

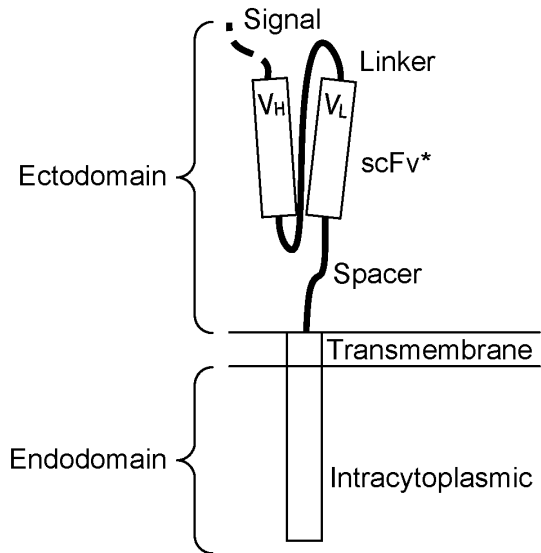


FIG. 1

* scFV can be V_H-V_L or V_L-V_H

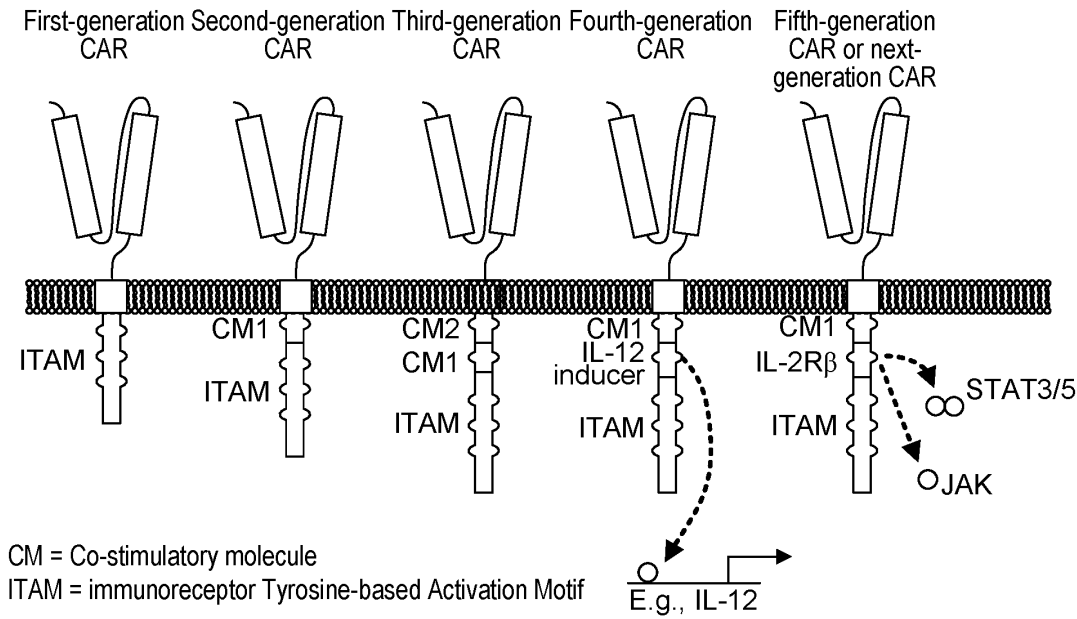


FIG. 2

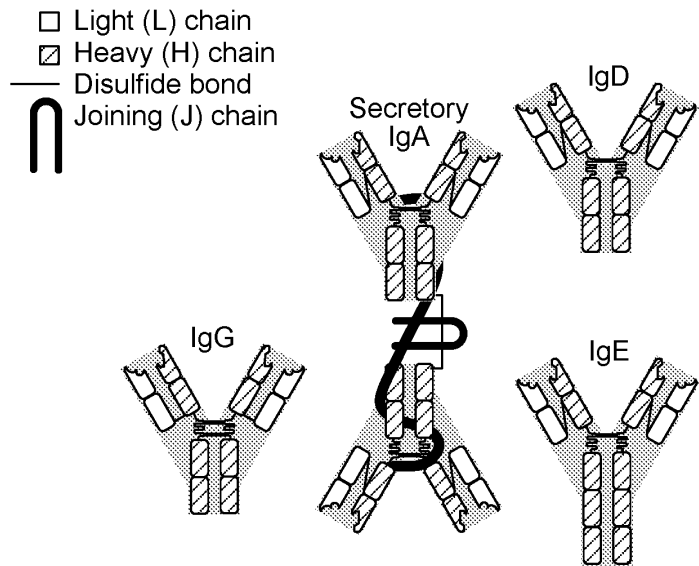


FIG. 3

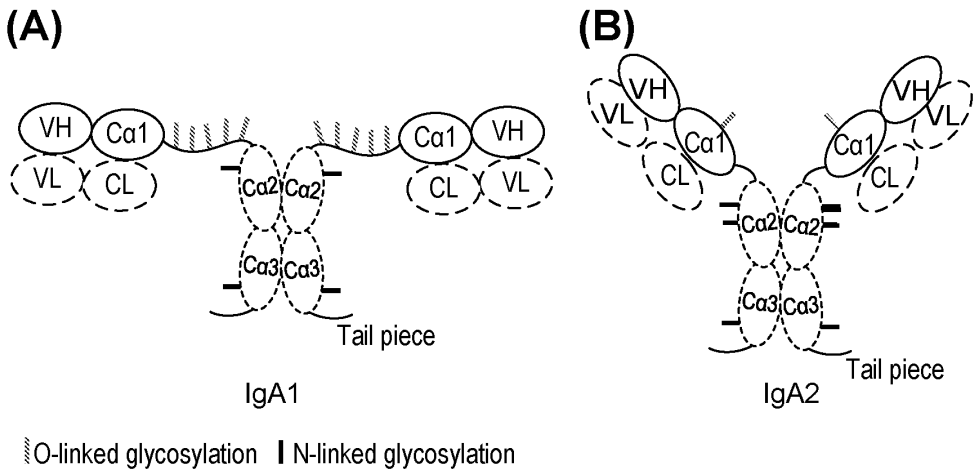


FIG. 4

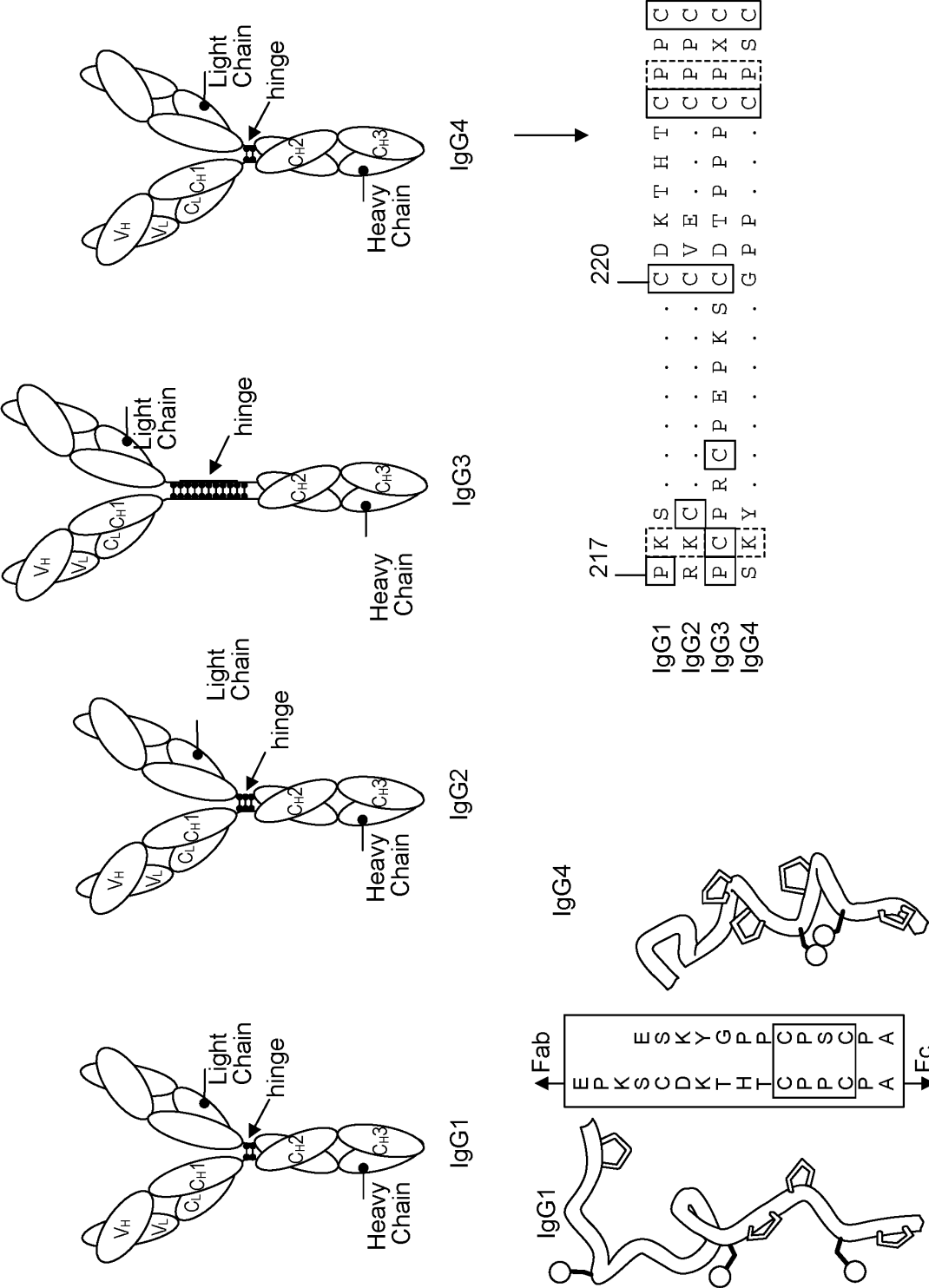


FIG. 5

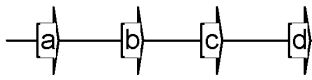
	
	Hinge exons 1-4
G3m(b*)	+ + + +
G3m(b*)	+ - - +
G3m(b*)	+ + + +
G3m(b*)	+ + + +
G3m(b*)	+ - + +
G3m(b*)	+ + + +
G3m(b**)	+ + + +
G3m(c3*)	+ - + +
G3m(c3c5*)	+ + + +
G3m(g*)	+ + + +
G3m(g*)	+ + + +
G3m(g*)	+ + + +
G3m(s*)	+ - + +
G3m(st*)	+ - + +
G3m(st)	+ - + +

FIG. 6

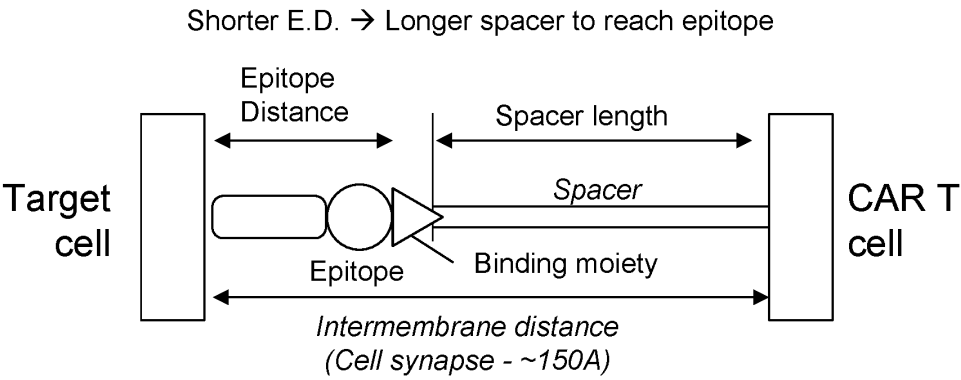


FIG. 7

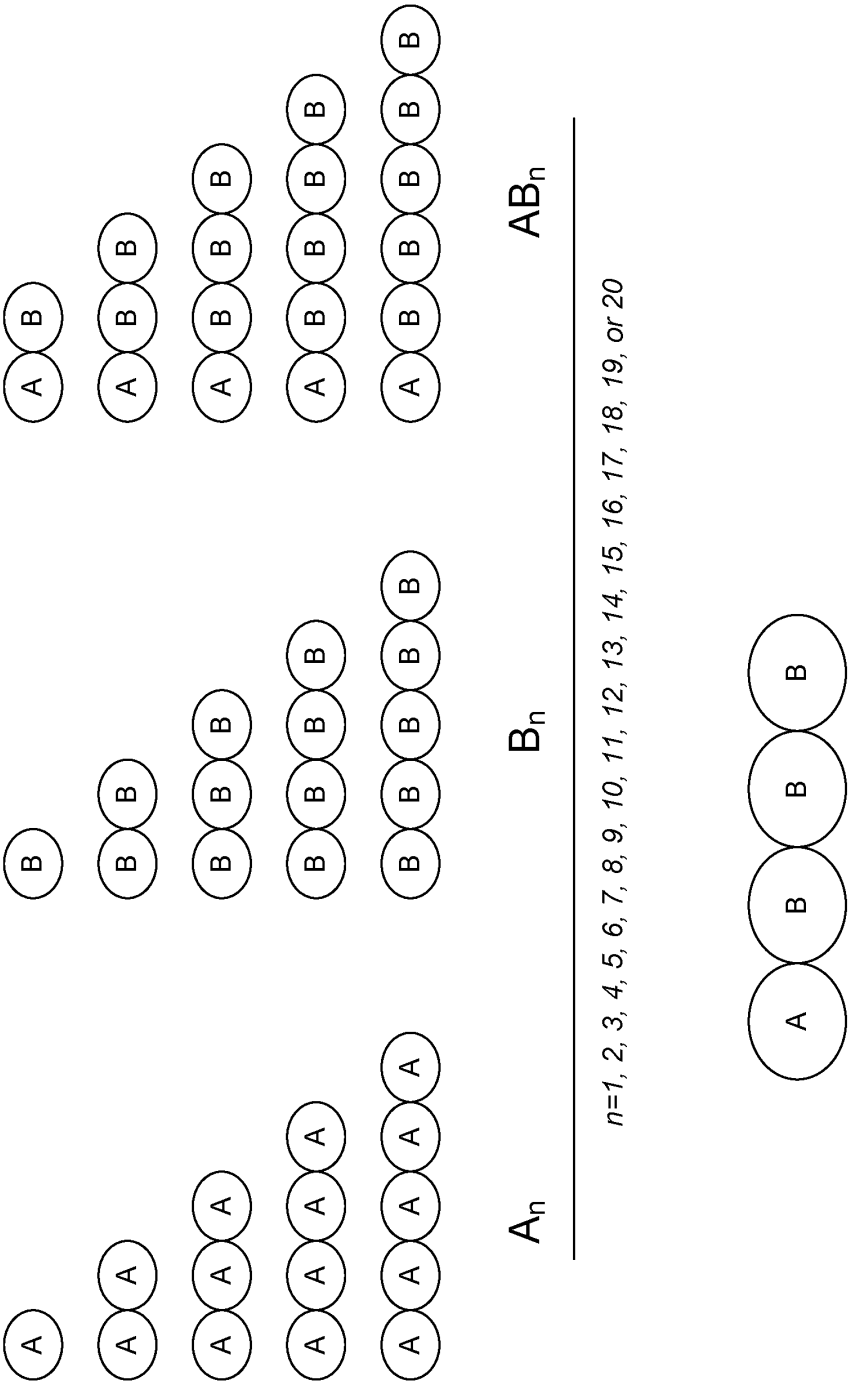


FIG. 8

Spacer	Type	Cysteines	SEQ ID NO	Sequence	SEQ ID NO	Optional Linker	aa with optional linker	aa without optional linker	Å (with linker)	Å (without linker)
1	mmIgG2	yes	4830	KPCPPCKCP	4818	GGSG	14	9	50.4	32.4
2	mmIgG2	yes	4831	KPCPPCKCPAP	4818	GGSG	16	11	57.6	39.6
3	mmIgG2	yes	4832	GPTIKPCPPCKCPAP	4818	GGSG	20	15	72	54
4	IgG3	yes	4833	ELKTLPLGDTHTCRCPEPKSCDTPPPCPCRCPEPKSCDTPPPCPCRCAP	4818	GGSG	69	64	248.4	230.4
5	IgG3	yes	4834	CPRCPEPKSCDTPPPCPCRCPEPKSCDTPPPCPCRCPEPKSCDTPPPCPCRCAP	4818	GGSG	57	52	205.2	187.2
6	IgG3	yes	4835	EPKSCDTPPPCPCRCPEPKSCDTPPPCPCRCPEPKSCDTPPPCPCRCAP	4818	GGSG	52	47	187.2	169.2
7	IgG3	yes	4836	EPKSCDTPPPCPCRCPEPKSCDTPPPCPCRCAP	4818	GGSG	37	32	133.2	115.2
8	IgG3	yes	4837	CPRCPEPKSCDTPPPCPCRCAP	4818	GGSG	27	22	97.2	79.2
9	IgG3	yes	4838	EPKSCDTPPPCPCRCAP	4818	GGSG	22	17	79.2	61.2
10	IgG1	yes	4839	EPKSCDKTHTCPCPCAP	4818	GGSG	22	17	79.2	61.2
11	IgG1	yes	4840	EPKSCDKTHTCPCPCAP	4818	GGSG	22	17	79.2	57.6
13	IgG3	yes	4841	PCRCPCAP	4818	GGSG	13	8	46.8	28.8
14	IgG2	yes	4842	PCAPPVAGPS	4818	GGSG	16	11	57.6	39.6
15	IgG1	no	4843	PPKPKOT	4818	GGSG	12	7	43.2	25.2
16	IgA1	yes	4844	TVPCVPSTPTPTSPSTPTPTSPSCCHP	4818	GGSG	33	28	118.8	100.8
17	IgA1	yes	4845	TVPCVPSTP	4818	GGSG	15	10	54	36
18	IgA1	no	4846	TPSPSTPTPT	4818	GGSG	15	10	54	36
19	IgA1	yes	4847	TPSPSCCHP	4818	GGSG	14	9	50.4	32.4
20	IgA2	yes	4848	VPCVPVPPPPCCHPR	4818	GGSG	20	15	72	54
21	IgA2	yes	4849	VPCVPP	4818	GGSG	12	7	43.2	25.2
22	IgA2	yes	4850	PPCCHP	4818	GGSG	12	7	43.2	25.2
23	IgA2	yes	4851	PCVPVPPPPCCHP	4818	GGSG	18	13	64.8	46.8
24	IgD	yes	4852	RWPESPKAQASSVPTAQPAEGSLAKATTATTRNTGRGGEKKKEKEKEEERETKTPE CPSHTQPLGVLLTP	4818	GGSG	82	77	295.2	277.2
25	IgD	yes	4853	KTEPCPSHTQPLGVLLTP	4818	GGSG	24	19	86.4	68.4
26	IgD	yes	4854	PECPSHTQPLGVLLTP	4818	GGSG	22	17	79.2	61.2
27	IgD	no	4855	RWPESPKAQASSVP	4818	GGSG	19	14	68.4	50.4
28	IgE	yes	4856	SVCSDRDFTP	4818	GGSG	15	10	54	36
29	IgM	no	4857	PLPVIAELPPKVSFVPPRDGFEGNP	4818	GGSG	31	26	111.6	93.6
30	IgM	no	4858	PLPVIAELPP	4818	GGSG	15	10	54	36
31	IgM	no	4859	ELPPKVSFVPP	4818	GGSG	17	12	61.2	43.2

FIG. 9

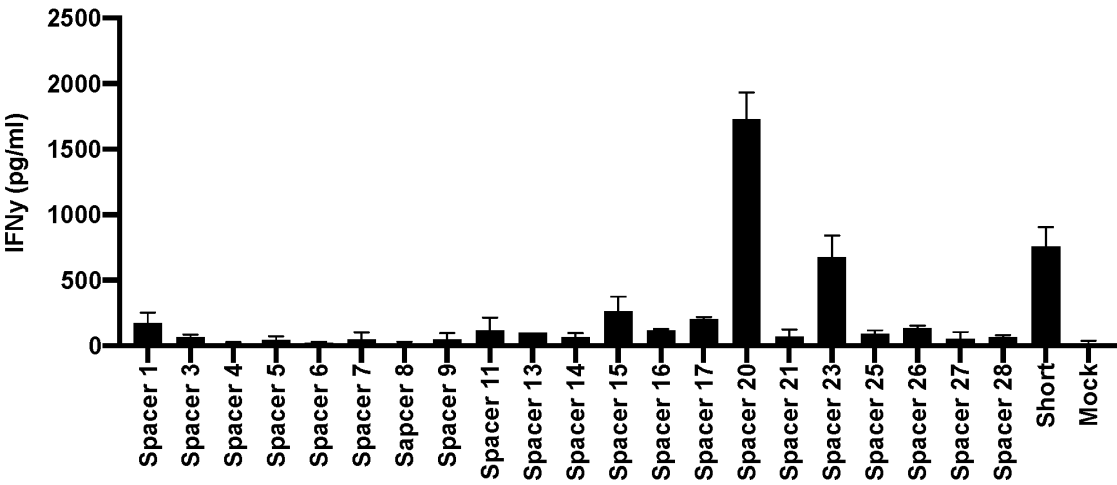


FIG. 10

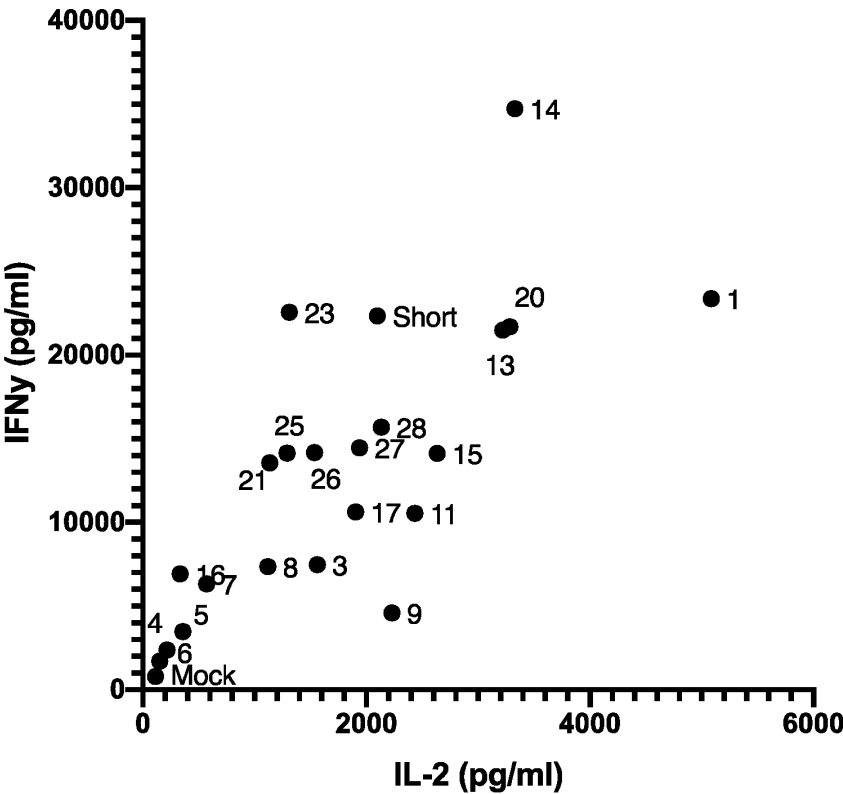


FIG. 11A

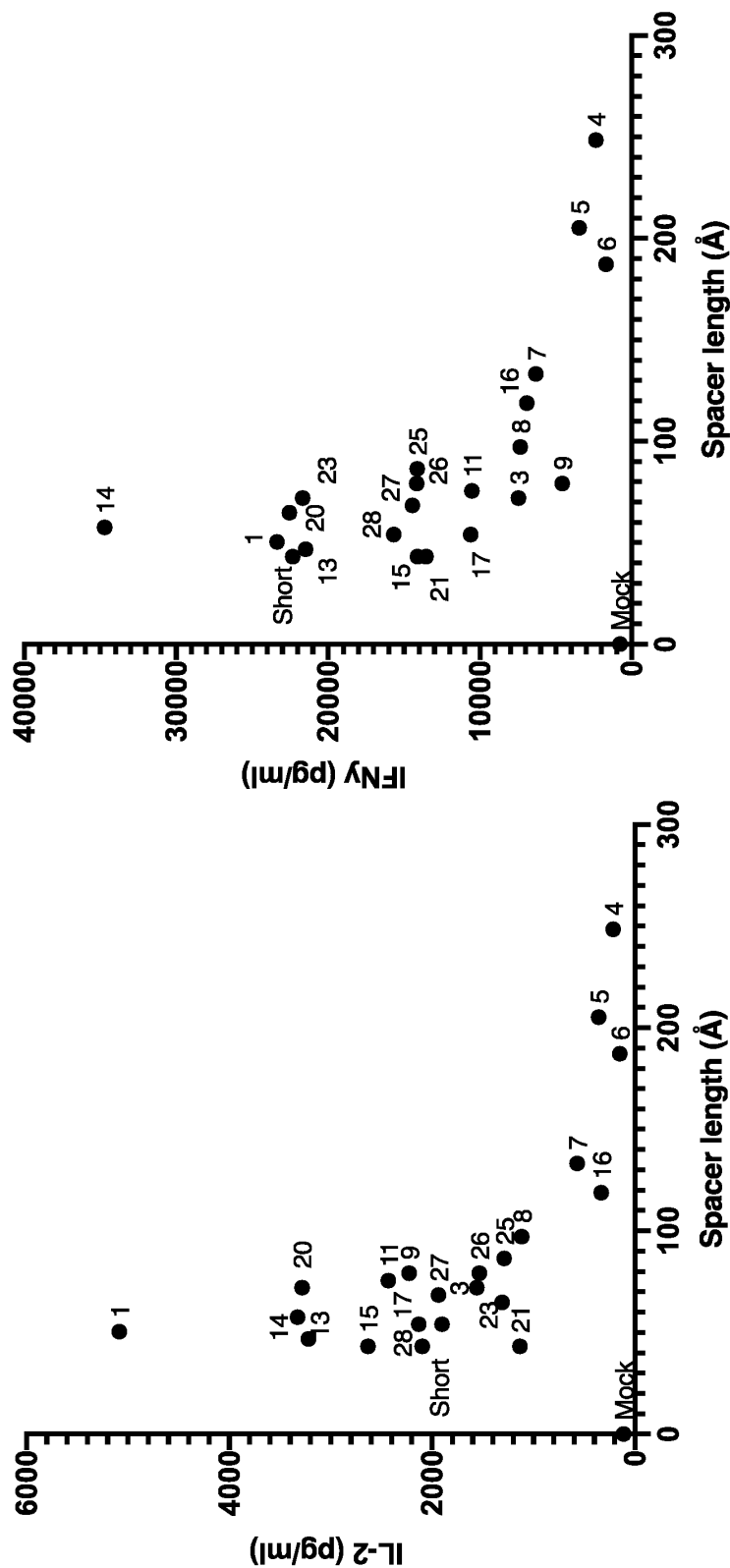


FIG. 11B

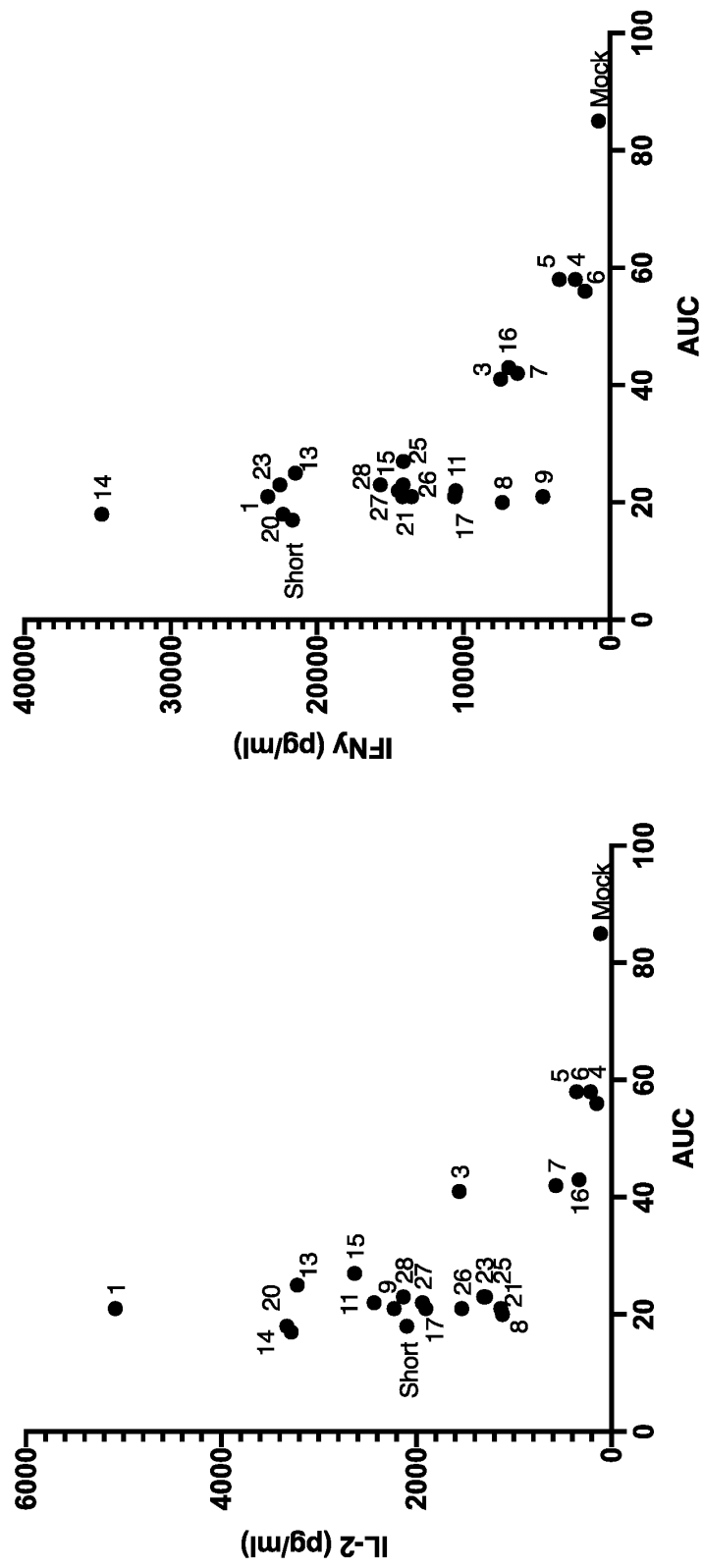


FIG. 11C

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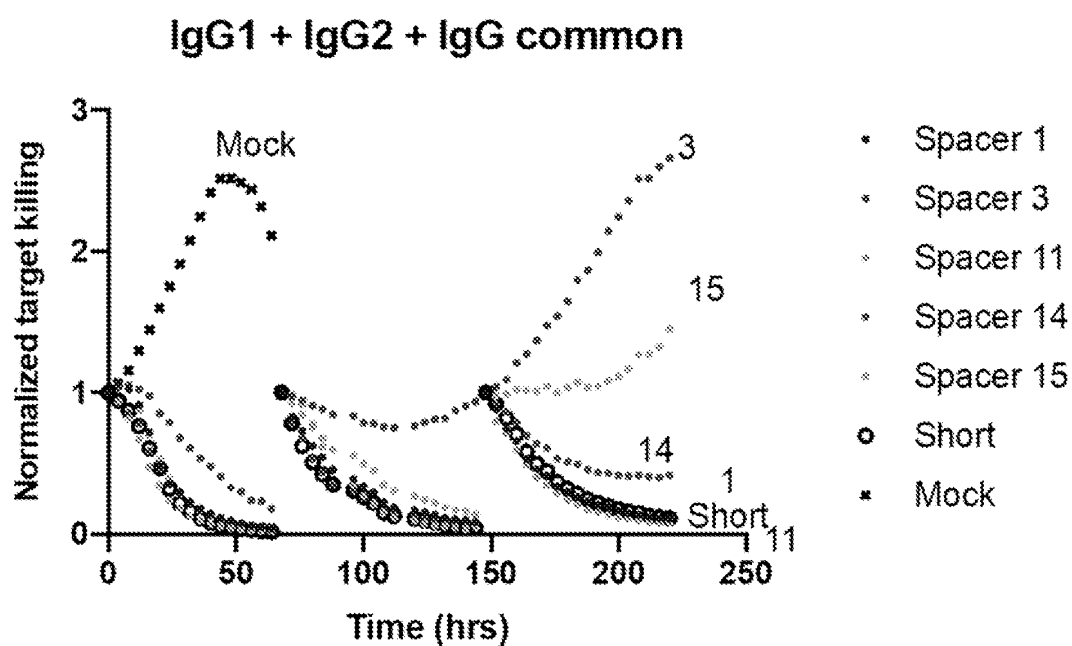


FIG. 12A

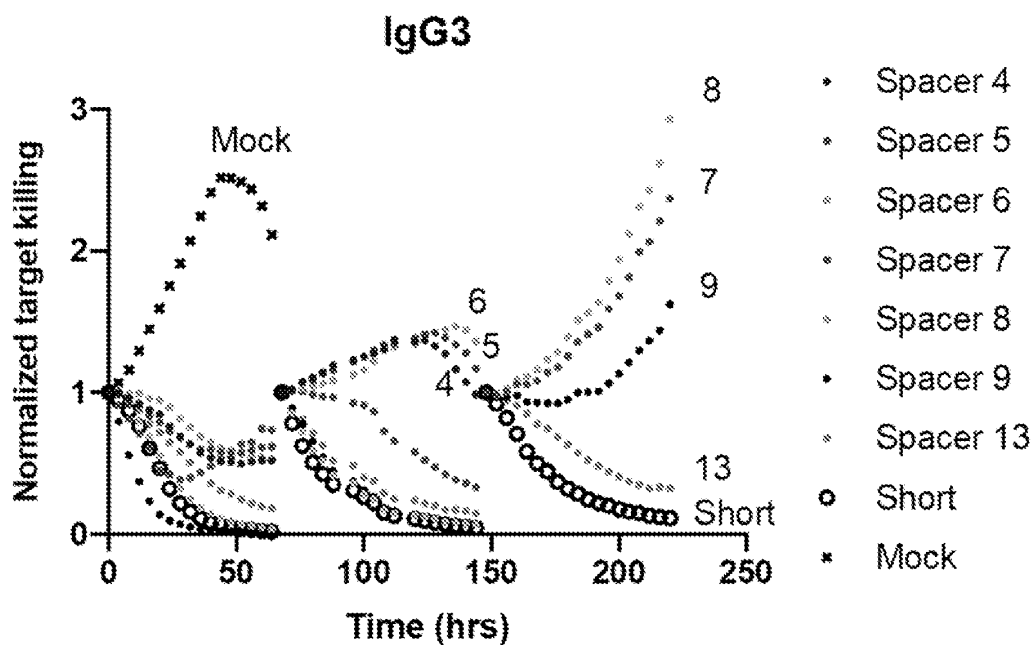


FIG. 12B

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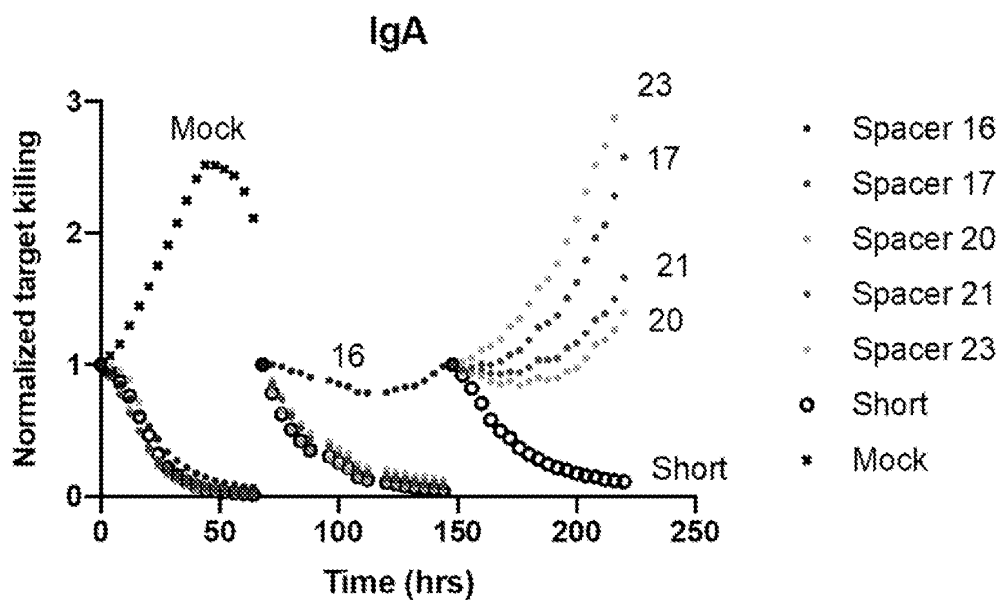


FIG. 12C

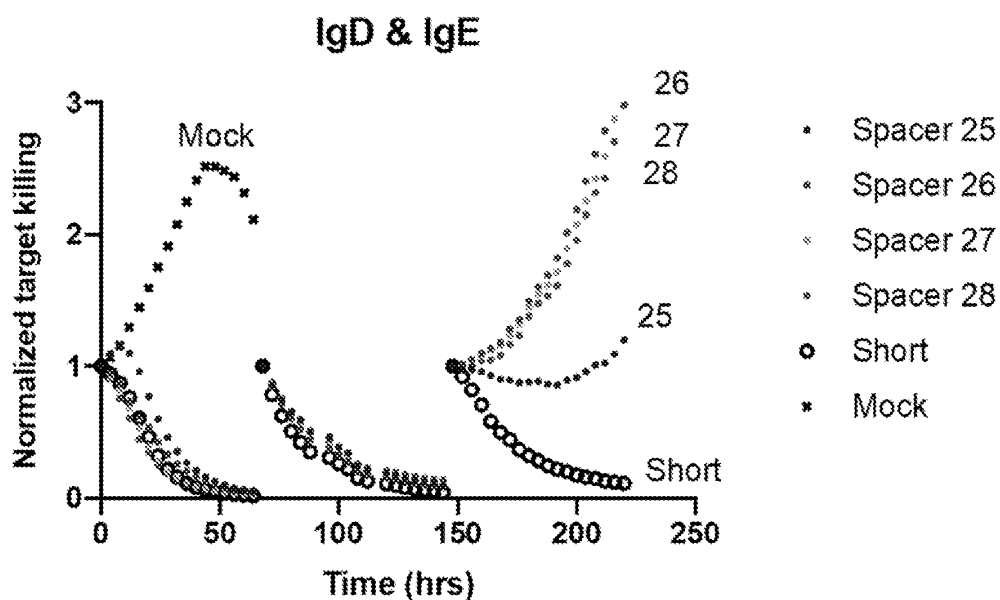


FIG. 12D

Top spacers	Spacer length (Å)
Spacer 1	50.4
Spacer 11	75.6
Spacer 13	45.8
Spacer 14	57.6
Short	43.2

*Full range of spacer lengths – 43.2 Å ~ 248.4 Å

FIG. 12E

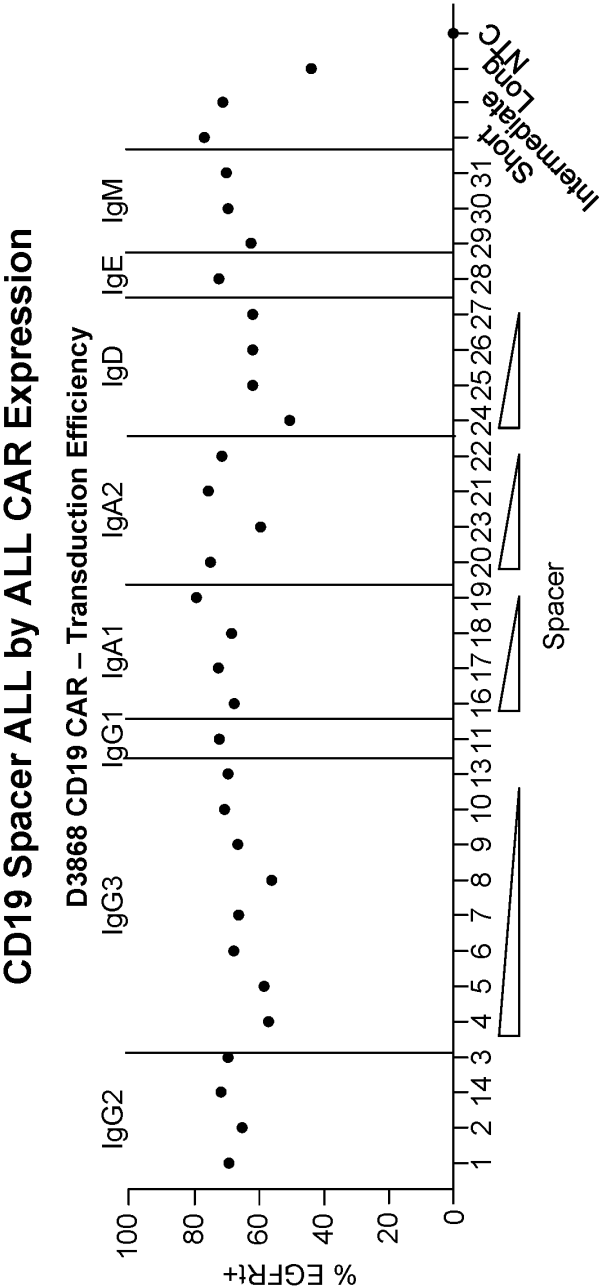


FIG. 13A

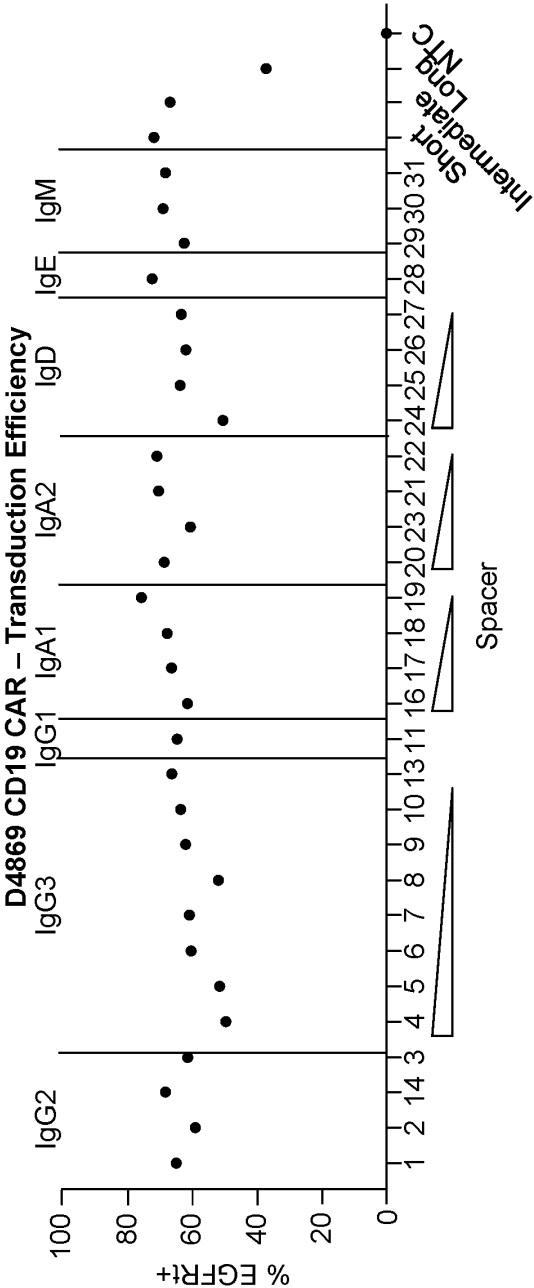


FIG. 13B

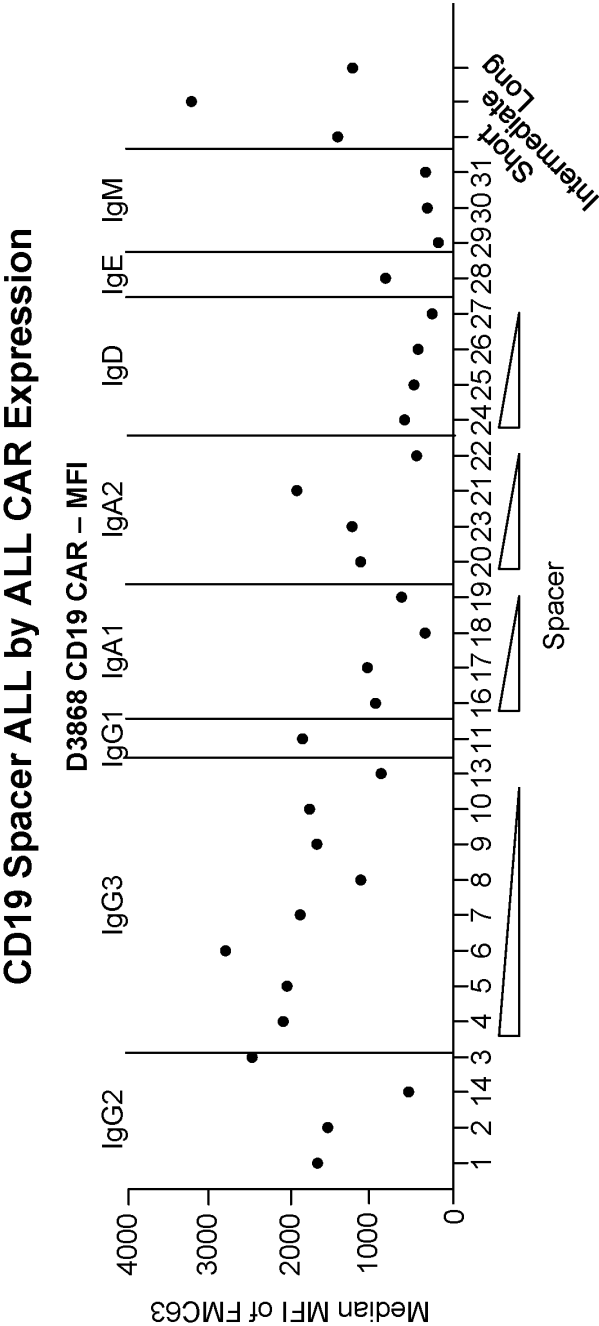


FIG. 13C

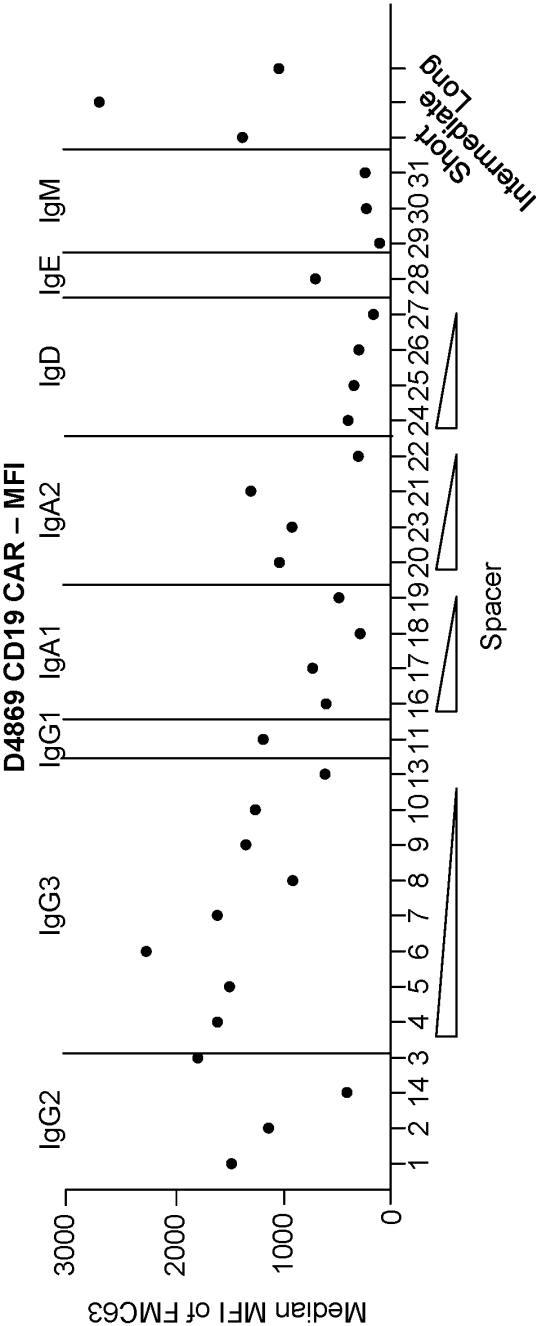


FIG. 13D

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Primary killing and AUC D3868 CAR-T : Raji-NLR 1:1

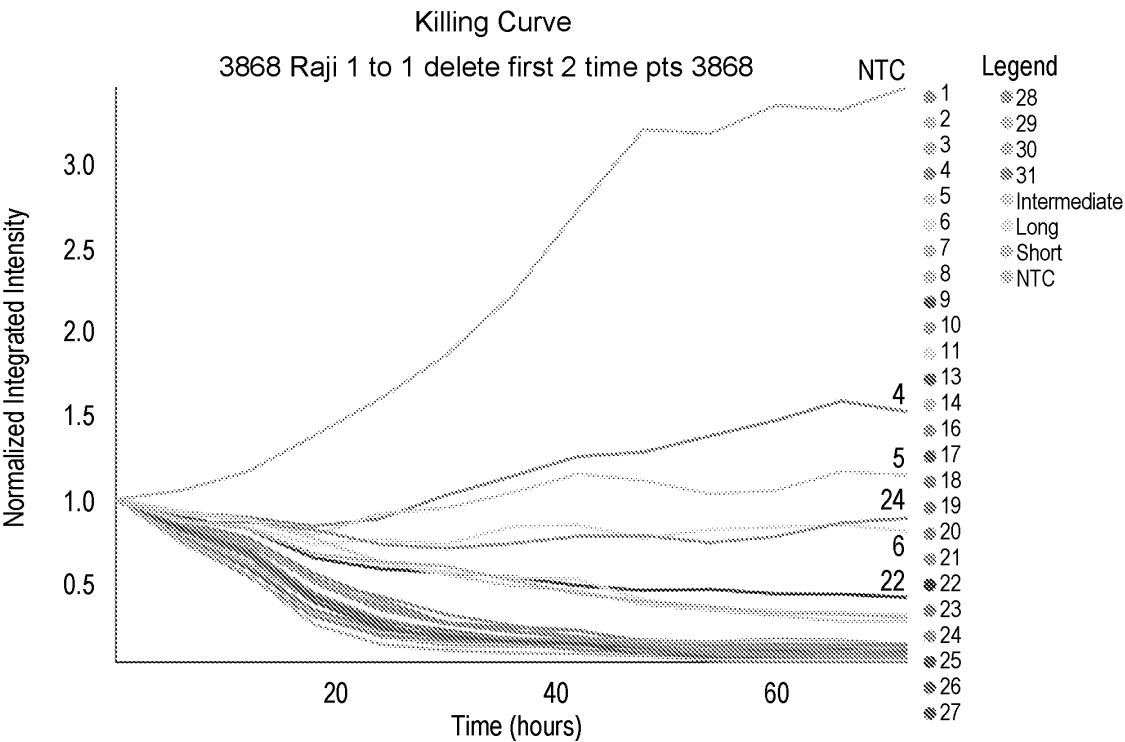


FIG. 14A

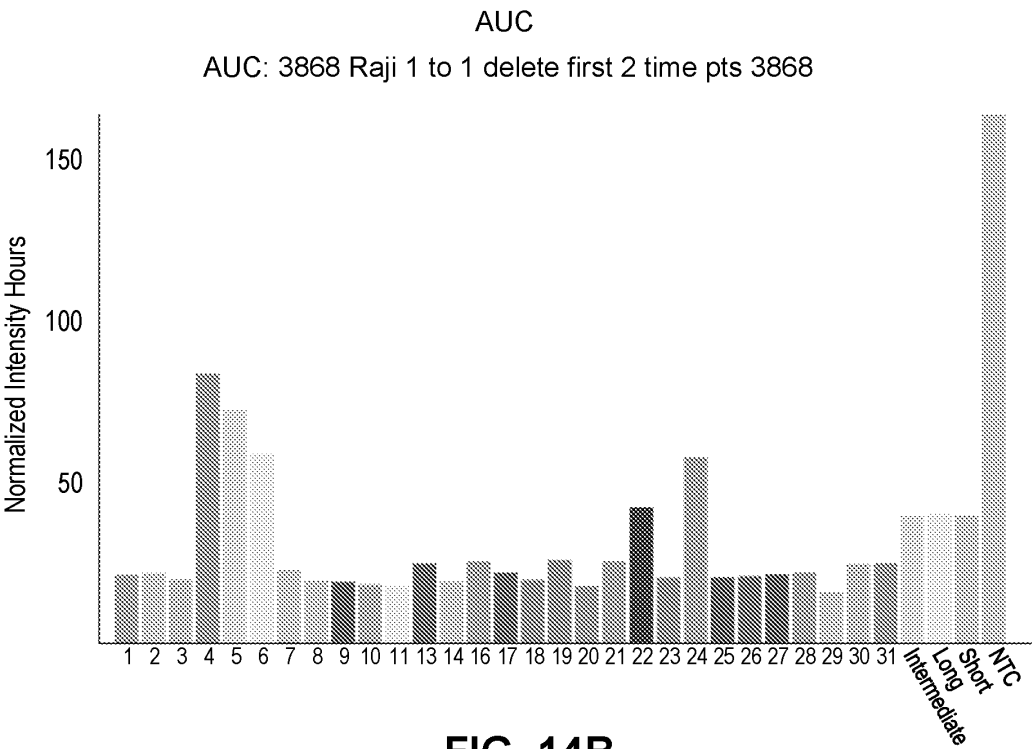
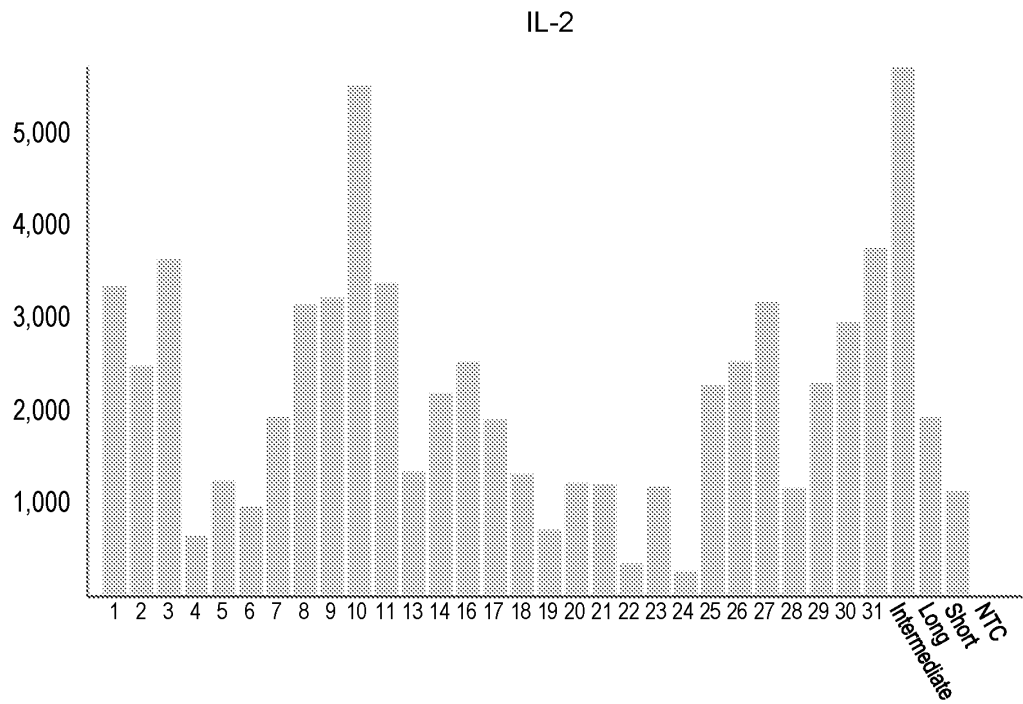
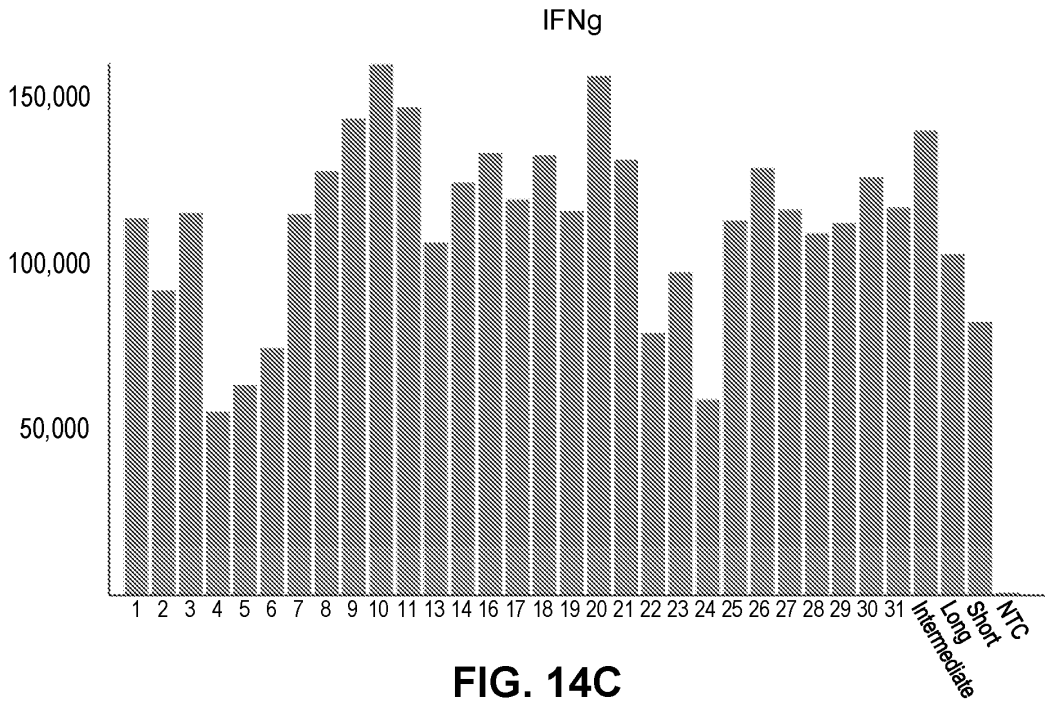


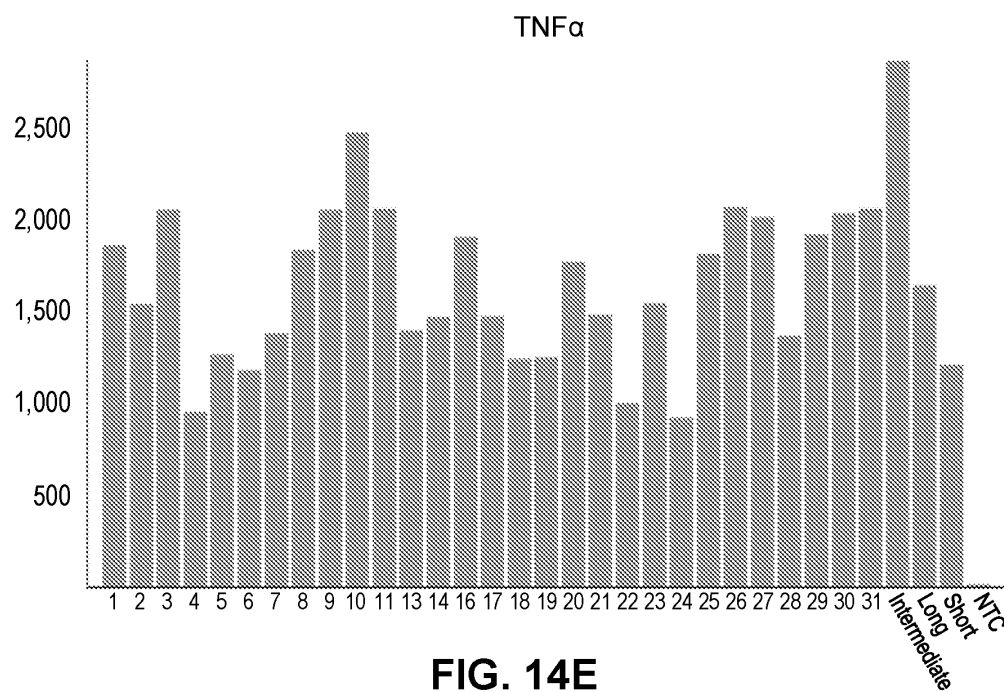
FIG. 14B

Primary killing and AUC D3868 CAR-T : Raji-NLR 1:1



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Primary killing and AUC D3868 CAR-T : Raji-NLR 1:1



Primary killing and AUC D4869 CAR-T : Raji-NLR 1:1

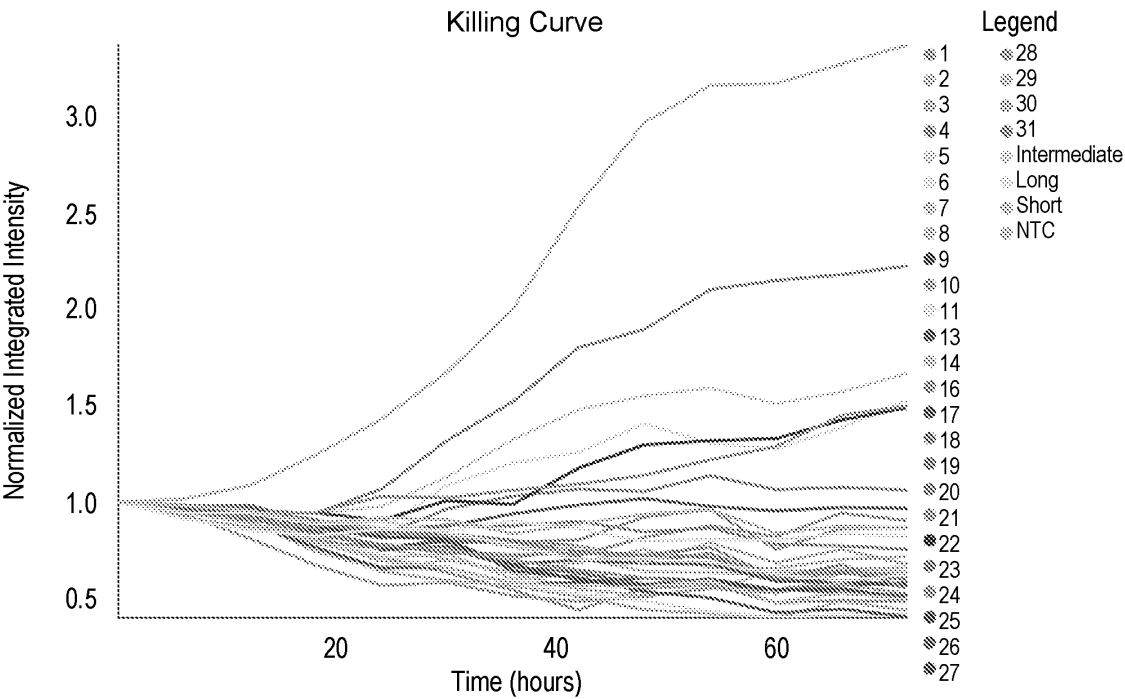


FIG. 15A

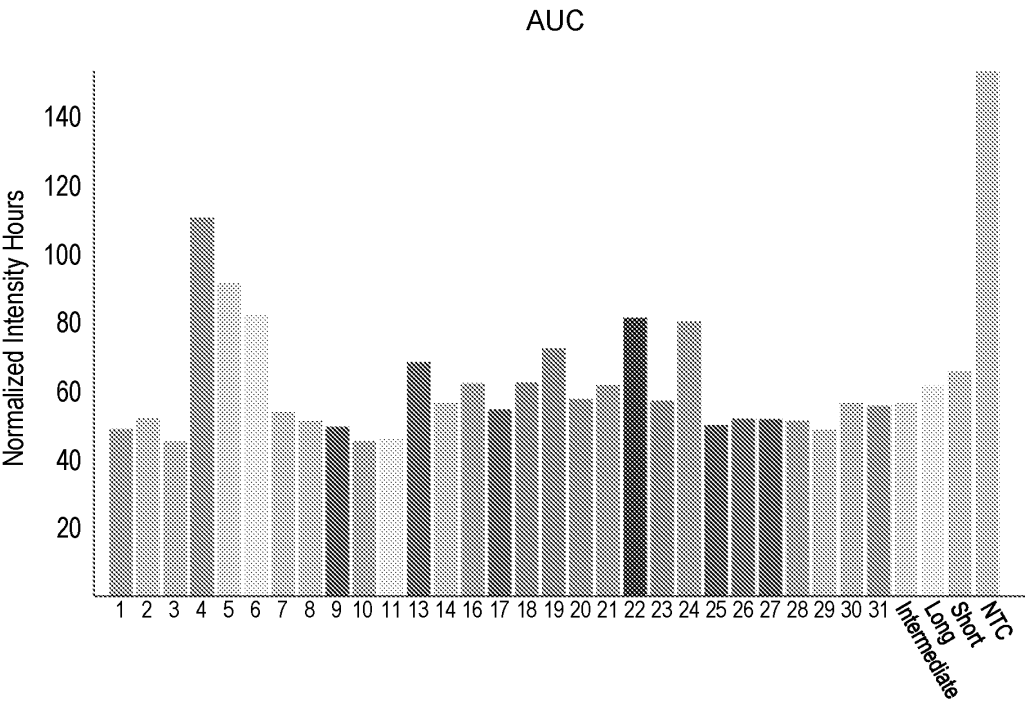
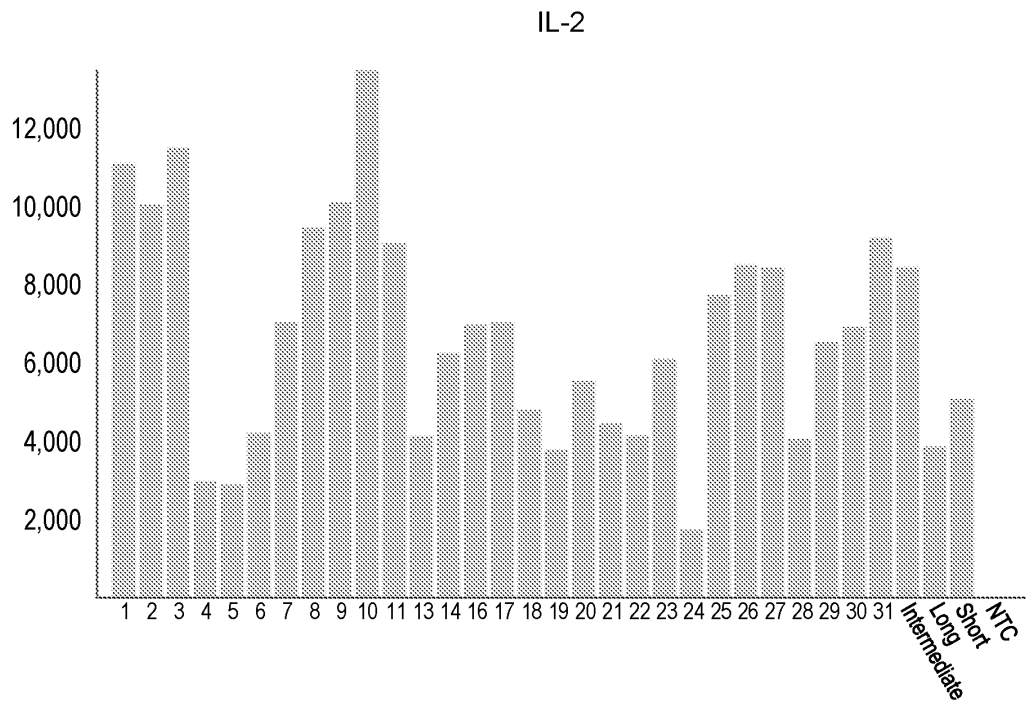
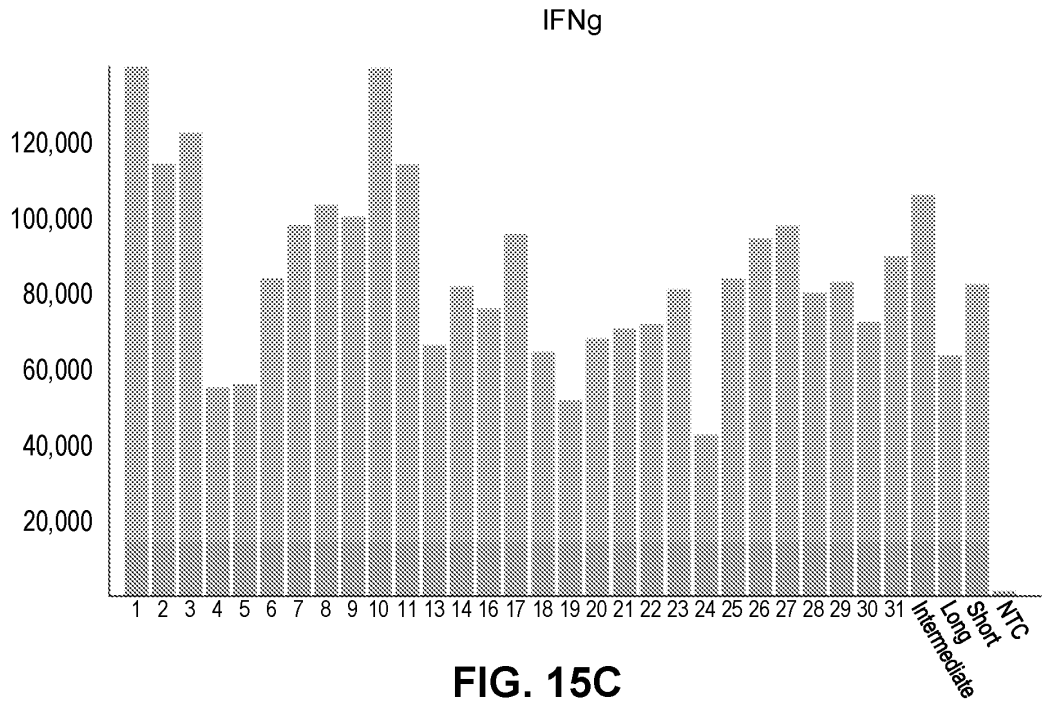
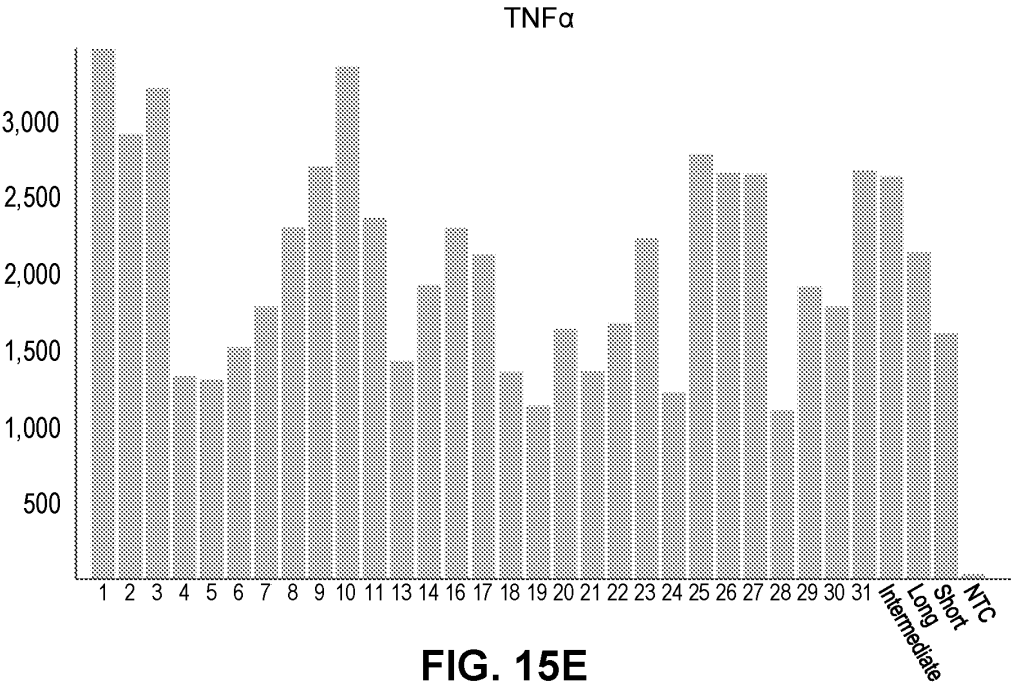


