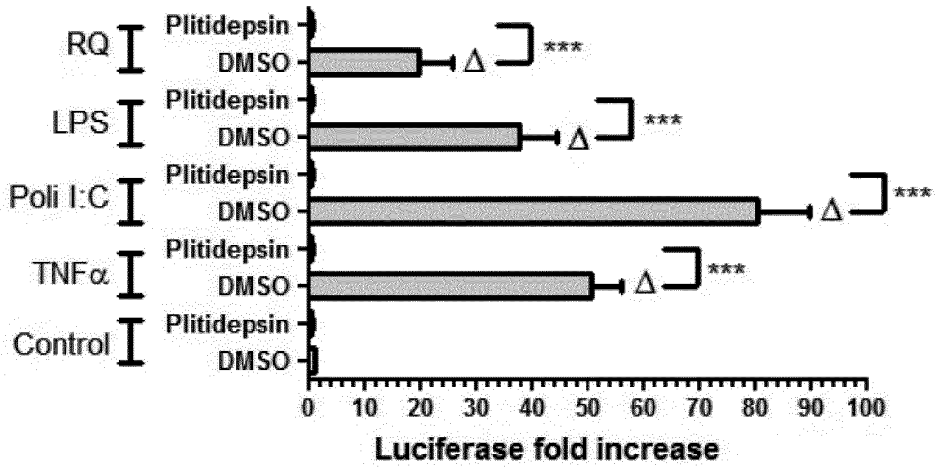

RQ
LPS
Poli I:C
TNFα
Control
Plitidepsin
DMSO
Δ
***
0 10 20 30 40 50 60 70 80 90 100
Luciferase fold increase

FIG. 1a

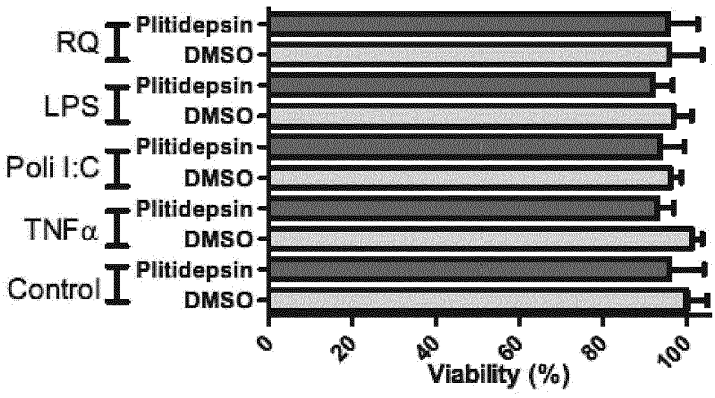

RQ
LPS
Poli I:C
TNFα
Control
Plitidepsin
DMSO
0 20 40 60 80 100
Viability (%)

FIG. 1b

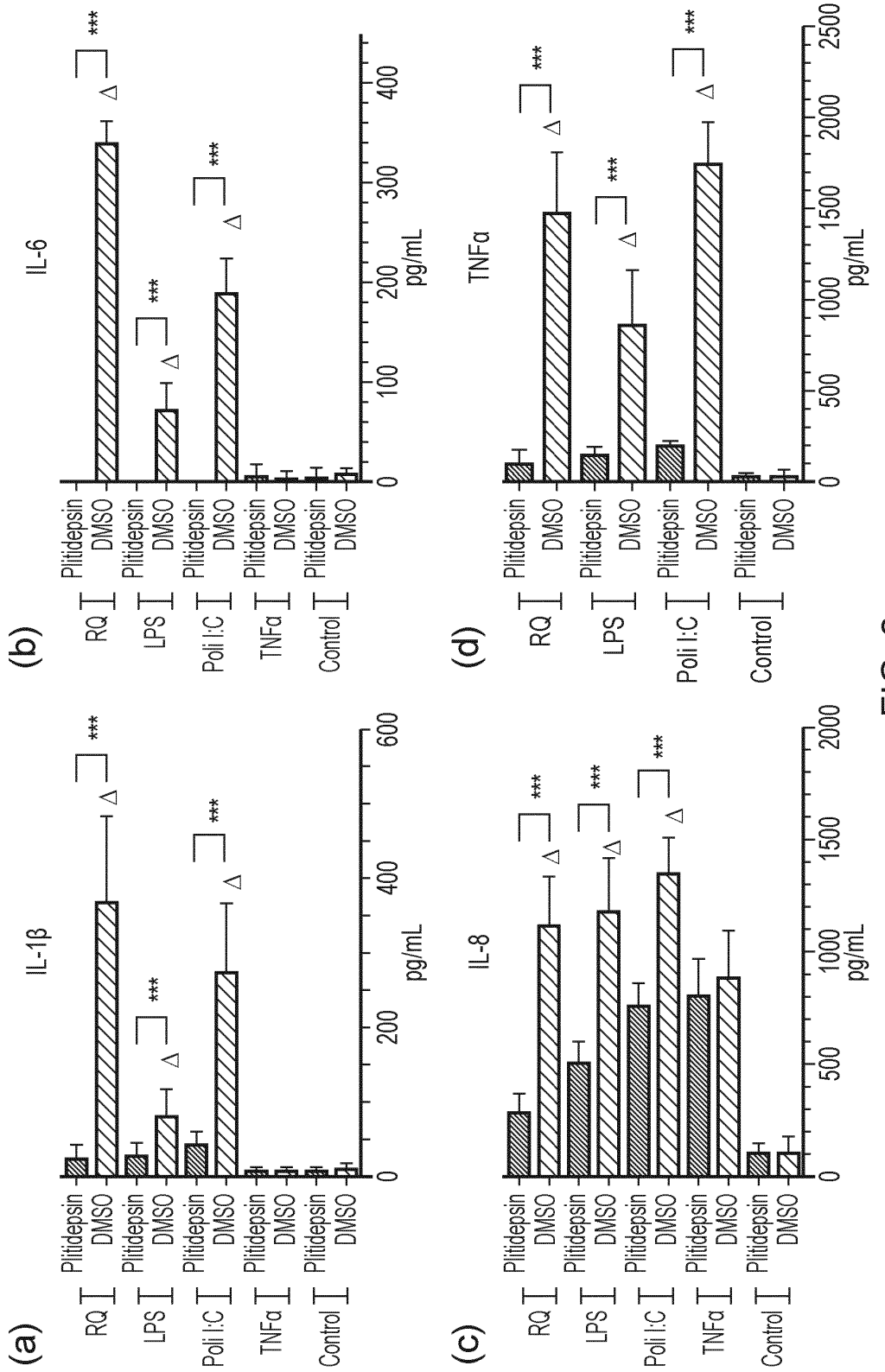

(a)
IL-1β
pg/mL
0
200
400
600
***
Δ
Plitidepsin
DMSO
RQ
LPS
Poli I:C
TNFα
Control
(b)
IL-6
pg/mL
0
100
200
300
400
***
Δ
(c)
IL-8
pg/mL
0
500
1000
1500
2000
***
Δ
(d)
TNFα
pg/mL
0
500
1000
1500
2000
2500
***
Δ

