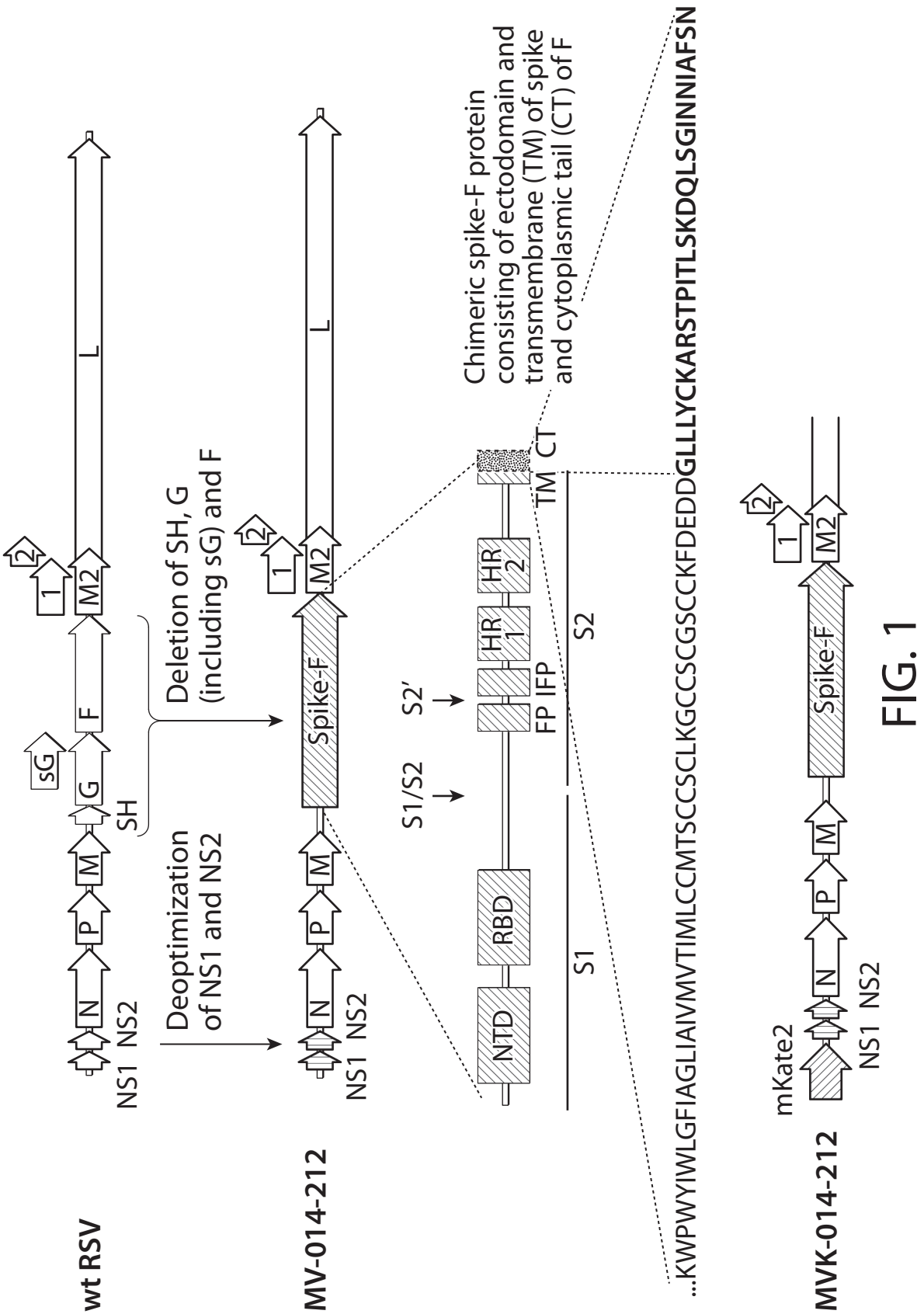

wt RSV
NS1 NS2
N
P
M
SH
G
sG
F
M2
L
1
2
Deoptimization of NS1 and NS2
Deletion of SH, G (including sG) and F
MV-014-212
Spike-F
S1/S2
S2'
S1
S2
NTD
RBD
HR 1
HR 2
FP
IFP
TM
CT
Chimeric spike-F protein consisting of ectodomain and transmembrane (TM) of spike and cytoplasmic tail (CT) of F
...KWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDDGLLLYCKARSTPITLSKDQLSGINNIAFSN
MVK-014-212
mKate2

FIG. 1

Junctions of the chimeric spike-F proteins

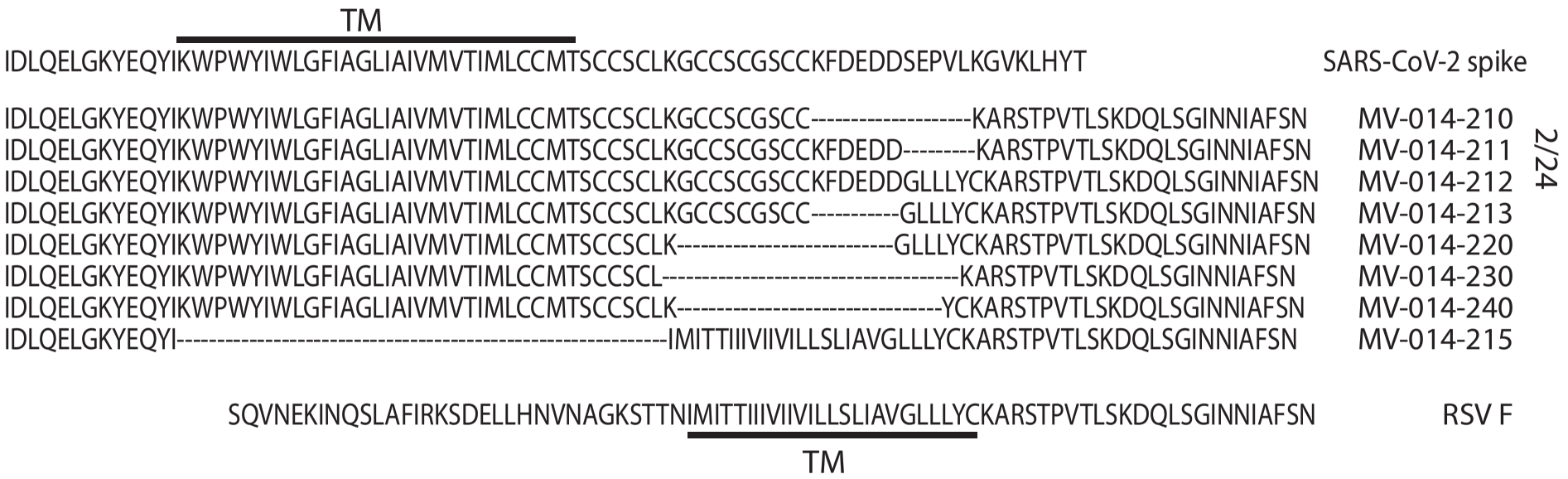



FIG. 2

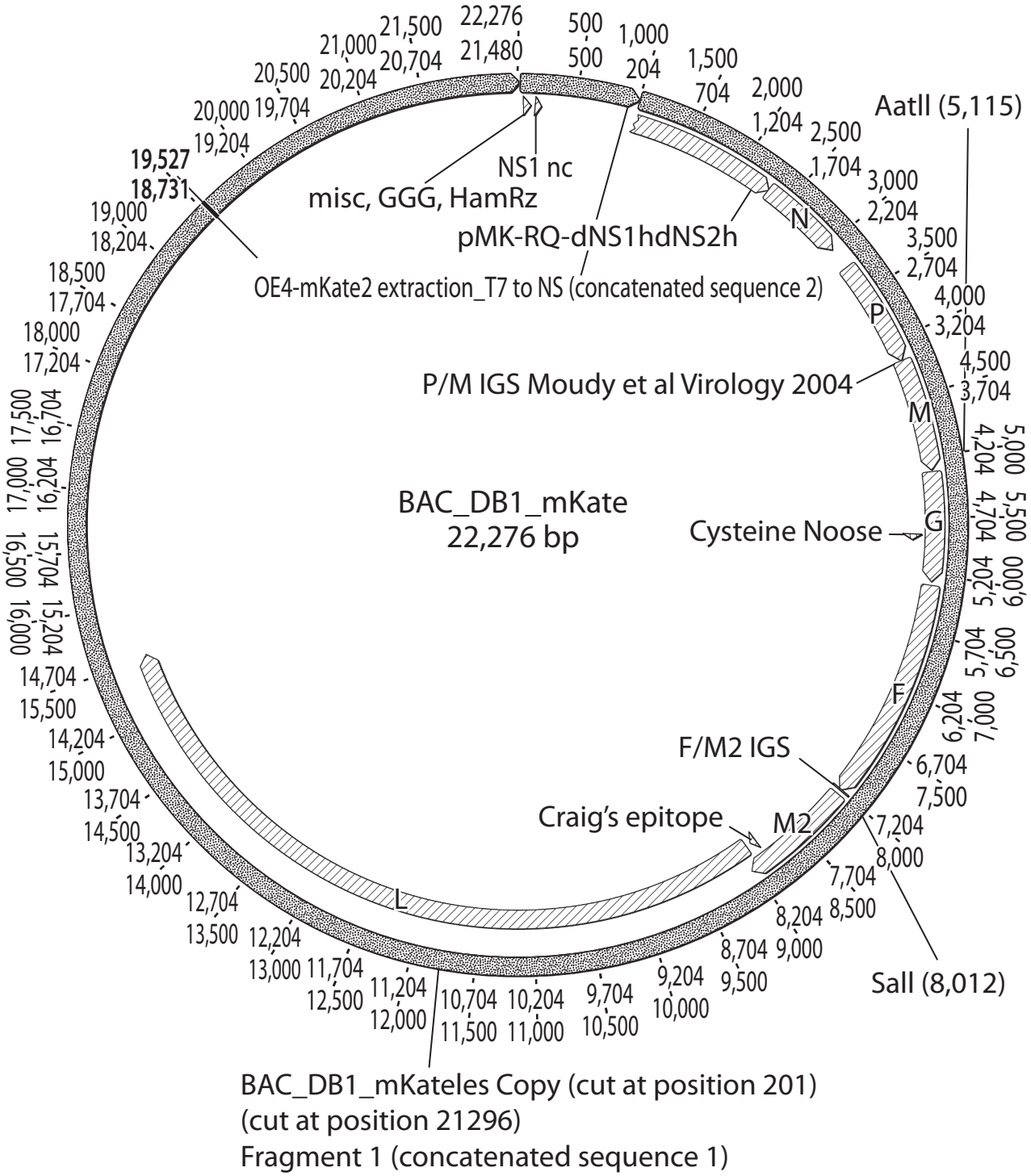
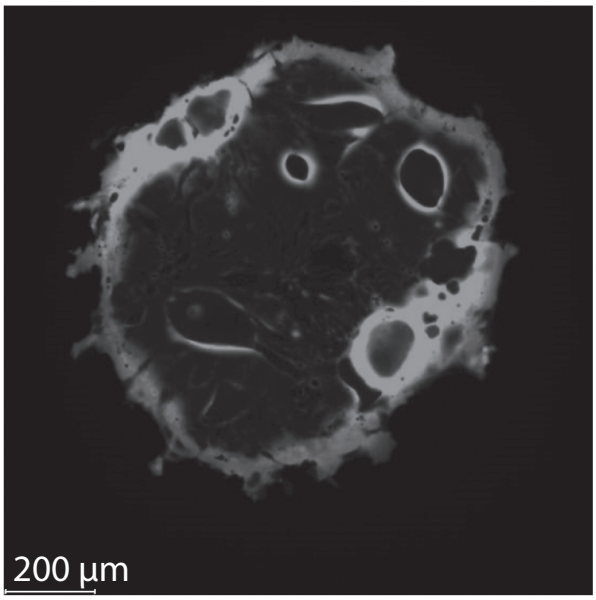

