

Supplemental information

Lipid nanoparticles enhance the efficacy of mRNA and protein subunit vaccines by inducing robust T follicular helper cell and humoral responses

Mohamad-Gabriel Alameh, István Tombácz, Emily Bettini, Katlyn Lederer, Sonia Ndeupen, Chutamath Sittplangkoon, Joel R. Wilmore, Brian T. Gaudette, Ousamah Y. Soliman, Matthew Pine, Philip Hicks, Tomaz B. Manzoni, James J. Knox, John L. Johnson, Dorottya Laczkó, Hiromi Muramatsu, Benjamin Davis, Wenzhao Meng, Aaron M. Rosenfeld, Shirin Strohmeier, Paulo J.C. Lin, Barbara L. Mui, Ying K. Tam, Katalin Karikó, Alain Jacquet, Florian Krammer, Paul Bates, Michael P. Cancro, Drew Weissman, Eline T. Luning Prak, David Allman, Botond Z. Igyártó, Michela Locci, and Norbert Pardi

SUPPLEMENTAL FIGURES AND TABLES

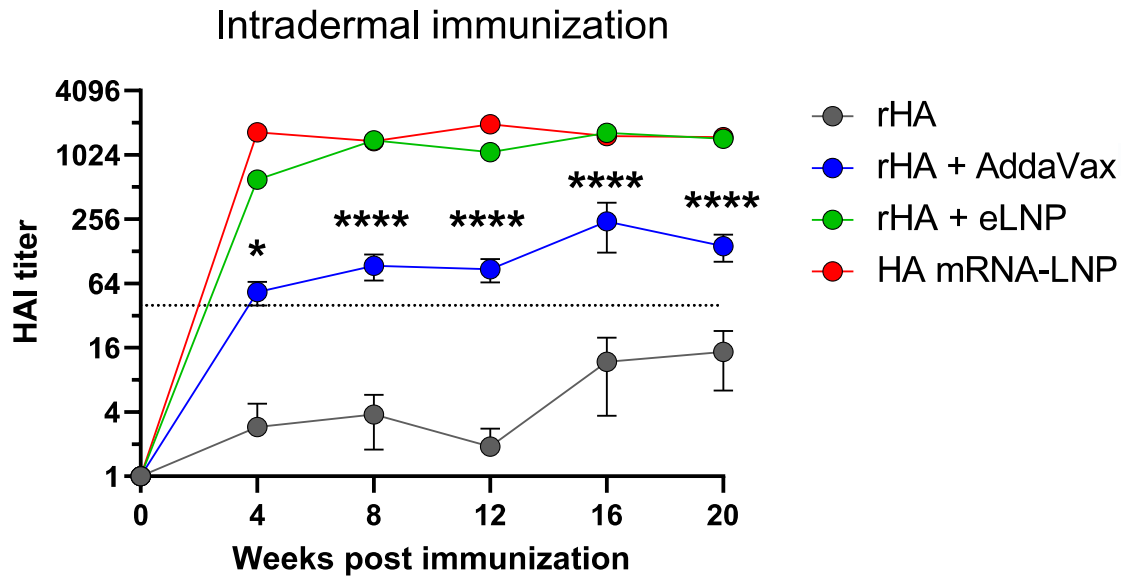


Figure S1. Related to Figure 1. LNP-adjuvanted vaccines induce durable humoral immune responses. (A) BALB/c mice received a single ID immunization with 10 μ g of rHA mixed with eLNP (equal amount of LNP as in 30 μ g mRNA-LNP) or AddaVax. Positive control animals received a single immunization with 10 μ g PR8 HA mRNA-LNP vaccine. PR8 HAI titers were followed for 20 weeks. n=10 mice/group. Symbols represent individual animals. The horizontal dotted line represents protective HAI value (1:40). Data are pooled from two independent experiments. Data shown are mean plus SEM. Statistical analysis: Two-way ANOVA with Bonferroni's multiple comparisons test; comparisons of AddaVax and eLNP groups are shown for each time point. * $P \leq 0.05$, **** $P \leq 0.0001$.

