

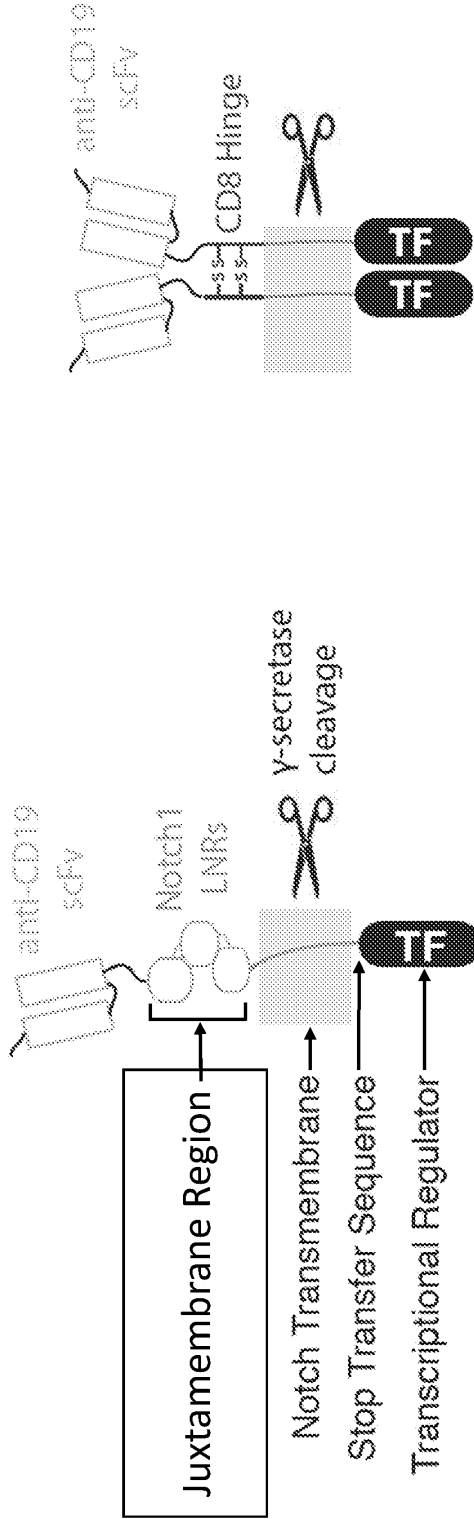


FIG. 1B

FIG. 1A

Primary Human CD4 T cells: anti-CD19 CD8hinge SynNotch Activation

SynNotch1 CD8 HingeNotch1 truncCD8 HingeNotch1

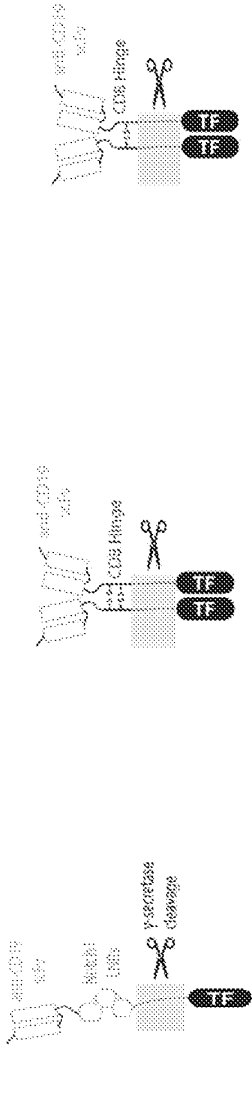


FIG. 2A

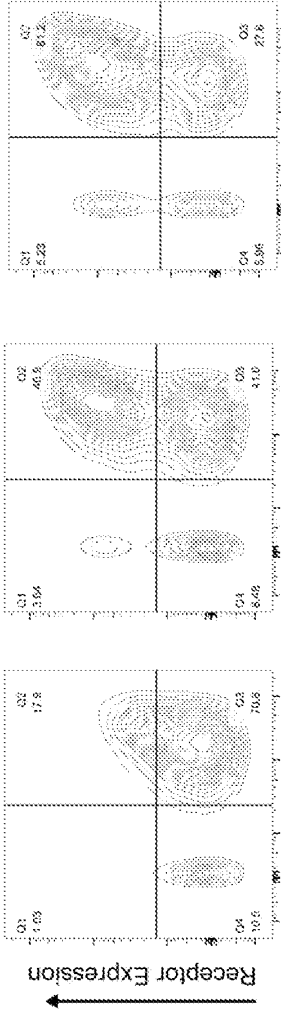


FIG. 2B

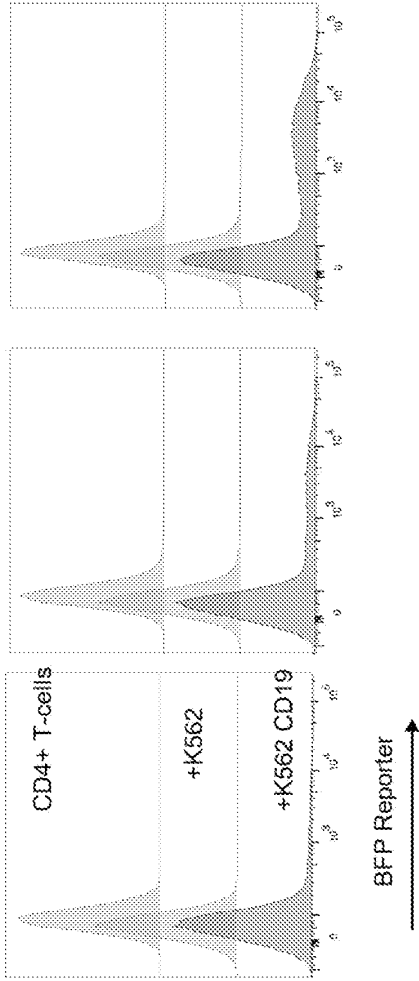


