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Supplementary Information File for:

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**An adenovirus-vectored COVID-19 vaccine confers
protection from SARS-COV-2 challenge in rhesus macaques**

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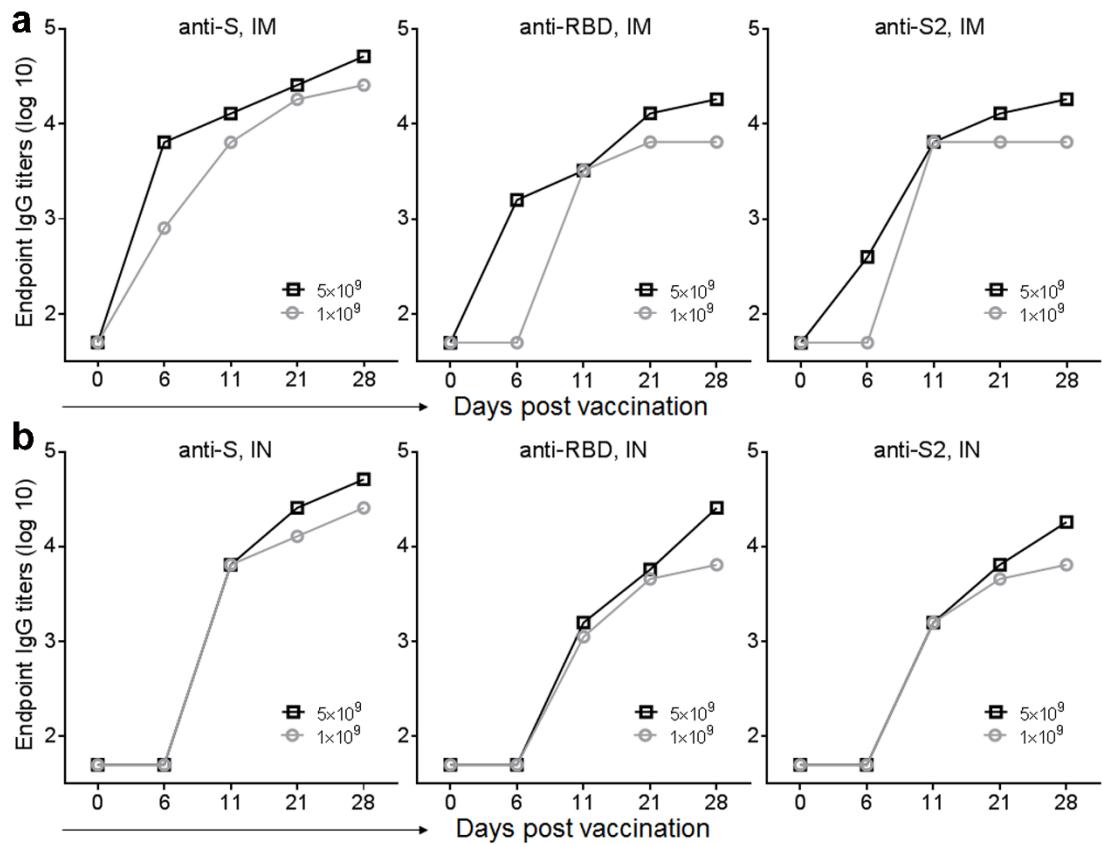
Feng L et al.

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10 **Supplementary Figures and the legends**

11 **Supplementary Figure 1**



12 **Supplementary Figure 1. Dynamics of S-binding IgG antibodies in Ad5-S-nb2**

13 **vaccinated mice. a** IgG antibodies against the S protein, RBD and the S2 subunit in

14 mice IM vaccinated with Ad5-S-nb2 on days 0, 6, 11, 21, and 28 after vaccination. **b**

15 IgG antibodies against the S protein, RBD and the S2 subunit in mice IN vaccinated

16 with Ad5-S-nb2 on days 0, 6, 11, 21, and 28 after vaccination. The serum samples from

