

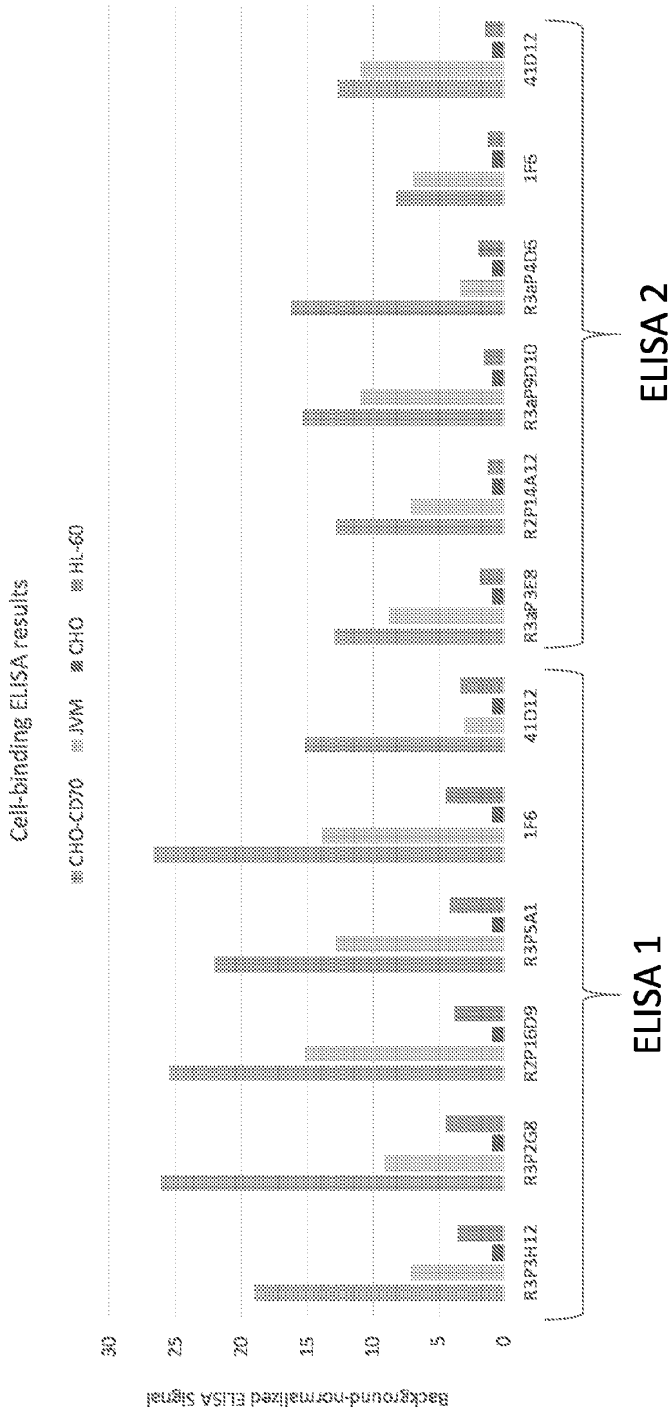


FIG. 1

Octet Binding Assay

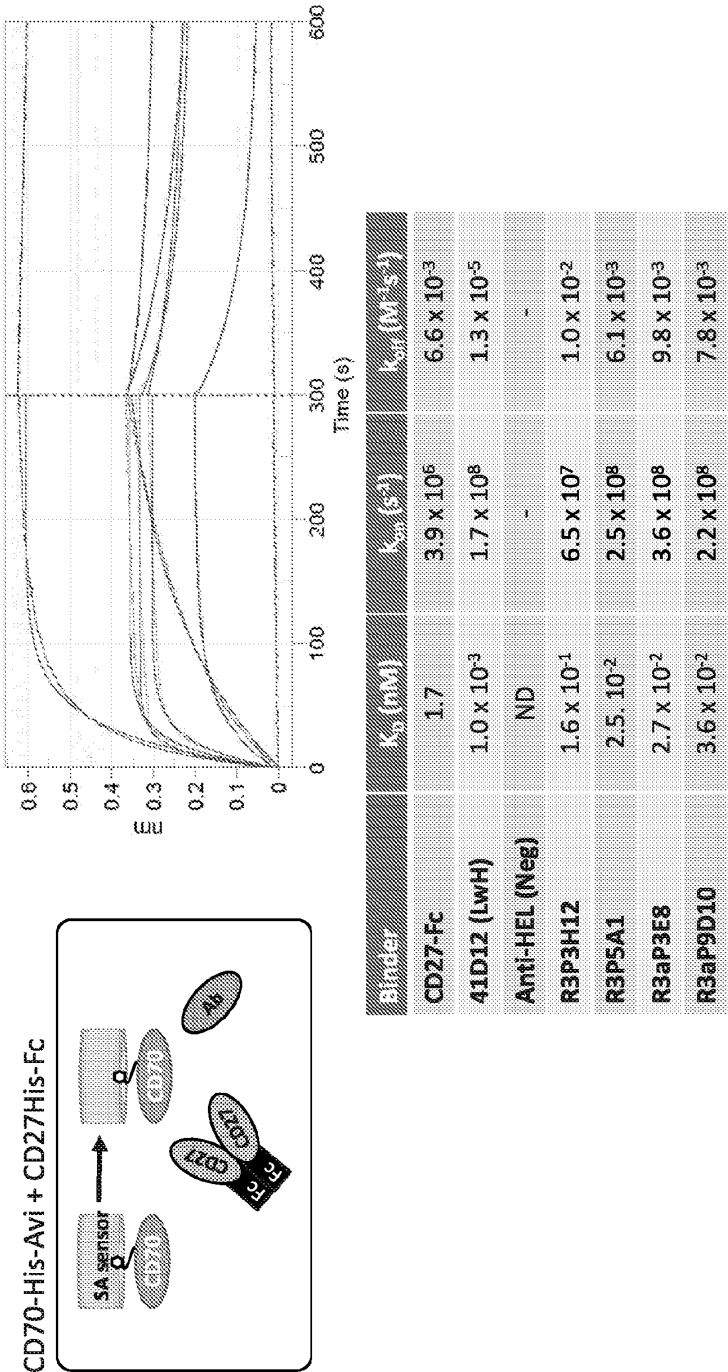


FIG. 2

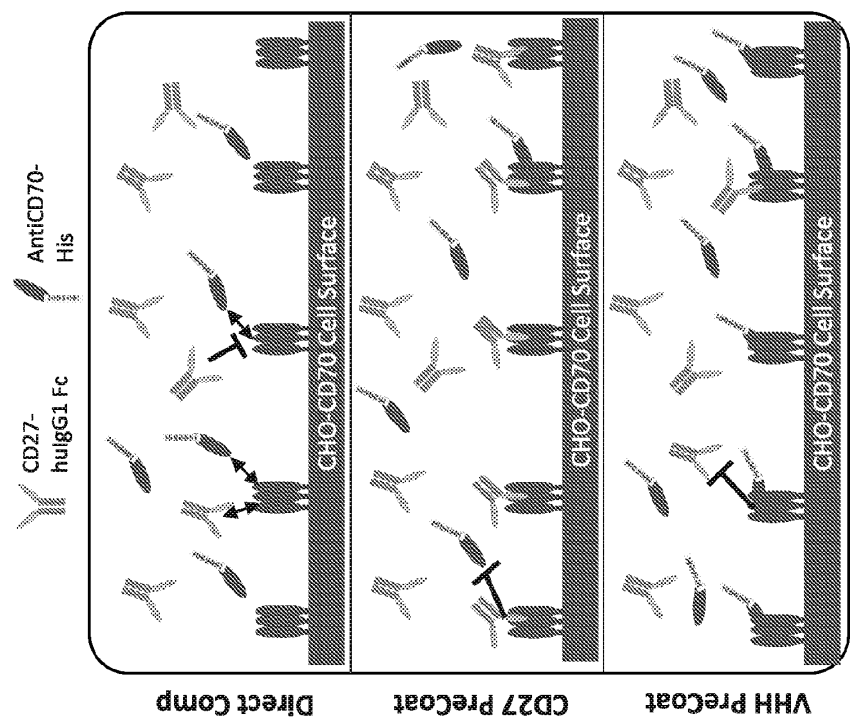


FIG. 4

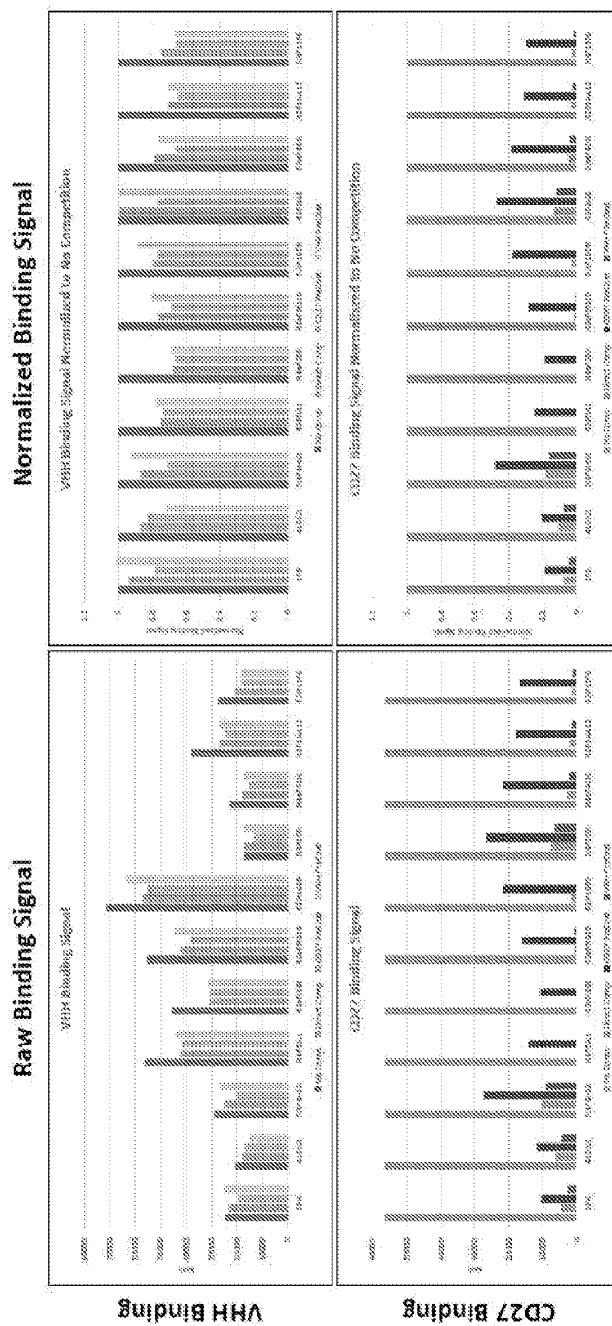
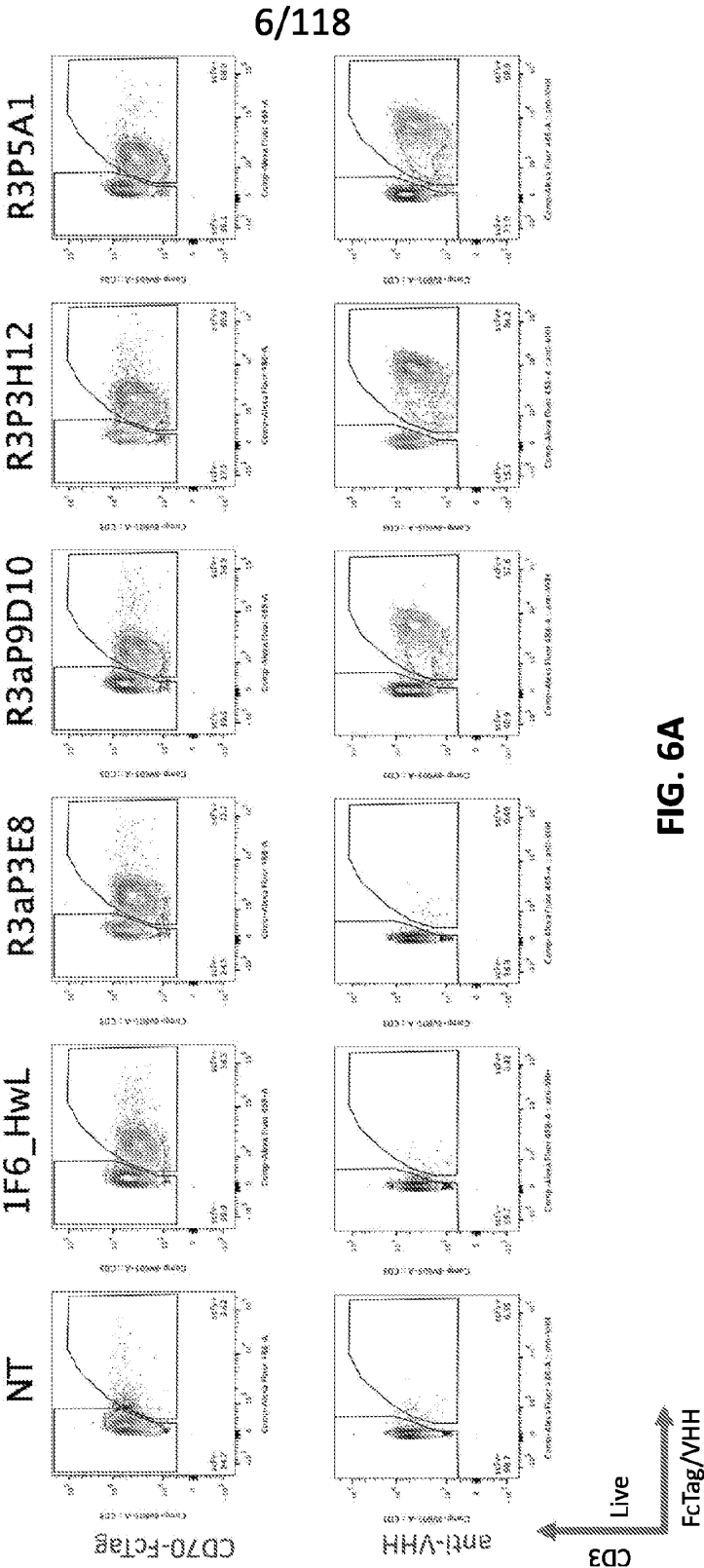


FIG. 5

TFP detection anti-VHH/CD70-Fc Tag



TFP detection anti-VHH

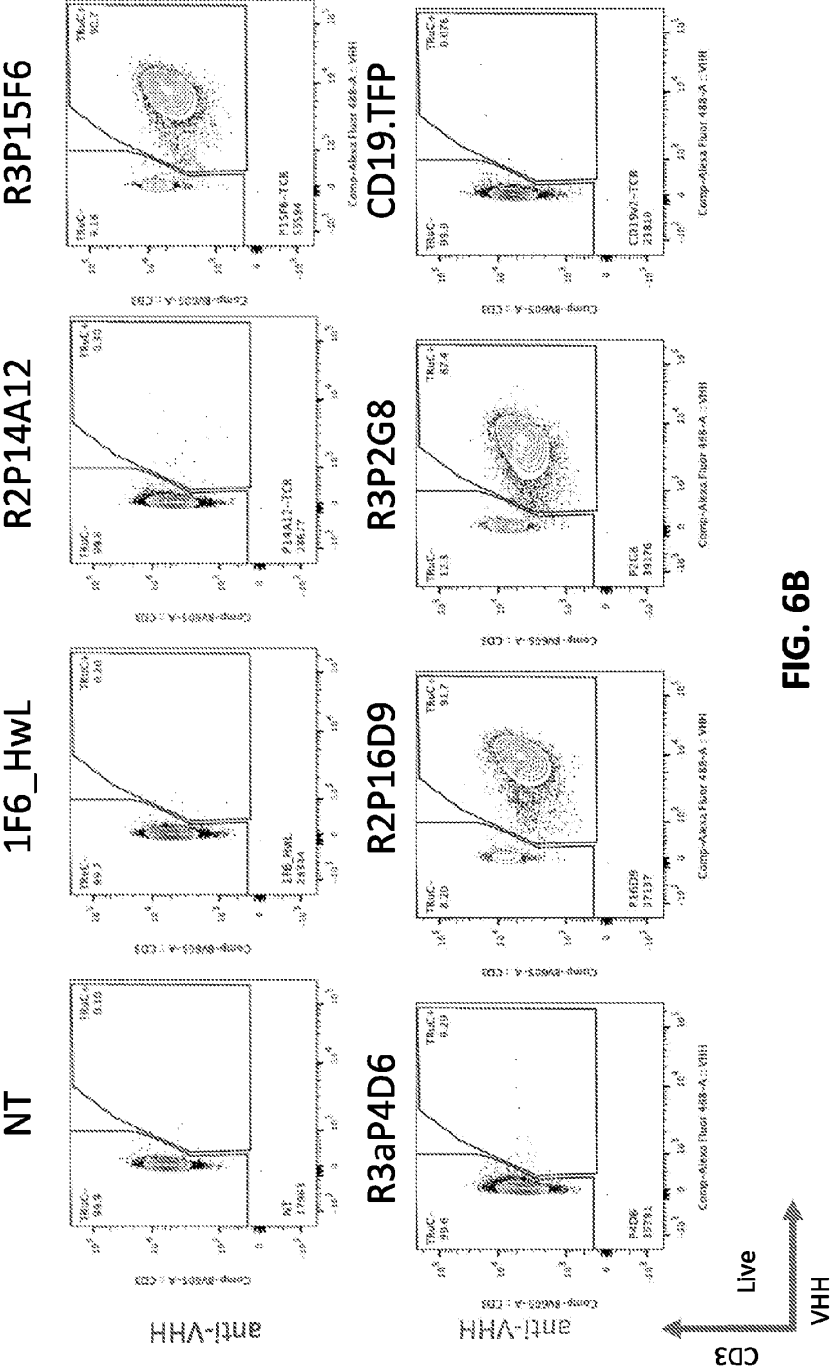
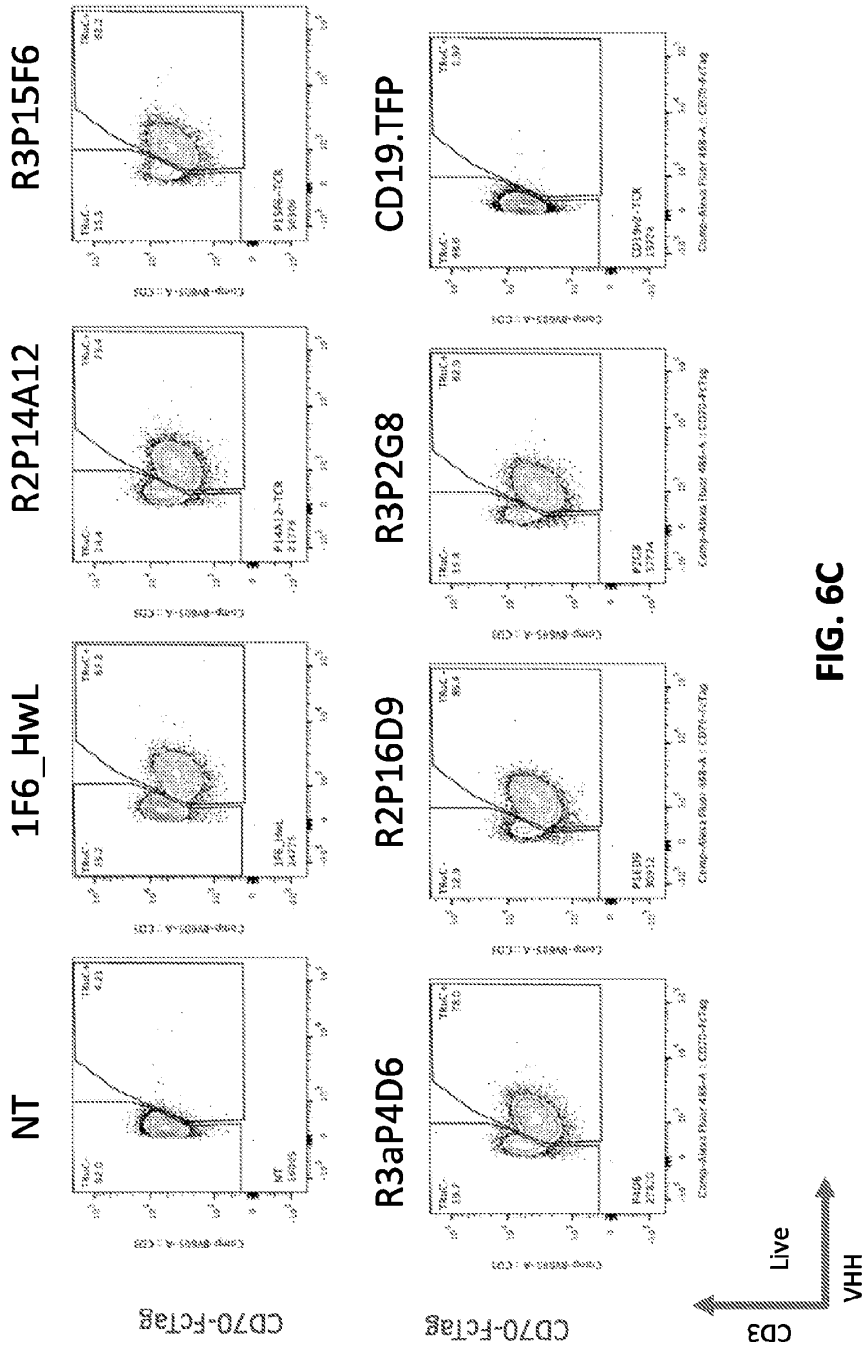


FIG. 6B

TFP detection CD70-Fc Tag



TFP+/- CD4 v CD8

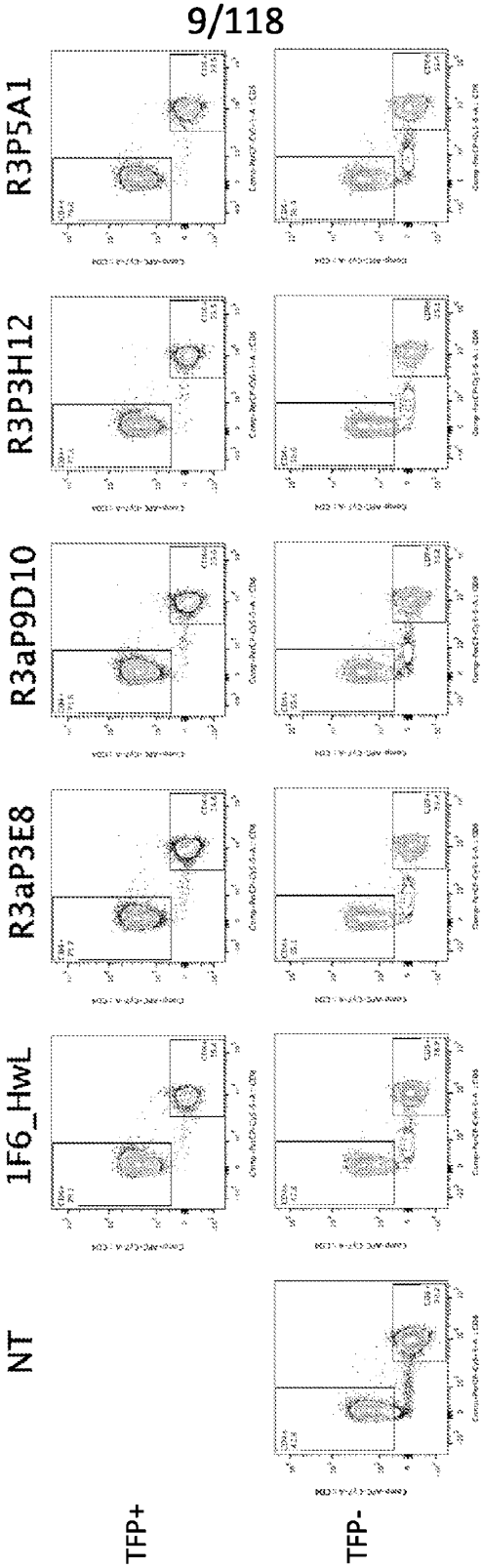
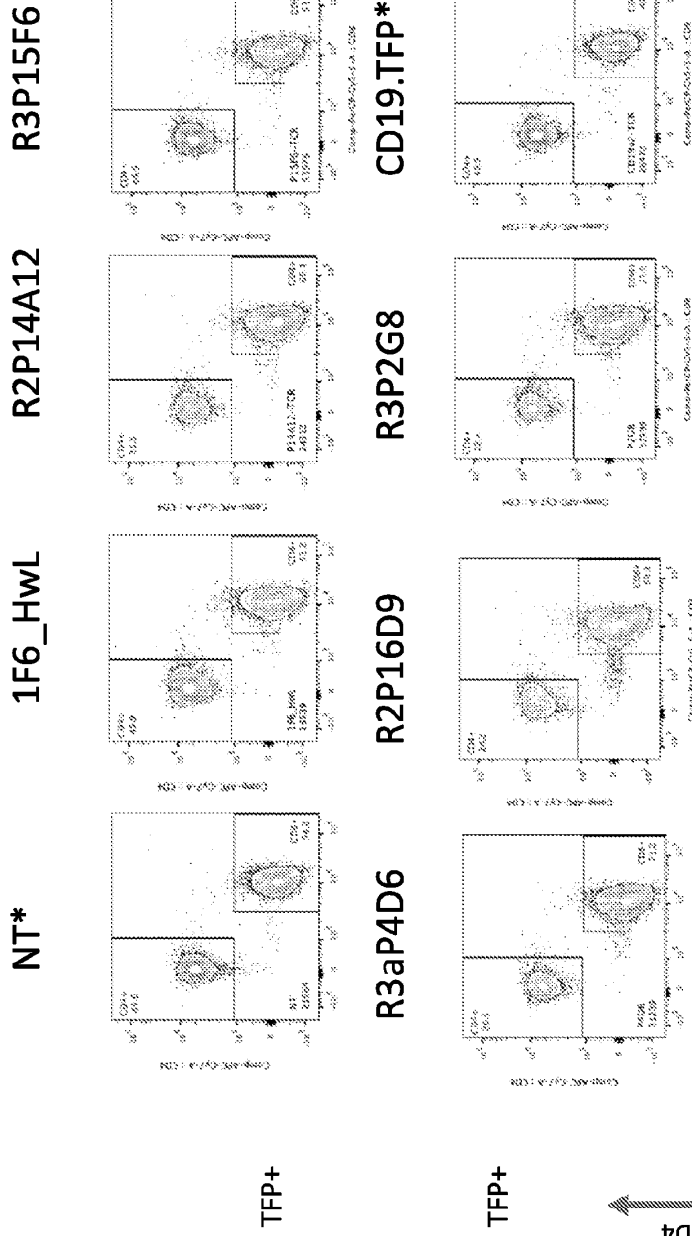


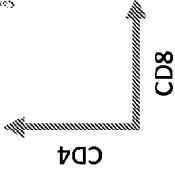
FIG. 7A

TFP+ CD4 v CD8



* Displays TCR

FIG. 7B



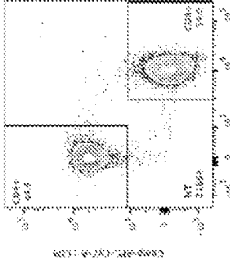
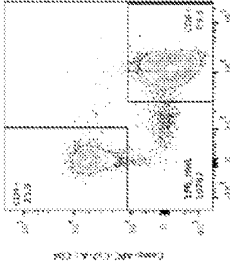
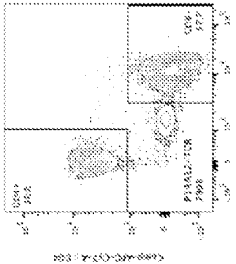
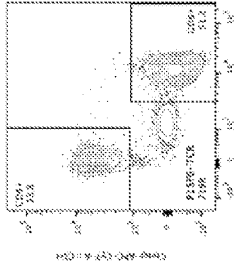
TFP- CD4 v CD8

R3P15F6

R2P14A12

1F6_HwL

NT



CD19.TFP

R3P2G8

R2P16D9

R3aP4D6

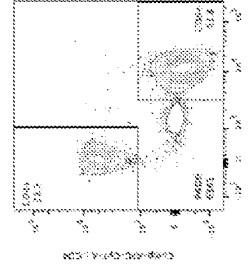
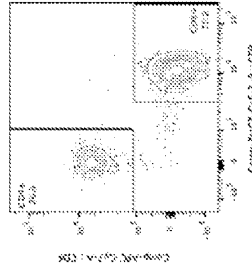
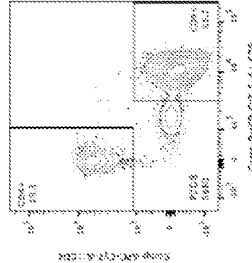
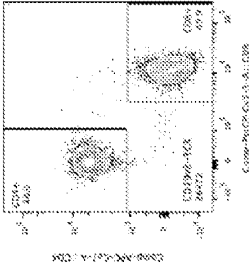
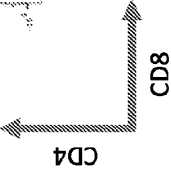
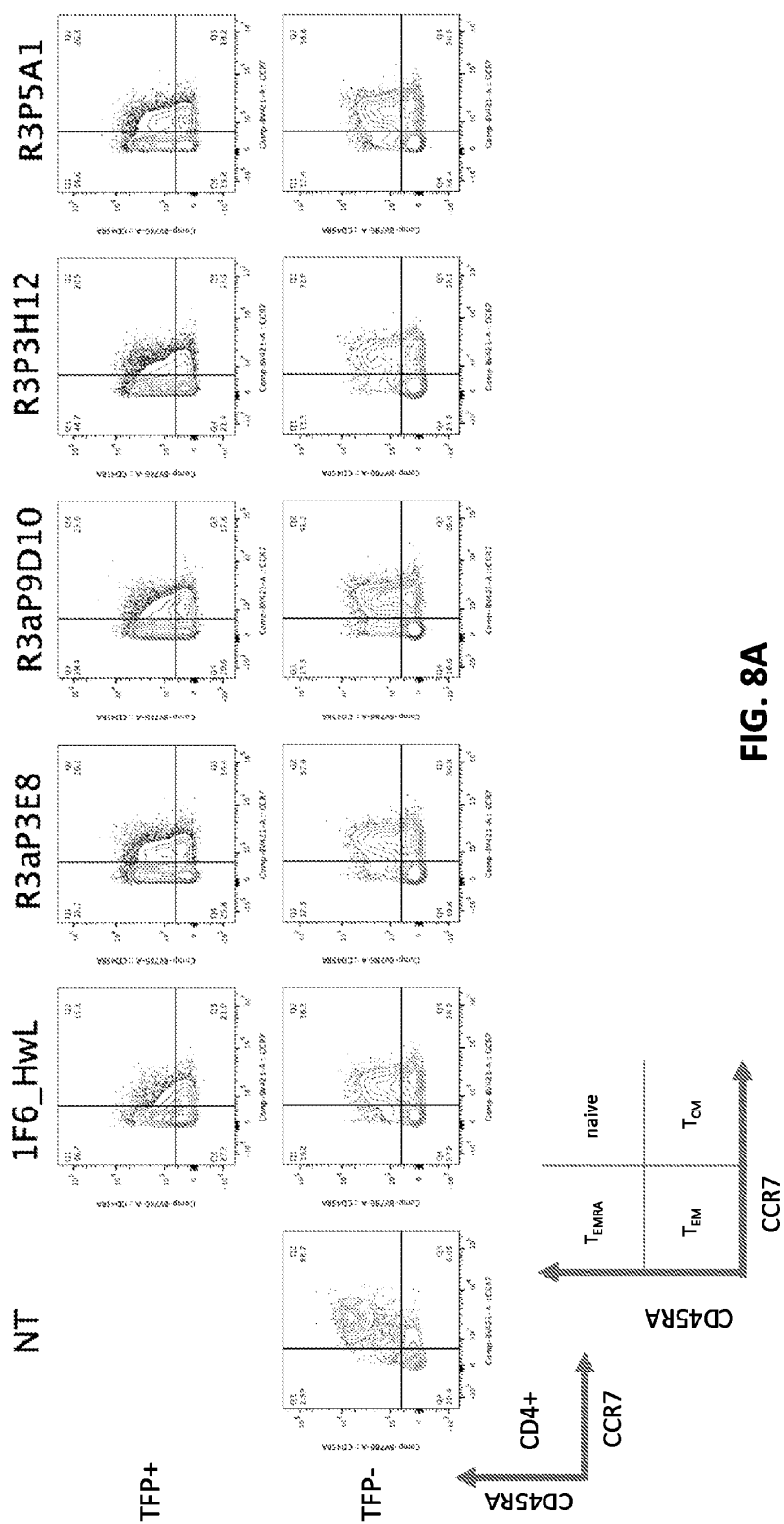


FIG. 7C



CD4+ TFP+/- T Cell Memory Status



CD4+ TFP- T Cell Memory Status

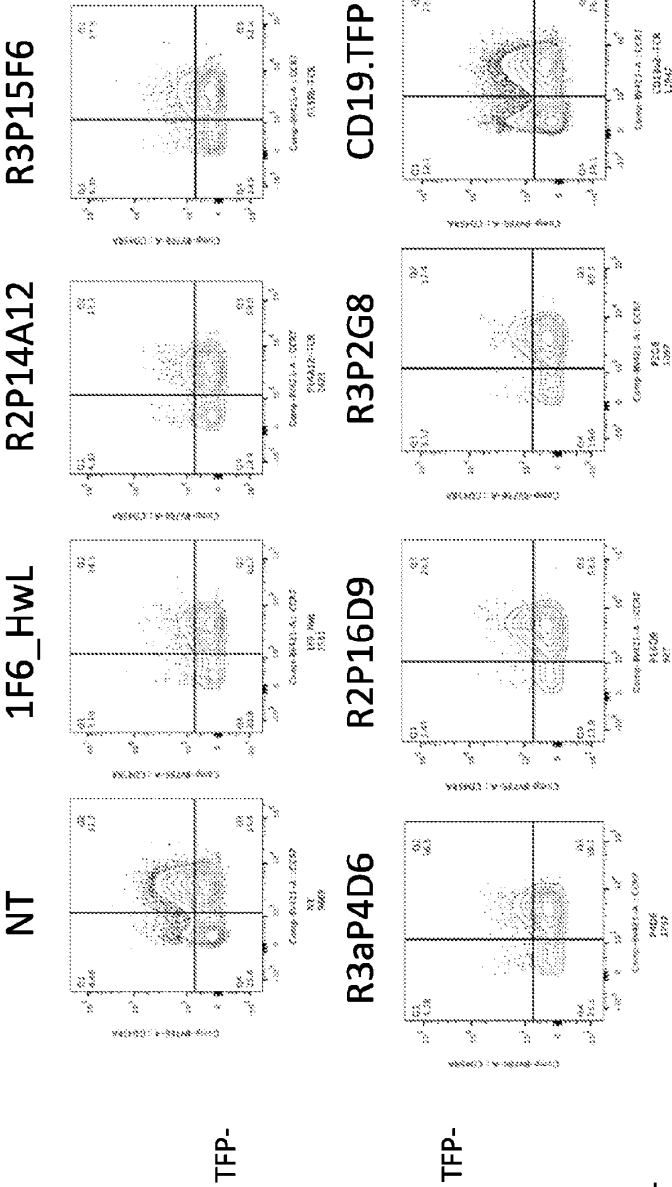
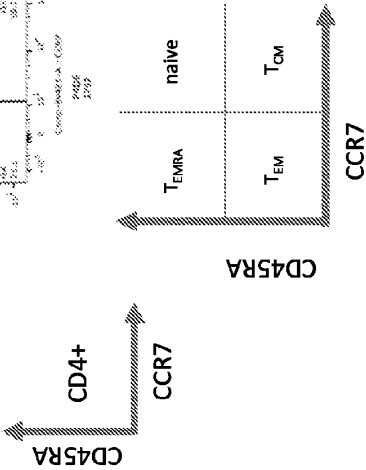
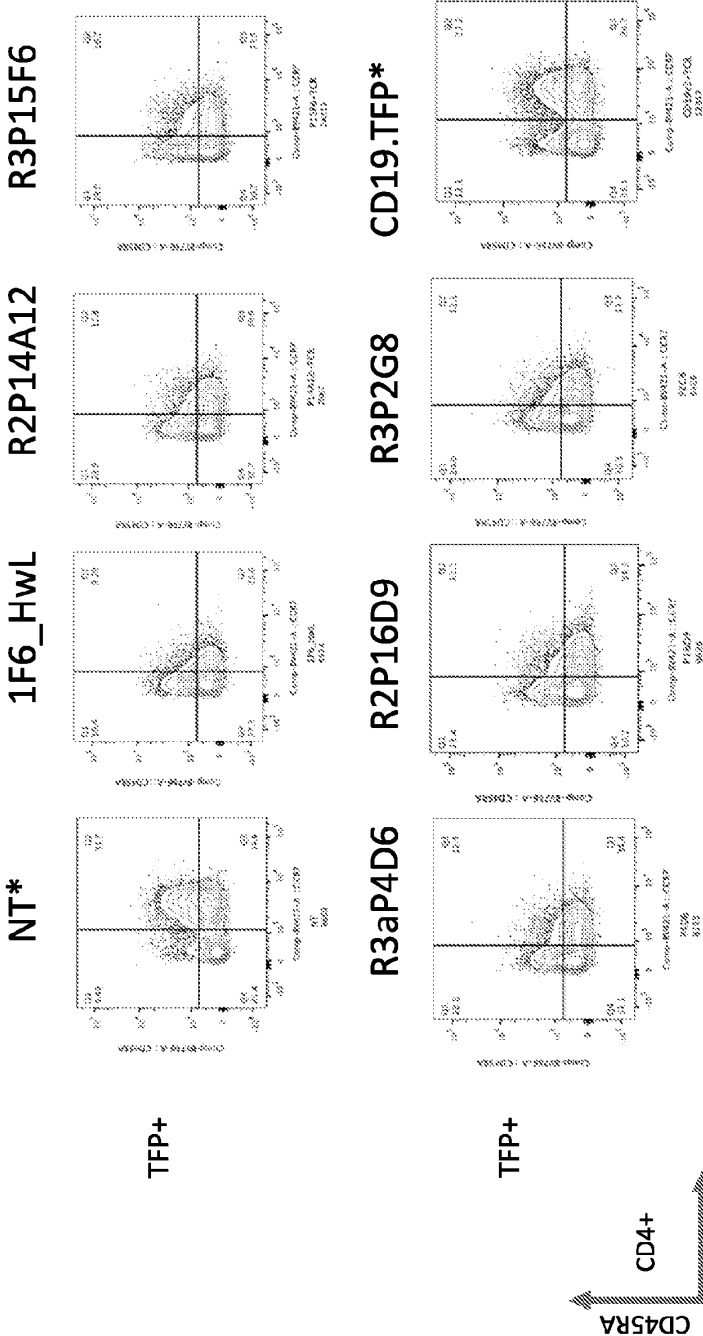


FIG. 8B



CD4+ TFP+ T Cell Memory Status



* Displays TCR

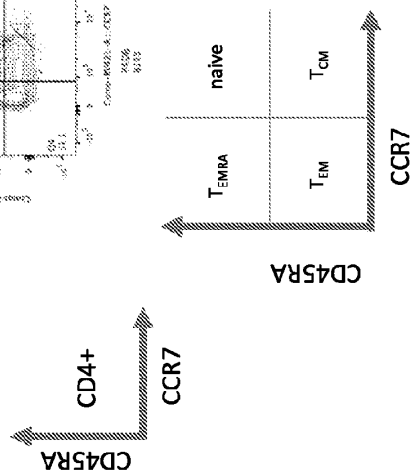


FIG. 8C

CD8+ TFP+/- T Cell Memory Status

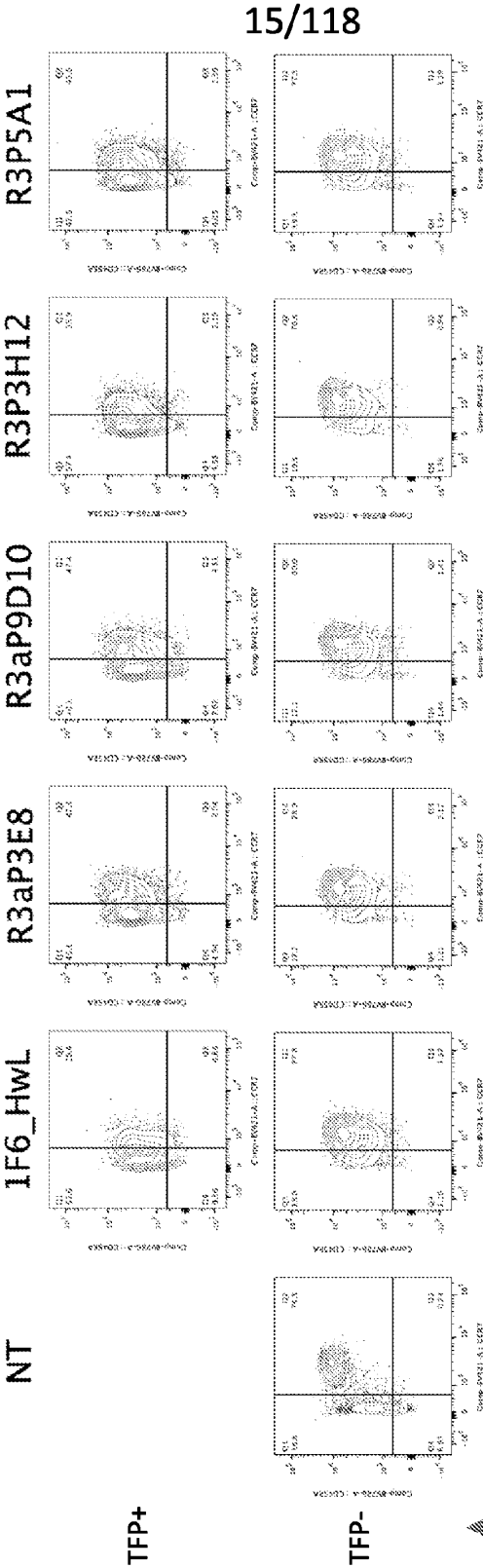
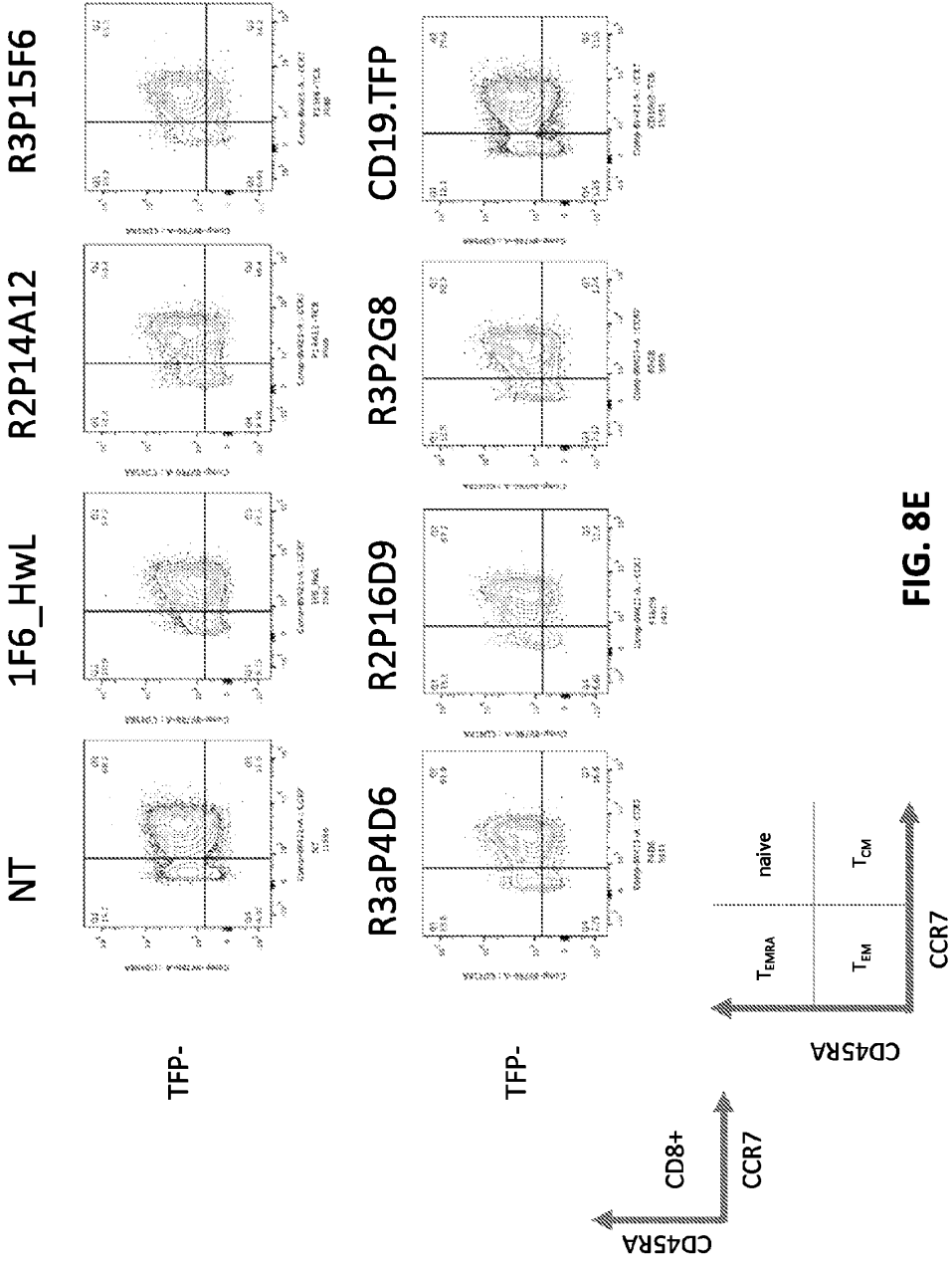


