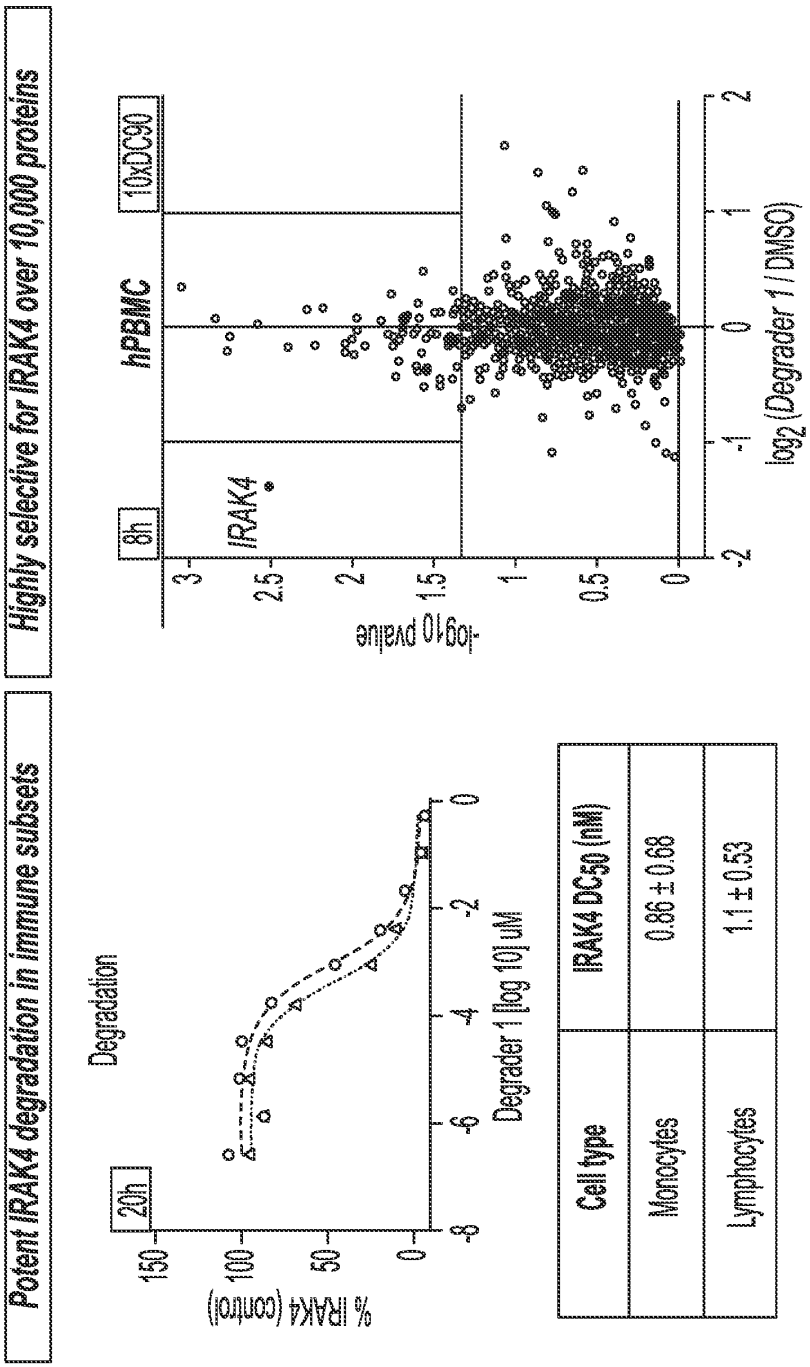

Potent IRAK4 degradation in immune subsets
Highly selective for IRAK4 over 10,000 proteins
20h
Degradation
% IRAK4 (control)
Degrader 1 [log 10] uM
8h
hPBMC
10xDC90
IRAK4
-log10 pvalue
log2 (Degrader 1 / DMSO)
Cell type
IRAK4 DC50 (nM)
Monocytes
0.86 ± 0.68
Lymphocytes
1.1 ± 0.53

FIG. 1

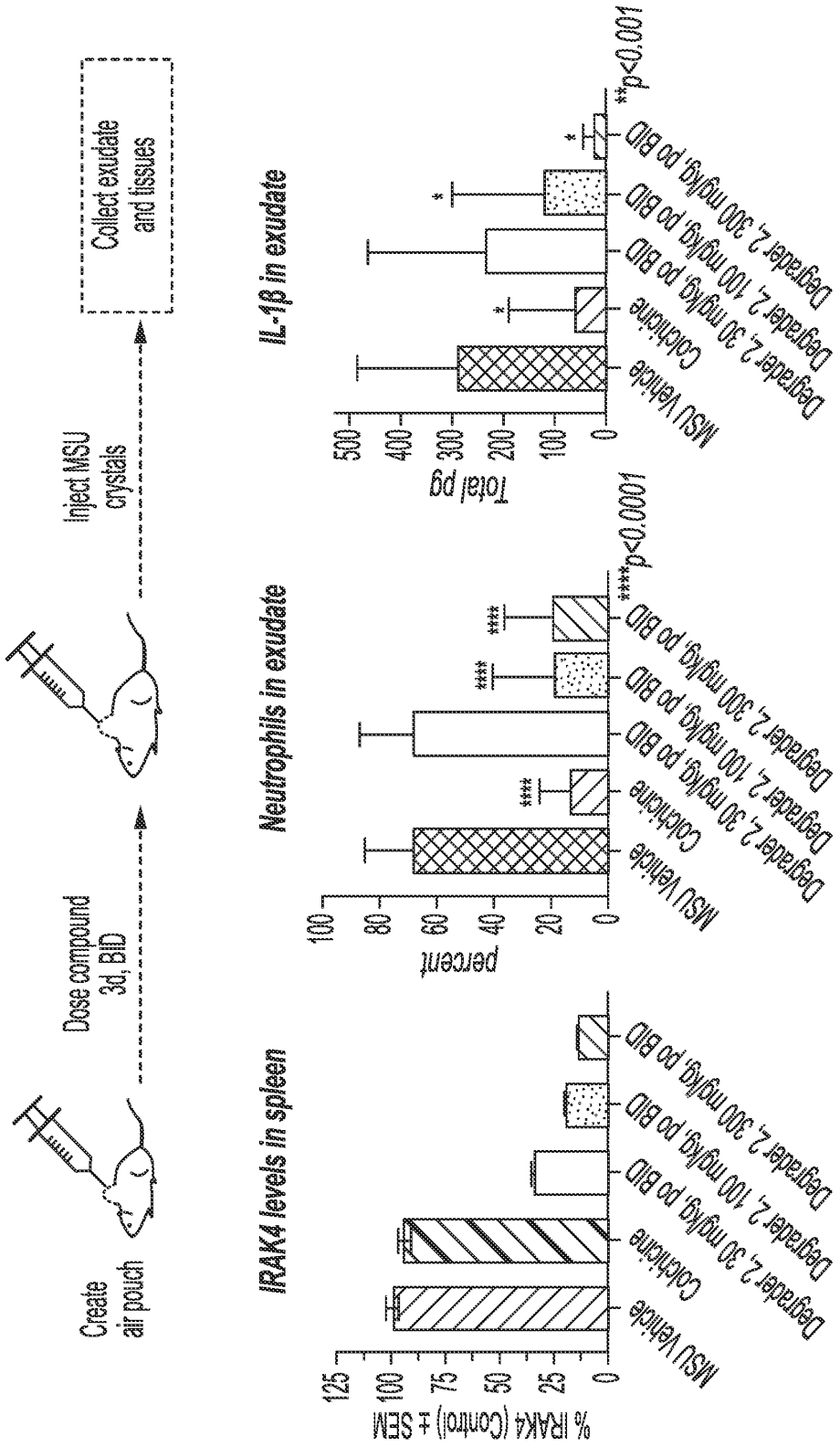

Create air pouch
Dose compound 3d, BID
Inject MSU crystals
Collect exudate and tissues
IRAK4 levels in spleen
% IRAK4 (Control) ± SEM
125
100
75
50
25
0
MSU Vehicle
Colchicine
Degrader 2, 30 mg/kg, po BID
Degrader 2, 100 mg/kg, po BID
Degrader 2, 300 mg/kg, po BID
Neutrophils in exudate
percent
100
80
60
40
20
0
****
****
****
****p<0.0001
IL-1β in exudate
Total pg
500
400
300
200
100
0
*
*
*
**p<0.001

FIG. 2

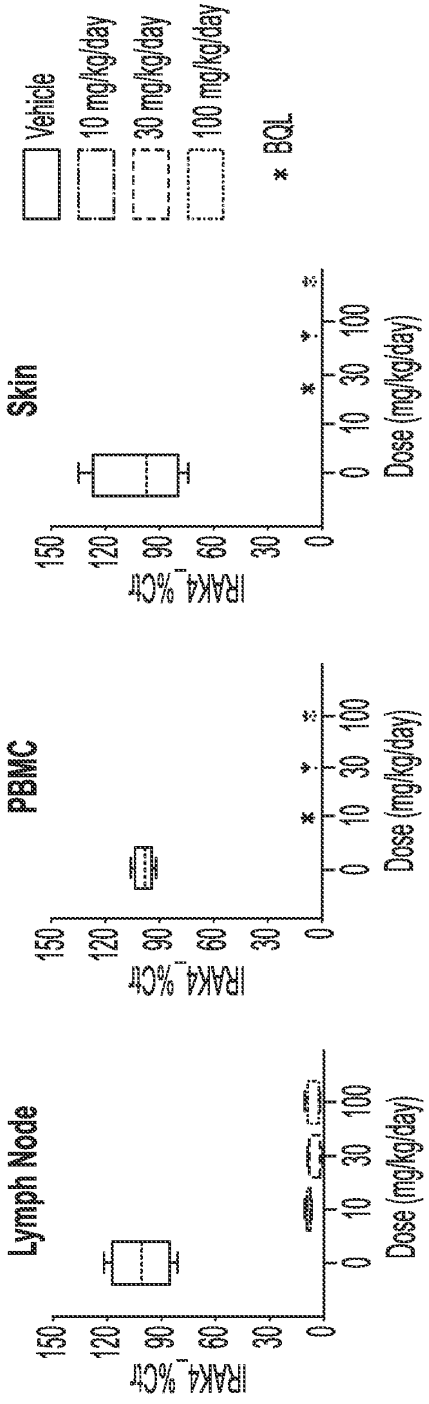

Vehicle
10 mg/kg/day
30 mg/kg/day
100 mg/kg/day
* BQL
Skin
PBMC
Lymph Node
IRAK4_%Ctr
Dose (mg/kg/day)
0
10
30
100
30
60
90
120
150

FIG. 3

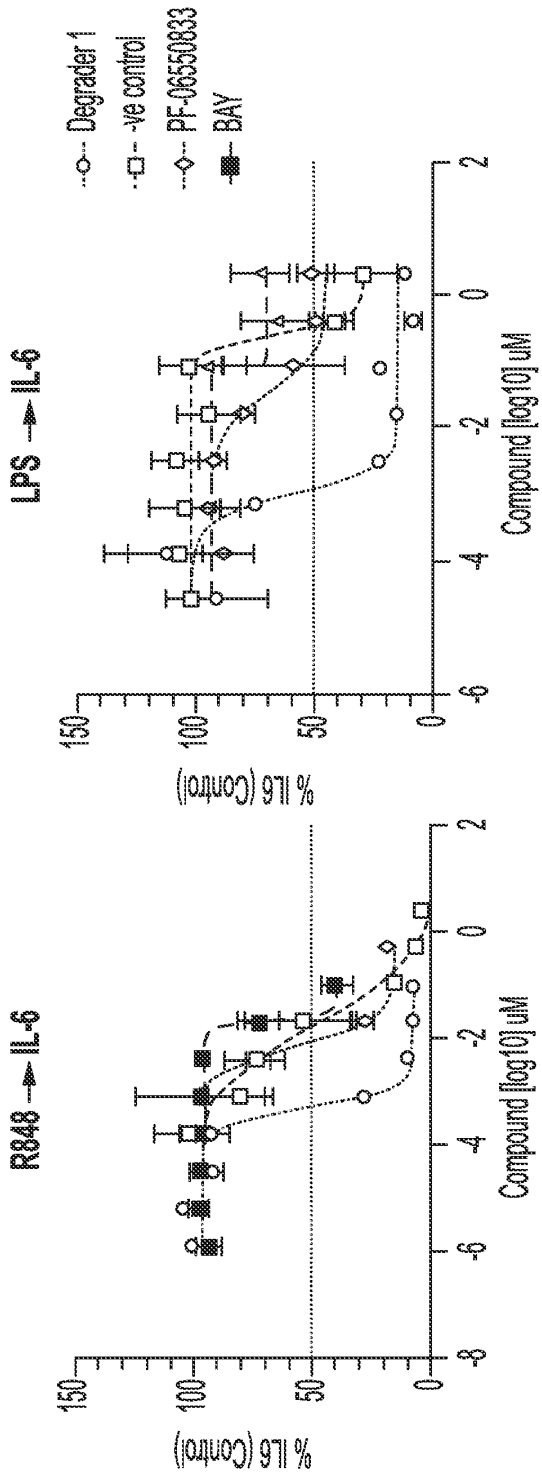

Degrader 1
-ve control
PF-06550833
BAY
LPS → IL-6
% IL6 (Contro)
Compound [log10] uM
R848 → IL-6
% IL6 (Contro)
Compound [log10] uM

