1 2	Supplemental Materials for
3	A single domain antibody inhibits SFTSV and mitigates virus-induced
4	pathogenesis in vivo
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25 26 27 28 29 30	Conflict of interest statement: The authors have declared no conflict of interest. A patent application on SNB02 was submitted by Y-Clone Medical science Co. Ltd., under PCT/CN2019/097350.
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Supplemental Figure 1. Camel anti-sera inhibit SFTSV to infect Vero cells. 4th
 anti-sera represents camel anti-sera after the 4th immunization o sGn (green line), pre bleed is the sera from the camel before the immunization of sGn.

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Supplemental Figure 2. The construction and verification of VHH phage library. (A) RNA extraction. Lane M: Ladder DL5000, Lane 1, RNA extraction from camel PBMC after final sGn immunization. (B) 1^{st} PCR product, M: Marker DL2000 Lane 1~12: Primer 1 pairs (12 tubes); (C) 2^{nd} PCR product, M: Marker DL5000 depicted as (A), Lane 1~8: Primer pairs (1 ~ 8); (D) Phage library characterization. Lane M: Marker DL2000 depicted as (B), the left lanes are single bacterial clones randomly picked up from bacterial phage library.

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Supplemental Figure 3. Analysis of sGn-VHH antibody phage library. The
 alignment of VHH sequences. 50 clones were randomly selected from phage library
 for sequencing and their sequences were converted into VHH amino acid sequences
 and aligned.

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Supplemental Figure 4. Sequence analysis of positive VHHs. **(A)** 50 Evolutionary relationships of 142 VHHs was analyzed using the Neighbor-Joining 51 method. The optimal tree with the sum of branch length = 6.61901583 is shown. The 52 tree is drawn to scale, with branch lengths in the same units as those of the 53 evolutionary distances used to infer the phylogenetic tree. The evolutionary 54 distances were computed using the Poisson correction method and are in the 55 units of the number of amino acid substitutions per site. The analysis involved 56 142 amino acid sequences of VHH. All positions containing gaps and missing data 57 were eliminated. There were a total of 93 58

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positions in the final dataset. Evolutionary analyses were conducted in MEGA6. The reliability values indicated at the branch nodes were determined using 1,000 bootstrap replications. Bootstrap value was labeled at nodes. (**B**) Clonal lineage analysis. Each slice represents one clonal lineage; the size of the slice is proportional to the number of clones in the lineage. The total number of clones is shown in the center of the pie. Lineage numbering can be found in Data file S1. Clonal lineages were assigned based on the identical amino acids. (**C**) CDRH1, CDR H2 and CDRH3 length distribution.

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70 Supplemental Figure 5. The SPR affinity profiles of SNB binding to sGn protein.

(A) Summary of affinity constants of SNBs binding with sGn as determined by SPR.
Ka, associate constant; kd, dissociate constant; KD, affinity constant; Rmax, the
maximum response unit. Kinetic binding curve of SNBs with sGn. Different color
curves represent different concentrations, sGn protein was bound by SNB02 (B),
SNB07 (C) and SNB112 (D).

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77 Supplemental Figure 6. Characterization of SNB02 binding with various subtypes

of sGn. (A) ELISA analysis of the reactivity of SNB02 with various subtypes of sGn.
sGn-A-E indicate the sGn protein from subtypes A-E, respectively. BSA binding with
SNB02 was taken as antibody negative control (Negative). Data represent mean ± SEM.
(B) Western-blot analysis of the reactivity of 0.5 µg/ml SNB02 with various subtypes
of 2 µg sGN. Lane M: Maker, lane1: sGn-A, lane 2: sGn-B, lane 3: sGn-C, lane 4: sGn-D, lane 5: sGn-E, lane 6: BSA protein taken as negative control.

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Supplemental Figure 7. Characterization of humanized SNB02. (A) ELISA analysis of the reactivity of SNB02 and humanized SNB02 (Hu-SNB02) with sGn. PBS binding with sGn was served as Blank control (Blank). (B) Neutralization activity of SNB against live SFTSV infection of Vero E6 cell. SNB-ctl, One VHH-huFc antibody without binding with sGn, was taken as antibody negative control. All experiments were repeated twice.

- Supplemental Table 1. Summary of sGn-VHH library panning against sGn protein.
- Supplemental Table 2. The representative bacterial supernatant binding with sGn
 - as determined by ELISA.
- Supplemental Table 3. Summary of CDR sequences of 142 positive VHH clones



Supplemental Figure 1. Camel anti-sera inhibit SFTSV to infect Vero cells. 4th anti-

sera represents camel anti-sera after the 4th immunization o sGn (green line), pre-bleed

is the sera from the camel before the immunization of sGn.



Supplemental Figure 2. The construction and verification of VHH phage library.
(A) RNA extraction. Lane M: Ladder DL5000, Lane 1, RNA extraction from camel
PBMC after final sGn immunization. (B) 1st PCR product, M: Marker DL2000 Lane
1~12: Primer 1 pairs (12 tubes); (C) 2nd PCR product, M: Marker DL5000 depicted as
(A), Lane 1~8: Primer pairs (1 ~ 8); (D) Phage library characterization. Lane M: Marker
DL2000 depicted as (B), the left lanes are single bacterial clones randomly picked up
from bacterial phage library.

