

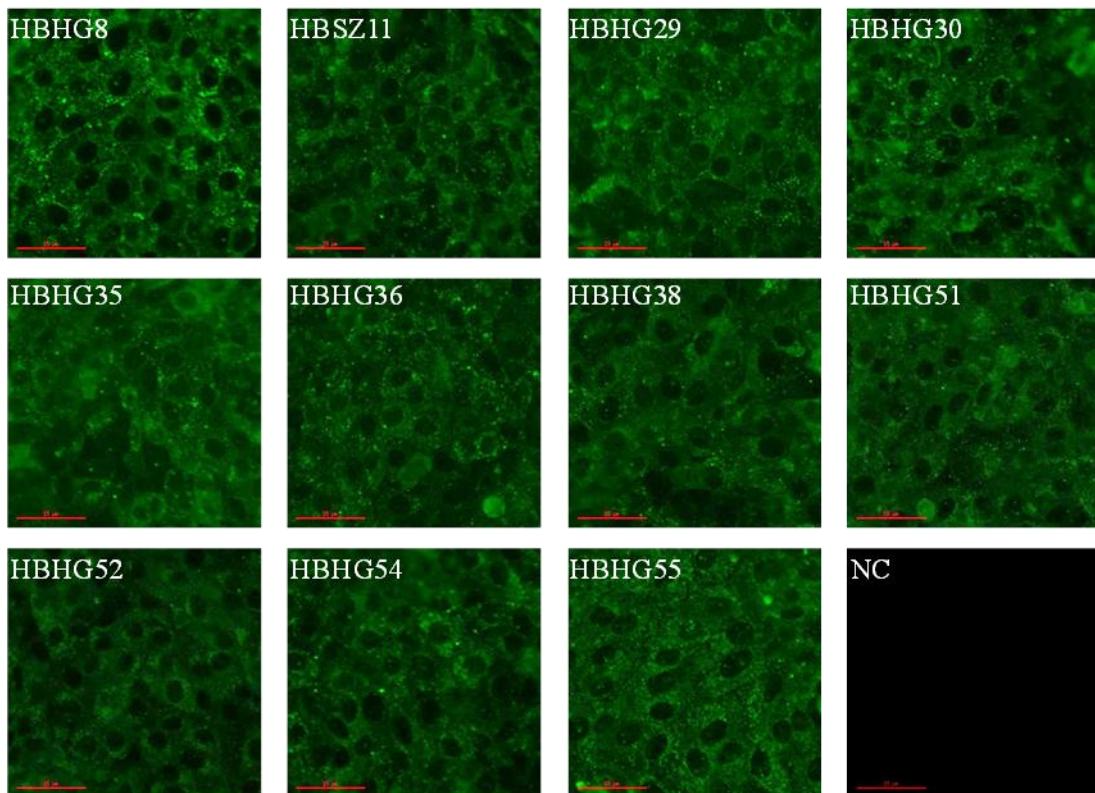
## Electronic Supplementary Material

### Novel SFTSV Phylogeny Reveals New Reassortment Events and Migration Routes

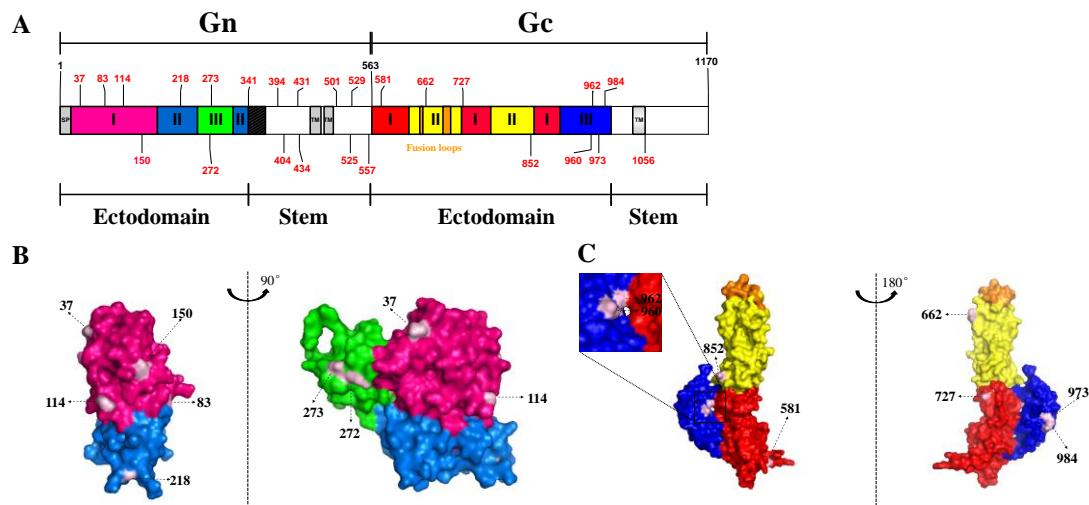
Xiaoli Wu<sup>1,2</sup> • Mingyue Li<sup>3</sup> • Yanfang Zhang<sup>1</sup> • Boyun Liang<sup>3</sup> • Junming Shi<sup>1,2</sup> • Yaohui Fang<sup>1,2</sup> • Zhengyuan Su<sup>1</sup> • Mengmeng Li<sup>3</sup> • Wenjing Zhang<sup>3</sup> • Ling Xu<sup>3</sup> • Jun Wang<sup>1</sup> • Qiaoli Wu<sup>1</sup> • Shuang Tang<sup>1</sup> • Hualin Wang<sup>1</sup> • Tao Zhang<sup>1</sup> • Cheng Peng<sup>3</sup> • Xin Zheng<sup>3</sup> • Fei Deng<sup>1</sup> • Shu Shen<sup>1</sup>