FIG. 2

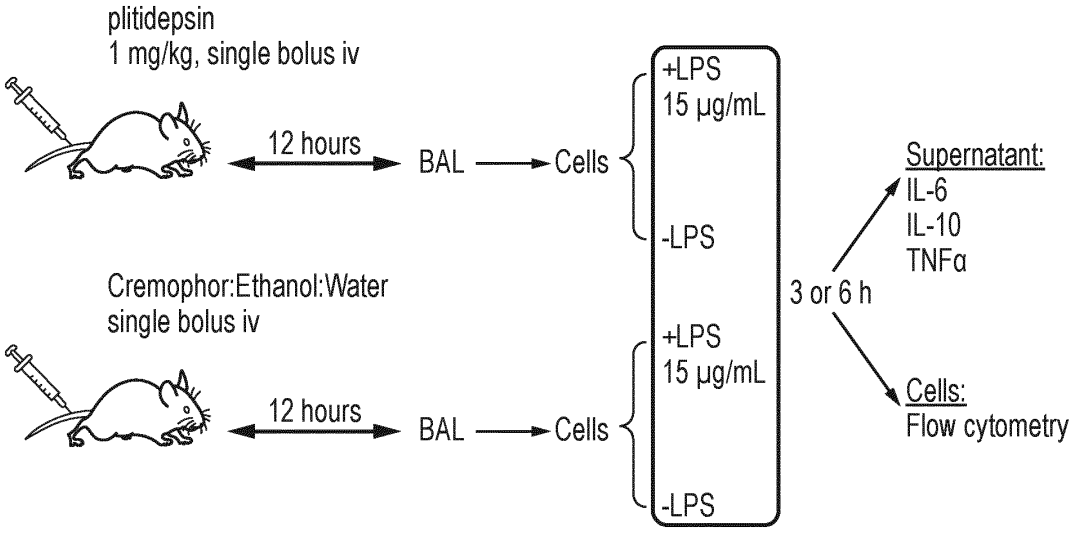

plitidepsin
1 mg/kg, single bolus iv
12 hours
BAL
Cells
+LPS
15 µg/mL
-LPS
Cremophor:Ethanol:Water
single bolus iv
12 hours
BAL
Cells
+LPS
15 µg/mL
-LPS
3 or 6 h
Supernatant:
IL-6
IL-10
TNFα
Cells:
Flow cytometry

FIG. 3(a)

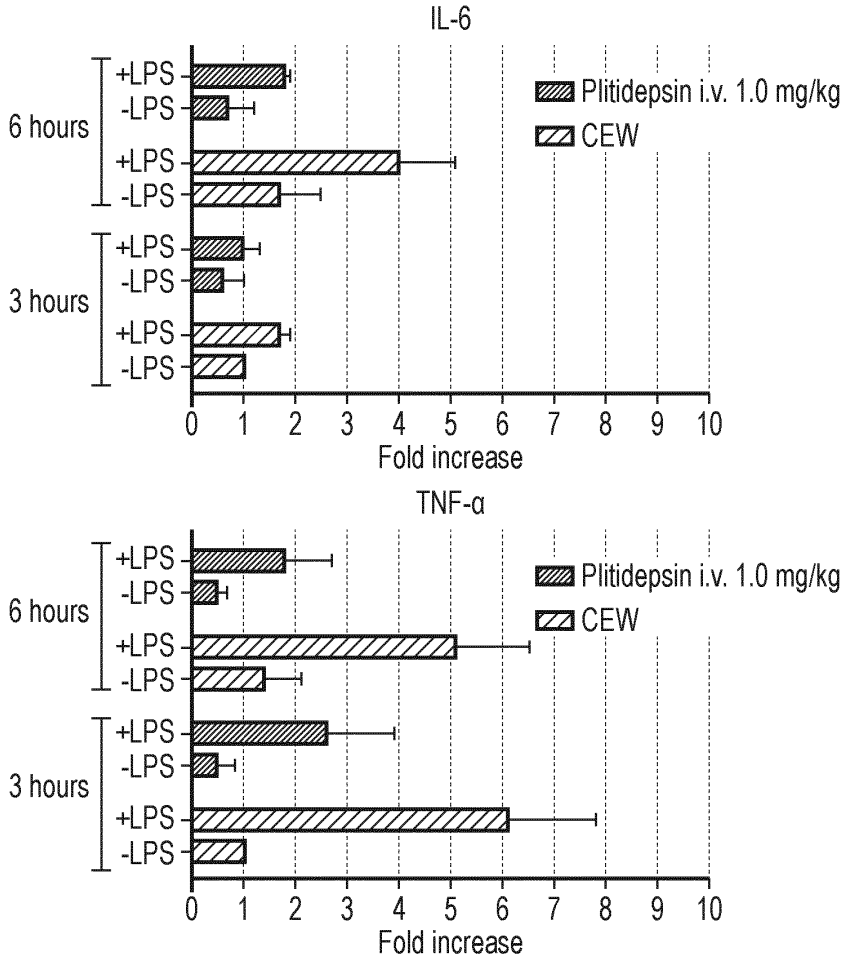

IL-6
6 hours
3 hours
+LPS
-LPS
Plitidepsin i.v. 1.0 mg/kg
CEW
0 1 2 3 4 5 6 7 8 9 10
Fold increase
TNF-α
6 hours
3 hours
+LPS
-LPS
Plitidepsin i.v. 1.0 mg/kg
CEW
0 1 2 3 4 5 6 7 8 9 10
Fold increase

FIG. 3(b)

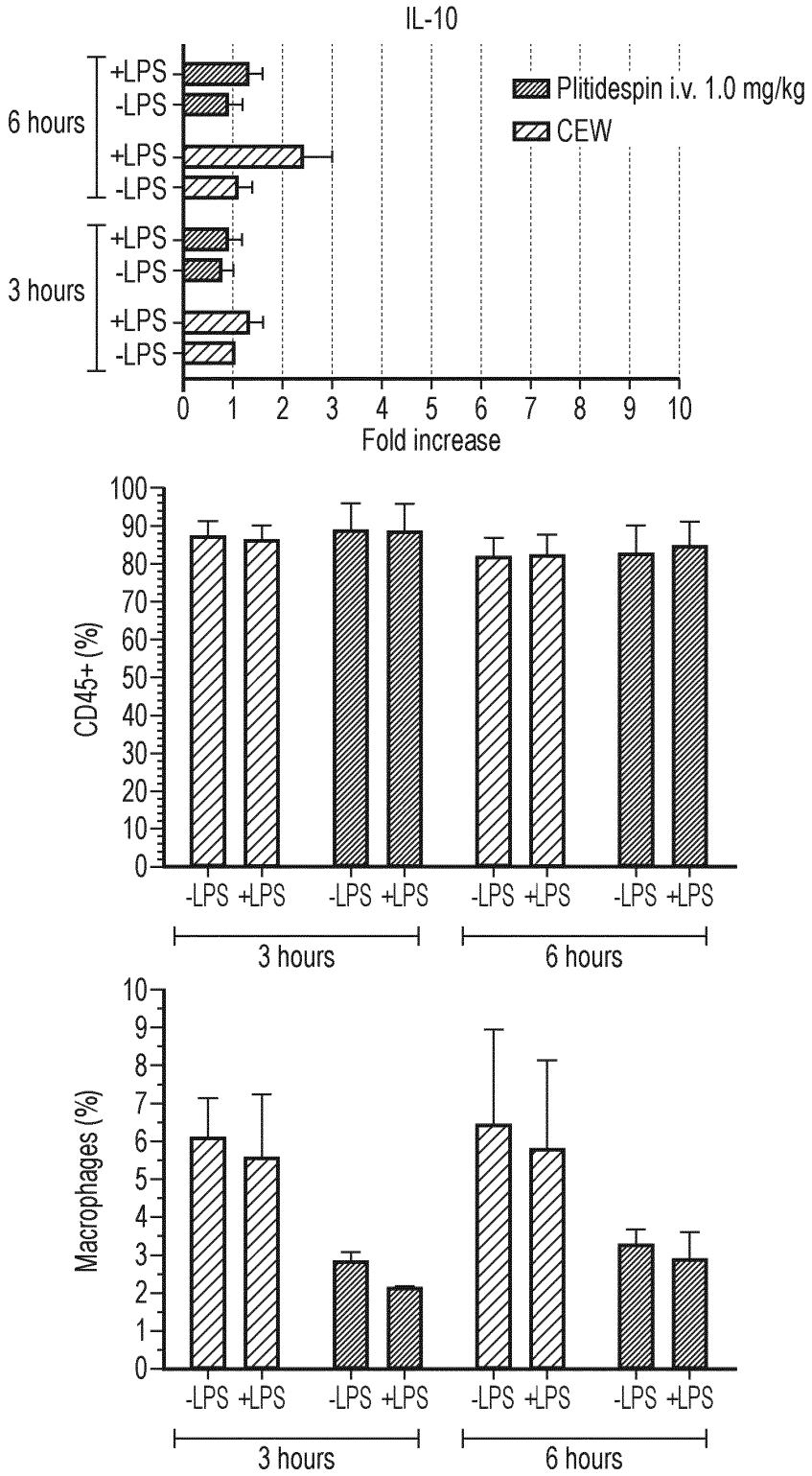


FIG. 3(b) (Continued)

