WT IL-2	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT		SEQ ID NO:1
H9 IL-2	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT	L80F, R81D, L85V, I86V, I92F	SEQ ID NO:2
H9_REA	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCASIISTLT	L80F, R81D, L85V, I86V, I92F L18R, Q22E, **Q126A**	SEQ ID NO:3
H9_REC	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCCSIISTLT	L80F, R81D, L85V, I86V, I92F L18R, Q22E,**Q126C**	SEQ ID NO:4
H9_RED	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCDSIISTLT	L80F, R81D, L85V, I86V, I92F L18R, Q22E, **Q126D**	SEQ ID NO:5
H9_REE	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCESIISTLT	L80F, R81D, L85V, I86V, I92F L18R, Q22E, **Q126E**	SEQ ID NO:6
H9_REG	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCGSIISTLT	L80F, R81D, L85V, I86V, I92F L18R, Q22E, **Q126G**	SEQ ID NO:7
H9_REH	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCHSIISTLT	L80F, R81D, L85V, I86V, I92F L18R, Q22E, **Q126H**	SEQ ID NO:8
H9_REI	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCISIISTLT	L80F, R81D, L85V, I86V, I92F L18R, Q22E, **Q126I**	SEQ ID NO:9
H9_REK	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCKSIISTLT	L80F, R81D, L85V, I86V, I92F L18R, Q22E, **Q126K**	SEQ ID NO:10
H9_REM	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCMSIISTLT	L80F, R81D, L85V, I86V, I92F L18R, Q22E, **Q126M**	SEQ ID NO:11
H9_RER	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCRSIISTLT	L80F, R81D, L85V, I86V, I92F L18R, Q22E, **Q126R**	SEQ ID NO:12
H9_RES	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCSSIISTLT	L80F, R81D, L85V, I86V, I92F L18R, Q22E, **Q126S**	SEQ ID NO:13
H9_RET	APTSSSTKKTQLQLEHLRLDLEMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFCTSIISTLT	L80F, R81D, L85V, I86V, I92F L18R, Q22E, **Q126T**	SEQ ID NO:14

FIG. 1

WT IL-2	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT		SEQ ID NO:1
H9 IL-2	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFH**FD**PRD**VV**SNINV**F**VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT	L80F, R81D, L85V, I86V, I92F	SEQ ID NO:2
WT_REH	APTSSSTKKTQLQLEHL**R**LDL**E**MILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFC**H**SIISTLT	L18R, Q22E, Q126H	SEQ ID NO:15
H9_REH	APTSSSTKKTQLQLEHL**R**LDL**E**MILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFH**FD**PRD**VV**SNINV**F**VLELKGSETTFMCEYADETATIVEFLNRWITFC**H**SIISTLT	L80F, R81D, L85V, I86V, I92F L18R, Q22E, Q126H	SEQ ID NO:8
WT_REM	APTSSSTKKTQLQLEHL**R**LDL**E**MILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQ SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFC**M**SIISTLT	L18R, Q22E, Q126M	SEQ ID NO:16

FIG. 2

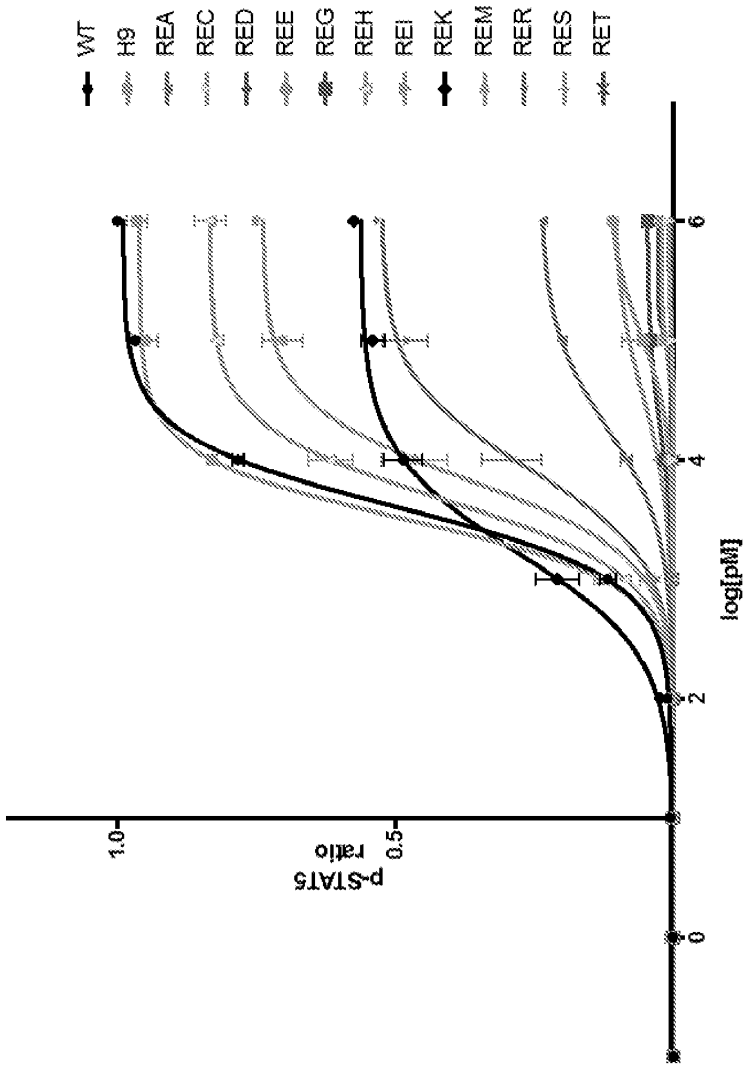
WT
H9
REA
REC
RED
REE
REG
REH
REI
REK
REM
RER
RES
RET
p-STAT5 ratio
1.0
0.5
0
2
4
6
log[pM]

FIG. 3

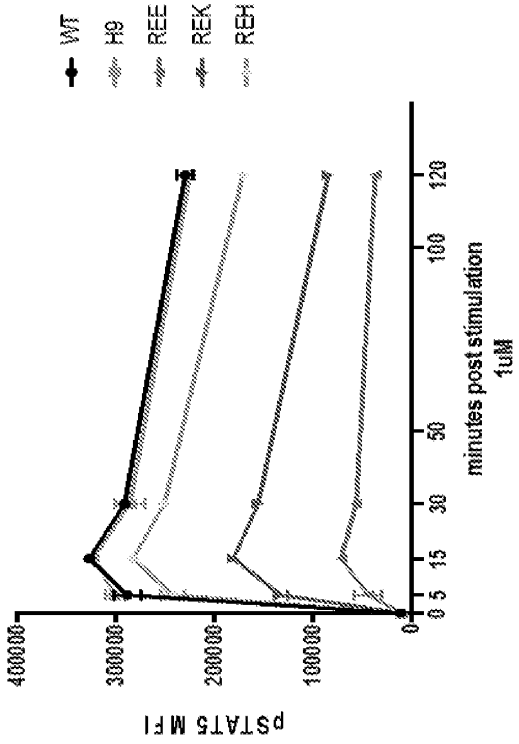
WT
H9
REE
REK
REH
pSTAT5 MFI
400000
300000
200000
100000
0
0 5 15 30 50 100 120
minutes post stimulation
1uM

FIG. 4B

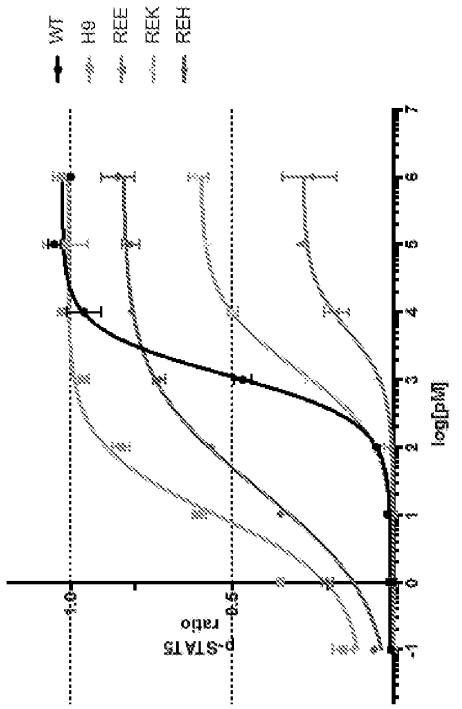
WT
H9
REE
REK
REH
p-STAT5
ratio
1.0
0.5
-1 0 1 2 3 4 5 6 7
log[pM]

