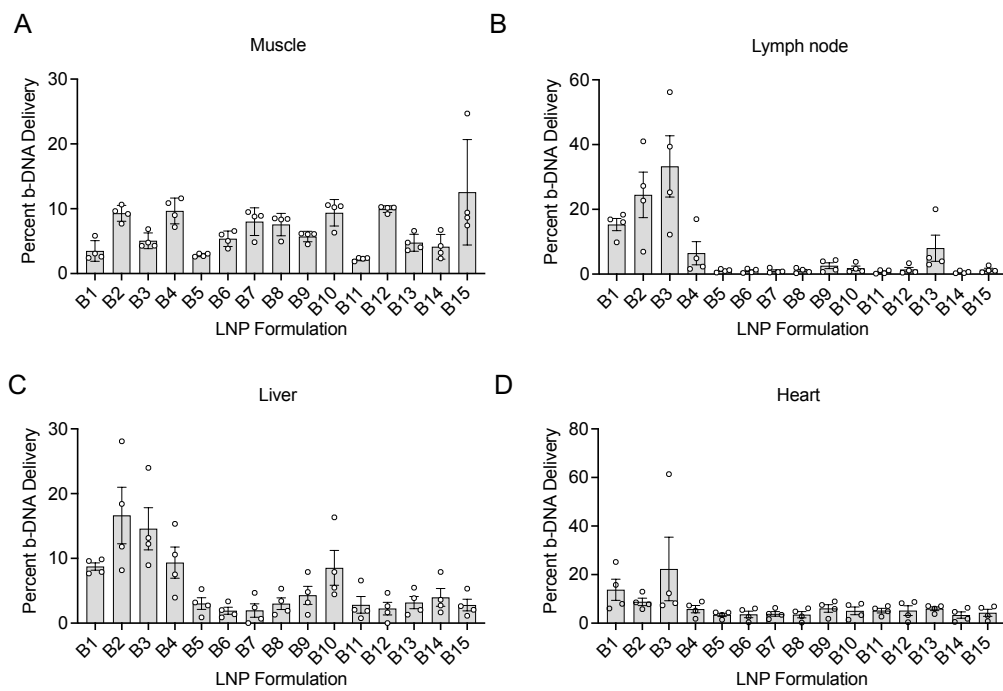
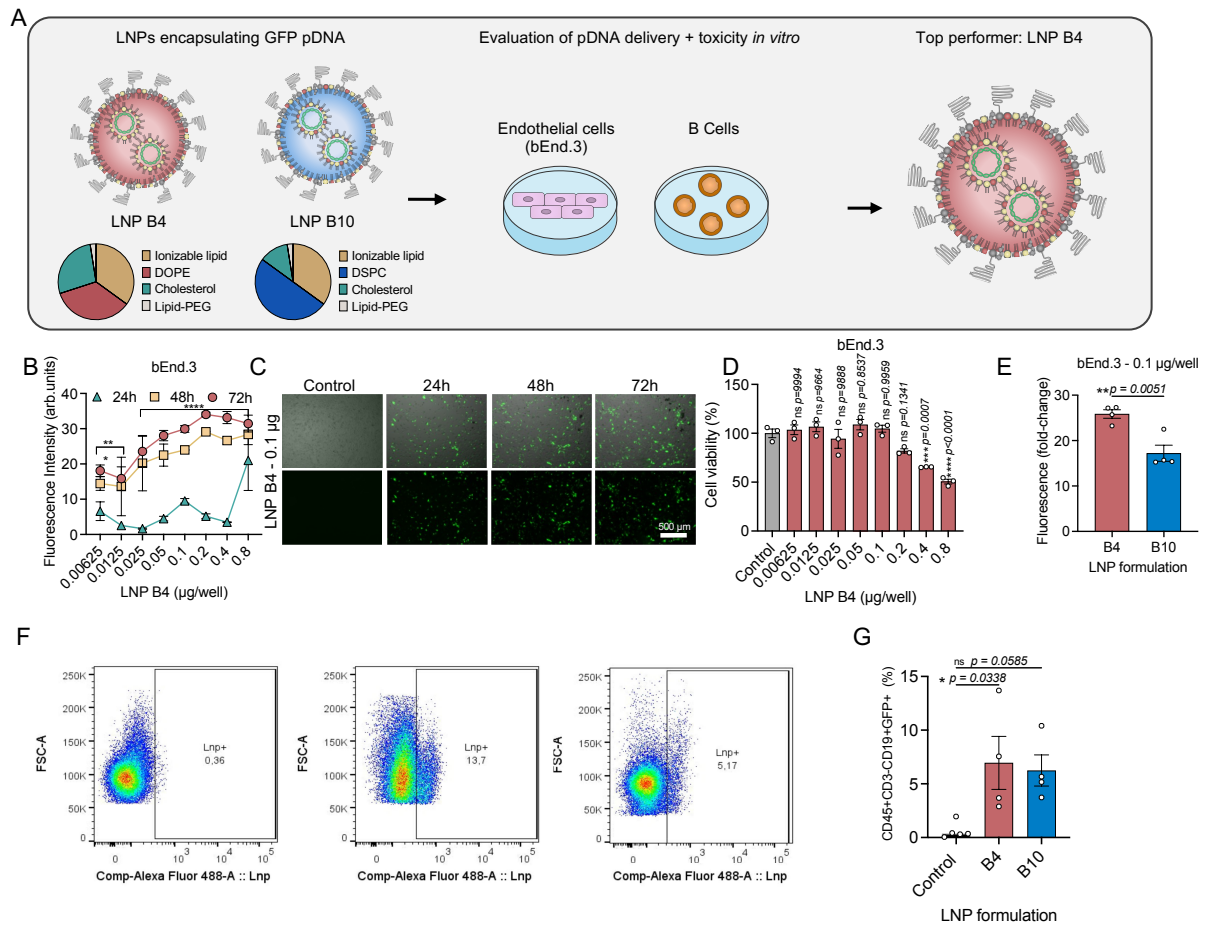


