THE LANCET

Supplementary appendix

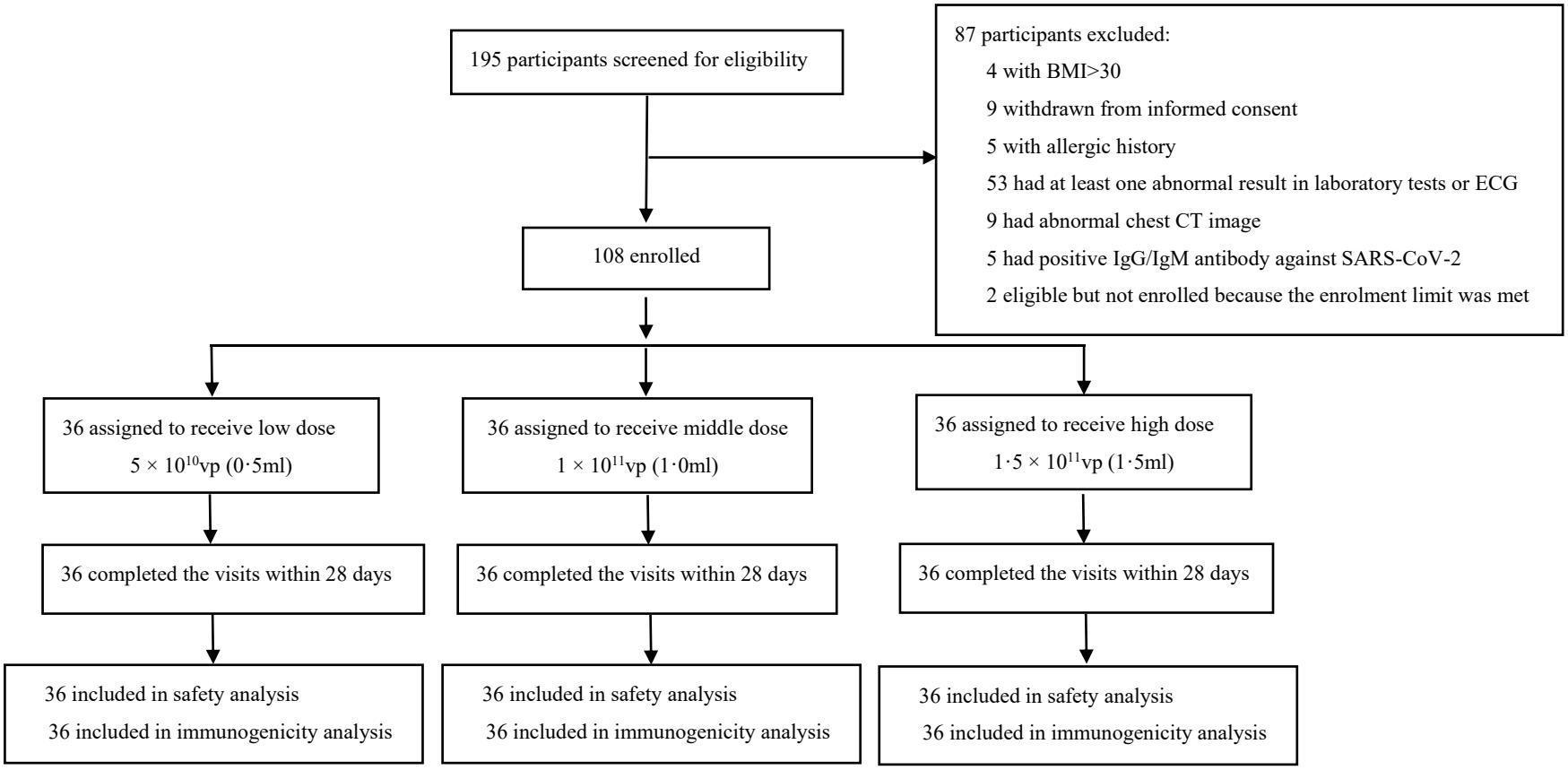
This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Zhu F-C, Li Y-H, Guan X-H, et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. *Lancet* 2020; published online May 22. https://doi.org/10.1016/S0140-6736(20)31208-3.

Appendix

Content

Appendix 1. Trial profile.



BMI=body mass index. ECG=electrocardiograph. CT=computerized tomography. IgG=immunoglobulin G. IgM=immunoglobulin M. SARS-CoV-2= severe acute respiratory syndrome coronavirus 2.

