Cell Reports Methods, Volume *2*

Supplemental information

Development of an efficient reproducible cell-cell transmission assay for rapid quantification of SARS-CoV-2 spike interaction with hACE2

George Ssenyange, Maya Kerfoot, Min Zhao, Shelli Farhadian, Sidi Chen, Lei Peng, Ping Ren, Charles S. Dela Cruz, Shaili Gupta, and Richard E. Sutton

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURES AND LEGENDS

Figure S1. Neutralization curves for post-vaccine and convalescent sera showing inhibition of S pseudotyped particle infection. Four-fold serial dilutions of convalescent (**A-C**) and post-vaccine (**E-G**) sera were pre-incubated with S pseudotyped particles for 1 h, then added to 293T-hACE2 target cells in triplicate. RLU was measured after 48 h, and IC-50 values calculated. One convalescent and one post-vaccine sample showed no significant inhibitory effect (**D & H**, respectively). Related to Figures 2, 3 and 5.

Figure S2. Reproducibility of cell-cell transmission assay. (**A-C**) Inhibition of cell-cell transmission by LCB1 performed 3 different times many weeks apart, IC-50 values were calculated for each experiment. (**D**) IC-50 values for the 3 experiments including the average IC-50 +/- SD. 1% DMSO in PBS was used as control. **(E, F)** Time-of-addition experiment using LCB1 to inhibit virus infection: 10-fold serial dilutions of LCB1 were added at -1, 0, +1 and +2 h relative to target cell addition. **(E)** pseudotyping; **(F)** cell-cell transmission. Related to Figure 6

Figure S3. Effect of tetherin on virus infection. Increasing amounts of tetherin plasmid DNA transfected into producer 293T cells (12-well plate for cell-cell transmission and 10 cm plate format for pseudotyping) inhibited cell-cell transmission of S **(A)** and VSV G **(B),** with a more profound inhibitory effect seen with pseudotyping with S **(B)** and VSV G **(D),** relative to cell-cell transmission. Effect of cholesterol derivatives on VSV G cell-cell transmission in transiently transfected cells: 10-fold serial dilutions of 25-hydroxycholesterol **(E)** and 27-hydroxycholesterol **(F)** were pre-incubated with VSV G-expressing producer cells for 1 hour; 293T target cells were then added, performed in triplicate, with RLU readout at 48 h. ns denotes not significant; *p-value <0.05; **p-value<0.01. Related to Figure 6.

Figure S4. Studies with S variants of concern (VOC). Serial dilutions of convalescent sera, post-vaccine sera, and peptide LCB1 were pre-incubated for 1 h with 293T producer cells transiently transfected with the different S VOC along with HIV-PV and

HIV-TV; 293T-hACE2 target cells were then added in triplicate and RLU measured at 48 h. **(A&C)** LCB1 inhibits cell-cell transmission of S VOC B.1.1.7 **(A)** and B.1.617.2 **(C)** but not B.1.351 **(B). (D-F)** Convalescent sera inhibits cell-cell transmission of spike VOCs. **(G-I)** Post-vaccine sera inhibits cell-cell transmission of S VOCs. Related to Figures 2, 3 and 4.

Figure S5. Immunoblotting and PCR of stable cell lines. (A-D) Expression of S, HIV Gag, and hACE2 proteins in stable cell lines were confirmed by Western blotting, with GAPDH immunoblots performed in parallel**. (E)** Confirmation of presence of HIV-TV by PCR. Ethidium bromide-stained horizontal agarose gel showing PCR product to confirm stable introduction of HIV-TV (inGLUC) in the 293T-Spike-TV stable producer cell line. Nested PCR was performed on extracted genomic DNA from 293T cells transiently and stably expressing HIV-TV. Related to Figures 5 and 6

