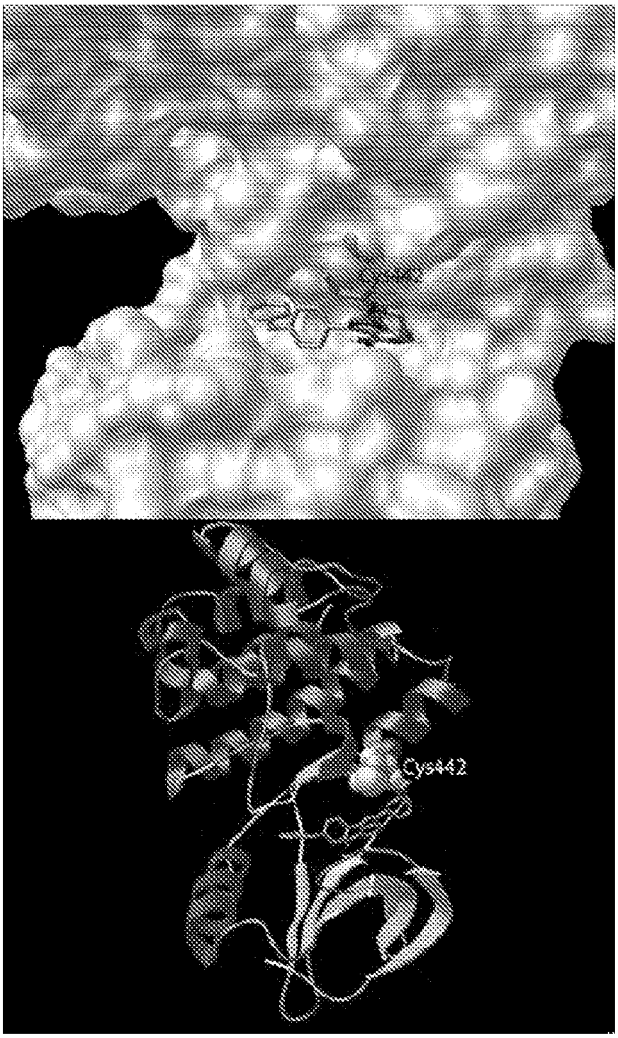
FIGURE 1A

a

	Tyrosine Kinase	Ibrutinib IC50(nM)
Irreversible Inhibition (Contain Cys-481)	ITK	2.2
	BTK	0.1
	Bmx	0.7
	Tec	0.4
	EGFR	4.5
	JAK3	3.8
	HER2	1.4
	HER4	<0.5
	Blk	<0.5
Reversible Inhibition	Lck	0.9
	C-src	148.5
	Lyn	0.9
	Yes	<0.5
	Csk	4.2
	VEGFR2	241

c



b

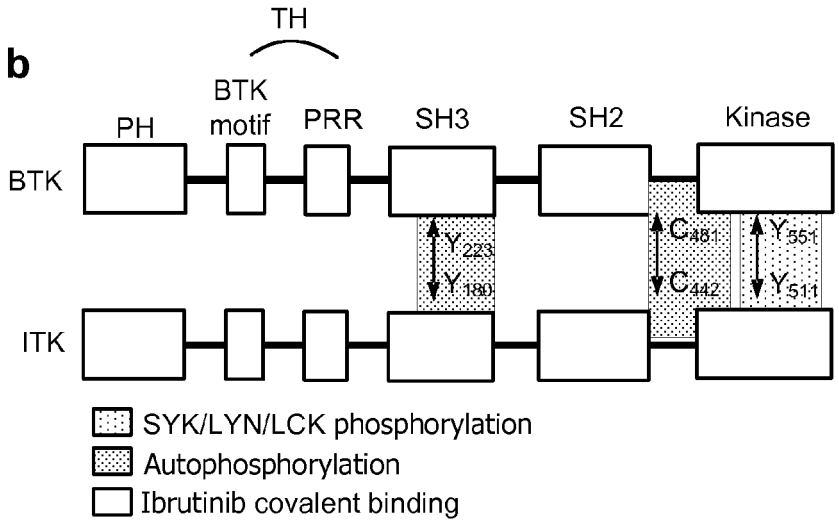


FIGURE 1A
(Continued)

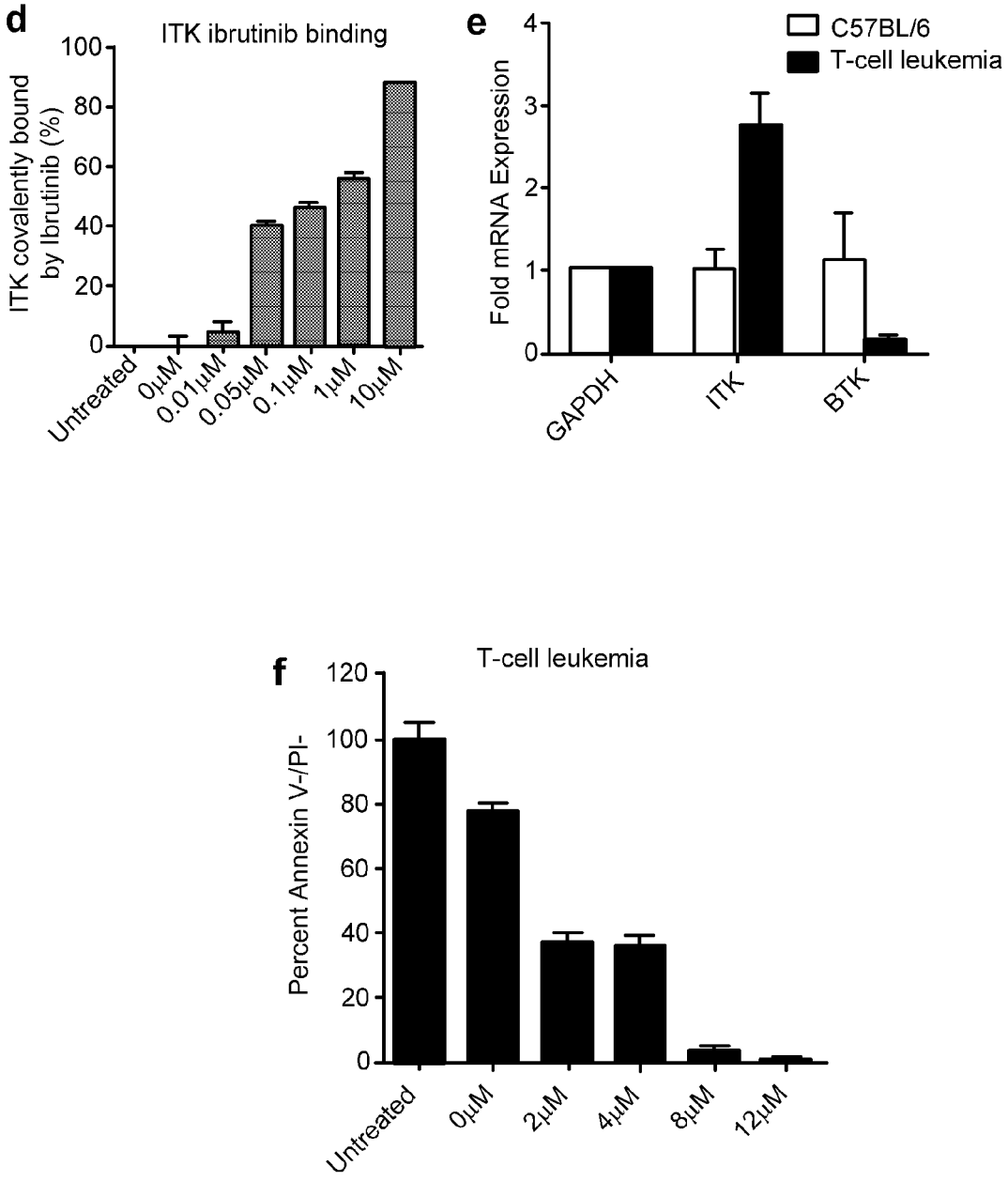


FIGURE 1B

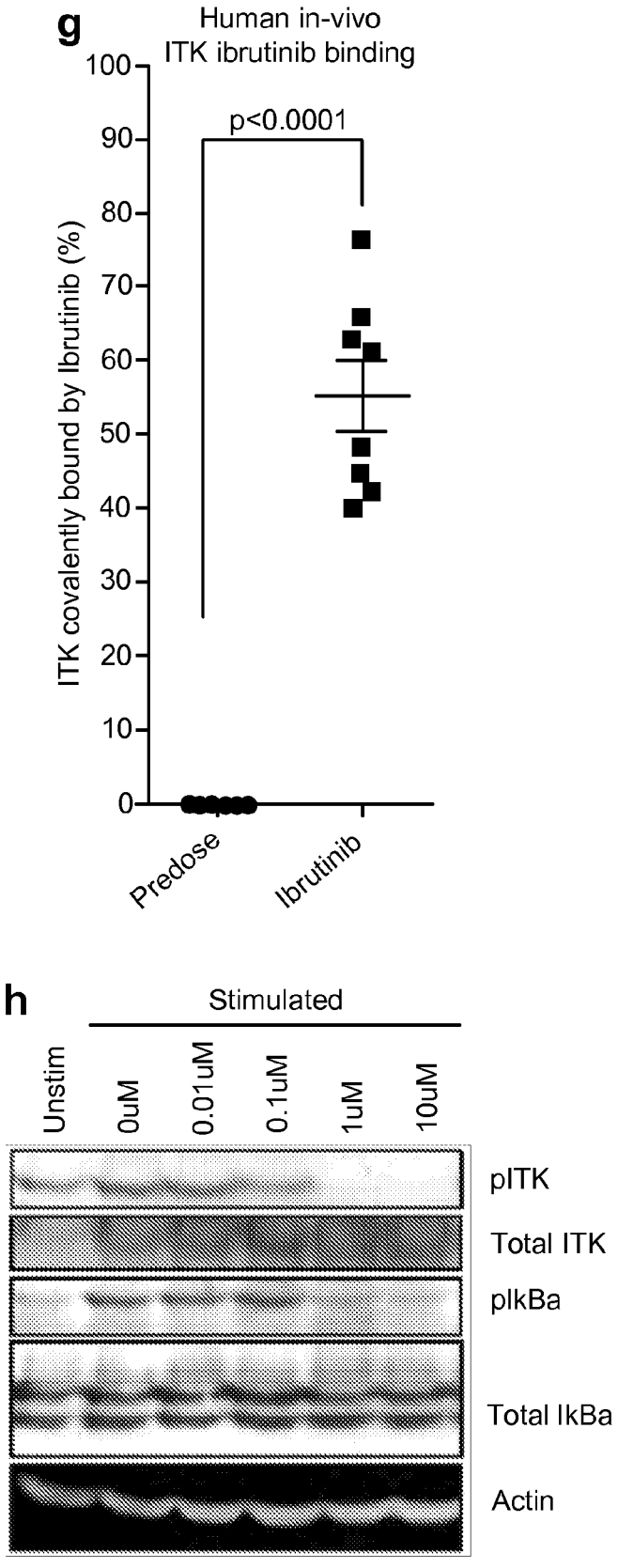

g
Human in-vivo
ITK ibrutinib binding
p<0.0001
ITK covalently bound by Ibrutinib (%)
100
90
80
70
60
50
40
30
20
10
0
Predose
Ibrutinib
h
Stimulated
Unstim
0uM
0.01uM
0.1uM
1uM
10uM
pITK
Total ITK
pIkBa
Total IkBa
Actin

FIGURE 1B
(Continued)

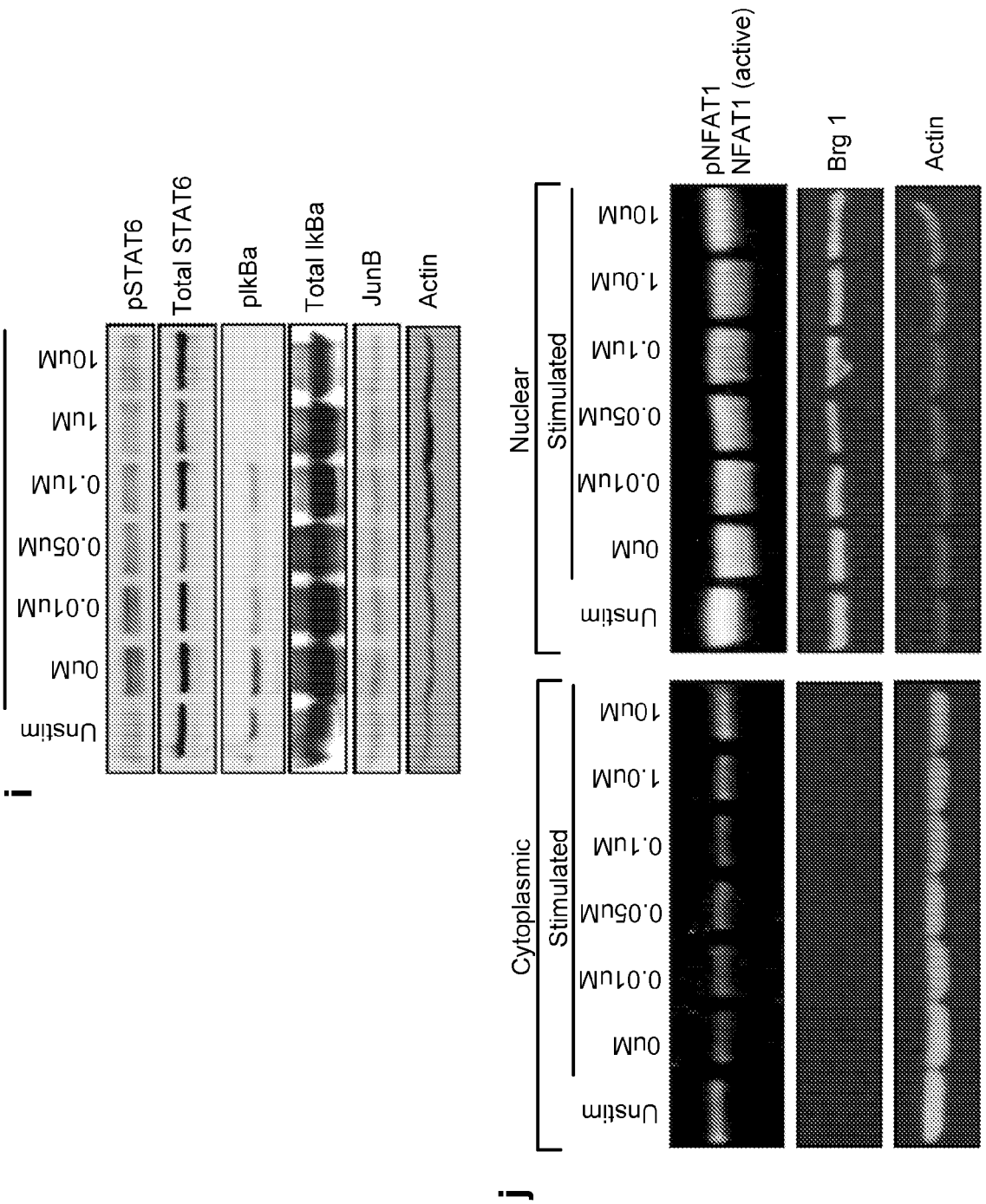


FIGURE 2

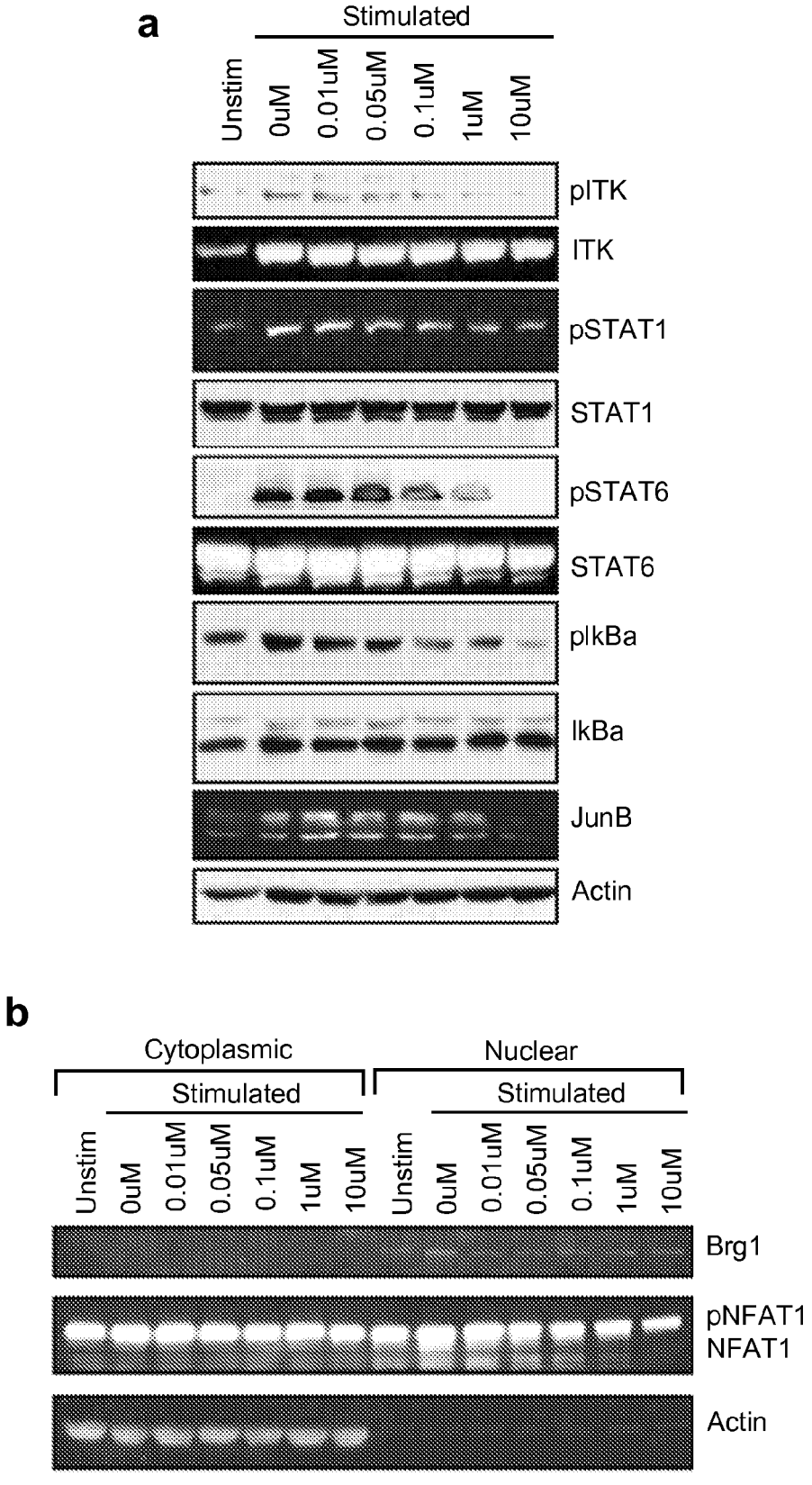
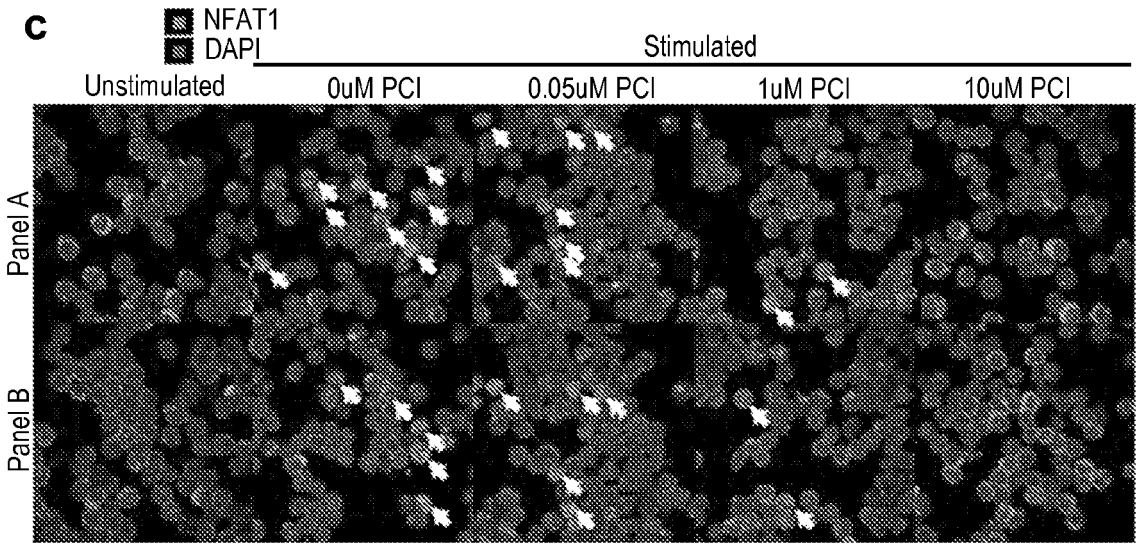

a
Stimulated
Unstim
0uM
0.01uM
0.05uM
0.1uM
1uM
10uM
pITK
ITK
pSTAT1
STAT1
pSTAT6
STAT6
pIkBa
IkBa
JunB
Actin
b
Cytoplasmic
Nuclear
Stimulated
Stimulated
Unstim
0uM
0.01uM
0.05uM
0.1uM
1uM
10uM
Unstim
0uM
0.01uM
0.05uM
0.1uM
1uM
10uM
Brg1
pNFAT1
NFAT1
Actin