FIG. 4A

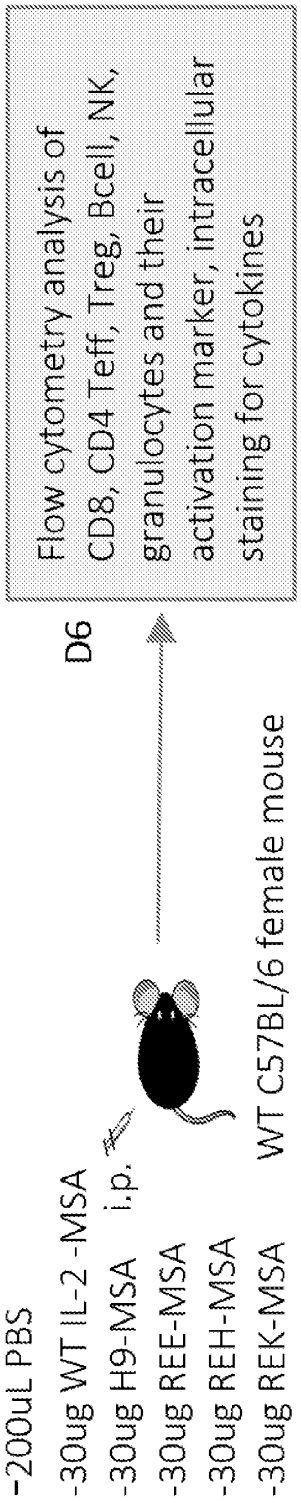
Flow cytometry analysis of CD8, CD4 Teff, Treg, Bcell, NK, granulocytes and their activation marker, intracellular staining for cytokines
D6
WT C57BL/6 female mouse
D0
-200uL PBS
-30ug WT IL-2 -MSA
-30ug H9-MSA
-30ug REE-MSA
-30ug REH-MSA
-30ug REK-MSA
i.p.

FIG. 5A

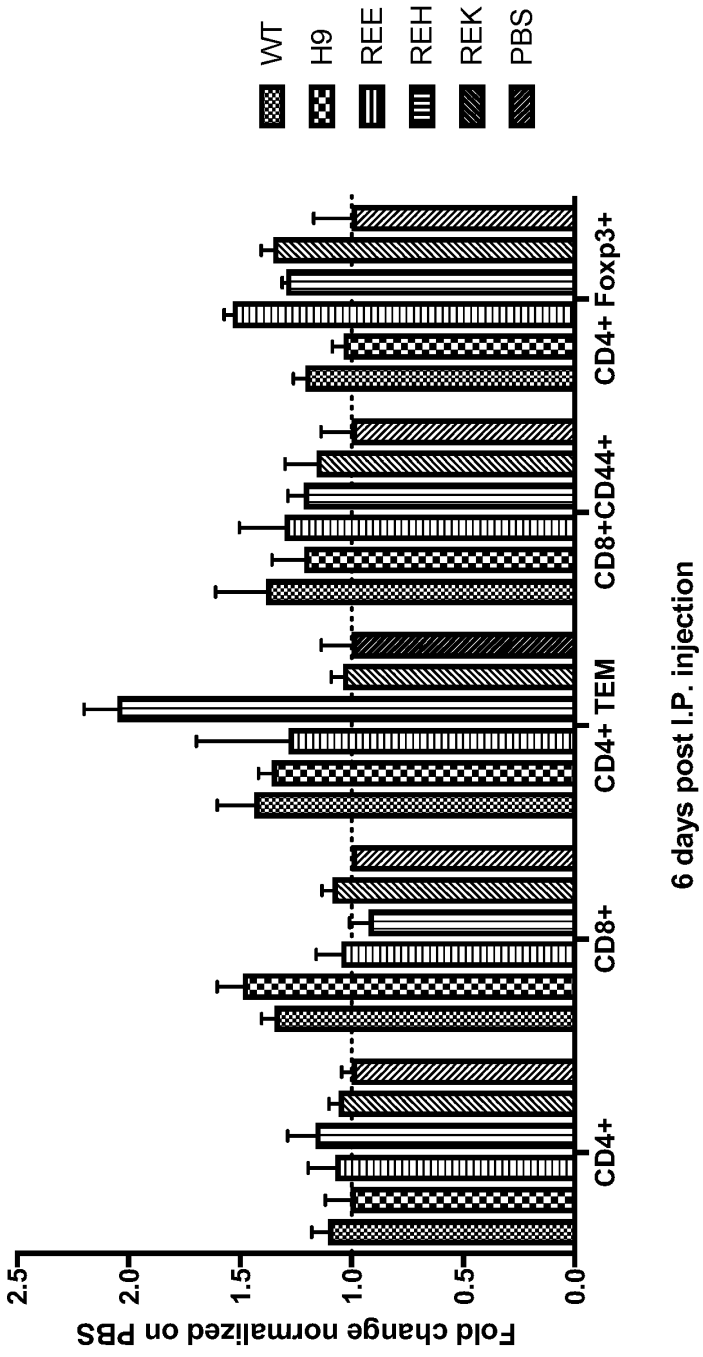
WT
H9
REE
REH
REK
PBS
Fold change normalized on PBS
2.5
2.0
1.5
1.0
0.5
0.0
CD4+
CD8+
CD4+ TEM
CD8+CD44+
CD4+ Foxp3+
6 days post I.P. injection

FIG. 5B

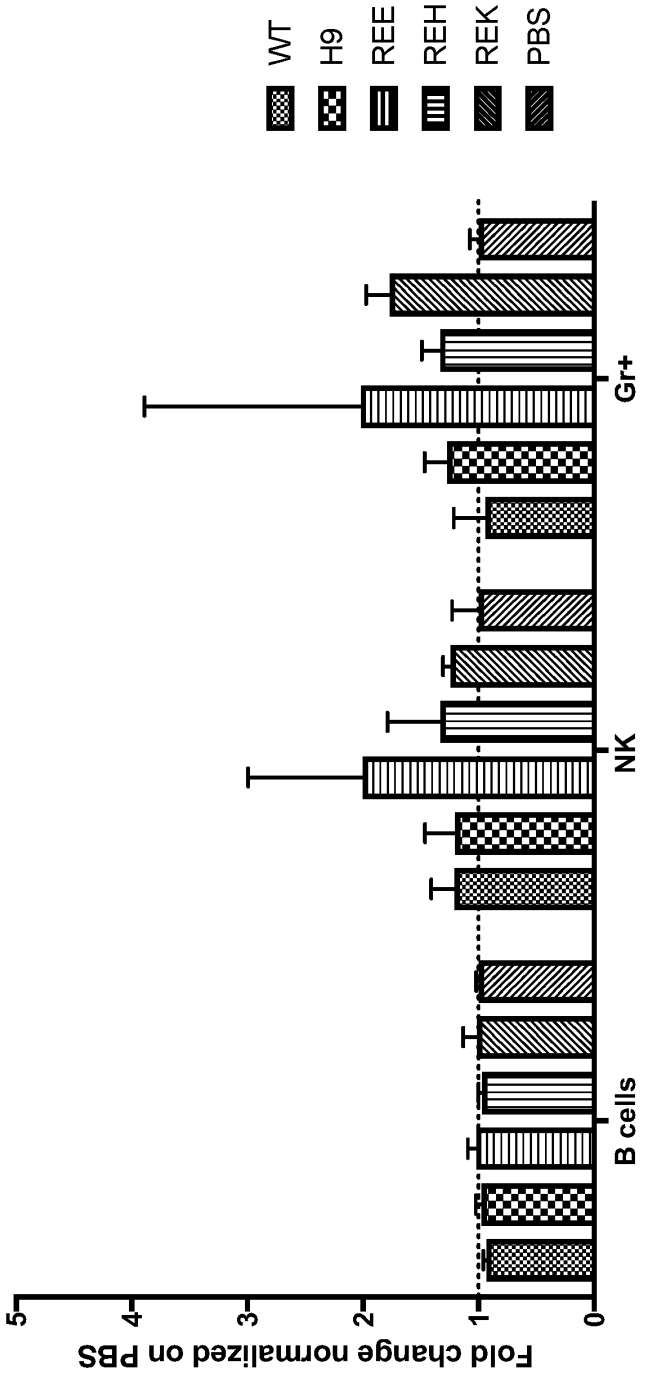
WT
H9
REE
REH
REK
PBS
Fold change normalized on PBS
0
1
2
3
4
5
B cells
NK
Gr+
6 days post I.P. injection

