A single dose SARS-CoV-2 simulating particle vaccine induces potent neutralizing activities

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1   **ABSTRACT**

2   **Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome**

3   **coronavirus 2 (SARS-CoV-2) for which a vaccine is urgently needed to control its spreading.**

4   **To facilitate the representation of a native-like immunogen without being infectious, here, we**

5   **reported a SARS-CoV-2 vaccine candidate (designated ShaCoVacc) by incorporating spike-**

6   **encoding mRNA inside and decorating spike protein on the surface of the virus simulating**

7   **particles (VSPs) derived from lentiviral particles. We characterized the mRNA copy number,**

8   **glycosylation status, transduction efficiency, and innate immune property of the new vaccine**

9   **platform. Importantly, we showed the ShaCoVacc induced strong spike-specific humoral**

10   **immune responses and potent neutralizing activities by a single injection. Additionally, we**

11   **disclosed the epitopes of spike-specific antibodies using peptide microarray and revealed**

12   **epitopes susceptible to specific neutralizing antibodies. These results support further**

13   **development of ShaCoVacc as a candidate vaccine for COVID-19 and VSP may serve as a new**

14   **vaccine platform for emerging infectious diseases.**

15

16   The coronavirus disease-19 (COVID-19) has been rapidly becoming a globe pandemic since its first

17   report in Wuhan, China, in the December of 2019[1]. The disease is caused by Severe Acute Respiratory

18   Syndrome Coronavirus 2 (SARS-CoV-2) which was effectively transmitted from human to human,

19   with influenza-like symptoms ranging from mild disease to severe lung injury and multi-organ failure,

20   eventually causing death, especially in aged patients with co-morbidities[2,3]. Vaccines are considered

21   to be one of the most effective ways to terminate the pandemic and help to restore the global

22   economy[4,5]. By May 12, at least six SARS-CoV-2 vaccine candidates have entered the clinical stage

23   basing on different platforms, i.e. mRNA, adenovirus, lentivirus, and plasmid DNA[6]. However,

24   published information regarding the neutralizing efficacy of each vaccine is still very limited except

25   an inactivated vaccine for which, however, multiple vaccinations are needed to get high EC50 titer[7,8].

26   In the past decades, numerous vaccine platforms have been approved for markets or clinical trials.

27   Live attenuated vaccines are weakened pathogens that induce strong humoral and cellular immune

28   response but also with infection risks especially for immune-compromised people[9,10]. Inactivated

29   vaccines are killed pathogens with intact structure and destroyed genetic materials – therefore, less

30   risky, but also less efficacy[9,10]. Protein subunit vaccine, viral vector vaccine, and nucleic acid vaccine

31   (DNA and mRNA) are generally safe, but difficult to reflect the conformational structure of viral

1   immunogen in its nature[11,12]. Virus-like particles (VLPs) are hulk particles with the unique ability to

2   present viral spikes in their natural conformation and elicit conformation-dependent neutralizing

3   antibodies[13-16]. A closer mimicking of a pathogen would be a VLP with spikes on its surface and

4   antigen-encoding nucleic acids inside. It is still unknown which vaccine platform will actually work

5   for SARS-CoV-2, making the development of new vaccine platforms of great importance. As

6   neutralizing antibodies have been detected in convalescents of COVID-19[17-20], a SARS-CoV-2

7   simulating vaccine may present the antigens to the immune systems in much the same way that it

8   would be presented by an authentic virus, thereby provoking a similar effective immune response.

9   In this study, we designed a candidate vaccine by encapsulating spike within and decorated onto the

10   surface of virus simulating particles (VSPs) derived from lentiviral particles in the form of mRNA

11   and protein, respectively, simulating the wild-type SARS-CoV-2. We hypothesized this could be

12   achieved by co-expression of a full-length spike during the production of mRNA-carrying lentiviral

13   particle which we have recently developed for time-restricted gene editing (Manuscript under

14   revision). To package the full-length spike mRNA into VSPs, we designed a spike construct

15   expressing the spike protein with 6X MS2 stem loop repeats on its transcripts which would allow the

16   spike mRNA to be packaged into VSPs via interaction with MS2 coat fused GagPol (Fig. 1a).

17   Meanwhile, as an envelope protein, the spike protein would automatically assemble into the

18   membrane of virus simulating particles (Fig. 1b).

