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Supplementary information

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Mucosal adenovirus vaccine boosting elicits IgA and durably prevents XBB.1.16 infection in nonhuman primates

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Group	Fold-increase of serum anti-XBB.1.16 IgG binding titers
mRNA-1273 (IM) x2 + mRNA-1273.222 (IM)	2.06
mRNA-1273 (IM) x2 + Bivalent ChAd (IN)	2.51
mRNA-1273 (IM) x2 + Bivalent ChAd (AE)	0.67
Bivalent ChAd (AE) x 1	4.03

Supplementary Table 1. Relative increase in serum titers to XBB.1.16 following challenge

Sera were collected pre- (week 48) and post- (day 15) XBB.1.16 challenge. Fold-increase in anti-XBB.1.16 IgG GMT in AU / mL described. AU below a value of 1 were replaced with a value of 1 prior to calculation. Related to **Fig. 2g**.

a

S-specific memory B cell frequency Week 8 (post-dose 2) vs. Week 36 (post-boost)				
Group	**BAL**		**PBMC**	
	p	*Fold-change*	*p*	*Fold-change*
IM boost	>0.05	1.07	0.0078	1.72
IN boost	>0.05	2.05	0.0313	0.45
AE boost	0.0313	19.99	>0.05	0.68

b

S-specific memory B cell frequency Week 36 (post-boost) for indicated group in comparison to IM boost group				
Group	**BAL**		**PBMC**	
	p	*Fold-change*	*p*	*Fold-change*
IM boost	-	-	-	-
IN boost	>0.05	0.76	0.0007	0.22
AE boost	0.0007	20.37	0.0007	0.25
AE prime	0.0040	13.31	0.0040	0.28

Supplementary Table 2. AE immunization preferentially expands antigen-specific memory B cell compartment in the lungs

(**a**) Two-sided Wilcoxon signed-rank tests conducted for S-specific memory B cell frequencies at week 36 (post-boost) compared to week 8 (post-dose 2) per group, and (**b**) two-sided Wilcoxon rank-sum tests conducted for each indicated vaccinated cohort in comparison to IM boosted benchmark at week 36 (post-boost). *p* values and fold-changes in geometric mean frequency indicated. S-specific frequencies of 0% were set to half the minimum of the non-zero data prior to calculation of fold-change. Statistically significant increased B cell frequencies are indicated in red while significant decreases are indicated in orange. Related to **Fig. 3**.

a

BAL IgG									
Group		IM boost		IN boost		AE boost		AE prime	
Comparison		p value	Fold-change	p value	Fold-change	p value	Fold-change	p value	Fold-change
WA1	Post-boost Wk 34 vs 28	0.0078	376.00	0.0313	89.51	0.0313	361.12	-	-
	Prechallenge Wk 48 vs 34	0.0078	0.20	>0.05	0.66	>0.05	0.70	>0.05	7.45
BA.5	Post-boost Wk 34 vs 28	0.0078	124.55	0.0313	25.45	0.0313	103.60	-	-
	Prechallenge Wk 48 vs 34	0.0078	0.23	>0.05	0.57	>0.05	0.73	>0.05	24.85
XBB.1.16	Post-boost Wk 34 vs 28	0.0078	88.25	0.0313	19.45	0.0313	87.08	-	-
	Prechallenge Wk 48 vs 34	0.0078	0.22	>0.05	0.56	>0.05	0.65	>0.05	19.77

b

BAL IgA									
Group		IM boost		IN boost		AE boost		AE prime	
Comparison		p value	Fold-change	p value	Fold-change	p value	Fold-change	p value	Fold-change
WA1	Post-boost Wk 34 vs 28	>0.05	2.64	0.0313	28.83	0.0313	1323.51	-	-
	Prechallenge Wk 48 vs 34	>0.05	0.58	>0.05	0.96	>0.05	1.04	>0.05	2.23
BA.5	Post-boost Wk 34 vs 28	0.0078	1.89	0.0313	13.00	0.0313	537.50	-	-
	Prechallenge Wk 48 vs 34	>0.05	0.67	>0.05	0.75	>0.05	1.44	>0.05	10.80
XBB.1.16	Post-boost Wk 34 vs 28	>0.05	1.00	0.0313	7.50	0.0313	346.74	-	-
	Prechallenge Wk 48 vs 34	>0.05	1.06	>0.05	0.89	>0.05	1.53	>0.05	12.62

