)
<b>Multiple organ failure treatment</b>	
Day 5–9	Monoammonium glycyrrhizinate and cysteine and sodium chloride injection (two 100 mL dose given intravenously)
Day 6–9	Vitacamphorae (50 mg given intravenously)
Day 6–9	Nalmefene hydrochloride (0.2 mg given intravenously)
Day 6–9	Mannitol (50 g given intravenously)
Day 7–9	Creatine phosphate sodium (1.0 g given intravenously)
Day 8–9	Compound diisopropylamine dichloroacetate (80 mg given intravenously)
Day 8–9	Coenzyme complex (0.2 mg given intravenously)

**Table S3.** The primers used to amplify the complete *S*, *M*, and *L* segments of SFTSV in the study.

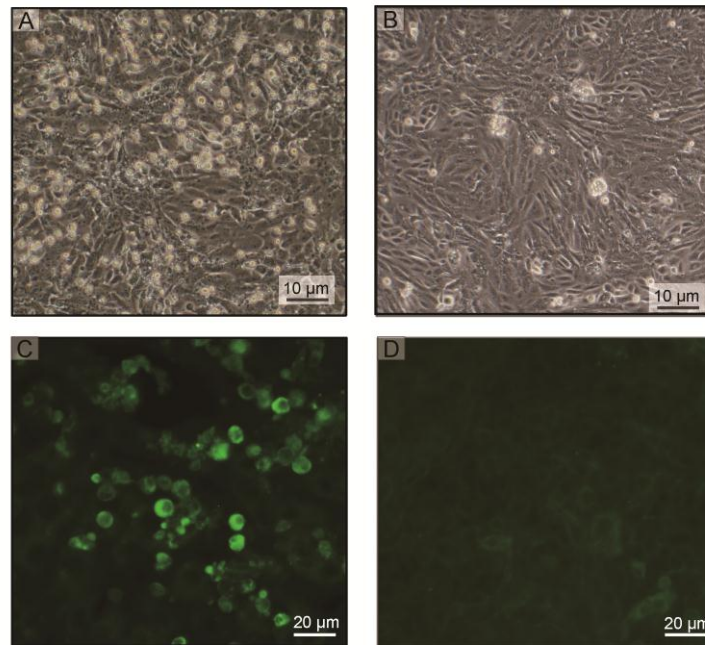
Genome segment	Interest fragment	Forward (Location) (5'-3')	Reverse (Location) (5'-3')
Small	1-1144	S- F1:ACACAAAGACCCCCT TCATTG (1-22)	S- R1:GGAGCCAGCAAGACAGAA GTTTAC (1144-1121)
	977-1744	S- F2:GTAAGCAGCAGCAGC AACCTCAG (977-999)	S- R2:ACACAAAGACCCCCAAAA AAGG (1744-1723)
Medium	1-1776	M- F1:ACACAGAGACGGCC AACAATGATG (1-24)	M- R1:ACATTCCTTCATATTTCCGC TCCC (1776-1753)
	1759- 3378	M- F2:GGAAATATGAAGGAA TGTGTCACA ACT (1759- 1785)	M- R2:ACACAAAGACCGGCCAAC ACTTCAATA (3378-3352)
Large	1-493	L- F1:ACACAGAGACGCC AGATGG (1-20)	L- R1:GATATCAACACGCCTTGAG ATTG (493-471)
	402-1449	L- F2:CCACTAGGAGCCATA ACATTG (402-422)	L- R2:GACTCATCTCCATACCTCG AATC (1449-1427)
	1427- 2501	L- F3:GATTTCGAGGTATGGA GATGAGTC (1427-1449)	L- R3:GGTTCTGCTTCCATTGTCTT C (2501-2481)
	2290- 3289	L- F4:AGAGGAGTCCACAG AACTGAATGCC (2290- 2314)	L- R4:GTGGAGGAGACTGGATGTA AAGTGC (3289-3265)
	3265- 3867	L- F5:GCACTTTACATCCAG TCTCCTCCAC (3265- 3289)	L- R5:GCAAATGCAGGGTTGTCCA TGAG (3867-3845)
	3353- 4517	L- F6:GAAAGCATCAGTGGG GTTGTG (3353-3373)	L- R6:CTATGCGGCTCCTGACAAT G (4517-4498)
	4339- 5352	L- F7:GCTGATGATGTCTTCT ATTTCTGCC (4339-4363)	L- R7:GCCACCCTGTTGTTGATCC C (5352-5333)
	5093- 6368	L- F8:GAGAGGGCACAGTC AGGCAC (5093-5112)	L- R8:ACACAAAGACCGCCCAGA TC (6368-6349)