Clone ID	CDR1	CDR2		CDR3	
'T1803131073.L225.seqH_A03.ab1' (1)	VK <mark>INESOGIS V</mark> ITOS <mark>LRISCAG</mark> YTLSNRI	SWERCAPCERBEVANICTIEGNE	YADSVK <mark>AR</mark> FTISJONAKNT <mark>I</mark> Y <mark>U</mark> ,MNSI.KPE <mark>D</mark> TAM <mark>TYC</mark>	ARSVAFYSCDSDYKYDI <mark>WQQS</mark>	WTVSSGTNEVCK
'T1803131074.L226.seqH_A04.ab1' (1)	VQLNESOGSVQAGES <mark>LRIS</mark> CAAPT-DTHSKDY	AWFREAPSKERBGVANIDIGDGRT	YYAASVITE <mark>R</mark> ETISCENAKBTVY <mark>L</mark> IMNSL/PR <mark>LIAV</mark> YYC	ATSKISWYQLLARFYTUWGQG	<u>IVTVSSG</u> TNEVCK
'T1803131079.L231.seqH_A09.ab1' (1)	VO <mark>LNESGOGSVOAGESLELSCA</mark> IERYRFRGIO	GWERCAPCKERTWVASTYTNGGTT	YADSVKG <mark>R</mark> FTISQDCXQNTVY <mark>L</mark> QMNSI.KPB <mark>D</mark> TAM <mark>Y</mark> YC	ADQSSYANRRCAISSE <mark>Y</mark> S <mark>YMGQG</mark>	<mark>(VTVSLG</mark> TNEVCK
'T1803131099.L251.seqH_C05.ab1' (1)	VQ <mark>INESOGEPVQAGISIRISCAAS</mark> T-YTYISNO	AWFRCAPCKERBGVA TYROOGT	YADSVKG <mark>R</mark> ETISCONAKNTVY <mark>L</mark> OMNSLKPE <mark>DIAMYYC</mark>	MSRRCSTATINTPRGNSDMBQC	<u>IVTVSSG</u> TNEVCK
'T1803131077.L229.seqH_A07.ab1' (1)	VK <mark>LNESGGSSVUNGESL</mark> ILS <mark>CVANGET</mark> SRGNG	AMERCAPCKERBGVAAFYSGRGNS	YDDSVKG <mark>R</mark> E I LSØGHKEDTVY <mark>L</mark> (MNSLKPE <mark>DSG</mark> A <mark>Y</mark> YC	MREGGCFPGLISAYIN MOOS	WIVPSAHHPEDP
'T1803131095.L247.seqH_E09.ab1' (1)	VQ <mark>EVESOGGVVQAGGSENES</mark> CVAGGSEATTNG	IMPRCAPSKERBGLAGI YITGAGYA	YVDSVKG <mark>r</mark> etisodkdKrTvy <mark>l</mark> lmnrikpe <mark>d</mark> tam <mark>y</mark> yc	AGVAGLDTGGGSSCHPRIAE NTNGQG	WTVSSAHHPEDP
'T1803131119.L271.seqH_E01.ab1' (1)	VK <mark>INESOOSSVQAGESI</mark> RIS <mark>CAABE</mark> YYYNSNDSND	IS <mark>WERCAPCKERBGVAMIYI</mark> DRGST	YADSVKG <mark>R</mark> FTISCONAKNLVY <mark>L</mark> CMCSIKPEDIAM <mark>YYC</mark>	NAVTGRYGG-TWYDENGVDYNEKG	WITSSAHHPEDP
'T1803131084.L236.seqH_B02.ab1' (1)	VQLEESGGSSVQAGESLELSCEVEKSTDIHTO	IS <mark>WEBCADGKER</mark> BGVAMISAQGVVP	EYRDSVKG <mark>R</mark> FTI <mark>T</mark> FENAKKMVY <mark>L</mark> MNSL/,PR <mark>OS</mark> AM <mark>Y</mark> YC	MHQRPL-CFSTWDRGDE <mark>M</mark> KS <mark>WGQG</mark>	WWSS <mark>A</mark> HHPEDP
'T1803131106.L258.seqH_C12.ab1' (1)	VQ <mark>LKESGOGSVQAGES<mark>LRLS</mark>CIALNYT</mark> YRTKY	GWF <mark>BCAR</mark> CKEREAVAASSTKGTFT	N <mark>g</mark> osver <mark>r</mark> ftisiovakntiy <mark>l</mark> (msnlepe <mark>d</mark> tam <mark>y</mark> yc	AKPAFPTSLRETD <mark>A</mark> GWGQG	WWSS
'T1803131081.L233.seqH_A11.ab1' (1)	VQ <mark>LNESQGGSVQAGESL</mark> RLS <mark>CAAMAYT</mark> NGWHO	IMF <mark>RCAPGKER</mark> BGVAHINTGOGSTY-YAE	YADSVKG <mark>R</mark> FTISCONDKRTVY <mark>L</mark> (MNSIKPE <mark>D</mark> TAM <mark>YYC</mark>	MGWYGGSCQRIYE <mark>WWWGQG</mark>	WTVSSEPKIPQP
'T1803131098.L250.seqH_C04.ab1' (1)	IQIN <mark>ESG</mark> GES <mark>V</mark> UNGES <mark>L</mark> RLS <mark>CAASA</mark> YTC	GWERCAPCKERRT	Y <mark>g</mark> osvkg <mark>r</mark> etisodiaknTv <mark>hl</mark> omnslker <mark>d</mark> tam <mark>yyc</mark>	MDRRYAGSWCYSRPAYE <mark>Y</mark> K <mark>YWGQG</mark>	WIVSSEPKIPQP
'T1803131089.L241.seqH_B07.ab1' (1)	VR <mark>LNESGOGLWUNGESLRLSCAA</mark> BGYTDSRYC	GNERCAPCKERBGVA TDN-NGRT	YADSVKG <mark>R</mark> ET <mark>V</mark> SQENTKNTVY <mark>L</mark> (MNSLKPE <mark>D</mark> TAM <mark>Y</mark> YC	ADRGEYVSCPYYR-LSA RTWOOG	WIVSSEPKIPQP
'T1803131082.L234.seqH_A12.ab1' (1)	VQ <mark>LVESQGESVQTDESLELS</mark> QAAEGYTYSSNE	IS <mark>MERCAPCER</mark> EVVACIATOSHST	YADSVKG <mark>R</mark> FTISCONAKNT <mark>TYL</mark> (MNSIKPE <mark>C</mark> TAM <mark>YYC</mark>	AENTYYVCTNPVDAAD <mark>E</mark> R <mark>INGOG</mark>	WTVSSEPKIPQP
'T1803131091.L243.seqH_B09.ab1' (1)	VQ <mark>LNESGOGS</mark> VUNGG <mark>TL</mark> SLS <mark>CAA</mark> GYLYRSSC	N <mark>WEROVINGKER</mark> BGVA VI ALI GVGRT	syadsvikg <mark>r</mark> eti sodovknTvt <mark>l</mark> omnslikpe <mark>d</mark> tam <mark>yy</mark> c	ADPTPCWAGNRYD <mark>y</mark> K <mark>ywgog</mark>	WTVSSEPKIPQP
