

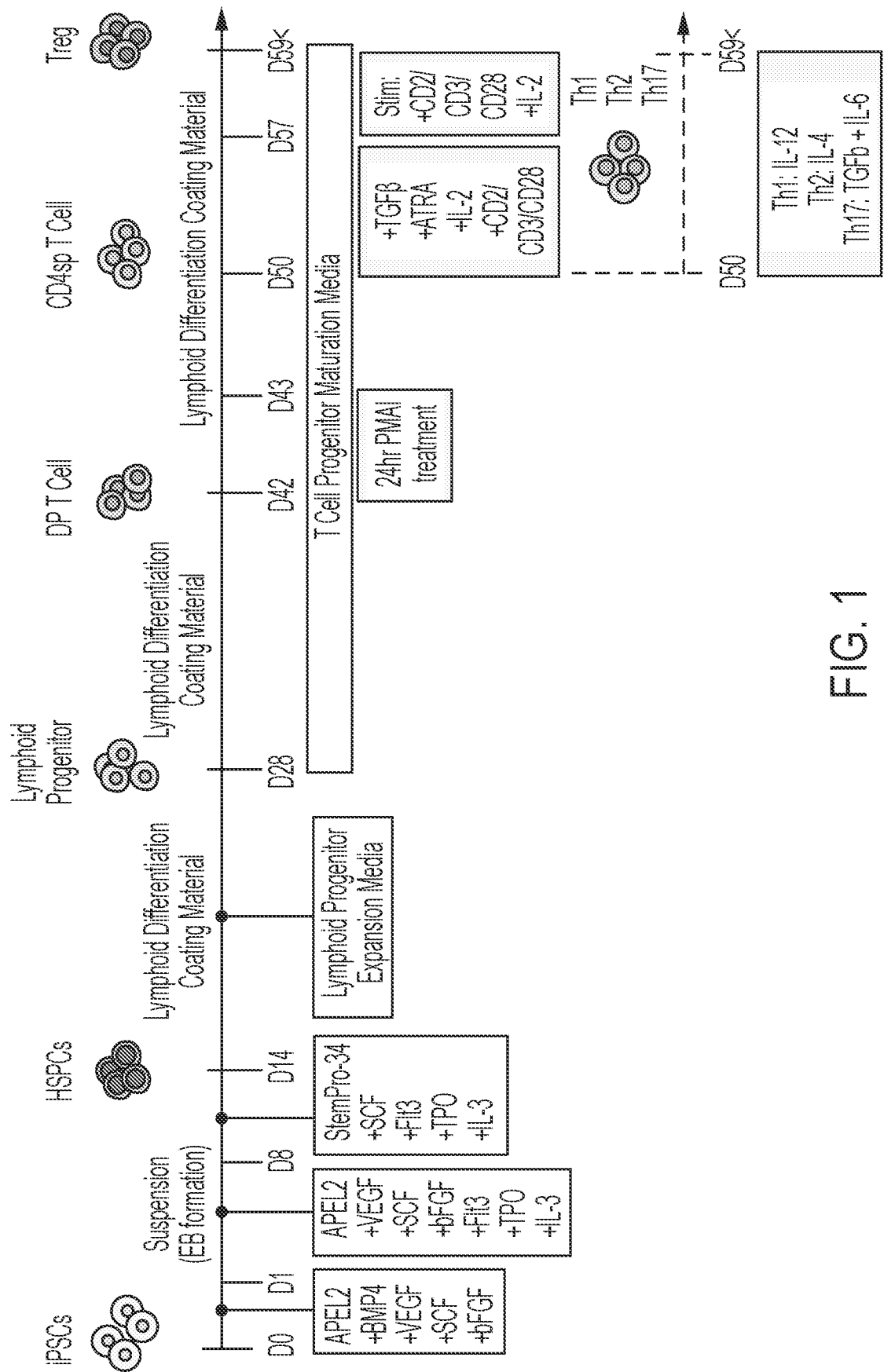


FIG. 1

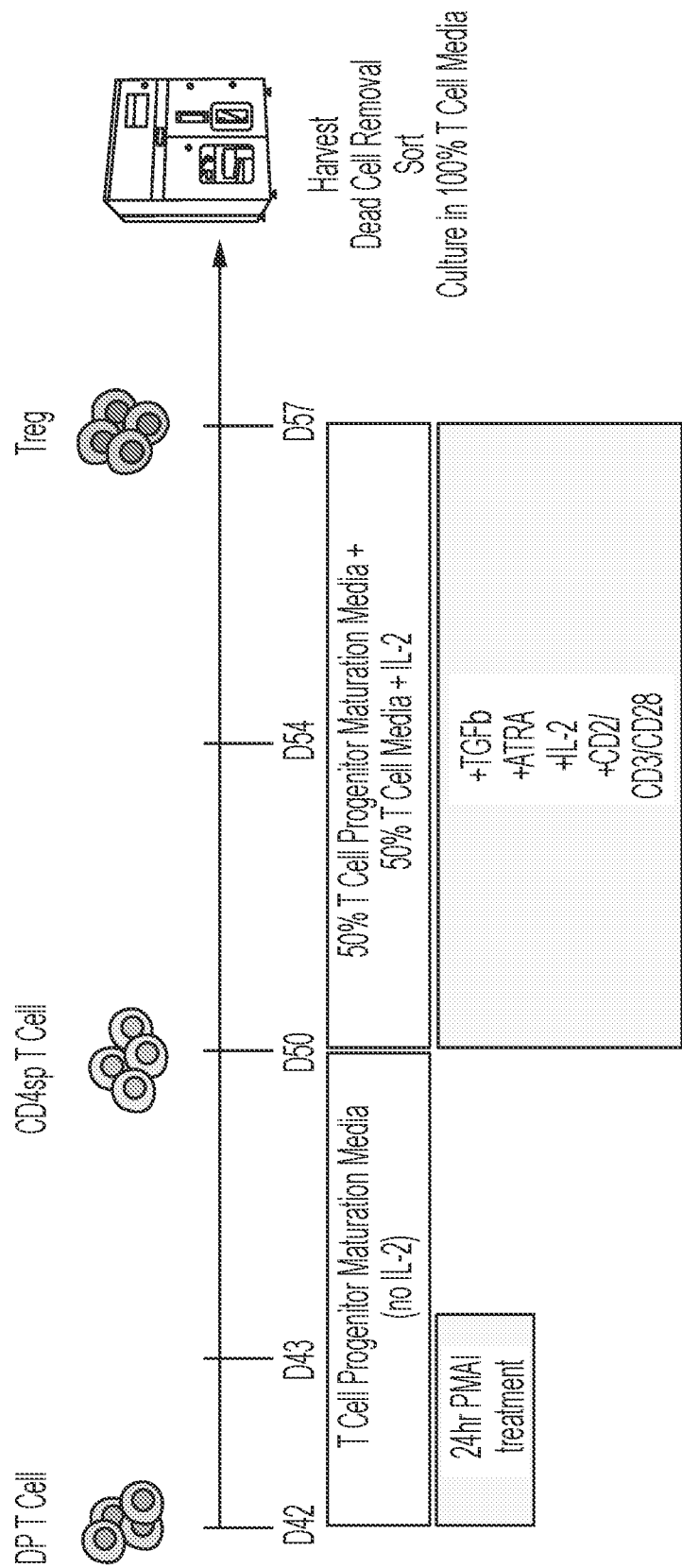


FIG. 2

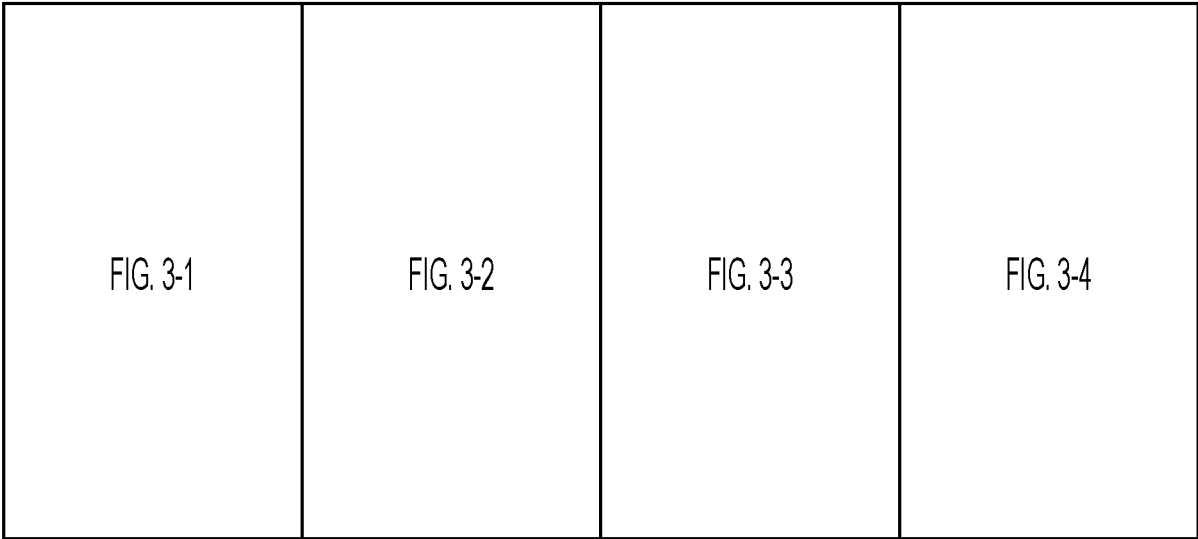


FIG. 3

Day 35 PMAI

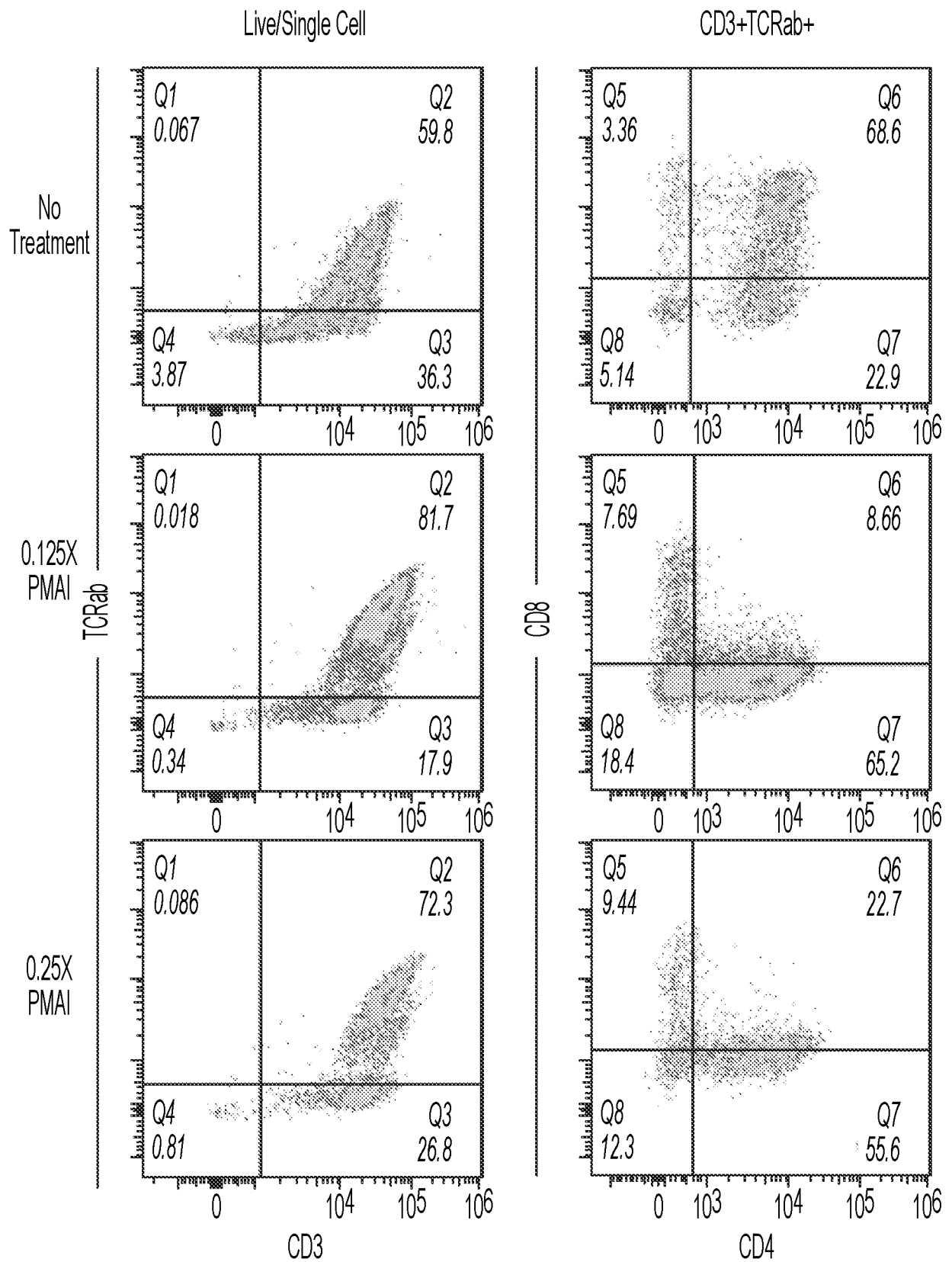


FIG. 3

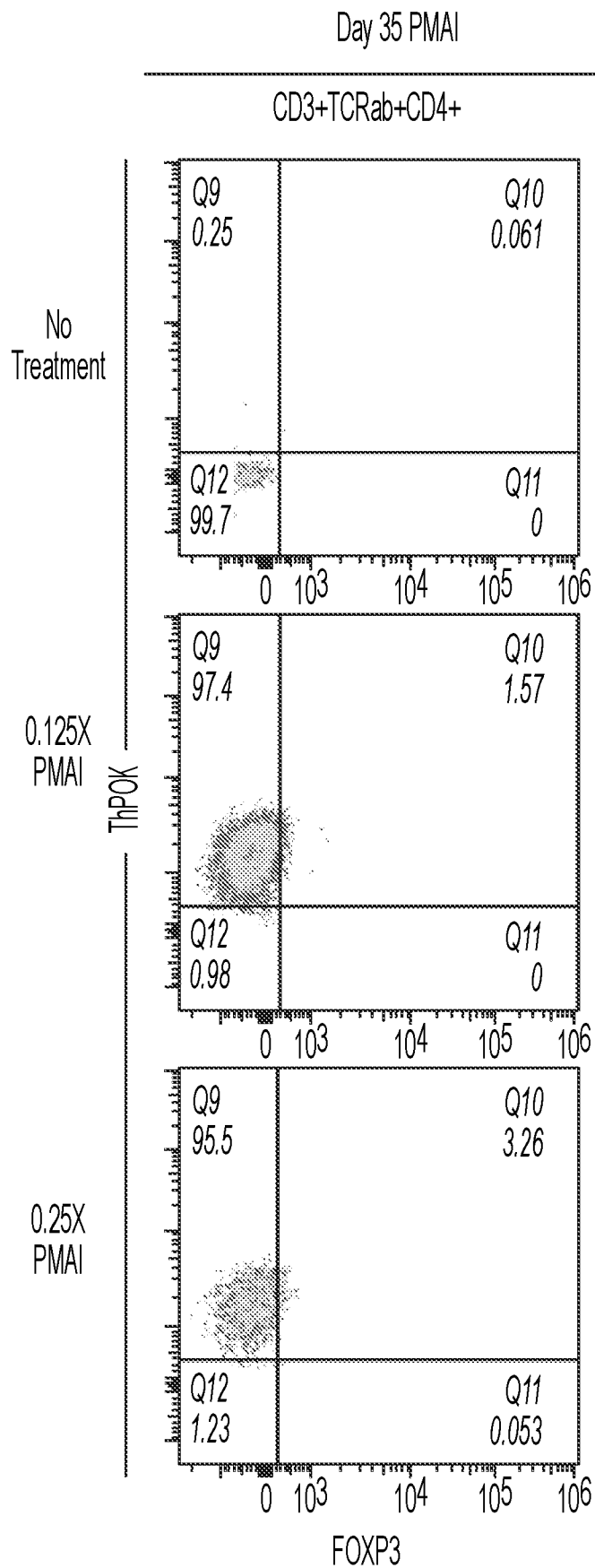


FIG. 3

Day 35 PMAI

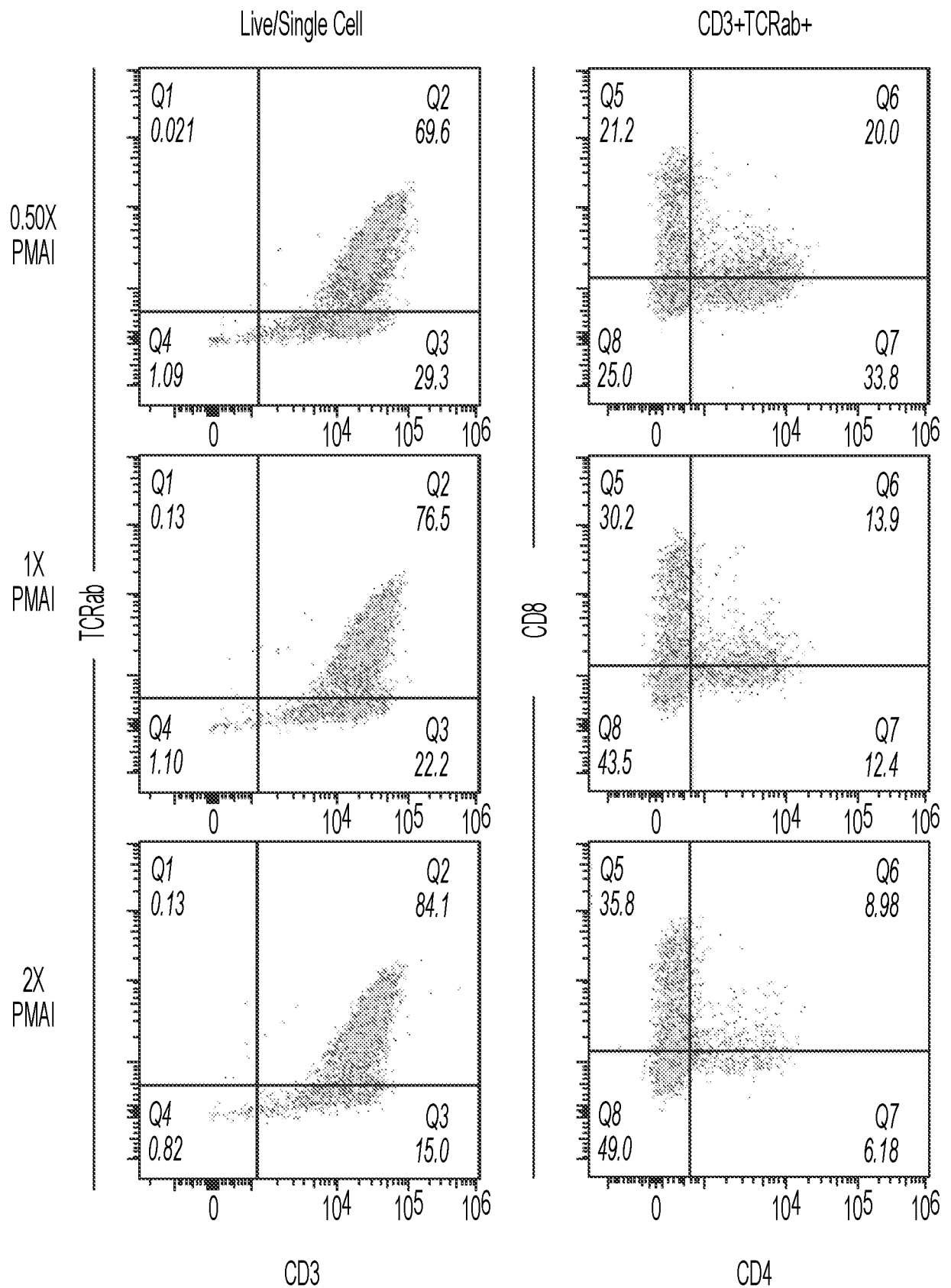


FIG. 3

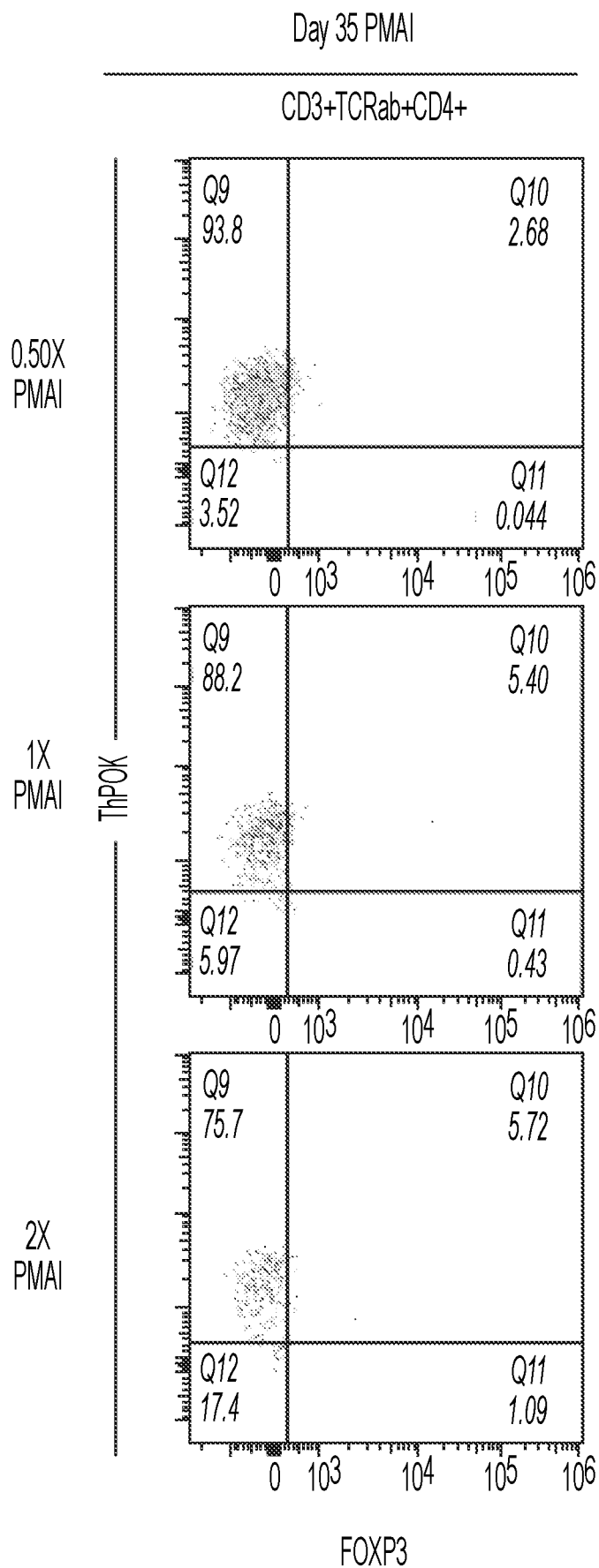


FIG. 3

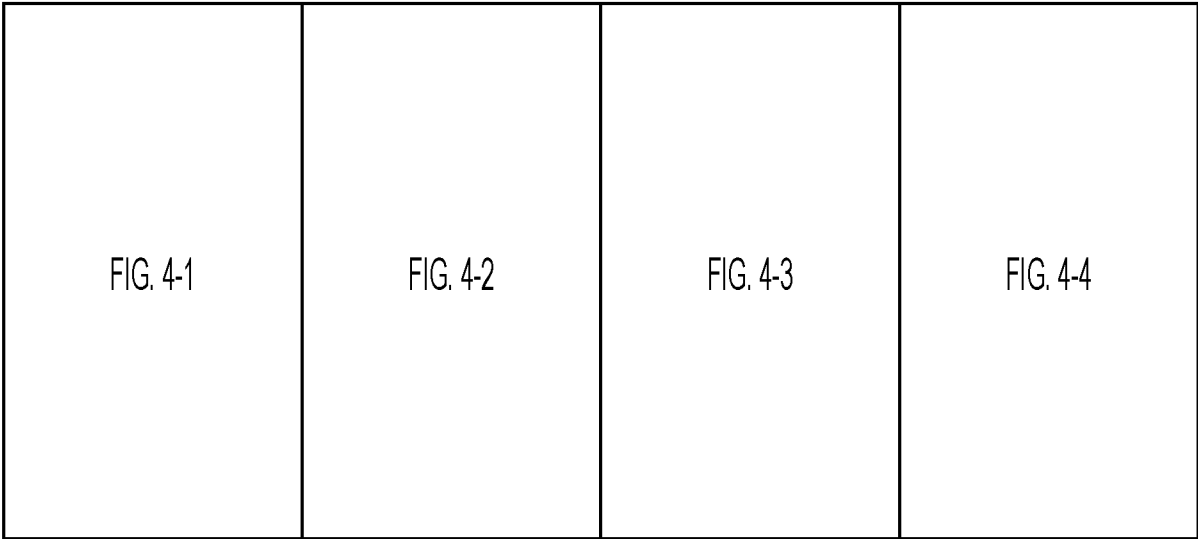


FIG. 4

Day 42 PMAI

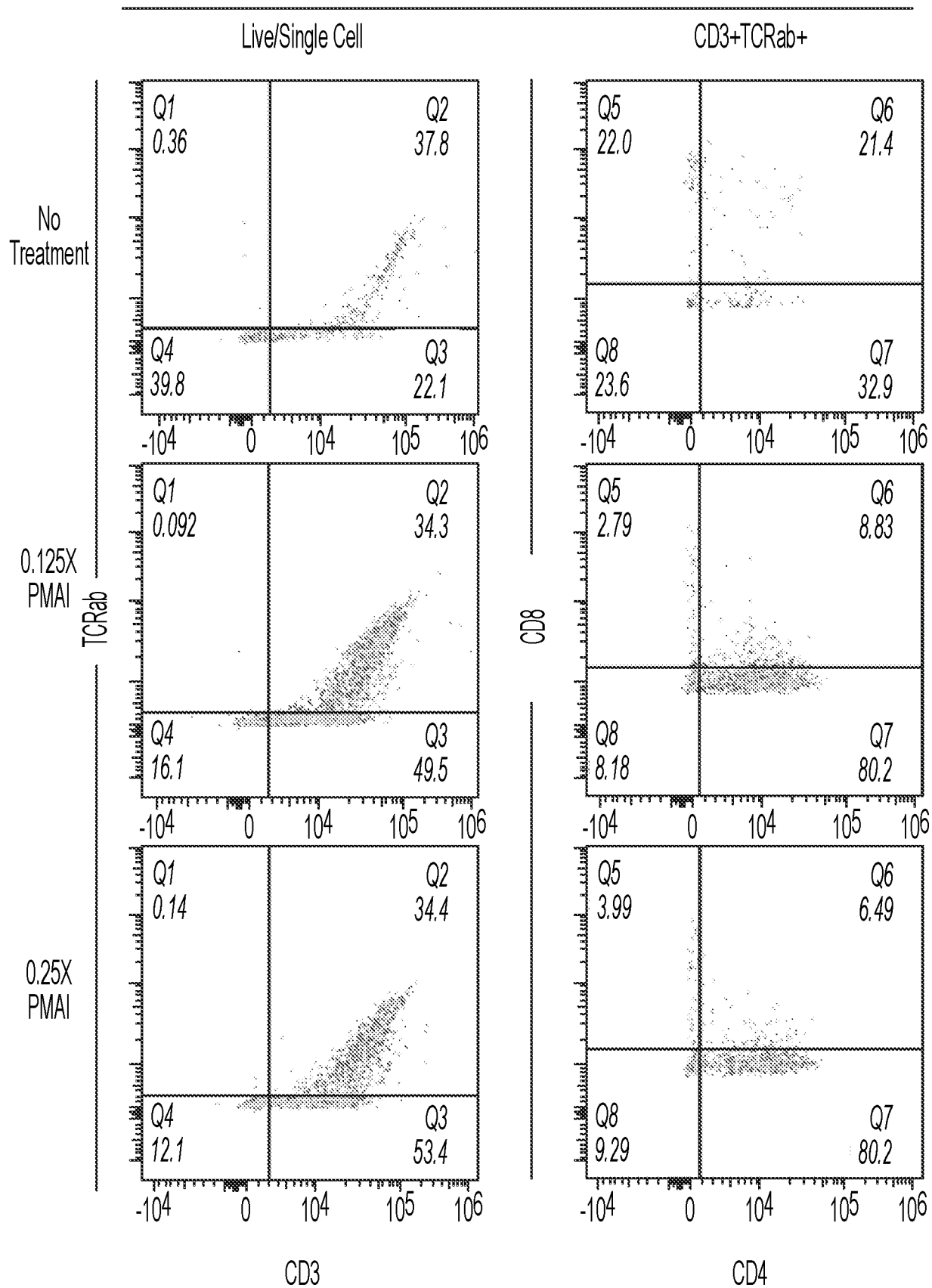


FIG. 4

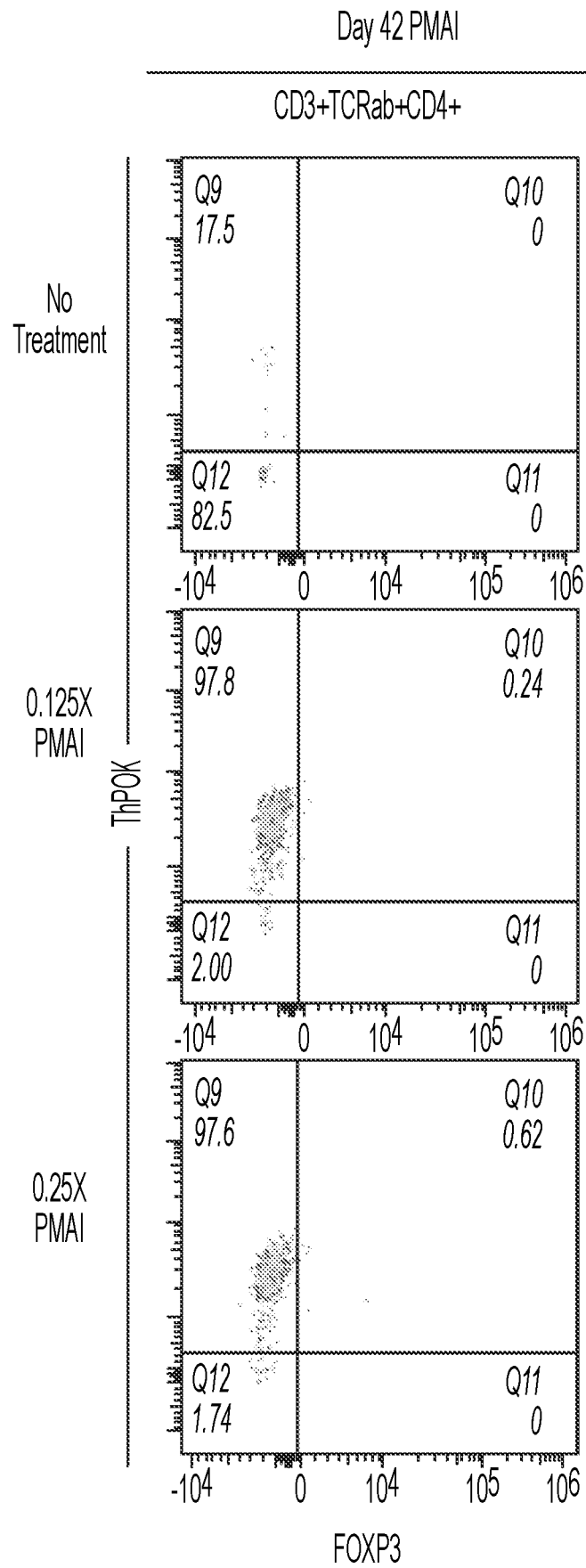


FIG. 4

Day 42 PMAI

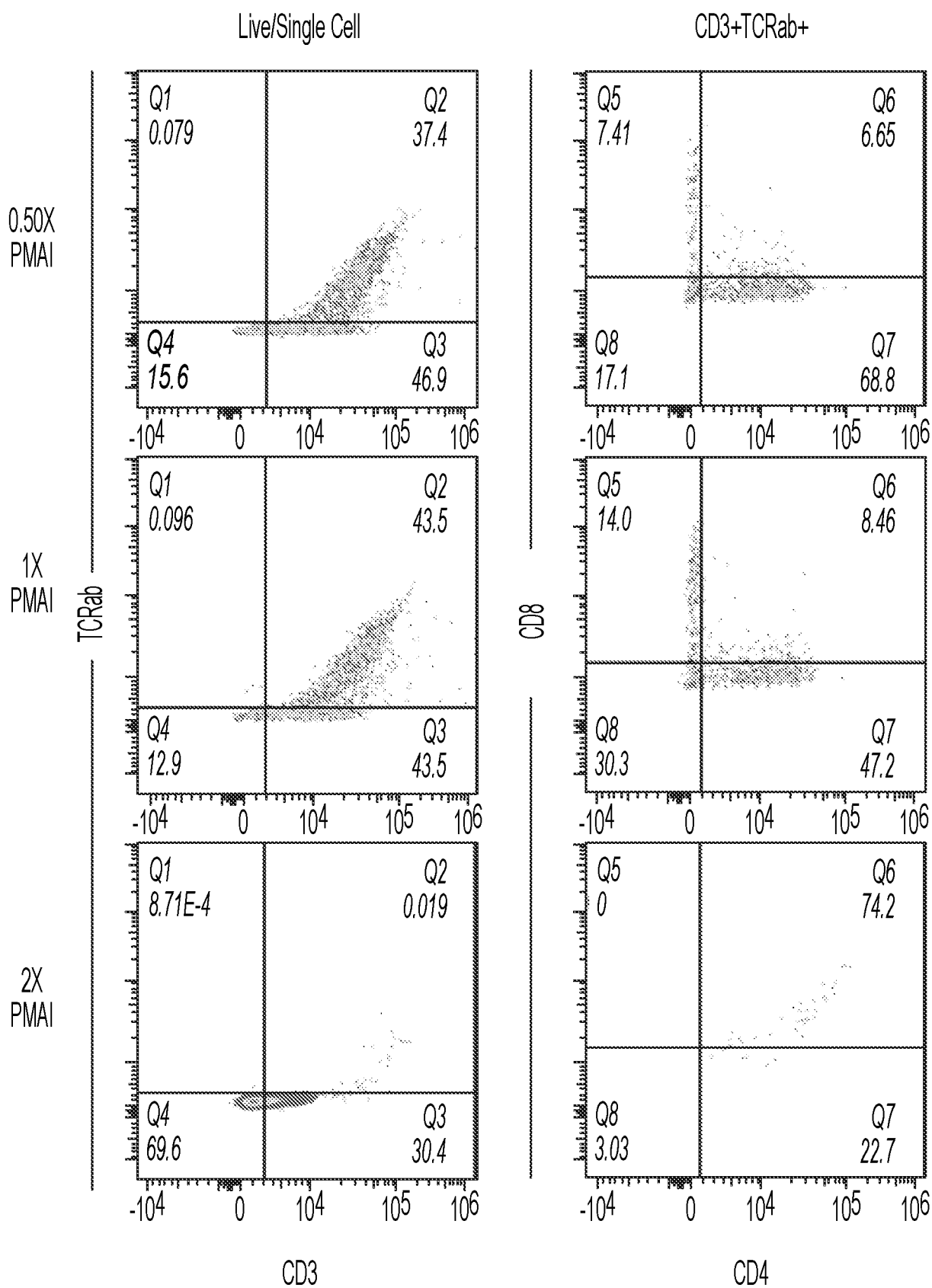


FIG. 4

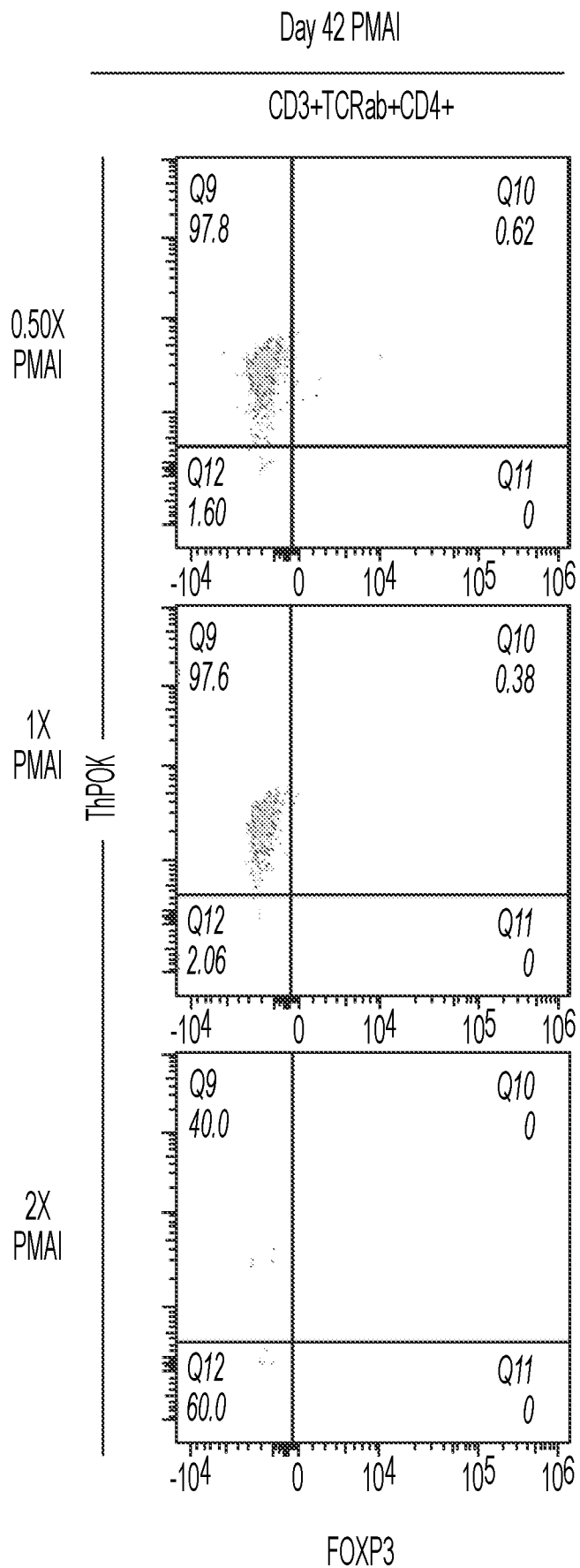