FIG. 4

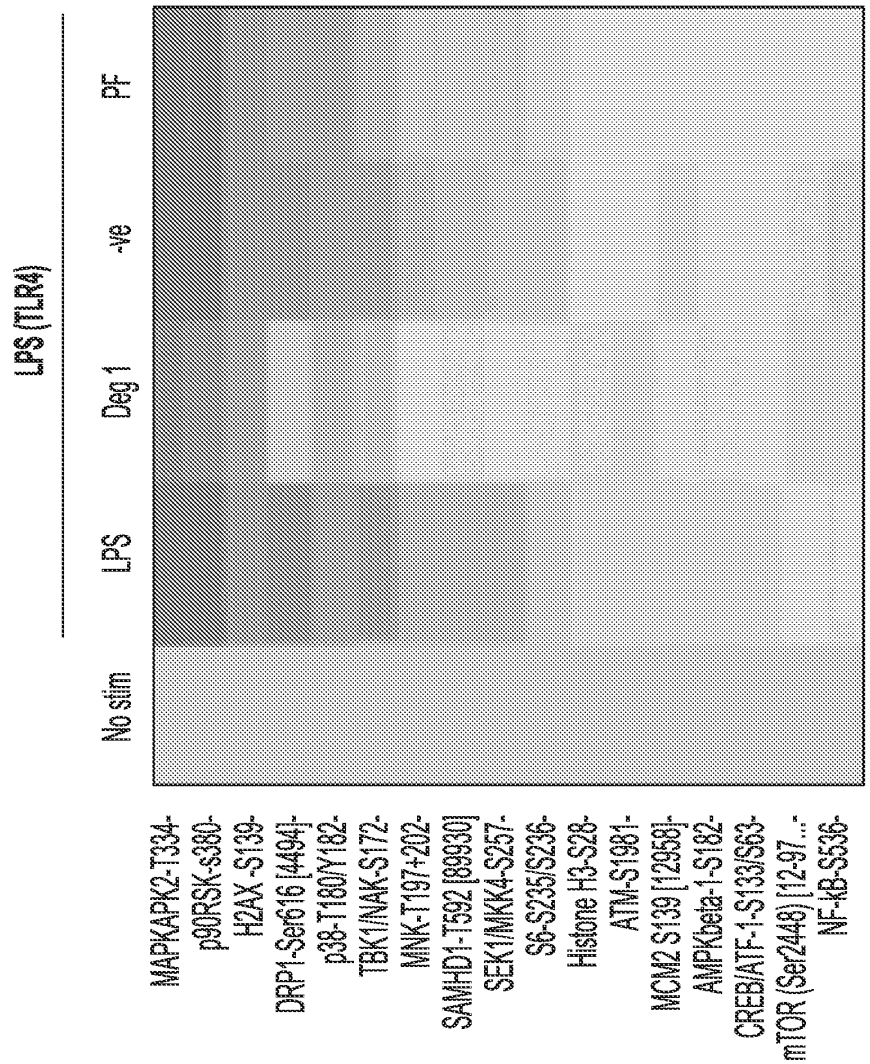
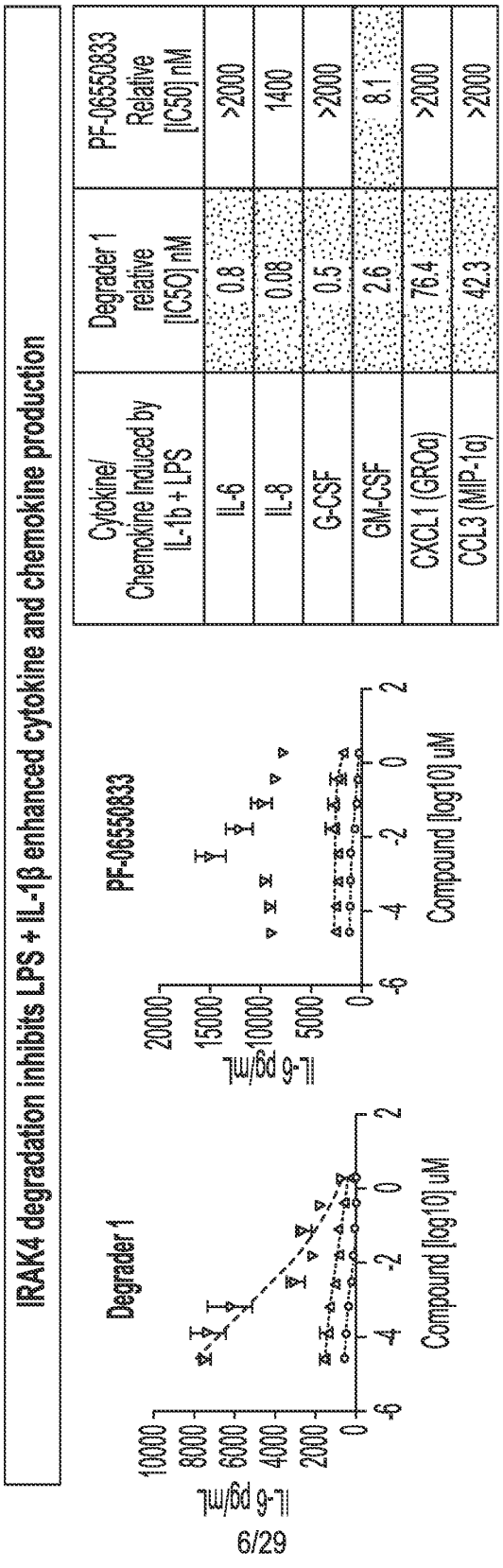


FIG. 4 CONTINUED



Cytokine/ Chemokine Induced by IL-1b + LPS	Degrader 1 relative [IC50] nM	PF-06550833 Relative [IC50] nM
IL-6	0.8	>2000
IL-8	0.08	1400
G-CSF	0.5	>2000
GM-CSF	2.6	8.1
CXCL1 (GROα)	76.4	>2000
CCL3 (MIP-1α)	42.3	>2000

FIG. 4 CONTINUED

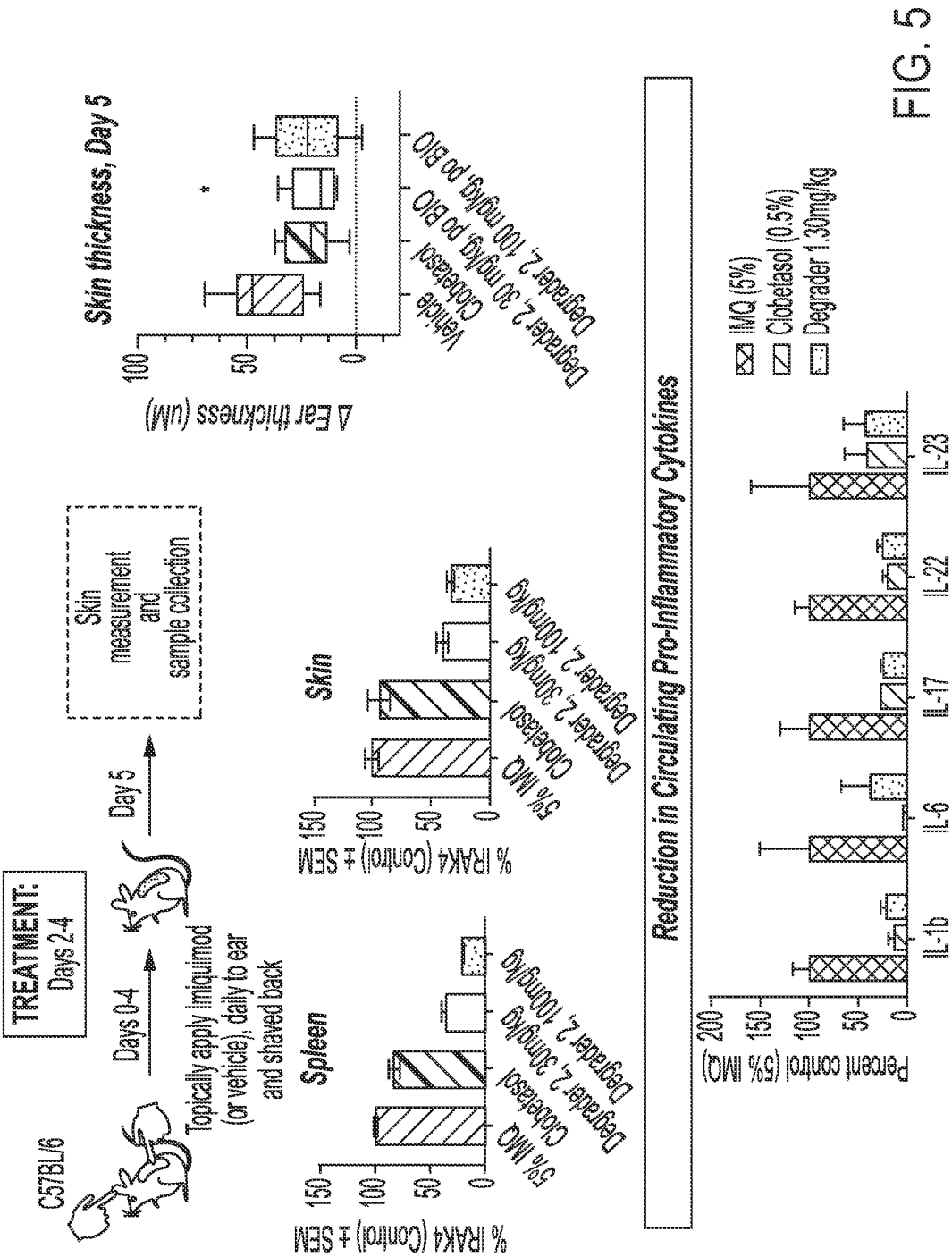

C57BL/6
TREATMENT:
Days 2-4
Topically apply Imiquimod
(or vehicle), daily to ear
and shaved back
Days 0-4
Day 5
Skin
measurement
and
sample collection
Skin thickness, Day 5
Δ Ear thickness (uM)
100
50
0
Vehicle
Clobetasol
Degrader 2, 30 mg/kg, po BID
Degrader 2, 100 mg/kg, po BID
Skin
% IRAK4 (Control) ± SEM
150
100
50
0
5% IMQ
Clobetasol
Degrader 2, 30mg/kg
Degrader 2, 10mg/kg
Spleen
% IRAK4 (Control) ± SEM
150
100
50
0
5% IMQ
Clobetasol
Degrader 2, 30mg/kg
Degrader 2, 10mg/kg
Reduction in Circulating Pro-Inflammatory Cytokines
IMQ (5%)
Clobetasol (0.5%)
Degrader 1.30mg/kg
Percent control (5% IMQ)
200
150
100
50
0
IL-1b
IL-6
IL-17
IL-22
IL-23