Appendix 2. List of severe (grade 3) adverse reactions reported within 28 days of follow-up.

ID of participants	Dose group	Symptom	Grade	Start time	Resolve time	Treatment
027	Low dose	Fever	3	Day 0 after vaccination	48h	No treatment
029	Low dose	Fever	3	Day 1 after vaccination	24h	No treatment
060	Middle dose	Fever	3	Day 1 after vaccination	48h	No treatment
067	Middle dose	Fever	3	Day 1 after vaccination	48h	No treatment
075	High dose	Fever	3	Day 1 after vaccination	48h	Self-purchased medicine
		Dyspnea	3	Day 1 after vaccination	24h	No treatment
		Fatigue	3	Day 1 after vaccination	48h	No treatment
		Muscle pain	3	Day 1 after vaccination	48h	No treatment
077	High dose	Fever	3	Day 1 after vaccination	24h	No treatment
078	High dose	Fatigue	3	Day 0 after vaccination	24h	No treatment
		Joint pain	3	Day 0 after vaccination	24h	No treatment
080	High dose	Fever	3	Day 1 after vaccination	48h	No treatment
082	High dose	Fever	3	Day 1 after vaccination	48h	No treatment
087	High dose	Fever	3	Day 1 after vaccination	48h	Self-purchased medicine

Appendix 3. Logistic regression analysis of the incidence of fever with the baseline characters, vaccine dose and pre-existing Ad5 antibodies.

			OR	
Model parameters	**Estimates**	**P value**	**Point estimates**	**95%CI by Wald**
Univariate analysis*				
Middle dose	0·00	>0·99	1·00	0·39, 2·55
High dose	0·56	0·24	1·75	0·69, 4·45
Age	0·939	0·049	2·56	1·01, 6·51
Sex	-0·52	0·18	0·60	0·28, 1·28
Pre-existing Ad5 antibodies (>200)	-1·31	0·0013	0·27	0·12, 0·60
Multivariate analysis†				
Intercept	0·09	0·85		
Middle dose	-0·04	0·95	0·97	0·35, 2·66
High dose	0·39	0·45	1·48	0·54, 4·07
Age	0·53	0·042	2·87	1·04, 7·94
Sex	-0·32	0·14	0·53	0·23, 1·23
Pre-existing Ad5 antibodies (>200)	-1·26	0·0028	0·28	0·12, 0·65

* The dose group (the low-dose group as the reference), age (stratified into 18-44, 45-60; using 45-60 years as reference), sex (female as reference), and the level of Ad5 antibodies pre-vaccination (≤200 as reference) are analysed in univariate logistic regression, respectively, before involved in a multivariable analysis. † The occurrence of adverse reactions (fever) is the dependent variable. The independent variables are the dose group (the low-dose group was the reference), age (stratified into 18-44, 45-60; using 45-60 years as reference), sex (female as reference), and the level of Ad5 antibodies pre-vaccination (≤200 as reference).

Appendix 4. Abnormal changes of laboratory tests on day 7 after vaccination.

Laboratory measures		Low dose (n=36)	Middle dose (n=36)	High dose (n=36)	Total
Platelet decrease	Any	1 (3)	1 (3)	2 (6)	4 (4)
	Grade 1	1 (3)	0 (0)	1 (3)	2 (2)
	Grade 2	0 (0)	1 (3)	1 (3)	2 (2)
ALT increase	Any	2 (6)	5 (14)	3 (8)	10 (9)
	Grade 1	2 (6)	5 (14)	3 (8)	10 (9)
AST increase	Any	0 (0)	0 (0)	1 (3)	1 (1)
	Grade 1	0 (0)	0 (0)	1 (3)	1 (1)
Hyperglycemia (fasting)	Any	1 (3)	1 (3)	2 (6)	4 (4)
	Grade 1	0 (0)	1 (3)	0 (0)	1 (1)
	Grade 2	1 (3)	0 (0)	2 (6)	3 (3)
Total bilirubin increase	Any	4 (11)	1 (3)	4 (11)	9 (8)
	Grade 1	4 (11)	0 (0)	3 (8)	7 (6)
	Grade 2	0 (0)	1 (3)	1 (3)	2 (2)

