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A tetrahedral framework nucleic acid based multifunctional nanocapsule for tumor prophylactic mRNA vaccination

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ABSTRACT

Synthetic antigen-encoding mRNA plays an increasingly significant role in tumor vaccine technology owing to its antigen-specific immune-activation. However, its immune efficacy is challenged by inferior delivery efficiency and demand for suitable adjuvants. Here, we develop a novel mRNA nanovaccine based on a multifunctional nanocapsule, which is a dual-adjuvant formulation composed of cytosine-phosphate-guanine motifs loaded tetrahedral framework nucleic acid (CpG-tFNA) and an immunopeptide murine β -defensin 2 (mDF2 β). This mRNA nanovaccine successfully achieves intracellular delivery, antigen expression and presentation of dendritic cells, and proliferation of antigen-specific T cells. In a tumor prophylactic vaccination model, it exerts an excellent inhibitory effect on lymphoma occurrence through cellular immunity. This mRNA nanovaccine has promising prophylactic applications in tumors and many other diseases.

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Nucleic acid vaccines, including DNA and RNA vaccines, have shown enormous promise in disease prevention and treatment [1,2]. Synthetic DNA or RNA is transported into cells, where they are transcribed and translated to eventually form endogenous antigen [3]. The pivotal advantage of nucleic acid vaccines is that they preferentially activate cellular immunity rather than humoral immunity, owing to the activation of CD8⁺ T cells derived from endogenous antigen presentation [4]. This is particularly significant to treating tumors and viral diseases [5,6].

Compared with DNA vaccines that may carry the risk of insertion into host genes and resultant gene variation [7], mRNA vaccines are generally degraded after protein translation, which implies superior biosafety [8]. Previous studies have highlighted the merits of mRNA vaccines and achieved preliminary success against various tumors and viral diseases, such as melanoma, prostate cancer, and COVID-19 [4,9].

However, extended application of mRNA vaccines remains challenged. mRNA is a long linear polymer of nucleotides and is negatively charged, which leads to poor cell permeability without ve-

hicle assistance [10,11]. Furthermore, the instability of synthetic mRNA necessitates modifications with specific nucleic acids, such as 5-methylcytidine [4]. Unfortunately, these modifications diminish the self-adjuvant effect of mRNA.

Although liposome based vehicles have been used for mRNA delivery [10,12,13], these vehicles have several drawbacks, including *in vivo* toxicity (especially liver toxicity) [3], lack of active selectivity towards antigen-presenting cells, and difficulty in co-delivery of multiple adjuvants [14]. Desirable vehicle/adjuvant formulations with the following features are still under exploration [15]: (1) enhancing mRNA delivery, (2) activating immunocytes (especially antigen-presenting cells), (3) preferentially inducing cellular immunity, (4) showing minimal *in vivo* toxicity.

Here, we report for the first time a dual-adjuvant multifunctional nanocapsule composed of cytosine-phosphate-guanine motifs loaded tetrahedral framework nucleic acid (CpG-tFNA) and murine β -defensin 2 (mDF2 β), for tumor prophylactic mRNA nanovaccine. tFNA is an emerging three-dimensional vehicle with excellent cell permeability and biosafety [16–24]. It drastically promotes the intracellular delivery of various oligonucleotide therapeutics [25], such as a representative adjuvant CpG [26]. Moreover, mDF2 β is a host defense peptide (HDP) that interacts with Toll-like receptor 4 (TLR4) expressed by dendritic cells (DCs) and