FIG. 5

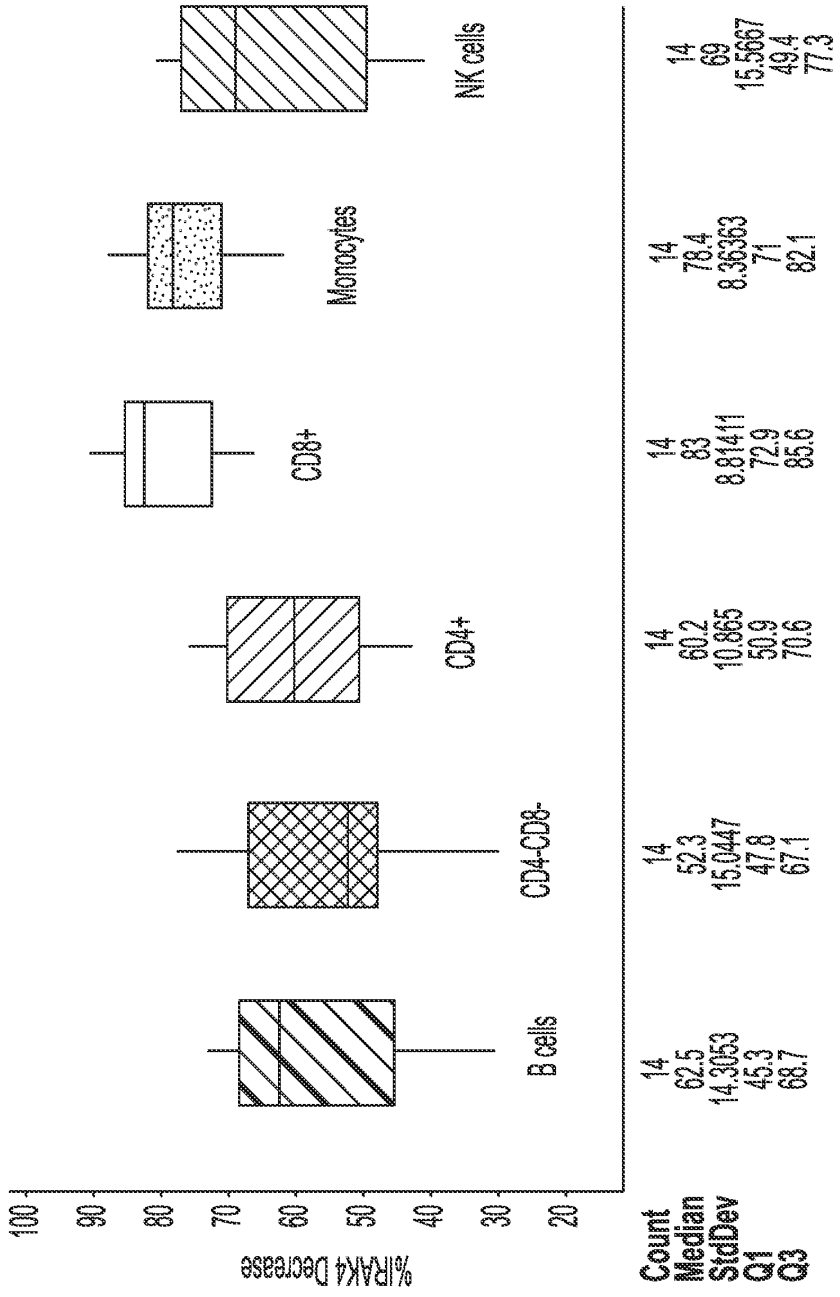

%IRAK4 Decrease
100
90
80
70
60
50
40
30
20
B cells
CD4-CD8-
CD4+
CD8+
Monocytes
NK cells
Count
Median
StdDev
Q1
Q3
14
62.5
14.3053
45.3
68.7
14
52.3
15.0447
47.8
67.1
14
60.2
10.865
50.9
70.6
14
83
8.81411
72.9
85.6
14
78.4
8.36363
71
82.1
14
69
15.5667
49.4
77.3

FIG. 6

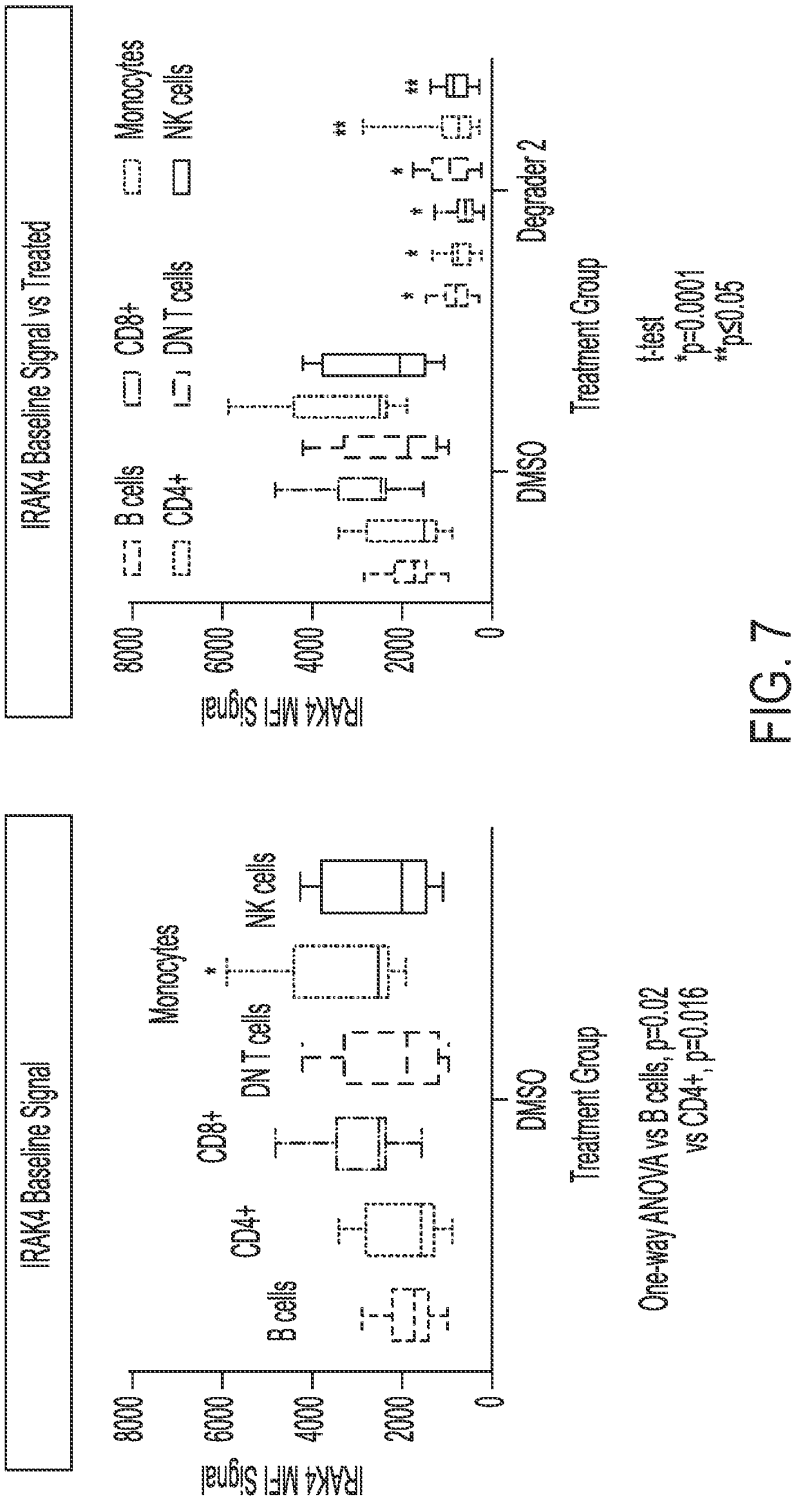

IRAK4 Baseline Signal
IRAK4 MFI Signal
8000
6000
4000
2000
0
B cells
CD4+
CD8+
DN T cells
Monocytes
NK cells
DMSO
Treatment Group
One-way ANOVA vs B cells, p=0.02
vs CD4+, p=0.016
IRAK4 Baseline Signal vs Treated
Degrader 2
t-test
*p=0.0001
**p≤0.05

FIG. 7

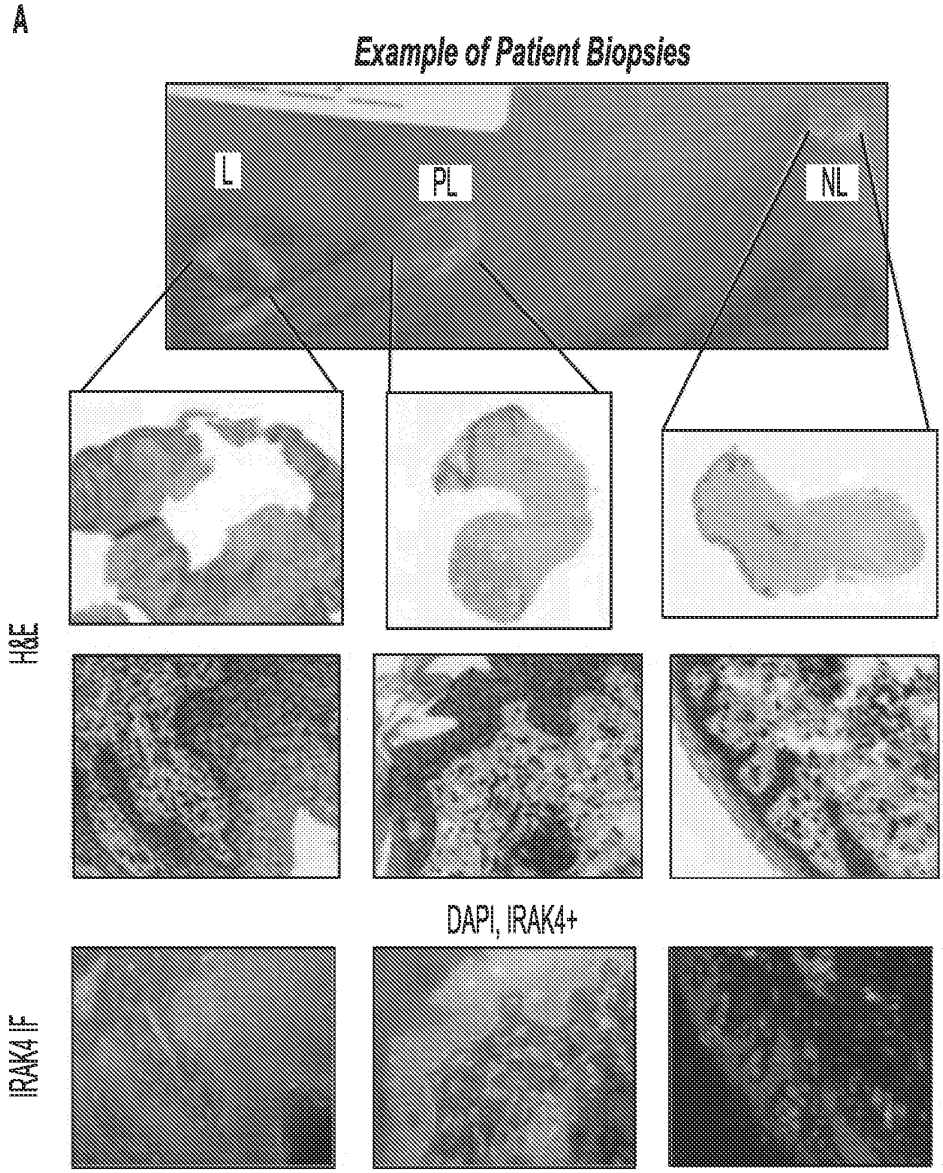


FIG. 8

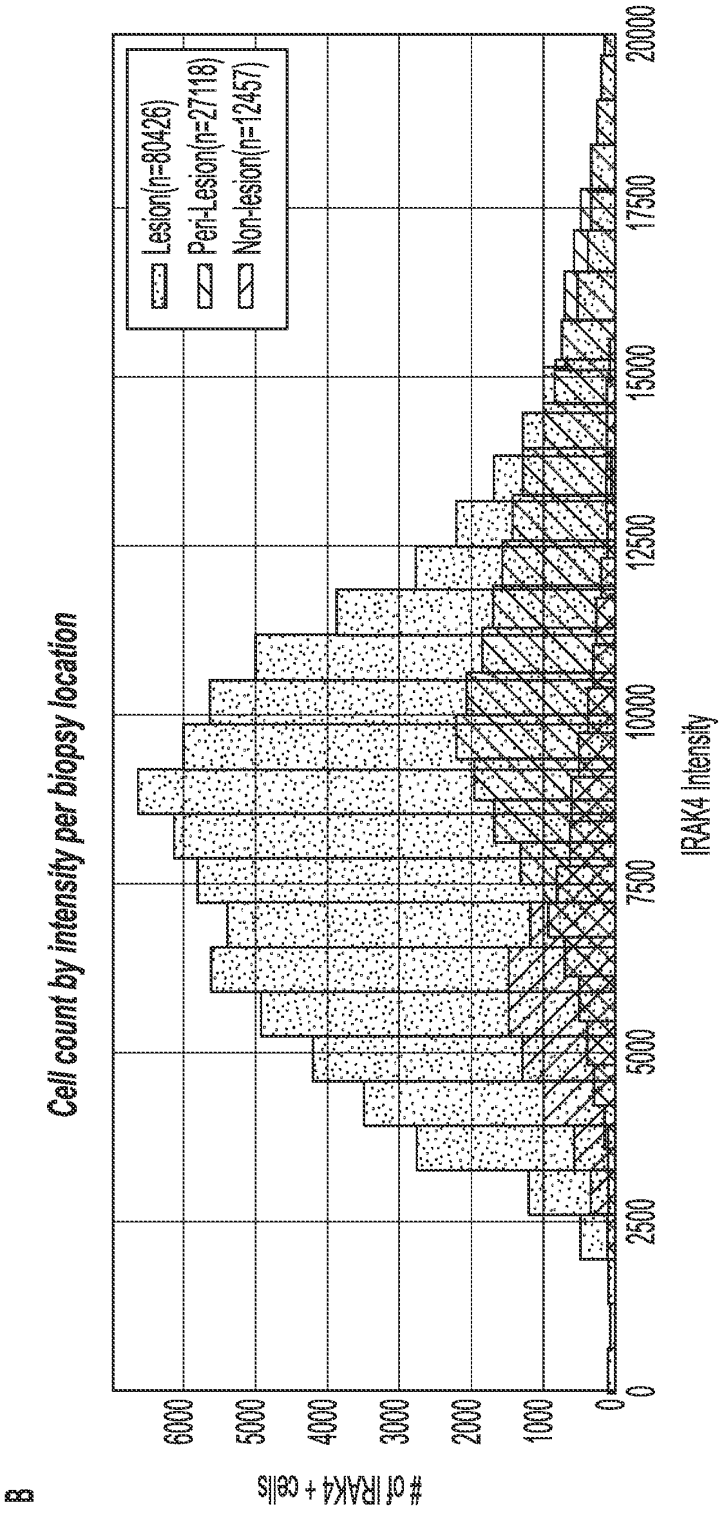

B
Cell count by intensity per biopsy location
# of IRAK4 + cells
IRAK4 Intensity
Lesion(n=80426)
Peri-Lesion(n=27118)
Non-lesion(n=12457)
0
1000
2000
3000
4000
5000
6000
2500
5000
7500
10000
12500
15000
17500
20000

