Supporting Information

Cationic Lipid-Assisted Nanoparticle for Delivery of mRNA Cancer Vaccine

Ya-Nan Fan[a] †, Min Li[a] †, Ying-Li Luo[a], Qian Chen[e], Li Wang[a], Hou-Bing Zhang[b], Song Shen[c,d,f], Zhen Gu[e] * and Jun Wang[c,d,f] *

[a] School of Life Sciences, University of Science & Technology of China, Hefei, Anhui 230027, P. R. China

[b] Hefei National Laboratory for Physical Sciences at the Microscale, University of Science and Technology of China, Hefei, Anhui 230027, China

[c] Institutes for Life Sciences, School of Biomedical Science and Engineering, South China University of Technology, Guangzhou, Guangdong 510006, P. R. China

[d] National Engineering Research Center for Tissue Restoration and Reconstruction, South China University of Technology, Guangzhou, Guangdong 510006, P. R. China

[e] Department of Bioengineering, California NanoSystems Institute and Center for Minimally Invasive Therapeutics, University of California, Los Angeles, Los Angeles, CA 90095, USA

[f] Key Laboratory of Biomedical Engineering of Guangdong Province, South China University of Technology, Guangzhou, Guangdong 510006, P. R. China

*Corresponding author:

E-mail: mcjwang@scut.edu.cn (Jun Wang) and guzhen@ucla.edu (Zhen Gu)

† These authors equally contributed to this manuscript.

Preparation of DNA template

Plasmid DNA were exacted and purified from pUC57 by QIAprep Spin Miniprep Kit according to the manufacturer's instructions and quantified by NanoDrop 2000. Genomic region flanking the target sites (~1.3k bp) were amplified by the corresponding primers with T7 promoter.

Expression of EGFP in DC2.4 cells

Transfection experiments were conducted on DC2.4 cell lines. The Mean Fluorescence Intensity of EGFP-positive cells after incubated over different time periods and with different concentrations of $CLAN_{mEGFP}$ (5.0, 3.75, 2.5 and 1.25 μg/mL) were examined by FACS, and analyzed by FlowJo V.

Differentiation of effector T cells *in vivo*.

C57BL/6 mice were injected with CLAN encapsulating mOVA (40 μg per mouse). Three days later, splenic and lymphoid cells were isolated and stained according to previous described method, processed and stained with anti-mouse CD3, CD4, CD8 (BioLegend, Inc., San Diego, USA). For the detection of differentiation of effector T cells (such as Tem and Tcm), they were stained with anti-mouse CD44 and CD62L (BioLegend, Inc., San Diego, USA), and then analyzed by BD FACSVerse™ flow cytometer.

Table S1: The DNA sequence of OVA with T7 promoter and amplification primers of OVA.

OVA sequence (with T7 promoter)	5'- TAATACGACTCACTATAGGGatgggctccatcggcgcagcaagcatggaattttgtttt gatgtattcaaggagctcaaagtccaccatgccaatgagaacatcttctactgccccattgccatcatgtca gctctagccatggtatacctgggtgcaaaagacagcaccaggacacagataaataaggttgttcgctttg ataaacttccaggattcggagacagtattgaagctcagtgtggcacatctgtaaacgttcactcttcactta gagacatcctcaaccaaatcaccaaaccaaatgatgtttattcgttcagccttgccagtagactttatgctga agagagatacccaatcctgccagaatacttgcagtgtgtgaaggaactgtatagaggaggcttggaacct atcaactttcaaacagctgcagatcaagccagagagctcatcaattcctgggtagaaagtcagacaaatg gaattatcagaaatgtccttcagccaagctccgtggattctcaaactgcaatggttctggttaatgccattgt cttcaaaggactgtgggagaaaacatttaaggatgaagacacacaagcaatgcctttcagagtgactga gcaagaaagcaaacctgtgcagatgatgtaccagattggtttatttagagtggcatcaatggcttctgaga aaatgaagatcctggagcttccatttgccagtgggacaatgagcatgttggtgctgttgcctgatgaagtct caggccttgagcagcttgagagtataatcaactttgaaaaactgactgaatggaccagttctaatgttatgg aagagaggaagatcaaagtgtacttacctcgcatgaagatggaggaaaaatacaacctcacatctgtctt aatggctatgggcattactgacgtgtttagctcttcagccaatctgtctggcatctcctcagcagagagcct gaagatatctcaagctgtccatgcagcacatgcagaaatcaatgaagcaggcagagaggtggtagggt cagcagaggctggagtggatgctgcaagcgtctctgaagaatttagggctgaccatccattcctcttctgt atcaagcacatcgcaaccaacgccgttctcttctttggcagatgtgtttccccttaaCGGGATCCG CGCG-3'
OVA F primer	5'-TAATACGACTCACTATAGGGATG-3'
OVA R primer	5'-TTAAGGGGAAACACATCTGC-3'