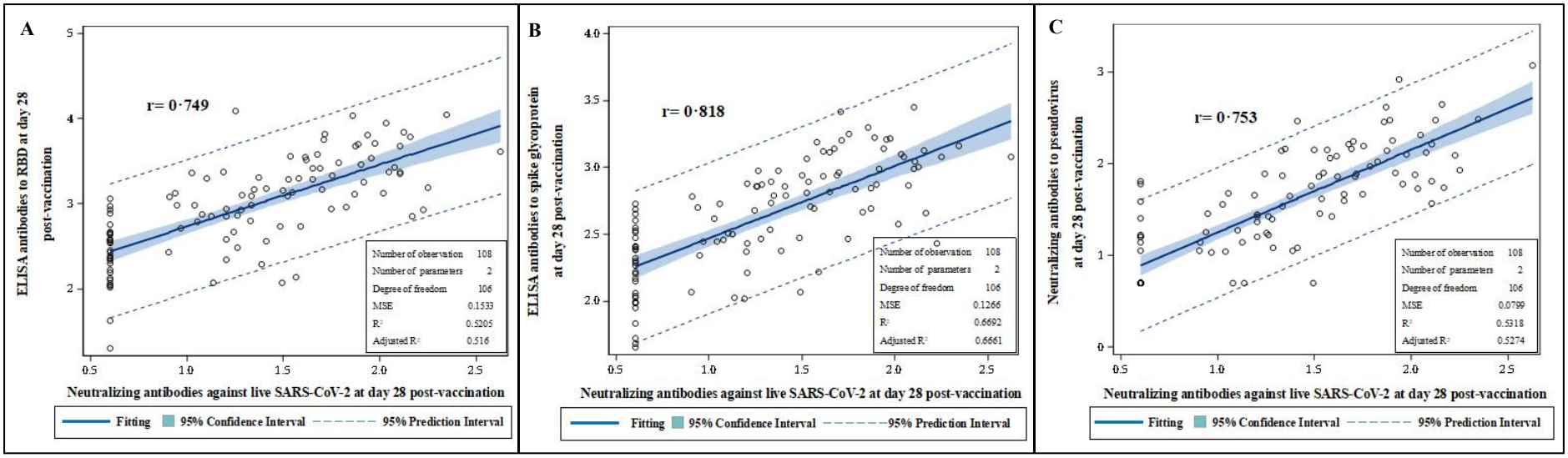
Data are n (%). ALT= alanine aminotransferase. AST= aspartate aminotransferase.

Appendix 5. Specific antibody responses to spike glycoprotein, neutralizing antibodies to pseudovirus at day 14 and 28 post-vaccination.

	Day 14				Day 28			
	Low dose (n=36)	Middle dose (n=36)	High dose (n=36)	P value	Low dose (n=36)	Middle dose (n=36)	High dose (n=36)	P value
ELISA antibodies to spike glycoprotein								
GMT	130·0 (83·0-203·7)	92·9 (64·9-132·9)	199·3 (141·7-280·2)	0·020*	385·1 (284·8-520·5)	345·6 (247·8-482·0)	596·4 (440·0-808·4)	0·034**
≥4-fold increase	15, 42%	22, 61%	25, 69%	0·050†	32, 89%	32, 89%	30, 83%	0·82
Neutralization antibodies to pseudovirus								
GMT	18·5 (11·1-31·1)	16·4 (10·8-24·9)	29·2 (18·9-45·3)	0·17	29·8 (18·4-48·1)	27·3 (16·9-44·3)	45·6 (28·4-73·0)	0·26
≥4-fold increase	16, 44%	17, 47%	21, 58%	0·46	21, 58%	19, 53%	25, 69%	0·34

Data are mean (95%*CI*) or n, %. n = number of participants. % = proportion of participants. ELISA=enzyme-linked immunosorbent assay. GMT= geometric mean antibody titer. The p values are the result of comparison between the three dose groups. The Student-Newman-Keuls test was used for multicomparison of GMTs. * The difference between high dose and middle dose was 149·2, with 95%CI 10·2~288·1. ** The difference between high dose and low dose was 280·2, with 95%CI 29·8~530·6; and the difference between high dose and low dose was 281·5, with 95%CI 31·1~531·9. † Only a margin of significance was found across the groups, and no significant difference was found after a Bonferroni correction for multicomparisons.

Appendix 6. The association between neutralizing antibodies to live SARS-CoV-2 and ELISA antibodies to receptor binding domain, spike glycoprotein, or pseudovirus-based neutralizing antibodies at day 28 post-vaccination.