FIG. 8

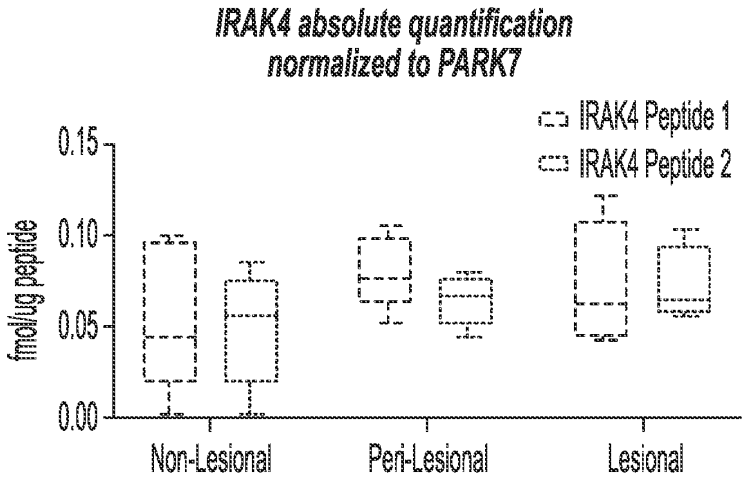

IRAK4 absolute quantification
normalized to PARK7
IRAK4 Peptide 1
IRAK4 Peptide 2
fmol/ug peptide
0.15
0.10
0.05
0.00
Non-Lesional
Peri-Lesional
Lesional

FIG. 9

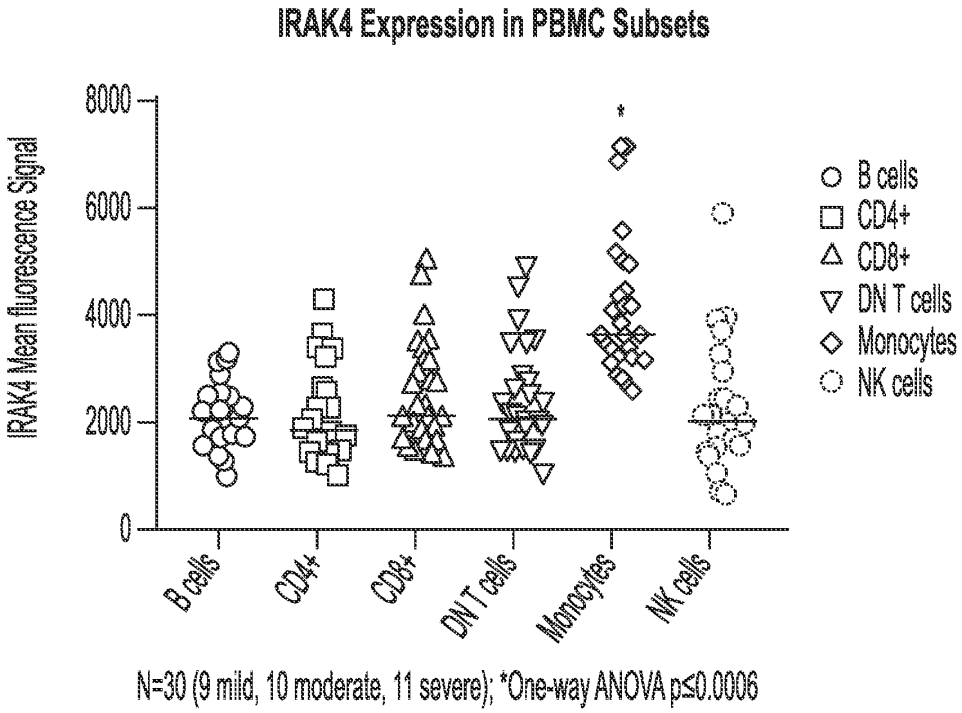

IRAK4 Expression in PBMC Subsets
IRAK4 Mean fluorescence Signal
8000
6000
4000
2000
0
B cells
CD4+
CD8+
DN T cells
Monocytes
NK cells
N=30 (9 mild, 10 moderate, 11 severe); *One-way ANOVA p≤0.0006

FIG. 10

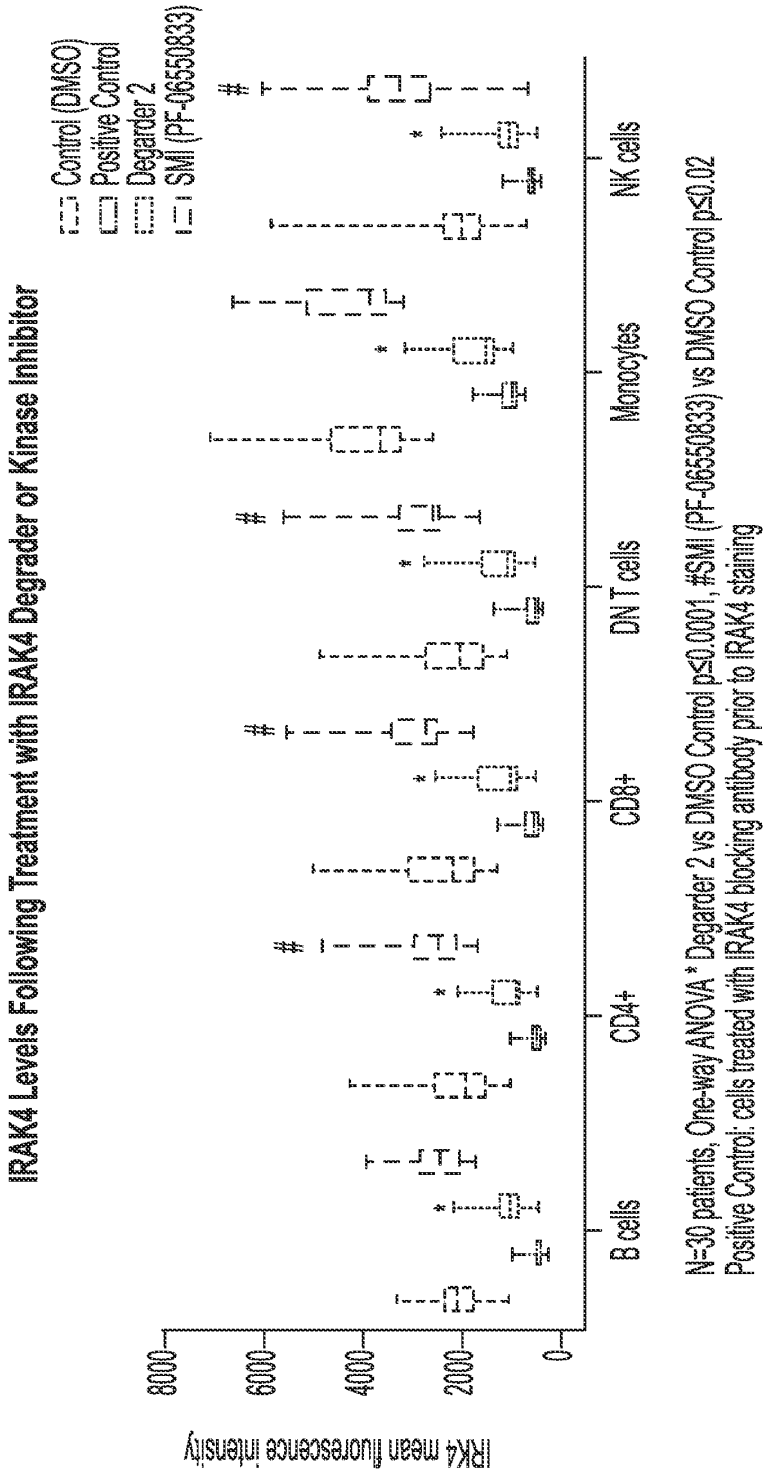
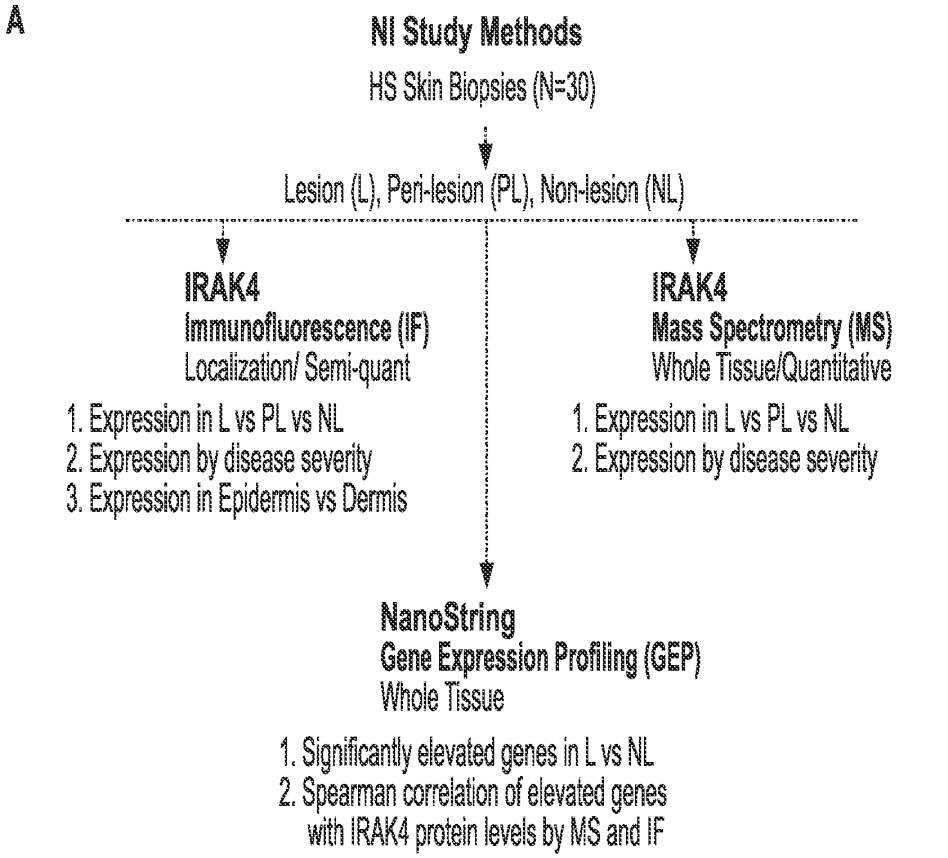

IRAK4 Levels Following Treatment with IRAK4 Degrader or Kinase Inhibitor
Control (DMSO)
Positive Control
Degarder 2
SMI (PF-06650833)
IRK4 mean fluorescence intensity
8000
6000
4000
2000
0
B cells
CD4+
CD8+
DN T cells
Monocytes
NK cells
N=30 patients, One-way ANOVA * Degarder 2 vs DMSO Control p≤0.0001, #SMI (PF-06650833) vs DMSO Control p≤0.02
Positive Control: cells treated with IRAK4 blocking antibody prior to IRAK4 staining

FIG. 11


A
NI Study Methods
HS Skin Biopsies (N=30)
Lesion (L), Peri-lesion (PL), Non-lesion (NL)
IRAK4
Immunofluorescence (IF)
Localization/ Semi-quant
1. Expression in L vs PL vs NL
2. Expression by disease severity
3. Expression in Epidermis vs Dermis
IRAK4
Mass Spectrometry (MS)
Whole Tissue/Quantitative
1. Expression in L vs PL vs NL
2. Expression by disease severity
NanoString
Gene Expression Profiling (GEP)
Whole Tissue
1. Significantly elevated genes in L vs NL
2. Spearman correlation of elevated genes with IRAK4 protein levels by MS and IF