1. State Key Laboratory of Virology and National Virus Resource Center, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China
2. University of Chinese Academy of Sciences, Beijing 100049, China
3. Department of Infectious Diseases, Institute of Infection and Immunology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

Supporting information to DOI: 10.1007/s12250-020-00289-0



**Fig. S1** Immunofluorescence assay of the new SFTSV strains isolated by blind passages. NC, negative control (uninfected Vero cells). Bars: 15  $\mu$ m.



**Fig. S2** Hotspot sites on GP. (A) Hotspot sites in the Gn and Gc domains. Hotspot positions are labeled in red. (B, C) Hotspot sites mapped in the 3D structures of (B) Gn and (C) Gc, respectively.

**Table S1.** Summary of the personal information of the 54 suspected SFTS patients enrolled in this study.

	Patients (%)
<b>Total</b>	<b>54</b>
Age	34–77
Gender	Female 28(51.9) Male 26(48.1)
Outcome	Survived 47(87.0) Fatal 3(5.6) Others 4(7.4)
Location	Hubei Province Huanggang 26(48.1) Suizhou 10(18.5) Xiaogan 6(11.1) Jingmen 1(1.9) Wuhan 1(1.9) Henan Province Xinyang 10(18.5)
Occupation	Farmer 26(48.1) Others 28(51.9)

**Table S2** Clinical features of the 11 SFTS patients that SFTSV was isolated from their serum samples.

Blood count	Case1	Case2	Case3	Case4	Case5	Case6	Case7	Case8	Case9	Case10	Case11	Normal range
PLT ( $\times 10^9/L$ )	79↓	52↓	39↓	57↓	40↓	115	83↓	21↓	10↓	88↓	51↓	100-300
WBC ( $\times 10^9/L$ )	1.54↓	6.98	2.81↓	2.26↓	2.46↓	6.99	12.5↑	5.39	5.04	5.29	1↓	4-9
ALT (U/L)	43↑	300↑	57↑	141↑	195↑	273↑	185↑	21	719↑	123↑	82↑	5-40
AST (U/L)	61↑	406↑	85↑	478↑	792↑	407↑	1202↑	27	2652↑	185↑	457↑	8-40
LDH (U/L)	526↑	484↑	625↑	1612↑	3336↑	N/A	11098↑	482↑	2838↑	774↑	1681↑	135-215
CK (U/L)	343↑	66	477↑	1418↑	3699↑	N/A	2184↑	109	4097↑	130	20162↑	18-198
CKMB (U/L)	N/A	18↑	N/A	64↑	22.4↑	N/A	6.2	2.2	28↑	24↑	N/A	0-6

PLT, platelets:  $100-300 \times 10^9/L$ ; WBC, white blood cells:  $4-9 \times 10^9/L$ ; ALT, alanine aminotransferase: 5-40U/L; AST, aspartate transaminase: 8-40U/L; LDH, lactate dehydrogenase: 135-215U/L; CK, creatine kinase: 18-198U/L; CKMB, creatine kinase isoenzyme: 0-6U/L; Clinical parameters lower than the normal range are indicated by “↓”, higher than the normal range are indicated by “↑”.