## Supplementary information

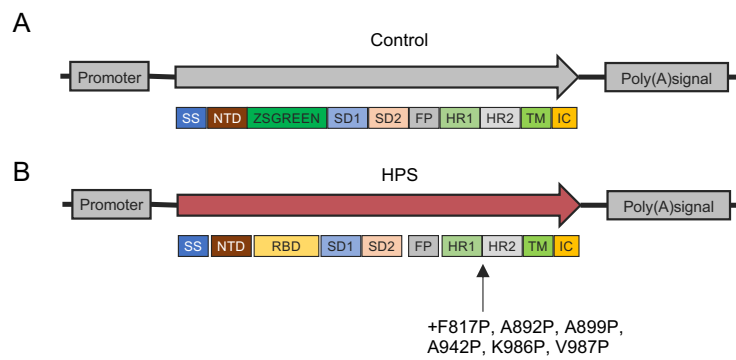
### Nanoparticle-based DNA vaccine protects against SARS-CoV-2 variants in female preclinical models



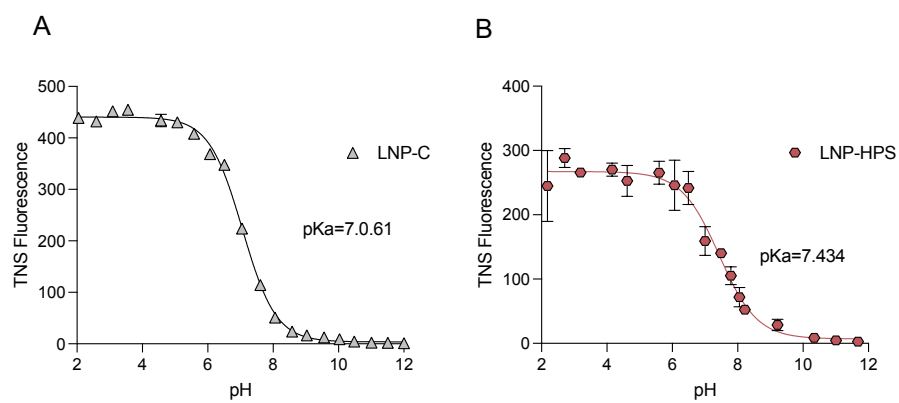
**Supplementary Fig. 1: In vivo delivery of LNPs encapsulating barcoded pDNA.** The bar graph illustrates the percentage quantification of LNP (B1–B15) delivery in different target tissues 4 hours after intramuscular injection ( $n = 4/\text{group}$ ). **(A)** Muscle and **(B)** Lymph node **(C)** Liver, **(D)** Heart. Data plotted as mean  $\pm$  SEM. Source data are provided as a Source Data file.



