

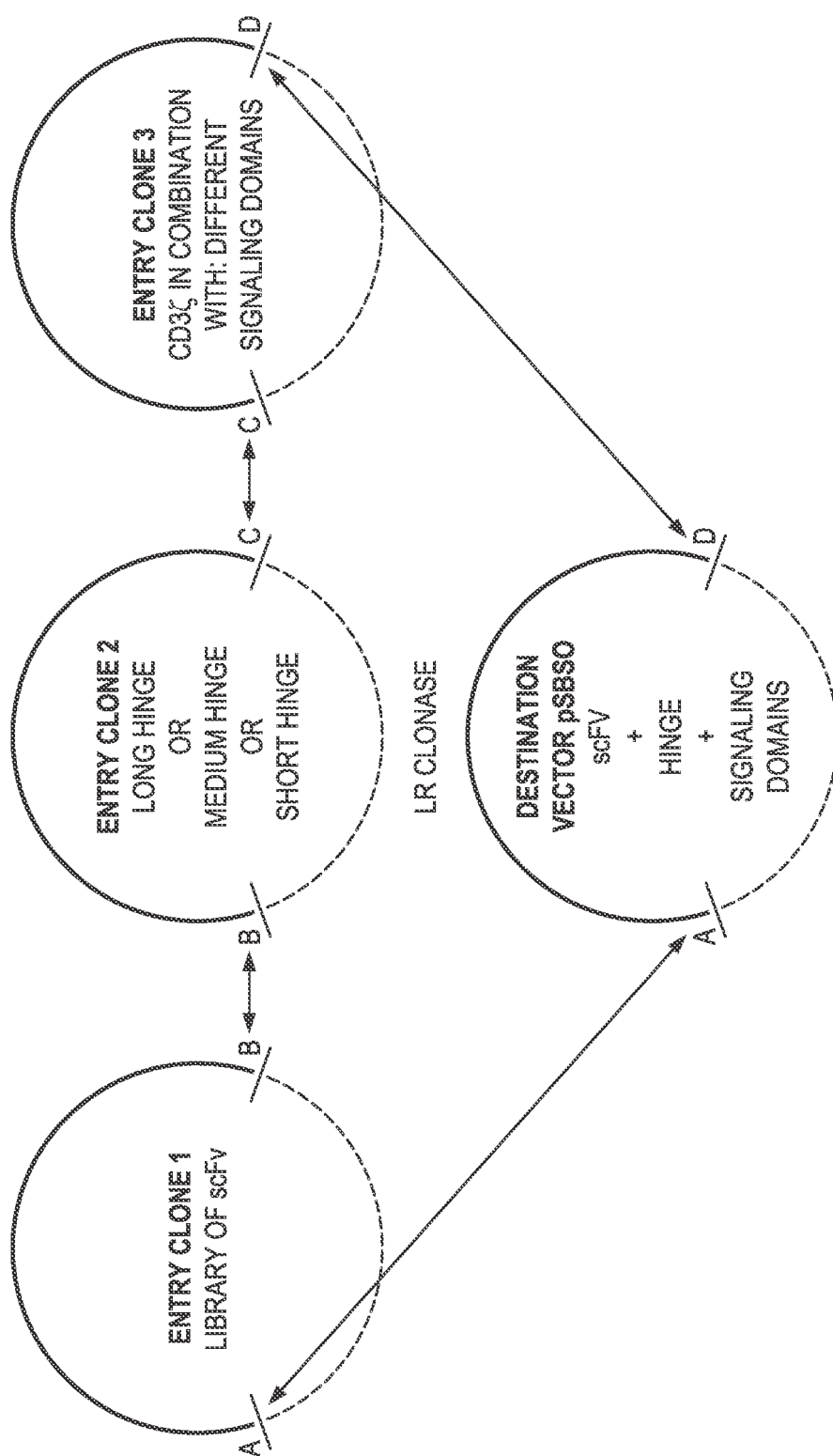


FIG. 1

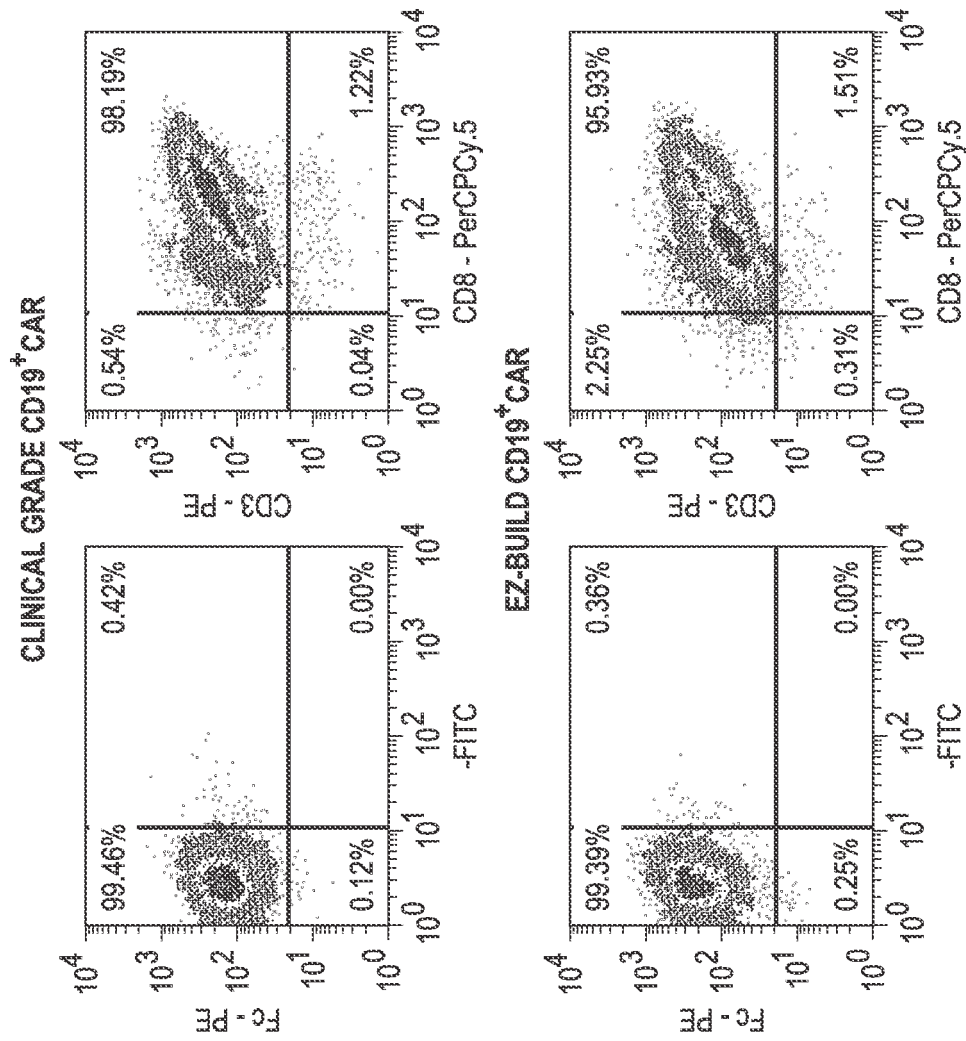


FIG. 2A

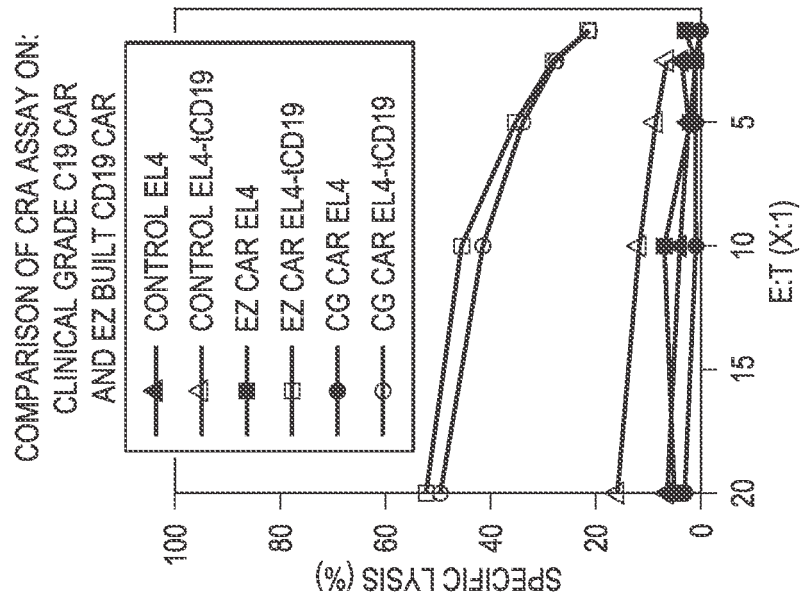


FIG. 2B

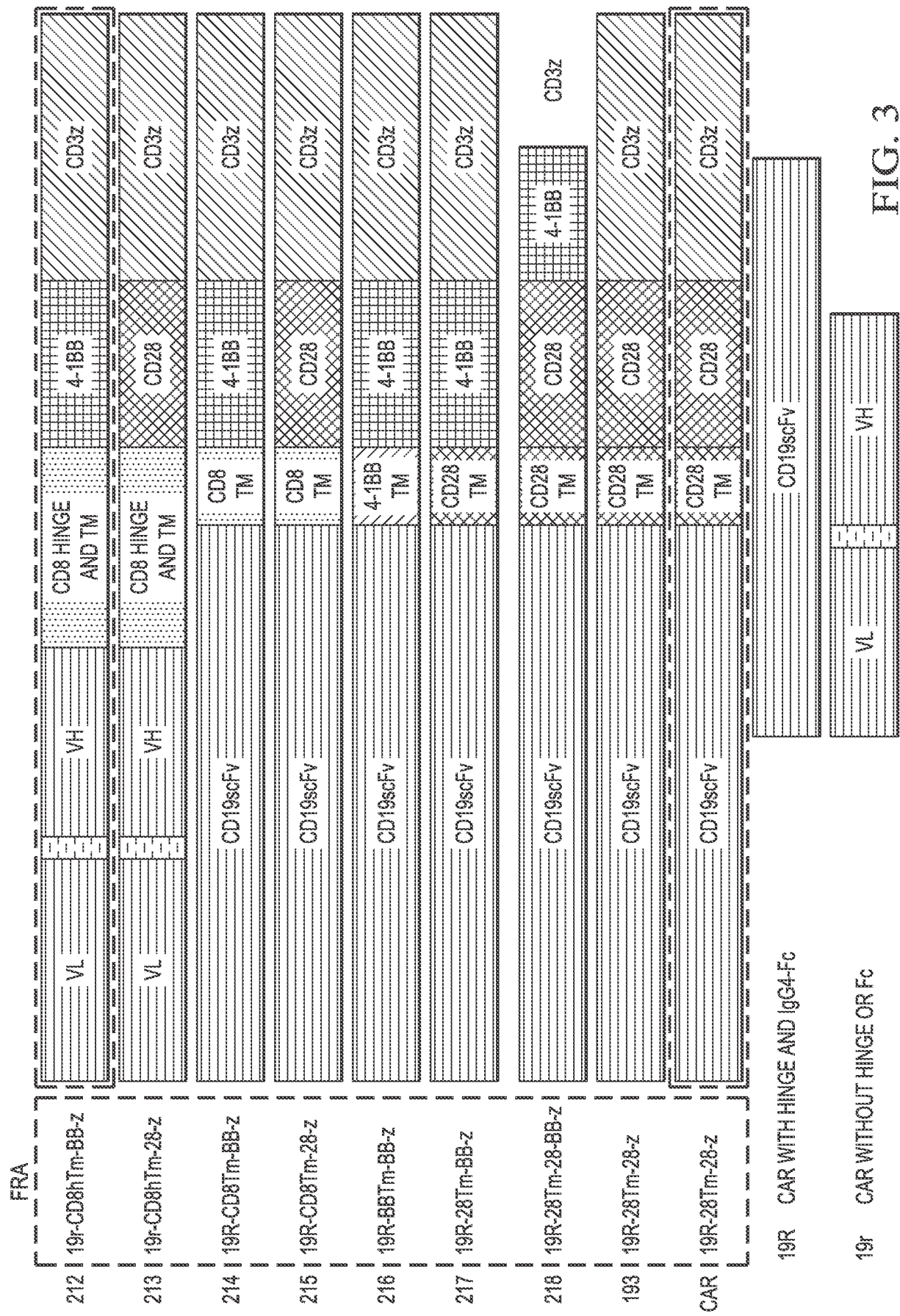


FIG. 3

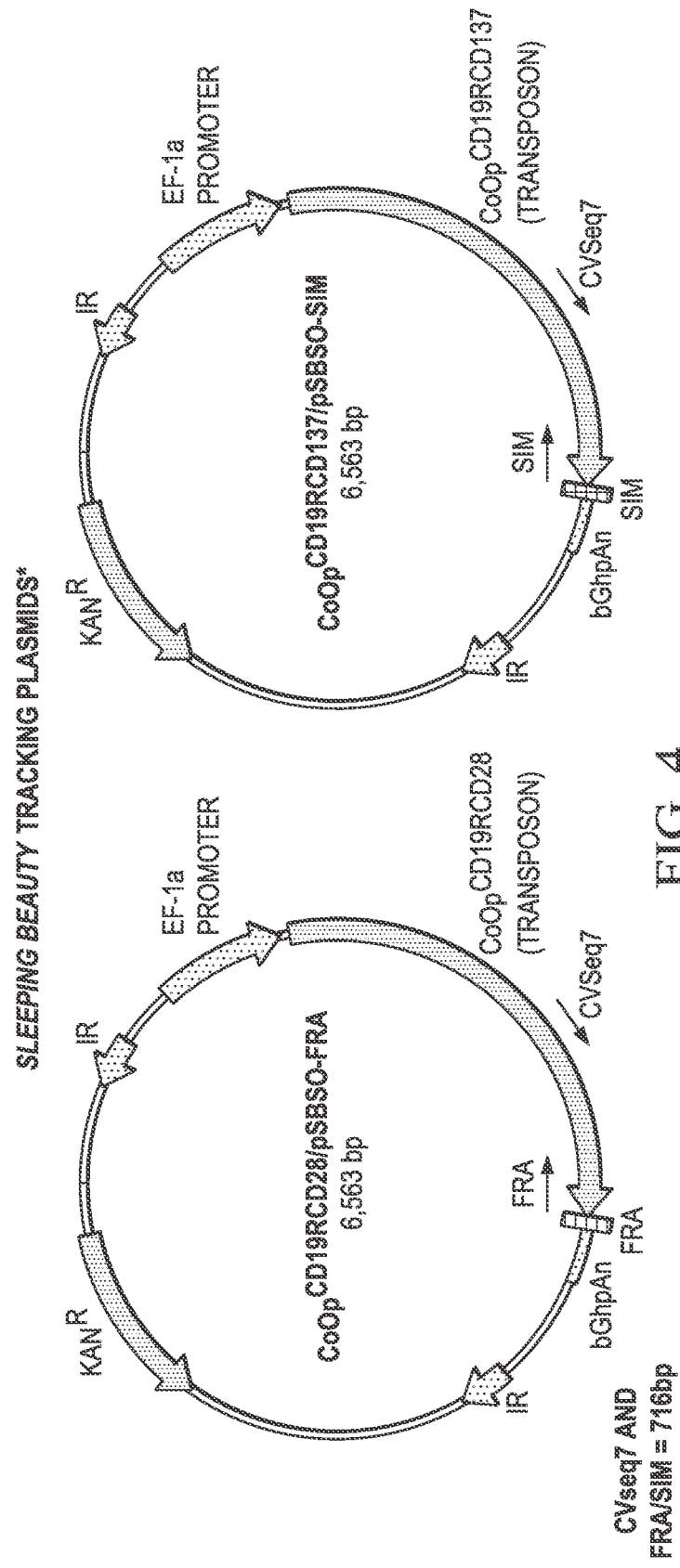


FIG. 4