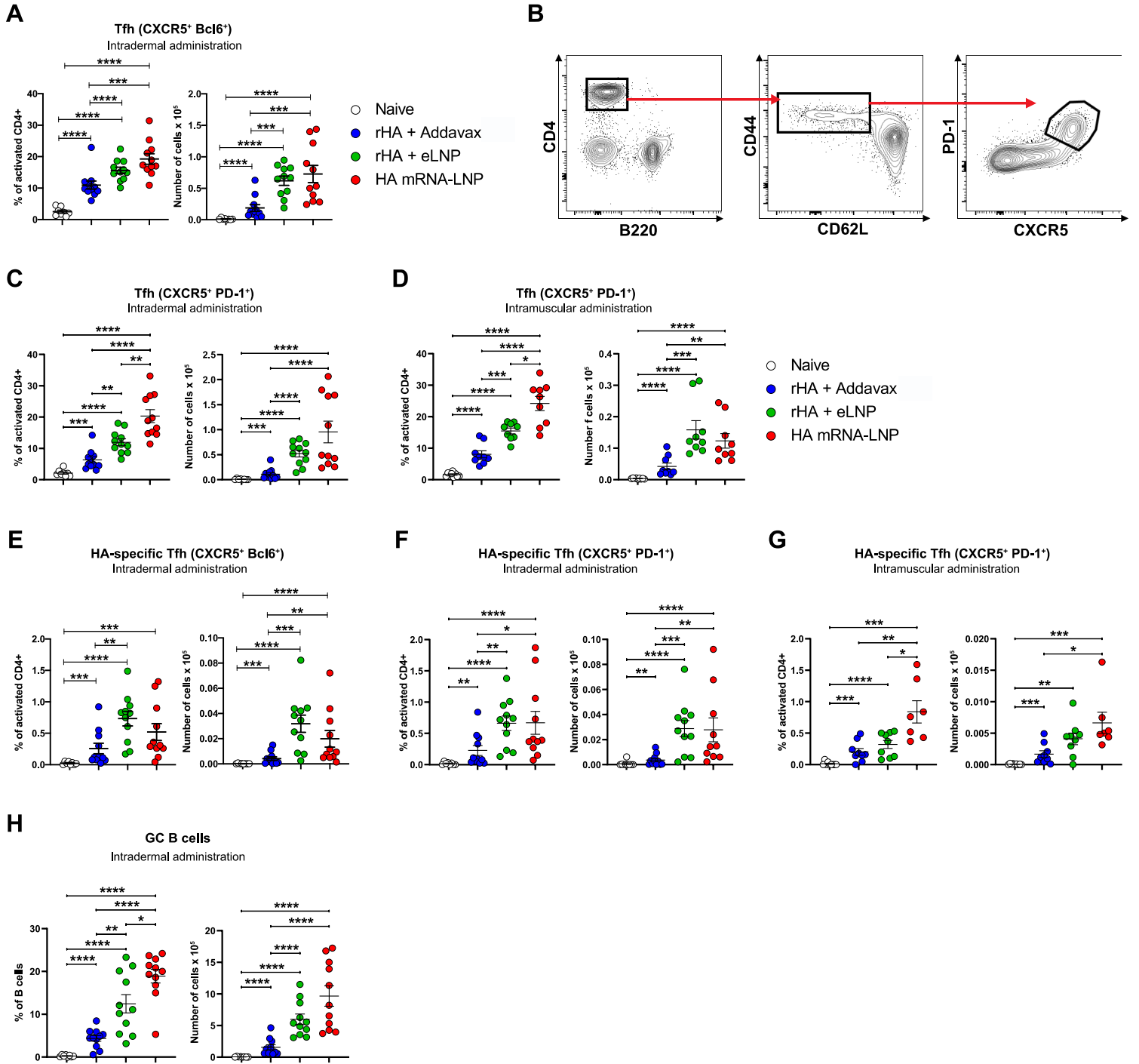


Figure S2. Related to Figure 2. LNP-adjuvanted vaccines induce potent antigen-specific Tfh cell responses. BALB/c mice received a single IM or ID immunization with 10 μ g of rHA mixed with eLNP or AddaVax. Positive control animals received a single immunization with 10 μ g PR8 HA mRNA-LNP vaccine. Twelve days later, Tfh and GC B cell responses were examined in draining lymph nodes. (A) Percentages and absolute numbers of Tfh cells (CD4⁺B220⁻CD44^{hi}CD62L⁻Bcl6⁺CXCR5⁺) after ID immunization. (B) Gating strategy for an alternative Tfh cell definition as CD4⁺B220⁻CD44^{hi}CD62L⁻PD-1⁺CXCR5⁺ Tfh cells. (C-D) Percentages and numbers of Tfh cells (defined as in B) after ID (C) or IM (D) immunizations. (E) Percentages and numbers of HA-specific Tfh cells (CD4⁺B220⁻CD44^{hi}CD62L⁻Bcl6⁺CXCR5⁺HA-MHCII tetramer⁺) after ID immunizations. (F-G) Percentages and numbers of HA-specific Tfh cells (CD4⁺B220⁻CD44^{hi}CD62L⁻PD-1⁺CXCR5⁺HA-MHCII tetramer⁺) after ID (F) or IM (G) immunizations. (H) Percentages and numbers of GC B cells (CD4⁺/CD8⁻CD19⁺FAS⁺GL7⁺) after ID immunizations. In (A) and (C-H), symbols represent individual animals. Data are shown as mean plus SEM. Data are combined from three independent experiments. Statistical analysis: Unpaired two-tailed Mann-Whitney U test was conducted. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