FIG. 15B

Primary killing and AUC D4869 CAR-T : Raji-NLR 1:1



Primary killing and AUC D4869 CAR-T : Raji-NLR 1:1



Primary killing and AUC D3868 CAR-T : Nalm6-NLR 1:1

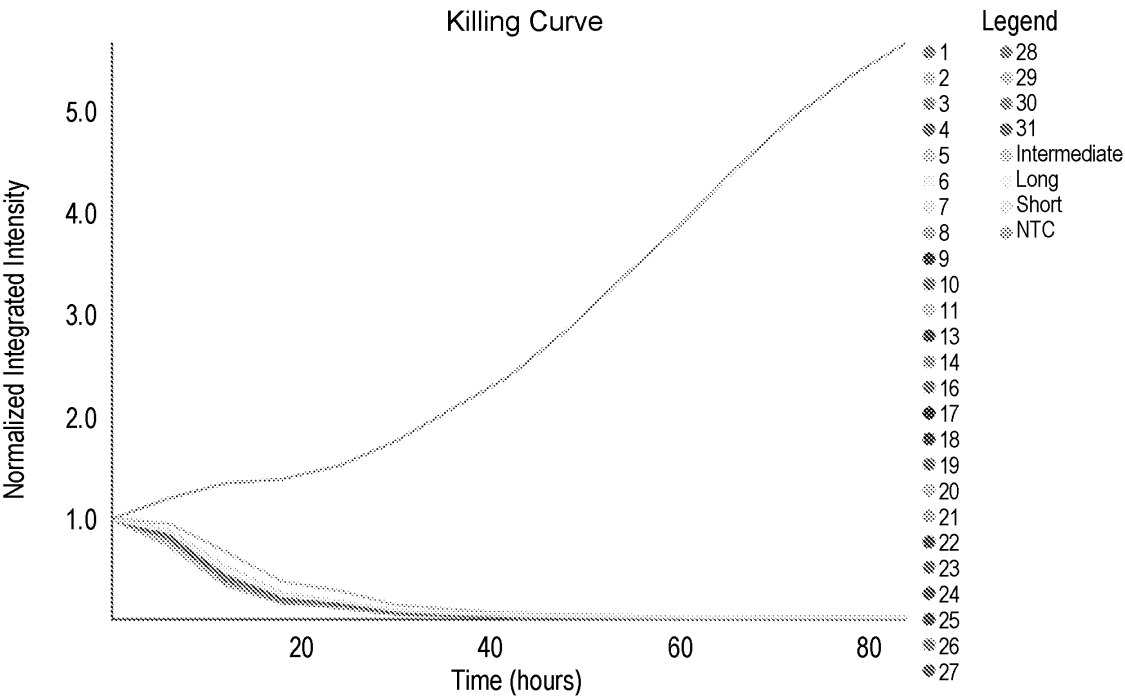


FIG. 16A

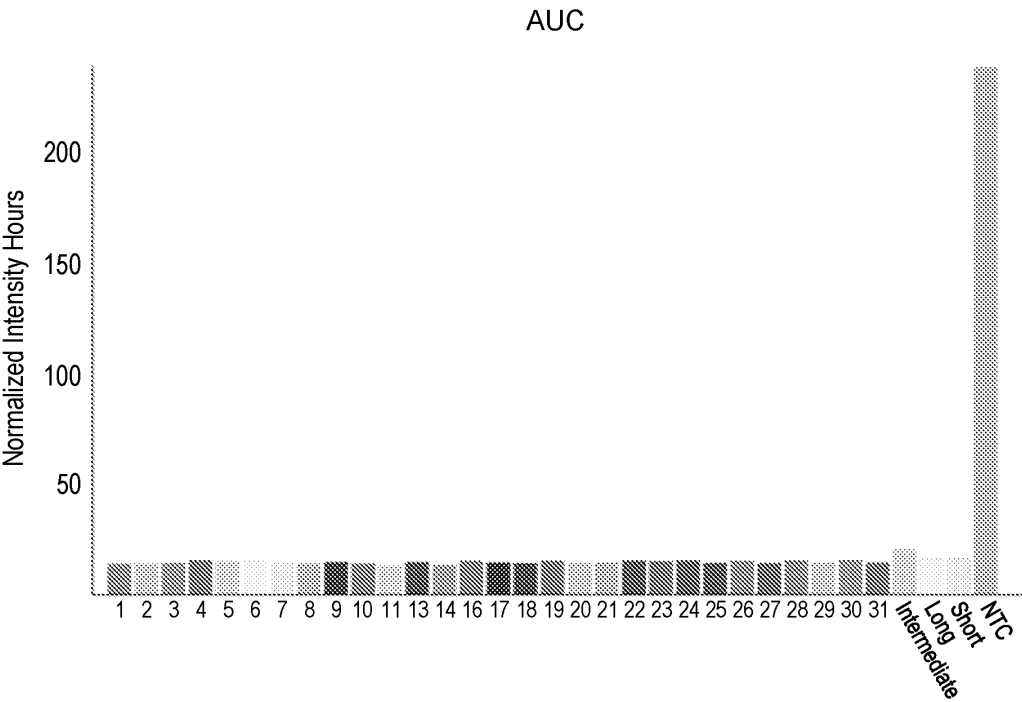
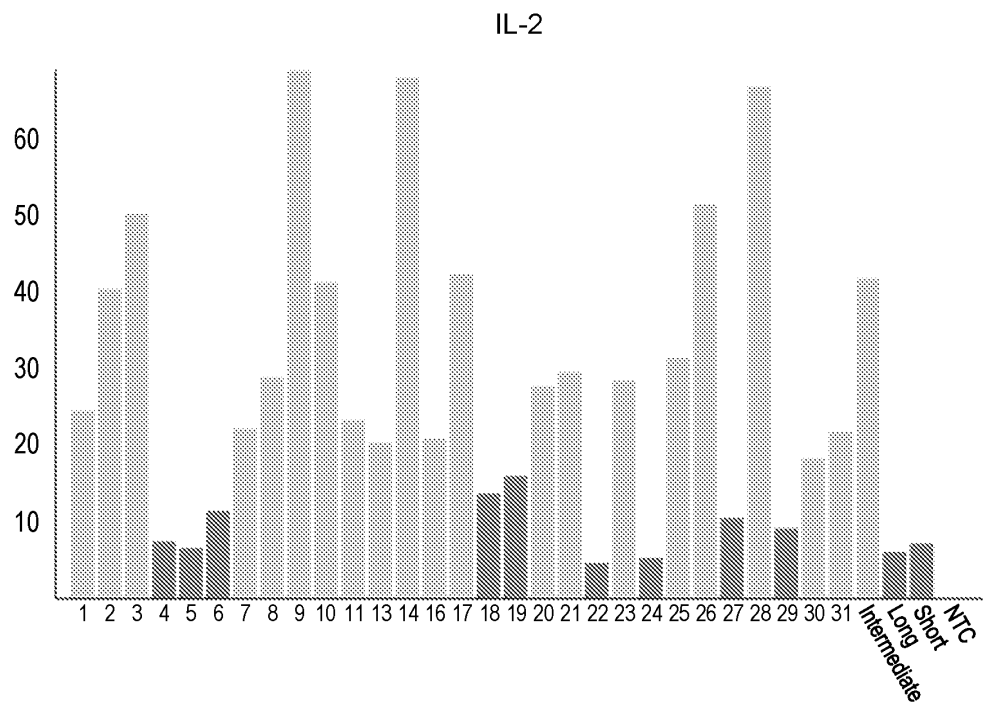
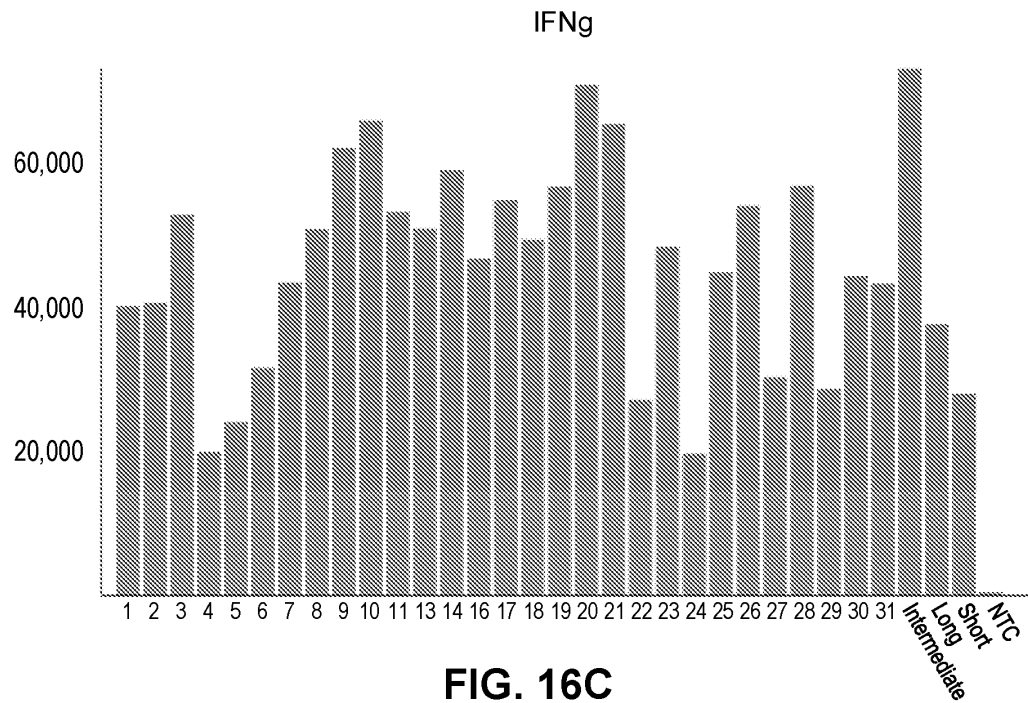


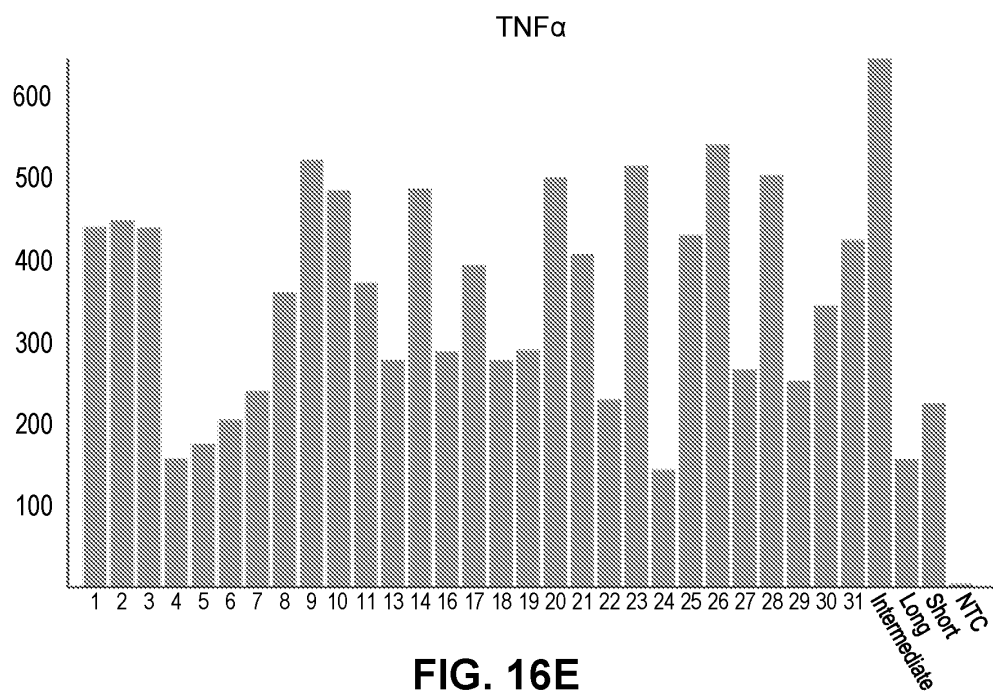
FIG. 16B

Primary killing and AUC D3868 CAR-T : Nalm6-NLR 1:1



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Primary killing and AUC D3868 CAR-T : Nalm6-NLR 1:1



Primary killing and AUC D4869 CAR-T : Nalm6-NLR 1:1

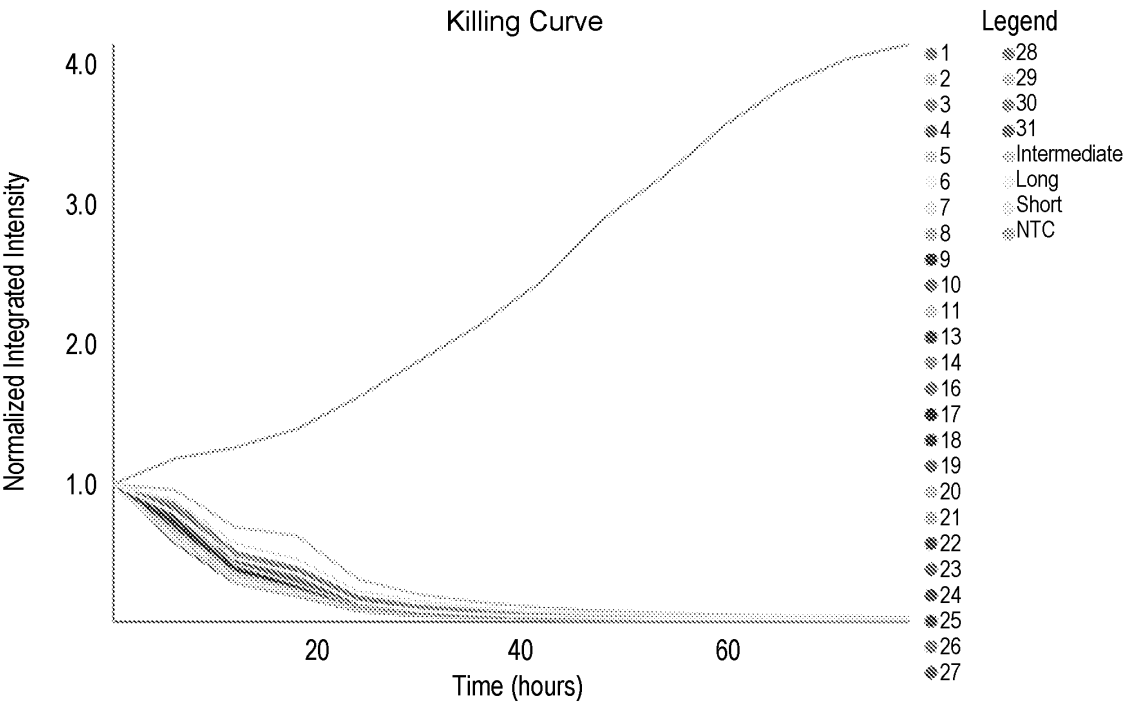


FIG. 17A

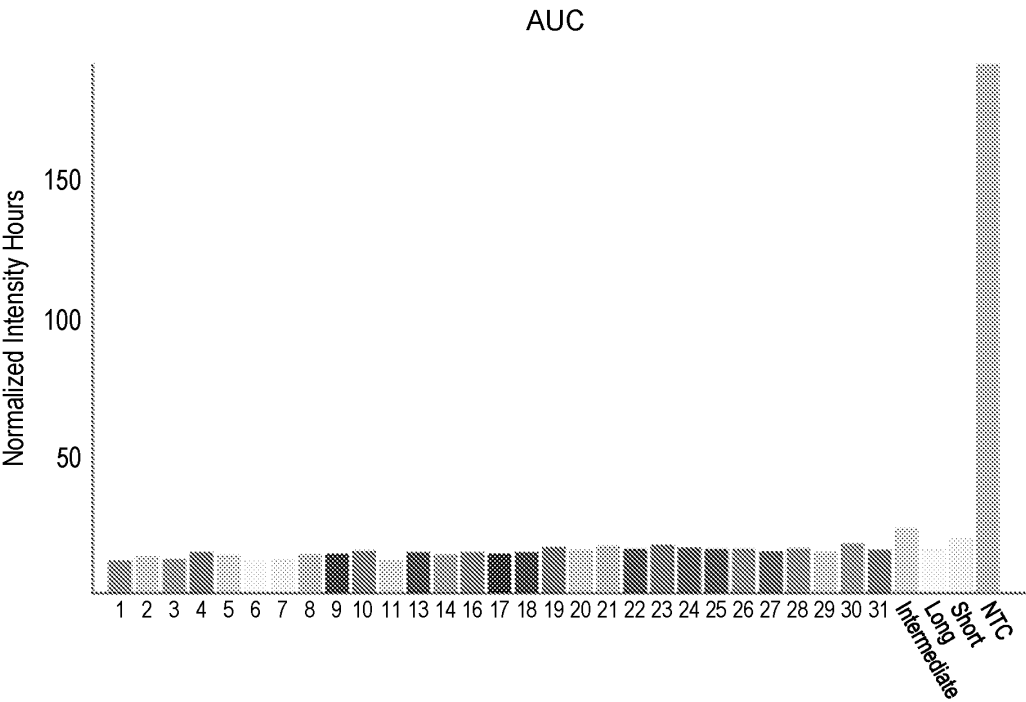
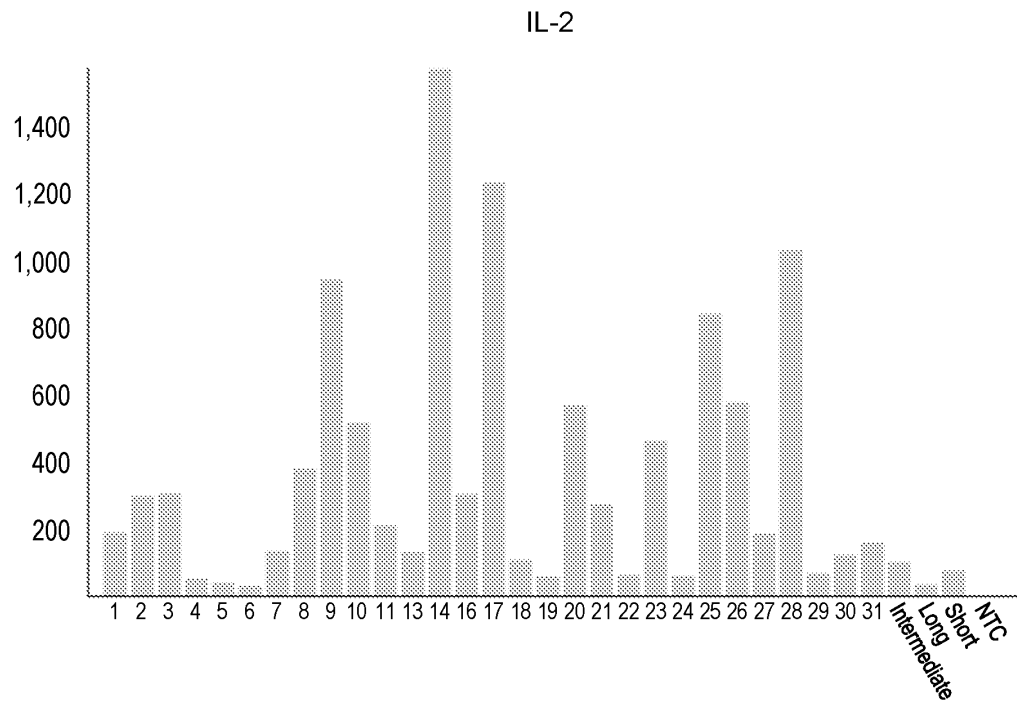
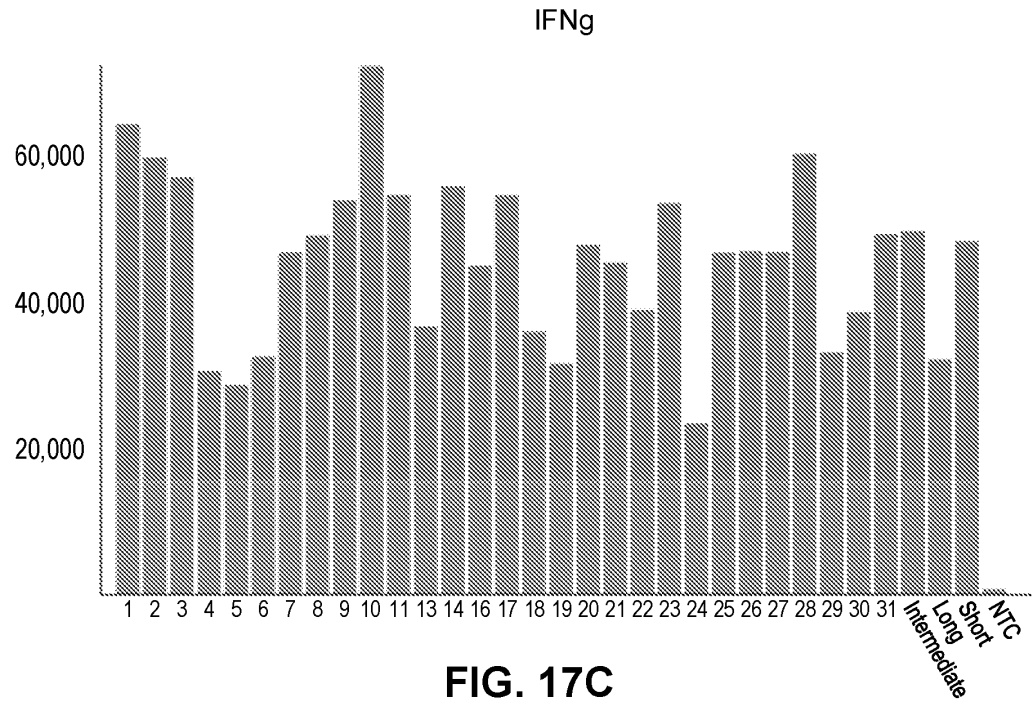


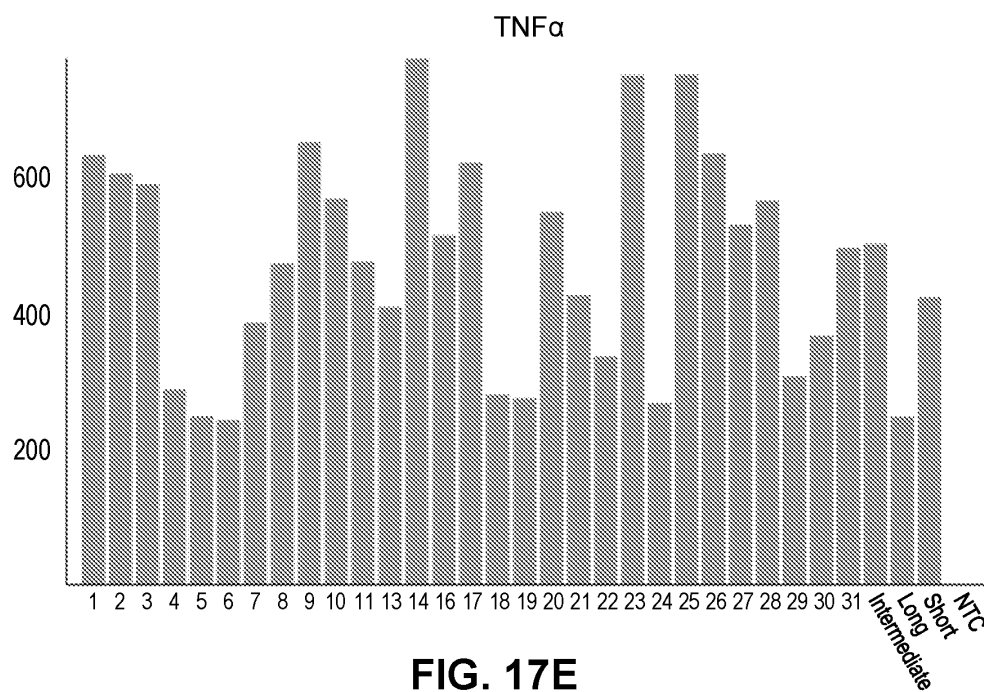
FIG. 17B

Primary killing and AUC D4869 CAR-T : Nalm6-NLR 1:1



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Primary killing and AUC D4869 CAR-T : Nalm6-NLR 1:1



Primary killing and AUC D3868 CAR-T : Raji_CD19_KO-NLR 1:1

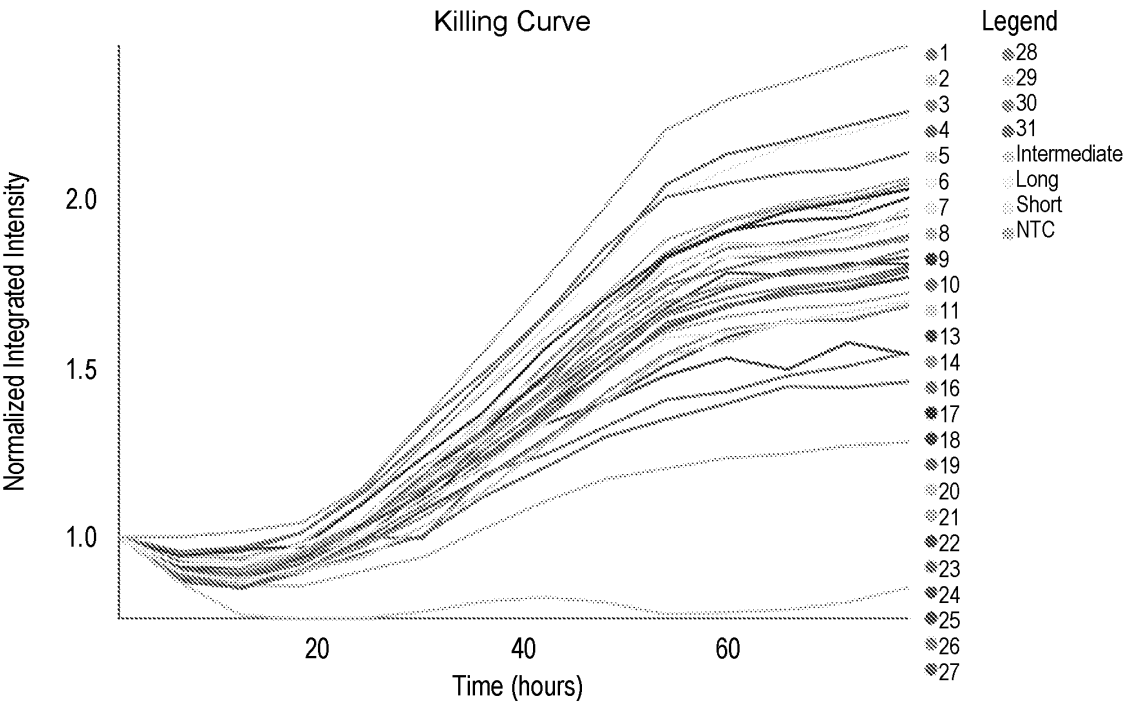


FIG. 18A

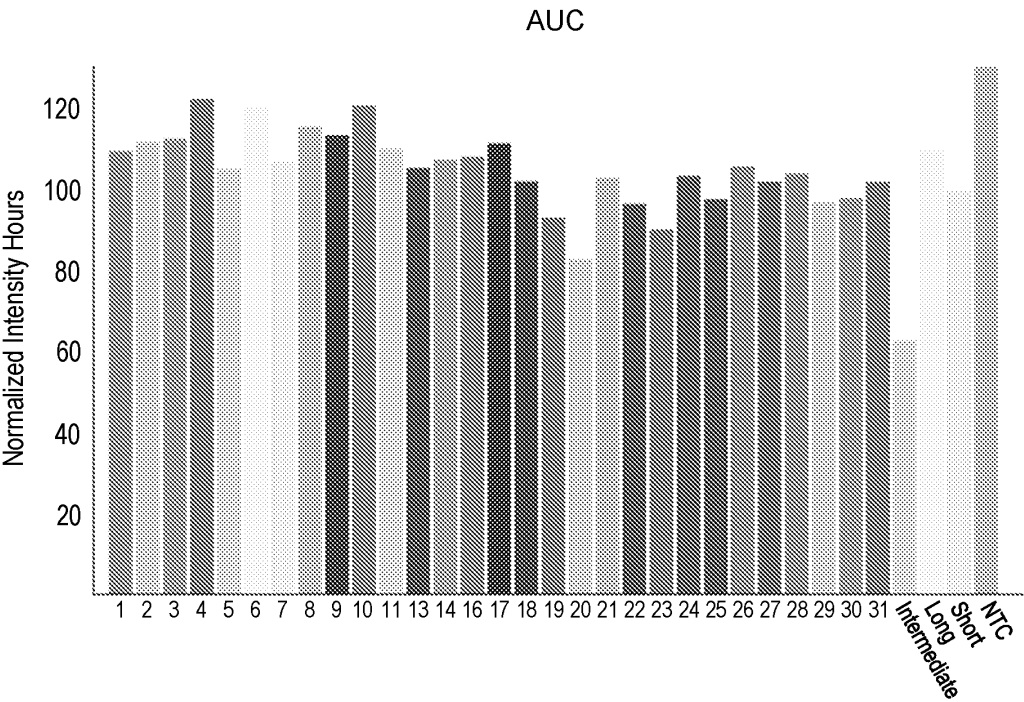
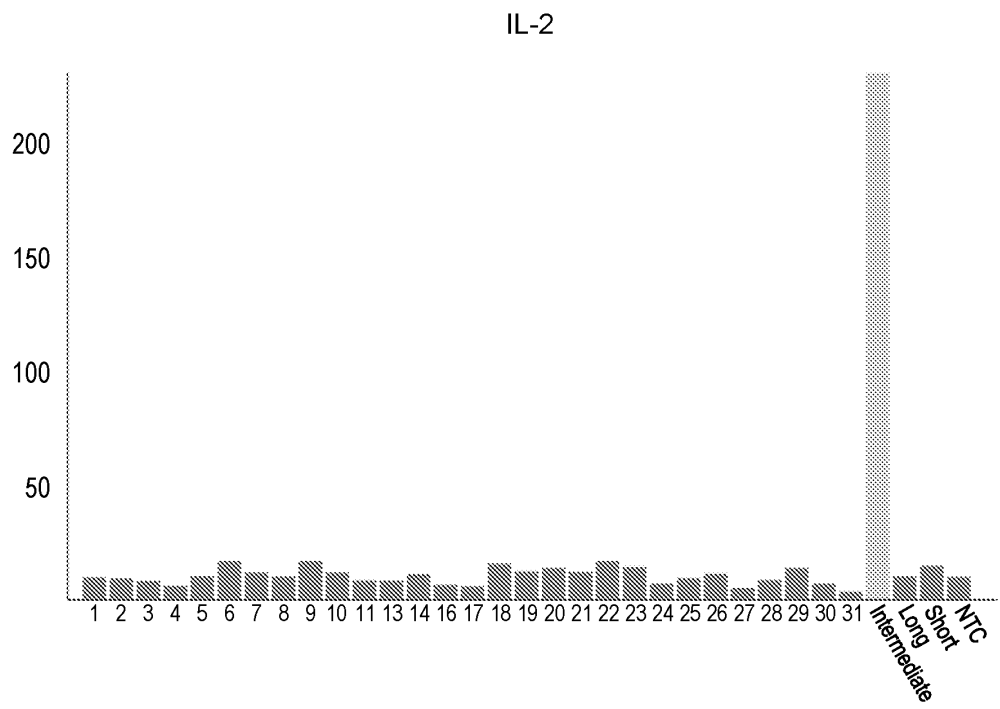
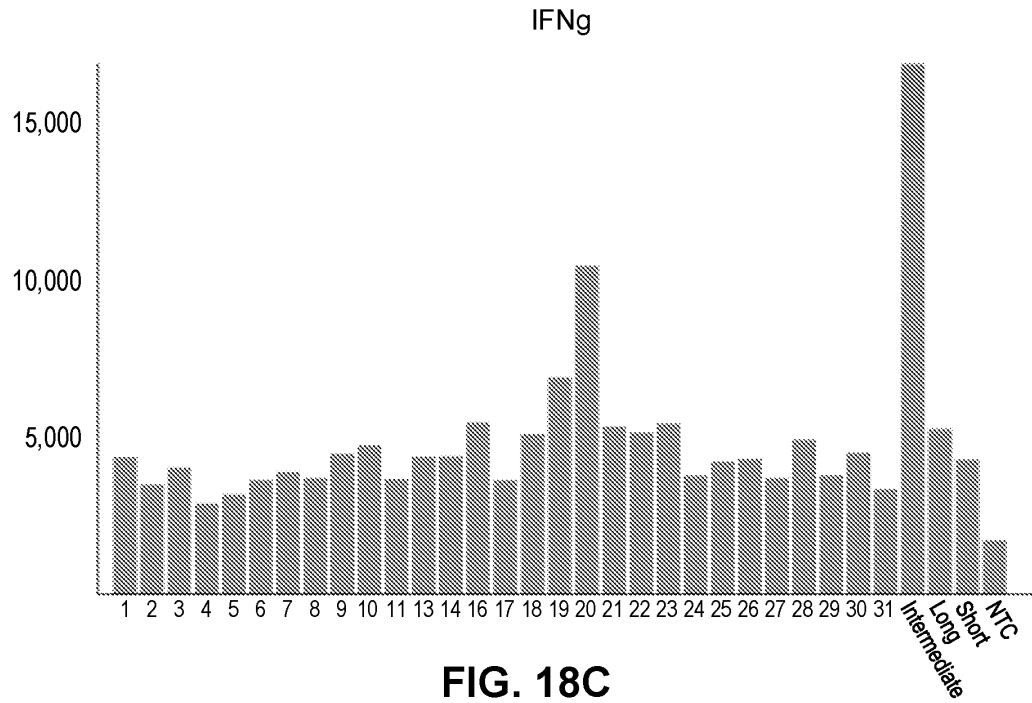


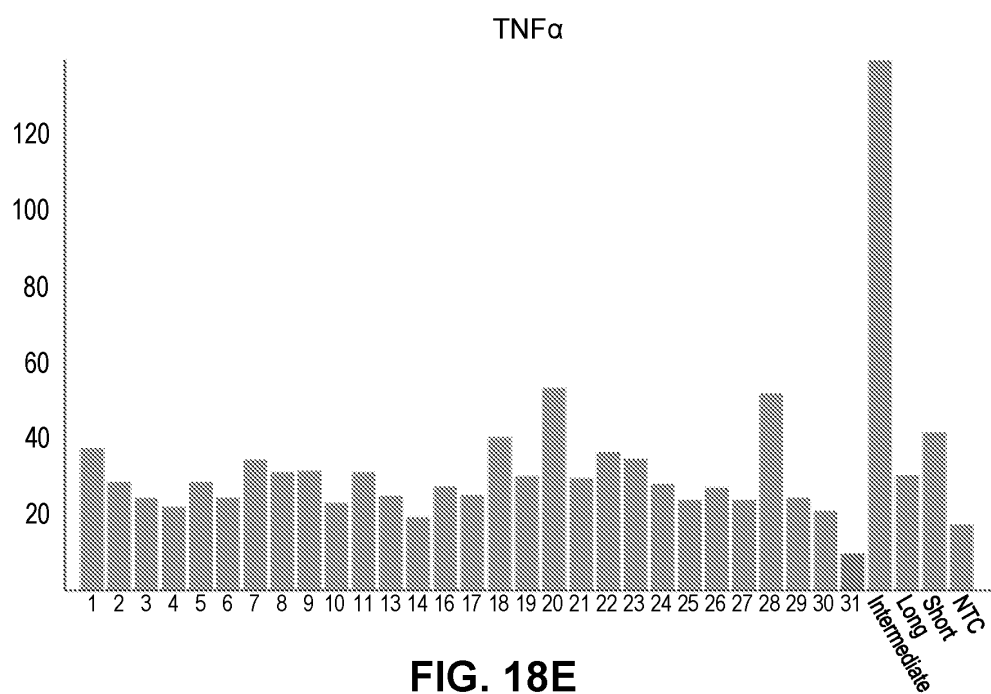
FIG. 18B

Primary killing and AUC D3868 CAR-T : Raji_CD19_KO-NLR 1:1



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Primary killing and AUC D4869 CAR-T : Nalm6-NLR 1:1



Primary killing and AUC D4869 CAR-T : Raji_CD19_KO-NLR 1:1

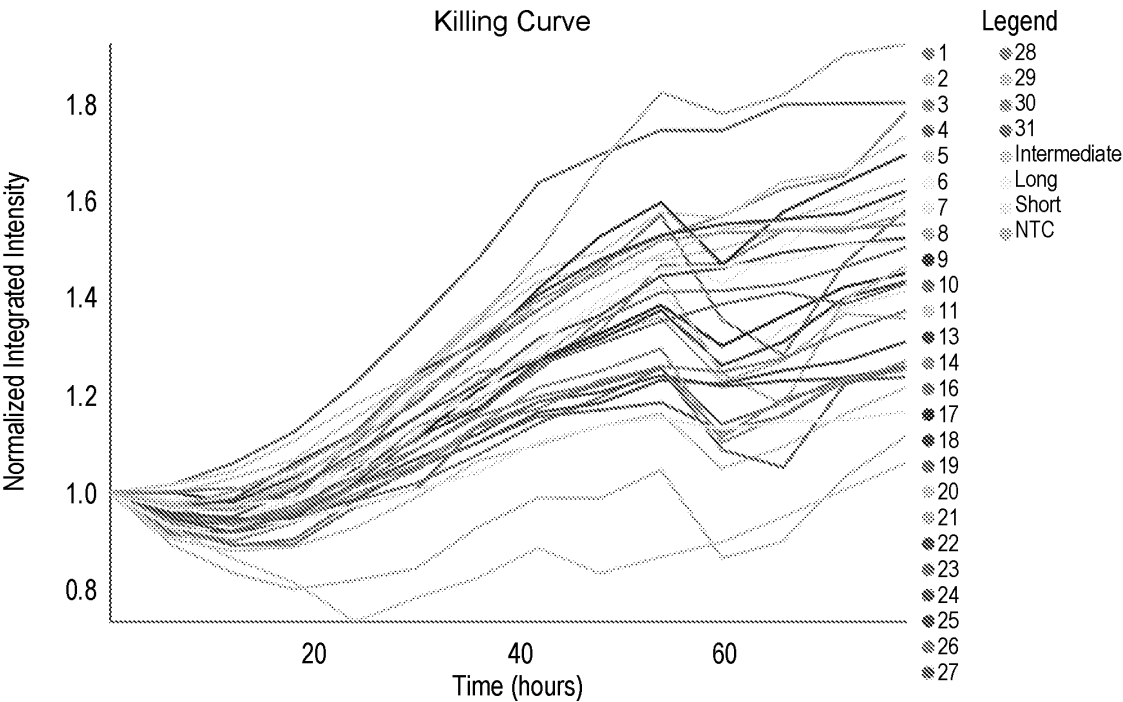


FIG. 19A

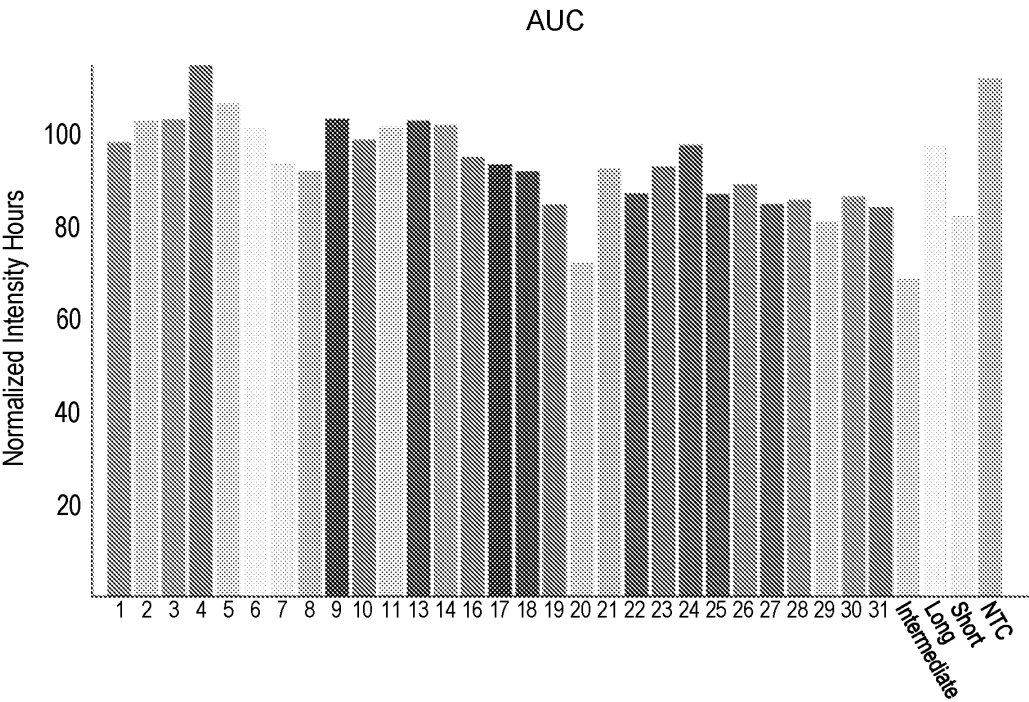
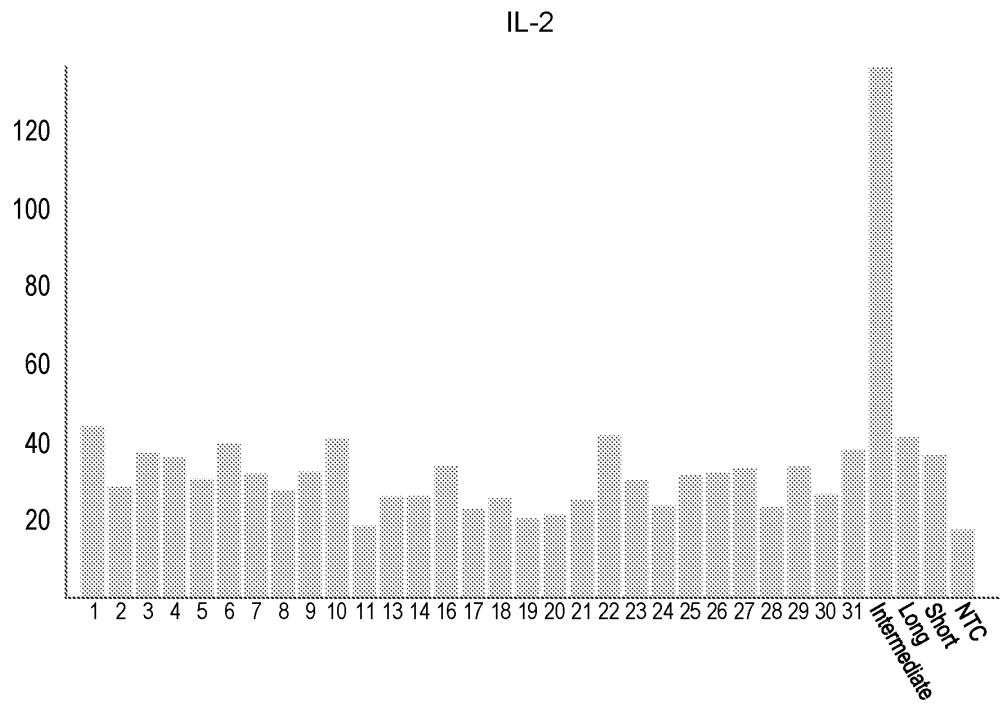
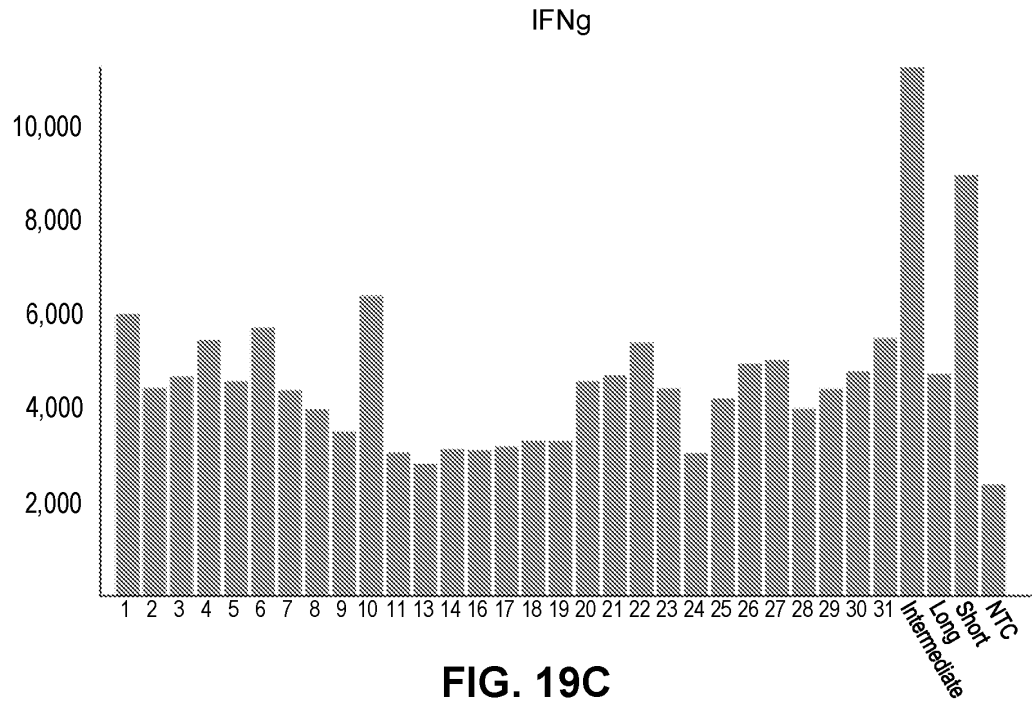


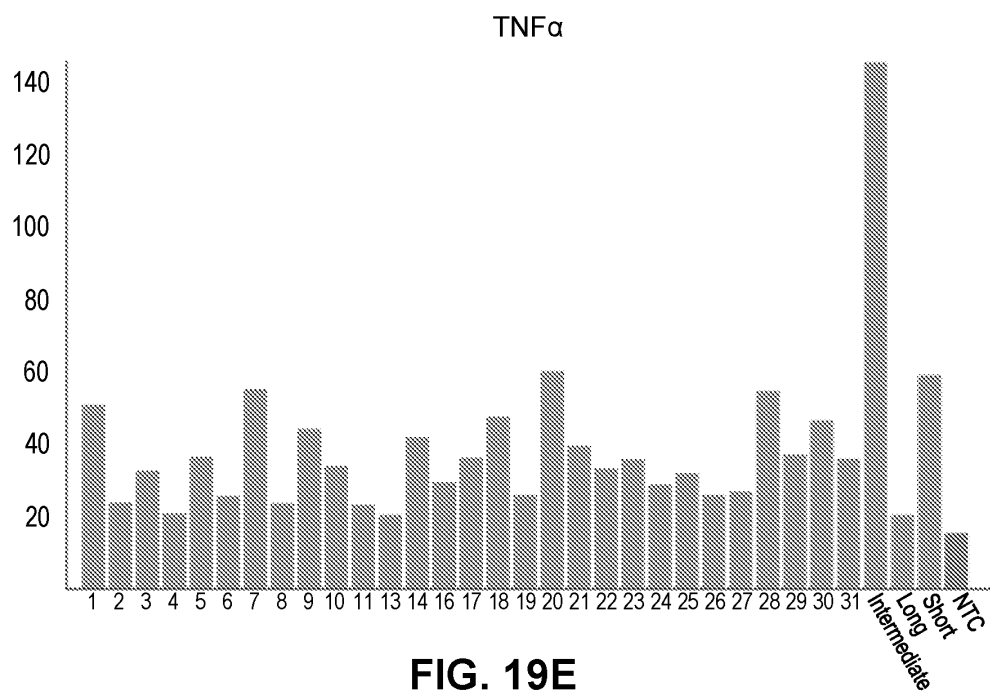
FIG. 19B

Primary killing and AUC D4869 CAR-T : Raji_CD19_KO-NLR 1:1

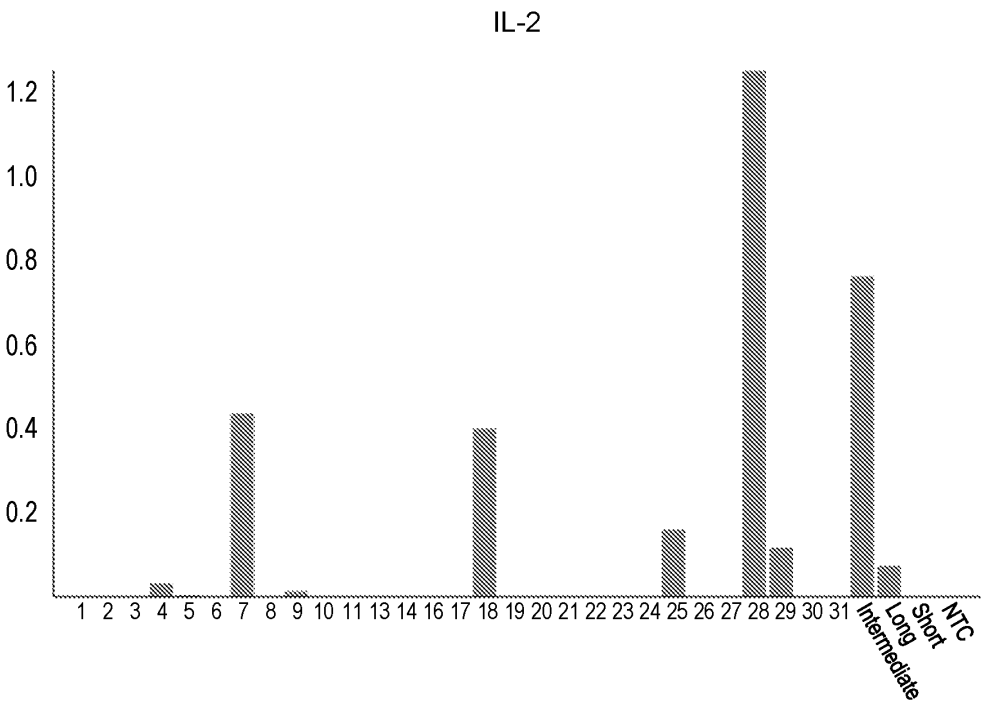
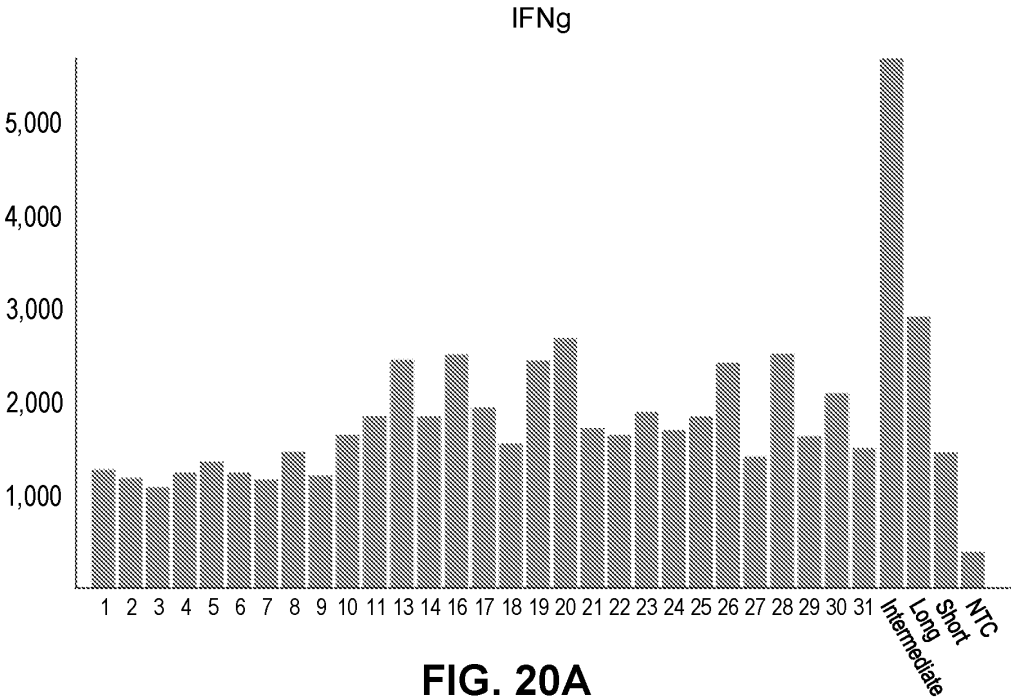


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Primary killing and AUC D4869 CAR-T : Raji_CD19_KO-NLR 1:1

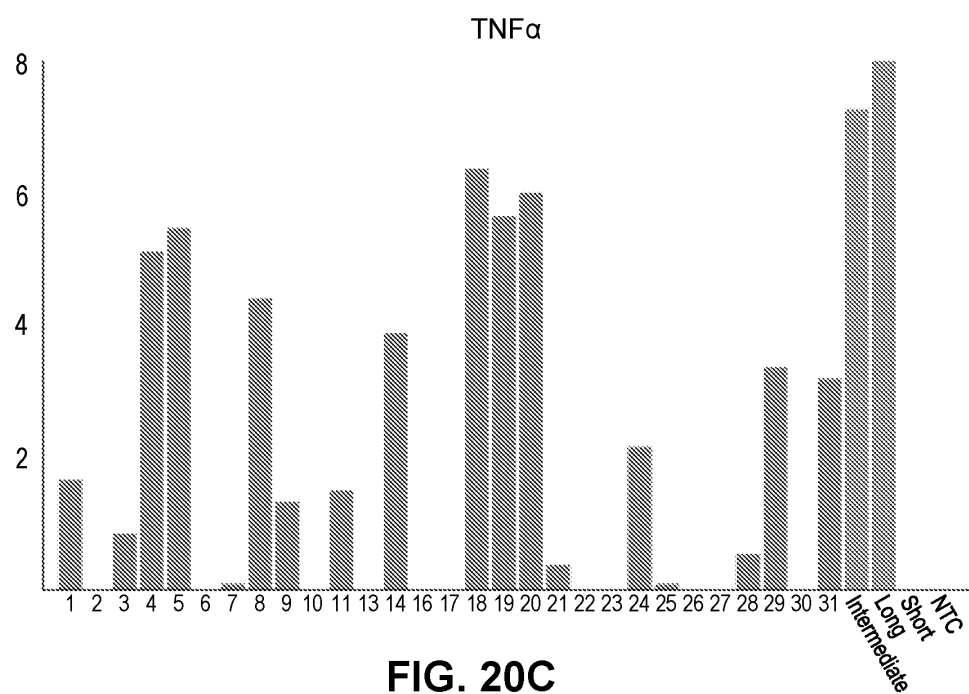


D3868 Target independent cytokines

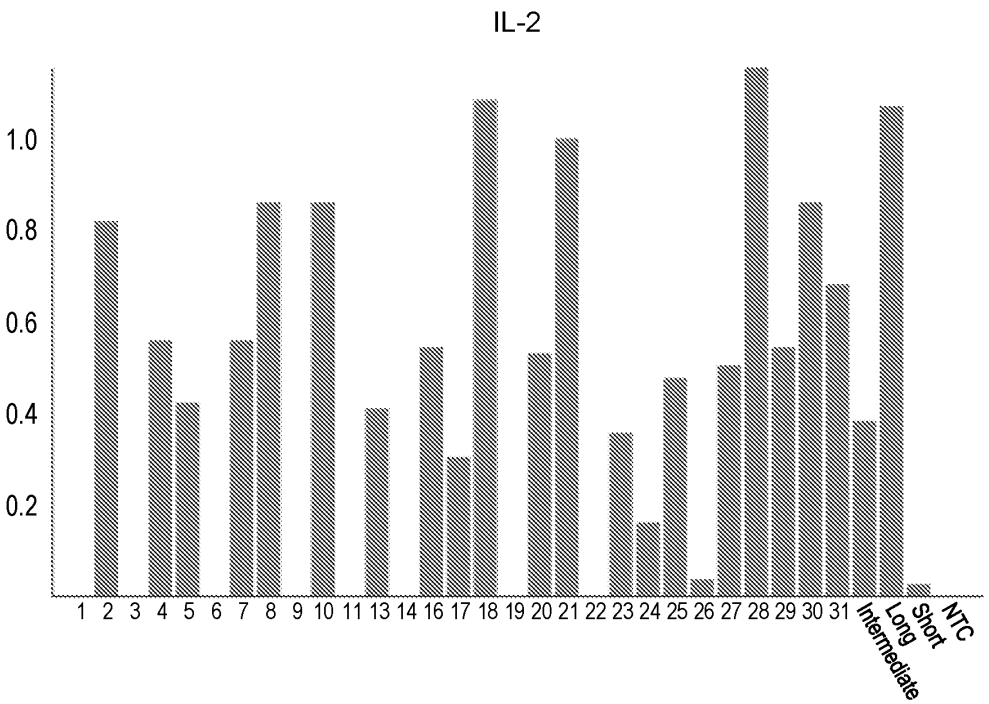
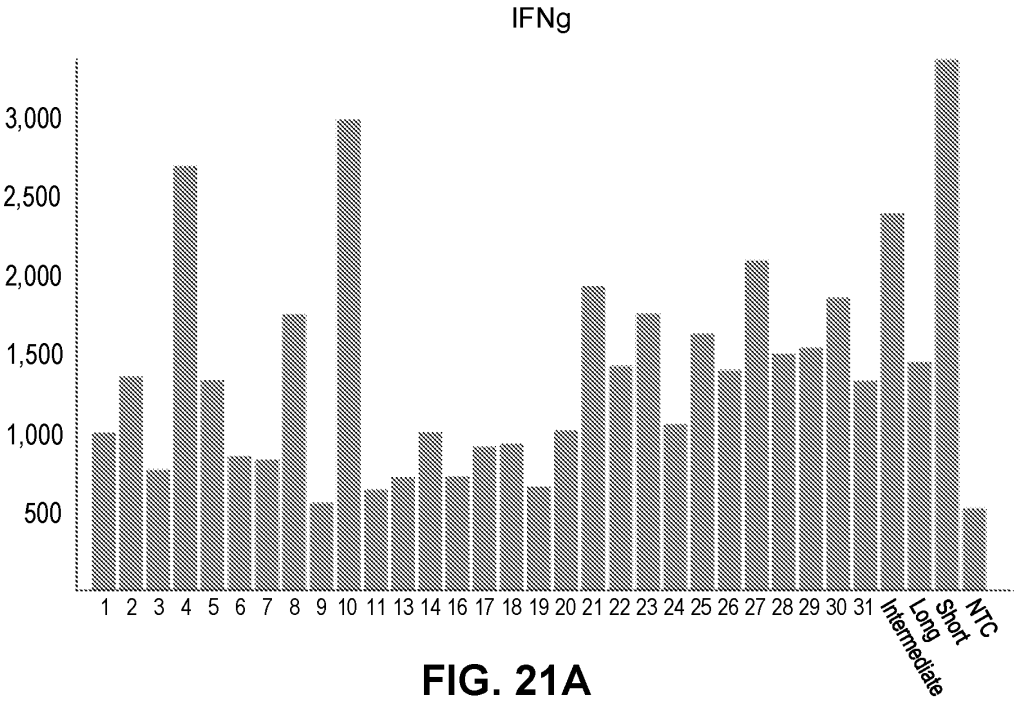


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D3868 Target independent cytokines

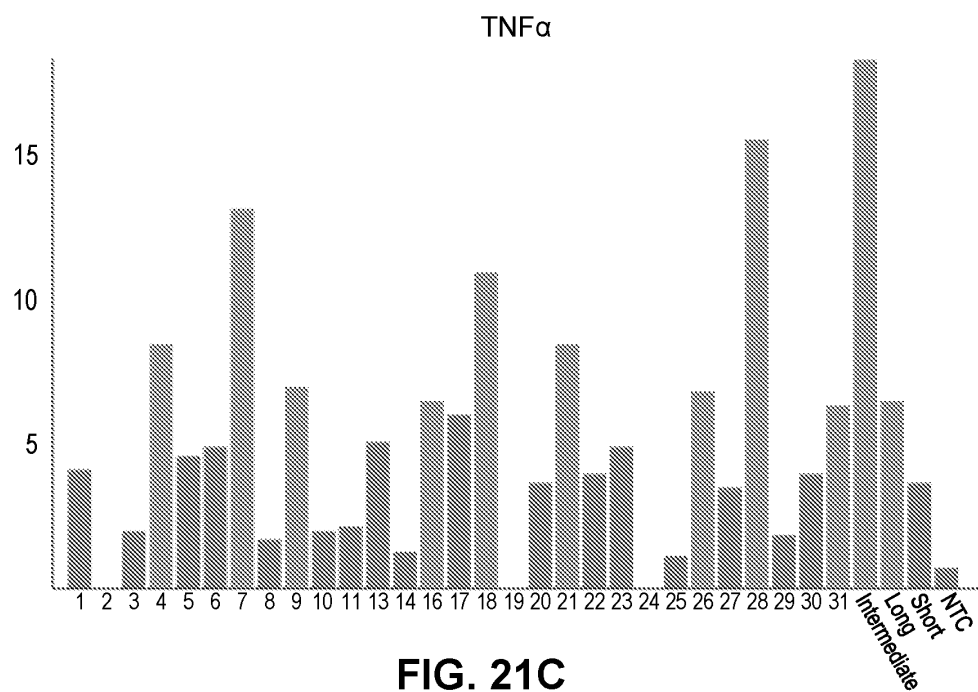


D4869 Target independent cytokines



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D4869 Target independent cytokines



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Cytokine vs AUC CD19 CAR : Raji = 1:1