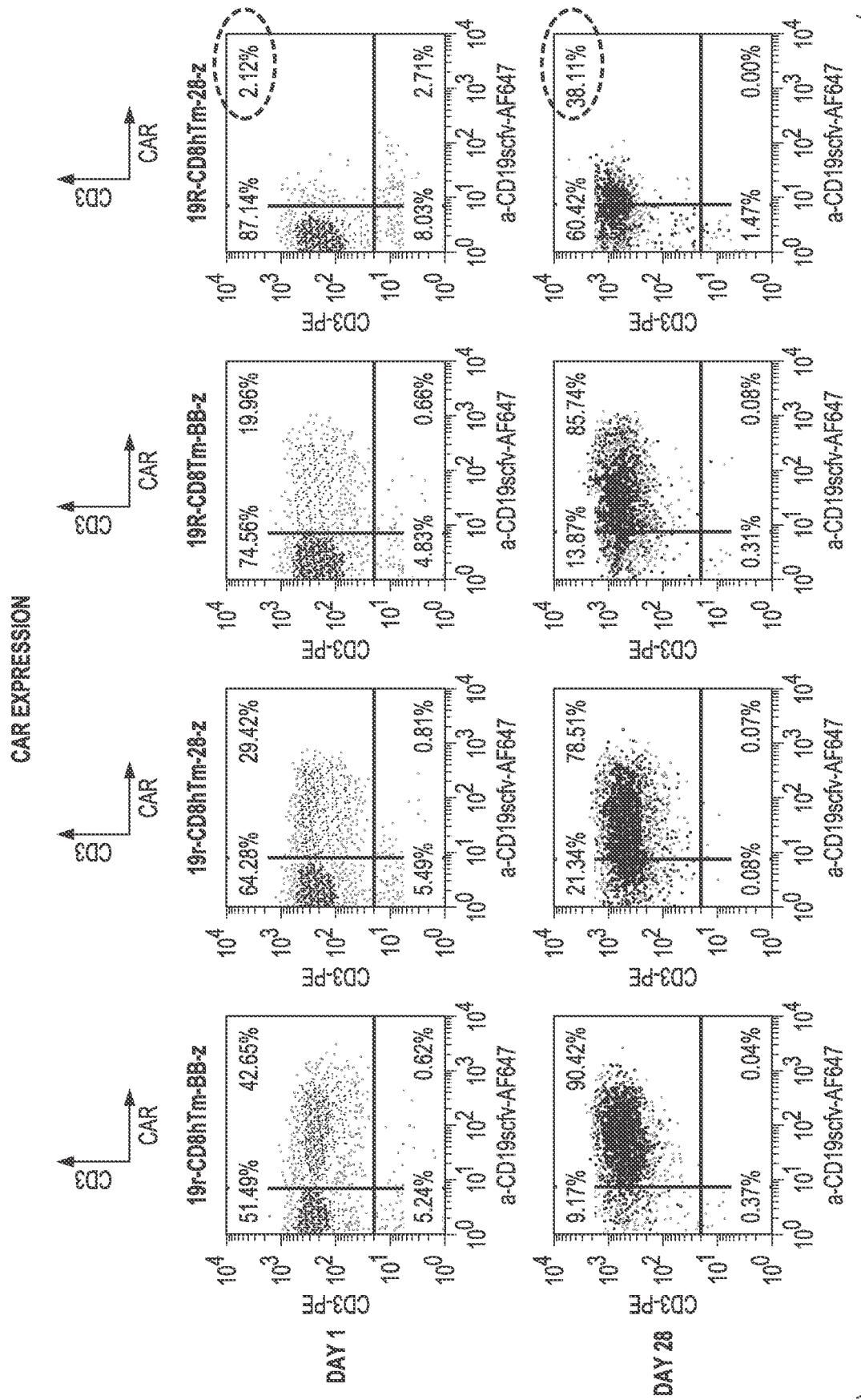


FIG. 5-1

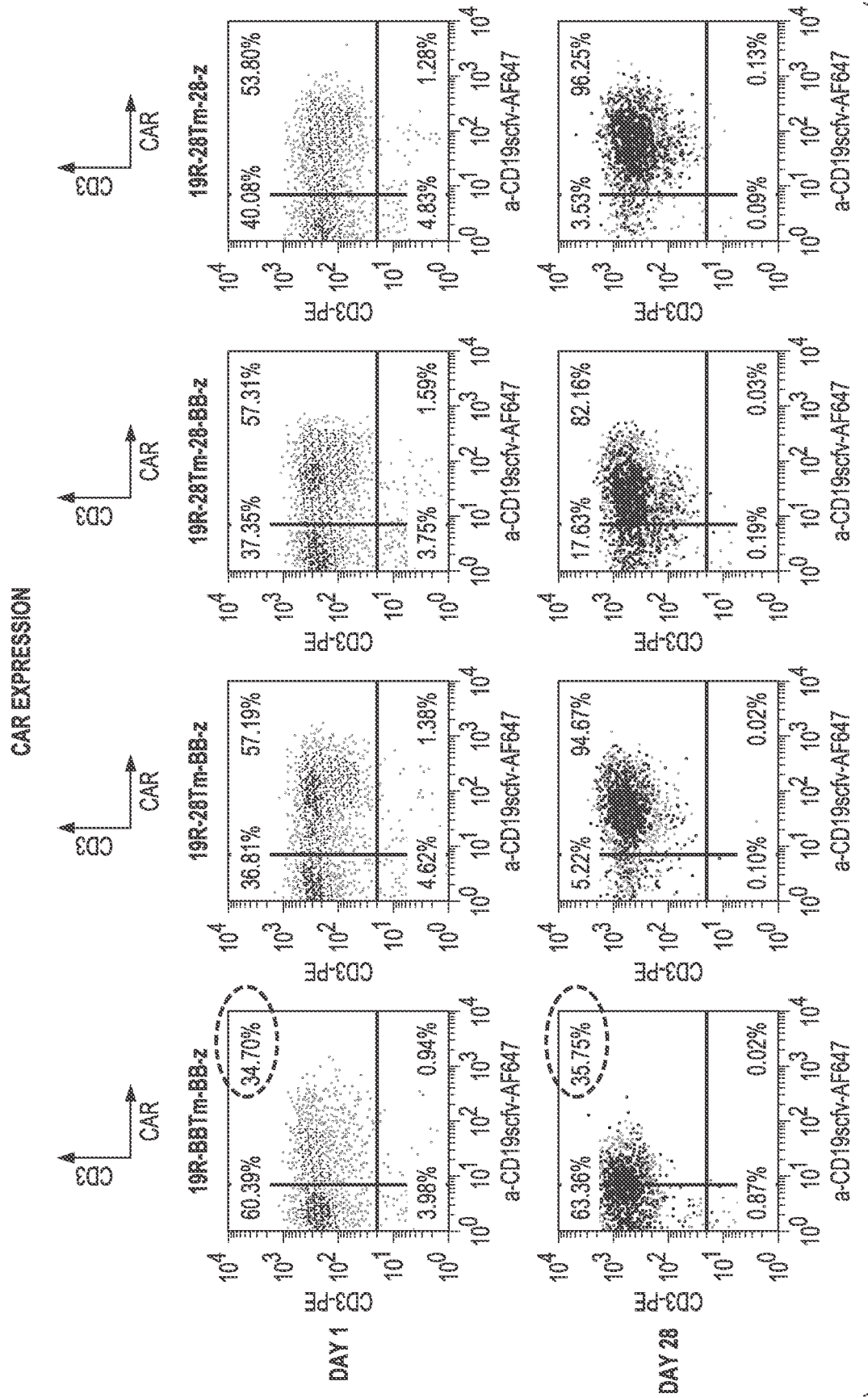


FIG. 5-2

7/43

FIG. 6

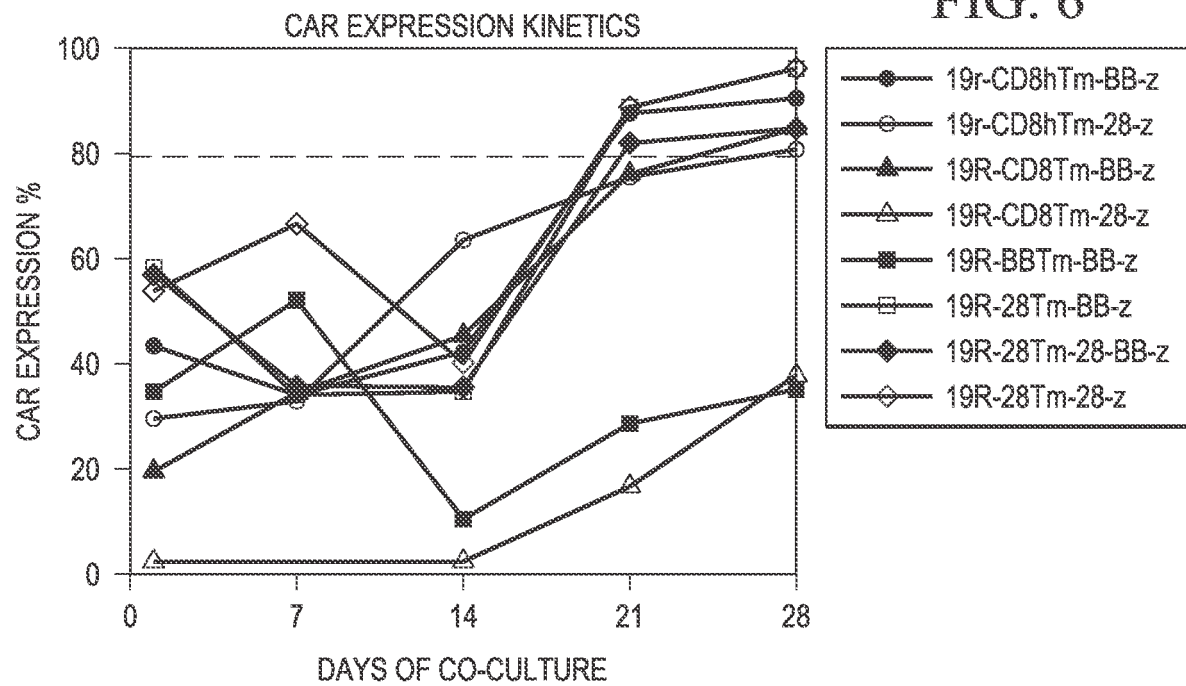
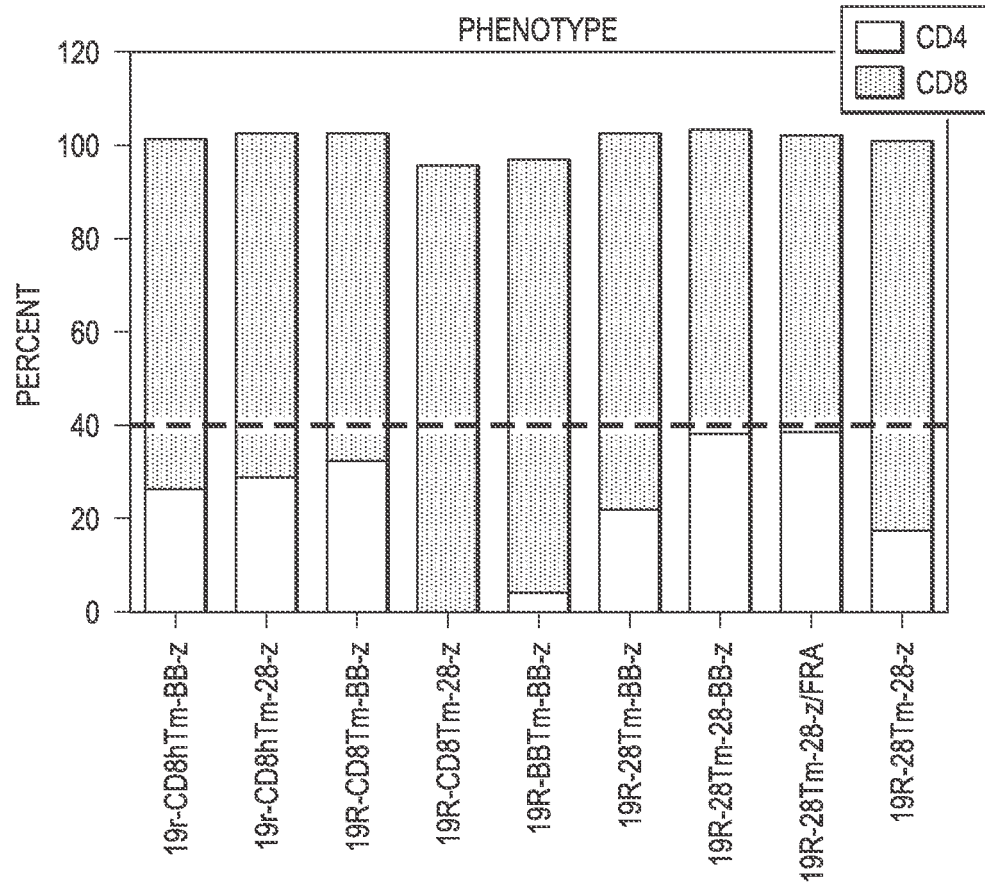


FIG. 7



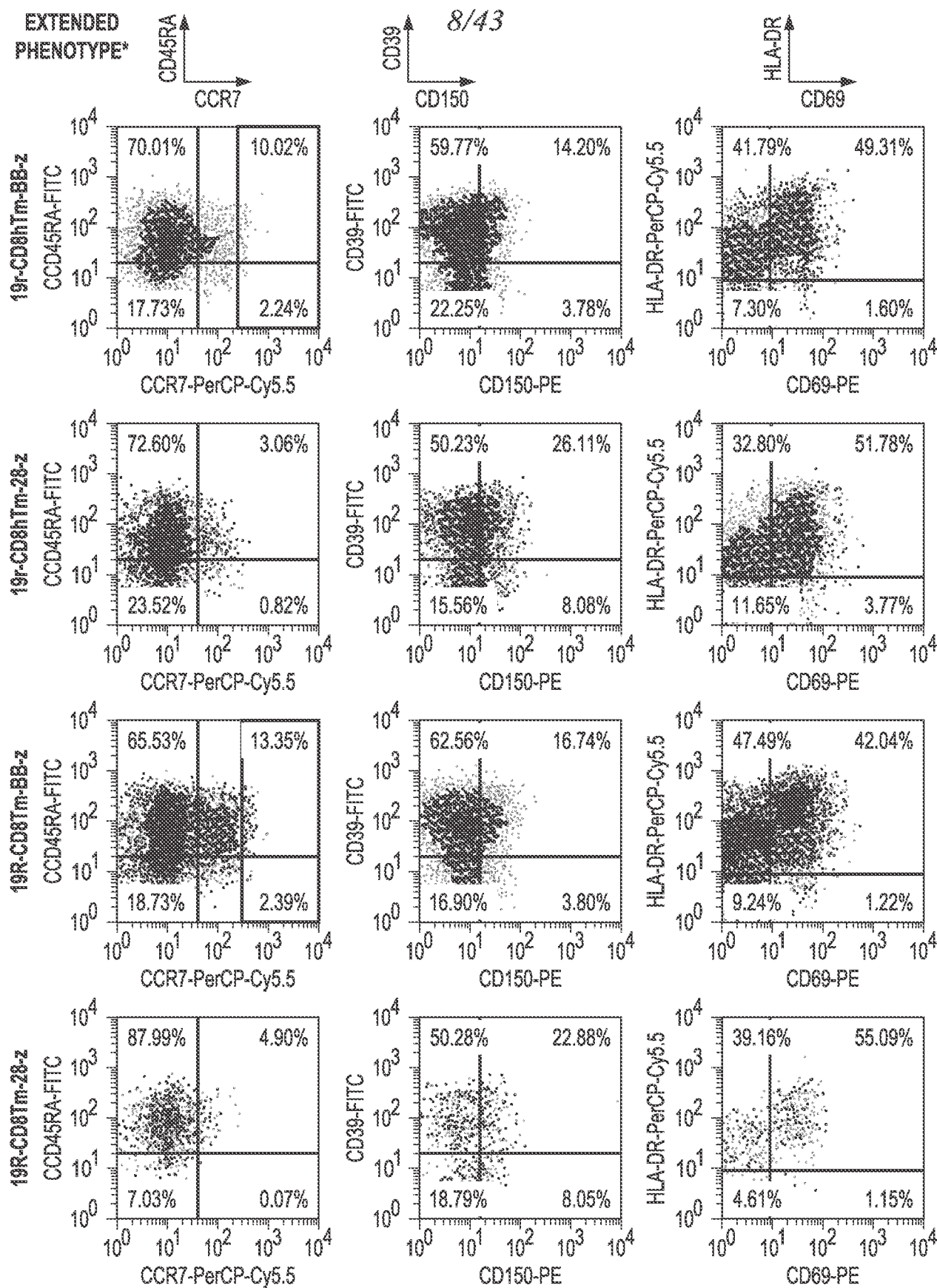
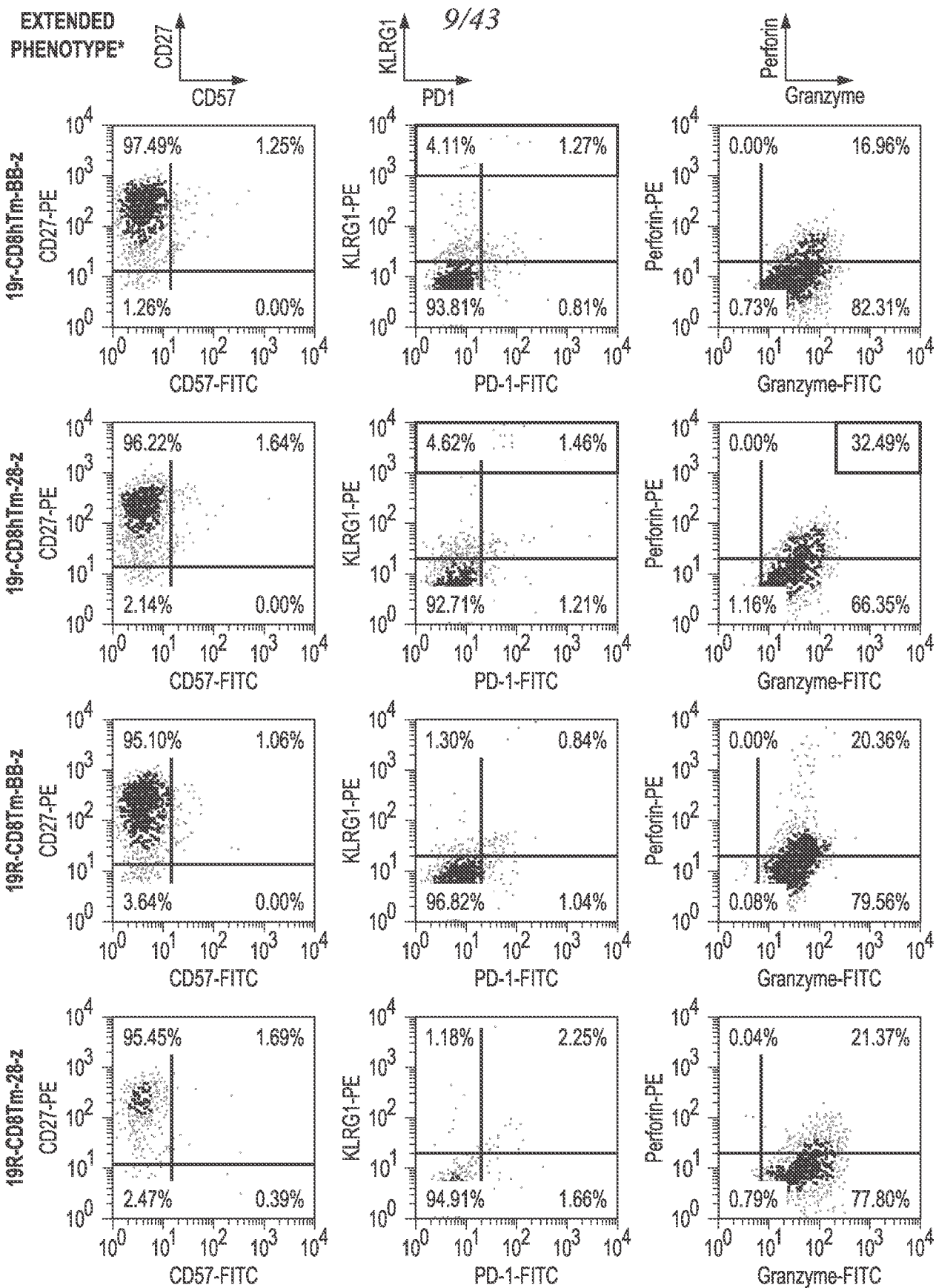