19   To make sure high transcription and translation in the producer cells, we used a CMV promoter to

20   drive spike expression (Fig. 1a). In addition, the spike sequence was codon-optimized and equipped

21   with a signal peptide from the human heavy chain of IgE to boost translation and immunogenicity

22   (Fig. 1a)[4]. We also introduced two proline substation mutations in the S2 of spike sequence which

23   have been reported to increase the expression of spike and enhance the production of neutralizing

24   antibodies (Fig. 1a)[21,22]. The resulting spike transcript contains a MS2 stem loop between the stop

25   codon and the polyA sequence so that it could be packaged into lentiviral particles via direct

26   interaction with its cognate MS2 coat protein localized in the N-terminus of GagPol polyprotein. As

27   VSV-G coated lentiviruses are efficiently taken up by antigen-presenting cells and show the high

28   immunogenicity of antigens[23], we also included the VSV-G encoding plasmid (pMD.2G) in the

29   production process so that the surface of resulting VSPs will be covered by both spike and VSV-G

30   (Fig. 1b).

31   To examine if spike mRNA has been packaged into lentiviral particle as designed, we performed RT-

32   qPCR and found 3 or 4 copies of spike mRNA on average for each VSP (Fig. 1c). To verify whether

1   spike proteins have been assembled into VSPs and their glycosylation status, we performed Western

2   blot on the lysates of VSP with integration-defective lentiviruses (IDLVs) as controls (Fig. 1d). We

3   found successful decoration of spikes both with and without mutations on the VSPs while more

4   mutant spikes could be loaded. As glycosylation impacts the immunogenicity and immunodominance

5   of a vaccine[24], we examined the glycosylation status of the spike on the surface of VSPs. Notably,

6   the S2 bands shifted downwards after PNGase F treatment indicating that the spikes on VSPs were

7   modified by N-linked glycosylations consistent with the resent finding for SARS-CoV-2 revealed by

8   mass spectrometric approach (Fig. 1d)[25]. Next, we transduced VSPs to 293T cells and evaluated the

9   expression of spikes. We harvested the cells 36 hr postinfection without boiling the samples for

10   Western blot to avoid potential aggregation of the spike protein. 293T cells were not infected by

11   SARS-CoV-2 unless supplemented with hACE2[26]. Here, we still observed the expression of spikes

12   in 293T cells from VSPs suggesting that VSV-G were co-assembled into VSPs, thereby, broadened

13   their tropism (Fig. 1e). We found two major bands for spikes which were likely glycosylated full-

14   length singlet spike and its dimeric/trimeric forms (Fig. 1e). Additionally, we confirmed the

15   expression of spike using confocal analysis of transfected or transduced 293T cells (Fig. 1f). To

16   examine the innate immune property of VSP, we used THP-1 derived macrophages as a model of

17   nucleic sensing and found no significant increase of the type I interferon (IFN) and IFN-stimulated

18   genes ISG-15, and retinoic acid-inducible gene I (RIG-I) (Fig. 1g-i). As VSP-spike-mut incorporates

19   both mRNA and protein of spike more efficiently than the wild-type counterpart, we therefore chose

20   it as a vaccine candidate (designated ShaCoVacc) for in vivo evaluation.

21   To access the immunogenicity of ShaCoVacc, we injected the vaccine candidate to C57BL/6J mice

22   (n=6 for each group) via footpad (Fig. 2a). We performed enzyme-linked immunosorbent assay

23   (ELISA) using the sera from mice two weeks after vaccination to get access to the spike-specific IgG.

24   As shown in Fig. 2b, we observed significant elicitation of the spike-specific IgG. To evaluate the

25   production of neutralizing antibodies, we performed the neutralizing assay using spike pseudotyped

26   HIV which encodes firefly luciferase - a well-established pseudovirus neutralization assay[27].

27   Interestingly, a single injection of ShaCoVacc in our study induced an immediate and potent immune

28   responses against SARS-CoV-2 in contrast to an inactivated vaccine which required at least two or

29   three doses of injections (Fig. 2c)[8]. We also adopted the spike pseudotyped lentivirus which encodes

30   GFP to transduce Huh-7 cells. We found pre-incubation with 1:40 diluted sera from vaccinated mice

31   almost completely abolished the fluorescence which was evident for placebo groups and positive

32   controls (Fig. 2d) in line with Fig. 2c. Interestingly, sera from the vaccinated mice did not inhibit the

1   transduction of VSV-G pseudotyped lentivirus in Huh-7 cells indicating the neutralizing antibody is

2   spike-specific (Fig. 2d). T cell immune response is usually important for the function of vaccines to

3   control virus infection[28]. However, in case of COVID-19, overproduction of cytokines has been