RBD= receptor binding domain. Panel A shows the association between binding antibodies to RBD and neutralizing antibodies to live virus at day 28 post-vaccination. Panel B shows the association between binding antibodies to spike glycoprotein and neutralizing antibodies to live SARS-CoV-2 at day 28 post-vaccination. Panel C shows the association between neutralizing antibodies to pseudovirus and live SARS-CoV-2 at day 28 post-vaccination.

Appendix 7. Specific antibody responses to receptor binding domain, spike glycoprotein, neutralizing antibodies to live SARS-CoV-2 or to pseudovirus at day 14 and 28 post-vaccination according to pre-existing Ad5.

	Day 14				Day 28			
	Low dose	**Middle dose**	**High dose**	**P value**	**Low dose**	**Middle dose**	**High dose**	**P value**
ELISA antibodies to RBD								
Pre-existing Ad5≤200								
n	16	17	20		16	17	20	
GMT	225·4	224·5	270·9	0·89	1013·3	1444·7	2275·9	0·16
	(92·9-546·8)	(139·1-362·4)	(142·5-514·9)		(468·2-2192·7)	(882·8-2364·3)	(1231·3-4206·6)	
≥4-fold increase	12, 75%	14, 82%	16, 80%	0·92	15, 94%	17, 100%	20, 100%	0·30
Pre-existing Ad5>200								
n	20	19	16		20	19	16	
GMT	32·2	40·7	54·3	0·39	413·5	478·2	820·0	0·15
	(21·0-49·4)	(21·1-78·3)	(30·9-95·4)		(273·4-625·2)	(259·5-881·0)	(473·4-1420·5)	
≥4-fold increase	4, 20%	4, 21%	6, 38%	0·50	20, 100%	17, 89%	16, 100%	0·20
ELISA antibodies to spike glycoprotein								
Pre-existing Ad5≤200								
n	16	17	20		16	17	20	
GMT	326·5	164·7	306·2	0·064	619·1	499·8	818·3	0·24
	(191·5-556·8)	(109·6-247·5)	(196·4-477·4)		(392·6-976·2)	(324·0-771·0)	(529·5-1264·4)	
≥4-fold increase	11, 69%	14, 82%	15, 75%	0·61	13, 81%	16, 94%	17, 85%	0·60
Pre-existing Ad5>200								
n	20	19	16		20	19	16	
GMT	62·3	55·7	116·5	0·073	263·3	248·4	401·6	0·19

	Day 14				Day 28			
	Low dose	Middle dose	High dose	P value	Low dose	Middle dose	High dose	P value
	(37·6-103·2)	(34·1-90·9)	(75·2-180·4)		(186·5-371·9)	(153·3-402·7)	(276·5-583·2)	
≥4-fold increase	4, 20%	8, 42%	10, 63%	0·034*	19, 95%	16, 84%	13, 81%	0·36
Neutralizing antibodies to live SARS-CoV-2								
Pre-existing Ad5≤200								
n	16	17	20		16	17	20	
GMT	16·1	17·3	25·3	0·40	30·6	27·8	50·2	0·26
	(9·0-28·8)	(9·5-31·7)	(15·8-40·6)		(16·9-55·6)	(14·6-53·0)	(30·0-84·0)	
≥4-fold increase	9, 56%	10, 59%	13, 65%	0·86	13, 81%	11, 65%	17, 85%	0·35
Pre-existing Ad5>200								
n	20	19	16		20	19	16	
GMT	4·8	5·7	5·3	0·72	8·0	10·0	20·8	0·031†
	(3·8-5·9)	(3·9-8·5)	(3·5-8·2)		(5·2-12·2)	(5·6-17·7)	(11·2-38·7)	
≥4-fold increase	1, 5%	1, 5%	2, 13%	0·67	5, 25%	7, 37%	10, 63%	0·070
Neutralization antibodies to pseudovirus								
Pre-existing Ad5≤200								
n	16	17	20		16	17	20	
GMT	46·1	34·1	58·9	0·43	61·2	45·5	63·8	0·71
	(19·1-111·5)	(19·5-59·6)	(36·0-96·6)		(31·4-119·3)	(23·4-88·7)	(34·4-118·5)	
≥4-fold increase	11, 69%	12, 71%	18, 90%	0·21	13, 81%	12, 71%	16, 80%	0·77
Pre-existing Ad5>200								
n	20	19	16		20	19	16	
GMT	8·9	8·5	12·2	0·51	16·8	17·3	29·9	0·39
	(5·8-13·9)	(5·4-13·5)	(7·1-20·9)		(9·1-30·7)	(8·8-34·2)	(14·1-63·3)	
≥4-fold increase	5, 25%	5, 26%	3, 19%	0·85	8, 40%	7, 37%	9, 56%	0·47

