Figure 1

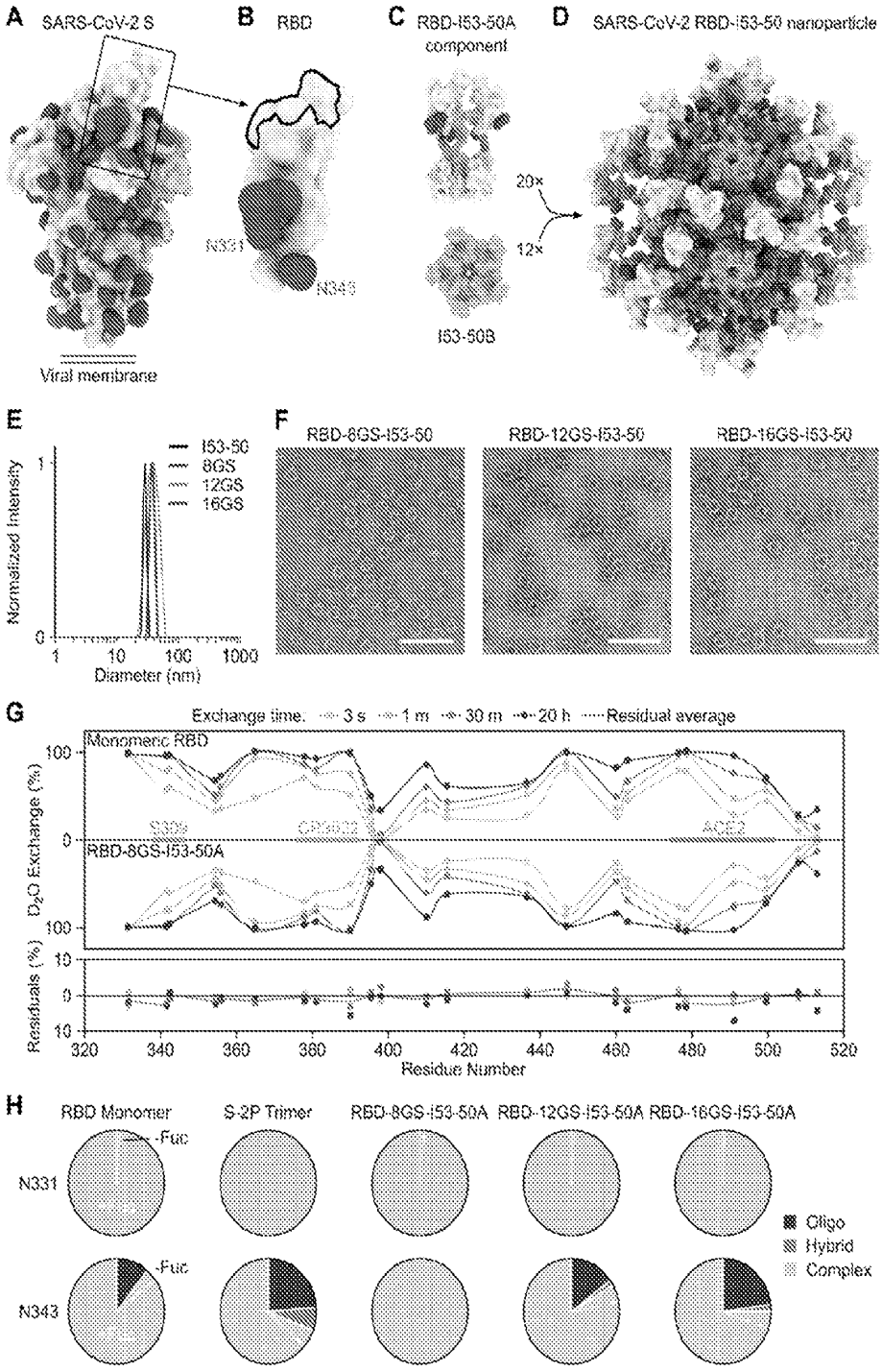

A
SARS-CoV-2 S
Viral membrane
B
RBD
N331
N343
C
RBD-I53-50A component
I53-50B
20×
12×
D
SARS-CoV-2 RBD-I53-50 nanoparticle
E
I53-50
8GS
12GS
16GS
Normalized Intensity
Diameter (nm)
F
RBD-8GS-I53-50
RBD-12GS-I53-50
RBD-16GS-I53-50
G
Exchange time. 3 s 1 m 30 m 20 h Residual average
Monomeric RBD
RBD-8GS-I53-50A
S309
CR3022
ACE2
D2O Exchange (%)
Residuals (%)
Residue Number
H
RBD Monomer
S-2P Trimer
RBD-8GS-I53-50A
RBD-12GS-I53-50A
RBD-16GS-I53-50A
-Fuc
N331
N343
Oligo
Hybrid
Complex

