FIG. 1

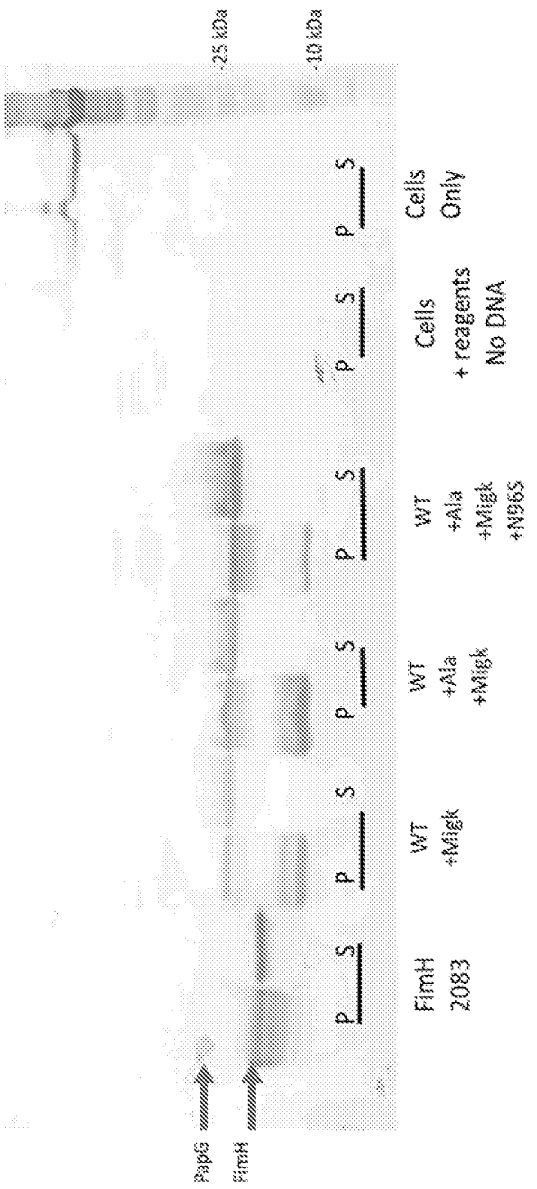

~25 kDa
~10 kDa
PapG
FimH
P S
FimH 2083
WT +Migk
WT +Ala +Migk
WT +Ala +Migk +N96S
Cells + reagents No DNA
Cells Only

FIG. 2A

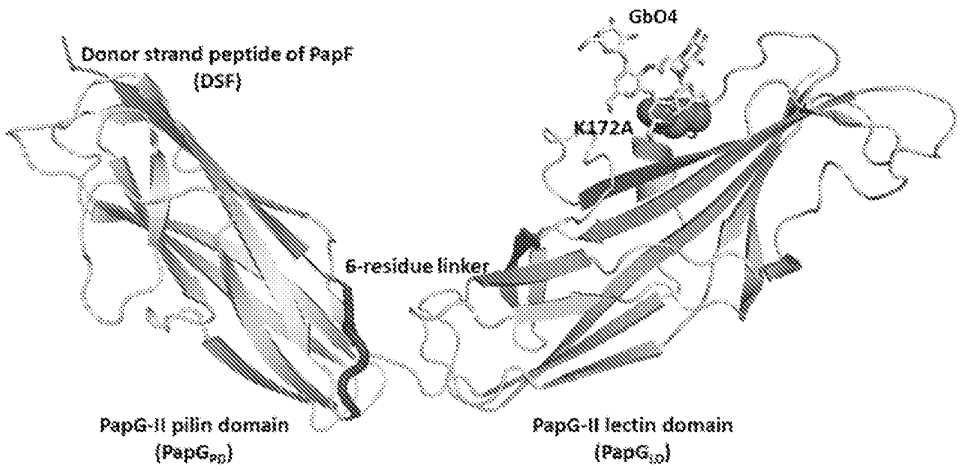

Donor strand peptide of PapF
(DSF)
Gb04
K172A
6-residue linker
PapG-II pilin domain
(PapG$_{PD}$)
PapG-II lectin domain
(PapG$_{LD}$)

FIG. 2B

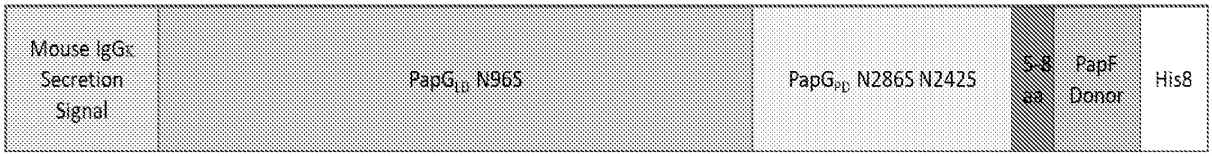

Mouse IgGκ Secretion Signal
PapG$_{LD}$ N96S
PapG$_{PD}$ N286S N242S
5-8 aa
PapF Donor
His8

FIG. 3

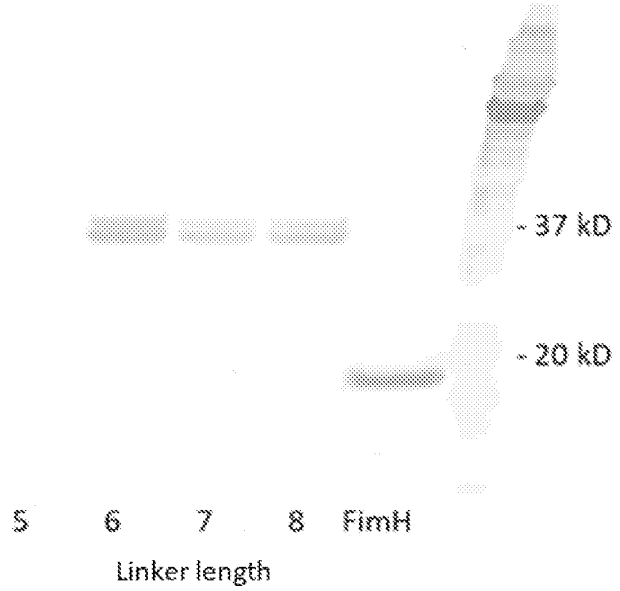


FIG. 4

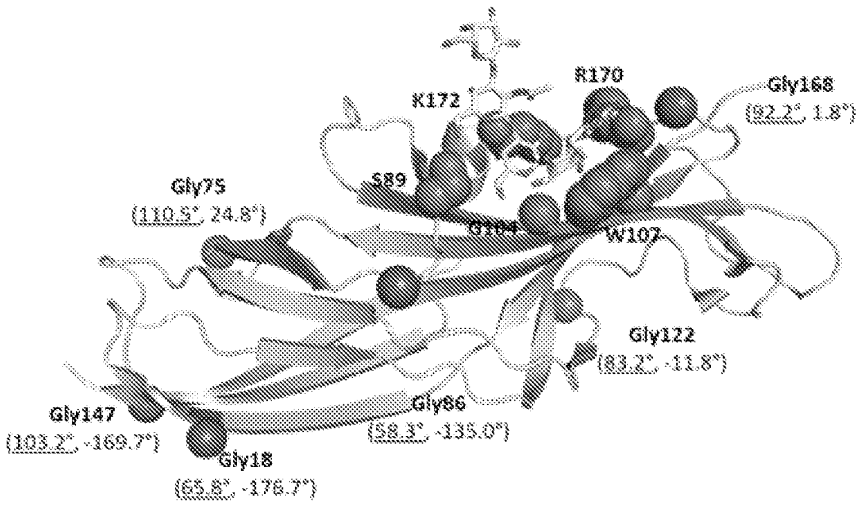

R170
Gly168
(92.2°, 1.8°)
K172
S89
Gly75
(110.5°, 24.8°)
G164
W107
Gly122
(83.2°, -11.8°)
Gly86
(58.3°, -135.0°)
Gly147
(103.2°, -169.7°)
Gly18
(65.8°, -176.7°)

