Immunity, Volume *53*

Supplemental Information

Nanoparticle Vaccines Based on the Receptor Binding Domain (RBD) and Heptad Repeat (HR) of SARS-CoV-2 Elicit Robust Protective Immune Responses

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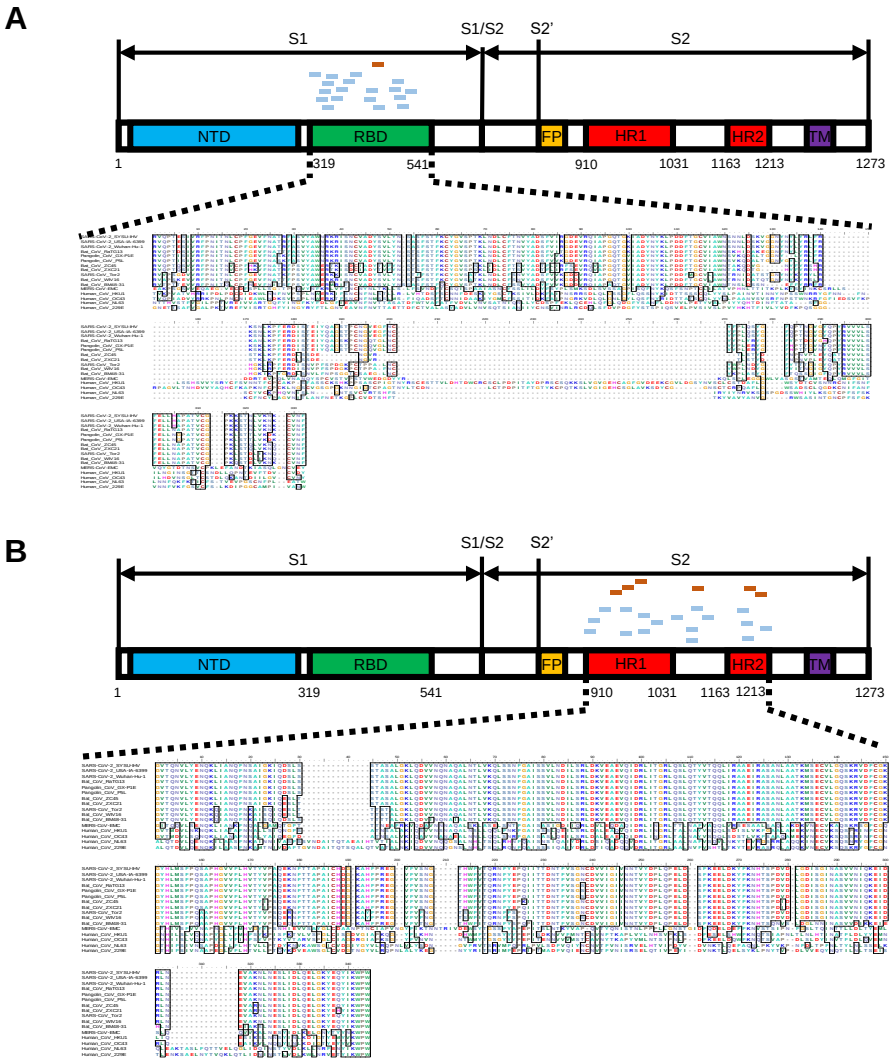
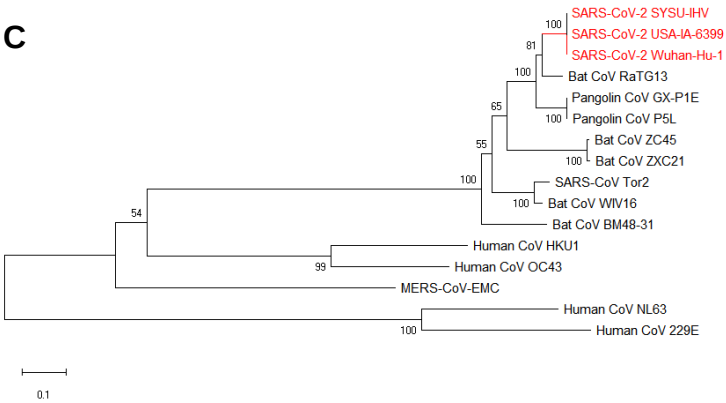
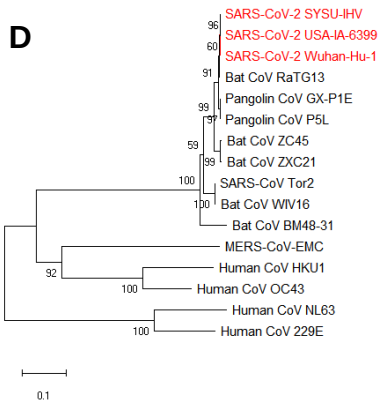

A
S1
S1/S2
S2'
S2
NTD
RBD
FP
HR1
HR2
TM
1
319
541
910
1031
1163
1213
1273
B
S1
S1/S2
S2'
S2
NTD
RBD
FP
HR1
HR2
TM
1
319
541
910
1031
1163
1213
1273


C
SARS-CoV-2 SYSU-IHV
SARS-CoV-2 USA-IA-6399
SARS-CoV-2 Wuhan-Hu-1
Bat CoV RaTG13
Pangolin CoV GX-P1E
Pangolin CoV P5L
Bat CoV ZC45
Bat CoV ZXC21
SARS-CoV Tor2
Bat CoV WIV16
Bat CoV BM48-31
Human CoV HKU1
Human CoV OC43
MERS-CoV-EMC
Human CoV NL63
Human CoV 229E
0.1


