Supplementary appendix 2

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

Supplementary appendix

**Supplementary Information: Materials and Methods**
To investigate whether convalescent individuals have reduced plasma virus neutralization titers against isolates of SARS-CoV-2 lineage P.1 compared to those against lineage B viruses. First, we calculated the paired differences in the neutralization titers between lineages P.1/28 and B, and those between P.1/30 and B. Subsequently we dichotomized the paired differences into "greater than 0" and "less or equal to 0" to produce count tables. The count table is the same for both paired differences, so we only need to conduct one hypothesis test. For the hypothesis test, we assumed that each sample was an independent draw from a much larger population, and every sample had the same probability of p of having a paired difference > 0. Consequently, we used a binomial distribution to model the count of "difference > 0". As an indication of statistical support, we calculated the p-value, which is the probability of observing at least as many "differences > 0" out of the total sample size, given that there is no difference in proportions/probabilities between "differences > 0" and "differences ≤ 0". We repeated the above procedure for the plasma from vaccinated individuals. The count tables produced is presented in Table S5. A Bonferroni multiple test correction was applied. In addition, we performed the Pearson's correlation between the two paired differences in the convalescent samples. All these analyses were performed using the R studio version 1.3.1073 (https://rstudio.com)
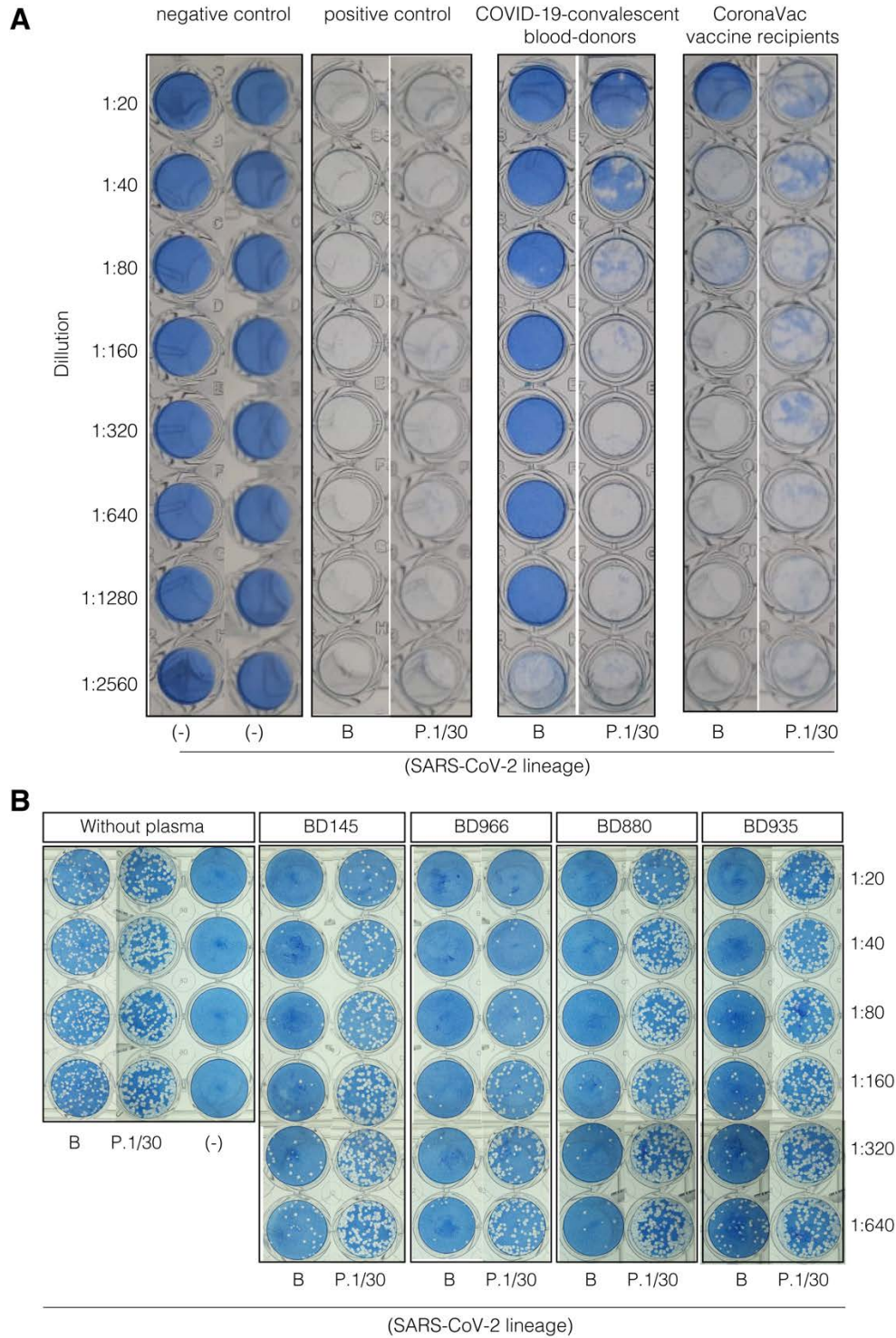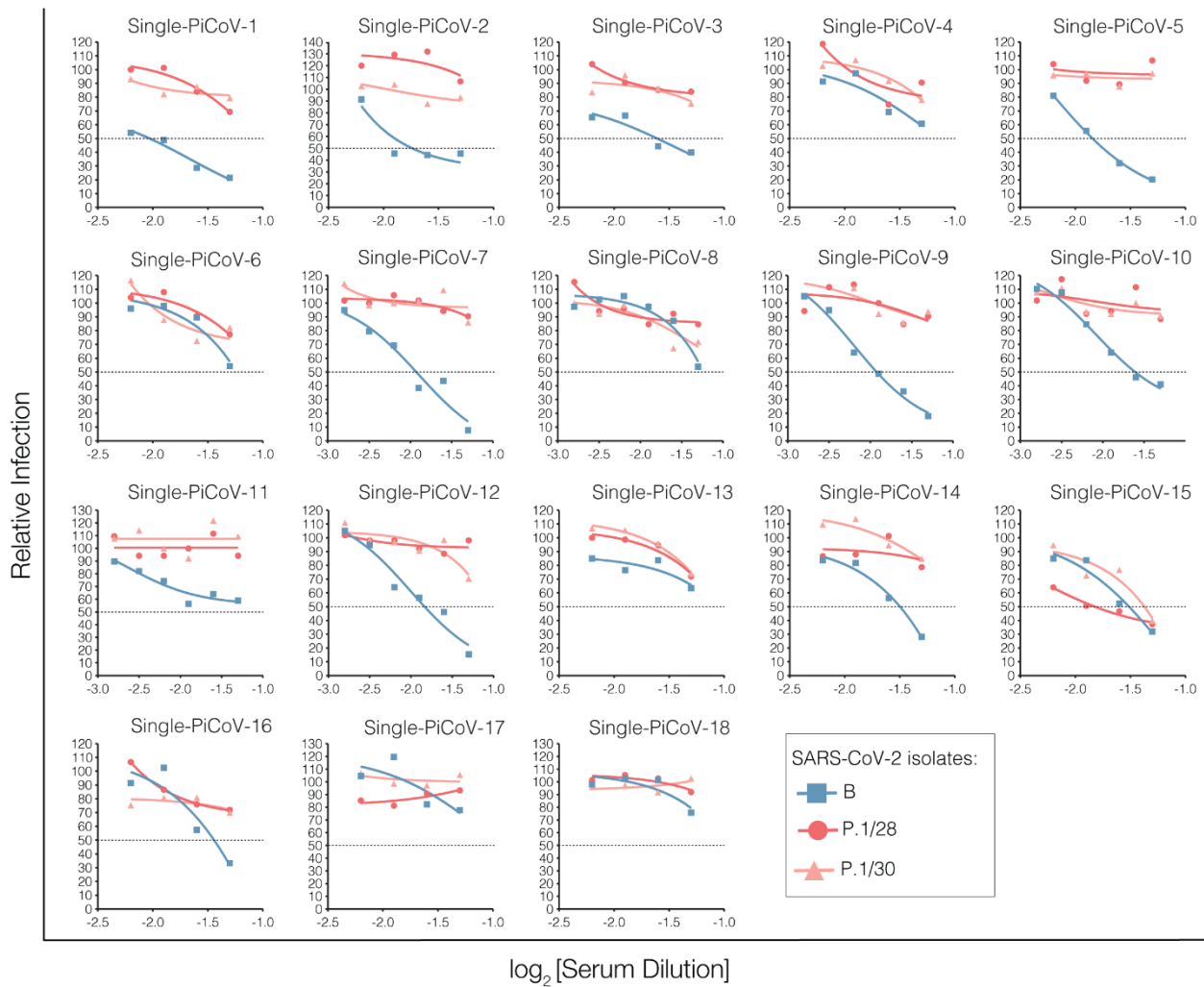
Supplementary Figure 1. Representative image of neutralizing antibody assays used in this study. (**A**) Virus neutralization test of plasma from COVID-19-convalescent blood-donors and CoronaVac vaccine recipients against isolates of P.1 and B lineages. (**B**) Plaque reduction neutralization test of plasma from COVID-19-convalescent blood-donors against isolates of P.1 and B lineages.
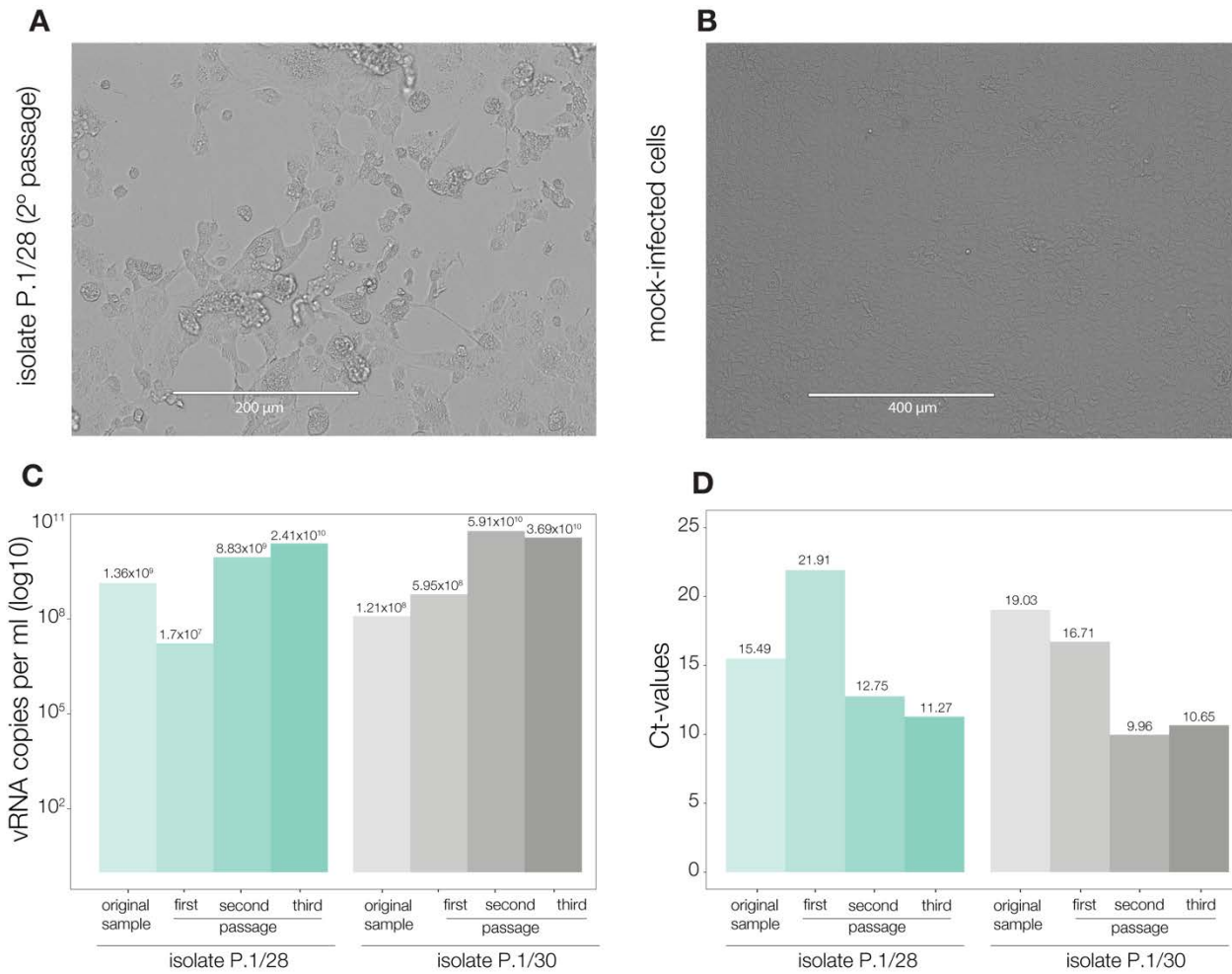