FIG. 4

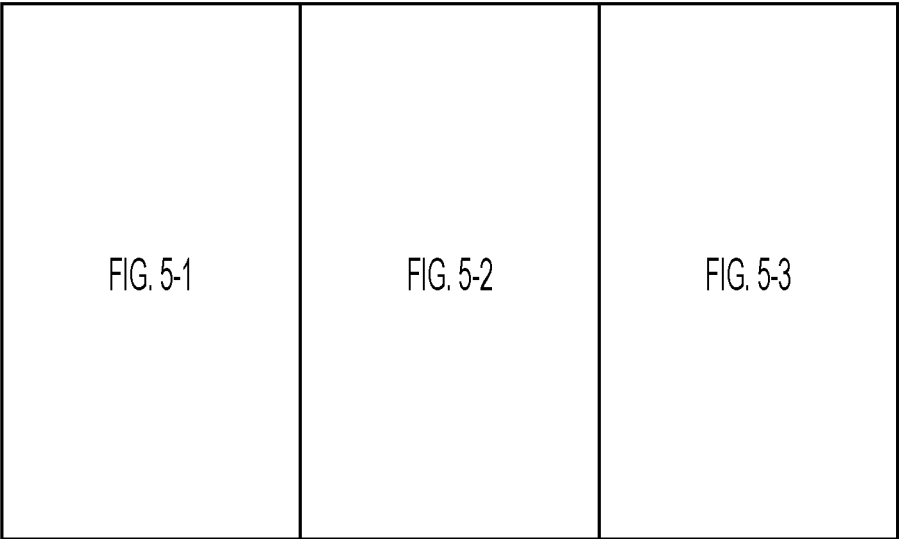


FIG. 5

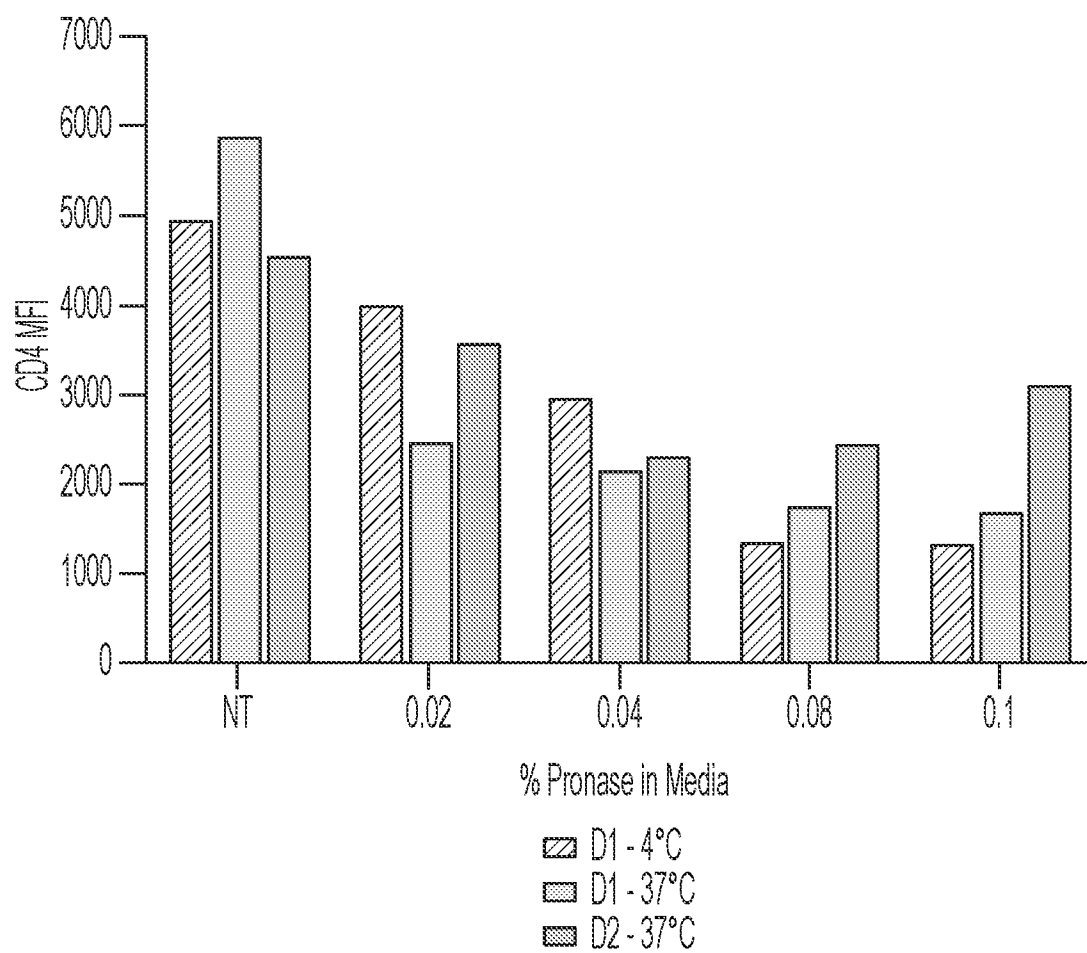


FIG. 5

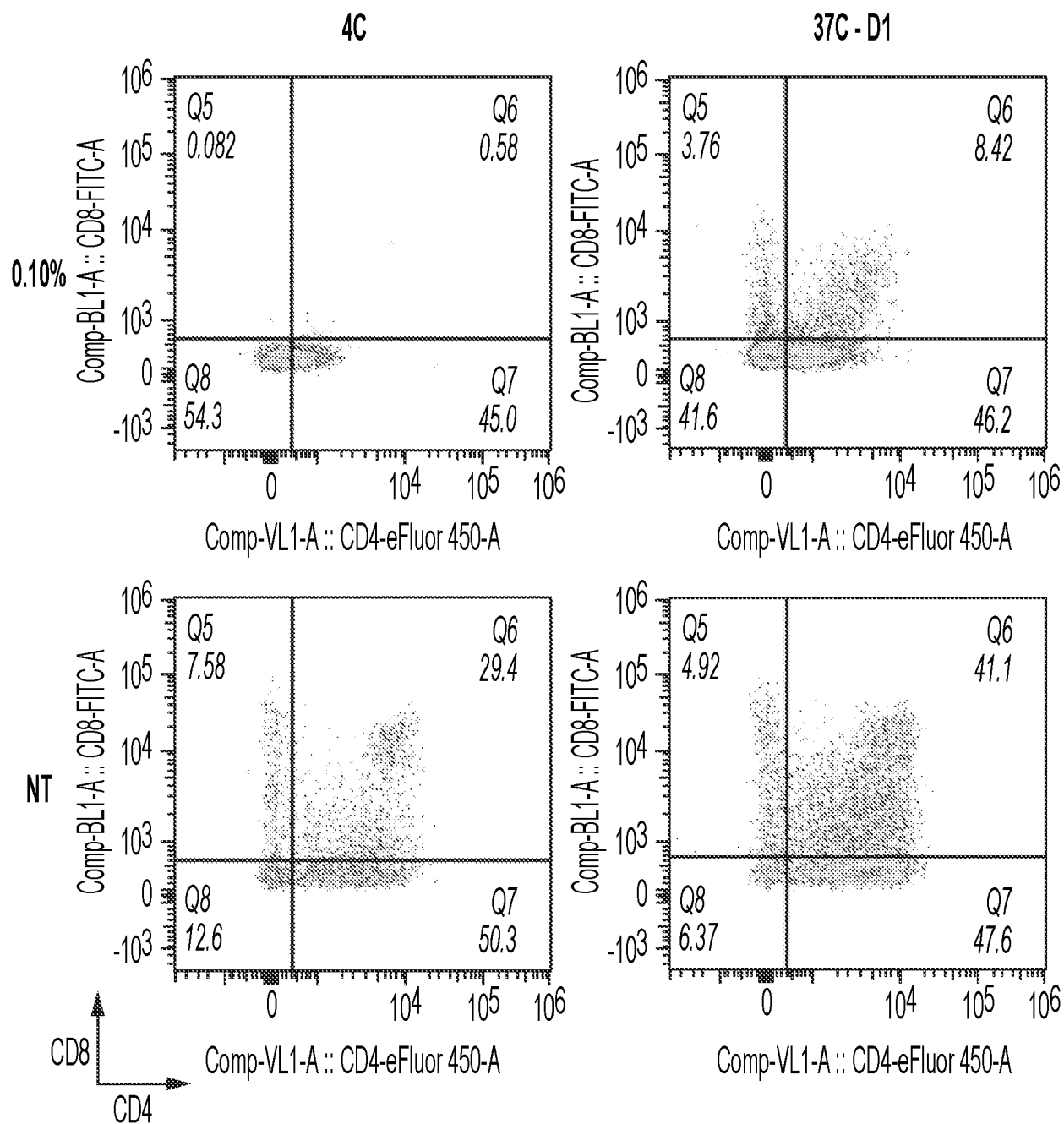


FIG. 5

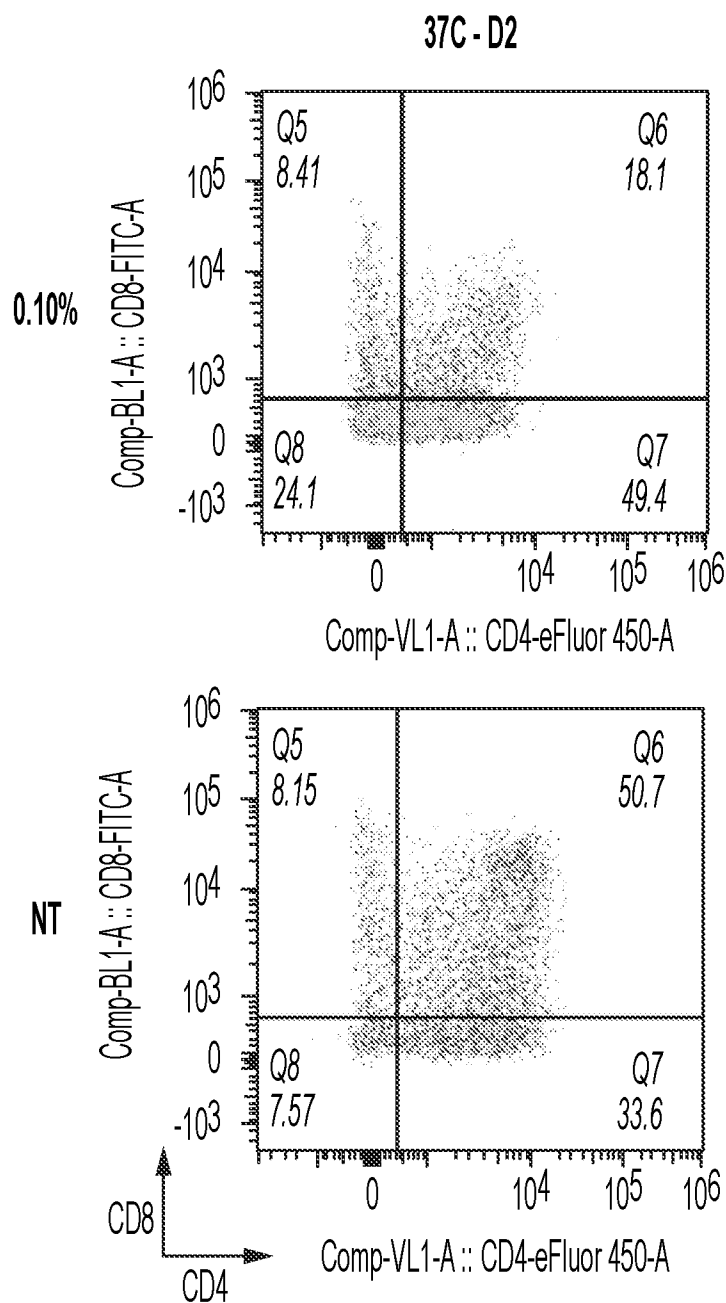


FIG. 5

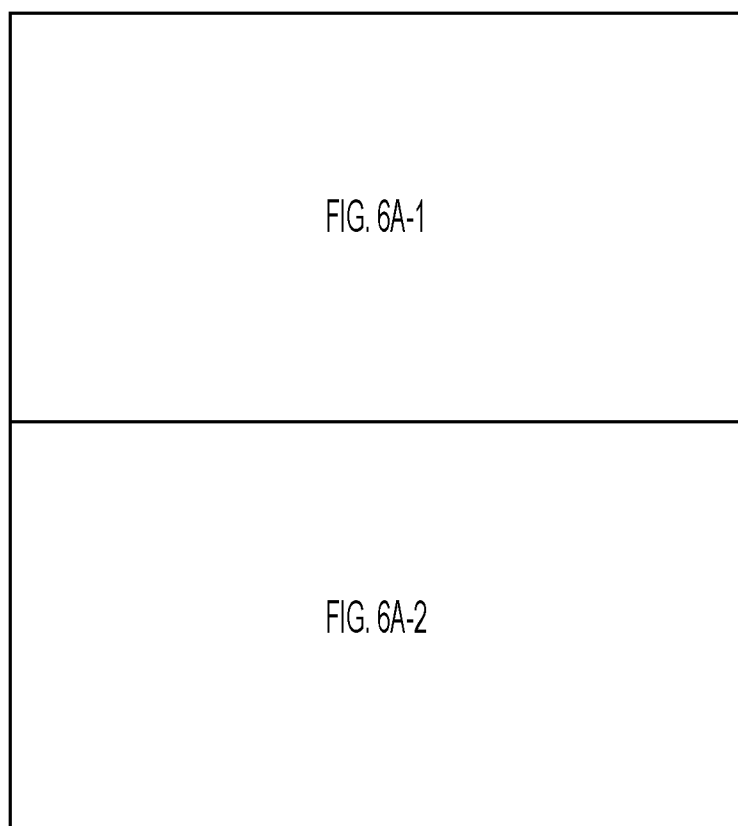


FIG. 6A

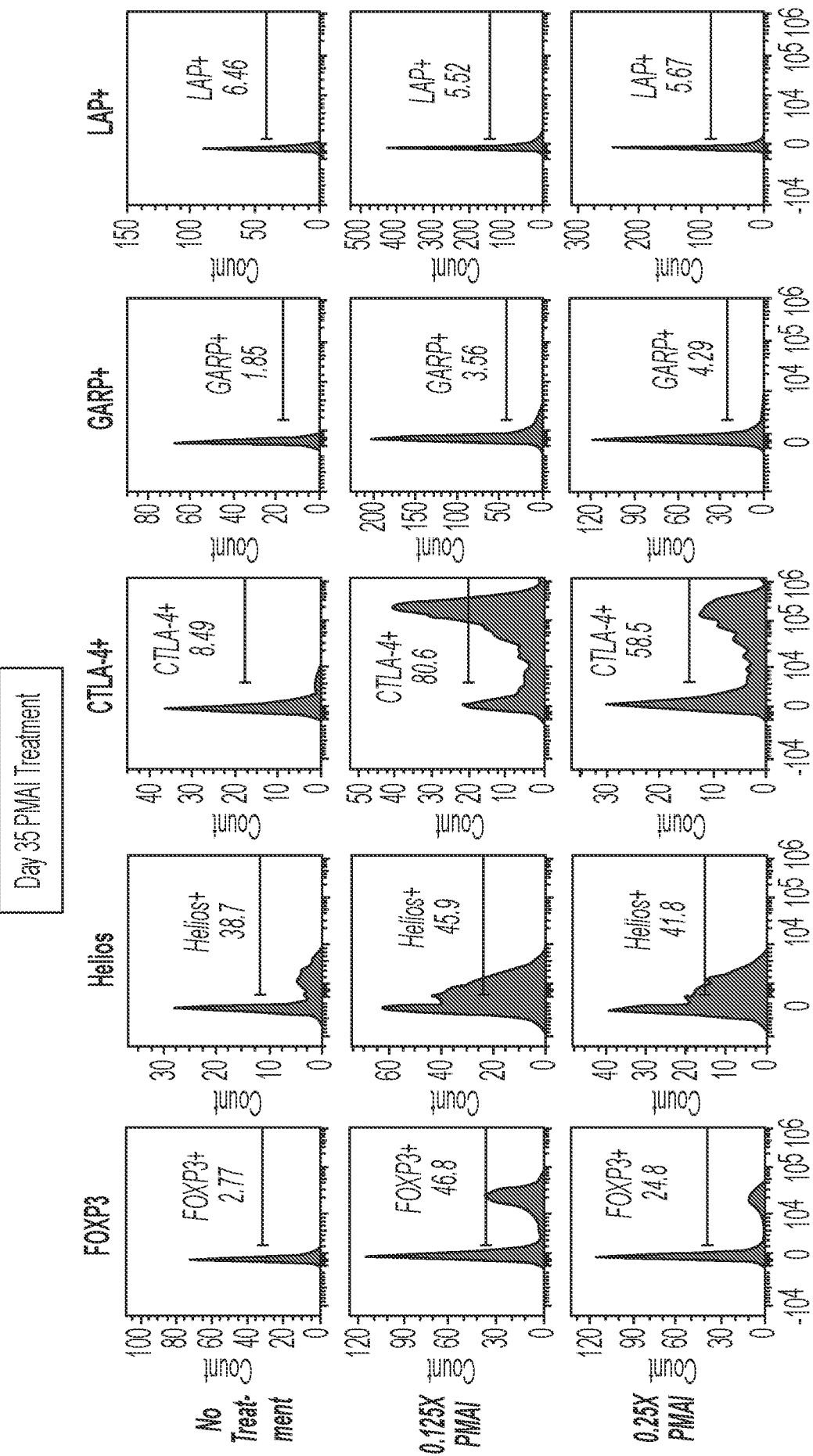


FIG. 6A
CONTINUED

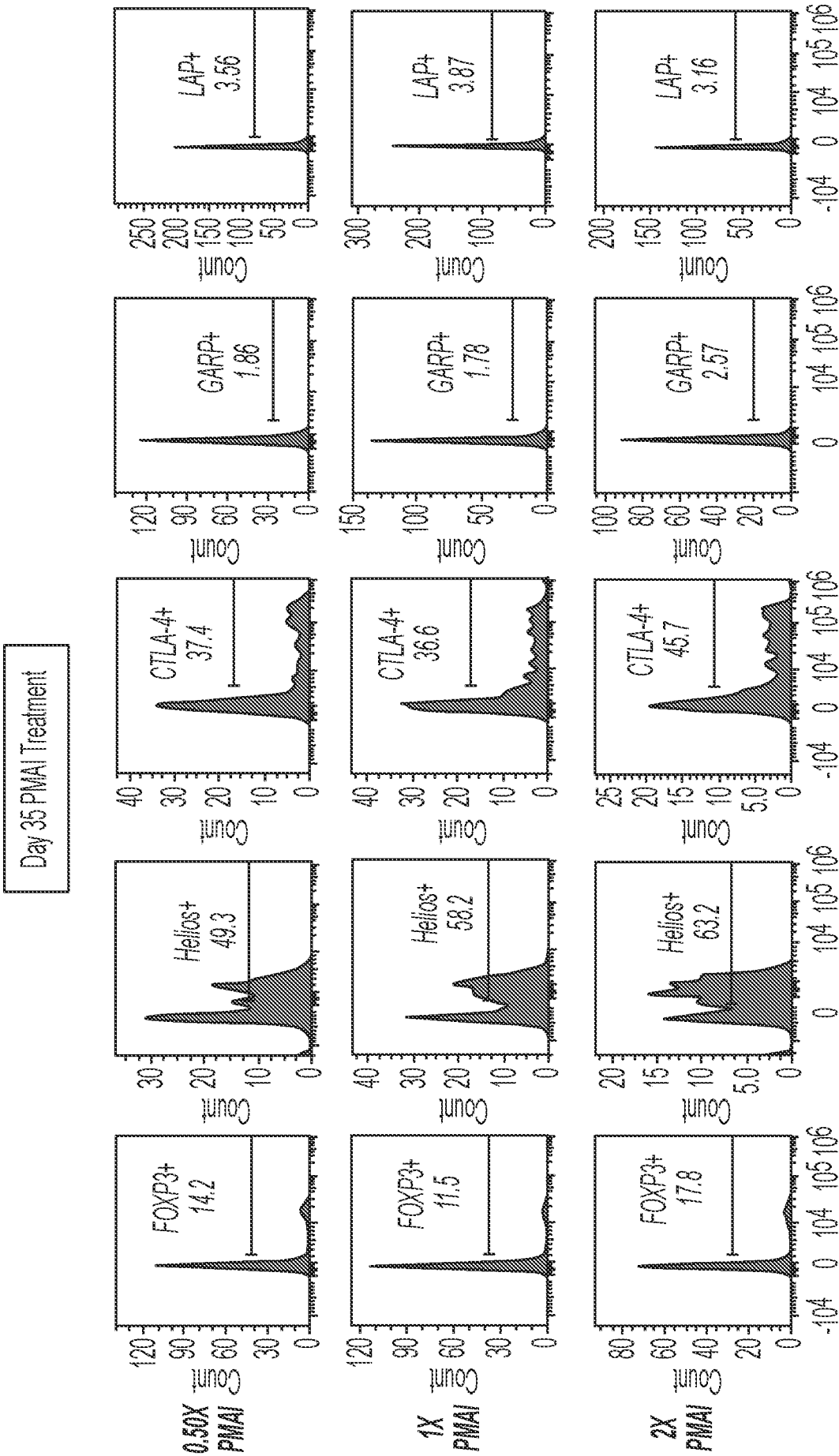


FIG. 6A

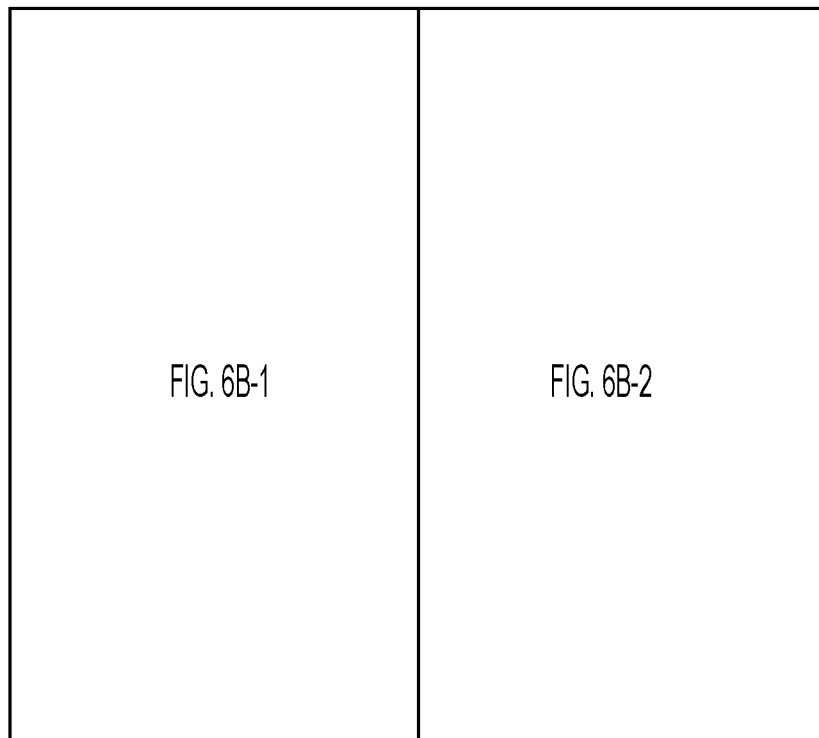


FIG. 6B

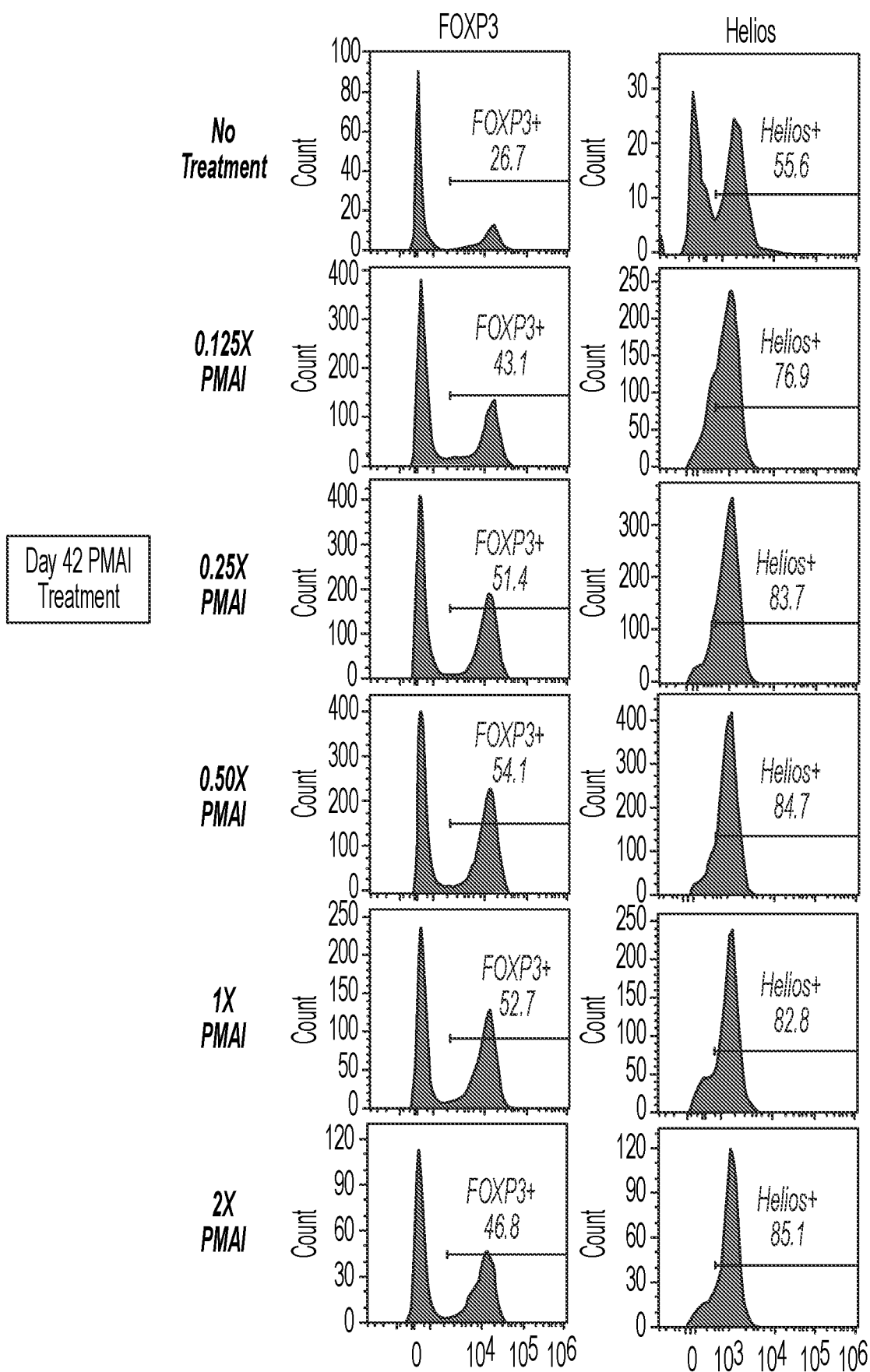


FIG. 6B

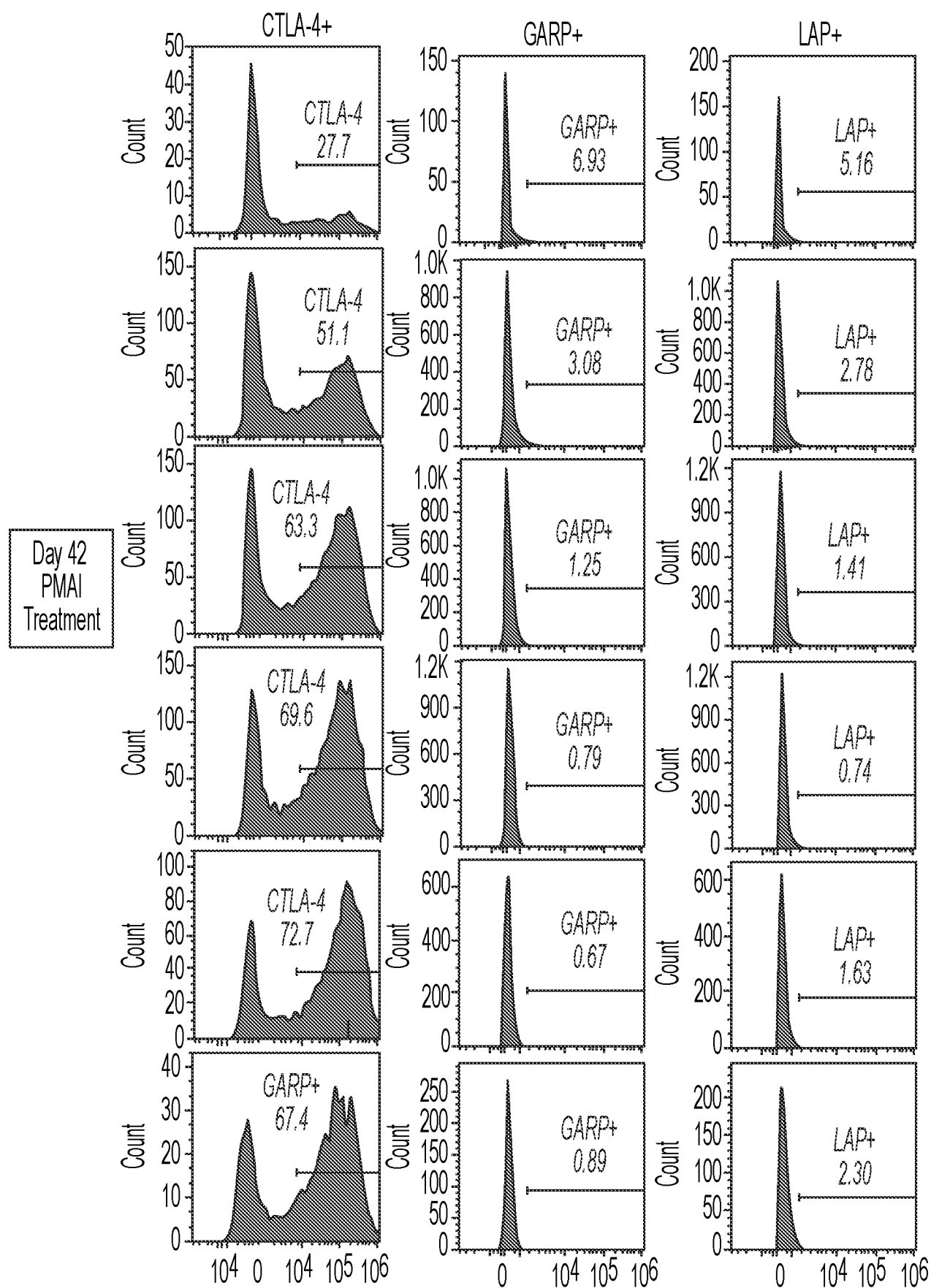


FIG. 6B

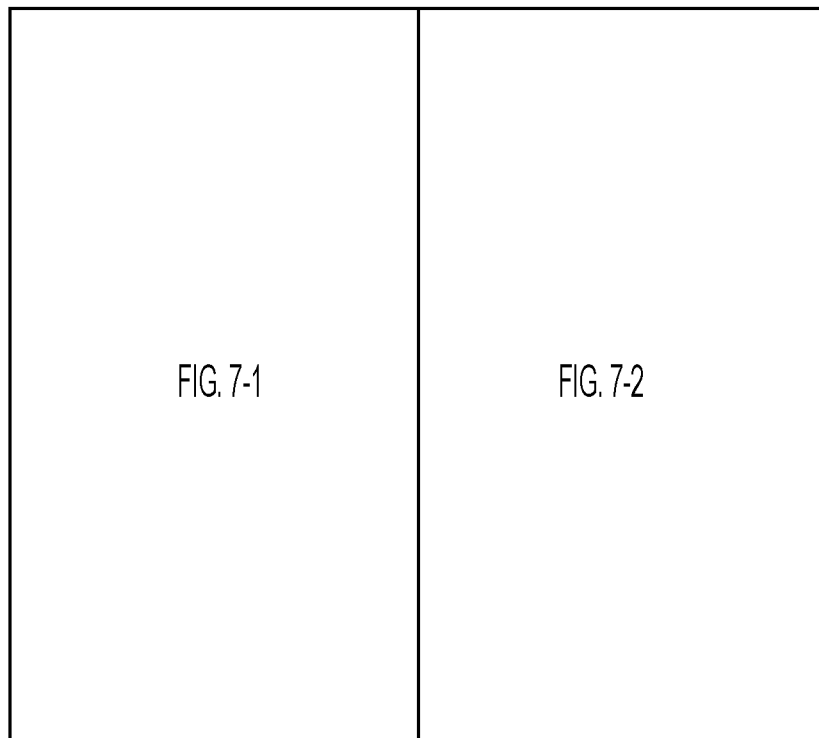
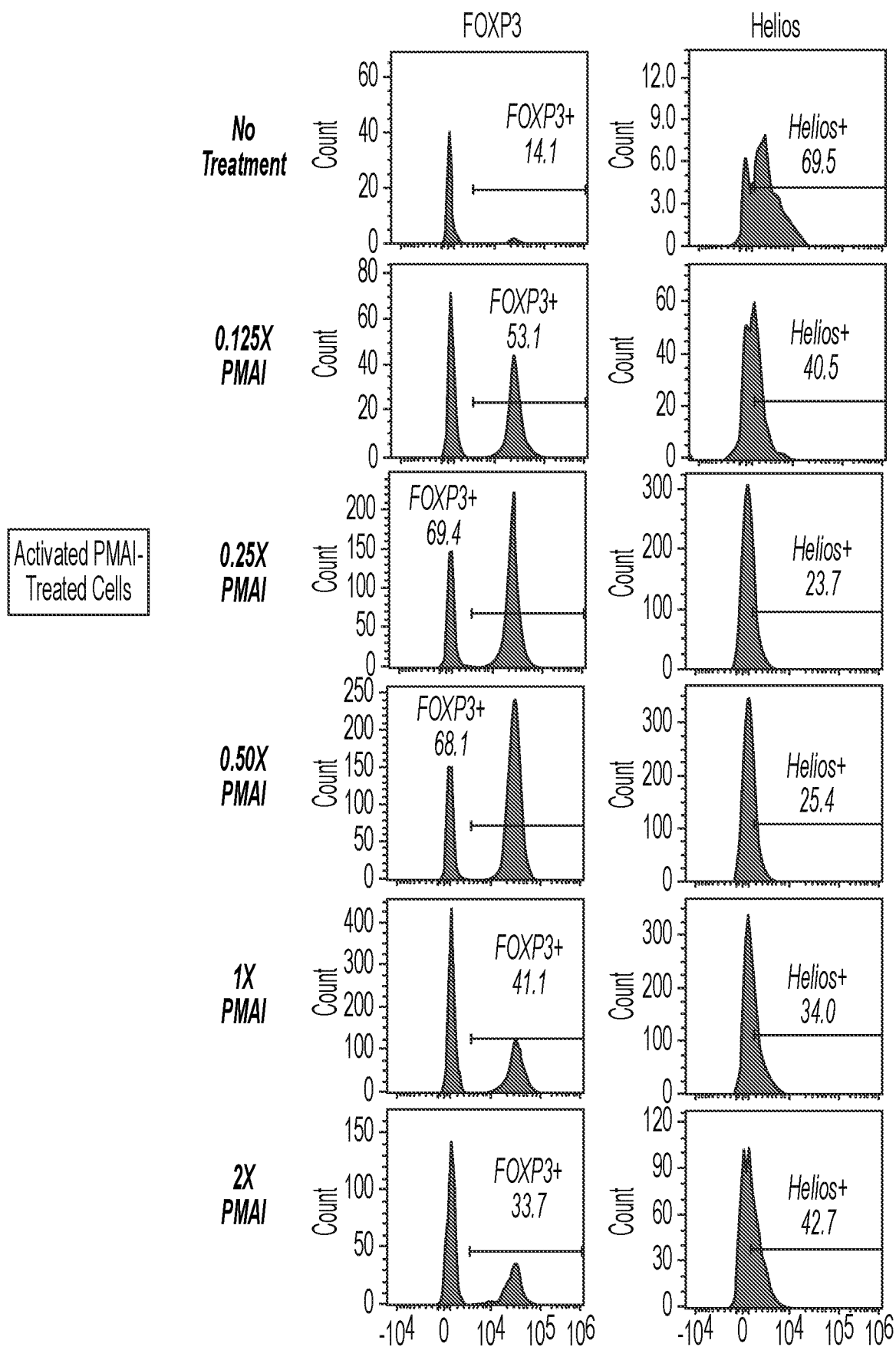