D
SARS-CoV-2 SYSU-IHV
SARS-CoV-2 USA-IA-6399
SARS-CoV-2 Wuhan-Hu-1
Bat CoV RaTG13
Pangolin CoV GX-P1E
Pangolin CoV P5L
Bat CoV ZC45
Bat CoV ZXC21
SARS-CoV Tor2
Bat CoV WIV16
Bat CoV BM48-31
MERS-CoV-EMC
Human CoV HKU1
Human CoV OC43
Human CoV NL63
Human CoV 229E
0.1

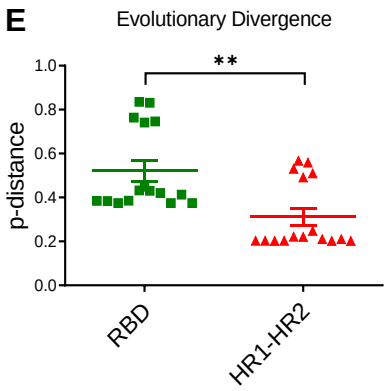

E
Evolutionary Divergence
**
p-distance
1.0
0.8
0.6
0.4
0.2
0.0
RBD
HR1-HR2

Figure S1. Related to Figure 1. Homology Analysis of RBD and HR among Coronaviruses

(A-B) Homology analysis of RBD and HR among coronaviruses which contained three SARS-CoV-2 strains (SYSU-IHV, USA-IA-6399 and Wuhan-Hu-1), six human pathogenic coronaviruses (SARS-CoV Tor2, MERS-CoV EMC, hCoV-HKU1, hCoV-OC43, hCoV-NL63 and hCoV-229E), five bat coronaviruses, and two pangolin coronaviruses. The alignments of sequences were built by using ClustalW method. All ambiguous positions were removed for each sequence pair. Orange and light blue bars within each schematic represented $CD8^+$ and $CD4^+$ T cell epitopes respectively. (C-D) The evolutionary history was inferred using the Neighbor-Joining method based on the protein sequences located in the regions of RBD:319-541(aa) (A) and HR1-HR2:910-1213(aa) (B) from the coronavirus Spike gene. The percentage of replicate trees in which the associated strains clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. (E) The average genetic distance between one given strain and the relevant entire population in the coronavirus phylogenetic tree was calculated using the p-distance method. The evolutionary analyses were performed in MEGA X software. Moreover, the two-tailed Mann-Whitney *U*-test was conducted with Prism 8.0 software for comparing the evolutionary divergence between the regions of RBD and HR1-HR2. Experiments were conducted independently in triplicates. Data represented as mean ± SEM (n=16). P-Value was calculated by two-tailed Mann-Whitney *U*-test. $^{**}p < 0.01$.

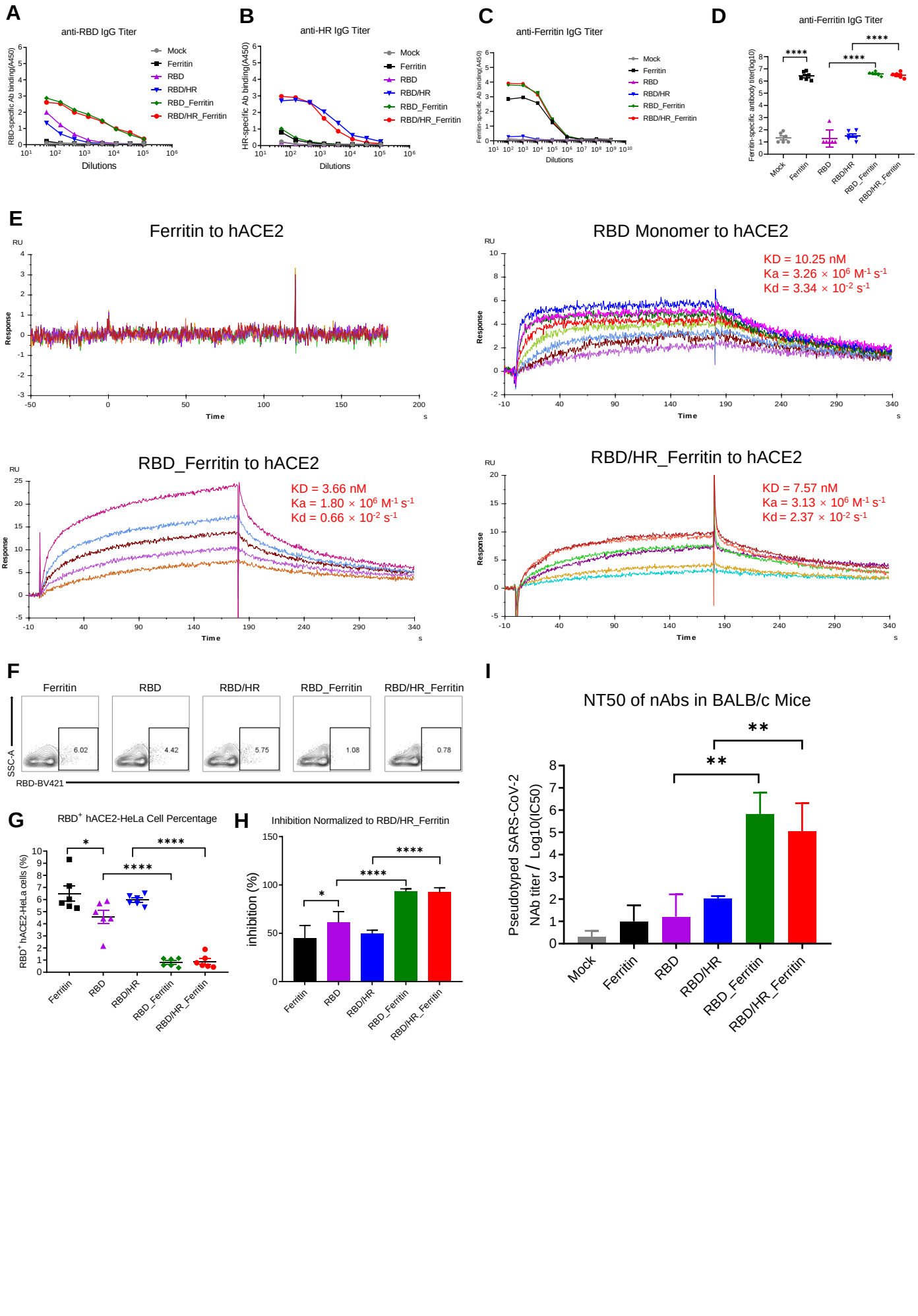
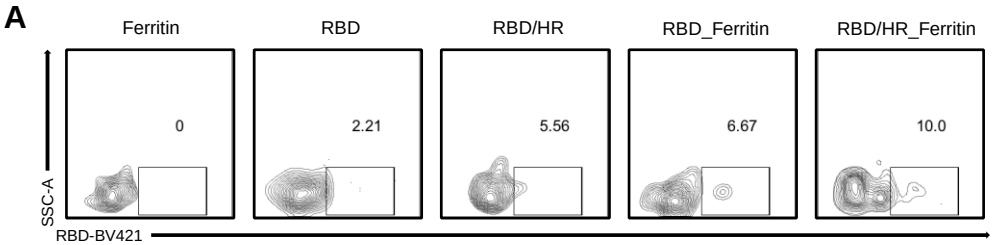
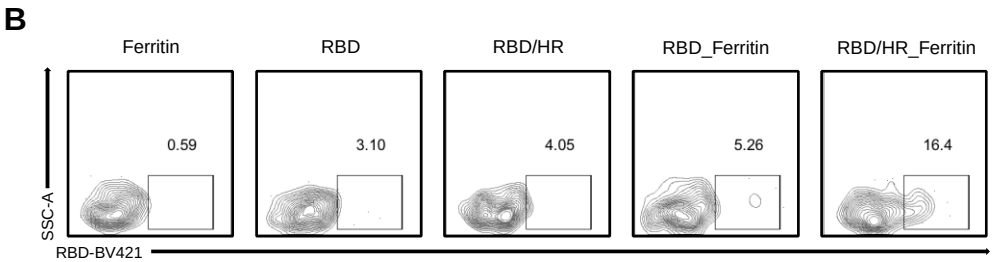
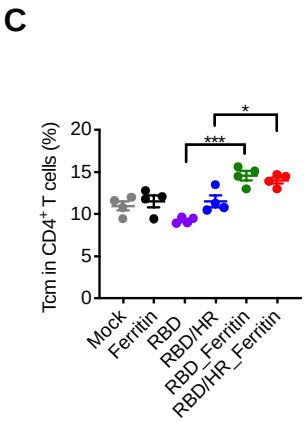

