Supplementary Information

SARS-like cluster of circulating bat coronavirus pose threat for human emergence.

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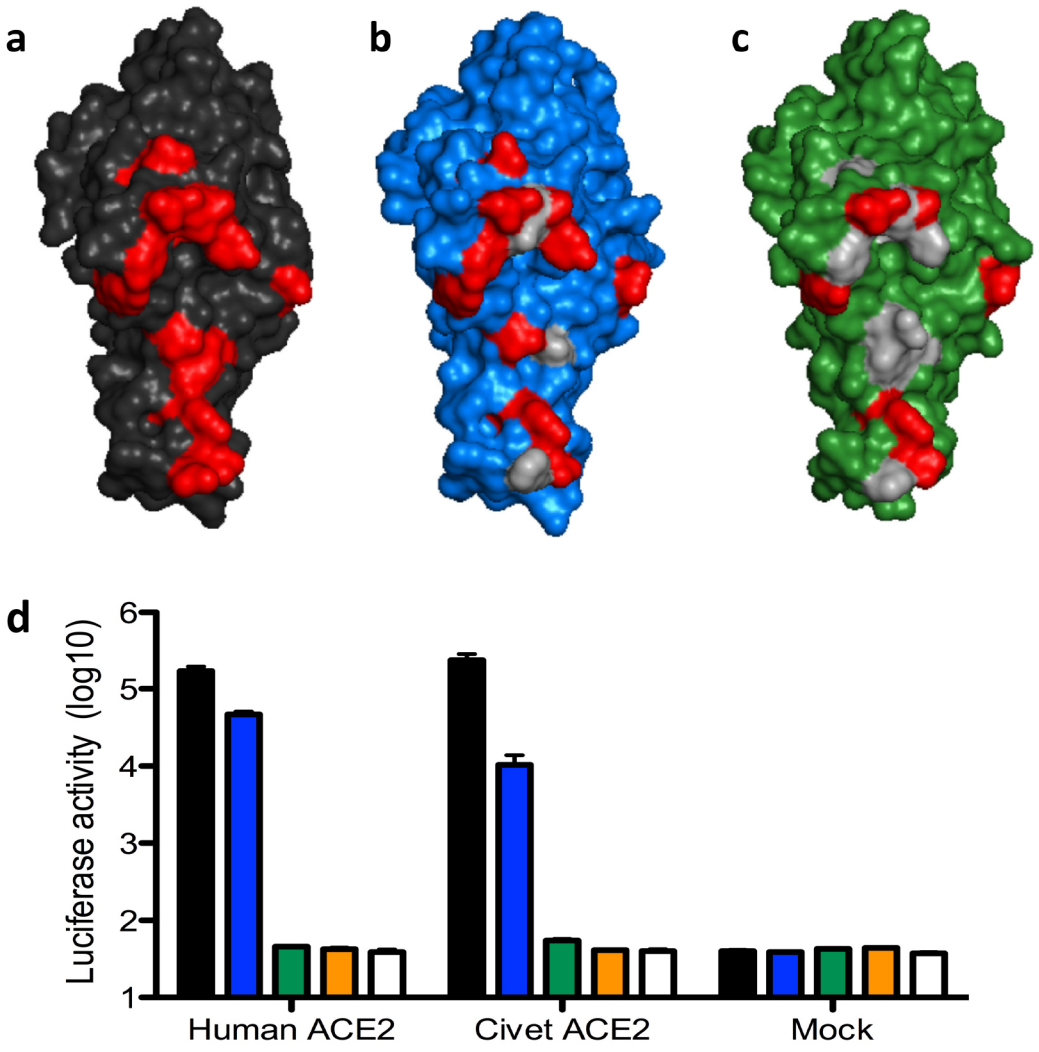

Supplementary Figure 1: Structural and pseudotyping based analysis of group 2b CoV spike proteins. Structure of the S1 domains of spike proteins from: (**a**) SARS-CoV (black), (**b**) WIV1 (blue), and (**c**) SHC014 (green), with red representing the 14 identified ACE2 contact residues. Changes in RBD contact residues for WIV1 and SHC014 are depicted in gray. d) SHC014 spike fails in pseudotype infectivity assay. Measurement of pseudovirus infectivity with expression of spikes from SARS-Urbani (black), WIV1-CoV (blue), SHC014-CoV (green), Bat CoV RP3 (orange), or mock (white). Entry measured in by determining luciferase activity for each pseudovirus in each cell type (n=3 for all groups). Cell lysates were prepared 48 hours post infection from HeLa-huACE2, HeLA-civetACE2, or mock transfected HeLA cells infected with pseudoviruses. The error bars indicate SEM.

