

Human GPR174 protein NP_115942.1
(SEQ ID NO:1)

MPANYTCTRPDGDNTDFRYFIYAVTYTVILVPGLIGNILALWVFYGYMKETKRAVIFMINLAIADLLQVL
SLPLRIFYYLNHDWPEFGPGLCMFCFYLYVNMVYASYFLVCISVRRFWFLMYPFRFHDCKQKYDLYISIA
GWLIIICLACVLFPLLRTSDDTSGNRTKCFVDLPTRNVNLAQSVMMMTIGELIGFVTPLLIVLYCTWKTVL
SLQDKYPMAQDLGEKQKALKMILTCAGVFLICFAPYHFSFPLDFLVKSNEIKSCLARRVILIFHSVALCL
ASLNSCLDPVIYYFSTNEFRRLSRQDLHDSIQLHAKSFVSNHTASTMTPELC

FIG. 1A

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Human GPR174 cDNA (NM_032553.1) SEQ ID NO:2

CACCATTAGGCAAGATAGTTTCTCTAGAGAGAAATCATGCTGTCTAAATTACACGTGTACCAGGCCAGATGG
AGACAATACAGATTTTCGATACTTTATTTATGCAGTGACATACACTGTCAATCTTGTGCCAGGTCTCATAG
GGAATATATTAGCCCTGTGGGTATTCTATGGTTATATGAAAGAAACAACGAGCTGTGATATTTATGATA
AACTTAGCCATTGCTGACTTACTACAAGTCTTCCCTGCCACTGAGGATCTTCTACTACTTGAATCATGA
CTGGCCATTGGGCCCTGGTCTCTGCATGTCTGTCTTCTACCTGAAGTATGTCAACATGTATGCAAGCATCT
ACTTCTTGGTCTGCATCAGTGTGCGACGATTTTGGTTTCTCATGTACCCCTTTCGCTTCCATGACTGCAAA
CAGAAATATGACCTGTACATCAGCATTGTCTGGCTGGCTGATCATCTGCCTTGCCTGTGTACTCTTTCCACT
CCTCAGAACCCAGTGATACCTCTGGCAATAGGACCAATGCTTTGTGGATCTTCTACAGGAATGTCA
ACCTGGCCCCAGTCCGTTGTATGATGACCAATTGGCGAGTTGATTGGGTTTGTAACTCCGCTTCTGATTGTC
CTATATTGTACCTGGAAGACGGTTTTATCACTGCAAGATAAATATCCCATGGCCCAAGATCTTGGAGAGAA
ACAGAAAGCCTTGAAGATGATTCTAACCTGTGCAGGGGTATTCCTAATTTGCTTTCACCTTATCATTTCA
GTTTTCCCTTAGATTTCCTGGTGAAGTCCAAATGAAATTAAAAGCTGCCTAGCCAGAAAGGTGATTTCTAATA
TTTCATTCTGTGGCATTGTGCTTGTCTAGTCTGAATTCATGTCTTGACCCAGTCATATACTACTTTTCCAC
TAATGAGTTCGGAAGACGGCTTTCAGACACAAGATTTGCATGACAGCATCCAACTCCATGCAAAATCCTTTG
TGAGTAACCATACAGCTTCACCATGACACCTGAATTATGCTAAAACAAAACCAAACTGAATGTGACCT
GAAATGCAAGTACATCAGAACATATCTGCAATACCCCAAGCCACAGGGAAGAACTTGCAAAACAACACAGCT
TTTCAGTTCCTCTATCTTACTGCTATGGGGAATTCACCTTCTTCAAAGCAGGACCTATTTGGAGCATTAC
GATCCACGATTATTGATGTGACATGTCCATGTAGTAATTTTCTTCAAGT

FIG. 1B

Mouse GPR174 protein NP_001028423.1
(SEQ ID NO:3)

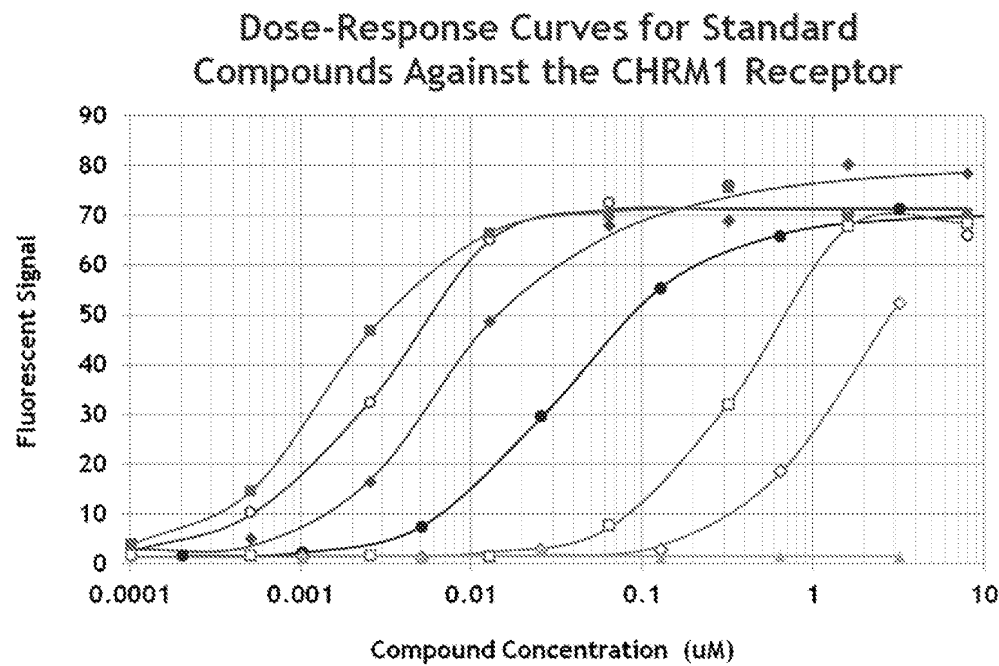
MTDNFTCNKTDGDNDFRYFIYAVTYTVILVPGLIGNILALWVFYGYMKETKRAVVF
MINLAIADLLQILSLPLRIFYYLNHDWPFPGPLCMFCFYLYVNMVYASIFYFLVCISV
RRFWFLMYPFRFNDCKQKYDLYISIIIGWLIICLACLLFPLLRNTDDTPGNRTKCFVD
LPIRNVNLAQSVAMITIGEVWGFVTPLMIVLYCTWKTALSQNKYPISQHLGEKKA
LKMILTCAGVFLVCFVPYHFSFPLDFLVKSNEIKSCFARRVILIFHSVALCLASLNS
CLDPVIYYFTTNEFRRRLSRQDLPDNIQLHTKSYKIASNHATSTVAAELC

FIG. 1C

Rat GPR174 protein NP_001100408.1
(SEQ ID NO:4)

MTDNFTCNKTDGDNNTDFRYFIYAVTYTVILVPLIGNILALWVFYGYMKETKRAVVFMINLAIADLLQIL
SLPLRIFYYLNHDWPFPGGLCMFCFYLYKYYNMYASIFYLVCSVRRFWFLMYPFRFNDCKQKYDLYISIV
GWLIIICLACLLFPLLRTNDDTPGNRTKCFVDLPIRNVNLAQSVAMITIGEYVGFITPLMIVLYCTWKTAL
SLQNKYPISQHLGEKKKALRMILTCAAVFLICFAPYHFSFPLDFLVKSNEIKSCLARRVILIFHSVALCL
ASLNSCLDPVIYYFTTNEFRRLSRQELPDSIQLQTKSYKIVNNHGASPTAAELC

FIG. 1D



Compound	Reported Potency (uM)
◆ Propantheline	0.2
○ Atropine	0.5
■ Scopolamine	1.0
● Pirenzepine	5.0
□ HHSiD	16.0
◇ Himbacine	60 - 120
▲ Vehicle	

FIG. 2

GPCR Signaling Profile Algorithm

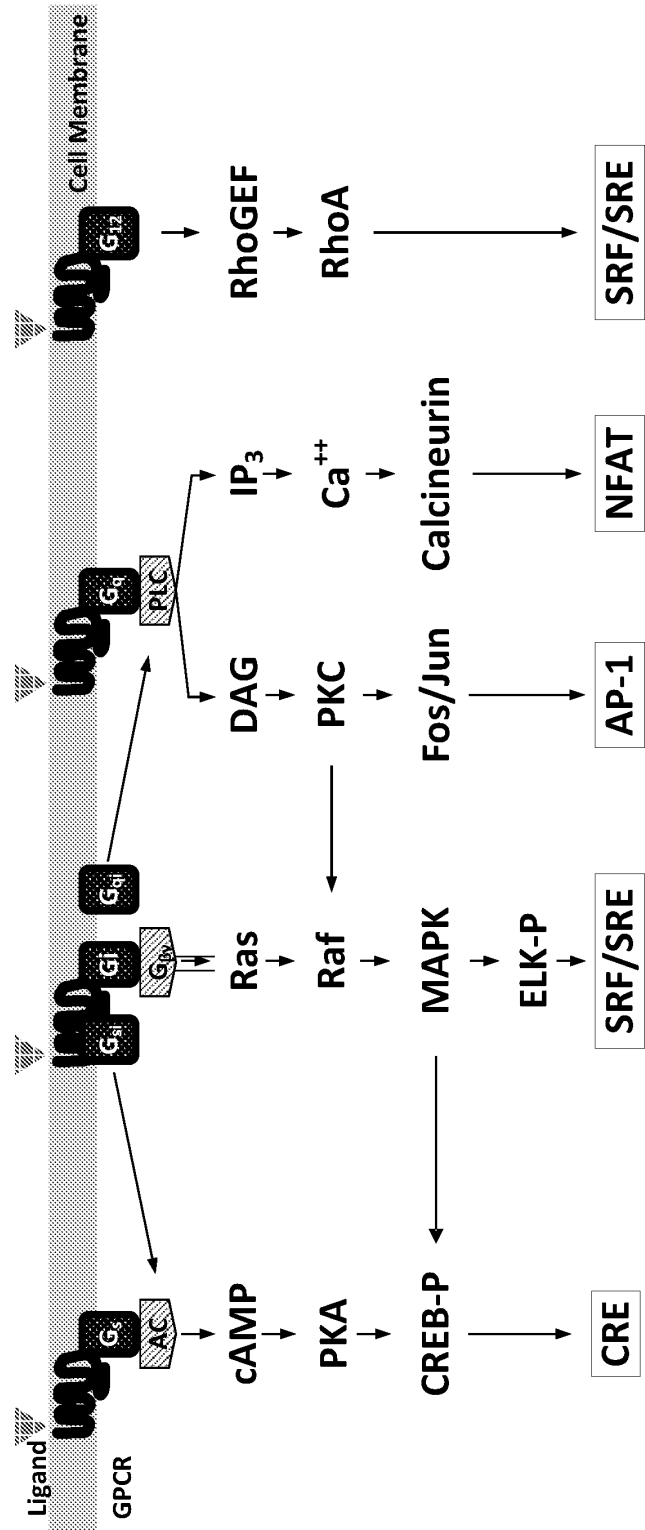


FIG. 3

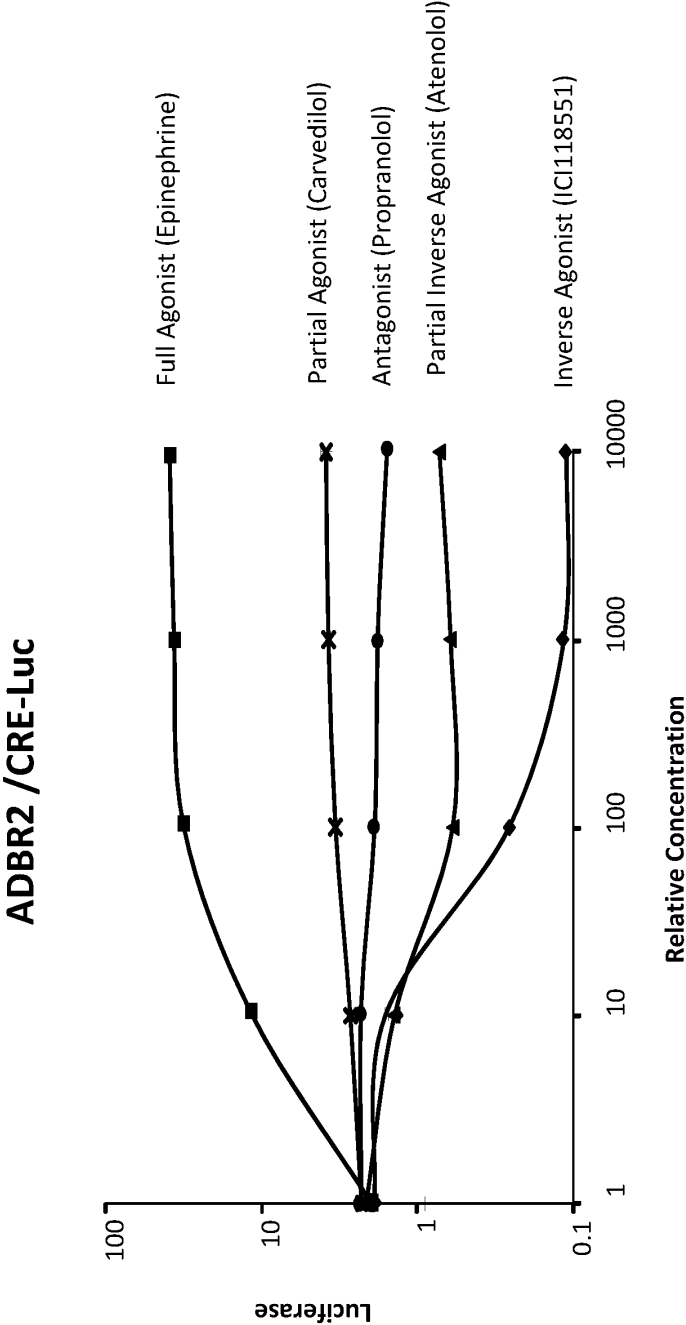


FIG. 4A

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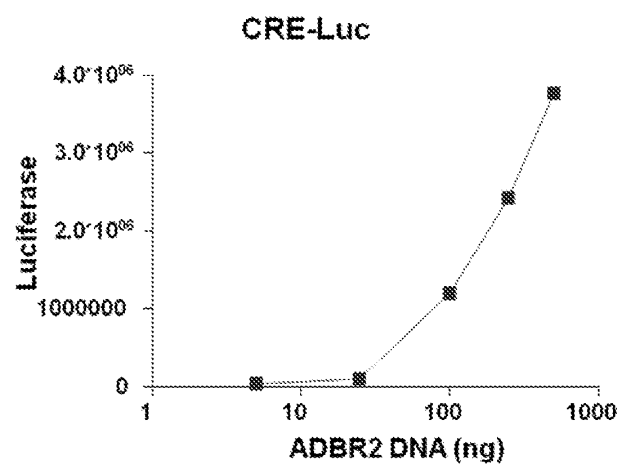


FIG. 4B

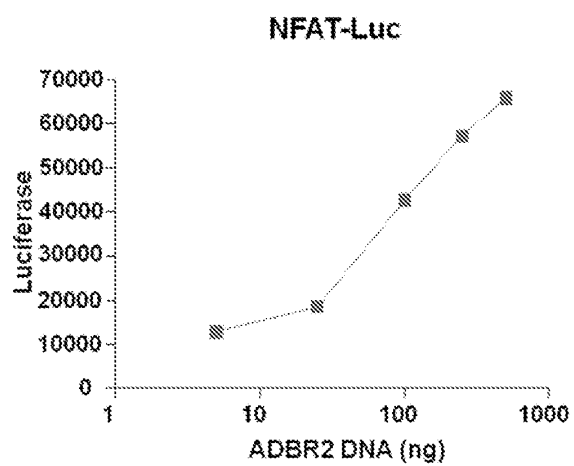


FIG. 4C

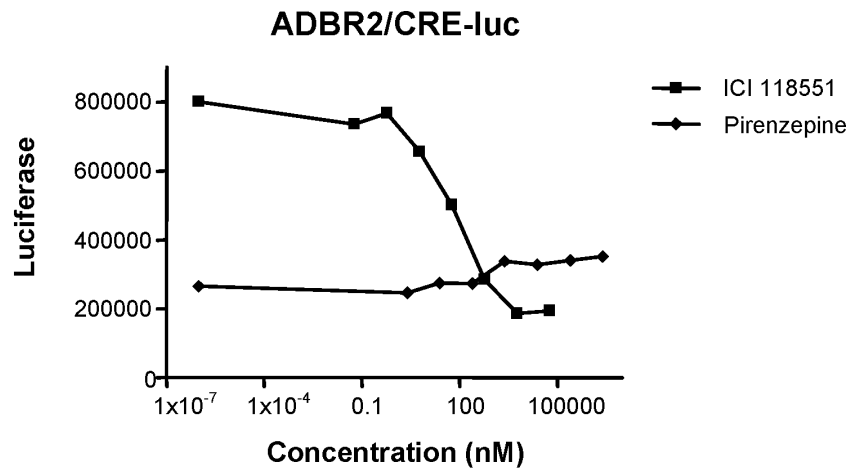


FIG. 4D

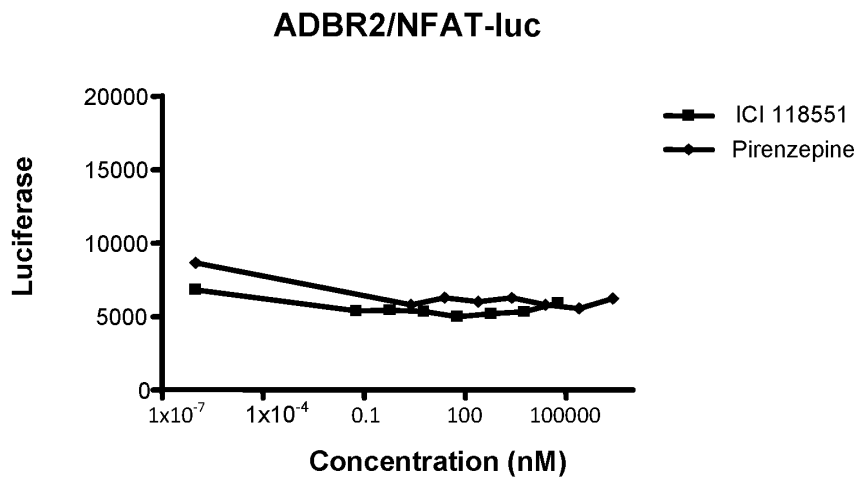


FIG. 4E

CRA Assay Results of Representative GPR174 interacting compounds

Compounds Against GPR174

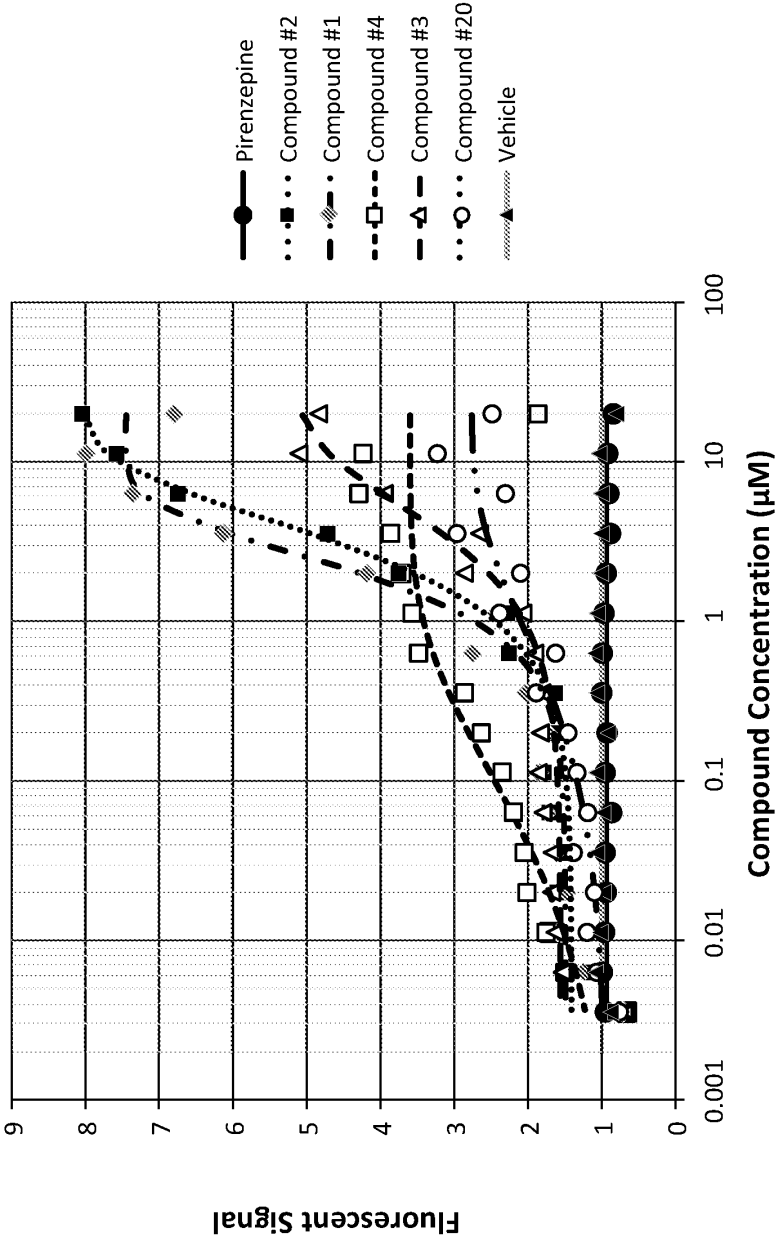


FIG. 5A

CRA Assay Results of Representative GPR174 interacting compounds

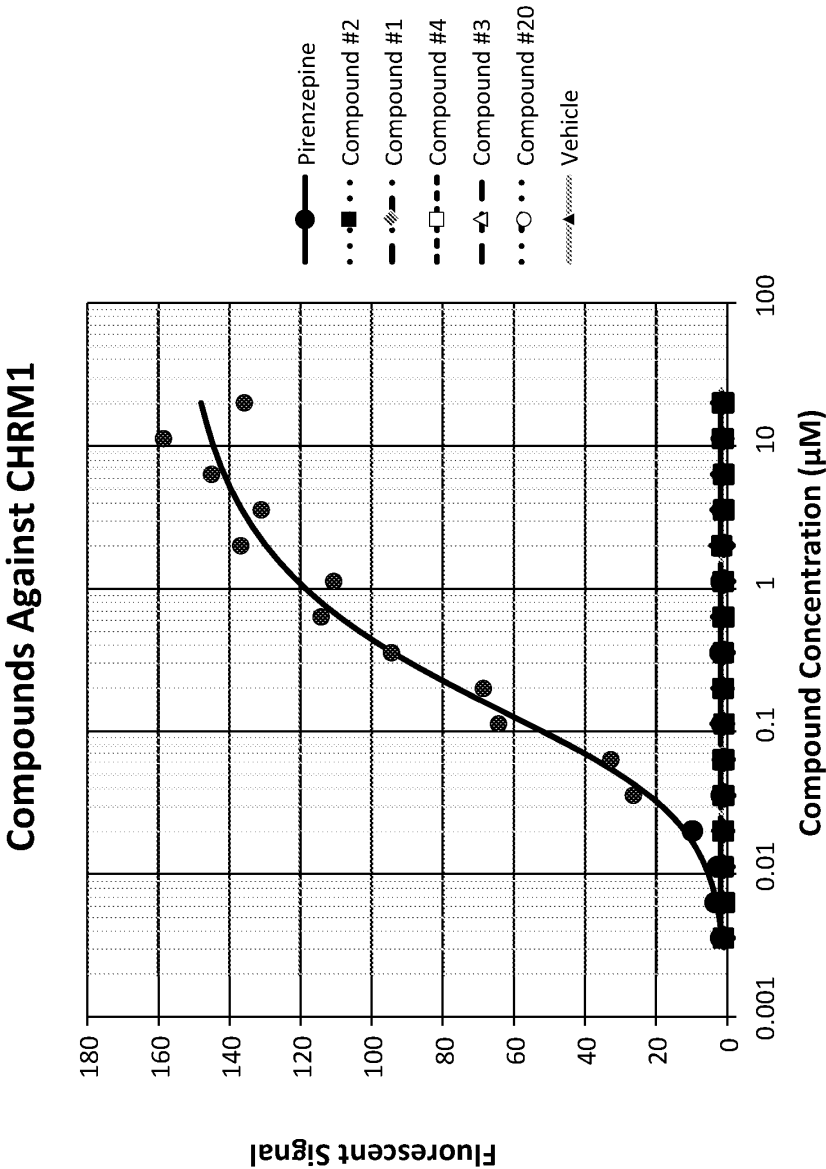


FIG. 5B

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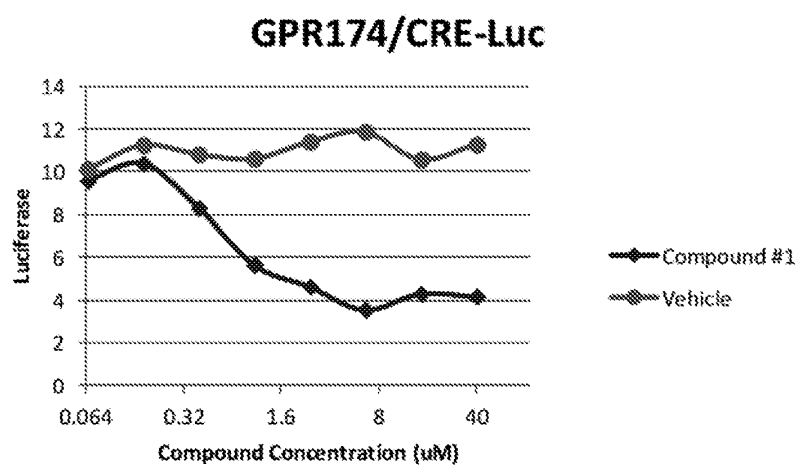


FIG. 6A

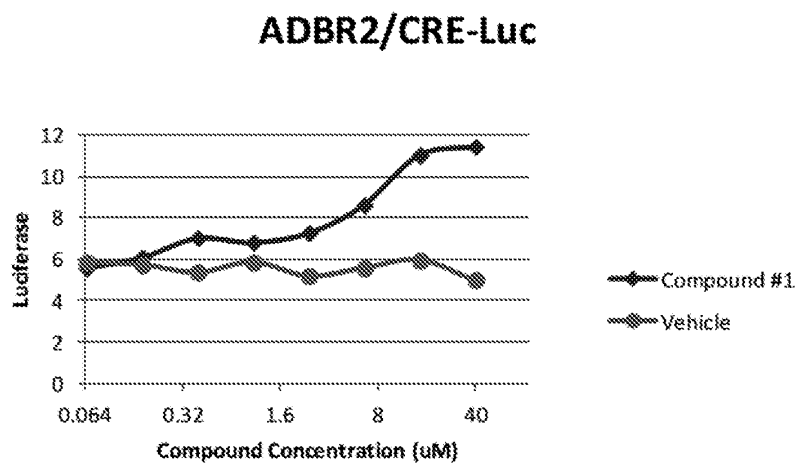


FIG. 6B

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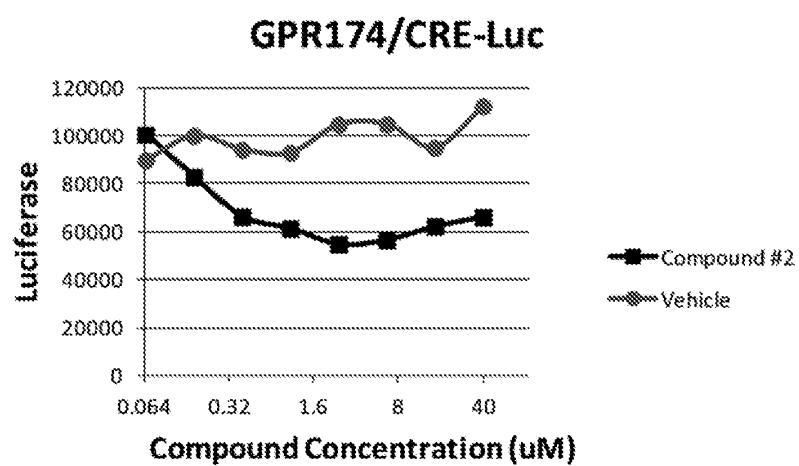


FIG. 7A

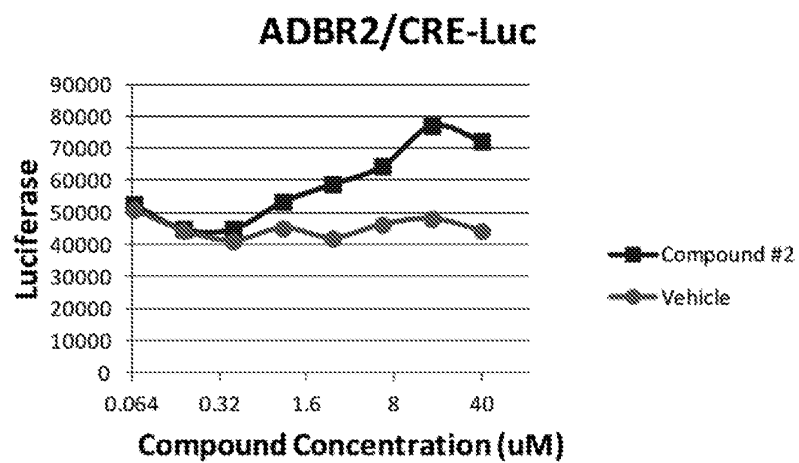


FIG. 7B

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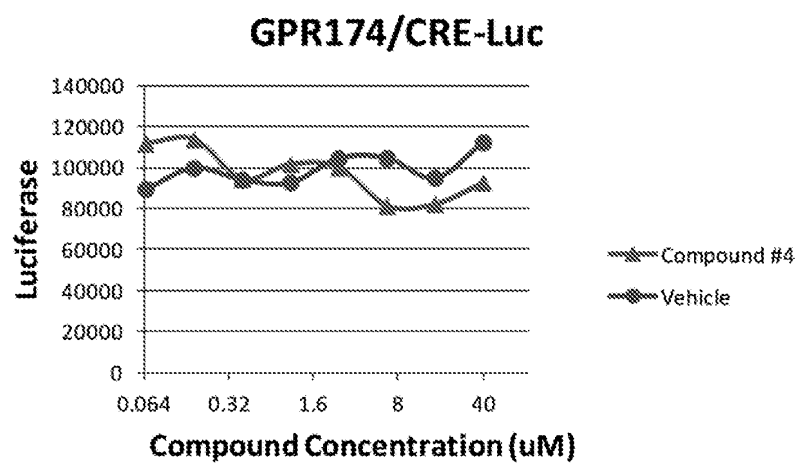


FIG. 8A

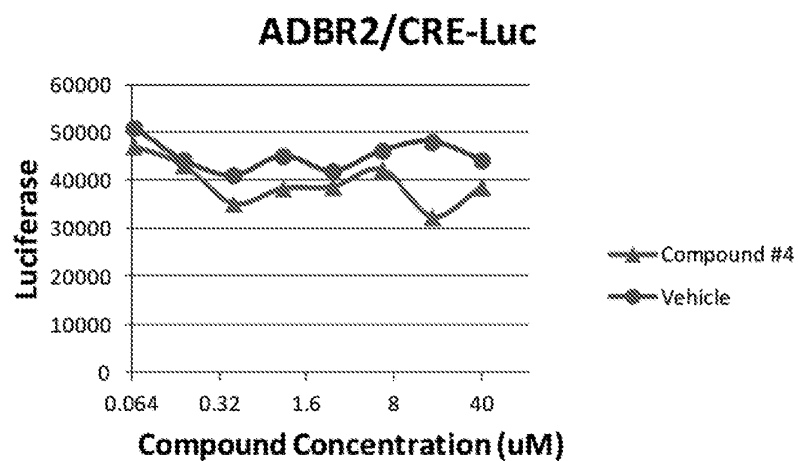


FIG. 8B

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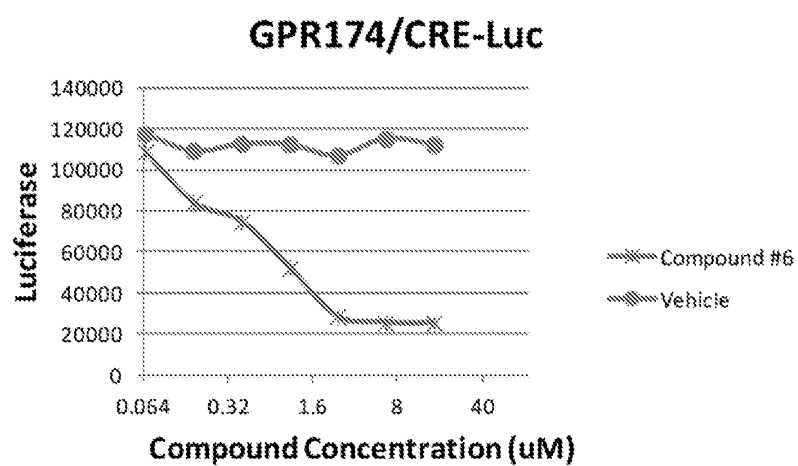


FIG. 9A

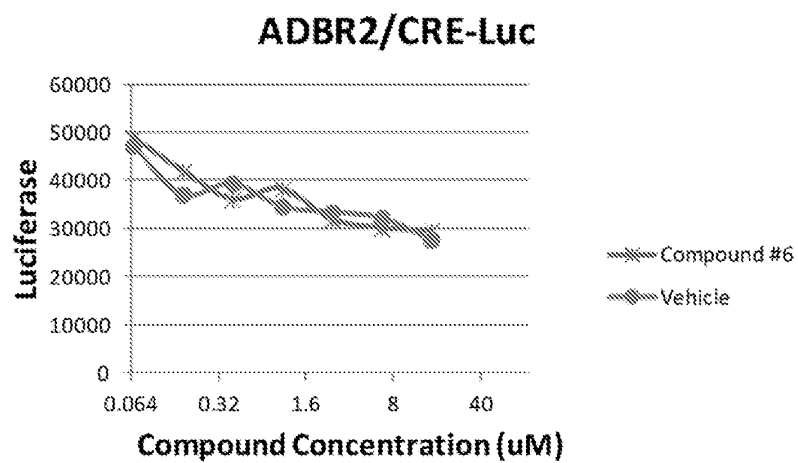


FIG. 9B

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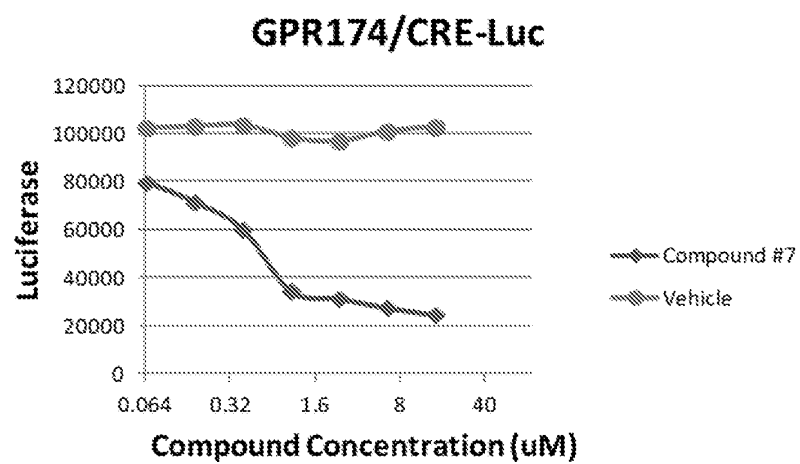


