

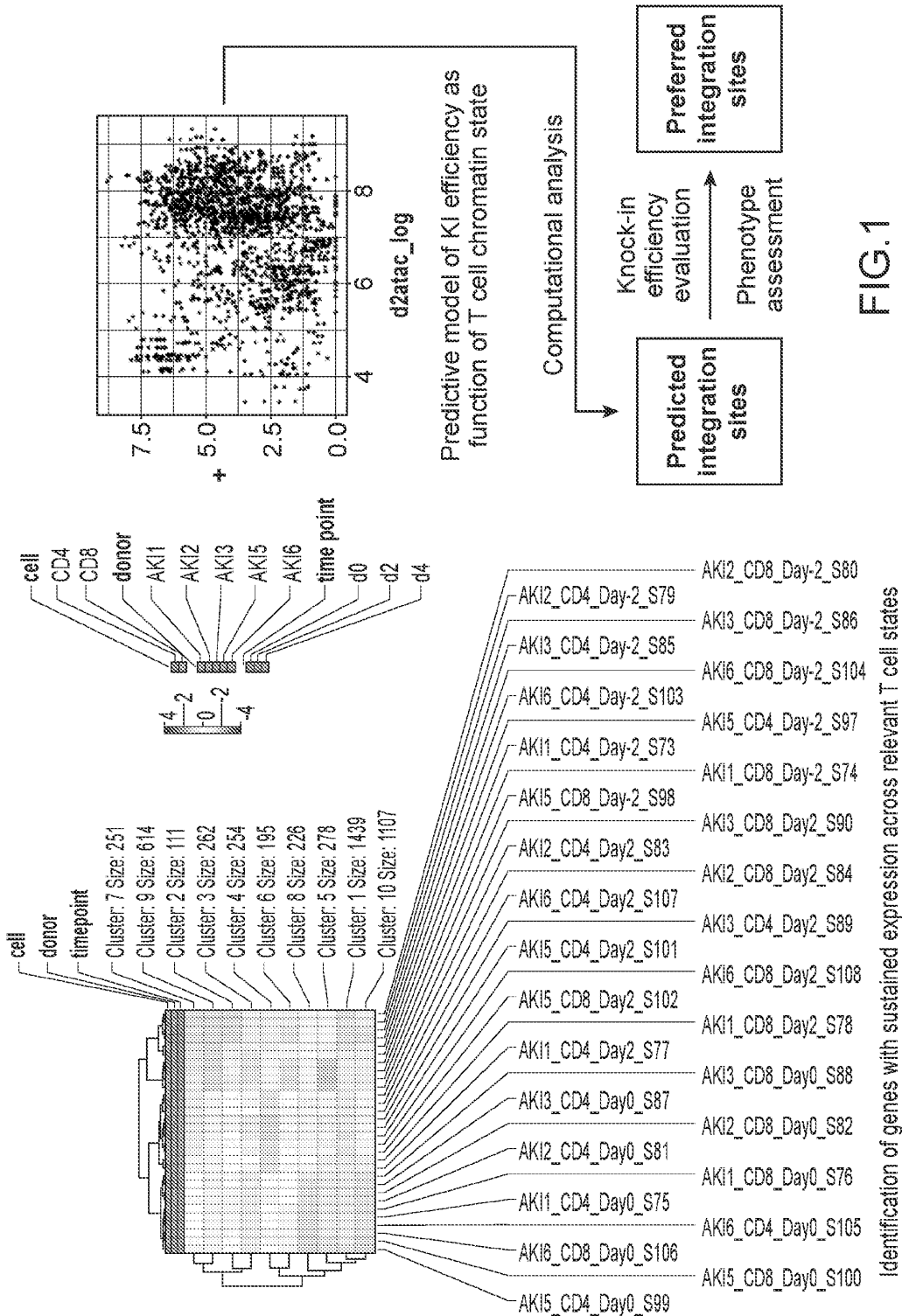


FIG.1

Identification of genes with sustained expression across relevant T cell states

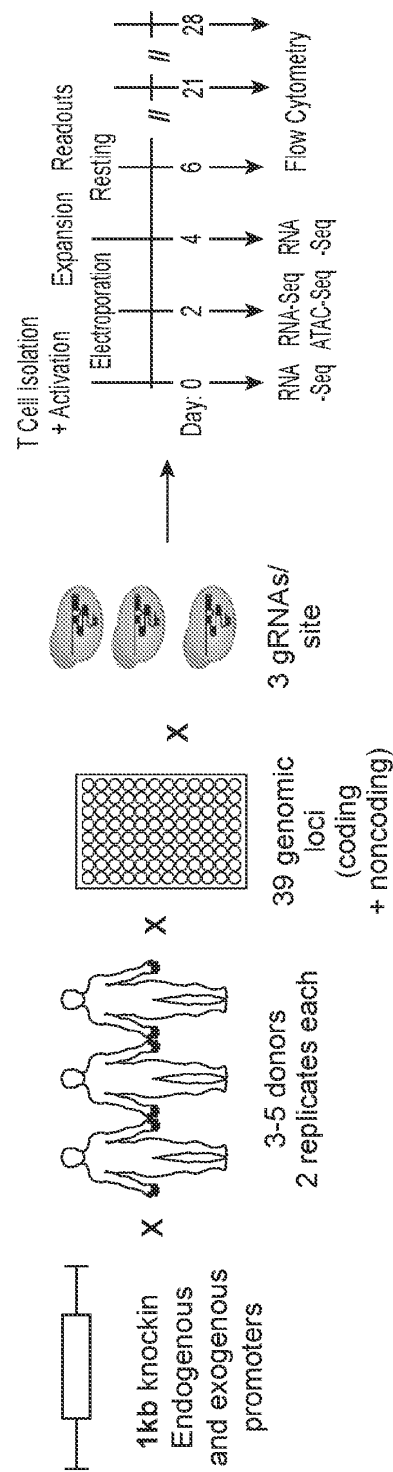
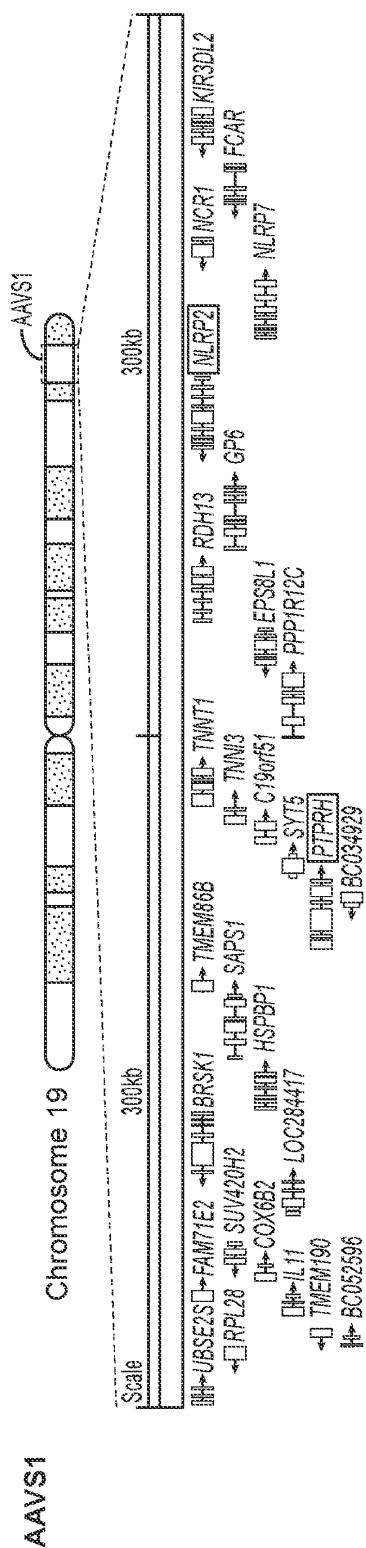
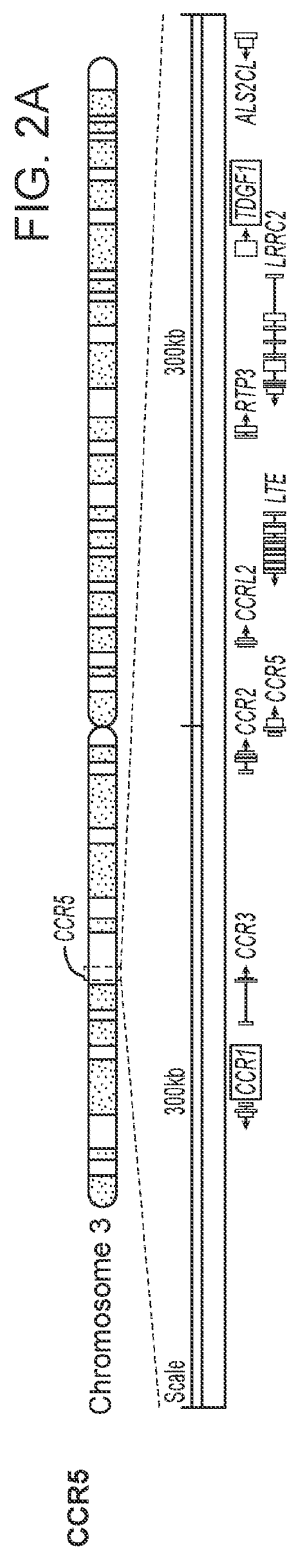
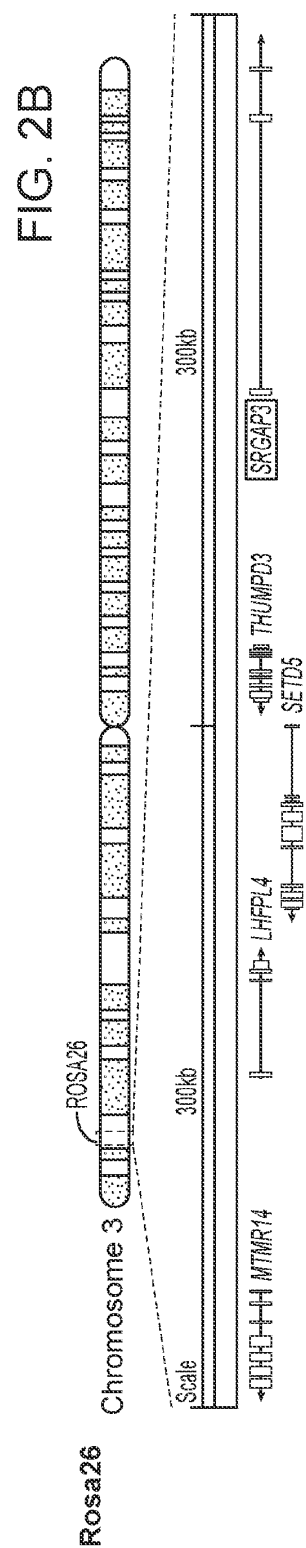
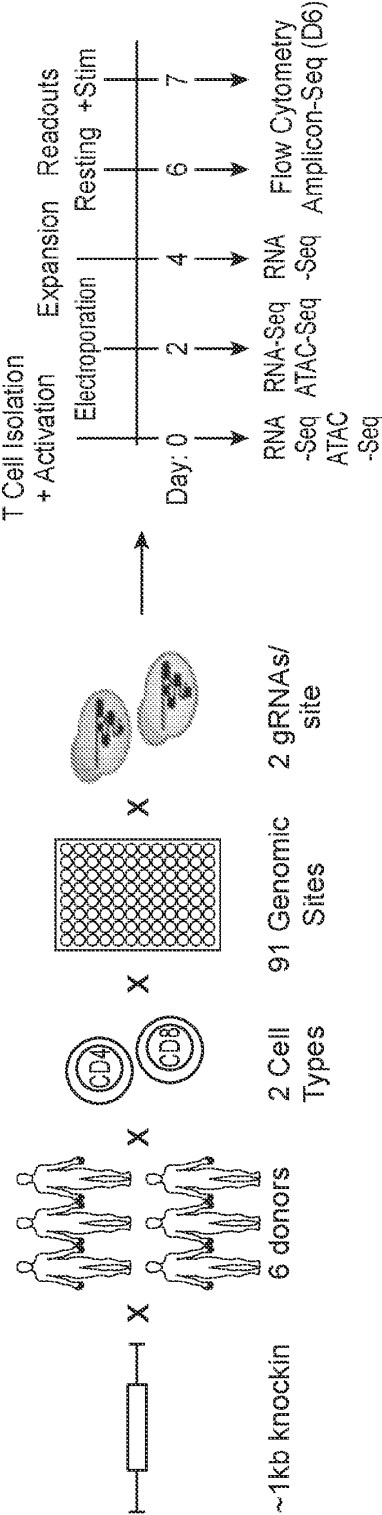


FIG.1 (Cont.)





- Roth, T. L., et al. 2019 data
 - 3 time points (d0, d2, d4), 6 donors, CD4+ and CD8+ cells
 - RNA-seq data
 - ATAC-seq data
 - KI efficiency data
 - sgRNAs editing data (not used for this analysis)

FIG. 3

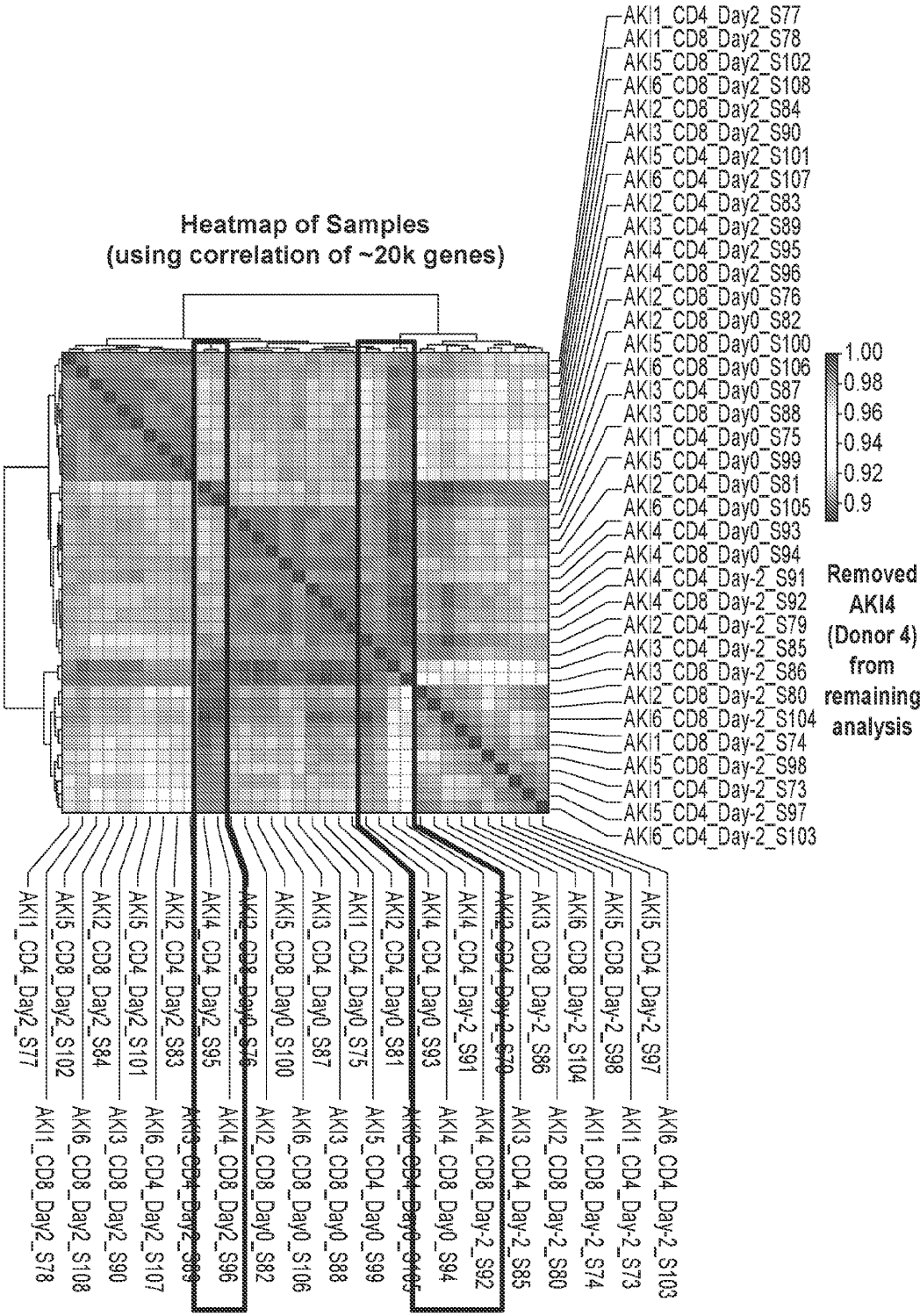


FIG. 4A

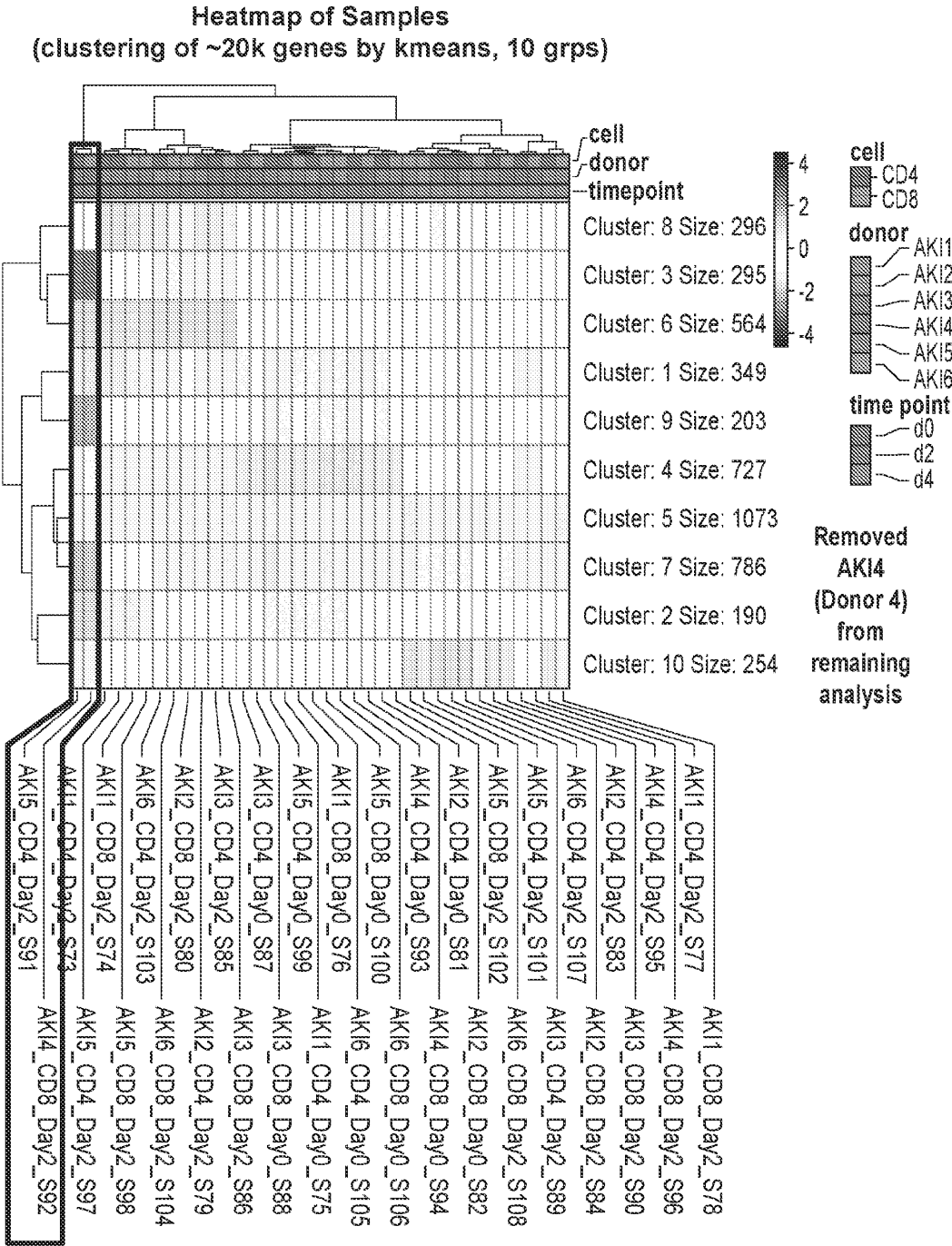


FIG. 4B

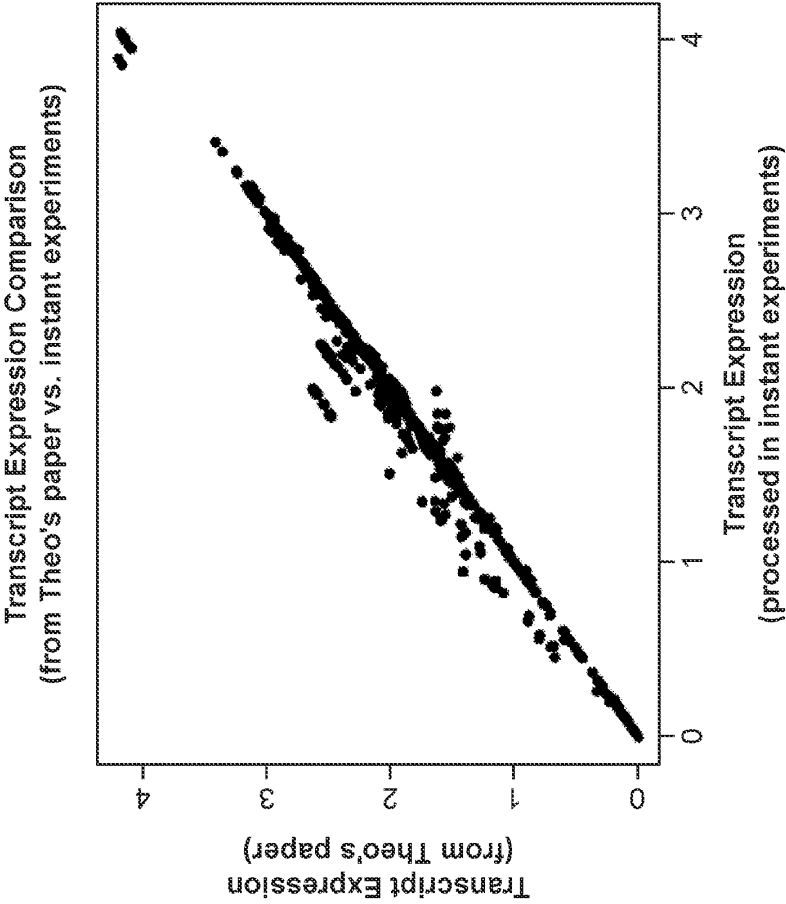


FIG. 5A

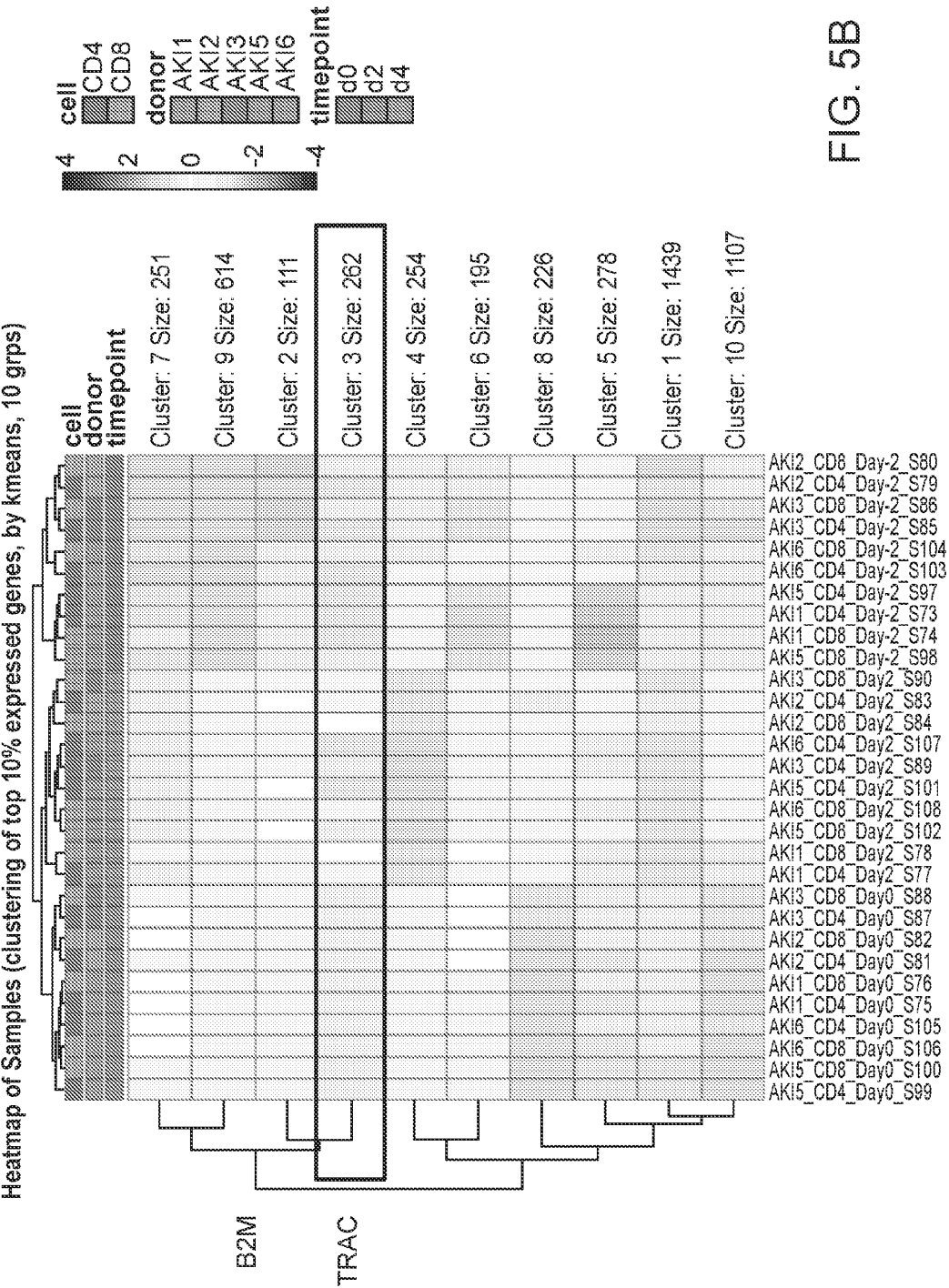


FIG. 5B

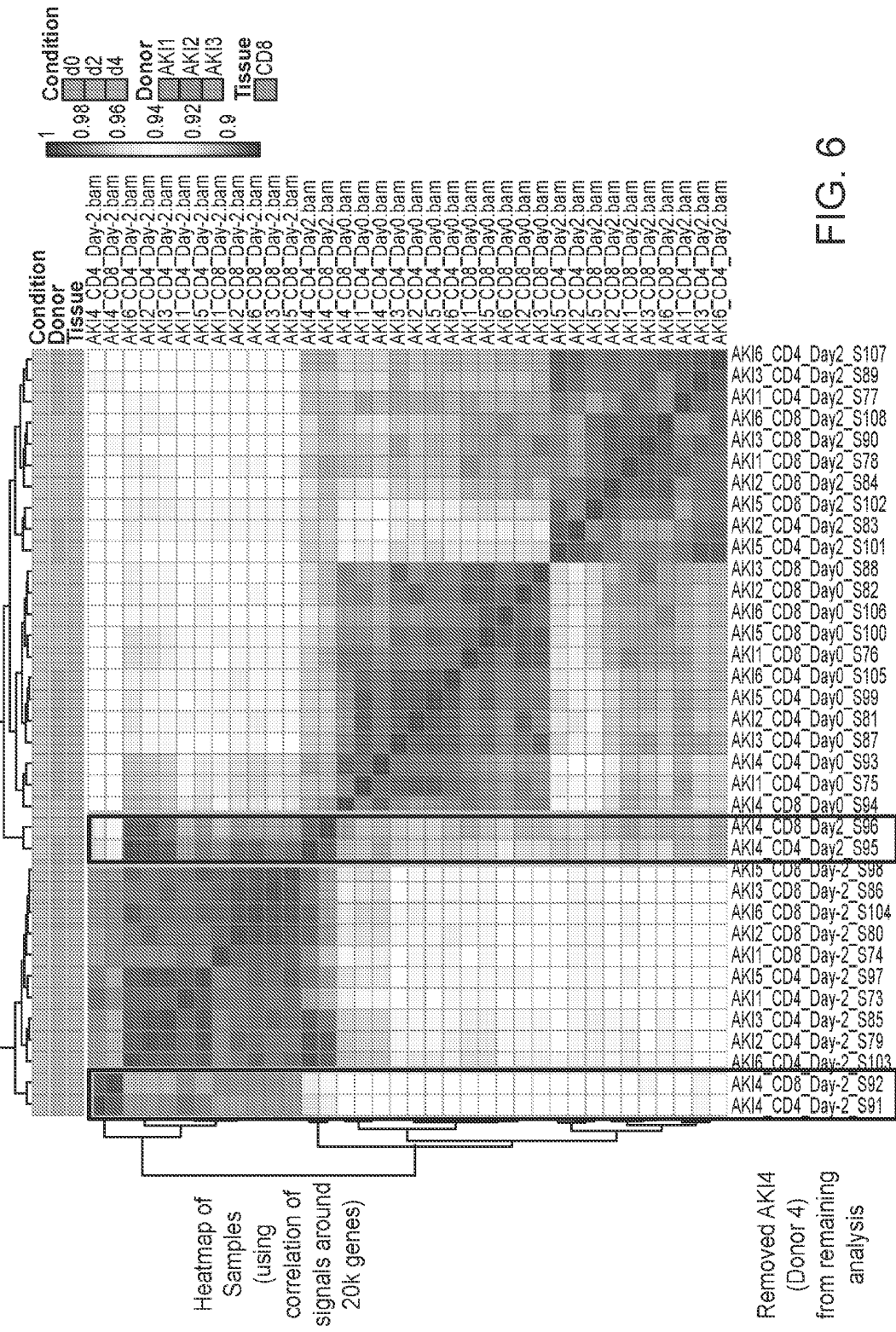
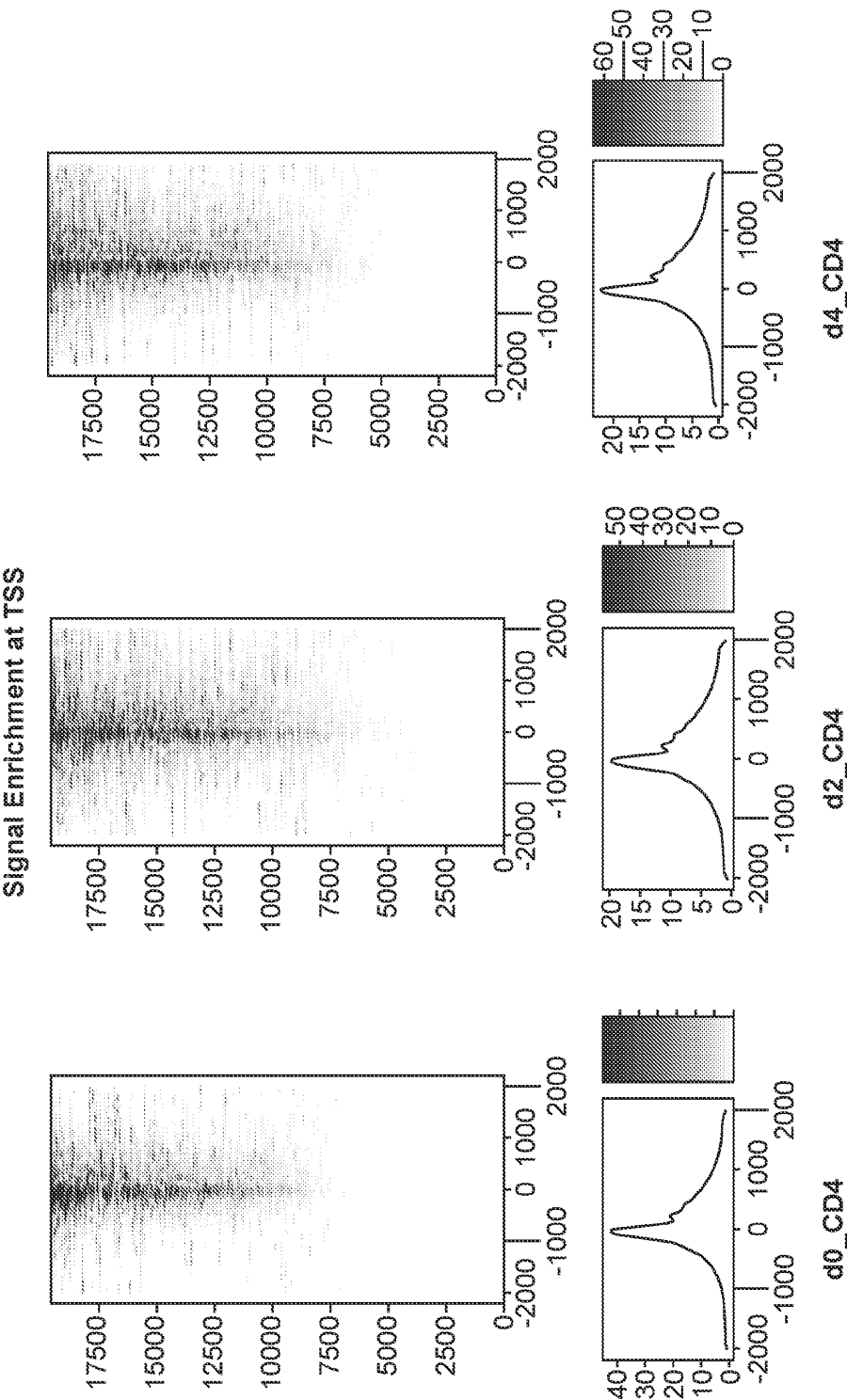