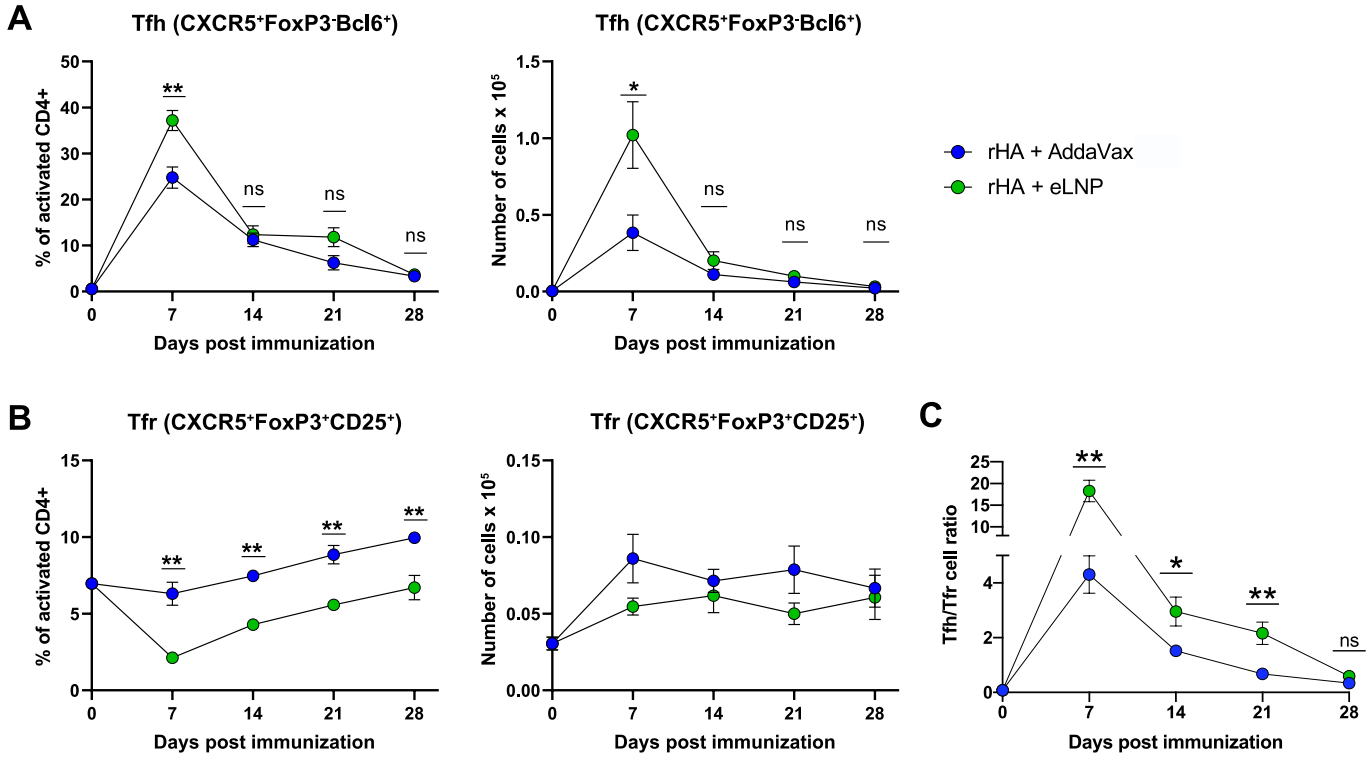


Figure S3. Related to Figure 2. Perturbation of Tfh/Tfr cell ratio resolves by 28 days post immunization. BALB/c mice received a single IM immunization with 10 μ g of rHA mixed with eLNP or AddaVax. At the timepoints indicated, Tfh and GC B cell responses were examined in draining lymph nodes by flow cytometry. (A) Kinetics of Tfh cell (B220-CD4⁺CD44^{hi}CD62L-CXCR5⁺FoxP3⁺CD25⁺Bcl6⁺) frequency and absolute number. (B) Kinetics of Tfr cell (B220-CD4⁺CD44^{hi}CD62L-CXCR5⁺FoxP3⁺CD25⁺) frequency and absolute number. (C) Kinetic of the Tfh/Tfr cell ratio. (A-C) n=6 mice/group. Data were combined from two independent experiments. Data shown are mean plus SEM. Statistical analysis: Unpaired two-tailed Mann-Whitney U test was conducted. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