FIG. 2C

Primary Human CD4 T cells: anti-CD19 CD8hinge SynNotch Activation

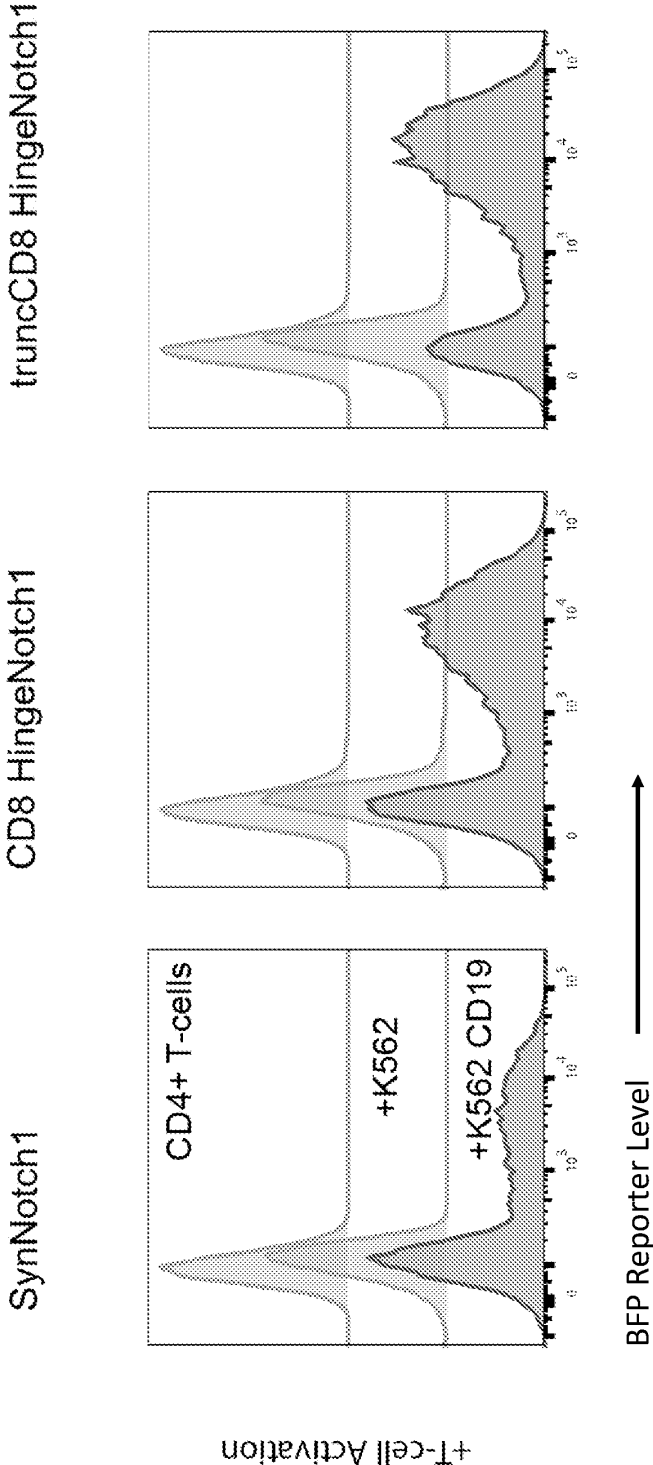


FIG. 3

CD8 Hinge SynNotch Optimization

FIG. 4A

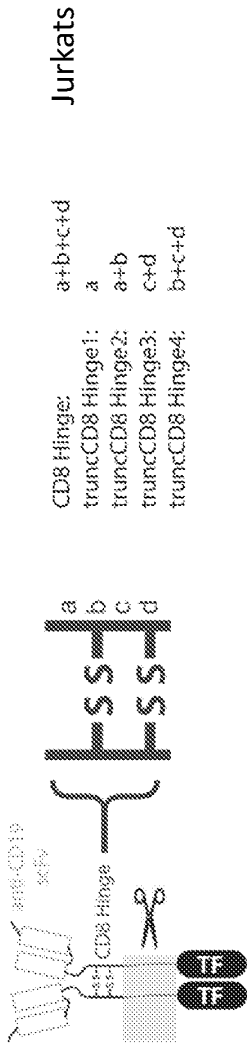


FIG. 4B

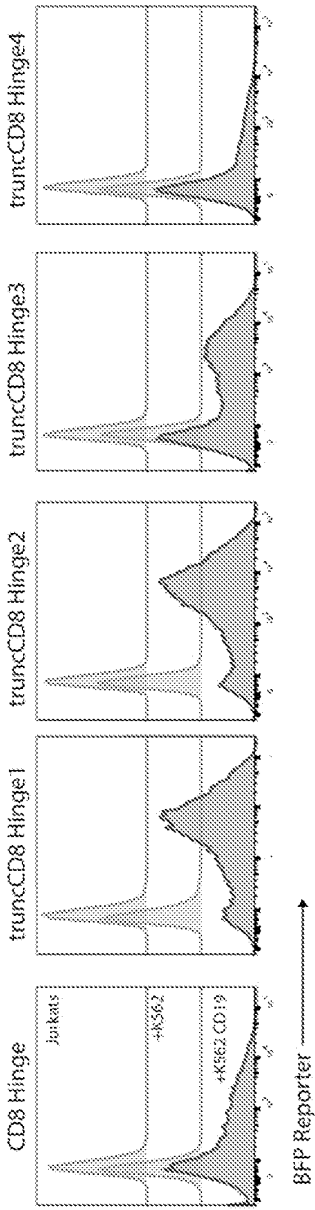
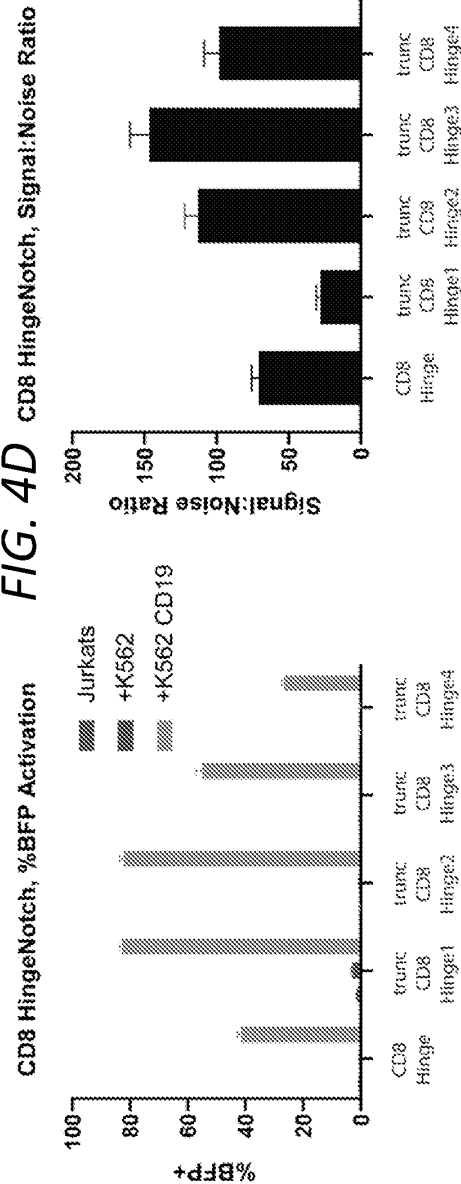