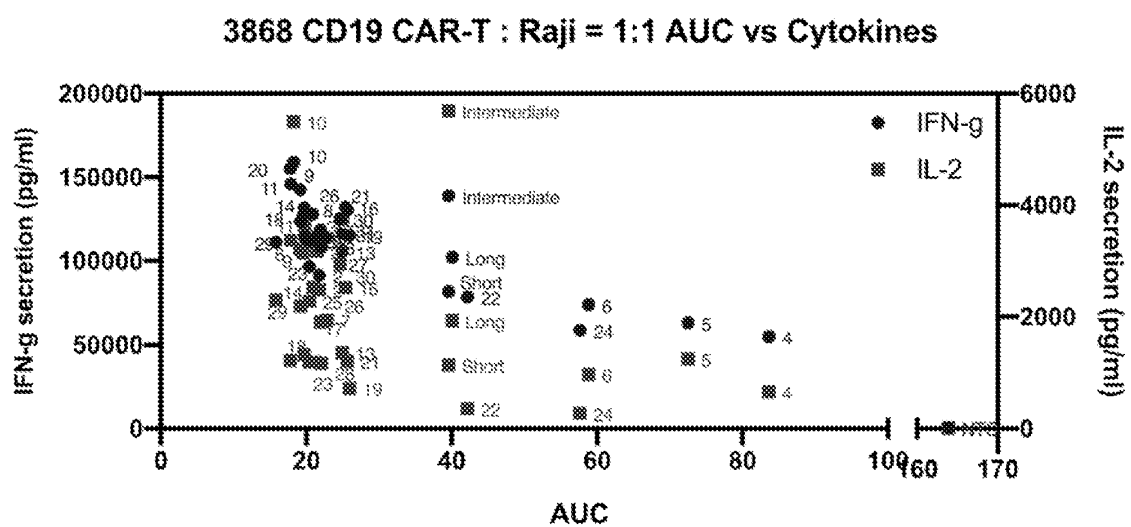


FIG. 22A

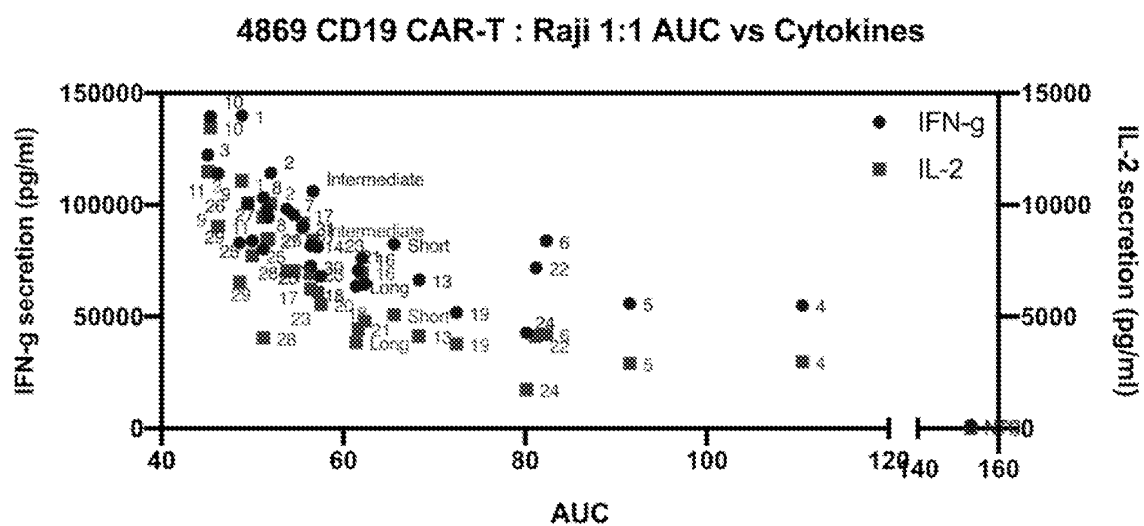


FIG. 22B

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Cytokine vs Spacer Length CD19 E:T = 1:1

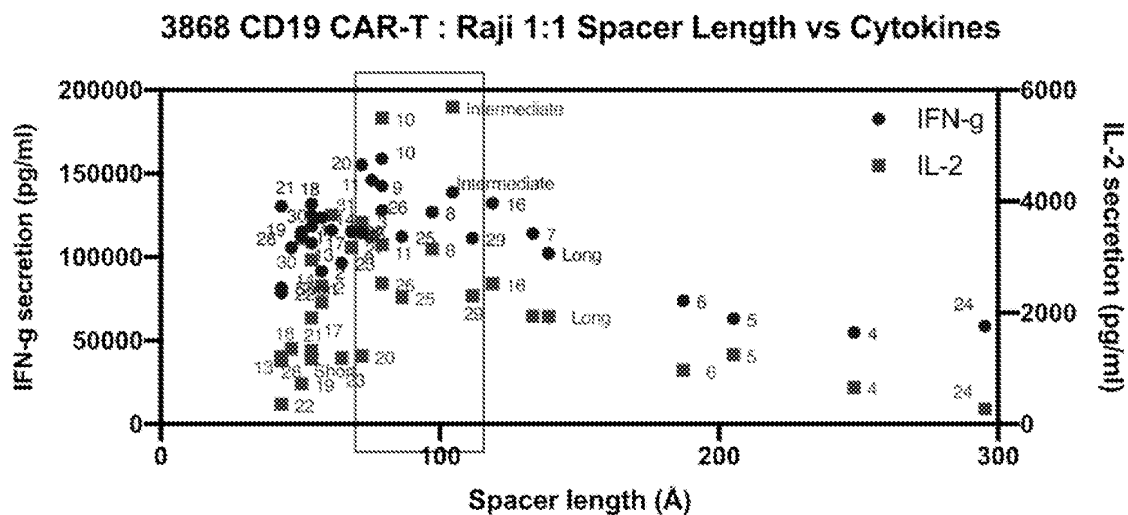


FIG. 23A

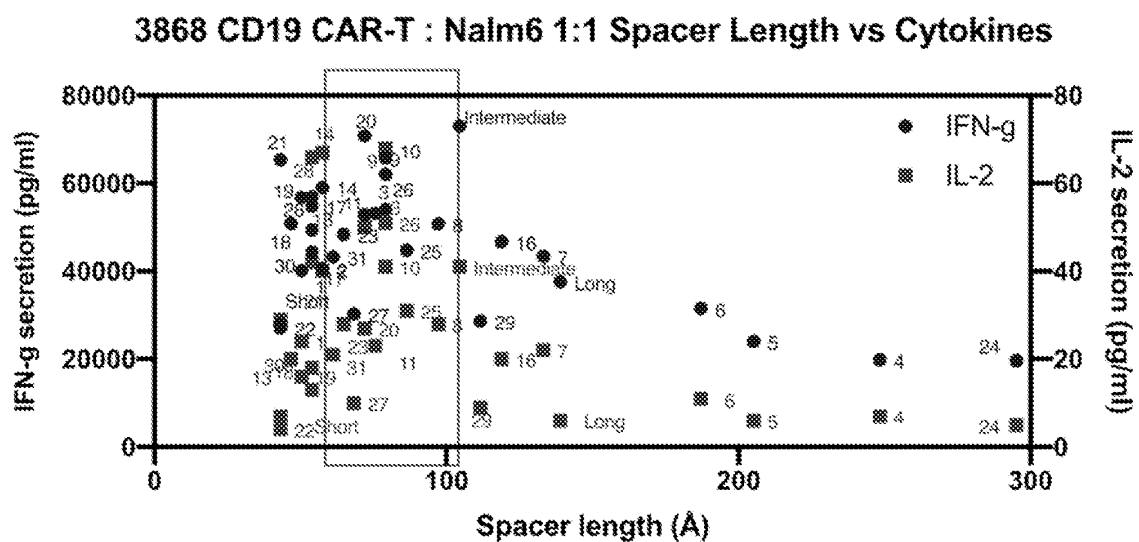


FIG. 23B

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Cytokine vs Spacer Length CD19 E:T = 1:1

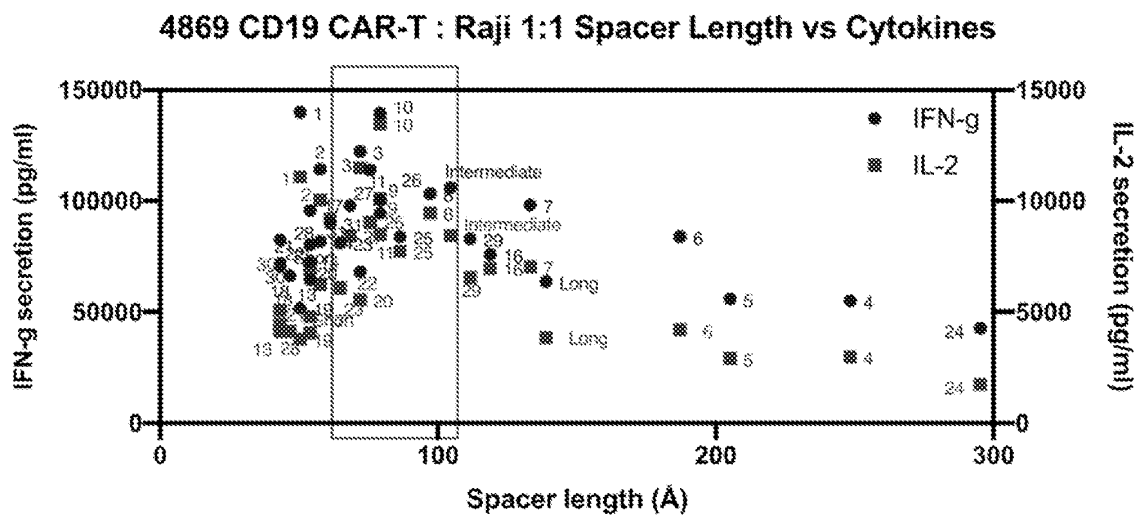


FIG. 23C

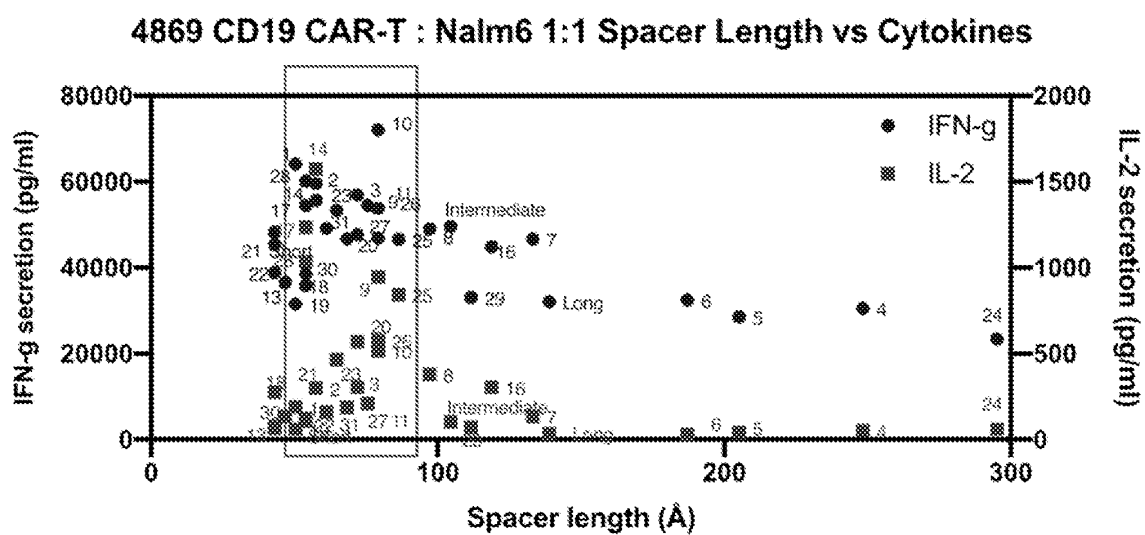


FIG. 23D

Spacer Length vs AUC CD19 CAR : Raji = 1:1

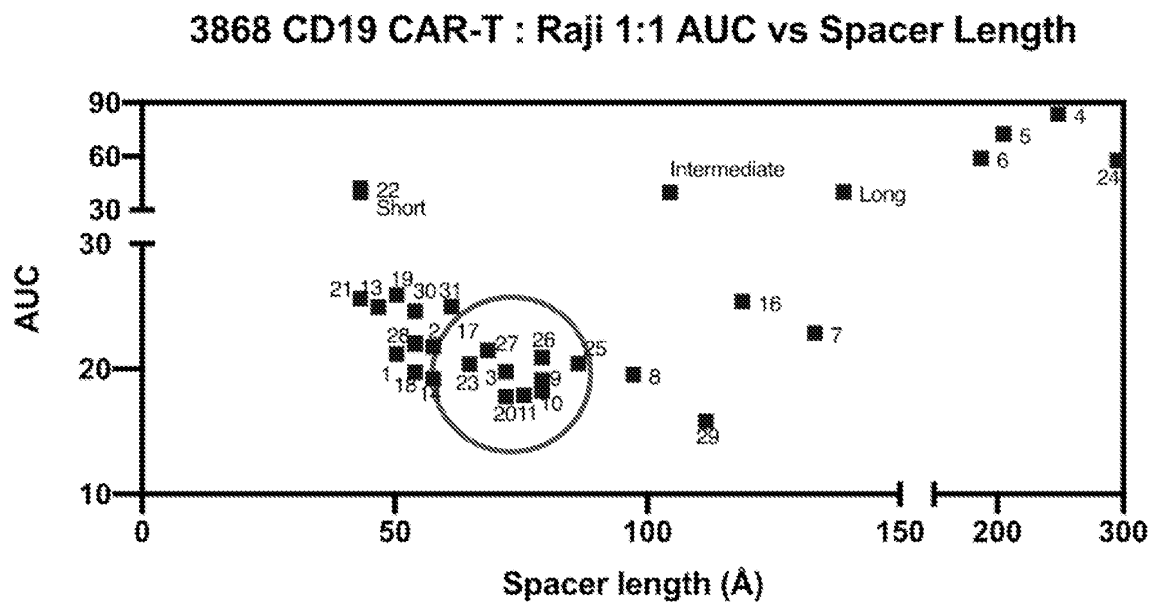


FIG. 24A

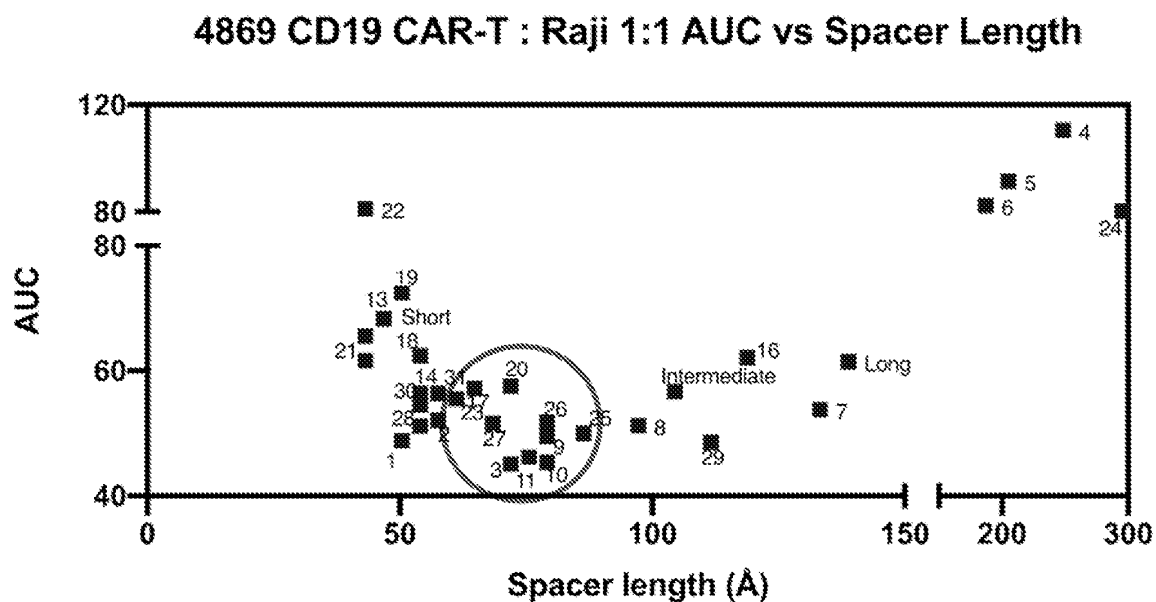


FIG. 24B

Sequential Kill Donor 3868

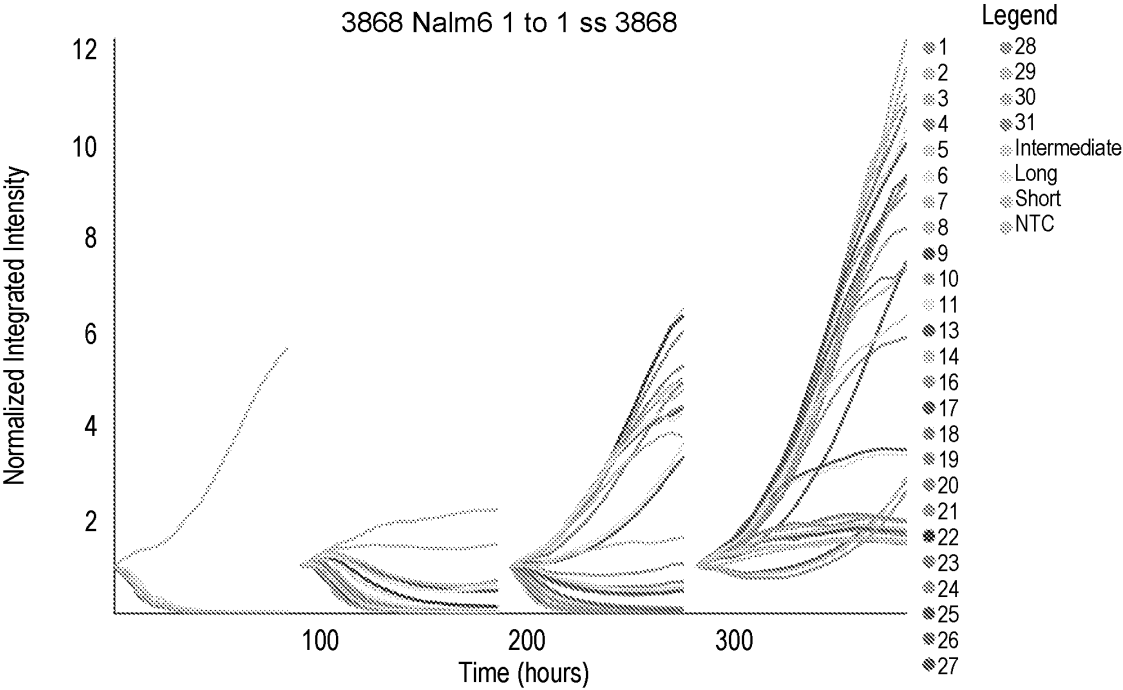


FIG. 25A

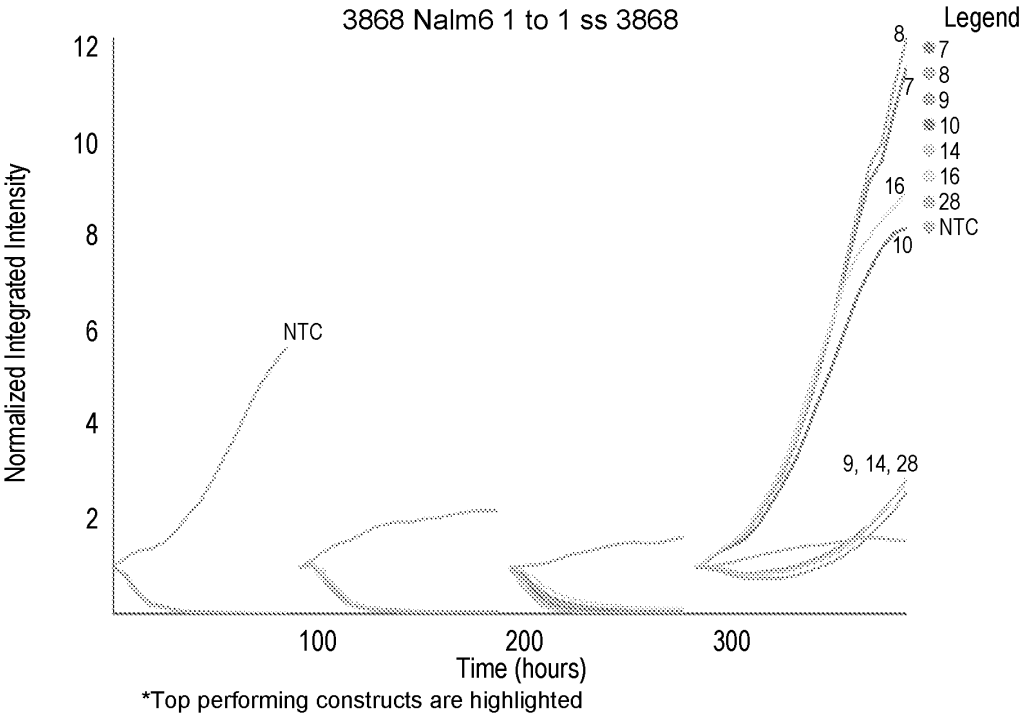


FIG. 25B

Sequential Kill Donor 3868

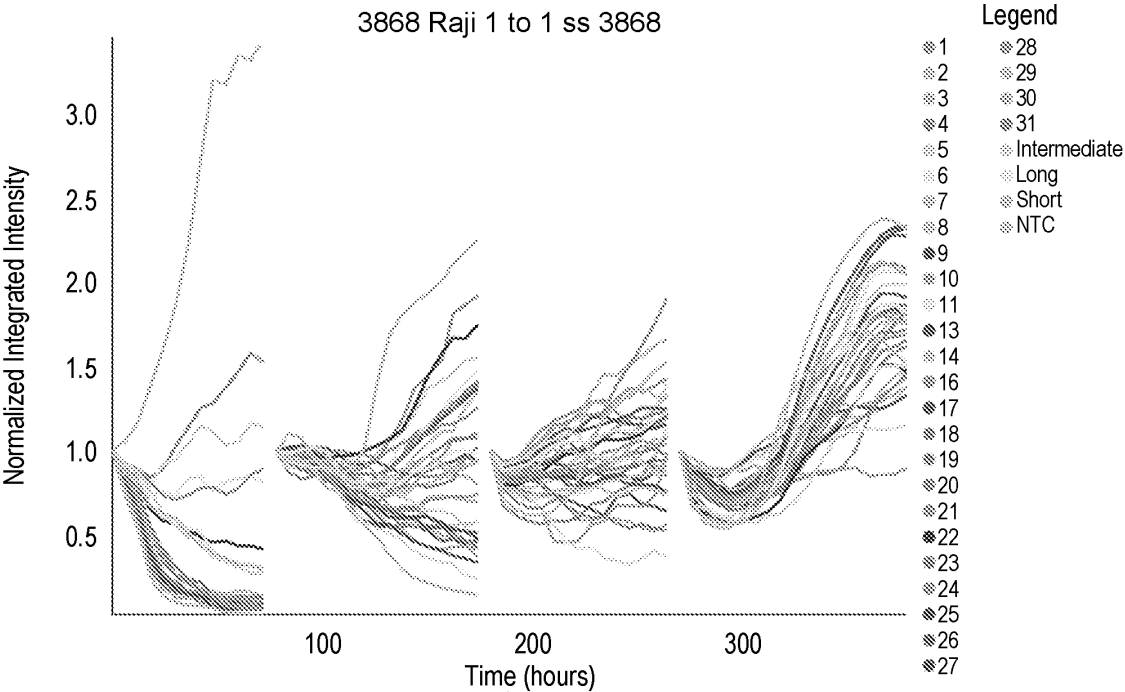
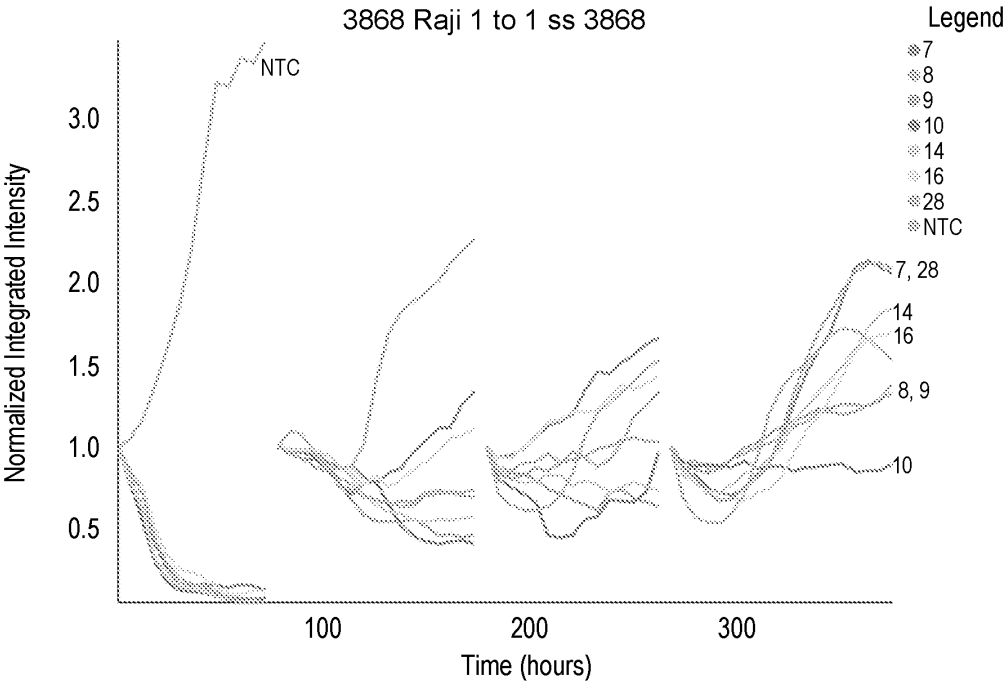


FIG. 25C



*Top performing constructs are highlighted

FIG. 25D

Sequential Kill Donor 4869

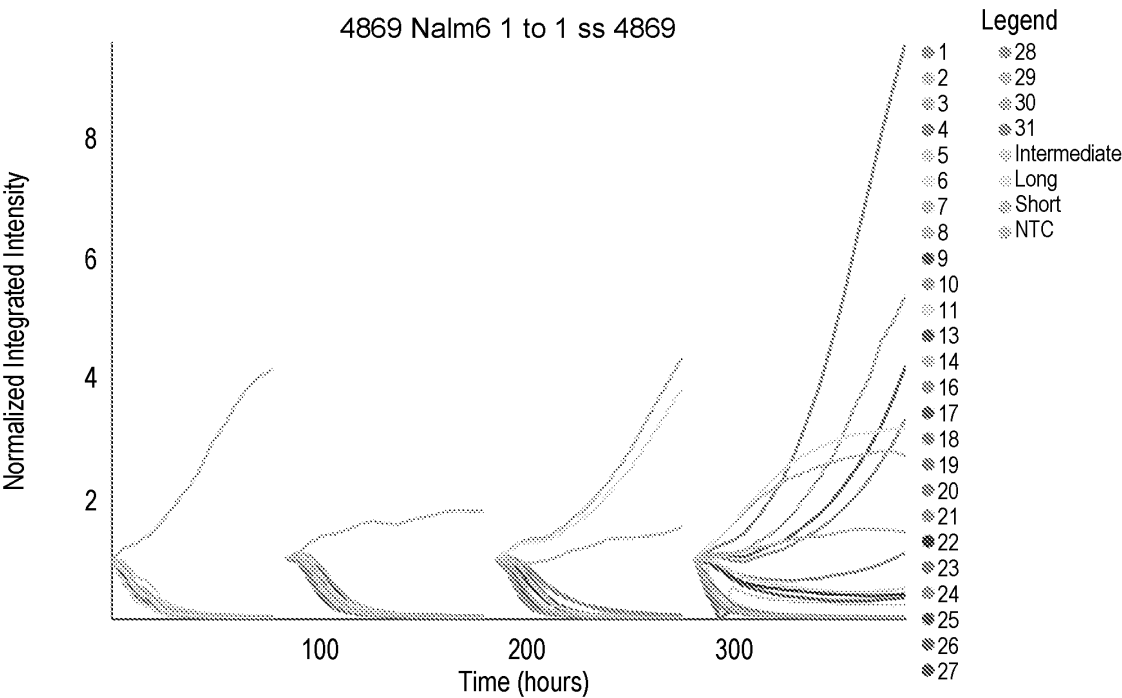


FIG. 26A

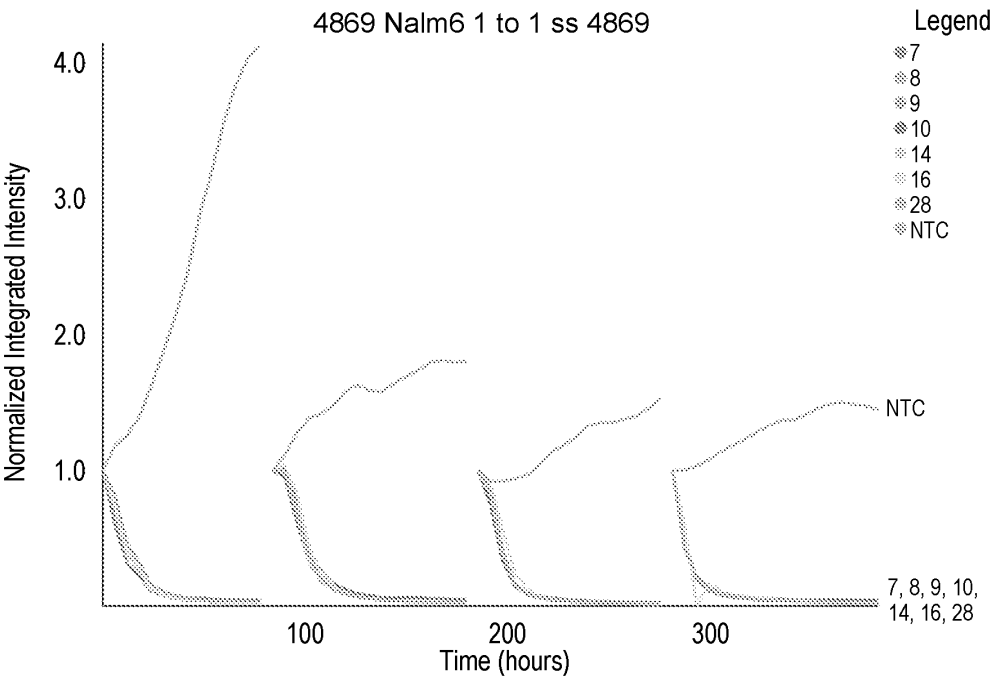


FIG. 26B

Sequential Kill Donor 4869

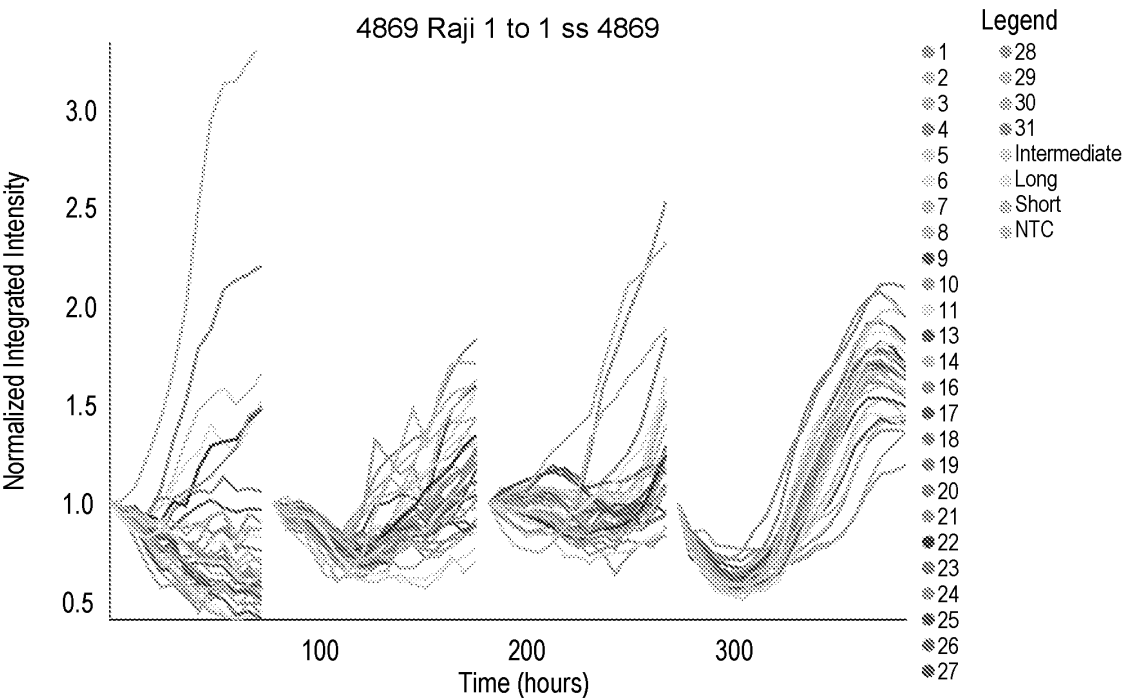


FIG. 26C

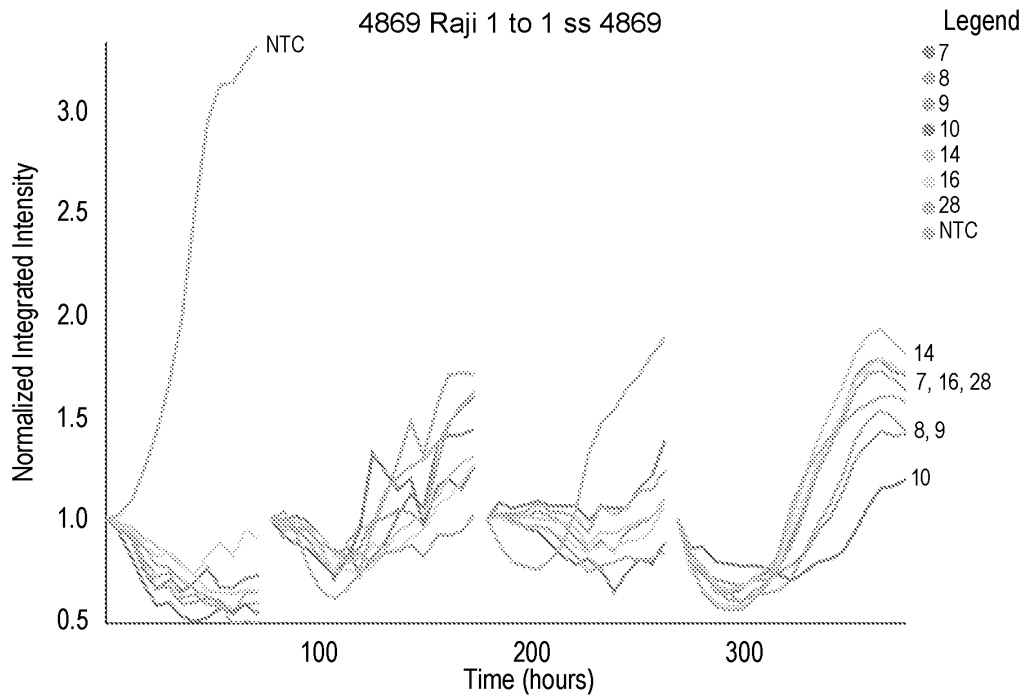


FIG. 26D

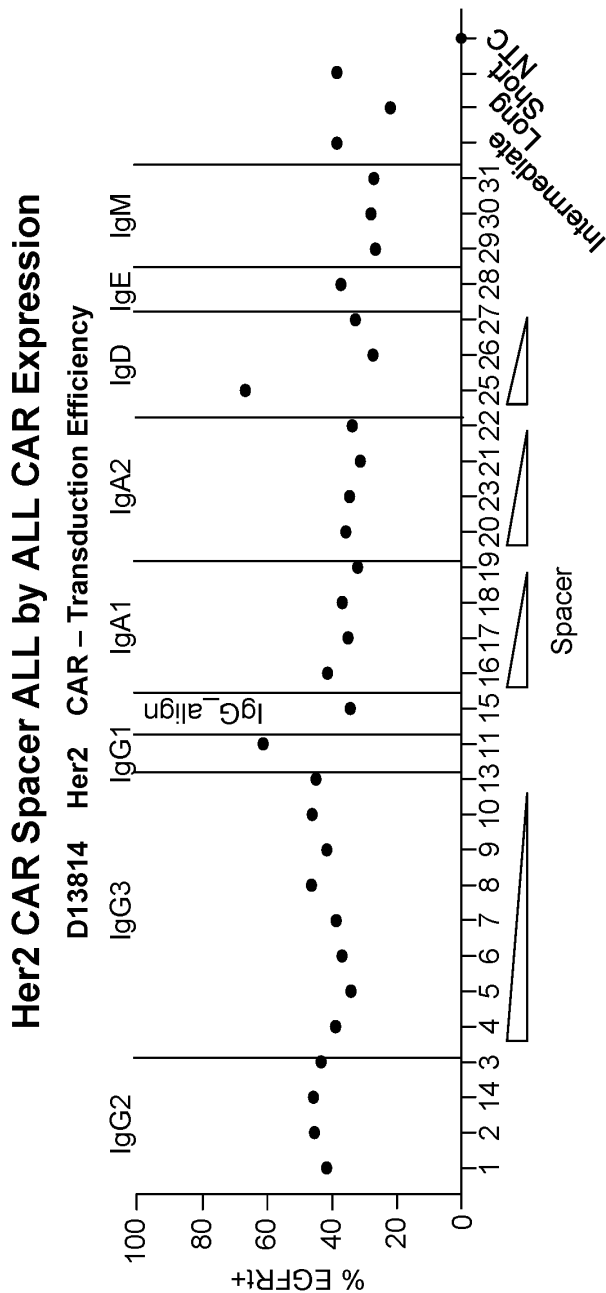


FIG. 27A

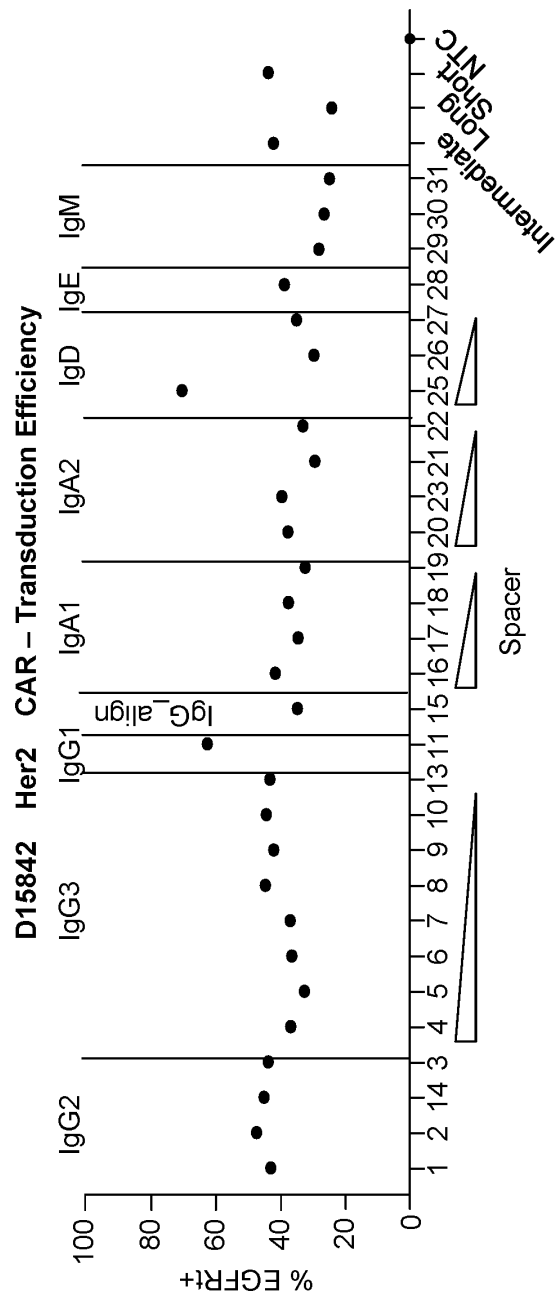


FIG. 27B

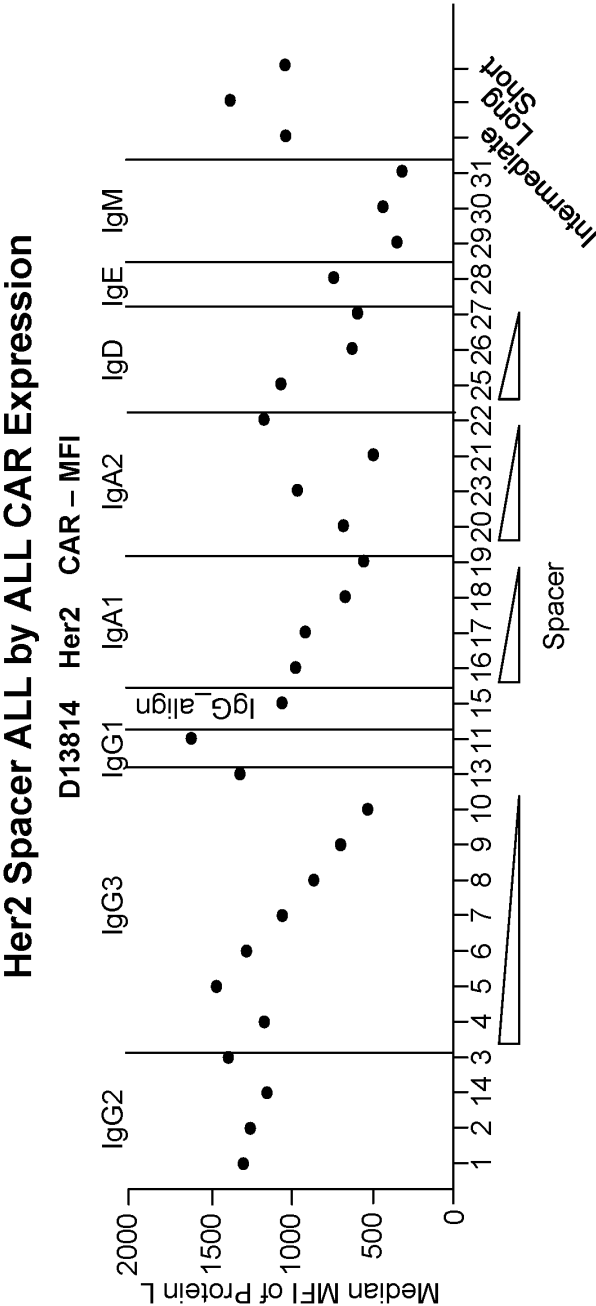


FIG. 27C

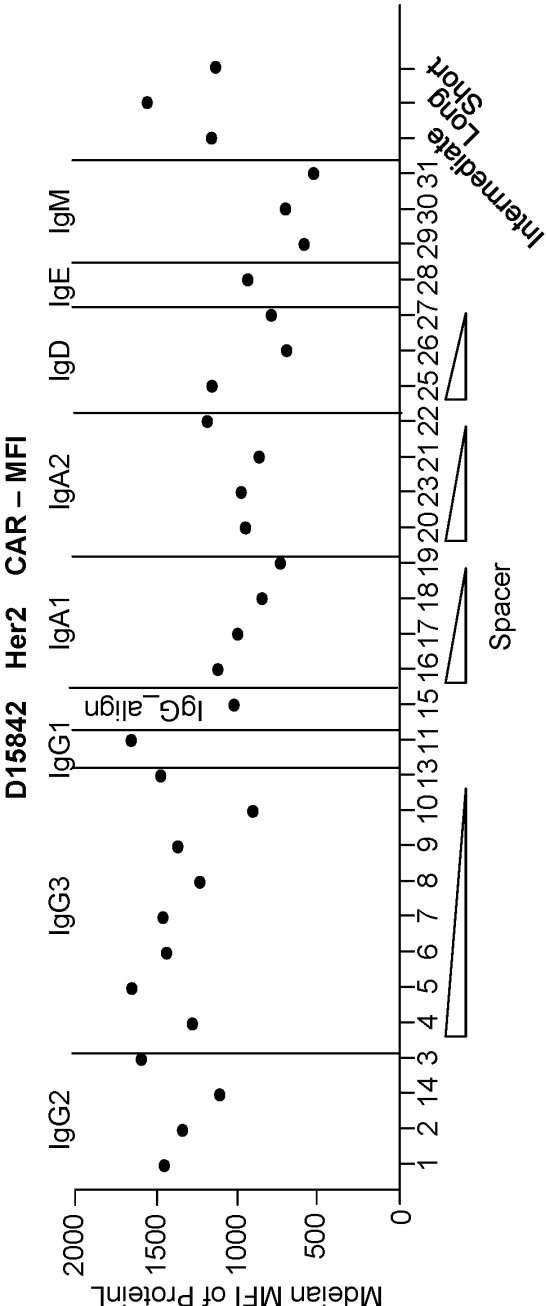


FIG. 27D

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Primary killing and AUC D13814 CAR-T : A549-NLR 1:1

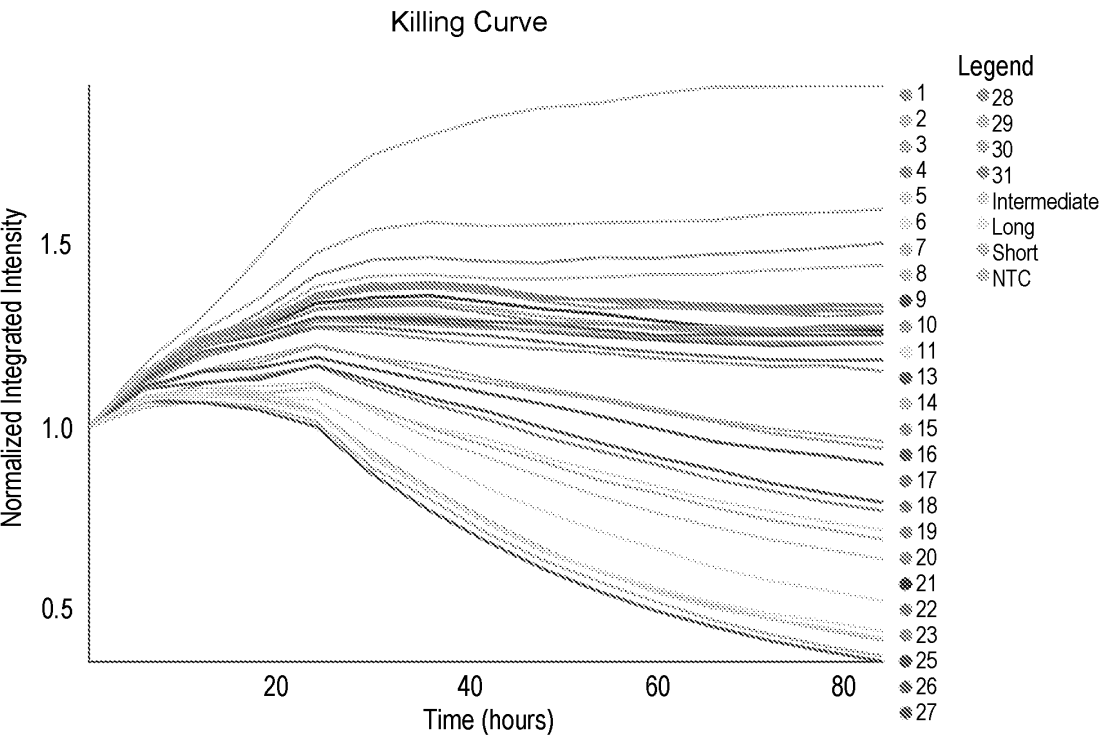


FIG. 28A

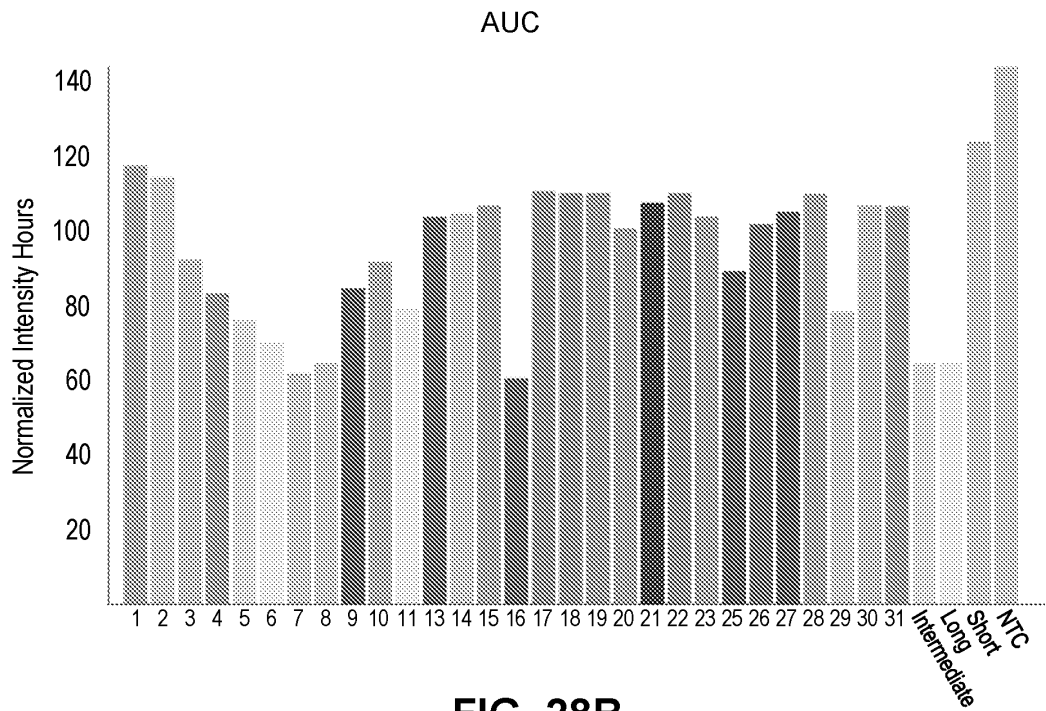
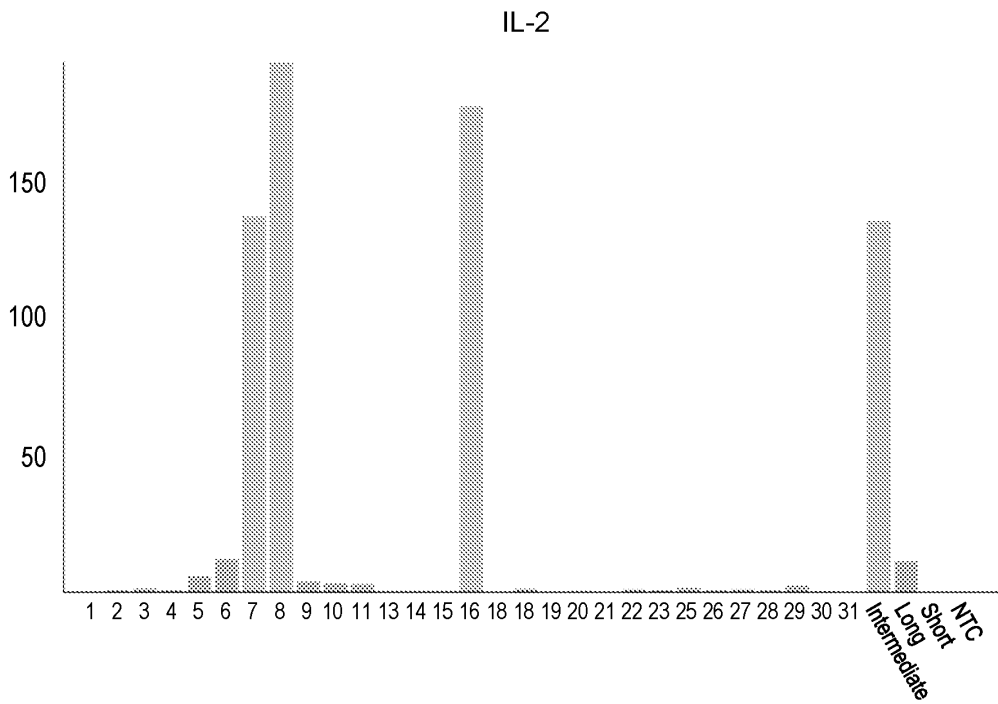
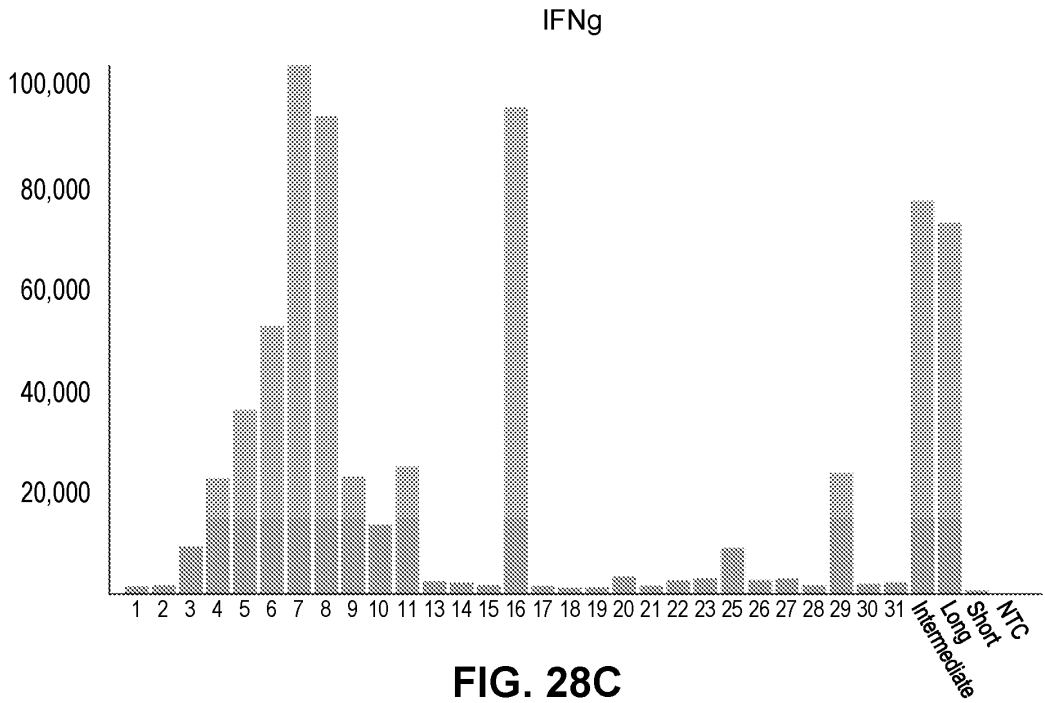


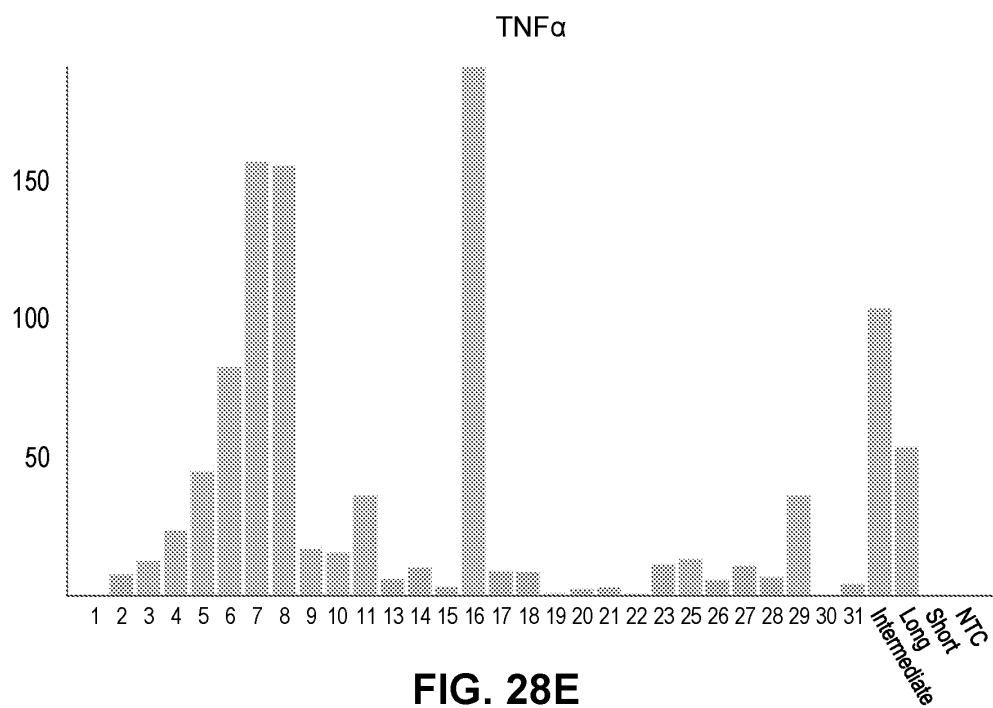
FIG. 28B

Primary killing and AUC D13814 CAR-T : A549-NLR 1:1



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Primary killing and AUC D13814 CAR-T : A549-NLR 1:1



Primary killing and AUC D15842 CAR-T : A549-NLR 1:1

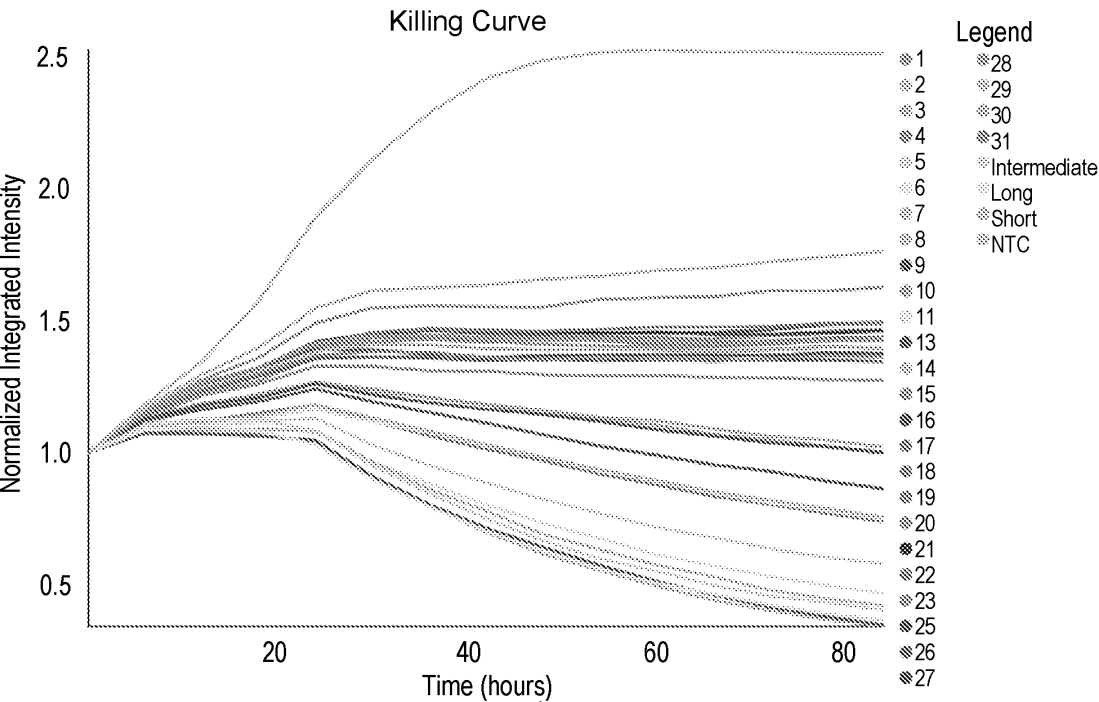


FIG. 29A

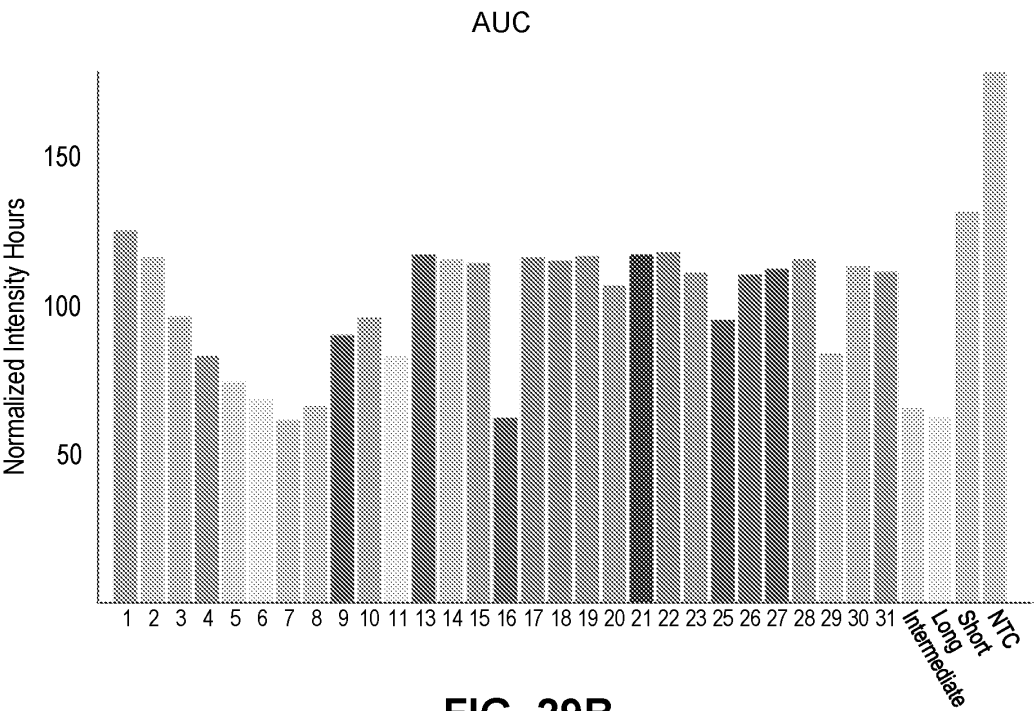
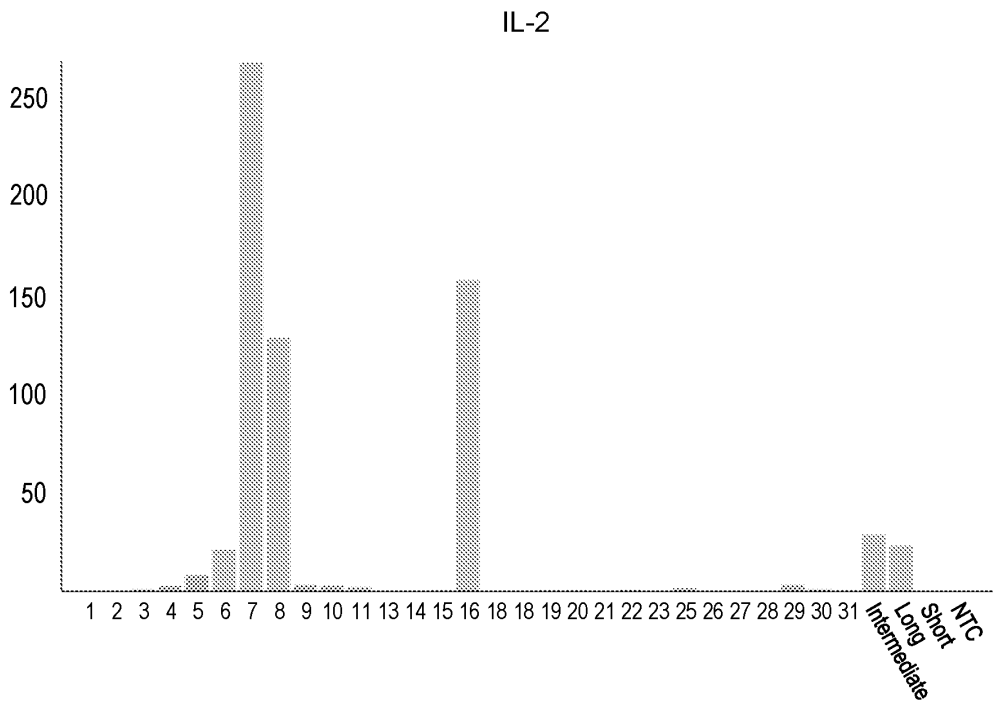
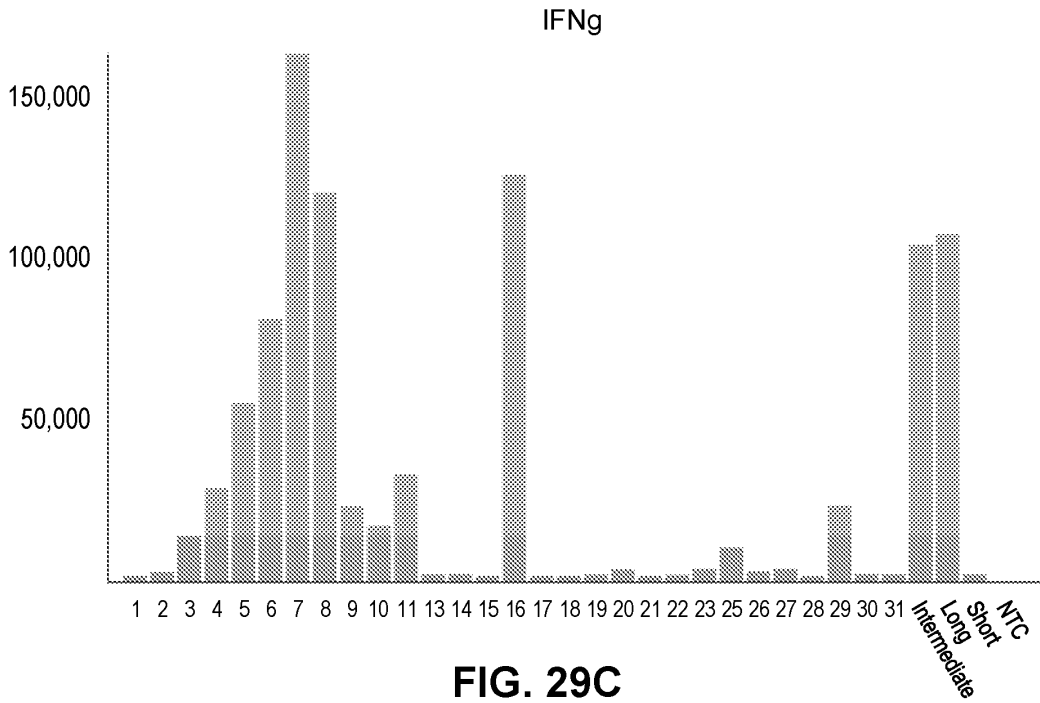


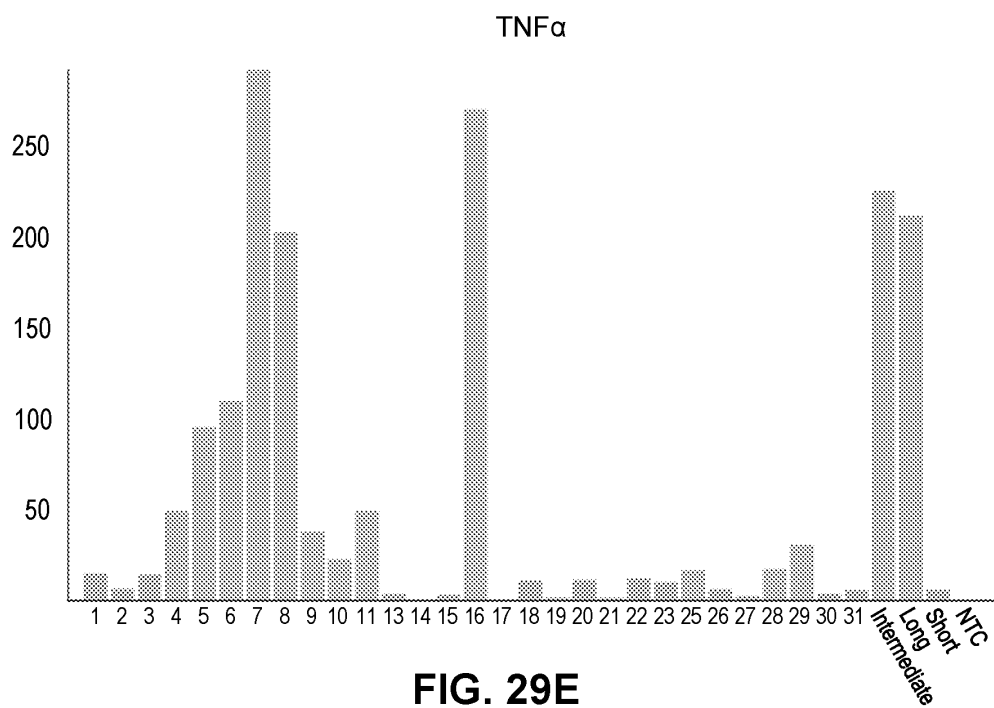
FIG. 29B

Primary killing and AUC D15842 CAR-T : A549-NLR 1:1



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Primary killing and AUC D15842 CAR-T : A549-NLR 1:1



