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Supplementary information

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A foldon-free prefusion F trimer vaccine for respiratory syncytial virus to reduce off-target immune responses

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Supplemental data to:

A foldon-free prefusion F vaccine for Respiratory Syncytial Virus by preventing trimerization-induced destabilization

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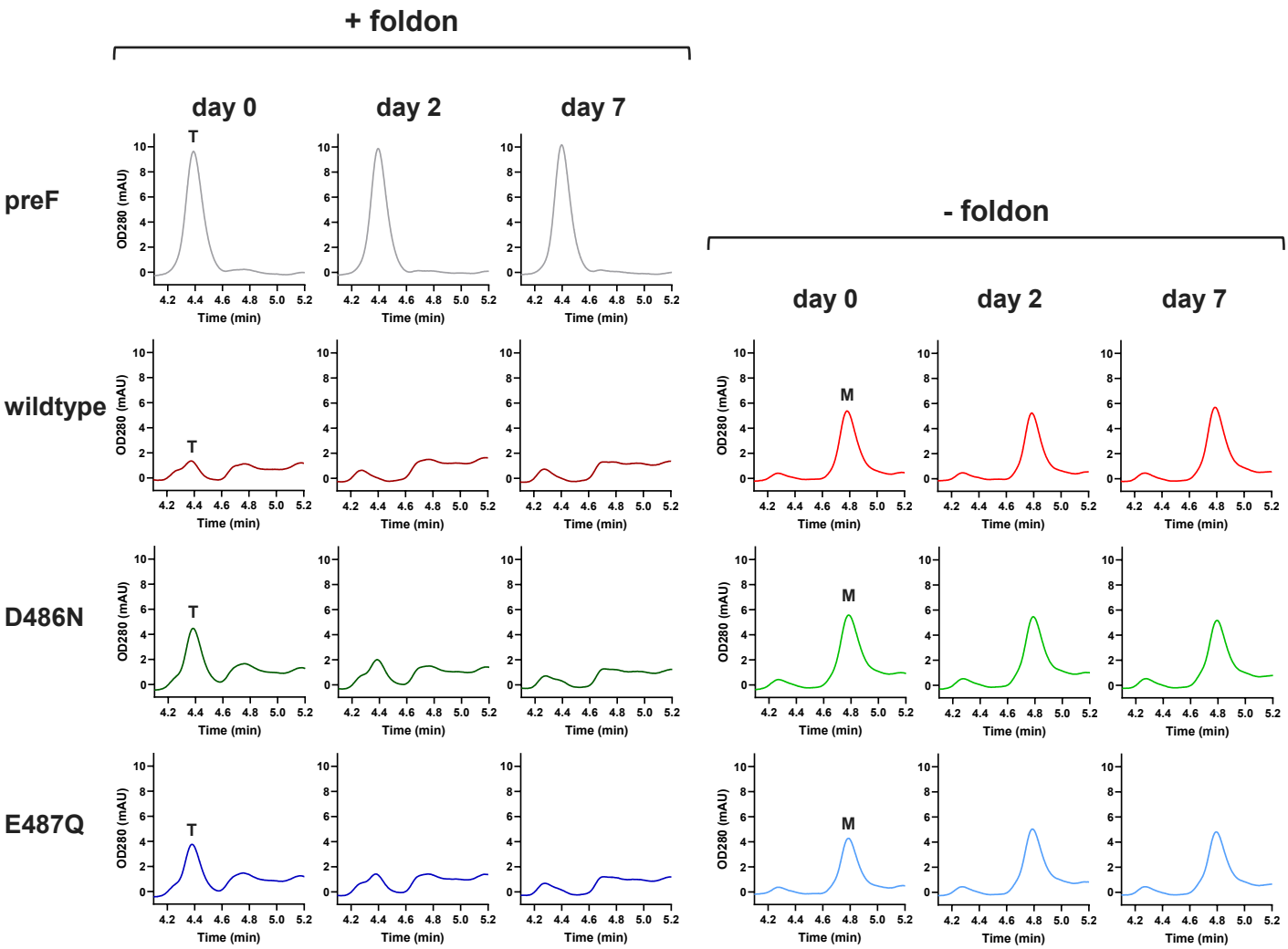
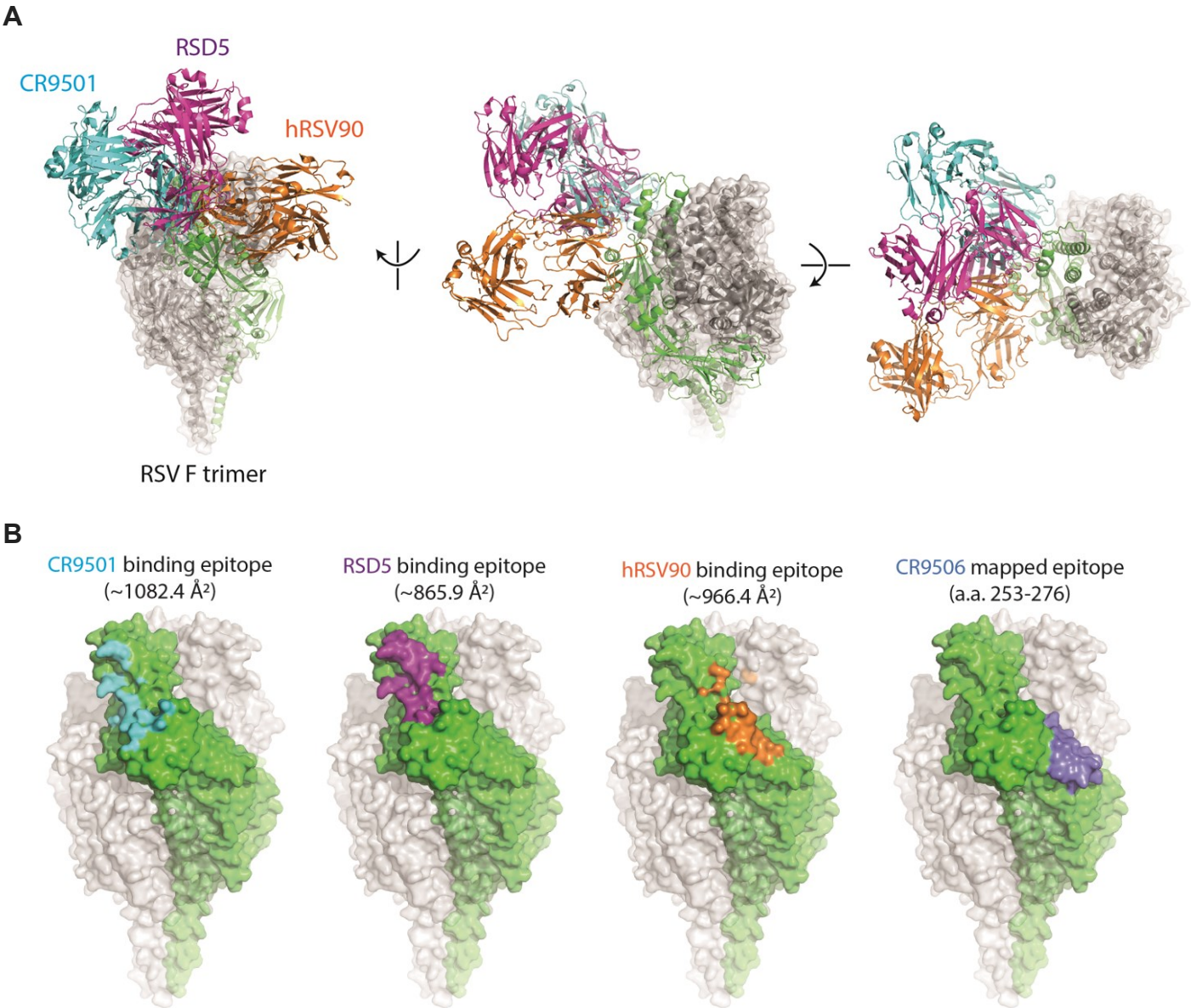
Figures S1 – S8