FIGURE 2
(Continued)



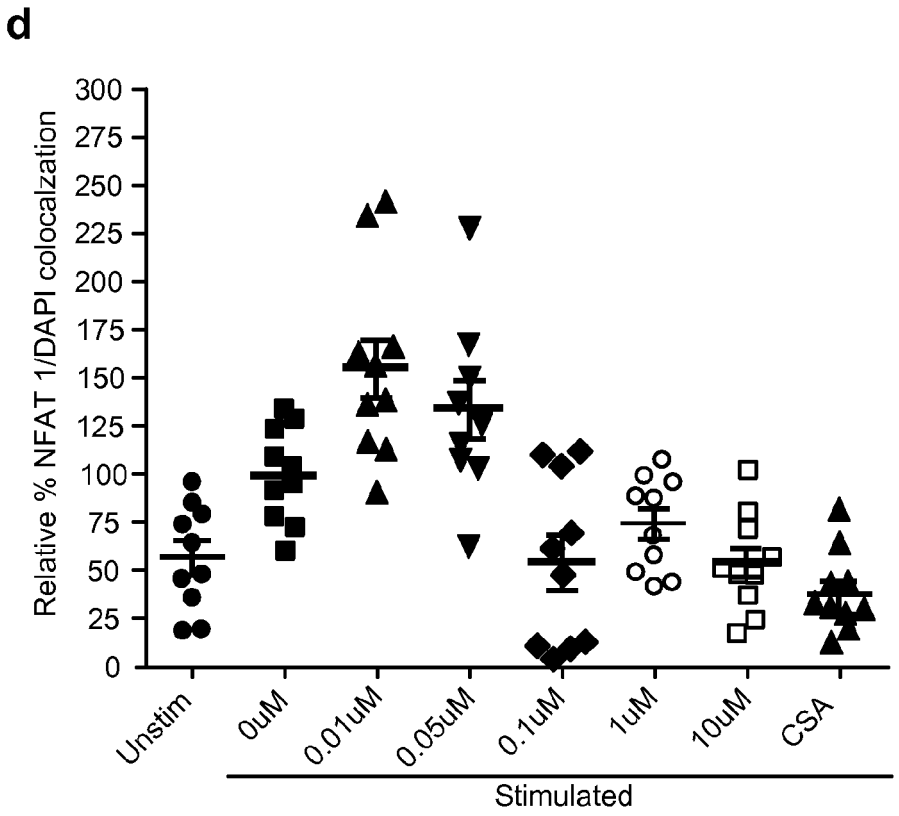


FIGURE 2
(Continued)

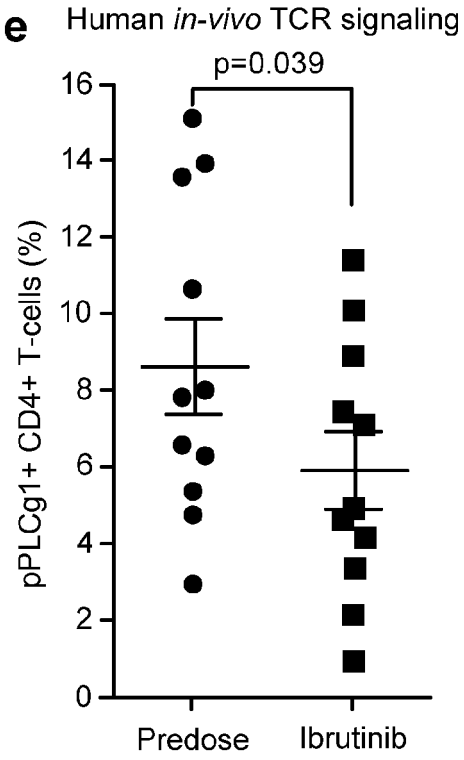


FIGURE 2
(Continued)

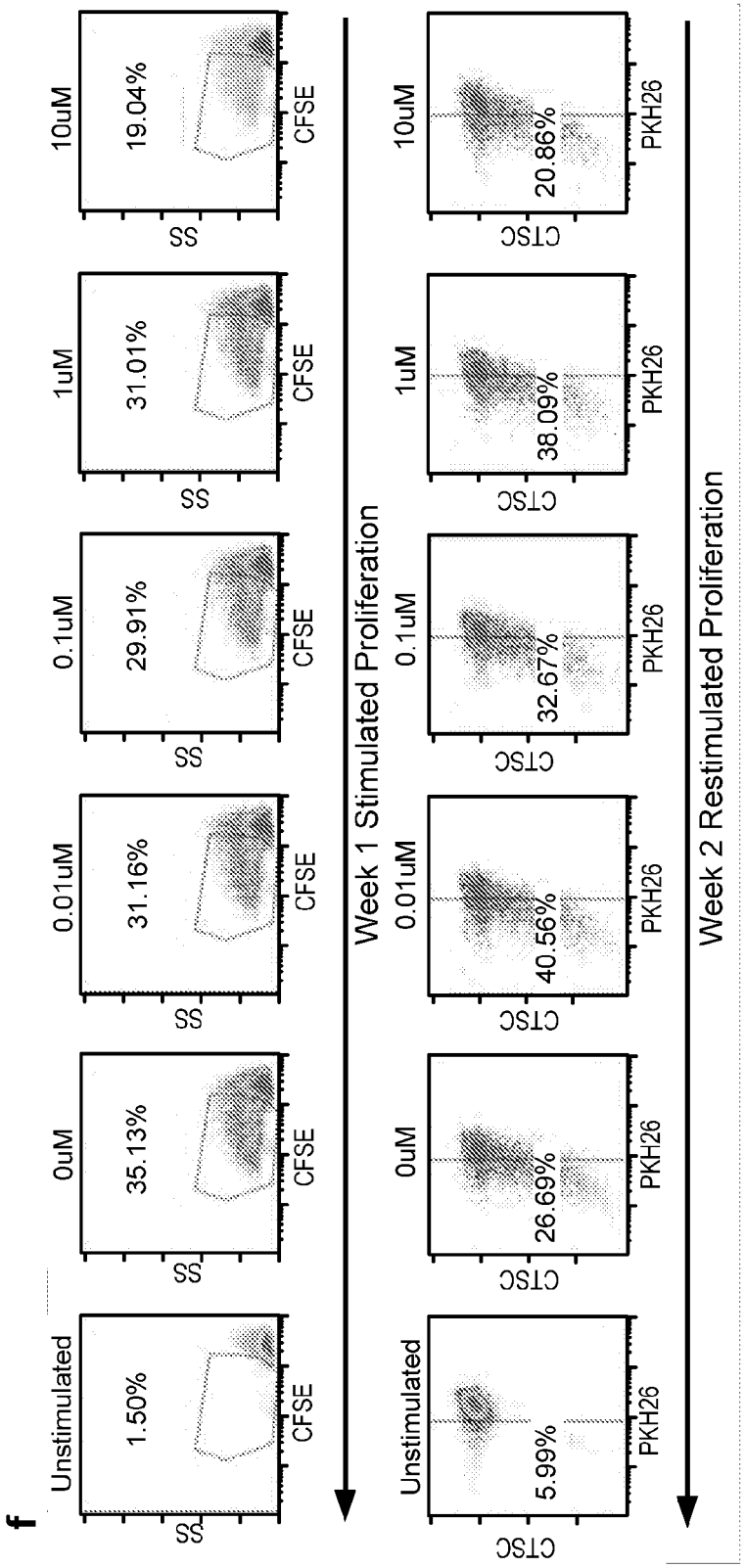
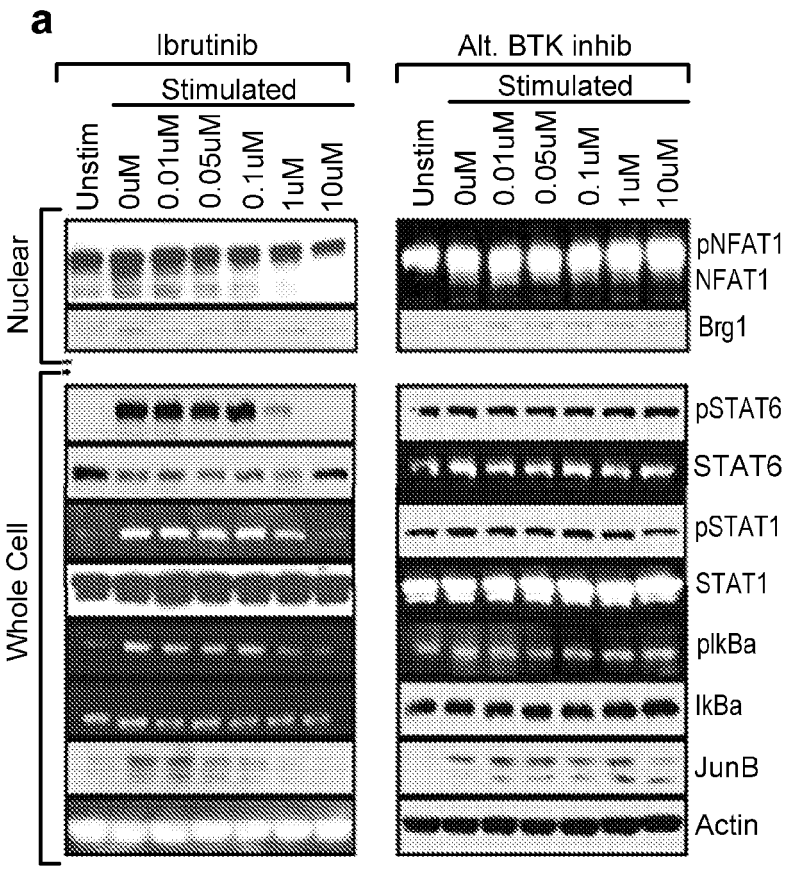


FIGURE 3


a
Ibrutinib
Alt. BTK inhib
Stimulated
Stimulated
Unstim
0uM
0.01uM
0.05uM
0.1uM
1uM
10uM
Nuclear
Whole Cell
pNFAT1
NFAT1
Brg1
pSTAT6
STAT6
pSTAT1
STAT1
plkBa
IkBa
JunB
Actin

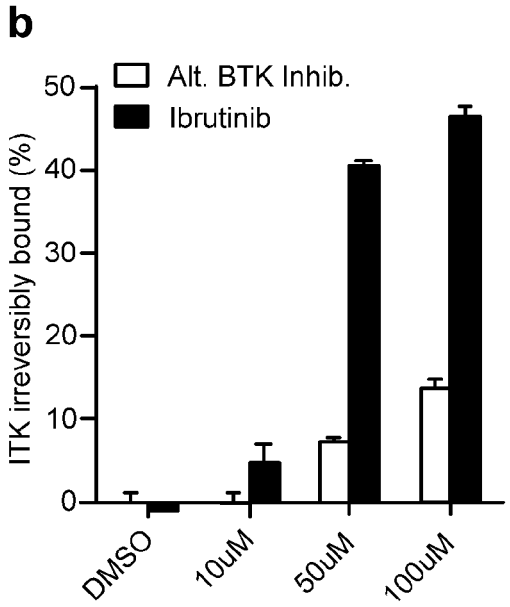

b
Alt. BTK Inhib.
Ibrutinib
ITK irreversibly bound (%)
50
40
30
20
10
0
DMSO
10uM
50uM
100uM

FIGURE 3
(Continued)

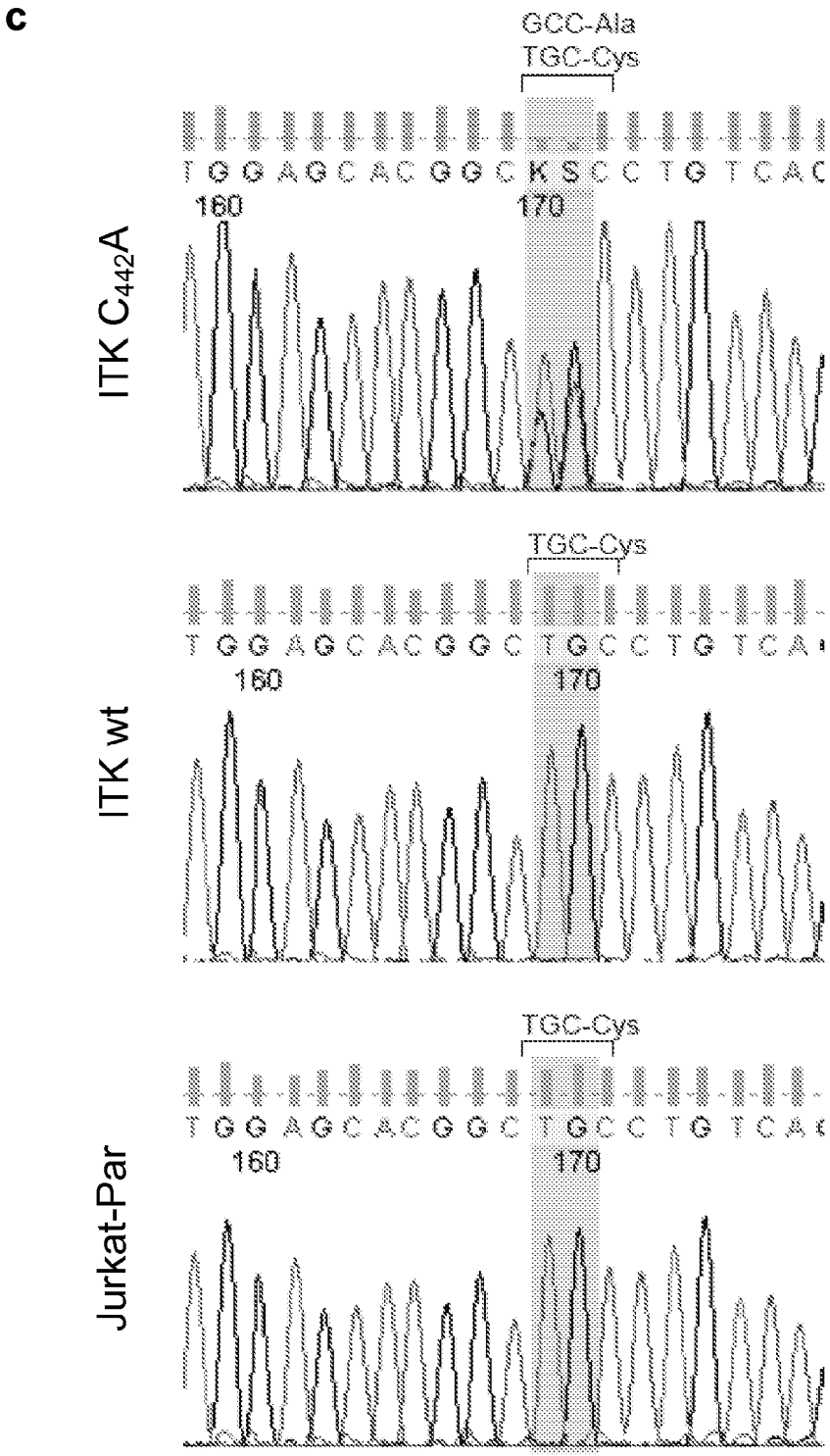
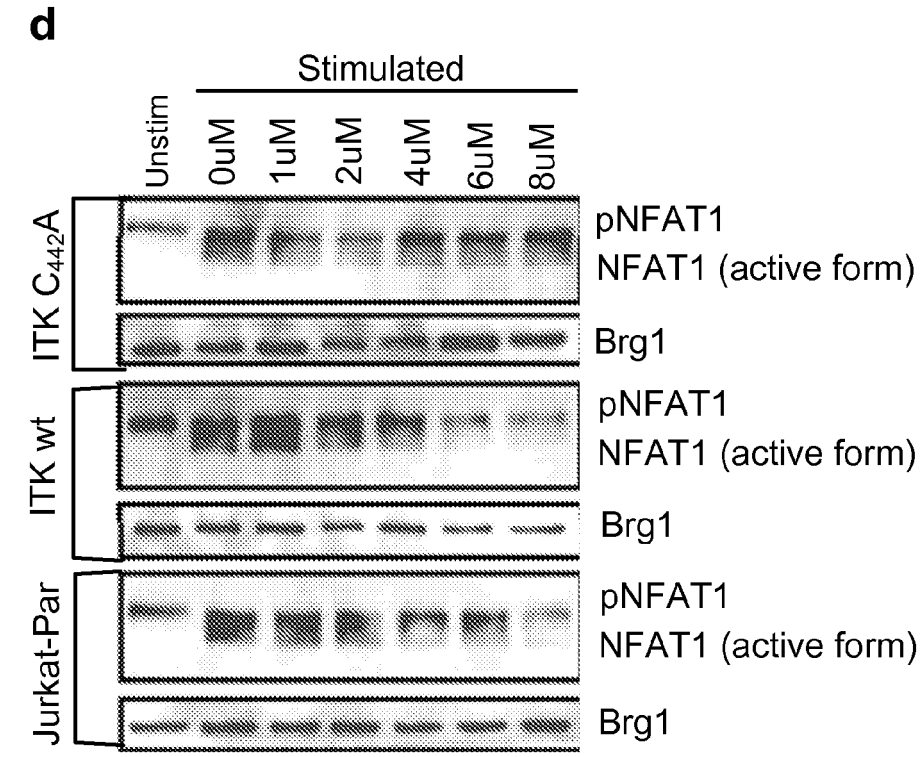