Figure S6. Transduction of stable 293T cells with HDAd-HIV-PV. (A-B) 293T-Spike-TV cells were transduced with increasing amounts HDAd-HIV-PV as indicated, performed in 12-well format, fixed, and stained using X-gal for lacZ expression. **(A)** Microscopy; **(B)** Photograph of actual plate. **(C)** Stable 293T-Spike-TV producer cells were transduced with indicated amounts of concentrated HDAd-HIV-PV; after 24 h cells were refed, co-cultured with 293T-hACE2 target cells in triplicate, and RLU measured after 48 h. **(D)** Stable 293T-Spike-TV producer cells were transduced HDAd-HIV-PV, at 48 h culture supernatant were harvested and used to transduce 293T-hACE2 target cells, pre-seeded the previous day in a separate plate, with indicated amounts of the supernatant and RLU measured at 48 h. Results normalized to µL/50,000 target cells. A positive control with producer cells directly co-cultured with the targets was included. **(E)** Stable 293T-Spike-TV producer cells expressing different spike variants of concern, as indicated, were transduced with HDAd-HIV-PV; after 24 h cells were refed, co-cultured with 293T-hACE2 target cells in triplicate, and RLU measured after 48 h. Transduction and luciferase assays were performed in 3 independent experiments; the mean and SD are shown. Related to Figures 5 and 6.

Figure S1. Neutralization curves for post-vaccine and convalescent sera showing inhibition of S pseudotyped particle infection. Related to Figures 2, 3 and 5.