'T1803131103.L255.seqH_C09.ab1' (1)	VQ <mark>LNESGOGS</mark> VQPGES <mark>L</mark> RLS <mark>O</mark> GTOGY <mark>S</mark> YSASD	NWERCAPCEGR EWVACI SOGRG LT	YADSVKG <mark>R</mark> ETISCOENKNT <mark>EYL</mark> CMINIKPE <mark>L</mark> TAM <mark>Y</mark> YC	AQPLAAMSEWCAQA'TGDVGR <mark>A</mark> G <mark>YWDQC</mark>	WTVSSEPKIPQP
'T1803131087.L239.seqH_B05.ab1' (1)	VQ <mark>1QESOGGSV</mark> QAGGS <mark>L</mark> ALS <mark>CAADGY</mark> VQATYH	IS <mark>WERCAPCKER</mark> BGWACTSICETS <mark>T</mark>	CASSVRIC <mark>P</mark> ETI SQIMAKNTVY <mark>U</mark> ,MNSU, PR <mark>UDGAY</mark> YC	INPRGRANOGSGSD A MCCCC	WIVSSEPKIPQP
'T1803131096.L248.seqH_C02.ab1' (1)	VQ <mark>LKESOGSS</mark> VQAGGS <mark>LRLS</mark> CAADGD <mark>S</mark> YSRRO	IS <mark>MERCAP<mark>G</mark>KERBGVAMIC<mark>II</mark>GDGRT</mark>	YAESVKG <mark>R</mark> ETI SQUNAQUNAKNI VY <mark>U</mark> , MNSI KPR <mark>U</mark> TUM <mark>YYC</mark>	GRFYVFGAGNYYLQGRA <mark>Y</mark> N <mark>yngog</mark>	WIVSSEPKIPQP
'T1803131115.L267.seqH_D09.ab1' (1)	VO <mark>LKESOOGS<mark>V</mark>ONGES<mark>LELS</mark>CAARGL<mark>S</mark>FSNVO</mark>	IS <mark>WERCAPCKER</mark> BGWAFYGGOGYT	TYAGSVKG <mark>R</mark> ETISIONAENT <mark>TY</mark> U(MASIKPE <mark>U</mark> UM <mark>YRC</mark>	AGGCIWVGNRPLDPGO <mark>T</mark> K MODG	WTVSPEPKIPQP
'T1803131102.L254.seqH_C08.ab1' (1)	VQ <mark>LKESGGGSVQMqQS<mark>L</mark>KLS<mark>CAASA</mark>YTYRGNN</mark>	AWERCAPCKERBGVALLTSTSTRQ	Y <mark>s</mark> esvkg <mark>r</mark> etisfenaknaly <mark>l</mark> imnsikpe <mark>d</mark> ta <mark>vyy</mark> c	AGTTR-LGSLLAPS <mark>Y</mark> L <mark>MCOG</mark>	WTVSSEPKIPQP
'T1803131114.L266.seqH_E10.ab1' (1)	VH <mark>LWESGOGSVQAGES<mark>L</mark>KLS<mark>C</mark>VA.9GL<mark>L</mark>YSSNC</mark>	WERCAPCKERBISVANIV GNGAT	YADSVKG <mark>R</mark> ETISLENAKNTVY <mark>L</mark> (MNSLKPE <mark>L/IAM</mark> YYC	ANARCROGEVLARAL <mark>I</mark> NNCEOG	WIVSSEPKIPQP
'T1803131105.L257.seqH_C11.ab1' (1)	VR <mark>LVESOGSVQAGOS</mark> LTLS <mark>CTA-EQU</mark> YRSAY	INFROSPORCE BOVA TYIPODIA	YVDFVKG <mark>R</mark> FTISODKAKKTVY <mark>LONSSIKPED</mark> TAM <mark>Y</mark> YC	WWNAMTGLDRTPDSPTNDQG	WTVSSEPKIPQP
'T1803131085.L237.seqH_B03.ab1' (1)	VK <mark>LVESOGSVOPOSSLALS</mark> CRVFGDLFTTYC	AWERCARGKEREGWASFTERWARTSSPKT	YDASVKG <mark>RVTISEDKAKNTIDL</mark> AMINIAP <mark>DOTAM</mark> YYC	AAQFGSRRSCGEWEPA	WTWSSEPKIPQP
'T1803131083.L235.seqH_B01.ab1' (1)	VK <mark>IVESOGOSVONGIS<mark>LAVS</mark>CIVSGPSSNO</mark>	ISWE <mark>BOT POKER</mark> BIGVAVI Y <mark>T</mark> GDGST	YANSVKG <mark>R</mark> FTISCENAKNTVY <mark>L</mark> AMNSIKPEDEAM <mark>YIC</mark>	AAPTTGIYDCYYGSWRRARMDINGKG	WTVSSGTNEVCK
'T1803131108.L260.seqH_D02.ab1' (1)	VR <mark>INESOGOSVONGOS<mark>L</mark>RIS<mark>CA</mark>ADGY<mark>T</mark>YSTNO</mark>	CMERCAPCKERBGVAMIYTCDGST	YACSVKG <mark>R</mark> FTISCONAKNTVY <mark>L</mark> OMNSIKPE <mark>C</mark> TAM <mark>YYC</mark>	GGWAVL9GTSMS ITTGA	SPRSF SP CNPRYPNX
'T1803131075.L227.seqH_A05.ab1' (1)	VQ <mark>LKESOGGSVQAGG<mark>L</mark>KLS<mark>CAADGYT</mark>NNRRØ</mark>	INTERCAPCKEREGVANTYIDDFIGVVGRT	YADFVNG <mark>R</mark> FTISCENAKNTVY <mark>L</mark> OMNSIKPE <mark>D</mark> TAM <mark>YYC</mark>	ARGEVMAGSQALRPYON NOCOC	WTVSSGTNOGCK
'T1803131086.L238.seqH_B04.ab1' (1)	VK <mark>INESGOSVONGSLEIS</mark> CKAGOIYSVNO	AMERCAPCKERREVACLETOGNT	YADSVKG <mark>R</mark> FTIS <u>O</u> DNAKNTVA <mark>LA</mark> MNSIKPE <mark>D</mark> TAM <mark>YE</mark> C	AADPSVCSDYAIGENDEN I MOOG	<mark>(VTVSSG</mark> <mark>TN</mark> QG <mark>CK</mark>
'T1803131092.L244.seqH_B10.ab1' (1)	VH <mark>INESOGGSVQAGGSL</mark> TIS <mark>CVASG</mark> YSVNY	NWERCAP <mark>GTDRBOVALTYRD</mark> GGTT	YADSVKG <mark>R</mark> FTISONNAKRTVY <mark>L</mark> ONNSIKPE <mark>D</mark> TAM <mark>YYC</mark>	AAYIAPSLFSSFMWFFADE <mark>YN WOOG</mark>	<mark>WTVSSG</mark> TNGG <mark>CK</mark>