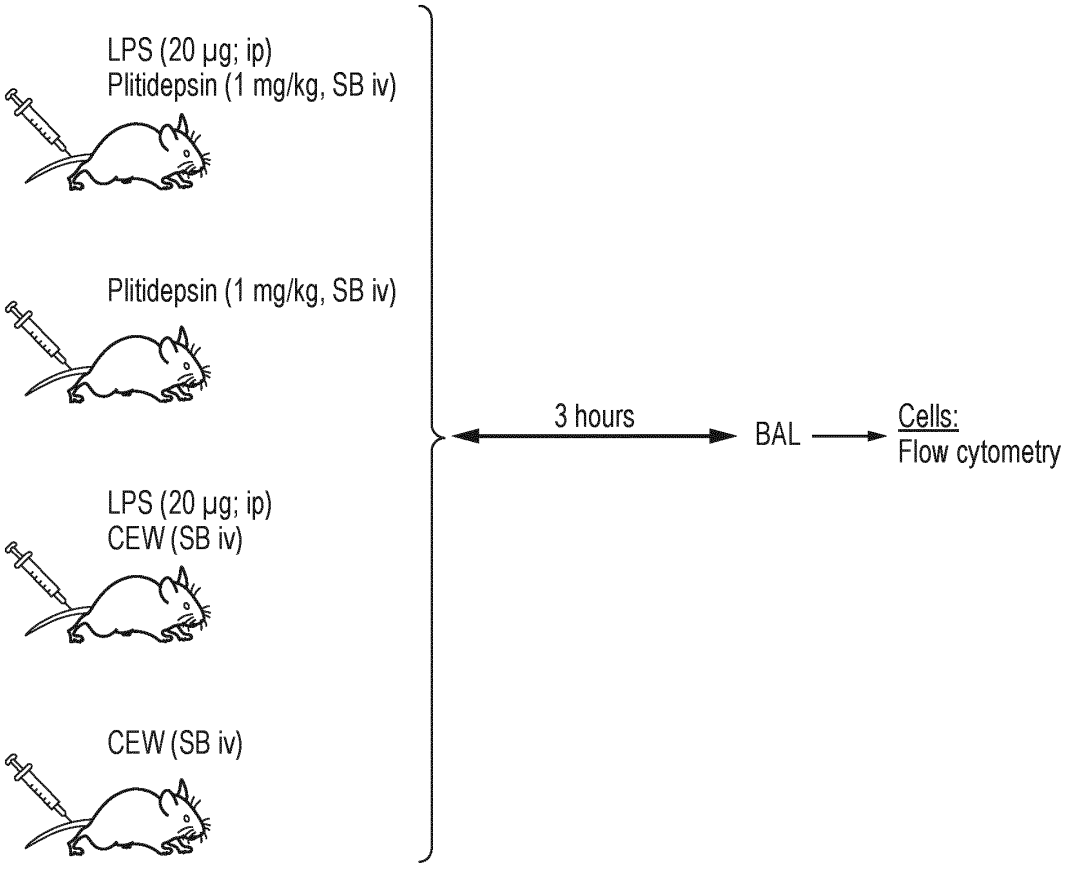

LPS (20 µg; ip)
Plitidepsin (1 mg/kg, SB iv)
Plitidepsin (1 mg/kg, SB iv)
LPS (20 µg; ip)
CEW (SB iv)
CEW (SB iv)
3 hours
BAL
Cells:
Flow cytometry

FIG. 4(a)

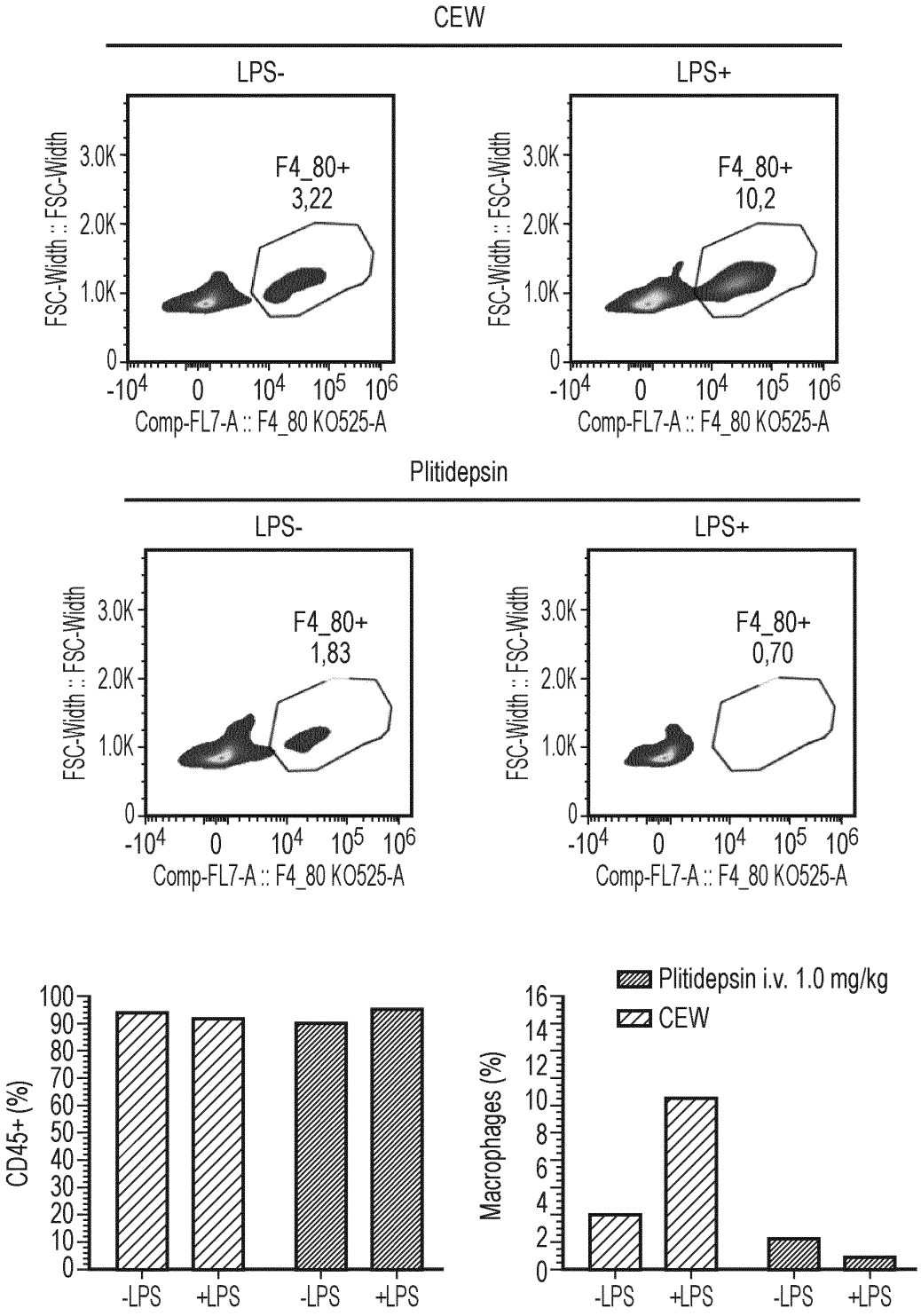

CEW
LPS-
LPS+
F4_80+
3,22
F4_80+
10,2
FSC-Width :: FSC-Width
Comp-FL7-A :: F4_80 KO525-A
Plitidepsin
F4_80+
1,83
F4_80+
0,70
CD45+ (%)
Macrophages (%)
Plitidepsin i.v. 1.0 mg/kg
CEW
-LPS
+LPS

FIG. 4(b)