c

NW IgG									
Group		IM boost		IN boost		AE boost		AE prime	
Comparison		p value	Fold-change	p value	Fold-change	p value	Fold-change	p value	Fold-change
WA1	Post-boost Wk 34 vs 28	0.0391	6.96	>0.05	1.90	>0.05	1.10	-	-
	Prechallenge Wk 48 vs 34	>0.05	0.51	>0.05	2.21	>0.05	3.11	>0.05	14.26
BA.5	Post-boost Wk 34 vs 28	0.0156	9.24	>0.05	1.99	>0.05	1.20	-	-
	Prechallenge Wk 48 vs 34	>0.05	0.44	>0.05	2.03	>0.05	2.91	>0.05	87.22
XBB.1.16	Post-boost Wk 34 vs 28	0.0391	4.58	>0.05	1.89	>0.05	1.12	-	-
	Prechallenge Wk 48 vs 34	>0.05	0.38	>0.05	1.95	>0.05	2.45	>0.05	24.94

d

NW IgA									
Group		IM boost		IN boost		AE boost		AE prime	
Comparison		p value	Fold-change	p value	Fold-change	p value	Fold-change	p value	Fold-change
WA1	Post-boost Wk 34 vs 28	>0.05	2.26	>0.05	12.63	>0.05	4.24	-	-
	Prechallenge Wk 48 vs 34	>0.05	0.37	0.0313	4.09	>0.05	1.34	>0.05	4.29
BA.5	Post-boost Wk 34 vs 28	>0.05	2.02	>0.05	10.97	>0.05	3.85	-	-
	Prechallenge Wk 48 vs 34	0.0078	0.25	0.0313	4.15	>0.05	1.51	>0.05	13.78
XBB.1.16	Post-boost Wk 34 vs 28	>0.05	1.87	>0.05	9.41	>0.05	2.99	-	-
	Prechallenge Wk 48 vs 34	0.0781	0.27	0.0313	4.27	>0.05	1.36	>0.05	2.48

Supplementary Table 3. Durable boost in mucosal antibodies following mucosal vaccination

BAL (**a-b**) and NW (**c-d**) were collected pre-boost (week 28), post-boost (week 34) and pre-challenge (week 48). IgG (**a, c**) and IgA (**b, d**) binding titers in AU / mL measured to WA1, BA.5 or XBB.1.16 S as indicated. AU below a value of 1 were replaced with a value of 1 before

calculation of fold-change in anti-S antibody GMT. AE prime cohort received no vaccine prior to the initial regimen at week 32 and thus responses were not measured at week 28. Note that pre-boost data is derived per individual group and is used for determination of fold-changes whereas fold-changes in the text reflect the combined values across all mRNA-primed cohorts prior to boost. Comparisons between timepoints were done using two-sided Wilcoxon signed-rank tests. Statistically significant increases in antibody titers are indicated in red while significant contractions are indicated in orange. Related to **Fig. 4**.

a

BAL ACE2 – variant S binding inhibition										
Group		IM boost		IN boost		AE boost		AE prime	
Comparison		p value	Fold-change	p value	Fold-change	p value	Fold-change	p value	Fold-change
WA1	Post-boost Wk 34 vs 28	0.0078	8.53	>0.05	1.71	0.0313	13.93	-	-
	Prechallenge Wk 48 vs 34	0.0078	0.30	>0.05	0.42	>0.05	0.94	>0.05	0.56
BA.5	Post-boost Wk 34 vs 28	>0.05	3.54	N.D.	1.00	>0.05	32.80	-	-
	Prechallenge Wk 48 vs 34	>0.05	0.28	N.D.	1.00	>0.05	0.89	>0.05	5.63
XBB.1.16	Post-boost Wk 34 vs 28	>0.05	0.70	0.0313	5.07	>0.05	2.35	-	-
	Prechallenge Wk 48 vs 34	>0.05	0.13	0.0313	2.37	>0.05	0.98	>0.05	0.62

b

NW ACE2 – variant S binding inhibition									
Group		IM boost		IN boost		AE boost		AE prime	
Comparison		p value	Fold-change	p value	Fold-change	p value	Fold-change	p value	Fold-change
WA1	Post-boost Wk 34 vs 28	>0.05	1.25	>0.05	0.65	>0.05	0.65	-	-
	Prechallenge Wk 48 vs 34	>0.05	0.85	0.0313	3.40	>0.05	2.18	>0.05	0.24

