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Supplemental information

Differential immunogenicity of homologous versus heterologous boost in Ad26.COV2.S vaccine recipients

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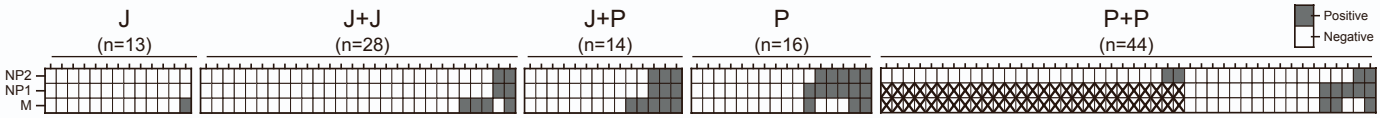

Figure S1: Identification of convalescent vaccinated individuals. Related to Figure 1

Heatmap shows the SARS-CoV-2 non-Spike-specific T cell responses quantified in vaccinated individuals using IFN-γ ELISpot. Individuals with at least one positive response (>20 SFC SFC/10^6 PBMCs) are categorized as potentially infected vaccinees. While individuals with no positive response are categorized as naïve vaccinees. "X" denotes structural peptide pools that were untested.

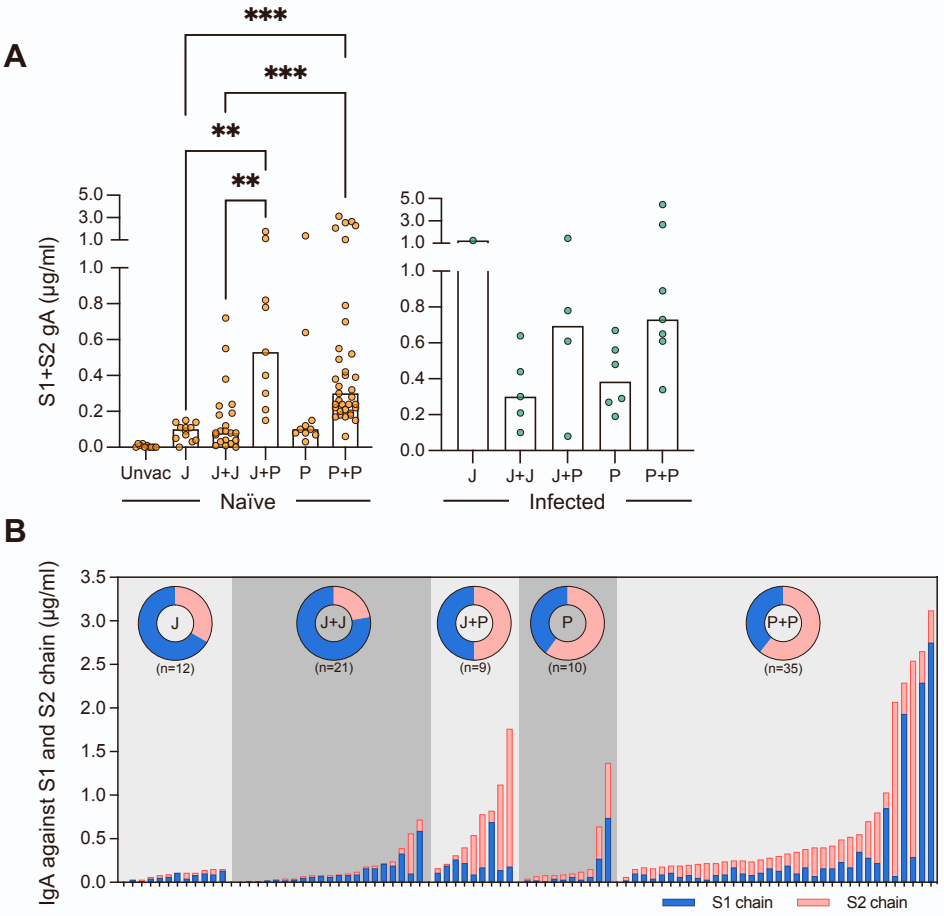

Figure S2: Quantitative and Qualitative profile of Spike-specific IgA antibodies. Related to Figure 2

(A) Spike-specific IgA antibody responses were tested in five cohorts of naïve vaccinated individuals: J (n=12), J+J (n=23), J+P (n=9), P (n=10) and P+P (n=37). Same analysis was carried out in SARS-CoV-2 convalescent vaccinated individuals: J (n=1), J+J (n=4), J+P (n=5), P (n=6) and P+P (n=7). A naïve unvaccinated cohort was used as a control, Unvac (n=10). Bars denote the median value of each group. Each dot represents an individual. Significant differences in each group were analysed by one-way ANOVA and the adjusted p-values (adjusted for multiple comparison) are shown. No significance is not shown, * = P≤0.05; ** = P≤0.01; *** = P≤0.001. **(B)** Stacked bars represent IgA antibody titers against the S1 (blue) and S2 (pink) chains of SARS-CoV-2 Spike antigen. Each column represents an individual. Donut plots represent the mean of percentage of IgA antibodies against S1 or S2.