Supplementary Figure 2. SARS-CoV-2 lineage P.1 (P.1/28 and P.1/30) isolated from nasopharyngeal samples of COVID-19 patients from Manaus city, Amazonas, Brazil. Isolation of P.1 lineage of SARS-CoV-2 was performed in Vero cells in a Biosafety Level 3 Facility of the Emerging Viruses Laboratory at the University of Campinas, and a typical cytopathic effect was observed after 72 hours post-infection in inoculated cells (**A**) in comparison with uninfected cells (**B**). The isolation was also confirmed by RT-qPCR for the envelope gene after RNA extraction of the cell supernatant, where is possible to observe an increase in viral load (**C**) and a decrease in the Ct-value (**D**) after the sustained passages in Vero cells. Images were obtained in an EVOS inverted microscope kindly provided by Thermo Fisher Scientific. Images showed were representative of P.1/28 isolate.
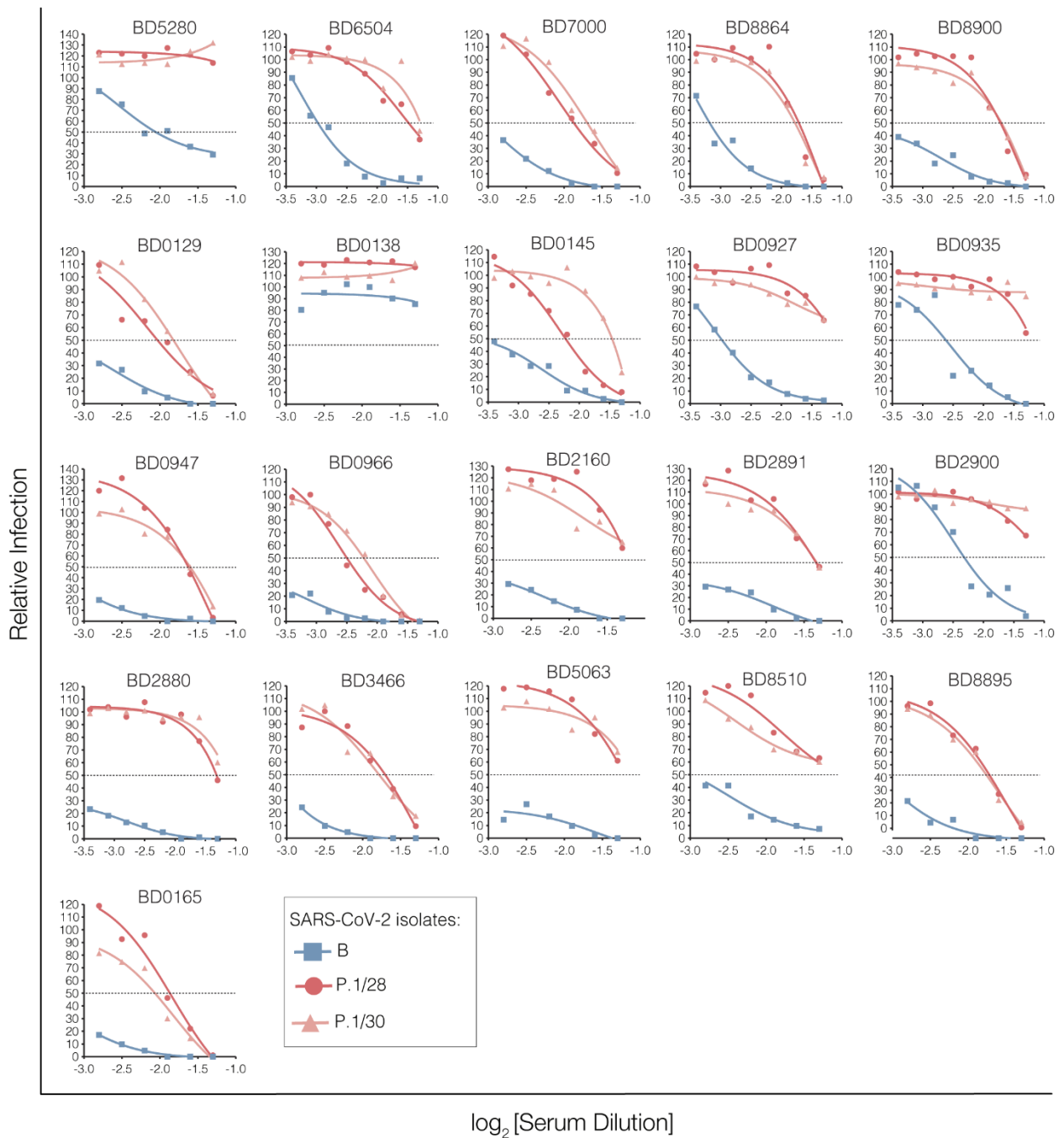
Supplementary Figure 3. PRNT$_{50}$ of each plasma of COVID-19-convalescent blood-donors (n=21). The PRNT$_{50}$ represents the dilution that showed 50% reduction in plaque formation in comparison with a control without plasma after linear regression analysis. The symbols represent the average of plasmas samples and error bars represent the standard errors.

