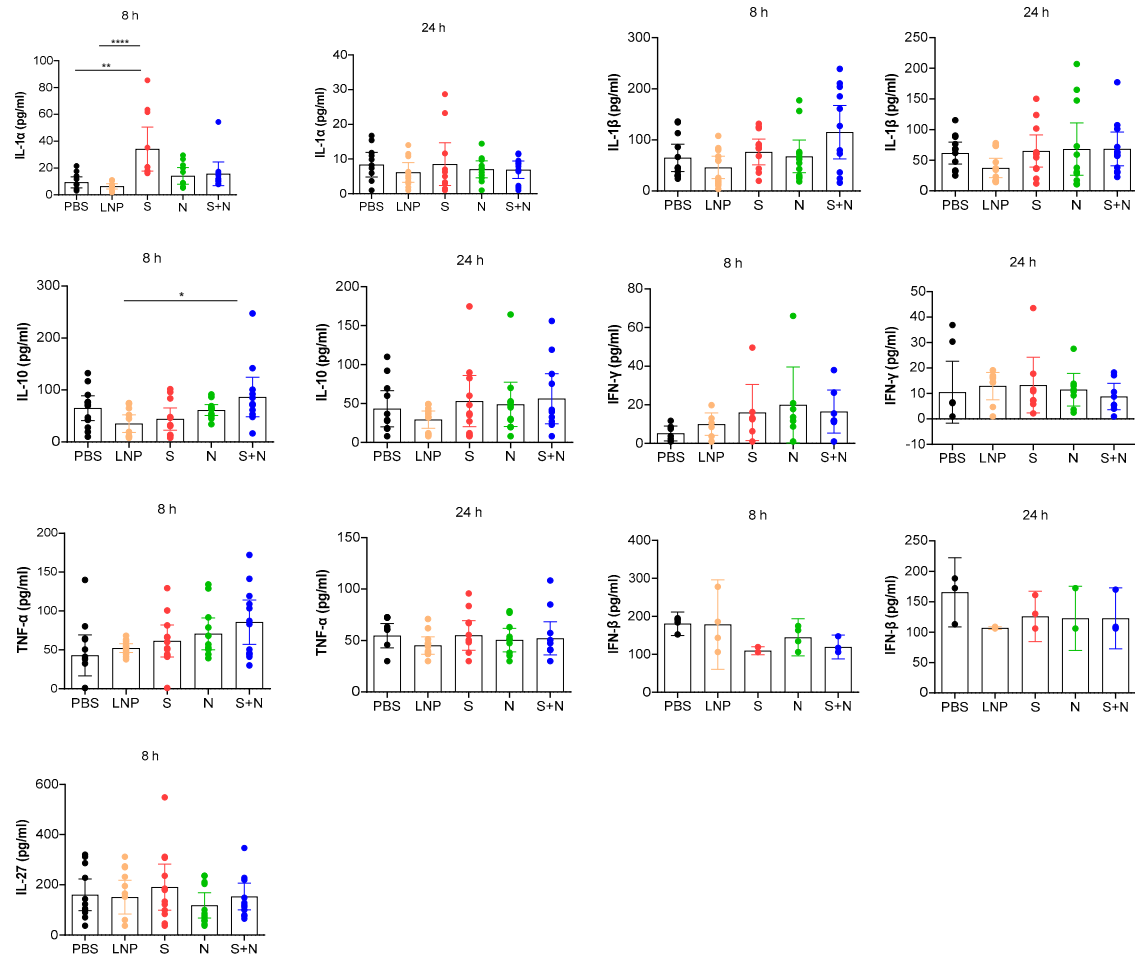


# Innate and adaptive immune parameters following mRNA vaccination in mice

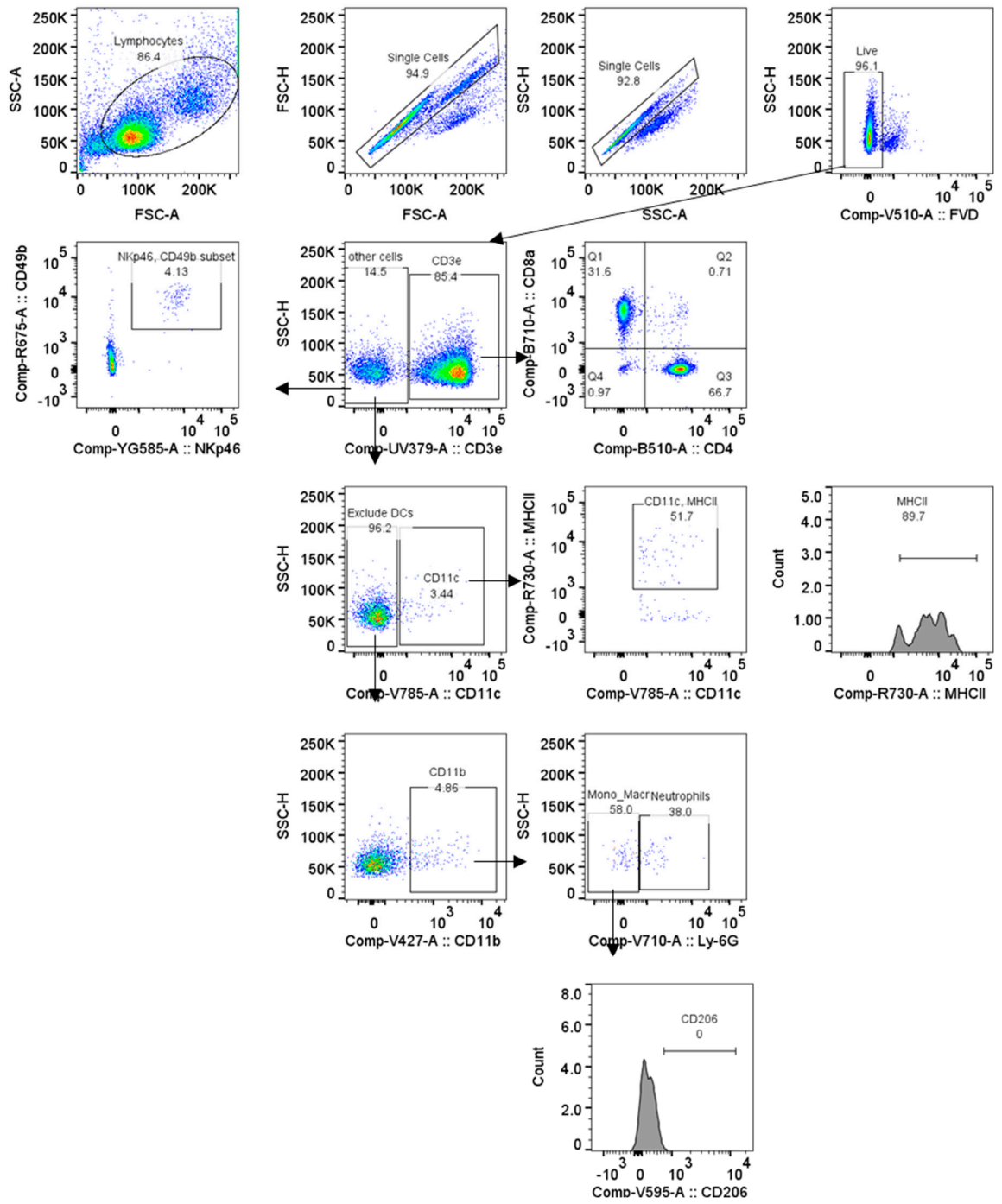
Srinivasa Reddy Bonam <sup>1\*</sup>, Nicholas C. Hazell <sup>1,2</sup>, Mano Joseph Mathew <sup>3,4</sup>, Yuejin Liang <sup>1</sup>, Xuxiang Zhang <sup>5</sup>, Zhi Wei <sup>5</sup>, Mohamad-Gabriel Alameh <sup>6</sup>, Drew Weissman <sup>6</sup>, Haitao Hu <sup>1,7,8,#,!</sup>