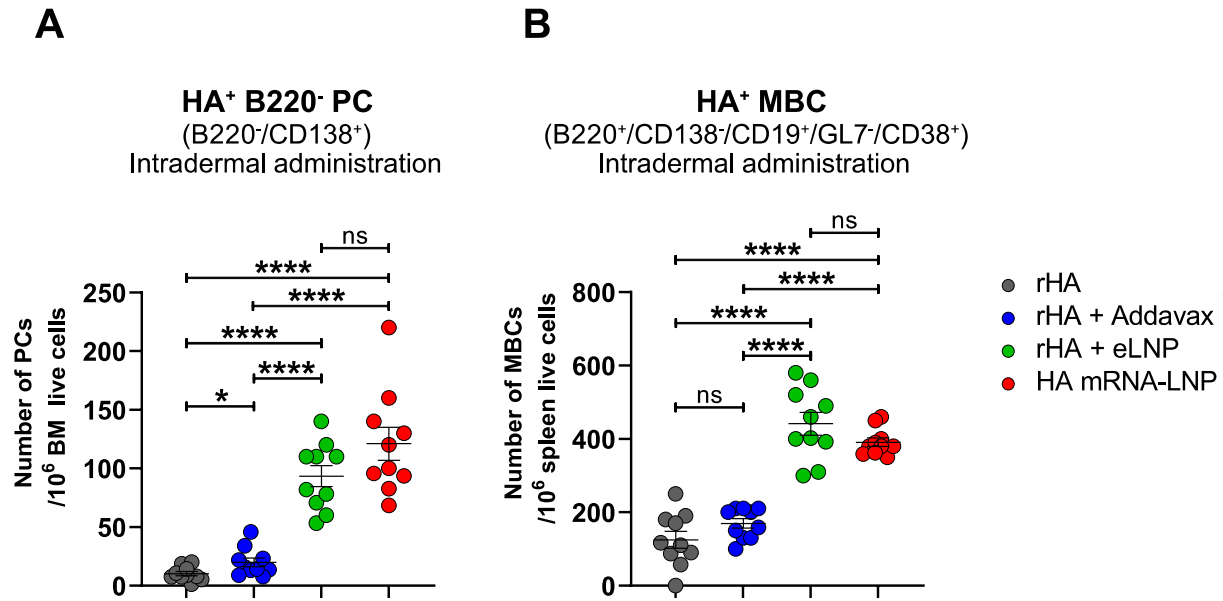
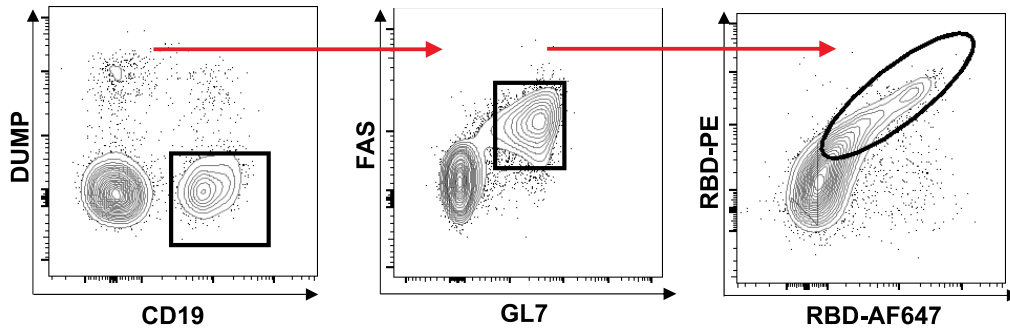
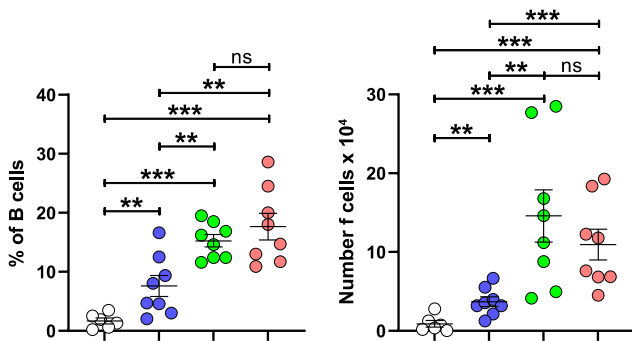