FIG. 7



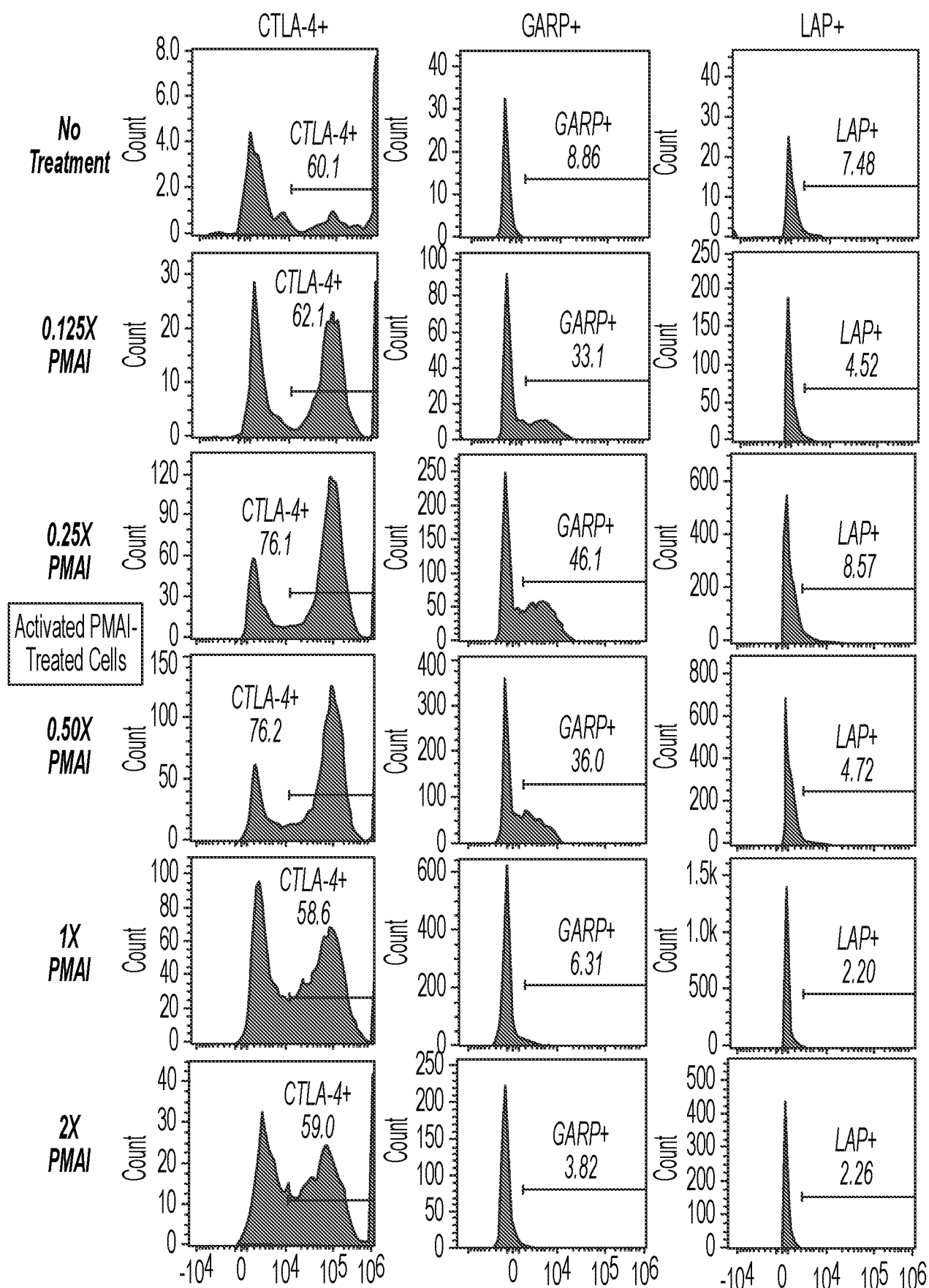


FIG. 7

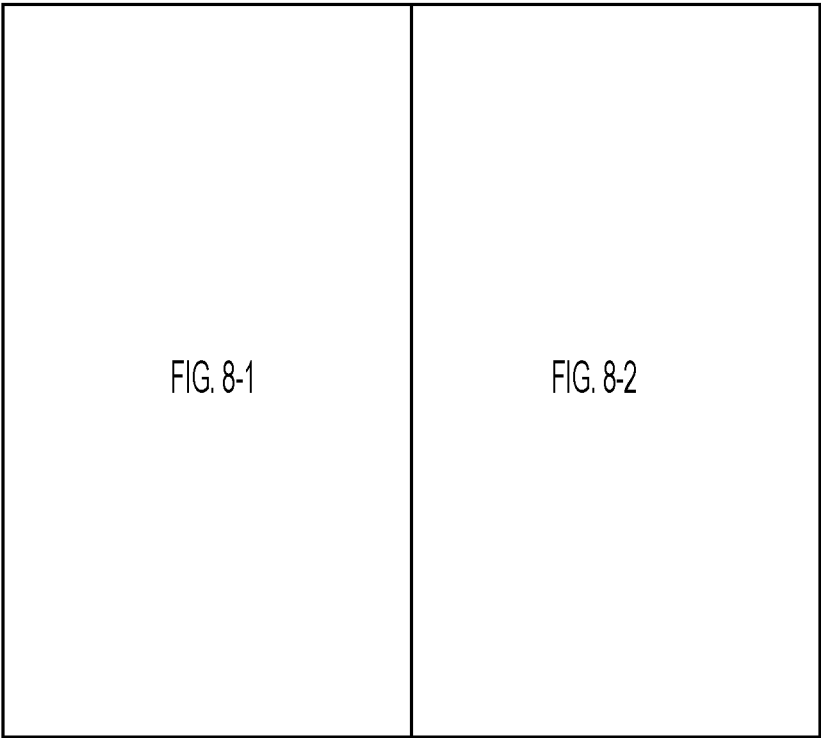


FIG. 8

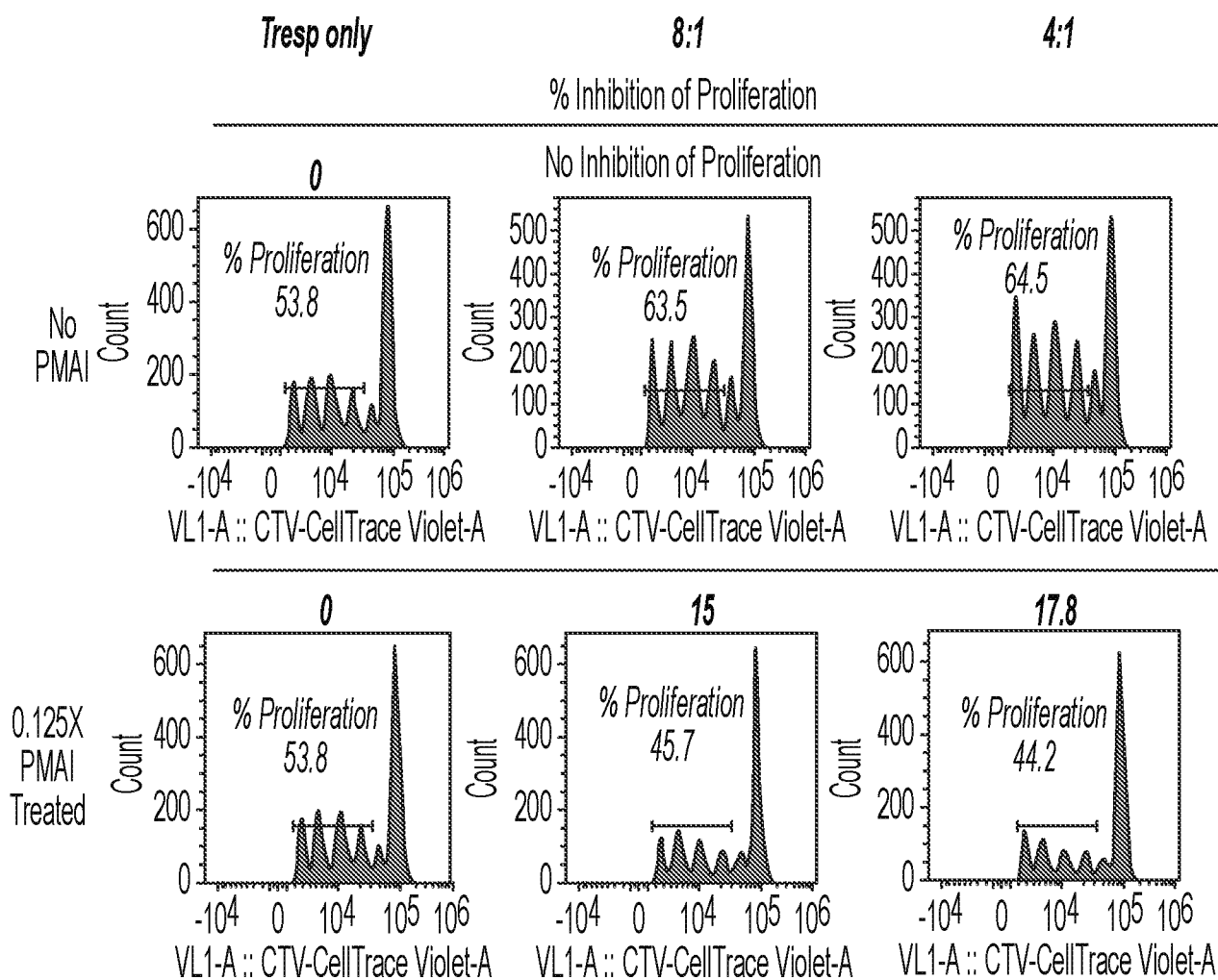


FIG. 8

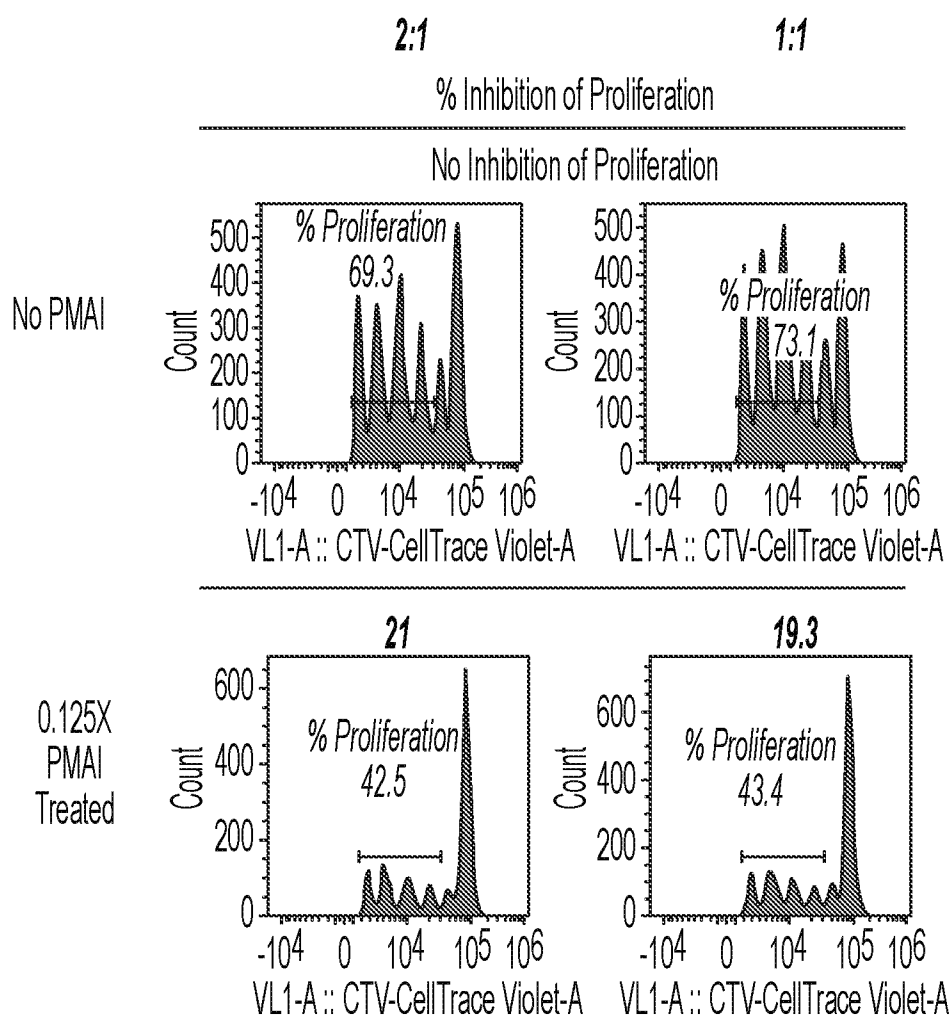


FIG. 8

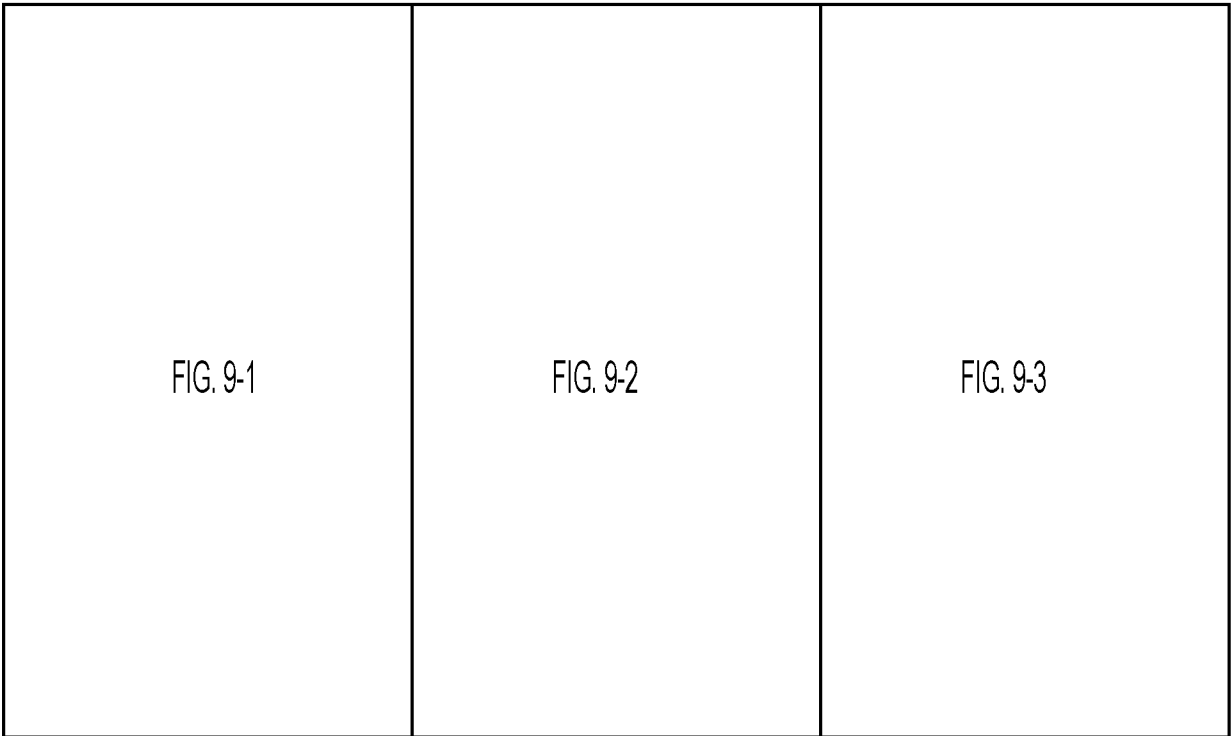


FIG. 9

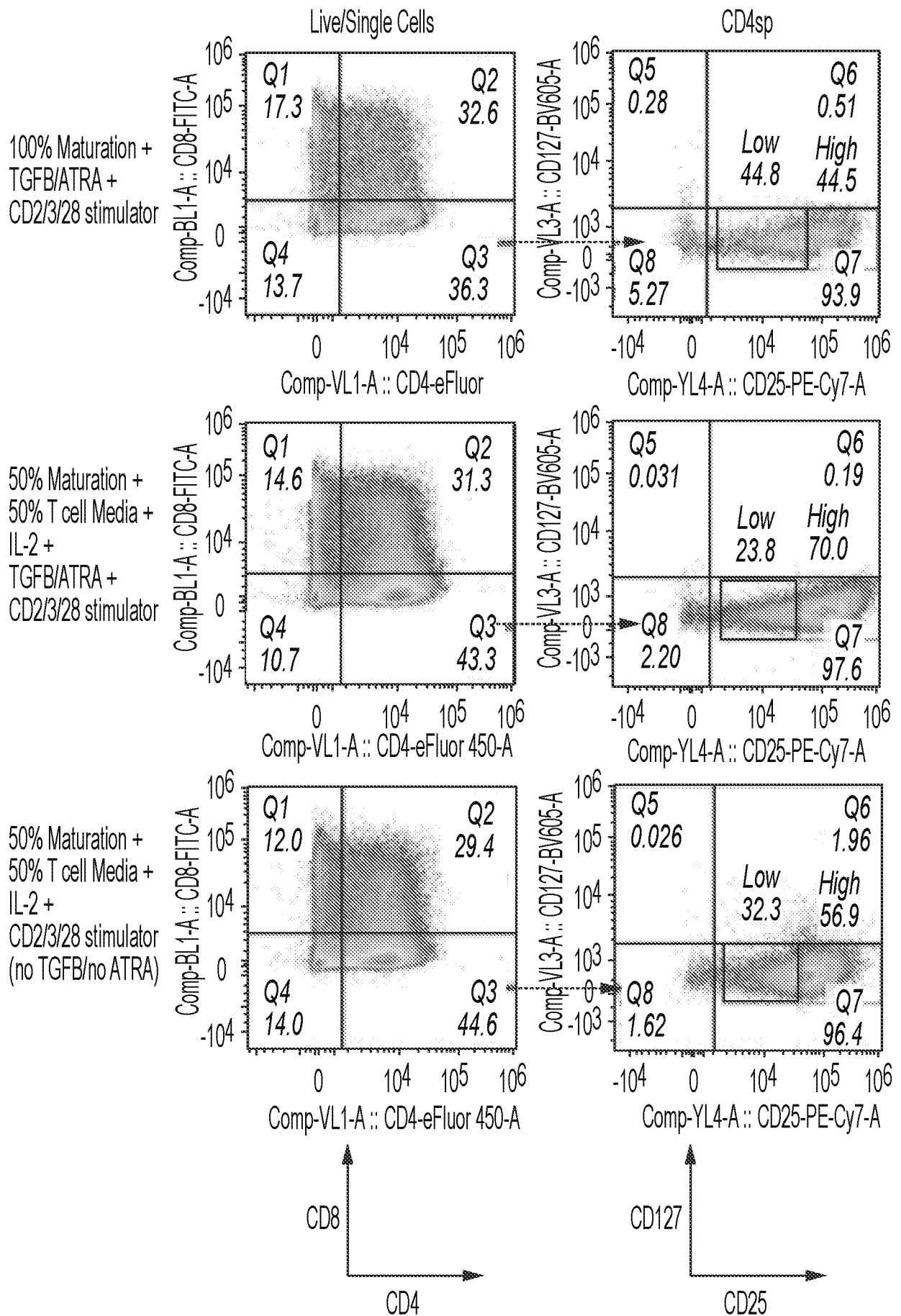


FIG. 9

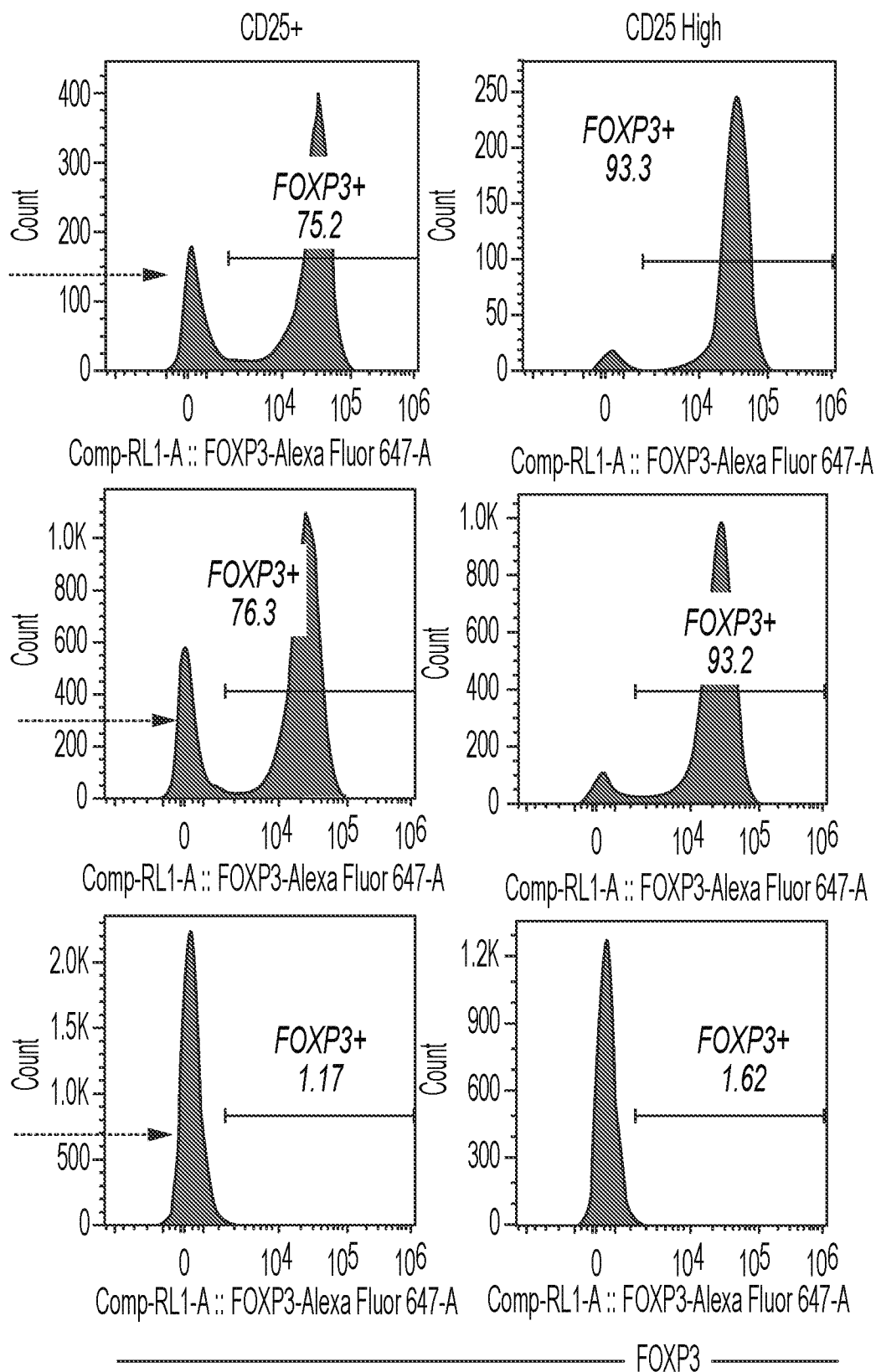


FIG.9

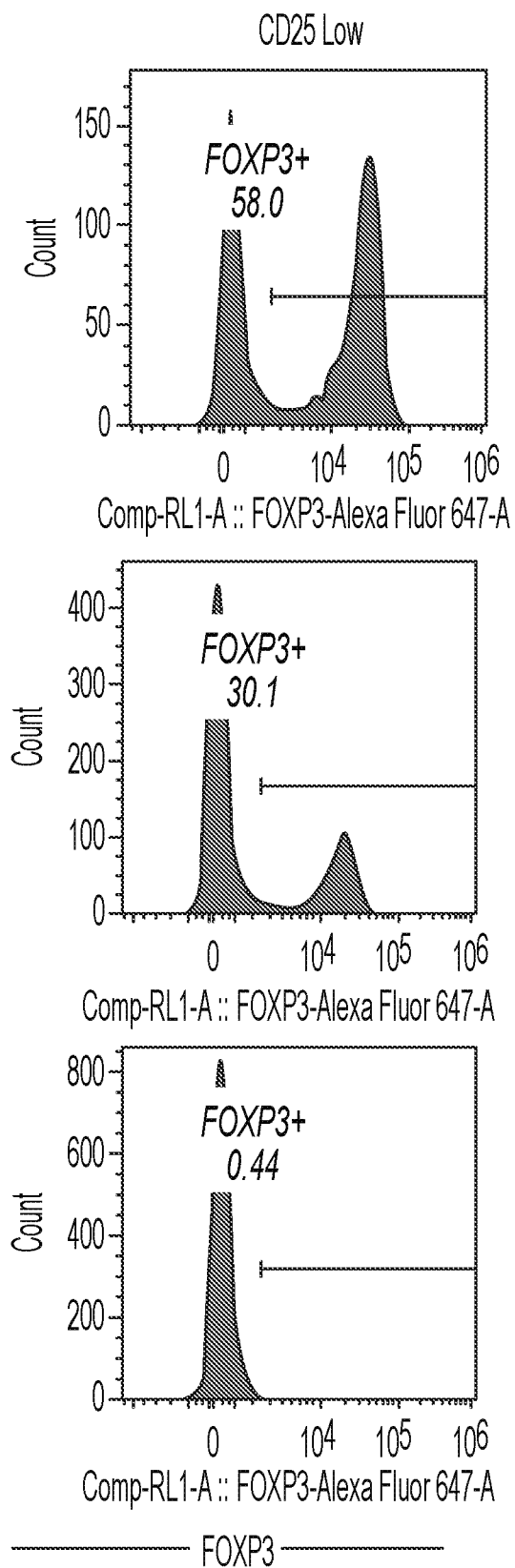


FIG.9

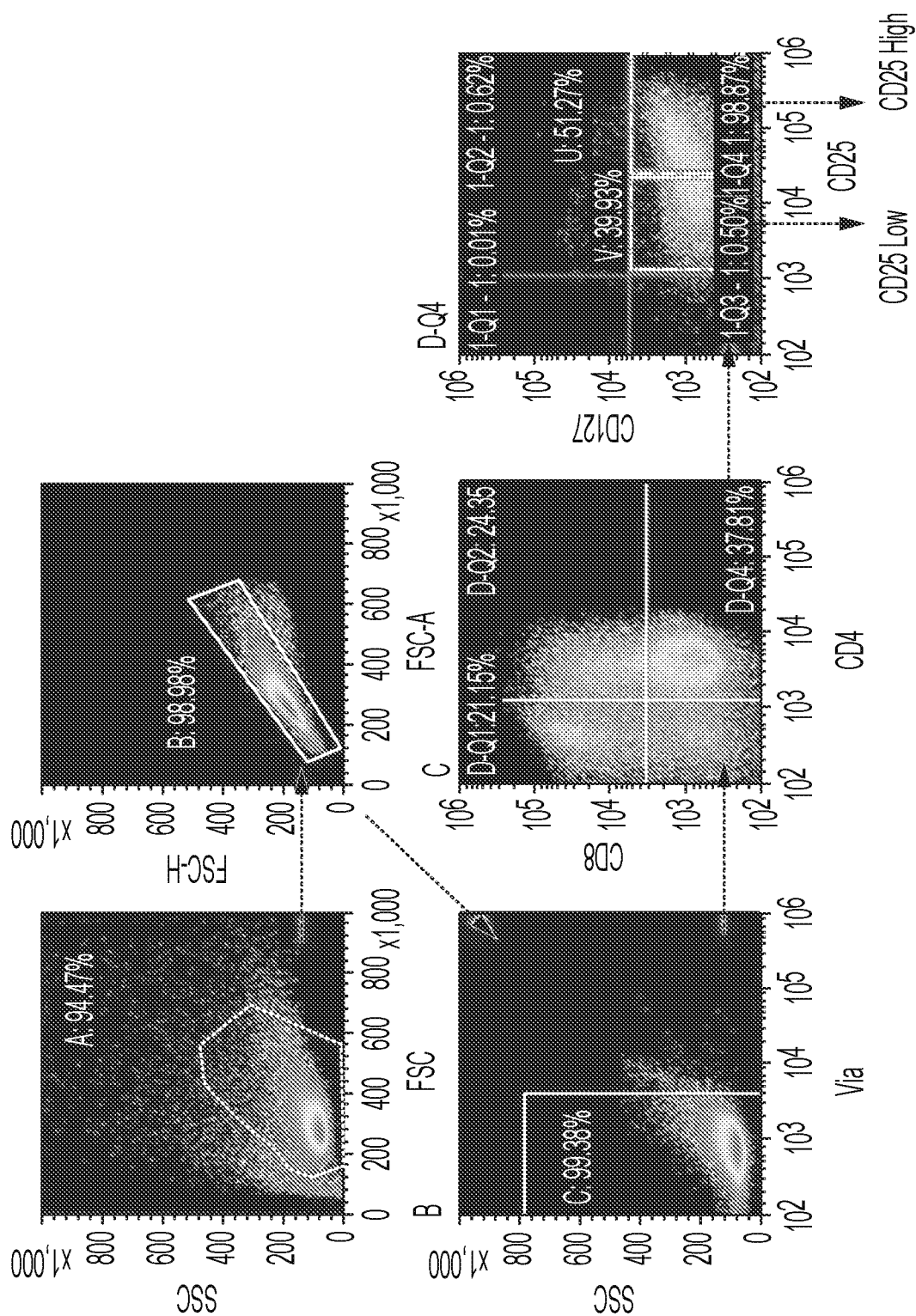


FIG. 10

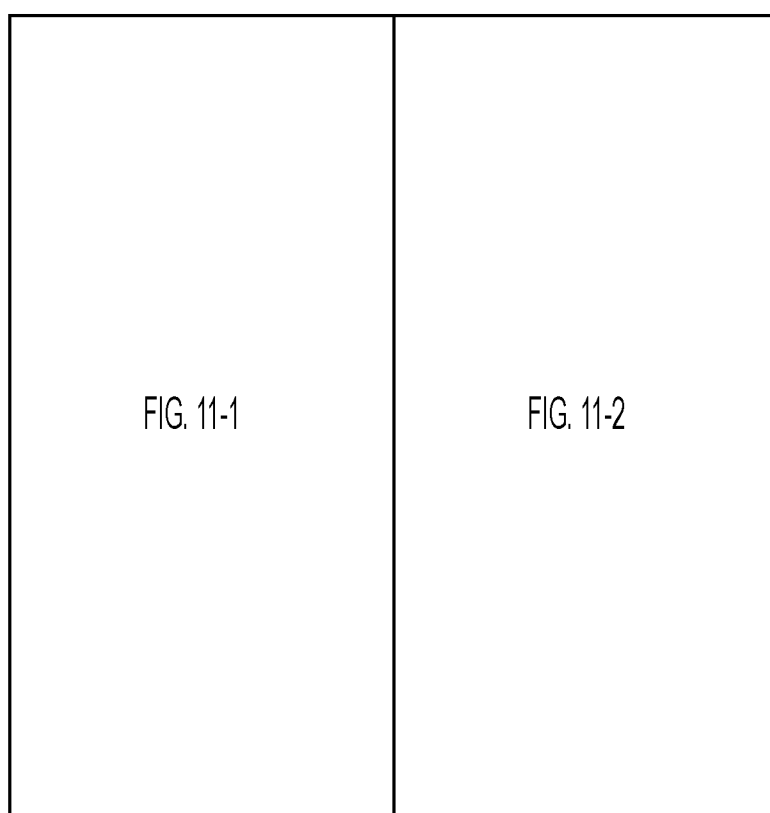


FIG. 11

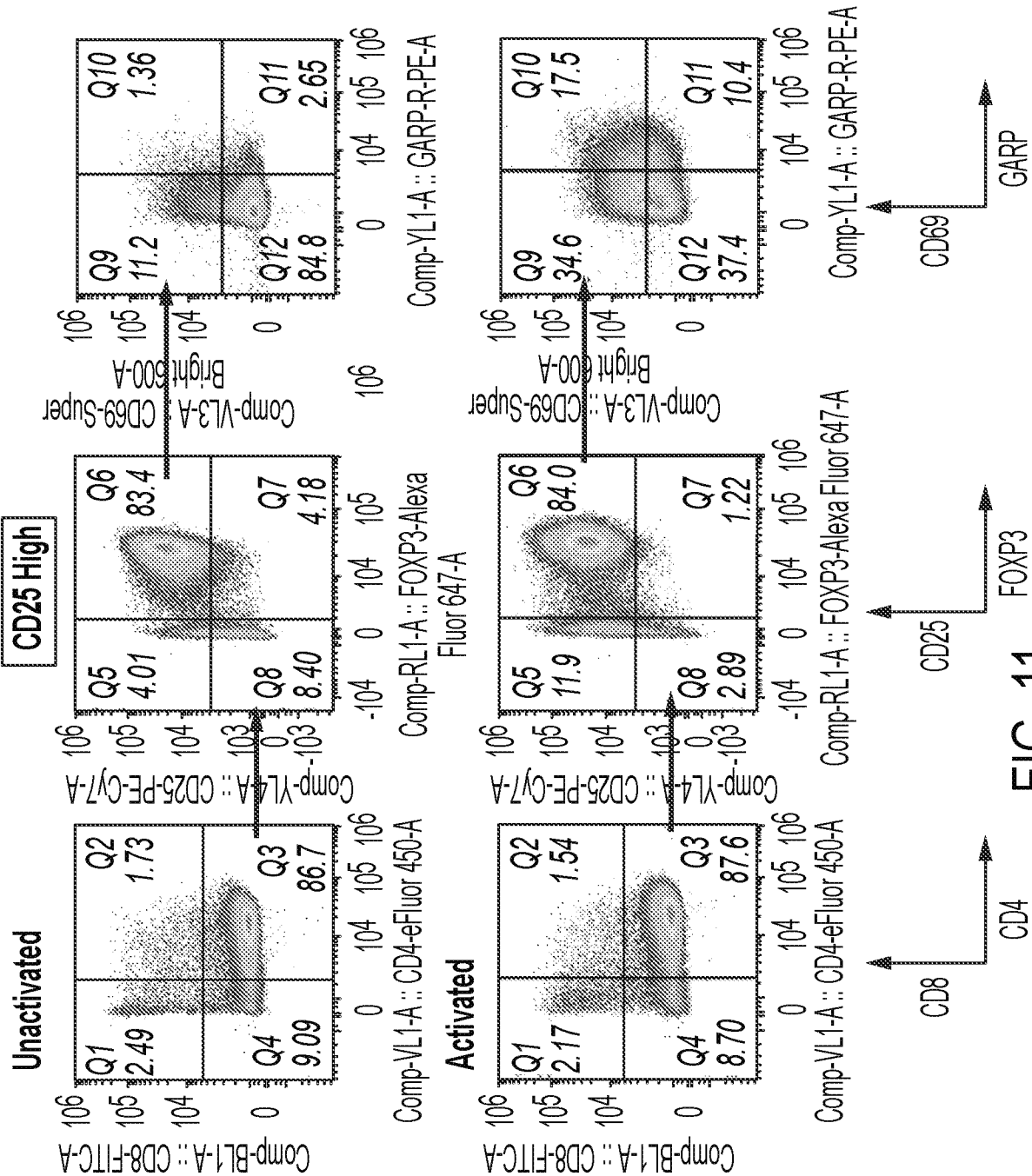


FIG. 11

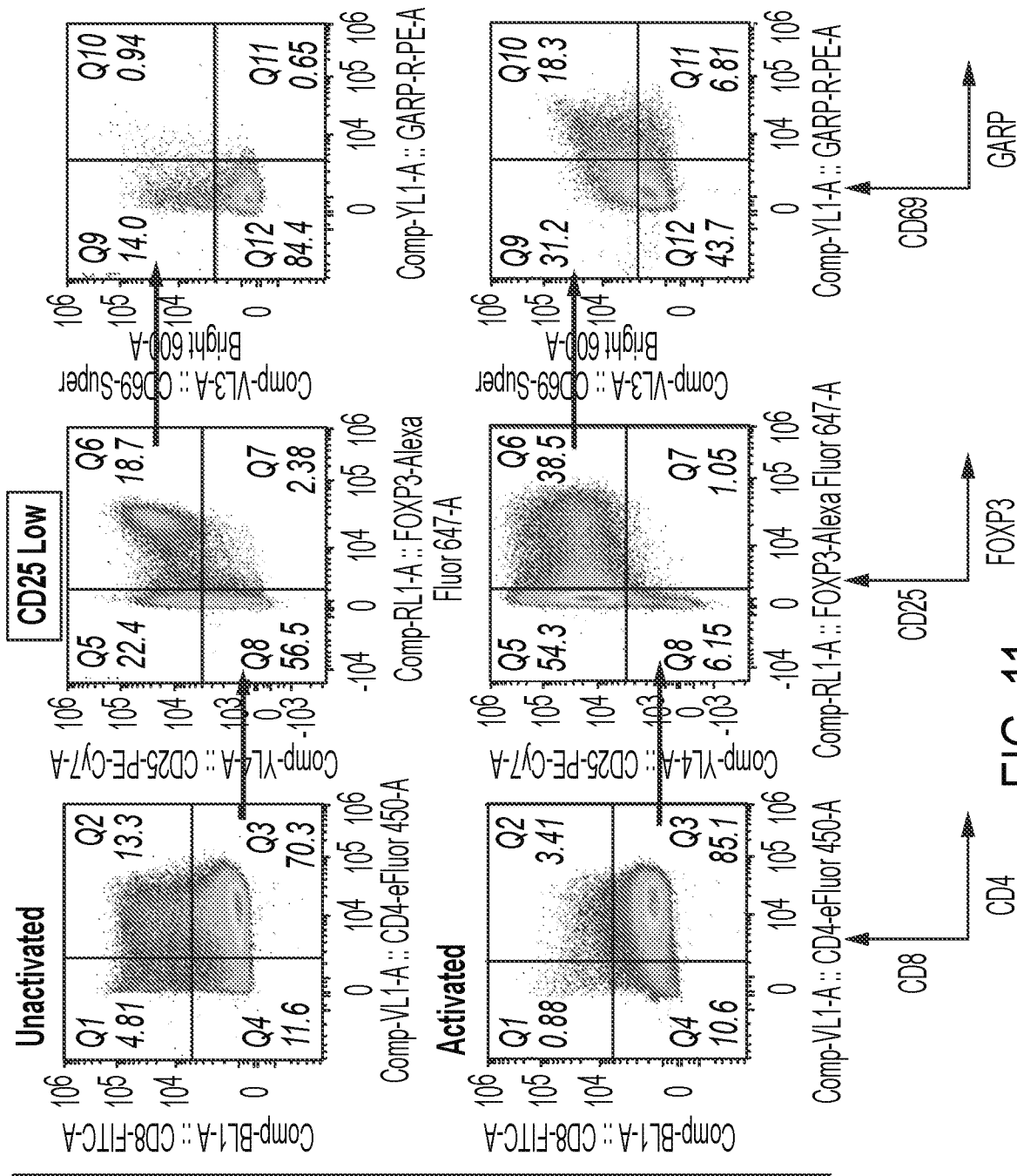


FIG. 11

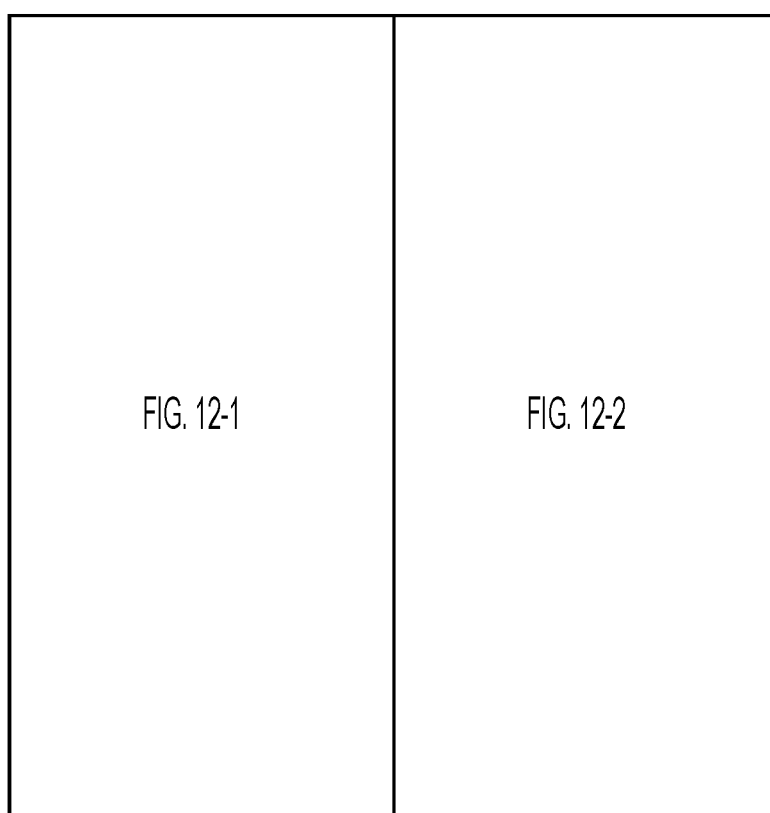


FIG. 12

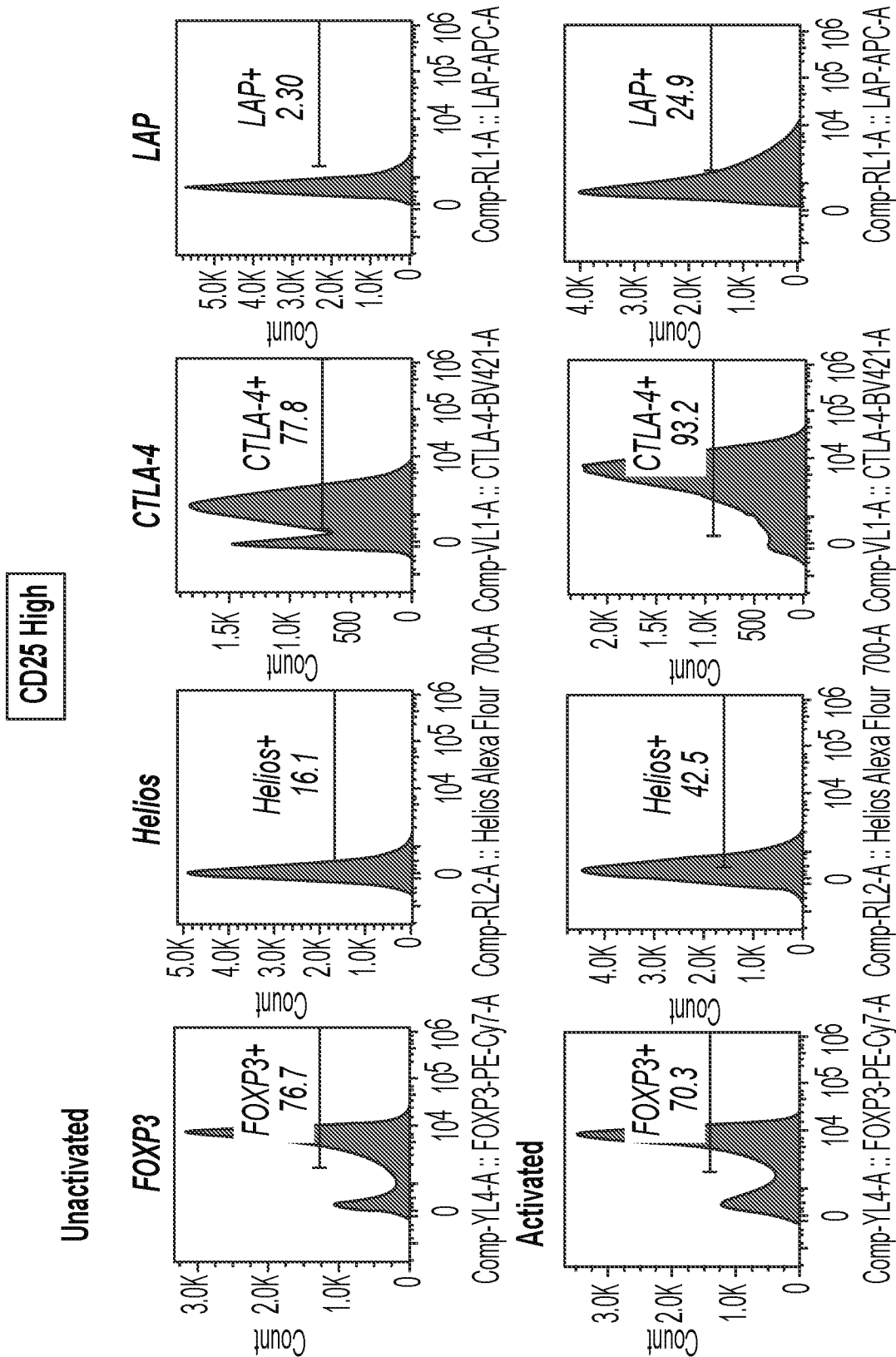
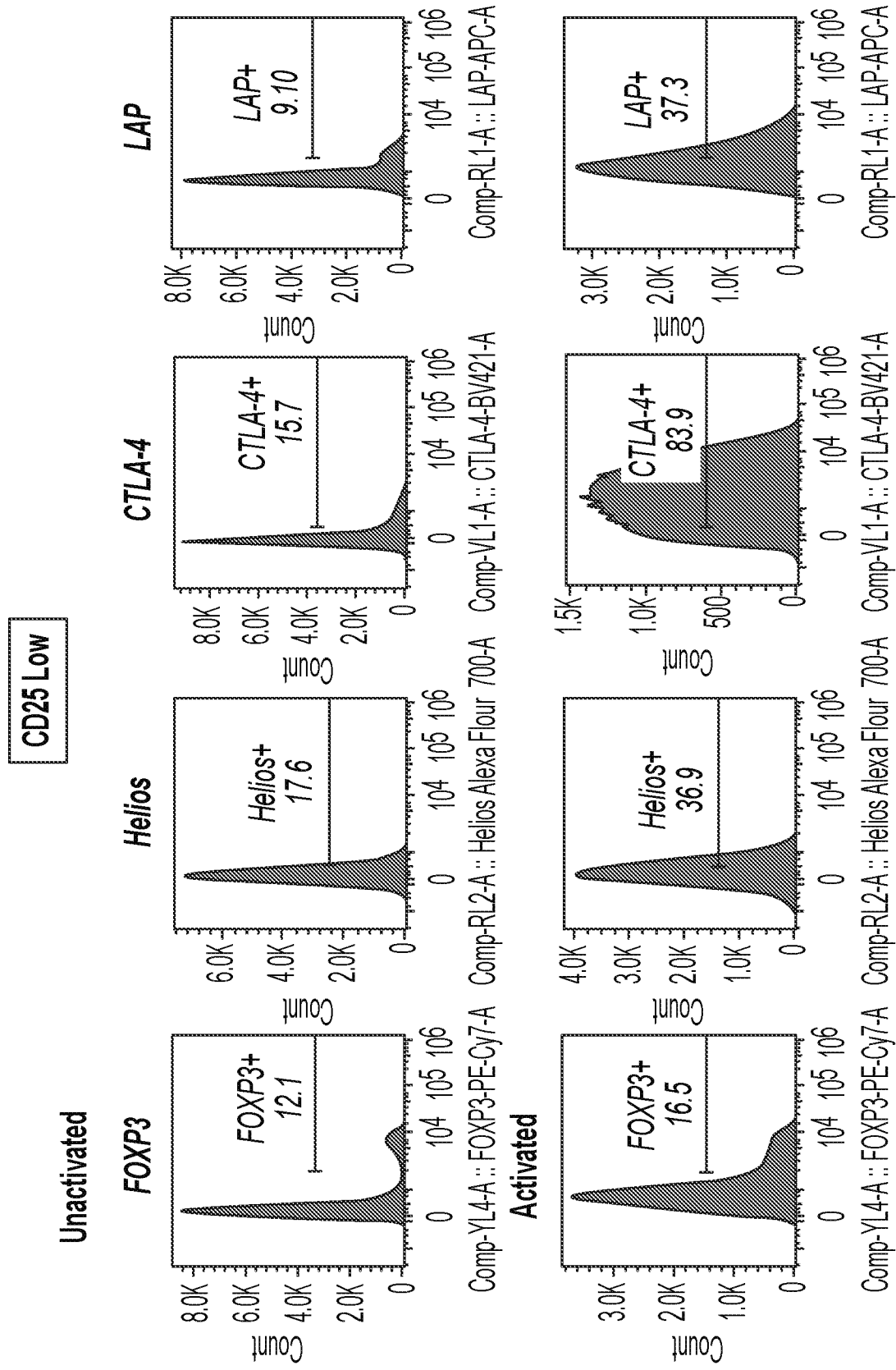