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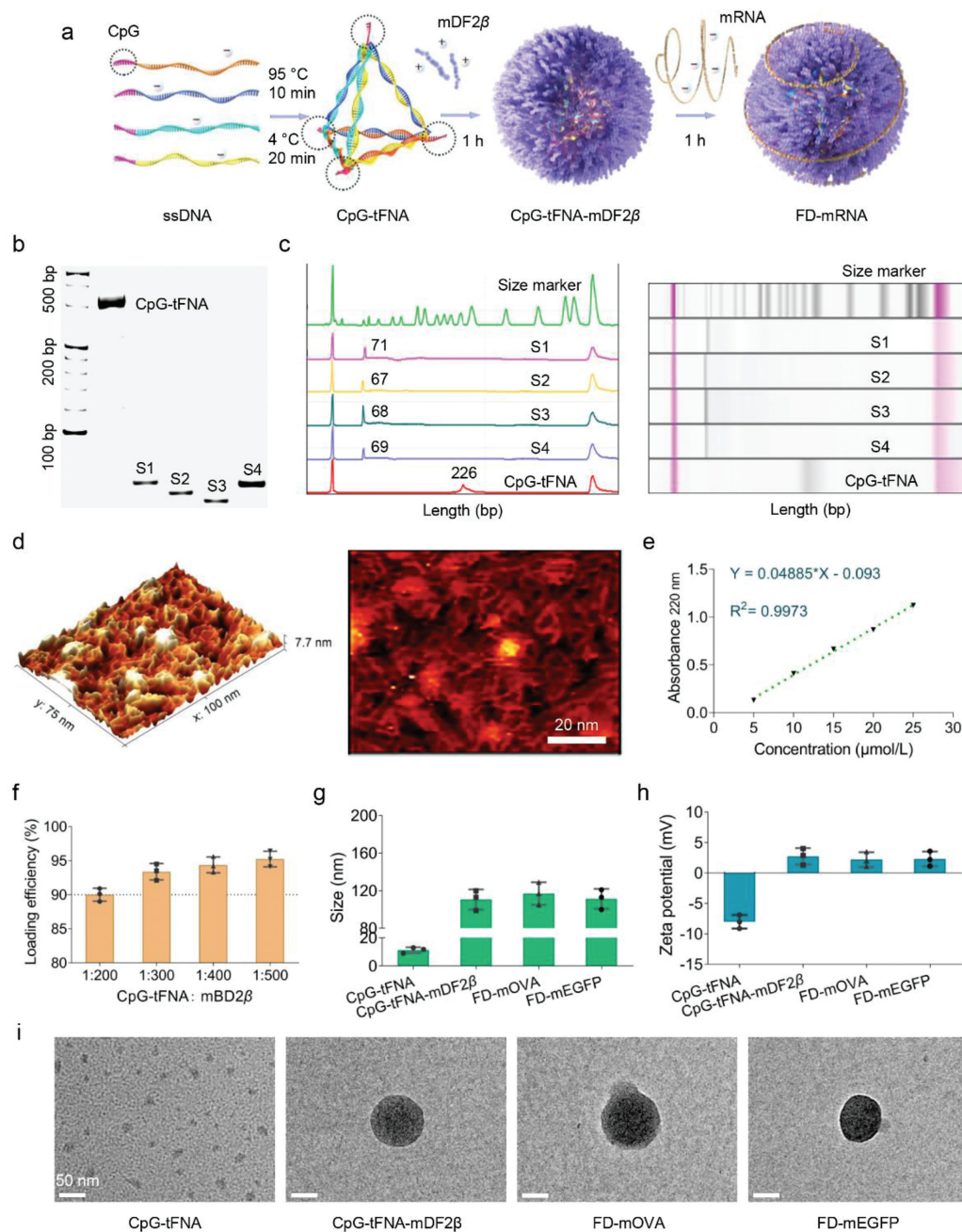


Fig. 1. Characterization of CpG-tFNA, dual-adjuvant nanocapsule, and mRNA nanovaccine. (a) Illustration of the synthesis process of CpG-tFNA, CpG-tFNA-mDF2 β and FD-mRNA (at the molar ratio of 1:300:1). (b) Analysis of the relative size of four ssDNAs and CpG-tFNA using 8% PAGE. (c) Analysis of the sequence length of four ssDNAs and CpG-tFNA using HPCE. (d) AFM images of CpG-tFNA. Scale bar = 20 nm. (e) Standard absorbance-concentration curve of mDF2 β . (f) Loading efficiency at different CpG-tFNA/mDF2 β ratios. (g) Particle size measurement of CpG-tFNA, CpG-tFNA-mDF2 β , FD-mOVA and FD-mEGFP using DLS. (h) ζ potential measurement of CpG-tFNA, CpG-tFNA-mDF2 β , FD-mOVA and FD-mEGFP using ELS. (i) TEM images of CpG-tFNA, CpG-tFNA-mDF2 β , FD-mOVA and FD-mEGFP. Scale bar = 50 nm.

potentiates specific immunity [27–30]. mDF2 β possesses positive targeting because TLR4 is expressed on the cell membrane of DCs [31], and it is positively charged that allows convenient combination with negatively charged mRNA and many other nanomaterials [32].

The efficacy of the developed nanovaccine is assessed *in vitro* using a DC2.4 cell model [33,34], and assessed *in vivo* using a prophylactic vaccination model of ovalbumin (OVA)-positive T cell lymphoma [10].

As previously reported [35,36], four single-stranded DNAs (ssDNAs) self-assembled to form CpG-tFNA within 30 min (Fig. 1a). Increases in molecular weight and sequence length were detected

using 8% polyacrylamide-gel electrophoresis (PAGE) and HPCE, respectively (Figs. 1b and c). In atomic force microscopy (AFM) images, CpG-tFNA clearly demonstrated a tetrahedral framework structure (Fig. 1d).

Based on electrostatic interactions, negatively charged CpG-tFNA and positively charged mDF2 β were combined to form a dual-adjuvant nanocapsule CpG-tFNA-mDF2 β . Negatively charged mRNA was self-assembled onto the positively charged nanocapsule, to eventually form a dual-adjuvant mRNA nanovaccine (Fig. 1a).

According to the standard absorbance-concentration curve of mDF2 β (Fig. 1e), CpG-tFNA was combined with mDF2 β (at the

molar ratio of 1:300) with the loading efficiency >90% (Fig. 1f), and finally combined with mRNA encoding OVA (mOVA) at the molar ratio of 1:300:1. This ratio was determined in consideration of the efficient concentration of mDF2 β and the controllable fabrication of the nanovaccine. The particle size increased from ~10 nm to ~120 nm (Fig. 1g) as measured using dynamic light scattering (DLS). The ζ potential increased from ~-10 mV to ~+3 mV (Fig. 1h) as measured using electrophoretic light scattering (ELS). In transmission electron microscopy (TEM) images (Fig. 1i), whereas CpG-tFNA typically demonstrated a triangle structure, the nanocapsule CpG-tFNA-mDF2 β and mOVA nanovaccine (FD-mOVA) both demonstrated a spherical structure with an enlarged diameter. CpG-tFNA-mDF2 β could also be combined with mRNA encoding enhanced green fluorescent protein (mEGFP), and the resultant FD-mEGFP yielded a TEM image similar to FD-mOVA.