4   correlated with the disease severity[29]. Therefore, cellular immunity must be cautious for any SARS-

5   CoV-2 vaccines. Here, we evaluated the T cell immune response here by simulating splenocytes with

6   a pool of spike-derived peptides. We did not found increased expression of IFN-γ and IL-2 suggesting

7   spike-specific cellular immune response was insignificant for ShaCoVacc (Fig. 2e-f). This is line

8   with a recent study with an inactivated SARS-CoV-2 vaccine which showed protection effects, but

9   found no notable changes in the percentages of lymphocytes and key cytokines in vaccinated

10   macaques[8]. Additionally, no weight loss caused by ShaCoVacc was found during the course of

11   vaccination suggesting no evident toxicity (Supplementary Fig. 1).

12   To dissect the linear epitope profile of the spike-specific antibodies in the vaccinated mice, we used

13   a newly developed peptide microarray which contains short peptides covering the full-length of a

14   spike. We found varying intensities of signals corresponding to certain spike peptides for the

15   vaccinated group while no signal was observed for the placebo treated mice (Fig. 2g). We also

16   quantified the signal intensity for antibodies against the S1 domain and receptor-binding domain

17   (RBD), respectively. The sera from vaccinated mice elicited significantly high signals for both

18   domains echoing our ELISA analysis of spike-specific antibodies and neutralizing assays (Fig. 2h).

19   To access to the panorama of epitopes, we made a heat map for of all vaccinated mice and found

20   epitope profiles for each vaccinated mouse were distinct (Fig. 2i and Supplementary Table 1).

21   However, we also found three epitopes (S2-22, S2-76, and S2-83) that were common for 66.7% of

22   the vaccinated mice. Interestingly, the S2-22 epitope also appeared in the majority of the

23   convalescents uncovered by our peptide microarray (manuscript in preparation). Antibody against

24   this epitope pulled from sera of convalescents has shown potent neutralizing activity[17]. Notably, S2-

25   76 and S2-83 epitopes are conserved epitopes and shared by SARS-CoV and SARS-CoV-2

26   (Supplementary Fig. 2).

27   We further revealed five neutralization epitopes locating on RBD, the domain responsible for the host

28   receptor recognition. The identified antibodies targeting these sites, therefore, can potentially block

29   the virus entry efficiently (Fig. 2j). Notably, former studies have shown that the murine

30   monoclonal/polyclonal antibodies that exhibit apparent binding to RBD from SARS-CoV exert no

31   neutralization activities against SARS-CoV-2, likely caused by their distinct structural features[30]. Our

32   findings thus pinpoint the key structural motifs in SARS-CoV-2-RBD that are susceptible to specific

5

1   neutralizing antibodies. Interestingly, we also identified S2-83 epitope (N1178-V1189) locating on

2   the HR2 region, which undergoes a dramatic refolding process during virus entry, leading to the

3   formation of a six-helix bundle structure that finally drives membrane fusion (Fig. 2h). Considering

4   the critical role of HR2 and its high sequence conservation among different coronaviruses

5   (Supplementary Fig. 2), it is conceivable that antibody targeting to HR2 may potentially induce a

6   broad neutralizing activity against various coronaviruses.

7   In compensation to the traditional vaccine platforms, our study provided a new vaccine platform by

8   simulating coronavirus surface protein and internal nucleic acids, therefore, combining features of

9   inactivated vaccines and mRNA vaccines. Due to the limited resource to SARS-CoV-2, we are

10 currently not able to re-challenge the vaccinated animals with an authentic virus. As the VSP platform

11 is based on lentiviral GagPol and no transfer vector is involved, it will not produce insertional and

12 replication competent lentiviruses. Additionally, the large-scale production and quality standard of

13 lentivirus that has been developed in CAR-T industry may facilitate the accessibility of VSP vaccine

14 to general populations. We further revealed the epitope profiles of vaccinated mice and the epitopes

15 susceptible to specific neutralizing antibodies, which may facilitate drug and antibody development.

16

17 **METHODS**

18 **Cell cultures**

19 293T and Huh-7 cells were cultured in DMEM (Gibco, USA) supplemented with 10% fetal bovine

20 serum (Gibco, USA) and 1% penicillin/streptomycin (P/S) (Thermo Fisher Scientific, USA). Primary

21 splenocytes and THP-1 cells were cultured in RPMI 1640 (Gibco, USA) with 10% fetal bovine serum

22 (Gibco, USA). THP-1 cells were differentiated into macrophage-like cells with 150 nM phorbol 12-

23 myristate 13-acetate (PMA) (Sigma) before the experiment.