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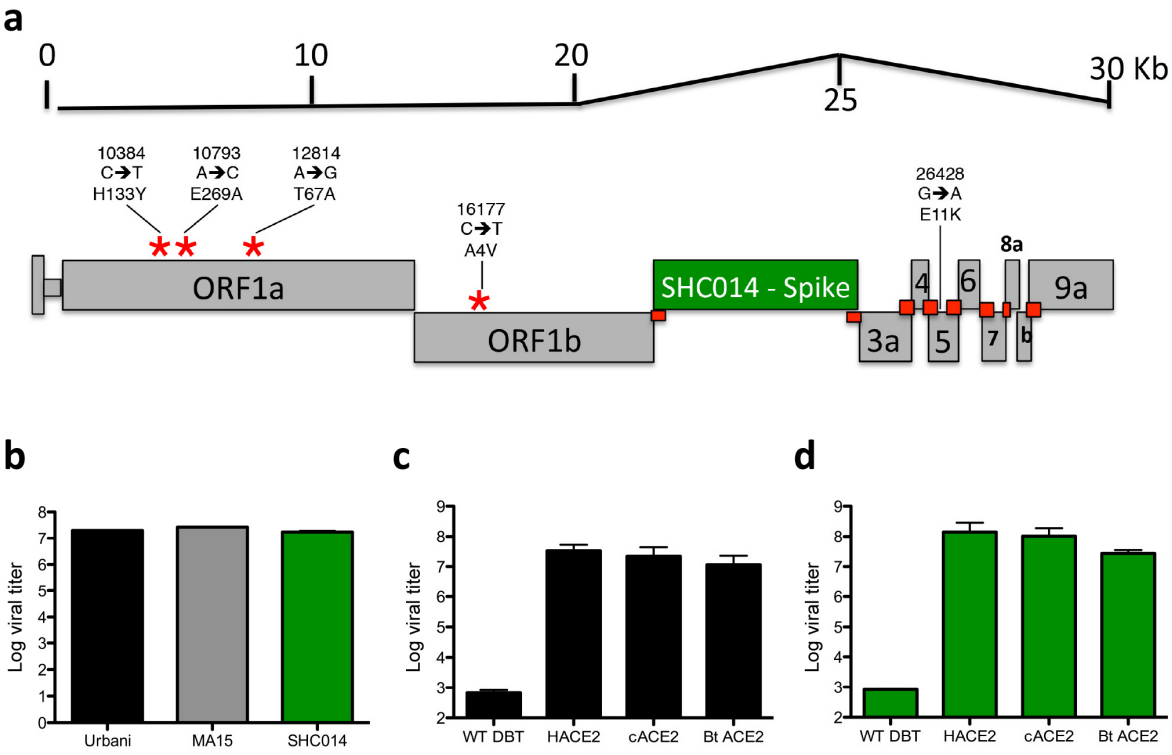

Supplementary Figure 2: Chimeric SHC014-MA15 CoV is viable and utilizes ACE2 orthologs for entry. (**a**) Organization of chimeric SARS-like virus which substitutes SHC014 (green) spike protein into the SARS-CoV mouse adapted (MA15) backbone. Amino acid changes between epidemic SARS-CoV Urbani and MA15 highlighted by stars. (**b**) End point titers 24 hours post infection following infection with wild-type Urbani (black, *n* = 1), mouse adapted MA15 (Gray, *n* = 1), or SHC014-MA15 CoV (green, *n* = 3). (**c**,**d**) End point titers of (**c**) SARS-CoV Urbani or (**d**) SHC014-MA15 in wild-type or DBT cells expressing human, civet, or bat ACE2 receptors (*n* = 3 for all groups. For each graphical figure, center value representative of group mean and error bars defined by SEM.