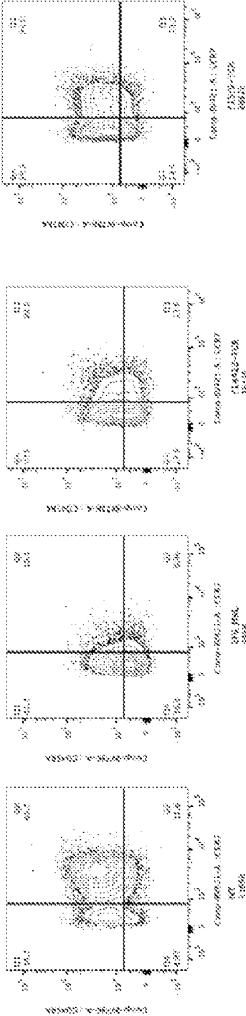
FIG. 8D

CD8+ TFP- T Cell Memory Status

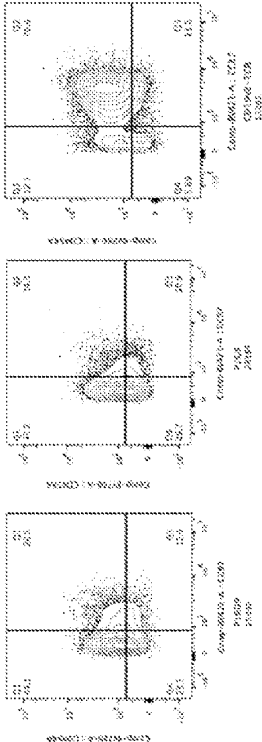


CD8+ TFP+ T Cell Memory Status

NT* 1F6_HwL R2P14A12 R3P15F6



CD19.TFP*

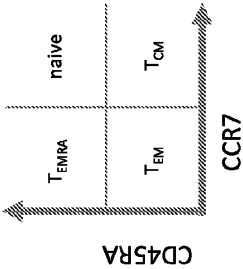
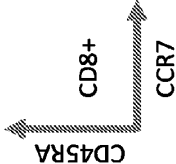


* Displays TCR

FIG. 8F

TFP+

TFP+



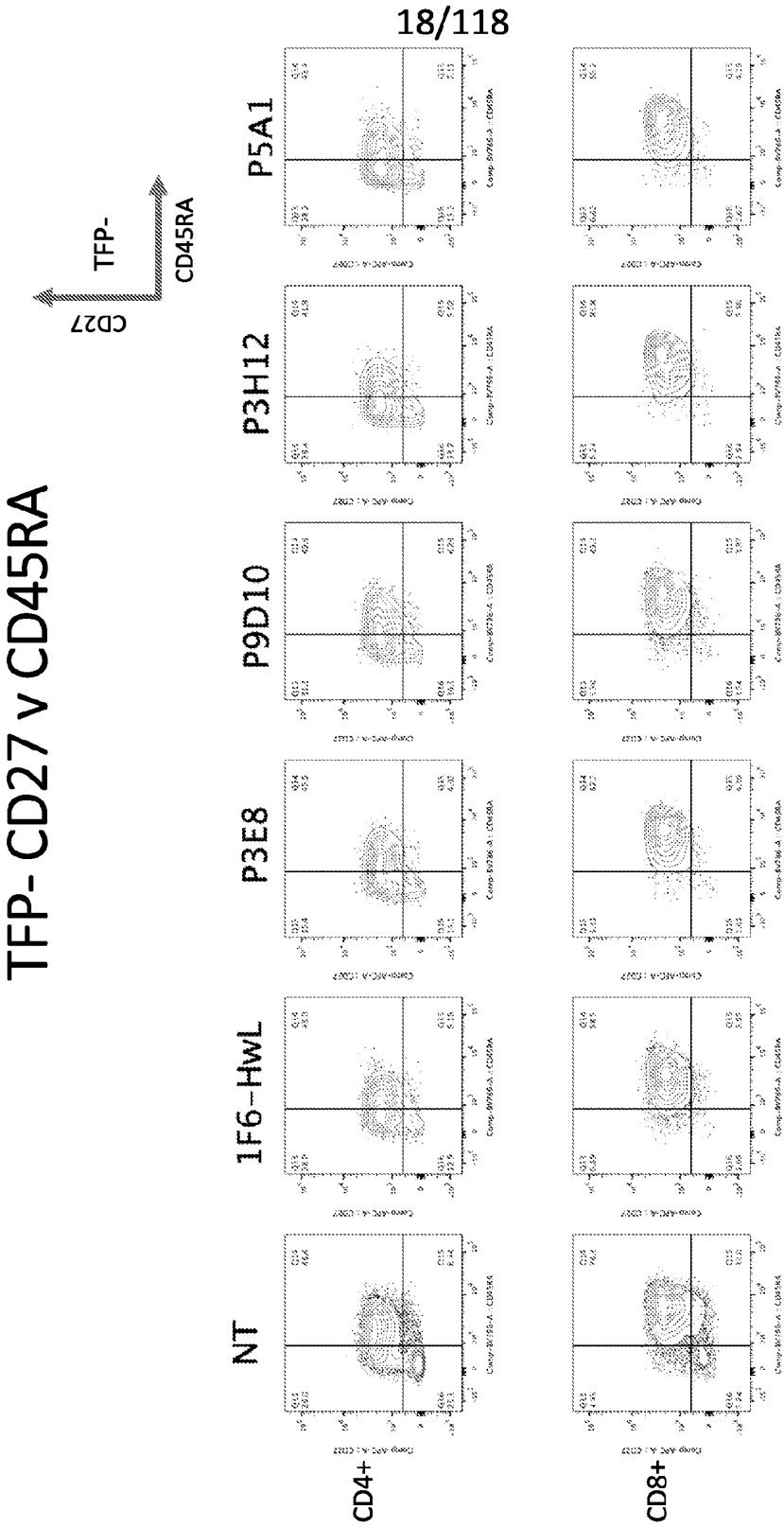


FIG. 9A

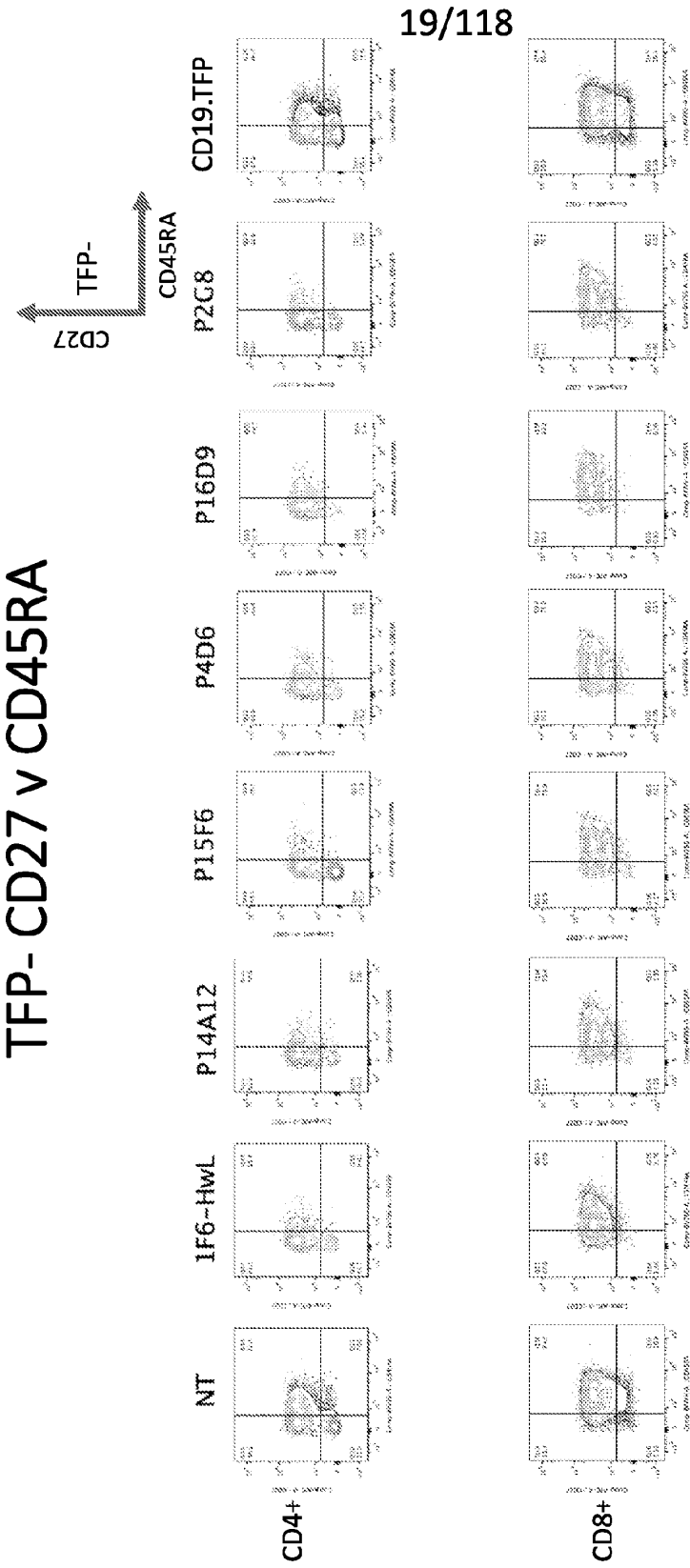
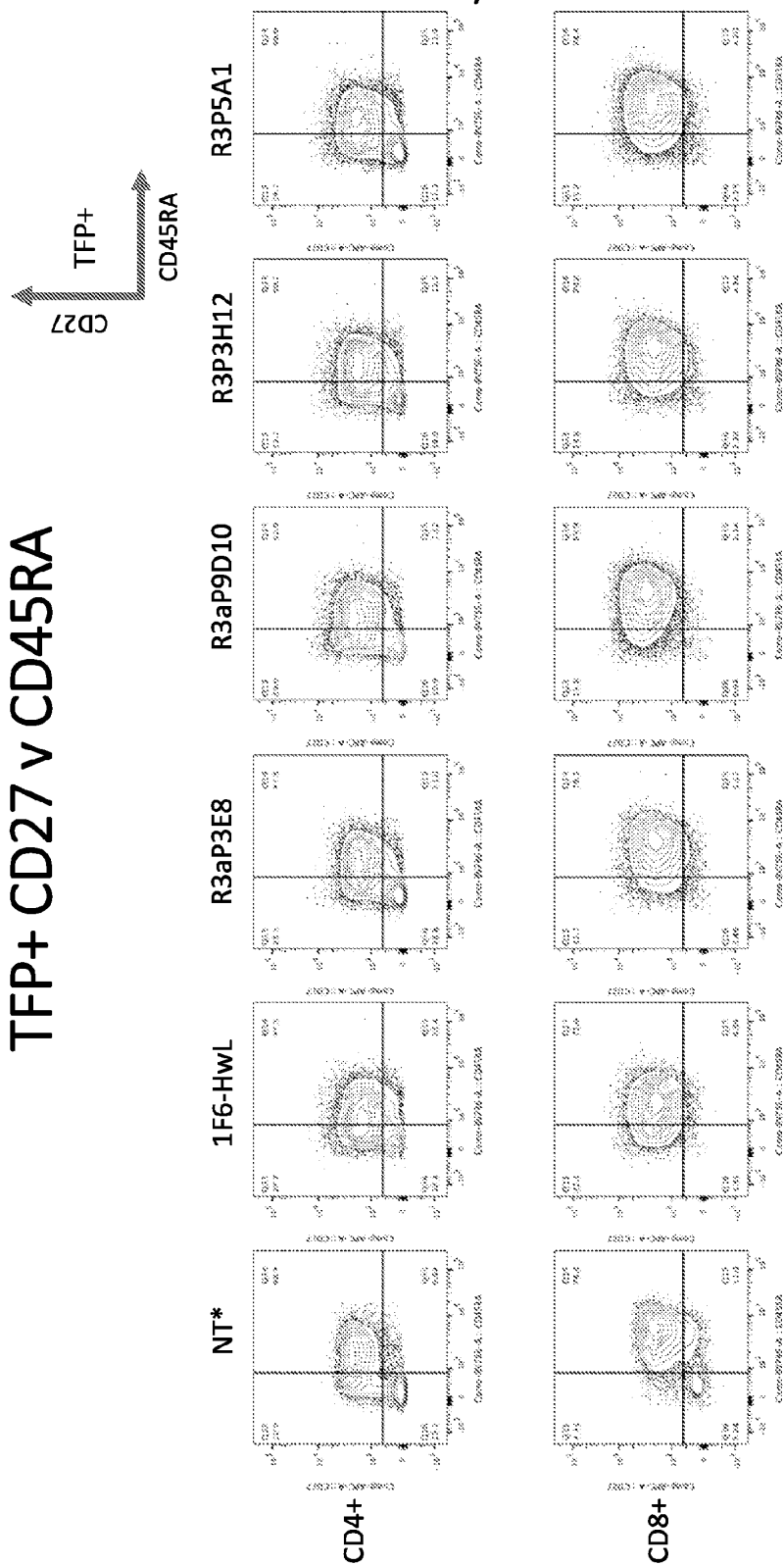


FIG. 9B



010925

FIG. 9C

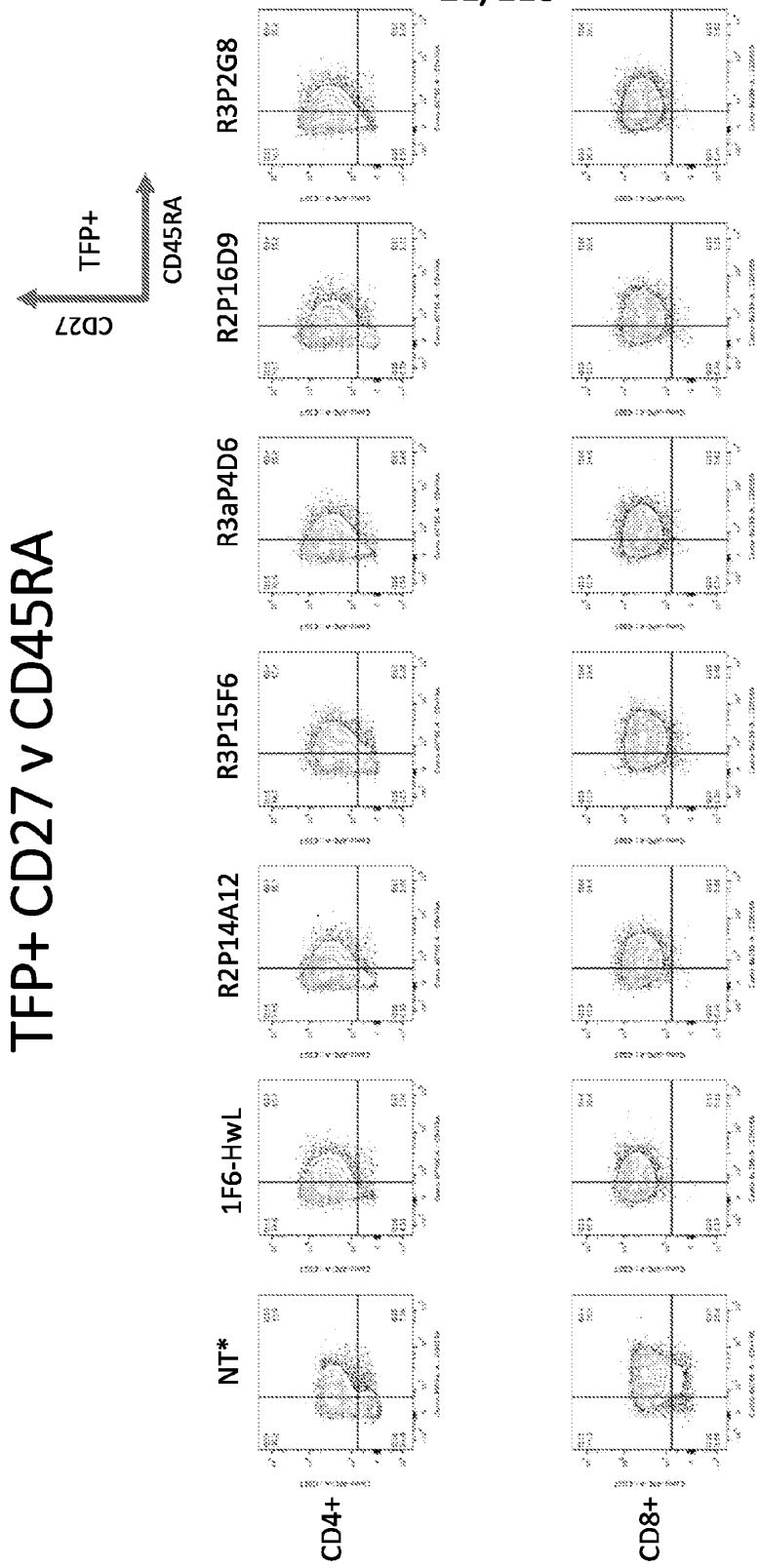


FIG. 9D

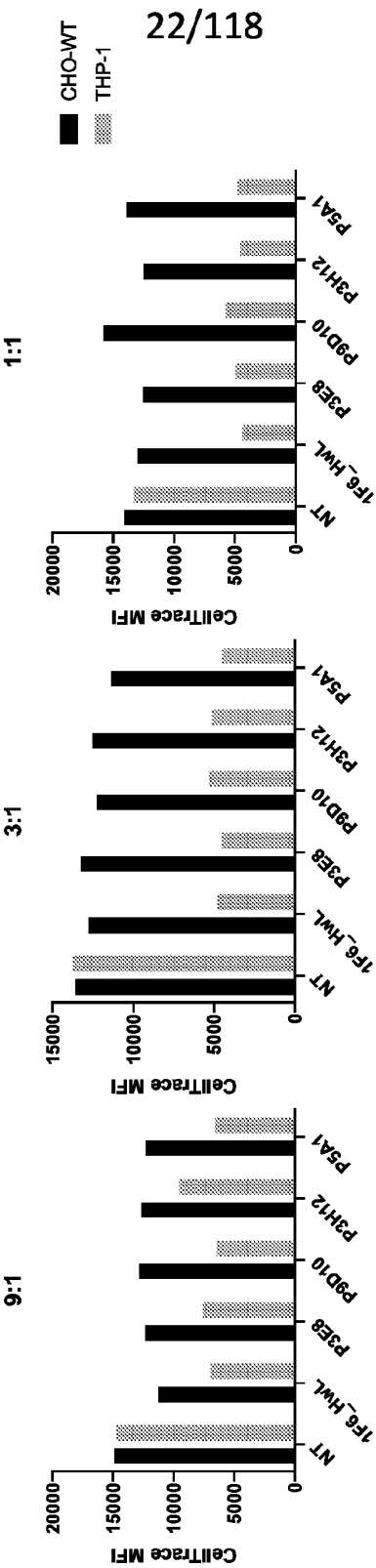


FIG. 10

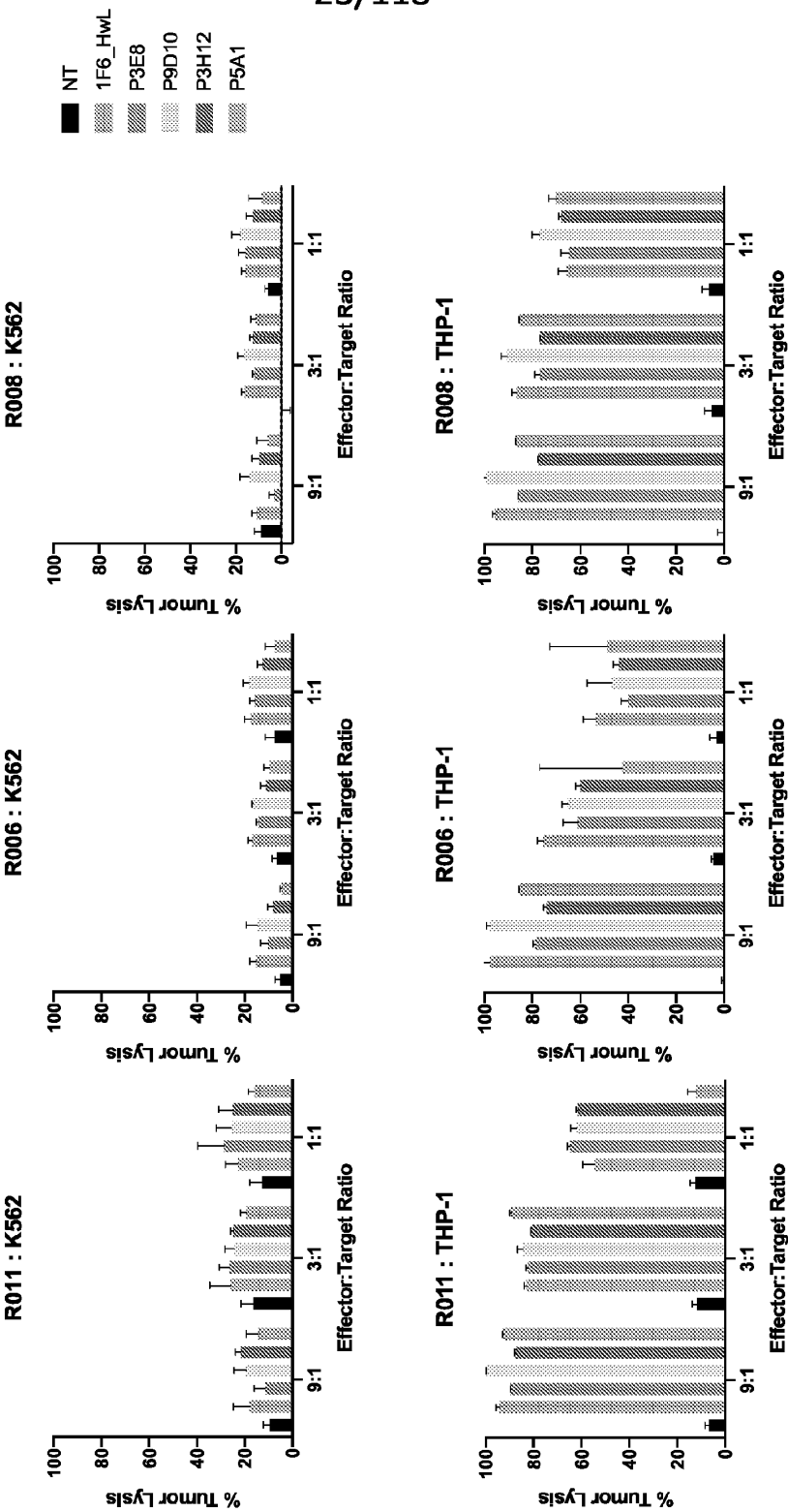


FIG. 11

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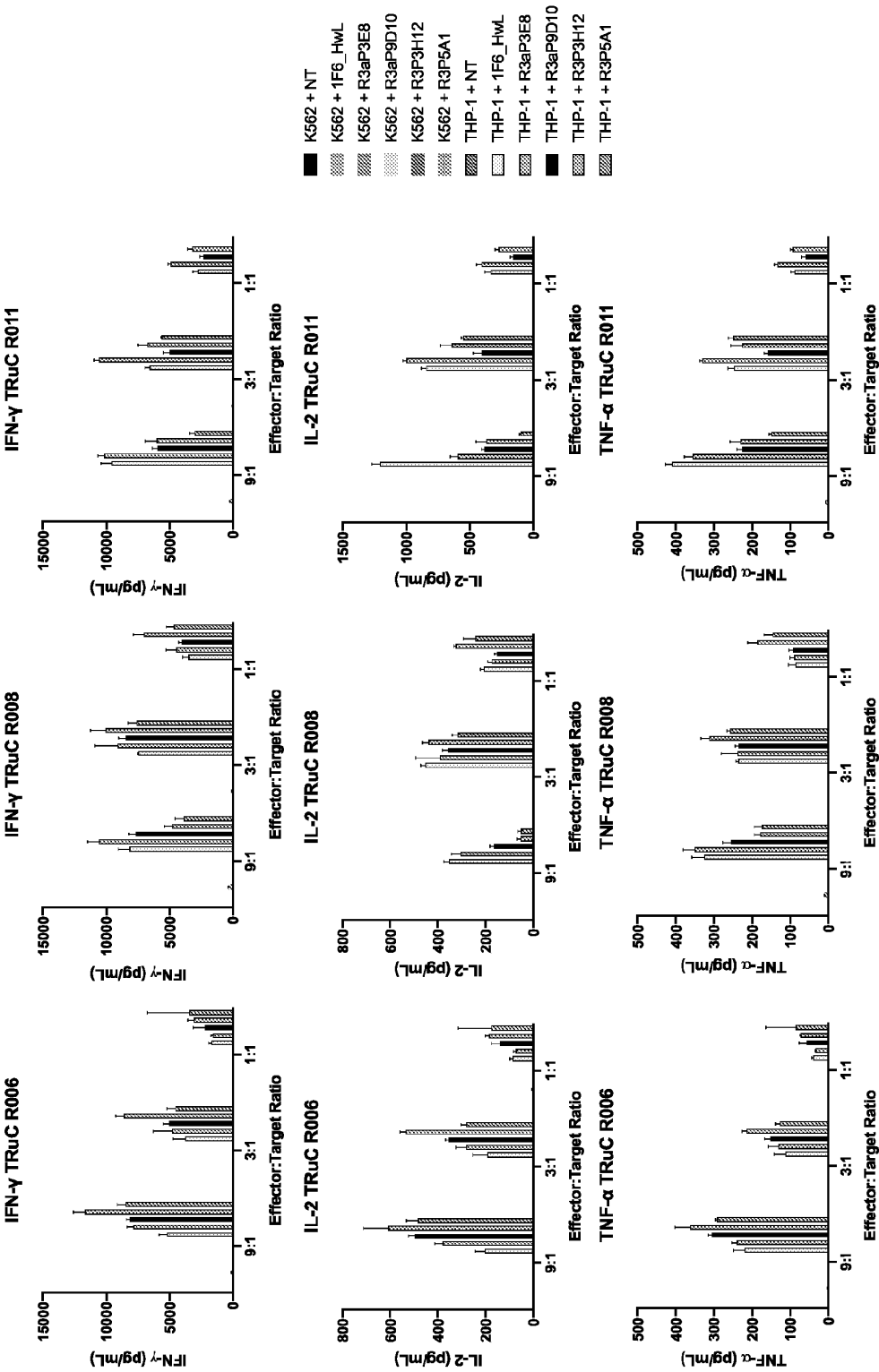


FIG. 12A

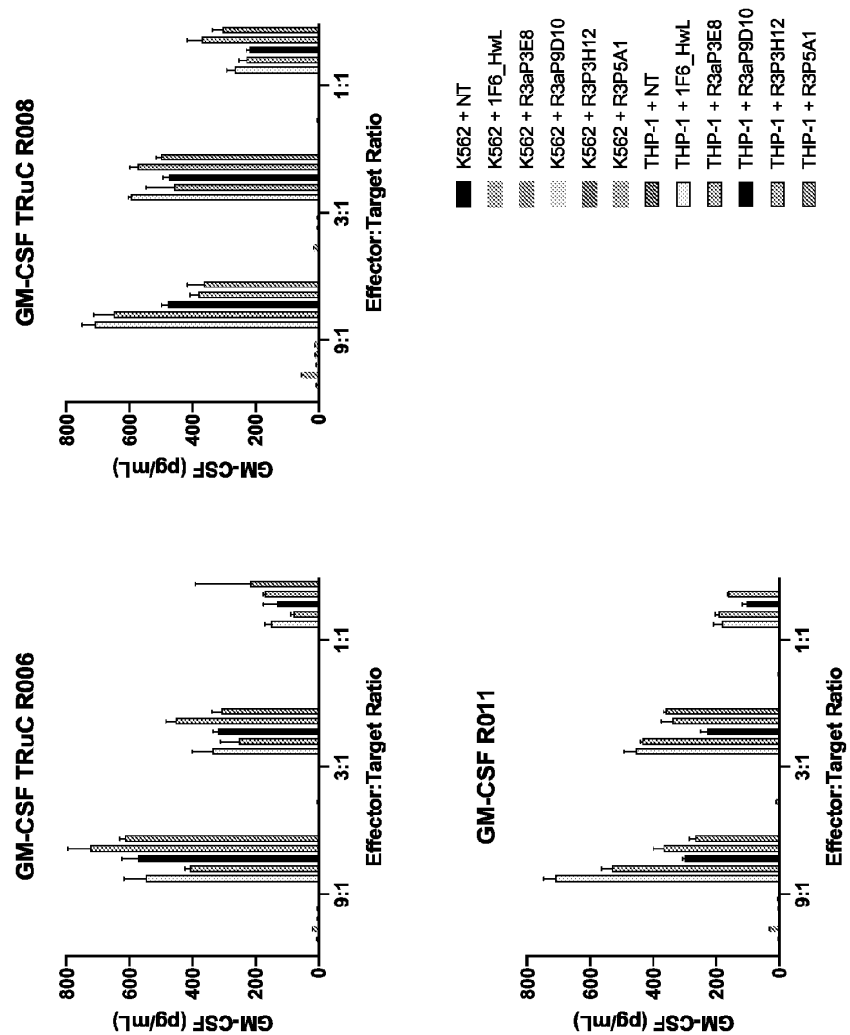


FIG. 12B

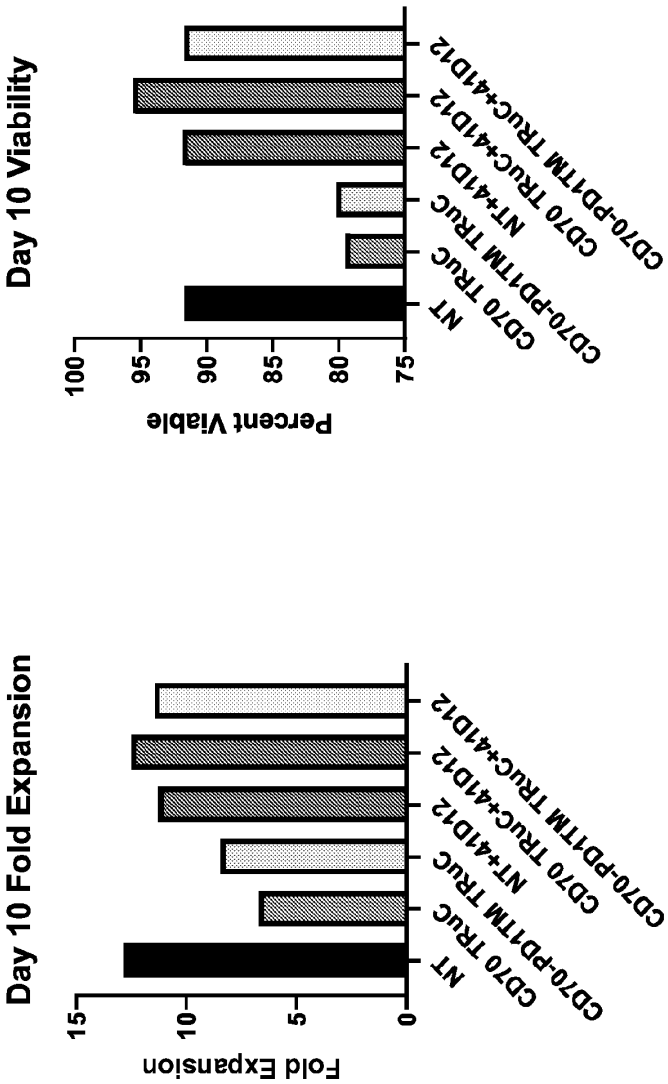


FIG. 13

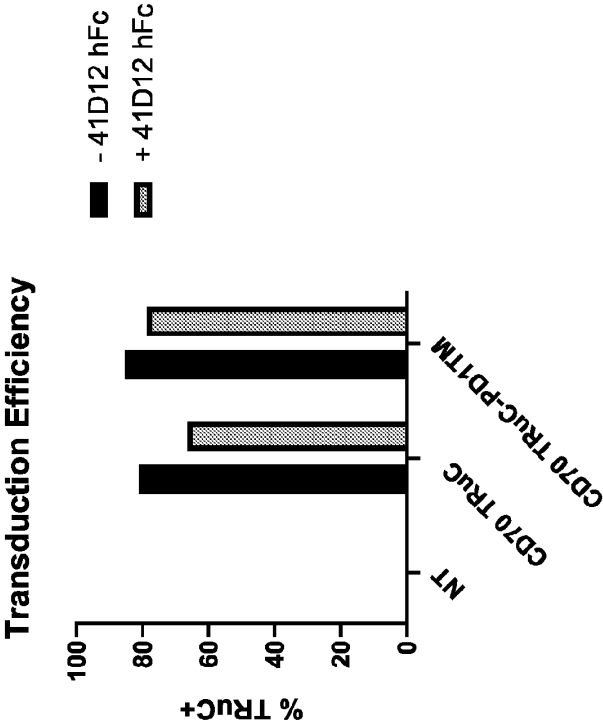


FIG. 14

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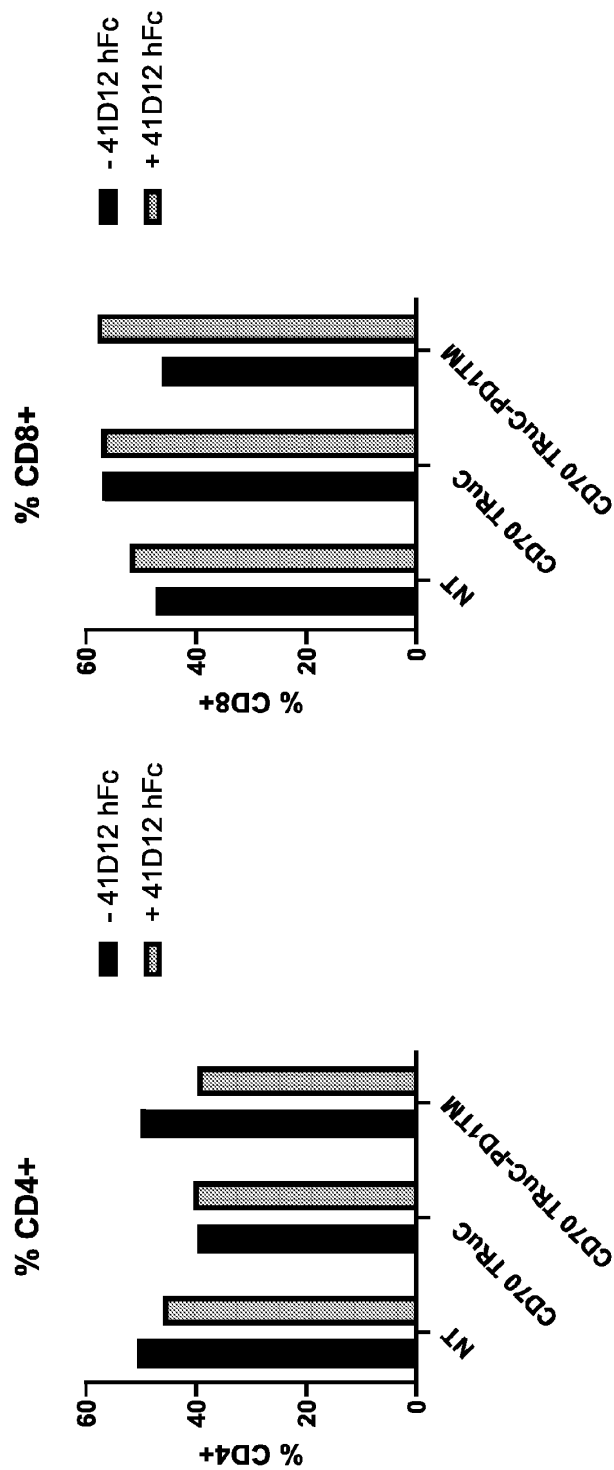


FIG. 15

Memory Phenotype

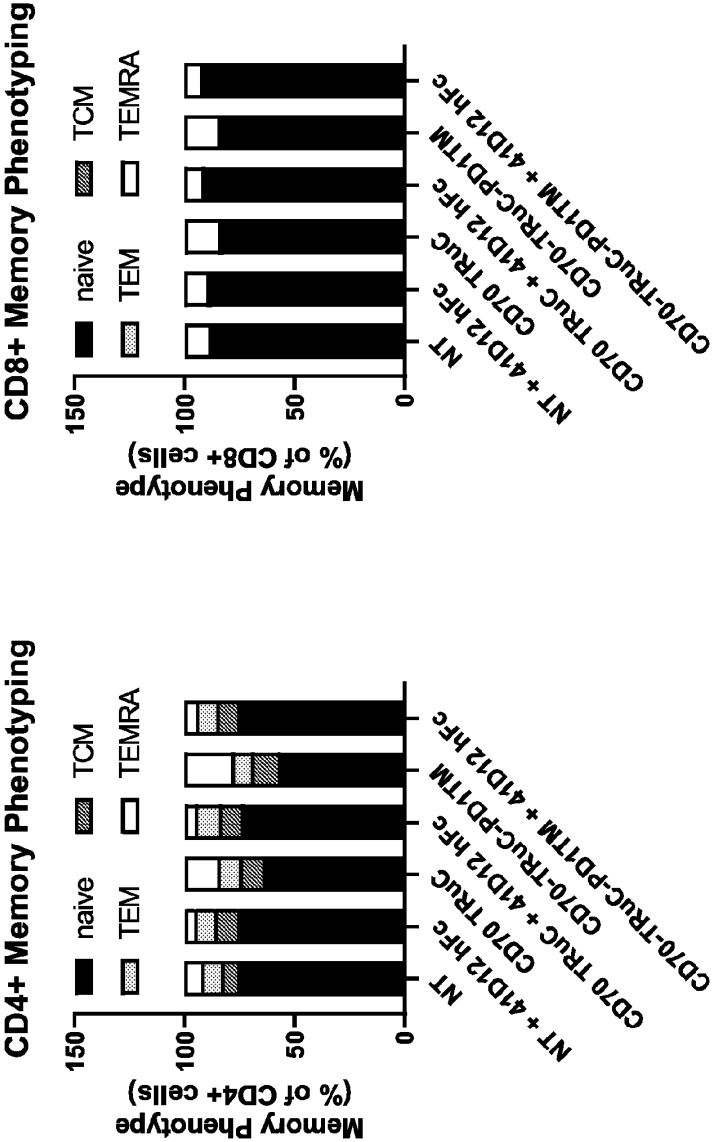


FIG. 16A

FIG. 16B

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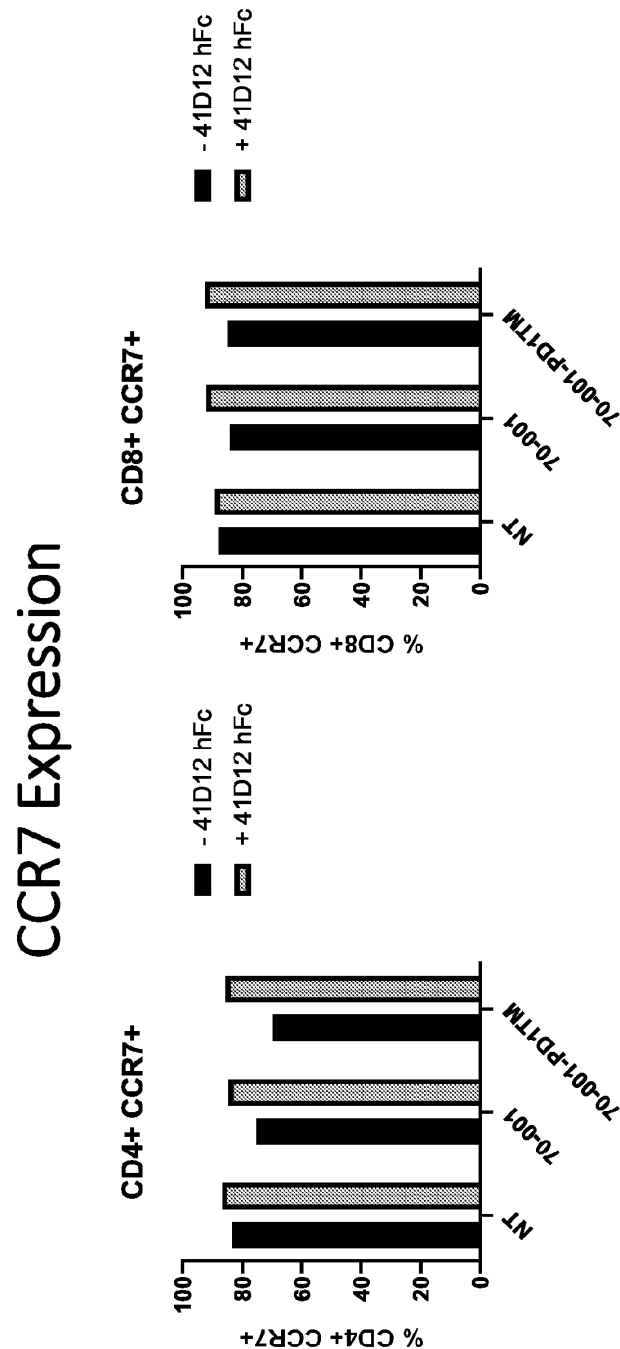