FIG. 10A

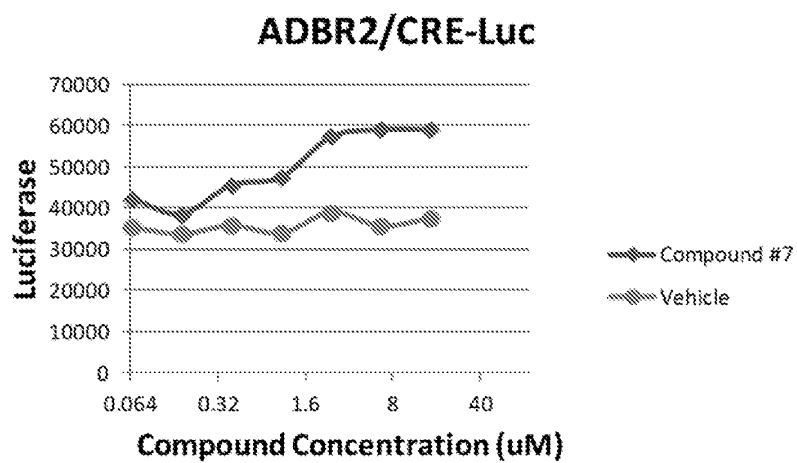


FIG. 10B

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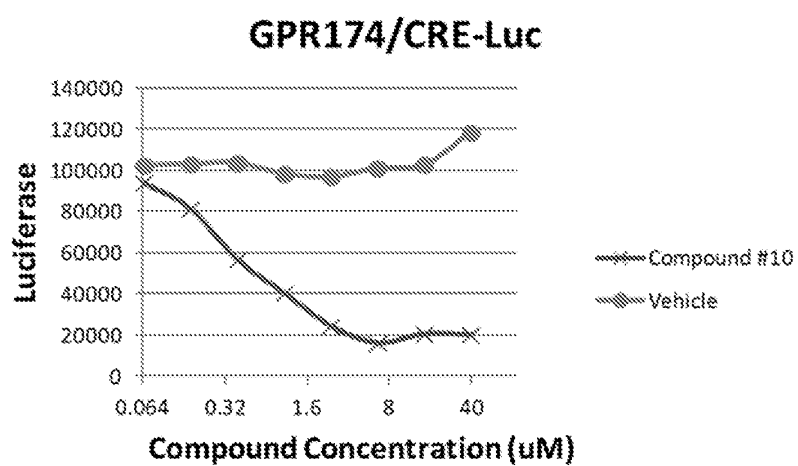


FIG. 11A

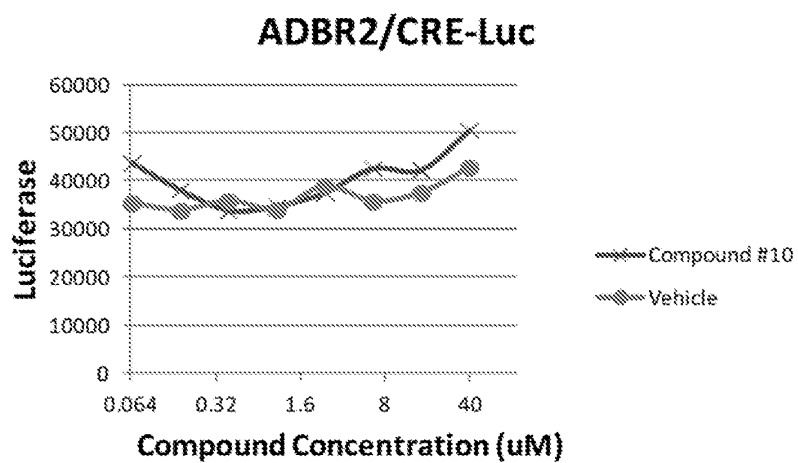


FIG. 11B

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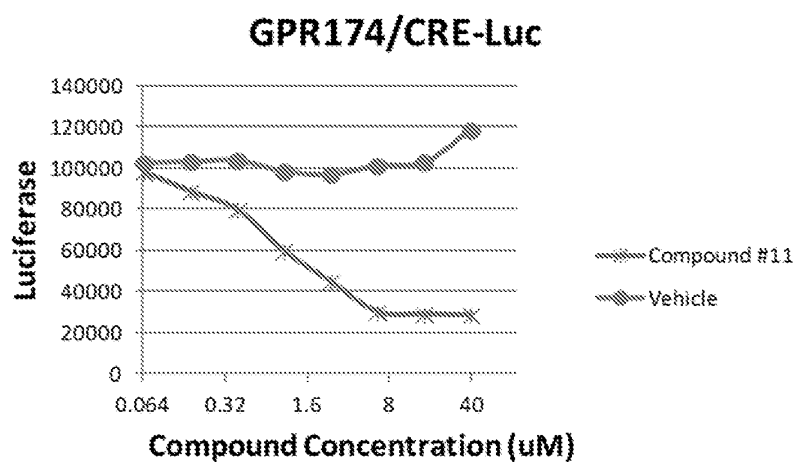


FIG. 12A

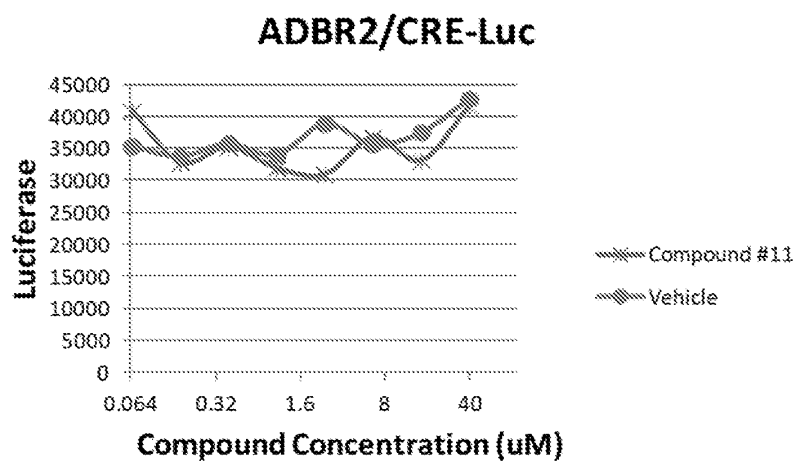


FIG. 12B

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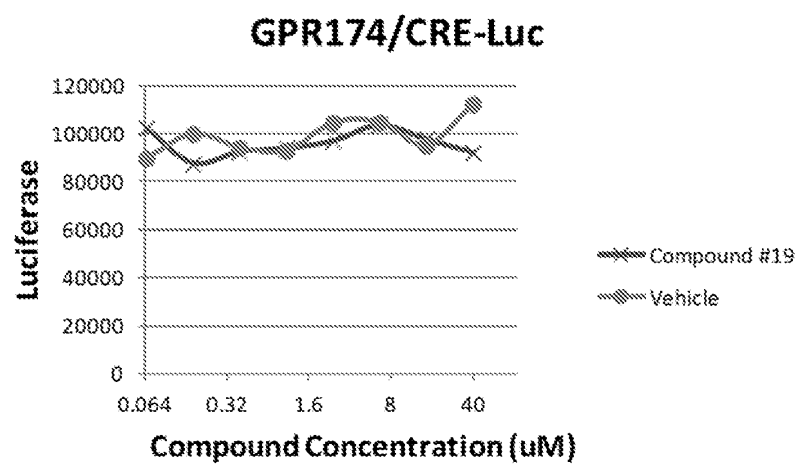


FIG. 13A

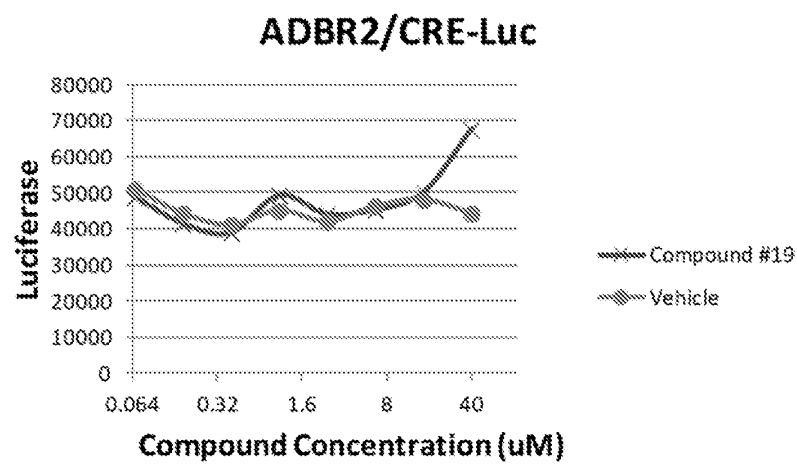


FIG. 13B

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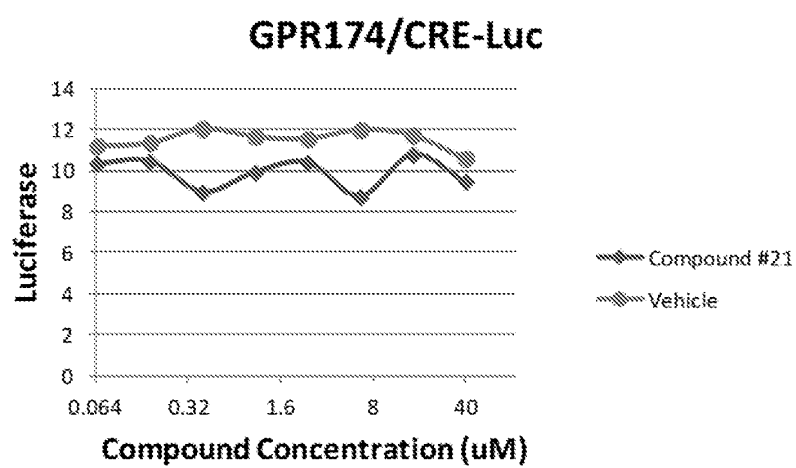


FIG. 14A

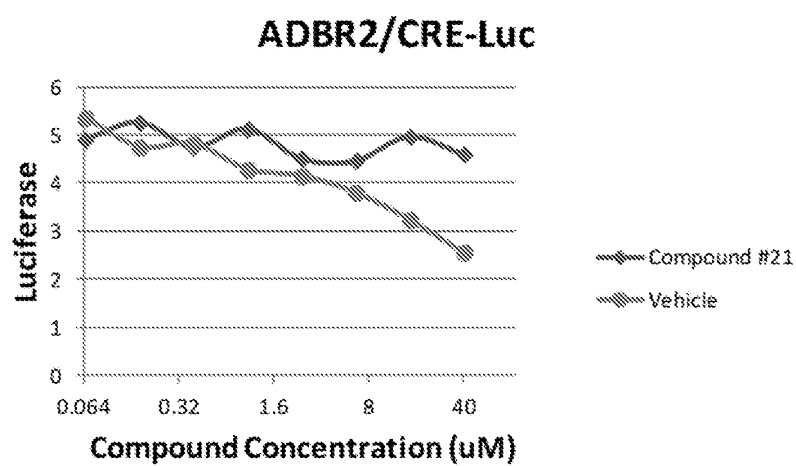


FIG. 14B

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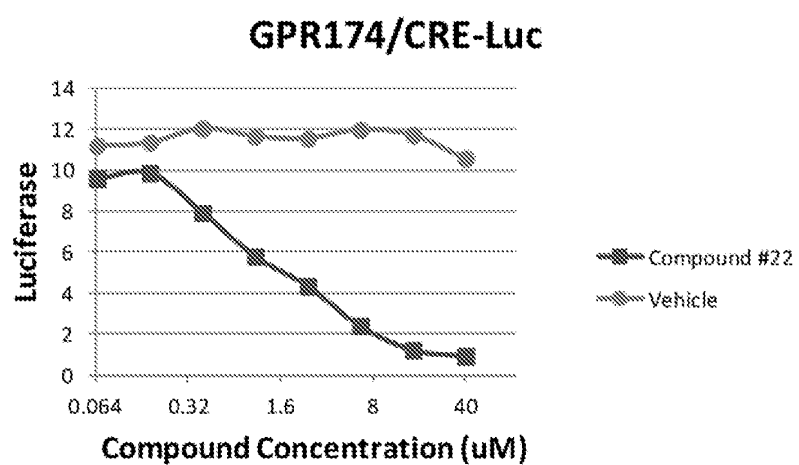


FIG. 15A

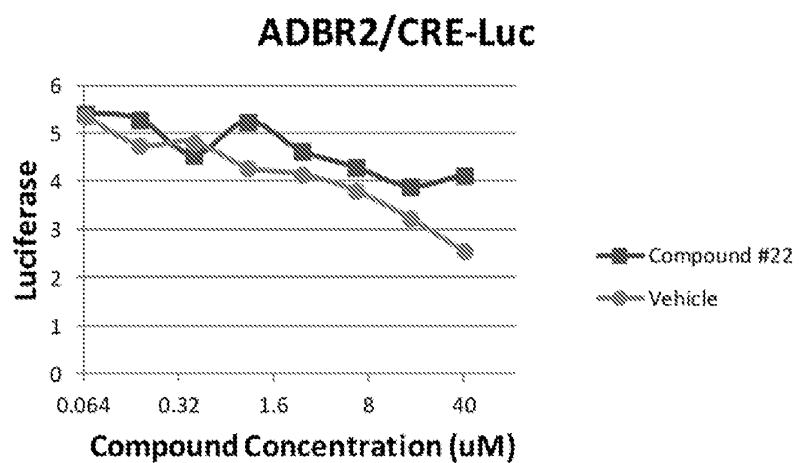


FIG. 15B

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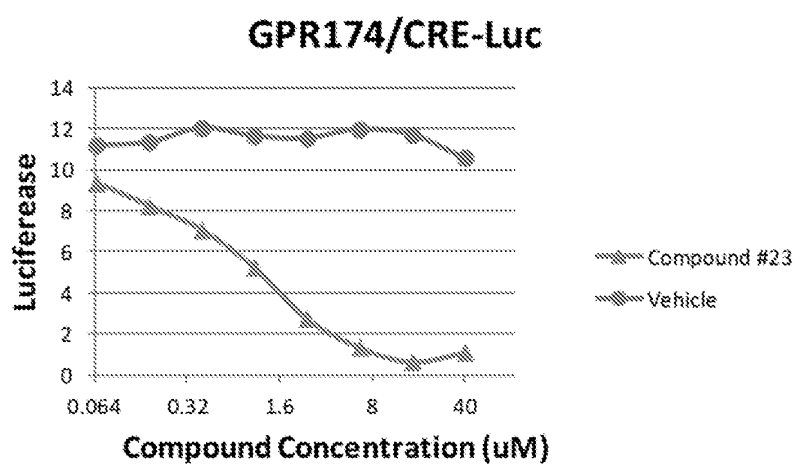


FIG. 16A

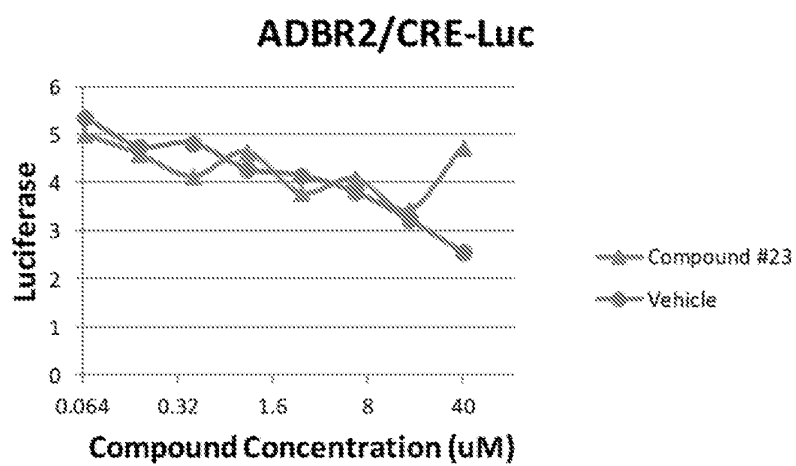


FIG. 16B

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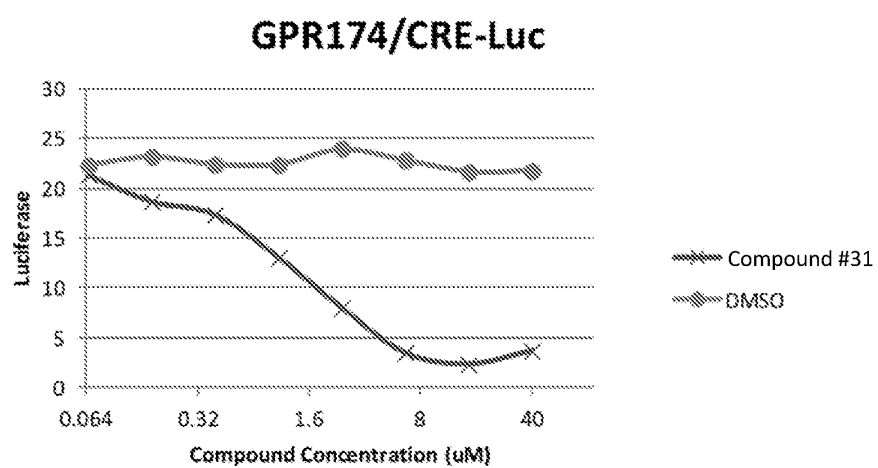


FIG. 17A

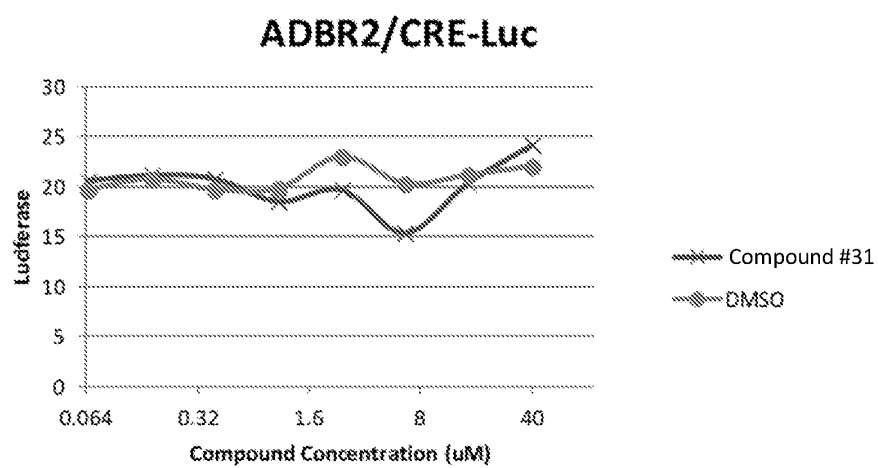


FIG. 17B

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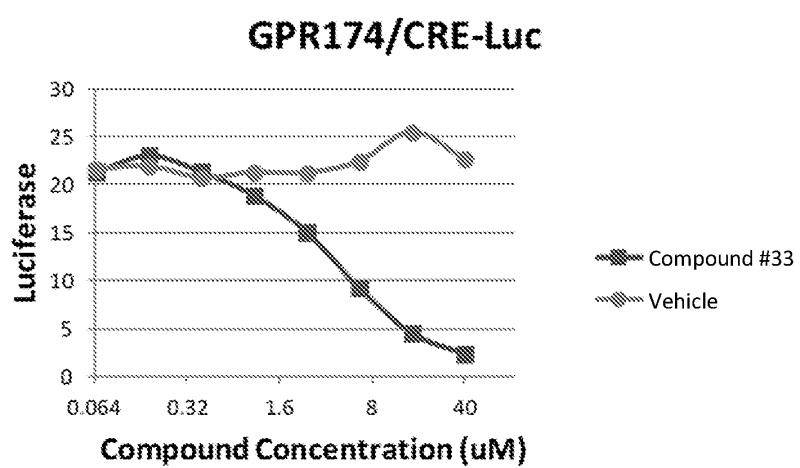


FIG. 18A

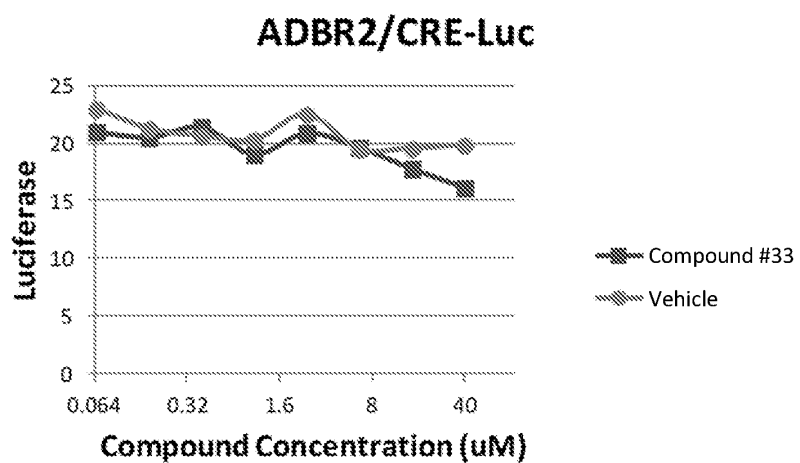


FIG. 18B

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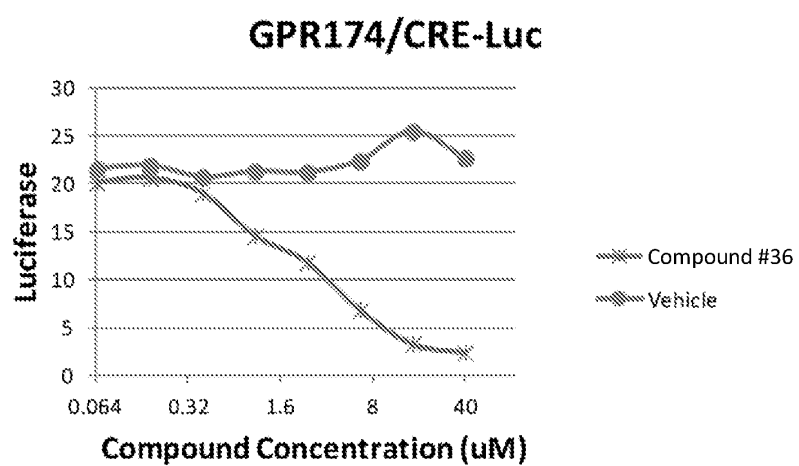


FIG. 19A

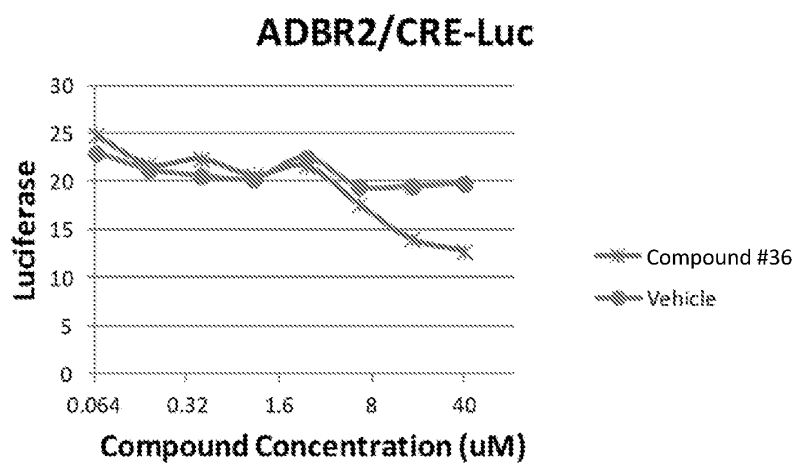


FIG. 19B

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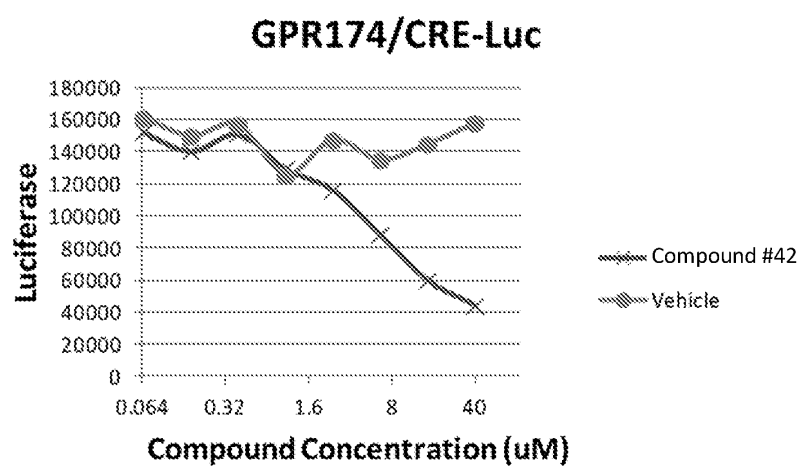


FIG. 20A

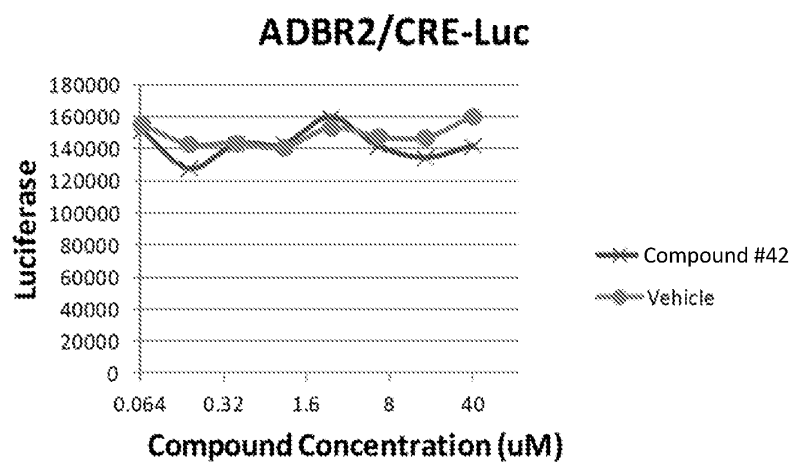


FIG. 20B

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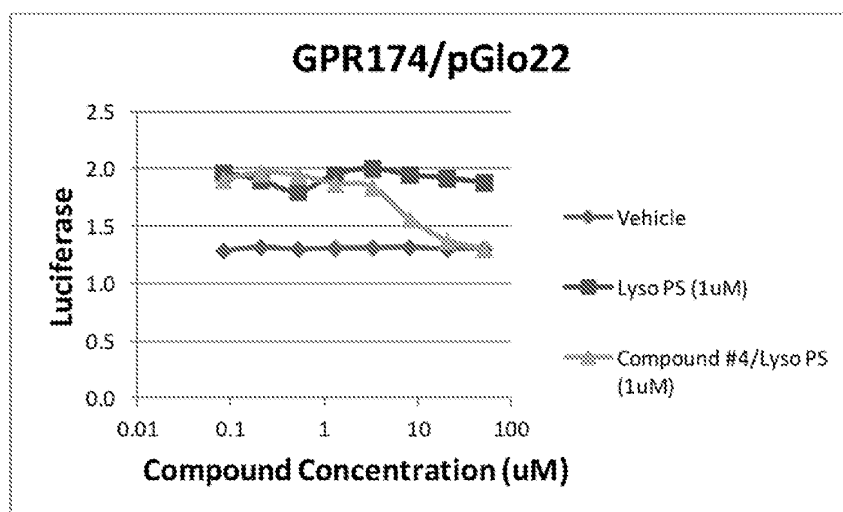


FIG. 21A

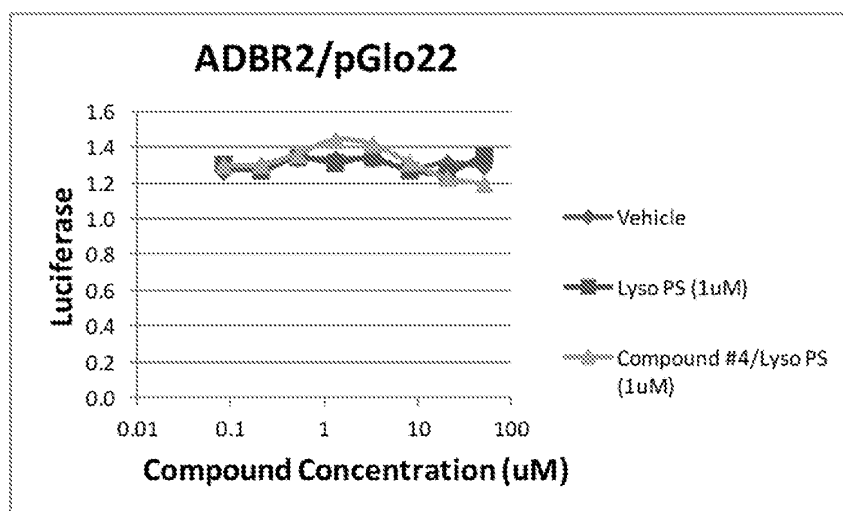


FIG. 21B

Relative transcript abundance of GPR174 in human tissues, as measured by qRT-PCR

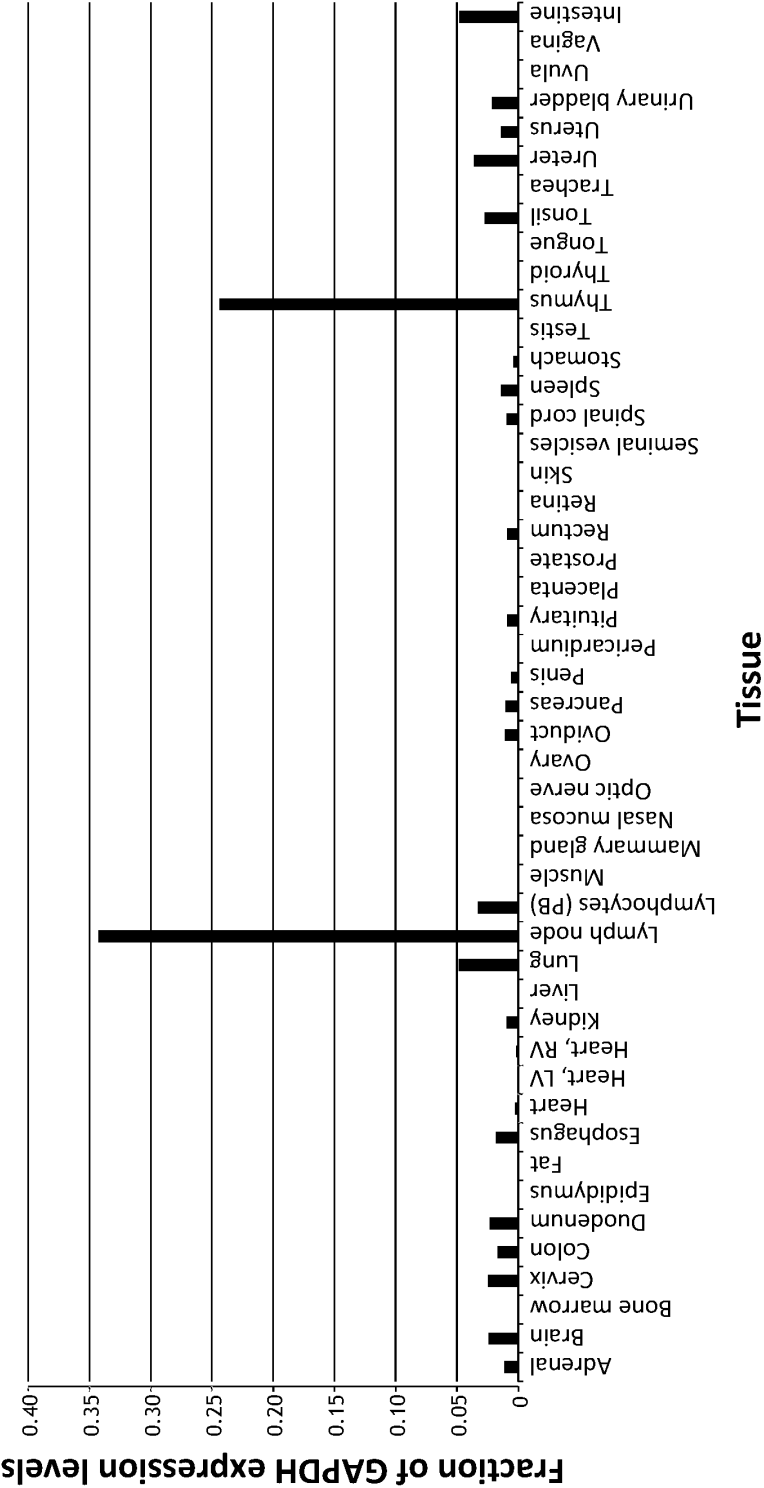


FIG. 22

Relative transcript abundance of GPR174 in human lymphoid cells, as measured by qRT-PCR

