Supplementary Information

A Single Amino Acid Mutation Alters the Neutralization Epitopes in the Respiratory Syncytial Virus Fusion Glycoprotein

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Supplementary Table 1. Proportion of sequencing reads containing a Leucine or Isoleucine at amino acid position 305.[a]

Passage No.	Leucine 305	Isoleucine 305
0a	100.00%	0.00%
0b	100.00%	0.00%
1a	100.00%	0.00%
1b	86.55% (663/766)	13.45% (103/766)
3a	91.45% (3371/3686)	8.55% (315/3686)
5b	42.03% (153/364)	57.97% (211/364)

[a] Sequencing reads obtained from the experiment described in Figure 1a with RSV-A2 under polyclonal anti-RSV selective pressure. Biological replicates are denoted ‘a’ and ‘b’ beside the passage number. The proportion of sequencing reads representing each amino acid are in parentheses where an alternate amino acid was observed.

Supplemental Table 2. RSV-A2 mutations under polyclonal anti-RSV selective pressure.

		passage number						
		0a	0b	1a	1b	3a	5b	
position No.	wt AA (codon)	alternate codon expressed (% of reads)						alt AA (codon)
66	K (aaa)				27.27%	18.00%	22.73%	E (gaa)
144	V (gtt)	38.65%	26.63%	30.30%	22.12%	10.01%	13.59%	A (gca)
152	V (gtt)				10.23%		31.82%	I (att)
276	N (aac)				13.18%	15.19%	36.92%	S (agc)
305+	L (cta)				13.45%	8.55%	57.97%	I (ata)
559	L (cta)				5.34%	1.50%	60.32%	L (ttg)

Mutations that arise at a >25% frequency in the evolution experiment described in Figure 1a are shown. The wildtype (wt) codon at each position is indicated on the left and was determined by sequencing of the original RW30 plasmid. Alternate (alt) codons are indicated on the right. Percentages indicate the proportion of reads expressing the alt codon. Each biological replicate of the experiment is denoted by ‘a’ or ‘b’ beside the passage number. Spaces have been left blank if alt codons were undetectable or if insufficient sequencing reads were obtained in the indicated passage number. + indicates positions that differ between RSV subtypes A and B.

Supplementary Table 3. Amino acid percentage similarities between RSV-A and RSV-B subtypes isolated from Alberta, Canada and Ohio, USA.

RSV gene	RSV A	RSV B	RSV A vs B
Whole genomes	99.4%	99.5%	87.6%
NS1	99.7%	100%	85.9%
NS2	99.5%	99.5%	89.7%
N	99.95%	99.8%	95.7%
P	99.8%	99.8%	90.6%
M	99.9%	99.8%	92.4%
SH	99.7%	98.3%	69.4%
G	94.6%	97.0%	46.6%
F	99.7%	99.6%	90.4%
M2-1	99.8%	98.9%	92.1%
M2-2	99.0%	98.3%	56.8%
L	99.8%	99.8%	91.8%