Table S1

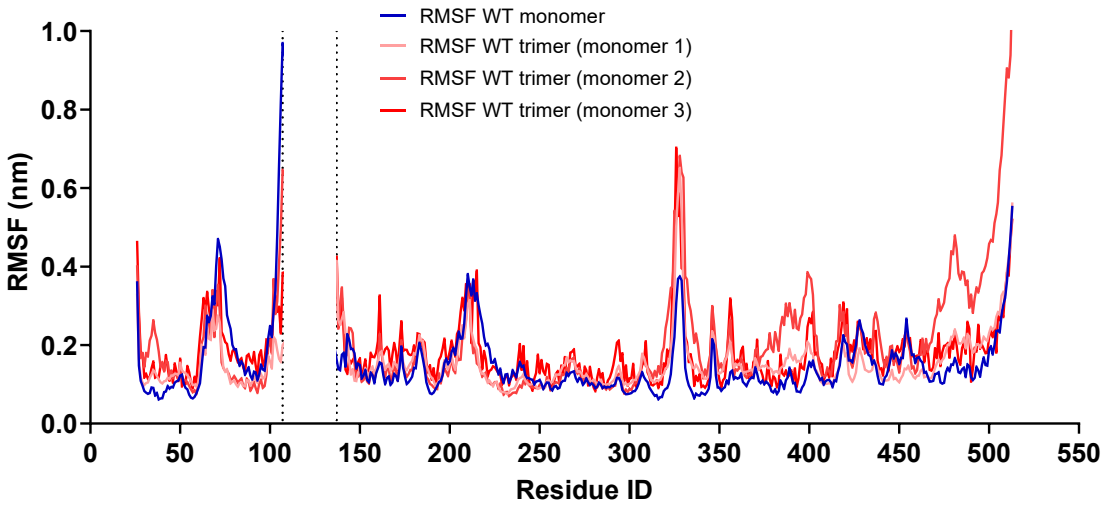


Supplementary Figure 1. Instability of RSV F trimers. Corresponding to data shown in Figure 1B. Analytical SEC analysis of RSV-A F protein variants expressed in the supernatant of Expi293F cells, as determined by analytical SEC. The expression pattern was measured three days after transfection at the day of harvest and after two and seven days storage at 4°C. As positive control for the preF trimer a stabilized (S215P, D486N) construct was taken along with a foldon ('preF'). Trimer (T) and monomer (M) fractions are indicated.

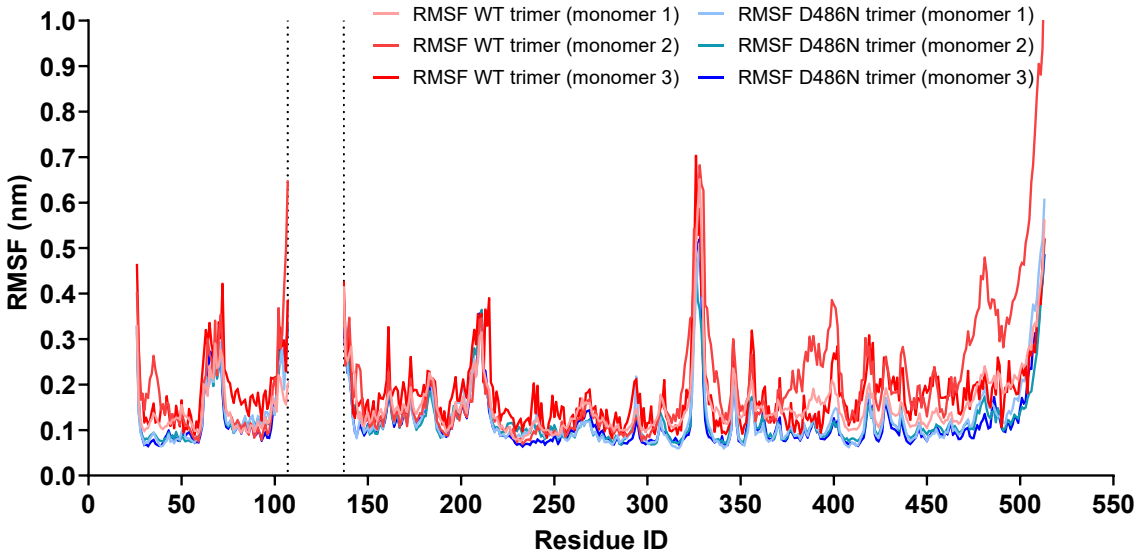


Supplementary Figure 2. Binding epitopes of monoclonal antibodies. (A) Ribbon presentation of the RSV F protein trimer in complex with antibodies CR9501, RSD5, and hRSV90 (shown in cyan, magenta, and orange, respectively). The binding RSV F monomer is shown in green and the other two in gray. (B) Surface representation of the RSV F protein highlighting the binding epitopes of the antibodies of (A) as well as the mapped epitope of CR9506.