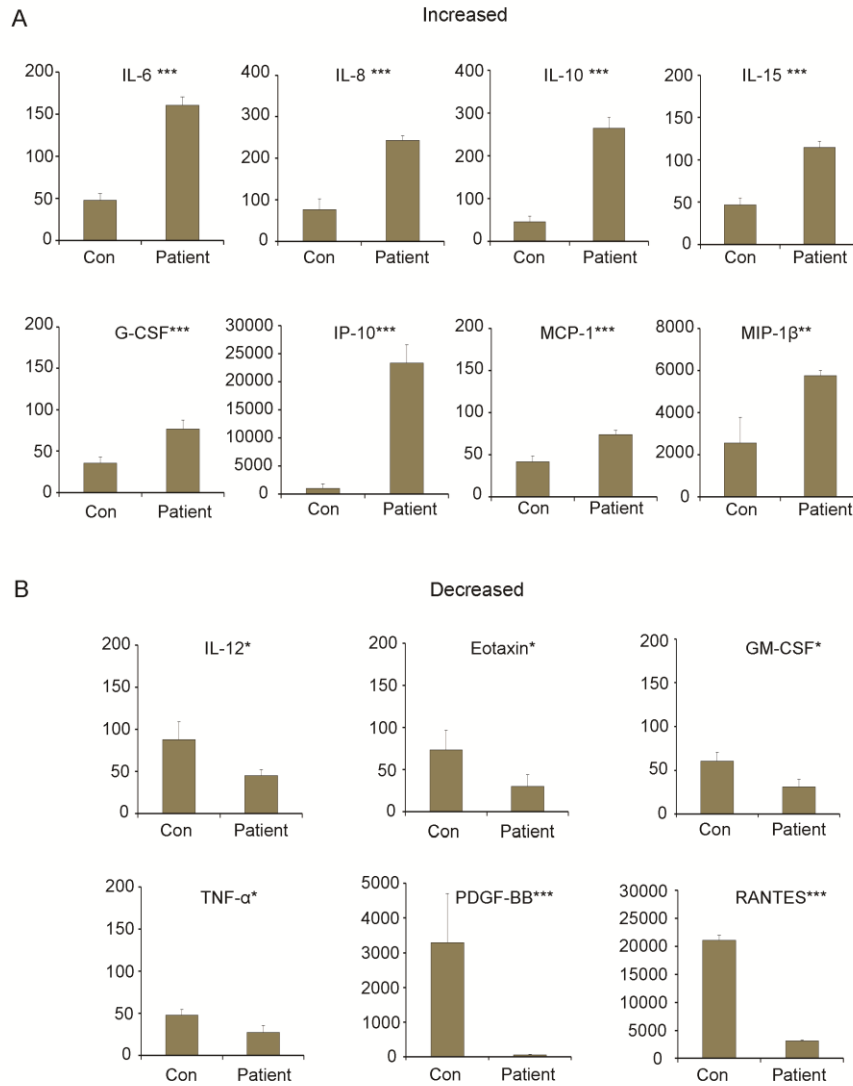
**Fig. S1.** Timeline of the clinical course of the patient and identification of causative pathogen. MODS, multiple organ dysfunction syndrome; MOF, multiple organ failure.