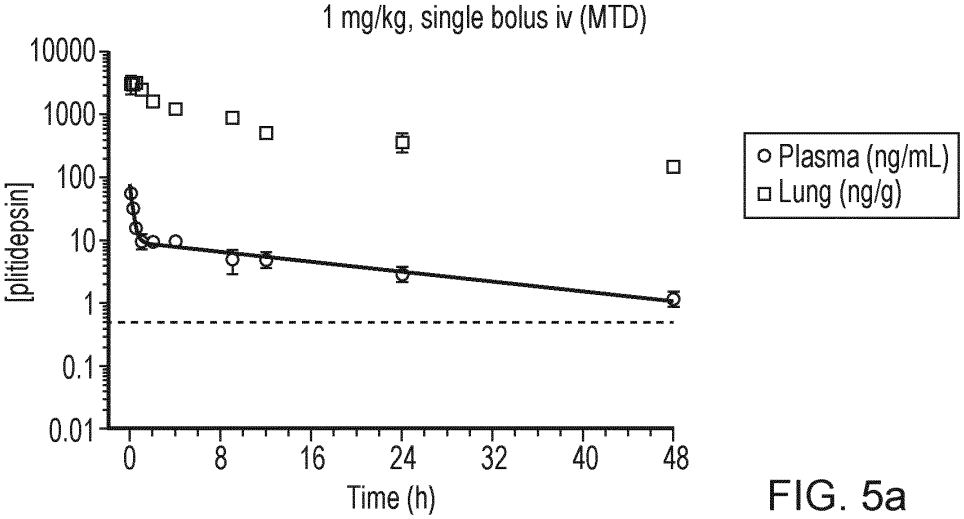


FIG. 5a

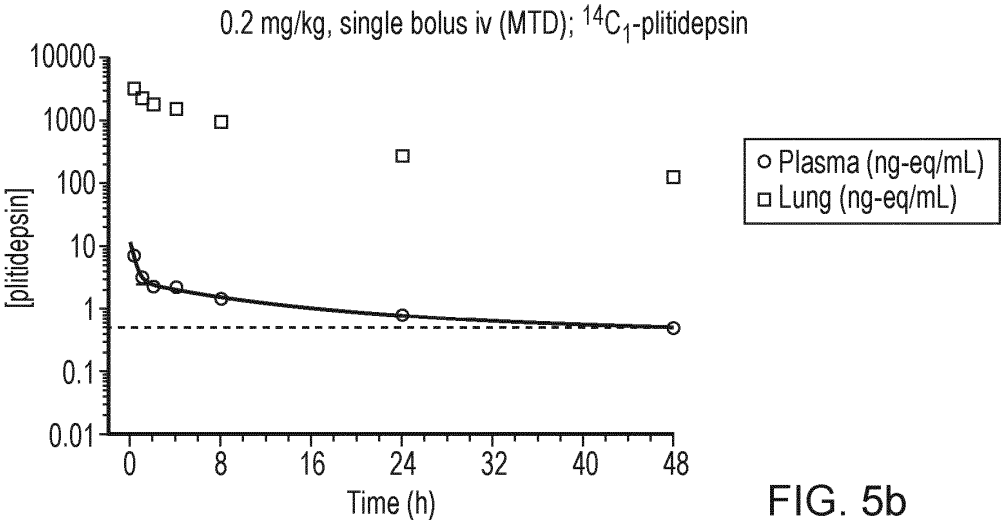


FIG. 5b

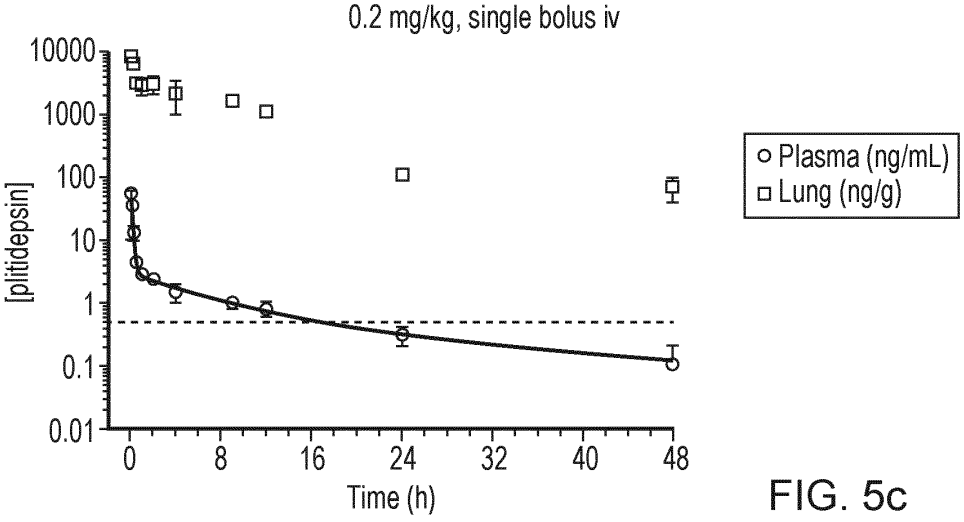


FIG. 5c

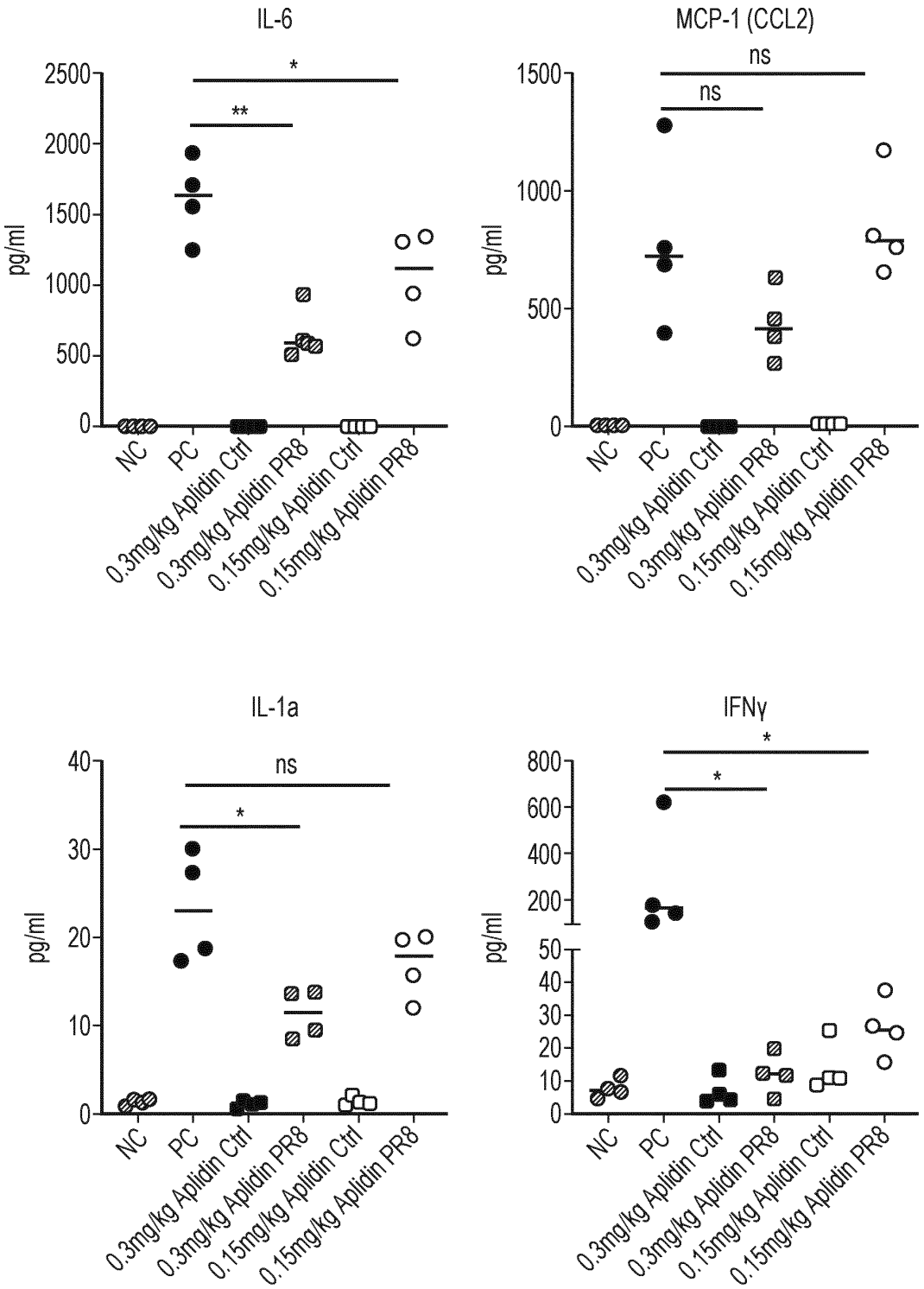

IL-6
pg/ml
2500
2000
1500
1000
500
0
*
**
NC
PC
0.3mg/kg Aplidin Ctrl
0.3mg/kg Aplidin PR8
0.15mg/kg Aplidin Ctrl
0.15mg/kg Aplidin PR8
MCP-1 (CCL2)
pg/ml
1500
1000
500
0
ns
ns
NC
PC
0.3mg/kg Aplidin Ctrl
0.3mg/kg Aplidin PR8
0.15mg/kg Aplidin Ctrl
0.15mg/kg Aplidin PR8
IL-1a
pg/ml
40
30
20
10
0
ns
*
NC
PC
0.3mg/kg Aplidin Ctrl
0.3mg/kg Aplidin PR8
0.15mg/kg Aplidin Ctrl
0.15mg/kg Aplidin PR8
IFNγ
pg/ml
800
600
400
200
50
40
30
20
10
0
*
*
NC
PC
0.3mg/kg Aplidin Ctrl
0.3mg/kg Aplidin PR8
0.15mg/kg Aplidin Ctrl
0.15mg/kg Aplidin PR8