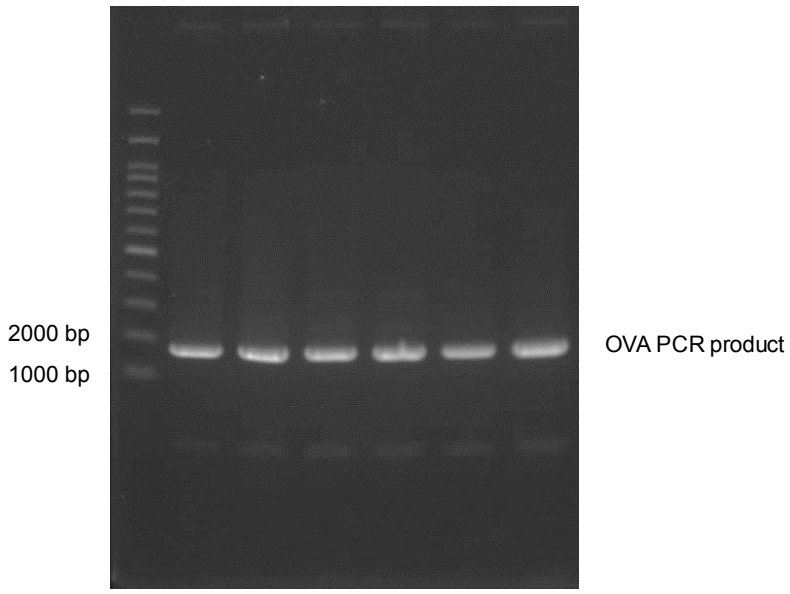

Fig. S1 Agarose gel electrophoresis of OVA PCR products amplified with T7 promoter primers.

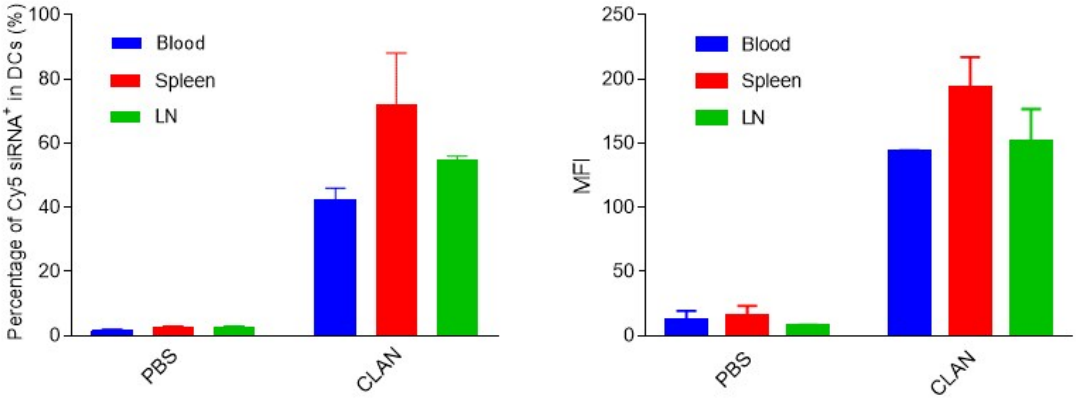

Fig. S2 Percentage (left) and MFI (right) of $Cy5^+$ DCs in blood, spleen and LN after *i.v.* injection of $CLAN_{Cy5\text{-}siRNA}$. Data are shown as means ± SD, n = 3.