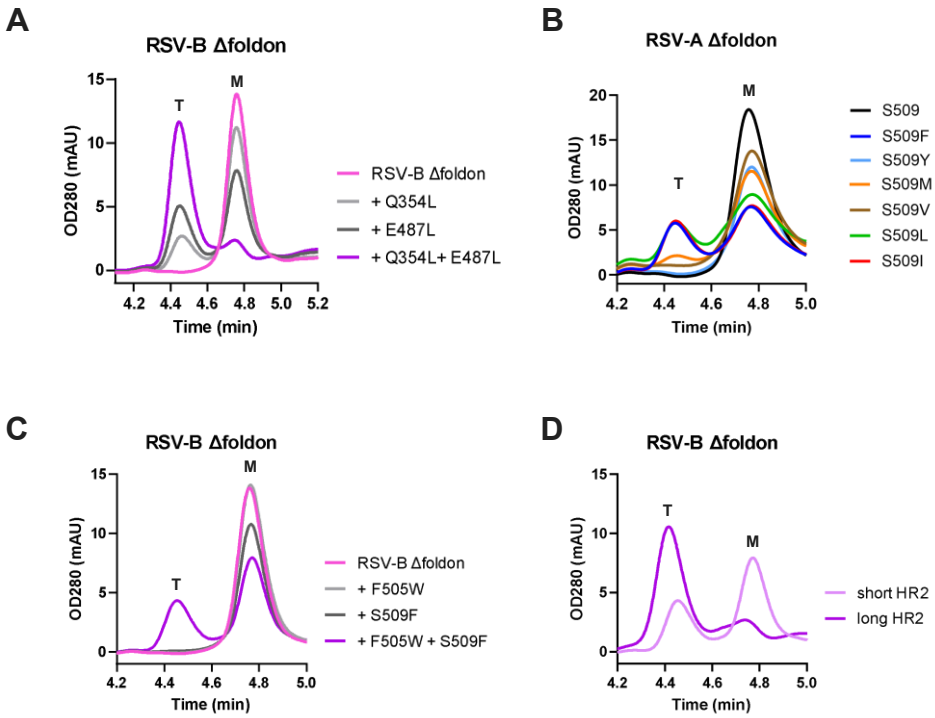
A Root mean square fluctuations (RMSF) of the wildtype monomer and trimer