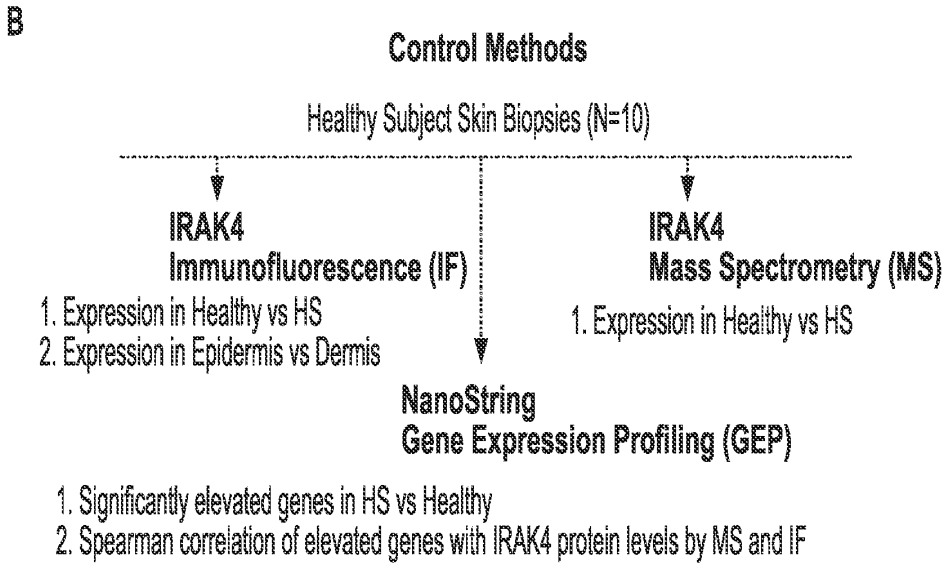

B
Control Methods
Healthy Subject Skin Biopsies (N=10)
IRAK4
Immunofluorescence (IF)
1. Expression in Healthy vs HS
2. Expression in Epidermis vs Dermis
IRAK4
Mass Spectrometry (MS)
1. Expression in Healthy vs HS
NanoString
Gene Expression Profiling (GEP)
1. Significantly elevated genes in HS vs Healthy
2. Spearman correlation of elevated genes with IRAK4 protein levels by MS and IF

FIG. 12

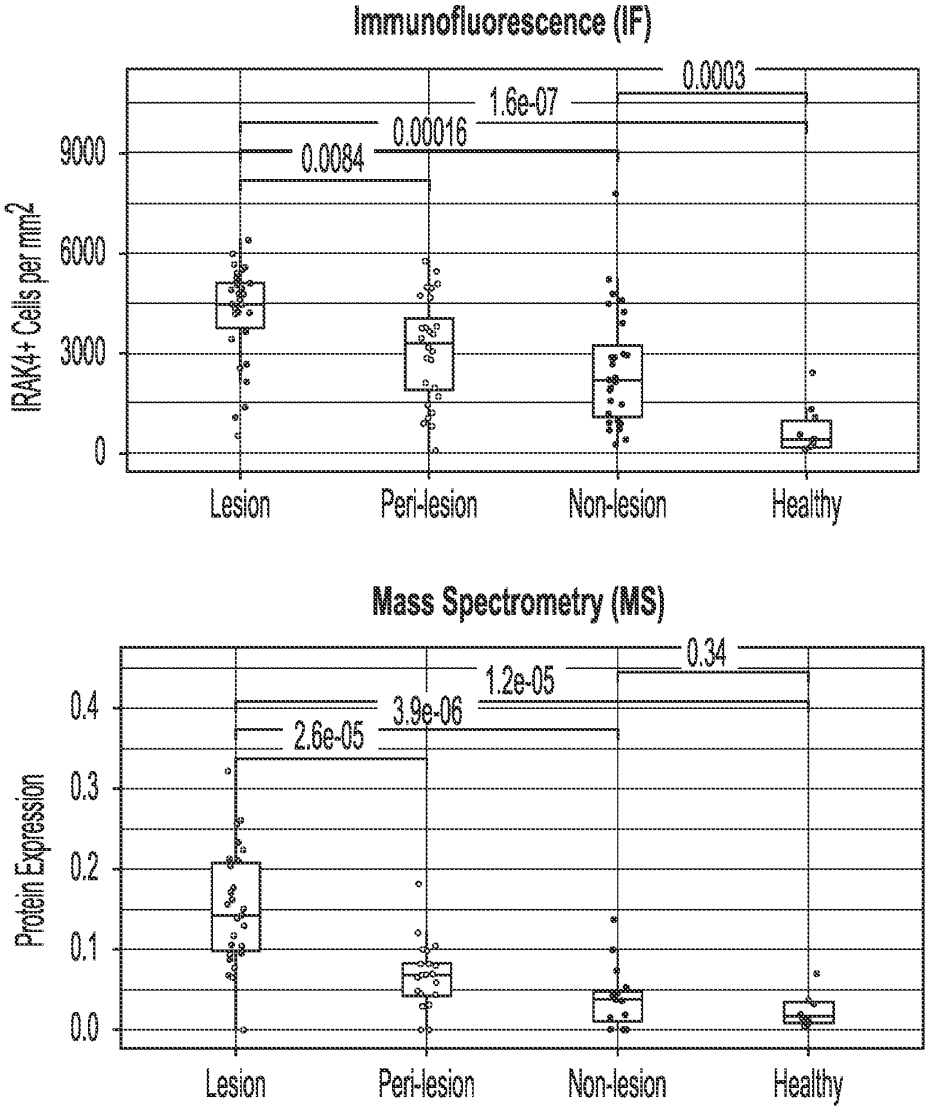

Immunofluorescence (IF)
IRAK4+ Cells per mm2
9000
6000
3000
0
0.0003
1.6e-07
0.00016
0.0084
Lesion
Peri-lesion
Non-lesion
Healthy
Mass Spectrometry (MS)
Protein Expression
0.4
0.3
0.2
0.1
0.0
0.34
1.2e-05
3.9e-06
2.6e-05
Lesion
Peri-lesion
Non-lesion
Healthy

FIG. 13

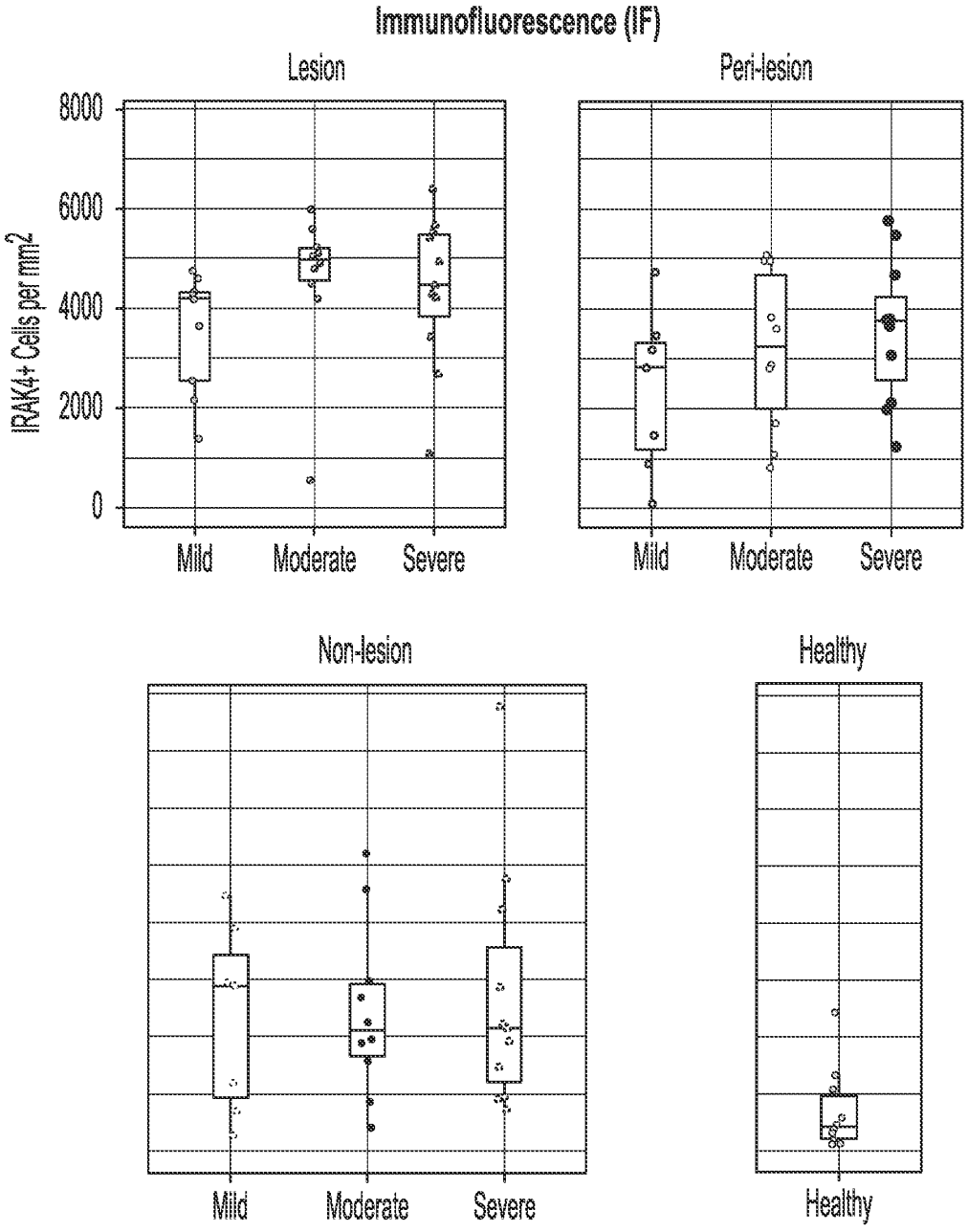


FIG. 13 CONTINUED

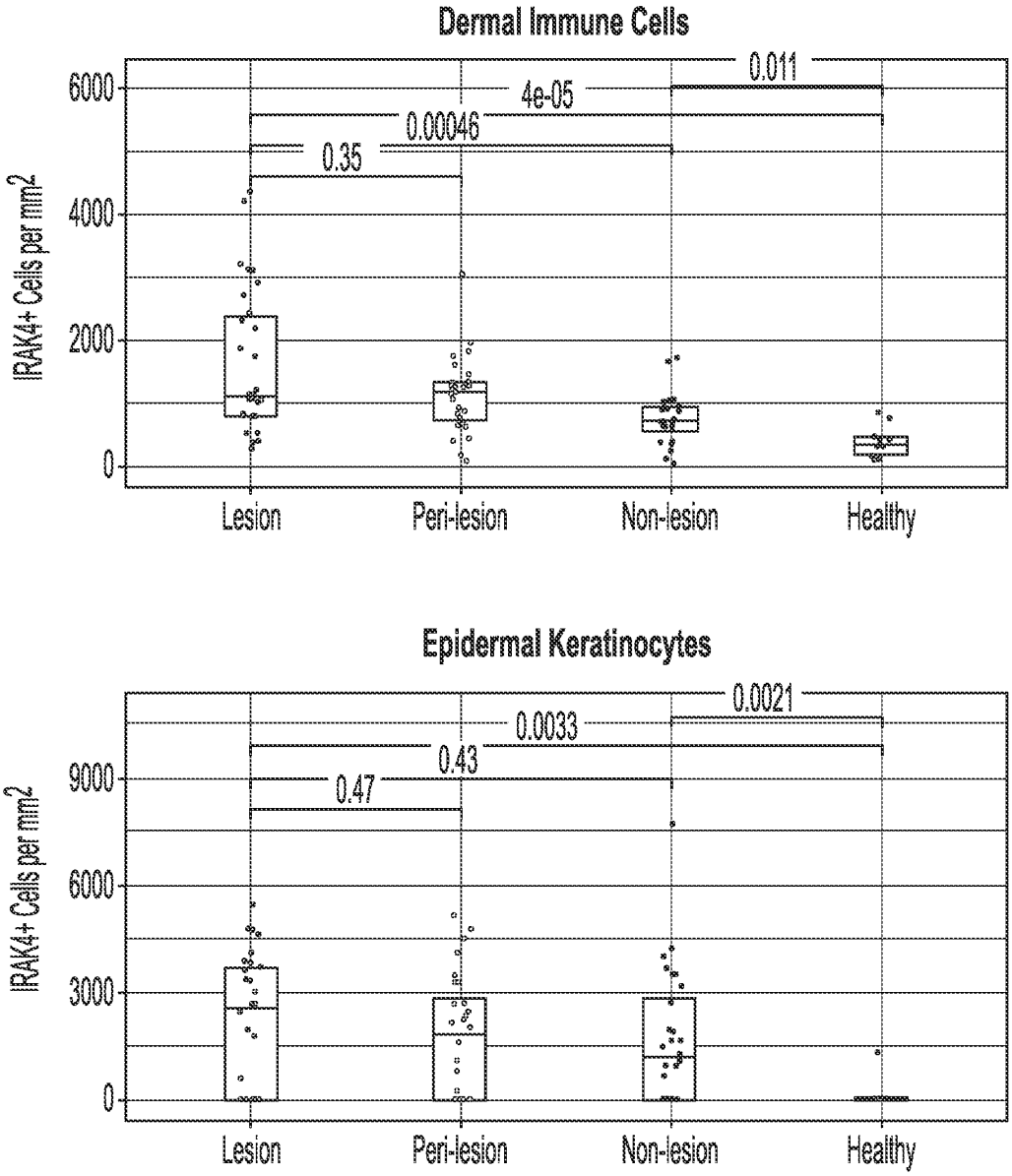

Dermal Immune Cells
IRAK4+ Cells per mm2
6000
4000
2000
0
0.011
4e-05
0.00046
0.35
Lesion
Peri-lesion
Non-lesion
Healthy
Epidermal Keratinocytes
IRAK4+ Cells per mm2
9000
6000
3000
0
0.0021
0.0033
0.43
0.47
Lesion
Peri-lesion
Non-lesion
Healthy