FIG. 6



TSS enrichment is highest at d0 and lowest at d2.
The # of peaks detected is the highest at d2

FIG. 7A

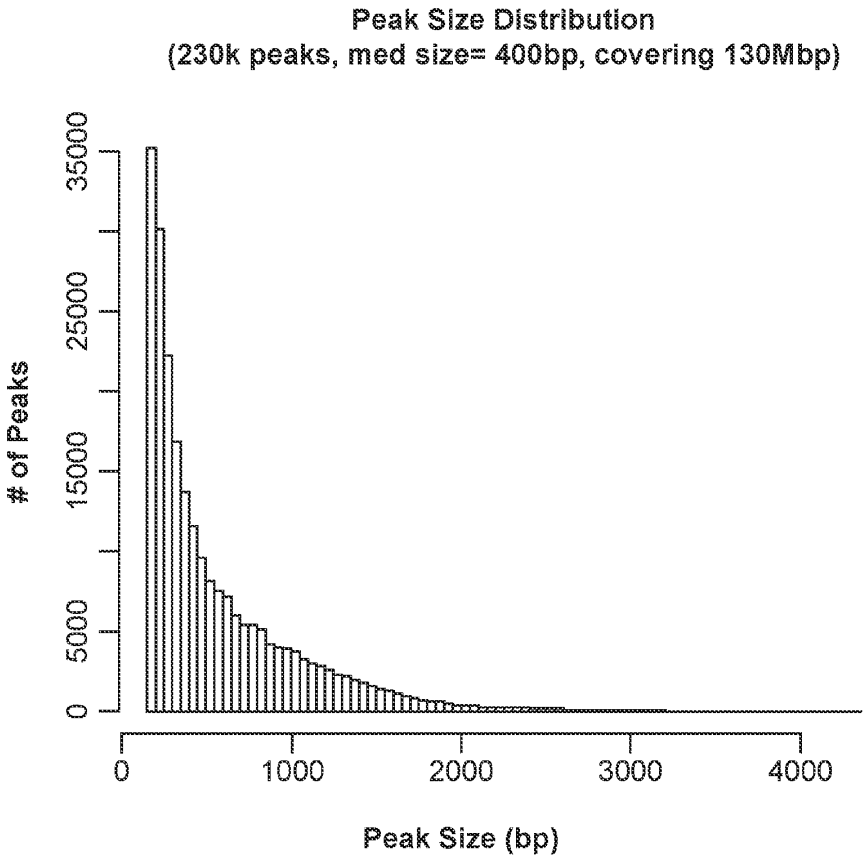


FIG. 7B

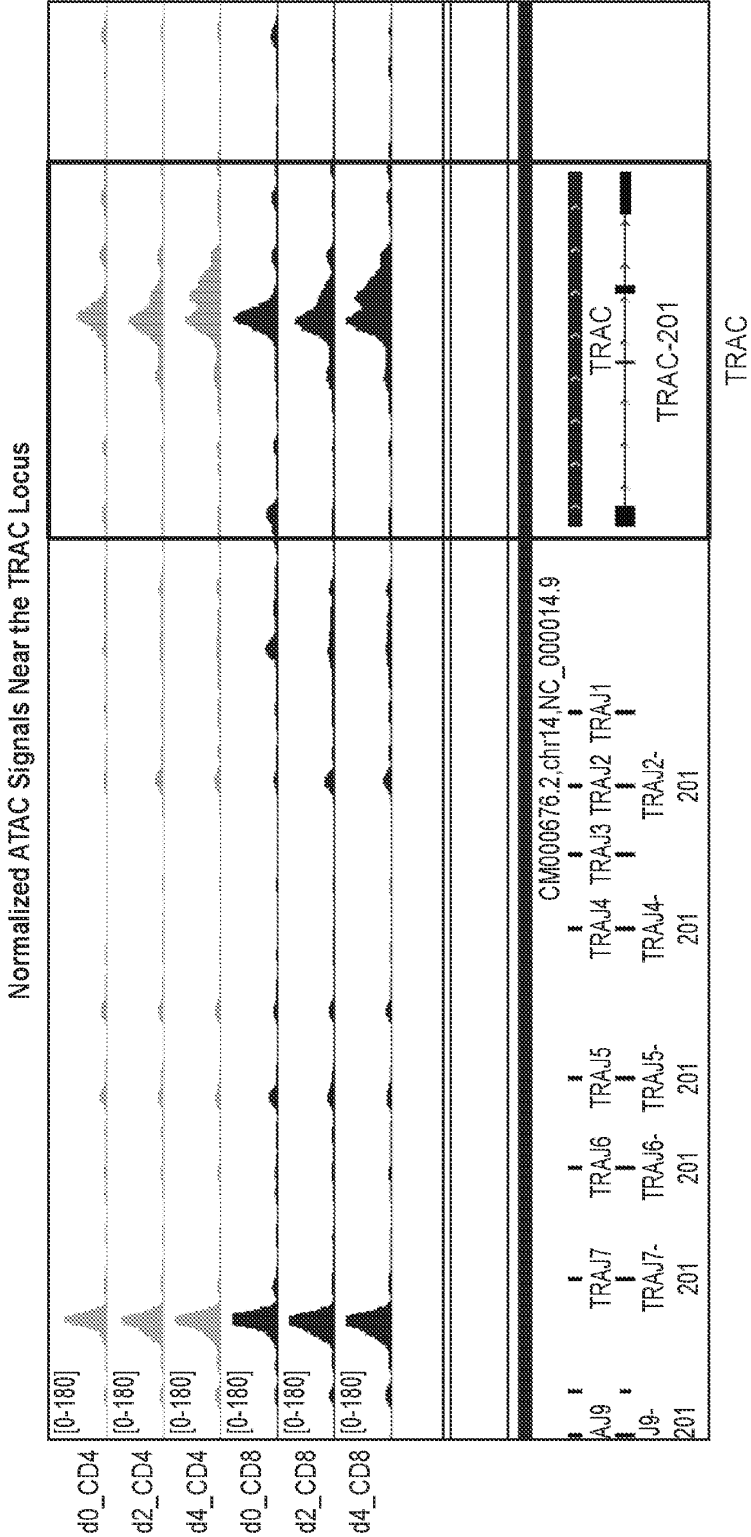
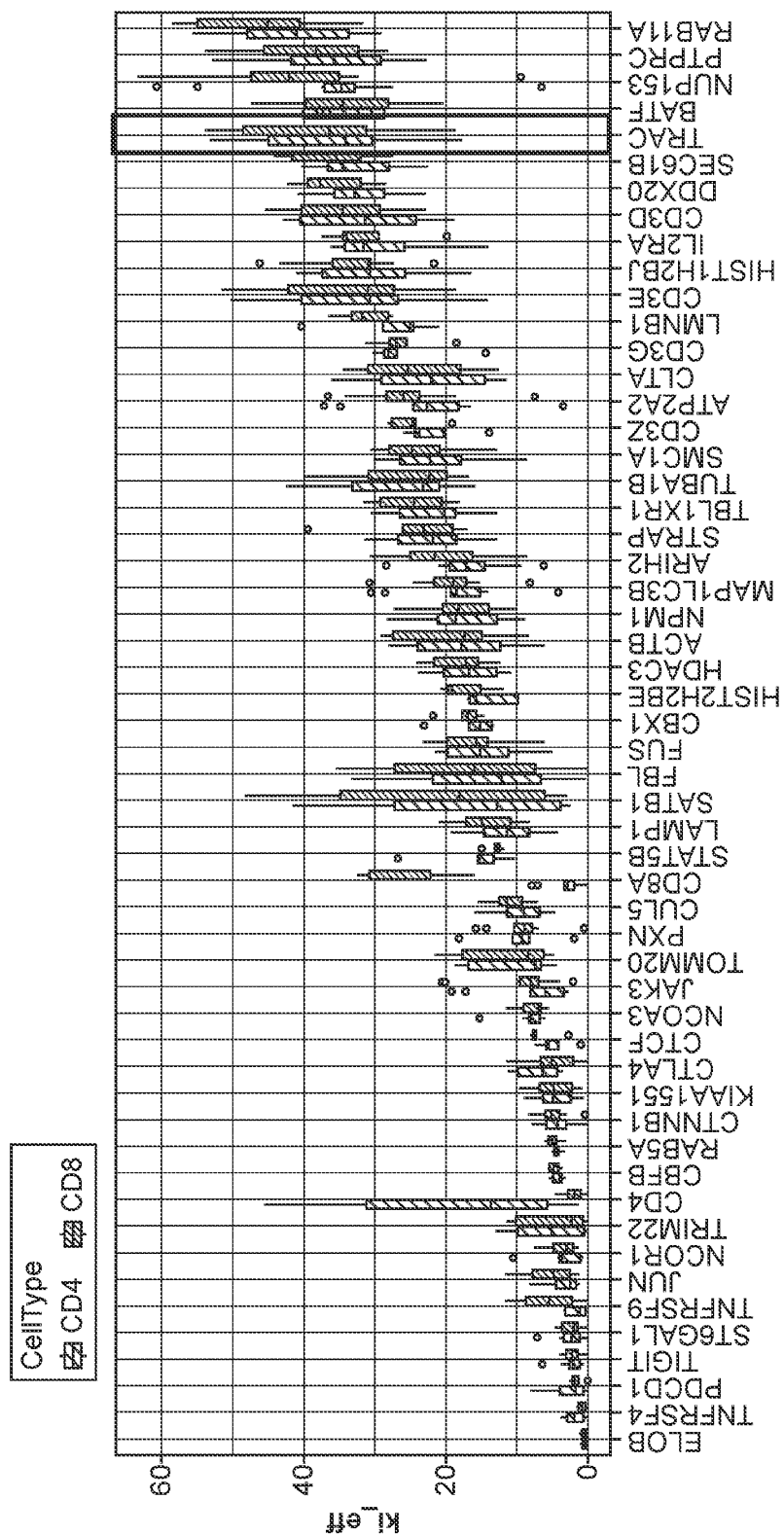


FIG. 8



9
G
L

Knock-In Efficiency with tNGFR reporter (5 donors, 2 gRNAs and 2 replicates/gRNAs)

