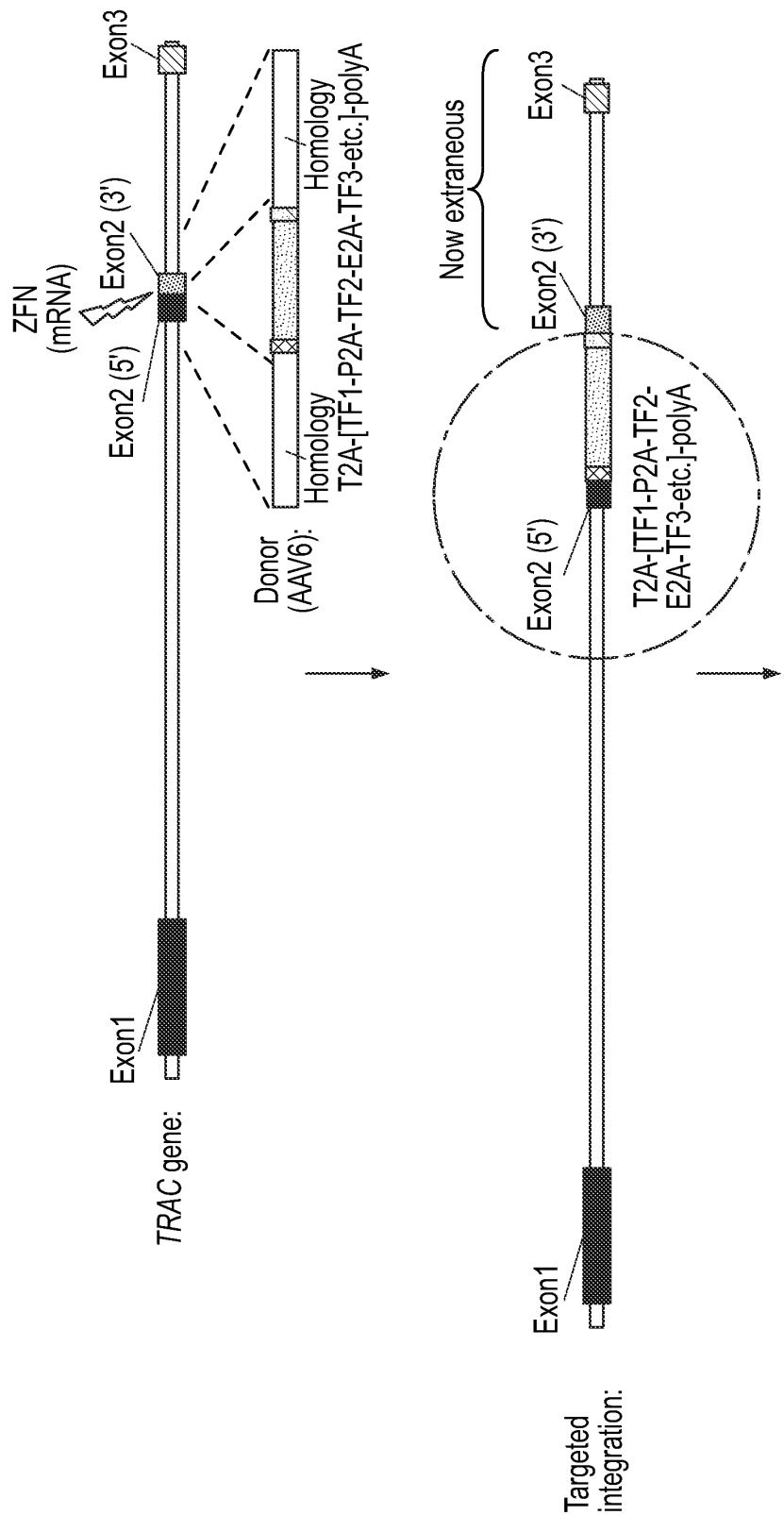




- * TRAC expression knocked out
- * Transgene (one or more Treg-induction factor – "TF") expression regulated by endogenous TRAC promoter

