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Supplemental information

Vaccine-mediated protection against *Merbecovirus* and *Sarbecovirus* challenge in mice

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Supplemental Information

Figure S1

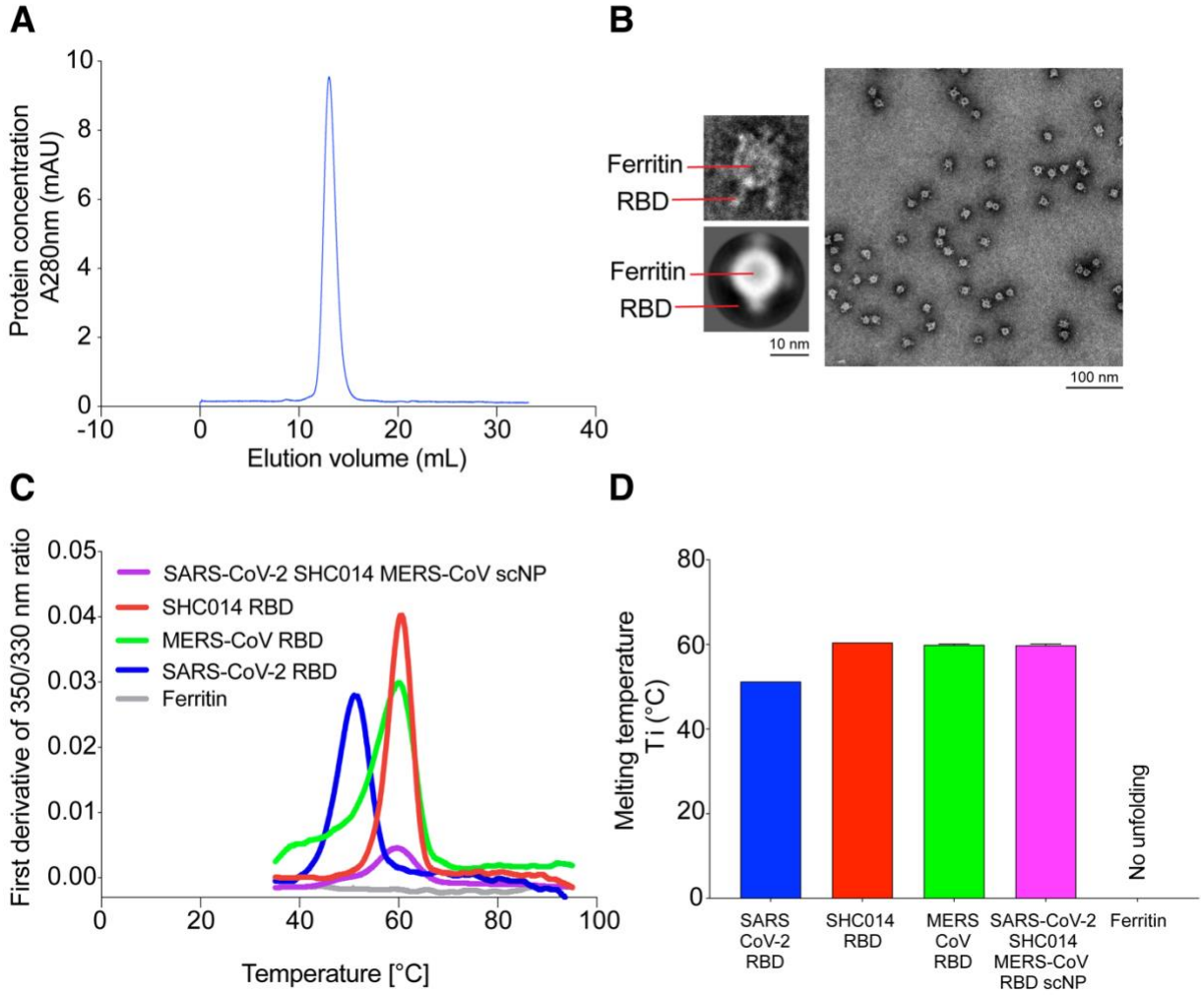

Figure S1. Structural and biophysical characterization of the trivalent SARS-CoV-2/SHC014/MERS RBD scNP. (A) Analytical size exclusion chromatography with a Superose6 column showing a homogenous protein nanoparticle at the expected elution volume. **(B)** Negative stain electron microscopy of trivalent RBD scNP. 2D class average is shown on the bottom left and a raw image of a single nanoparticle is shown on the top left. The raw image