A
anti-RBD IgG Titer
RBD-specific Ab binding(A450)
Dilutions
Mock
Ferritin
RBD
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
B
anti-HR IgG Titer
HR-specific Ab binding(A450)
Dilutions
C
anti-Ferritin IgG Titer
Ferritin-specific Ab binding(A450)
Dilutions
D
anti-Ferritin IgG Titer
Ferritin-specific antibody titer(log10)
****
E
Ferritin to hACE2
RBD Monomer to hACE2
KD = 10.25 nM
Ka = 3.26 × 10^6 M^-1 s^-1
Kd = 3.34 × 10^-2 s^-1
RBD_Ferritin to hACE2
KD = 3.66 nM
Ka = 1.80 × 10^6 M^-1 s^-1
Kd = 0.66 × 10^-2 s^-1
RBD/HR_Ferritin to hACE2
KD = 7.57 nM
Ka = 3.13 × 10^6 M^-1 s^-1
Kd = 2.37 × 10^-2 s^-1
RU
Response
Time
s
F
Ferritin
RBD
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
SSC-A
RBD-BV421
6.02
4.42
5.75
1.08
0.78
G
RBD+ hACE2-HeLa Cell Percentage
RBD+ hACE2-HeLa cells (%)
H
Inhibition Normalized to RBD/HR_Ferritin
inhibition (%)
I
NT50 of nAbs in BALB/c Mice
Pseudotyped SARS-CoV-2
NAb titer / Log10(IC50)
**

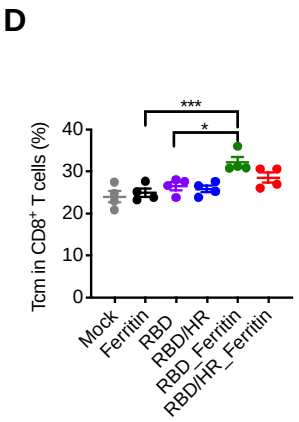
Figure S2. Related to Figure 2. Humoral Immune Responses in Nanoparticles Vaccinated BALB/c Mice