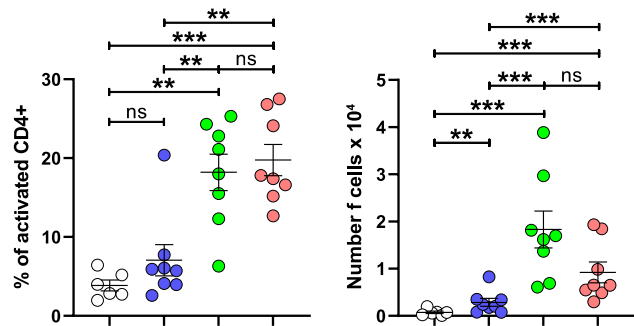
Figure S4. Related to Figure 3. LNP-adjuvanted vaccines induce antigen-specific LLPC and MBC responses. BALB/c mice received a single ID immunization with 10 µg of rHA mixed with eLNP or AddaVax. Positive control animals received a single immunization with PR8 HA mRNA-LNP vaccine. Twenty weeks later PR8 HA-specific (A) LLPC (IgD-Dump-B220⁻CD138⁺PR8 HA⁺) and (B) MBC (IgD-Dump-CD138⁻B220⁺CD19⁺CD38⁺GL7⁻PR8 HA⁺) responses were examined in bone marrow and spleen, respectively. n=8 mice/group. Symbols represent individual animals. Data shown are mean plus SEM. Data are pooled from two independent experiments. Statistical analysis: Unpaired two-tailed Mann-Whitney U test was conducted. *P ≤ 0.05, ****P ≤ 0.0001.

A**B**

GC B cells
Intramuscular Immunization

**C**

Tfh (CXCR5⁺ PD-1⁺)
Intramuscular Immunization



○ Naive
● rRBD + Addavax
● rRBD + eLNP
● RBD mRNA-LNP

Figure S5. Related to Figure 4. LNP-adjuvanted SARS-CoV-2 vaccines induce potent GC B and Tfh cell responses. BALB/c mice received a single IM immunization with 10 μ g of rRBD mixed with eLNP or AddaVax. Positive control animals received a single immunization with 10 μ g of RBD mRNA-LNP. Twelve days later GC B and Tfh cell responses were examined in dLNs. (A) Gating strategy for defining RBD-specific GC B cells (CD4/CD8⁻CD19⁺FAS⁺GL7⁺RBD⁺). (B) Percentages and numbers of GC B cells (CD4/CD8⁻CD19⁺FAS⁺GL7⁺) after immunization. (C) Percentages and numbers of Tfh cells (CD4⁺B220⁻CD44^{hi}CD62L⁻PD-1⁺CXCR5⁺) after immunization. n=8 mice/group. Symbols represent individual animals. (B-C) Data are shown as mean plus SEM. Data are pooled from three independent experiments. Statistical analysis: Unpaired two-tailed Mann-Whitney U test was conducted. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

