

Supplementary Materials for

**Design and preclinical assessment of mRNA-1345 prefusion F glycoprotein-encoding mRNA vaccine
for respiratory syncytial virus**

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Supplementary Table 1. Serum neutralizing antibody GMFR at 1 month after vaccination with mRNA-1777, mRNA-1172, and mRNA-1345 in healthy adults

		mRNA-1777 ³⁰		mRNA-1172 ³¹		mRNA-1345 ^{45, 46}	
		RSV-A nAb GMFR (95% CI)	RSV-B nAb GMFR (95% CI)	RSV-A nAb GMFR range	RSV-B nAb GMFR range	RSV-A nAb GMFR (95% CI)	RSV-B GMFR (95% CI)
Younger adults	Placebo	1.2 (0.9, 1.6)	1.2 (0.9, 1.5)			0.9 (0.8, 1.1)	0.9 (0.7, 1.1)
	25 µg	1.6 (1.3, 1.9)	1.8 (1.5, 2.2)			N/A	N/A
	50 µg	N/A	N/A	4.0-6.4 ^b	2.4-.3.6 ^b	20.5 (13.6, 30.9)	14.4 (9.6, 21.8)
	100 µg	2.7 (2.1, 3.4)	2.4 (1.8, 3.4)			22.3 (14.9, 33.5)	11.7 (6.7, 20.3)
	200 µg	3.9 (3.1, 4.9)	3.1 (2.3, 4.3)			20.0 (14.0, 28.6)	13.9 (8.1, 23.9)
Older adults ^a	Placebo	1.2 (1.0, 1.4)	1.2 (0.9, 1.6)			1.2 (1.0, 1.3)	1.1 (1.0, 1.3)
	12.5 µg	N/A	N/A			10.2 (7.2, 14.5)	5.3 (3.7, 7.5)
	25 µg	1.7 (1.3, 2.3)	1.7 (1.3, 2.3)			12.2 (8.9, 16.6)	6.6 (4.9, 8.9)
	50 µg	N/A	N/A	3.7-7.8 ^c	2.5-4.3 ^c	12.0 (8.8, 16.5)	9.0 (6.8, 11.8)
	100 µg	4.3 (3.2, 5.9)	2.2 (1.7, 2.7)			14.1 (10.2, 19.5)	9.6 (7.3, 12.6)
	200 µg	3.0 (2.4, 3.9)	3.1 (2.2, 4.4)			16.5 (12.3, 22.3)	12.5 (9.1, 17.2)
	300 µg	3.6 (2.5, 5.1)	2.9 (2.2, 4.0)			N/A	N/A

^aOlder adults were aged 65-79 years for mRNA-1345 and 60-79 years for mRNA-1172 and mRNA-1777.

^bGMFR range across all administered doses (100 µg, 200 µg, and 300 µg).

^cGMFR range across all administered doses (25 µg, 100 µg, 200 µg, and 300 µg).

