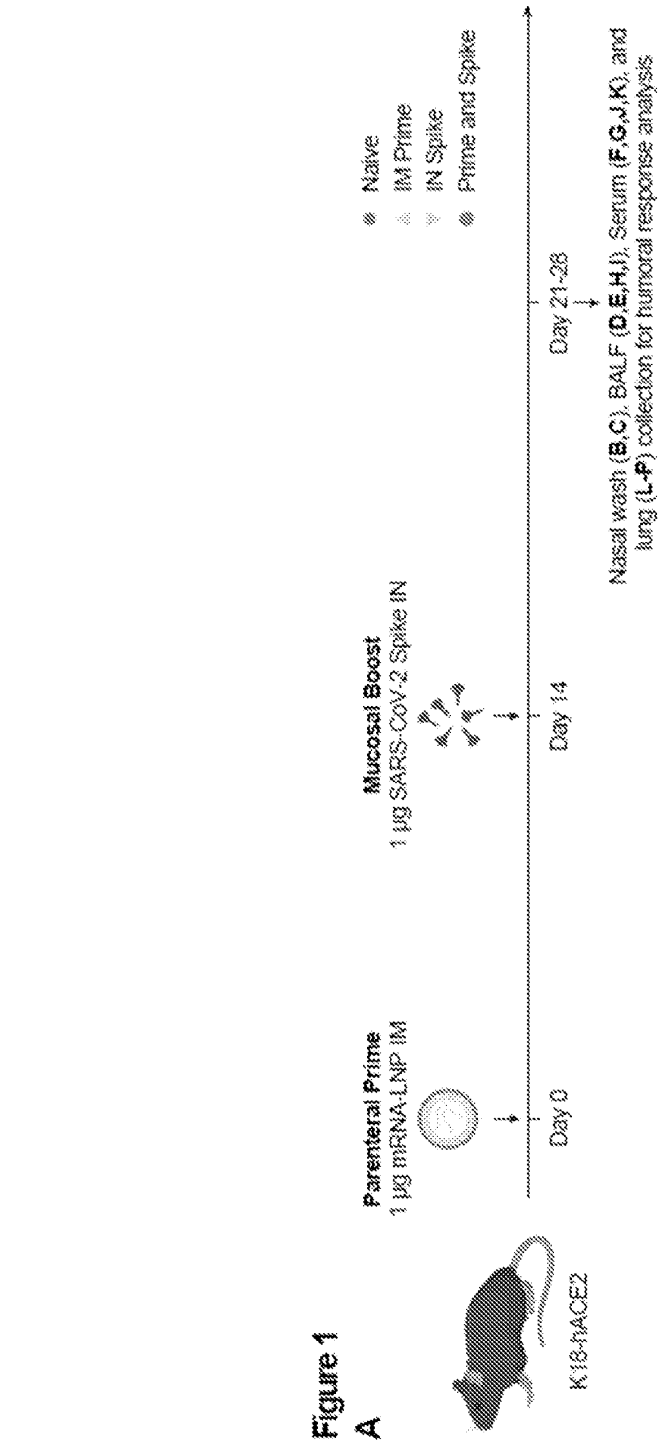
Figure 1
A
K18-hACE2
Parenteral Prime
1 µg mRNA-LNP IM
Mucosal Boost
1 µg SARS-CoV-2 Spike IN
Naive
IM Prime
IN Spike
Prime and Spike
Day 0
Day 14
Day 21-28
Nasal wash (B,C), BALF (D,E,H,I), Serum (F,G,J,K), and lung (L-P) collection for humoral response analysis

FIG. 1A

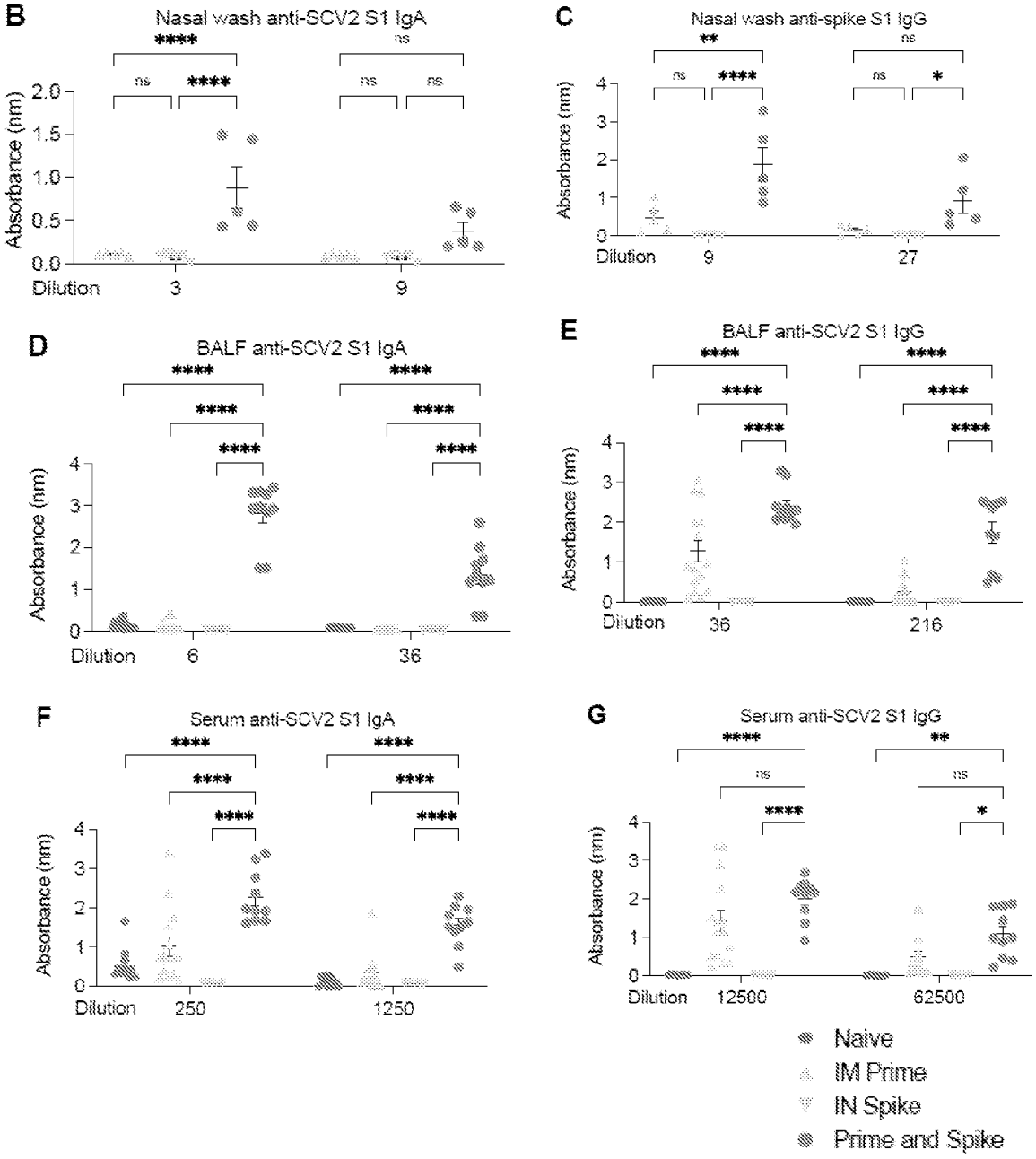
B
Nasal wash anti-SCV2 S1 IgA
Absorbance (nm)
Dilution
3
9
C
Nasal wash anti-spike S1 IgG
Absorbance (nm)
Dilution
9
27
D
BALF anti-SCV2 S1 IgA
Absorbance (nm)
Dilution
6
36
E
BALF anti-SCV2 S1 IgG
Absorbance (nm)
Dilution
36
216
F
Serum anti-SCV2 S1 IgA
Absorbance (nm)
Dilution
250
1250
G
Serum anti-SCV2 S1 IgG
Absorbance (nm)
Dilution
12500
62500
Naive
IM Prime
IN Spike
Prime and Spike

FIG. 1B

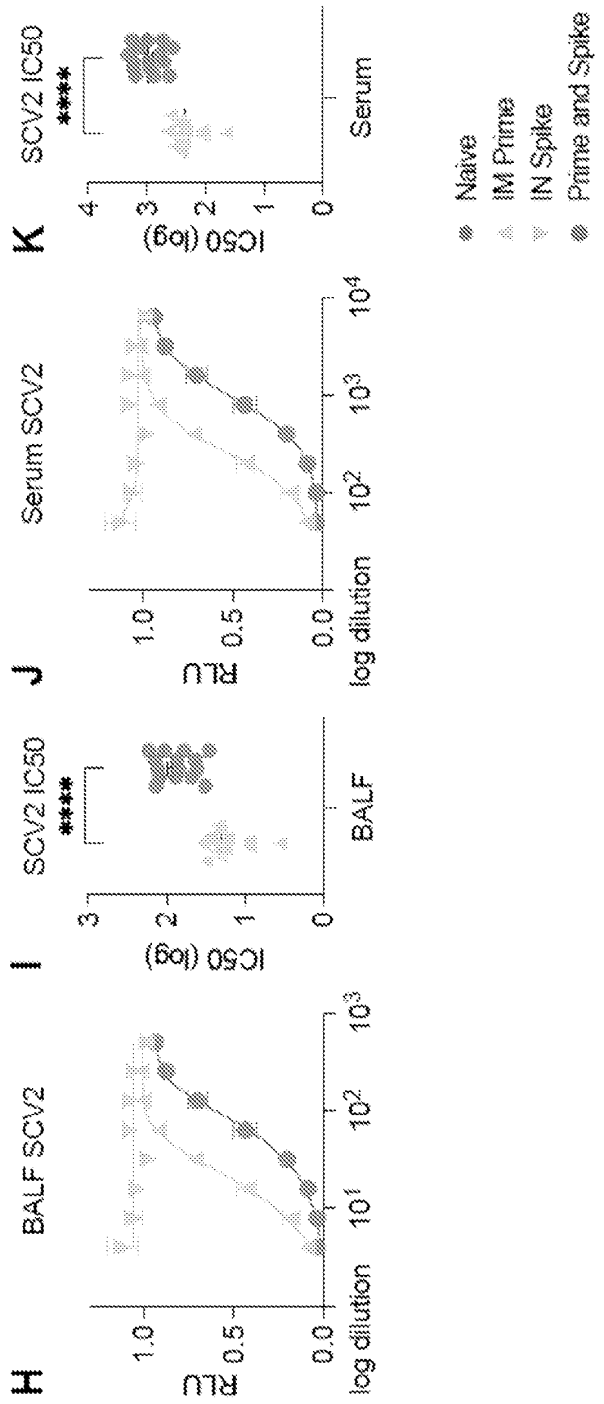
H
BALF SCV2
RLU
log dilution
I
SCV2 IC50
****
IC50 (log)
BALF
J
Serum SCV2
RLU
log dilution
K
SCV2 IC50
****
IC50 (log)
Serum
Naive
IM Prime
IN Spike
Prime and Spike