FIG. 5C

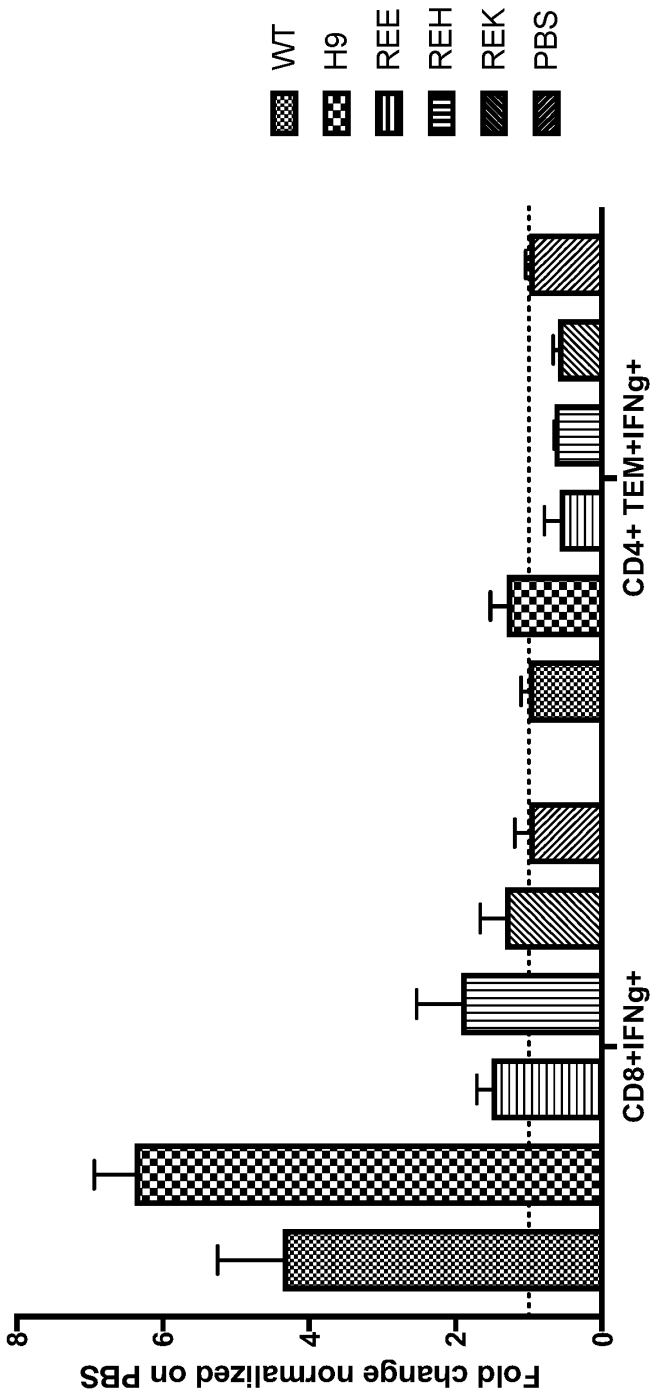
WT
H9
REE
REH
REK
PBS
Fold change normalized on PBS
0
2
4
6
8
CD8+IFNg+
CD4+ TEM+IFNg+
6 days post I.P. injection

FIG. 5D

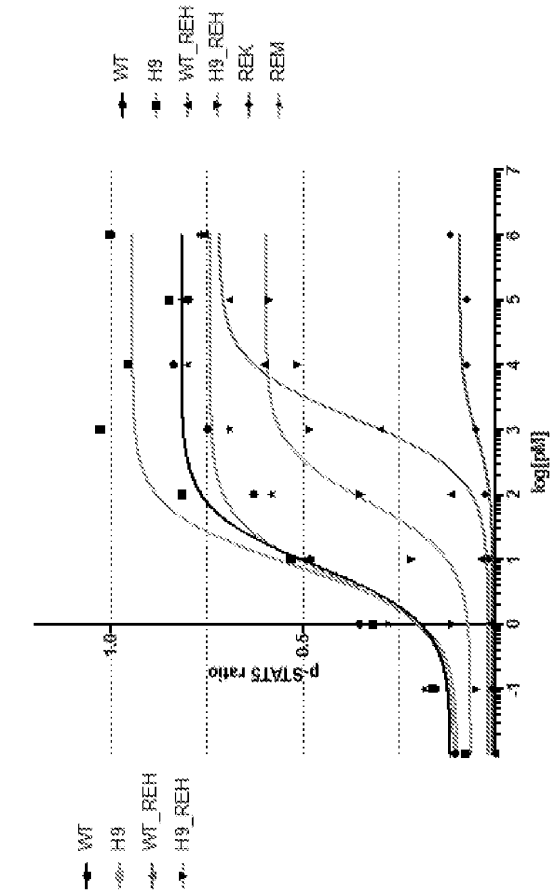
WT
H9
WT_REH
H9_REH
REK
REM
p-STAT5 ratio
log[pM]

FIG. 6B

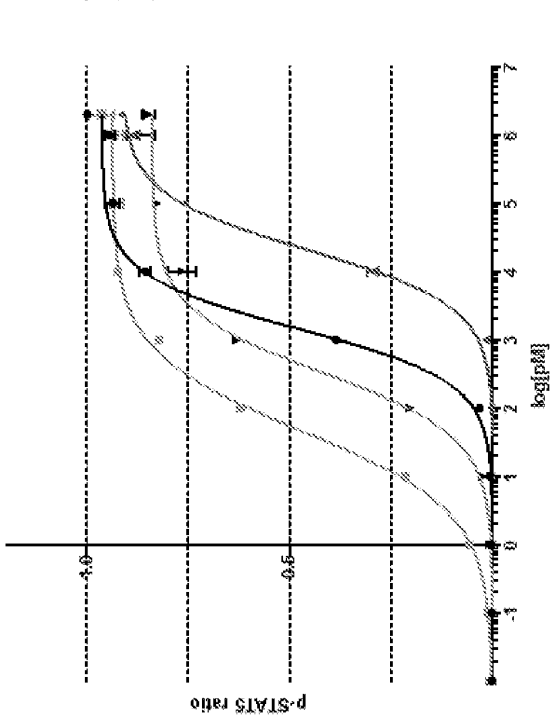
WT
H9
WT_REH
H9_REH
p-STAT5 ratio
log[pM]

FIG. 6A

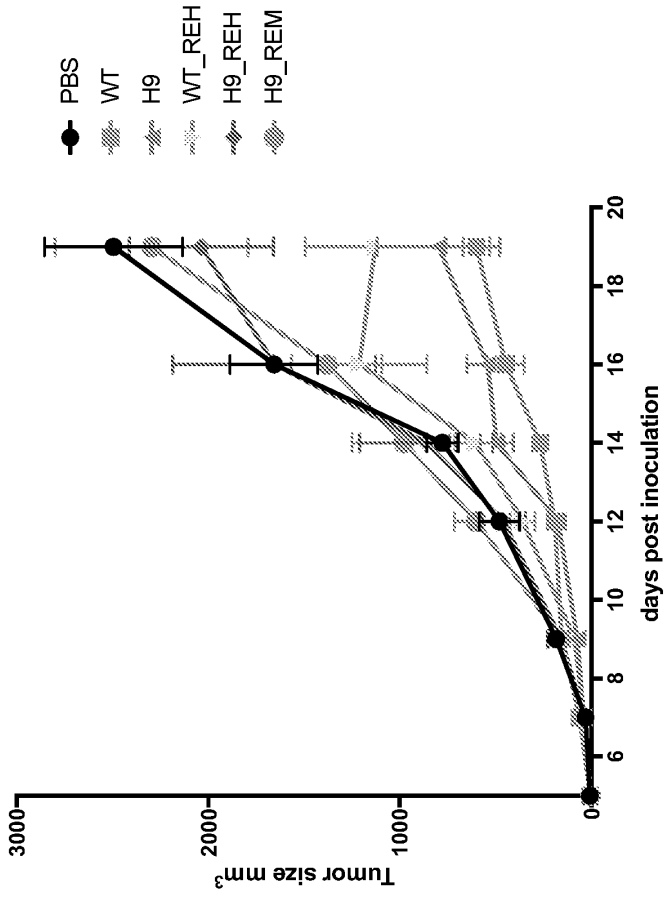
PBS
WT
H9
WT_REH
H9_REH
H9_REM
Tumor size mm3
3000
2000
1000
0
0
6
8
10
12
14
16
18
20
days post inoculation