24

25 **Plasmids**

26 pCCL-PGK-spike-flag and pCCL-PGK-spike-mut-flag were constructed by replacing the GFP gene

27 in pCCL-PGK-eGFP with spike or mutant spike (K1003P and V1004P) gene. pCMV-spike-mut-

28 6XMS2, pCMV-spike-6XMS2-flag, pCMV-spike-mut-6XMS2-flag were generated by inserting

29 6XMS2 stem loop repeats between the stop codon of spike or mutant spike gene and polyA sequence

30 while the whole expression cassette is under the control of CMV promoter.

31

32 **Production of VSP, IDLVs, and pseudovirus**

1 VSP, IDLV, and pseudovirus were produced by 293T cells in 15-cm dishes. Cells were seeded in the

2 15-cm dish at a density of 10^7/dish 24 hr before calcium phosphate transfection. The media were

3 refreshed 12 hr after transfection. 48 hr and 72 hr post-transfection, supernatants were filtered through

4 a 0.45-μm filter (Millipore) and ultracentrifuged at 4°C for 2 hr. Pellets were re-suspended in PBS

5 and stored at −80°C. To produce GFP-expressing spike pseudovirus and IDLV (IDLV-spike or

6 IDLV-spike-mut), cells were transfected with 9.07 μg pMD.2G (or corresponding spike plasmids),

7 7.26 μg pRSV-Rev, 31.46 μg pMDlg/pRRE-D64V, 31.46 μg pCCL-PGK-eGFP (or pCCL-PGK-

8 spike-flag or pCCL-PGK-spike-mut-flag). To produce VSP-spike (or VSP-spike-mut), cells were

9 transfected with 9.07 μg pMD.2G, 31.46 μg pMS2M-PH-gagpol-D64V, 31.46 μg pCMV-spike-

10 6XMS2, or pCMV-spike-mut-6XMS2, or their flag tag versions. To produce GFP-expressing spike

11 pseudovirus, cells were transfected with 9.07 μg pCMV-spike, 7.26 μg pRSV-Rev, 31.46 μg

12 pMDlg/pRRE and 31.46 μg pCCL-PGK-eGFP. To produce luciferase-encoding spike pseudovirus,

13 293T cells were transfected with 20 μg pcDNA3.1-SARS-Cov2-spike and 20 μg pNL4-3.luc.RE.

14

15 **Western blot**

16 To detect spike protein associated with VSP or IDLV, we use Western Blot to detect spike protein

17 with/without treatment of PNGase F (NEB). 100 ng particles were incubated with Glycoprotein

18 Denaturing Buffer at 98°C for 10 min. After adding GlycoBuffer 2 and NP-40 (10%), the mixtures

19 were incubated with/without PNGase F at 37°C for 2 hr. Mixtures were then incubated with SDS

20 loading buffer (Beyotime Biotechnology) before sample loading. To detect spike protein expressed

21 in cells, 293T cells were lysed in RIPA 36 hr after transfected with VSP or IDLV. The lysates were

22 incubated with SDS loading buffer supplemented with 2.5% β-Mercaptoethanol at 37°C for 30 min

23 without boiling. The proteins were separated by SDS-polyacrylamide gel electrophoresis and

24 transferred to the PVDF membrane. The membrane was blocked by 5% fat-free milk dissolved in

25 TBS/0.05% Tween-20 for 1 hr then cut off according to the marker and incubated with anti-flag

26 monoclonal antibody (Sigma) overnight at 4°C. The membranes were incubated with anti-mouse

27 secondary antibodies (Cell Signaling Technology) for 1 hr at room temperature. Proteins were

28 visualized by hypersensitive ECL chemiluminescence (Beyotime Biotechnology).

29

30 **Quantitative PCR**

31 To detected spike mRNA numbers carried by VSP, total RNAs from all samples were extracted using

32 the Viral DNA/RNA extraction kit (TaKaRa) followed by cDNA synthesization using the HiScript

1   Q RT SuperMix for qPCR (Vazyme, China) according to the manufacturer's protocol. RT-qPCR was

2   performed using qPCR SYBR Green Master Mix (Vazyme) following the manufacturer's protocol.

3   Plasmid pLV-PGK-S-mut diluted into copies of 10^3, 10^4, 10^5, 10^6, 10^7 per microliter were used to

4   make a standard curve for absolute quantification. Primer sequences are as follows, forward primer:

5   5'-ACAGATGAGATGATCGCCCAG-3', reverse primer: 5'-TCTGCATGGCGAAAGGGATC-3'.