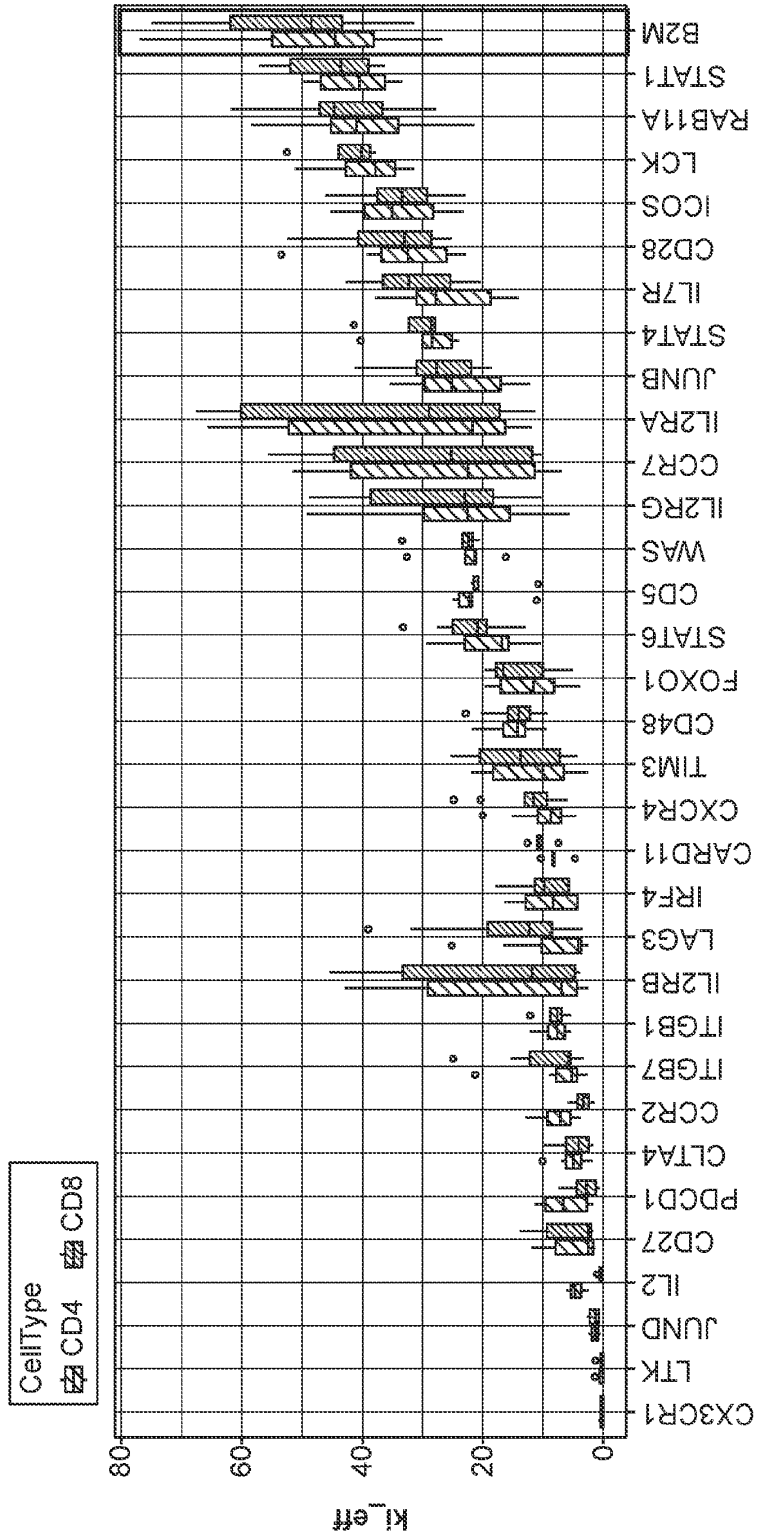


FIG. 10

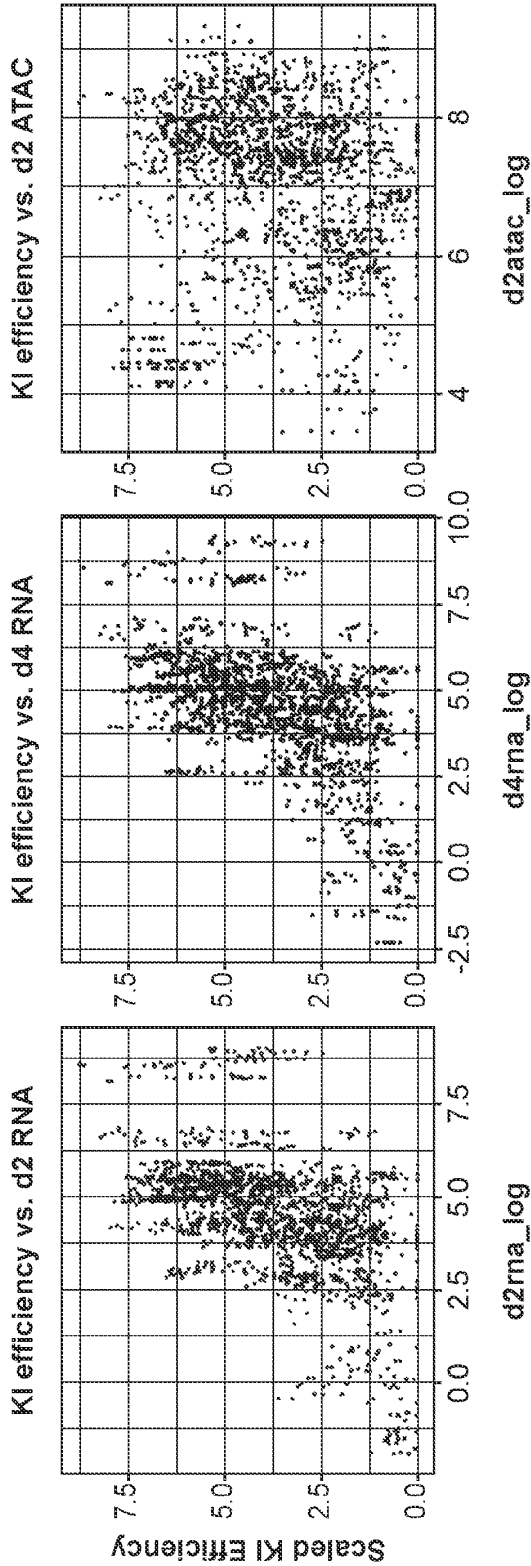


FIG. 11

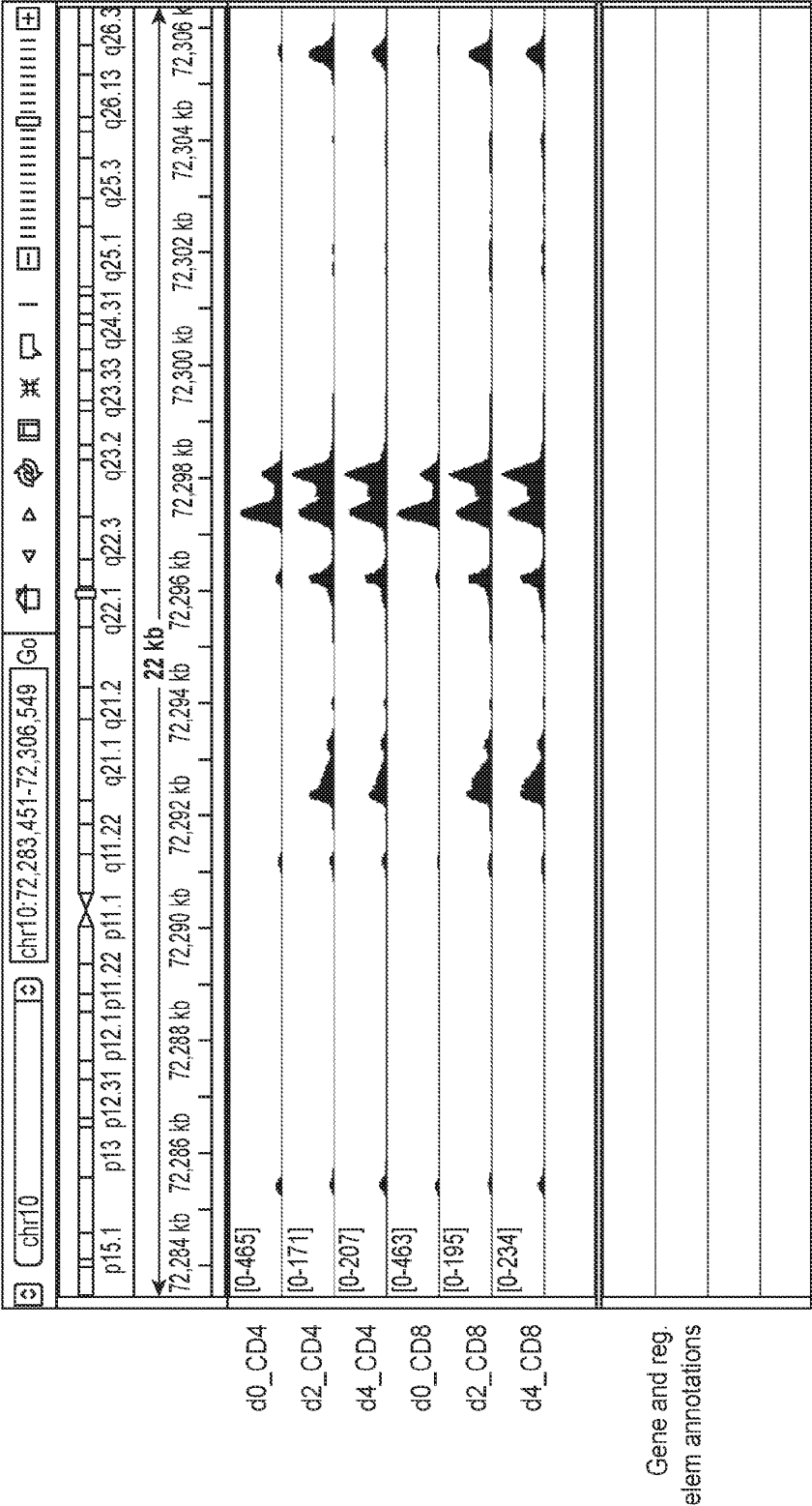


FIG. 12

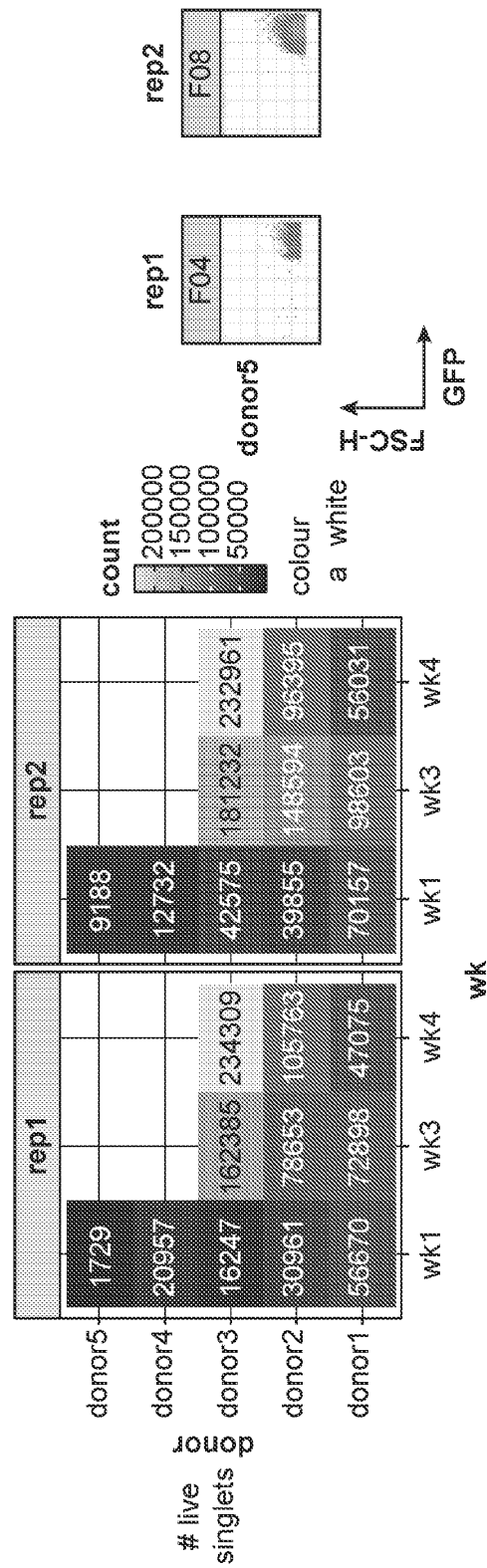


FIG. 13A

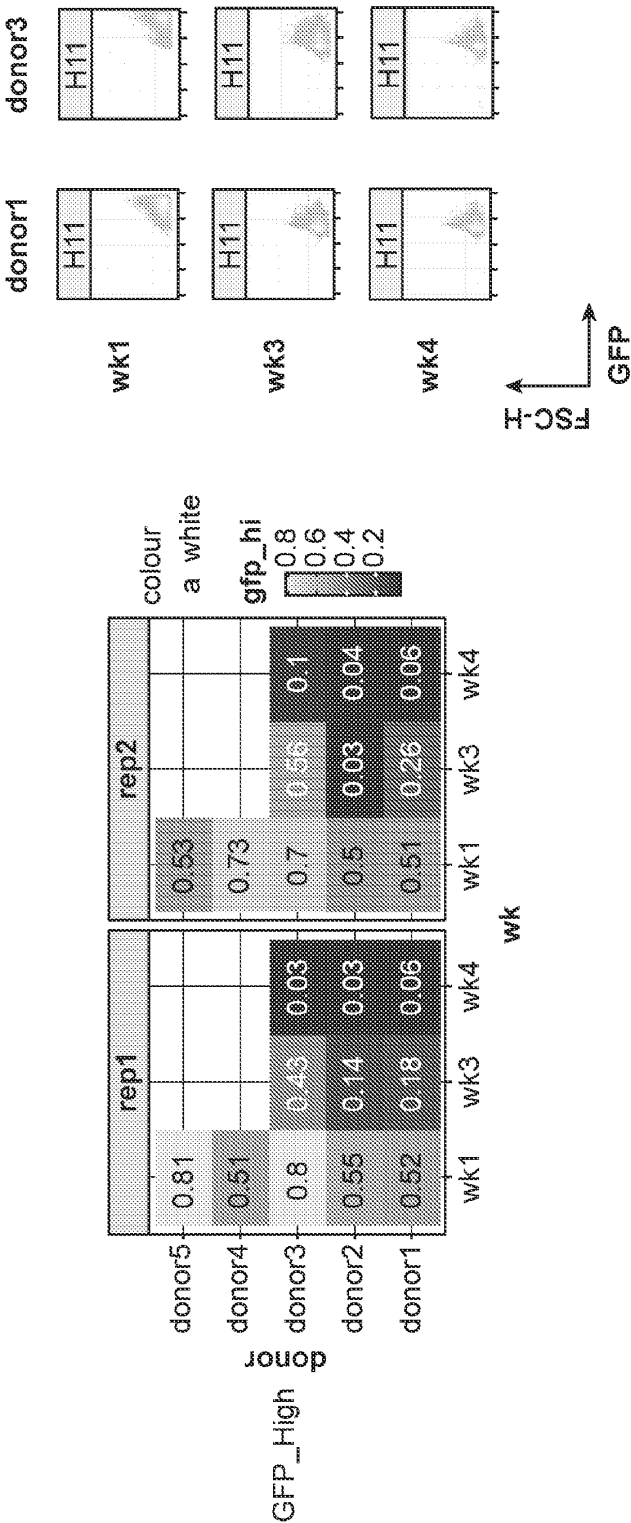


FIG. 13B

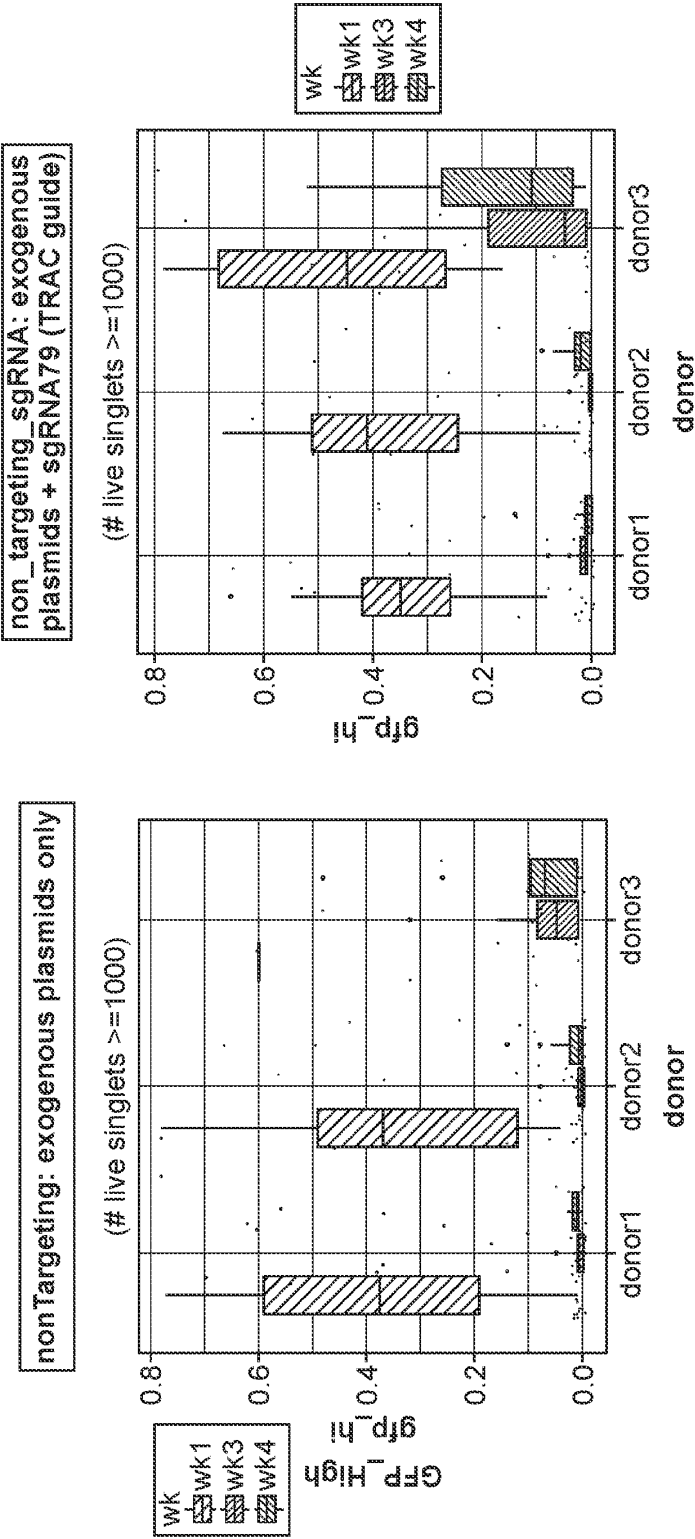


FIG. 14A

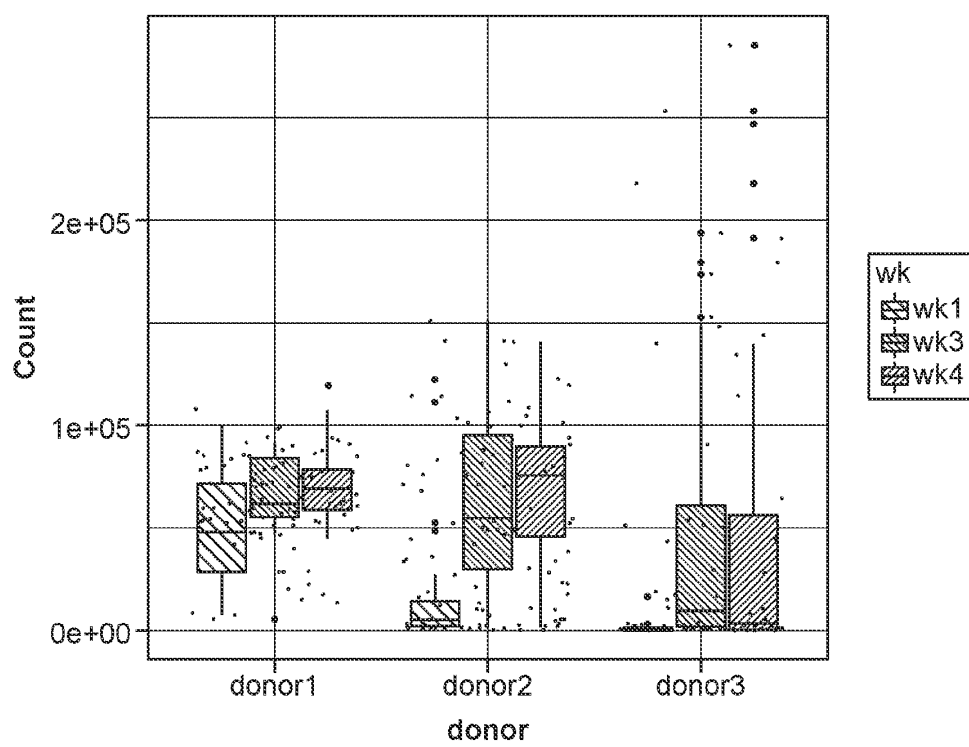


FIG. 14B

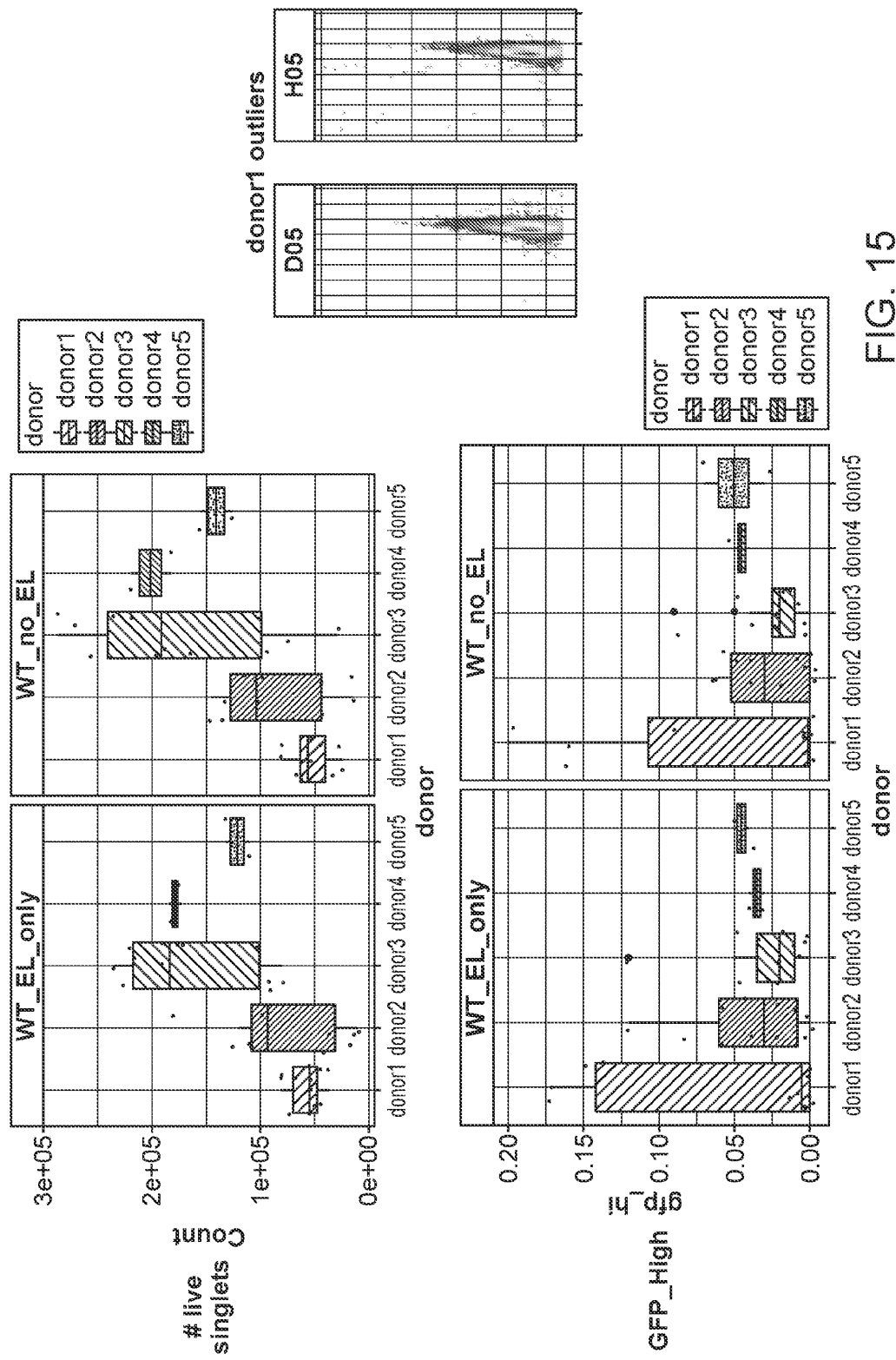


FIG. 15

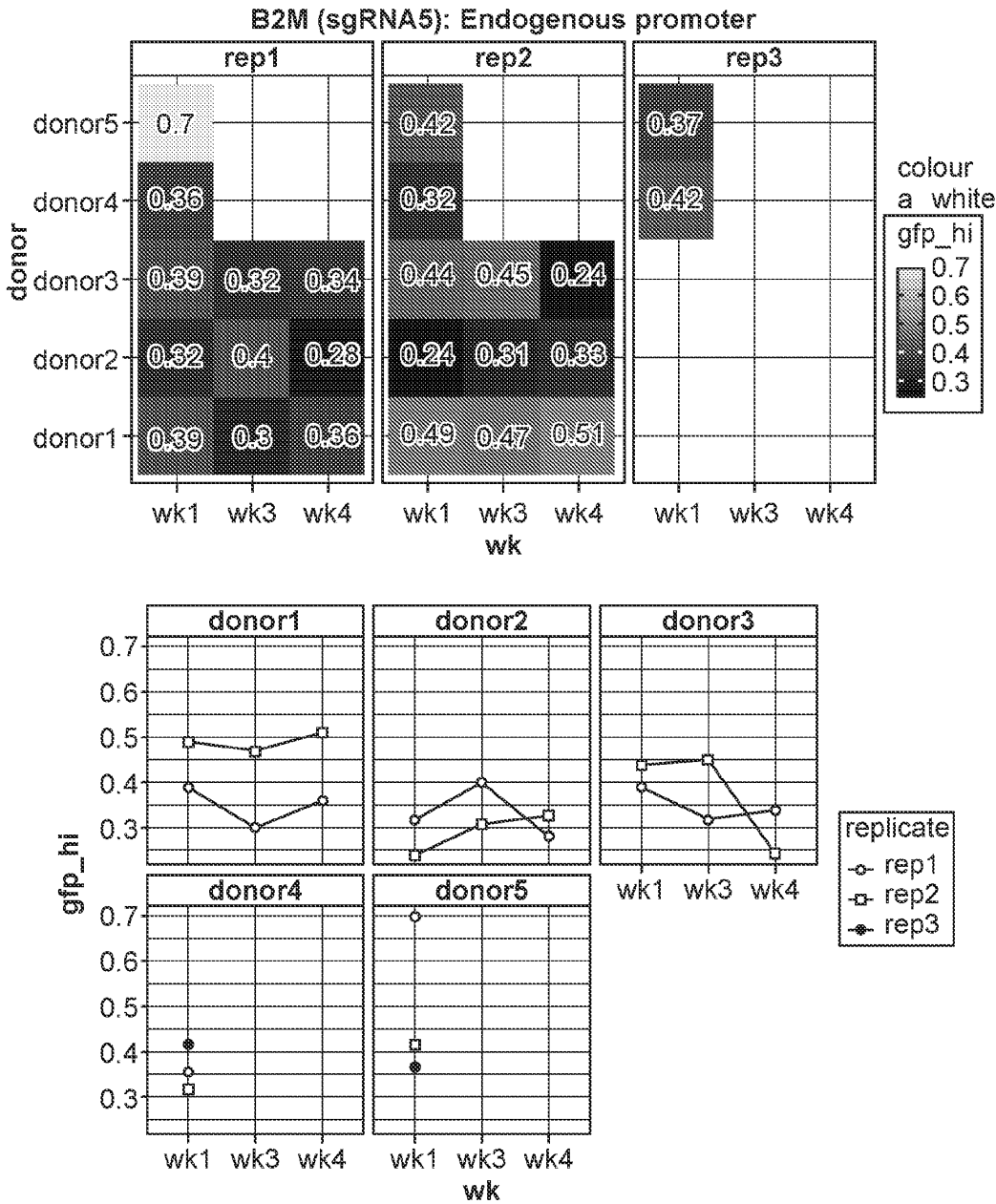
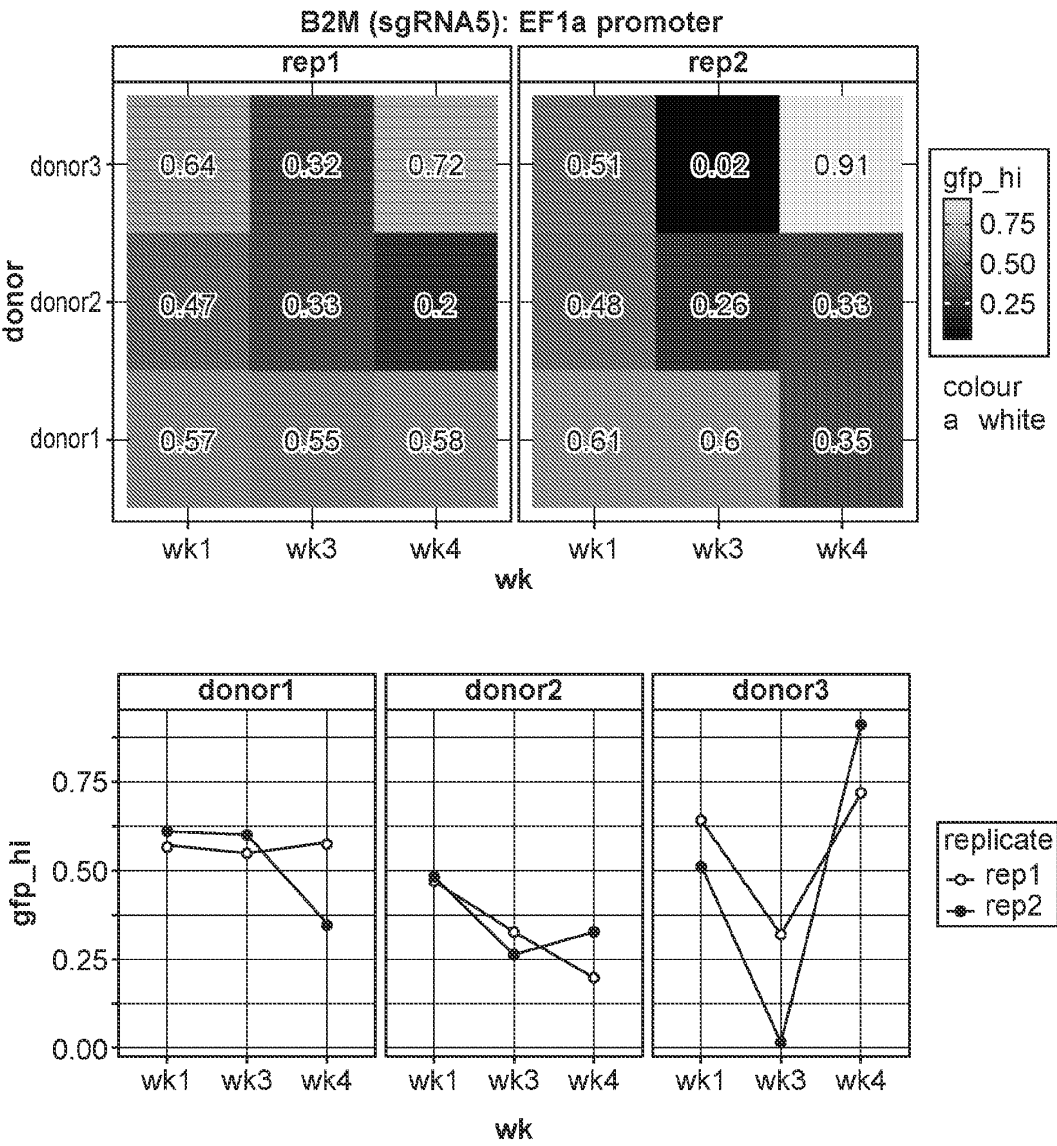


FIG. 16



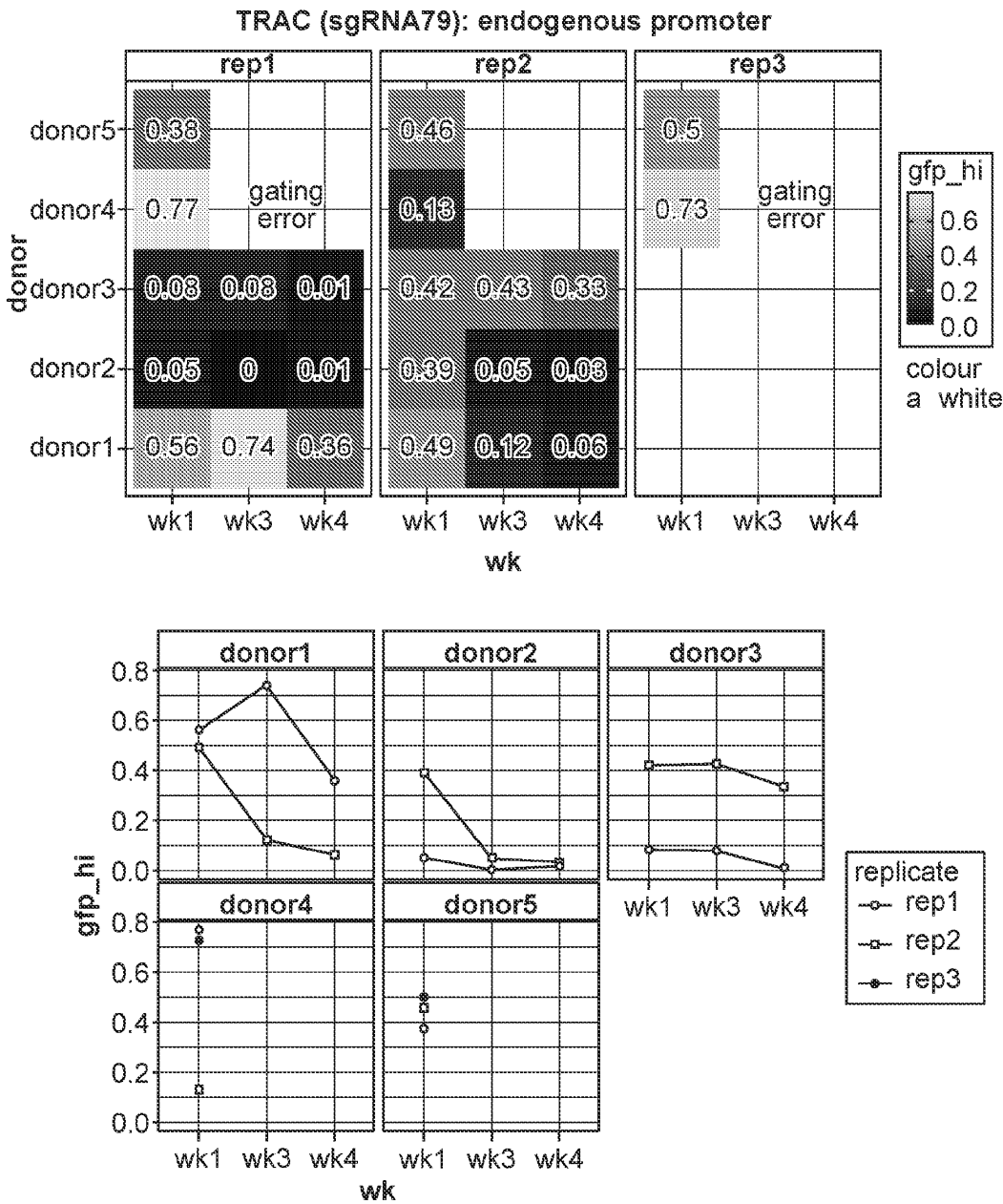


FIG. 18

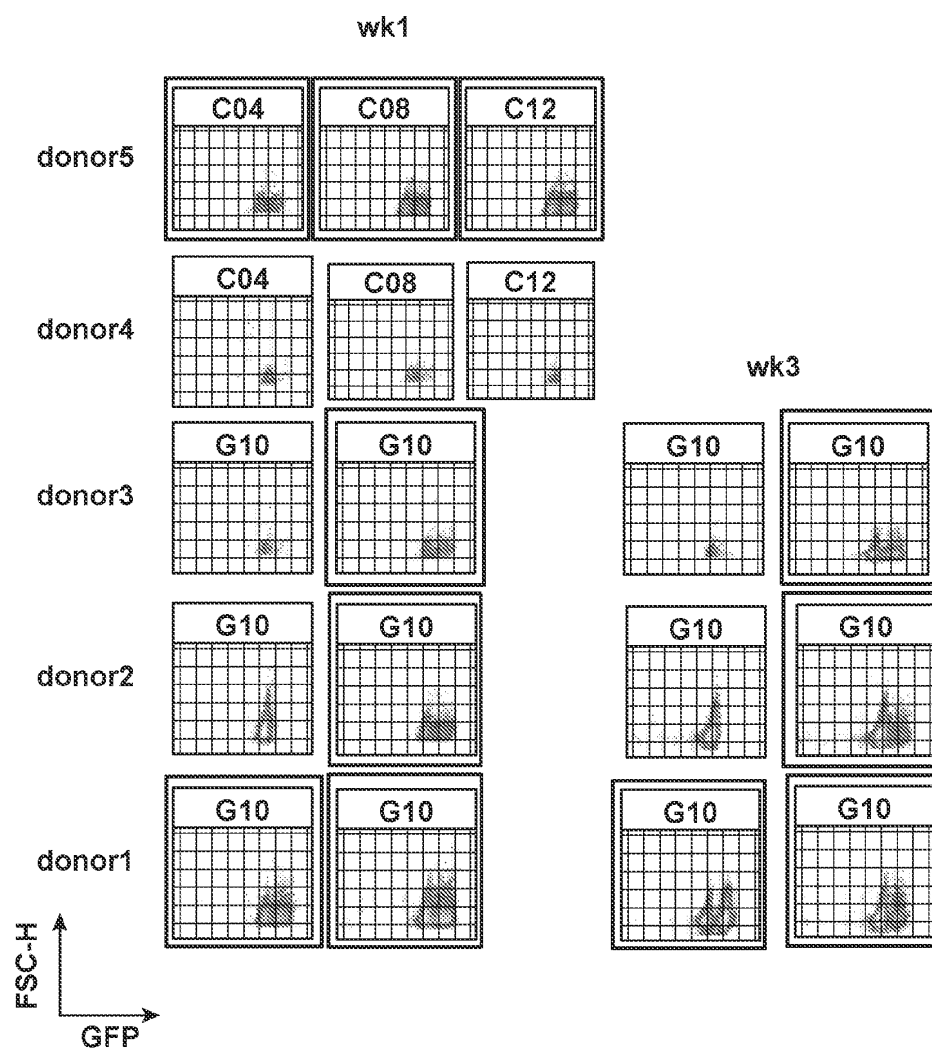


FIG. 18 (Cont.)

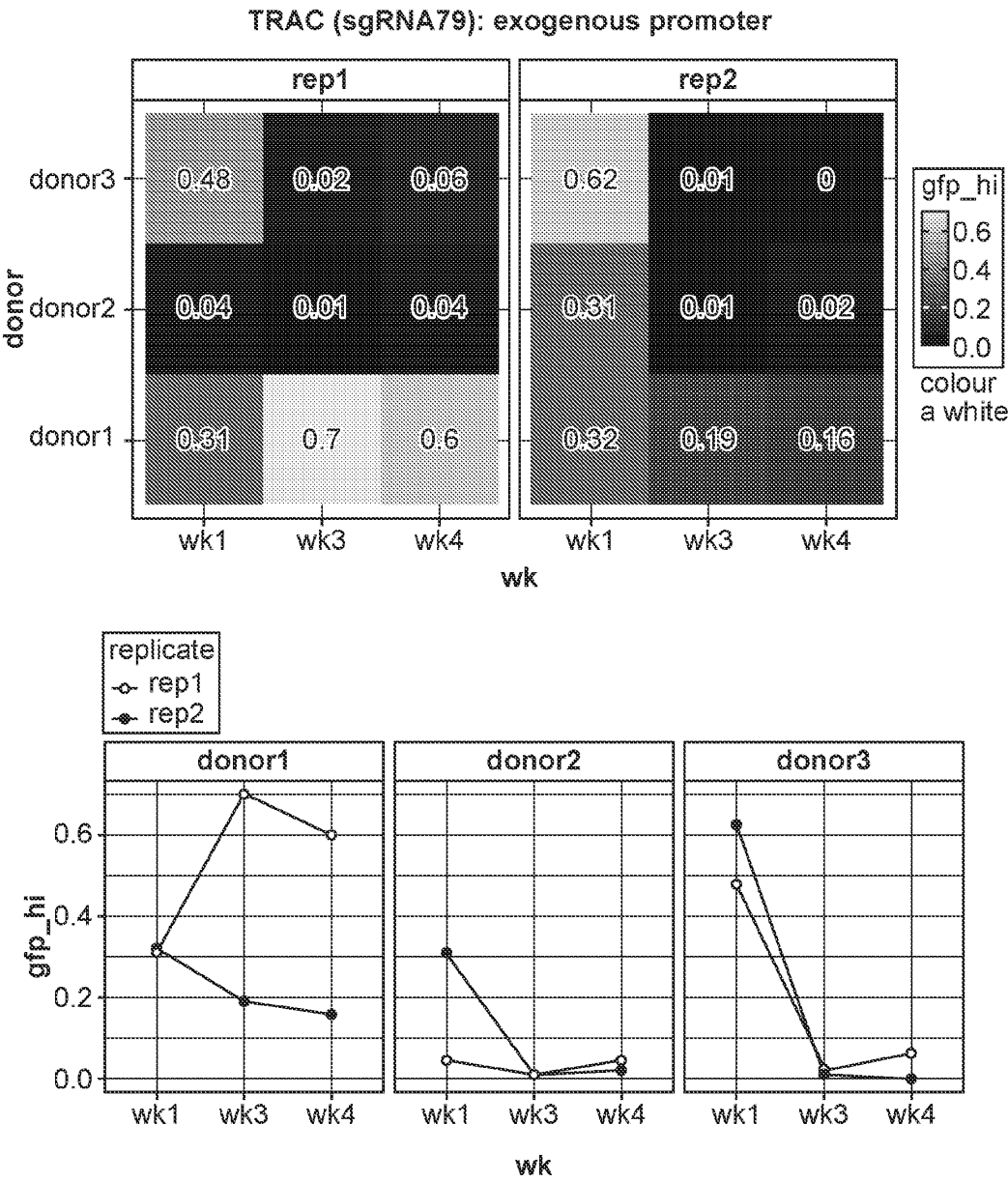
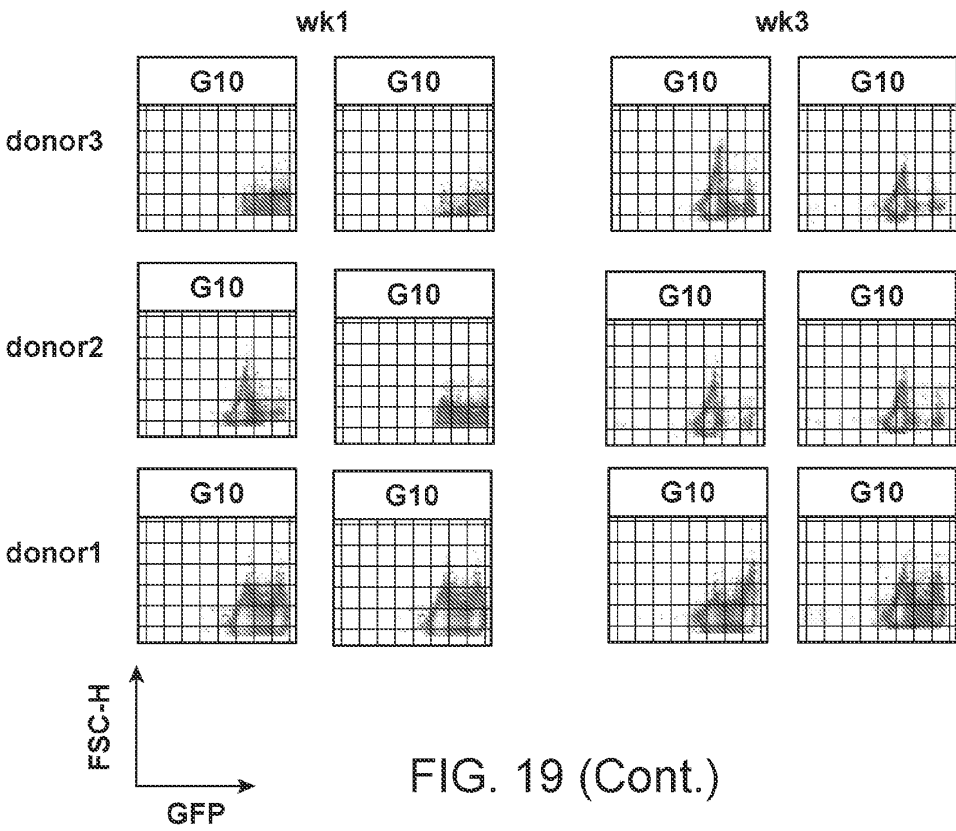


FIG. 19



sgRNA_79 (Endogenous): TCR vs GFP among all donors and time points

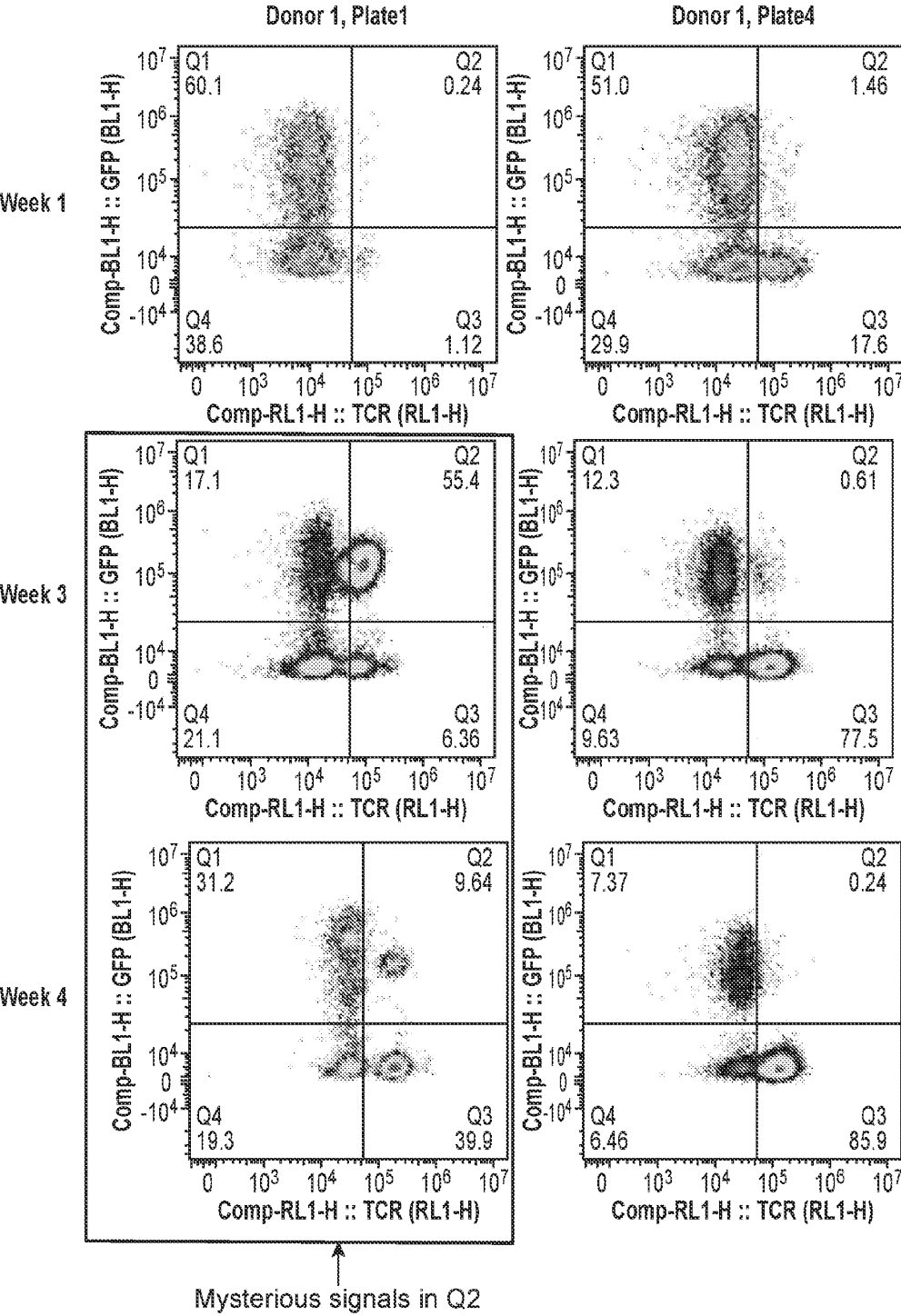


FIG. 20

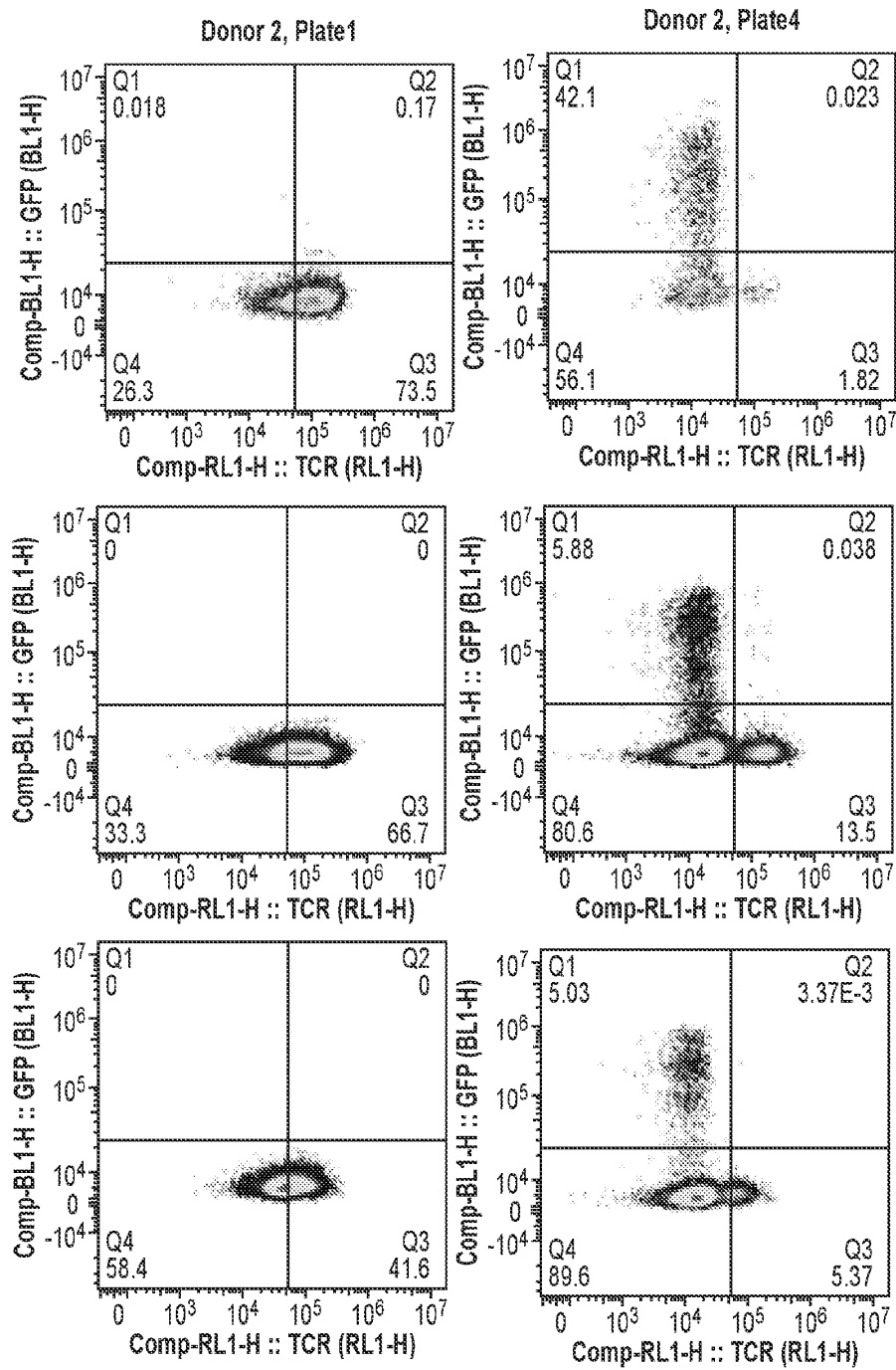


FIG. 20 (Cont. 1)