FIG. 12



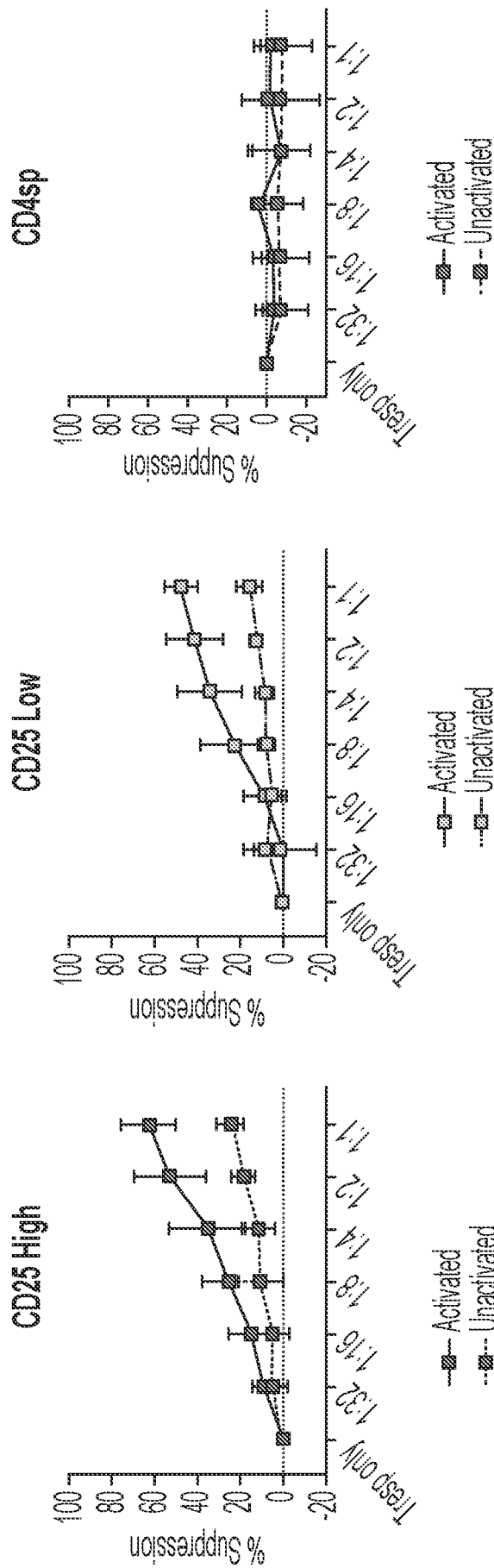


FIG. 13

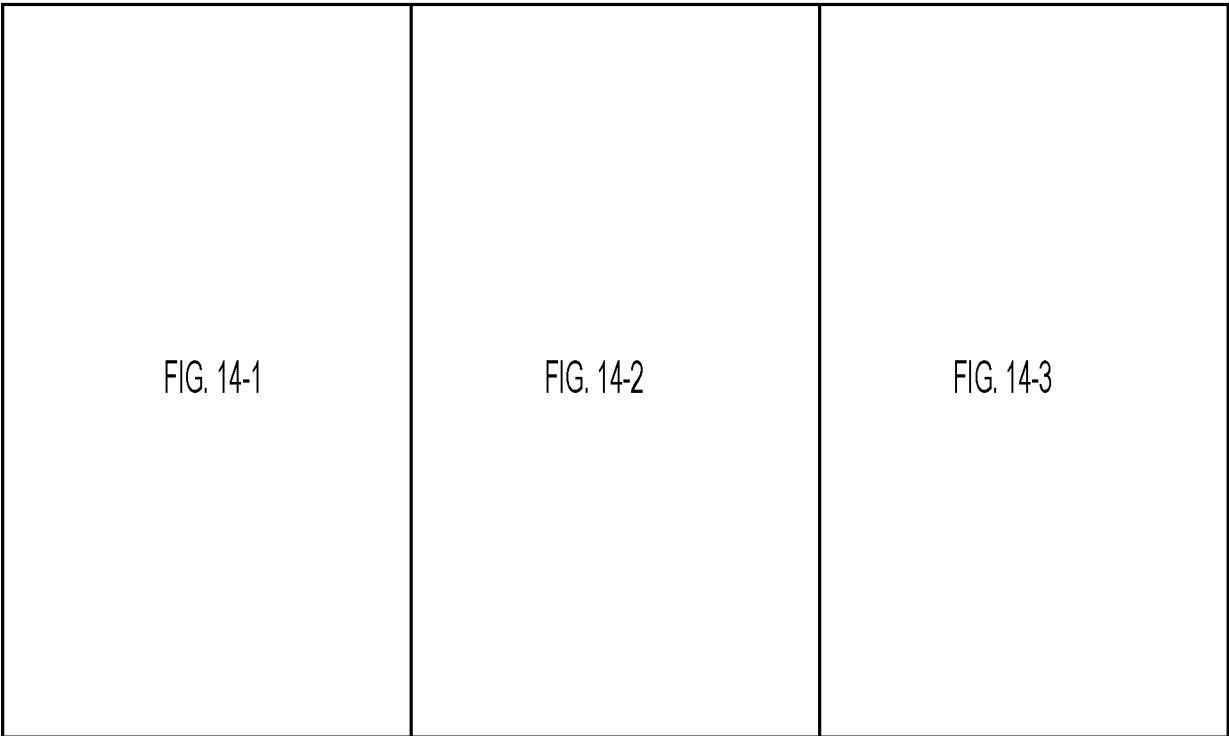


FIG. 14

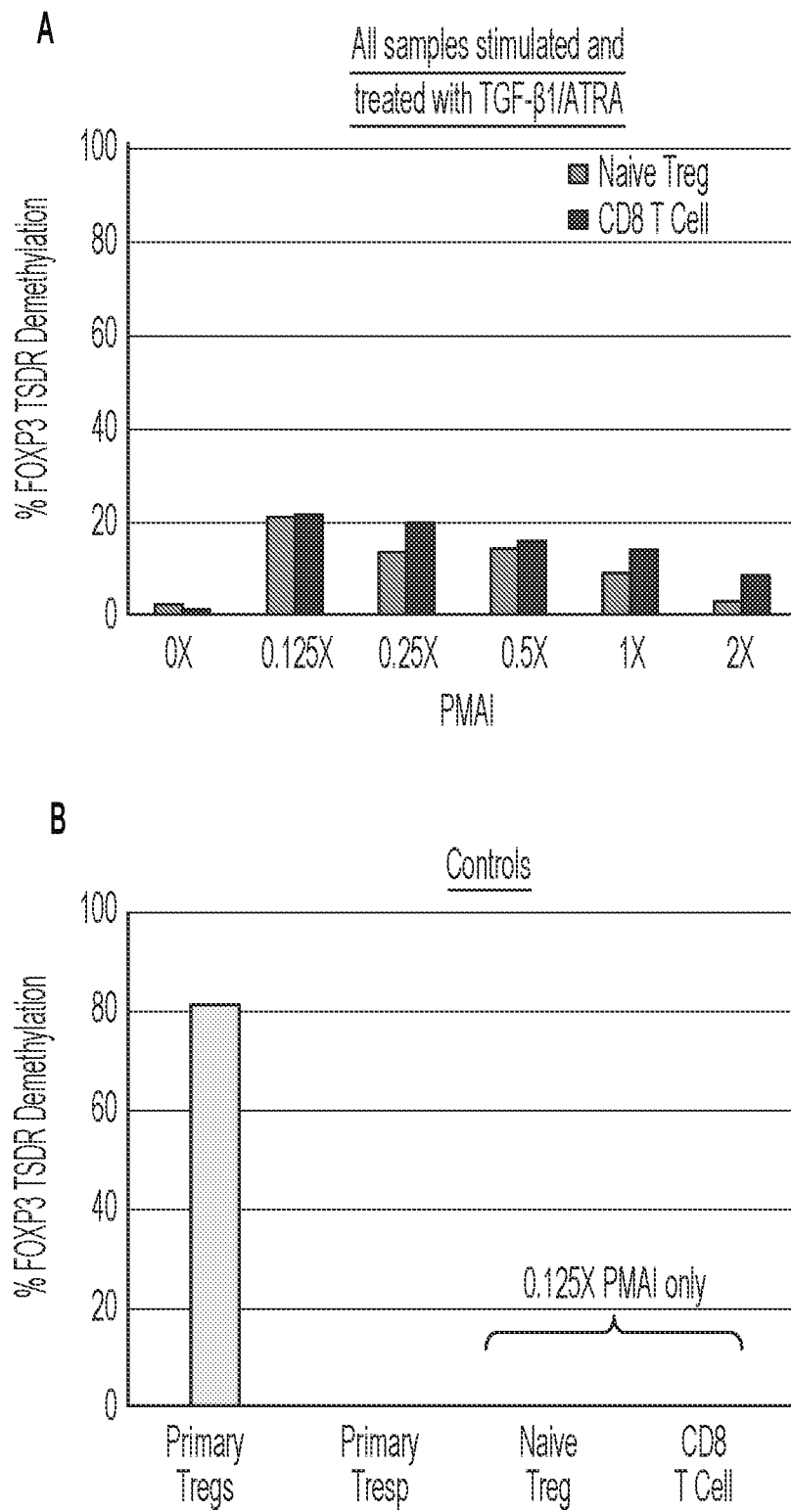


FIG.14

C

All samples stimulated and
treated with TGF- β 1/ATRA

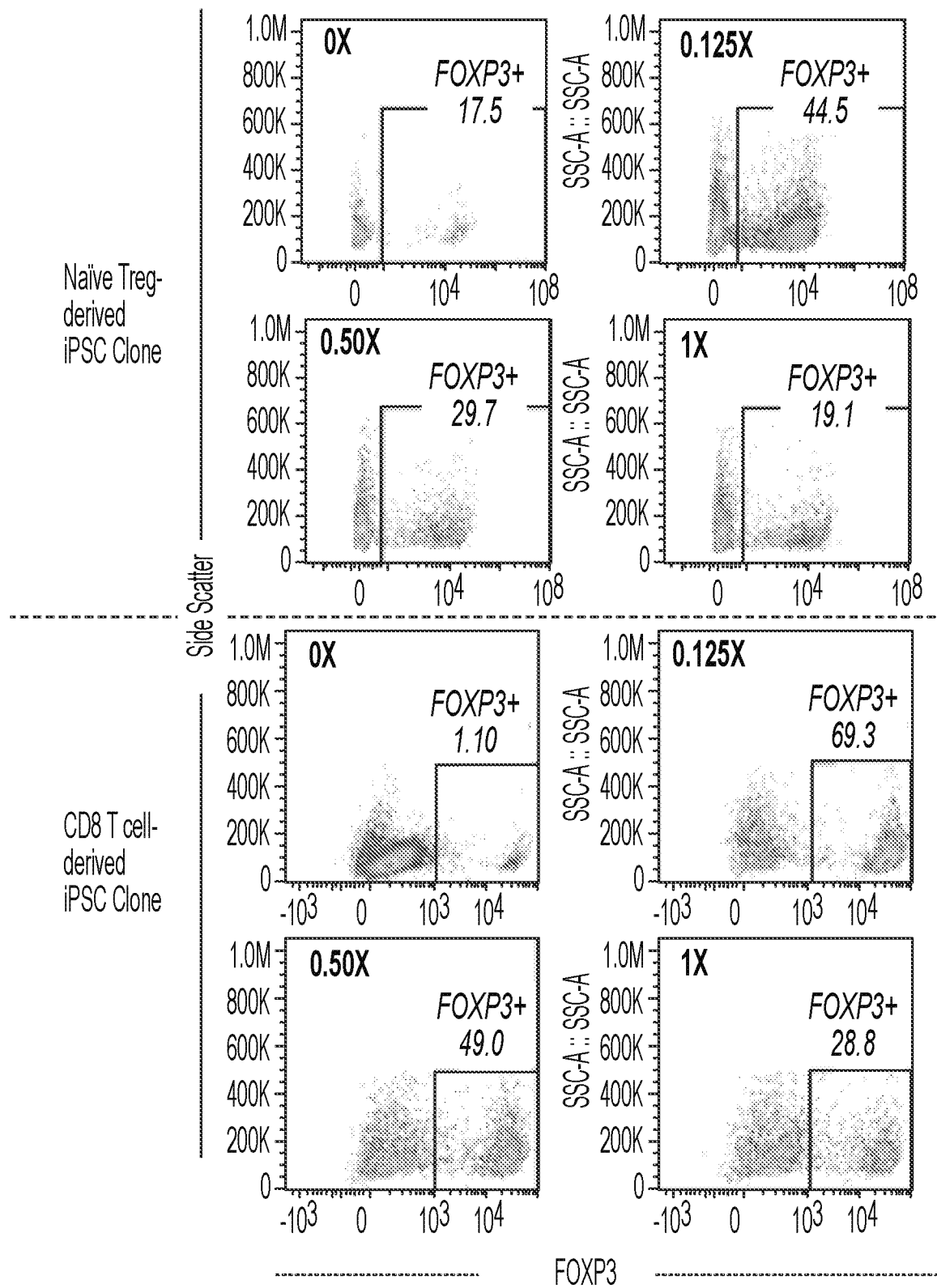


FIG.14

C

All samples stimulated and
treated with TGF- β 1/ATRA

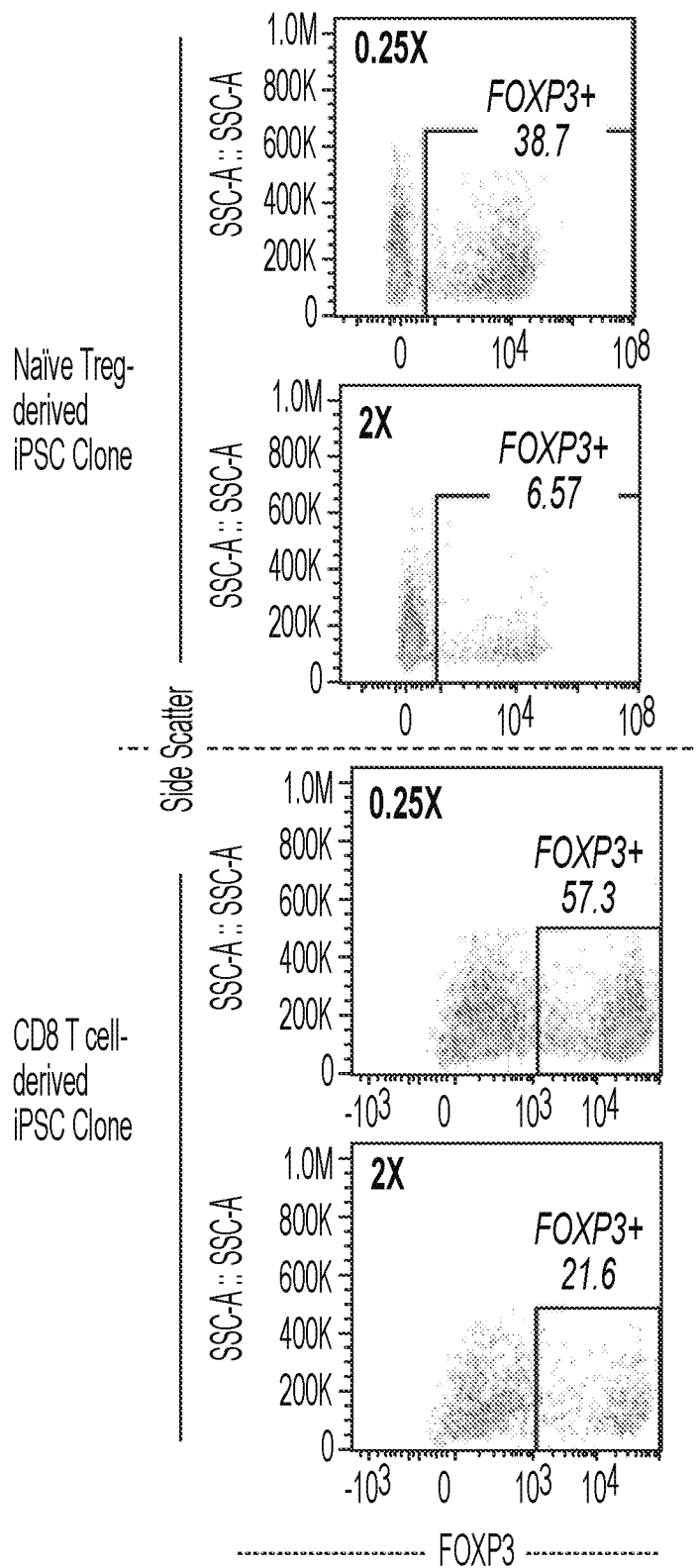


FIG.14

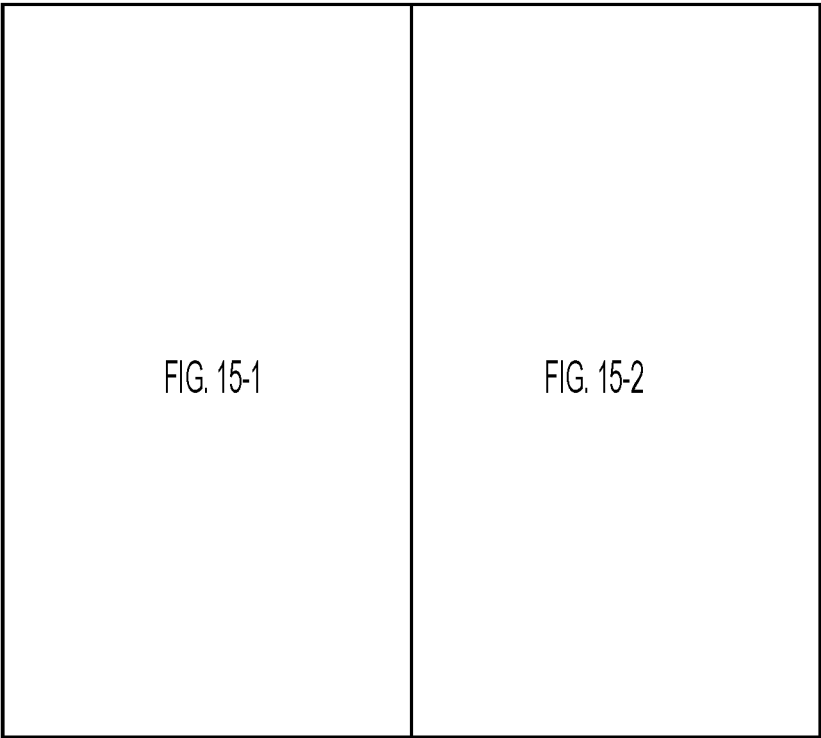
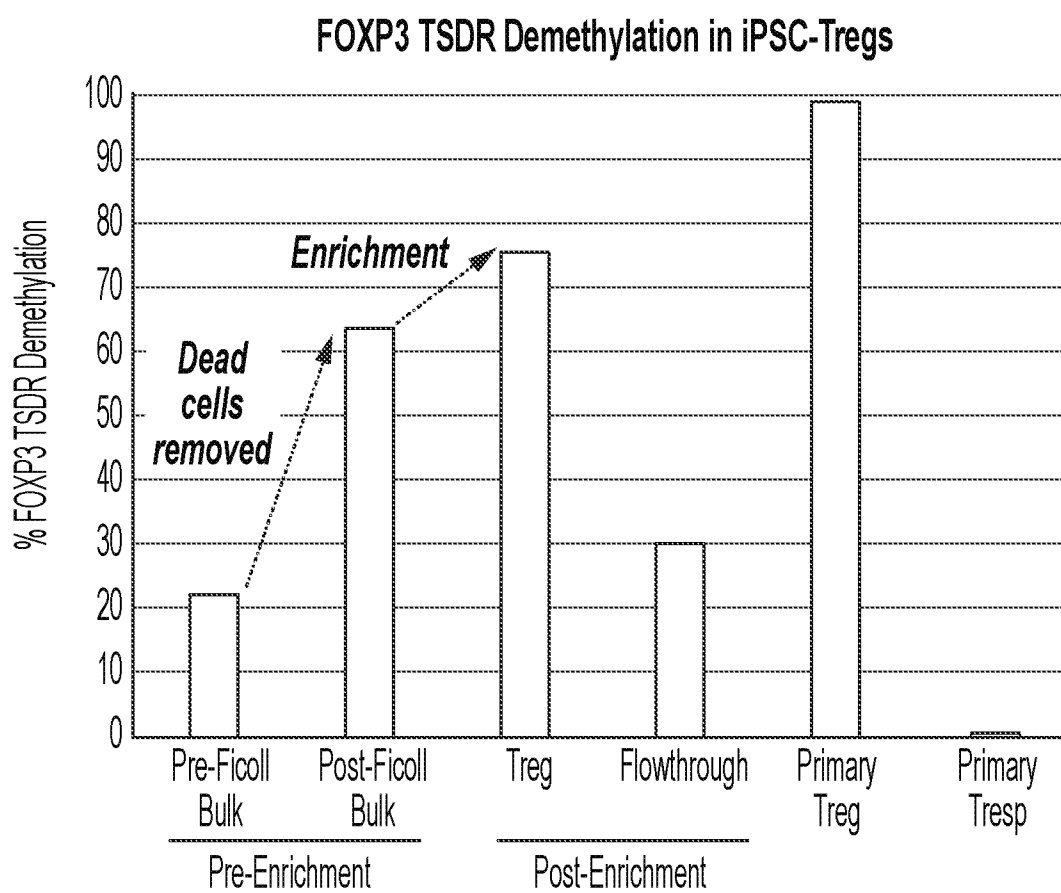
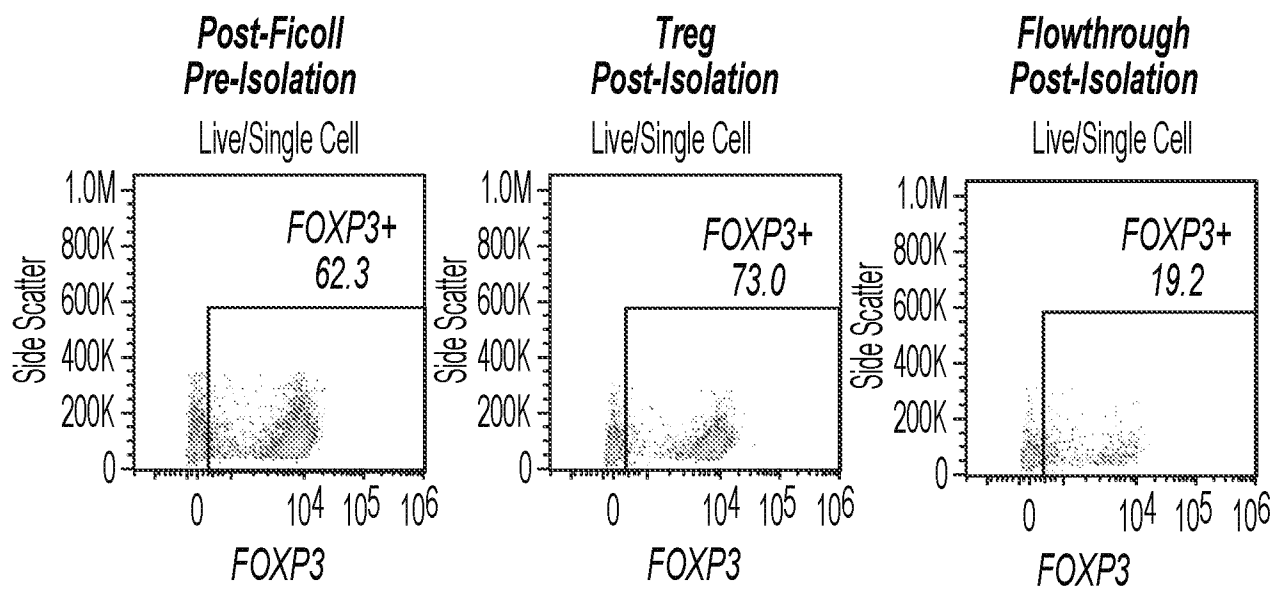