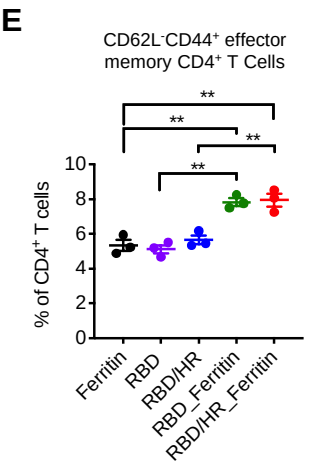
(A-B) SARS-CoV-2 RBD- and HR-specific IgG titers of immunized BALB/c mice at week 6 were detected by ELISA. IgG antibody titers of serum were determined by serial dilution. (C-D) *H. pylori* ferritin-specific IgG titers of immunized BALB/c mice at week 6 were detected by ELISA and further determined by serial dilutions. The titers were represented as the reciprocal of the endpoint serum dilution (n=6). (E) Representative BIAcore plots of Ferritin, RBD monomer, RBD_Ferritin and RBD/HR_Ferritin bound to hACE2. The Ka, Kd, and KD values were calculated by the software BIAevaluation. The KD value shown was a mean of three independent experiments. (F) Serum inhibition of SARS-CoV-2 RBD binding to hACE2-HeLa cells among different groups of immunized BALB/c mice were analyzed by flow cytometry. RBD proteins were tagged by BV421. (G-H) Serum of different mice mediated RBD blocking was represented by both the percentages of RBD^+ hACE2-HeLa cells (G) and corresponding inhibition normalized to RBD/HR-Ferritin (H) (n=6). (I) Groups of serially diluted serum were detected for neutralizing antibody against pseudotyped SARS-CoV-2. Data represented NT50 of nAbs in each group. Experiments were conducted independently in triplicates. Data represented as mean ± SEM. Adjusted p-Values were calculated by one-way ANOVA with Tukey's multiple comparisons test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

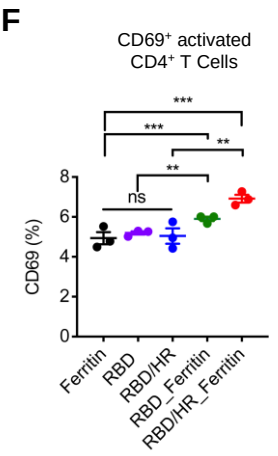

A
Ferritin
RBD
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
0
2.21
5.56
6.67
10.0
SSC-A
RBD-BV421

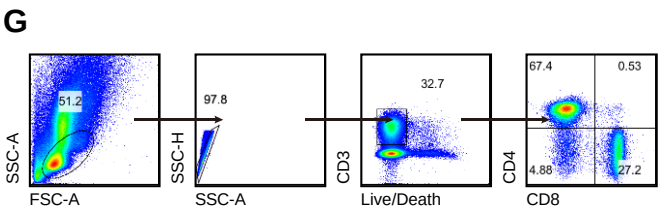

B
Ferritin
RBD
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
0.59
3.10
4.05
5.26
16.4
SSC-A
RBD-BV421

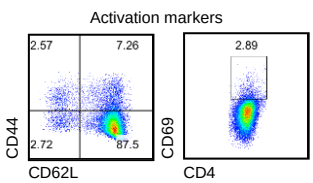

C
Tcm in CD4+ T cells (%)
Mock
Ferritin
RBD
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin

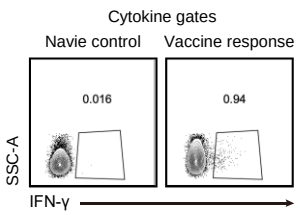

D
Tcm in CD8+ T cells (%)
Mock
Ferritin
RBD
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin

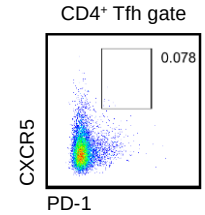

E
CD62L-CD44+ effector memory CD4+ T Cells
% of CD4+ T cells
Ferritin
RBD
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin


F
CD69+ activated CD4+ T Cells
CD69 (%)
ns
Ferritin
RBD
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin


G
51.2
97.8
32.7
67.4
0.53
4.88
27.2
SSC-A
FSC-A
SSC-H
SSC-A
CD3
Live/Death
CD4
CD8