22,276
21,480
500
500
1,000
204
1,500
704
2,000
1,204
2,500
1,704
3,000
2,204
3,500
2,704
4,000
3,204
4,500
3,704
5,000
4,204
5,500
4,704
6,000
5,204
6,500
5,704
7,000
6,204
7,500
6,704
8,000
7,204
8,500
7,704
9,000
8,204
9,500
8,704
10,000
9,204
10,500
9,704
11,000
10,204
11,500
10,704
12,000
11,204
12,500
11,704
13,000
12,204
13,500
12,704
14,000
13,204
14,500
13,704
15,000
14,204
15,500
14,704
16,000
15,204
16,500
15,704
17,000
16,204
17,500
16,704
18,000
17,204
18,500
17,704
19,000
18,204
19,527
18,731
19,204
20,000
19,704
20,500
20,204
21,000
20,704
21,500
AatII (5,115)
NS1 nc
misc, GGG, HamRz
pMK-RQ-dNS1hdNS2h
N
OE4-mKate2 extraction_T7 to NS (concatenated sequence 2)
P
P/M IGS Moudy et al Virology 2004
M
BAC_DB1_mKate
22,276 bp
Cysteine Noose
G
F
F/M2 IGS
Craig's epitope
M2
L
SalI (8,012)
BAC_DB1_mKateles Copy (cut at position 201)
(cut at position 21296)
Fragment 1 (concatenated sequence 1)

FIG. 3



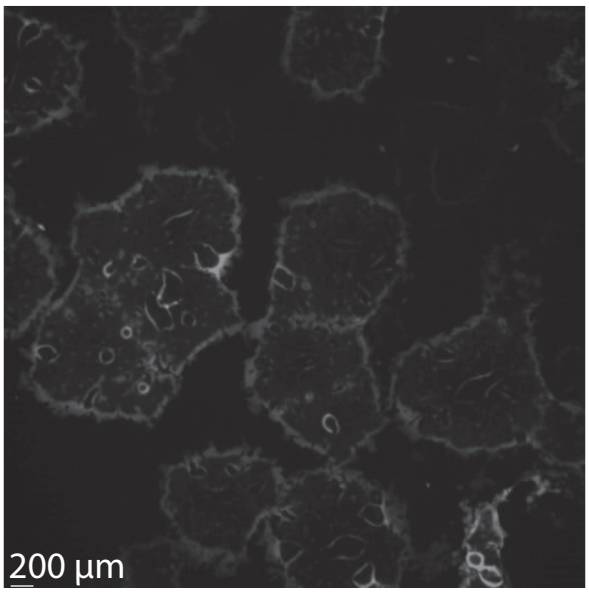

10X Magnification, TRITC



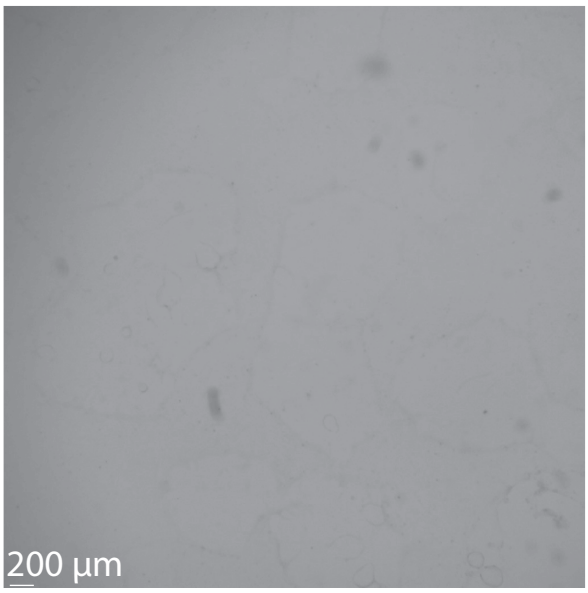

10X Magnification, Brightfield

FIG. 4A

MV-014-210 spread
2.5X brightfield, TRITC

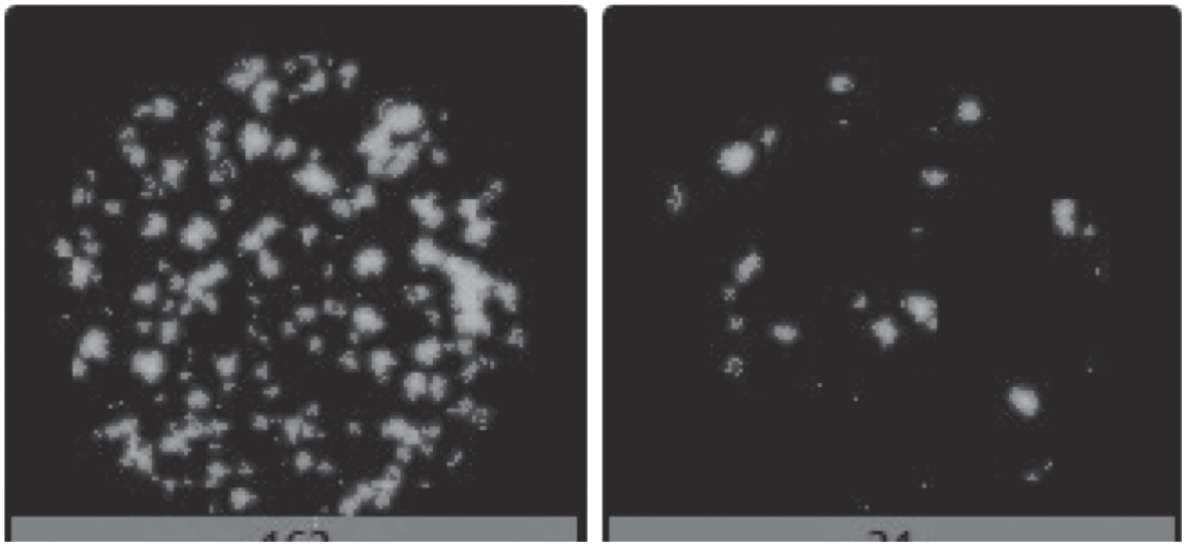



2.5X Magnification, TRITC




2.5X Magnification, Brightfield

FIG. 4B



1:10 dilution, Celigo Image

1:100 dilution, Celigo Image

FIG. 4C

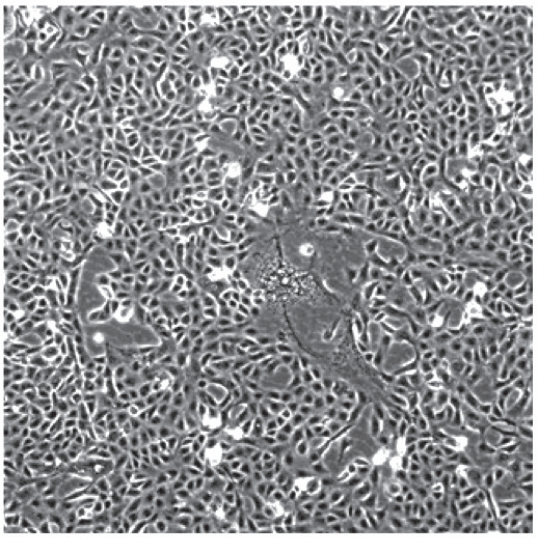

MV-014-212
BAC
T7
pA2-
M2-1opt
pA2-Nopt
pA2-
Popt
pA2-Lopt
Electroporation
in Vero cells
Expansion by
passage