FIG. 17

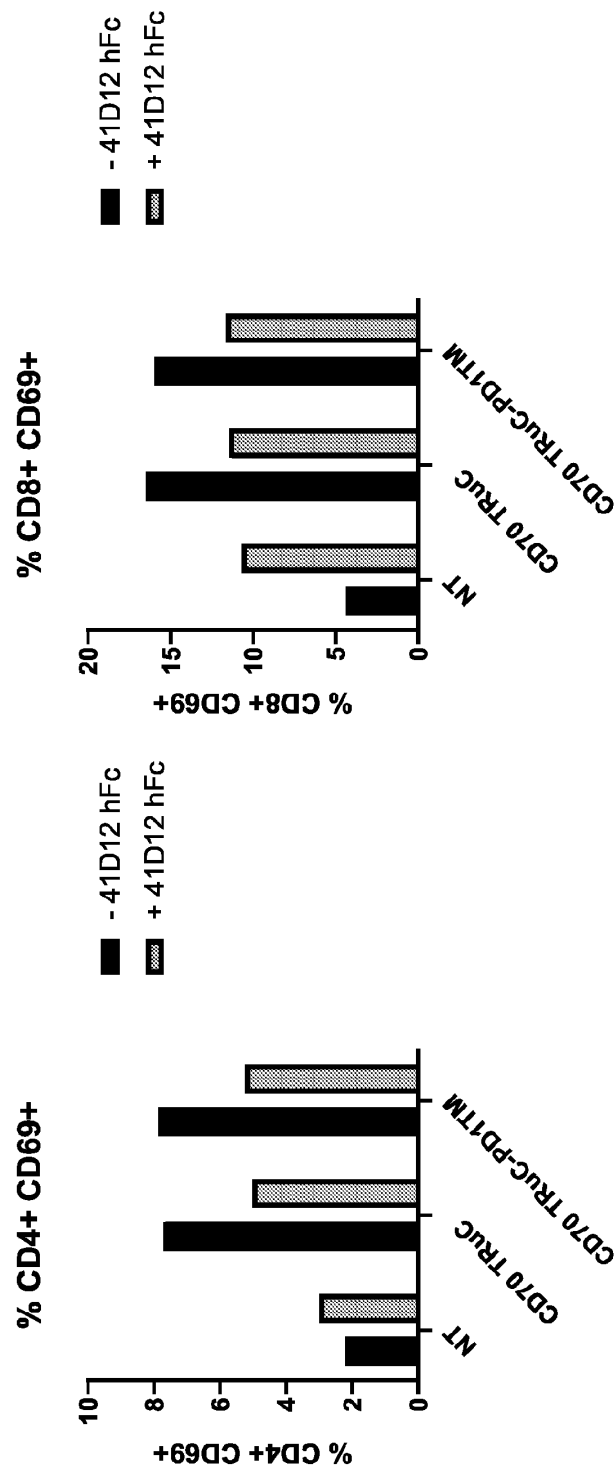


FIG. 18

CD4+ CD70 versus CD27 – 41D12 blocks CD70

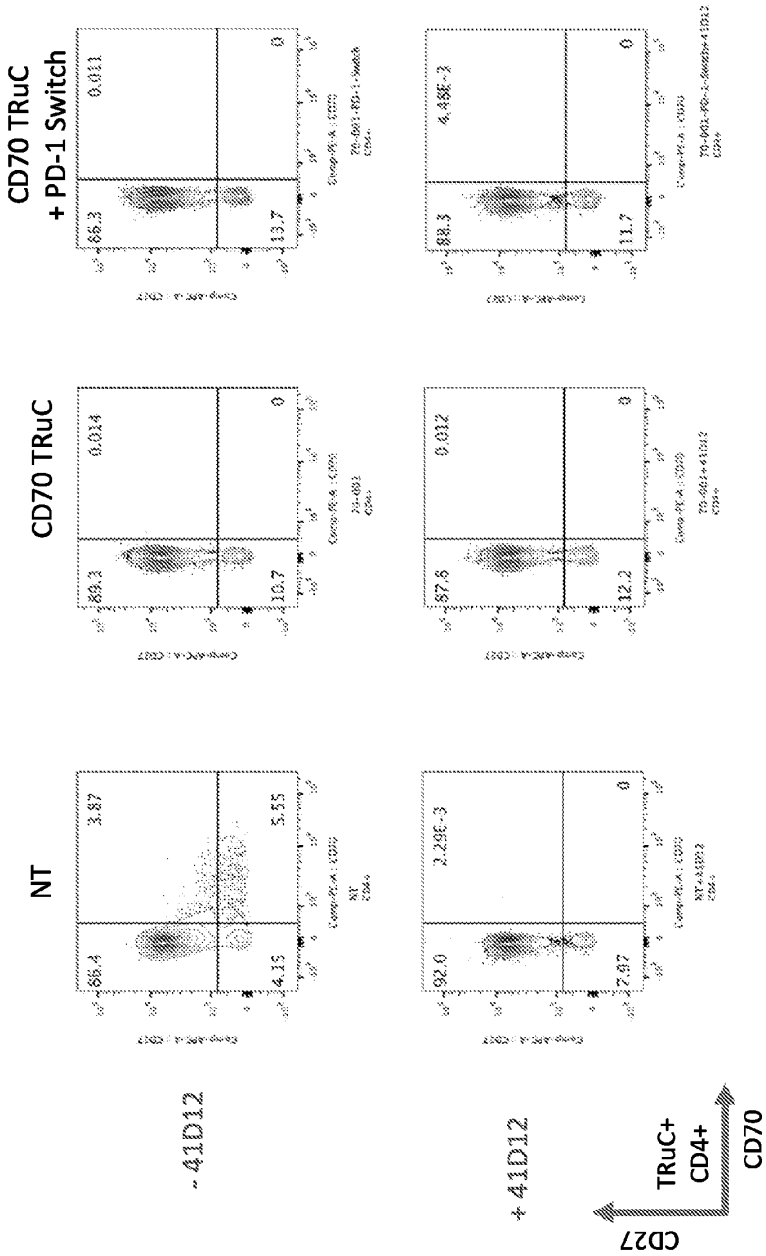


FIG. 19A

CD8+ CD70 versus CD27

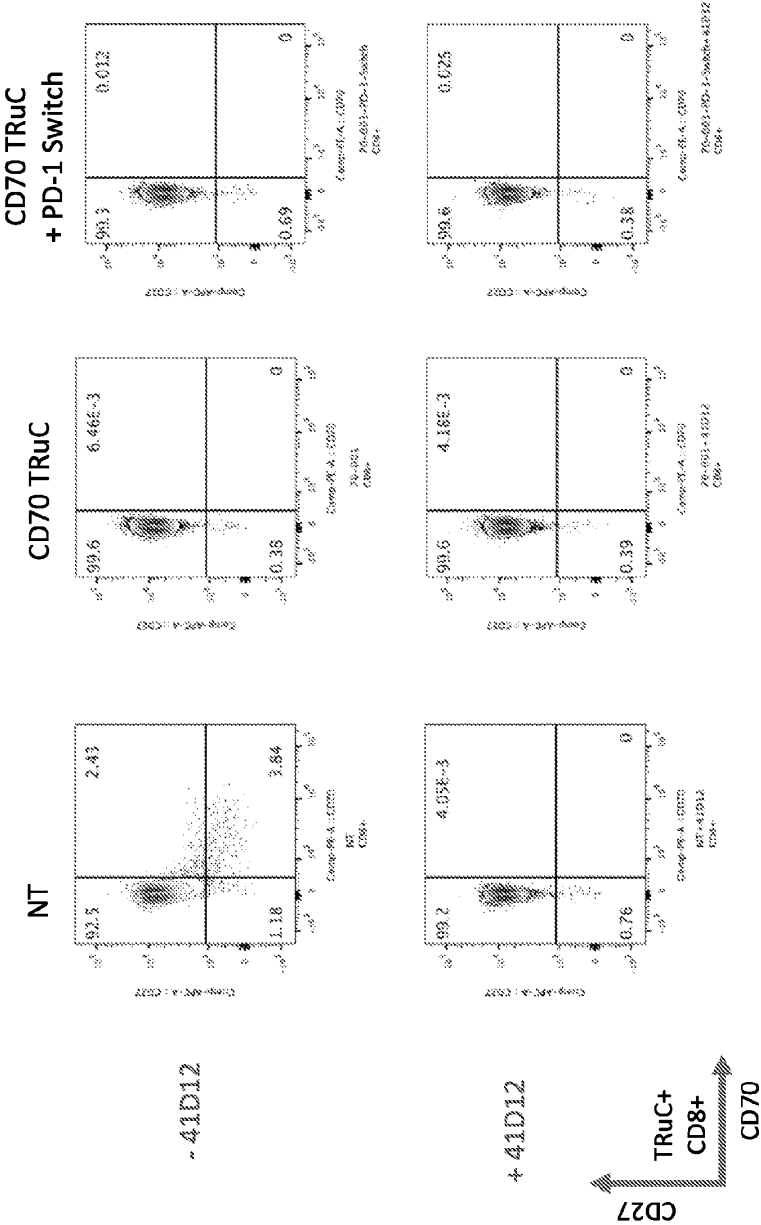


FIG. 19B

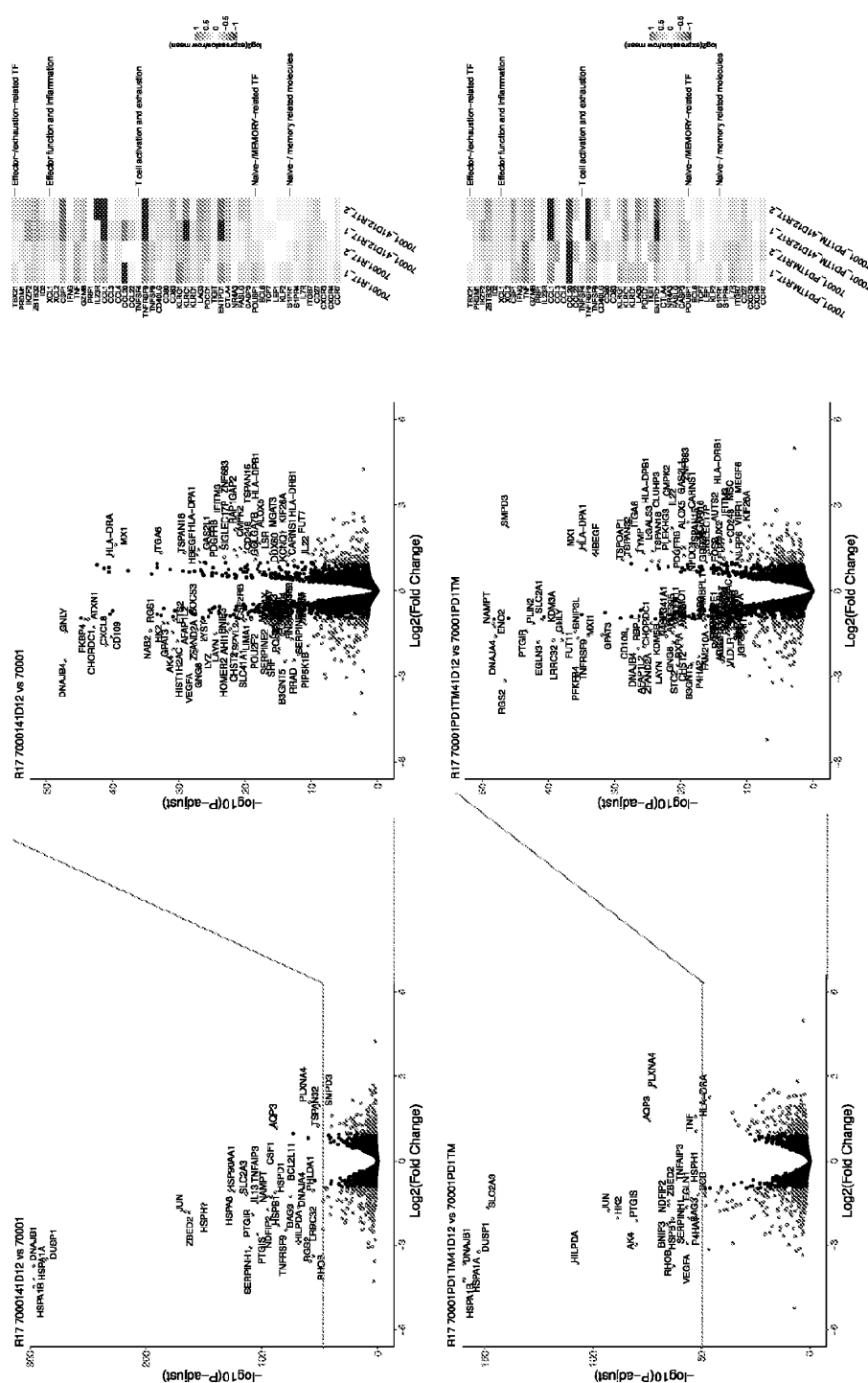


FIG. 20

24 Hour Cytotoxicity

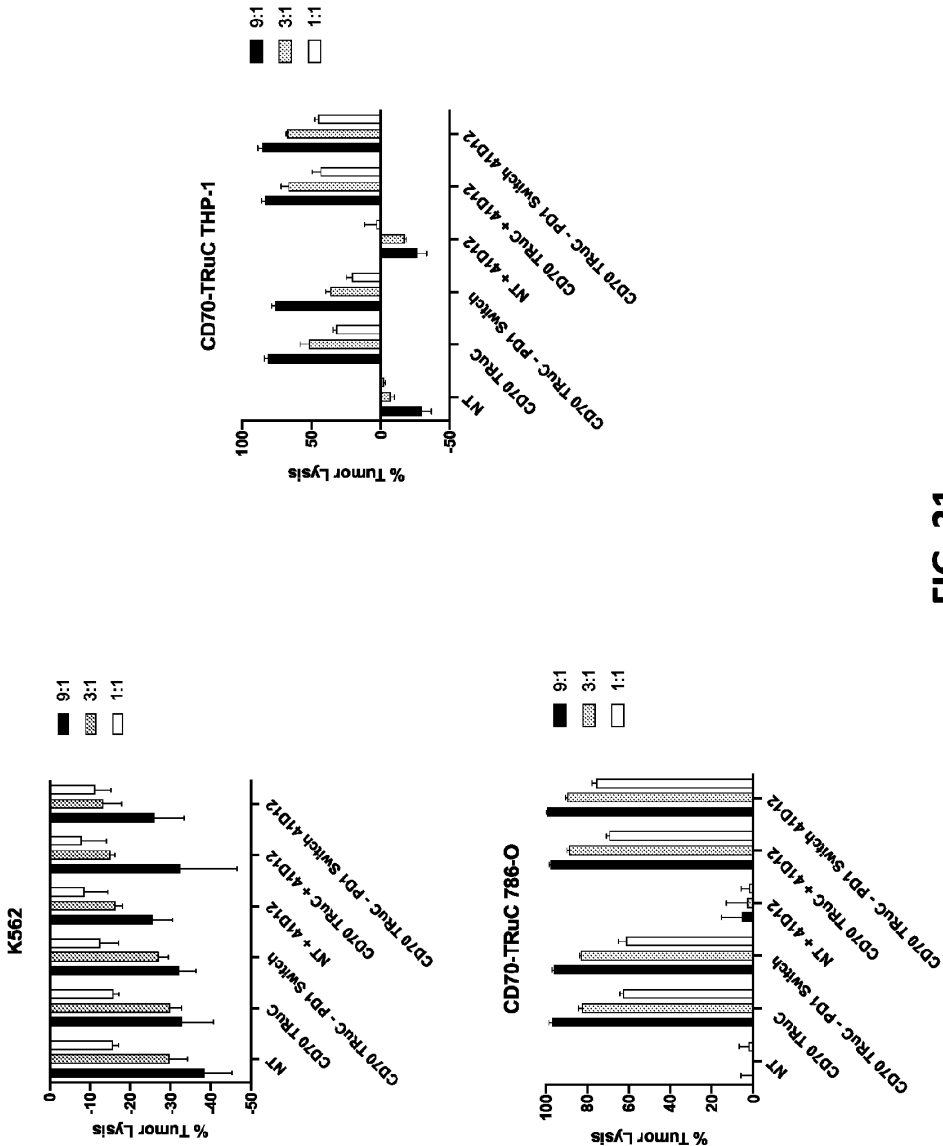


FIG. 21

24 Hour GM-CSF

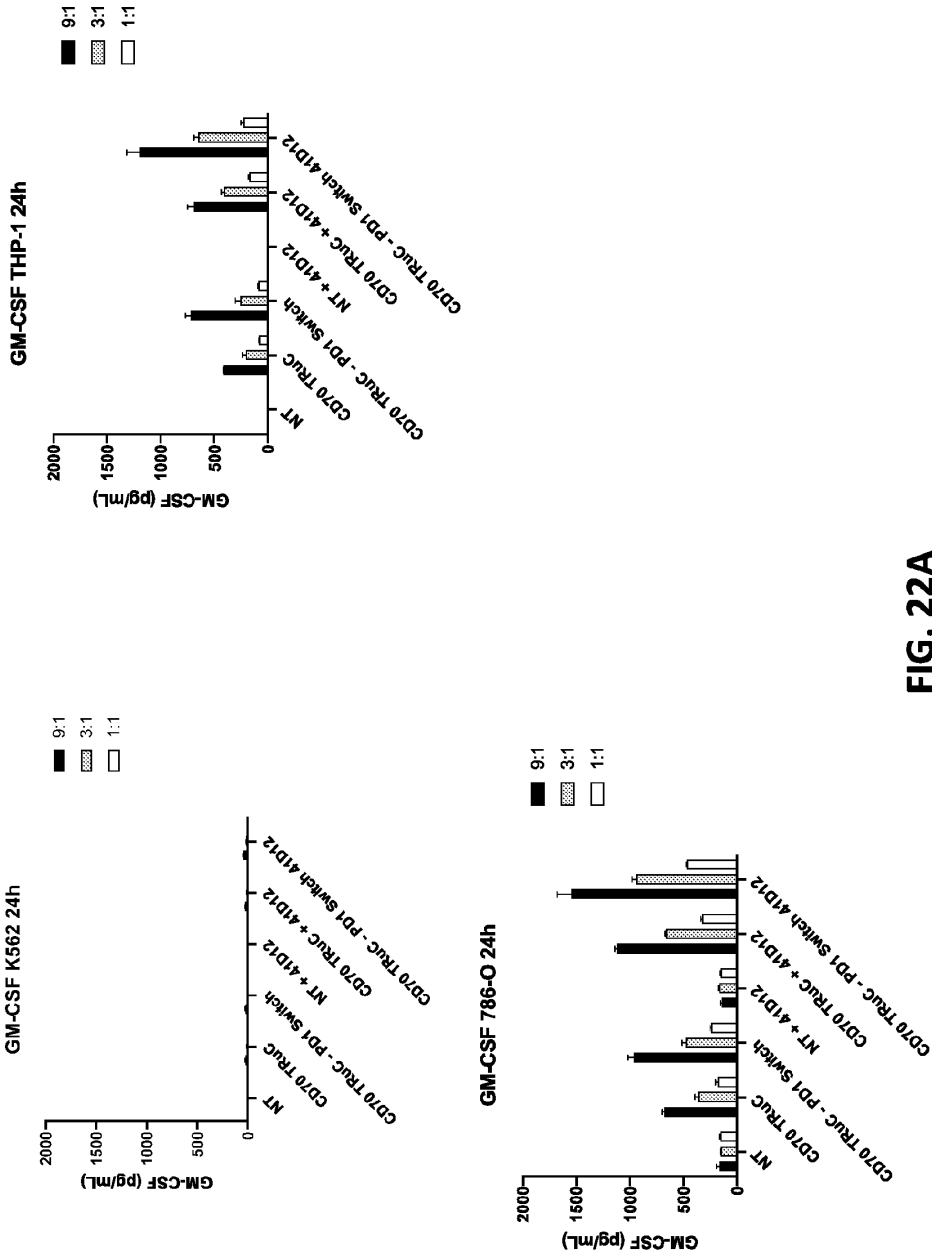


FIG. 22A

72 Hour GM-CSF

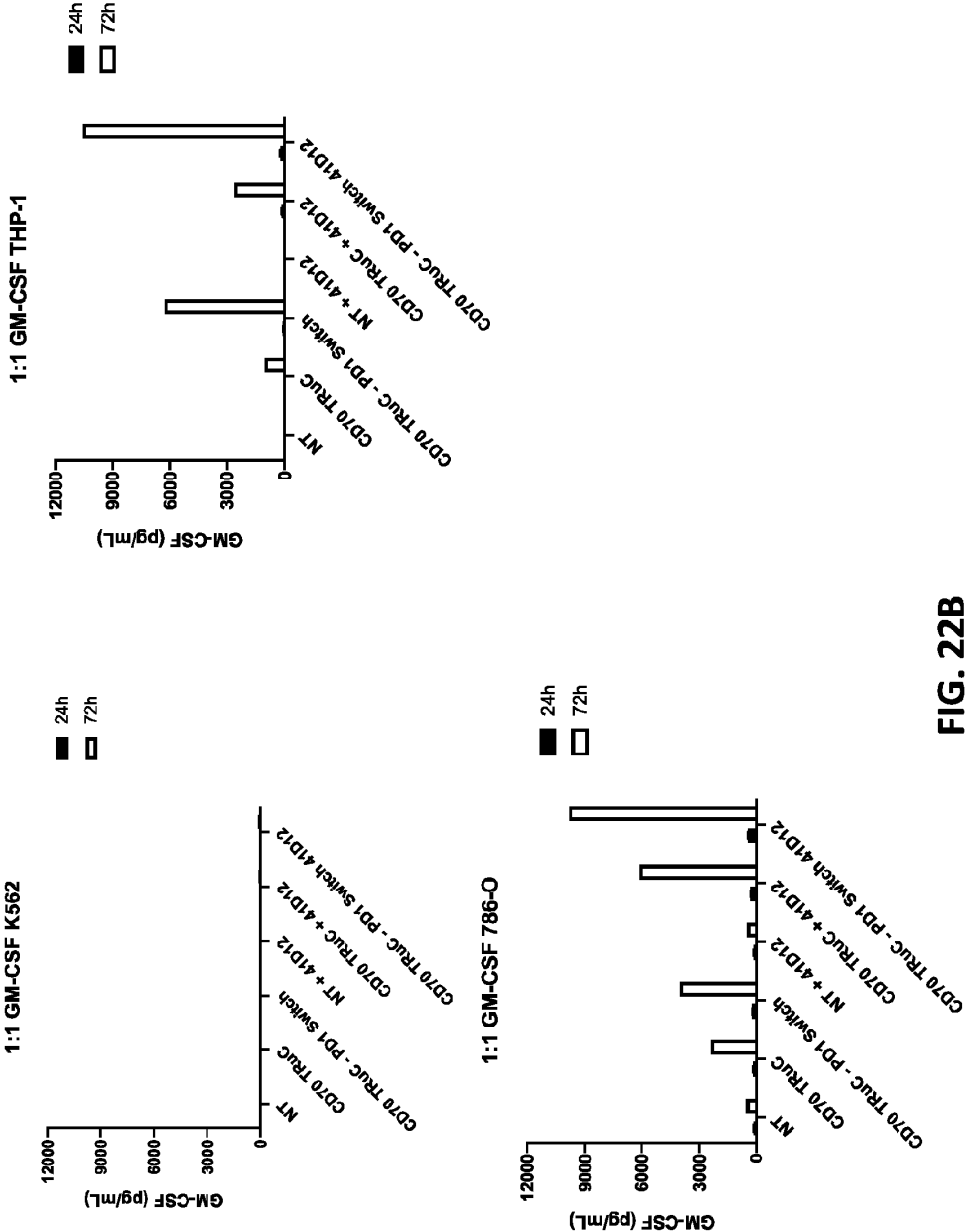


FIG. 22B

24 Hour IFN-gamma

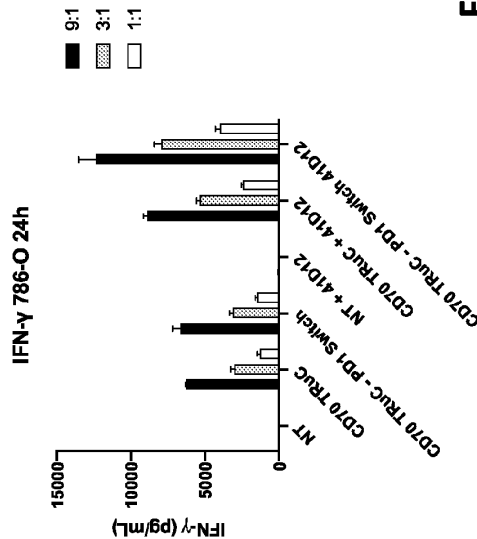
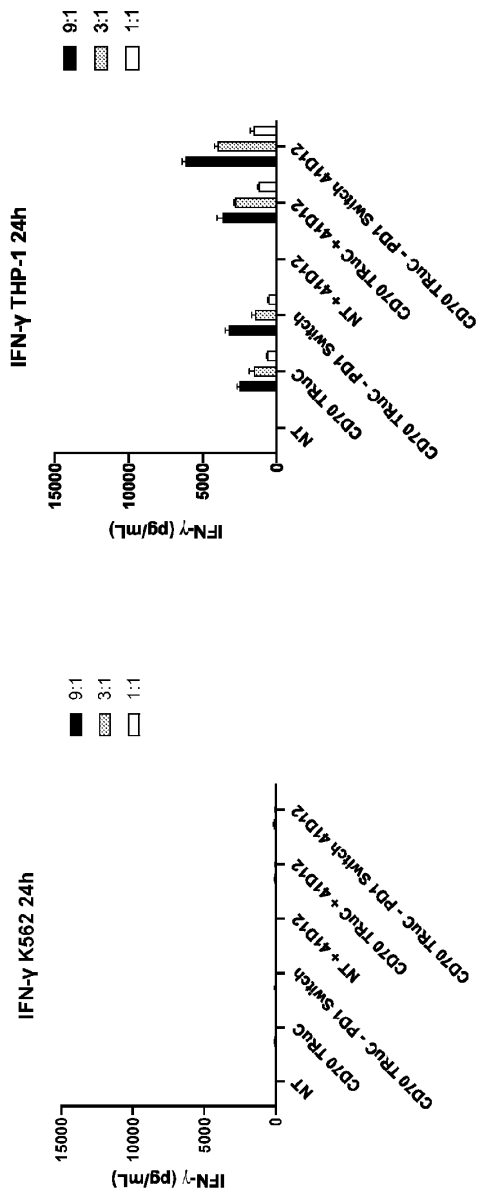


FIG. 22C

72 Hour IFN-gamma

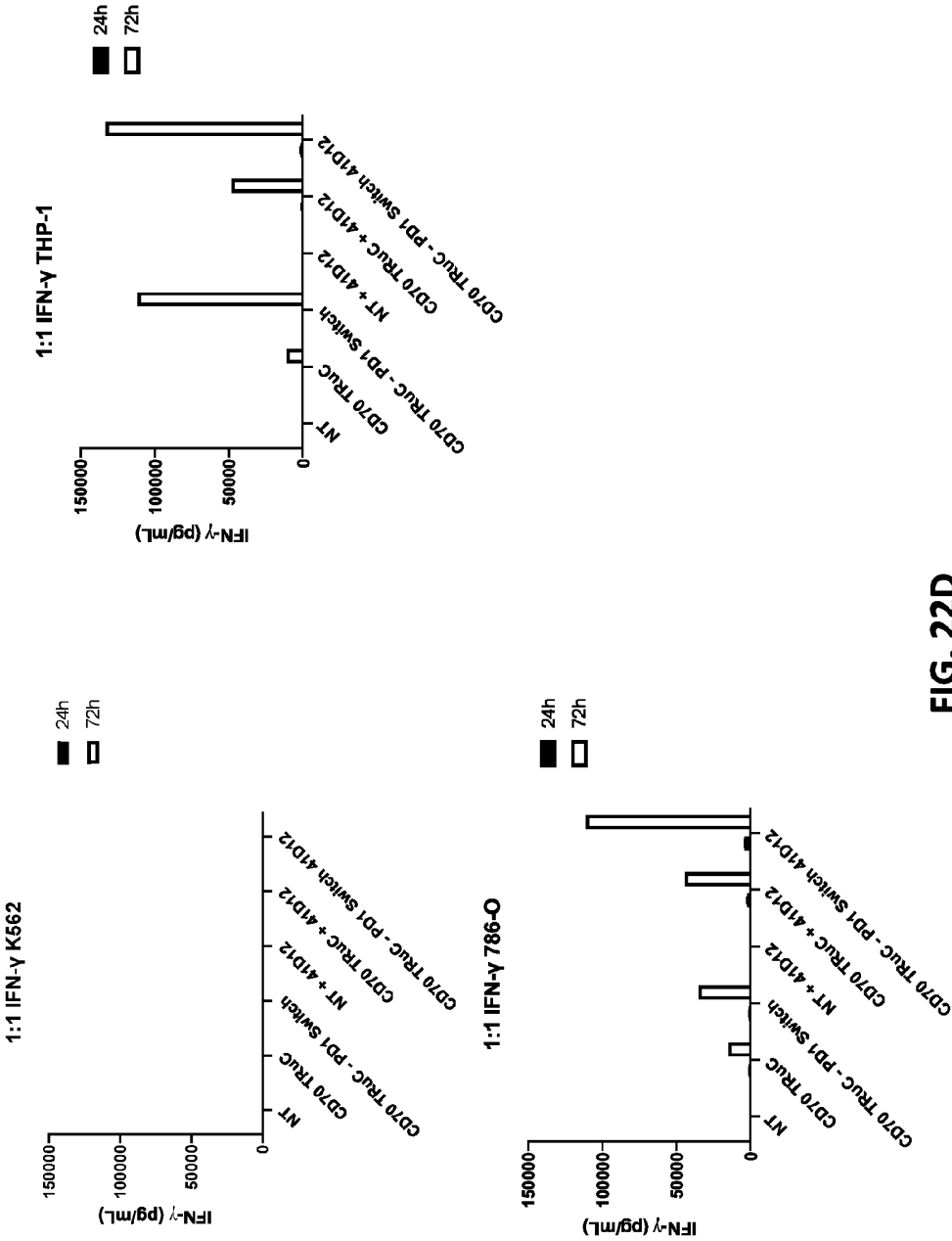


FIG. 22D

24 Hour IL-2

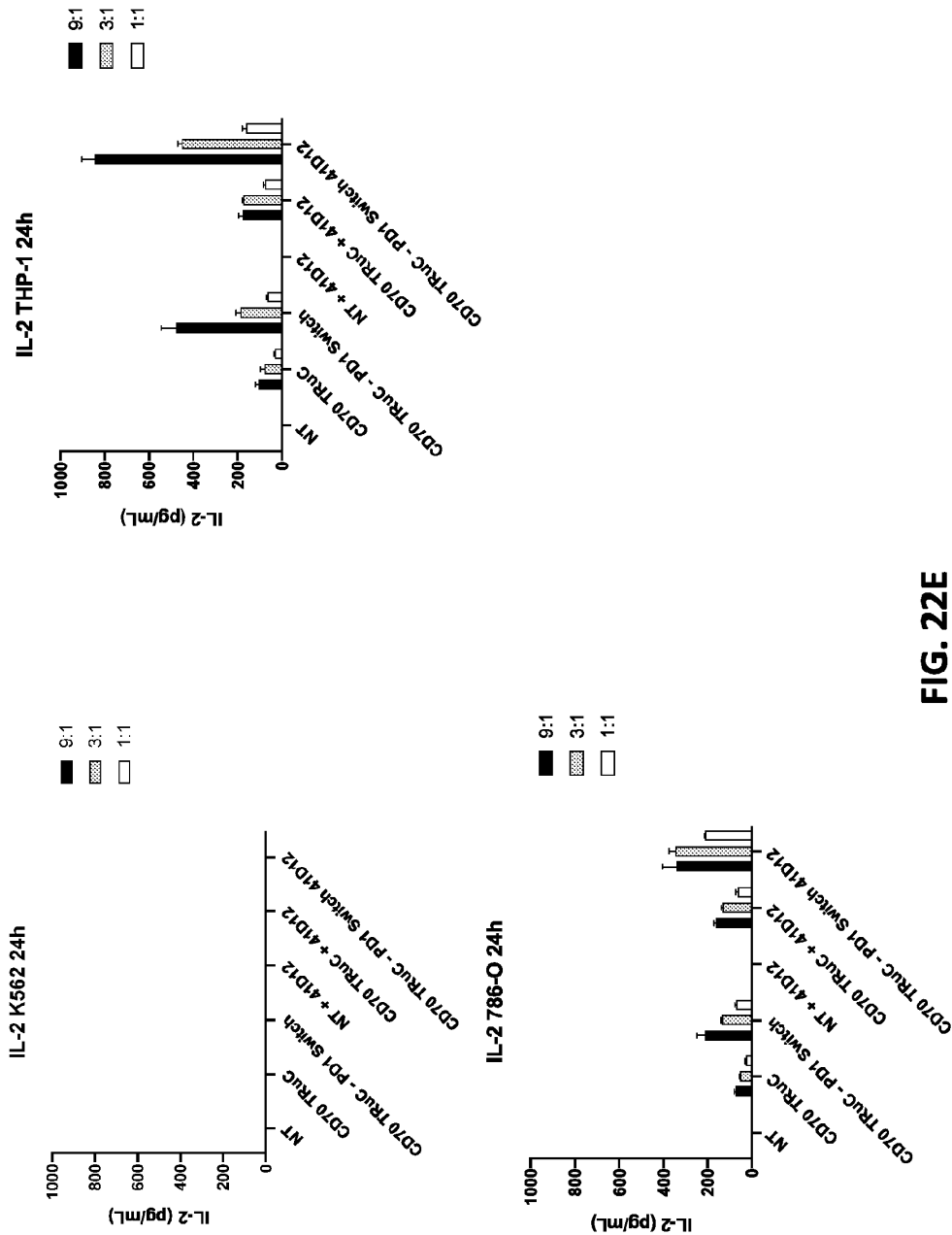


FIG. 22E

72 Hour IL-2

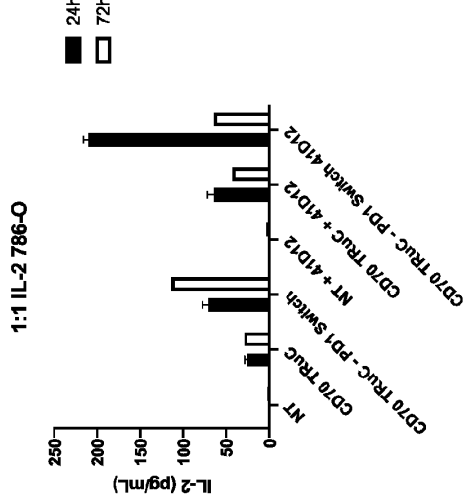
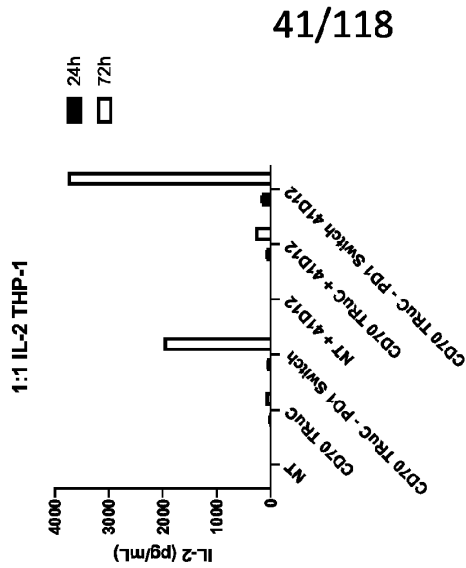
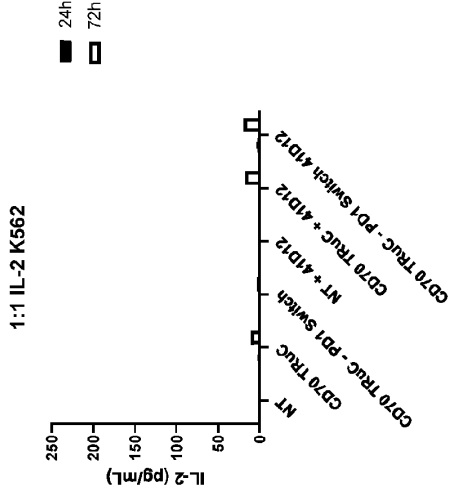


FIG. 22F

24 Hour TNF-alpha

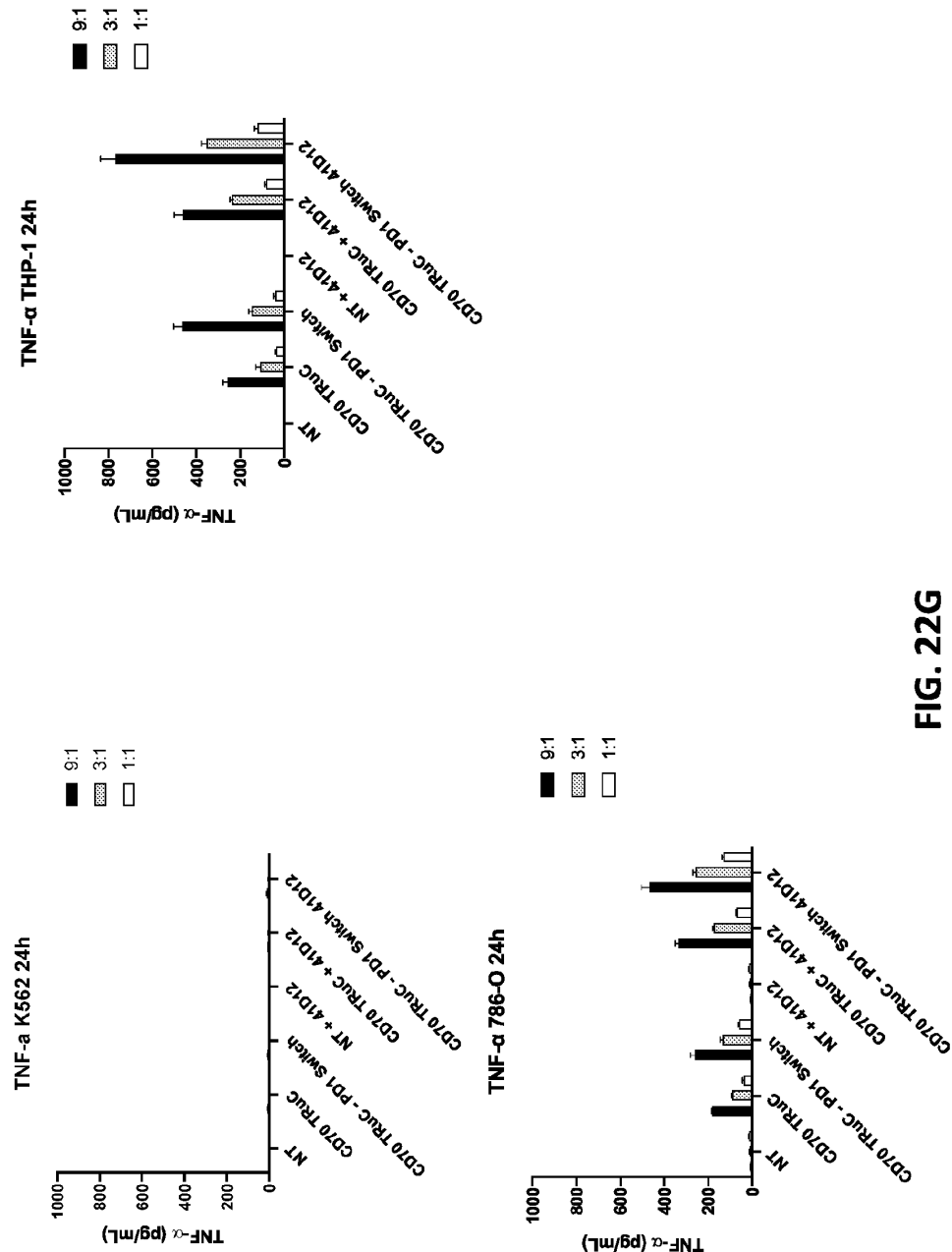


FIG. 22G

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72 Hour TNF-alpha

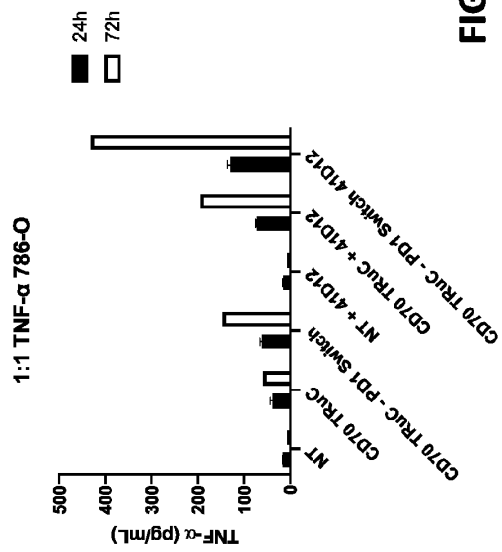
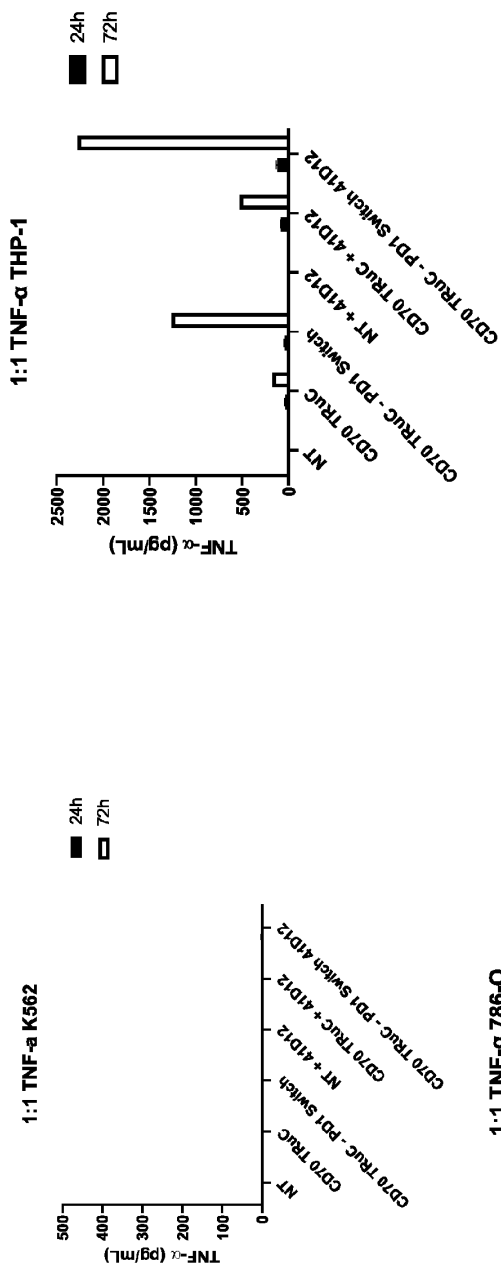


