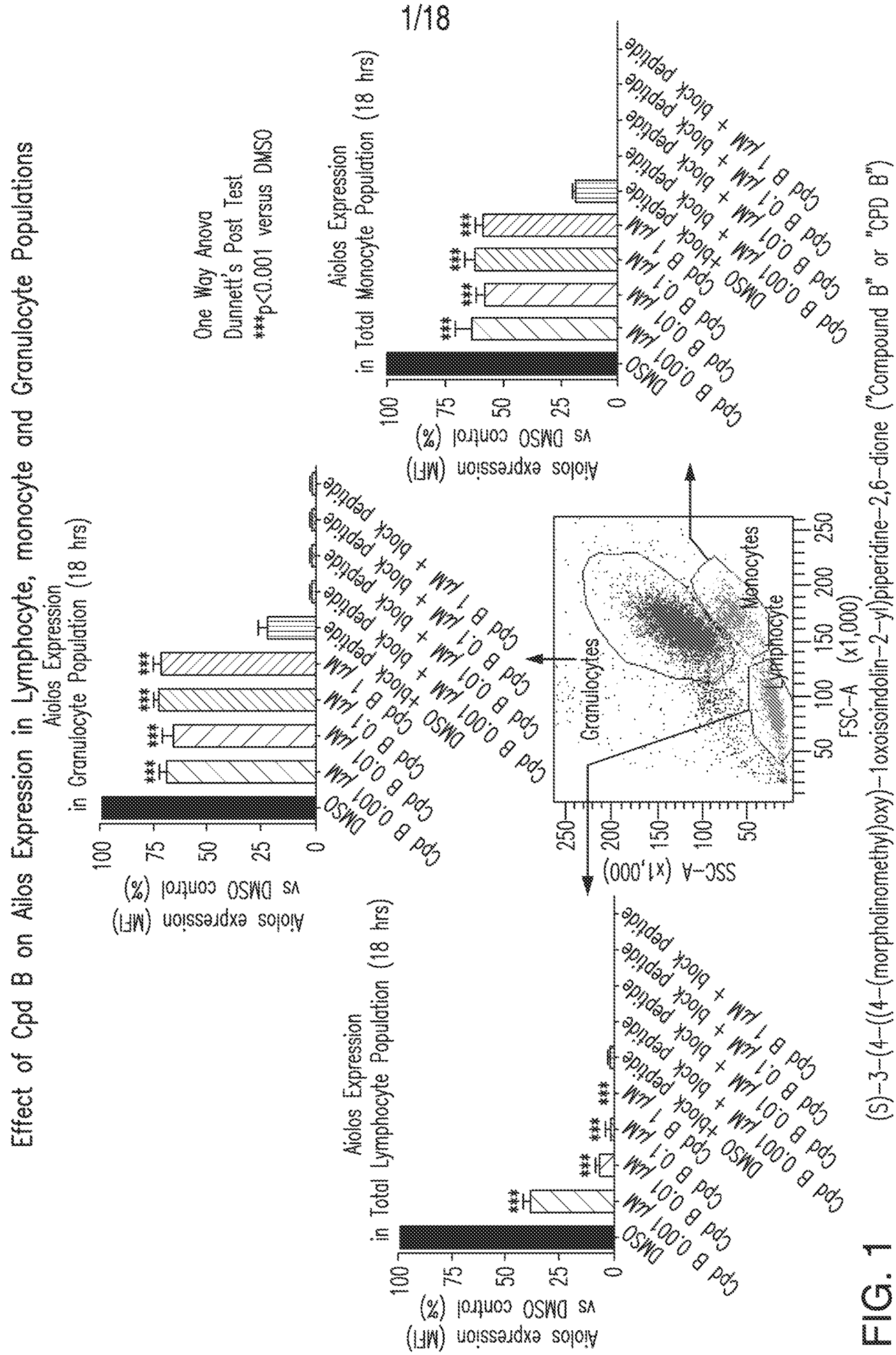

Effect of Cpd B on Aiolos Expression in Lymphocyte, monocyte and Granulocyte Populations
Aiolos Expression
in Granulocyte Population (18 hrs)
Aiolos expression (MFI)
vs DMSO control (%)
One Way Anova
Dunnett's Post Test
***p<0.001 versus DMSO
DMSO
Cpd B 0.001 µM
Cpd B 0.01 µM
Cpd B 0.1 µM
Cpd B 1 µM
DMSO +block peptide
Cpd B 0.001 µM + block peptide
Cpd B 0.01 µM + block peptide
Cpd B 0.1 µM + block peptide
Cpd B 1 µM + block peptide
Granulocytes
Monocytes
Lymphocyte
SSC-A (x1,000)
FSC-A (x1,000)
Aiolos Expression
in Total Monocyte Population (18 hrs)
Aiolos Expression
in Total Lymphocyte Population (18 hrs)
(S)-3-(4-((4-(morpholinomethyl)oxy)-1oxoisoindolin-2-yl)piperidine-2,6-dione ("Compound B" or "CPD B")

FIG. 1

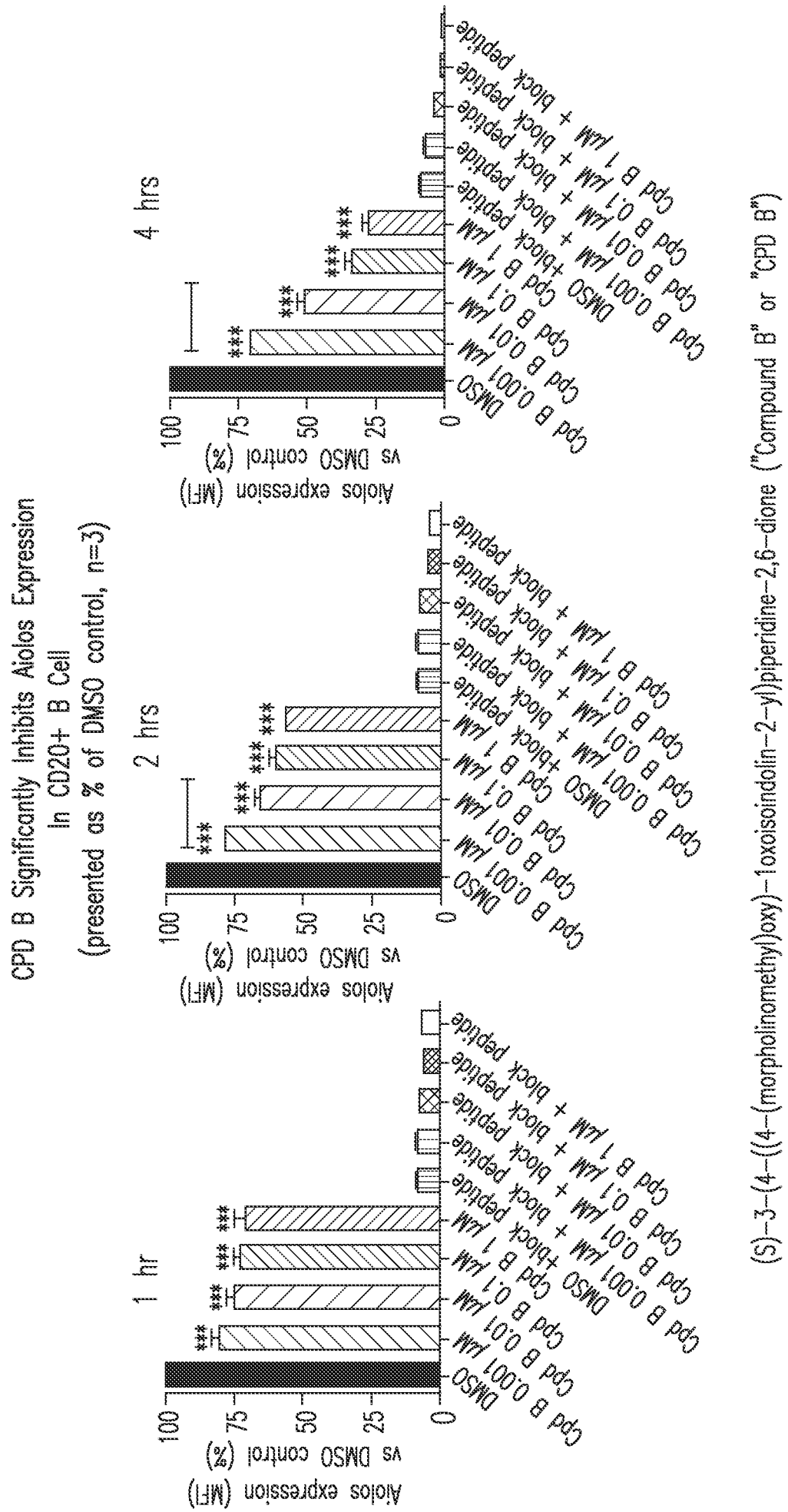


FIG. 2

(S)-3-(4-((4-(morpholinomethyl)oxy)-1oxoisoindolin-2-yl)piperidine-2,6-dione ("Compound B" or "CPD B")

