

Supplementary Materials for  
**Structural and biochemical characteristics of mRNA nanoparticles determine anti-SARS-CoV-2 humoral and cellular immune responses**

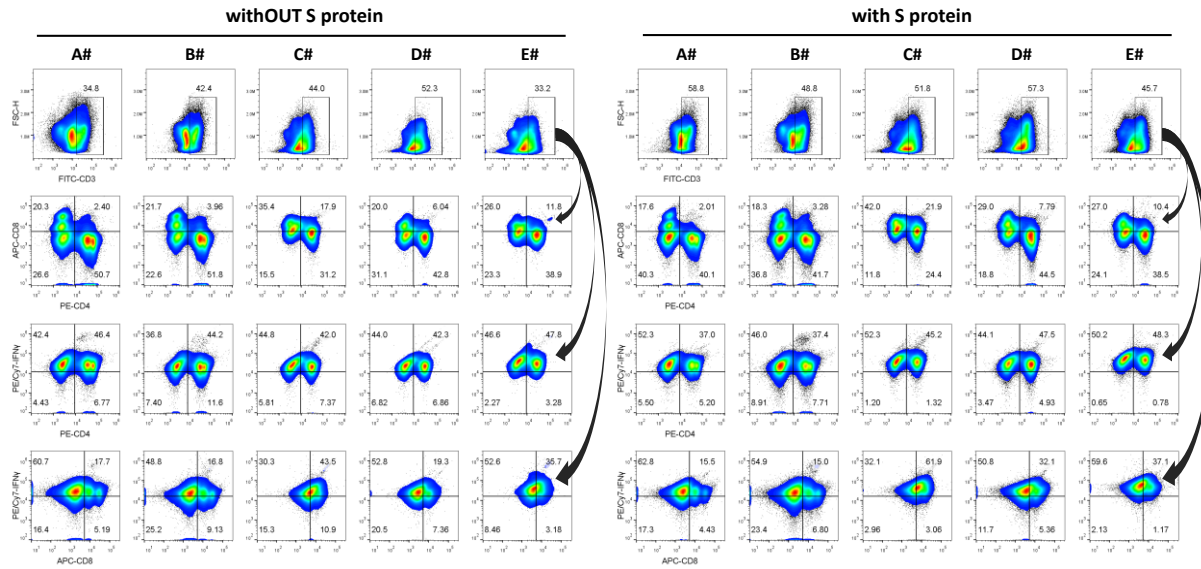
Yingying Shi *et al.*

Corresponding author: Yunqing Qiu, [qiuyq@zju.edu.cn](mailto:qiuyq@zju.edu.cn); Jian You, [youjiandoc@zju.edu.cn](mailto:youjiandoc@zju.edu.cn);  
Yan Lou, [yanlou@zju.edu.cn](mailto:yanlou@zju.edu.cn)

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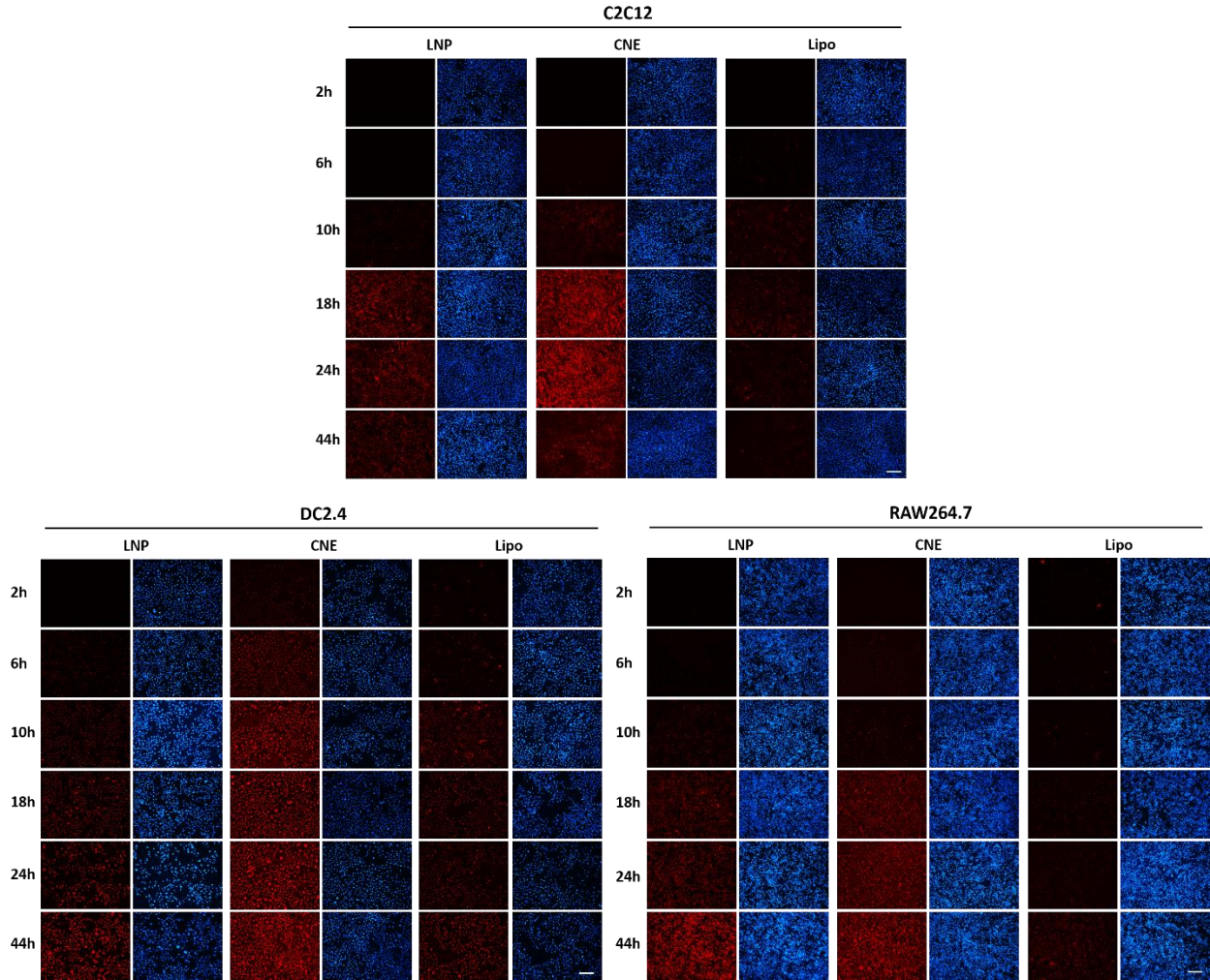
**This PDF file includes:**

