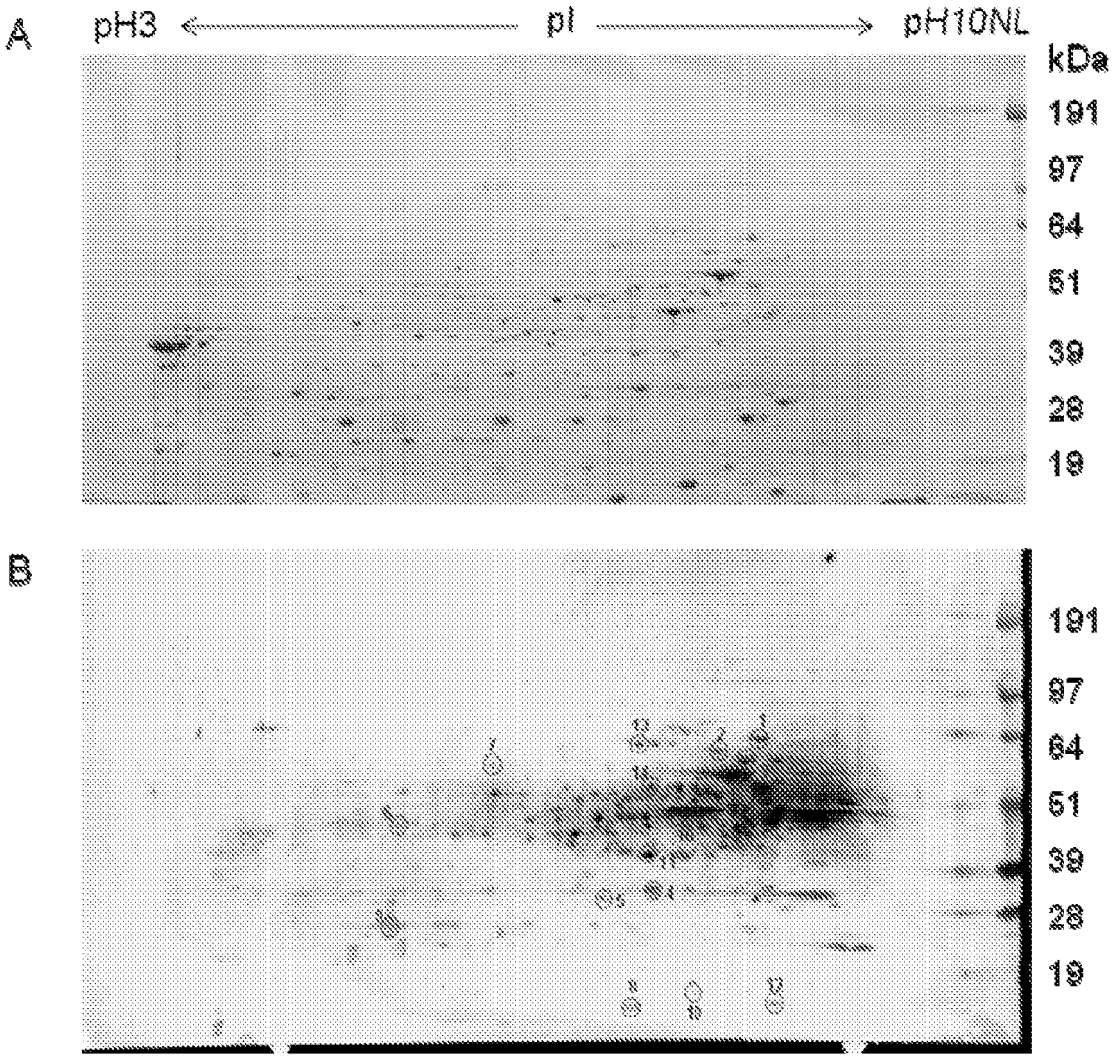


FIG. 1

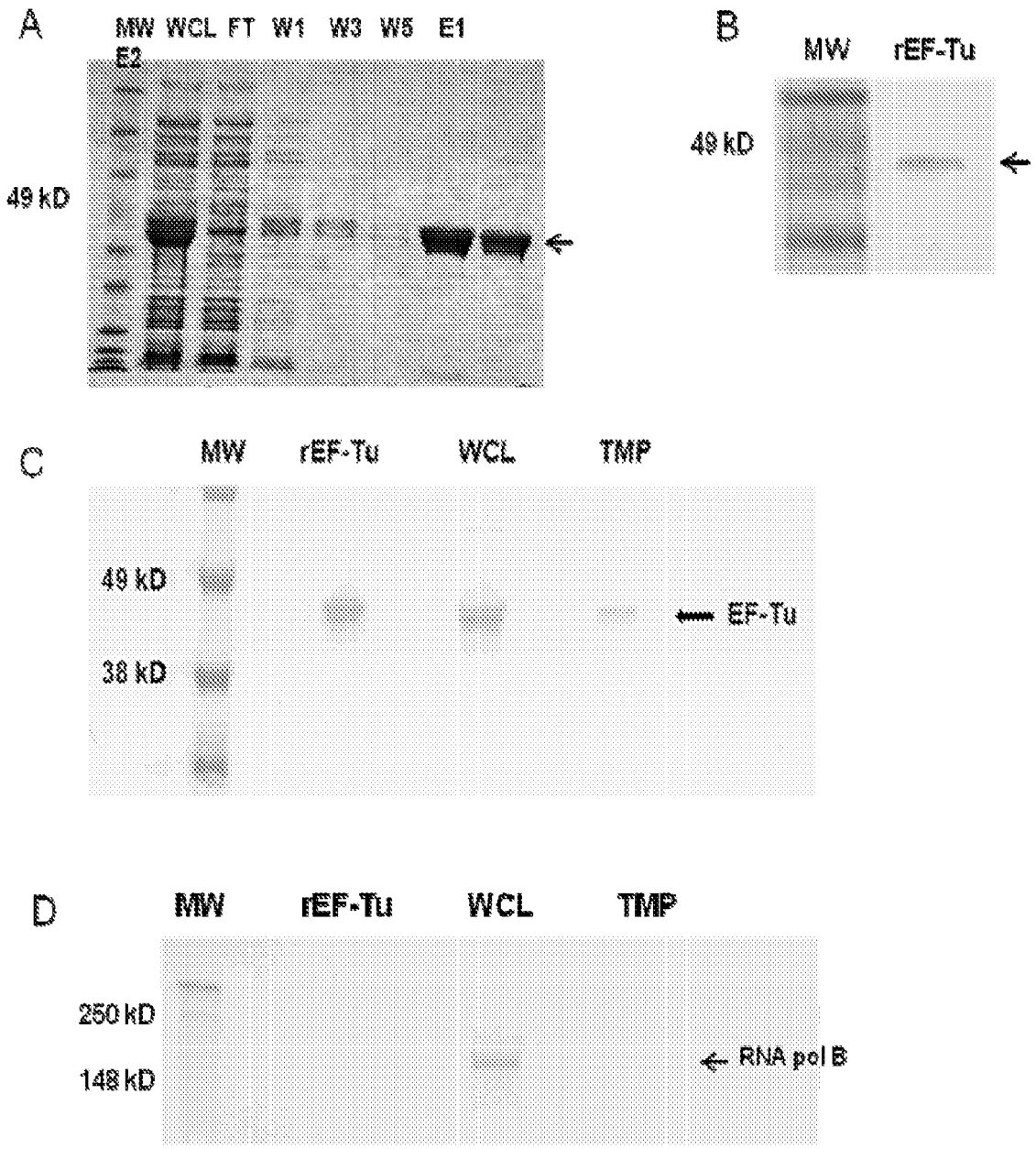

A
MW WCL FT W1 W3 W5 E1
E2
49 kD
B
MW rEF-Tu
49 kD
C
MW rEF-Tu WCL TMP
49 kD
38 kD
EF-Tu
D
MW rEF-Tu WCL TMP
250 kD
148 kD
RNA pol B