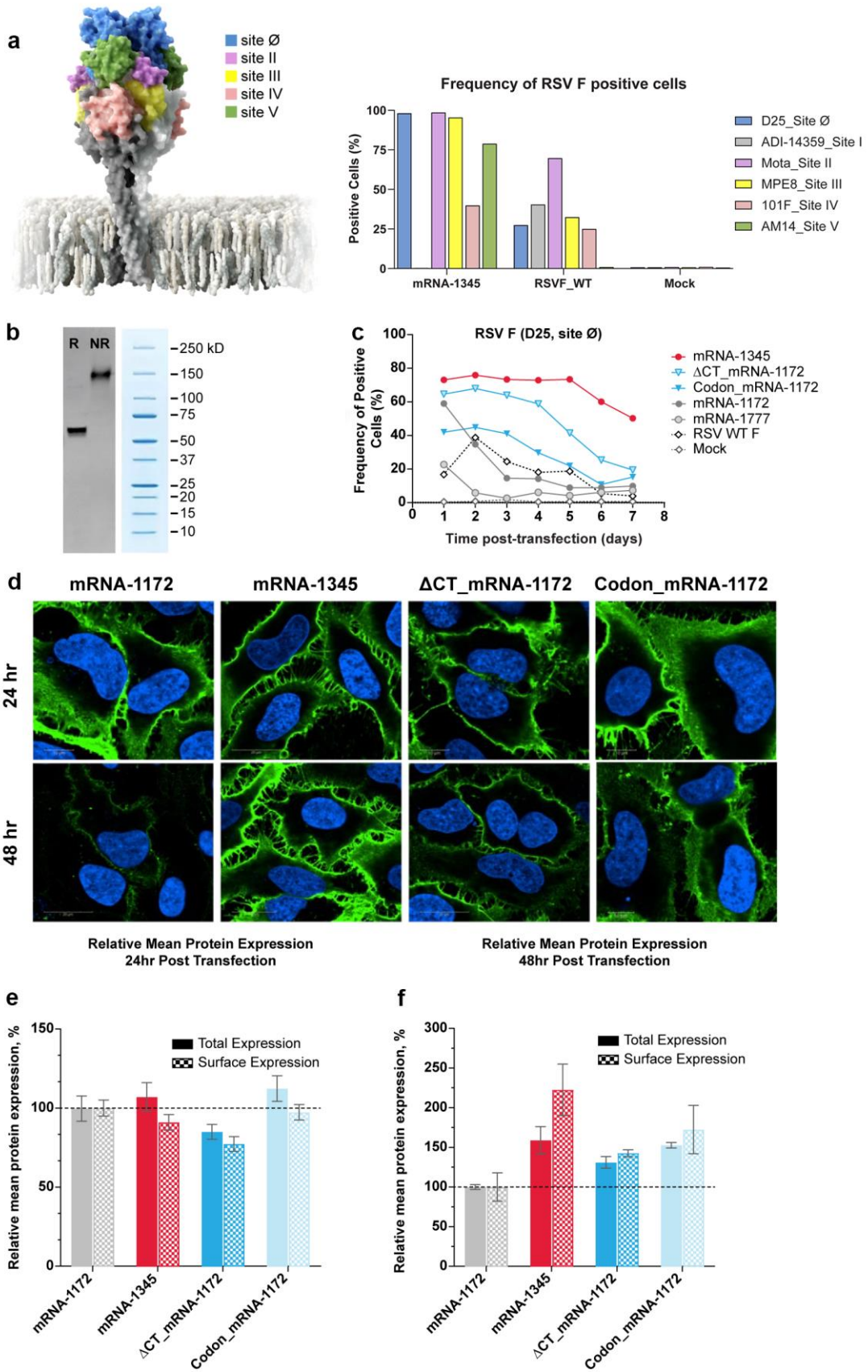
GMFR geometric mean fold rise, LNP lipid nanoparticle, nAb neutralizing antibody, N/A not assessed, RSV respiratory syncytial virus.

Supplementary Figures

Supplementary Figure 1. Experimental characterization of RSV F mRNA vaccine candidates.