of the carbon grid is shown on the right. **(C and D)** Differential scanning fluorimetry of the individual components and assembled trivalent RBD scNP. Melting temperature is defined as the inflection temperature (T_i) on the 350 nm/330 nm ratio curve. Symbols represent values from independent experiments with bars representing the mean of the two independent experiments.

Figure S2

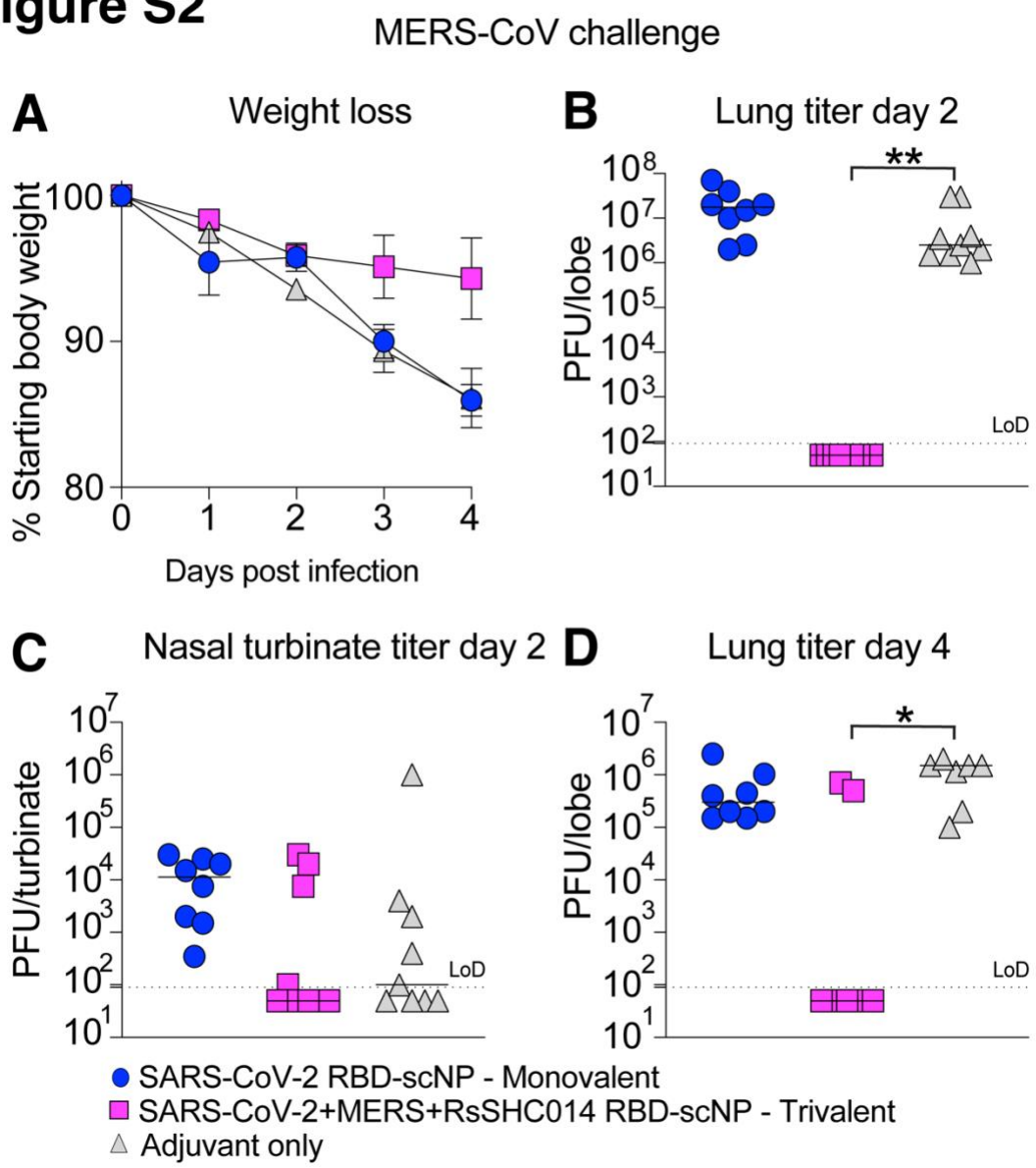
MERS-CoV challenge
A
Weight loss
% Starting body weight
100
90
80
0
1
2
3
4
Days post infection
B
Lung titer day 2
PFU/lobe
**
LoD
C
Nasal turbinate titer day 2
PFU/turbinate
LoD
D
Lung titer day 4
PFU/lobe
*
LoD
SARS-CoV-2 RBD-scNP - Monovalent
SARS-CoV-2+MERS+RsSHC014 RBD-scNP - Trivalent
Adjuvant only