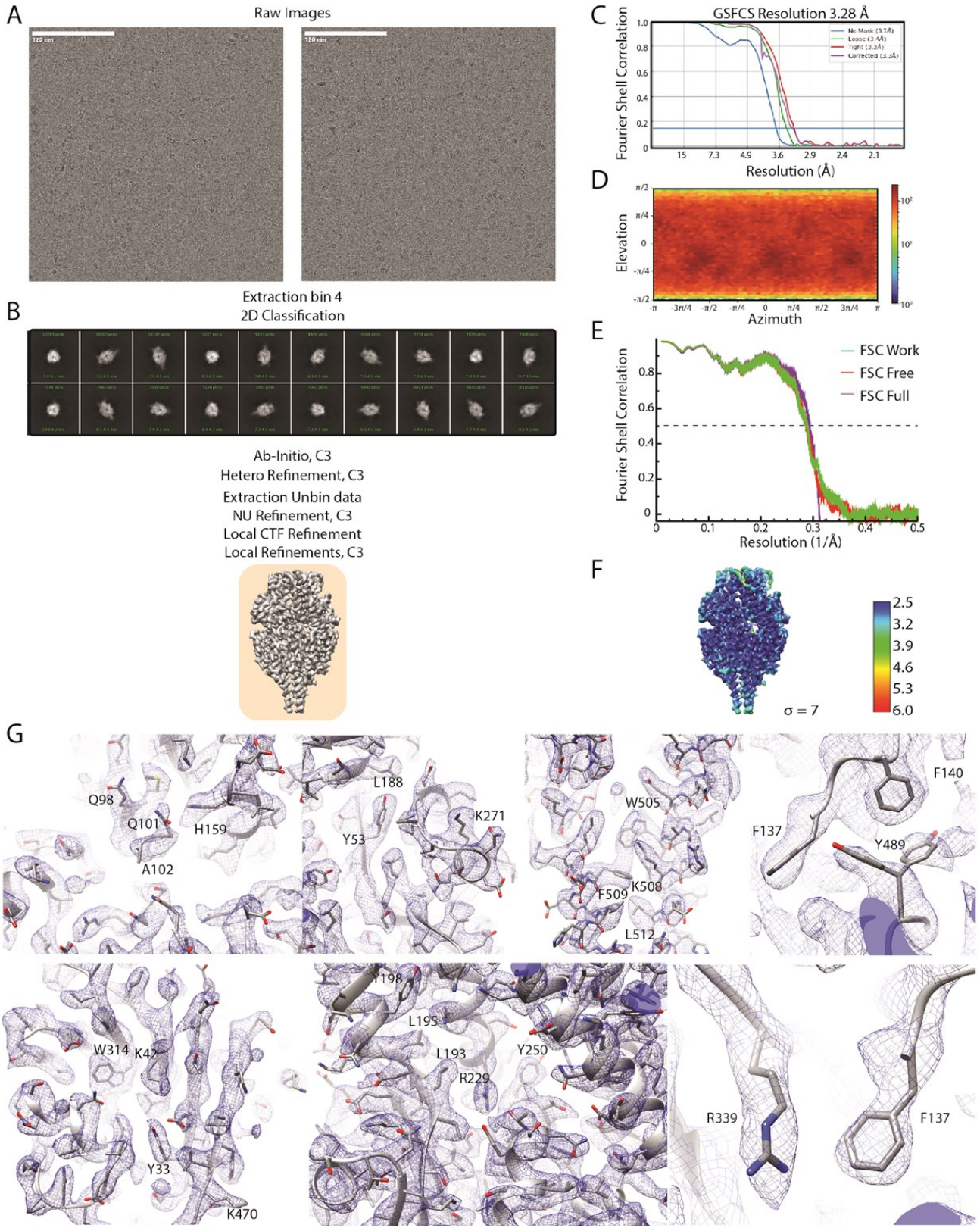
B Root mean square fluctuations (RMSF) of the wildtype and D486N mutant trimers