Supplementary Figure 4. PRNT$_{50}$ of each plasma of single-dose CoronaVac recipients (n=18). The PRNT$_{50}$ represents the dilution that showed 50% reduction in plaque formation in comparison with a control without plasma after linear regression analysis. The symbols represent the average of plasmas samples and error bars represent the standard errors.

Supplementary Figure 5. PRNT$_{50}$ of each plasma of two-dose CoronaVac recipients collected ~3 weeks after vaccination (n=38). The plasma was collected between 17 to 38 days (median = 21 days) after second dose. The PRNT$_{50}$ represents the dilution that showed 50% reduction in plaque formation in comparison with a control without plasma after linear regression analysis. The symbols represent the average of plasmas samples and error bars represent the standard errors.
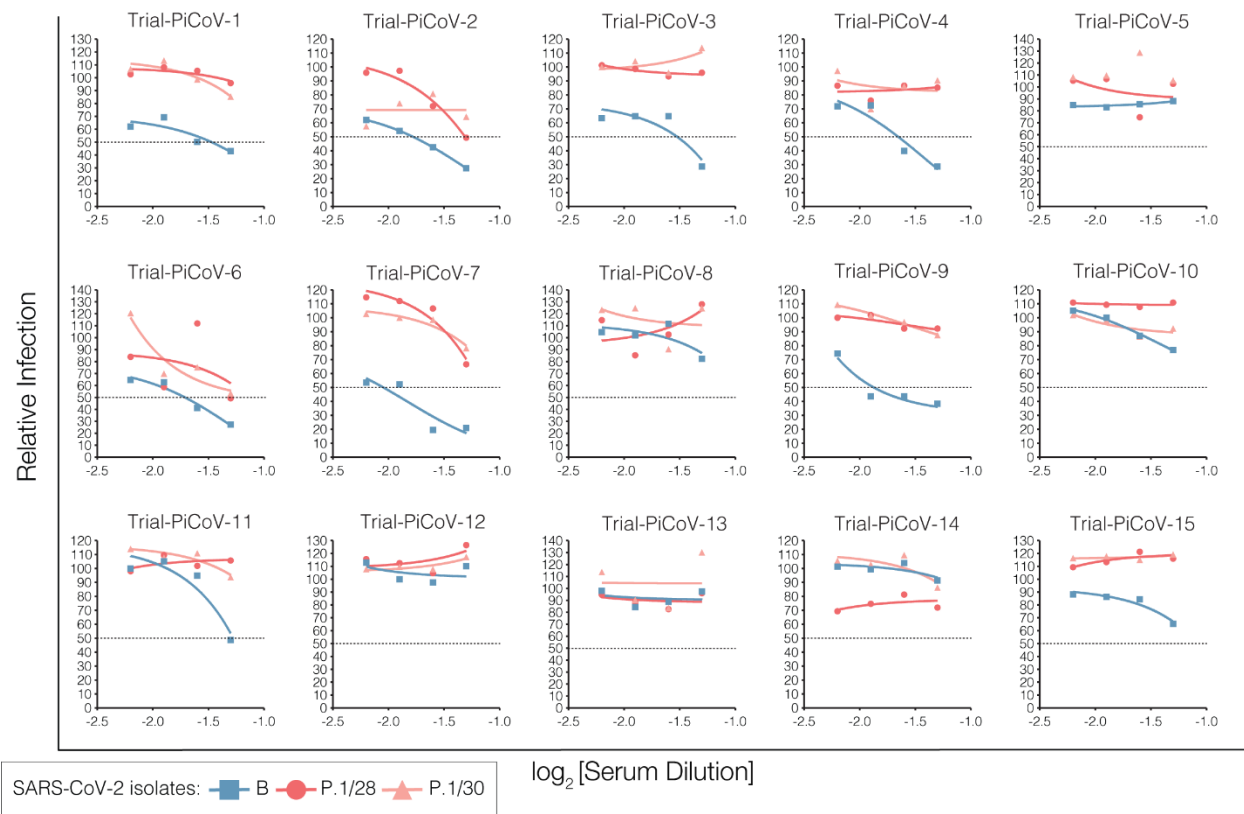
Supplementary Figure 6. PRNT$_{50}$ of each plasma of two-dose CoronaVac recipients collected ~5 months after vaccination (n=38). The plasma was collected between 134 to 260 days (median = 158 days) after second dose. The PRNT$_{50}$ represents the dilution that showed 50% reduction in plaque formation in comparison with a control without plasma after linear regression analysis. The symbols represent the average of plasmas samples and error bars represent the standard errors.

