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Novel Vaccine Design Strategies Against SARS-CoV-2 Variants: From Conserved Region Targeting to Multiepitope Approaches

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Abstract: SARS-CoV-2, the causative agent of COVID-19, represents a significant global health challenge. The continuous emergence of variants with immune-evasion capabilities, particularly Delta, Beta, and Omicron, has raised concerns about the long-term efficacy of current vaccines. These variants harbor mutations in the spike protein that alter viral binding affinity and enable escape from vaccine-induced neutralizing antibodies. This research examines vaccine design strategies targeting breakthrough variants, with particular focus on antibody-tolerant functions and mutational adaptability characteristics. We analyze modifications in spike protein configuration and function, especially within the receptor-binding domain (RBD), which primarily contributes to immune escape. Additionally, we evaluate how these mutations impact vaccine development and propose broad-spectrum protection strategies. By integrating insights from molecular evolution, structural biology, and immunology, this study provides a comprehensive framework for understanding SARS-CoV-2 and offers novel perspectives on future vaccine design. Our findings underscore the importance of developing mutation-responsive vaccines and therapeutic approaches to address continuously evolving viral threats.

Keywords: SARS-CoV-2 variants, Vaccine design, Immune escape, Conserved region vaccines, Multiepitope vaccines

1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China in December 2019, precipitating an unprecedented global pandemic that continues to pose significant public health challenges in 2025 and beyond [1]. According to the World Health Organization (WHO), SARS-CoV-2 has resulted in over 776.8 million confirmed COVID-19 cases and more than 7 million deaths as of November 10, 2024. This extraordinary global impact underscores the critical importance of understanding viral evolution and developing effective countermeasures[2].

Widespread vaccination programs have significantly reduced SARS-CoV-2 mortality rates while the virus maintains its characteristic high transmissibility. Since the authorization of the first COVID-19 vaccine on July 22, 2020, approximately 13.59 billion vaccine doses have been administered globally as of February 2024 [3]. The current vaccine landscape encompasses multiple technological platforms, including mRNA-based vaccines, adenoviral vectors (Ad26.COV2.S), inactivated

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vaccines, and protein subunit vaccines [4]. Each platform presents distinct advantages and limitations in clinical application. While mRNA vaccines represent cutting-edge technology, they face challenges related to stability and potential immunological side effects. Adenoviral vector vaccines demonstrate enhanced stability profiles and versatility across diverse population demographics. Traditional inactivated vaccines, though requiring a three-dose regimen, provide robust protection through well-established immunological mechanisms. Recent evidence suggests that heterologous prime-boost strategies combining mRNA and inactivated vaccines may optimize immunological responses through complementary activation pathways[5].

The rapid evolutionary dynamics of SARS-CoV-2 have generated multiple variants of concern (VOCs), including Alpha, Beta, and Omicron, characterized by enhanced transmissibility, immune evasion capabilities, and breakthrough infection potential6]. The occurrence of breakthrough infections in vaccinated individuals has raised significant concerns regarding vaccine efficacy and necessitates the development of next-generation vaccines targeting emergent variants. This research aims to analyze the molecular determinants of viral adaptability, characterize mutational signatures associated with immune escape, and develop predictive frameworks for vaccine design strategies targeting potential future variants. Additionally, we seek to evaluate proactive vaccine development approaches that can be implemented before variant emergence.

Our investigation specifically focuses on understanding immune escape mechanisms in breakthrough variants, particularly Omicron and Delta, and their implications for current vaccine efficacy. This research will elucidate the molecular mechanisms underlying breakthrough variant immune escape and inform the rational design of broad-spectrum vaccines targeting conserved viral epitopes. The manuscript systematically addresses these objectives through a structured approach, beginning with a comprehensive introduction contextualizing the SARS-CoV-2 pandemic and current vaccine landscape. Subsequent sections examine the fundamental principles of existing vaccine platforms, analyze the impact of viral mutations on vaccine neutralization efficacy, propose approaches for developing broadly protective vaccines targeting conserved regions, and synthesize key findings and implications for future vaccine development strategies.