FIG. 1C

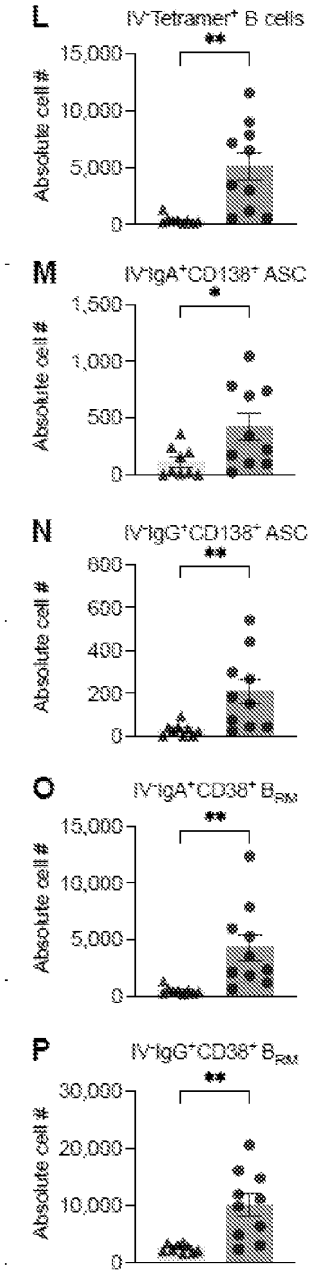
L
IV⁻Tetramer⁺ B cells
**
15,000
10,000
5,000
0
Absolute cell #
M
IV⁻IgA⁺CD138⁺ ASC
*
1,500
1,000
500
0
Absolute cell #
N
IV⁻IgG⁺CD138⁺ ASC
**
800
600
400
200
0
Absolute cell #
O
IV⁻IgA⁺CD38⁺ B$_{RM}$
**
15,000
10,000
5,000
0
Absolute cell #
P
IV⁻IgG⁺CD38⁺ B$_{RM}$
**
30,000
20,000
10,000
0
Absolute cell #

FIG. 1D

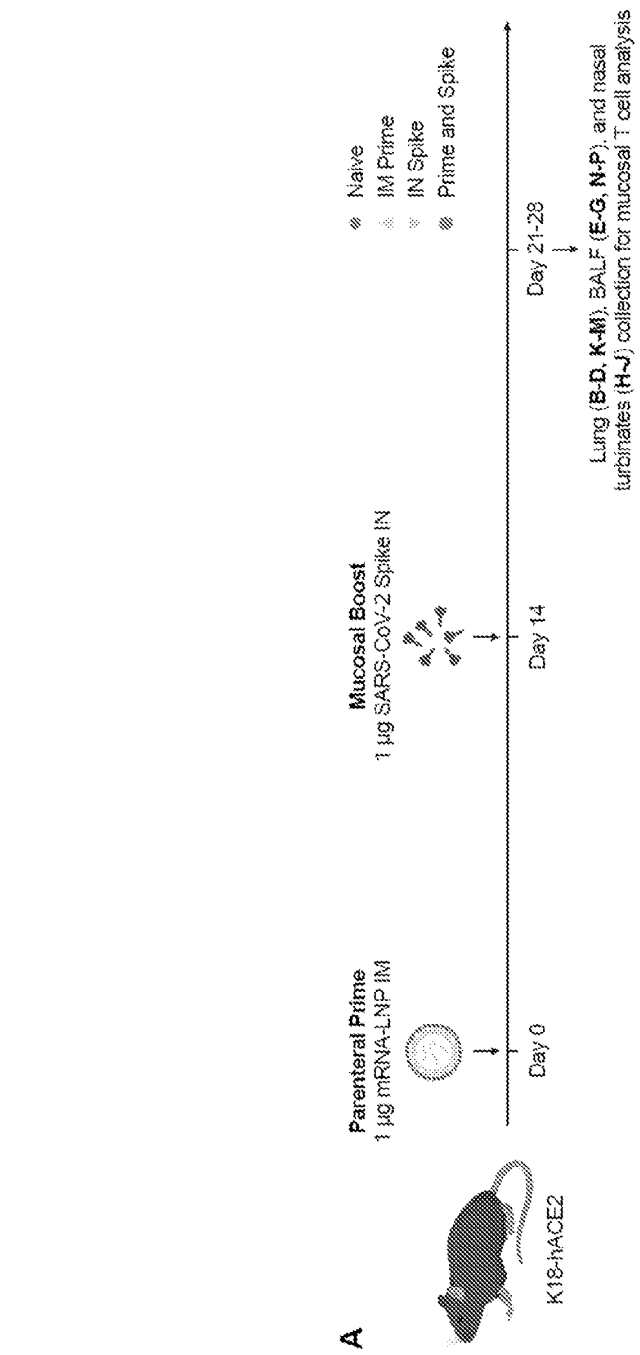
A
K18-hACE2
Parenteral Prime
1 µg mRNA-LNP IM
Mucosal Boost
1 µg SARS-CoV-2 Spike IN
Day 0
Day 14
Day 21-28
Naive
IM Prime
IN Spike
Prime and Spike
Lung (B-D, K-M), BALF (E-G, N-P), and nasal turbinates (H-J) collection for mucosal T cell analysis

FIG. 2A

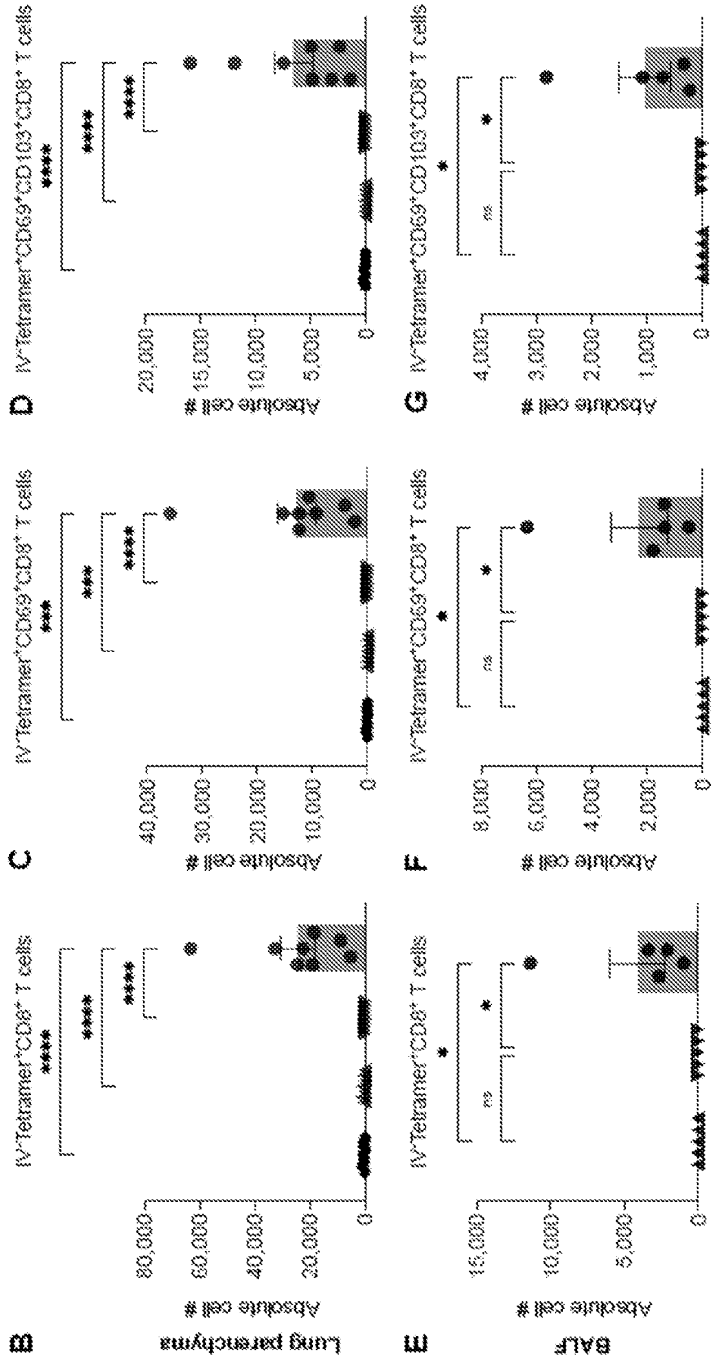
B
IV-Tetramer+CD8+ T cells
Lung parenchyma
Absolute cell #
80,000
60,000
40,000
20,000
0
C
IV-Tetramer+CD69+CD8+ T cells
Absolute cell #
40,000
30,000
20,000
10,000
0
D
IV-Tetramer+CD69+CD103+CD8+ T cells
Absolute cell #
20,000
15,000
10,000
5,000
0
E
IV-Tetramer+CD8+ T cells
BALF
Absolute cell #
15,000
10,000
5,000
0
F
IV-Tetramer+CD69+CD8+ T cells
Absolute cell #
8,000
6,000
4,000
2,000
0
G
IV-Tetramer+CD69+CD103+CD8+ T cells
Absolute cell #
4,000
3,000
2,000
1,000
0

FIG. 2B

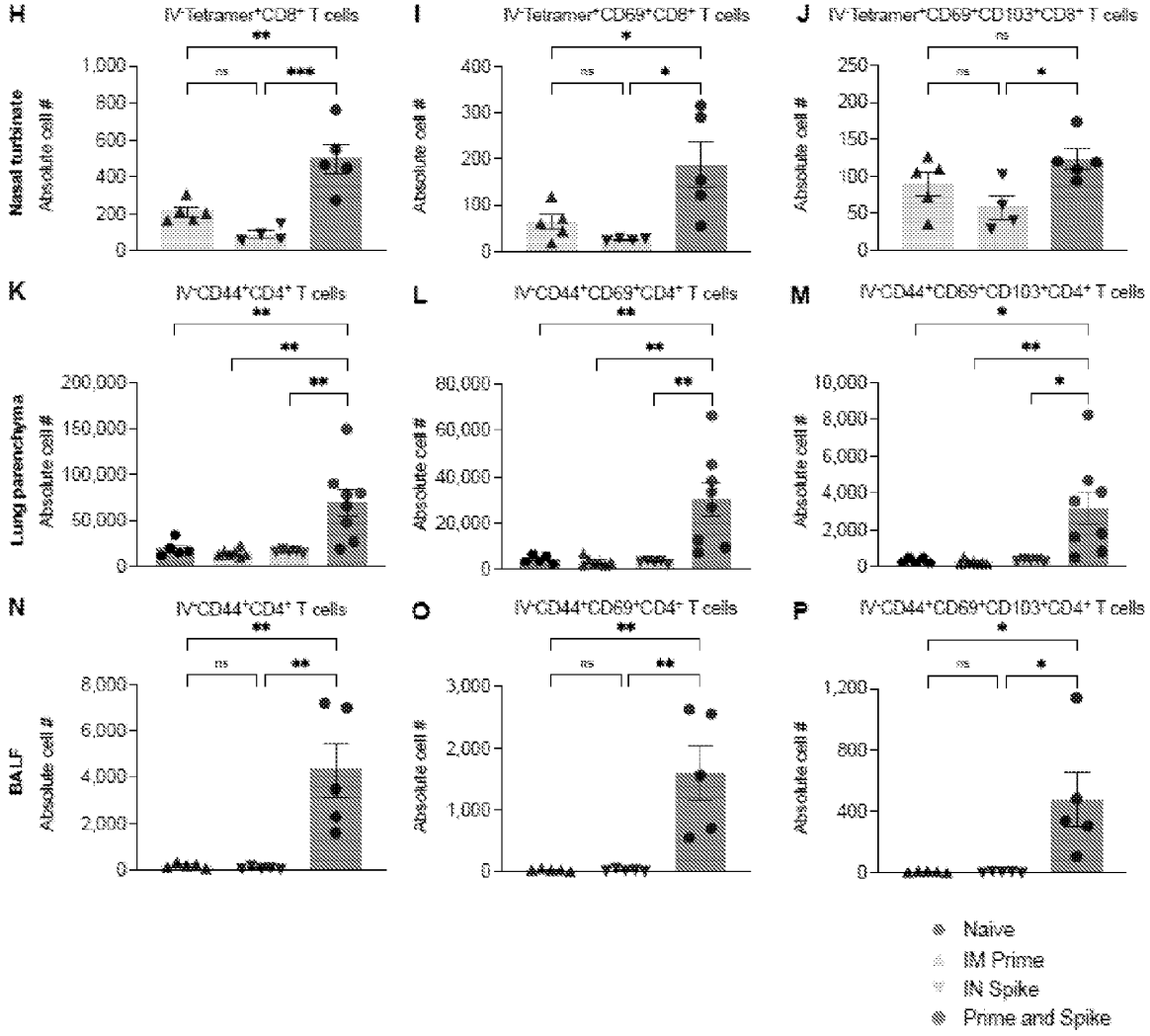
H
IV-Tetramer+CD8+ T cells
Nasal turbinate
Absolute cell #
I
IV-Tetramer+CD69+CD8+ T cells
J
IV-Tetramer+CD69+CD103+CD8+ T cells
K
IV-CD44+CD4+ T cells
Lung parenchyma
L
IV-CD44+CD69+CD4+ T cells
M
IV-CD44+CD69+CD103+CD4+ T cells
N
IV-CD44+CD4+ T cells
BALF
O
IV-CD44+CD69+CD4+ T cells
P
IV-CD44+CD69+CD103+CD4+ T cells
Naive
IM Prime
IN Spike
Prime and Spike