FIG. 5

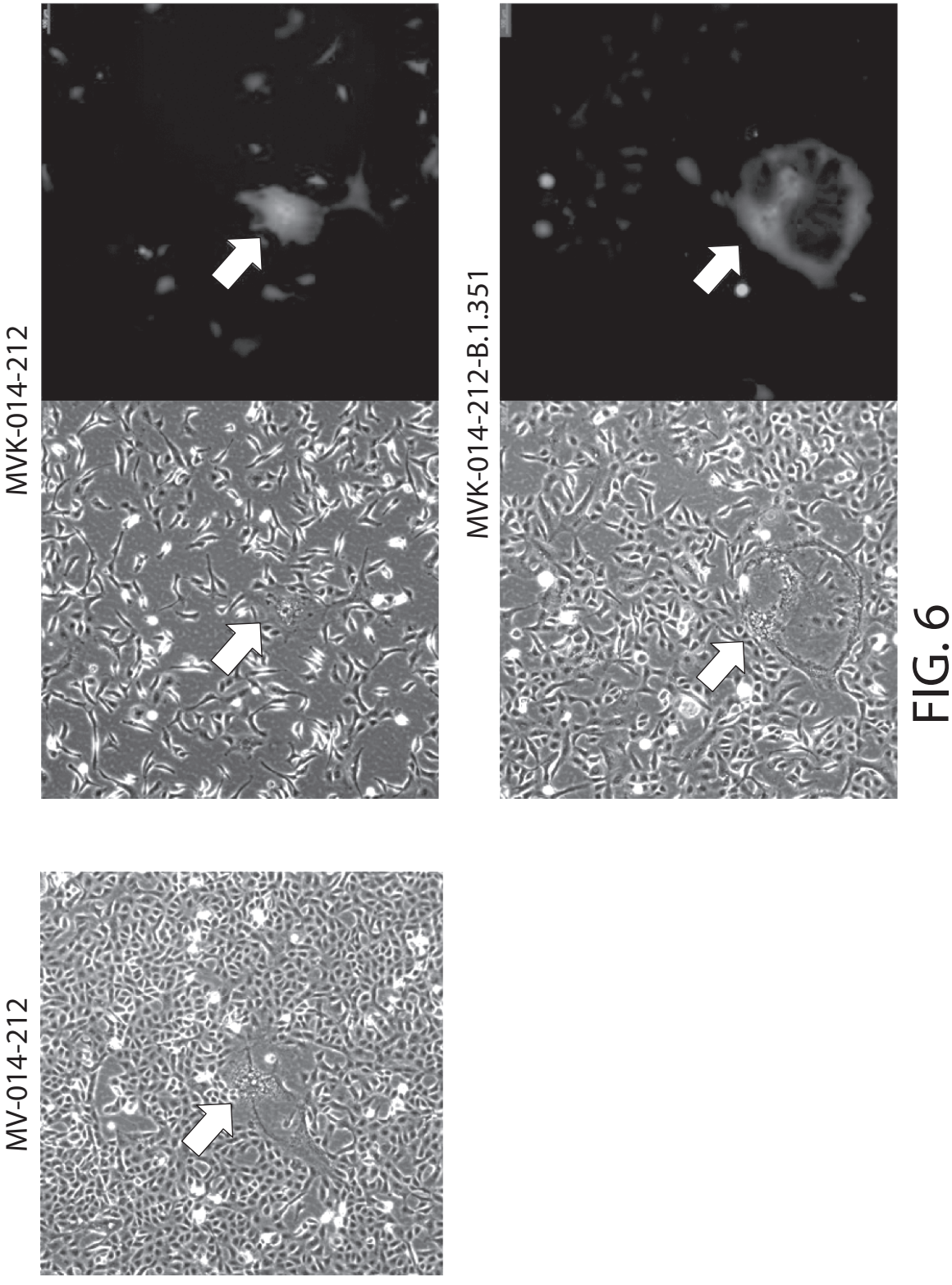



FIG. 6

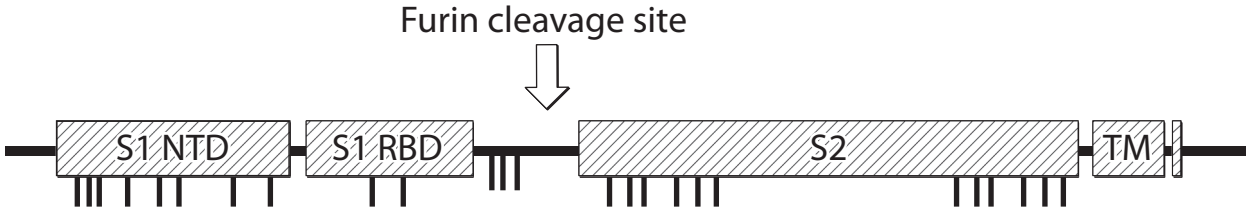



FIG. 7

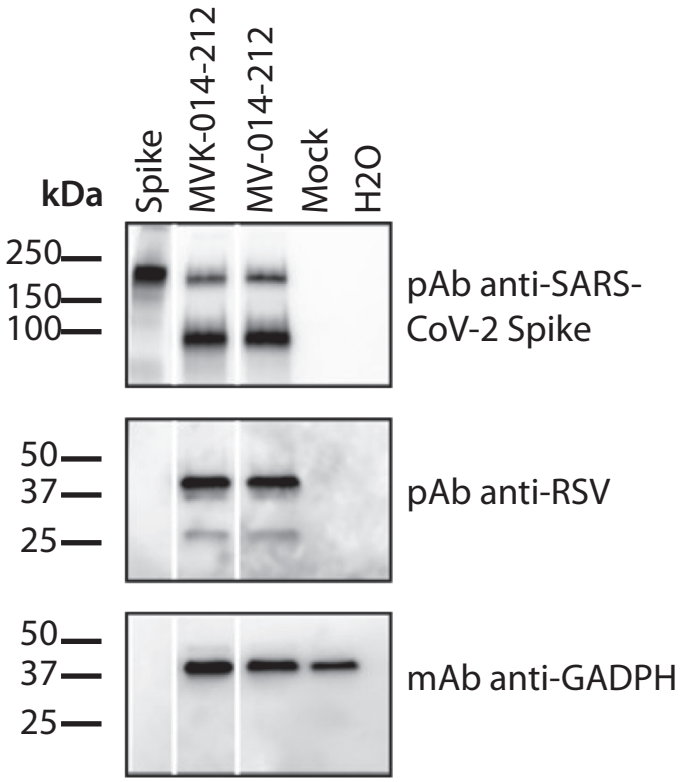

kDa
Spike
MVK-014-212
MV-014-212
Mock
H2O
250
150
100
pAb anti-SARS-CoV-2 Spike
50
37
25
pAb anti-RSV
50
37
25
mAb anti-GADPH

FIG. 8A

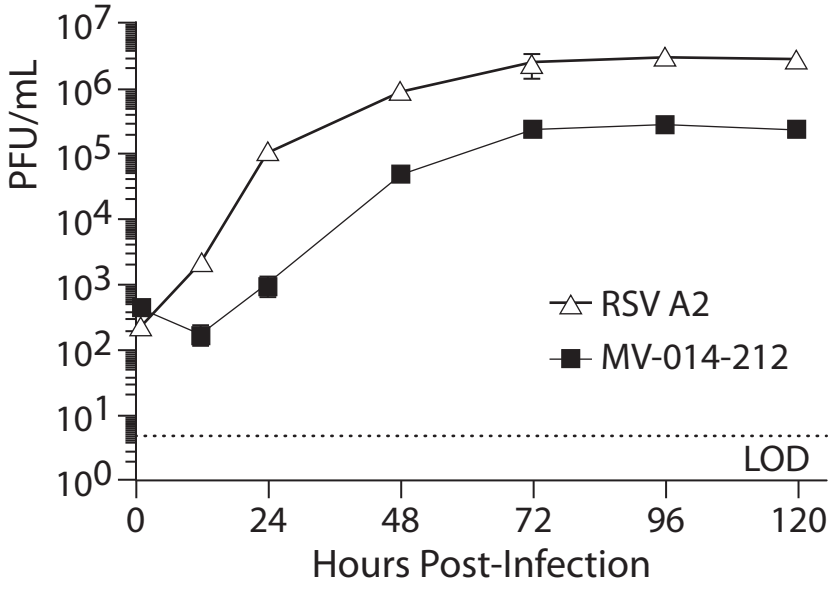

PFU/mL
$10^7$
$10^6$
$10^5$
$10^4$
$10^3$
$10^2$
$10^1$
$10^0$
RSV A2
MV-014-212
LOD
0
24
48
72
96
120
Hours Post-Infection

FIG. 8B

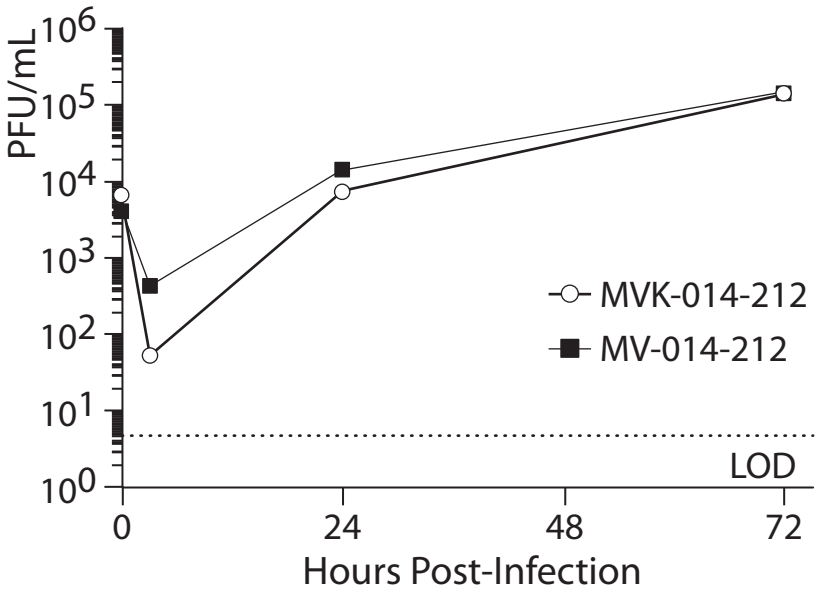

PFU/mL
$10^6$
$10^5$
$10^4$
$10^3$
$10^2$
$10^1$
$10^0$
MVK-014-212
MV-014-212
LOD
0
24
48
72
Hours Post-Infection