FIG. 22H

CD70 CRISPR KO TRuCs –Day 7
CD70 versus CD3

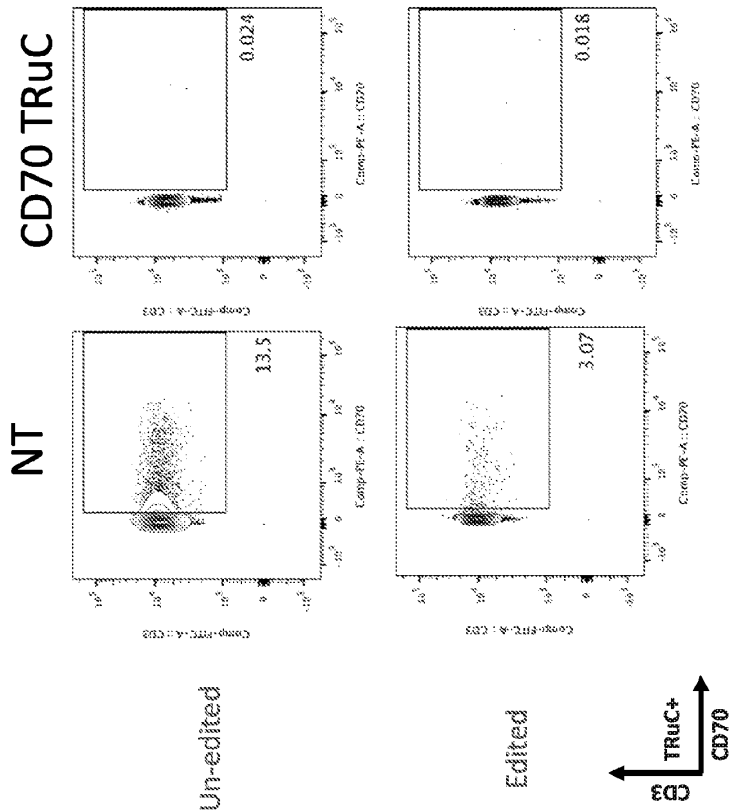


FIG. 23A

CD70 CRISPR KO TRuCs –Day 9
CD70 versus CD3

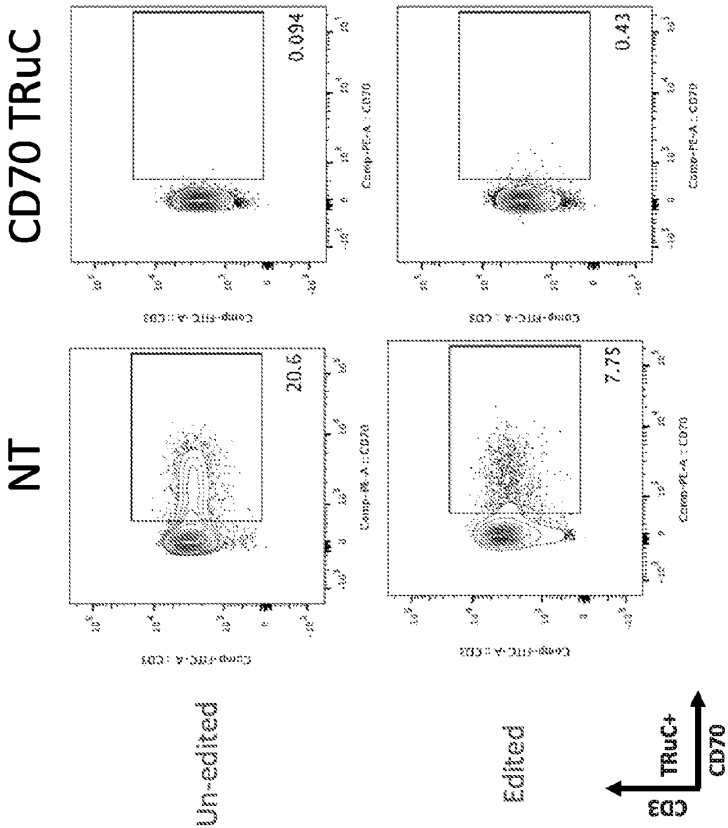


FIG. 23B

Day 10 TRuC Phenotyping – Donor R017

Anti-VHH vs CD3

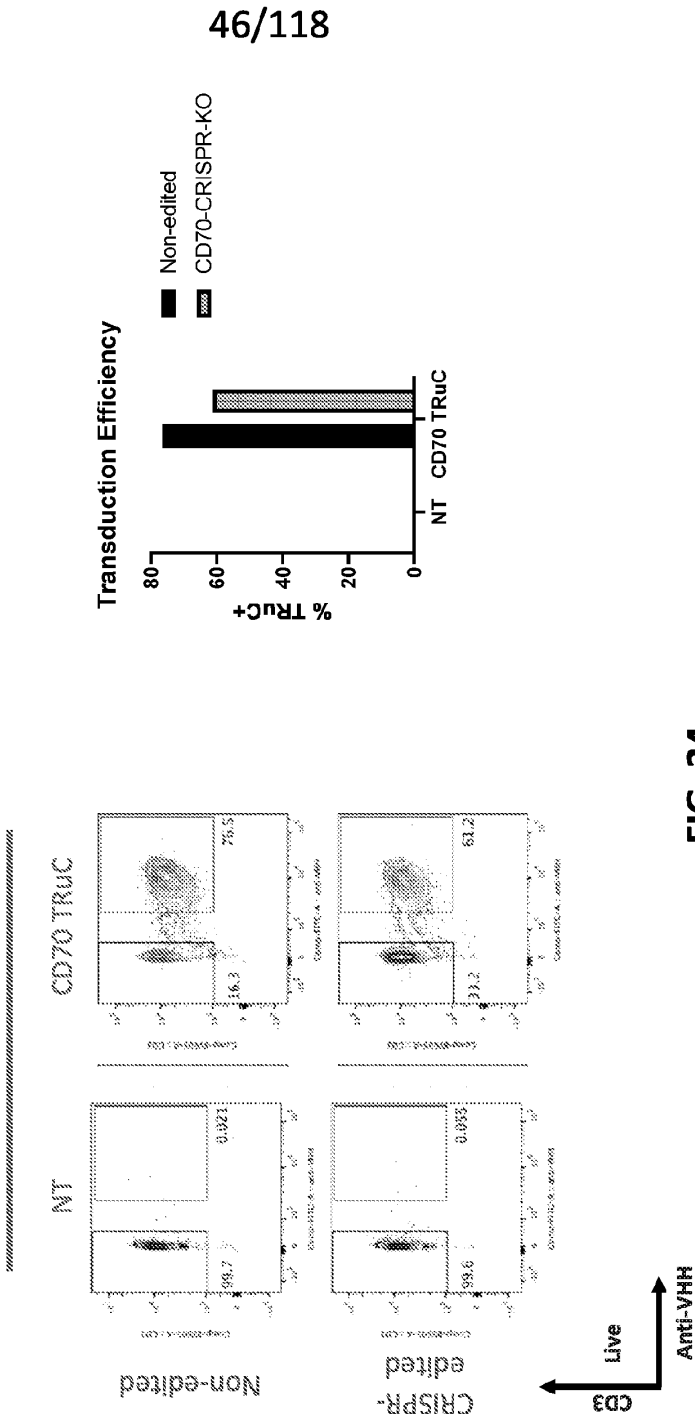


FIG. 24

Day 10 TRuC Phenotyping – CD4+ vs CD8+ T cells

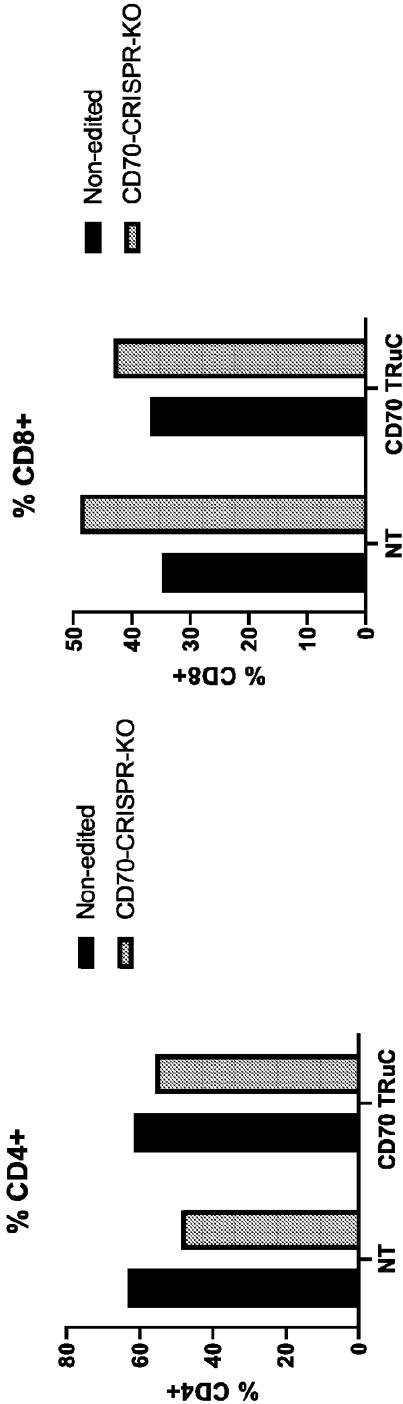


FIG. 25

Day 10 TRuC Phenotyping: CD70 versus CD27

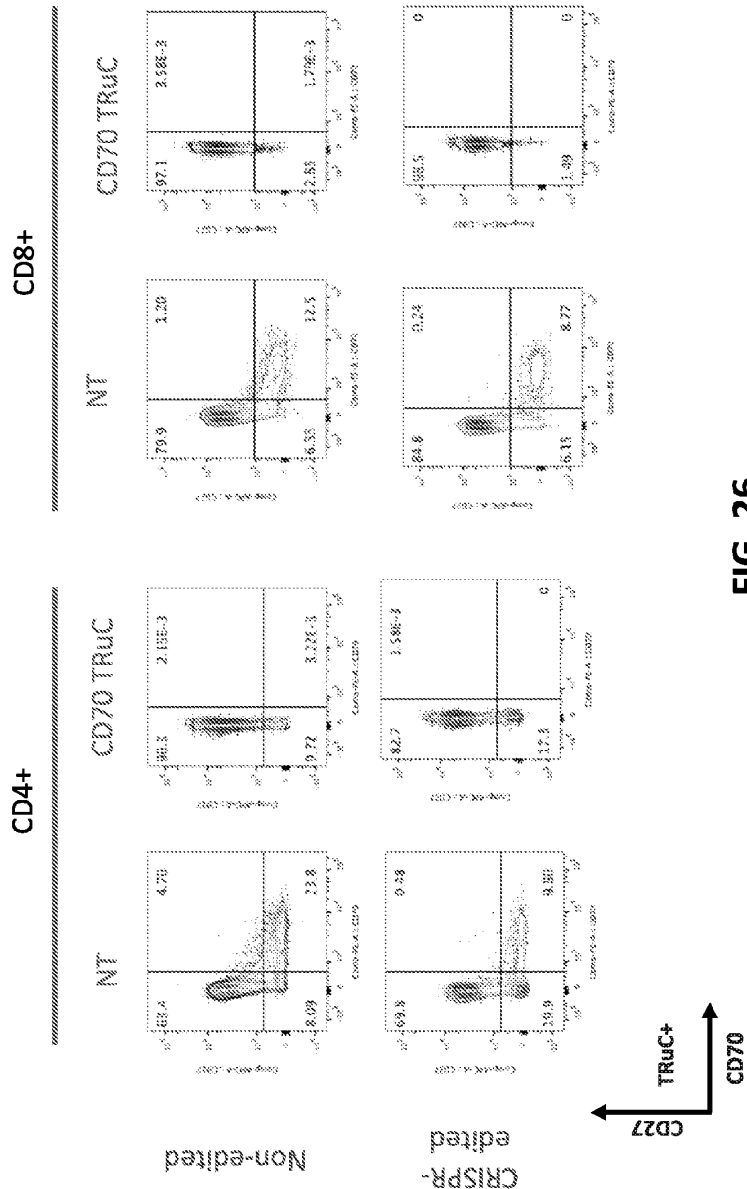


FIG. 26

Memory Phenotype

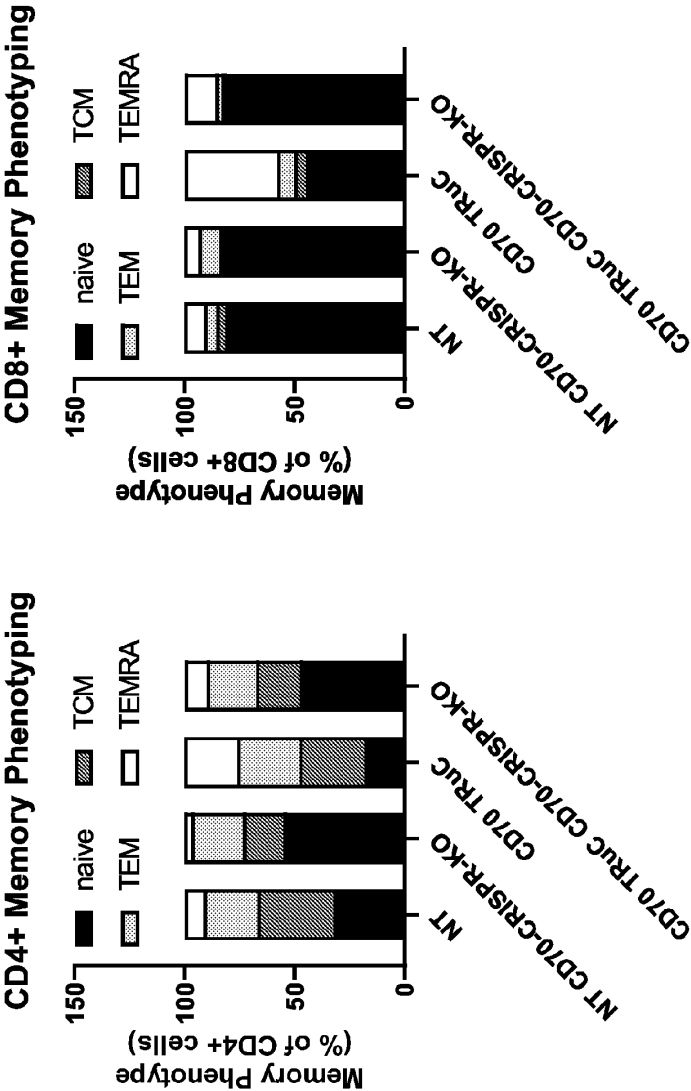


FIG. 27A

FIG. 27B

Day 10 TRuC Phenotyping: CD28 versus CD69

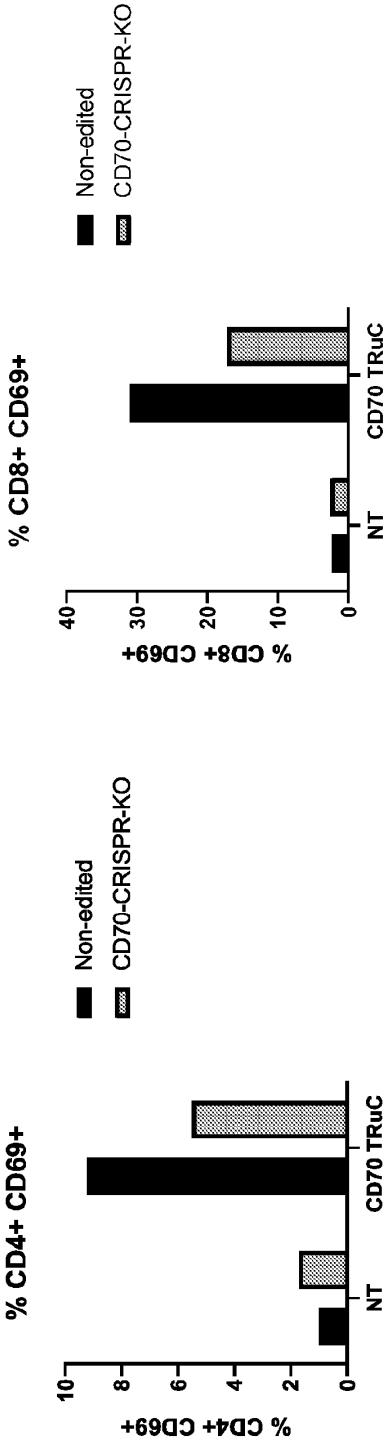


FIG. 28

Generation of Jurkat WT and CD3e KO 70-001 TRuCs

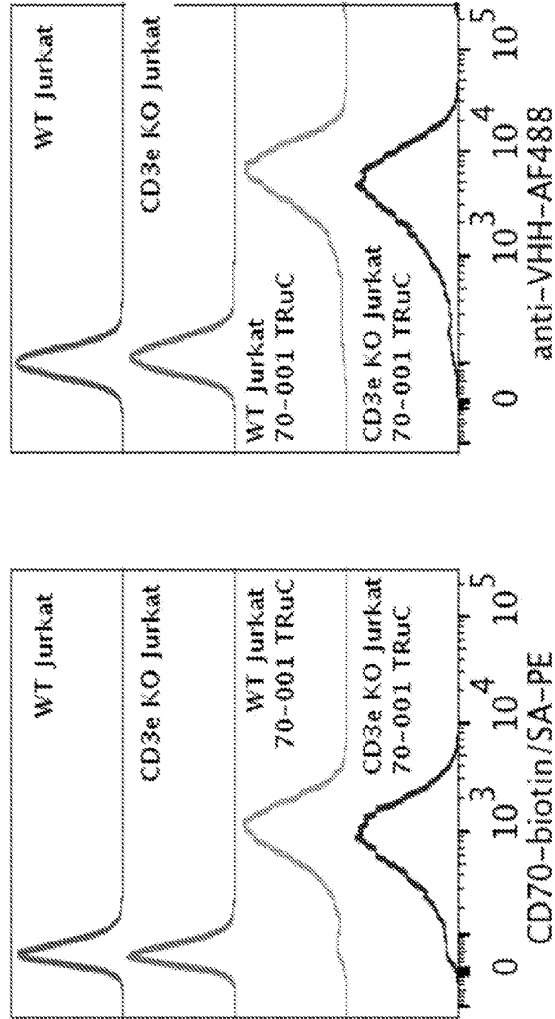


FIG. 29

CD69 signaling in 70-001 TRuC Jurkats and control by α CD70 41d12

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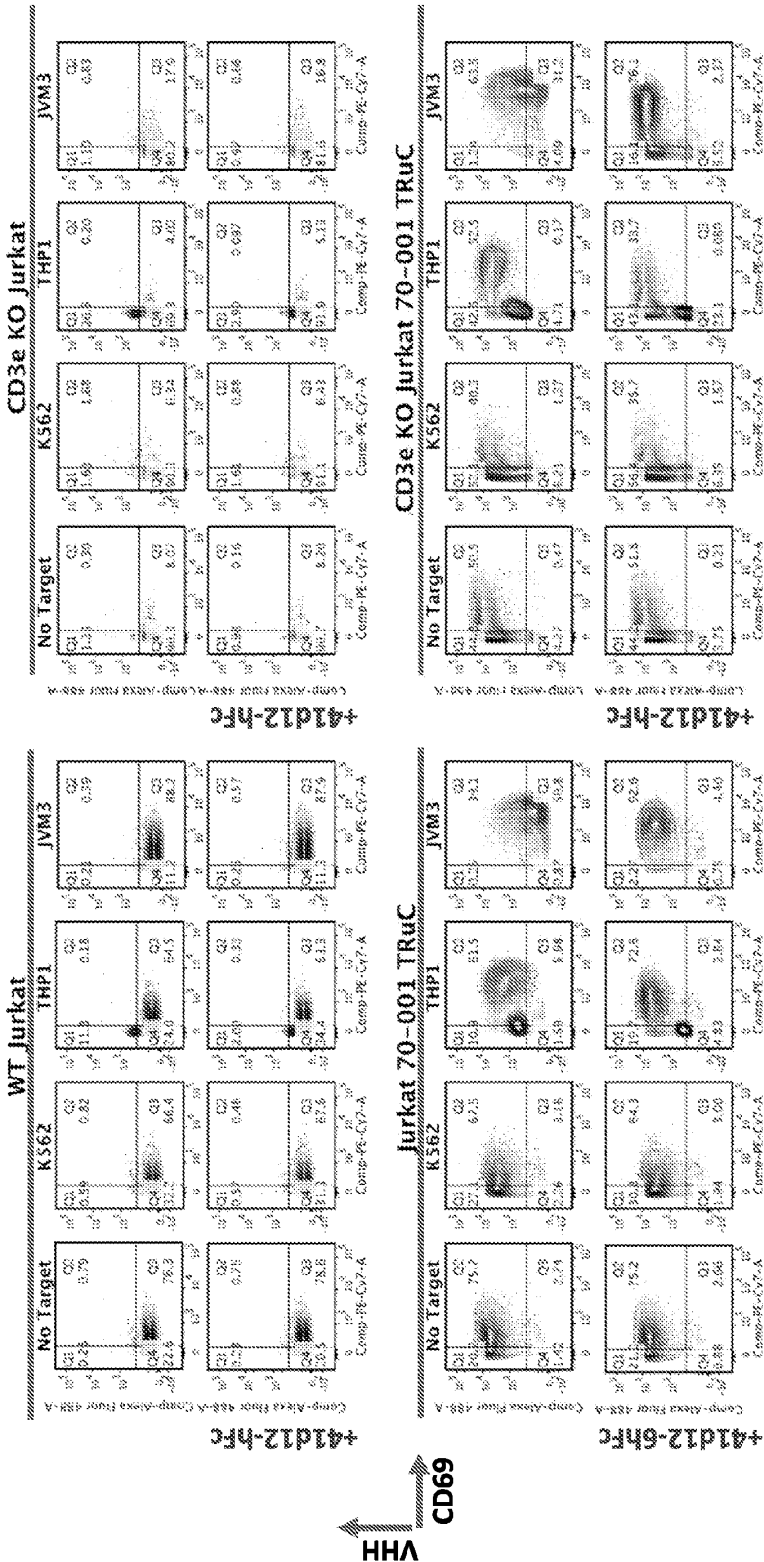


FIG. 30

*1:1 E:I co-culture, 16 hours

41d12 blocks target cell-dependent induction of CD69 in 70-001 TRuCs

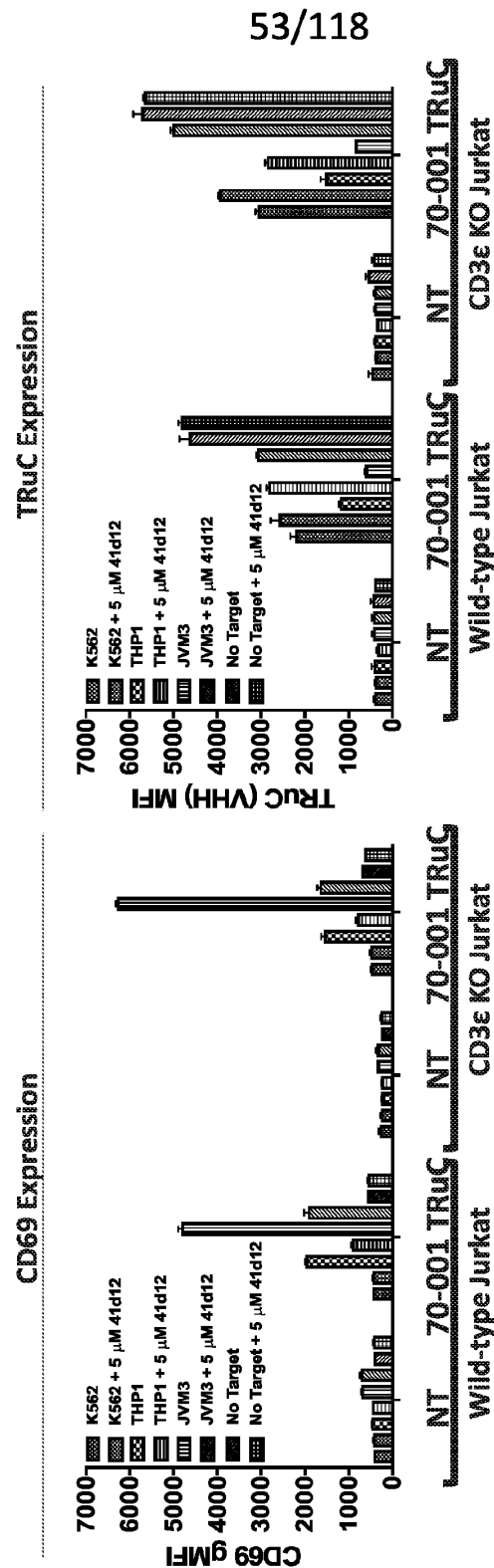
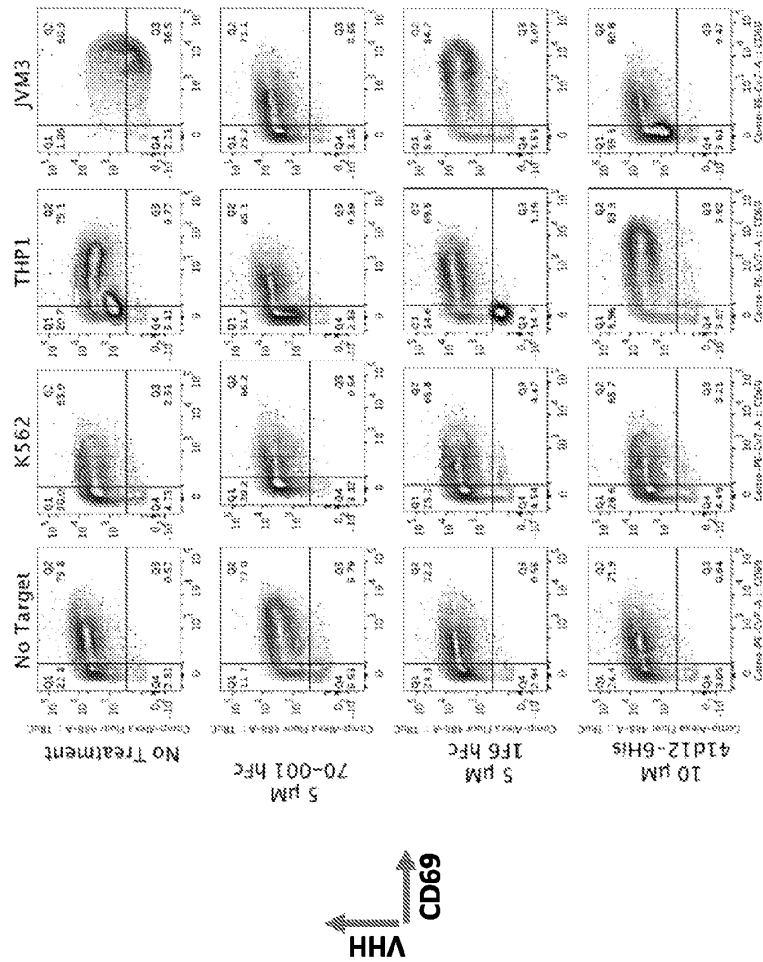


FIG. 31

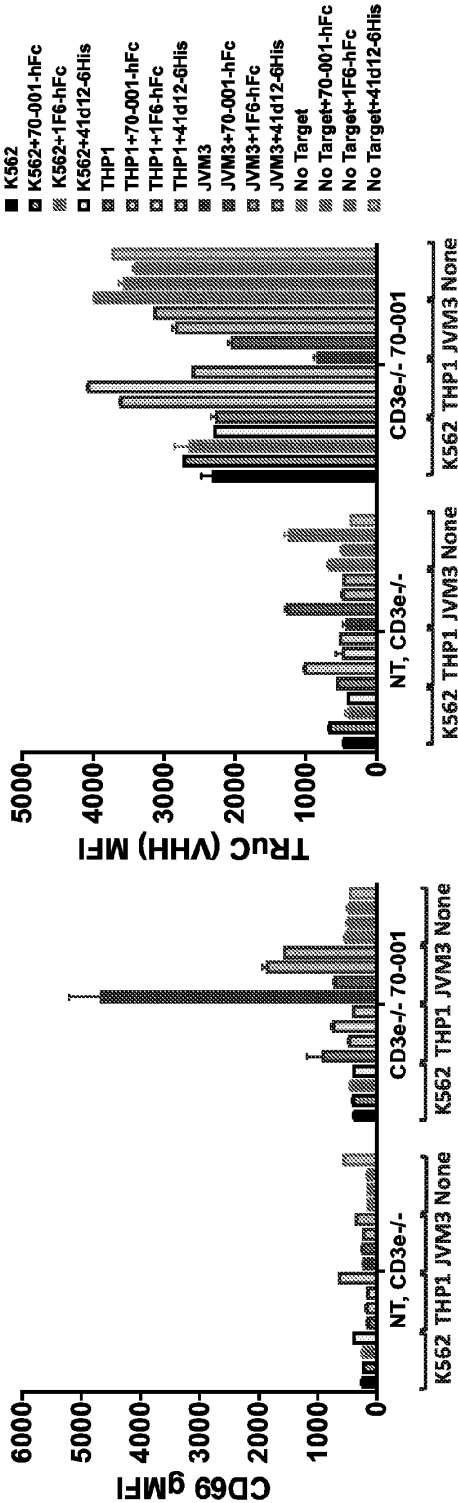
70-001-hFc and 1F6-hFc fusions block target cell-dependent induction of CD69 in CD3e KO 70-001 Jurkat TRuCs



*1:1 E:T co-culture, 16 hours

FIG. 32

70-001 and 1F6 hFc fusions block target cell-dependent induction of CD69 in 70-001 TRuCs



*1:1 E:T co-culture, 16 hours

FIG. 33

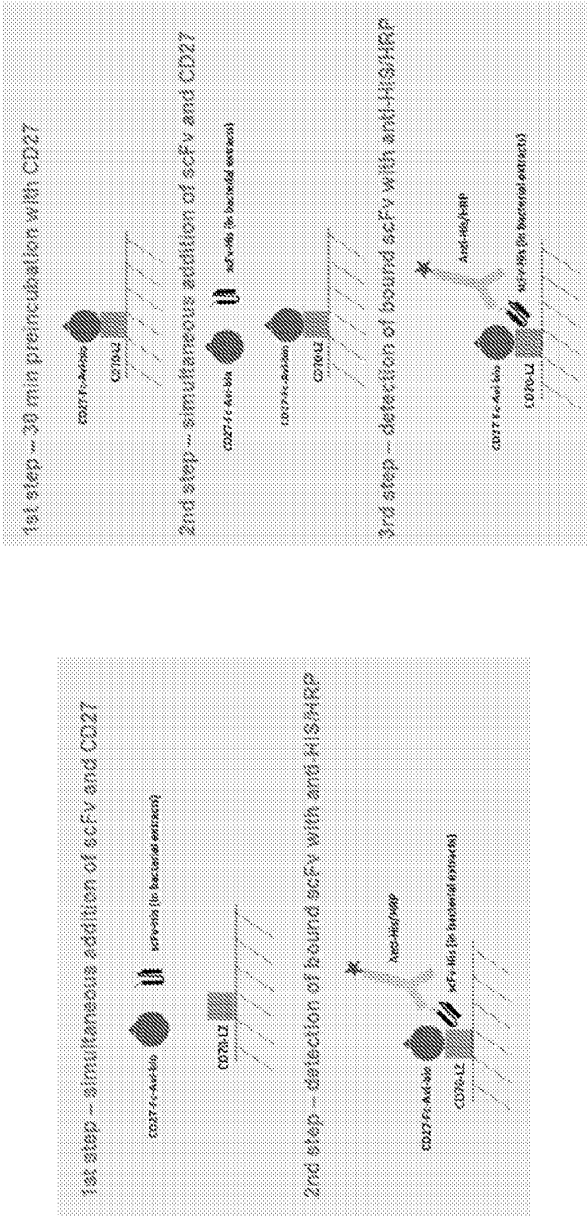


FIG. 34

Steady-state titration of CD70 with α CD70 scFvs

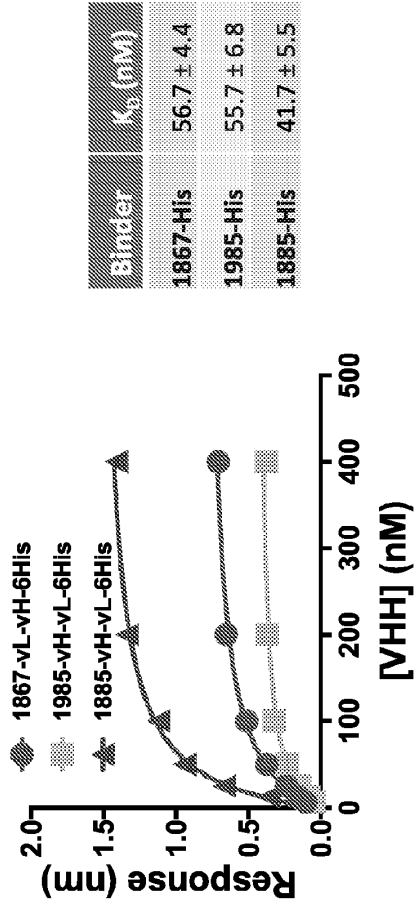
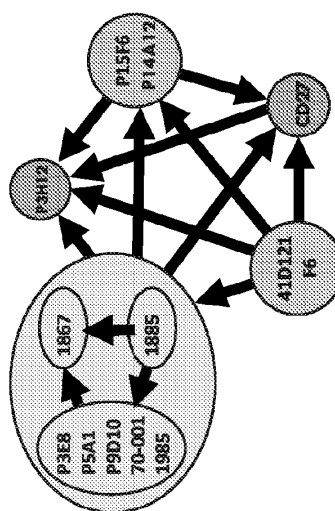
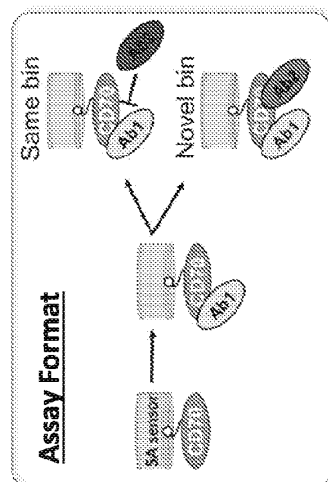


FIG. 35

α CD70 ligand, VHH, and scFv epitope binning



		Antibody 2						
		1867-His	70-001-His	1885-His	P3H12-His	CD27-His	1885-His	41d12-His
Antibody 1	1867-His	0.03	0.01	0.01	-0.02	0.02	0.46	0.15
	70-001-His	0.03	0.01	0.05	0.03	0.04	0.69	0.22
	1885-His	0.15	0.04	0.02	0.01	0.10	0.74	0.22
	P3H12-His	0.34	0.10	0.05	0.09	0.16	1.01	0.49
	CD27-His	0.28	0.05	0.10	0.07	0.03	0.85	0.40
	1885-His	-0.08	-0.12	-0.08	-0.10	-0.08	0.07	0.45
	41d12-His	0.03	-0.02	0.02	0.01	-0.01	-0.02	0.04

Binder	K_b (nM)
1857-His	56.7 ± 4.4
70-001-His	7.9 ± 1.3
1985-His	55.7 ± 6.8
P3H12-His	26.2 ± 7.5
CD27-His	129.8 ± 18.9
1885-His	41.7 ± 5.5

Epitope mapping VHHs

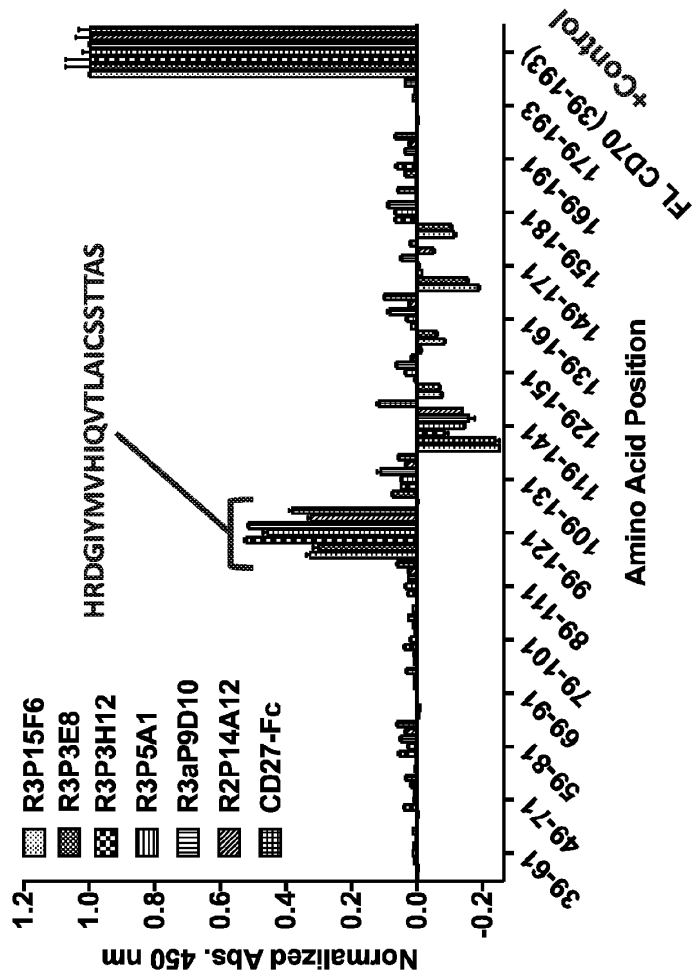


FIG. 37A

Epitope mapping of scFvs

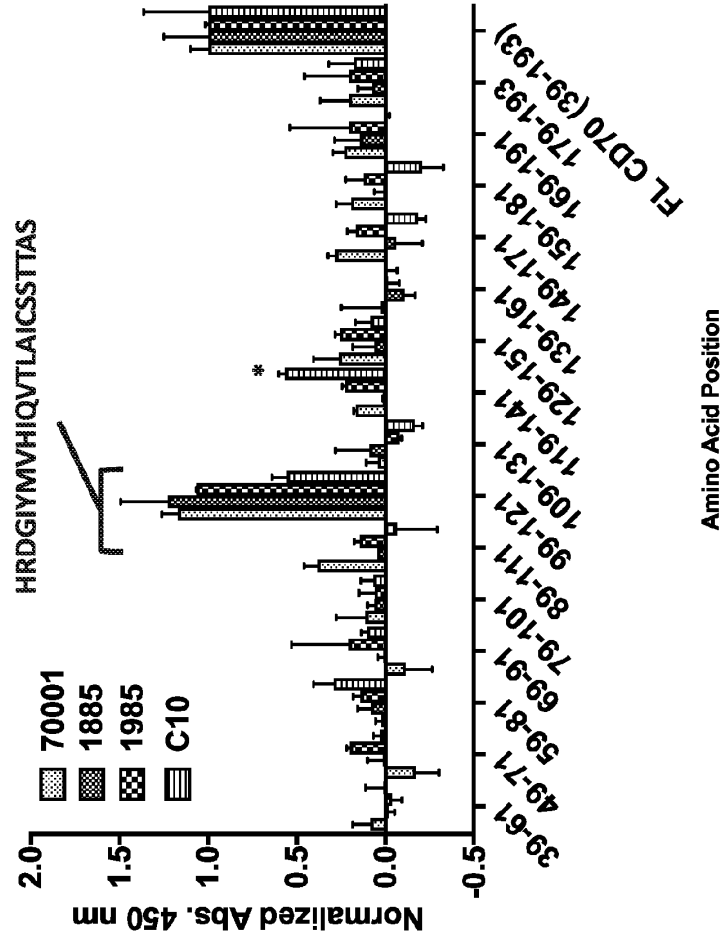
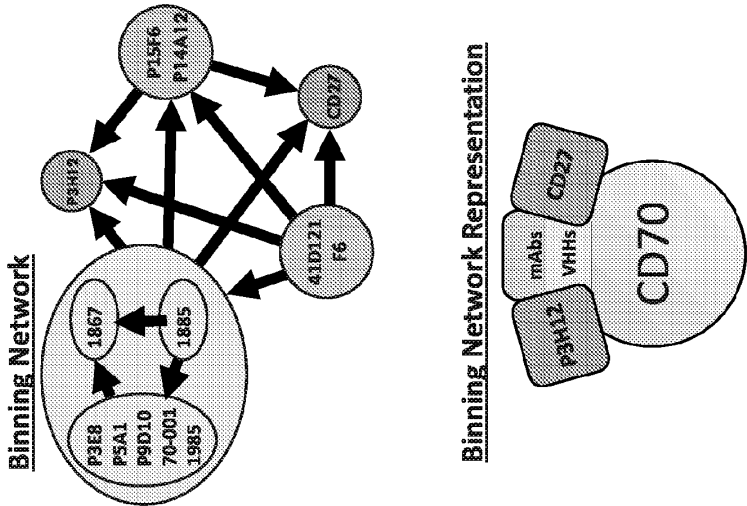


FIG. 37B

Comparison of αCD70 Binders

	Type	K _d (nM)	CD27 blocking	Epitope
41D12	scFv	0.02-0.34	Yes	HRDGIYMVHIQVTLAICSSTTAS
1885	scFv	41.7	Yes	HRDGIYMVHIQVTLAICSSTTAS
1985	scFv	55.7	Yes	HRDGIYMVHIQVTLAICSSTTAS
C10 (1867)	scFv	56.7	Yes	HRDGIYMVHIQVTLAICSSTTAS
70-001	VHH	4.1	Yes	ASRHHPTTLAVGICSPASRSISL
P15F6	VHH	11.6	Yes	HRDGIYMVHIQVTLAICSSTTAS
P3E8	VHH	8.3	Yes	HRDGIYMVHIQVTLAICSSTTAS
P5A1	VHH	4.9	Yes	HRDGIYMVHIQVTLAICSSTTAS
P3H12	VHH	19.5	No	HRDGIYMVHIQVTLAICSSTTAS
CD27Fc	Rec	27.1	Yes	HRDGIYMVHIQVTLAICSSTTAS

FIG. 37C



Virus transduction restores surface TCR expression on CD3ε KO Jurkat and elevates CD69 expression on TRuC+ (CD3+) populations