FIG. 2

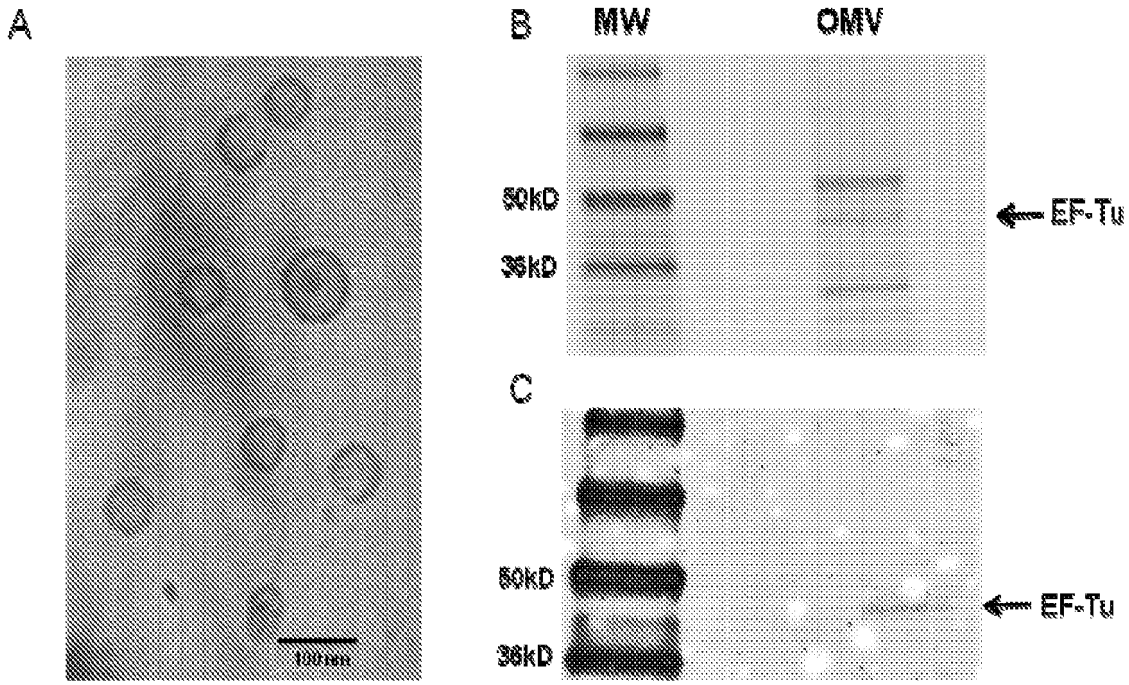


FIG. 3

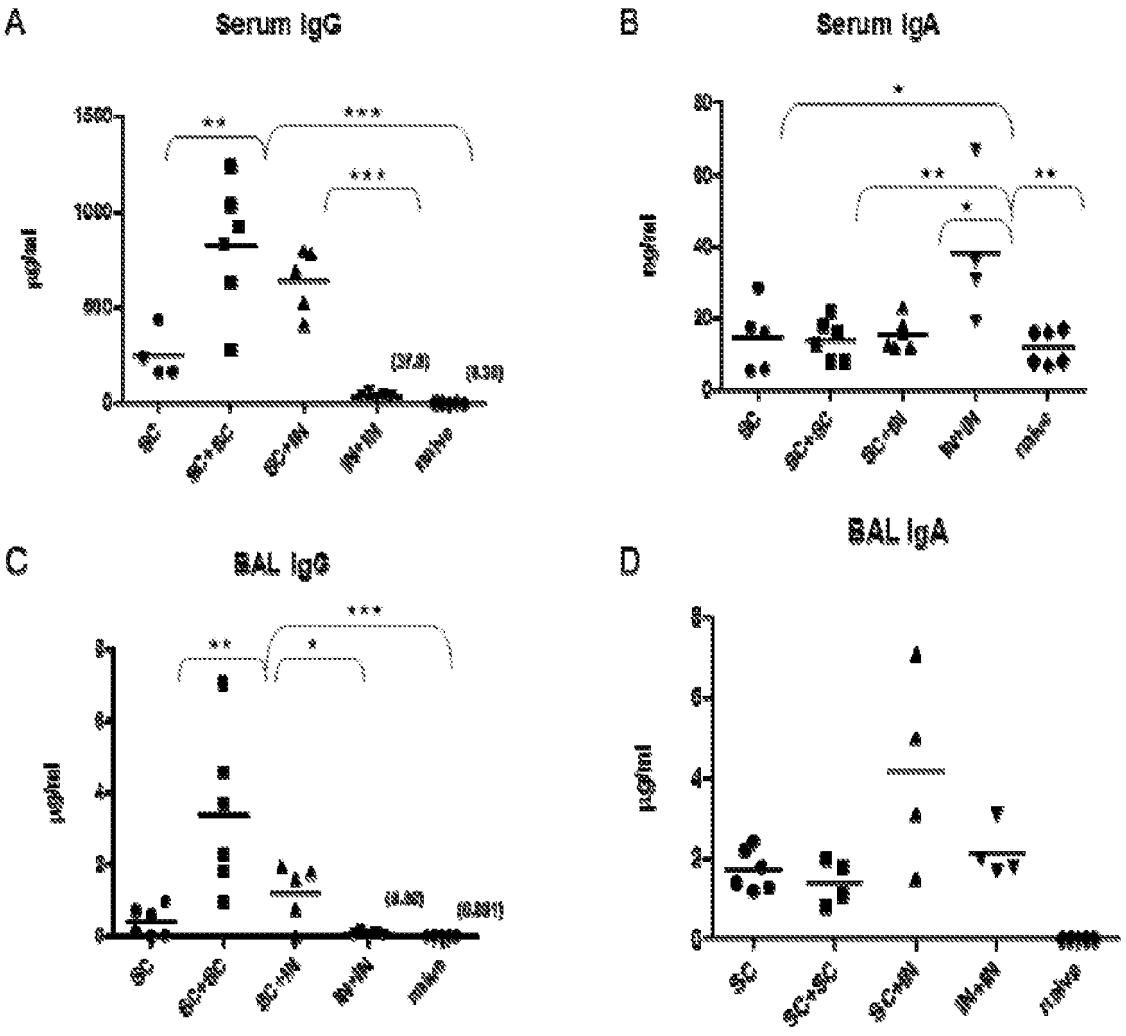


FIG. 4

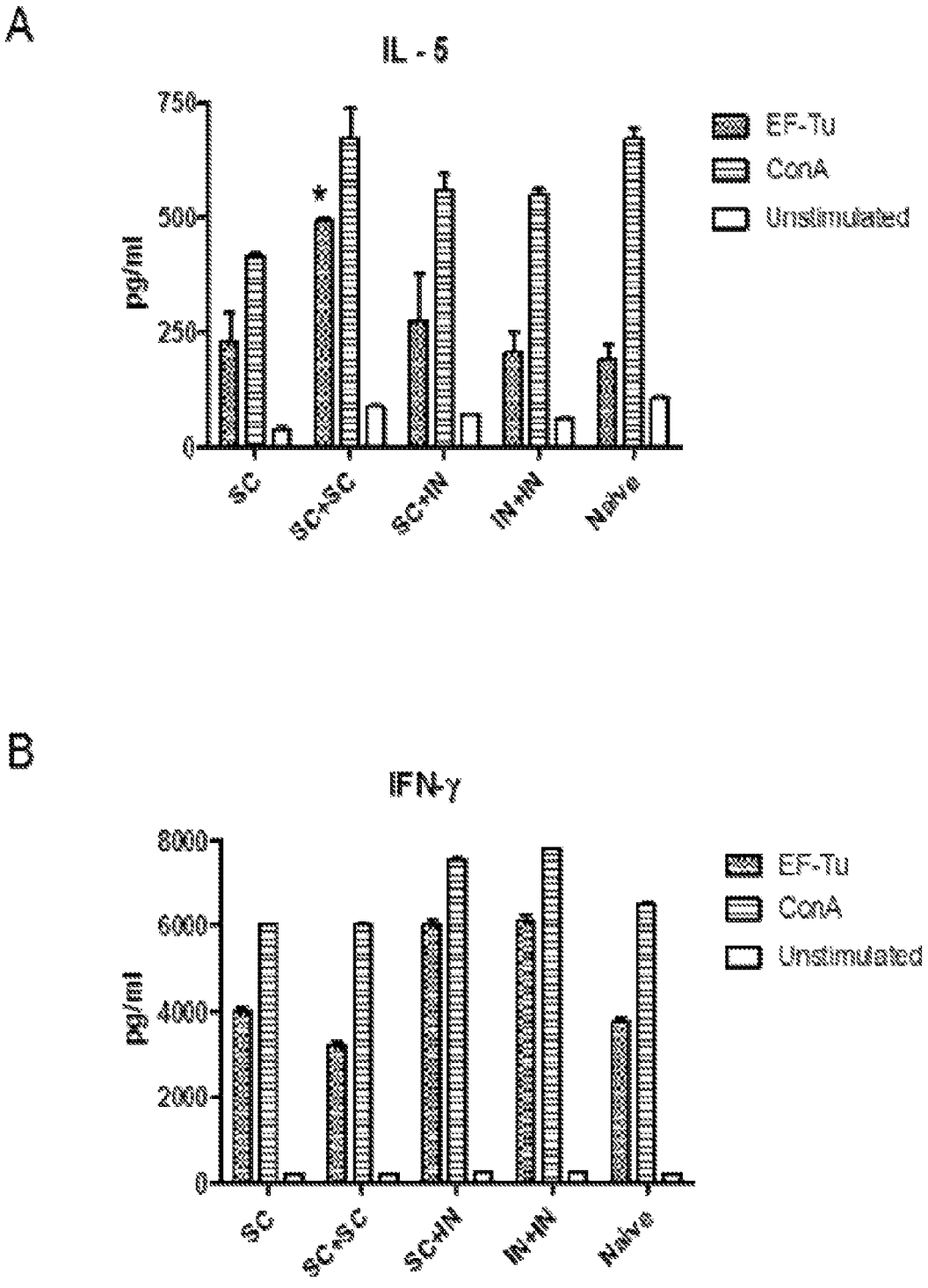

A
IL - 5
750
500
250
0
pg/ml
*
SC
SC+SC
SC+IN
IN+IN
Naïve
EF-Tu
ConA
Unstimulated
B
IFN-γ
8000
6000
4000
2000
0
pg/ml
SC
SC+SC
SC+IN
IN+IN
Naïve
EF-Tu
ConA
Unstimulated

FIG. 5

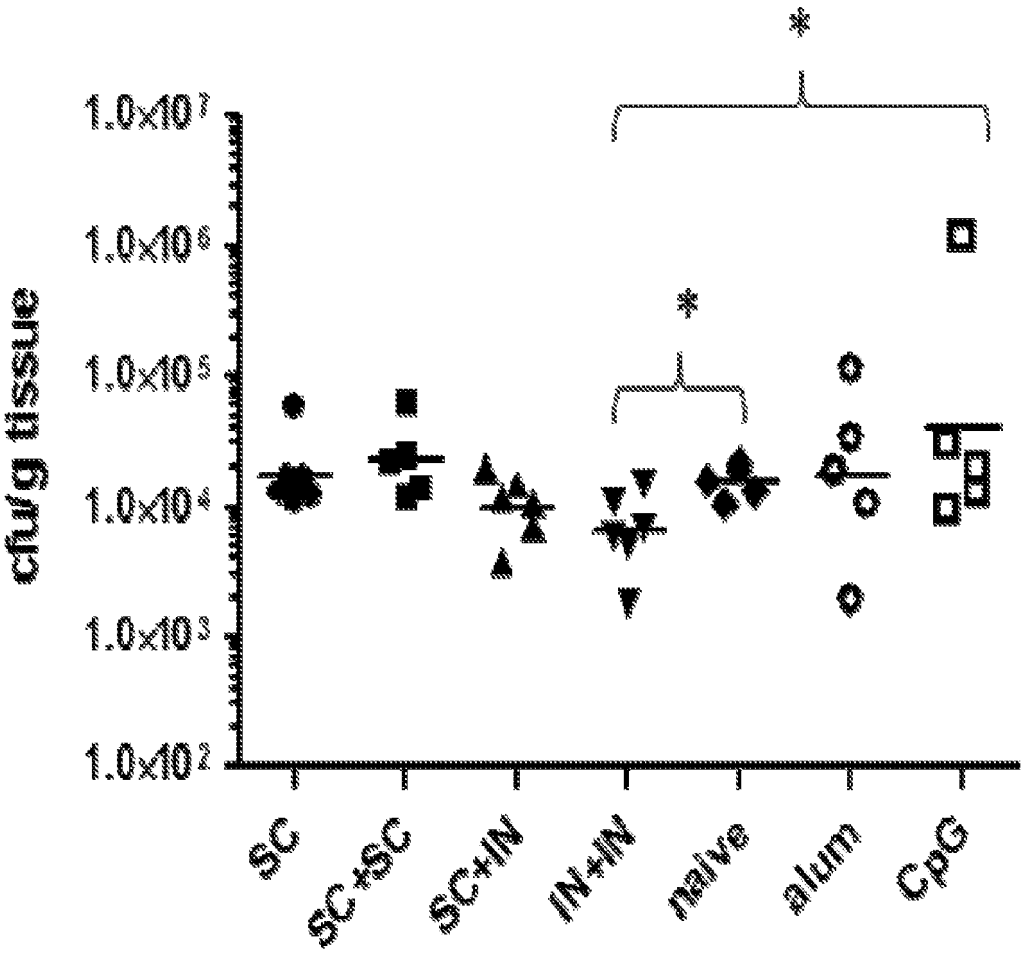

cfu/g tissue
1.0×10⁷
1.0×10⁶
1.0×10⁵
1.0×10⁴
1.0×10³
1.0×10²
*
*
SC
SC+SC
SC+IN
IN+IN
naïve
alum
CpG

FIG. 6

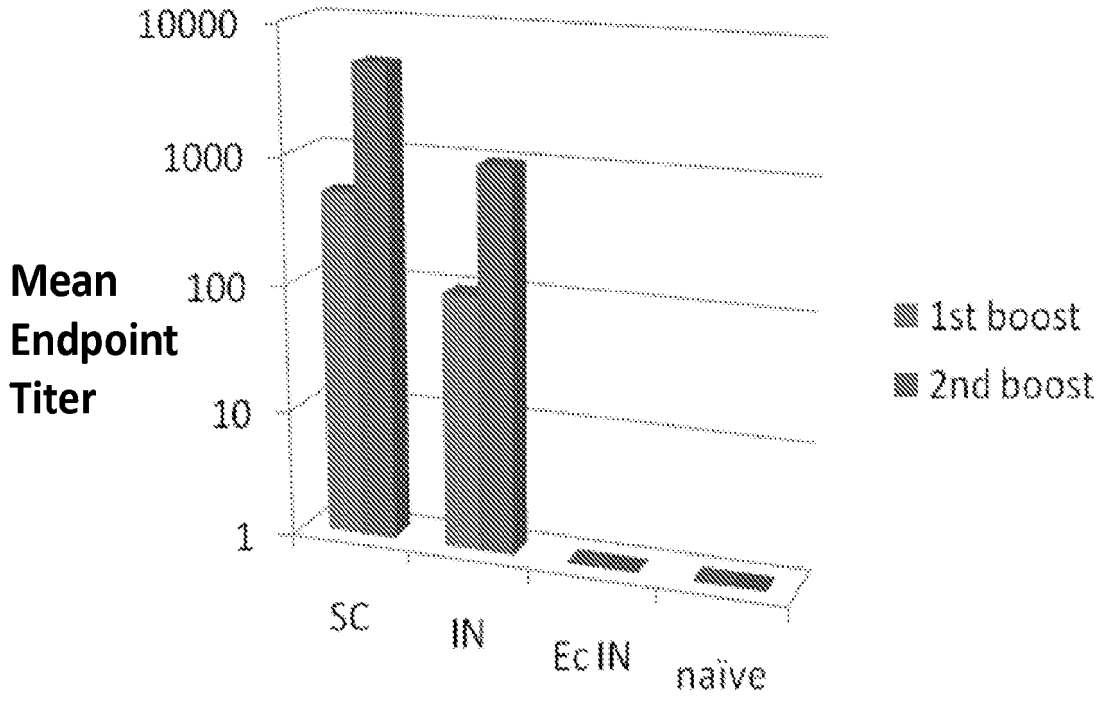

10000
1000
100
10
1
Mean Endpoint Titer
SC
IN
Ec IN
naïve
1st boost
2nd boost

FIG. 7

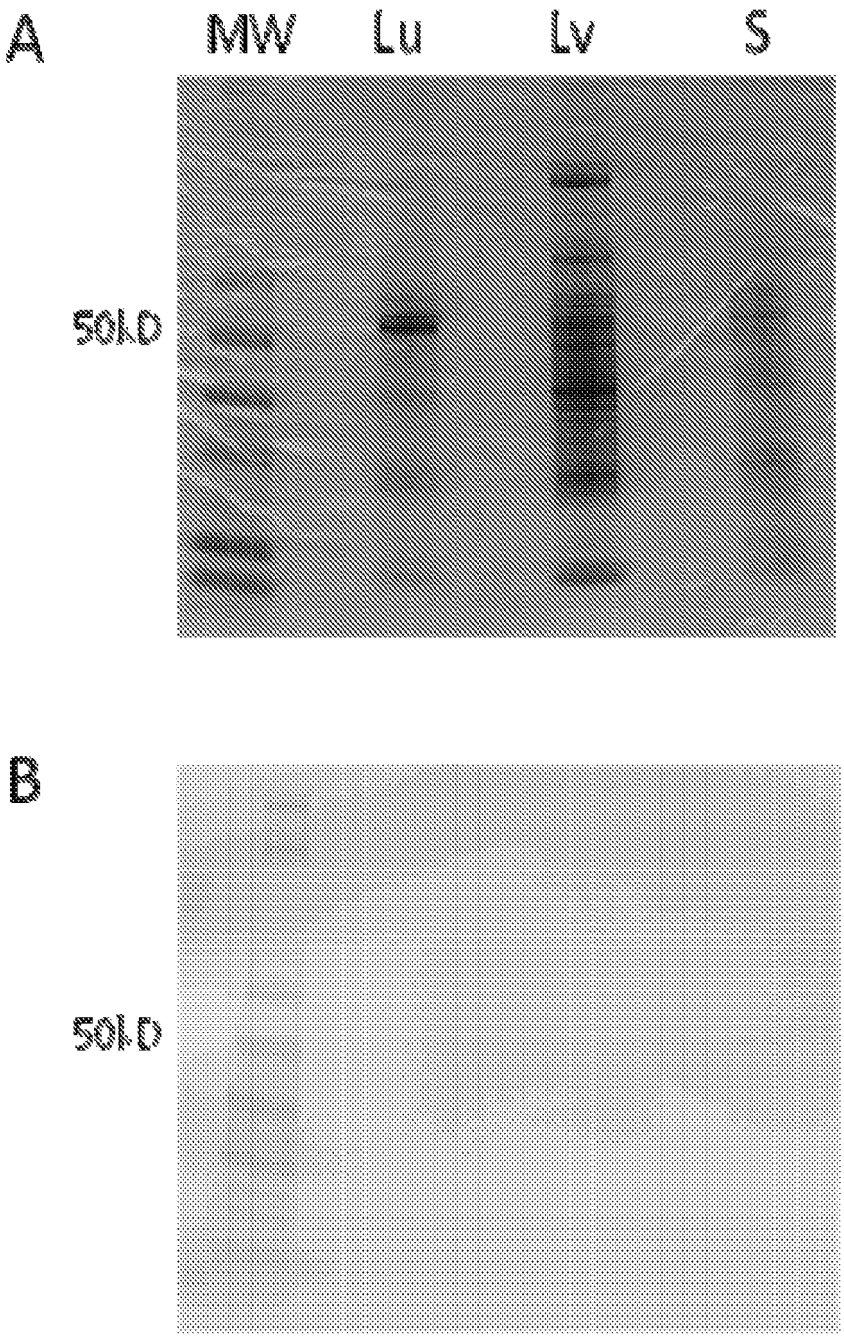


FIG. 8

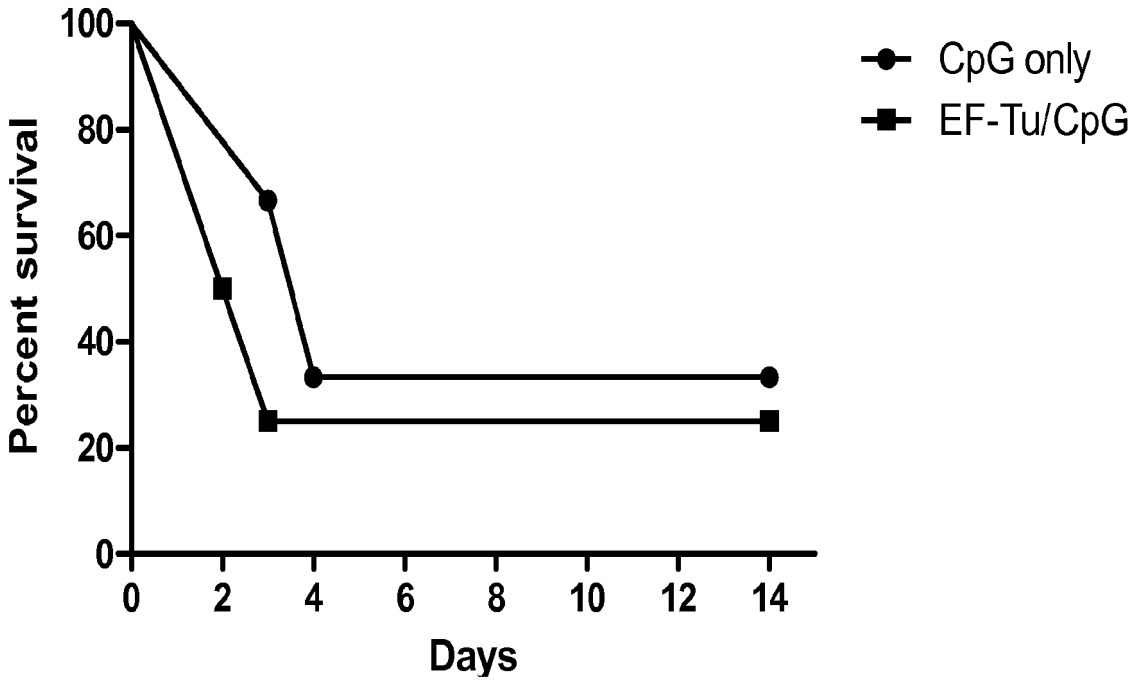

100
80
60
40
20
0
Percent survival
0
2
4
6
8
10
12
14
Days
CpG only
EF-Tu/CpG

FIG. 9

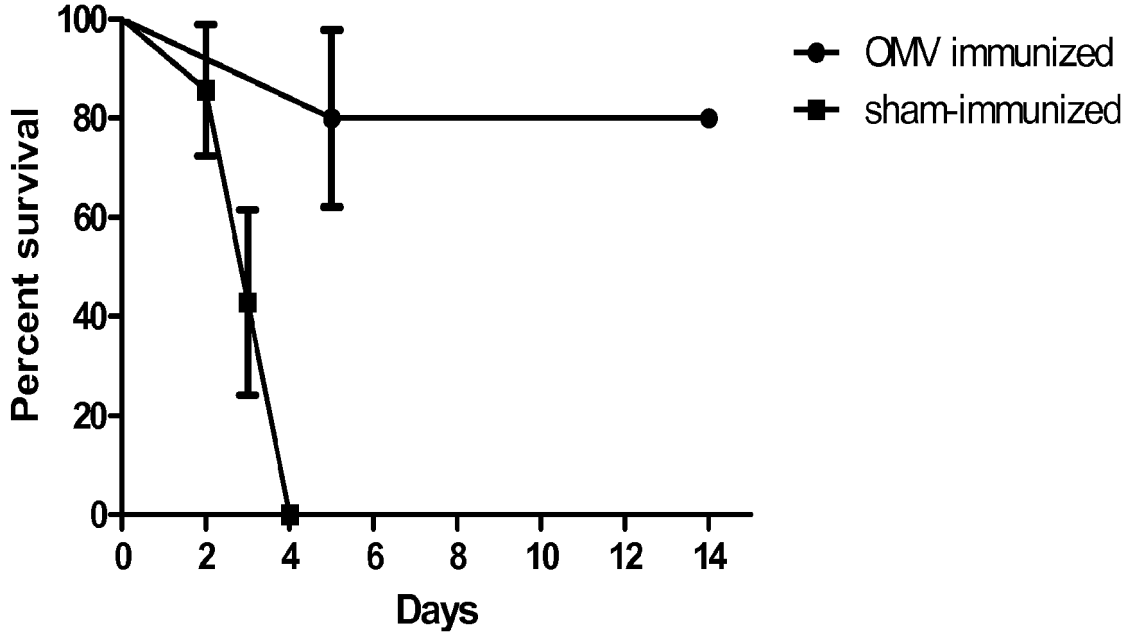

100
80
60
40
20
0
Percent survival
0
2
4
6
8
10
12
14
Days
OMV immunized
sham-immunized