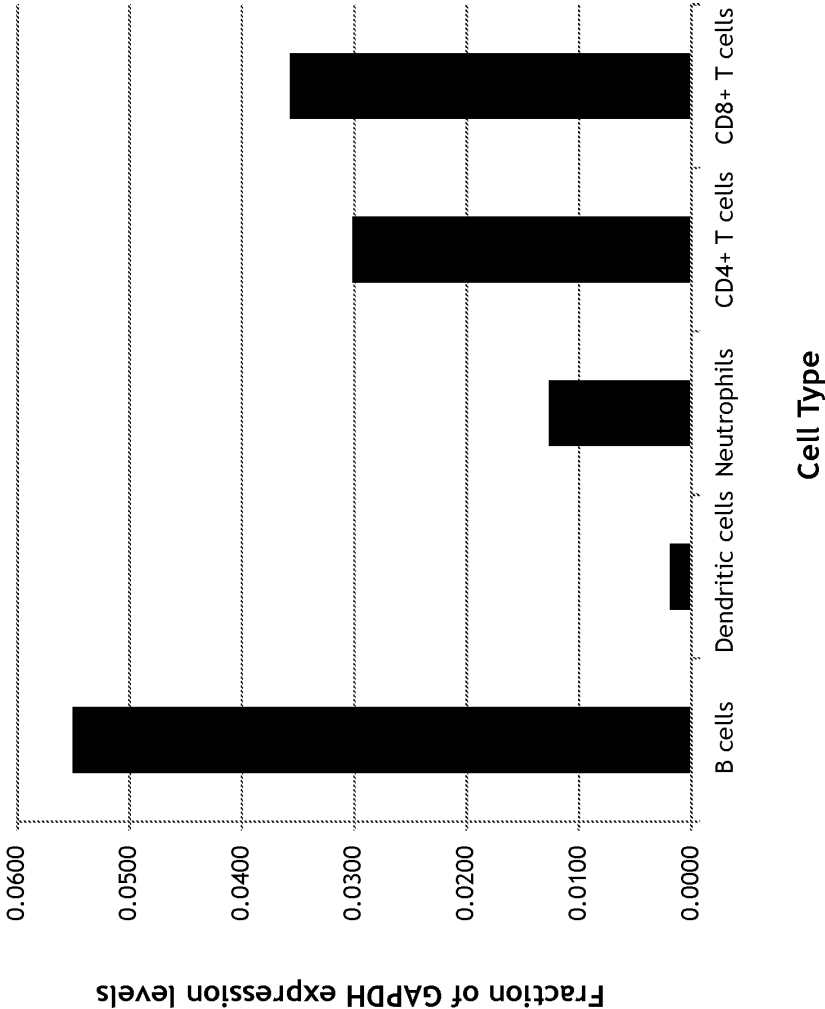


FIG. 23

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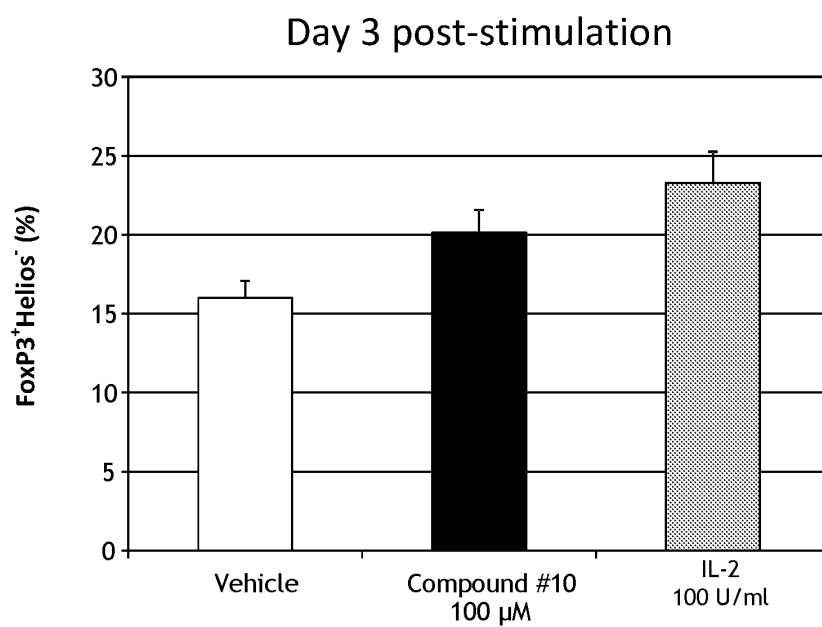


FIG. 24A

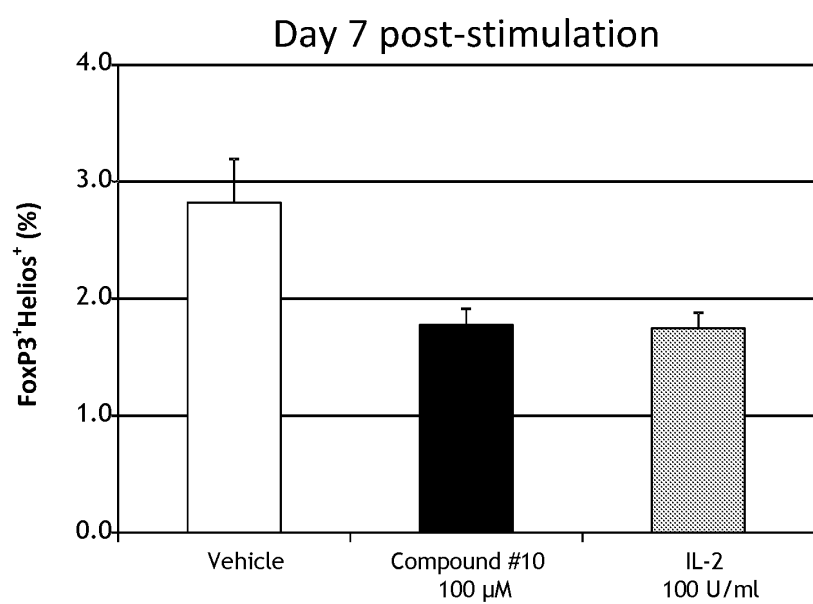


FIG. 24B

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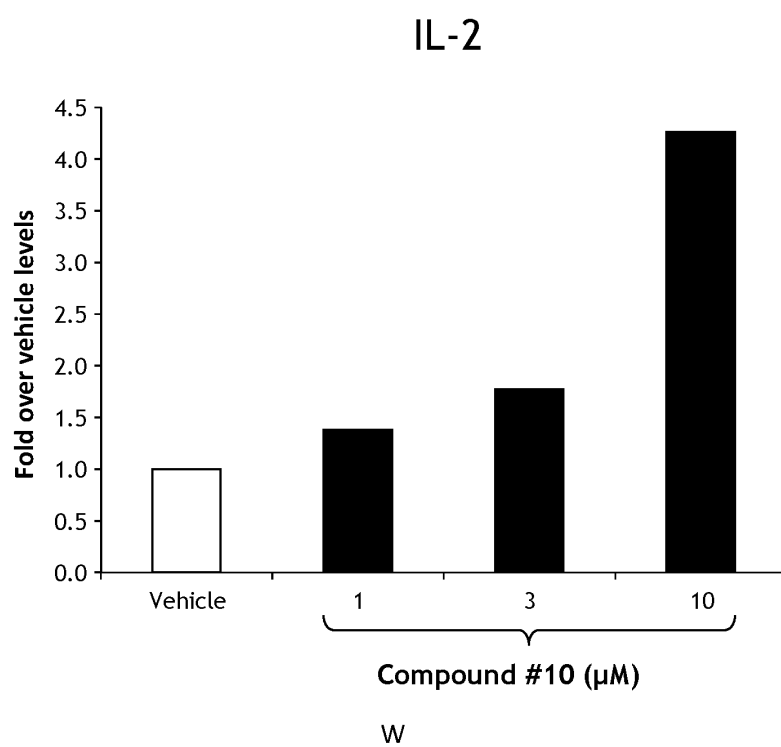


FIG. 25

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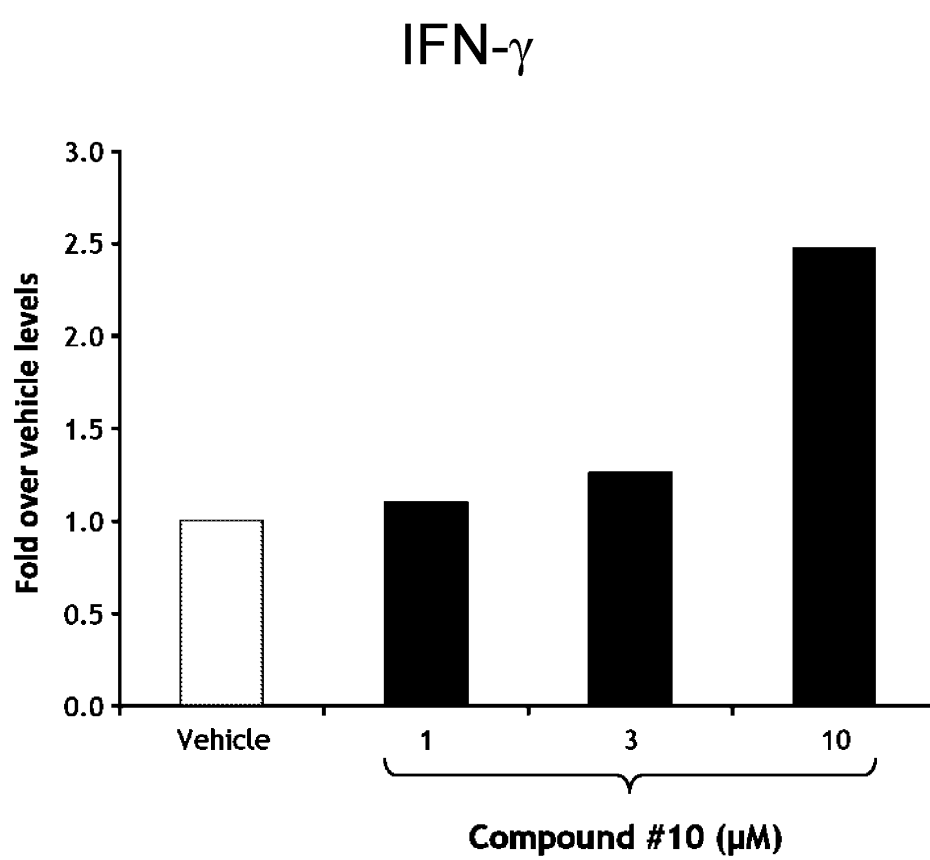


FIG. 26

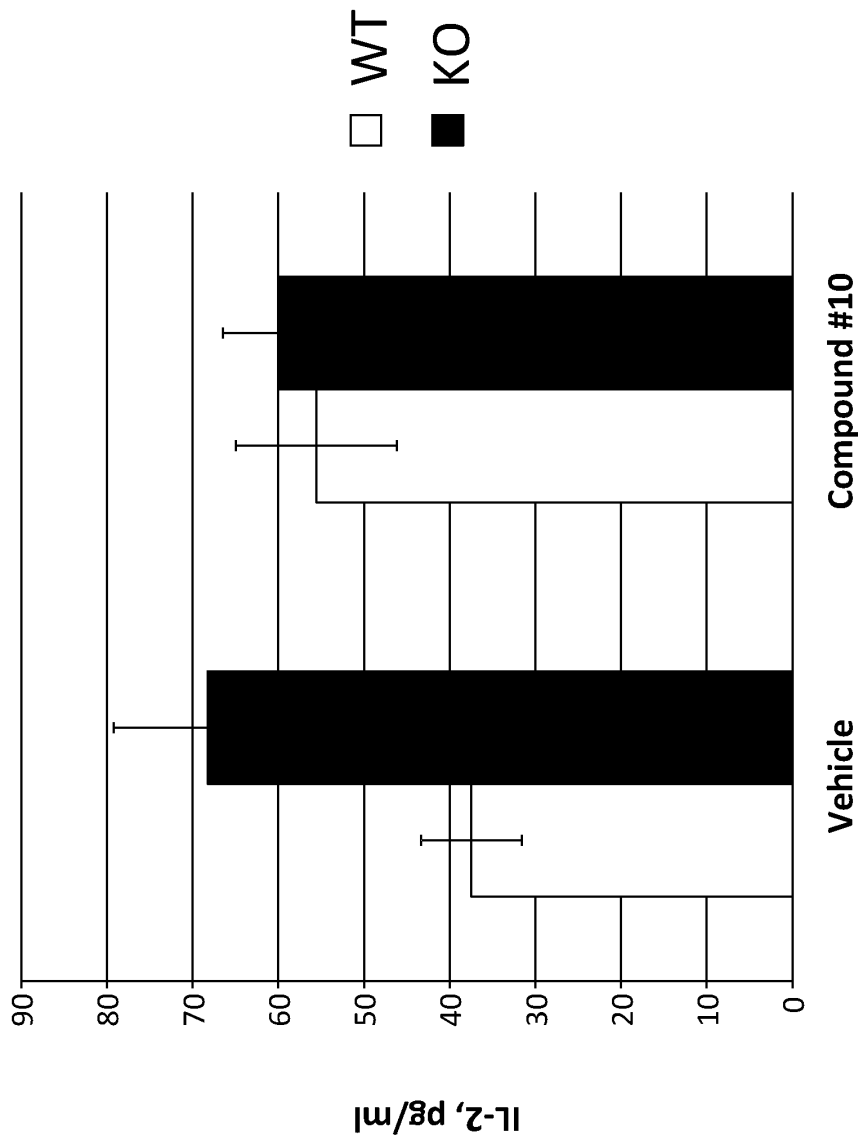
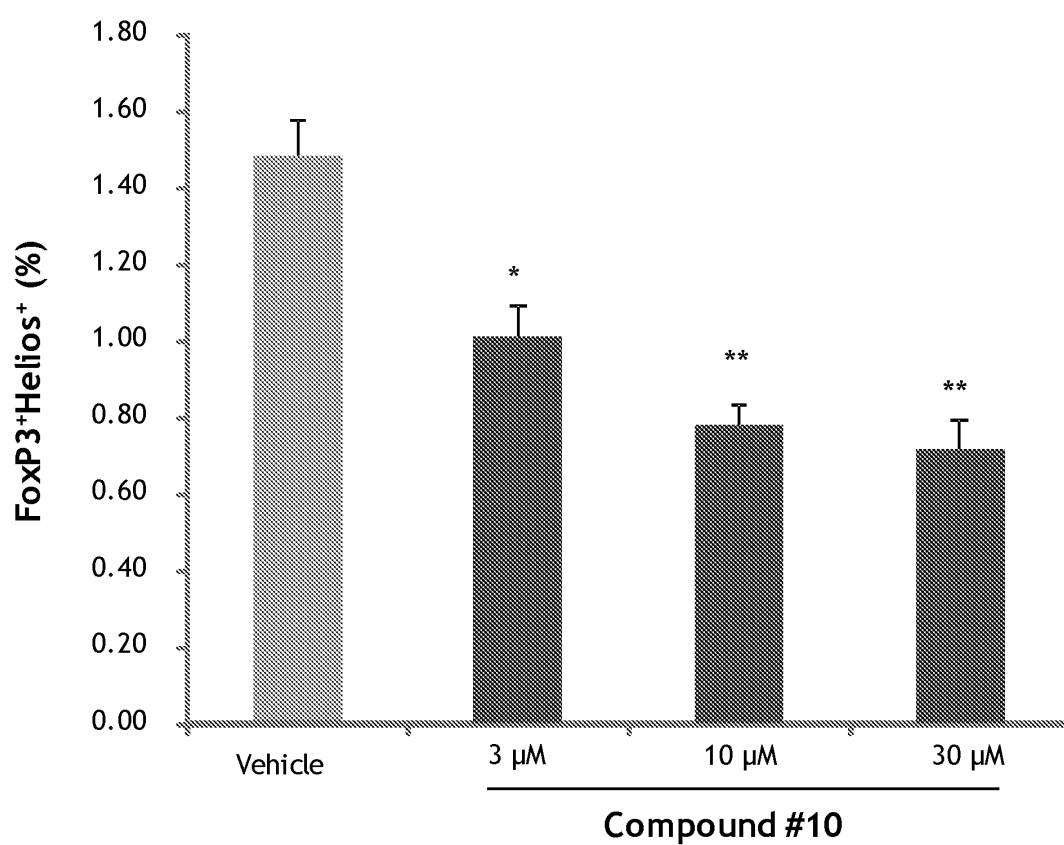


FIG. 27

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Human Treg Cells (Day 7 post-stimulation)



$N=3$
* $P<0.05$
** $P<0.01$

FIG. 28

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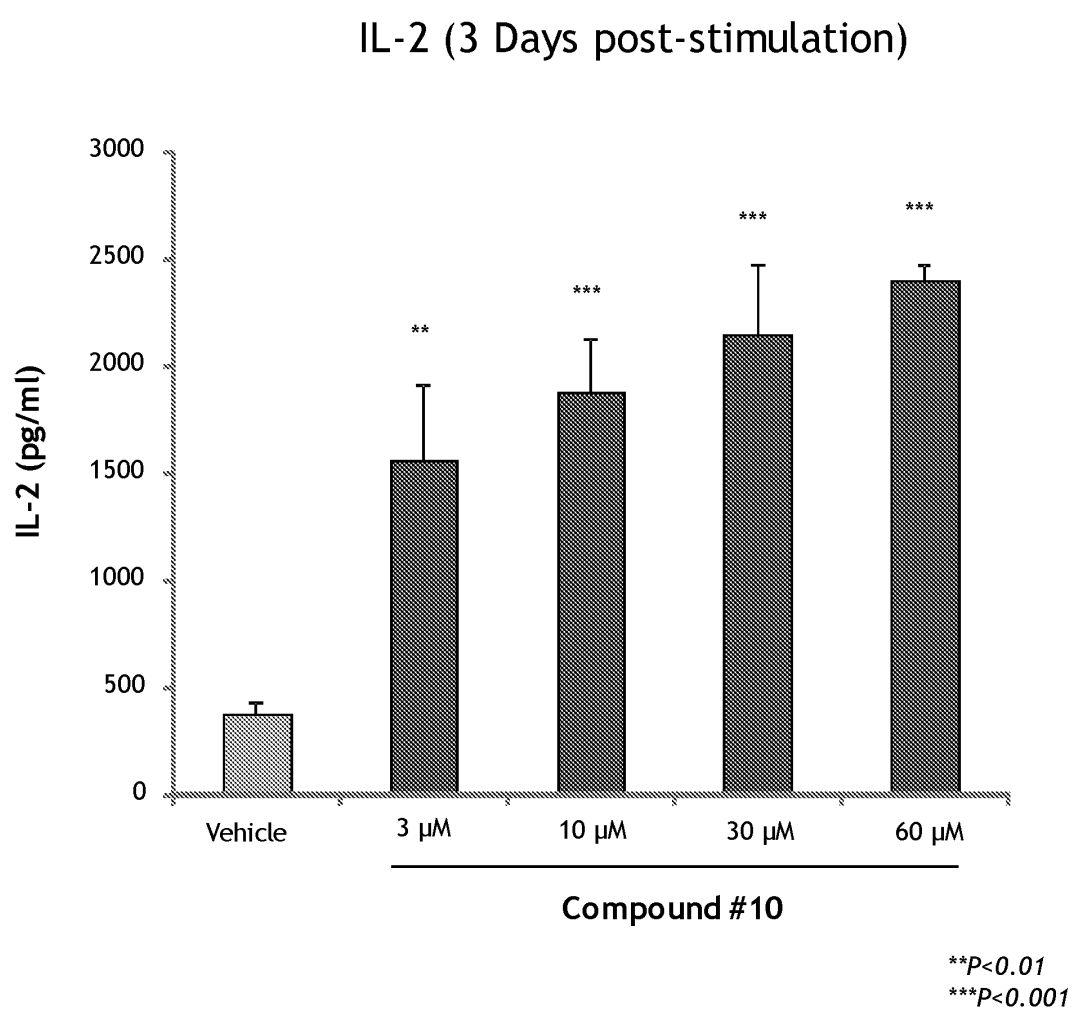


FIG. 29

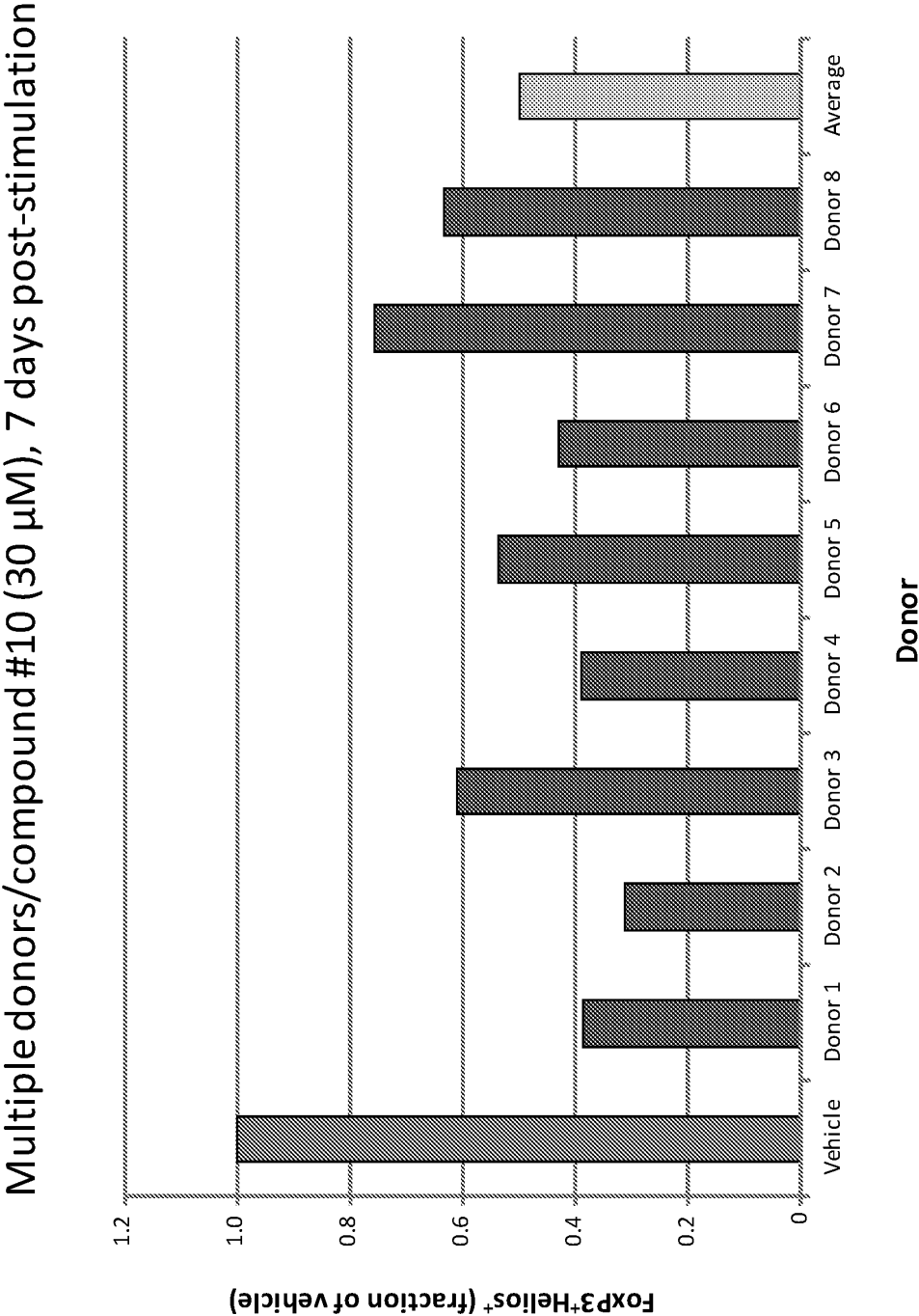


FIG. 30

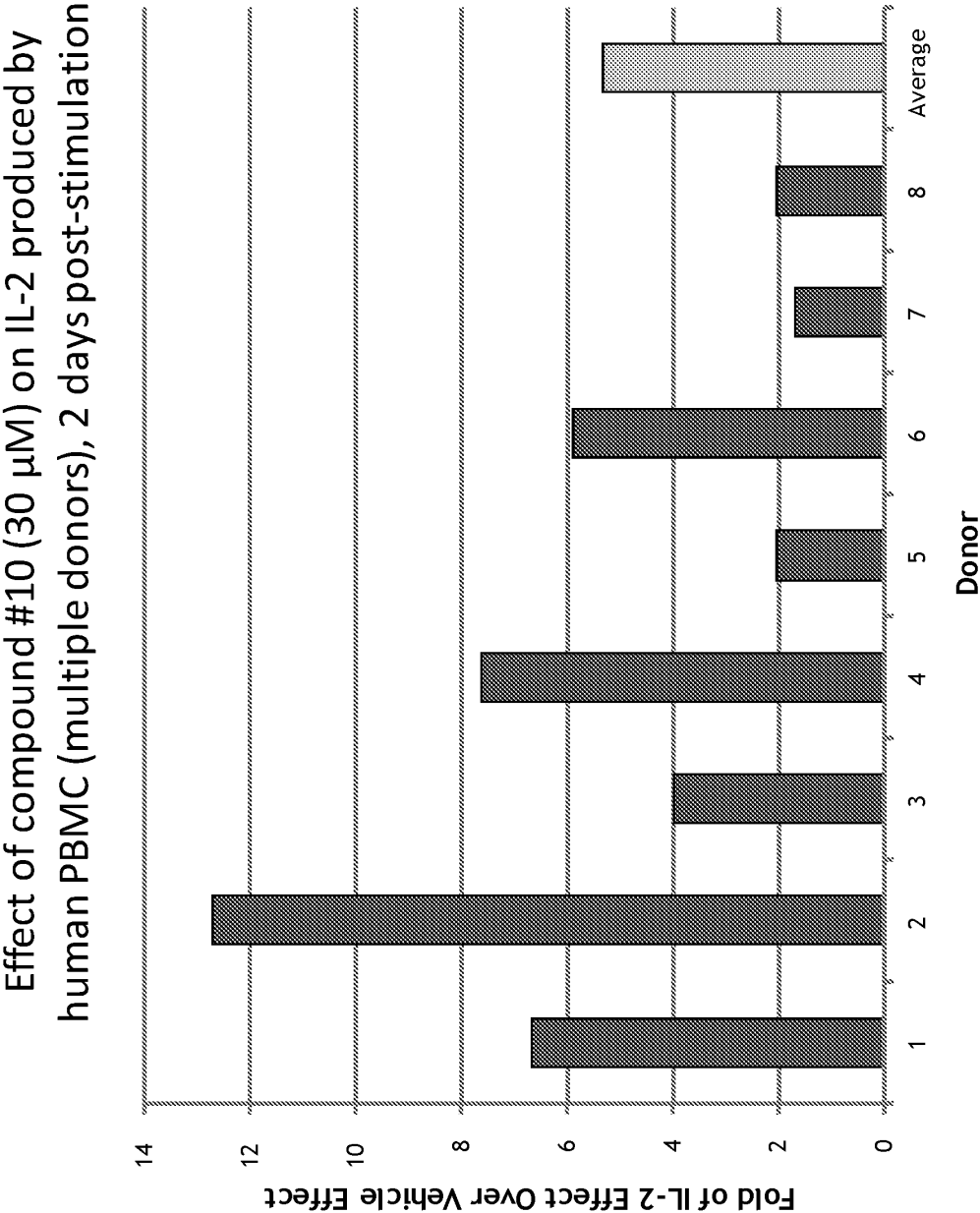


FIG. 31

Effect of compound #10 (30 μ M) on cytokines produced by pooled human PBMCs from multiple donors, 2 days post-stimulation

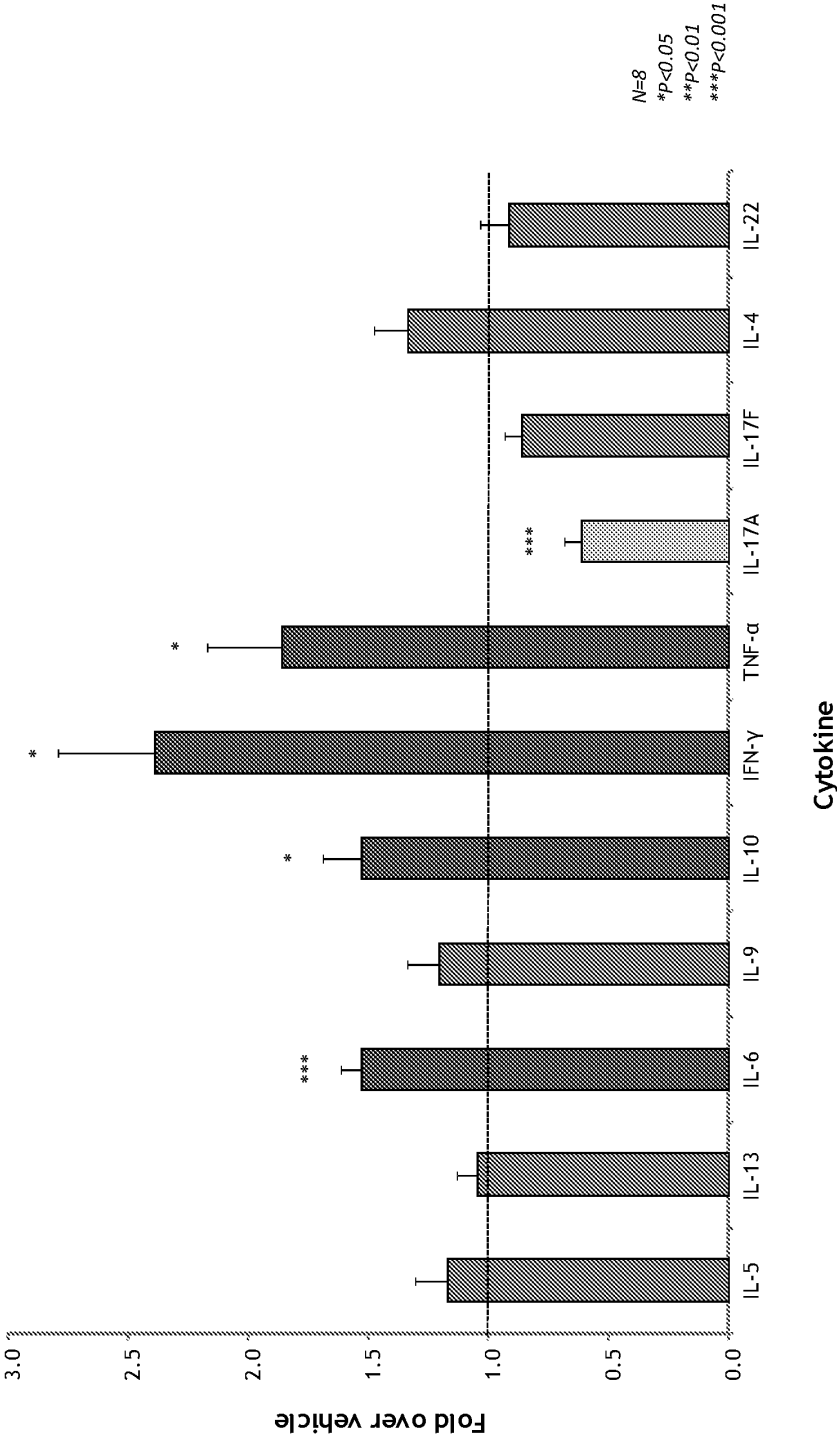


FIG. 32

Effects of compound #10 (30 μ M) on cytokine levels produced in mixed culture splenocytes from different mouse strains (co-cultured for 6 days)

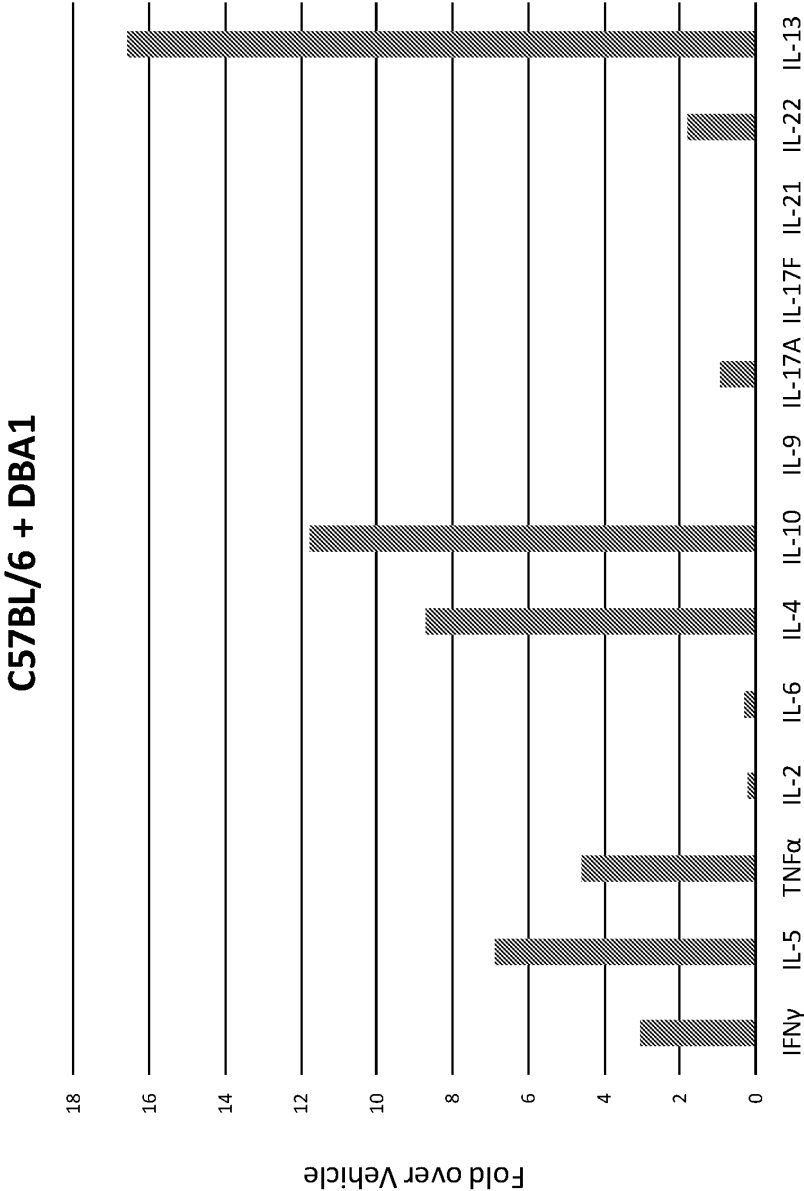


FIG. 33

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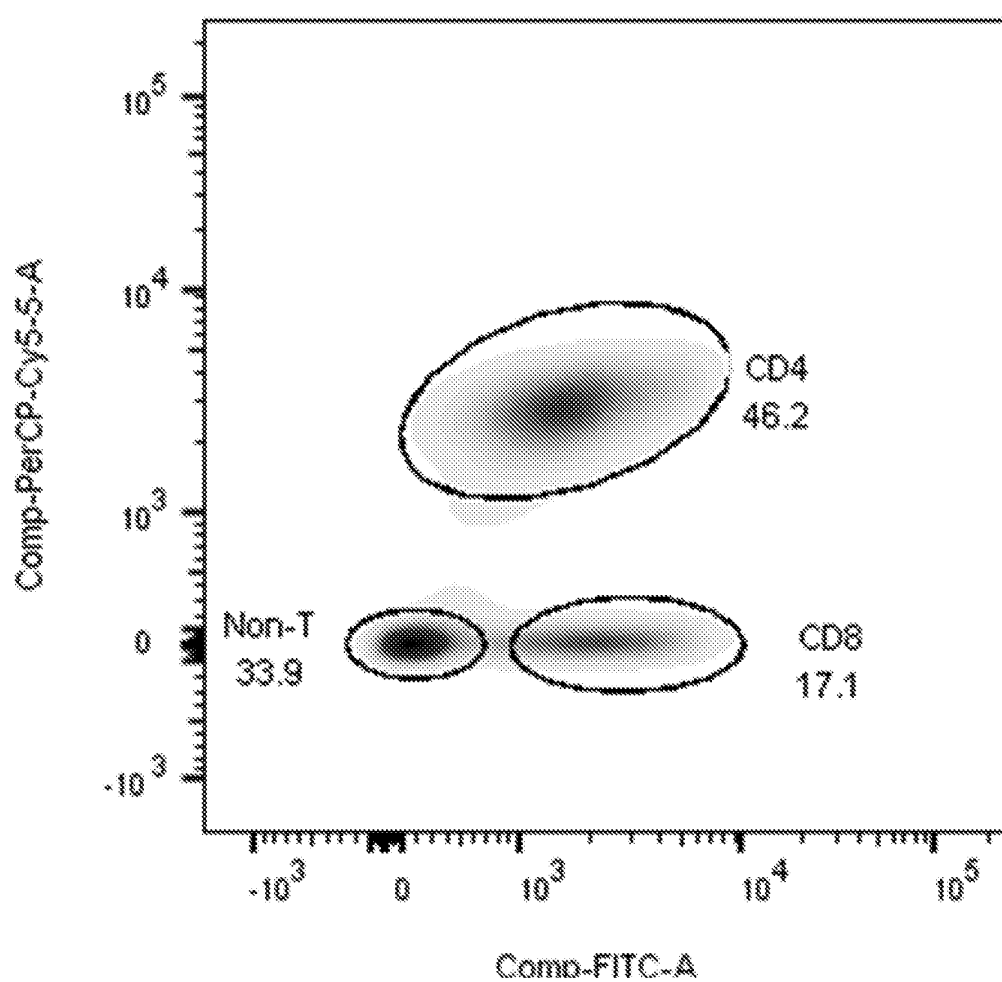