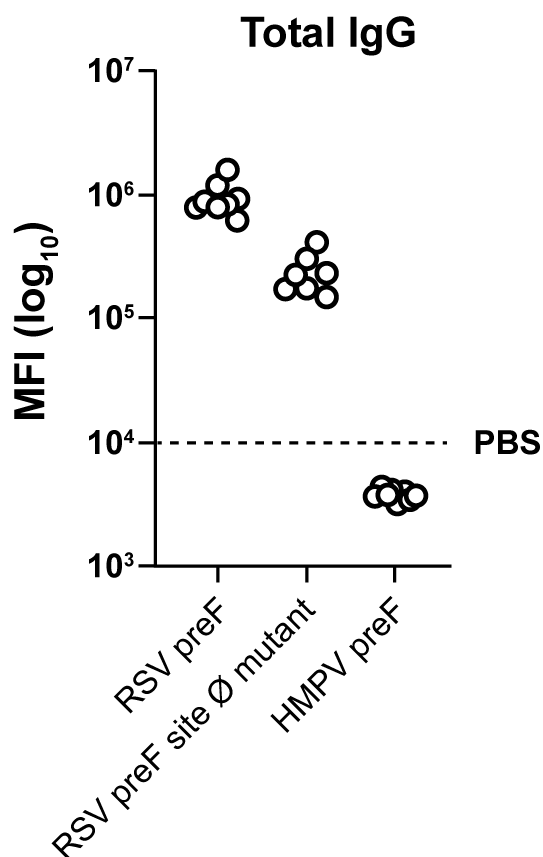
a The preF conformation of the mRNA-1345 encoded fusion protein was experimentally confirmed by transfecting Expi293T cells with RNA (500 ng per 1 million cells) and staining with a panel of RSV-F monoclonal antibodies 48 hours later followed by flow cytometry. PreF-specific mAbs AM14 (site V, trimer specific), D25 (site \emptyset), and MPE8 (site III) bind a substantial percentage of cells transfected with mRNA-1345, but bind a much lower percentage of cells transfected with mRNA encoding the native (wild-type) F protein (containing none of the preF-stabilizing mutations). In contrast, postF-specific mAb ADI-14359 (site I) binds the WT F protein, but not the mRNA-1345. Monoclonal antibodies motavizumab (site II) and 101F (site IV), specific for regions of the F protein that are structurally homologous between the preF and postF conformations, bind both mRNA-1345 and WT RSV-F proteins. These results indicate that the F protein encoded by mRNA-1345 is folded in a trimeric, prefusion conformation. **b** Western blot performed under reducing (R) and non-reducing (NR) conditions, showing the formation of interprotomer disulfide bonds in the preF glycoprotein antigen expressed from mRNA-1345. **c** Frequency of preF protein-positive cells through 7 days after mRNA transfection, using the antibody D25 and normalized to mock transfection. **d** Representative fluorescent microscopy images of HeLa cells transfected with mRNA-1172, mRNA-1345, Δ CT_mRNA-1172, and codon_mRNA-1172. Images were taken at 24 and 48 hours post-transfection to show surface expression. All images are set to the same scale (63x). Green staining indicates preF protein, and blue staining indicates the cell nucleus. Mean \pm standard error of the preF protein expression at 24 hours **e** and 48 hours **f** on the cell surface and total expression normalized to mRNA-1172. Dashed line is 100% (i.e., the level for mRNA-1172; other constructs were normalized to mRNA-1172).

Abbreviations: Δ CT deletion of cytoplasmic tail, *mAb* monoclonal antibody, *NR* non-reducing, *R* reducing; *RSV* respiratory syncytial virus, *WT* wild-type.



Supplementary Figure 2. Specificity of serum antibody response in BALB/c mice vaccinated with mRNA-1345 at day 36 (14 days after dose 2). A portion of preF IgG antibodies induced by mRNA-1345 are directed to the antigenic site zero (\emptyset). Antigenic site \emptyset specificity was evaluated using RSV F protein with amino acid substitutions K65N-N67T-P205N-V207T-K209N-S211T (site \emptyset mutation) that create three N-linked glycosylation sites and block the binding of site-specific mAb D25. Serum from mRNA-1345 vaccinated mice does not bind HMPV-A preF protein, demonstrating the specificity of the response.