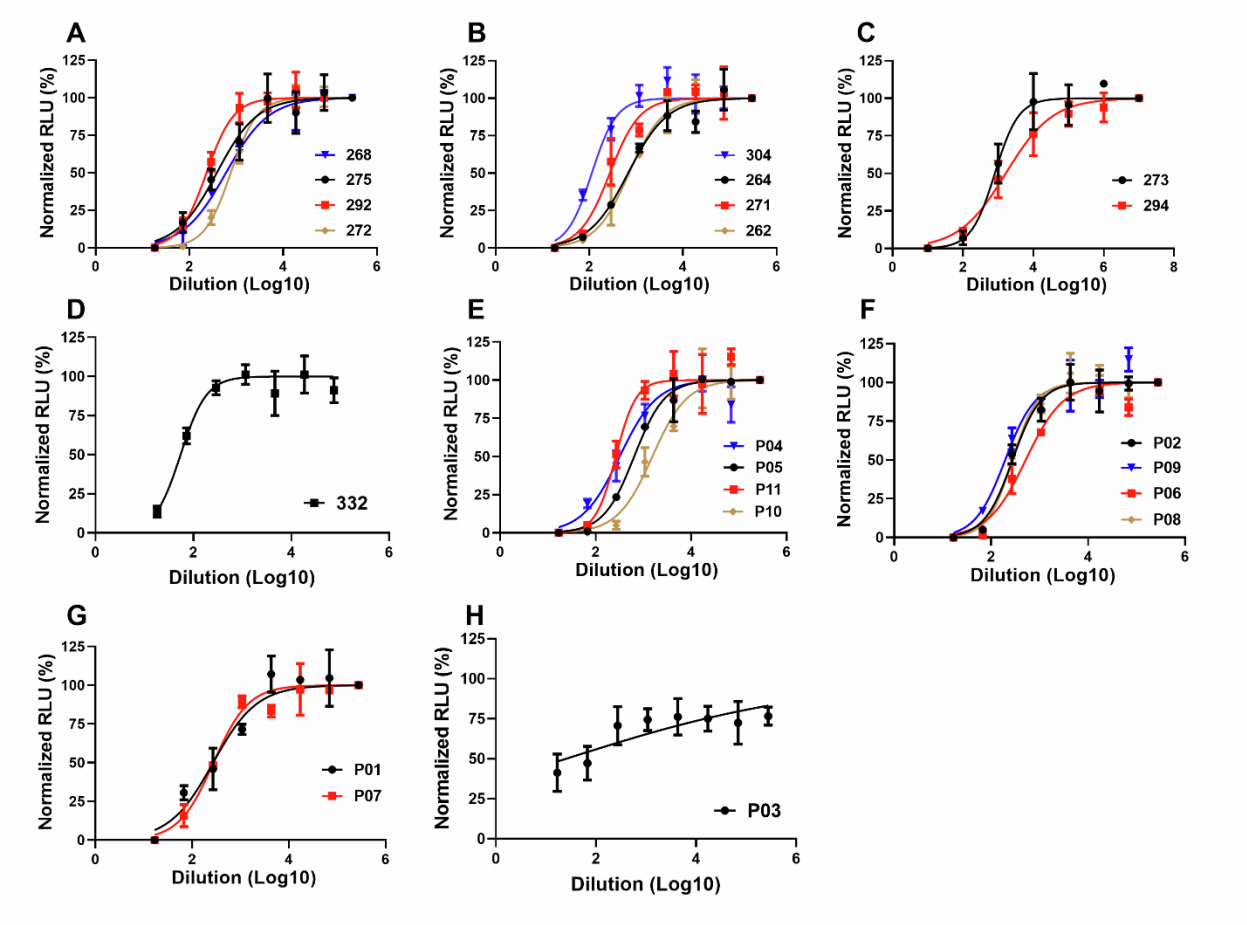


Figure S2. Reproducibility of cell-cell transmission assay. Related to Figure 6

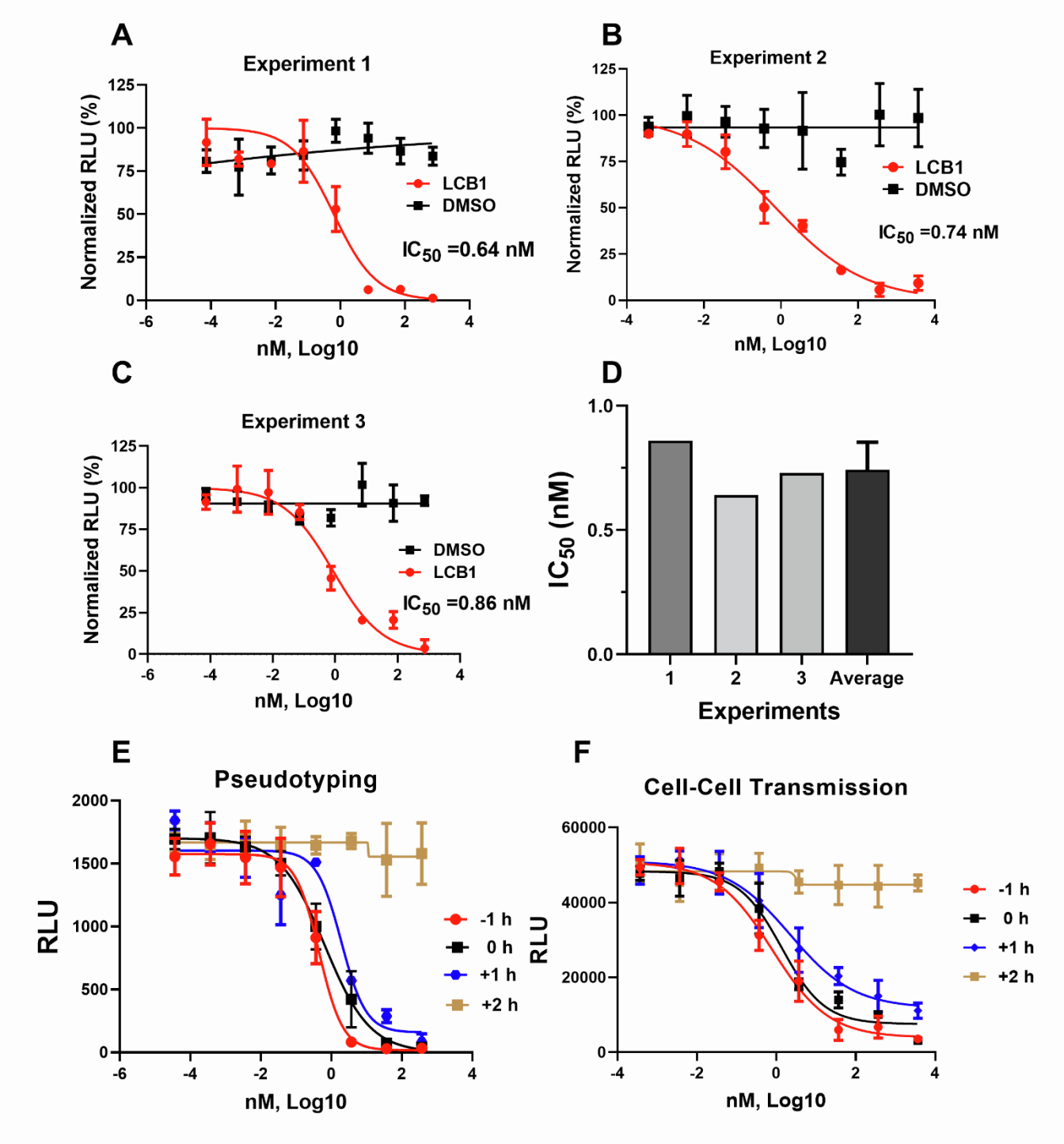


Figure S3. Effect of tetherin on virus infection. Related to Figure 6.

