Dear Editor,

Excessive inflammatory responses lead to increased mortality from the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Nearly all deceased patients infected with SARS-CoV-2 are found to have cytokine storm syndrome and viral sepsis. They are prone to superinfections exacerbating the course of disease. Therefore, preventing hyperinflammation is critical for avoiding this cytokine storm syndrome. Currently, specific immunomodulators are under clinical research or application, including targeting inflammatory cytokines (sarilumab, anakinra, tocilizumab, infliximab, adalimumab) and inflammatory pathway (baricitinib, ruxolitinib). However, they may also increase the risk of superinfections. Therefore, it is urgent to develop highly selective anti-inflammatory drugs which don’t cause superinfections to effective treatment of SARS-CoV-2.

The role of innate immune receptors in the response to SARS-CoV-2 has been explored. It was demonstrated that the spike protein of SARS-CoV-2 (S), a trimeric protein and highly glycosylated class I fusion protein, is capable of interacting with and activating TLR4 to induce inflammatory cytokines (IL-1β and IL-6) production. Therefore, specifically blocking the S-TLR4 interaction to inhibit inflammatory responses should be a novel therapeutic protocol. Recently, several DNA aptamers have been reported with neutralization activity against SARS-CoV-2 by blocking the interaction of the RBD domain of spike protein and ACE2. Nevertheless, aptamers that can inhibit inflammatory responses have not been developed. Hence, we screened spike protein against DNA aptamers, aiming to selectively inhibit inflammation caused by SARS-CoV-2.

After 12 rounds of selection, aptamer ST-6 clearly stands out from other candidate sequences due to its high inhibition of blocking the S-TLR4 interaction (Supplementary Fig. S1a–e). Interestingly, aptamer ST-6 is Guanine(G)-rich sequences and can be assembled into G-quadruplexes (Fig. 1a and Supplementary Fig. S1f, g). According to this structure, we truncated and optimized this aptamer, and obtained two truncated aptamers (ST-6-1 and ST-6-2) (Supplementary Table S1). Disruption of the G-quadruplex significantly reduces the binding ability (Supplementary Fig. S1h). Subsequently, their characteristics were analyzed. The dissociation constants (K_D) of those three aptamers were determined to be in the range of 36 nM to 80 nM (Fig. 1b). Consistently, the results of flow cytometry and fluorescent images also demonstrate that those aptamers have high binding affinity (Fig. 1c and Supplementary Fig. S2a). Additionally, they bind only to spike proteins of SARS-CoV-2, neither spike proteins of other viruses (SARS-CoV, MERS, HCoV-HKU1) nor other proteins (IgG, PDGF-AA, ACE2, TLR4, BSA, RBD) (Supplementary Fig. S2b). This indicates that these aptamers have ideal selectivity, reducing the potential for off-target cytotoxicity. Furthermore, they also showed high stability and low immunogenicity in mice (Supplementary Fig. S2c–e). Overall, those characteristics show that those aptamers have good pharmaceutical potential.

We evaluated the blocking capacity of those aptamers. In the prevention experiment with simulated SARS-CoV-2 prevention reagents, those aptamers replaced about 70% TLR4 bound to the spike protein (Fig. 1d). Moreover, to simulate the window period and infected patients, those aptamers also showed significant blockade in competition and substitution experiments, respectively (Supplementary Fig. S3a, b). Additionally, to explore the structural basis for aptamer blocking the S-TLR4 interaction, molecular dynamics simulations showed that the aptamer and TLR4 were bound to the same epitope of spike protein, and TLR4 didn’t interact with RBD (Supplementary Fig. S3c, d). Furthermore, since the aptamer doesn’t bind to RBD, it doesn’t block the S-ACE2 interaction (Supplementary Fig. S3e, f). Those have verified the details of the aptamer suppressing S-TLR4 interaction. Taken together, those aptamers showed their potential therapeutic properties.

