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Supplemental information

Lipid nanoparticle encapsulation of a Delta spike-CD40L DNA vaccine improves effectiveness against Omicron challenge in Syrian hamsters

Levi Tamming, Diana Duque, Jegarubee Bavananthasivam, Anh Tran, Casey Lansdell, Grant Frahm, Jianguo Wu, Emily E.F. Fekete, Marybeth Creskey, Sathya N. Thulasi Raman, Emmanuel Laryea, Wanyue Zhang, Annabelle Pfeifle, Caroline Gravel, Andrew Stalker, Anwar M. Hashem, Wangxue Chen, Matthew Stuible, Yves Durocher, David Safronetz, Jingxin Cao, Lisheng Wang, Simon Sauve, Michael Rosu-Myles, Xu Zhang, Michael J.W. Johnston, and Xuguang Li

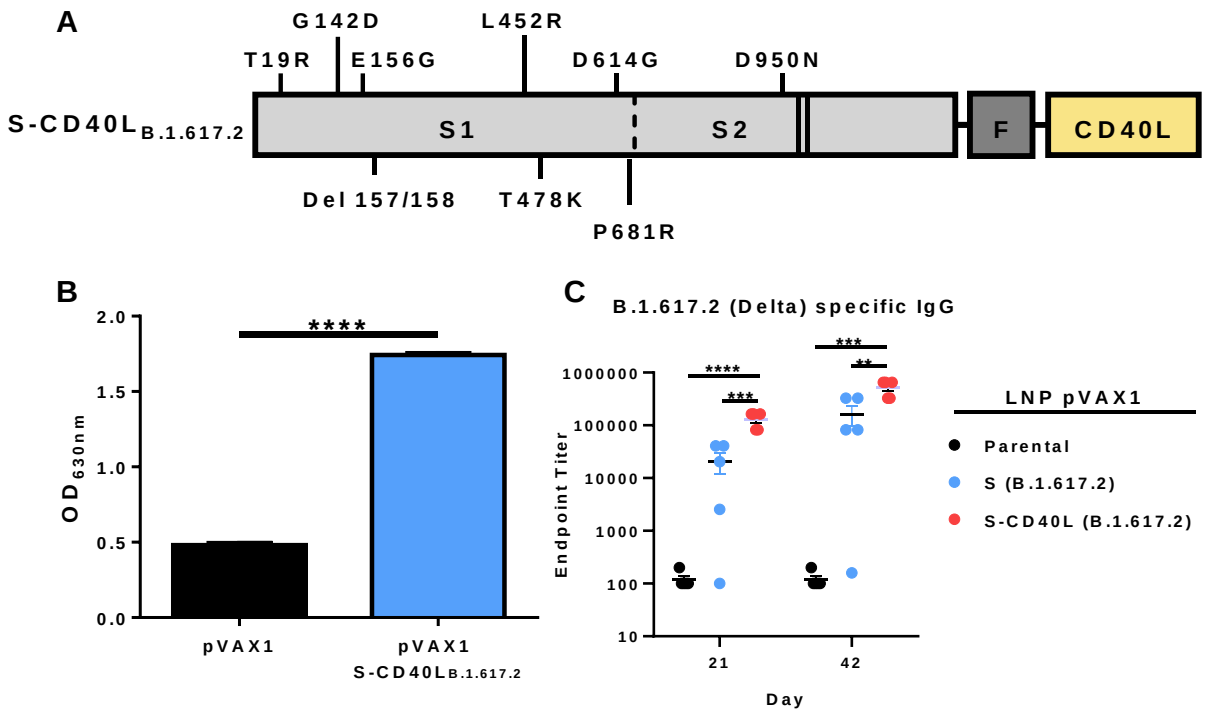

Figure S1. DNA Vaccine Design and Characterization. **(A)** The SARS-CoV-2 S ectodomain was prefusion stabilized *via* two proline mutations (solid lines) and the replacement of the furin cleavage site with "GSAS" (dotted line). The antigen was fused to a T4 fibritin trimerization motif (F) and the ectodomain of Syrian hamster CD40L (yellow). Nine mutations (T19R, G142D, E156G, Del 157/158, L452R, T478K, D614G, P681R, D950N) from the B.1.617.2 (Delta) variant were incorporated in the vaccine antigen. The codon-optimized DNA sequence encoding the fusion antigen was subcloned into a pVAX1 vector using KpnI and XhoI restriction enzymes. **(B)** CD40L reporter cells were stimulated for 24 hours with cell culture supernatant collected from HEK293T cells transfected with pVAX1 or pVAX1 S-CD40L$_{B.1.617.2}$. After the stimulation, SEAP expression in the reporter cell culture supernatant was measured using QUANTI-Blue™ reagent. Abs$_{630nm}$ values were measured after a 30-minute incubation. Data shown is mean ± SEM; n = 3 per group, ****p < 0.0001 (unpaired two-tailed t-test). **(C)** Male Syrian hamsters were immunized intramuscularly on day 0 and 28 with either 100-µg of pVAX1, or 5 of SM1-20 LNP-encapsulated pVAX1 S-CD40L$_{B.1.617.2}$. DNA-LNPs were generated using a NanoAssemblr™ Ignite™ instrument with NxGen™ cartridges equipped with toroidal structures. ELISA determination of total B.1.617.2 spike-specific IgG in the sera of immunized hamsters on Day 21 and 42. Data shown is mean ± SEM; n = 5 per group, **p < 0.01, ***p < 0.001, ****p < 0.0001.