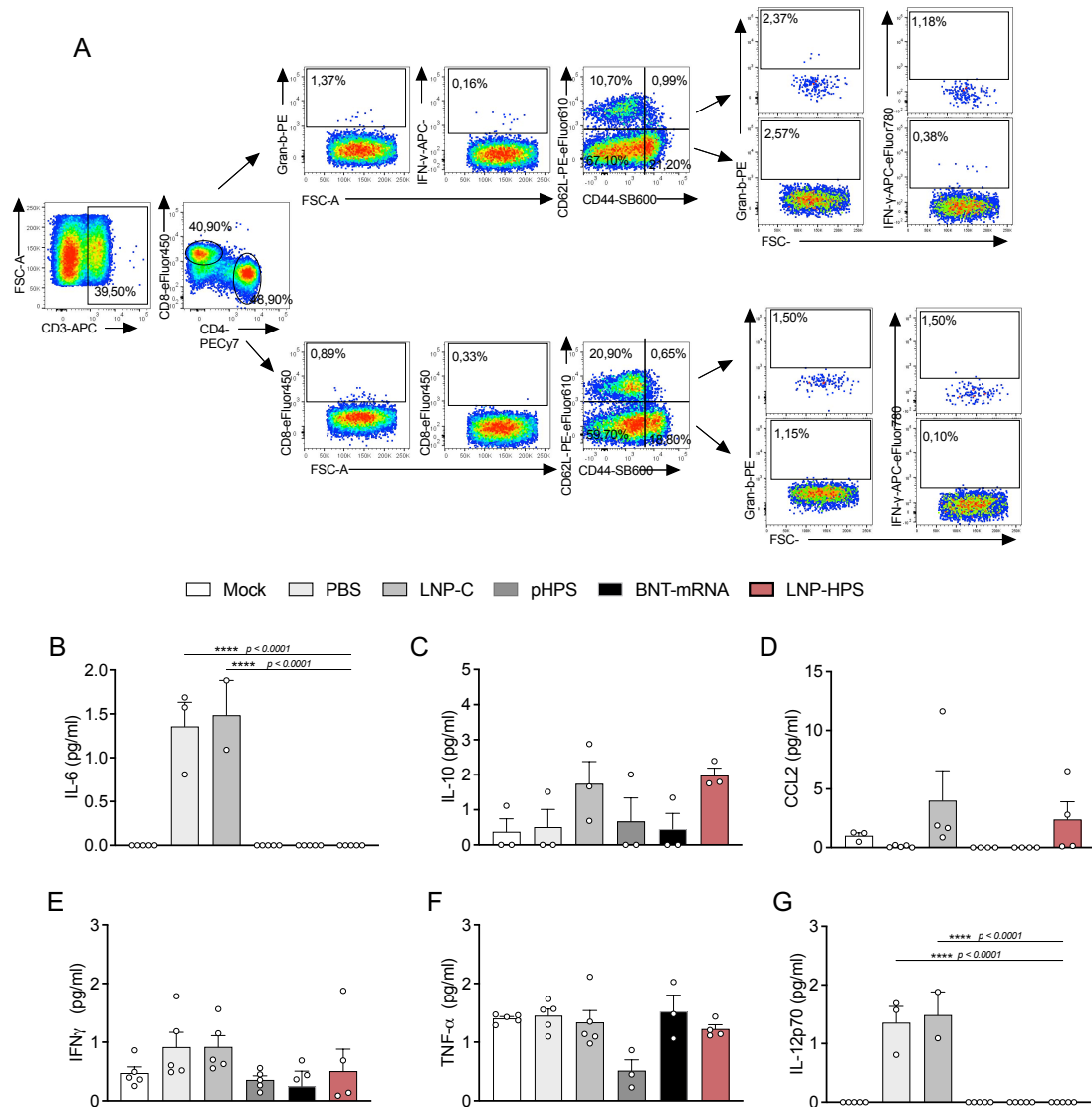
**Supplementary Fig. 2: Top-performing LNPs induced enhanced in vitro GFP expression in endothelial and B cells.** (A) Schematic of the 2 top-performing LNPs encapsulating DNA encoding GFP for in vitro screening of transfection efficiency in endothelial and B cells. (B) Quantification of GFP expression was measured after 24 h, 48 h, and 72h. (C) Representative GFP fluorescence (Bottom) and overlaid on brightfield (Top) photomicrographs after treatment of LNP B4 in endothelial cells (n = 4/group). (D) cell viability was measured after 72 h in endothelial cells transfected with LNP B4 at different doses (n = 3/group). (E) Quantification of GFP expression after treatment with B4 and B10 LNPs in endothelial cells. (F) Representative density plots illustrating the gating strategy for CD45+CD3-CD19+GFP+ in splenocytes treated with B4 or B10. (G) Quantification of GFP+ expression following treatment with B4 and B10 LNPs in B cells from mouse splenocytes. Data are presented as mean  $\pm$  SEM (n = 4/group). (B, F, D, G) One-way ANOVA with Dunnet's post hoc test for 72h compared to control; ns not significant, \*p<0.05, \*\*p<0.01\*\*\*p<0.001 \*\*\*\*p<0.0001. (E, G) Two-tailed unpaired t-test; \*p<0.05, \*\*p<0.01. Source data are provided as a Source Data file.