FIGURE 3
(Continued)



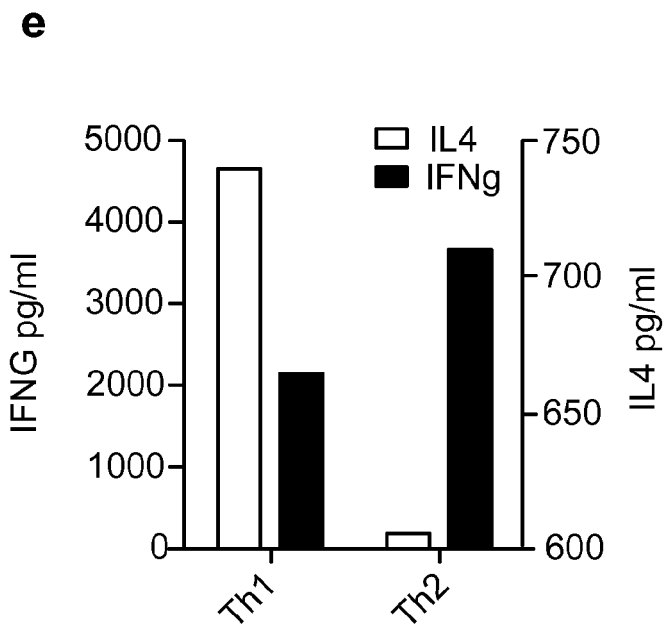
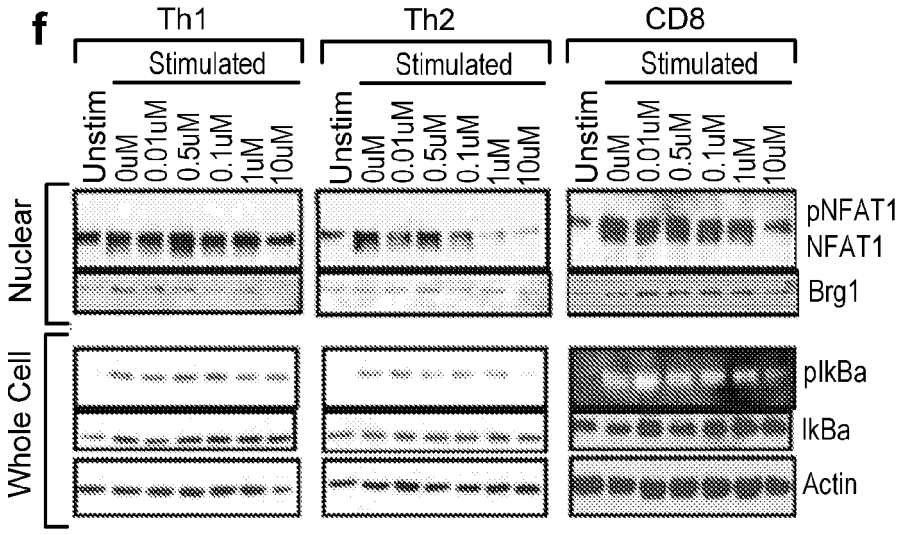


FIGURE 3
(Continued)



g

Cell Line	GAPDH (Ct)	RLK (Ct)
Parental Jurkat	13.15	∞
Empty Vector	12.5	∞
RLK-Jurkat	12.7	24.6

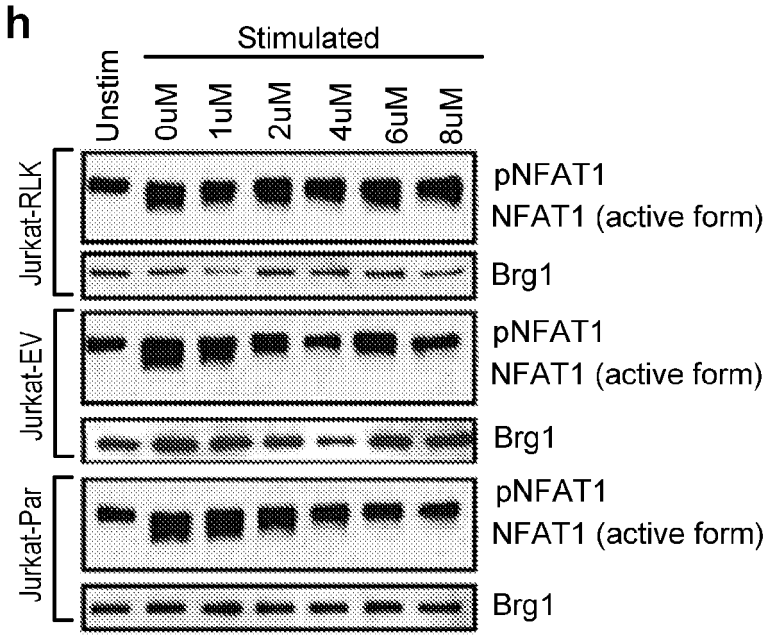
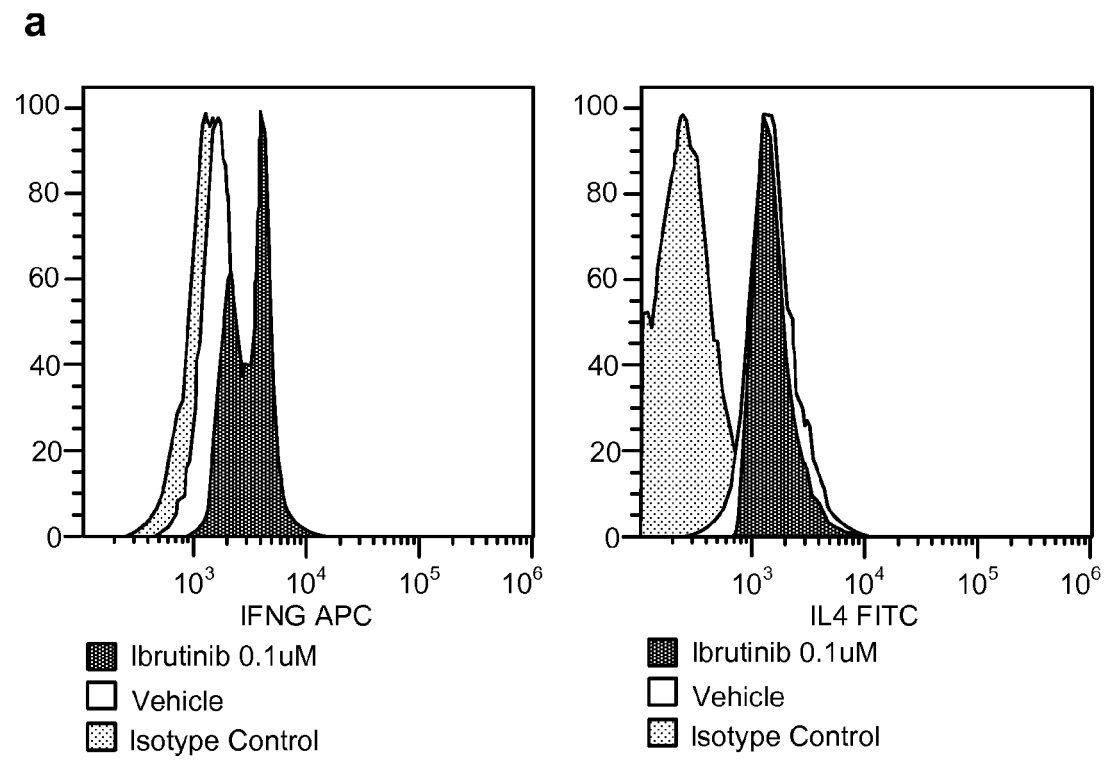


FIGURE 4


a
100
80
60
40
20
0
10^3
10^4
10^5
10^6
IFNG APC
Ibrutinib 0.1uM
Vehicle
Isotype Control
IL4 FITC
Ibrutinib 0.1uM
Vehicle
Isotype Control

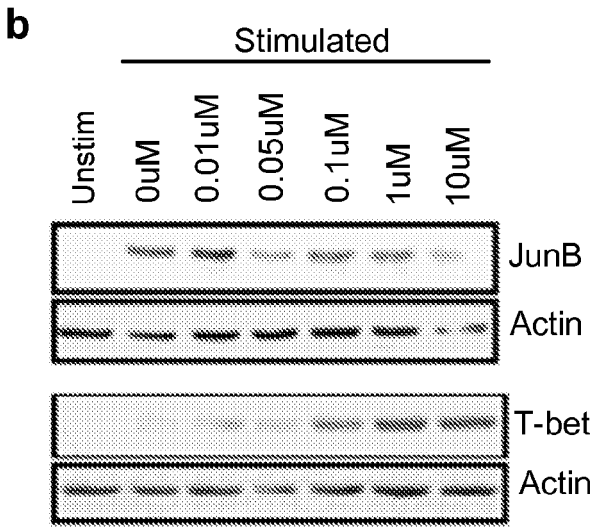

b
Stimulated
Unstim
0uM
0.01uM
0.05uM
0.1uM
1uM
10uM
JunB
Actin
T-bet
Actin

FIGURE 4
(Continued)

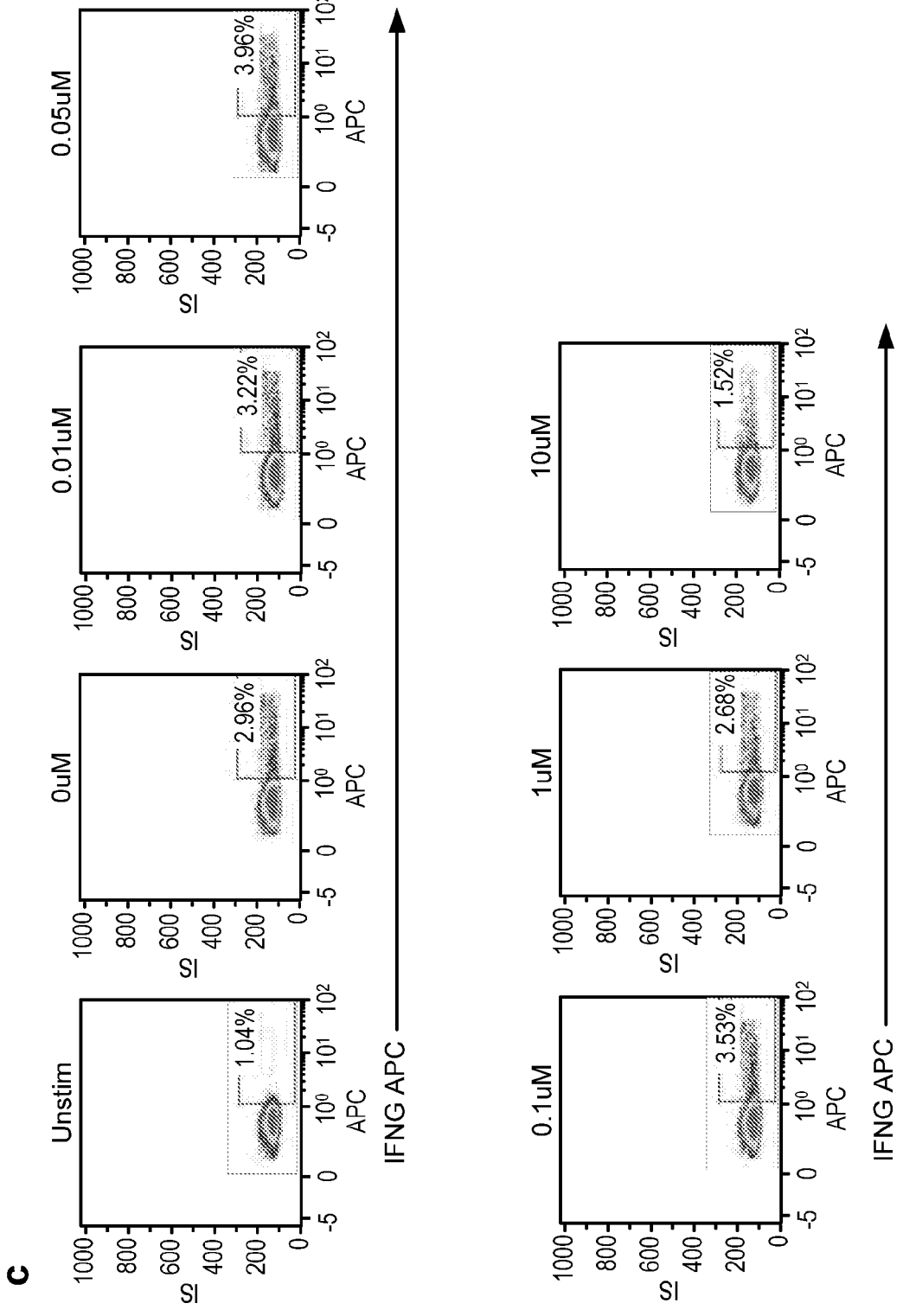


FIGURE 4
(Continued)

C
(Continued)

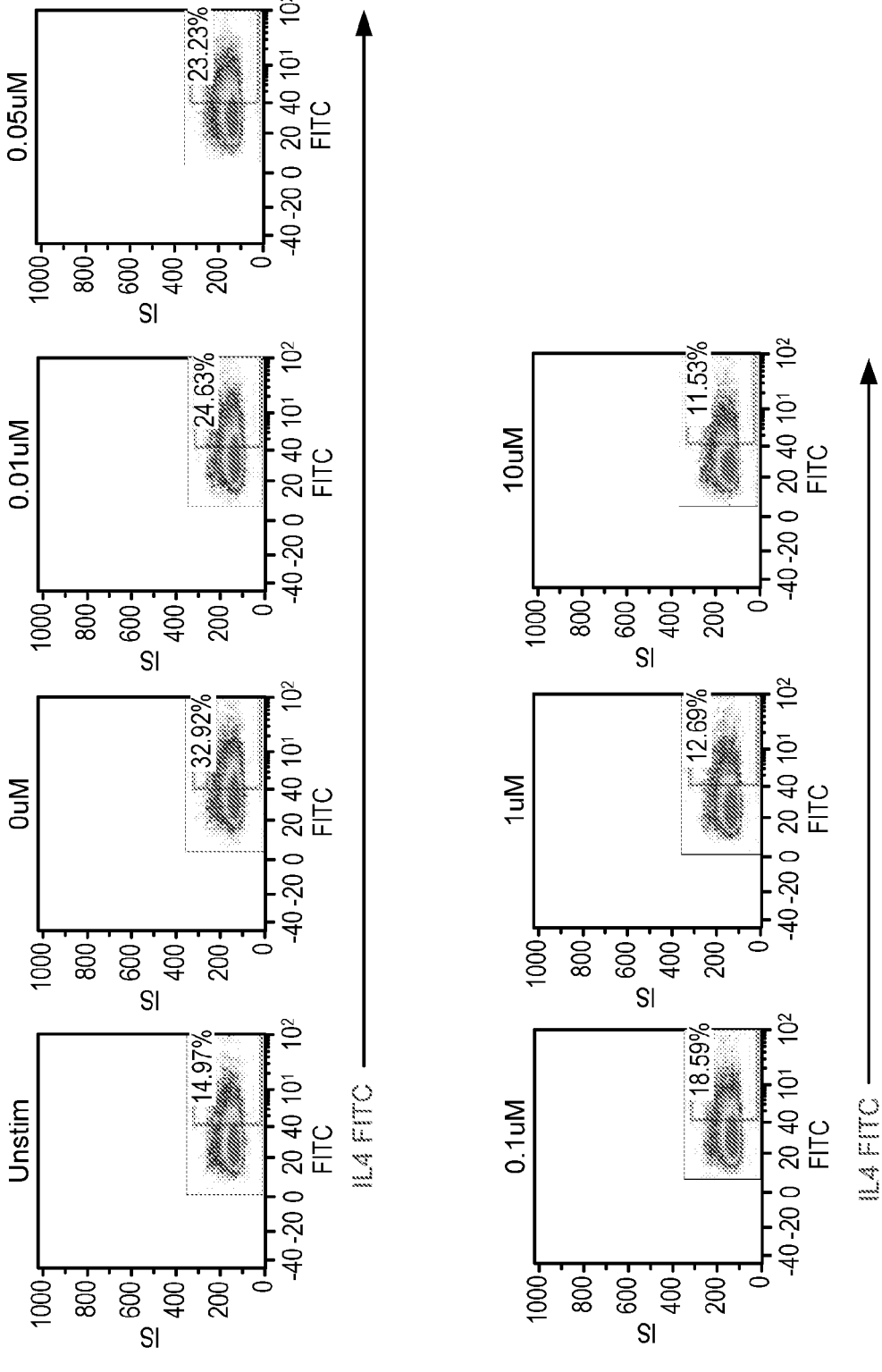
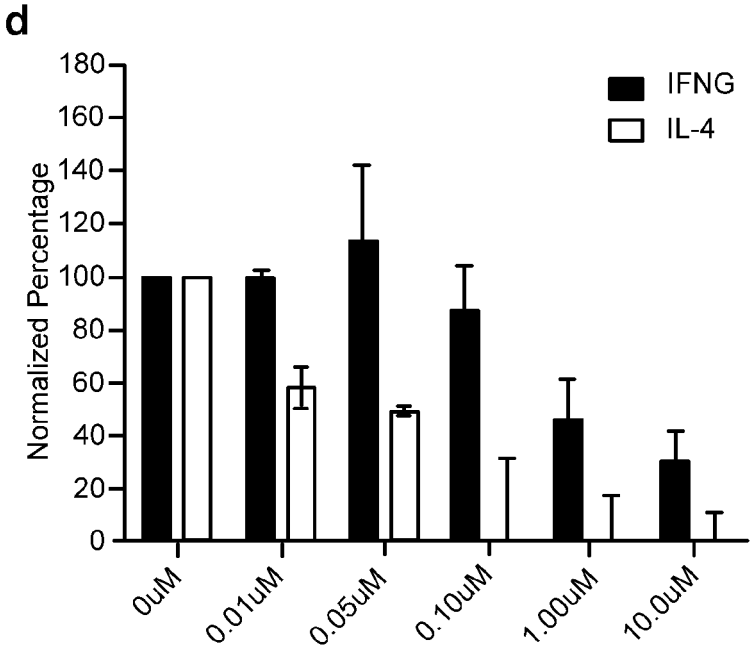


FIGURE 4
(Continued)