Through this systematic investigation, we aim to contribute crucial insights into SARS-CoV-2 variant evolution and vaccine development, ultimately advancing our capability to respond effectively to emerging viral threats. The findings will have significant implications for public health strategies and the future development of broad-spectrum coronavirus vaccines.

2. Theoretical Research

The development of SARS-CoV-2 vaccines has yielded three primary technological platforms. mRNA-based vaccines operate by delivering specific genetic instructions that guide host cells to produce viral S protein[7]. Upon intramuscular administration, myocytes synthesize and display S protein fragments on their surface, thereby inducing protective antibody responses against SARS-CoV-2 [8]. Carrier vaccines utilize modified viral vectors to incorporate SARS-CoV-2 components, specifically engineering cellular machinery to replicate the S protein. This process triggers immune system activation through antibody production and T-cell recruitment. In contrast, protein subunit vaccines contain purified S proteins, which directly stimulate immune recognition and subsequent generation of protective antibodies and cellular immune responses [9].

Vaccine development strategies targeting the Spike protein implement four distinct methodological approaches, all utilizing full-length Spike protein as the primary antigen. This comprehensive strategy enables the induction of broad-spectrum immune responses against multiple Spike protein epitopes, encompassing both neutralizing antibodies and cellular immune components, thus providing effective viral neutralization [10]. The receptor binding domain (RBD) approach involves expressing the RBD-encoding gene in various expression systems, including insect cells and

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Escherichia coli, to generate recombinant RBD protein antigens. Experimental validation in animal models demonstrates that RBD-based vaccines successfully induce protective antibody responses capable of viral neutralization [11].

Despite significant advances in COVID-19 vaccine development and implementation, critical challenges persist regarding RBD-mediated antibody escape mechanisms. This phenomenon manifests through three distinct pathways: (1) alteration of antibody binding sites that diminishes antibody-RBD interaction efficacy, leading to reduced neutralization; (2) conformational changes in protein structure that impair antibody recognition; and (3) enhanced receptor binding affinity. Notably, specific RBD mutations can strengthen the interaction between viral Spike protein and host cellular receptors, facilitating more efficient viral entry.

The evolution of SARS-CoV-2 variants presents ongoing challenges for vaccine development, necessitating focused research on antibody tolerance and mutational adaptability. These investigations are crucial for developing next-generation vaccines capable of providing broad-spectrum protection against emerging viral variants.

3. Research Challenges

The global vaccine rollout has revealed several critical challenges. Despite high vaccination rates in Western nations, breakthrough infections in fully immunized individuals continue to rise globally [12]. This phenomenon is attributed to two primary factors: the emergence of SARS-CoV-2 variants of concern (VOCs), particularly Omicron subvariants that demonstrate reduced susceptibility to vaccine-induced antibodies [13], and the temporal decline in serum antibody levels. Studies indicate that spike protein-specific antibody concentrations significantly decrease six months post-vaccination [14], potentially compromising infection prevention and transmission control [15,16].

The spike protein, the primary target for neutralizing antibodies in both convalescent sera and vaccine-induced immunity [17], plays a crucial role in viral entry through its interaction with ACE2 [18]. This process is facilitated by transmembrane protease serine 2 (TMPRSS2), which cleaves S1 and S2 at the S2′ site adjacent to the furin cleavage site (FCS), optimizing cellular infection [19]. The receptor-binding domain (RBD) within S1 mediates binding to the host cell ACE2 receptor, facilitating viral membrane-host cell membrane fusion [20].

The emergence of variants such as Omicron and Delta has significantly impacted vaccine efficacy. Notably, Omicron's Δ143-145 mutation alters the N3 loop structure, potentially enabling escape from anti-NTD neutralizing antibodies. This necessitates urgent investigation into Omicron's impact on the neutralization capacity of diverse anti-RBD antibody classes and epitopes.

While vaccination remains crucial for global disease control, variant emergence has compromised vaccine effectiveness. Although current vaccines maintain protection against severe disease, their efficacy against emerging variants is suboptimal. Future vaccine development must specifically target variant characteristics, including immune escape mechanisms and enhanced cellular entry capabilities demonstrated by Omicron, Delta, and other variants.