Furthermore, mDF2 β could be directly combined with mOVA (300:1), and the resultant single-adjuvant nanovaccine D-mOVA demonstrated a chain-like structure in the TEM image (Fig. S1 in Supporting information). The photographs of CpG-tFNA, D-mOVA and FD-mOVA solutions (Fig. S2 in Supporting information) suggested that these products were relatively stable.

In summary, fabricated using an efficient strategy of temperature control and electrostatic interactions, our dual-adjuvant mRNA nanovaccine was mildly positively charged and approximately 120 nm in diameter. mRNA vaccine principally necessitates intracellular expression of the desired antigen. Here, mEGFP was delivered alone or with the nanocapsule CpG-tFNA-mDF2 β , and the expression level of EGFP was assessed. Using confocal imaging (Fig. 2a) and quantitative flow-cytometry analysis (Fig. S3 in Supporting information), it was found that both D-mEGFP and FD-mEGFP led to successful expression of EGFP manifested by green fluorescence, whereas mEGFP alone was largely ineffective.

Furthermore, compared with D-mOVA, FD-mOVA elicited superior cell migration (Fig. 2b, statistical results in Fig. 2e) as assessed using the scratch test, and superior cell viability (Fig. 2f) as measured using Cell counting kit-8 (CCK-8). In terms of cellular morphology as assessed by optical microscopy (Fig. 2c) and SEM (Fig. 2d), both D-mOVA and FD-mOVA led to a relatively irregular cellular configuration with synapse-like features, which clearly indicated DC maturation.

Next and more importantly, essential biomolecules for antigen presentation were investigated, including secreted cytokines, co-stimulatory molecules, and antigen-MHC-1 complex. Compared with D-mOVA, FD-mOVA elicited increased secretion of C-C motif ligand (CCL)-18, a chemokine for lymphocytes, and IL-12 (p70), which induces cellular immunity (Fig. 2g), as revealed by enzyme-linked immunosorbent assay (ELISA). Notably, both D-mOVA and FD-mOVA exerted a minimal effect on proinflammatory tumor necrosis factor (TNF)- α and interleukin (IL)-1 β secretion (Fig. 2g).

Proinflammatory cytokine secretion is previously regarded as an indication of antigen-presenting cell maturation and adaptive immunity initiation [10,11]. However, a recent study has suggested that the adverse effect of TNF- α -caused non-specific immunity may outweigh its positive modulatory effect on adaptive immunity [37]. Therefore, the homeostasis of proinflammatory factors in our results is considered beneficial to the balance of efficacy and toxicity of the mRNA nanovaccine. This balance is possibly attributed to the biocompatibility of tFNA [16,35,38,39] and the bidirectional modulation effect of mDF2 β [28]. HDPs are capable of both anti-inflammation and proinflammation, which is dependent on the synergistic effect of other biomolecules and the degree of immunocyte activation [29,32].

Moreover, compared with D-mOVA, FD-mOVA elicited improved expression of the co-stimulatory molecules CD40 and CD86, and the antigen-MHC-1 complex H-2k^b SIINFEKL (OVA₂₅₇₋₂₆₄) (~29% to ~37%, ~37% to ~44%, ~35% to ~41%, respectively) (Fig. 2h). This

lays the foundation for DC-T cell interactions and antigen presentation.

In summary, FD-mOVA could stimulate the *in vitro* maturation and antigen presentation of DCs more efficiently than D-mOVA, whereas mOVA without any vehicle was ineffective.

Based on the aforementioned *in vitro* results, we next assessed the *in vivo* immune response to single injection of the mRNA nanovaccine. A fundamental principle for mRNA vaccination is preferential enrichment in immunity-relevant organs, such as the spleen [40]. Using fluorescent Cy5-mEGFP for *in vivo* imaging, we found that the mRNA nanovaccine gradually dispersed throughout the whole body from the liver 5 min after intraperitoneal injection (Fig. 3a). Twelve hours post-injection, luminescence analysis of separated organs showed that D-Cy5-mEGFP and FD-Cy5-mEGFP, but not Cy5-mEGFP without any vehicle, selectively accumulated in the spleen (Fig. 3a).

We next explored the proliferation of OVA-specific CD8⁺ T cells that are essential for cellular immunity. Five days post-vaccination, splenocytes were collected with a desirable live cell percentage (Fig. S4 in Supporting information) and analyzed. In contrast with D-mOVA, FD-mOVA elicited a higher percentage of OVA-specific CD8⁺ T cells in splenic lymphocytes (~5.6% to ~7.6%, representative flow-cytometry results in Fig. 3b, statistical results in Fig. 3d). Seven days post-vaccination, analysis of peripheral blood cells also demonstrated a higher percentage of OVA-specific CD8⁺ T cells in peripheral lymphocytes in response to FD-mOVA (~4.2% to ~6.5%, representative flow-cytometry results in Fig. 3c, statistical results in Fig. 3e).