**Table S3.** Accession numbers and geographical data of SFTSV strains used in this study.

**Table S4.** Visual comparison of the strain placement discrepancies in the different phylogenetic trees.

Inconsistent segment	Strain	Location	Date	Genotypes		
				L	M	S
<b>S</b>	JS2014-39	JS	2014	C2	C2	C4
	HB2014-22	HB	2014	C2	C2	C3
	HBSZ55	HB	2017	C2	C2	C3
	YSC3	HN	2011	C2	C2	C4
	HYS_L-HZM	HYS	Unknown	C3	C3	C2
	NB38	ZJ	2013	C4	C4	J2
	HBHG38	HB	2017	C4	C4	C3
	JS2014- <i>H.longicornis</i> -01	JS	2014	J3	J3	J2
	JS2011-69	JS	2011	J3	J3	J2
	JS2014-16	JS	2014	J3	J3	J2
	HB2014-31	HB	2014	J3	J3	J2
	HB2014-37	HB	2014	J3	J3	J2
	CB1	SK	2014	J3	J3	J2
<b>M</b>	2011YPQ12	HN	2012	C2	C4	C2
	LN2012-42	LN	2012	C3	C2	C3
	LN2012-34	LN	2012	C3	C2	C3
	LN2012-14	LN	2012	C3	C2	C3
	LN2012-41	LN	2012	C3	C2	C3
	2011YSC60	HN	2011	C4	C2	C4
<b>L</b>	2011YSC45	HN	2011	C2	C3	C3
	LN2012-58	LN	2012	C3	C2	C2
	2011YSC52	HN	2011	C4	C3	C3
	NB31	ZJ	2013	C6	J2	J2
	SPL100A	JA	2013	C6	J2	J2
	SPL097A	JA	2013	C6	J2	J2
	SPL057A	JA	2013	C6	J2	J2
	Rodent02	ZJ	2014	C6	J2	J2
	ZJ2013-06	ZJ	2013	C6	J2	J2
	NB24	ZJ	2013	C6	J2	J2
	NB39	ZJ	2014	C6	J2	J2
	Rodent01	ZJ	2014	C6	J2	J2
<b>L/M/S</b>	AHL	AH	2011	C5	C4	C3
	KADGH4	SK	2013	C6	J1	J2
	ZJ2014-01	ZJ	2014	C6	J1	J2
	NB32	ZJ	2013	C6	C4	J2

Unknown indicates that the isolation date of the strains were unclear.

HB-Hubei; JS-Jiangsu; HN-Henan; HYS-Huaiyangshan; ZJ-Zhejiang; LN-Liaoning; AH-Anhui; JA-Japan; SK-South Korea.

**Table S5.** SFTSV reassortment events, as determined by RDP package.

Reassorted segment	Event	Reassorted sequence (L/M/S)	Major parental sequence (L/M/S)	Minor parental sequence (L/M/S)	RDP RCS	Tools
S	1	HBSZ55_HB_2017 C2/C2/C3	HBHG8_HB_2017 C3/C3/C3	HBSZ52_HB_2017 C2/C2/C2	0.738	R,G,M,S,T
	2	JS2014-39_JS_2014 C2/C2/C4	JS2014-Hedgehog01_JS_2014 C4/C4/C4	JS2012-goat01_JS_2012 C2/C2/C2	0.743	R,G,M,S,T
	3	YSC3_HN_2011 C2/C2/C4	YXX2_HN_2011 C4/C4/C4	ZL13-32_HN_2013 C2/C2/C2	0.708	R,G,M,C,S,T
	4	HYS_L-HZM_HYS C3/C3/C2	HYS_L-WWG-HYS C2/C2/C2	2012YSH9_HN_2012 C3/C3/C3	0.731	R,G,B,M,C,S,T
	5	HBHG38_HB_2017 C4/C4/C3	JS2013-71_JS_2013 C4/C4/C4	HBHG8_HB_2017 C3/C3/C3	0.675	R,G,B,M,C,S,T
	6	NB38_ZJ_2013 C4/C4/J2	ZJ2013-07_ZJ_2013 C4/C4/C4	NB31_ZJ_2013 C6/J2/J2	0.655	G,B,M,C,S,T
M	7	2011YSC60_HN_2011 C4/C2/C4	HNXY_245_HN_2011 C2/C2/C2	HL/Larvae/G2 C4/C4/C4	0.724	R,G,B,M,C,S,T
	8	2011YPQ12_HN_2012 C2/C4/C2	2012YSH107_HN_2012 C2/C2/C2	HL/Larvae/G2 C4/C4/C4	0.746	R,G,B,M,C,S,T
	9	LN2012-41_LN_2012 C3/C2/C3	JS2011-062_JS_2012 C3/C3/C3	BX-2010_HN_2010 C2/C2/C2	0.521	R,G,B,M,C,S,T
		LN2012-42_LN_2012 C3/C2/C3				
		LN2012-34_LN_2012 C3/C2/C3				
		LN2012-14_LN_2012 C3/C2/C3				
L	10	2011YSC45_HN_2011 C2/C3/C3	HNXY_31_HN_2011 C2/C2/C2	2011YSC52_HN_2011 C4/C3/C3	0.59	R,G,B,M,S,T
	11	2011YSC52_HN_2011 C4/C3/C3	HNXY_212_HN_2011 C3/C3/C3	SFTS-38_HB_2017 C4/C4/C3	0.718	R,G,B,M,S,T
	12	LN2012-58_LN_2012 C3/C2/C2	JS2011-062_JS_2012 C3/C3/C3	LN2_LN_2010 C2/C2/C2	0.679	R,G,B,M,C,S,T
	13	ZJ2013-06_ZJ_2013 C6/J2/J2	SPL057A_JA_2013 C6/J2/J2	Unknown(NB32_ZJ_2013) C6/C4/J2	0.647	R,G,M,C,T
L/M/S	14	AHL_AH_2011 C5/C4/C3	Unknown(JS2013-44_JS_2013) C2/C2/C2	KASJH_SK_2013 C3/C3/C3	0.403	R,G,B,M,C,S
	15	NB32_ZJ_2013 C6/C4/J2	NB39_ZJ_2014 C6/J2/J2	NB38_ZJ_2013 C4/C4/J2	0.735	R,G,T