FIG. 7A

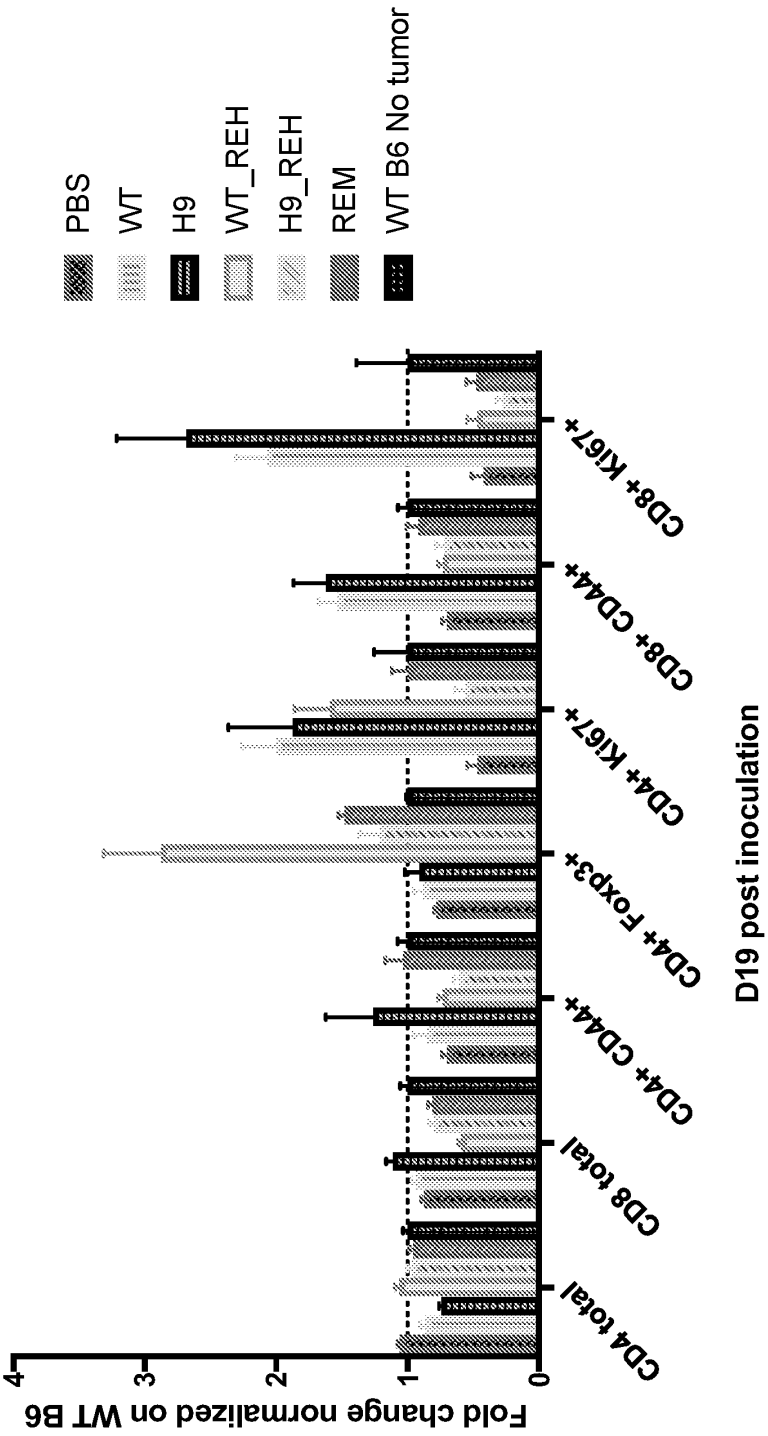
PBS
WT
H9
WT_REH
H9_REH
REM
WT B6 No tumor
Fold change normalized on WT B6
0
1
2
3
4
CD4 total
CD8 total
CD4+ CD44+
CD4+ Foxp3+
CD4+ Ki67+
CD8+ CD44+
CD8+ Ki67+
D19 post inoculation

FIG. 7B

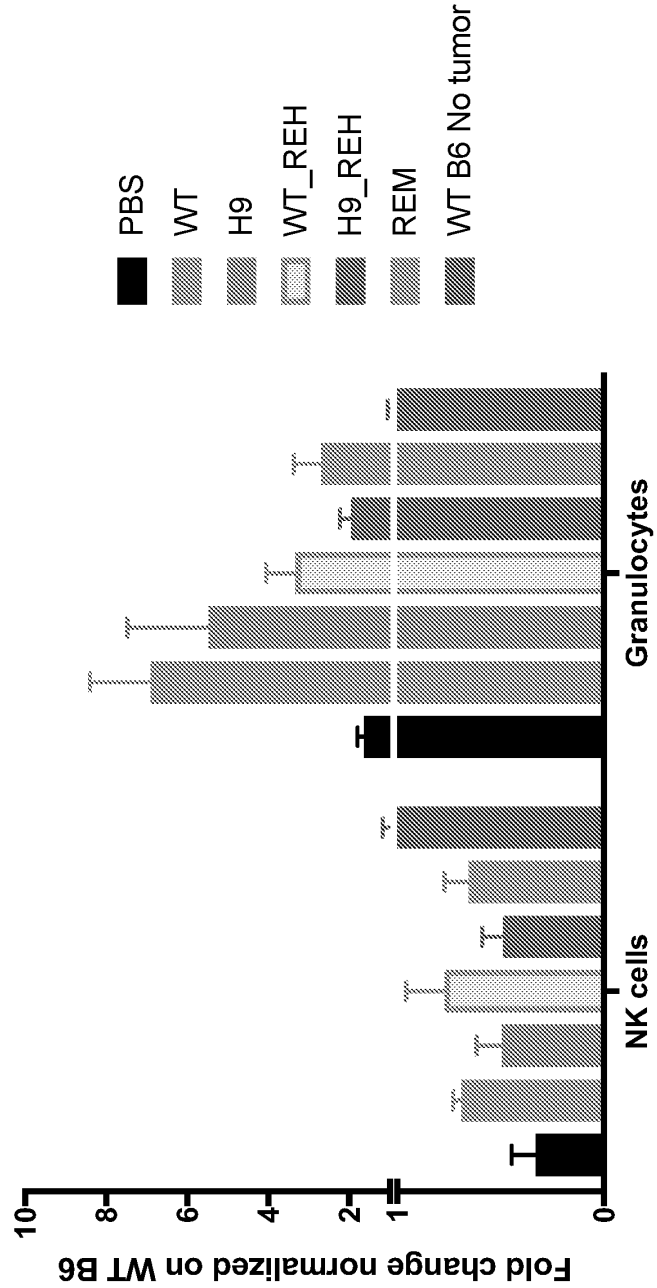
PBS
WT
H9
WT_REH
H9_REH
REM
WT B6 No tumor
Fold change normalized on WT B6
10
8
6
4
2
1
0
NK cells
Granulocytes
D19 post inoculation