We also assessed the body temperature of mice 12 h post-injection (Fig. 3f), which was rarely noticed in previous studies. The body temperature of all the mice treated with the mRNA nanovaccine remained at approximately 37 °C, suggesting that the mRNA nanovaccine was relatively biosafe with minimal likelihood of systemic inflammation.

Theoretically, mRNA vaccines are effective for both tumor treatment and prevention [4]. mRNA vaccines used for tumor treatment necessitate the synergistic effect of other therapeutics such as anti-PD-1 antibody [10,12], and immunotherapy is still an alternative strategy to traditional chemotherapeutics. We suppose that mRNA vaccines are preferentially promising for tumor prevention, based on the concept that prevention is better than cure [41]. Here, we used a prophylactic vaccination model of OVA-positive T cell lymphoma to determine the tumor prophylactic efficacy of the developed mRNA nanovaccine. All animal experiments were approved and strictly conducted following the guidelines of the Animal Ethics Committee of Sichuan University.

Following the time scheme in Fig. 4a, the tumor volume curve within 19 days was recorded (Fig. 4b). Both D-mOVA and FD-mOVA suppressed tumor growth compared with the control (representative photographs of tumor size on day 9 in Fig. 4e). More specifically, the average tumor occurrence was delayed from ~8 days to ~15 days (Fig. 4c), and the average tumor weight at the end of observation was decreased to ~40% (statistical results in Fig. 4d, photographs of all the isolated tumors in Fig. 4f).

Subsequently, all the mice were sacrificed on day 19 for histological assessment, which was rarely reported in the literature. First, hematoxylin-eosin (H&E) staining was used to assess the degree of tumor death (Fig. S5a in Supporting information). The control group exhibited typical pathological mitosis (yellow arrows) with an inapparent degree of tumor necrosis and apoptosis; the mOVA group showed similar features (yellow arrows). In comparison, the D-mOVA group demonstrated a higher degree of tumor apoptosis (blue arrows), and the FD-mOVA group showed a higher degree of both apoptosis (blue arrows) and necrosis (red arrows; ~10% folds vs. control). Furthermore, FD-mOVA elicited an optimal degree of apoptosis demonstrated by TdT-mediated dUTP Nick-End