To evaluate the anti-inflammatory potential, monocytes and neutrophils cells were stimulated by spike proteins, with or without pre-bound aptamer, respectively. Resatorvid, a selective TLR4 inhibitor that inhibits cytokines production, was used as the positive control. As expected, those three aptamers remarkably reduce cytokines (IL-1β, IL-6, TNF-α and IFN-β) production in THP-1 cells (a cell line of human monocytes) (Fig. 1e and Supplementary Fig. S4a, b). We also treated HL-60 cells (a promyelocytic leukemia cell line) with all-trans retinoic acid (ATRA) which directed HL-60 cells to differentiate into neutrophils. Likewise, the same results were observed in ATRA-differentiated HL-60 cells (Fig. 1f and Supplementary Fig. S4c). More interestingly, they also reduce IL-1β production in THP-1 cells which are stimulated by spike proteins of SARS-CoV-2 variants, including the Delta, Lambda and Omicron variants (Supplementary Fig. S4d–f), demonstrating that those aptamers exert ideal universality and robust anti-inflammatory potency. Subsequently, aptamers were assessed for inhibition of authentic SARS-CoV-2 induced inflammation. They inhibit cytokines (IL-1β, IFN-β, IL-6, IL-8, TNF-α) production in a dose-dependent manner. The IC50 is estimated to be in the range of 47.89 to 65.52 nM and they reduced the amounts of cytokines by approximately 75 %, indicating that those aptamers displayed a high anti-inflammatory potency against authentic SARS-CoV-2 virus (Fig. 1h and Supplementary Fig. S4g).

A major challenge in designing anti-inflammatory agents is to balance efficacy and safety, especially to ensure that it doesn’t affect the host’s defense against other bacterial or viral infections. Hence, we wondered whether those aptamers could impair the host’s immune function. Lipopolysaccharide (LPS) stimulated immune responses are mediated by Toll-like receptors on the surface of host cells was used to simulate secondary infection (Fig. 1l). There is no cytokine production in THP-1 cells pretreated with Resatorvid and spike proteins, suggesting that TLR4 inhibitors impair the normal immune function of the host. Nevertheless, fortunately, THP-1 cells pretreated with aptamers and spike

Received: 16 January 2022 Revised: 2 March 2022 Accepted: 13 March 2022
Published online: 11 April 2022
proteins reproduced cytokines under LPS stimulation, indicating that the TLR4 signal transduction pathway maintained normal immune responses. Consistently, this phenomenon is also observed in ATRA-differentiated HL-60 cells. Overall, those results demonstrate that those three aptamers are highly selective inhibitors and don’t impair the host’s immune function to cause superinfection.

In summary, three aptamers that effectively prevent inflammation by specifically blocking S-TLR4 interaction are developed. They remarkably reduced the release of cytokines in monocytes and
neutrophils, regardless of whether the stimulus came from spike proteins of wild-type or SARS-CoV-2 variants. They also exhibited robust anti-inflammatory potential against authentic SARS-CoV-2. Notably, due to their high selectivity and low immunogenicity, the possibility of these aptamers causing superinfection is very low. Therefore, these three DNA aptamers will provide highly selective anti-inflammatory agents without causing superinfection and may reduce inflammation symptoms before they become severe. Combining these aptamers with antiviral infection drugs may be used as a potential treatment against SARS-CoV-2.

DATA AVAILABILITY
All data are available from the corresponding author.

ACKNOWLEDGEMENTS
This study was supported by the National Key Research and Development Project (2017YFA0504300), Chengdu Science and Technology Program (2021-YF05-01681-SN).

AUTHOR CONTRIBUTIONS
Conceptualization, Y.Z., X.S. and J.Z.; funding acquisition, X.S., Y.Z. and L.L.; investigation, Y.G., Y.W., S.Z. with the help of D.Y. and F.L.; data analysis, Y.Z., G.Y.; software and formal analysis, Y.L.; writing original draft, Y.Z. and G.Y.; reviewing and editing, X.S., Y.Z. All authors approved the final manuscript.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41392-022-00968-2.

Competing interests: The authors declare no competing interests.

1Center for Functional Genomics and Bioinformatics, College of Life Science, Sichuan University, Chengdu, Sichuan 610064, P.R. China and 5State Key Laboratory of Respiratory Disease, Guangzhou Institute of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong 510182, China

These authors contributed equally: Gang Yang, Shengnan Zhang, Yuchun Wang, Ling Li

Correspondence: Jincun Zhao (zhaojincun@gird.cn) or Xu Song (xusong@scu.edu.cn) or Yongyun Zhao (yongyun@163.com)

REFERENCES
1. Huang, C., Wang, Y. & Li, X. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 496–499 (2020).
2. Henderson, L. A. et al. On the alert for cytokine storm: immunopathology in COVID-19. Arthritis Rheumatol. 72, 1059–1063 (2020).
3. Rizk, J. G. et al. Pharmaco-immunomodulatory therapy in COVID-19. Drugs 80, 1267–1292 (2020).
4. Zhao, Y. et al. SARS-CoV-2 spike protein interacts with and activates TLR41. Cell Res. 31, 818–820 (2021).
5. Yang, G. et al. Identification of SARS-CoV-2 against aptamer with high neutralization activity by blocking the RBD domain of spike protein 1. Signal Transduct. Target Ther. 6, 227 (2021).

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.