FIG. 2C

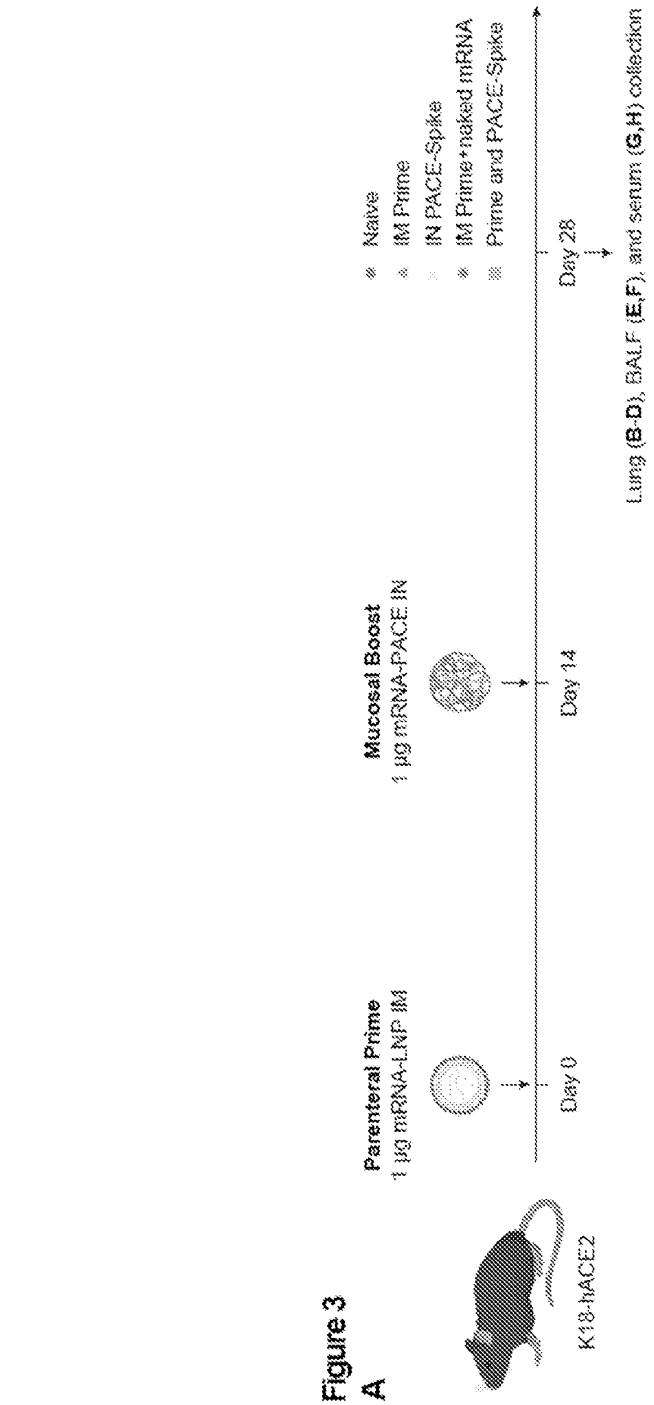
Figure 3
A
Parenteral Prime
1 µg mRNA-LNP IM
Mucosal Boost
1 µg mRNA-PACE IN
Day 0
Day 14
Day 28
K18-hACE2
Naive
IM Prime
IN PACE-Spike
IM Prime+naked mRNA
Prime and PACE-Spike
Lung (B-D), BALF (E,F), and serum (G,H) collection

FIG. 3A

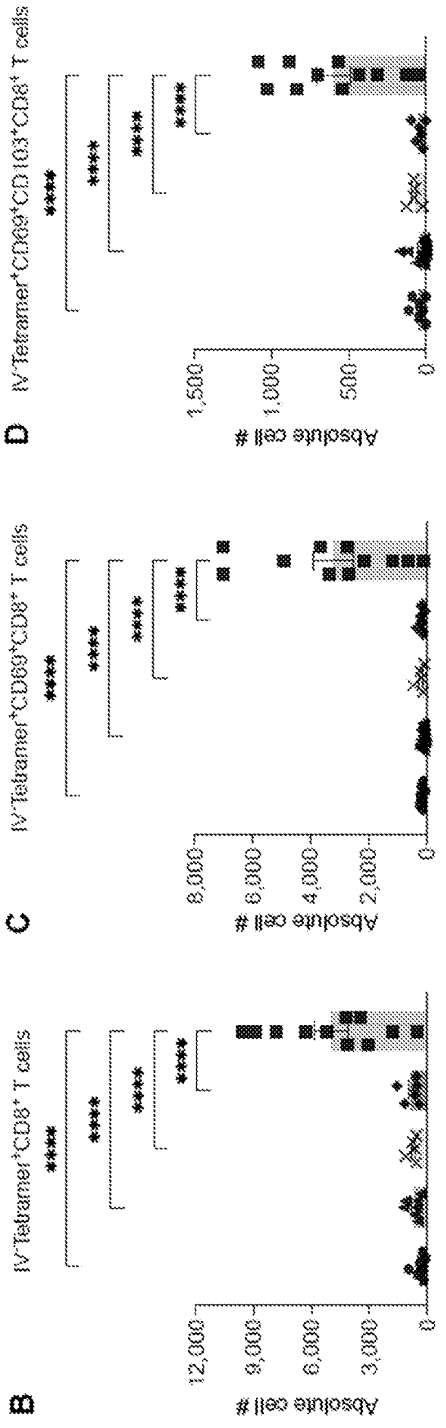
B
IV Tetramer+CD8+ T cells
Absolute cell #
12,000
9,000
6,000
3,000
0
****
****
****
****
C
IV Tetramer+CD69+CD8+ T cells
Absolute cell #
8,000
6,000
4,000
2,000
0
****
****
****
****
D
IV Tetramer+CD69+CD103+CD8+ T cells
Absolute cell #
1,500
1,000
500
0
****
****
****
****

FIG. 3B

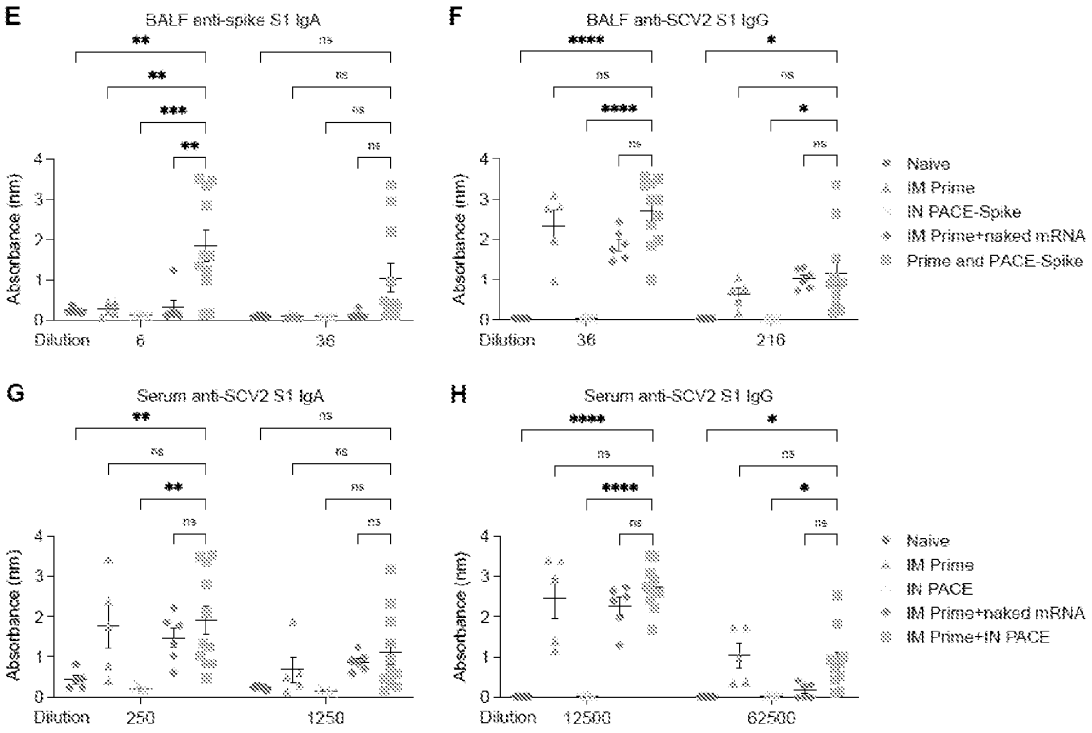
E
BALF anti-spike S1 IgA
Absorbance (nm)
Dilution
6
36
F
BALF anti-SCV2 S1 IgG
Absorbance (nm)
Dilution
36
216
Naive
IM Prime
IN PACE-Spike
IM Prime+naked mRNA
Prime and PACE-Spike
G
Serum anti-SCV2 S1 IgA
Absorbance (nm)
Dilution
250
1250
H
Serum anti-SCV2 S1 IgG
Absorbance (nm)
Dilution
12500
62500
Naive
IM Prime
IN PACE
IM Prime+naked mRNA
IM Prime+IN PACE