FIG. 5A

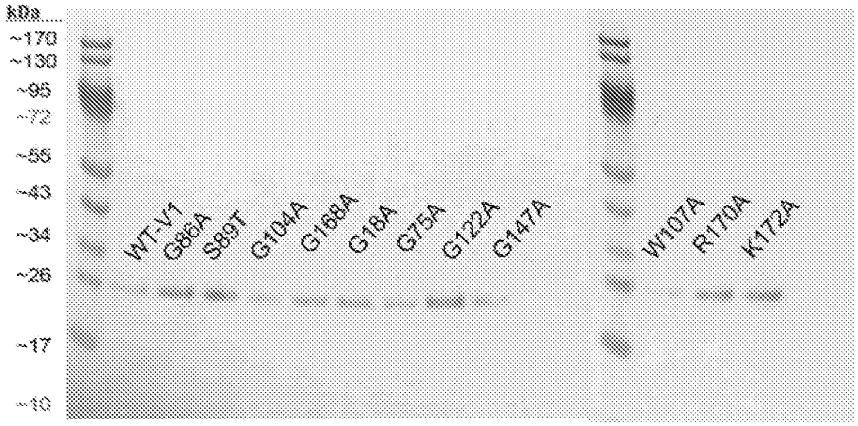

kDa
~170
~130
~95
~72
~55
~43
~34
~26
~17
~10
WT-V1
G86A
S89T
G104A
G168A
G16A
G75A
G122A
G147A
W107A
R170A
K172A

FIG. 5B

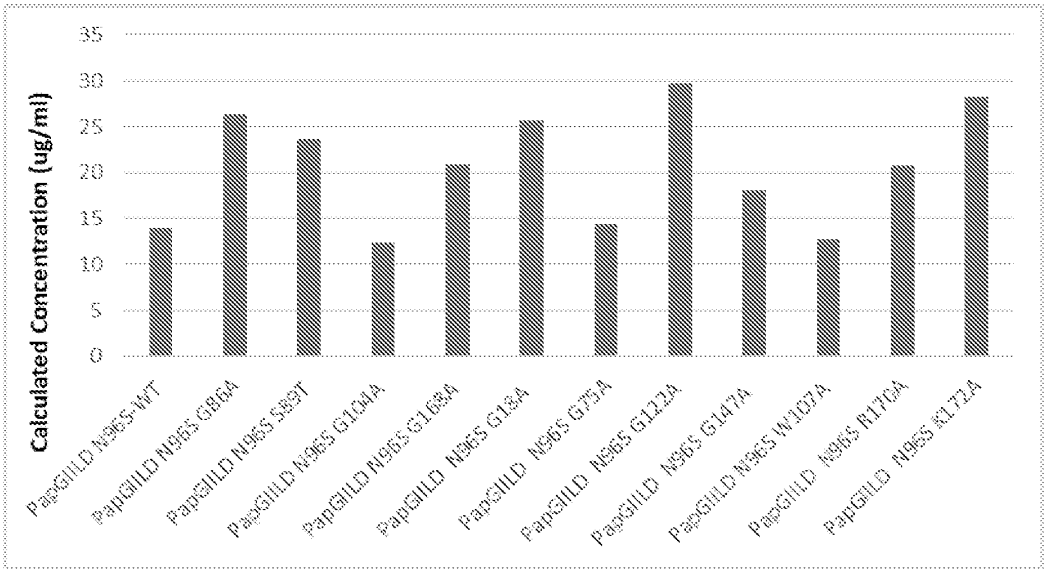

Calculated Concentration (ug/ml)
35
30
25
20
15
10
5
0
PapGILD N965-WT
PapGILD N965 G86A
PapGILD N965 S89T
PapGILD N965 G104A
PapGILD N965 G168A
PapGILD N965 G13A
PapGILD N965 G75A
PapGILD N965 G122A
PapGILD N965 G147A
PapGILD N965 W107A
PapGILD N965 R170A
PapGILD N965 K172A