6

7   **Mice**

8   6-8 weeks old, male, specific-pathogen-free (SPF) C57BL/6 mice were inoculated with VSP, IDLV,

9   or PBS by foot-pad injection. Animals were sacrificed by cervical dislocation under isoflurane. The

10   animal study has complied with the guidelines of the Institutional Animal Care and Use Committee

11   (IACUC) of the Shanghai Jiao Tong University.

12

13   **Splenocytes isolation**

14   Spleens were removed aseptically, placed in RPM 1640 medium, gently homogenized, and passed

15   through the cell strainer (Jet Bio-Filtration) to generate a single-cell suspension. Erythrocytes were

16   rapidly washed and lysed by the RBC lysis buffer (Sangon Biotech), and the splenocytes were

17   resuspended in 1mL RPMI 1640 medium.

18

19   **ELISA**

20   HIV p24 ELISA (Beijing Biodragon Immunotechnologies) was used to measure the p24 level of the

21   lentiviral particles according to the manufacturer. To detect SARS-CoV-2-spike specific antibodies

22   *in vivo*, serum from the animals were used to test the spike-specific IgG by Mouse IgG ELISA (Bethyl)

23   with a few modifications. 200 ng recombinant spike proteins were coated in 96-well ELISA plates

24   overnight at 4°C in a carbonating buffer (pH 9.5). The plates were blocked with PBS containing 0.05%

25   Tween 20 (PBS-T) and 2% bovine serum albumin (BSA) for 1 hr. Serum samples were diluted 1: 4

26   using PBS incubated for two hours, then washed and test follow the manufacturer's instructions. To

27   find the involvement of cellular immunity, the cytokine production in the splenic cells upon treatment

28   of spike peptides in vitro was measured. For the IFN-γ and IL-2 assay, the splenocytes were plated

29   in a 96-well plate at a density of 5×10^5 cells/200 μL. Cells were incubated with a pool of SARS-CoV-

30   2 spike peptides of 1 μg/mL final concentrations for each peptide for 24 hr, or 5 μg/mL concanavalin

31   A (ConA) (Sigma) and culture medium as controls. A pool of 210 peptides (15-17-mer) overlapping

32   by 10 spanning the entire SARS-CoV-2 spike protein were synthesized and kindly provided by Dr.

8

1   Shengce Tao from Shanghai Jiao Tong University, China. Peptides were pretreated with 100 µg/mL

2   polymyxin B (Beyotime) for 30 min on ice before stimulation to exclude the LPS contamination.

3   Cytokine IFN-γ and IL-2 in the supernatants were detected using the corresponding ELISA Kits

4   according to the manufacturer's instruction (MultiSciences Biotech). A standard curve was

5   established according to the OD values, and antibody concentrations were calculated.

6

7   **Neutralization assay**

8   To determine the serum neutralization activity against GFP-expressing spike pseudovirus. Vaccinated

9   mouse serum (40×dilutions) were incubated with GFP-expressing spike pseudovirus at 37°C for 1 hr

10   before adding the mixtures to Huh-7 cells (4×10^4 cells per well in 48-well plates). The media were

11   changed after 12 hr and photos were token at 48 hr post infection. For luciferase-encoding spike

12   pseudovirus neutralization assay. Serial dilutions of ShaCovacc or placebo vaccinated mouse serum

13   were incubated with luciferase-encoding pseudovirus at 37°C for 1 hr before adding to Huh-7 cells

14   (10^4 cells per well in 96-well plates). The culture media were refreshed 12 hr post-infection, which

15   was followed by an additional 48 hr incubation. Huh-7 cells were subsequently lysed with 50 µL lysis

16   reagent (Promega), and 30 µL lysates were transferred to 96-well Costar flat-bottom luminometer

17   plates (Corning Costar) for the detection of relative light units using the Firefly Luciferase Assay Kit

18   (Promega) with an Ultra 384 luminometer (Tecan). A nonlinear regression analysis was performed

19   on the resulting curves using Prism (GraphPad) to calculate half-maximal inhibitory concentration

20   (EC50) values.