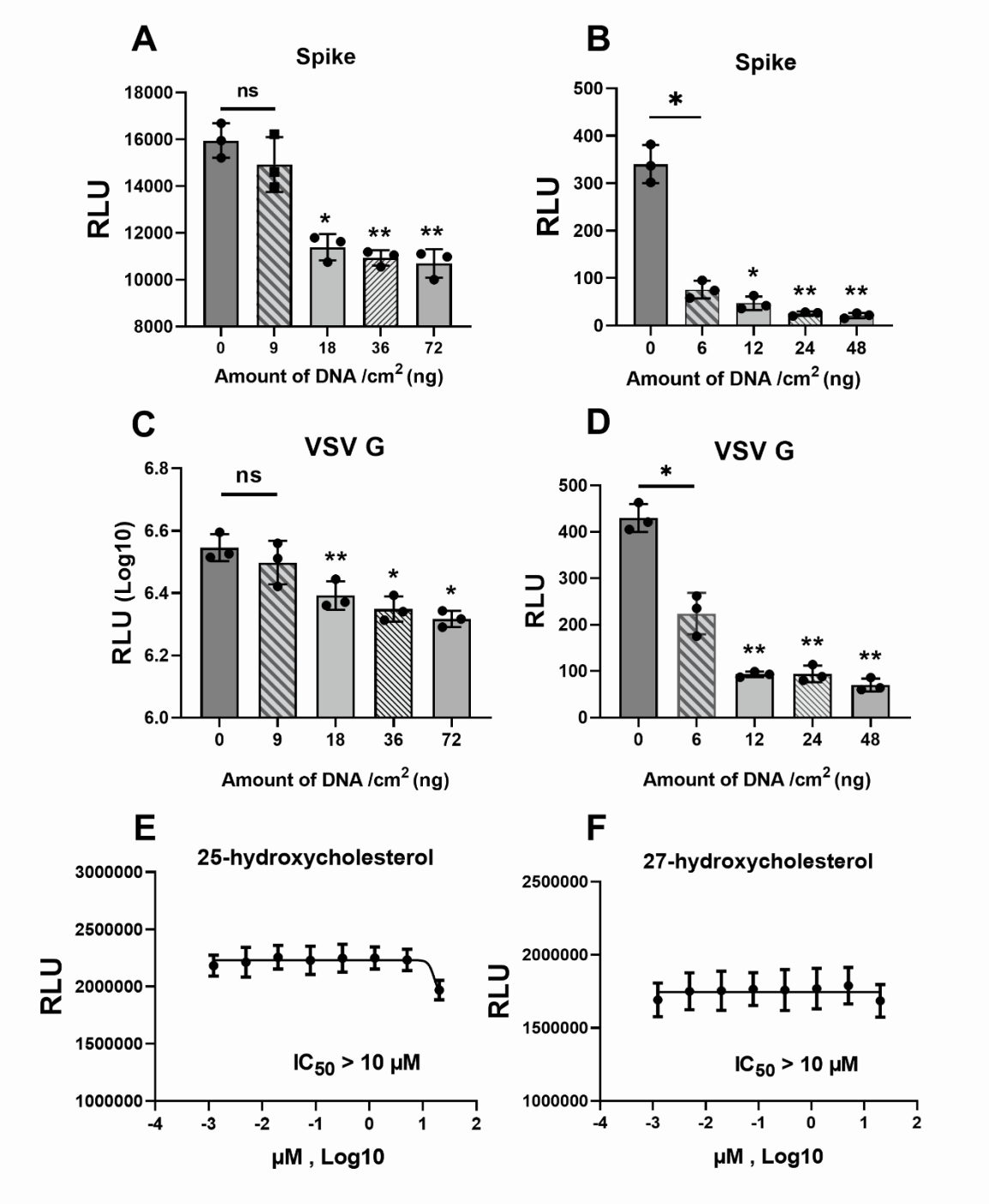


Figure S4. Studies with S variants of concern (VOC). Related to Figures 2, 3 and 4.

