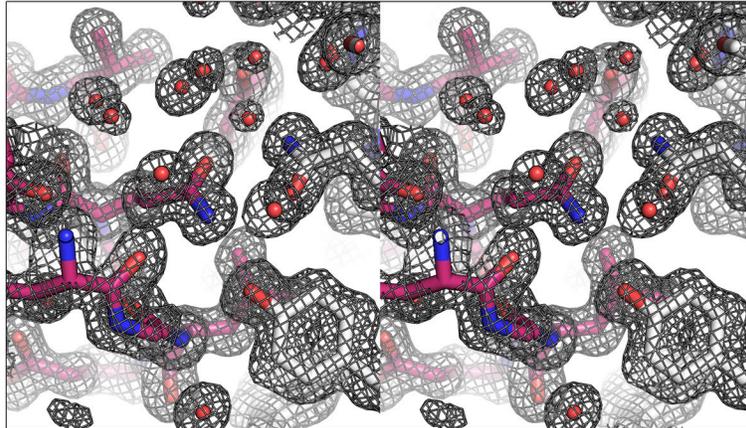
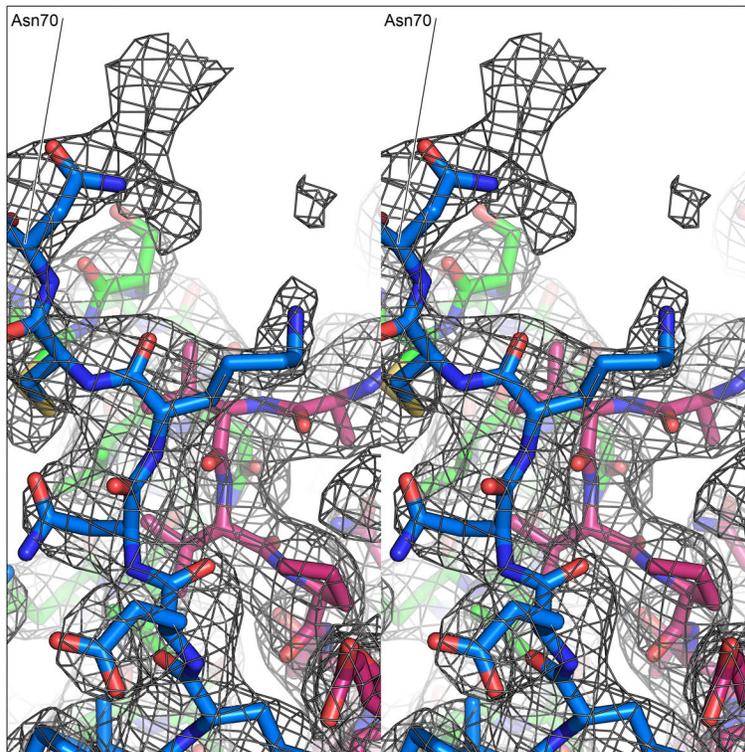


**Supplementary Figure 1 | Surface plasmon resonance experiments. (A)** Sensorgrams for binding of Fab 5C4 (*upper*) and D25 (*lower*) to prefusion-stabilized RSV F derived from strain A2. **(B)** Same as (A), except RSV F derived from strain 9320. For all panels, equilibrium dissociation constants ( $K_D$ ) are listed. Red lines represent best fit of the data to a 1:1 binding model. **(C)** Kinetic rate constants derived from the best fits of the model.