FIG. 4C



TruncCD8Hinge2 SynNotch1 Optimization
Regulation by PKC signaling

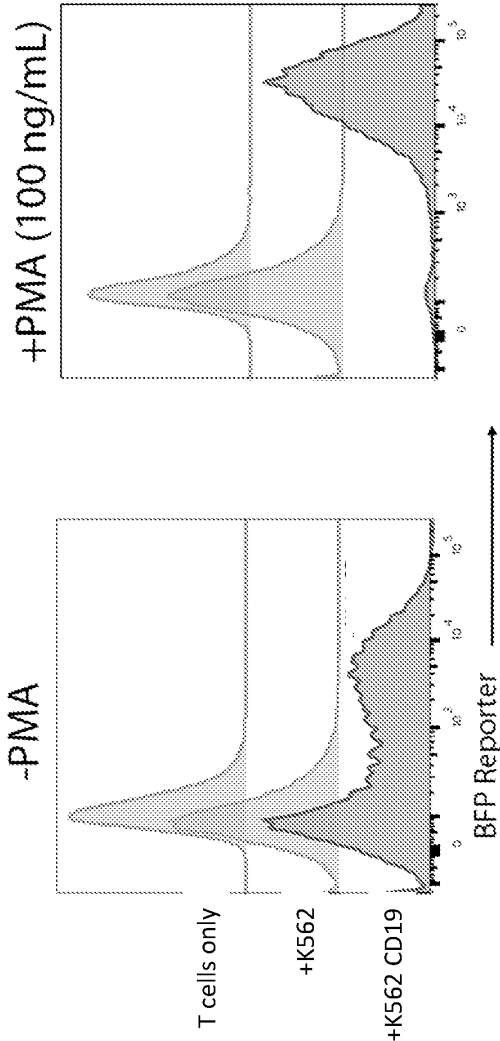


FIG. 5

Hinge SynNotch1 Variant
Jurkat T cells

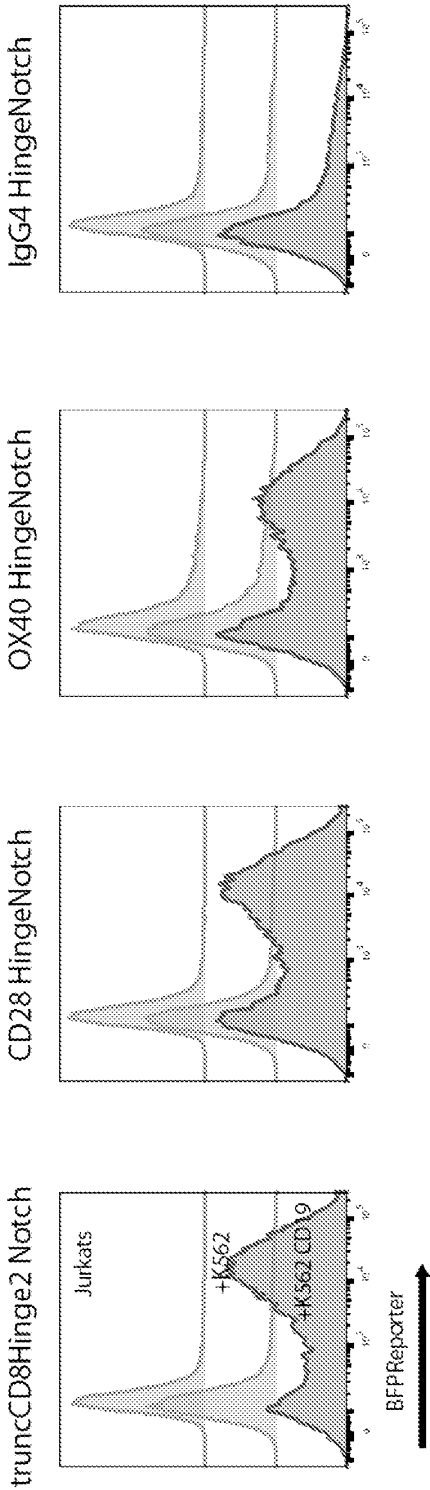


FIG. 6A

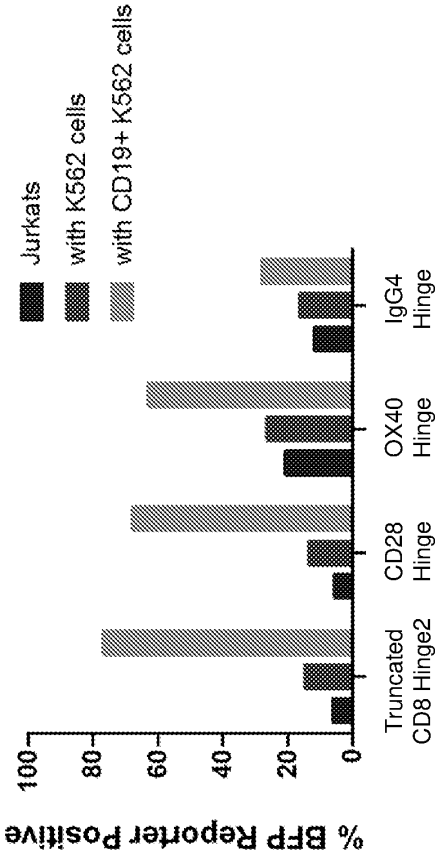


FIG. 6B

TruncCD8Hinge2 SynNotch1
Swapping the Ligand-binding Domain

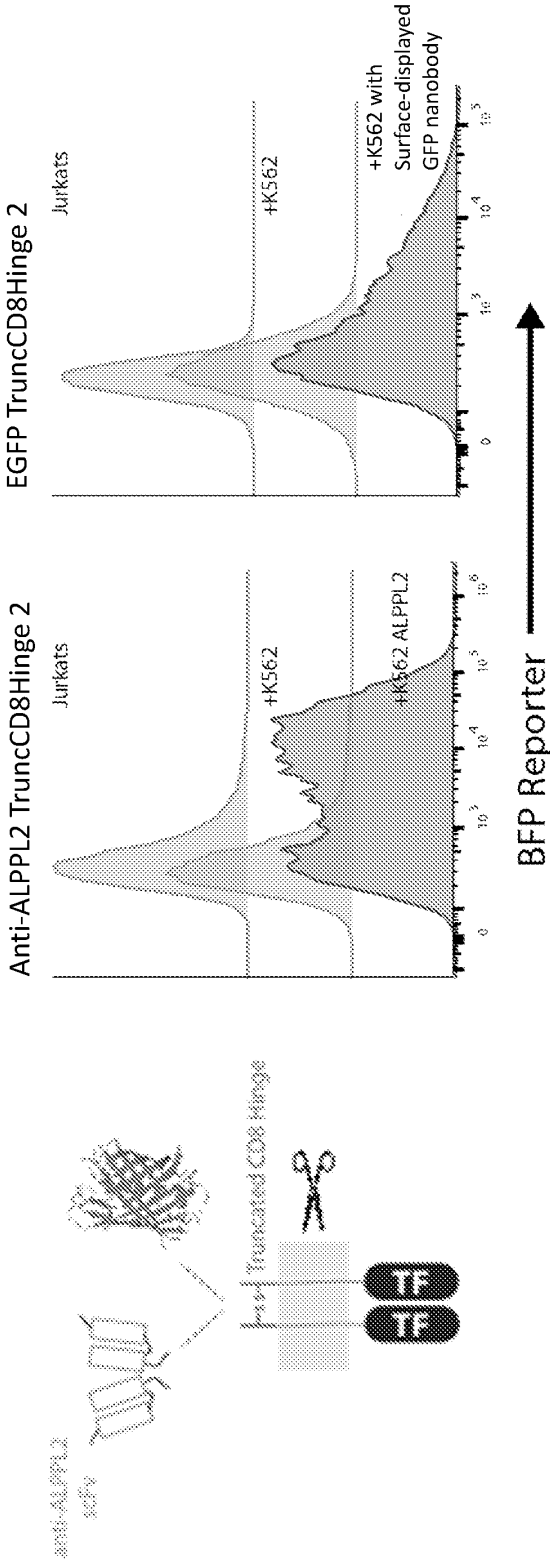


FIG. 7A

FIG. 7B

TruncCD8Hinge2 SynNotch1 Stop Transfer Variant

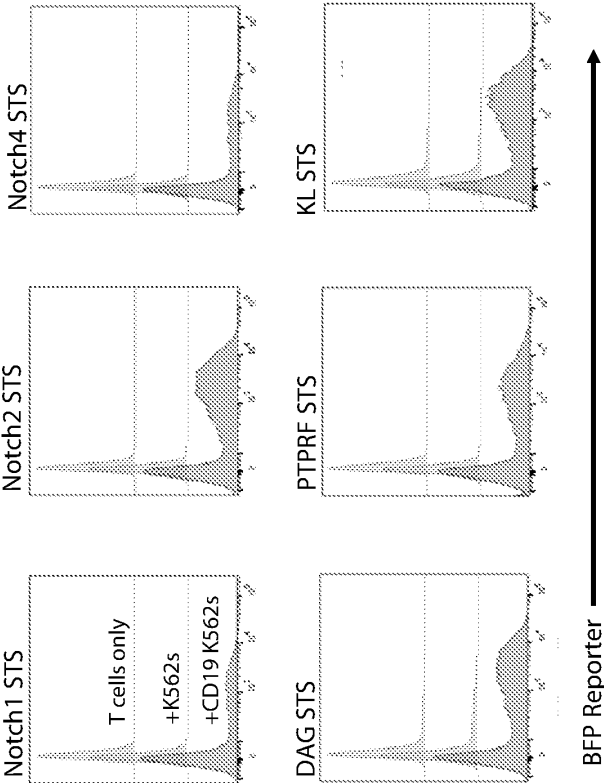


FIG. 8A

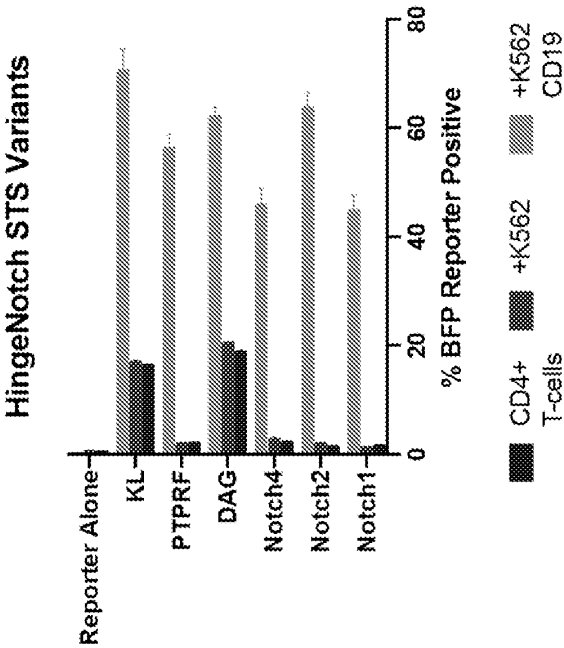


FIG. 8B

CD8Hinge SynNotch1 Optimization
with co-expressed CAR
Confirmation in CD45+ T cells