FIG. 15

A**FIG. 15**

B**FIG. 15**

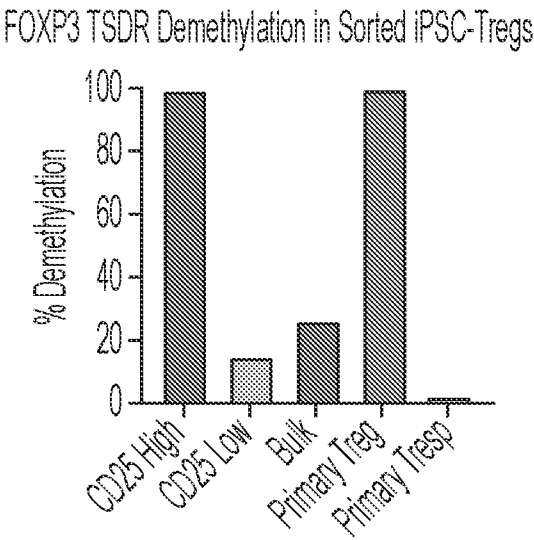


FIG. 16

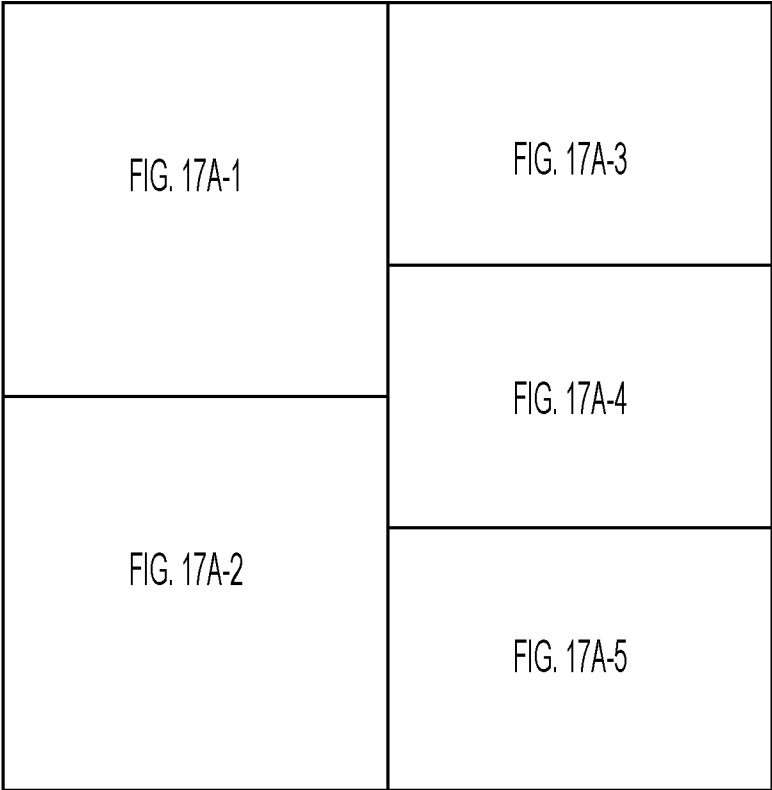


FIG. 17A

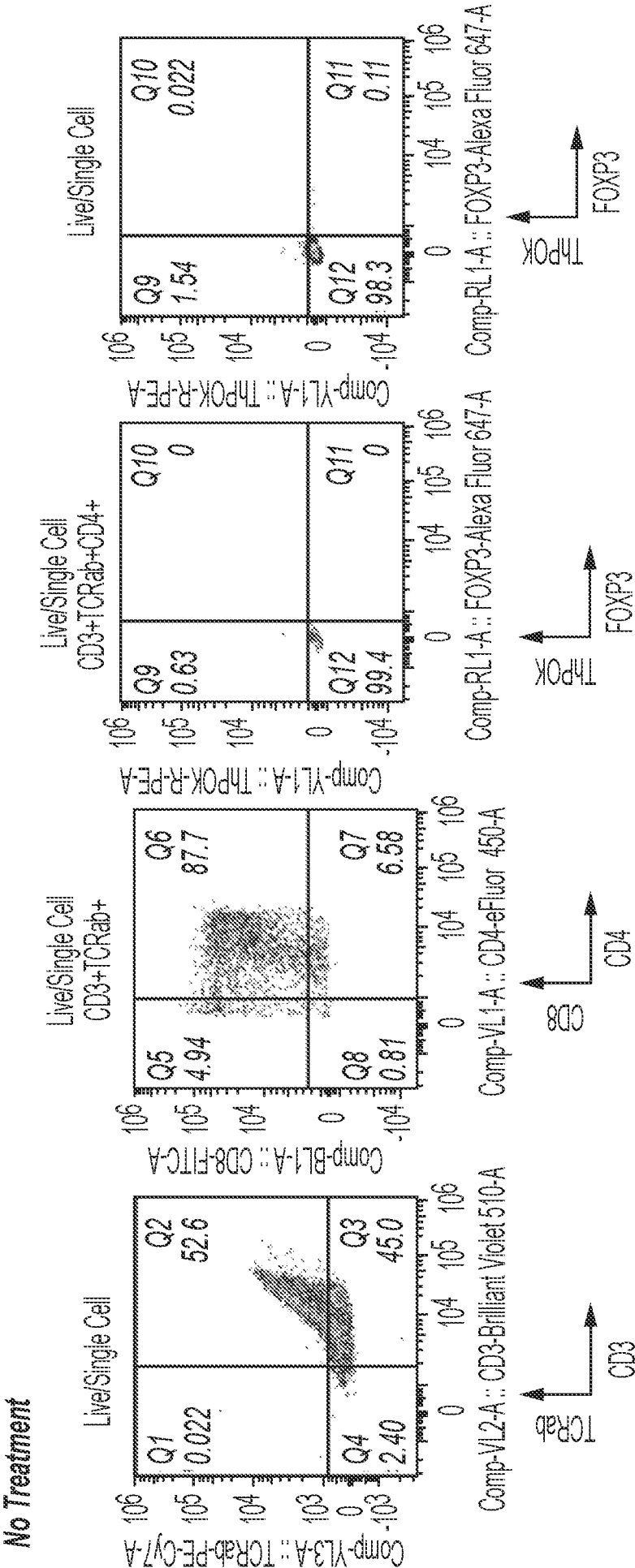


FIG. 17A

0.125X PMAI

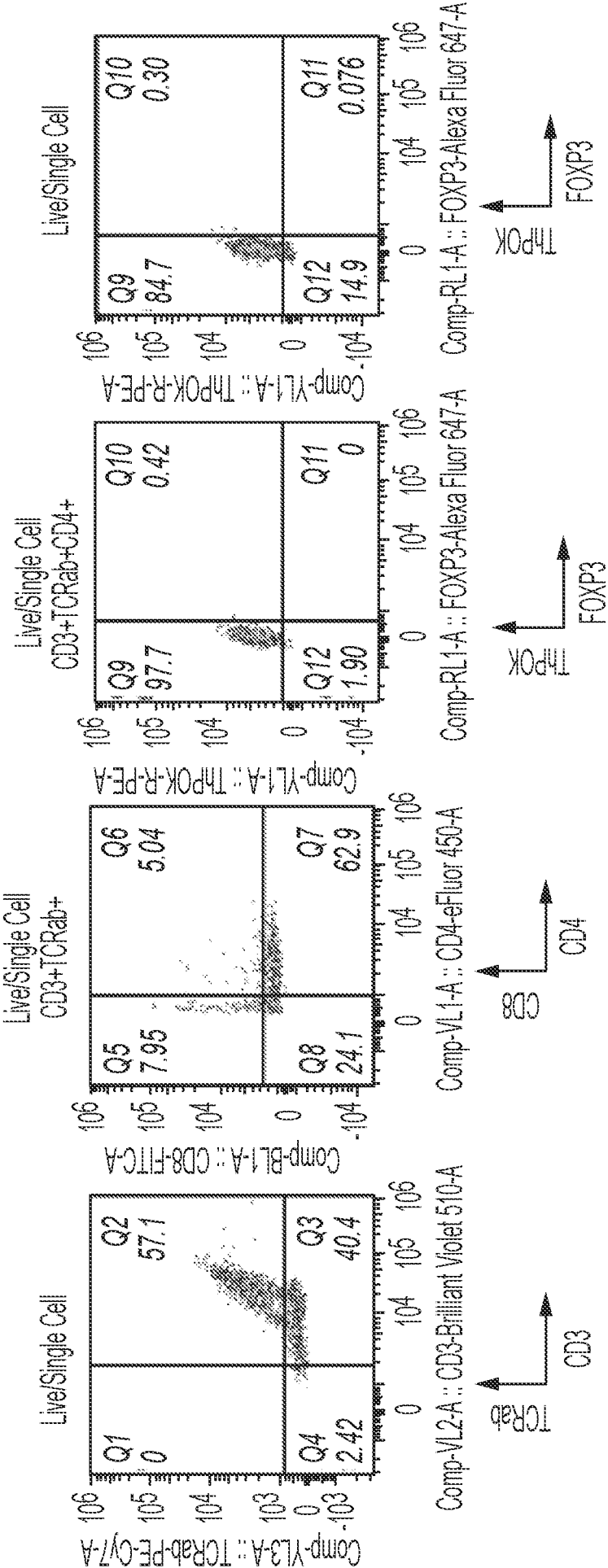


FIG. 17A

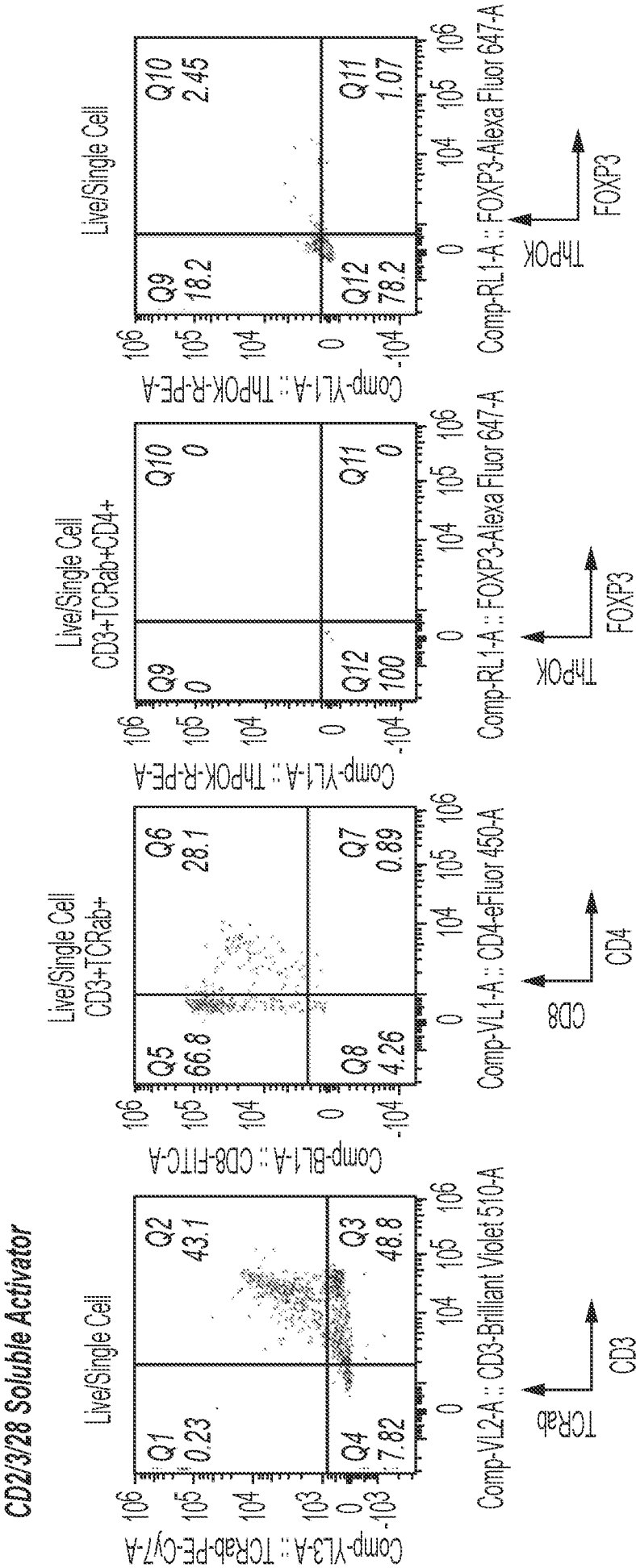


FIG. 17A

CD3/28 Soluble Activator

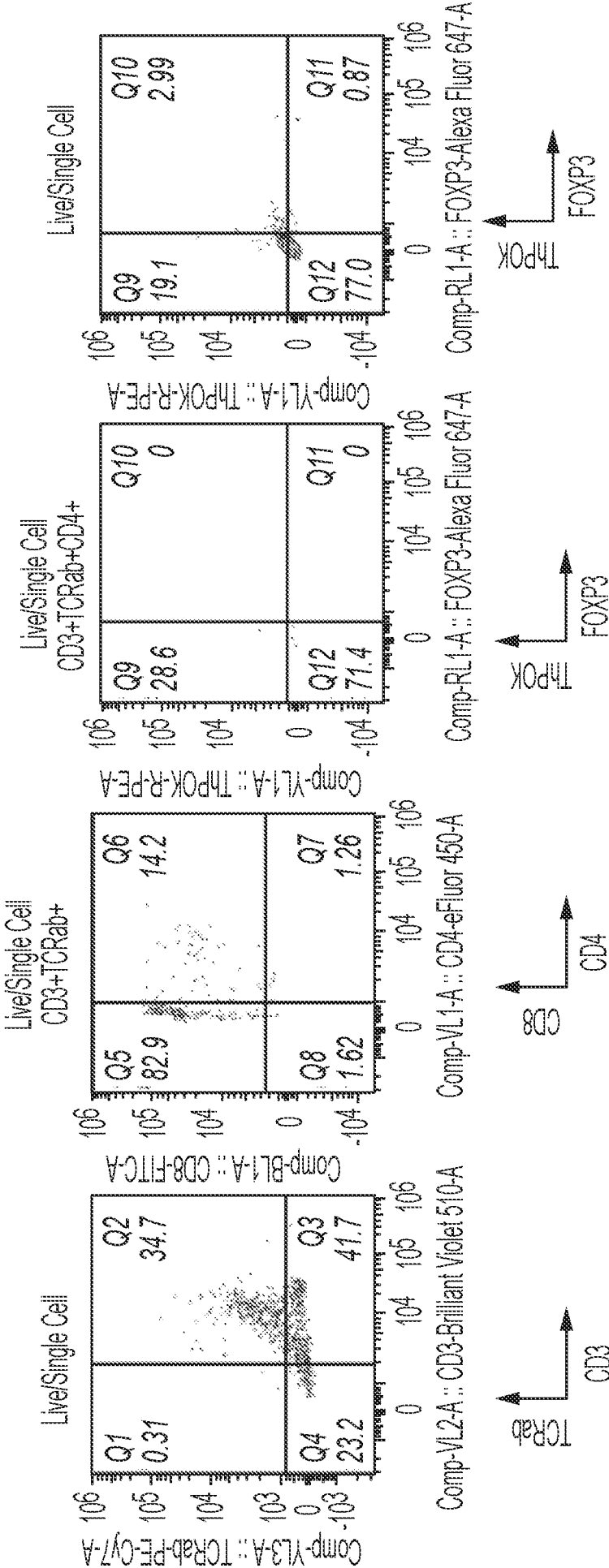


FIG. 17A

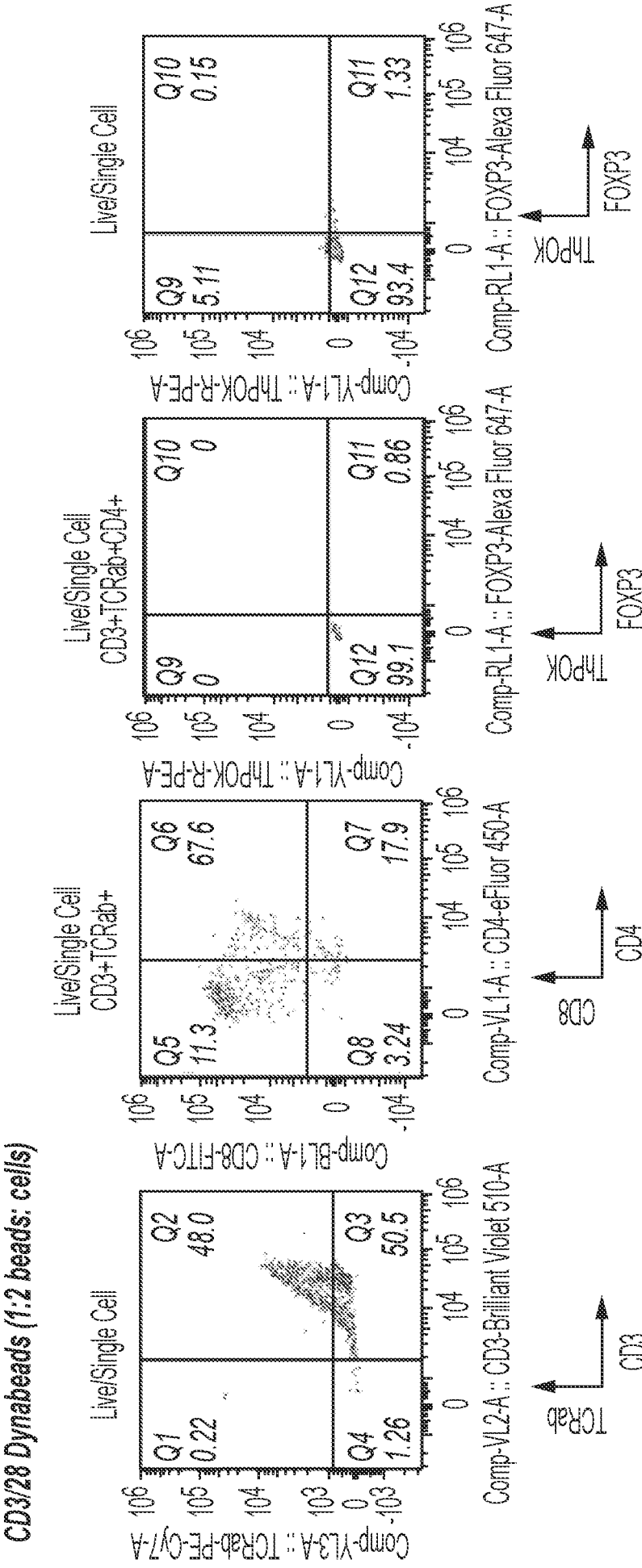


FIG. 17A

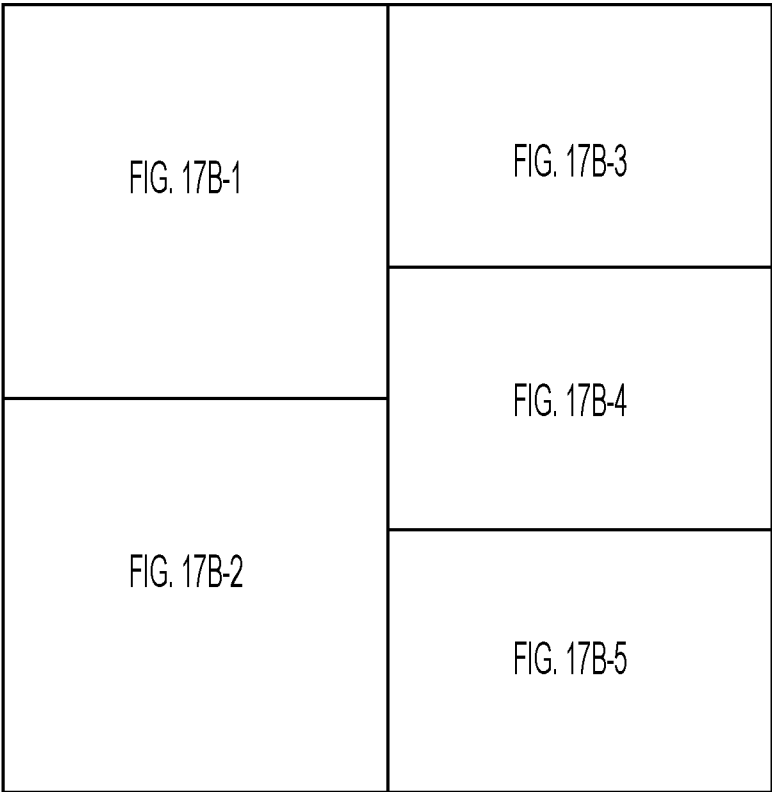


FIG. 17B

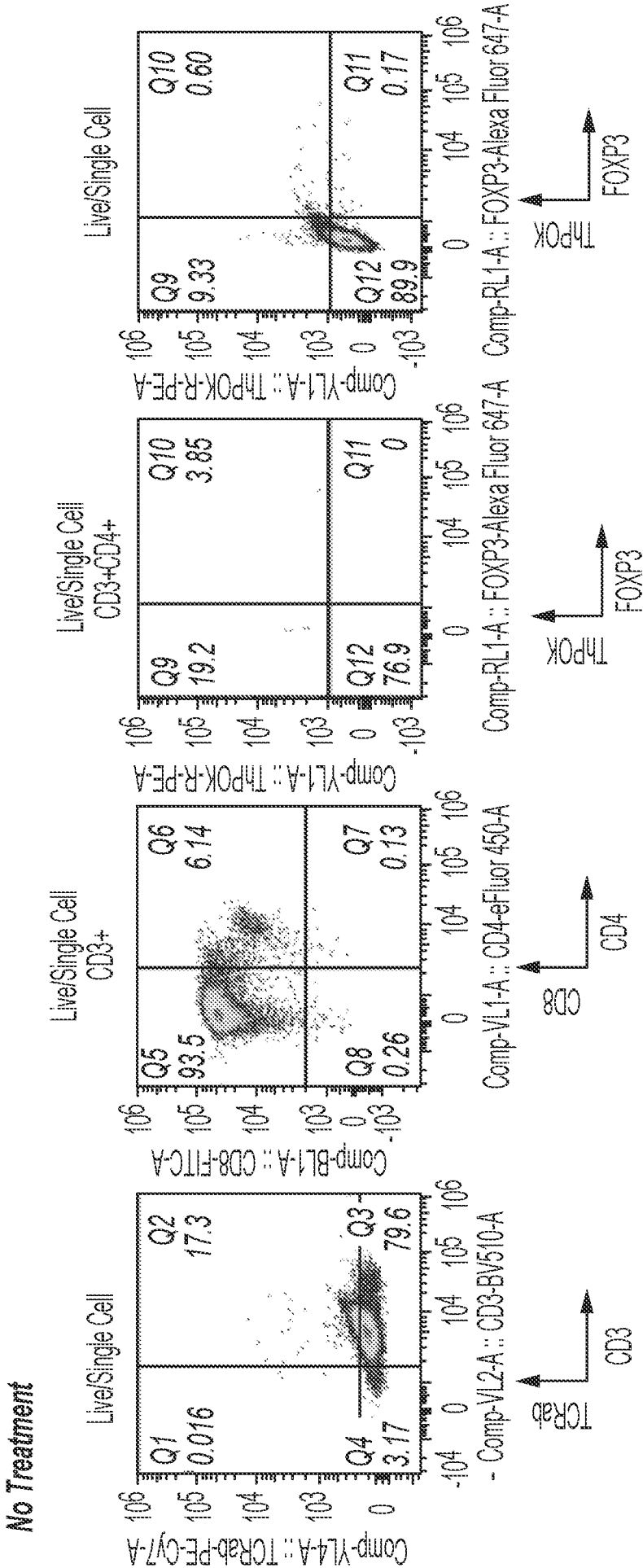


FIG. 17B

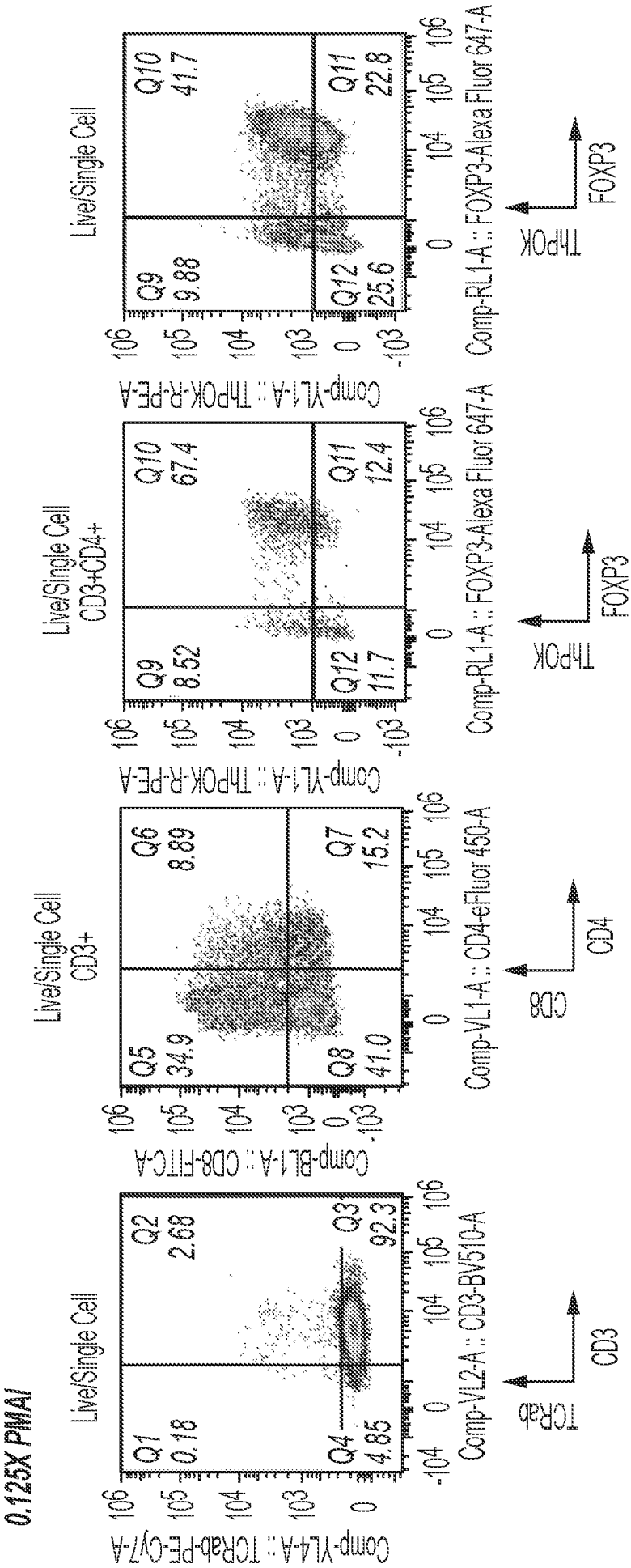


FIG. 17B

CD2/3/28 Soluble Activator

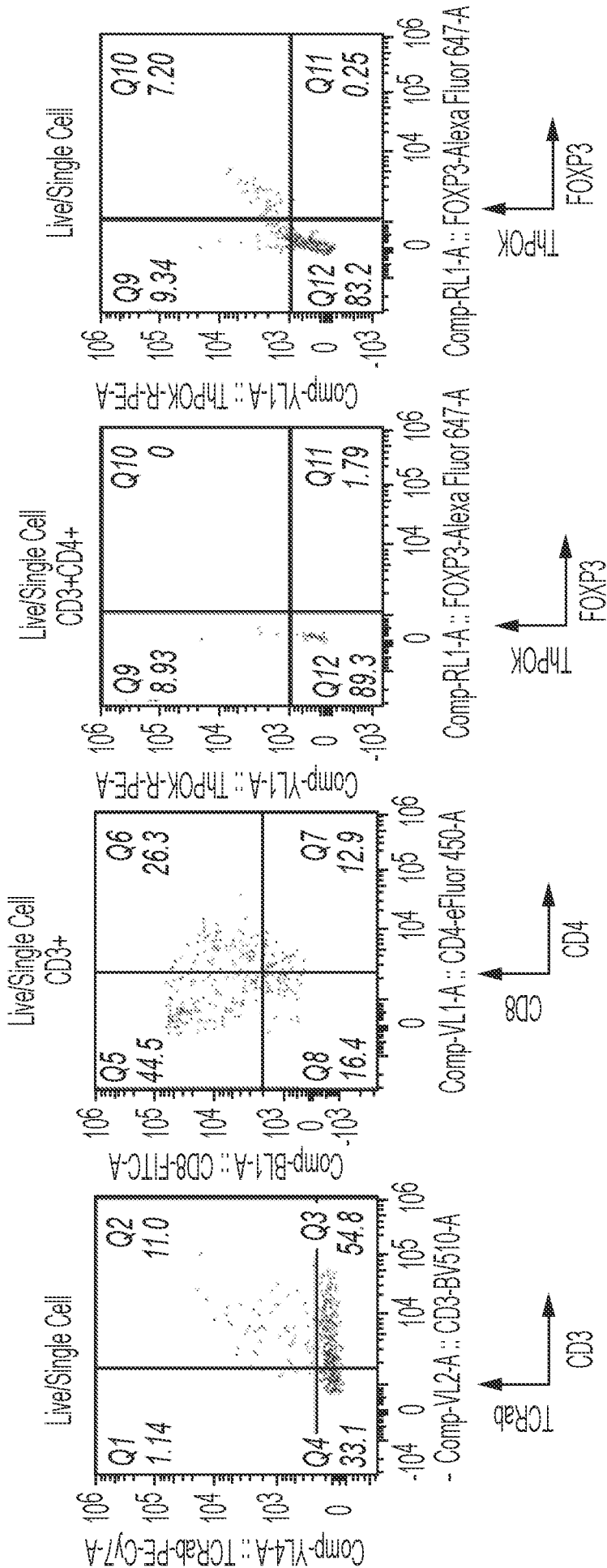


FIG. 17B

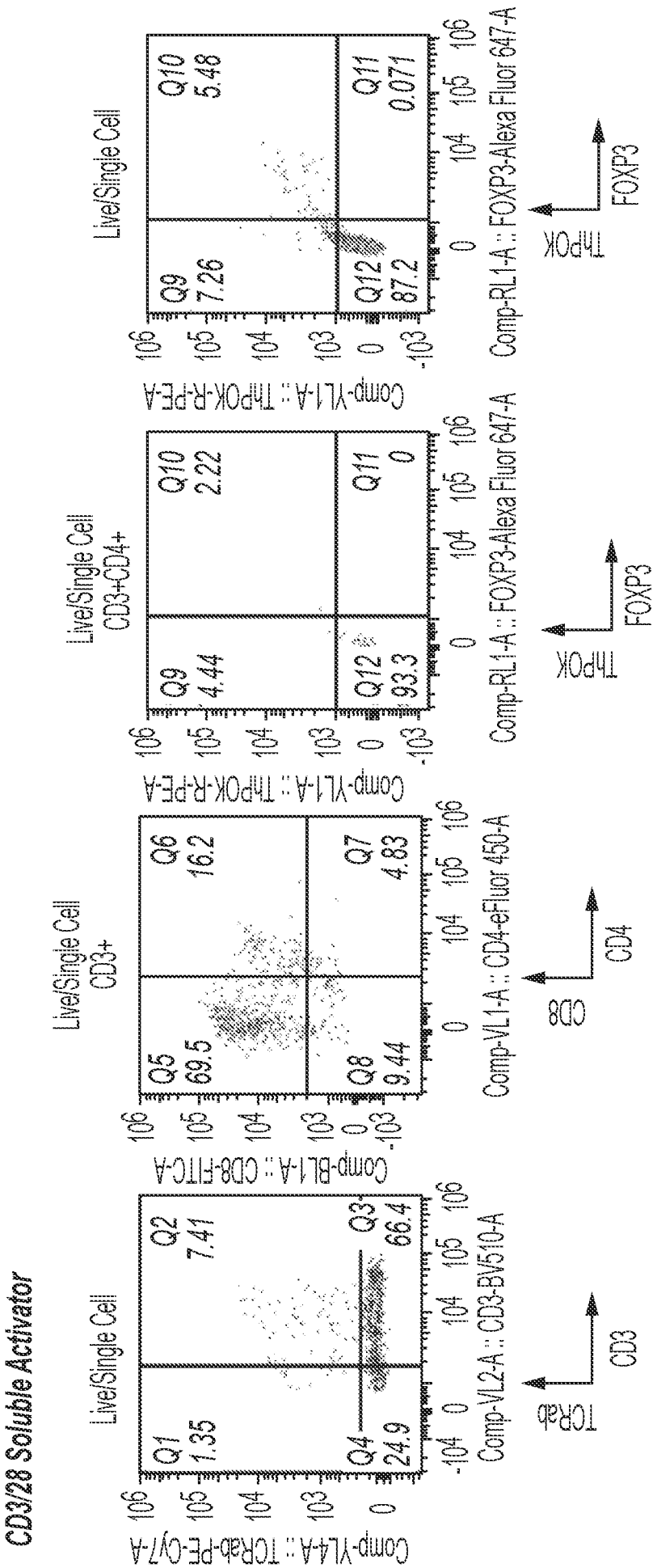


FIG. 17B

CD3/28 Dynabeads (1:2 beads: cells)