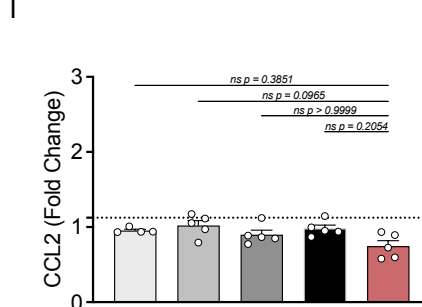
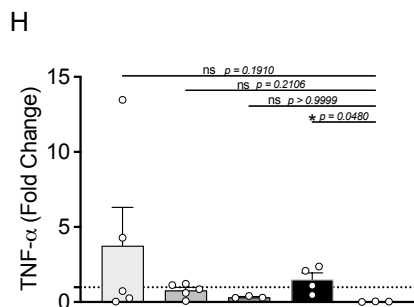
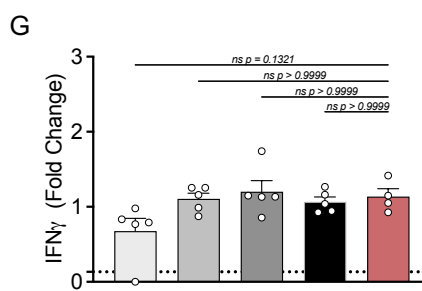
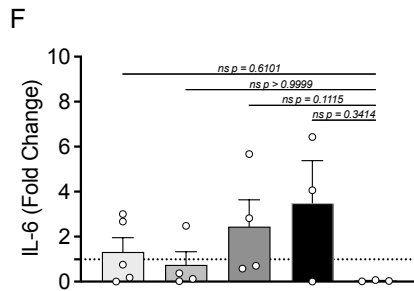
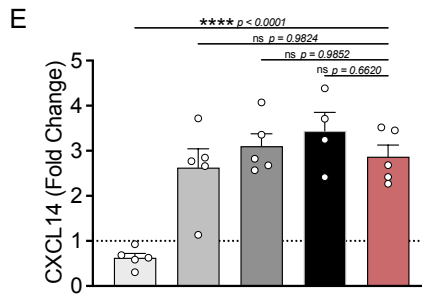
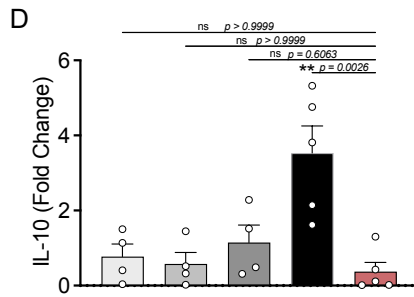
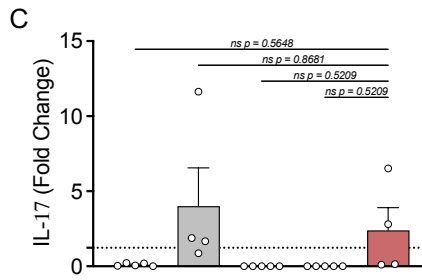
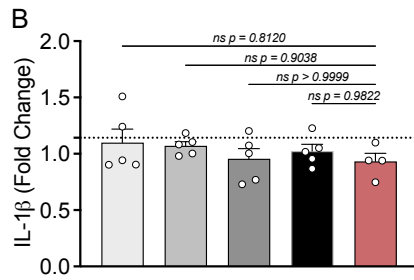
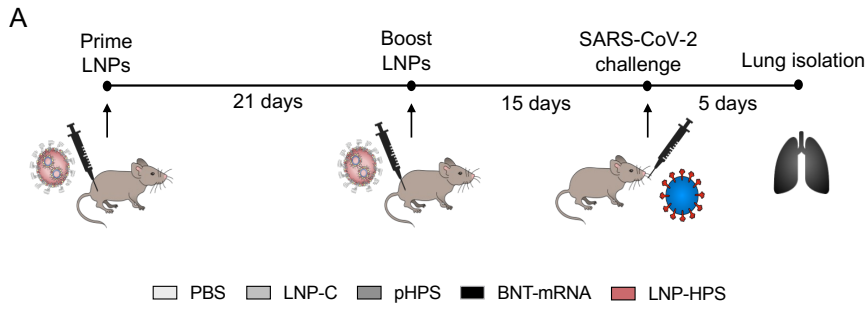
**Supplementary Fig. 3: Plasmid DNAs (A)** pZsGreen-N1 (Clontech laboratories, no. 632448) encoding ZsGreen, a green fluorescent protein (GFP) **(B)** recombinant HexaPro Spike plasmid (Addgene, no. 154754). Source data are provided as a Source Data file.