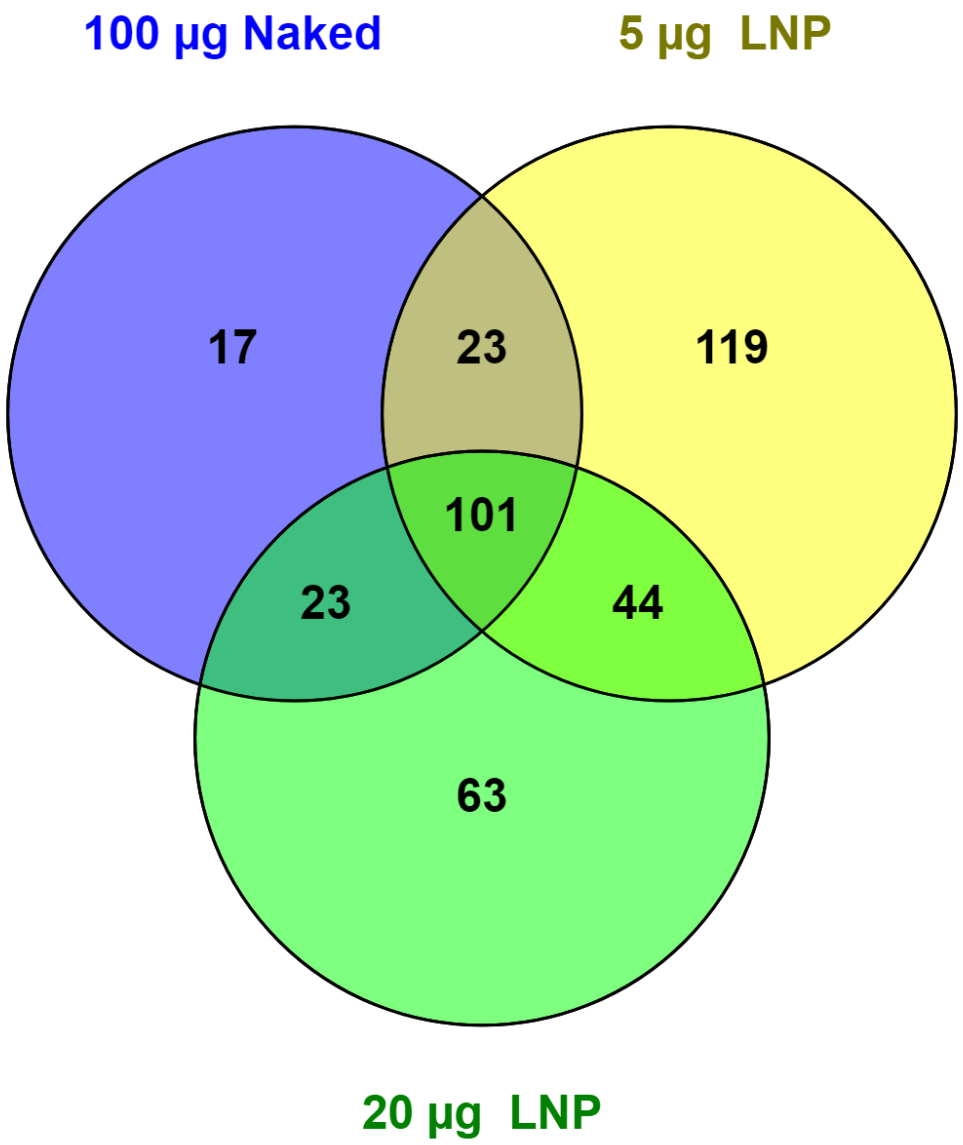

Figure S2: Venn Diagram of Differentially Expressed Proteins. Venn diagram showing overlap in DEP proteins between pVAX1 vector and pVAX1 S-CD40L$_{B.1.617.2}$ vaccinated groups. Venn diagram generated using Venny 2.1 [Oliveros, J.C. (2007-2015) Venny. An interactive tool for comparing lists with Venn's diagrams. https://bioinfogp.cnb.csic.es/tools/venny/index.html]