Primary killing and AUC D13814 CAR-T : T47D-NLR 1:1

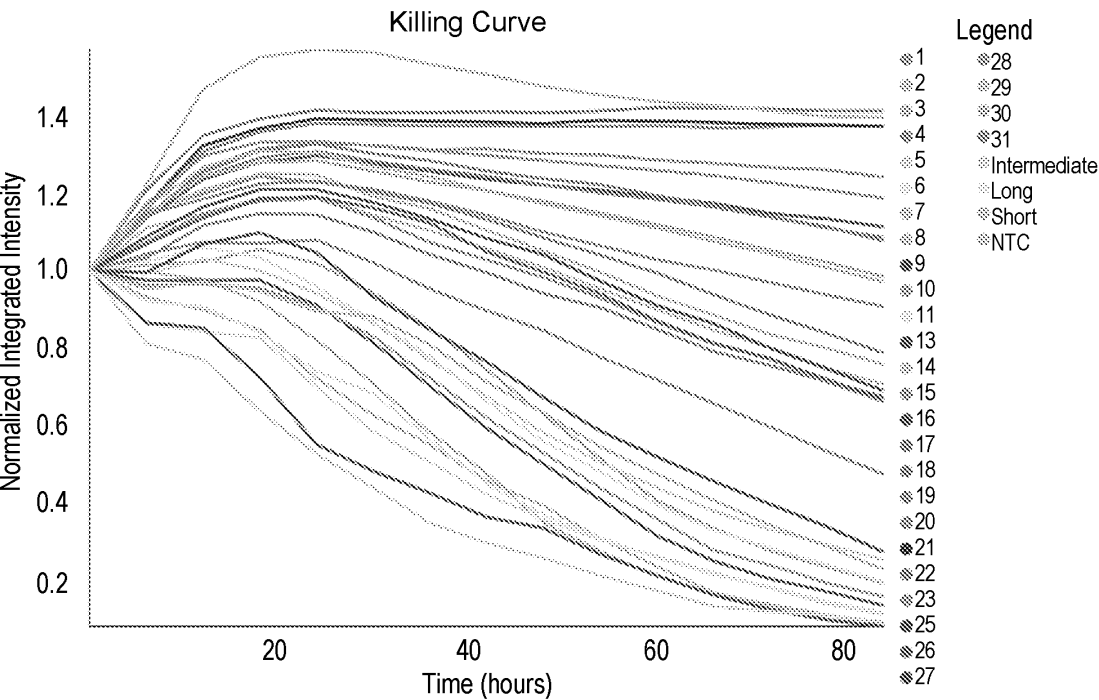


FIG. 30A

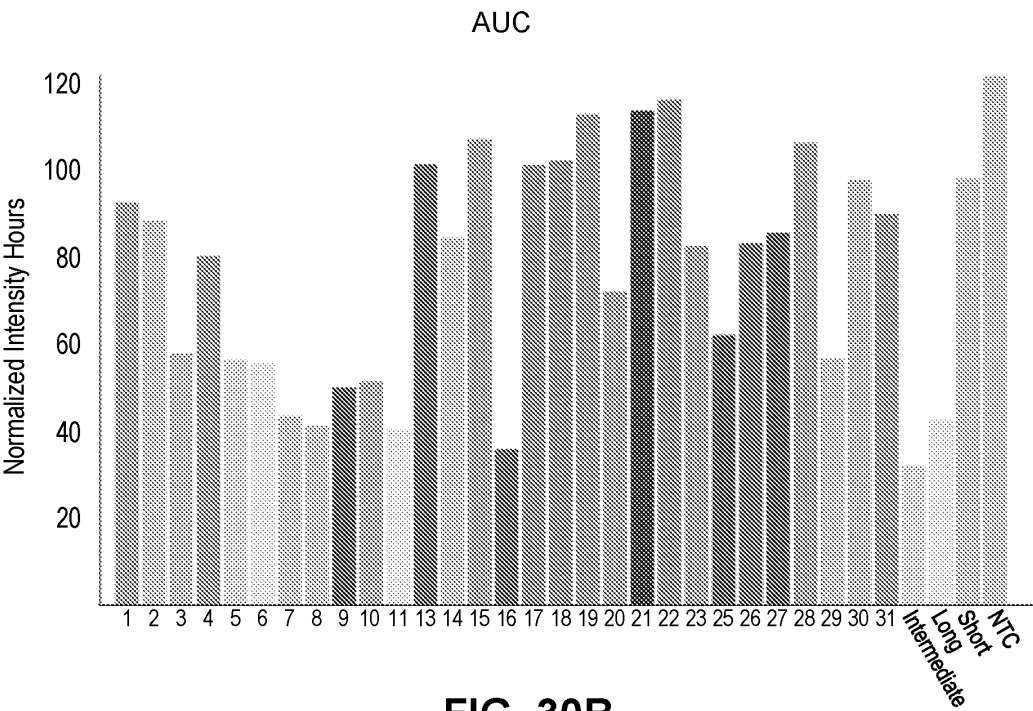
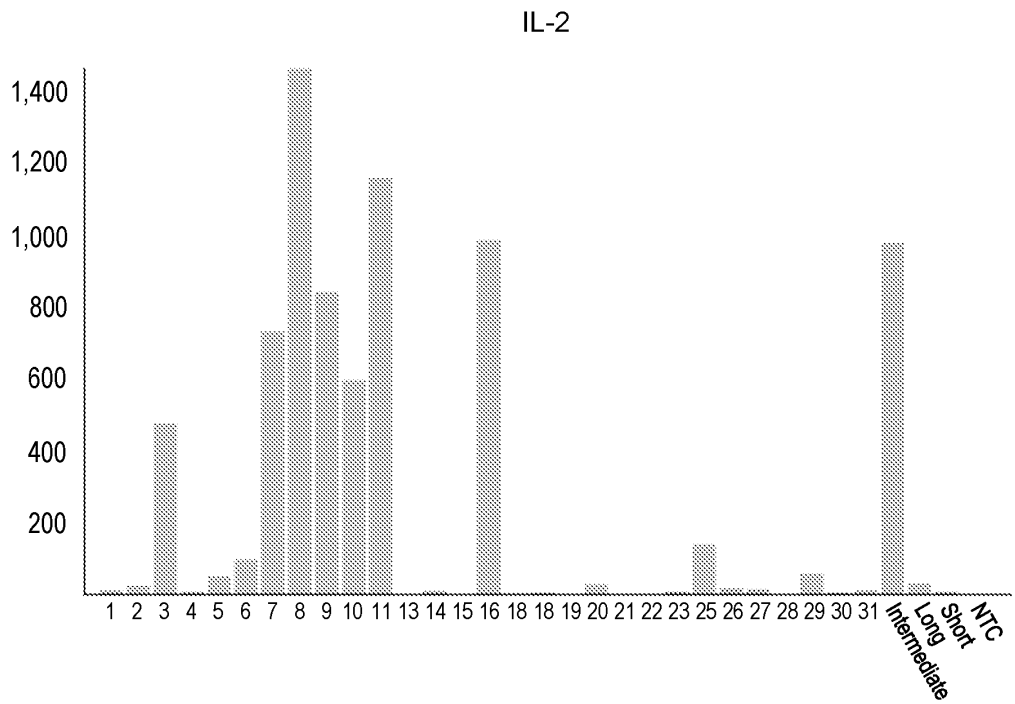
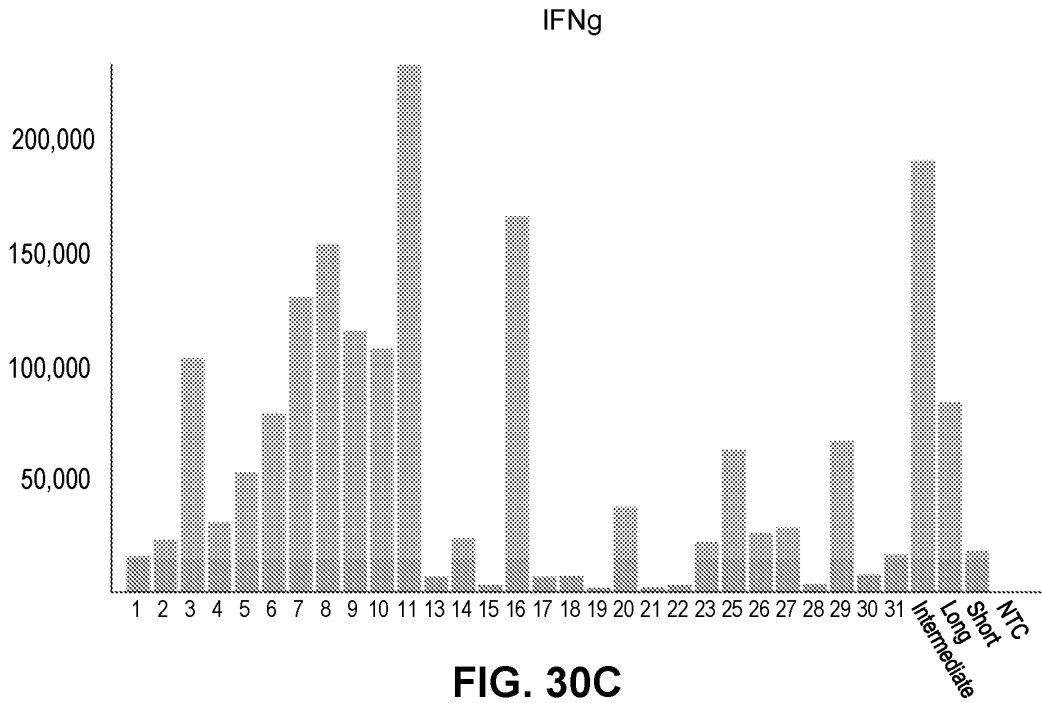


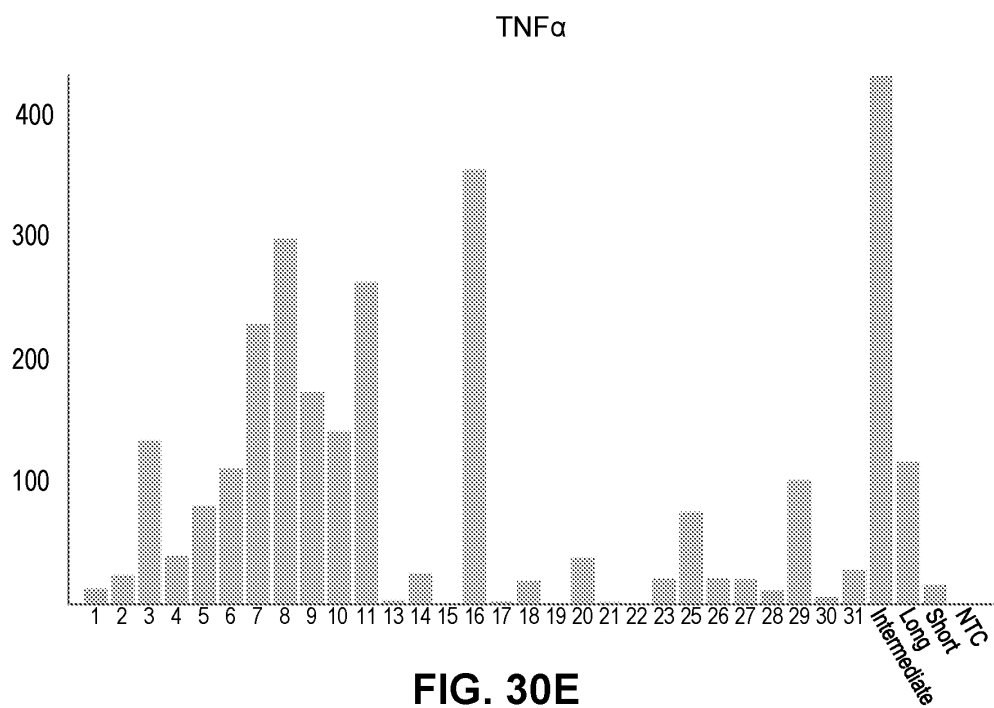
FIG. 30B

Primary killing and AUC D13814 CAR-T : T47D-NLR 1:1



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Primary killing and AUC D13814 CAR-T : T47D-NLR 1:1



Primary killing and AUC D15842 CAR-T : T47D-NLR 1:1

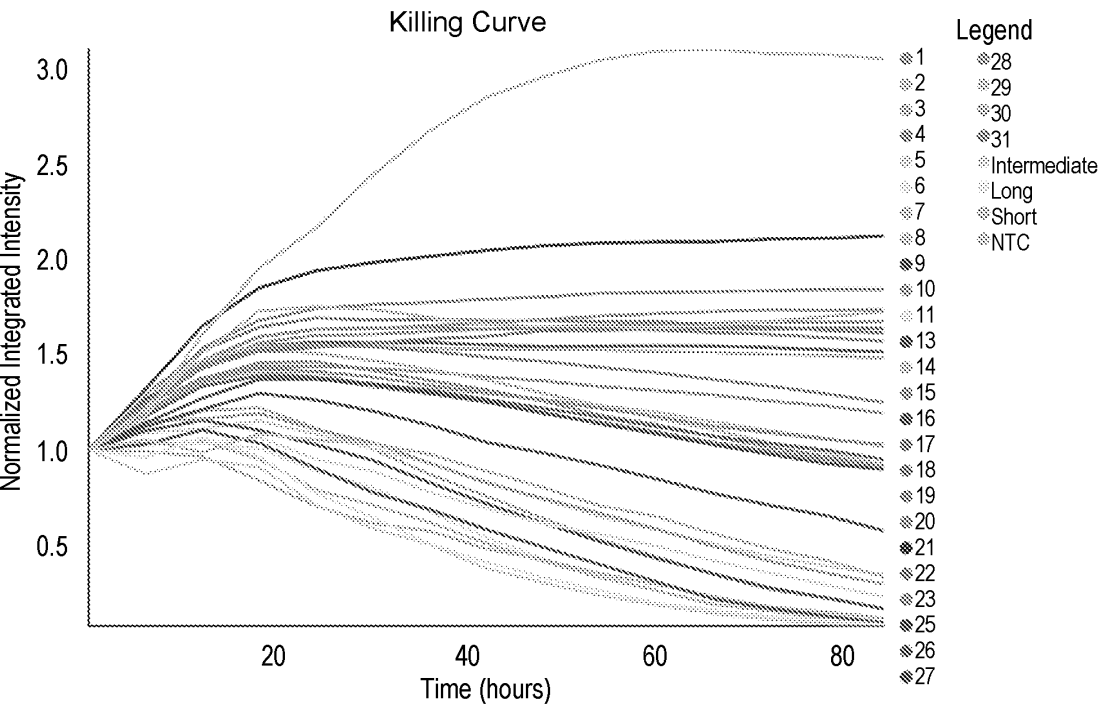


FIG. 31A

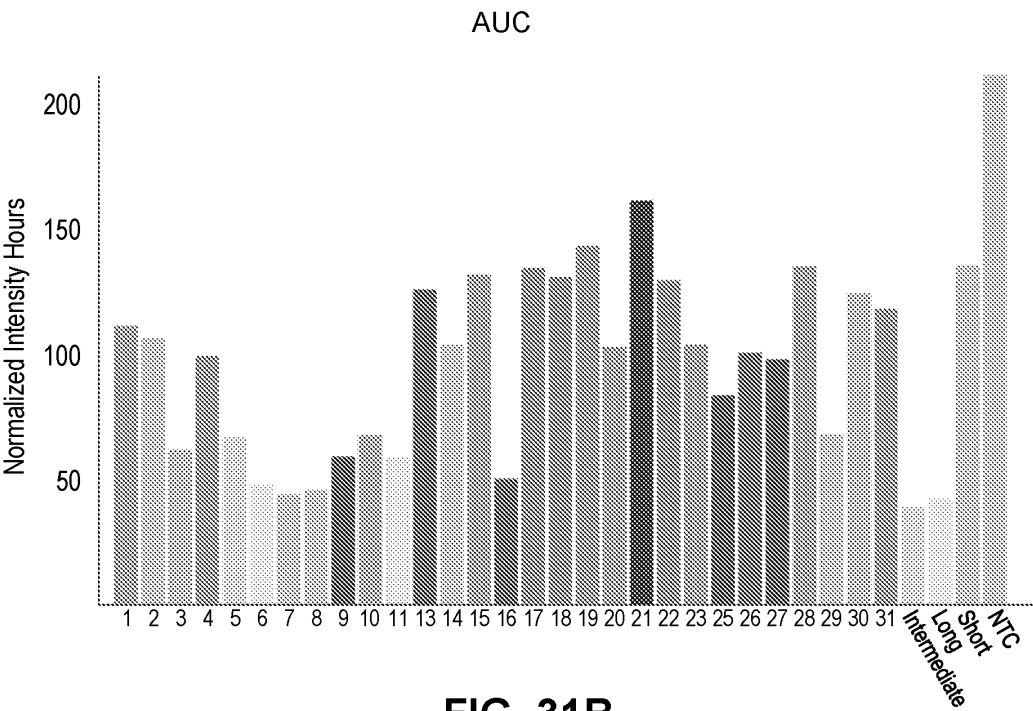
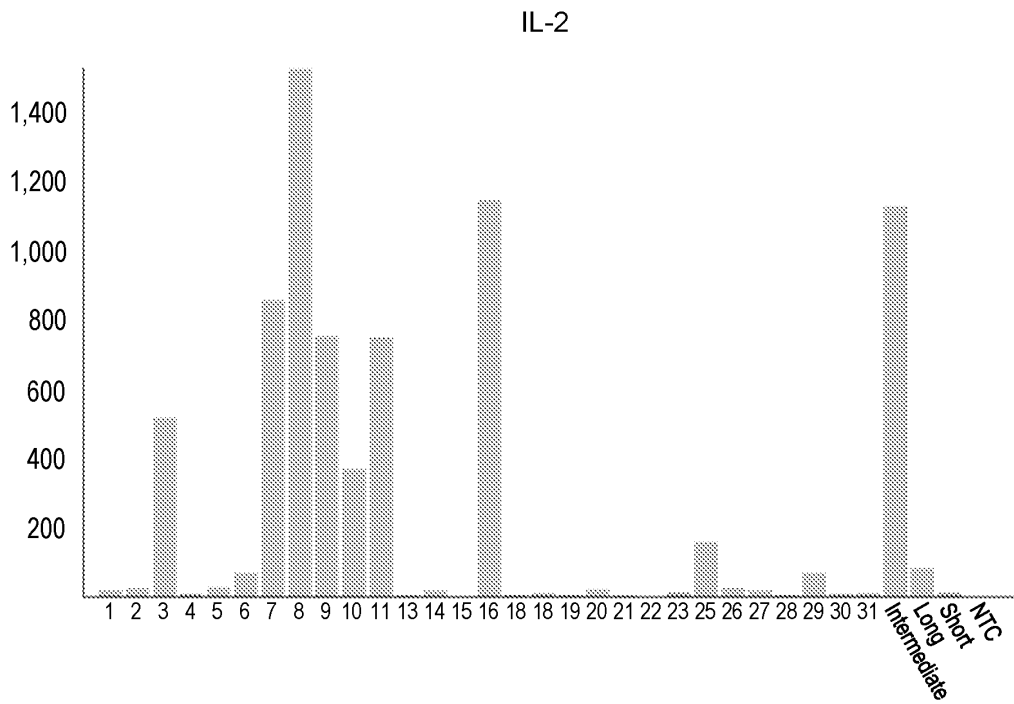
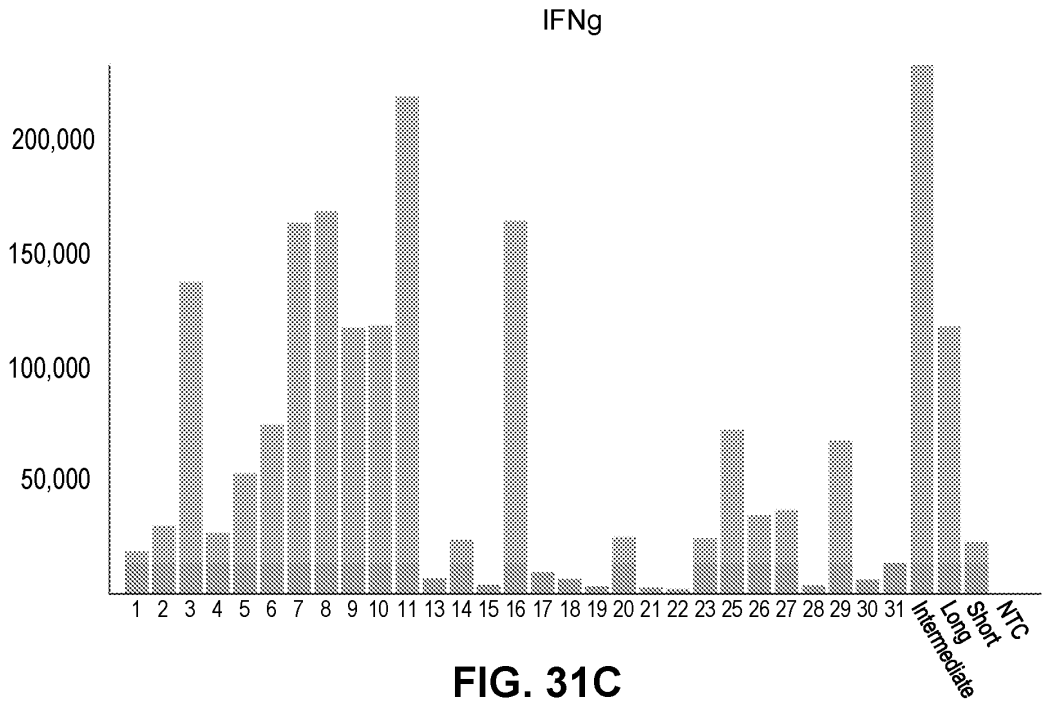


FIG. 31B

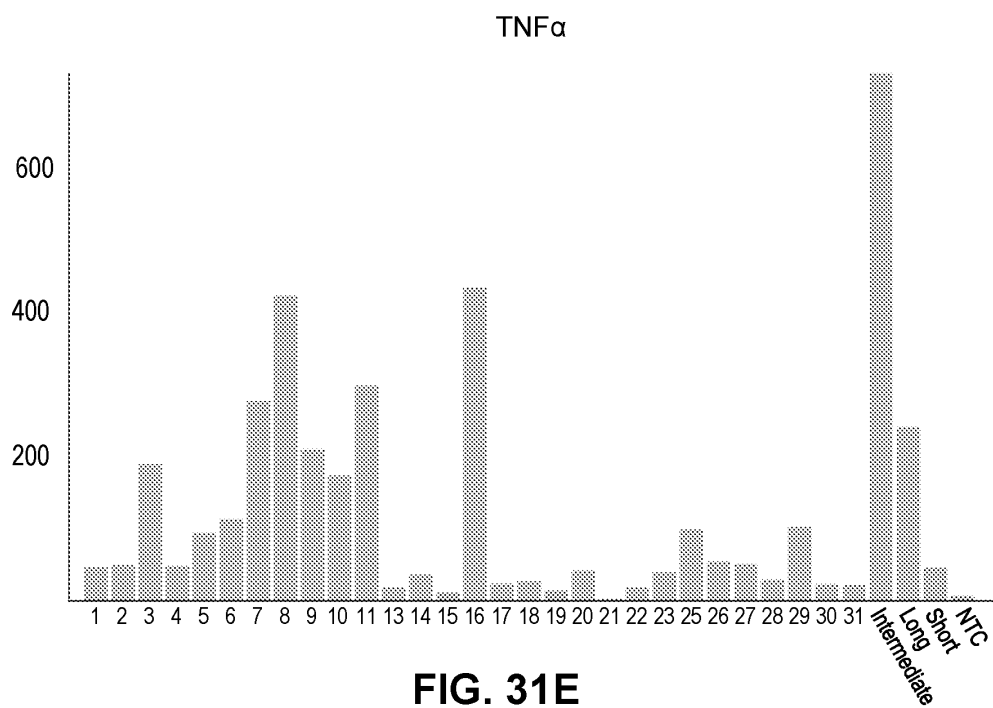
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Primary killing and AUC D15842 CAR-T : T47D-NLR 1:1



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Primary killing and AUC D15842 CAR-T : T47D-NLR 1:1



Primary killing and AUC D13814 CAR-T : T47D-Her2KO-NLR 1:1

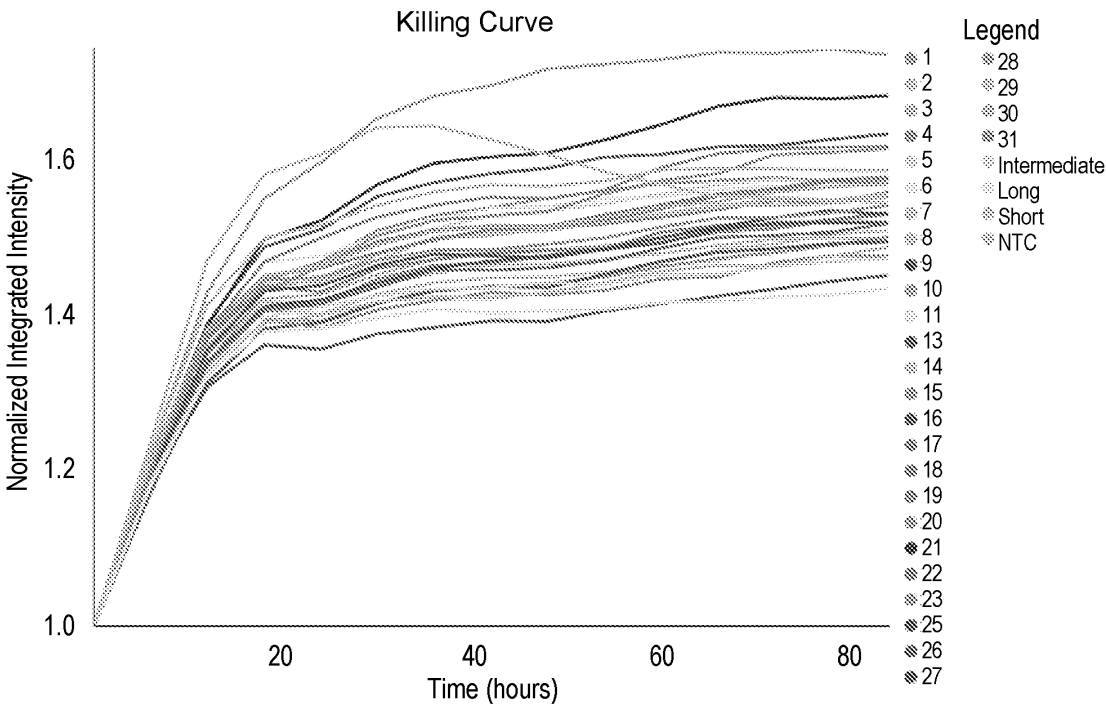


FIG. 32A

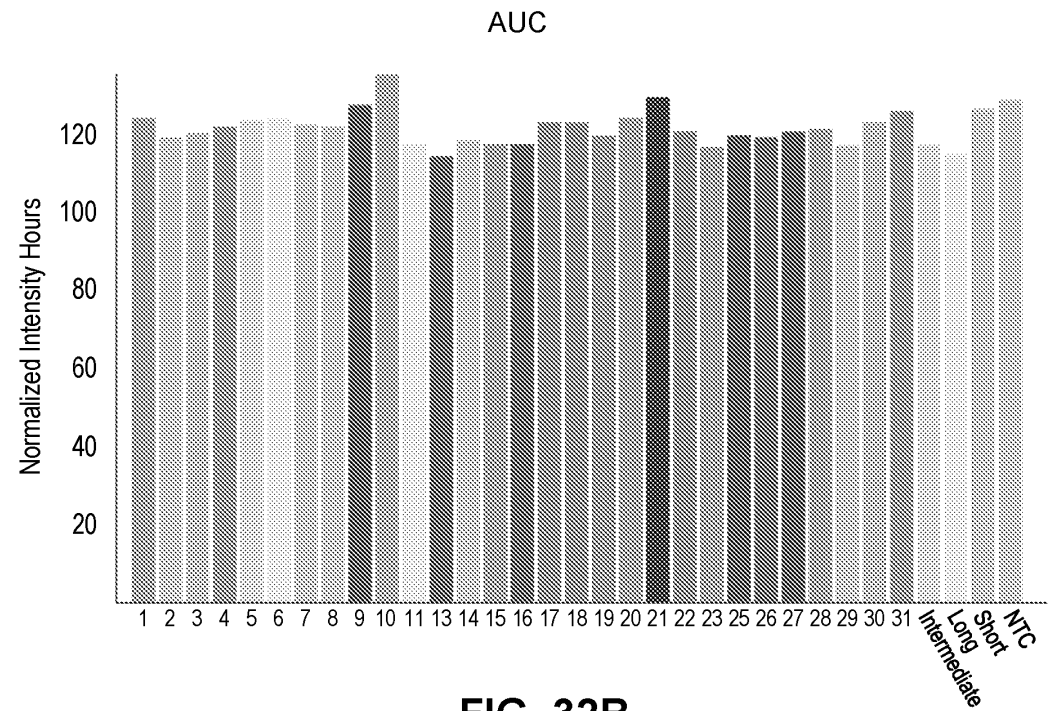
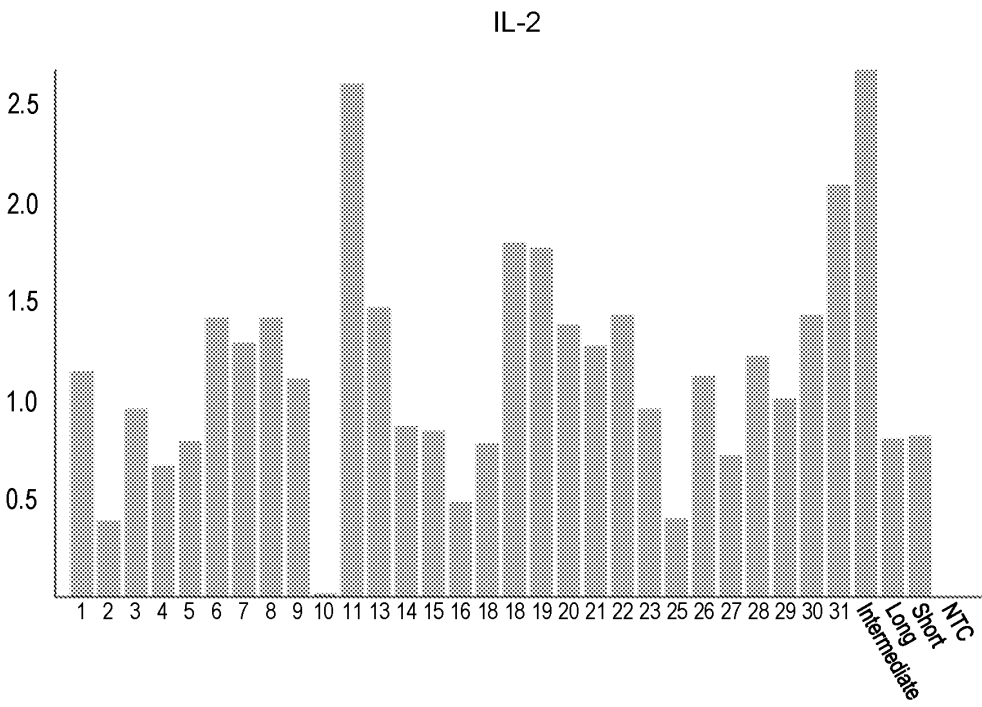
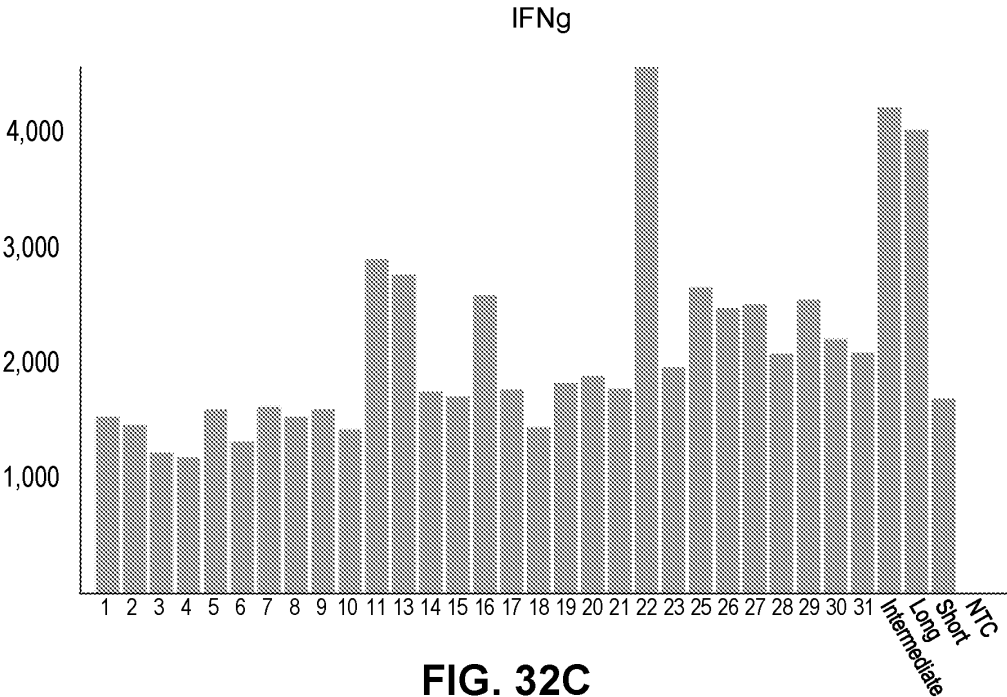


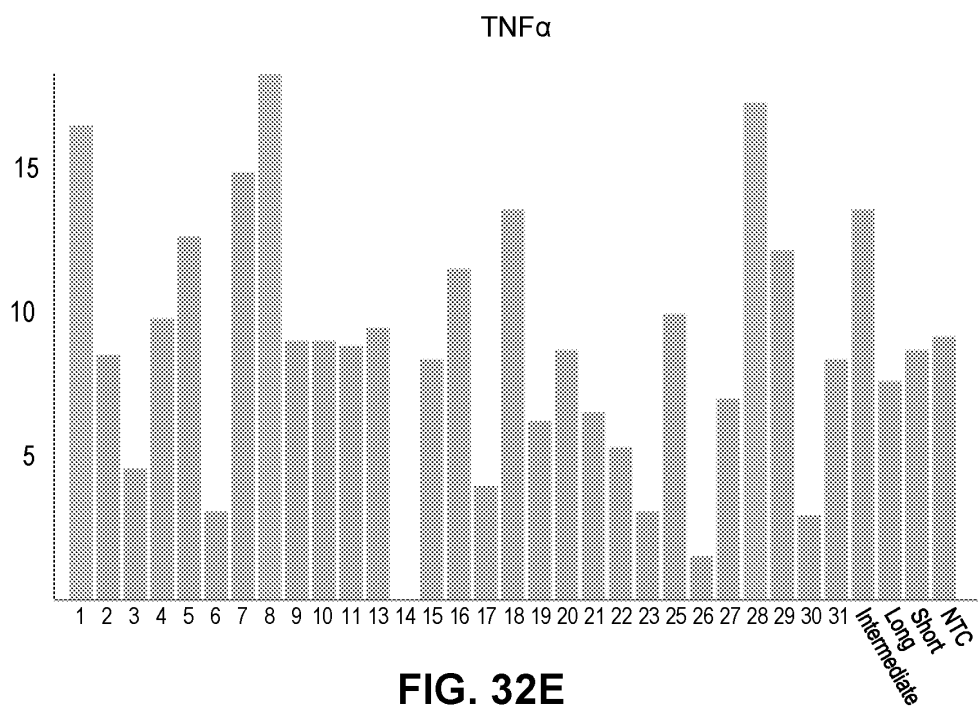
FIG. 32B

Primary killing and AUC D13814 CAR-T : T47D-Her2KO-NLR 1:1



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Primary killing and AUC D13814 CAR-T : T47D-Her2KO-NLR 1:1



Primary killing and AUC D15842 CAR-T : T47D-Her2KO-NLR 1:1

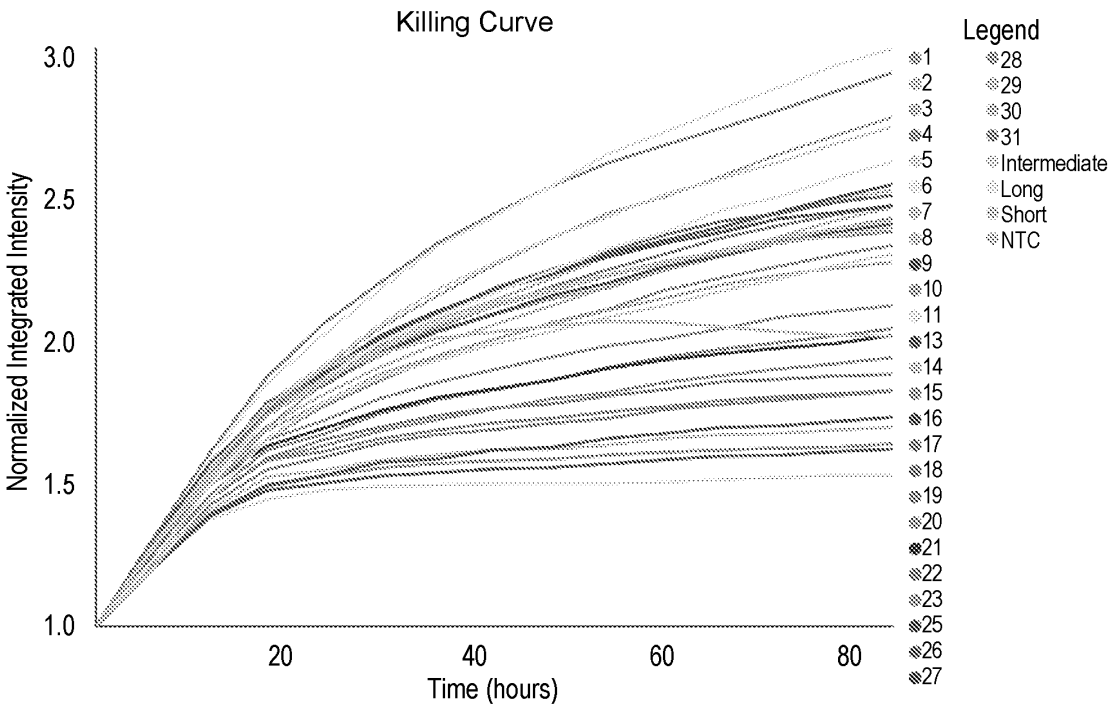


FIG. 33A

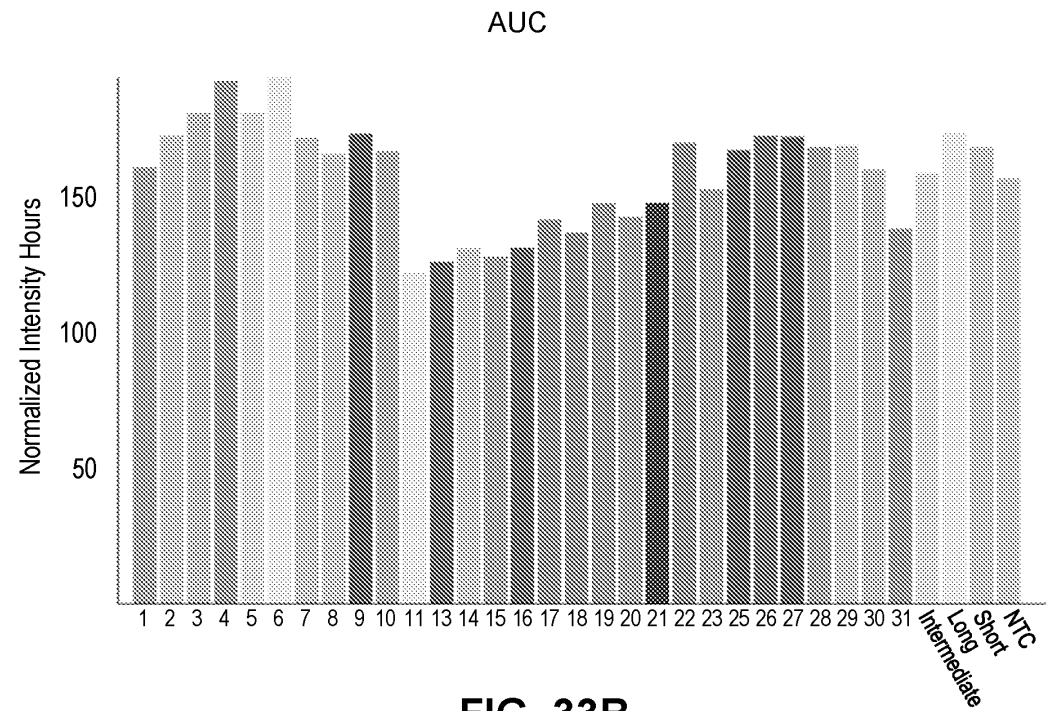
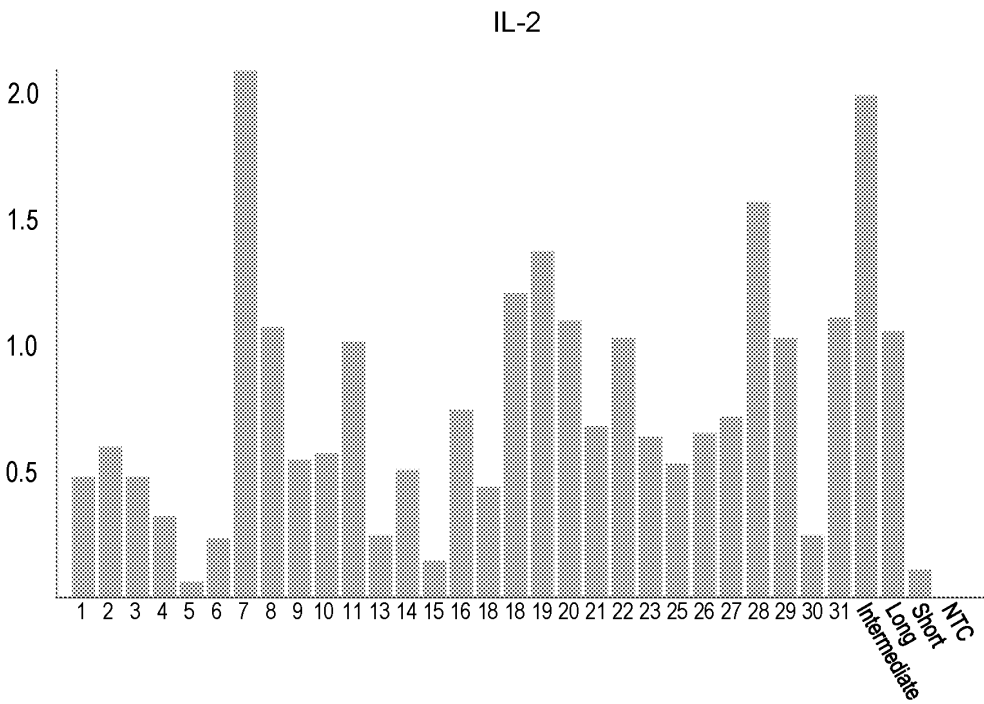
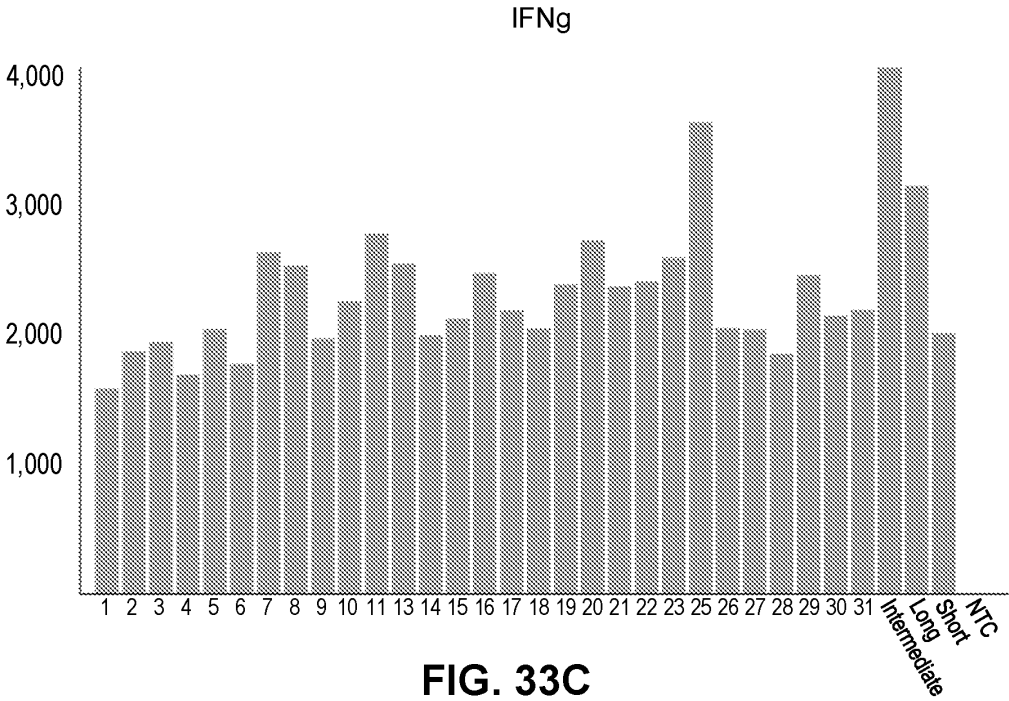


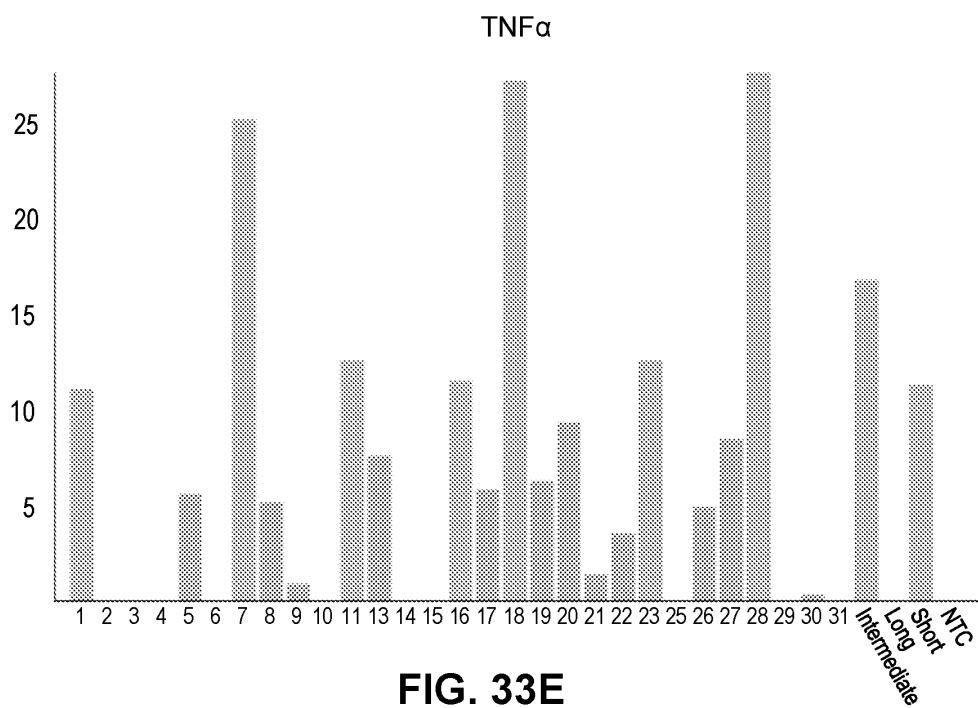
FIG. 33B

Primary killing and AUC D15842 CAR-T : T47D-Her2KO-NLR 1:1



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Primary killing and AUC D15842 CAR-T : T47D-Her2KO-NLR 1:1



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D13814 Target independent cytokines

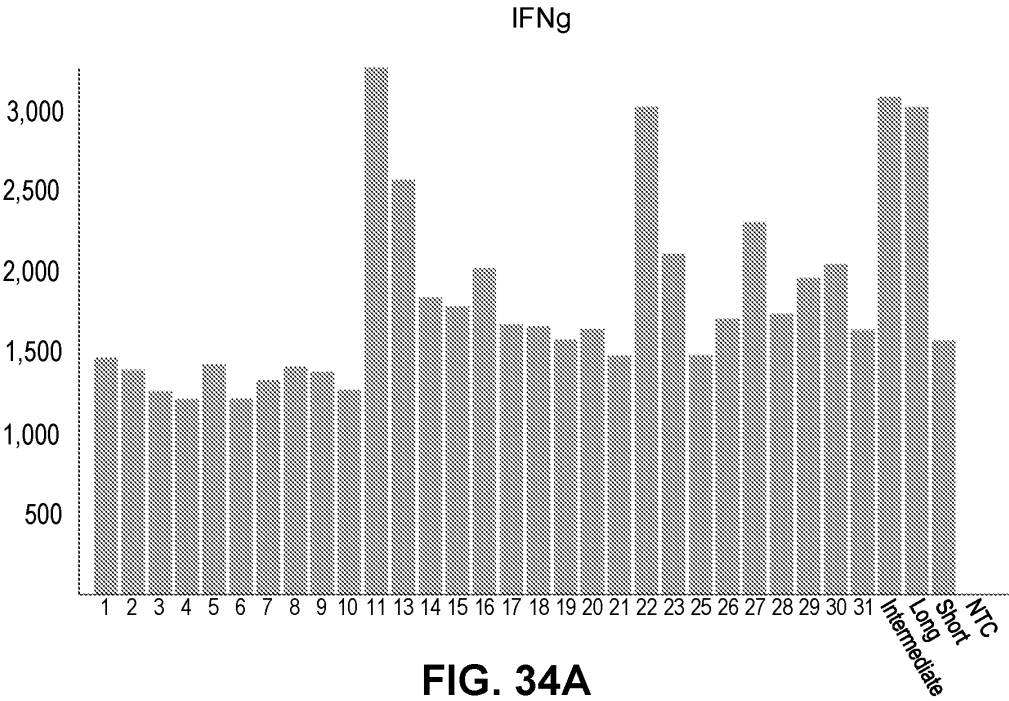


FIG. 34A

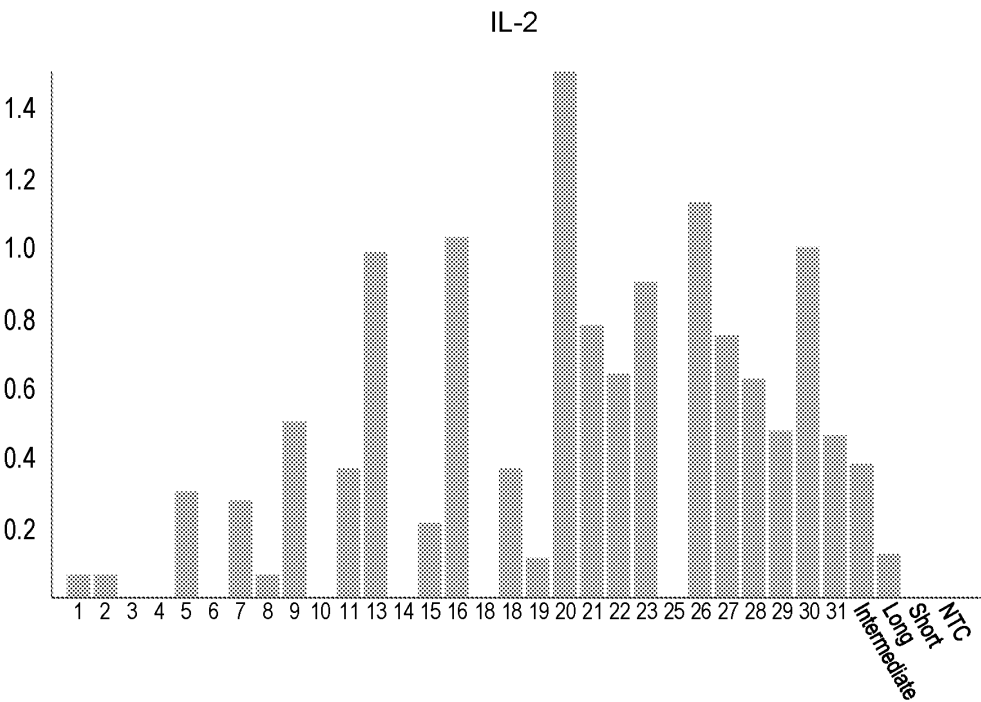
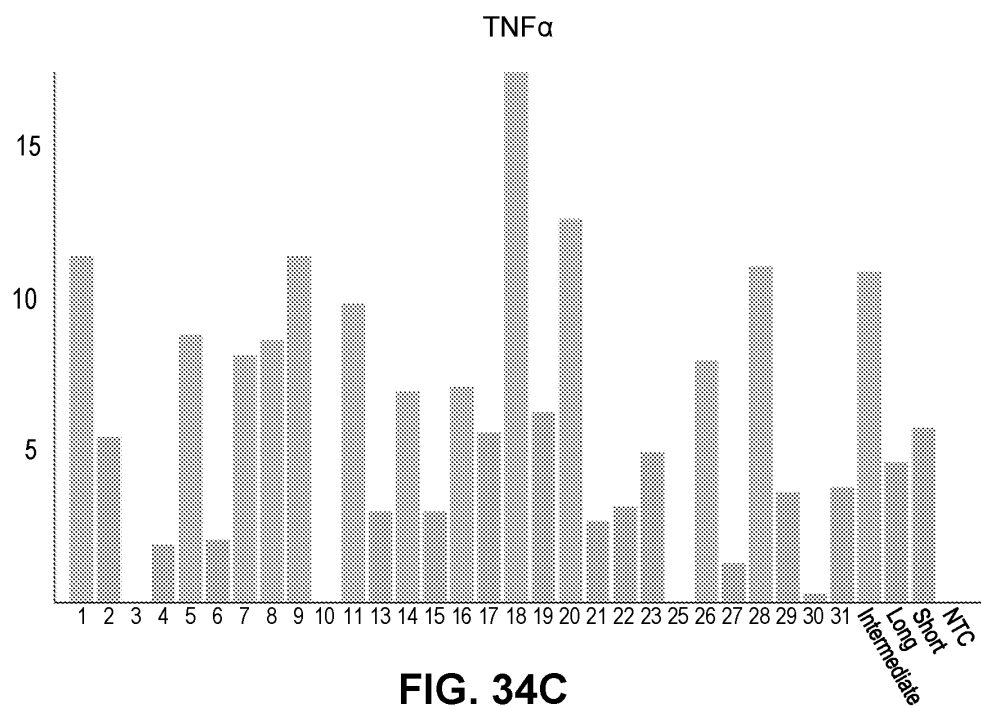


FIG. 34B

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D13814 Target independent cytokines



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D15842 Target independent cytokines

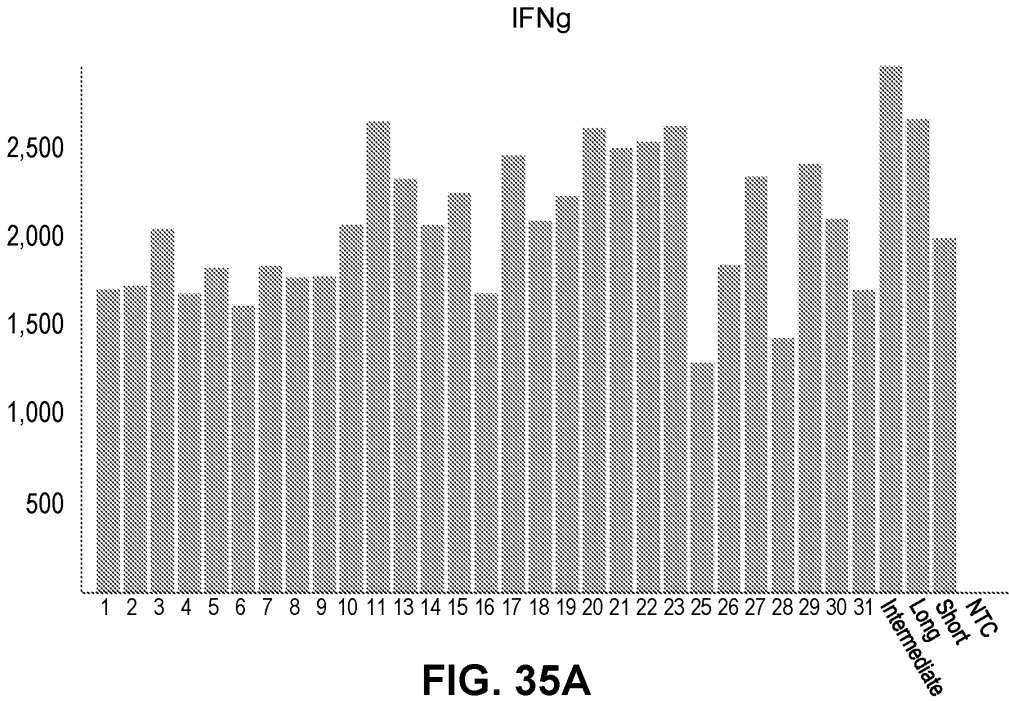


FIG. 35A

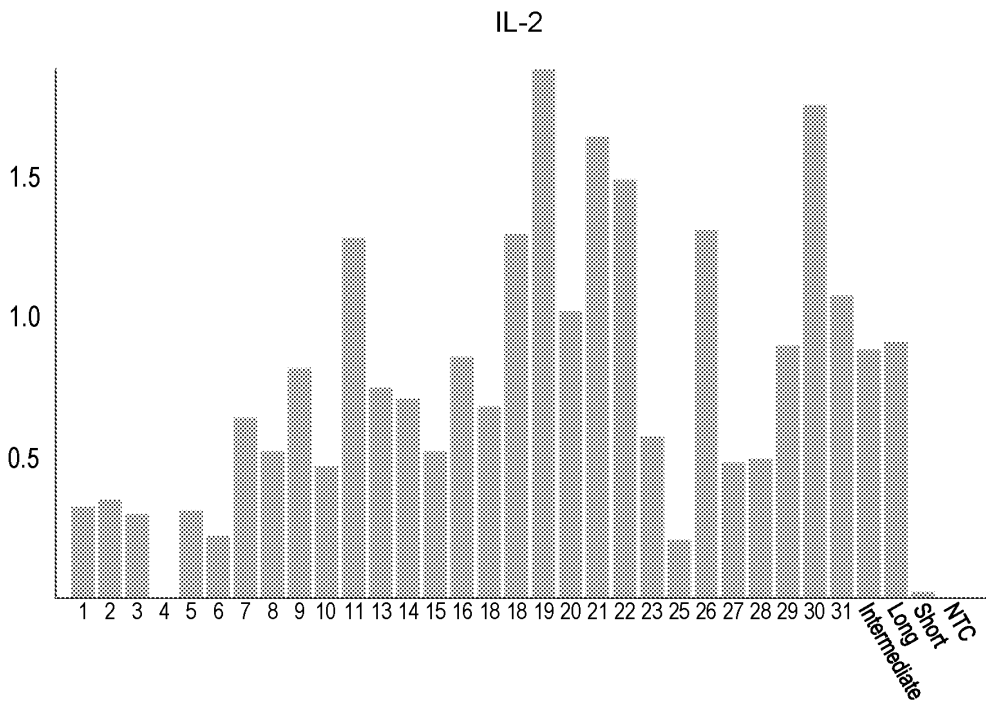
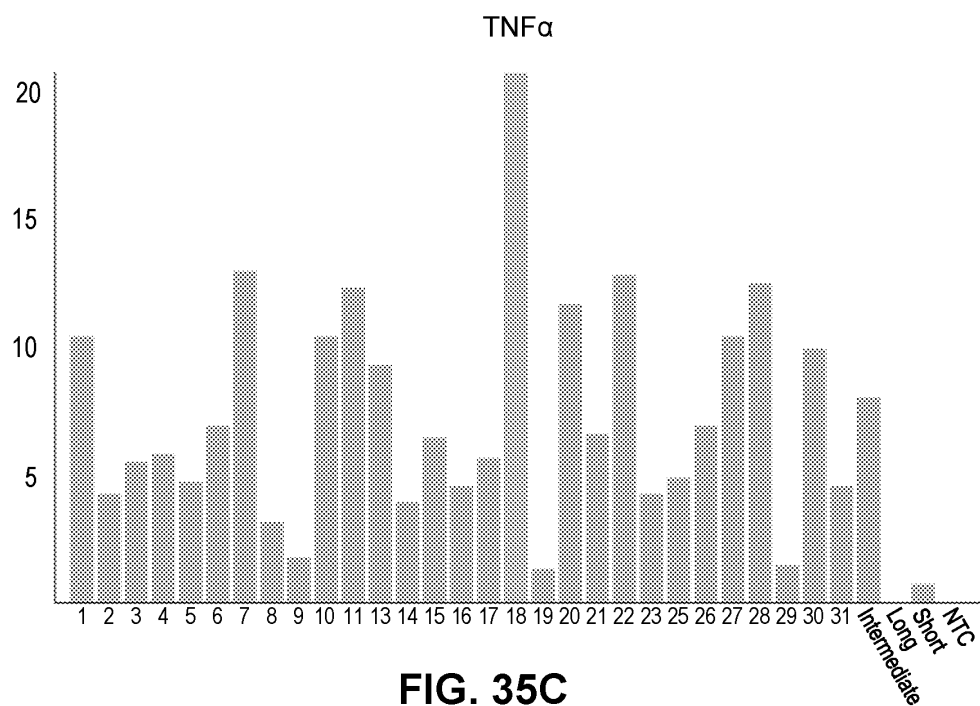


FIG. 35B

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D15842 Target independent cytokines