'T1803131078.L230.seqH_A08.ab1' (1)	VR <mark>LNESOGGSVOAGGS<mark>L</mark>RLS<mark>CAA</mark> GY<mark>T</mark>YSSYS</mark>	IN <mark>RECTIPENER EGVA</mark> TE <mark>S</mark> SGST	SYADSVKG <mark>R</mark> FTISKDNATNT <mark>I</mark> Y <mark>L</mark> (MNSIKPR <mark>D</mark> TAM <mark>Y</mark> YC	AAPLVHSDYFRRARPTDLTWGQG	WTVSSGTNGGCK
'T1803131076.L228.seqH_A06.ab1' (1)	IQINESOCIATIONSIS <mark>INIS</mark> CVARGINGGPCT	NTATE OVER STATES AND STATES	YEDSVKO <mark>R</mark> FITSOCNAKNTVN <mark>L</mark> (MTSIKLE <mark>D/AT</mark> YYC	MECINVPLHGGLAD	IVTVSSGTNEVCK
'T1803131097.L249.seqH_C03.ab1' (1)	IQINESOGSVQAGIS <mark>I.RIS</mark> CTATGL <mark>E</mark> SNRCG	AWER CANCE FWYS ST SED S	EY IDSVKG <mark>RETISED</mark> KPVDTVY <mark>L(MNSL) PADTAWYLC</mark>	KTDSAYGGNPFTCN WEOG	IVTVSSGTNQGCK
'T1803131090.L242.seqH_B08.ab1' (1)	VR <mark>LVESÖGGSVONGIS<mark>L</mark>RIS<mark>C</mark>LATAD<mark>T</mark>YNNNÖ</mark>	OMERCAPCKERBISVA CLADGST	HTDAVKGRESISLINANNTVYLOMNSIKPECTAMYKC	ALKRGLQGGLTAQGRRMTAMQALGVMAAG	<mark>JVTVSSG</mark> <mark>TN</mark> EV <mark>CK</mark>
'T1803131100.L252.seqH_C06.ab1' (1) -	VH <mark>INESOGGSVUNGGS<mark>LEIS</mark>CEATELGGENVNO</mark>	OMERCAPCKERREVAURYFADGSA	SADSVKG <mark>RETISEDNVANTEY</mark> LLANSLRPE <mark>DTAMYRO</mark>	YCPWGVDDRRWNVADE	WTVSEGTNOGCK
'T1803131088.L240.seqH_B06.ab1' (1)	VK <mark>IVESOGSVQAGIS</mark> INIS <mark>CAAGG</mark> -YTFSDSD	CWERCSPGKERPAVACTYSGPRGSSA	YAASVKG <mark>R</mark> ETISODNÄENT <mark>IHL</mark> EMINIKPE <mark>D</mark> TAM <mark>YRC</mark>	ASKAQTSTDRRKTRAITSSTVCVPSD	WTVSSGTNEVCK
'T1803131118.L270.seqH_D12.ab1' (1)	IQINESCOSS <mark>VOAGESLELS</mark> CAVEGYTYSRYC	IMEROTPOGOTRDIAMEDOSTL	YADSVKG <mark>R</mark> FTISCENCKNTET <mark>L</mark> IMINI <i>K</i> PEDTAMYYC	ASRGRLCPKNFLGNFAG	WIVSSDGSSSPPPPQVTVSSGTNEVCK
'T1803131093.L245.seqH_B11.ab1' (1)	VR <mark>INESOGSSVONGESLTISC</mark> VHECDYFSDRF	WEROSOS-KERBOVANDRDGIT	YADSVKG <mark>RETIWEDTAKNILYLOMNSINPEDAAMYYC</mark>	MGLPYNFGLGTVASONK MGOG	WTVSEGTNEVCK
'T1803131120.L272.seqH_E02.ab1' (1)	VOLOESOOSS <mark>V</mark> KAGIS <mark>LELSCELSO</mark> NIRSRYU	INFROGTG-KEREAVA WENDCSTN	YTESVKC <mark>RETTREDCAKNILTLANTELKPECTAMTYC</mark>	WGCRFRRLKLWQQPDE	WTVSSGTNOGCK
'T1803131094.L246.seqH_B12.ab1' (1)	VHINESOCIAVOPORSILRISCEACO-FTISNYD	TWAR CAPERAL (WVSTIRSGVT AVT	YADSVOGRETISEDNAKIITLYLODNSLKVEDTAMYYC	LGLSIGGSROOG	WTVSSGTNEVCK
'T1803131112.L264.seqH_D06.ab1' (1)	VOLKESGP SLVKPSOTLSLTCTV CG-SITTSGYG	ISWIRDTPGKGLFAMCALAWTUS	YNESLKS <mark>ETTLEADTSKNLESLALSSVTPEDTAVY</mark> O	RHGGGSWYQMRYYYGMD	WTISSEPKIPOP
'T1803131113.L265.segH_D07.ab1' (1)	VOLEESGPELVKPSOTLSLACTVESG-SITTSDYG	NWIRDPRENGL	YSPSLKSKTSTSFDTSKNOFS <mark>LHESSVTPFDTAVTYC</mark>	RSWGSNOP MEDOG	WTVSSGTNEVCK
'T1803131116.L268.segH_D10.ab1' (1)	VOLCESCERLARPOTISIACTVE EPIATSSIYE	ITWIR PPG/GL	YMEHLASESSISMUTSKNQESLO SSUTPENSIONTO	RLRRTYVAG IVDPDS JAPR CON	WTVSSGTNGGCK
'T1803131117.L269.segH D11.ab1' (1)	VOLVESCIES VOAGETLELS CITES FTLDGSD	WRLTPONECTR	YSTSVAR FTISPITIAN KEYLLMKPARPENTERYC	TNEDGSDESGTRRIVPDNCG	WAVSSGTNEVCK
'T1803131101.L253.segH C07.ab1' (1)	VRIVESCOSS VOVOISI RISCIASI - SFFONHI	CMERCSPOREHBOVASTESCOGT	YSTSAKCETINSE-ACATINLCANSIKPEDATTEC	AFRYSTEGCSPVTWOS	WLWSSGTNEVCK
'T1803131107.L259.seaH D01.ab1' (1)	VKINESOGSVOAGSLEISCVARG-YTYRSKO	WEROGSCHER	NLESVKGETISCENAKITVYLOMISLKPEDTATYKC	WGPACSADPLAINING OF	WSVSSGTNEVCK
'T1803131110.L262.segH_D04.ab1' (1)	VOLFESORS VARISTRUS AV SCHRYSA YO	NWERCARS-KGRBOVANDEOCSTS	YVDSVKGRETTISCENAKNTEYLOMNSLKPEDTAMYYC	GARCPYGGSWLPNDYNYAGOG	TV <mark>SVSSC</mark> TNEVCK
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Supplemental Figure 3. Analysis of sGn-VHH antibody phage library. The alignment of VHH sequences. 50 clones were randomly selected from phage library for sequencing and their sequences were converted into VHH amino acid sequences and aligned.