Figure S2. Protective efficacy of monovalent vs trivalent RBD scNP vaccines against MERS-CoV challenge in 2X vaccinated mice. (**A**) Weight loss following MERS-CoV intranasal challenge in monovalent, trivalent, and adjuvant-only vaccinated mice. (**B**) Infectious virus replication in the lung at day 2 post infection. (**C**) Infectious virus replication at day 2 post infection in nasal turbinates. (**D**) Infectious virus replication at day 4 post infection. A Kruskal-Wallis test with a Dunn's multiple comparison correction test was used for calculating statistical significance in all panels. *P < 0.05, **P < 0.005, ***P < 0.0005, and ****P < 0.0001

Figure S3

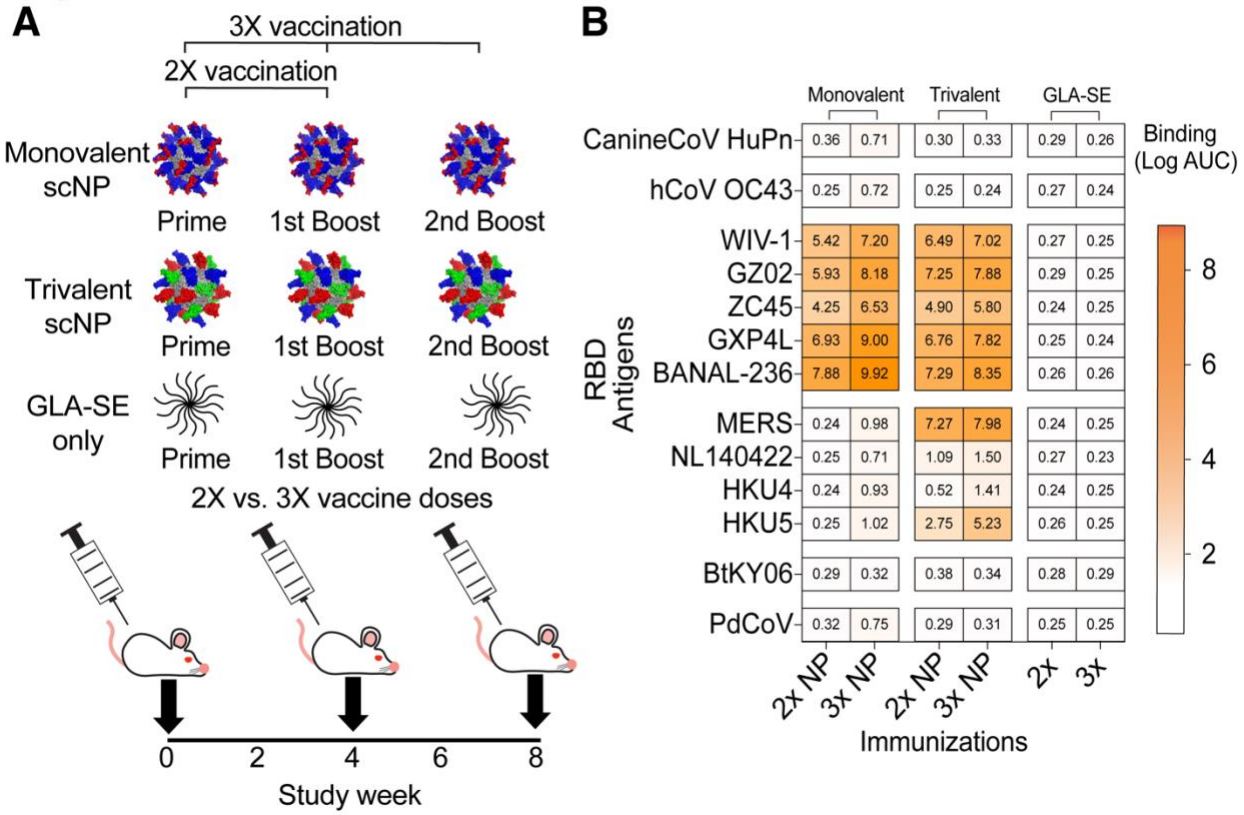
A
3X vaccination
2X vaccination
Monovalent scNP
Prime
1st Boost
2nd Boost
Trivalent scNP
Prime
1st Boost
2nd Boost
GLA-SE only
Prime
1st Boost
2nd Boost
2X vs. 3X vaccine doses
0
2
4
6
8
Study week
B
Monovalent
Trivalent
GLA-SE
CanineCoV HuPn 0.36 0.71 0.30 0.33 0.29 0.26
hCoV OC43 0.25 0.72 0.25 0.24 0.27 0.24
WIV-1 5.42 7.20 6.49 7.02 0.27 0.25
GZ02 5.93 8.18 7.25 7.88 0.29 0.25
ZC45 4.25 6.53 4.90 5.80 0.24 0.25
GXP4L 6.93 9.00 6.76 7.82 0.25 0.24
BANAL-236 7.88 9.92 7.29 8.35 0.26 0.26
MERS 0.24 0.98 7.27 7.98 0.24 0.25
NL140422 0.25 0.71 1.09 1.50 0.27 0.23
HKU4 0.24 0.93 0.52 1.41 0.24 0.25
HKU5 0.25 1.02 2.75 5.23 0.26 0.25