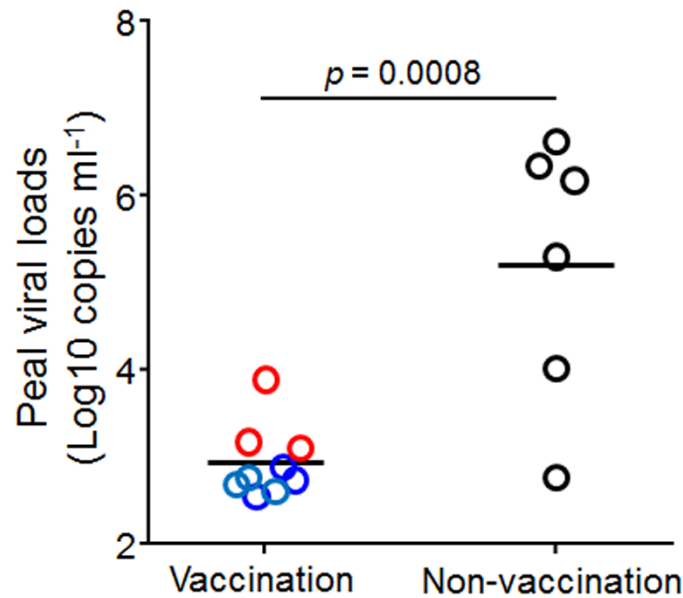
17 each mouse in one group (n = 5) were equally pooled and examined by ELISA. The

18 data points indicate the mean value of two technical replicates. Source data are provided

19 as a Source Data file.

20 21

22 **Supplementary Figure 2**



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24 **Supplementary Figure 2. Peak viral loads in the pharyngeal swabs of vaccinated**

25 **macaques and non-vaccinated macaques.** Each circle represents the peak viral load

26 of one macaque. Blue circle, macaques that received IM vaccination with 1×10^{11} vp

27 Ad5-S-nb2; Red circles, macaques that received IN vaccination with 5×10^{10} vp Ad5-S-

28 nb2; Dark cyan circles, macaques that received IM vaccination with 1×10^{10} vp Ad5-S-

29 nb2; Black circles, non-vaccinated macaques. Black lines reflect the mean viral loads.

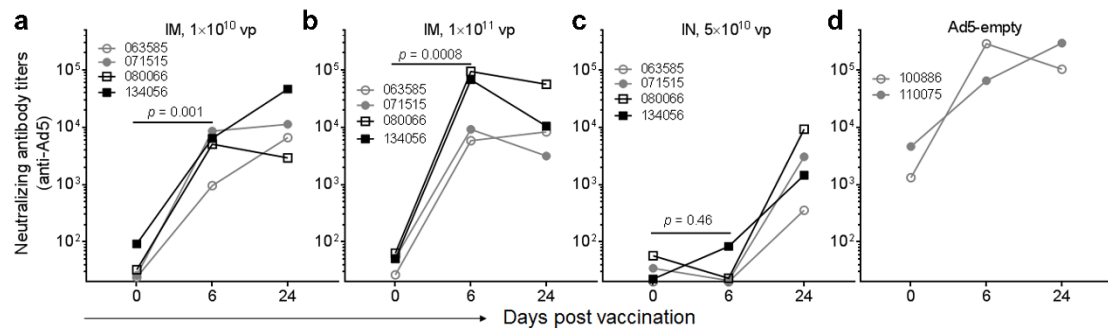
30 Comparison between vaccinated ($n = 9$) and non-vaccinated ($n = 6$) macaques was

31 conducted using Student's t-test (unpaired, two-tailed). Source data are provided as a

32 Source Data file.

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34 **Supplementary Figure 3**