FIG. 8C

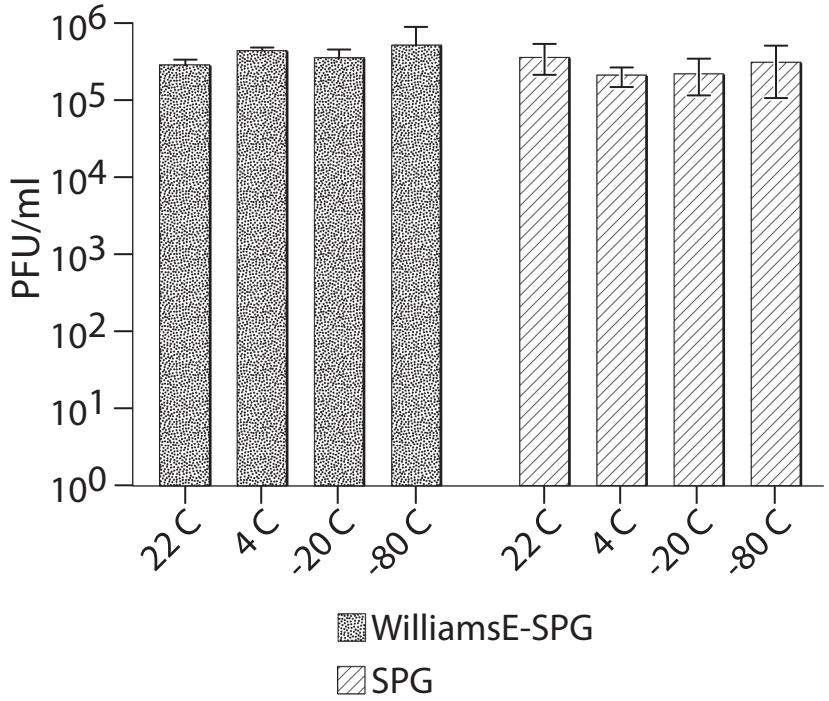

PFU/ml
$10^6$
$10^5$
$10^4$
$10^3$
$10^2$
$10^1$
$10^0$
22 C
4 C
-20 C
-80 C
22 C
4 C
-20 C
-80 C
WilliamsE-SPG
SPG

FIG. 8D

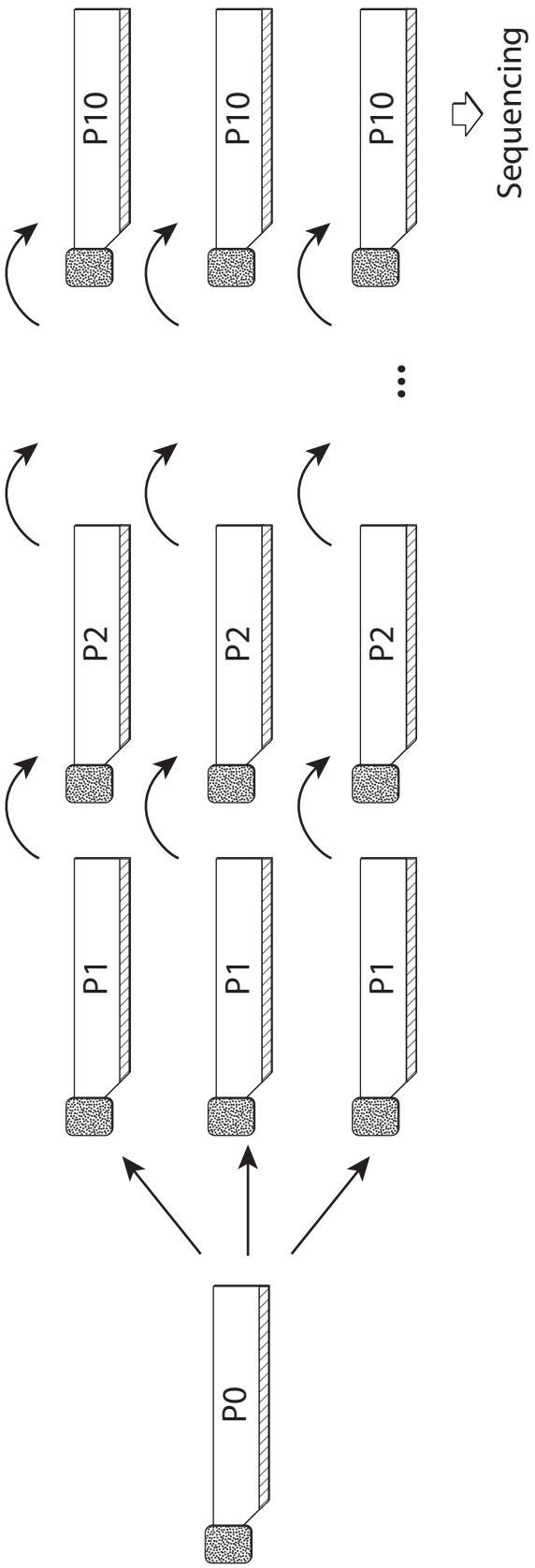
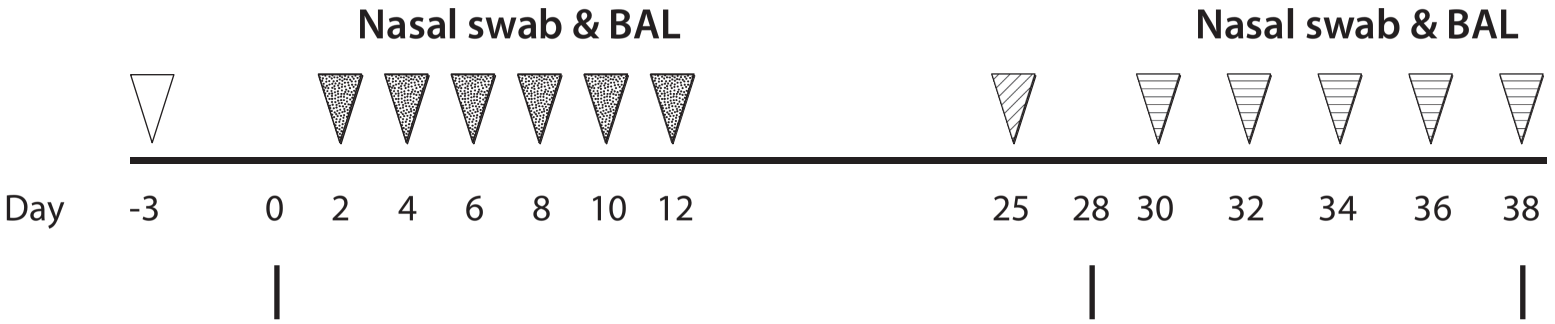