FIG. 8A-1



**FIG. 8A-2**

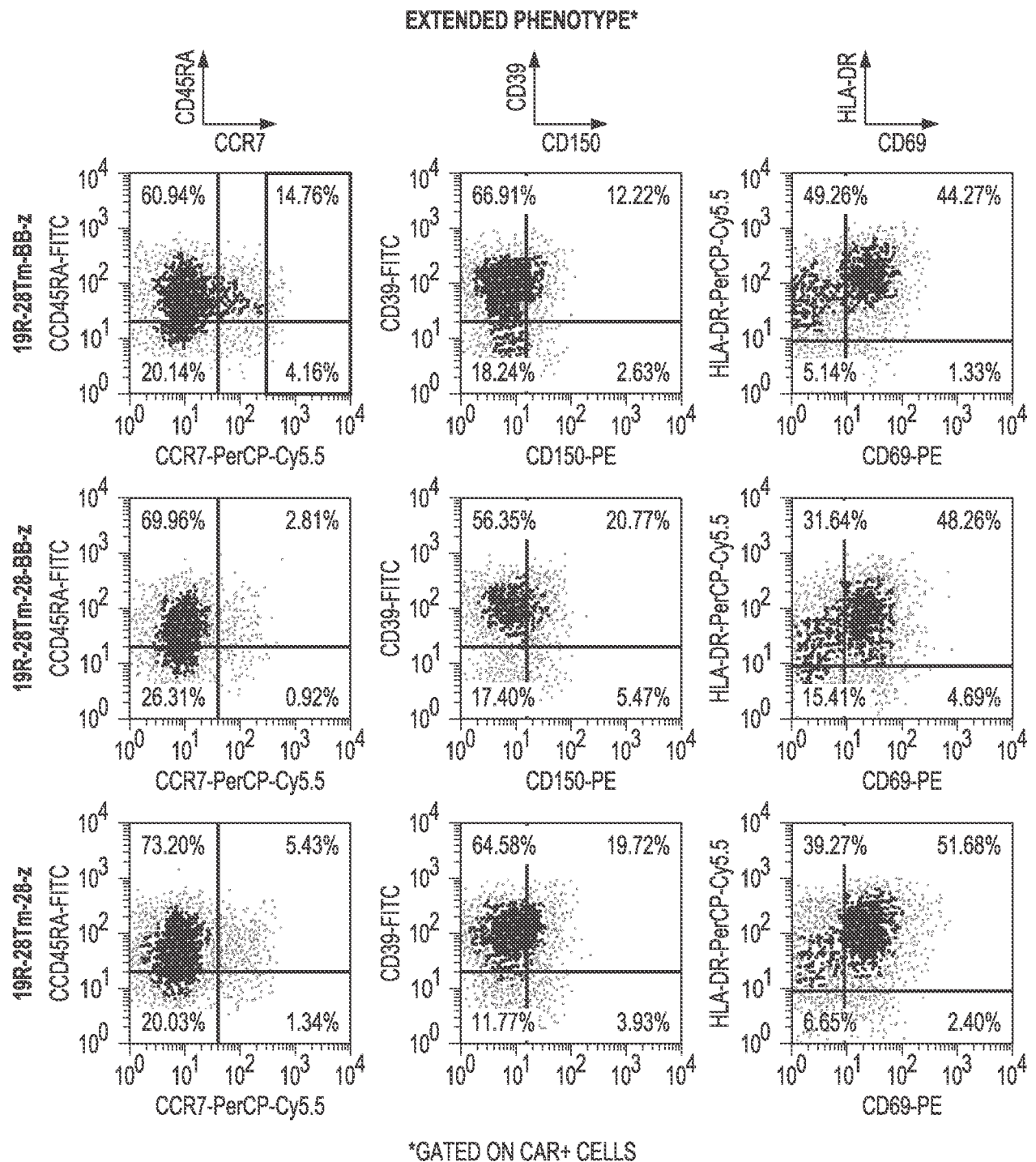


FIG. 8B-1

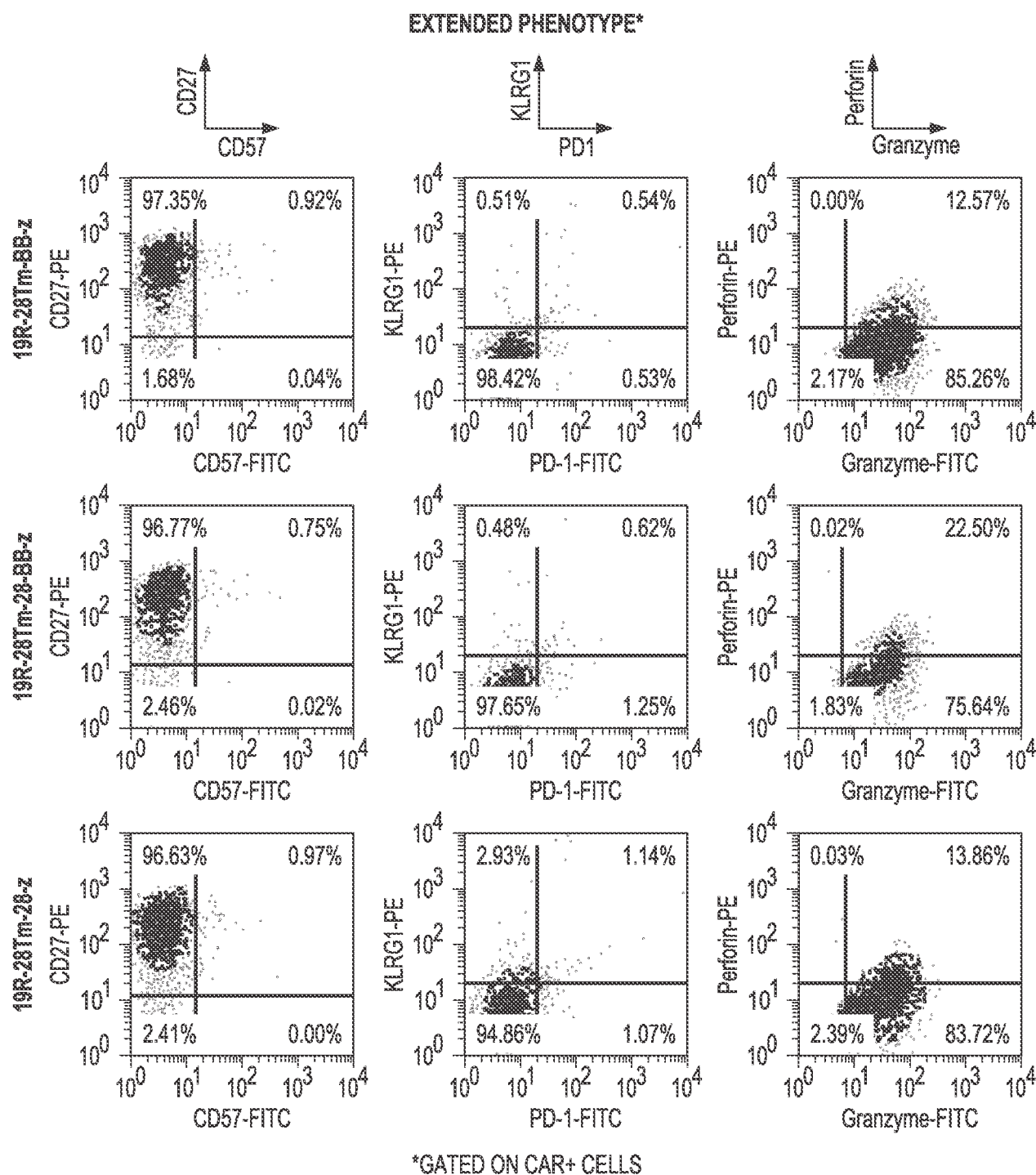


FIG. 8B-2

WESTERN BLOT ANALYSIS\*

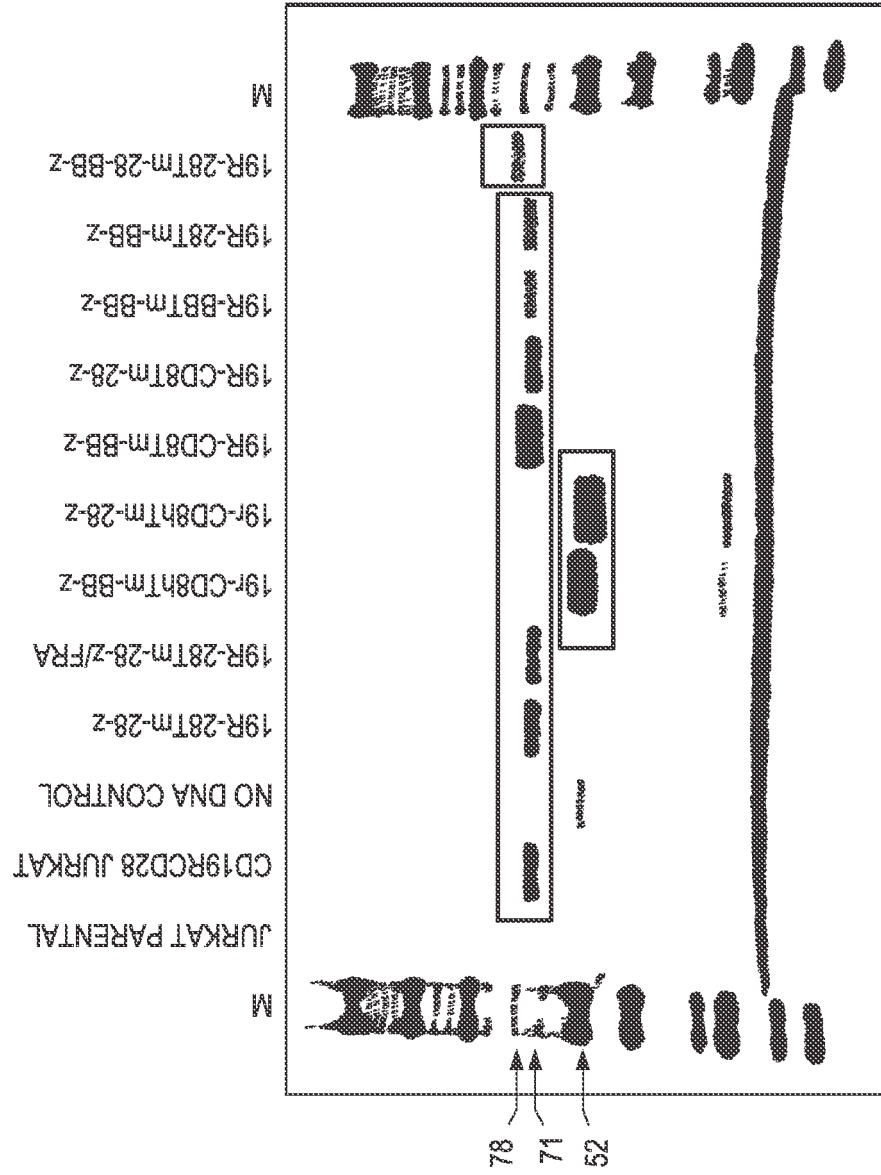
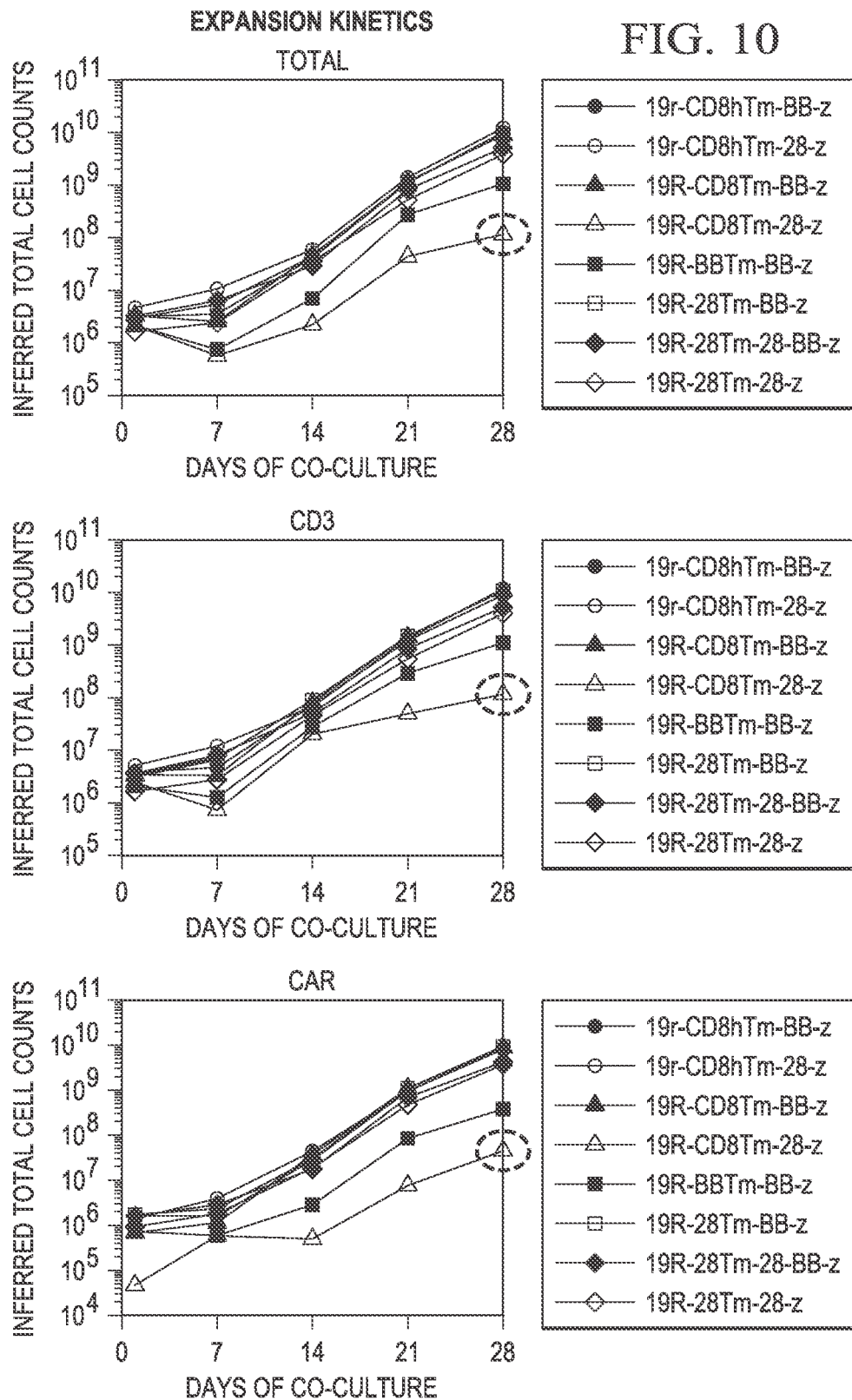


FIG. 9

| CONSTRUCT         | MOLECULAR WT (kDS) |      |
|-------------------|--------------------|------|
|                   | EXPTD              | OESD |
| 19R-28Tm-28-z     |                    | 71.4 |
| 19R-28Tm-28-z/FRA | 73.1               | 70.1 |
| 19r-CD8hTm-BB-z   | 52.2               | 51.4 |
| 19r-CD8hTm-28-z   | 52.1               | 51.9 |
| 19R-CD8Tm-BB-z    | 73.3               | 73.5 |
| 19R-CD8Tm-28-z    | 73.2               | 70.7 |
| 19R-BBTm-BB-z     | 73.3               | 72.8 |
| 19R-28Tm-BB-z     | 73.2               | 72.8 |
| 19R-28Tm-28-BB-z  | 78.0               | 77.9 |



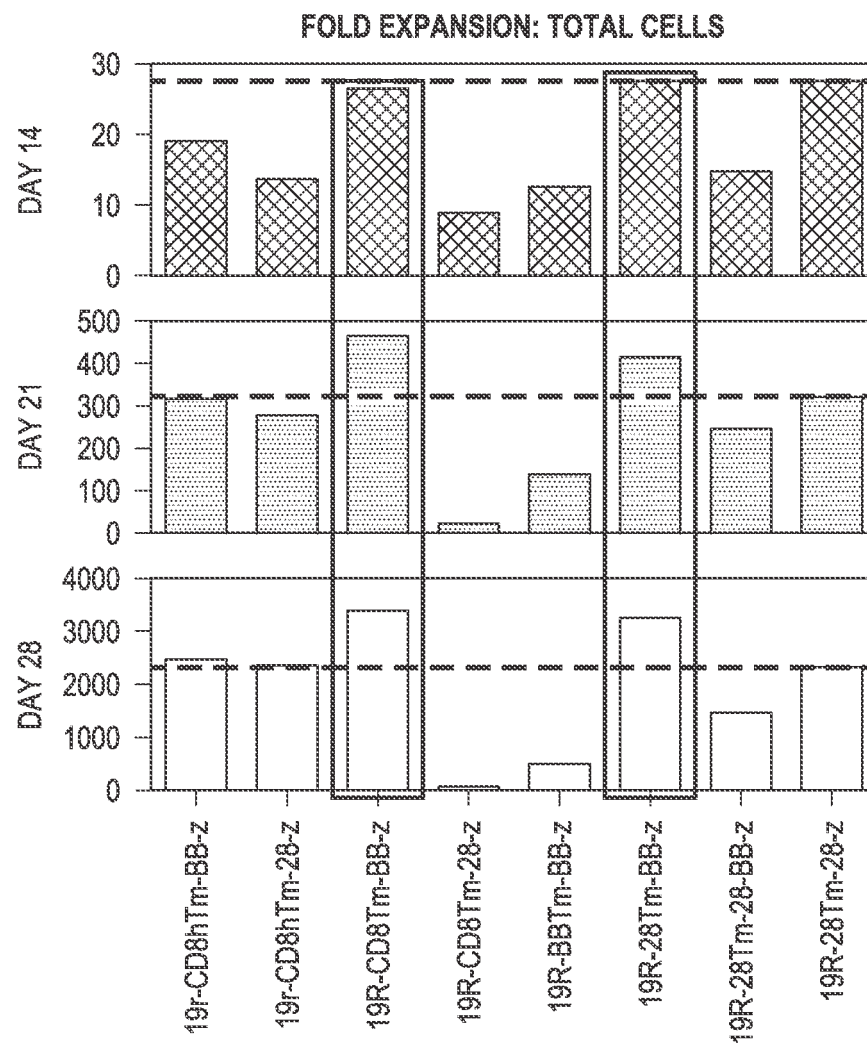


FIG. 11

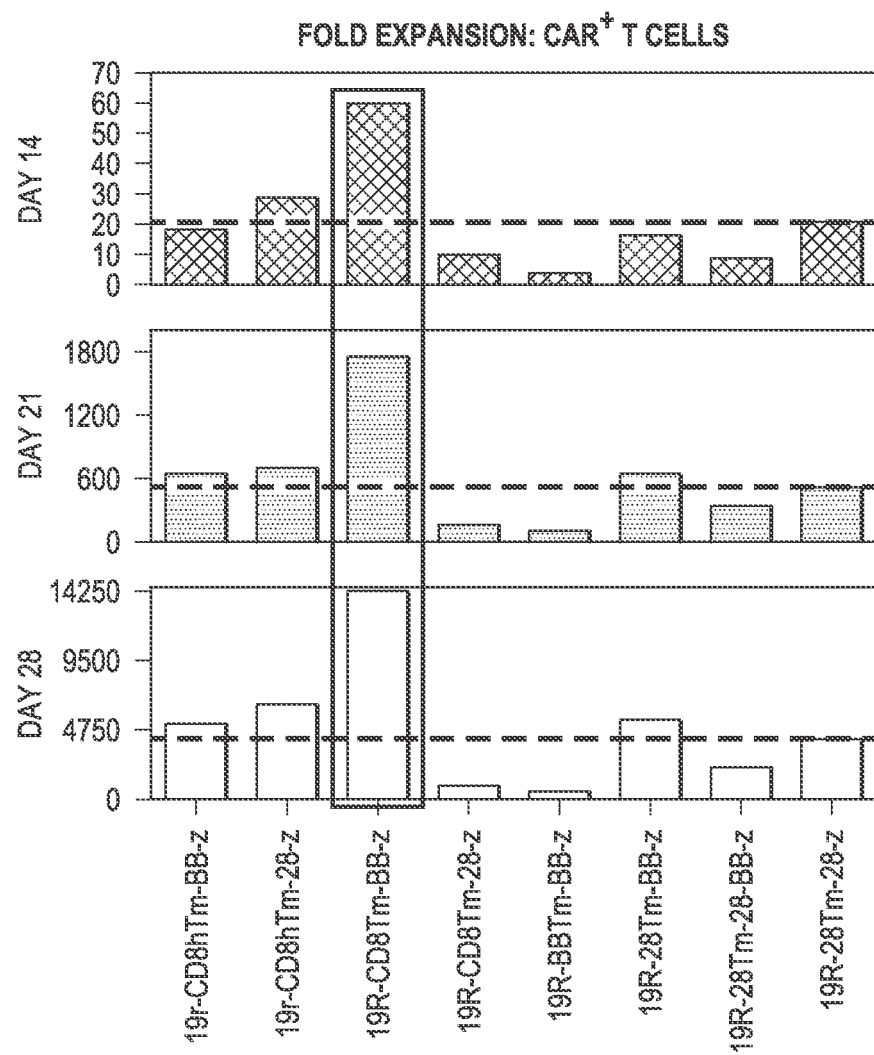
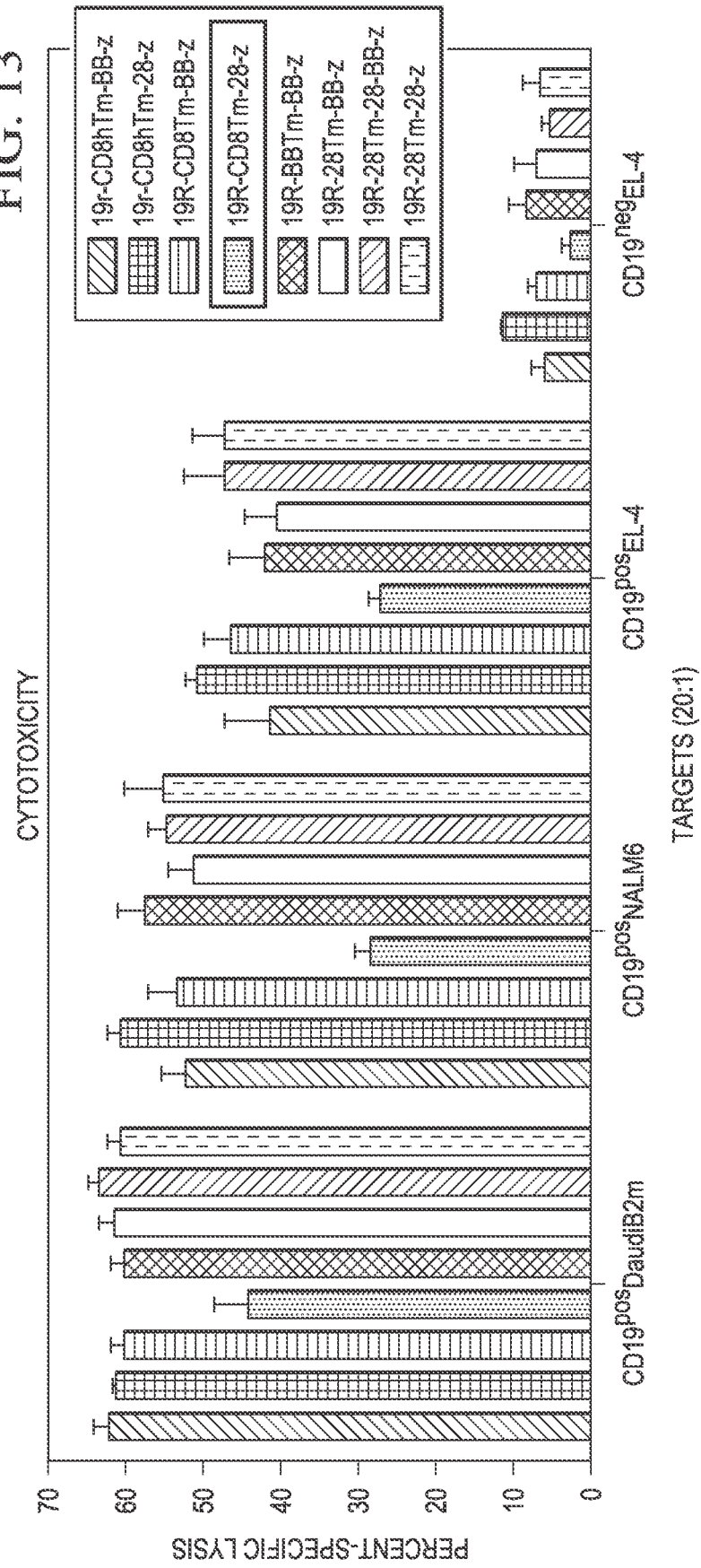
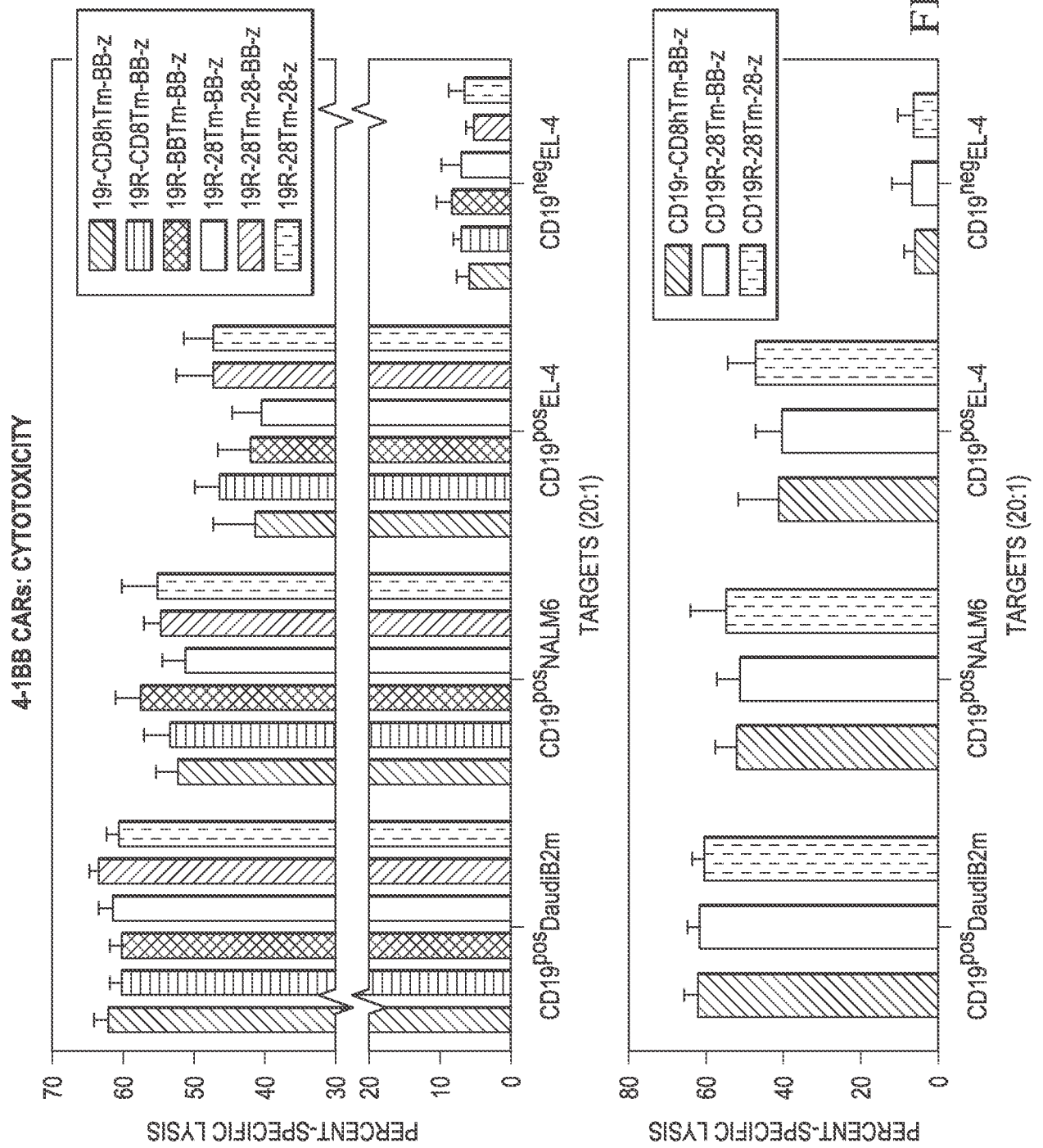