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FIG. 9A

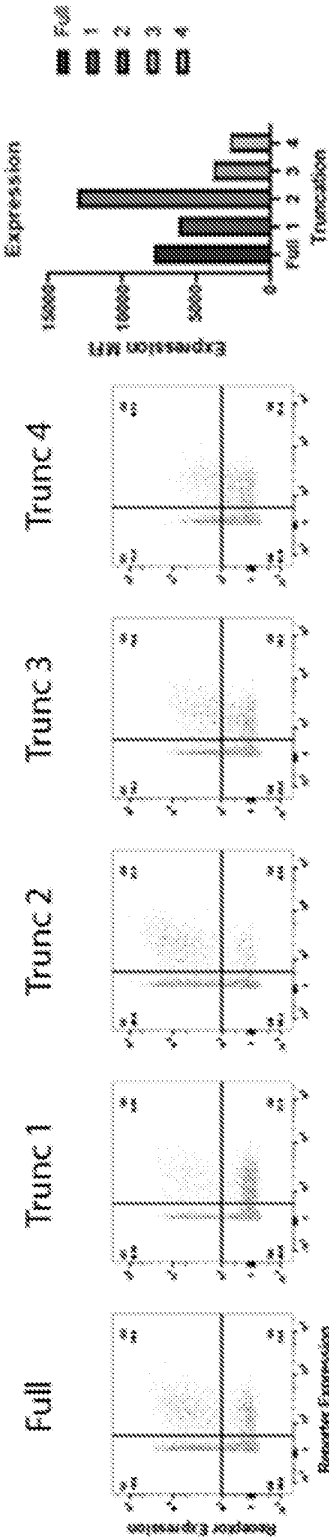


FIG. 9B

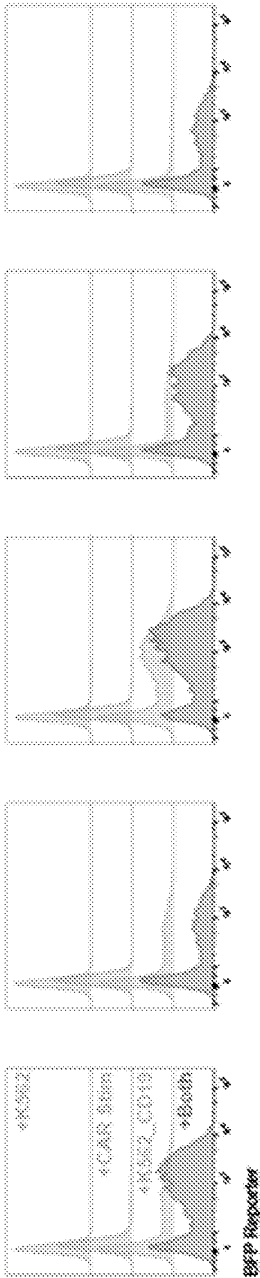


FIG. 9C

Activation with other binding domains and dependence on proteolytic activity

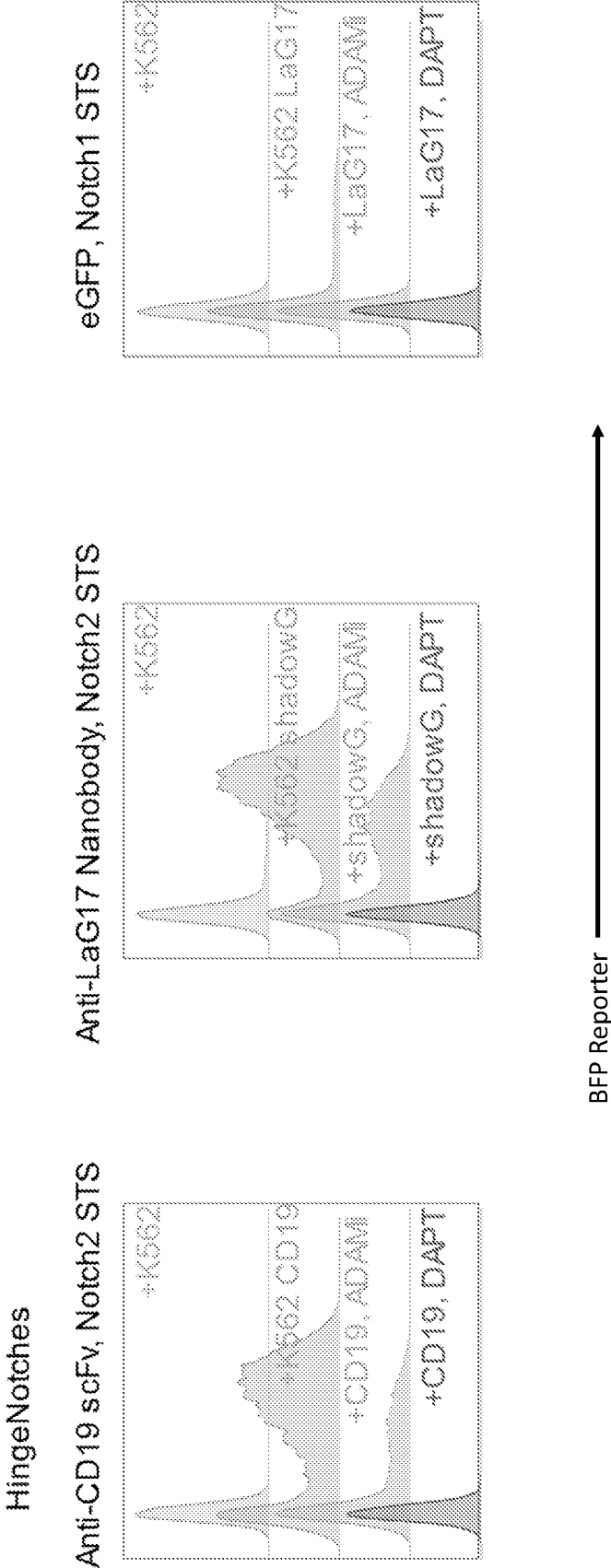


FIG. 10

HingeNotch with Notch2 STS receptor activation with other binding domains

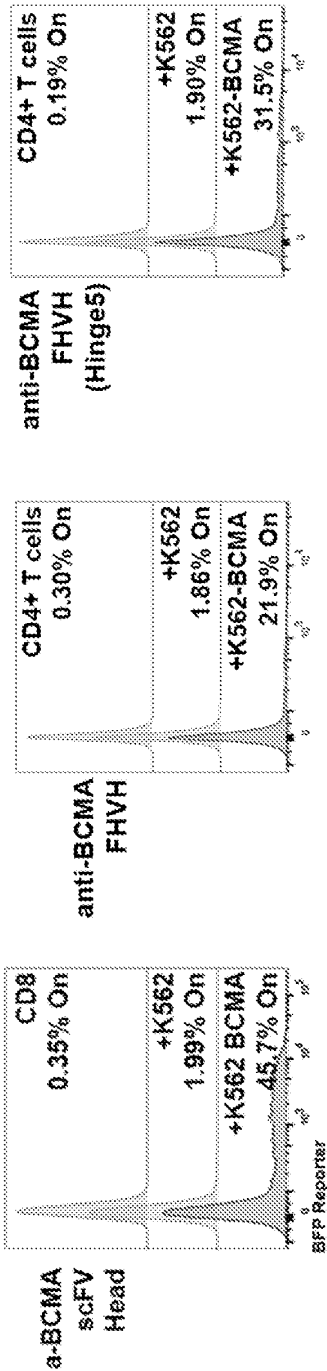


FIG. 11A