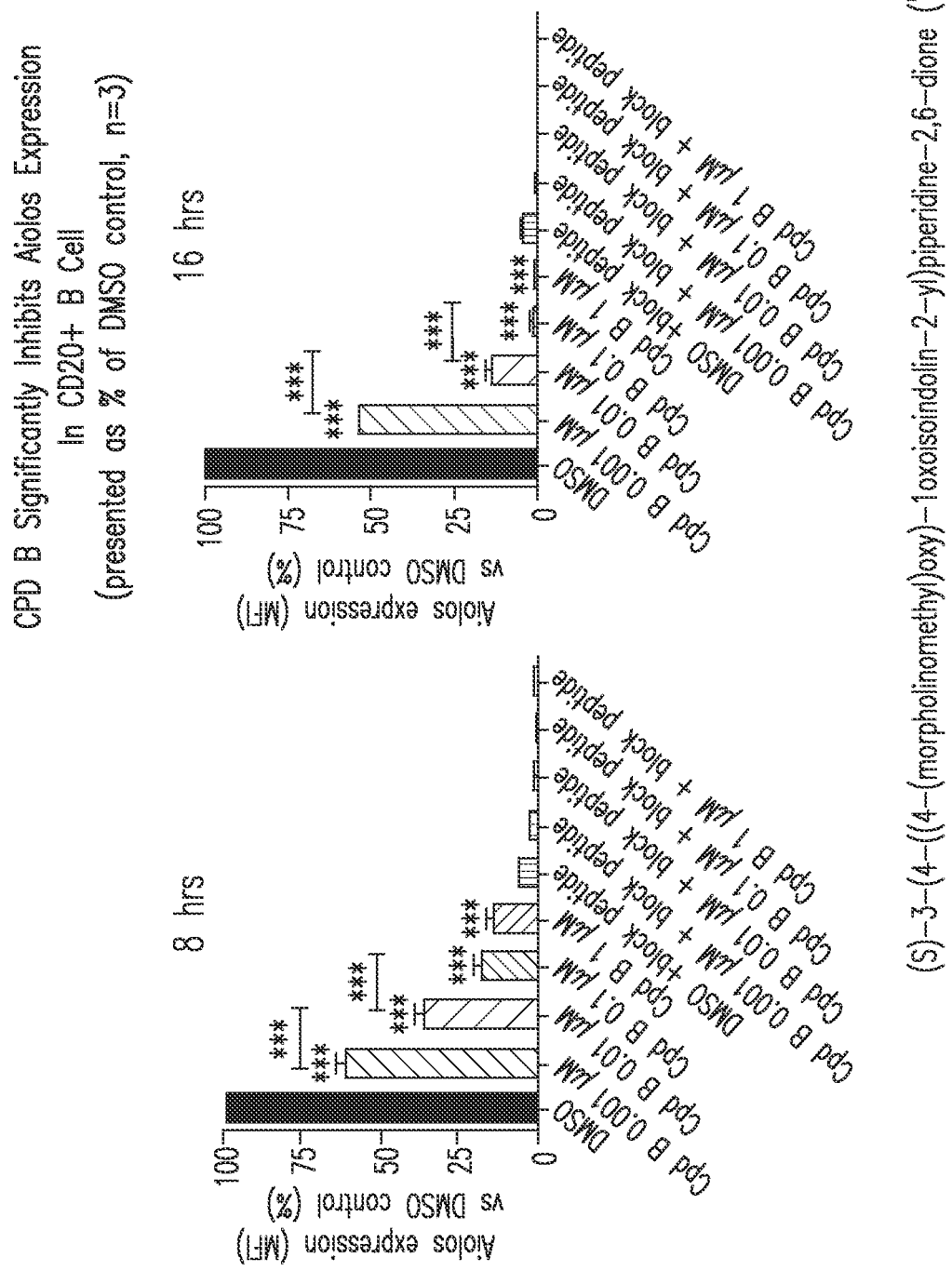


FIG. 2 continued

(S)-3-(4-((4-(morpholinomethyl)oxy)-1oxoisoindolin-2-yl)piperidine-2,6-dione ("Compound B" or "CPD B")

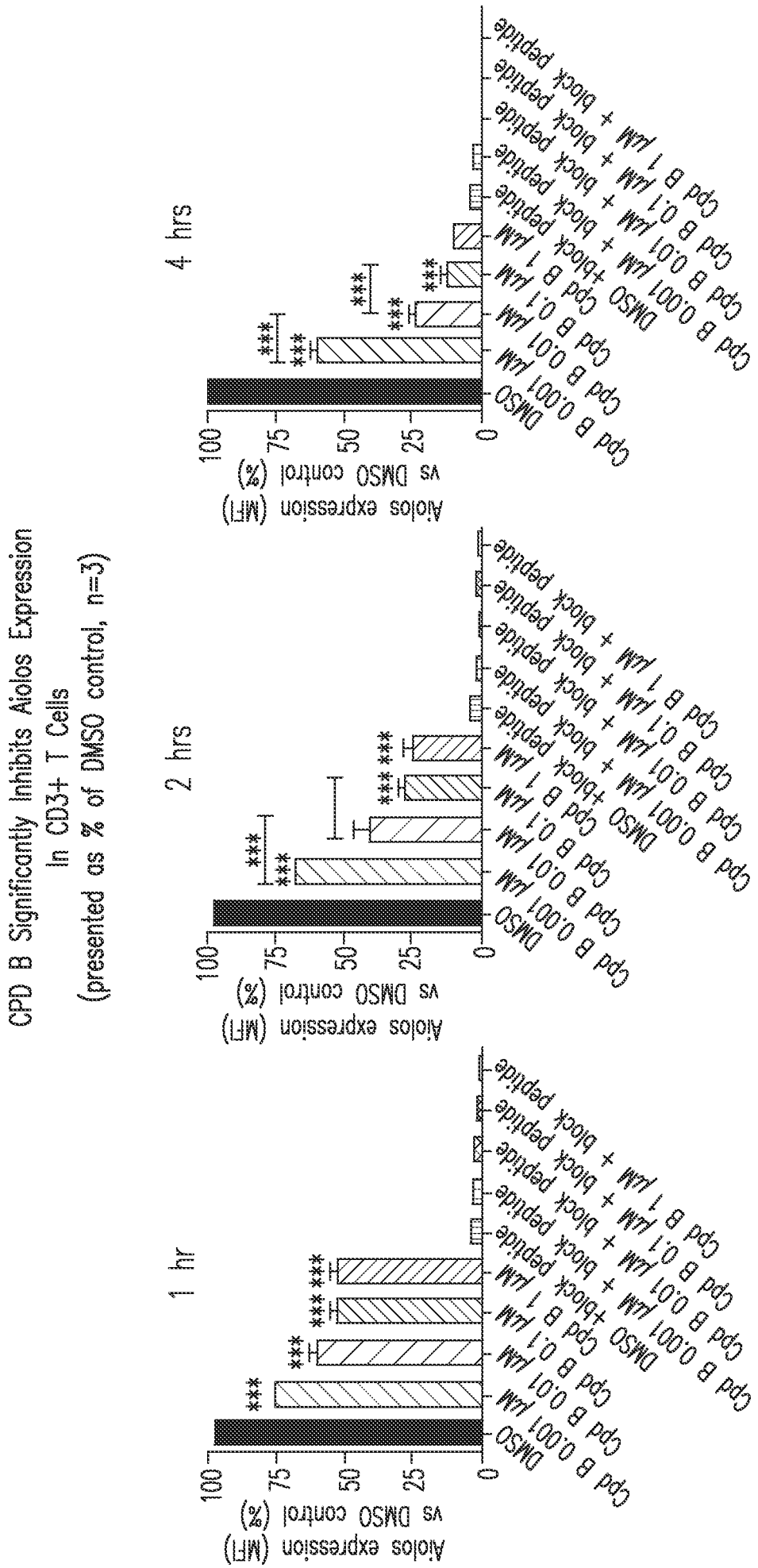


FIG. 3

(S)-3-(4-((4-(morpholinomethyl)oxy)-1oxoisoindolin-2-yl)piperidine-2,6-dione ("Compound B" or "CPD B")