FIG. 34A

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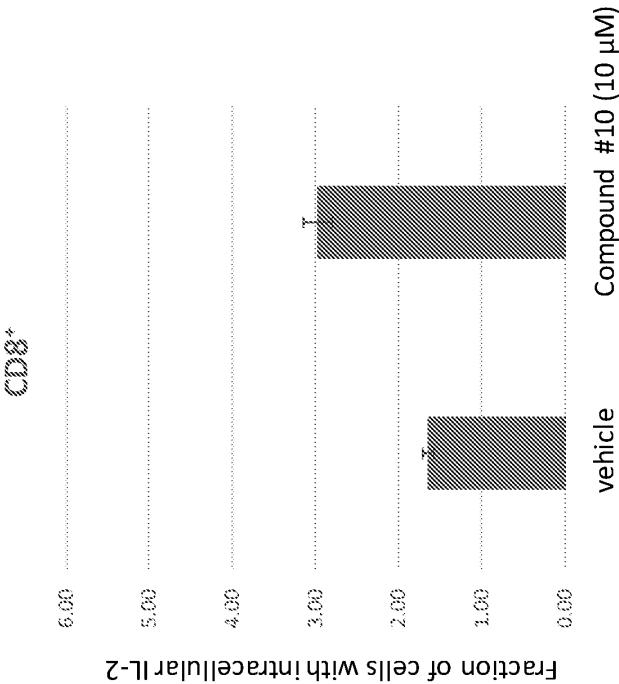


FIG. 34C

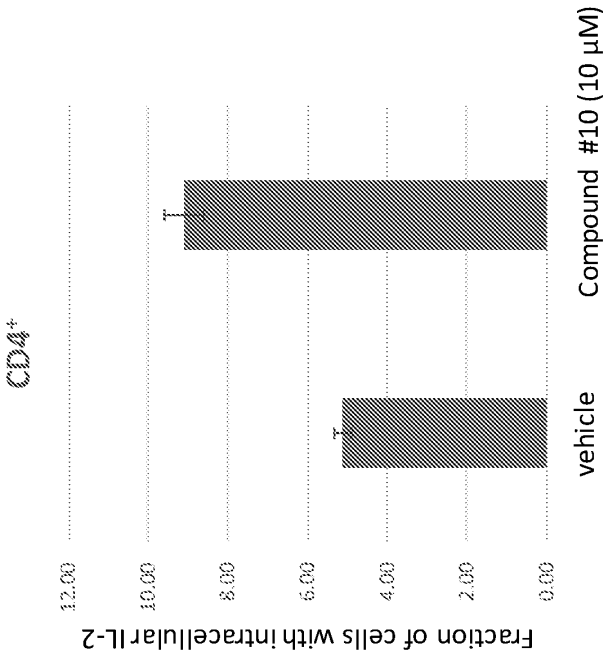


FIG. 34B

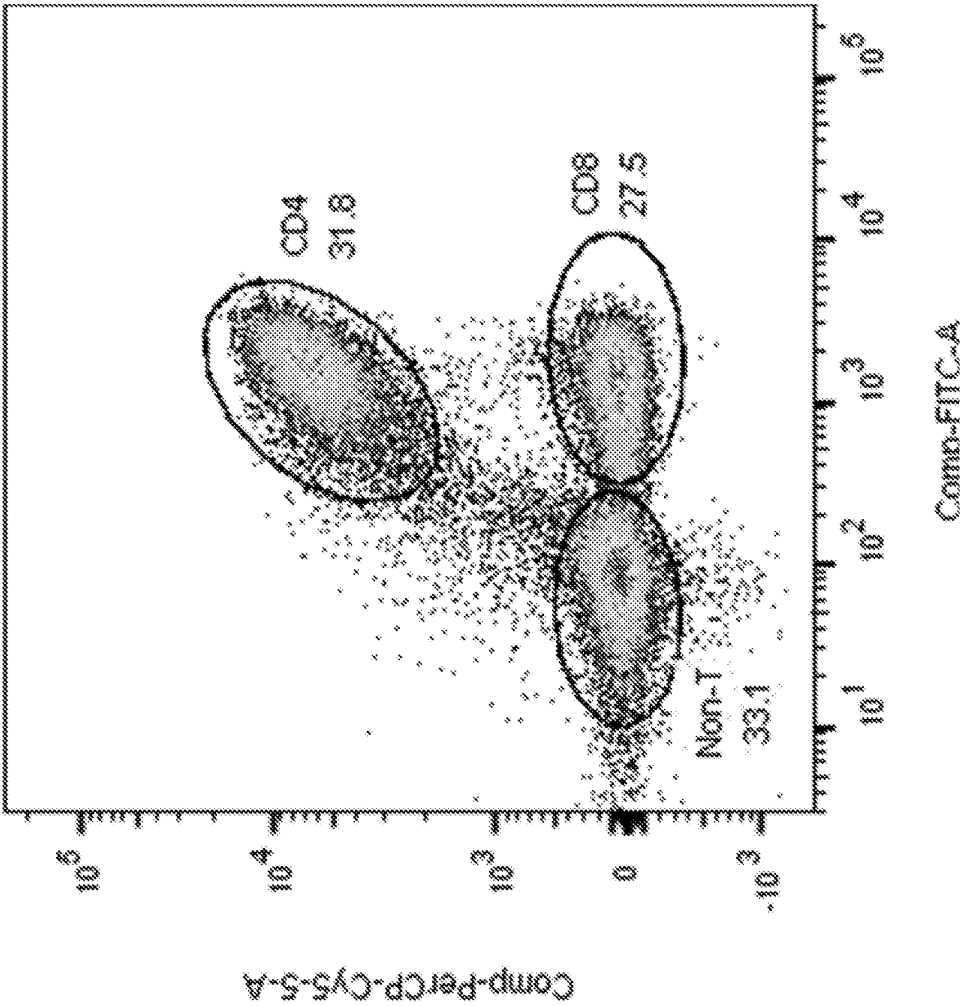


FIG. 35A

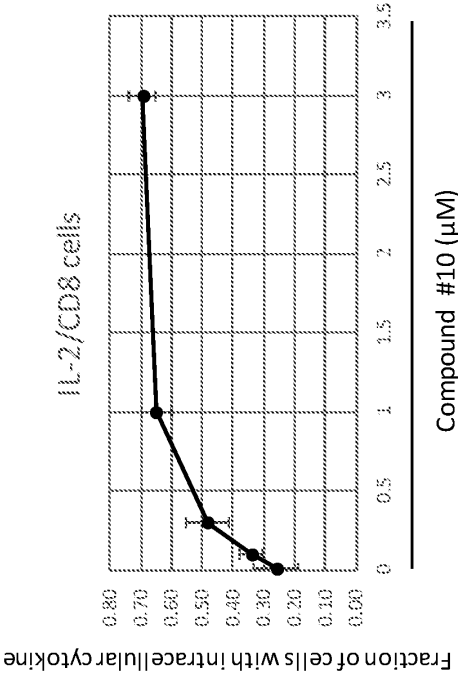


FIG. 35C

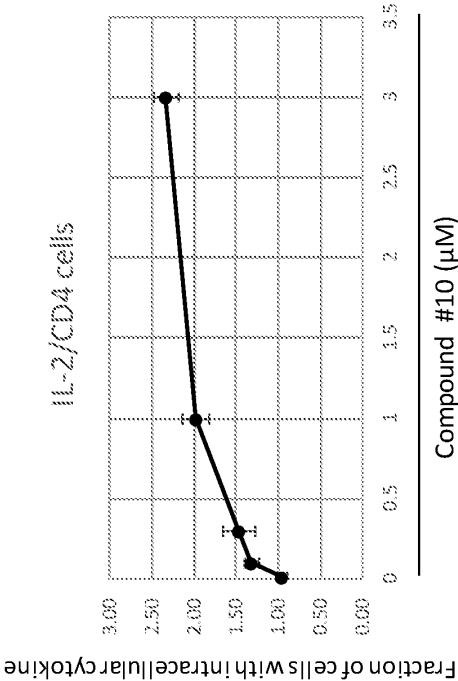


FIG. 35B

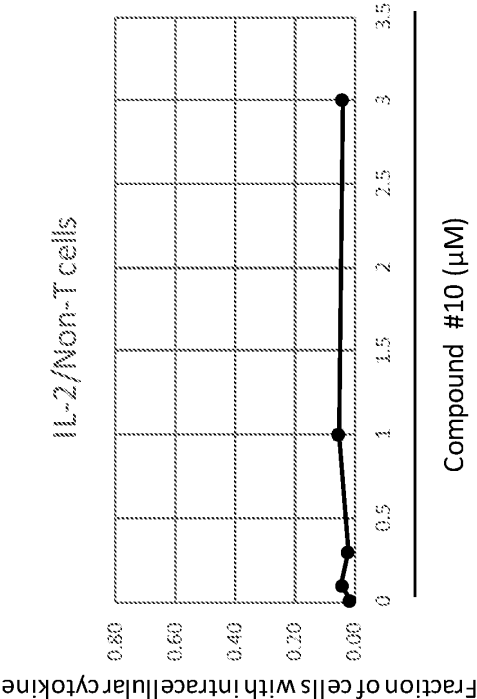


FIG. 35D

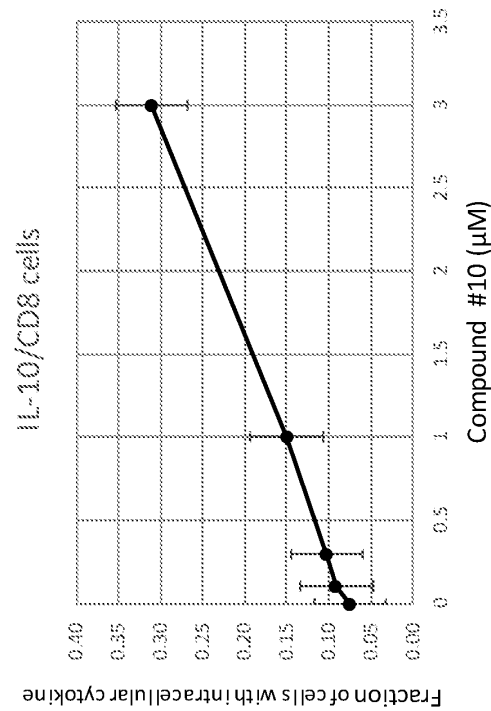


FIG. 35F

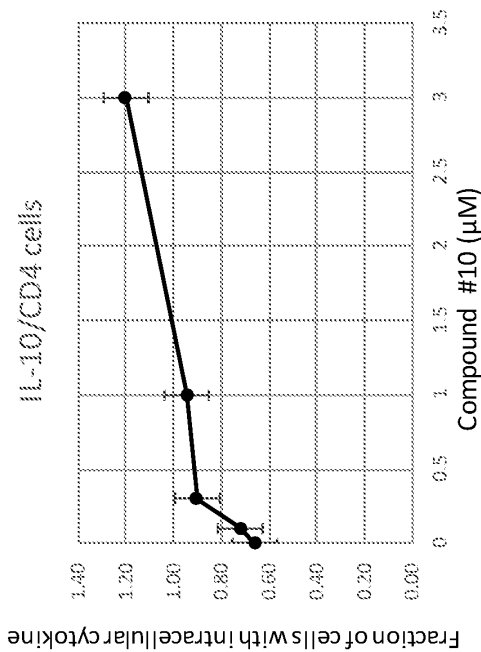


FIG. 35E

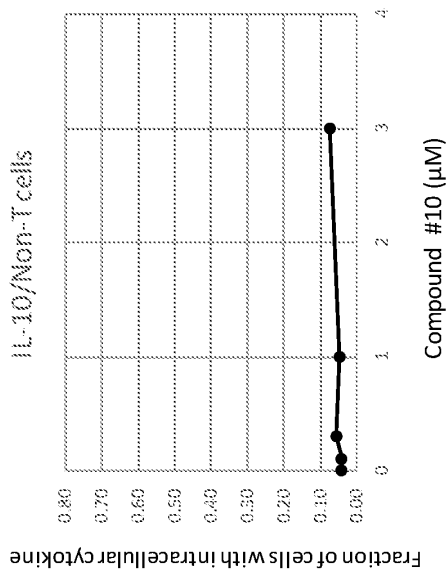
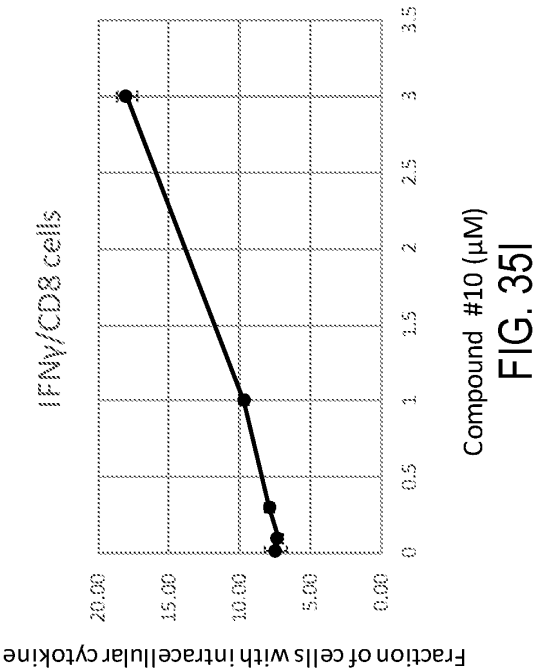
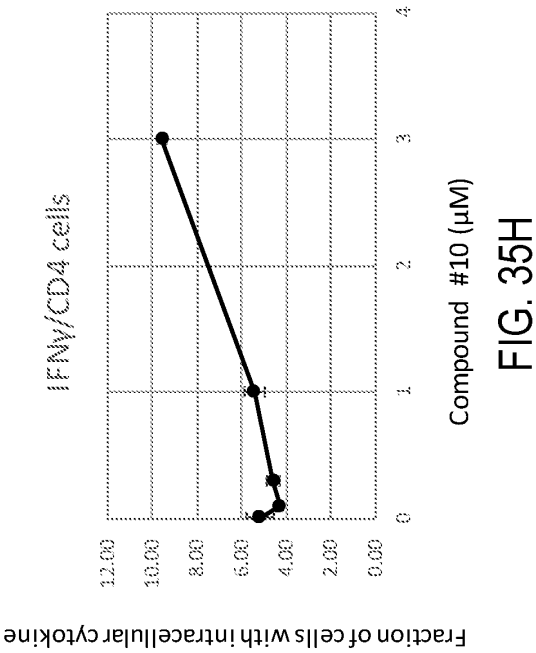
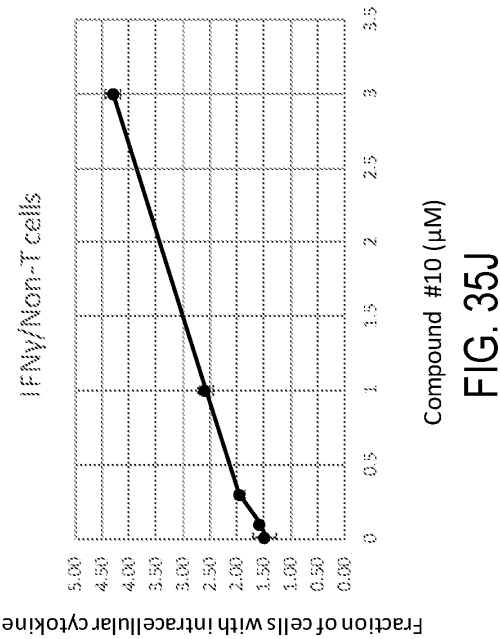


FIG. 35G



Induction of TNF- α by compound #10

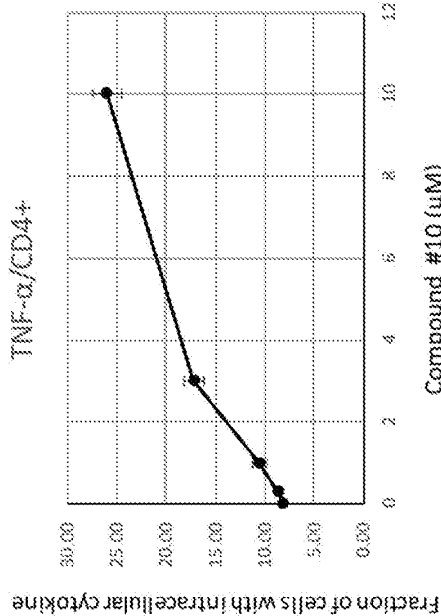


FIG. 35K

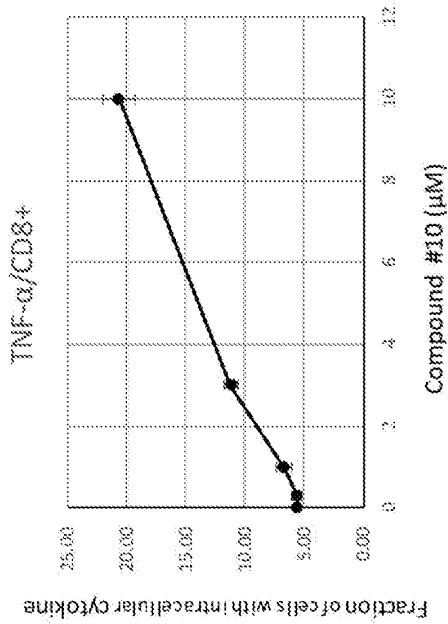


FIG. 35L

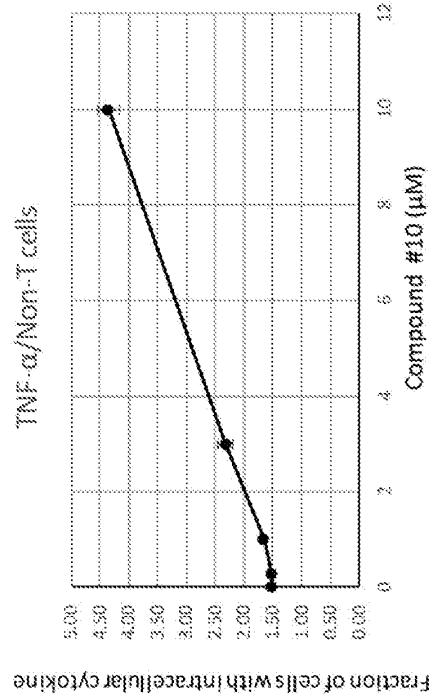


FIG. 35M

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Additional analysis of non-T cell population from FIG. 35A

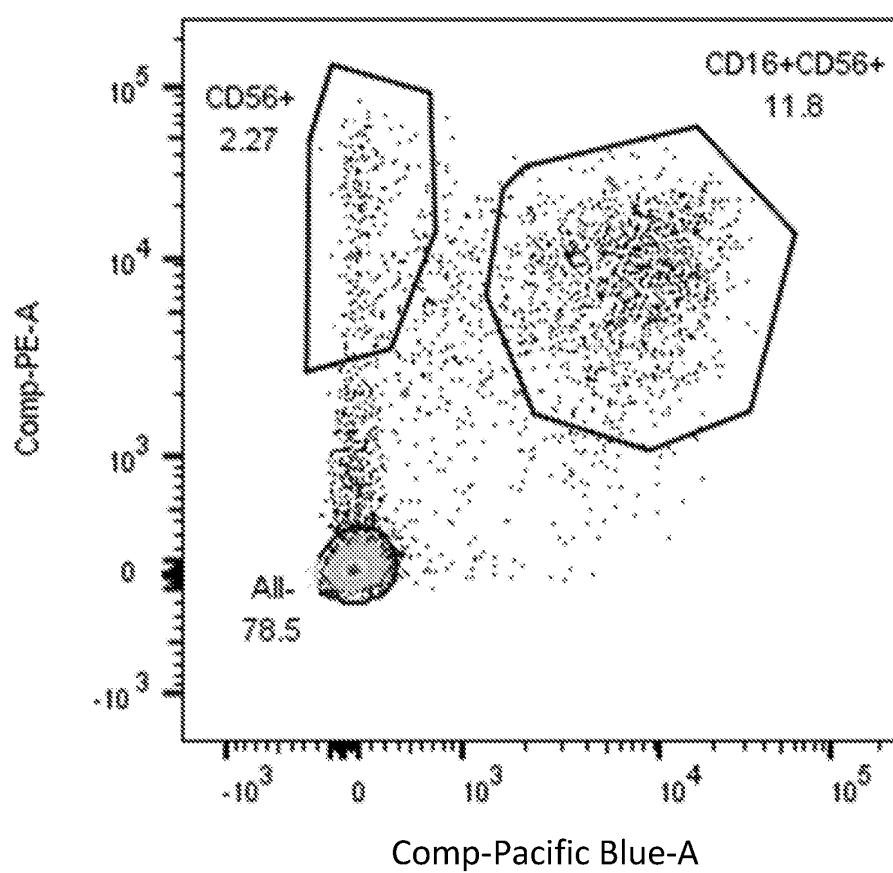


FIG. 36A

Induction of IFN γ by compound #10 in NK cells

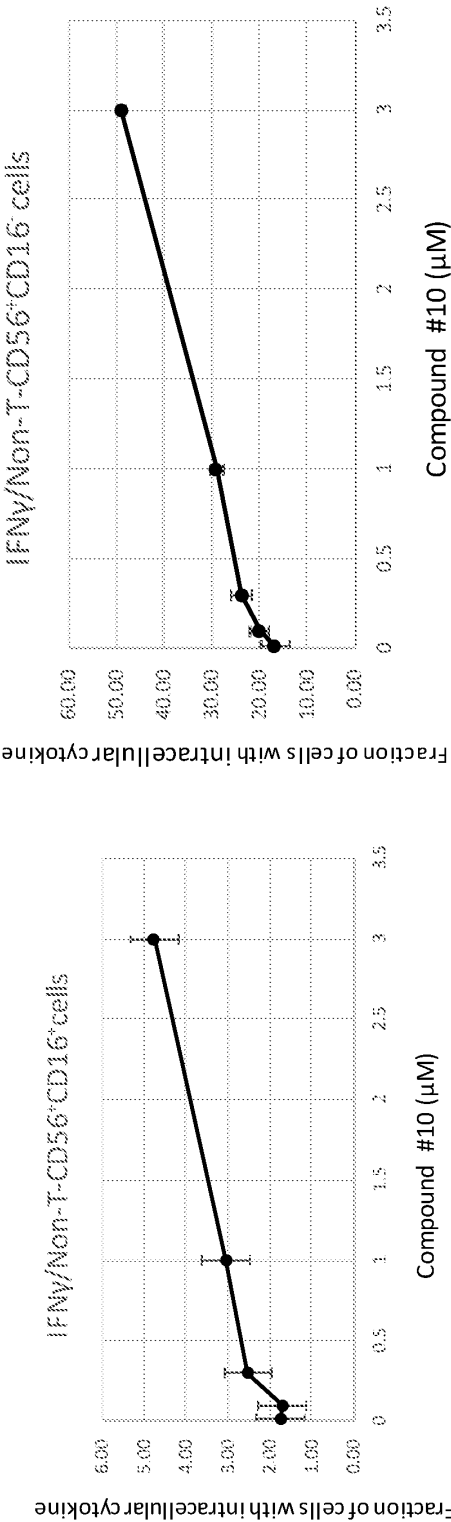
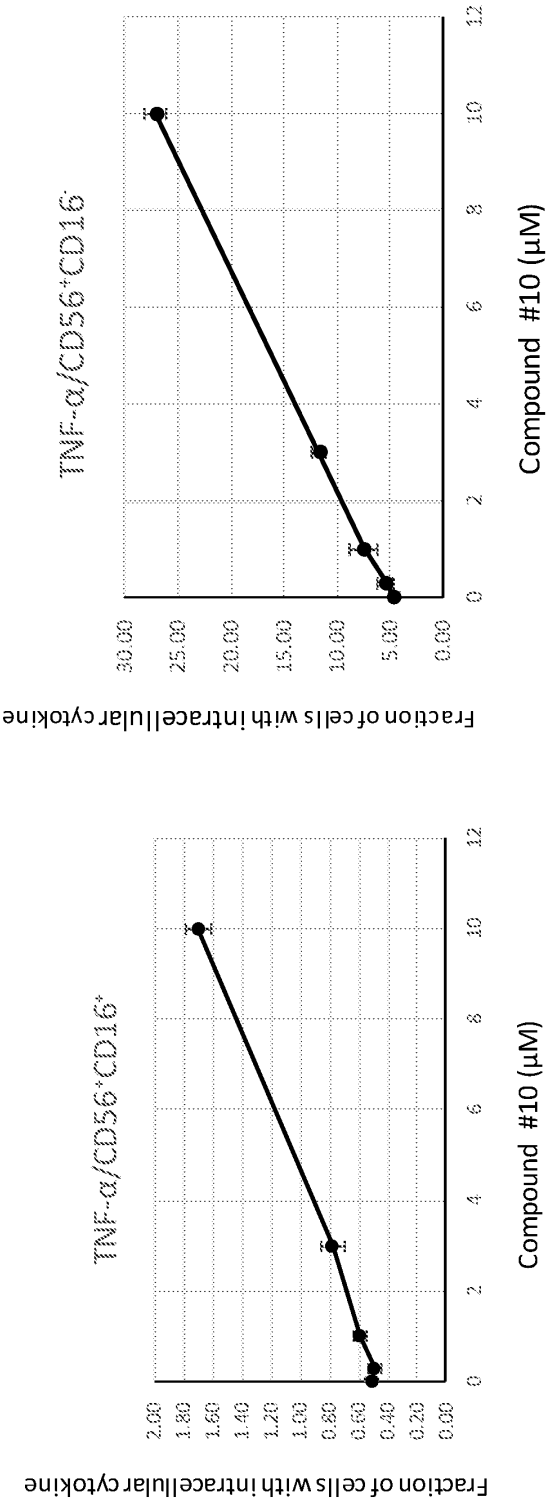


FIG. 36C

FIG. 36B

Induction of TNF- α by compound #10 in NK cells



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Effect of GPR174 on in Vivo-Expanded Flu-Specific CD8⁺ Cells
Upon Flu PR8 Infection of Mice

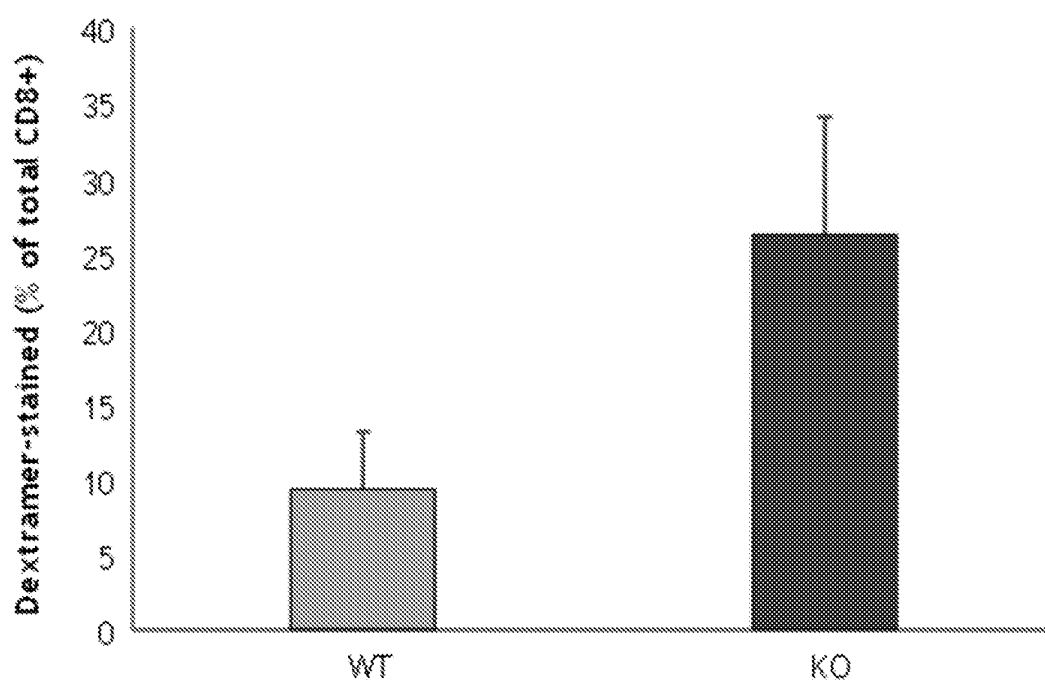


FIG. 37

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Dextramer Staining

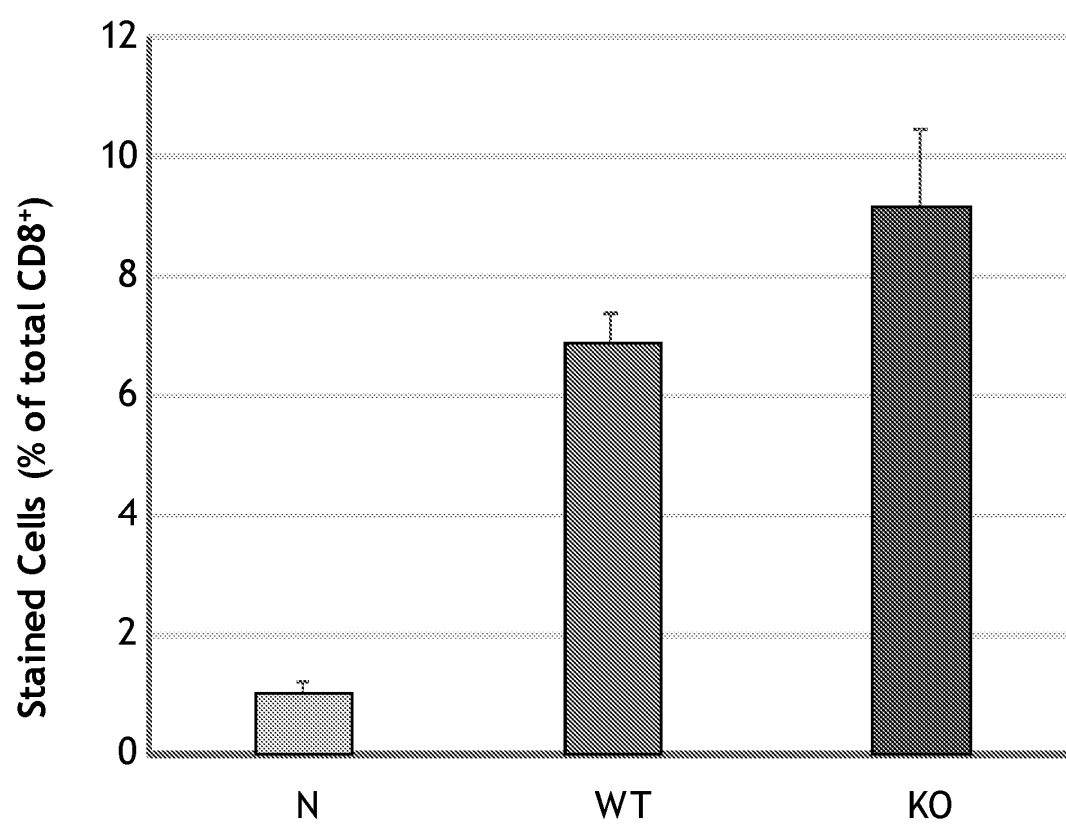


FIG. 38

Effect of GPR174i on CTLA4 Expression on Human Naïve T Cells
(one day post-stimulation)

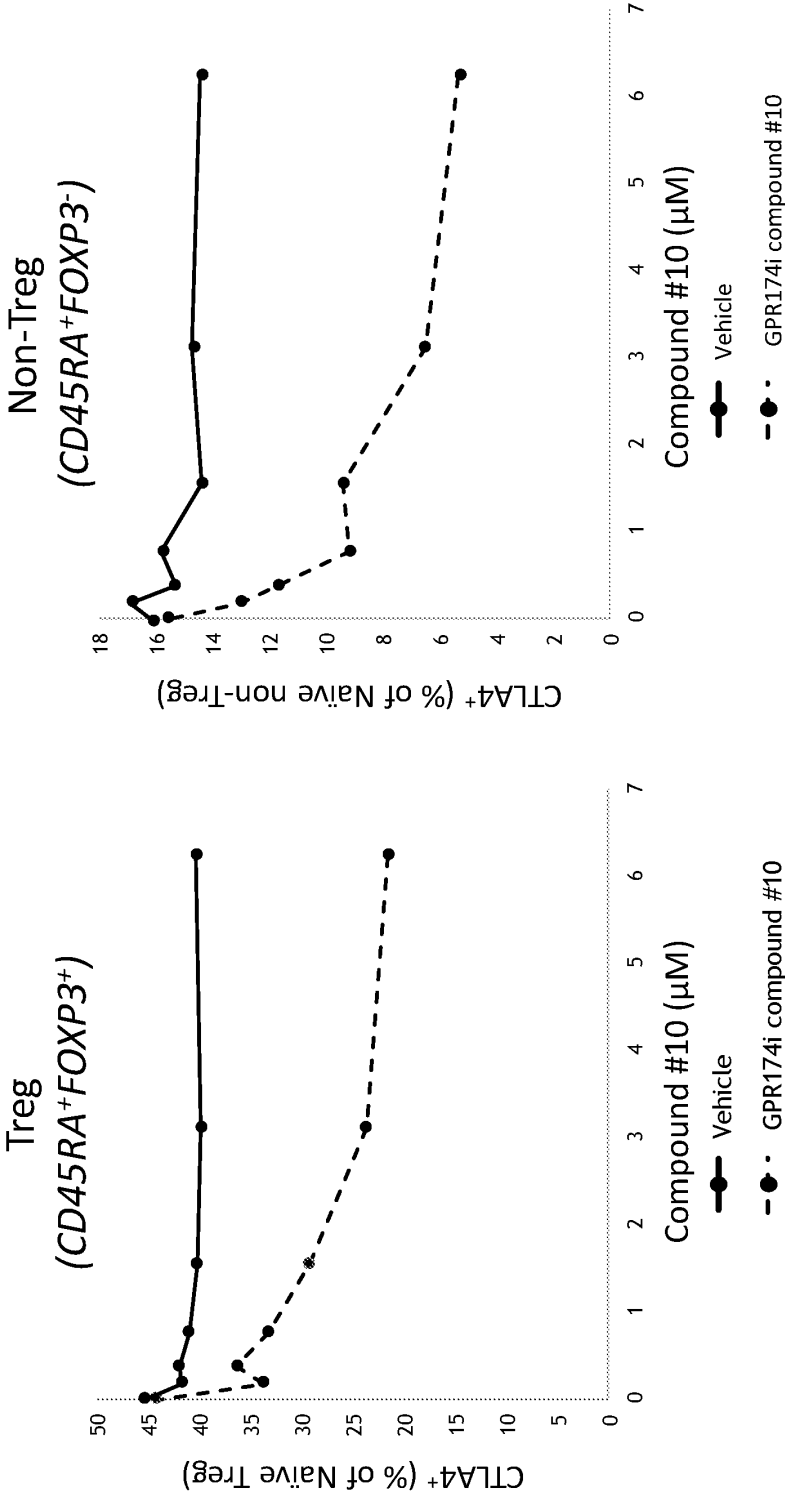


FIG. 39A

FIG. 39B

Effect of GPR174i on CTLA4 Expression on Human Memory T Cells
(one day post-stimulation)