FIG. 9




MV-014-212 n = 4
*wt* RSV A2 n = 4
Mock (PBS) n = 3

Pre-challenge sera for neutralizing Ab and IgG

Replication of MV-014-212 and *wt* RSV A2 in nasal swab and BAL

Nasal swab and sera for IgA post vaccination

Replication of *wt* SARS-CoV-2 challenge

FIG. 10

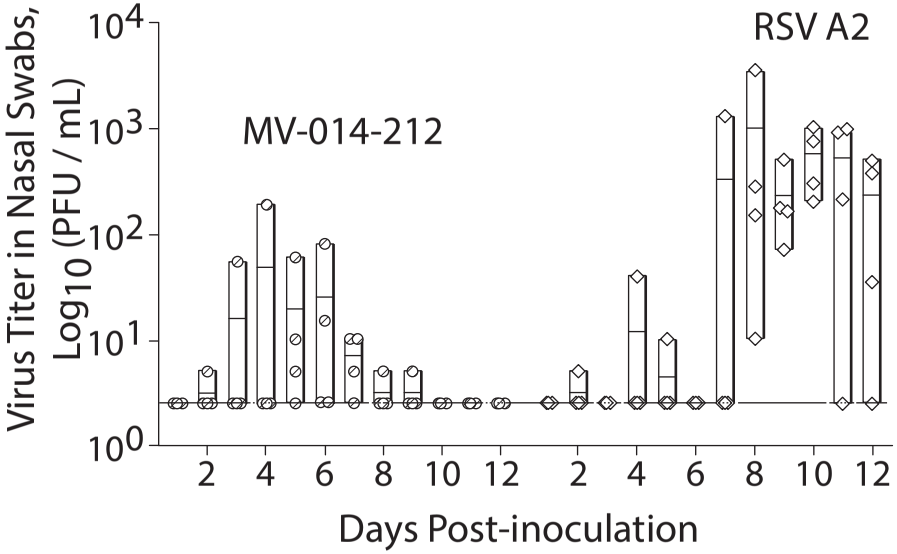



FIG. 11A

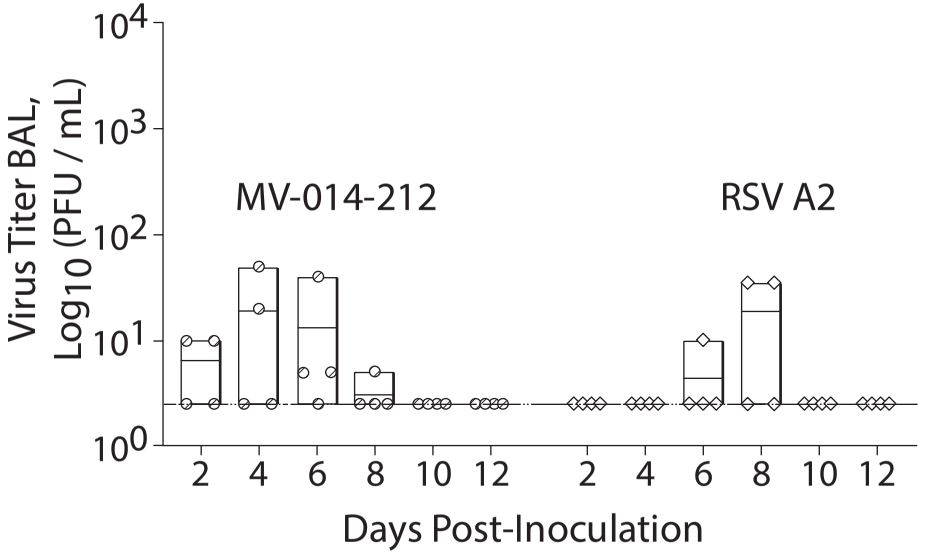



FIG. 11B

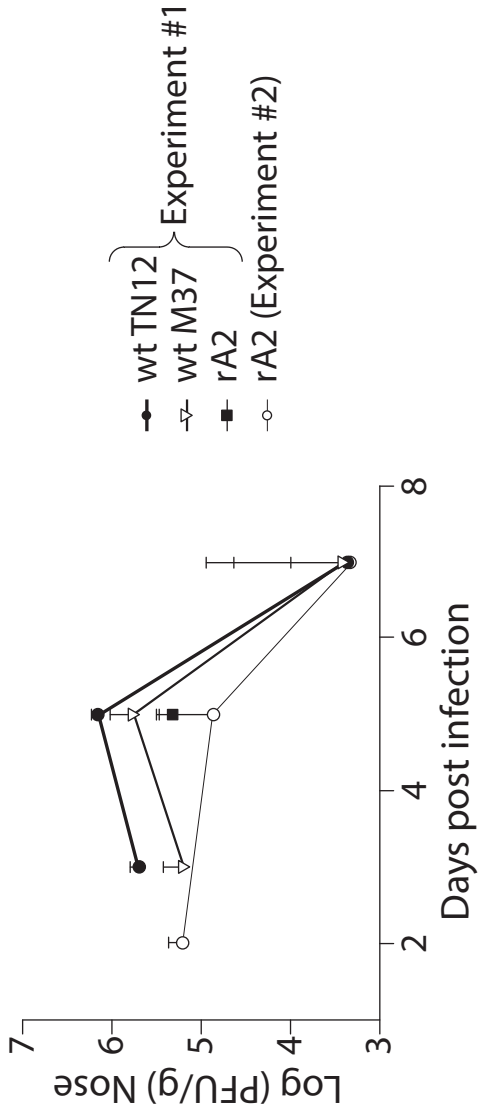



FIG. 12A

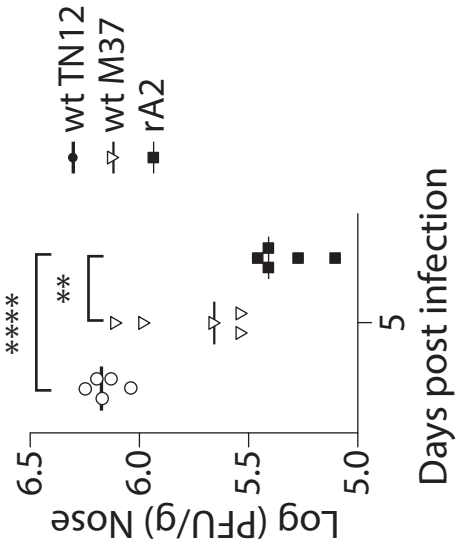



FIG. 12B




FIG. 12C

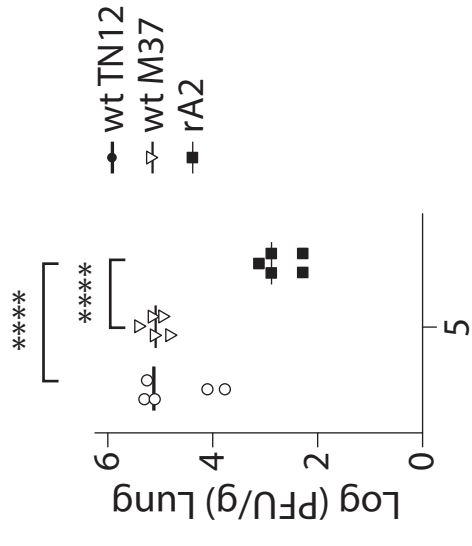



FIG. 12D

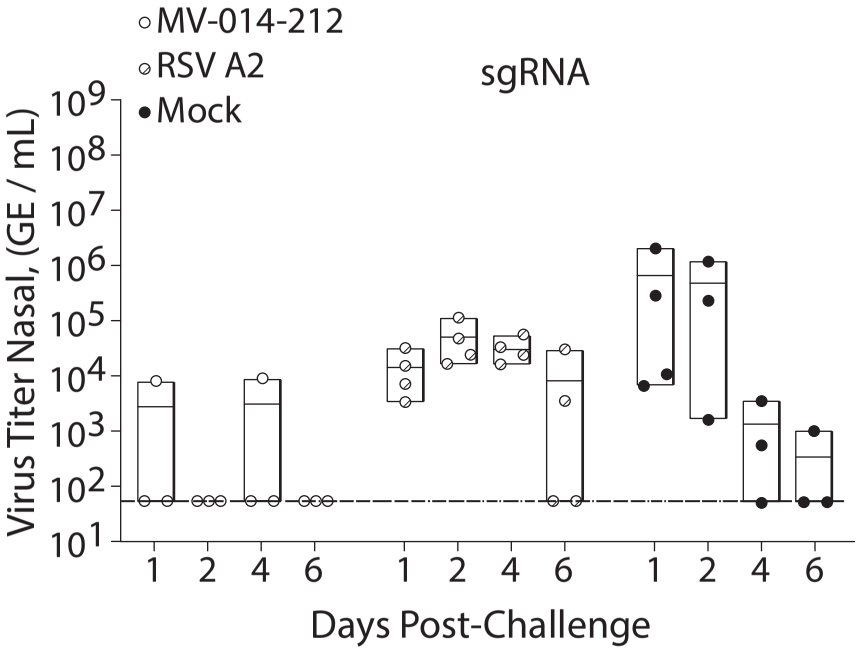



FIG. 13A

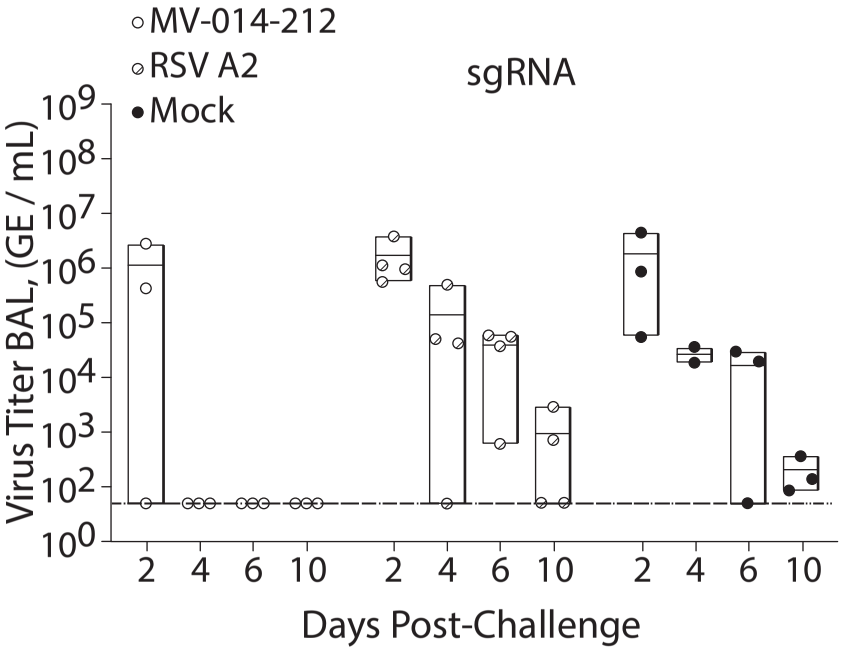



FIG. 13B