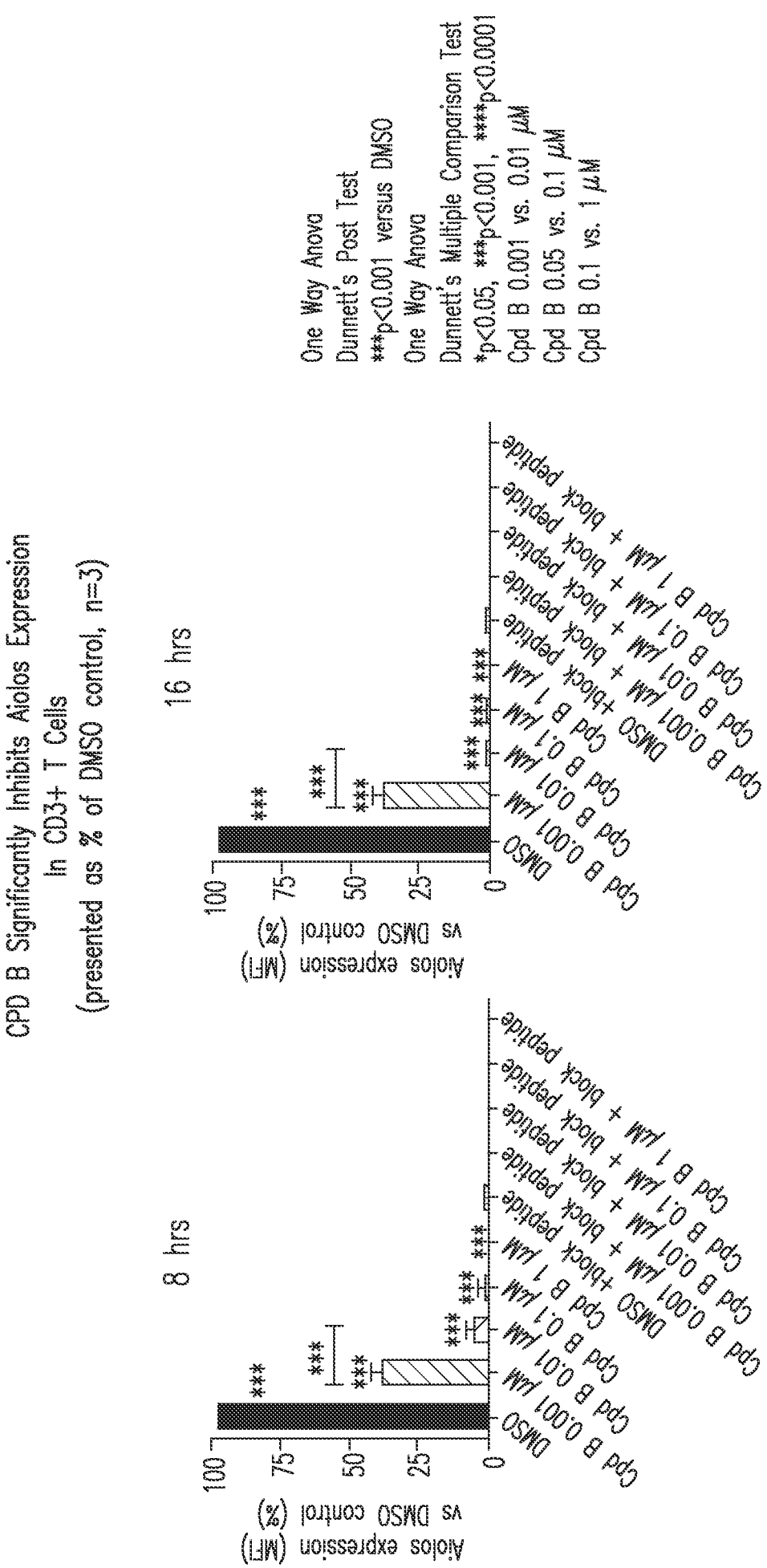


FIG. 3 Continued

(S)-3-(4-((4-(morpholinomethyl)oxy)-1oxoisoindolin-2-yl)piperidine-2,6-dione ("Compound B" or "CPD B")

Aiolos westren blotting in human whole blood
Donor1
Donor1
Donor1
DMSO
Pom
CPD A
CPD B
U2932
hWBC from BioReclamation: Lot# BRH532756
hWBC from BioReclamation: Lot# BRH532757
hWBC from BioReclamation: Lot# BRH532758
Aiolos
Actin
75 kDa
50 kDa
37 kDa
50 kDa
37 kDa
whole blood samples were treated with compounds at 250 nM for 18 h and then subjected to PBMC preparation and IB
3-(5-amino-2-methyl-4-oxo-4H-quinazolin-3-yl)-piperidine-2,6-dione ("Compound A" or CPD A")
(S)-3-(4-((4-(morpholinomethyl)oxy)-1oxoisoindolin-2-yl)piperidine-2,6-dione ("Compound B" or "CPD B")
Pomalidomide ("Pom")

FIG. 4

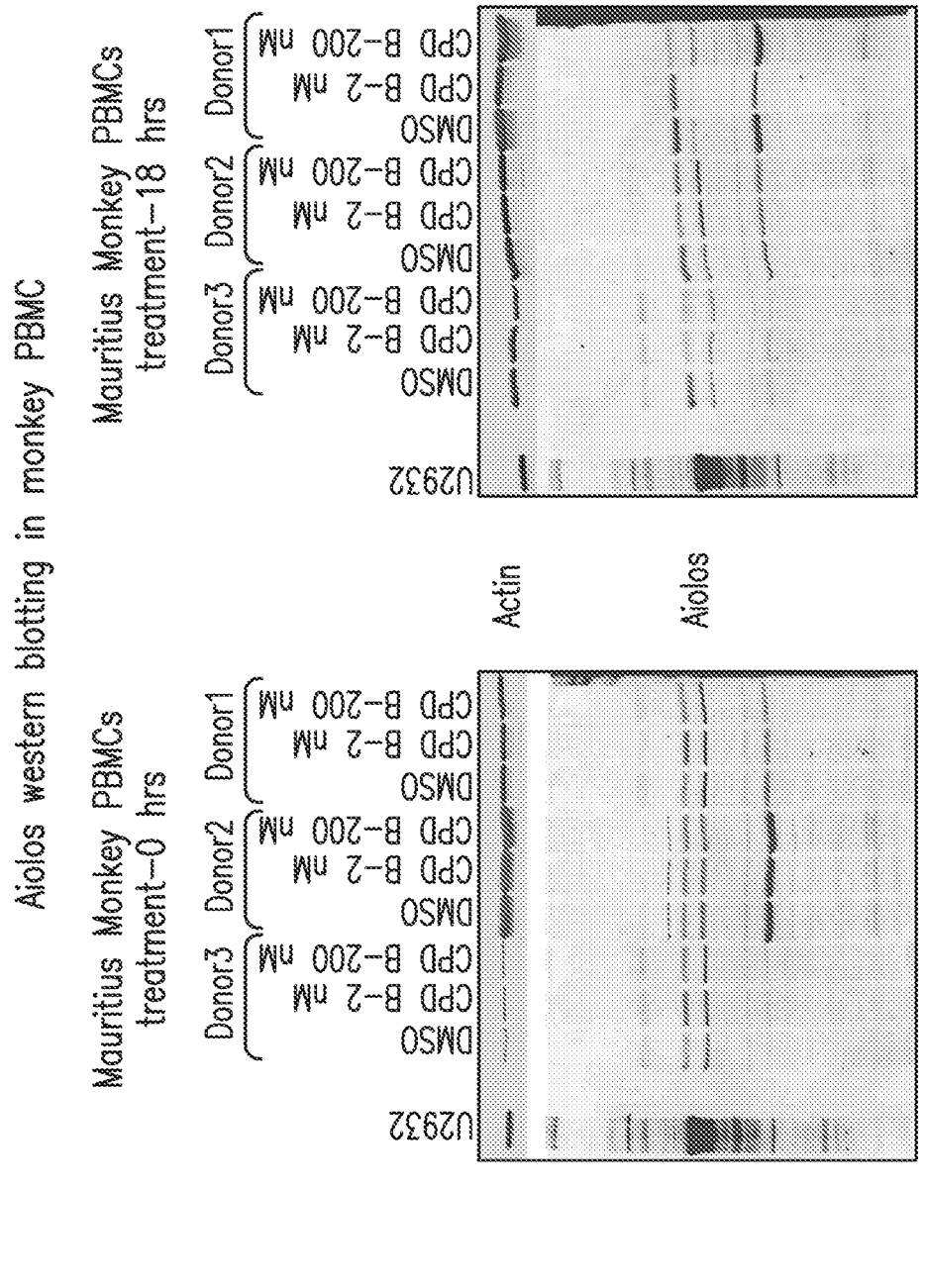

Aiolos western blotting in monkey PBMC
Mauritius Monkey PBMCs treatment-0 hrs
Mauritius Monkey PBMCs treatment-18 hrs
Donor1
Donor2
Donor3
DMSO
CPD B-2 nM
CPD B-200 nM
U2932
Actin
Aiolos
~25 μg proteins per lane, Aiolos Ab 1:200 dilution
(S)-3-(4-((4-(morpholinomethyl)benzyl)oxy)-1-oxoisoindolin-2-yl)piperidine-2,6-dione ("Compound B" or "CPD B")