Figure 2

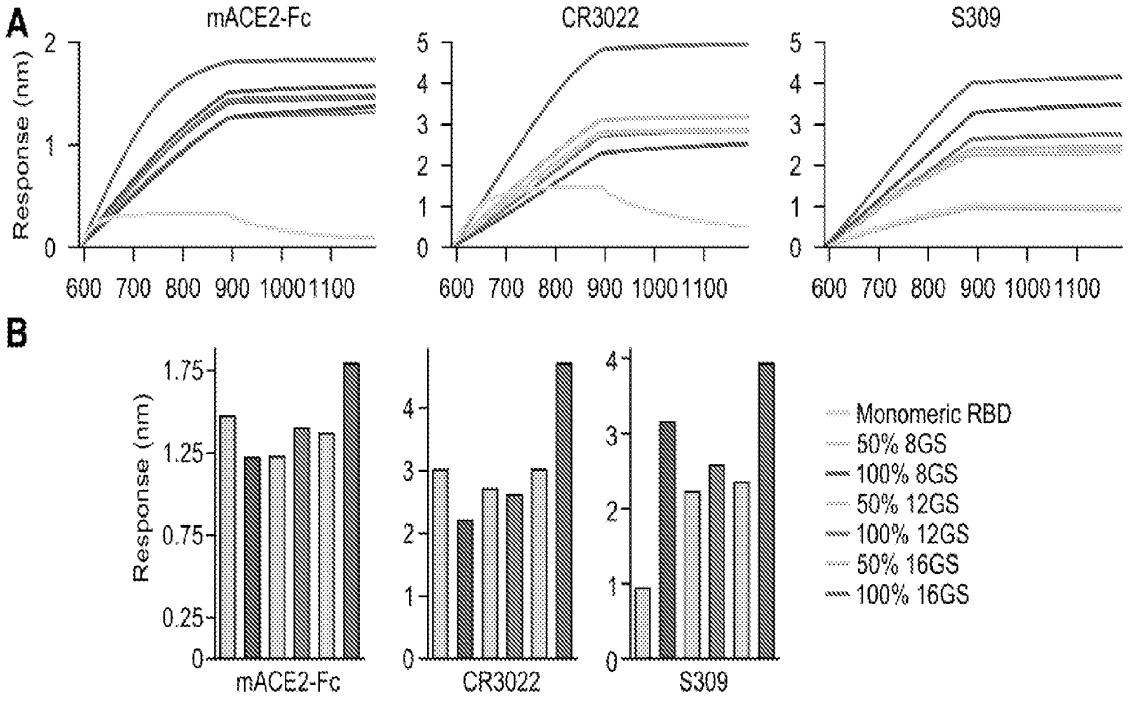

A
mACE2-Fc
CR3022
S309
Response (nm)
600 700 800 900 1000 1100
B
Response (nm)
mACE2-Fc
CR3022
S309
Monomeric RBD
50% 8GS
100% 8GS
50% 12GS
100% 12GS
50% 16GS
100% 16GS

Figure 3

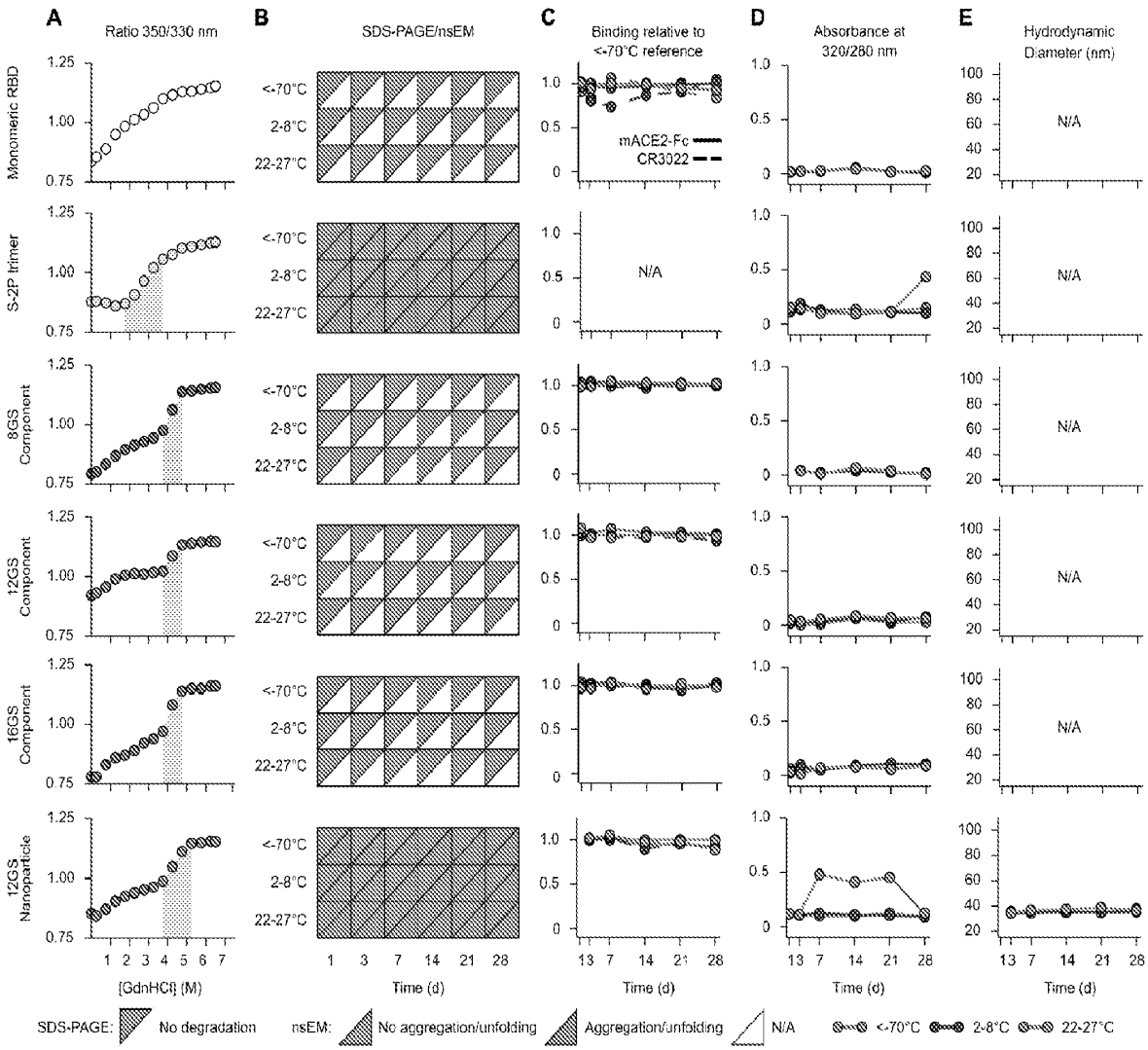

A
Ratio 350/330 nm
B
SDS-PAGE/nsEM
C
Binding relative to <-70°C reference
D
Absorbance at 320/280 nm
E
Hydrodynamic Diameter (nm)
Monomeric RBD
S-2P trimer
8GS Component
12GS Component
16GS Component
12GS Nanoparticle
<-70°C
2-8°C
22-27°C
mACE2-Fc
CR3022
N/A
[GdnHCl] (M)
Time (d)
SDS-PAGE: No degradation
nsEM: No aggregation/unfolding
Aggregation/unfolding
N/A
<-70°C
2-8°C
22-27°C