FIG. 3C

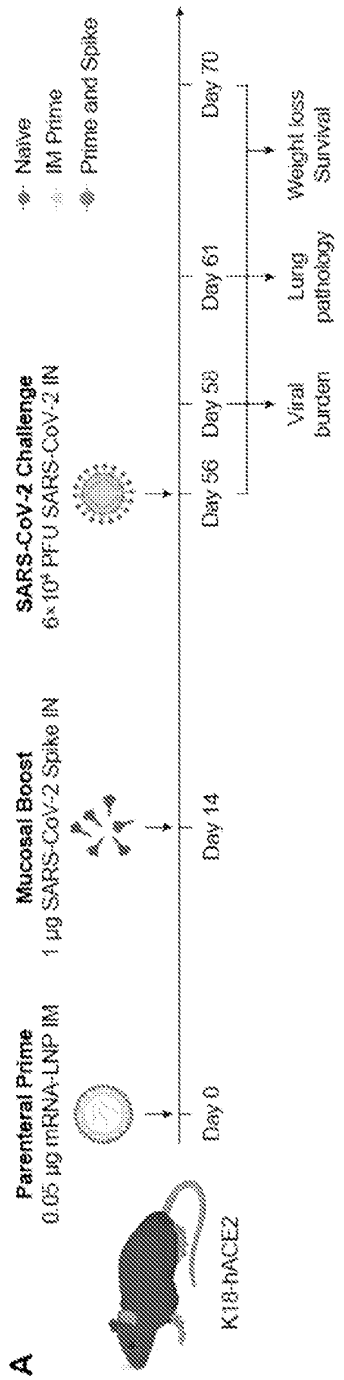
A
K18-hACE2
Parenteral Prime
0.05 µg mRNA-LNP IM
Mucosal Boost
1 µg SARS-CoV-2 Spike IN
SARS-CoV-2 Challenge
6×10⁵ PFU SARS-CoV-2 IN
Naive
IM Prime
Prime and Spike
Day 0
Day 14
Day 56
Day 58
Day 61
Day 70
Viral burden
Lung pathology
Weight loss
Survival

FIG. 4A

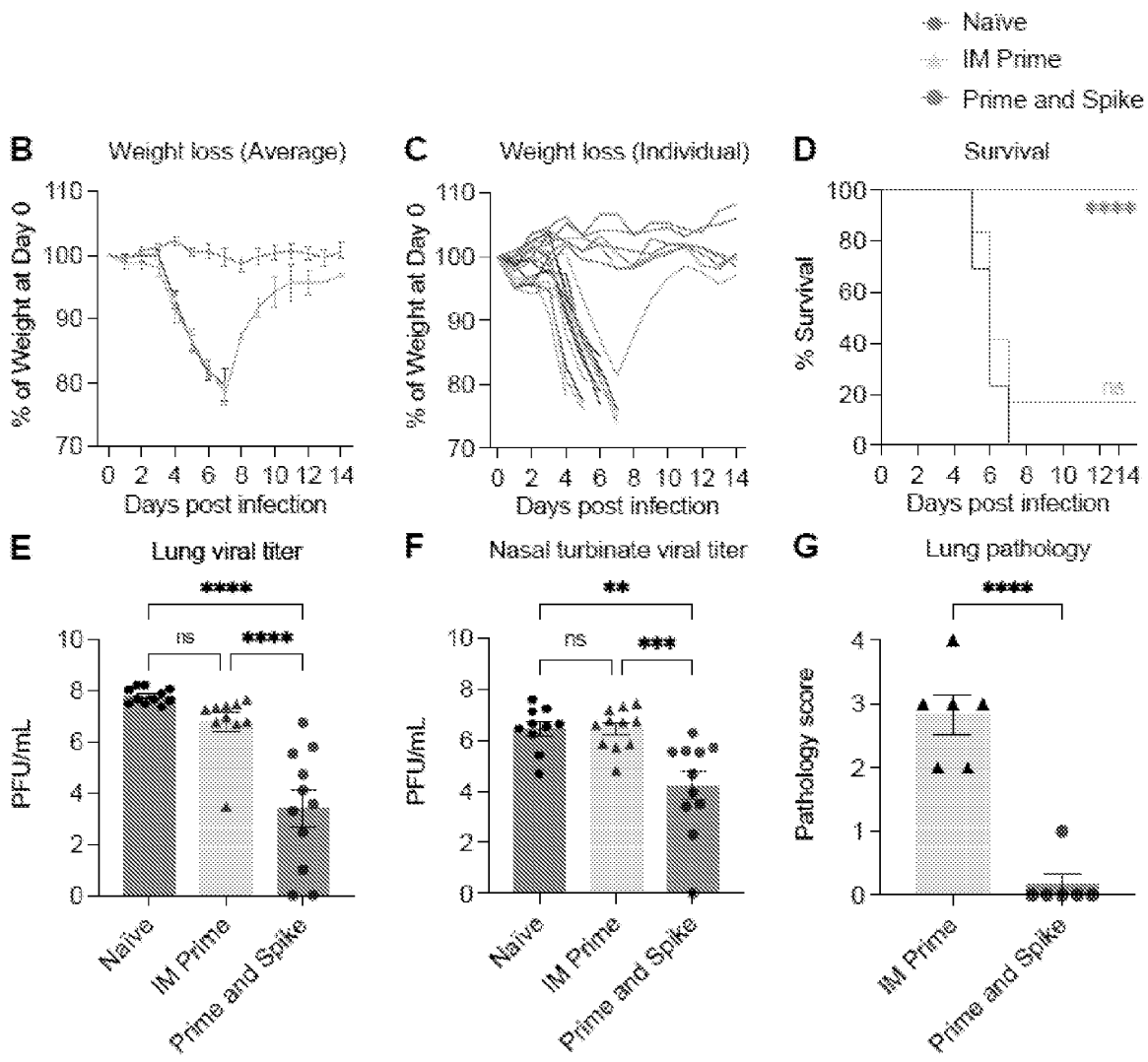
Naive
IM Prime
Prime and Spike
B
Weight loss (Average)
% of Weight at Day 0
Days post infection
C
Weight loss (Individual)
% of Weight at Day 0
Days post infection
D
Survival
% Survival
ns
Days post infection
E
Lung viral titer
PFU/mL
ns
Naive
IM Prime
Prime and Spike
F
Nasal turbinate viral titer
PFU/mL
ns
Naive
IM Prime
Prime and Spike
G
Lung pathology
Pathology score
IM Prime
Prime and Spike

FIG. 4B

H

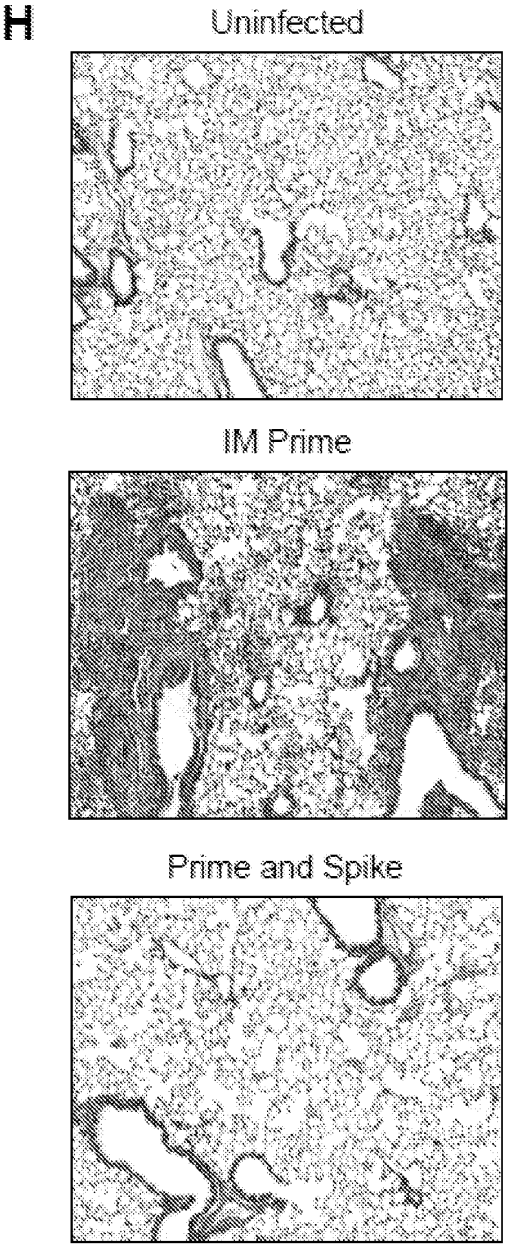

FIG. 4C

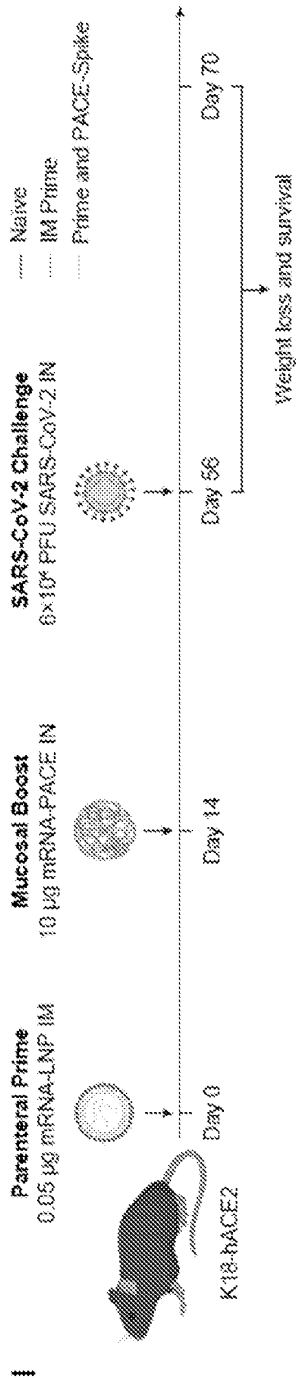
Parenteral Prime
0.05 µg mRNA-LNP IM
Mucosal Boost
10 µg mRNA-PACE IN
SARS-CoV-2 Challenge
6×10⁴ PFU SARS-CoV-2 IN
Naive
IM Prime
Prime and PACE-Spike
Day 0
Day 14
Day 56
Day 70
Weight loss and survival
K18-hACE2

FIG. 4D

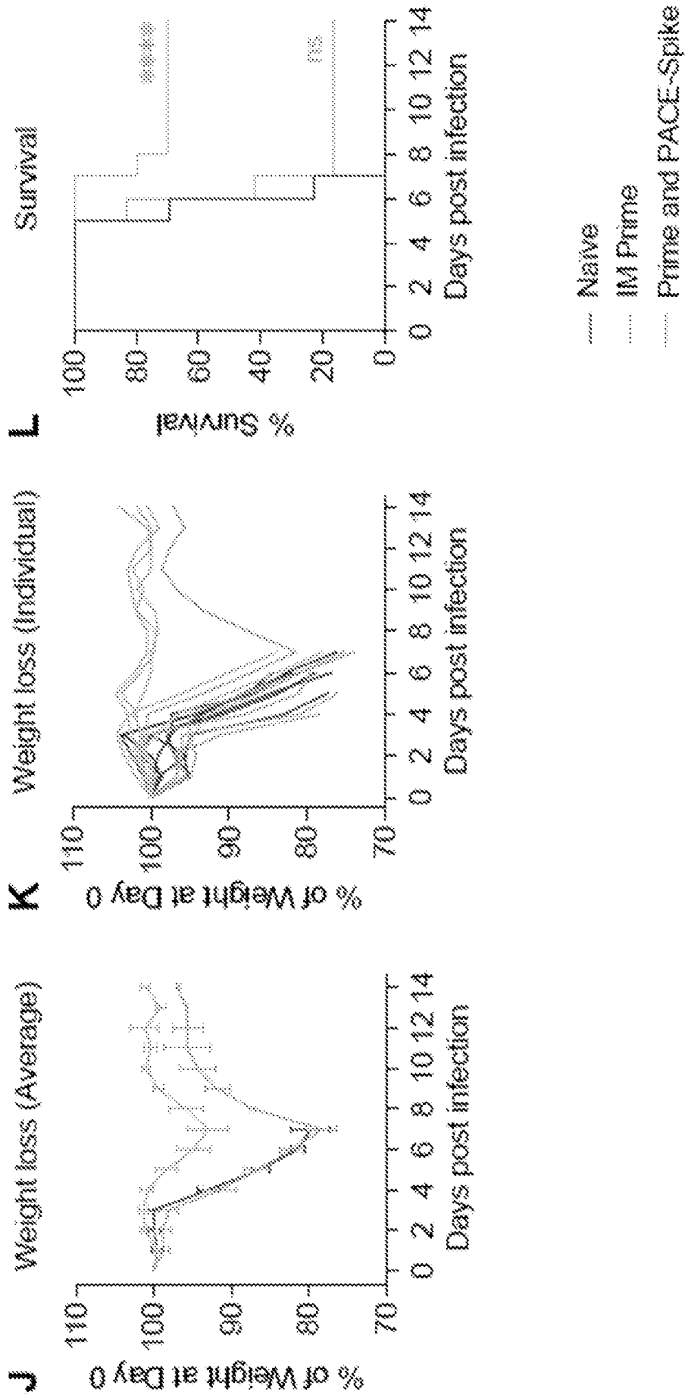
J
Weight loss (Average)
% of Weight at Day 0
110
100
90
80
70
0 2 4 6 8 10 12 14
Days post infection
K
Weight loss (Individual)
% of Weight at Day 0
110
100
90
80
70
0 2 4 6 8 10 12 14
Days post infection
L
Survival
% Survival
100
80
60
40
20
0
0 2 4 6 8 10 12 14
Days post infection
ns
****
Naive
IM Prime
Prime and PACE-Spike