21

22   **Immunofluorescence imaging**

23   293T cells were seed to 48-well plates with 0.1 mg/mL poly-D-lysine coated cover glasses at a destiny

24   of 4×10^4/well. On the next day, cells were transduced by 150 ng IDLVs or VSPs, or transfected by

25   0.6 µg pCMV-spike-6XMS2 or pCMV-spike-mut-6XMS2 plasmids. Cells were fixed using 4%

26   paraformaldehyde 36 hr after transduction and transfection. Cells were then stained by anti-flag tag

27   antibody (Proteintech) followed by Alexa Fluor 555 IgG incubation (Cell Signaling Technology) and

28   nuclei staining with DAPI (Beyotime Biotechnology). The imaging was performed on a confocal

29   microscope (A1Si, Nikon) to verify the expression of Spike proteins.

30

31   **Statistics**

1 Data are presented as mean ± S.D. in all experiments. One-way analysis of variance (ANOVA) or

2 student's t-tests were performed to determine the P values. *indicates statistical significance (*$P<$

3 0.05, **$P<$ 0.01, ***$P<$ 0.001, n.s.=non-significant).

4

Data availability

6 Data generated or analysed during this study are available from the corresponding author on

7 reasonable request.

8

Acknowledgement

17

Conflict of interest

19 The authors declare no conflict of interest.

20

Author contribution

22 D.Y, S.L., and Y.C. conceived the study and designed the experiments; D.Y., S.L., X.T, Y.L., Z.X.,

23 J.H., X.Z., X.W., and J.H. performed the experiments; all the authors analysed the data; D.Y., S.L.,

24 T.Y., and Y.C. wrote the manuscript with the help from all the authors.

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REFERENCES

1. Wu, F.*, et al.* A new coronavirus associated with human respiratory disease in China. *Nature* **579**, 265-269 (2020).

2. Zhou, F.*, et al.* Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The Lancet* **395**, 1054-1062 (2020).

3. Zhang, J.-j.*, et al.* Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* **n/a**(2020).

4. Richner, J.M.*, et al.* Modified mRNA Vaccines Protect against Zika Virus Infection. *Cell* **169**, 176 (2017).

5. Pardi, N.*, et al.* Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. *Nature* **543**, 248-251 (2017).

6. Thanh Le, T.*, et al.* The COVID-19 vaccine development landscape. *Nat. Rev. Drug Discov.* **19**, 305-306 (2020).

7. Kim, E.*, et al.* Microneedle array delivered recombinant coronavirus vaccines: Immunogenicity and rapid translational development. *EBioMedicine* **0**, 102743 (2020).

8. Gao, Q.*, et al.* Rapid development of an inactivated vaccine candidate for SARS-CoV-2. *Science*, eabc1932 (2020).

9. Rappuoli, R., Pizza, M., Del Giudice, G. & De Gregorio, E. Vaccines, new opportunities for a new society. *Proceedings of the National Academy of Sciences* **111**, 12288 (2014).

10. Plotkin, S.A. Vaccines: past, present and future. *Nat. Med.* **11**, S5-S11 (2005).

11. Pardi, N., Hogan, M.J., Porter, F.W. & Weissman, D. mRNA vaccines - a new era in vaccinology. *Nat. Rev. Drug Discov.* **17**, 261-279 (2018).

12. Shang, W., Yang, Y., Rao, Y. & Rao, X. The outbreak of SARS-CoV-2 pneumonia calls for viral vaccines. *npj Vaccines* **5**, 18 (2020).

13. Kushnir, N., Streatfield, S.J. & Yusibov, V. Virus-like particles as a highly efficient vaccine platform: Diversity of targets and production systems and advances in clinical development. *Vaccine* **31**, 58-83 (2012).

14. Noad, R. & Roy, P. Virus-like particles as immunogens. *Trends Microbiol.* **11**, 438-444 (2003).

15. Urakami, A.*, et al.* Development of a Novel Virus-Like Particle Vaccine Platform That Mimics the Immature Form of Alphavirus. *Clin. Vaccine Immunol.* **24**, e00090-00017 (2017).

16. Masavuli, M.G., Wijesundara, D.K., Torresi, J., Gowans, E.J. & Grubor-Bauk, B. Preclinical Development and Production of Virus-Like Particles As Vaccine Candidates for Hepatitis C. *Frontiers in Microbiology* **8**(2017).

17. Poh, C.M.*, et al.* Potent neutralizing antibodies in the sera of convalescent COVID-19 patients are directed against conserved linear epitopes on the SARS-CoV-2 spike protein. *bioRxiv*, 2020.2003.2030.015461 (2020).

18. Duan, K.*, et al.* Effectiveness of convalescent plasma therapy in severe COVID-19 patients. *Proceedings of the National Academy of Sciences*, 202004168 (2020).