Figure 4

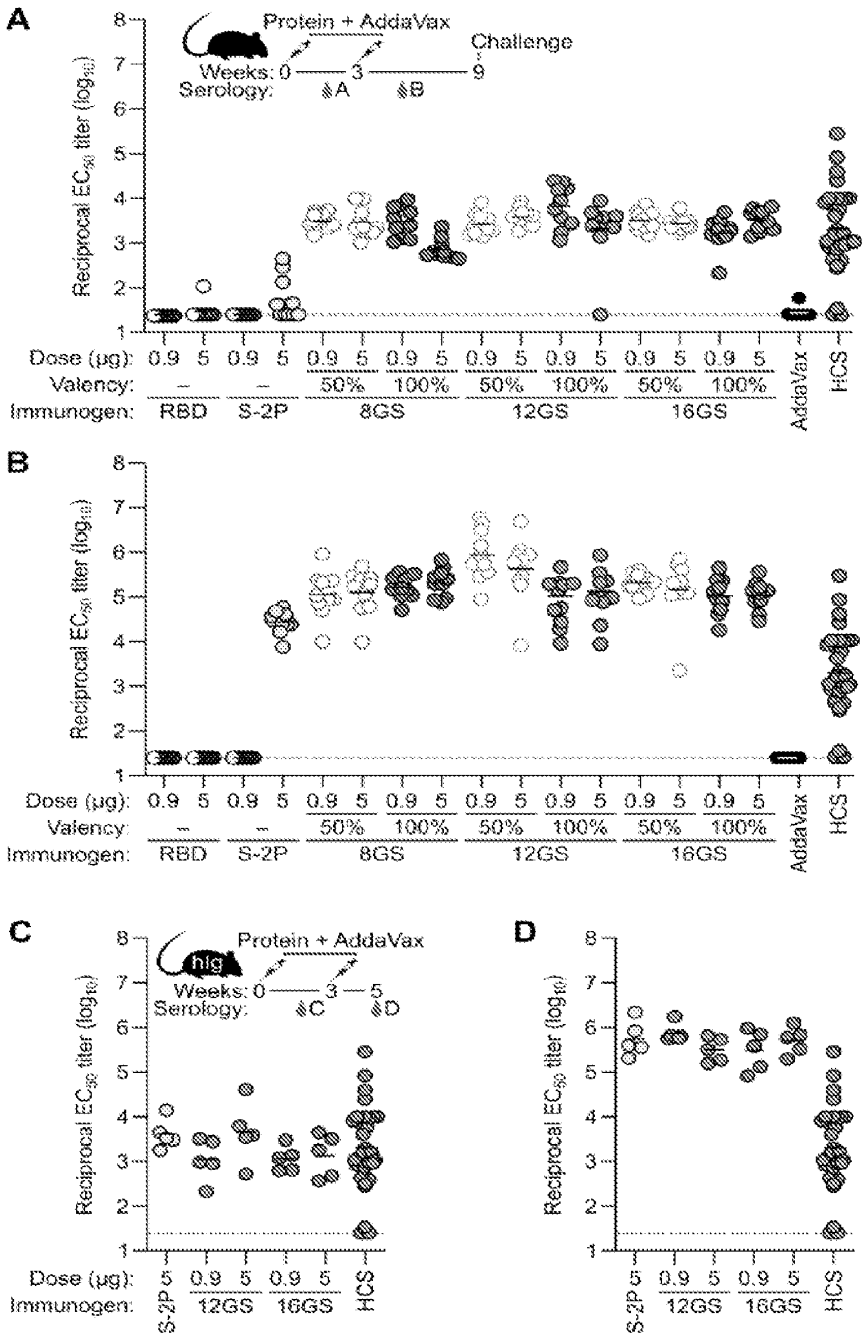

A
Protein + AddaVax
Challenge
Weeks: 0 3 9
Serology: A B
Reciprocal EC50 titer (log10)
Dose (μg): 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5
Valency: – – 50% 100% 50% 100% 50% 100%
Immunogen: RBD S-2P 8GS 12GS 16GS AddaVax HCS
B
Reciprocal EC50 titer (log10)
Dose (μg): 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5
Valency: – – 50% 100% 50% 100% 50% 100%
Immunogen: RBD S-2P 8GS 12GS 16GS AddaVax HCS
C
hIg
Protein + AddaVax
Weeks: 0 3 5
Serology: C D
Reciprocal EC50 titer (log10)
Dose (μg): 5 0.9 5 0.9 5 HCS
Immunogen: S-2P 12GS 16GS
D
Reciprocal EC50 titer (log10)
5 0.9 5 0.9 5
S-2P 12GS 16GS HCS