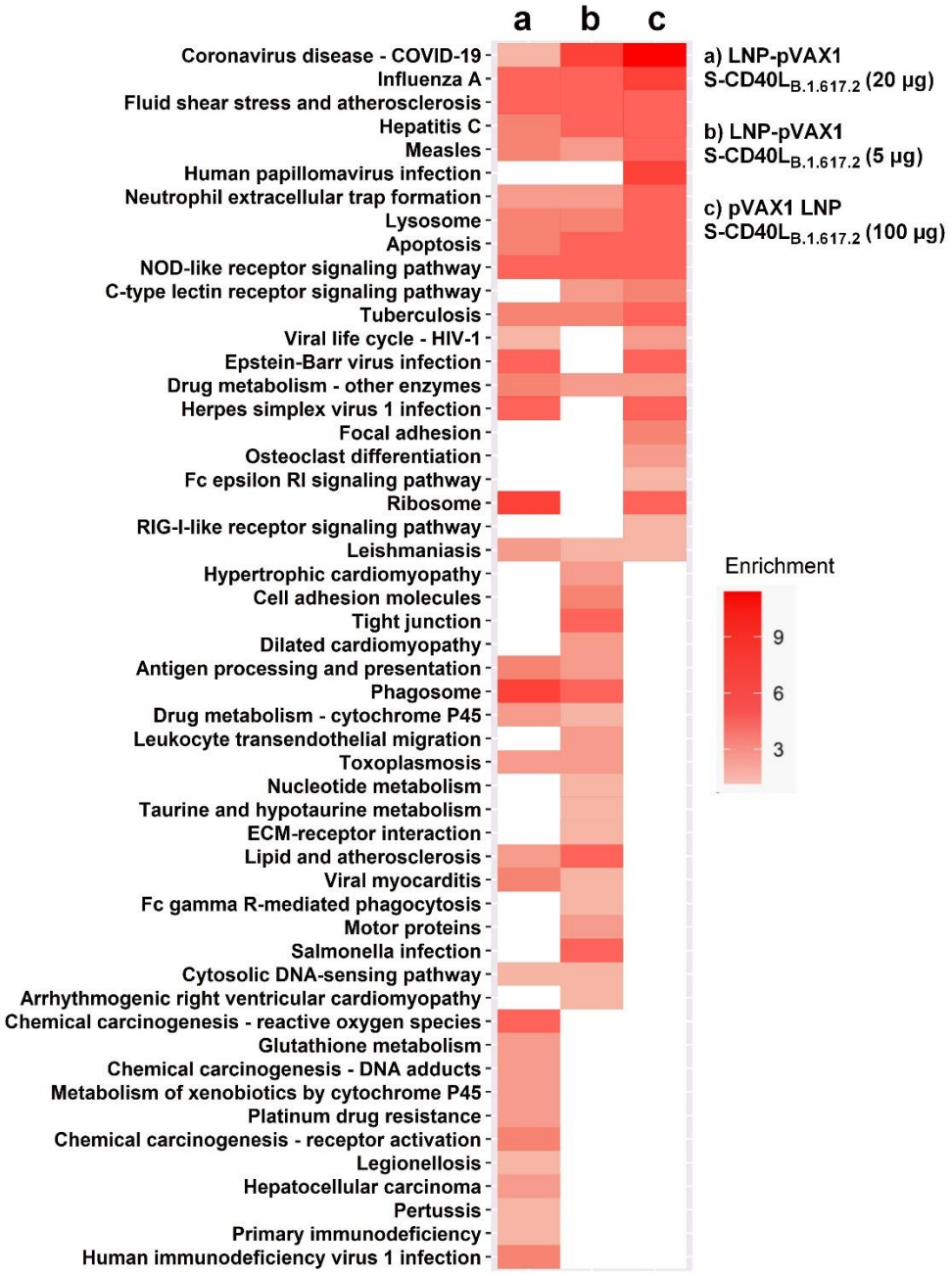

Figure S3. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Heatmap of KEGG enrichment analysis of the differentially expressed proteins obtained from pairwise comparisons between pVAX1 and pVAX1 S-CD40L$_{B.1.617.2}$ vaccinated groups. Color refers to the enrichment score.

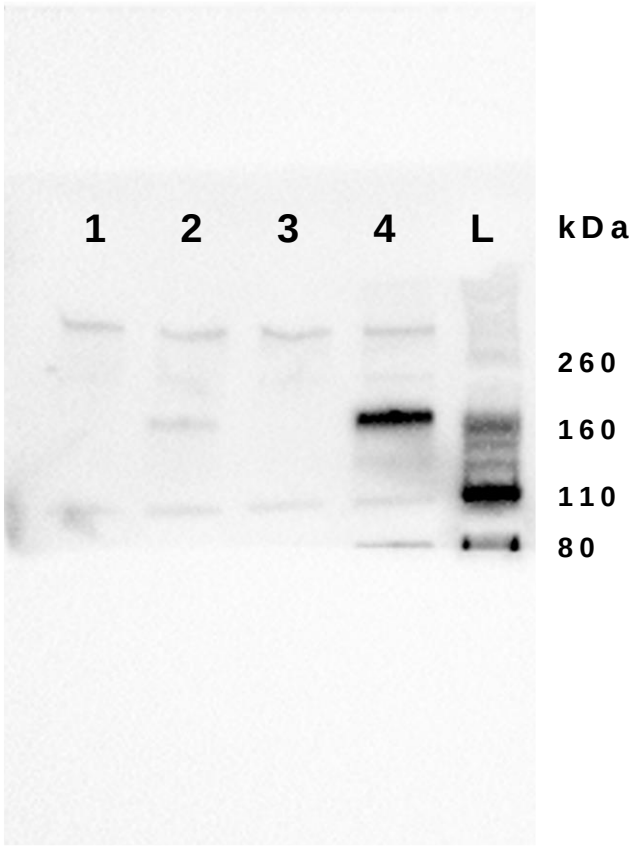

Figure S4: Spike Western Blot Images. SARS-CoV-2 (2019-nCoV) Spike Antibody, Rabbit PAb, Antigen Affinity Purified (Cat: 40591-T62, Sino Biologics). 1– KC2 pVAX1, 2- KC2 LNP pVAX1 S-CD40L$_{B.1.617.2}$, 3- SM-102 LNP pVAX1, 4- SM-102 LNP pVAX1 S-CD40L$_{B.1.617.2}$, L – Novex Sharp Pre-stained Protein Standard. Chemiluminescence image of the membrane. kDa, kiloDaltons.