FIG. 10

```
Burkholderia thailandensis E264   -------MAKEKFERTKPHVNVGTIGHVDHGKTTLTAAIATVLSAKFGGE 43
Burkholderia pseudomallei K96243  -------MAKEKFERTKPHVNVGTIGHVDHGKTTLTAAIATVLSAKFGGE 43
Burkholderia mallei ATCC 23344    -------MAKEKFERTKPHVNVGTIGHVDHGKTTLTAAIATVLSAKFGGE 43
E. coli str. K-12 substr. MG1655  -------MSKEKFERTKPHVNVGTIGHVDHGKTTLTAAITTVLAKTYGGA 43
Homo sapiens                      MVNFTVDQIRAIMDKKANIRNMSVIAHVDHGKSTLTDSLVCKAGIIASAR 50
                                         :  :::.    *:..*.******:*** ::.   .   ..

Burkholderia thailandensis E264   AKKYDEIDAAPEEKARGITIN--TAHIEYETANRHY---------- 77
Burkholderia pseudomallei K96243  AKKYDEIDAAPEEKARGITIN--TAHIEYETANRHY---------- 77
Burkholderia mallei ATCC 23344    AKKYDEIDAAPEEKARGITIN--TAHIEYETANRHY---------- 77
E. coli str. K-12 substr. MG1655  ARAFDQIDNAPEEKARGITIN--TSHVEYDTPTRHY---------- 77
Homo sapiens                      AGETRFTDTRKDEQERCITIKSTAISLFYELSENDLNFIKQSKDGAGFLI 100
                                  *     *   :*: * ***:  :  : *: . ..

Burkholderia thailandensis E264   AHVDCPGHADYVKNMITGAAQMDGAILVCSAADGPMPQTR---------- 117
Burkholderia pseudomallei K96243  AHVDCPGHADYVKNMITGAAQMDGAILVCSAADGPMPQTR---------- 117
Burkholderia mallei ATCC 23344    AHVDCPGHADYVKNMITGAAQMDGAILVCSAADGPMPQTR---------- 117
E. coli str. K-12 substr. MG1655  AHVDCPGHADYVKNMITGAAQMDGAILVVAATDGPMPQTR---------- 117
Homo sapiens                      NLIDSPGHVDFSSEVTAALRVTDGALVVVDCVSGVCVQTETVLRQAIAER 150
                                    :*.***.*: .:: :.    ***::*  ...*   **.

Burkholderia thailandensis E264   ------------------------------------------------
Burkholderia pseudomallei K96243  ------------------------------------------------
Burkholderia mallei ATCC 23344    ------------------------------------------------
E. coli str. K-12 substr. MG1655  ------------------------------------------------
Homo sapiens                      IKPVLMMNKMDRALLELQLEPEELYQTFQRIVENVNVIISTYGEGESGPM 200


Burkholderia thailandensis E264   EHILLARQVGVP------------------YIIVFLNK----------- 137
Burkholderia pseudomallei K96243  EHILLARQVGVP------------------YIIVFLNK----------- 137
Burkholderia mallei ATCC 23344    EHILLARQVGVP------------------YIIVFLNK----------- 137
E. coli str. K-12 substr. MG1655  EHILLGRQVGVP------------------YIIVFLNK----------- 137
Homo sapiens                      GNIMIDPVLGTVGFGSGLHGWAFTLKQFAEMYVAKFAAKGEGQLGPAERA 250
                                   :*::   :*.                   *:  *  *
```

FIG. 11A

```
Burkholderia thailandensis E264   ------------------------------------CDMVDD----- 143
Burkholderia pseudomallei K96243  ------------------------------------CDMVDD----- 143
Burkholderia mallei ATCC 23344    ------------------------------------CDMVDD----- 143
E. coli str. K-12 substr. MG1655  ------------------------------------CDMVDD----- 143
Homo sapiens                      KKVEDMMKKLWGDRYFDPANGKFSKSATSPEGKKLPRTFCQLILDPIFKV 300
                                                                      *::: *

Burkholderia thailandensis E264   -AELLELVEMEVRELLSKYDFPGDD-------TPIIKGSAKLALEGDKGE 185
Burkholderia pseudomallei K96243  -AELLELVEMEVRELLSKYDFPGDD-------TPIIKGSAKLALEGDKGE 185
Burkholderia mallei ATCC 23344    -AELLELVEMEVRELLSKYDFPGDD-------TPIIKGSAKLALEGDKGE 185
E. coli str. K-12 substr. MG1655  -EELLELVEMEVRELLSQYDFPGDD-------TPIVRGSALKALEGD--A 183
Homo sapiens                      FDAIMNFKKEETAKLIEKLDIKLDSEDKDKEGKPLLKAVMRRWLPAG-DA 349
                                     :::: : *. :*:.: *:  *.      .*:::.    * ..

Burkholderia thailandensis E264   LGEVAIMNLADALDT--------YIPTPER---------AVDGAFLMPVE 218
Burkholderia pseudomallei K96243  LGEVAIMNLADALDT--------YIPTPER---------AVDGAFLMPVE 218
Burkholderia mallei ATCC 23344    LGEVAIMNLADALDT--------YIPTPER---------AVDGAFLMPVE 218
E. coli str. K-12 substr. MG1655  EWEAKILELAGFLDS--------YIPEPER---------AIDKPFLLPIE 216
Homo sapiens                      LLQMITIHLPSPVTAQKYRCELLYEGPPDDEAAMGIKSCDPKGPLMMYIS 399
                                    :   :.*.. : :       *   *:           . .::: :.

Burkholderia thailandensis E264   DVFSISGRG-TVVTGRVERGVIKVGEEIEIVG------------------ 249
Burkholderia pseudomallei K96243  DVFSISGRG-TVVTGRVERGVIKVGEEIEIVG------------------ 249
Burkholderia mallei ATCC 23344    DVFSISGRG-TVVTGRVERGVIKVGEEIEIVG------------------ 249
E. coli str. K-12 substr. MG1655  DVFSISGRG-TVVTGRVERGIIKVGEEVEIVG------------------ 247
Homo sapiens                      KMVPTSDKGRFYAFGRVFSGLVSTGLKVRIMGPNYTPGKKEDLYLKPIQR 449
                                  .:.. *.:*   . ***  *::..* ::.*:*

Burkholderia thailandensis E264   --------IKATAKTTCT------GVEMFR-------------------- 265
Burkholderia pseudomallei K96243  --------IKATAKTTCT------GVEMFR-------------------- 265
Burkholderia mallei ATCC 23344    --------IKATAKTTCT------GVEMFR-------------------- 265
E. coli str. K-12 substr. MG1655  --------IKETQKSTCT------GVEMFR-------------------- 263
Homo sapiens                      TILMMGRYVEPIEDVPCGNIVGLVGVDQFLVKTGTITTFEHAHNMRVMKF 499
                                         ::   . .*      **: *
```

FIG. 11B

```
Burkholderia thailandensis E264   ------KLLDQGQAGDNVGILLRGTK---REDVERGQVLAKPG--SITPH 304
Burkholderia pseudomallei K96243  ------KLLDQGQAGDNVGILLRGTK---REDVERGQVLAKPG--SITPH 304
Burkholderia mallei ATCC 23344    ------KLLDQGQAGDNVGILLRGTK---REDVERGQVLAKPG--SITPH 304
E. coli str. K-12 substr. MG1655  ------KLLDEGRAGENVGVLLRGIK---REEIERGQVLAKPG--TIKPH 302
Homo sapiens                      SVSPVVRVAVEAKNPADLPKLVEGLKRLAKSDPMVQCIIEESGEHIIAGA 549
                                        ::  :.:   ::  *:.* *   :.:     :: :.*   *

Burkholderia thailandensis E264   THFTAEVYVLSKDEGGRHTPFFNN-----YRP------------------ 331
Burkholderia pseudomallei K96243  THFTAEVYVLSKDEGGRHTPFFNN-----YRP------------------ 331
Burkholderia mallei ATCC 23344    THFTAEVYVLSKDEGGRHTPFFNN-----YRP------------------ 331
E. coli str. K-12 substr. MG1655  TKFESEVYILSKDEGGRHTPFFKG-----YRP------------------ 329
Homo sapiens                      GELHLEICLKDLEEDHACIPIKKSDPVVSYRETVSEESNVLCLSKSPNKH 599
                                   .:  *: : . :*.    *: :.     **

Burkholderia thailandensis E264   -QFYFRTTDVTGSIELPKDKEMVMPGDNVSITVKLIAP------------ 368
Burkholderia pseudomallei K96243  -QFYFRTTDVTGSIELPKDKEMVMPGDNVSITVKLIAP------------ 368
Burkholderia mallei ATCC 23344    -QFYFRTTDVTGSIELPKDKEMVMPGDNVSITVKLIAP------------ 368
E. coli str. K-12 substr. MG1655  -QFYFRTTDVTGTIELPEGVEMVMPGDNIKMVVTLIHP------------ 366
Homo sapiens                      NRLYMKARPFPDGLAEDIDKGEVSARQELKQRARYLAEKYEWDVAEARKI 649
                                   ::*:::  ... :    .   * . :::.  .  :

Burkholderia thailandensis E264   --------------------------IAMEEGLRFAIRE---------- 381
Burkholderia pseudomallei K96243  --------------------------IAMEEGLRFAIRE---------- 381
Burkholderia mallei ATCC 23344    --------------------------IAMEEGLRFAIRE---------- 381
E. coli str. K-12 substr. MG1655  --------------------------IAMDDGLRFAIRE---------- 379
Homo sapiens                      WCFGPDGTGPNILTDITKGVQYLNEIKDSVVAGFQWATKEGALCEENMRG 699
                                                            ::  *:::* :*

Burkholderia thailandensis E264   ------------------GGRTVGAG---VVAKIIE------------- 396
Burkholderia pseudomallei K96243  ------------------GGRTVGAG---VVAKIIE------------- 396
Burkholderia mallei ATCC 23344    ------------------GGRTVGAG---VVAKIIE------------- 396
E. coli str. K-12 substr. MG1655  ------------------GGRTVGAG---VVAKVLS------------- 394
Homo sapiens                      VRFDVHDVTLHADAIHRGGGQIIPTARRCLYASVLTAQPRLMEPIYLVEI 749
                                                    **: : :.   : *.::
```

FIG. 11C

```
Burkholderia thailandensis E264  ------------------------------------------------
Burkholderia pseudomallei K96243 ------------------------------------------------
Burkholderia mallei ATCC 23344   ------------------------------------------------
E. coli str. K-12 substr. MG1655 ------------------------------------------------
Homo sapiens                     QCPEQVVGGIYGVLNRKRGHVFEESQVAGTPMFVVKAYLPVNESFGFTAD 799

Burkholderia thailandensis E264  ------------------------------------------------
Burkholderia pseudomallei K96243 ------------------------------------------------
Burkholderia mallei ATCC 23344   ------------------------------------------------
E. coli str. K-12 substr. MG1655 ------------------------------------------------
Homo sapiens                     LRSNTGGQAFPQCVFDHWQILPGDPFDNSSRPSQVVAETRKRKGLKEGIP 849

Burkholderia thailandensis E264  ---------
Burkholderia pseudomallei K96243 ---------
Burkholderia mallei ATCC 23344   ---------
E. coli str. K-12 substr. MG1655 ---------
Homo sapiens                     ALDNFLDKL 858
```

FIG. 11D

```
Burkholderia pseudomallei K96243    MAKEKFERTKPHVNVGTIGHVDHGKTTLTAAIATVLSAKFGGEAKKYDEI 50
B. pseudomallei Pasteur 52237       MAKEKFERTKPHVNVGTIGHVDHGKTTLTAAIATVLSAKFGGEAKKYDEI 50
Burkholderia pseudomallei 406e      MAKEKFERTKPHVNVGTIGHVDHGKTTLTAAIATVLSAKFGGEAKKYDEI 50
Burkholderia pseudomallei 1106a     MAKEKFERTKPHVNVGTIGHVDHGKTTLTAAIATVLSAKFGGEAKKYDEI 50
Burkholderia pseudomallei MSHR346   MAKEKFERTKPHVNVGTIGHVDHGKTTLTAAIATVLSAKFGGEAKKYDEI 50
                                    **************************************************

Burkholderia pseudomallei K96243    DAAPEEKARGITINTAHIEYETANRHYAHVDCPGHADYVKNMITGAAQMD 100
B. pseudomallei Pasteur 52237       DAAPEEKARGITINTAHIEYETANRHYAHVDCPGHADYVKNMITGAAQMD 100
Burkholderia pseudomallei 406e      DAAPEEKARGITINTAHIEYETANRHYAHVDCPGHADYVKNMITGAAQMD 100
Burkholderia pseudomallei 1106a     DAAPEEKARGITINTAHIEYETANRHYAHVDCPGHADYVKNMITGAAQMD 100
Burkholderia pseudomallei MSHR346   DAAPEEKARGITINTAHIEYETANRHYAHVDCPGHADYVKNMITGAAQMD 100
                                    **************************************************

Burkholderia pseudomallei K96243    GAILVCSAADGPMPQTREHILLARQVGVPYIIVFLNKCDMVDDAELLELV 150
B. pseudomallei Pasteur 52237       GAILVCSAADGPMPQTREHILLARQVGVPYIIVFLNKCDMVDDAELLELV 150
Burkholderia pseudomallei 406e      GAILVCSAADGPMPQTREHILLARQVGVPYIIVFLNKCDMVDDAELLELV 150
Burkholderia pseudomallei 1106a     GAILVCSAADGPMPQTREHILLARQVGVPYIIVFLNKCDMVDDAELLELV 150
Burkholderia pseudomallei MSHR346   GAILVCSAADGPMPQTREHILLARQVGVPYIIVFLNKCDMVDDAELLELV 150
                                    **************************************************

Burkholderia pseudomallei K96243    EMEVRELLSKYDFPGDDTPIIKGSAKLALEGDKGELGEVAIMNLADALDT 200
B. pseudomallei Pasteur 52237       EMEVRELLSKYDFPGDDTPIIKGSAKLALEGDKGELGEVAIMNLADALDT 200
Burkholderia pseudomallei 406e      EMEVRELLSKYDFPGDDTPIIKGSAKLALEGDKGELGEVAIMNLADALDT 200
Burkholderia pseudomallei 1106a     EMEVRELLSKYDFPGDDTPIIKGSAKLALEGDKGELGEVAIMNLADALDT 200
Burkholderia pseudomallei MSHR346   EMEVRELLSKYDFPGDDTPIIKGSAKLALEGDKGELGEVAIMNLADALDT 200
                                    **************************************************

Burkholderia pseudomallei K96243    YIPTPERAVDGAFLMPVEDVFSISGRGTVVTGRVERGVIKVGEEIEIVGI 250
B. pseudomallei Pasteur 52237       YIPTPERAVDGAFLMPVEDVFSISGRGTVVTGRVERGVIKVGEEIEIVGI 250
Burkholderia pseudomallei 406e      YIPTPERAVDGAFLMPVEDVFSISGRGTVVTGRVERGVIKVGEEIEIVGI 250
Burkholderia pseudomallei 1106a     YIPTPERAVDGAFLMPVEDVFSISGRGTVVTGRVERGVIKVGEEIEIVGI 250
Burkholderia pseudomallei MSHR346   YIPTPERAVDGAFLMPVEDVFSISGRGTVVTGRVERGVIKVGEEIEIVGI 250
                                    **************************************************
```