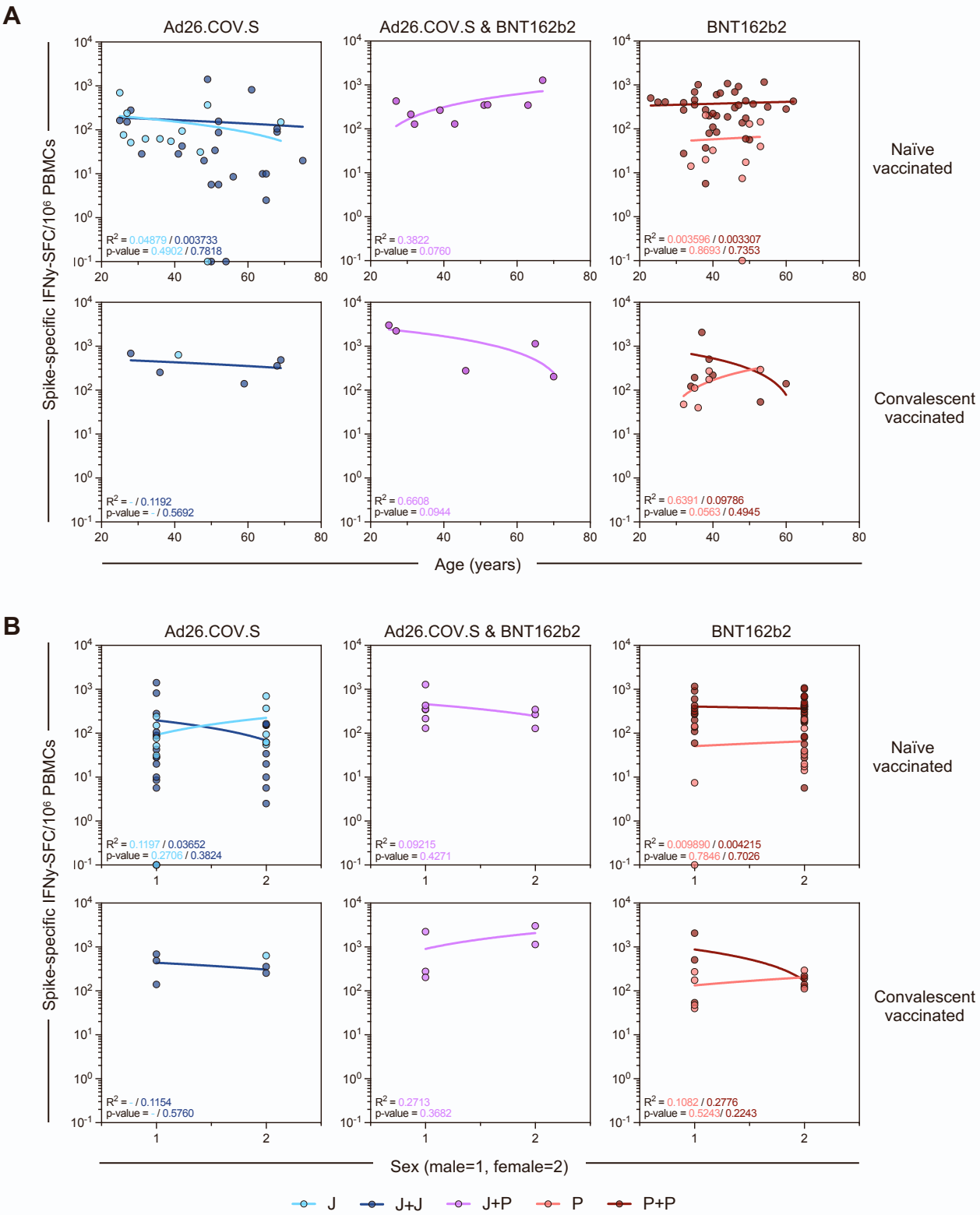

Figure S3: Correlation of Spike-specific T cell frequency with age or sex. Related to Figure 2

Linear regression analysis between Spike-specific T cell frequency and **(A)** age or **(B)** sex of vaccine recipients who are naïve (top panel) or convalescent (bottom panel) vaccinated individuals. Goodness of fit and p-value are shown in plots.

Table S1: **Age vs Spike-specific T cell responses. Related to Figure 2**

Data shows heterogeneity amongst the vaccinated individuals. X denotes convalescent vaccinated individuals while O denotes non-responders (as determined based on 25th percentile of total responses).