Table S1. Information and VNT$_{50}$ results of COVID-19-convalescent blood-donors.

ID	age	sex	Diagnosis	Symptoms durations (days)	Time between symptoms and collection (days)	Hospitalization	Plasma collection	IgM	IgG	P.1/28 [1]	P.1/30 [1]	B [1]
BD5280	31	F	RT-qPCR	14	52	no	21/05/20	0.88	2.82	<20	<20	<20
BD6504	48	M	RT-qPCR	11	42	no	01/06/20	7.92	3.54	30	<20	220
BD7000	33	F	RT-qPCR	19	44	no	11/06/20	5.48	8.15	60	35	560
BD8864	31	F	RT-qPCR	22	66	no	26/06/20	2.11	4.66	45	35	440
BD8900	43	M	RT-qPCR	0*	71	no	07/07/20	20.42	6.02	<20	50	320
BD0129	34	M	serology	4	69	no	08/07/20	0.74	5.73	25	60	320
BD0138	32	F	RT-qPCR	16	60	no	09/07/20	2.48	0.01	<20	<20	320
BD0145	58	M	serology	10	102	no	10/07/20	1.88	5.47	30	95	1040
BD0927	44	M	RT-qPCR	2	68	no	24/07/20	16.34	6.31	<20	30	400
BD0935	28	F	serology	10	56	no	24/07/20	13.27	7.04	<20	<20	180
BD0947	18	M	serology	3	84	no	21/07/20	3.64	7.14	55	55	260
BD0966	29	F	RT-qPCR	13	43	yes	24/07/20	22.16	8.10	320	120	2560
BD2160	34	F	RT-qPCR	9	73	no	30/07/20	9.23	6.07	<20	20	120
BD2891	57	M	serology	17	10	no	04/08/20	1.39	5.5	60	<20	90
BD2900	36	F	serology	0*	28	no	05/08/20	1.94	7.61	<20	<20	110
BD2880	34	M	RT-qPCR	13	45	no	10/08/20	45.86	5.62	<20	<20	1600
BD3466	43	F	serology	16	68	no	13/08/20	3.06	8.36	40	35	360
BD5063	25	F	serology	34	69	yes	27/08/20	0.29	2.55	30	20	180
BD8510	41	M	RT-qPCR	16	65	no	29/08/20	7.87	0.63	<20	<20	105
BD8895	31	F	RT-PCR	4	96	no	03/07/20	3.05	2.01	35	30	160
BD0165	34	F	serology	12	31	no	14/07/20	0.6	7.81	70	40	240