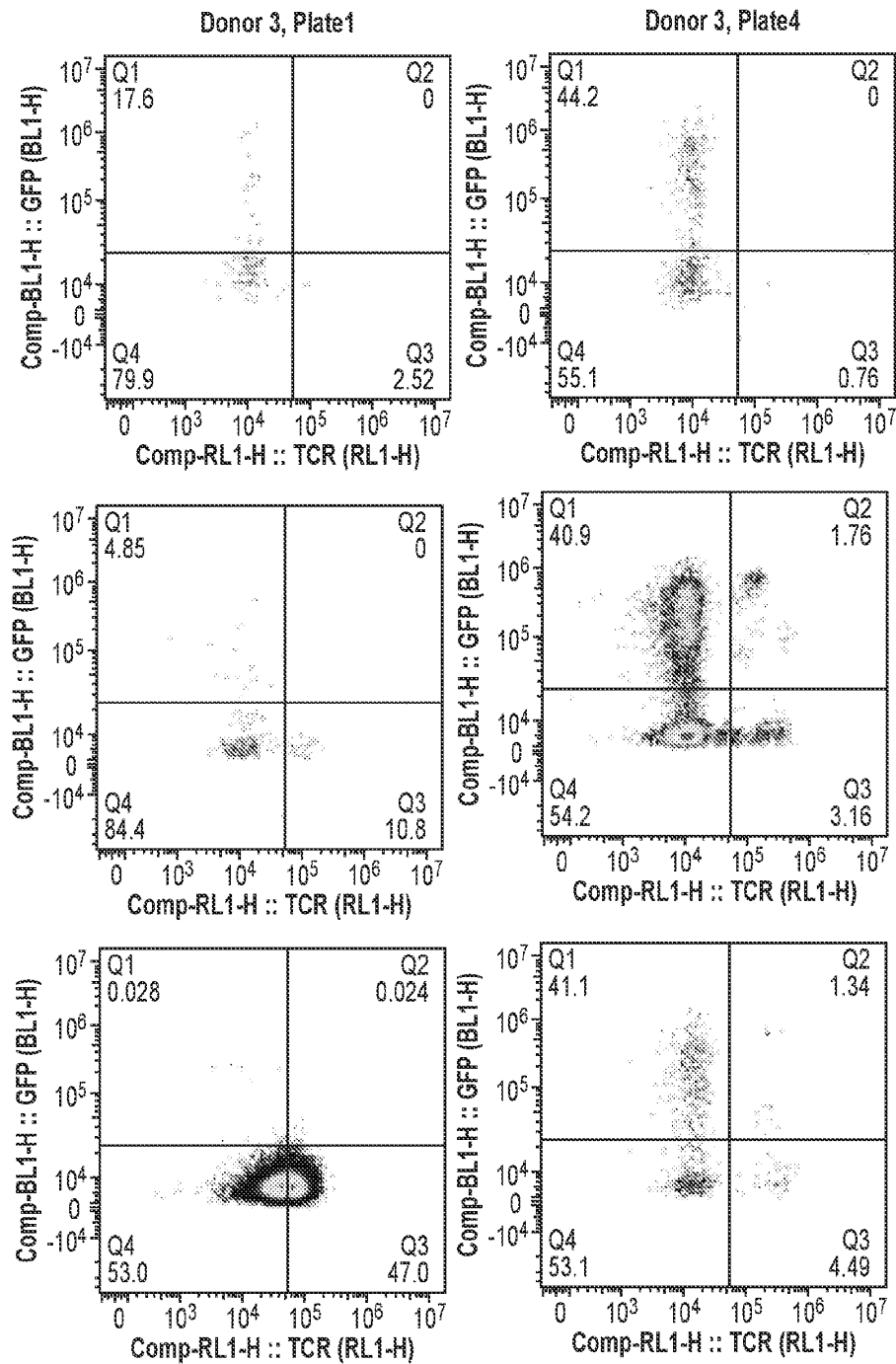


FIG. 20 (Cont. 2)

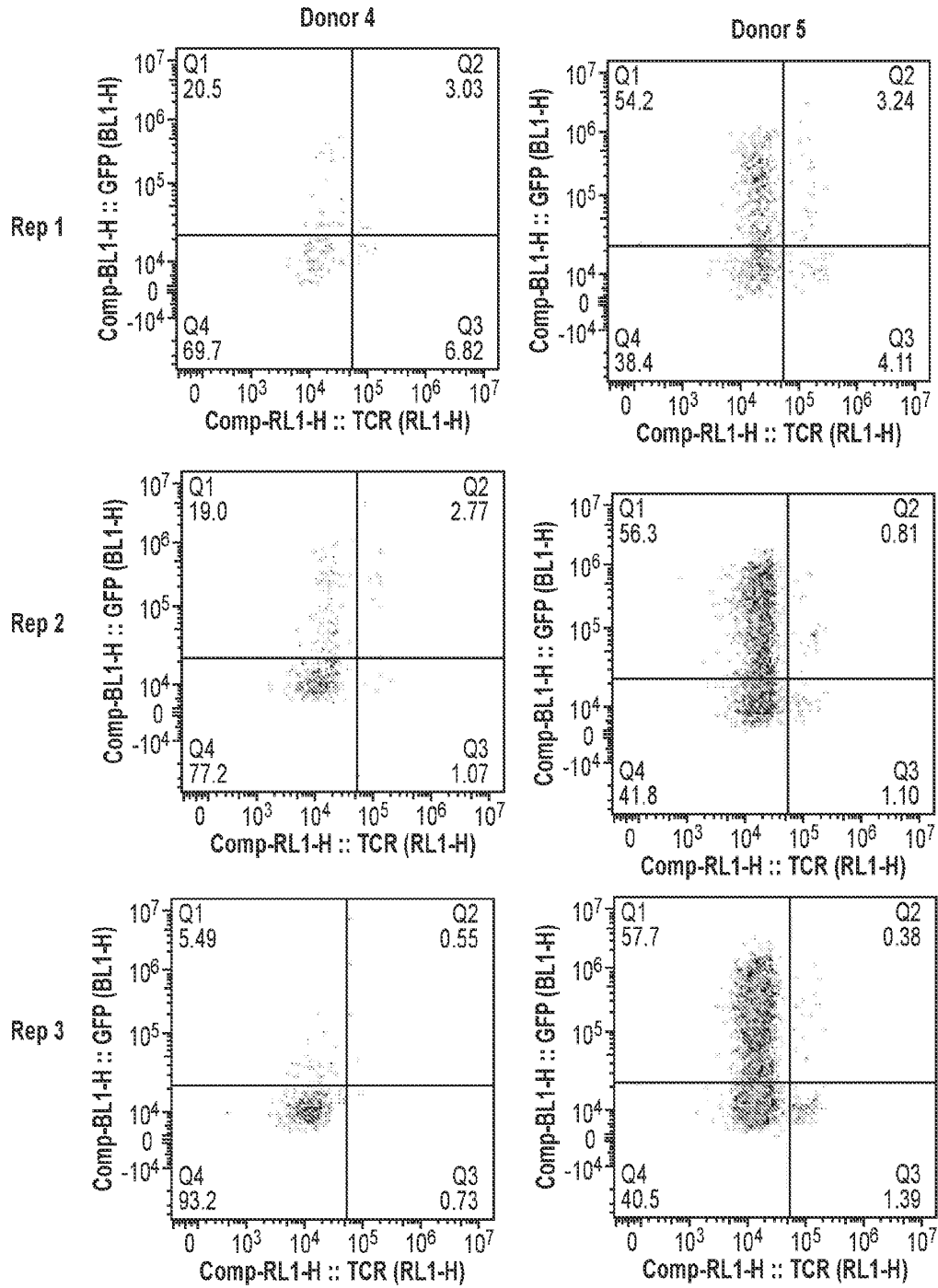


FIG. 20 (Cont. 3)

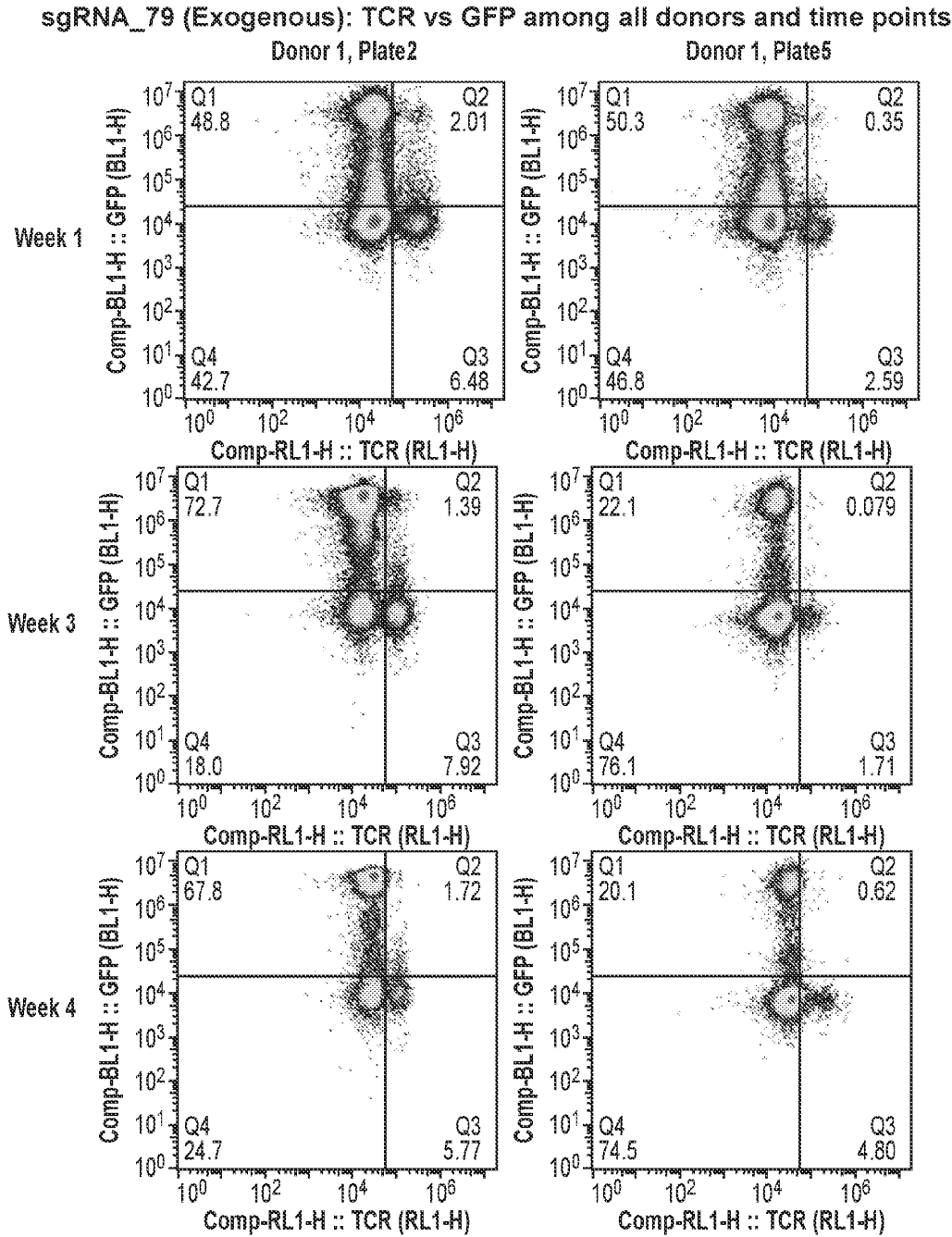


FIG. 21

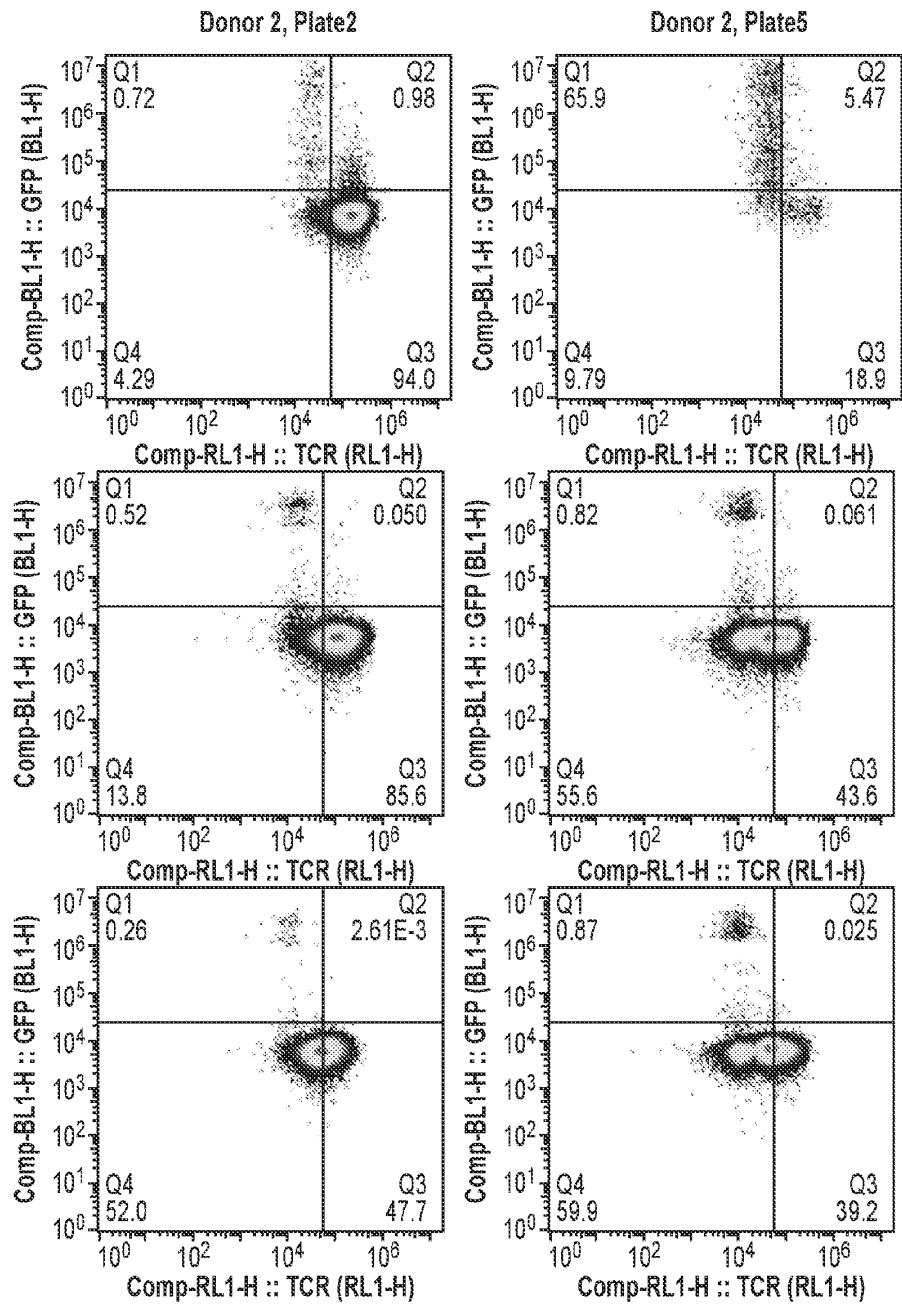


FIG. 21 (Cont. 1)

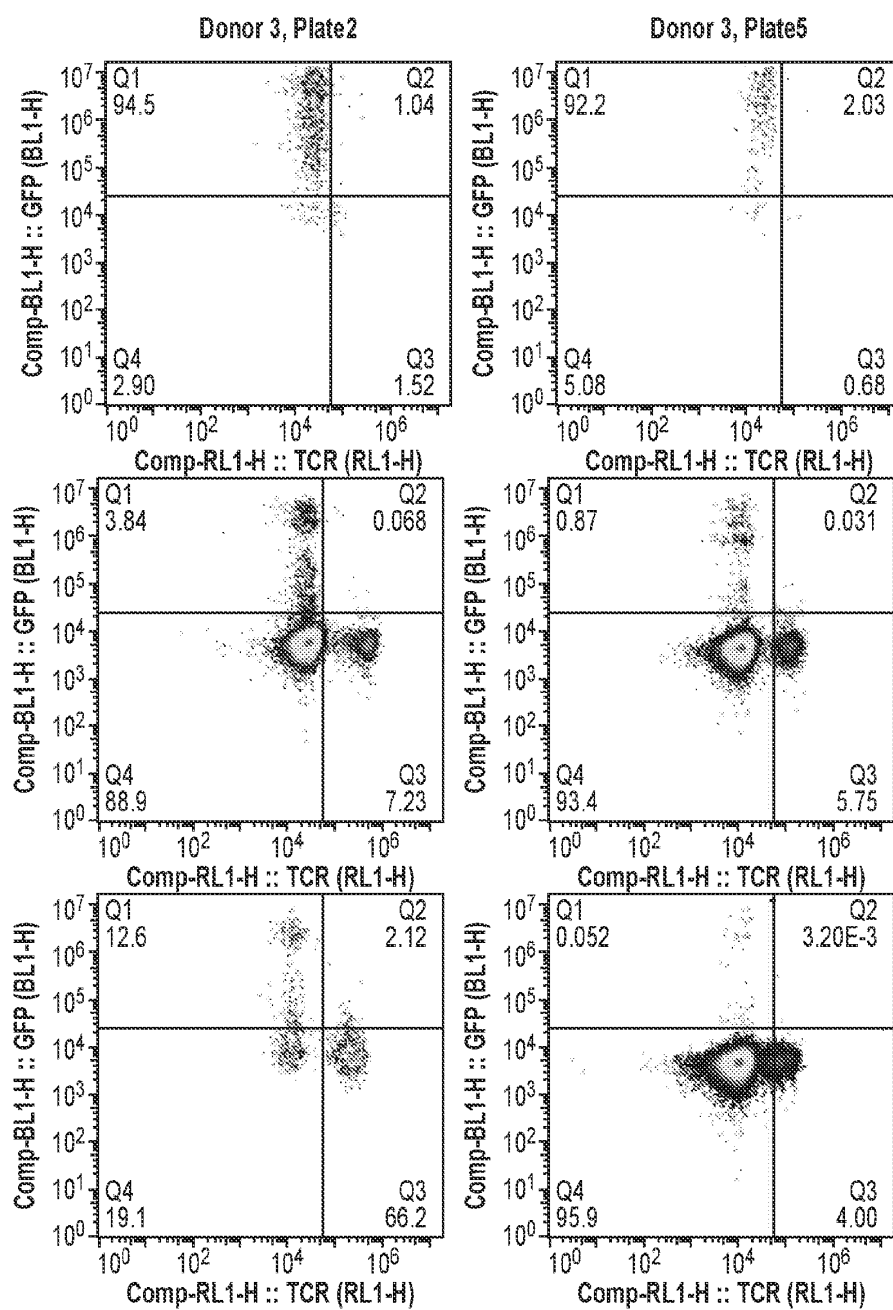


FIG. 21 (Cont. 2)

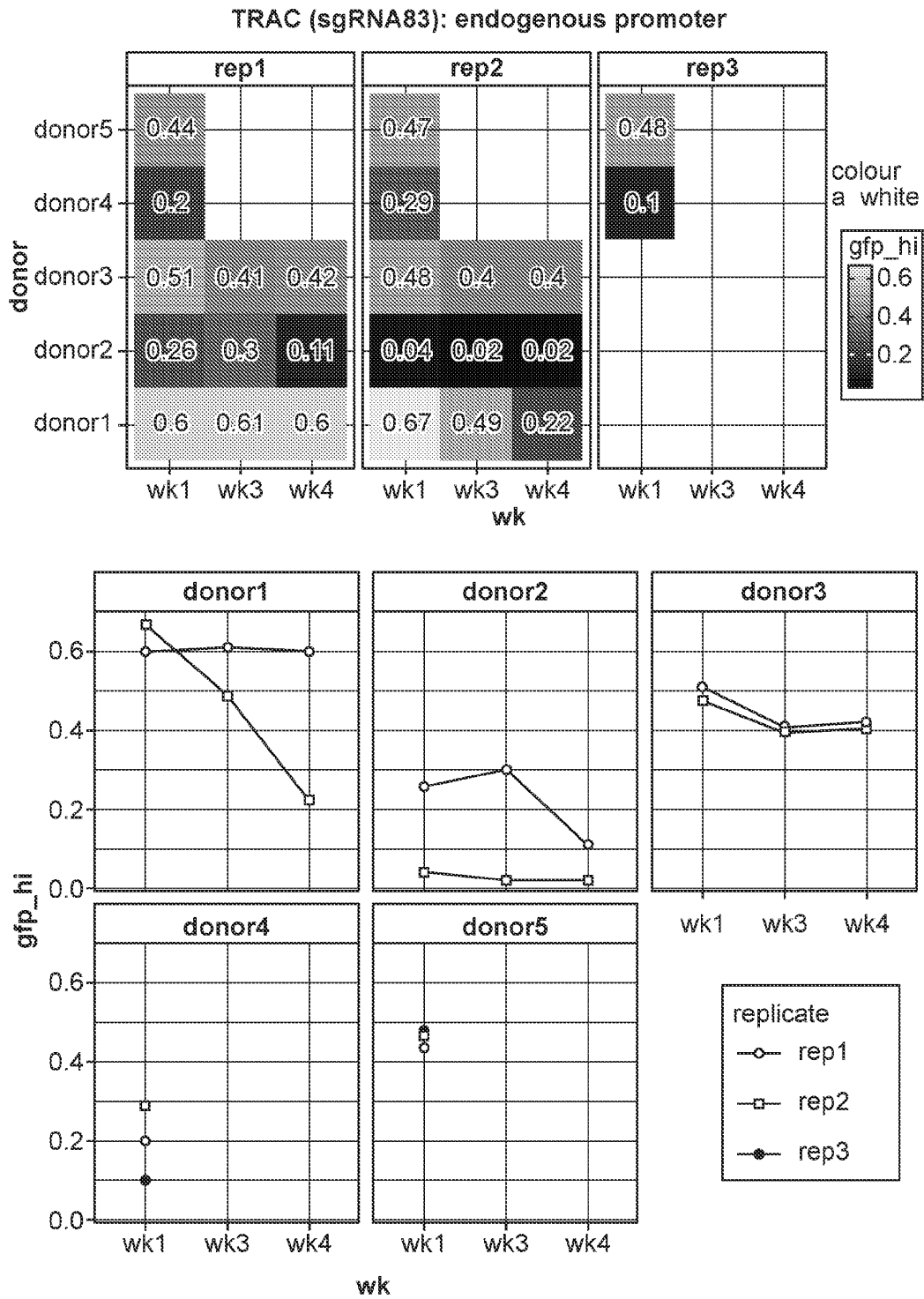


FIG. 22

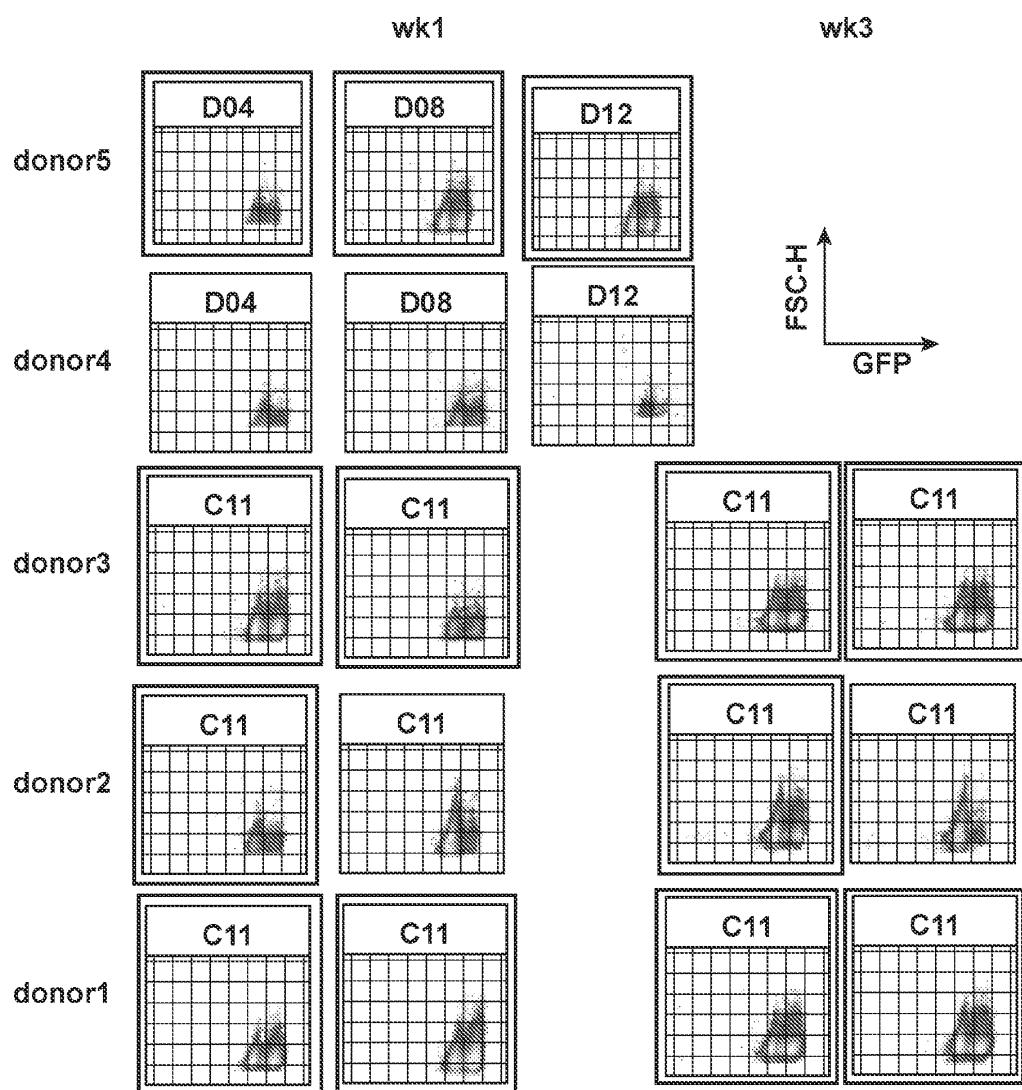
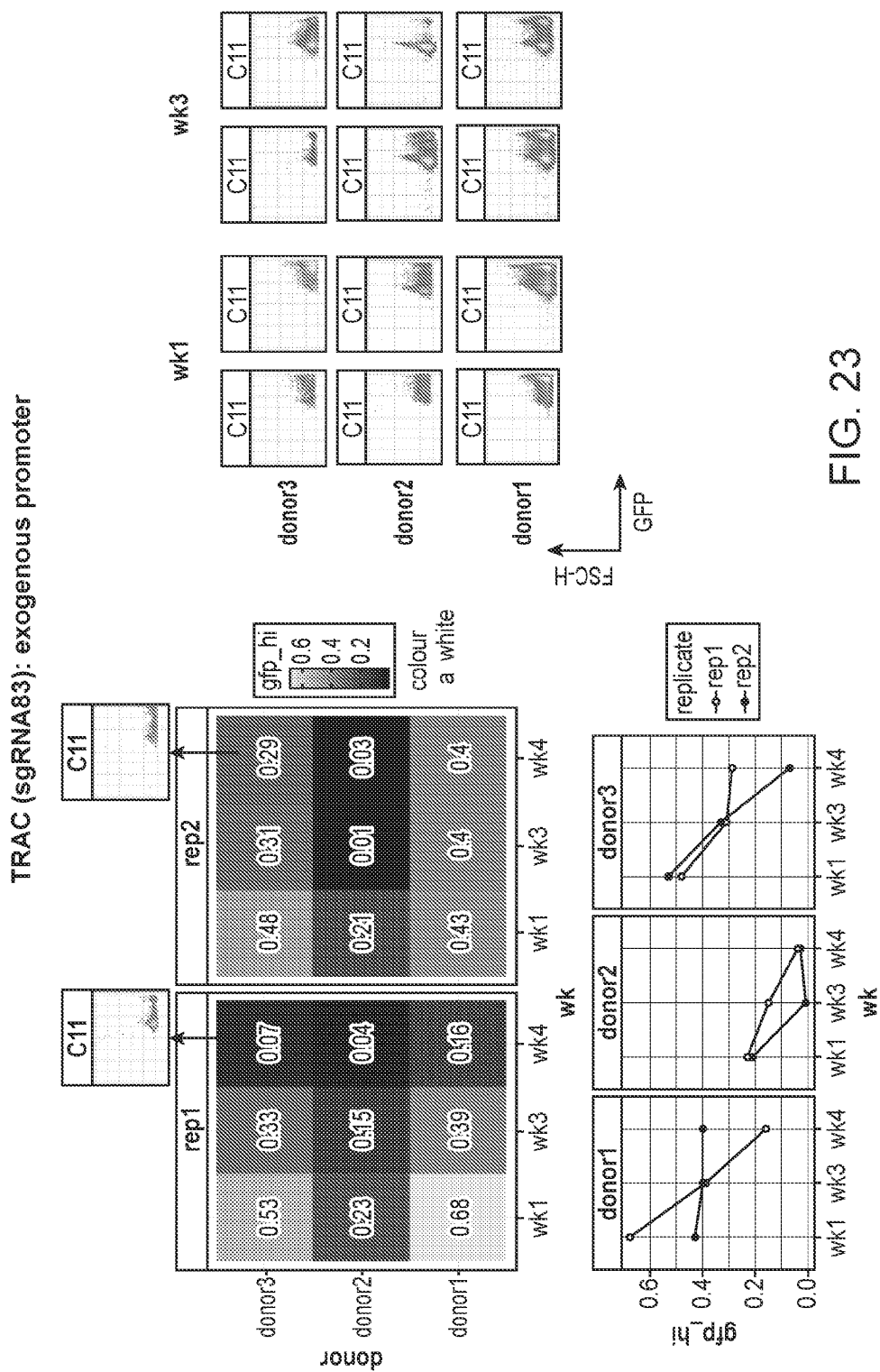