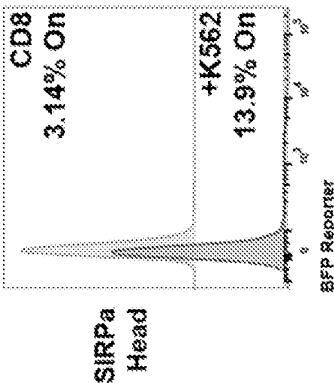


FIG. 11B

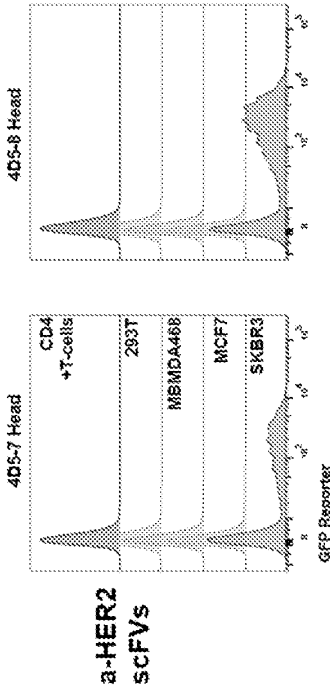


FIG. 11C

HingeNotch activation with different STS and promoters

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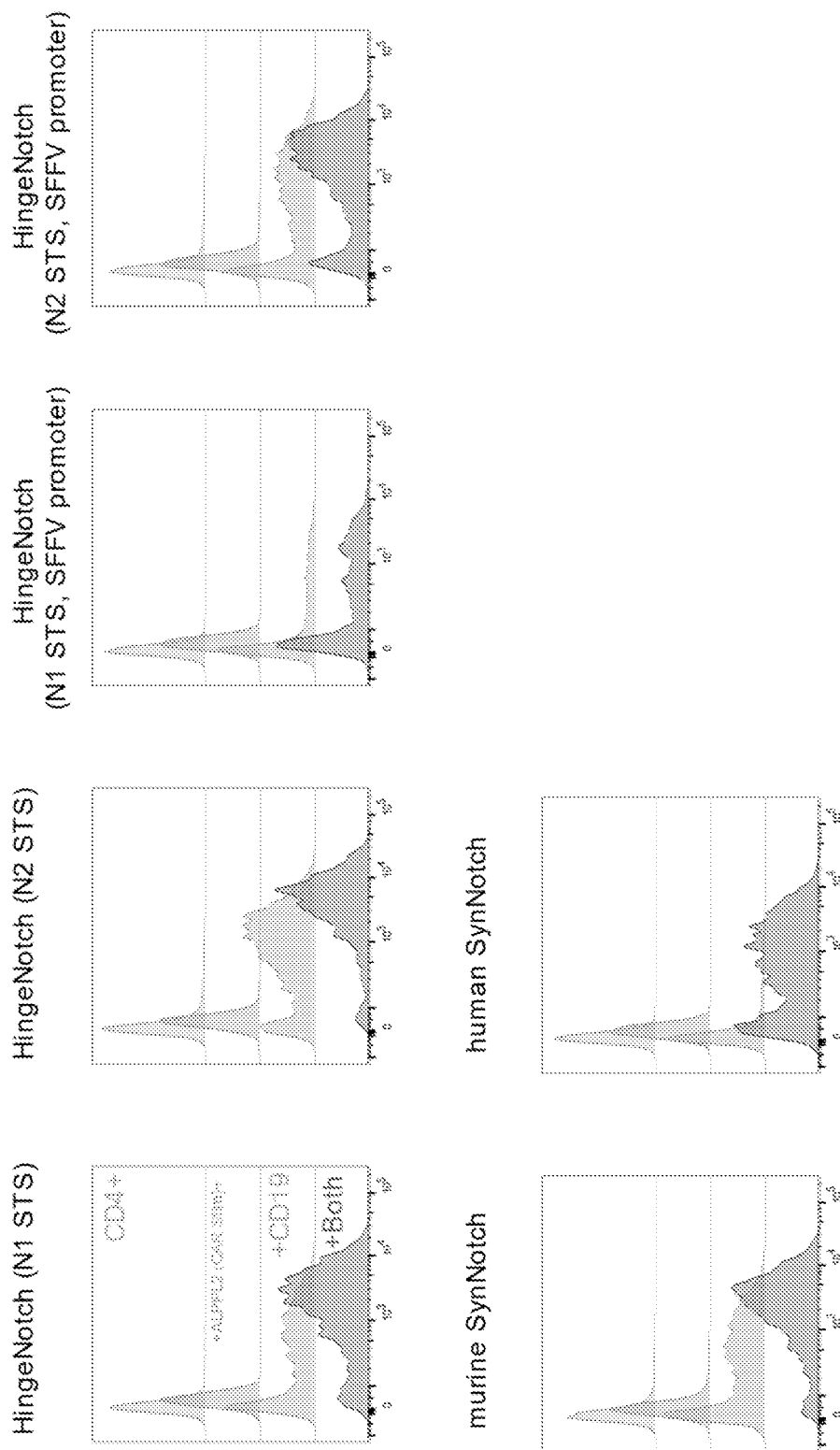


FIG. 12

Hinge synNotch alanine scan

FIG. 13A

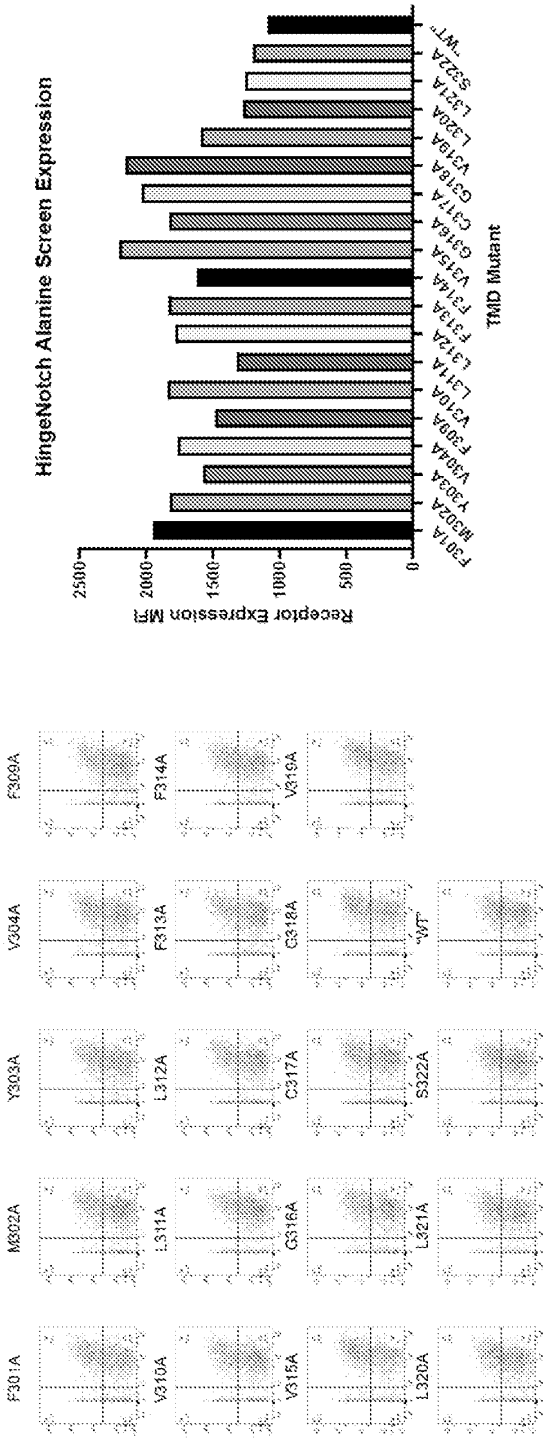
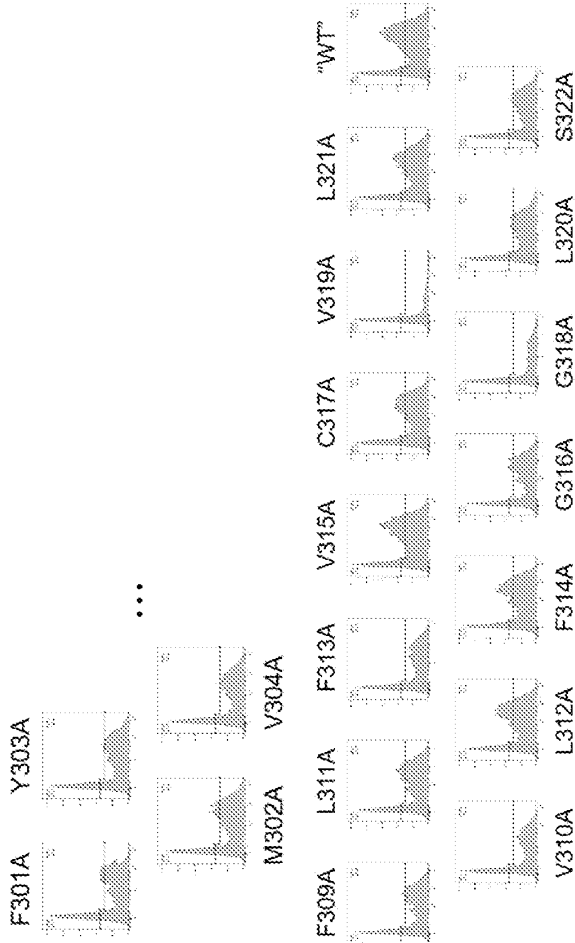


FIG. 13A

FIG. 13B



...