Activation markers
2.57
7.26
2.72
87.5
2.89
CD44
CD62L
CD69
CD4


Cytokine gates
Navie control
Vaccine response
0.016
0.94
SSC-A
IFN-γ


CD4+ Tfh gate
0.078
CXCR5
PD-1

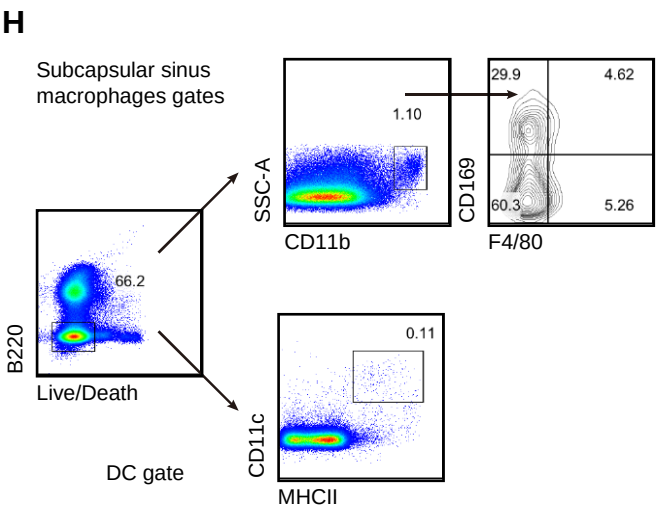
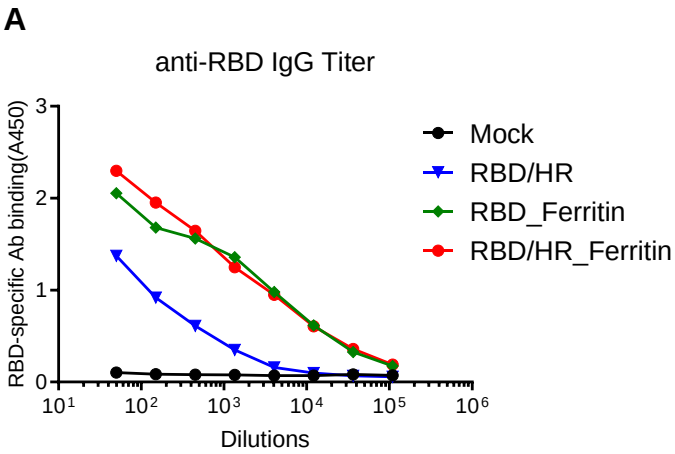
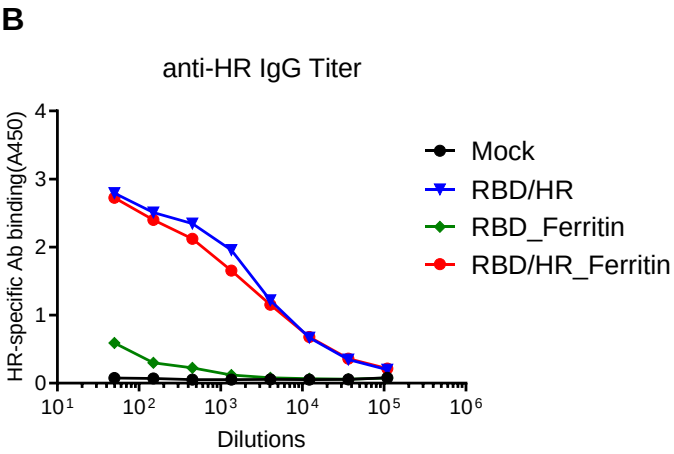
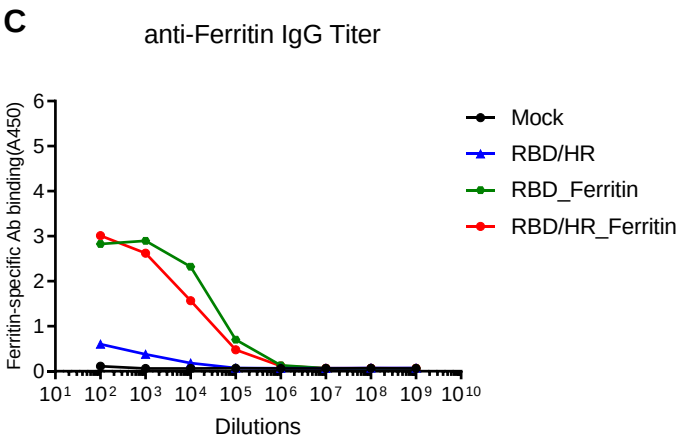

H
Subcapsular sinus macrophages gates
1.10
29.9
4.62
60.3
5.26
SSC-A
CD11b
CD169
F4/80
66.2
B220
Live/Death
DC gate
0.11
CD11c
MHCII

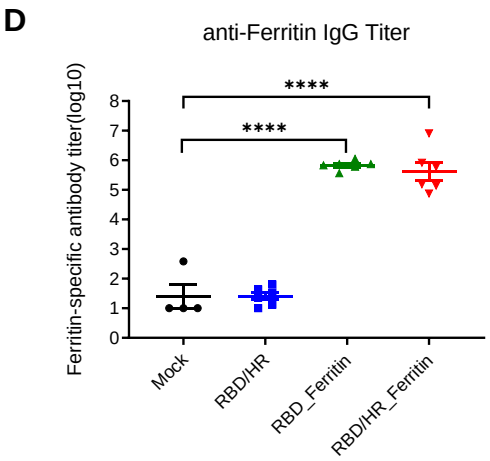
Figure S3. Related to Figure 2, Figure 3 and Figure 4. B and T Cell Immune Responses, Antigen Presentation and T/B Coordination

(A-B) Representative flow cytometry plots of RBD-specific IgG1 and IgG2b memory B cells. (C-D) The percentage of $CD4^+$ and $CD8^+$ central memory T cells which were isolated from spleen of 10 weeks-immunized mice were analyzed by flow cytometry (n=4). (E-F) The percentage of $CD62L^-CD44^+$ and the percentage of $CD69^+$ cells within $CD4^+$ T cells, which were isolated from spleen of 10-days immunized mice were analyzed by flow cytometry (n=3). (G) Gating strategy for activation marker staining and intracellular cytokine staining in $CD8^+$ and $CD4^+$ T cells. Activation marker were displayed. $CD8^+$ or $CD4^+$ T cells as indicated. Intracellular cytokine gating examples are spleen from a naïve mouse and a representative $CD8^+$ T cell cytokine response to antigens. (H) Gating strategy for DC and macrophage. Inguinal LNs were digested into single cells, $B220^-$ non-B cells were identified into two distinct populations: $CD11c^{hi}MHCII^+$DCs, and $CD11b^+F4/80^-CD169^+$ subscapular sinus macrophages. Experiments were conducted independently in triplicates. Data represented as mean ± SEM. Adjusted p-Values were calculated by one-way ANOVA with Tukey's multiple comparisons test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.


A
anti-RBD IgG Titer
RBD-specific Ab binding(A450)
Dilutions
Mock
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin


B
anti-HR IgG Titer
HR-specific Ab binding(A450)
Dilutions
Mock
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin


C
anti-Ferritin IgG Titer
Ferritin-specific Ab binding(A450)
Dilutions
Mock
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin


D
anti-Ferritin IgG Titer
Ferritin-specific antibody titer(log10)
****
****
Mock
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin