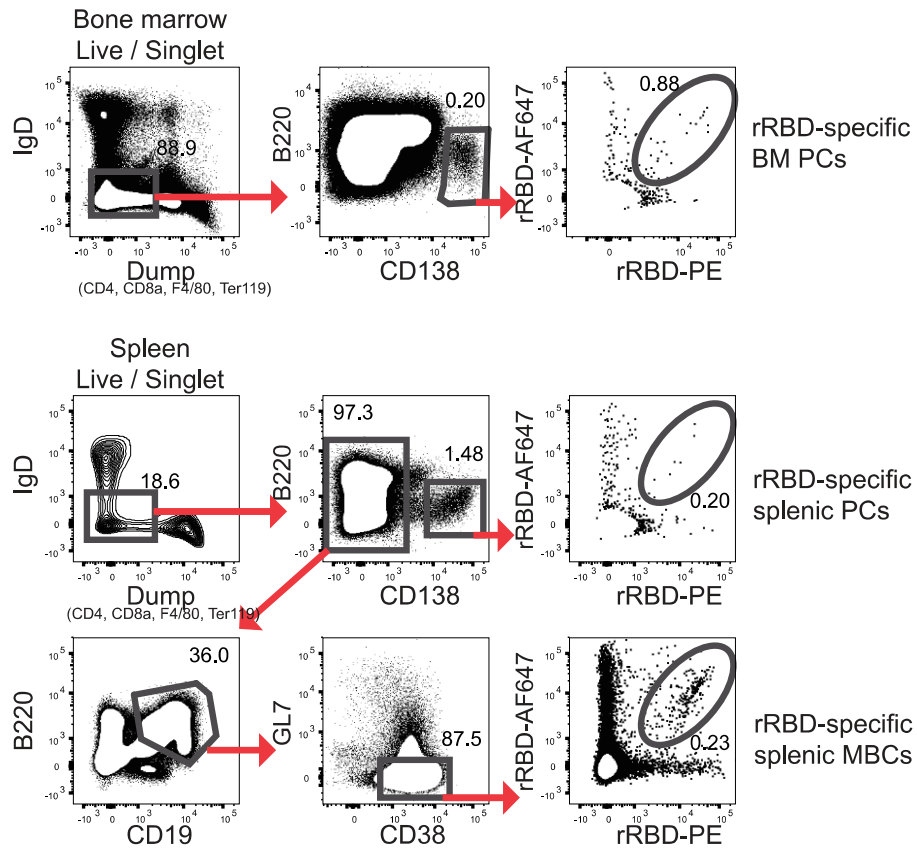


Figure S6. Related to Figure 4. Gating strategy for measuring RBD-specific LLPC and MBC responses. Representative gating strategy for antigen-specific LLPCs (IgD-Dump-B220-CD138⁺RBD⁺) in bone marrow and MBCs (IgD-Dump-CD138-B220⁺CD19⁺CD38⁺GL7-RBD⁺) in spleen.