FIG. 5

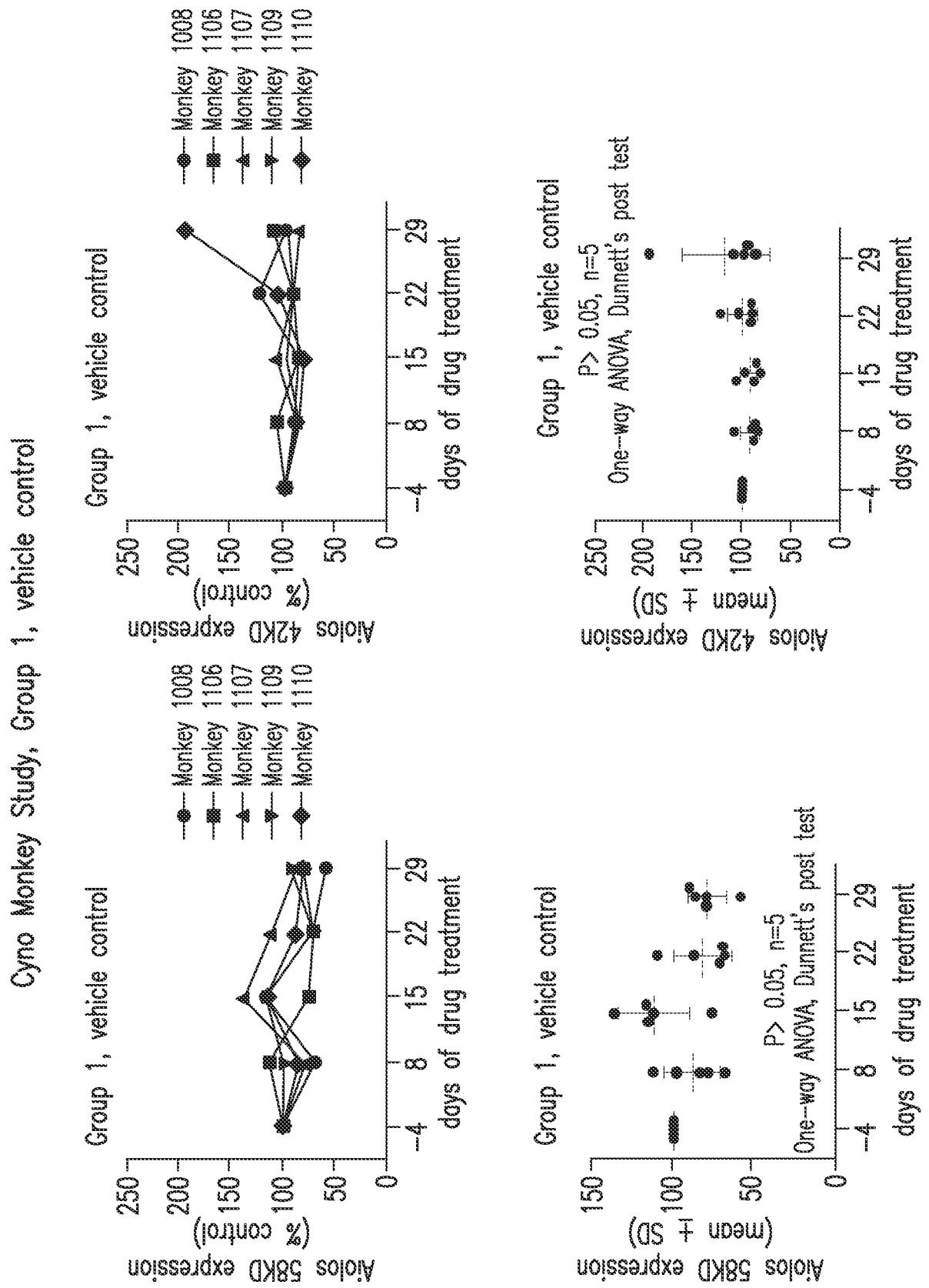

Cyno Monkey Study, Group 1, vehicle control
Group 1, vehicle control
Aiolos 58KD expression
(% control)
days of drug treatment
Monkey 1008
Monkey 1106
Monkey 1107
Monkey 1109
Monkey 1110
Group 1, vehicle control
Aiolos 42KD expression
(% control)
days of drug treatment
Monkey 1008
Monkey 1106
Monkey 1107
Monkey 1109
Monkey 1110
Group 1, vehicle control
P> 0.05, n=5
One-way ANOVA, Dunnett's post test
Aiolos 42KD expression
(mean ± SD)
days of drug treatment
Group 1, vehicle control
P> 0.05, n=5
One-way ANOVA, Dunnett's post test
Aiolos 58KD expression
(mean ± SD)
days of drug treatment

FIG. 6

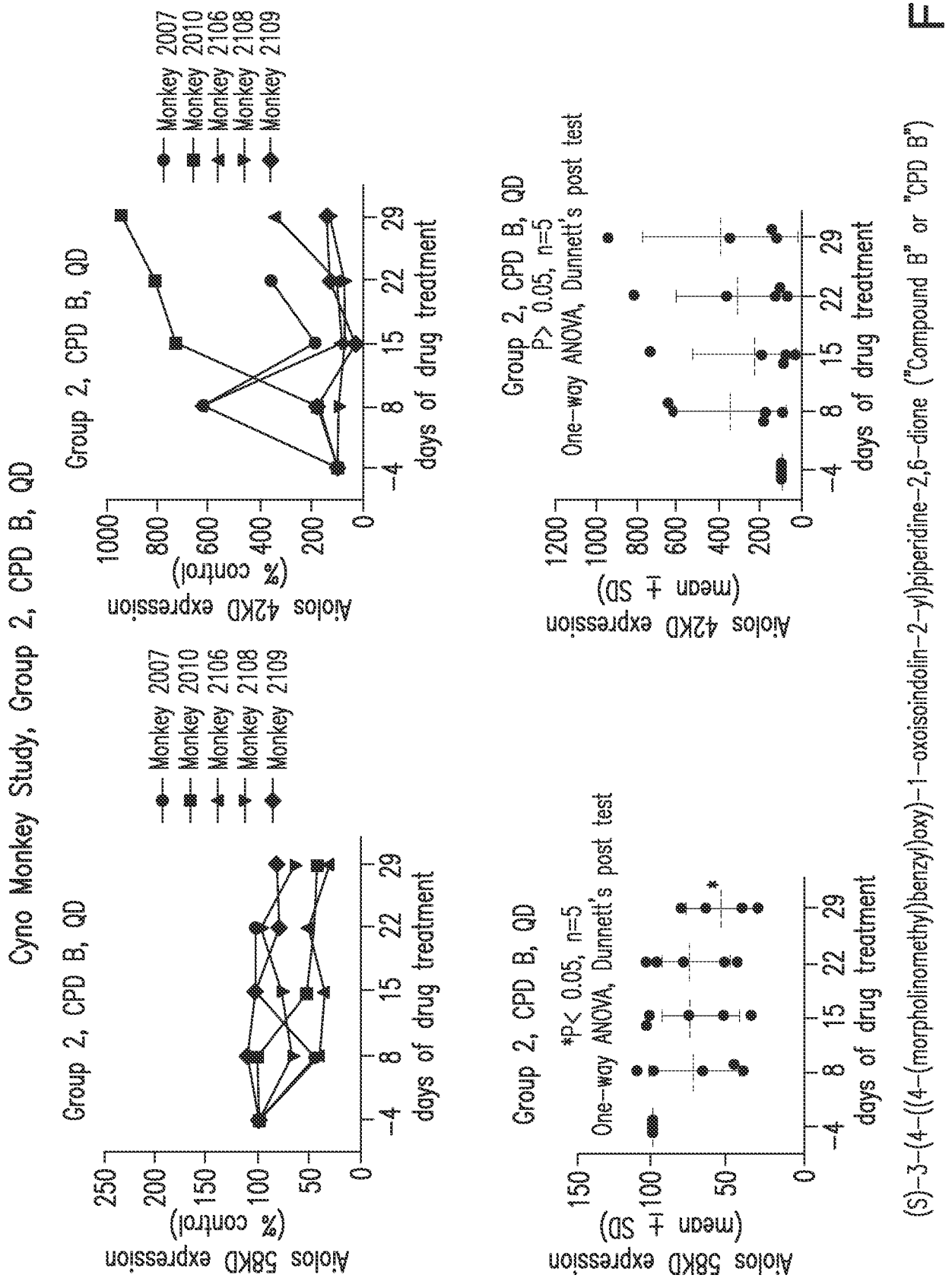


FIG. 7

(S)-3-(4-((4-(morpholinomethyl)benzyl)oxy)-1-oxoisoindolin-2-yl)piperidine-2,6-dione ("Compound B" or "CPD B")