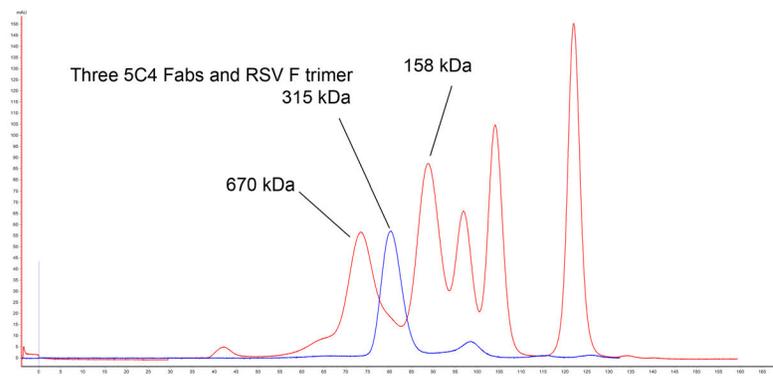
A



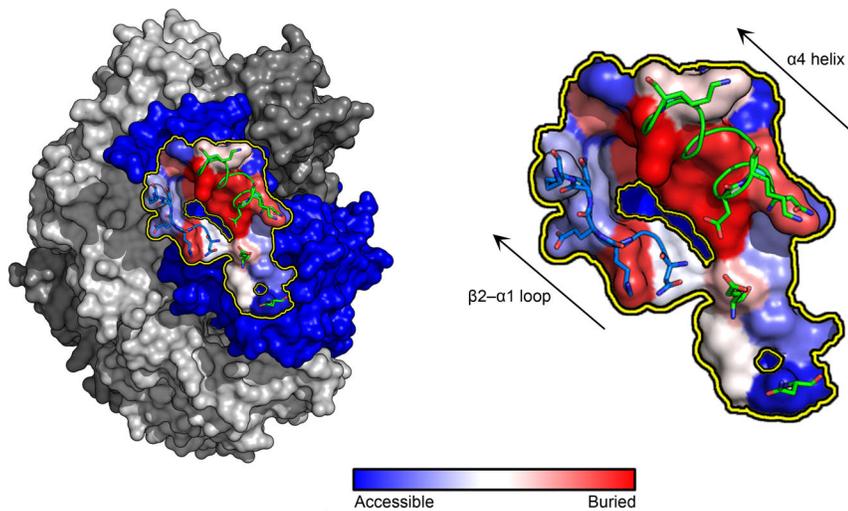
B



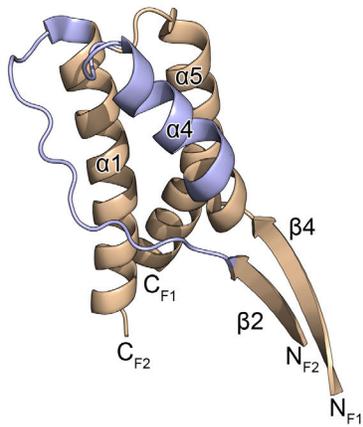
**Supplementary Figure 2 | Stereo images of electron density.** Stereo images of a portion of (A) the 5C4 Fab crystal structure and (B) the 5C4-RSV F complex crystal structure depicted as in Figure 2 with the  $2F_o-F_c$  electron density maps contoured at  $1\sigma$  shown as a black mesh.



**Supplementary Figure 3 | 5C4–RSV F complex has an approximate molecular weight of 315 kDa, consistent with a trimeric RSV F bound to three Fabs.** 5C4–RSV F complex is shown as a blue trace and molecular weight standards (BioRad) are overlaid as a red trace with 670 kDa (bovine thyroglobulin) and 158 kDa (bovine  $\gamma$ -globulin) labeled for comparison.



**Supplementary Figure 4 | Surface of RSV F buried by 5C4.** (*Left*) Two RSV F protomers are shown in gray and the remaining RSV F protomer is colored to show buried surface area (BSA). BSA is shown from blue to white to red (accessible to buried), calculated as the fraction of accessible surface area (ASA) on the RSV F protomer buried by 5C4 (BSA/ASA). The  $\alpha$ 4 helix of RSV F is colored green and the loop between  $\beta$ 2 and  $\alpha$ 1 is colored blue, with select side chains shown as sticks. (*Right*) enlargement of the 5C4 binding footprint depicted as above.