FIG. 6

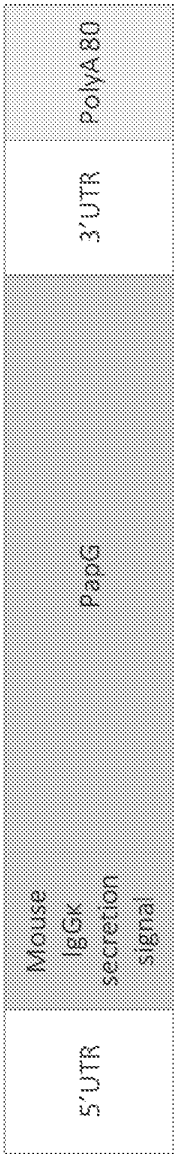


FIG. 7A

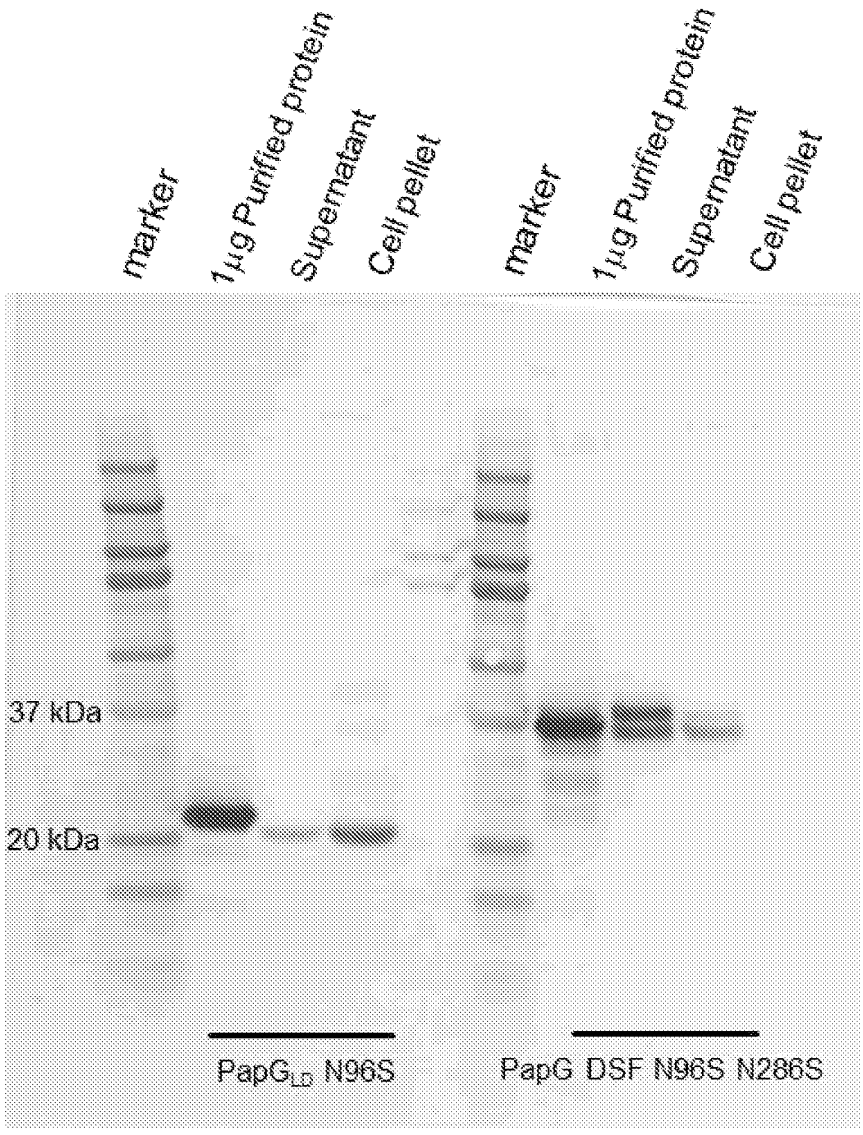

marker
1 µg Purified protein
Supernatant
Cell pellet
marker
1 µg Purified protein
Supernatant
Cell pellet
37 kDa
20 kDa
PapG$_{LD}$ N96S
PapG DSF N96S N286S

FIG. 7B

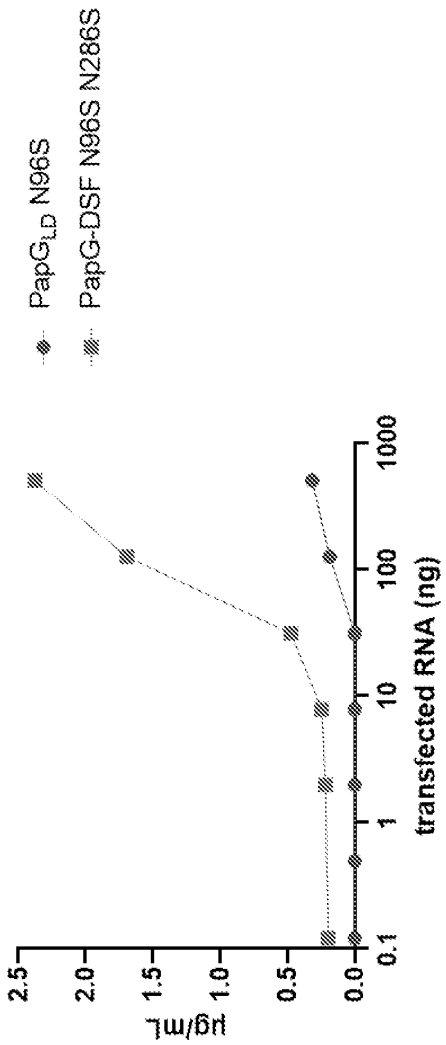

PapG$_{LD}$ N96S
PapG-DSF N96S N286S
µg/mL
2.5
2.0
1.5
1.0
0.5
0.0
0.1
1
10
100
1000
transfected RNA (ng)

FIG. 8

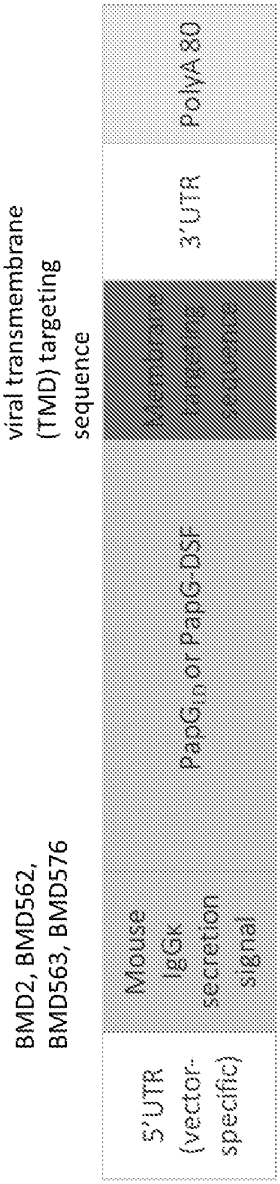

5'UTR (vector-specific)
Mouse IgGκ secretion signal
PapG$_{II}$ or PapG-DSF
DAF-GPI, Thy1-GPI, viral transmembrane (TMD) targeting sequence
3'UTR
PolyA 80
BMD2, BMD562, BMD563, BMD576