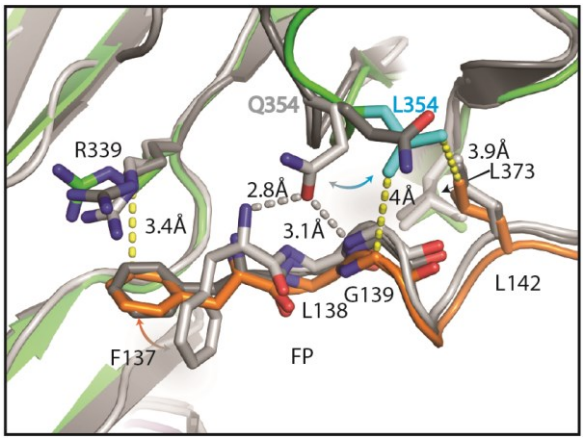
Supplementary Figure 3. Analysis of the root mean square fluctuations from molecular dynamics simulations. (A) Root mean square fluctuations (RMSF) of the wildtype (WT) monomer and trimer as a function of residue number. Three monomers of the trimer simulations were plotted separately. (B) Comparison of the RMSF for wildtype trimer and D486N mutant trimer. RMSFs of each monomer within the trimers were plotted separately.



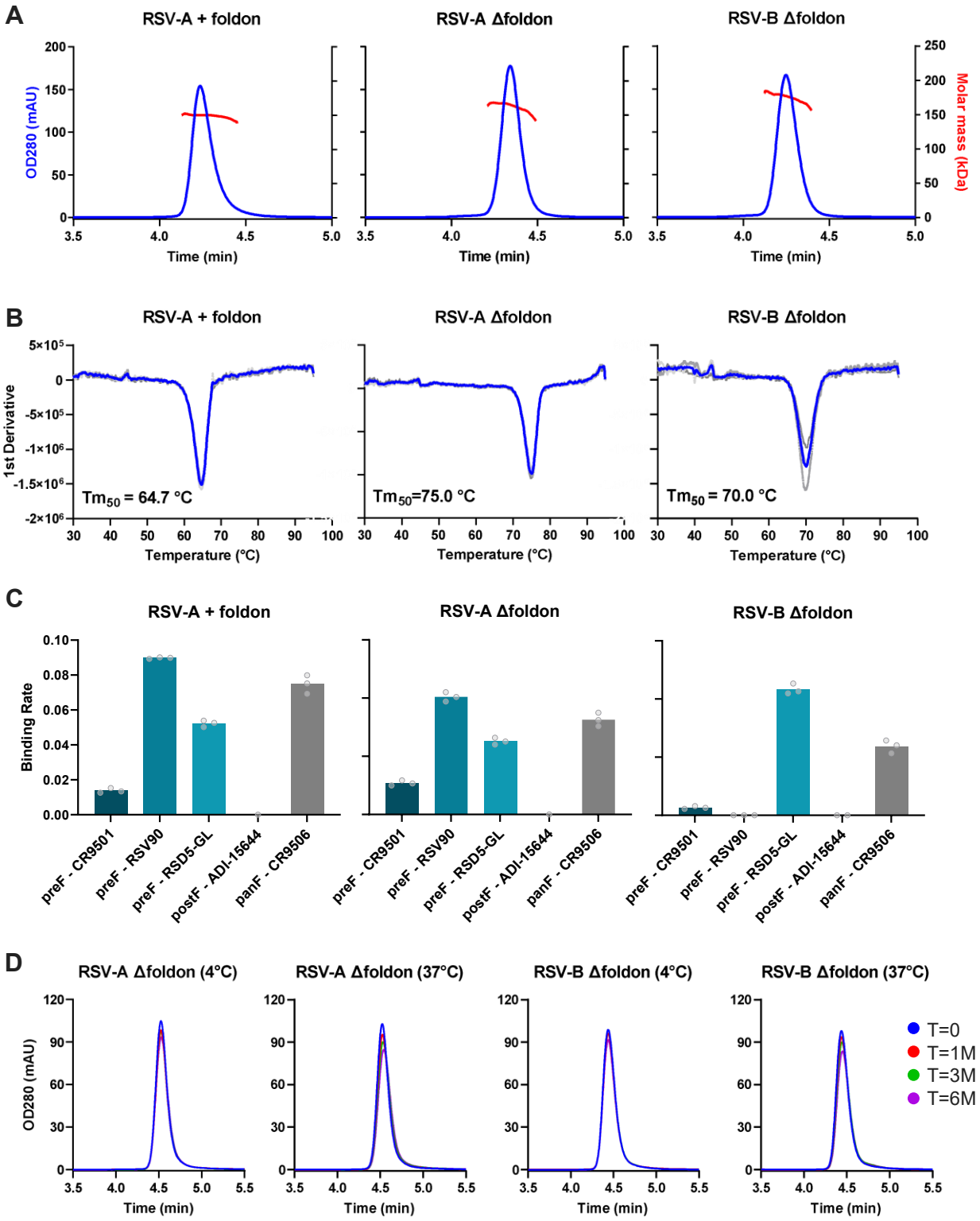
Supplementary Figure 4. Stable prefusion RSV F without a foldon domain through stabilization of the base and modification of the HR2 region. Analytical SEC analysis of RSV F proteins without a foldon domain (Δfoldon), as determined by analytical SEC in the supernatant of Expi293F cells. The approximate retention time of trimeric (T) or monomeric (M) F is indicated. (A) RSV-B Δfoldon backbone includes residues 1-513 and carries substitutions P101Q, I152M, L203I, S215P, D486N and D489Y. The additive effect of substitutions Q354L and/or E487L on trimerization is evaluated. (B) RSV-A Δfoldon backbone includes residues 1-524 and carries substitutions N67I, S215P, and D486N. The impact of hydrophobic substitutions at position S509 is assessed. (C) RSV-B Δfoldon backbone as described in (A). The additive effect of substitutions F505W and/or S509F on trimerization is evaluated. (D) RSV-B Δfoldon with either a short HR2 (ectodomain residues 1-513) or a long HR2 (ectodomain residues 1-524) and carrying substitutions P101Q, I152M, L203I, S215P, D486N, D489Y, F505W and S509F.