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Supplemental Figure 4. Sequence analysis of positive VHHs. (A) Evolutionary relationships of 142 VHHs was analyzed using the Neighbor-Joining method. The optimal tree with the sum of branch length = 6.61901583 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances

124 used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid 125 substitutions per site. The analysis involved 142 amino acid sequences of VHH. All 126 positions containing gaps and missing data were eliminated. There were a total of 93 127 positions in the final dataset. Evolutionary analyses were conducted in MEGA6. The 128 reliability values indicated at the branch nodes were determined using 1,000 bootstrap 129 replications. Bootstrap value was labeled at nodes. (B) Clonal lineage analysis. Each 130 131 slice represents one clonal lineage; the size of the slice is proportional to the number of clones in the lineage. The total number of clones is shown in the center of the pie. 132 Lineage numbering can be found in Data file S1. Clonal lineages were assigned based 133 on the identical amino acids. (C) CDRH1, CDR H2 and CDRH3 length distribution. 134



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Supplemental Figure 5. The SPR affinity profiles of SNB binding to sGn protein.
(A) Summary of affinity constants of SNBs binding with sGn as determined by SPR.
Ka, associate constant; kd, dissociate constant; KD, affinity constant; Rmax, the
maximum response unit. Kinetic binding curve of SNBs with sGn. Different color
curves represent different concentrations, sGn protein was bound by SNB02 (B),
SNB07 (C) and SNB112 (D).



Supplemental Figure 6. Characterization of SNB02 binding with various subtypes
of sGn. (A) ELISA analysis of the reactivity of SNB02 with various subtypes of sGn.
sGn-A-E indicate the sGn protein from subtypes A-E, respectively. BSA binding with
SNB02 was taken as antibody negative control (Negative). Data represent mean ± SEM.
(B) Western-blot analysis of the reactivity of 0.5 µg/ml SNB02 with various subtypes
of 2 µg sGN. Lane M: Maker, lane1: sGn-A, lane 2: sGn-B, lane 3: sGn-C, lane 4: sGnD, lane 5: sGn-E, lane 6: BSA protein taken as negative control.

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Supplemental Figure 7. Characterization of humanized SNB02. (A) ELISA analysis of the reactivity of SNB02 and humanized SNB02 (Hu-SNB02) with sGn. PBS binding with sGn was served as Blank control (Blank). (B) Neutralization activity of SNB against live SFTSV infection of Vero E6 cell. SNB-ctl, One VHH-huFc antibody without binding with sGn, was taken as antibody negative control. All experiments were repeated twice.