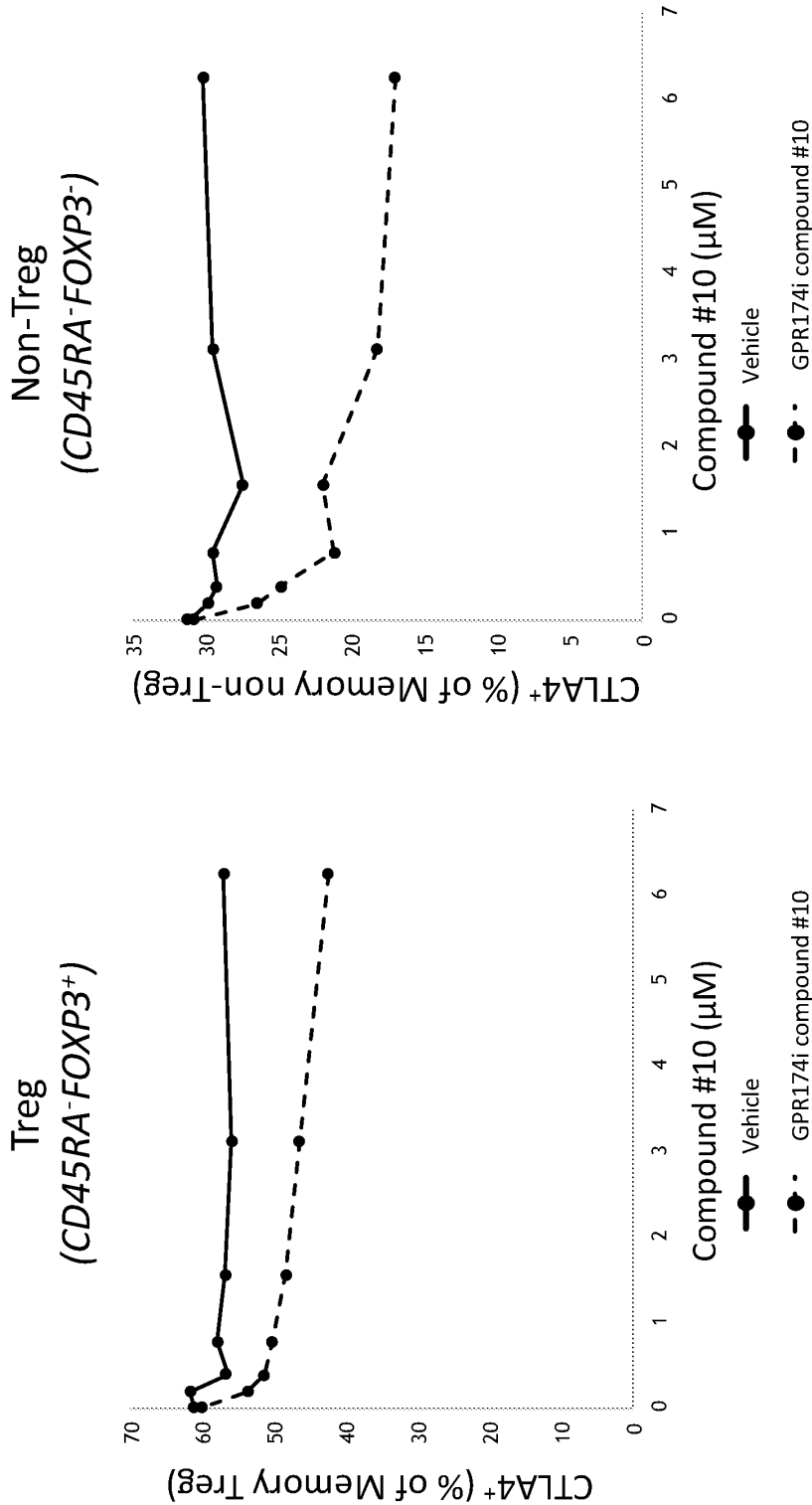


FIG. 40A

FIG. 40B

Effect of GPR174i on PD-L1 Expression on Human CD4 T Cells
(one day post-stimulation)

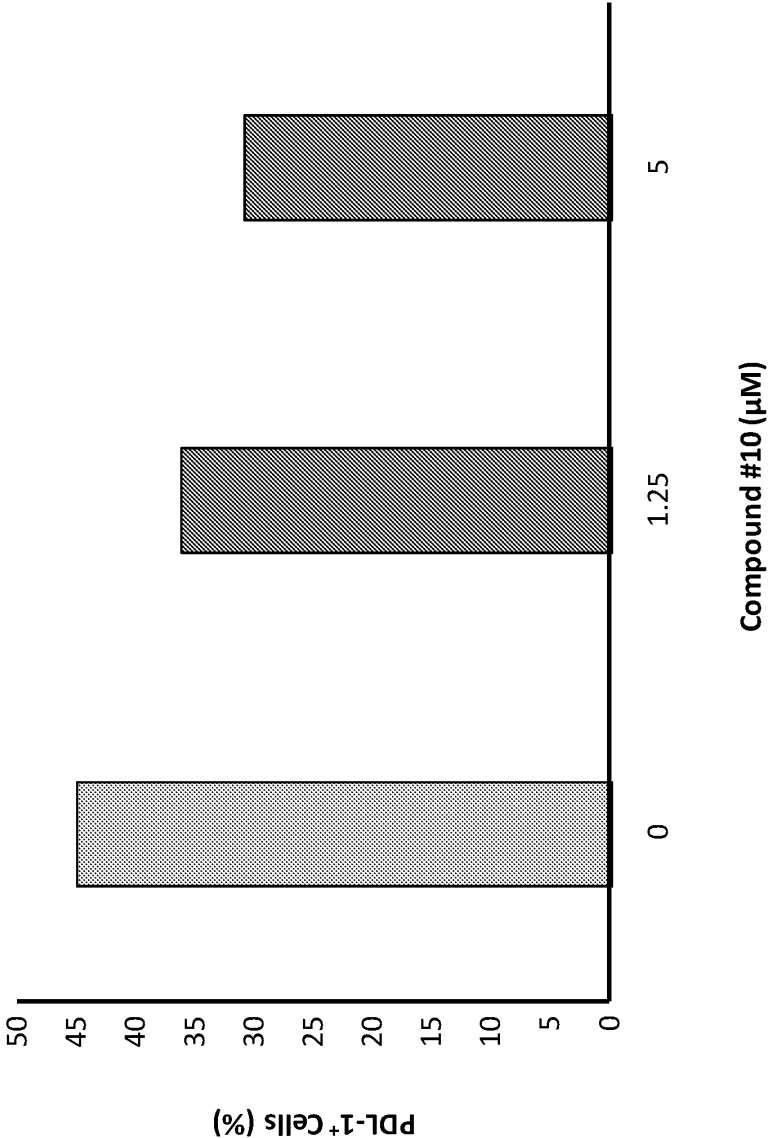


FIG. 41

**Effect of GPR174i on TIGIT Expression on Human CD8 T Cells
(one day post-stimulation)**

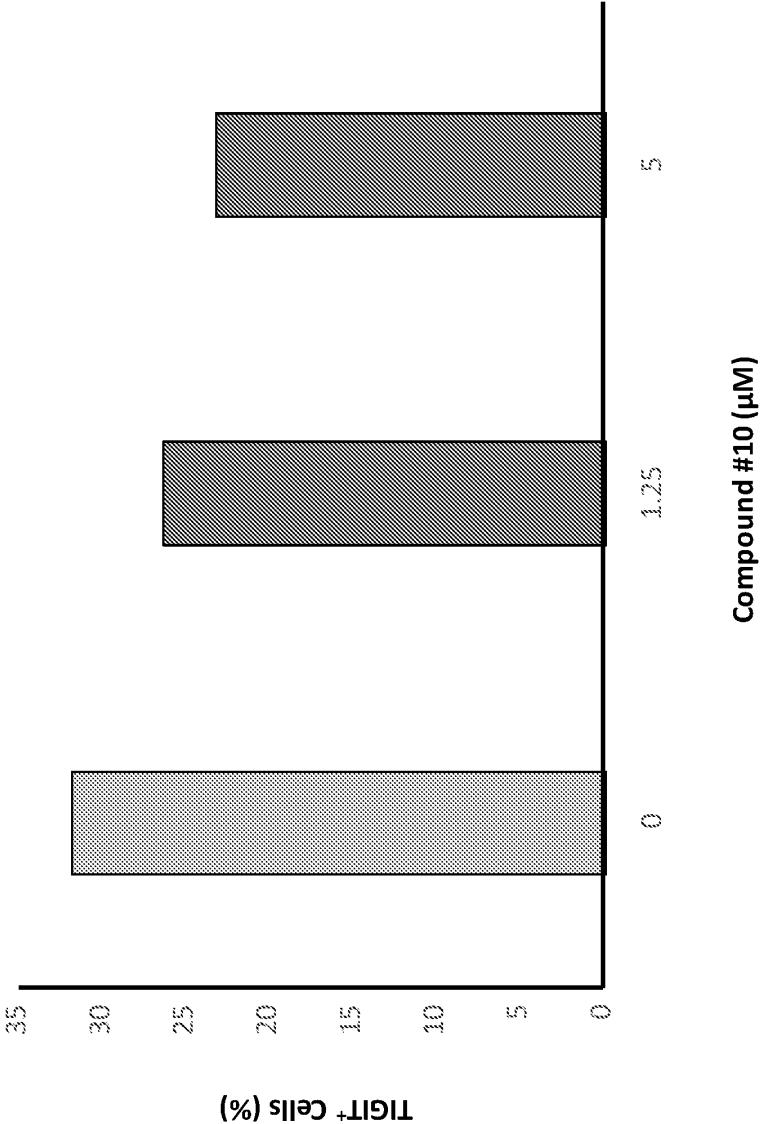


FIG. 42

**Effect of GPR174i on AREG Expression on Human CD4 T Cells
(one day post-stimulation)**

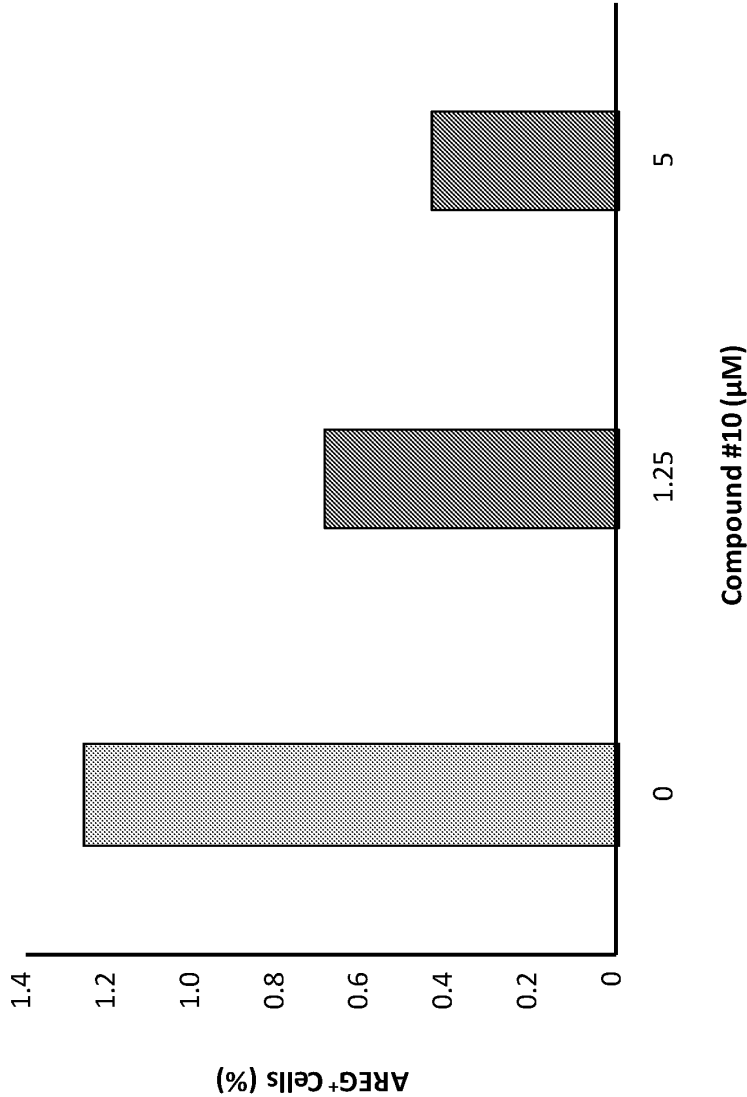


FIG. 43

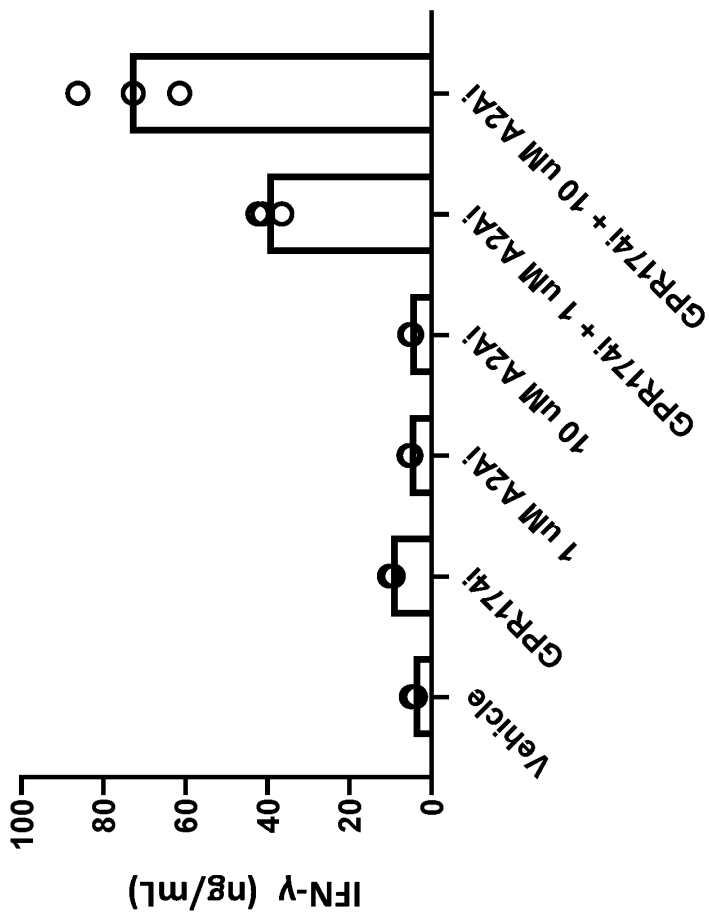


FIG. 44

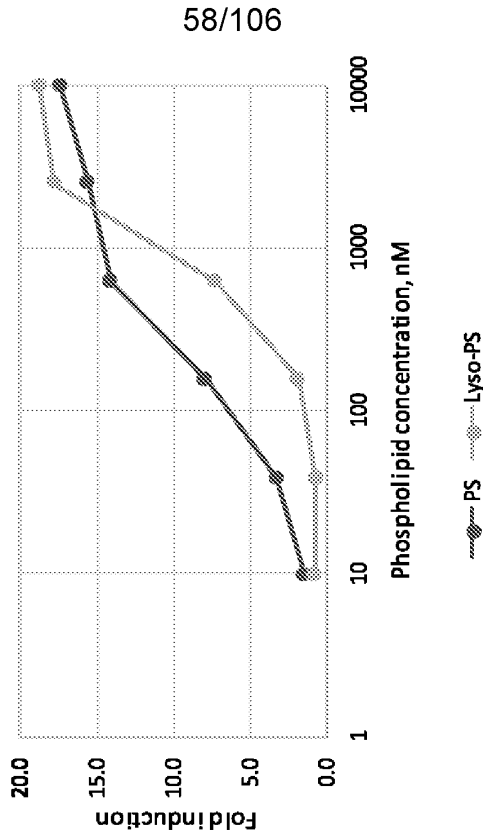


FIG. 45B

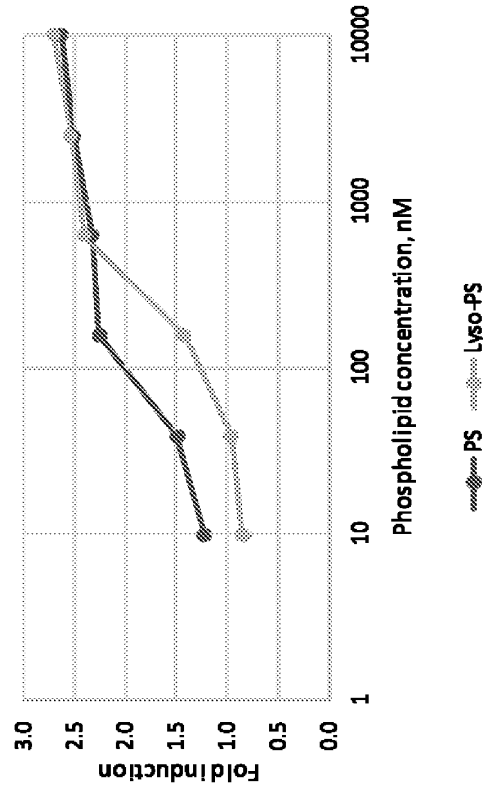


FIG. 45A

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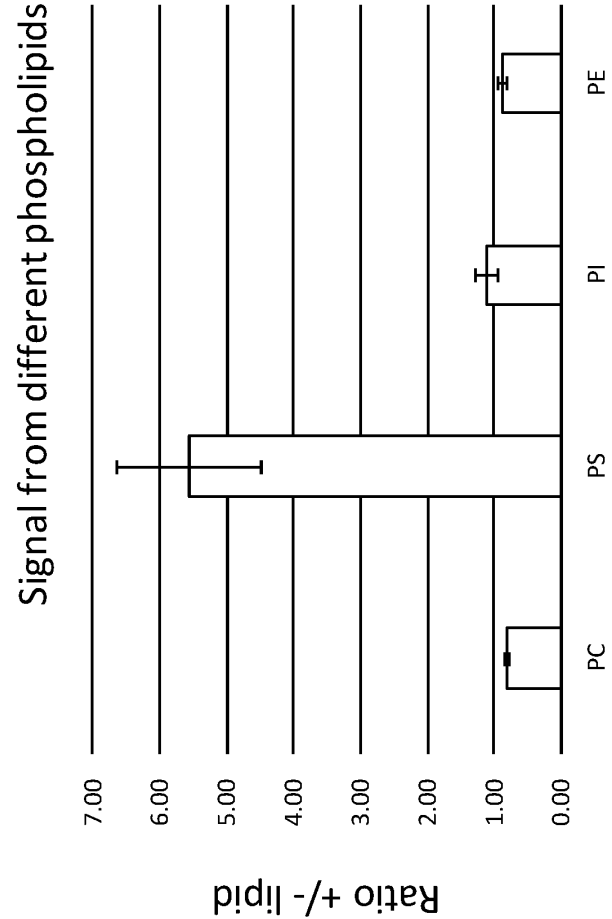


FIG. 46

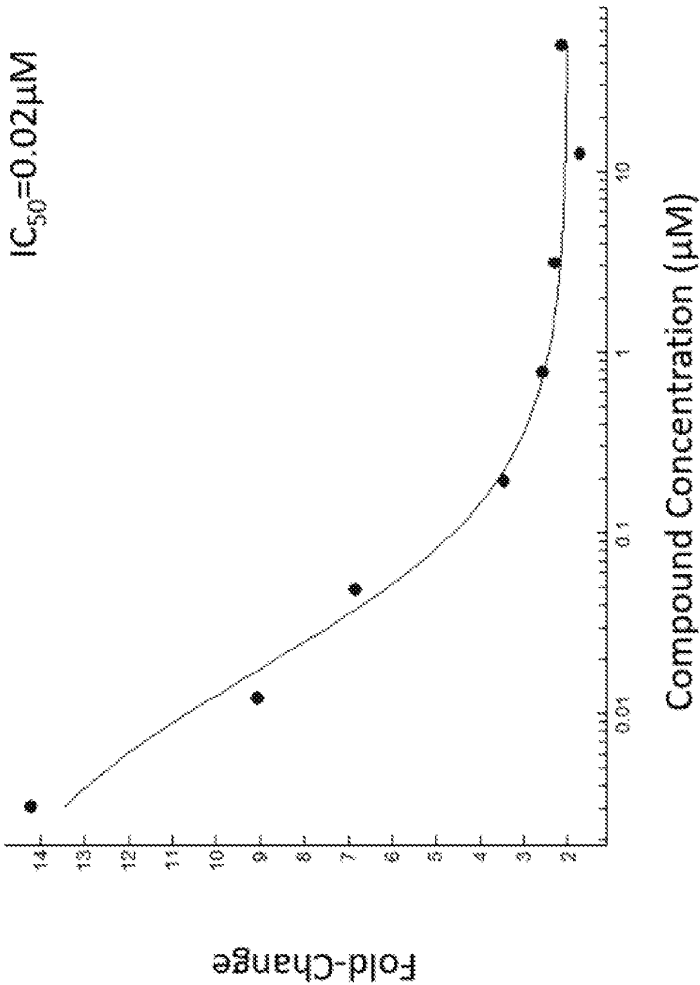


FIG. 47A

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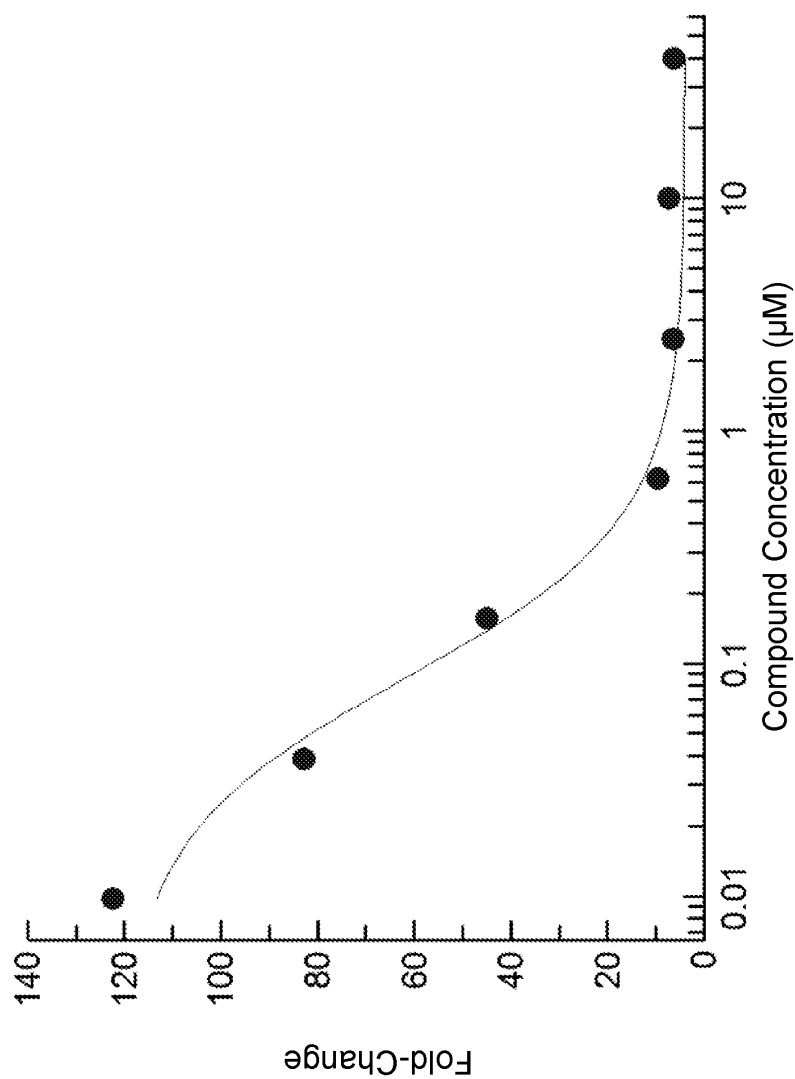


FIG. 47B

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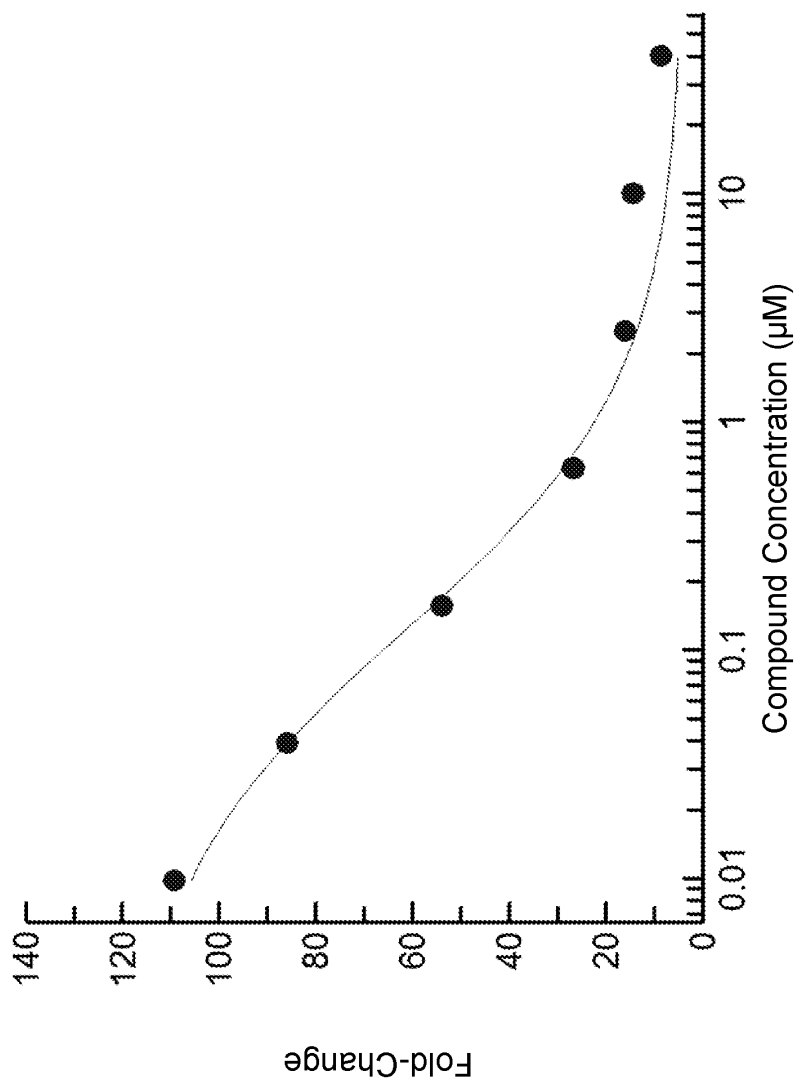


FIG. 47C

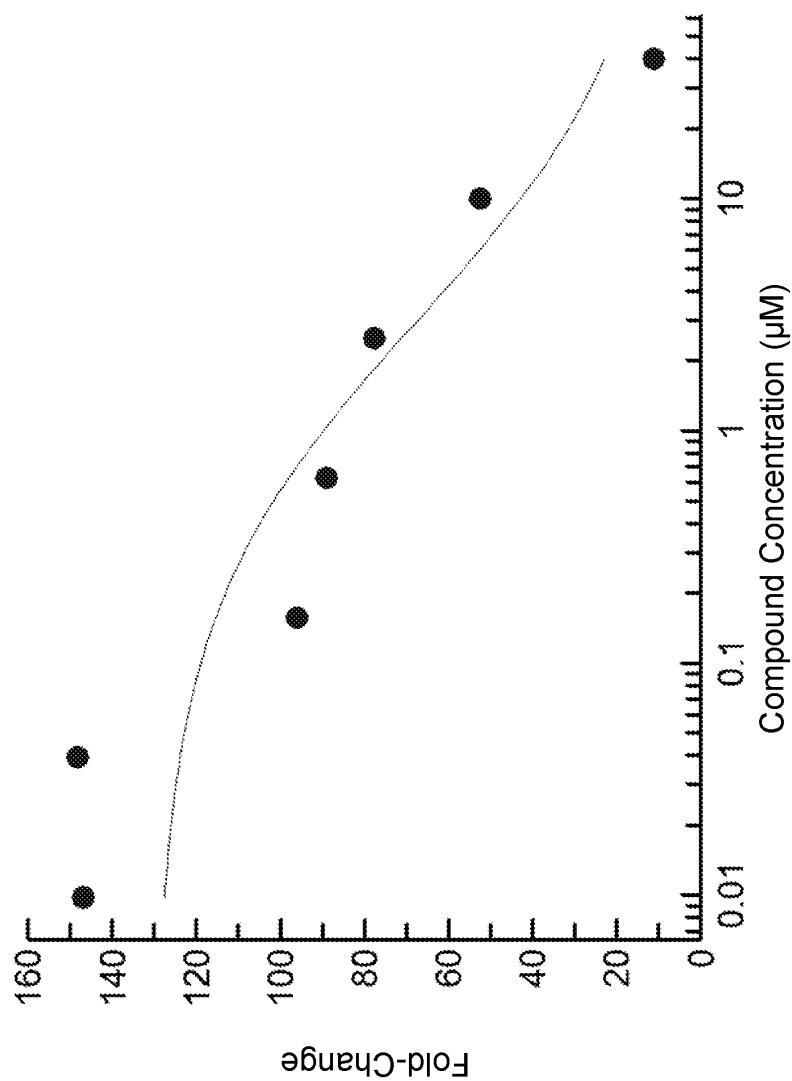


FIG. 47D

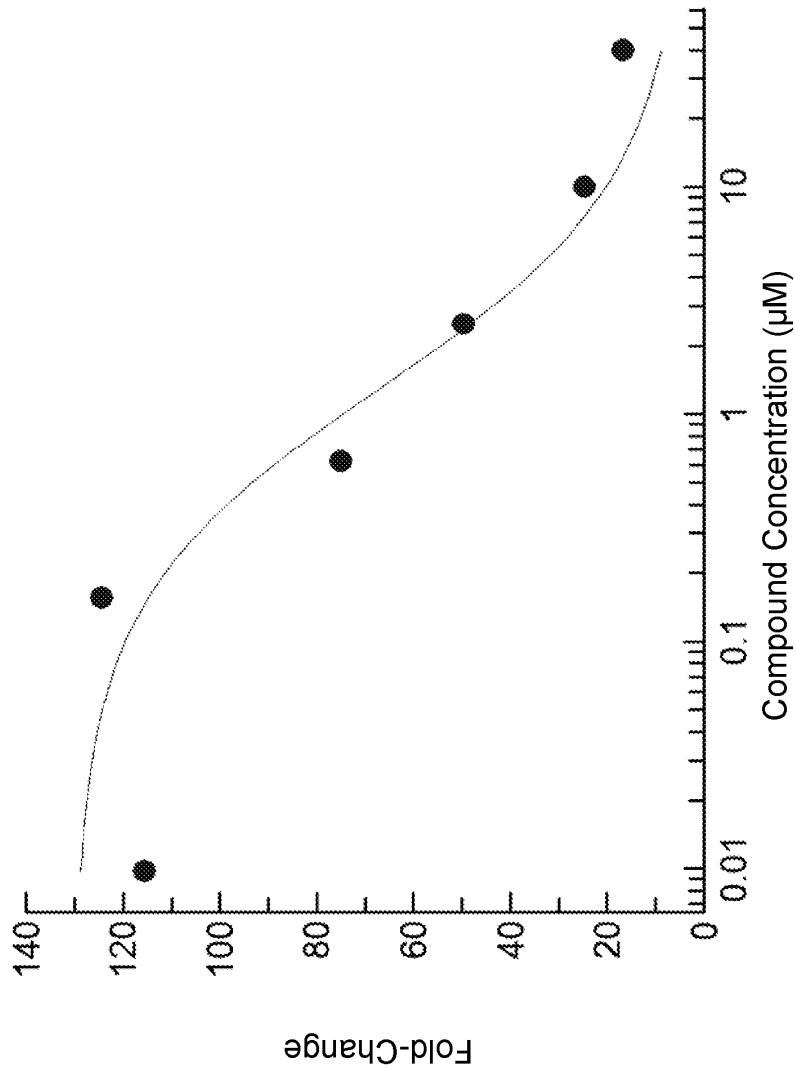


FIG. 47E

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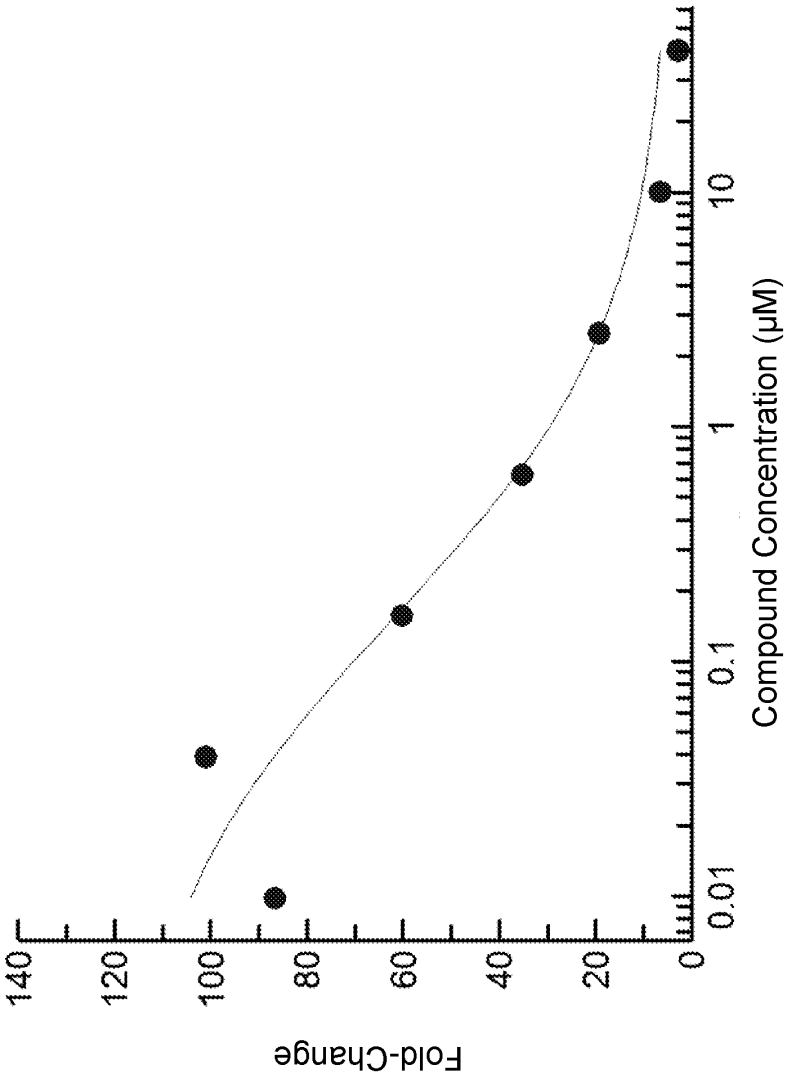