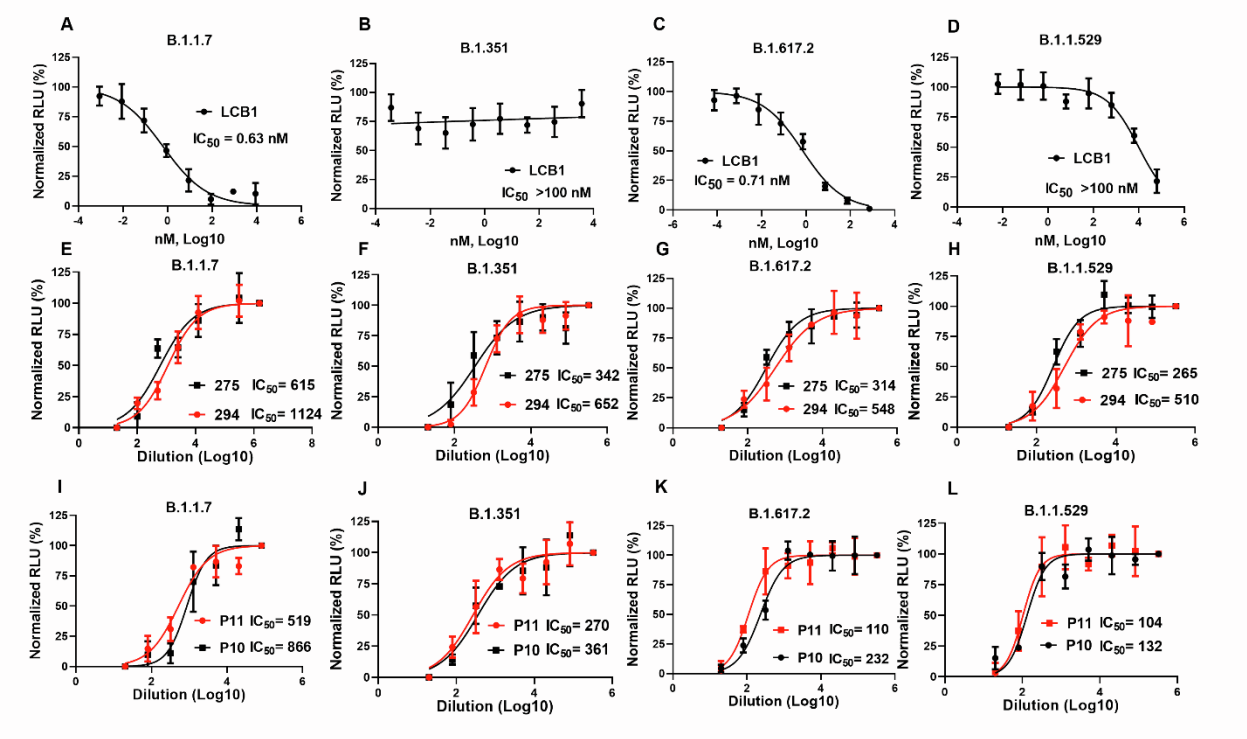


Figure S5. Immunoblotting and PCR of stable cell lines. Related to Figures 5 and 6.