19. Ju, B.*, et al.* Potent human neutralizing antibodies elicited by SARS-CoV-2 infection. *bioRxiv*, 2020.2003.2021.990770 (2020).

20. Wu, F.*, et al.* Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. *medRxiv*, 2020.2003.2030.20047365 (2020).

21. Walls, A.C.*, et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* **181**, 281-292.e286 (2020).

22. Pallesen, J*., et al.* Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. *Proceedings of the National Academy of Sciences* **114**, E7348 (2017).

23. Hu, B., Tai, A. & Wang, P. Immunization delivered by lentiviral vectors for cancer and infectious diseases. *Immunol. Rev.* **239**, 45-61 (2011).

24. Chang, D. & Zaia, J. Why Glycosylation Matters in Building a Better Flu Vaccine. *Molecular &amp; Cellular Proteomics* **18**, 2348 (2019).

25. Watanabe, Y., Allen, J.D., Wrapp, D., McLellan, J.S. & Crispin, M. Site-specific glycan analysis of the SARS-CoV-2 spike. *Science*, eabb9983 (2020).

26. Crawford, K.H.D*., et al.* Protocol and reagents for pseudotyping lentiviral particles with SARS-CoV-2 Spike protein for neutralization assays. *bioRxiv*, 2020.2004.2020.051219 (2020).

27. Ying, T*., et al.* Exceptionally Potent Neutralization of Middle East Respiratory Syndrome Coronavirus by Human Monoclonal Antibodies. *J. Virol.* **88**, 7796 (2014).

28. Prompetchara, E., Ketloy, C. & Palaga, T. Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. *Asian Pac. J. Allergy Immunol.* **38**, 1-9 (2020).

29. Zheng, H.-Y*., et al.* Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. *Cell. Mol. Immunol.* **17**, 541-543 (2020).

30. Wang, Q*., et al.* Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. *Cell*.

Legends

Figure 1. Construct and characterization of a SARS-Cov-2 simulating particle vaccine. a, Construction of mRNA-encoding plasmid which transcribes a MS2 stem loop-containing spike mRNA. The spike mRNA and protein will be packaging into lentiviral particles via the RNA-coat protein interaction and self-assembly, respectively. NTD, N-terminal domain; RBD, receptor binding domain; SD1 and SD2, subdomain 1 and 2; FP, fusion peptide; HR1 and HR2, heptad repeat 1 and 2; TM, transmembrane domain; CT, cytoplasmic tail. **b**, Illustration of the production process of the SARS-CoV-2 vaccine using VSP platform. **c**, Copy number of spike mRNA in each VSP particle. The copy number was detected by absolute quantification RT-qPCR and normalized to IDLV-spike-mut (2 copies RNA per virion). **d**, Western blot analysis of the spike protein in the virion treated with/without PNGase F. IDLV use as a control. 100 ng p24 for each vector. **e**, Western blot analysis of the spike protein expression. 293T cells were collected 36 hr after transfection or transduction. 300 ng p24 virus for each well. **f**. Confocal analysis of spike protein expression. 293T cells were fixed 36 hr after transfection or transduction. **g-i**, Innate immune response induced by VSP in THP-1 derived

1   macrophages. Cells were harvested for IFNB1, ISG15 and RIG-I analysis by RT-qPCR 6 hr after

2   transduction 150 ng p24 per well for each virus and 1.5 µg poly I:C per well as positive controls. S

3   represents spike. One-way ANOVA with Dunnett's *post hoc* tests were performed, ***P< 0.001,

4   n.s.=non-significant.

5

6   **Figure 2. Analysis of neutralizing activity of sera from ShaCoVacc vaccinated mice and linear**

7   **epitope profiling with peptide microarray. a,** Illustration of the working plan. The sera and

8   splenocytes were collected 14 days after footpad ShaCoVacc injection for further analysis. **b**, ELISA

9   analysis of spike specific IgG. **c** and **d**, Neutralization activity of vaccinated sera evaluated by

10 luciferase assay and confocal microscopy. A firefly luciferase-encoding and GFP-expressing SARS-

11 CoV2 pseudovirus was used, respectively, to transduce Huh-7 cells. **e** and **f**, Evaluation of the spike-

12 specific cellular immune response stimulating with a spike peptide pool. **g-i**, Peptide microarray

13 analysis of epitopes. **g**, Representative images of spike protein peptide microarray. S1 protein and

14 RBD were included in the microarray as controls. Highly frequent positive peptides were labeled. **h**.

15 Antibody responses against S1 protein or RBD in vaccinated mice. Signal intensity was averaged

16 fluorescent intensity of tinplated spots for each array. **i** Heatmap of antibody responses against