FIG. 47F

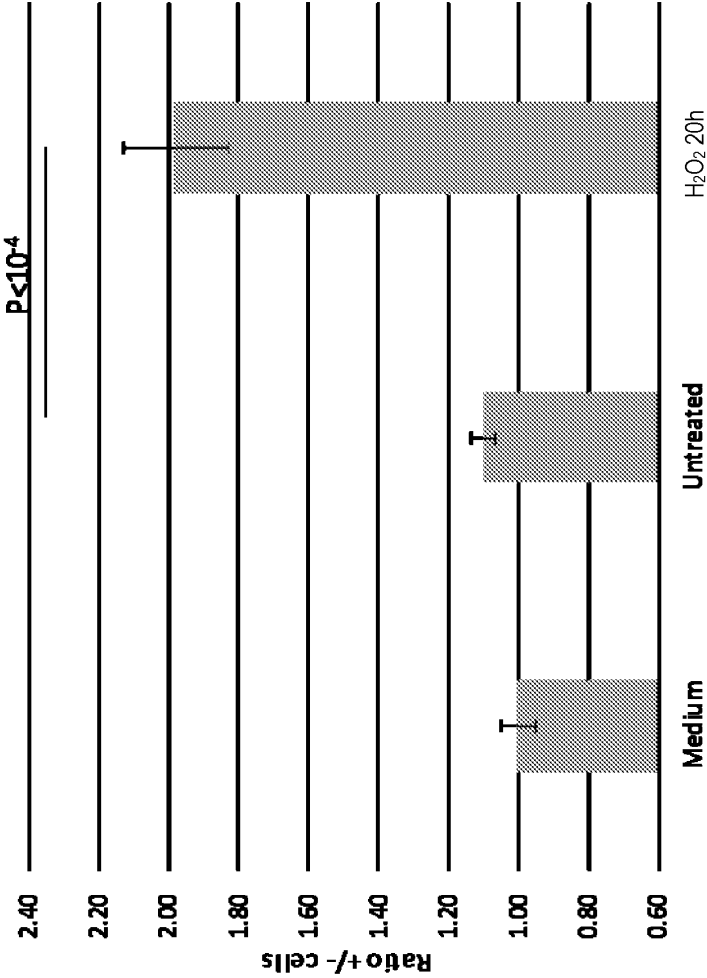


FIG. 48A

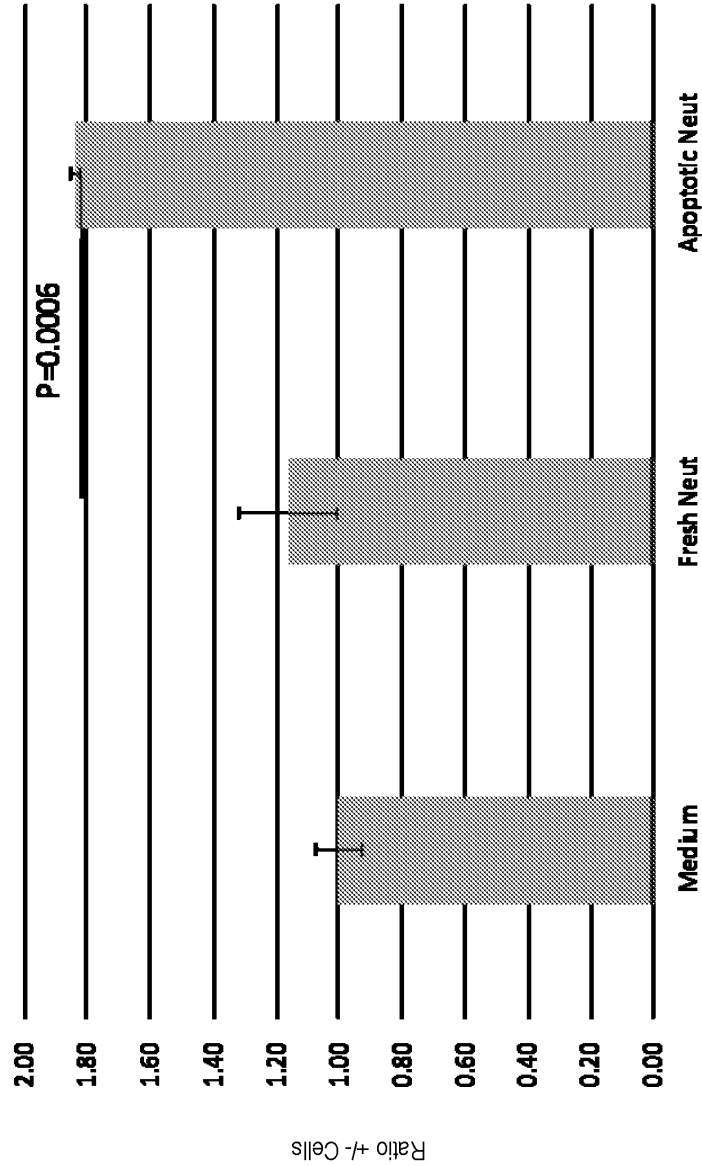


FIG. 48B

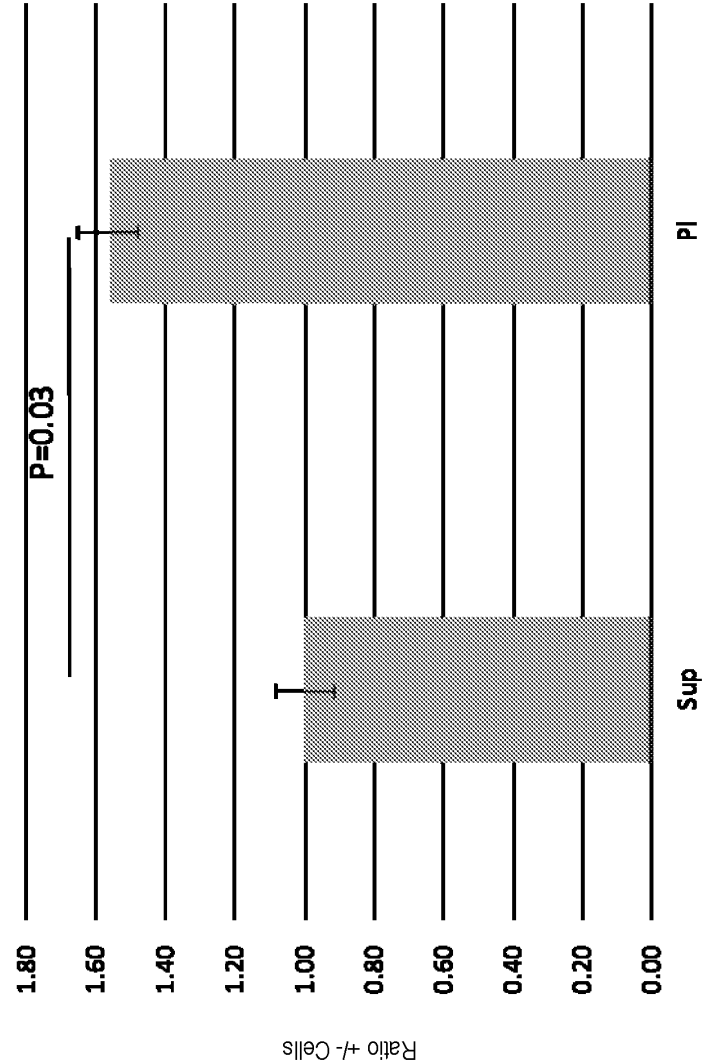


FIG. 48C

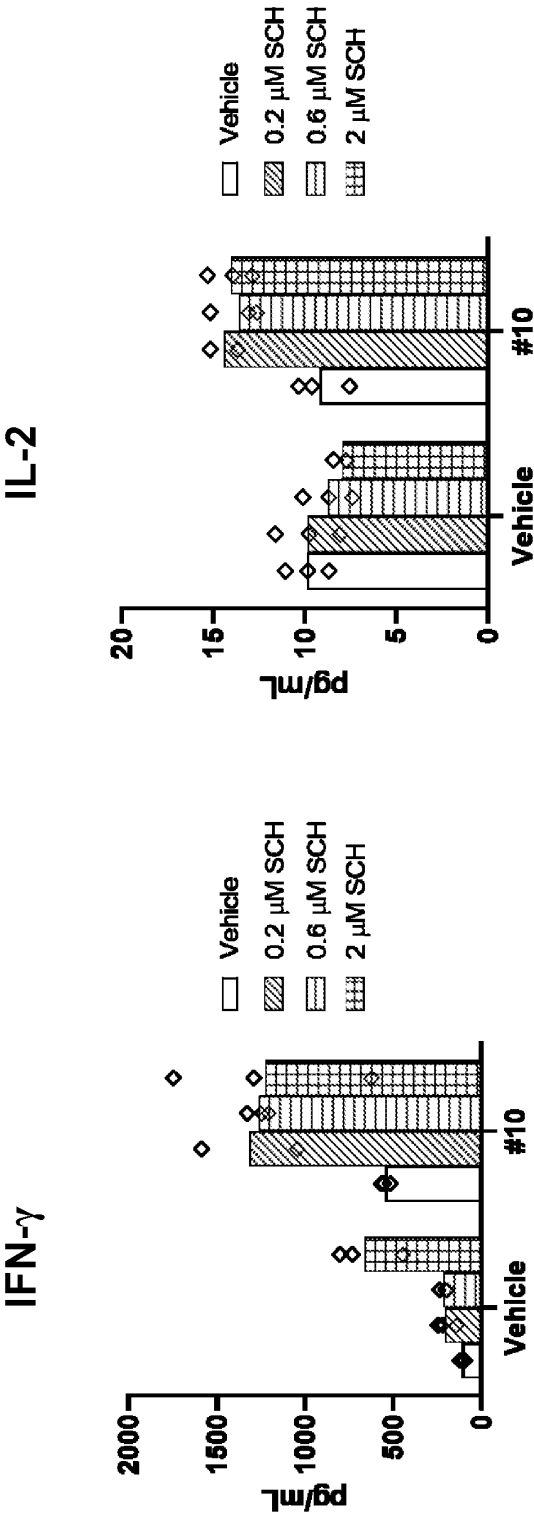


FIG. 49B

FIG. 49A

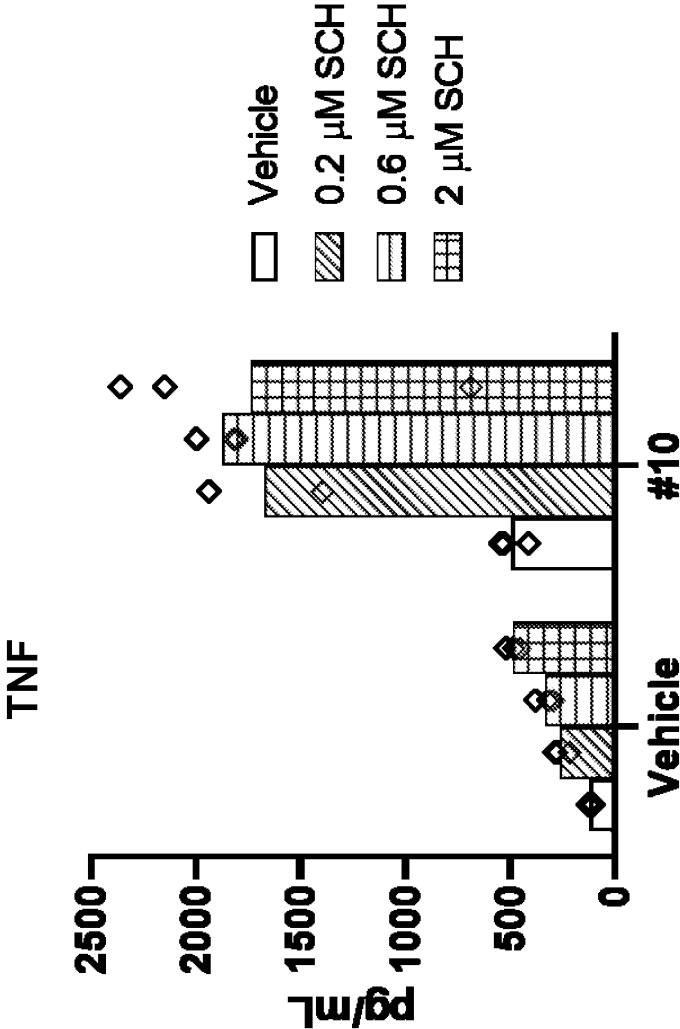


FIG. 49C

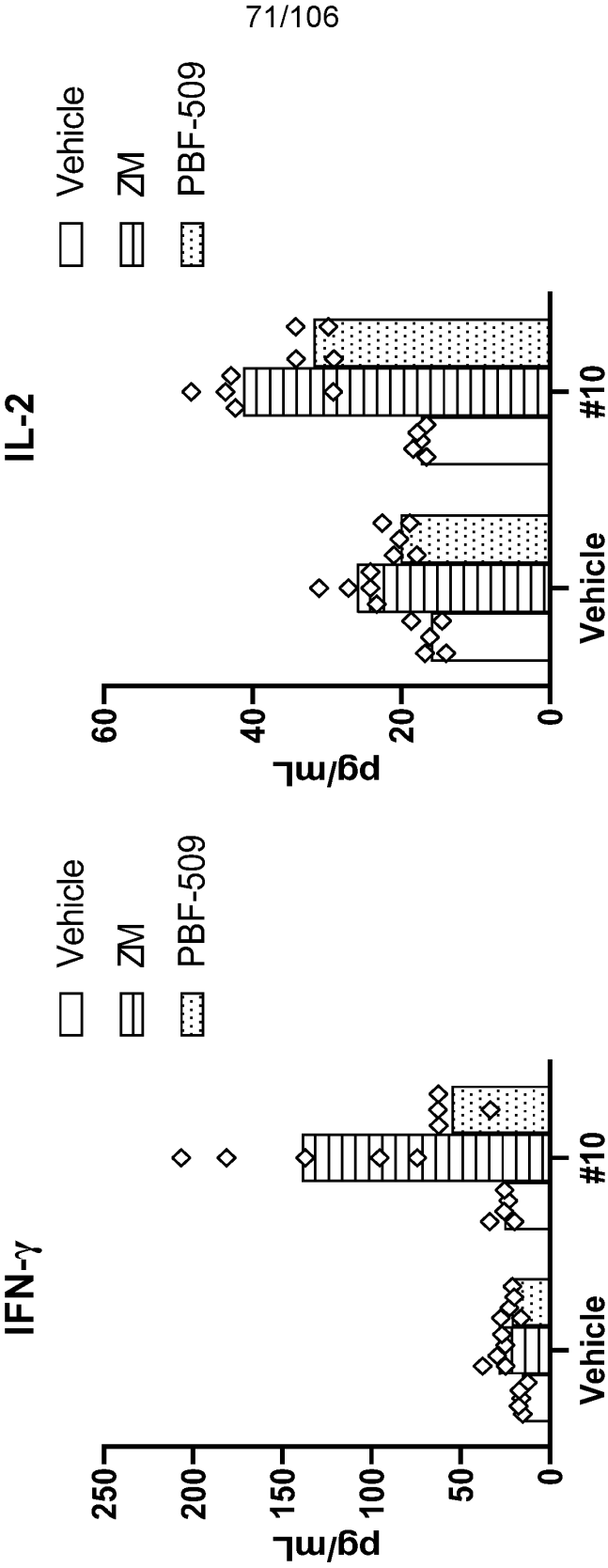
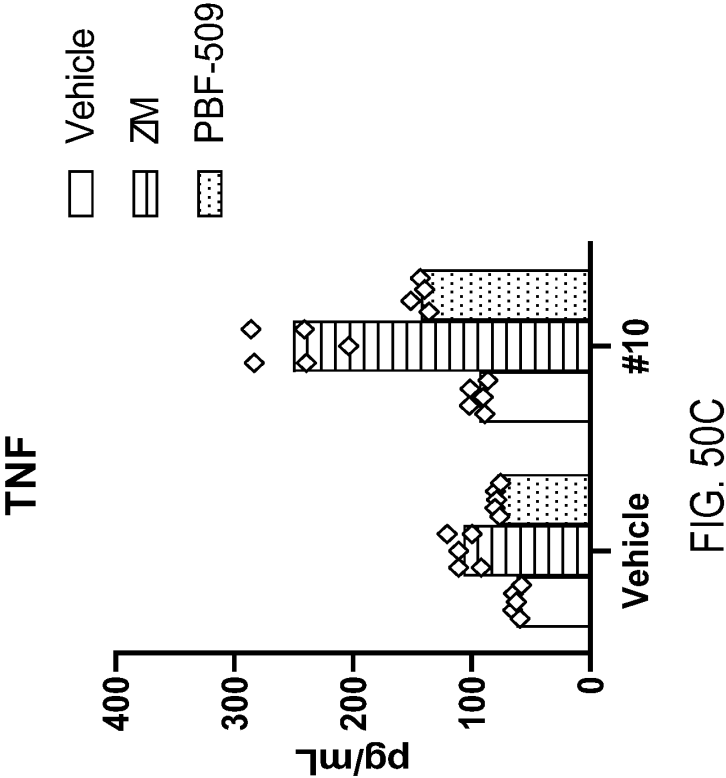


FIG. 50A

FIG. 50B



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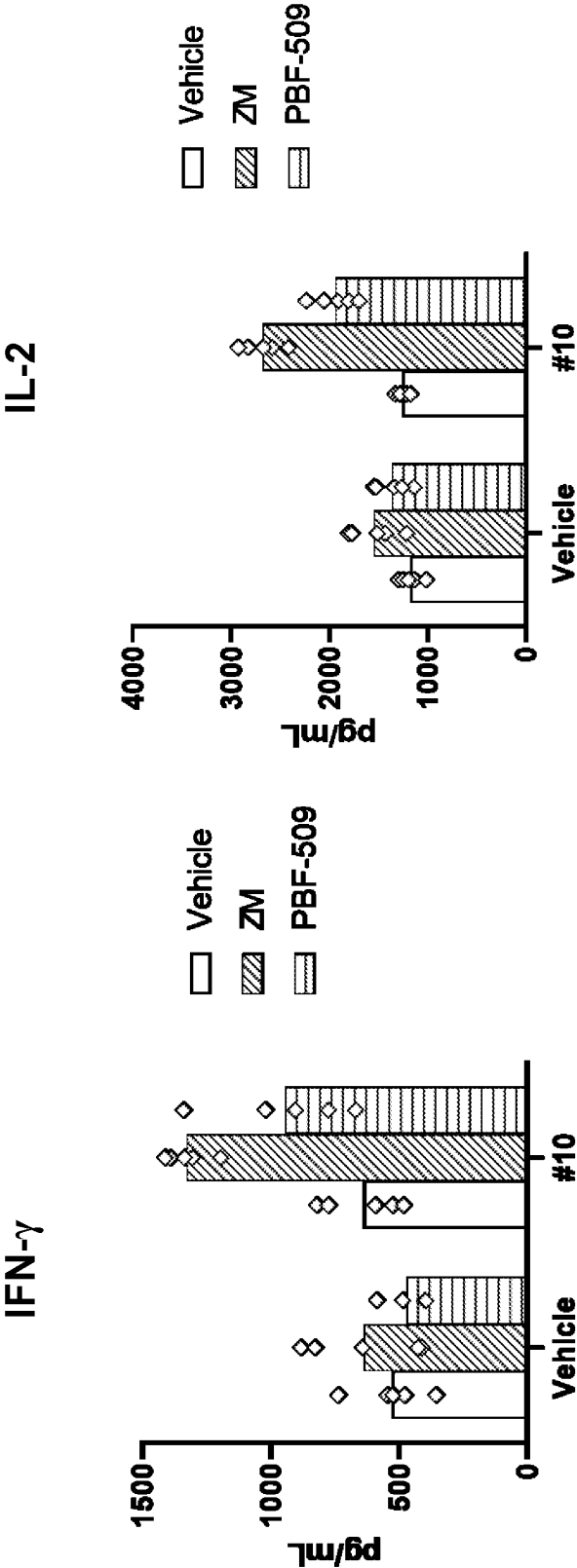


