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Supplemental Information

Efficient Targeting and Activation of Antigen-Presenting Cells In Vivo after Modified mRNA Vaccine Administration in Rhesus Macaques

Frank Liang, Gustaf Lindgren, Ang Lin, Elizabeth A. Thompson, Sebastian Ols, Josefine Röhss, Shinu John, Kimberly Hassett, Olga Yuzhakov, Kapil Bahl, Luis A. Brito, Hugh Salter, Giuseppe Ciaramella, and Karin Loré

SUPPLEMENTAL INFORMATION

Figure S1

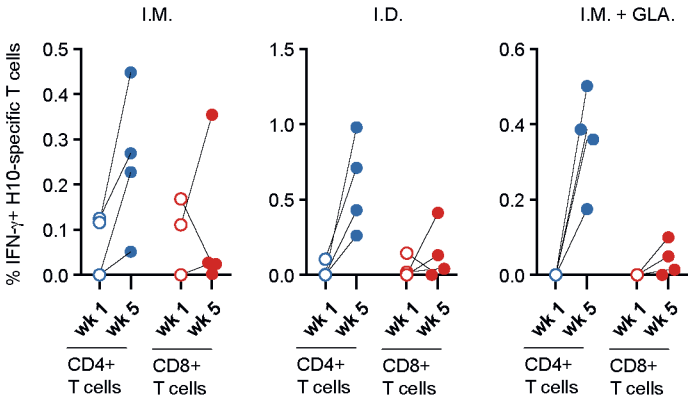

Figure S1. IFN-γ responses by H10-specific CD4+ and CD8+ T cells after prime and boost immunization. Rhesus macaques received either intramuscular (I.M.) or intradermal (I.D.) prime and boost immunizations with LNP/H10 mRNA with our without GLA adjuvant at week 0 and 4 respectively. PBMCs collected one week after prime (open) or boost (filled) were stimulated with H10 peptides for analysis of H10-specific IFN-γ production by total memory CD4+ T cells (blue) vs. CD8+ T cells (red) (n=4/group). Each connected circles show the percentages of IFN-γ+ T cells from an individual animal at the indicated week.