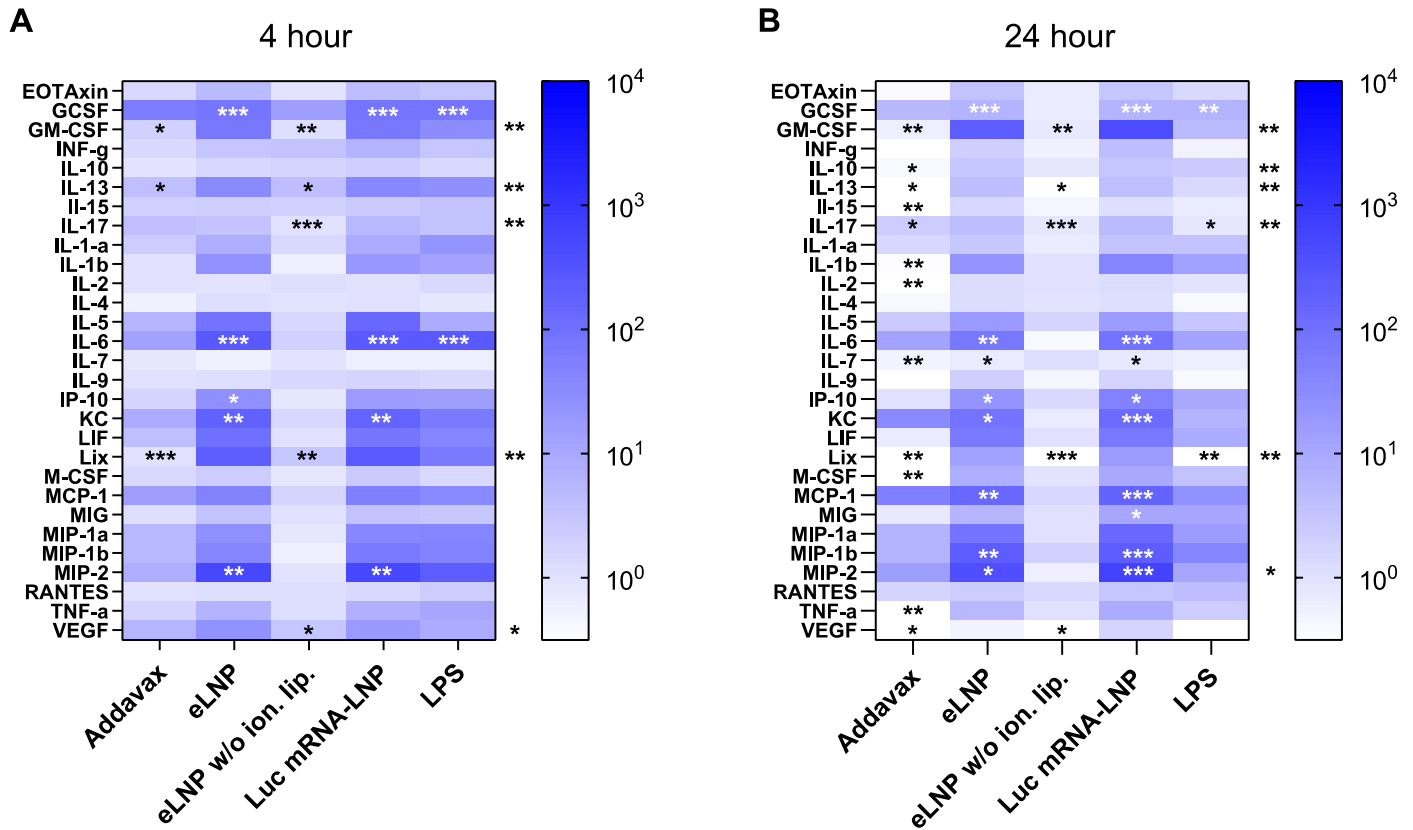


Figure S7. Related to Figure 7. Cytokine and chemokine expression in adjuvant-injected mice. BALB/c mice received a single ID immunization with 30 μ l of eLNPs with or without the ionizable lipid, 10 μ g of Luc mRNA-LNP or 30 μ l of AddaVax, and levels of selected cytokines were measured in dLN lysates with Luminex 4 or 24 hours later. Positive control animals received a single injection with LPS (1 mg/kg of body weight). (A, B) Heat map depicting cytokine expression as a ratio of the cytokine concentration in the adjuvant-treated groups to the cytokine concentration in the PBS control group at 4 hours (A) and 24 hours (B) post immunization. Three samples per group were measured, except 4h PBS, 24h LPS and 24h Addavax, where only two samples per group were analyzed. Asterisks mark the number of samples with readings above (white) or below (black) accurate signal detection range. Asterisks outside the heat map represent out of range values for the PBS control. Concentration values of the highest or lowest standard were used in these cases.

Stain	Fluorochrome	Clone	Vendor	Catalogue #
Live/Dead	-	-	Biologend	423101
B220	APC	RA3-6B2	Tonbo Biosci.	20-0452-U100
IgM	PerCP/F710	II/41	Invitrogen	46-5790-82
CD19	BV711	6D5	Biologend	115555
CD138	BV421	281-2	Biologend	142508
CD4	PE-Cy7	GK1.5	Biologend	100409
CD8 α	PE-Cy7	53-6.7	Biologend	100722
Ter-199	PE-Cy7	Ter-199	Biologend	116222
F4/80	PE-Cy7	BM8	Biologend	123114
IgD	APC-Cy7	11-26c.2a	Biologend	405716
GL7	AF488	GL7	Biologend	144612
CD38	AF700	90	Invitrogen	56-0381-82
rHA probe	PE	-	Labeled in-house	See methods

Stain	Fluorochrome	Clone	Vendor	Catalogue #
Live/Dead	-	-	Biologend	423101
B220	BUV496	RA3-6B2	BD Biosciences	612950
IgM	PerCP/F710	II/41	Invitrogen	46-5790-82
CD19	BV711	6D5	Biologend	115555
CD138	BV421	281-2	Biologend	142508
CD4	PE-Cy7	GK1.5	Biologend	100409
CD8 α	PE-Cy7	53-6.7	Biologend	100722
Ter-199	PE-Cy7	Ter-199	Biologend	116222
F4/80	PE-Cy7	BM8	Biologend	123114
IgD	APC-Cy7	11-26c.2a	Biologend	405716
GL7	AF488	GL7	Biologend	144612
CD38	AF700	90	Invitrogen	56-0381-82
rRBD probe	PE	-	Labeled in-house	See methods
rRBD probe	AF647	-	Labeled in-house	See methods

Table S1. Related to Figures 3, 4 and S6. LLPC and MBC panel for HA and RBD experiments.

Stain	Fluorochrome	Clone	Vendor	Catalogue #
CD19	BV785	6D5	Biolegend	115543
B220	PE	RA3-6B2	Biolegend	103207
CD4	PE-Cy5	H29.19	Biolegend	130312
CD8 α	PE-Cy5	53-6.7	BD Biosciences	553034
F4/80	PE-Cy5	BM8	eBioScience	15-4801-82
Gr-1	PE-Cy5	RB6-8C5	Biolegend	108410
IgD	BV605	11-26c.2a	Biolegend	405727
IgM	PE-CF594	R6-60.2	BD Biosciences	562565
rHA probe	BV421	-	Labeled in-house	See methods
rHA probe	AF647	-	Labeled in-house	See methods

Table S2. Related to Figure 3. B cell sorting panel.