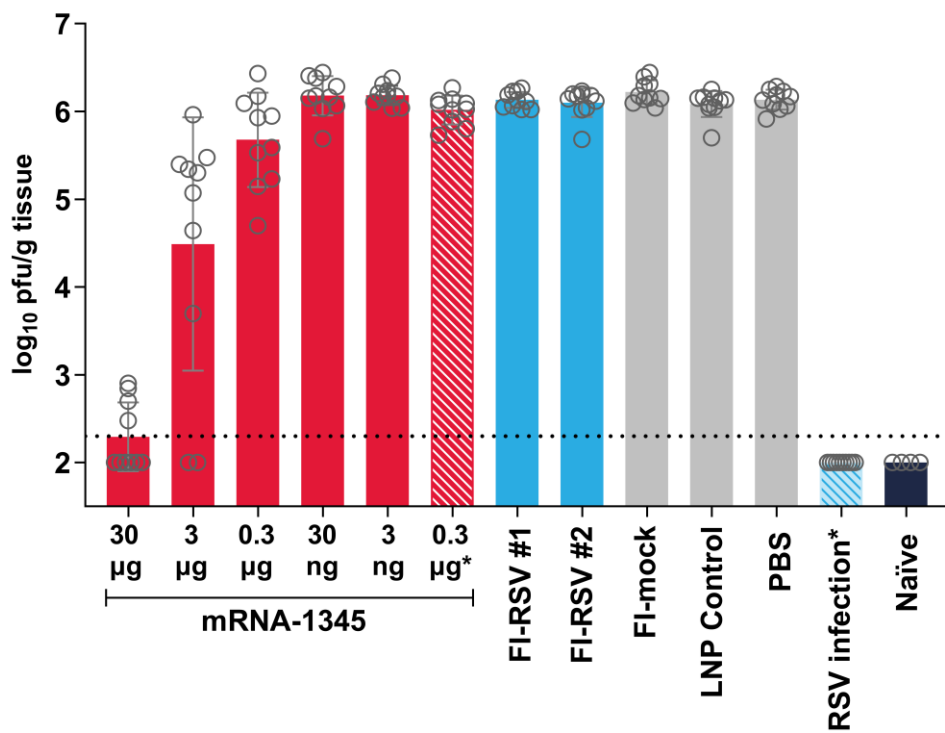
Abbreviations: IgG immunoglobulin G, HMPV human metapneumovirus, mAb monoclonal antibody, MFI median fluorescent intensity, preF prefusion F protein, PBS phosphate-buffered saline, RSV respiratory syncytial virus.



Supplementary Figure 3. Nose viral titers after RSV challenge post vaccination with mRNA-1345 in cotton rats. Mean \pm standard deviation nose viral load 5 days after RSV challenge. Naïve, N=4; all other groups, N=10.

*Single vaccination (day 0); the remaining groups received two doses (days 0 and 28).