Figure 5

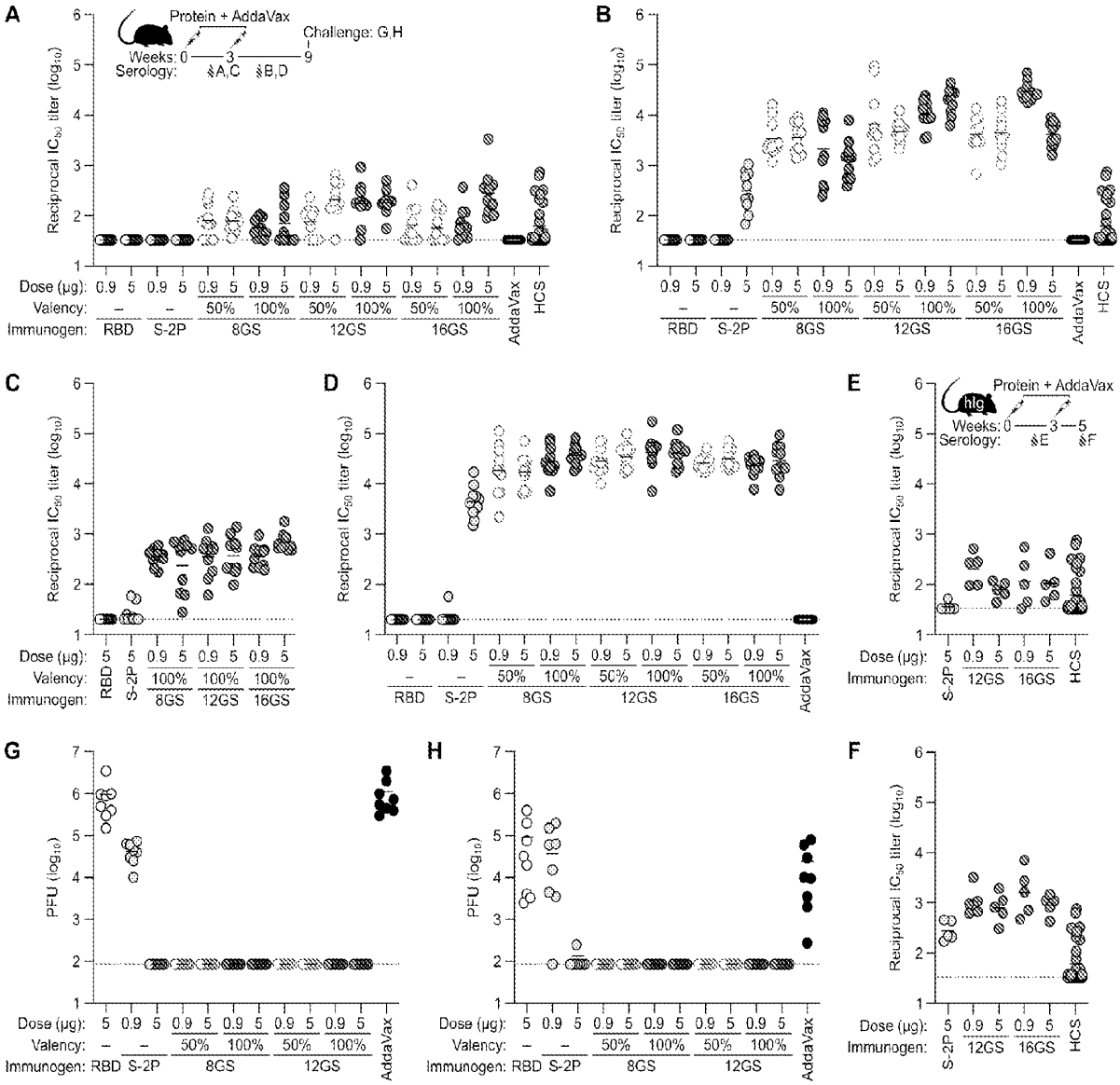

A
Protein + AddaVax
Challenge: G,H
Weeks: 0 3 9
Serology: ∛A,C ∛B,D
Reciprocal IC50 titer (log10)
Dose (μg): 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5
Valency: – – 50% 100% 50% 100% 50% 100%
Immunogen: RBD S-2P 8GS 12GS 16GS AddaVax HCS
B
Reciprocal IC50 titer (log10)
0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5
– – 50% 100% 50% 100% 50% 100%
RBD S-2P 8GS 12GS 16GS AddaVax HCS
C
Reciprocal IC50 titer (log10)
Dose (μg): 5 5 0.9 5 0.9 5 0.9 5
Valency: 100% 100% 100%
Immunogen: RBD S-2P 8GS 12GS 16GS
D
Reciprocal IC50 titer (log10)
0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5
– – 50% 100% 50% 100% 50% 100%
RBD S-2P 8GS 12GS 16GS AddaVax
E
Protein + AddaVax
hIg
Weeks: 0 3 5
Serology: ∛E ∛F
Reciprocal IC50 titer (log10)
Dose (μg): 5 0.9 5 0.9 5
Immunogen: S-2P 12GS 16GS HCS
G
PFU (log10)
Dose (μg): 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5
Valency: – – 50% 100% 50% 100%
Immunogen: RBD S-2P 8GS 12GS AddaVax
H
PFU (log10)
Dose (μg): 5 0.9 5 0.9 5 0.9 5 0.9 5 0.9 5
Valency: – – 50% 100% 50% 100%
Immunogen: RBD S-2P 8GS 12GS AddaVax
F
Reciprocal IC50 titer (log10)
Dose (μg): 5 0.9 5 0.9 5 0.9 5
Immunogen: S-2P 12GS 16GS HCS