FIG. 51B

FIG. 51A

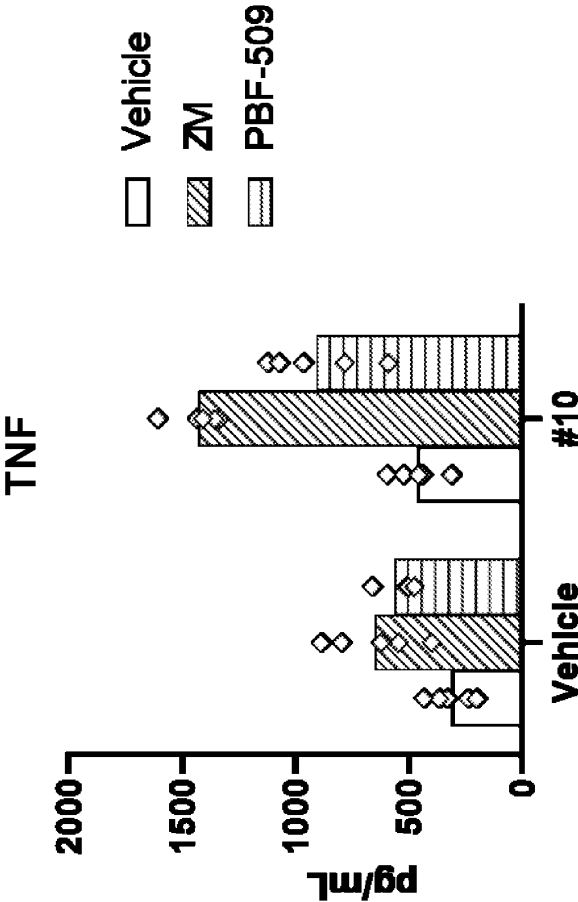


FIG. 51C

IL-2

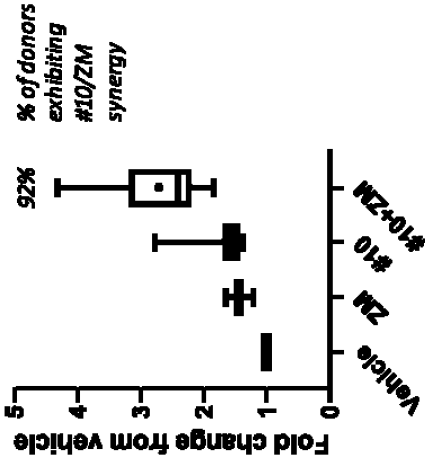


FIG. 52B

IFN- γ

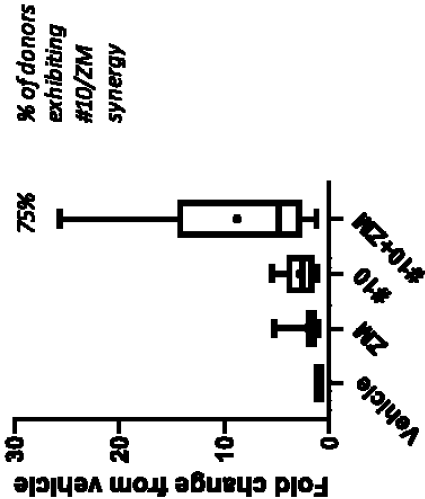


FIG. 52A

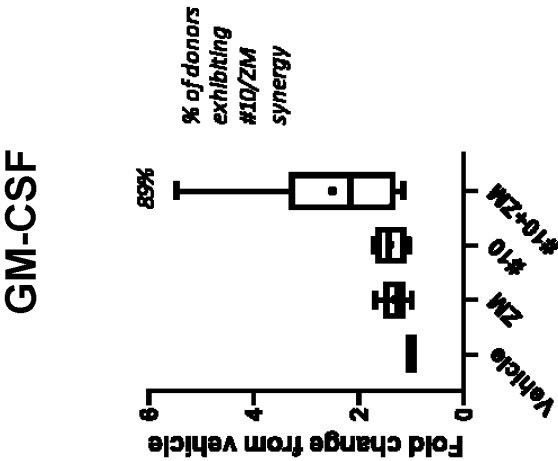


FIG. 52D

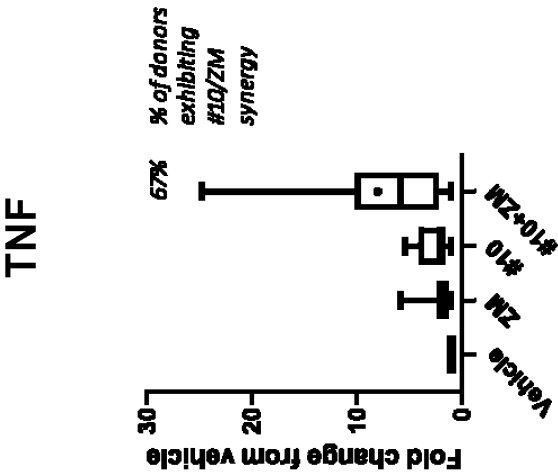


FIG. 52C

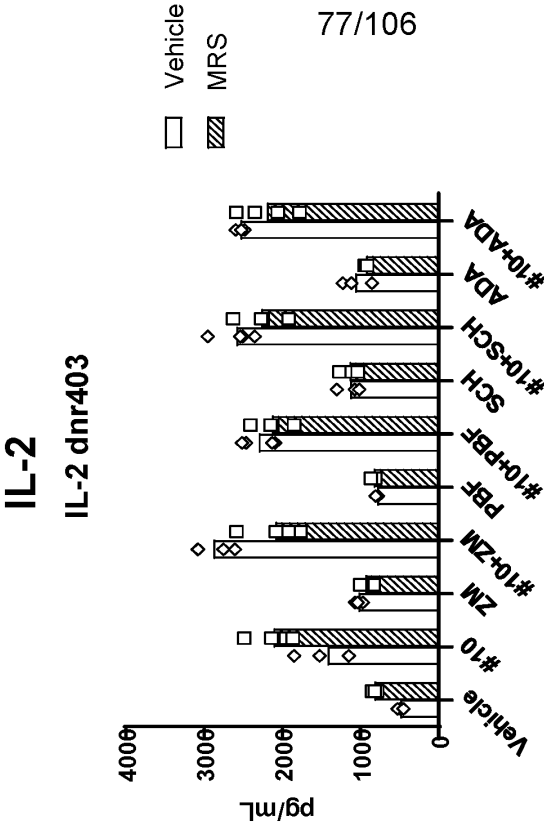


FIG. 53B

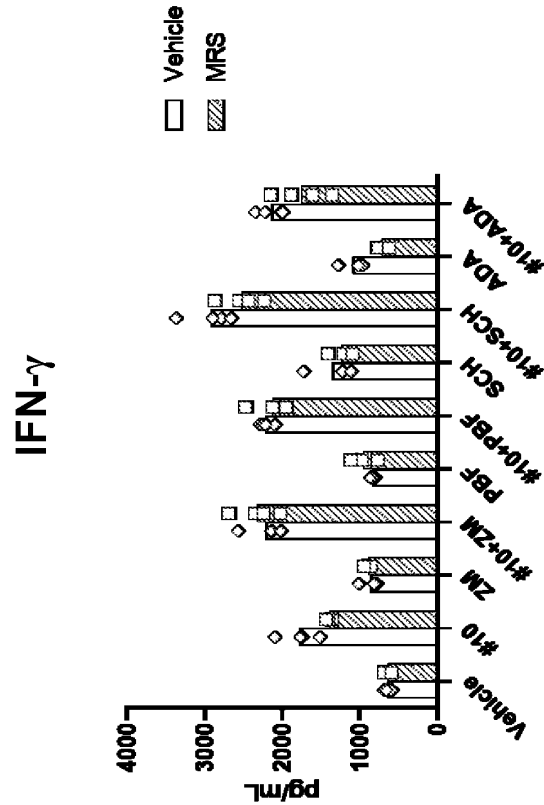


FIG. 53A

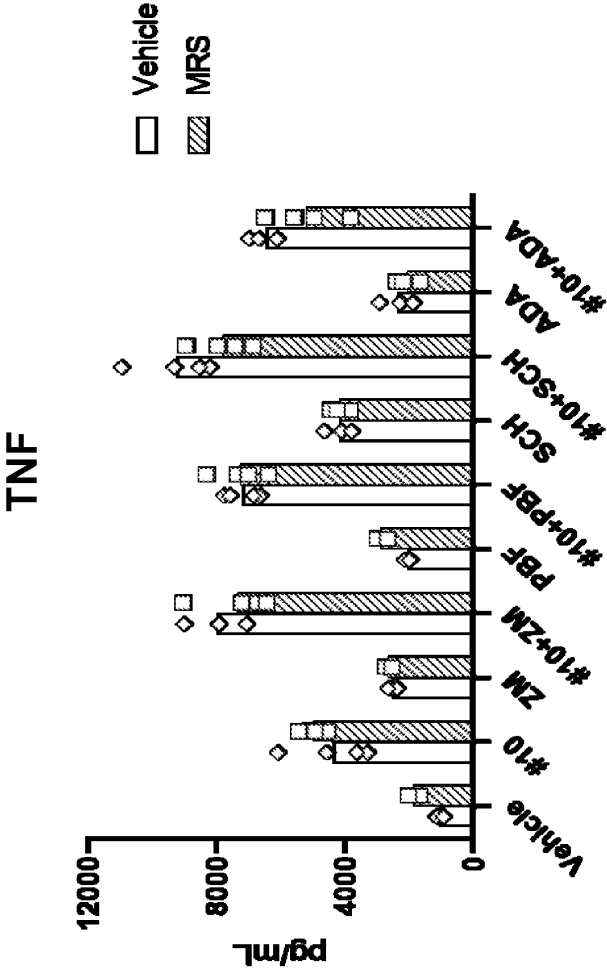


FIG. 53C

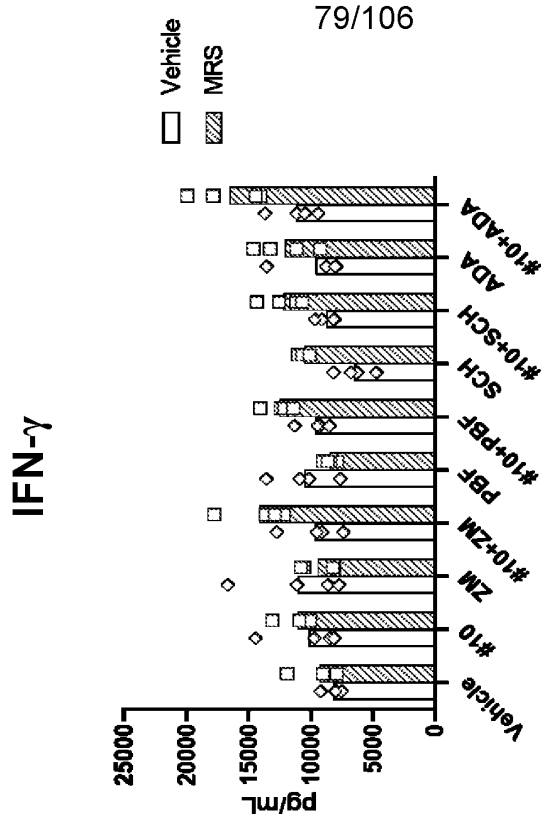


FIG. 54B

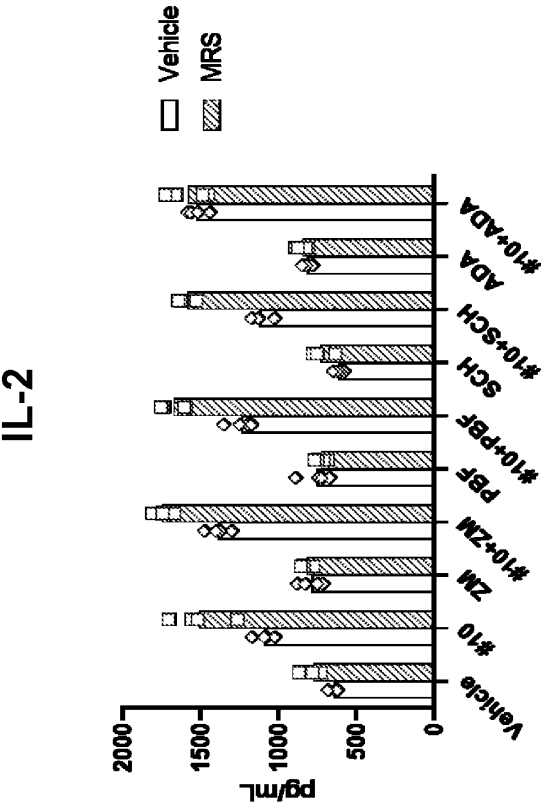


FIG. 54A

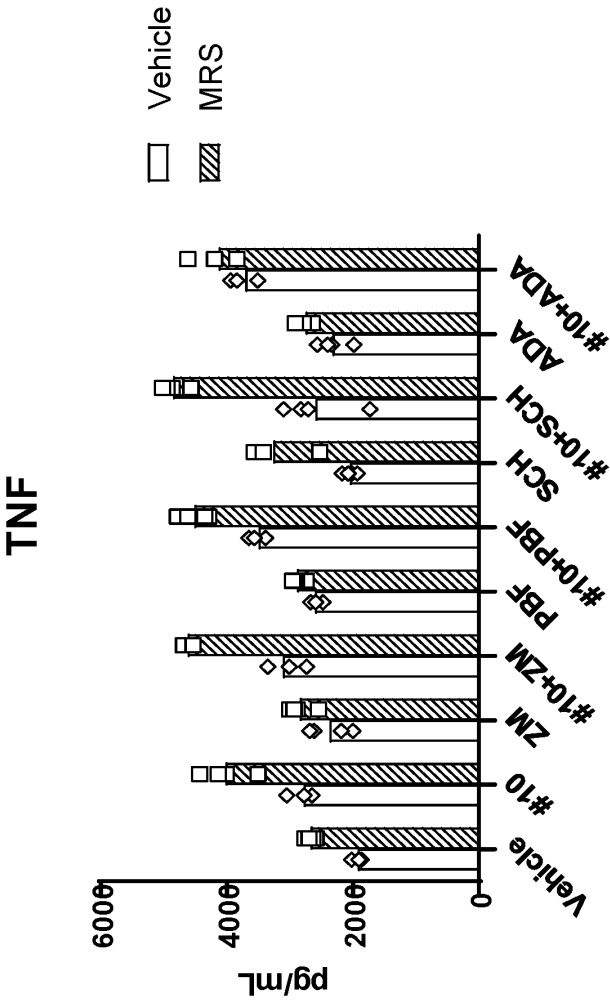


FIG. 54C

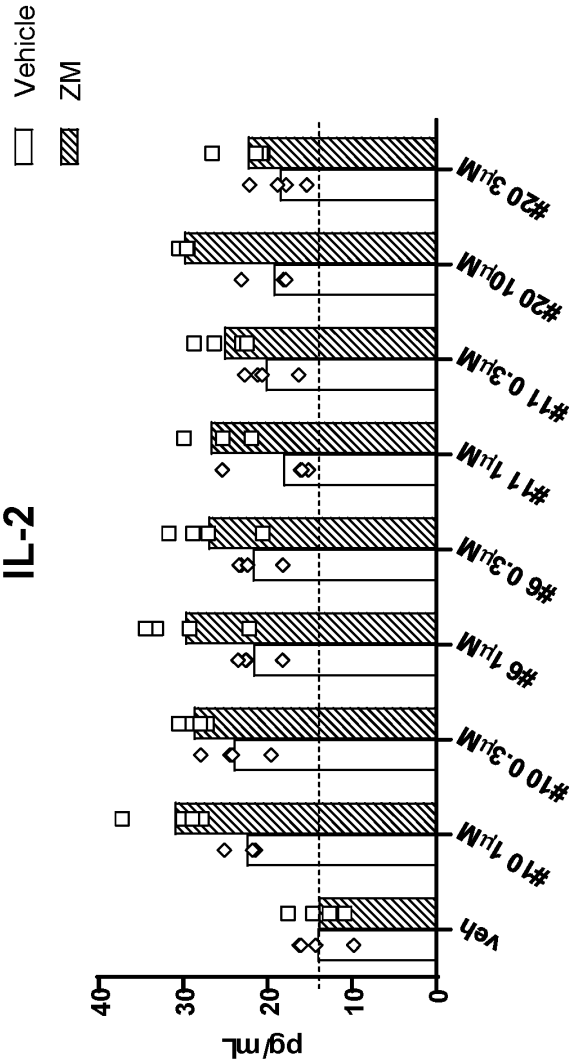


FIG. 55A

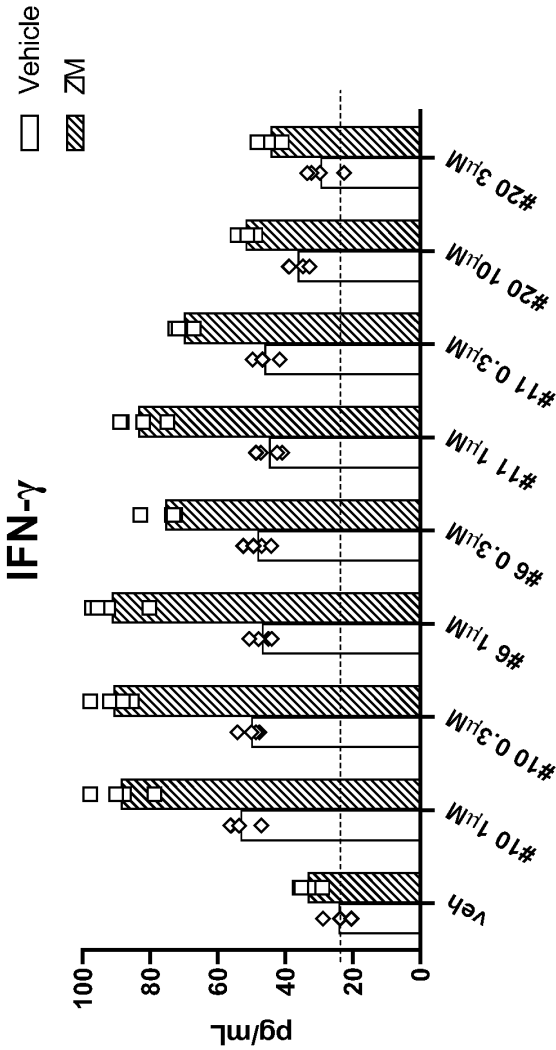


FIG. 55B

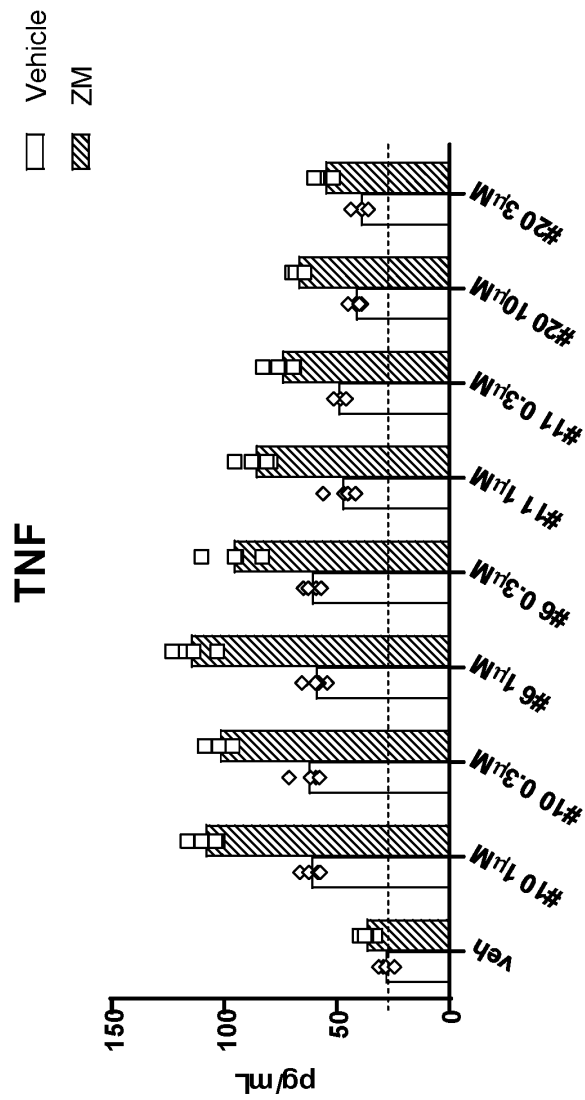


FIG. 55C

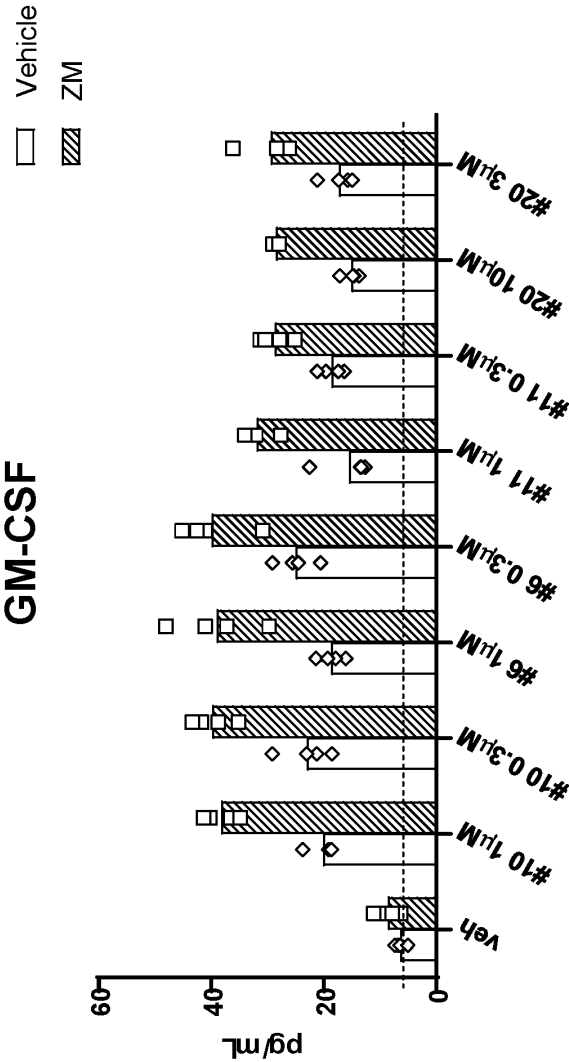


FIG. 55D

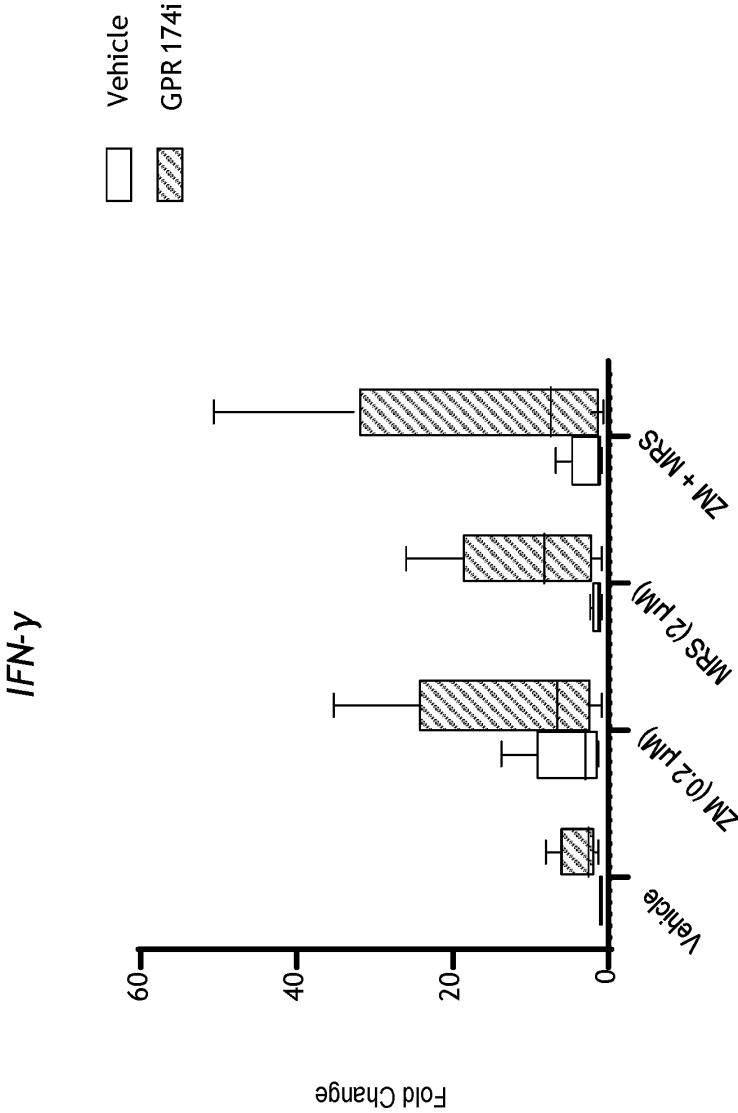


FIG. 56A

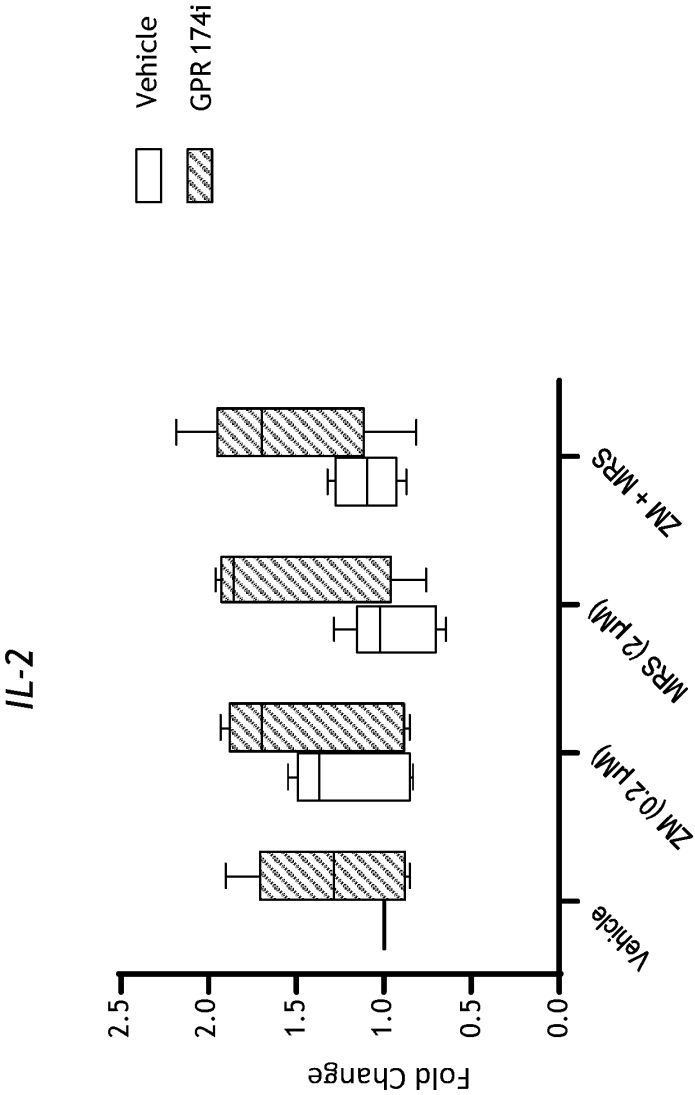


FIG. 56B

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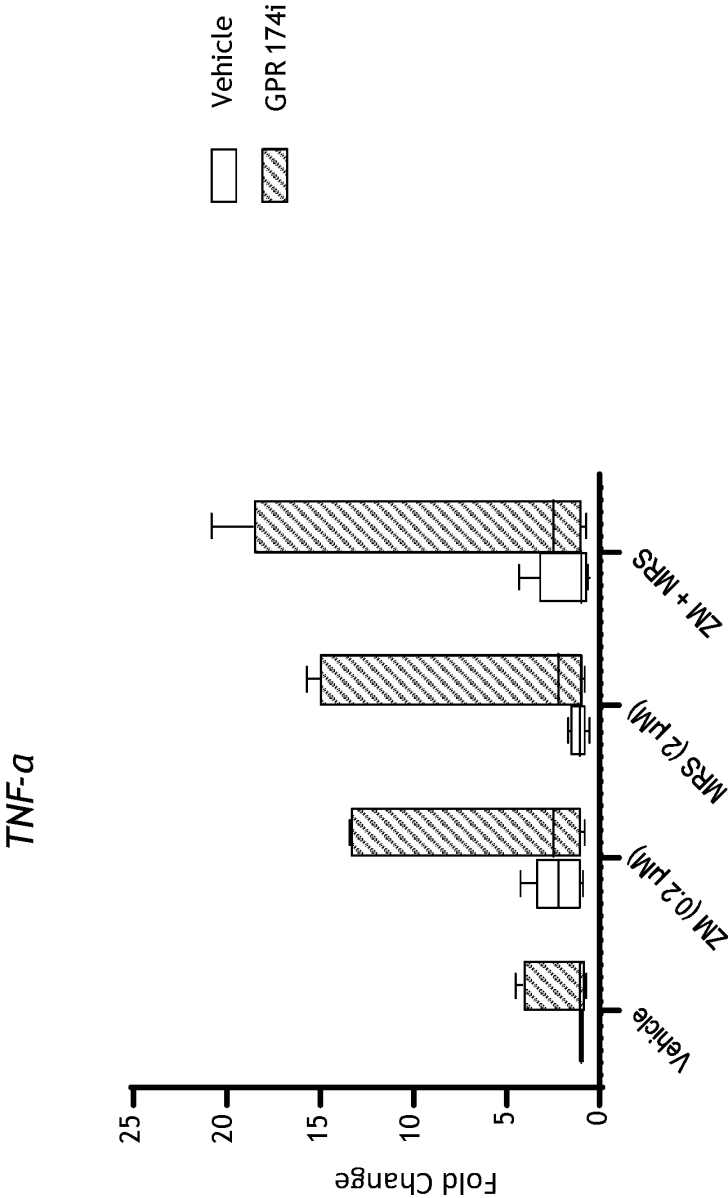


FIG. 56C

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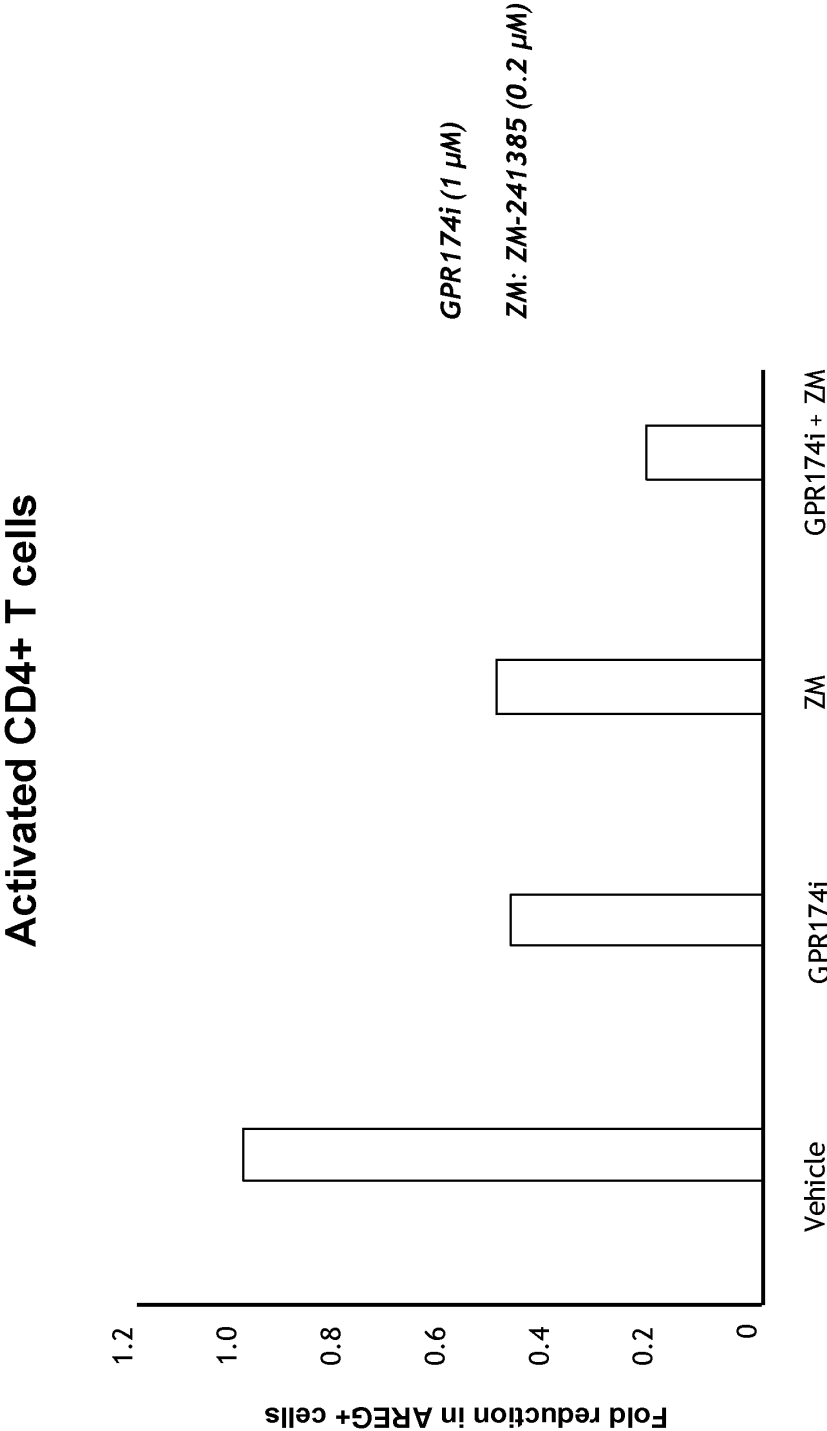


FIG. 57

Both GPR174 and Adenosine Receptors
Respond to Products of Cell Stress & Death

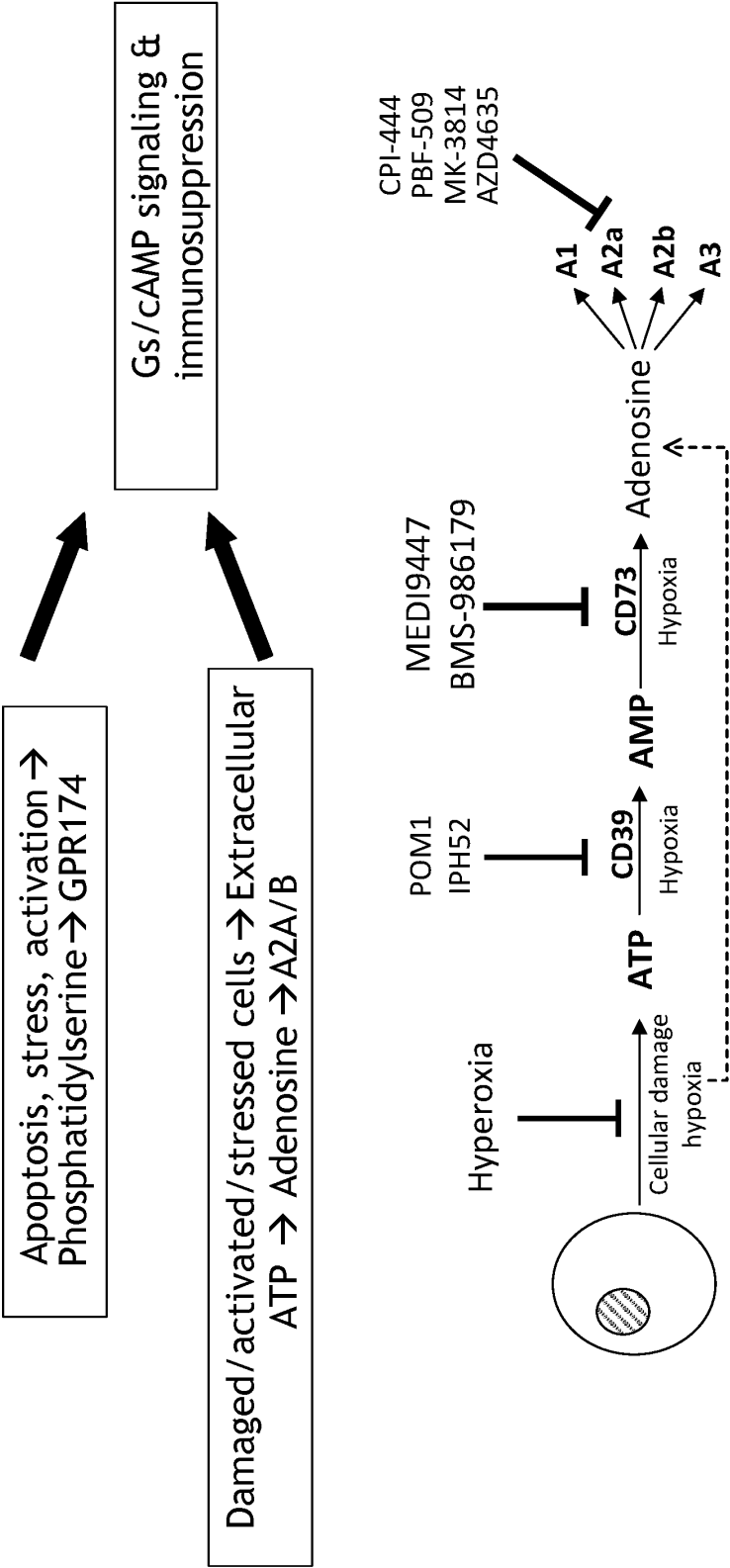


FIG. 58

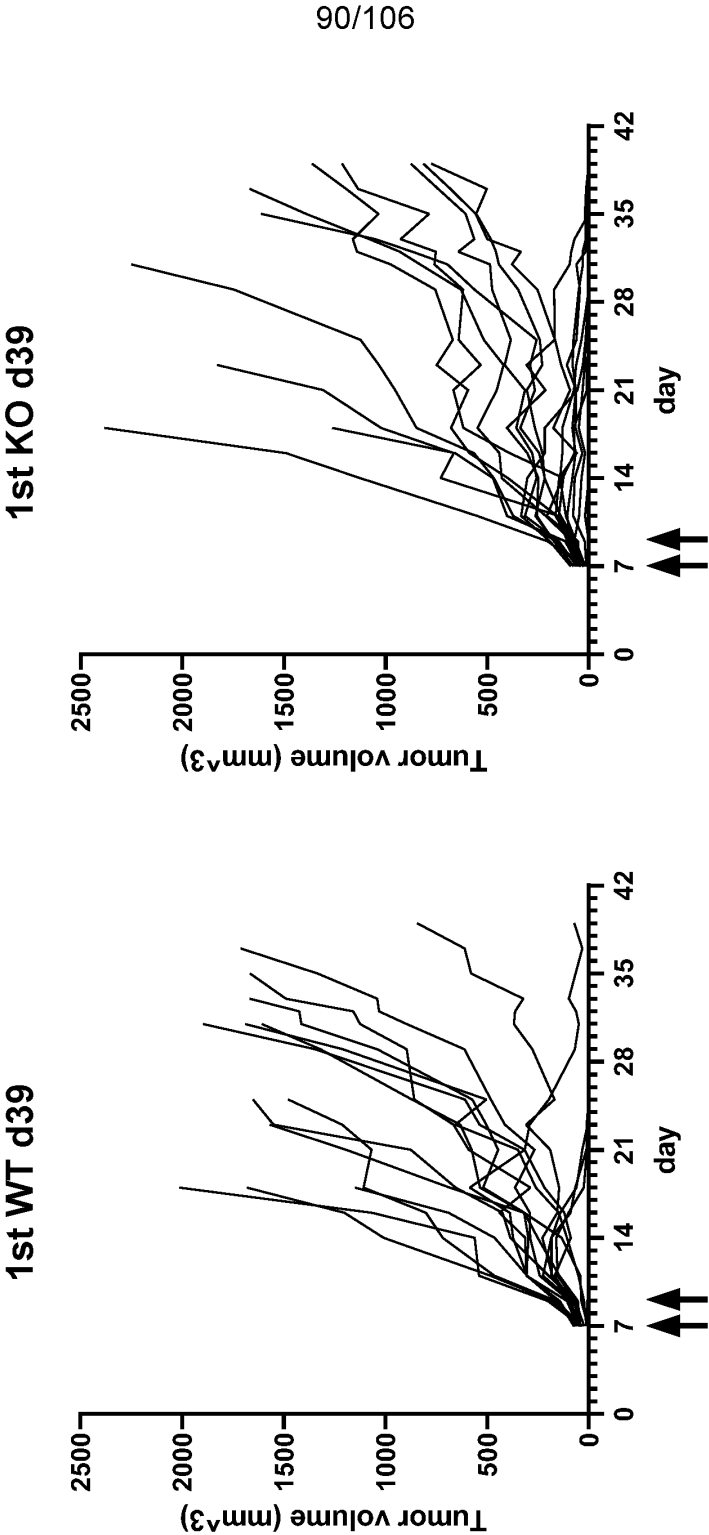


FIG. 59B

FIG. 59A

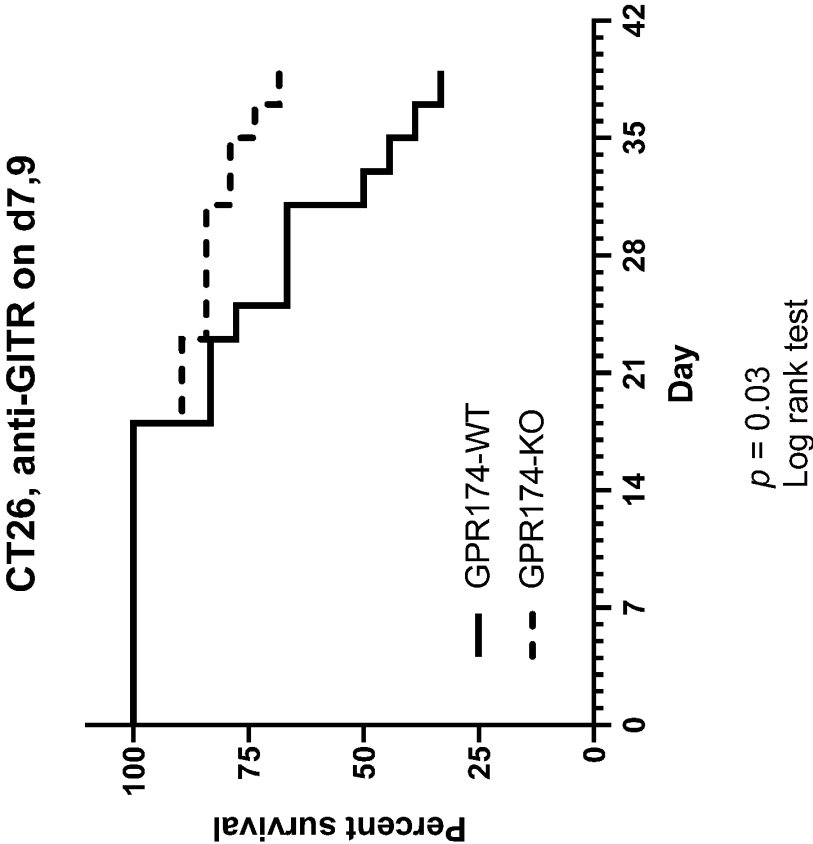


FIG. 60

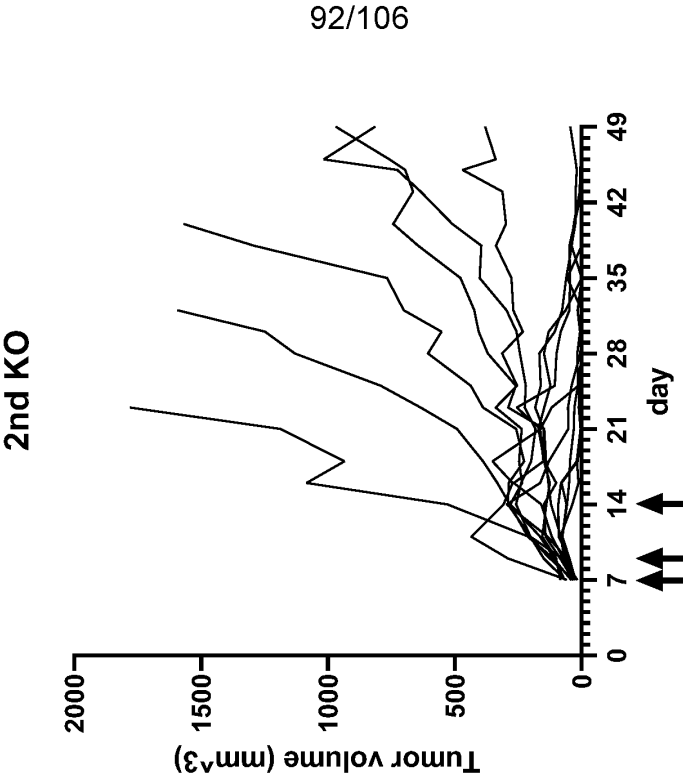


FIG. 61B

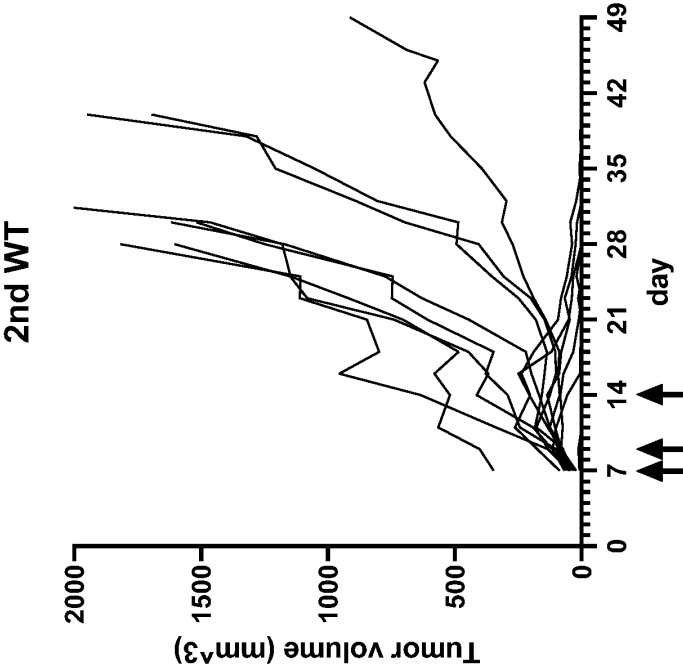


FIG. 61A

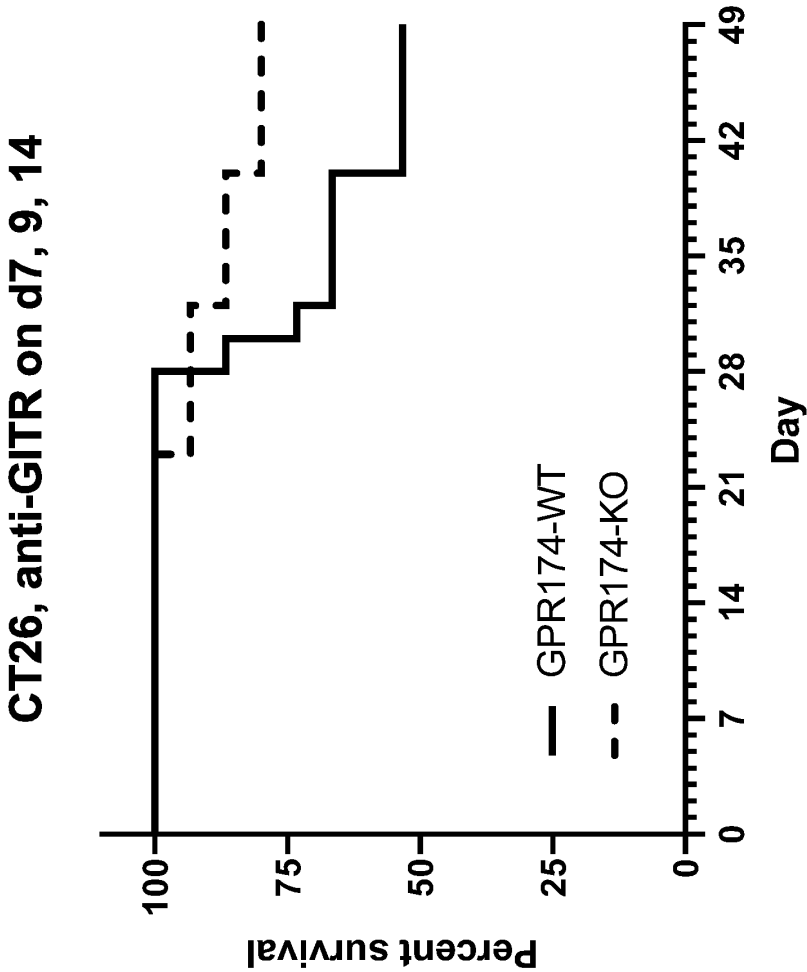


FIG. 62

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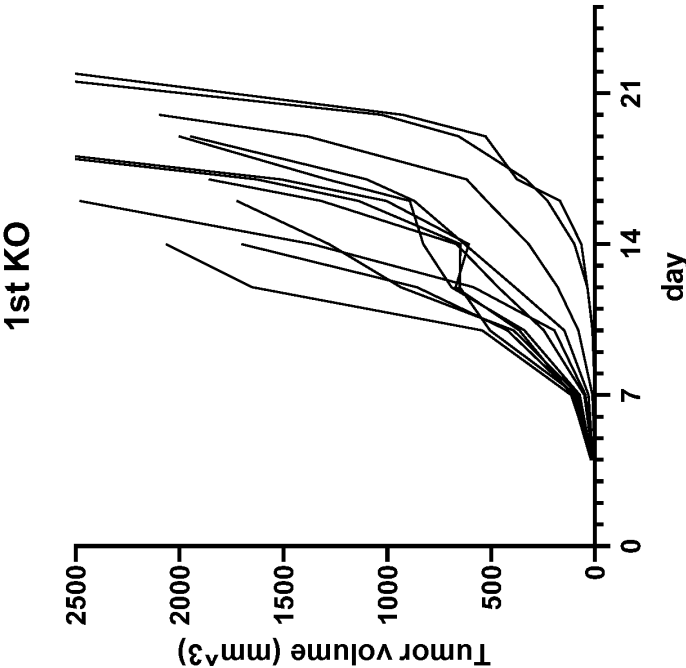