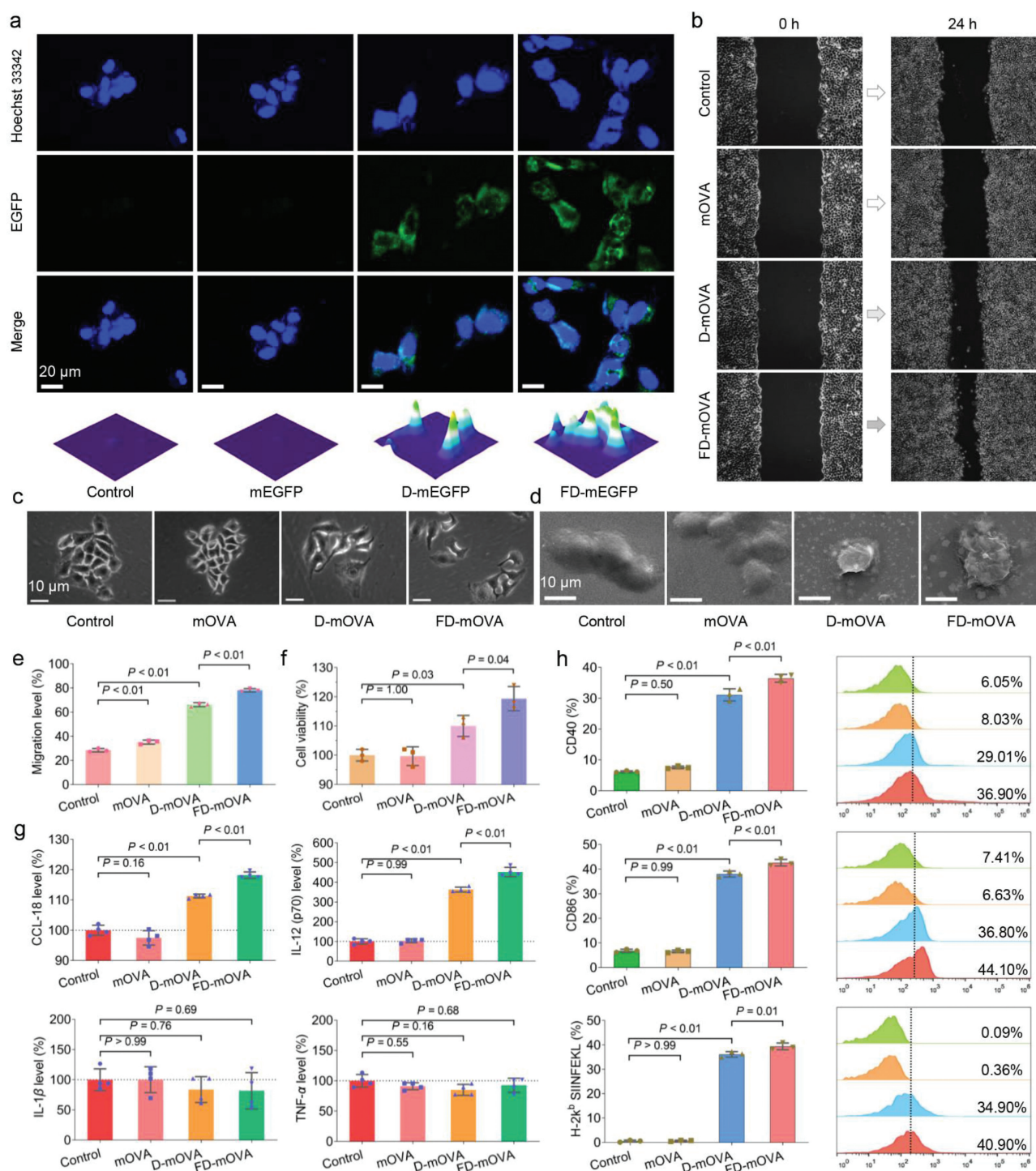


Fig. 2. *In vitro* maturation and antigen presentation of DCs. (a) Confocal images of nucleus (stained by Hoechst 33342, blue fluorescence) and EGFP (green fluorescence), and three-dimensional thermal images of EGFP. Scale bar = 20 μ m. (b) Representative results of cell migration assessed by the scratch test. (c) Cellular morphology observation using optical microscopy. Scale bar = 10 μ m. (d) Cellular morphology observation using SEM. Scale bar = 10 μ m. (e) Statistical analysis of cell migration ($n=3$). (f) Cell viability assessed by CCK-8 ($n=3$). (g) Quantitative analysis of cytokine secretion of CCL-18, IL-12 (p70), IL-1 β and TNF- α by ELISA ($n=4$). (h) Quantitative analysis of CD40 ($n=3$), CD86 ($n=3$) and H-2K^b SIINFEKL ($n=3$) using flow-cytometry and representative flow-cytometry results.

labeling analysis (Fig. S5b in Supporting information, white arrows, ~ 10 folds vs. control) and caspase-3 immunohistochemical analysis (Fig. S5c in Supporting information, green arrows, ~ 9 folds vs. control).

Importantly, CD8 immunohistochemical analysis revealed that FD-mOVA elicited optimal infiltration of CD8⁺ cells (Fig. S5d in Supporting information, gray arrows, ~ 16 folds vs. control), which

explains the optimal tumor-prophylactic effect of FD-mOVA. Taken together, these results suggest that FD-mOVA carries the highest likelihood of prolonging the survival of tumor-bearing mice [42,43].

H&E staining was also used to determine the systemic toxicity of our mRNA nanovaccine (Fig. S6 in Supporting information). There was no obvious change in the histological feature of the