FIG. 1

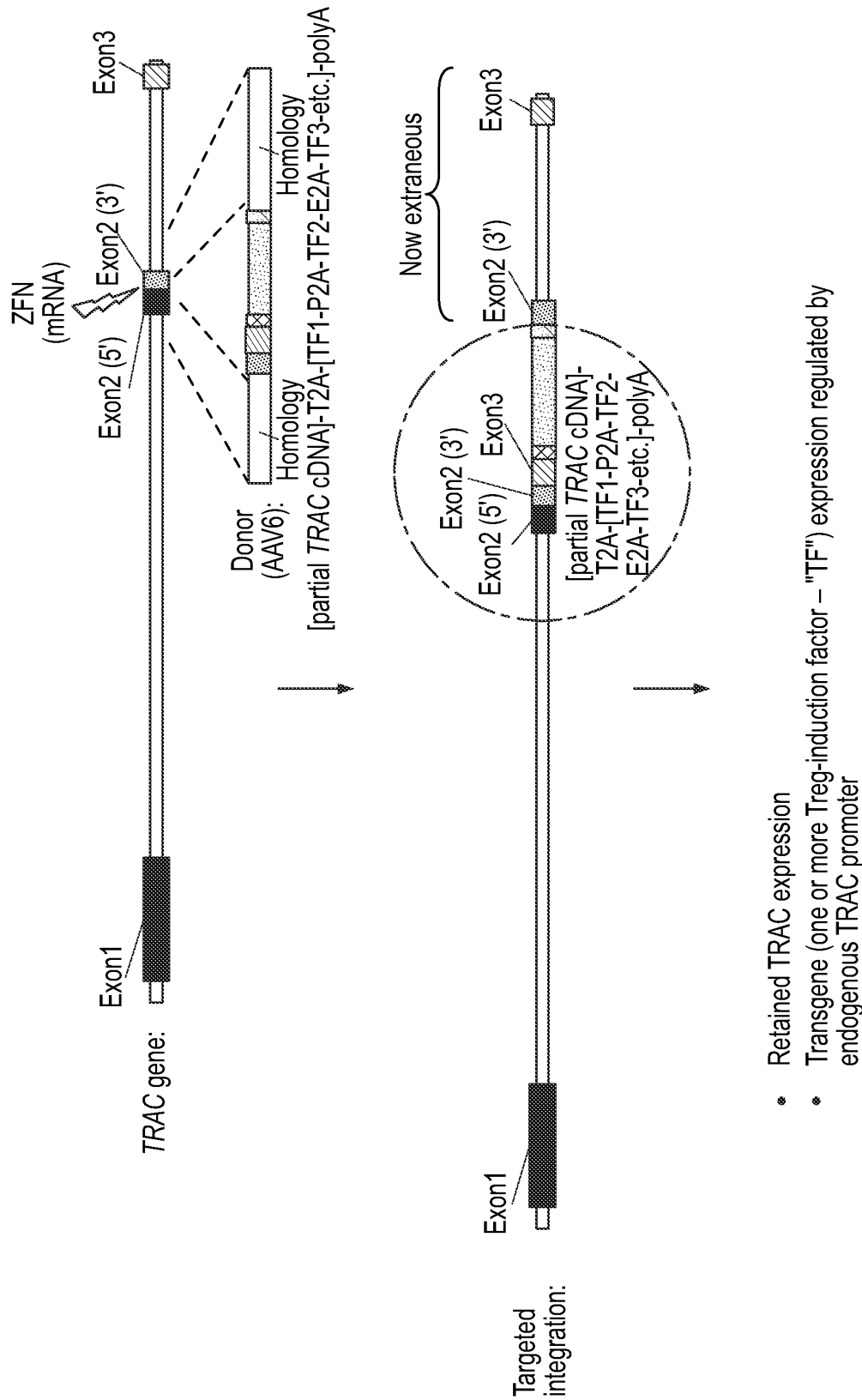


FIG. 2

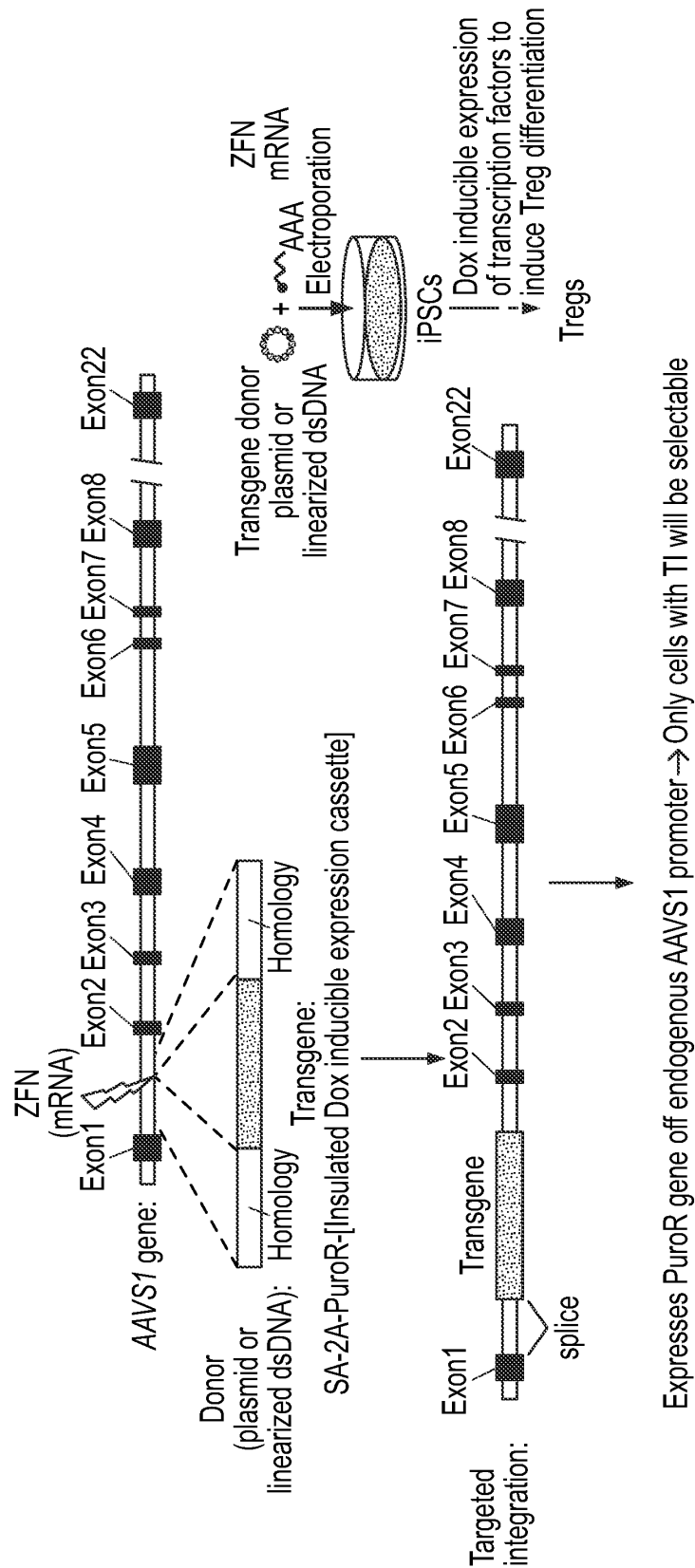


FIG. 3