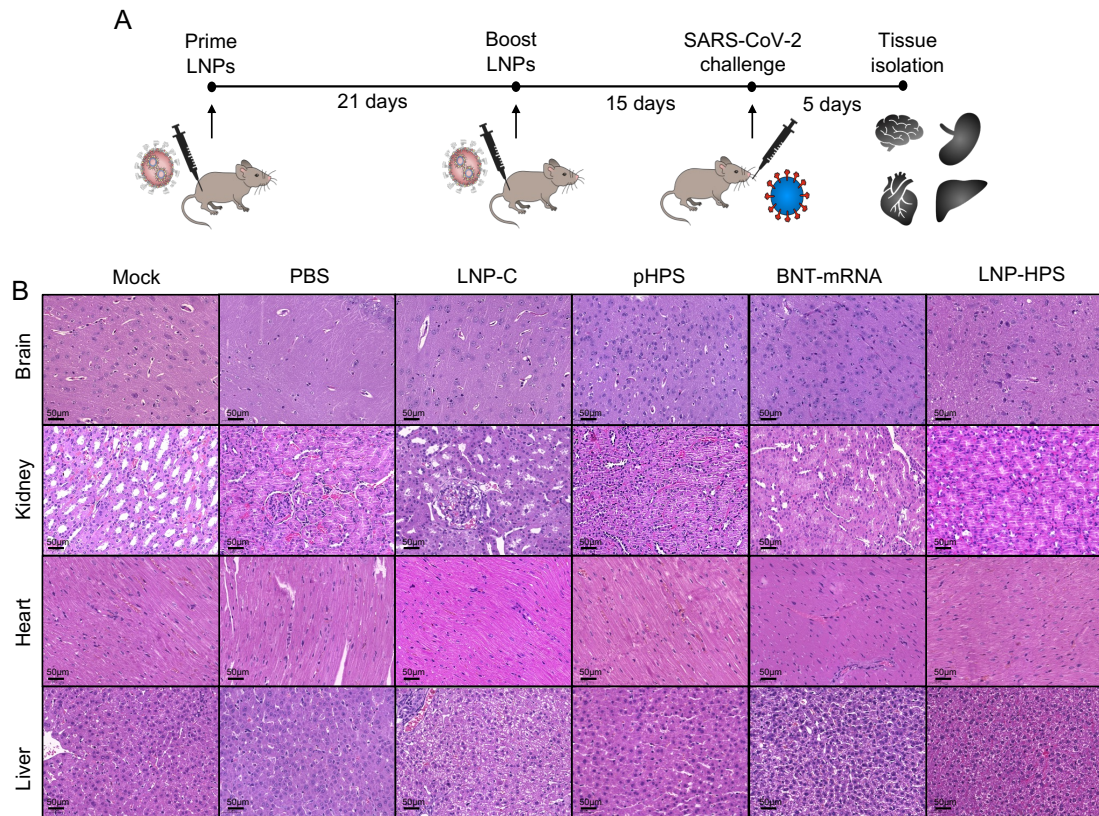
**Supplementary Fig. 4: pKa measurements of LNP-C (A) and LNP-HPS (B) via TNS fluorescence assay.** ( $n = 6/\text{group}$ ). Data are presented as mean  $\pm$  SEM. Source data are provided as a Source Data file.