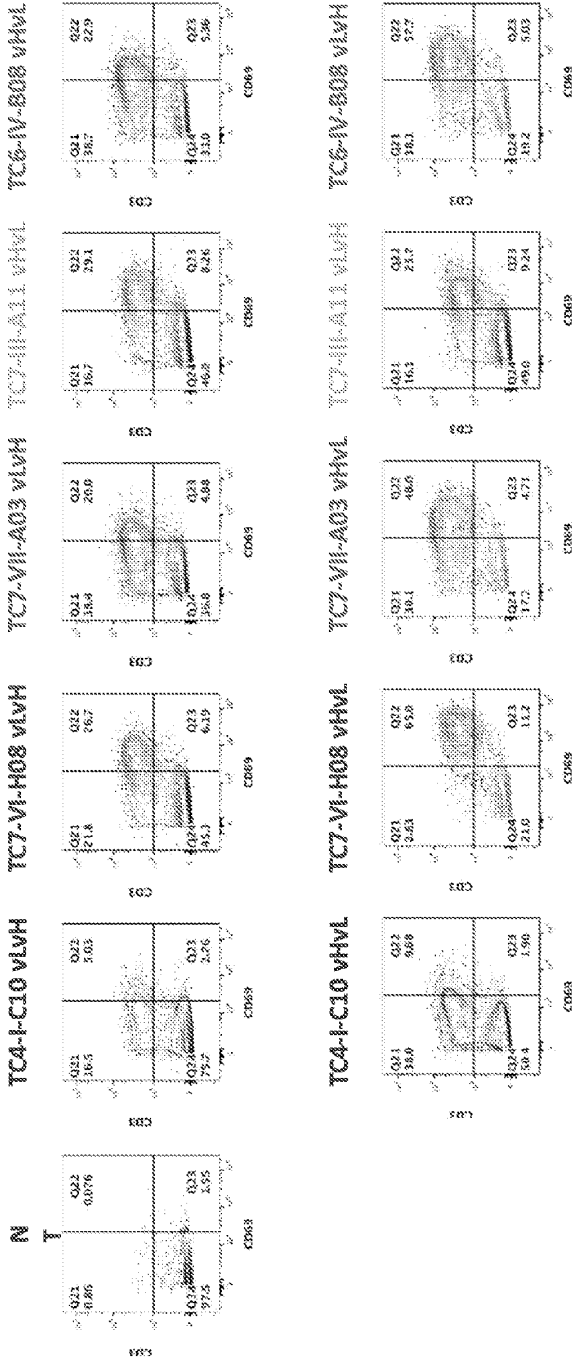


FIG. 38

Human scFv TRuC mediated CD70-specific cell activation (Jurkats)

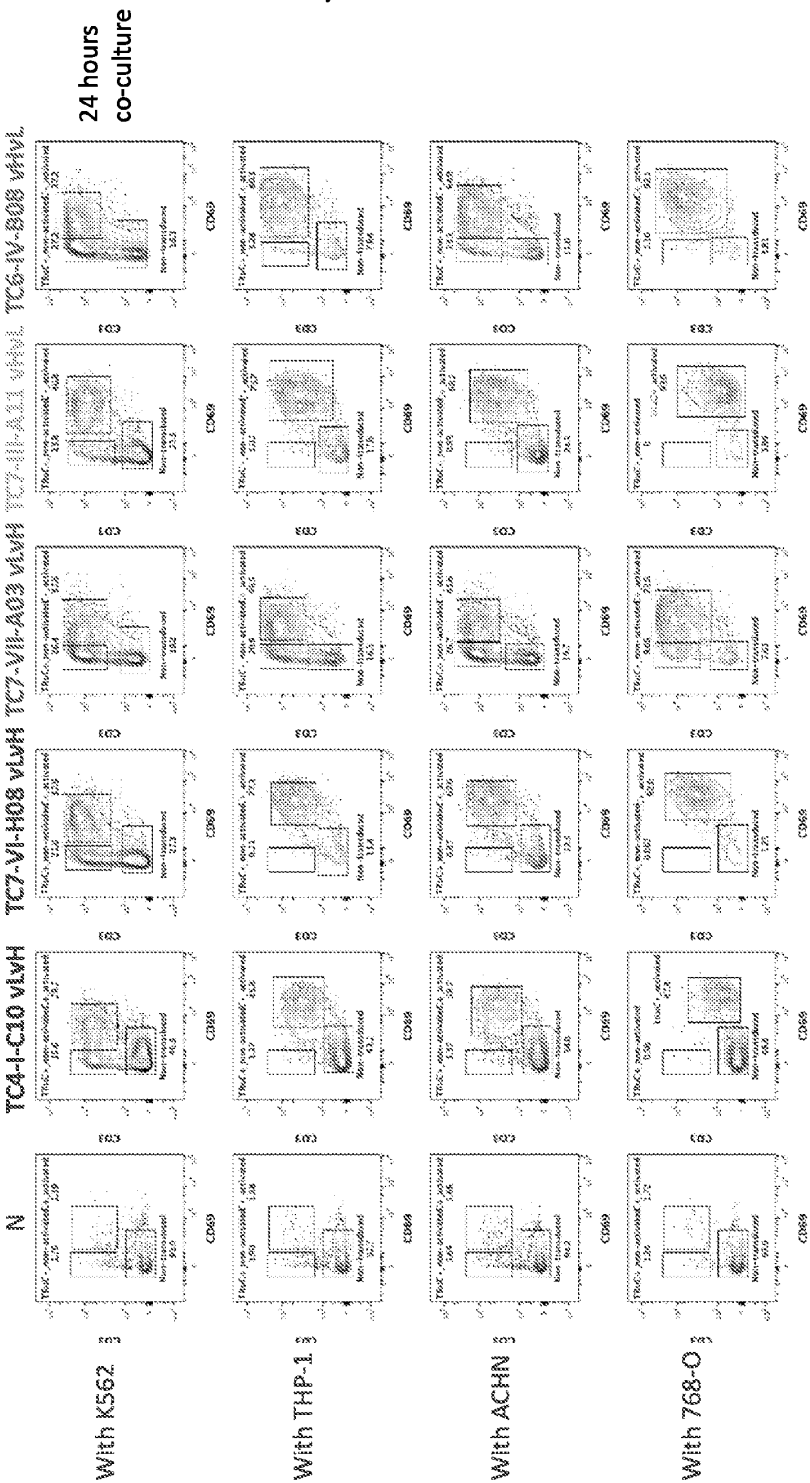


FIG. 39A

Human scFv TRuC mediated CD70-specific cell activation (Jurkats)

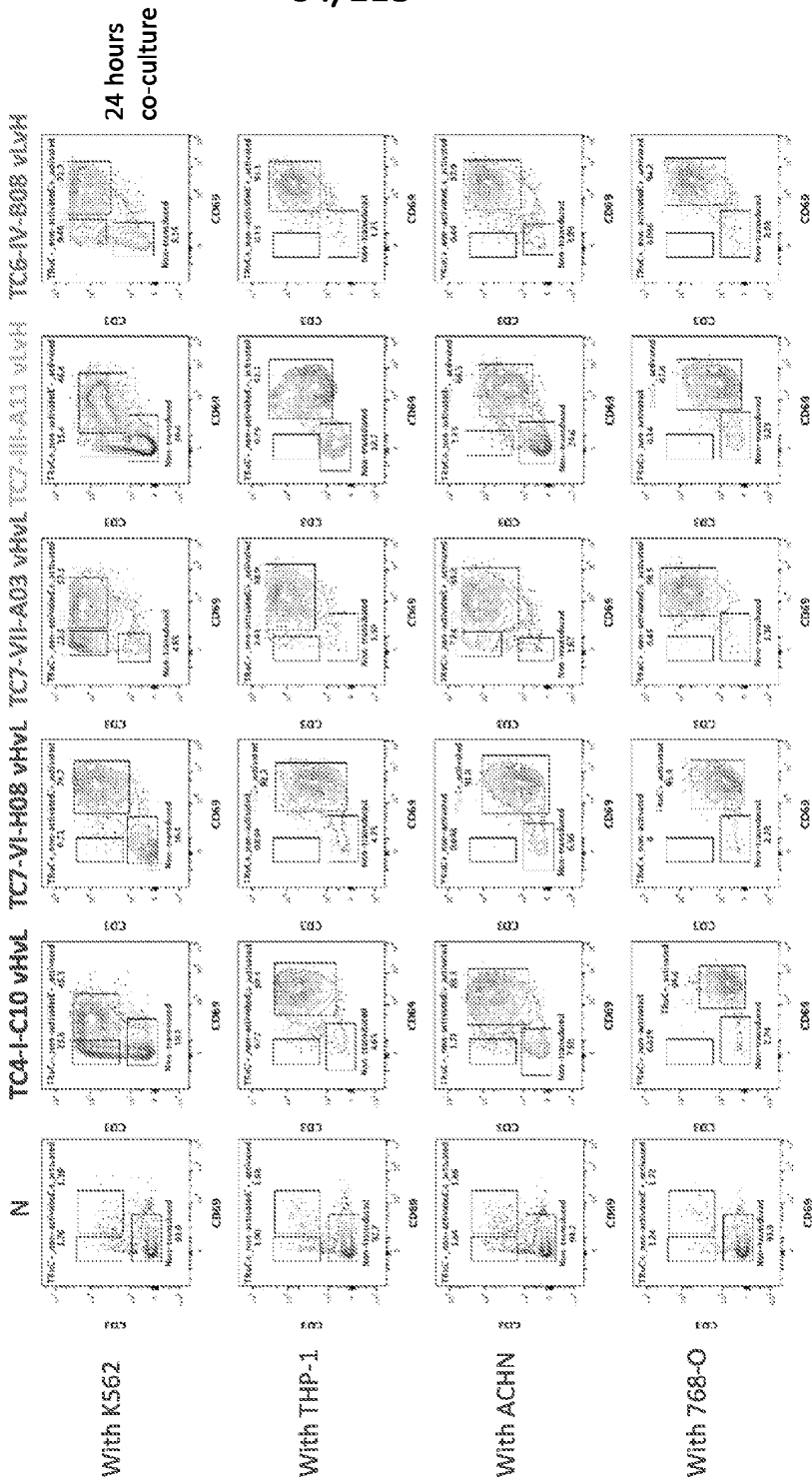


FIG. 39B

Cytokine production from the same co-culture (Jurkats)

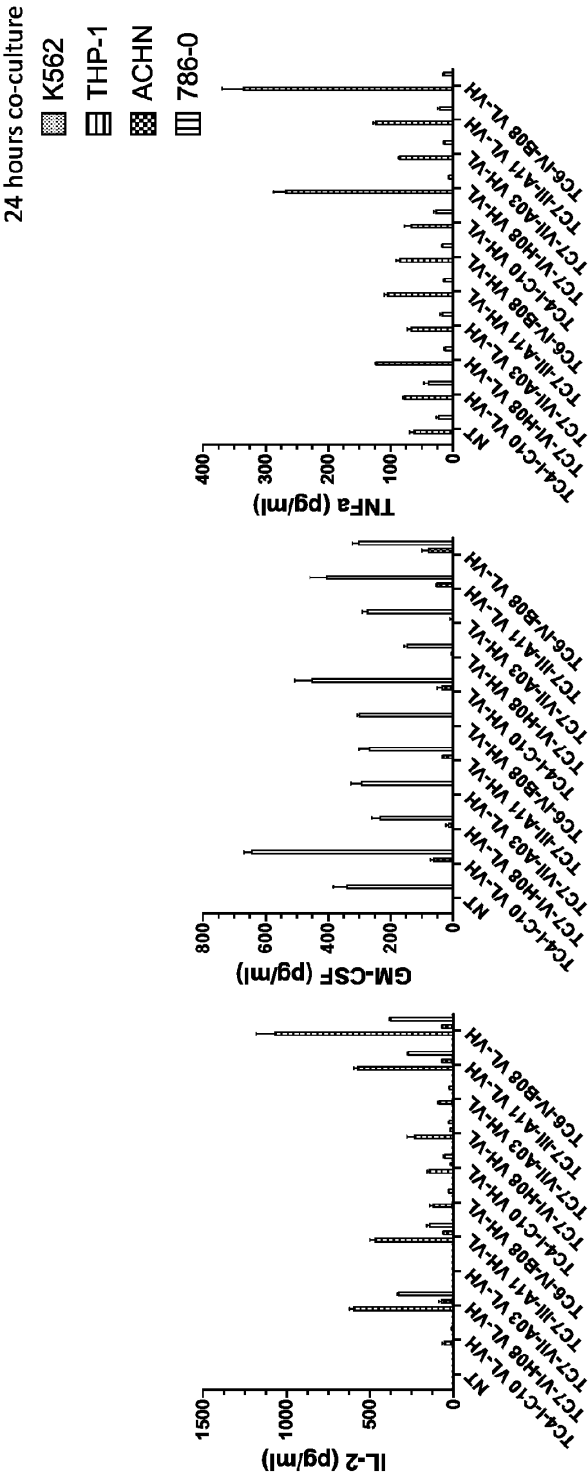
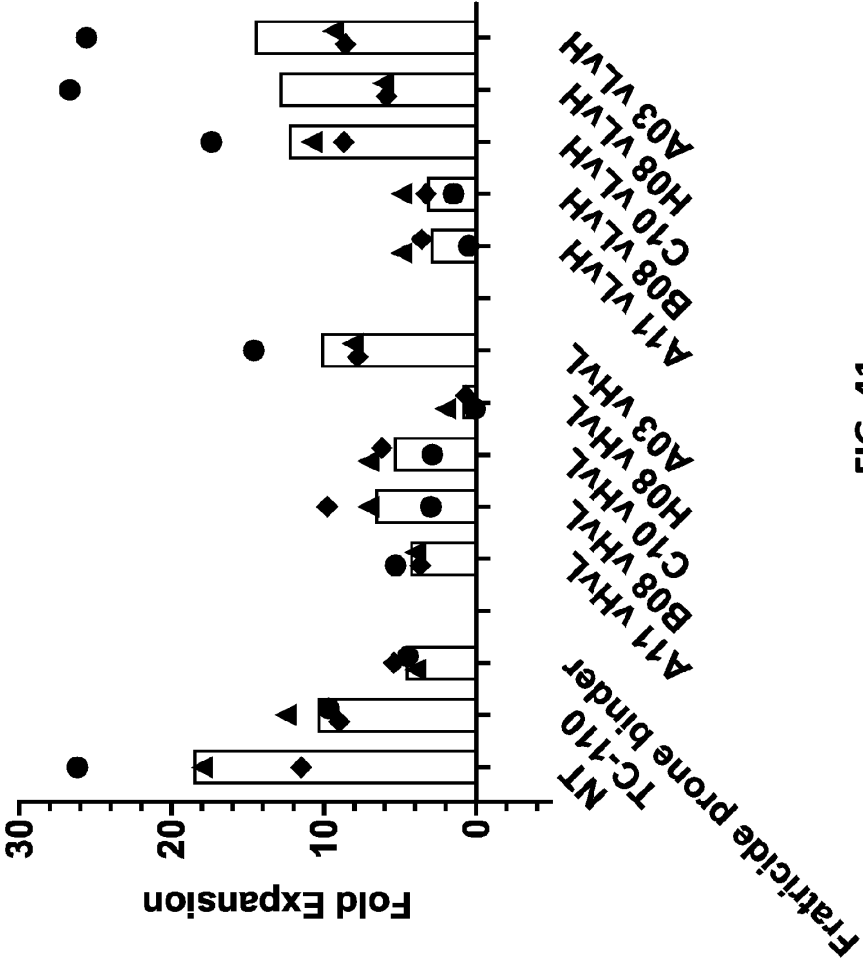


FIG. 40



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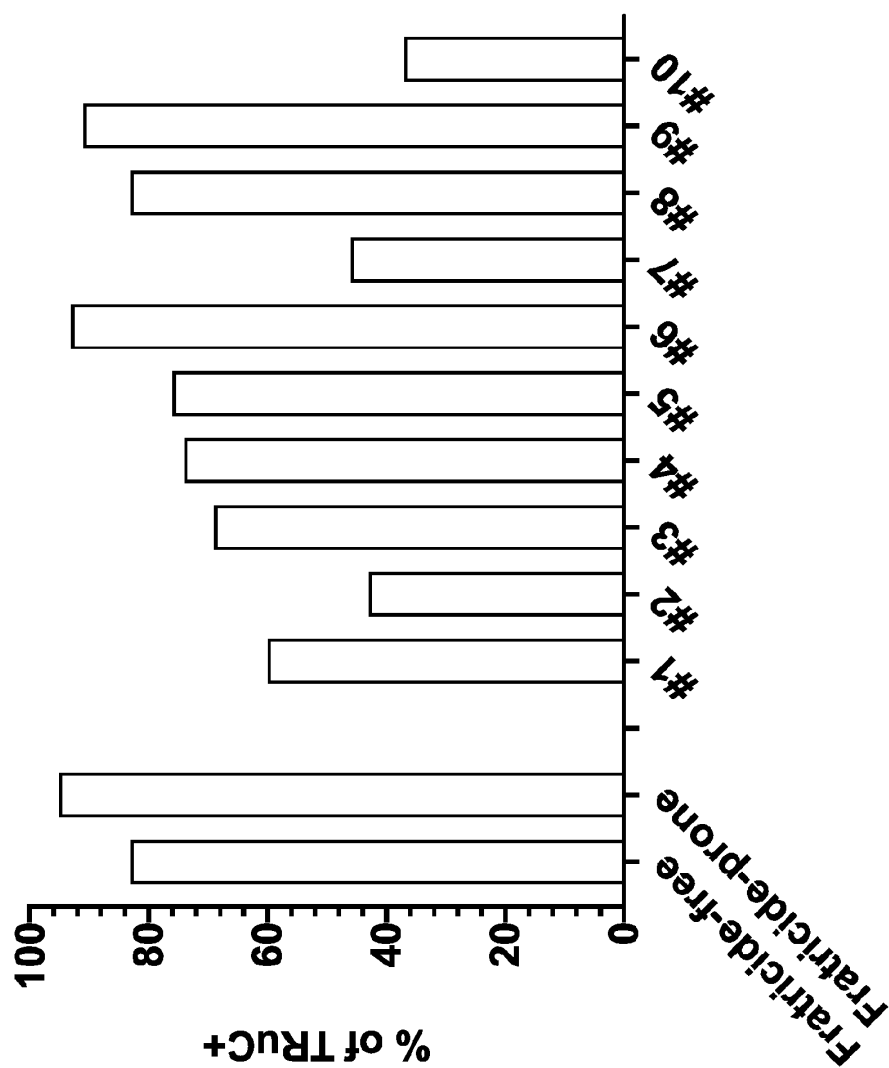


FIG. 42

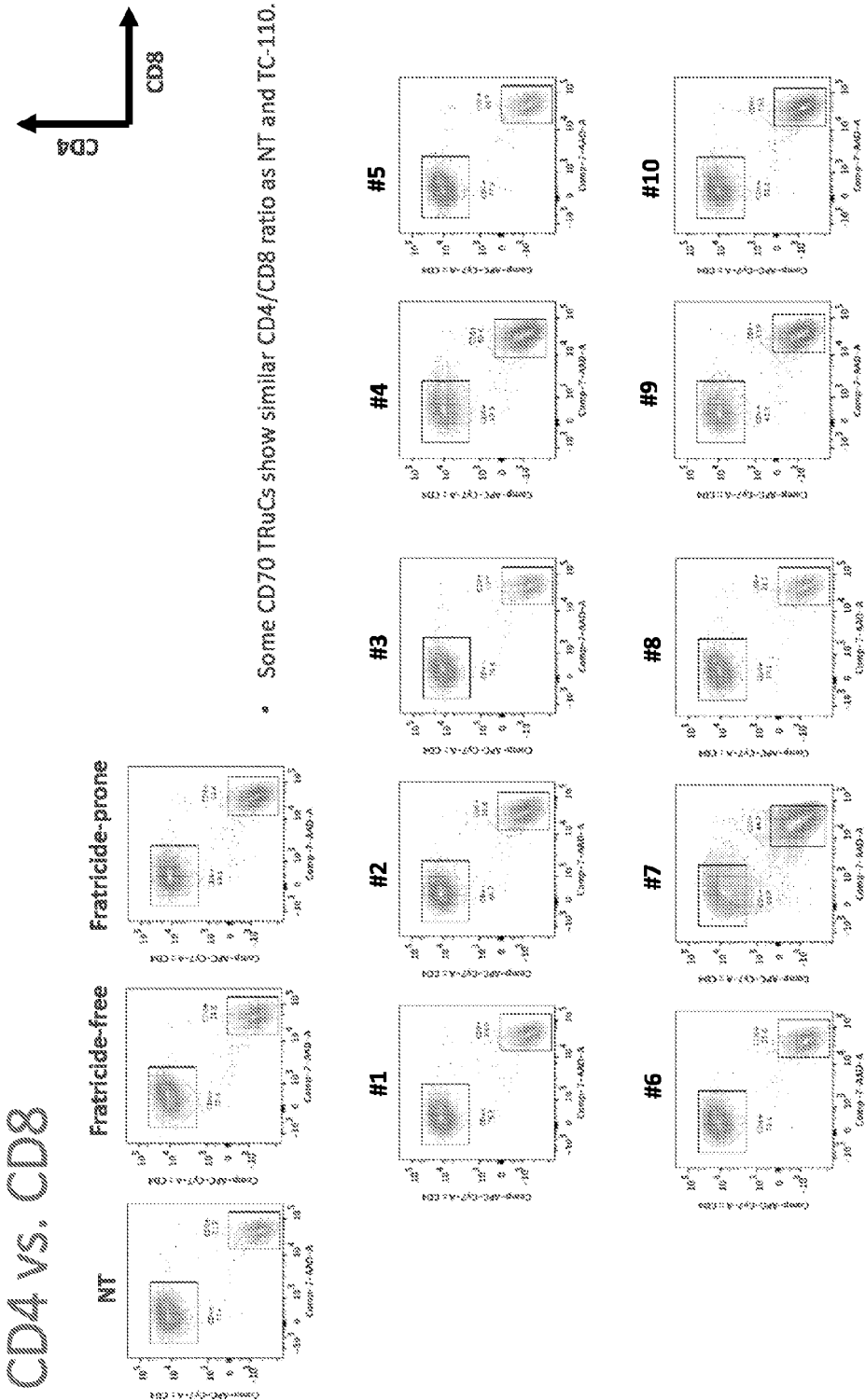


FIG. 43

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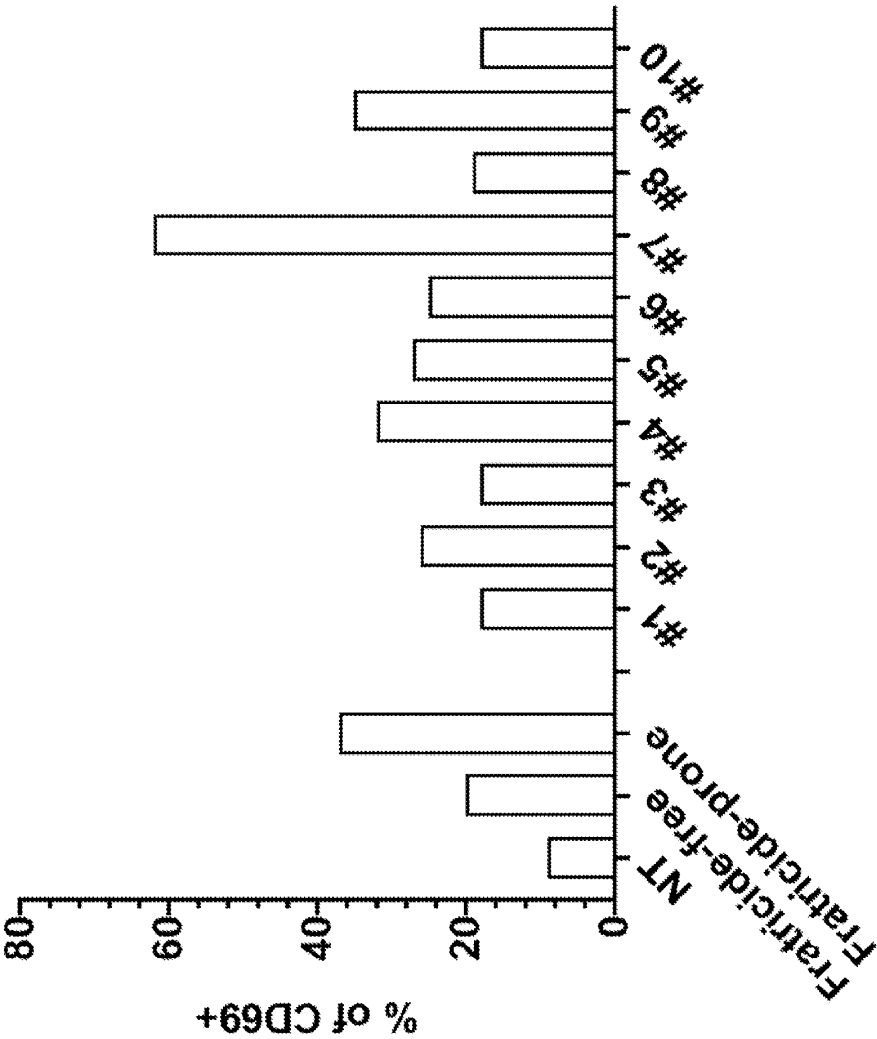


FIG. 44

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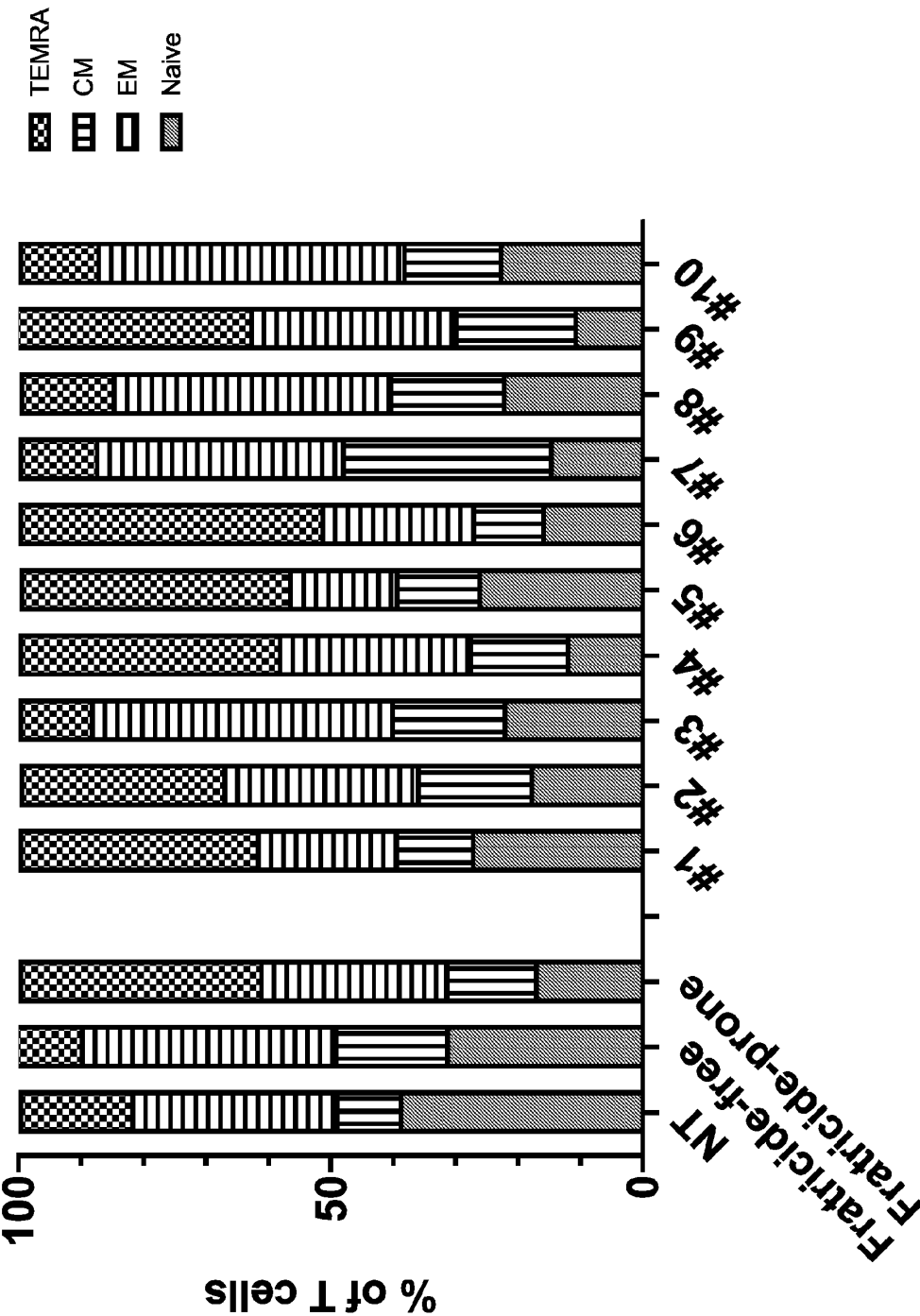


FIG. 45

	Fratricide-free (non-CD70 targeting TRuC)	Fratricide- prone (CD70 targeting TRuC)	CD70-sepcifc TRuC candidates									
			#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Expansion	Normal	Low	*	*	*	X	X	X	X	*	X	X
TRuC expression	High	High	*	*	*	*	*	*	*	*	*	*
Activation	Low	High	*	X	*	X	X	X	X	*	X	*
Differentiation	High Naïve Low TEMRA	Low Naïve High TEMRA	*	X	*	X	*	X	X	*	X	*

FIG. 46

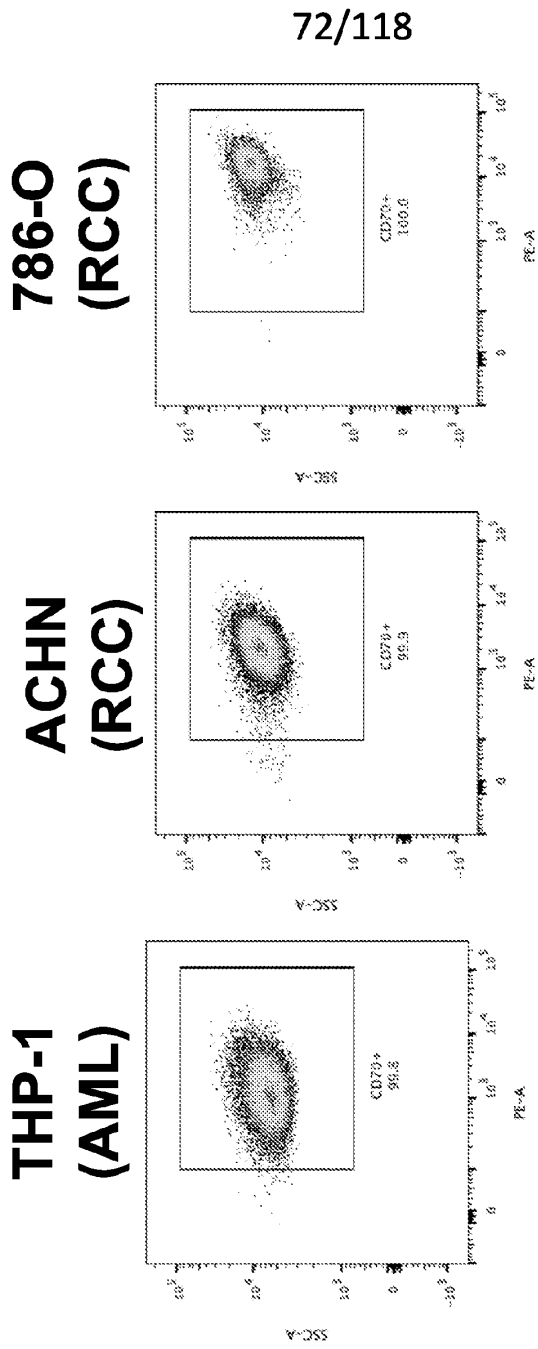


FIG. 47

TC4-I-C10ε vLVH and TC7-VI-H08ε vLVH TRuCs show high potency against CD70-expressing cell lines