The global vaccine distribution inequity presents additional challenges. Disproportionate vaccination rates worldwide create conditions conducive to increased immune escape risk through enhanced viral transmission and evolution. In regions with low vaccination coverage, unrestricted viral transmission facilitates adaptive mutations, potentially accelerating variant evolution toward increased transmissibility and pathogenicity. These emerging variants complicate prevention and control measures, presenting unprecedented challenges to global healthcare systems.

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4. Solutions to the problems

Vaccination remains the most effective strategy for limiting the spread of SARS-CoV-2 and other viral pathogens. Developing new vaccines for variants is necessary for future pandemic preparedness, while biological and computational modeling provides valuable tools to predict SARS-CoV-2 viral dynamics. Current vaccine design strategies focus on two promising approaches: universal vaccines targeting conserved regions and multiepitope vaccines.

Universal vaccines aim to target highly conserved regions of viral proteins to reduce the possibility of immune escape through mutation. When developing vaccines against viruses with multiple strains or variants, researchers exploit conserved epitopes that remain consistent across different viral strains [21]. For example, in influenza vaccine development, a common approach for creating universal vaccines has been to target conserved epitopes on the hemagglutinin (HA stalk) or the matrix ectodomain (M2e) [22-28].

For SARS-CoV-2, the S2 subunit contains highly conserved epitopes capable of inducing broadly neutralizing antibodies and eliciting robust humoral and cell-mediated immune responses. However, these conserved regions in S2 can be weak immunogens or remain inaccessible to the immune system. Through strategic vaccine design, these antigens can be modified to enhance their immunogenicity. While S2 shows significant promise, neutralizing epitopes on the S1 subunit remain critically important. Therefore, when designing a multi-epitope universal antigen targeting SARS-CoV-2 variants, it is essential to incorporate the highly conserved HR2 domain alongside other identified conserved epitopes on the NTD, RBD, FP, and HR1 regions [21].

Multiepitope vaccines demonstrate high structural stability and can induce specific immune responses, representing a potential strategy against SARS-CoV-2. The development of multiepitope vaccines primarily involves T-cell epitope prediction (including cytotoxic T lymphocyte (CTL) and helper T lymphocyte (HTL) epitope prediction) and B-cell epitope prediction. The latter is particularly important for IFN-γ epitope prediction, which plays a significant role in antiviral, anti-tumor, and immune regulatory activities.

In recent studies, immunoinformatic tools have been used to construct multi-epitope vaccines against SARS-CoV-2 comprising CTL, HTL, and IFN-γ epitopes capable of triggering strong immune responses. These designed multi-epitope vaccines have demonstrated both antigenic and immunogenic properties [29], suggesting their potential effectiveness for future vaccine development strategies.

5. Building Biology and Computational Modeling

With the high mutation rate of SARS-CoV-2, predicting viral evolution trends is both crucial and complex. Developing data models that can anticipate mutational patterns, especially recent mutations, provides essential blueprints for diagnosis, treatment strategies, and vaccine development[30].

The process of building biological and computational models begins with formulating precise research questions. These questions typically center on genomic locations, so establishing genomic coordinates constitutes the first step. Next, developing predictive functions incorporating key independent variables such as historical mutation rates and daily mutational frequencies creates the foundation for modeling. These functions analyze historical patterns to generate forward-looking predictions.

The second phase leverages machine learning methodologies for data modeling and performance evaluation, including the capability to anticipate unforeseen mutations. While predicting real-time frequency and characteristics of mutations remains challenging due to their complexity and unpredictability, the data transformation strategy developed by Zhou and Hu [31] offers a promising framework for this analysis.

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6. Global Vaccine Allocation and Vaccination Strategies

6.1. Fair Resource Allocation

6.1.1. Establish a Global Vaccine Allocation Fund

Governments, international organizations, and charitable foundations should jointly contribute to establishing a dedicated fund. Vaccine procurement resources should be allocated according to scientific criteria, incorporating factors such as population size, economic development level, and epidemic severity. Priority support should be directed to low-income countries experiencing severe outbreaks to ensure vaccine affordability and accessibility.