Figure S2

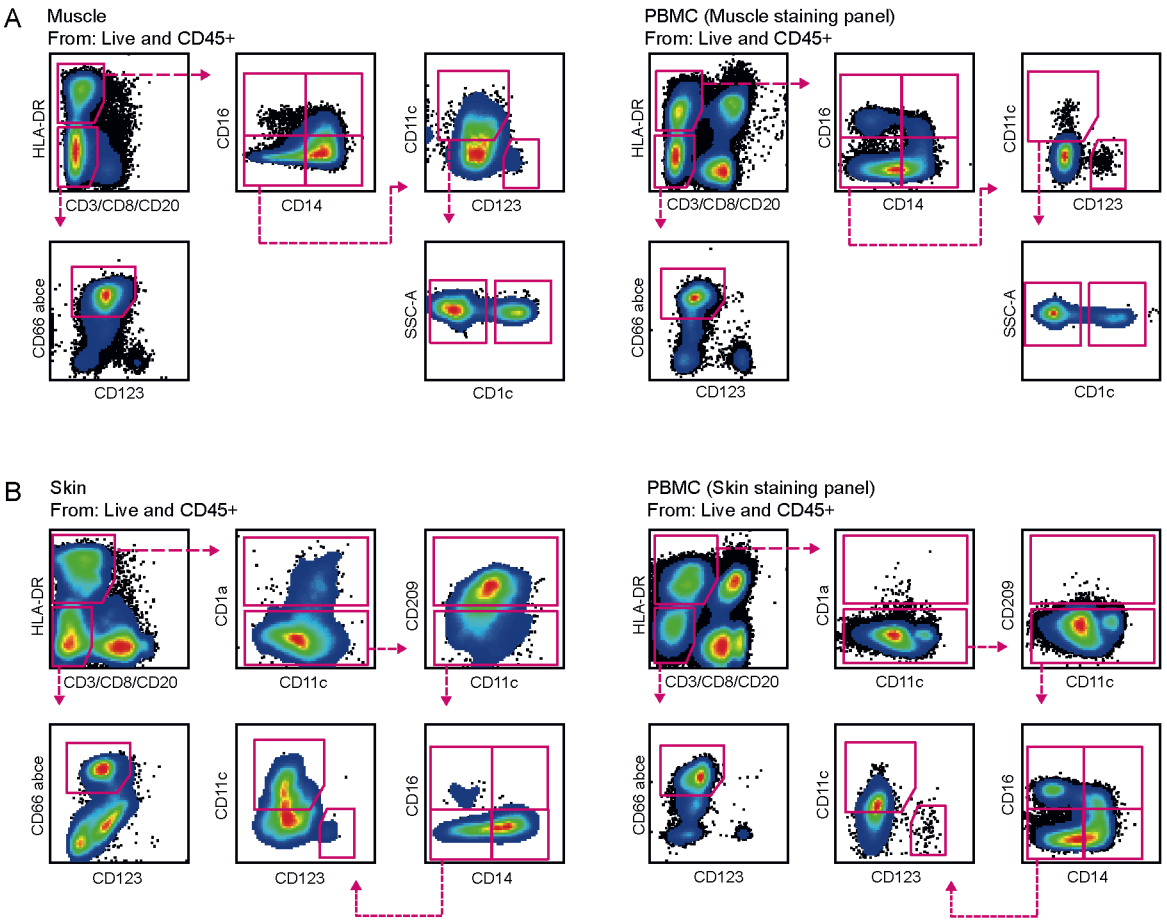

Figure S2. Gating strategy of rhesus macaque immune cell subsets at immunization sites. (A and B) Cell suspensions obtained from processed muscle (A) and skin (B) biopsies collected at the LNP/mRNA injection sites were stained with indicated panels. PBMCs were stained in parallel for reference.

Figure S3

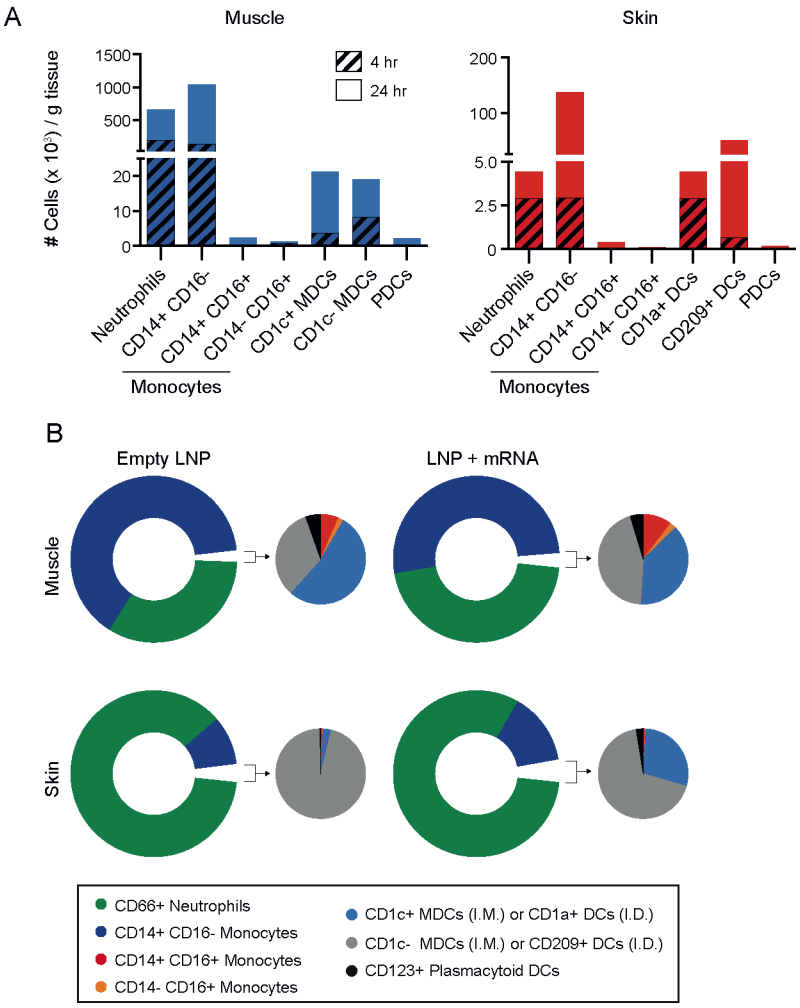

Figure S3. Immune cell infiltrating the sites of empty LNP vs. LNP/mRNA administration. Muscle and skin injected with LNP without mRNA content (Empty LNP) or LNP/mRNA were sampled for phenotyping of infiltrating immune cells. (A) The number of specific cell subsets in the empty LNP-injected muscle (blue) or skin (red) at 4 hrs (hatched bars, n=3/group) or 24 hrs (n=2/group). (B) Pie charts representing the proportions of different cell subsets mobilized to the muscle or skin at 24 hrs post-injection with empty LNP vs. LNP/mRNA (n=5/group). Proportions of infiltrating cell subsets found in lower frequencies are shown by the smaller pie charts and the doughnut charts depict the more abundant cell types.