c

BAL immunoglobulin class depletion									
Group		IM boost		IN boost		AE boost		AE prime	
Comparison		p value	Fold-change	p value	Fold-change	p value	Fold-change	p value	Fold-change
IgG Depletion	WA1	0.0078	0.11	>0.05	0.93	>0.05	0.37	>0.05	1.16
	BA.5	>0.05	0.54	>0.05	0.84	>0.05	0.49	>0.05	0.62
	XBB.1.16	0.0078	0.21	N.D.	1.00	0.0313	0.21	>0.05	0.86
IgA Depletion	WA1	>0.05	1.48	>0.05	1.85	>0.05	0.87	>0.05	0.57
	BA.5	>0.05	1.68	>0.05	0.88	>0.05	0.56	>0.05	1.19
	XBB.1.16	0.0391	0.57	N.D.	1.00	0.0313	0.37	>0.05	0.90

d

NW immunoglobulin class depletion									
Group		IM boost		IN boost		AE boost		AE prime	
Comparison		p value	Fold-change	p value	Fold-change	p value	Fold-change	p value	Fold-change
IgG Depletion	WA1	0.0156	0.32	>0.05	0.93	0.0313	0.62	>0.05	1.24
IgA Depletion	WA1	>0.05	0.74	0.0313	0.13	>0.05	0.55	>0.05	0.69

Supplementary Table 4. IgG and IgA contribute to protective airway responses in mucosally vaccinated NHP

BAL (**a, c**) and NW (**b, d**) were collected pre-boost (week 28), post-boost (week 34) and pre-challenge (week 48) for longitudinal analysis in **a-b** and at a single late memory timepoint (week 44) for immunoglobulin class depletion in **c-d**. WA1, BA.5 and XBB.1.16 S binding to ACE2 was measured with and without the addition of mucosal fluids to determine percentage inhibition as a surrogate for neutralizing antibodies. (**a-b**) Fold-changes in median binding inhibition shown. Note that pre-boost data is derived per individual group and is used for determination of fold-changes whereas fold-changes in the text reflect the combined values across all mRNA-primed cohorts prior to boost. (**c-d**) Fold-changes in median binding inhibition shown for complete fluid and depleted fluid. Any group with median inhibition below 1% was given a value of 1% before calculation of fold-change. AE prime cohort received no vaccine prior to the initial regimen at week 32 and thus fold-changes between pre- and post-boost timepoints are not reported. N.D. or "Not Determined" refers to samples for which there were insufficient differences (less than 3 unique values) between timepoints. Comparisons between timepoints were done using two-sided Wilcoxon signed-rank tests. Statistically significant increases in inhibitory responses are indicated in red while significant reductions are indicated in orange. Related to **Fig. 5**.

a

S-specific T_H1 frequency								
Tissue	PBMC				BAL			
Group	IM boost	IN boost	AE boost	AE prime	IM boost	IN boost	AE boost	AE prime
Comparison	Fold-change				Fold-change			
Post-boost Wk 34 vs 6	0.96	0.74	1.09	-	0.56	1.59	12.81	-
Pre-challenge Wk 48 vs 34	0.89	1.00	1.19	1.00	1.21	0.74	1.97	1.06
Post-challenge Day 4 vs Wk 48	0.95	1.00	0.92	N.D.	1.07	1.30	0.65	0.36
Post-challenge Day 15 vs Wk 48	1.24	1.06	0.85	1.66	1.73	5.66	0.90	1.58

b

S-specific $CD8^+$ T cell frequency								
Tissue	PBMC				BAL			
Group	IM boost	IN boost	AE boost	AE prime	IM boost	IN boost	AE boost	AE prime
Comparison	Fold-change				Fold-change			
Post-boost Wk 34 vs 6	0.83	1.00	1.13	-	0.95	1.11	7.75	-
Pre-challenge Wk 48 vs 34	0.93	1.00	1.53	1.40	0.63	0.96	9.27	2.03
Post-challenge Day 4 vs Wk 48	1.48	1.00	0.75	N.D.	1.18	1.11	0.70	0.40
Post-challenge Day 15 vs Wk 48	1.41	1.02	0.76	0.86	2.53	4.44	0.73	0.54