FIG. 22 (Cont.)



sgRNA_83 (Endogenous): TCR vs GFP among all donors and time points

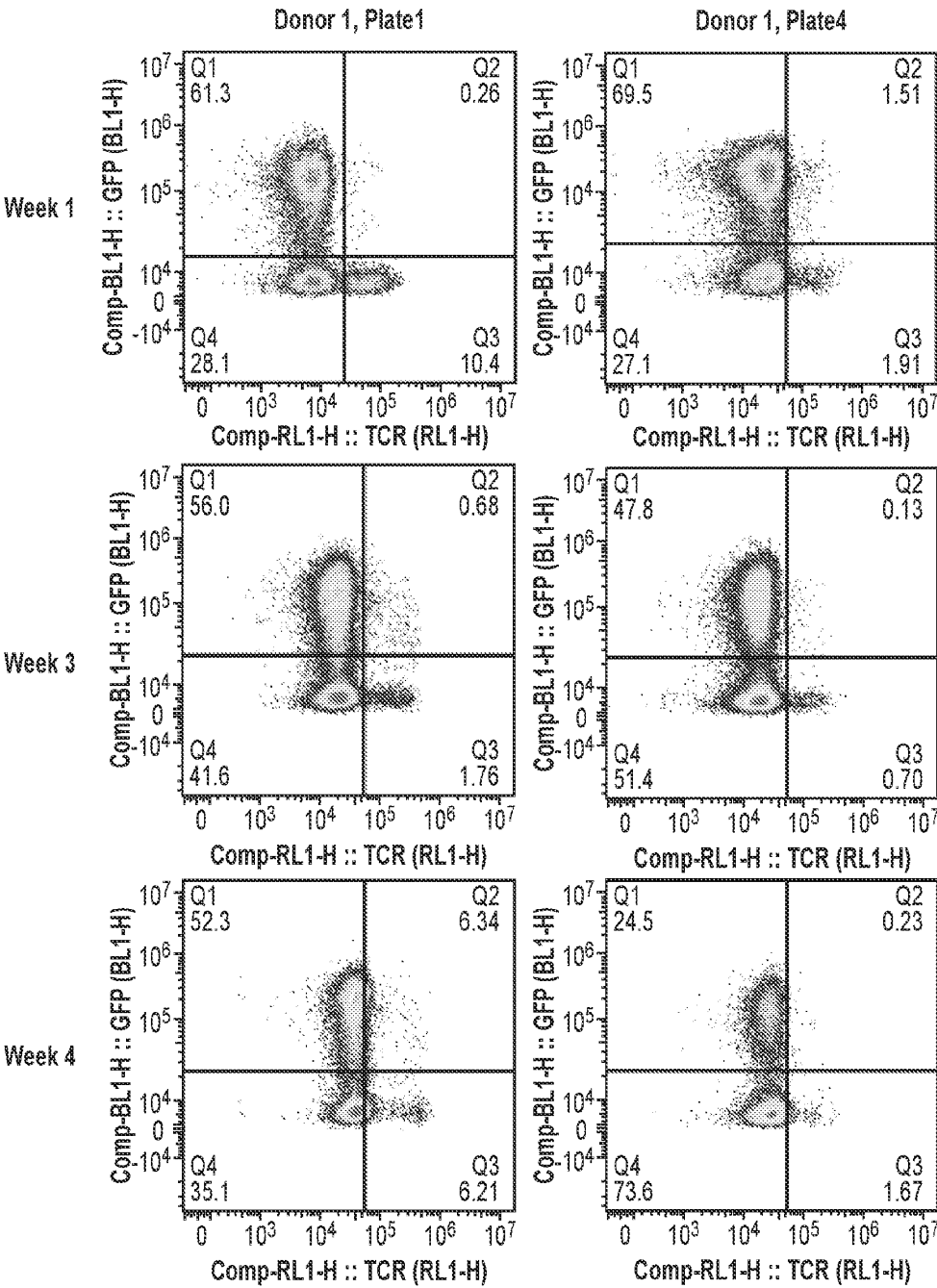
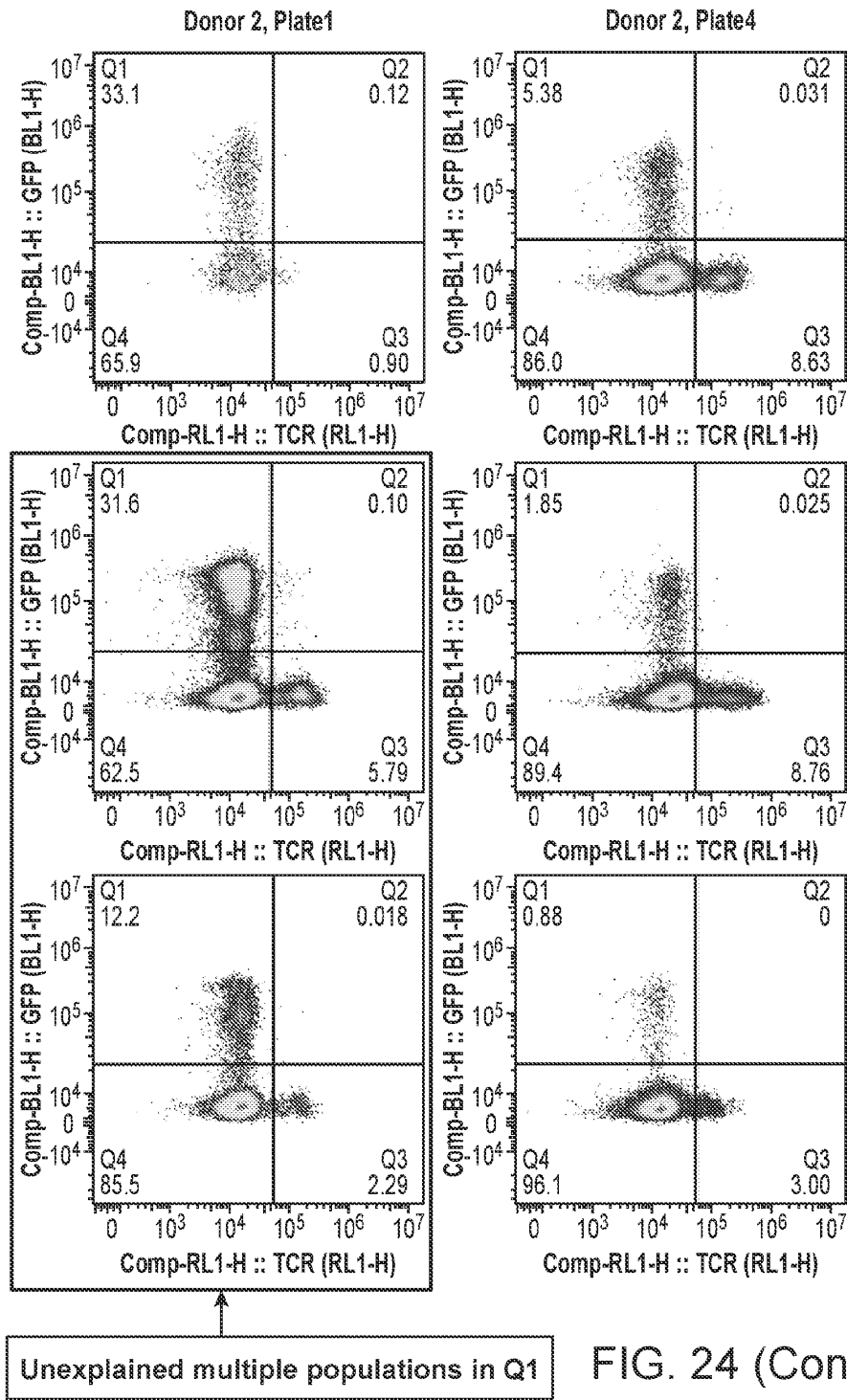


FIG. 24



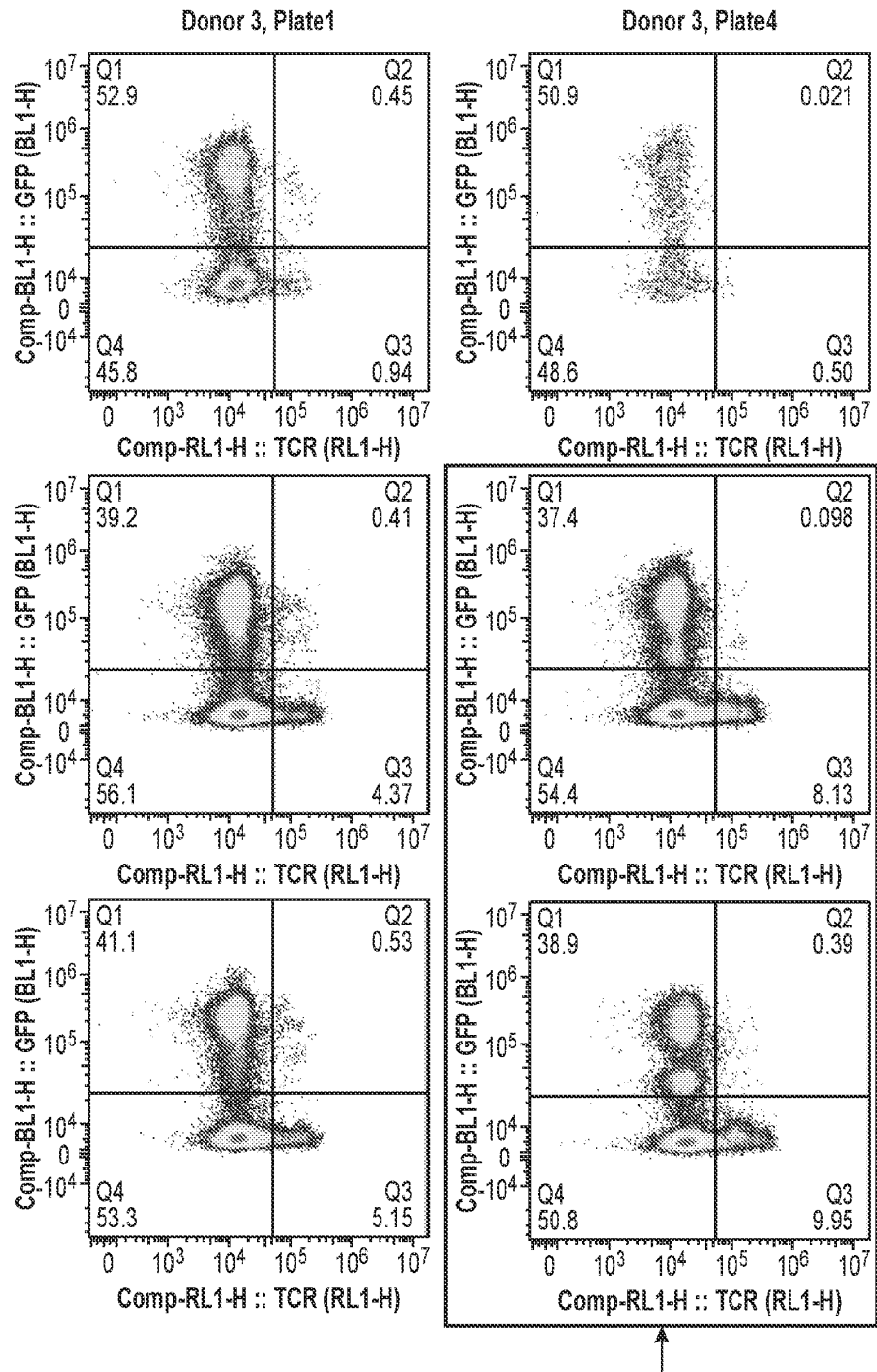


FIG. 24 (Cont. 2)

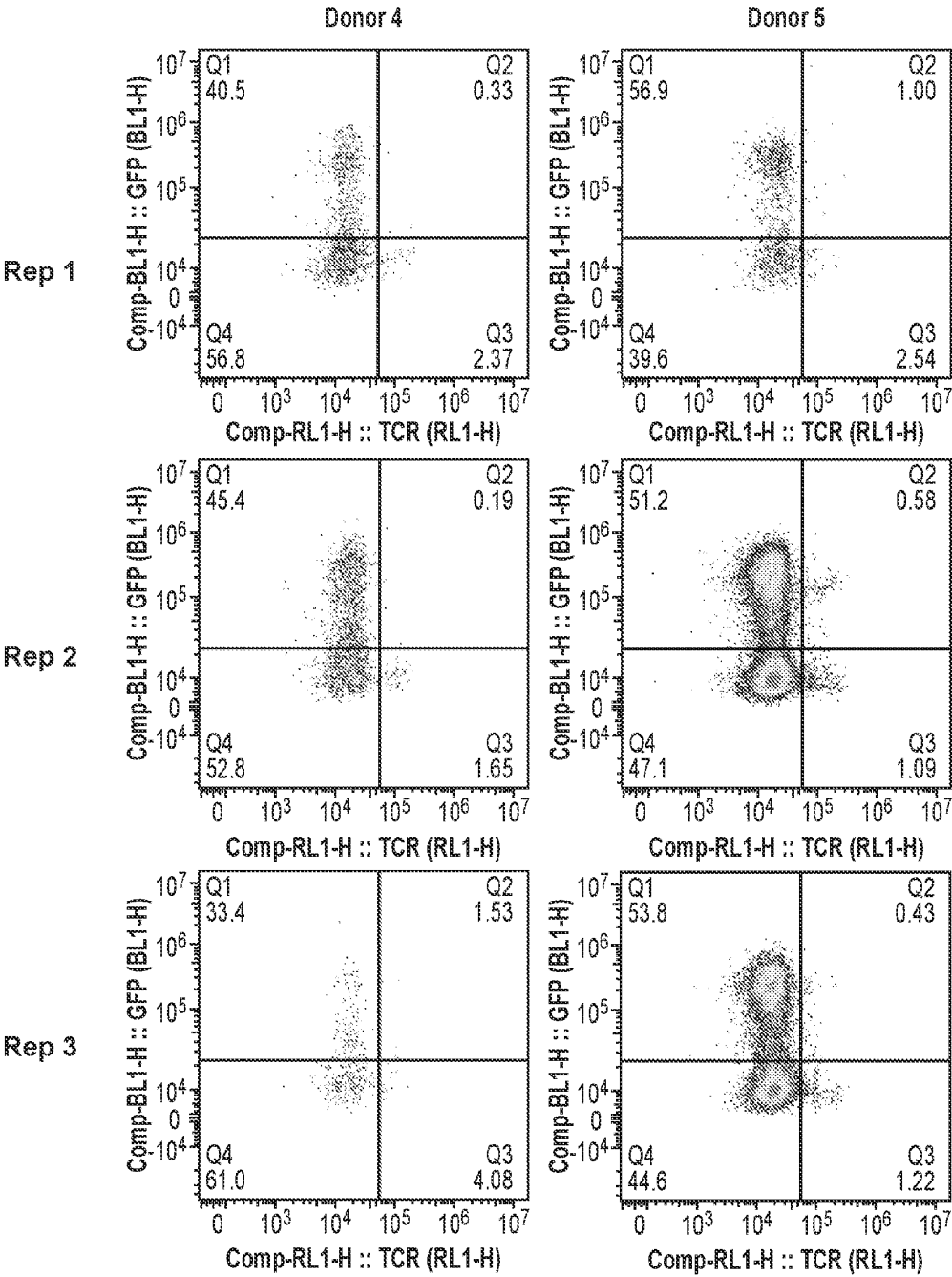


FIG. 24 (Cont. 3)

sgRNA_83 (Exogenous): TCR vs GFP among all donors and time points

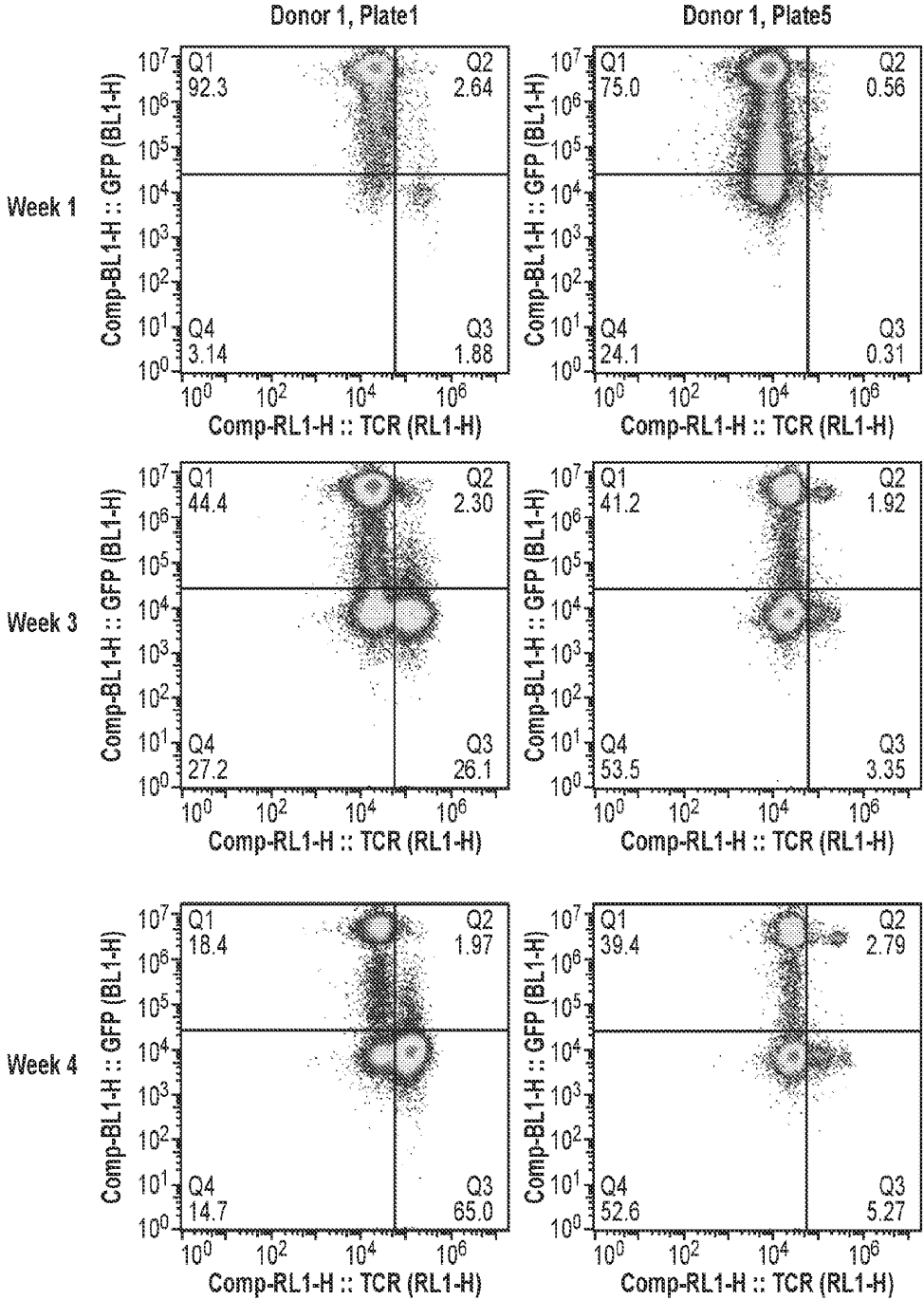


FIG. 25

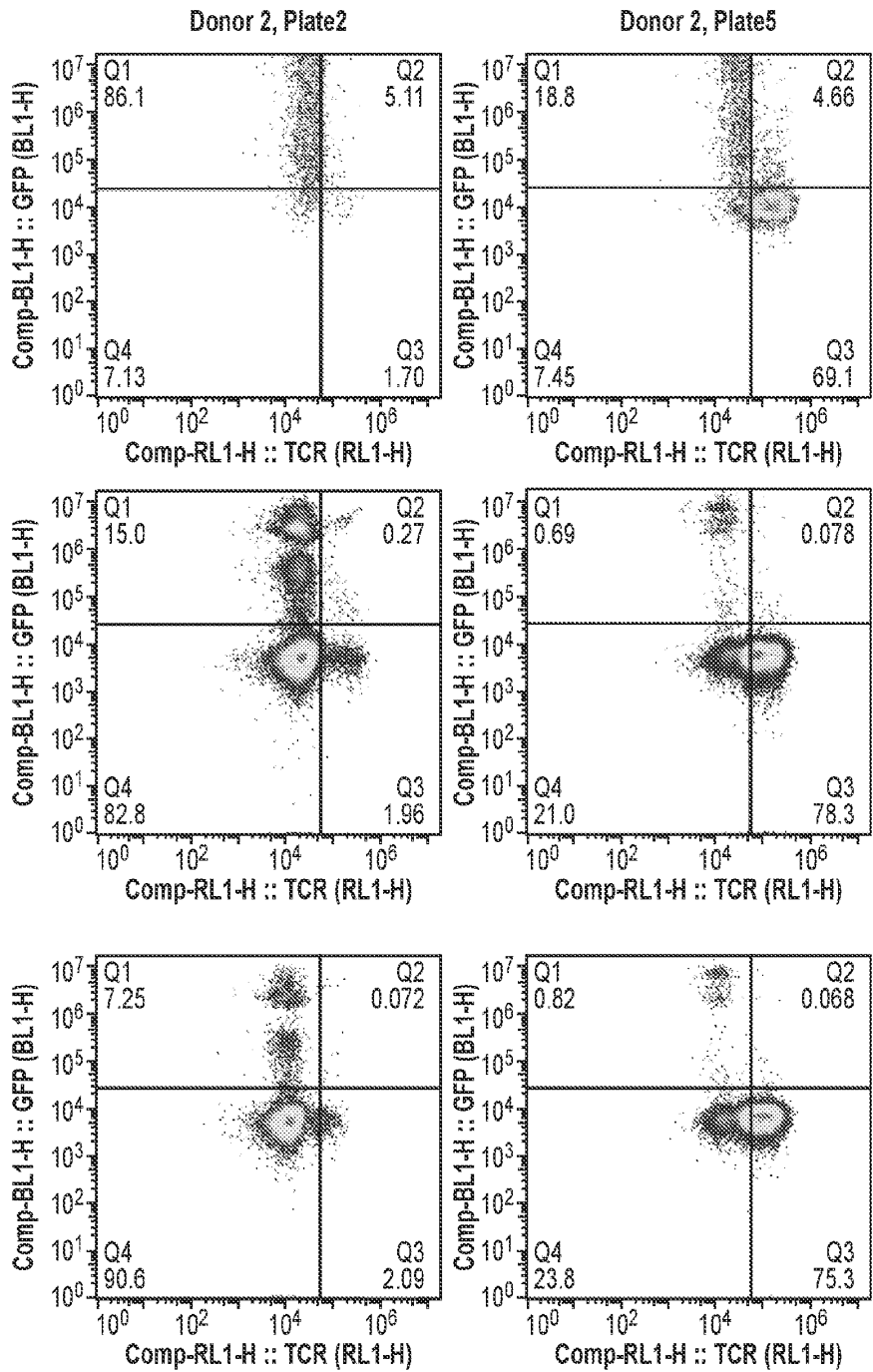


FIG. 25 (Cont. 1)

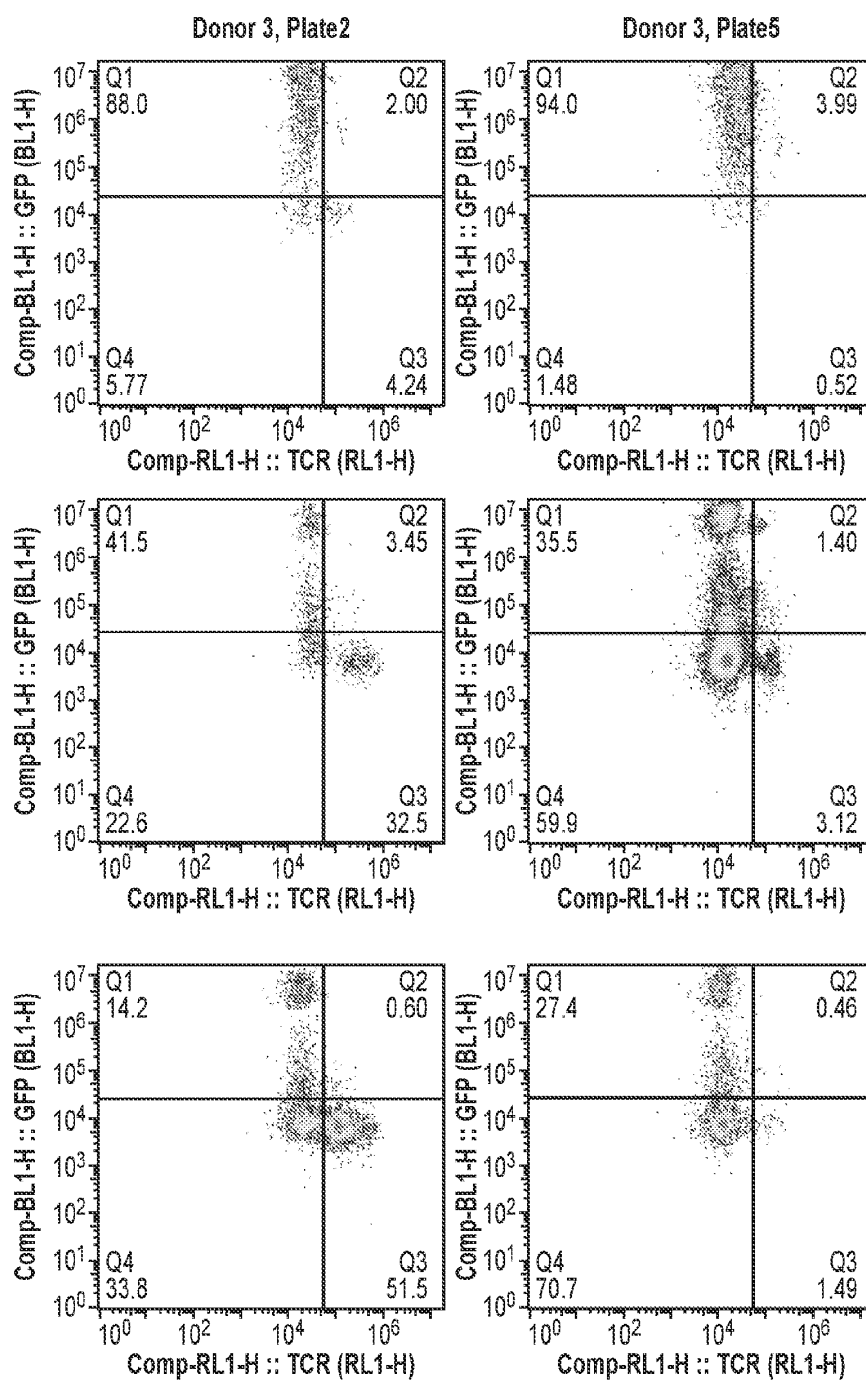


FIG. 25 (Cont. 2)

