

**Supplemental information**

**Development of safe and highly  
protective live-attenuated SARS-CoV-2  
vaccine candidates by genome recoding**

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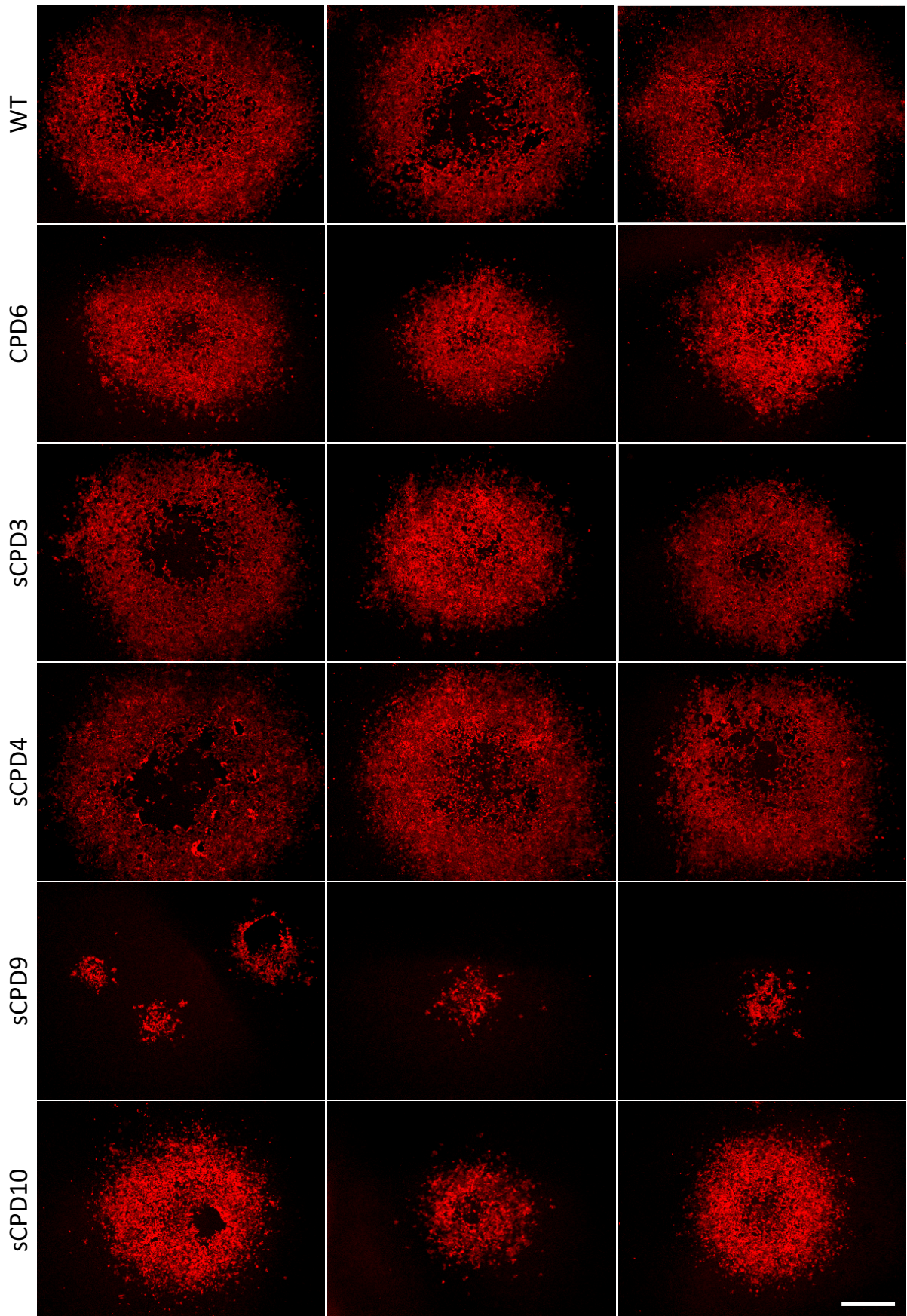
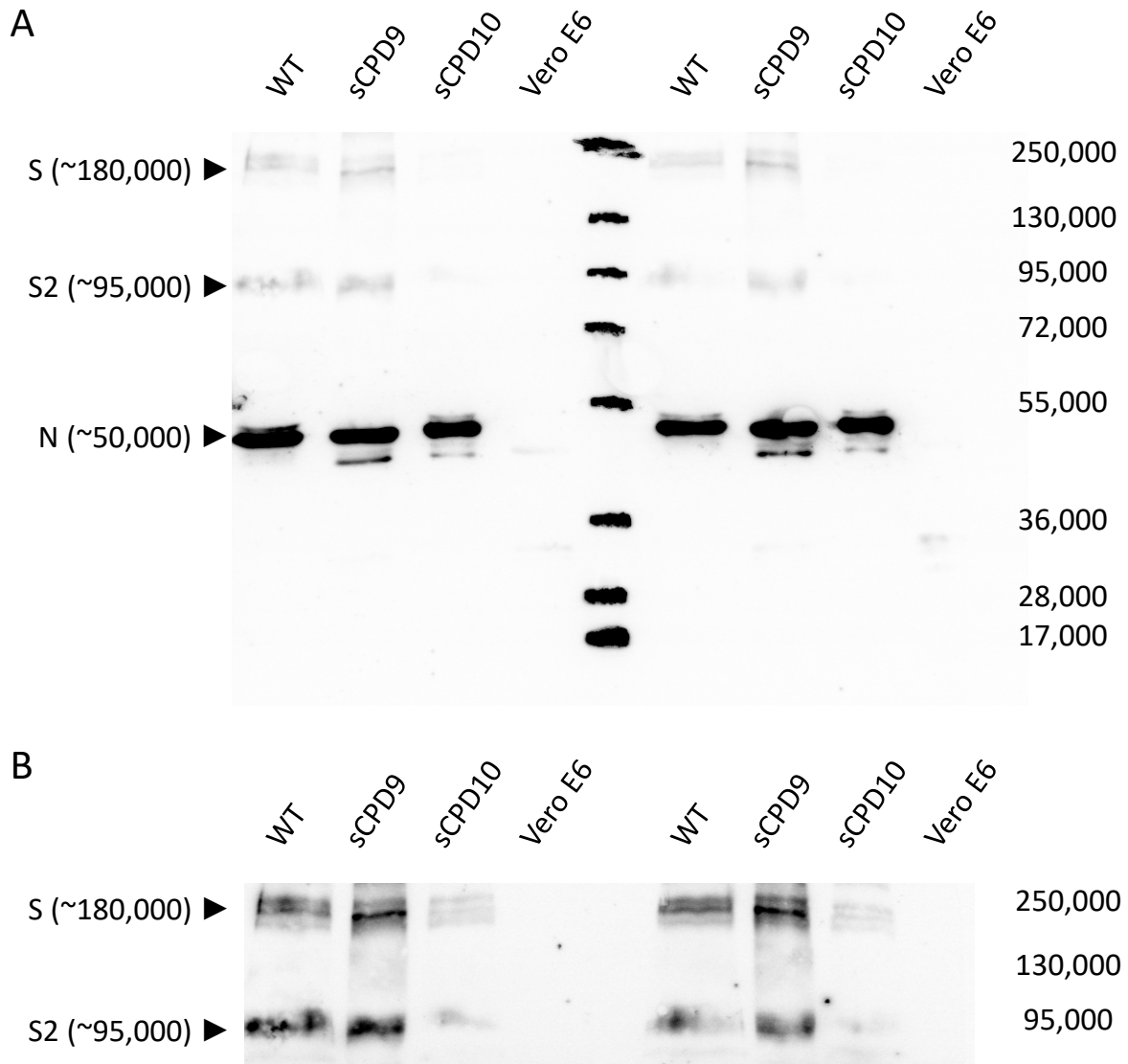