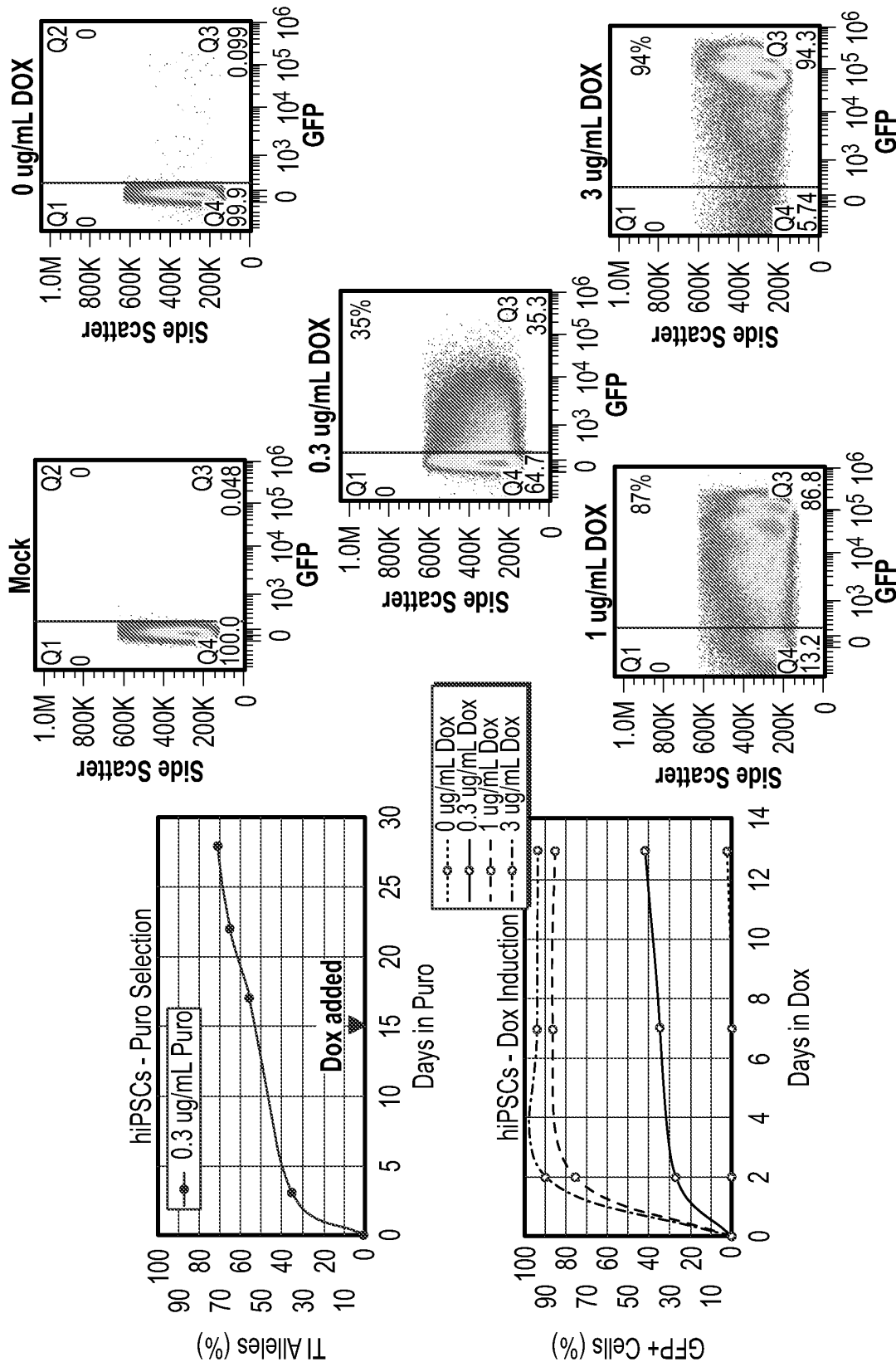
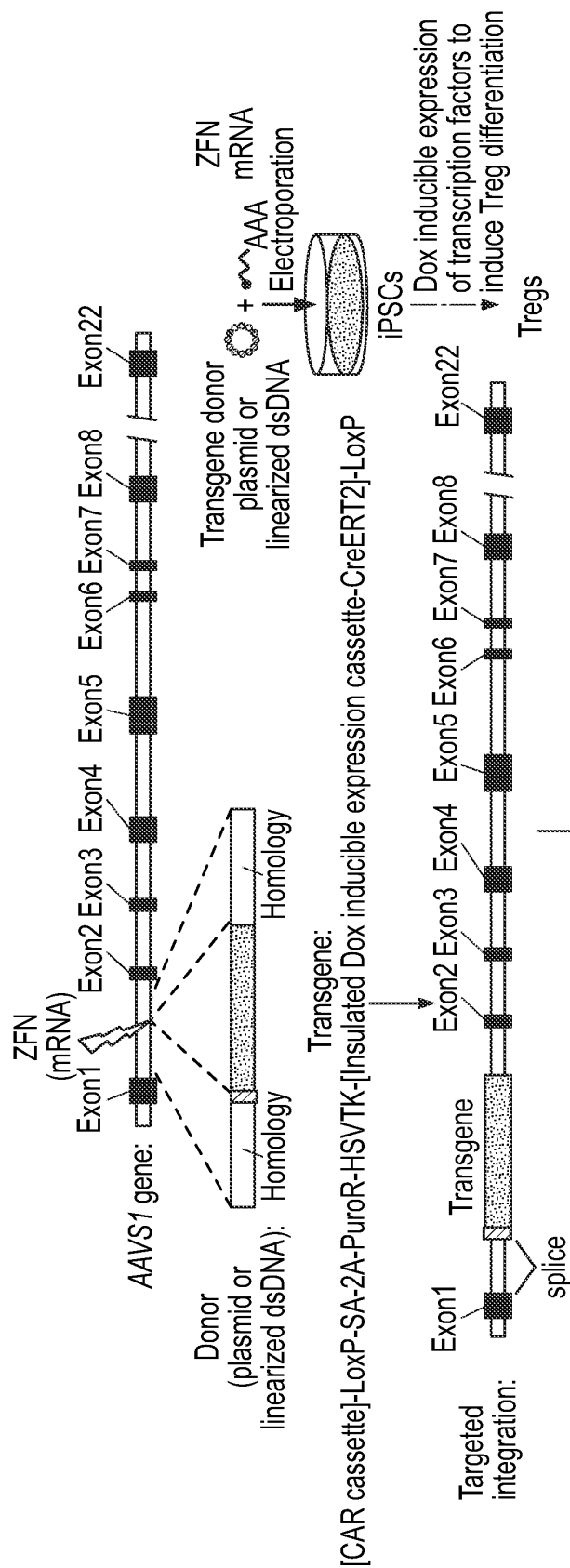
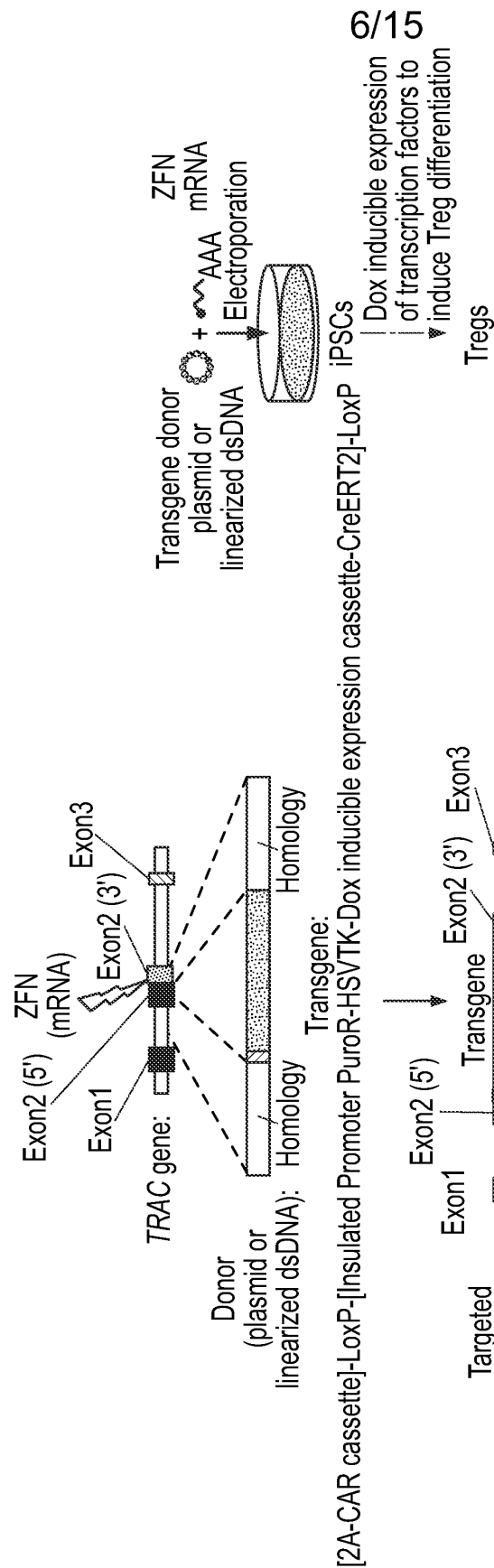