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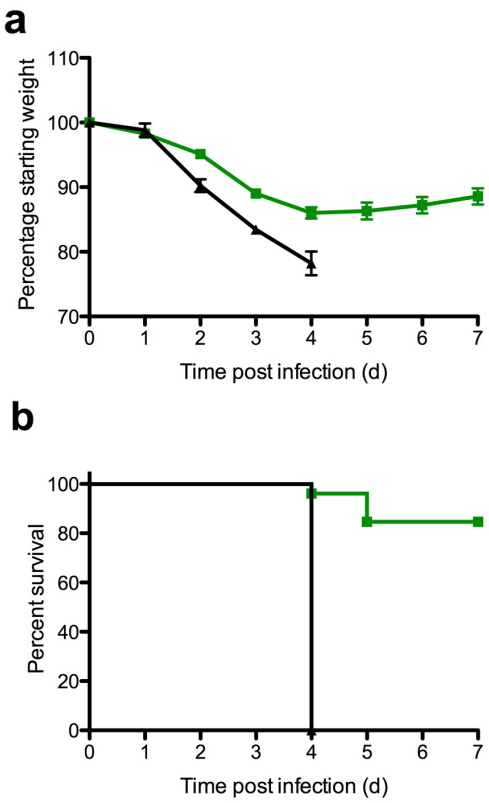

Supplementary Figure 3: Chimeric SHC014-MA15 induces robust disease in aged animals. In vivo infection of 12 month old Balb/c mice infected with 1×10^4 PFU of SARS-CoV MA15 (black, *n* = 4) or SHC014-MA15 (green, *n* = 29) via the *i.n.* route. (**a**) Weight loss and (**b**) lethality. For each graphical figure, center value representative of group mean and error bars defined by SEM.

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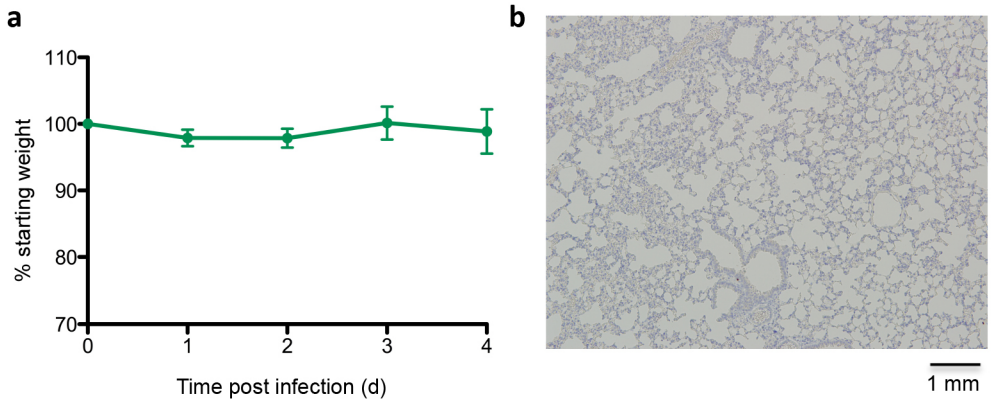

Supplementary Figure 4: SARS-like chimeric viruses require ACE2 for in vivo replication and pathogenesis. *In vivo* infection of 10 week old *Ace2*$^{-/-}$ mice infected with 1 × 10^4 PFU of SHC014-MA15 (green, *n* = 6) via the *i.n.* route. (**a**) Weight loss and (**b**) representative day 2 anti-SARS N protein staining for SHC014-MA15. For graphical weight loss figure, center value representative of group mean and error bars defined by SEM.