FIG. 7C

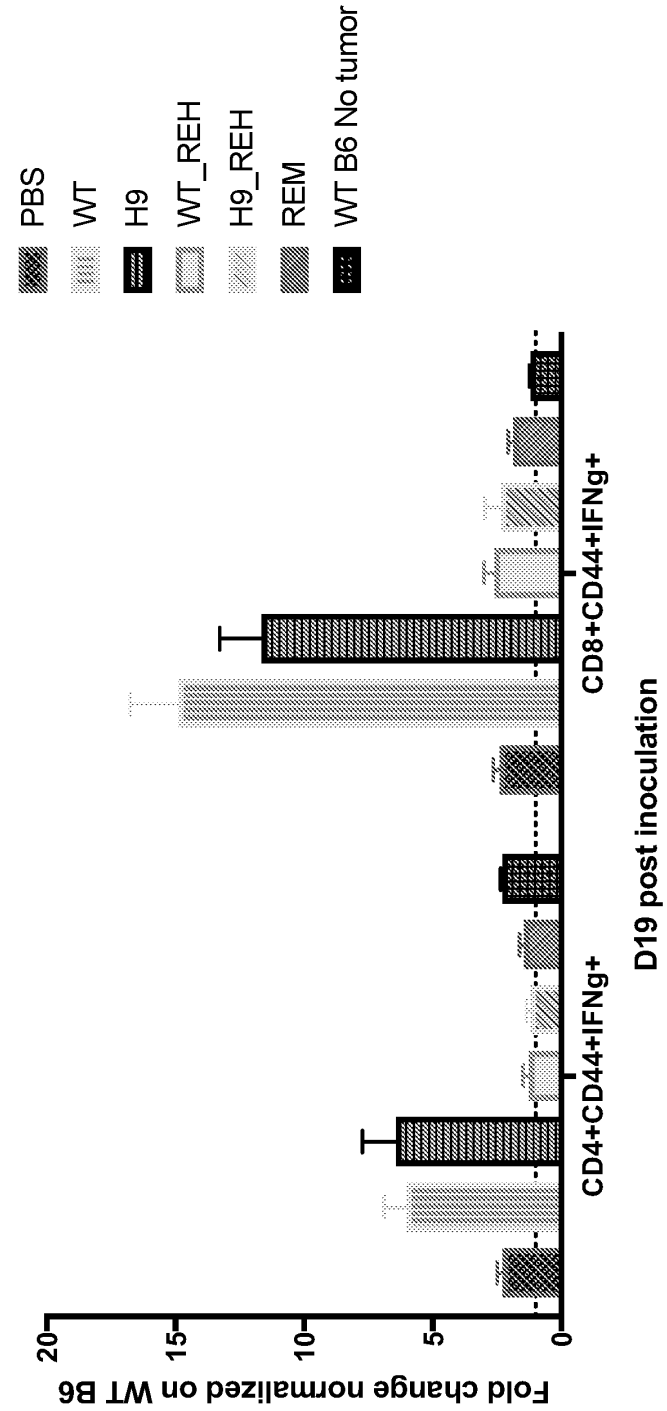
PBS
WT
H9
WT_REH
H9_REH
REM
WT B6 No tumor
Fold change normalized on WT B6
20
15
10
5
0
CD4+CD44+IFNg+
CD8+CD44+IFNg+
D19 post inoculation

FIG. 7D

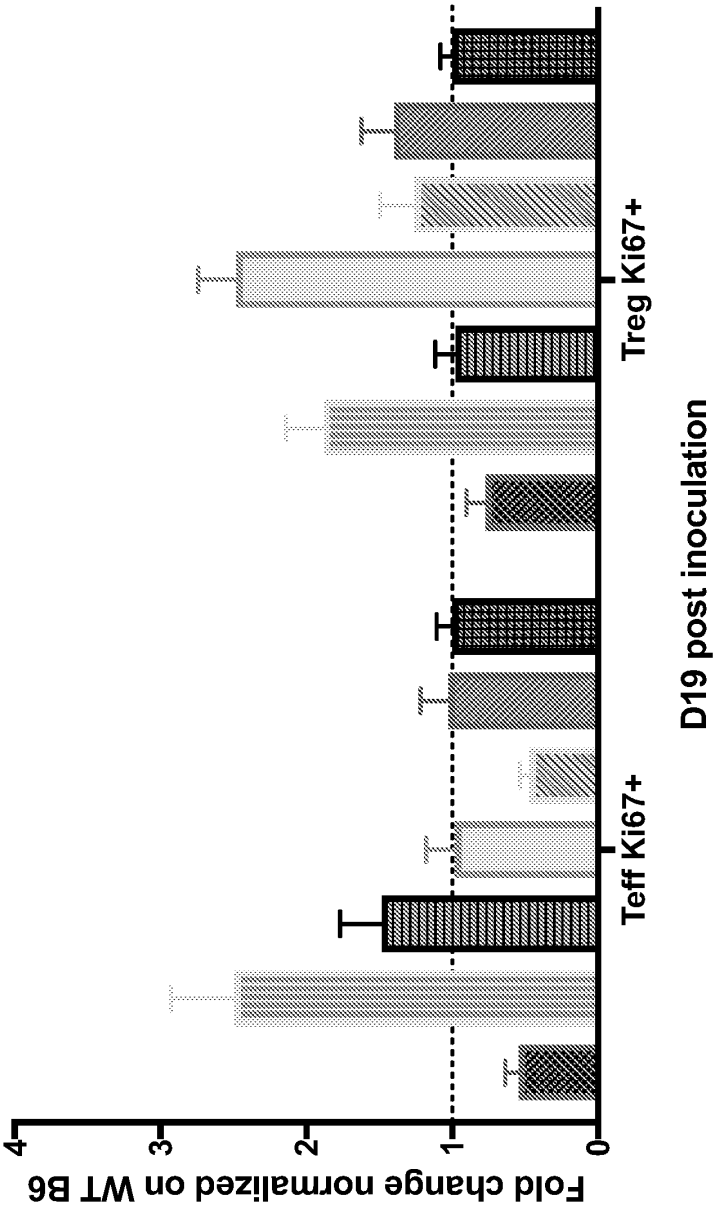
Fold change normalized on WT B6
0
1
2
3
4
Teff Ki67+
Treg Ki67+
D19 post inoculation