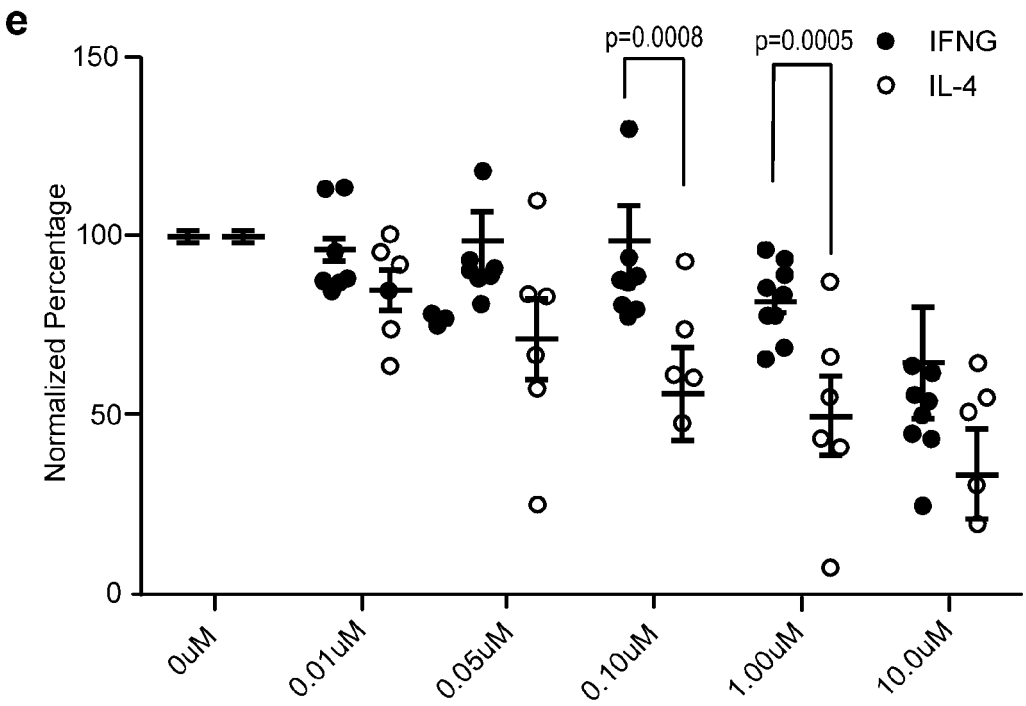


FIGURE 5

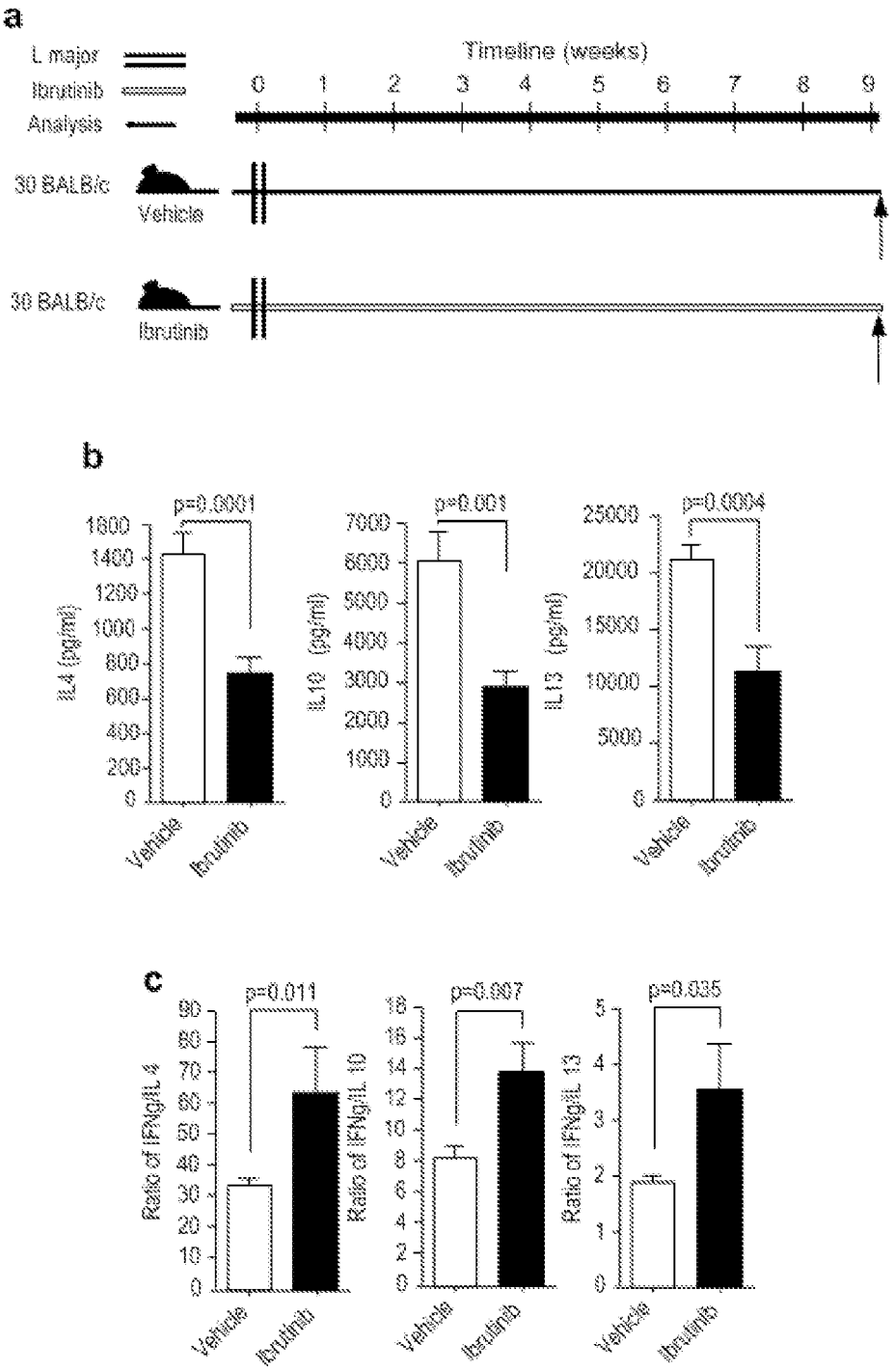

a
L major
Ibrutinib
Analysis
Timeline (weeks)
0 1 2 3 4 5 6 7 8 9
30 BALB/c
Vehicle
30 BALB/c
Ibrutinib
b
p=0.0001
IL4 (pg/ml)
1600 1400 1200 1000 800 600 400 200 0
Vehicle
Ibrutinib
p=0.001
IL10 (pg/ml)
7000 6000 5000 4000 3000 2000 1000 0
Vehicle
Ibrutinib
p=0.0004
IL13 (pg/ml)
25000 20000 15000 10000 5000 0
Vehicle
Ibrutinib
c
p=0.011
Ratio of IFNg/IL 4
90 80 70 60 50 40 30 20 10 0
Vehicle
Ibrutinib
p=0.007
Ratio of IFNg/IL 10
18 16 14 12 10 8 6 4 2 0
Vehicle
Ibrutinib
p=0.035
Ratio of IFNg/IL 13
5 4 3 2 1 0
Vehicle
Ibrutinib

FIGURE 5
(Continued)

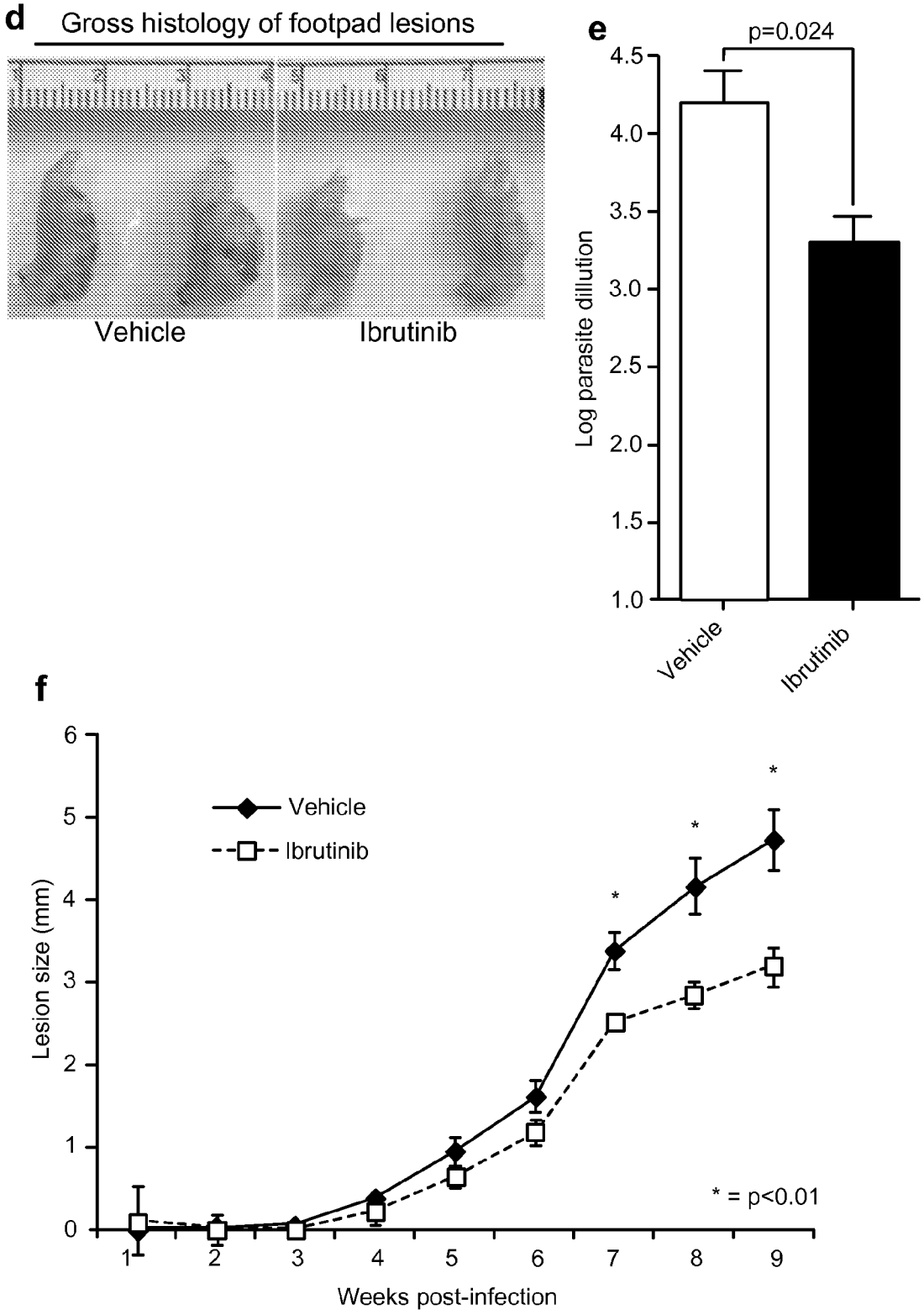
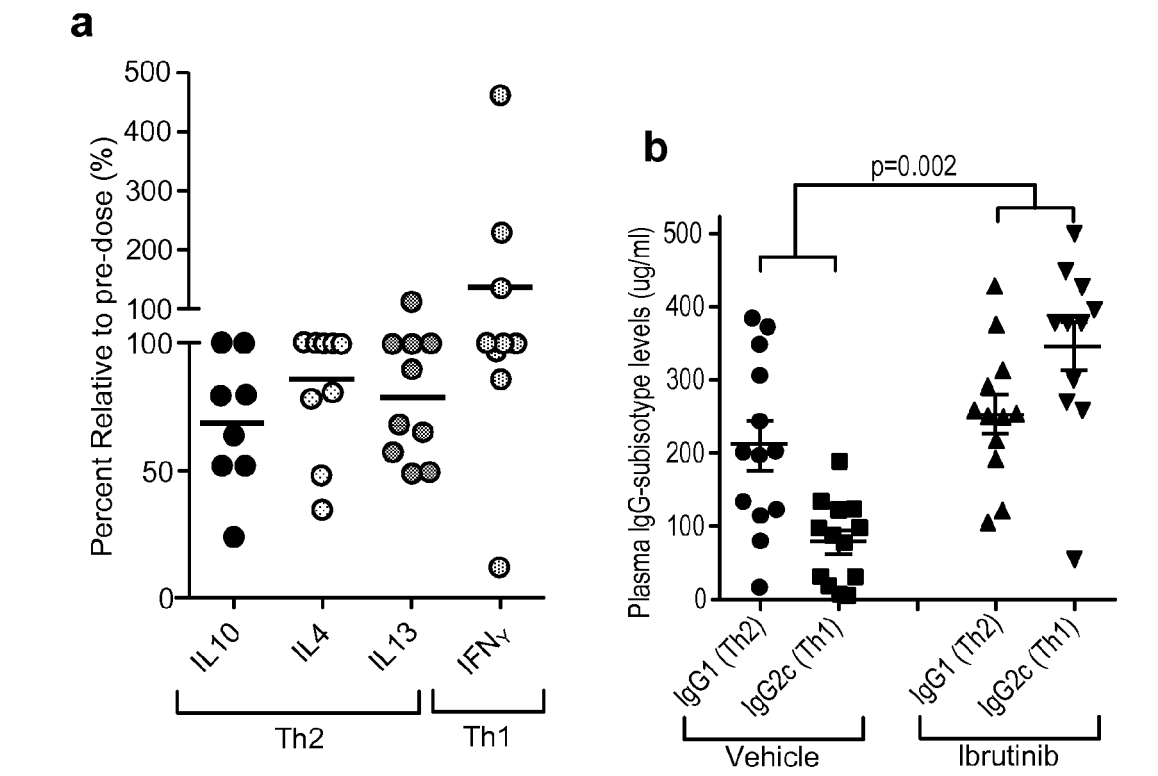


FIGURE 6A


a
Percent Relative to pre-dose (%)
500
400
300
200
100
100
50
0
IL10
IL4
IL13
IFNγ
Th2
Th1
b
p=0.002
Plasma IgG-subisotype levels (ug/ml)
500
400
300
200
100
0
IgG1 (Th2)
IgG2c (Th1)
IgG1 (Th2)
IgG2c (Th1)
Vehicle
Ibrutinib

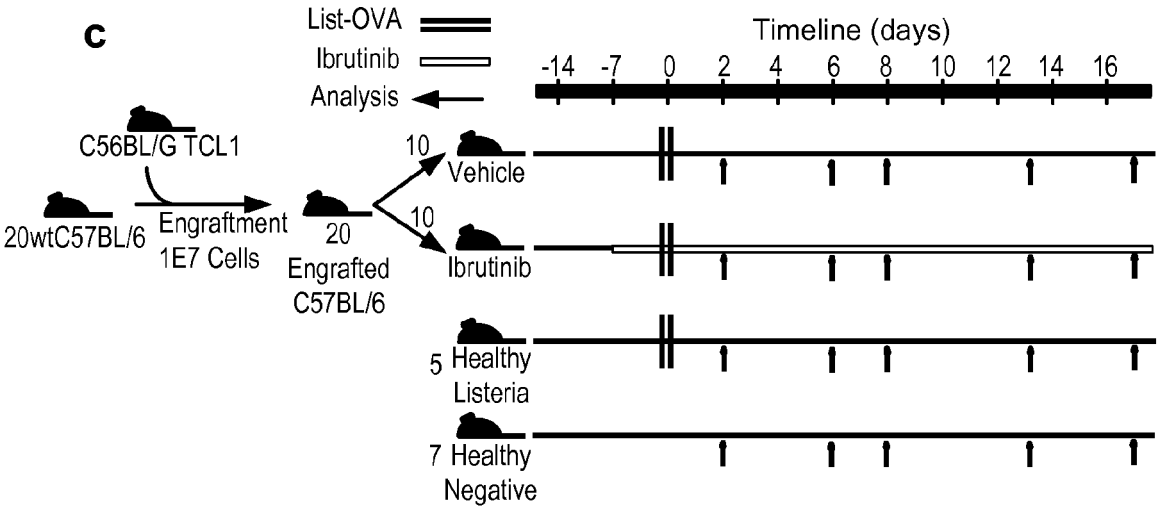

c
List-OVA
Ibrutinib
Analysis
Timeline (days)
-14 -7 0 2 4 6 8 10 12 14 16
C56BL/G TCL1
20wtC57BL/6
Engraftment
1E7 Cells
20
Engrafted
C57BL/6
10
Vehicle
10
Ibrutinib
5 Healthy
Listeria
7 Healthy
Negative

FIGURE 6A
(Continued)

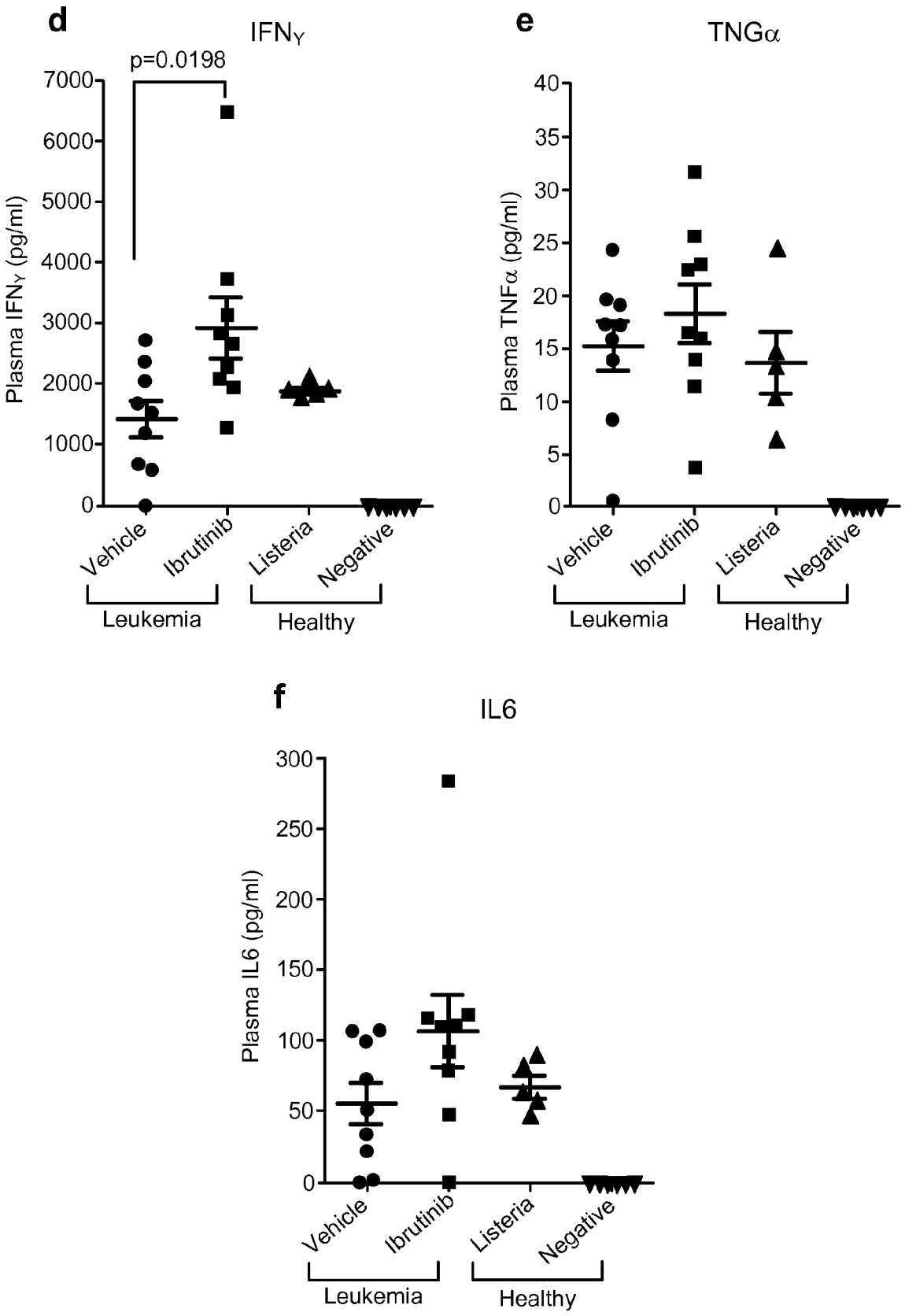


FIGURE 6A
(Continued)