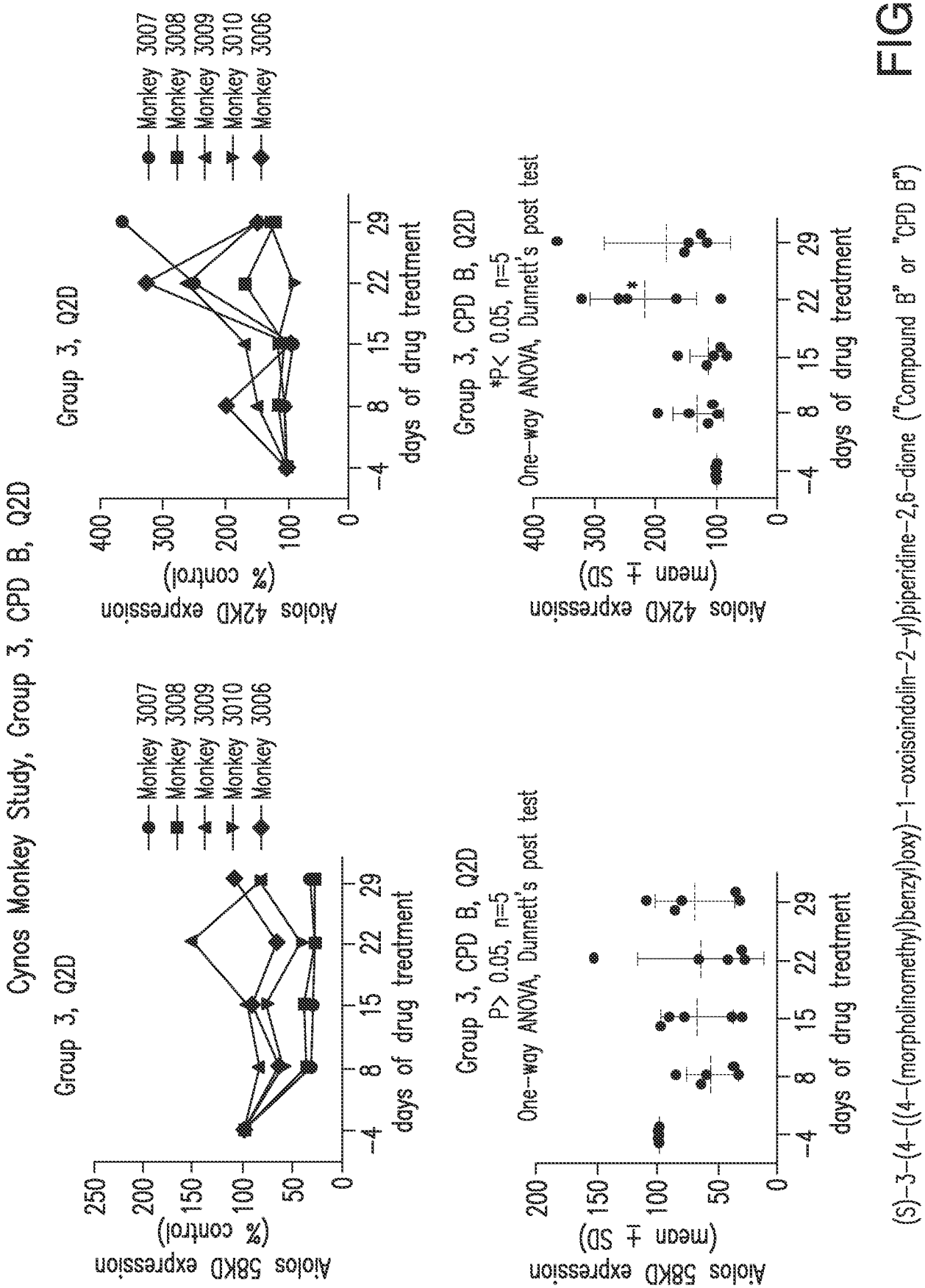


FIG. 8

(S)-3-(4-((4-(morpholinomethyl)benzyl)oxy)-1-oxoisoindolin-2-yl)piperidine-2,6-dione ("Compound B" or "CPD B")

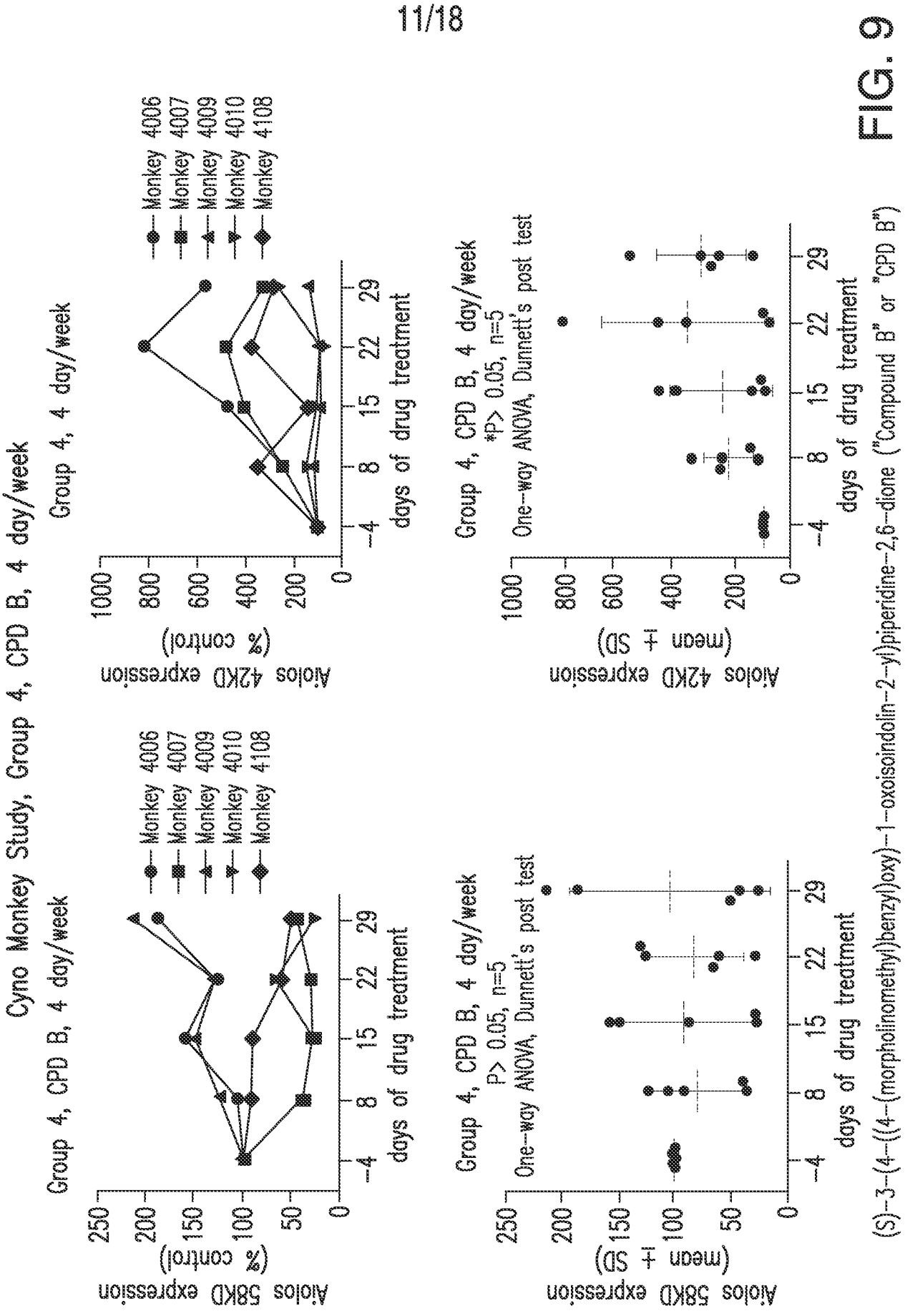


FIG. 9

(S)-3-(4-((4-(morpholinomethyl)benzyl)oxy)-1-oxoisoindolin-2-yl)piperidine-2,6-dione ("Compound B" or "CPD B")