FIG. 4E

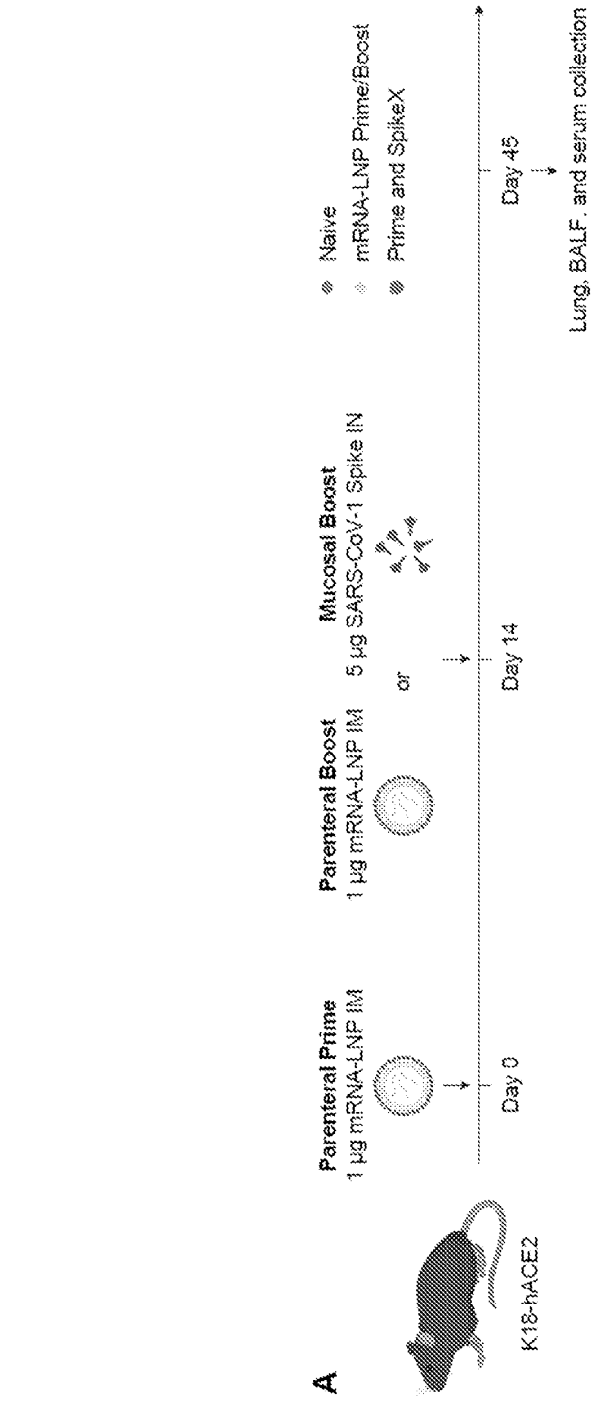
A
K18-hACE2
Parenteral Prime
1 µg mRNA-LNP IM
Day 0
Parenteral Boost
1 µg mRNA-LNP IM
or
Day 14
Mucosal Boost
5 µg SARS-CoV-1 Spike IN
Day 45
Lung, BALF, and serum collection
Naive
mRNA-LNP Prime/Boost
Prime and SpikeX

FIG. 5A

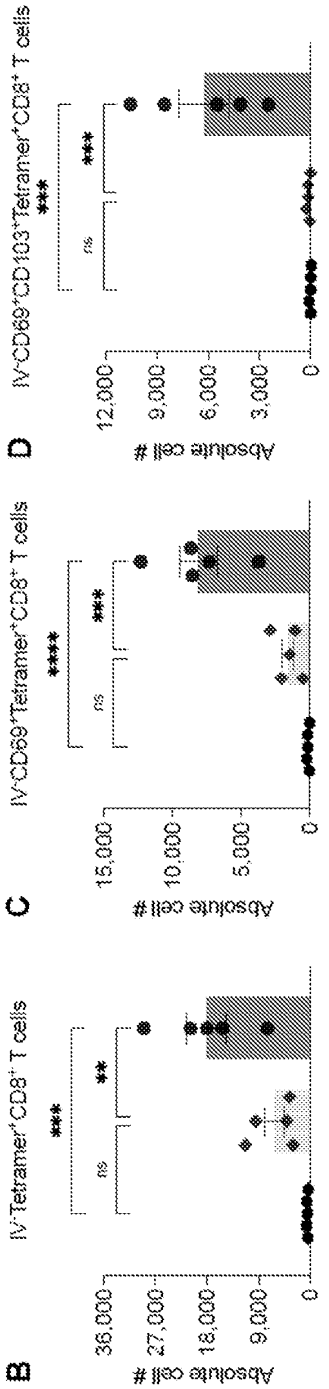
B
IV-Tetramer+CD8+ T cells
Absolute cell #
36,000
27,000
18,000
9,000
0
***
ns
**
C
IV-CD69+Tetramer+CD8+ T cells
Absolute cell #
15,000
10,000
5,000
0
****
ns
***
D
IV-CD69+CD103+Tetramer+CD8+ T cells
Absolute cell #
12,000
9,000
6,000
3,000
0
***
ns
***

FIG. 5B

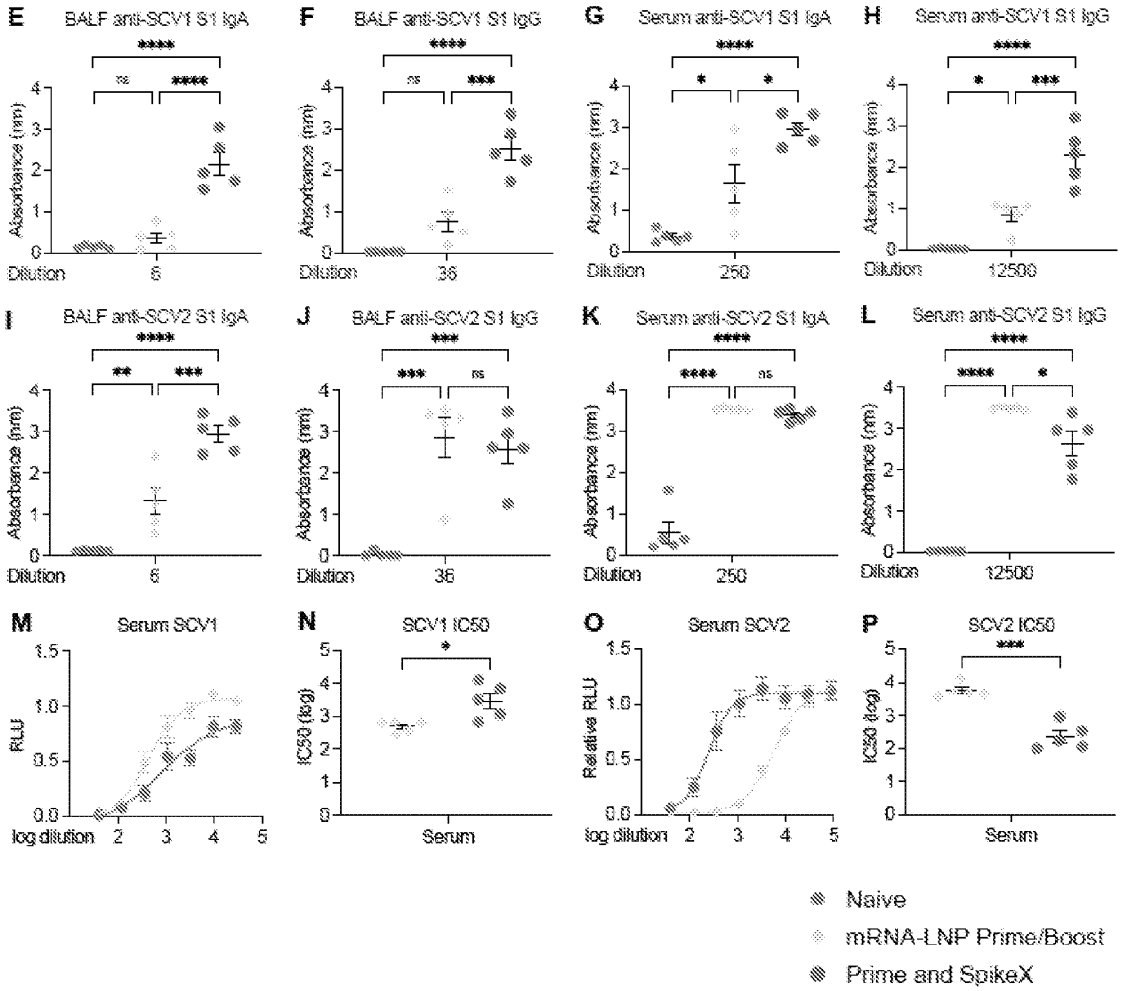
E
BALF anti-SCV1 S1 IgA
F
BALF anti-SCV1 S1 IgG
G
Serum anti-SCV1 S1 IgA
H
Serum anti-SCV1 S1 IgG
I
BALF anti-SCV2 S1 IgA
J
BALF anti-SCV2 S1 IgG
K
Serum anti-SCV2 S1 IgA
L
Serum anti-SCV2 S1 IgG
Absorbance (nm)
Dilution
6
36
250
12500
M
Serum SCV1
RLU
log dilution
N
SCV1 IC50
IC50 (log)
Serum
O
Serum SCV2
Relative RLU
P
SCV2 IC50
Naive
mRNA-LNP Prime/Boost
Prime and SpikeX