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Cytokine vs AUC

13814 Her 2 CAR-T : A549 1:1 AUC vs Cytokines

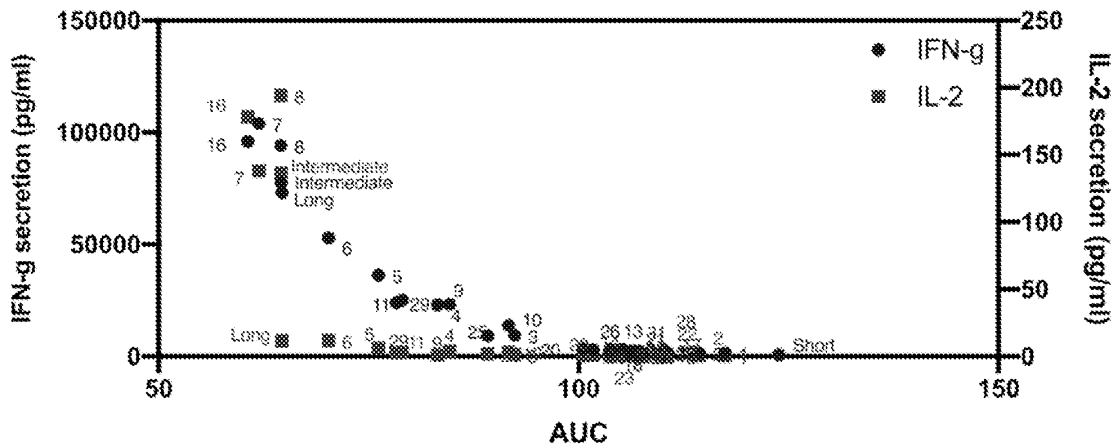


FIG. 36A

13814 Her 2 CAR-T : A47D 1:1 AUC vs Cytokines

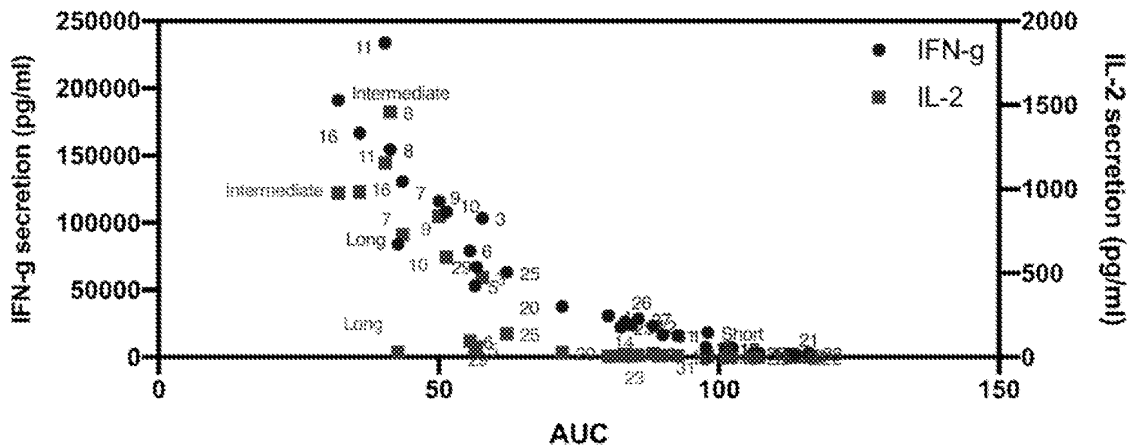


FIG. 36B

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Cytokine vs AUC

15842 Her 2 CAR-T : A549 1:1 AUC vs Cytokines

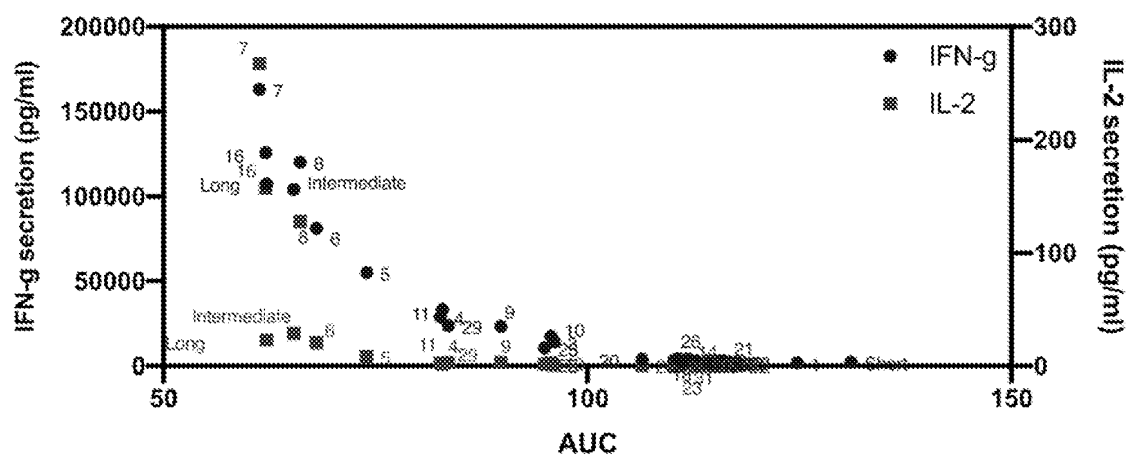


FIG. 36C

15842 Her 2 CAR-T : A47D 1:1 AUC vs Cytokines

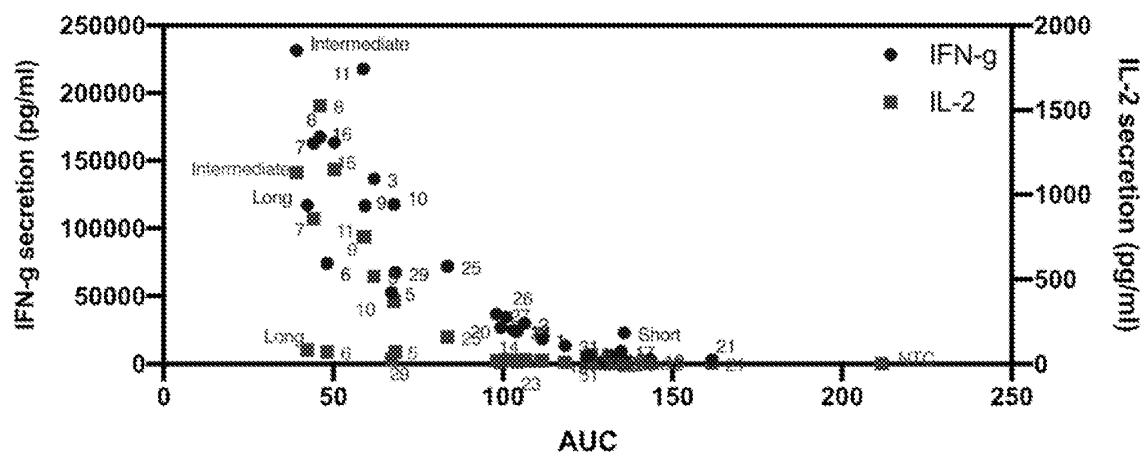


FIG. 36D

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Cytokine vs Spacer Length

13814 Her 2 CAR-T : A549 1:1 Spacer Length vs Cytokines s

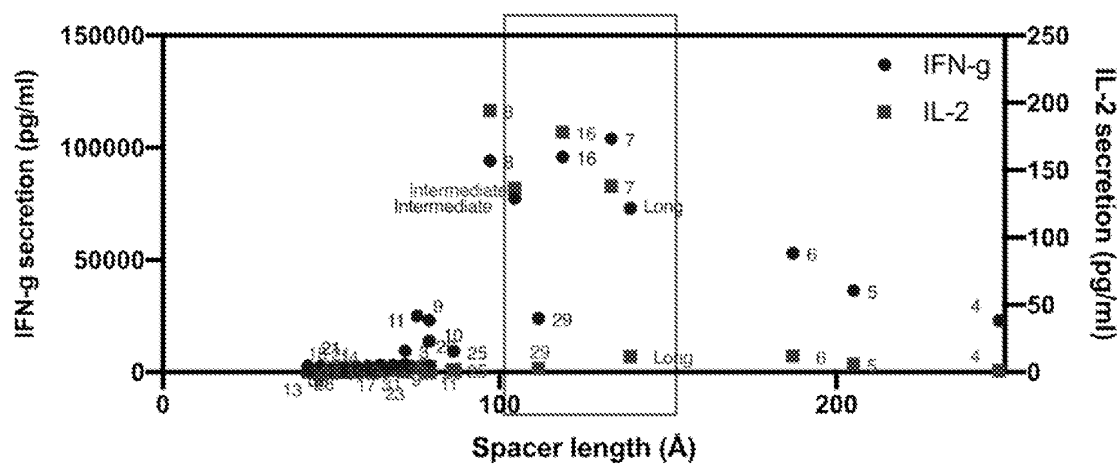


FIG. 37A

13814 Her 2 CAR-T : A47D 1:1 Spacer Length vs Cytokines s

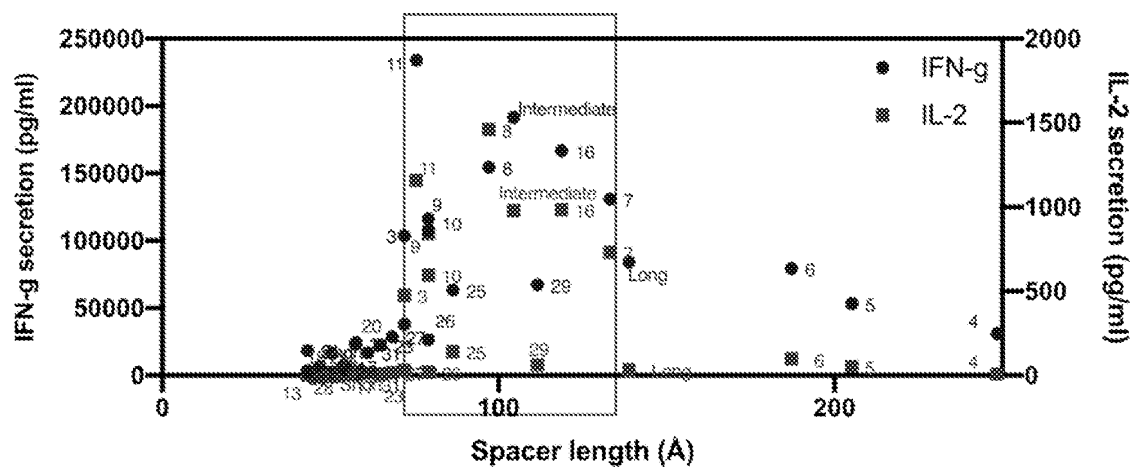


FIG. 37B

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Cytokine vs Spacer Length

15842 Her 2 CAR-T : A549 1:1 Spacer Length vs Cytokines

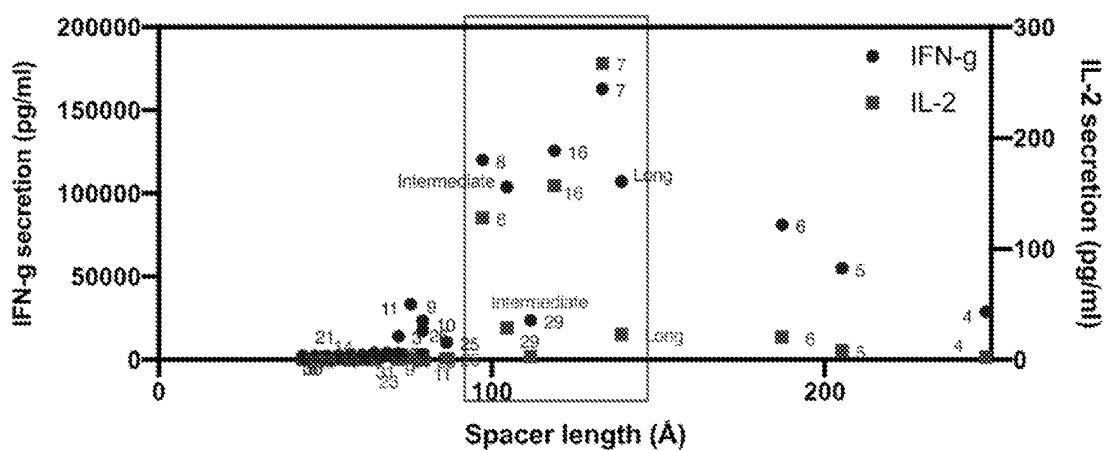


FIG. 37C

15842 Her 2 CAR-T : A47D 1:1 Spacer Length vs Cytokines

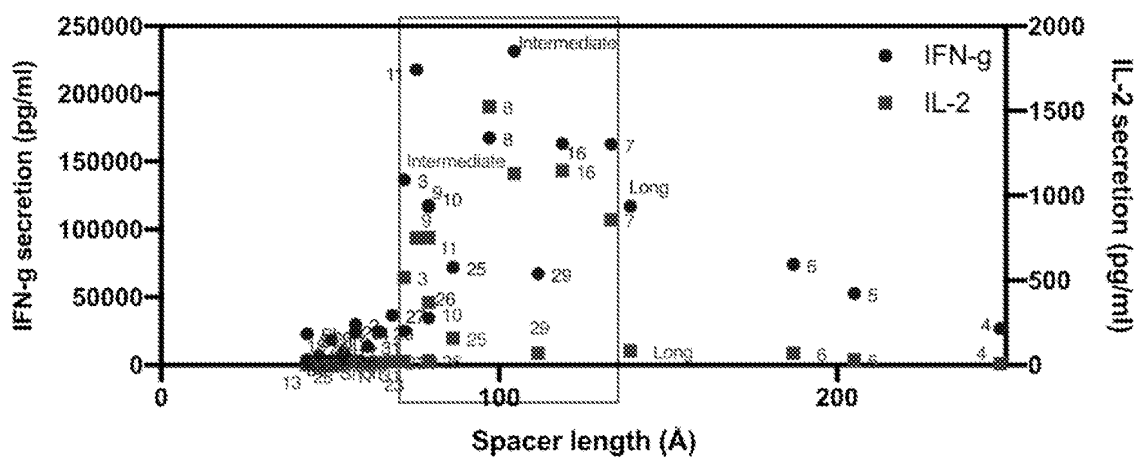


FIG. 37D

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Spacer Length vs AUC

13814 Her 2 CAR-T : A549 1:1 AUC vs Spacer Length

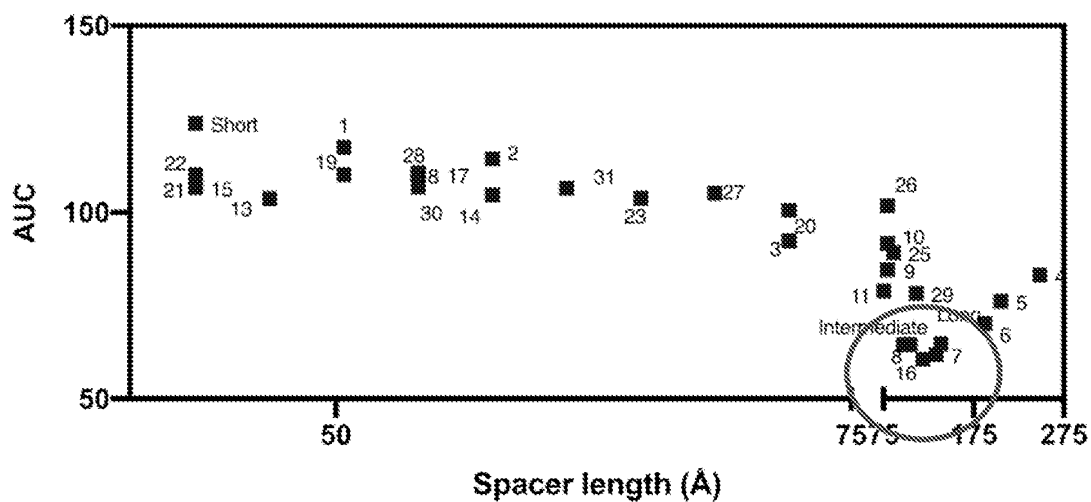


FIG. 38A

13814 Her 2 CAR-T : A47D 1:1 AUC vs Spacer Length

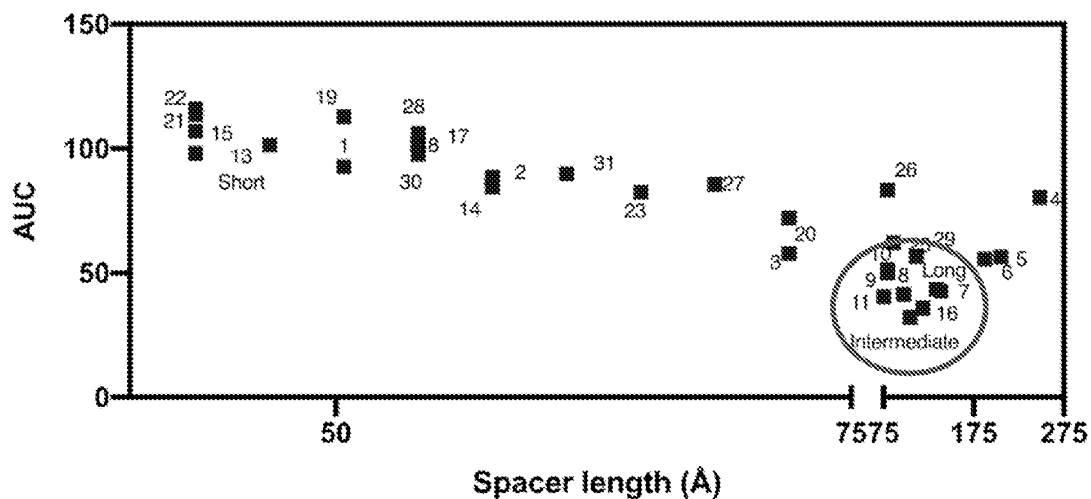


FIG. 38B

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Spacer Length vs AUC

13814 Her 2 CAR-T : A549 1:1 AUC vs Spacer Length

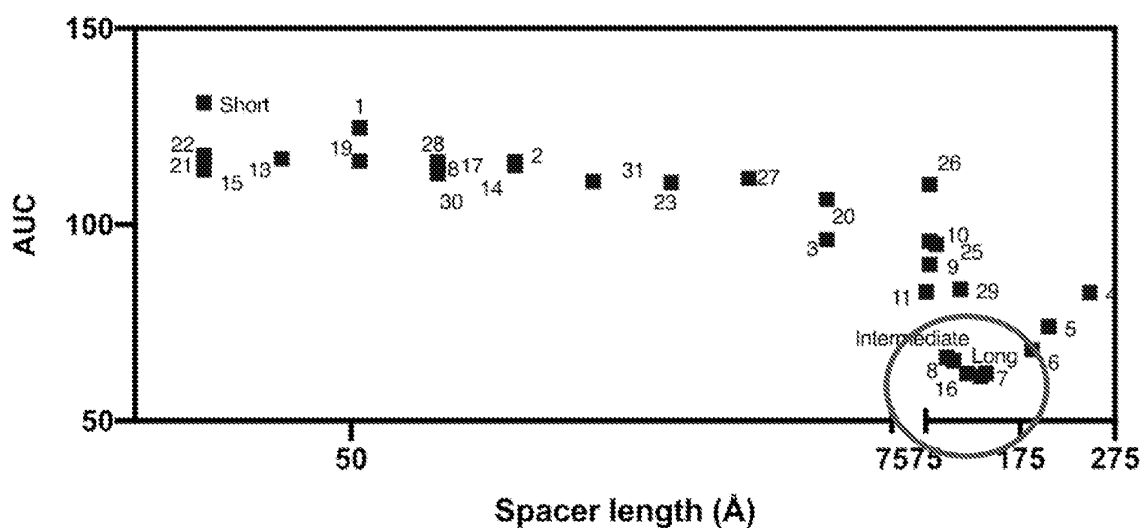


FIG. 38C

15842 Her 2 CAR-T : A47D 1:1 AUC vs Spacer Length

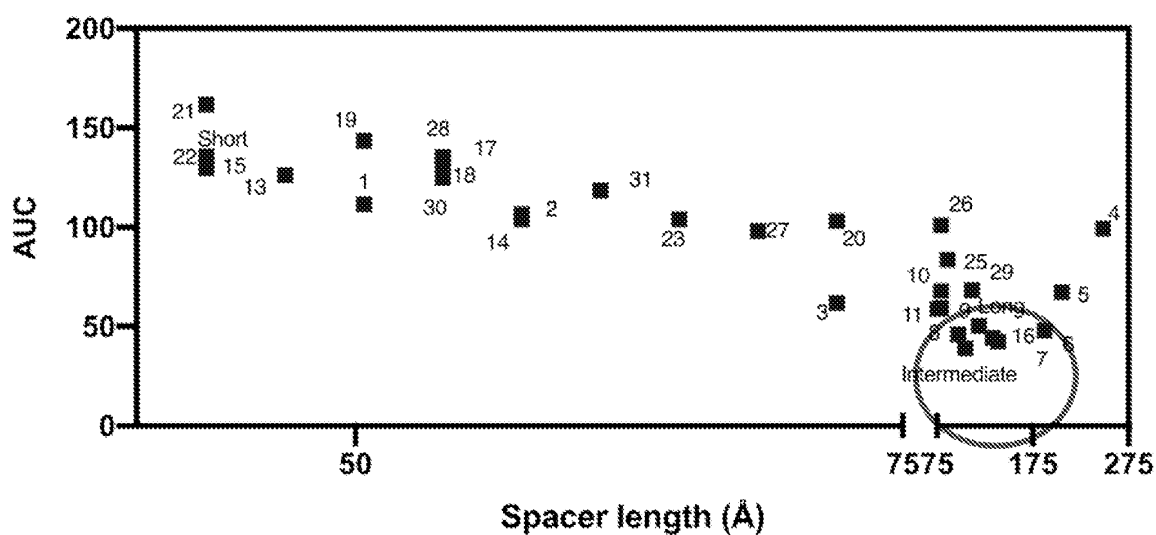


FIG. 38D

Sequential Kill Donor 13814

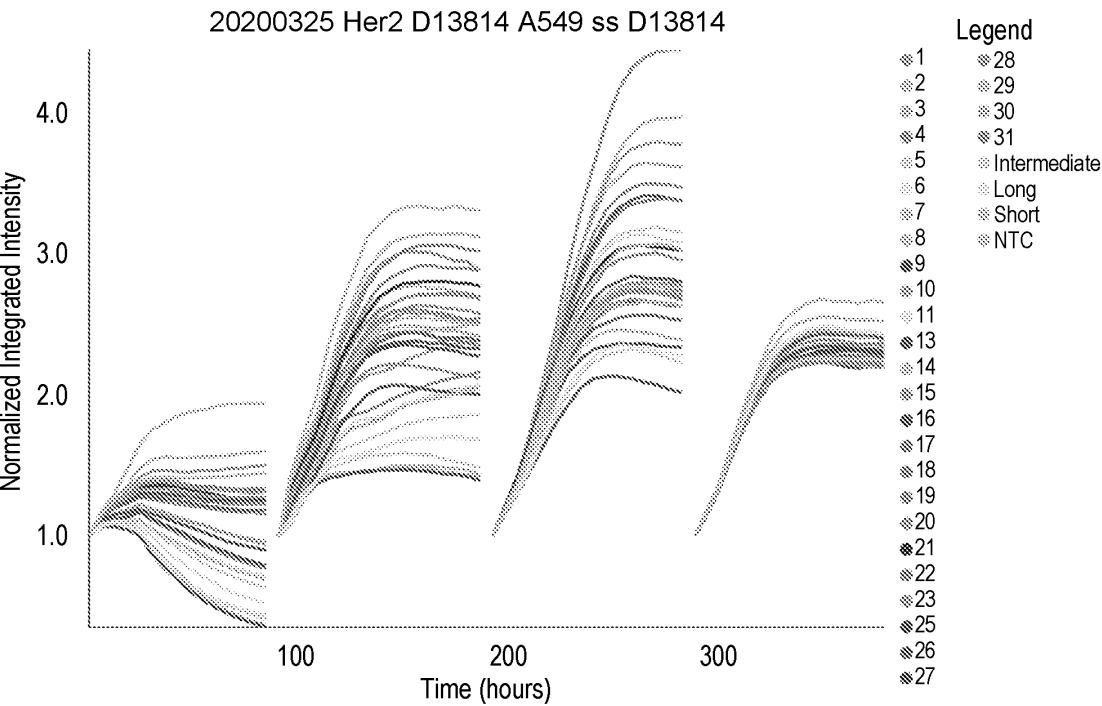


FIG. 39A

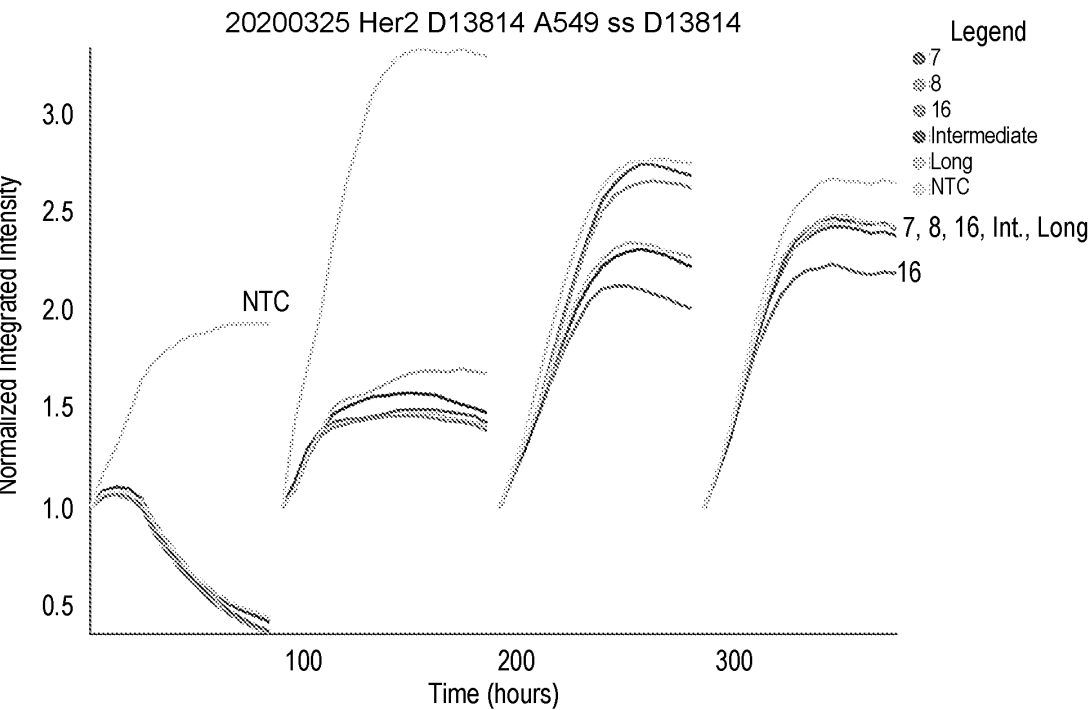


FIG. 39B

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Sequential Kill Donor 13814

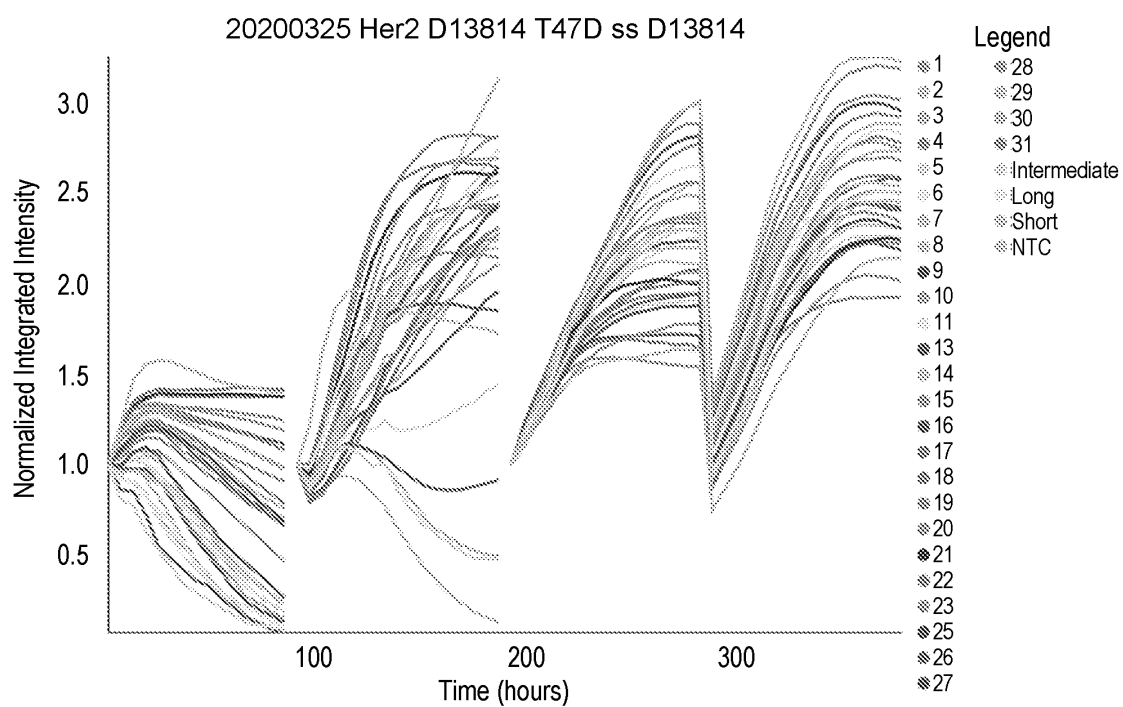


FIG. 39C

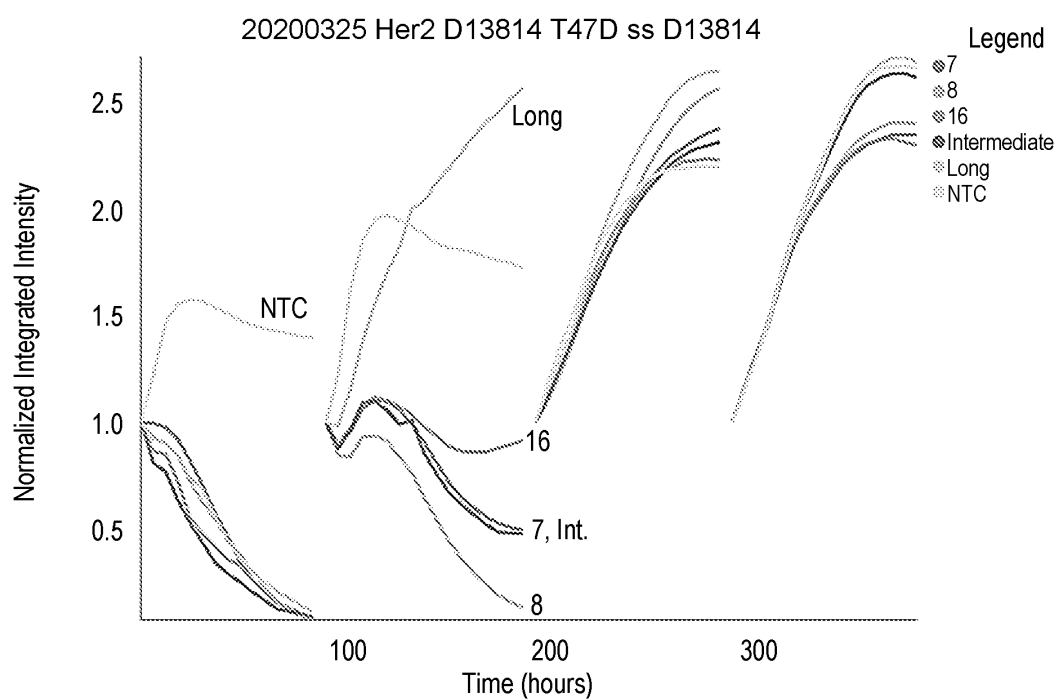


FIG. 39D

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Sequential Kill Donor 15842

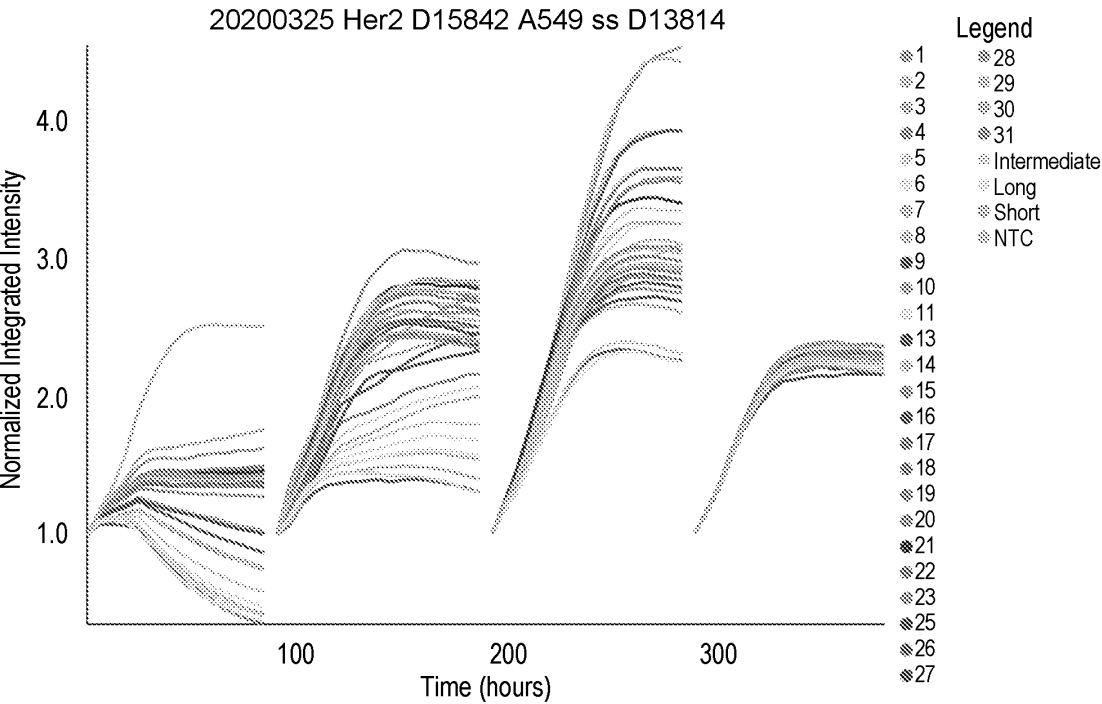


FIG. 40A

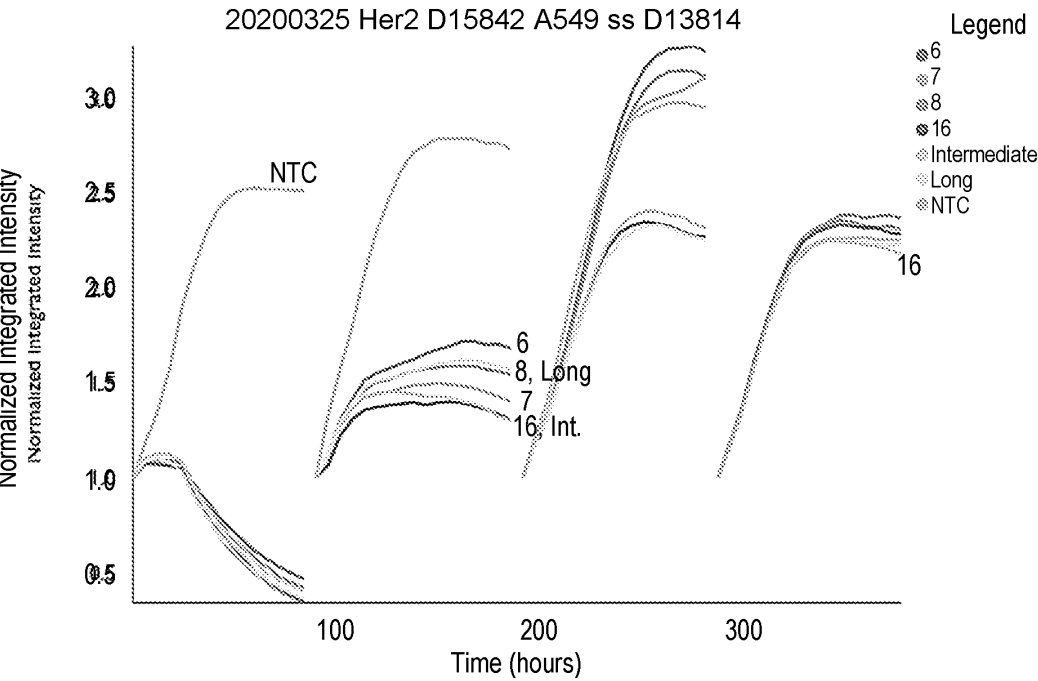


FIG. 40B

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Sequential Kill Donor 15842

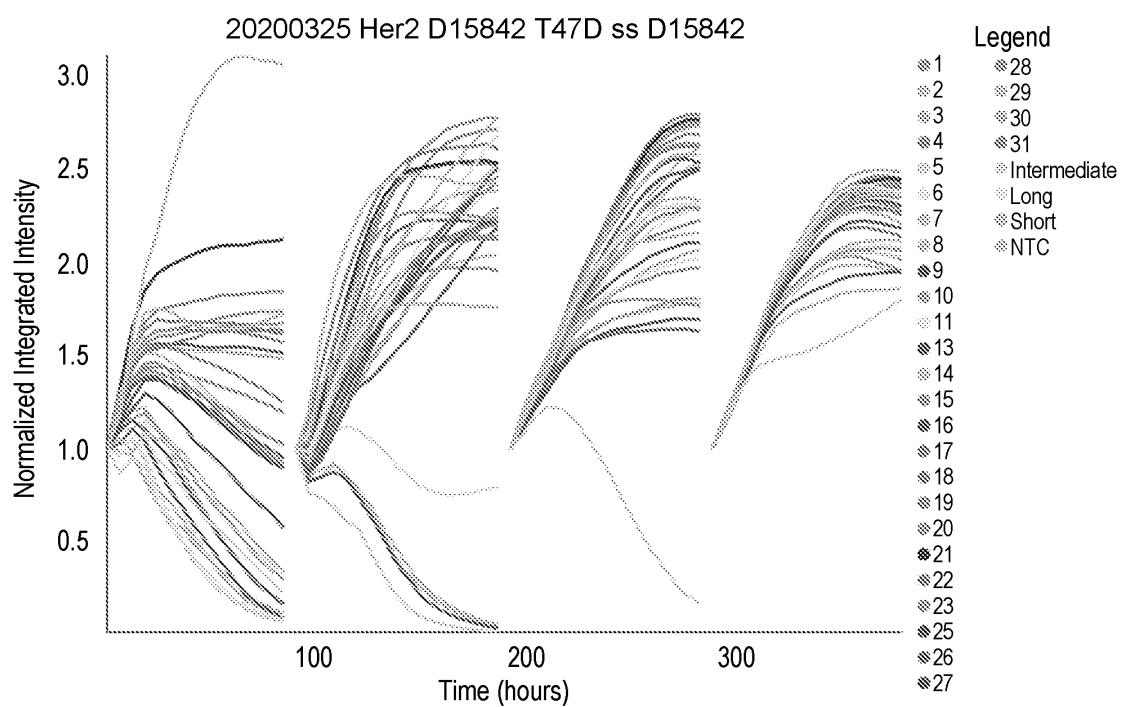


FIG. 40C

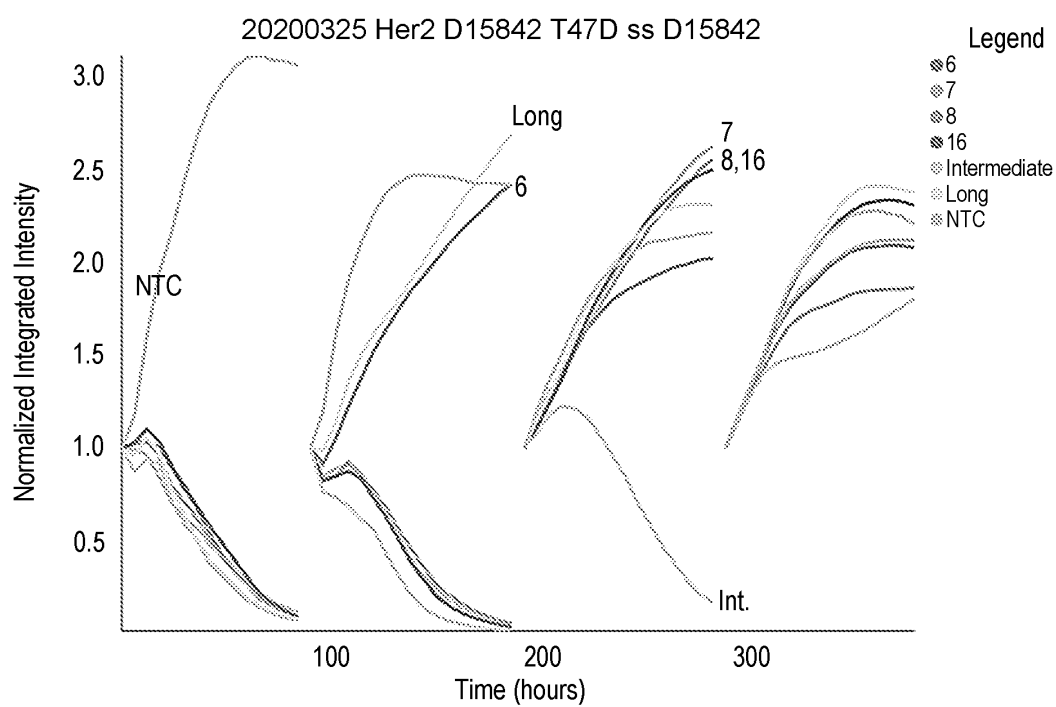
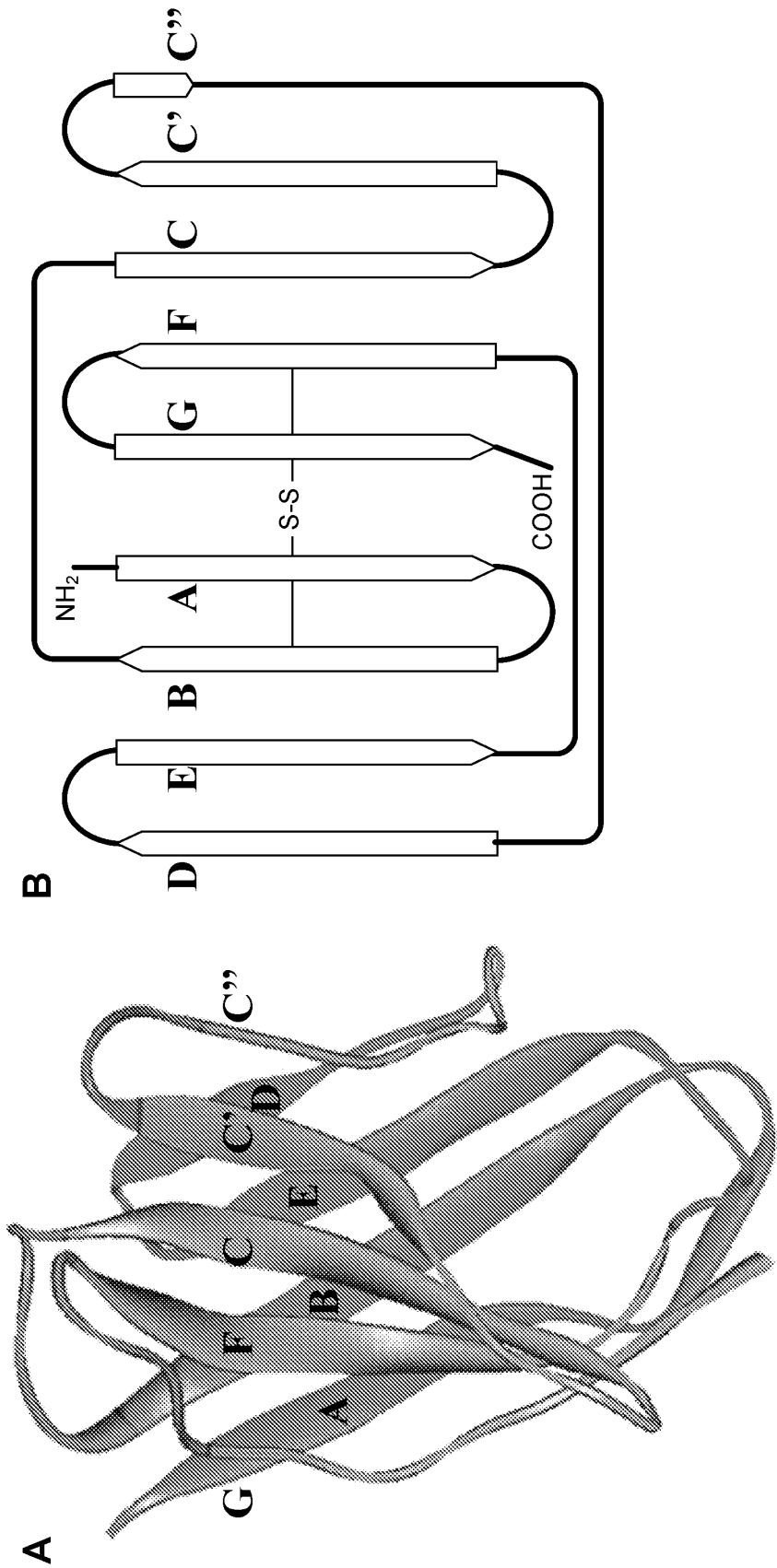


FIG. 40D



Multiple sequence alignment & secondary structure of human immunoglobulin heavy chain isotypes

C - coil secondary structure (loop)
E - extended secondary structure (β strand)
H - helical secondary structure (α helix)
* - single, fully conserved residue
: - property conservation - scoring > 0.5 in the Gonnet PAM 250 matrix
. - no property conservation - scoring < 0.5 in the Gonnet PAM 250 matrix

```

CONFORMATION      CCCCCCEEECCCCC-CCCCCEEEEEEECCCCC-CEEEEECEEE-CCCCCCE
sp|P01880|IGHD_HUMAN APTKAPDVFPIISGCR-HPKDNSPVVLACLIIGYHPTS-VTVTWYMGTS--QPQRTFPE 56
sp|P01871|IGHM_HUMAN GSASAPTLFPLVSCEN-SPSUTSSVAVGCLAQDFLPDS-ITFSWYKYNNSDSSSTRGFPS 58
sp|P01876|IGHA1_HUMAN ASPTSPKVFPLSLCST-QPDG--INVVIACLVQGFPPQEPPLSVTWSESGQ--VTARNFPP 55
sp|P01877|IGHA2_HUMAN ASPTSPKVFPLSLDST-QPDG--INVVIACLVQGFPPQEPPLSVTWSESGQ--VTARNFPP 55
sp|P01854|IGHE_HUMAN ASTQSPSVFPLTRCCKNIPSNATSVTLGCLATGYFPEP-VNVTWDTGSLN--GTTMTLPA 57
sp|P01857|IGHG1_HUMAN ASTKGPSVFPLAPSSKSTS--GGTAALGCLVKDYFPEP-VTVSWNSGALT--SGVHTFPA 55
sp|P01860|IGHG3_HUMAN ASTKGPSVFPLAPCSRSTS--GGTAALGCLVKDYFPEP-VTVSWNSGALT--SGVHTFPA 55
sp|P01859|IGHG2_HUMAN ASTKGPSVFPLAPCSRSTS--ESTAALGCLVKDYFPEP-VTVSWNSGALT--SGVHTFPA 55
sp|P01861|IGHG4_HUMAN ASTKGPSVFPLAPCSRSTS--ESTAALGCLVKDYFPEP-VTVSWNSGALT--SGVHTFPA 55
                        .  . *  : **  . : *  ;  . *  ; *
DOMAIN                | START OF CH1

CONFORMATION      E-EECCCCEEEEEEEEEECCCCC-CCEEEEEECCCCCEEE-----
sp|P01880|IGHD_HUMAN I-QRHDSYMYTSSQLSTPLQWRQ--GEYKCVVQHTASKSKKEIF-----98
sp|P01871|IGHM_HUMAN V--LRGGKYAATSQVLLPSKINMGTDENVCKVQHPNGNKEK--N--VPLPVIAELPPK 112
sp|P01876|IGHA1_HUMAN SQDASGDLVYTTSSQLTLPA-----74
sp|P01877|IGHA2_HUMAN SQDASGDLVYTTSSQLTLPA-----74
sp|P01854|IGHE_HUMAN TTLTLGHWATISLLTV-SGAWAK--QMFTRVAHTPSSTDWQNKTFSVCSRDFTPPT 113
sp|P01857|IGHG1_HUMAN V-LQSSGLYSLSSVVTVPSSSLGT---QTYICNVNHPKPSNTKVQKVE-----99
sp|P01860|IGHG3_HUMAN V-LQSSGLYSLSSVVTVPSSSLGT---QTYICNVNHPKPSNTKVQKVELKTPLGOTTHTC 111
sp|P01859|IGHG2_HUMAN V-LQSSGLYSLSSVVTVPSSNFGT---QTYTCNVNHPKPSNTKVQKVE-----101
sp|P01861|IGHG4_HUMAN V-LQSSGLYSLSSVVTVPSSSLGT---KTYTCNVNHPKPSNTKVQKRVESK-----101
                        .  . *  *  :
DOMAIN                | END OF CH1 |

CONFORMATION      -----ECCCCCCCCCCCCCCCCCCCCC-----
sp|P01880|IGHD_HUMAN -----RWPESPKAGASSVPTAQFQAEGLA 123
sp|P01871|IGHM_HUMAN VS-VFVPPRDGFGNPRKSKLICQATGFSPRQIQVSWLREKQVGSQVTTDQVQAEAKES 171
sp|P01876|IGHA1_HUMAN -----74
sp|P01877|IGHA2_HUMAN -----74
sp|P01854|IGHE_HUMAN VK-ILQSSCDGGGHPPTIQLCLVSGYTPGTINITWLEDGQVMDVLSASTTQEGE--170
sp|P01857|IGHG1_HUMAN -----99
sp|P01860|IGHG3_HUMAN PRCPPEPRSCDTPPPCPR-----CPE-----P-----K-----133
sp|P01859|IGHG2_HUMAN -----101
sp|P01861|IGHG4_HUMAN -----101

DOMAIN

CONFORMATION      CCCCCCCCCC-CCCCCCCC-CEEECCCCCCCCCCCCCCCC-----
sp|P01880|IGHD_HUMAN KATTAPATTRNT----GRGGEKKK--EKEKEEQEERETKTPECPST-----165
sp|P01871|IGHM_HUMAN GPTTYKVTSTLTIXESDNLQSMFTCRVDHRLT--FOQNASMCMVPOQ-----218
sp|P01876|IGHA1_HUMAN -----TQCLAGKSVTCHVKHYTH-----SQVLTGENTSTPTSTPT-----117
sp|P01877|IGHA2_HUMAN -----TQCPDGKSVTCHVKHYTHS--SQVLTGENTSTPTSTPT-----105
sp|P01854|IGHE_HUMAN --LASTQSELTLQXHWLSDRTYTCQVYQGH--FEDSTKKCAD-SNP-----214
sp|P01857|IGHG1_HUMAN -----PPQVQKTHTPPPPAPELL-----118
sp|P01860|IGHG3_HUMAN -----DTEPRCKGCEPRKSDTPPPCPHFAPELL-----165
sp|P01859|IGHG2_HUMAN -----CLEVFPFCAPPE-----114
sp|P01861|IGHG4_HUMAN -----NGEPFCAPPEFL-----115

DOMAIN                | START OF CH2

```

FIG. 42

SUBSTITUTE SHEET (RULE 26)

CONFORMATION

```

sp|P01880|IGHD_HUMAN      -----CCCEEECC--CCCCCCCCCCCCCCCCCCCC--EEEEEECCCCCCCCCCCC
sp|P01871|IGHM_HUMAN      -----C-----AWQDILNRDKATFTCFVVSQDKD--AHLTHVEAGKVPTGGVEE 217
sp|P01876|IGHA1_HUMAN      -----DTAIRVFAIPF--SFASIFLTKSTKLTCLVDTLTYD--SVTISWTRNGEAVK--THT 271
sp|P01877|IGHA2_HUMAN      -----C-----ALEDLILGSEANLTCTLTGLRDAS--GVTFWTPTSSGKSAV--QGP 174
sp|P01854|IGHE_HUMAN      -----C-----ALEDLILGSEANLTCTLTGLRDAS--GATFTWTPSSGKSAV--QGP 161
sp|P01857|IGHG1_HUMAN      -----C-----C-----DLFTRKSPITITCLVVDLAPSKGTNNLTWSRASGKPVN--HST 287
sp|P01859|IGHG2_HUMAN      -----C-----C-----KDTLNLISRTPEVTCVVDVSHDEPQVFMWYVDGVEVHN--AKT 172
sp|P01860|IGHG3_HUMAN      -----C-----C-----KDTLNLISRTPEVTCVVDVSHDEPQVFMWYVDGVEVHN--AKT 219
sp|P01859|IGHG2_HUMAN      -----C-----C-----KDTLNLISRTPEVTCVVDVSHDEPQVFMWYVDGVEVHN--AKT 168
sp|P01861|IGHG4_HUMAN      -----C-----C-----KDTLNLISRTPEVTCVVDVSHDEPQVFMWYVDGVEVHN--AKT 169

```

DOMAIN

CONFORMATION

```

sp|P01880|IGHD_HUMAN      CCEEECCCEEEECCEEEECCECCCHNNEEEEEEECCECCCCCEEEEEECCCCCCCCCCCC
sp|P01871|IGHM_HUMAN      GLEENHSNGSQSHSLTLPRSLNAGTSVTCVTLN-----RMLALREPAAGAPVKLSL 277
sp|P01876|IGHA1_HUMAN      NISESHPNATFSAVGEASICEDWNGSERGFTCTVTHDTLPSPKQTIISKPKGVALHRPQV 331
sp|P01877|IGHA2_HUMAN      --PERDLGCGYSVSSVLPQCAEPNMGKTFCTCTAAV-----LTATLSKS--GNTFRPEV 230
sp|P01854|IGHE_HUMAN      --PERDLGCGYSVSSVLPQCAEPNMGKTFCTCTAAV-----LTANITKS--GNTFRPEV 217
sp|P01857|IGHG1_HUMAN      KKEEKQRNGTLTVTSITLPGTRDWEIGEIVQCVRVTHPHLPALMRSTTKTSGP--RAAPEV 326
sp|P01859|IGHG2_HUMAN      KPREEQYNSTYRWVSIVTLVHKGDLNGKEYKCKVSH-----IEKTISKAKGQ--PREPQV 231
sp|P01860|IGHG3_HUMAN      KPREEQYNSTYRWVSIVTLVHKGDLNGKEYKCKVSH-----IEKTISKTKGQ--PREPQV 278
sp|P01859|IGHG2_HUMAN      KPREEQYNSTYRWVSIVTLVHKGDLNGKEYKCKVSH-----IEKTISKTKGQ--PREPQV 227
sp|P01861|IGHG4_HUMAN      KPREEQYNSTYRWVSIVTLVHKGDLNGKEYKCKVSHKGLPSSIEKTISKAKGQ--PREPQV 228

```

DOMAIN

CONFORMATION

```

sp|P01880|IGHD_HUMAN      CCCCCCCCC--CCCEEEEEEECCCCCCCCCEEEECCEEECCCCCCCCCCCC--CCCCC
sp|P01871|IGHM_HUMAN      LLAPSSDPP--EAASHILCEVSGFSPPNELLMLLEDQREVNYSGFA-----PRST 332
sp|P01876|IGHA1_HUMAN      NYLPPAREQNLRESATITCLVTGFSPADVFQVQMGKQPLSPKEQVYTSAPHPPEQAP--G 390
sp|P01877|IGHA2_HUMAN      HLLPPPESEELALNELVTLTCLARGFSPKQDLVRLMQSQELPREKYLTHWASQEPSSQIT 290
sp|P01854|IGHE_HUMAN      HLLPPPESEELALNELVTLTCLARGFSPKQDLVRLMQSQELPREKYLTHWASQEPSSQIT 277
sp|P01857|IGHG1_HUMAN      YAFATPEWPGS--RDKRTLACLIGNFMPEDITSQNLHWEVQLPQARHSTTQPRKTKG---S 382
sp|P01859|IGHG2_HUMAN      YTLPPSRDELIT--KNQVSLTCLVKGFPYSDIAVEWESNGQ---ETL-----VLDSQ---G 285
sp|P01860|IGHG3_HUMAN      YTLPPSRDEMT--KNQVSLTCLVKGFPYSDIAVEWESNGQ---ETL-----VLDSQ---G 332
sp|P01859|IGHG2_HUMAN      YTLPPSRDEMT--KNQVSLTCLVKGFPYSDIAVEWESNGQ---ETL-----VLDSQ---G 281
sp|P01861|IGHG4_HUMAN      YTLPPSQEEMT--KNQVSLTCLVKGFPYSDIAVEWESNGQ---ETL-----VLDSQ---G 282

```

DOMAIN

CONFORMATION

```

sp|P01880|IGHD_HUMAN      CCEEEEEECECCCCCCCCCEEEEEECCCCCEEEEEECEEECCCC-----CC--
sp|P01871|IGHM_HUMAN      TFWASVLRVPAPPSPQATYTCVVSHEDSRTLLRASRSLEVSYVT-----DH-- 380
sp|P01876|IGHA1_HUMAN      RYFAHSILTYSEEDWNTGETTYTCVVAHEALPN--RVTERTVKSTGKPTLYNWSLVMSQTA 449
sp|P01877|IGHA2_HUMAN      TFAYTSILRVAAEDWKGOTFSCHMGHEALPL--AFTQKTIIDRLAGKPTHWVSVMMAED 349
sp|P01854|IGHE_HUMAN      TFAYTSILRVAAEDWKGOTFSCHMGHEALPL--AFTQKTIIDRLAGKPTHWVSVMMAED 336
sp|P01857|IGHG1_HUMAN      GFFVFSRLVETRAEWQKQKDFICRAVHEAASPSQTVQKAVSVNPGK-----428
sp|P01859|IGHG2_HUMAN      SFFLYSKLTVDKSRWQGNVFSVSNVHEALHN--HYTQKSLSLSPGK-----330
sp|P01860|IGHG3_HUMAN      SFFLYSKLTVDKSRWQGNVFSVSNVHEALHN--HYTQKSLSLSPGK-----377
sp|P01859|IGHG2_HUMAN      SFFLYSKLTVDKSRWQGNVFSVSNVHEALHN--HYTQKSLSLSPGK-----326
sp|P01861|IGHG4_HUMAN      SFFLYSKLTVDKSRWQGNVFSVSNVHEALHN--HYTQKSLSLSLGK-----327

```

DOMAIN

FIG. 42 (continued)

R11 CAR Transduction efficiency Donor 1 and 2

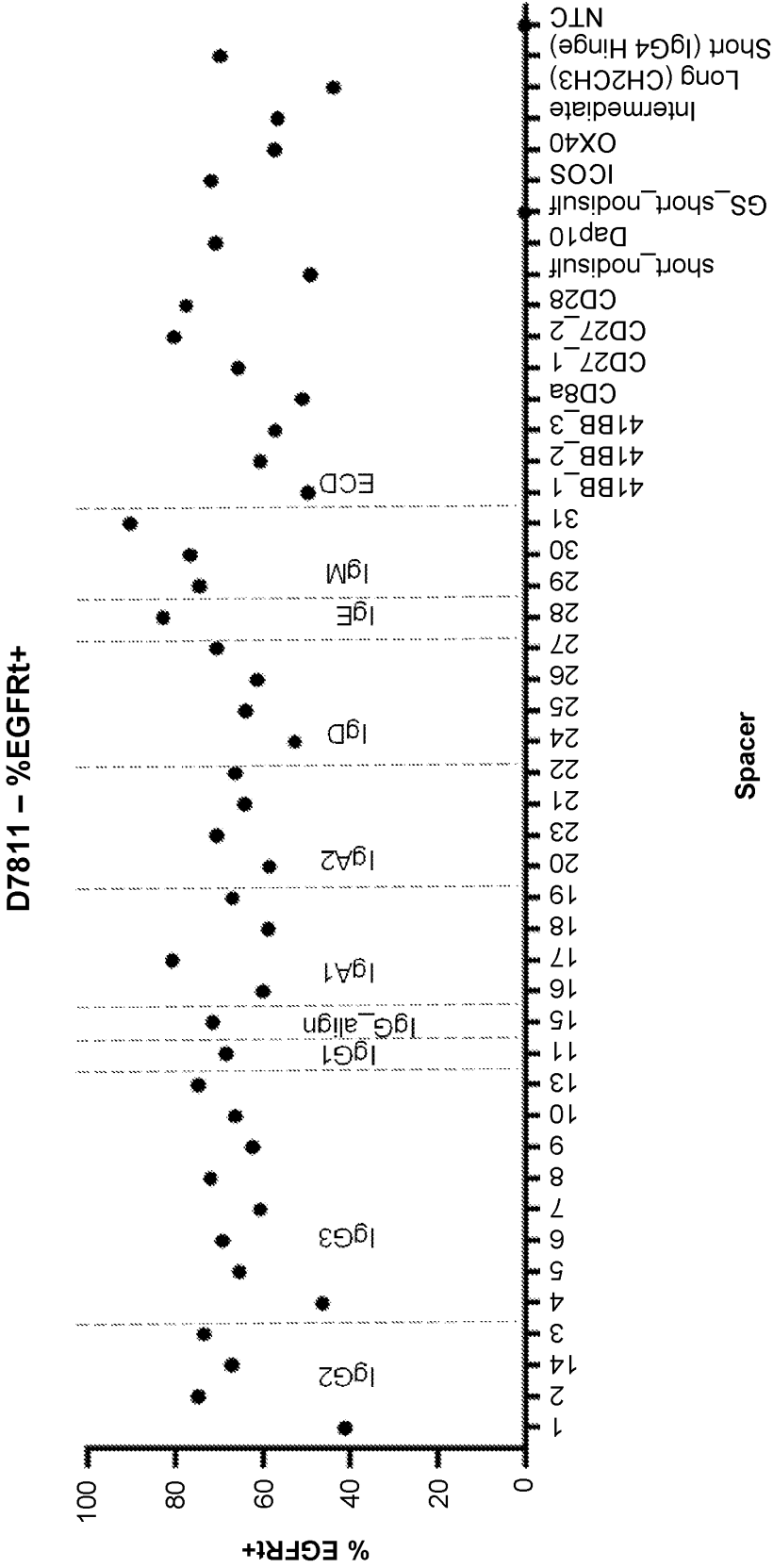


FIG. 44A

R11 CAR Transduction efficiency Donor 1 and 2

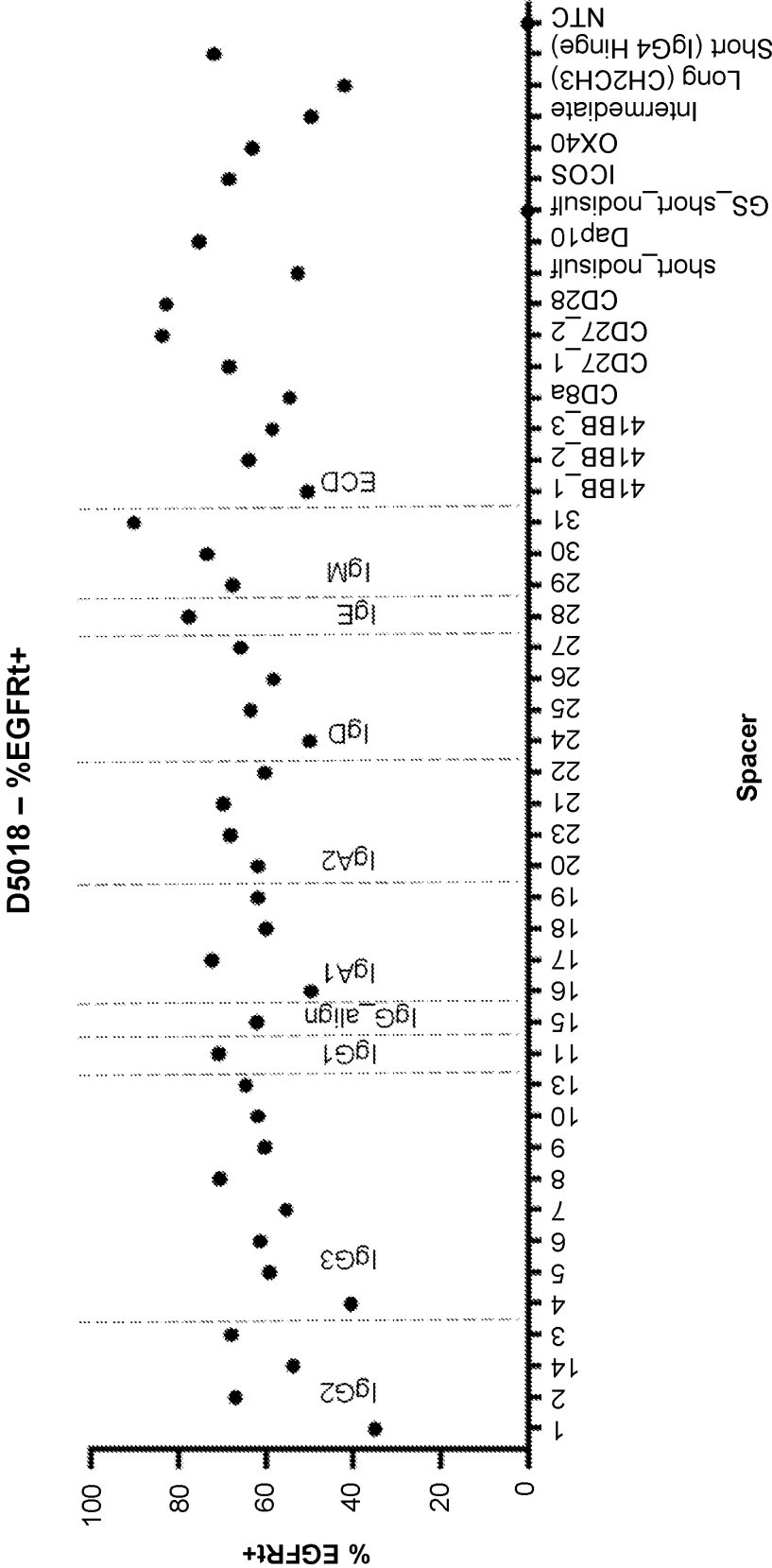


FIG. 44B

R11 CAR Transduction efficiency Donor 1 and 2

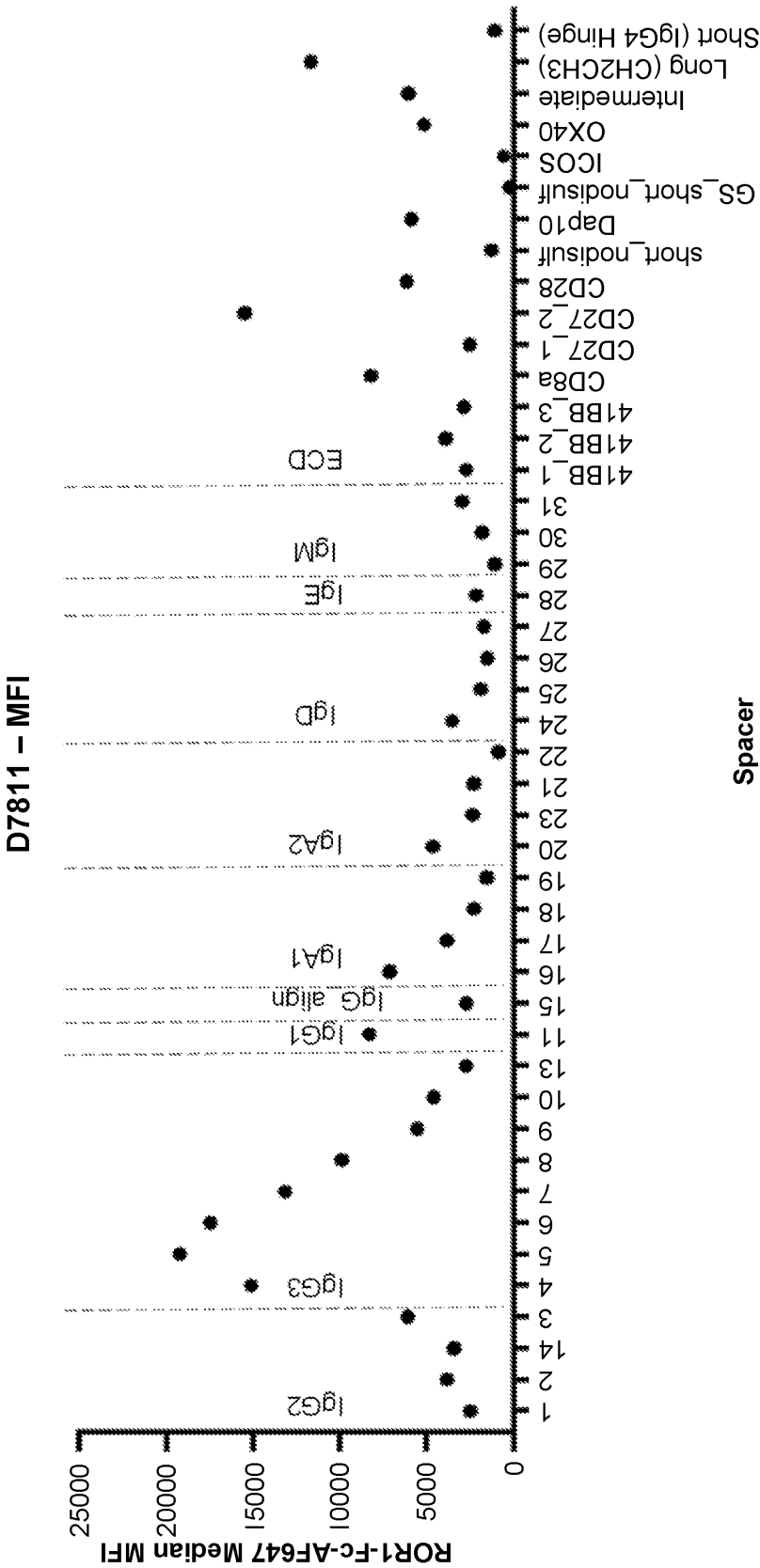


FIG. 44C

R11 CAR Transduction efficiency Donor 1 and 2

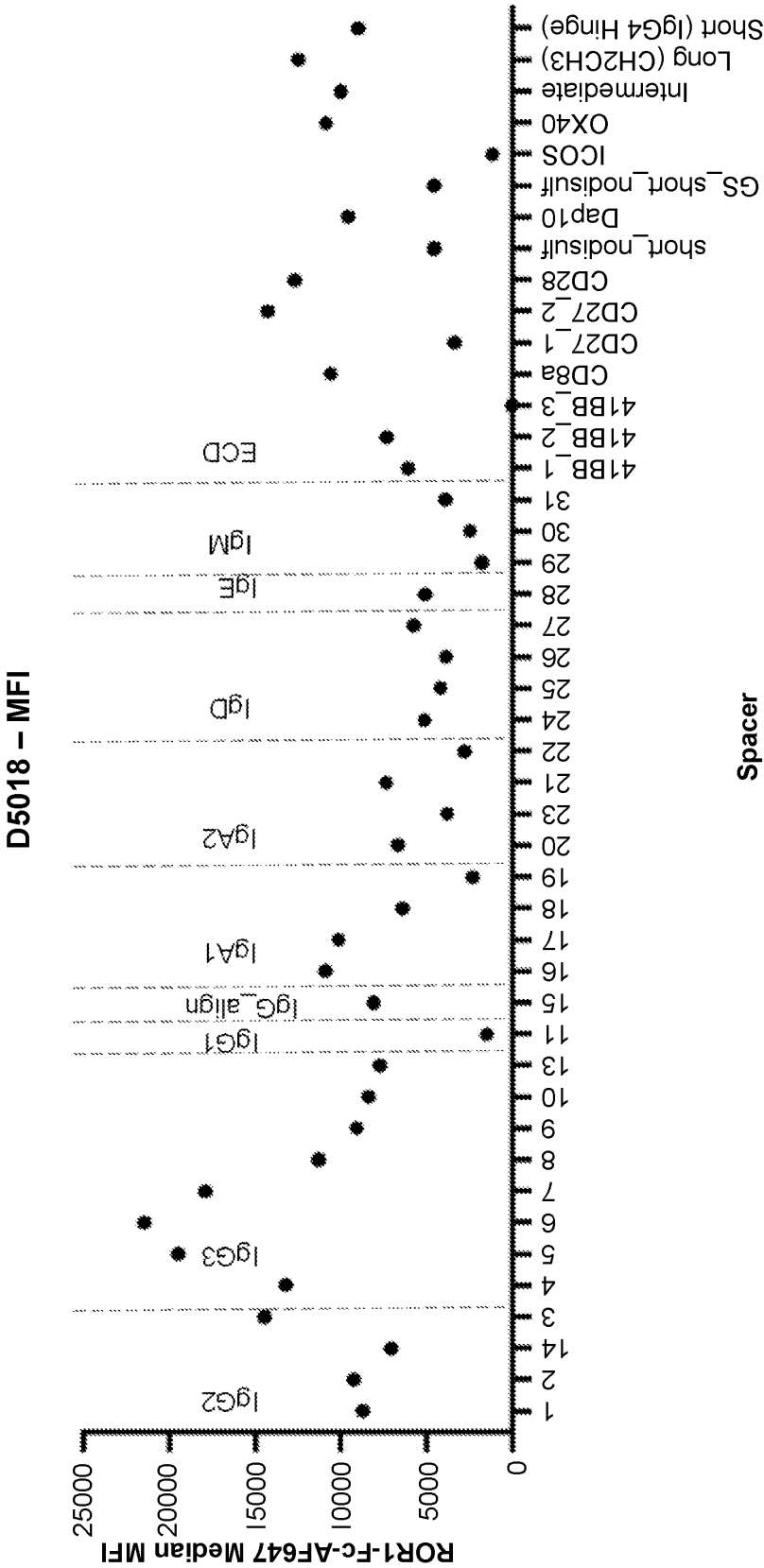


FIG. 44D

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Primary killing and AUC D7811 R11 CAR-T : A549-NLR 1:1