FIG. 14

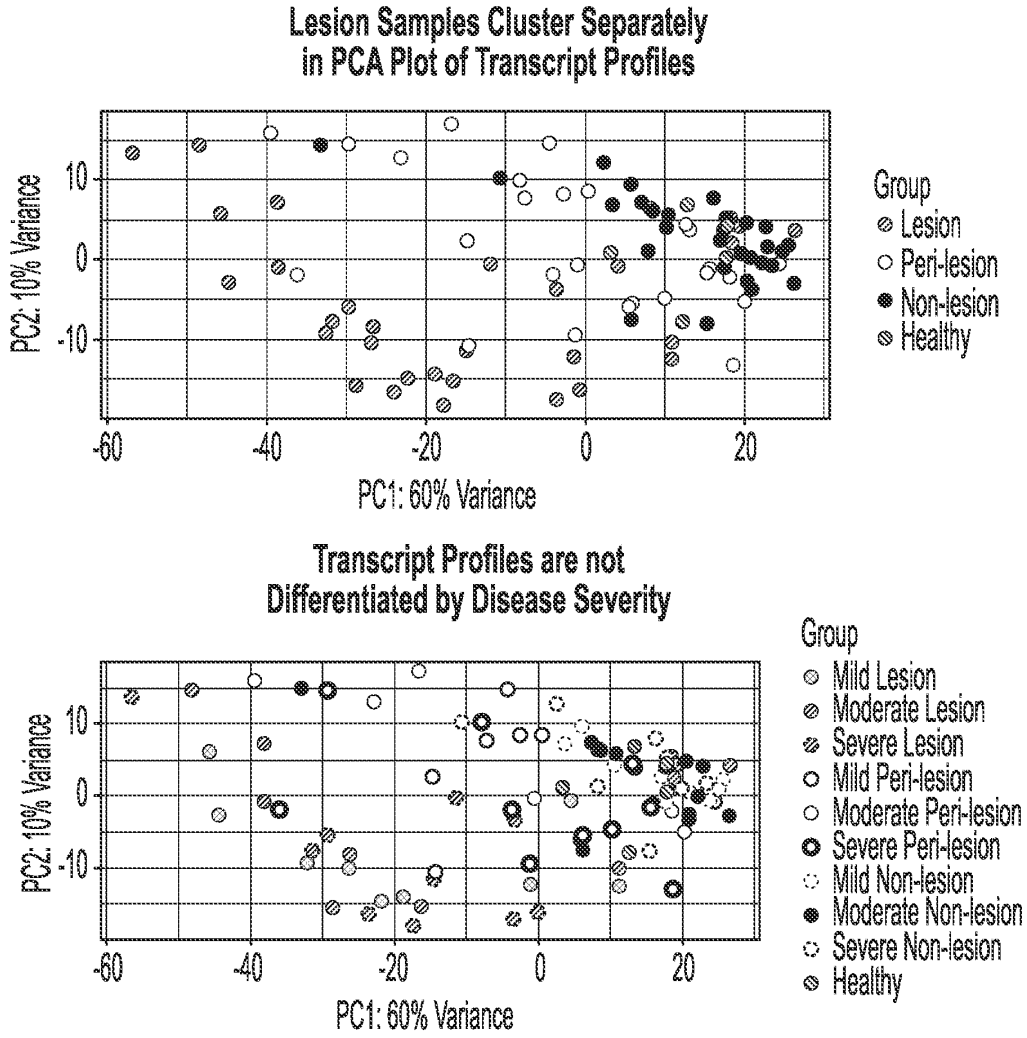

Lesion Samples Cluster Separately
in PCA Plot of Transcript Profiles
PC2: 10% Variance
10
0
-10
-60
-40
-20
0
20
PC1: 60% Variance
Group
Lesion
Peri-lesion
Non-lesion
Healthy
Transcript Profiles are not
Differentiated by Disease Severity
PC2: 10% Variance
10
0
-10
-60
-40
-20
0
20
PC1: 60% Variance
Group
Mild Lesion
Moderate Lesion
Severe Lesion
Mild Peri-lesion
Moderate Peri-lesion
Severe Peri-lesion
Mild Non-lesion
Moderate Non-lesion
Severe Non-lesion
Healthy

FIG. 15

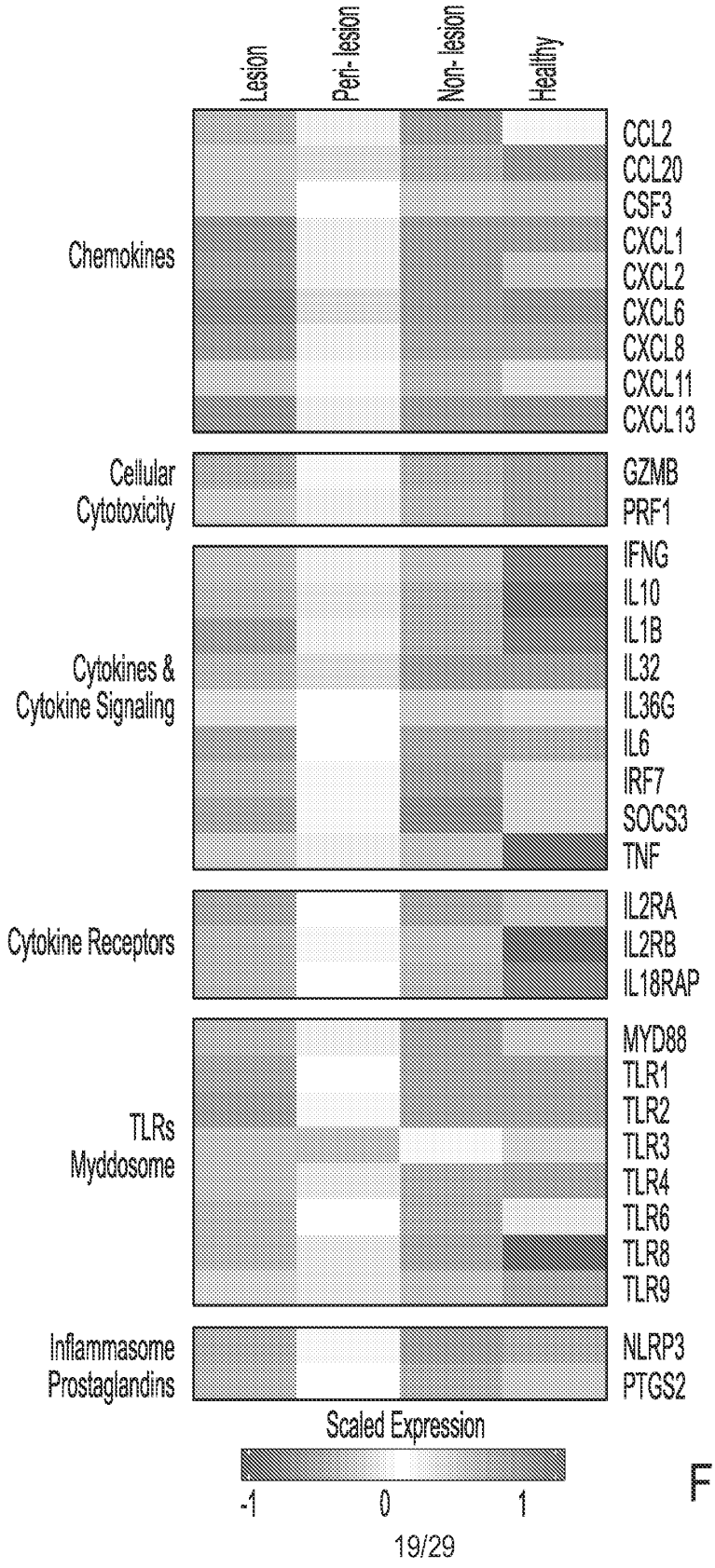

Lesion
Peri- lesion
Non- lesion
Healthy
Chemokines
CCL2
CCL20
CSF3
CXCL1
CXCL2
CXCL6
CXCL8
CXCL11
CXCL13
Cellular Cytotoxicity
GZMB
PRF1
Cytokines & Cytokine Signaling
IFNG
IL10
IL1B
IL32
IL36G
IL6
IRF7
SOCS3
TNF
Cytokine Receptors
IL2RA
IL2RB
IL18RAP
TLRs Myddosome
MYD88
TLR1
TLR2
TLR3
TLR4
TLR6
TLR8
TLR9
Inflammasome Prostaglandins
NLRP3
PTGS2
Scaled Expression
-1
0
1