FIG. 7E

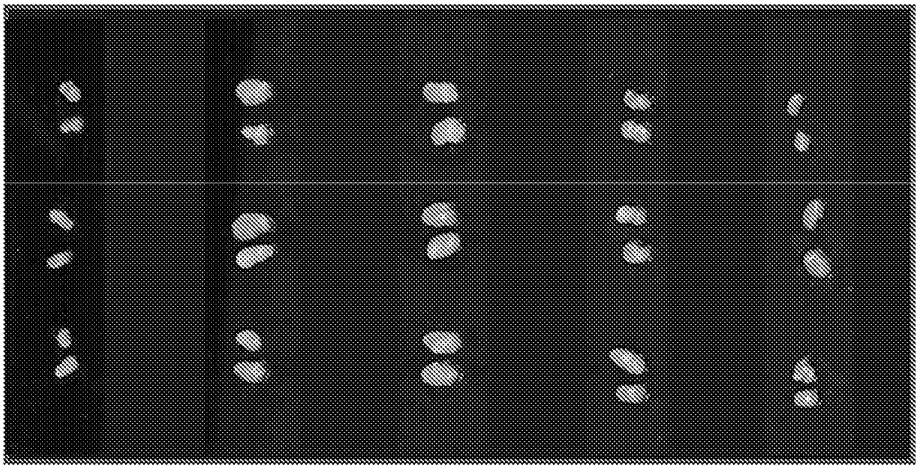

FIG. 8B

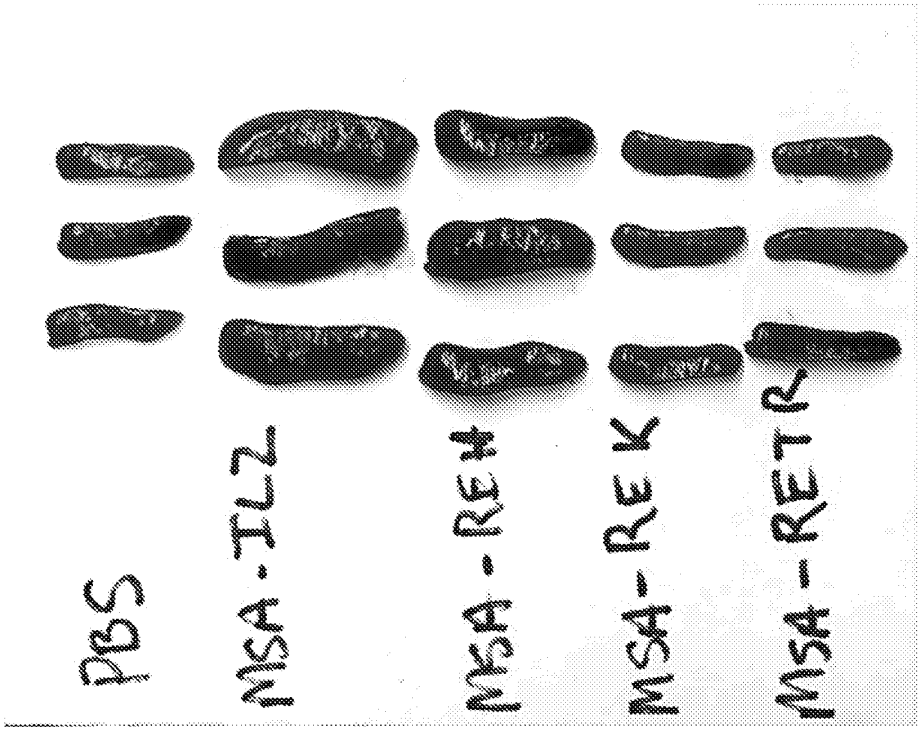

FIG. 8A

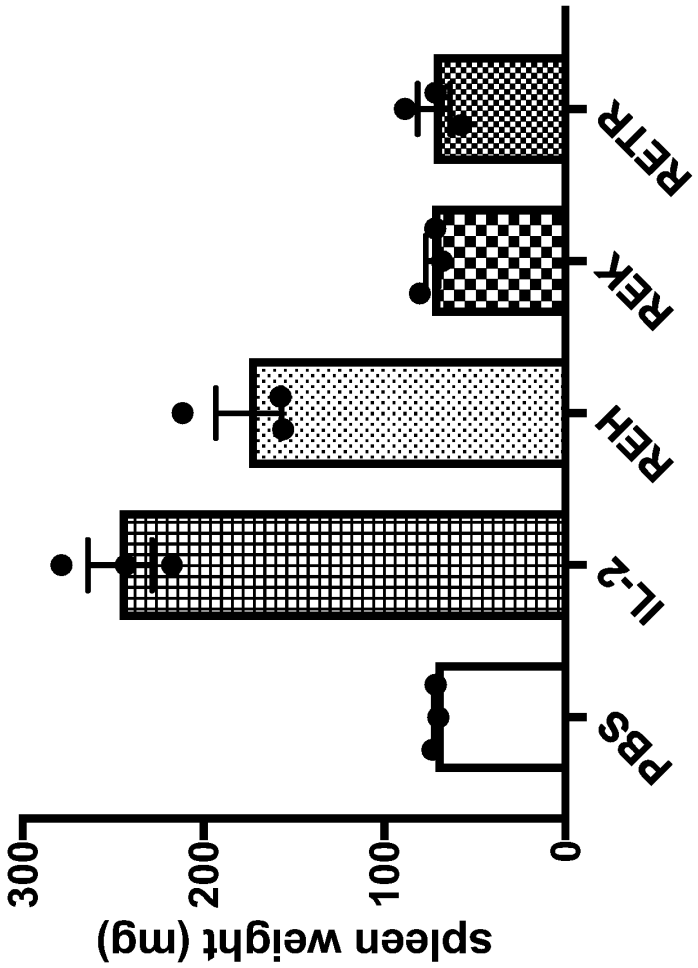

FIG. 8C

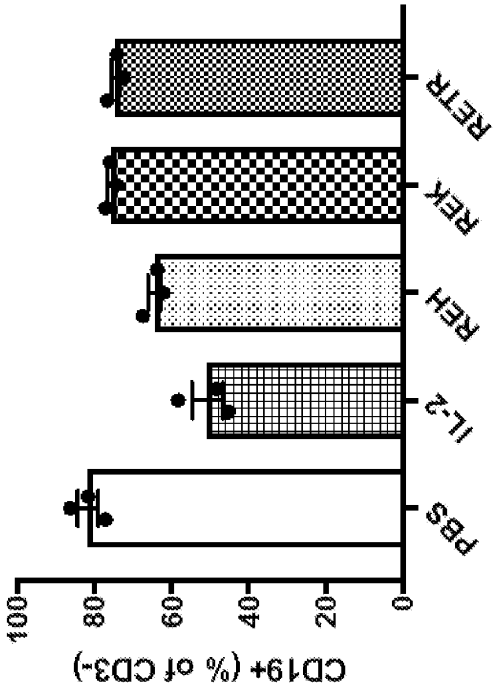
CD19+ (% of CD3-)
0
20
40
60
80
100
PBS
IL-2
REH
REK
RETR

FIG. 8E

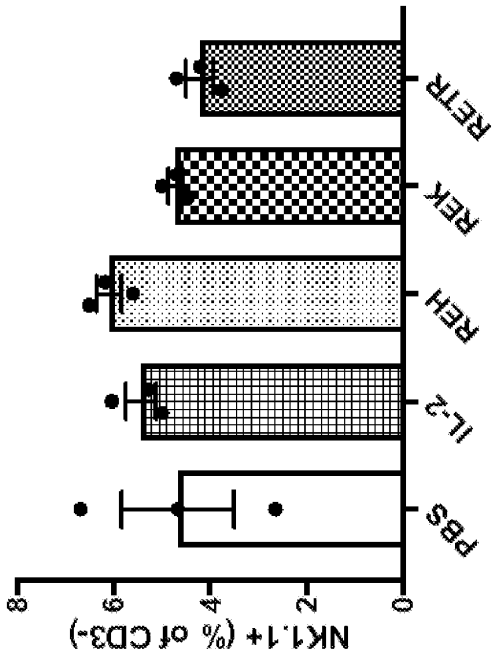
NK1.1+ (% of CD3-)
0
2
4
6
8
PBS
IL-2
REH
REK
RETR

FIG. 8D

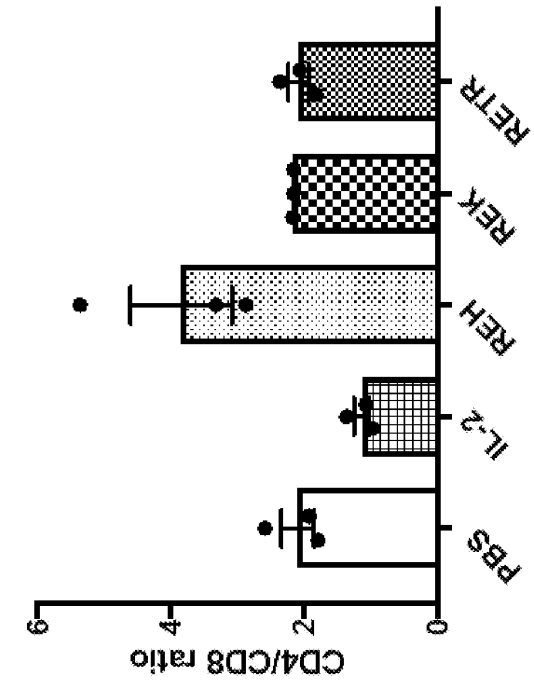
CD4/CD8 ratio
0
2
4
6
PBS
IL-2
REH
REK
RETR

FIG. 8G

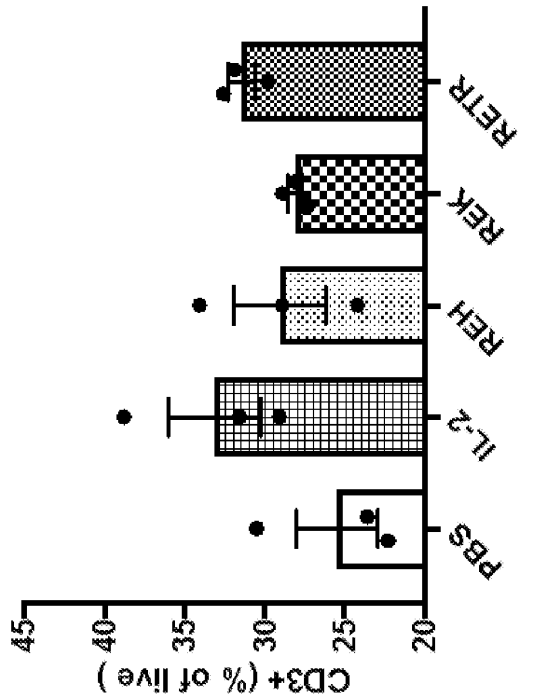
CD3+ (% of live)
20
25
30
35
40
45
PBS
IL-2
REH
REK
RETR