	Study ID	Age	X - Infected O - NonResponder	Total Spike
J	#107	41	X	638.40
	#017	25		702.50
	#108	26		76.95
	#113	27		239.40
	#120	28		51.30
	#025	32		62.50
	#023	36		62.50
	#026	39		55.00
	#121	42		94.05
	#096	47	O	31.35
	#093	49	O	0.00
	#106	49		367.65
	#095	69		151.05

	Study ID	Age	X - Infected O - NonResponder	Total Spike
P	#034	32	X	47.50
	#037	35	X	112.50
	#033	36	X	40.00
	#022	39	X	175.00
	#039	39	X	272.50
	#014	53	X	295.00
	#087	34	O	14.25
	#035	38		205.00
	#036	38	O	20.00
	#032	40	O	32.50
	#013	48	O	0.00
	#058	48	O	7.50
	#038	49	O	17.50
	#059	50		130.00
	#011	53		145.00
	#057	53		40.00

	Study ID	Age	X - Infected O - NonResponder	Total Spike
J+J	#024	28	X	687.50
	#088	36	X	253.65
	#063	59	X	140.00
	#040	68	X	360.00
	#044	69	X	487.50
	#041	25		165.00
	#028	27		152.50
	#053	28		280.00
	#091	31	O	28.50
	#089	41	O	28.50
	#100	42		42.75
	#060	48	O	19.95
	#085	49		1422.15
	#099	50	O	0.00
	#101	50	O	5.70
	#084	51	O	34.20
	#052	52		157.14
	#064	52		87.50
	#110	52	O	5.70
	#092	54	O	0.00
	#090	56	O	8.55
	#046	61		820.00
	#042	64	O	10.00
	#045	65	O	10.00
	#056	65	O	2.50
	#051	68		90.00
	#061	68		105.45
	#027	75	O	20.00

	Study ID	Age	X - Infected O - NonResponder	Total Spike
P+P	#123	34	X	122.55
	#116	35	X	193.80
	AT	37	X	2075
	#118	39	X	510.15
	#115	53	X	54.15
	G-033	40	X	220
	G-027	60	X	140.00
	JO	23		505.00
	G-006	25		405.00
	G-007	27		410.00
	#112	32		270.75
	G-003	32		395.00
	G-025	32	O	27.50
	G-015	35		355
	G-012	35		697.50
	G-032	35		457.5
	KK	36		1015
	#109	38	O	5.70
	#122	38		37.05
	G-022	38		272.50
	G-011	39		200.00
	G-009	39		80.00
	#111	40		225.15
	G-020	40		110.00
	G-005	41		602.50
	G-018	41		200.00
	G-008	41		85.00
	NLB	42		652.5
	G-004	44		187.50
	Ade	44		1077.5
	G-002	46		692.50
	G-014	46		305.00
	G-023	47		920.00
	S-014	47		347.50
	G-017	48		137.50
	#117	49		59.85
	#124	49		176.70
	G-030	49		437.50
	#114	50		57.00
	G-019	51		370.00
	G-031	54		1170
	G-016	55		315
	AB	60		282.5
	S-129	62		427.50

	Study ID	Age	X - Infected O - NonResponder	Total Spike
J+P	#031	25	X	3020.00
	#043	27	X	2237.50
	#012	46	X	277.50
	#020	65	X	1137.50
	#047	70	X	202.86
	#098	27		433.20
	#019	31		215.00
	#104	32		128.25
	#050	39		267.50
	#049	43		130.00
	#119	51		347.70
	#103	52		356.25
	#018	63		347.50
	#030	67		1282.50

Table S2: **List of antibodies used for Spike-specific B cell phenotyping. Related to STAR Methods.**

Antibody	Catalogue Number	Concentration
Brilliant Violet 605™ anti-human CD3 Antibody	317322	2:50
PE-CF594 Mouse Anti-Human CD10	562396	2:50
BV510 Mouse Anti-Human CD19	562947	2:50
BV421 Mouse Anti-Human CD21	562966	1:50
BV650 Mouse Anti-Human CD27	563228	3:50
PE/Cyanine7 anti-human CD38 Antibody	356608	2:50
PerCP/Cyanine5.5 anti-human CD40 Antibody	334316	2:50
FITC anti-human CD71 Antibody	334104	3:50
APC-Cy™7 Mouse Anti-Human CD69	557756	1:50
BUV737 Mouse Anti-Human CD95	564710	1:50
Alexa Fluor® 700 anti-human IgD Antibody	348230	2:50
BV786 Mouse Anti-Human IgG	564230	2:50
Brilliant Violet 711™ anti-human IgM Antibody	314540	2:50
LIVE/DEAD™ Fixable Blue Dead	L23105	2:1000