FIG. 12A

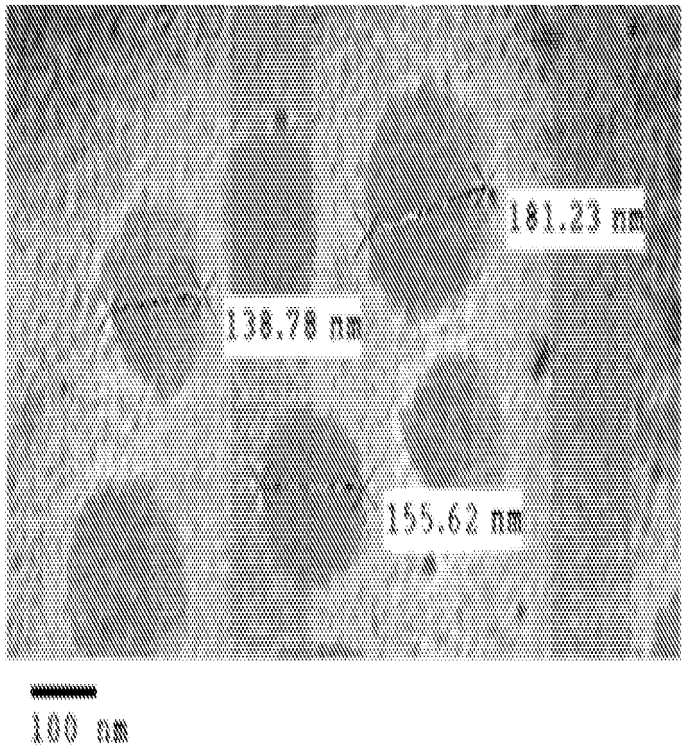
```
Burkholderia pseudomallei K96243    KATAKTTCTGVEMFRKLLDQGQAGDNVGILLRGTKREDVERGQVLAKPGS 300
B. pseudomallei Pasteur 52237       KATAKTTCTGVEMFRKLLDQGQAGDNVGILLRGTKREDVERGQVLAKPGS 300
Burkholderia pseudomallei 406e      KATAKTTCTGVEMFRKLLDQGQAGDNVGILLRGTKREDVERGQVLAKPGS 300
Burkholderia pseudomallei 1106a     KATAKTTCTGVEMFRKLLDQGQAGDNVGILLRGTKREDVERGQVLAKPGS 300
Burkholderia pseudomallei MSHR346   KATAKTTCTGVEMFRKLLDQGQAGDNVGILLRGTKREDVERGQVLAKPGS 300
                                    **************************************************

Burkholderia pseudomallei K96243    ITPHTHFTAEVYVLSKDEGGRHTPFFNNYRPQFYFRTTDVTGSIELPKDK 350
B. pseudomallei Pasteur 52237       ITPHTHFTAEVYVLSKDEGGRHTPFFNNYRPQFYFRTTDVTGSIELPKDK 350
Burkholderia pseudomallei 406e      ITPHTHFTAEVYVLSKDEGGRHTPFFNNYRPQFYFRTTDVTGSIELPKDK 350
Burkholderia pseudomallei 1106a     ITPHTHFTAEVYVLSKDEGGRHTPFFNNYRPQFYFRTTDVTGSIELPKDK 350
Burkholderia pseudomallei MSHR346   ITPHTHFTAEVYVLSKDEGGRHTPFFNNYRPQFYFRTTDVTGSIELPKDK 350
                                    **************************************************

Burkholderia pseudomallei K96243    EMVMPGDNVSITVKLIAPIAMEEGLRFAIREGGRTVGAGVVAKIIE 396
B. pseudomallei Pasteur 52237       EMVMPGDNVSITVKLIAPIAMEEGLRFAIREGGRTVGAGVVAKIIE 396
Burkholderia pseudomallei 406e      EMVMPGDNVSITVKLIAPIAMEEGLRFAIREGGRTVGAGVVAKIIE 396
Burkholderia pseudomallei 1106a     EMVMPGDNVSITVKLIAPIAMEEGLRFAIREGGRTVGAGVVAKIIE 396
Burkholderia pseudomallei MSHR346   EMVMPGDNVSITVKLIAPIAMEEGLRFAIREGGRTVGAGVVAKIIE 396
                                    *********************************************
```

FIG. 12B

A


181.23 nm
138.78 nm
155.62 nm
100 nm

B

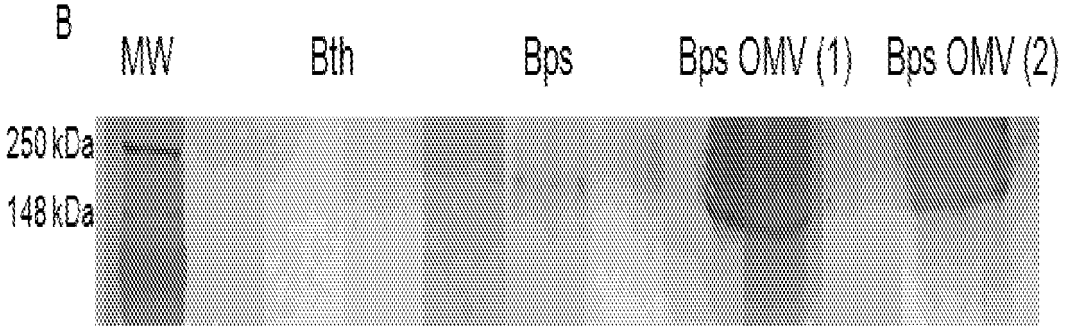

MW
Bth
Bps
Bps OMV (1)
Bps OMV (2)
250 kDa
148 kDa

FIG. 13

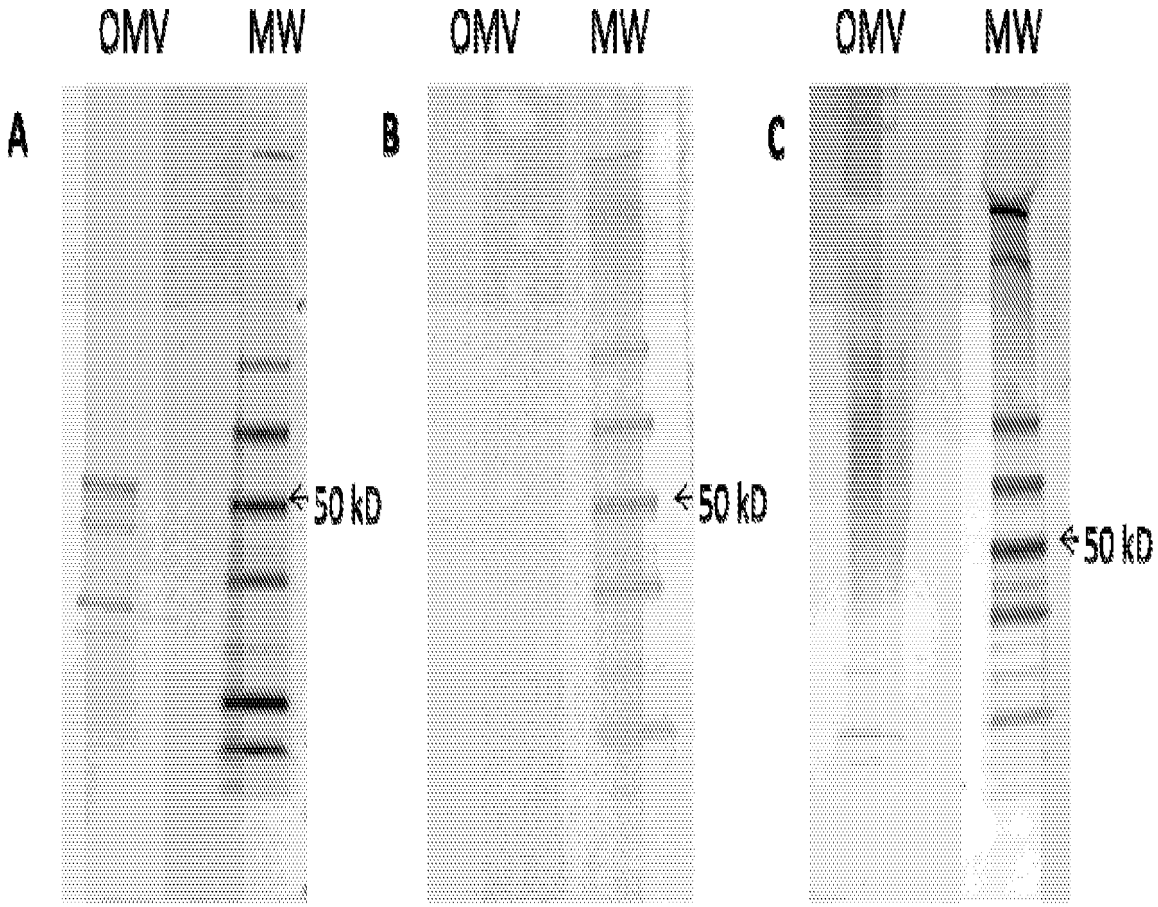


FIG. 14

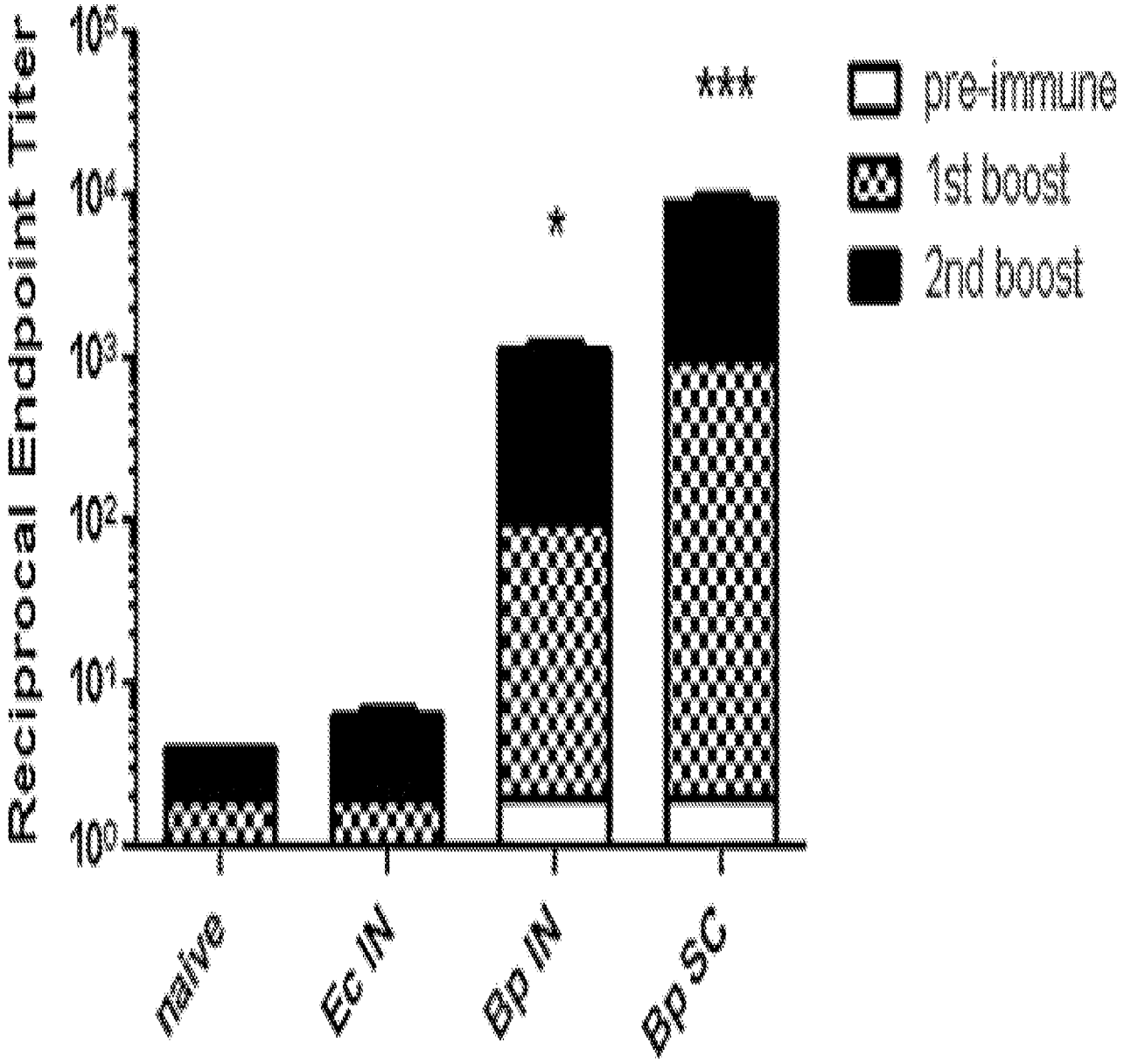

Reciprocal Endpoint Titer
10^5
10^4
10^3
10^2
10^1
10^0
***
*
pre-immune
1st boost
2nd boost
naive
Ec IN
Bp IN
Bp SC