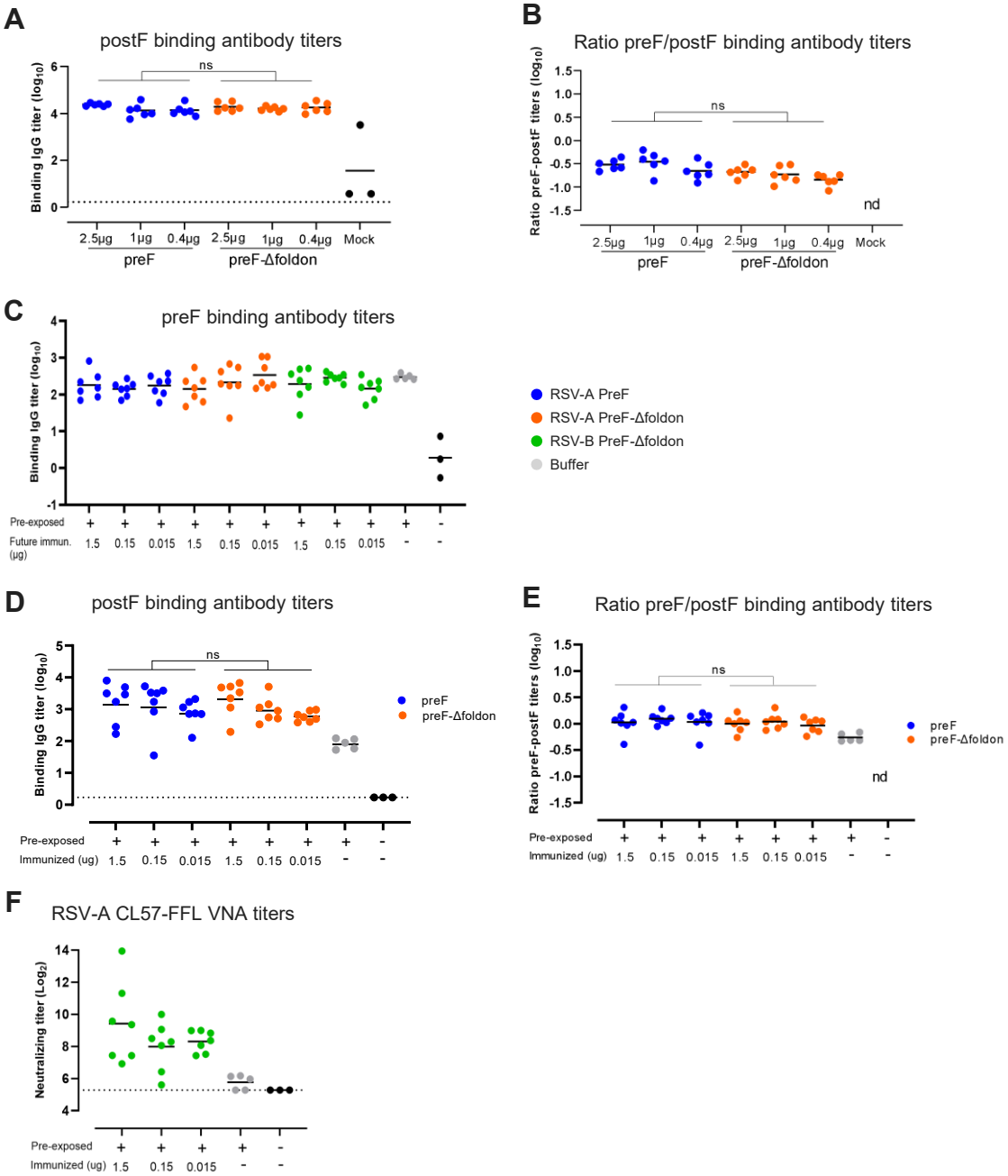
Supplementary Figure 5. Cryo-EM analysis of RSV-A preF Δfoldon prefusion trimer. (A) Reference raw images. Data collection was performed three times, a representative experiment is shown. (B) Flow chart of the cryo-EM data processing procedure. Details can be found in the Methods section. (C) Fourier shell correlation (FSC) curves of the RSV prefusion trimer complex structure with FSC as a function of resolution using cryoSPARC Local refinement outputs. (D) Angular orientation distribution of the particles used in the final reconstructions. The particle distribution is indicated by different color shades. (E) Comparison of the FSC curves between model and half map 1 (work), model and half map 2 (free), and model and full map are plotted in green, red, and magenta, respectively. (F) Local resolution of the map and colored as indicated. (G) Local cryo-EM densities at various regions of the RSV prefusion trimer complex structure.



Supplementary Figure 6. Stabilizing effect of Q354L. Overlay of two wildtype F proteins (light grey: PDB ID 4MMS, dark grey: PDB ID 4MMV), with stabilized RSV Pre-F (green) with the fusion peptide (orange) and stabilizing substitution Q354L (cyan) highlighted. The yellow dotted lines show the interactions within the stabilized RSV Pre-F structure between R339 and F137, and between 354L and G139/L373, with the latter substitution stabilizing the turn in the fusion peptide. The grey dotted lines show the interactions from the wildtype RSF Pre-F structure(PDBID:4MMS) between Q354 and main chains of G139/F137. Flexibility of the wildtype fusion peptide is illustrated by different orientations of F137 and Q354 side chains for representative examples PDB ID 4MMV (dark grey) and PDB ID 4MMS (light grey) [17].