FIG. 9

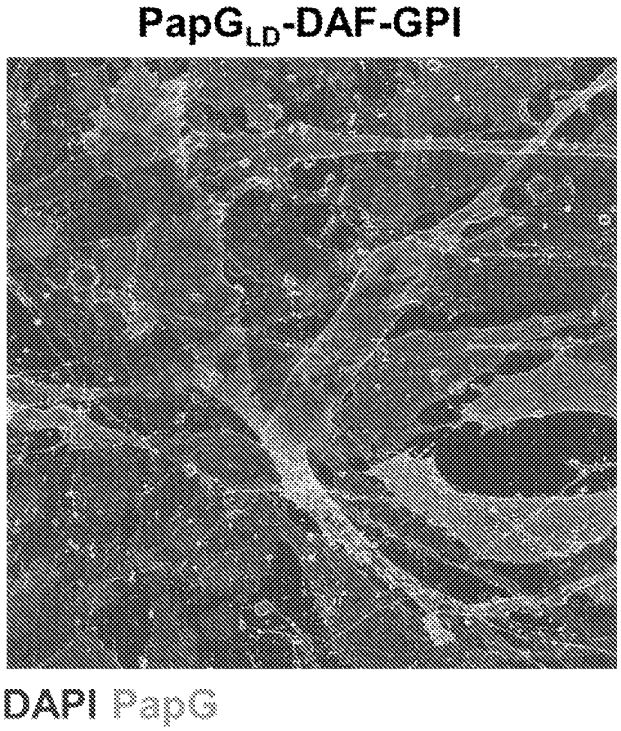


FIG. 10

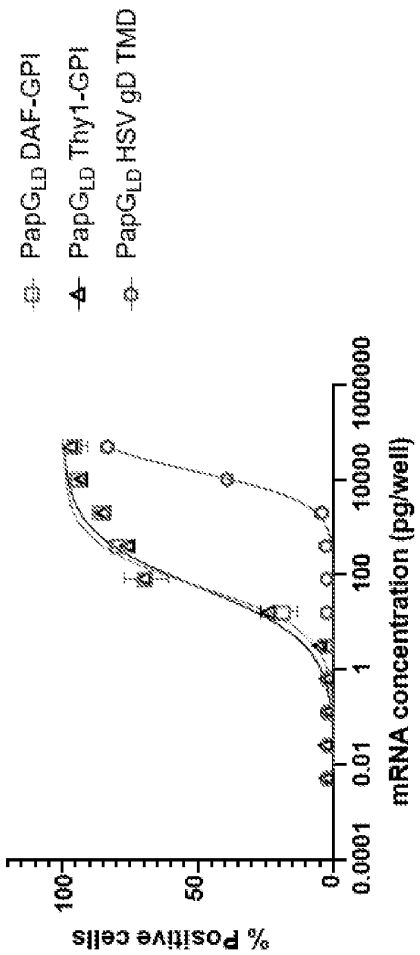

PapG$_{LD}$ DAF-GPI
PapG$_{LD}$ Thy1-GPI
PapG$_{LD}$ HSV gD TMD
% Positive cells
100
50
0
0.0001
0.01
1
100
10000
1000000
mRNA concentration (pg/well)

FIG. 11

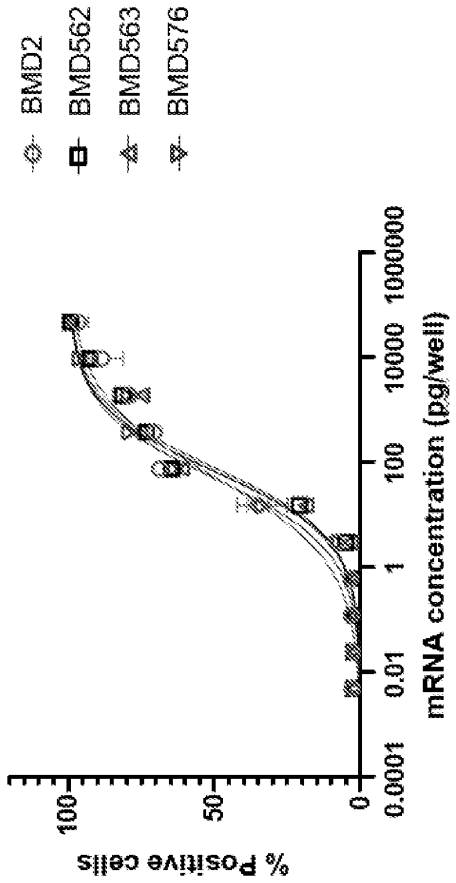

BMD2
BMD562
BMD563
BMD576
% Positive cells
100
50
0
0.0001
0.01
1
100
10000
1000000
mRNA concentration (pg/well)

FIG. 12

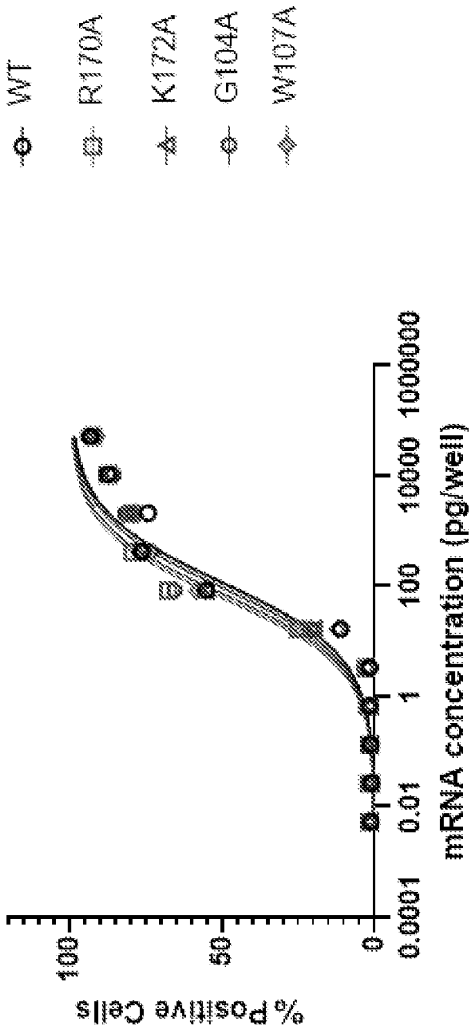

WT
R170A
K172A
G104A
W107A
% Positive Cells
100
50
0
0.0001
0.01
1
100
10000
1000000
mRNA concentration (pg/well)

FIG. 13A

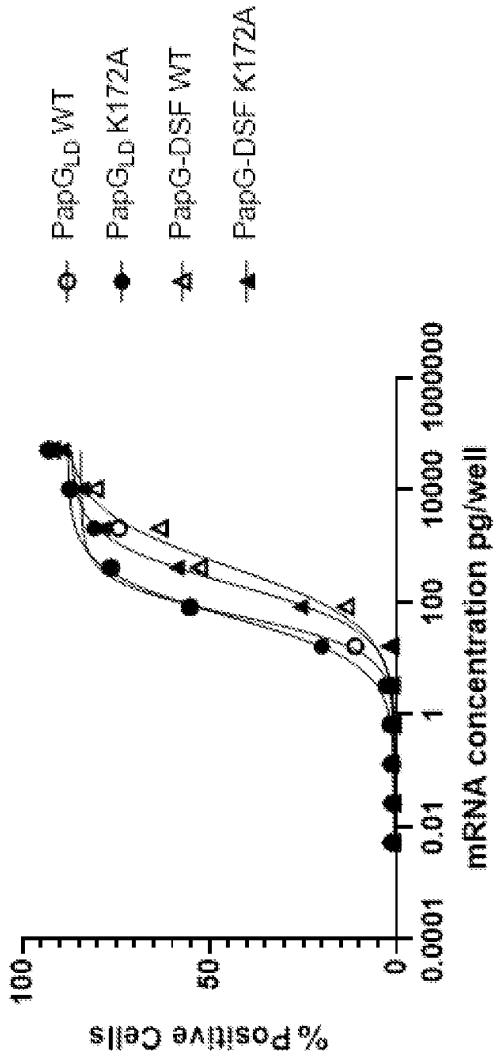

PapG$_{LD}$ WT
PapG$_{LD}$ K172A
PapG-DSF WT
PapG-DSF K172A
% Positive Cells
100
50
0
0.0001
0.01
1
100
10000
1000000
mRNA concentration pg/well