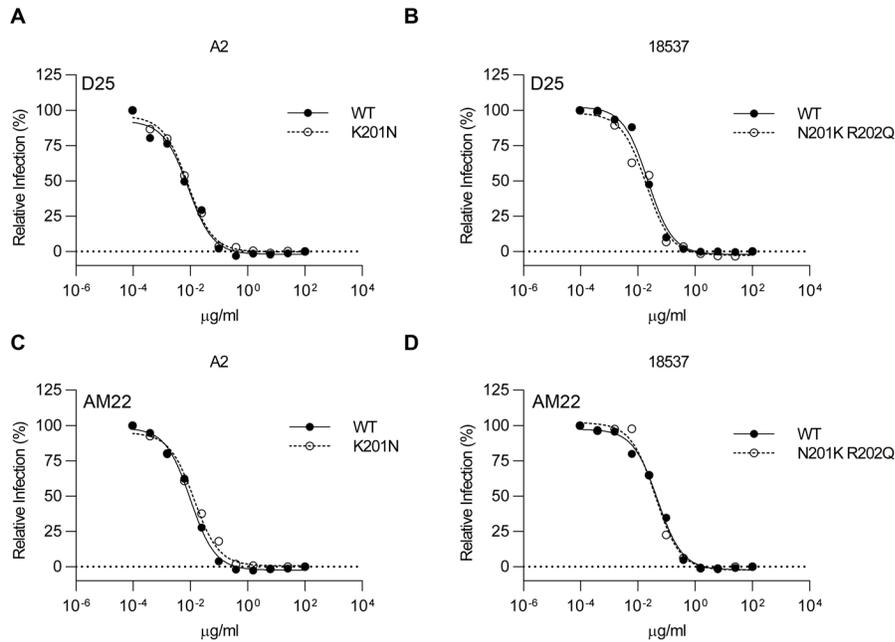


<b>F1:</b>		200	201	202	203	204	205	206	207	208	209	210	211	212	213
A2:	D	K	Q	L	L	P	I	V	N	K	Q	S	C	S	
A (Long):	D	K	Q	L	L	P	I	V	N	K	Q	S	C	R	
B18537:	N	N	R	L	L	P	I	V	N	Q	Q	S	C	R	
B9320:	N	N	Q	L	L	P	I	L	N	Q	Q	S	C	R	

<b>F2:</b>		60	61	62	63	64	65	66	67	68	69	70	71	72	73	74
A2:	E	L	S	N	I	K	E	N	K	C	N	G	T	D	A	
A (Long):	E	L	S	N	I	K	E	N	K	C	N	G	T	D	A	
B18537:	E	L	S	N	I	K	E	T	K	C	N	G	T	D	T	
B9320:	E	L	S	N	I	K	E	T	K	C	N	G	T	D	T	

**Supplementary Figure 5 | Five subtype-specific mutations within site Ø.** (*Left*) Site Ø epitope highlighted in light blue on a portion of a pre-F protomer colored wheat with annotated secondary structure. Carboxyl (C) and amino (N) termini are also labeled. (*Right*) Sequence of site Ø among select RSV strains. Residues that vary between subtype A and B are shaded in blue. Positions containing strain-specific variations are shaded in red.



**Supplemental Figure 6 | D25 and AM22 neutralize wild-type and position 201 mutant viruses of both subtypes with similar potency. (A)** The  $EC_{50}$  of D25 against A2 wild-type and K201N mutant virus is  $0.010 \pm 0.002 \mu\text{g/ml}$  (Mean $\pm$ -SD) and  $0.008 \pm 0.0008 \mu\text{g/ml}$ , respectively. Data is representative of 3 independent experiments. **(B)** The  $EC_{50}$  of D25 against 18537 wild-type and N201K mutant virus is  $0.029 \pm 0.008 \mu\text{g/ml}$  and  $0.023 \pm 0.005 \mu\text{g/ml}$ , respectively. Data is representative of 3 independent experiments. **(C)** The  $EC_{50}$  of AM22 against A2 wild-type and K201N mutant virus is  $0.015 \pm 0.005 \mu\text{g/ml}$  and  $0.016 \pm 0.003 \mu\text{g/ml}$ , respectively. Data is representative of 3 independent experiments. **(D)** The  $EC_{50}$  of AM22 against 18537 wild-type and N201K mutant virus is  $0.054 \pm 0.005 \mu\text{g/ml}$  and  $0.046 \pm 0.003 \mu\text{g/ml}$ , respectively. Data are representative of three independent experiments.

**Supplementary Table 1** Crystallographic data collection and refinement statistics

	5C4 Fab	RSV F with 5C4 Fab
<b>PDB ID</b>	5W24	5W23
<b>Data Collection</b>		
Space group	<i>C</i> 2	<i>P</i> 4 <sub>1</sub> 2 <sub>1</sub> 2
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	117.0, 63.0, 56.5	183.4, 183.4, 275.9
$\alpha$ , $\beta$ , $\gamma$ (°)	90, 98.6, 90	90, 90, 90
Wavelength (Å)	1.000	0.979
Resolution (Å)	37.5–1.5 (1.53–1.50)*	34.1–3.4 (3.48–3.40)
<i>R</i> <sub>merge</sub>	0.044 (0.234)	0.380 (1.596)
<i>R</i> <sub>meas</sub>	0.051 (0.275)	0.406 (1.707)
<i>R</i> <sub>pim</sub>	0.026 (0.143)	0.140 (0.585)
<i>I</i> / $\sigma$ <i>I</i>	14.9 (3.8)	7.4 (1.9)
CC(1/2)	0.998 (0.938)	0.968 (0.462)
Completeness (%)	93.7 (69.7)	99.1 (99.2)
Redundancy	3.7 (3.5)	7.3 (7.2)
<b>Refinement</b>		
Resolution (Å)	32.9–1.5 (1.52–1.50)	34.1–3.4 (3.45–3.40)
Unique reflections	60,747 (2,024)	64,427 (2,773)
<i>R</i> <sub>work</sub> / <i>R</i> <sub>free</sub> (%)	17.0/20.3	19.8/23.9
No. atoms		
Protein	3,313	20,304
Solvent	6	8
Water	608	0
<i>B</i> -factors		
Protein	21.1	63.3
Solvent	25.3	106.0
Water	32.2	-
R.m.s. deviations		
Bond lengths (Å)	0.006	0.007
Bond angles (°)	0.85	1.05
Ramachandran		
Favored (%)	97.9	96.8
Allowed (%)	2.1	3.1
Outliers (%)	0	0
Clashscore	2.3	6.5