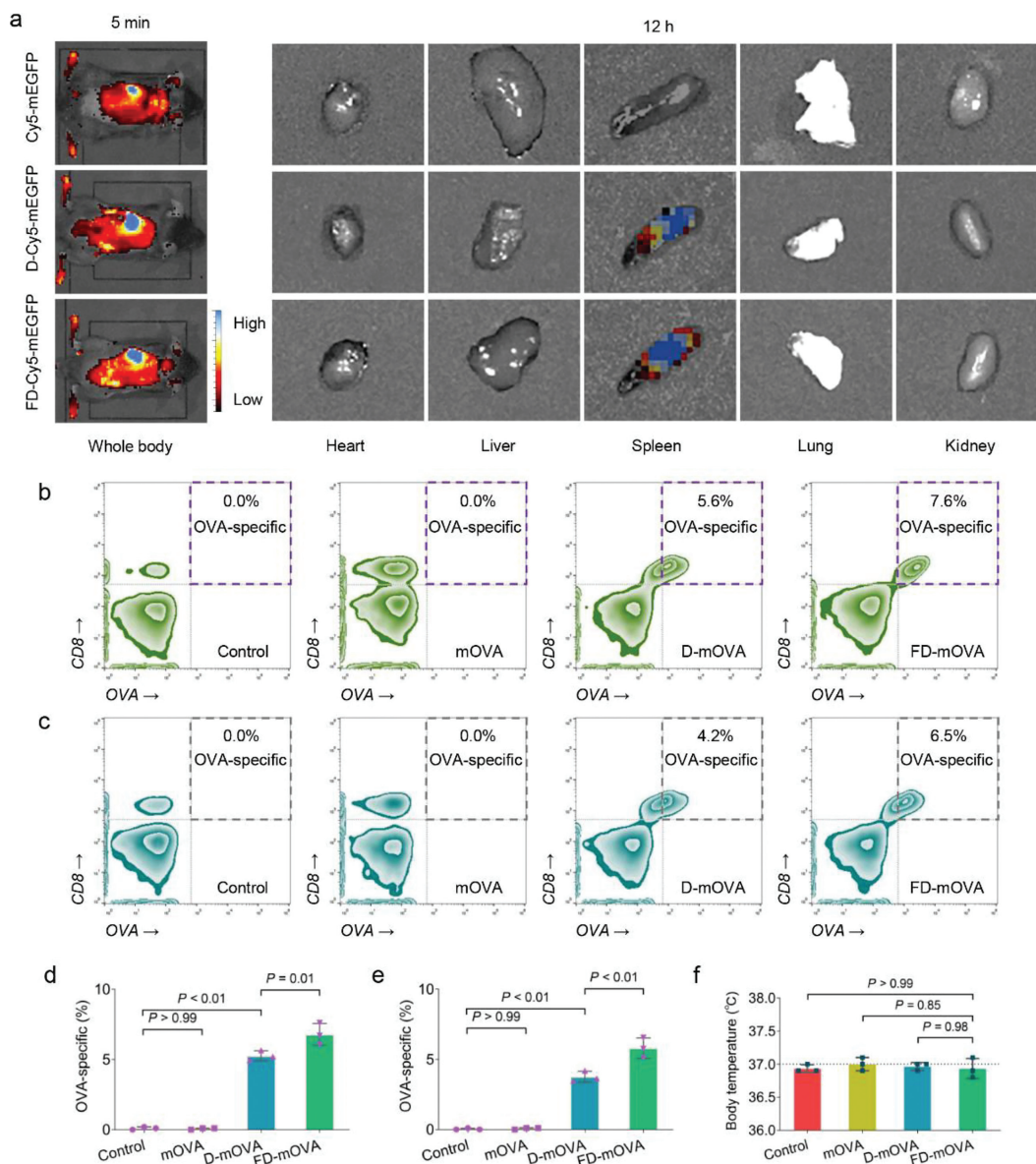


Fig. 3. Biodistribution imaging and immune response of single injection of the mRNA nanovaccine. (a) *In vivo* biodistribution imaging 5 min post-vaccination and isolated organ imaging (heart, liver, spleen, lung, and kidney) 12 h post-vaccination. (b) Representative flow-cytometry results of OVA-specific CD8⁺ T cells in splenic lymphocytes 5 days post-vaccination. (c) Representative flow-cytometry results of OVA-specific CD8⁺ T cells in peripheral lymphocytes 7 days post-vaccination. (d, e) Quantitative analysis of OVA-specific CD8⁺ T cells in splenic lymphocytes ($n=3$) and peripheral lymphocytes ($n=3$), respectively. (f) Quantitative analysis of the body temperature 12 h post-vaccination ($n=3$).

heart, liver, lung, or kidney, which implied the superior *in vivo* biosafety of our mRNA nanovaccine, while liposome vehicles may cause liver damage according to the literature [5]. In addition, the developed nanocapsule is believed to be biodegradable because mDF2 β is an endogenous biomolecule and tFNA based nanostructures are typically subjected to renal clearance [16,44].

To the best of our knowledge, this is the first study to report a dual-adjuvant nanocapsule CpG-tFNA-mDF2 β for mRNA vaccination, which is reasonably designed, multifunctional, and biosafe. The mechanism of action of this mRNA nanovaccine in tumor prevention is summarized in Scheme S1 (Supporting information). This nanovaccine is internalized by DCs wherein it promotes DC maturation. Matured DCs migrate for antigen presentation to T cells, together with cytokine secretion and expression of costimulatory molecules. Activated antigen-specific CD8⁺ T cells migrate peripherally and infiltrate early tumor tissues, recognize and attack tumor cells, and finally inhibit tumor occurrence and growth. This

nanovaccine demonstrates superior prophylactic efficacy compared with its single-adjuvant counterpart, whereas mRNA without a vehicle hardly achieves immune function.

Notably, our nanovaccine design provides an example for how mRNA is located outside the whole vaccine rather than being incorporated inside. Traditional vehicles, such as liposomes, deliver mRNA in an encapsulated form [3]. This typically ensures the stability of “vulnerable” mRNA under complex physiological conditions. However, a preliminary concern is that an overprotective effect exerted by vehicles may impede free interactions between mRNA and transcription-related molecules, thus suppressing mRNA transcription [4]. Since the developed nanovaccine proves effective, we believe that it is an invaluable topic to weigh the “sheltered” and “naked” condition for mRNA delivery in future studies. Regarding the “naked” concept reported here, it is worth further integration with the concept of “dynamic release” [45,46] for optimized interactions between mRNA and transcription-related molecules. For-