Figure 6

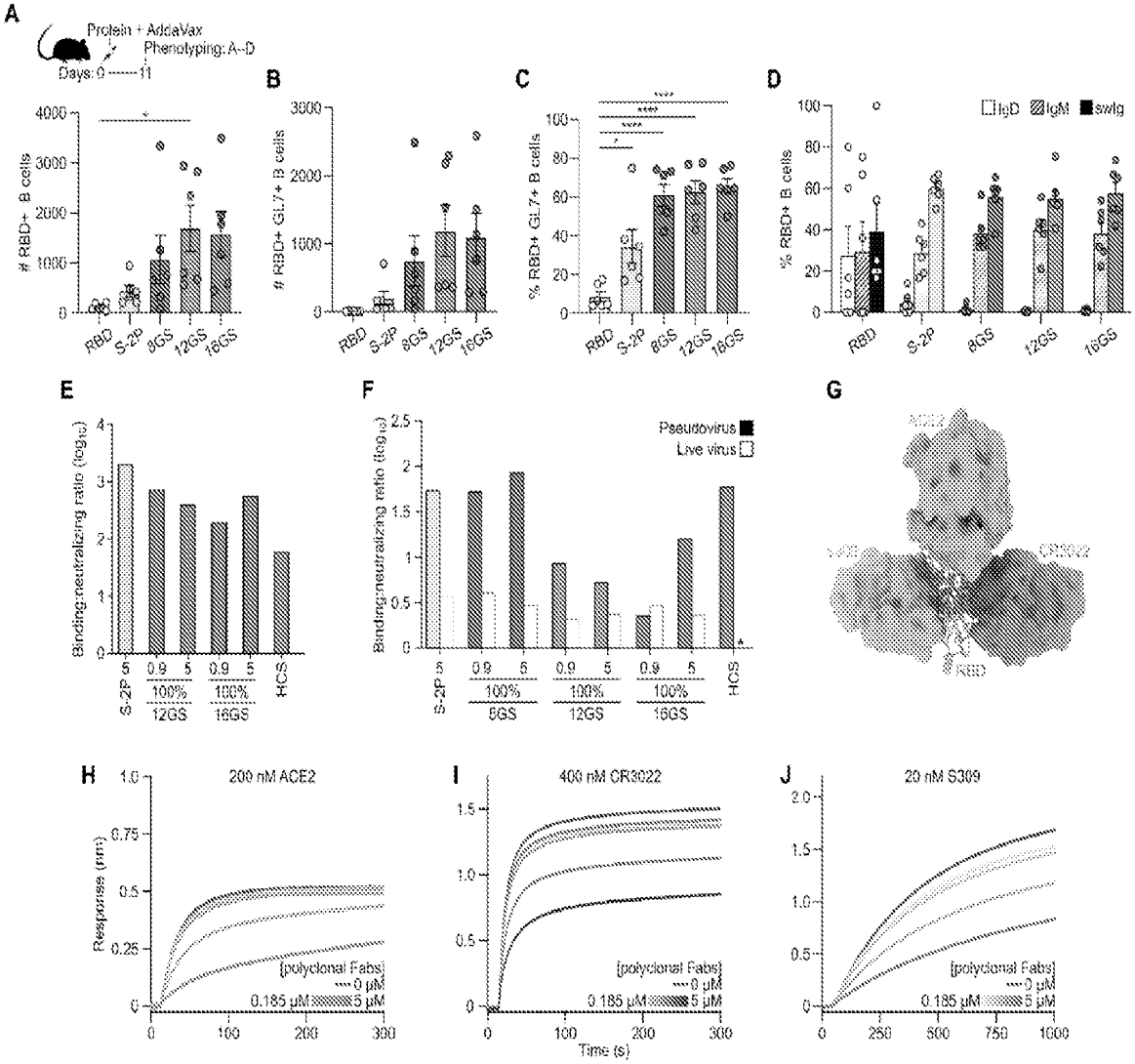

A
Protein + AddaVax
Phenotyping: A–D
Days: 0 11
B
C
D
RBD+ B cells
RBD+ GL7+ B cells
% RBD+ GL7+ B cells
% RBD+ B cells
IgD IgM swIg
RBD S-2P 8GS 12GS 16GS
E
F
G
Binding:neutralizing ratio (log10)
Pseudovirus
Live virus
S-2P
100%
12GS
16GS
8GS
HCS
ACE2
CR3022
RBD
H
I
J
200 nM ACE2
400 nM CR3022
20 nM S309
Response (nm)
Time (s)
[polyclonal Fabs]
0 µM
0.185 µM
5 µM

Figure 7

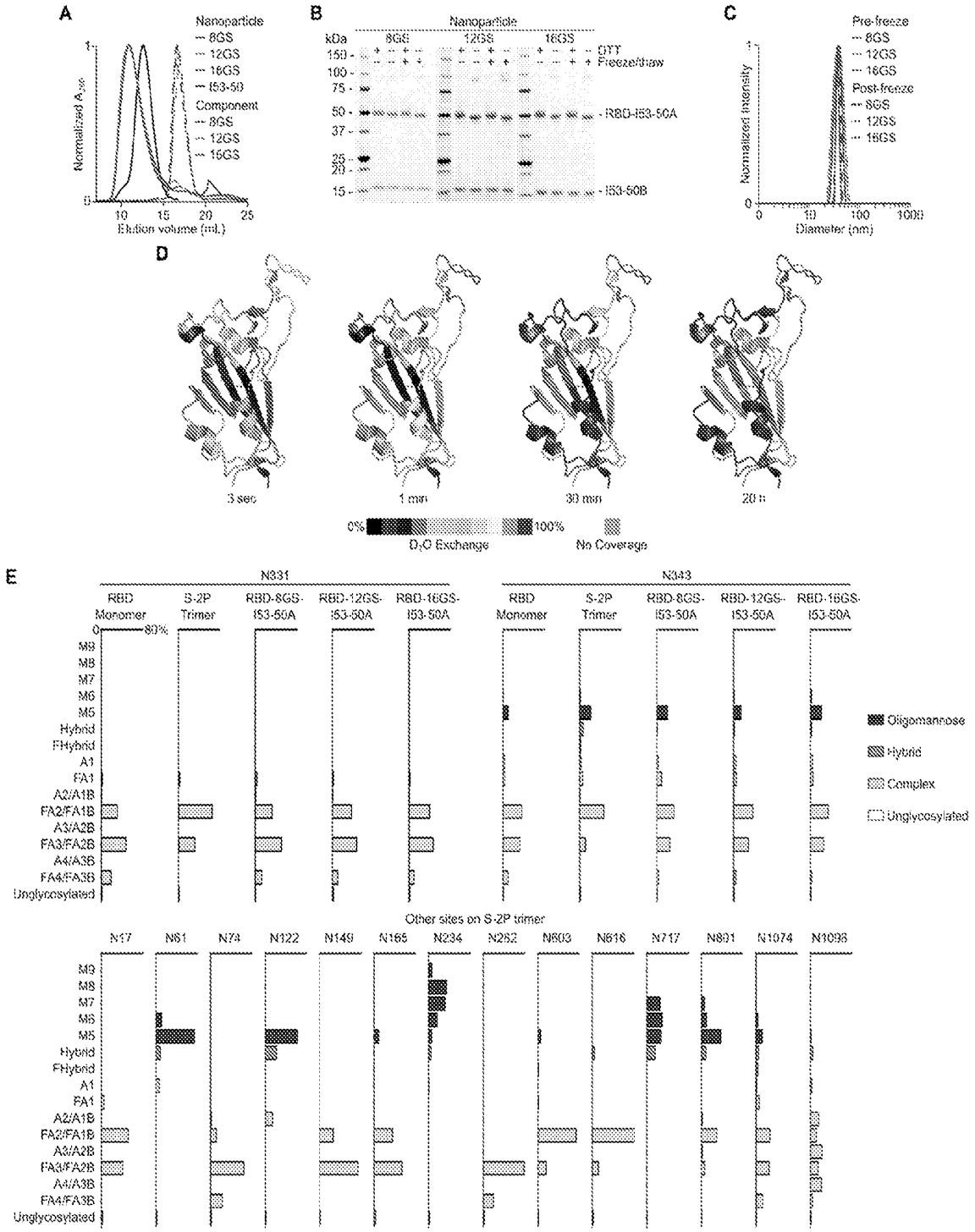

A
Nanoparticle
8GS
12GS
16GS
I53-50
Component
8GS
12GS
16GS
Normalized A280
Elution volume (mL)
B
Nanoparticle
kDa
8GS
12GS
16GS
DTT
Freeze/thaw
150
100
75
50
37
25
20
15
RBD-I53-50A
I53-50B
C
Pre-freeze
8GS
12GS
16GS
Post-freeze
8GS
12GS
16GS
Normalized intensity
Diameter (nm)
D
3 sec
1 min
30 min
20 h
0%
100%
D2O Exchange
No Coverage
E
N331
N343
RBD Monomer
S-2P Trimer
RBD-8GS-I53-50A
RBD-12GS-I53-50A
RBD-16GS-I53-50A
0
80%
M9
M8
M7
M6
M5
Hybrid
FHybrid
A1
FA1
A2/A1B
FA2/FA1B
A3/A2B
FA3/FA2B
A4/A3B
FA4/FA3B
Unglycosylated
Oligomannose
Hybrid
Complex
Unglycosylated
Other sites on S-2P trimer
N17
N61
N74
N122
N149
N165
N234
N262
N603
N616
N717
N801
N1074
N1098