FIG. 5C

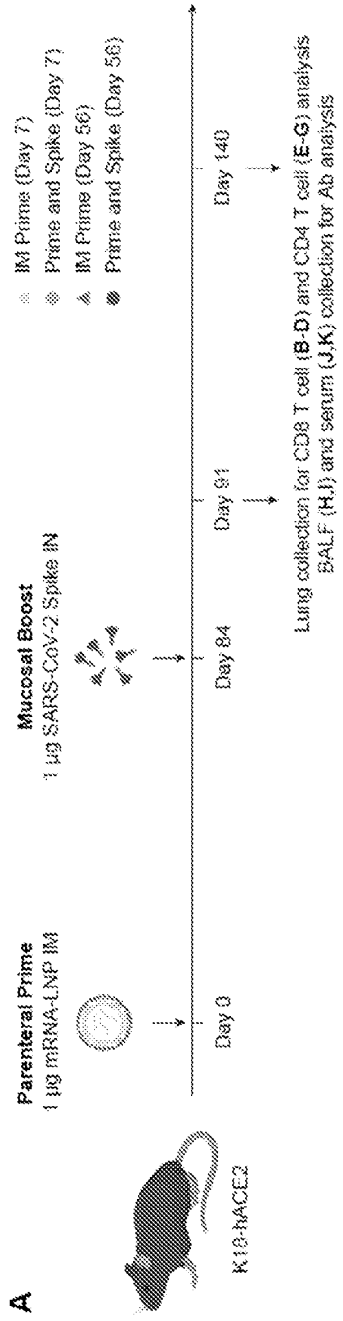
A
Parenteral Prime
1 µg mRNA-LNP IM
Mucosal Boost
1 µg SARS-CoV-2 Spike IN
IM Prime (Day 7)
Prime and Spike (Day 7)
IM Prime (Day 56)
Prime and Spike (Day 56)
K18-hACE2
Day 0
Day 84
Day 91
Day 140
Lung collection for CD8 T cell (B-D) and CD4 T cell (E-G) analysis
BALF (H,I) and serum (J,K) collection for Ab analysis

FIG. 6A

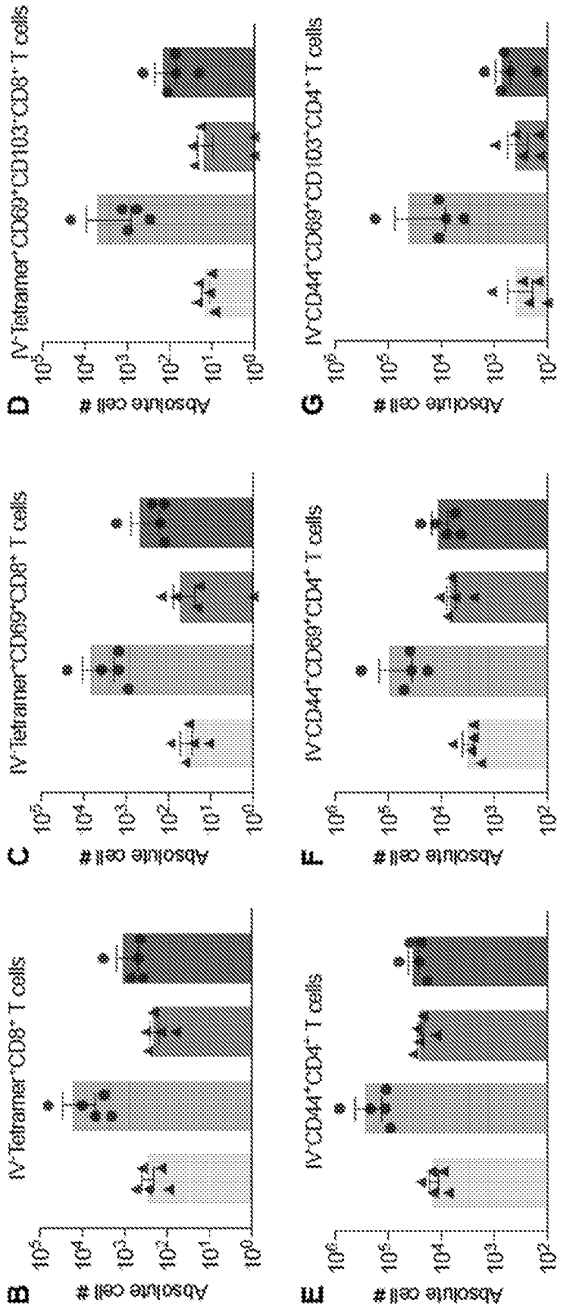
B
IV Tetramer+CD8+ T cells
Absolute cell #
C
IV Tetramer+CD69+CD8+ T cells
Absolute cell #
D
IV Tetramer+CD69+CD103+CD8+ T cells
Absolute cell #
E
IV CD44+CD4+ T cells
Absolute cell #
F
IV CD44+CD69+CD4+ T cells
Absolute cell #
G
IV CD44+CD69+CD103+CD4+ T cells
Absolute cell #

FIG. 6B

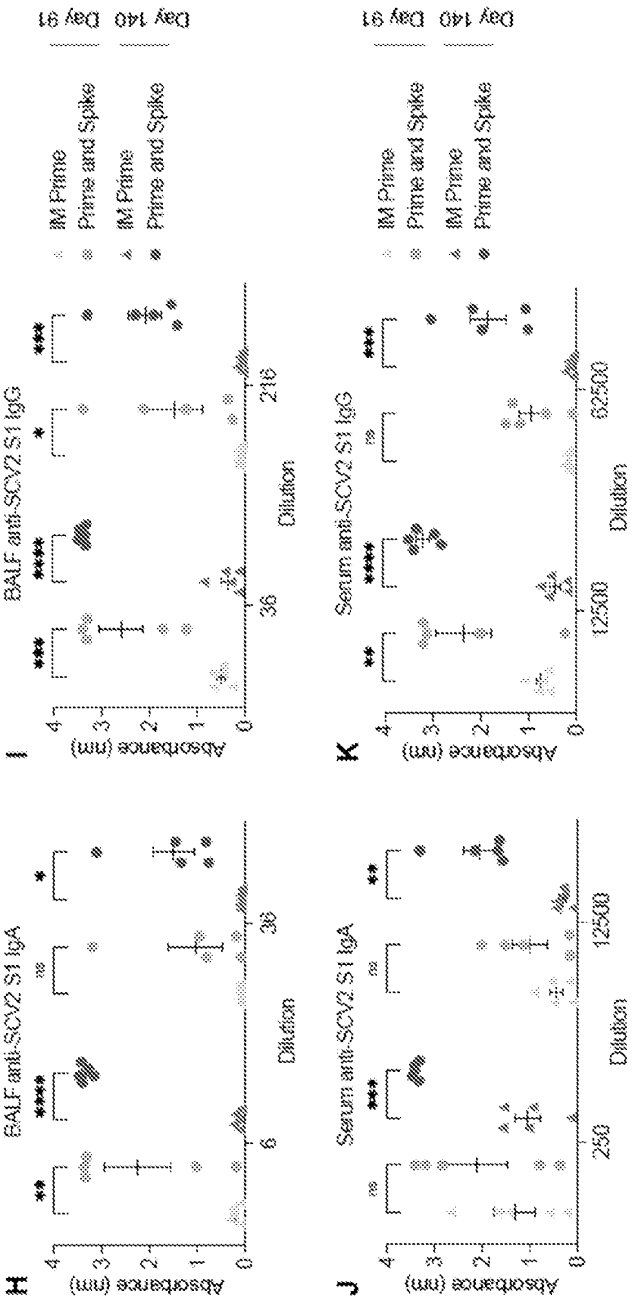
H
BALF anti-SCV2 S1 IgA
Absorbance (nm)
Dilution
J
Serum anti-SCV2 S1 IgA
Absorbance (nm)
Dilution
I
BALF anti-SCV2 S1 IgG
Absorbance (nm)
Dilution
K
Serum anti-SCV2 S1 IgG
Absorbance (nm)
Dilution
IM Prime
Prime and Spike
IM Prime
Prime and Spike
Day 91
Day 140

FIG. 6C

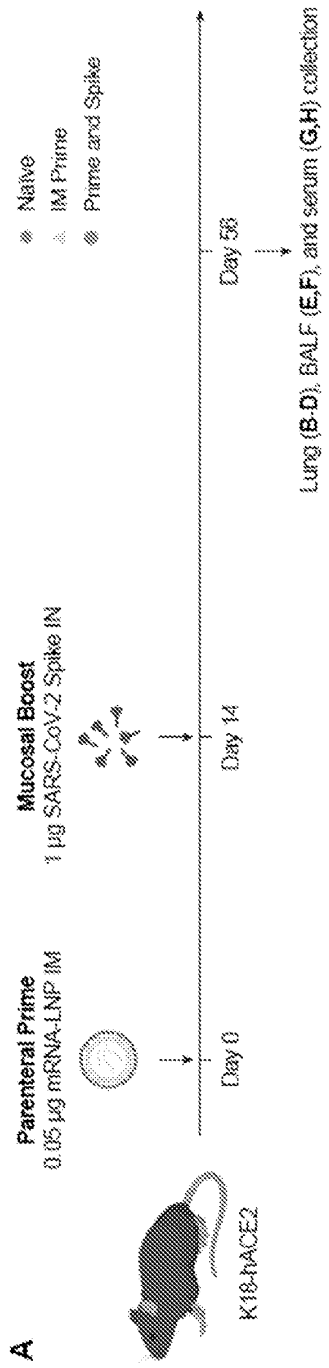
A
Parenteral Prime
0.05 µg mRNA-LNP IM
Mucosal Boost
1 µg SARS-CoV-2 Spike IN
Naive
IM Prime
Prime and Spike
Day 0
Day 14
Day 56
K18-hACE2
Lung (B-D), BALF (E,F), and serum (G,H) collection

FIG. 7A

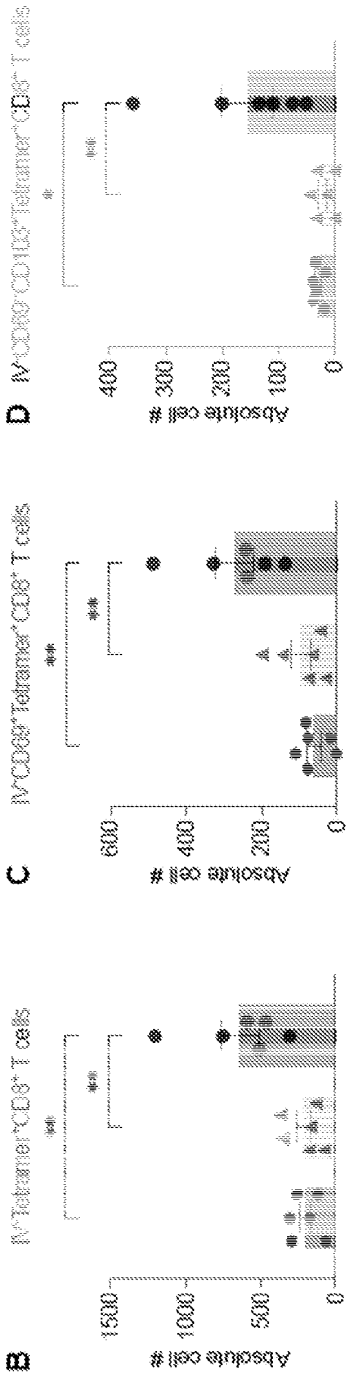
B
IV Tetramer+CD8+ T cells
Absolute cell #
1500
1000
500
0
C
IV CD69+ Tetramer+CD8+ T cells
Absolute cell #
600
400
200
0
D
IV CD69+ CD103+ Tetramer+ CD8+ T cells
Absolute cell #
400
300
200
100
0