F301A Y303A

M302A V304A

F309A L311A

F313A V315A

C317A V319A

L321A L320A

F314A F313A

G318A V319A

S322A WT

WT

WT

WT

WT

WT

WT

WT

WT

WT

WT

WT

WT

WT

WT

WT

WT

WT

WT

WT

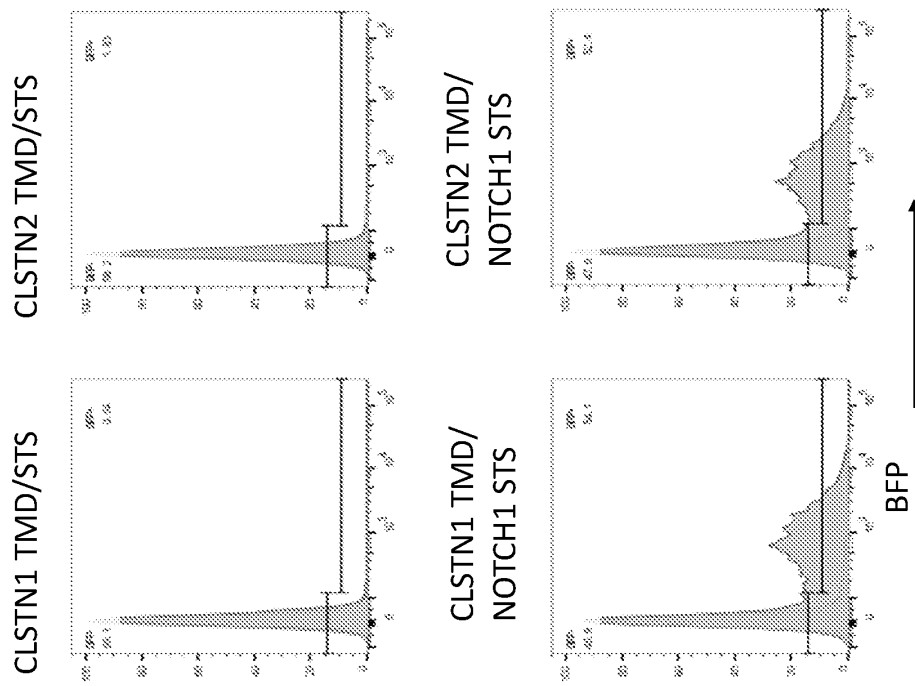
WT

WT

WT

SynCLSTN1/2 Receptors

-CAR
Activity



+CAR
Activity

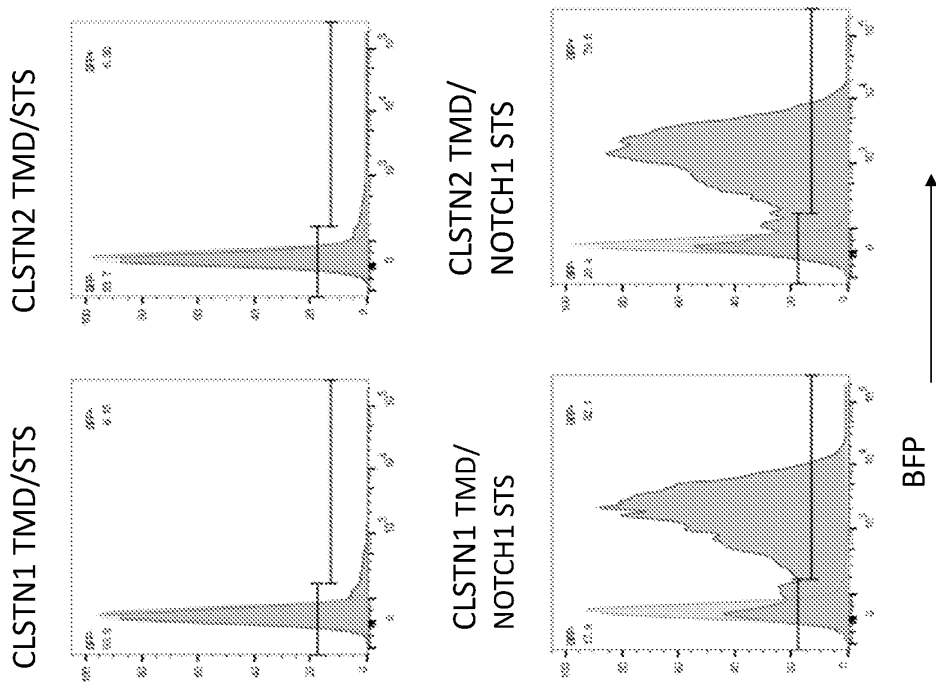


FIG. 14

Tunable, ligand-dependent expansion of T cells using HingeNotch-controlled expression of an engineered cytokine

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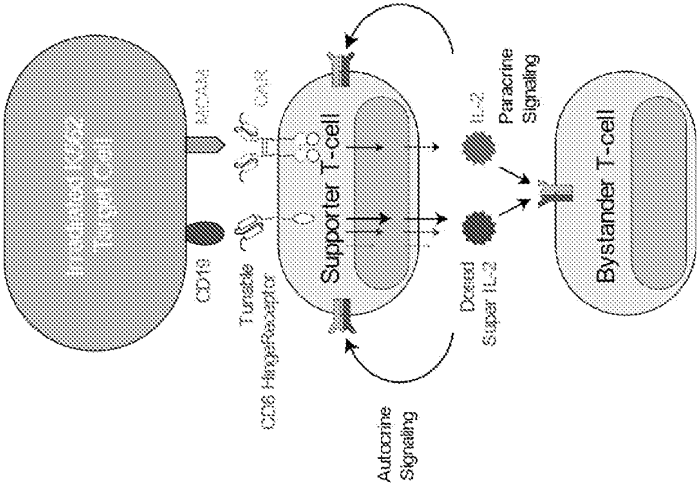


FIG. 15A

Receptor Expression of HingeNotch STS variants

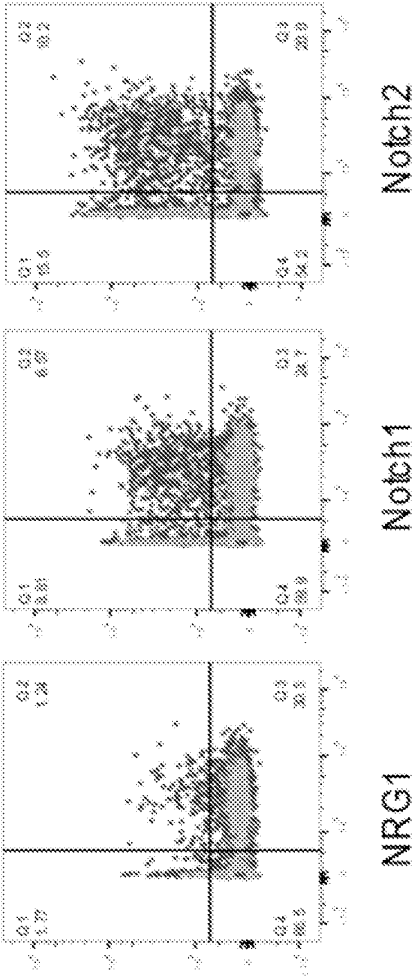


FIG. 15B

Tunable, ligand-dependent expansion of T cells using
HingeNotch-controlled expression of an engineered cytokine

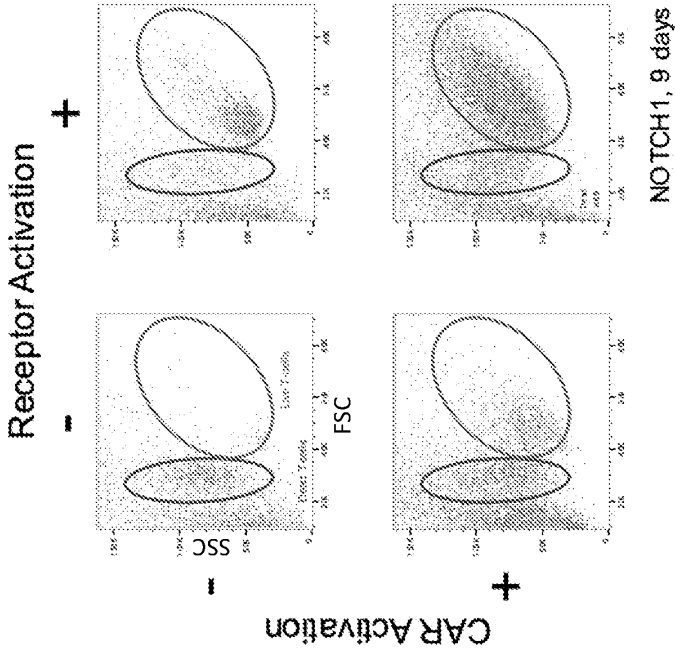


FIG. 16

Tunable, ligand-dependent expansion of T cells using
HingeNotch-controlled expression of an engineered cytokine