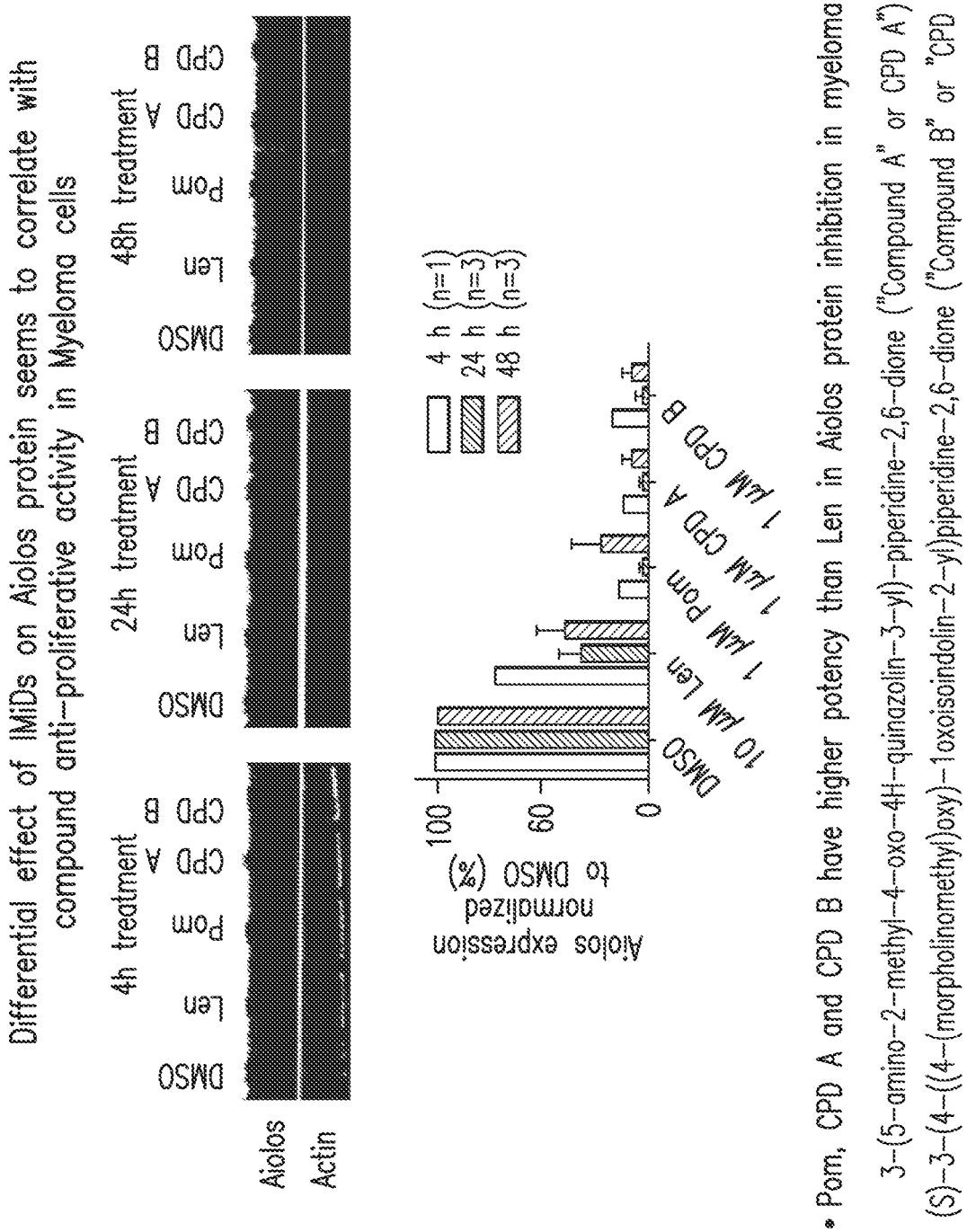


FIG. 10

* Pom, CPD A and CPD B have higher potency than Len in Aiolos protein inhibition in myeloma cells

3-(5-amino-2-methyl-4-oxo-4H-quinazolin-3-yl)-piperidine-2,6-dione ("Compound A" or CPD A")

(S)-3-(4-((4-(morpholinomethyl)oxy)-1oxoisoindolin-2-yl)piperidine-2,6-dione ("Compound B" or "CPD B")

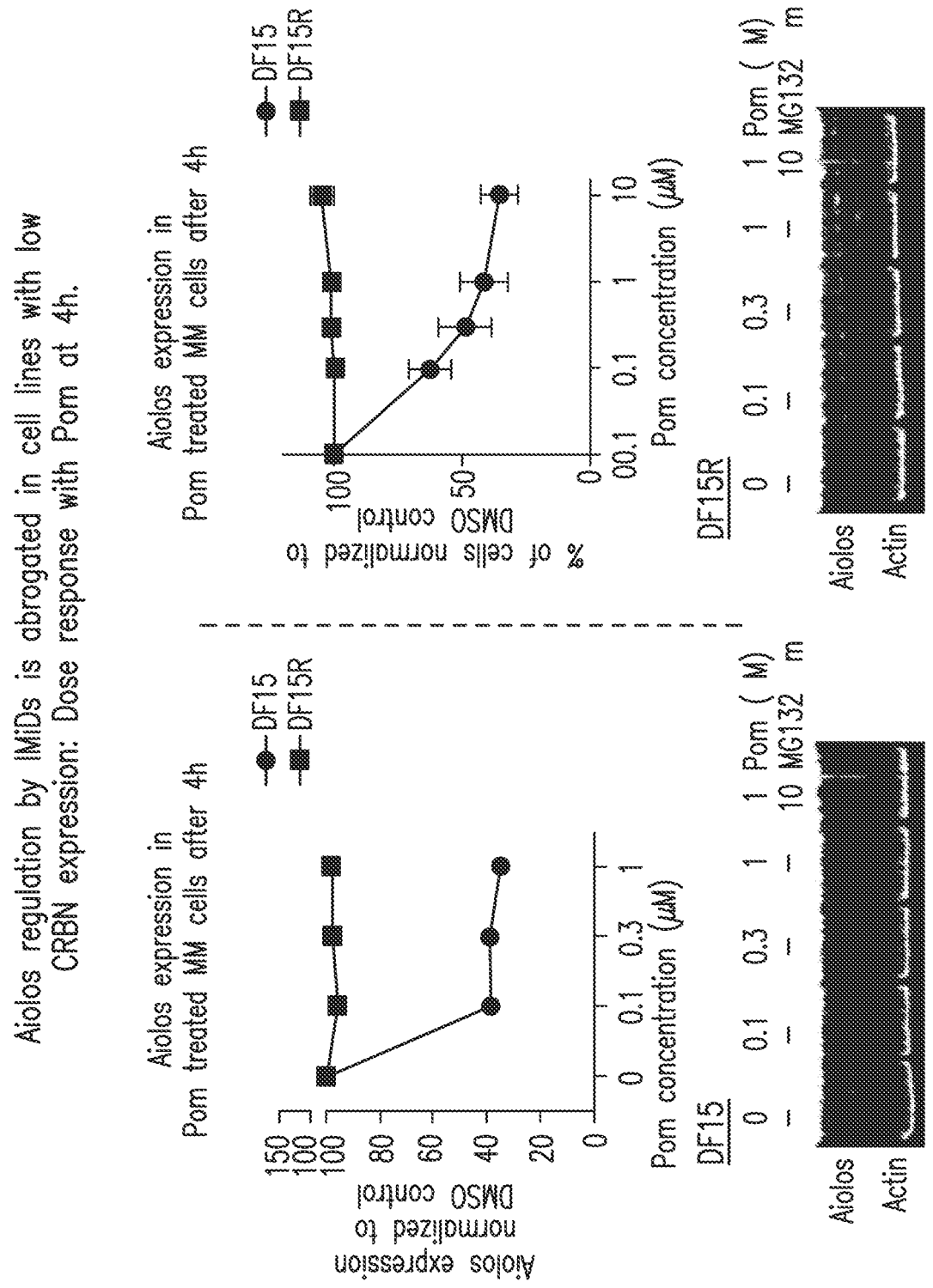

Aiolos regulation by IMiDs is abrogated in cell lines with low CRBN expression: Dose response with Pom at 4h.
Aiolos expression in Pom treated MM cells after 4h
DF15
DF15R
Aiolos expression normalized to DMSO control
Pom concentration (μM)
DF15
DF15R
0 0.1 0.3 1 1 Pom (M)
– – – – 10 MG132 m
Aiolos
Actin
Aiolos expression in Pom treated MM cells after 4h
DF15
DF15R
% of cells normalized to DMSO control
Pom concentration (μM)
DF15R
0 0.1 0.3 1 1 Pom (M)
– – – – 10 MG132 m
Aiolos
Actin
Pomalidomide ("Pom")

FIG. 11

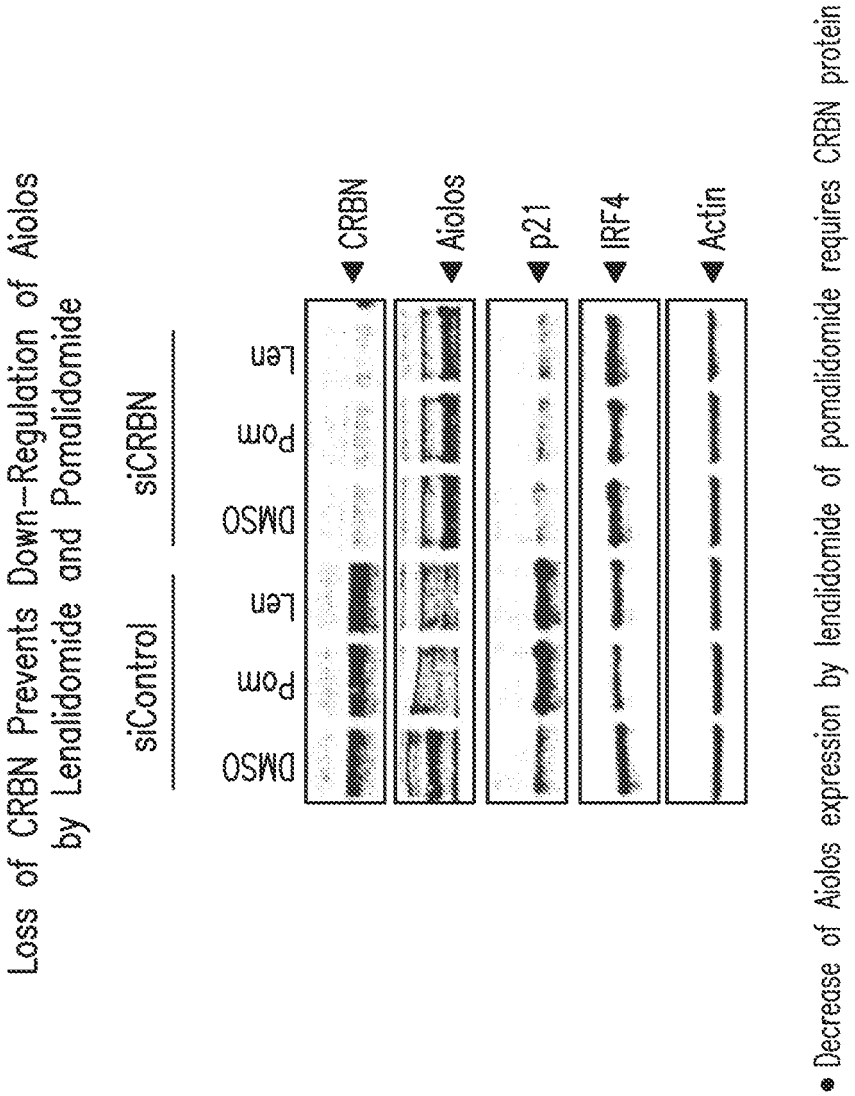

Loss of CRBN Prevents Down-Regulation of Aiolos by Lenalidomide and Pomalidomide
siControl
siCRBN
DMSO
Pom
Len
DMSO
Pom
Len
CRBN
Aiolos
p21
IRF4
Actin
• Decrease of Aiolos expression by lenalidomide of pomalidomide requires CRBN protein