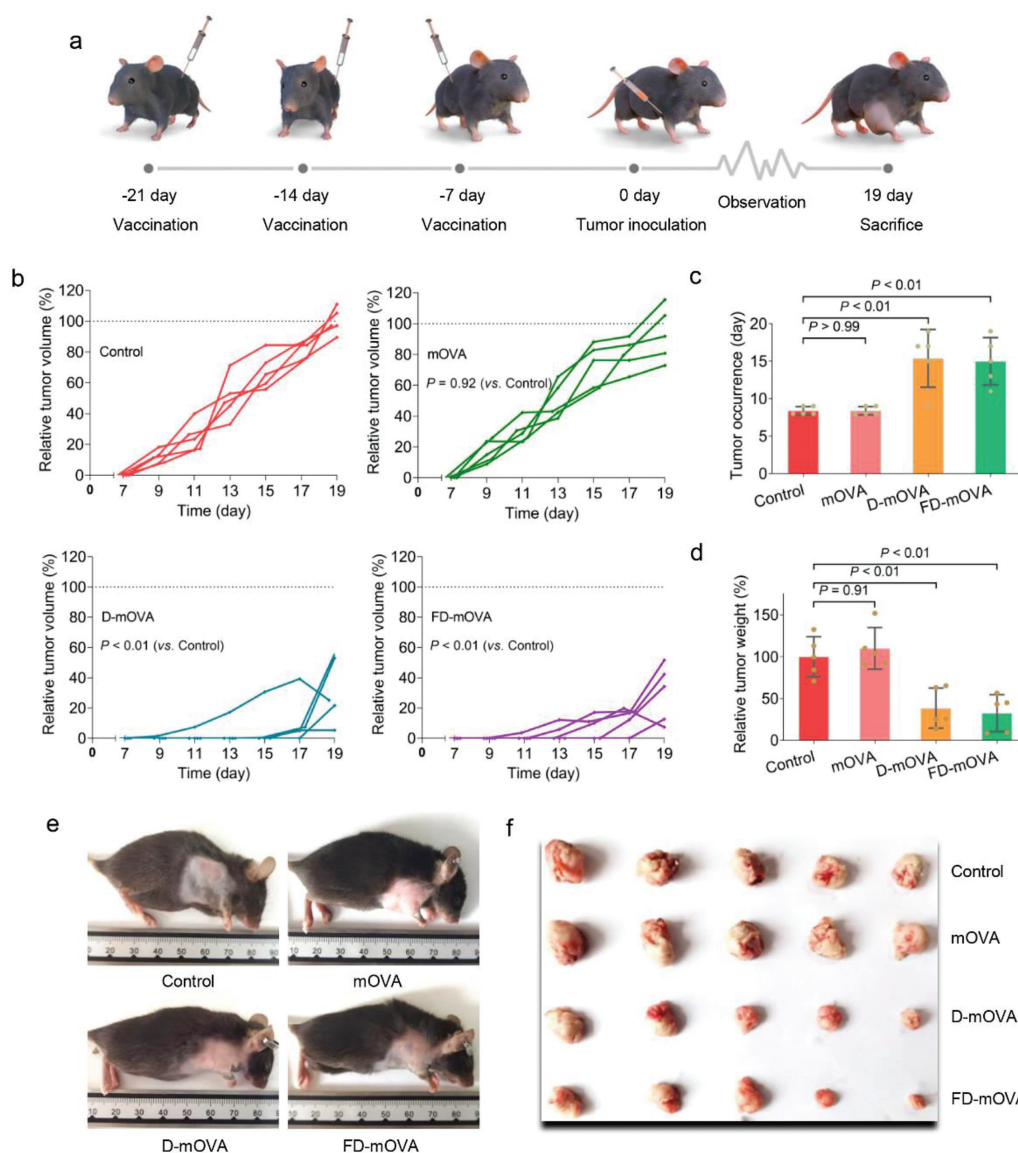


Fig. 4. Observation of the prophylactic vaccination model of OVA-positive T cell lymphoma. (a) Time scheme of the prophylactic vaccination model. (b) Tumor volume measurement after tumor inoculation ($n=5$). (c) Comparison of the average time for tumor occurrence ($n=5$). (d) Comparison of the weight of isolated tumor tissues at the deadline of observation ($n=5$). (e) Representative photographs of *in vivo* tumor size on day 9. (f) Photographs of isolated tumor tissues at the deadline of observation ($n=5$).

tunately, both tFNA [45,47] and HDP [48] can be designed with stimuli-responsiveness to physiochemical conditions (such as pH) or biomarkers, which provides opportunities for intracellular dynamic release of mRNA.

In conclusion, our tFNA based multifunctional nanocapsule provides a promising platform for prophylactic mRNA vaccination, for tumors diseases (such as melanoma and prostate cancer) and viral diseases (such as HIV and COVID-19).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ccl.2022.107987.

References

- [1] J.A. Kulkarni, D. Witzigmann, S.B. Thomson, et al., *Nat. Nanotechnol.* 16 (2021) 630–643.