FIG. 13B

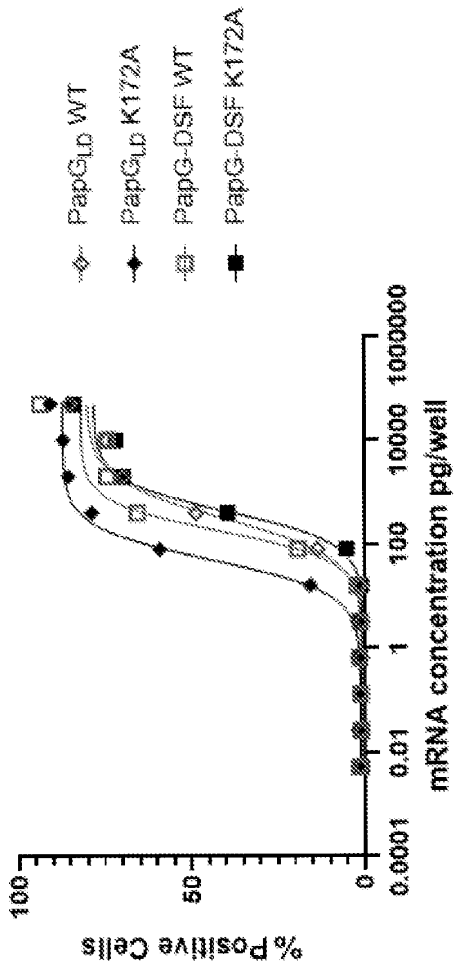

PapG$_{LD}$ WT
PapG$_{LD}$ K172A
PapG-DSF WT
PapG-DSF K172A
% Positive Cells
100
50
0
0.0001
0.01
1
100
10000
1000000
mRNA concentration pg/well

FIG. 14

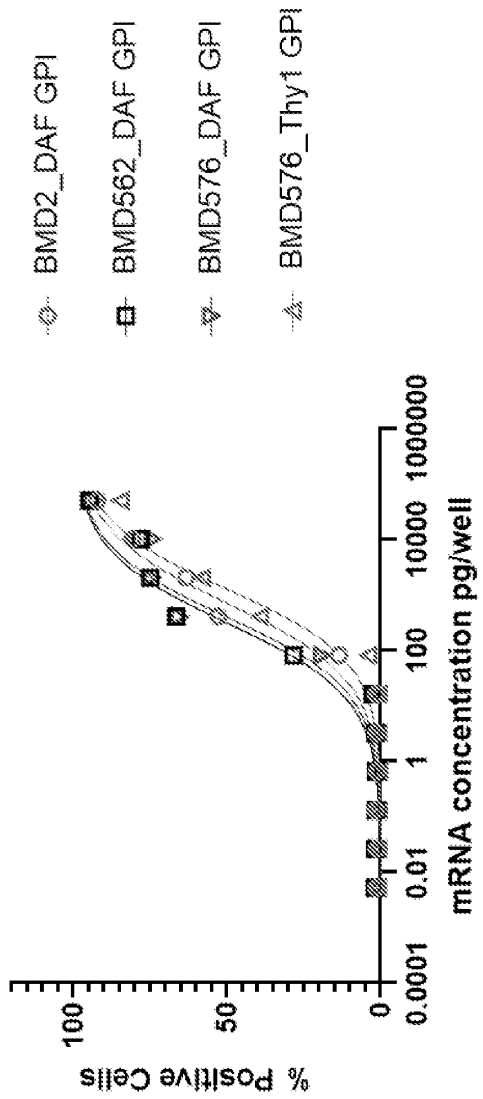

BMD2_DAF GPI
BMD562_DAF GPI
BMD576_DAF GPI
BMD576_Thy1 GPI
% Positive Cells
100
50
0
0.0001
0.01
1
100
10000
1000000
mRNA concentration pg/well

FIG. 15

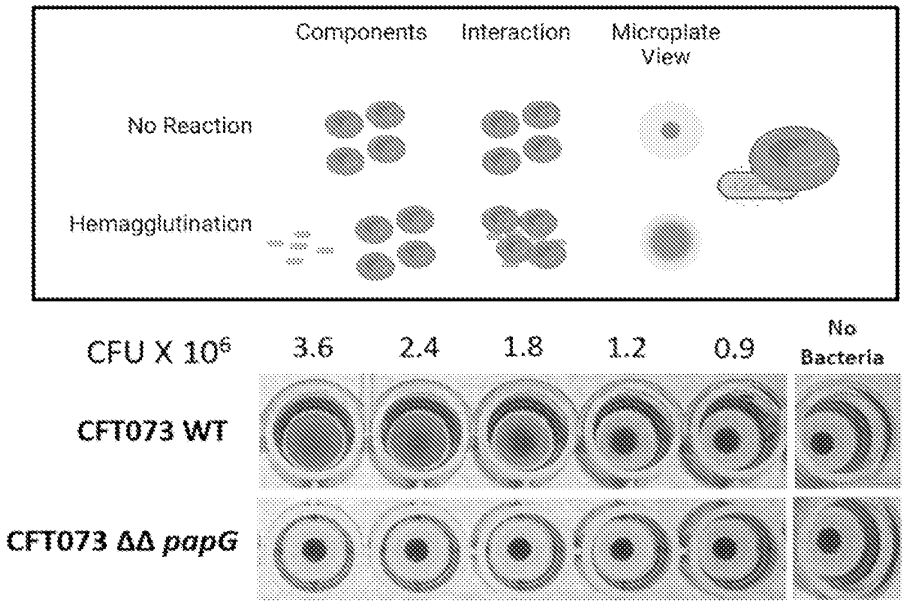

Components
Interaction
Microplate View
No Reaction
Hemagglutination
CFU X 10^6
3.6
2.4
1.8
1.2
0.9
No Bacteria
CFT073 WT
CFT073 ΔΔ papG

FIG. 16A

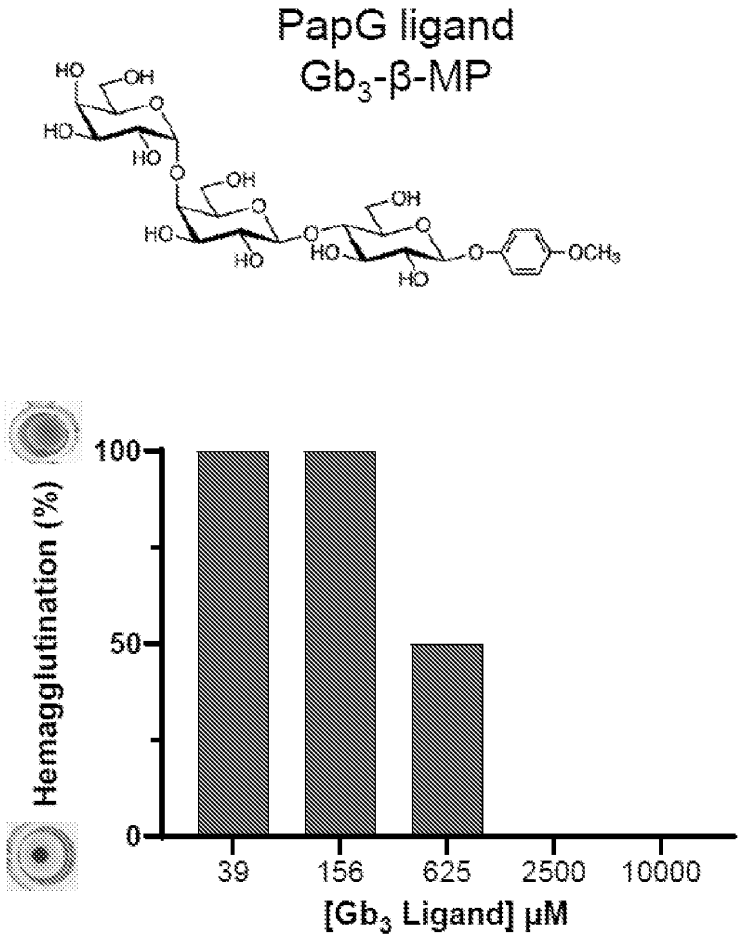

PapG ligand
Gb3-β-MP
HO
OH
O
HO
HO
OH
O
HO
HO
OH
O
HO
HO
O
OCH3
Hemagglutination (%)
100
50
0
39
156
625
2500
10000
[Gb3 Ligand] µM