FIG. 4



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. 5



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. 6

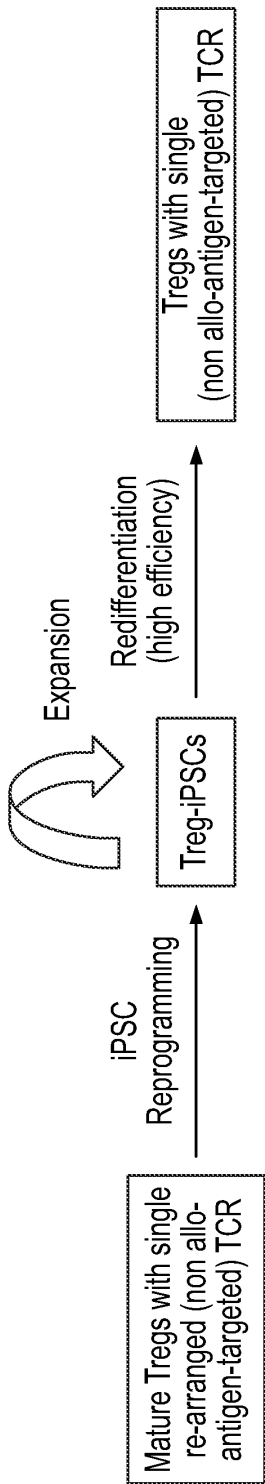