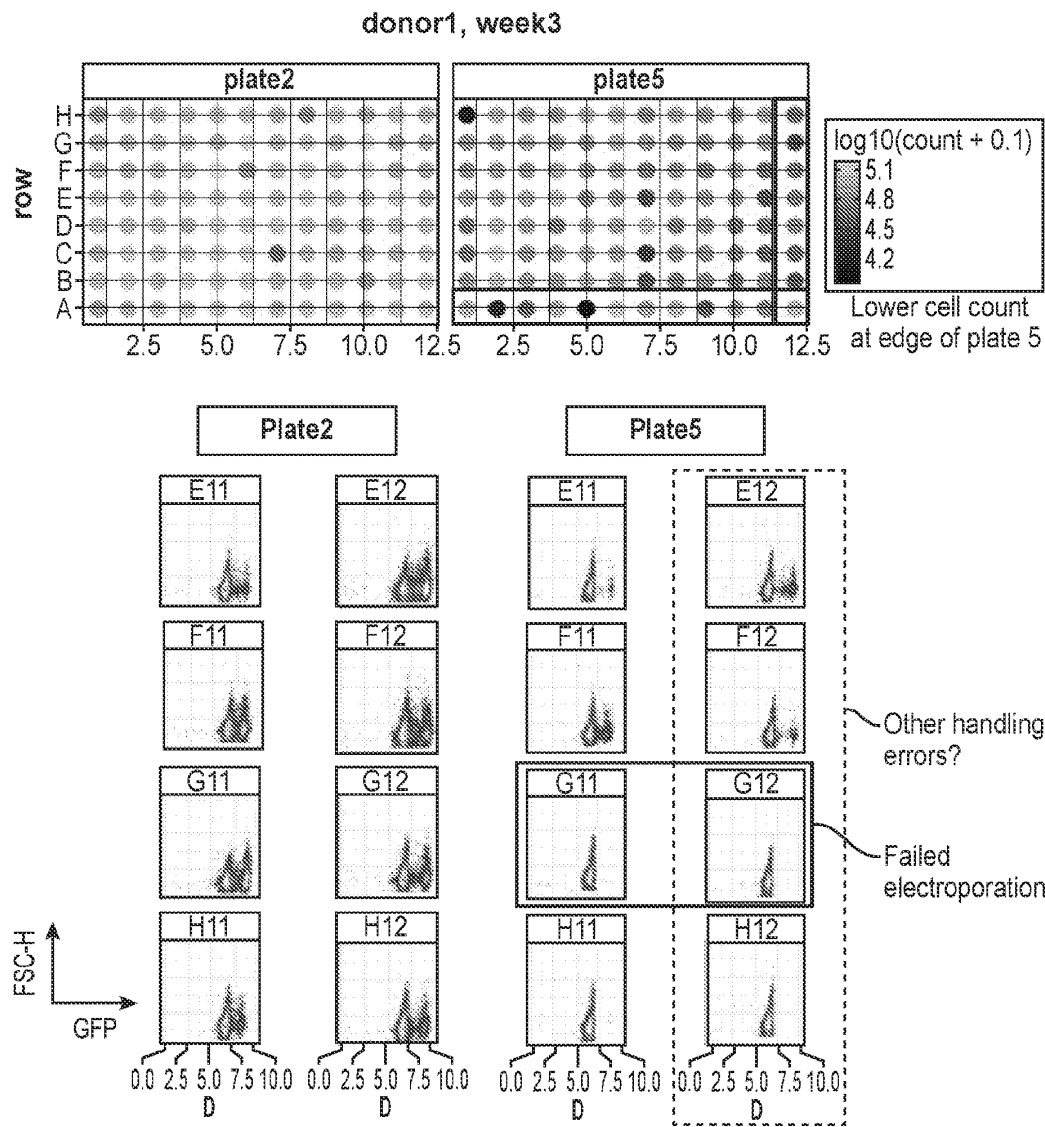


FIG. 26

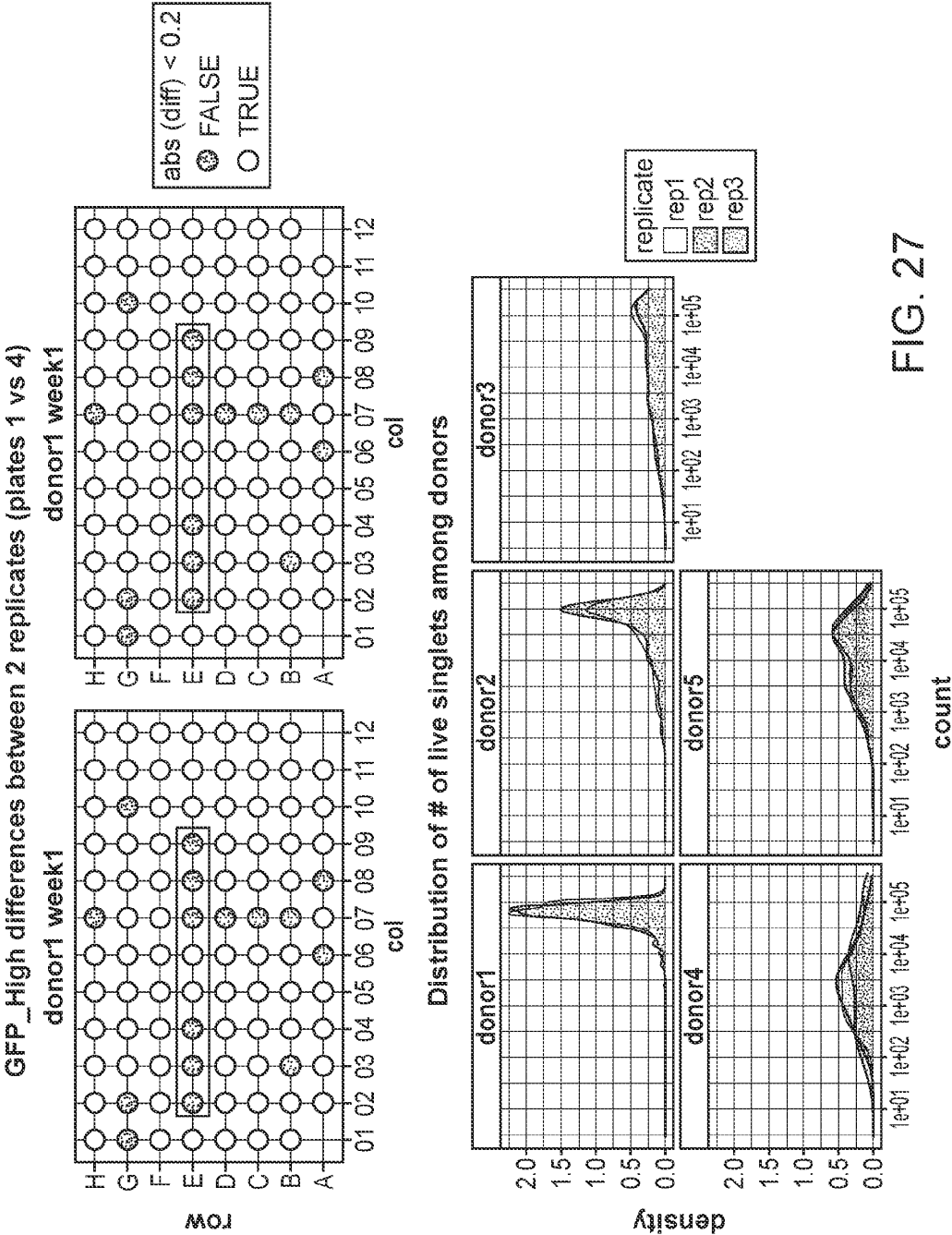


FIG. 27

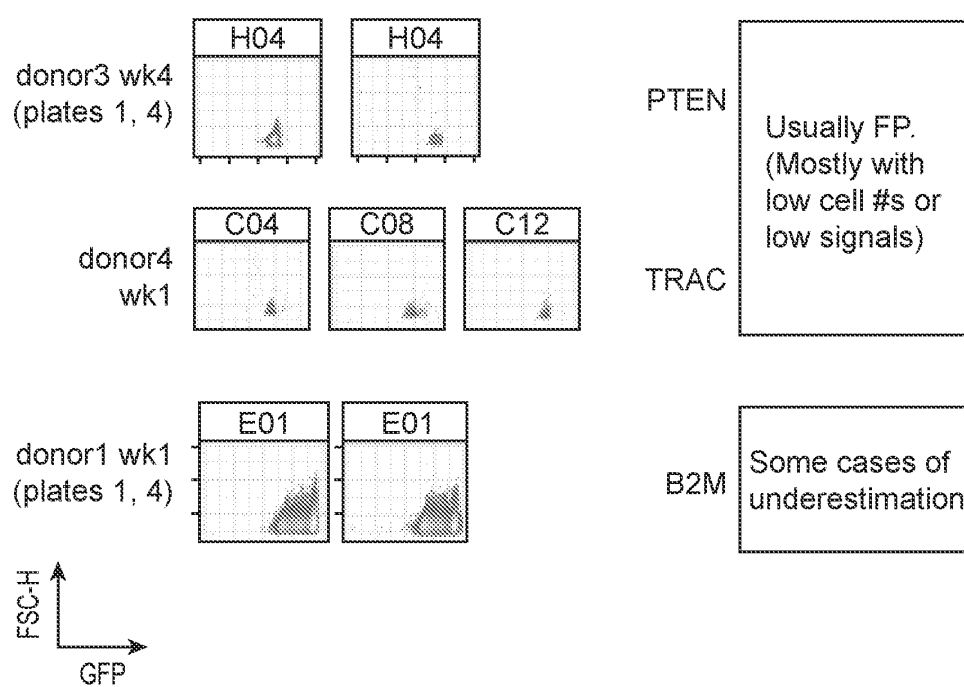


FIG. 28

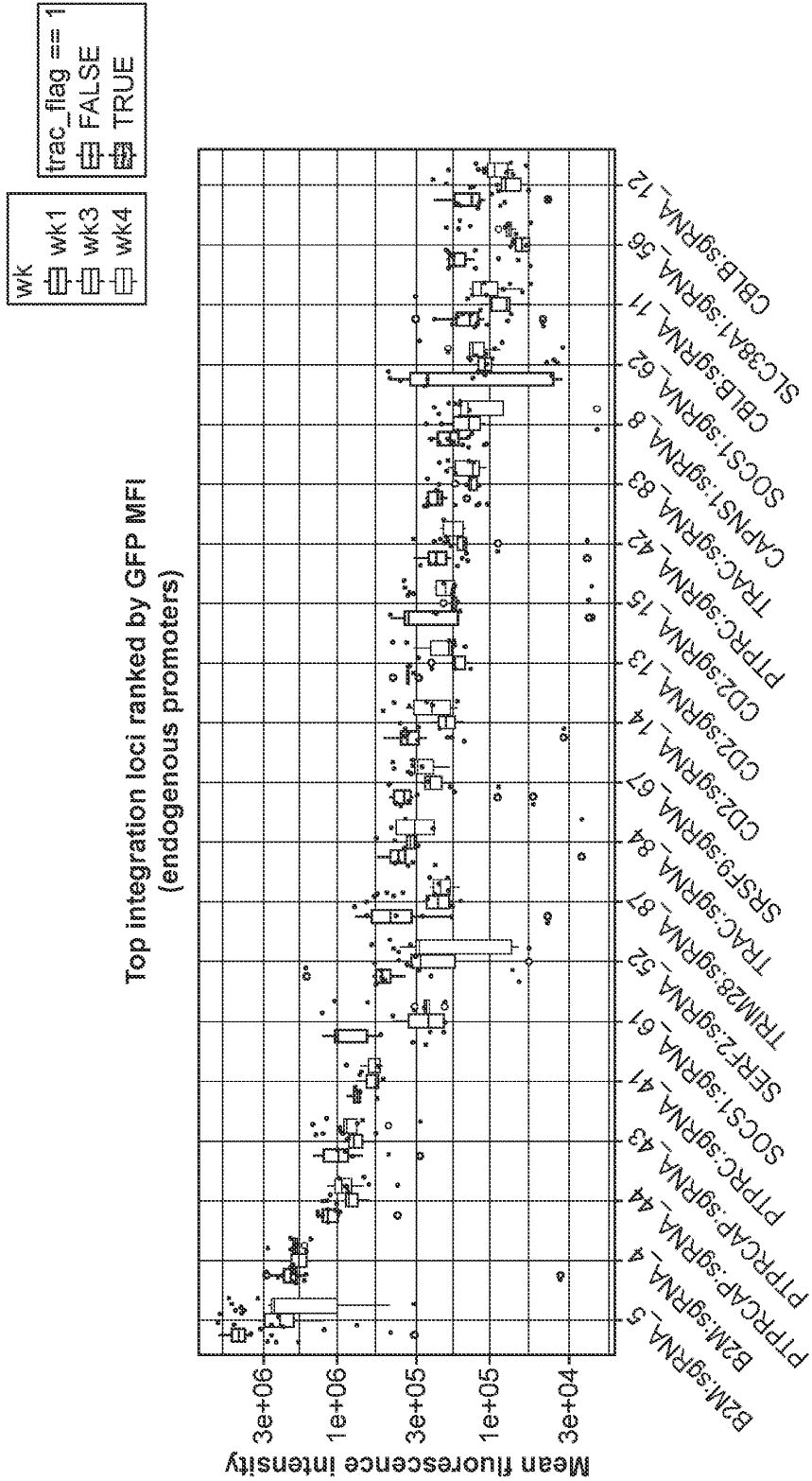


FIG. 29

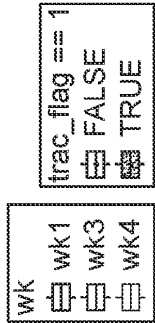
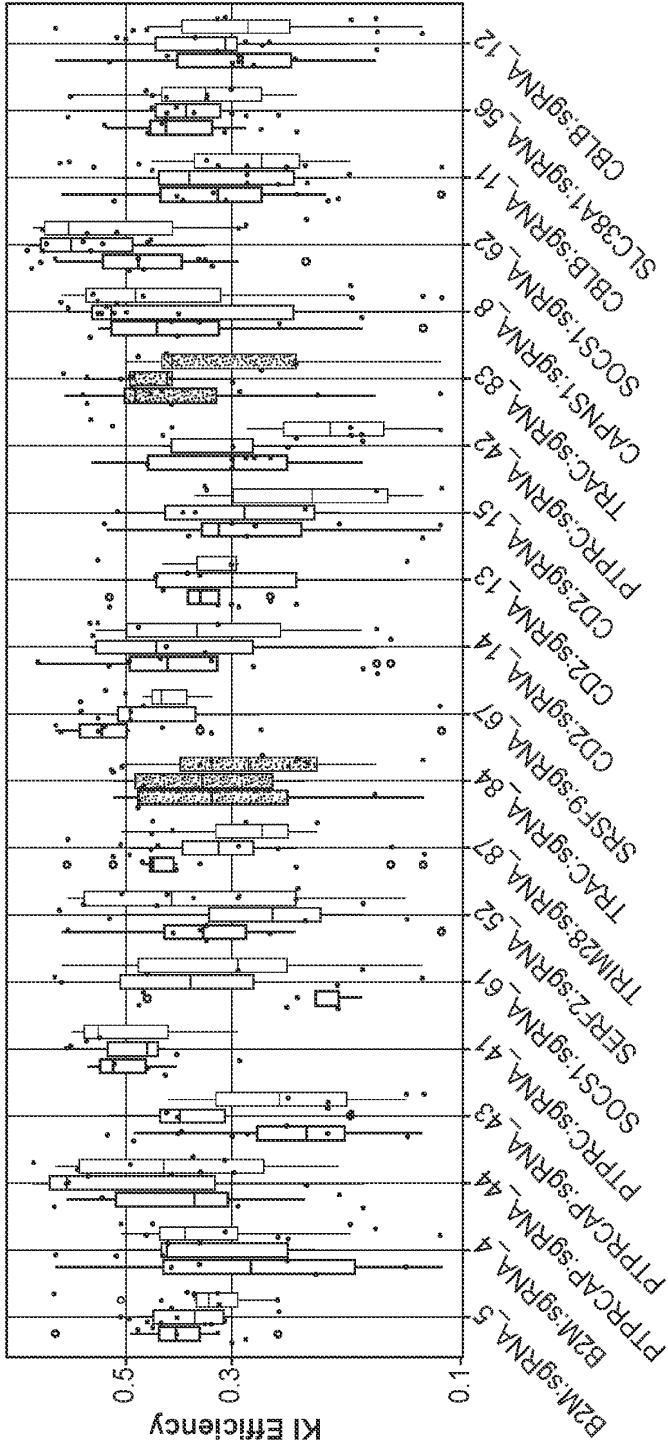
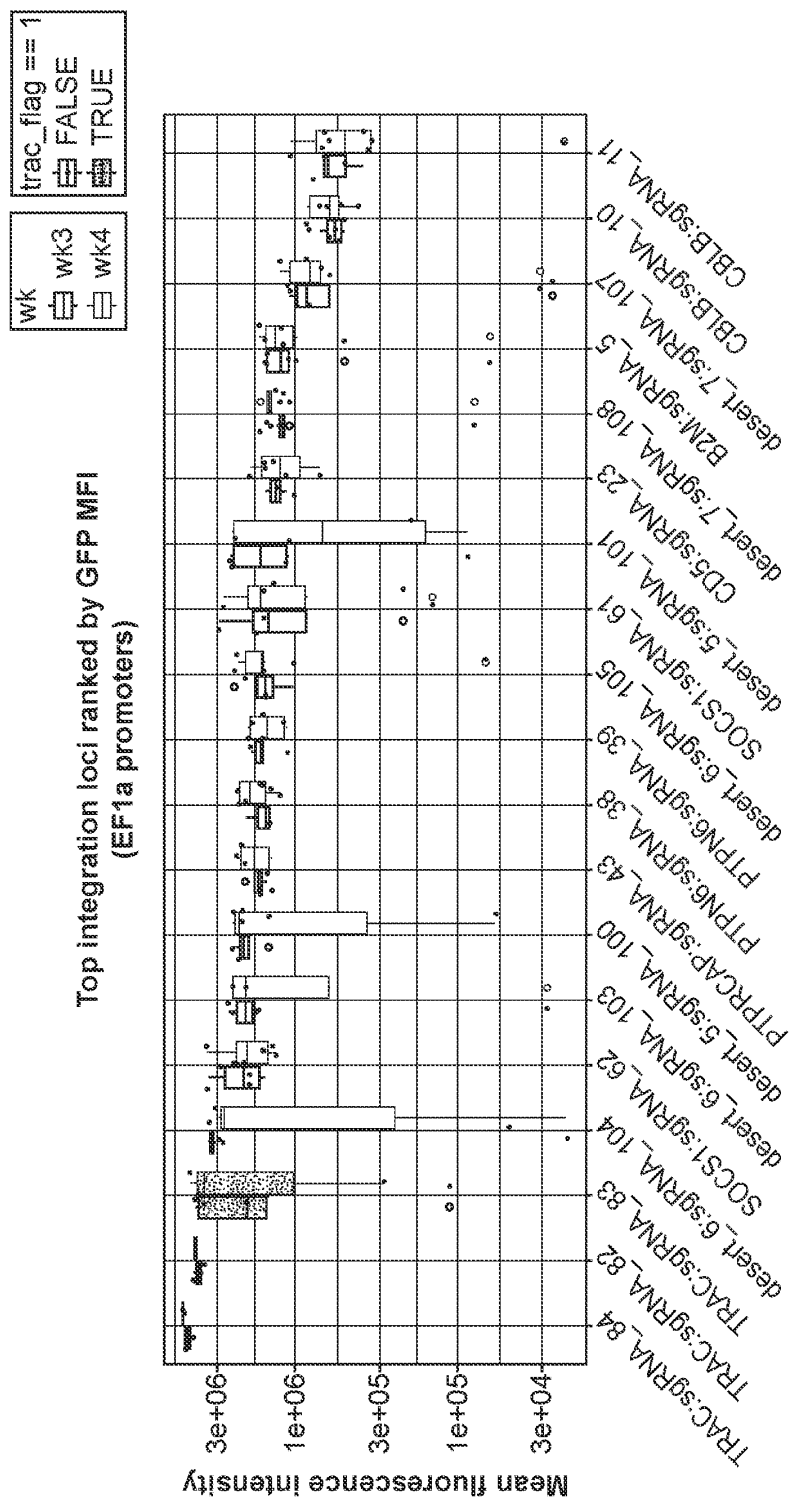


FIG. 29 (Cont.)



30

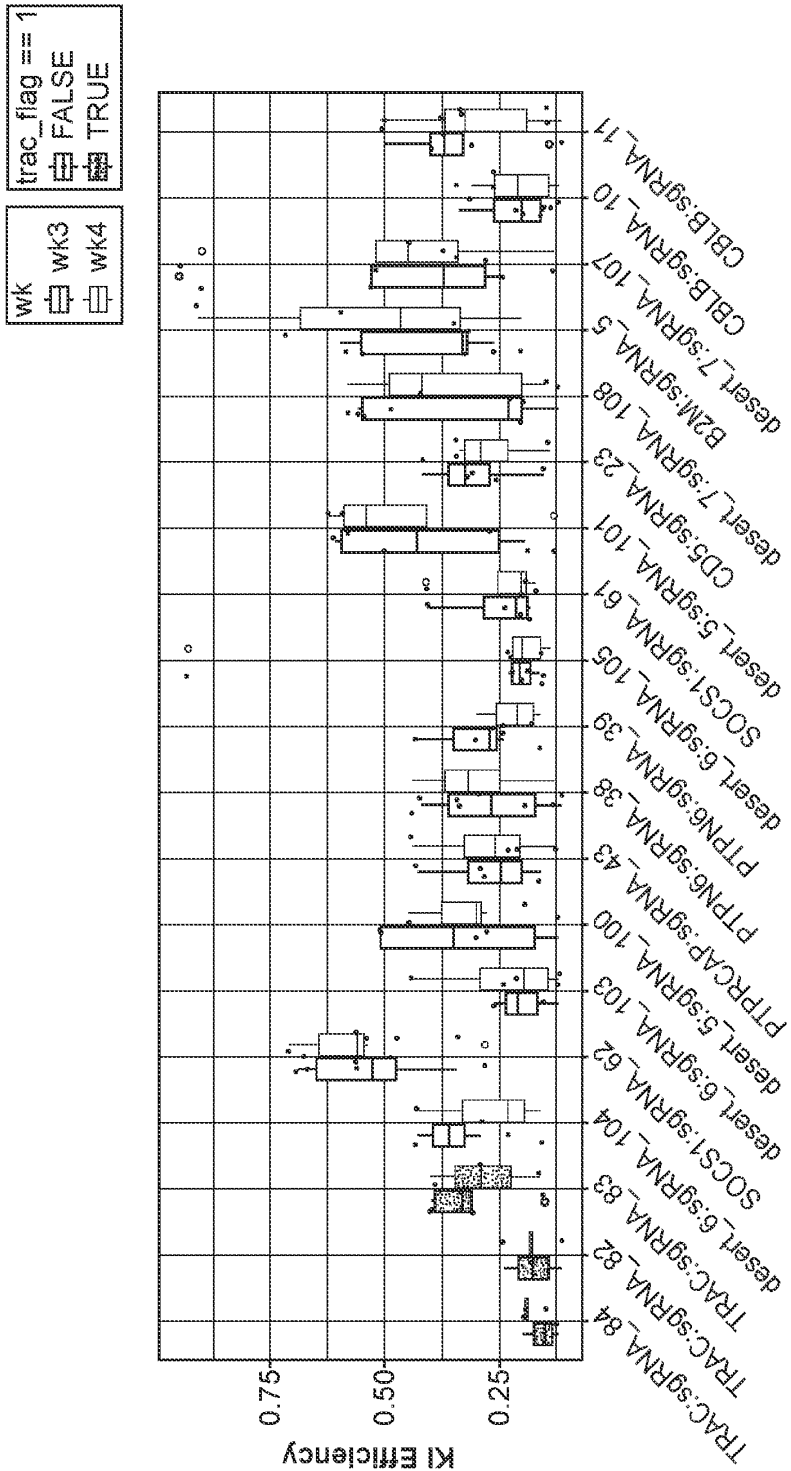


FIG. 30 (Cont.)

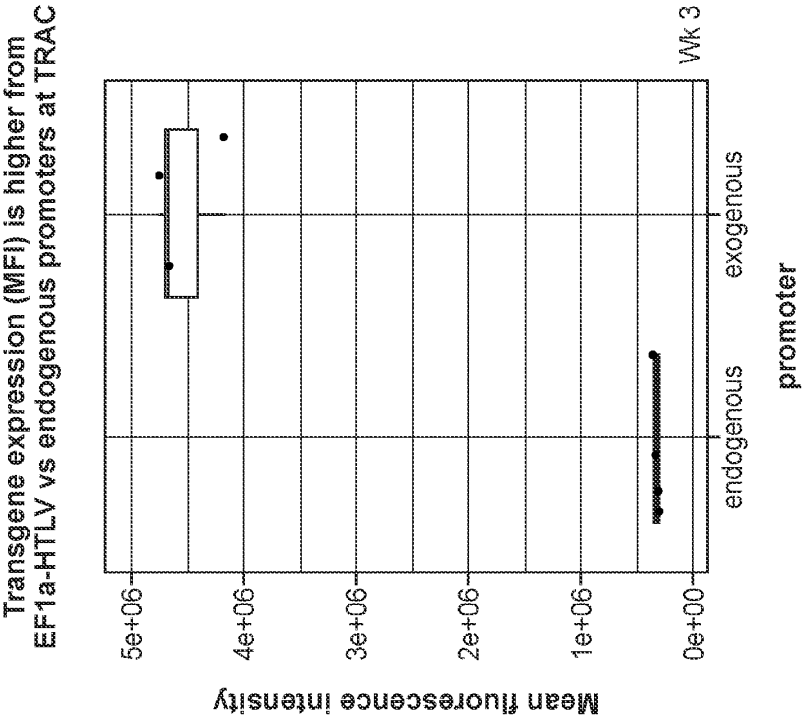


FIG. 31A

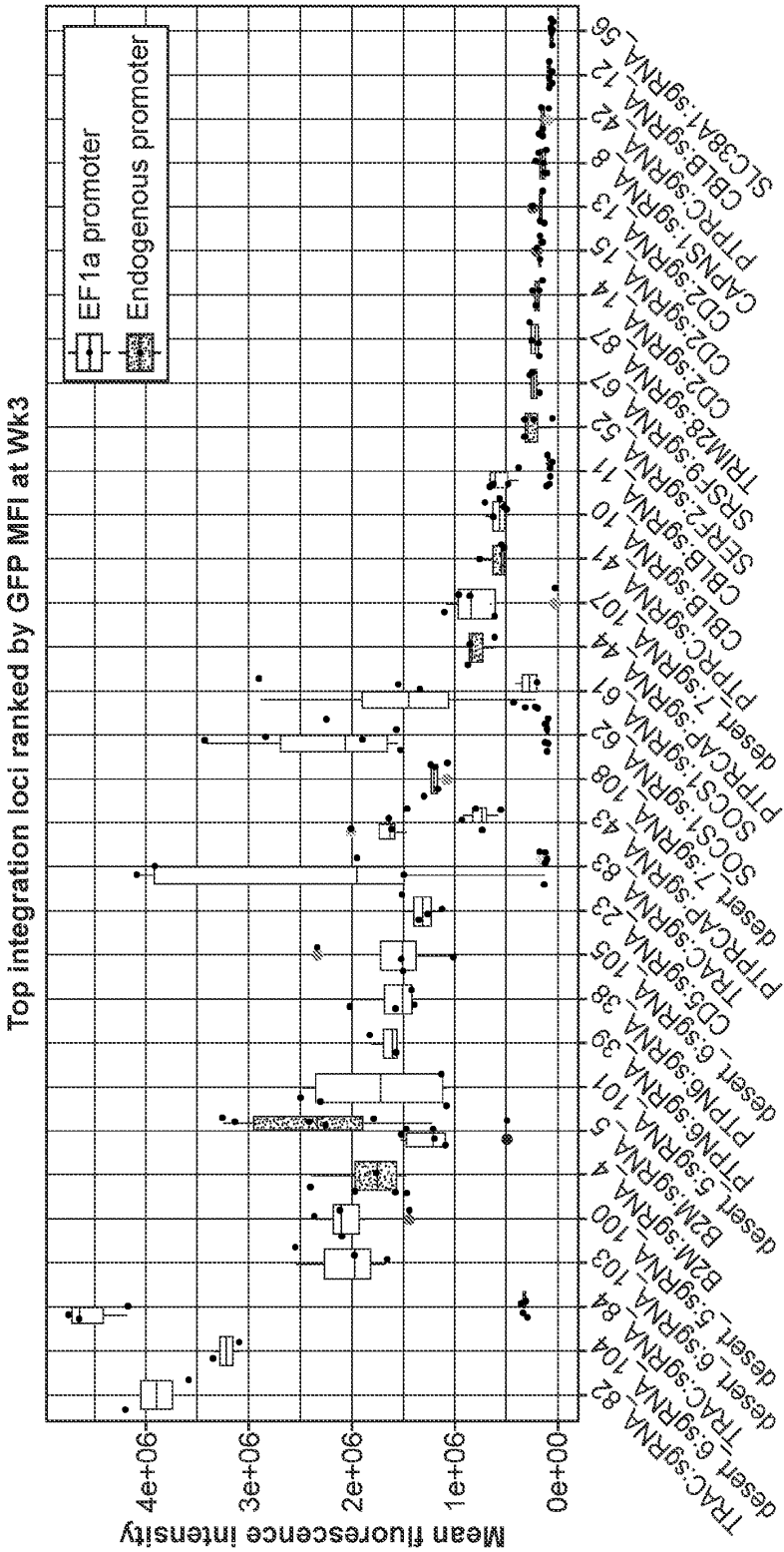


FIG. 31B

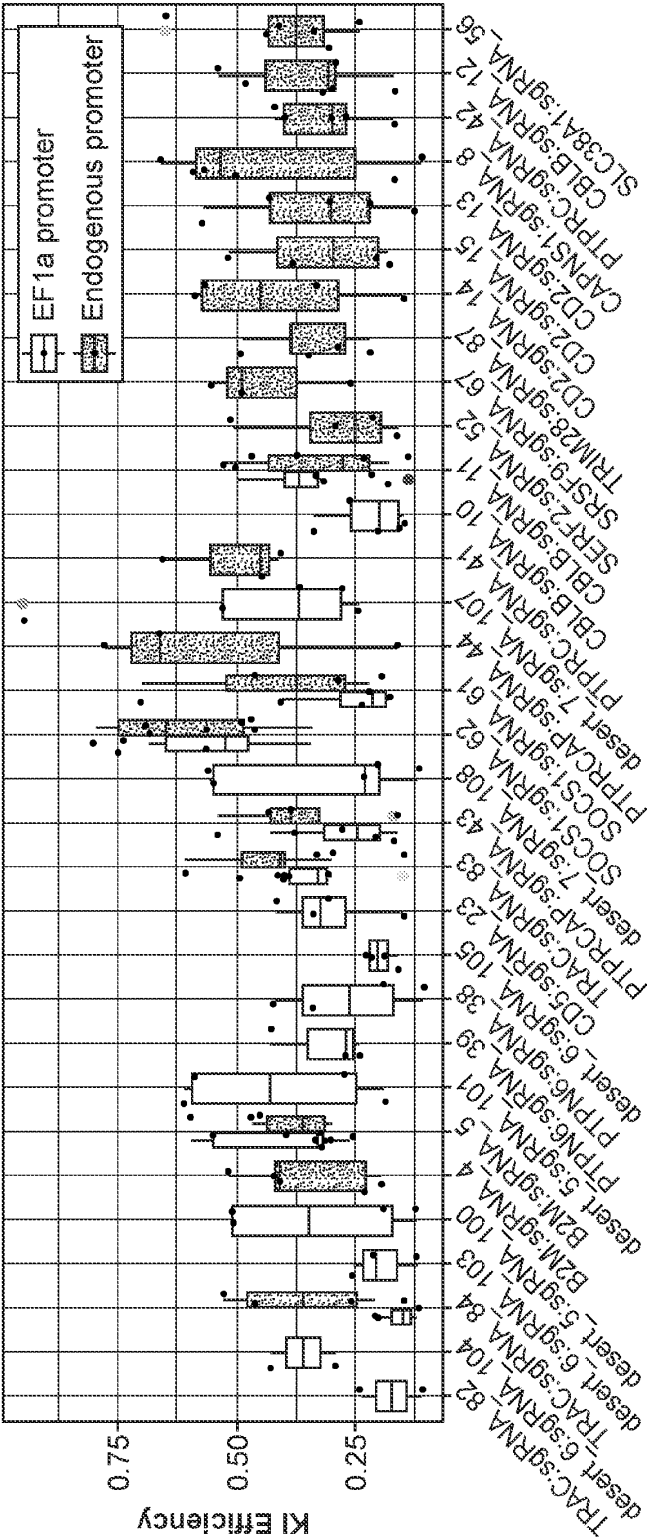


FIG. 31B (Cont.)