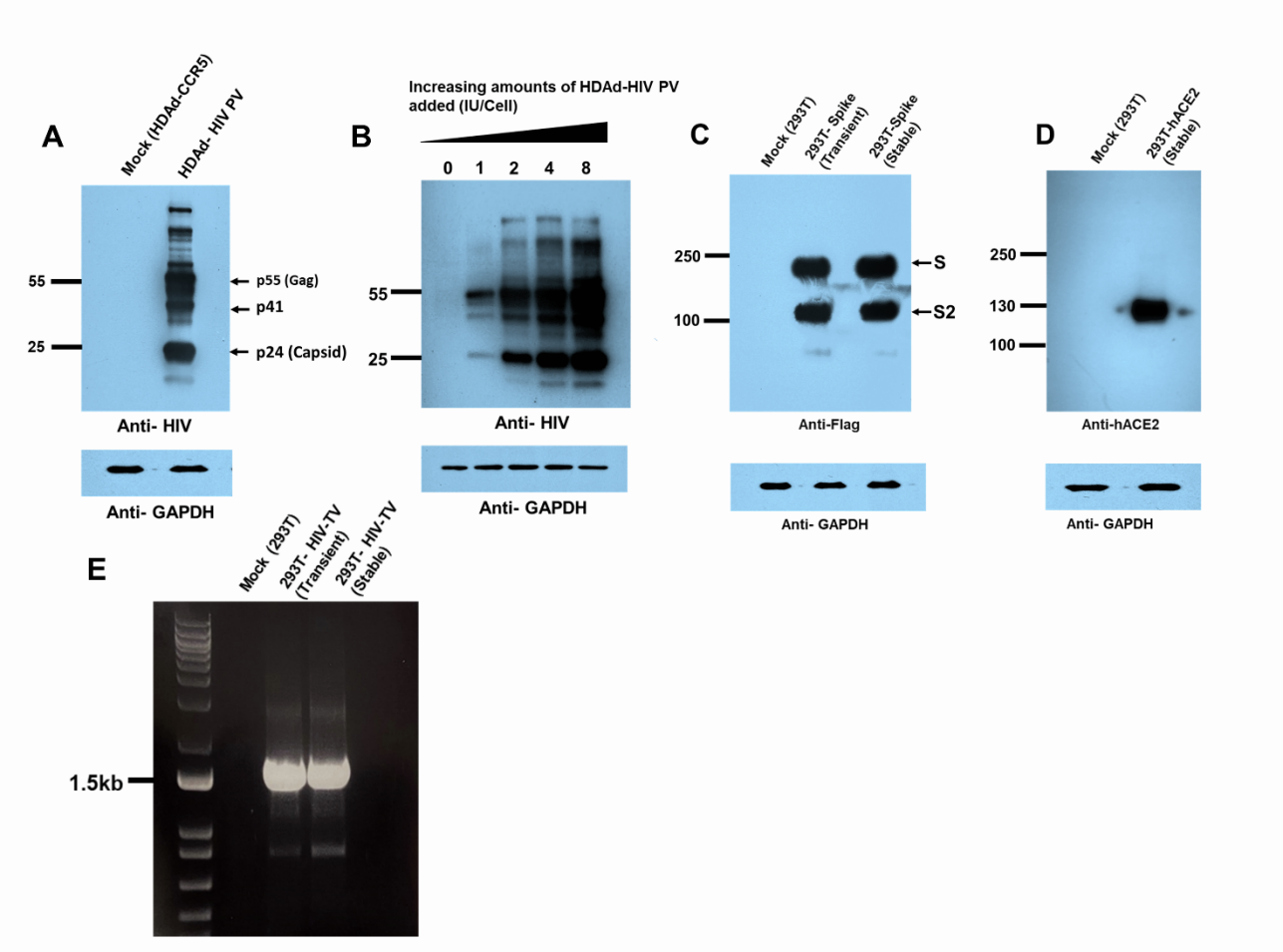
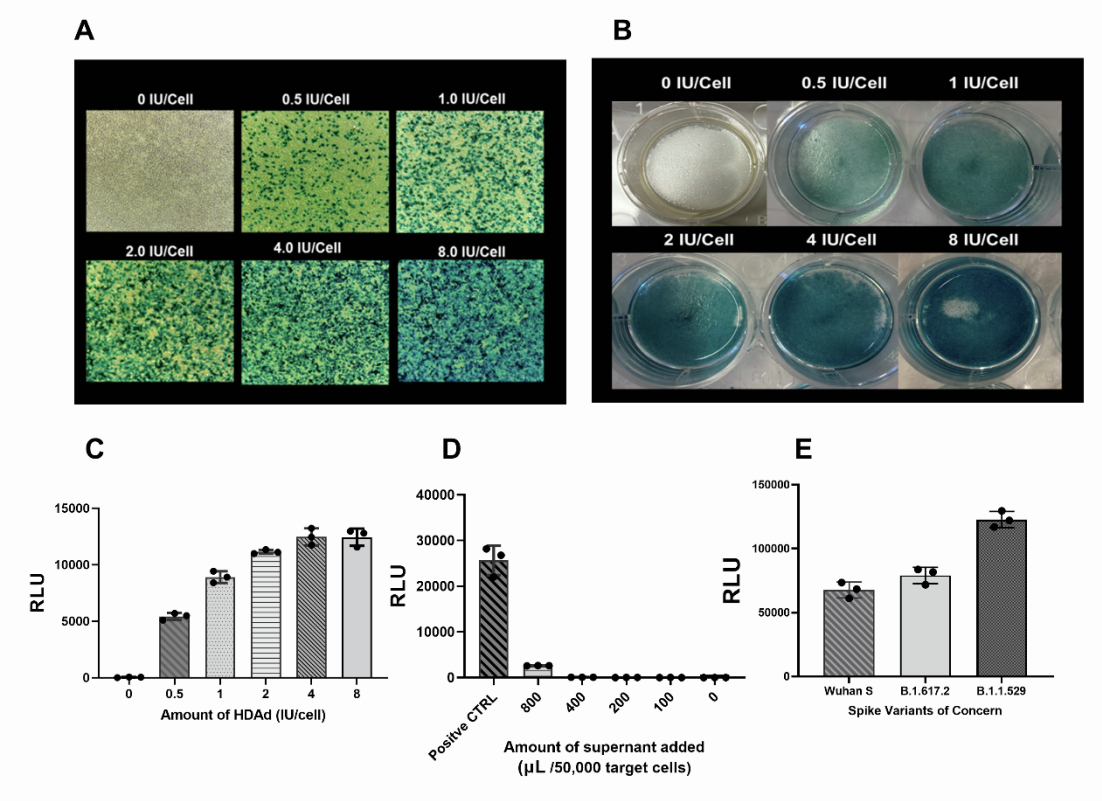