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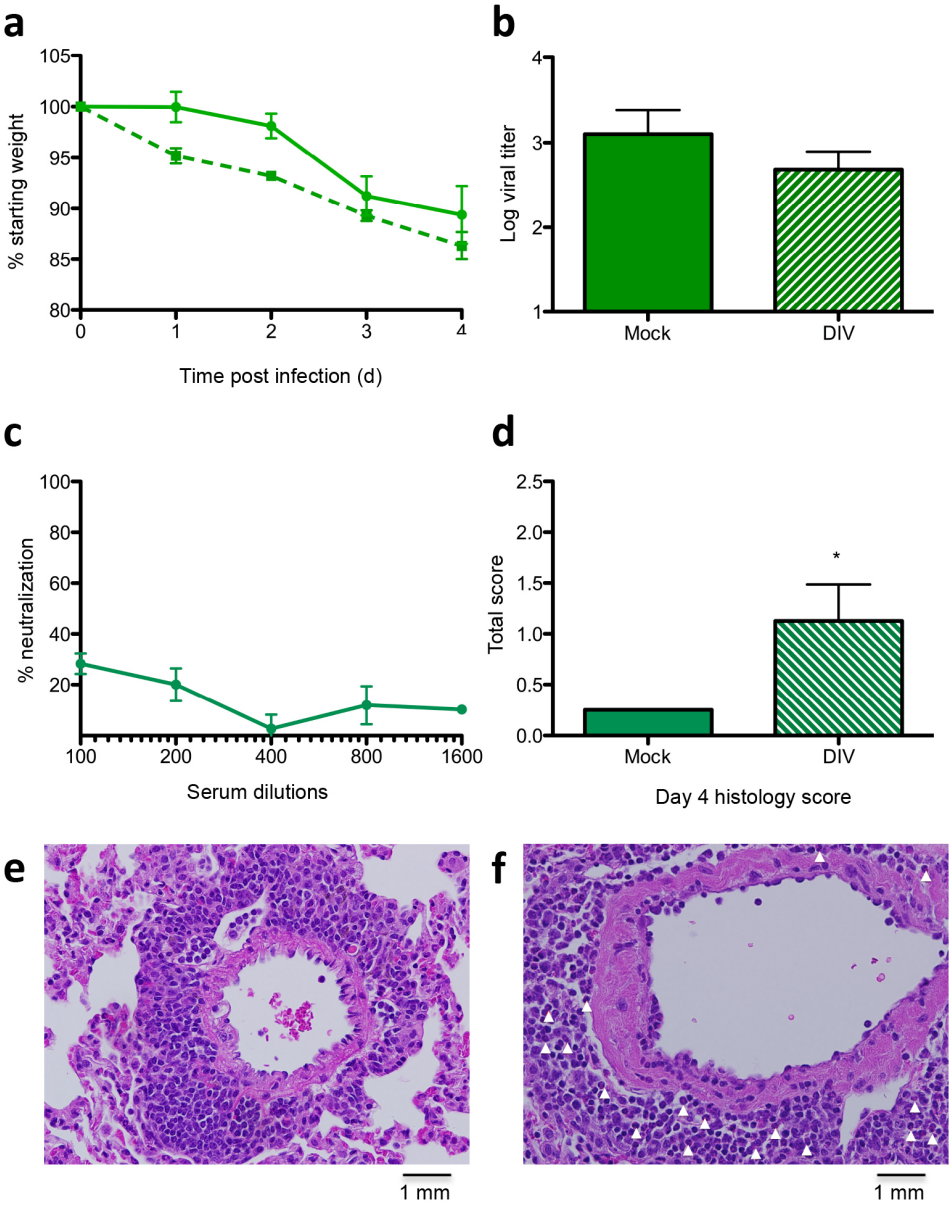

Supplementary Figure 5: Double-inactivated whole SARS-CoV vaccine fails to protect aged animals from chimeric SARS-like virus infection. Twelve month-old mice were vaccinated and boosted with DIV (dotted line, *n* = 4) or PBS (solid line, *n* = 3) and infected 21 days post boost with 10^4 PFU of SHC014-MA15 via the *i.n.* route. (**a**) Weight loss following SHC014-MA15 challenge and (**b**) viral replication in the lung 4 DPI. (**c**) Neutralization of SHC014-MA15 (green, *n* = 3) with serum from aged, DIV vaccinated mice. (**d**–**h**) Histopathology lung sections stained for H&E from DIV and mock-vaccinated mice. (**d**) Eosinophil score (scale 0-4) following DIV or mock vaccination 4 DPI. (**e,f**) Representative H&E lung sections for (**e**) mock vaccinated SHC014-MA15 and (**f**) DIV SHC014-MA15 infected mice. White triangles indicate individual eosinophil locations. For each graphical figure, center value representative of group mean and error bars defined by SEM. *p*-values based on 2-tailed Student's T-test of individual time points and are marked as indicated * < 0.05.