Data are mean (95%*CI*) or n, %. n = number of participants. % = proportion of participants. ELISA= enzyme-linked immunosorbent assay. RBD= receptor binding domain. GMT= geometric mean antibody titer. The p values are the result of comparison between the three dose groups. * The difference between high dose and low dose was 0·43%, with 95%CI 0·13%~0.72%. † The difference between high dose and low dose was 23·4, with 95%CI 4·3~42·5.

Appendix 8. Logistic regression analysis of seroconversion rate of neutralizing antibodies to live SARS-CoV-2 at day 28 post-vaccination with the baseline characters, vaccine dose and pre-existing Ad5 antibodies.

			OR	
Model parameters	**Estimates**	**P value**	**Point estimates**	**95%CI by Wald**
Univariate analysis*				
Middle dose	0·00	>0·99	1·00	0·40, 2·52
High dose	1·10	0·031	3·00	1·11, 8·14
Age	1·17	0·011	3·22	1·30, 7·96
Sex	-0·47	0·23	0·62	0·29, 1·35
Pre-existing Ad5 antibodies (>200)	-1·63	0·0001	0·20	0·08, 0·45
Multivariable analysis†				
Intercept	0·65	0·17		
Middle dose	-0·06	0·91	0·94	0·33, 2·67
High dose	1·02	0·074	2·76	0·91, 8·44
Age	0·68	0·011	3·88	1·37, 11·01
Sex	-0·32	0·17	0·53	0·21, 1·31
Pre-existing Ad5 antibodies (>200)	-1·67	0·0003	0·19	0·08, 0·47

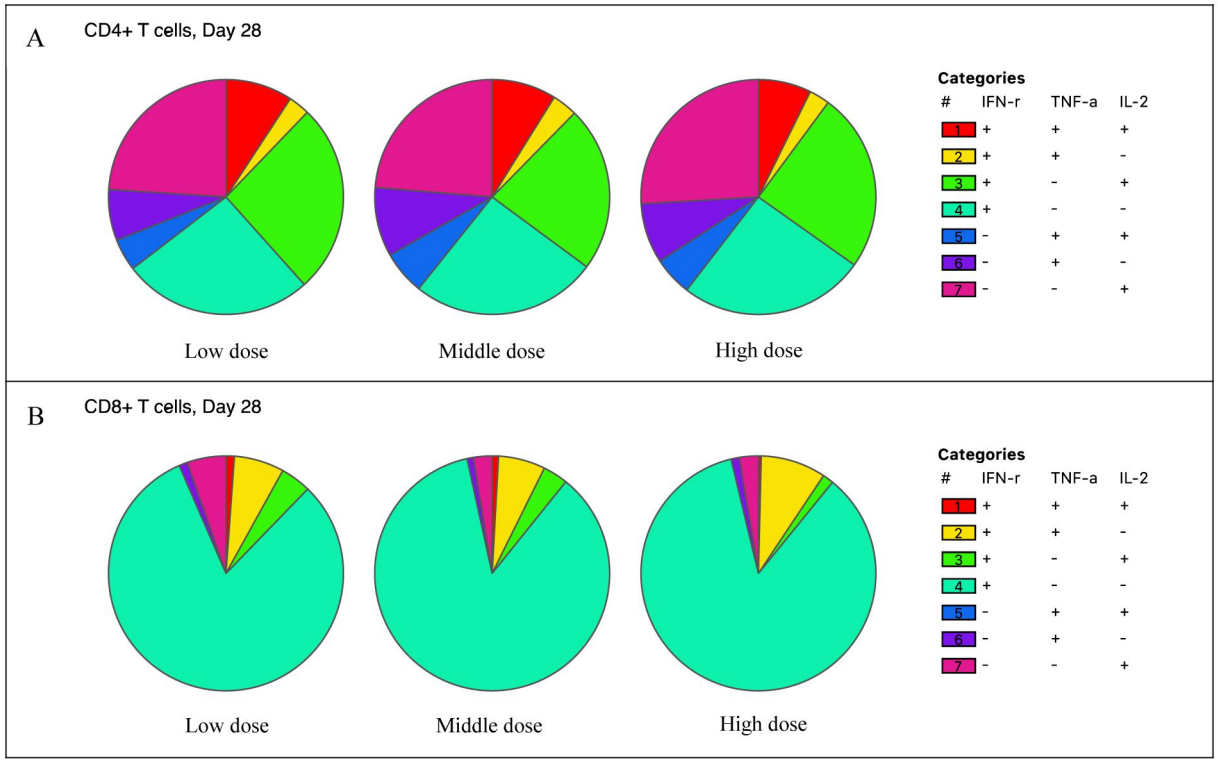
*The dose group (the low-dose group as the reference), age (stratified into 18-44, 45-60; using 45-60 years as reference), sex (female as reference), and the level of Ad5 antibodies pre-vaccination (≤200 as reference) are analysed in univariate logistic regression, respectively, before involved in a multivariable analysis. † The independent variables are the dose group (the low-dose group as the reference), age (stratified into 18-44, 45-60; using 45-60 years as reference), sex (female as reference), and the level of Ad5 antibodies pre-vaccination (≤200 as reference) included in a multivariable analysis.