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36 **Supplementary Figure 3. Neutralizing antibodies against Ad5 in macaques before**
37 **and after vaccination. a** Dynamics of anti-Ad5 neutralizing antibodies in macaques
38 that received IM vaccination with 1×10¹¹ vp Ad5-S-nb2. **b** Dynamics of anti-Ad5
39 neutralizing antibodies in macaques that received IM vaccination with 1×10¹⁰ vp Ad5-
40 S-nb2. **c** Dynamics of anti-Ad5 neutralizing antibodies in macaques that received IN
41 vaccination with 5×10¹⁰ vp Ad5-S-nb2. **d** Dynamics of anti-Ad5 neutralizing antibodies
42 in macaques that received IM vaccination with 1×10¹¹ vp Ad5-empty. Macaque serum
43 samples were assessed using a neutralization assay based on an Ad5-SEAP reporter
44 virus. Each data point represents the mean value of two technical replicates.
45 Comparison between different time points were conducted using Student's t-test (paired,
46 two-tails, n = 4). Source data are provided as a Source Data file.

48 **Supplementary Table 1.**

Macaque ID	Vaccination		Body weight (kg)	Age (year)	Sex	Immunisation history						
	Dose	Route				2013	2014	2015	2016	2017	2018	2019
063585	Ad5-S-nb2 1×10 ¹⁰ vp	IM	12.2	14	male	IIV(H7N7,H7N9) ^a	IIV(H7N7,H7N9)	IIV(H7N9)	—	IIV(H5N6)	IIV(H5N6)	—
071515			12.1	13	male	IIV(H7N9)	IIV(H7N9)	—	—	IIV(H5N6)	IIV(H5N6)	—
080066			8.5	12	female	IIV(H7N9)	IIV(H7N9)	—	—	IIV(H5N6)	IIV(H5N6)	—
134056			6.2	7	female	—	—	—	—	—	—	IIV(H1N1,H7N9), Ad2-EBOLA GP ^b
100109	Ad5-S-nb2 1×10 ¹¹ vp	IM	9.7	10	male	—	—	—	—	IIV(H5N6)	IIV(H5N6)	—
116004			16.7	9	male	—	—	—	—	IIV(H5N6)	IIV(H5N6)	—
116008			6.6	9	female	—	—	—	—	IIV(H5N6)	IIV(H5N6)	—
130460			5.7	7	female	—	—	—	—	—	—	IIV(H1N1,H7N9), Ad2-EBOLA GP
071539	Ad5-S-nb2 5×10 ¹⁰ vp	IN	10.2	13	male	IIV(H7N9)	IIV(H7N9)	—	—	IIV(H5N6)	IIV(H5N6)	—
110113			9.3	9	male	—	—	—	—	IIV(H5N6)	IIV(H5N6)	—
140052			6.2	6	female	—	—	—	—	—	—	IIV(H1N1,H7N9), Ad2-EBOLA GP
134018			7.1	7	female	—	—	—	—	—	—	IIV(H1N1,H7N9), Ad2-EBOLA GP
100886	Ad5-empty 1×10 ¹¹ vp	IM	7.3	10	female	—	EBOLA VLP ^c	EBOLA VLP, Ad2-EBOLA GP	EBOLA GP ^d , Ad2-EBOLA GP	EBOLA GP	EBOLA GP, IIV(H7N9)	Ad5-empty
110075			6.8	9	male	—	EBOLA VLP	EBOLA VLP	EBOLA GP, EBOLA VLP	EBOLA GP, Ad2-EBOLA GP	EBOLA GP, IIV(H7N9)	Ad5-empty
C1	Non- vaccinated	—	6.7	7	female				—			
C2			9.3	9	male				—			
C3			6.3	6	female				—			
C4			9.0	7	male				—			
D1			6.6	7	female				—			
D2			10.1	9	male				—			

- 50 ^aIIV, Inactivated influenza virus.
- 51 ^bAn adenovirus type 2 vector expressing the glycoprotein (GP) of Ebola virus.
- 52 ^cVirus like particles harboring the GP protein of Ebola virus.
- 53 ^dPurified GP protein of Ebola virus.