FIG. 6

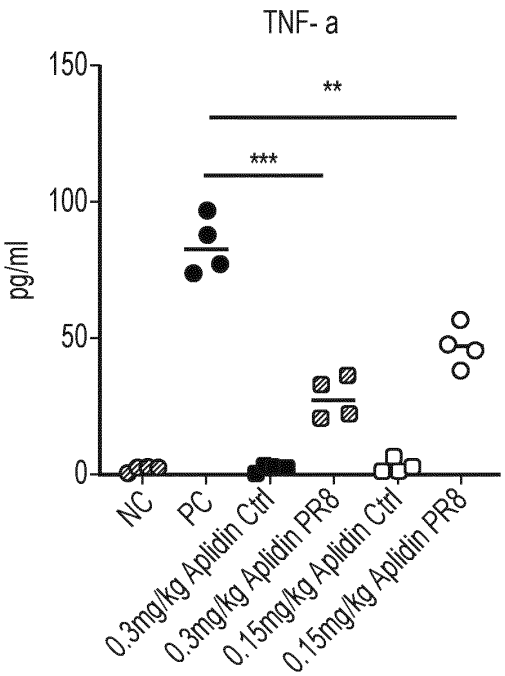


FIG. 6 (continued)

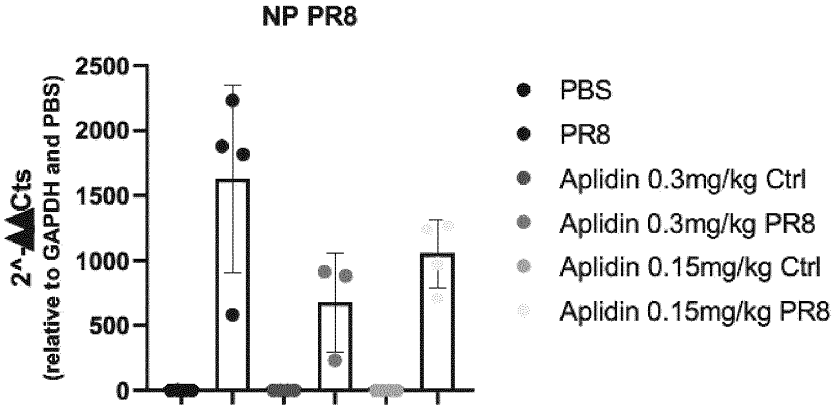


FIG. 7

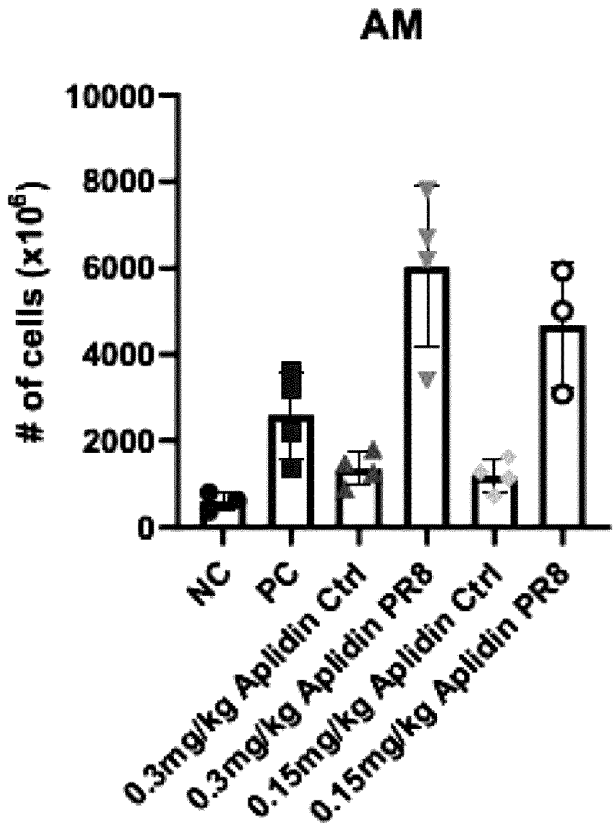

AM
# of cells (x10^6)
10000
8000
6000
4000
2000
0
NC
PC
0.3mg/kg Aplidin Ctrl
0.3mg/kg Aplidin PR8
0.15mg/kg Aplidin Ctrl
0.15mg/kg Aplidin PR8

FIG. 8

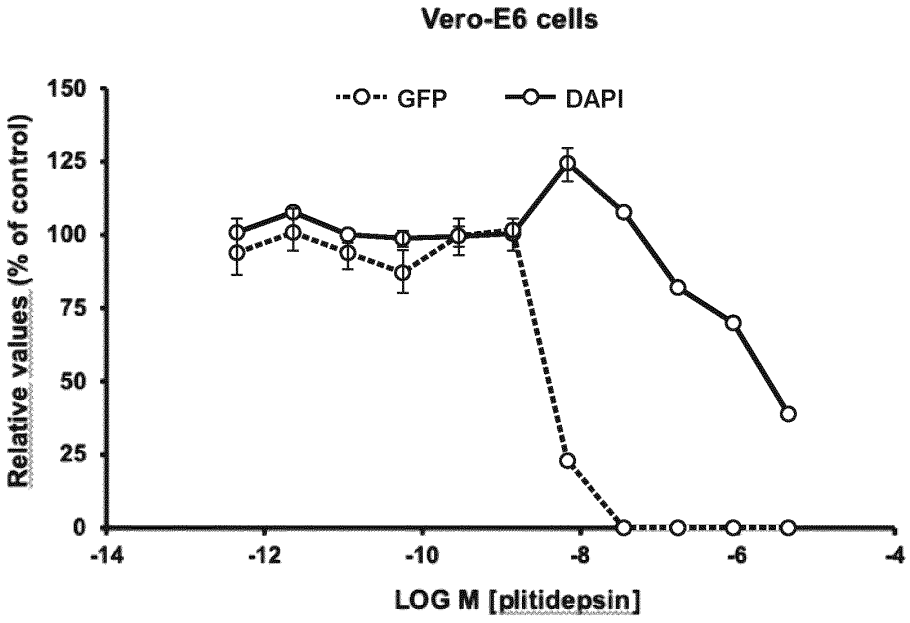

Vero-E6 cells
GFP
DAPI
Relative values (% of control)
150
125
100
75
50
25
0
-14
-12
-10
-8
-6
-4
LOG M [plitidepsin]