6.1.2. Optimize the Vaccine Supply Chain

This requires strengthening international logistics cooperation, establishing dedicated vaccine transportation channels, and coordinating with customs authorities to streamline procedures. Building regional vaccine reserve centers in proximity to production facilities facilitates timely distribution, reducing delivery timeframes and associated costs.

6.2. Enhance Vaccination Capabilities

6.2.1. Train Professional Vaccinators

Countries should organize comprehensive vaccination skill training programs for healthcare personnel, with particular emphasis on those serving in underdeveloped regions. Vaccination protocols and adverse reaction management knowledge should be disseminated through both online and offline channels to elevate overall vaccination competency.

6.2.2. Improve Vaccination Infrastructure

Temporary vaccination sites should be established in remote and densely populated low-resource areas, equipped with appropriate refrigeration and medical supplies. Mobile vaccination units can provide door-to-door services in communities and villages, enhancing convenience and accessibility for residents.

7. Conclusions

SARS-CoV-2 variants such as Delta and Omicron harbor significant mutations in the spike protein, particularly within the receptor-binding domain (RBD). These mutations alter viral binding affinity, enabling immune escape and substantially compromising current vaccine efficacy. To address these challenges, this research proposes two promising vaccine design strategies. Universal vaccines target highly conserved viral regions, specifically the conserved epitopes of the S2 subunit, to minimize immune escape risk. Complementarily, multiepitope vaccines, developed through sophisticated T and B cell epitope prediction, demonstrate capacity to induce robust immune responses.

Vaccine design innovation remains critical as emerging variants continue to challenge global health systems. Future vaccines must demonstrate adaptability to maintain effectiveness against evolving viral strains, thereby protecting public health and mitigating pandemic impact.

Future vaccine development efforts should be tightly integrated with global epidemiological surveillance. Scientists must continuously monitor viral mutational adaptability while considering worldwide immunization coverage disparities. This necessitates optimizing vaccine allocation strategies to ensure equitable distribution and enhancing vaccination capabilities across diverse global

settings. Through these coordinated approaches, the international community can mount more effective responses to emerging variants and better control the global transmission of SARS-CoV-2.

References

[1] Zhou P., Yang X.L., Wang X.G., Hu B., Zhang L., Zhang W., Si H.R., Zhu Y., Li B., Huang C.L., et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579:270–273. doi: 10.1038/s41586-020-2012-7. [DOI] [PMC free article] [PubMed] [Google Scholar]

[2] Sharma A, Ahmad Farouk I, Lal SK. COVID-19: A Review on the Novel Coronavirus Disease Evolution, Transmission, Detection, Control and Prevention. Viruses. 2021 Jan 29;13(2):202. doi: 10.3390/v13020202. PMID: 33572857; PMCID: PMC7911532.

[3] WHO WHO Coronavirus (COVID-19) Dashboard. [(accessed on 12 March 2024)]. Available online: https://data.who.int/dashboards/covid19/

[4] Sun Y, Huang W, Xiang H, Nie J. SARS-CoV-2 Neutralization Assays Used in Clinical Trials: A Narrative Review. Vaccines (Basel). 2024 May 18;12(5):554. doi: 10.3390/vaccines12050554. PMID: 38793805; PMCID: PMC11125816.

[5] Chang J. Adenovirus Vectors: Excellent Tools for Vaccine Development. Immune Netw. 2021 Feb 15;21(1):e6. doi: 10.4110/in.2021.21.e6. PMID: 33728099; PMCID: PMC7937504.

[6] Flores-Alanis A, Delgado G, Espinosa-Camacho LF, Rodríguez-Gómez F, Cruz-Rangel A, Sandner-Miranda L, Cravioto A, Morales-Espinosa R. Two Years of Evolutionary Dynamics of SARS-CoV-2 in Mexico, With Emphasis on the Variants of Concern. Front Microbiol. 2022 Jul 5;13:886585. doi: 10.3389/fmicb.2022.886585. PMID: 35865920; PMCID: PMC9294468.