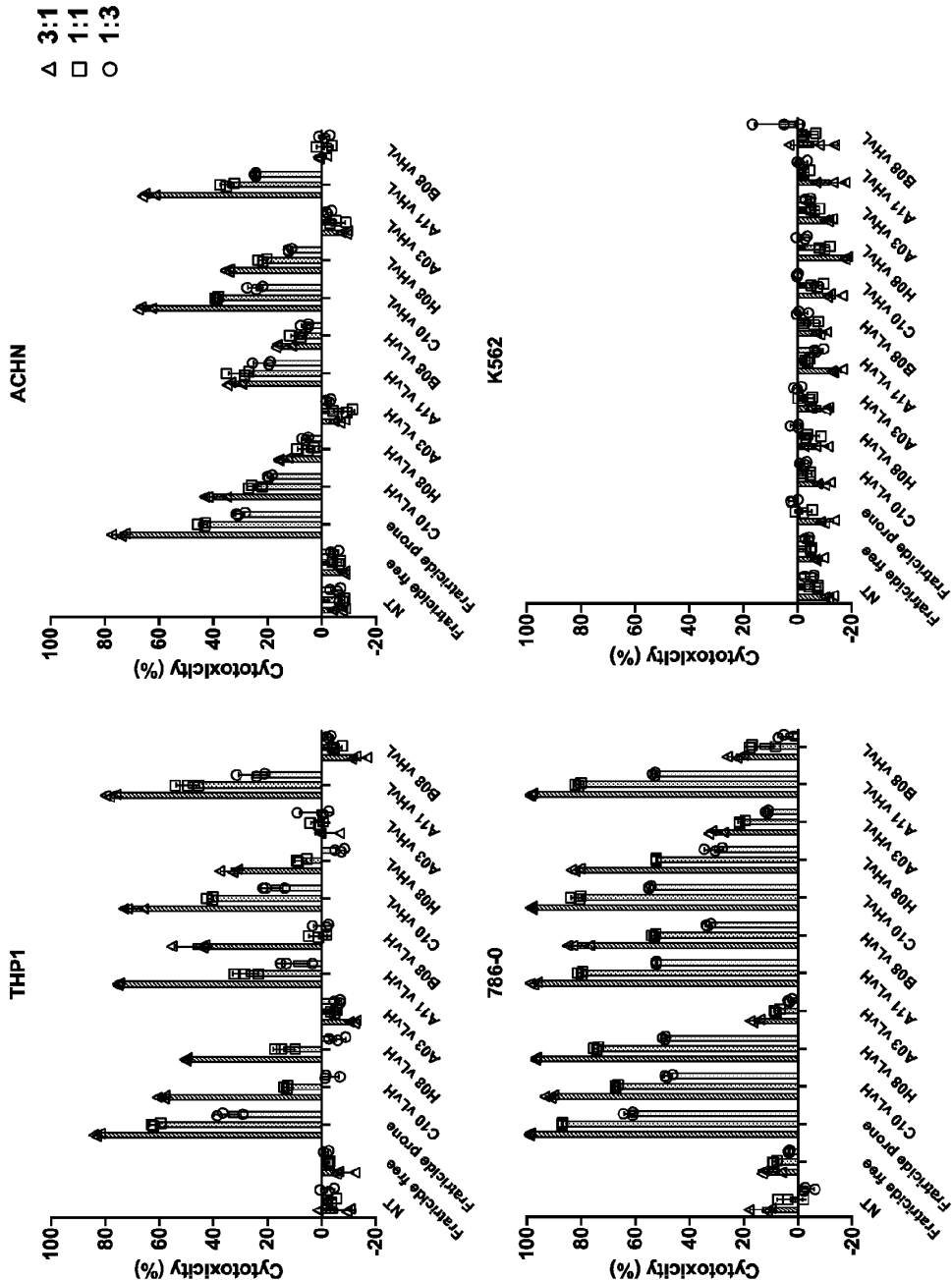


FIG. 48

TC4-I-C10ε vLvH produces high IFN γ in CD70-expressing cell lines

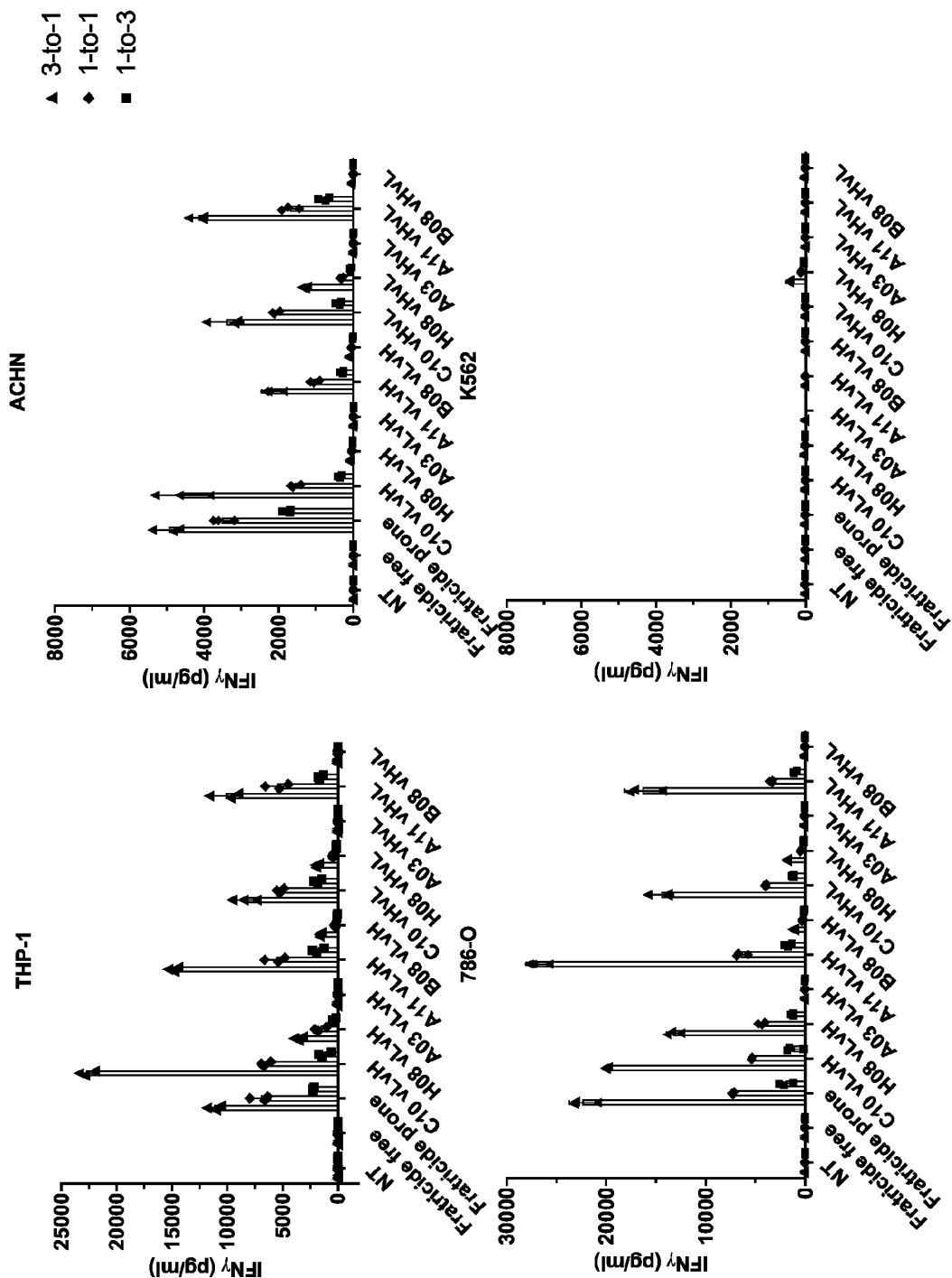


FIG. 49A

TC4-I-C10ε vLvH produces high IL-2 in CD70-expressing cell lines

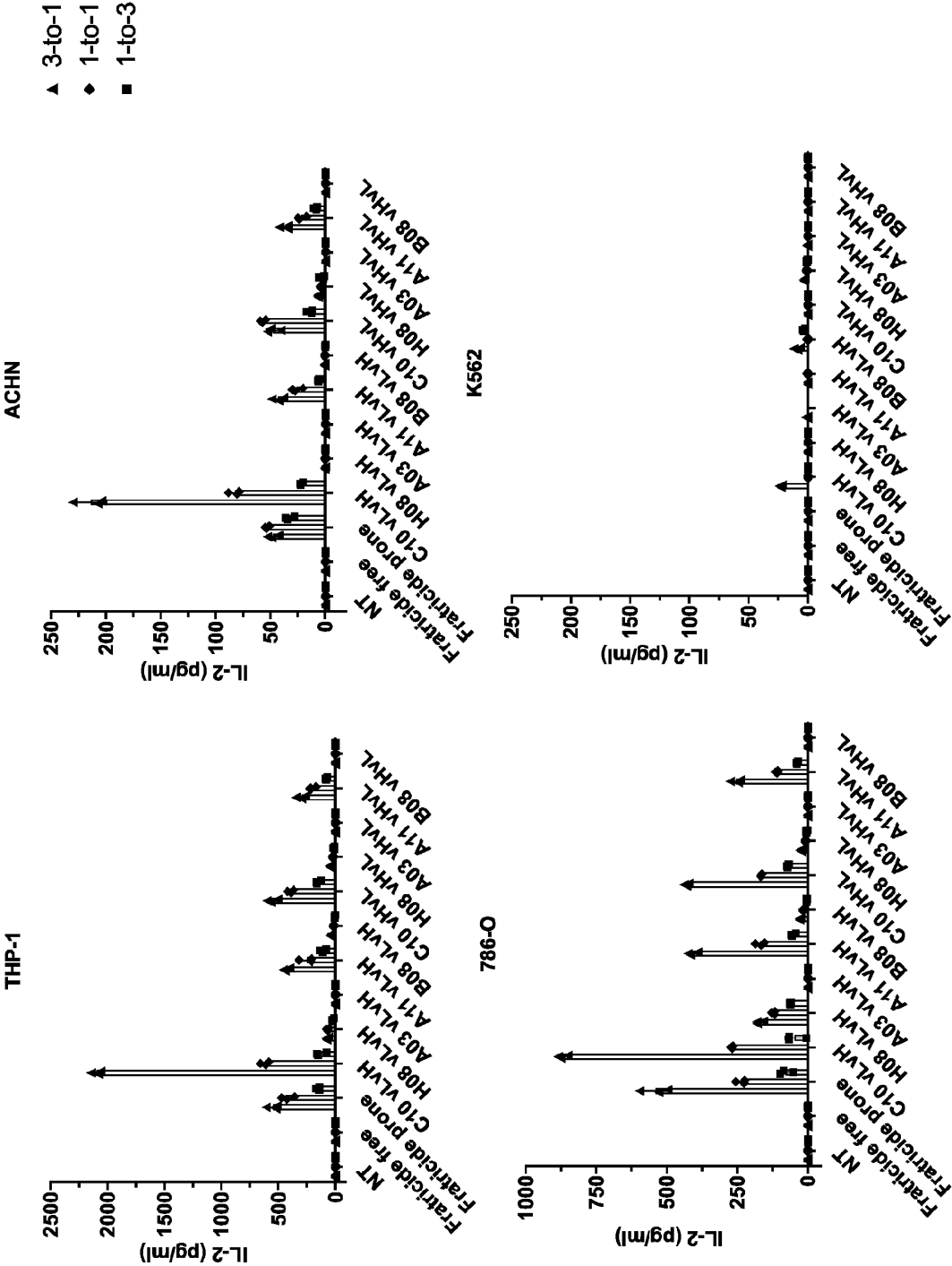


FIG. 49B

TC4-I-C10ε vLvH produces high TNFα in CD70-expressing cell lines

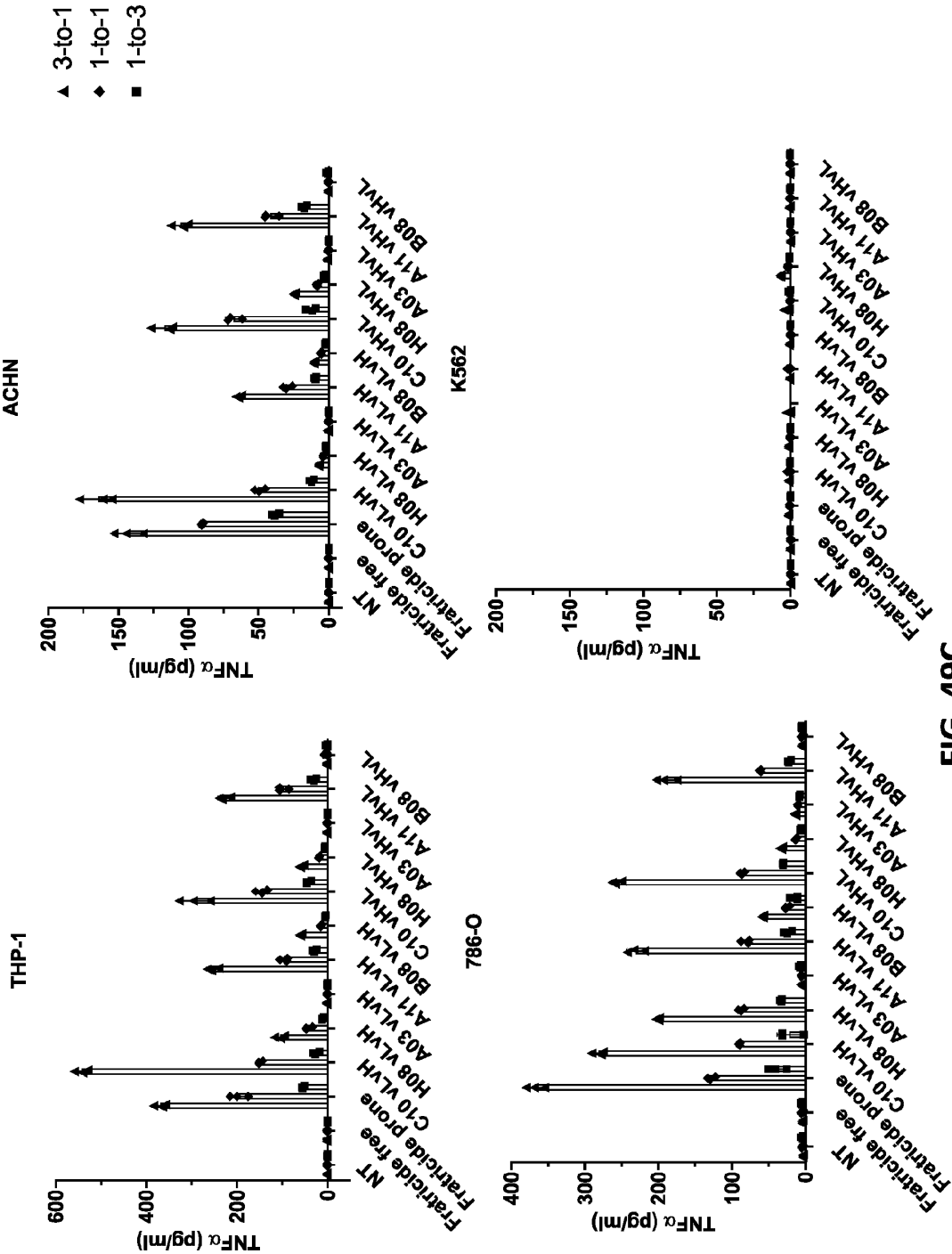


FIG. 49C

TC4-I-C10ε vLvH produces high GM-CSF in CD70-expressing cell lines

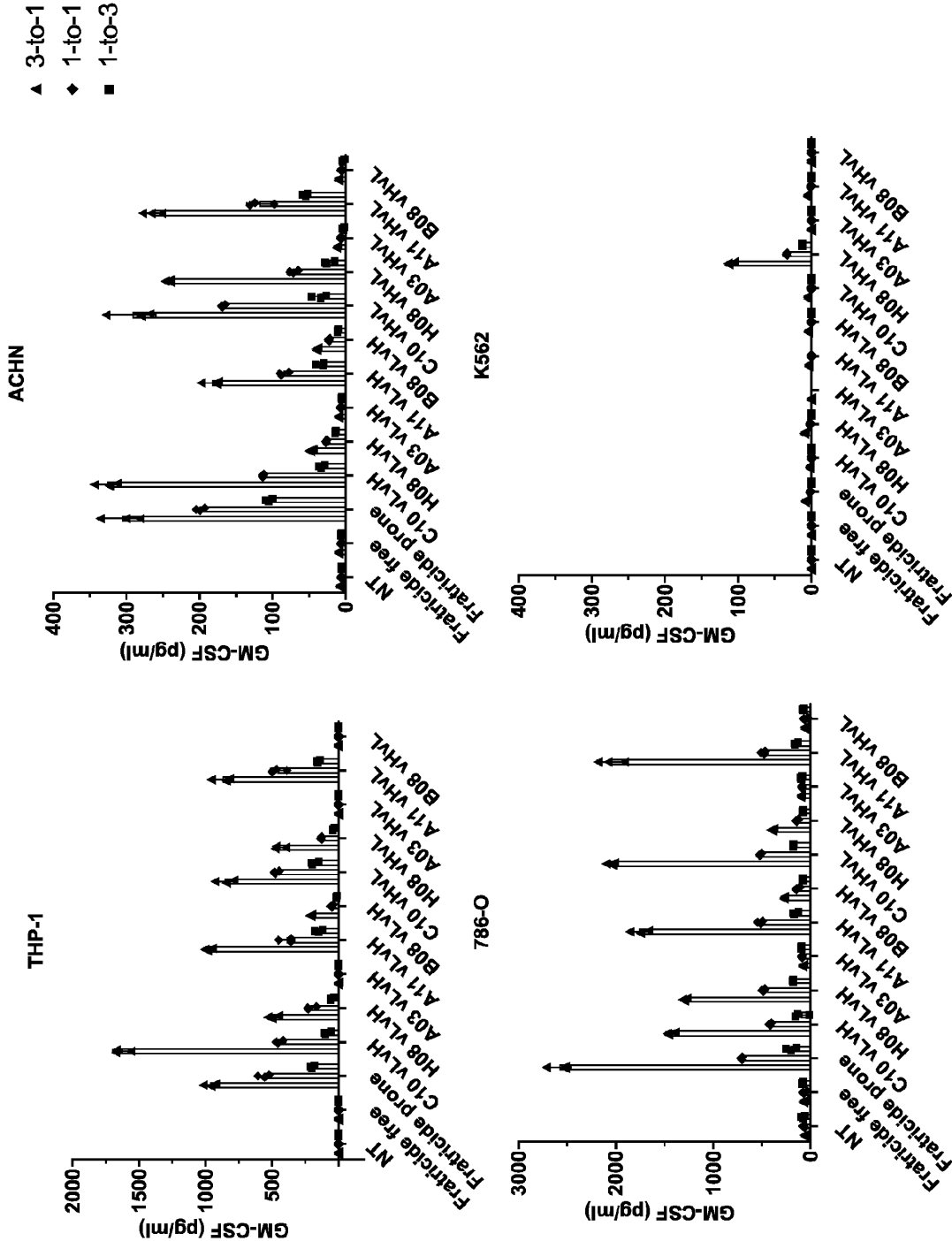
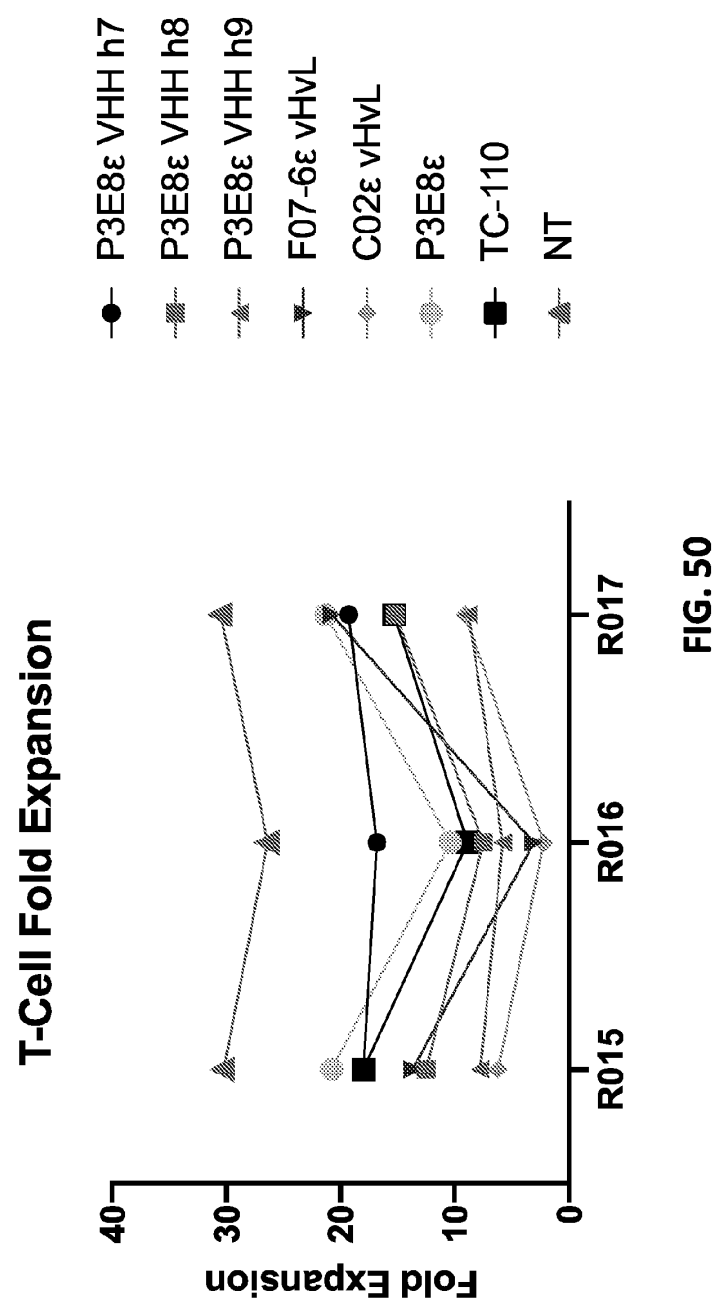
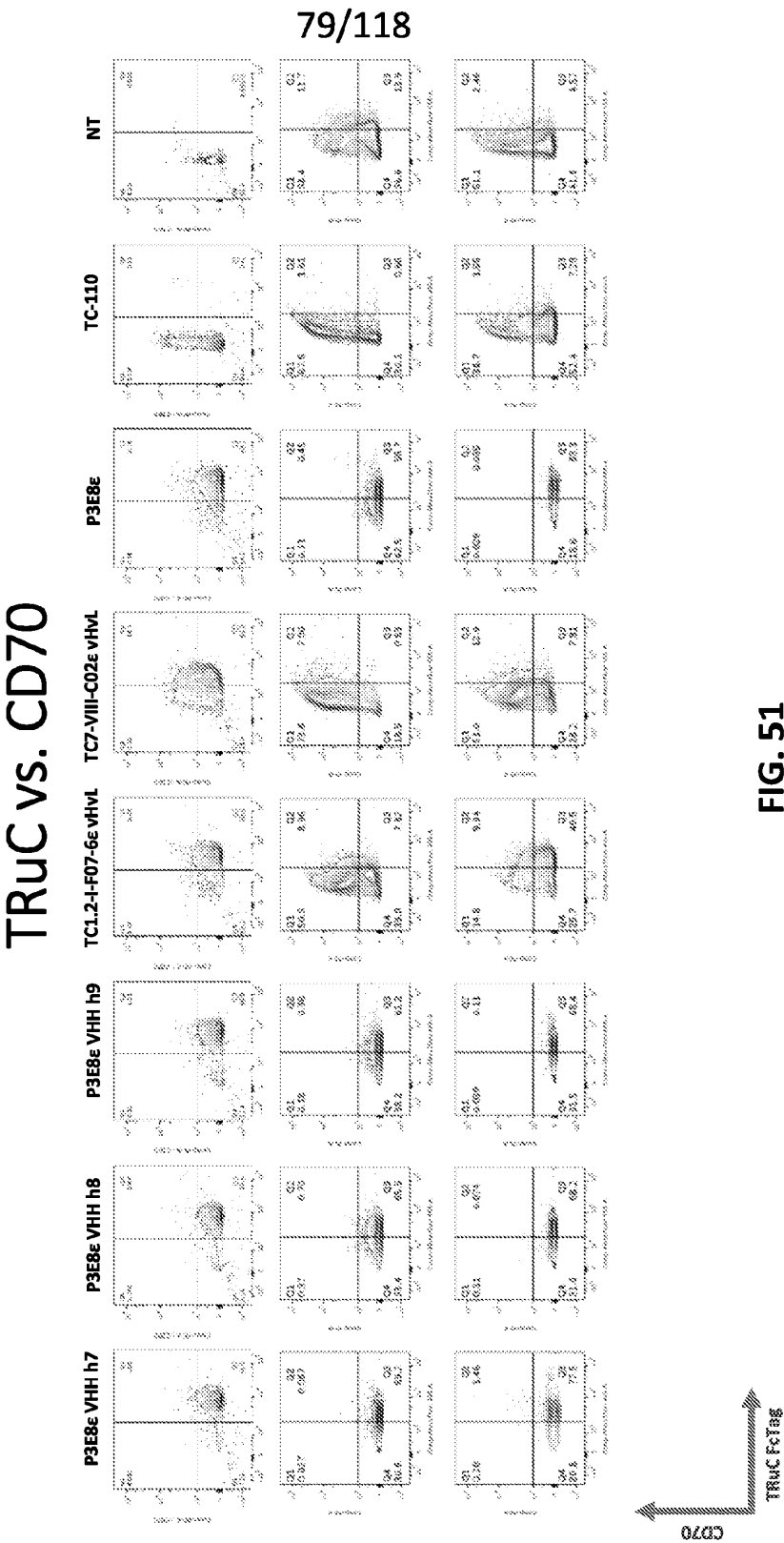


FIG. 49D

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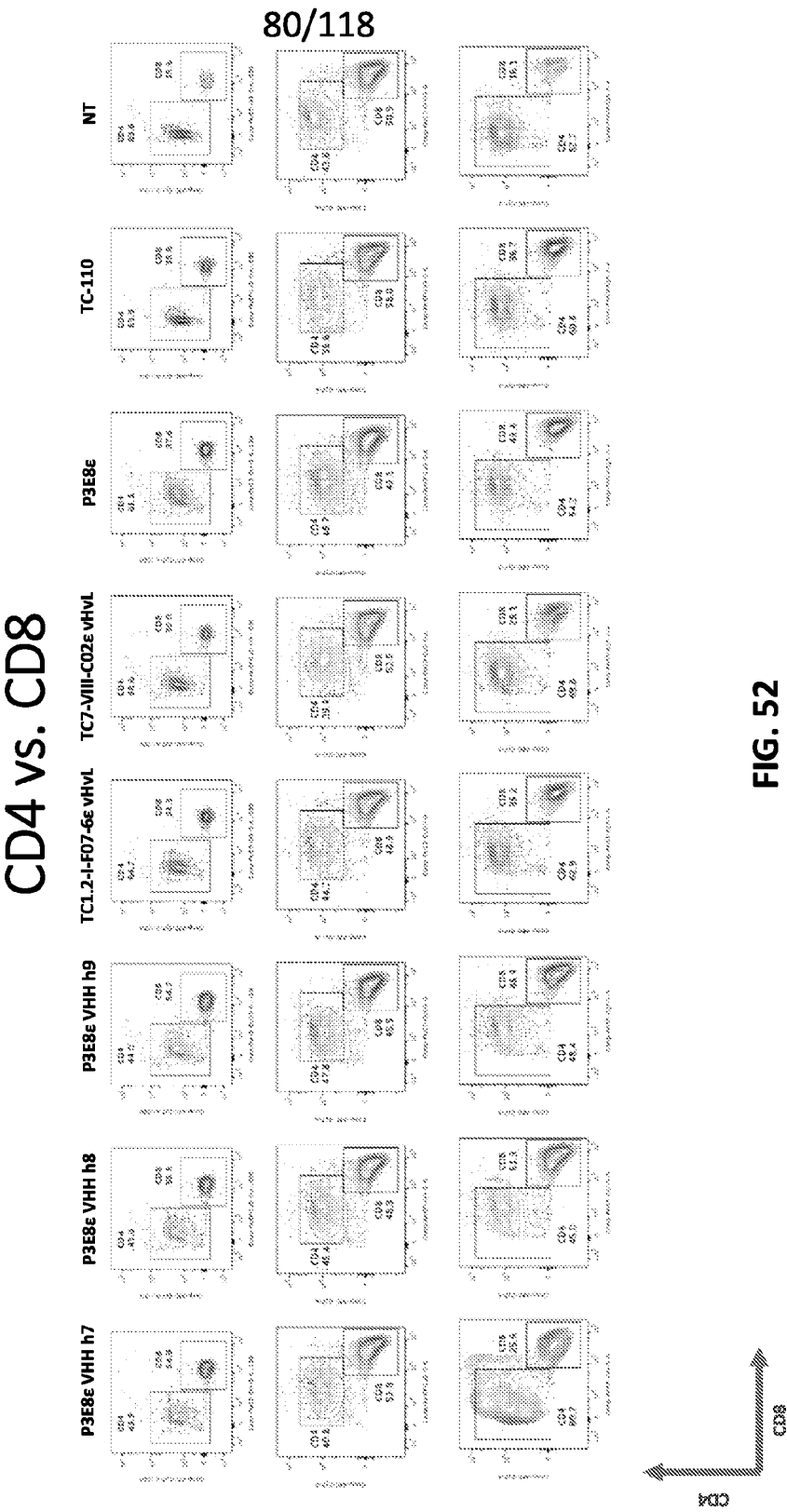
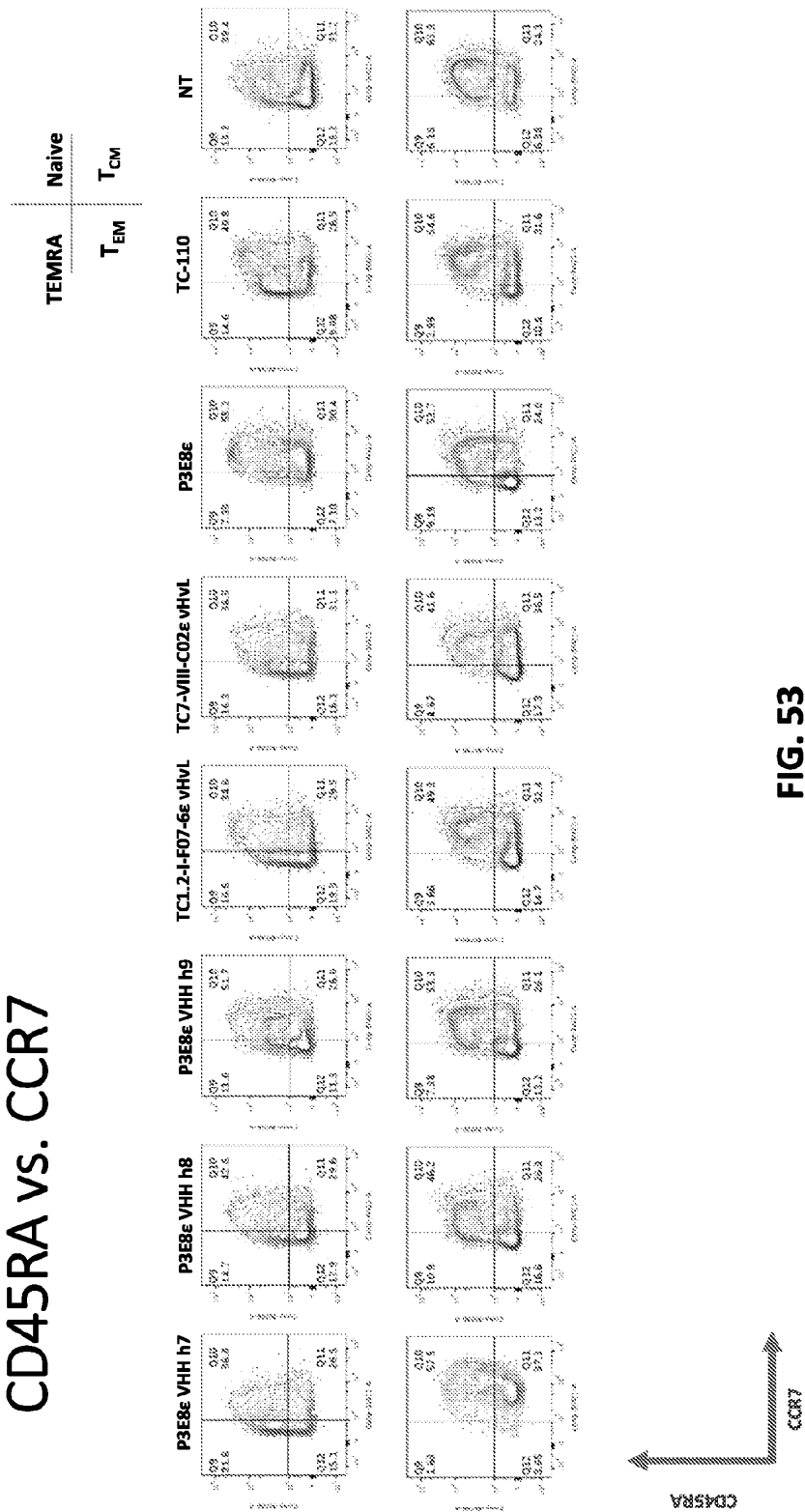


FIG. 52



TRuC vs. CD69

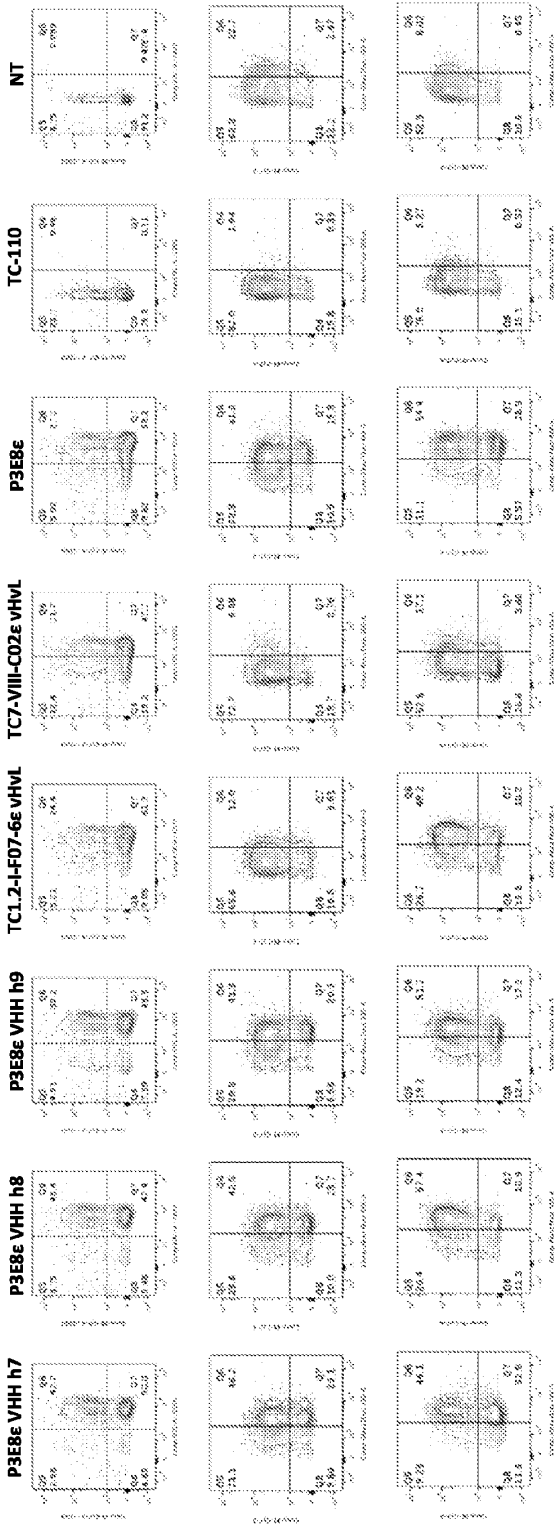


FIG. 54

Results: 24-hour Cytotoxicity

- ▲ 3-to-1
- 1-to-1
- 1-to-3

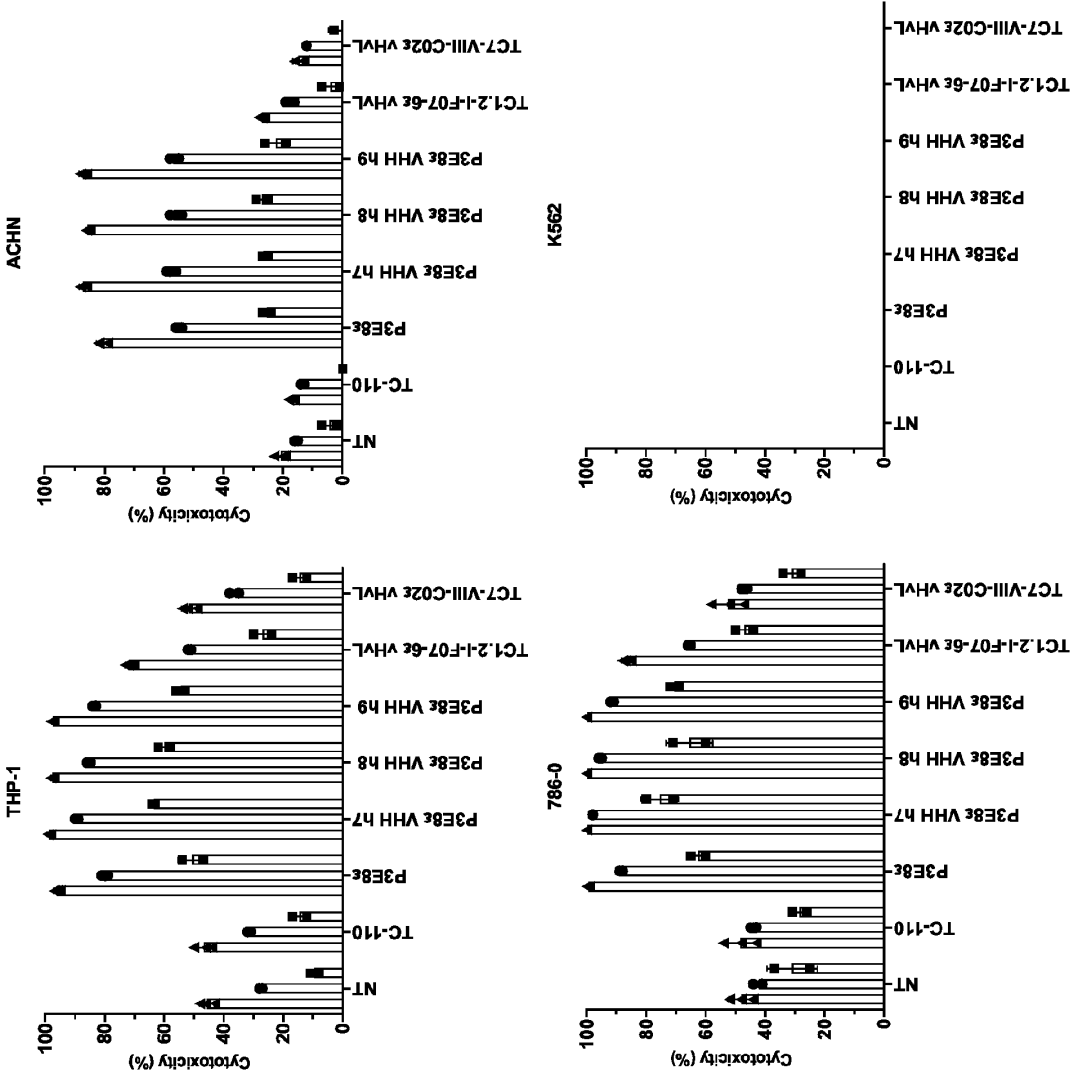


FIG. 55

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Results: Cytokine production

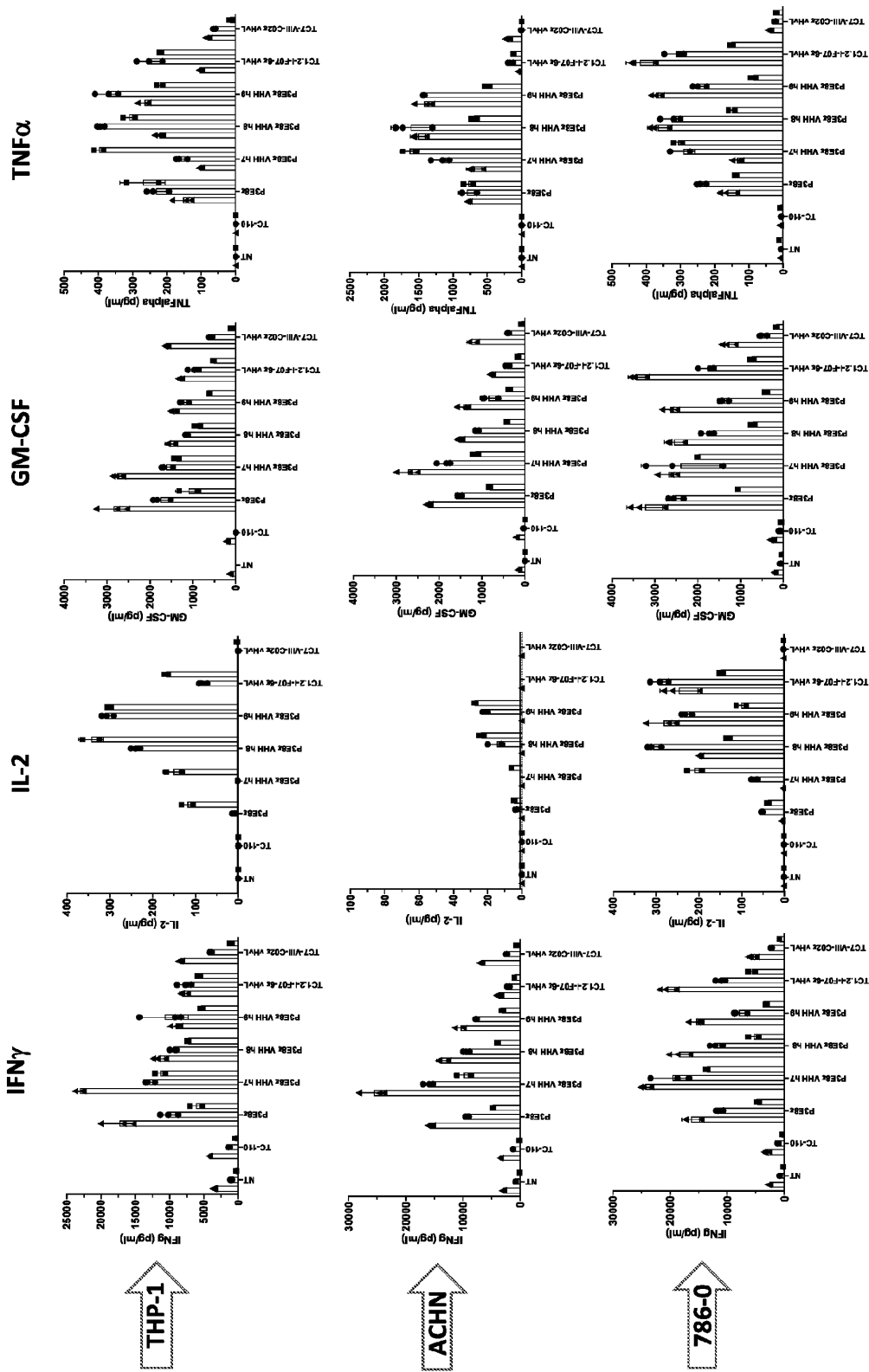


FIG. 56

Expansion of humanized 70-001 VHH TRuCs in Primary T Cells

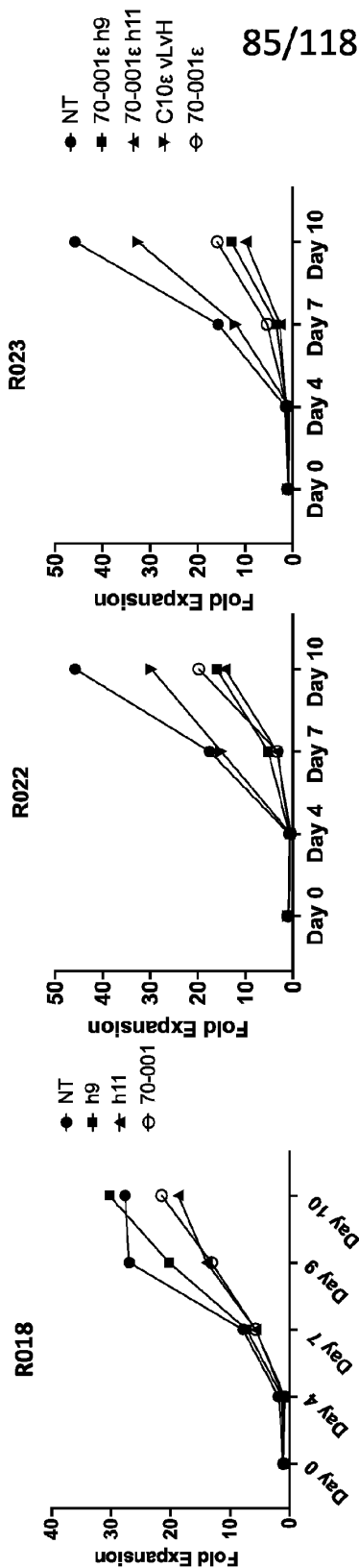


FIG. 57

Expression of 70-001 VHH TRuCs in Primary Cells

R018

anti-VHH

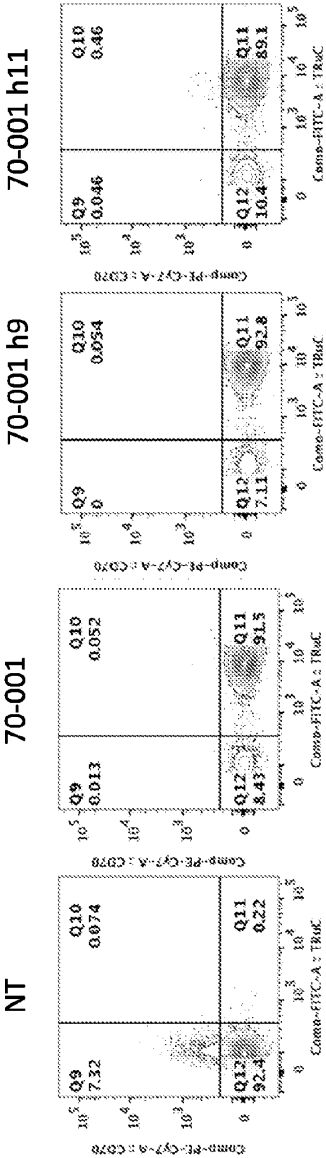


FIG. 58

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CD4/CD8

R018

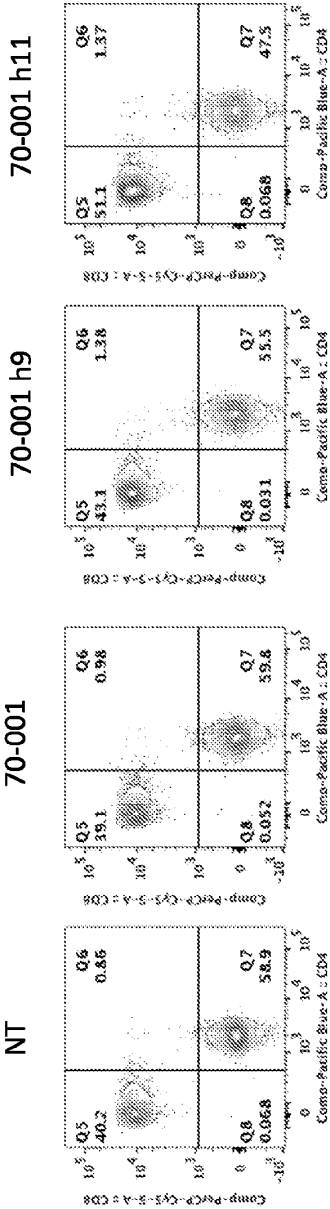


FIG. 59

Memory Phenotype CD3+

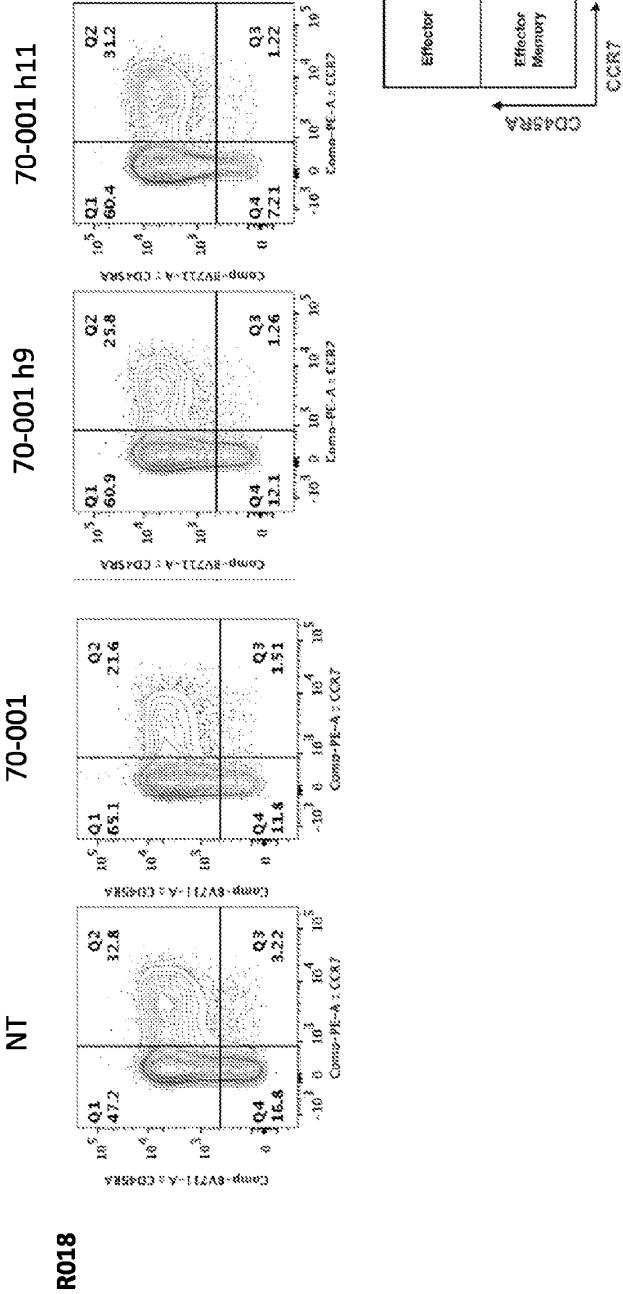
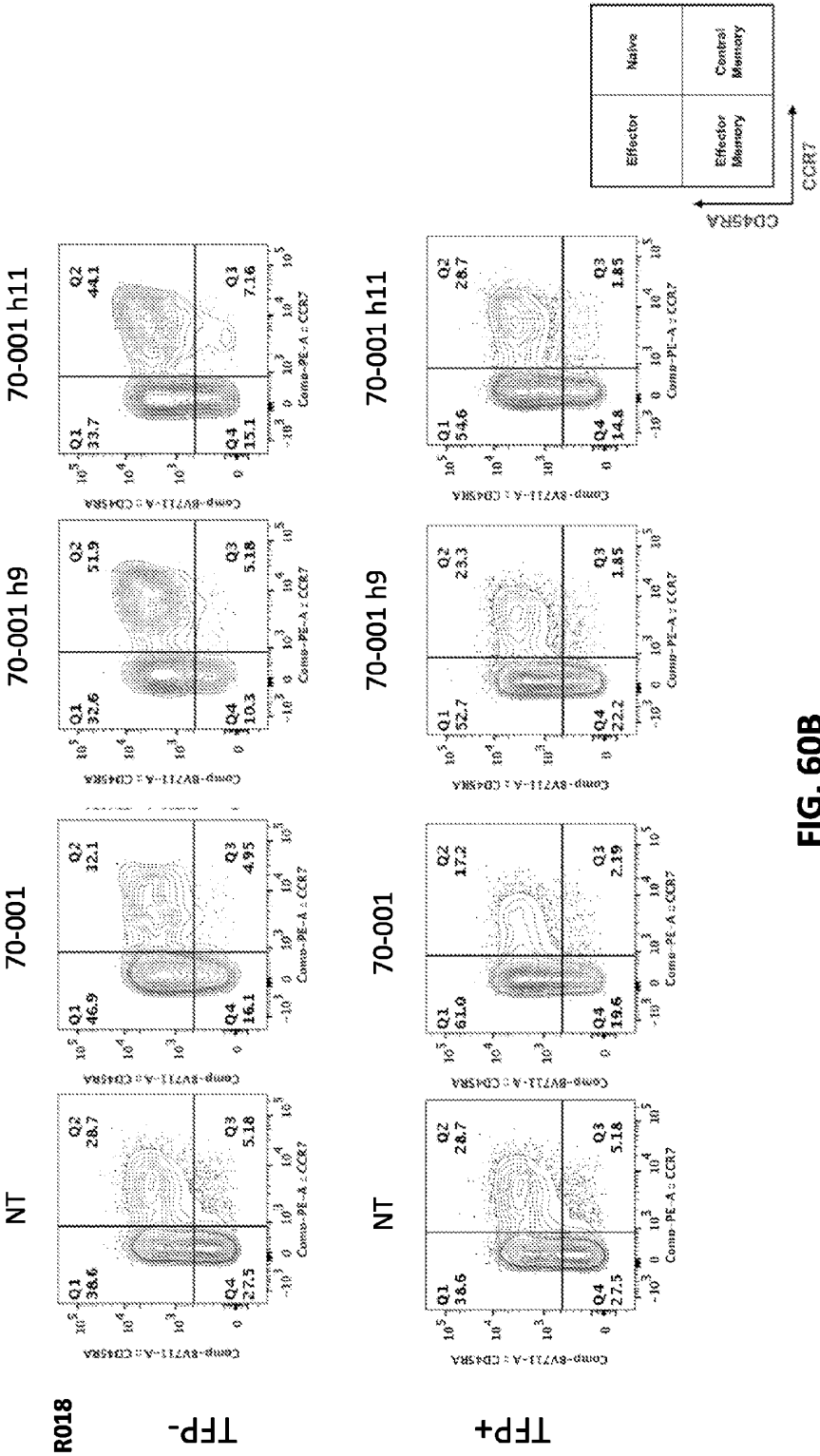