Legend: RT-qPCR, real-time quantitative polymerase chain reaction. * asymptomatic cases. F, female. M, male. IgM, Immunoglobulin M. IgG, Immunoglobulin G. [1] VNT titer.

Table S2. Information and VNT$_{50}$ values from CoronaVac vaccinated individuals with single-dose and two-dose collected ~3 weeks after immunization.

sample ID		Age (years)	Sex	1º dose of CoronaVac vaccine							2º dose of CoronaVac vaccine						
1º dose	2º dose			Immunization	Plasma collection	IgM	IgG	P.1/28[1]	P.1/30[1]	B[1]	Immunization	Plasma collection	IgM	IgG	P.1/28[1]	P.1/30[1]	B[1]
Single-PiCoV-1	Two-PiCoV-1	25	M	20/01/21	10/02/21	0.49	1.22	<20	<20	30	10/02/21	02/03/21	0.92	8.2	90	33	130
Single-PiCoV-2	Two-PiCoV-2	25	M	20/01/21	10/02/21	0.07	0.55	<20	<20	30	10/02/21	02/03/21	1.07	0.25	<20	<20	<20
Single-PiCoV-3	Two-PiCoV-3	35	F	20/01/21	10/02/21	0.06	0.41	<20	<20	20	10/02/21	02/03/21	0.43	2.35	<20	30	180
Single-PiCoV-4	Two-PiCoV-4	29	F	20/01/21	10/02/21	0.02	0.57	<20	<20	<20	10/02/21	02/03/21	0.46	1.31	<20	<20	240
Single-PiCoV-5	Two-PiCoV-5	30	F	20/01/21	09/02/21	0.34	0.73	<20	<20	30	10/02/21	03/03/21	0.41	3.9	<20	<20	40
Single-PiCoV-6	Two-PiCoV-6	36	M	20/01/21	10/02/21	0.09	0.09	<20	<20	20	10/02/21	03/03/21	0.13	0.24	<20	20	<20
Single-PiCoV-7	Two-PiCoV-7	24	F	18/01/21	08/02/21	0.13	3.84	<20	<20	50	09/02/21	01/03/21	1.94	3.14	20	20	680
Single-PiCoV-8	Two-PiCoV-8	27	F	20/01/21	10/02/21	0.21	1.23	<20	<20	<20	10/02/21	05/03/21	1.11	2.05	<20	<20	70
Single-PiCoV-9	Two-PiCoV-9	29	F	20/01/21	10/02/21	0.02	0.5	30	<20	30	10/02/21	02/03/21	0.61	0.5	<20	<20	35
Single-PiCoV-10	Two-PiCoV-10	29	F	20/01/21	10/02/21	0.58	1.31	<20	<20	20	10/02/21	03/03/21	0.89	2.46	<20	<20	270
Single-PiCoV-11	Two-PiCoV-11	28	F	20/01/21	10/02/21	0.01	0.43	<20	<20	<20	10/02/21	02/03/21	0.54	0.34	45	20	80
Single-PiCoV-12	Two-PiCoV-12	37	F	20/01/21	10/02/21	0.19	0.76	<20	<20	20	11/02/21	02/03/21	0.98	1.62	45	<20	80
Single-PiCoV-13	Two-PiCoV-13	42	M	20/01/21	11/02/21	0.14	0.84	<20	<20	20	10/02/21	03/03/21	0.52	0.47	<20	20	<20
Single-PiCoV-14	Two-PiCoV-14	26	F	20/01/21	10/02/21	0.29	0.14	<20	<20	50	10/02/21	02/03/21	0.93	2.94	45	<20	90
Single-PiCoV-15	Two-PiCoV-15	38	M	20/01/21	10/02/21	0.01	0.93	<20	<20	30	10/02/21	05/03/21	3.37	0.4	<20	<20	20
Single-PiCoV-16	Two-PiCoV-16	26	F	20/01/21	10/02/21	0.08	0.69	<20	<20	30	10/02/21	02/03/21	0.94	4.25	35	20	170
Single-PiCoV-17	Two-PiCoV-17	28	M	21/01/21	11/02/21	0.02	0.11	<20	<20	20	12/02/21	04/03/21	0.18	0.1	<20	<20	40
Single-PiCoV-18	Two-PiCoV-18	42	F	20/01/21	12/02/21	0.03	0.25	<20	<20	<20	10/02/21	04/03/21	1.16	0.31	25	25	<20
N/A	Two-PiCoV-19	46	F	19/01/21	N/P	N/P	N/P	N/P	N/P	N/P	09/02/21	04/03/21	1.77	0.34	<20	20	<20
N/A	Two-PiCoV-20	43	F	19/01/21	N/P	N/P	N/P	N/P	N/P	N/P	09/02/21	04/03/21	0.59	0.66	20	<20	50