[7] Bettini E, Locci M. SARS-CoV-2 mRNA Vaccines: Immunological Mechanism and Beyond. Vaccines (Basel). 2021 Feb 12;9(2):147. doi: 10.3390/vaccines9020147. PMID: 33673048; PMCID: PMC7918810.

[8] Understanding mRNA COVID-19 vaccines. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/mrna.html. Accessed Jan. 6, 2021.

[9] The different types of COVID-19 vaccines. World Health Organization. https://www.who.int/news-room/feature-stories/detail/the-race-for-a-covid-19-vaccine-explained. Accessed Feb. 3, 2021.

[10] Delacher, M., Simon, M., Sanderink, L., Hotz-Wagenblatt, A., Wuttke, M., Schambeck, K., Schmidleithner, L., Bittner, S., Pant, A., Ritter, U., et al. (2021). Single-cell chromatin accessibility landscape identifies tissue repair program in human regulatory T cells.Immunity54, 702–720.e17. https://doi.org/10.1016/j.immuni.2021.03.007

[11] Yang J, et al. A vaccine targeting the RBD of the S protein of SARS - CoV - 2 induces protective immunity[J].

[12] M. Bergwerk, T. Gonen, Y. Lustig, S. Amit, M. Lipsitch, C. Cohen, M. Mandelboim, E. Gal Levin, C. Rubin, V. Indenbaum, I. Tal, M. Zavitan, N. Zuckerman, A. Bar-Chaim, Y. Kreiss, G. Regev-Yochay, Covid-19 breakthrough infections in vaccinated health care workers. N. Engl. J. Med. 385, 1474–1484 (2021).

[13] Y. Araf, F. Akter, Y.-D. Tang, R. Fatemi, M. S. A. Parvez, C. Zheng, M. G. Hossain, Omicron variant of SARS-CoV-2: Genomics, transmissibility, and responses to current COVID-19 vaccines. J. Med. Virol. 94, 1825–1832 (2022).

[14] E. G. Levin, Y. Lustig, C. Cohen, R. Fluss, V. Indenbaum, S. Amit, R. Doolman, K. Asraf, E. Mendelson, A. Ziv, C. Rubin, L. Freedman, Y. Kreiss, G. Regev-Yochay, Waning immune humoral response to BNT162b2 covid-19 vaccine over 6 months. N. Engl. J. Med. 385, e84 (2021).

[15] S. Feng, D. J. Phillips, T. White, H. Sayal, P. K. Aley, S. Bibi, C. Dold, M. Fuskova, S. C. Gilbert, I. Hirsch, H. E. Humphries, B. Jepson, E. J. Kelly, E. Plested, K. Shoemaker, K. M. Thomas, J. Vekemans, T. L. Villafana, T. Lambe, A. J. Pollard, M. Voysey; Oxford COVID Vaccine Trial Group, Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. Nat. Med. 27, 2032–2040 (2021).

[16] P. B. Gilbert, D. C. Montefiori, A. B. McDermott, Y. Fong, D. Benkeser, W. Deng, H. Zhou, C. R. Houchens, K. Martins, L. Jayashankar, F. Castellino, B. Flach, B. C. Lin, S. O'Connell, C. McDanal, A. Eaton, M. Sarzotti-Kelsoe, Y. Lu, C. Yu, B. Borate, L. W. P. van der Laan, N. S. Hejazi, C. Huynh, J. Miller, H. M. El Sahly, L. R. Baden, M. Baron, L. De La Cruz, C. Gay, S. Kalams, C. F. Kelley, M. P. Andrasik, J. G. Kublin, L. Corey, K. M. Neuzil, L. N. Carpp, R. Pajon, D. Follmann, R. O. Donis, R. A. Koup; Immune Assays Team§; Moderna, Inc. Team§; Coronavirus Vaccine Prevention Network (CoVPN)/Coronavirus Efficacy (COVE) Team§; United States Government (USG)/CoVPN Biostatistics Team§, Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. Science 375, 43–50 (2021).