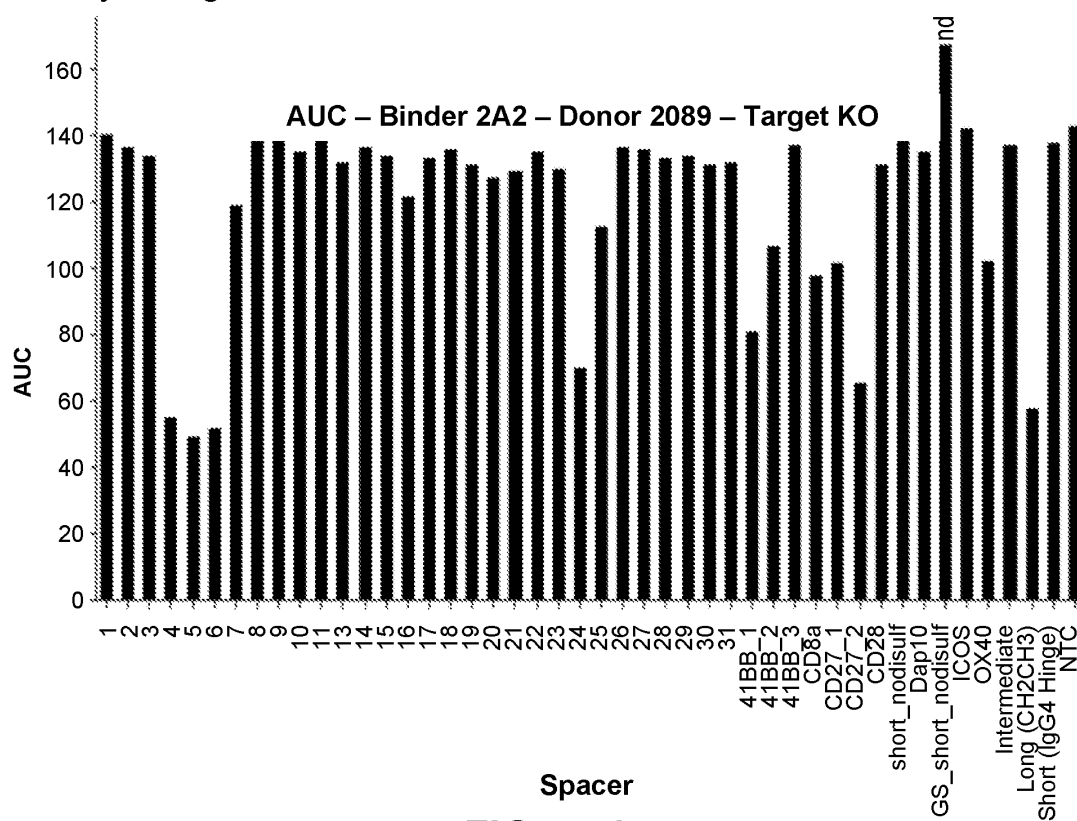


FIG. 45A

Primary killing and AUC D7811 R11 CAR-T : A549-NLR 1:1

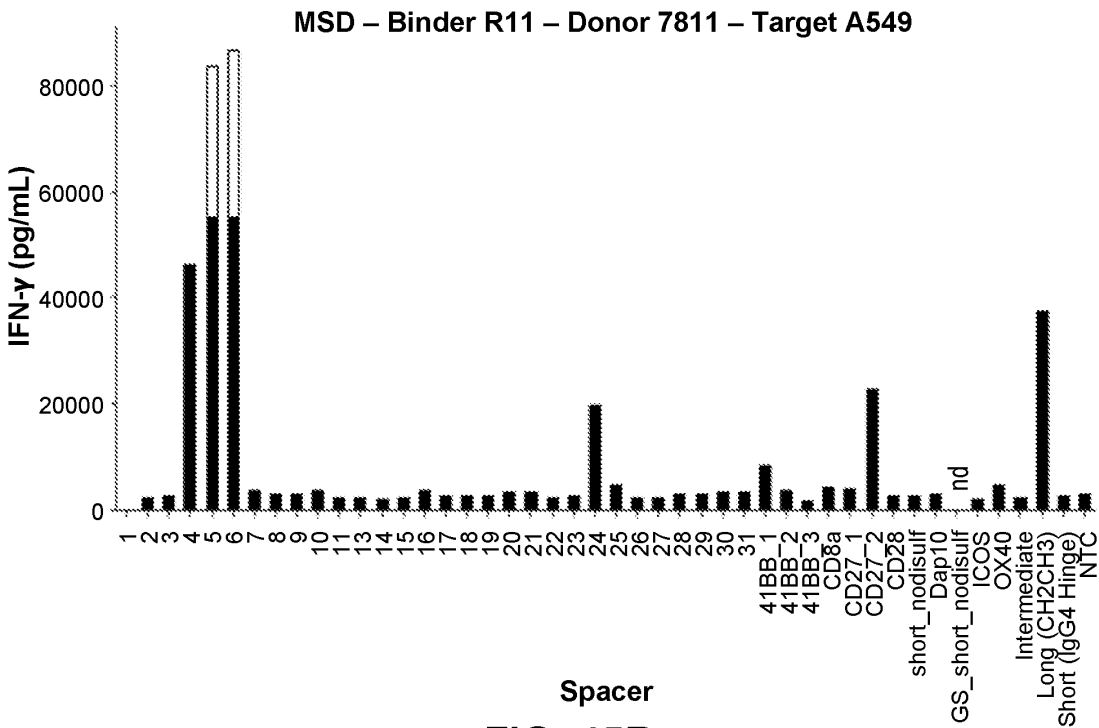


FIG. 45B

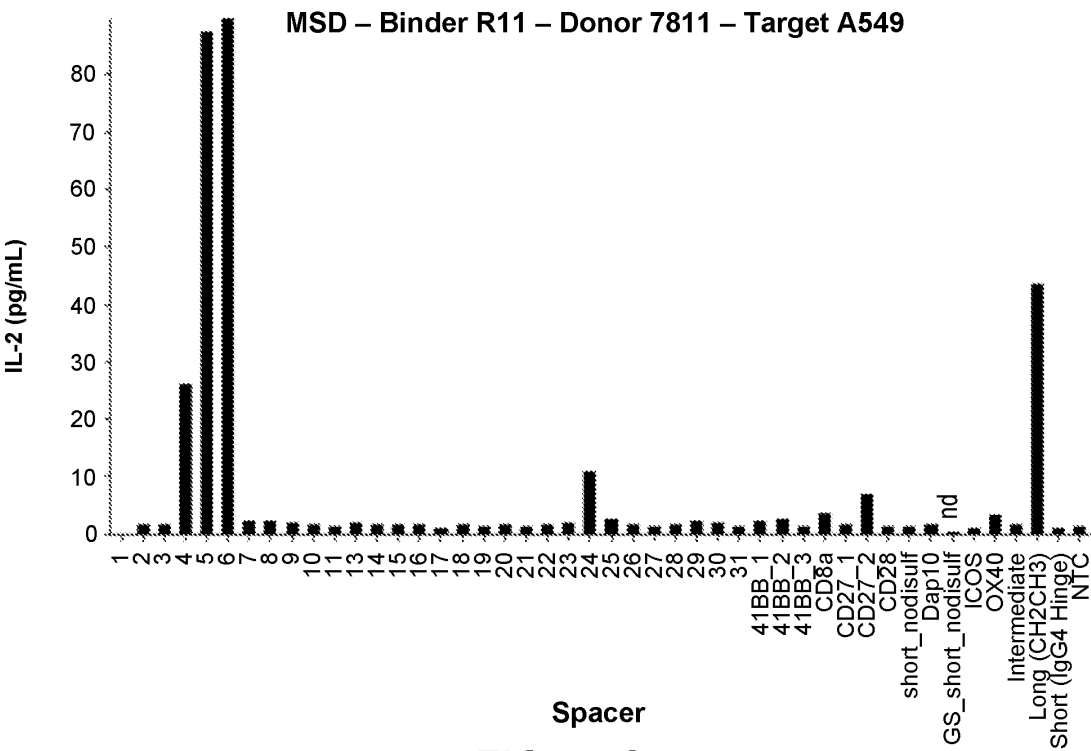


FIG. 45C

Primary killing and AUC D5018 R11 CAR-T : A549-NLR 1:1

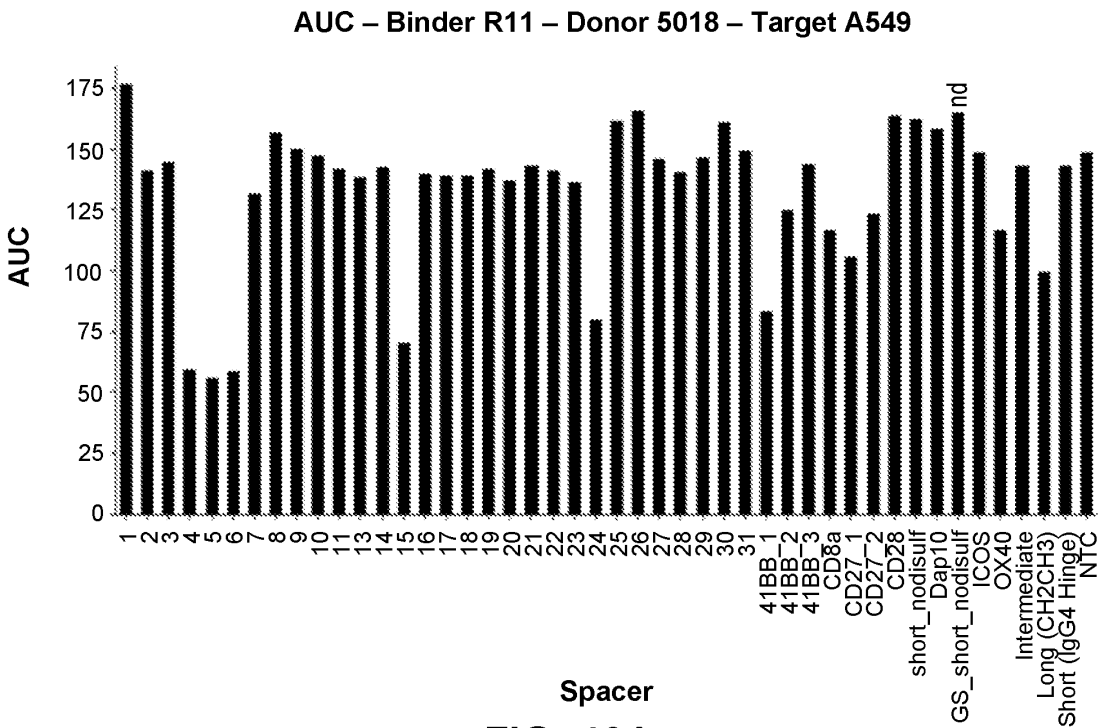


FIG. 46A

Primary killing and AUC D5018 R11 CAR-T : A549-NLR 1:1

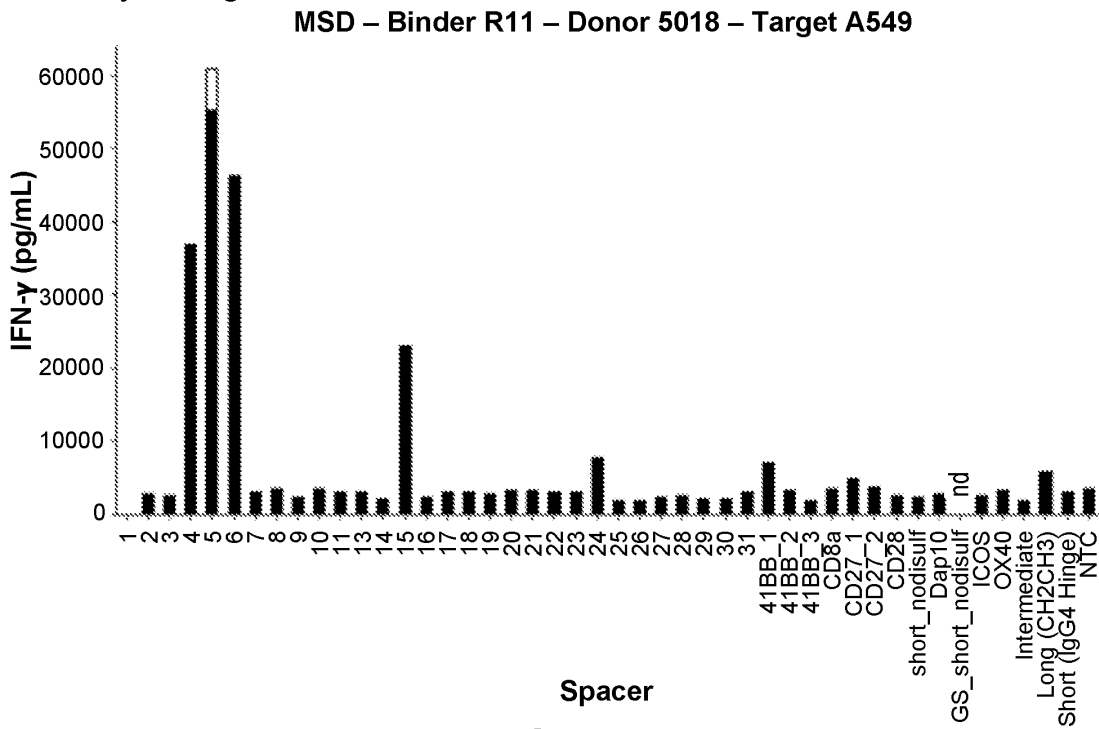


FIG. 46B

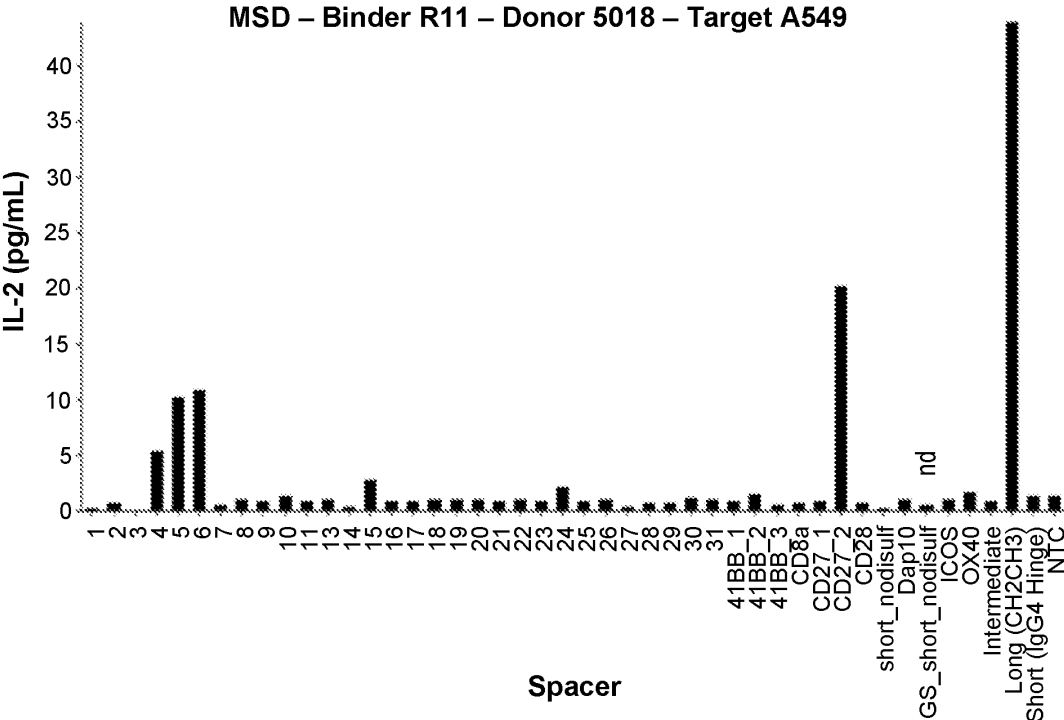


FIG. 46C

Primary killing and AUC D7811 R11 CAR-T : H1975-NLR 1:1

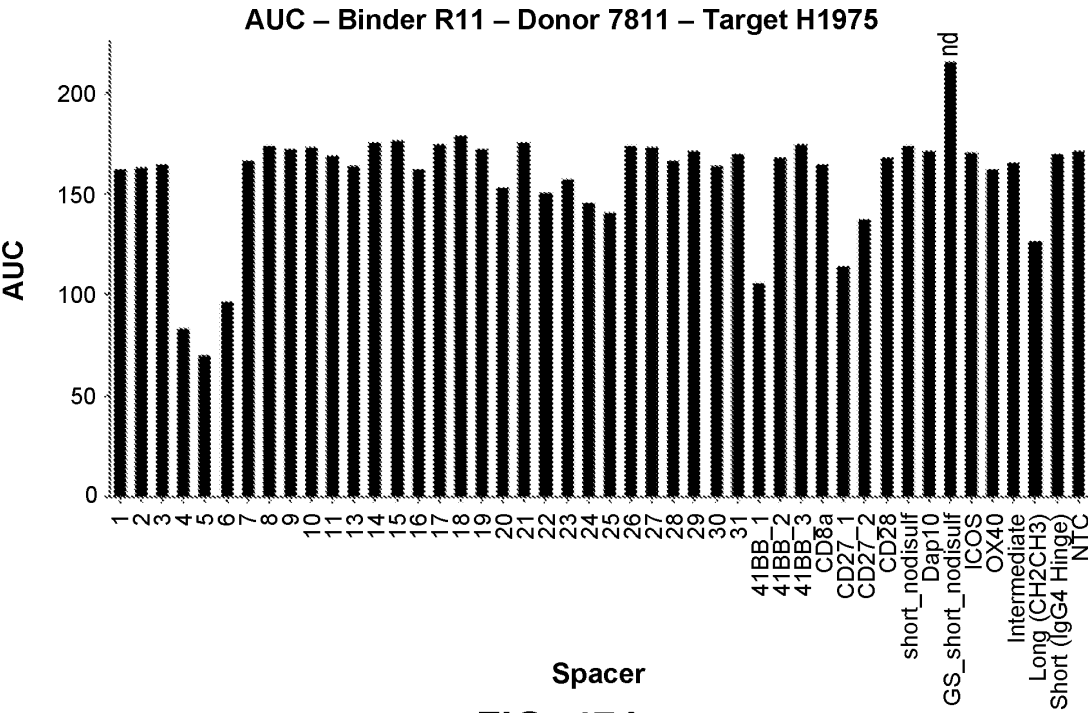


FIG. 47A

Primary killing and AUC D7811 R11 CAR-T : H1975-NLR 1:1

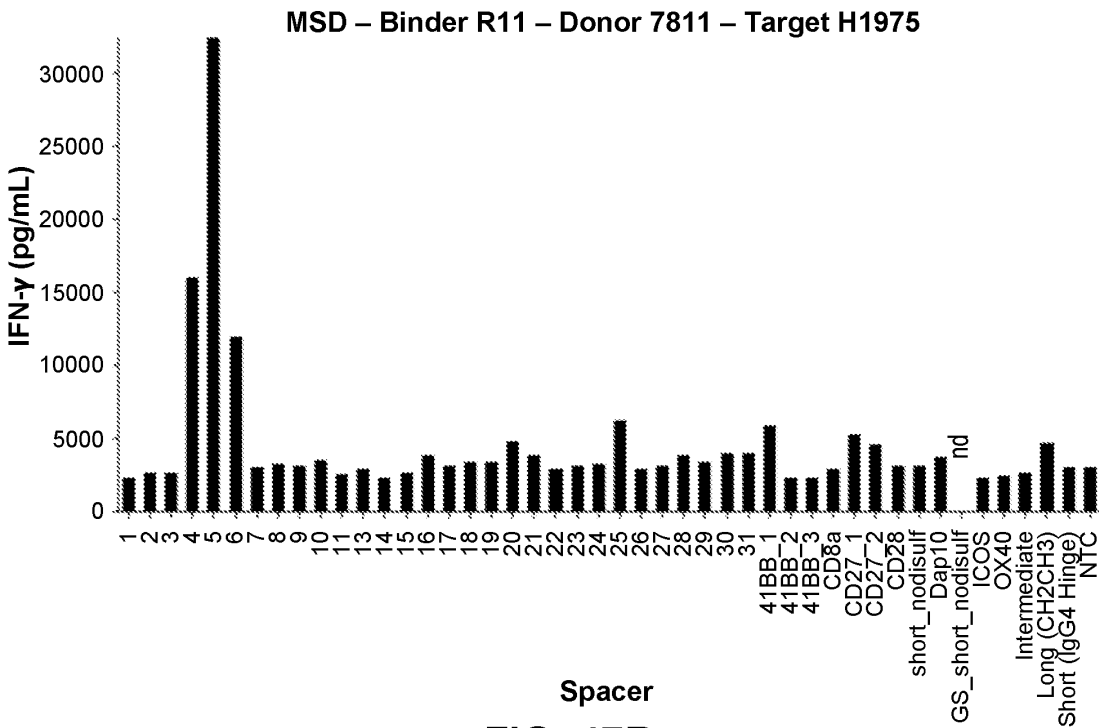


FIG. 47B

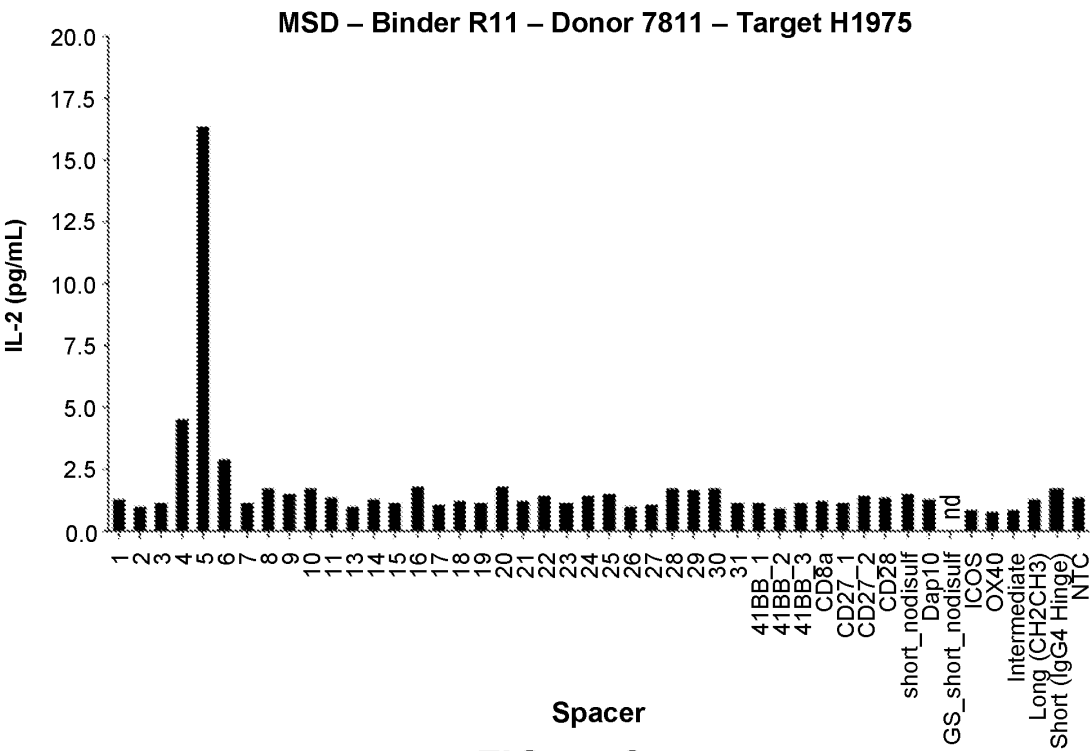


FIG. 47C



Primary killing and AUC D7811 R11 CAR-T : A549-ROR1KO-NLR 1:1

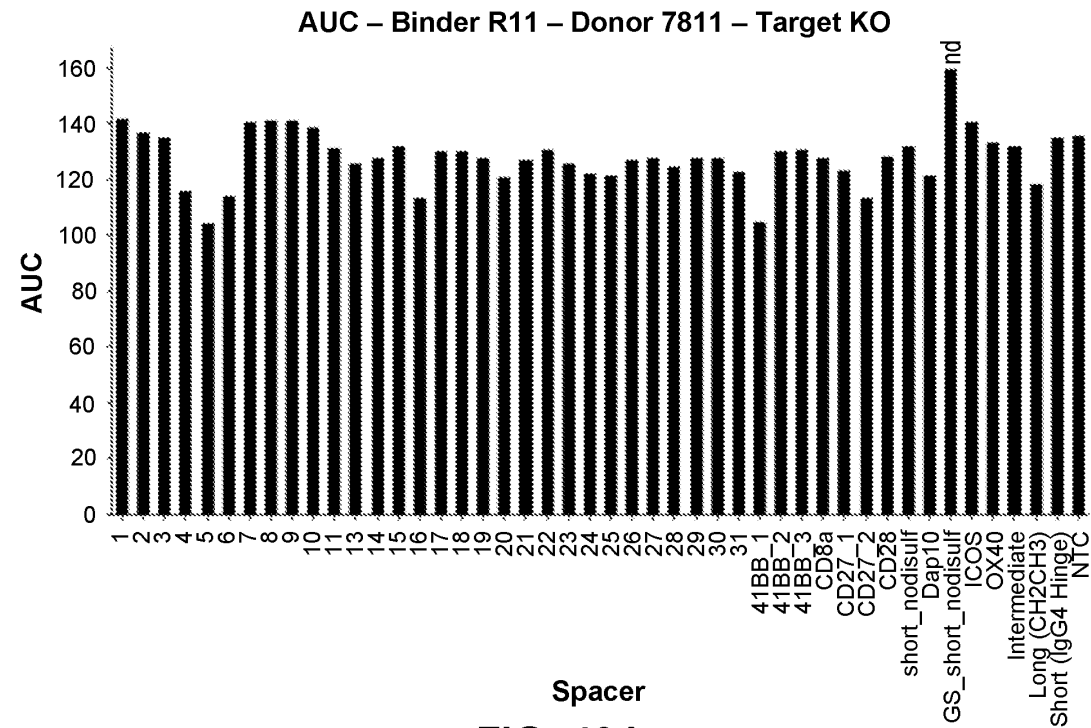


FIG. 49A

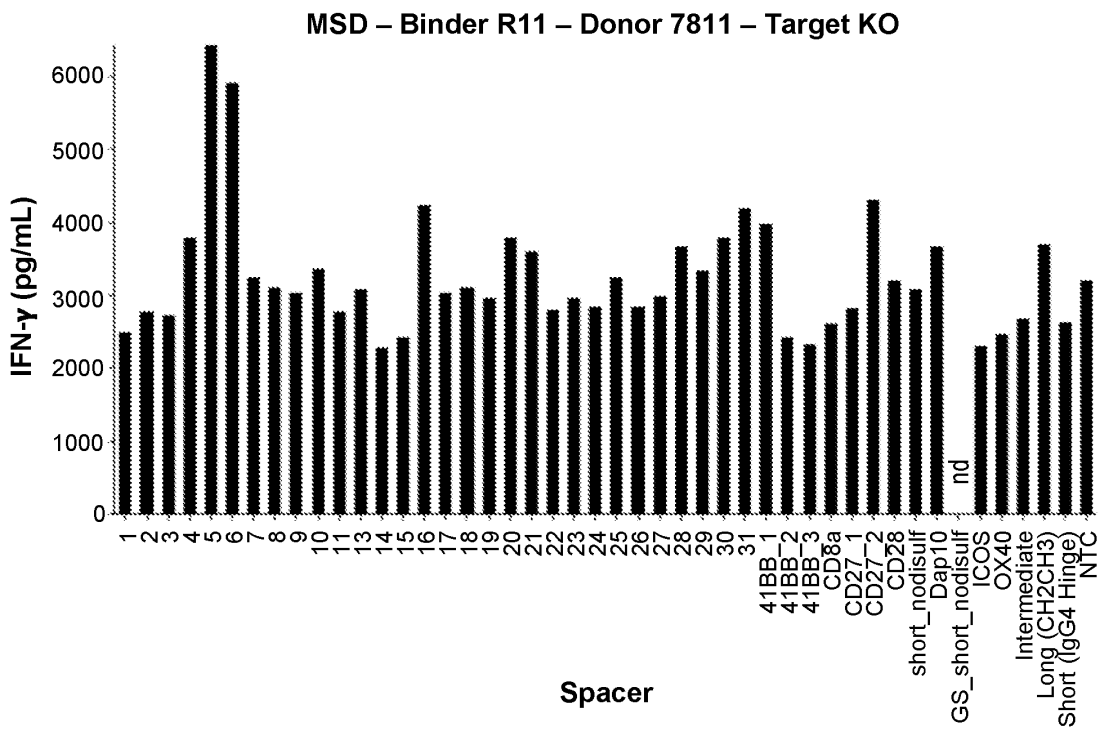
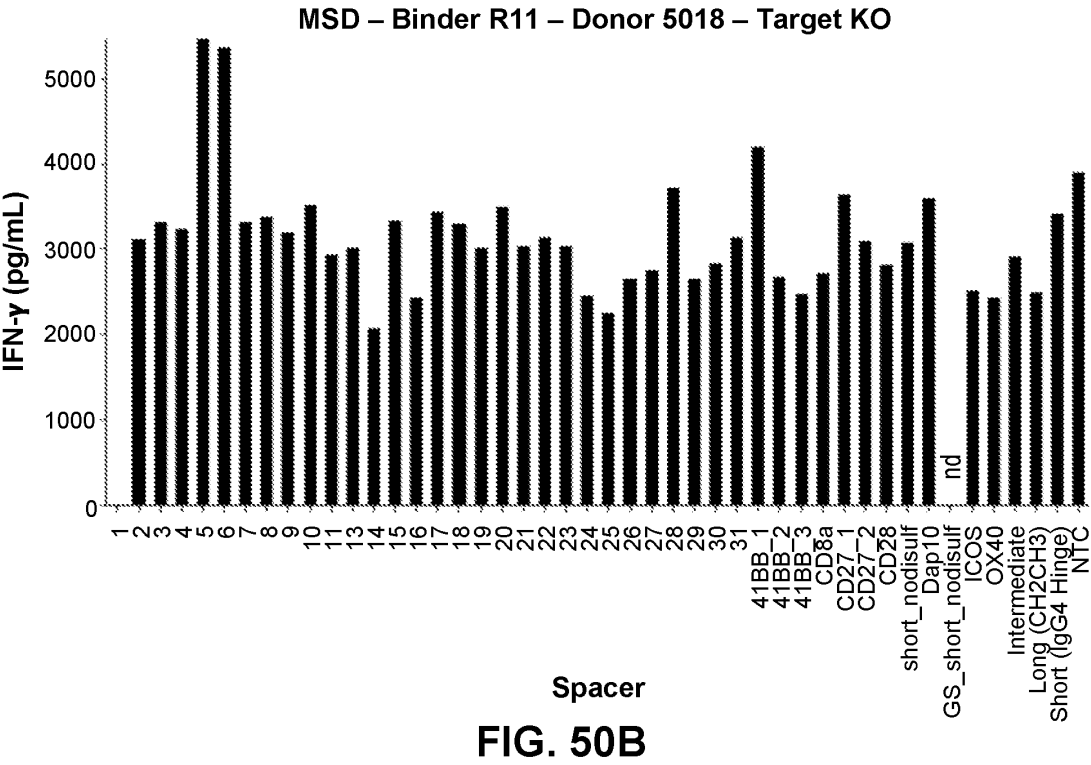
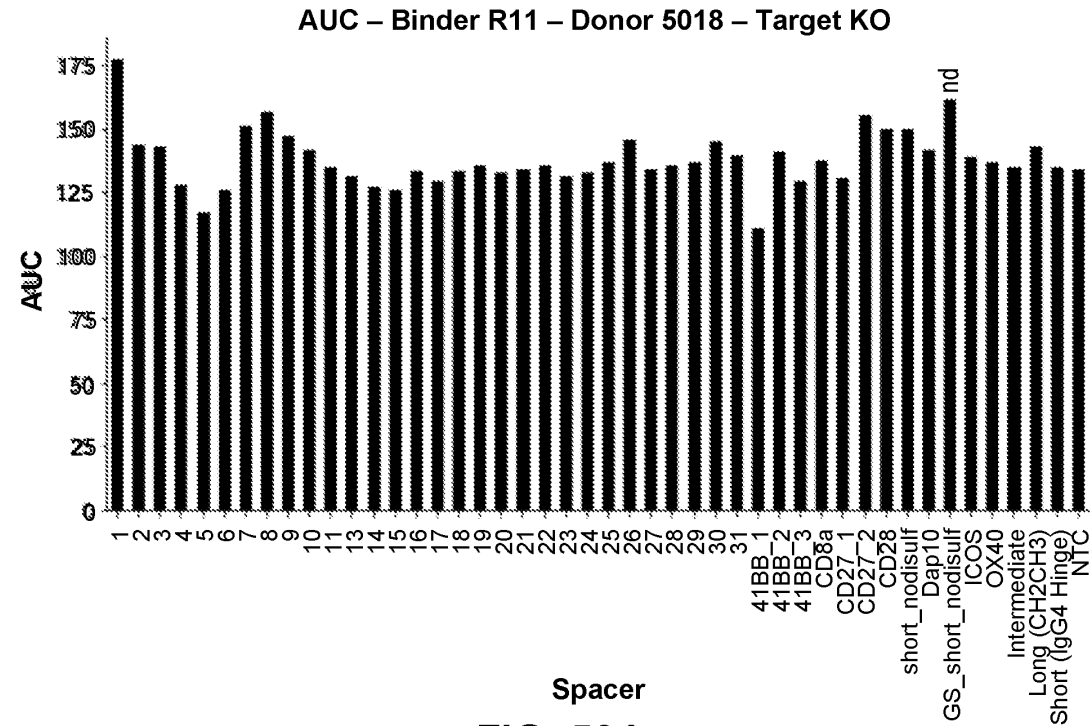