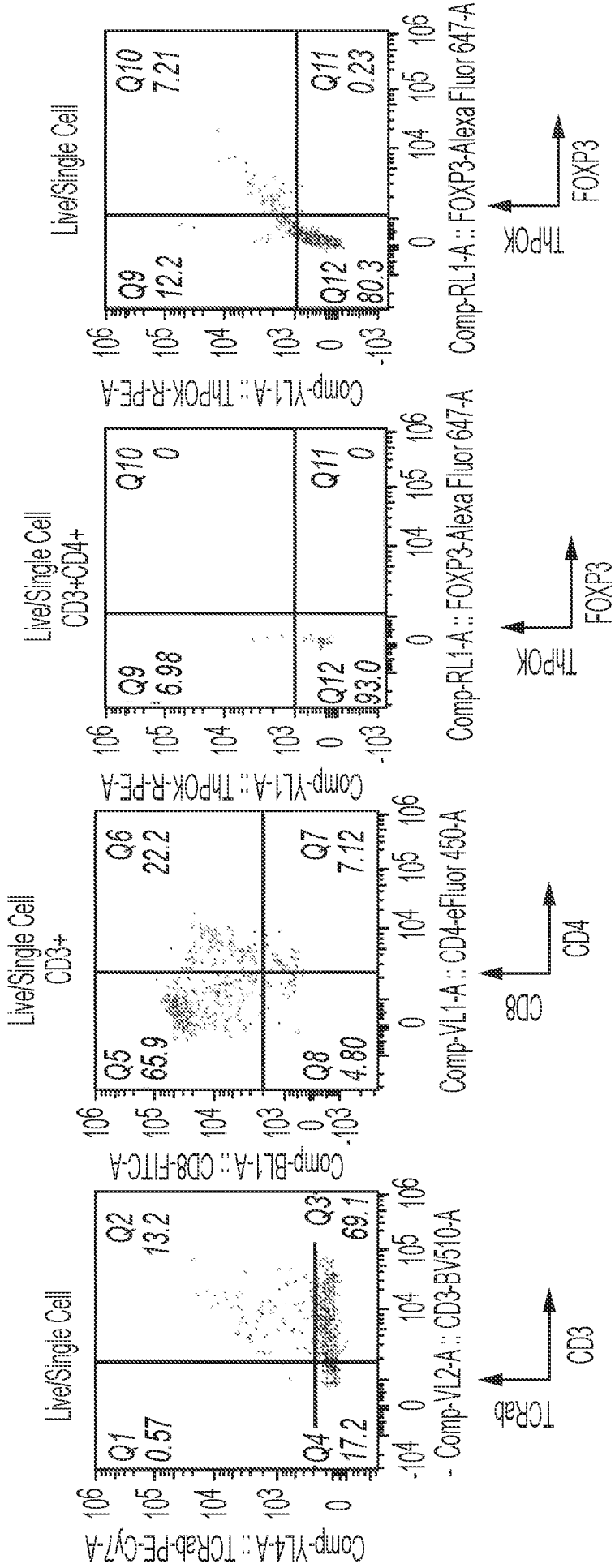


FIG. 17B

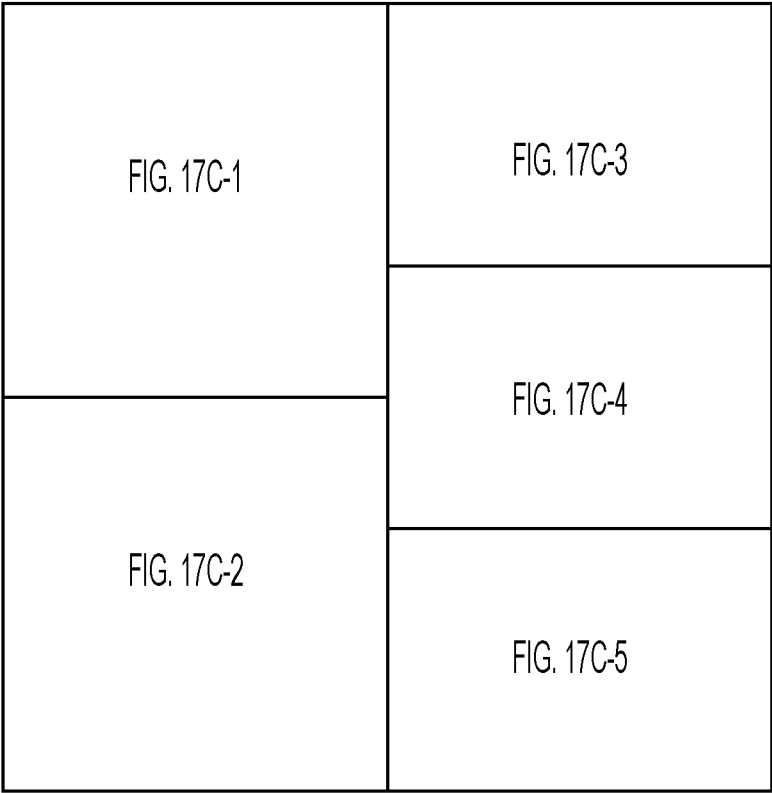


FIG. 17C

No Treatment

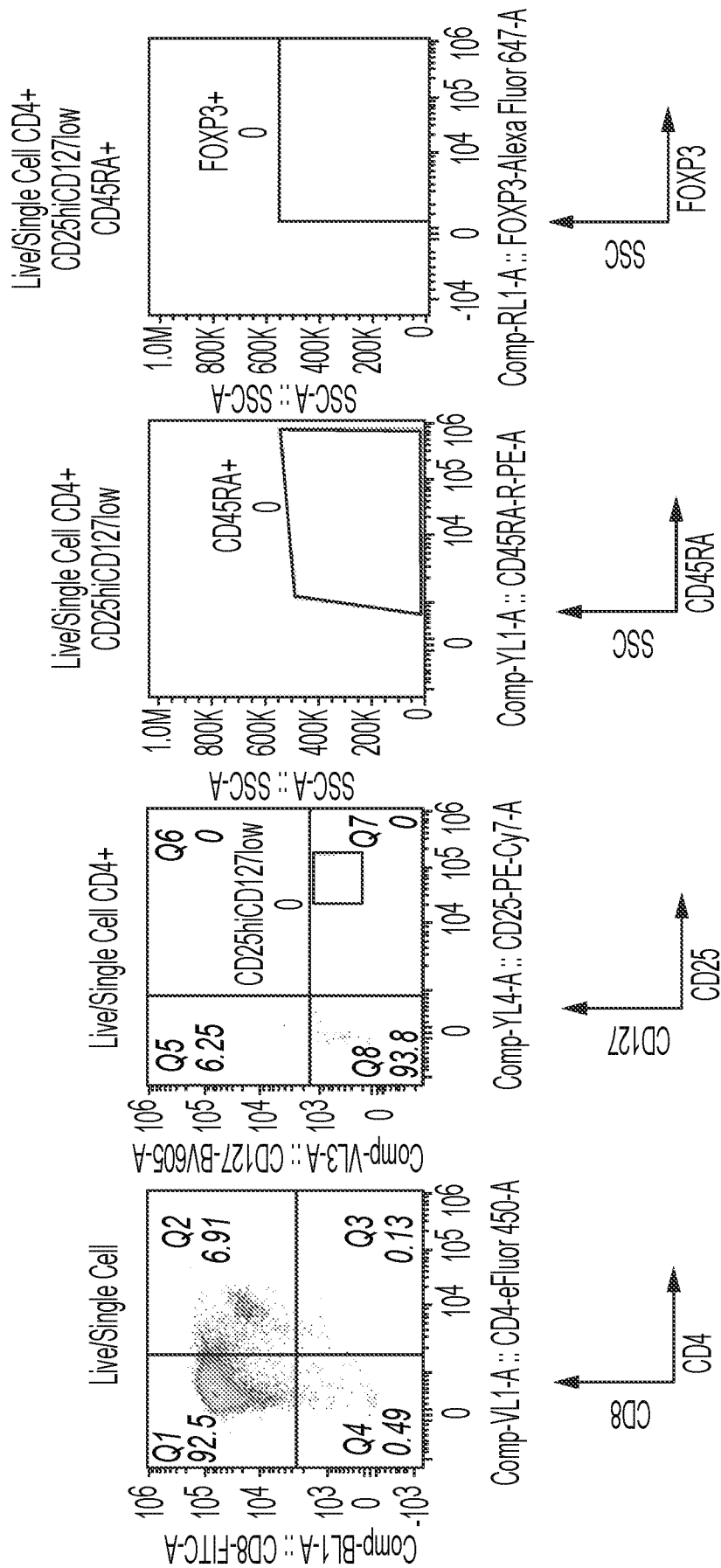


FIG. 17C

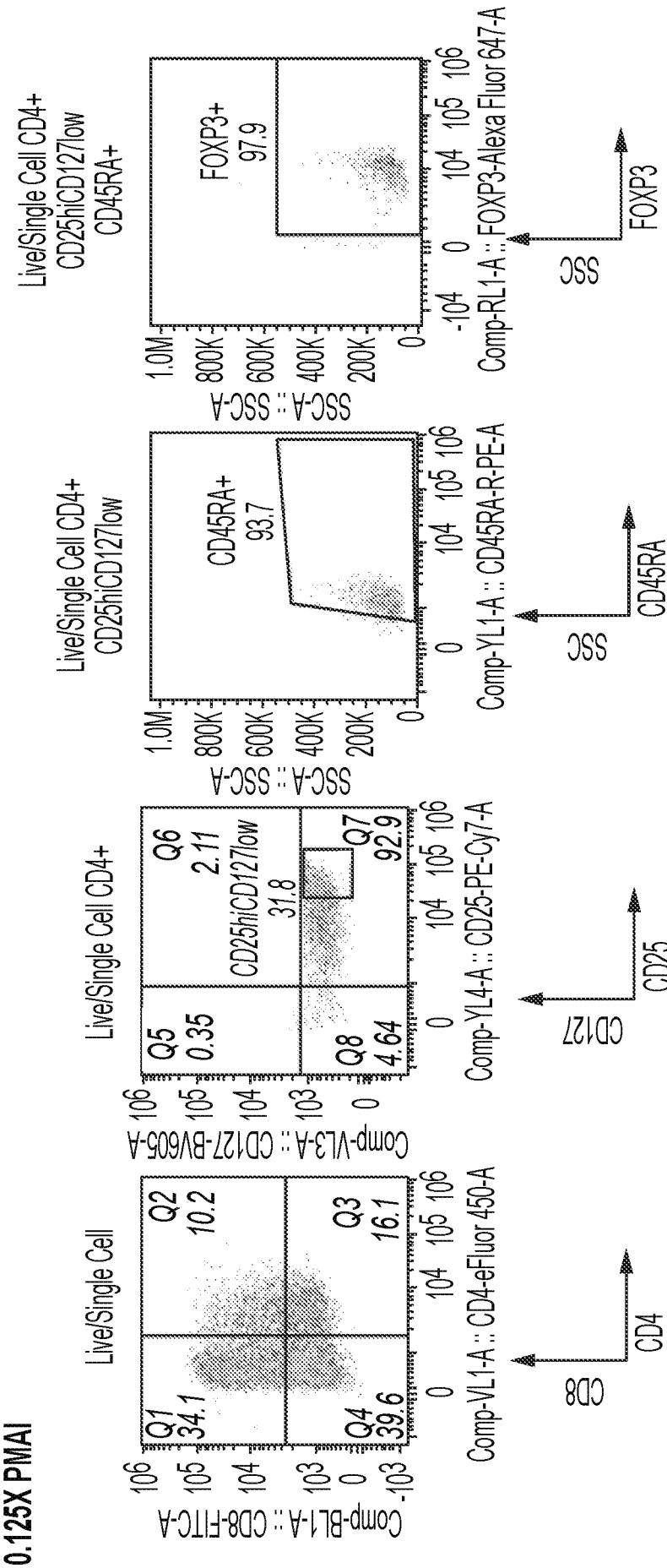


FIG. 17C

CD2/3/28 Soluble Activator

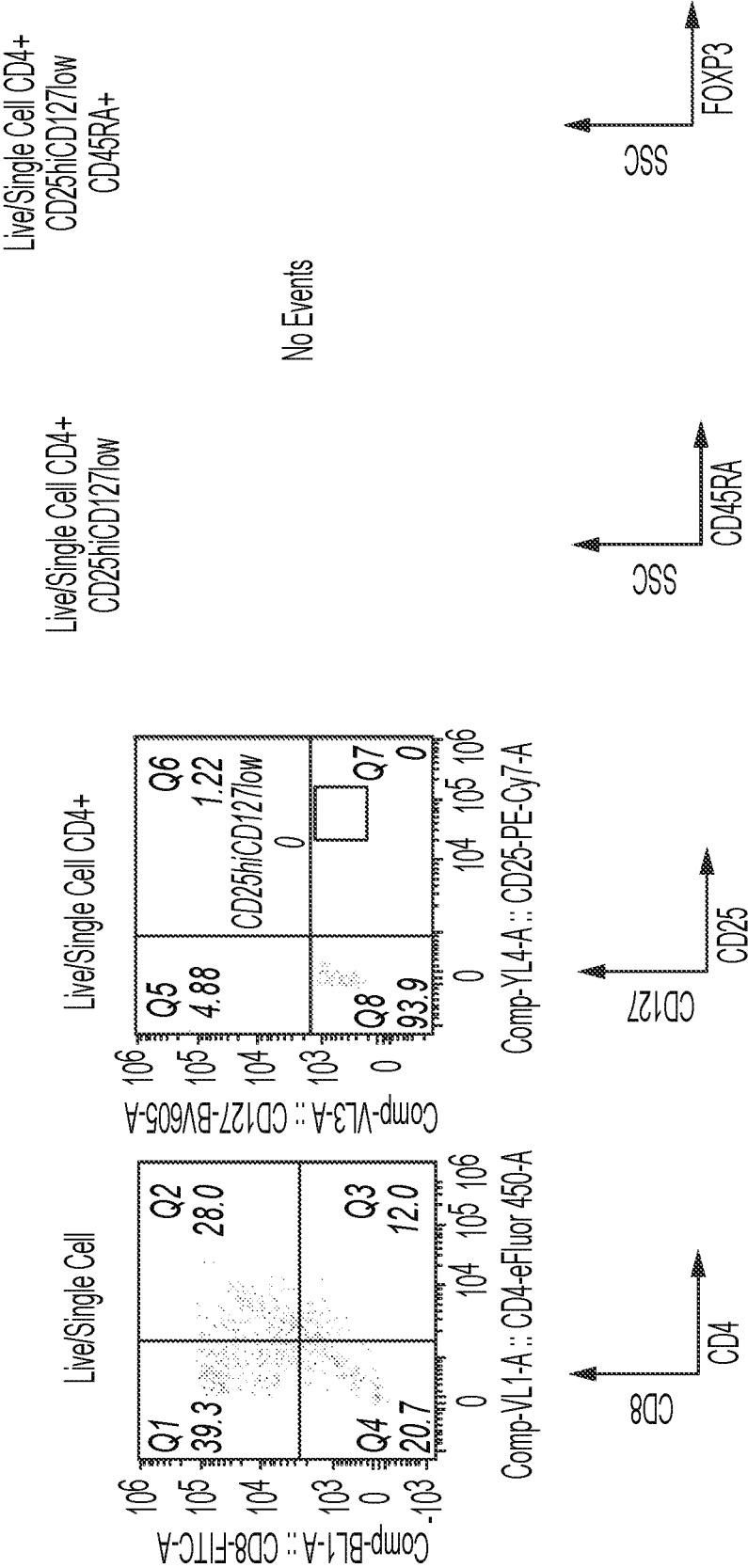


FIG. 17C

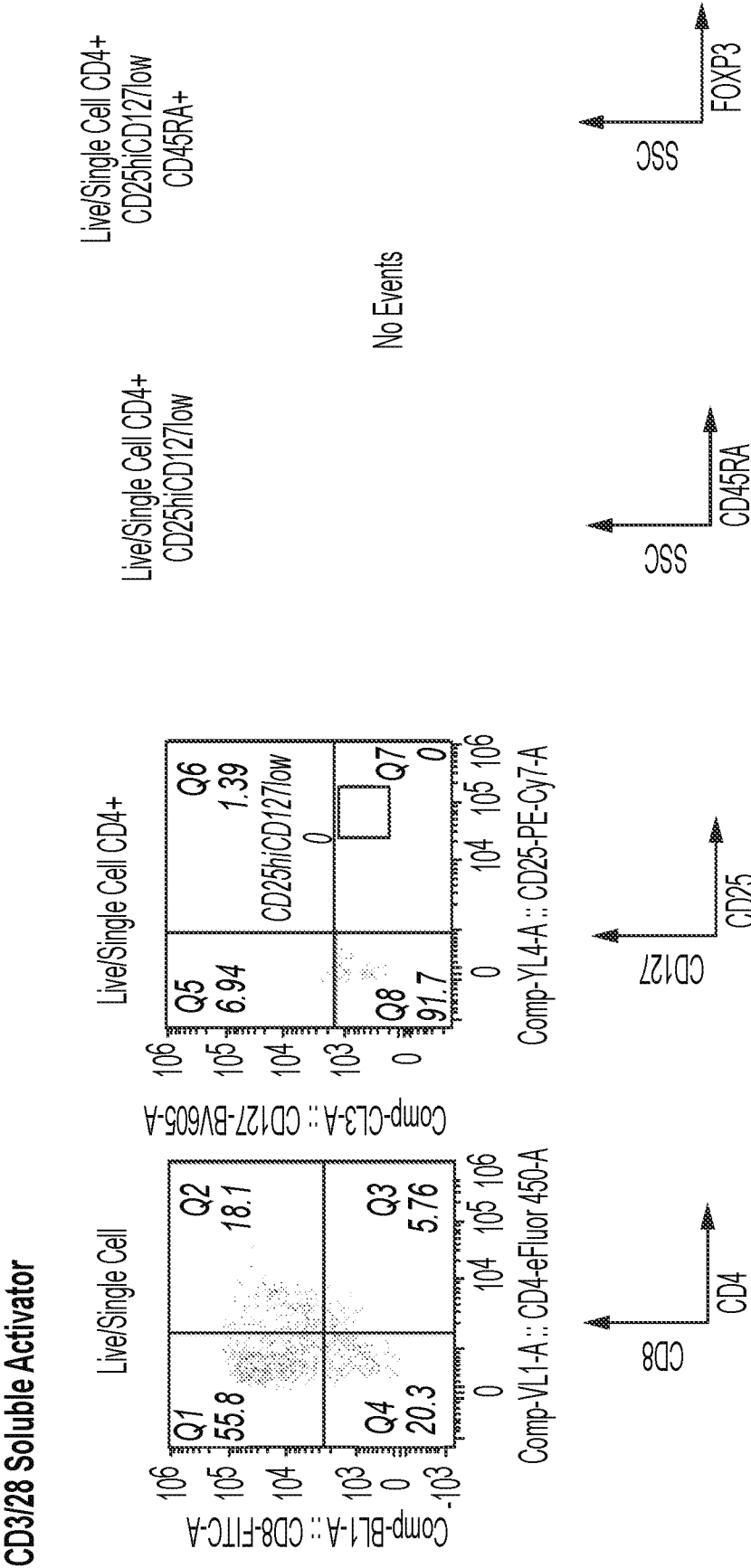


FIG. 17C

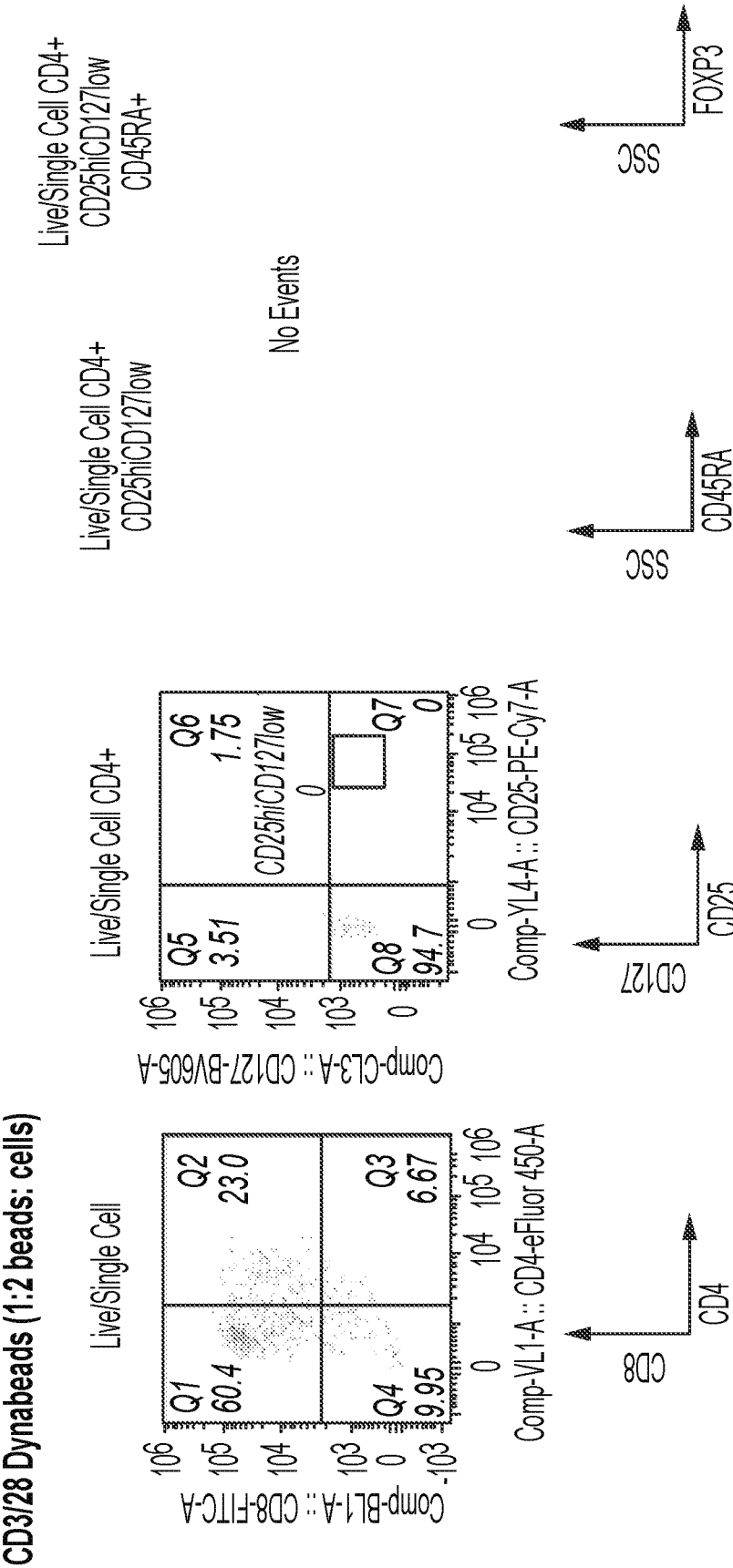


FIG. 17C

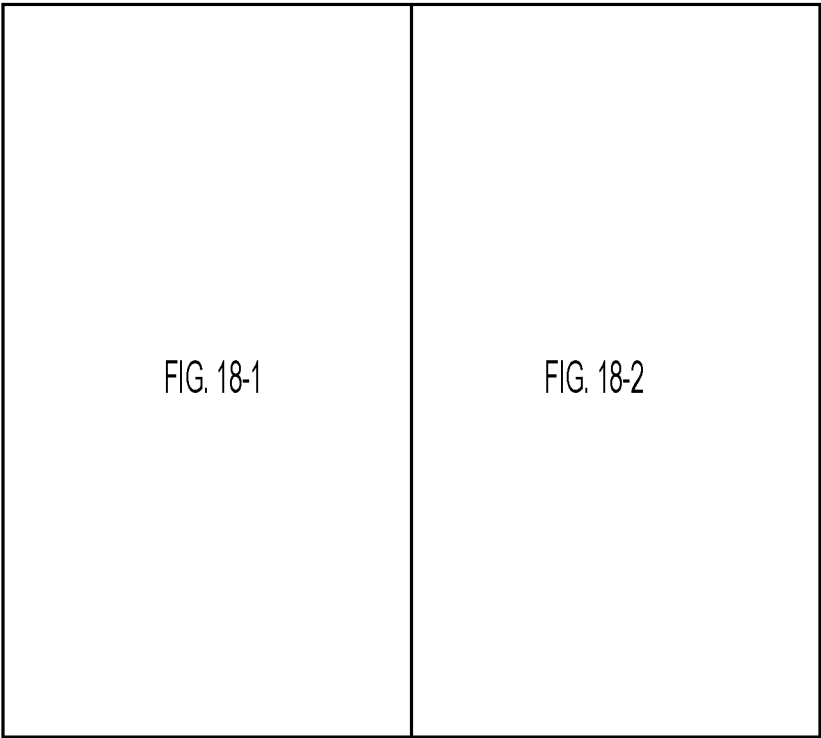


FIG. 18

A

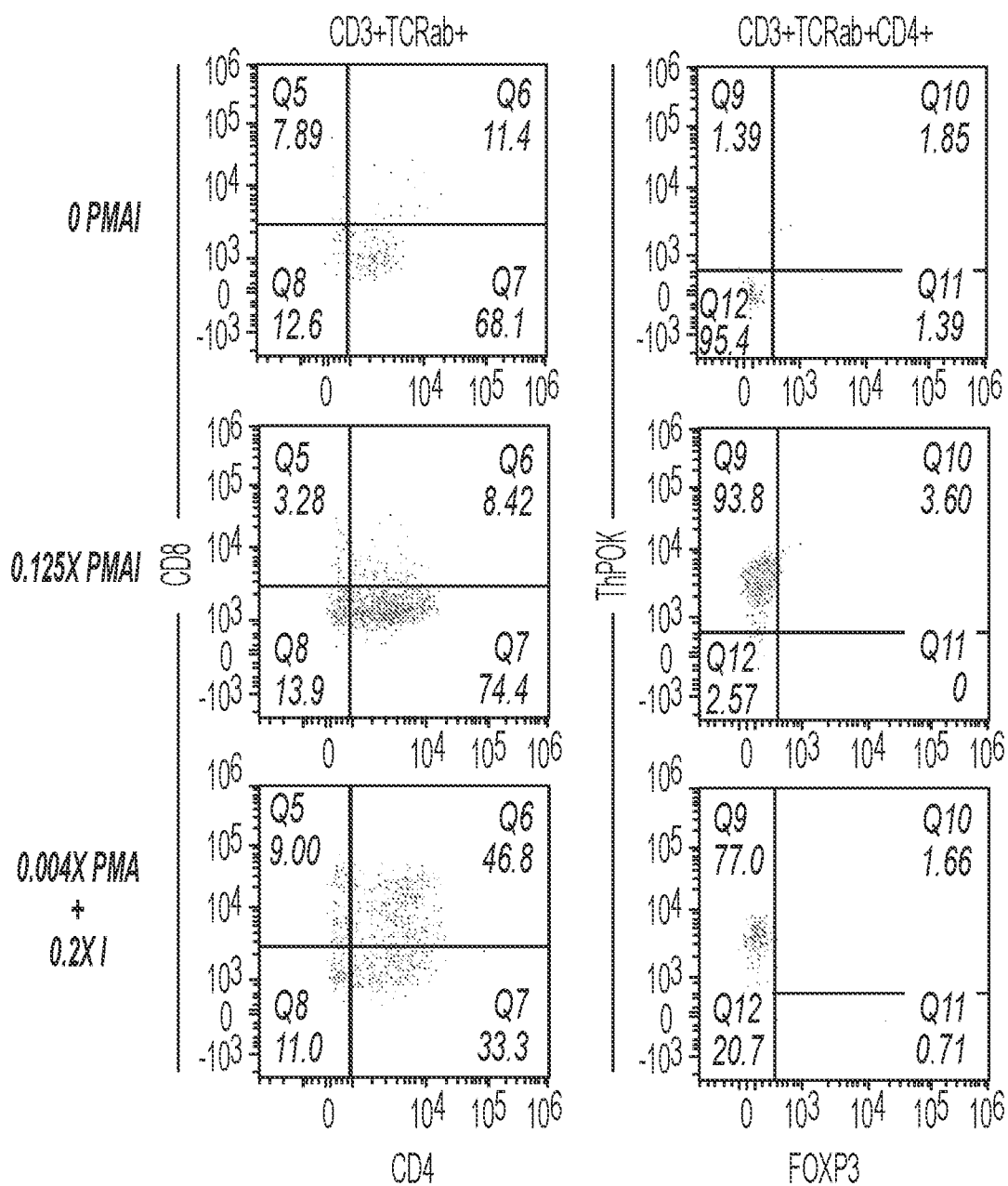


FIG. 18

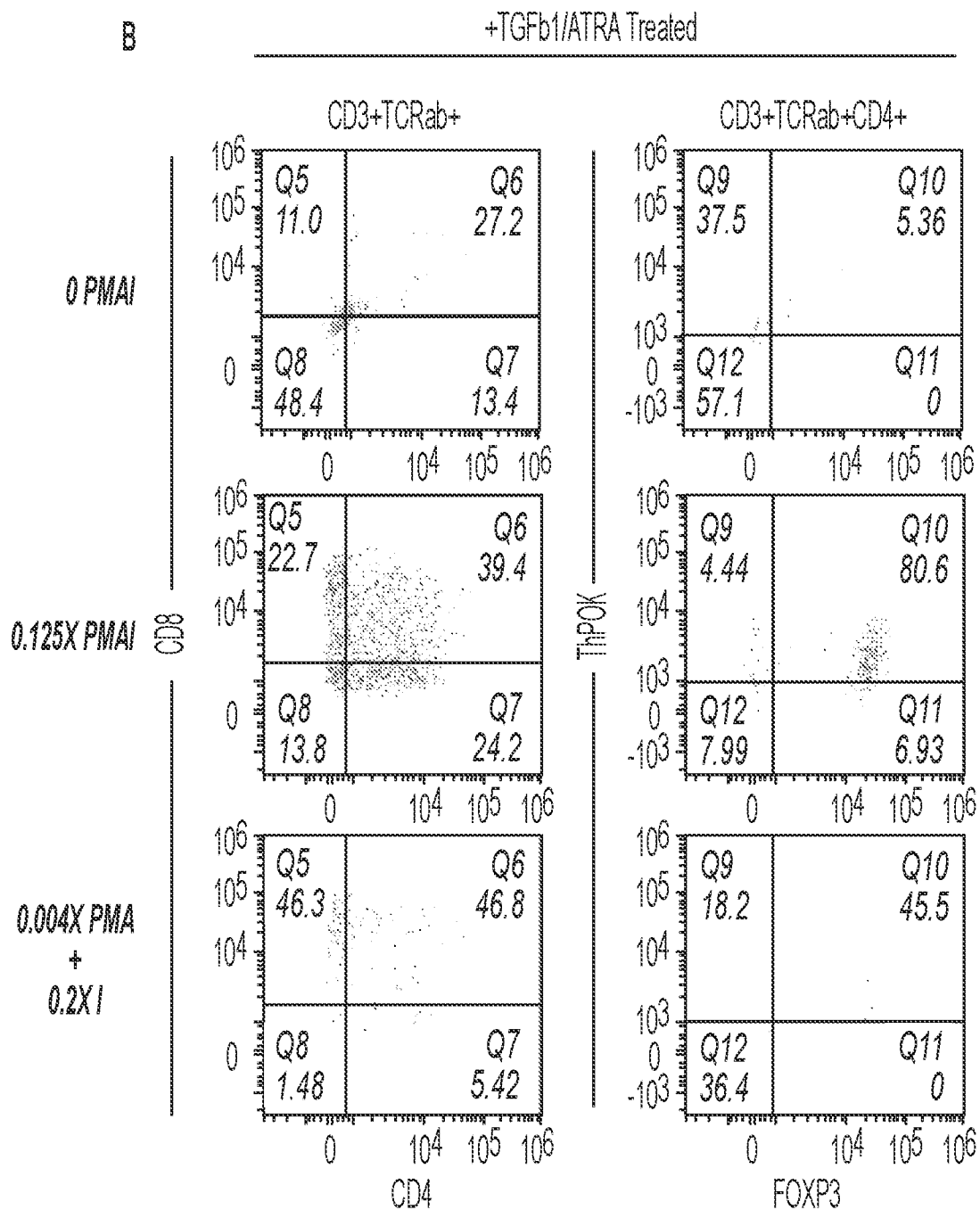