Figs. S1 to S4



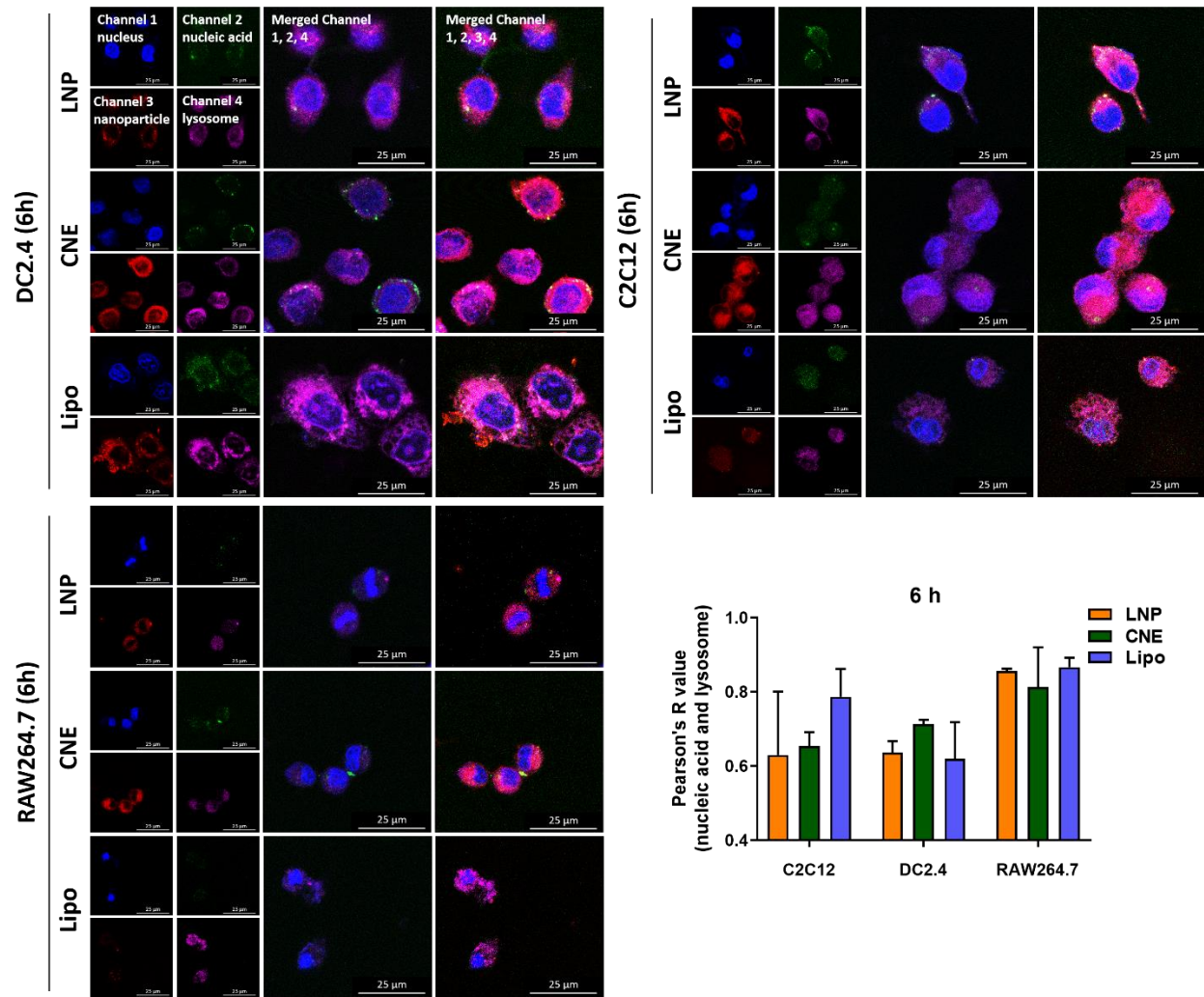
**Fig. S1.**

**Representative flow cytometric pictures of data showing the frequency of CD8<sup>+</sup> T cells, CD4<sup>+</sup> T cells, IFN- $\gamma$ <sup>+</sup> CD8<sup>+</sup> T cells and IFN- $\gamma$ <sup>+</sup> CD4<sup>+</sup> T cells in splenic lymphocytes ex vivo stimulated with (right) or without (left) S protein for 48 hours.**



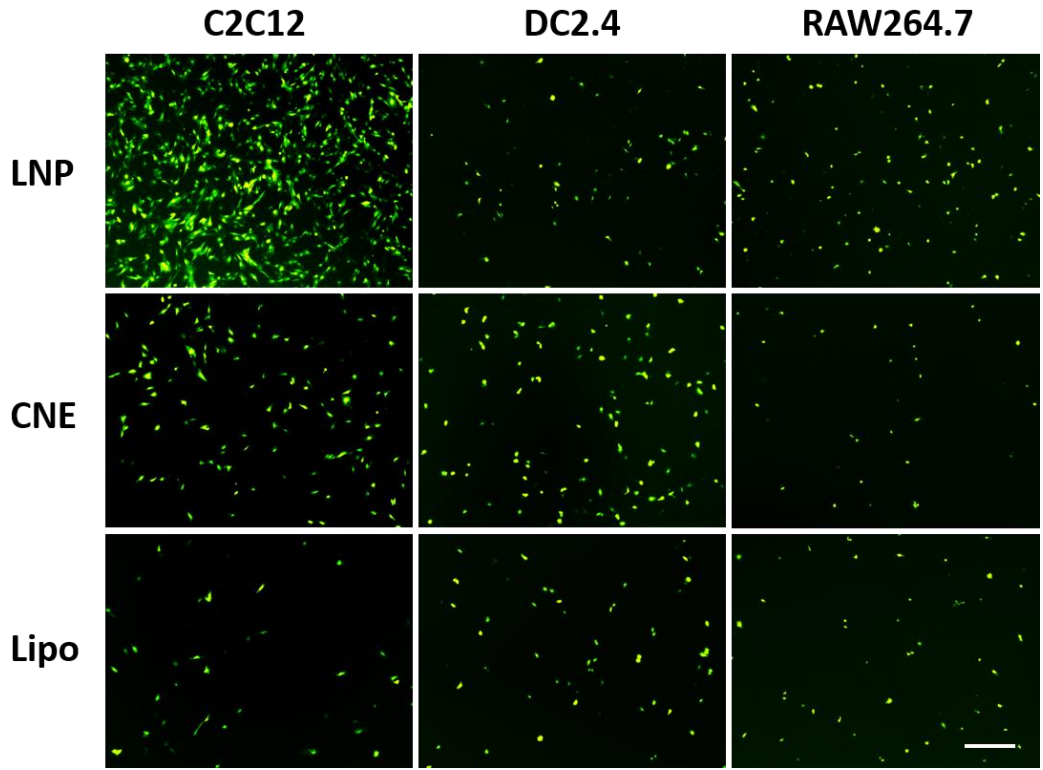
**Fig. S2.**

**Cellular uptake of different nanoparticles by C2C12, DC2.4, and RAW264.7.** Fluorescence images of C2C12, DC2.4, and RAW264.7 treated with LNP, CNE, or Lipo for 2, 6, 10, 18, 24 or 44 hours. Red: DiI-labelled nanoparticles, blue: nucleus. Scale bar: 200  $\mu$ m.



**Fig. S3.**

**Lysosome escape of LNP, CNE and Lipo by myocytes and APCs at 6 hours.** Confocal microscopic observation of the lysosome escape of nanoparticles in C2C12, DC2.4, and RAW264.7 at 6 hours post treatment. Scale bar, 25 μm. Images were analyzed to calculate the colocalization ratio of lysosomes and nanoparticles based on the Pearson's correlation coefficient ( $n = 3$ ). All error bars were expressed as  $\pm$  SD.



**Fig. S4.**

**Fluorescence pictures of in vitro transfected C2C12, DC2.4, and RAW264.7 with different eGFP mRNA nanoparticles at 24 hours. Scale bar: 200  $\mu$ m.**