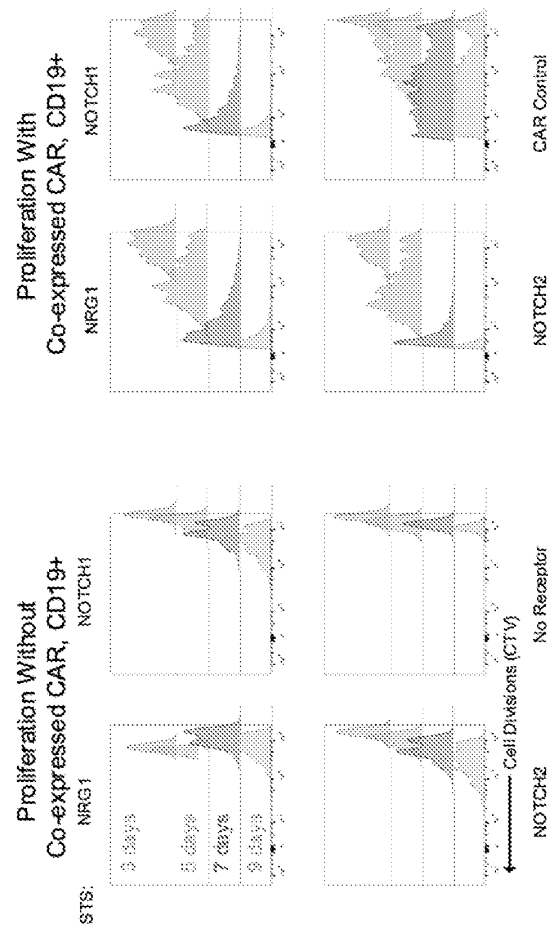


FIG. 17

Tunable, ligand-dependent expansion of T cells using
HingeNotch-controlled expression of an engineered cytokine

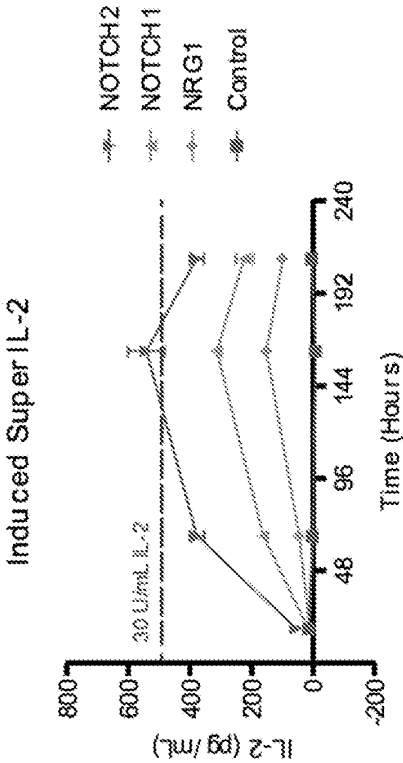


FIG. 18A

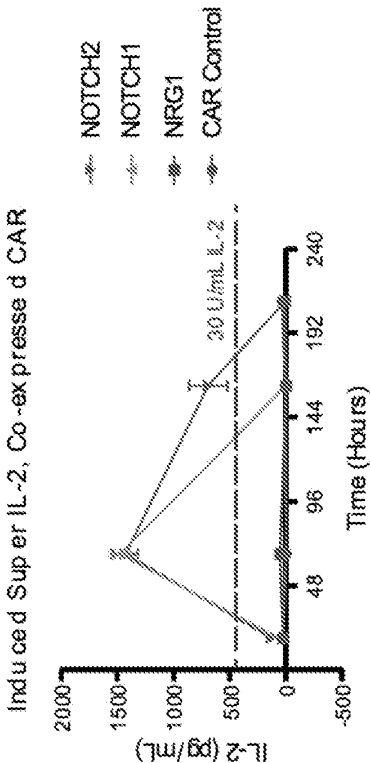


FIG. 18B

Tunable, ligand-dependent expansion of T cells using
HingeNotch-controlled expression of an engineered cytokine

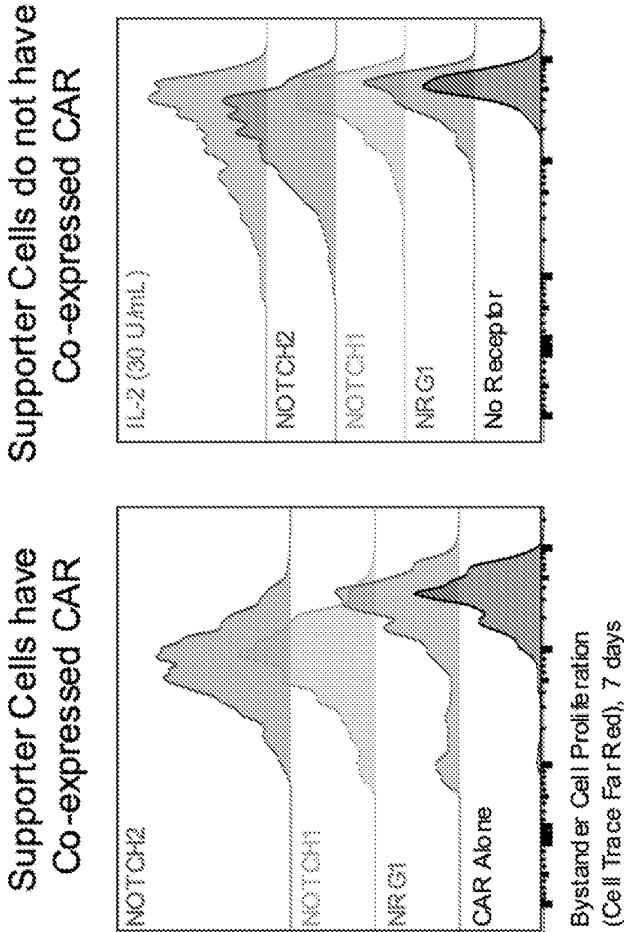


FIG. 19

HingeNotch receptor circuits can function on a single lentiviral vector

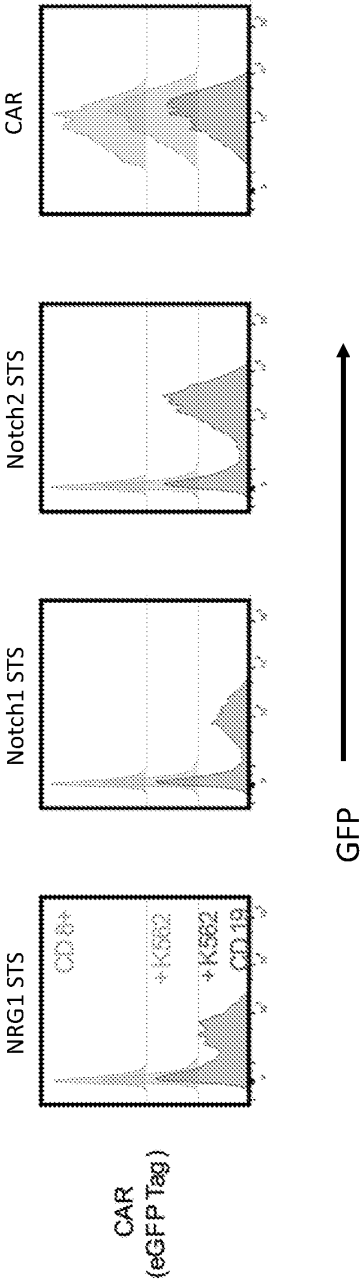


FIG. 20

CAR induction from HingeNotch single vector constructs can specifically eliminate dual-antigen target cells