FIG. 7B

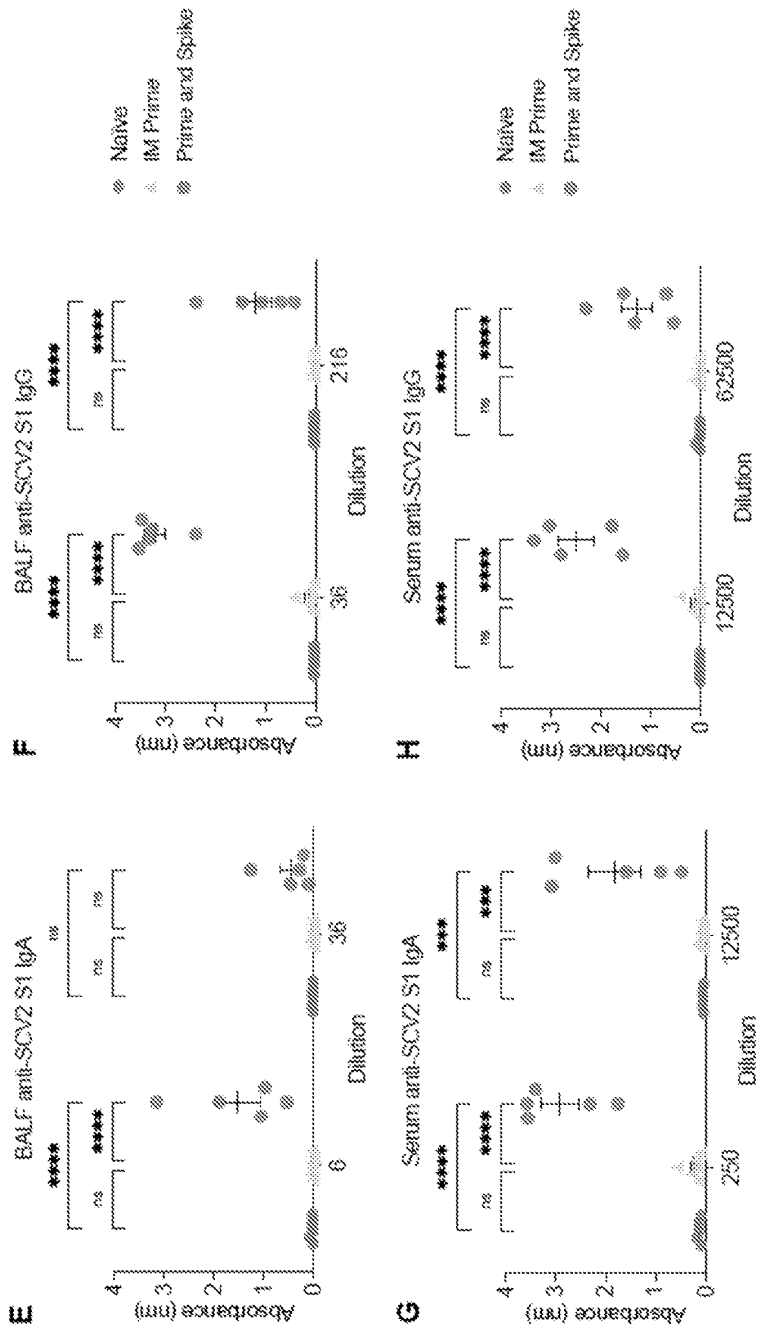
E
BALF anti-SCV2 S1 IgA
Absorbance (nm)
Dilution
0
36
F
BALF anti-SCV2 S1 IgG
Absorbance (nm)
Dilution
0
36
216
G
Serum anti-SCV2 S1 IgA
Absorbance (nm)
Dilution
250
12500
H
Serum anti-SCV2 S1 IgG
Absorbance (nm)
Dilution
12500
62500
Naive
IM Prime
Prime and Spike
ns
****
***

FIG. 7C

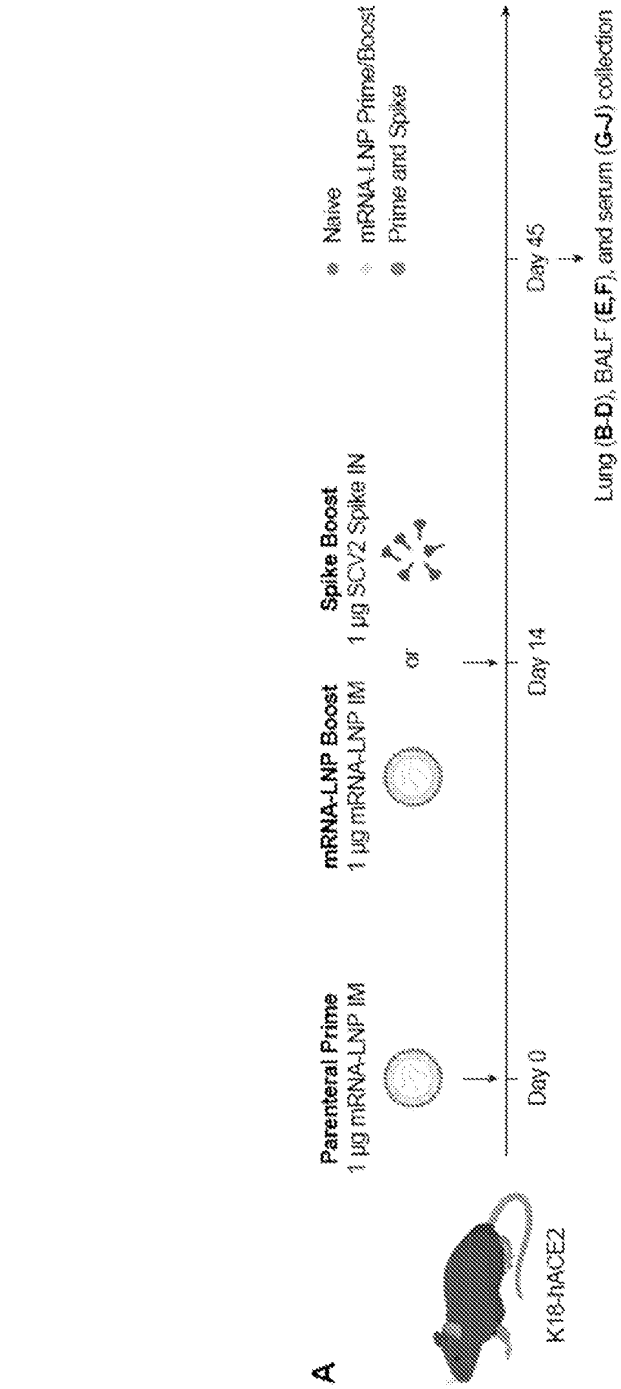
A
K18-hACE2
Parenteral Prime
1 µg mRNA-LNP IM
Day 0
mRNA-LNP Boost
1 µg mRNA-LNP IM
or
Spike Boost
1 µg SCV2 Spike IN
Day 14
Day 45
Lung (B-D), BALF (E,F), and serum (G-J) collection
Naive
mRNA-LNP Prime/Boost
Prime and Spike

FIG. 8A

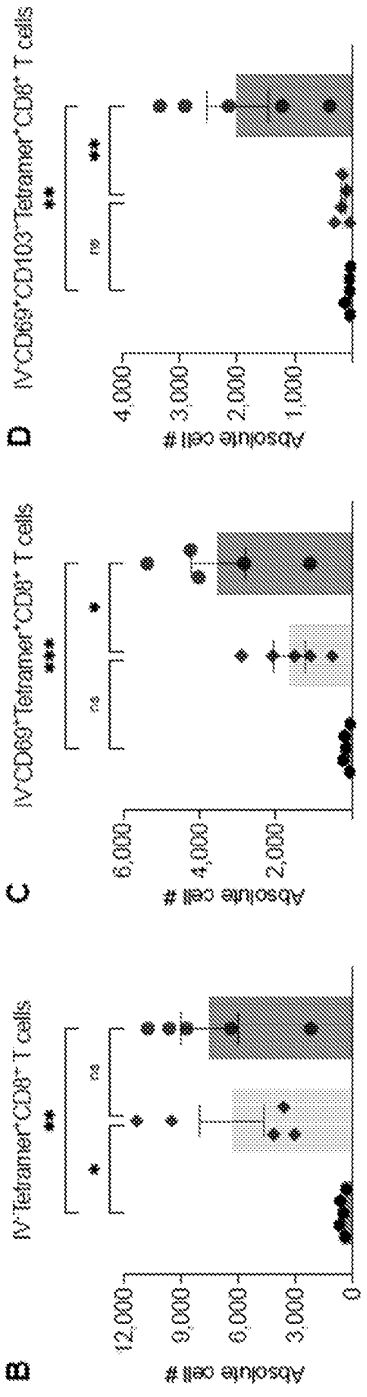
B
IV⁺Tetramer⁺CD8⁺ T cells
Absolute cell #
12,000
9,000
6,000
3,000
0
C
IV⁺CD69⁺Tetramer⁺CD8⁺ T cells
Absolute cell #
6,000
4,000
2,000
D
IV⁺CD69⁺CD103⁺Tetramer⁺CD8⁺ T cells
Absolute cell #
4,000
3,000
2,000
1,000

FIG. 8B

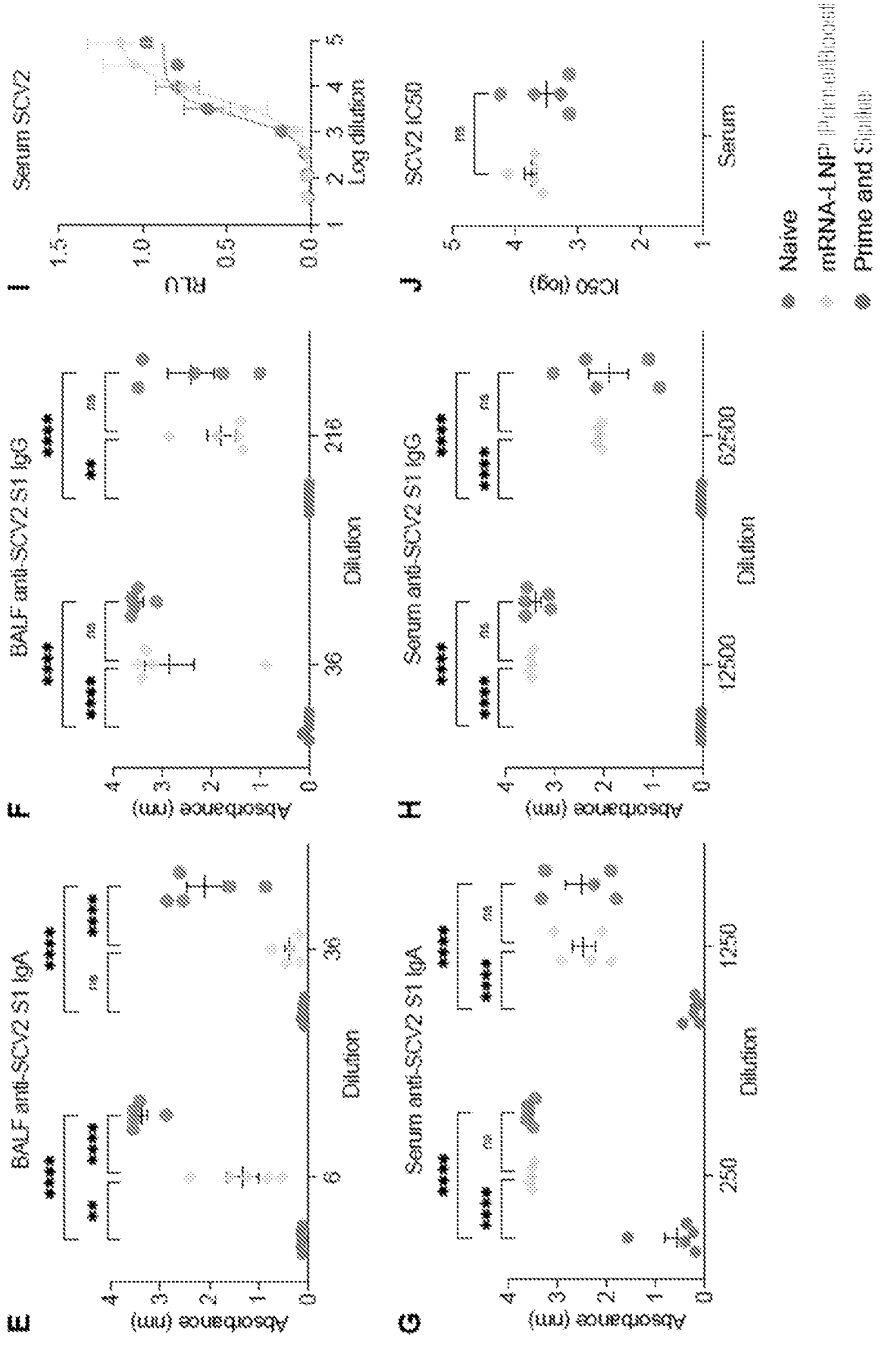
E
BALF anti-SCV2 S1 IgA
Absorbance (nm)
Dilution
F
BALF anti-SCV2 S1 IgG
Absorbance (nm)
Dilution
G
Serum anti-SCV2 S1 IgA
Absorbance (nm)
Dilution
H
Serum anti-SCV2 S1 IgG
Absorbance (nm)
Dilution
I
Serum SCV2
RLU
Log dilution
J
SCV2 IC50
IC50 (log)
Serum
Naive
mRNA-LNP Prime-eBoost
Prime and Spike