FIG. 60A

Memory Phenotype CD4+



Memory Phenotype CD8+

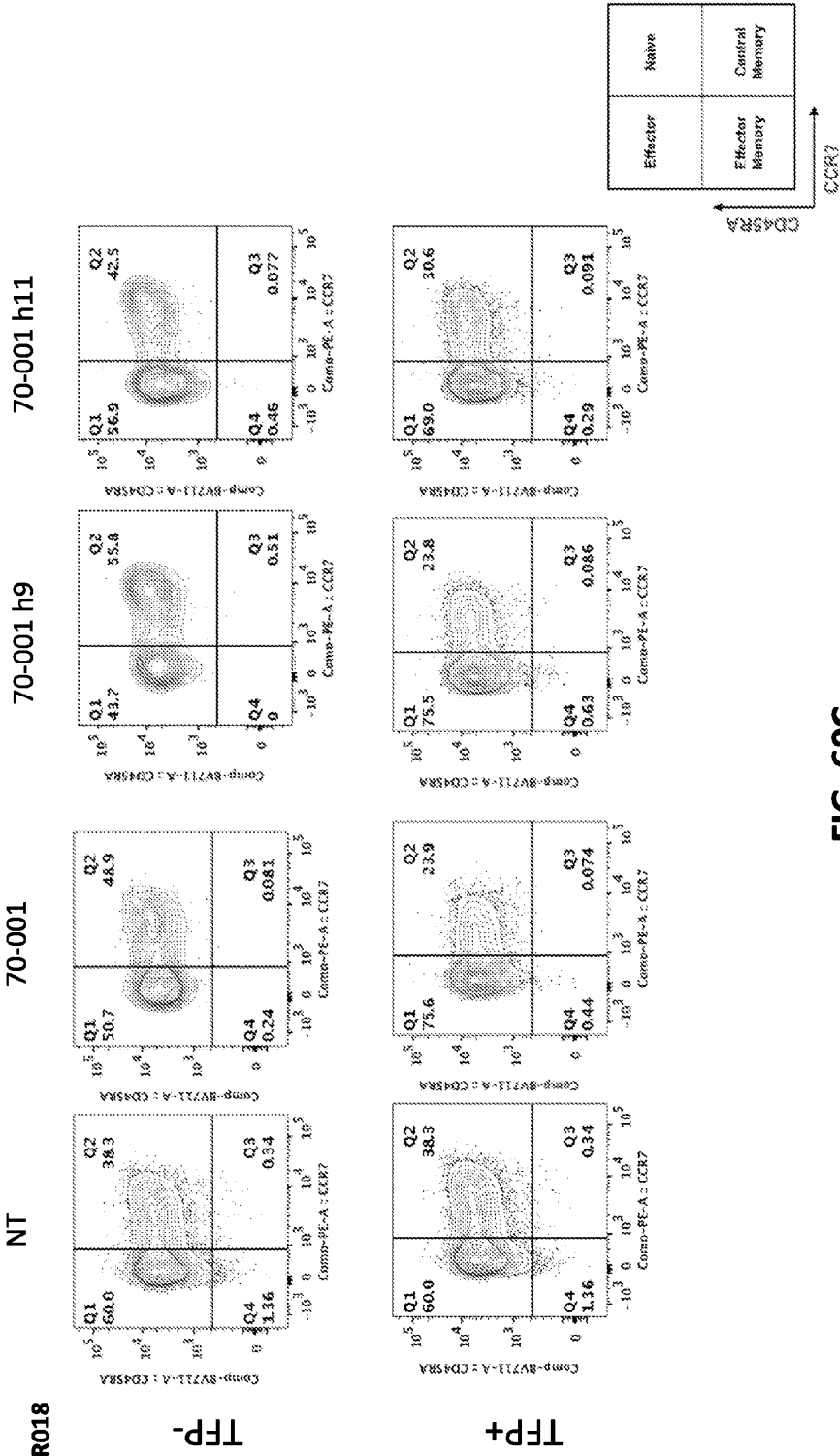


FIG. 60C

Robust killing is seen in all CD70+ cell lines

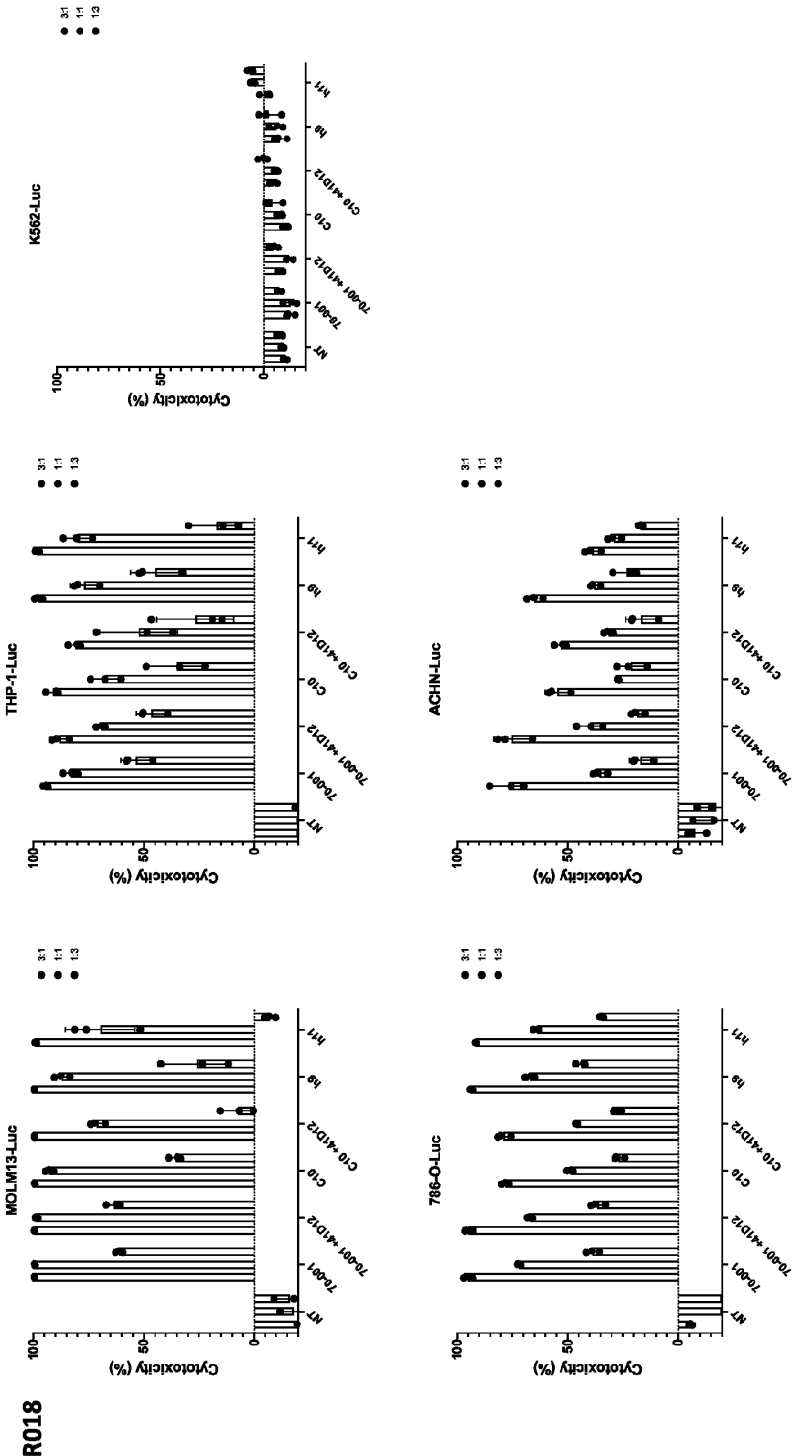


FIG. 61

24-hours Cytokine Production

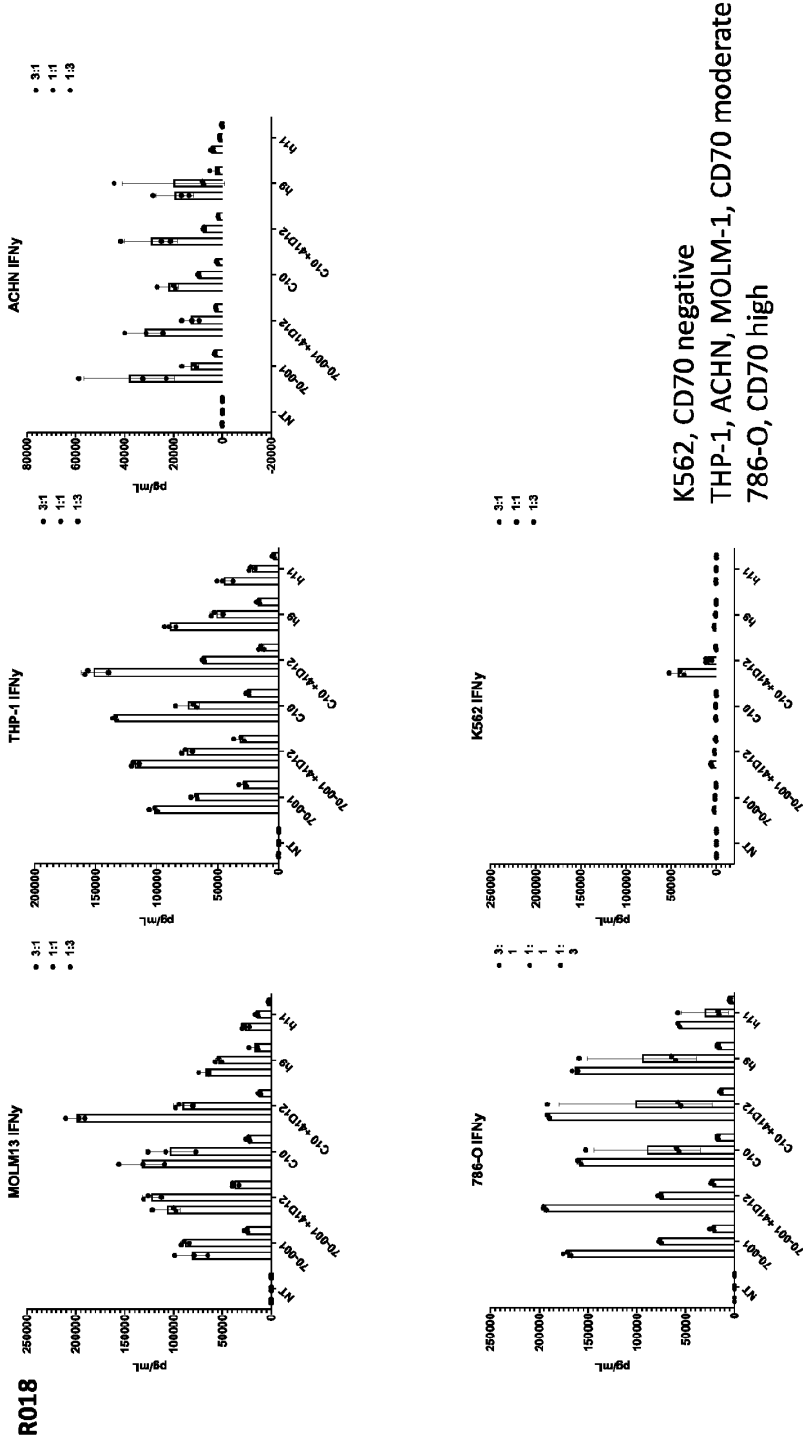


FIG. 62A

24-hours Cytokine Production

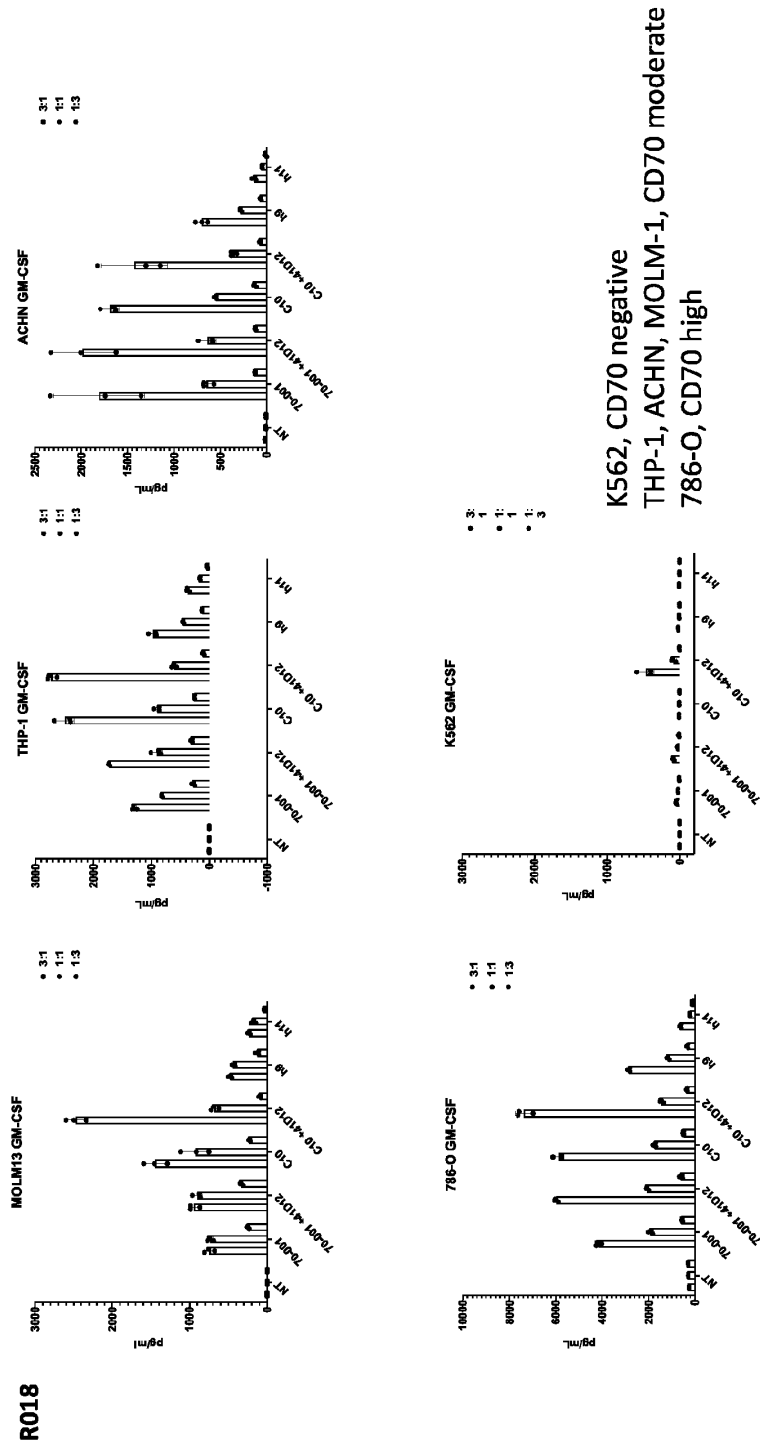


FIG. 62B

24-hours Cytokine Production

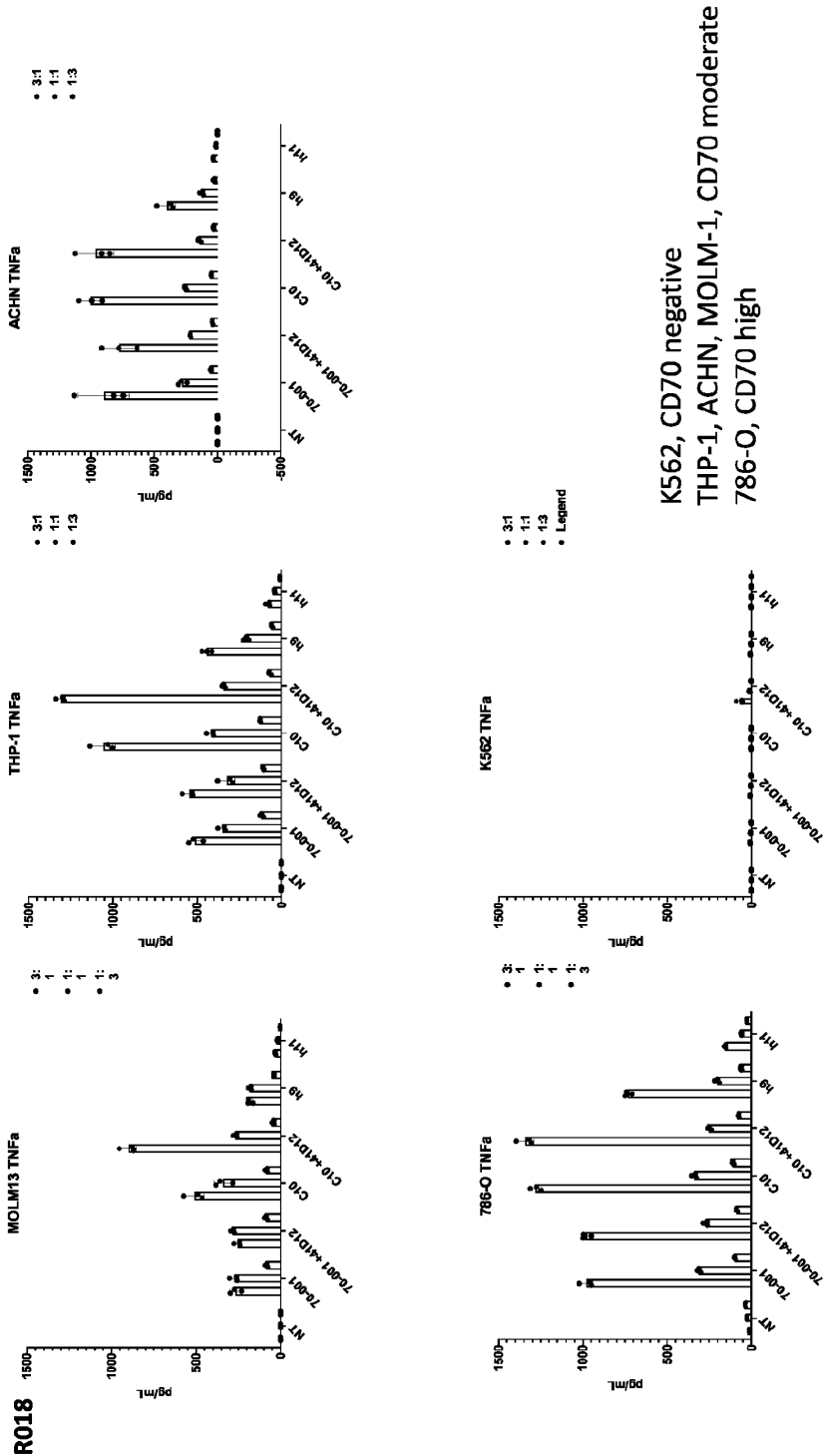


FIG. 62D

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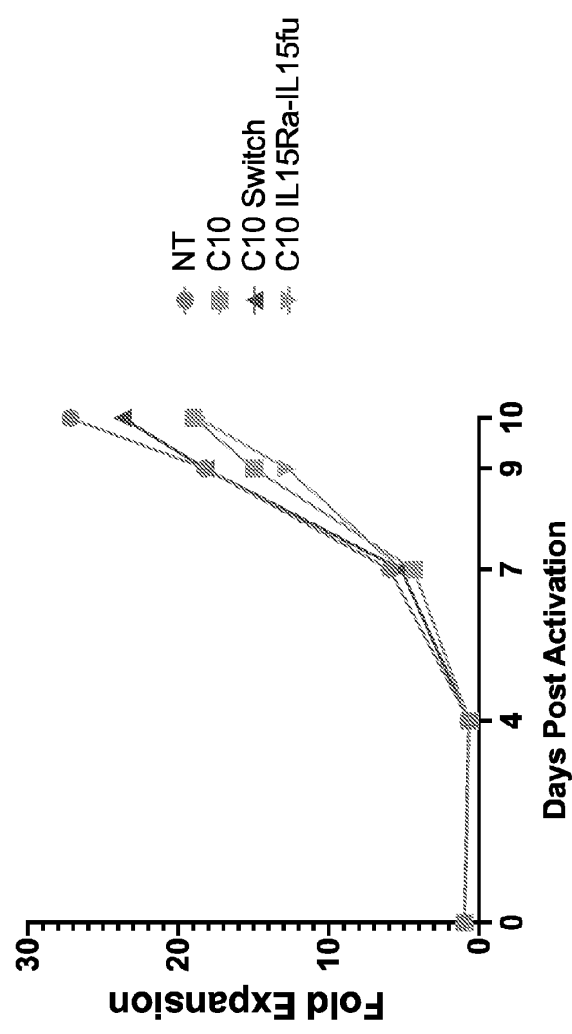


FIG. 63

Transduction

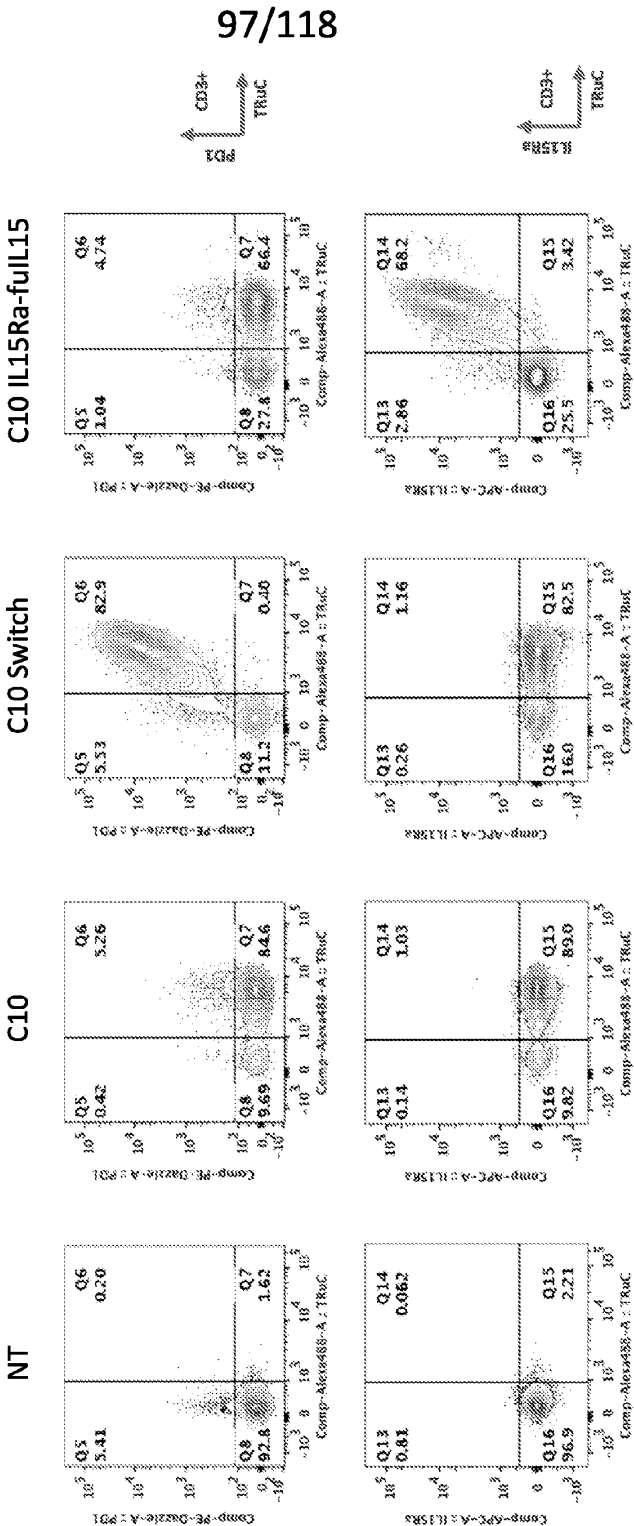


FIG. 64

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CD4+ T cells

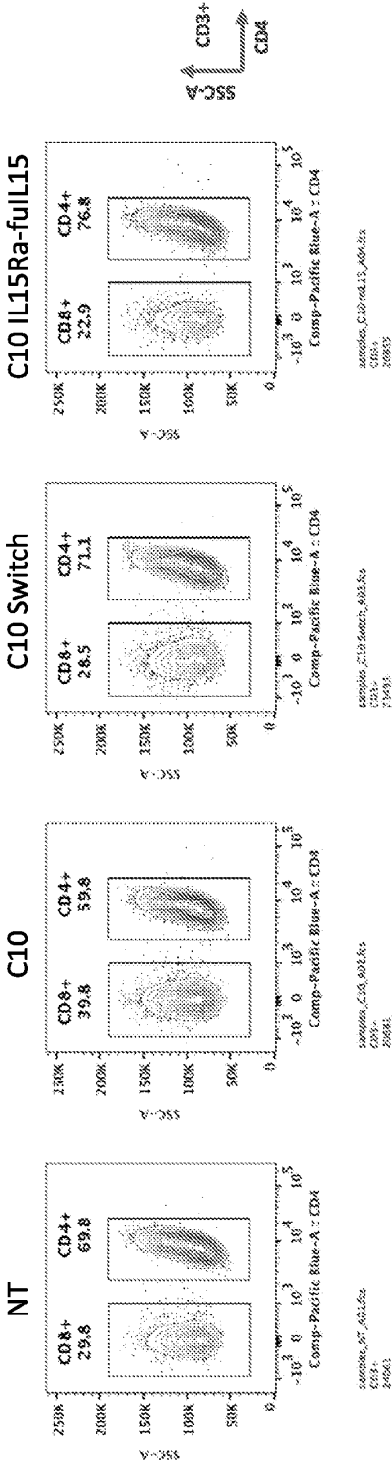


FIG. 65

Memory Phenotype

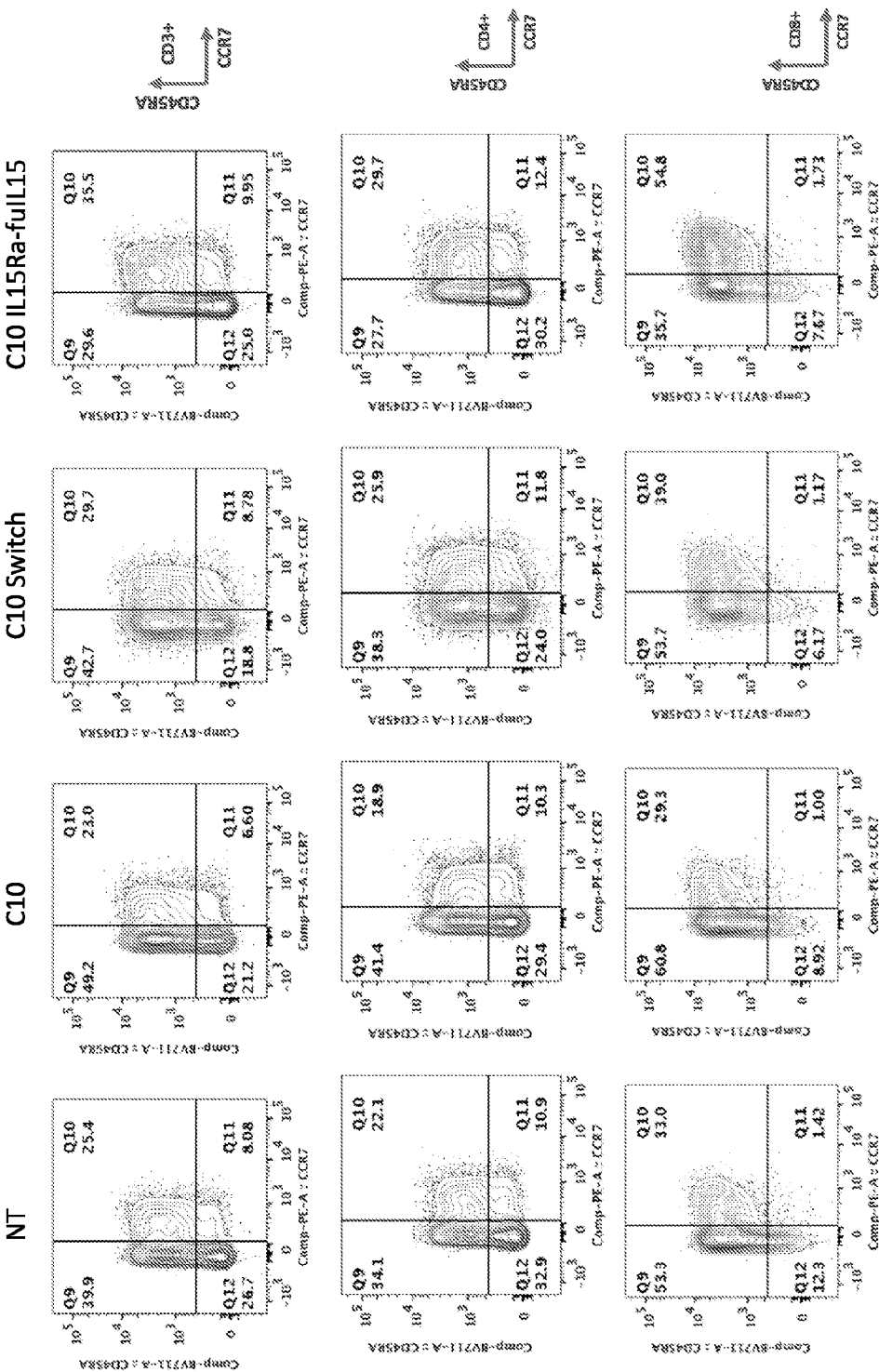
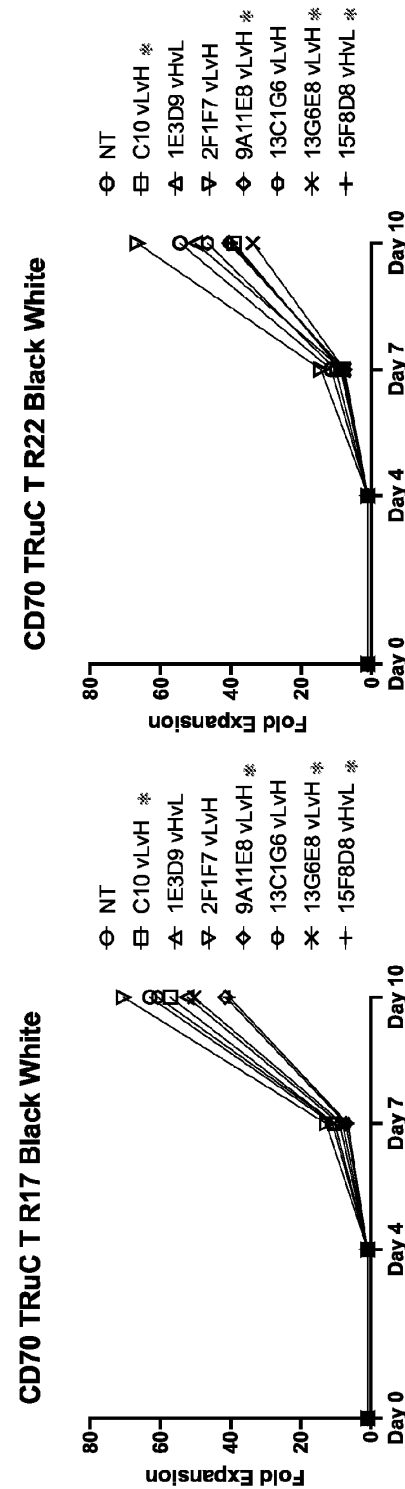


FIG. 66

CD70 TRuC T cells expansion



* TRuC T cells showed fratricide and cytotoxicity

FIG. 67

CD70 TRuC expression (Donor R17)

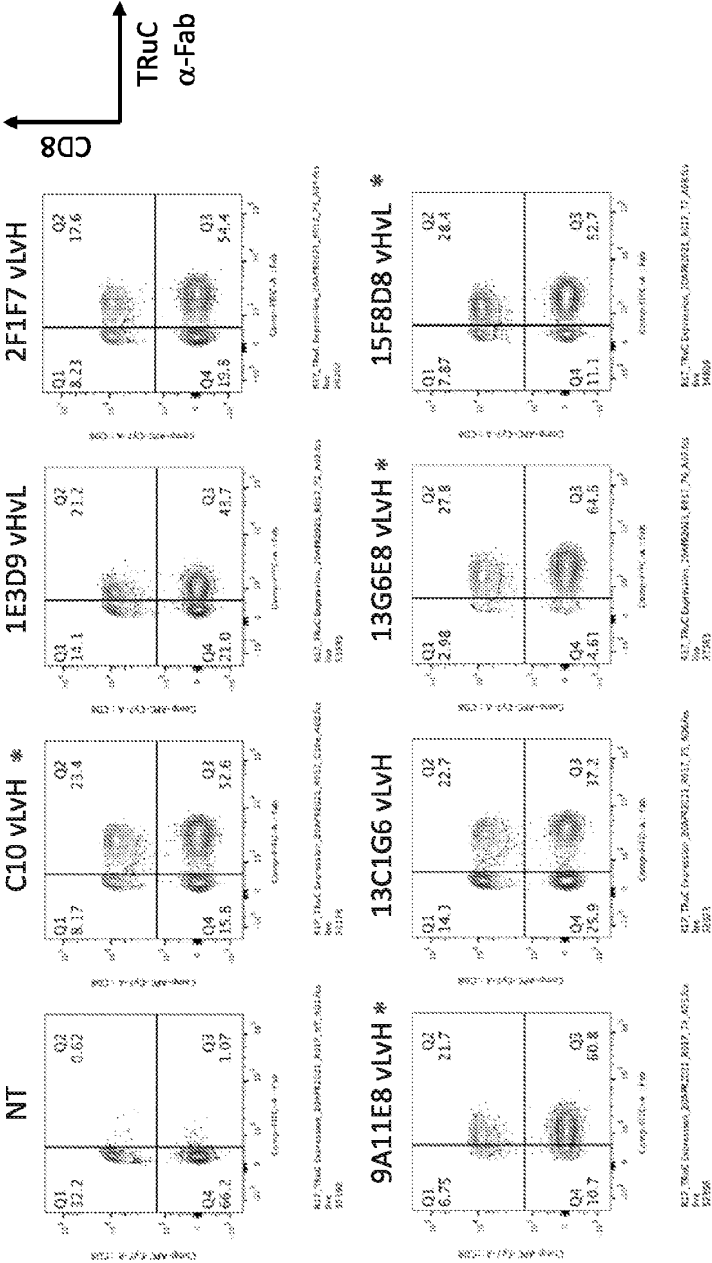


FIG. 68A

CD70 TRuC expression (Donor R22)

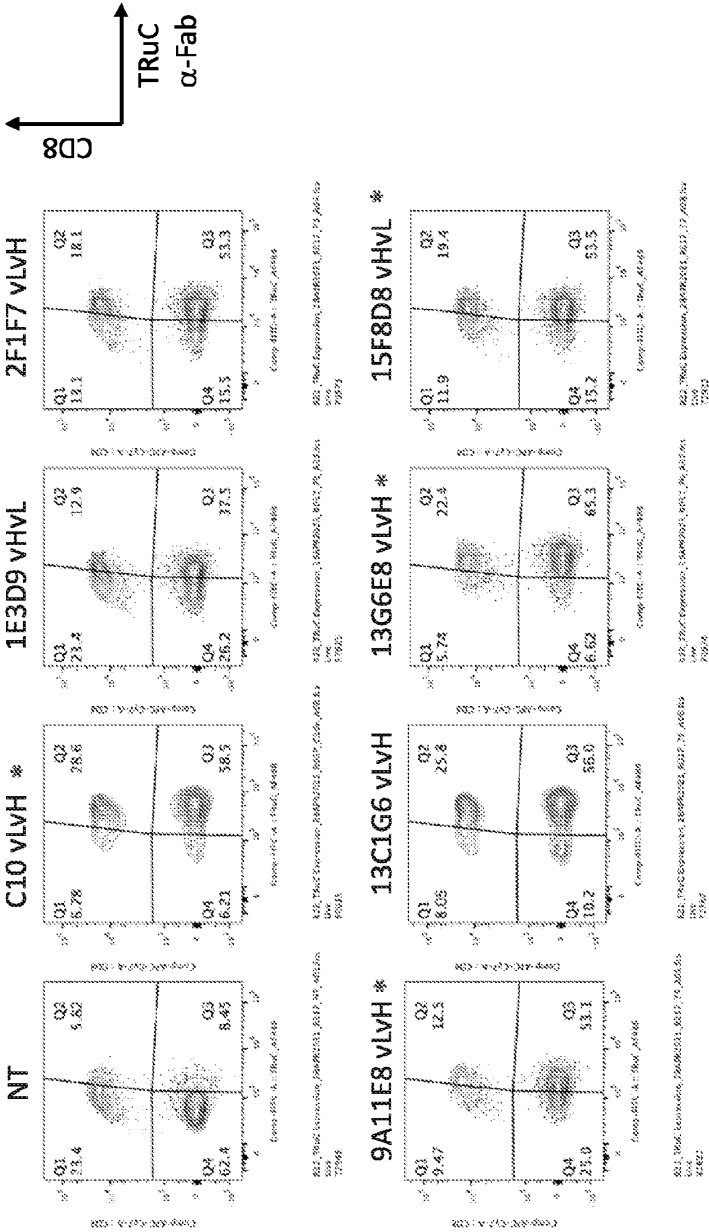


FIG. 68B

CD70 expression (R17)

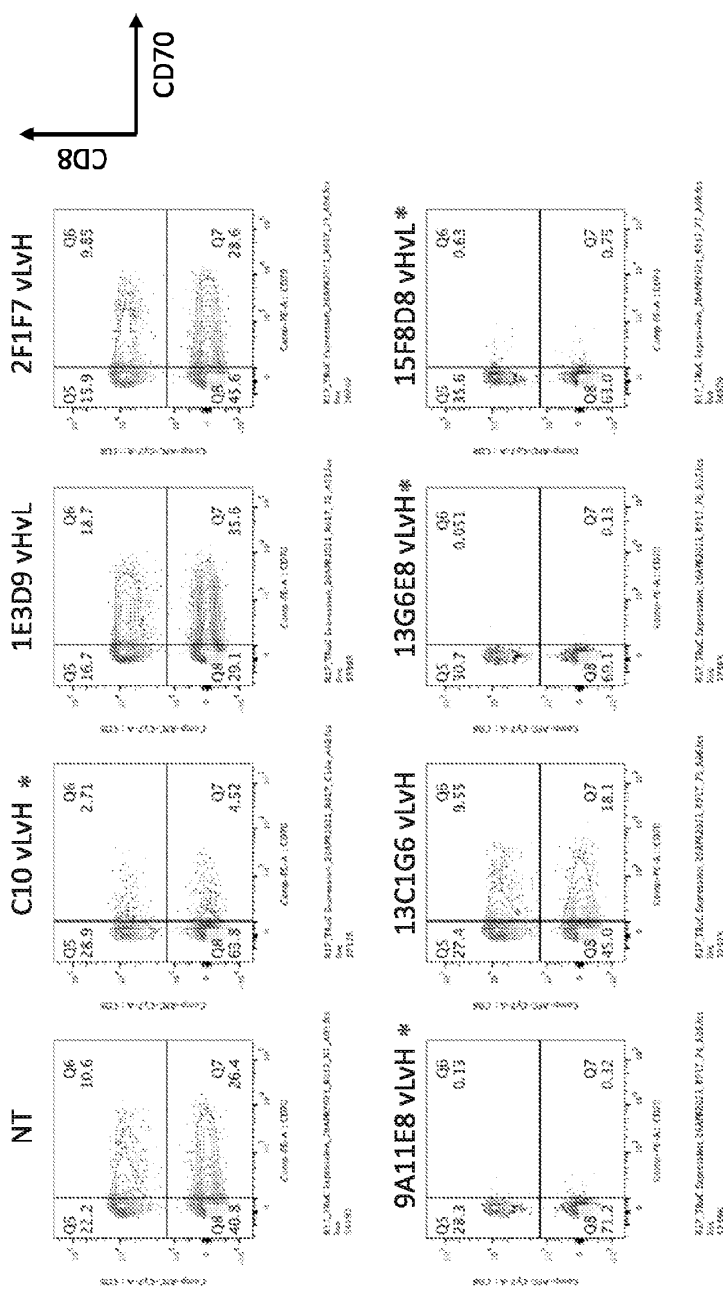


FIG. 69A

CD70 expression (R22)

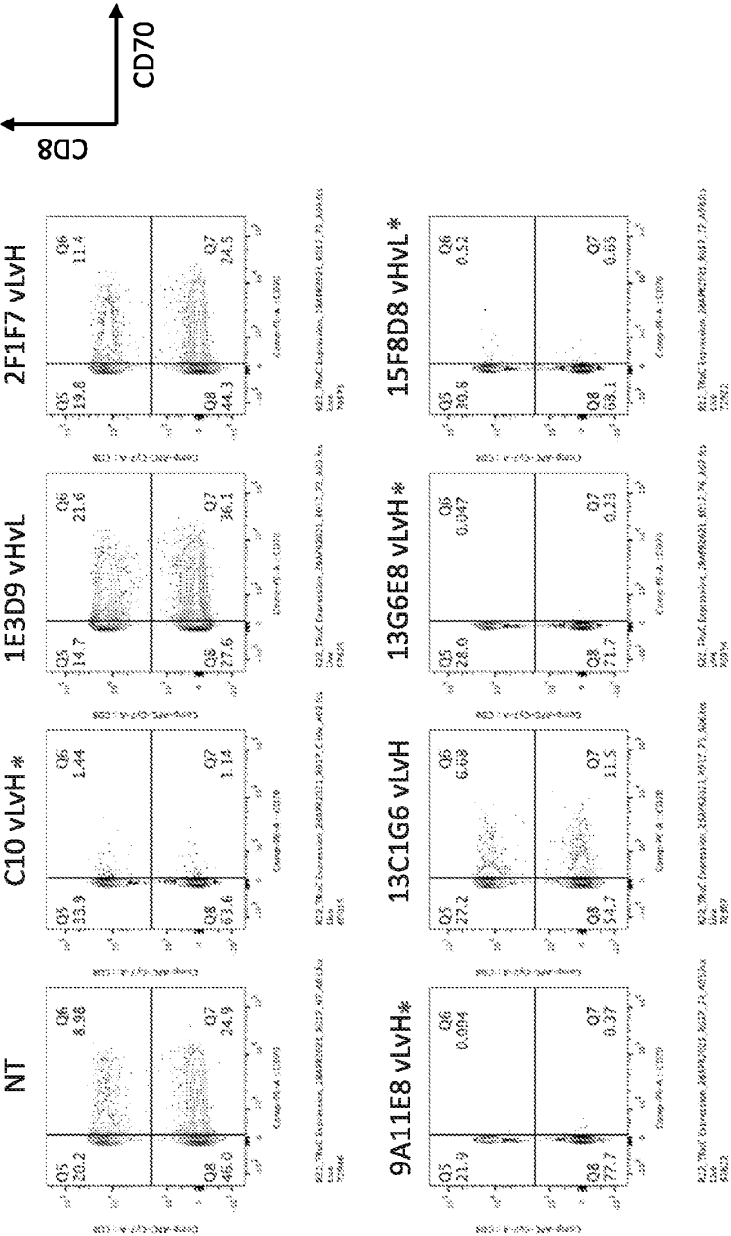


FIG. 69B

CD8 memory/effector phenotyping (R17)