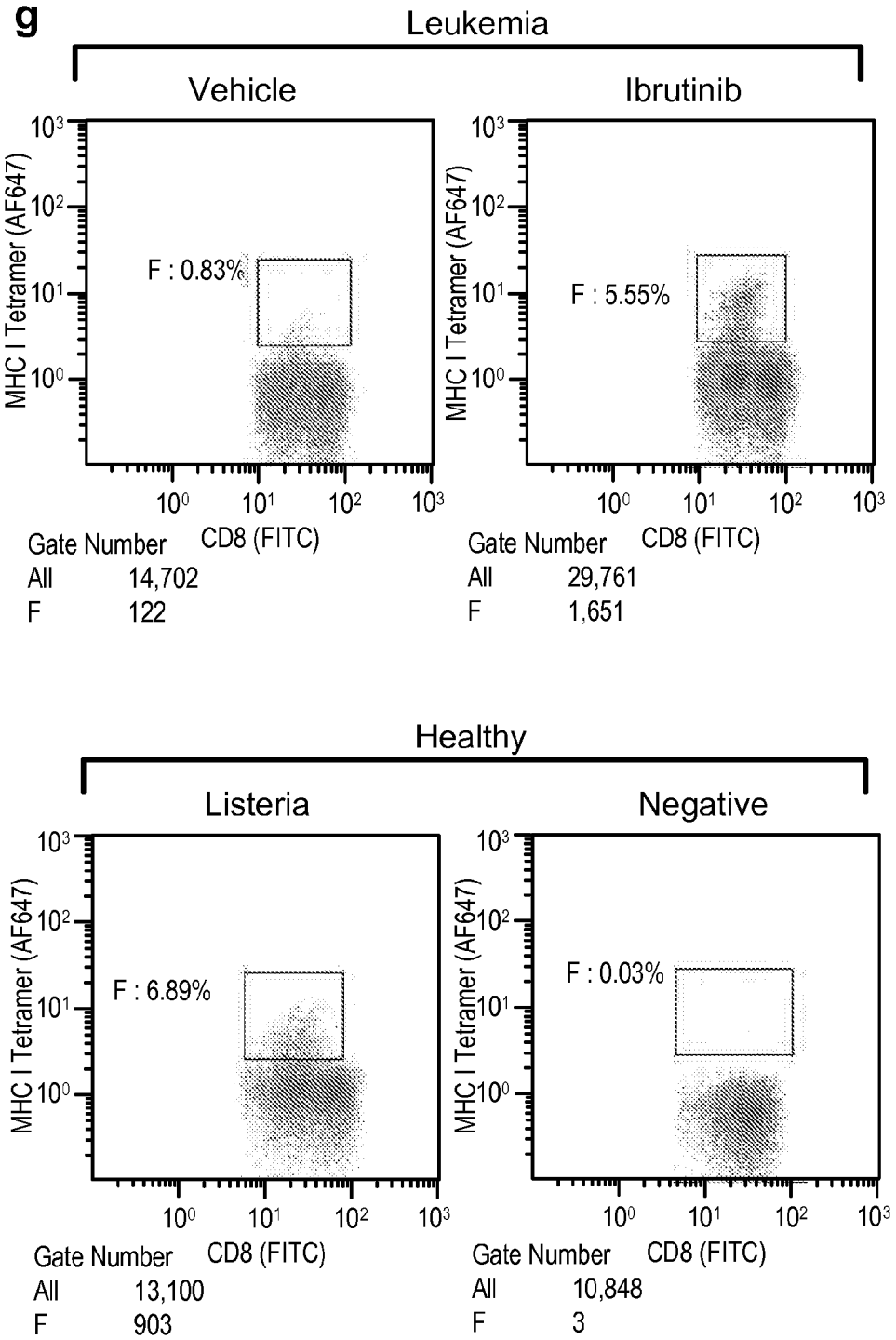


FIGURE 6B

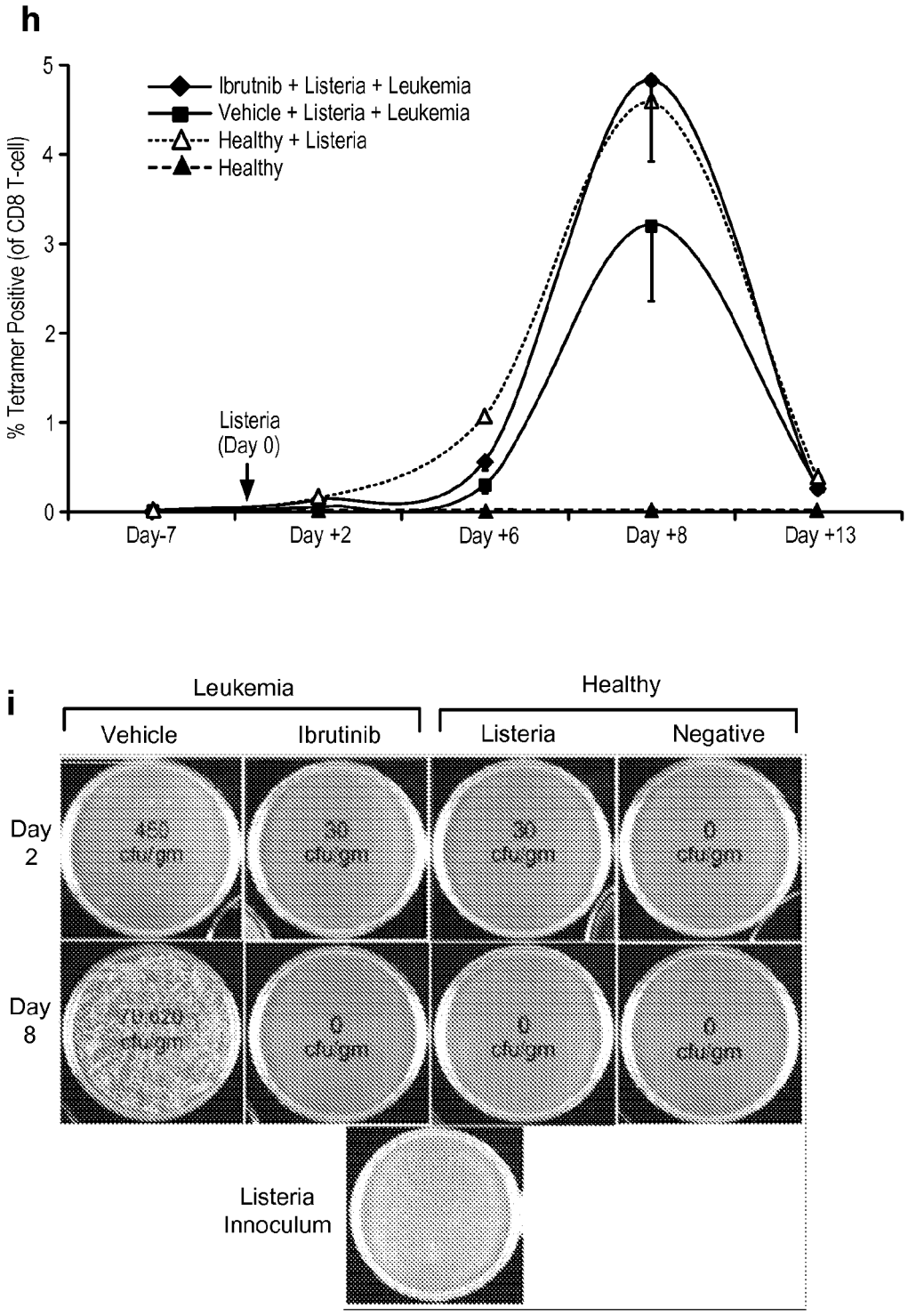

h
Ibrutnib + Listeria + Leukemia
Vehicle + Listeria + Leukemia
Healthy + Listeria
Healthy
% Tetramer Positive (of CD8 T-cell)
0
1
2
3
4
5
Listeria
(Day 0)
Day-7
Day +2
Day +6
Day +8
Day +13
i
Leukemia
Healthy
Vehicle
Ibrutinib
Listeria
Negative
Day 2
450 cfu/gm
38 cfu/gm
30 cfu/gm
0 cfu/gm
Day 8
Tu 620 cfu/gm
0 cfu/gm
0 cfu/gm
0 cfu/gm
Listeria Innoculum

Figure 7

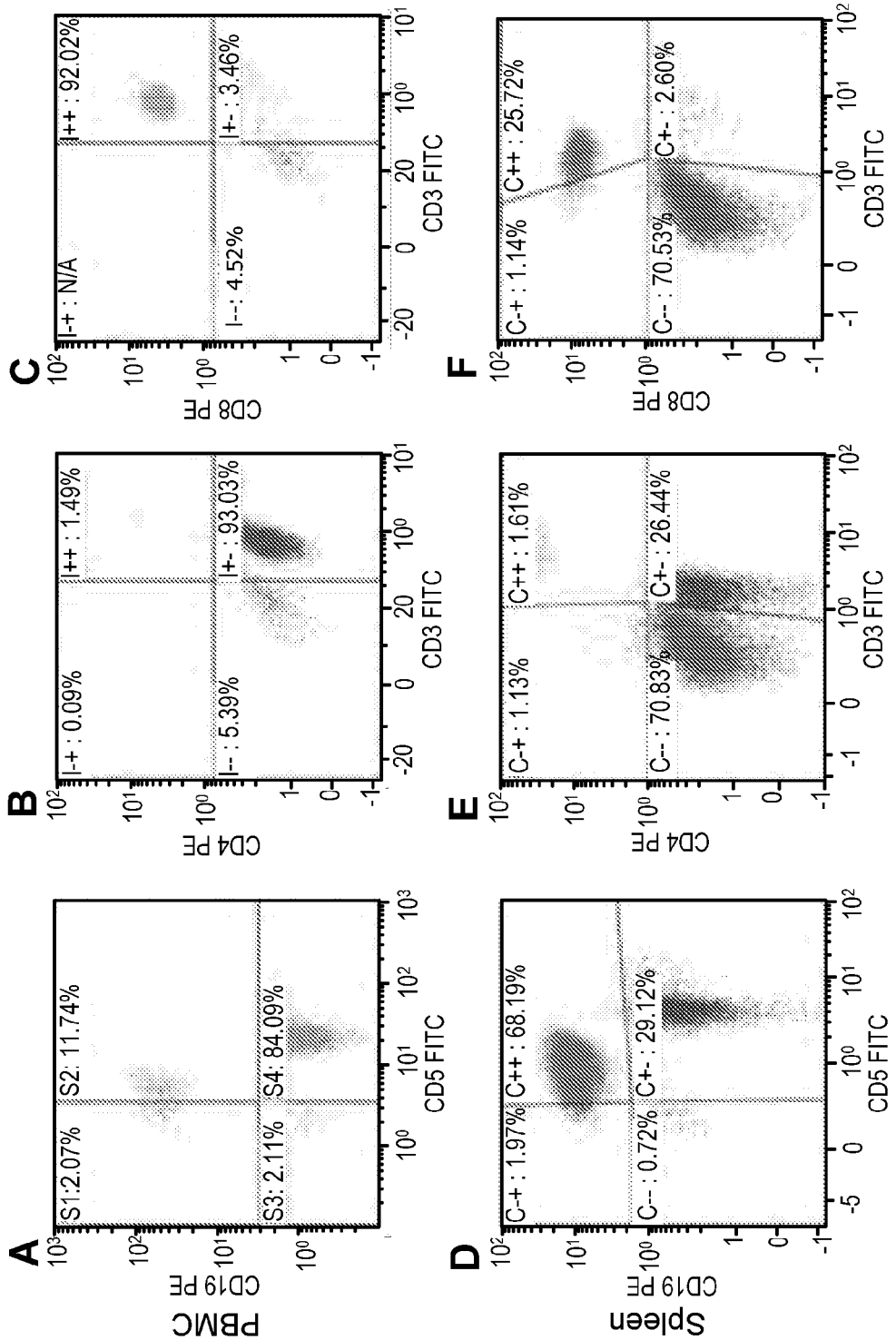

A
PBMC
CD19 PE
CD5 FITC
S1:2.07%
S2: 11.74%
S3: 2.11%
S4: 84.09%
B
CD4 PE
CD3 FITC
I-+ : 0.09%
I++ : 1.49%
I-- : 5.39%
I+- : 93.03%
C
CD8 PE
CD3 FITC
I-+ : N/A
I++ : 92.02%
I--: 4.52%
I+- : 3.46%
D
Spleen
CD19 PE
CD5 FITC
C-+ : 1.97%
C++ : 68.19%
C-- : 0.72%
C+- : 29.12%
E
CD4 PE
CD3 FITC
C-+ : 1.13%
C++ : 1.61%
C-- : 70.83%
C+- : 26.44%
F
CD8 PE
CD3 FITC
C-+ : 1.14%
C++ : 25.72%
C-- : 70.53%
C+- : 2.60%

Figure 7
(Continued)