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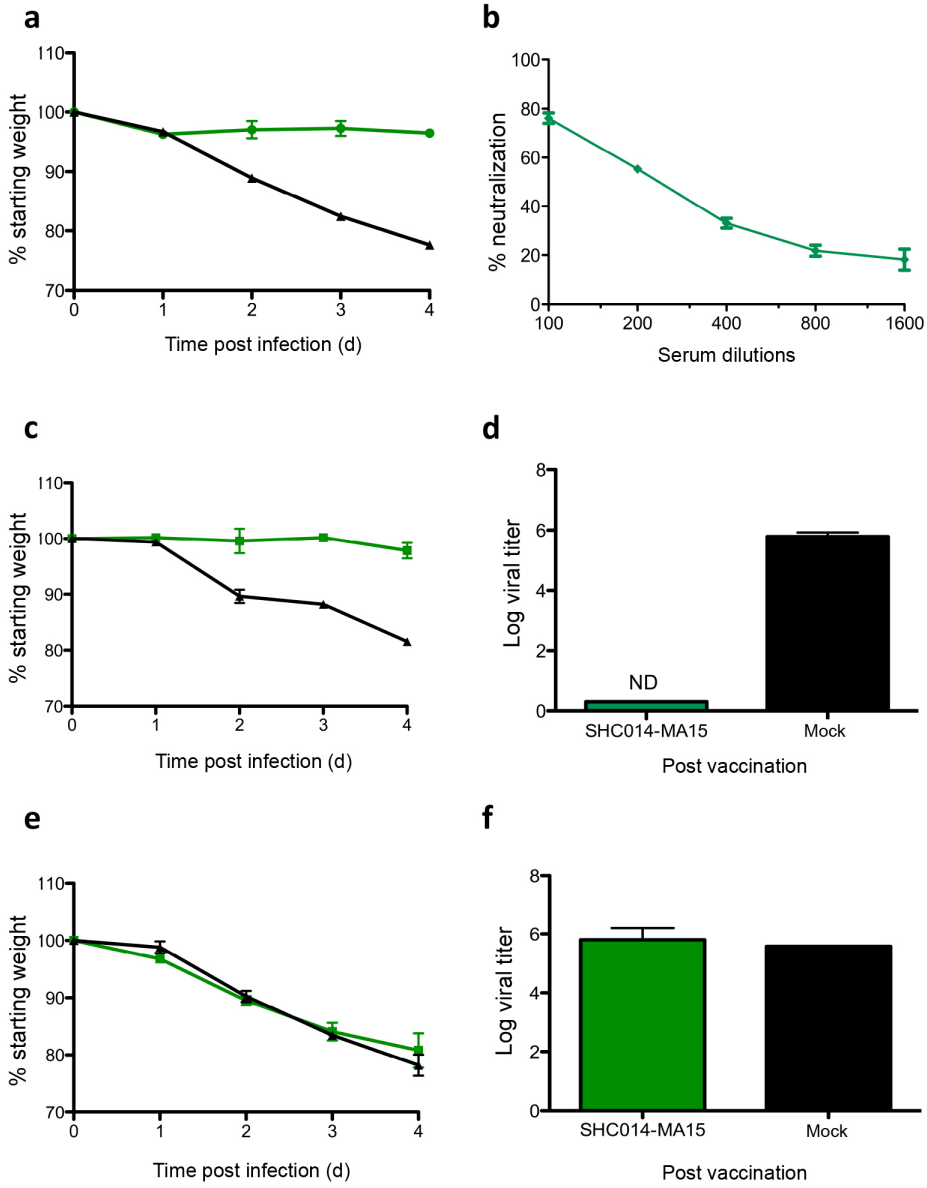

Supplementary Figure 6: Chimeric SARS-like viruses provide incomplete protection from lethal SARS-CoV challenge. (**a**) Ten-week-old mice vaccinated with 1 × 10^4 PFU of SHC014-MA15 (green) or mock-infected with PBS (black) were challenged with 1 × 10^5 SARS-CoV MA15 and evaluated for weight loss over a 4-day time course (*n* = 4 for SHC014-MA15 vaccinated, *n* = 5 for PBS vaccinated). B) Serum neutralization of SARS-CoV from 10-week-old mice vaccinated with SHC014-MA15 (*n* = 3). (**c**,**d**) Twelve-month-old mice vaccinated with 1 × 10^4 PFU of SHC014-MA15 (green) or mock-vaccinated with PBS (black) were challenged with 1 × 10^5 PFU SARS-CoV MA15 and evaluated for (**c**) weight loss and(**d**) viral replication (*n* = 4 for SHC014-MA15 vaccinated, *n* = 5 for PBS vaccinated). (**e**,**f**) Twelve-month-old mice vaccinated with 1 × 10^2 PFU of SHC014-MA15 (green) or mock-vaccinated with PBS (black) were challenged with 1 × 10^5 PFU SARS-CoV MA15 and evaluated for (**e**) weight loss (*n* = 4 for SHC014-MA15 vaccinated, *n* = 5 for PBS vaccinated) and (**f**) viral replication (*n* = 3 for SHC014-MA15 vaccinated, *n* = 2 for PBS vaccinated). For each graphical figure, center value representative of group mean and error bars defined by SEM.