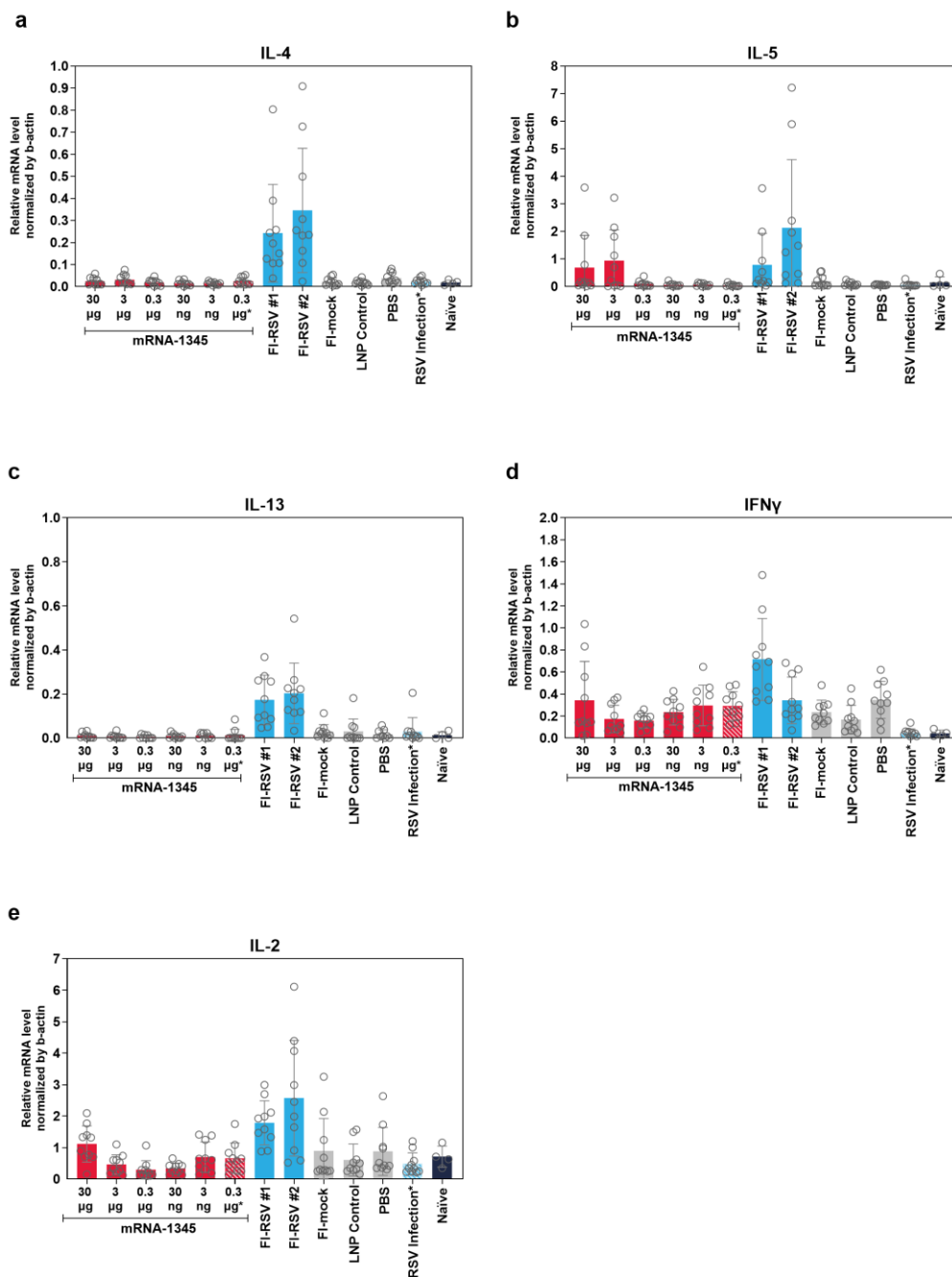
Abbreviations: FI formalin-inactivated, LNP lipid nanoparticle, PBS phosphate-buffered saline, RSV respiratory syncytial virus.



Supplementary Figure 4. Post-infection characterization of lung cytokine mRNA profiles in cotton rats vaccinated with different doses of mRNA-1345 or other vaccines. a IL-4. b IL-5. c IL-13. d INF γ . e IL-2.

*Single vaccination (day 0); the remaining groups received two doses (days 0 and 28).

Abbreviations: IL interleukin, INF γ interferon gamma, FI formalin-inactivated, LNP lipid nanoparticle, PBS phosphate-buffered saline, *preF* prefusion F protein, RSV respiratory syncytial virus.



Supplementary Figure 5. Post-infection lung histopathology in cotton rats vaccinated with different doses of mRNA-1345 or other vaccines. a Alveolitis (cells within the alveolar spaces). **b** Interstitial Pneumonia (inflammatory cell infiltration and thickening of alveolar walls). **c** Perivasculitis (inflammatory cell infiltration around the small blood vessels). **d** Peribronchiolitis (inflammatory cell infiltration around the bronchioles).

*Single vaccination (day 0); the remaining groups received two doses (days 0 and 28).

Abbreviations: FI formalin-inactivated, *LNP* lipid nanoparticle, *PBS* phosphate-buffered saline, *RSV* respiratory syncytial virus.