FIG. 15

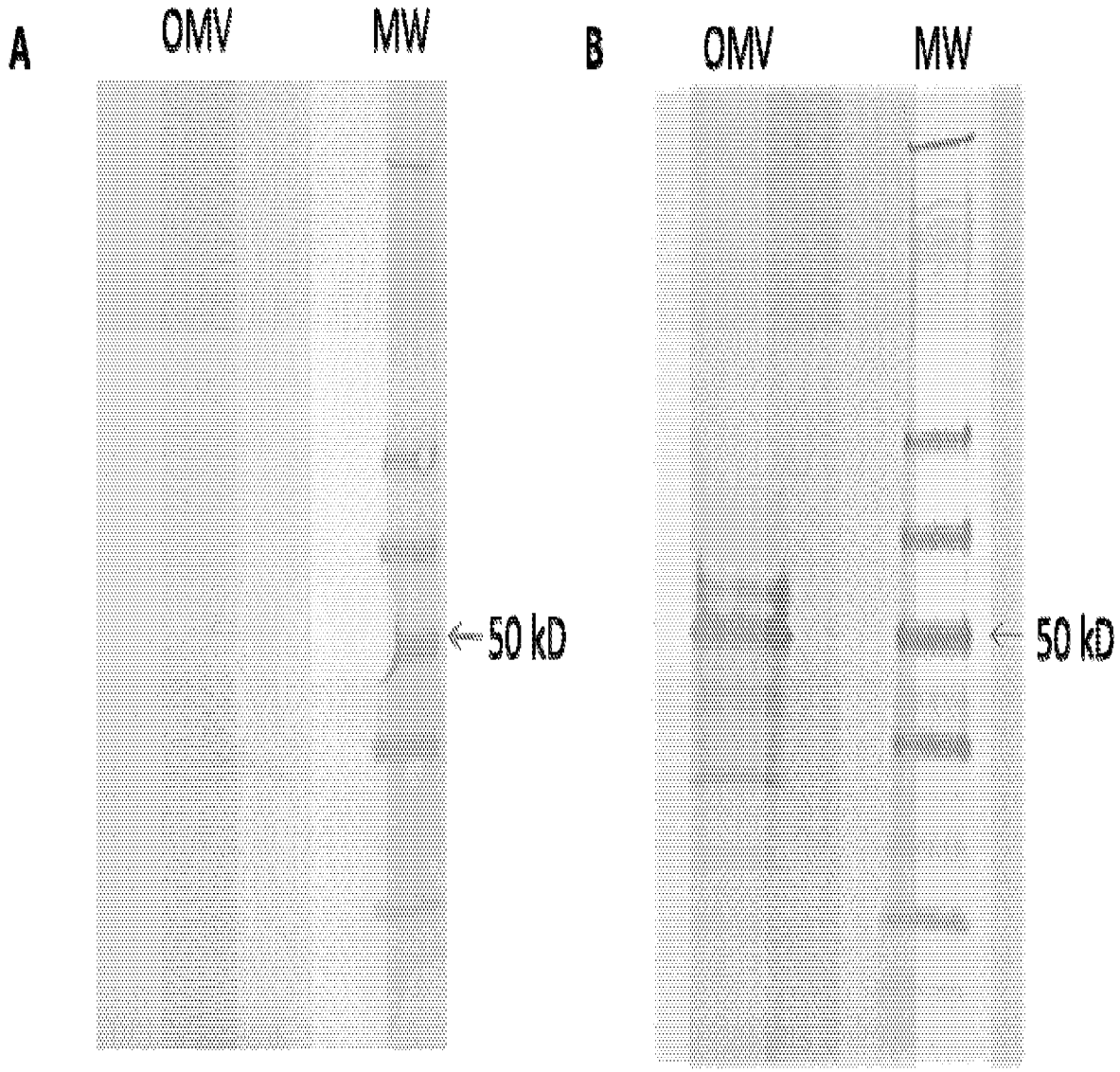


FIG. 16

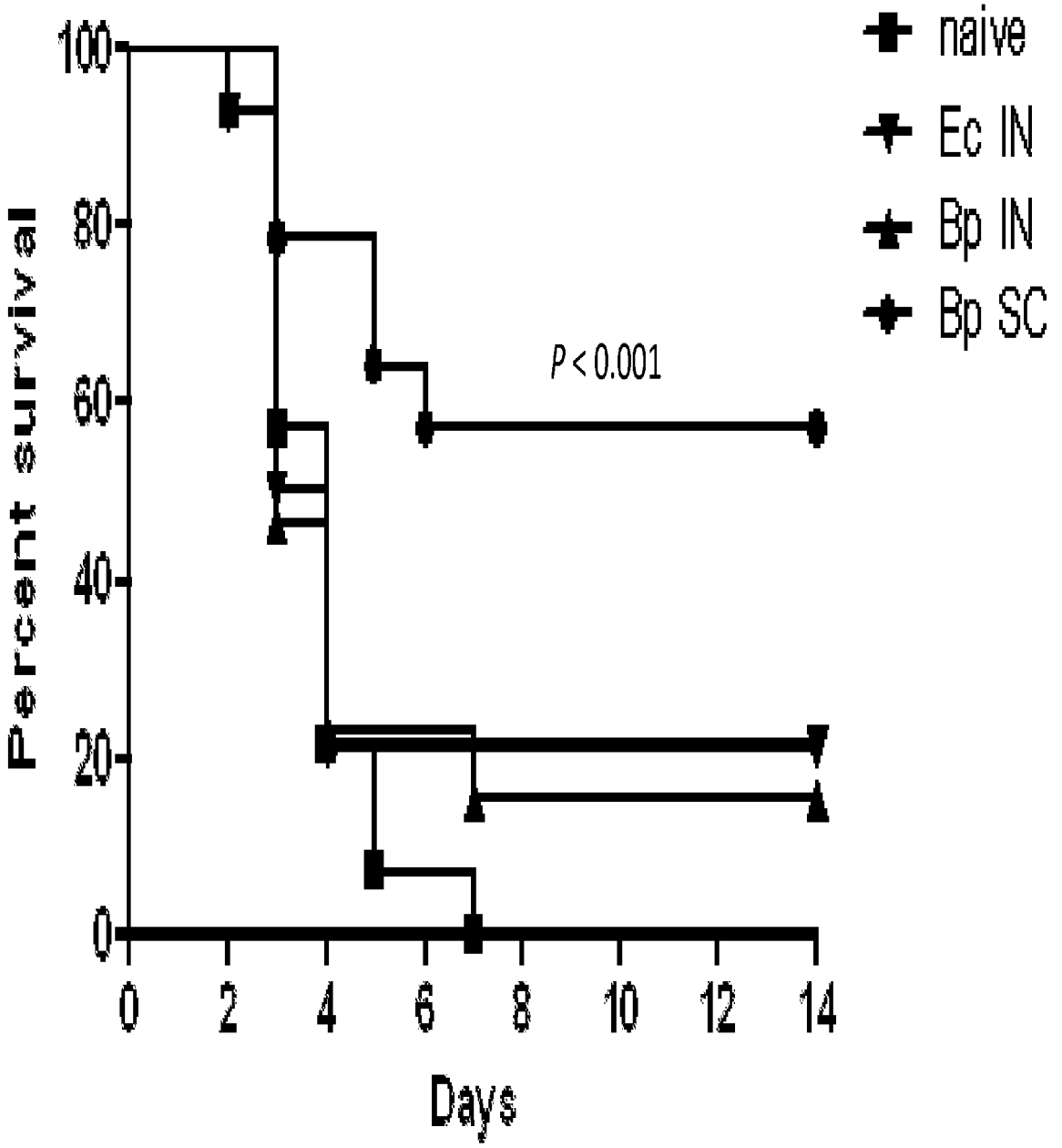

naive
Ec IN
Bp IN
Bp SC
P < 0.001
Percent survival
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Days

FIG. 17

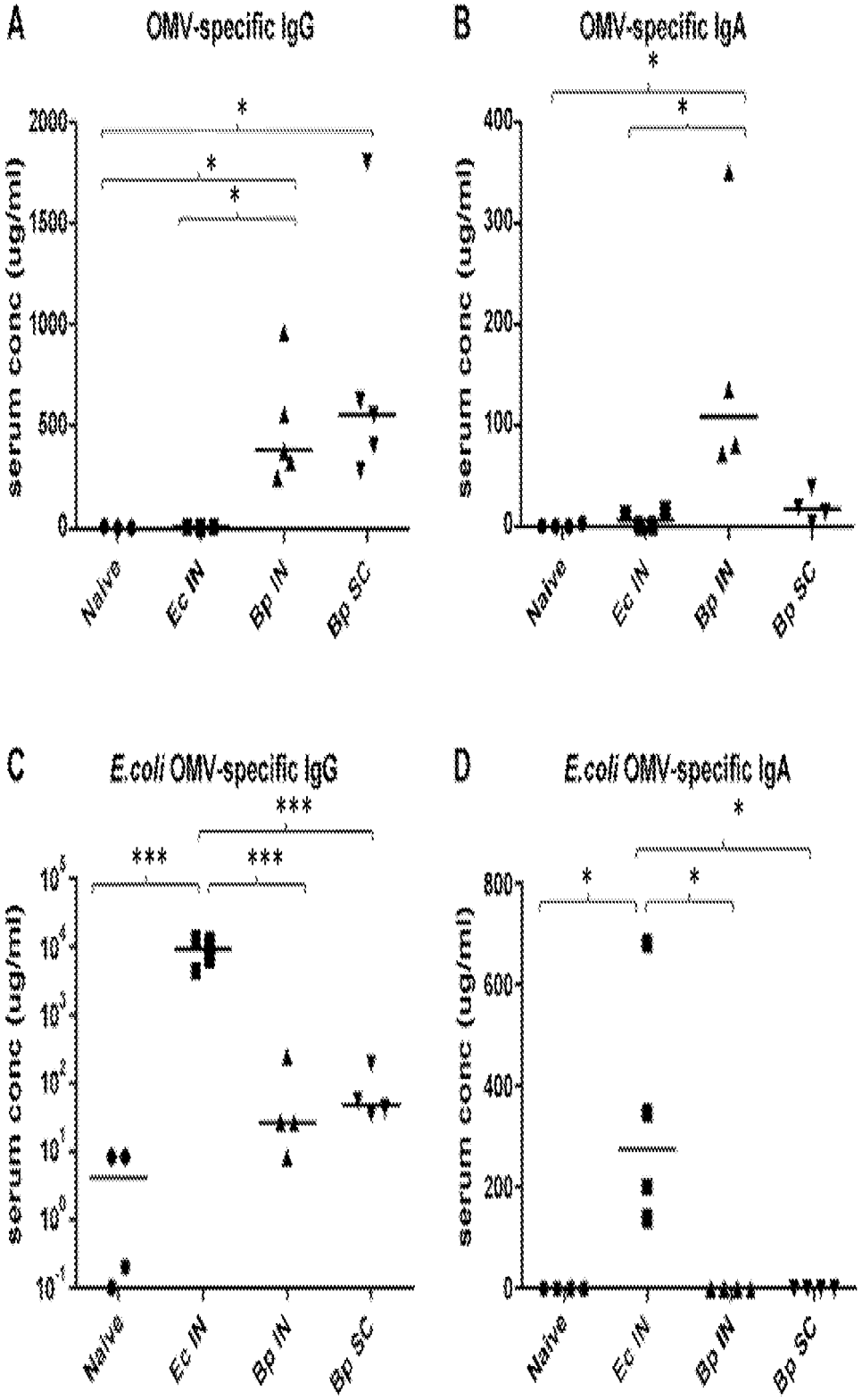

A
OMV-specific IgG
serum conc (ug/ml)
2000
1500
1000
500
0
Naive
Ec IN
Bp IN
Bp SC
B
OMV-specific IgA
serum conc (ug/ml)
400
300
200
100
0
Naive
Ec IN
Bp IN
Bp SC
C
E.coli OMV-specific IgG
serum conc (ug/ml)
Naive
Ec IN
Bp IN
Bp SC
D
E.coli OMV-specific IgA
serum conc (ug/ml)
800
600
400
200
0
Naive
Ec IN
Bp IN
Bp SC

FIG. 18

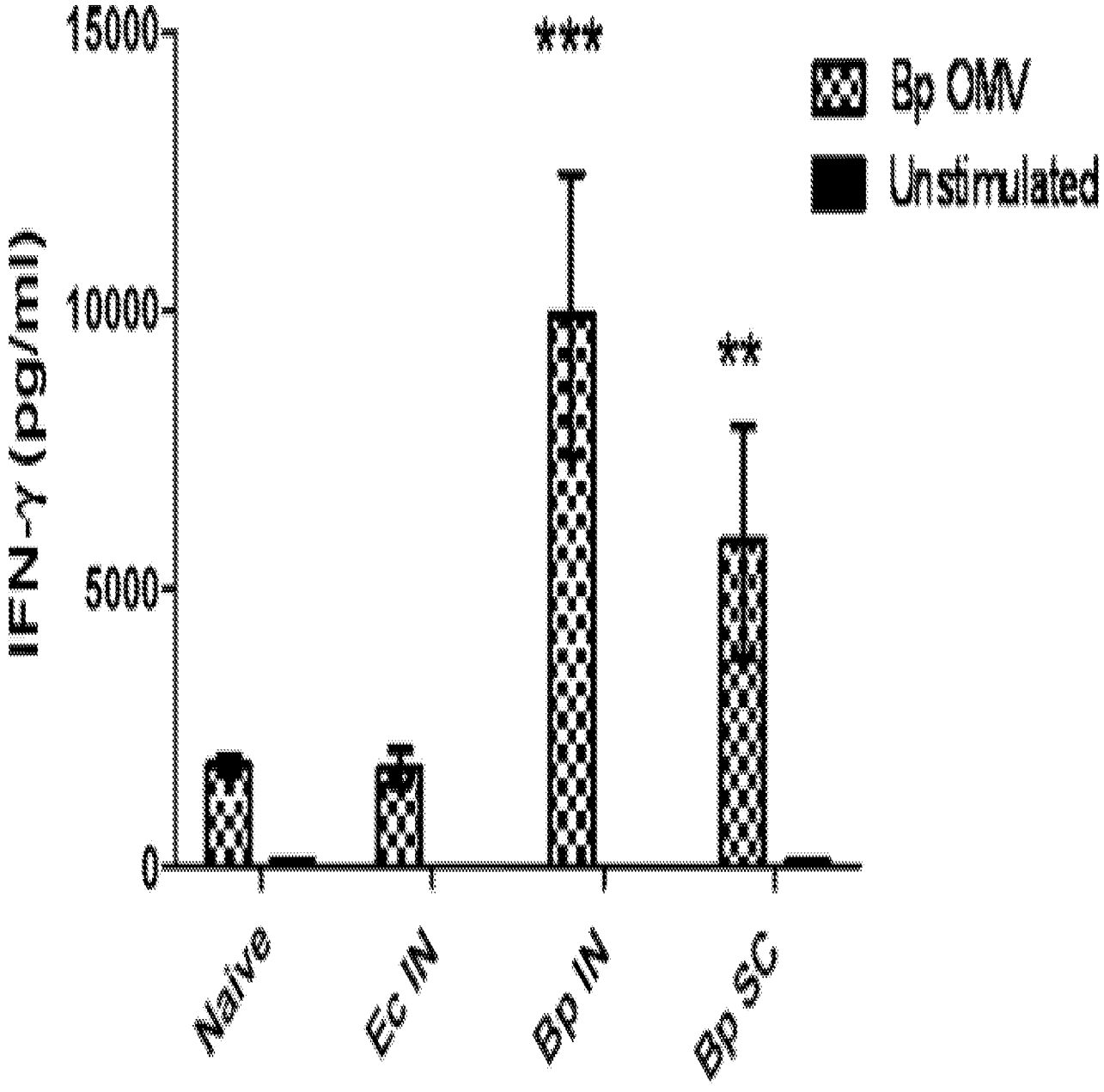

15000
10000
5000
0
IFN-γ (pg/ml)
***
**
Bp OMV
Unstimulated
Naive
Ec IN
Bp IN
Bp SC