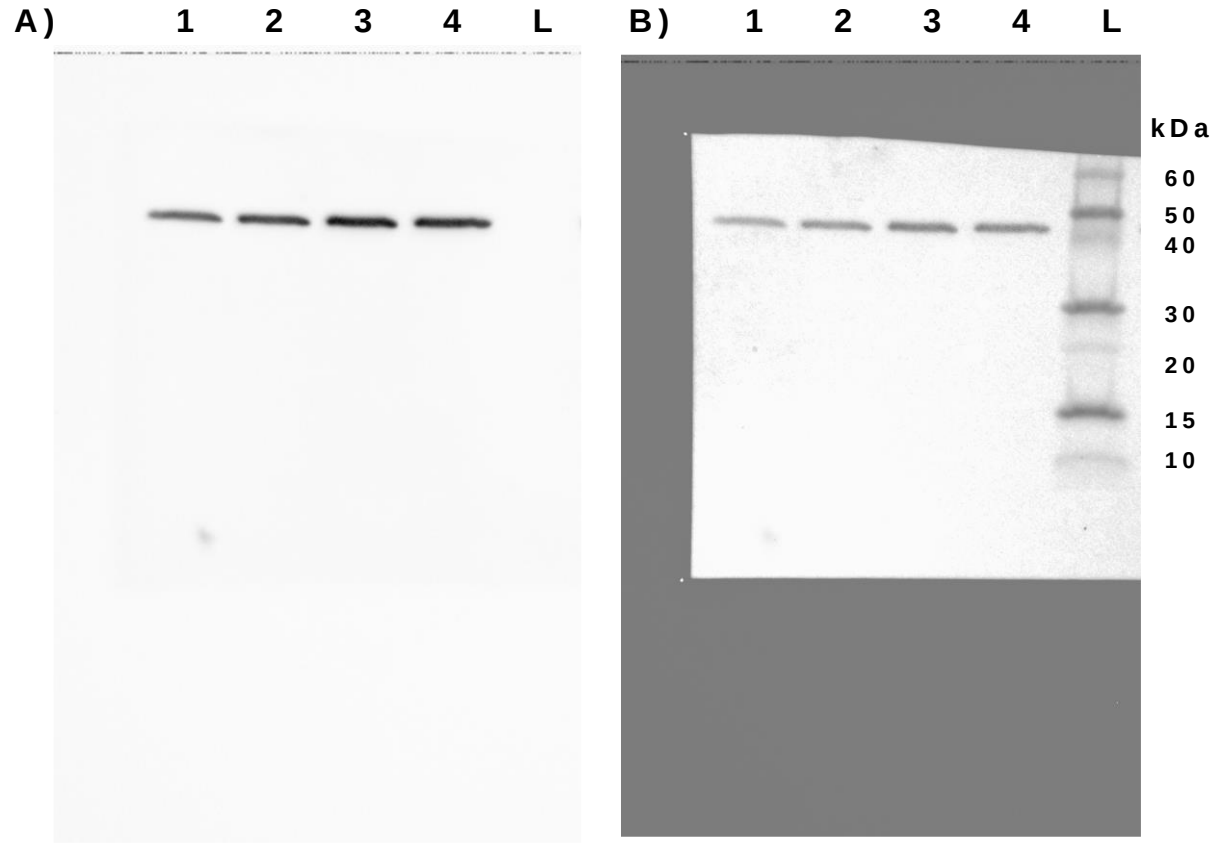

Figure S5: B-actin Western Blot Image. β-Actin Antibody (Cat: 4967, Cell Signaling). 1– KC2 pVAX1, 2- KC2 LNP pVAX1 S-CD40L$_{B.1.617.2}$, 3- SM-102 LNP pVAX1, 4- SM-102 LNP pVAX1 S-CD40L$_{B.1.617.2}$, L – Novex Sharp Pre-stained Protein Standard. kDa, kiloDaltons. **(A)** Chemiluminescence image of the membrane. **(B)** Composite chemiluminescence and colormetric image of the membrane. kDa, kiloDaltons.