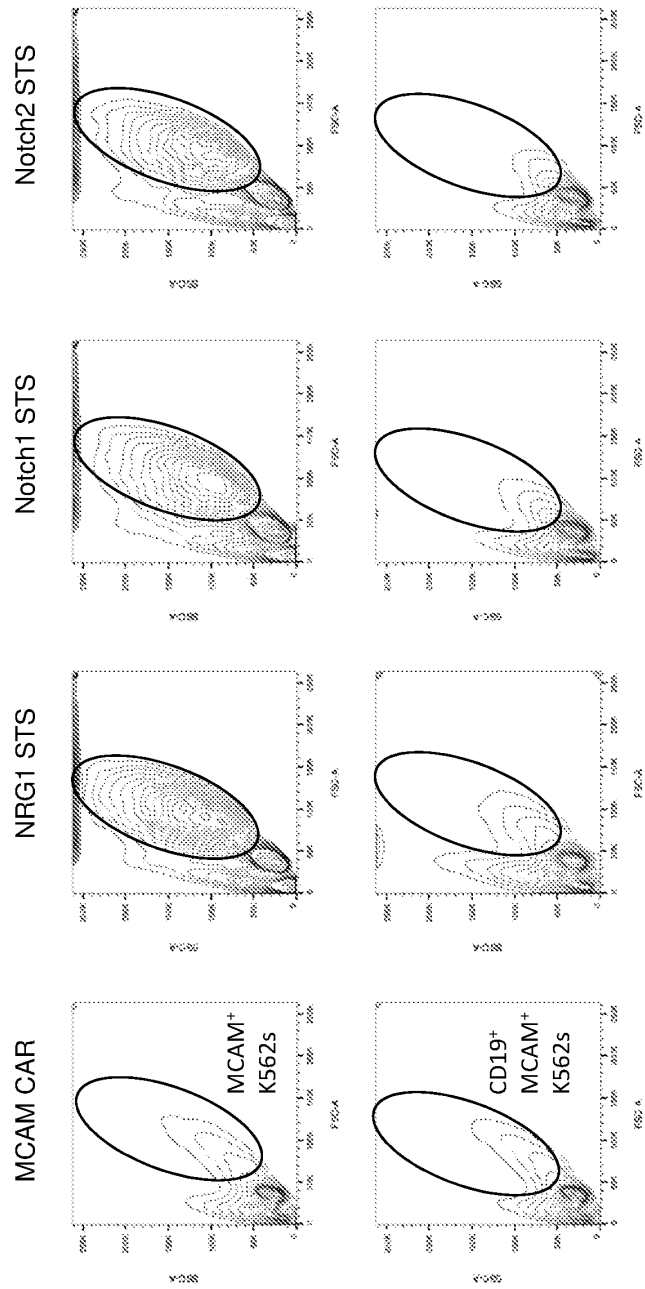


FIG. 21

CAR induction from HingeNotch single vector constructs can specifically eliminate dual-antigen target cells

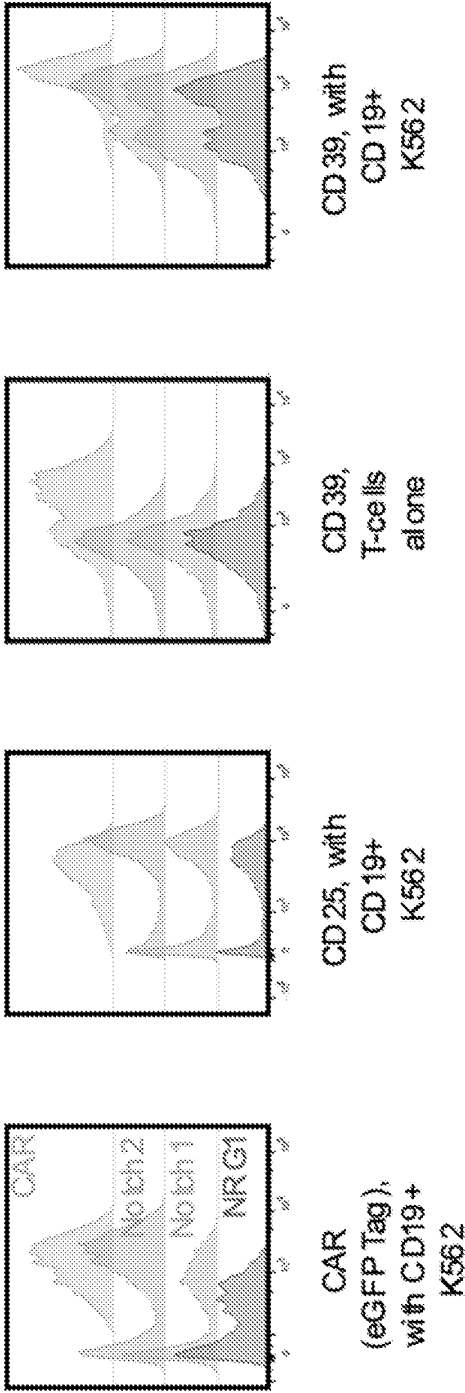


FIG. 22

Figure 1 illustrates the experimental model and the results of the study. The schematic diagram shows a Tumor cell expressing CD19 and BCMA, interacting with a T cell expressing CD19 and BCMA. The T cell is also shown interacting with a BCMA-CAR T cell. The graph displays % Survival (n=5) and Tumor volume (mm³) over 20 days. The legend indicates: No treatment (n=5) - solid black line, Untreated (n=5) - dotted line, BCMA-CAR (n=5) - dashed line, CD19 hinge-knock (n=5) - solid grey line, and BCMA-CAR (n=5) - hatched area.

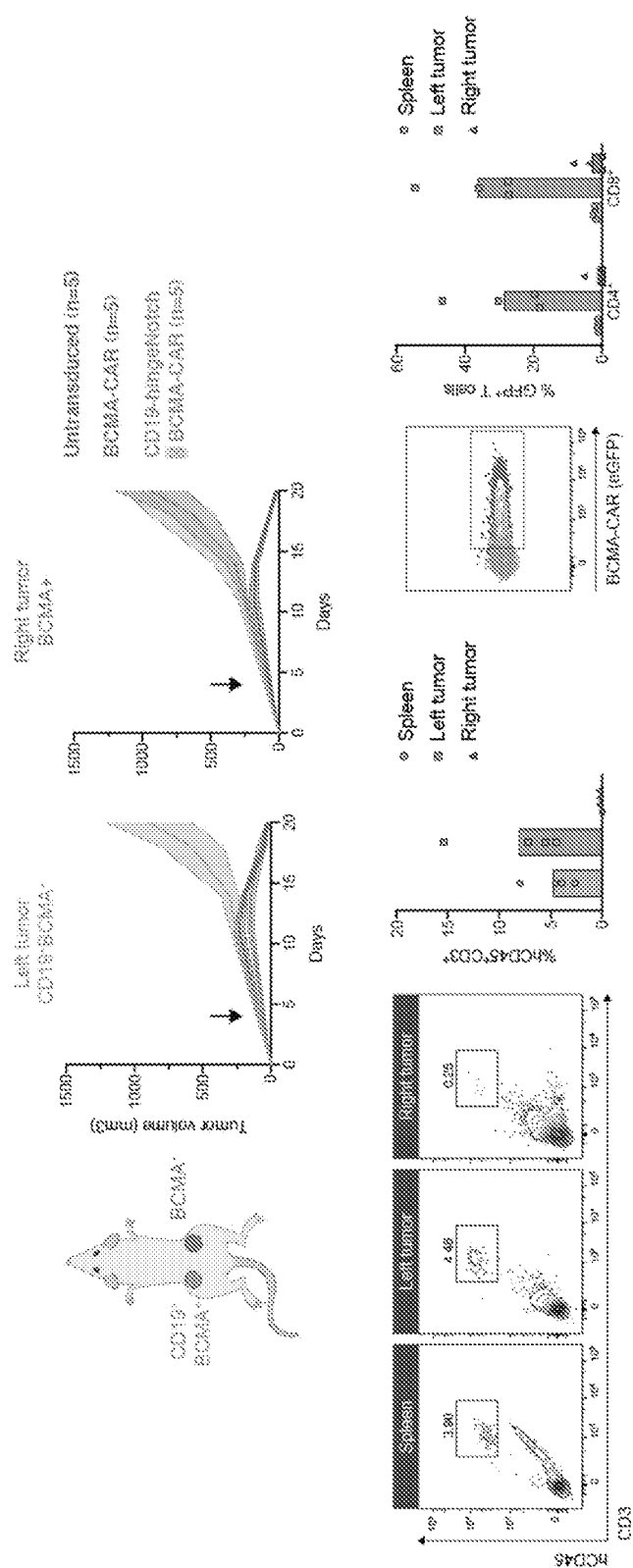


FIG. 23