FIG. 16B

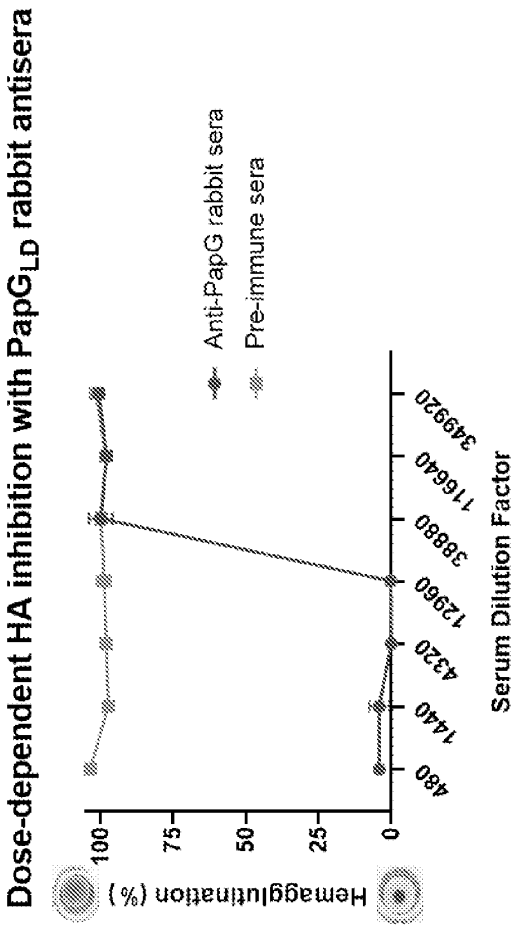

Dose-dependent HA inhibition with PapG$_{LD}$ rabbit antisera
Hemagglutination (%)
100
75
50
25
0
480
1440
4320
12960
38880
116640
349920
Serum Dilution Factor
Anti-PapG rabbit sera
Pre-immune sera

FIG. 17

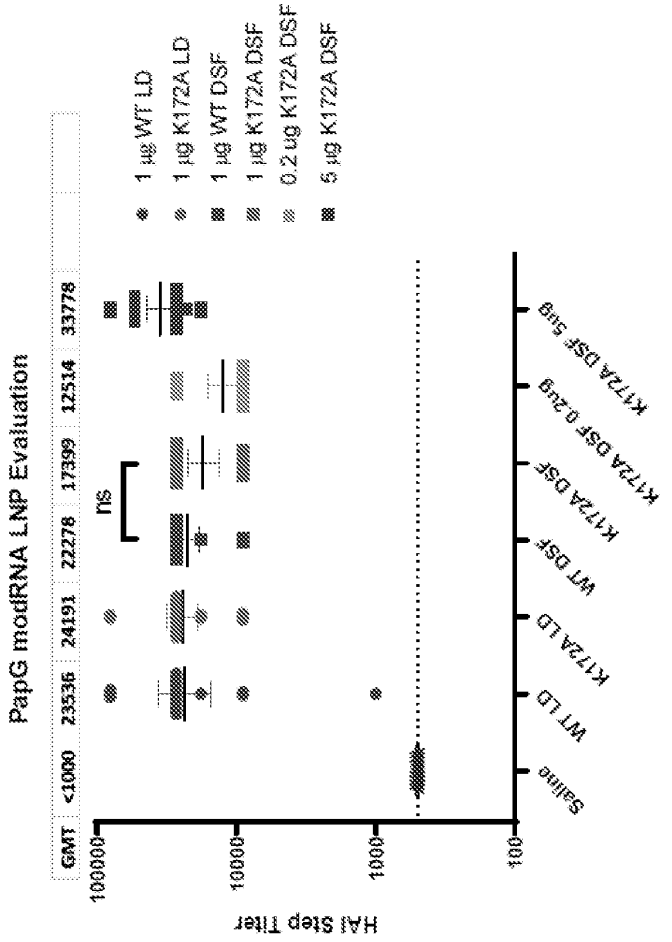

PapG modRNA LNP Evaluation
GMT
<1000
23536
24191
22278
17399
12514
33778
ns
1 µg WT LD
1 µg K172A LD
1 µg WT DSF
1 µg K172A DSF
0.2 ug K172A DSF
5 µg K172A DSF
HAI Step Titer
100000
10000
1000
100
Saline
WT LD
K172A LD
WT DSF
K172A DSF
K172A DSF 0.2ug
K172A DSF 5ug