Appendix 9. Specific neutralizing antibodies to Ad5 vector at day 14 and 28 post-vaccination according to pre-existing Ad5.

	Day 14				Day 28			
	Low dose (n=36)	Middle dose (n=36)	High dose (n=36)	P value	Low dose (n=36)	Middle dose (n=36)	High dose (n=36)	P value
Total								
GMT	840·6 (332·7-2123·5)	1429·6 (642·5-3181·0)	1208·9 (595·5-2454·0)	0·64	672·0 (254·9-1771·2)	1041·2 (467·6-2318·1)	898·7 (412·6-1957·2)	0·76
Pre-existing Ad5≤200								
GMT<	71·9 (20·7-249·3)	340·6 (82·7-1403·6)	323·7 (130·5-802·6)	0·10	49·3 (14·1-172·4)	238·4 (60·5-939·5)	202·0 (76·4-534·2)	0·12
Pre-existing Ad5>200								
GMT	6008·9 (4537·2-7957·9)	5159·1 (3745·1-7106·9)	6275·9 (4833·1-8149·5)	0·59	5434·1 (4029·6-7328·0)	3893·5 (2625·4-5774·1)	5808·1 (4600·0-7333·6)	0·16

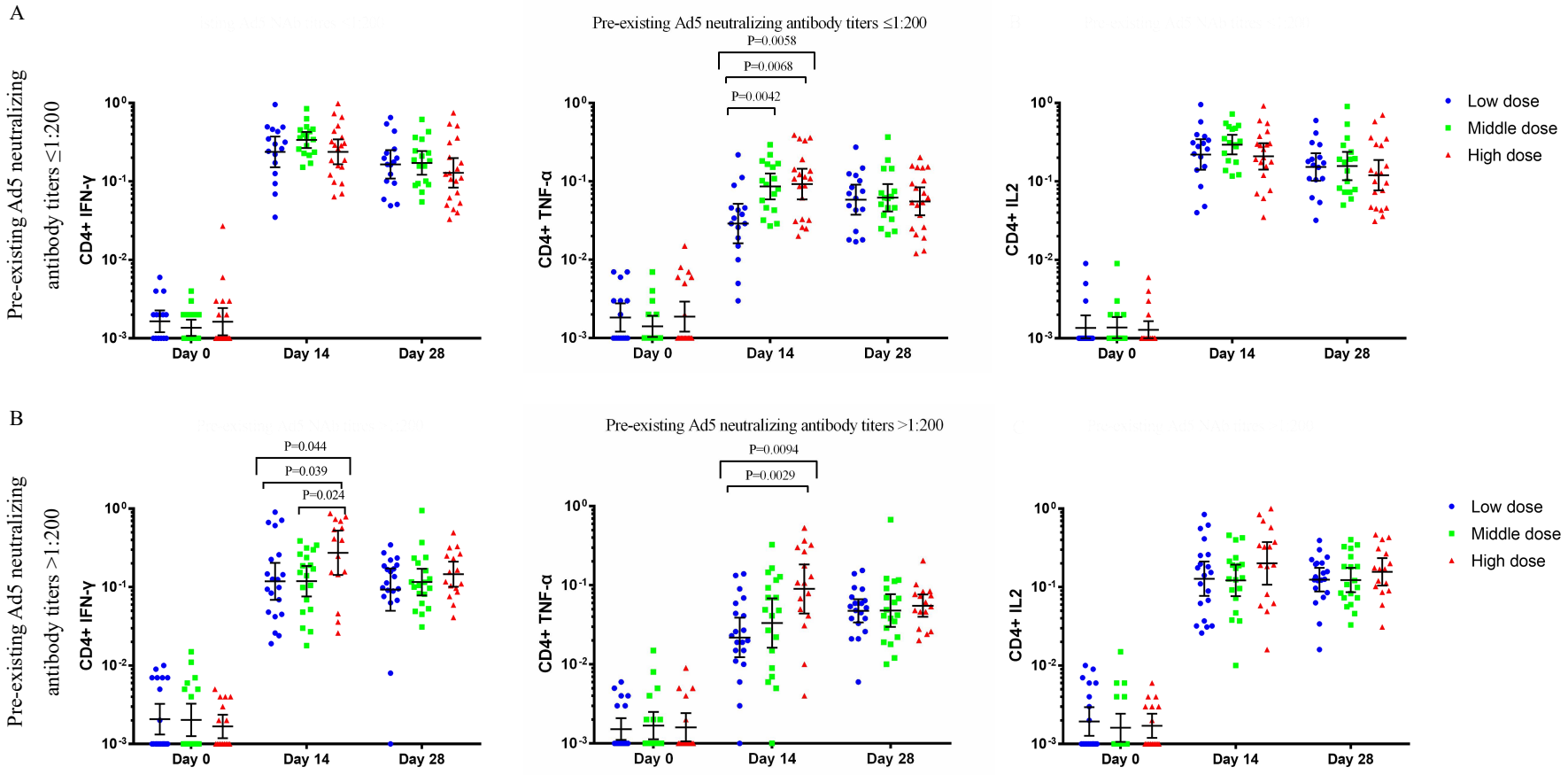
Data are mean (95%*CI*) or n, %. n = number of participants. % = proportion of participants. Ad5= adenovirus type 5. GMT= geometric mean antibody titer. The p values are the result of comparison between the three dose groups.

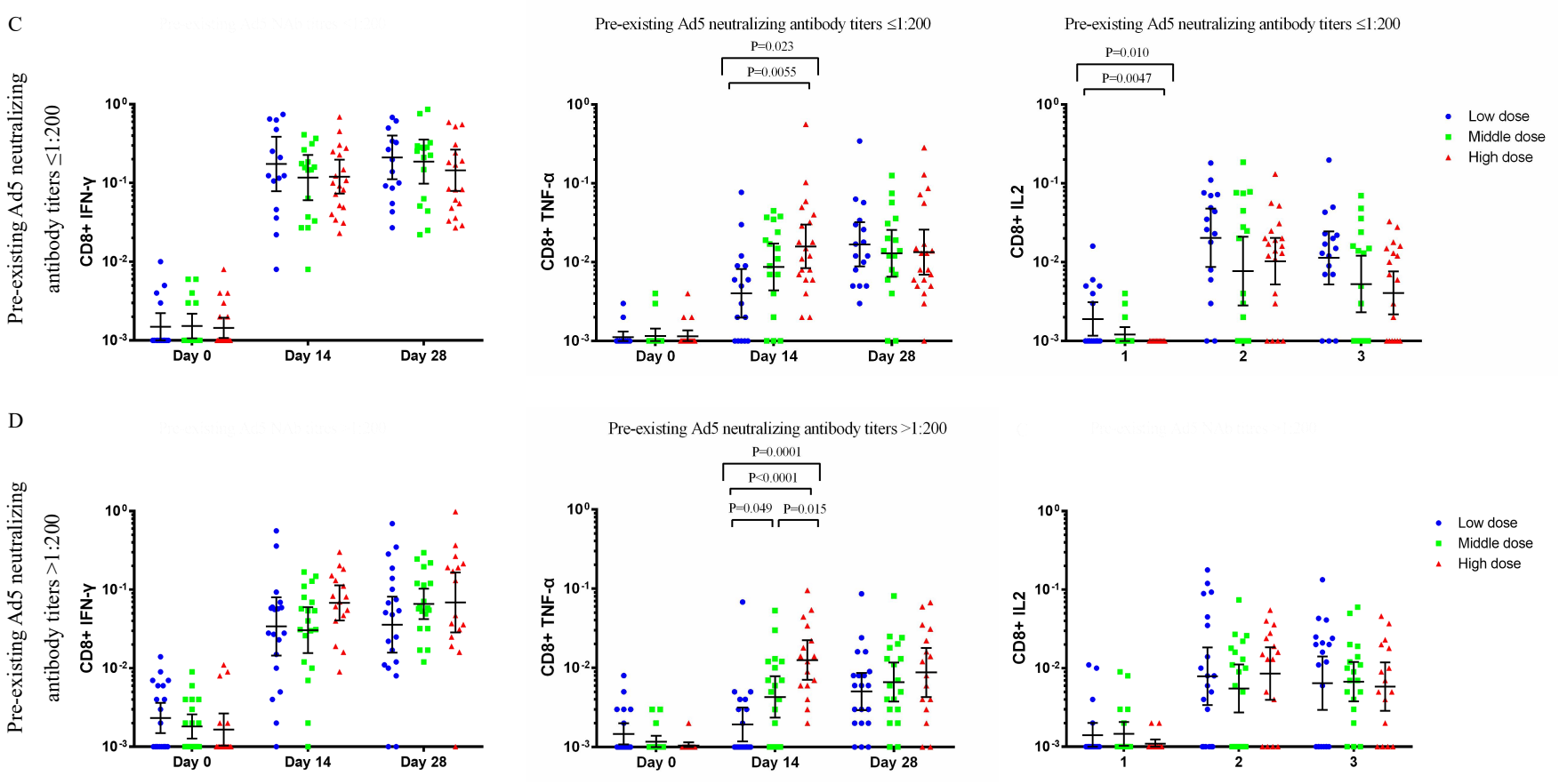
Appendix 10. T cells producing combination of INF-γ, TNF-α, and IL-2 at day 28 post-vaccination measured by flow cytometry.