Stain	Fluorochrome	Clone	Vendor	Catalogue #
CXCR5	Biotin	SPRCL5	eBioscience	13-7185-82
Streptavidin	BV421	-	BioLegend	405225
B220	BV650	RA3-6B2	BioLegend	103241
CD4	PerCP-Cy5.5	RM4-5	BioLegend	100540
CD44	FITC	IM7	eBioscience	11-0441-82
CD62L	BUV395	MEL-14	BD Biosciences	740218
PD-1	PE-Cy7	RMP1-30	BioLegend	109110
Bcl6	AF647	K112-91	BD Biosciences	624024
Live/Dead	eFluor 780	-	eBiosciences	65-0865-14
Tetramer	PE	-	-	See methods

Table S3. Related to Figures 2, S2 and S4. Tfh panel

Stain	Fluorochrome	Clone	Vendor	Catalogue #
CXCR5	Biotin	SPRCL5	eBioscience	13-7185-82
Streptavidin	BV421	-	BioLegend	405225
B220	BV650	RA3-6B2	BioLegend	103241
CD4	PerCP-Cy5.5	RM4-5	BioLegend	100540
CD44	BV605	IM7	BioLegend	103047
CD62L	BUV395	MEL-14	BD	740218
PD-1	PE	RMP1-30	BioLegend	109104
Bcl6	AF647	K112-91	BD	624024
Live/Dead	eFluor 780	-	eBioscience	65-0865-14

Table S4 related to Figures 6 and 7. Tfh panel

Stain	Fluorochrome	Clone	Vendor	Catalogue #
CXCR5	Biotin	SPRCL5	eBioscience	13-7185-82
Streptavidin	BV421	-	BioLegend	405225
B220	BV650	RA3-6B2	BioLegend	103241
CD4	PerCP-Cy5.5	RM4-5	BioLegend	100540
CD44	BV605	IM7	BioLegend	103047
CD62L	BUV395	MEL-14	BD Biosciences	740218
PD-1	PE	RMP1-30	BioLegend	109104
CD25	AF488	PC61.5	eBioscience	53-0251-82
Bcl6	AF647	K112-91	BD Biosciences	624024
FoxP3	PE-Cy7	FJK-16s	eBioscience	25-2773-82
Live/Dead	eFluor 780	-	eBioscience	65-0865-14

Table S5 related to Figure S3. Tfr panel

Stain	Fluorochrome	Clone	Vendor	Catalogue #
CD138	Biotin	281-2	BD Biosciences	553713
Streptavidin	BV650	-	BioLegend	405232
CD3e	BUV395	145-2c11	BD Biosciences	563565
CD19	BV605	6D5	BioLegend	115540
GL7	PerCP-Cy5.5	GL7	BioLegend	144610
FAS	PE	Jo2	BD Biosciences	554258
IgD	PE-Cy7	11-26c.2a	BioLegend	405720
HA Tetramer 1	AF488	-	Labeled in-house	See methods
HA Tetramer 2	AF647	-	Labeled in-house	See methods
RBD Tetramer	BV421	-	Labeled in-house	See methods
Live/Dead	eFluor 780	-	eBioscience	65-0865-14

Table S6 related to Figures 6 and 7. GC B cell panel

Stain	Fluorochrome	Clone	Vendor	Catalogue #
CD138	Biotin	281-2	BD Biosciences	553713
Streptavidin	BV650	-	BioLegend	405232
CD4	PerCP-Cy5.5	RM4-5	BioLegend	100540
CD8a	PerCP-Cy5.5	53-6.7	BioLegend	100734
CD19	PE-Cy7	eBio1D3	eBioscience	25-0193-82
GL7	eFluor 660	GL7	eBioscience	50-5902-82
FAS	PE	Jo2	BD Biosciences	554258
IgD	eFluor 450	11-26c	eBioscience	48-5993-82
HA Probe 1	AF488	-	Labeled in-house	See methods
HA Probe 2	AF647	-	Labeled in-house	See methods
RBD Probe	BV421	-	Labeled in-house	See methods
Live/Dead	eFluor 780	-	eBioscience	65-0865-14

Table S7 related to Figures 2, S2 and S4. GC B cell panel