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Supplementary Table 1: Spike amino acid identity of residues that interact directly with human ACE2 from SARS-CoV Urbani, SARS-CoV MA15, WIV1-CoV, and SHC014-CoV. Colored residues represent changes relative to the epidemic Urbani strain. Numbers with (*) indicate residues previously identified as important host range determinants.

Strain	402	426	436	441	442*	472*	473	475	479*	484	486	487*	488	491*
Urbani	T	R	Y	R	Y	L	N	Y	N	Y	T	T	G	Y
MA15	T	R	H	R	Y	L	N	Y	N	Y	T	T	G	Y
WIV1	T	R	Y	R	S	F	N	Y	N	Y	T	N	G	Y
SHC014	T	N	Y	R	W	P	N	Y	R	F	T	A	G	H

Supplementary Table 2: Histology scoring following challenge of 10 week old mice with SARS-like chimeric viruses.

			H&E Staining Score (0-4)													SARS Anti-N Stain (0-4)		
	Virus	Day	Denudation	Debris	Inflammation	Total Airway Disease	Perivascular cuffing	Edema	Vasculature	Interstitial Septum	Airspace inflammation	Alveoli Parenchyma	Hyaline Membrane	Exudates	DAD Total	Eosinophil	Airway	Parenchyma	Overall
1.1	SHC014-MA15	D2	0.25	0.50	0.00	0.75	0.75	0.00	0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.50	2.00	1.75
1.2	SHC014-MA15	D2	0.00	0.25	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.75	0.75
1.3	SHC014-MA15	D2	0.25	0.50	0.25	1.00	0.50	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.25	1.50	1.25
1.4	SHC014-MA15	D2	1.00	1.25	0.25	2.50	0.75	0.00	0.75	0.75	0.00	0.75	0.00	0.75	0.75	0.00	1.25	2.25	2.00
	SHC014-MA15	D2	0.38	0.63	0.13	1.13	0.50	0.00	0.50	0.19	0.00	0.19	0.00	0.19	0.19	0.00	1.13	1.63	1.44
2.1	SHC014-MA15	D4	1.00	1.25	0.75	3.00	0.75	0.25	1.00	1.75	0.75	2.50	0.00	0.75	0.75	0.25	0.25	0.50	0.50
2.2	SHC014-MA15	D4	0.50	0.75	1.00	2.25	0.75	0.00	0.75	1.00	0.50	1.50	0.00	0.25	0.25	0.25	0.25	1.00	0.75
2.3	SHC014-MA15	D4	0.75	1.00	0.75	2.50	1.25	0.50	1.75	1.00	1.00	2.00	0.00	1.00	1.00	0.50			
2.4	SHC014-MA15	D4	0.75	1.00	1.00	2.75	1.00	0.00	1.00	1.00	1.25	2.25	0.75	0.75	1.50	0.50	0.50	0.75	0.75
	SHC014-MA15	D4	0.75	1.00	0.88	2.63	0.94	0.19	1.13	1.19	0.88	2.06	0.19	0.69	0.88	0.38	0.33	0.75	0.67
7.1	SARS-MA15	D2	1.00	1.25	0.25	2.50	0.75	0.00	0.75	0.50	0.00	0.50	0.00	0.00	0.00	0.50	2.50	2.50	2.50
7.2	SARS-MA15	D2	1.25	1.75	0.75	3.75	1.00	0.25	1.25	0.50	0.00	0.50	0.00	0.00	0.00	0.25	1.75	1.75	1.75
7.3	SARS-MA15	D2	1.00	1.00	0.50	2.50	0.75	0.00	0.75	0.75	0.00	0.75	0.00	0.75	0.75	0.25	2.00	2.00	2.00
	SARS-MA15	D2	1.08	1.33	0.50	2.92	0.83	0.08	0.92	0.58	0.00	0.58	0.00	0.25	0.25	0.33	2.08	2.08	2.08
8.1	SARS-MA15	D4	1.00	0.50	1.00	2.50	1.00	0.50	1.50	1.00	1.00	2.00	0.00	0.75	0.75	0.00	0.75	1.00	1.00
8.2	SARS-MA15	D4	0.50	0.50	0.75	1.75	1.00	0.50	1.50	1.00	1.00	2.00	0.75	0.75	1.50	0.50	1.00	1.25	1.25
8.3	SARS-MA15	D4	0.75	0.50	0.75	2.00	1.00	0.50	1.50	0.75	0.75	1.50	1.75	1.00	2.75	0.75	0.75	1.50	1.25
	SARS-MA15	D4	0.75	0.50	0.83	2.08	1.00	0.50	1.50	0.92	0.92	1.83	0.83	0.83	1.67	0.42	0.83	1.25	1.17
ACE2-/- KO																			
9.2	SHC014-MA15	D2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10.3	SHC014-MA15	D2	0.25	0.25	0.00	0.50	0.25	0.00	0.25	0.25	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10.4	SHC014-MA15	D2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SHC014-MA15	D2	0.08	0.08	0.00	0.17	0.08	0.00	0.08	0.08	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00