FIG. 8F

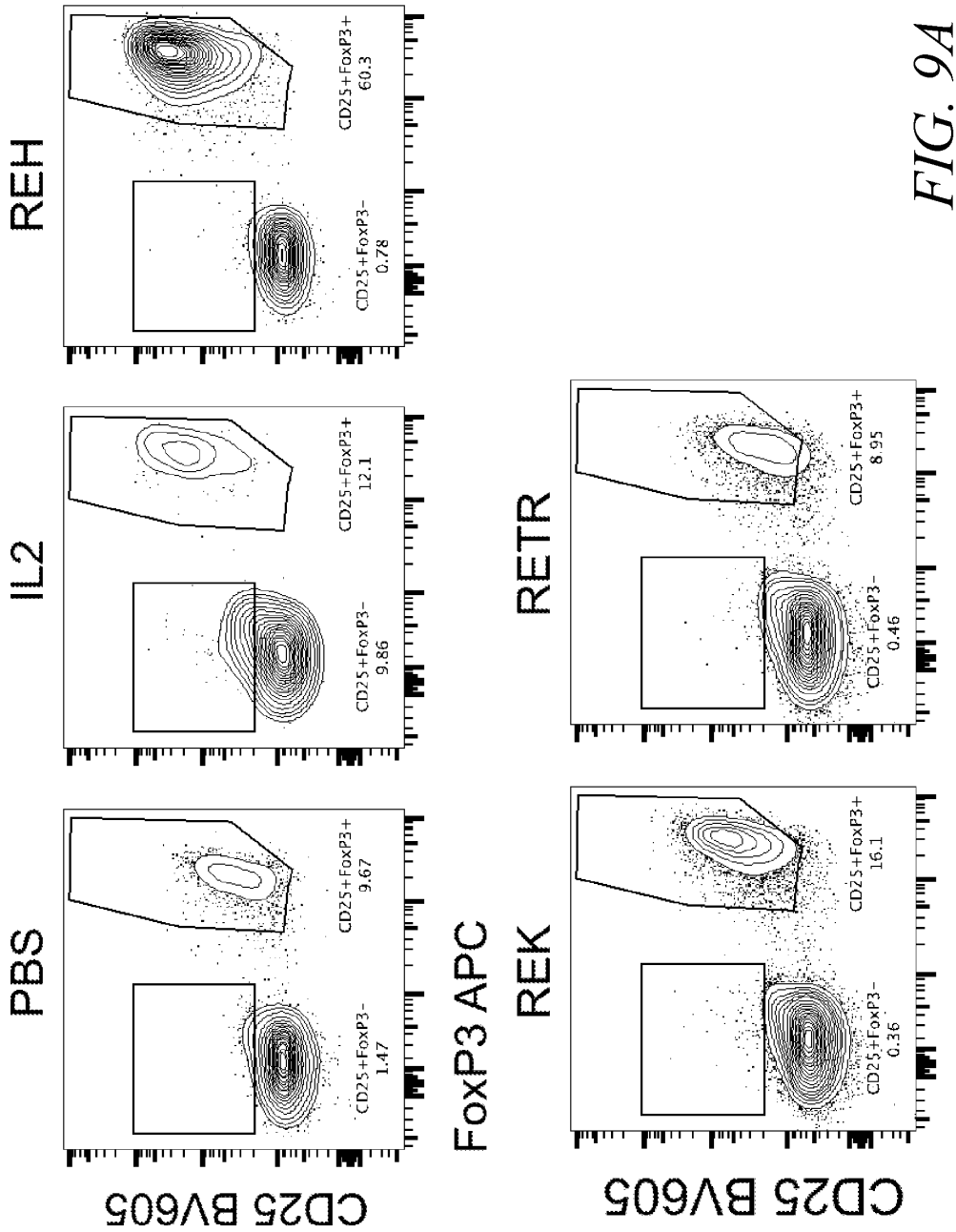
PBS
IL2
REH
RETR
REK
FoxP3 APC
CD25 BV605
CD25+FoxP3+ 9.67
CD25+FoxP3- 1.47
CD25+FoxP3+ 12.1
CD25+FoxP3- 9.86
CD25+FoxP3+ 60.3
CD25+FoxP3- 0.78
CD25+FoxP3+ 8.95
CD25+FoxP3- 0.46
CD25+FoxP3+ 16.1
CD25+FoxP3- 0.36

FIG. 9A

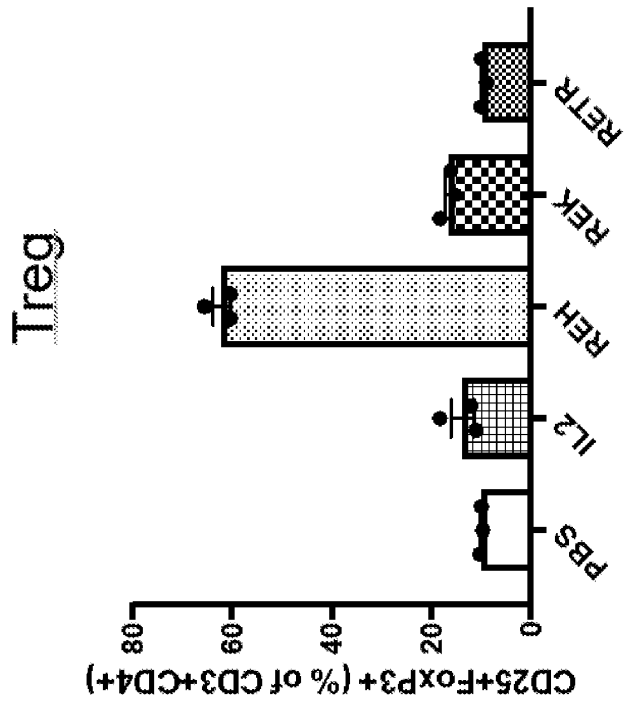
Treg
CD25+FoxP3+ (% of CD3+CD4+)
0
20
40
60
80
PBS
IL2
REH
REK
RETR

FIG. 9C

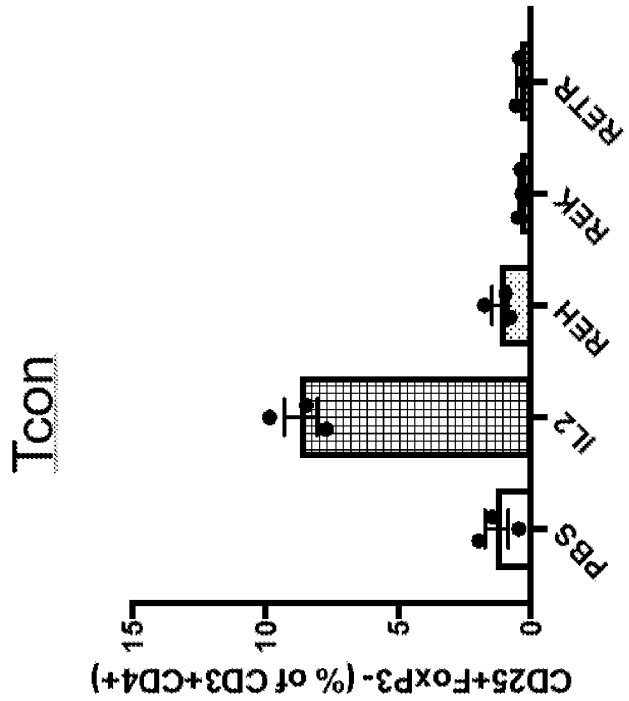
Tcon
CD25+FoxP3- (% of CD3+CD4+)
0
5
10
15
PBS
IL2
REH
REK
RETR