Top integration loci (endogenous + EF1a promoters) ranked by GFP MFI
(wk3 and 4, donors 1-3)

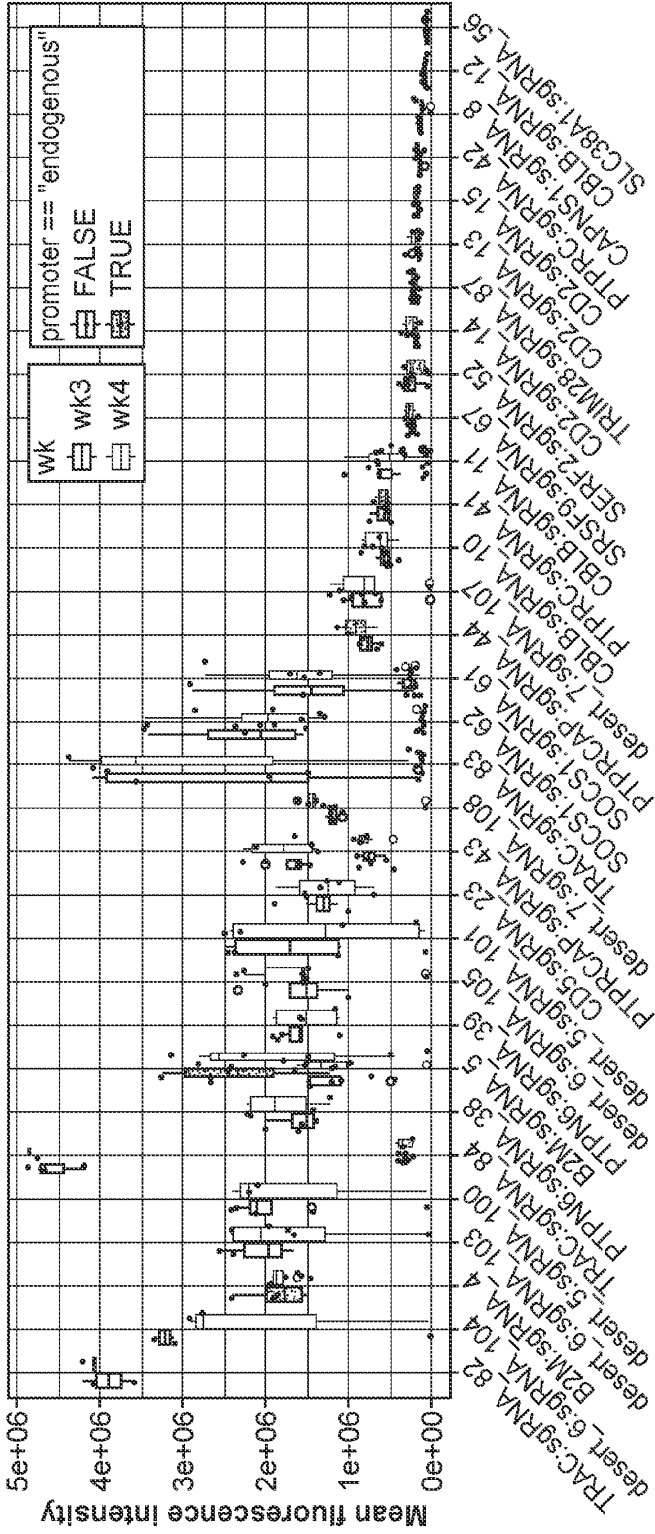


FIG. 31C

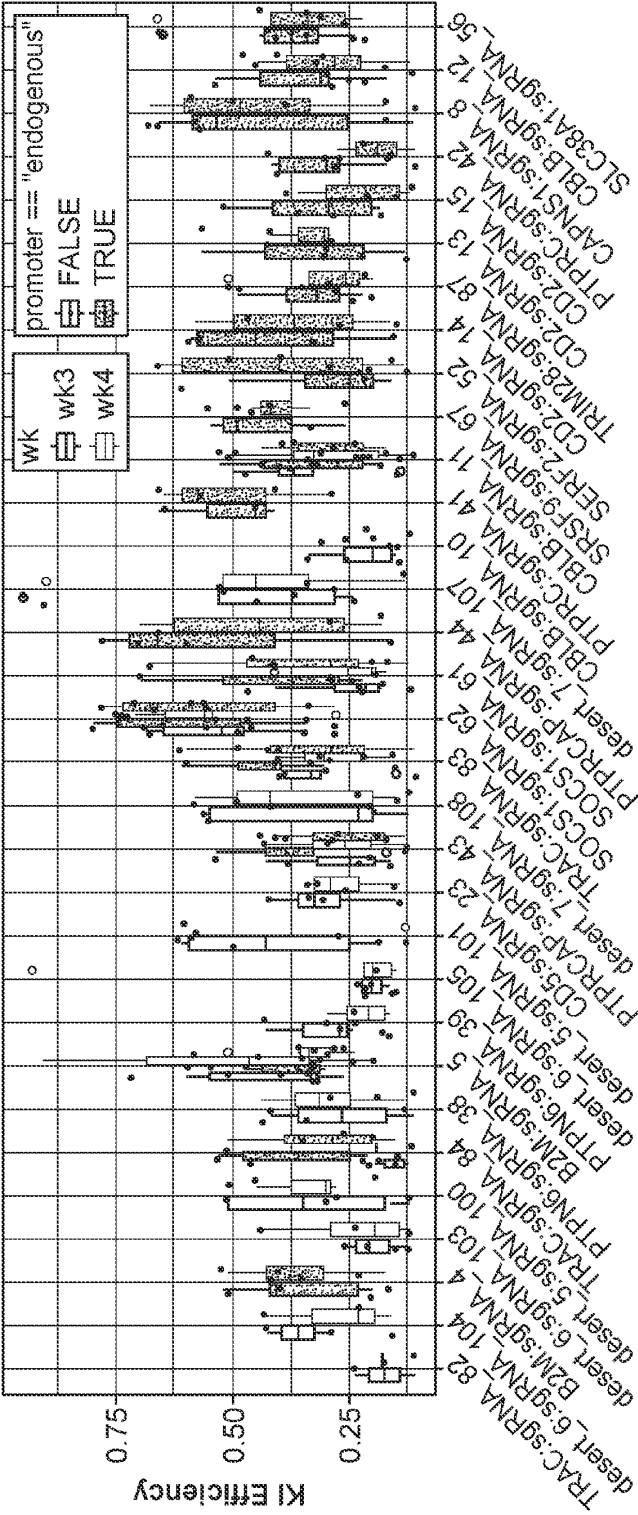


FIG. 31C (Cont.)