FIG. 9

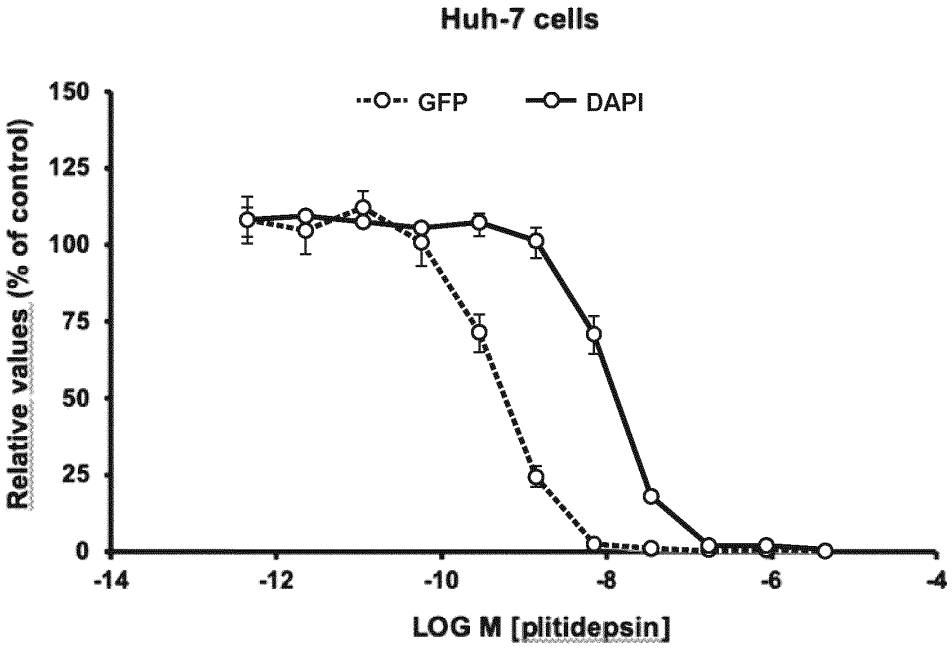


FIG. 10

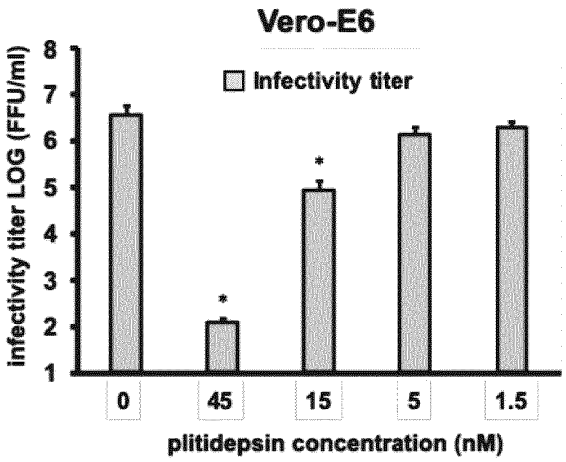


FIG. 11(a)

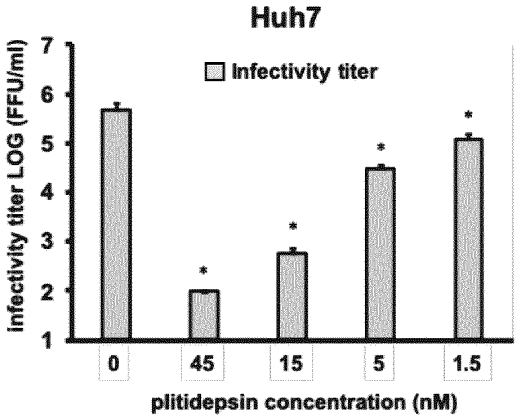


FIG. 11(b)

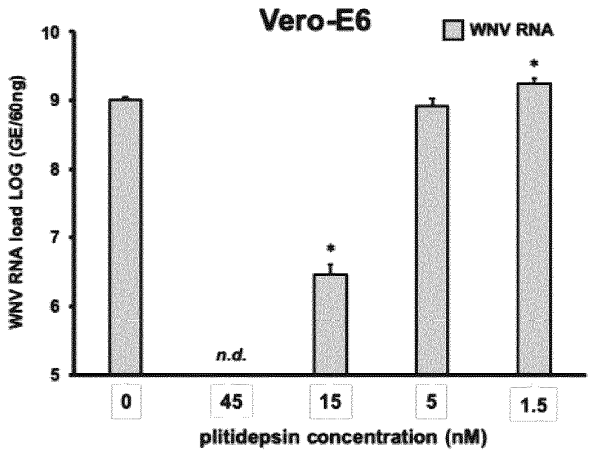


FIG. 12(a)

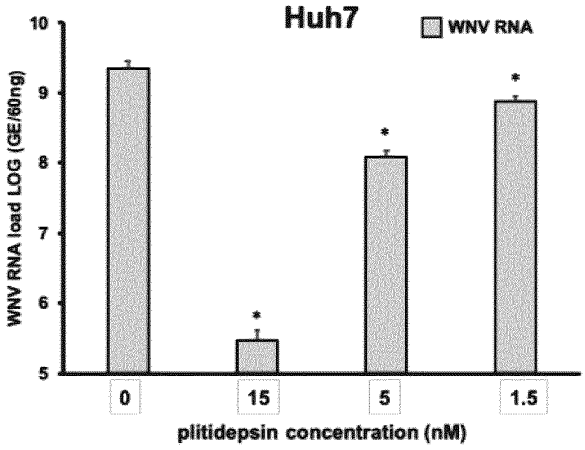


FIG. 12(b)

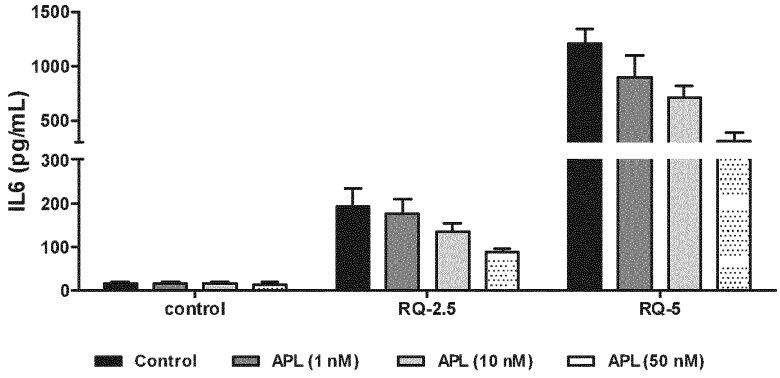


FIG. 13(a)

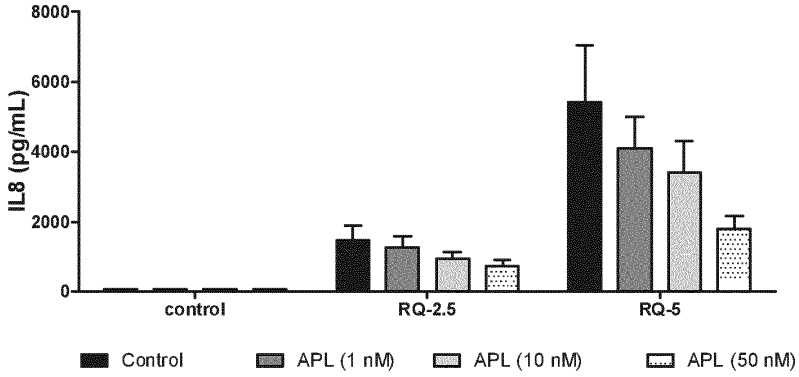


FIG. 13(b)

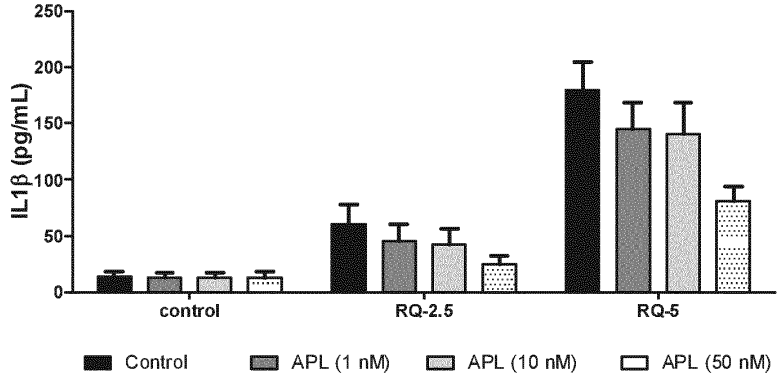


FIG. 13(c)