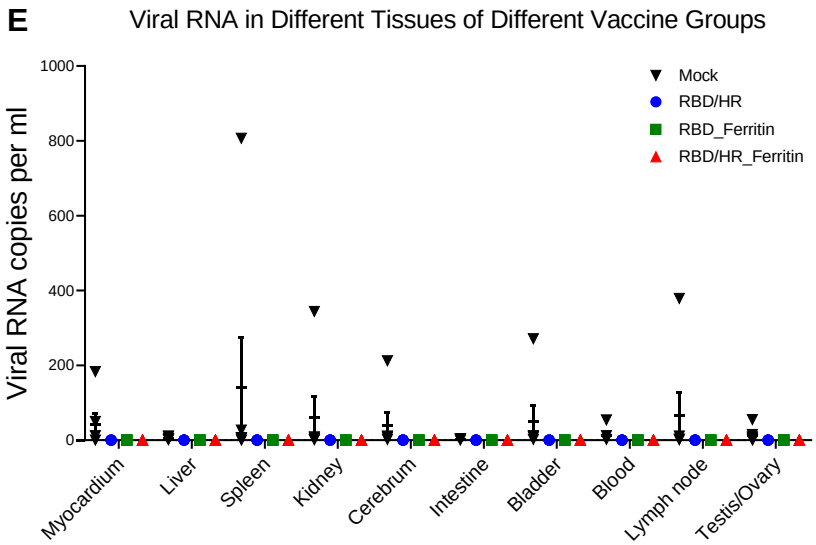

E
Viral RNA in Different Tissues of Different Vaccine Groups
Viral RNA copies per ml
Mock
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
Myocardium
Liver
Spleen
Kidney
Cerebrum
Intestine
Bladder
Blood
Lymph node
Testis/Ovary

Figure S4. Related to Figure 6. Protection of Nanoparticle Vaccines against SARS-CoV-2 in hACE2 Mice

(A-B) SARS-CoV-2 RBD- and HR-specific IgG titers of immunized hACE2 mice at week 6 were detected by ELISA. IgG antibody titers of serum were determined by serial dilution. (C-D) *H. pylori.* Ferritin-specific IgG titers of immunized hACE2 mice at week 6 were detected by ELISA and further determined by serial dilutions. The titers were represented as the reciprocal of the endpoint serum dilution. (E) Viral RNA copies in different tissues of each mice were determined by qRT-PCR and plotted as copies per ml. Experiments were conducted independently in triplicates. Data represented as mean ± SEM (n=4 for mock, n=6 for RBD/HR, RBD nanoparticle and RBD/HR nanoparticle groups). Adjusted p-Values were calculated by one-way ANOVA with Tukey's multiple comparisons test. ****$p < 0.0001$.

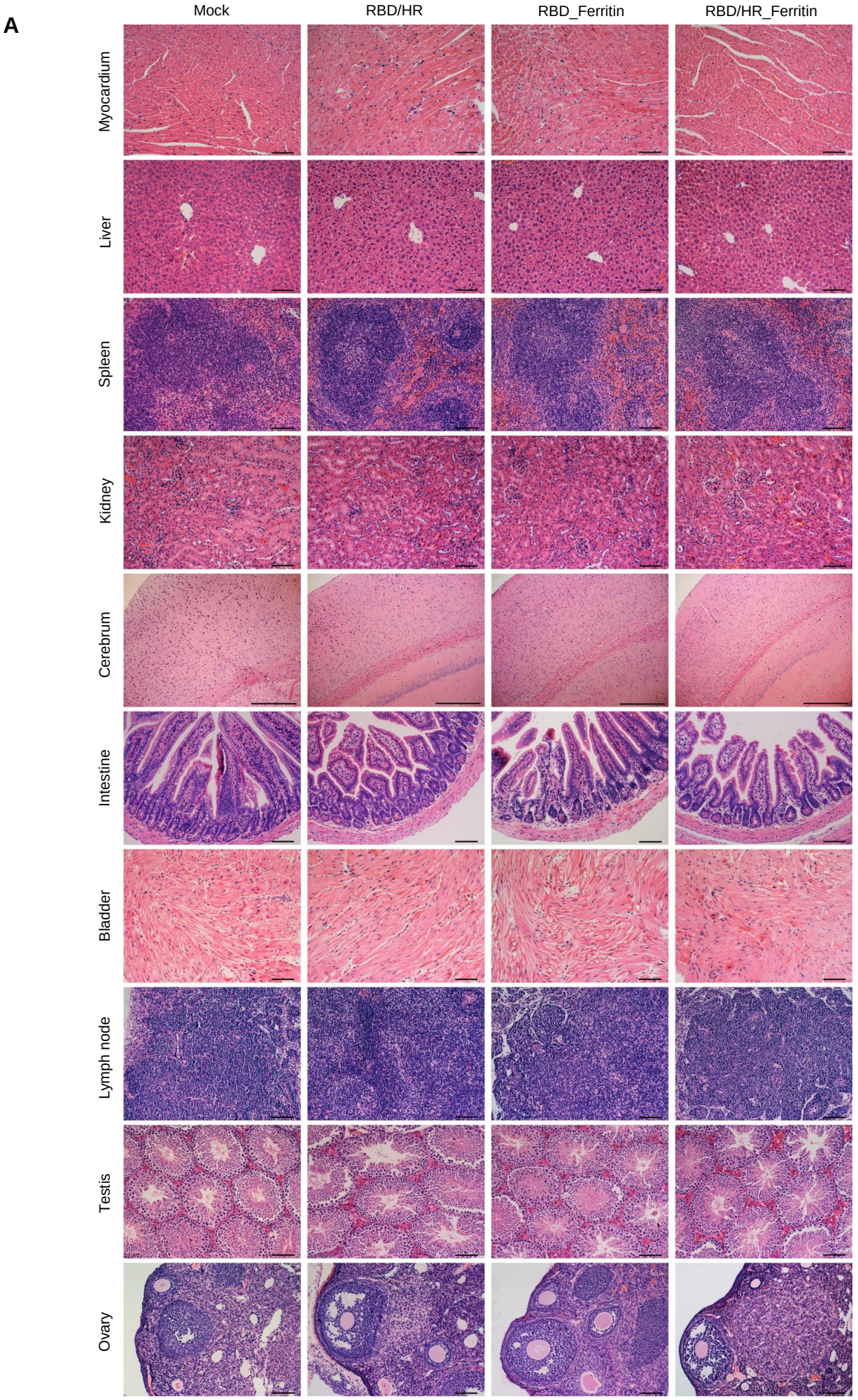

A
Mock
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
Myocardium
Liver
Spleen
Kidney
Cerebrum
Intestine
Bladder
Lymph node
Testis
Ovary

Figure S5. Related to Figure 6. Histopathological Analysis of Different Tissues of Vaccinated Mice

(A) HE staining of different tissues of vaccinated mice. Tissues contained myocardium, liver, spleen, kidney, cerebrum, intestine, bladder, lymph node, and testis or ovary. Scale bars represented 20 μm.