Figure S4

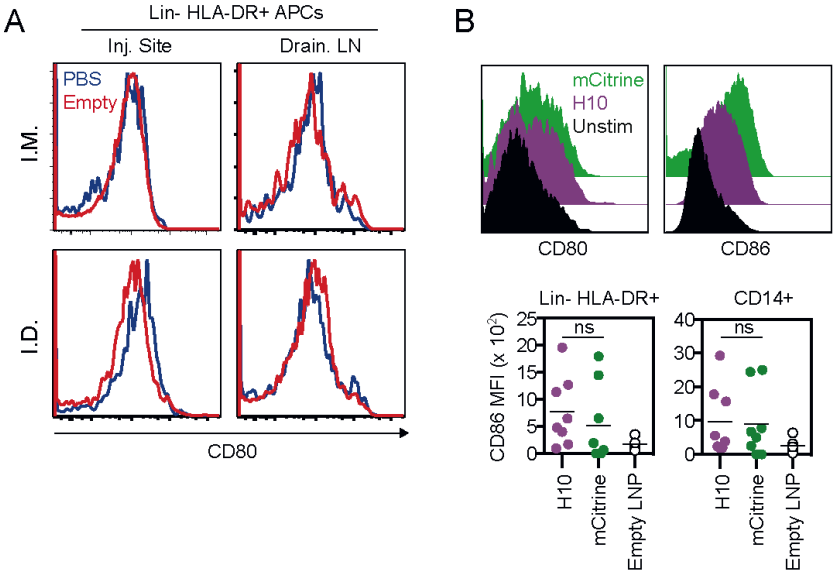

Figure S4. Cellular activation by vaccine mRNA is independent of the encoded protein. (A) Histogram on CD80 expression by HLA-DR+ Lin (CD3/CD8/CD20)- APCs at the PBS (blue) vs. Empty LNP (red) sites from the same animals. (B) Histograms of CD80 or CD86 expression by HLA-DR+ Lin- APCs after *in vitro* stimulation with LNP containing mRNA encoding H10 or mCitrine. Compiled data on background (i.e. media only) subtracted CD86 MFI values from stimulated HLA-DR+ Lin- APCs or CD14+ monocytes, (n = 8). Bars represent the mean, ns (not significant). Wilcoxon signed rank test.