160	Supplemental Table 1. S	ummary of sGn-VHH librar	y panning against sGn protein.
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Project	Ag	Round	Input(pfu)	Output (pfu)	Enrichment	Positive rate(%)
	sGn protein 20ug/ml; 150ul/well;	1st	1x10^12	1.38x10^6	1	/
sGn-VHH library	sGn protein 100ug/ml; 150ul/well;	2nd	2x10^12 (1st amplified phage 3.2x10^13)	2.7x10^7	3.75	24.5%
	sGn protein 50ug/ml; 150ul/well;	3rd	1.2x10^12 (2nd amplified phage 1.21x10^13)	1.44x10^8	8.89	67%

163 Supplemental Table 2. The representative bacterial supernatant binding with sGn

164 as determined by ELISA.

	$\langle \rangle$	1	2	3	4	5	6	7	8	9	10	11	12
	А	0.300	0.231	0.161	0.126	0.126	0.100	0.360	0.203	0.068	3.027	3.034	0.115
	В	0.384	0.163	0.182	0.175	0.109	0.335	0.804	0.094	0.153	0.188	0.219	0.076
	С	0.303	0.279	0.255	0.217	1.619	0.370	0.057	0.259	0.078	0.105	0.179	0.062
sGn	D	0.091	0.315	0.055	0.102	0.094	0.914	0.157	0.094	0.142	0.065	0.078	0.120
	Е	0.422	0.085	3.383	0.545	0.060	0.268	0.096	0.191	1.876	0.090	0.258	0.106
	F	0.212	2.312	0.478	3.607	0.180	0.574	0.168	0.159	0.072	0.066	0.186	0.096
	G	0.174	0.073	0.129	3.282	0.185	0.198	0.229	0.203	0.220	0.246	0.078	0.589
	Н	0.145	0.206	0.075	0.102	0.532	0.301	0.141	0.965	0.148	2.982	0.243	0.205
	$\langle \rangle$	1	2	3	4	5	6	7	8	9	10	11	12
	А	0.088	0.079	0.068	0.076	0.071	0.075	0.090	0.077	0.059	0.090	0.121	0.068
	В	0.096	0.062	0.065	0.090	0.058	0.082	0.131	0.060	0.065	0.070	0.109	0.055
	С	0.123	0.076	0.080	0.073	0.104	0.097	0.053	0.083	0.063	0.060	0.069	0.051
Blank	D	0.057	0.087	0.050	0.058	0.064	0.170	0.070	0.060	0.072	0.059	0.060	0.066
	E	0.098	0.055	0.096	0.097	0.057	0.078	0.065	0.099	0.110	0.058	0.076	0.065
	F	0.100	0.121	0.091	0.091	0.083	0.115	0.067	0.081	0.054	0.054	0.066	0.062
	G	0.070	0.059	0.094	0.088	0.104	0.092	0.101	0.076	0.090	0.074	0.060	0.172
	H	0.069	0.102	0.059	0.064	0.138	0.137	0.082	0.244	0.084	0.111	0.153	0.084
	$\langle \rangle$	1	2	3	4	5	6	7	8	9	10	11	12
	A	3.428	2.927	2.370	1.663	1.789	1.318	4.021	2.643	1.152	33. 637	24.993	1.682
	В	3.988	2.607	2.797	1.940	1.866	4.083	6.127	1.566	2.364	2.687	2.005	1.395
	С	2.457	3.664	3.184	2.962	15.552	3.829	1.073	3.136	1.246	1.764	2.579	1.224
sGn/Blank	D	1.592	3.628	1.097	1.768	1.487	5.392	2.249	1.580	1.981	1.095	1.287	1.810
	E	4.314	1.560	35.347	5.646	1.060	3.422	1.481	1.935	17.105	1.537	3.404	1.620
	F	2.127	19.172	5.252	39.589	2.164	4.979	2.513	1.962	1.340	1.237	2.807	1.561
	G	2.476	1.247	1.375	37.460	1.784	2.157	2.272	2.660	2.447	3.310	1.318	3.422
	Н	2 006	2 008	1 287	1 605	3 844	2 188	1 736	3 057	1 764	26 087	1 503	2 440

Note: Each readout represents bacterial supernatant binding with sGn protein (upper table) and blank control (middle table). The lower table is the readout of sGn binding/the readout of blank binding from the same bacterial supernatant.