\*Values in parentheses are for highest-resolution shell.

**Supplementary Data 1. RSV F DS-Cav1 nucleotide sequences.**

**RSV A2\_F DS-Cav1, codon optimized**

ATGGAACTGCTGATCCTGAAGGCCAACGCAATTACCACTATCCTGACTGCCGTGACT  
TTTTGTTTCGCAAGCGGACAGAACATTACAGAGGAGTTCTACCAGTCAACATGCAGC  
GCCGTGTCCAAGGGATACCTGTCCGCTCTGCGAACCGGGTGGTATACATCTGTGATT  
ACTATCGAGCTGAGTAACATCAAGGAAAACAAATGTAATGGCACCGACGCAAAGGT  
GAAACTGATCAAGCAGGAGCTGGATAAGTACAAAAATGCCGTGACAGAACTGCAGC  
TGCTGATGCAGAGTACACCAGCAACTAACAAATCGCGCCAGGAGAGAGCTGCCCCGA  
TTCATGAACTATACCCTGAACAATGCTAAGAAAACCAATGTGACACTGTCTAAGAA  
ACGCAAGCGGCGCTTCCTGGGGTTCCTGCTGGGCGTGGGATCTGCCATCGCTAGTGG  
GGTGGCCGTCTGCAAAGTCCTGCACCTGGAGGGCGAAGTGAACAAGATCAAATCAG  
CTCTGCTGAGCACTAACAAAGGCAGTGGTCAGTCTGTCAAATGGAGTGAGCGTCCTGA  
CCTTTAAGGTGCTGGACCTGAAAAATTATATTGATAAGCAGCTGCTGCCTATCCTGA  
ACAAACAGAGCTGTTCCATTTCTAATATCGAGACAGTGATCGAGTTCAGCAGAAG  
AACAAATAGACTGCTGGAGATTACTCGGGAGTTCAGCGTGAACGCTGGCGTCACCAC  
ACCCGTGTCAACCTACATGCTGACAAATCCGAGCTGCTGTCTCTGATTAACGACAT  
GCCTATCACCAATGATCAGAAGAACTGATGTCTAACAAATGTGCAGATCGTCAGAC  
AGCAGAGTTATTCAATTATGTGCATCATTAAAGGAGGAGGTCCTGGCCTACGTGGTCC  
AGCTGCCACTGTATGGAGTGATCGACACCCCTGCTGGAAACTGCATACAAGCCCTC  
TGTGCACTACCAACACAAAGGAGGGGTCCAATATCTGCCTGACTCGAACCGACAGG  
GGGTGGTACTGTGATAACGCAGGCAGCGTGTCTTCTTTCCCCAGGCCGAGACCTGC  
AAGGTCCAGAGCAACCGGGTGTCTGTGACACTATGAATAGTCTGACCCTGCCTTCA  
GAAGTCAACCTGTGCAATGTGGACATCTTTAACCCAAAGTACGATTGTAAGATCATG  
ACTTCCAAGACCGATGTCAGCTCCTCTGTGATTACTAGCCTGGGAGCCATCGTGTCC  
TGCTACGGGAAGACAAAGTGTACTGCTTCTAACAAAAACCGCGGCATCATTAAAGAC  
CTTTTCAAACGGATGCGACTATGTCAGCAACAAGGGCGTGGATAACAGTGTGAGTCG  
GAAACACTCTGTACTATGTCAATAAGCAGGAGGGGAAAAGCCTGTACGTGAAGGGC  
GAACCAATCATTAACTTCTATGACCCCTGGTCTTCCCTAGCGACGAGTTTGATGCA  
TCTATTAGTCAGGTGAACGAAAAAATCAATCAGTCCCTGGCCTTTATTAGGAAGTCT  
GATGAGCTGCTGCACAACGTGAATGCTGGCAAATCCACAACATAACATCATGATCAC  
CACAATCATCATCGTGATTATCGTCATTCTGCTGTCTCTGATCGCTGTGGGACTGCTG  
CTGTACTGTAAGGCAAGAAGTACCCCGTGACCCTGAGCAAAGACCAGCTGAGCGG  
CATCAATAACATCGCCTTCTCTAACTAATGA