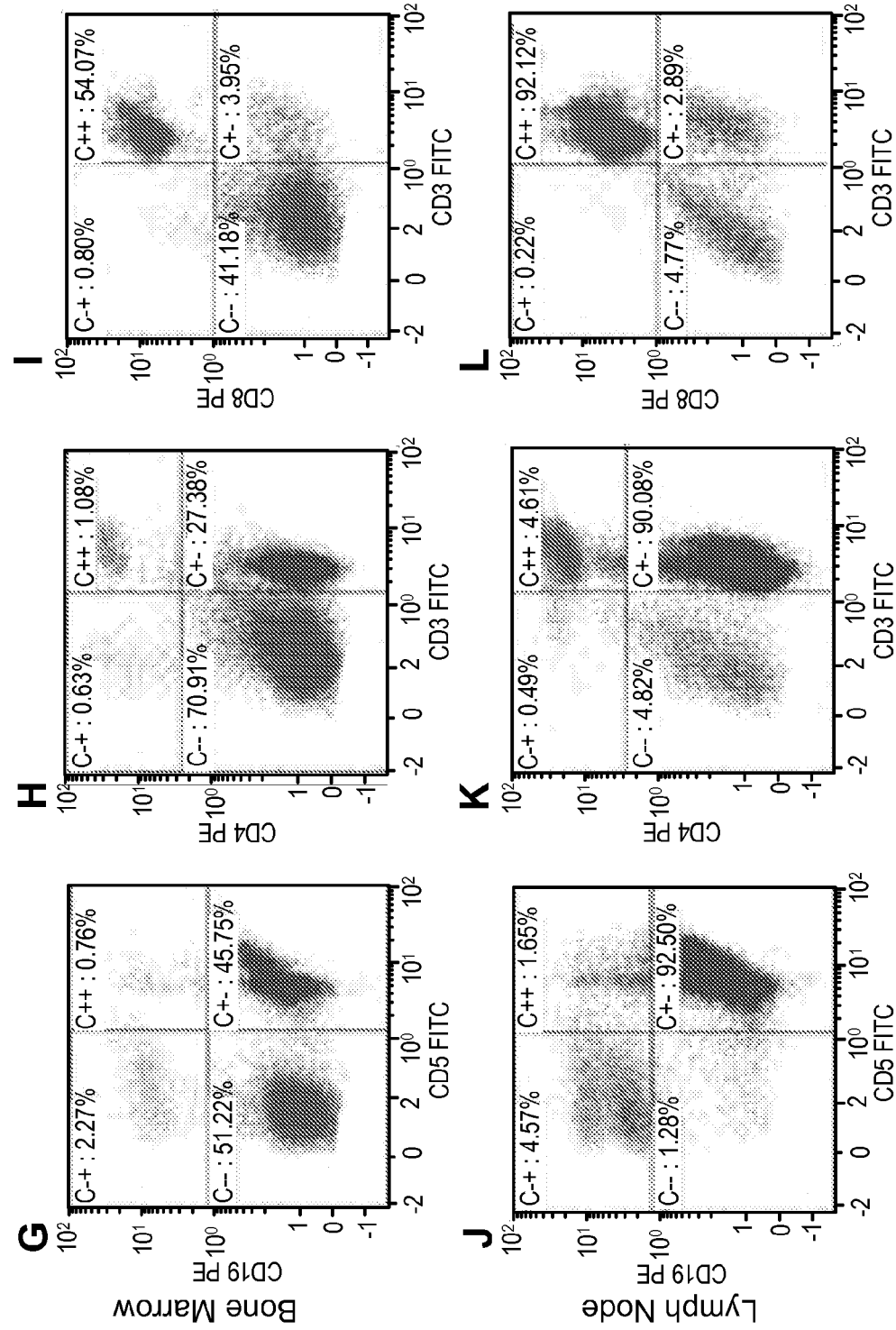


FIGURE 8

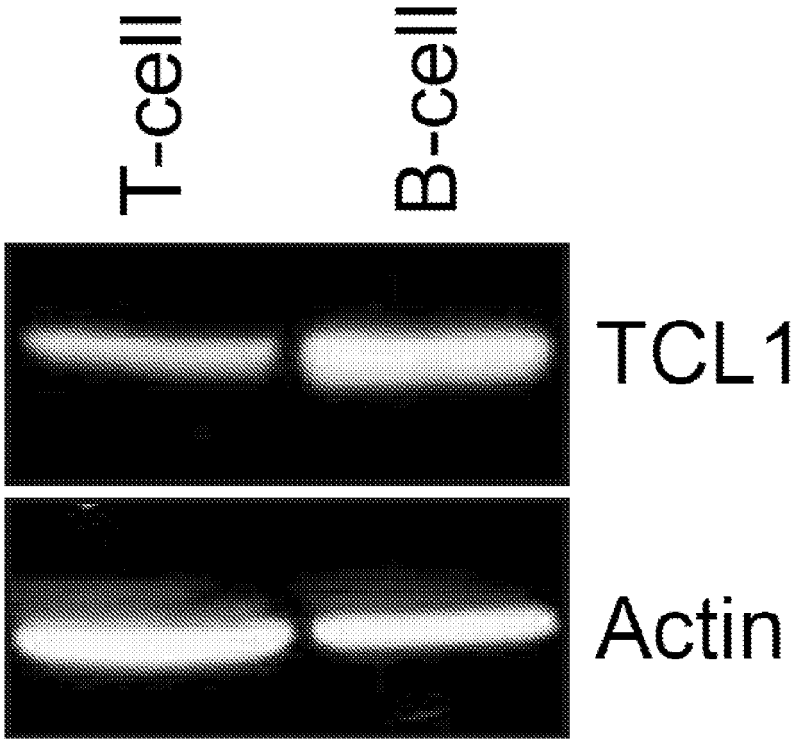


FIGURE 9

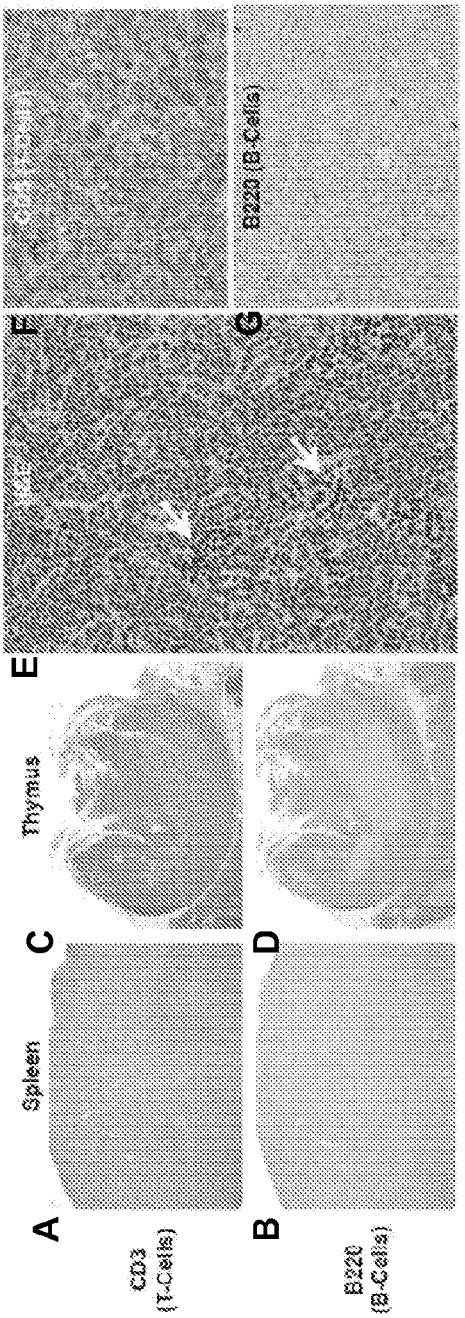


FIGURE 10

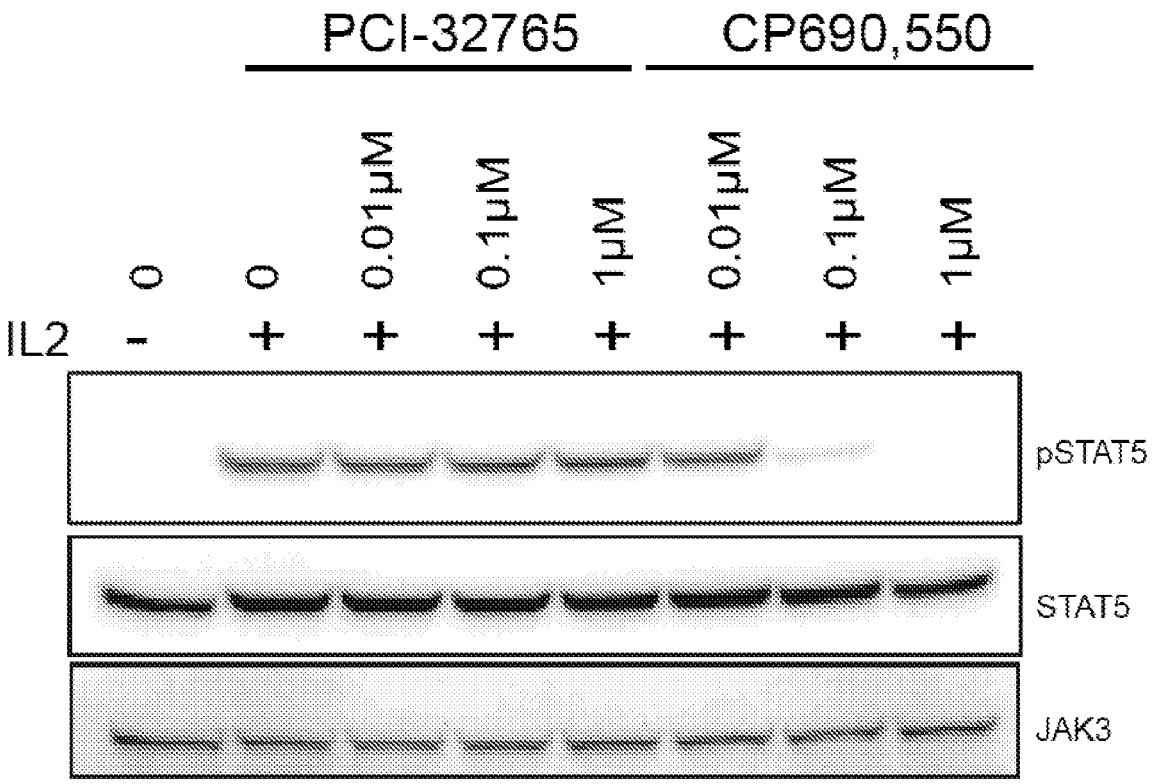

PCI-32765
CP690,550
0
0
0.01μM
0.1μM
1μM
0.01μM
0.1μM
1μM
IL2
-
+
+
+
+
+
+
+
pSTAT5
STAT5
JAK3

FIGURE 11

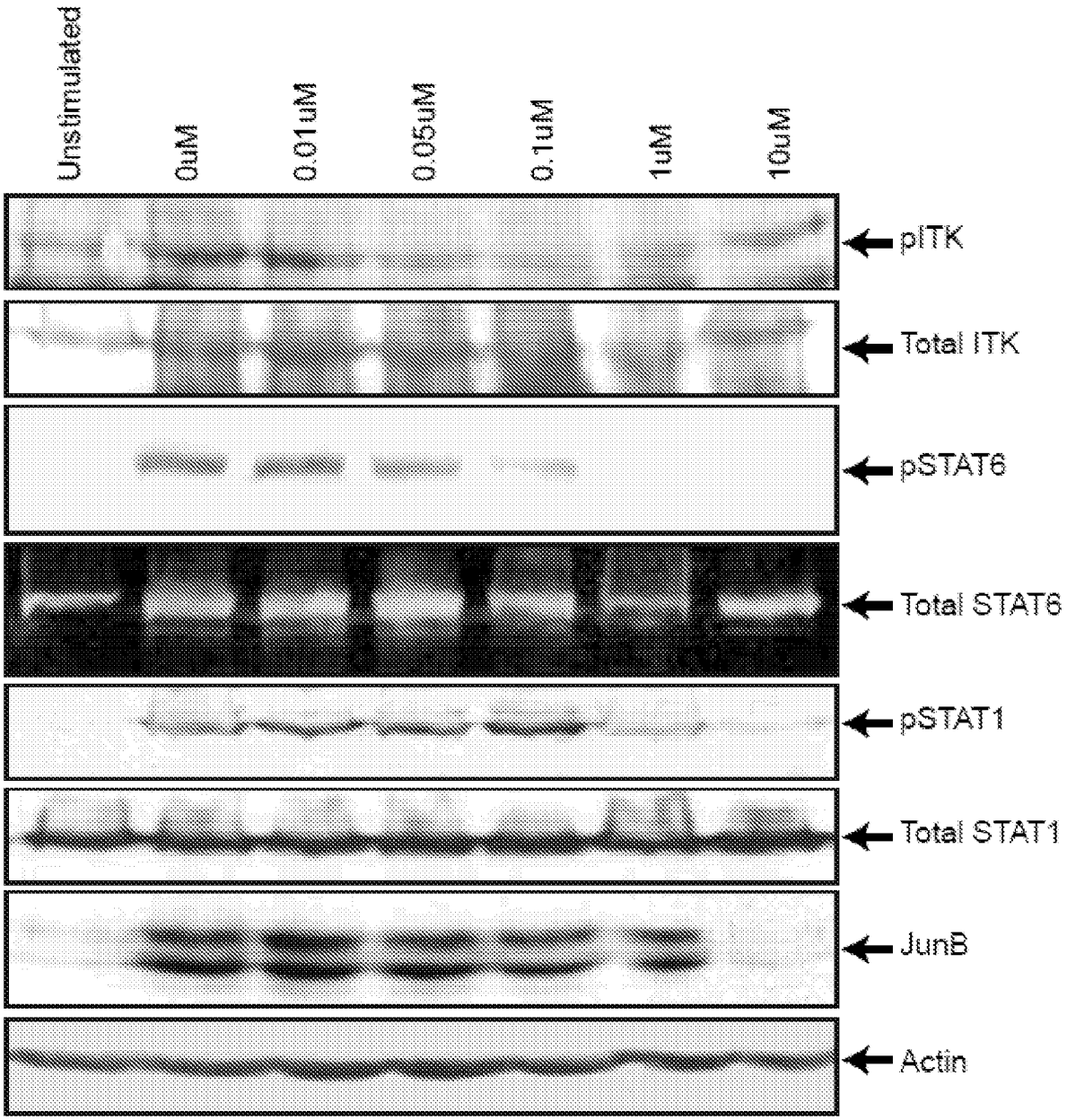

Unstimulated
0uM
0.01uM
0.05uM
0.1uM
1uM
10uM
pITK
Total ITK
pSTAT6
Total STAT6
pSTAT1
Total STAT1
JunB
Actin

FIGURE 12

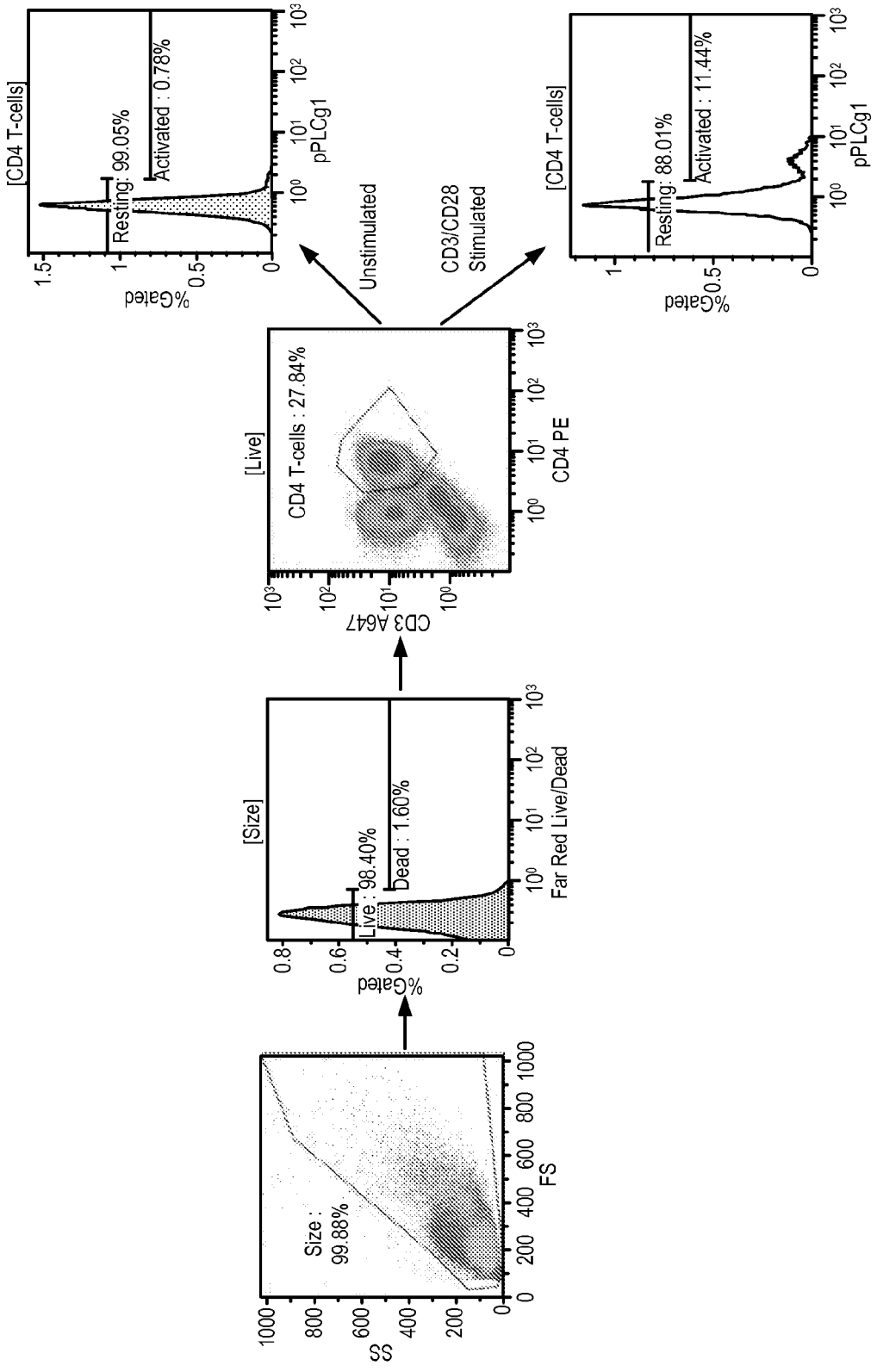

SS
FS
Size : 99.88%
[Size]
%Gated
Live : 98.40%
Dead : 1.60%
Far Red Live/Dead
[Live]
CD3 A647
CD4 T-cells : 27.84%
CD4 PE
Unstimulated
CD3/CD28 Stimulated
[CD4 T-cells]
Resting: 99.05%
Activated : 0.78%
pPLCg1
[CD4 T-cells]
Resting: 88.01%
Activated : 11.44%
pPLCg1

FIGURE 13

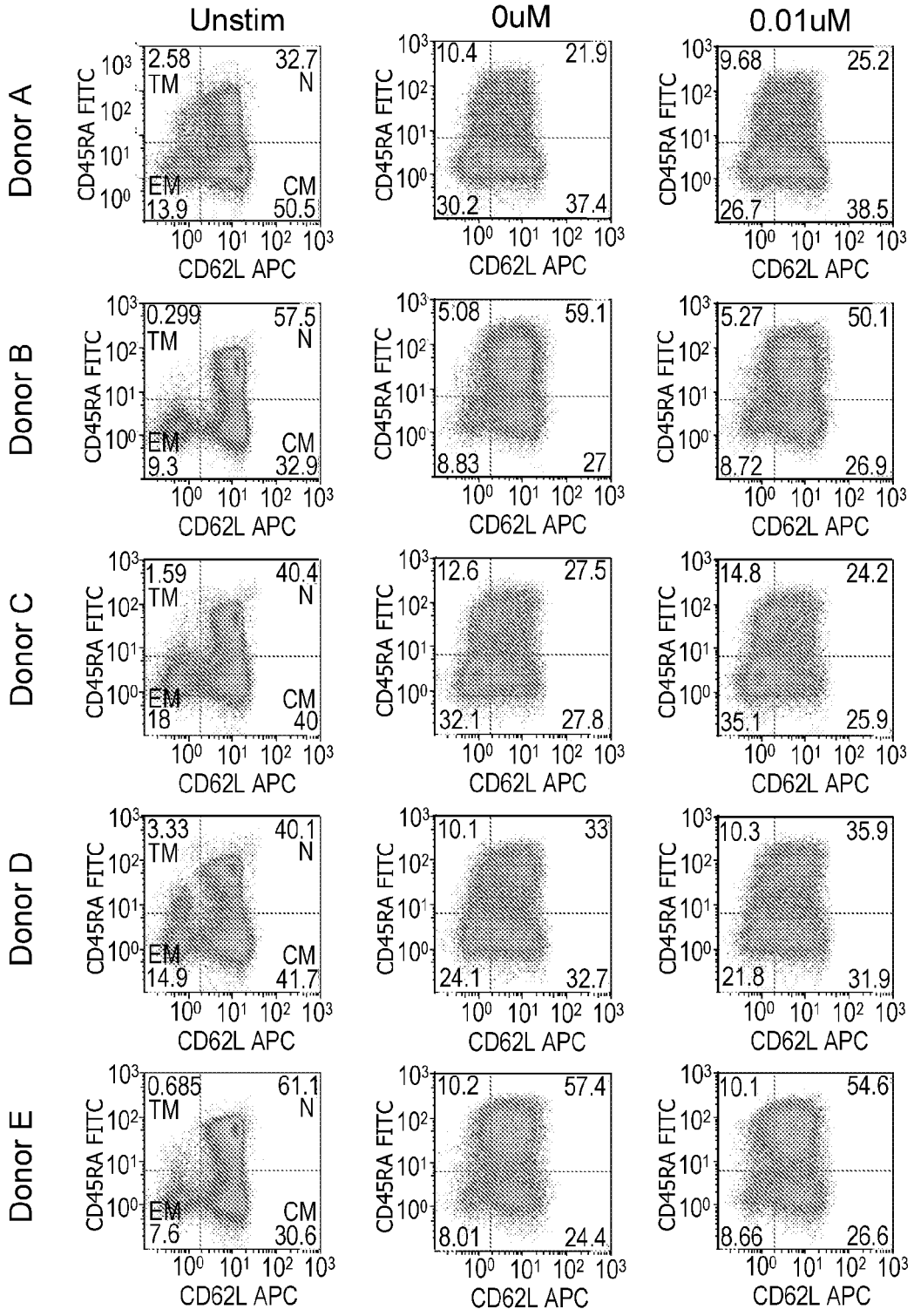

Unstim
0uM
0.01uM
Donor A
Donor B
Donor C
Donor D
Donor E
CD45RA FITC
CD62L APC
2.58
TM
32.7
N
EM
13.9
CM
50.5
10.4
21.9
30.2
37.4
9.68
25.2
26.7
38.5
0.299
TM
57.5
N
EM
9.3
CM
32.9
5.08
59.1
8.83
27
5.27
50.1
8.72
26.9
1.59
TM
40.4
N
EM
18
CM
40
12.6
27.5
32.1
27.8
14.8
24.2
35.1
25.9
3.33
TM
40.1
N
EM
14.9
CM
41.7
10.1
33
24.1
32.7
10.3
35.9
21.8
31.9
0.685
TM
61.1
N
EM
7.6
CM
30.6
10.2
57.4
8.01
24.4
10.1
54.6
8.66
26.6

FIGURE 13
(Continued)