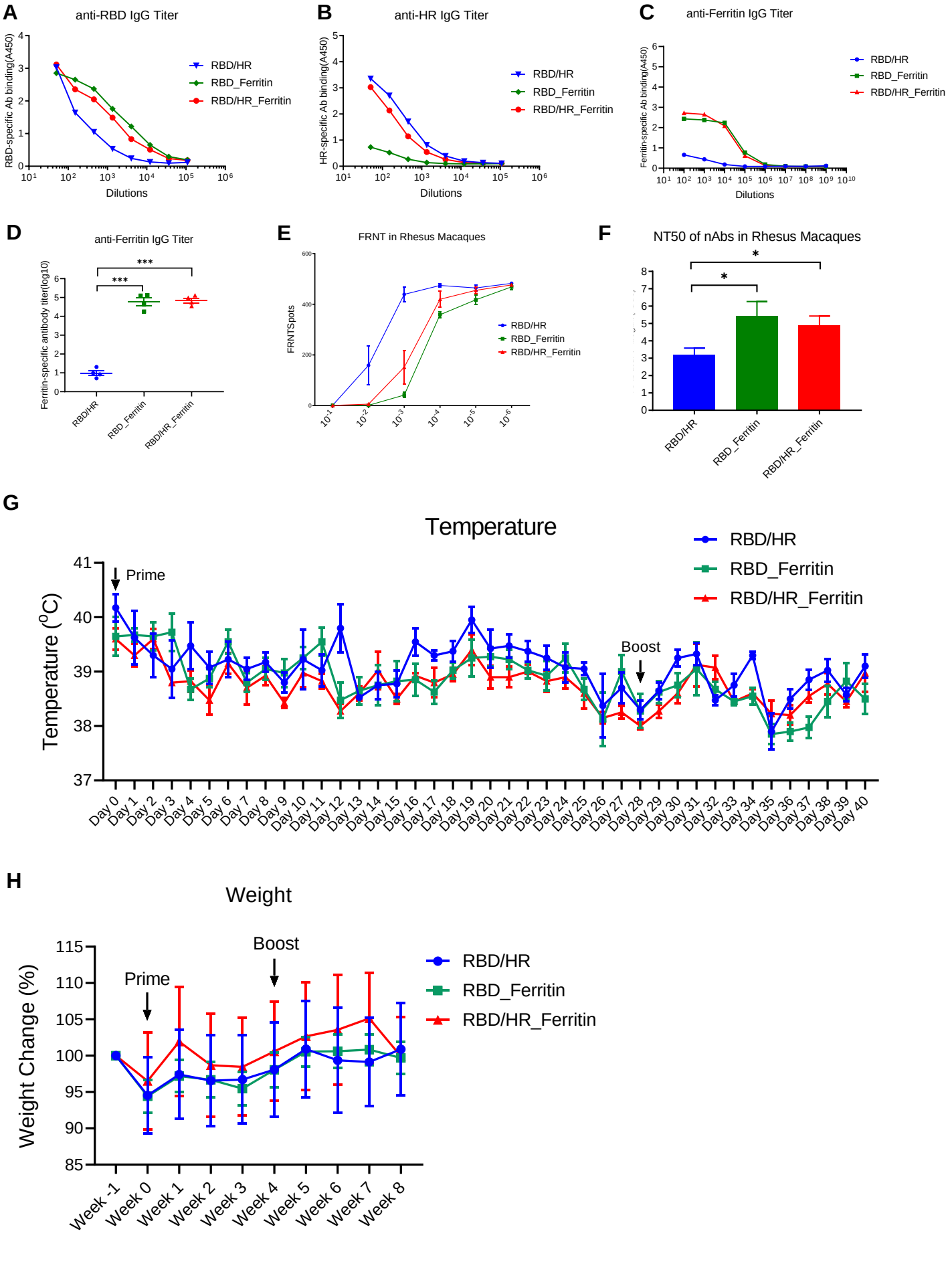

A
anti-RBD IgG Titer
RBD-specific Ab binding(A450)
Dilutions
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
B
anti-HR IgG Titer
HR-specific Ab binding(A450)
Dilutions
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
C
anti-Ferritin IgG Titer
Ferritin-specific Ab binding(A450)
Dilutions
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
D
anti-Ferritin IgG Titer
Ferritin-specific antibody titer(log10)
***
***
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
E
FRNT in Rhesus Macaques
FRNTSpots
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
F
NT50 of nAbs in Rhesus Macaques
*
*
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
G
Temperature
Temperature (°C)
Prime
Boost
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
H
Weight
Weight Change (%)
Prime
Boost
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin

Figure S6. Related to Figure 7. The Immunogenicity of Nanoparticle Vaccines in Rhesus Macaques

(A-B) SARS-CoV-2 RBD- and HR-specific IgG titers of immunized rhesus macaques at week 6 were detected by ELISA. IgG antibody titers of serum were determined by serial dilution. (C-D) Ferritin-specific IgG titers of immunized monkeys at week 6 were detected by ELISA and further determined by serial dilutions. The titers were represented as the reciprocal of the endpoint serum dilution. (E) Serial dilutions of serum were analyzed by FRNT for neutralizing authentic SARS-CoV-2. (F) Groups of serially diluted serum were detected for neutralizing antibody against pseudotyped SARS-CoV-2. Data represented NT50 of nAbs in each group. (G) Body temperatures of each monkey across the whole prime/boost vaccination period. (H) Body weights of each monkey were measured every week. Experiments were conducted independently in triplicates. Data represented as mean ± SEM (n=4). Adjusted p-Values were calculated by one-way ANOVA with Tukey's multiple comparisons test. *$p < 0.05$, ***$p < 0.001$.