FIG. 9B

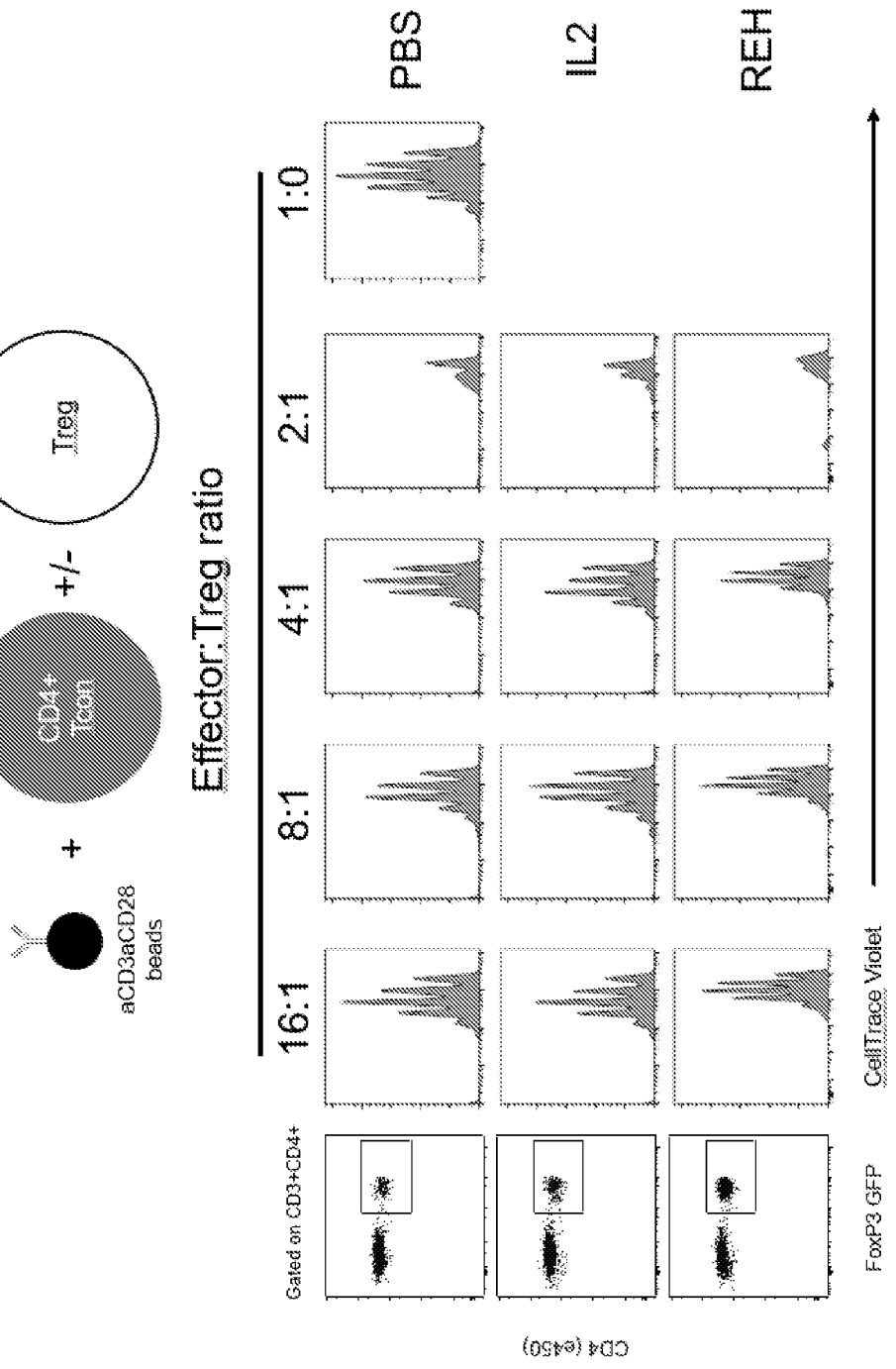
aCD3aCD28 beads
+
CD4+ Tcon
+/-
Treg
Effector:Treg ratio
16:1
8:1
4:1
2:1
1:0
PBS
IL2
REH
Gated on CD3+CD4+
CD4 (e450)
FoxP3 GFP
CellTrace Violet

FIG. 10

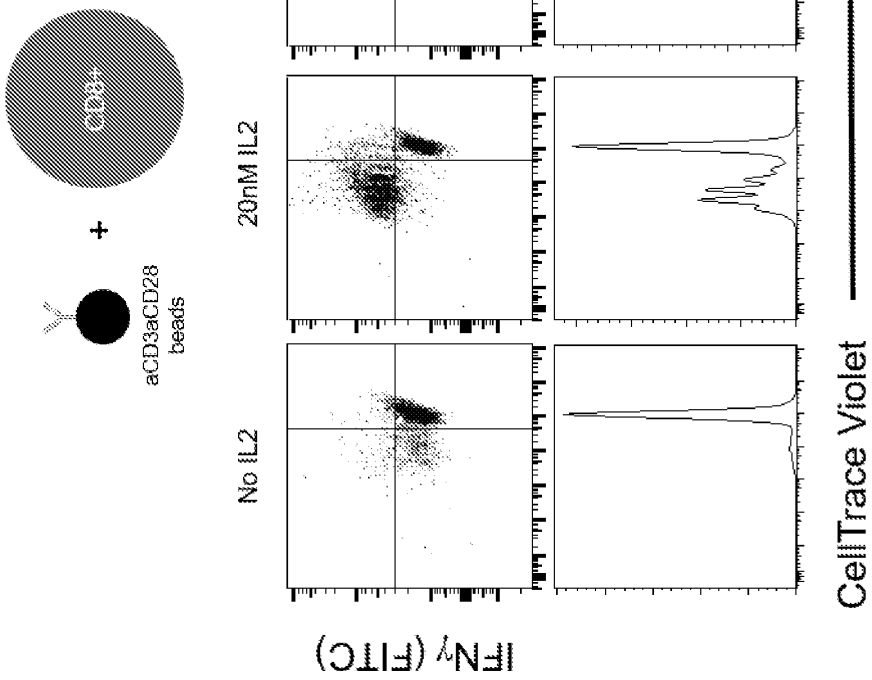
CD37
+
aCD3aCD28
beads
No IL2
20nM IL2
20nM REH
IFNγ (FITC)
CellTrace Violet

FIG. 11

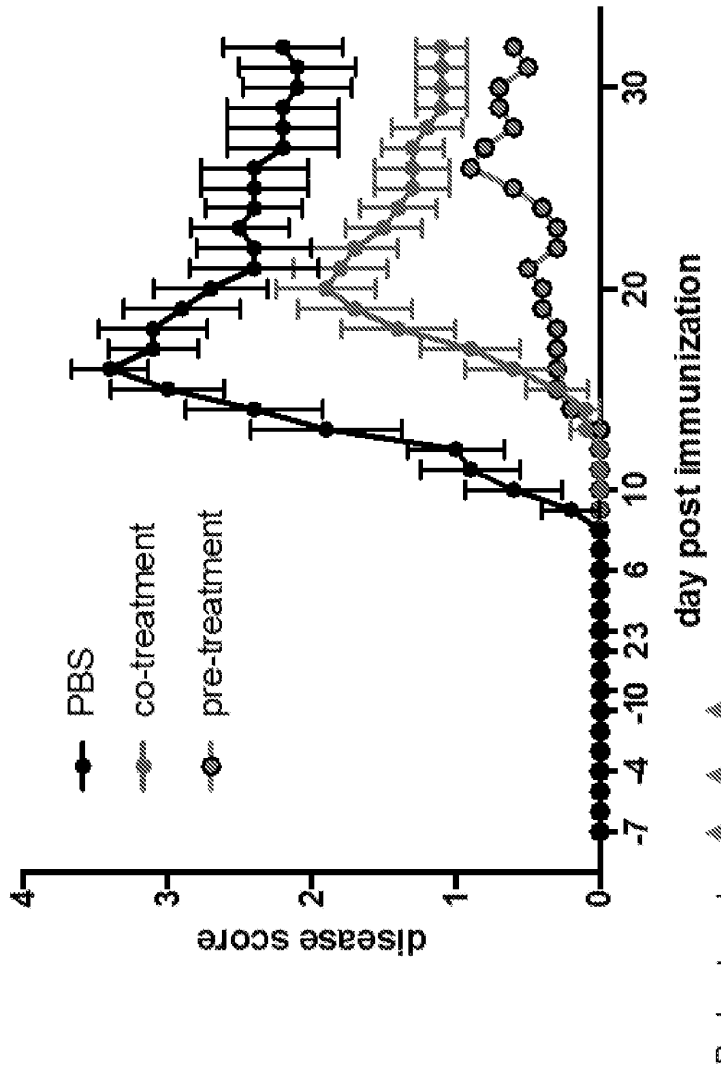
PBS
co-treatment
pre-treatment
disease score
0
1
2
3
4
-7
-4
-10
23
6
10
20
30
day post immunization
Pretreatment
Cotreatment
MOG in CFA
PTX

FIG. 12