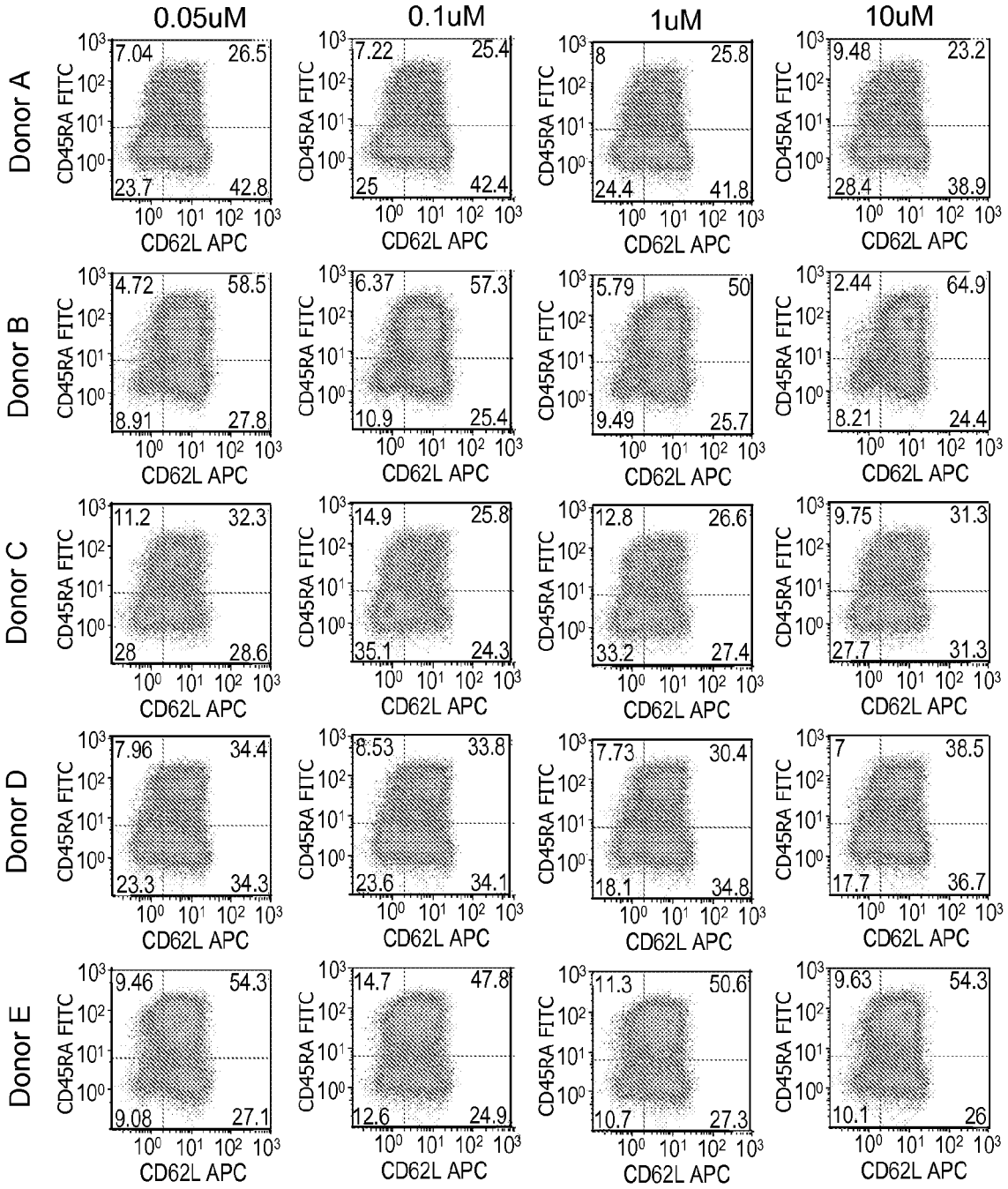
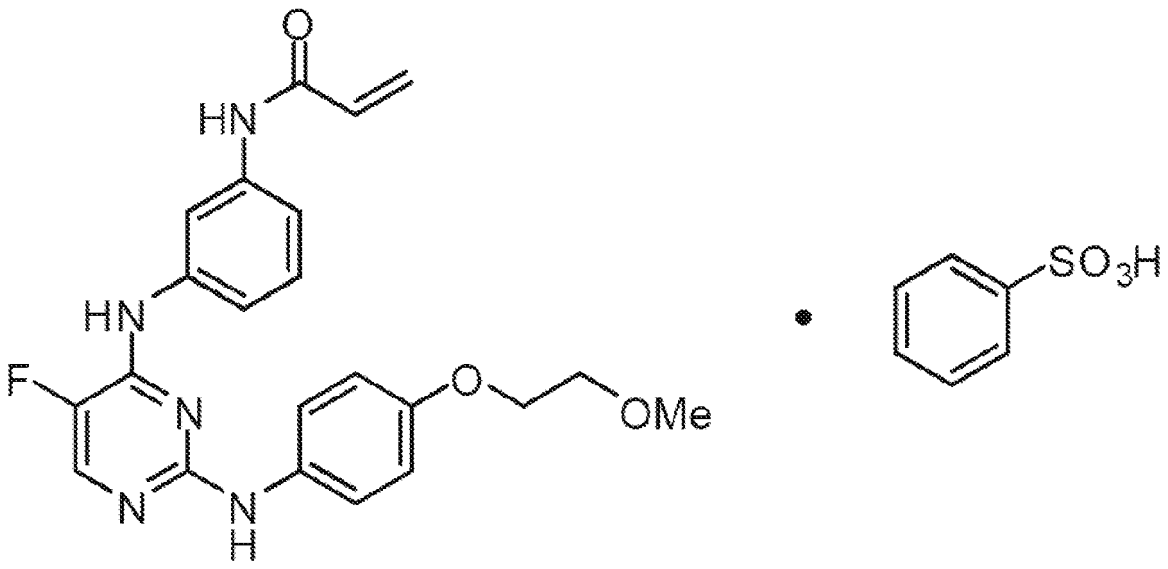


FIGURE 14

	Tyrosine Kinase	Ibrutinib IC 50 (nM)	Alt BTK inhib. IC50 (nM)	PCI-45292 IC50(nM)
Irreversible Inhibition (Contain Cys-481)	ITK	2.2	22.5	139
	BTK	0.1	1.8	0.78
	Bmx	0.7	1.2	0.77
	Tec	0.4	2.2	32
	EGFR	4.5	108.9	4,156
	JAK3	3.8	0.9	>10,000
	HER2	1.4	141	3,256
	HER4	<0.5	7.5	3.9
	Blk	<0.5	20.5	2.6
Reversible Inhibition	Lck	0.9	352.9	4.3
	C-src	148.5	378.2	147
	Lyn	0.9	904	40
	Yes	<0.5	88.3	0.94
	Csk	4.2	3931	3.2
	VEGFR2	241	nd	4,437

FIGURE 15



Alt BTK inhib.

Chemical Formula: $C_{22}H_{22}FN_5O_3$
Exact Mass: 423.17
Molecular Weight: 423.44
Formula Weight: 581.62

FIGURE 16

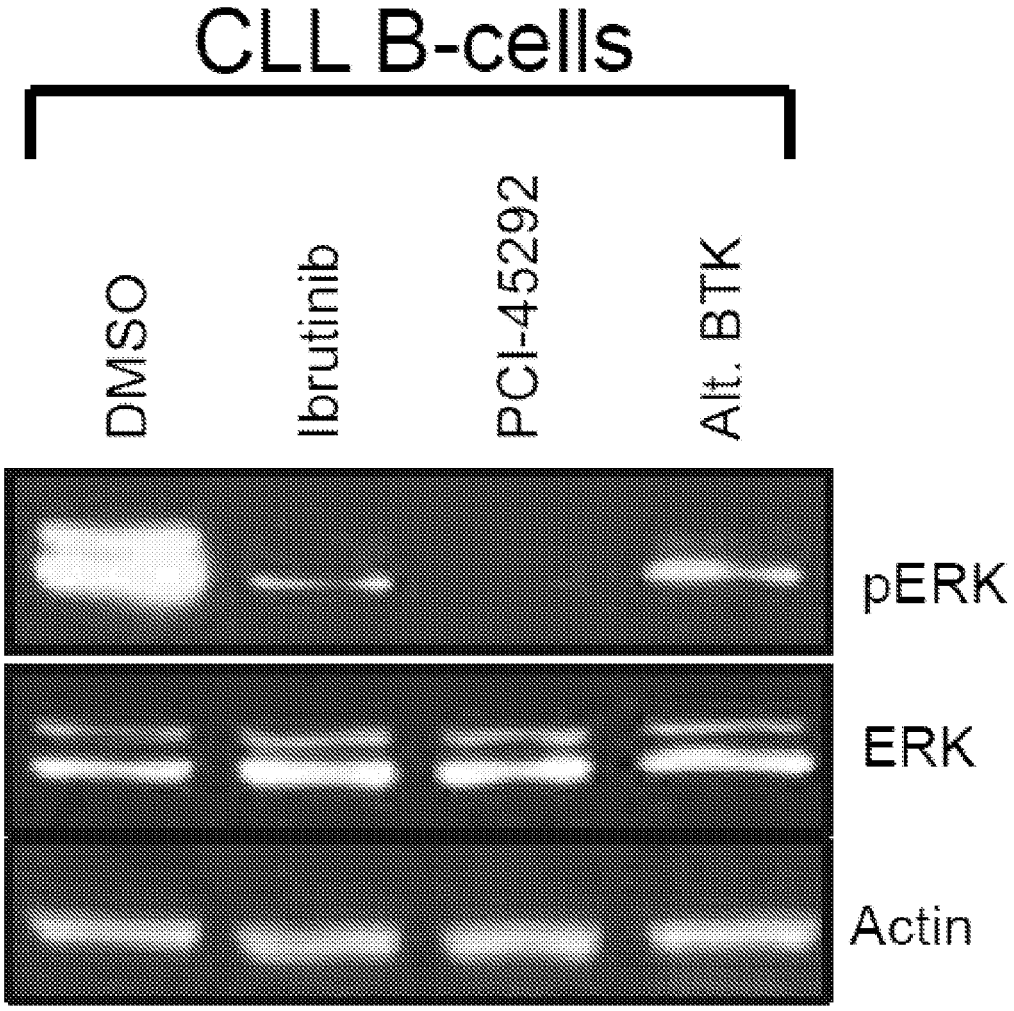


FIGURE 17

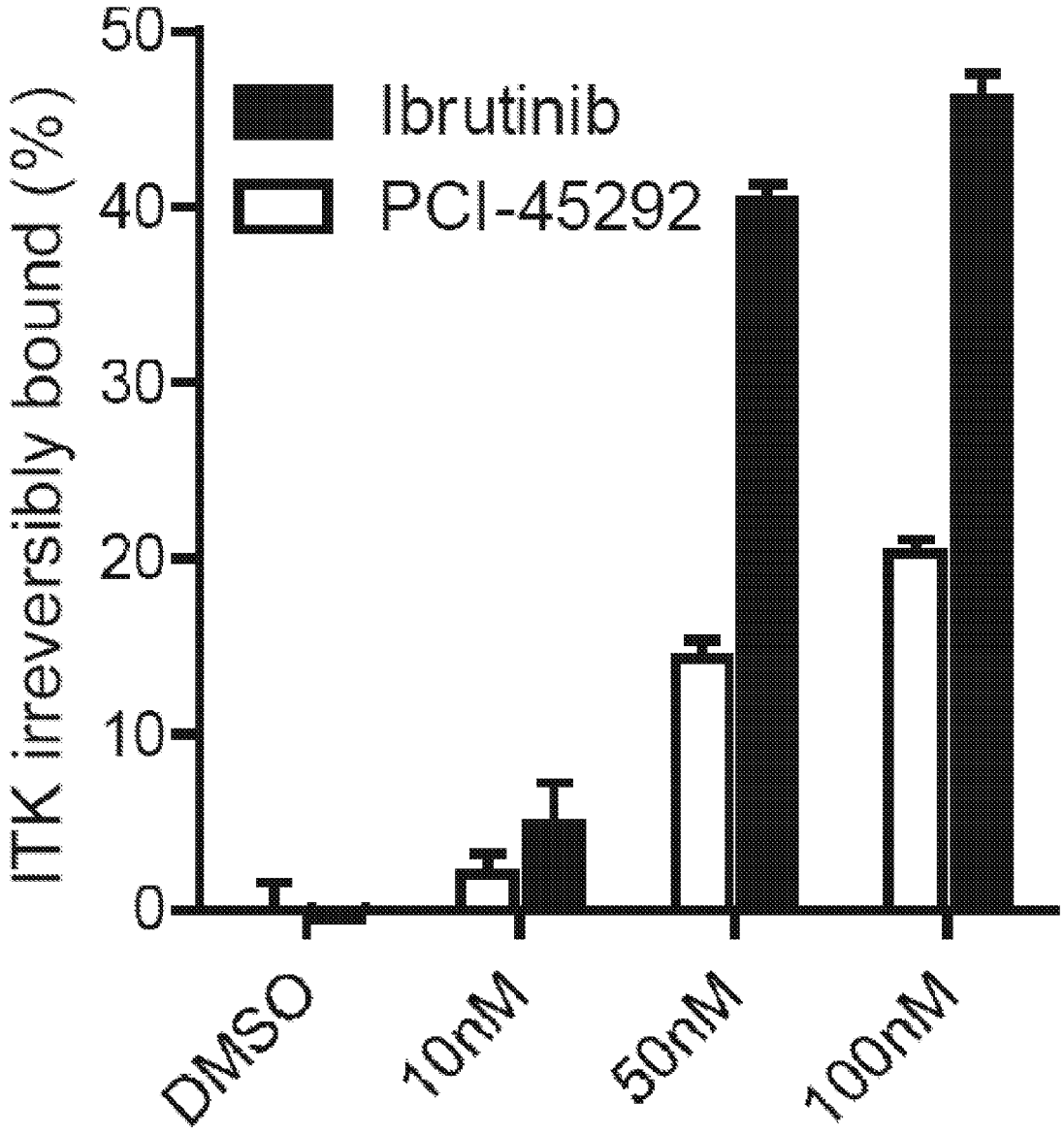

ITK irreversibly bound (%)
50
40
30
20
10
0
Ibrutinib
PCI-45292
DMSO
10nM
50nM
100nM

FIGURE 18

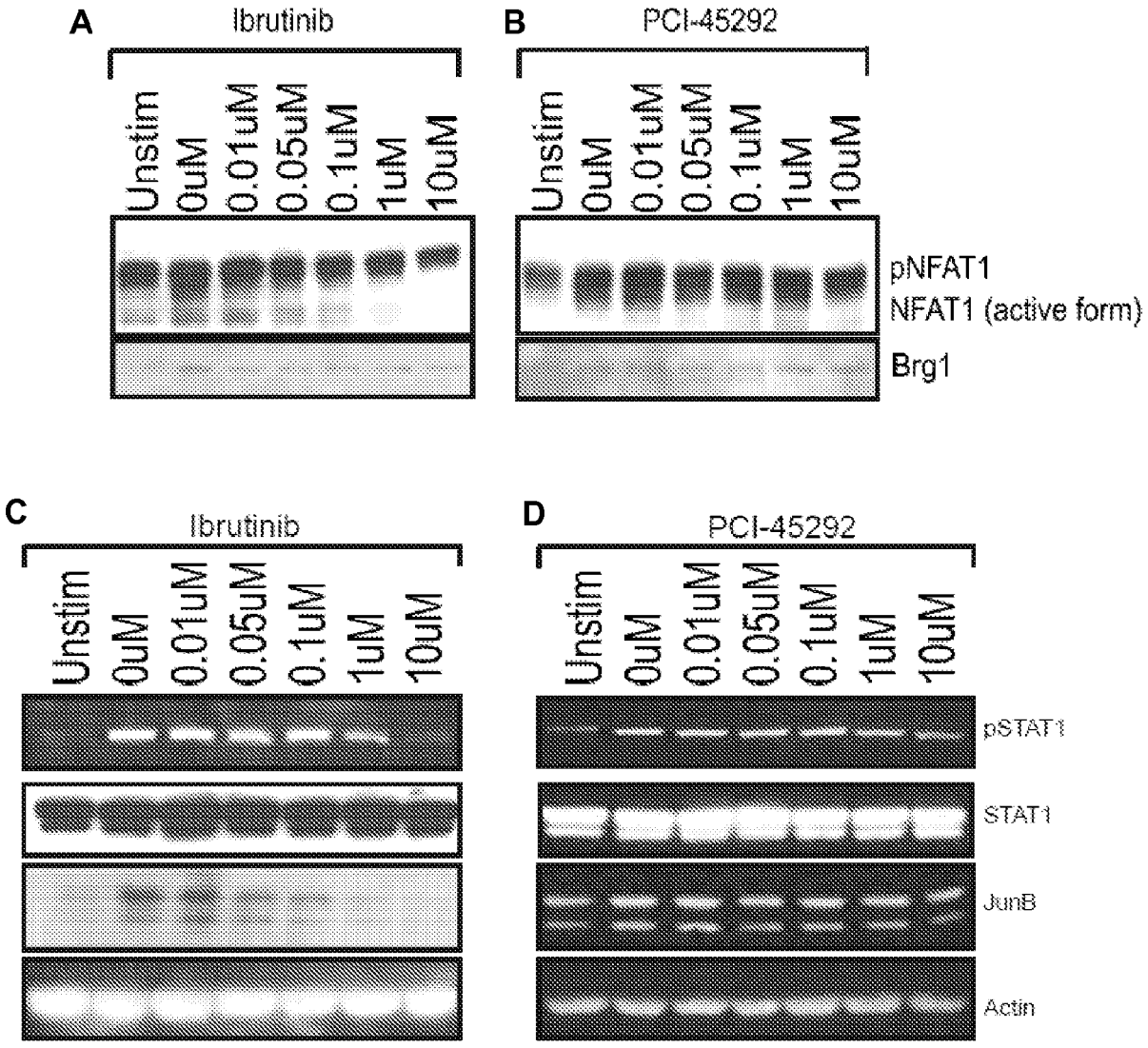

A
Ibrutinib
Unstim
0uM
0.01uM
0.05uM
0.1uM
1uM
10uM
B
PCI-45292
Unstim
0uM
0.01uM
0.05uM
0.1uM
1uM
10uM
pNFAT1
NFAT1 (active form)
Brg1
C
Ibrutinib
Unstim
0uM
0.01uM
0.05uM
0.1uM
1uM
10uM
D
PCI-45292
Unstim
0uM
0.01uM
0.05uM
0.1uM
1uM
10uM
pSTAT1
STAT1
JunB
Actin