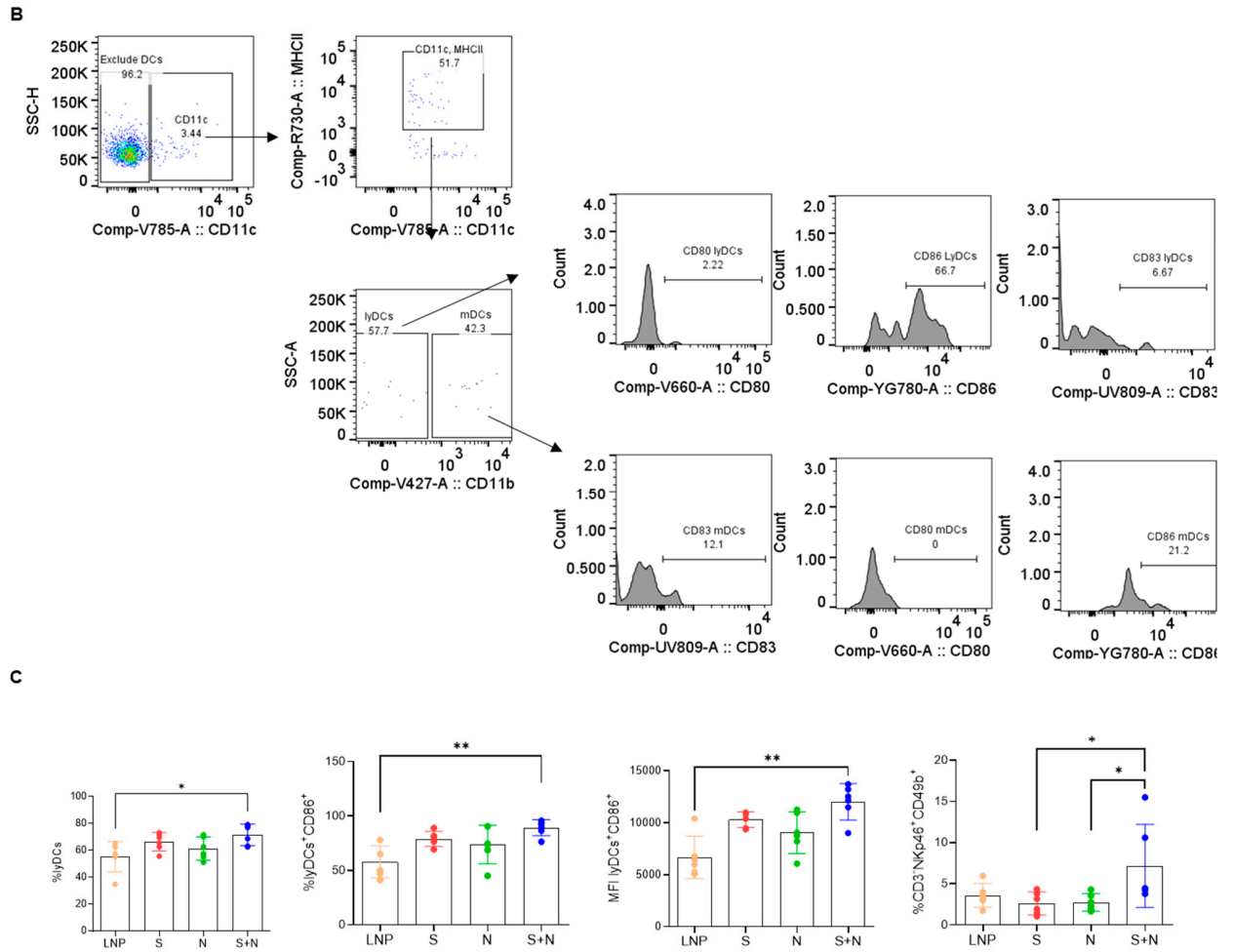
- <sup>1</sup> Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555, USA; bsrpharmacy90@gmail.com (S.R.B.); yu2liang@UTMB.EDU (Y.L.); haihu@UTMB.EDU (H.H.)
- <sup>2</sup> Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555, USA; nchazell@utmb.edu (N.C.H.)
- <sup>3</sup> EFREI Research Lab | Panthéon Assas University, 30-32 Avenue de la République, 94800 Villejuif, France
- <sup>4</sup> Laboratoire Génomique, Bioinformatique, et Chimie Moléculaire, EA7528, Conservatoire National des Arts et Métiers, HESAM Université, 2 rue Conté 75003 – Paris, France; mano.mathew@efrei.fr (M.J.M.)
- <sup>5</sup> Department of Computer Science, New Jersey Institute of Technology, Newark, NJ 07102, USA; xz445@njit.edu (X.Z.); zhiwei04@gmail.com (Z.W.)
- <sup>6</sup> Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA; Mg.Alameh@pennmedicine.upenn.edu (M.G.A.); drewww@pennmedicine.upenn.edu (D.W.)
- <sup>7</sup> Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX 77555, USA.
- <sup>8</sup> Sealy Institute for Vaccine Sciences, University of Texas Medical Branch, Galveston, TX 77555, USA.
- <sup>#</sup> Correspondence: Haitao Hu, PhD; haihu@UTMB.edu; Srinivasa Reddy Bonam, PhD; bsrpharmacy90@gmail.com
- <sup>!</sup> Lead Contact.



**Figure S1.** mRNA-LNP vaccination in mice induces robust innate immune response. Balb/c mice were injected with either LNP or mRNA-S or mRNA-N or mRNA-S+N intramuscularly. B. Serum cytokines were measured after 8 and 24 hours; n = 4-12. Nonparametric Tests-Kruskal-Wallis One-Way ANOVA with Dunn's multiple comparisons post hoc test was used for statistical analysis. \*P < 0.05.