FIG. 16

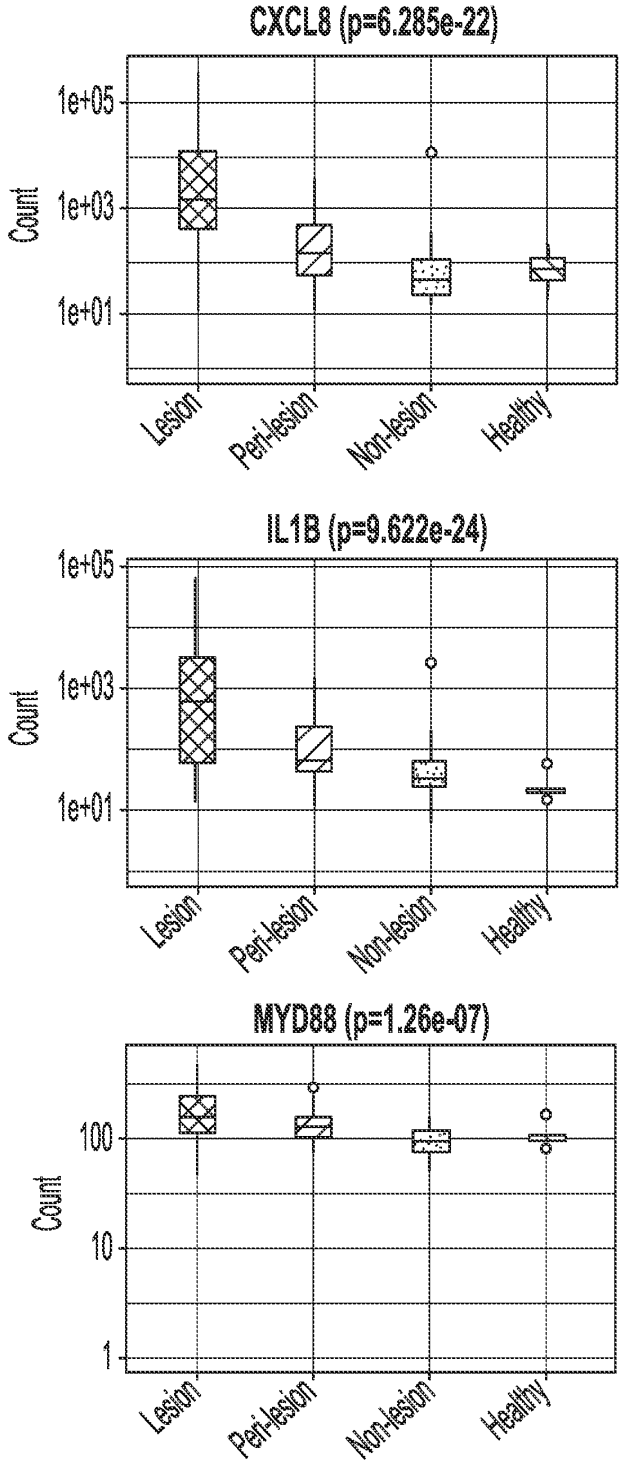


FIG. 16 CONTINUED

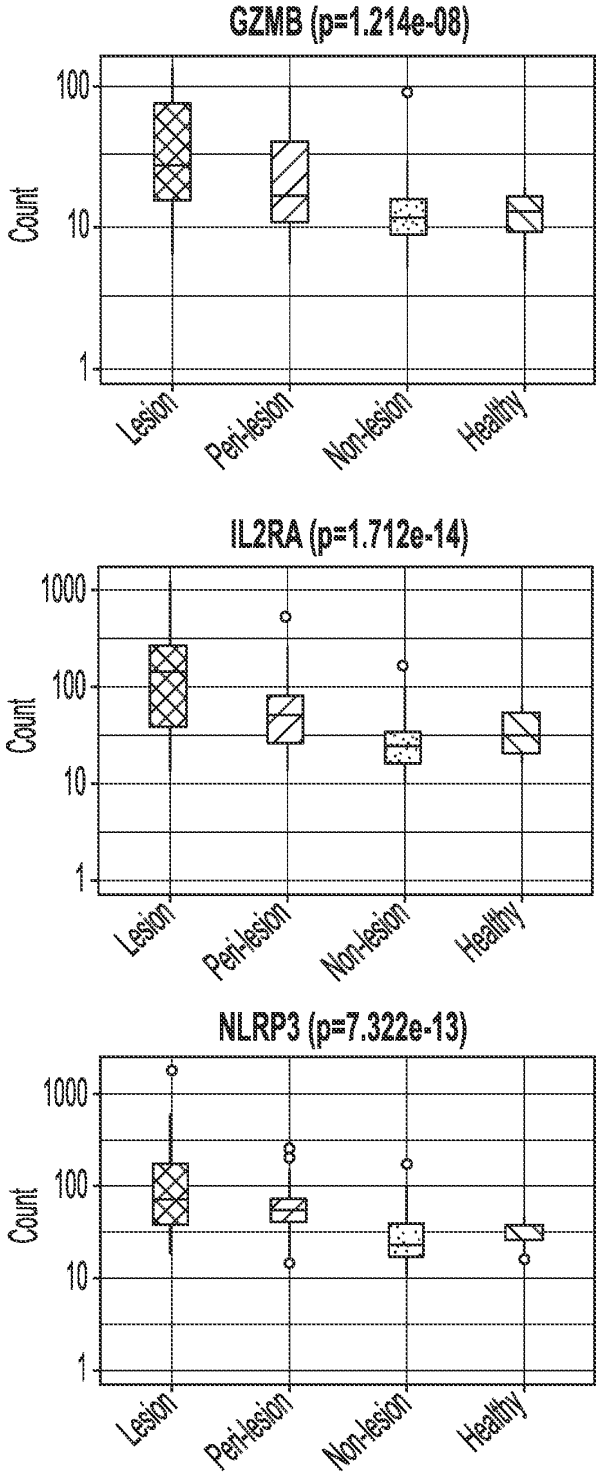


FIG. 16 CONTINUED

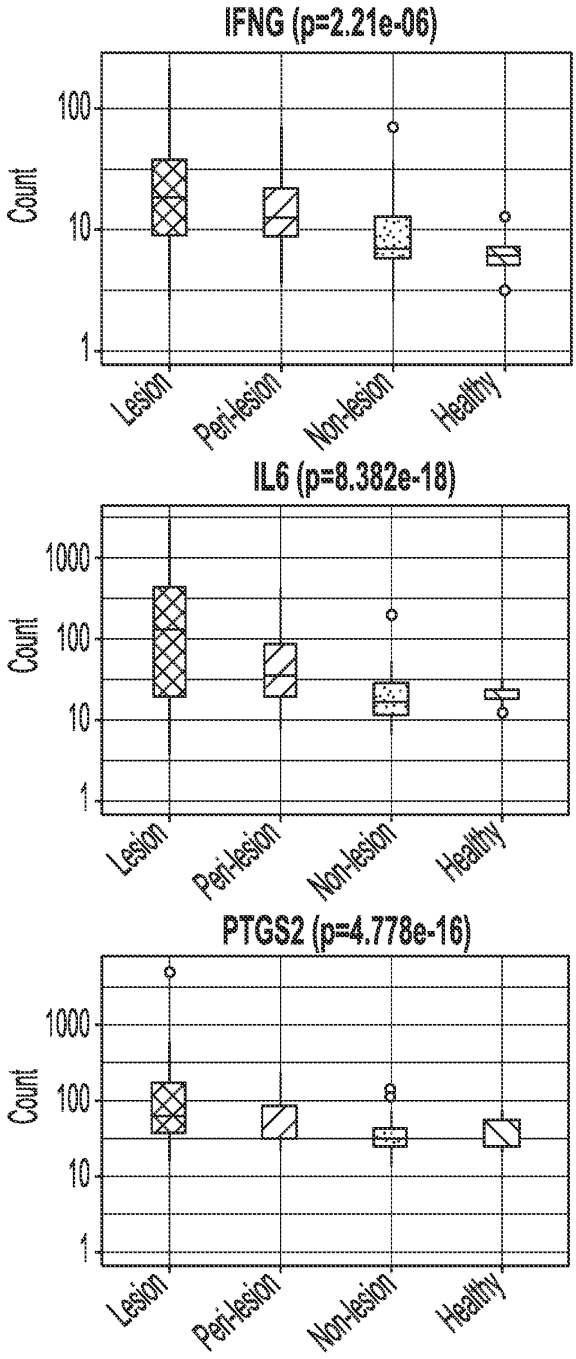


FIG. 16 CONTINUED

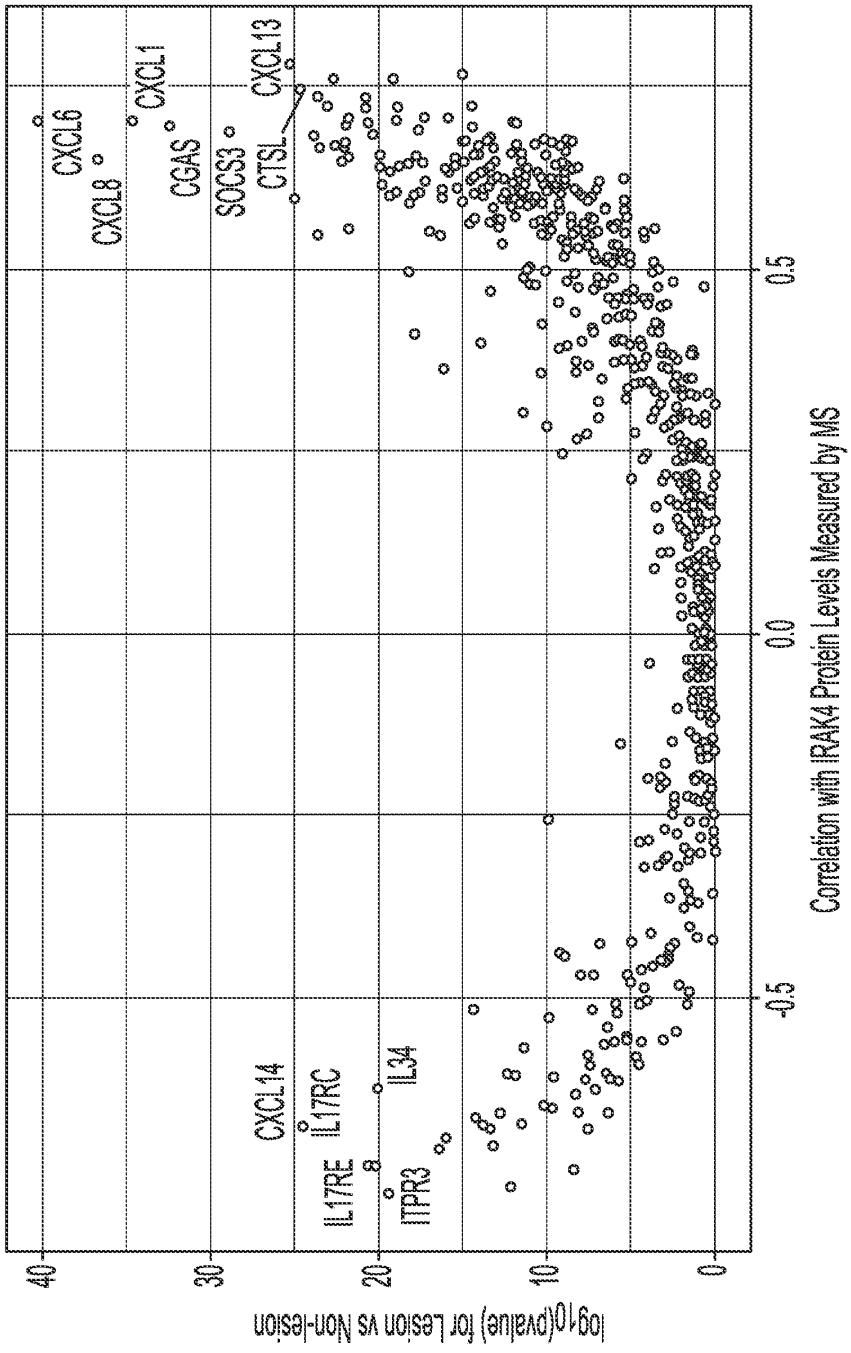

CXCL6
CXCL8
CXCL1
CGAS
SOCS3
CTSL
CXCL13
CXCL14
IL17RC
IL17RE
ITPR3
IL34
40
30
20
10
0
-0.5
0.0
0.5
log10(pvalue) for Lesion vs Non-lesion
Correlation with IRAK4 Protein Levels Measured by MS