FIG. 63B

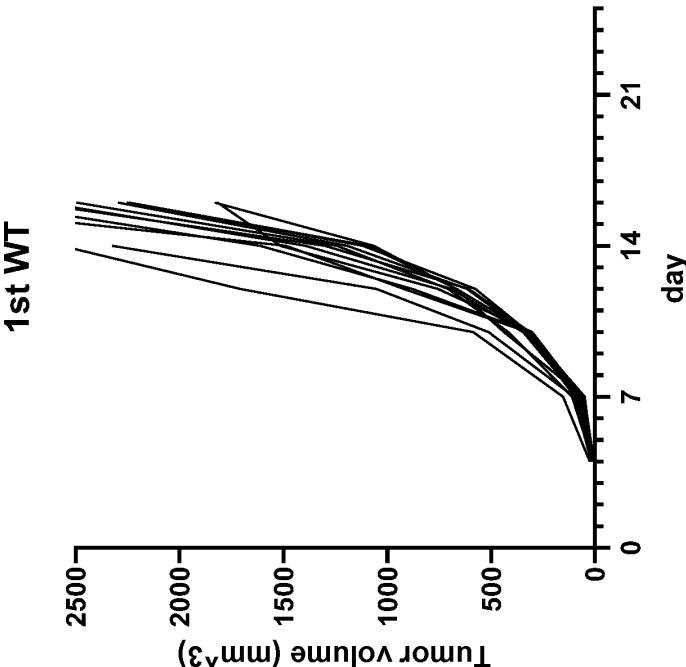


FIG. 63A

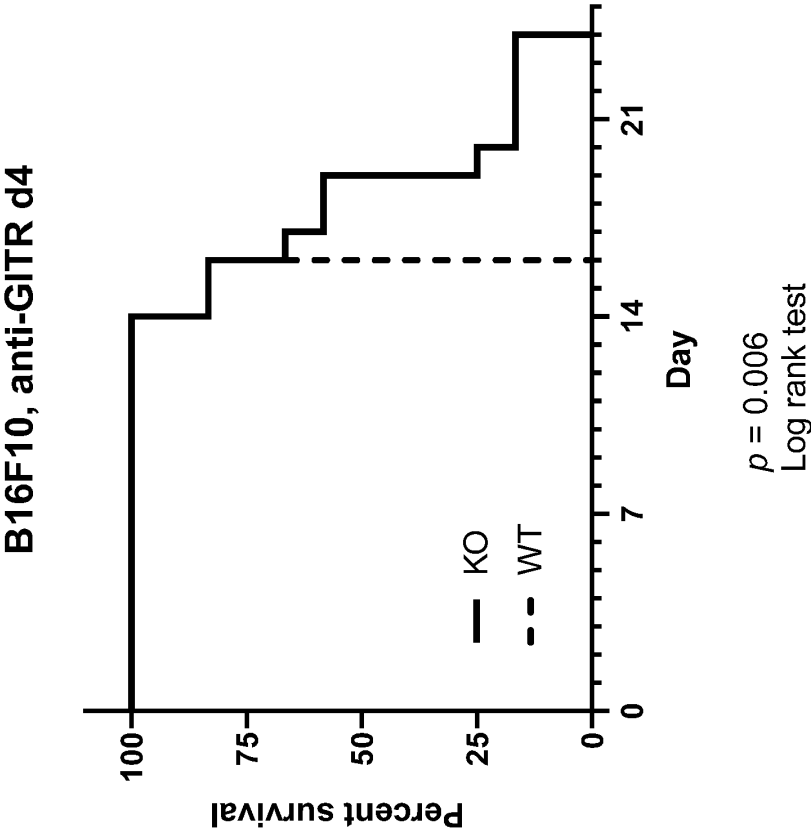


FIG. 64

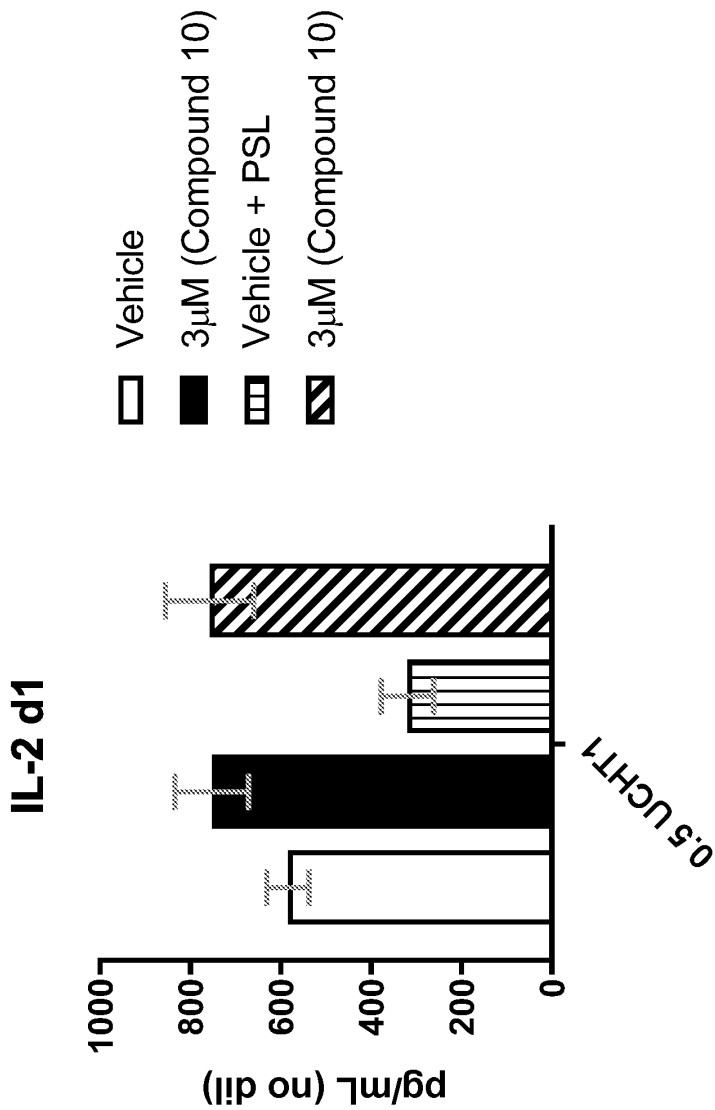


FIG. 65

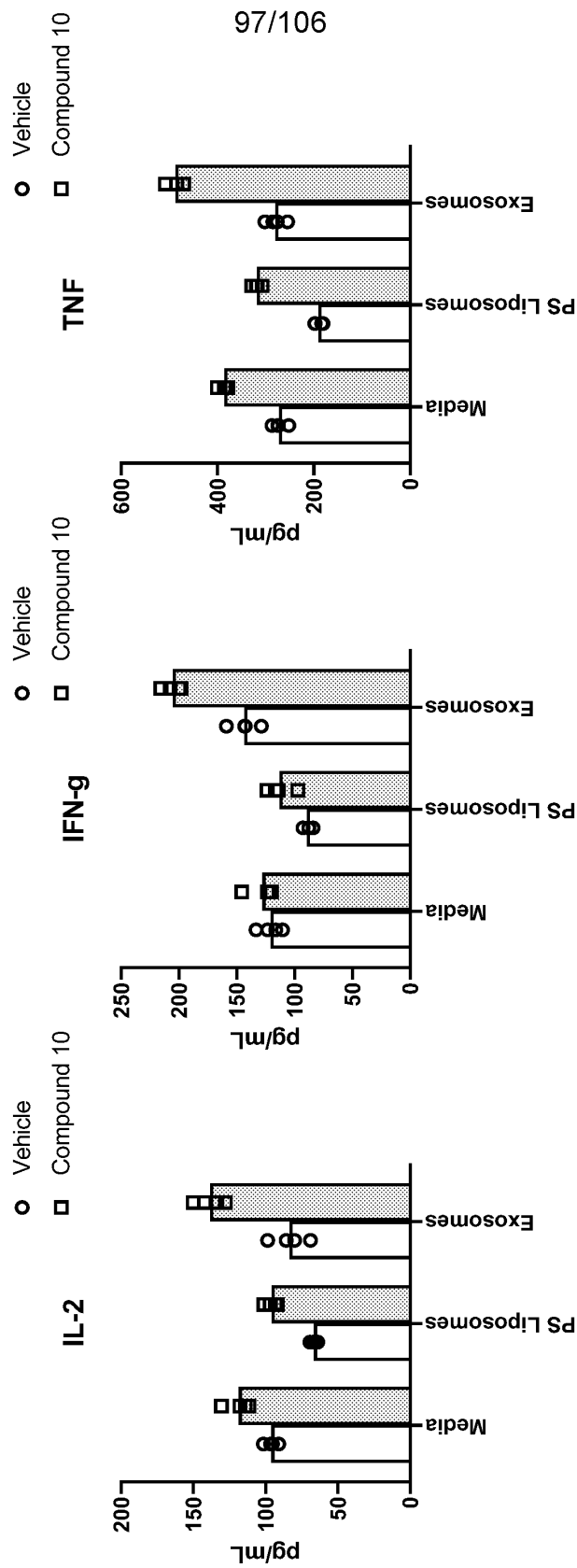


FIG. 66A

FIG. 66B

FIG. 66C

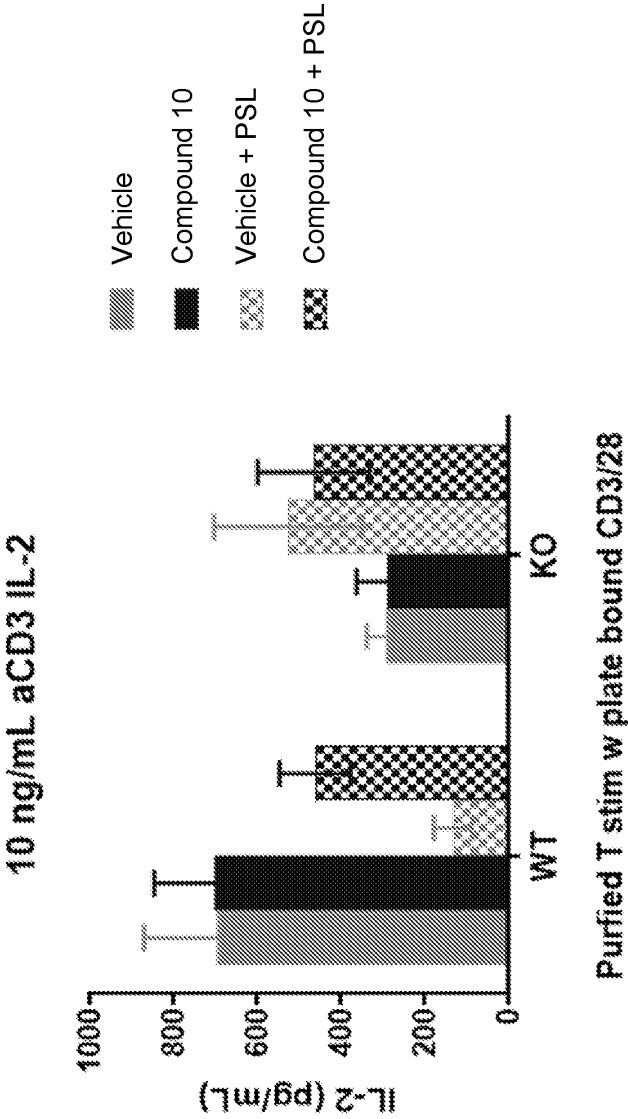


FIG. 67

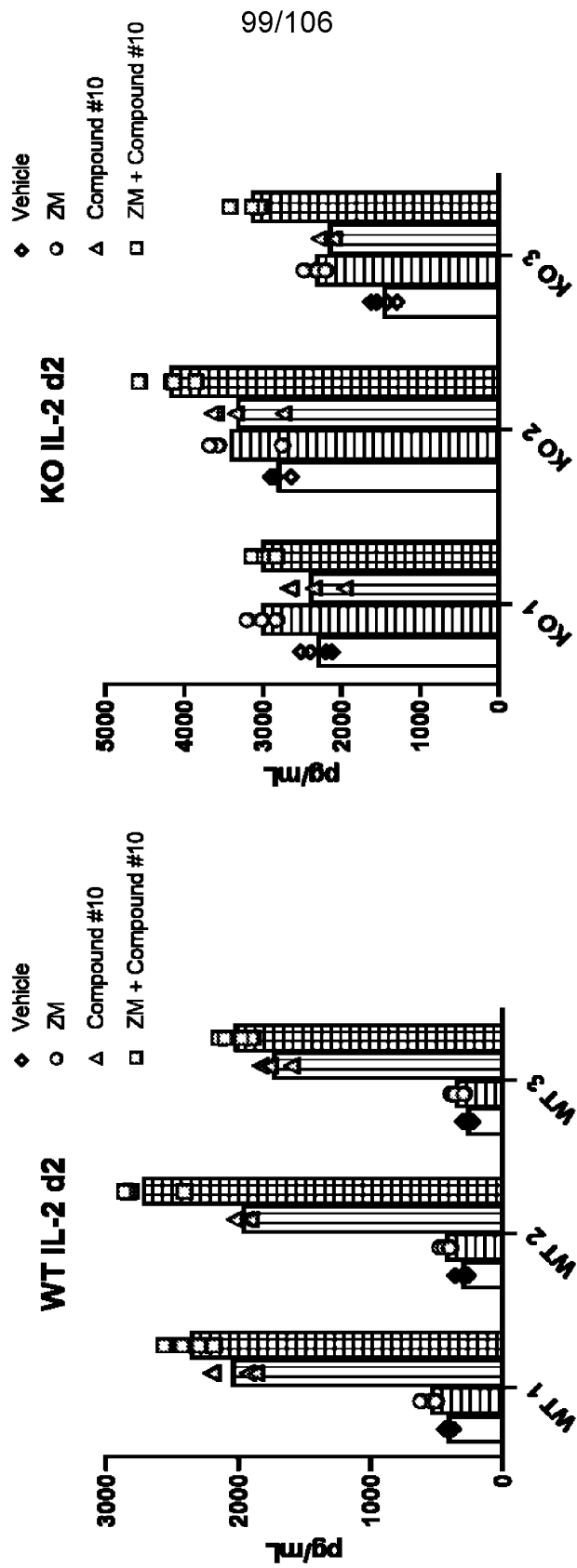


FIG. 68B

FIG. 68A

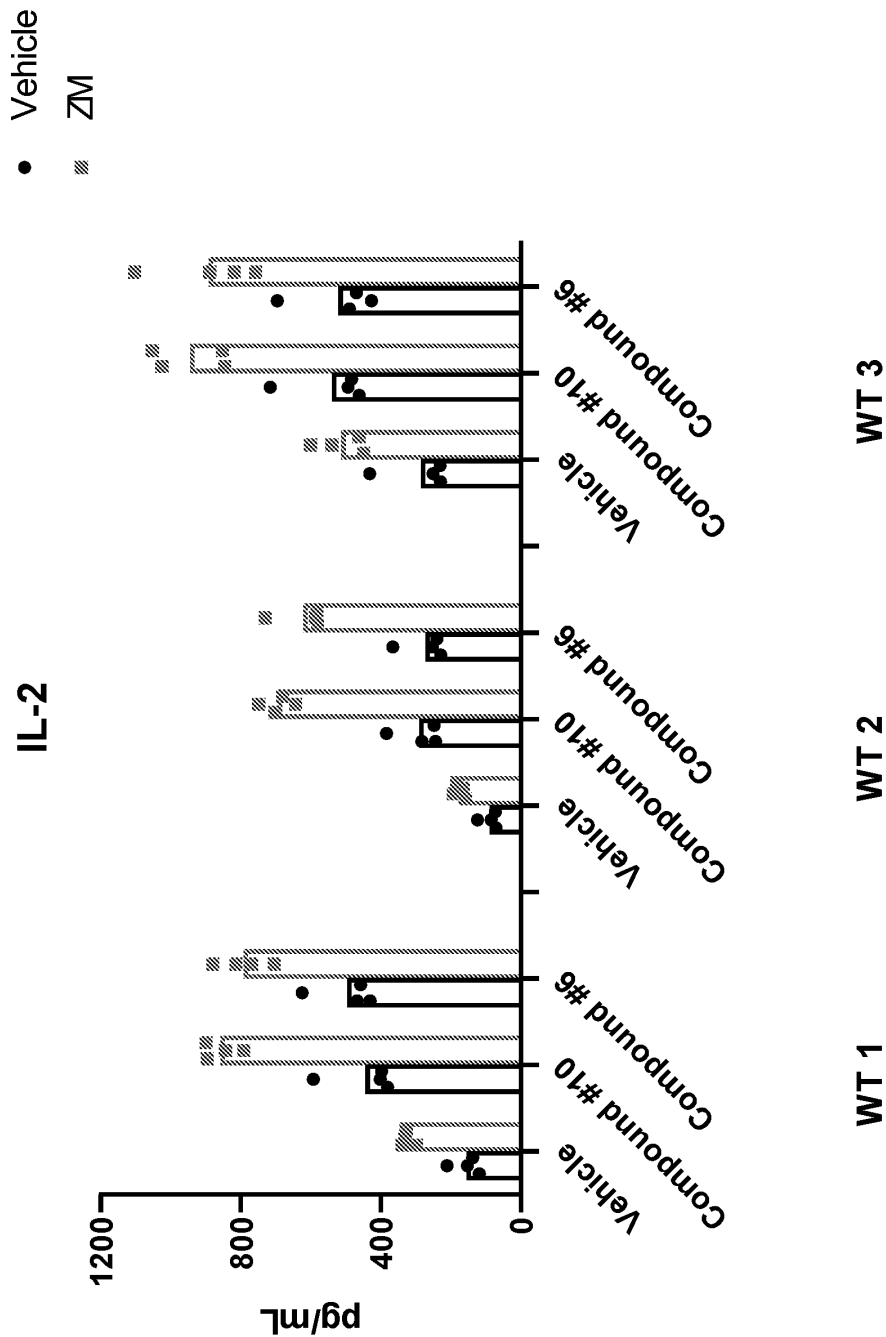


FIG. 69

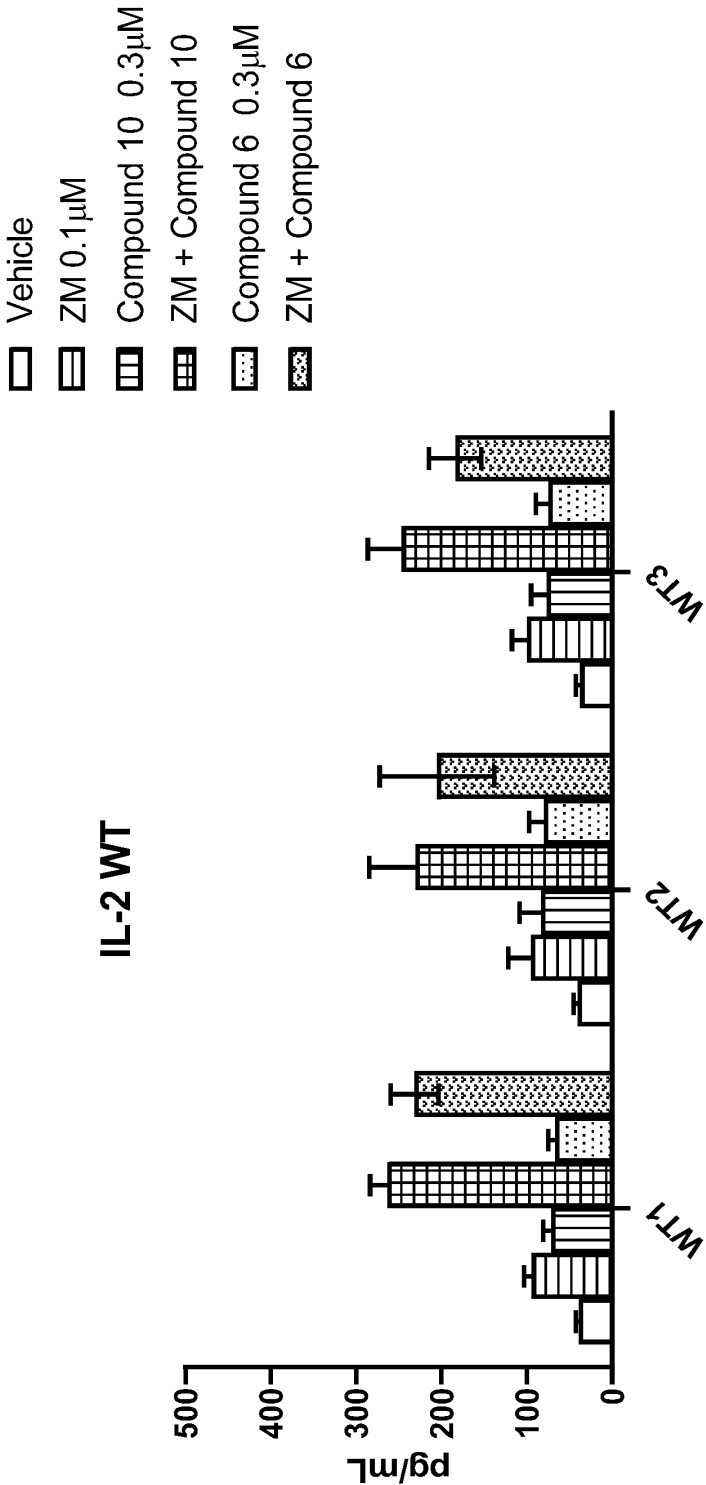


FIG. 70A

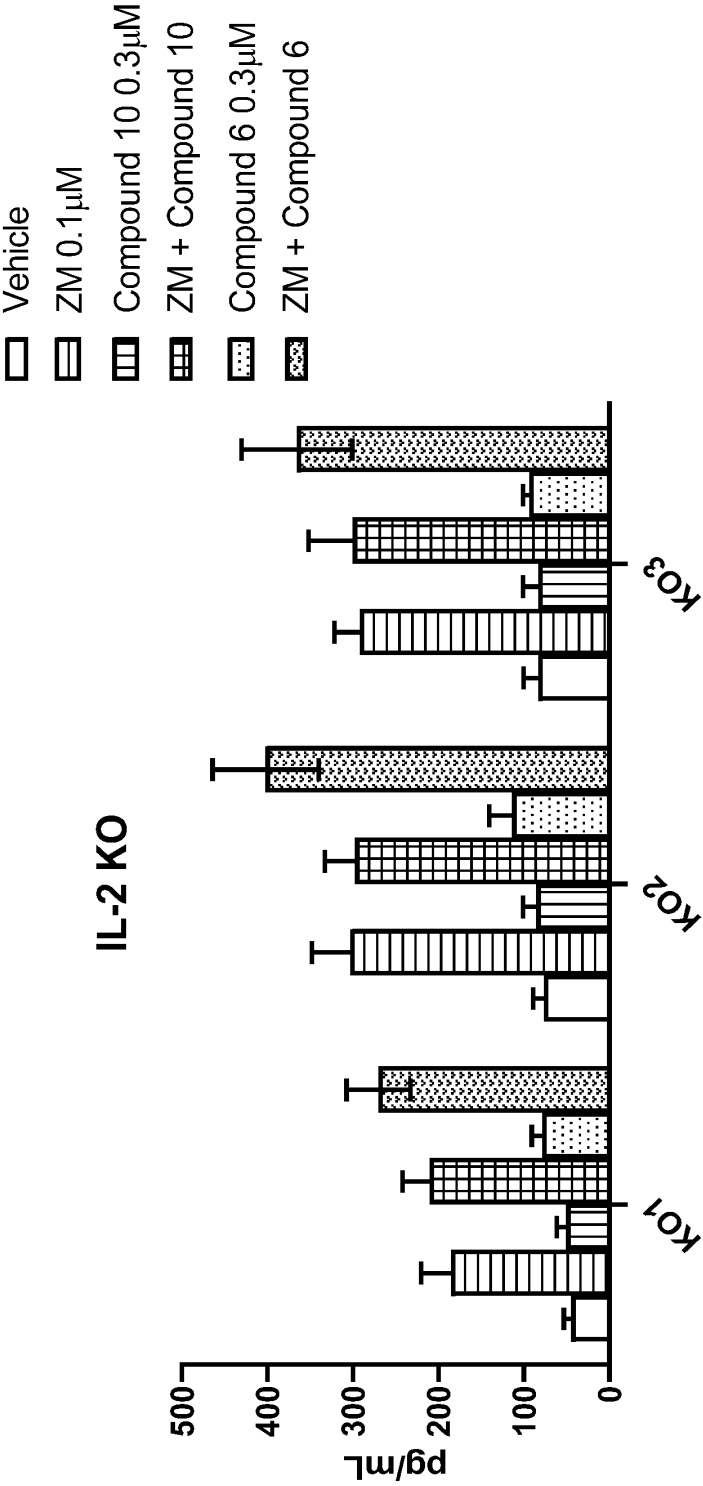


FIG. 70B

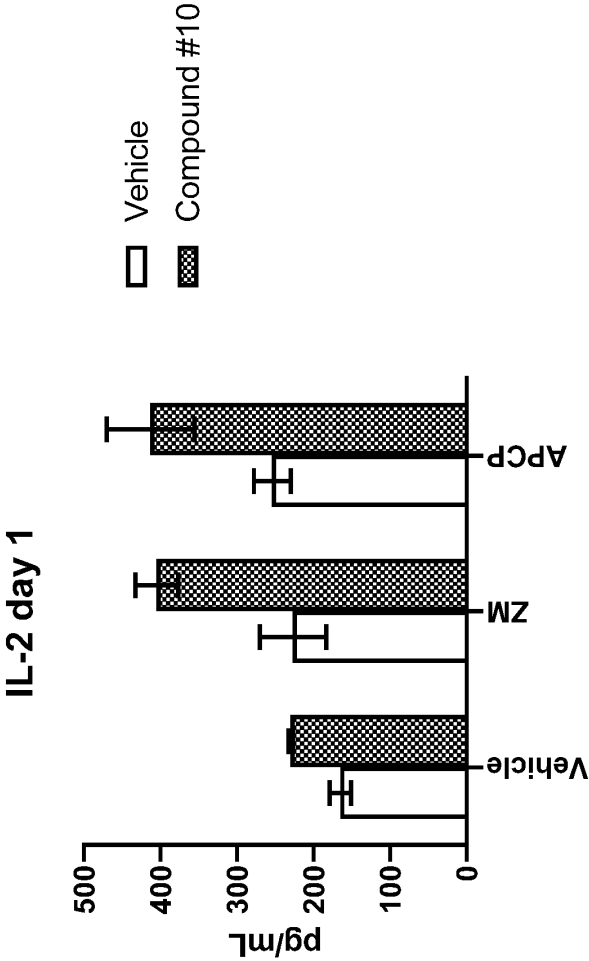


FIG. 71

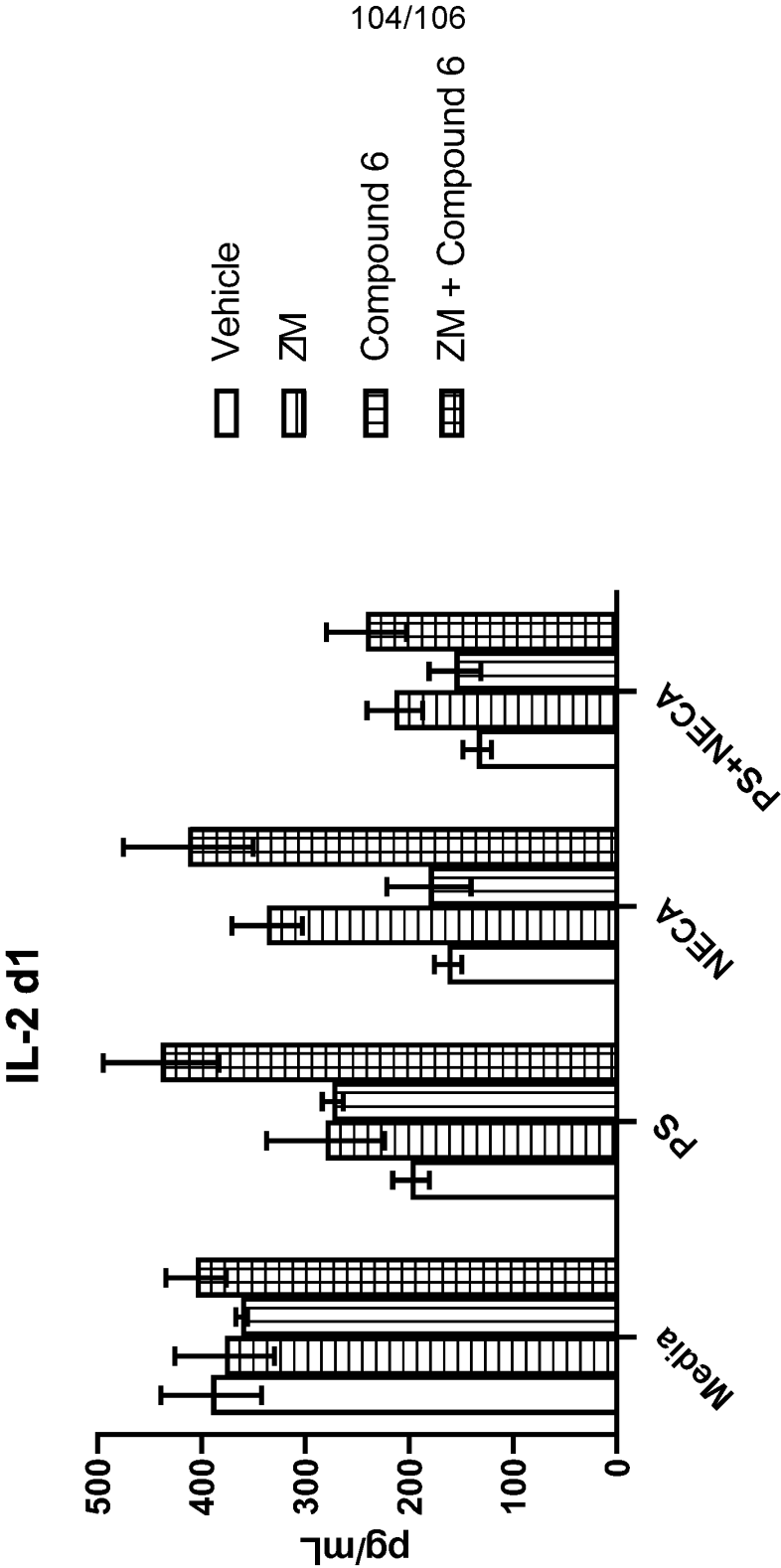


FIG. 72A



FIG. 72B

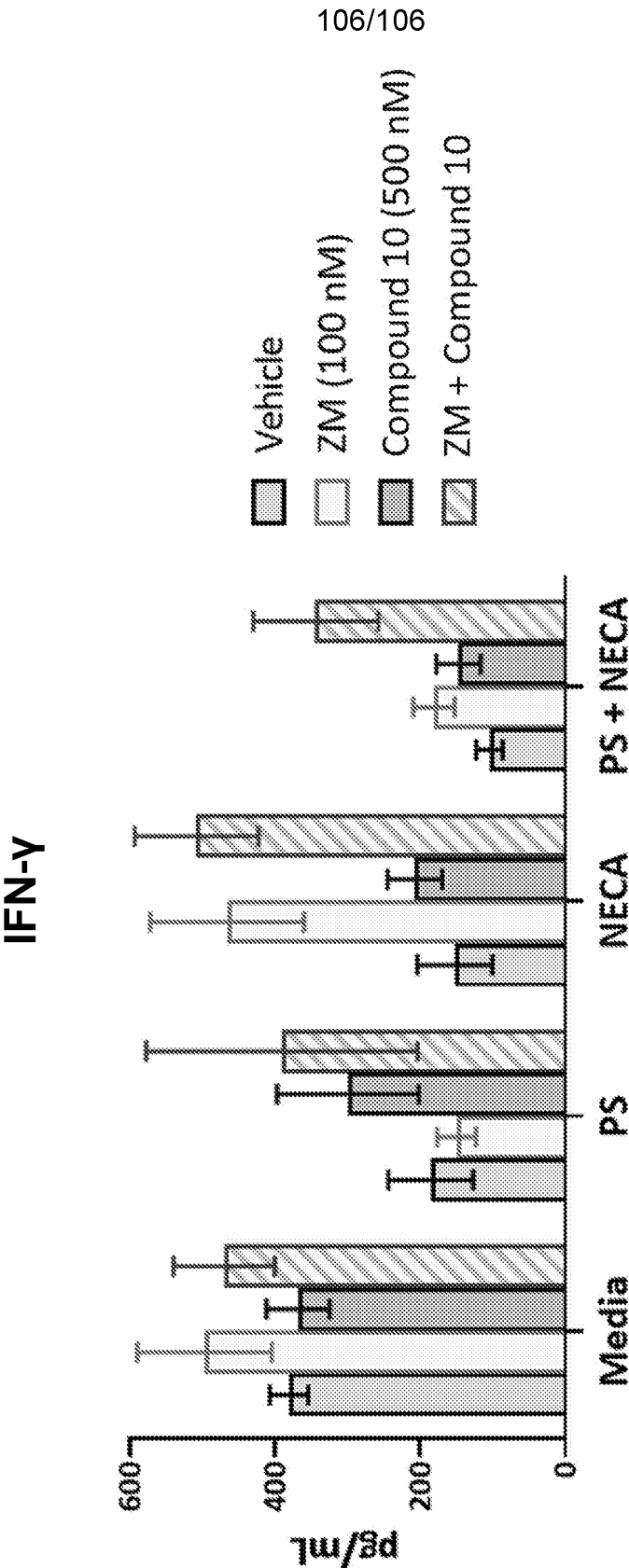


FIG. 72C