FIG. 12

316





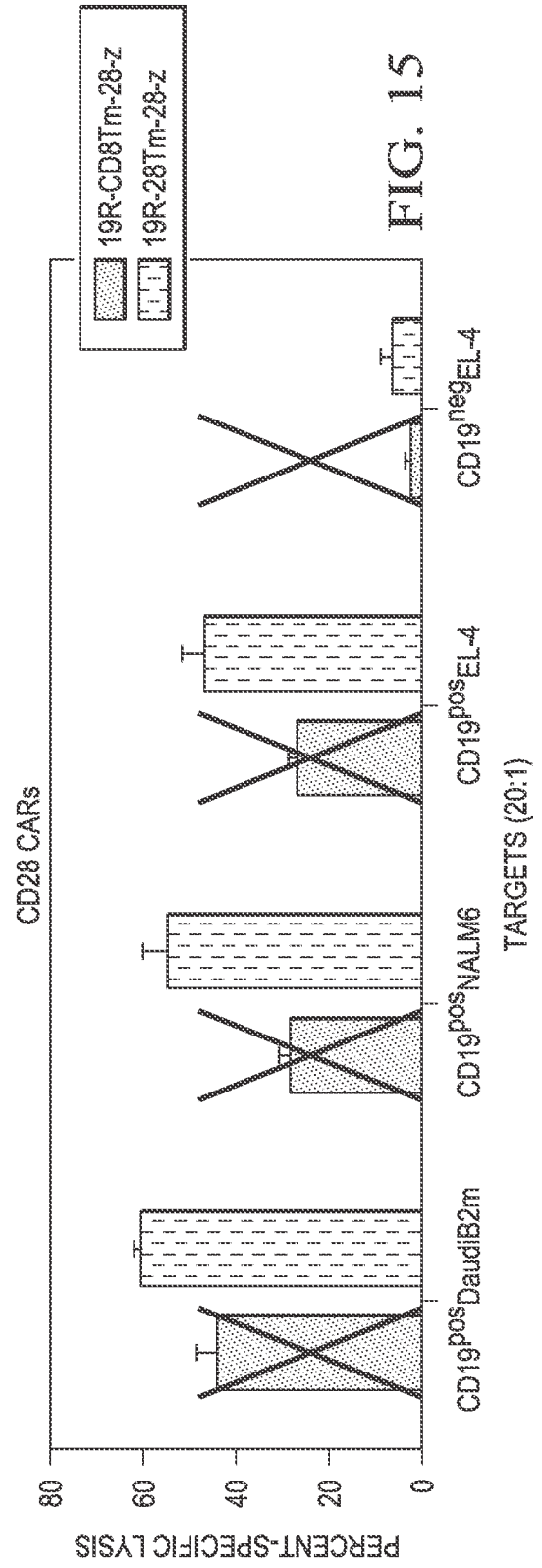
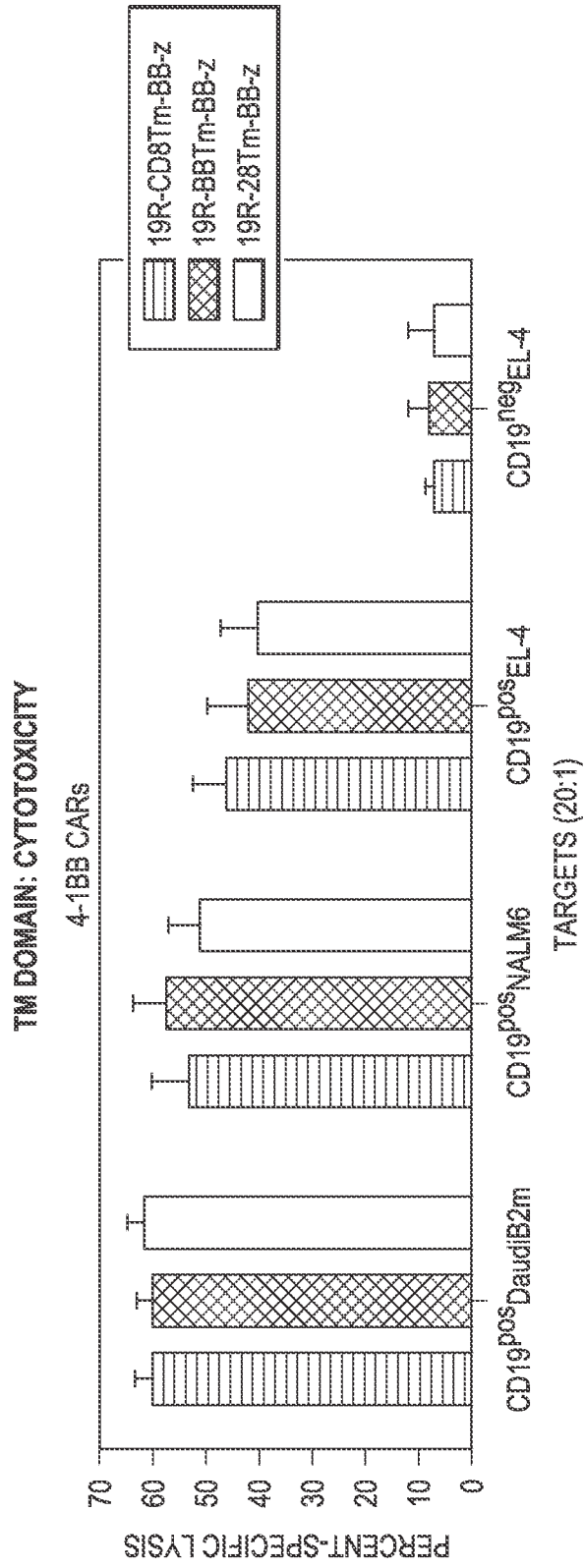
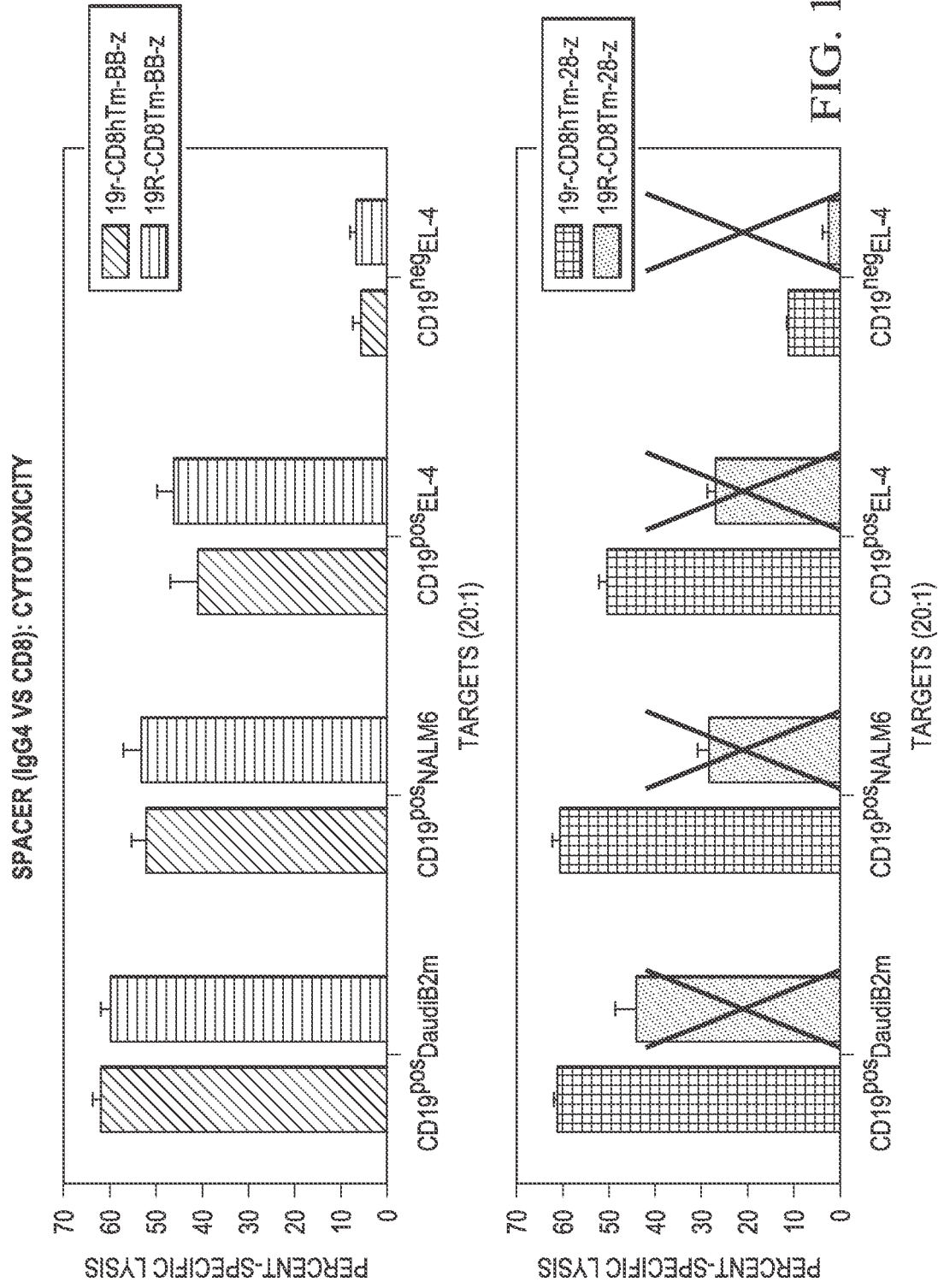
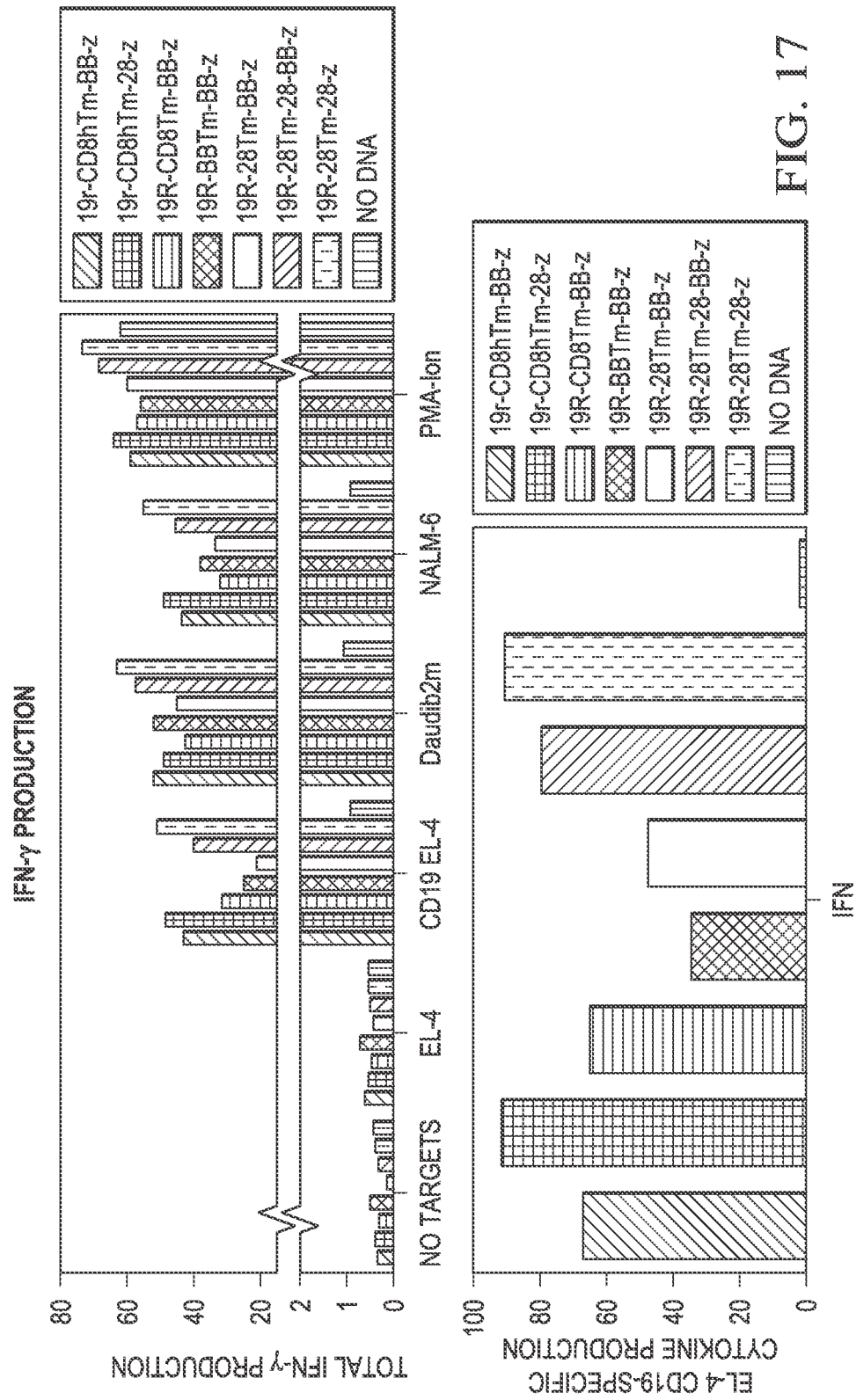


FIG. 15





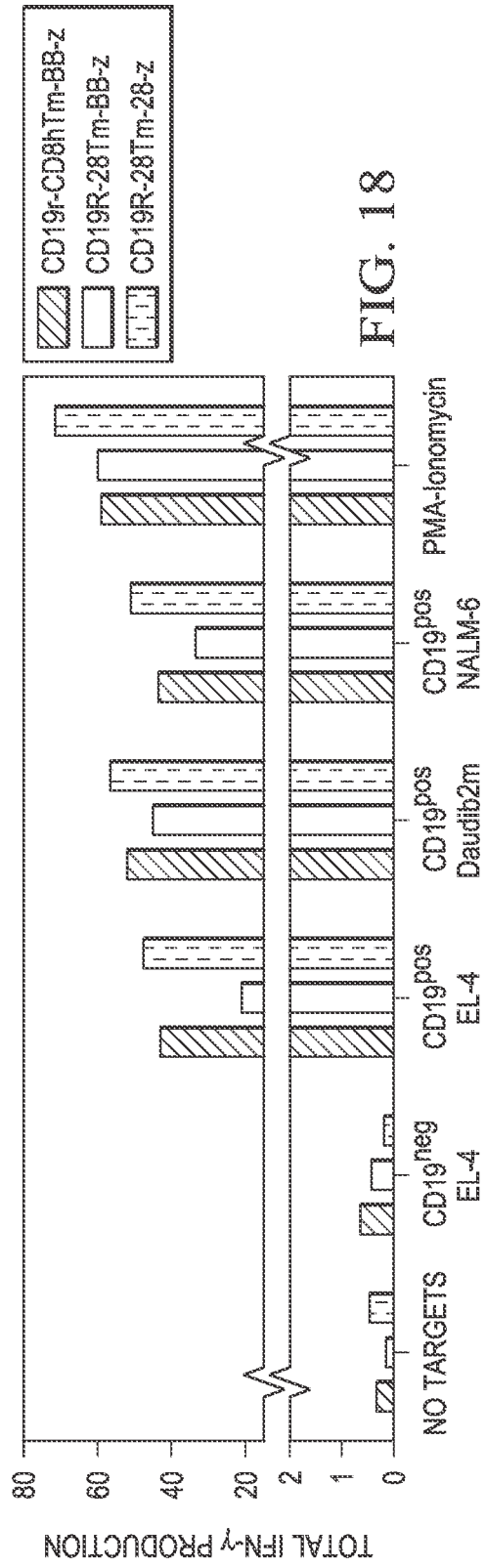
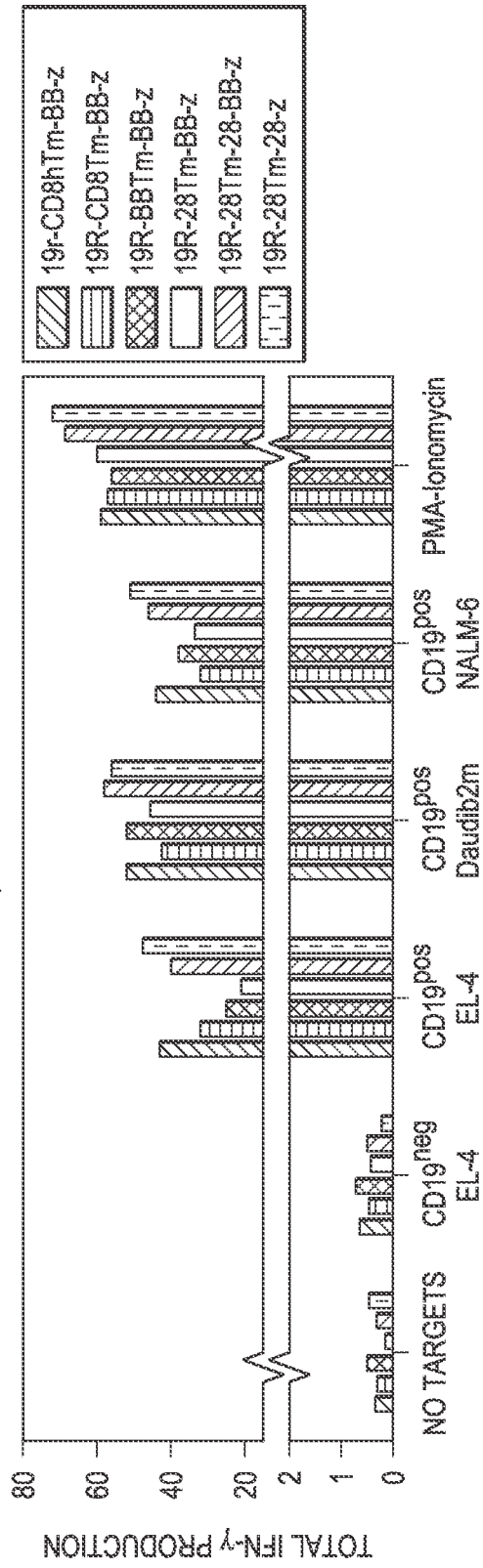
4-1BB CARs: IFN- $\gamma$  PRODUCTION