FIG. 8C

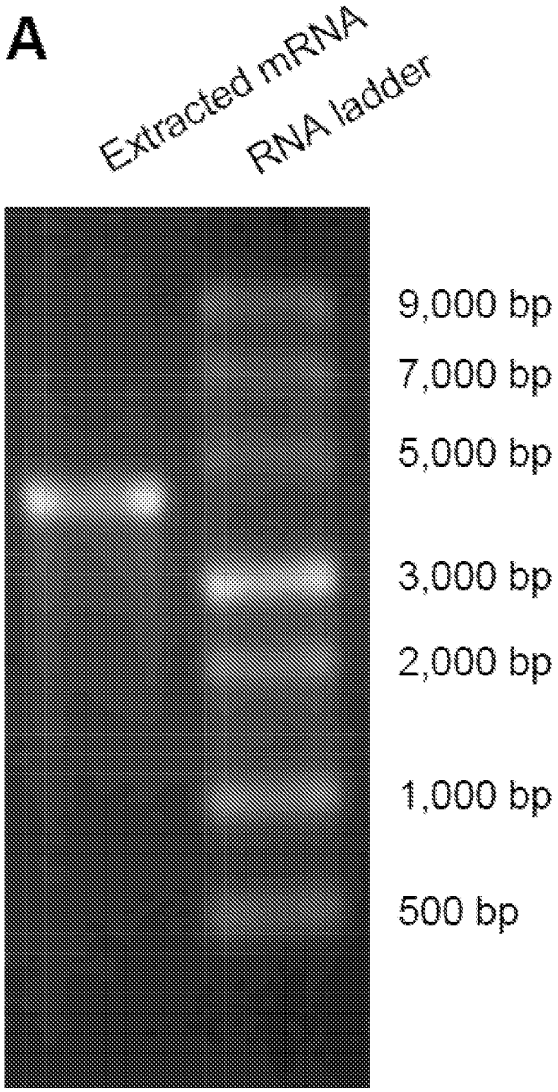
A
Extracted mRNA
RNA ladder
9,000 bp
7,000 bp
5,000 bp
3,000 bp
2,000 bp
1,000 bp
500 bp

FIG. 9

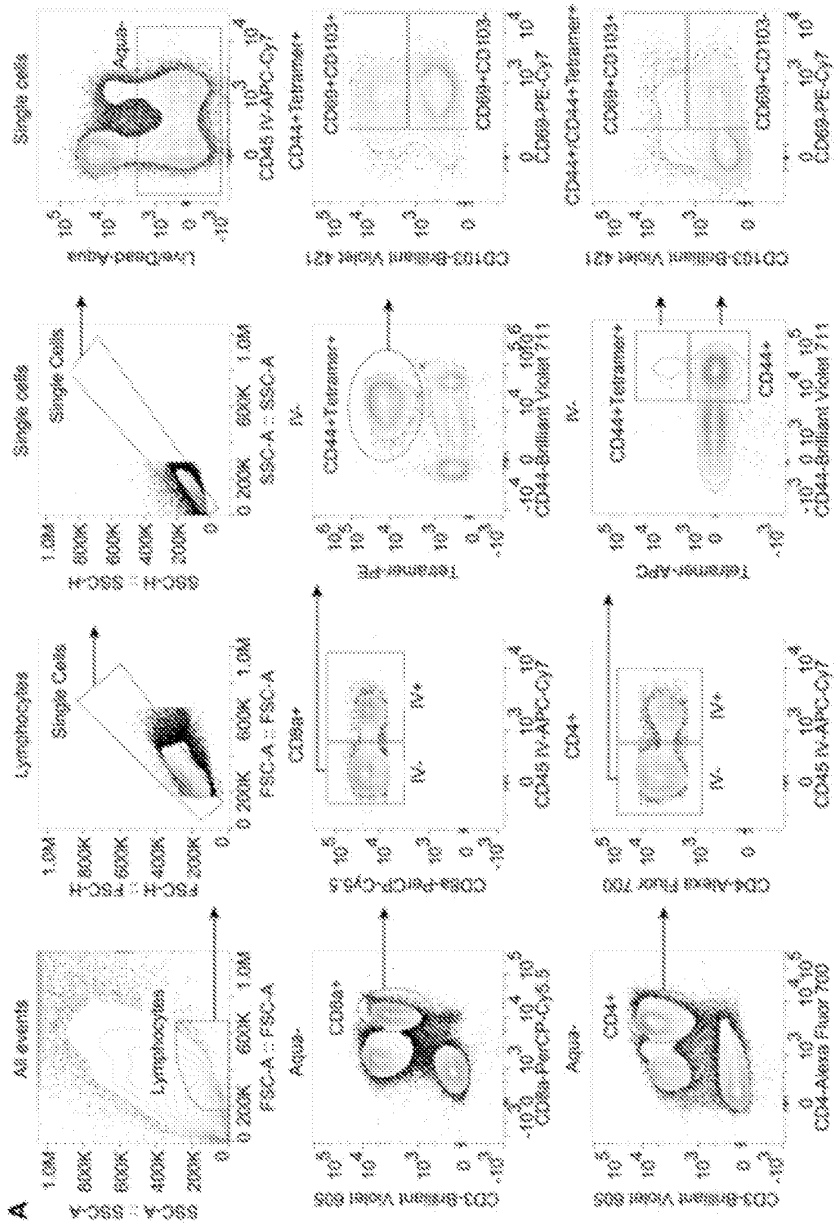
A
All events
Lymphocytes
Single cells
Aqua-
CD8a+
CD4+
CD44+Tetramer+
CD69+CD103+
CD69+CD103-
CD44+/CD44+Tetramer+
CD3-Brilliant Violet 605
CD4-Alexa Fluor 700
CD8a-PerCP-Cy5.5
CD45 IV-APC-Cy7
Tetramer-PE
Tetramer-APC
CD44-Brilliant Violet 711
CD103-Brilliant Violet 421
CD69-PE-Cy7
Live/Dead-Aqua
SSC-A :: SSC-A
SSC-H :: SSC-H
FSC-A :: FSC-A
FSC-H :: FSC-H
IV-
IV+

FIG. 10A

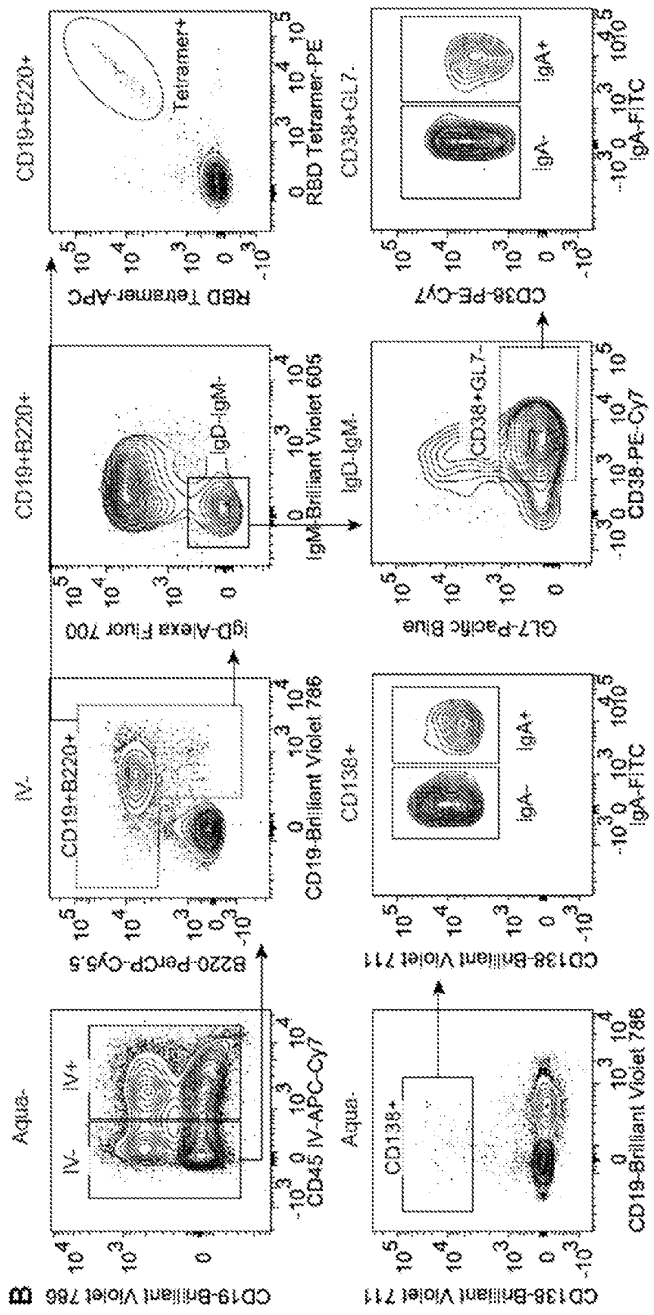
B
Aqua-
IV-
IV+
CD45 IV-APC-Cy7
CD19-Brilliant Violet 786
B220-PerCP-Cy5.5
CD19+B220+
IgD-Alexa Fluor 700
IgD-IgM-
IgM-Brilliant Violet 605
RBD Tetramer-APC
Tetramer+
RBD Tetramer-PE
CD138+
CD138-Brilliant Violet 711
IgA-
IgA+
IgA-FITC
GL7-Pacific Blue
CD38+GL7-
CD38-PE-Cy7

FIG. 10B