**Supplementary Fig. 5: Gating strategy and serum cytokine and chemokines measurements.** (A) Representative density plots showing the gating strategy. T cell subsets were gated on CD45+ live cells following the gate for single cells (FSC-H x FSC-A). CD3-positive cells were further subdivided into CD4 and CD8 T cells. Within each subset, memory subpopulations were assessed, including central memory (CD44+CD62L+), effector/effector memory (CD44+CD62L-), and naive cells (CD44-CD62L+). IFN-gamma and granzyme-b expression were evaluated in all subsets. (B-G) Serum cytokines and chemokines were quantified in K18-ACE-2 mice were immunized with controls and LNP-HPS and boosted with equivalent doses 3 weeks later. Immunized mice were challenged with lethal  $6 \times 10^4$  PFU of SARS-CoV-2 variants Gamma lineage (P.1). Levels of cytokines in serum of mice were measured by at 5 days post-infection (n = 5/group). Data are presented as mean  $\pm$  SEM. One-way ANOVA followed by Dunnett's multiple comparison test. \*\*\*\*p<0.0001. Source data are provided as a Source Data file.

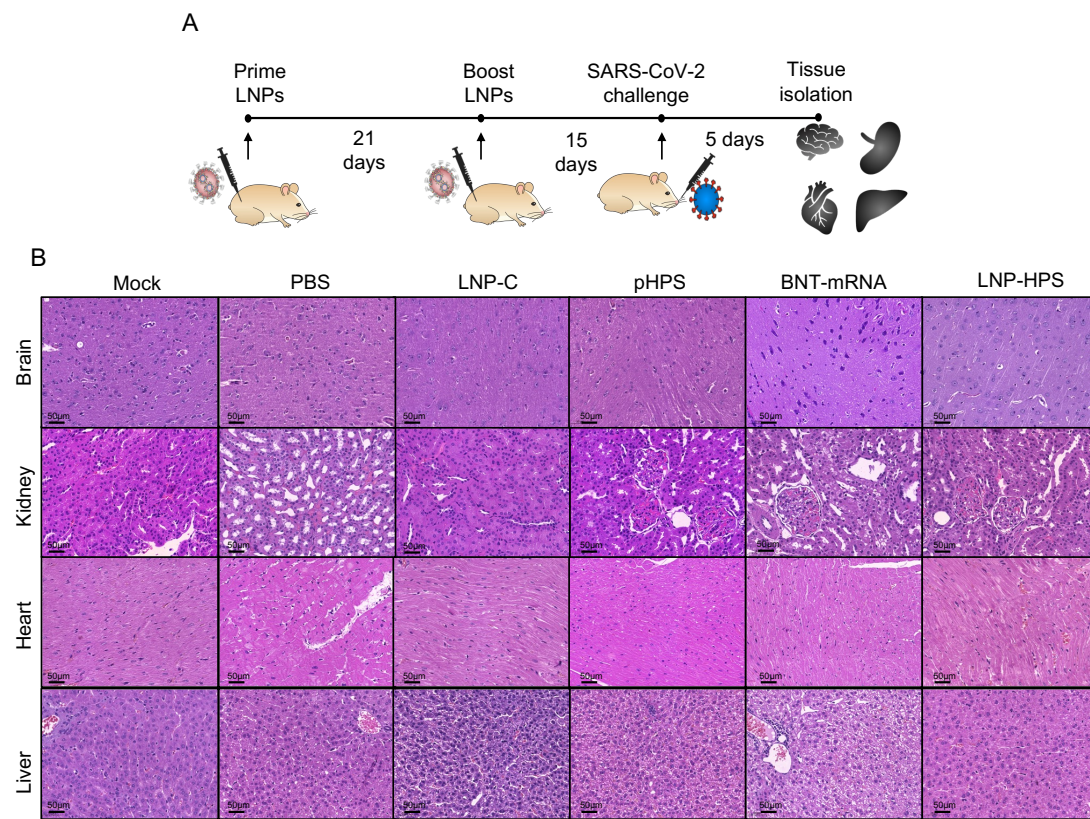


**Supplementary Fig. 6: Levels of cytokines.** (A) Scheme of immunization: K18-ACE-2 mice were immunized with controls and LNP-HPS, and boosted with equivalent doses 3 weeks later. Fold change in the gene expression of the indicated cytokines and chemokines (B) IL-1 $\beta$ , (C) IL-17, (D) IL-10, (E) CXCL14, (F) IL-6, (G) IFN $\gamma$ , (H) TNF, and (I) CCL2, as determined by RT-qPCR, in lung homogenates of immunized and controls K18-hACE-2 mice infection (Mock n = 4; PBS, LNP-C, pHPS, BNT-mRNA, and LNP-HPS n = 5). HPRT was used as a reference gene. Data are presented as mean  $\pm$  SEM. One-way ANOVA followed by Tukey's multiple comparison test. \*p<0.05, \*\*p<0.01, \*\*\*\*p<0.0001. Source data are provided as a Source Data file.