- [2] A.J. Barbier, A.Y. Jiang, P. Zhang, R. Wooster, D.G. Anderson, *Nat. Biotechnol.* 40 (2022) 840–854.
- [3] K.A. Hajj, K.A. Whitehead, *Nat. Rev. Mater.* 2 (2017) 17056.
- [4] N. Pardi, M.J. Hogan, F.W. Porter, D. Weissman, *Nat. Rev. Drug Discov.* 17 (2018) 261–279.
- [5] F. Chen, Y. Wang, J. Gao, et al., *Biomaterials* 270 (2021) 120709.
- [6] J.S. Chahal, O.F. Khan, C.L. Cooper, et al., *Proc. Natl. Acad. Sci. U. S. A.* 113 (2016) E4133–E4142.
- [7] M. Lim, A.Z.M. Badruddoza, J. Firdous, et al., *Pharmaceutics* 12 (2020) 30.
- [8] L. Tan, X. Sun, *Nano Res.* 11 (2018) 5338–5354.
- [9] L.A. Jackson, E.J. Anderson, N.G. Roupheal, P.C. Roberts, J.H. Beigel, *New Engl. J. Med.* 383 (2020) 1920–1931.
- [10] R. Verbeke, I. Lentacker, K. Breckpot, et al., *ACS Nano* 13 (2019) 1655–1669.
- [11] A.L. Coolen, C. Lacroix, P. Mercier-Gouy, et al., *Biomaterials* 195 (2019) 23–37.
- [12] L. Miao, L. Li, Y. Huang, et al., *Nat. Biotechnol.* 37 (2019) 1174–1185.
- [13] X. Hou, T. Zaks, R. Langer, Y. Dong, *Nat. Rev. Mater.* 6 (2021) 1078–1094.
- [14] J. Yang, S. Arya, P. Lung, et al., *Nanoscale* 11 (2019) 21782–21789.
- [15] Y. Li, X. Ma, Y. Yue, et al., *Adv. Mater.* 34 (2022) 2109984.
- [16] Q. Zhang, S. Lin, L. Wang, et al., *Chem. Eng. J.* 413 (2021) 127426.
- [17] Y. Liu, Y. Sun, S. Li, et al., *Nano Lett.* 20 (2020) 3602–3610.
- [18] T. Zhang, T. Tian, Y. Lin, *Adv. Mater.* 34 (2022) 2107820.
- [19] T. Tian, D. Xiao, T. Zhang, et al., *Adv. Funct. Mater.* 31 (2021) 2007342.
- [20] Y. Gao, X. Chen, T. Tian, et al., *Adv. Mater.* 34 (2022) 2201731.
- [21] J. Li, Y. Yao, Y. Wang, et al., *Adv. Mater.* 34 (2022) 2202513.
- [22] S. Li, Y. Liu, T. Zhang, et al., *Adv. Mater.* 34 (2022) 2204287.
- [23] Z. Liu, X. Chen, W. Ma, et al., *Adv. Funct. Mater.* 32 (2022) 2204587.
- [24] J. Zhu, Y. Yang, W. Ma, et al., *Nano Lett.* 22 (2022) 2381–2390.
- [25] Y. Liu, Z. Liu, W. Cui, et al., *APL Mater.* 8 (2020) 100701.
- [26] G. Zhu, L. Mei, H.D. Vishwasrao, et al., *Nat. Commun.* 8 (2017) 1482.
- [27] A. Biragyn, P.A. Ruffini, C.A. Leifer, et al., *Science* 298 (2002) 1025–1029.
- [28] S.C. Mansour, O.M. Pena, R.E. Hancock, *Trends Immunol.* 35 (2014) 443–450.
- [29] N. Mookherjee, M.A. Anderson, H.P. Haagsman, D.J. Davidson, *Nat. Rev. Drug Discov.* 19 (2020) 311–332.
- [30] X. Ji, Q. Li, H. Song, C. Fan, *Adv. Mater.* 34 (2022) 2201562.
- [31] X. Yuan, J. Wang, M. Cheng, X. Zhang, *Int. Immunopharmacol.* 55 (2018) 133–141.
- [32] A.L. Hilchie, K. Wuerth, R.E. Hancock, *Nat. Chem. Biol.* 9 (2013) 761–768.
- [33] Y. Yoshizaki, E. Yuba, N. Sakaguchi, et al., *Biomaterials* 141 (2017) 272–283.
- [34] M. Morishita, Y. Takahashi, A. Matsumoto, M. Nishikawa, Y. Takakura, *Biomaterials* 111 (2016) 55–65.
- [35] M. Zhang, X. Zhang, T. Tian, et al., *Bioact. Mater.* 8 (2022) 368–380.
- [36] W. Ma, Y. Yang, J. Zhu, et al., *Adv. Mater.* 34 (2022) 2109609.
- [37] E. Perez-Ruiz, L. Minute, I. Otano, et al., *Nature* 569 (2019) 428–432.
- [38] X. Qin, L. Xiao, N. Li, et al., *Bioact. Mater.* 14 (2022) 134–144.
- [39] Y. Wen, M. Zhang, Y. Yao, et al., *Chin. Chem. Lett.* 34 (2023) 107549.
- [40] R. Firdessa-Fite, R.J. Creusot, *Mol. Ther. Methods Clin. Dev.* 16 (2020) 50–62.
- [41] F. Islami, R.L. Siegel, A. Jemal, *Nat. Rev. Clin. Oncol.* 17 (2020) 631–649.
- [42] S. Liu, Q. Jiang, X. Zhao, et al., *Nat. Mater.* 20 (2020) 421–430.
- [43] M. Luo, H. Wang, Z. Wang, et al., *Nat. Nanotechnol.* 12 (2017) 648–654.
- [44] D. Jiang, H.J. Im, M.E. Boleyn, et al., *Nano Res.* 12 (2019) 637–642.
- [45] T. Tian, D. Xiao, T. Zhang, et al., *Adv. Funct. Mater.* 31 (2020) 2007342.
- [46] M. Xiao, W. Lai, F. Wang, et al., *J. Am. Chem. Soc.* 141 (2019) 20354–20364.
- [47] S. Li, Y. Liu, T. Tian, et al., *Small* 17 (2021) e2104359.
- [48] J. Lee, E.T. Oh, H. Lee, et al., *Bioconjug. Chem.* 31 (2020) 43–50.