FIGURE 19

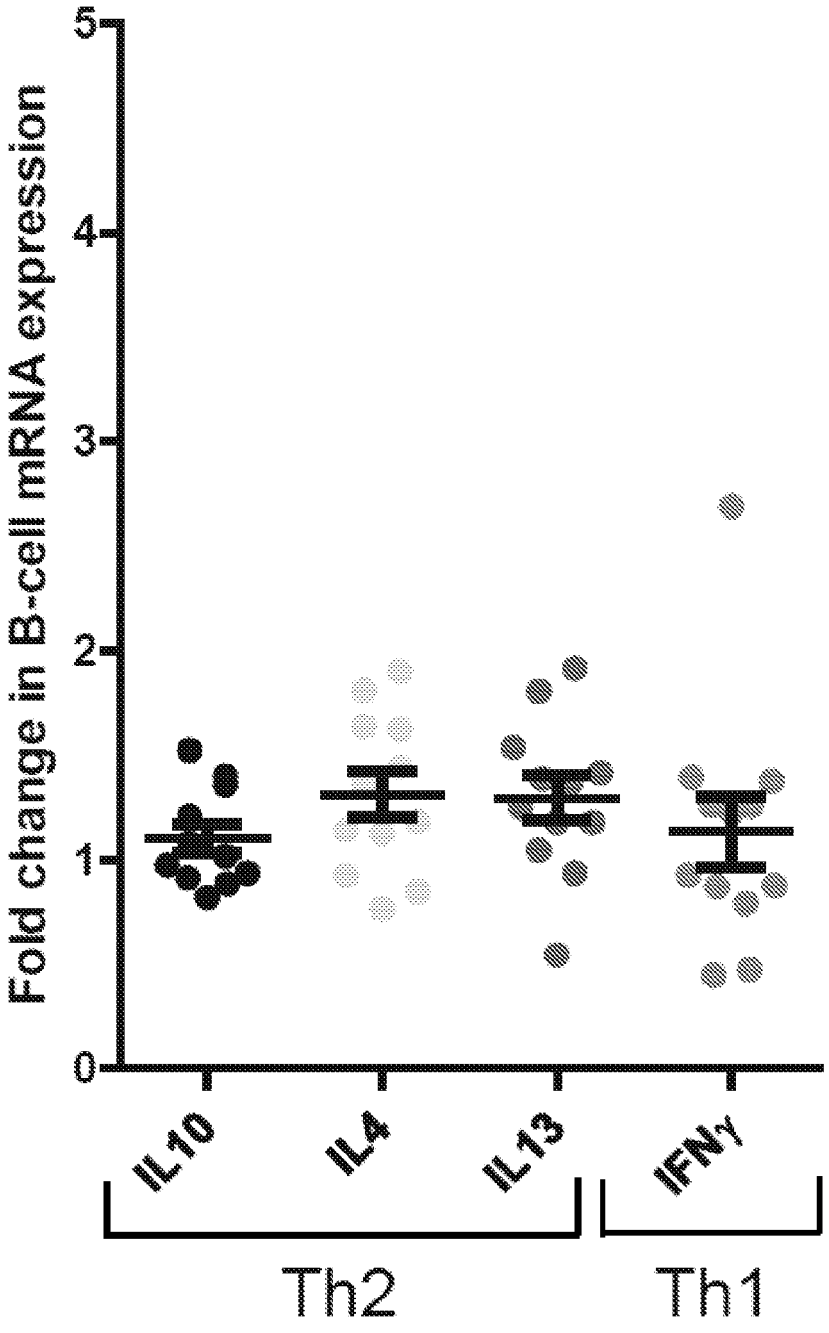

Fold change in B-cell mRNA expression
0
1
2
3
4
5
IL10
IL4
IL13
IFNγ
Th2
Th1

FIGURE 20

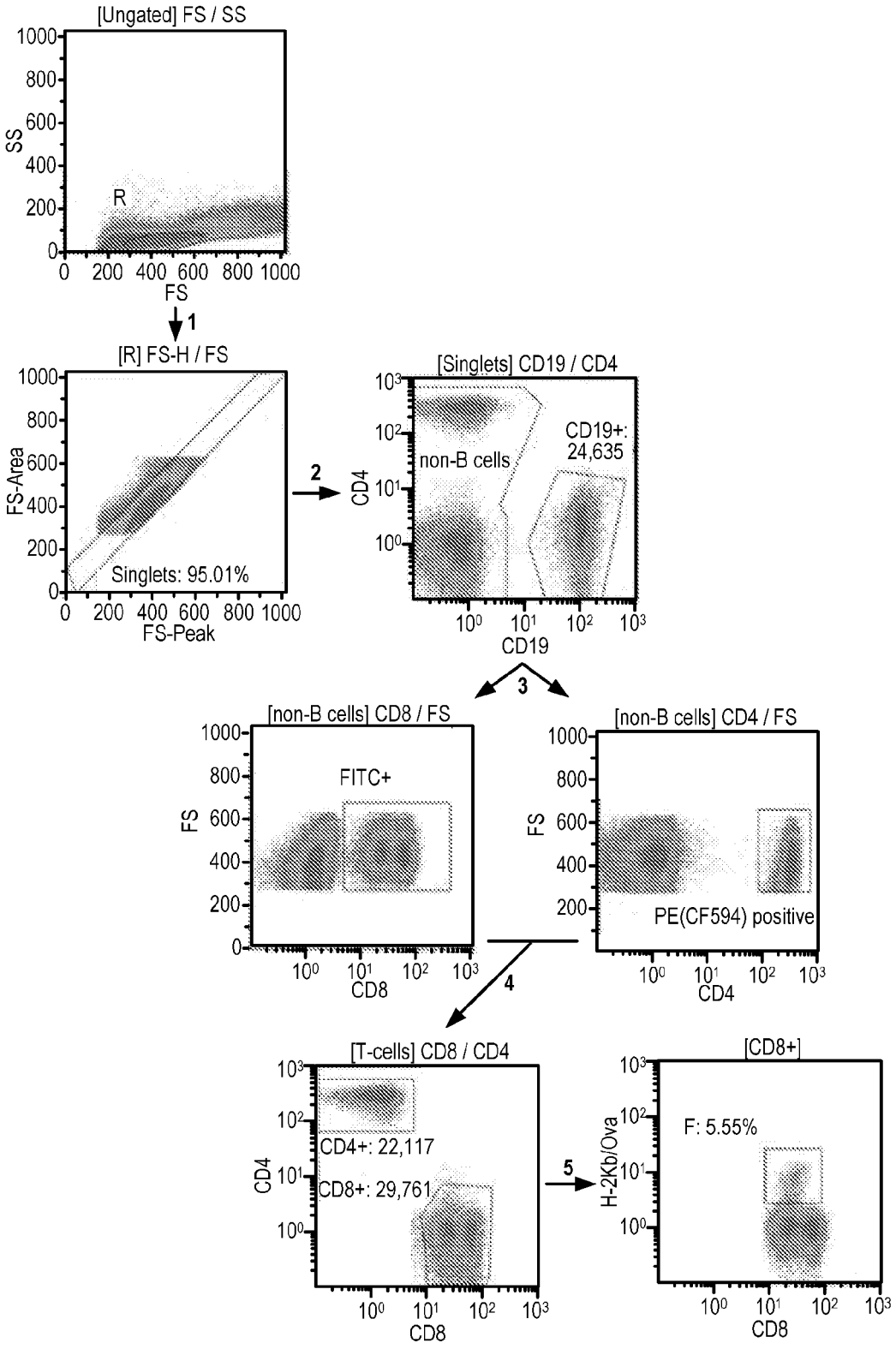

[Ungated] FS / SS
SS
FS
R
1
[R] FS-H / FS
FS-Area
FS-Peak
Singlets: 95.01%
2
[Singlets] CD19 / CD4
CD4
CD19
non-B cells
CD19+:
24,635
3
[non-B cells] CD8 / FS
FS
CD8
FITC+
[non-B cells] CD4 / FS
CD4
PE(CF594) positive
4
[T-cells] CD8 / CD4
CD4
CD8
CD4+: 22,117
CD8+: 29,761
5
[CD8+]
H-2Kb/Ova
CD8
F: 5.55%

FIGURE 21A

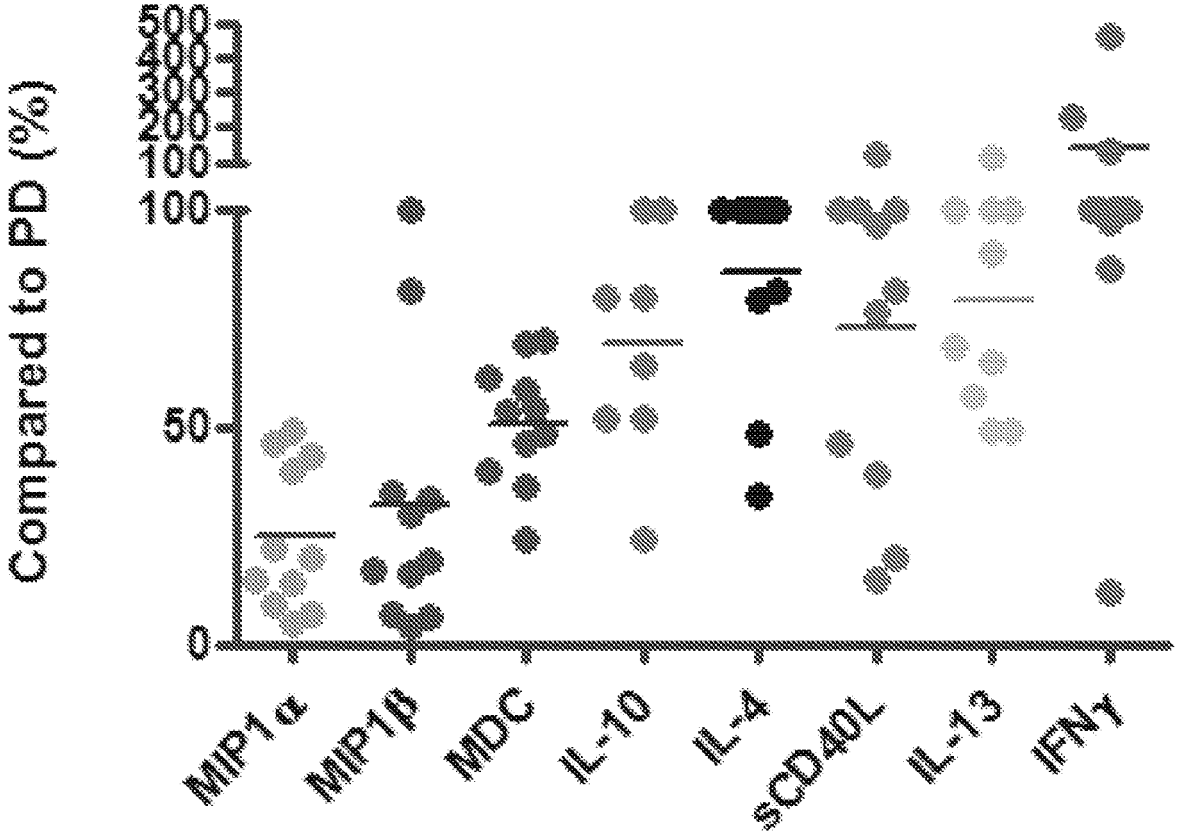

Compared to PD (%)
500
400
300
200
100
100
50
0
MIP1α
MIP1β
MDC
IL-10
IL-4
sCD40L
IL-13
IFNγ

FIGURE 21B

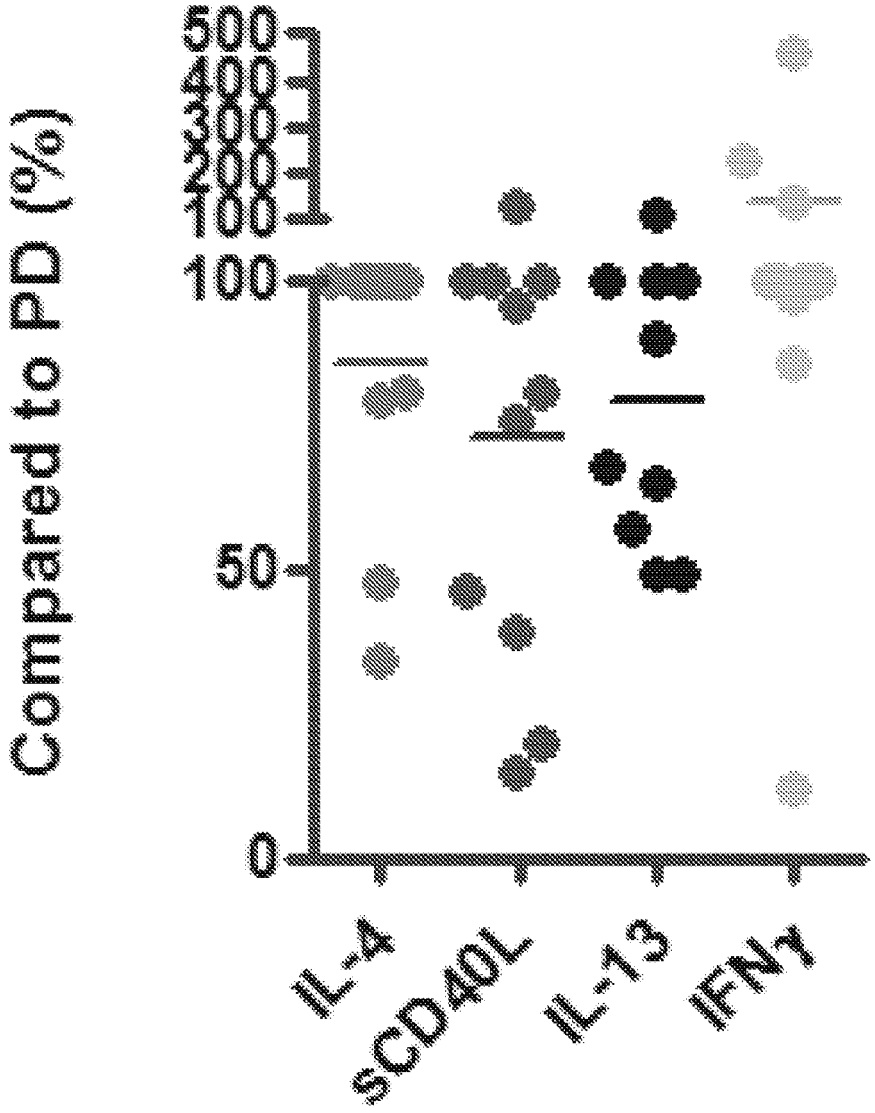

Compared to PD (%)
500
400
300
200
100
100
50
0
IL-4
sCD40L
IL-13
IFNγ

FIGURE 22

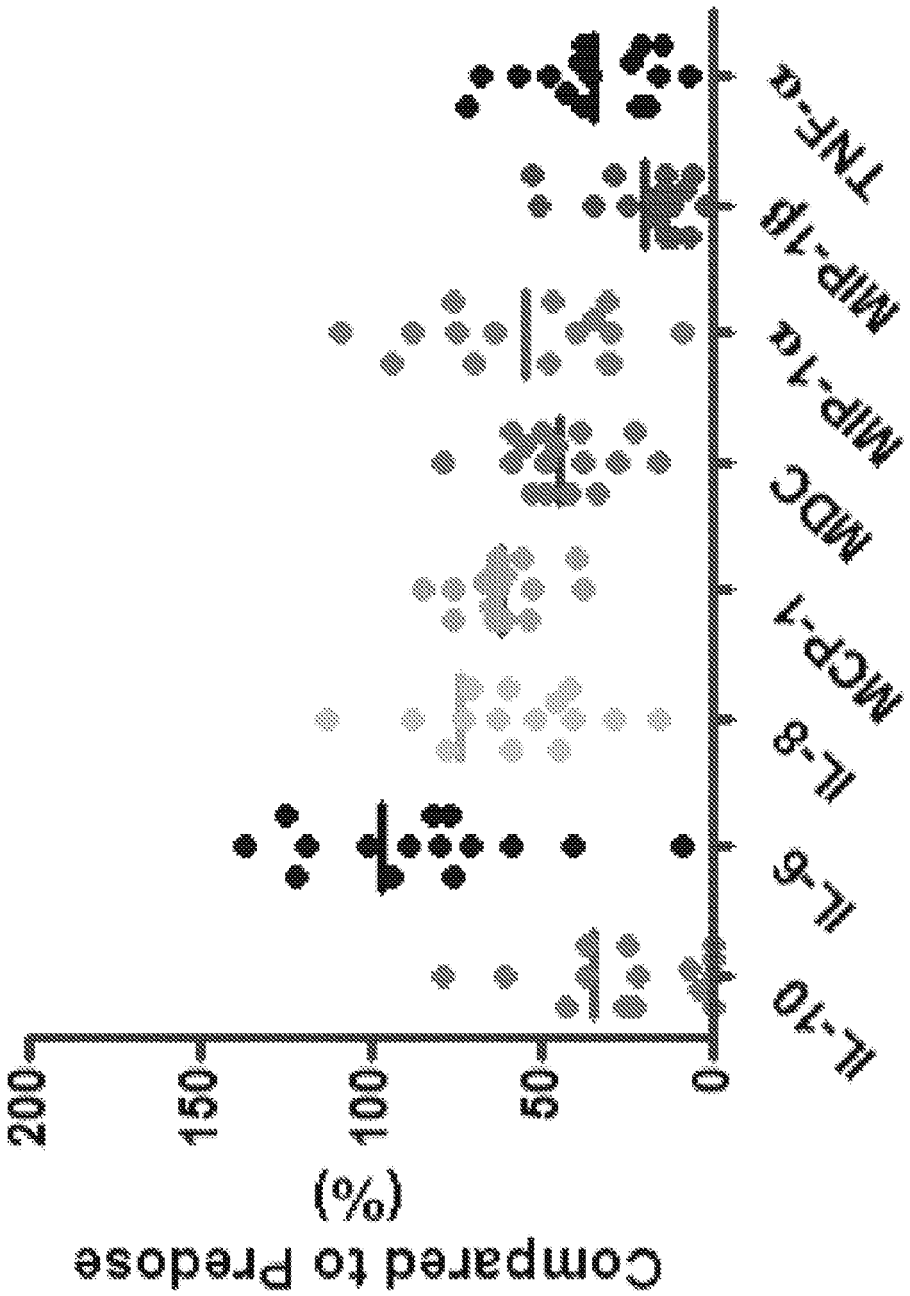
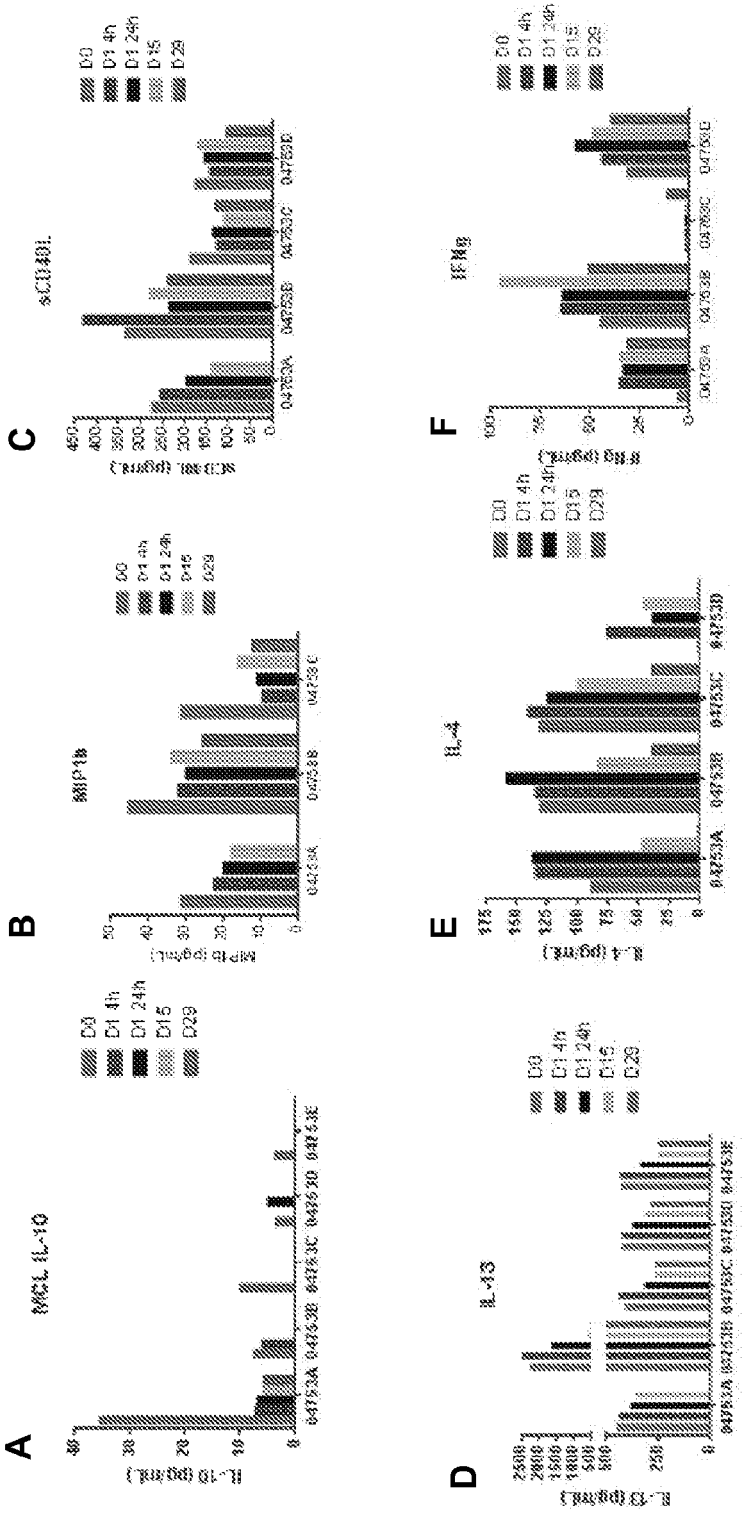

Compared to Predose
(%)
200
150
100
50
0
IL-10
IL-6
IL-8
MCP-1
MDC
MIP-1α
MIP-1β
TNF-α

FIGURE 23


A
MCP1b
B
MIP1b
C
sCD40L
D
IL-13
E
IL-4
F
IFNg
D0
D1 4h
D1 24h
D15
D29