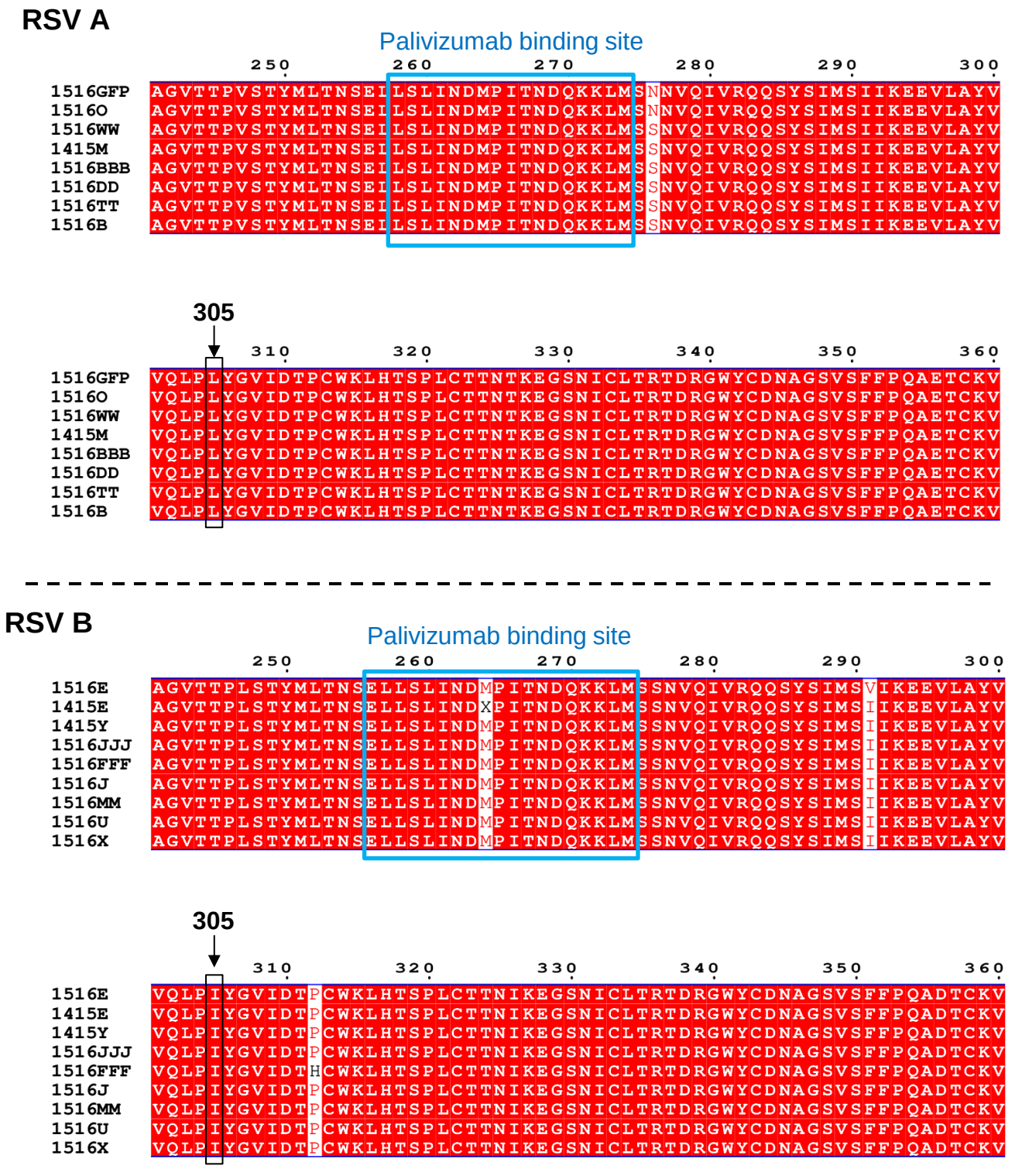
RSV-A and -B isolates (n=34) were sequenced via NGS and aligned with Geneious Prime Sotware. Whole RSV genomes and each of the 11 RSV genes were individually compared both among and between the RSV subtypes. F protein statistics are highlighted in grey. Percent similarities between RSV-A and -B were calculated as follows: AB=(2)(Total)-(1/2)(AA+BB).

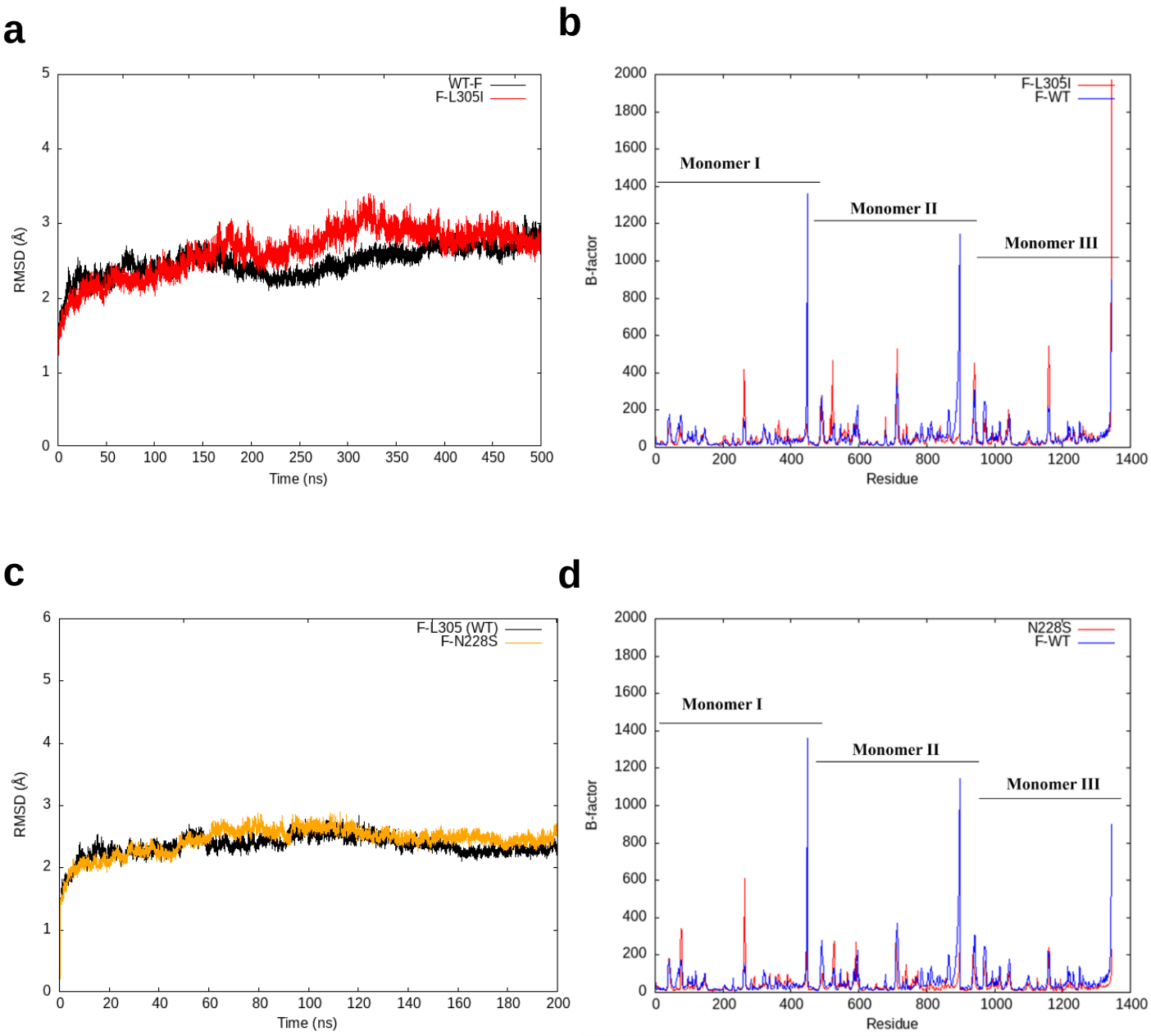
Supplemental Table 4. Proportion of NCBI published RSV-F sequences containing differences in amino acid consensus sequences between RSVA and RSVB subtypes.