Supplementary Figure 7. Characterization of RSV-A and RSV-B preF with or without a foldon domain. (A) SEC-MALS of RSV-A F trimer with (+ foldon) or without (Δfoldon) a foldon domain and RSV-B trimer without a foldon domain (Δfoldon). Δfoldon variants have a long HR2 (ectodomain residues 1-524) and contain stabilizing substitutions P101S, I152M, S215P, Q354L, D486N, E487L, D489Y, F505W and S509F. RSV-A F with foldon includes residues 1-513 and has stabilizing substitutions S215P, and D486N. The blue line indicates the OD280 signal and the red line the MALS signal. (B) Melting temperature (Tm50) of F variants described in (A), determined by differential scanning fluorimetry. Data is reported as average (blue line) of n=3 technical replicates (grey lines). (C) Antigenicity profile of RSV F variants described in (A), measured with biolayer interferometry. The specificity of the anti-RSV F antibodies is indicated as binding to the prefusion (preF) or postfusion (postF) conformation, or as pan-specific (panF), indicating binding to both prefusion and postfusion conformations. Binding rate is reported as average of three technical replicates and individual data points are represented as open circles. (D) Long-term stability of RSV F variants described in (A) as determined by SEC-MALS on proteins stored for 0, 1, 3, or 6 months at either 4°C or 37°C.



Supplementary Figure 8. Antibody responses in RSV pre-exposed and naïve mice.

(A, B) Balb/c mice were immunized at week 0 and week 4 with formulation buffer (Mock, n=3), or with $AS01_E$ adjuvanted preF or preF-Δfoldon protein in the doses indicated (n=6 per group). Serum samples were isolated 2 weeks after the second immunization. (A) PostF binding antibody titers were determined and (B) the ratio of preF and postF binding antibody titers, both expressed as relative potency, of samples having both values above LLOQ were calculated.

(C-F) Balb/c mice were intranasally pre-exposed with RSV A 18-001989, and immunized with unadjuvanted preF, RSV-A preF-Δfoldon or RSV-B preF-Δfoldon (n=7 per group) or were mock immunized (n=5) 20 weeks after pre-exposure. Naïve non pre-exposed mice (n=3) were included as control. (C) To confirm pre-exposure, preF binding antibody titers were determined in serum isolated at week 8 after pre-exposure, prior to immunization. (D) PostF binding antibody titers were determined in serum samples isolated 6 weeks after immunization, and (E) the ratio of preF and postF binding antibody titers of samples having both values above LLOQ were calculated. (F) RSV-A CL57 neutralizing antibody titers were determined in animals immunized with RSV-B preF-Δfoldon 20 weeks after pre-exposure or control animals using a FireFly luciferase (FFL) virus neutralization assay. Titers are given as the log2 of the IC90.

The black bars represent the mean of response of each group and the dotted line refers to the lower limit of qualification (LLOQ). All measurements under LLOQ were set at LLOQ. ns = not statistically significant. Statistical testing (two-sided) was performed across dose using a Tobit model (panels A, B), or using a Tobit model with Dunnett correction for multiple comparisons (panels D, E).

Supplementary Table 1. Cryo-EM data collection, refinement and validation statistics

	RSV-A preF-Δfoldon (EMDB-44117) (PDB 9B2X)
Data collection and processing	
Magnification	150,000x
Voltage (kV)	200
Electron exposure (e–/Å^2)	40.0
Defocus range (μm)	-0.8 to -2.4
Pixel size (Å)	0.91
Symmetry imposed	C3
Initial particle images (no.)	812,532
Final particle images (no.)	282,698
Map resolution (Å)	3.28
FSC threshold	0.143
Map resolution range (Å)	3.1-5.2
Refinement	
Initial model used (PDB code)	7UJA
Model resolution (Å)	3.23
FSC threshold	0.5
Model resolution range (Å)	3.1-4.2
Map sharpening *B* factor (Å^2)	-143.7
Model composition	
Non-hydrogen atoms	
Protein residues	1,380
Ligands	6
B factors (Å^2)	
Protein	38.8
Ligand	82.3
R.m.s. deviations	
Bond lengths (Å)	0.0038
Bond angles (°)	0.96
Validation	
MolProbity score	1.61
Clashscore	4.02
Poor rotamers (%)	0.47
Ramachandran plot	
Favored (%)	93.52
Allowed (%)	6.48
Disallowed (%)	0.00