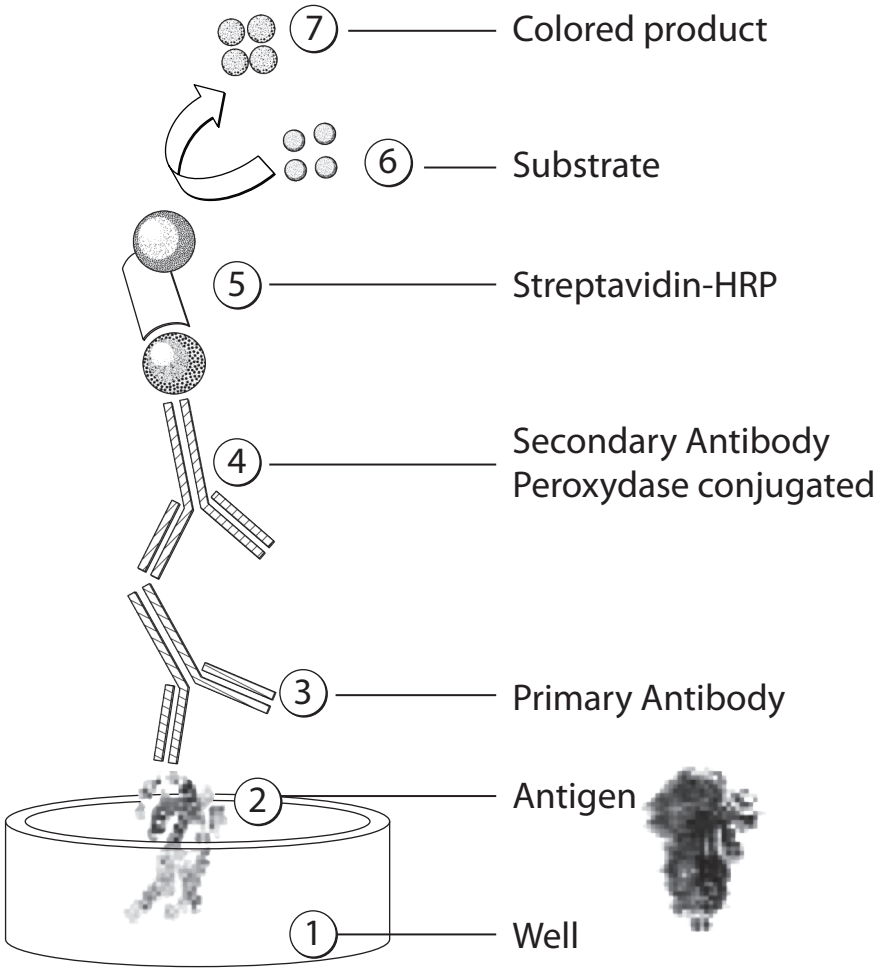

7
Colored product
6
Substrate
5
Streptavidin-HRP
4
Secondary Antibody
Peroxydase conjugated
3
Primary Antibody
2
Antigen
1
Well

FIG. 14A

For positive control, replace Spike with IgA capture Ab

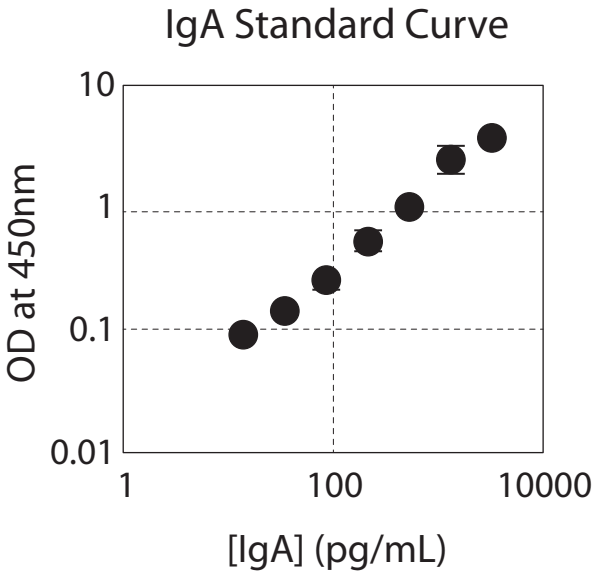



FIG. 14B

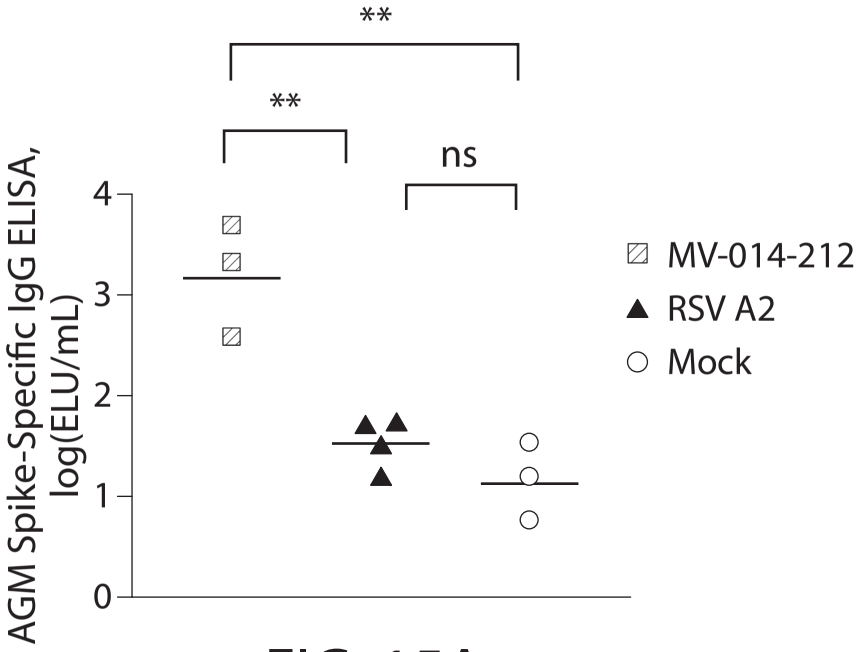



FIG. 15A

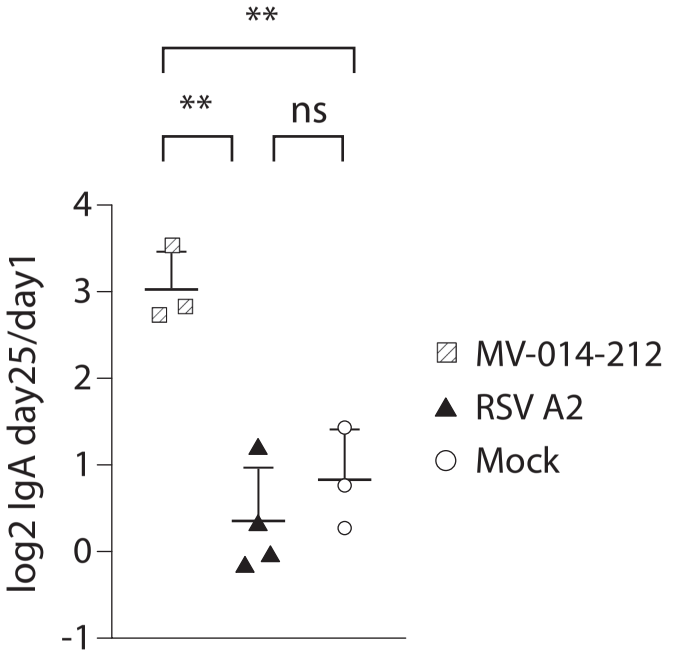



FIG. 15B

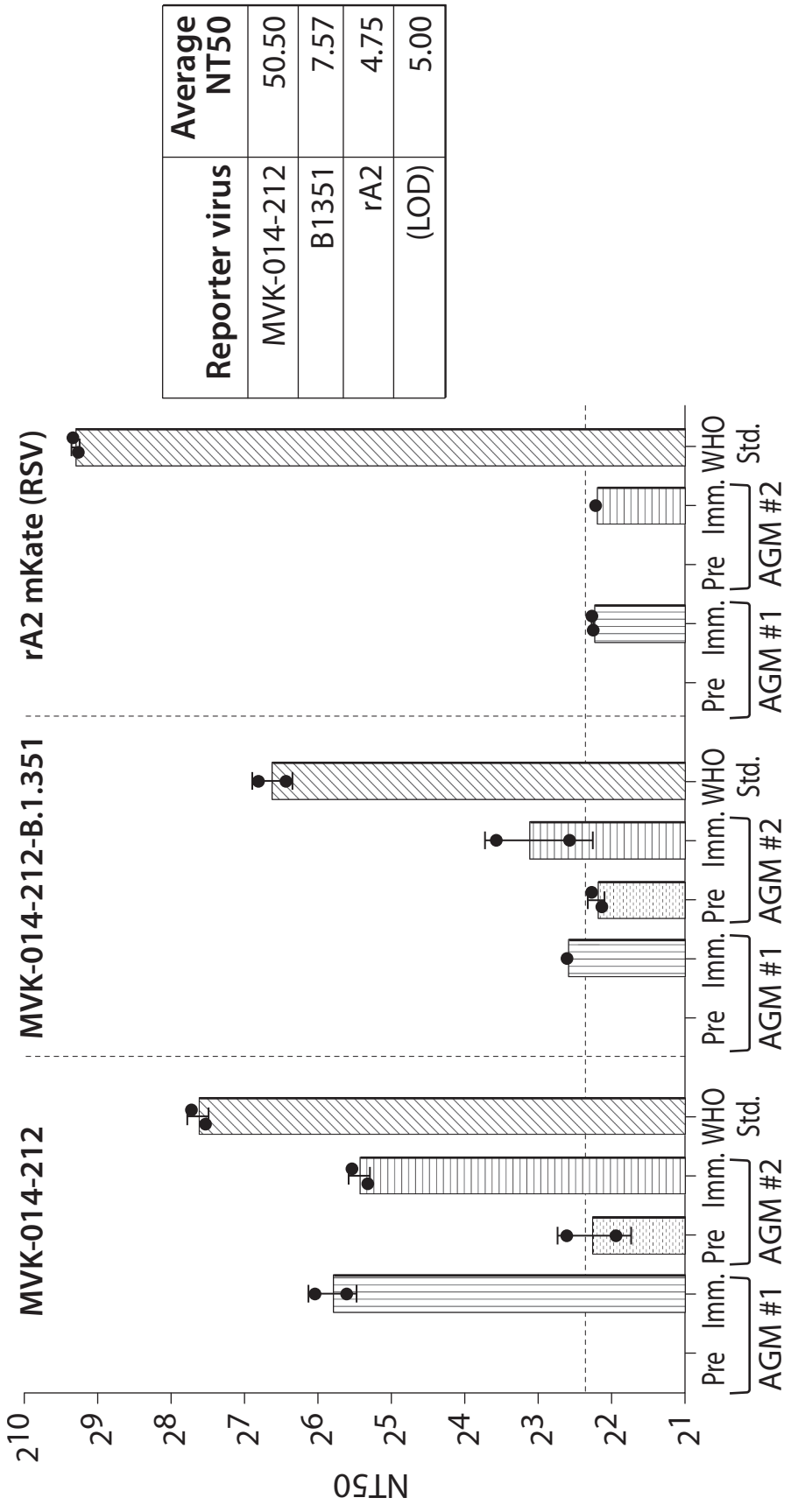

Reporter virus
Average NT50
MVK-014-212
50.50
B1351
7.57
rA2
4.75
(LOD)
5.00
MVK-014-212
MVK-014-212-B.1.351
rA2 mKate (RSV)
NT50
$2^{10}$
$2^{9}$
$2^{8}$
$2^{7}$
$2^{6}$
$2^{5}$
$2^{4}$
$2^{3}$
$2^{2}$
$2^{1}$
Pre
Imm.
AGM #1
Pre
Imm.
AGM #2
WHO Std.