Position No.	Amino Acid	RSVA		RSVB	
276	N	697	30.13%	44	3.07%
	S	1602	69.26%	1386	96.65%
305	L	2295	99.22%	9	0.63%
	I	8	0.35%	1416	98.74%

3747 complete RSV-F sequences were downloaded from The Virus Pathogen Resource (ViPR). Only sequences already sub typed into RSVA (2313) and RSVB (1434) were included in the analysis. Amino acid counts at the indicated positions were determined using Geneious Prime software. Total counts of sequence and percentage of total sequences containing a given amino acid are indicated for each RSV subtype.

Supplemental Table 5. RSVA and B clinical isolate sequences of RSV-F from amino acid positions 241-360. Clinical RSV isolates originating from Ohio, USA were subtyped via RT-qPCR and sequenced by NGS. Sequences were processed and aligned in Geneious Prime software. The trimmed portion of sequences is indicated for each isolate. Isoleucine and Leucine residues occupying position 305 are indicated in a black box. The Palivizumab binding site is indicated in a blue box. The RSV season from which the isolate was obtained is indicated in brackets beside the isolate name as follows: 1415 represents the 2014-2015 RSV season.





Supplementary Figure 1. Analysis of RSV-F MD simulation. a, Root mean square deviation (RMSD) from initial structure coordinates for RSV-F WT and RSV-F^{L305I} mutant for 500 ns simulation time. **b,** Atomic fluctuation as a function of B-factor and residues of WT and RSV-F^{L305I} protein (residue numbering exceeds 574 amino acids due to continous numbering of the residues by the AMBER package during the MD simulation). **c,** RMSD trend of the RSV-F^{N228S} mutant during a 200 ns simulation vs WT. **d,** Atomic fluctuation as a function of the RSV F trimer backbone of the RSV-F^{N228S} mutant (red) compared to the WT (blue).

Supplementary Table 6. Constants of the binding kinetics between monoclonal antibodies and DS-Cav1 recombinant proteins.

(mAb site)	constants	DS-Cav1^{L305}	DS-Cav1^{I305}
D25 (site Ø)	K_D (M)	8.35×10^{-10}	4.02×10^{-9}
	k_{on} ($M^{-1}s^{-1}$)	2.71×10^{5}	1.59×10^{5}
	k_{off} (s^{-1})	2.27×10^{-4}	6.94×10^{-4}
	X^2	0.27	0.11
	R_{max} (RU)	14.15	7.22
PZMB (site II)	K_D (M)	2.86×10^{-10}	1.03×10^{-10}
	k_{on} ($M^{-1}s^{-1}$)	5.29×10^{5}	6.45×10^{5}
	k_{off} (s^{-1})	1.51×10^{-4}	6.66×10^{-5}
	X^2	0.48	1.00
	R_{max} (RU)	20.53	41.73
101F (site IV)	K_D (M)	5.17×10^{-10}	3.15×10^{-10}
	k_{on} ($M^{-1}s^{-1}$)	6.71×10^{5}	8.21×10^{5}
	k_{off} (s^{-1})	3.47×10^{-4}	2.59×10^{-4}
	X^2	1.08	1.48
	R_{max} (RU)	33.60	38.18

Equilibrium dissociation constant (K_D), Chi-squared value (X^2), maximum response (R_{max}), dissociation constant (k_{off}), and association constant (k_{on}) from the binding affinity interactions of monoclonal antibodies D25, Palivizumab, and 101F to DS-Cav1^{L305} and DS-Cav1^{I305} recombinant proteins.

Supplemental Movie 1. Molecular Dynamics of the RSV F trimer. Overlay of the RSV-F^{L305} (gold) and RSV-F^{L305I} (green) MD simulations shows the conformational changes over time between the two trimers. The movie was generated using Chimera.