FIG. 7

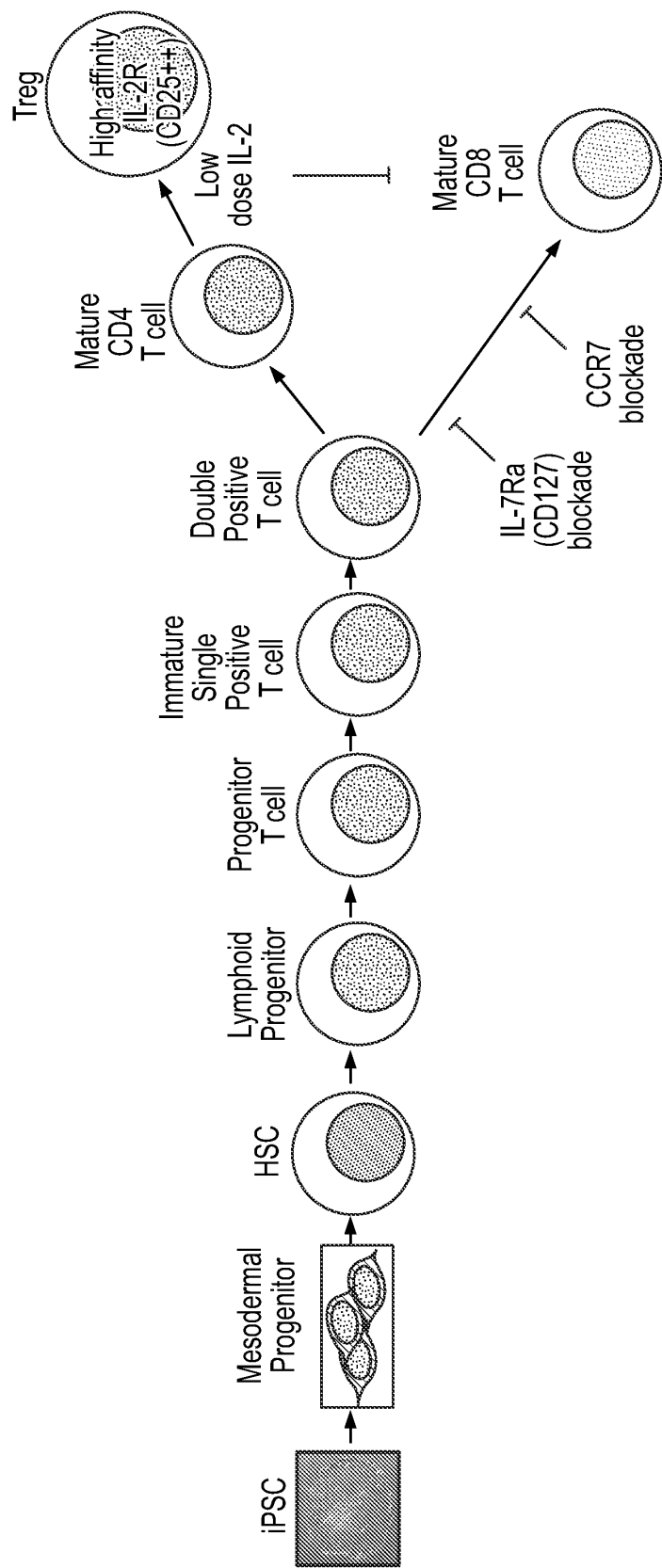


FIG. 8

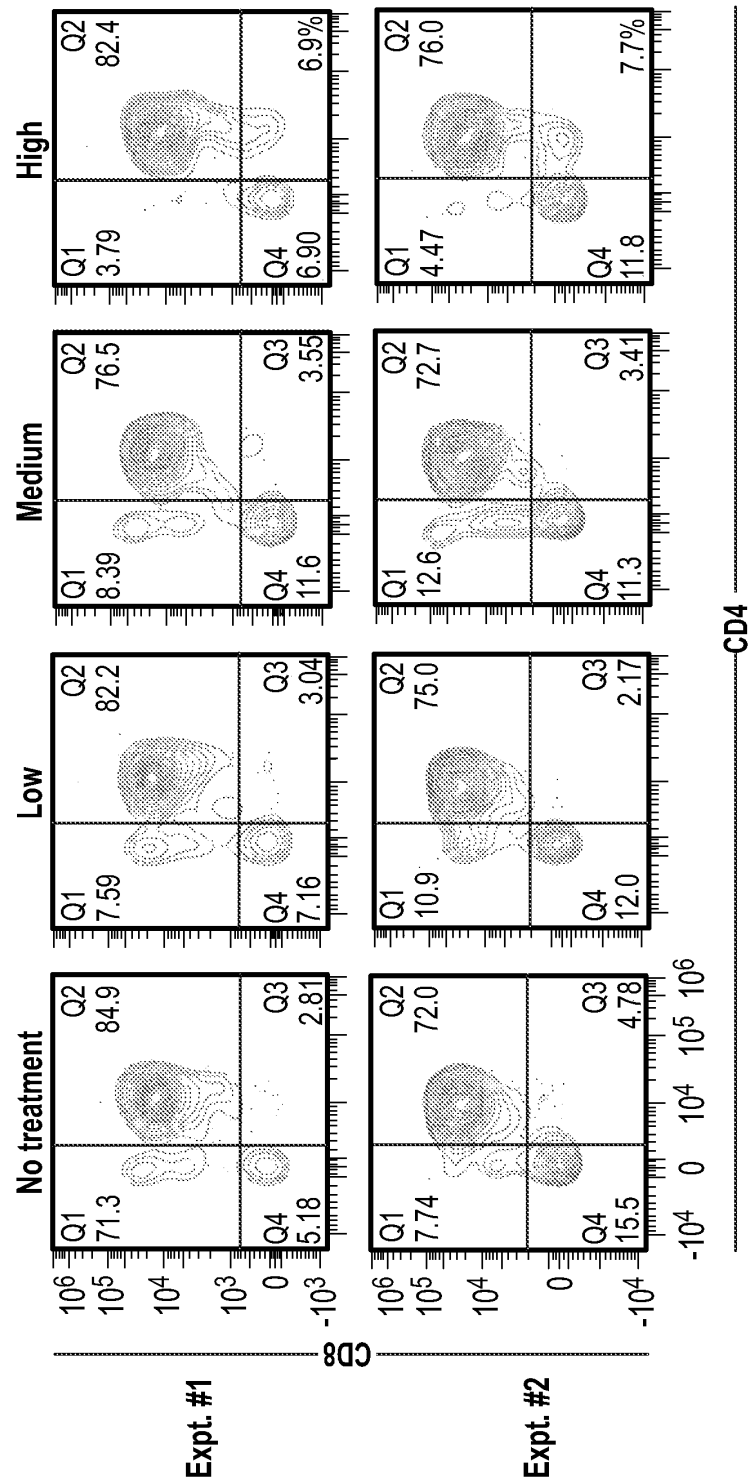


FIG. 9

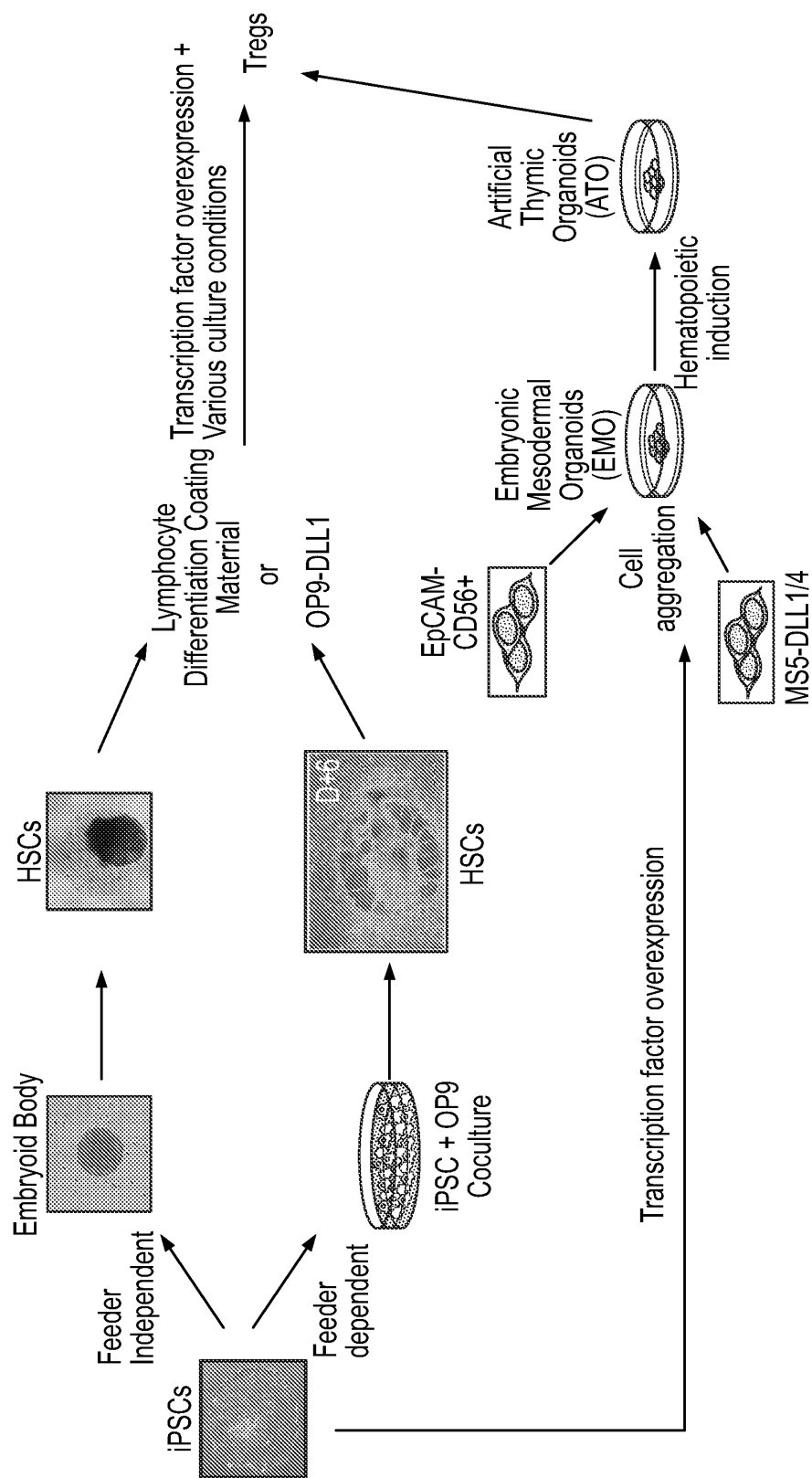


FIG. 10