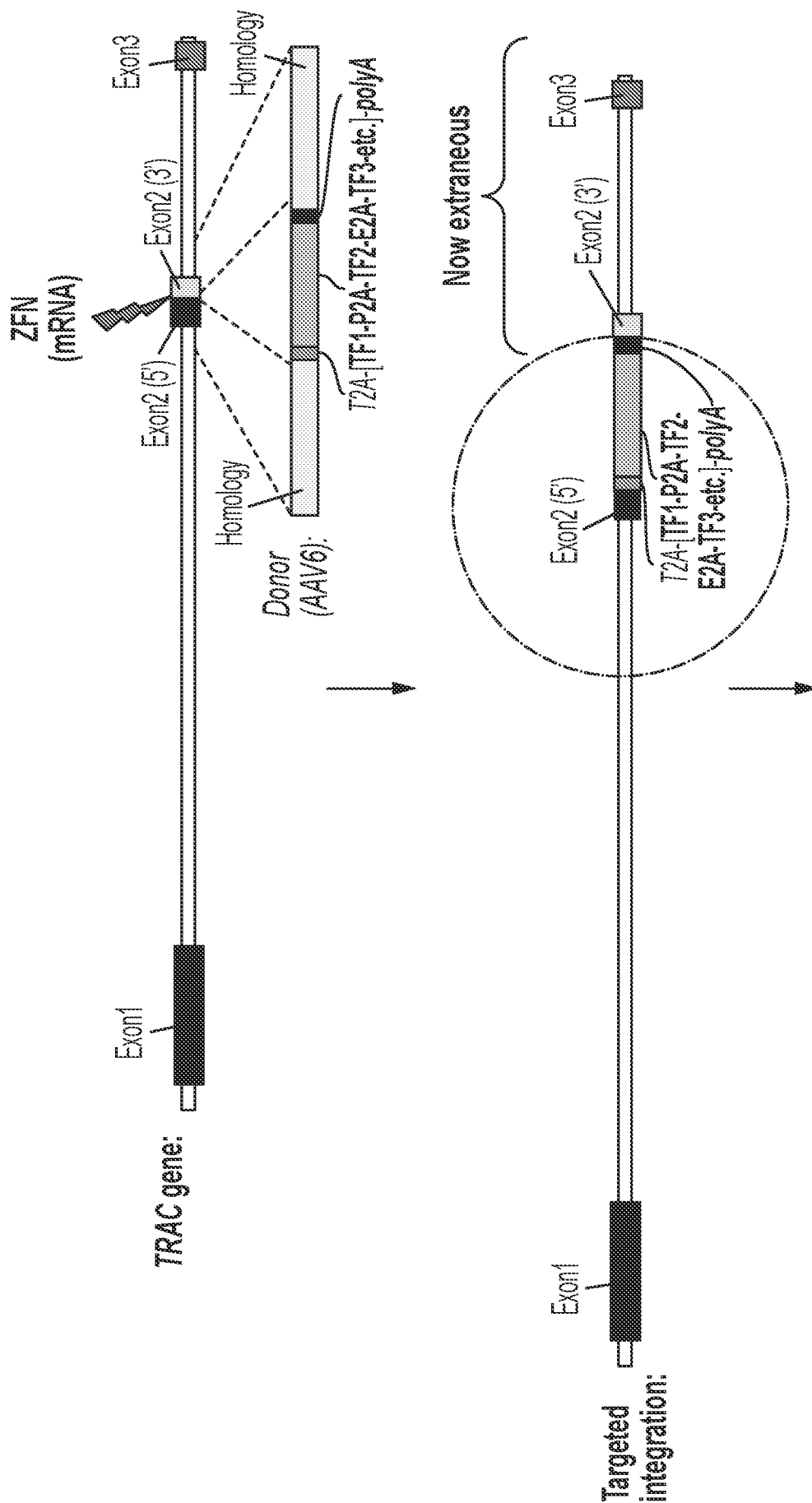


FIG. 18



- TRAC expression knocked out
- Transgene (one or more Treg-induction factor ~ "TF") expression regulated by endogenous TRAC promoter

FIG. 19

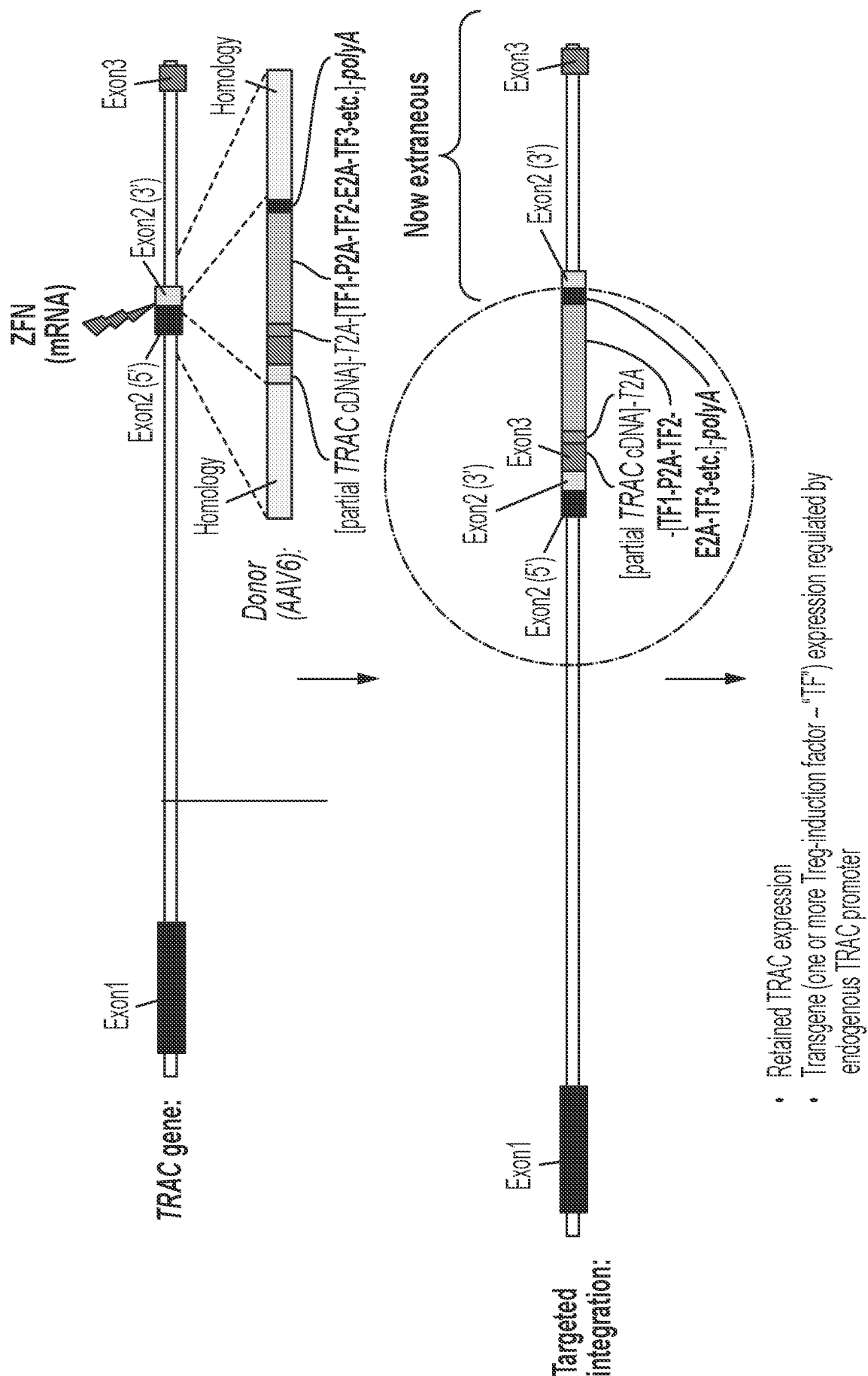


FIG. 20

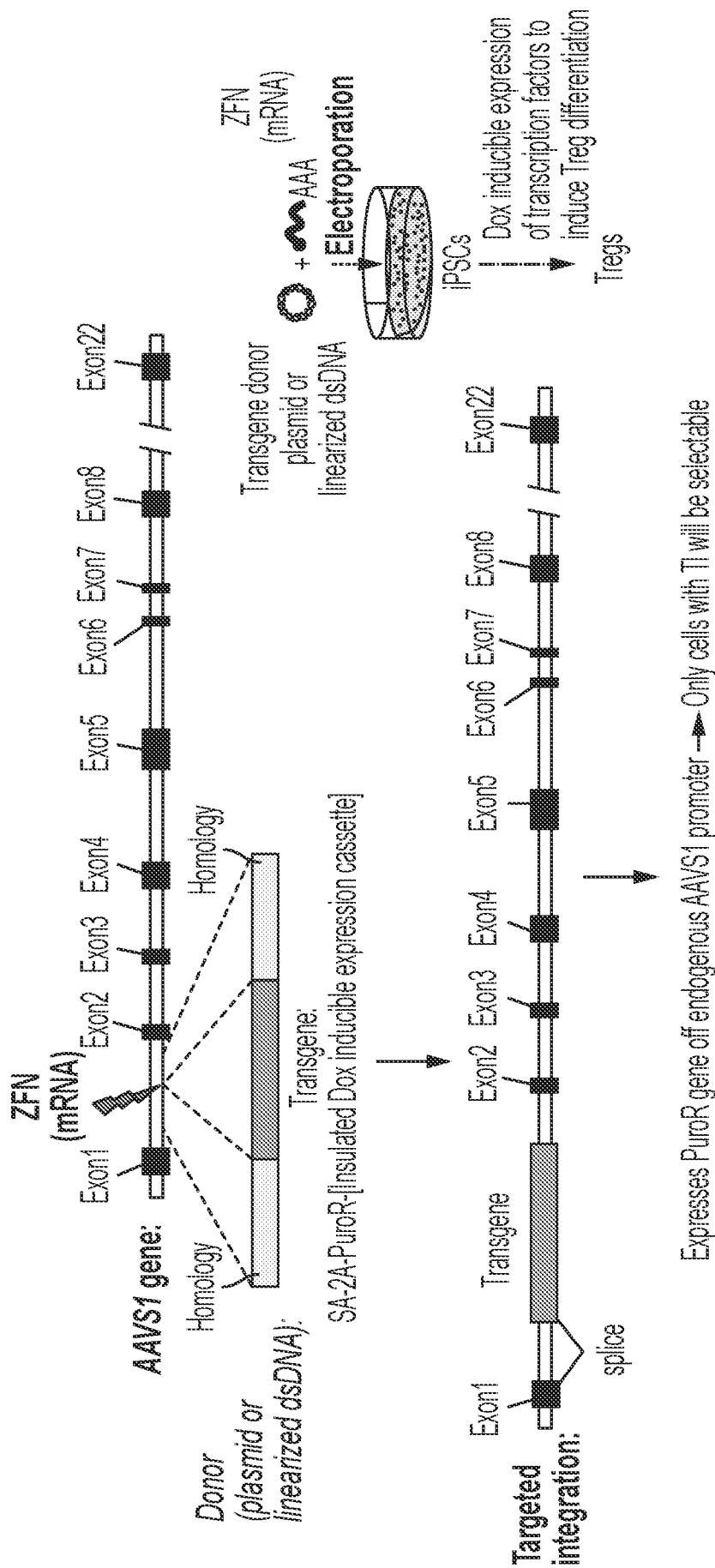


FIG. 21

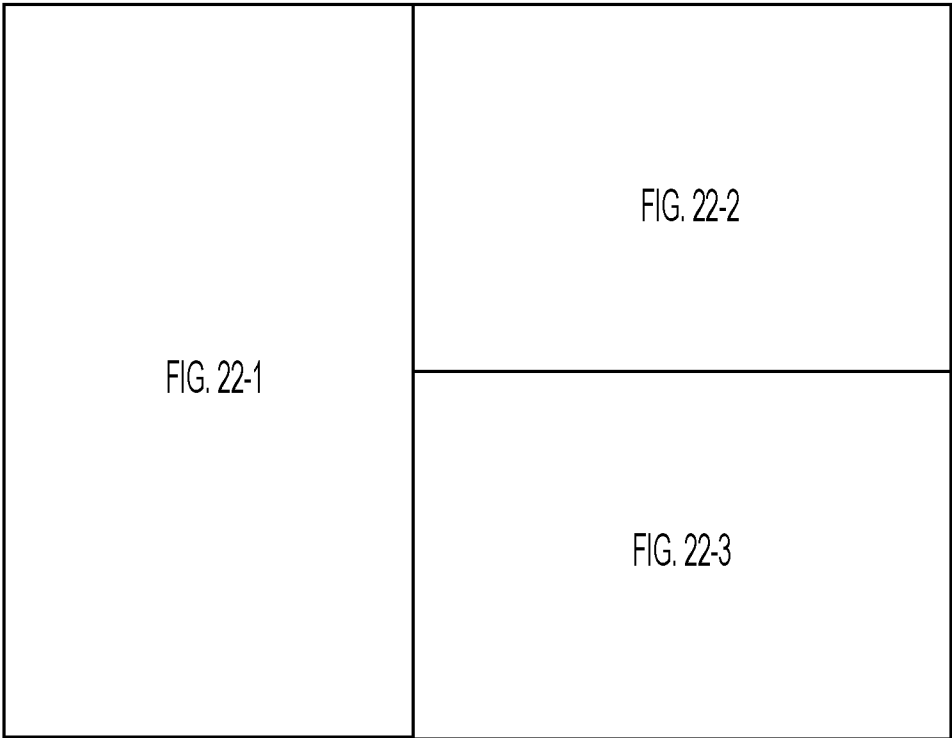


FIG. 22

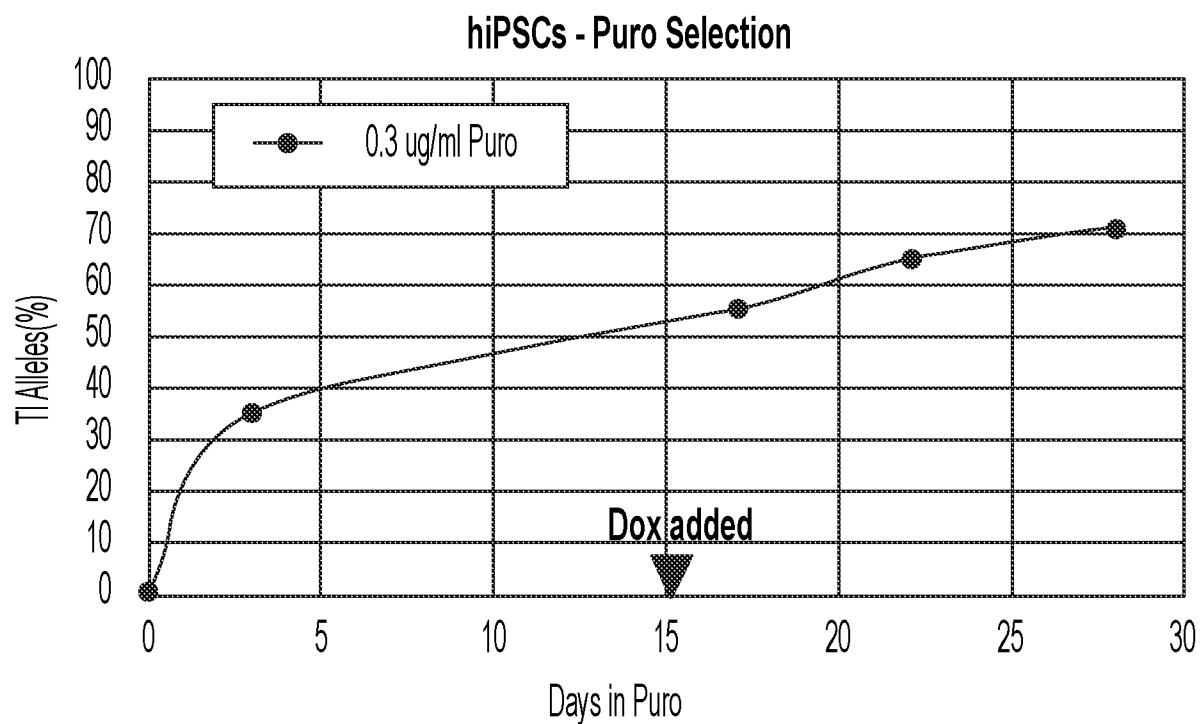
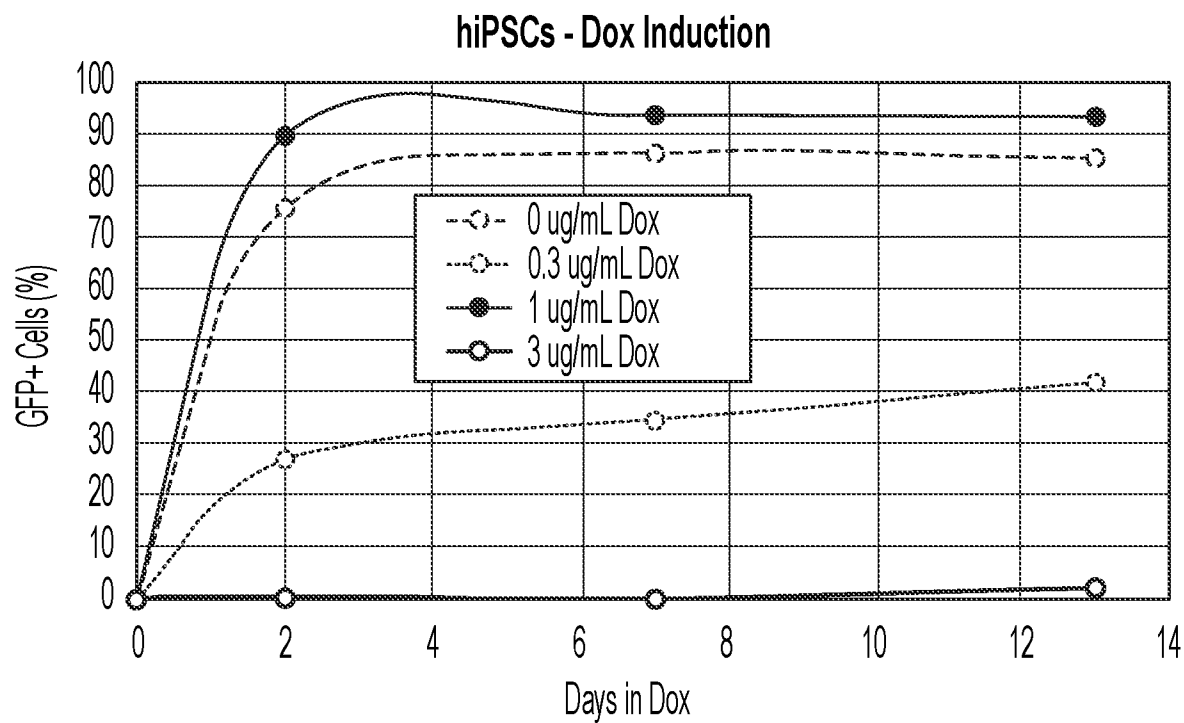


FIG. 22

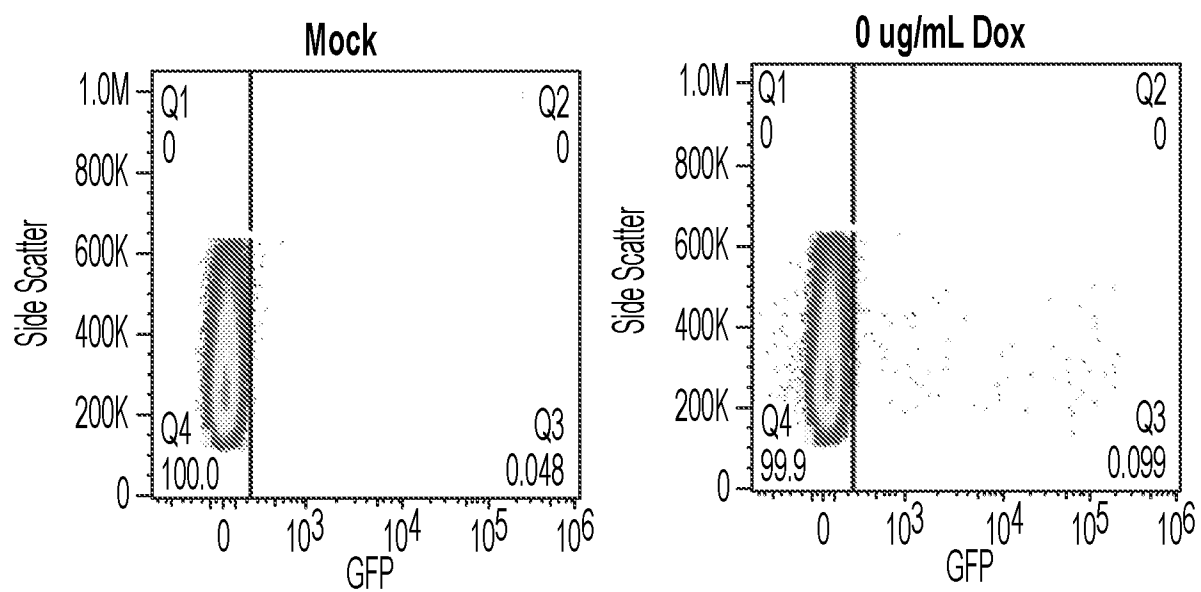


FIG. 22

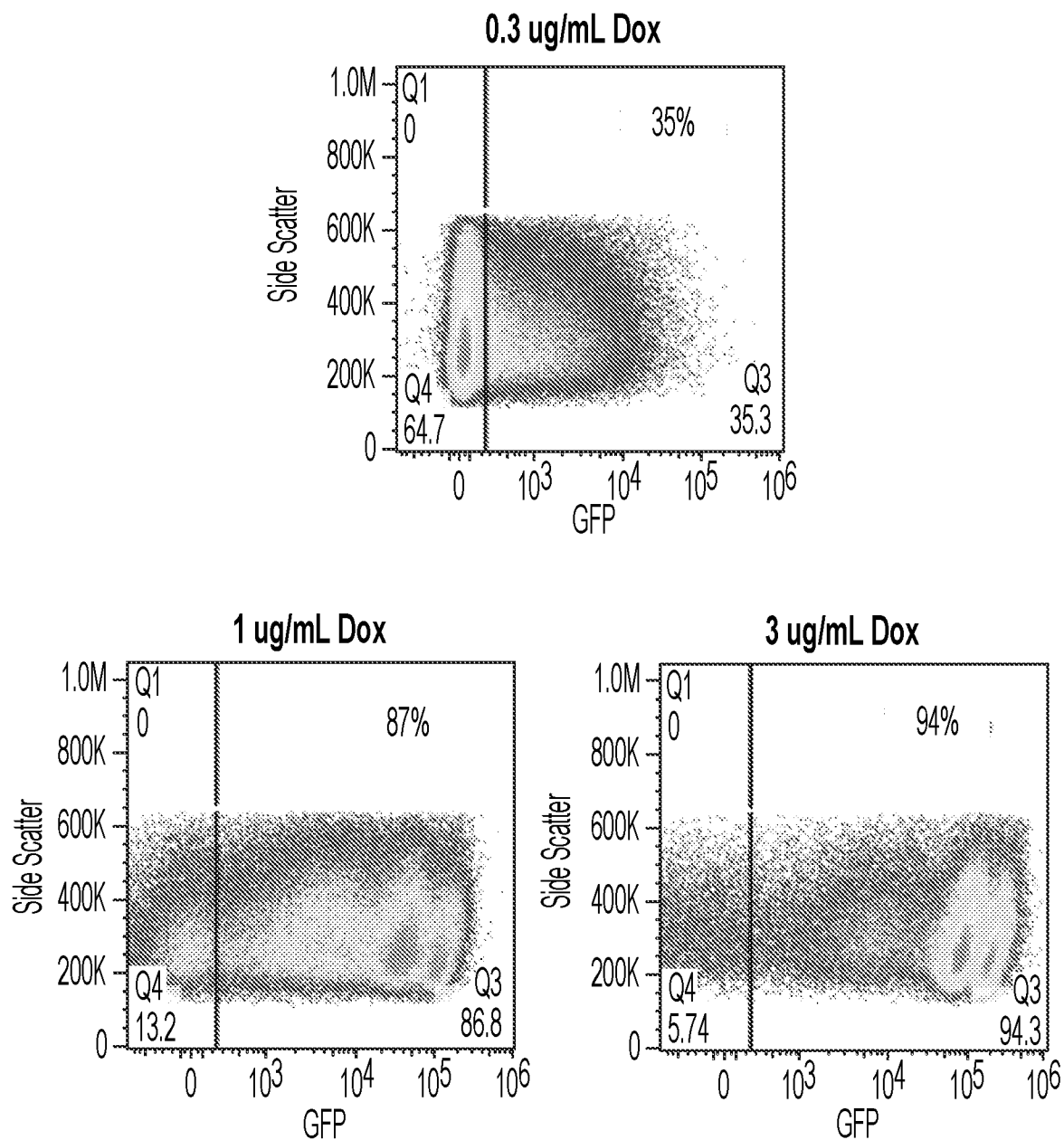
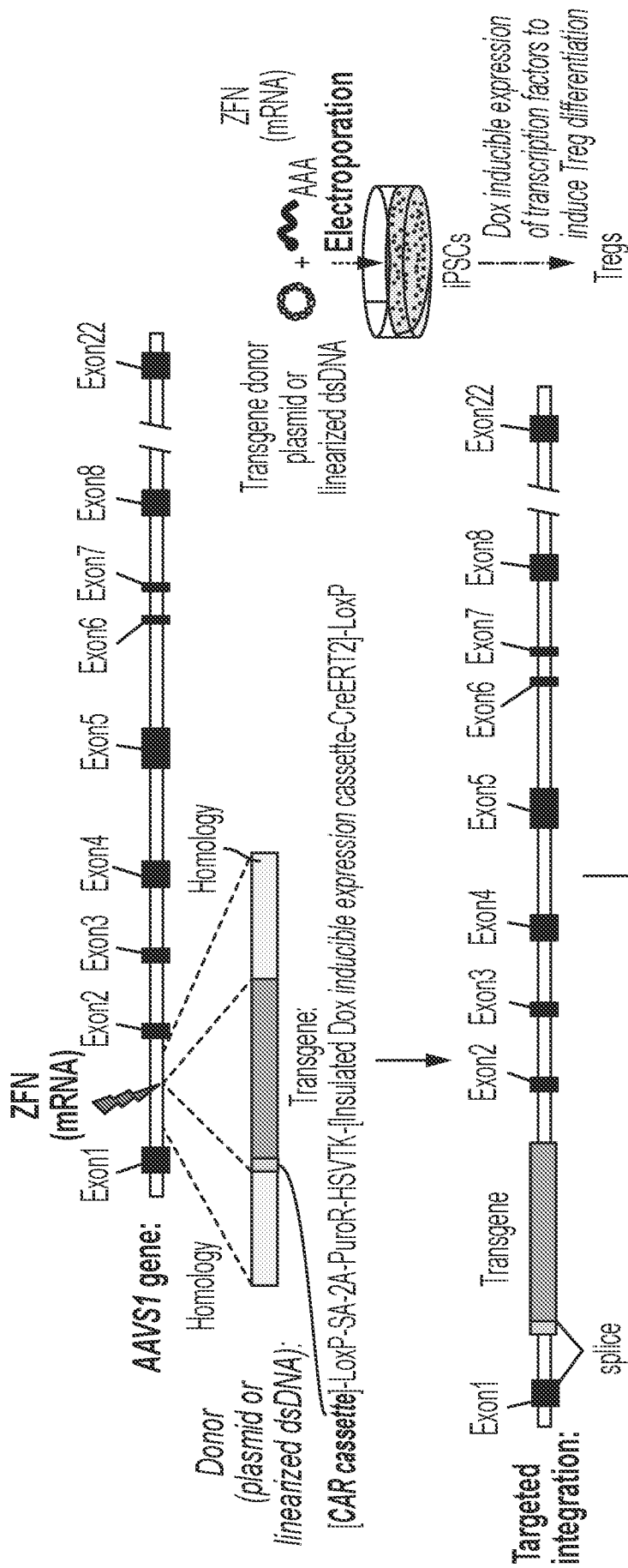