FIG. 18

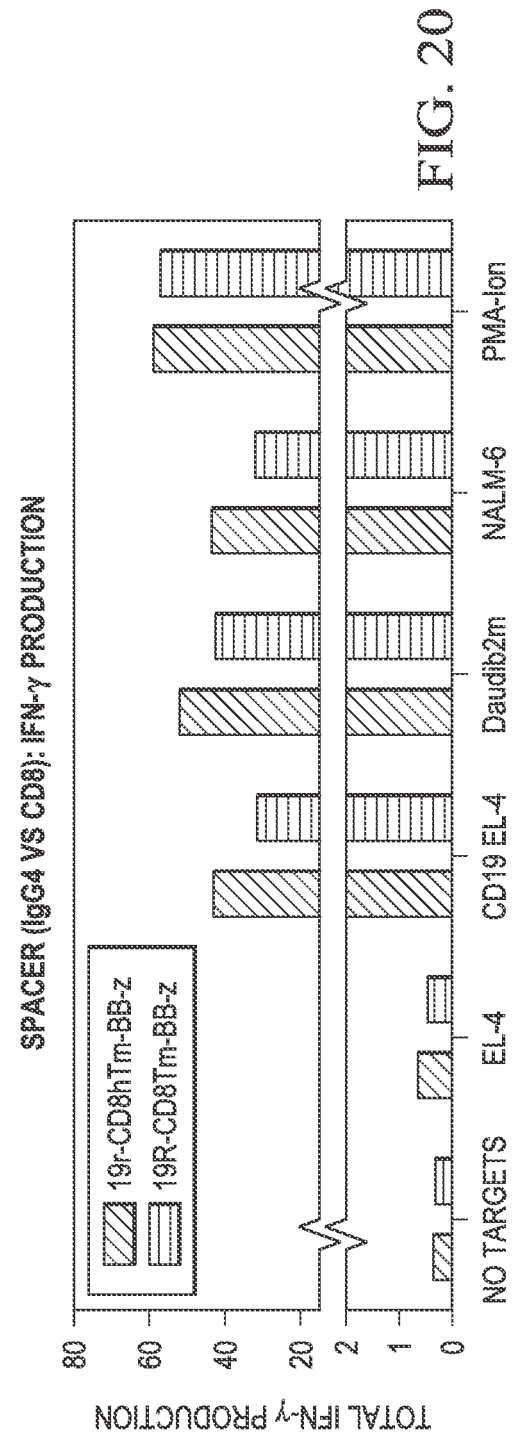
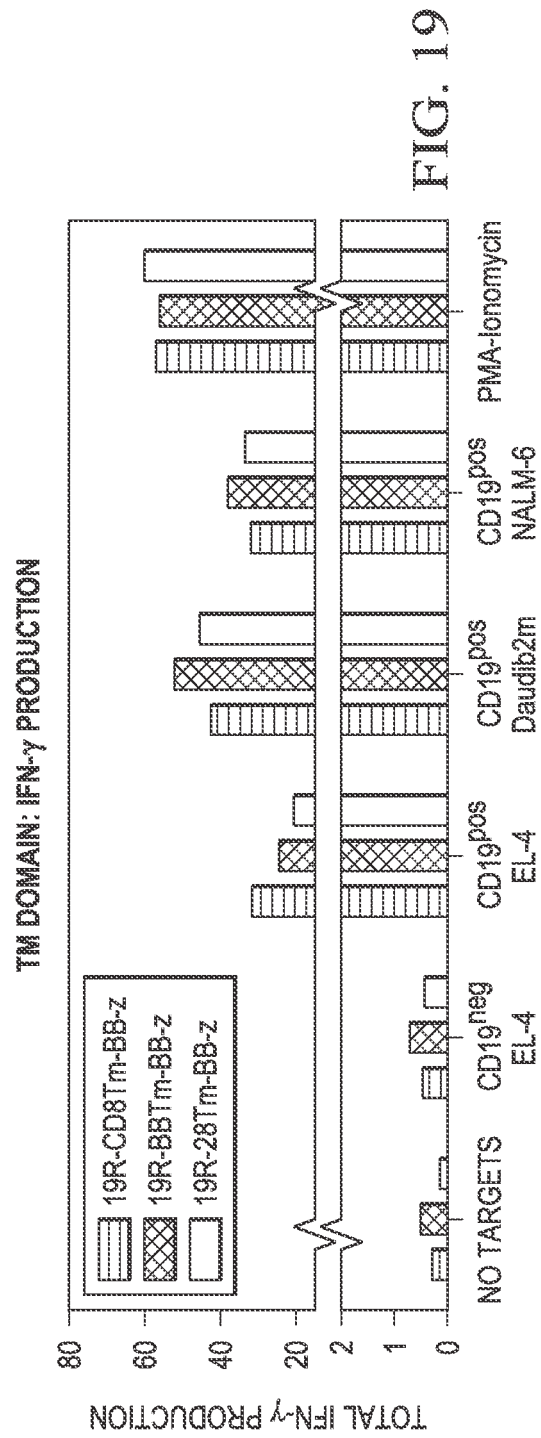
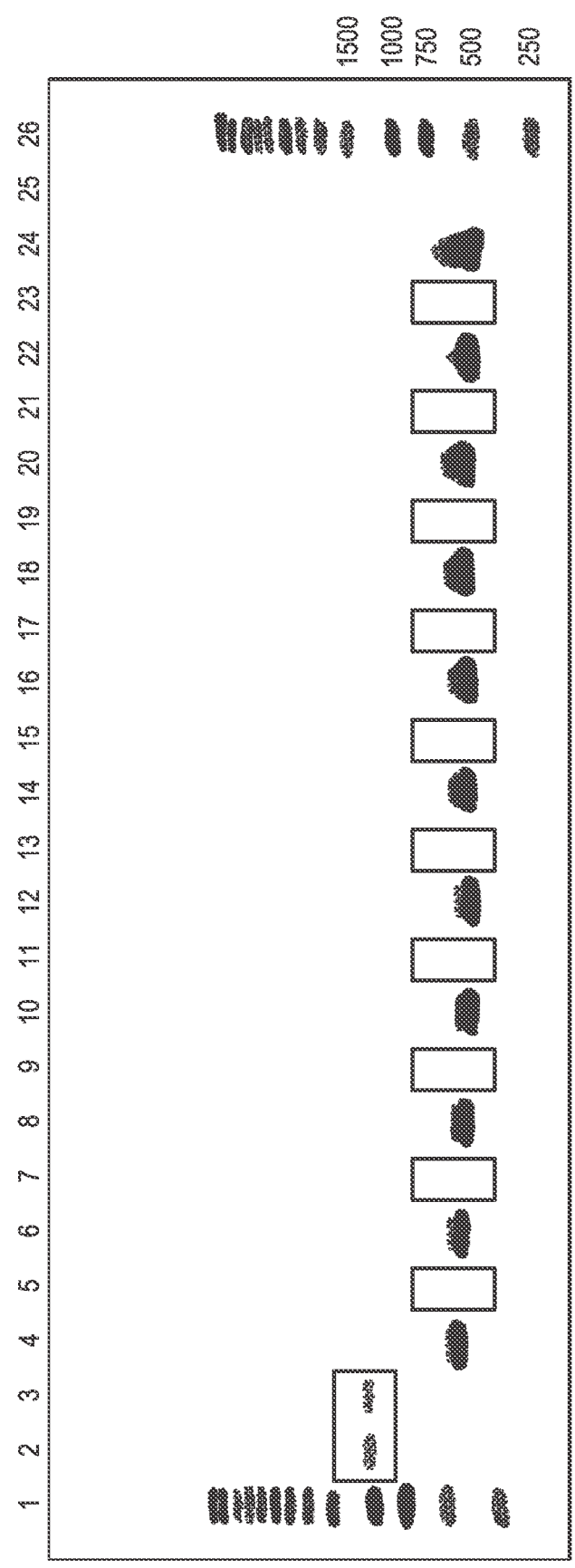


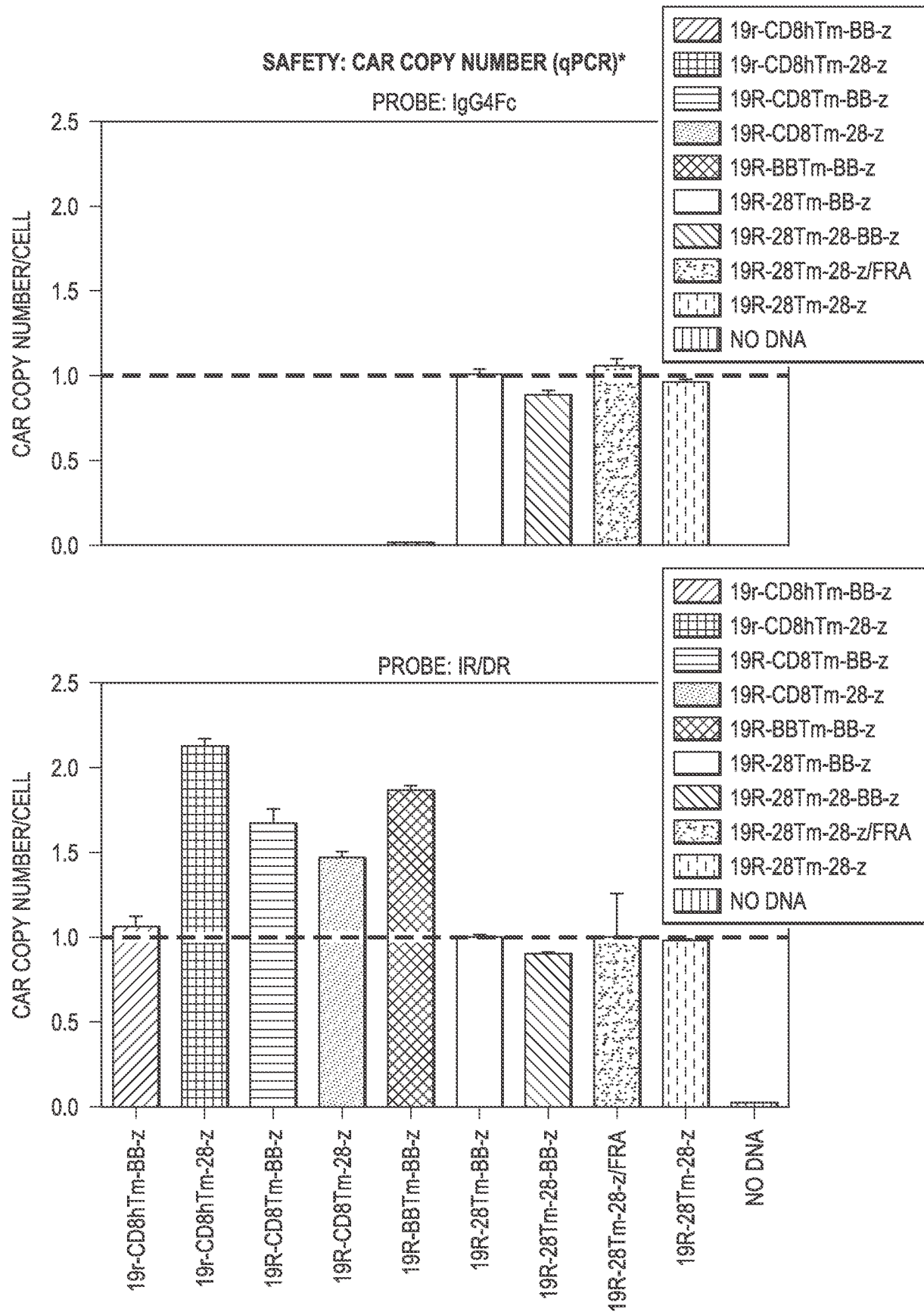
FIG. 21

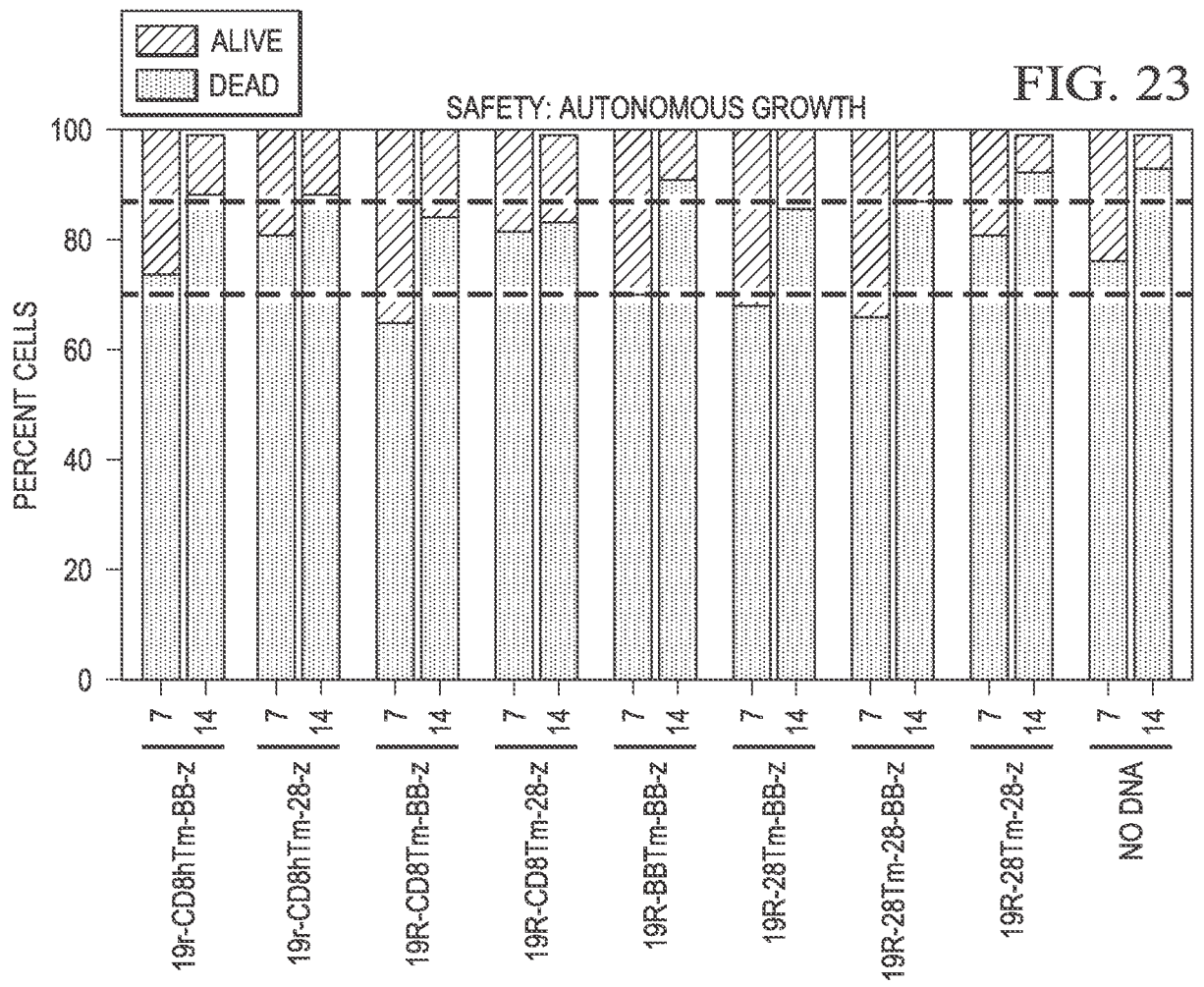
SAFETY: SB11 PCR\*

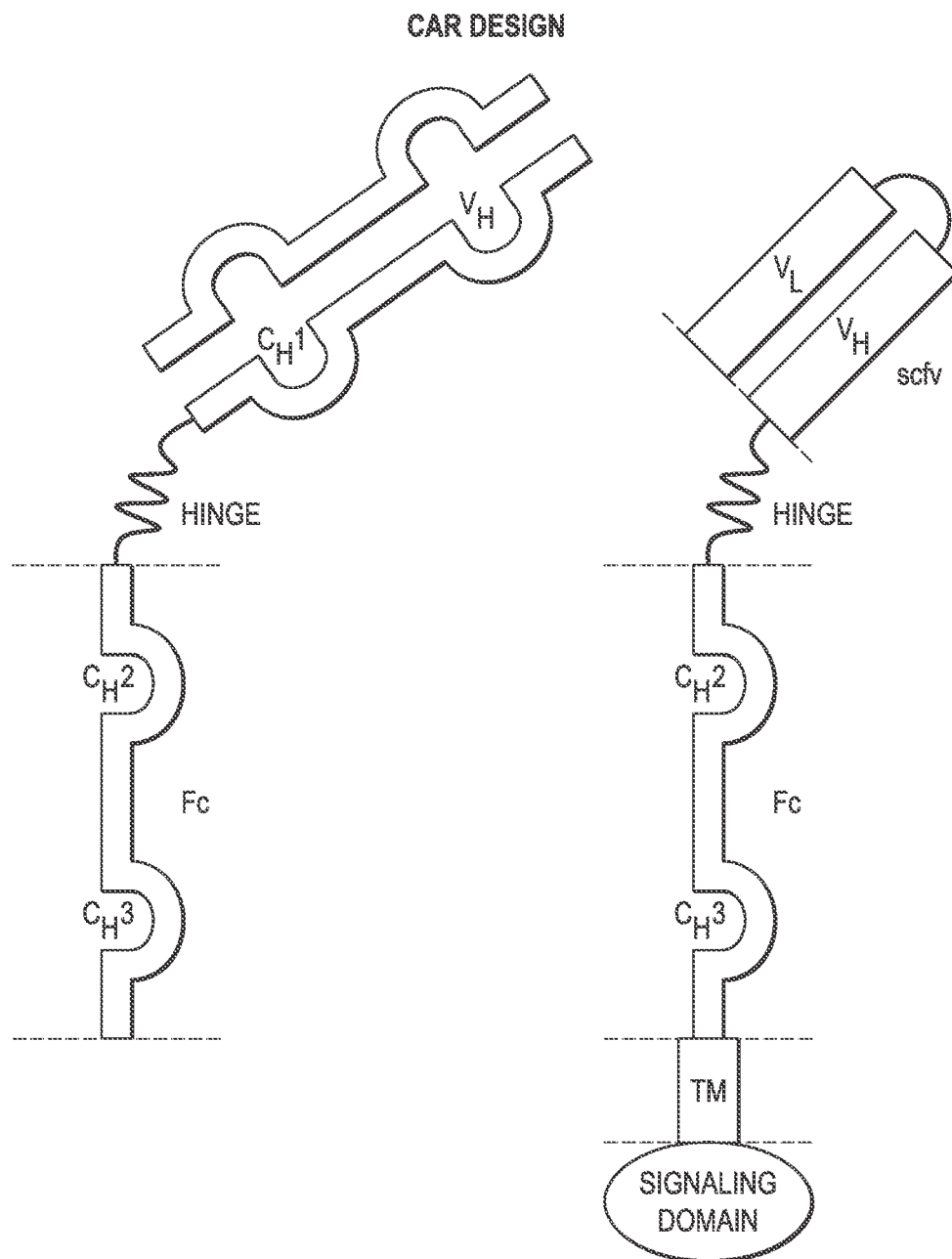


| SB11 PRIMERS      |                             | GAPDH PRIMERS              |  |
|-------------------|-----------------------------|----------------------------|--|
| 1,26 = 1kb LADDER | 2 = SB11-IRES2-EGFP PLASMID | 4 = JURKAT/SB11-IRES2-EGFP |  |
| 25 = BLANK        | 3 = JURKAT/SB11-IRES2-EGFP  | 6 = NO DNA                 |  |
|                   | 5 = NO DNA                  | 8 = 217                    |  |
|                   | 7 = 217                     | 10 = 215                   |  |
|                   | 9 = 215                     | 12 = 213                   |  |
|                   | 11 = 213                    | 14 = 218                   |  |
|                   | 13 = 218                    | 16 = 214                   |  |
|                   | 15 = 214                    | 18 = CAR                   |  |
|                   | 17 = CAR                    | 20 = 193                   |  |
|                   | 19 = 193                    | 22 = 216                   |  |
|                   | 21 = 216                    | 24 = 212                   |  |
|                   | 23 = 212                    |                            |  |

FIG. 22





**FIG. 24**

CD3 $\zeta$ 

HOMO SAPIENS CD247 MOLECULE (CD247), TRANSCRIPT VARIANT 1, mRNA  
NCBI REFERENCE SEQUENCE: NM\_198053.2

Query 1 RVKFSRSADAPAYQQGQNQLYNELNLRREEYDVLDKRRGRDPEMGGKP-RRKNPQEGLY 59  
Sbjct RVKFSRSADAPAYQQGQNQLYNELNLRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLY  
Query 60 NELQDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR 112  
Sbjct NELQDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQA

T-CELL SURFACE GLYCOPROTEIN CD3 ZETA CHAIN ISOFORM 1 PRECURSOR [HOMO SAPIENS]  
NCBI REFERENCE SEQUENCE: NP\_932170.1

Query 1 RVKFSRSADAPAYQQGQNQLYNELNLRREEYDVLDKRRGRDPEMGGKP-RRKNPQEGLY 59  
Sbjct 52 RVKFSRSADAPAYQQGQNQLYNELNLRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLY 111  
Query 60 NELQDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR 112  
Sbjct 112 NELQDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR 164

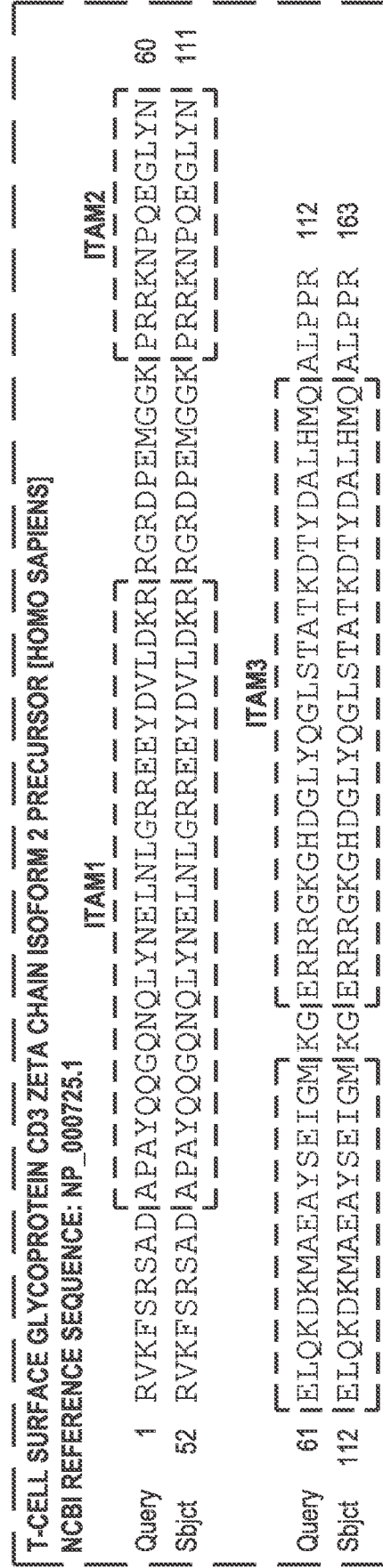
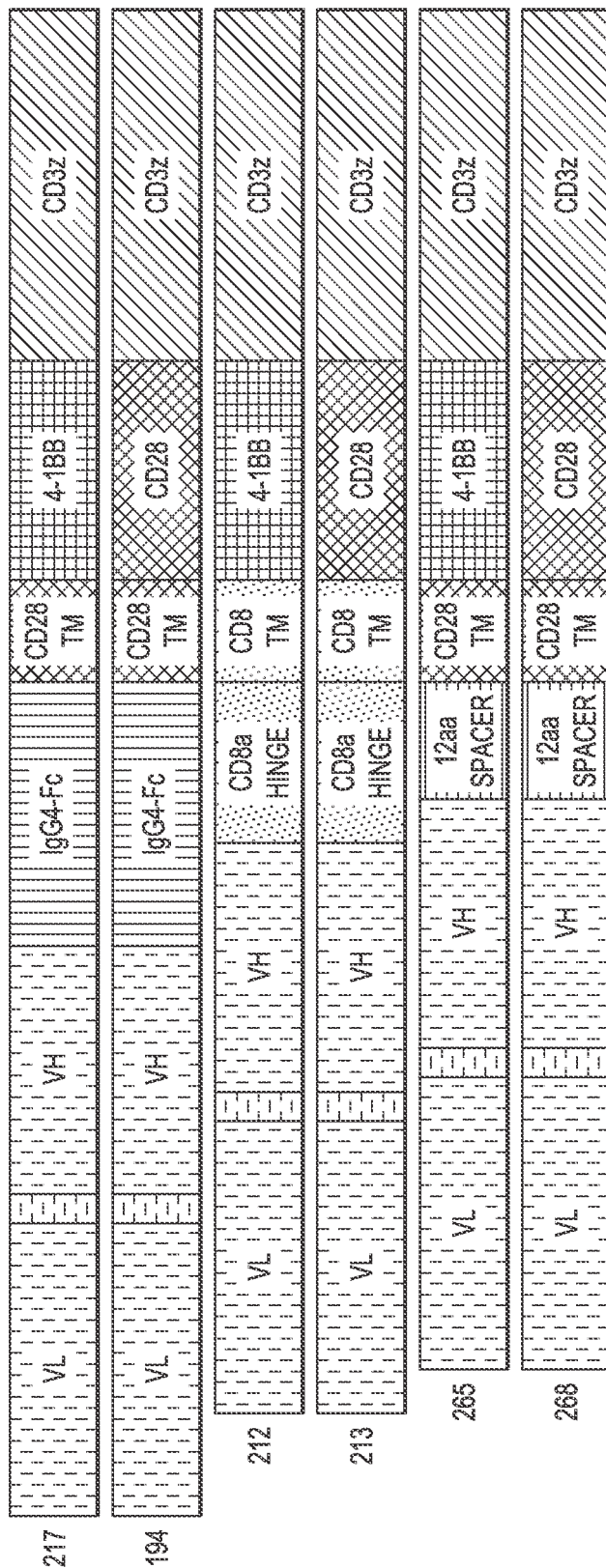


FIG. 25

FIG. 26

CARs\*

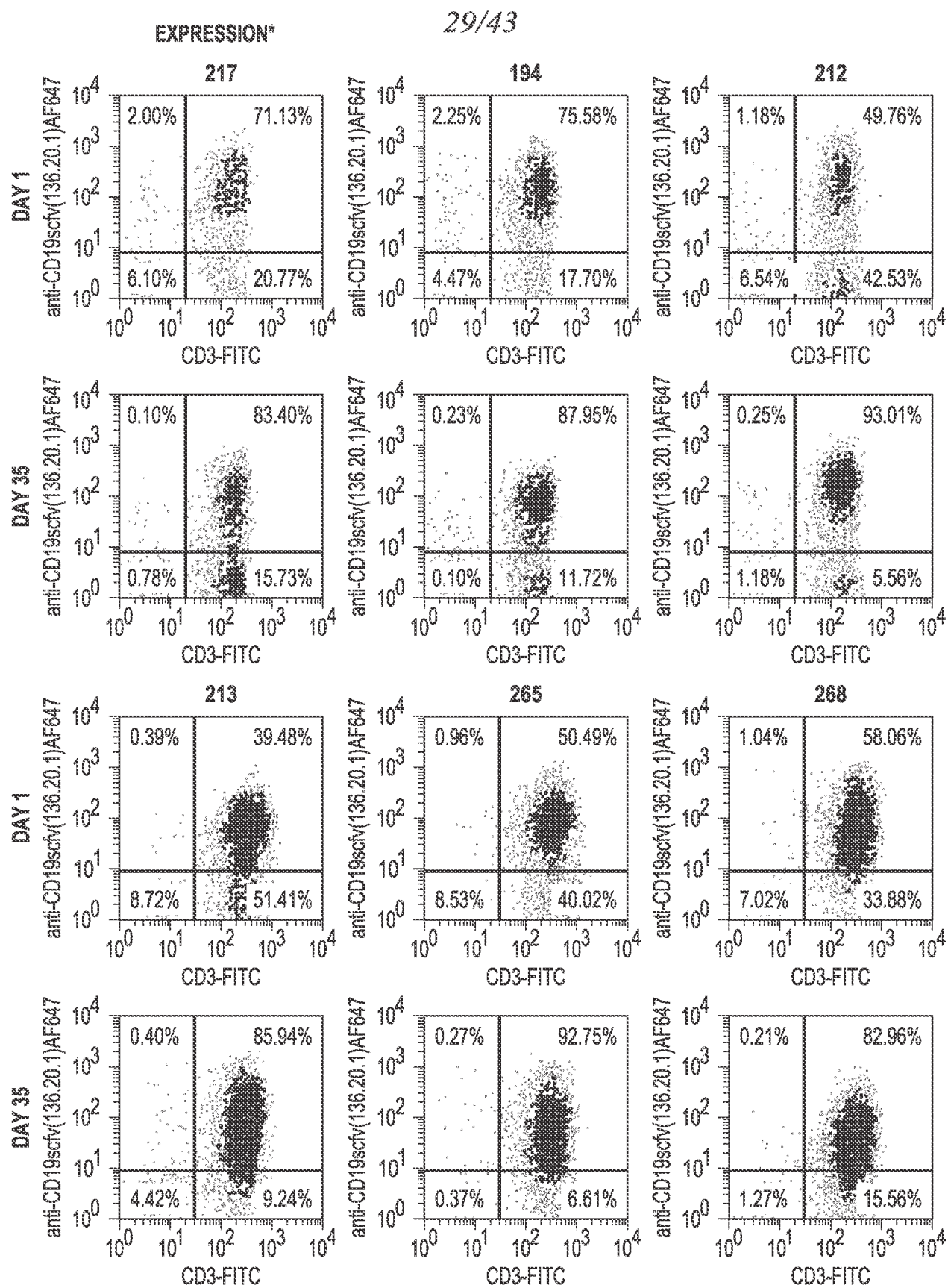


ESKYGPPCPPCP = IgG4 Fc HINGE = 12aa SPACER

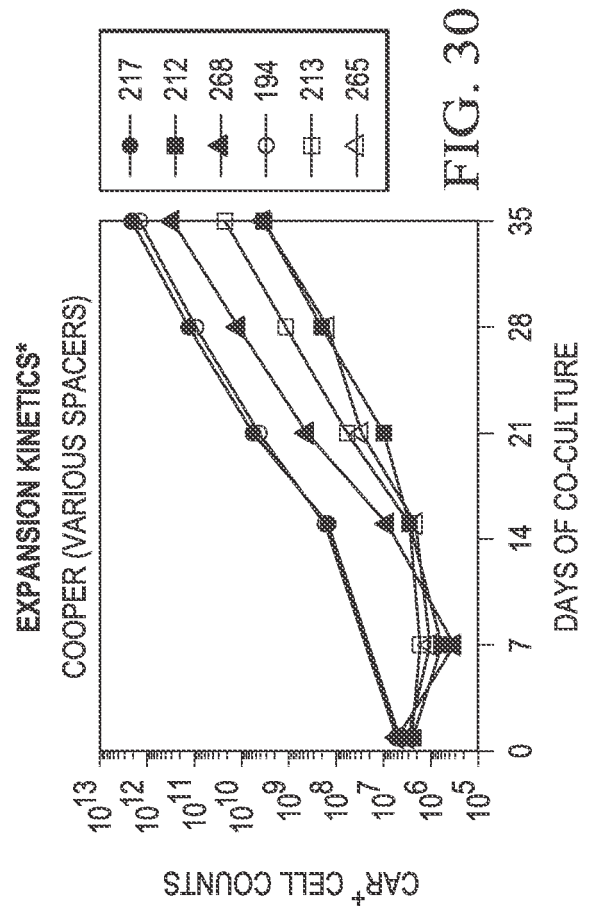
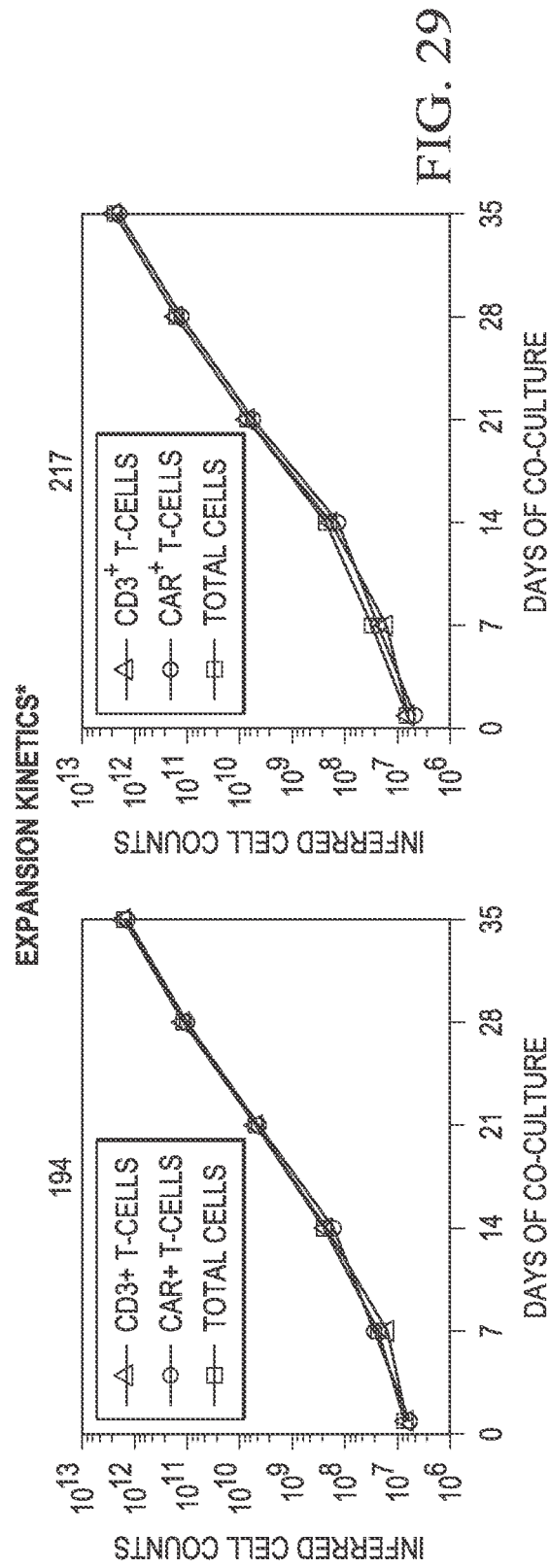
FIG. 27

CARs

| CAR →        | COOPER |       |
|--------------|--------|-------|
| SPACER       | CD28   | CD137 |
| Fc (230aa)   | 194    | 217   |
| CD8 (47aa)   | 213    | 212   |
| SHORT (12aa) | 265    | 268   |
| CD3ζ         | WT     | WT    |