FIG. 19

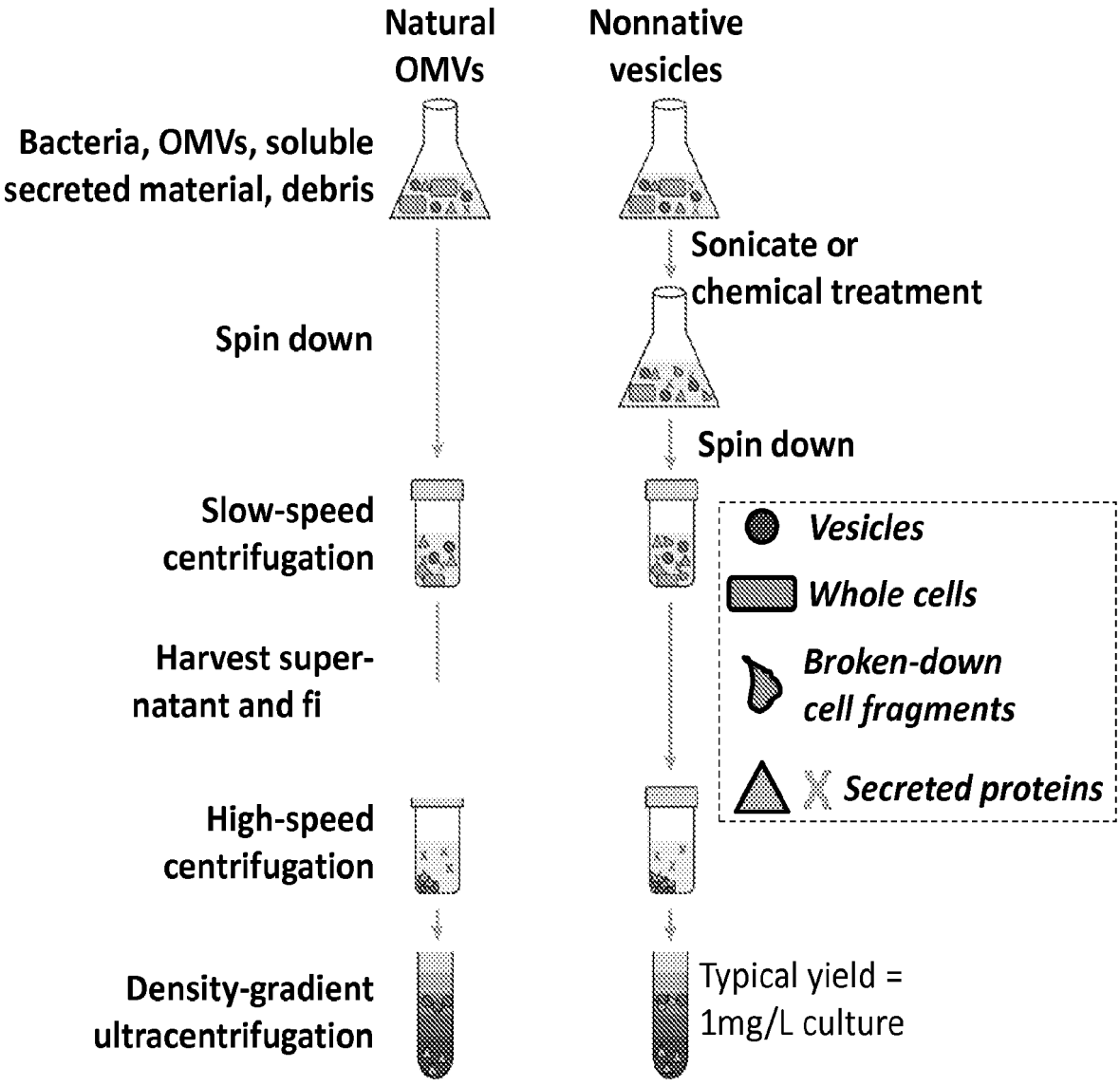

Natural OMVs
Nonnative vesicles
Bacteria, OMVs, soluble secreted material, debris
Sonicate or chemical treatment
Spin down
Spin down
Slow-speed centrifugation
Harvest super-natant and fi
High-speed centrifugation
Density-gradient ultracentrifugation
Vesicles
Whole cells
Broken-down cell fragments
Secreted proteins
Typical yield = 1mg/L culture

FIG. 20

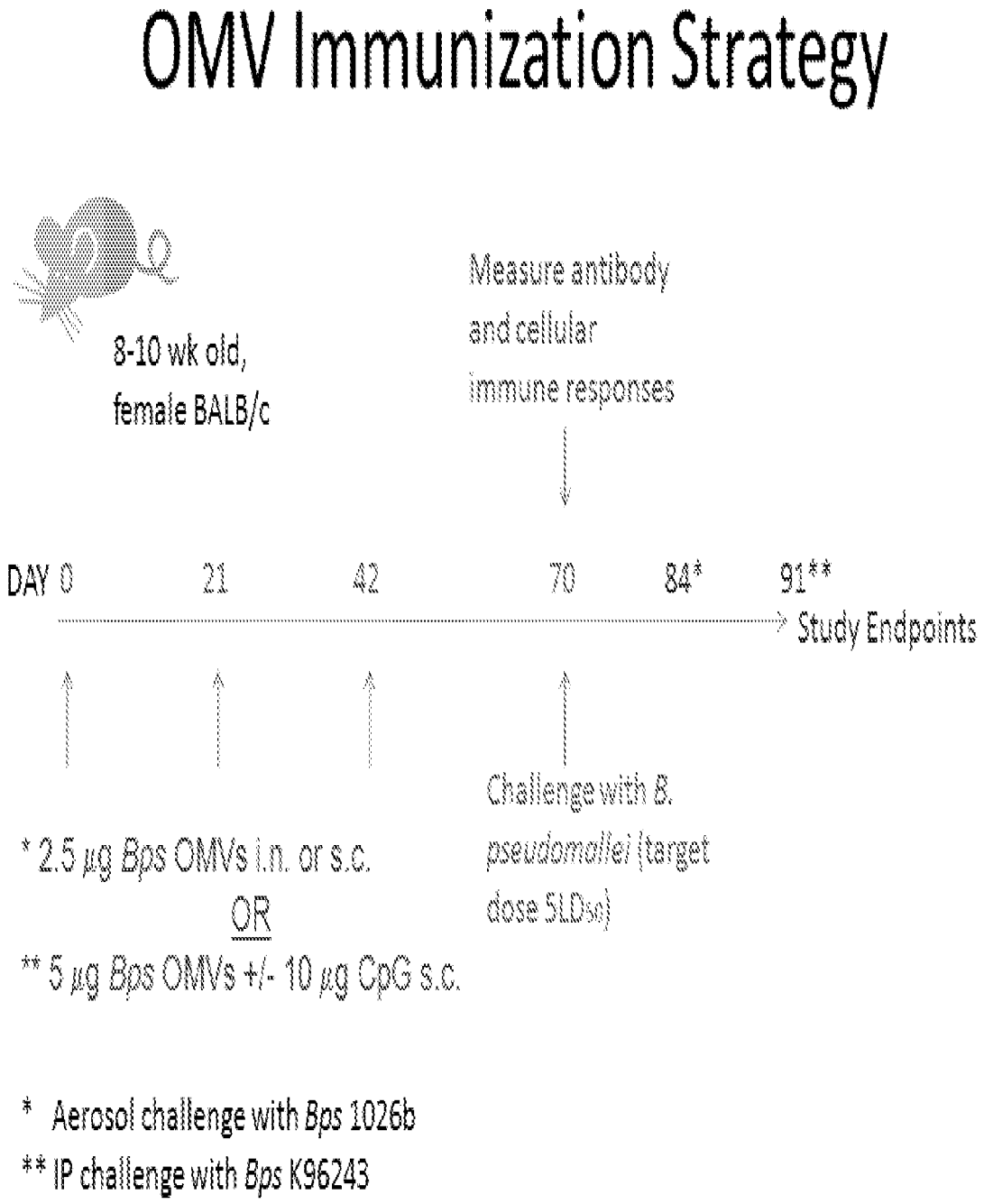

OMV Immunization Strategy
8-10 wk old,
female BALB/c
Measure antibody
and cellular
immune responses
DAY 0
21
42
70
84*
91**
Study Endpoints
* 2.5 μg Bps OMVs i.n. or s.c.
OR
** 5 μg Bps OMVs +/- 10 μg CpG s.c.
Challenge with B.
pseudomallei (target
dose SLD50)
* Aerosol challenge with Bps 1026b
** IP challenge with Bps K96243

FIG. 21

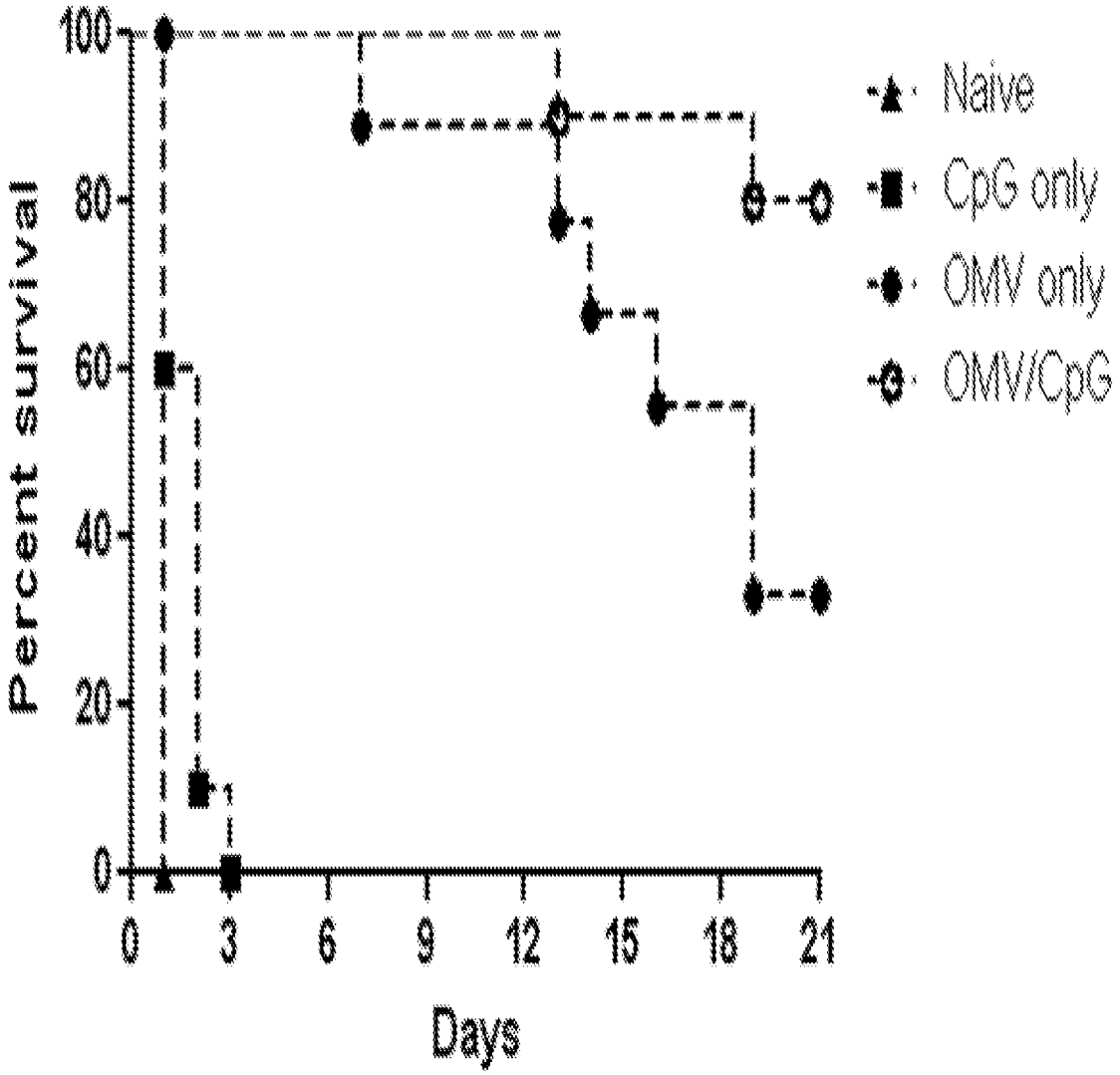

Percent survival
100
80
60
40
20
0
0
3
6
9
12
15
18
21
Days
Naive
CpG only
OMV only
OMV/CpG