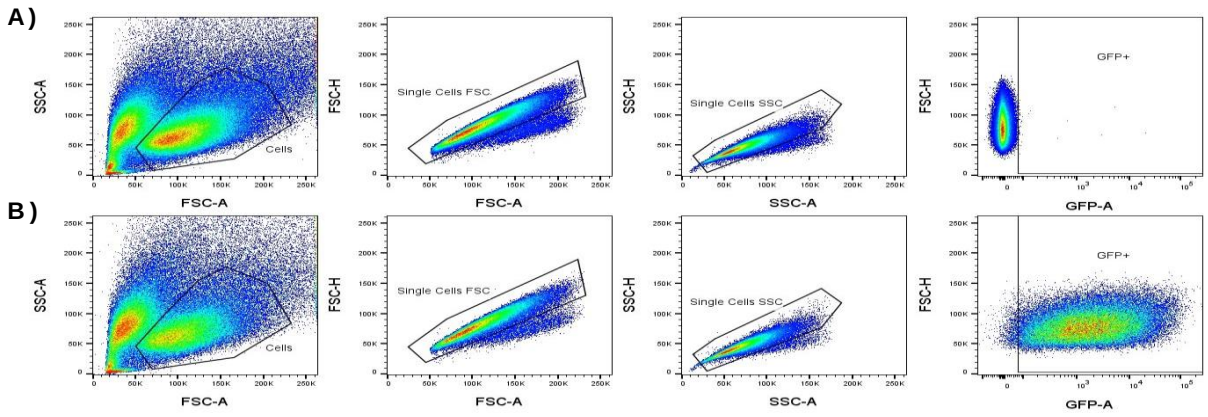

Figure S6. In Vitro GFP Transfection Gating Strategy. Representative flow cytometry gates for the analysis of GFP+ HEK293T cells transfected with **(A)** pVAX1 or **(B)** pVAX1 GFP.

Table S1. KC2 LNP Characterization. DNA-LNPs were generated using a NanoAssemblr BT™ instrument with a microfluidics cartridge containing a staggered herringbone mixing unit. Mean particle diameter measured by nanoparticle tracking analysis (NTA). Encapsulation efficiency determined by SYBR Gold assay. SD, standard deviation.

Dose	**Cohort**	**LNP Diameter (Mean ± SD , nm)**	**Encapsulation Efficiency (%)**
Prime	1	102.2 ± 35	>99
	2	98.3 ± 43	98.3
Boost	1	90.8 ± 34	96.1
	2	110 ± 63	97.8

Table S2. KC2 and SM-102 LNP Characterization. DNA-LNPs were generated using a NanoAssemblr BT™ instrument with a microfluidics cartridge containing a staggered herringbone mixing unit. Mean particle diameter measured by NTA. Encapsulation efficiency determined by SYBR Gold assay. SD, standard deviation.

Ionizable Lipid Component	Dose	Cohort	LNP Diameter (Mean ± SD , nm)	Encapsulation Efficiency (%)
KC2	Prime	1	83.7 ± 22	91.5
		2	84.0 ± 23	93.8
	Boost	1	90.9 ± 29	90.5
		2	84.8 ± 24.5	93.1
SM-102	Prime	1	79.0 ± 22	89.1
		2	82.1 ± 21	83.0
	Boost	1	85.0 ± 23.6	89.5
		2	80.9 ± 19	92.7

Table S3. Mass-spectrometry pairwise comparisons. Related to Figure 5. Protein expression in Syrian hamster lung tissue samples were compared pairwise using a pairwise t-test. Proteins with an absolute fold change >1.5 and an adjusted p-value <0.05 were defined as differentially expressed proteins (DEPs).

Table S4. GO and KEGG Analysis. Related to Figure 5. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed on the DAVID Bioinformatics database with all parameter settings at their default values.

Table S5. Ingenuity Pathway Analysis. Related to Figure 5. DEPs identified through pairwise t-tests were imported into Ingenuity Pathway Analysis (QIAGEN) and used to predict the activation or inhibition of canonical pathways.