ID		SNBID	CDR1	CDR2	
P02 P03	G02 G03 G05		GHTLSSNC GPEYNTHC	IYTGGGHTY VYPHLTY	AADLSPYDCYTGSLDMASRFT
P04	G06		GDTSTAYY	IYRGGRATV	AAGLAGGSGYLPLNWAGFRY
P05	G07		GDTYSSSC	ICSDG-SAA	AARRTWHAGFPY
P06	G10		GDTSTAYY	IYRGGHSTV	AAGLDGGSGYLPLNWAGFRY
P07	G11		GFTSC	IYTRDGRPY	AANRRAY-PYGGDCRLRHAEFDY
P08 P09 P10	G12 G13 G14		GESENAYC	IDSEGRID IDS-GGSSS	AADVPGRREVRGLGPCDRMYDY AAKVPFGRGSCAYSTAHWFPY
P11	G15		GDTSTAYY	IYRGGRATV	AAGLDGGSGYLPLNWAGFRY
P12	G16		GDTSTAYY	IYRGGRSTV	AAGLDGGSGYLPLNWDGFRY
P13	G17		GYTFSNTC	IDR-SGSAS	VADLSAWCRAVRPG-VITYNY
P14	G19		GRTYRTYC	ITA-LSATS	AADPRDPNGSRTDCTVLTSKDLYNS
P15 P16	G20 G22		GFTSC	IYTRHGGTTV IYTRDGRPY	AAGPGCSWSSFAY AANRRAY-PYGGDCRLRHAEFDY
P18 P19	G23 G24 G25		GFTLSMYD GDTSTAYY	IYTSGRRPW IERGGRSTV	AAVIGVDIR
P20	G27		EYTGSRNC	ISILYSGITVSY	ARWGDCDSASWSRTWYAV
P21	G28		GYTYNNYR	ISAGGD-HTY	ARSRASLWSGNWYRSLSEDEYNS
P22	G30		GDTSTAYY	IYRGGRSTV	AAGHDGGSGYLPLNWDGFRY
P23	G31		GLYYPPLC	IDR-DGSTS	AAADAQRGRTCFFGARY
P24 P25	G36 G38 G39		GDTSTAYY	ICSDG-STS IYRGGRSTV	AVRWYWDAGFKY AAGHDGRSGYLPLNWAGFRY
P27 P28	G41 G42		GSAYSTNC GYTYSSNC	IYRDDGTTY	AADSTPSDCYSGSWLEKYPSEYSY AADVVSYYSDYVCTDAADFGY
P29	G43	SNB43	GDTSTAYY	IYRGGRSTV	AAGLDGGSGYLPLNWAGFRY
P30	G45		GFTFNAYD	IFRSGRTSW	AAVIGVDIRRY
P31 P32	G46 G47		GFTLSMYD VYTYRGNN	IFTSGRRPW ITSTGT-RQY	AAVIGVDIR
P34 P35	G48 G50 G52		RYTPTITR GDTSTAYY	IYRGGHSTV IYTRGDRTF IYBGGBSTV	AAGEDGGSGTEPENWAGERT AAGEMYGETRSP-NWVNY AAGEDGGSGYEPENWAGE
P36	G55		VYTSSTMW	IYRGNGATG	AKKR-TYDCYSGTCS
P37	G57		GDTYSSSC	ICSDG-SAA	AARRTWHAGFPY
P38 P39	G58 G60		GFTFNAYD GYTSTAYY	IFRSGRTSW IWRGGHSTL	AAVIGVDIRRY
P40 P41 P42	G62 G63		GDTSTAYY	IYRGGRSTV IYRGGHSTV	AAGLDGGSGYLPLNWAGFRY AAGLDGGSGYLPLNWAGFRY AAGLDGGSGYLPLNWAGF
P43	G66		GDTSTAYY	IYRGGRSTV	AAGLRGGSGYLPLNWAGFRY
P44	G66.1		GETSSNYD	IFSSGRRPW	AAVIGVDIRRY
P45	G67		GDTSTAYY	IYRGGRSTV	AAGHDGGSGYLPLNWAGFRY
P46	G67.1		GFTFNAYD	IFRSGRTSW	AAVIGVDIRRY
P47	G68	SNB109	GFSENAYC	IDS-GGSSS	AAKVPFGRGSCAYSTAHWFPY
P48	G68.1		GFTLSMYD	IFTSGRRPW	AAVIGVDIRY
P49 P50 P51	G69 G69.1	SNB07	GFTSC GDTSTAYY	IYTRGGHSTV IYTRDGRPY	AAGLDGGSGYLPLNWAGFRY AANRRAY-PYGGDCRLRHAEFDY AAGLDGGSGYLPLNWDGFY
P52	G71.1		GFTSSSCG	LHTDGLTS	KTVKDPTSPPGCSRGY
P53	G72		GFTLSMYD	IFTSGRRSW	AAVIGVDIRGY
P54 P55	G73 G74	SNB74	GYTYSSVC GDTSTAYY	IYTATGRTY	AADL-LIGACSQMRTNFDY AAGLDGGSGYLPLNWAGFRY
P56 P57	G75 G76	SNID77	GYTYNTAY GDTSTAYY	IDSDGSTS IFRGGRSTV	AANPYSPGAGRELLSYPYTD AAGLDGGSGYLPLNWDGFRY AAGLVGGSDYEPLNWTGE
P59	G78	SNB78	GDTSTAYY	IYRGGRSTV	AAGLDGGSGYLPLNWAGFRY
P60	G79	SNB79	GGTSTAYY	IYRGGHSTV	AAGLDGGSGYLPLNWAGFRY
P61	G80	SNB87	GYAYSTYC	IDS-DGVTD	AANQGS-GDYC-YMAMLIYGMFY
P62	G81	SNB95	GFTSC	IYTRDGRPY	AANRRAY-PYGGDCRLRHAEFDY
P63	G82		GYTYNEYS	INSVGRTR	AARDGSWFLSLVPATYGY
P64	G86		GYNFNNVC	IYTSIGRTY	AADL-QIGSCSQMRRYNYAY
P66 P67	G87 G88 G89		VYTSSTMW	IYRGNGATG	AAGR-TYDCYPGTCS
P68	G90		GSTDSRRC	IYRGSGTTH	AASLRARWVQRGAPLLPSFYGY
P69	G91		GYPYSSKTYTNNC	IYLANGATY	AAG-PWVATPEIANEYNY
P70	G92		RYSSSRRC	IYHGDGTTY	VAG-IWTCGRSALTDPNNY
P71	G93		GYIYNDYF	IGSDGTTK	AATFGLFWESWEWYKNWHY
P72 P73 P74	G94 G95 G96		TTTNY	IFSSRDYTD	AADP-GVLCGRSW-VGRFP
P75	G97	SNB171	GYTYYNSNC	IYTRDSRTY	AAAHEPGSWTD-IEARGKISDDPFGY