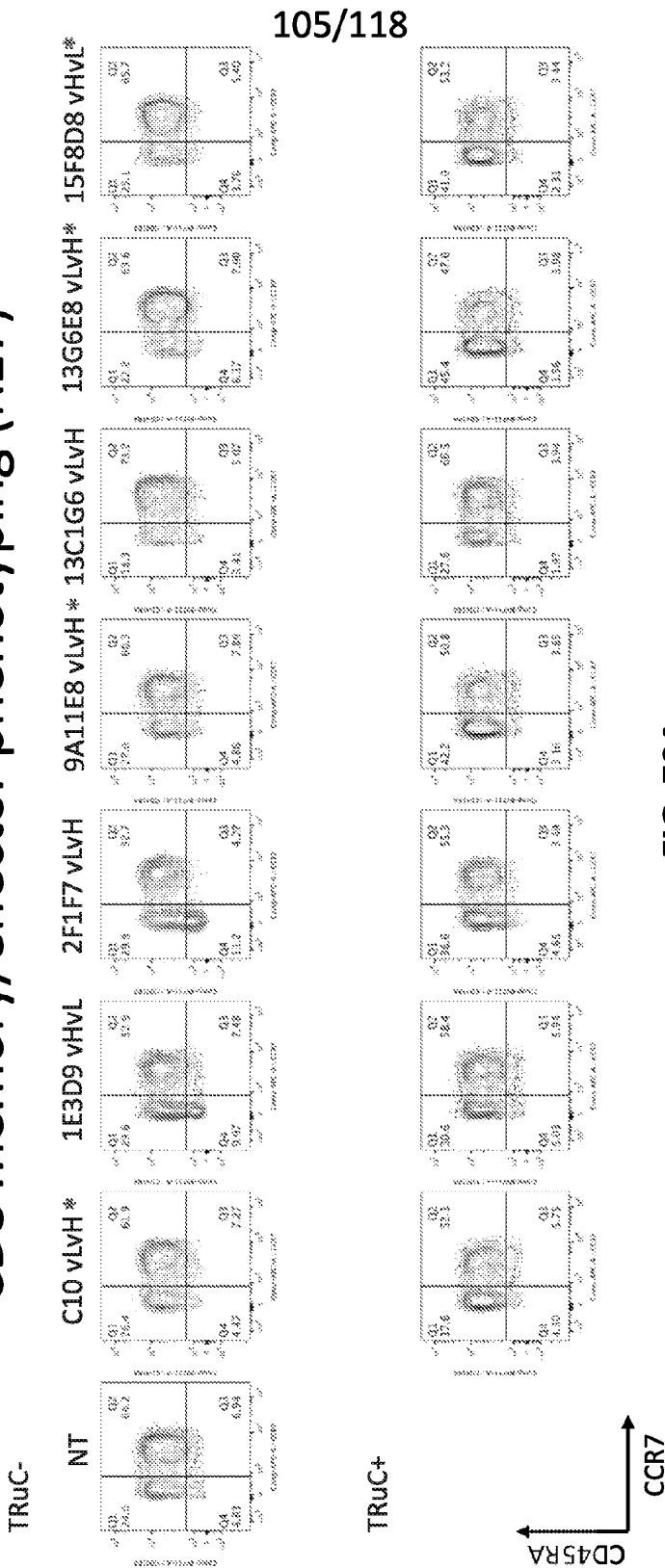
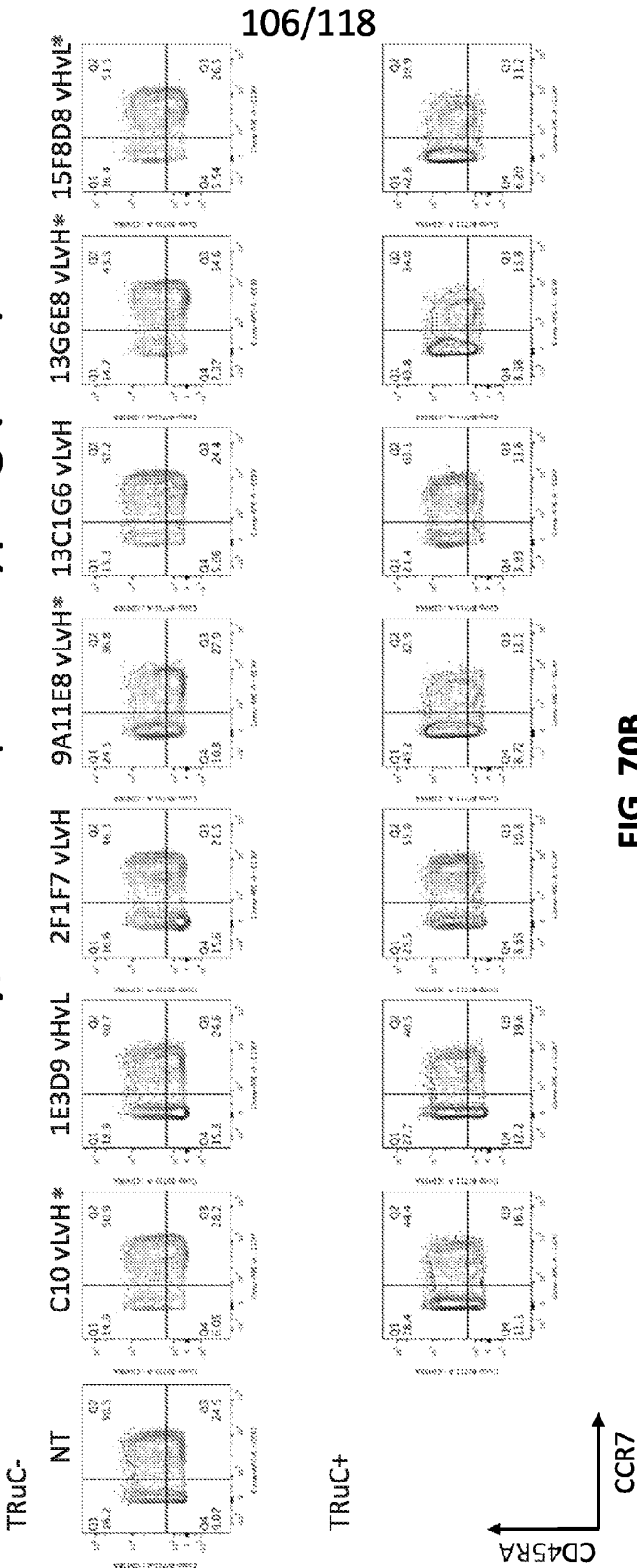


FIG. 70A

CD4 memory/effector phenotyping (R17)



CD8 memory/effector phenotyping (R22)

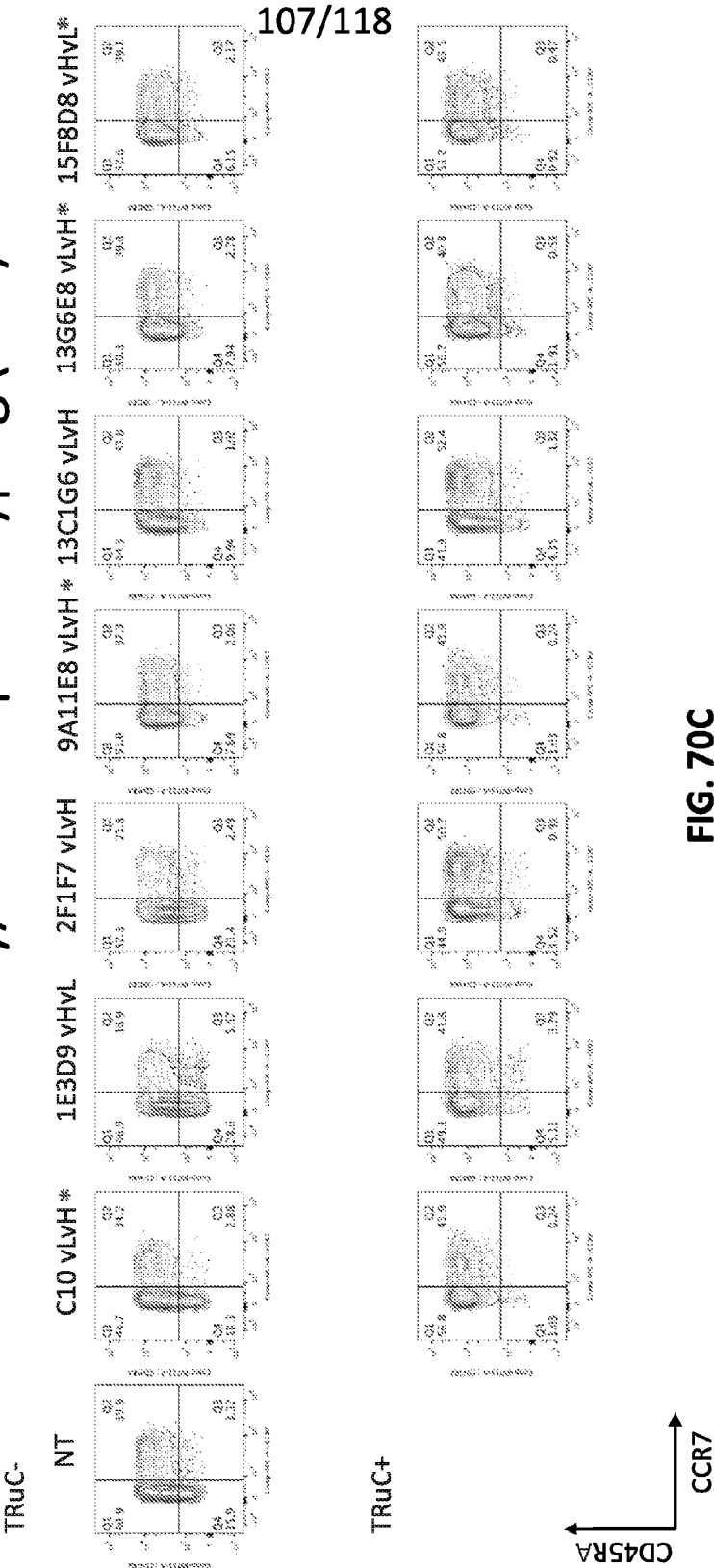
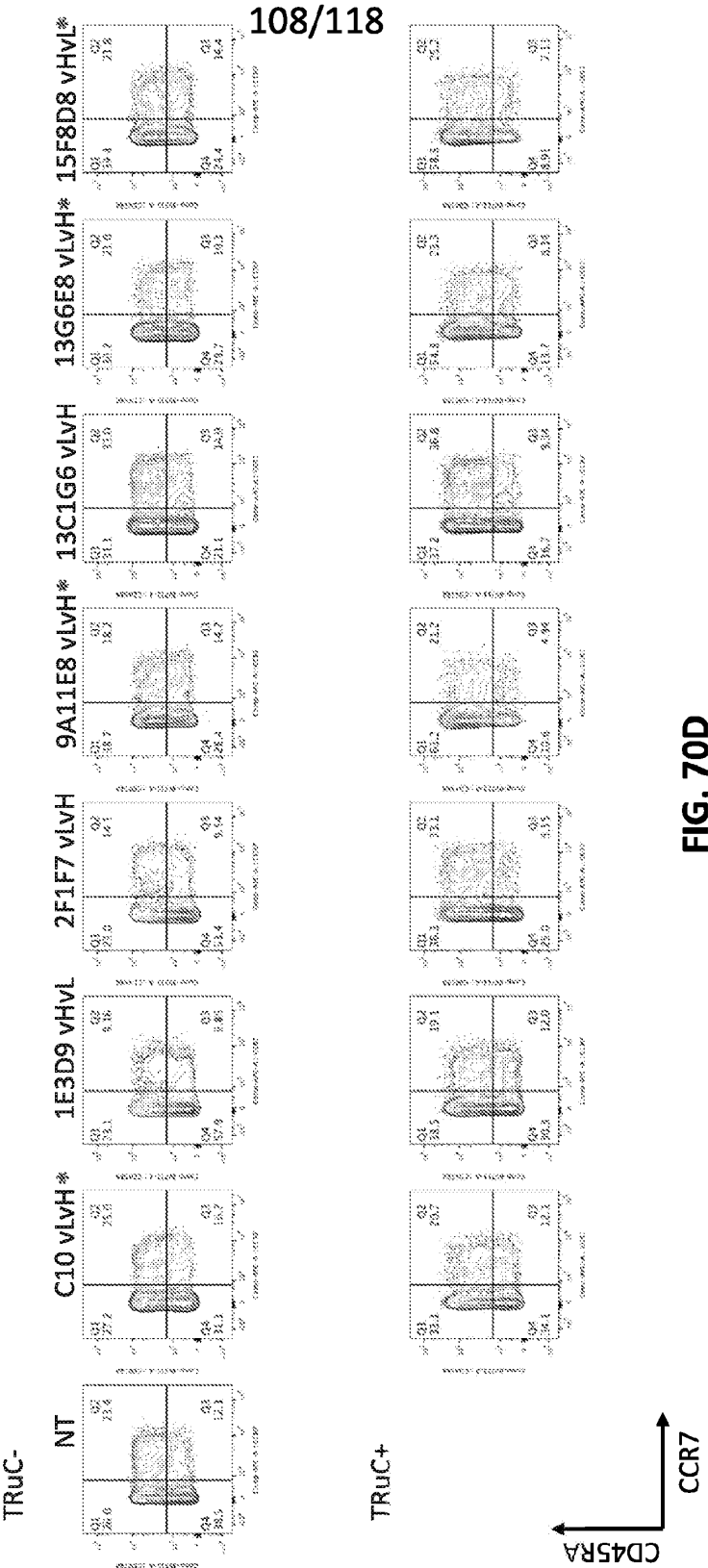


FIG. 70C

CD4 memory/effector phenotyping (R22)



CD70 TRuC T cell cytotoxicity (R17)

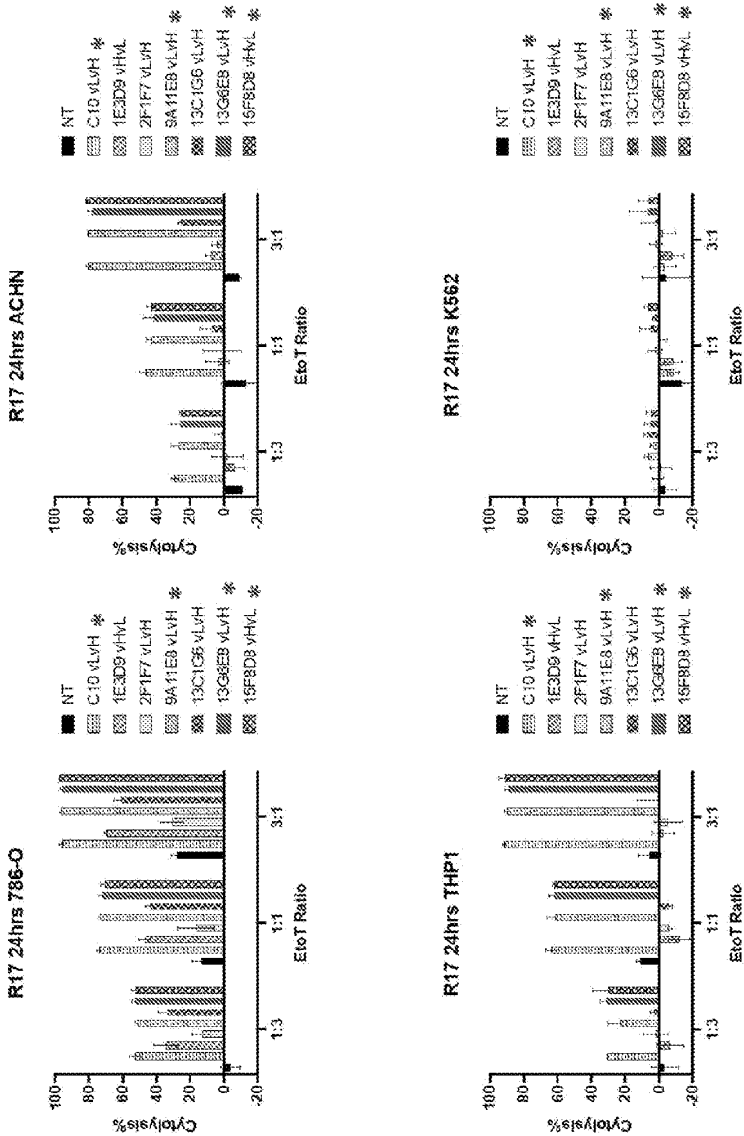


FIG. 71A

CD70 TRuCT cell cytotoxicity (R22)

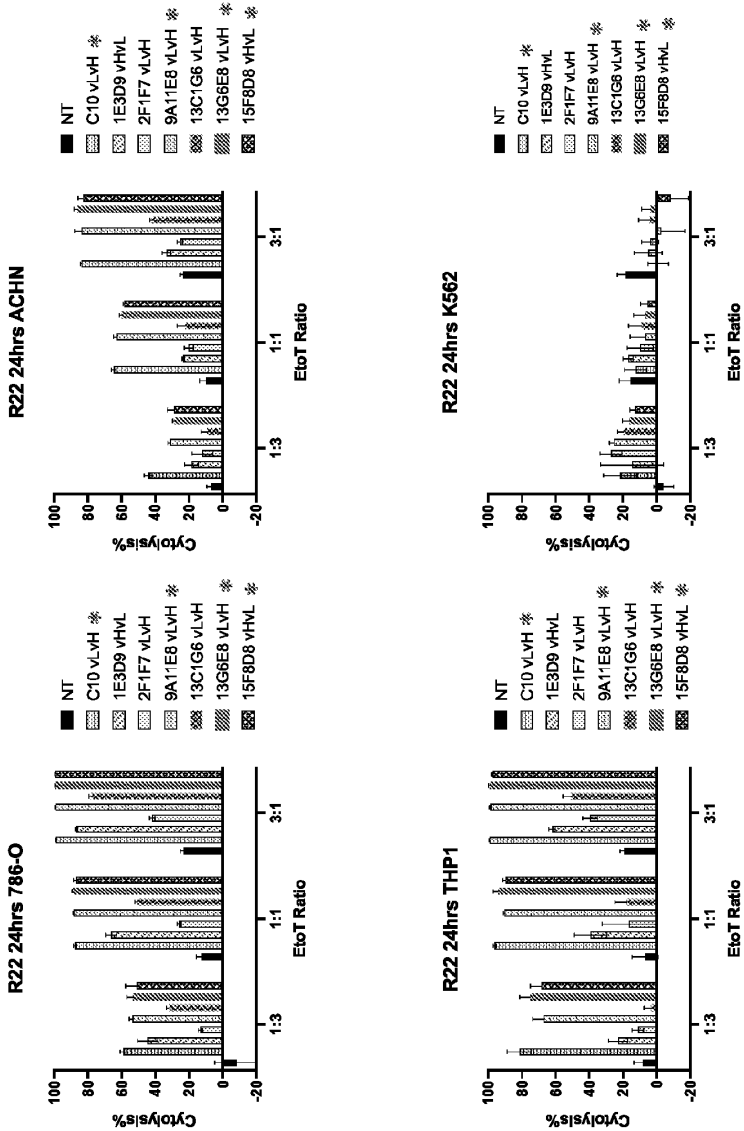


FIG. 71B

CD70 TRuCs Result in 786-0 Tumor Regression

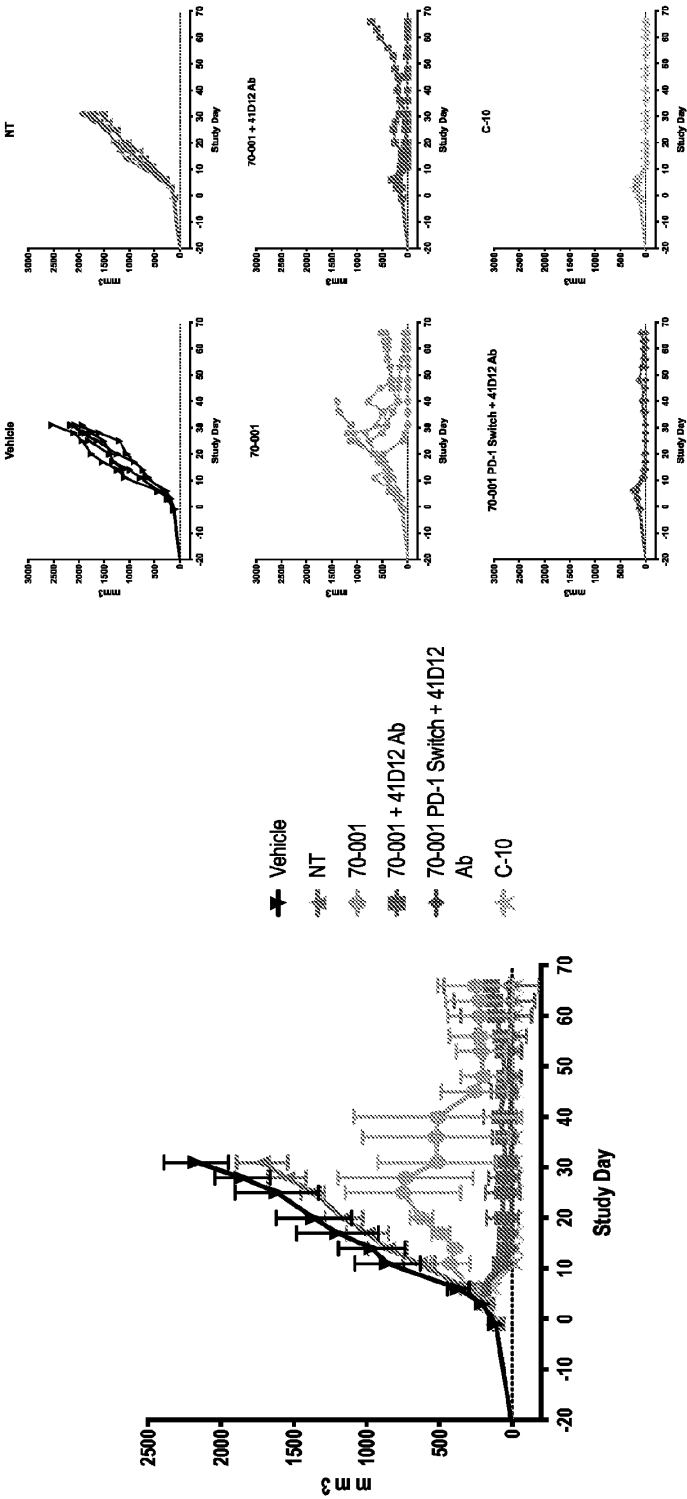


FIG. 72A

Tumor-free Mice Rechallenged with SC 786-0

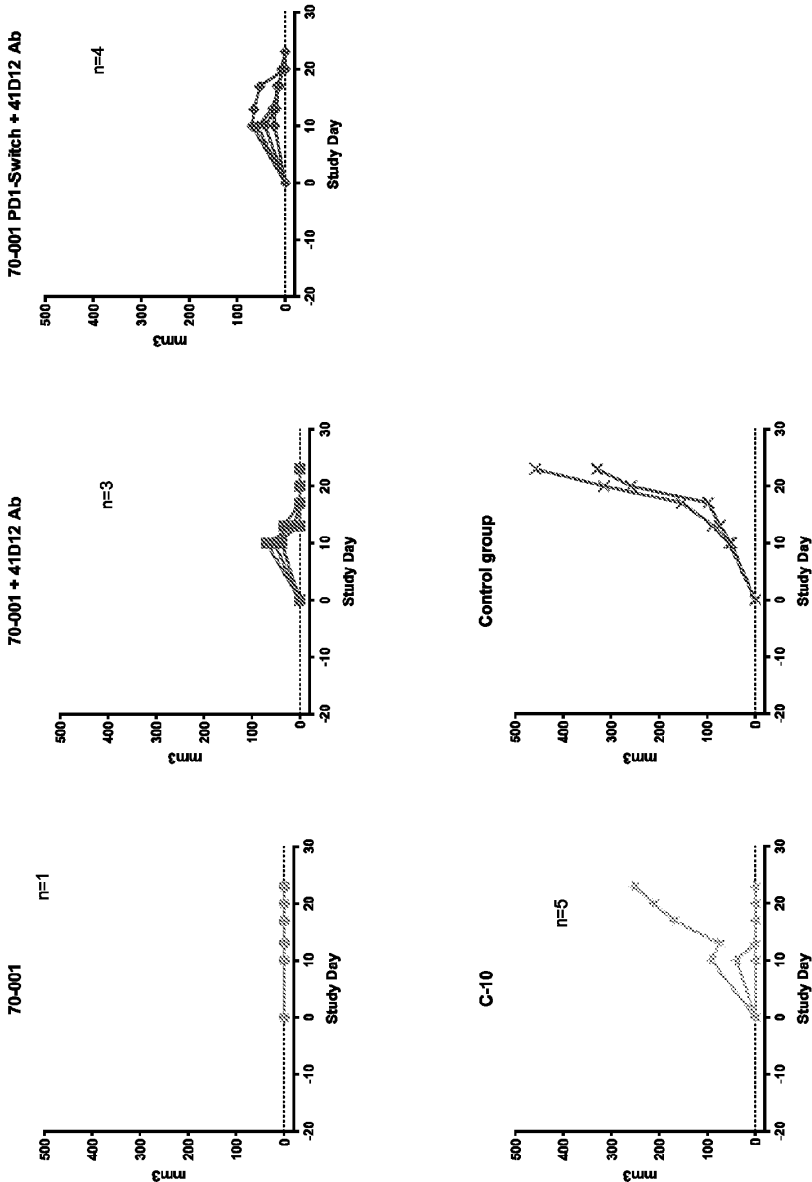


FIG. 72B

Raji Tumor Efficacy

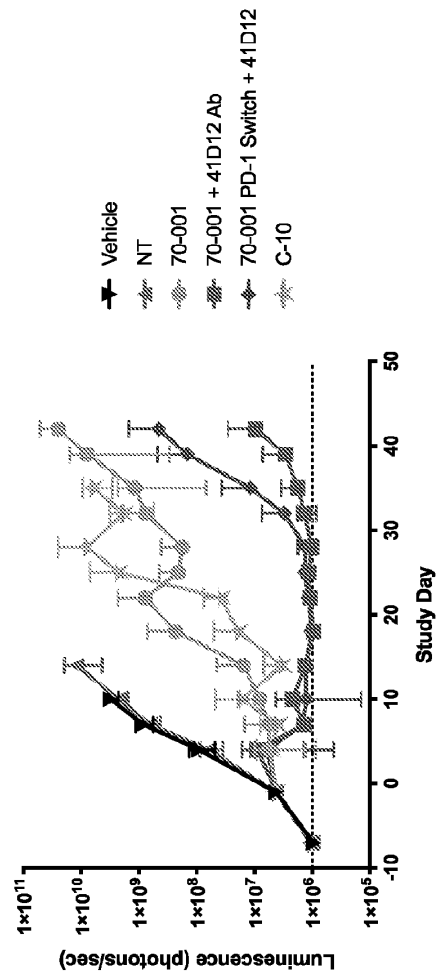


FIG. 73A

Raji Tumor Efficacy

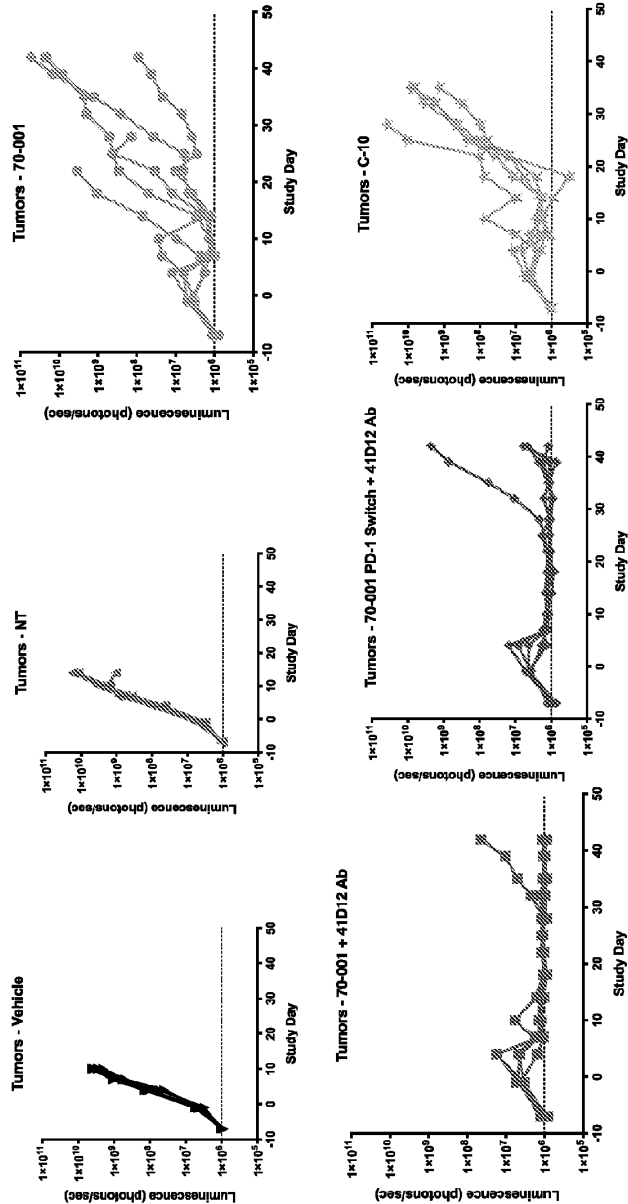


FIG. 73B

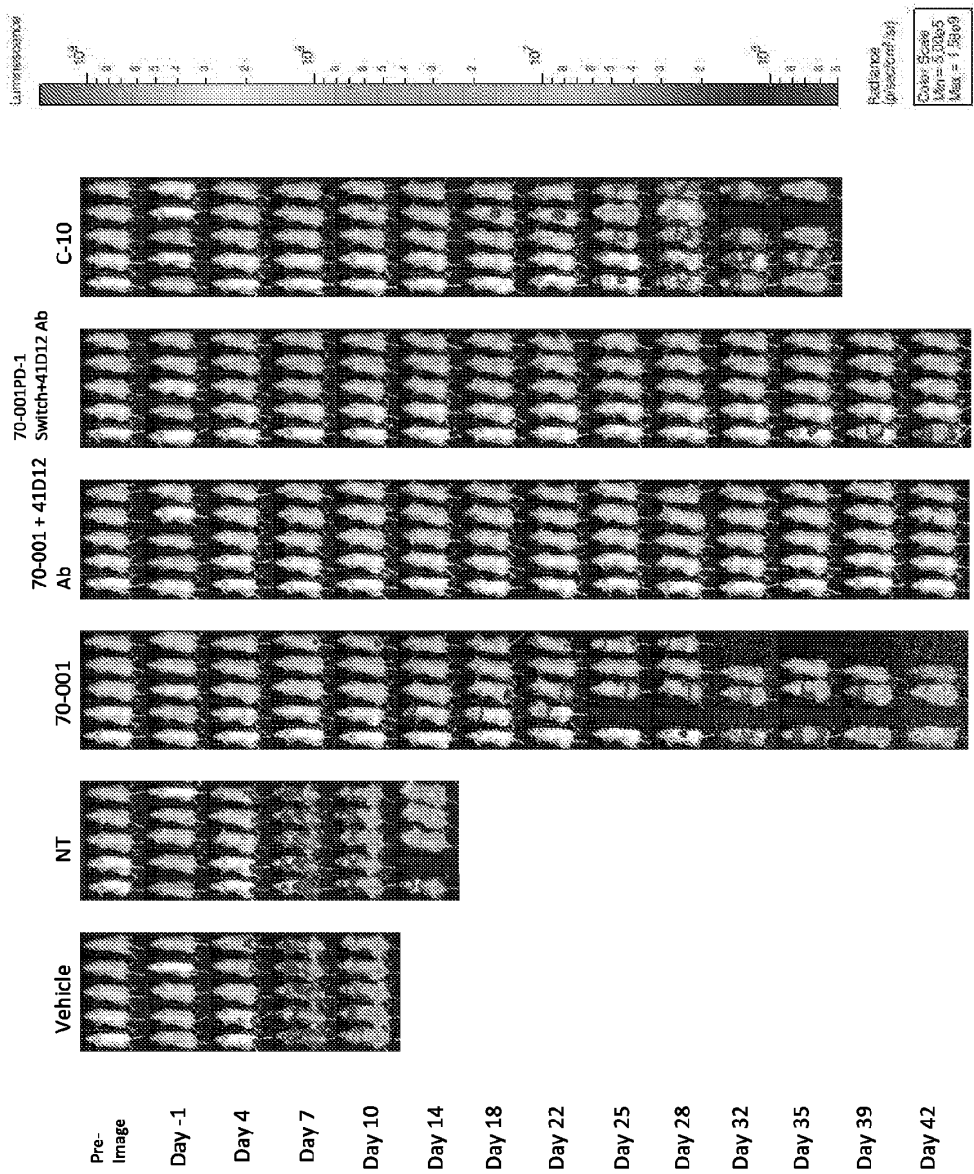


FIG. 73C

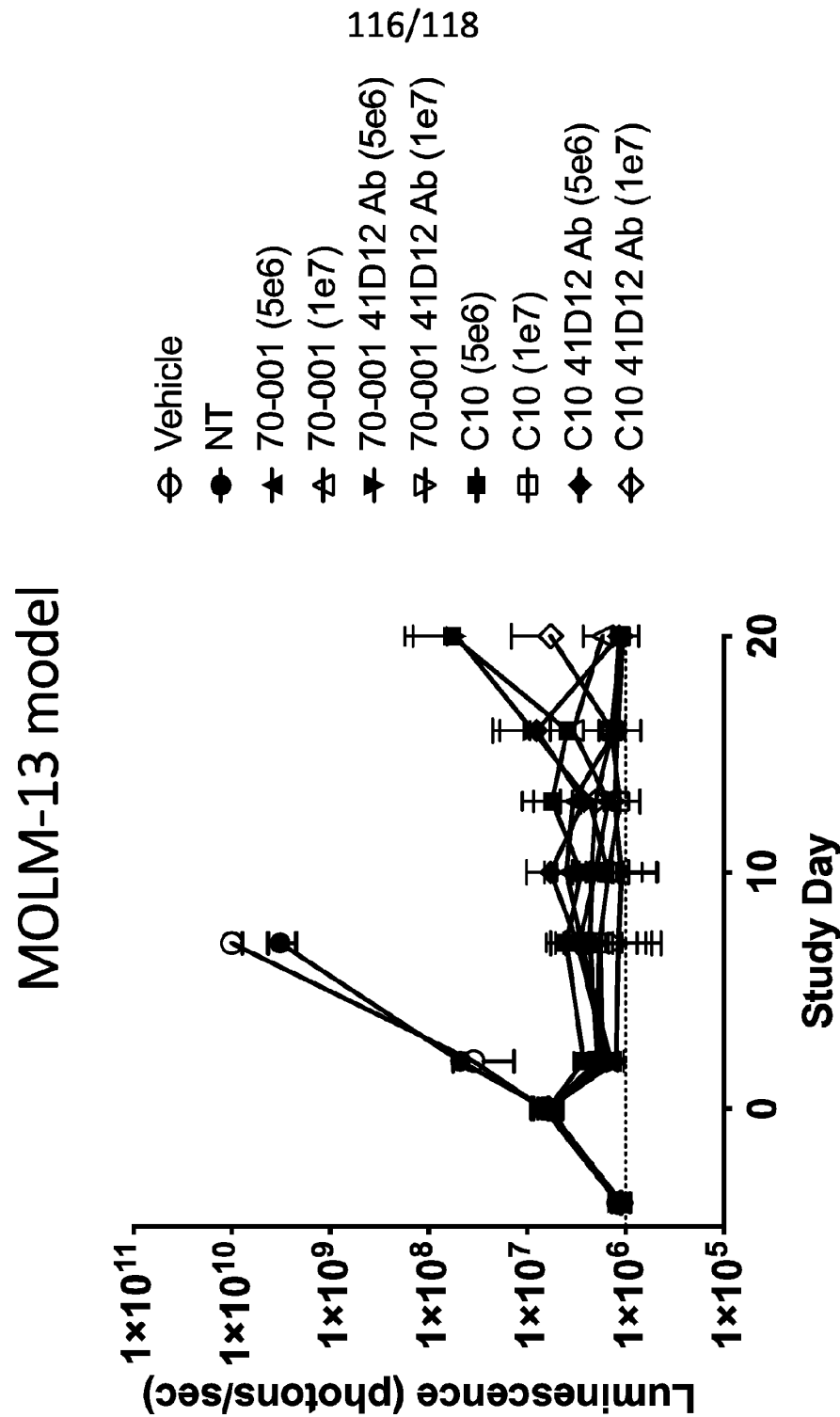


FIG. 74A

1e7 TFP T cells

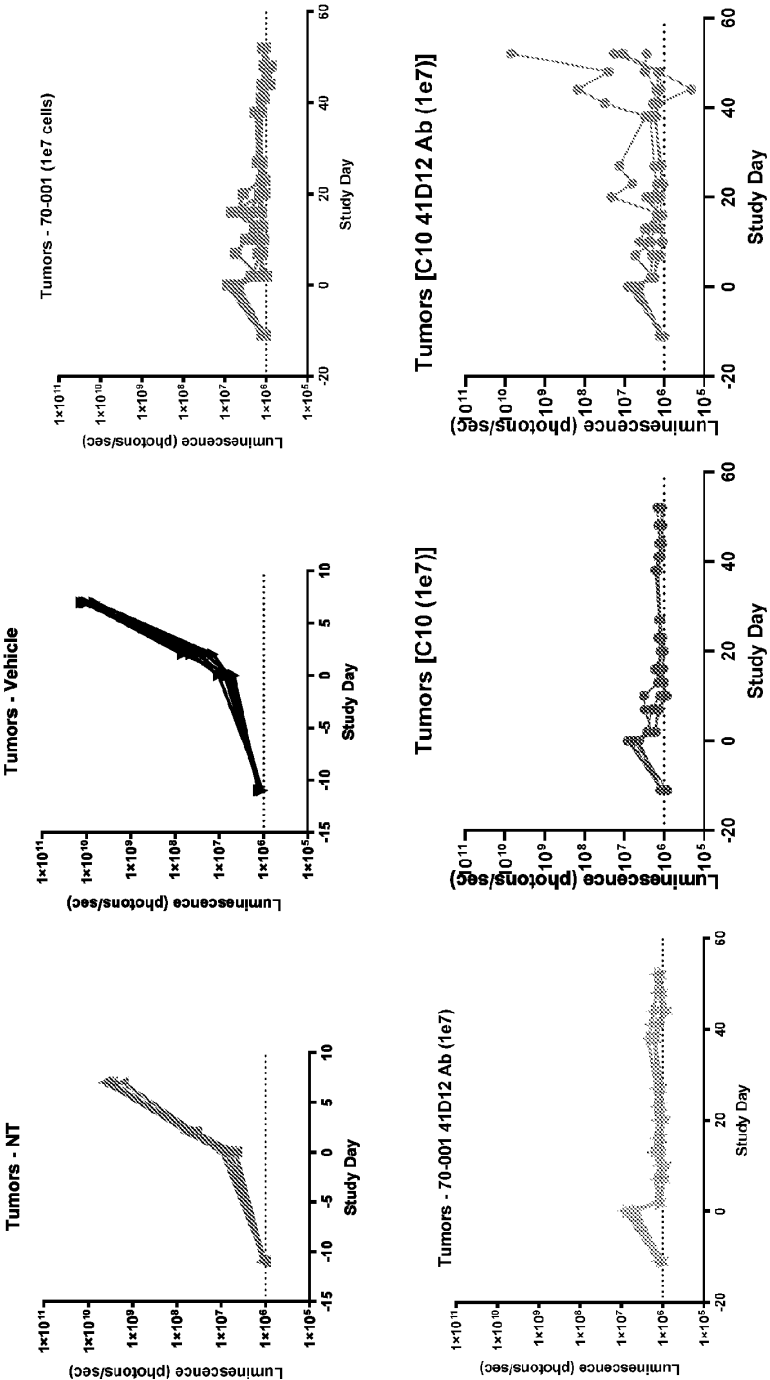


FIG. 74B

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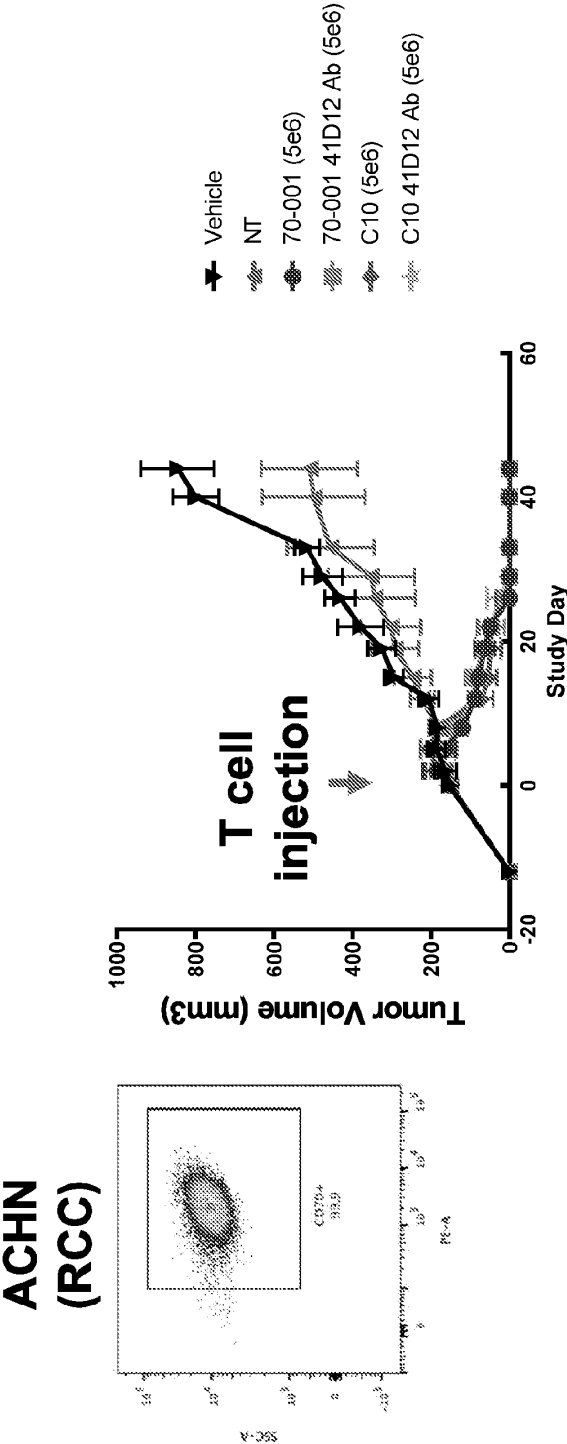


FIG. 75