Panel A shows the proportions of CD4+ T cells producing any combination of INF-γ, TNF-α, and IL-2 at day 28 post-vaccination. Panel B shows the proportions of CD8+ T cells producing any combination of INF-γ, TNF-α, and IL-2 at day 28 post-vaccination.

Appendix 11. Stratified analyses of the T cell responses based on the pre-existing Ad5 neutralizing antibody titers measured by flow cytometry.





Panel A shows the specific T cell response with secretion of IFN-γ, tumor necrosis factor α (TNF-α), and interleukin-2 from CD4+ T cells at day 0, 14, and 28 in participants with low pre-existing Ad5 neutralizing antibody titers (≤1: 200) in three dose groups. Panel B shows the specific T cell response with secretion of IFN-γ, TNF-α, and interleukin-2 from CD4+ T cells at day 0, 14, and 28 in participants with high pre-existing Ad5 neutralizing antibody titers (>1: 200) in three dose groups. Panel C shows the specific T cell response with secretion of IFN-γ, TNF-α, and interleukin-2 from CD8+ T cells at day 0, 14, and 28 in participants with low pre-existing Ad5 neutralizing

antibody titers (≤1: 200) in three dose groups. Panel D shows the specific T cell response with secretion of IFN-γ, TNF-α, and interleukin-2 from CD8+ T cells at day 0, 14, and 28 in participants with high pre-existing Ad5 neutralizing antibody titers (>1: 200) in three dose groups.

Appendix 12. Proportion of participants with either seroconversion of neutralizing antibodies to live SARS-CoV-2 or a positive T cell response measured by INF-γ ELISpot at day 14 and 28 post-vaccination.

		Low dose (n=36)	Middle dose (n=36)	High dose (n=36)	Total (n=108)
Day 14 post-vaccination					
Seroconversion	**n (%)**	30 (83)	35 (97)	35 (97)	100 (93)
Seroconversion rate CI	**95%CI**	68·1, 92·1	85·8, 99·5	85·8, 99·5	86·1, 96·2
Day 28 post-vaccination					
Seroconversion	**n (%)**	28 (78)	33 (92)	36 (100)	97 (90)
Seroconversion rate CI	**95%CI**	61·9, 88·3	78·2, 97·1	90·4, 100·0	82·7, 94·2

n (%), n = number of participants. % = proportion of participants. A positive antibody response (seroconversion) was defined as at least a 4-fold increase in post-vaccination titer from baseline. The ELISpot T cell responses were considered positive if at least 2-fold increase in the numbers of IFN-γ-secreting T cells was found post-vaccination compared to the baseline.