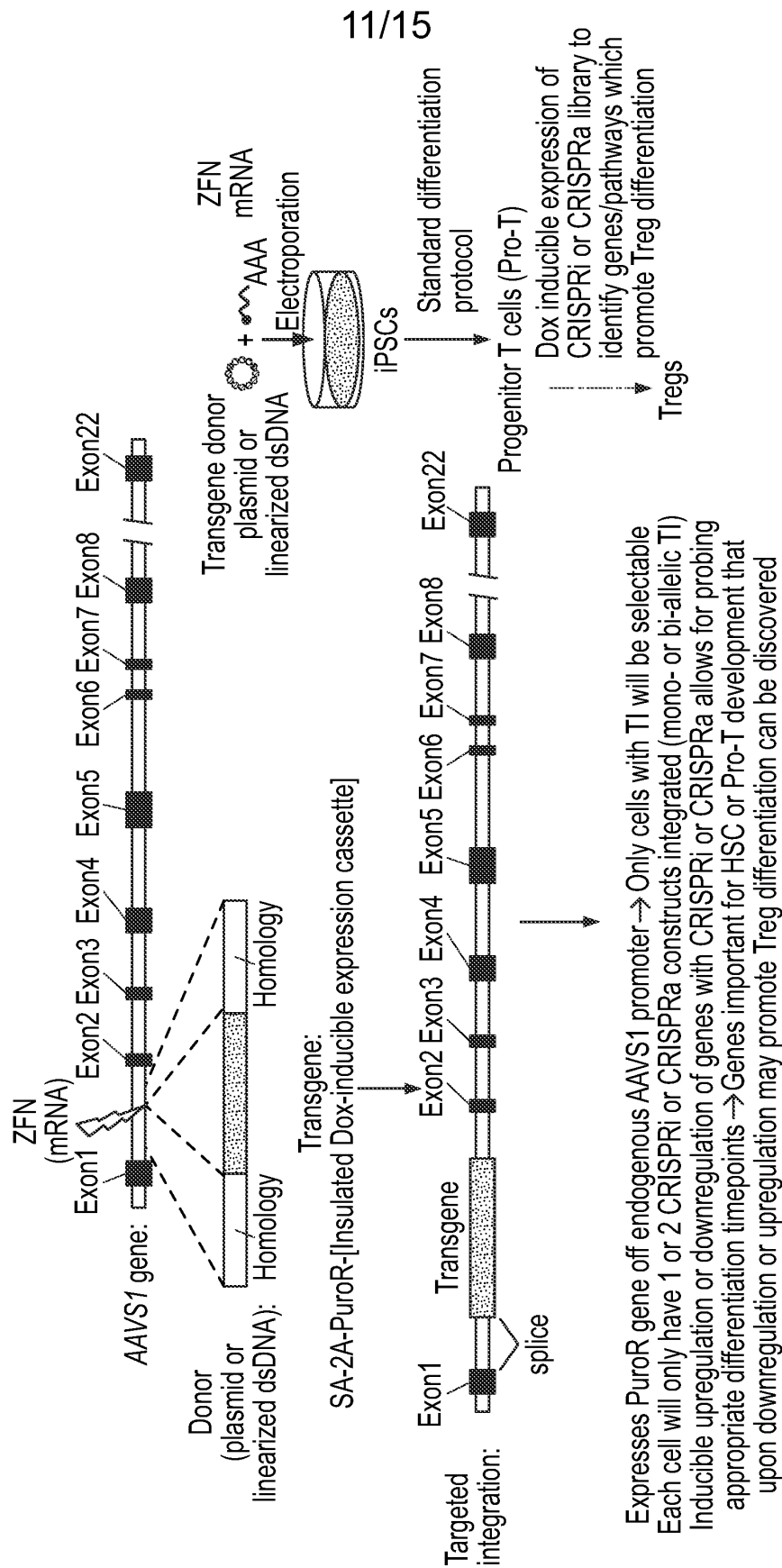


FIG. 11

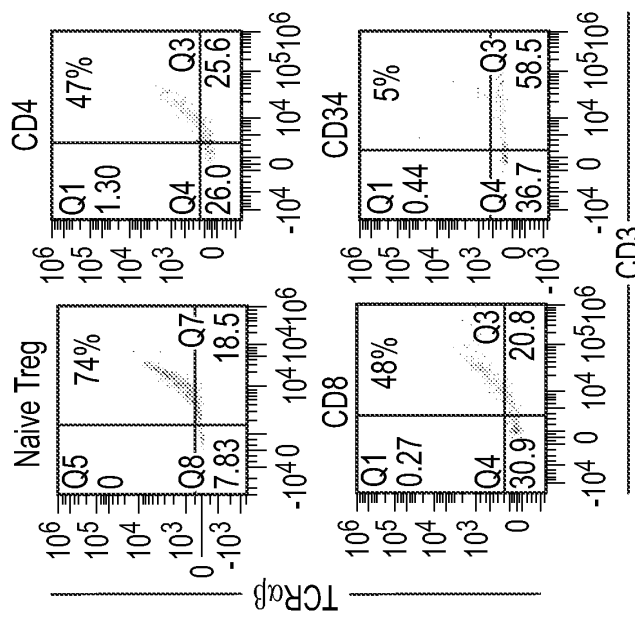


FIG. 12B

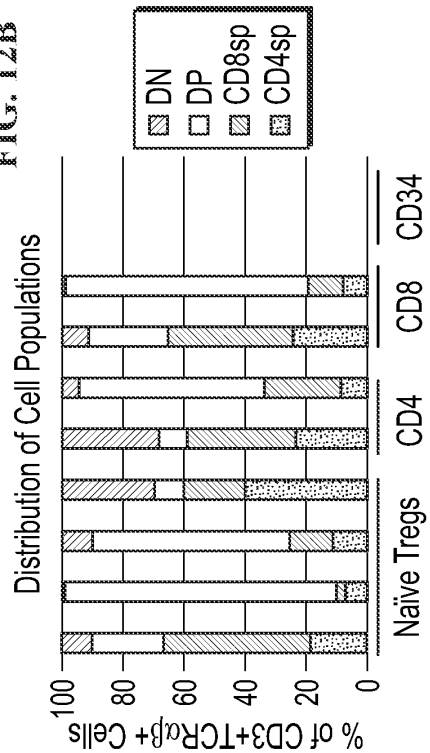


FIG. 12D