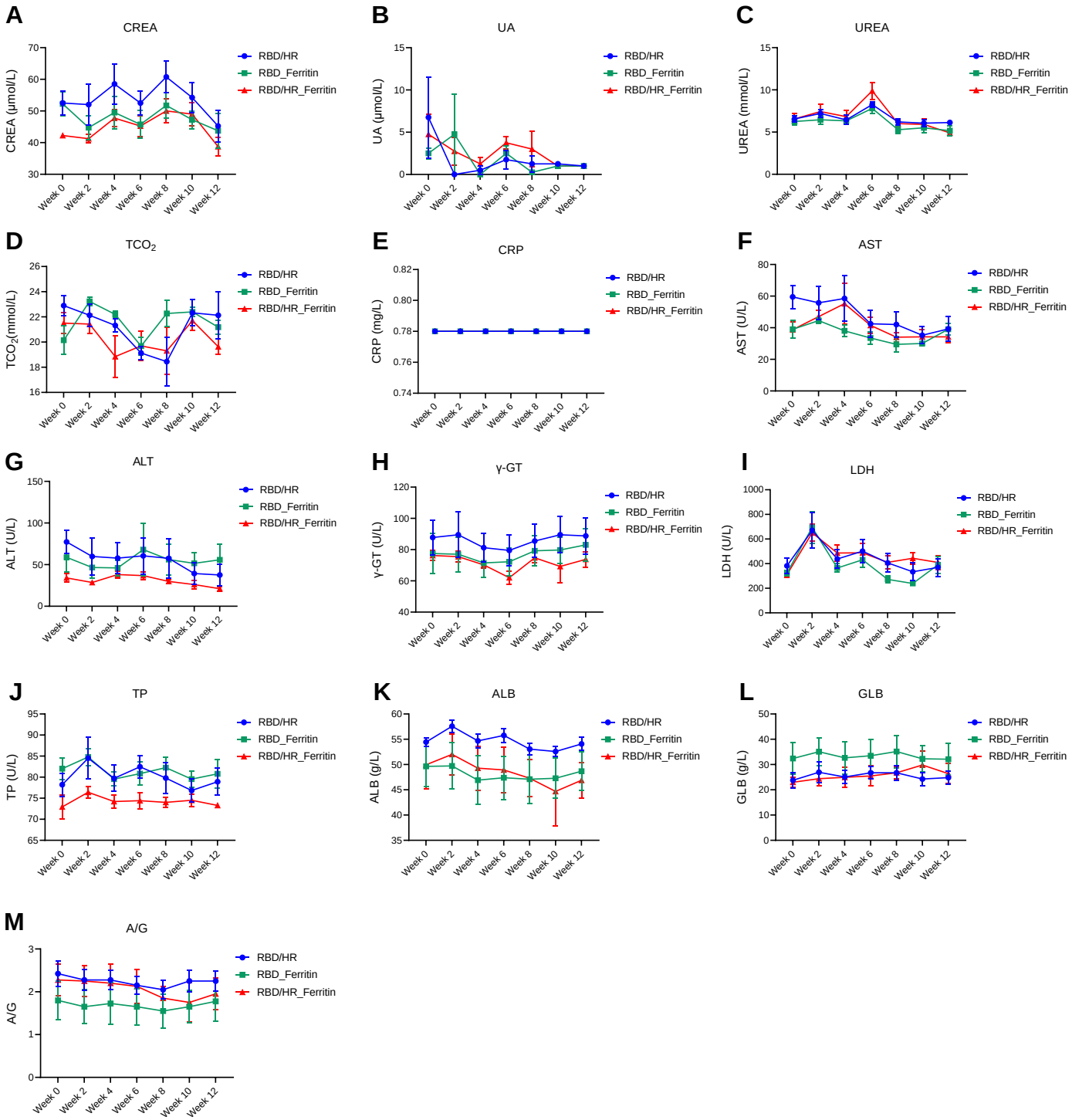

A
CREA
CREA (μmol/L)
B
UA
UA (μmol/L)
C
UREA
UREA (mmol/L)
D
TCO2
TCO2(mmol/L)
E
CRP
CRP (mg/L)
F
AST
AST (U/L)
G
ALT
ALT (U/L)
H
γ-GT
γ-GT (U/L)
I
LDH
LDH (U/L)
J
TP
TP (U/L)
K
ALB
ALB (g/L)
L
GLB
GLB (g/L)
M
A/G
A/G
RBD/HR
RBD_Ferritin
RBD/HR_Ferritin
Week 0
Week 2
Week 4
Week 6
Week 8
Week 10
Week 12

Figure S7. Related to Figure 7. Blood Biochemical Indexes of Each Vaccinated Monkey

(A-M) Blood biochemical indexes of each vaccinated monkey. The parameters contained the concentrations of creatinine (CREA), uric acid (UA), blood urea (UREA), total CO_2 (TCO_2), C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GT), lactate dehydrogenase (LDH), total protein (TP), albumin (ALB), globulin (GLB) and albumin/globulin ratio (A/G). Blood biochemical analysis was conducted every two weeks.