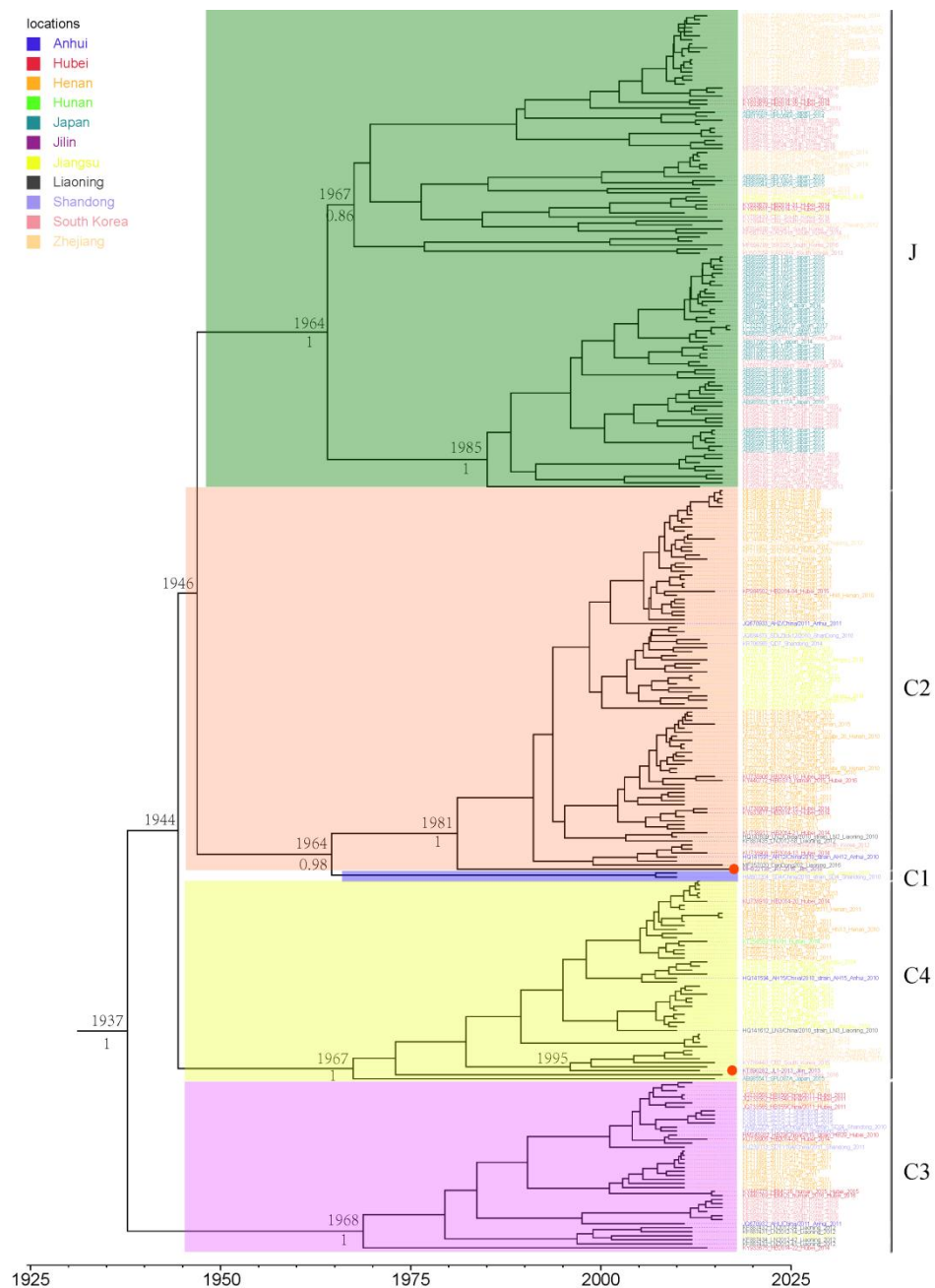
**Fig. S2.** Laboratory values for the patient during the hospitalization. Panel A shows the absolute values for the white-cell count and differential count for the patient during the hospitalization. Panel B shows hematocrit values (green) and platelet counts (yellow) during the patients' hospitalization. The dashed lines indicate normal values.



**Fig. S3.** Morphologic features and molecular detection of isolated SFTSV. Virus-induced CPE that are visualized on light microscopy in Vero cells infected with the patient's serum and urine samples after 4 days of inoculation (A), and Panel B shows the uninfected cell control. SFTSV was grown in Vero cells and used to detect IgM antibodies by immunofluorescence assay from serum sample obtained from the patient (C), and Panel D shows the uninfected cells. Negatively stained virions that purified from SFTSV-infected Vero cells show typical structural characteristics of bunyaviruses.



**Fig. S4.** Comparison of cytokine production in the patient and healthy individuals. The cytokine levels were compared between the patient and 7 healthy donors. Panel A showed the increased cytokine level and Panel B showed the decreased cytokine level. Statistical significance was defined as \* $P < 0.05$ , \*\* $P < 0.005$ , \*\*\* $P < 0.001$ .



**Fig. S5.** Time-scaled Bayesian phylogenetic tree based on the *S* segment of SFTSVs. Bayesian analysis was performed with the BEAST software packages using the HKY+I+G nucleotide substitution, relaxed lognormal molecular clock, and constant population size models. The independent analysis was performed using 50 million generations, with sampling every 10,000 generations. The time to the most recent common ancestor (TMRCA), date and location are depicted at the main nodes. Posterior probabilities are listed below the branches for supported nodes, and the values more than 0.7 are indicated. Sequences are identified by their GenBank accession number, strain name, origin, and isolated date. The Jilin strains including JA1-2018 from human and JL1-2013 from tick are marked with red dots. Genotypes (C1, C2, C3, C4 and J) are marked with different colors. SFTSV strain names are labeled on each branch and marked with different colors according to the location legend at the top-left corner. Blue, Anhui; Red, Hubei; Orange, Henan; Green, Hunan; Navy blue, Japan; Purple, Jilin; Yellow, Jiangsu; Dark, Liaoning; Light blue: Shandong; Light red, South Korea; Light orange, Zhejiang. The sequence data are shown in Supplementary Table S3.