BtKY06 0.29 0.32 0.38 0.34 0.28 0.29
PdCoV 0.32 0.75 0.29 0.31 0.25 0.25
RBD Antigens
2x NP
3x NP
2x NP
3x NP
2x
3x
Immunizations
Binding (Log AUC)
8
6
4
2

Figure S3. Immunogenicity of monovalent vs trivalent RBD scNP vaccines in 2X vs 3X vaccinated mice. (**A**) vaccination schema with monovalent scNP, trivalent scNP, and adjuvant only (GLA-SE). (**B**) LogAUC IgG binding comparison of 2X (prime-boost) vs 3X (prime-boost-boost) vaccinated mice with monovalent, trivalent, and adjuvant-only against genetically divergent RBDs from Group 1 (canineCoV-HuPn). Group 2a (OC43), Group 2b (WIV-1, GZ02, ZC45, GXP4L, and BANAL-236), Group 2c (MERS-CoV, NL140422, HKU4, and HKU5), Group 2d (BtKY06), and Group 4 (Porcine deltacoronavirus Haiti).

Supplementary Table 1. Mass spectrometry quantification of receptor binding domain (RBD) abundance in trivalent RBD nanoparticle vaccine.

Protein Name	Abundance: Lot#1: Sample	Abundance: Lot#2: Sample	Abundance: Lot#3: Sample	Average	Standard Deviation	Ratio Lot#1 to MERS	Ratio Lot#2 to MERS	Ratio Lot#3 to MERS	Avg Ratio to MERS	Standard Deviation Ratio to MERS
MERS-CoV RBD	739055837	909817735	894822235	847898602	94558325.36	1	1	1	1	0
SARS-CoV-2 RBD	868638091	938426136	844043588	883702605	48961423.89	1.2	1.0	0.9	1.1	0.1
RsSHC014 RBD	1185580726	1054705449	948483177	1062923117	118762197.5	1.6	1.2	1.1	1.3	0.3