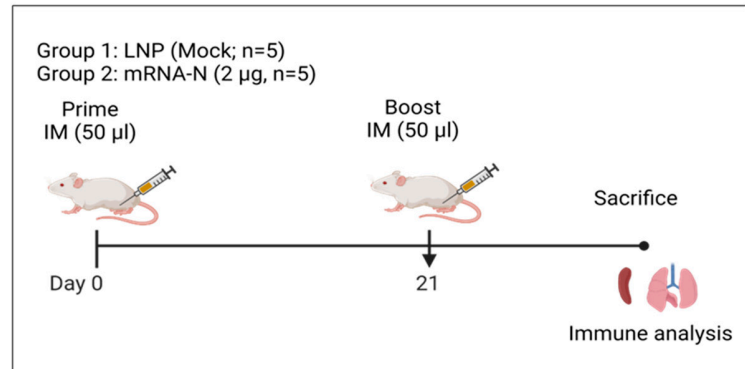
**A**



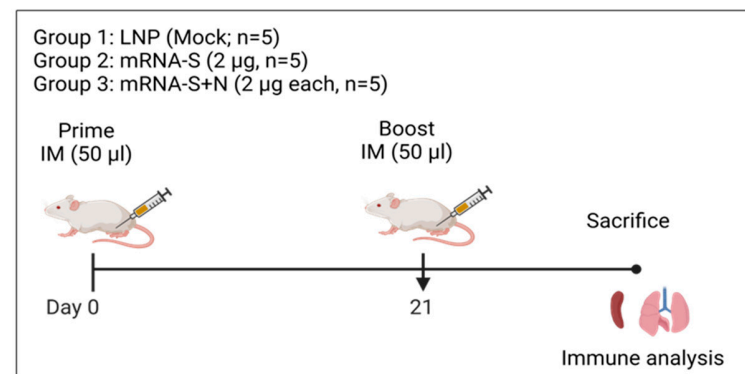


**Figure S2.** Immune phenotyping of lymph nodes from mRNA-LNP vaccinated mice. A-B. Gating strategies of innate and adaptive immune cells. C. Activation status of DCs and NK cells were measured by co-stimulatory marker CD86 and NKp46<sup>+</sup>CD49b<sup>+</sup>, respectively; n = 4-6. Data were presented as mean with 95% confidence interval. Nonparametric Tests-Kruskal-Wallis One-Way ANOVA with Dunn's multiple comparisons post hoc test was used for statistical analysis. \*P < 0.05 and \*\*P < 0.01.

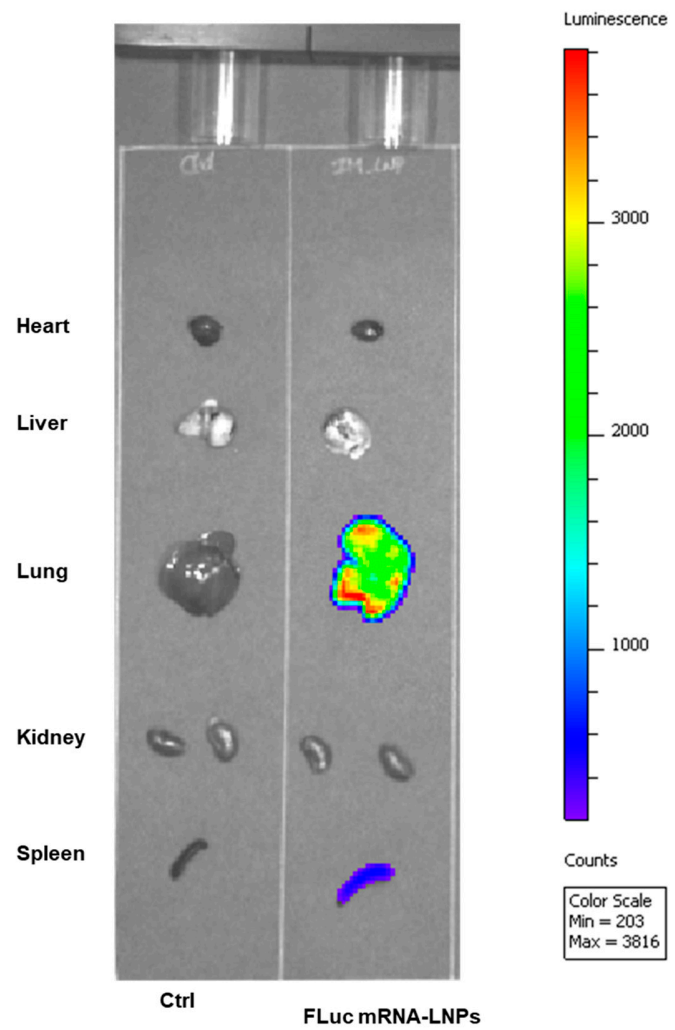
**A**



**B**



**Figure S3.** mRNA-LNP vaccination design. A-B. Balb/c mice were injected with either LNP or mRNA-S or mRNA-N or mRNA-S+N intramuscularly (as indicated in the illustration) on day 0, and booster dose at day 21. Two weeks later booster dose, mice were sacrificed and harvested the spleen and lungs.



**Figure S4.** *In vivo* FLuc mRNA-LNP delivery. Representative ex vivo images of heart, lung, liver, spleen, and kidneys collected from either nontreated or FLuc mRNA-LNP treated mice.