**Supplementary Fig. 7: Histopathological analysis of P.1 SARS-CoV-2 infection in K18-hACE2 mice. (A)** Scheme of immunization. K18-hACE2 mice were immunized with controls and LNP-HPS and boosted with equivalent doses 3 weeks later. Immunized mice were challenged with lethal  $6 \times 10^4$  PFU of SARS-CoV-2 variants Gamma lineage (P.1). Brain, kidney, heart and liver were harvested at 5 days post-infection for all immunized groups for histopathological analysis. **(B)** Histopathological analysis at 20 x magnification of brain, kidney, heart and liver at 5 days post-infection with the P.1 strain ( $n = 5/\text{group}$ ), reveals no significant histological changes between the groups. Source data are provided as a Source Data file.





**Supplementary Fig. 8: Histopathological analysis of P.1 SARS-CoV-2 infection in hamsters.** **(A)** Scheme of immunization. Syrian hamsters were immunized with controls and LNP-HPS and boosted with equivalent doses 3 weeks later. Immunized hamsters were challenged with  $6 \times 10^5$  PFU of SARS-CoV-2 variants Gamma lineage (P.1). Brain, kidney, heart and liver were harvested at 5 days post-infection for all immunized groups for histopathological analysis. **(B)** Histopathological analysis at 20 x magnification of brain, kidney, heart and liver at 5 days post-infection with the P.1 strain ( $n = 5/\text{group}$ ), reveals no significant histological changes between the groups. Source data are provided as a Source Data file.

**Supplementary Table 1:** Composition of ionizable lipid nanoparticle formulations

LNP formulation	Ionizable lipid:b-DNA ratio	Molar ratio %				
		Ionizable lipids	Helper lipids	Cholesterol	Lipid-PEG	
B1	10:1	35	15	47,5	2,5	DOPE
B2	10:1	35	20	42,5	2,5	
B3	10:1	35	25	37,5	2,5	
B4	10:1	35	35	27,5	2,5	
B5	10:1	35	50	12,5	2,5	
B6	10:1	35	15	47,5	2,5	DSPC
B7	10:1	35	20	42,5	2,5	
B8	10:1	35	25	37,5	2,5	
B9	10:1	35	35	27,5	2,5	
B10	10:1	35	50	12,5	2,5	
B11	10:1	35	15	47,5	2,5	DOTAP
B12	10:1	35	20	42,5	2,5	
B13	10:1	35	25	37,5	2,5	
B14	10:1	35	35	27,5	2,5	
B15	10:1	35	50	12,5	2,5	

**Abbreviations:** LNP: ionizable lipid-nanoparticles; b-DNA: barcoded DNA; DOPE: 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine; DSPC:1,2-distearoyl-sn-glycero-3-phosphocholine; DOTAP:1,2-dioleoyl-3-trimethylammonium-propane.