Figure S1. Representative images of virus foci formed by the parental SARS-CoV-2 (WT) and recoded viruses. Related to Figure 2. Bar, 1 mm.



**Figure S2. sCPD10 virus produces less spike protein in infected Vero E6 cells than parental or sCPD9 viruses. Related to Figures 1 and 2.**

(A) Western blot analysis of viral protein production in infected cells by SARS-CoV-2 (WT), sCPD9 and sCPD10 viruses. Vero E6 cells were infected with WT, sCPD9 or sCPD10 viruses. After 48 h, infected cells were lysed, cell lysates separated by SDS-PAGE under reducing conditions, and proteins were transferred to PVDF membranes. Membranes were cut into two parts. The upper membrane part, containing proteins with higher molecular weight, was incubated with a mouse monoclonal anti-S antibody. Note that the antibody binds to the S2 subunit of the spike protein and thus can recognize both the uncleaved spike protein (MW ~180,000) and its S2 subunit (MW ~95,000). The lower membrane part containing lower molecular weight proteins was incubated with a mouse monoclonal antibody recognizing the N protein (MW ~90,000).

(B) The image of the upper part of the membrane containing the uncleaved spike protein and its subunits, obtained after a longer exposure. Data are representative of three independent experiments.

**Table S1. Properties of the parental (WT) and recoded, codon pair deoptimized (CPD) sequences. Related to Figure 1.**

Sequence	Length (bp)	Codons	GC (%)	CPS (WT)	CPS (CPD)	UpA (WT)	UpA (CPD)	CpG (WT)	CpG (CPD)
CPD2	2,007	669	39.0	0.054	-0.361	118	195	27	107
CPD3	2,676	892	36.5	0.070	-0.282	227	324	20	109
CPD4	2,400	800	34.4	0.029	-0.297	239	317	31	105
CPD5	2,667	889	36.6	0.041	-0.307	253	335	20	115
CPD6	1,836 (1,482 + 354)	612	39.5	0.036	-0.337	152	225	25	94
CPD7	2,790	930	37.5	0.042	-0.323	250	347	41	162
CPD8	2,406	802	37.9	0.059	-0.344	205	293	26	132
CPD9	2,508 (1,146 + 1,362)	836	34.8	0.056	-0.321	221	310	26	116
CPD10	2,046	682	38.6	0.099	-0.302	123	216	16	110
sCPD3	999	333	36.8	0.077	-0.294	76	122	11	42
sCPD4	999	333	34.0	0.014	-0.278	114	143	11	34
sCPD5	999	333	35.8	0.035	-0.320	91	127	6	44
sCPD8	1,011	337	36.4	0.094	-0.302	88	119	7	54
sCPD9	1,146	382	34.4	0.056	-0.302	104	138	14	51
sCPD10	999	333	38.1	0.109	-0.340	51	103	5	49

WT and CPD sequences contain exactly the same codons, but the position of the synonymous codons in the corresponding sequences is different. CPD increases the number of UpA and CpG dinucleotides in deoptimized sequences. Length, length of recoded sequences in recoded fragments; GC, GC-content; CPS, average codon pair score of the WT and CPD sequences; UpA and CpG, number of UpA and CpG dinucleotides.

**Table S2. The most relevant ongoing clinical trials registered with ClinicalTrials.gov. Related to Figure 1.**

Identifier	Sponsor/Institution	Vaccine
<b>Non-replicating virus vector vaccines</b>		
NCT04530396	Gamaleya Research Institute of Epidemiology and Microbiology	Sputnik V
NCT04741061	Gamaleya Research Institute of Epidemiology and Microbiology	Sputnik-Light
NCT04516746	AstraZeneca	AZD1222
NCT04536051	AstraZeneca	AZD1222
NCT04341389	Institute of Biotechnology, Academy of Military Medical Sciences, PLA of China	Ad5-nCoV vaccine
NCT04526990	CanSino Biologics Inc.	Ad5-nCoV
NCT04840992	CanSino Biologics Inc.	Ad5-nCoV
NCT04614948	Janssen Vaccines & Prevention B.V.	d26.COVS.2
NCT04838795	Janssen Vaccines & Prevention B.V.	d26.COVS.2
NCT04791423	ReiThera Srl	GRAd-COV2
<b>mRNA vaccines</b>		
NCT04754594	BioNTech SE	mRNA vaccine BNT162b2
NCT04368728	BioNTech SE	mRNA vaccines BNT162b1, BNT162b2 and BNT162b2SA
NCT04813796	ModernaTX, Inc.	mRNA-1283 and mRNA-1273 vaccines
NCT04785144	ModernaTX, Inc.	mRNA-1273.351 vaccine
NCT04811664	National Institute of Allergy and Infectious Diseases	mRNA-1273 vaccine
NCT04470427	ModernaTX, Inc.	mRNA-1273 vaccine
NCT04796896	ModernaTX, Inc.	mRNA-1273 vaccine
NCT04649151	ModernaTX, Inc.	mRNA-1273 vaccine
NCT04652102	CureVac AG	mRNA vaccine CVnCoV
NCT04860258	CureVac AG	mRNA vaccine CVnCoV
NCT04674189	CureVac AG	mRNA vaccine CVnCoV
NCT04847102	Walvax Biotechnology Co., Ltd.	mRNA vaccine ARCoV
<b>Inactivated or subunit virus vaccines</b>		
NCT04582344	Health Institutes of Turkey, Sinovac	inactivated SARS-CoV-2
NCT04747821	Butantan Institute, Sinovac	inactivated SARS-CoV-2
NCT04659239	Chinese Academy of Medical Sciences	inactivated SARS-CoV-2
NCT04510207	China National Biotec Group Company	inactivated SARS-CoV-2, BBIBP-CorV
NCT04560881	Laboratorio Elea Phoenix S.A.	inactivated SARS-CoV-2, BBIBP-CorV
NCT04611802	Novavax	SARS-CoV-2 rS with Matrix-M1 adjuvant
NCT04583995	Novavax	SARS-CoV-2 rS with Matrix-M1 adjuvant
NCT04636697	Medicago	recombinant coronavirus-like particle CoVLP
NCT04852705	Shenzhen Kangtai Biological Products Co.	inactivated SARS-CoV-2
NCT04641481	Bharat Biotech International Ltd.	BBV152
NCT04683224	Vaxxinity, Inc.	subunit vaccine UB-612
<b>Live attenuated virus vaccines</b>		
NCT04619628	Codagenix, Inc	COVI-VAC