The strains listed in the table were named with format “Strains name\_Location\_Year”. HYS\_L-HZM\_HYS and HYS\_L-WWG-HYS the isolation years were unclear.

**Table S6.** Migration routes occurred between provinces in China, Japan, and South Korea.

	One-way migration	Two-way migration
C1	SD-HB SD-JS SK-SD	
C2	SD-HB SD-JS SK-SD JA-HN AH-SK JS-HB HB-LN	HB-HN HB-AH
C3	HB-JX HB-AH	HB-SK HB-HN HB-SD
C4	HB-JS HB-AH JS-LN ZJ-JA SK-JA	HB-HN HB-SK ZJ-SK
C5	SK-HB SK-JA	
J1		SK-JA
J2	SK-JA SK-HB	SK-JA
J3	SK-HB SK-ZJ SK-JA	

SD, Shandong Province; HB, Hubei Province; JS, Jiangsu Province; SK, South Korea; JA, Japan; HN, Henan Province; AH, Anhui Province; LN, Liaoning Province; ZJ, Zhejiang Province.

**Table S7.** Amino acid variations identified in the SFTSV RdRp, GP, NSs, and NP proteins.

Viral protein	Positions	Properties of amino acids <sup>&amp;</sup>			
		Acidic	Alkaline	Hydrophilic	Hydrophobic
<b>RdRp (n=44)</b>	2	D		N	
	11			N/T/S	
	128			S/T	
	140			T	A
	240		R/K		
	243		R	G	
	249	E	K		
	251	E	K		
	284			S/N	
	307		K/R		
	314	D		G/S	
	317			S	A
	343			S/T	
	352			Y	F
	381				V/I
	389	D		G	
	397	E/D			
	398		K/R		
	444		K/R		
	448	E/D			
	449			T	A
	453			Y	F

457	E/D	
479		I/V
566		Y
586	D/E	F
828		N/S
835		R/K
884		K/R
1008		T
1038		S/T
1039		K/R
1045		S/T
1061	E/D	V
1116		N/S/C
1199		I/V
1208		T/S
1266		T
1433		A
1447		I/V
1659		I/M
1684		R/K
1717		V/I
1825		K/R
1837		V/I
1906		K/R
<b>GP (n=50)</b>	11	I/V
	13	F/L

18		S/G	
21		S/T	
37	D	N/G/S	A
83		Y	F
114	E	G	
150		T/S	I
218	R	G/S	
272	E	K/R	
273		T	A
337			L/M
340		N/S	
341		Q/S	P
364			I/V
385		T/S	
394	H	G	
404		T	A
431	D	N	
434	D	G	
479			I/V
491			I/M/V
495			I/M
501		T/S	A
506			M/V
525	R	G	
529	R/H	Q	
532			V/I

537		A/V
557	T	I/V
562	S/G	
577	K/R	
581	E	G
587		V/I
662	S	P
727	T	A
801		V/I
852	S/T	A/V
926	K/R	
934		S/N
960	T/S	I
962	R	S
973	R	Q
984	H/K	Q
988	K/R	
1011		T/S
1053		L/M
1056	S	F
1058		F/L
1065		V/I
NSs (n=17)		
7	S/T	F
16		L/M
30	Y	F
38		V/I

143		G/S	
144	R	Q	
145	R/K		
172		T	I
201		Y	
207		S	P
235			L/M
238	D/E		
239	D/E		
245	H	Q	
249	H	Y	
264			I/M
276	D	N	
281	R/K		
NP (n=2)	52	K/R	
	95	K/R	

&Sites using amino acids of different properties are shaded.