FIG. 22

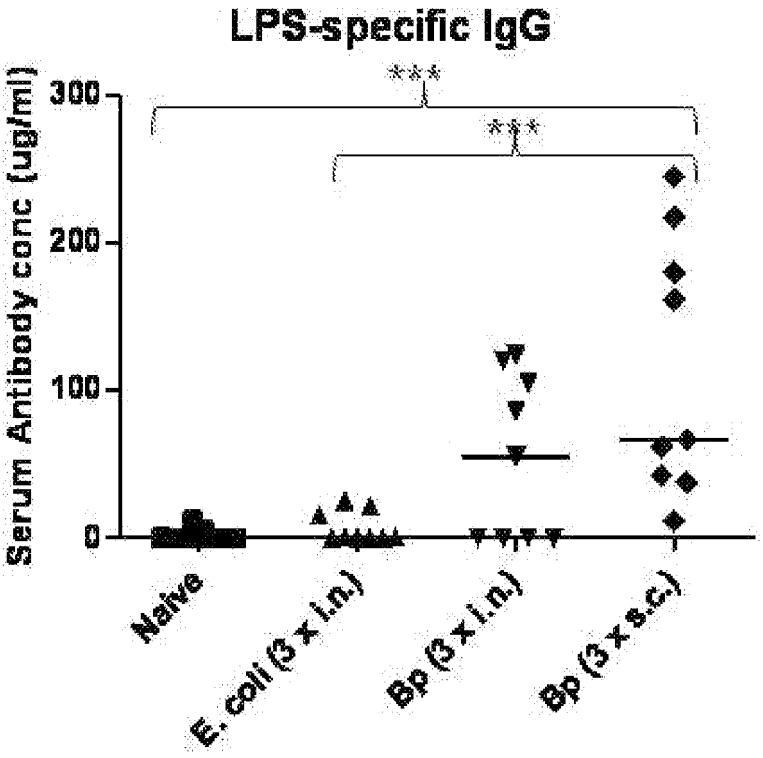


FIG. 23A

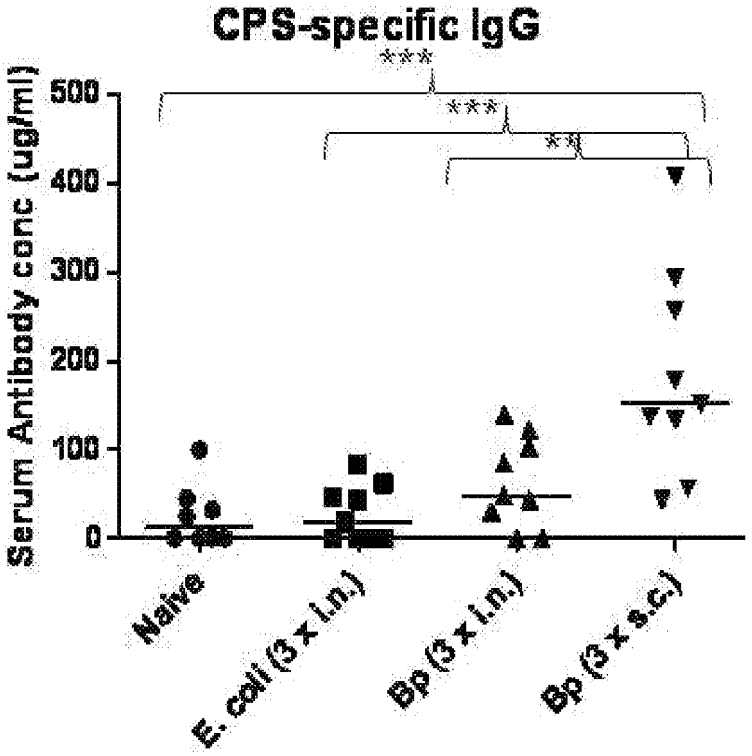


FIG. 23B

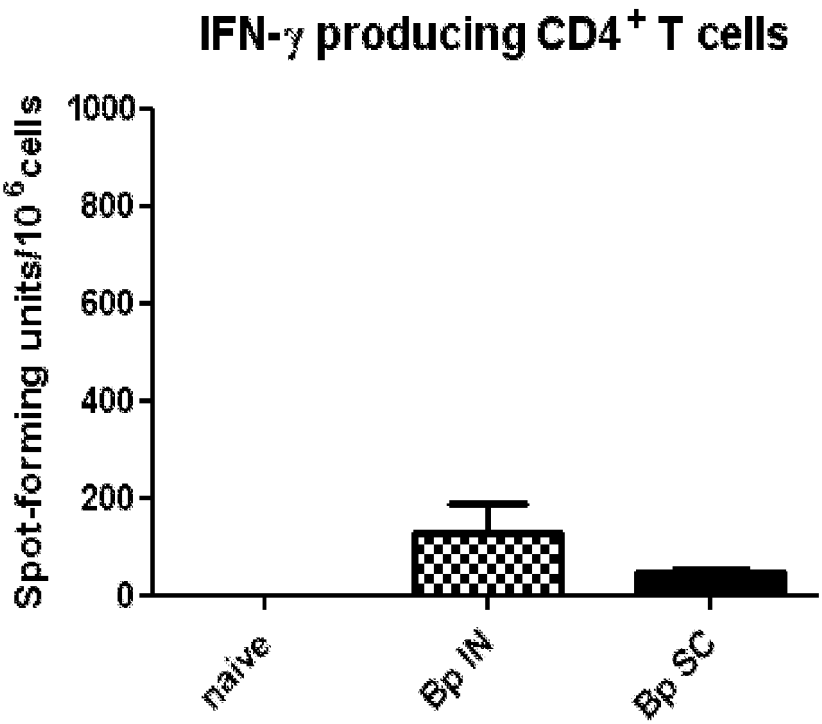


FIG. 24A

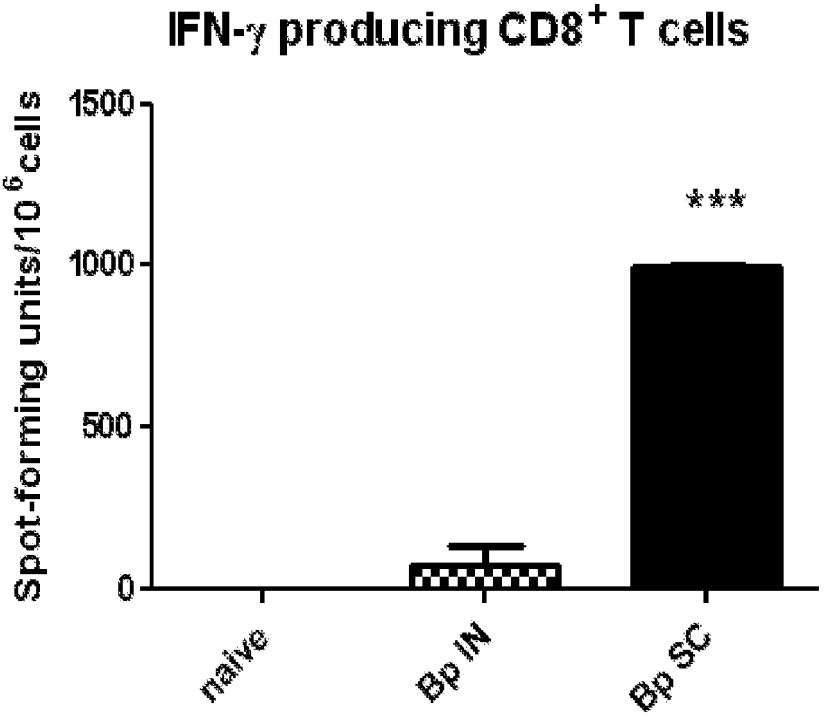


FIG. 24B

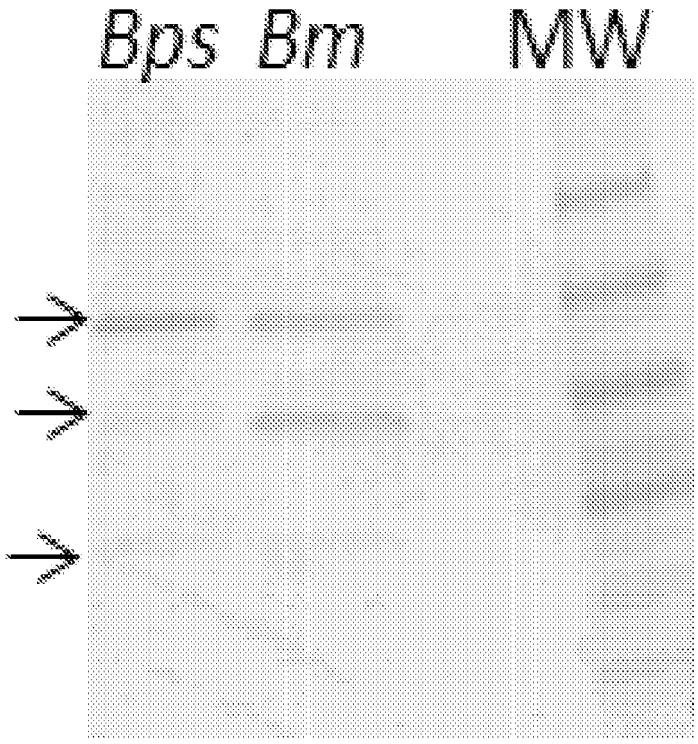


FIG. 25

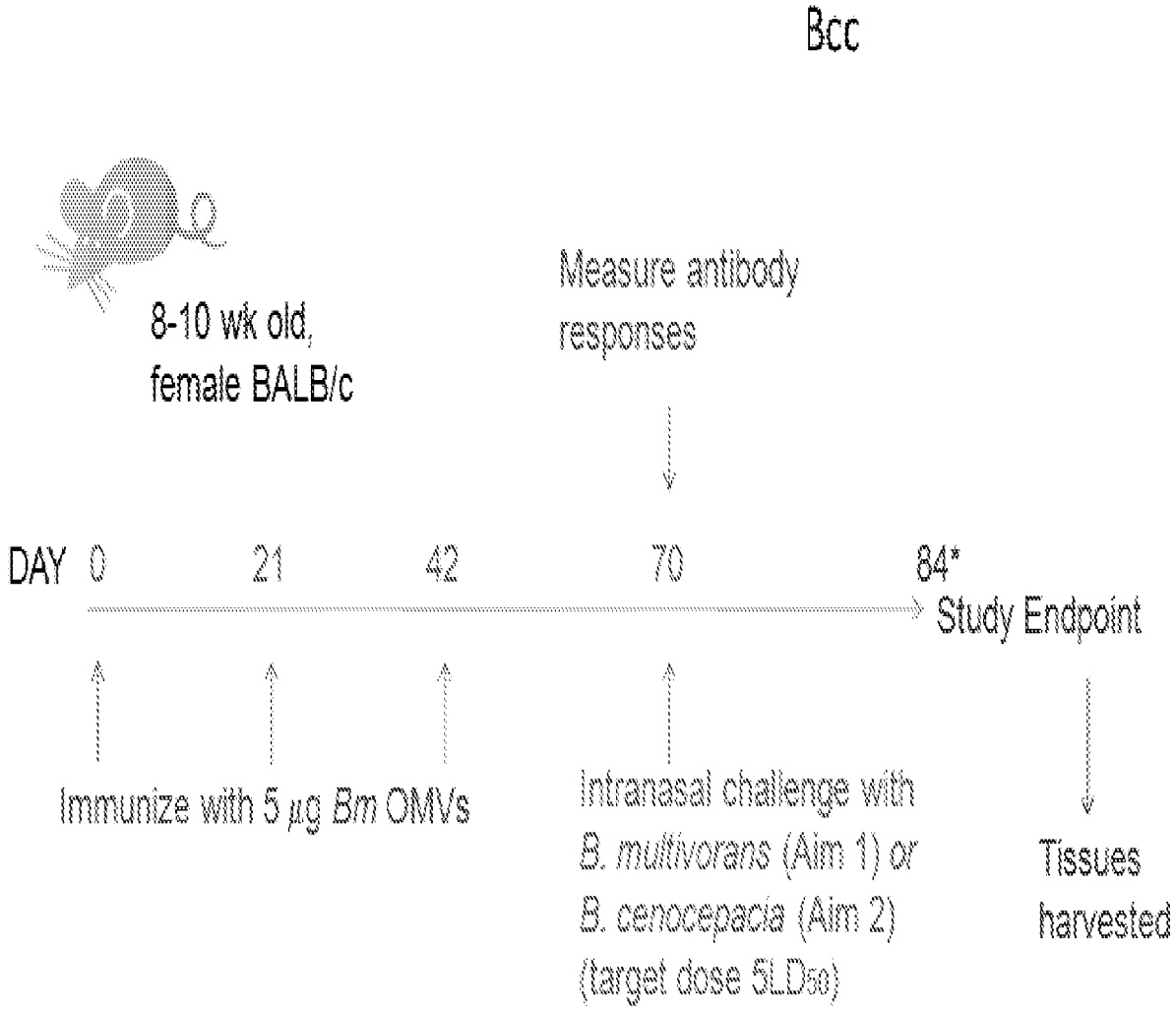

Bcc
8-10 wk old,
female BALB/c
Measure antibody
responses
DAY 0
21
42
70
84*
Study Endpoint
Immunize with 5 μg Bm OMVs
Intranasal challenge with
B. multivorans (Aim 1) or
B. cenocepacia (Aim 2)
(target dose 5LD50)
Tissues
harvested

FIG. 26

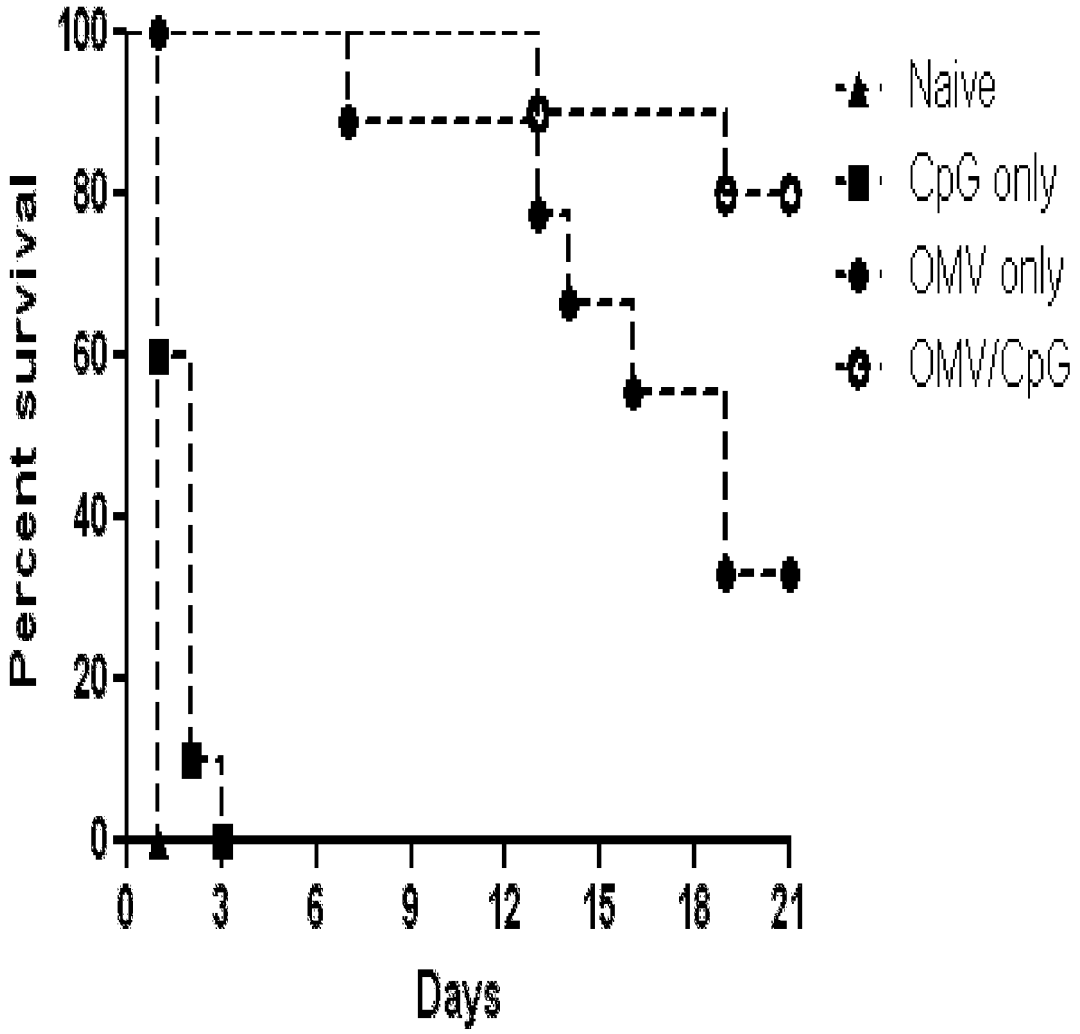

Percent survival
100
80
60
40
20
0
0
3
6
9
12
15
18
21
Days
Naive
CpG only
OMV only
OMV/CpG