**FIG. 28**



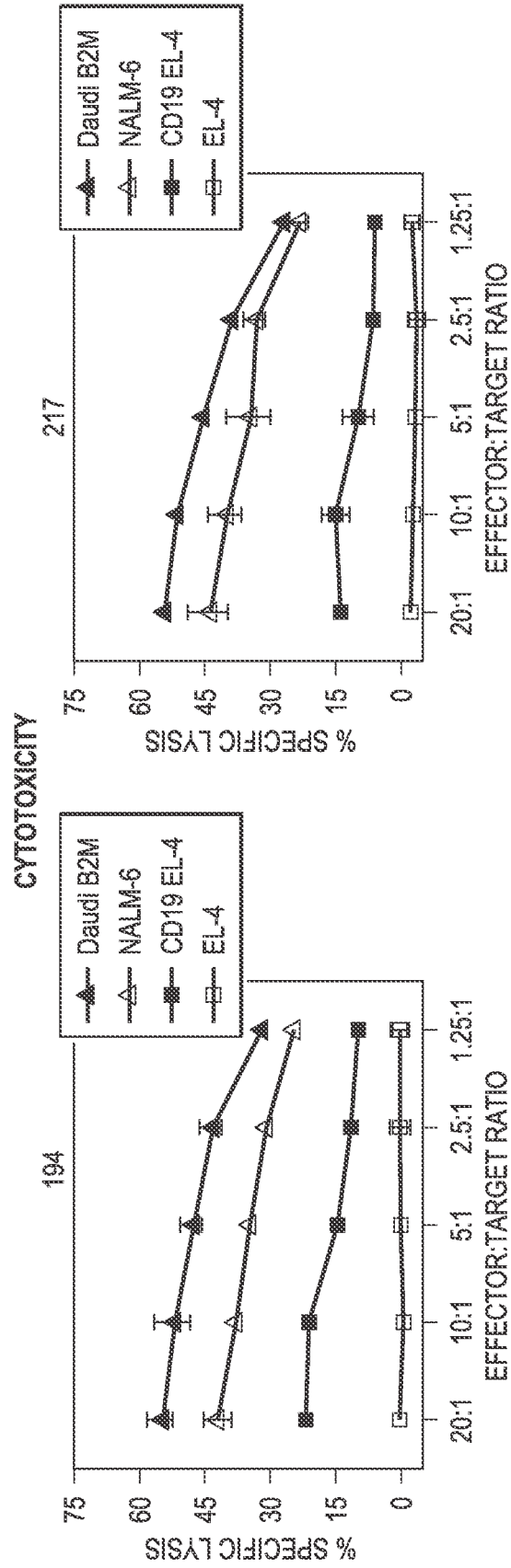


FIG. 31

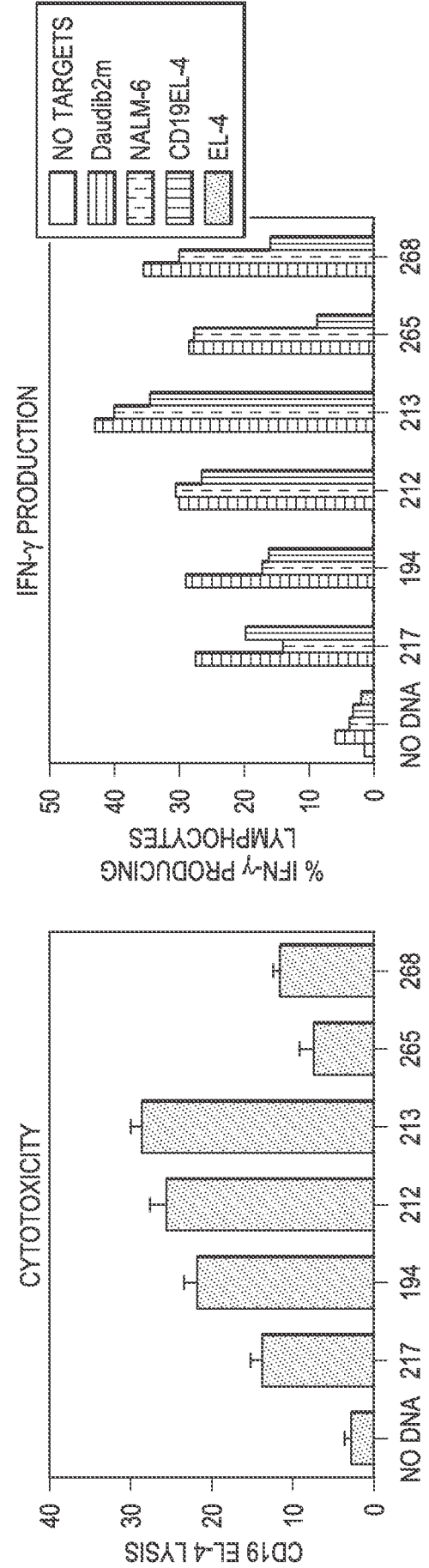


FIG. 32

FIG. 34

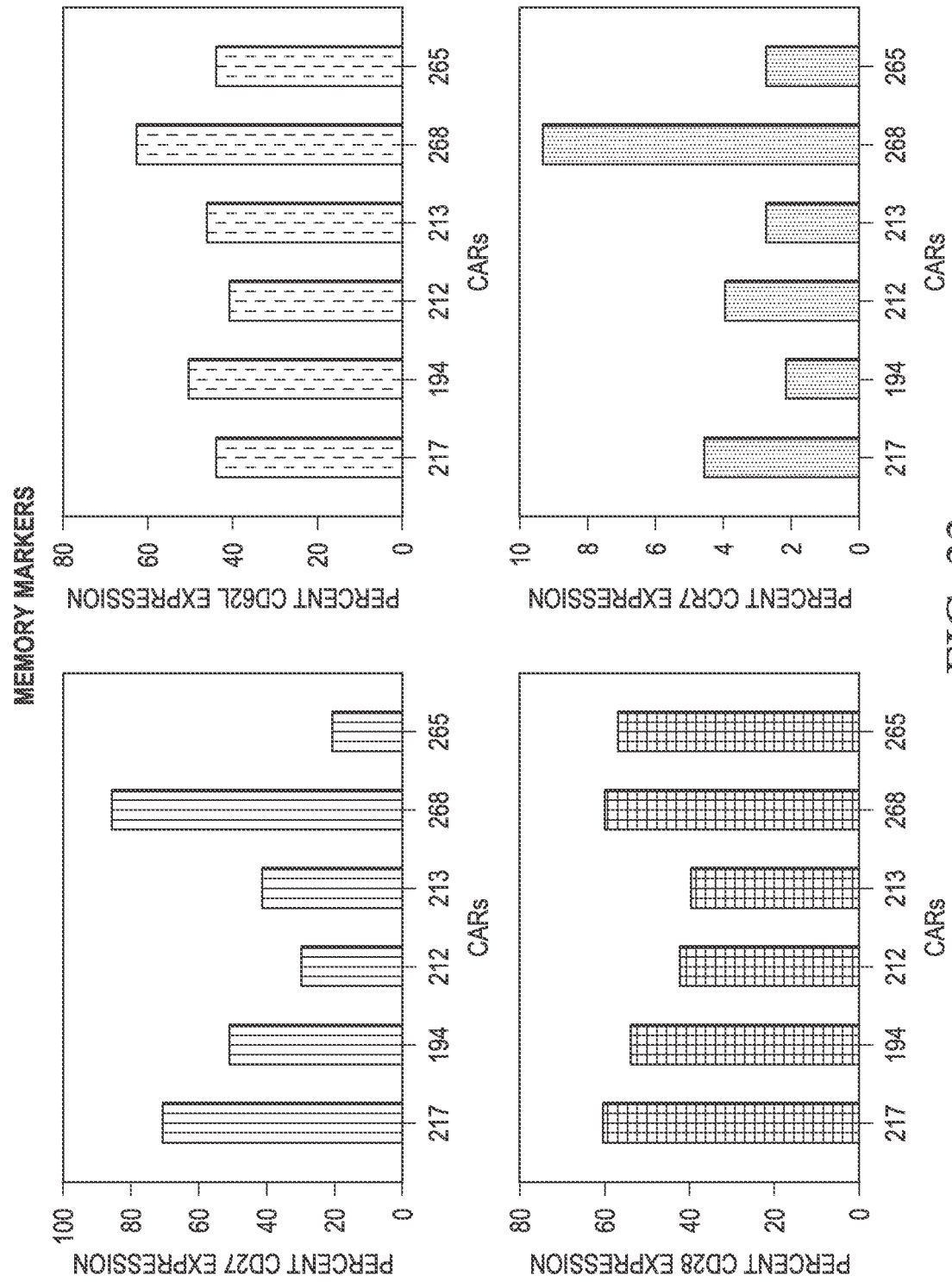


FIG. 33

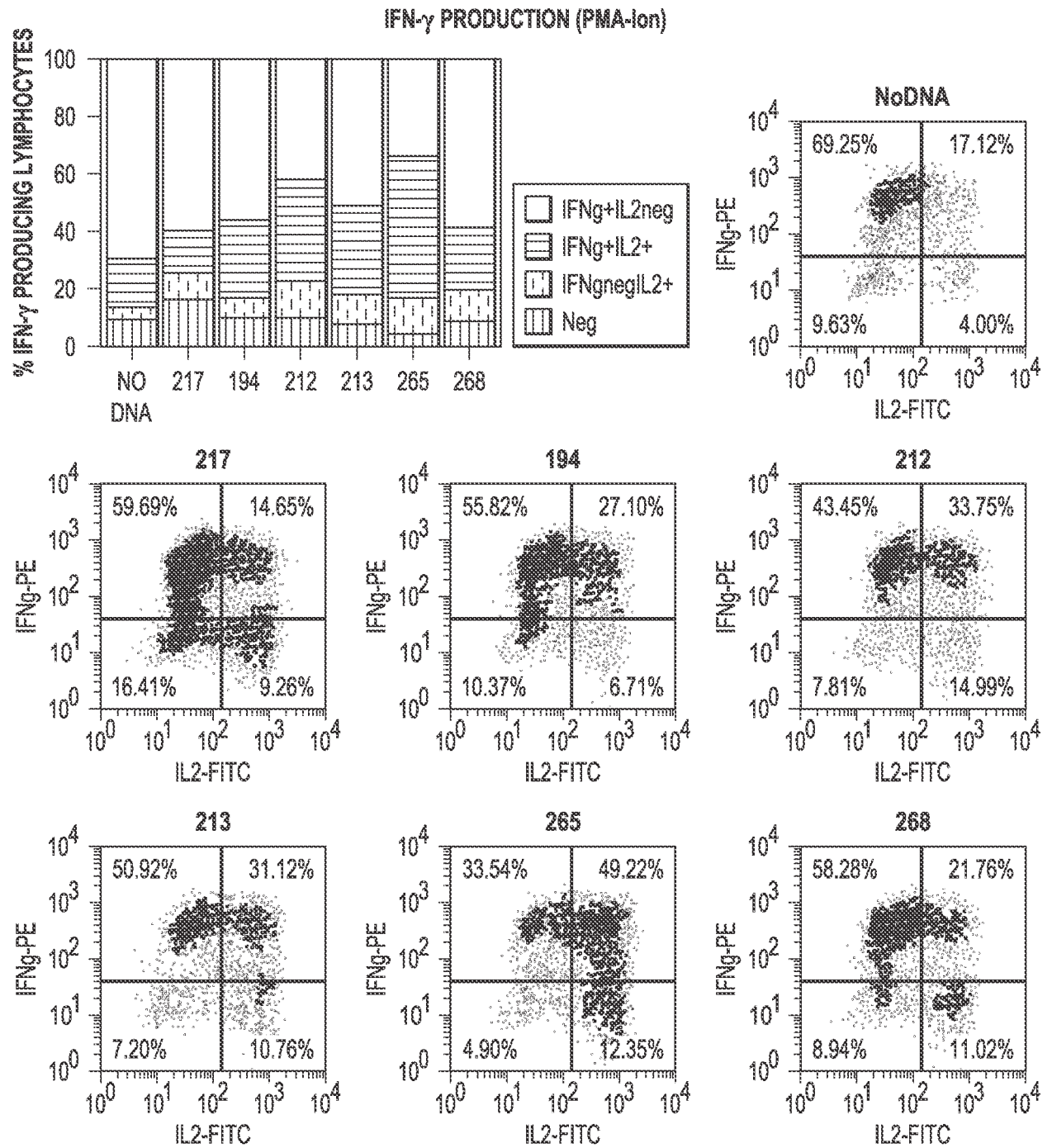


FIG. 35

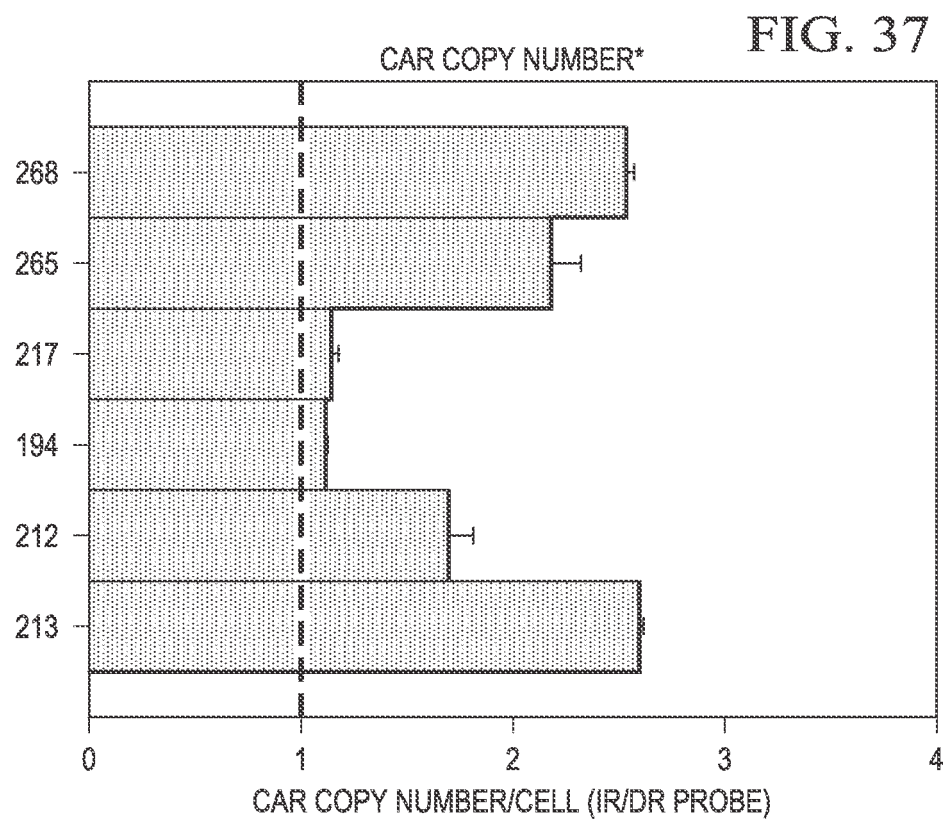
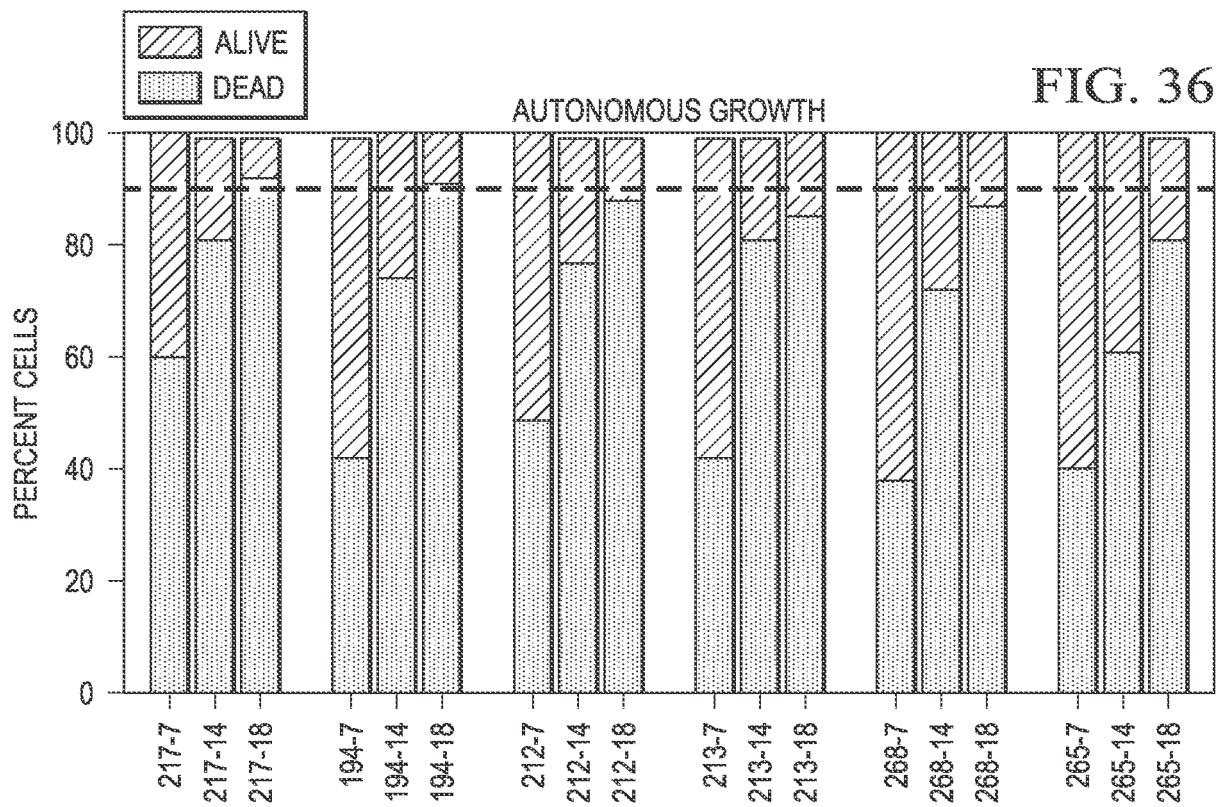


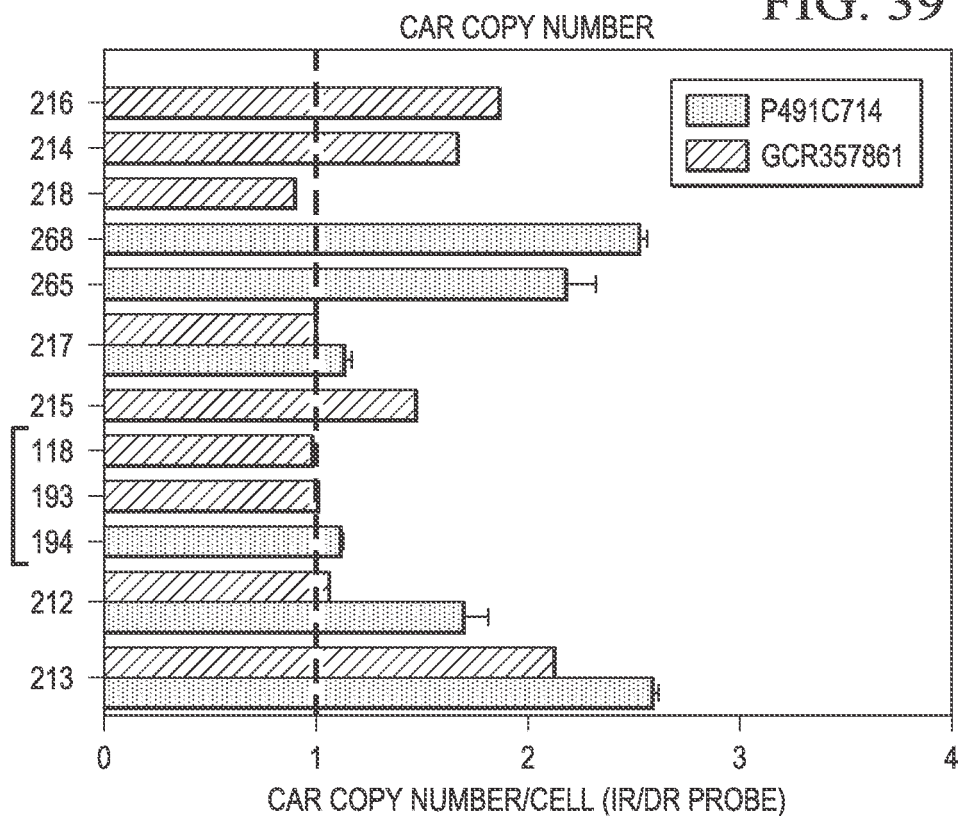
FIG. 38

|                                                                                   |                 |
|-----------------------------------------------------------------------------------|-----------------|
|  | DONOR P491C714  |
|  | DONOR GCR357861 |

CAR COPY NUMBER

| DOMAINS                 | COOPER             |                                        |       |                    |                     |               |                |
|-------------------------|--------------------|----------------------------------------|-------|--------------------|---------------------|---------------|----------------|
| INTRACELLULAR SIGNALING | CD28               |                                        |       | CD137              |                     |               | CD28+<br>CD137 |
| TM/SPACER               | CD8                | CD28                                   | CD137 | CD8                | CD28                | CD137         | CD28           |
| Fc (230aa)              | 215<br>(1.48)      | 194 (1.12)<br>193 (1.01)<br>118 (0.98) |       | 214<br>(1.67)      | 217<br>(1.01, 1.14) | 216<br>(1.87) | 218<br>(0.90)  |
| CD8 (47aa)              | 213<br>(2.13, 2.6) |                                        |       | 212<br>(1.06, 1.7) |                     |               |                |
| SHORT (12aa)            |                    | 265<br>(2.18)                          |       |                    | 268<br>(2.53)       |               |                |
| CD3 $\zeta$             | WT                 | WT                                     | WT    | WT                 | WT                  | WT            | WT             |

FIG. 39



TRANSFECTION OF 293 HEK CELLS  
CD19R CAR WITH Ig4 HINGES AND DIFFERENT ENDODOMAINS:

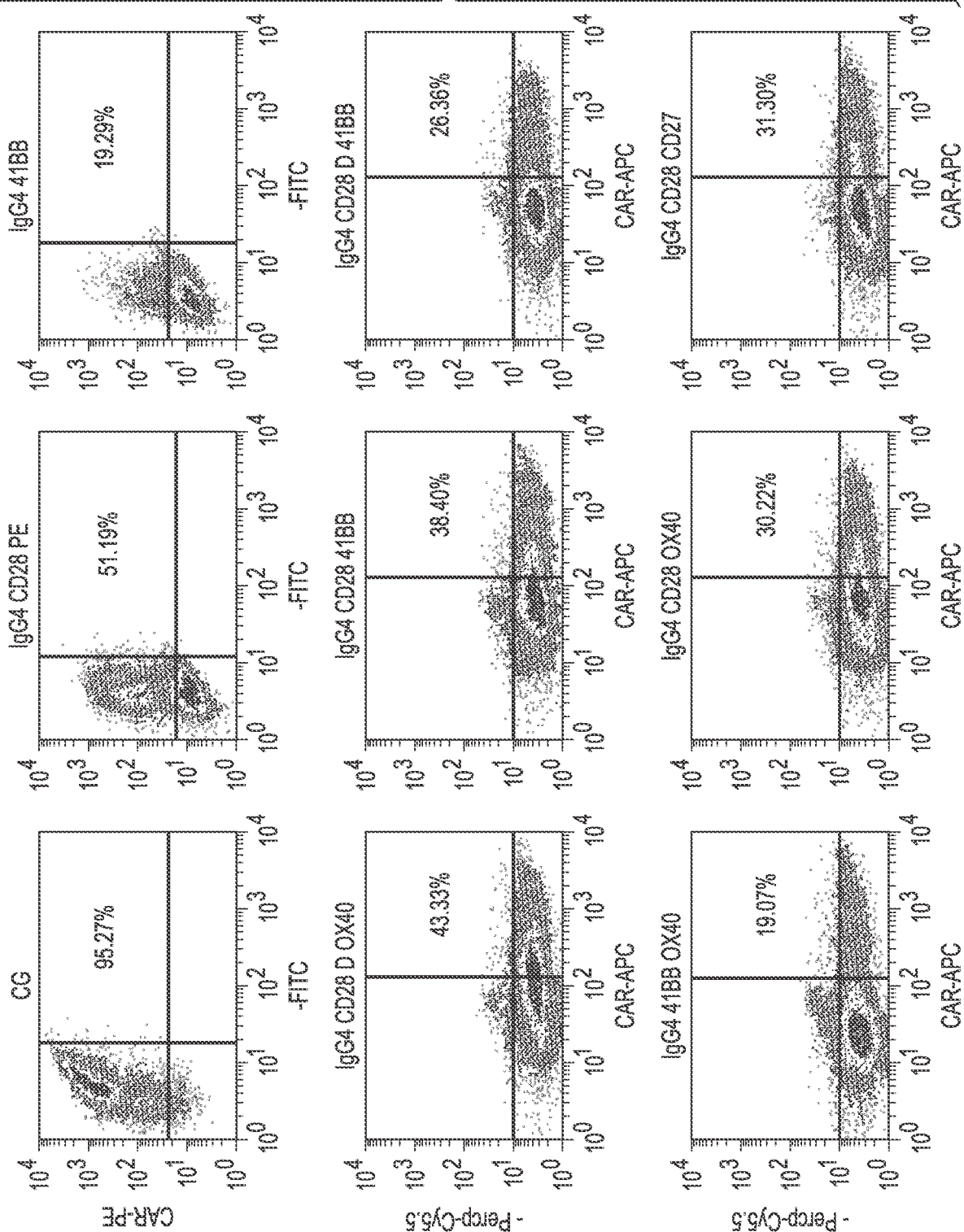


FIG. 40A

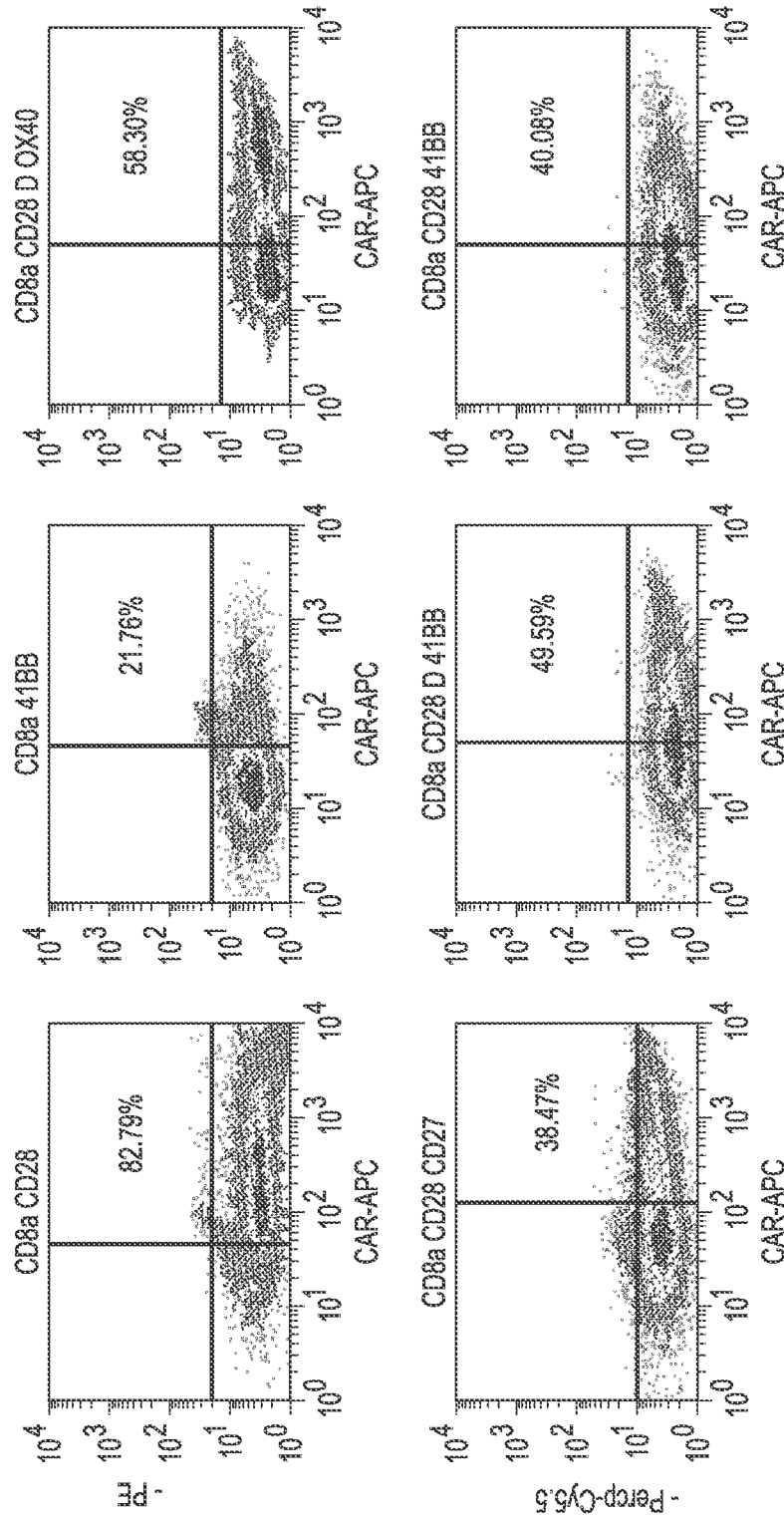
CD19R CAR WITH CD8 $\alpha$  HINGES AND DIFFERENT ENDODOMAINS:

FIG. 40B

## CD19R CAR WITH t-12AA AND t-20AA (SHORT) HINGES AND DIFFERENT ENDODOMAINS:

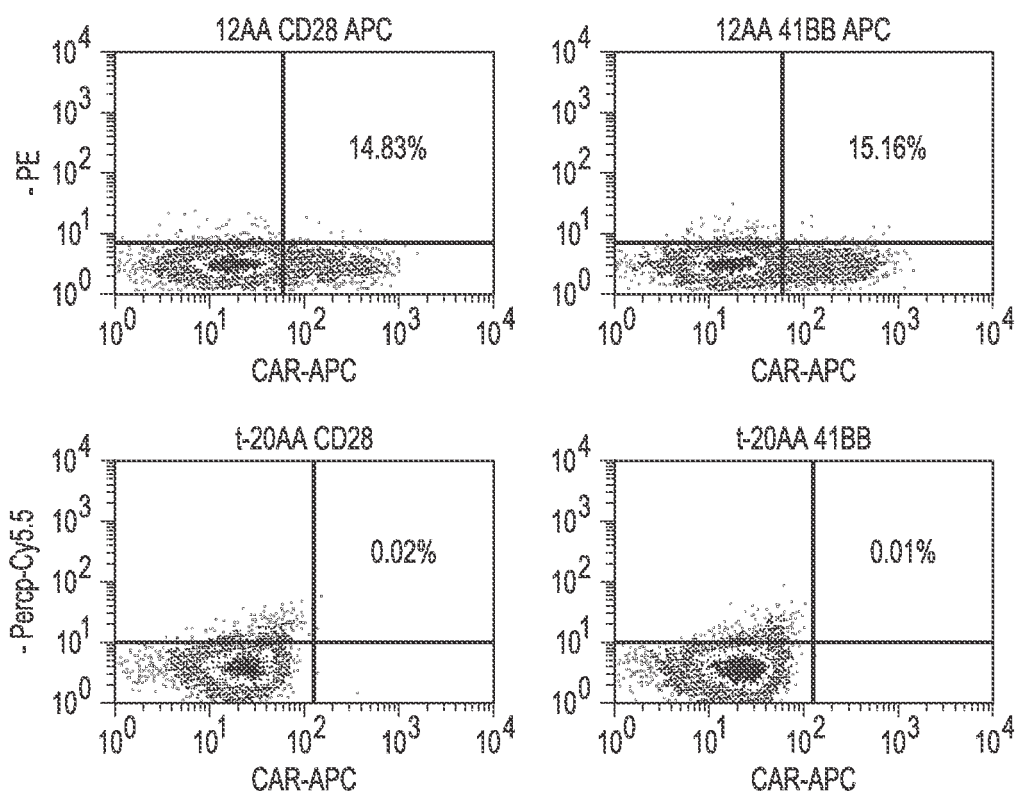


FIG. 40C

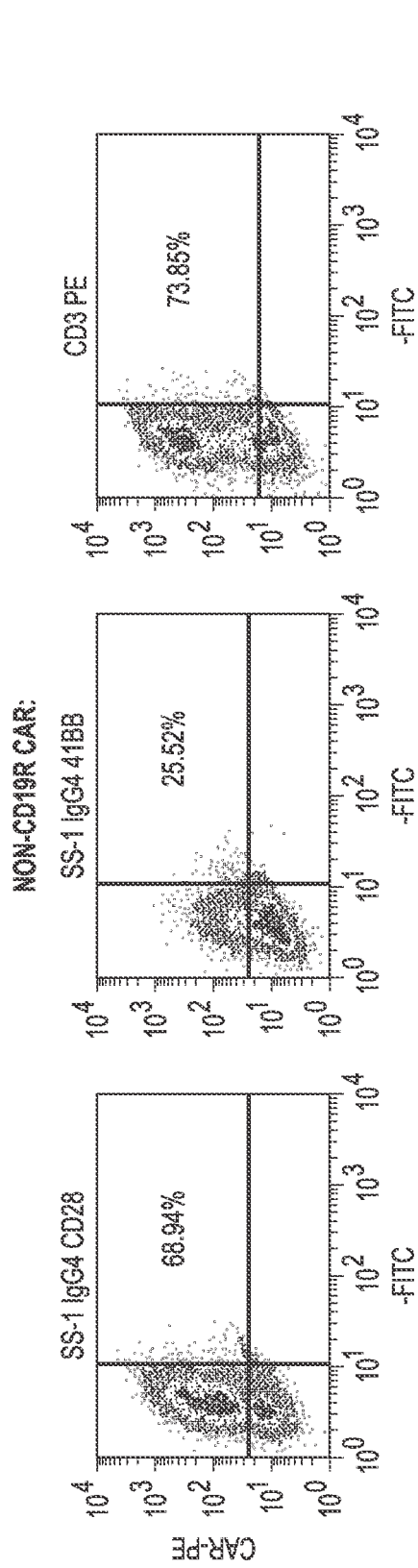


FIG. 40D

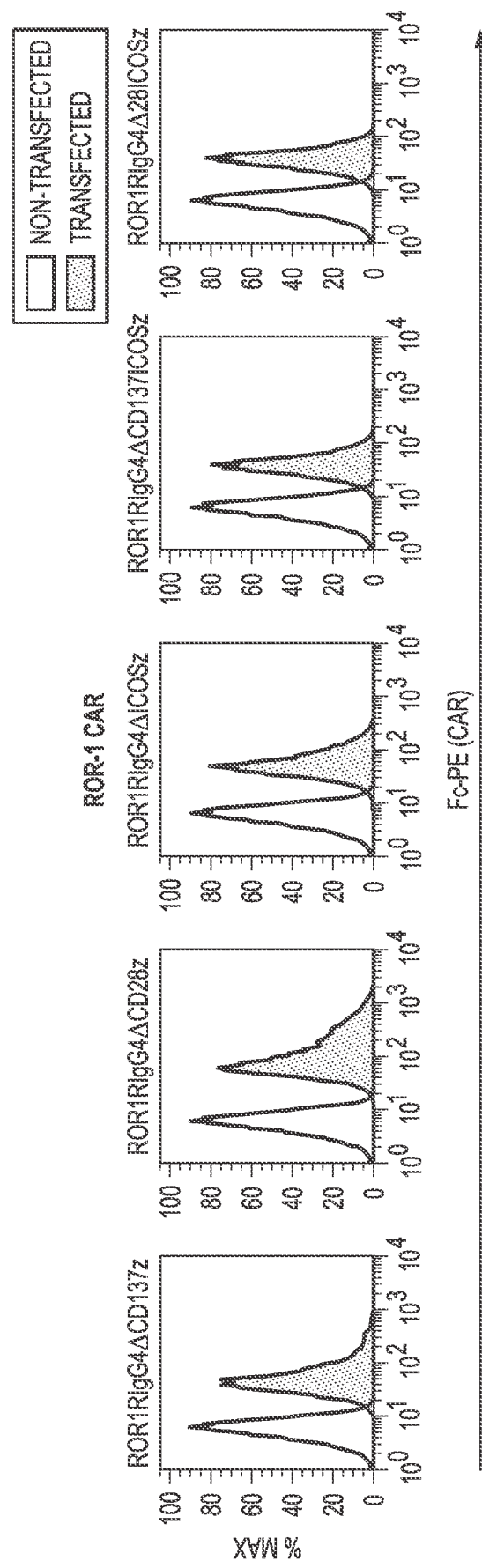


FIG. 40E

## GATING STRATEGY FOR IDENTIFYING TARGET AND EFFECTOR CELLS EXPRESSING GFP:

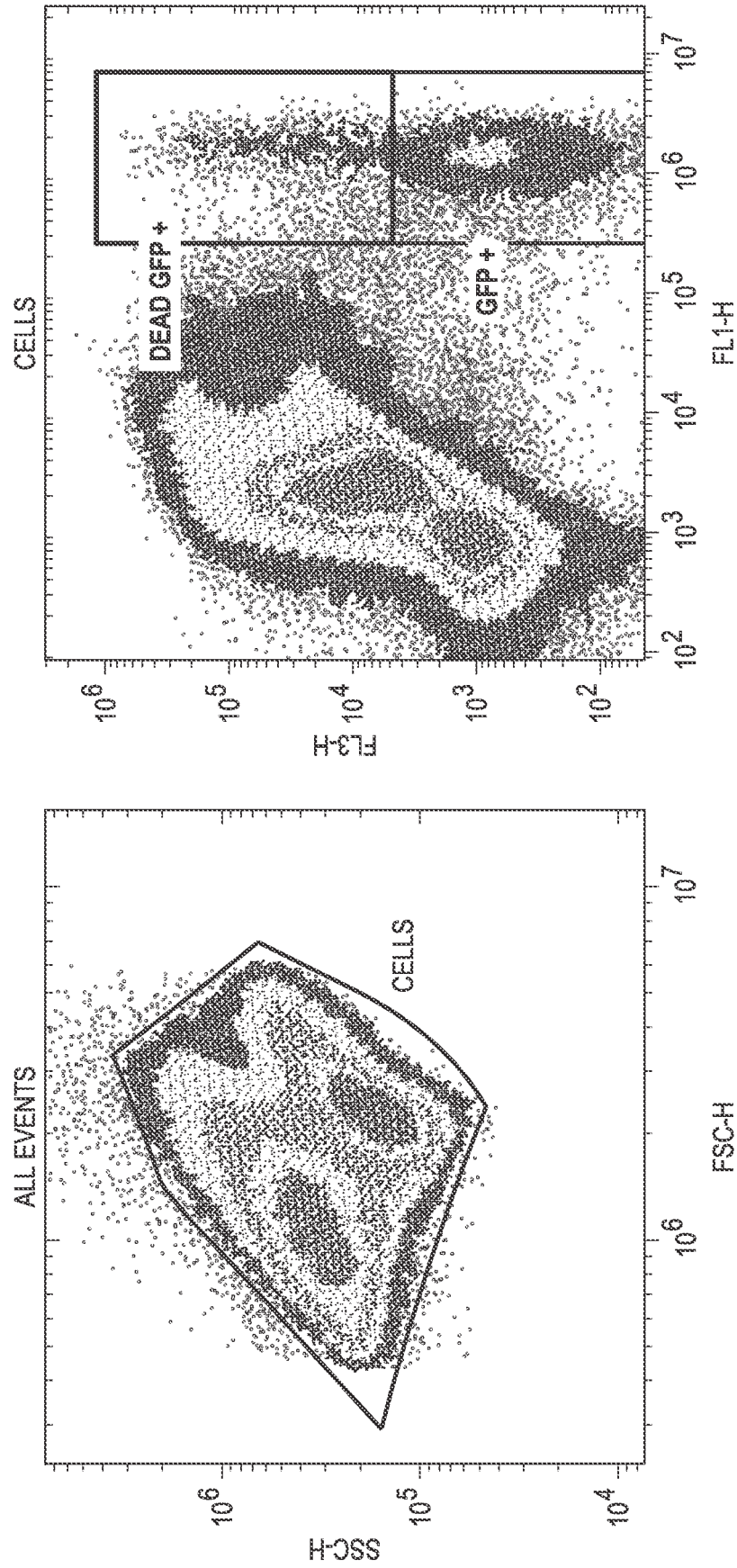


FIG. 41A-1

## REAL TIME VISUALIZATION OF DATA ACQUISITION:

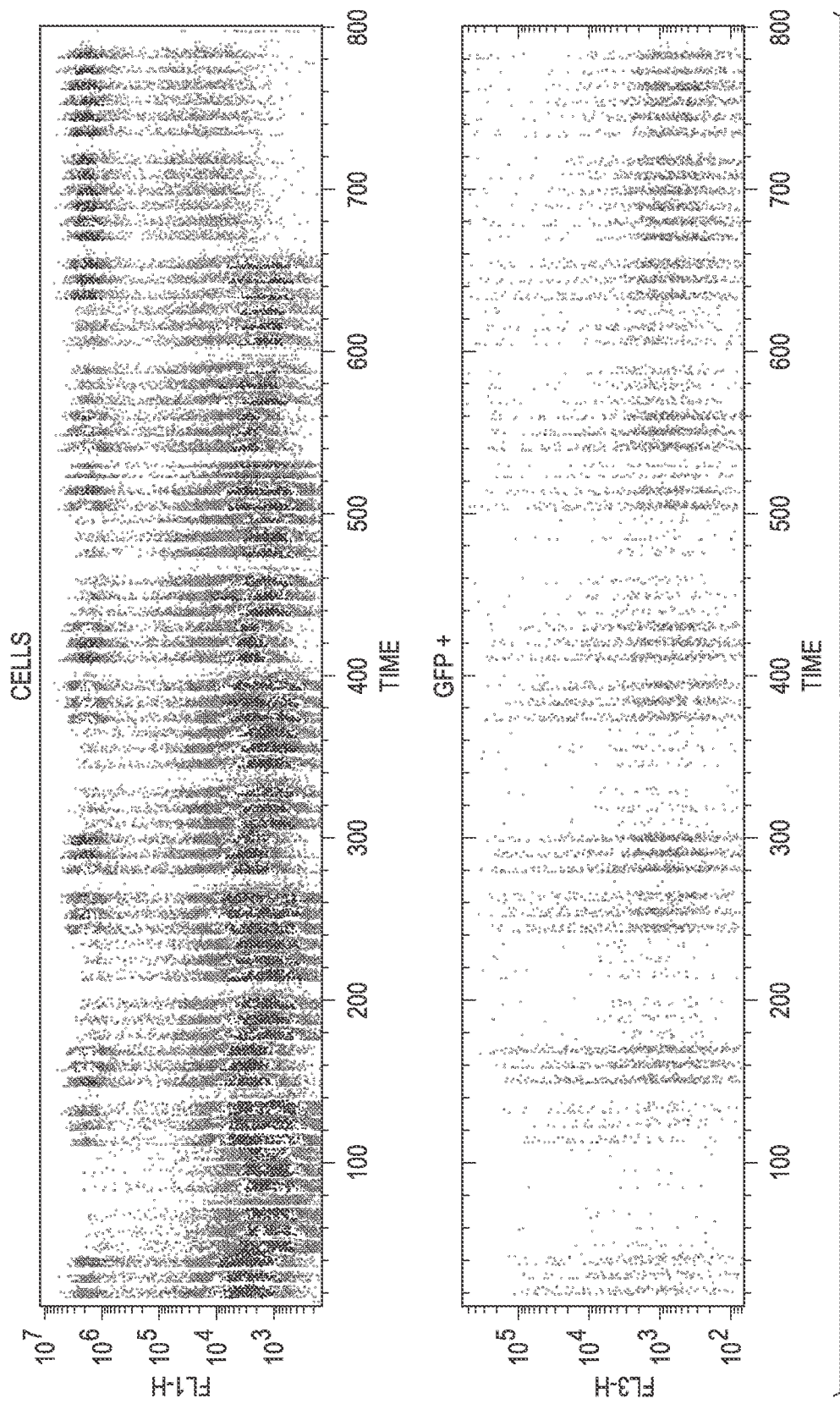


FIG. 41A-2

COMPARISON OF KILLING ASSAY ON: EZ CAR WITH DIFFERENT HINGES ON IntelliCyt's iQue™

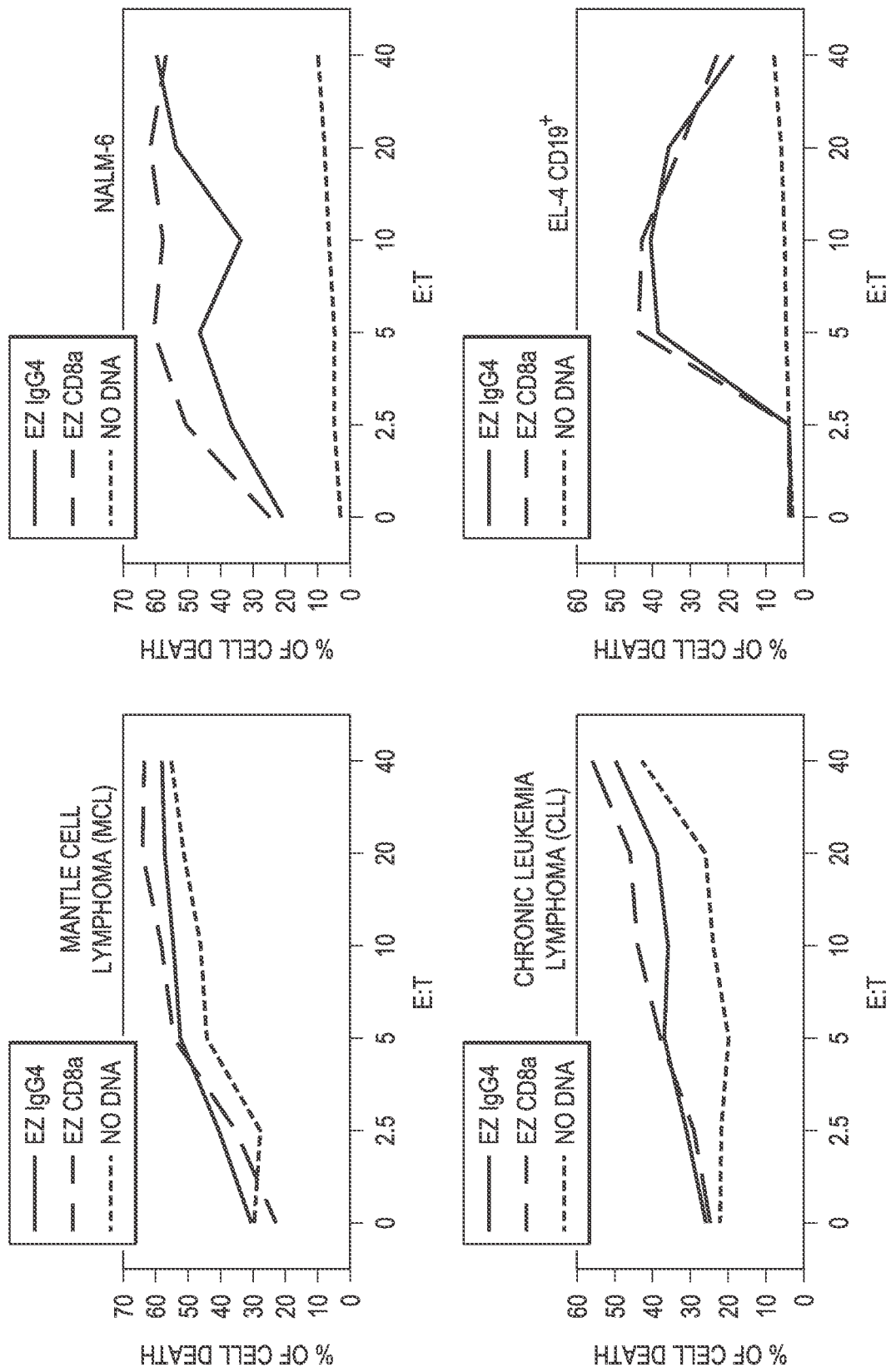


FIG. 41B