Figure 8

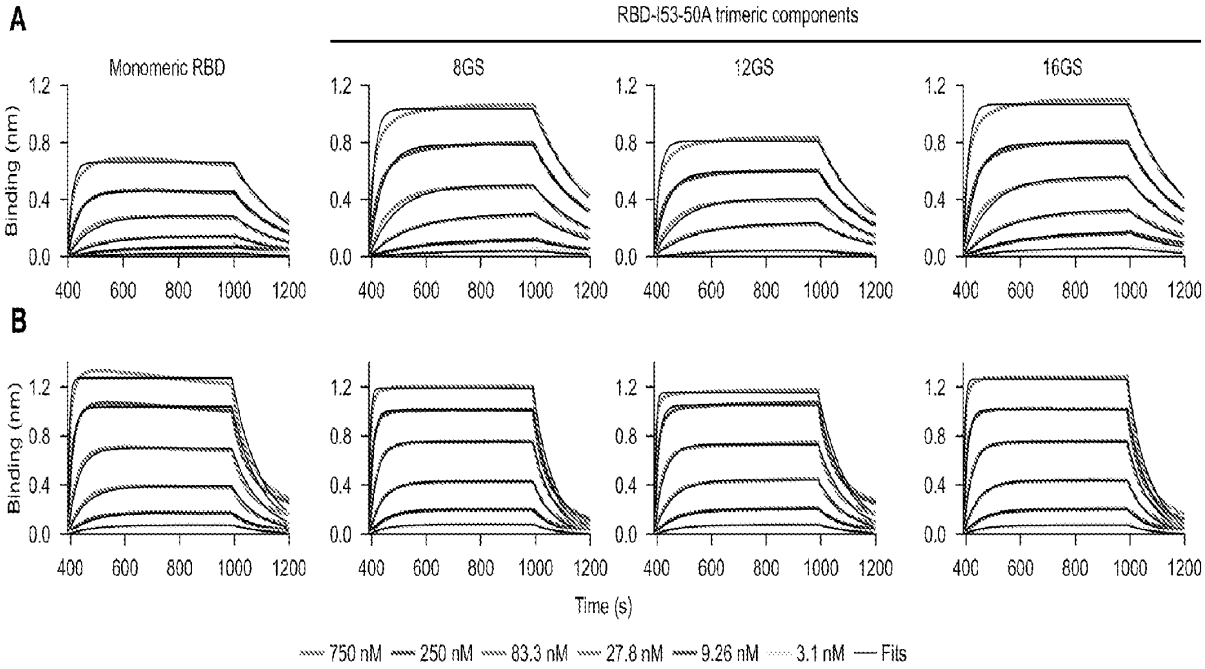

A
RBD-I53-50A trimeric components
Monomeric RBD
8GS
12GS
16GS
Binding (nm)
1.2
0.8
0.4
0.0
400
600
800
1000
1200
B
Time (s)
750 nM
250 nM
83.3 nM
27.8 nM
9.26 nM
3.1 nM
Fits

Figure 9

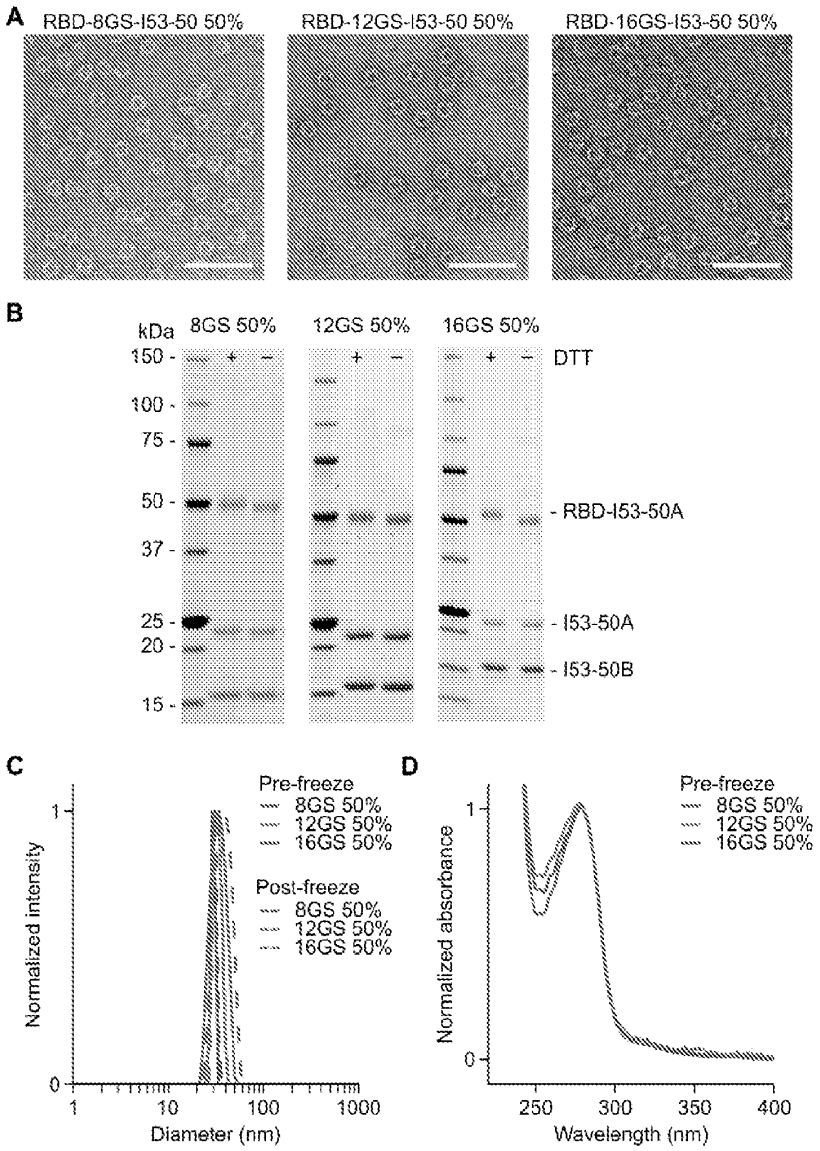

A
RBD-8GS-I53-50 50%
RBD-12GS-I53-50 50%
RBD-16GS-I53-50 50%
B
kDa
8GS 50%
12GS 50%
16GS 50%
DTT
150
100
75
50
37
25
20
15
- RBD-I53-50A
- I53-50A
- I53-50B
C
Pre-freeze
8GS 50%
12GS 50%
16GS 50%
Post-freeze
8GS 50%
12GS 50%
16GS 50%
Normalized intensity
Diameter (nm)
D
Pre-freeze
8GS 50%
12GS 50%
16GS 50%
Normalized absorbance
Wavelength (nm)