FIG. 27

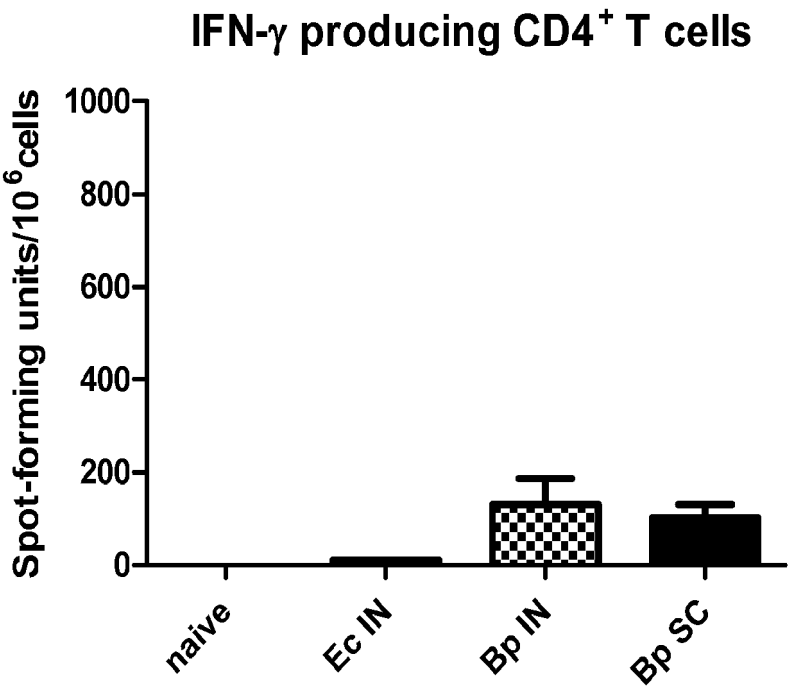


FIG. 28A

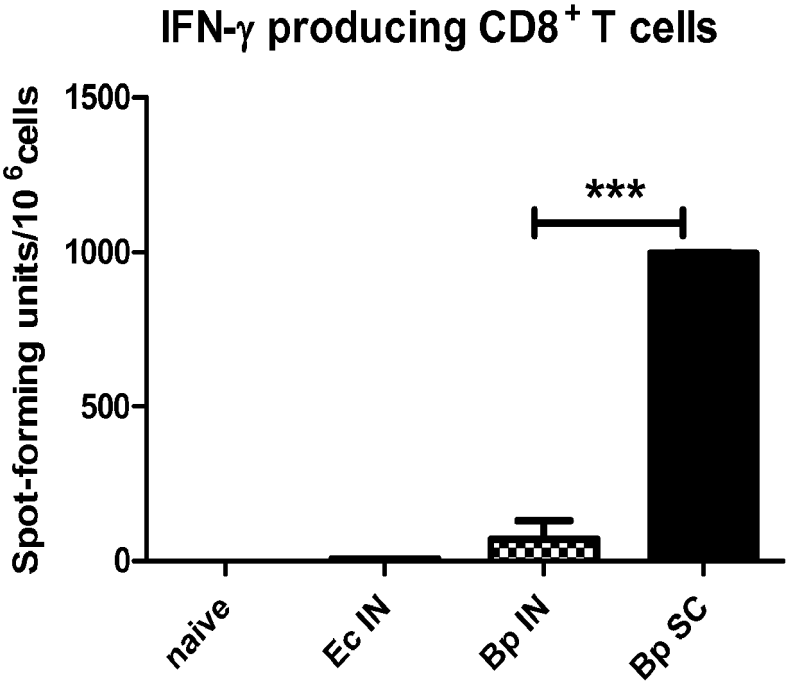


FIG. 28B

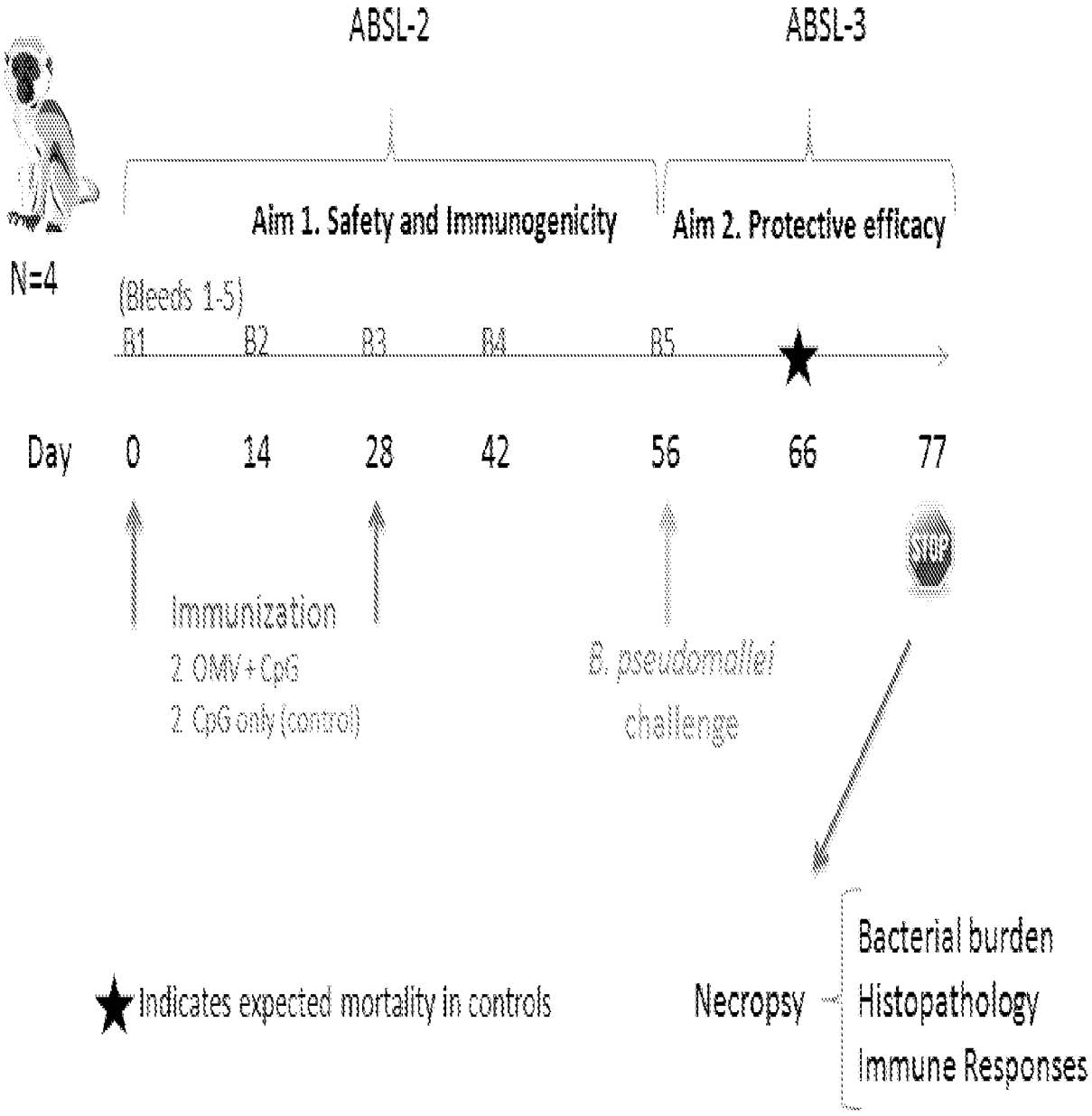

ABSL-2
ABSL-3
Aim 1. Safety and Immunogenicity
Aim 2. Protective efficacy
N=4
(Bleeds 1-5)
B1
B2
B3
B4
B5
Day
0
14
28
42
56
66
77
Immunization
2 OMV + CpG
2 CpG only (control)
B. pseudomallei
challenge
Indicates expected mortality in controls
Necropsy
Bacterial burden
Histopathology
Immune Responses

FIG. 29

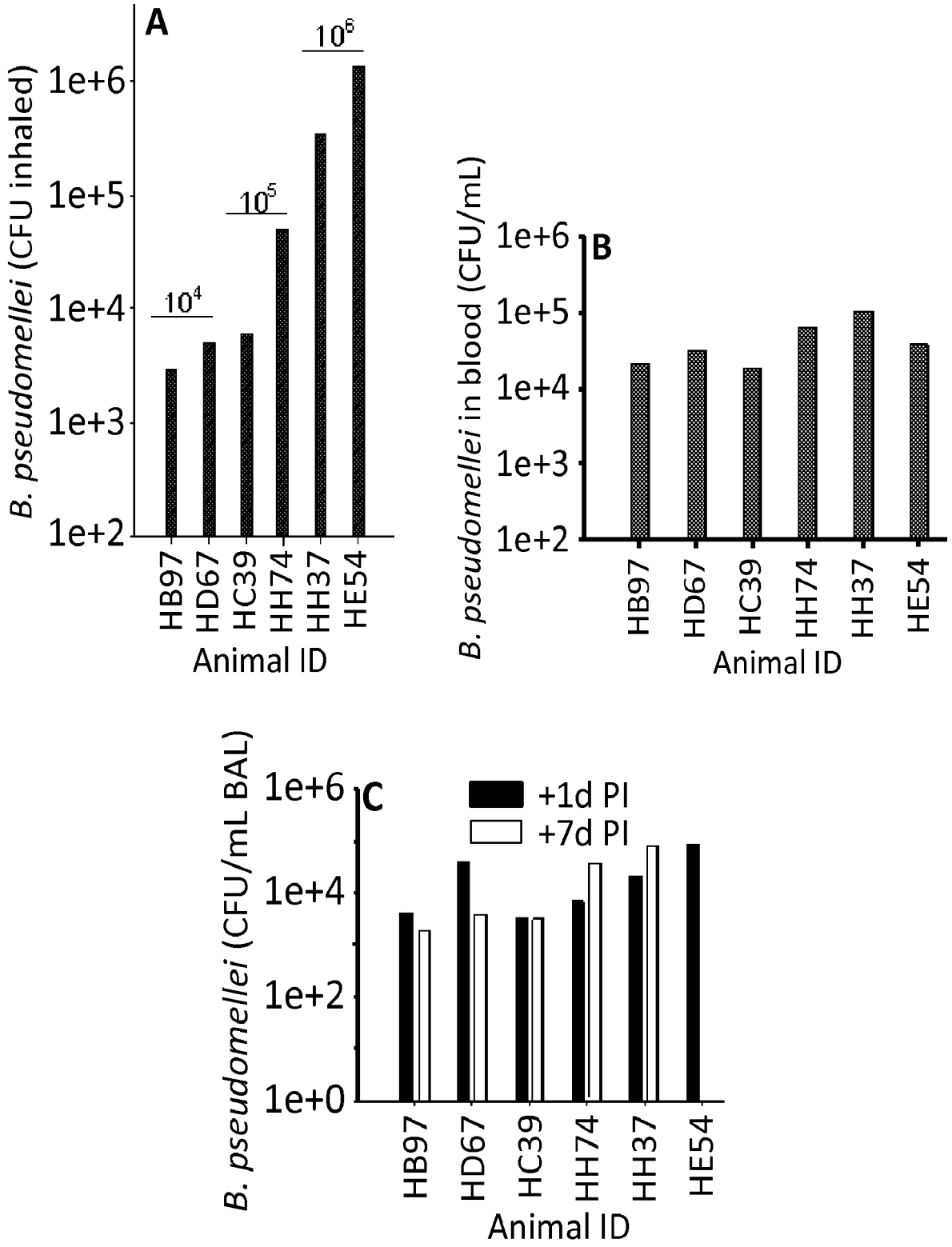

A
B. pseudomellei (CFU inhaled)
1e+6
1e+5
1e+4
1e+3
1e+2
10^6
10^5
10^4
HB97
HD67
HC39
HH74
HH37
HE54
Animal ID
B
B. pseudomellei in blood (CFU/mL)
1e+6
1e+5
1e+4
1e+3
1e+2
HB97
HD67
HC39
HH74
HH37
HE54
Animal ID
C
B. pseudomellei (CFU/mL BAL)
1e+6
1e+4
1e+2
1e+0
+1d PI
+7d PI
HB97
HD67
HC39
HH74
HH37
HE54
Animal ID

FIG. 30

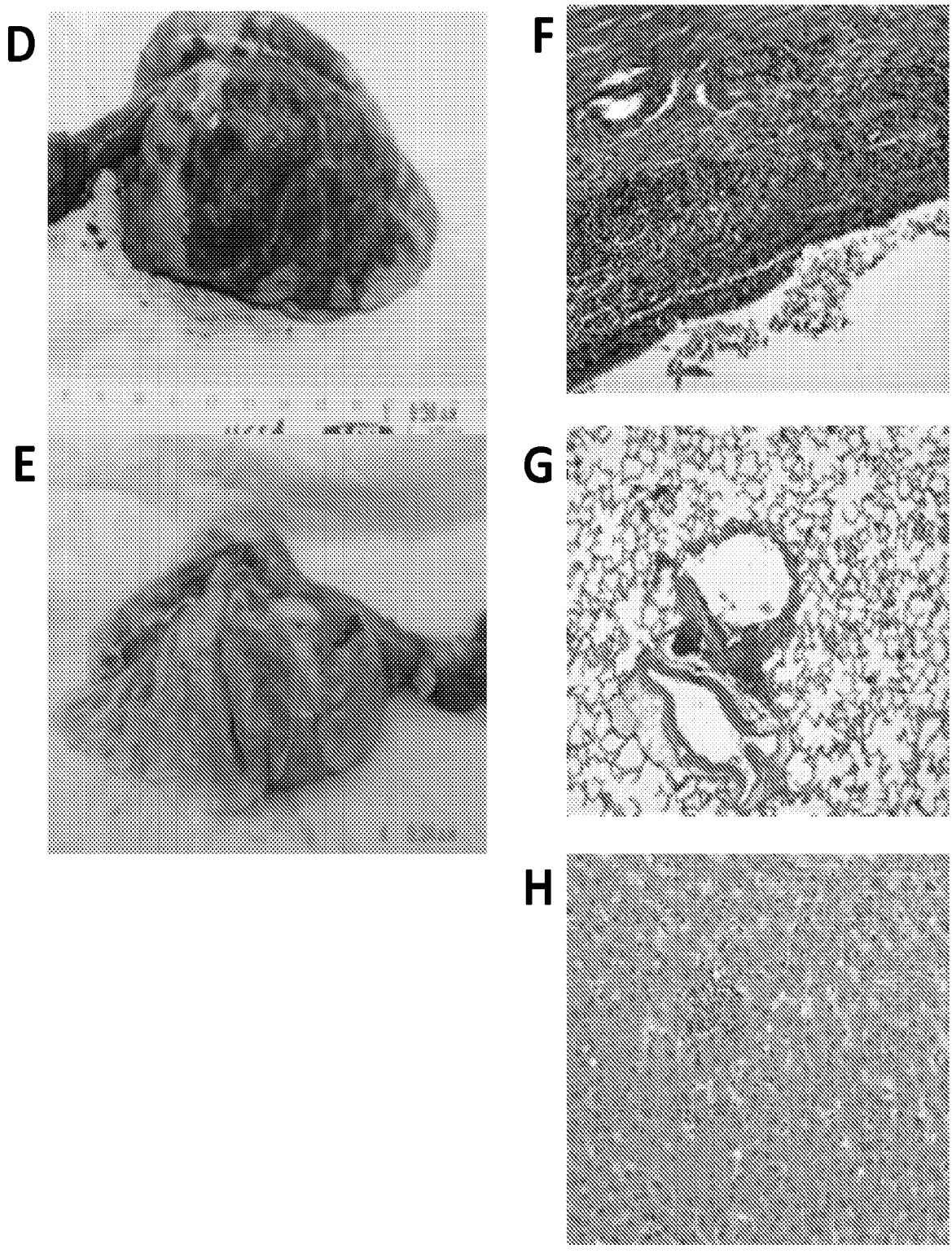


FIG. 30

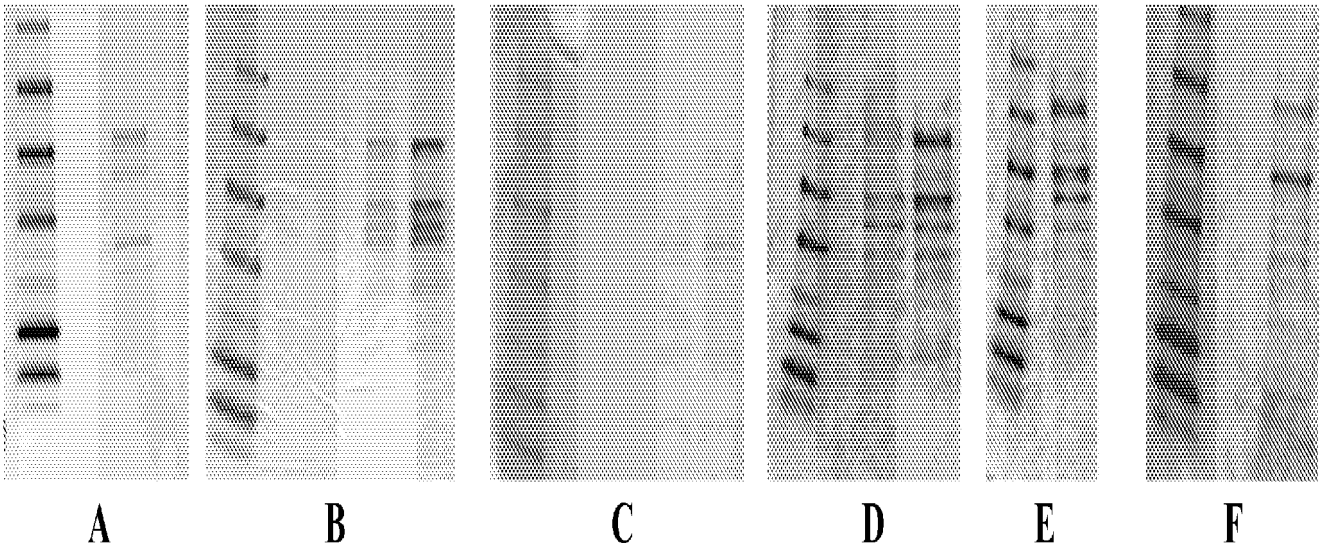


FIG. 31