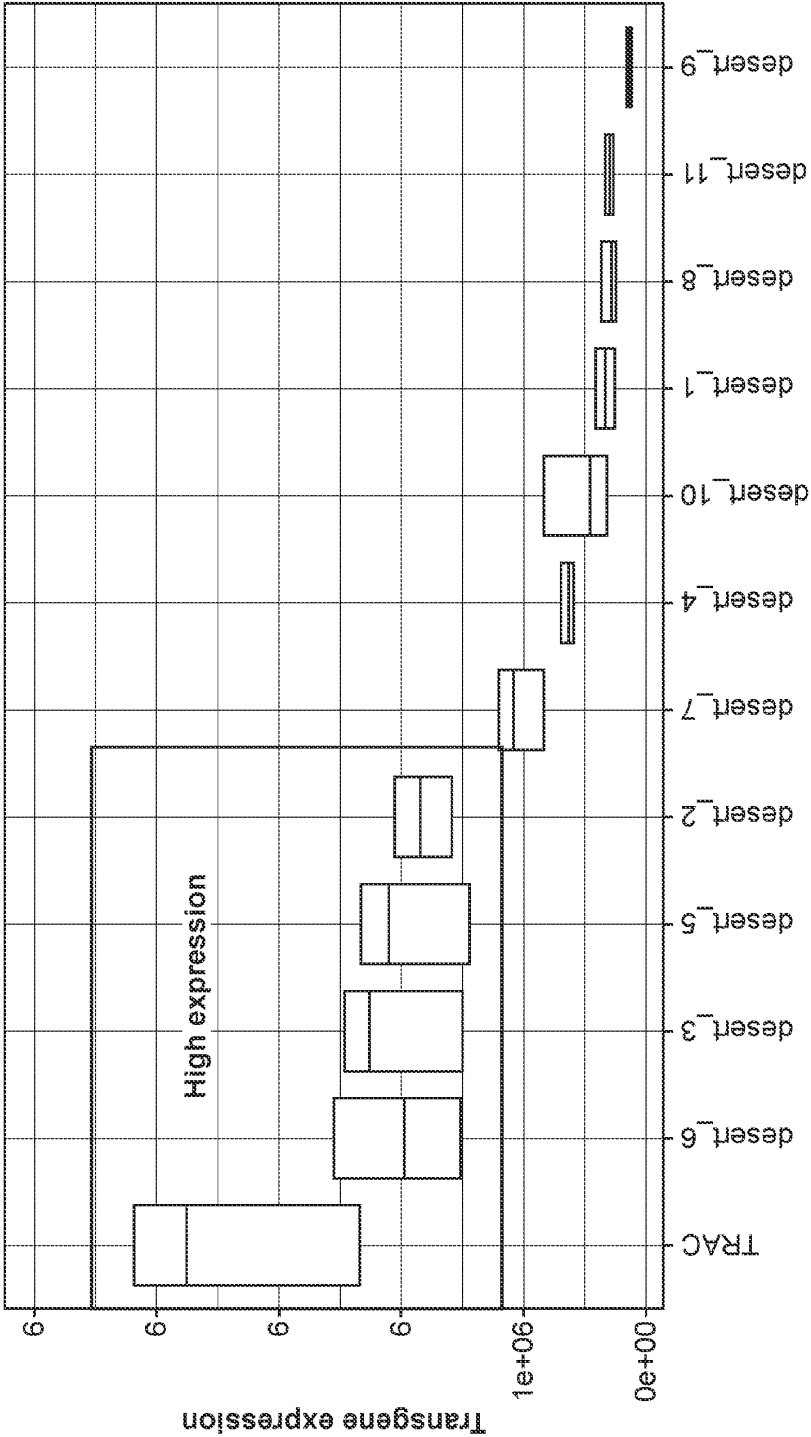


FIG. 32A

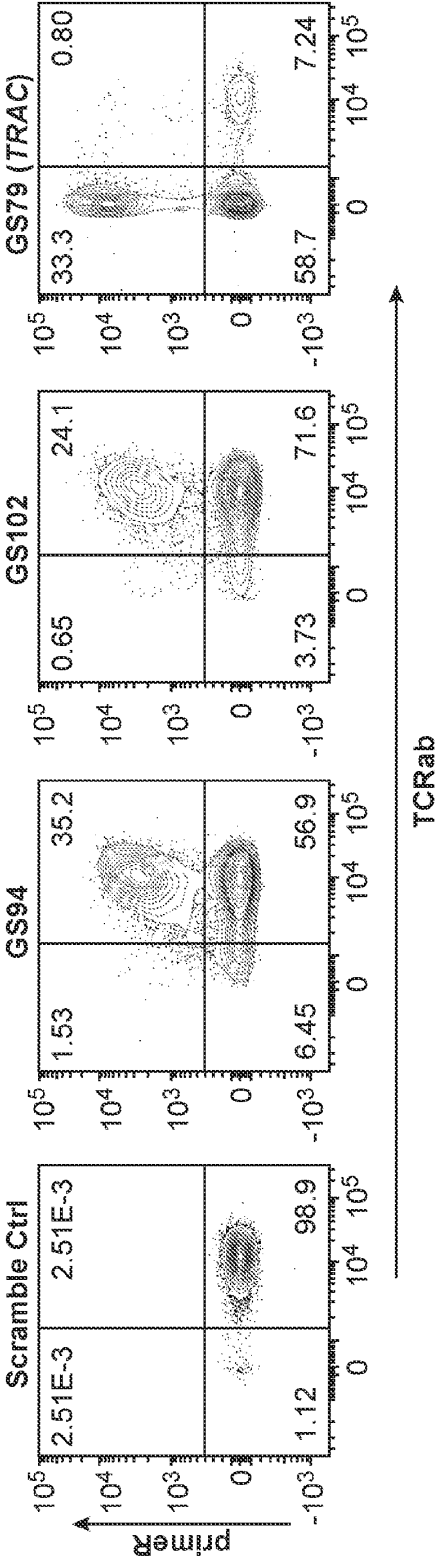


FIG. 32B

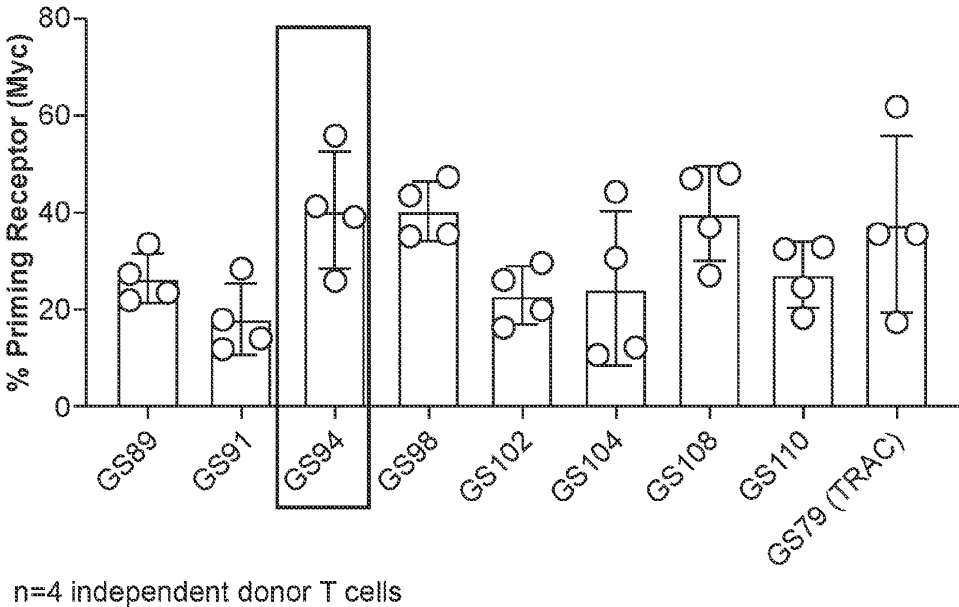


FIG. 33A

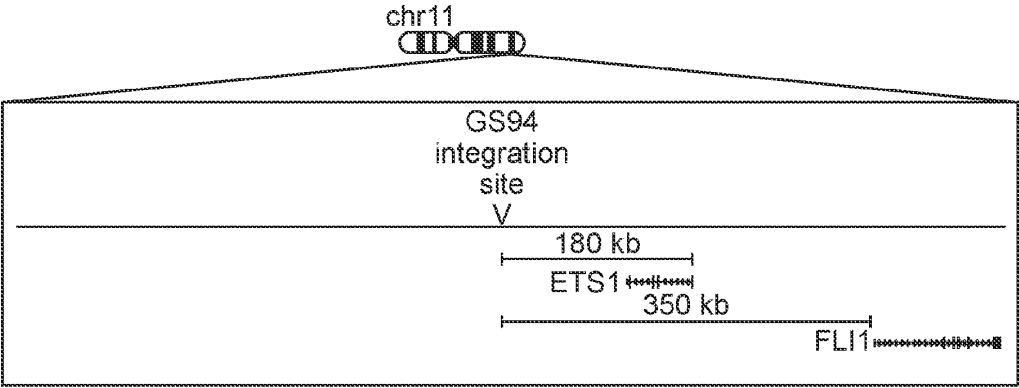


FIG. 33B

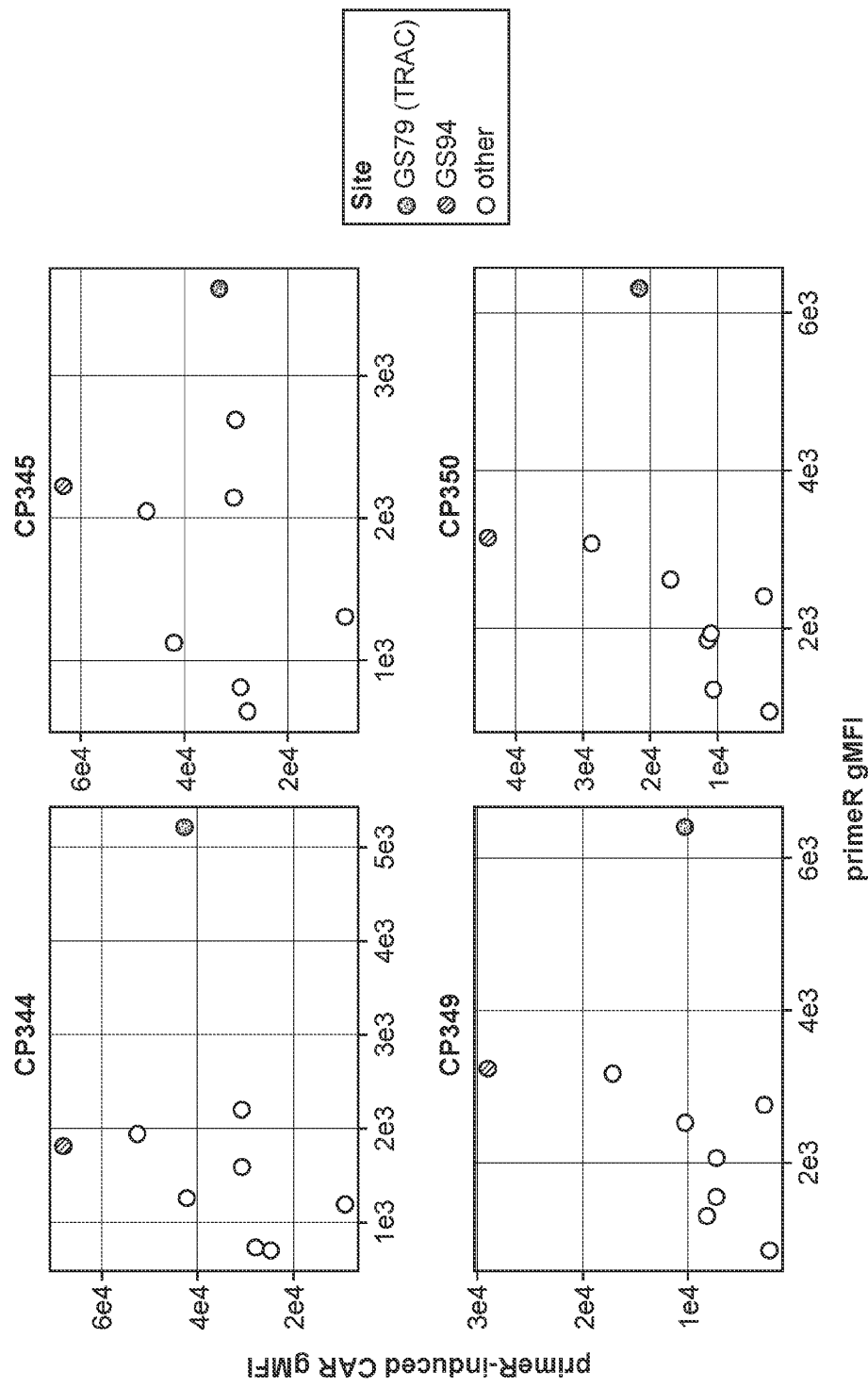


FIG. 34A

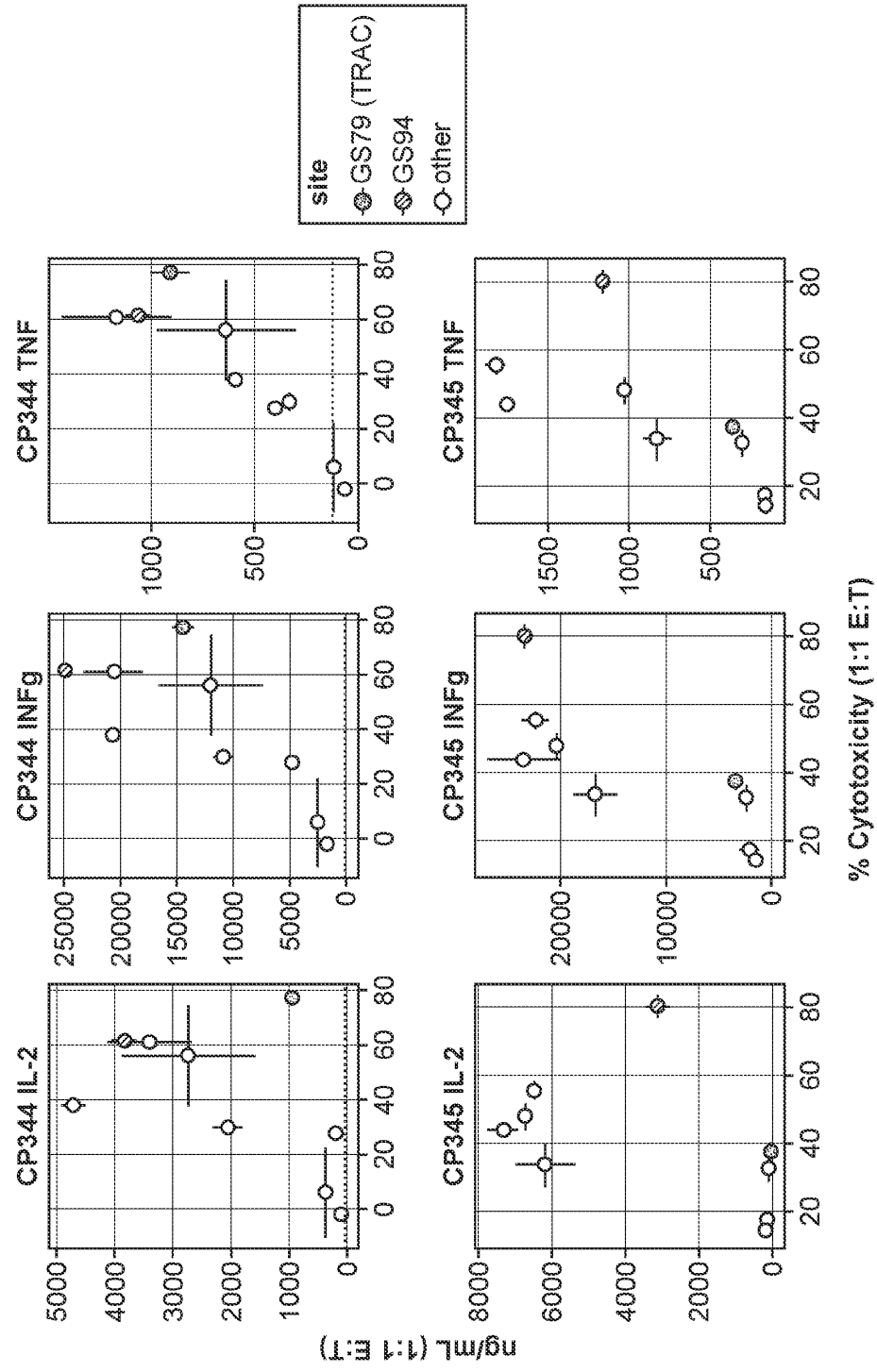


FIG. 34B

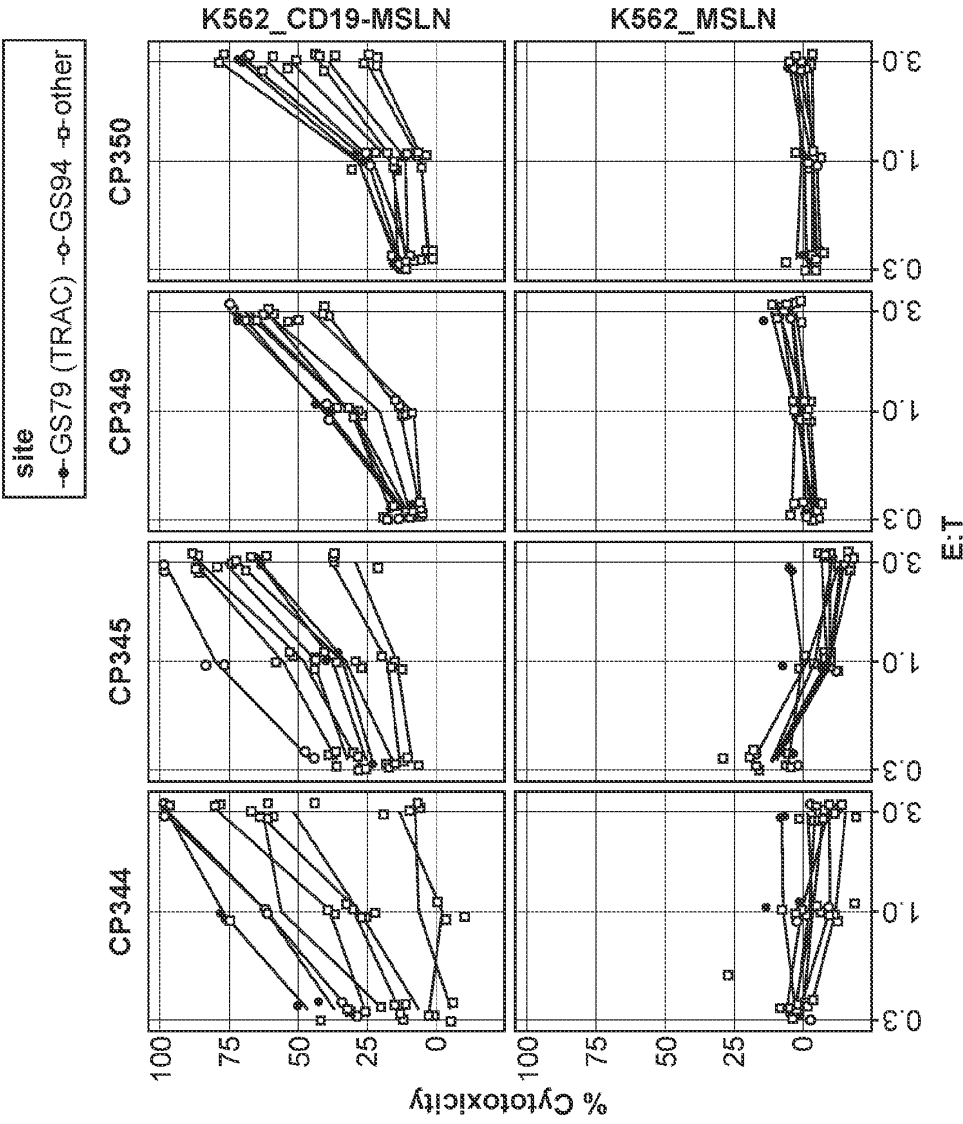


FIG. 35B

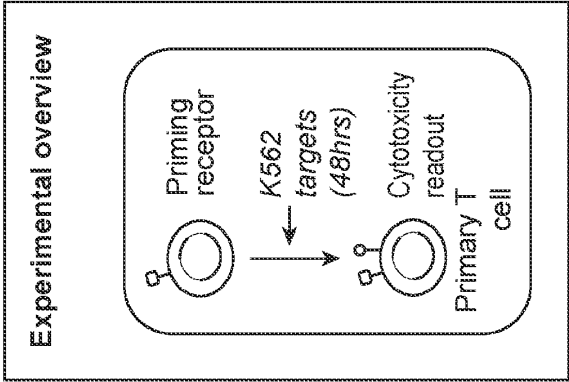


FIG. 35A

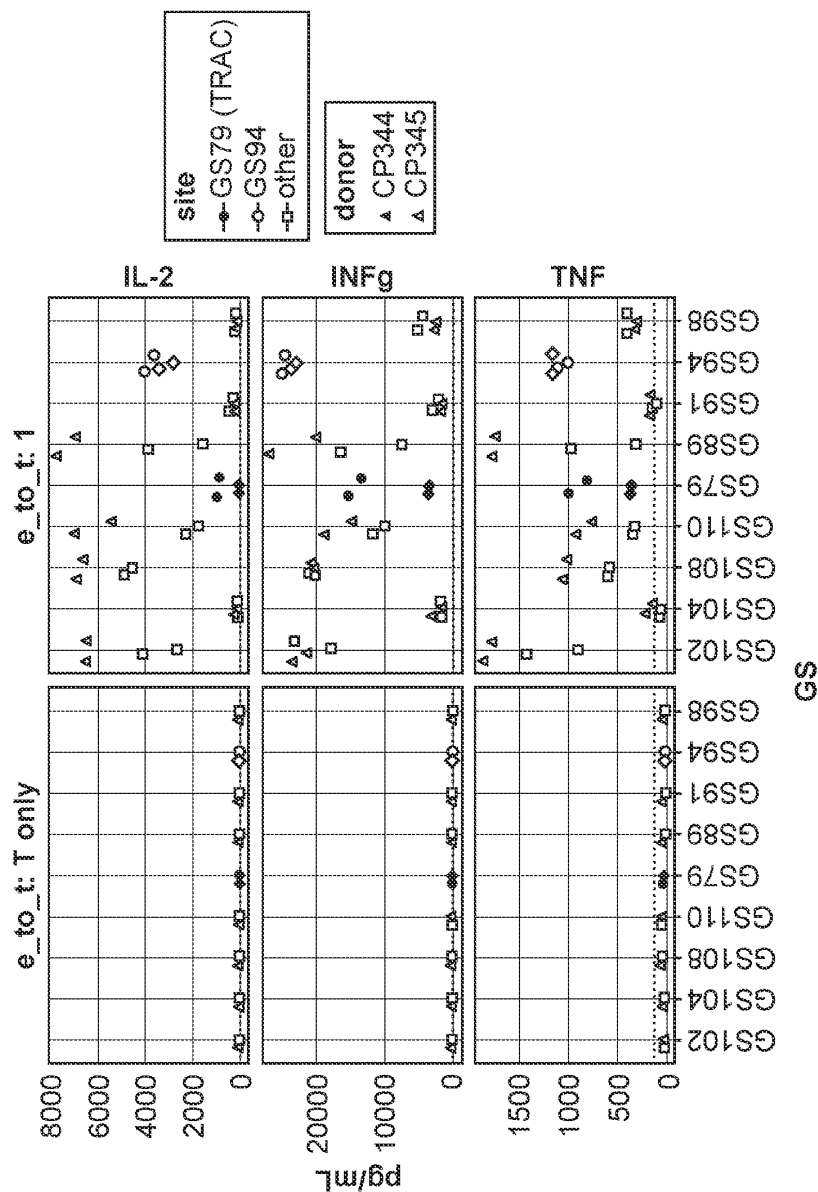


FIG. 36A

FIG. 36B

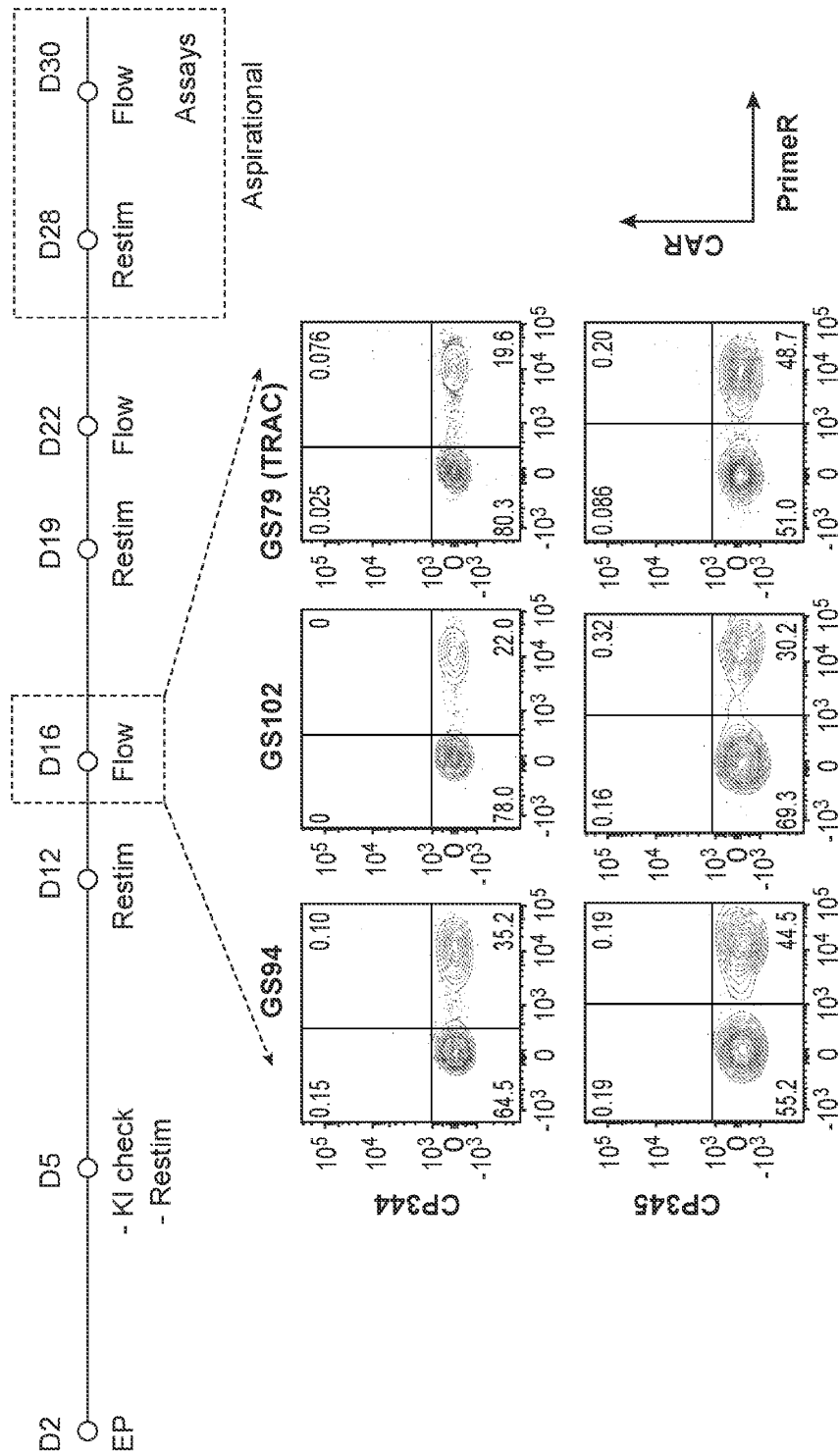


FIG. 37

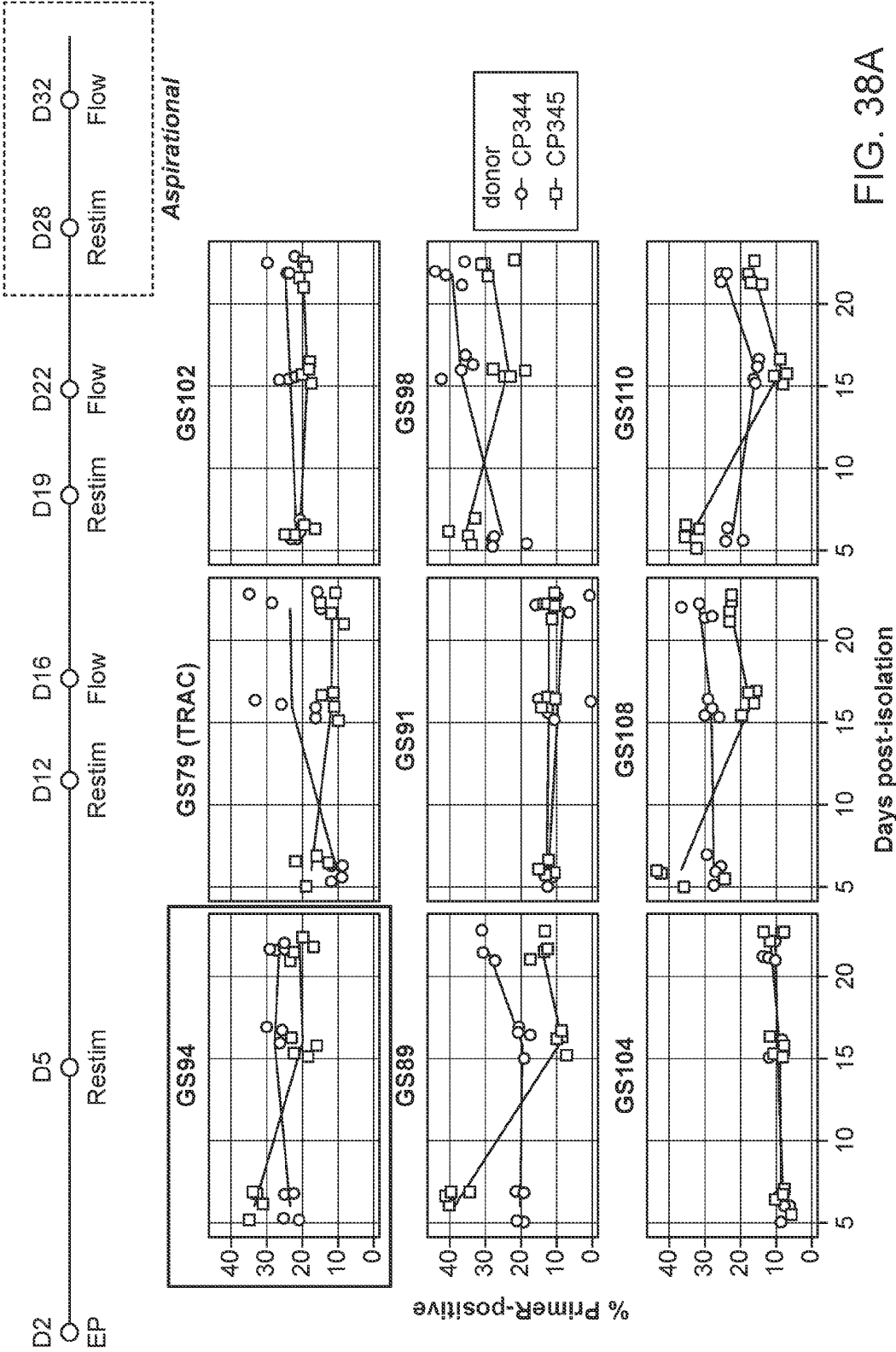
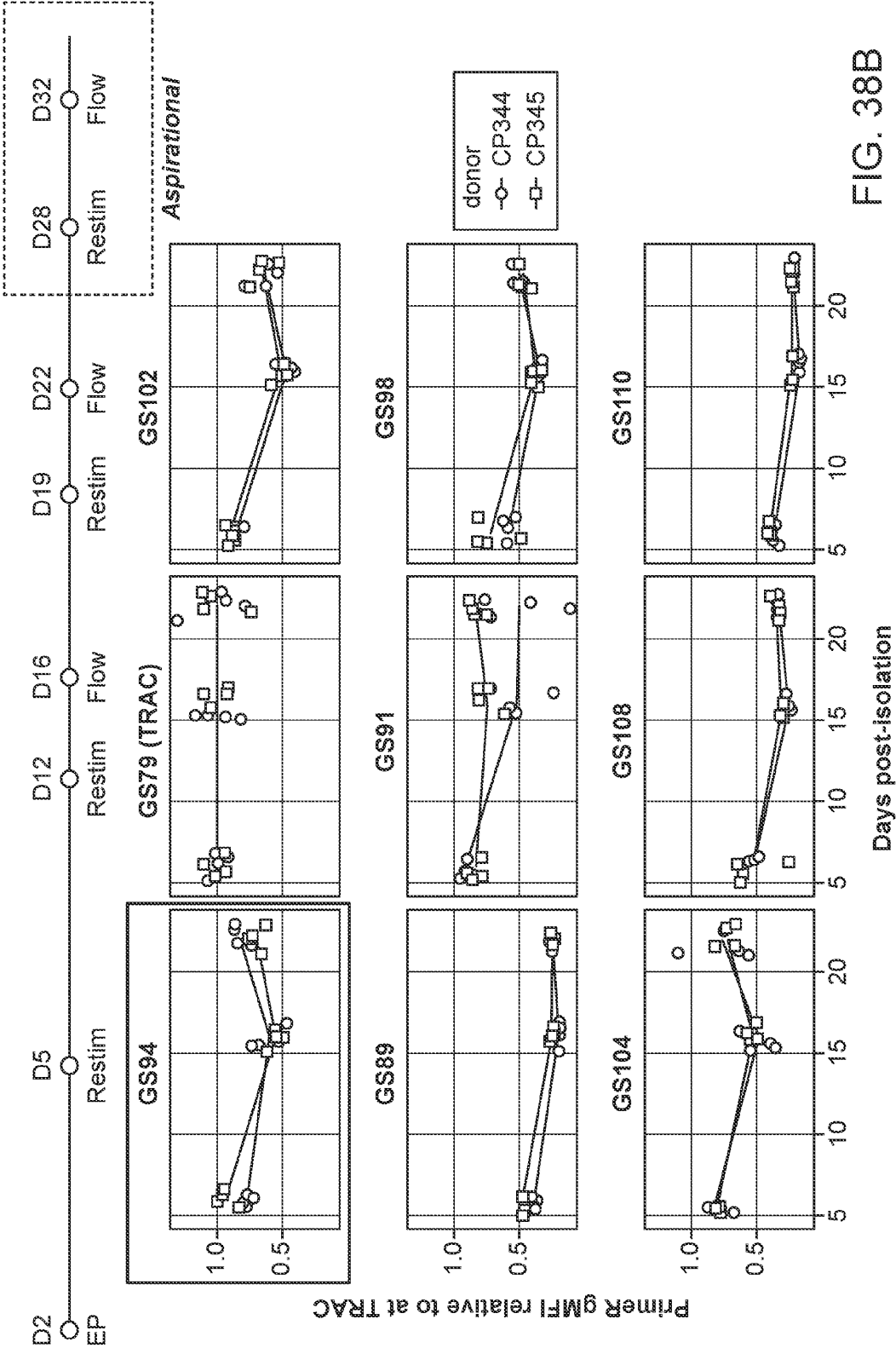
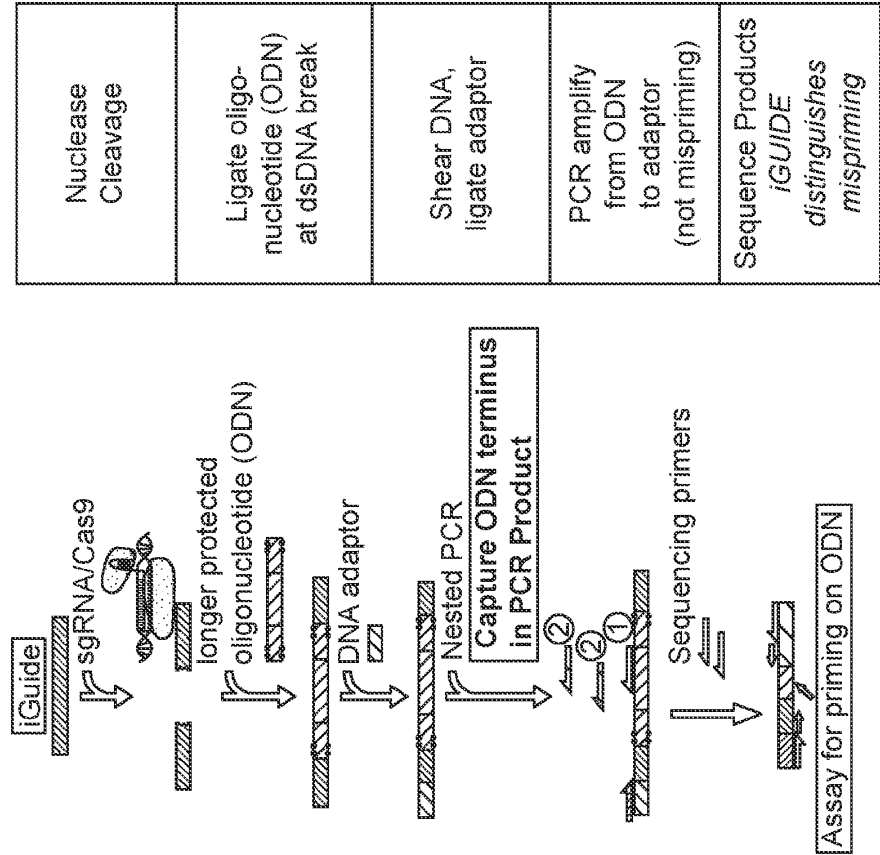


FIG. 38A



iGuide-Seq assay used to determine on-target efficiencies



Nobles et al. Genome Biol 2019

FIG. 39A

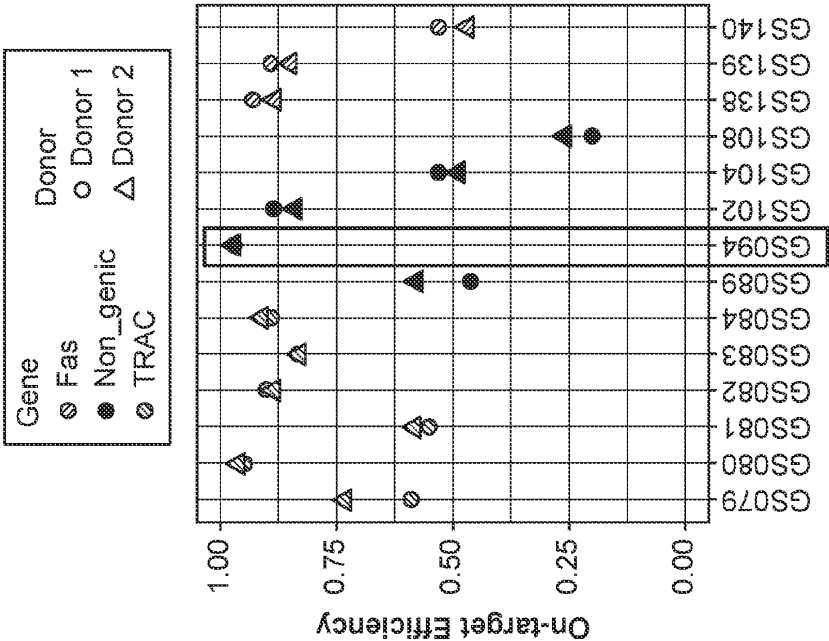


FIG. 39B

iGUIDE-seq 20pm 96W sgRNA_94 (G)

Target: sgRNA_94

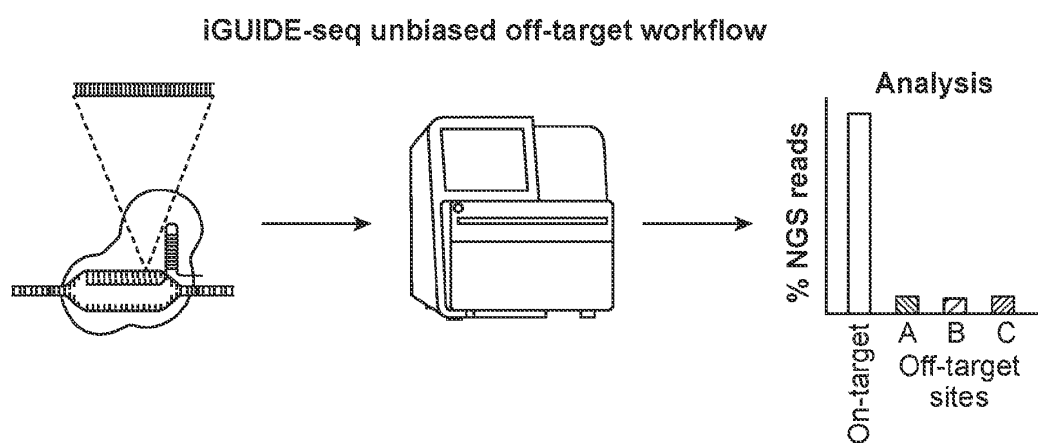
GAGCCATGCTTGGCTTACGANGG	Mismatch	Target	Abund.	MESL	Gene_ID
.....G..	0	On	2,336	95.1	ETS1~
A.T.....T.C.TA.	6	Off	7	23.9	RNU6-259P
C.....TA..CC.C.T.T.C	6	Off	5	32.3	RN7SKP254
..TGA...A...CA.T..	6	Off	5	23.7	CYCSP17
..T.T..A..T..A.T..	6	Off	4	95.1	RNU4-8P
..A.C...C...C.G.TA.	6	Off	4	36.6	TBC1D14*
..T..TCC...G.A..G.C	6	Off	4	35.4	MDFI*
.....A...TCCG..C.CT.	6	Off	3	32.3	L0C101928823*
CCT.....GCC.T.A	6	Off	3	30.0	MYBBP1A*~
..TA..A.....C.GC.GT.	6	Off	3	29.4	HSPD1P15

iGUIDE-seq 20pm 96W sgRNA_94 (Donor 2) (G)

Target: sgRNA_94

GAGCCATGCTTGGCTTACGANGG	Mismatch	Target	Abund	MESL	Gene_ID
.....G..	0	On	2,164	95.5	ETS1~
.....C.GAG.....C.C.GA.	6	Off	4	37.3	ZFPM1*
..TA...T...TA.....C.CA.	6	Off	2	27.3	OR6D1P
.....C.....GC.CA.CT.C	6	Off	2	25.2	NPHP4*
.....GTC.....GAG.....TT.	6	Off	1	80.5	ELN~
A.....GAT.....A..T...A.T	6	Off	1	56.9	RAPGEF4*
C.....CT..A..T.CA.T	6	Off	1	43.2	TMEM181*
.....G.C.T..C...A.CA.T	6	Off	1	37.3	LDHD
..CA...C.T..A..T.AA.	6	Off	1	37.3	LINC01392
C...AG..A.G.....C...C..	6	Off	1	32.6	C14orf180

FIG. 39C



GS94 gRNA extremely specific, and superior to all other gRNAs evaluated

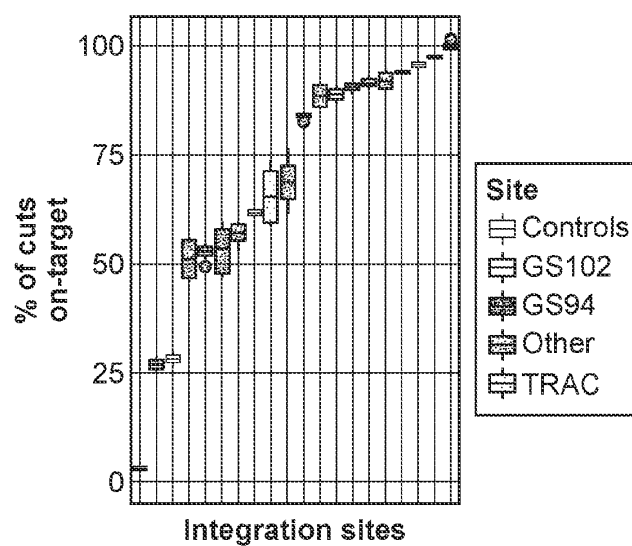


FIG. 40

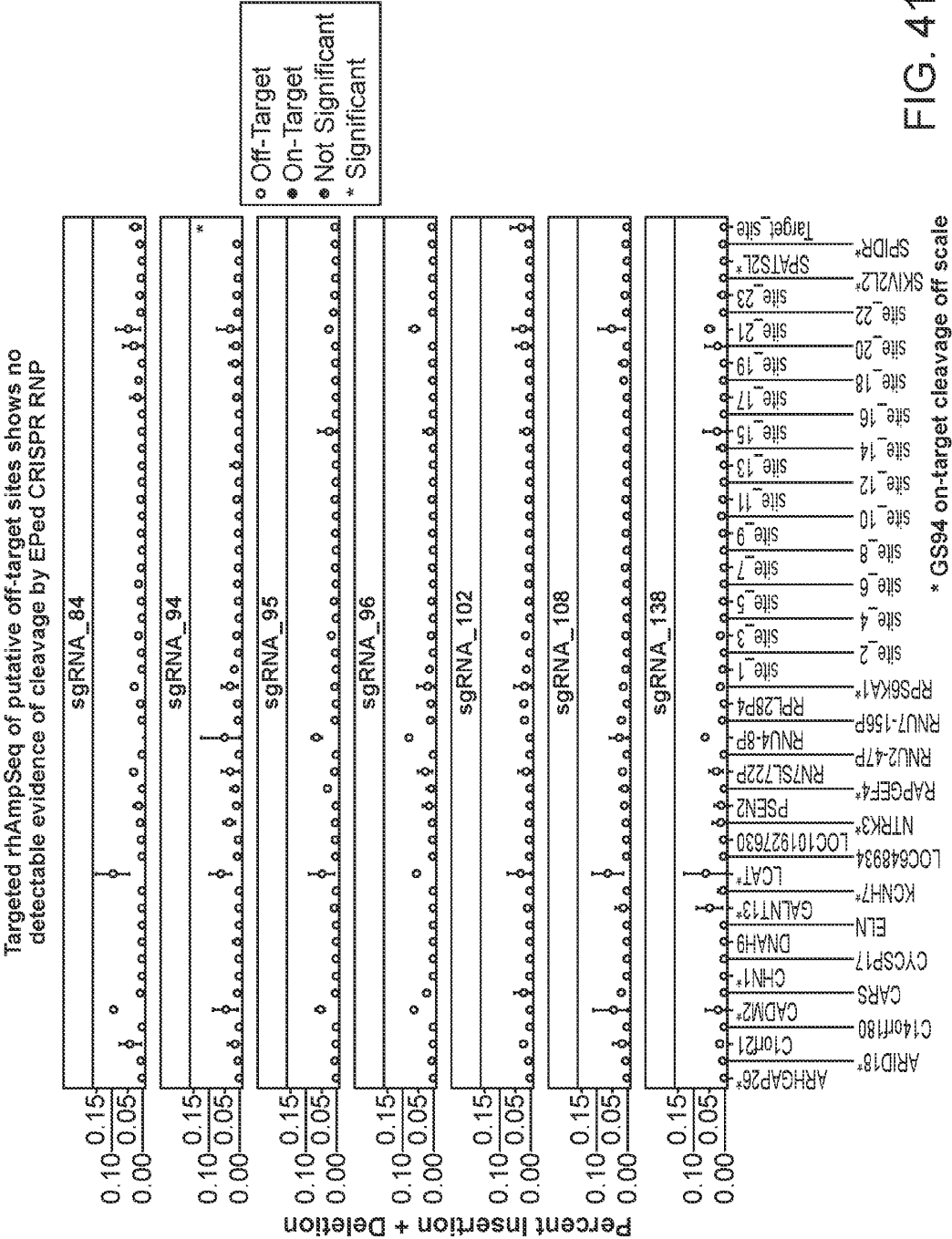


FIG. 41

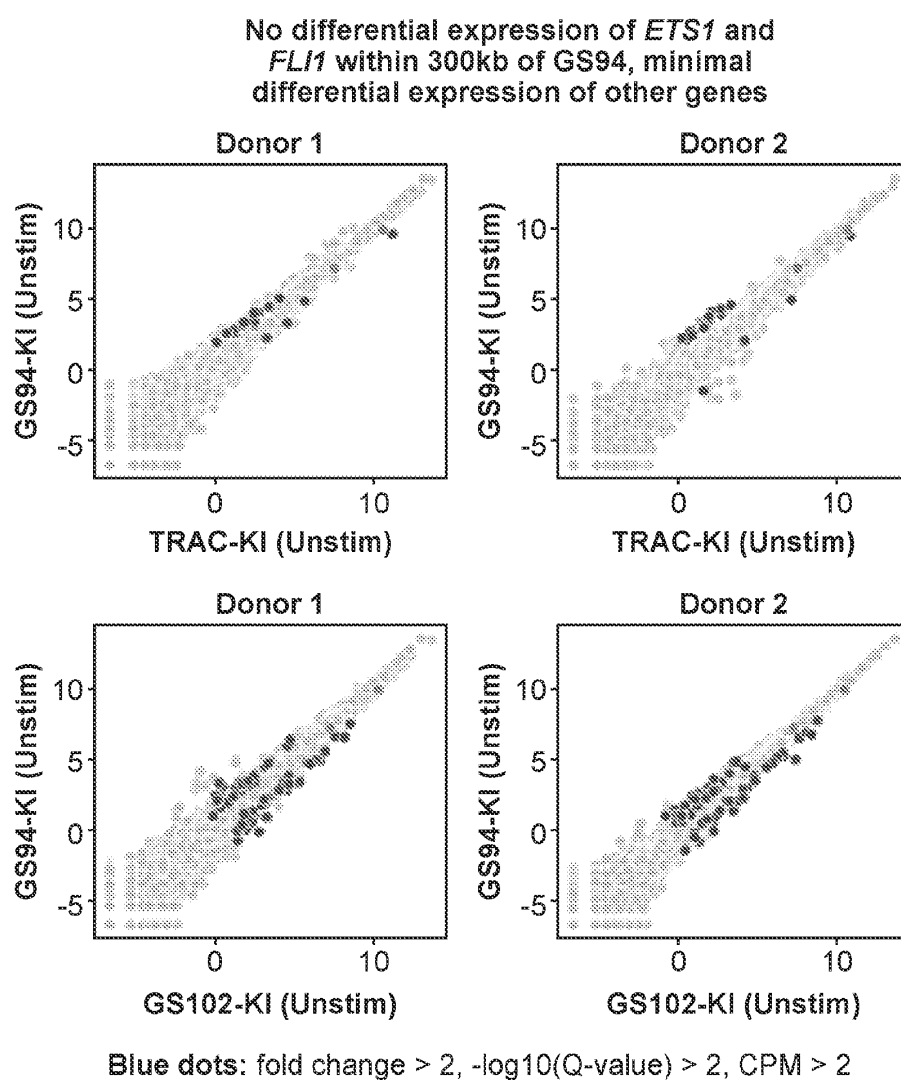


FIG. 42

No evidence of cytokine-independent growth in GS94 KI
primary T cells (short-term assay)

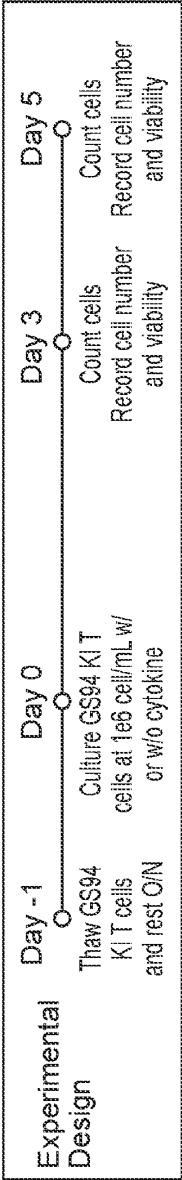
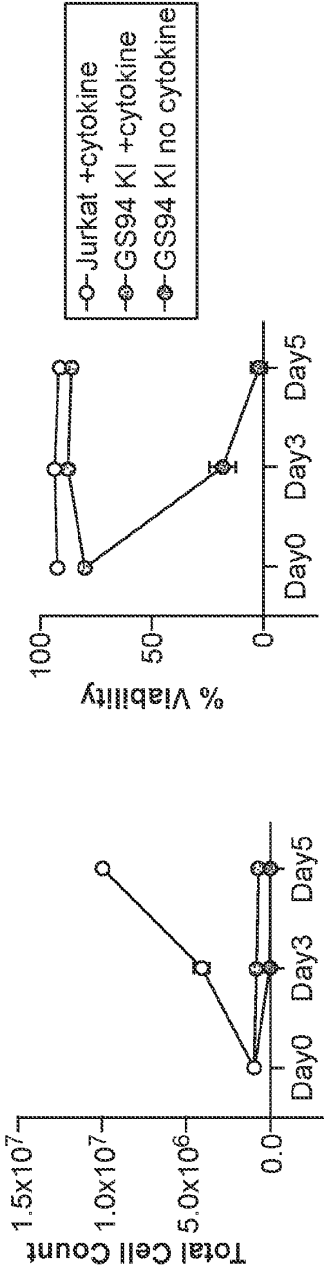


FIG. 43

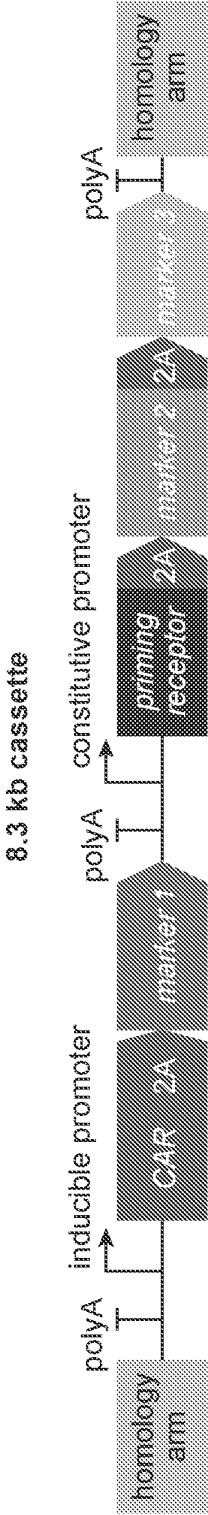


FIG. 44

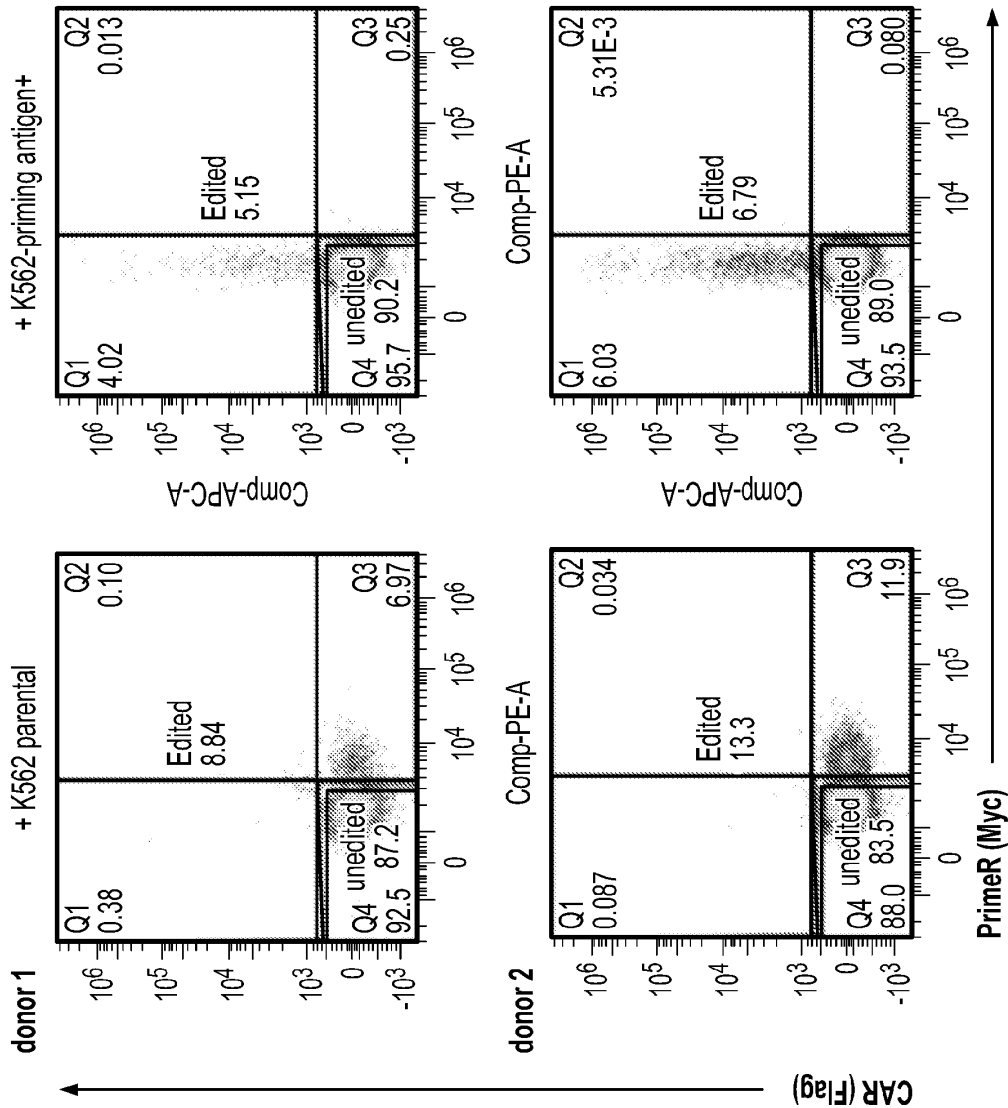


FIG. 45

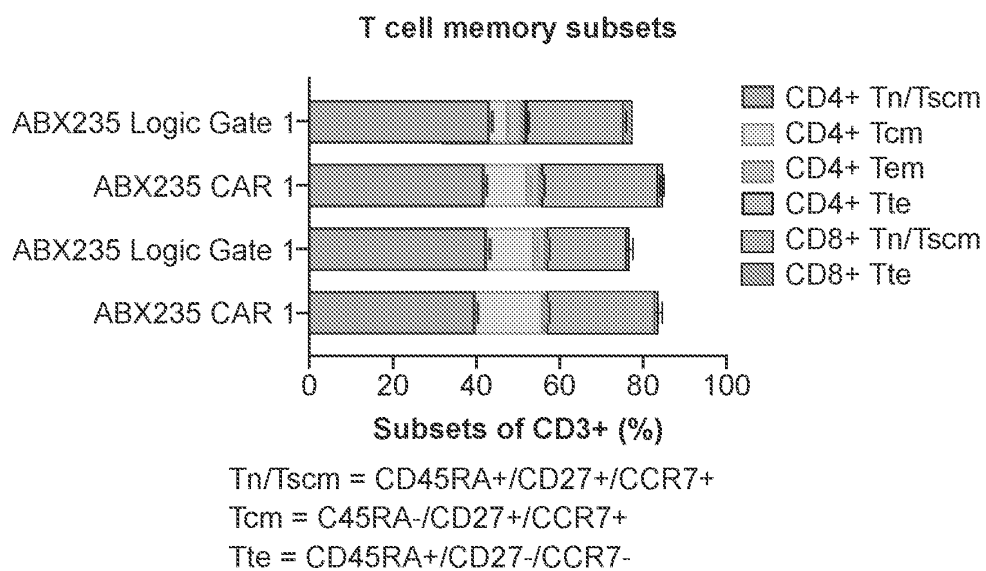


FIG. 46