N/A	Two-PiCoV-21	40	F	10/02/21	N/P	N/P	N/P	N/P	N/P	N/P	02/03/21	24/03/21	1.96	2.45	<20	25	30
N/A	Two-PiCoV-22	36	F	19/01/21	N/P	N/P	N/P	N/P	N/P	N/P	09/02/21	04/03/21	0.11	0.5	<20	160	<20
N/A	Two-PiCoV-23	41	M	22/01/21	N/P	N/P	N/P	N/P	N/P	N/P	12/02/21	04/03/21	0.46	1.16	25	<20	<20
N/A	Two-PiCoV-24	25	F	07/02/21	N/P	N/P	N/P	N/P	N/P	N/P	27/02/21	25/03/21	1	6.79	25	<20	20
N/A	Two-PiCoV-25	42	M	23/01/21	N/P	N/P	N/P	N/P	N/P	N/P	16/02/21	26/03/21	0.48	0.31	50	55	2560
N/A	Two-PiCoV-26	56	F	19/01/21	N/P	N/P	N/P	N/P	N/P	N/P	09/02/21	01/03/21	0.71	3.99	25	<20	20
N/A	Two-PiCoV-27	30	F	07/02/21	N/P	N/P	N/P	N/P	N/P	N/P	27/02/21	24/03/21	0.47	0.2	<20	<20	<20
N/A	Two-PiCoV-28	24	F	20/01/21	N/P	N/P	N/P	N/P	N/P	N/P	10/02/21	02/03/21	1.91	1.53	25	50	960
N/A	Two-PiCoV-29	37	F	19/01/21	N/P	N/P	N/P	N/P	N/P	N/P	09/02/21	04/03/21	0.85	4.02	140	210	960
N/A	Two-PiCoV-30	26	F	09/02/21	N/P	N/P	N/P	N/P	N/P	N/P	02/03/21	19/03/21	0.78	4.09	50	20	120
N/A	Two-PiCoV-31	24	F	07/02/21	N/P	N/P	N/P	N/P	N/P	N/P	27/02/21	24/03/21	0.37	0.41	20	<20	680
N/A	Two-PiCoV-32	57	F	04/02/21	N/P	N/P	N/P	N/P	N/P	N/P	03/03/21	23/03/21	0.88	0.21	<20	30	800
N/A	Two-PiCoV-33	68	F	19/01/21	N/P	N/P	N/P	N/P	N/P	N/P	09/02/21	04/03/21	0.44	0.08	<20	<20	<20
N/A	Two-PiCoV-34	24	M	04/02/21	N/P	N/P	N/P	N/P	N/P	N/P	26/02/21	24/03/21	0.32	1.09	<20	30	640
N/A	Two-PiCoV-35	26	M	09/02/21	N/P	N/P	N/P	N/P	N/P	N/P	01/03/21	24/03/21	0.64	2.69	<20	<20	<20
N/A	Two-PiCoV-36	29	F	04/02/21	N/P	N/P	N/P	N/P	N/P	N/P	24/02/21	19/03/21	2.17	2.17	25	<20	120
N/A	Two-PiCoV-37	32	F	21/01/21	N/P	N/P	N/P	N/P	N/P	N/P	12/02/21	04/03/21	0.31	0.37	<20	<20	680
N/A	Two-PiCoV-38	24	F	08/02/21	N/P	N/P	N/P	N/P	N/P	N/P	28/02/21	24/03/21	0.33	0.78	<20	85	1920

Legend: N/A, not applicable. N/P, not performed. F, female. M, male. IgM, Immunoglobulin M. IgG, Immunoglobulin G. [1]VNT titer.

Table S3. Information and VNT$_{50}$ values from CoronaVac vaccinated individuals with two-dose collected 5 months after second-dose.