Figure S6. Transduction of stable 293T cells with HDAd-HIV-PV. Related to Figures 5 and 6.



SUPPLEMENTAL TABLES WITH TITLES

Table S1.

Demographic and relevant clinical information of the subjects from whom COVID-19 convalescent sera samples were collected. Related to Figure 2.

Subject ID	Date of sample collection (after symptom onset)	Age	Sex	Race	BMI[a]	Co-morbidities				Severity of disease		Outcome
						Chronic heart disease?	Chronic lung disease?	High blood pressure	Other co-morbid conditions	moderate	severe	
262	21	50	M	hispanic	42			+	diabetes		Intubated[d]	deceased post 1 month
264	7	73	M	black	30			+		Floor[c]		D/C[e] post 1 month
268	8	40	M	asian	29					Floor[c]		D/C[e] at 1 week
271	10	95	F	white	27			+	GERD[j], hypothyroidism	Floor[c]		Deceased post 1 month
272	4	76	M	white	34			+	diabetes	Floor[c]		D/C[e] after 2 days
273	3	66	F	black	37	Afib[f], CHF[b]	COPD[i]	+			Intubated[d]	D/C after 1 month
275	11	86	M	hispanic	25				Diabetes, Alzheimer's dementia	Floor[c]		D/C[e] after 1 week
292	5	69	M	black	27			+		Floor[c]		D/C[e] after 1 week
294	12	65	M	white	46		COPD[i]	+	Diabetes, Hx PE[k]		Intubated[d]	D/C[e] after 2 months
304	16	59	M	Black	16				Hx pancreatitis, alcohol use disorder		Intubated[d]	D/C[e] after 3 weeks

332	8	94	F	black	36	CHF[b]	COPD[i], OSA[g]	+	Breast cancer, GERD[j]		Bipap[h]	Deceased after 6 days

Footnotes:

[a]BMI denotes body mass index

[b]CHF denotes congestive heart failure

[c]Floor denotes subject remained on a regular in-patient unit

[d]Intubated denotes subject was endotracheally intubated in the intensive care unit

[e]D/C denotes discharge from the hospital

[f]Afib denotes atrial fibrillation

[g]OSA denotes obstructive sleep apnea

[h]BIPAP denotes subject required Bilevel Positive Airway Pressure required but not endoctracheal intubation

[i]COPD denotes chronic obstructive pulmonary disease

[j]GERD denotes Gastroesophageal reflux disease

[k]PE denotes pulmonary embolism

Table S2.

Demographic and relevant clinical information of the subjects from whom post-vaccine sera samples were collected. Related to Figures 3 & 5.

Subject ID*	Age	Sex	Race/Ethnicity	BMI[a]	Co-morbidities		
					Chronic heart disease?	Chronic lung disease?	Other co-morbid conditions
P01	65	F	white	27.4	CAD[b]	COPD[c]	
P02	63	M	white	23.3			Hypertension
P03	57	F	white	34.1			Diabetes
P04	56	F	white	26.5			
P05	63	M	white	29.5			
P06	56	F	white	29.2			
P07	38	F	white	27.4			
P08	46	M	white	28.1			
P09	31	F	white-hispanic	23.4			
P10	55	F	white-hispanic	32.1			
P11	40	F	Asian	19.3			

Footnotes:

[a]BMI denotes body mass index

[b]CAD denotes coronary artery disease

[c]COPD denotes chronic obstructive pulmonary disease

*All subjected received the Pfizer vaccine one month prior to blood draw