FIG. 15C

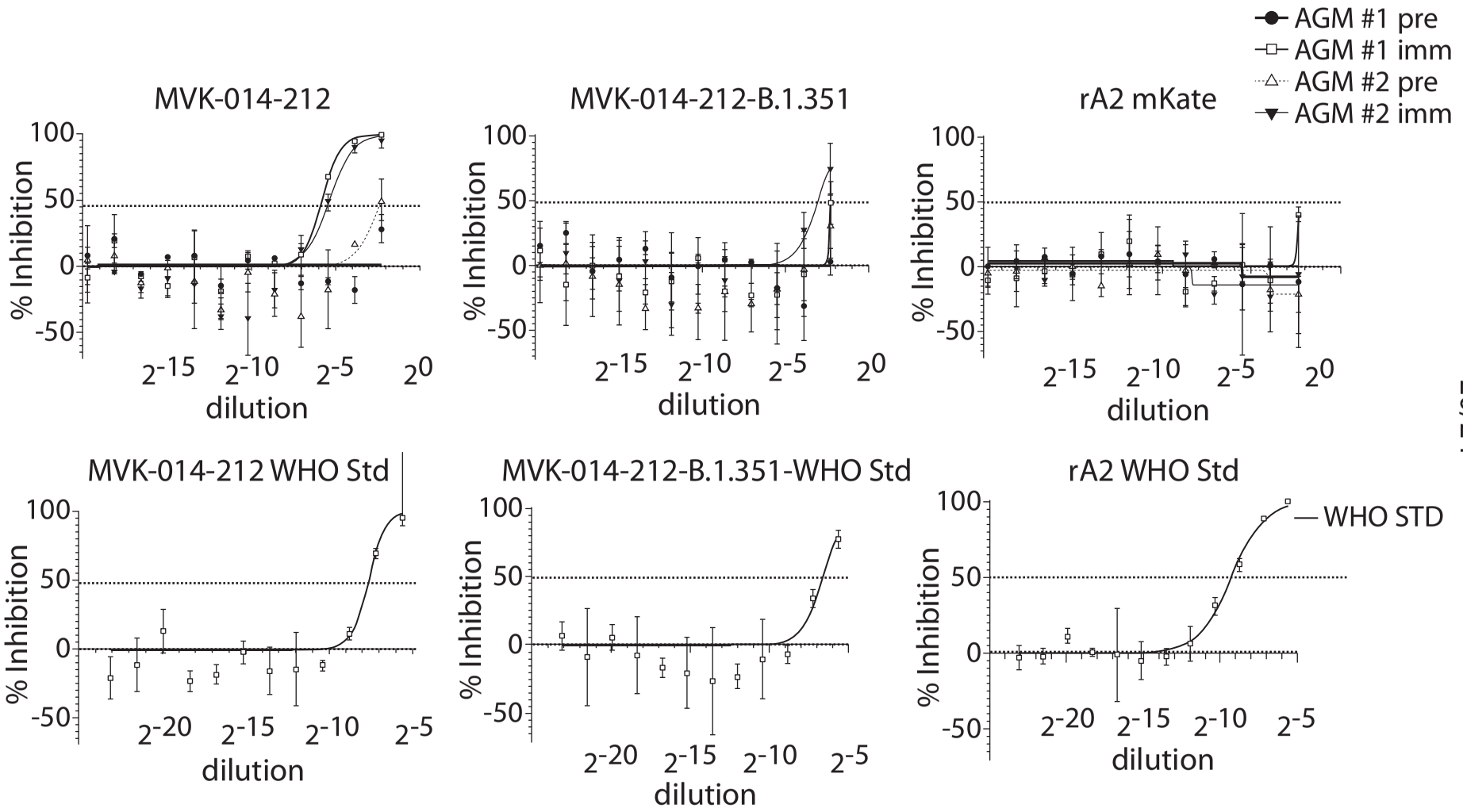

AGM #1 pre
AGM #1 imm
AGM #2 pre
AGM #2 imm
MVK-014-212
MVK-014-212-B.1.351
rA2 mKate
MVK-014-212 WHO Std
MVK-014-212-B.1.351-WHO Std
rA2 WHO Std
WHO STD
% Inhibition
dilution
100
50
0
-50
2-15
2-10
2-5
20
2-20

FIG. 16

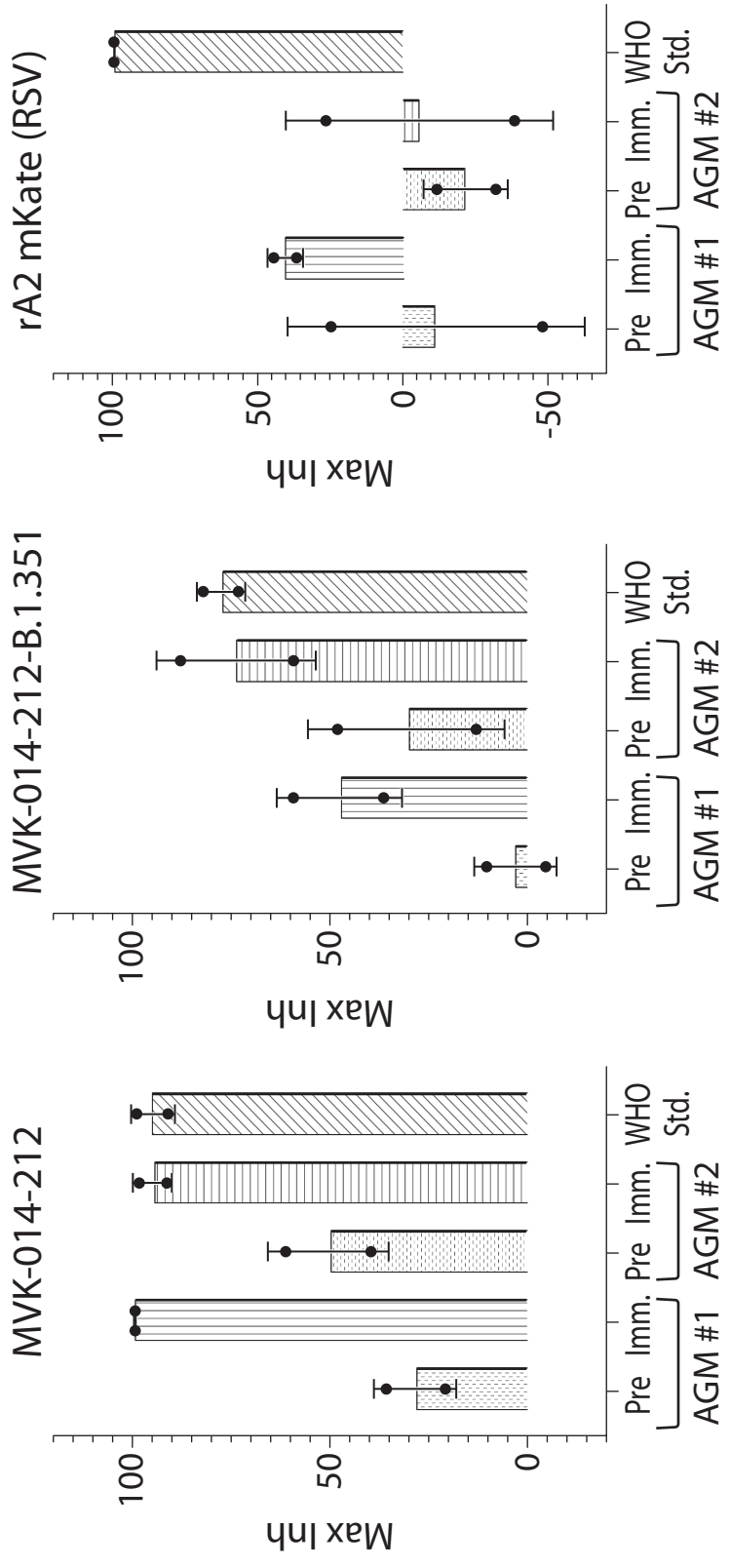

MVK-014-212
MVK-014-212-B.1.351
rA2 mKate (RSV)
Max Inh
100
50
0
-50
Pre Imm. Pre Imm. WHO Std.
AGM #1 AGM #2