**RSV 18537\_F DS-Cav1, codon optimized**

ATGGAAGCTGCTGATTCATCGGAGTAGCGCAATTTTCCTGACACTGGCCGTGAACGCA  
CTGTACCTGACCTCATCCCAGAACATTACCGAGGAATTCTACCAGAGCACATGCTCC  
GCAGTGTCTAGGGGATACTTTAGCGCCCTGAGAACTGGGTGGTATAACCAGCGTGATT  
ACAATCGAGCTGTCCAATATCAAGGAAACCAAATGTAACGGAACAGACACTAAGGT  
GAAACTGATCAAGCAGGAGCTGGATAAGTATAAAAATGCCGTACAGAACTGCAGC  
TGCTGATGCAGAACTCCAGCCGCTAACAATCGAGCTAGGAGAGAGGCACCCCAG  
TACATGAACTATACCATCAATACCACAAAGAACCTGAATGTGAGCATCTCCAAGAA  
ACGCAAACGGCGCTTCCTGGGGTTTCTGCTGGGCGTGGGAAGCGCAATTGCCTCCGG  
GATCGCTGTGTGCAAGGTCCTGCACCTGGAGGGCGAAGTGAACAAGATCAAAAATG  
CCCTGCTGTCCACCAACAAGGCTGTGGTCTCTCTGAGTAACGGCGTGTCTGTCCTGA  
CATTCAAGGTGCTGGACCTGAAAACTACATTAACAATCGGCTGCTGCCTATCCTGA  
ATCAGCAGTCTTGTCGATTAGTAACATCGAGACTGTGATCGAATTCCAGCAGATGA  
ATAGTCGCCTGCTGGAGATCACCCGAGAATTTTCAGTGAACGCCGGCGTCACTACCC  
CTCTGAGCACTTACATGCTGACCAATTCAGAGCTGCTGAGCCTGATTAACGACATGC  
CAATCACCAATGATCAGAAGAACTGATGAGCTCCAACGTGCAGATCGTCAGGCAG  
CAGTCATATAGCATTATGTGCATCATTAAAGGAGGAAGTGCTGGCTTACGTGGTCCAG  
CTGCCAATCTACGGCGTCATCGACACTCCCTGCTGGAAGCTGCATACCAGTCCTCTG  
TGTACAACATAACATCAAGGAGGGAAGCAATATCTGCCTGACCAGAACAGACCGGGG  
GTGGTACTGTGATAACGCTGGCTCCGTGTCTTTCTTTCCCCAGGCAGACACATGCAA  
GGTGCAGTCCAATAGAGTCTTCTGTGATACAATGAACTCTCTGACTCTGCCTAGTGA  
AGTGTCACTGTGCAACACAGACATCTTTAATTCCAAGTACGATTGTAAGATCATGAC  
TTCTAAGACCGATATTTCTAGTTCAGTGATCACTAGTCTGGGAGCAATCGTCTCATG  
CTACGGGAAGACAAAATGTAAGTGCCTCCAACAAGAATCGGGGCATCATCAAGACCT  
TTTCTAATGGATGCGACTATGTCAGTAACAAGGGCGTCGATACCGTGTCTGTCTGGAA  
ACACACTGTACTATGTGAATAAGCTGGAGGGGAAAAACCTGTACGTCAAGGGCGAA  
CCAATCATTAACTACTATGACCCCTGGTGTTCCTAGCGACGAGTTTGATGCTAGT  
ATTTACAGGTCAACGAAAAGATCAATCAGTCACTGGCATTCAATTCGAAGGAGCGA  
TGAGCTGCTGCACAACGTGAATACCGGCAAAAGCACCACAAACATCATGATCACTA  
CCATCATCATCGTGATTATCGTGGTCTGCTGAGCCTGATTGCCATCGGACTGCTGCT  
GTATTGTAAGGCTAAAAACACCCCGTGACACTGTCCAAGGATCAGCTGTCTGGGAT  
TAACAATATCGCCTTTTCCAATGATGA