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Supplementary Table 3: Histology and antigen scoring following challenge of year old mice with SARS-like chimeric viruses.

			H&E Staining Score (0-4)														SARS Anti-N Stain (0-4)		
ID	Virus	Day	Denudation	Debris	Inflammation	Total Airway Disease	Perivascular cuffing	Edema	Vasculature	Interstitial Septum	Airspace inflammation	Alveoli Parenchyma	Hyaline Membrane	Exudates	DAD Total	Eosinophil	Airway	Parenchyma	Overall
4.1	SHC014-MA15	D2	0.50	0.50	1.25	2.25	1.75	0.50	2.25	1.00	0.75	1.75	0.00	0.25	0.25	0.75	0.75	2.00	2.00
4.2	SHC014-MA15	D2	0.25	0.75	0.50	1.50	1.25	0.50	1.75	1.00	0.75	1.75	0.00	0.25	0.25	0.25	0.25	1.50	1.50
4.3	SHC014-MA15	D2	0.50	1.00	0.75	2.25	1.25	0.75	2.00	1.00	0.75	1.75	0.25	1.00	1.25	0.50	1.00	1.50	1.50
4.4	SHC014-MA15	D2	0.25	0.50	0.50	1.25	1.00	0.50	1.50	1.25	0.25	1.50	0.00	0.50	0.50	0.50	0.75	1.75	1.50
4.5	SHC014-MA15	D2	0.50	1.25	1.25	3.00	1.00	0.50	1.50	0.75	0.75	1.50	0.00	0.50	0.50	0.50	1.25	1.75	1.75
	SHC014-MA15	D2	0.38	0.88	0.75	2.00	1.13	0.56	1.69	1.00	0.63	1.63	0.06	0.56	0.63	0.44	0.81	1.63	1.56
5.1	SHC014-MA15	D4	0.50	0.50	0.75	1.75	1.50	0.75	2.25	1.25	1.25	2.50	0.50	1.00	1.50	0.25	0.25	1.00	1.00
5.2	SHC014-MA15	D4	0.25	0.50	1.00	1.75	1.25	1.00	2.25	1.00	1.25	2.25	0.25	1.25	1.50	0.50	0.25	1.00	1.00
5.3	SHC014-MA15	D4	0.25	0.25	0.75	1.25	1.25	0.75	2.00	1.25	1.25	2.50	0.25	1.25	1.50	0.25	0.25	1.75	1.75
5.4	SHC014-MA15	D4	0.50	1.25	0.75	2.50	1.75	1.00	2.75	1.75	1.75	3.50	0.50	1.75	2.25	0.50	0.25	2.00	2.00
5.5	SHC014-MA15	D4	0.50	1.00	1.25	2.75	1.75	1.00	2.75	1.50	1.50	3.00	0.50	1.50	2.00	0.50	0.00	1.75	1.75
	SHC014-MA15	D4	0.40	0.70	0.90	2.00	1.50	0.90	2.40	1.35	1.40	2.75	0.40	1.35	1.75	0.40	0.20	1.50	1.50
6.1	SHC014-MA15	D7	0.50	0.75	0.75	2.00	1.25	0.75	2.00	1.25	1.50	2.75	1.00	1.25	2.25	0.50	0.00	0.25	0.25
6.2	SHC014-MA15	D7	1.00	1.25	1.00	3.25	1.75	1.00	2.75	1.75	1.75	3.50	0.75	2.00	2.75	0.50	0.25	0.50	0.50
6.3	SHC014-MA15	D7	1.00	1.25	1.00	3.25	1.50	1.00	2.50	1.75	1.75	3.50	0.75	1.50	2.25	0.25	0.00	0.25	0.25
6.4	SHC014-MA15	D7	0.75	2.00	2.25	5.00	2.75	1.25	4.00	1.75	2.25	4.00	1.00	2.25	3.25	0.25	0.25	0.25	0.25
6.5	SHC014-MA15	D7	0.50	1.75	2.25	4.50	3.00	1.00	4.00	2.50	2.25	4.75	0.75	2.75	3.50	0.25	0.25	0.25	0.25
	SHC014-MA15	D7	0.75	1.40	1.45	3.60	2.05	1.00	3.05	1.80	1.90	3.70	0.85	1.95	2.80	0.35	0.15	0.30	0.30
7.1	Mock	D7	0.25	0.25	0.25	0.75	0.25	0.00	0.25	0.75	0.50	0.25	0.00	0.00	0.00	0.25	0.00	0.00	0.00
7.2	Mock	D7	0.00	0.00	0.25	0.25	0.25	0.00	0.25	0.50	0.00	0.50	0.00	0.00	0.00	0.25	0.00	0.00	0.00
	Mock	D7	0.13	0.13	0.25	0.50	0.25	0.00	0.25	0.63	0.25	0.38	0.00	0.00	0.00	0.25	0.00	0.00	0.00