FIG. 17

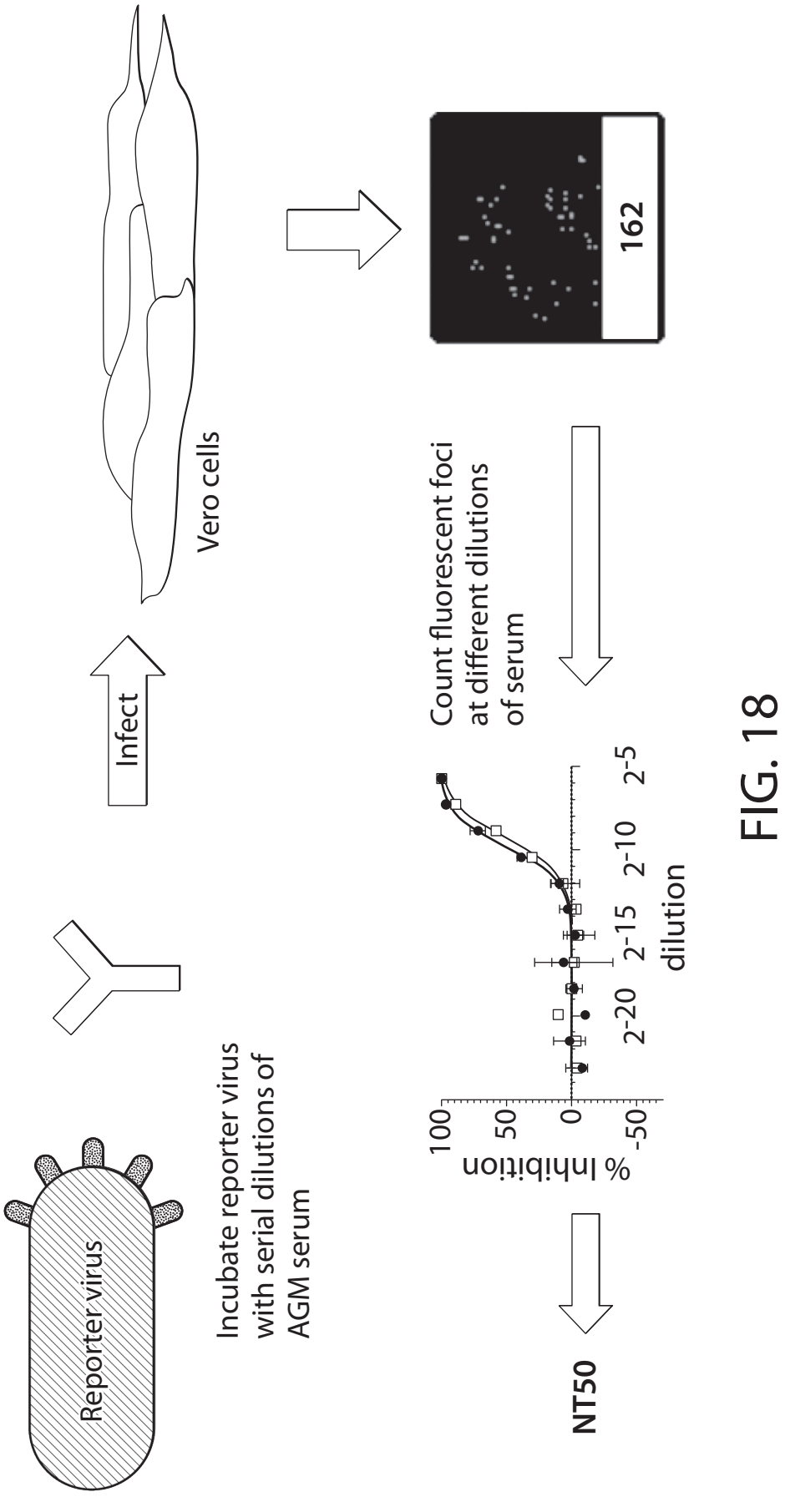

Reporter virus
Incubate reporter virus with serial dilutions of AGM serum
Infect
Vero cells
162
Count fluorescent foci at different dilutions of serum
100
50
0
-50
% Inhibition
2-20
2-15
2-10
2-5
dilution
NT50

FIG. 18

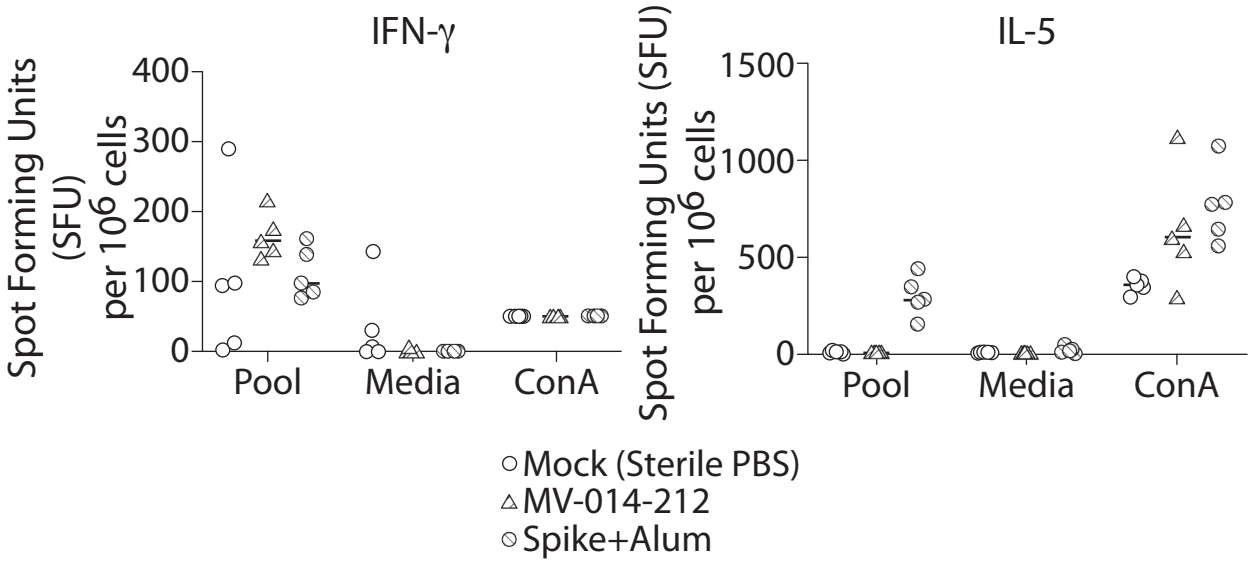



FIG. 19A

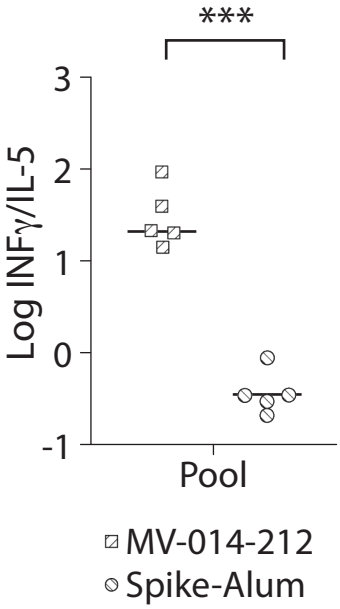



FIG. 19B

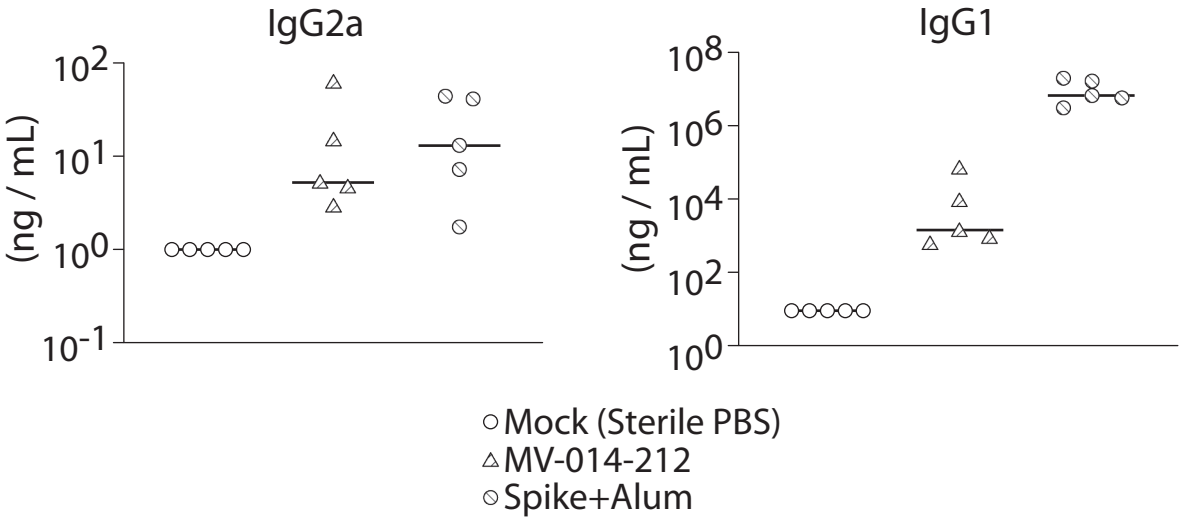



FIG. 19C

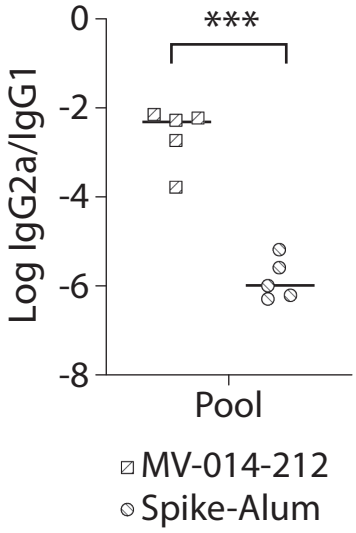



FIG. 19D