**Supplementary Table 2: Oligonucleotide sequences of b-DNA**

LNP formulation	b-DNA added	Oligonucleotide sequences
B1	TAACGCACCT	A*G*A*CGTGTGCTCTTCCGATCT TAACGCACCT NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B2	GAGGGTACTT	A*G*A*CGTGTGCTCTTCCGATCT GAGGGTACTT NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B3	TGTCTCCCAT	A*G*A*CGTGTGCTCTTCCGATCT TGTCTCCCAT NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B4	GGAGAAACAG	A*G*A*CGTGTGCTCTTCCGATCT GGAGAAACAG NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B5	ATGATCGTCG	A*G*A*CGTGTGCTCTTCCGATCT ATGATCGTCG NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B6	TTGCAGCCTT	A*G*A*CGTGTGCTCTTCCGATCT TTGCAGCCTT NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B7	CGTACAAACG	A*G*A*CGTGTGCTCTTCCGATCT CGTACAAACG NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B8	ATCCATGAGG	A*G*A*CGTGTGCTCTTCCGATCT ATCCATGAGG NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B9	TTCCACGATG	A*G*A*CGTGTGCTCTTCCGATCT TTCCACGATG NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B10	GAATGCTGAC	A*G*A*CGTGTGCTCTTCCGATCT GAATGCTGAC NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B11	TCTCGCCTTT	A*G*A*CGTGTGCTCTTCCGATCT TCTCGCCTTT NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B12	GCTGGGAATT	A*G*A*CGTGTGCTCTTCCGATCT GCTGGGAATT NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B13	GACACGTTCT	A*G*A*CGTGTGCTCTTCCGATCT GACACGTTCT NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B14	TTCGCATCTG	A*G*A*CGTGTGCTCTTCCGATCT TTCGCATCTG NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T
B15	CAGATCAGAG	A*G*A*CGTGTGCTCTTCCGATCT CAGATCAGAG NNNNNNNNNN AGATCGGAAGAGCGTCG*T*G*T

**Abbreviations:** LNP: ionizable lipid-nanoparticles; b-DNA: barcoded DNA.

**Supplementary Table 3: Characterization of b-DNA encapsulated LNPs**

LNP formulation	Hydrodynamic diameter (nm)	Polydispersity index (PDI)	Zeta Potential (mV)	
<b>B1</b>	106 ± 2	0.114 ± 0.009	-9.37 ± 9.15	<b>DOPE</b>
<b>B2</b>	117 ± 5	0.187 ± 0.017	-6.14 ± 6.02	
<b>B3</b>	111 ± 4	0.181 ± 0.015	-2.2 ± 6.64	
<b>B4</b>	115 ± 3	0.138 ± 0.030	-1.41 ± 7.26	
<b>B5</b>	127 ± 2	0.141 ± 0.002	-8.9 ± 8.75	
<b>B6</b>	108 ± 3	0.242 ± 0.008	-10.05 ± 7.85	<b>DSPC</b>
<b>B7</b>	96 ± 8	0.221 ± 0.011	-12.35 ± 6.85	
<b>B8</b>	171 ± 1	0.229 ± 0.009	-9.67 ± 11.2	
<b>B9</b>	233 ± 6	0.224 ± 0.018	-5.31 ± 5.41	
<b>B10</b>	183 ± 10	0.235 ± 0.020	1.46 ± 5.08	
<b>B11</b>	118 ± 1	0.200 ± 0.002	-7.84 ± 6.97	<b>DOTAP</b>
<b>B12</b>	115 ± 3	0.124 ± 0.011	-23.03 ± 7.61	
<b>B13</b>	110 ± 1	0.102 ± 0.021	27.53 ± 4.41	
<b>B14</b>	162 ± 11	0.253 ± 0.016	16.8 ± 9.55	
<b>B15</b>	84 ± 5	0.261 ± 0.038	20.37 ± 6.01	

**Abbreviations:** b-DNA: barcoded DNA; LNP: ionizable lipid-nanoparticles; nm: nanometer; mV: millivolts; PDI: polydispersity index; DOPE: 1,3-bis(sn-3'-phosphatidyl)-sn-glycerol-2; DSPC: 1,3-bis(sn-3'-phosphatidyl)-sn-glycerol-2; DOTAP: 1,3-bis(3-trimethylammoniumpropyl)-sn-glycerol-2.