Figure S5

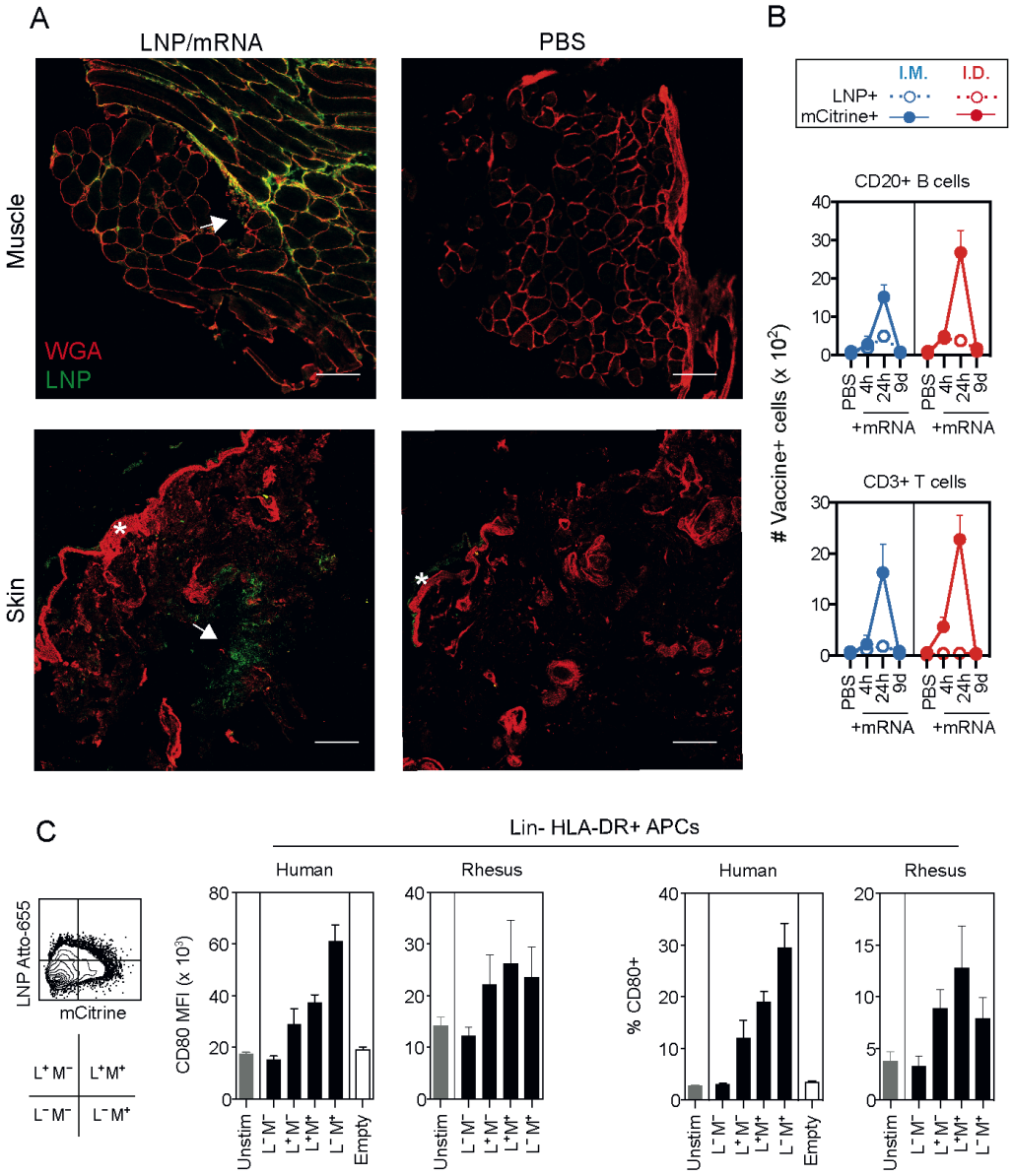

Figure. S5. LNP/mRNA distribution and targeting of lymphocytes in vivo and APCs in vitro. (A) Images of muscle and skin at 4 hrs post LNP/mRNA injection. Arrows indicate the distribution of LNPs labeled with Atto-647or 655 (green) and cell membrane is visualized with wheat germ agglutinin (red). Asterisk denotes epidermis of the skin. Scale bars: 200 mm. (B) LNP+ and mCitrine+ T cells and B cells in the draining LNs at the indicated time points. Open and closed circles denote mean±SEM of LNP+ and mCitrine+ cells respectively. (C) CD80 expression *in vitro* on four populations of human (n = 5) and rhesus (n = 4) HLA-DR+ Lin- APCs according to vaccine uptake and translation as shown in the gating key. Mean ±SEM shown.

Table S1. Fluorescently labeled antibodies for flow cytometry.

Marker	Clone	Company
CD1a	SK9	BD Biosciences
CD1c (BDCA-1)	AD5-8E7	Miltenyi Biotec
CD11c	3.9	Biolegend
CD123	7G3	BD Biosciences
CD14	M5E2	Biolegend
CD16	3G8	BD Biosciences
CD20	L27	BD Biosciences
CD209 (DC-SIGN)	DCN46	BD Biosciences
CD3	SP34-2	BD Biosciences
CD4	L200	BD Biosciences
CD45	D058-1283	BD Biosciences
CD45RA	L48	BD Biosciences
CD66 biotin	TET2	Miltenyi Biotec
Steptavidin-BV421	-	Biolegend
CD8	RPA-T8	BD Biosciences
CD80	L307.4	BD Biosciences
CD86	IT2.2	BD Biosciences
HLA-DR	TU36	Invitrogen
IFN alpha	LT27:295	Miltenyi Biotec
IFN gamma	4S.B3	BD Biosciences
IL-2	MQ1-17H12	BD Biosciences
TNF	MAb11	Biolegend