P76	G98		GDTSTAYY	IYRGGRATV	AAGLAGGSGYLPLNWAGFRY
P77	G99	SNB112	GGSVTTGNYY	ITASGSTY	ARASFRGSWFFEGY
P78	G100		GYTENTRC	ISAGGISID	AAARRWYYENSCLKVLRSPGDY
P79 P80 P81	G100.1 G101 G102	SNB29 SNB35	GFTSNSCG	IFSDGETA	AAGPPSGPLRACHESVFGY KTVRDPASPPSCSGGY AADR-VI GROSBRU SDEGY
P82	G103	511200	GYTHSSDS	IYTGDGSTH	AAGTYY-SDYDPPRY-EYKY
P83	G104		RSVNRDTC	ISAQGVIPG	AAKERPLCGSFWERGDEYAS
P84	G105		AFISNNHC	IDS-AGSTR	AAVSWACWRLSGTGFNY
P85	G106		GFTFSSYD	IRSGGGNTY	AADLGVDDYSDYLDHPFGY
P86 P87	G107 G108 G109	SNIP02	GFTFDDSD GYRC	FVTGAGSTY	AADIGGSWPRKPHPDPNFGGECGGYGMAF AADSSSLPCYPRAAQFPRLRY
P89 P90	G110 G111	SNB111	GATYNINF GDTSTAYY	IDNNGWST	AARPAPVSGITRFRLNRSLLPNEYNS AAGLDGGSGYLPLNWAGFRY
P91	G112	SNB113	GDTSTAYY	IFRGGRSTV	AAGLDGGSGYLPLNWDGFRY
P92	G113		GDTSTAYY	IYRGGRSTV	AAGLDGGSGYLPLNWANFRY
P93 P94	G117 G117.1	SNB117	VYTSSTMW	IDTNGS-TS IYRGNGATG	AAEGGWRDYVRSWGRNFGY AAGR-TYDCYPGTCS
P96 P97	G120 G121 G122		GETESSYY GETYSSSC	IYSDG-SAA	AADLWRGPPFGGYWSPTKSEFAY AARBTWHAGEPY
P98	G123		GFTSC	IYTRDGRPY	AANRRAY-PYGGDCRVRHAEFDY
P99	G126		GDTYSSSC	ICSDG-SAA	AARRTWHAGFPY
P100	G128	SNB130	GDTSTAYY	IYRGGRSTV	AAGLDGGSGYLPLNWAGFRY
P101	G130		GFTSC	IYTRDGRPY	AANRRAY-PYGGDCRLRHAEFDY
P102 P103 P104	G133 G134		GDTSTAYY GDTSTAYY	IYRGGHSTV	AAGLDGGSGYLPLNWAGFRY AAGLDGGSGYLPLNWAGFRY
P105	G138		GYTYSSAC	ICSDG-SSA	AARHYWSAGFPY
P106	G139		G-MYSNTC	IYTGIGSTY	AADR-VLGRCSRRILSDFGY
P107	G141		GDTSTAYY	IYRGGRSTV	AAGLDGGSGYLPLNWAGFRY
P108	G142		GDTSTAYY	IYRGGHSTV	AAGLDGGSGYLPLNWAGFRY
P109 P110 P111	G143 G144 G145		GDTSTAYY GGSITTNYYG	IYRGGRSTV IYRGGRSTV	AAGEDGGSGYLPENWAGFRY AAGEDGGSGYLPENWAGFRY ARSSPRTV/AGEGD
P112	G147	SNB148	GDTSTAYY	IYRGGRSTV	AAGLDGGSGYLPLNWAGFRY
P113	G148		GDSSTAYY	IYRGGRSTV	AAGLDGGNGYLPLNWAGFRY
P114	G149		GNTYTSSC	ICSDG-STA	AARRTWHAGFPY
P115	G150		GLYYPPLC	IDR-DGSTS	AAADAQRGRTCFFGA
P116 P117 P119	G151 G153 G154		G-MYSNTC GLYYPLC		ARGPTGUISVSGCYRKGGVDNY AADR-VLGRCSRRLLSDFGY AAADAORGRTCFEGA
P119	G155	SNB176	GDTYSSSC	ICSDG-SAA	AARRTWHAGFPY
P120	G156		GFTSNSCG	IHTDGSTS	KTVRDPTSPRSCSGGY
P121 P122	G157 G158		GLWHPPLC GDTYSSSC	IDR-DGSTS ICSDG-SAA	AAADAQRGRTCFFGARY
P123 P124 P125	G159 G160	SNB166	GYTYGSYC GLYYSPLC BYTS	SNINGGSTY IDR-DGSTS	AAYHSGSWCYLP-HLGSYGY AAA-DAQRGRTCFFGAGY
P126	G167	SNB168	GYTYSSNC	VYNDGGHTY	AASFRPTWFCRGLAPYKYNL
P127	G168		GYRYNACS	IFTRGTTK	TSAS-LTWDGGNWYCPTHGY
P128	G169	SNB170	VYTSSTMW	IYRGNGATG	AAGR-TYDCYSGTCS
P129	G170		GNPSGRKF	LYLGGSITY	AAGAYRASFTY
P130	G173		GDTSTAYY	IYRGGRSTV	AAGLDGGSGYLPLNWAGFRŸ
P131	G175		GDTYSSSC	ICSDG-SAA	AARRTWHAGFPY
P133	G179		GDTSTAYY	IFRGGRSTV	AAGHDGGSGYLPLNWAGFRY
P134	G180		GFTSC	IYTRDGRPY	AANRRPY-PYGGDCRLRHAFFDY
P135	G183		EDTSTAYY	IYRGGHSTV	AAGLDGGSGYLPLNWAGFRY
P136	G184		GDTSTAYY	IYRGGRSTV	AAGLDGGSGYLPLAWAGFRY
P137	G185		GDTSTAYY	IFRGGRSTV	AAGLDGGSGYLPLNWDGFRY
P138	G186		GDTSTAYY	IYRGGRSTV	AAGLDGGSGYLPLNWARFRY
P139 P140 P141	G190 G191 G194		GYTYSSHC	LDG-DGRVR	AAGVAGGSGYLPLNWAGFRY AAGFAGCYGSSWYGSADFGY AAGLDGGSGYLPLNWAGE
P142	G195		GLYYPPLC	IDR-DGSTS	AAADAQRGRTCFFGA

173 Supplemental Table 3. Summary of CDR sequences of 142 positive VHH clones