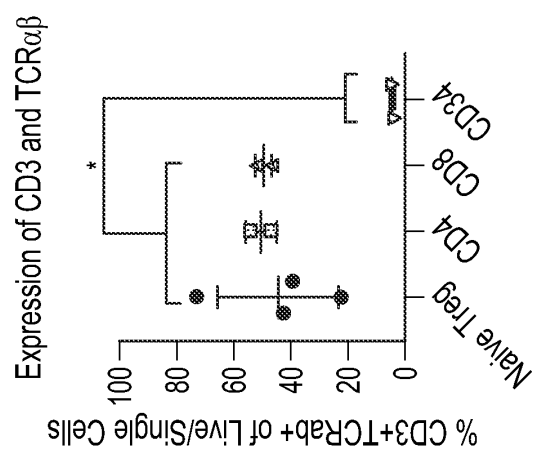


FIG. 12A

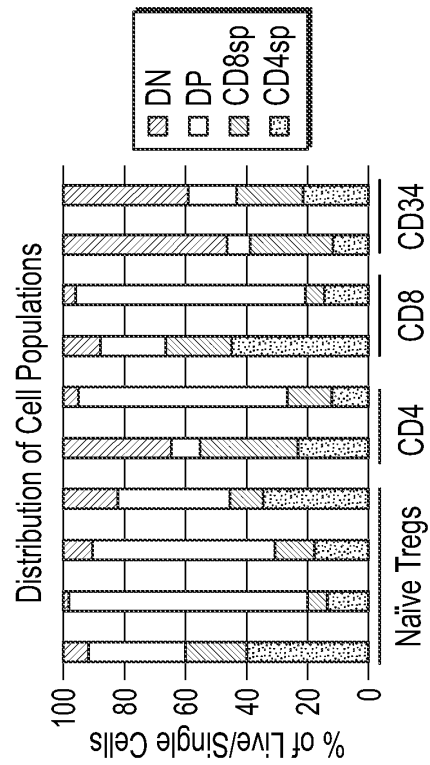


FIG. 12C

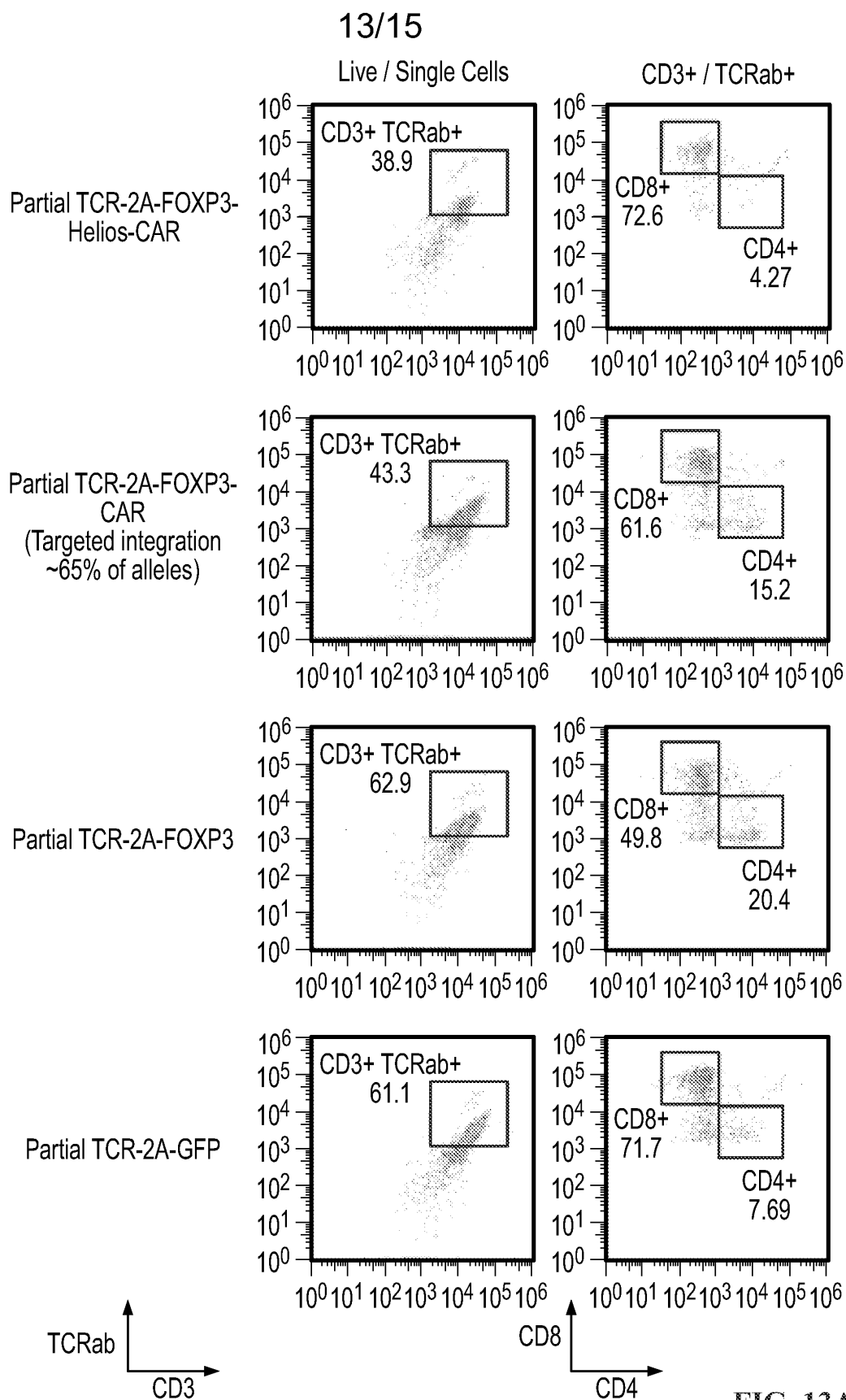
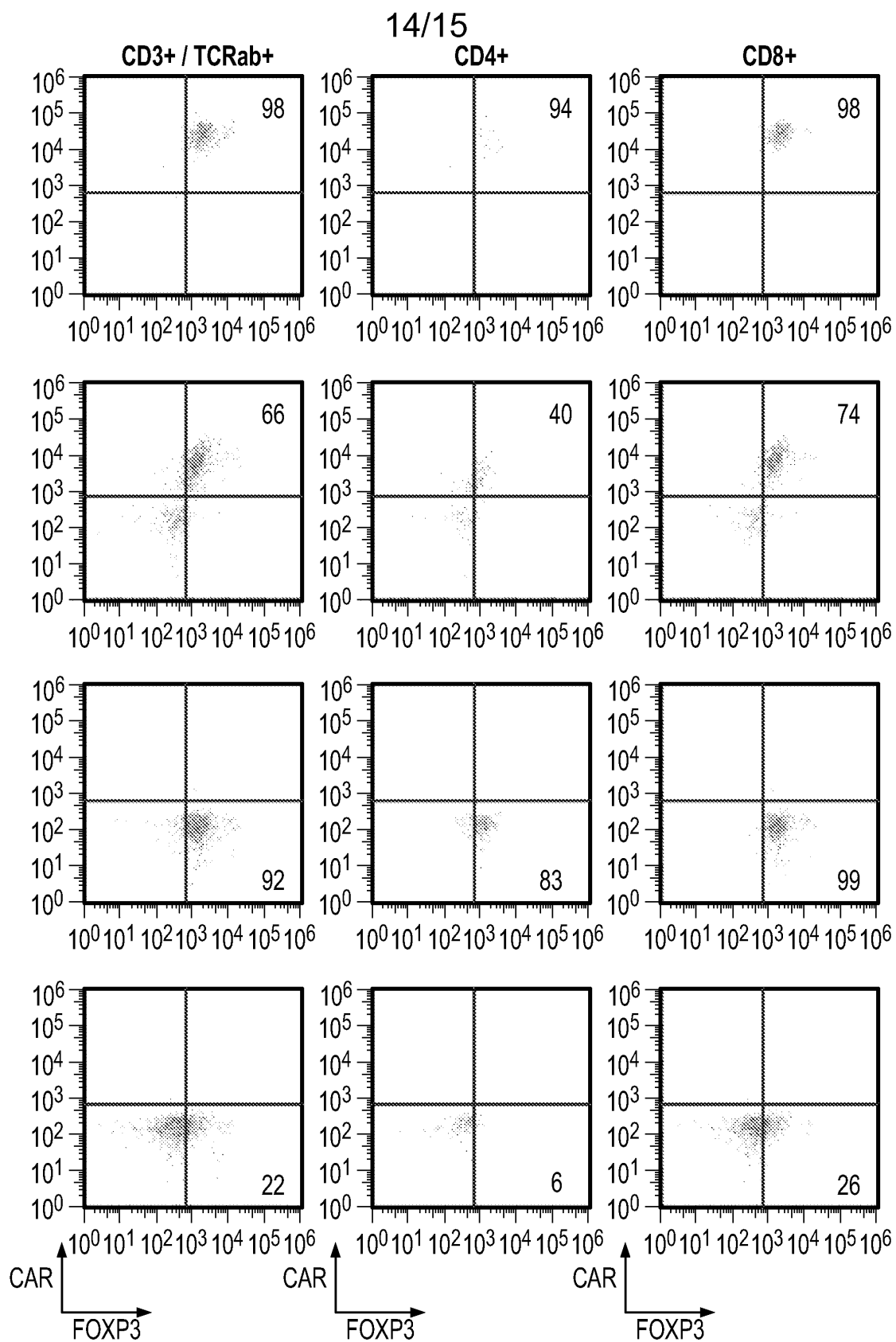