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Supplementary Table 4, Histology scoring following vaccination and challenge with SARS-like chimeric viruses.

			H&E Staining Score (0-4)														SARS Anti-N Stain (0-4)		
Vaccine	**Virus Challenge**	**ID**	**Denudation**	**Debris**	**Inflammation**	**Total airway disease**	**Perivascular Cuffing**	**Edema**	**Vasculature**	**Interstitial Septum**	**Airspace inflammation**	**Alveoli Parenchyma**	**Hyaline Membrane**	**Exudates**	**DAD Total**	**Eosinophil**	**Airway**	**Parenchyma**	**Total**
DIV	SHC014-MA15	2.1	0.00	0.50	0.75	1.25	1.00	0.00	1.00	0.50	0.25	0.75	0.00	0.25	0.25	1.00	0.00	0.75	0.75
DIV	SHC014-MA15	2.2	0.25	0.75	1.50	2.50	1.75	1.00	2.75	1.25	1.00	2.25	0.25	0.75	1.00	1.25	0.50	1.25	1.25
DIV	SHC014-MA15	2.3	0.00	1.50	1.50	3.00	2.25	0.75	3.00	0.75	0.50	1.25	0.00	0.50	0.50	2.00	0.25	0.25	0.25
DIV	SHC014-MA15	2.4	0.00	0.50	1.00	1.50	1.00	0.25	1.25	0.25	0.25	0.50	0.00	0.00	0.00	0.25	0.00	0.25	0.50
DIV	**SHC014-MA15**		**0.06**	**0.81**	**1.19**	**2.06**	**1.50**	**0.50**	**2.00**	**0.69**	**0.50**	**1.19**	**0.06**	**0.38**	**0.44**	**1.13**	**0.19**	**0.63**	**0.69**
Mock	SHC014-MA15	4.1	0.00	0.00	0.50	0.50	1.75	0.25	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.25	0.25
Mock	SHC014-MA15	4.2	0.25	0.50	0.50	1.25	1.00	0.25	1.25	1.50	1.25	2.75	0.50	0.50	1.00	0.25			
Mock	SHC014-MA15	4.3	0.25	0.50	0.50	1.25	0.75	0.25	1.00	0.50	0.50	1.00	0.00	0.00	0.00	0.25	0.00	0.75	0.75
Mock	SHC014-MA15	4.4	0.25	0.75	1.50	2.50	1.75	1.00	2.75	2.00	1.75	3.75	0.50	1.75	2.25	0.25	0.00	1.50	1.50
Mock	**SHC014-MA15**		**0.19**	**0.44**	**0.75**	**1.38**	**1.31**	**0.44**	**1.75**	**1.00**	**0.88**	**1.88**	**0.25**	**0.56**	**0.81**	**0.19**	**0.08**	**0.83**	**0.83**
Mock	Mock	3.2	0.00	0.00	0.25	0.25	0.25	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.25	0.00	0.00	0.00	0.00
Mock	Mock	5.2	0.00	0.25	1.00	1.25	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00
Mock	Mock	5.3	0.00	0.00		0.00	0.25	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mock	**Mock**		**0.00**	**0.08**	**0.63**	**0.50**	**0.50**	**0.00**	**0.42**	**0.00**	**0.00**	**0.00**	**0.08**	**0.00**	**0.08**	**0.00**	**0.08**	**0.00**	**0.00**

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