FIG. 12

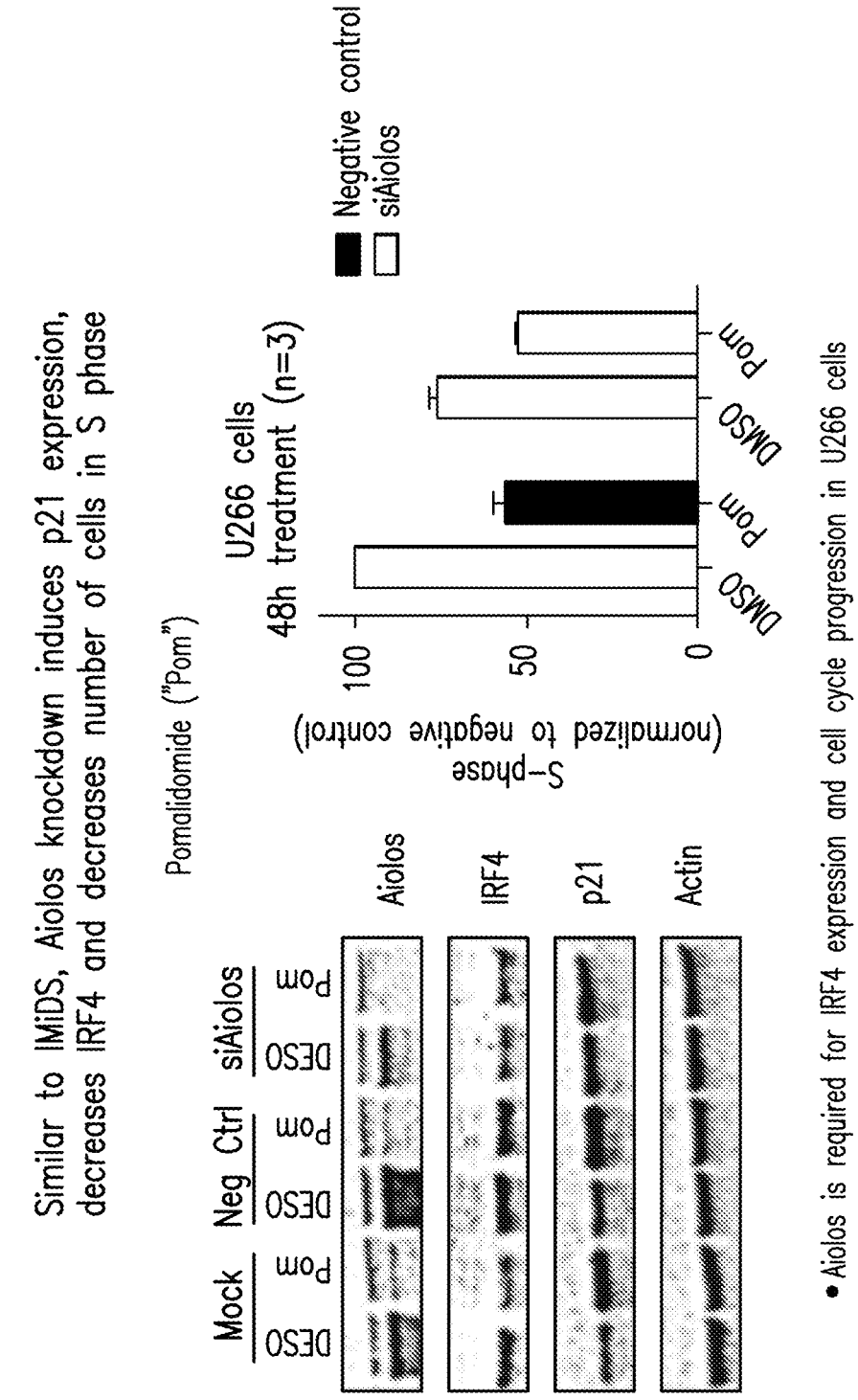

Similar to IMiDS, Aiolos knockdown induces p21 expression, decreases IRF4 and decreases number of cells in S phase
Pomalidomide ("Pom")
Mock
Neg Ctrl
siAiolos
DESO
Pom
Aiolos
IRF4
p21
Actin
U266 cells
48h treatment (n=3)
Negative control
siAiolos
S-phase
(normalized to negative control)
100
50
0
DMSO
Pom
• Aiolos is required for IRF4 expression and cell cycle progression in U266 cells

FIG. 13

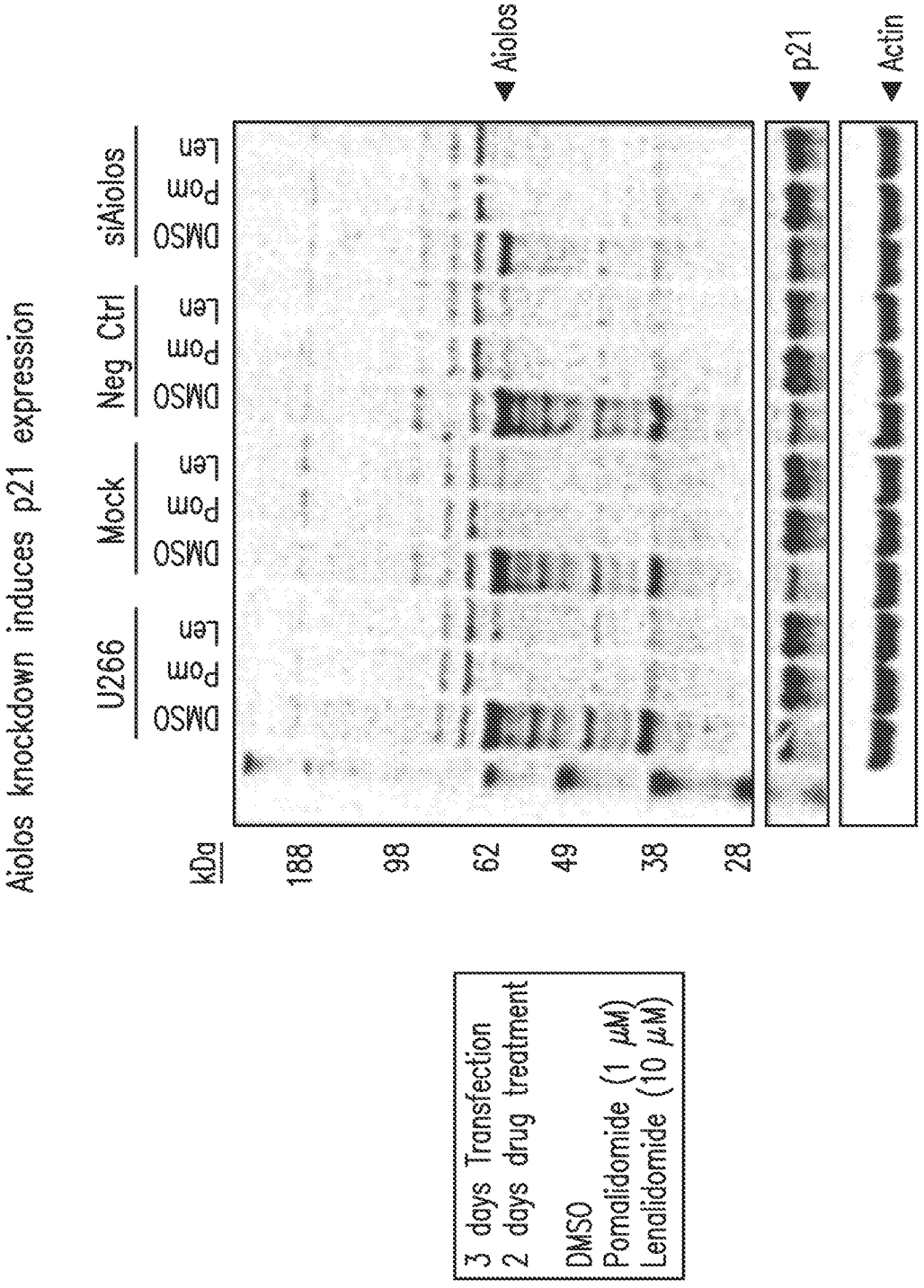

Aiolos knockdown induces p21 expression
U266
Mock
Neg Ctrl
siAiolos
DMSO
Pom
Len
kDa
188
98
62
49
38
28
Aiolos
p21
Actin
3 days Transfection
2 days drug treatment
DMSO
Pomalidomide (1 μM)
Lenalidomide (10 μM)