FIG. 13A



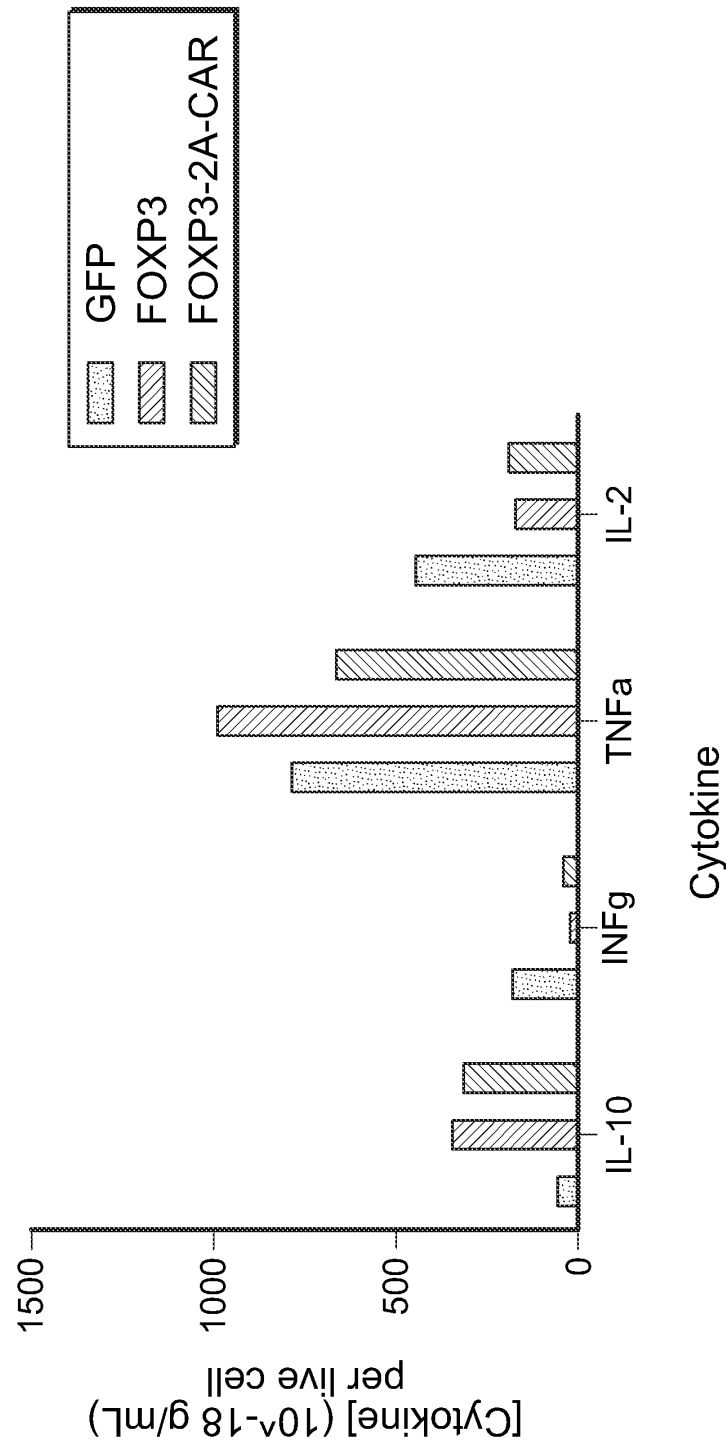


FIG. 13B