FIG. 18

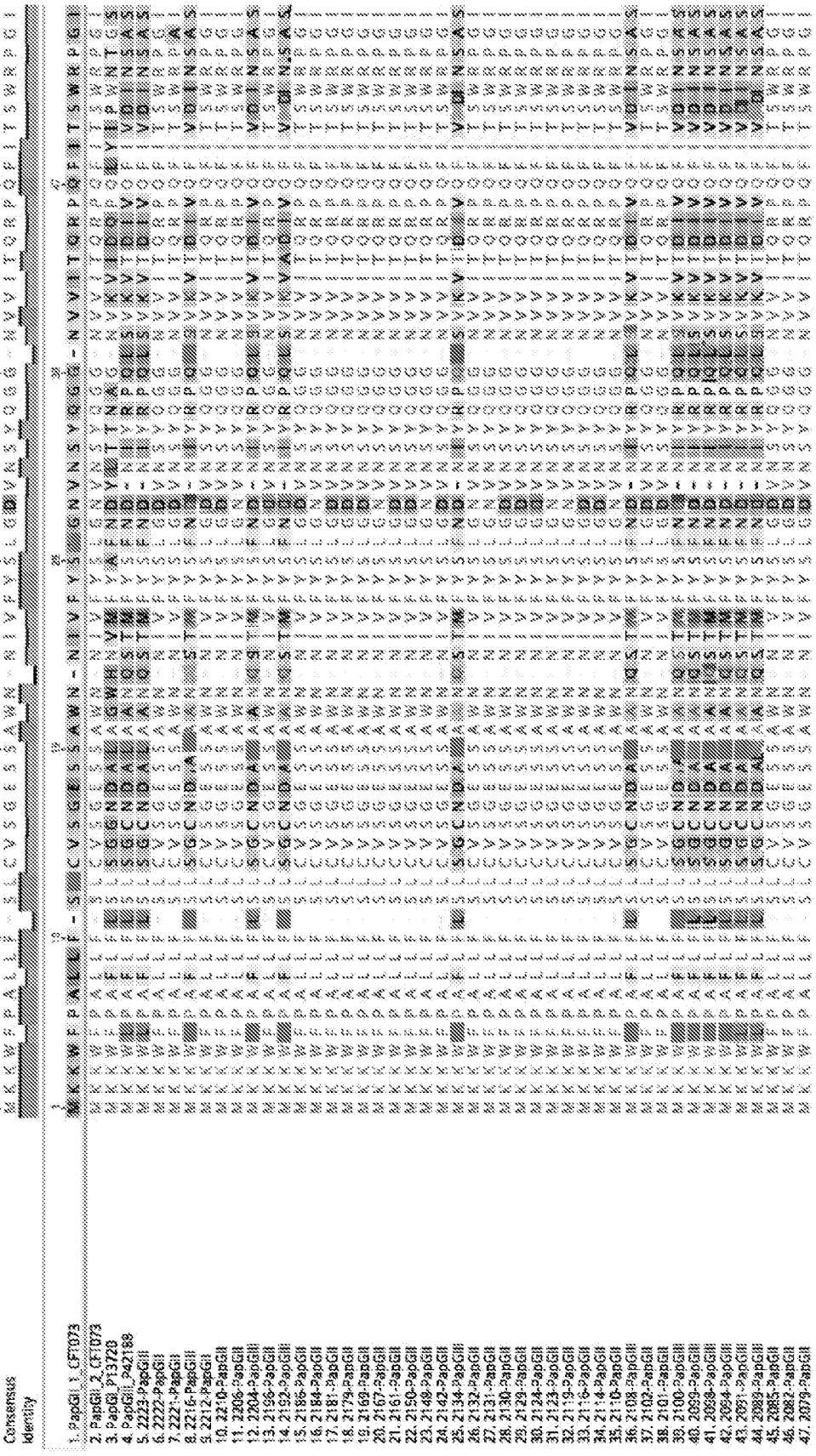


FIG. 18 (CONT.)

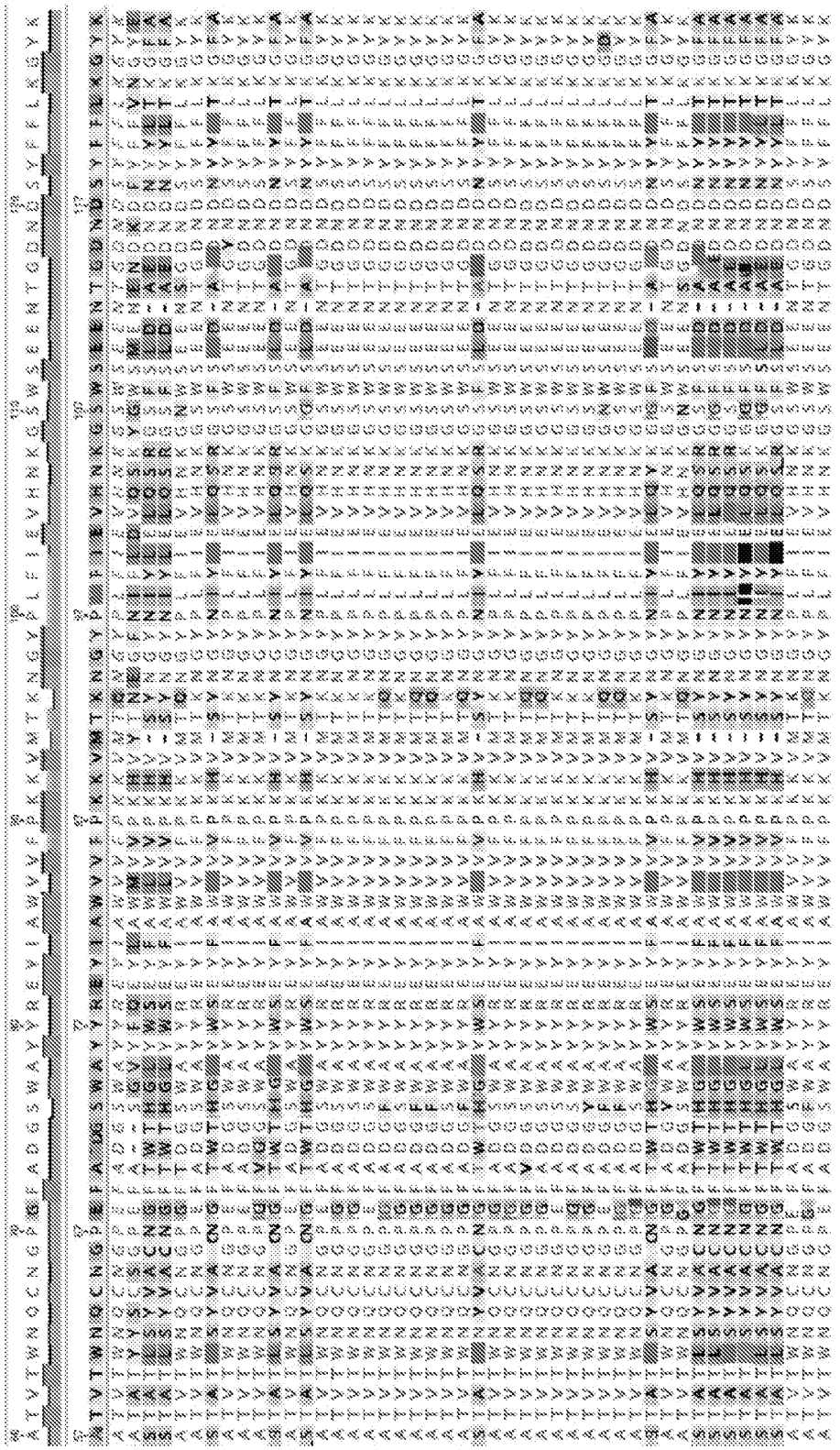


FIG. 18 (CONT.)