17 peptides. Gray grid indicates negative response. **j**, Analysing the epitopes of ShaCoVacc induced

18 spike-specific antibodies on spike protein. 6 mice used for each group, 1.5 µg ShaCoVacc or 50 µL

19 PBS were injected via footpad into each mouse. Error bars represent ±S.D. Unpaired two-tailed

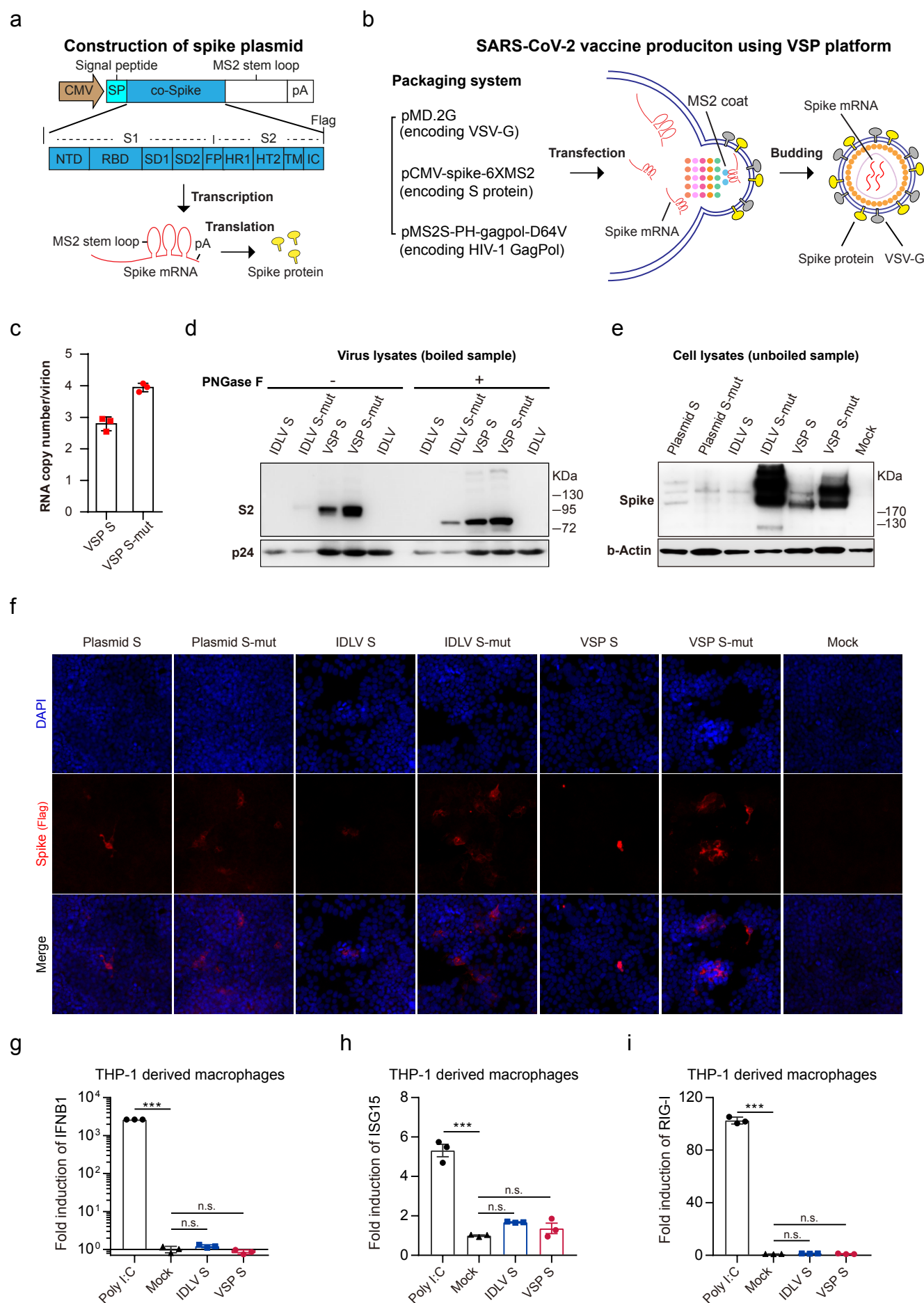20 student's t-tests (**b**, **c** and **h**), *P<0.05, ***P< 0.001.

21

Figure 1

Figure 2