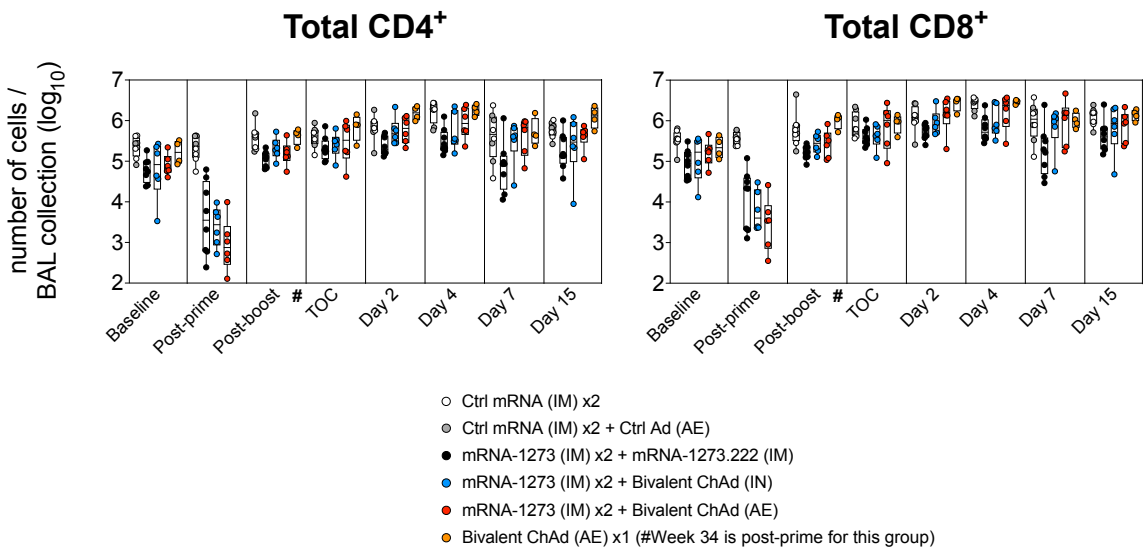
c

S-specific T_H2 and T_{FH} frequencies								
Tissue	PBMC – T_H2				PBMC - T_{FH}			
Group	IM boost	IN boost	AE boost	AE prime	IM boost	IN boost	AE boost	AE prime
Comparison	Fold-change				Fold-change			
Post-boost Wk 34 vs 6	1.00	1.00	1.00	-	0.72	0.40	1.43	-
Pre-challenge Wk 48 vs 34	1.00	1.00	1.00	1.00	0.46	0.63	0.85	0.60

Post-challenge Day 4 vs Wk 48	1.00	1.00	1.00	N.D.	0.62	0.69	0.46	N.D.
Post-challenge Day 15 vs Wk 48	1.00	1.00	1.00	1.00	1.83	2.32	0.57	1.04

Supplementary Table 5. AE immunization preferentially expands antigen-specific memory T cell compartment in the lungs

PBMC and BAL fluid were collected at weeks 6 (post-prime), 34 (post-boost) and 48 (pre-challenge) as well as on days 4 and 15 post-challenge. Lymphocytes were stimulated with SARS-CoV-2 S1 and S2 peptide pools (WA1) and then measured by intracellular staining. (**a, c**) Fold-changes for indicated timepoints in percentage of memory $CD4^+$ T cells with (**a**) T_H1 markers (IL-2, TNF or IFNγ), (**c**) T_H2 markers (IL-4 or IL-13) or T_{FH} cells expressing CD40L following stimulation. (**b**) Fold-changes for indicated timepoints in percentage of memory $CD8^+$ T cells expressing IL-2, TNF or IFNγ following stimulation. Frequencies below 0.1% were set to 0.1% prior to calculation of fold-change in geometric mean S-specific frequency. AE prime cohort received no vaccine prior to the initial regimen at week 32 and thus fold-changes between pre- and post-boost timepoints are not reported. N.D. or "Not Determined" refers to comparisons in which there were less than 2 samples contributing to calculation of group frequency for one of the timepoints. Related to **Fig. 6**.



Supplementary Figure 1. Non-specific T cell recruitment into the lungs following IM, IN or AE immunization

BAL fluid was collected pre-vaccination (baseline) and at weeks 6 (post-prime), 34 (post-boost) and 48 (time of challenge, TOC) as well as on days 2, 4, 7 and 15 post-challenge. Absolute number of total $CD4^+$ and $CD8^+$ T cells (memory and naïve) determined by flow cytometry. Circles, boxes and horizontal lines represent individual animals, interquartile range and median, respectively, while minima and maxima are denoted at whisker termini. # indicates that while sample collection for AE prime cohort (orange) occurred on week 34, week 34 was two weeks following the single AE prime rather than two weeks following a boost as in all other groups. Immunizations include control mRNA (ctrl mRNA) via IM route, control adenovirus vector (ctrl Ad) via AE route, mRNA-1273 via IM route, mRNA-1273.222 via IM route, and bivalent ChAd-SARS-CoV-2-S via AE or IN route. Number of NHP per group (*n*) are as follows: control = 8; IM boost = 8; IN boost = 6; AE boost = 6; AE prime = 4.