FIG. 14

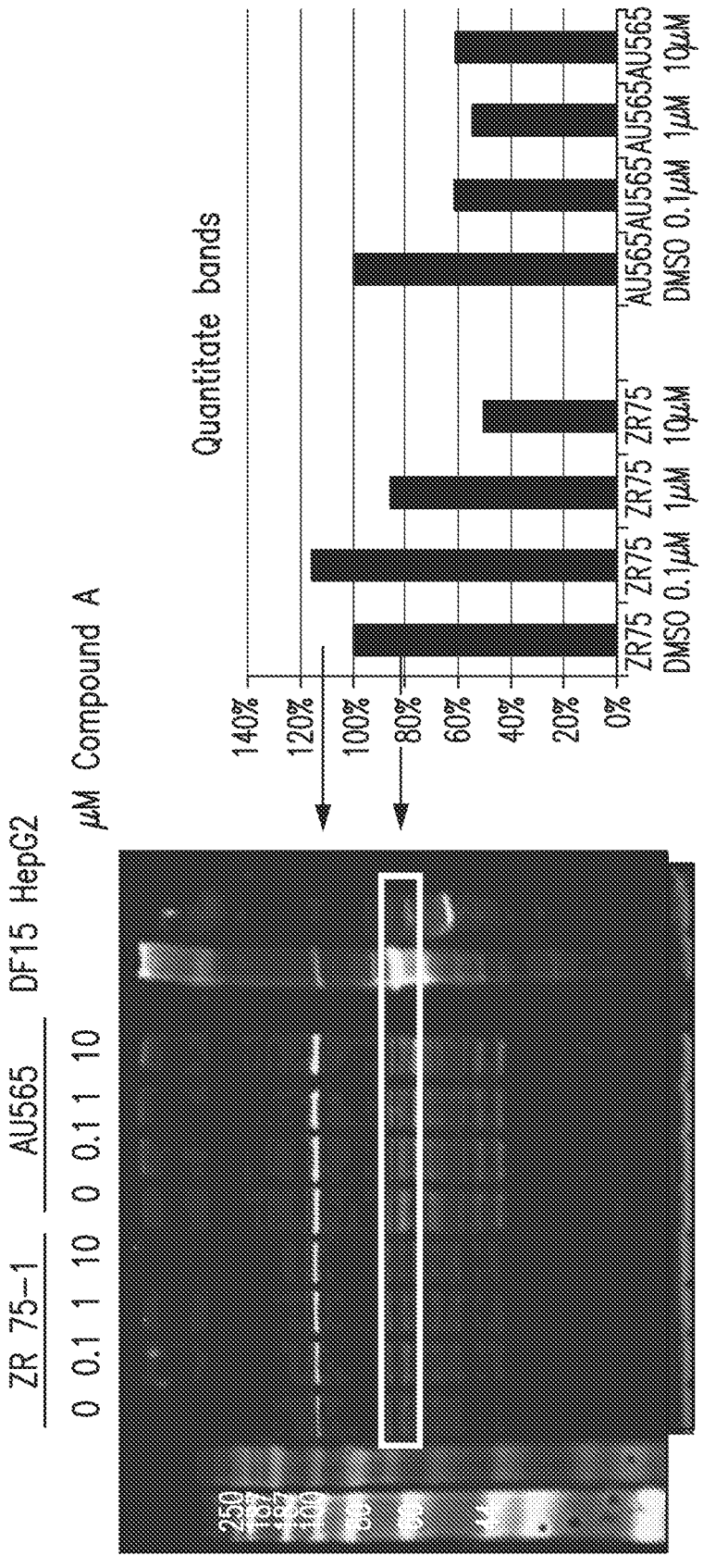
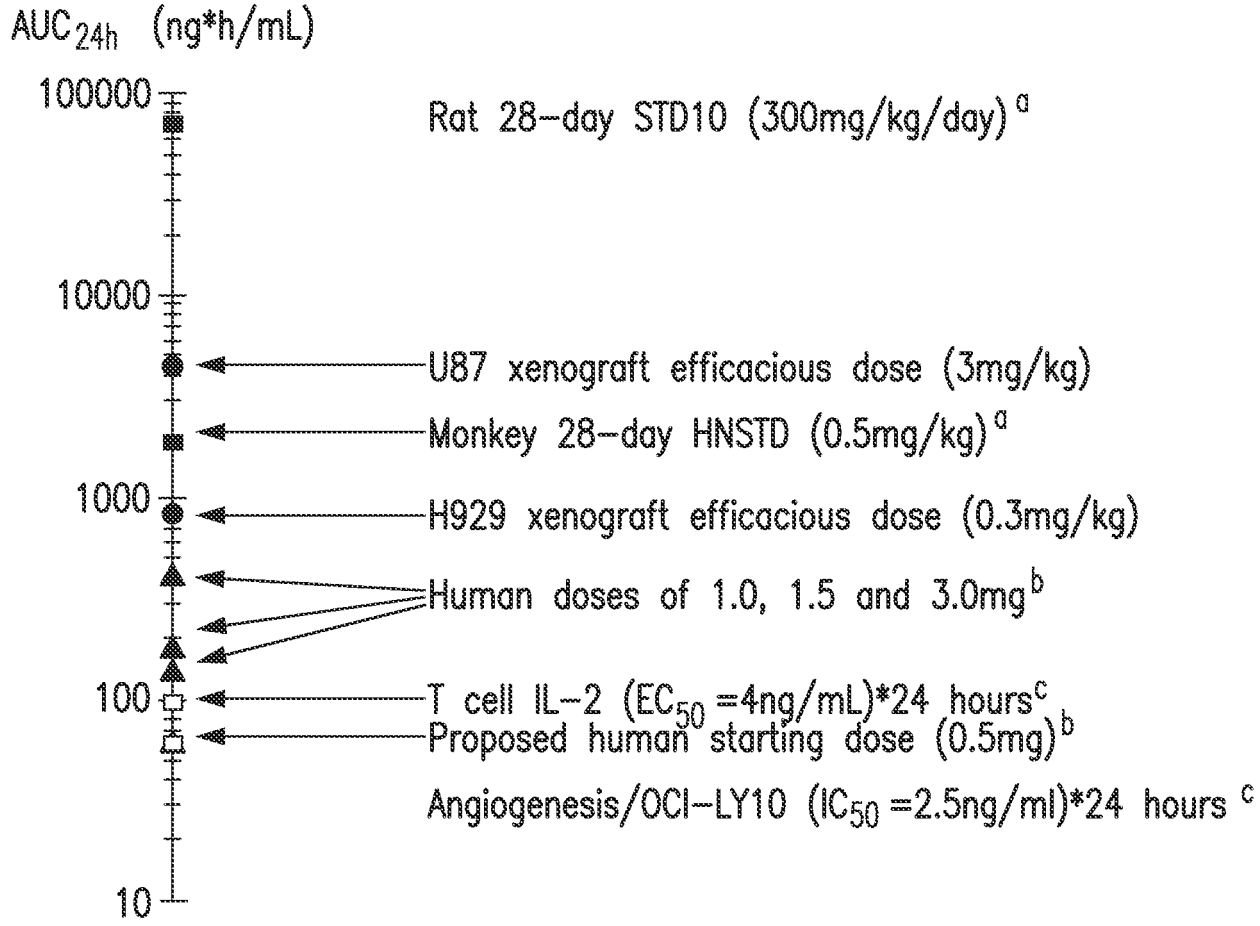

ZR 75-1
0 0.1 1 10
AU565
0 0.1 1 10
DF15 HepG2
μM Compound A
250
150
100
75
50
37
25
20
15
Quantitate bands
140%
120%
100%
80%
60%
40%
20%
0%
ZR75 DMSO
ZR75 0.1μM
ZR75 1μM
ZR75 10μM
AU565 DMSO
AU565 0.1μM
AU565 1μM
AU565 10μM

FIG. 15



(AUC 24hr)=area under the curve from 0 to 34 hr; HNSTD=highest non-serverely toxic dose; HED=human equivalent dose; STD10=severely toxic dose in 10% of the animal.

[a] Day 23 (monkey) or Day 28 (rat) AUG$_{24hr}$ values from 28-day toxicity studies using sex with lowest AUC.

[b] Human AUC values are predicted based on allometric scaling.

[c] AUC values for in vitro data were calculated as IC50* 24 hours.

FIG. 16