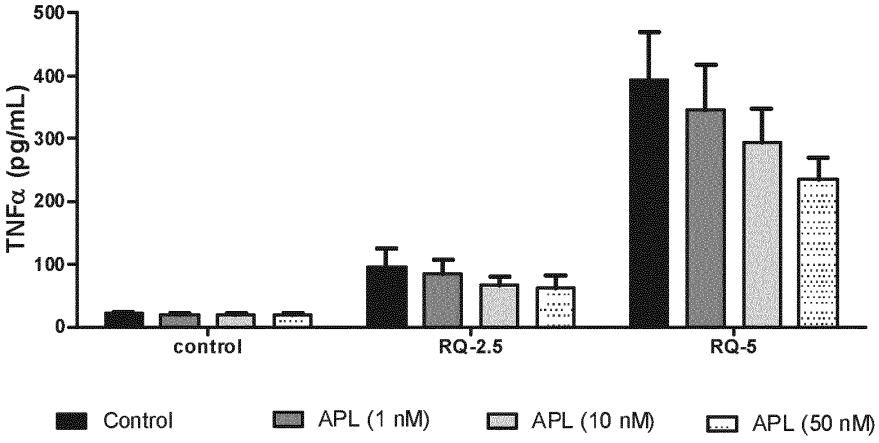


FIG. 13(d)

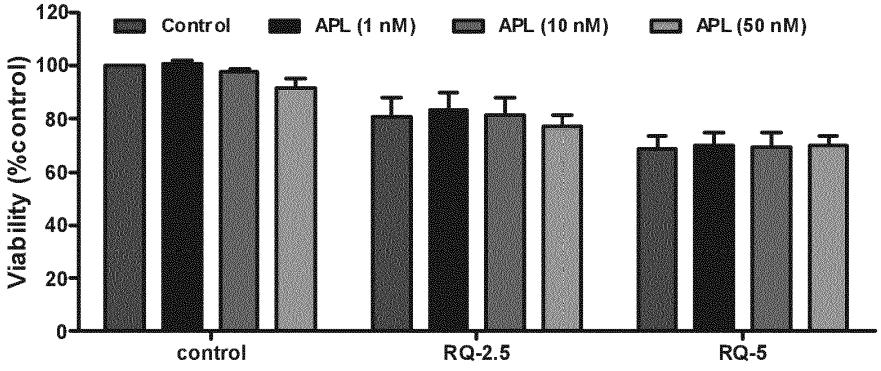


FIG. 13(e)

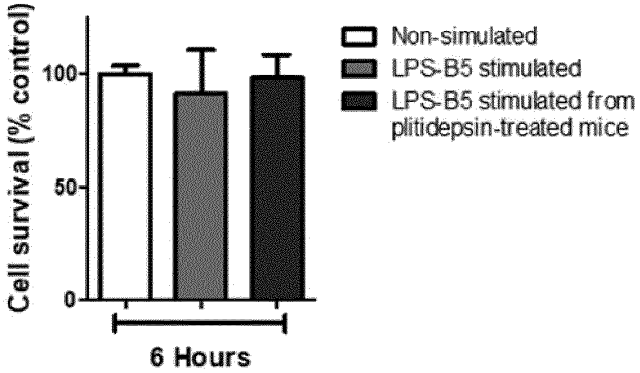


FIG. 14(a)

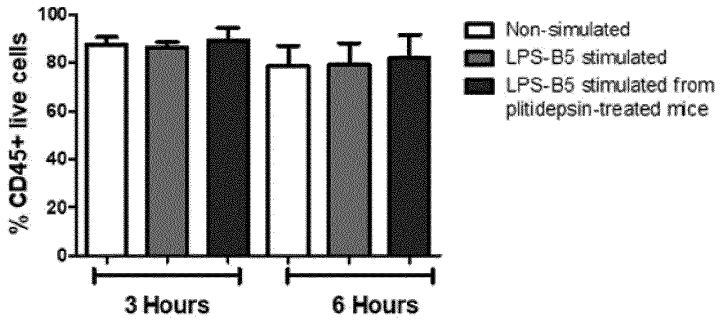


FIG. 14(b)

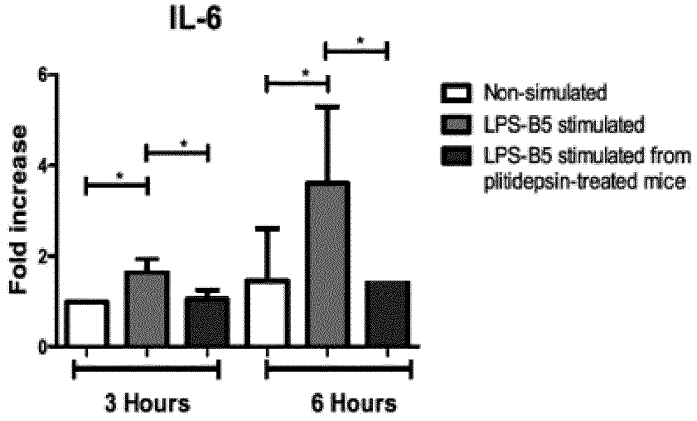


FIG. 14(c)

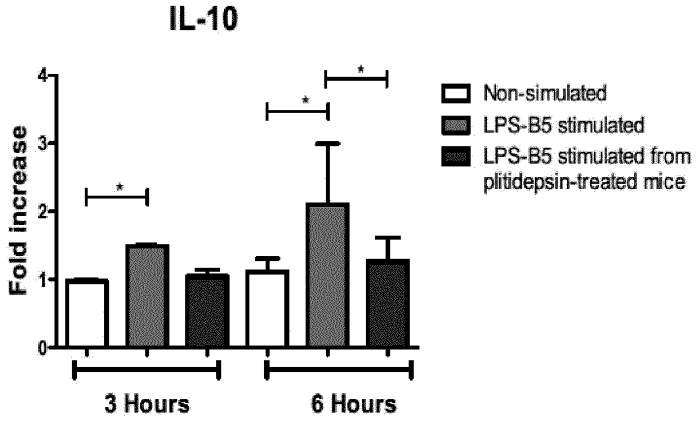


FIG. 14(d)

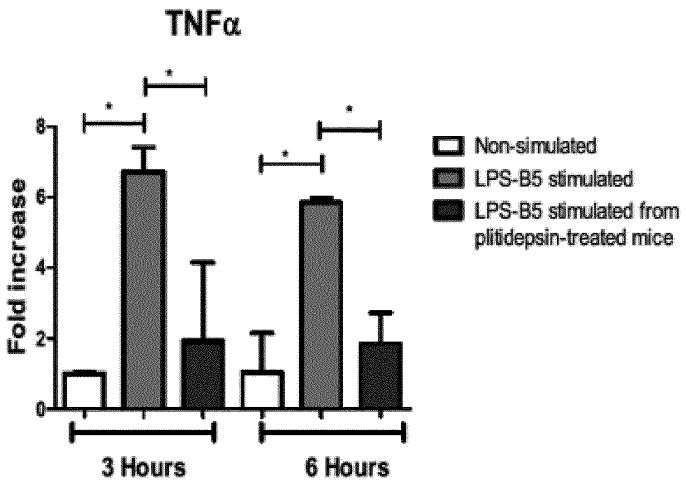


FIG. 14(e)