FIG. 17

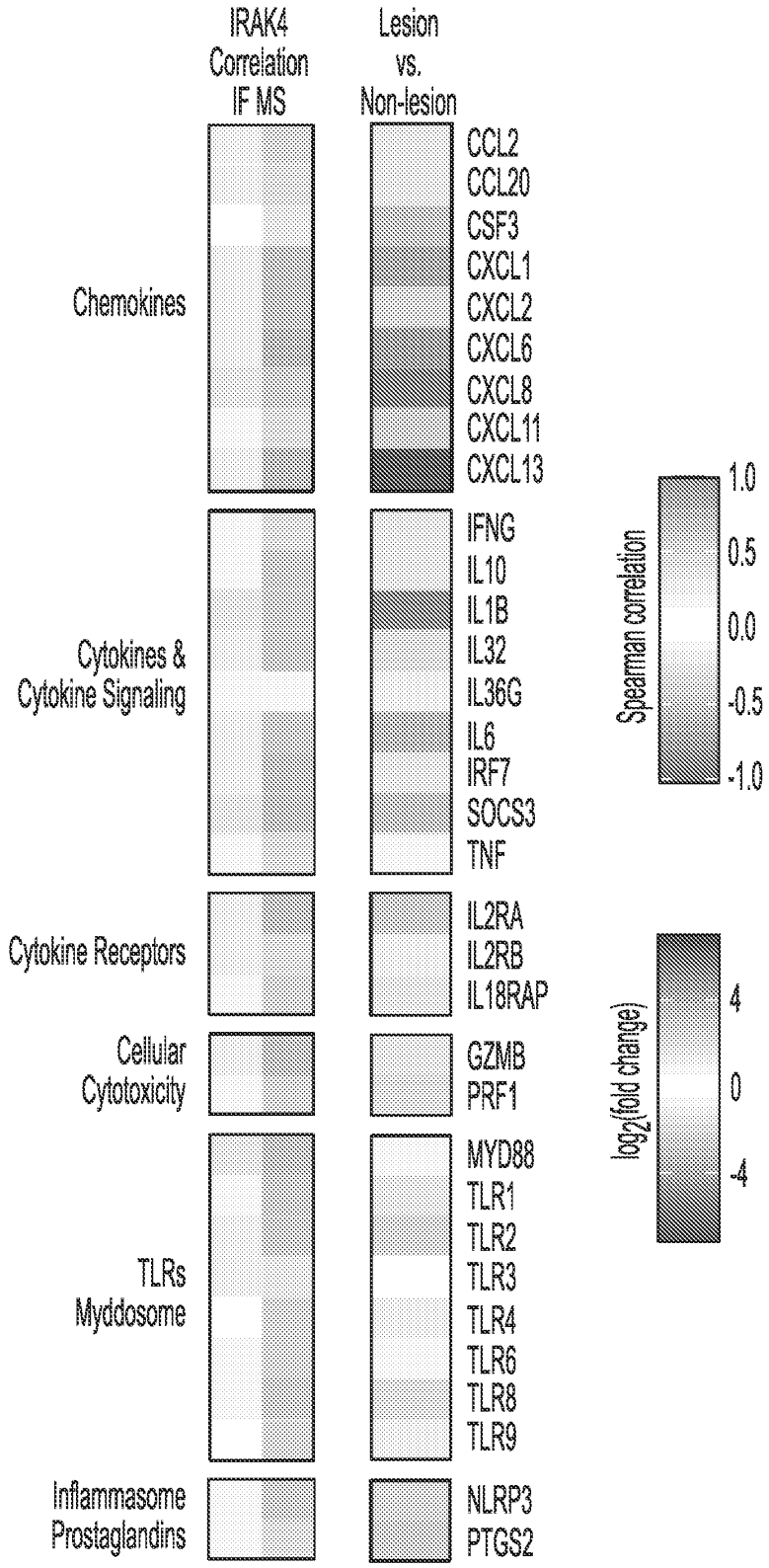


FIG. 17 CONTINUED

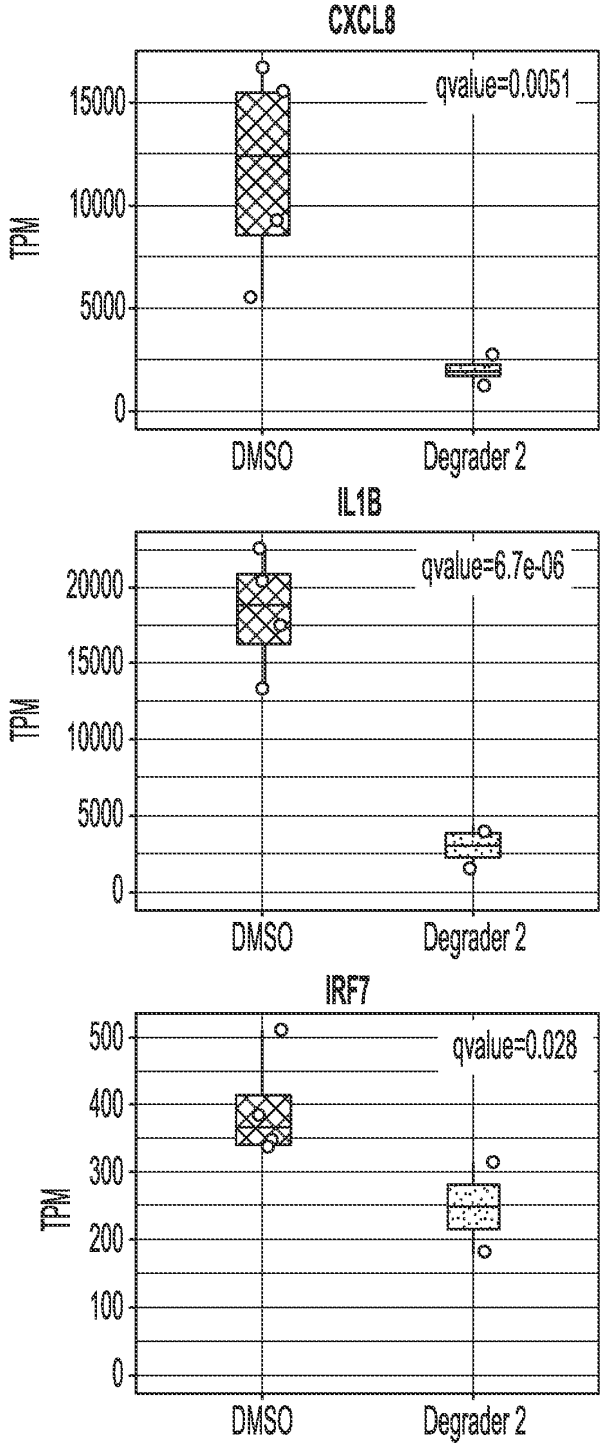

CXCL8
qvalue=0.0051
TPM
15000
10000
5000
0
DMSO
Degrader 2
IL1B
qvalue=6.7e-06
TPM
20000
15000
10000
5000
0
DMSO
Degrader 2
IRF7
qvalue=0.028
TPM
500
400
300
200
100
0
DMSO
Degrader 2

FIG. 18

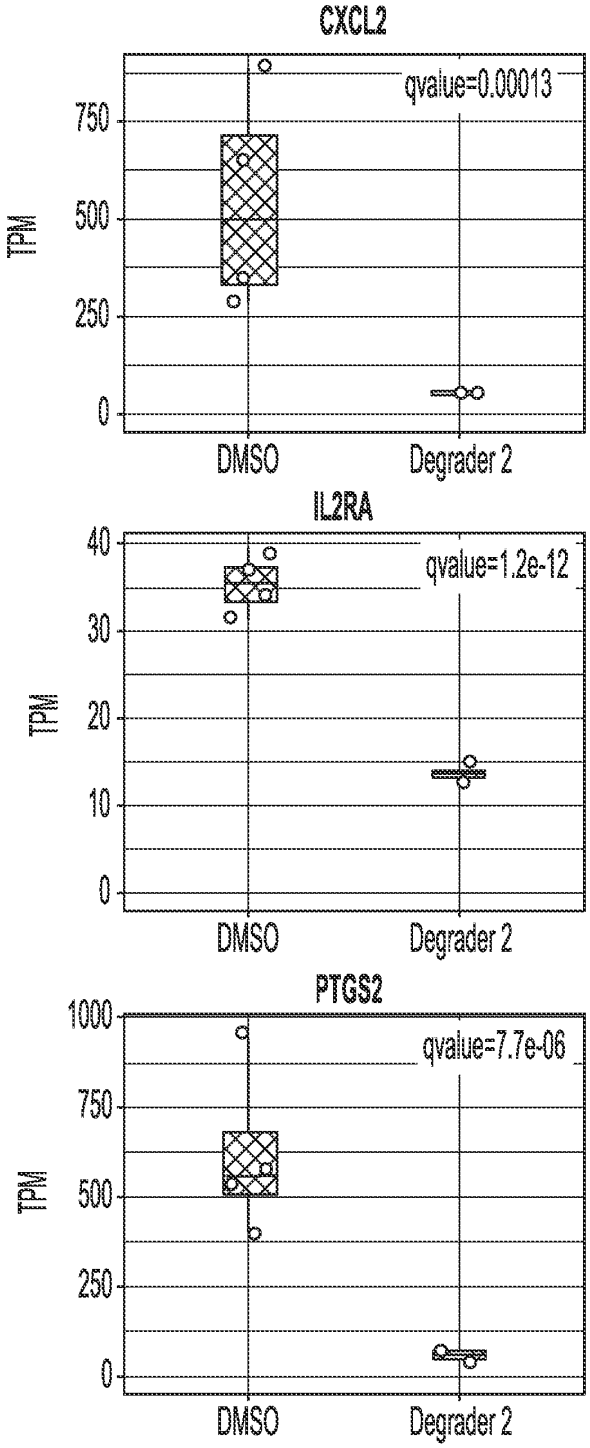


FIG. 18 CONTINUED

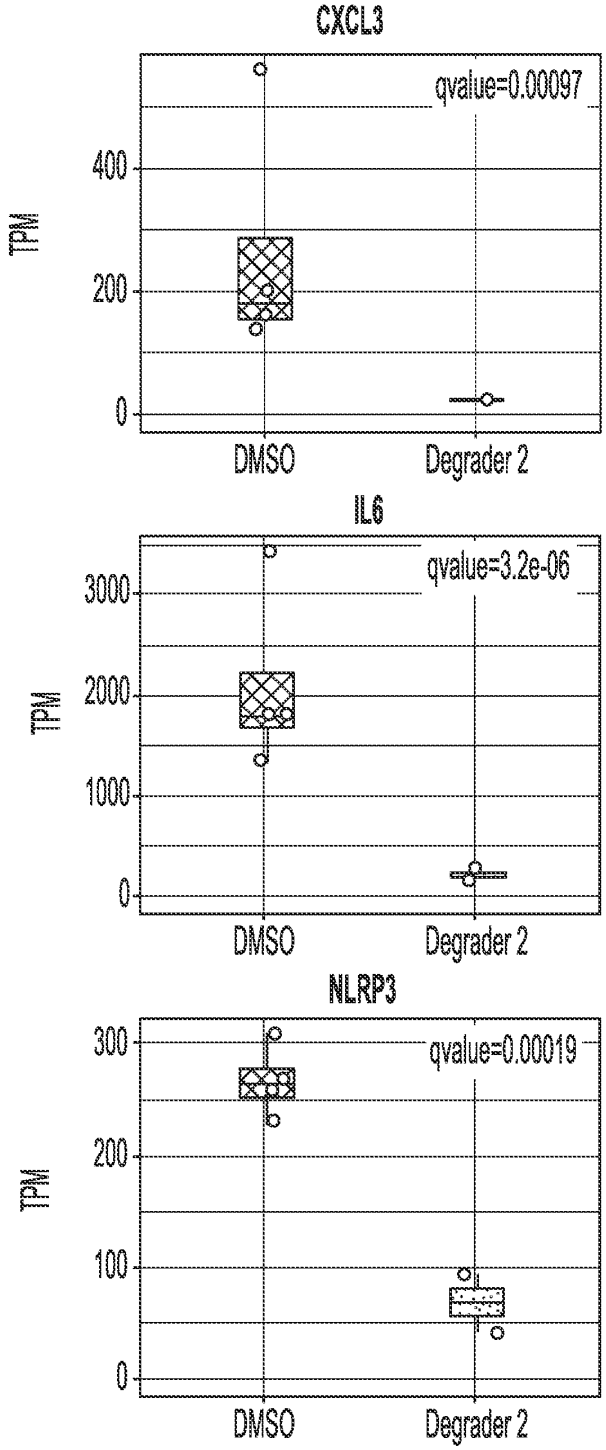


FIG. 18 CONTINUED

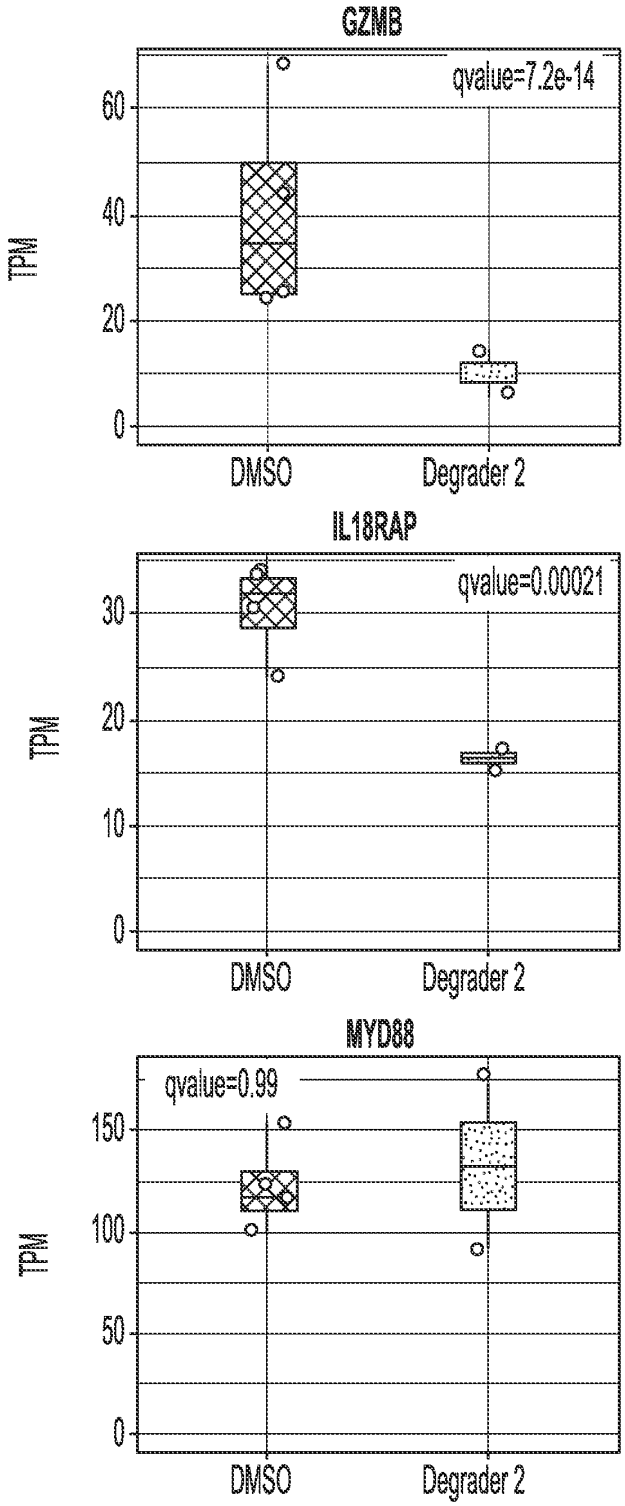


FIG. 18 CONTINUED

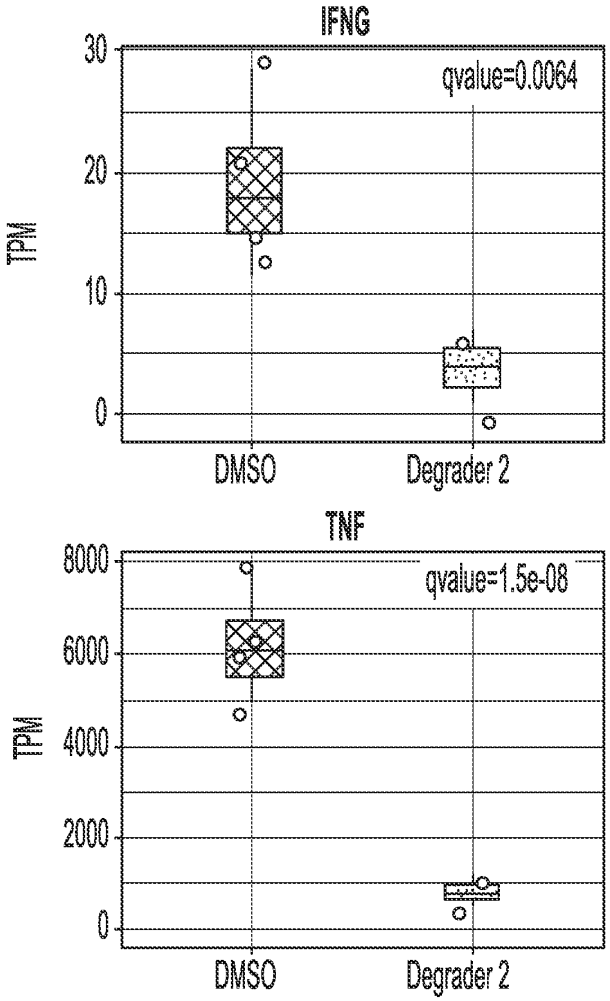


FIG. 18 CONTINUED