[17] Cao, Y., Wang, J., Jian, F. et al. Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. Nature 602, 657–663 (2022). https://doi.org/10.1038/s41586-021-04385-3

[18] L. Subissi, C.C. Posthuma, A. Collet, et al. One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities Proc. Natl. Acad. Sci. USA, 111 (37) (2014), pp. E3900 E3909, 10.1073/pnas.1323705111

[19] *Md. Aminul Islam, A review of SARS-CoV-2 variants and vaccines: Viral properties, mutations, vaccine efficacy, and safety, Infectious Medicine, Volume 2, Issue 4, 2023, Pages 247-261, ISSN 2772-431X, https://doi.org/10.1016/j.imj.2023.08.005.*

[20] *L. Subissi, C.C. Posthuma, A. Collet, et al.One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities Proc. Natl. Acad. Sci. USA, 111 (37) (2014), pp. E3900-E3909, 10.1073/pnas.1323705111*

[21] *Olukitibi, T.A.; Ao, Z.; Warner, B.; Unat, R.; Kobasa, D.; Yao, X. Significance of Conserved Regions in Coronavirus Spike Protein for Developing a Novel Vaccine against SARS-CoV-2 Infection. Vaccines 2023, 11, 545. https://doi.org/10.3390/vaccines11030545*

[22] *Erbelding, E.J.; Post, D.J.; Stemmy, E.J.; Roberts, P.C.; Augustine, A.D.; Ferguson, S.; Paules, C.I.; Graham, B.S.; Fauci, A.S. A universal influenza vaccine: The strategic plan for the National Institute of Allergy and Infectious Diseases. J. Infect. Dis. 2018, 218, 347–354. [Google Scholar] [CrossRef]*

[23] *Nachbagauer, R.; Krammer, F. Universal influenza virus vaccines and therapeutic antibodies. Clin. Microbiol. Infect. 2017, 23, 222–228. [Google Scholar] [CrossRef] [PubMed] [Green Version]*

[24] *Neirynck, S.; Deroo, T.; Saelens, X.; Vanlandschoot, P.; Jou, W.M.; Fiers, W. A universal influenza A vaccine based on the extracellular domain of the M2 protein. Nat. Med. 1999, 5, 1157–1163. [Google Scholar] [CrossRef]*

[25] *Saelens, X. The role of matrix protein 2 ectodomain in the development of universal influenza vaccines. J. Infect. Dis. 2019, 219, S68–S74. [Google Scholar] [CrossRef] [PubMed] [Green Version]*

[26] *Uranowska, K.; Tyborowska, J.; Jurek, A.; Szewczyk, B.a.; Gromadzka, B. Hemagglutinin stalk domain from H5N1 strain as a potentially universal antigen. Acta Biochim. Pol. 2014, 61, 541–550. [Google Scholar] [CrossRef] [Green Version]*

[27] *Turley, C.B.; Rupp, R.E.; Johnson, C.; Taylor, D.N.; Wolfson, J.; Tussey, L.; Kavita, U.; Stanberry, L.; Shaw, A. Safety and immunogenicity of a recombinant M2e–flagellin influenza vaccine (STF2. 4xM2e) in healthy adults. Vaccine 2011, 29, 5145–5152. [Google Scholar] [CrossRef]*

[28] *Olukitibi, T.; Ao, Z.; Azizi, H.; Mahmoudi, M.; Coombs, K.; Kobasa, D.; Kobinger, G.; Yao, X. Development and characterization of influenza M2 ectodomain and/or hemagglutinin stalk-based dendritic cell-targeting vaccines. Front. Microbiol. 2022, 13, 937192–937206. [Google Scholar] [CrossRef]*

[29] *Kar, T., Narsaria, U., Basak, S. et al. A candidate multi-epitope vaccine against SARS-CoV-2. Sci Rep 10, 10895 (2020). https://doi.org/10.1038/s41598-020-67749-1*

[30] *Thadani, N.N., Gurev, S., Notin, P. et al. Learning from prepandemic data to forecast viral escape. Nature 622, 818–825 (2023). https://doi.org/10.1038/s41586-023-06617-0*

[31] *Xinyu Zhou, Kevin Hu, Minmin Pan, Yajie Li, Chi Zhang, Sha Cao bioRxiv 2023.12.19.572480; doi: https://doi.org/10.1101/2023.12.19.572480*