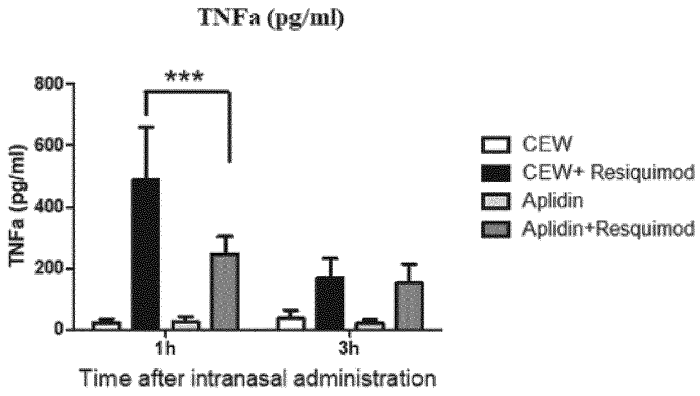


FIG. 15

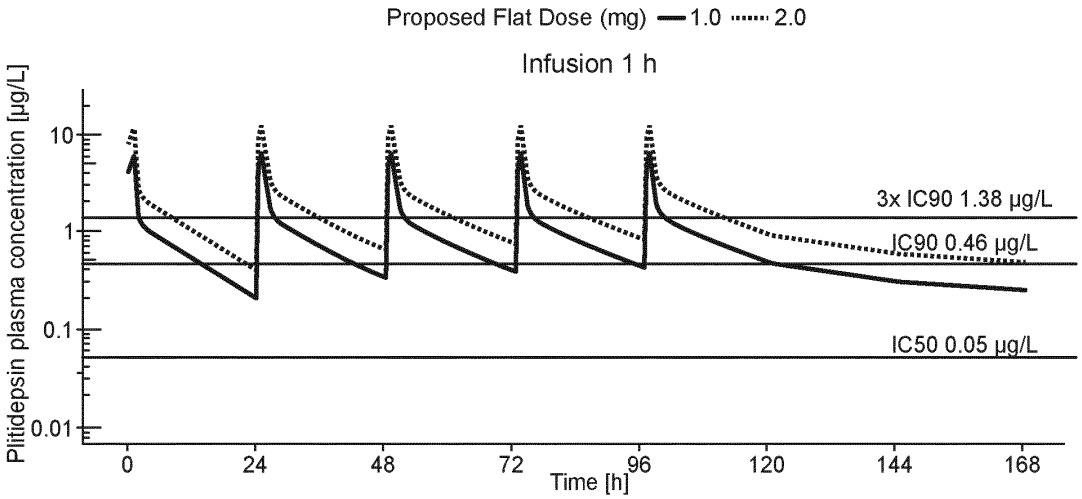

Proposed Flat Dose (mg) 1.0 2.0
Infusion 1 h
Plitidepsin plasma concentration [µg/L]
10
1
0.1
0.01
3x IC90 1.38 µg/L
IC90 0.46 µg/L
IC50 0.05 µg/L
0
24
48
72
96
120
144
168
Time [h]

FIG. 16

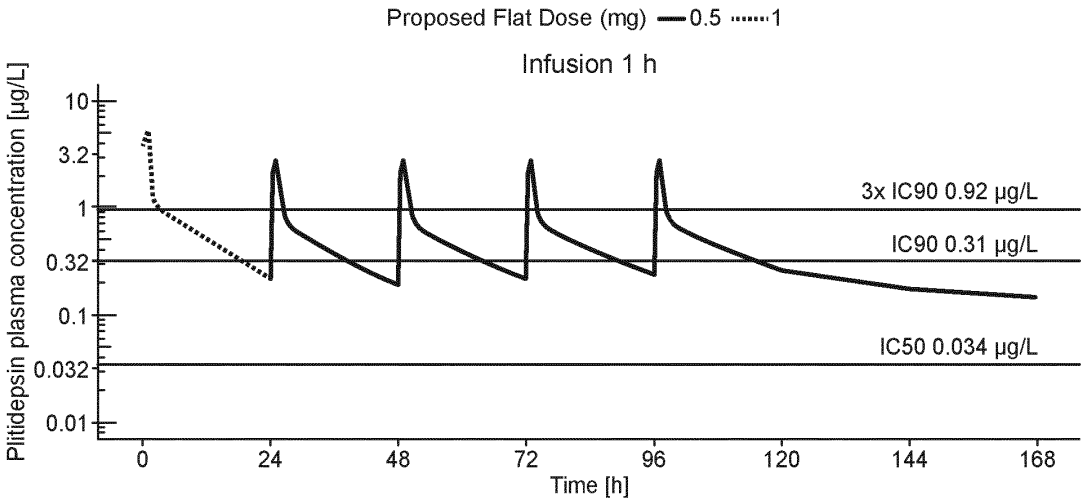

Proposed Flat Dose (mg) 0.5 1
Infusion 1 h
Plitidepsin plasma concentration [µg/L]
10
3.2
1
0.32
0.1
0.032
0.01
3x IC90 0.92 µg/L
IC90 0.31 µg/L
IC50 0.034 µg/L
0
24
48
72
96
120
144
168
Time [h]

FIG. 17

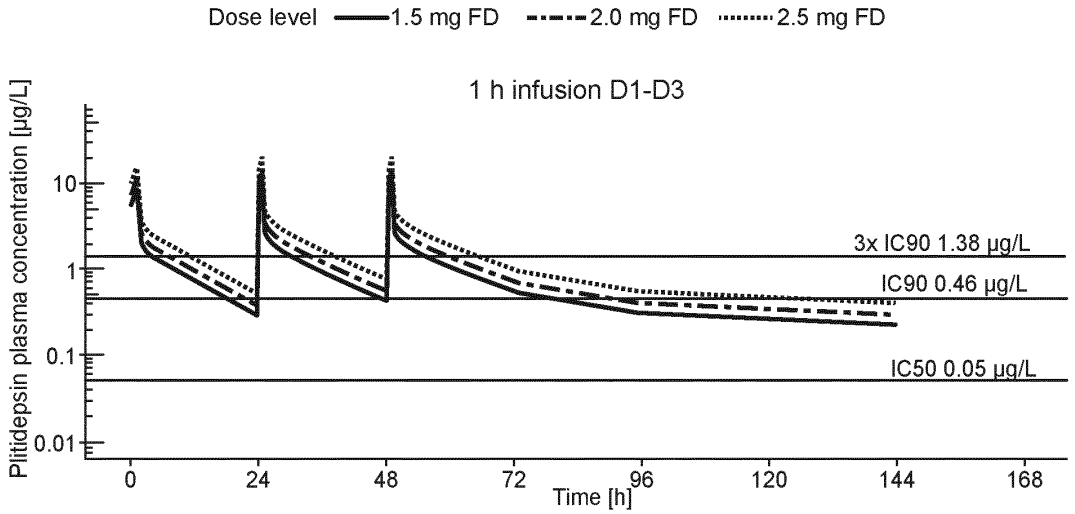

Dose level 1.5 mg FD 2.0 mg FD 2.5 mg FD
1 h infusion D1-D3
Plitidepsin plasma concentration [µg/L]
10
1
0.1
0.01
3x IC90 1.38 µg/L
IC90 0.46 µg/L
IC50 0.05 µg/L
0
24
48
72
96
120
144
168
Time [h]

FIG. 18

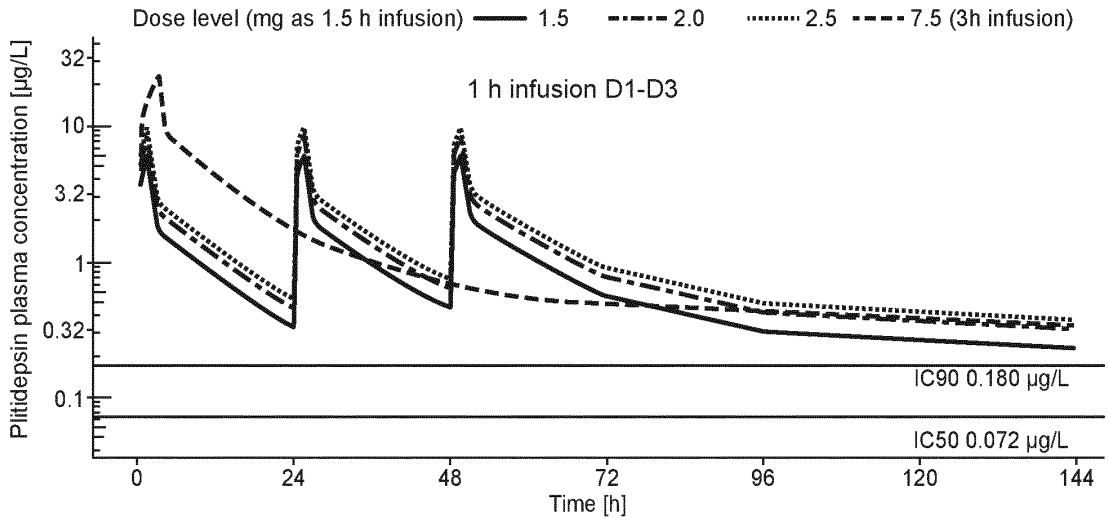

Dose level (mg as 1.5 h infusion) 1.5 2.0 2.5 7.5 (3h infusion)
1 h infusion D1-D3
Plitidepsin plasma concentration [µg/L]
32
10
3.2
1
0.32
0.1
IC90 0.180 µg/L
IC50 0.072 µg/L
0
24
48
72
96
120
144
Time [h]

FIG. 19