FIG. 49B

Primary killing and AUC D5018 R11 CAR-T : A549-ROR1KO-NLR 1:1



D7811 R11 CAR-T Target independent cytokines

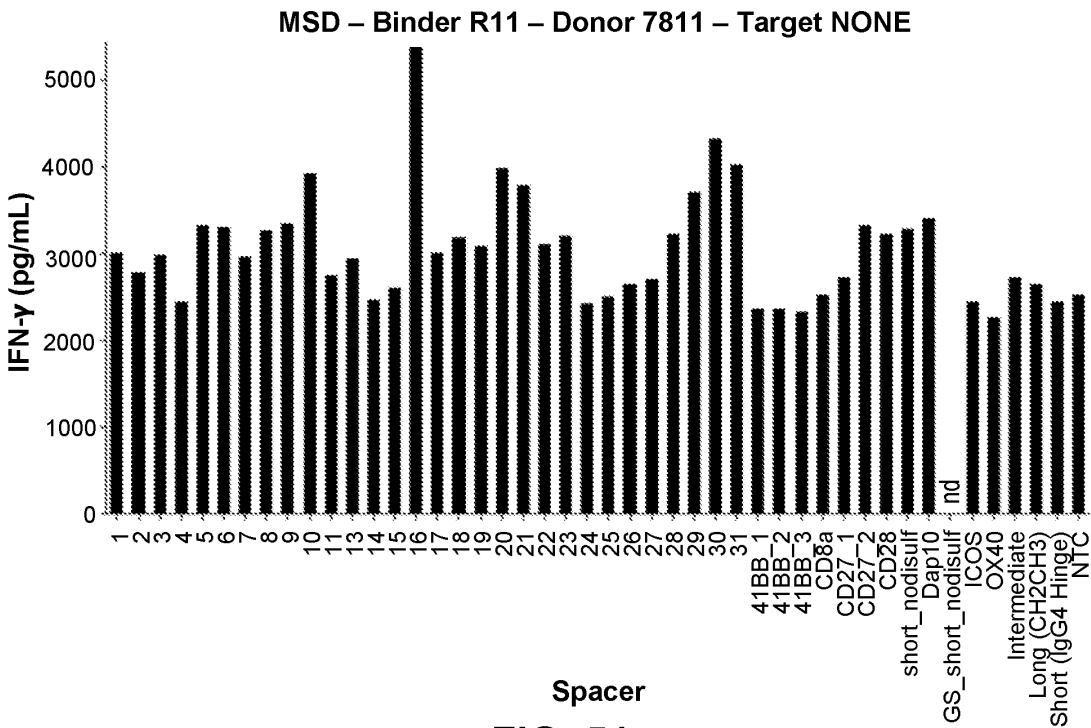


FIG. 51

D5018 R11 CAR-T Target independent cytokines

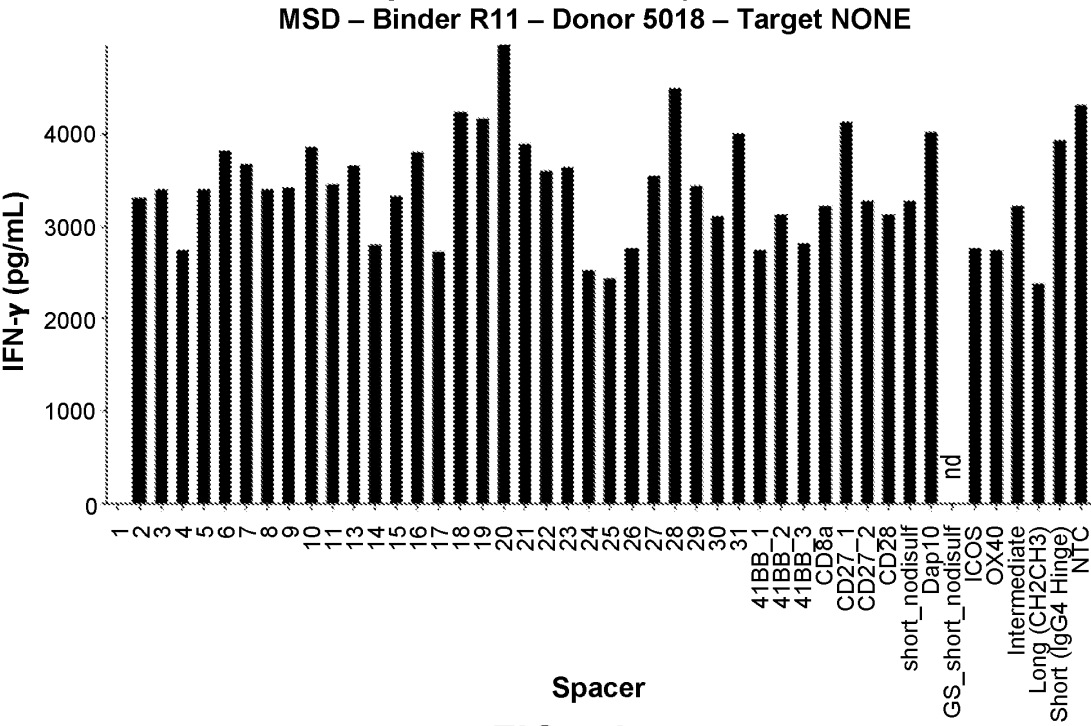


FIG. 52

D7811 R11 CAR-T Scatter plots suggest optimal spacer length for R11 is ~200Å

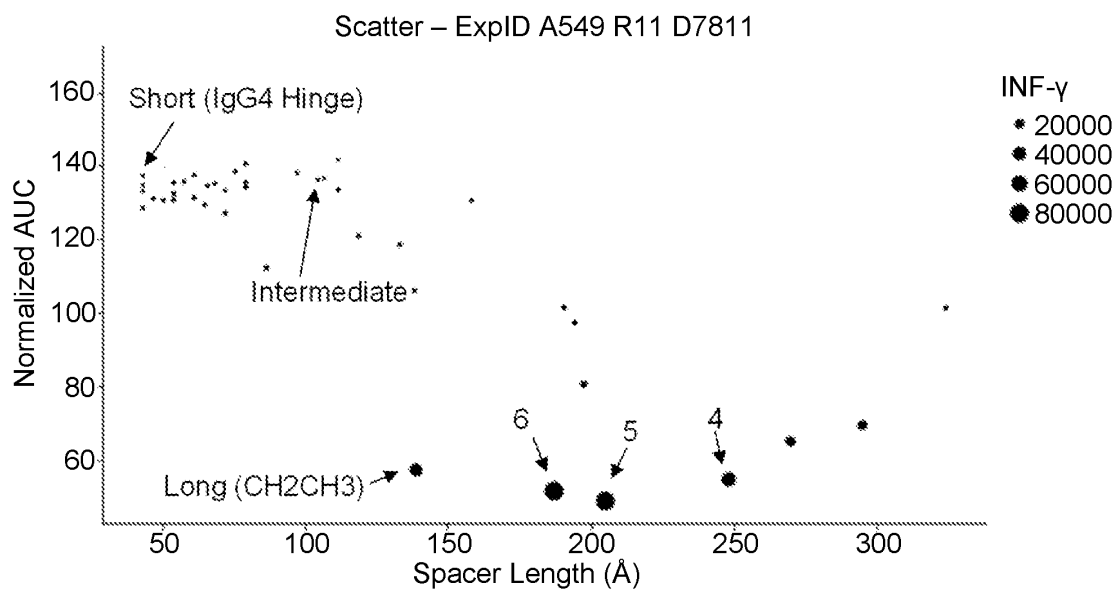


FIG. 53A

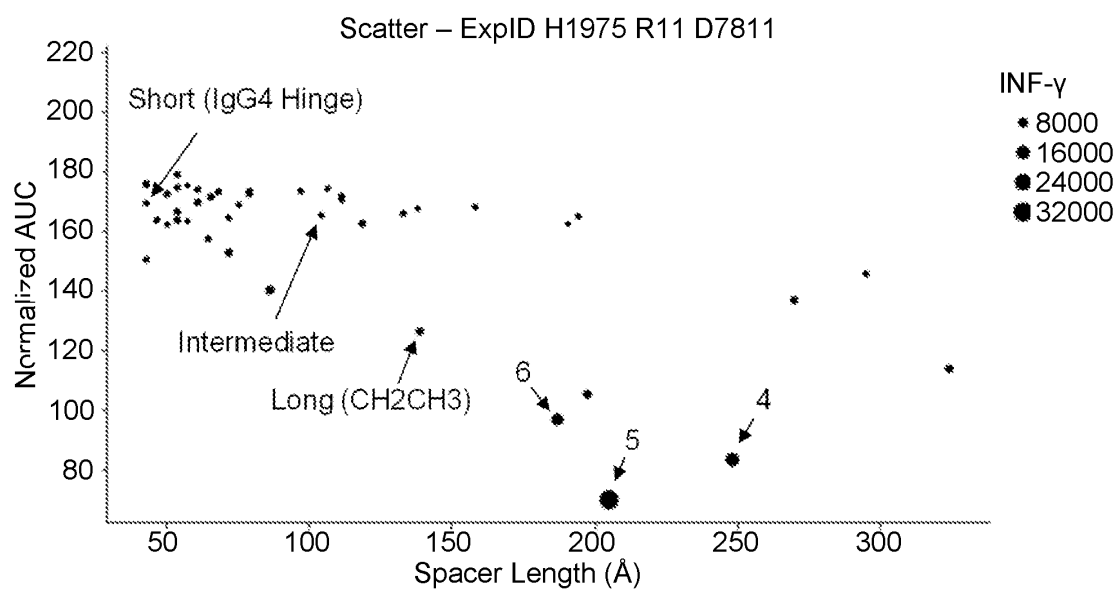


FIG. 53B

D5018 R11 CAR-T Scatter plots suggest optimal spacer length for R11 is ~200Å

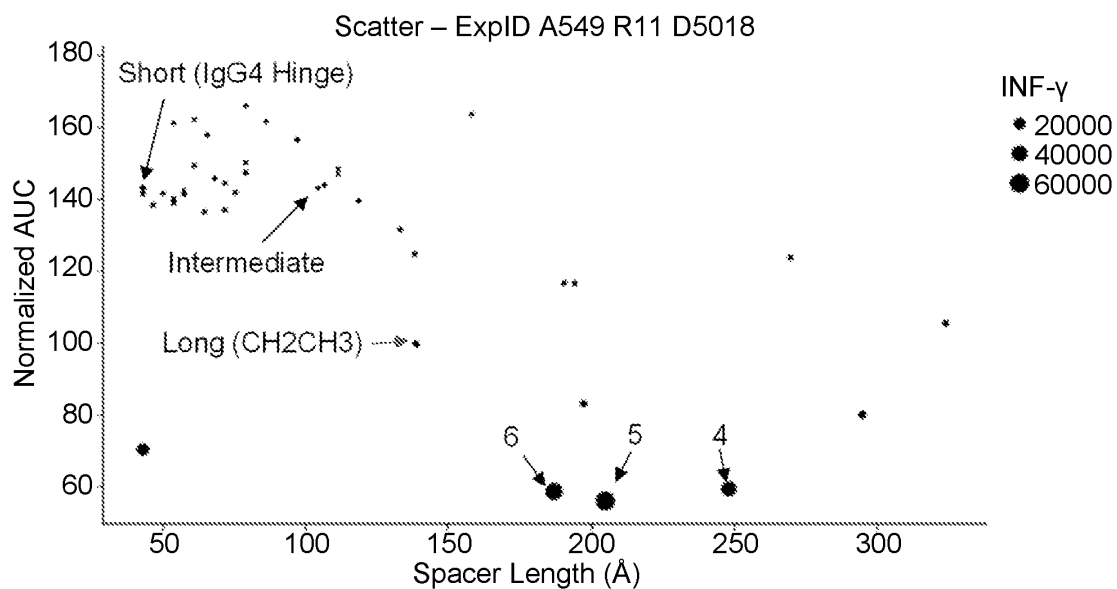


FIG. 54A

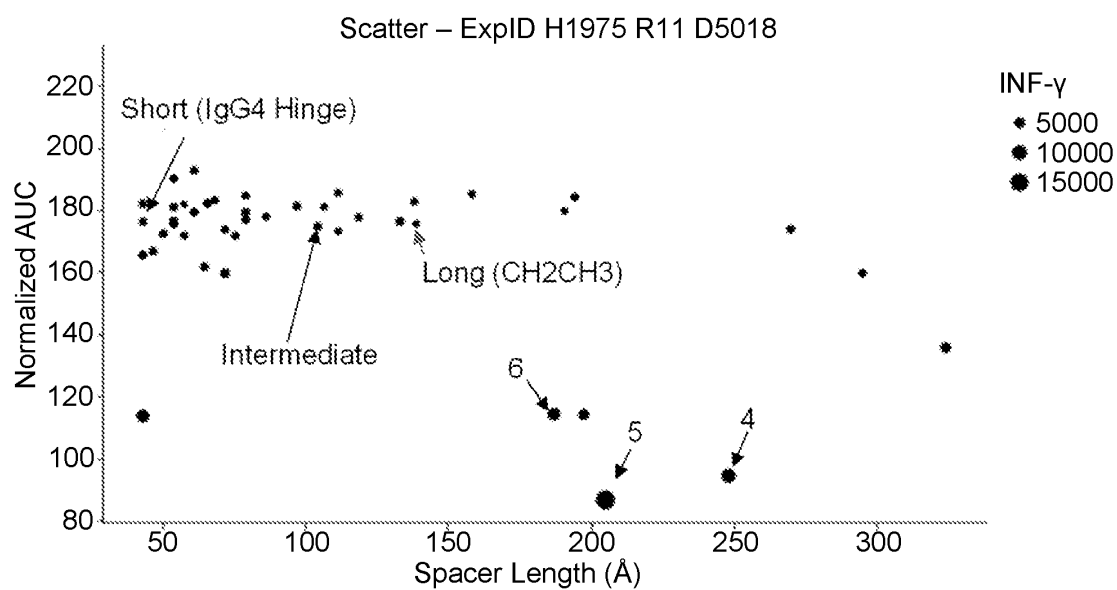


FIG. 54B

R12 CAR Transduction efficiency Donor 1 and 2

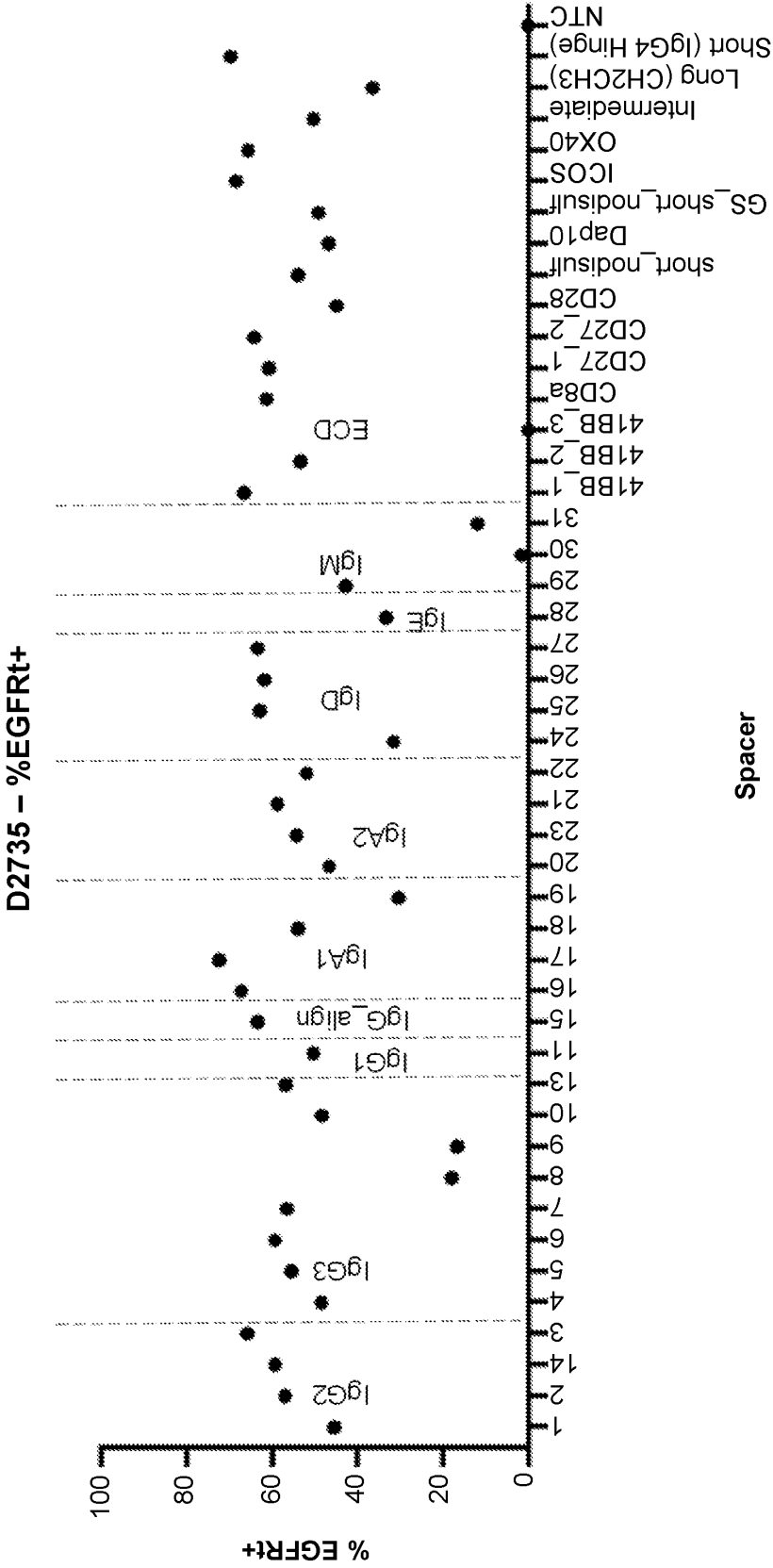


FIG. 55A

R12 CAR Transduction efficiency Donor 1 and 2

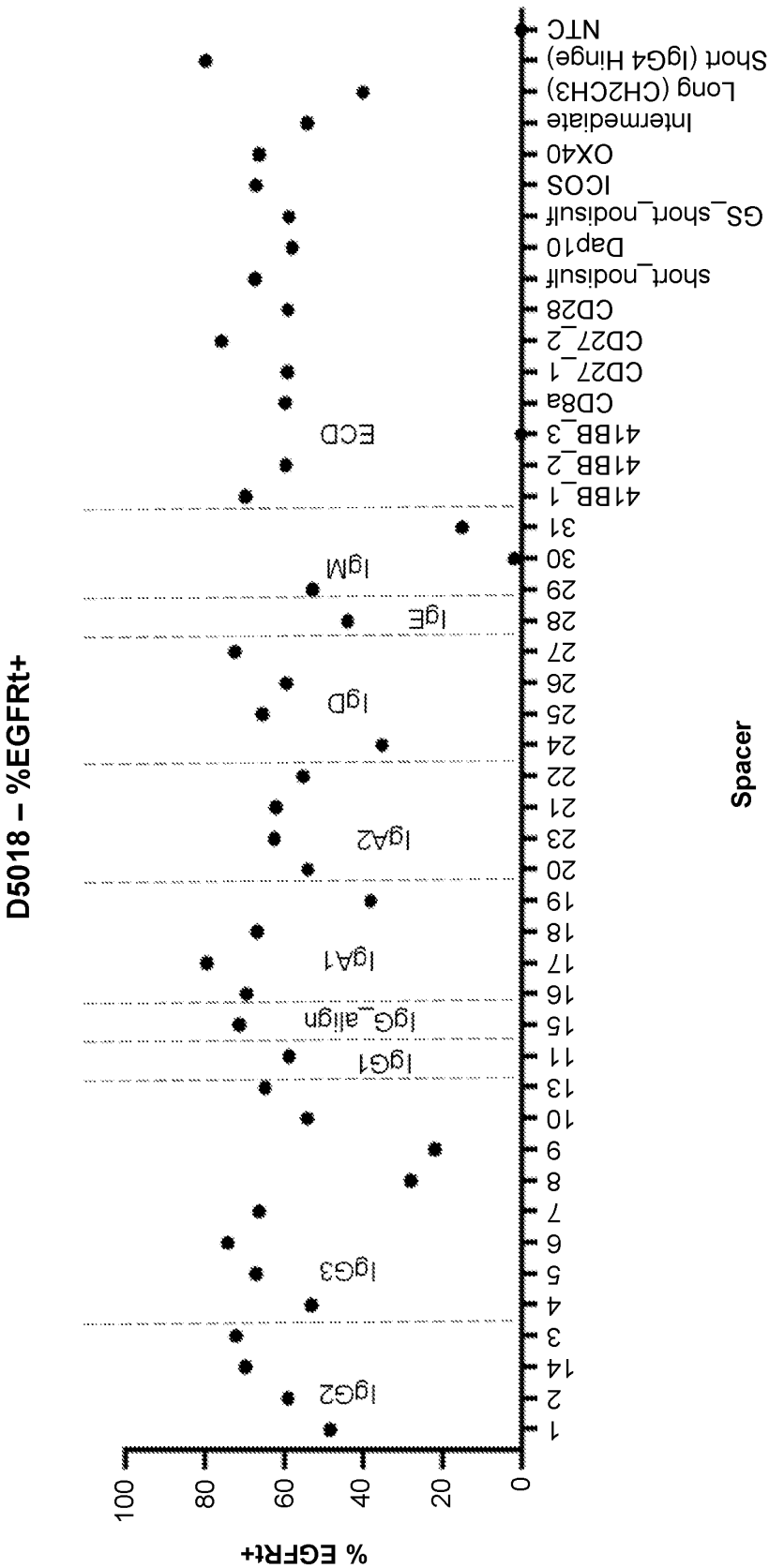


FIG. 55B

R12 CAR Transduction efficiency Donor 1 and 2

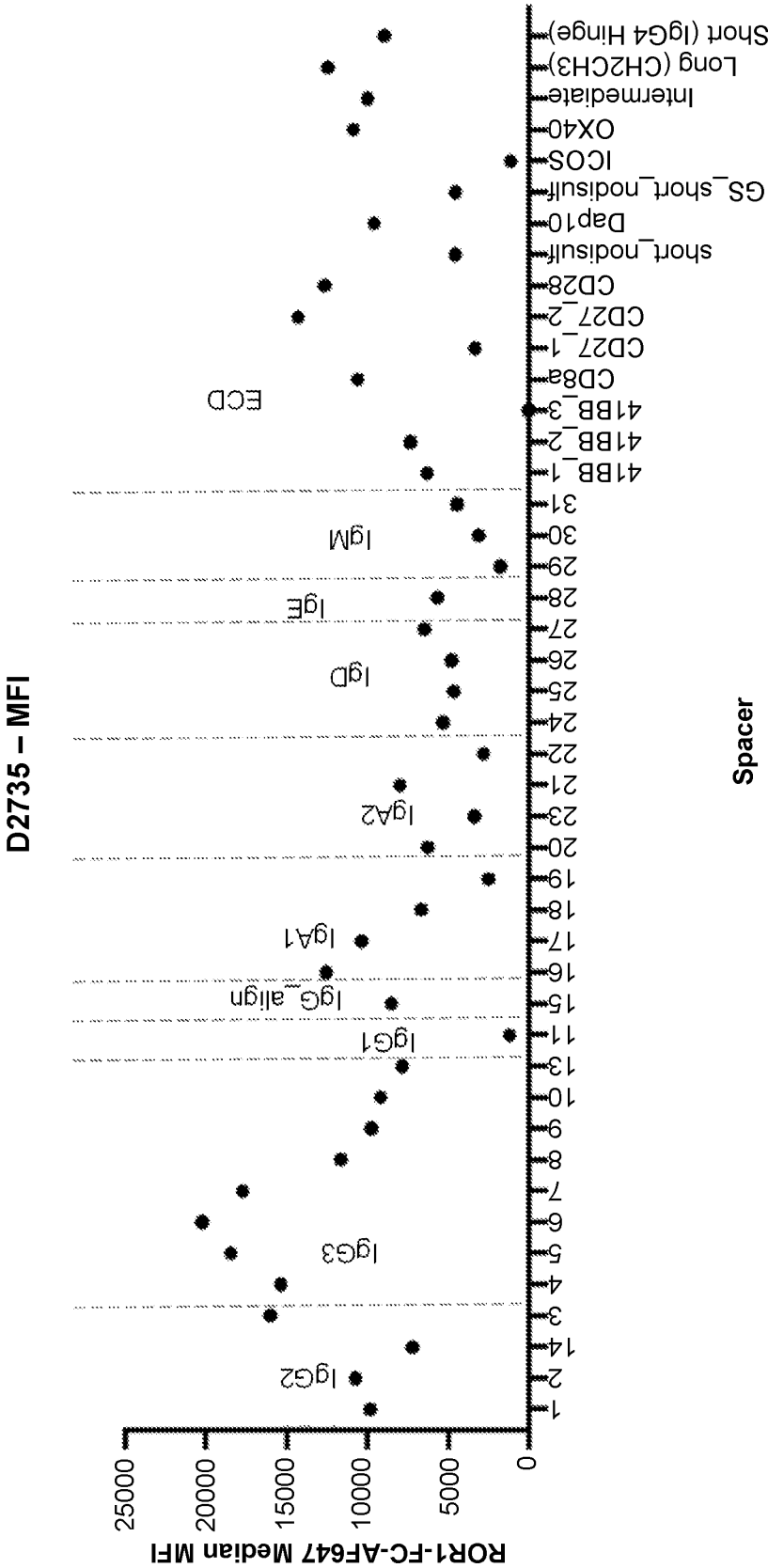


FIG. 55C

R12 CAR Transduction efficiency Donor 1 and 2

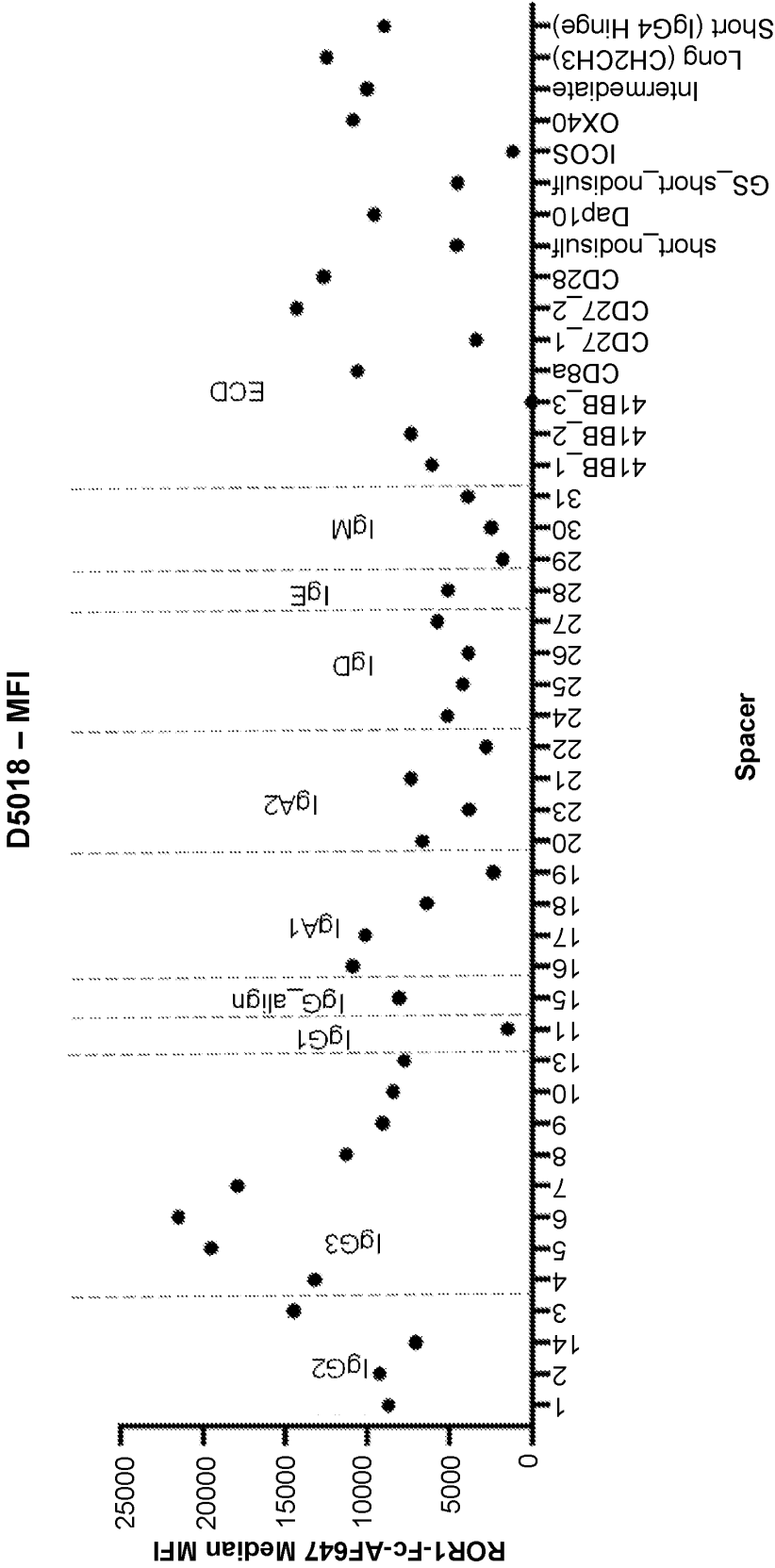


FIG. 55D

Primary killing and AUC D2735 R12 CAR-T : A549-NLR 1:1

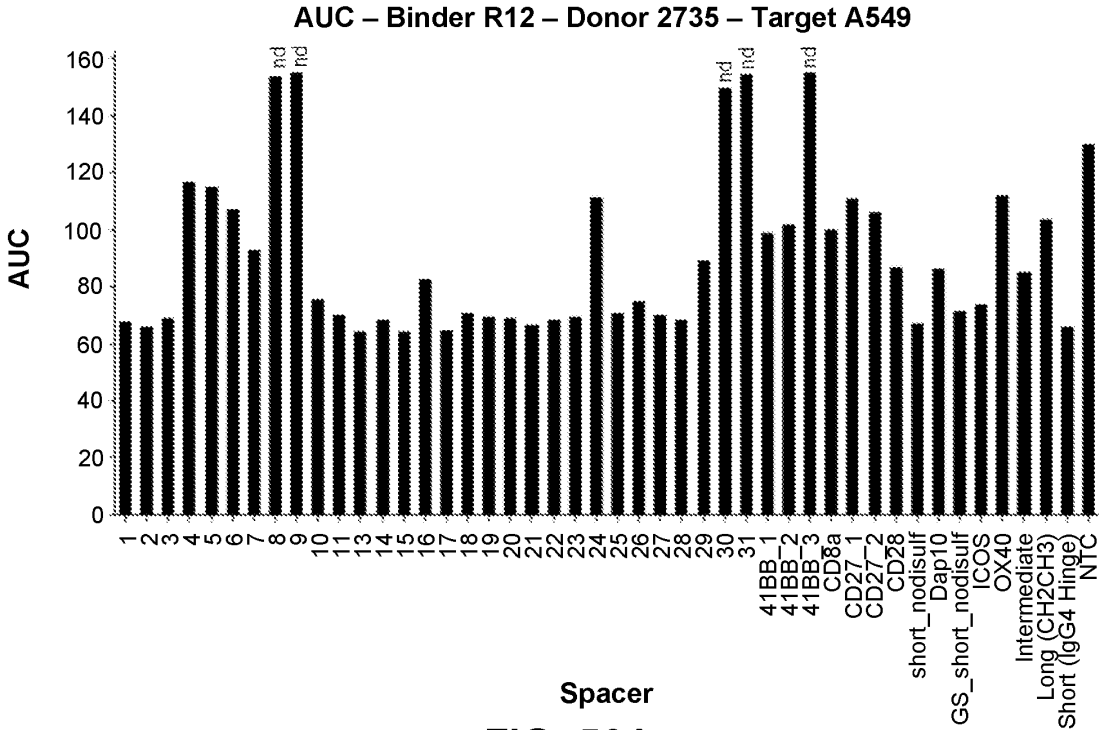


FIG. 56A

Primary killing and AUC D2735 R12 CAR-T : A549-NLR 1:1

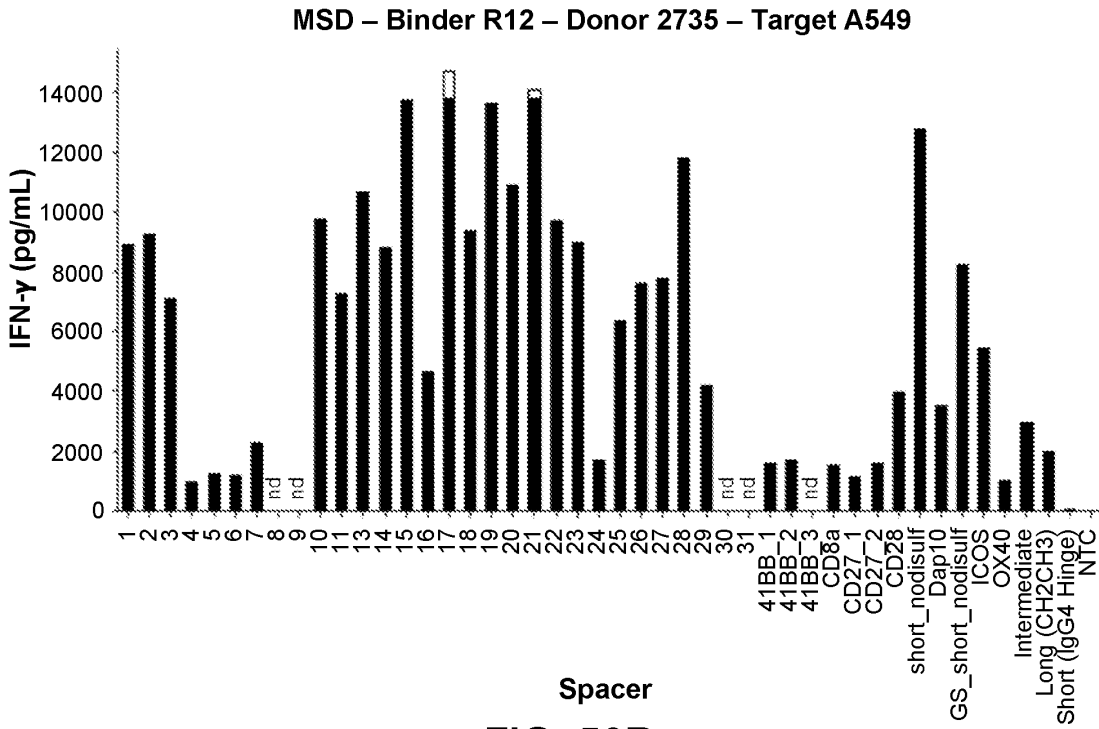


FIG. 56B

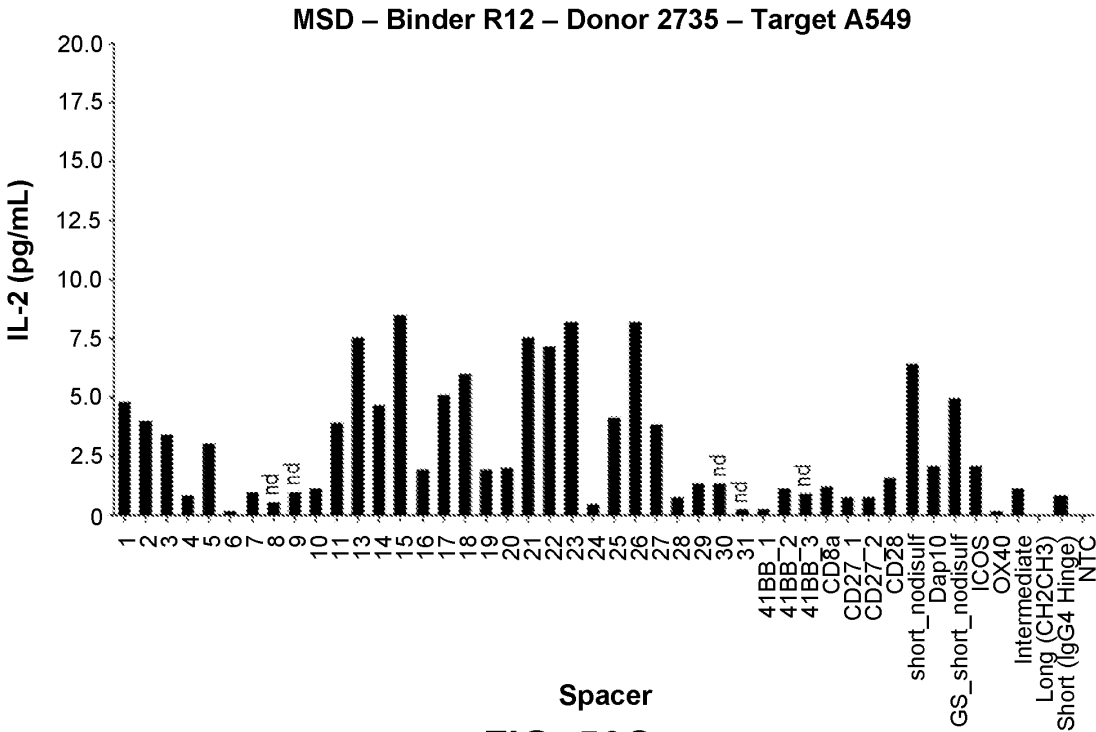


FIG. 56C

Primary killing and AUC D5018 R12 CAR-T : A549-NLR 1:1

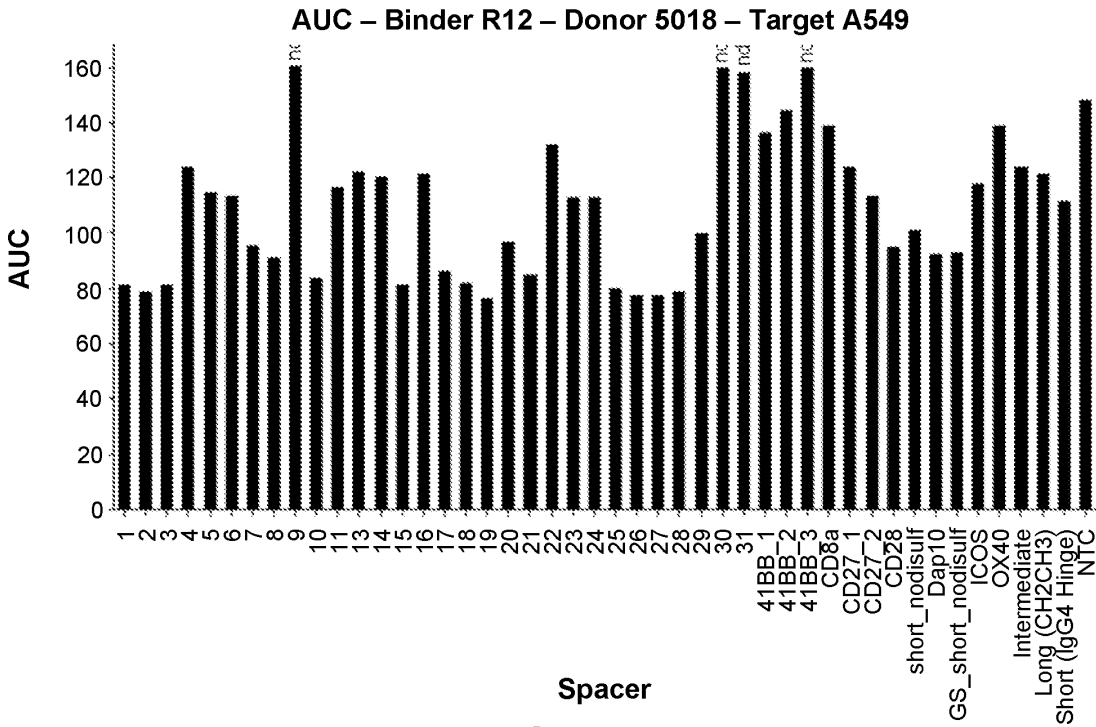


FIG. 57A

Primary killing and AUC D5018 R12 CAR-T : A549-NLR 1:1

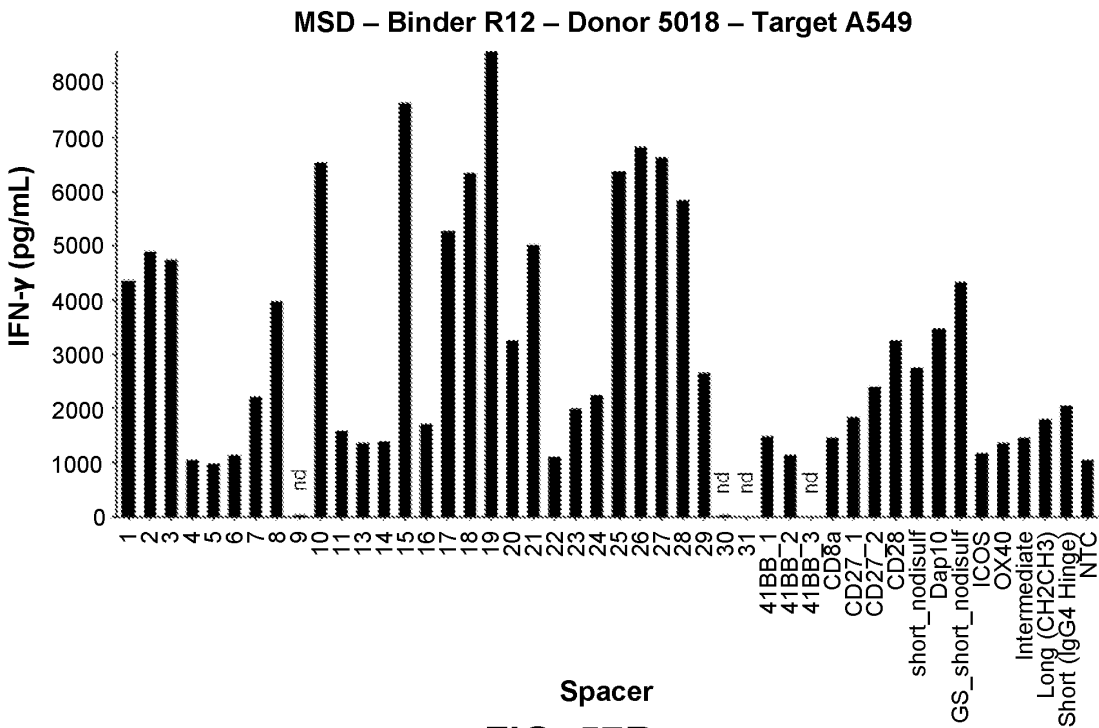


FIG. 57B

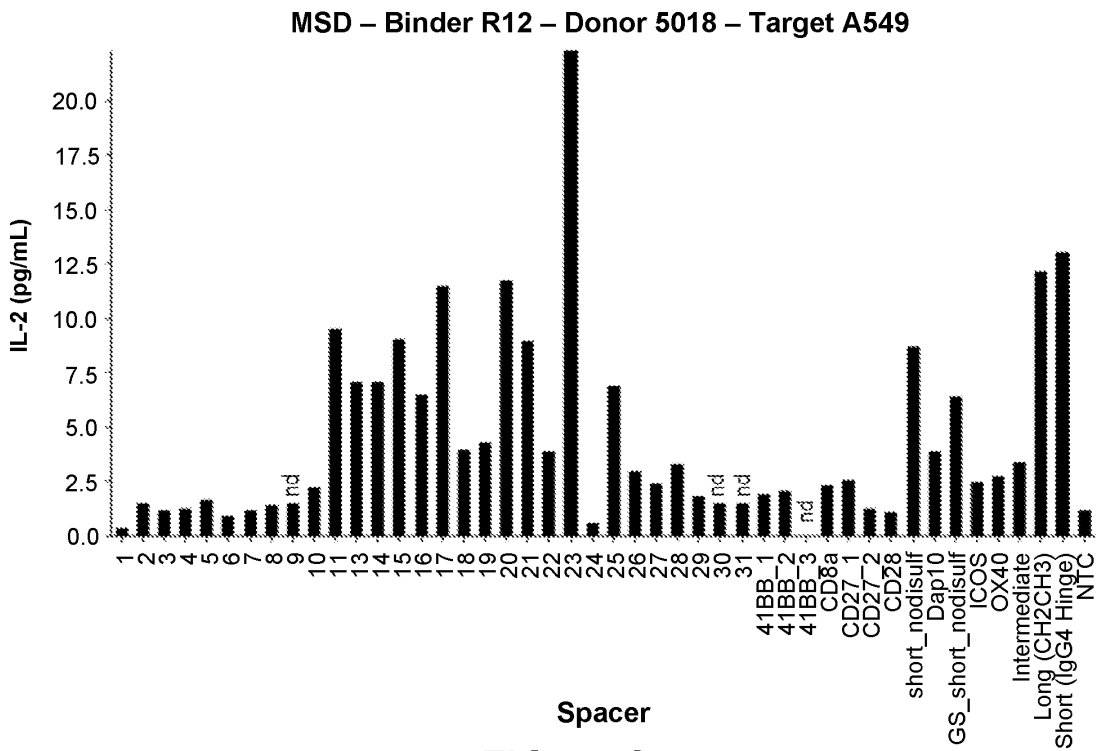


FIG. 57C

Primary killing and AUC D2735 R12 CAR-T : H1975-NLR 1:1

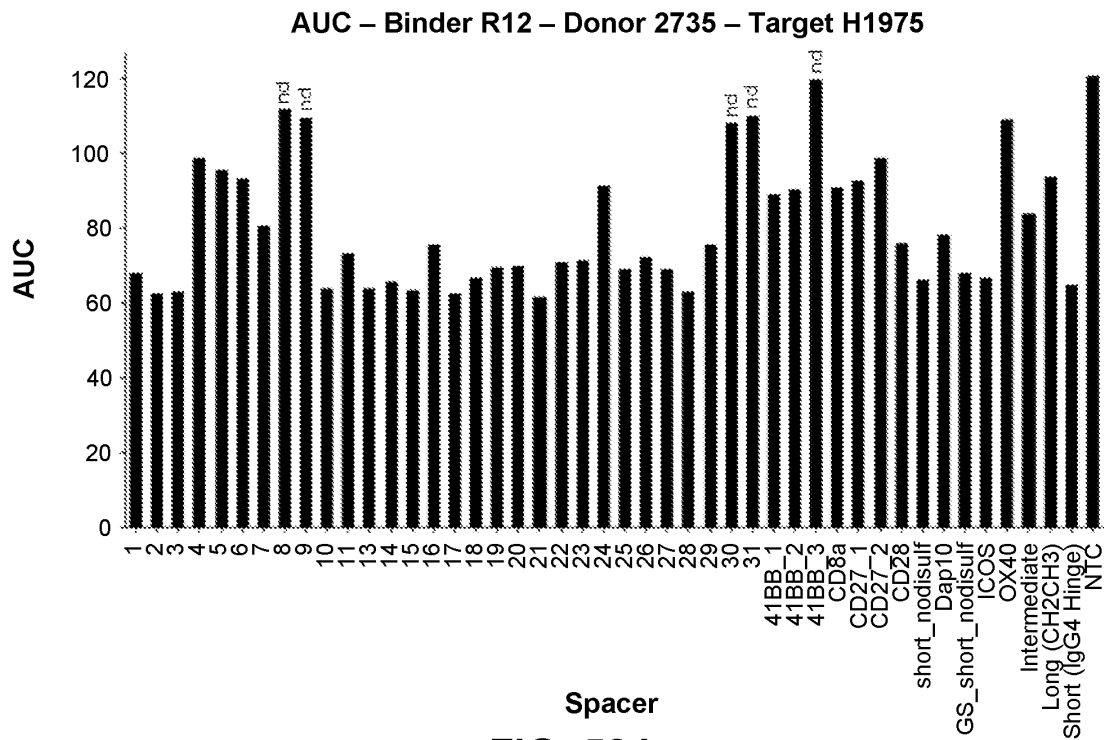


FIG. 58A

Primary killing and AUC D2735 R12 CAR-T : H1975-NLR 1:1

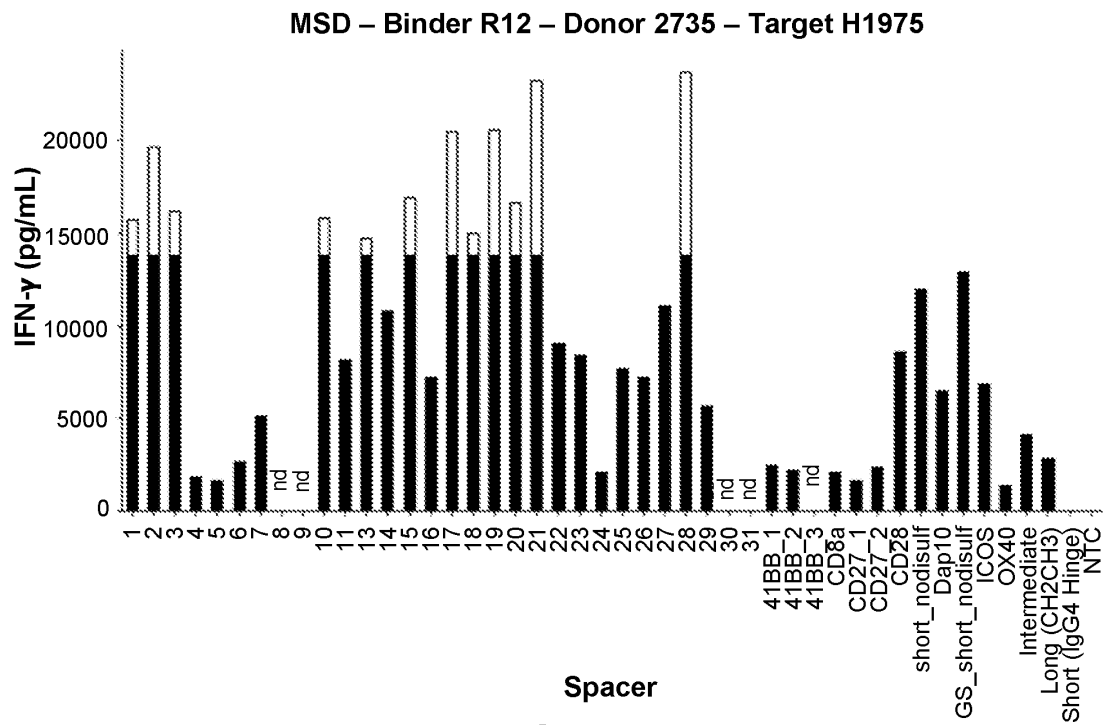


FIG. 58B

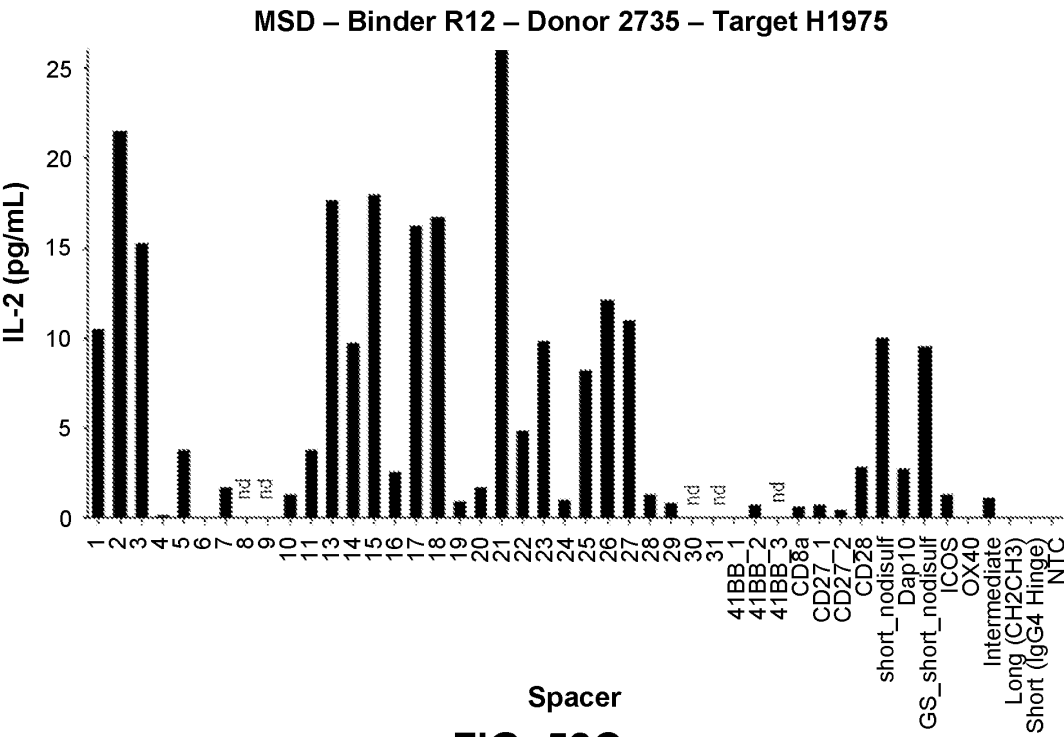


FIG. 58C

Primary killing and AUC D5018 R12 CAR-T : H1975-NLR 1:1
AUC – Binder R12 – Donor 5018 – Target H1975

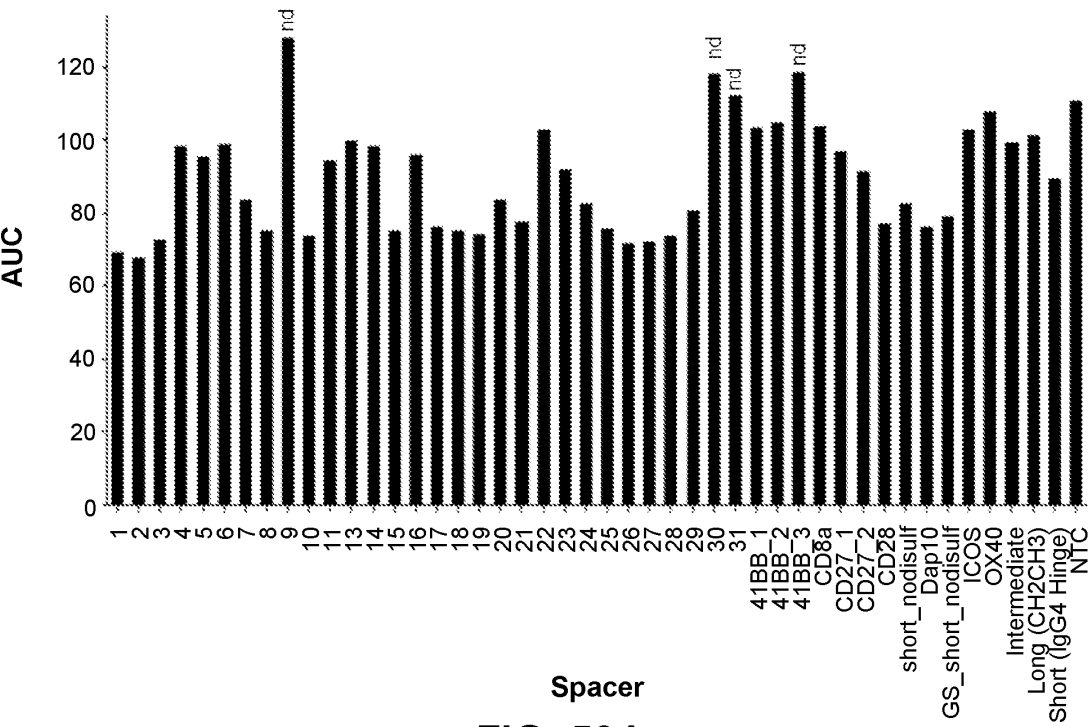


FIG. 59A

Primary killing and AUC D5018 R12 CAR-T : H1975-NLR 1:1

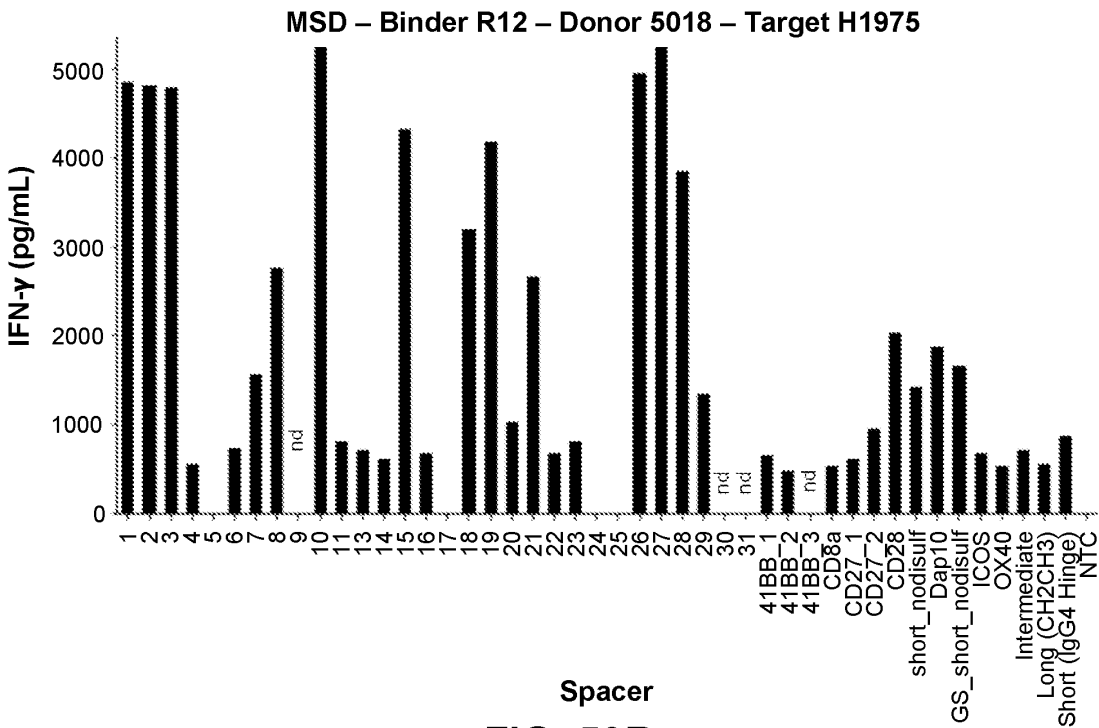


FIG. 59B

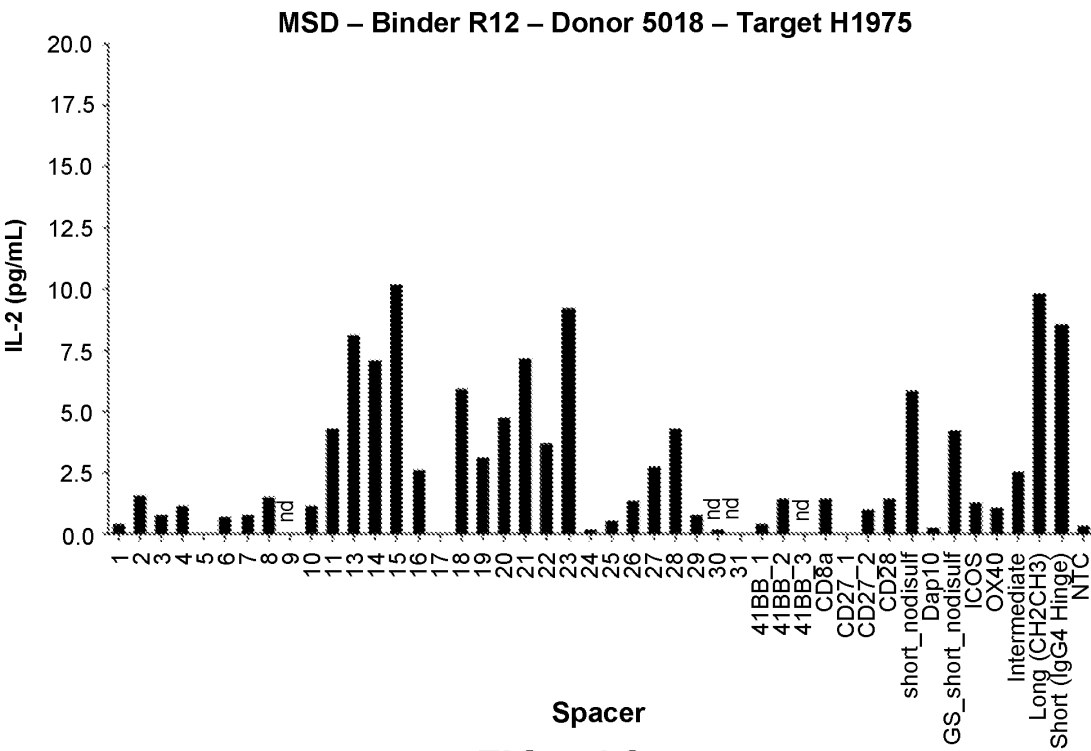


FIG. 59C

Primary killing and AUC D2735 R12 CAR-T : A549-ROR1KO-NLR 1:1

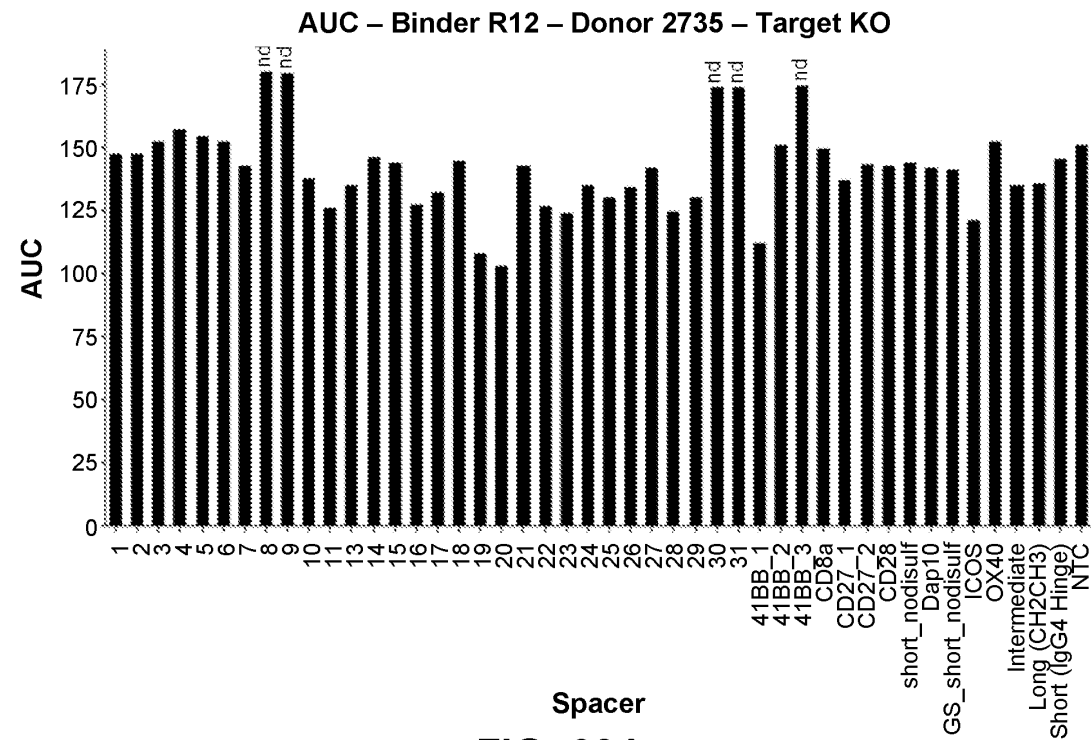


FIG. 60A

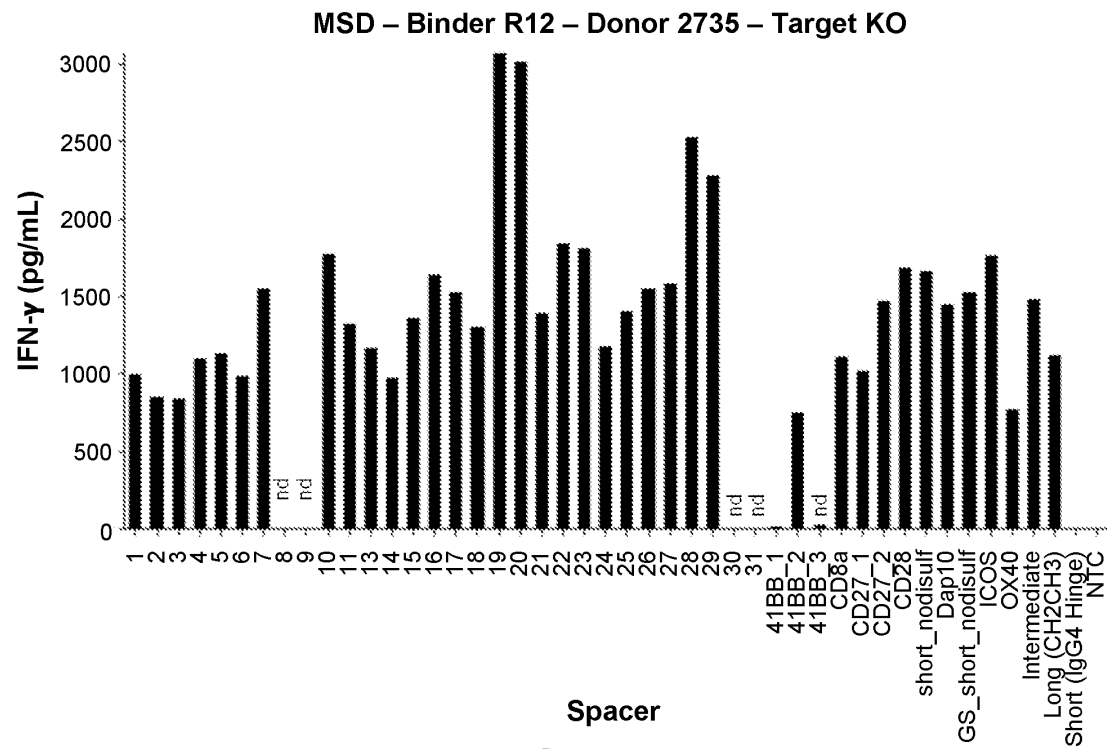


FIG. 60B

Primary killing and AUC D5018 R12 CAR-T : A549-ROR1KO-NLR 1:1

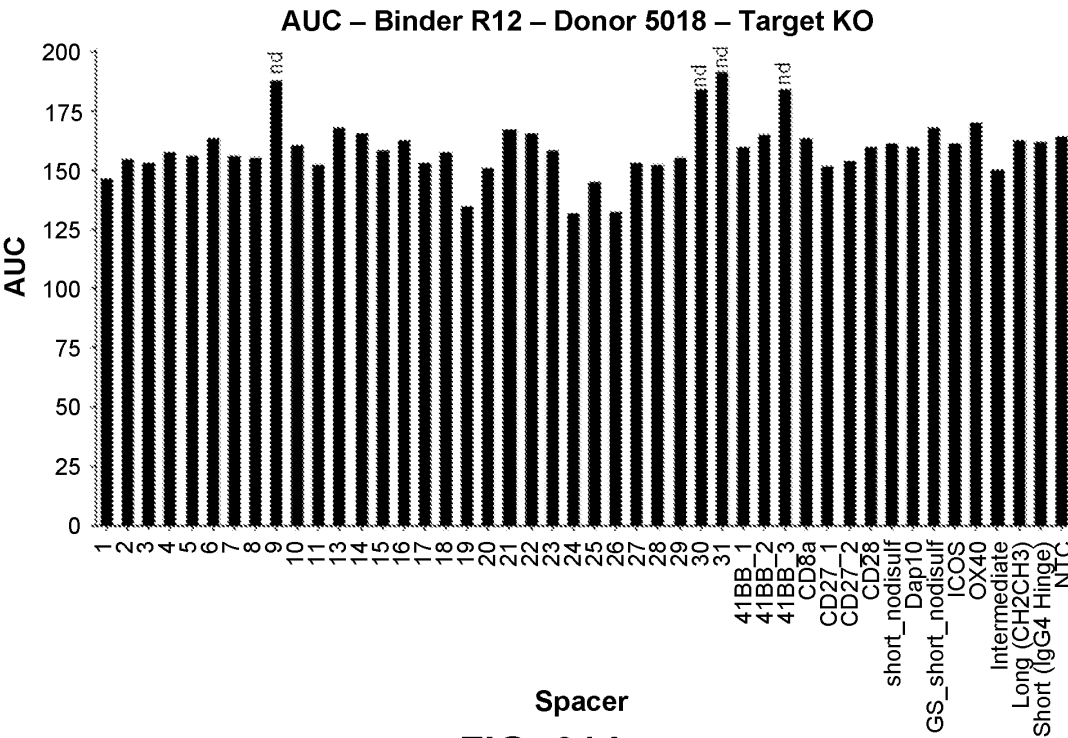


FIG. 61A

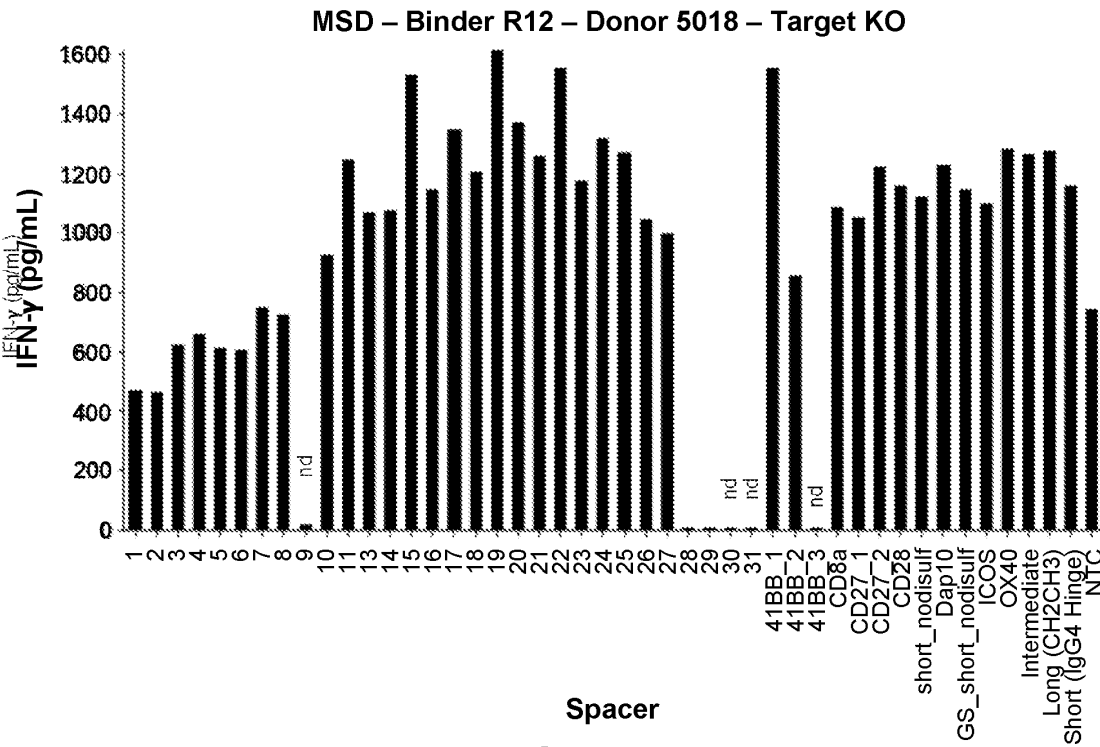


FIG. 61B

D2735 R12 CAR-T Target independent cytokines

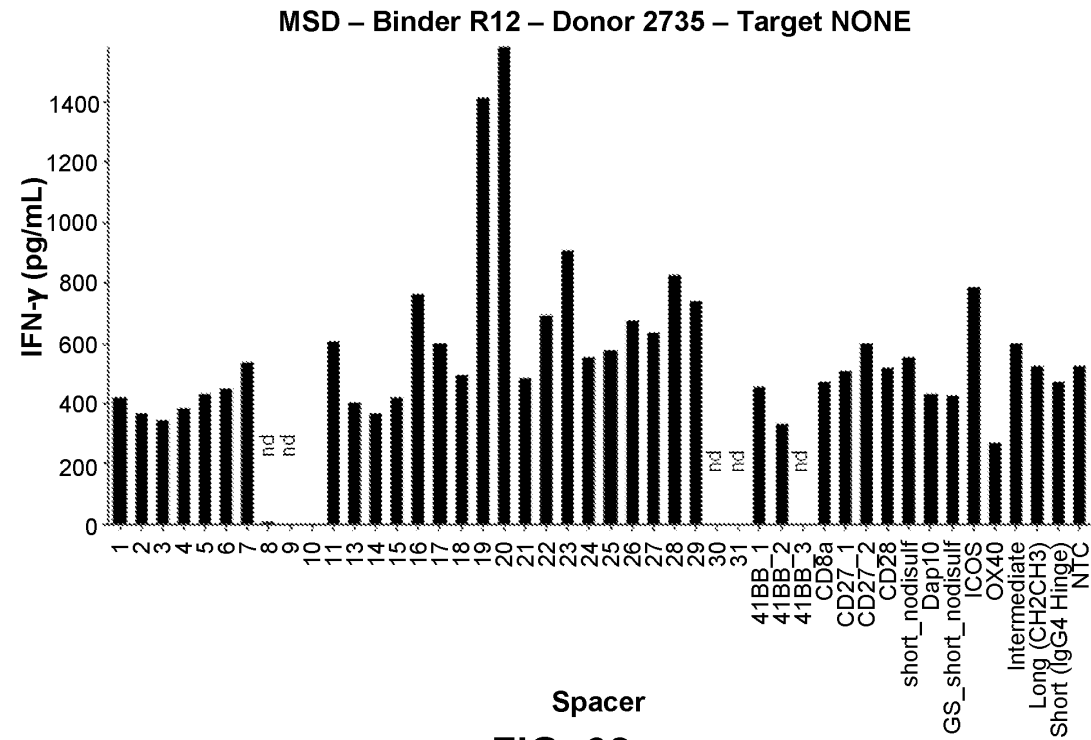


FIG. 62

D5018 R12 CAR-T Target independent cytokines

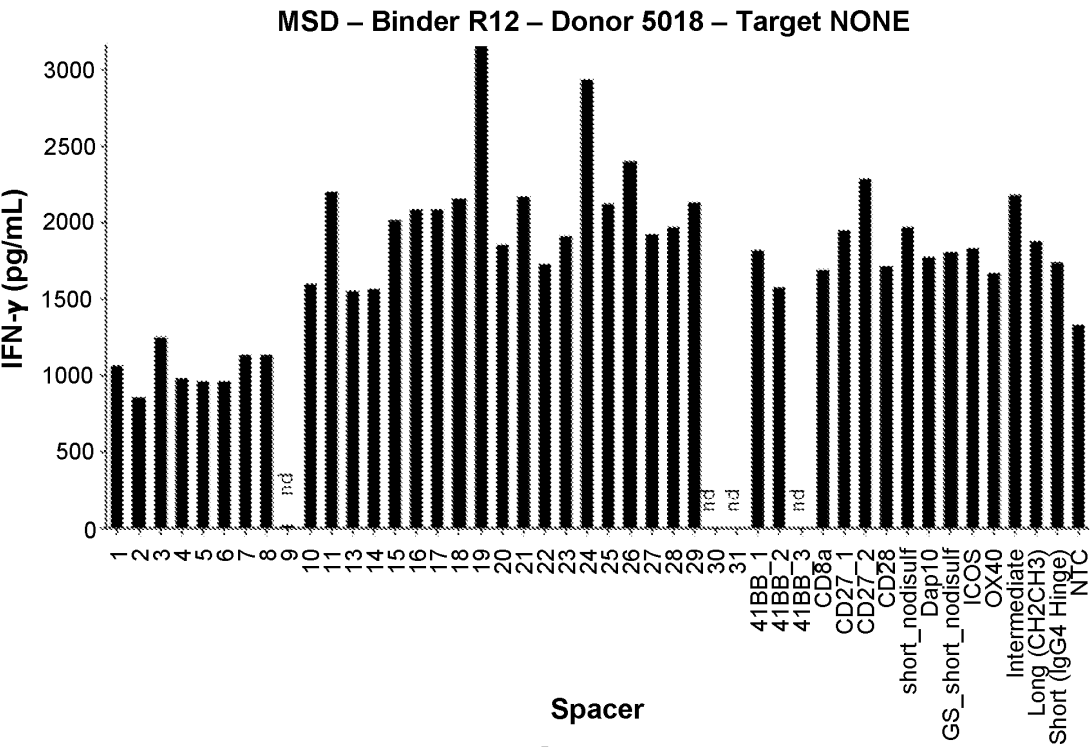


FIG. 63

D2735 R12 CAR-T Scatter plots suggest optimal spacer length for R12 is ~45Å

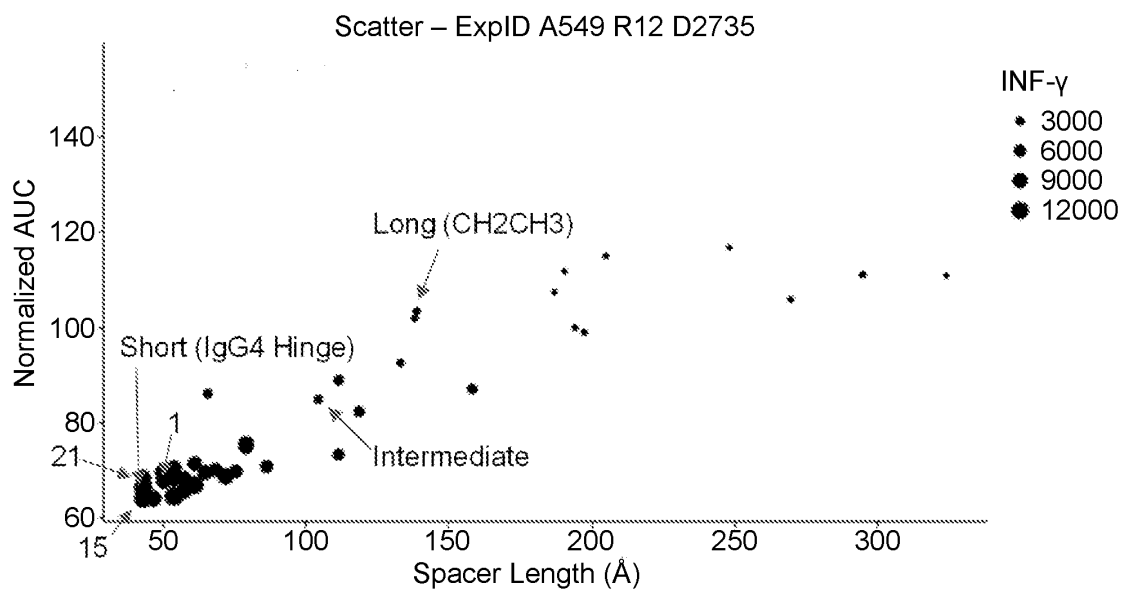


FIG. 64A

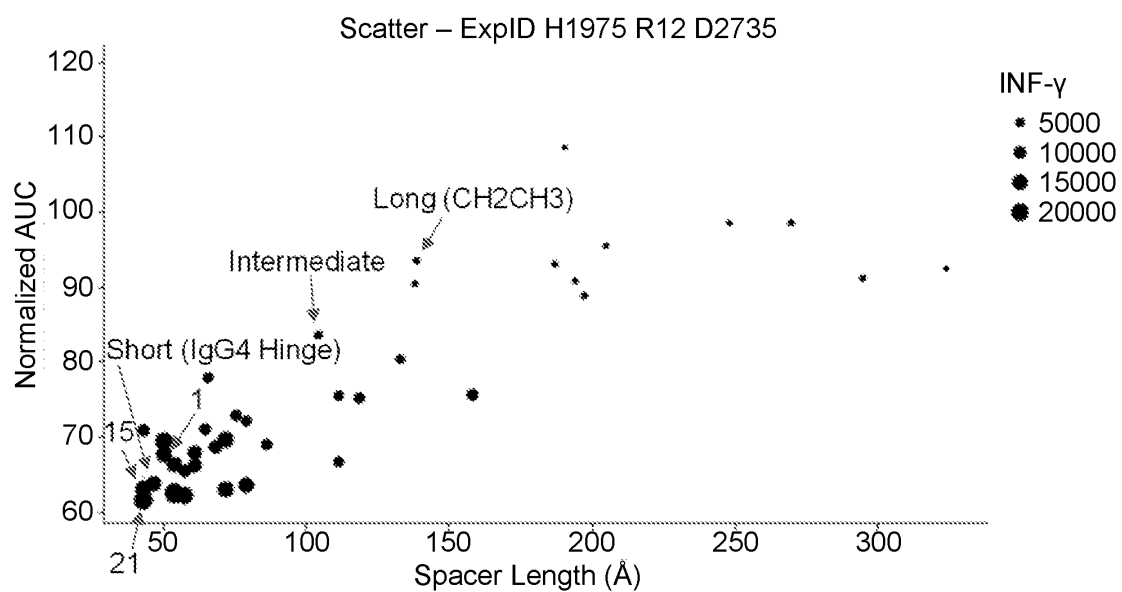


FIG. 64B

D5018 R12 CAR-T Scatter plots suggest optimal spacer length for R12 is ~45Å

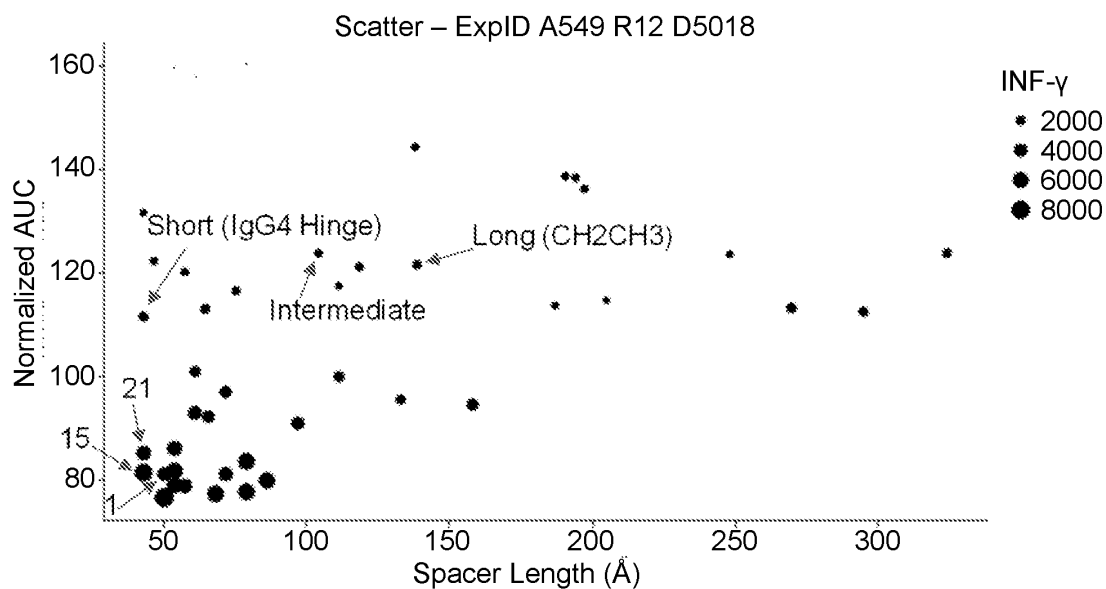


FIG. 65A

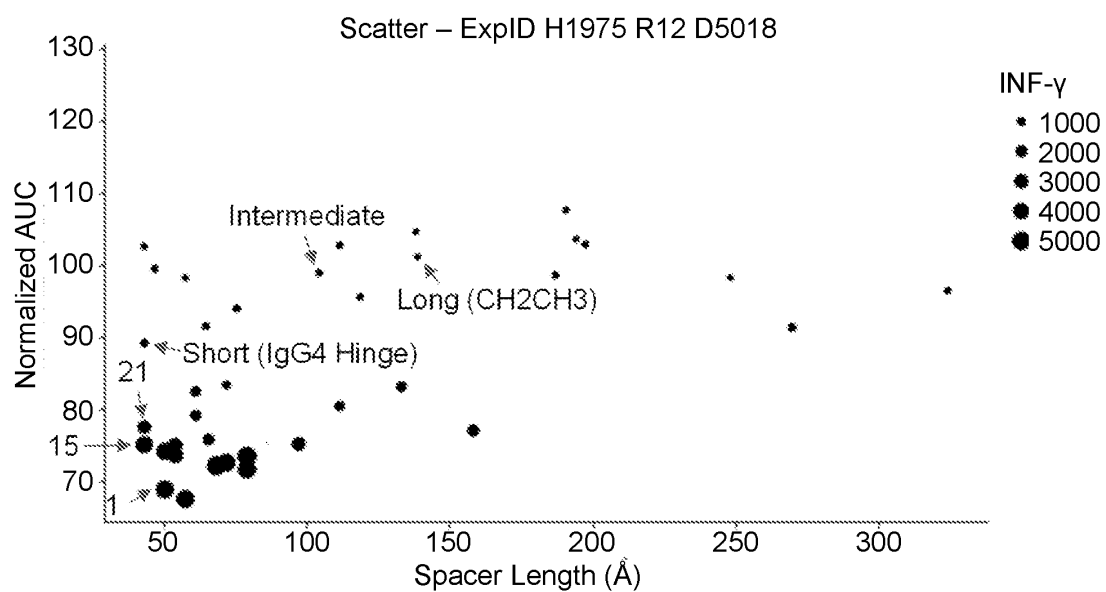


FIG. 65B

2A2 CAR Transduction efficiency Donor 1 and 2

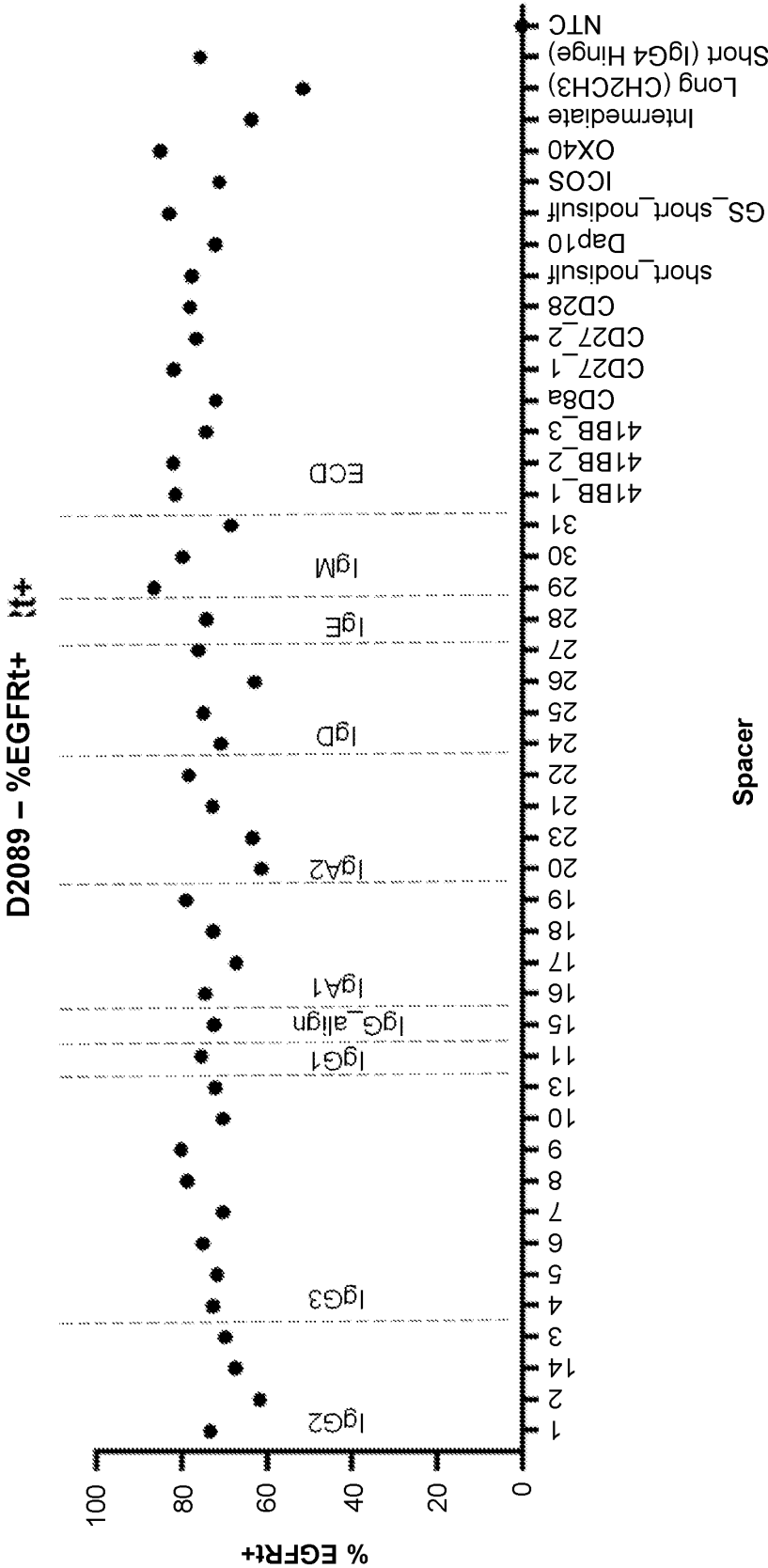
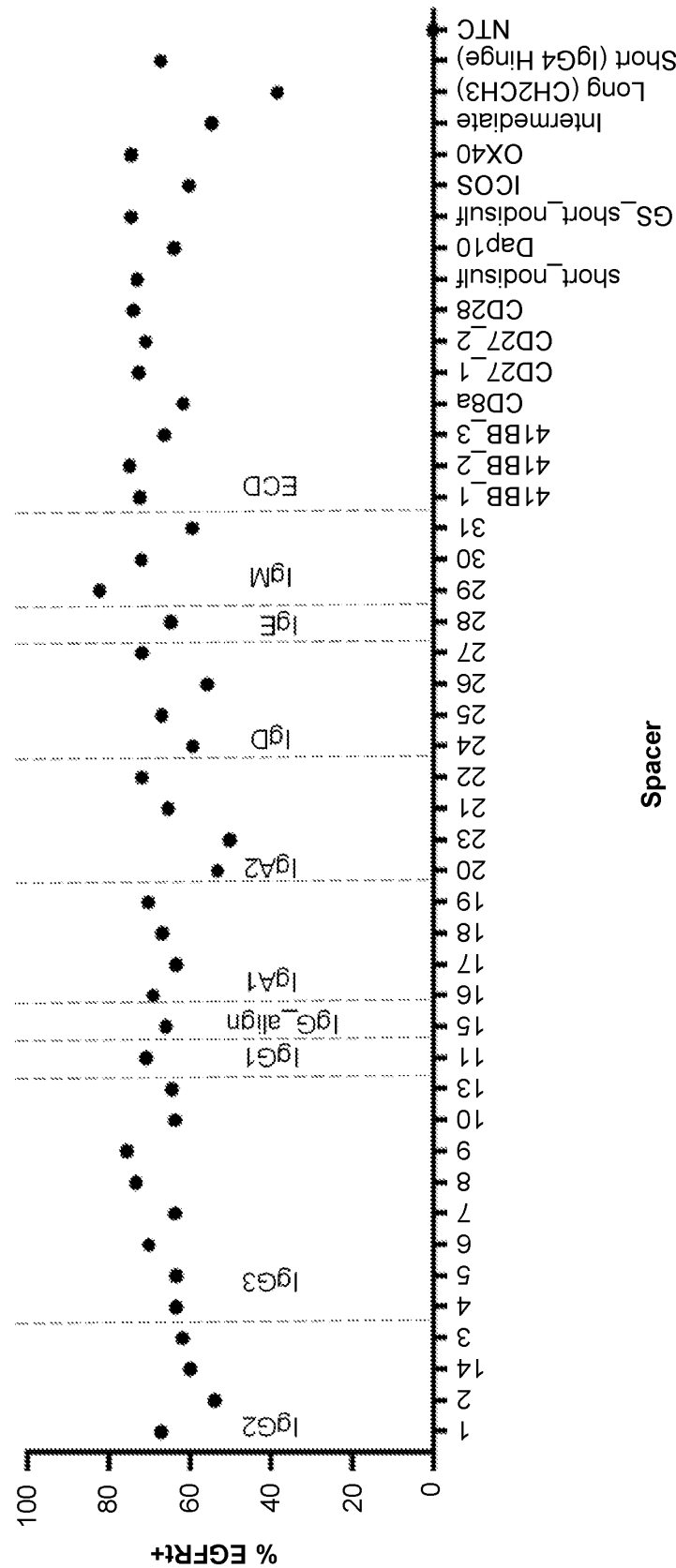