ID	Age (years)	sex	1º dose	2º dose	Plasma collection	IgM	IgG	P.1/ 28 [1]	P.1/30 [1]	B [1]
Trial-PiCoV-1	59	F	13/08/20	28/08/20	28/01/21	0.29	0.33	*<20*	*<20*	20
Trial-PiCoV-2	40	F	14/08/20	28/08/20	28/01/21	0.26	0.06	*<20*	*<20*	35
Trial-PiCoV-3	29	M	14/08/20	28/08/20	02/02/21	0.06	0.38	*<20*	*<20*	20
Trial-PiCoV-4	38	M	14/08/20	28/08/20	02/02/21	0.06	0.08	*<20*	*<20*	90
Trial-PiCoV-5	34	M	14/08/20	28/08/20	02/02/21	0.15	0.36	*<20*	*<20*	*<20*
Trial-PiCoV-6	36	F	14/08/20	28/08/20	02/02/21	0.12	0.16	*<20*	*<20*	30
Trial-PiCoV-7	32	M	14/08/20	28/08/20	03/02/21	0.87	0.31	*<20*	*<20*	25
Trial-PiCoV-8	34	F	14/08/20	28/08/20	03/02/21	0.43	0.29	*<20*	*<20*	*<20*
Trial-PiCoV-9	35	F	20/08/20	03/06/20	18/02/21	0.10	0.31	*<20*	*<20*	30
Trial-PiCoV-10	31	F	03/09/20	18/09/20	12/02/21	0.14	1.09	*<20*	*<20*	60
Trial-PiCoV-11	28	F	21/08/20	04/09/20	18/02/21	0.08	0.25	*<20*	*<20*	*<20*
Trial-PiCoV-12	34	M	17/09/20	30/09/20	11/02/21	0.04	0.07	*<20*	*<20*	*<20*
Trial-PiCoV-13	42	F	17/08/20	31/08/20	19/02/21	0.12	0.13	*<20*	*<20*	*<20*
Trial-PiCoV-14	39	F	18/08/20	27/08/20	19/02/21	0.04	0.85	*<20*	*<20*	*<20*
Trial-PiCoV-15	32	F	18/08/20	27/08/20	19/02/21	0.31	0.09	*<20*	*<20*	*<20*

Legend: F, female. M, male. IgM, Immunoglobulin M. IgG, Immunoglobulin G. [1]VNT titer.

Table S4. Information of genome sequencing of SARS-CoV-2 lineage P.1 used in neutralizing antibody assays.

Amino acid	Protein	Position	P.1/28*	P.1/30*
aa	*orf1ab*	*S1188L*	undetermined	confirmed
aa	*orf1ab*	*K1795Q*	confirmed	confirmed
deletion	*11288*	*9 (aa)*	confirmed	confirmed
aa	*S*	*L18F*	confirmed	confirmed
aa	*S*	*T20N*	confirmed	confirmed
aa	*S*	*P26S*	confirmed	confirmed
aa	*S*	*D138Y*	confirmed	confirmed
aa	*S*	*R190S*	undetermined	confirmed
aa	*S*	*K417T*	confirmed	confirmed
aa	*S*	*E484K*	confirmed	confirmed
aa	*S*	*N501Y*	confirmed	confirmed
aa	*S*	*H655Y*	confirmed	confirmed
aa	*S*	*T1027I*	confirmed	confirmed
aa	*orf3a*	*G174C*	absence	absence
aa	*orf8*	*E92K*	confirmed	confirmed
aa	*N*	*P80R*	confirmed	confirmed
aa	*S*	*D614G*	confirmed	confirmed
aa	*S*	*V1176F*	confirmed	confirmed
aa	*orf*3a	S253P	confirmed	confirmed
aa	*N*	*R203K*	confirmed	confirmed
aa	*N*	*G204R*	confirmed	confirmed
aa	*N*	*S250P*	confirmed	absence
aa	nsp3	S370L	undetermined	confirmed
aa	nsp3	K977Q	confirmed	confirmed
aa	nsp3	S1735F	absence	confirmed
aa	nsp6	L260F	absence	confirmed
aa	nsp12	P323L	confirmed	confirmed
aa	nsp13	E341D	confirmed	confirmed

Legend: * sequences are available on GISAID (accession number: EPI_ISL_1708317 and EPI_ISL_1708318; https://www.gisaid.org). The coding regions are those annotated on Wuhan-Hu-1 (GenBank accession number: NC_045512).

Table S5. Count tables of paired differences.

Groups	Isolates paired	less or equal to 0 ("≤ 0")	greater than 0 ("> 0")
COVID-19-convalescent blood-donors	B and P.1/28	1	20
	B and P.1/30	1	20
Single-dose CoronaVac (~3 week after)	B and P.1/28	5	13
	B and P.1/30	4	14
Two-doses CoronaVac (~3 week after)	B and P.1/28	12	26
	B and P.1/30	10	28
Two-doses CoronaVac (~5 months after)	B and P.1/28	7	8
	B and P.1/30	7	8