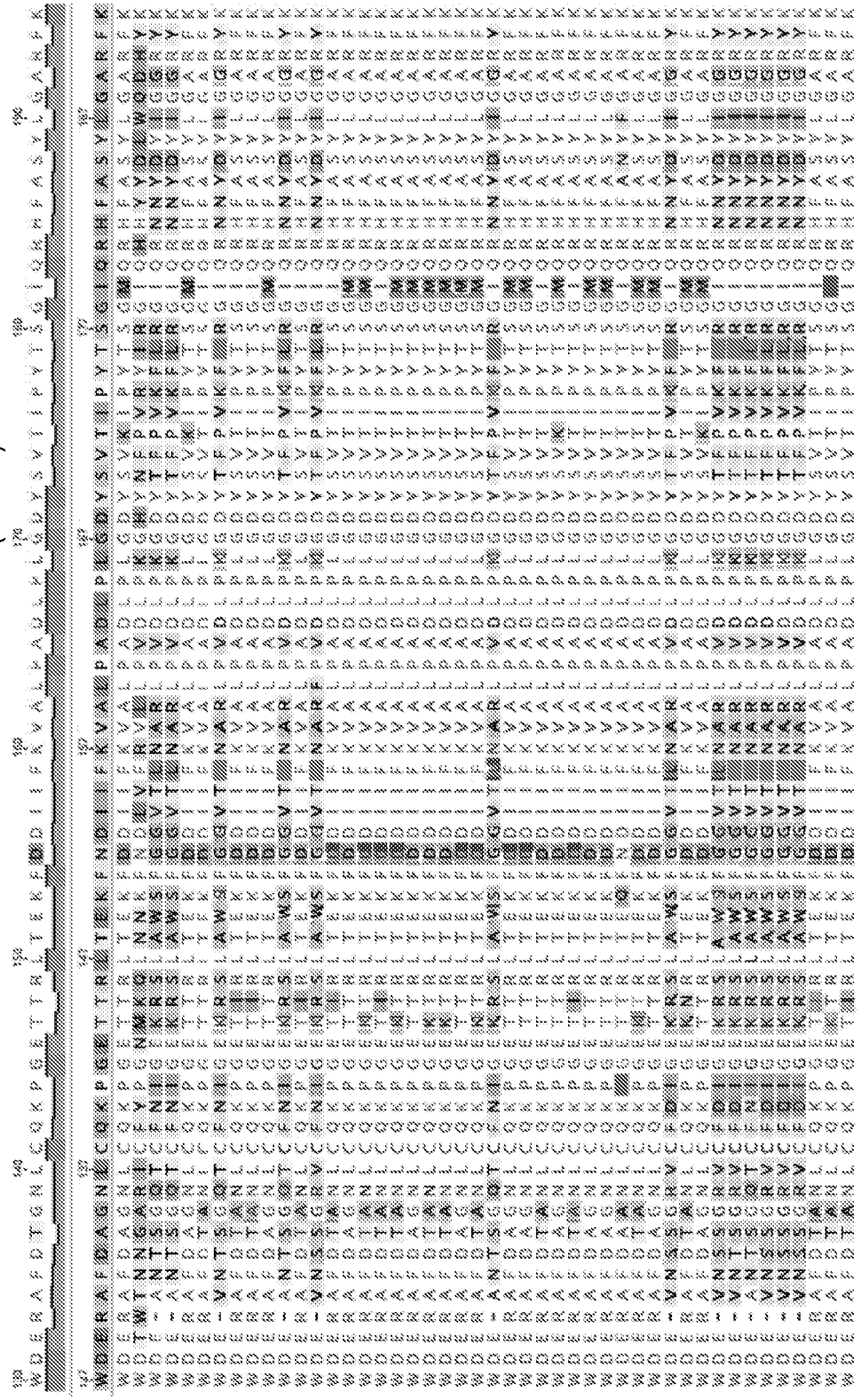


FIG. 18 (CONT.)

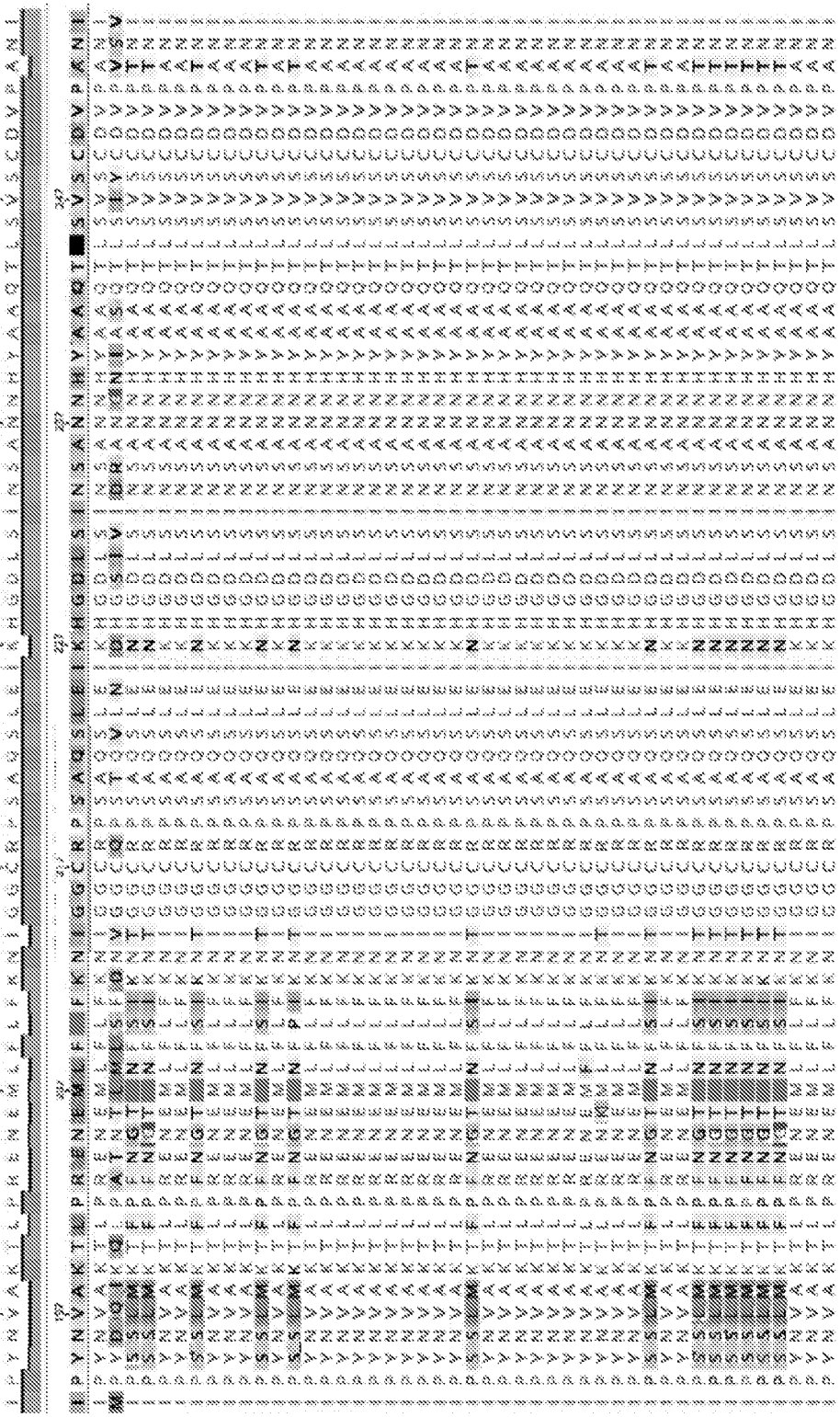


FIG. 18 (CONT.)