Figure 10

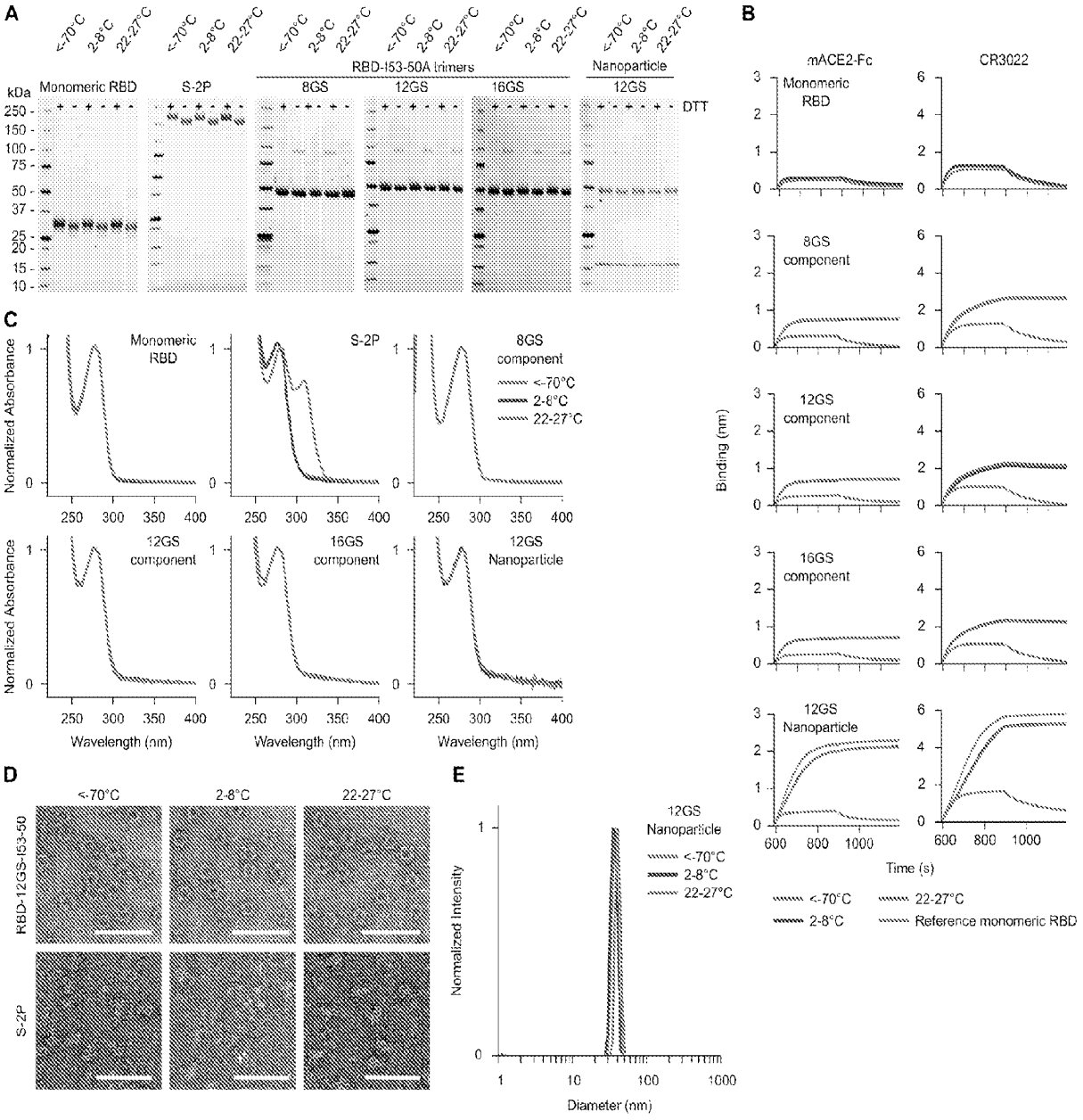

A
<-70°C
2-8°C
22-27°C
RBD-I53-50A trimers
Nanoparticle
kDa
Monomeric RBD
S-2P
8GS
12GS
16GS
12GS
DTT
250
150
100
75
50
37
25
20
15
10
B
mACE2-Fc
CR3022
Monomeric RBD
8GS component
12GS component
16GS component
12GS Nanoparticle
Binding (nm)
Time (s)
<-70°C
2-8°C
22-27°C
Reference monomeric RBD
C
Normalized Absorbance
Monomeric RBD
S-2P
8GS component
12GS component
16GS component
12GS Nanoparticle
Wavelength (nm)
D
RBD-12GS-I53-50
S-2P
E
Normalized Intensity
Diameter (nm)