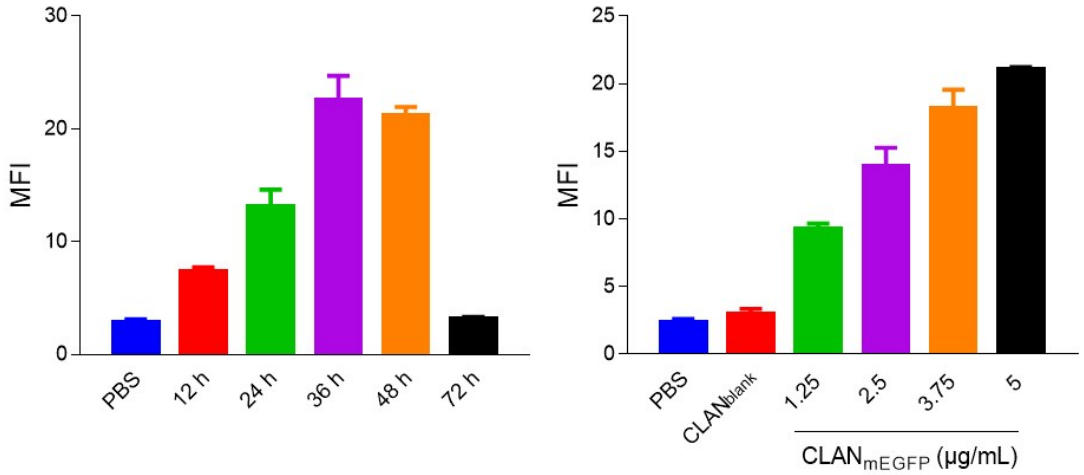

Fig. S3 Time-dependent monitoring (left) and dose-dependent monitoring (right) of the mean fluorescence intensity (MFI) of EGFP expression in DC2.4 after treated with $CLAN_{mEGFP}$. Data are shown as means ± SD, n = 3.

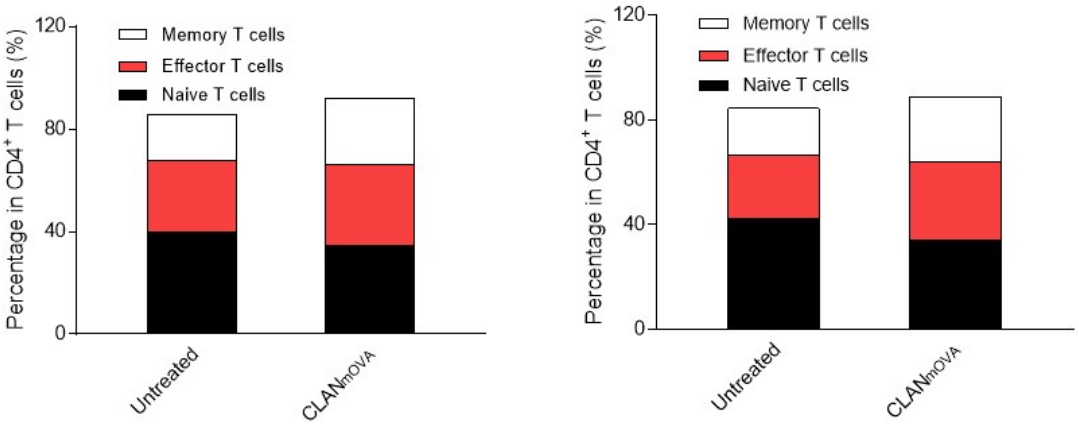

Fig. S4 Delivery of mOVA by CLAN vector ($CLAN_{mOVA}$) enables the activation of effector T cells and memory cells in spleen (left) and LN (right). Data are shown as means ± SD, n = 3.