FIG. 22



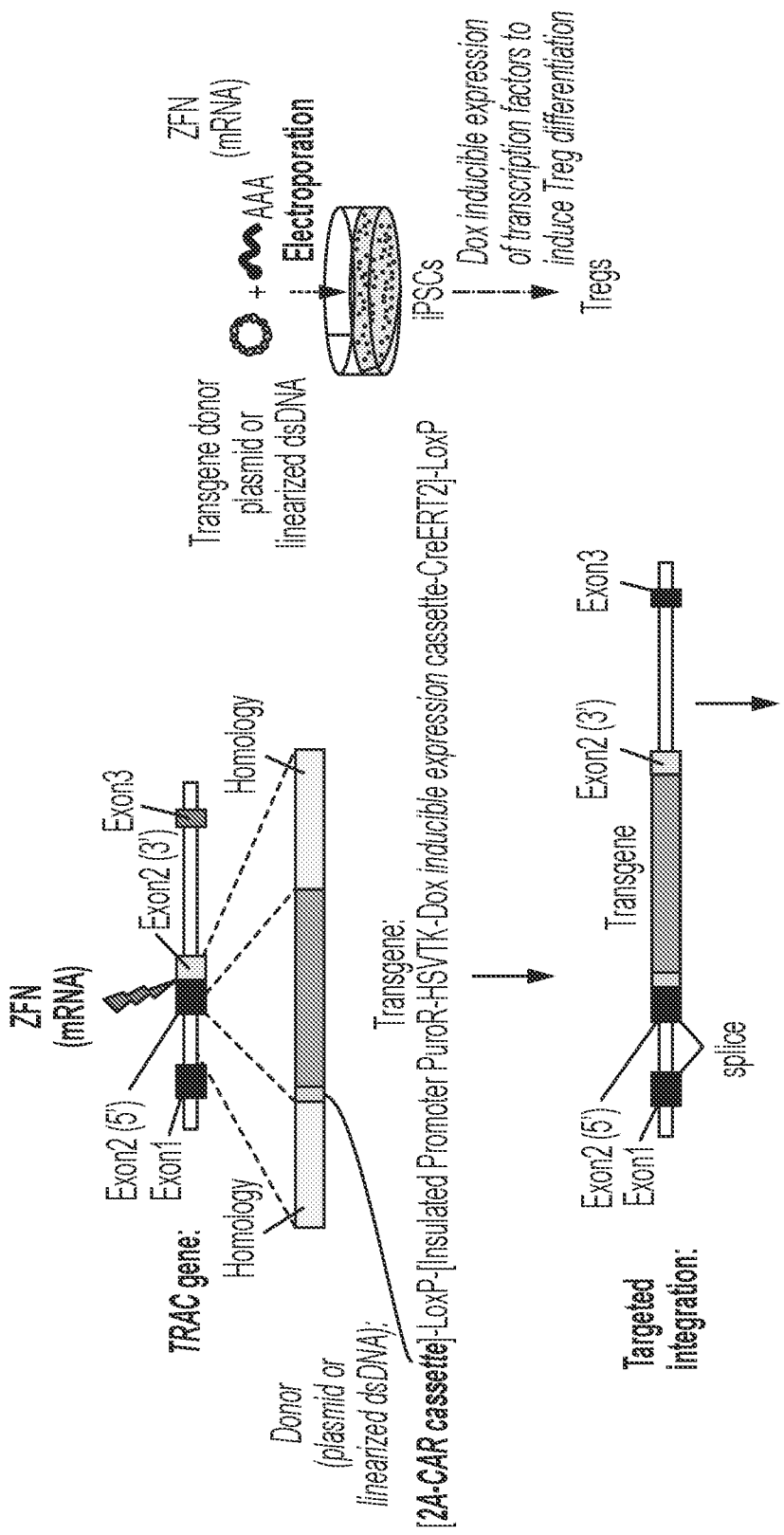
Expresses PuroR gene off endogenous AAVS1 promoter → Only cells with TI will be selectable

4-OH-Tamoxifen-inducible Cre recombinase → can excise selection/Dox-induction cassette after Tregs have formed

Negative selection using ganciclovir-inducible suicide gene (HSVTK) possible to eliminate cells which have not undergone Cre excision

CAR or other transgene requiring constitutive expression remains with scarless removal of cassette to induce Treg differentiation

FIG. 23



PuroR gene driven off its own promoter within donor construct → Only T1 cells will be resistant after episomal donor DNA dilutes out after several cell divisions
4-OH-Tamoxifen-inducible Cre recombinase → can excise selection/Dox-induction cassette after Tregs have formed
Negative selection using ganciclovir-inducible suicide gene (HSVTK) possible to eliminate cells which have not undergone Cre excision
CAR or other transgene driven off endogenous TRAC promoter remains with scarless removal of cassette to induce Treg differentiation

FIG. 24

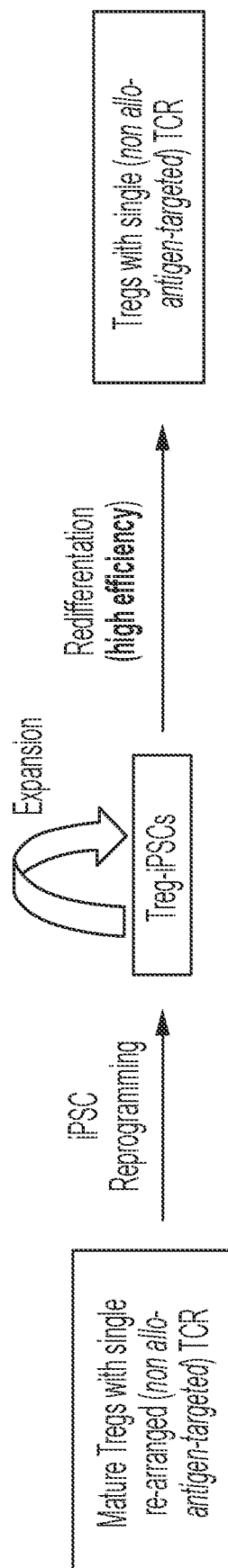


FIG. 25

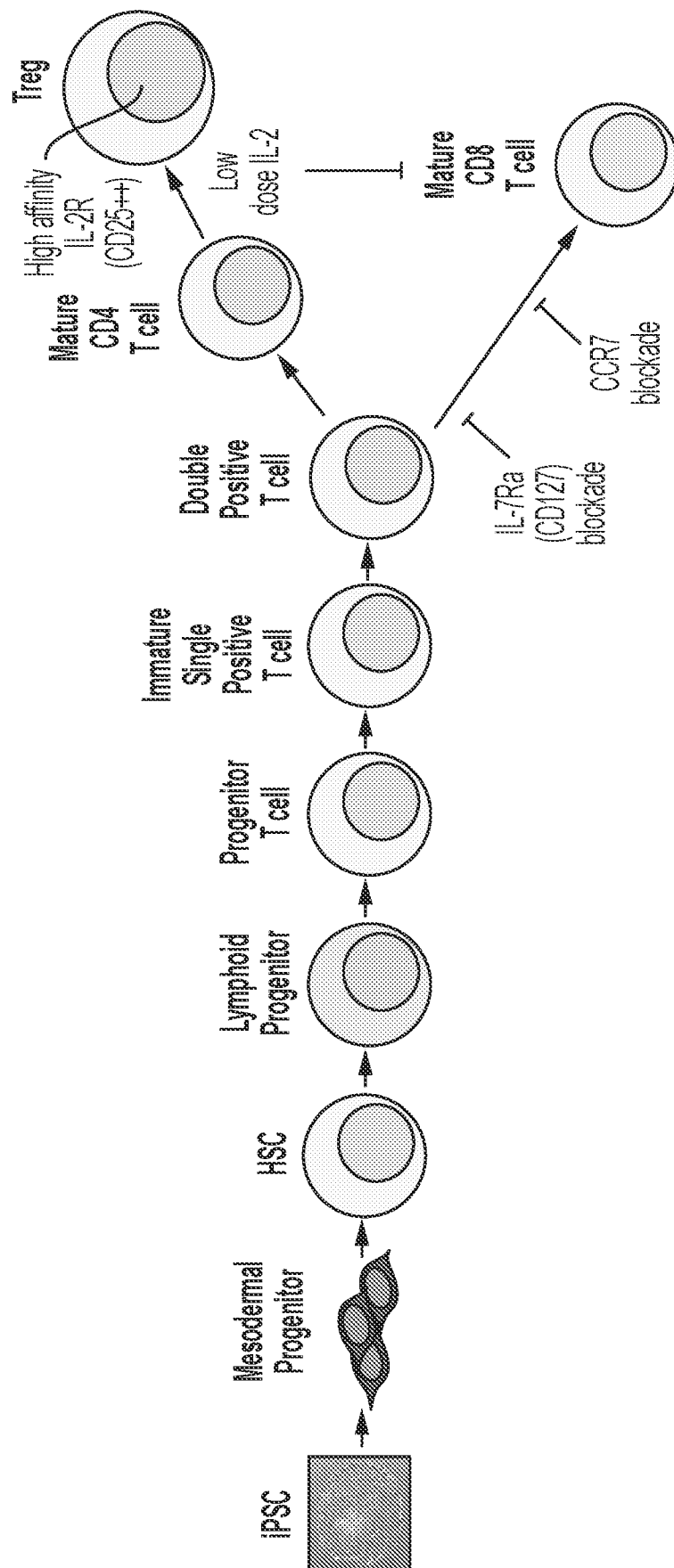


FIG. 26

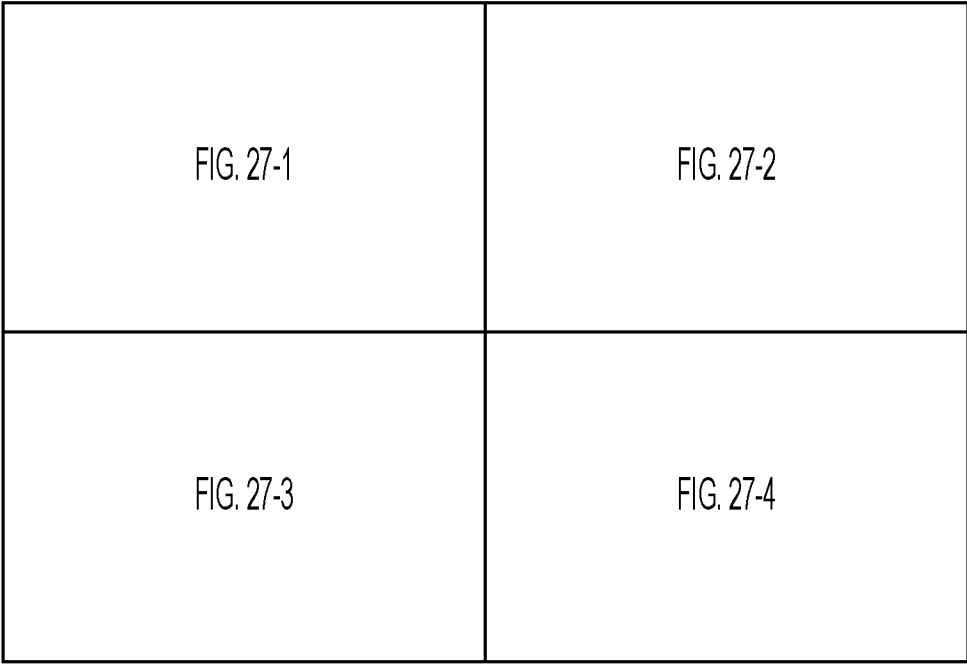


FIG. 27

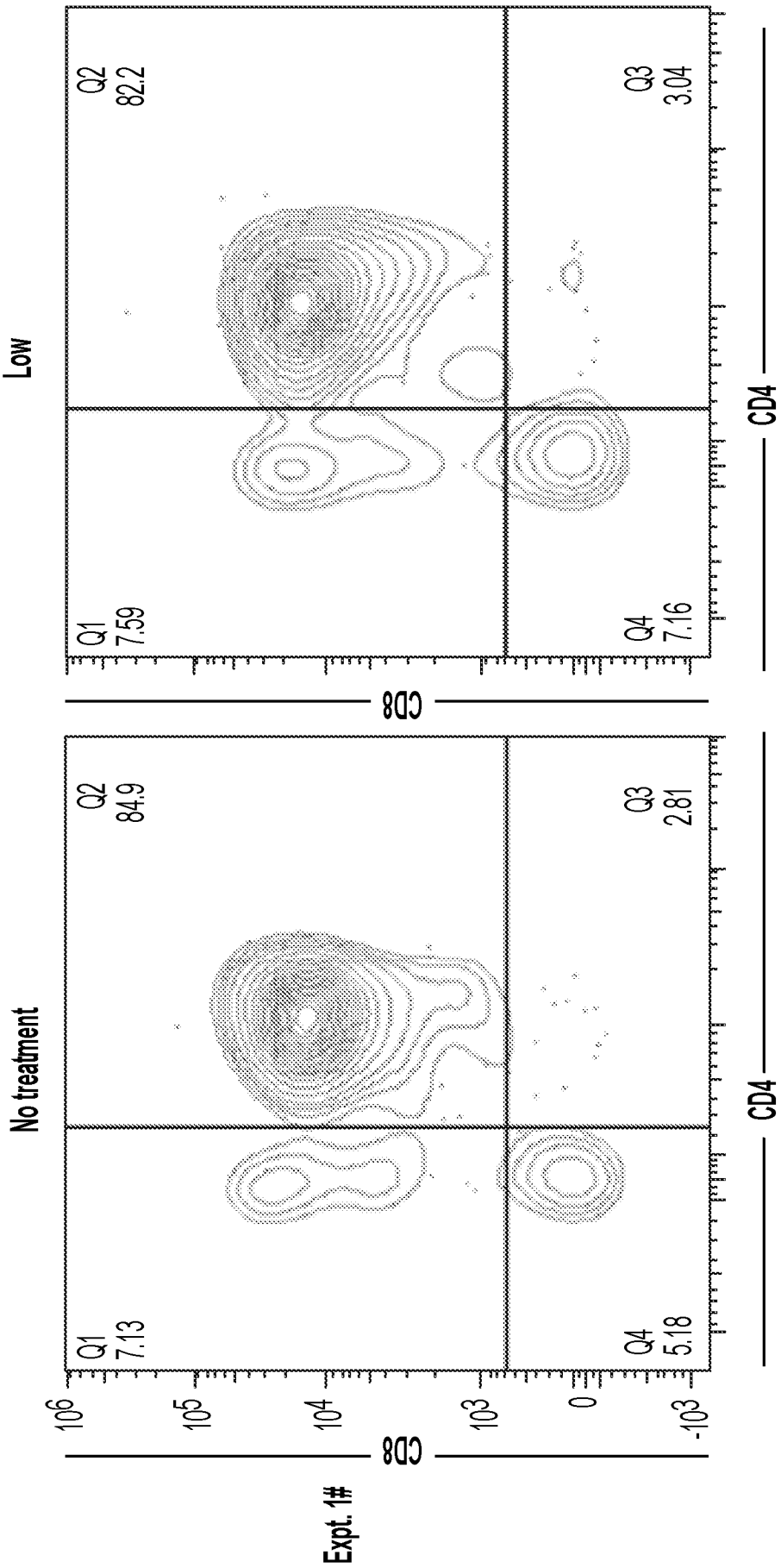


FIG. 27

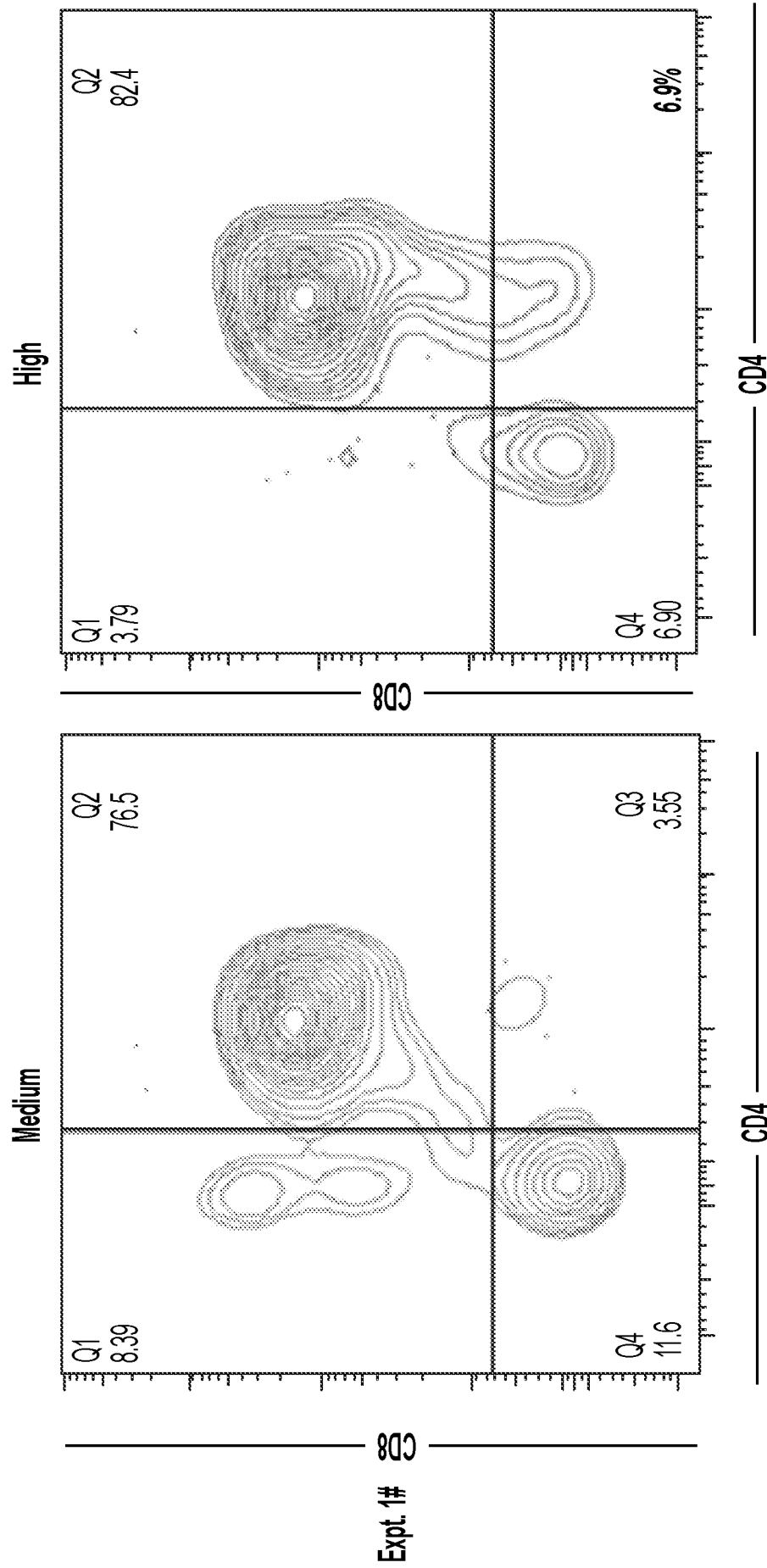


FIG. 27

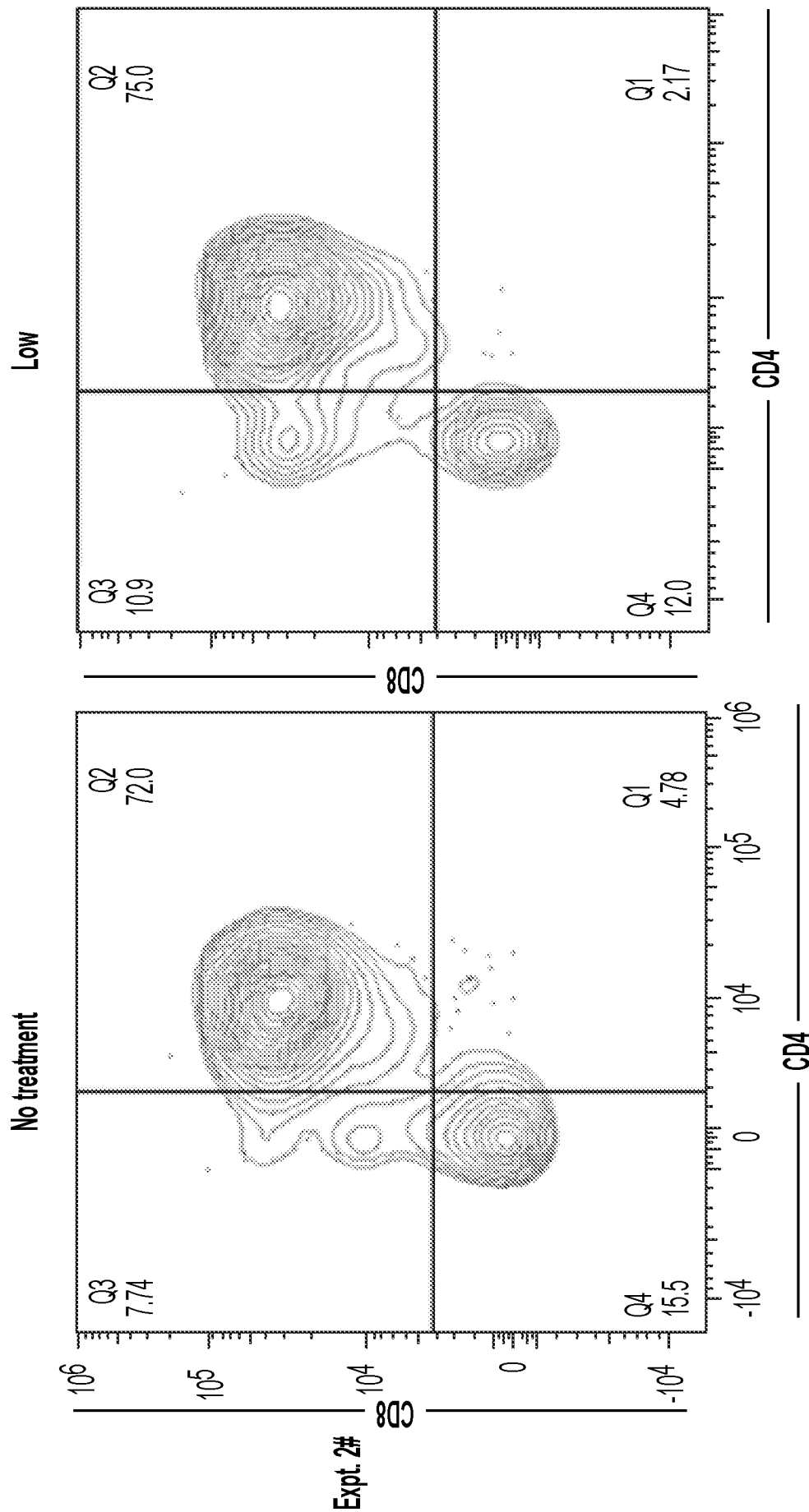


FIG. 27

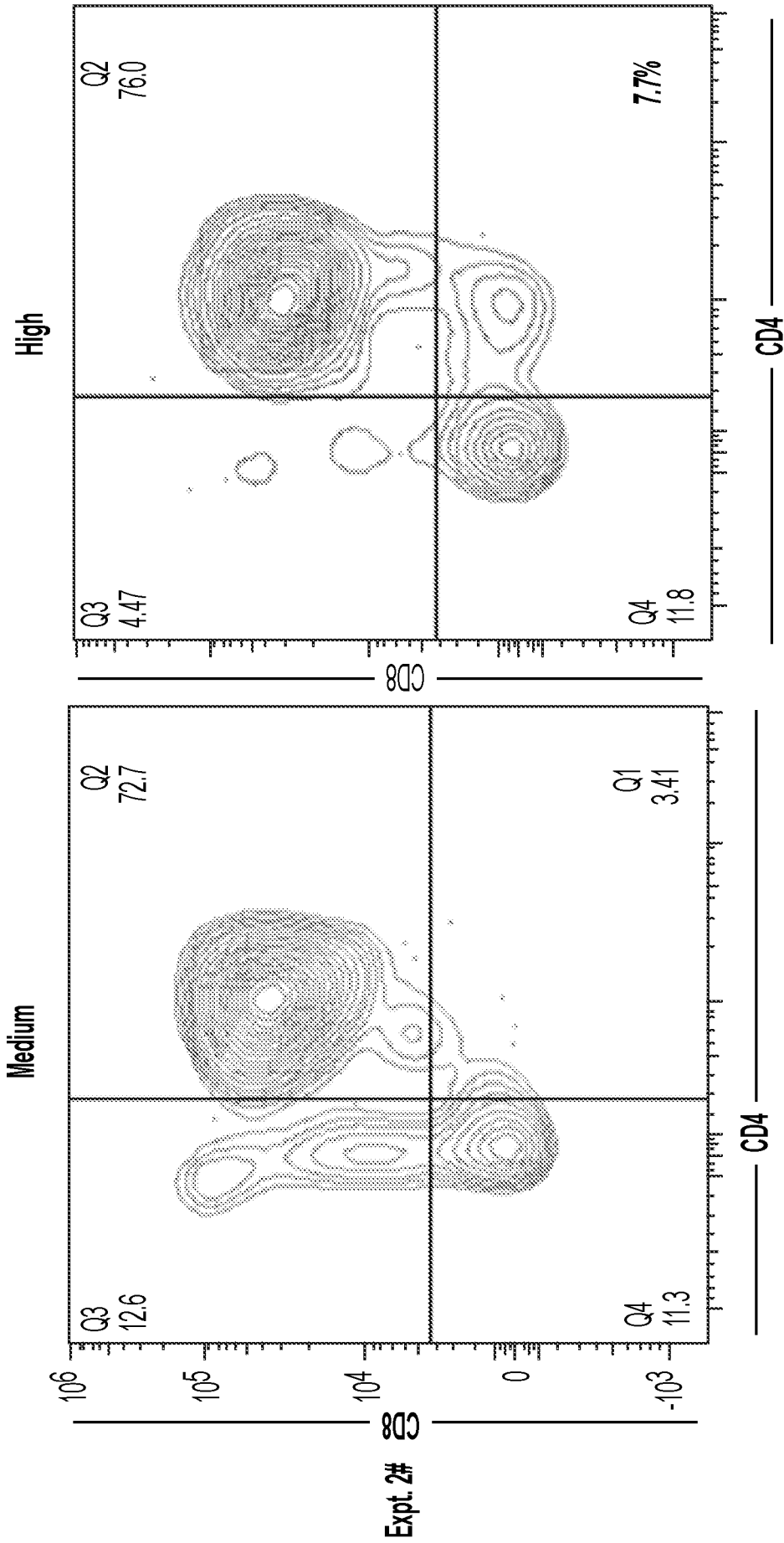


FIG. 27

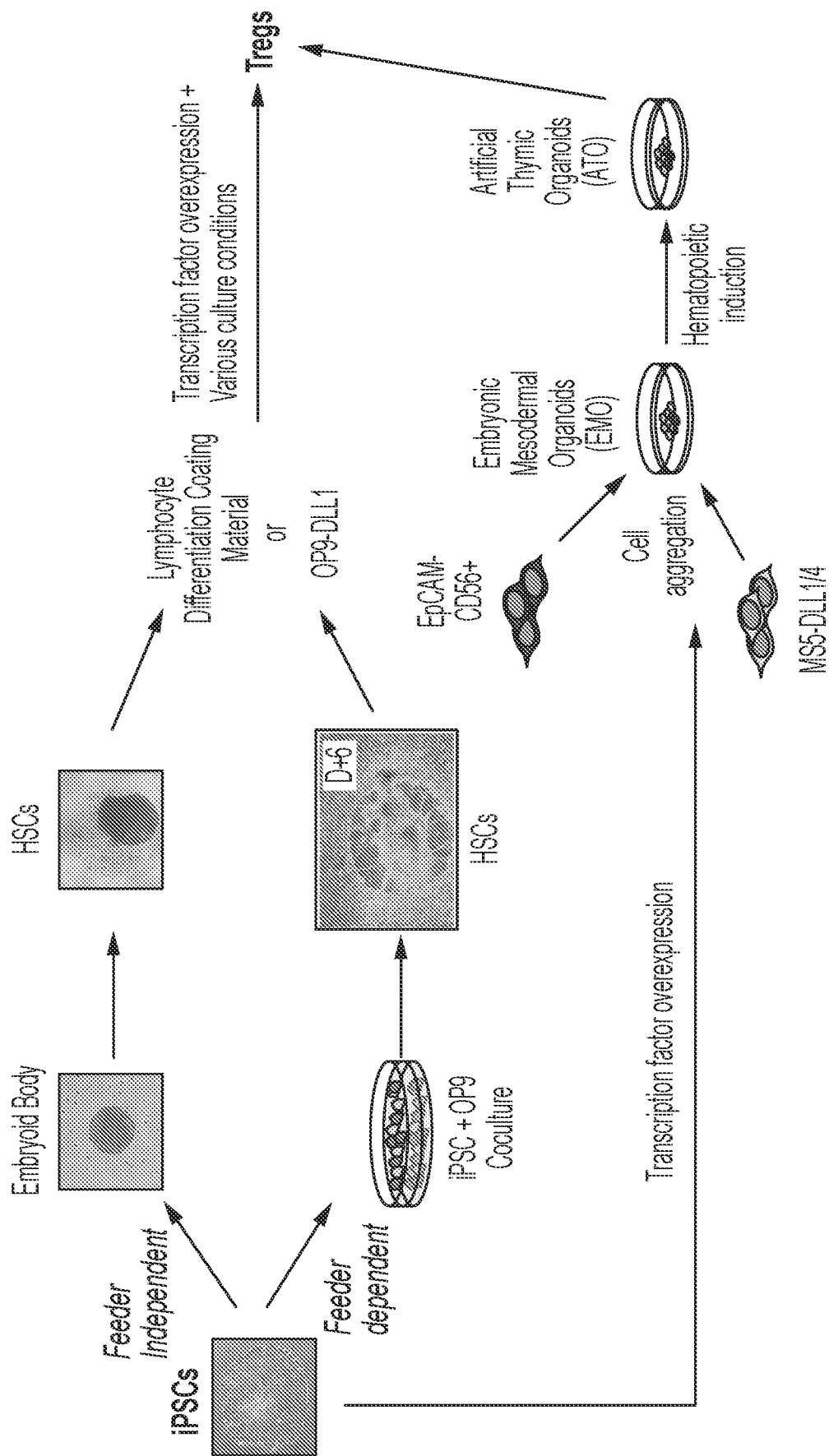


FIG. 28