Figure 11

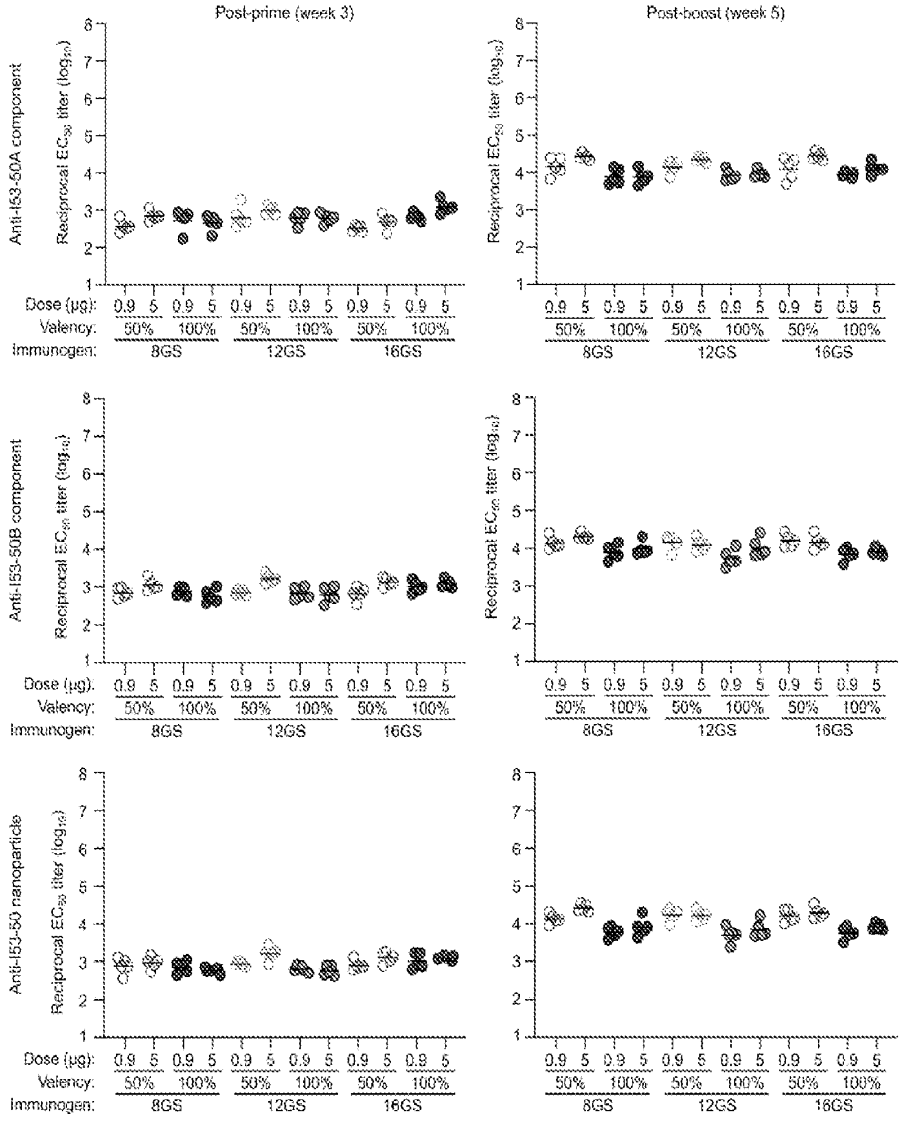
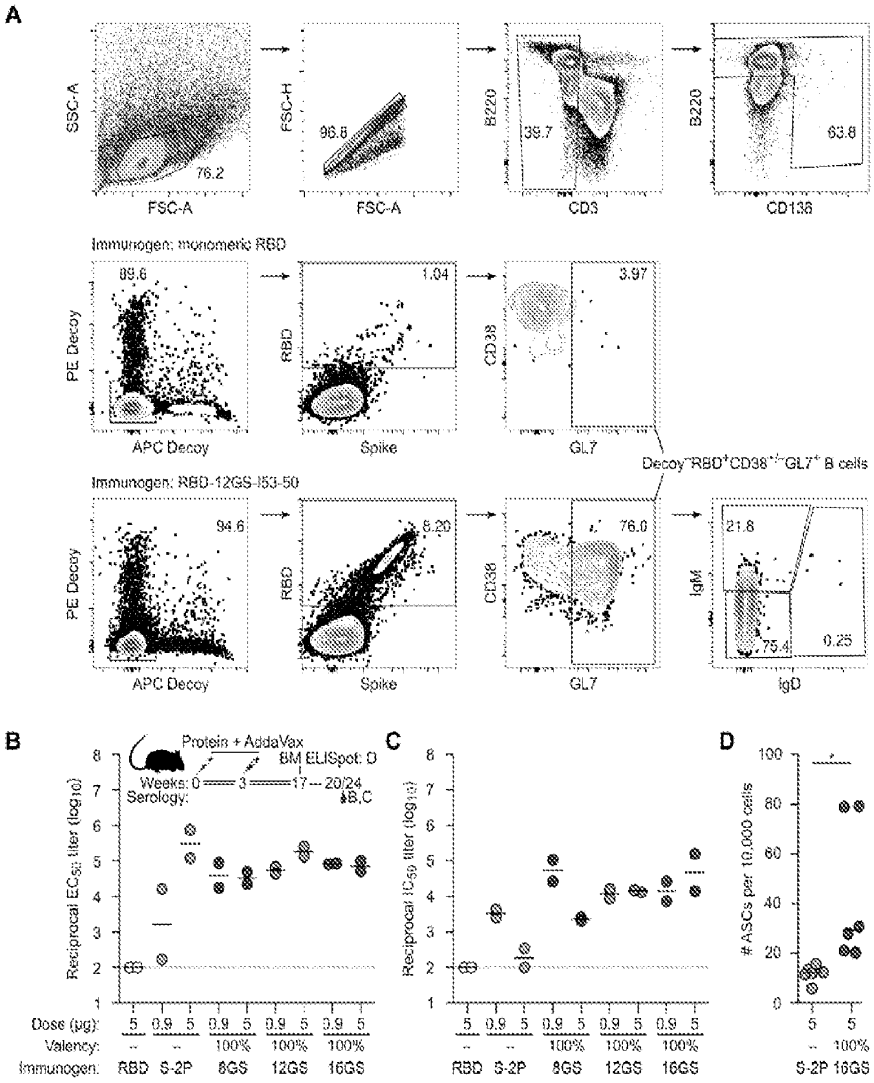

Post-prime (week 3)
Post-boost (week 5)
Anti-I53-50A component
Anti-I53-50B component
Anti-I53-50 nanoparticle
Reciprocal EC50 titer (log10)
Dose (μg):
Valency:
Immunogen:
0.9 5
50% 100%
8GS 12GS 16GS

Figure 12


A
SSC-A
FSC-A
76.2
FSC-H
FSC-A
96.6
B220
CD3
39.7
B220
CD138
63.8
Immunogen: monomeric RBD
PE Decoy
APC Decoy
89.6
RBD
Spike
1.04
CD38
GL7
3.97
Decoy⁻RBD⁺CD38⁺/⁻GL7⁺ B cells
Immunogen: RBD-12GS-I53-50
PE Decoy
APC Decoy
94.6
RBD
Spike
8.20
CD38
GL7
76.0
IgM
IgD
21.8
75.4
0.25
B
Protein + AddaVax
BM ELISpot: D
Weeks: 0 3 17 20/24
Serology: ↓B,C
Reciprocal EC50 titer (log10)
Dose (μg): 5 0.9 5 0.9 5 0.9 5 0.9 5
Valency: -- -- 100% 100% 100%
Immunogen: RBD S-2P 8GS 12GS 16GS
C
Reciprocal IC50 titer (log10)
RBD S-2P 8GS 12GS 16GS
D
ASCs per 10,000 cells
5 5
-- 100%
S-2P 16GS