FIG. 66A

2A2 CAR Transduction efficiency Donor 1 and 2

D5018 – %EGFRt+



Spacer

FIG. 66B

2A2 CAR Transduction efficiency Donor 1 and 2

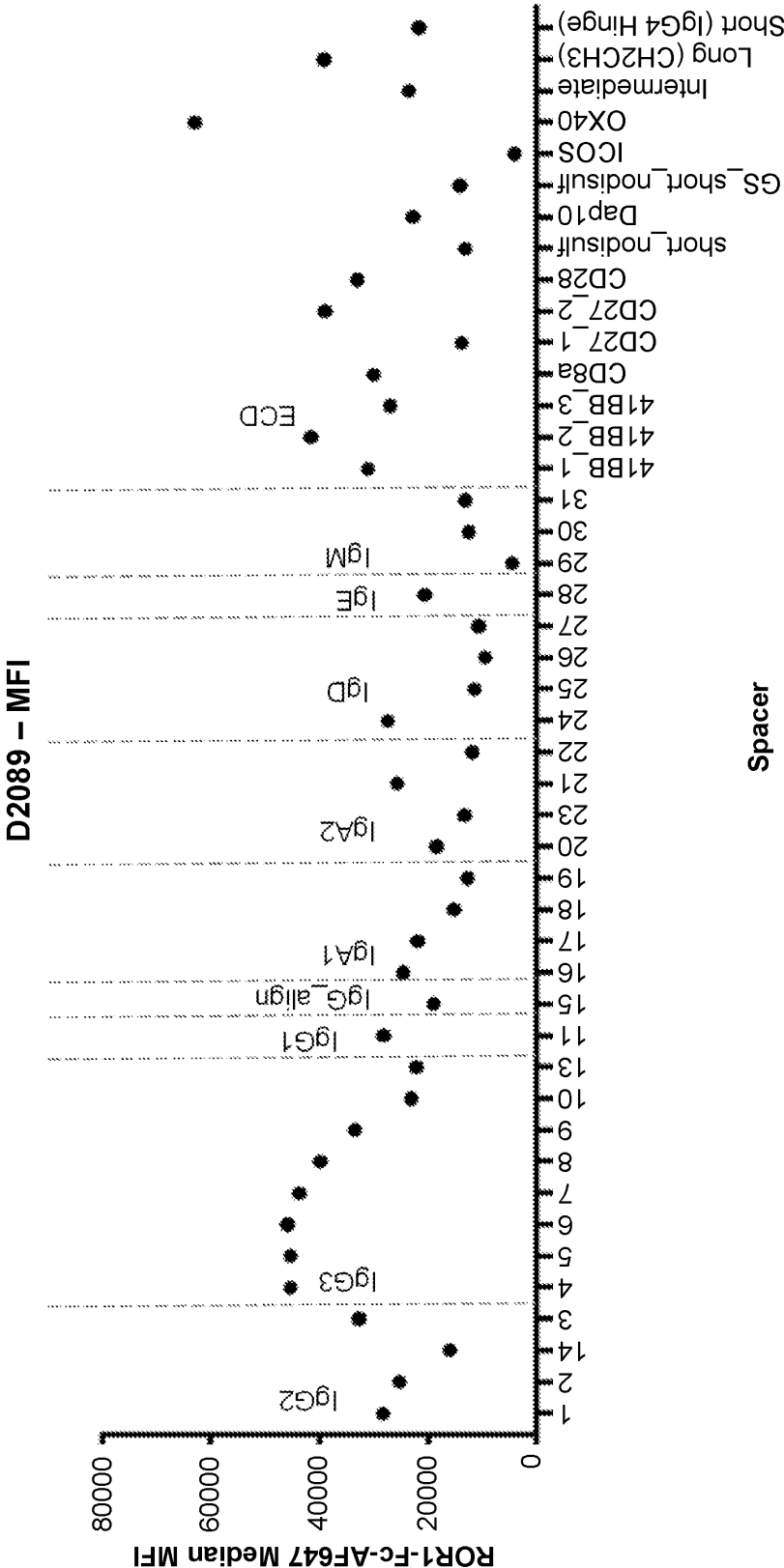


FIG. 66C

2A2 CAR Transduction efficiency Donor 1 and 2

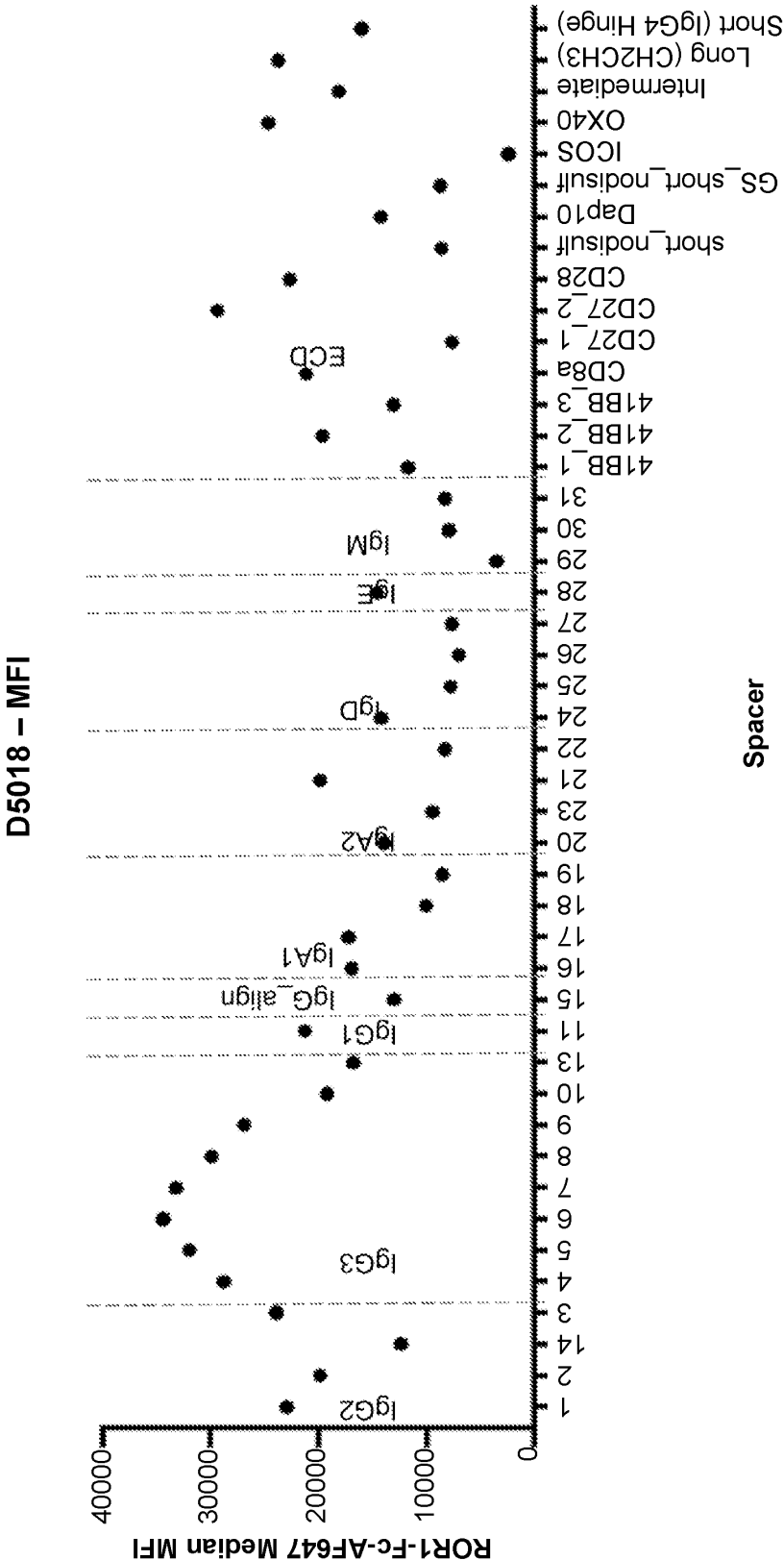


FIG. 66D

Primary killing and AUC D2089 2A2 CAR-T : A549-NLR 1:1

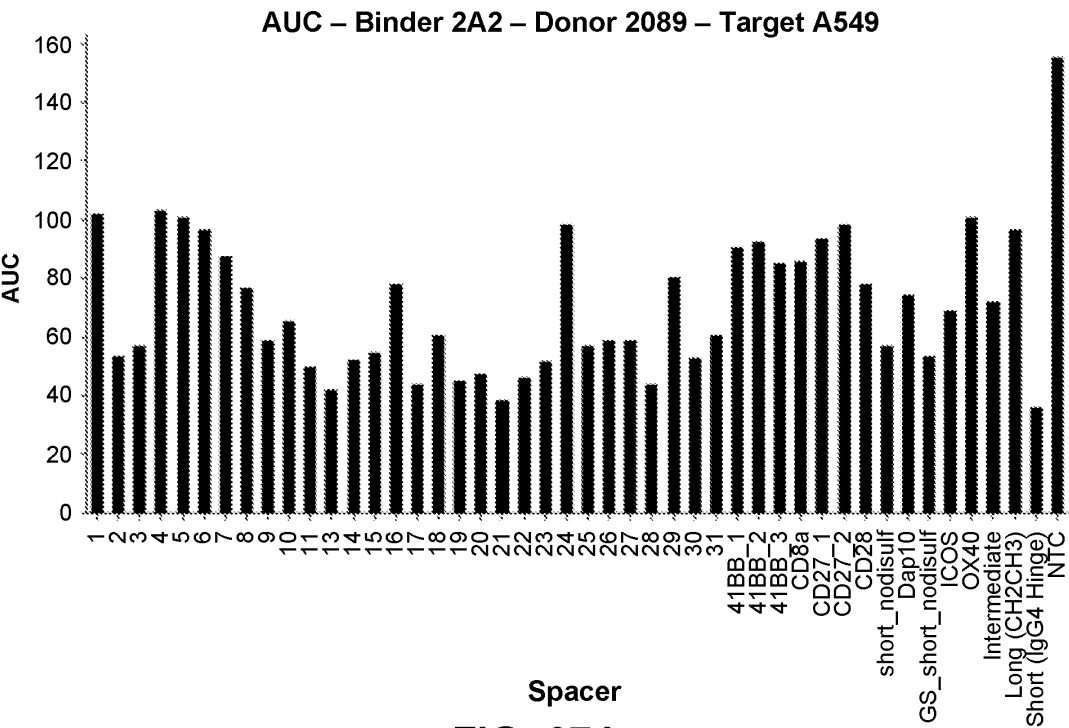


FIG. 67A



FIG. 67B

Primary killing and AUC D5018 2A2 CAR-T : A549-NLR 1:1

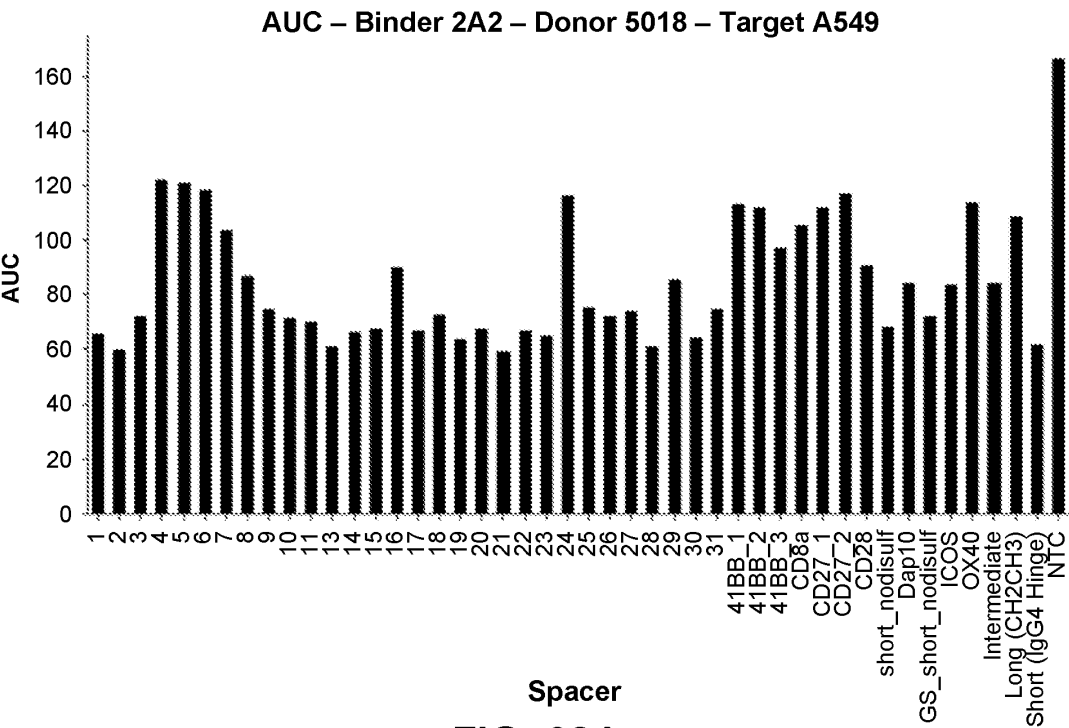


FIG. 68A



FIG. 68B

Primary killing and AUC D5018 2A2 CAR-T : A549-NLR 1:1

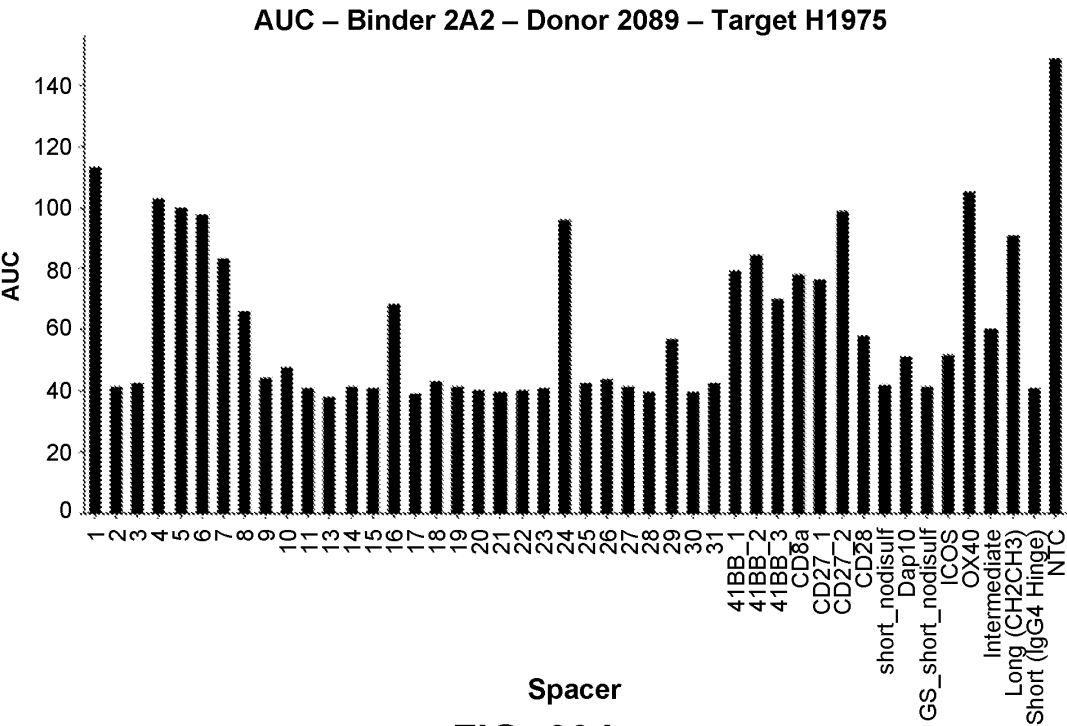


FIG. 69A

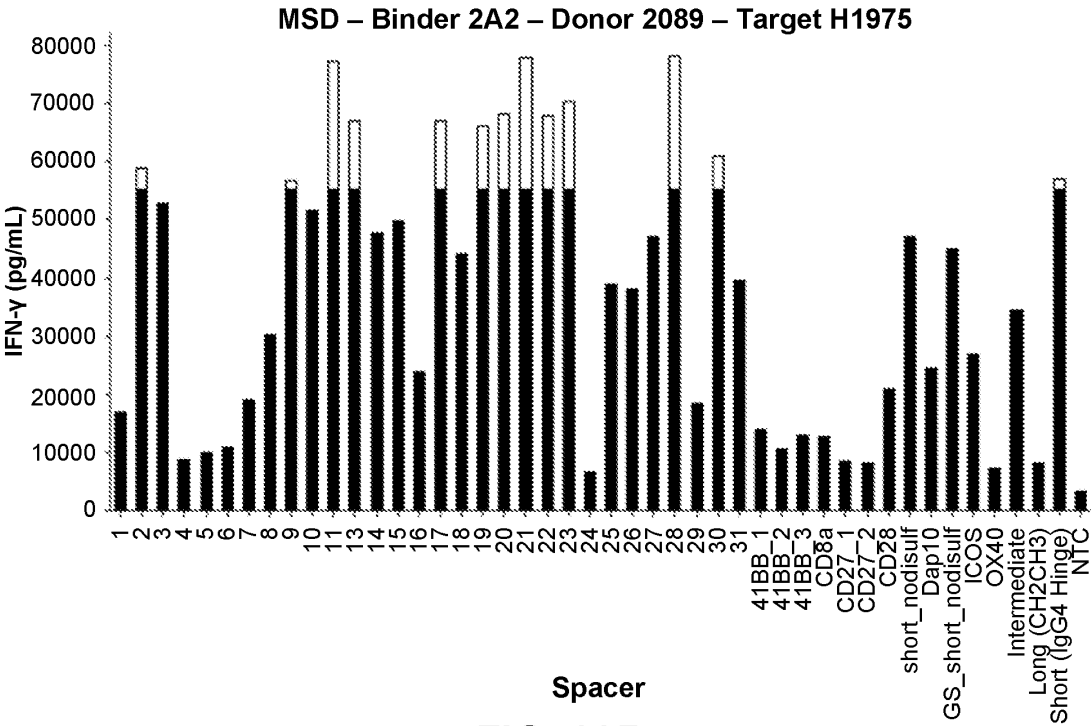


FIG. 69B

Primary killing and AUC D5018 2A2 CAR-T : H1975-NLR 1:1

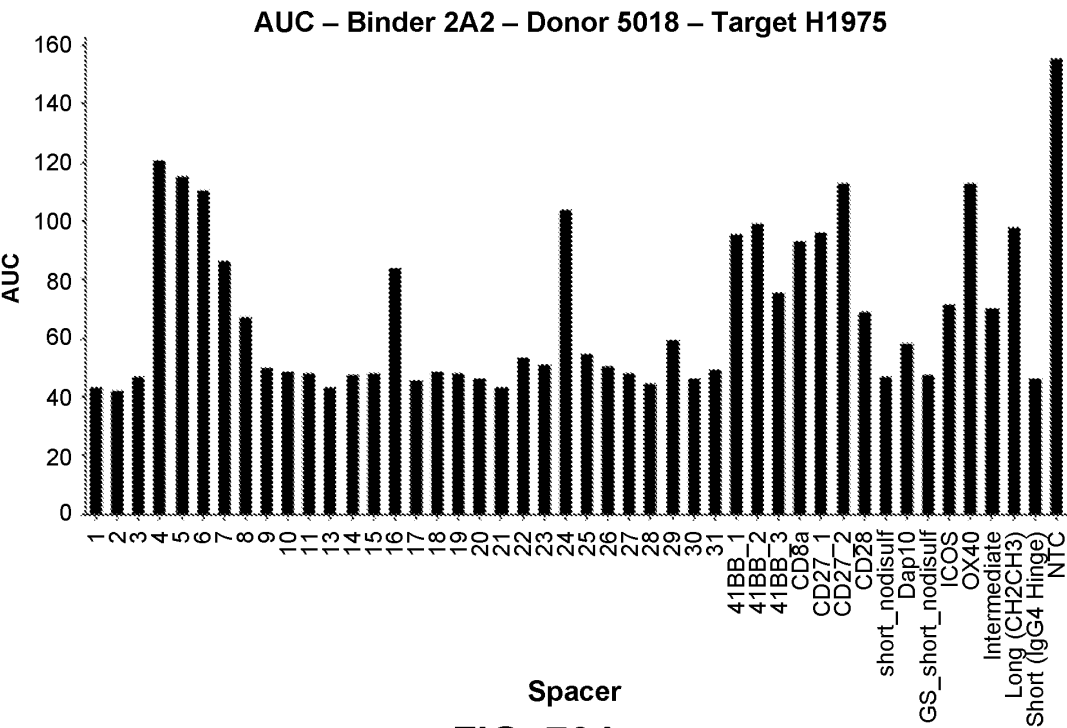


FIG. 70A

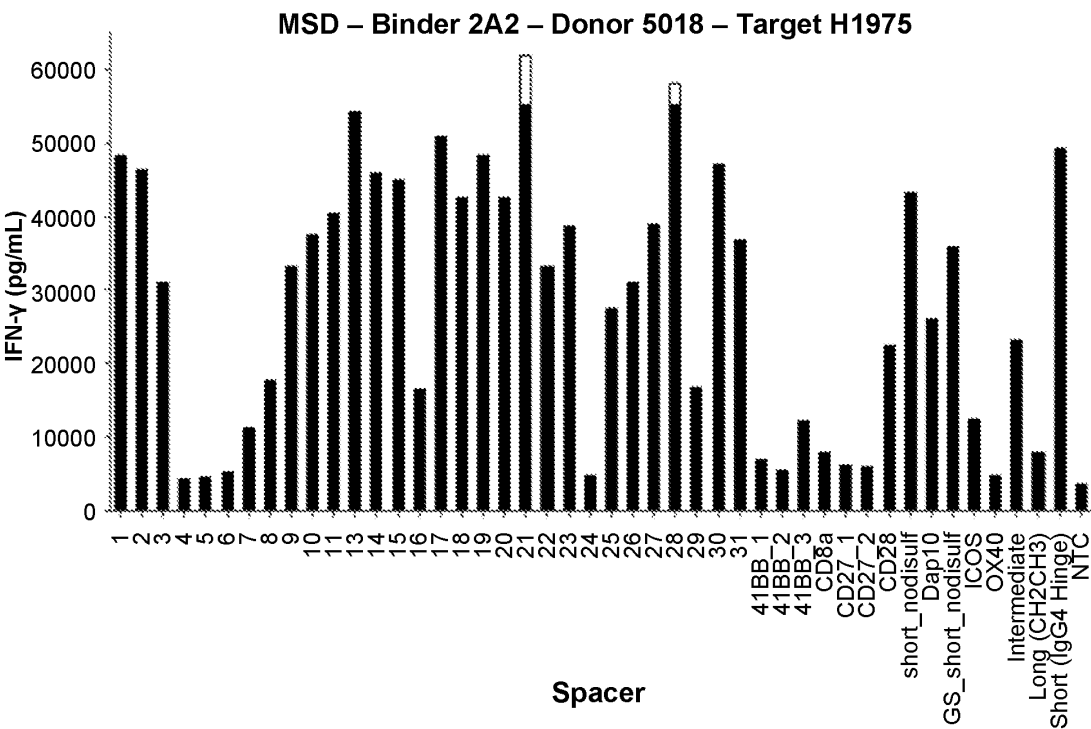


FIG. 70B

Primary killing and AUC D2089 2A2 CAR-T : A549-ROR1KO-NLR 1:1

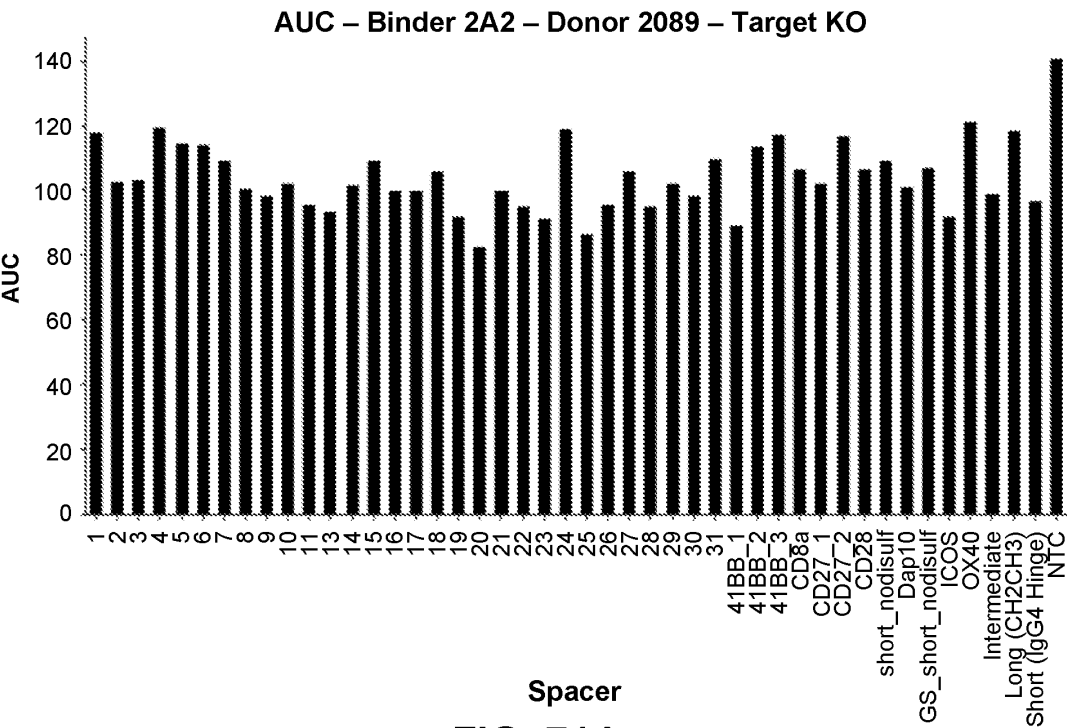


FIG. 71A

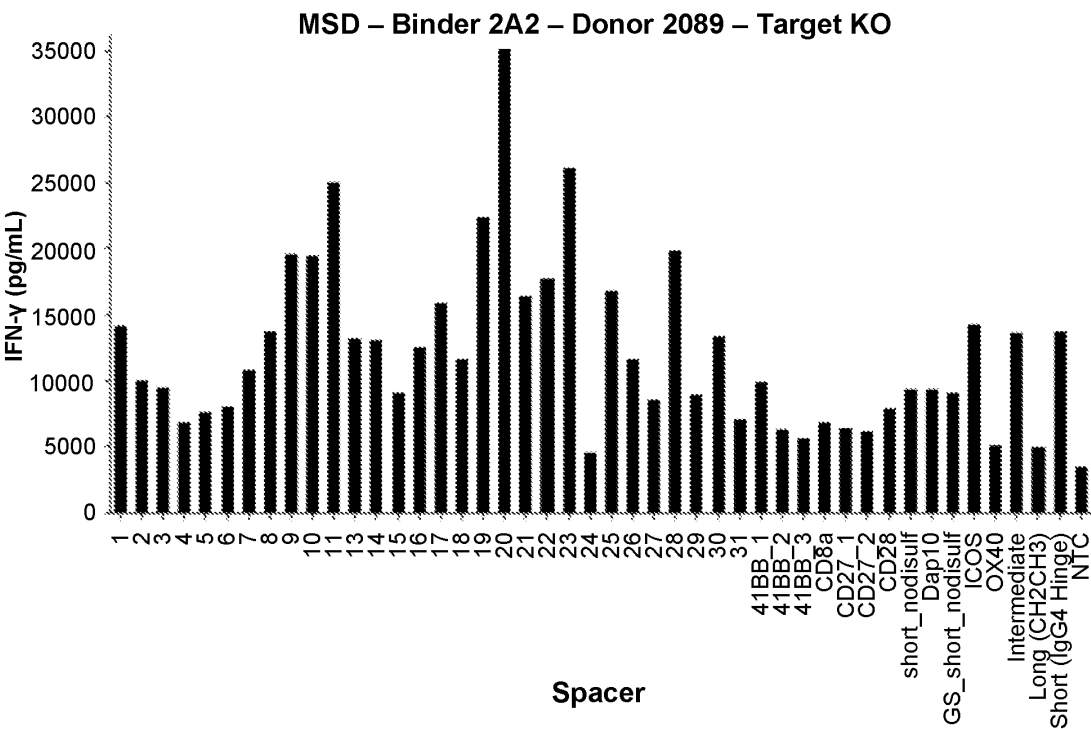


FIG. 71B

Primary killing and AUC D5018 2A2 CAR-T : A549-ROR1KO-NLR 1:1

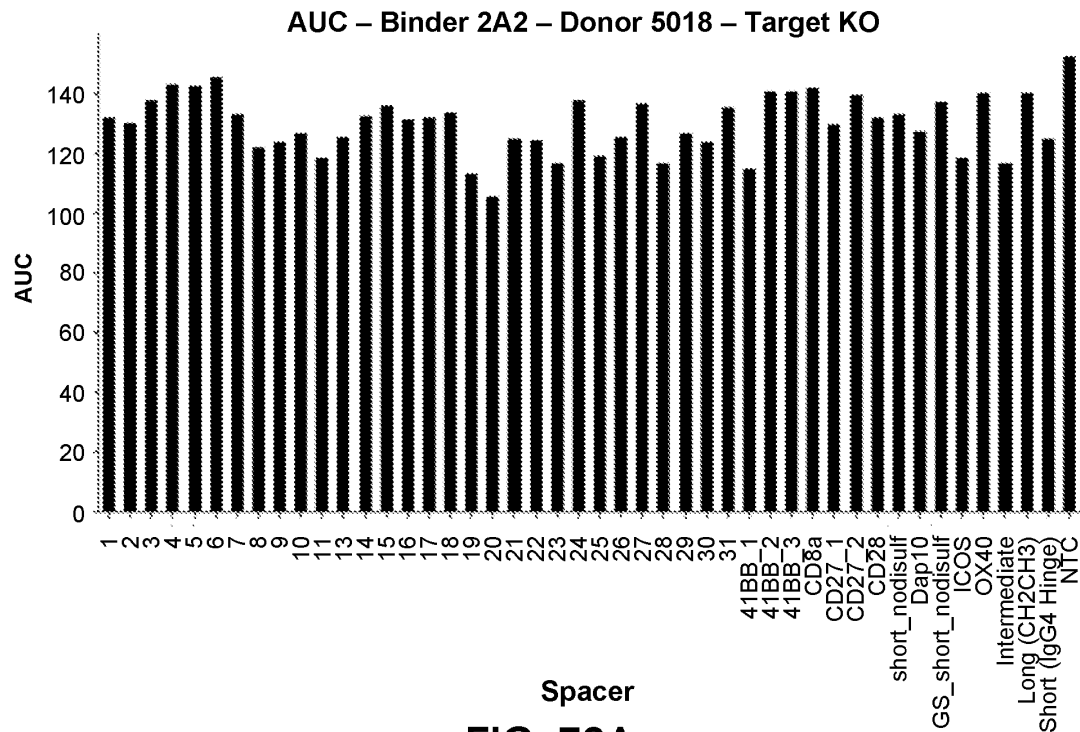


FIG. 72A

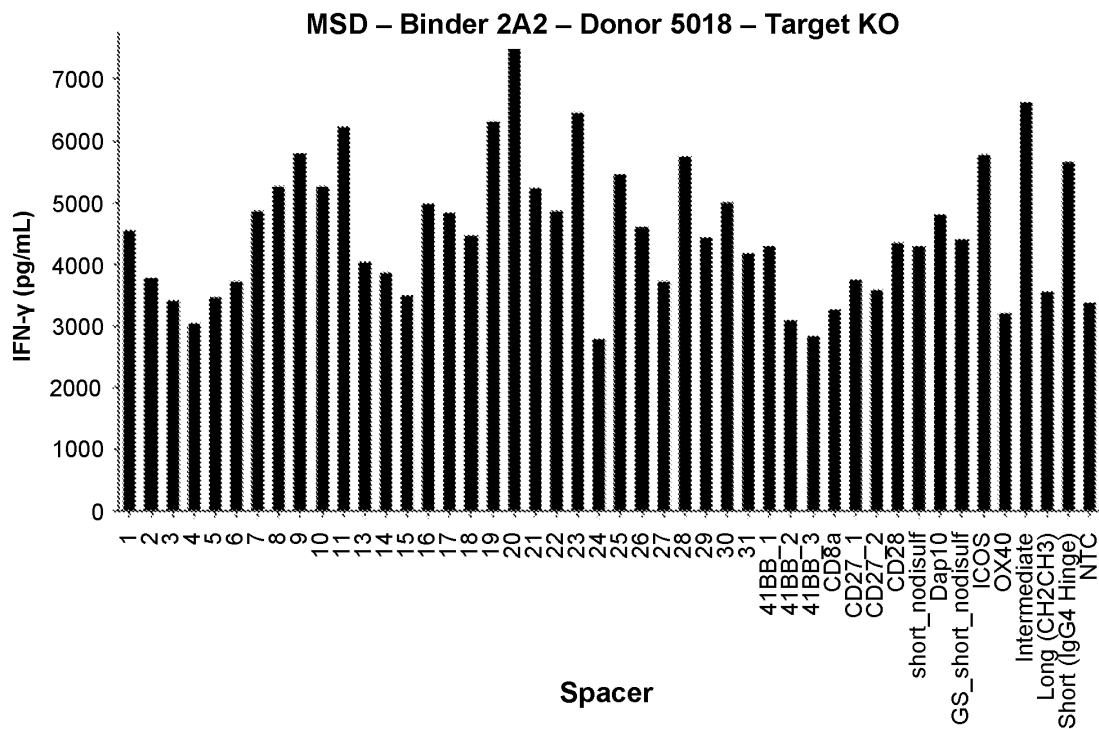


FIG. 72B

D2089 2A2 CAR-T Target independent cytokines

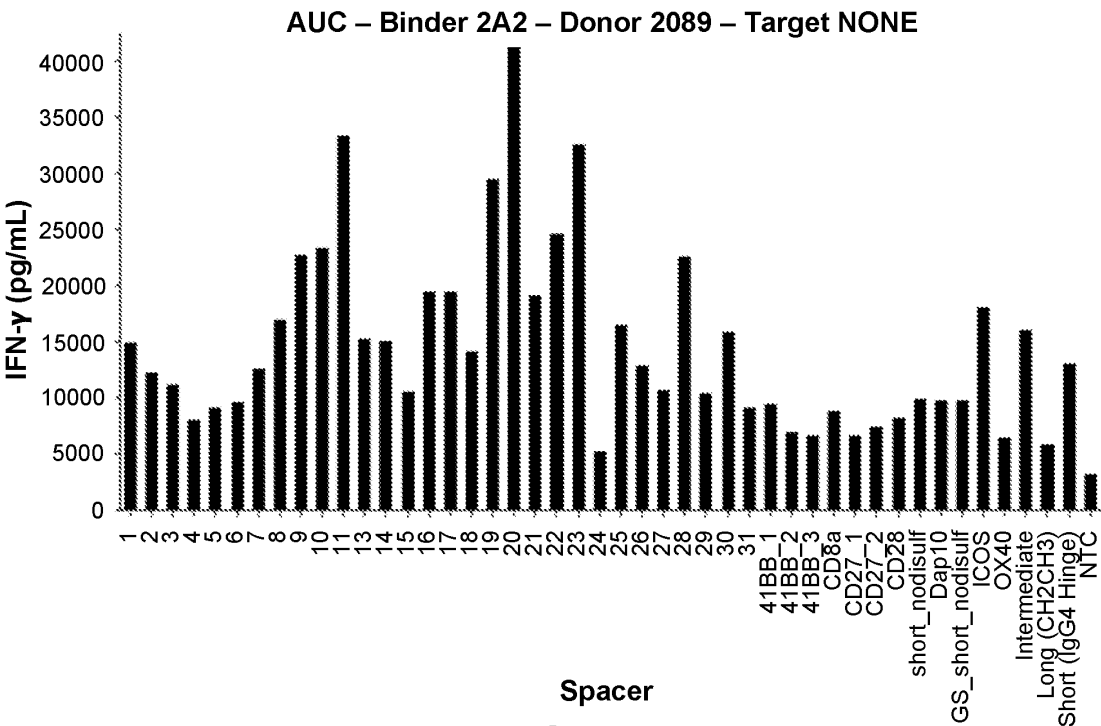


FIG. 73

D5018 2A2 CAR-T Target independent cytokines

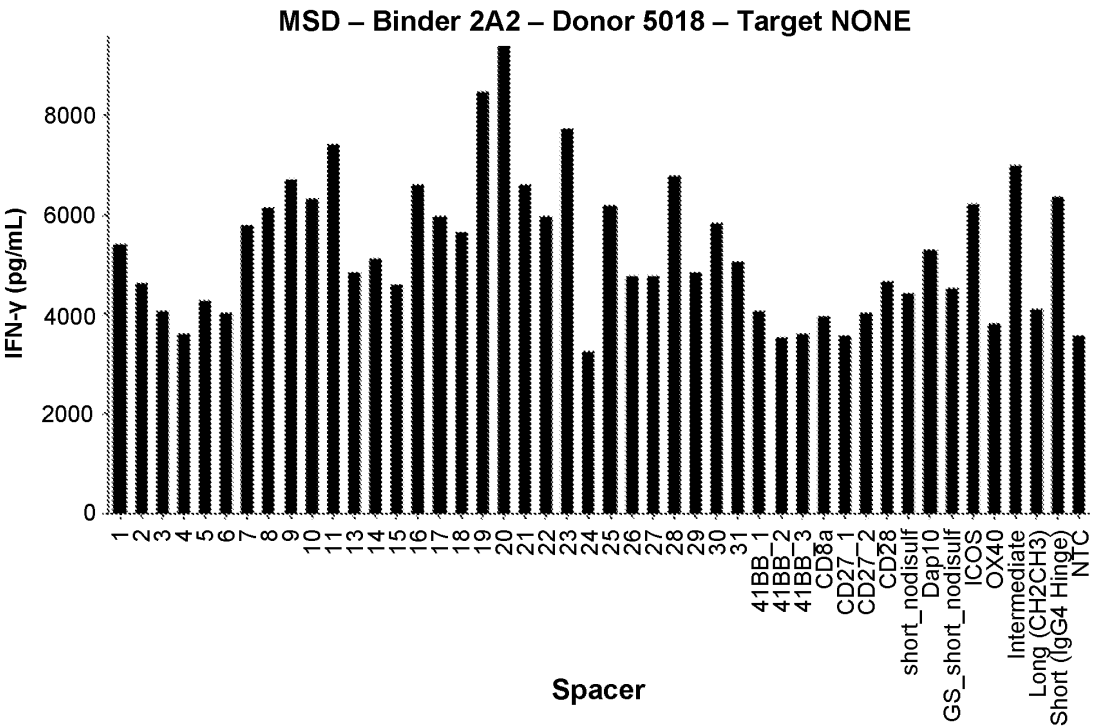


FIG. 74

D2089 2A2 CAR-T Scatter plots suggest optimal spacer length for 2A2 is ~45Å

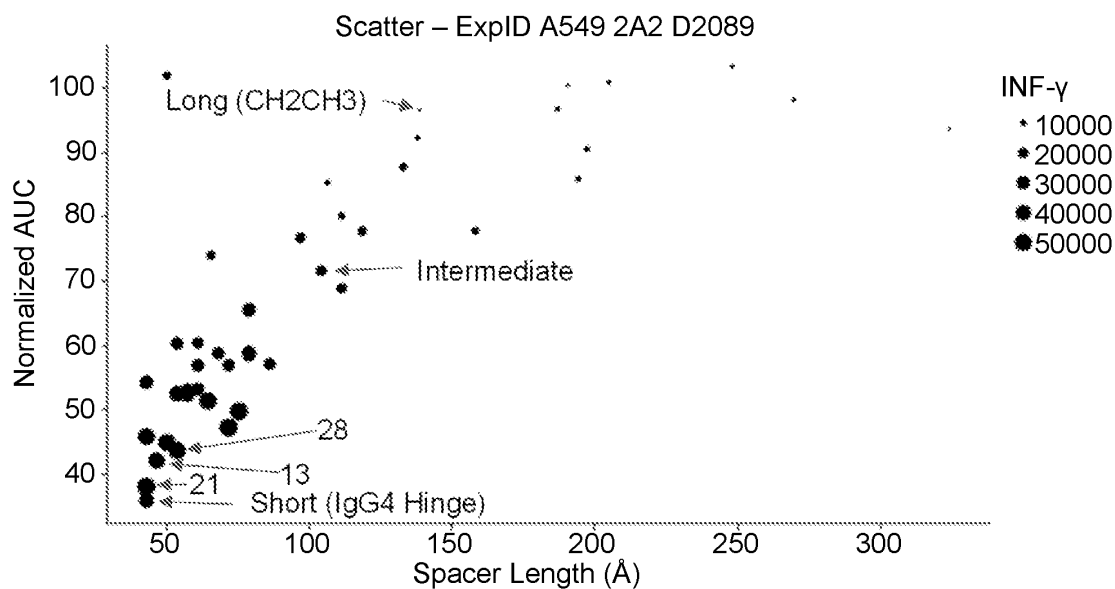


FIG. 75A

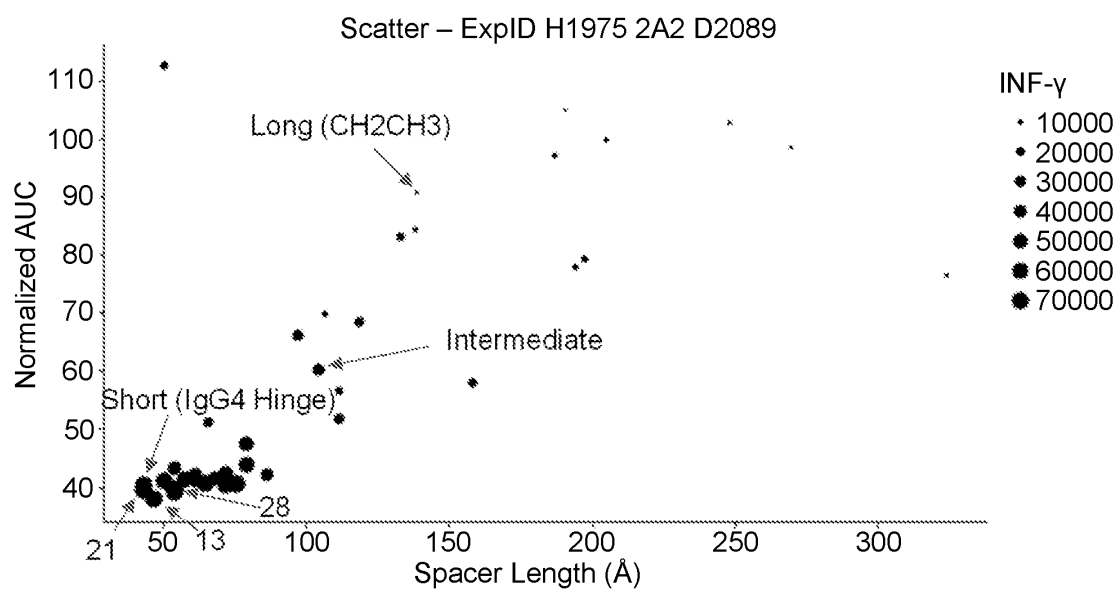


FIG. 75B

D5018 2A2 CAR-T Scatter plots suggest optimal spacer length for 2A2 is ~45Å

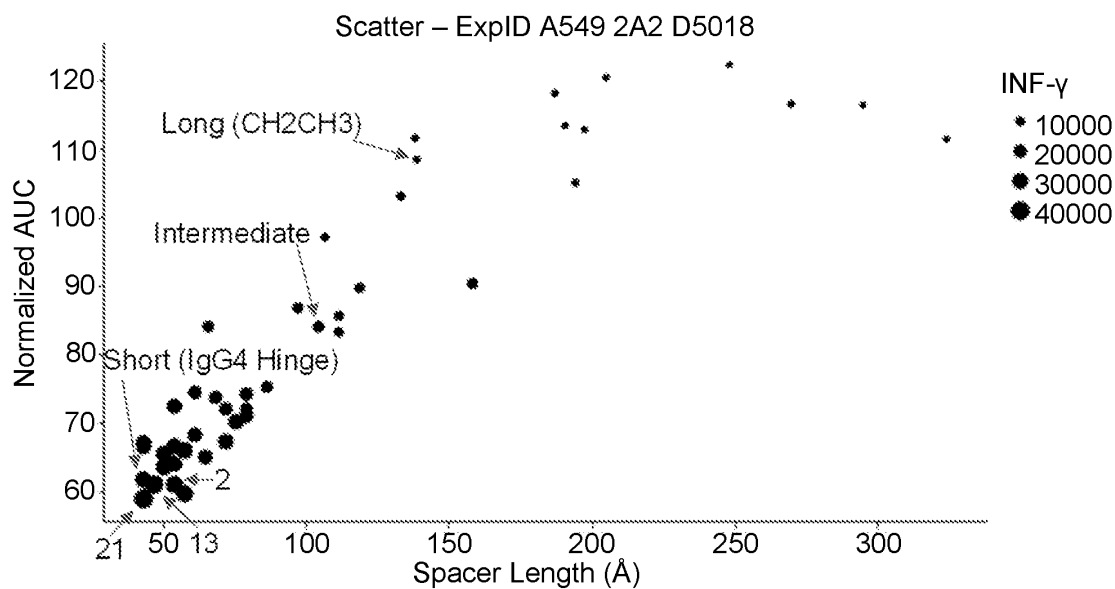


FIG. 76A

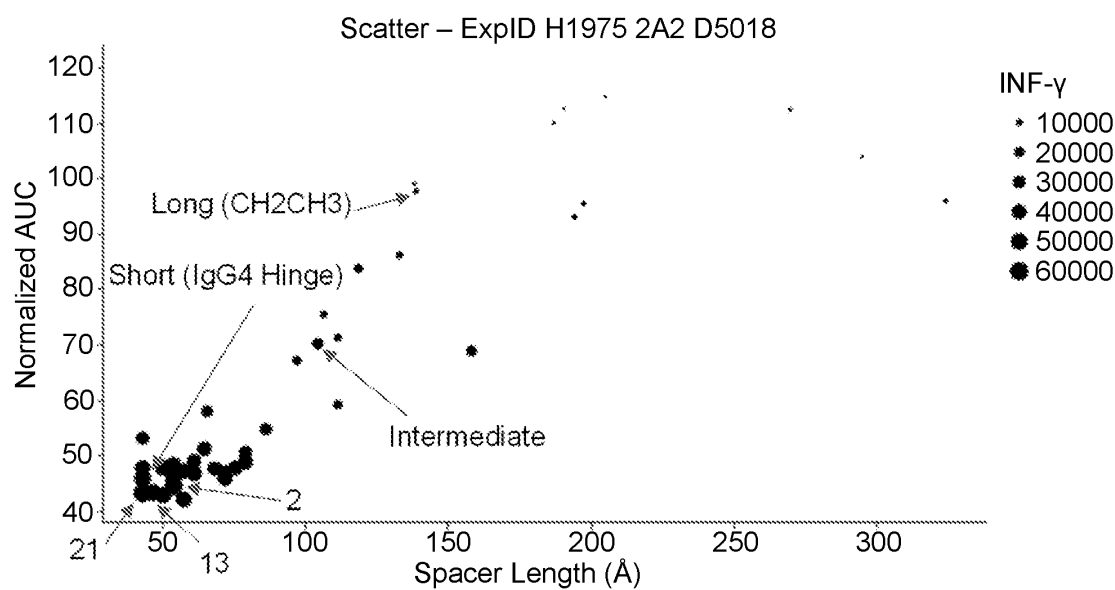


FIG. 76B

Representative bubble plot by donor/binder/target reveals binder specific optimal spacer length

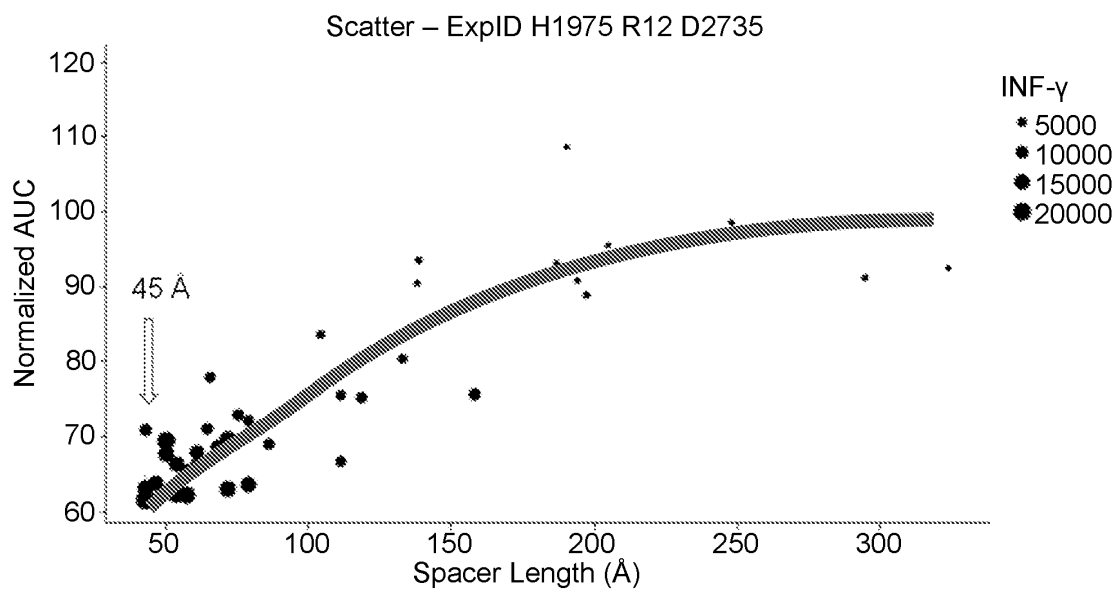


FIG. 77A

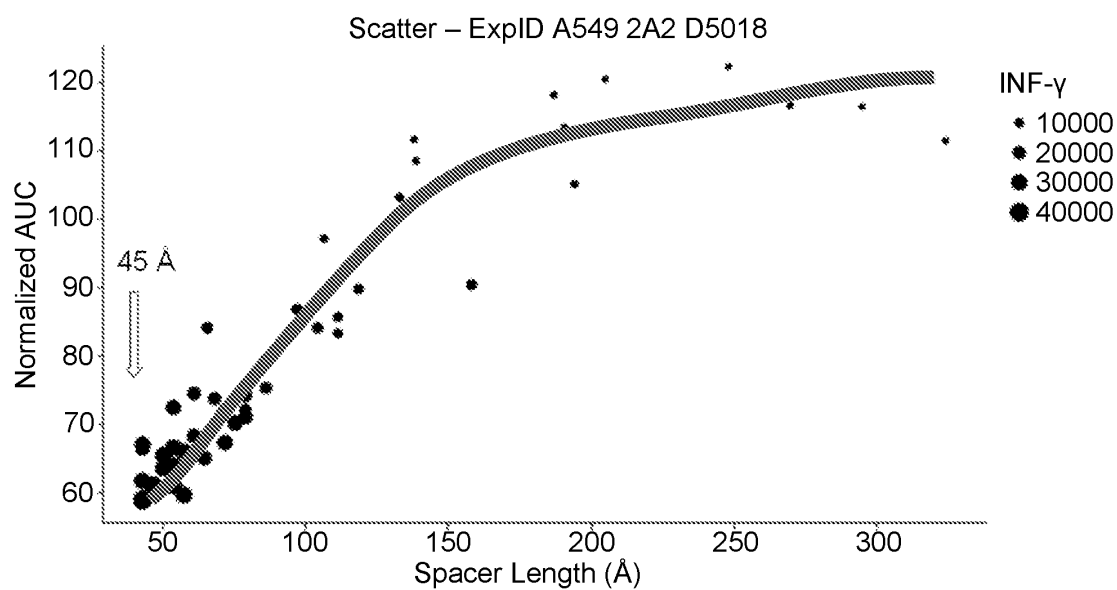
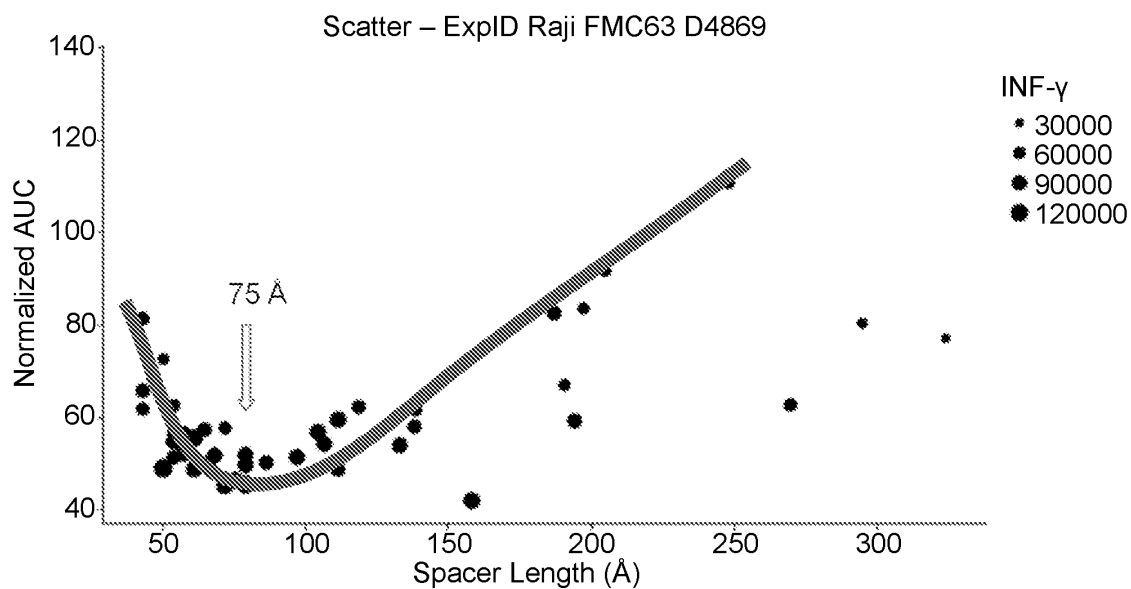
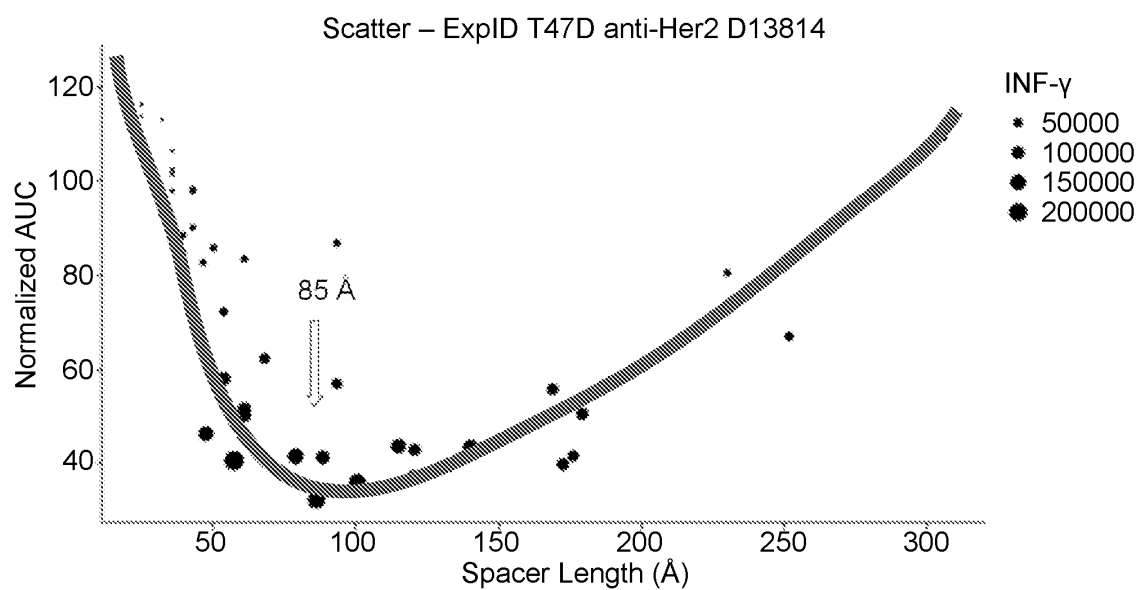


FIG. 77B

Representative bubble plot by donor/binder/target reveals binder specific optimal spacer length

**FIG. 77C****FIG. 77D**

Representative bubble plot by donor/binder/target reveals binder specific optimal spacer length

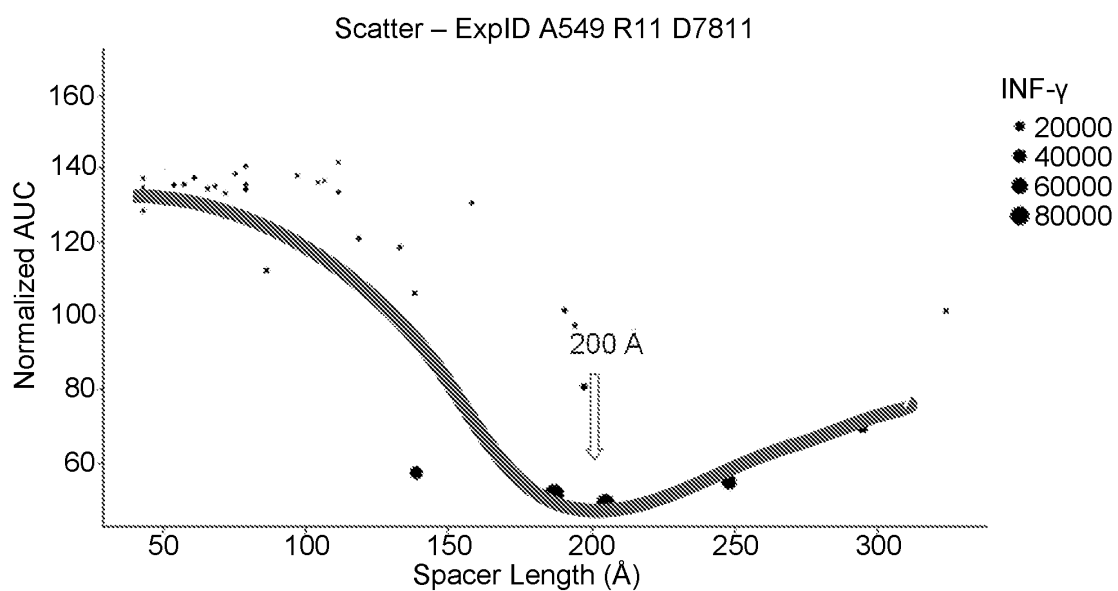
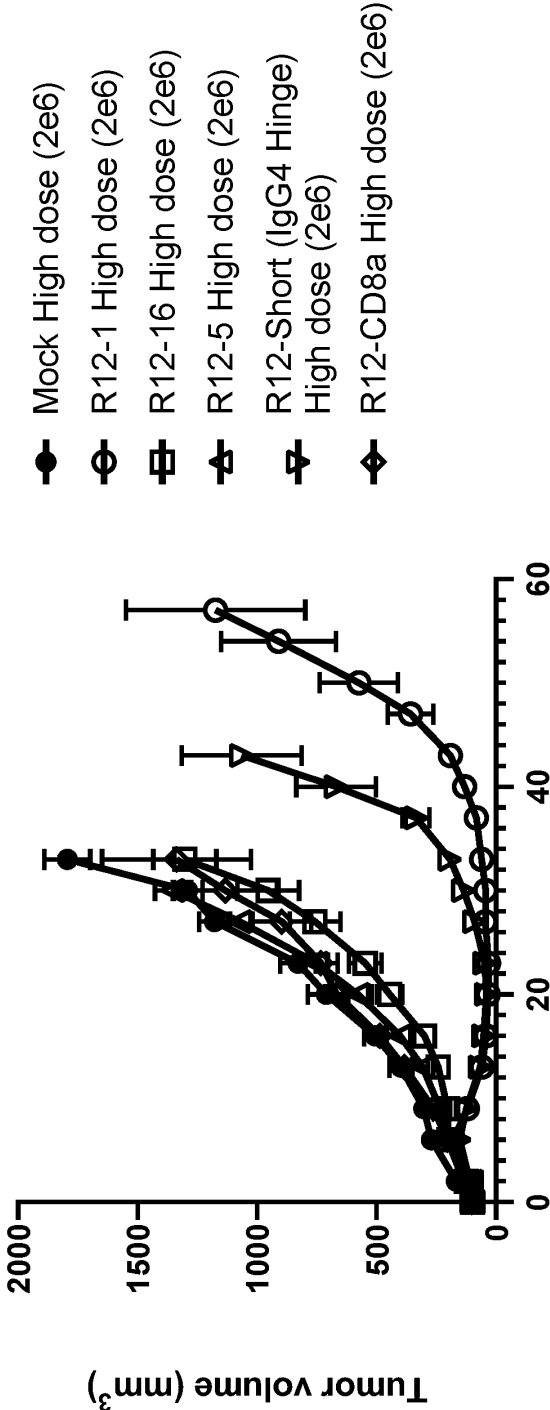


FIG. 77E

L0123 H1975 Donor 3035586
Mean Tumor Volume High dose



days after T cell injection

FIG. 78A

L0123 H1975 Donor 3035586
Body Weight High dose

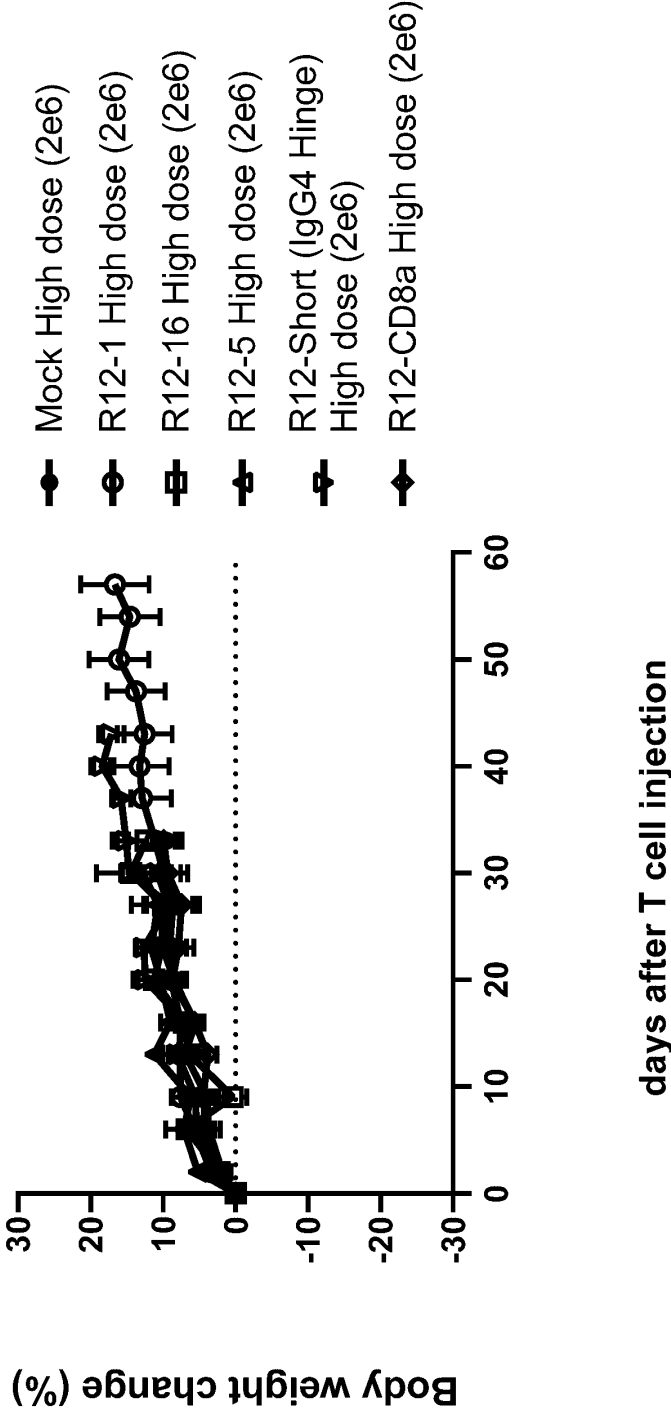


FIG. 78B

L0123 H1975 Donor 3035586
Mean Tumor Volume Low dose

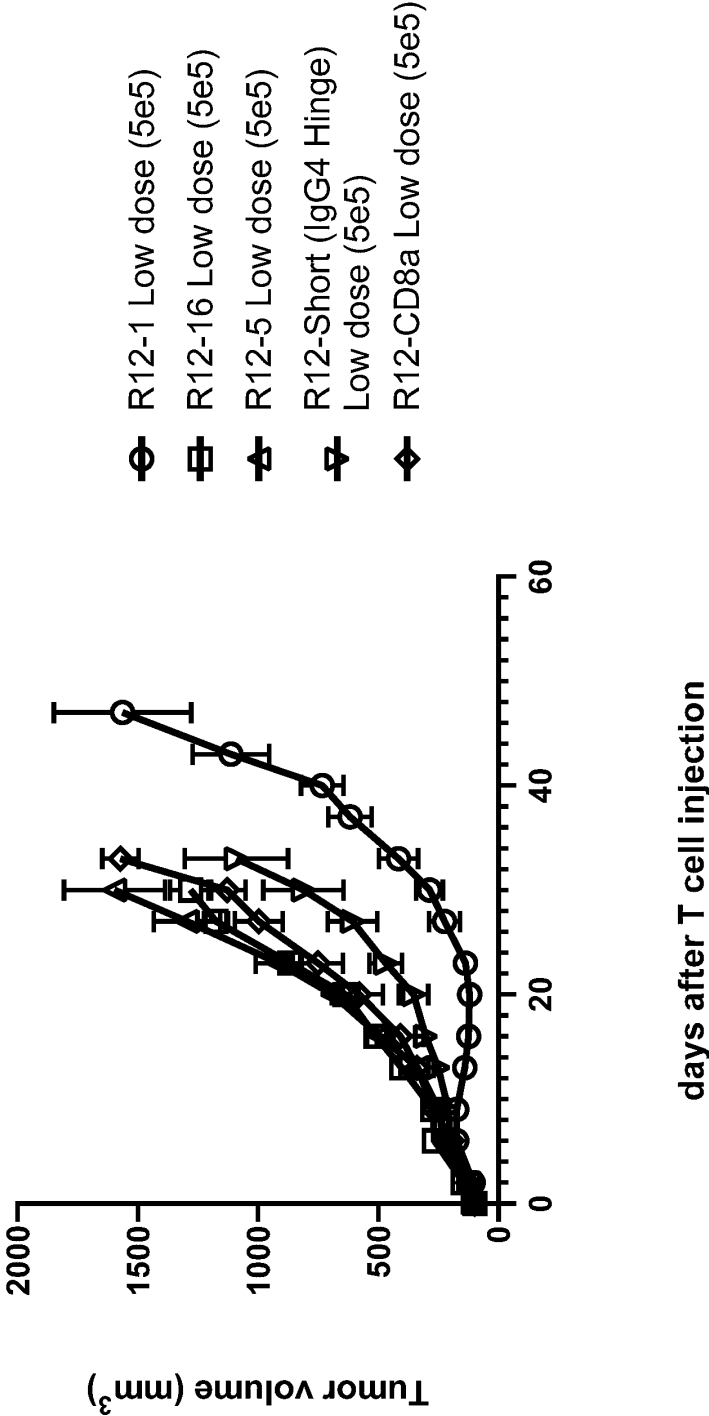


FIG. 79A

L0123 H1975 Donor 3035586
Body Weight Low dose

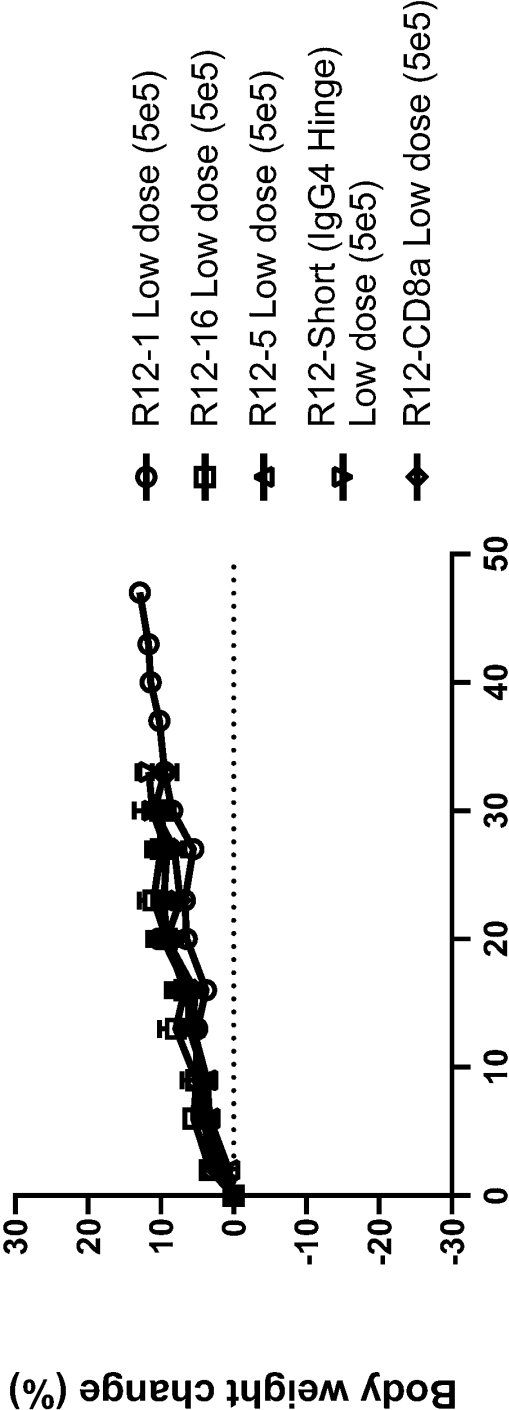


FIG. 79B

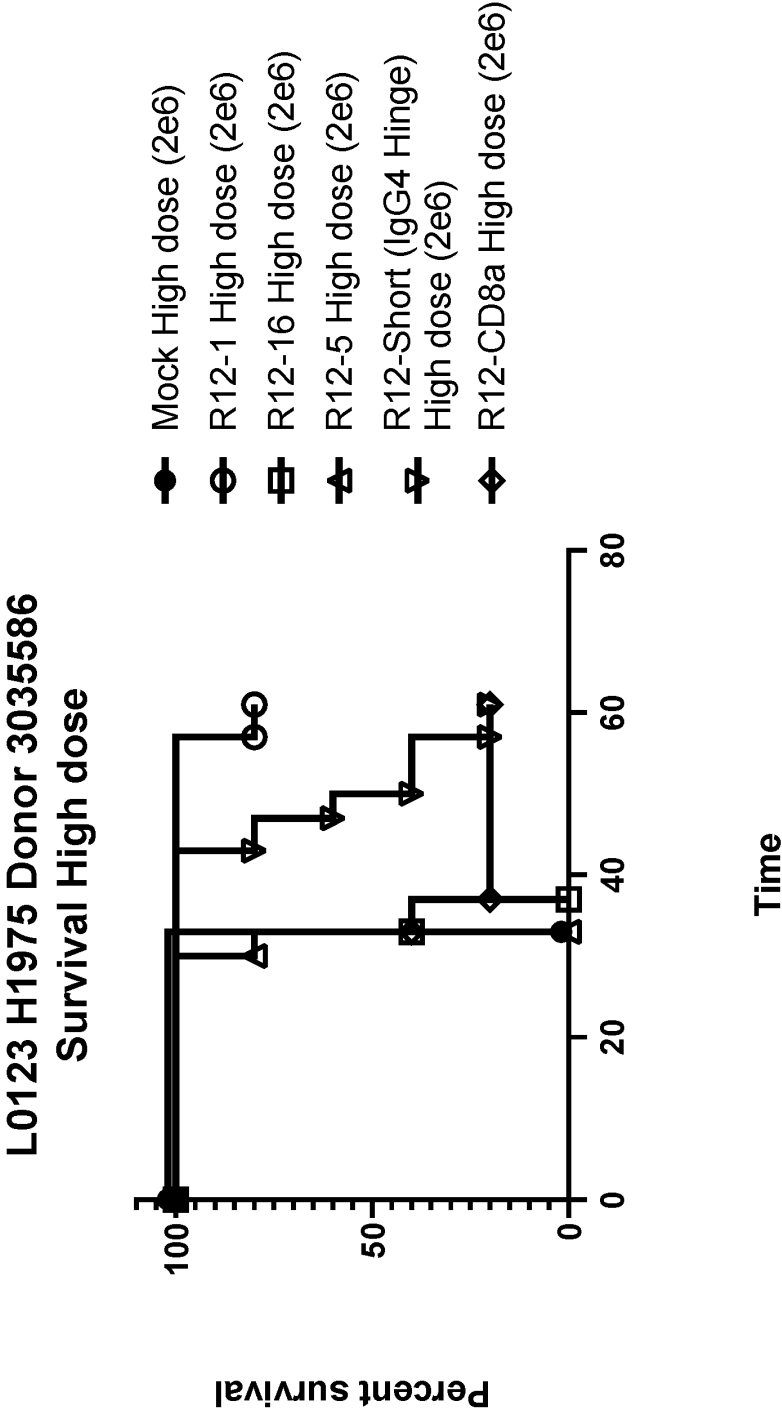


FIG. 80

L0123 H1975 Donor 3035586
Survival Low dose

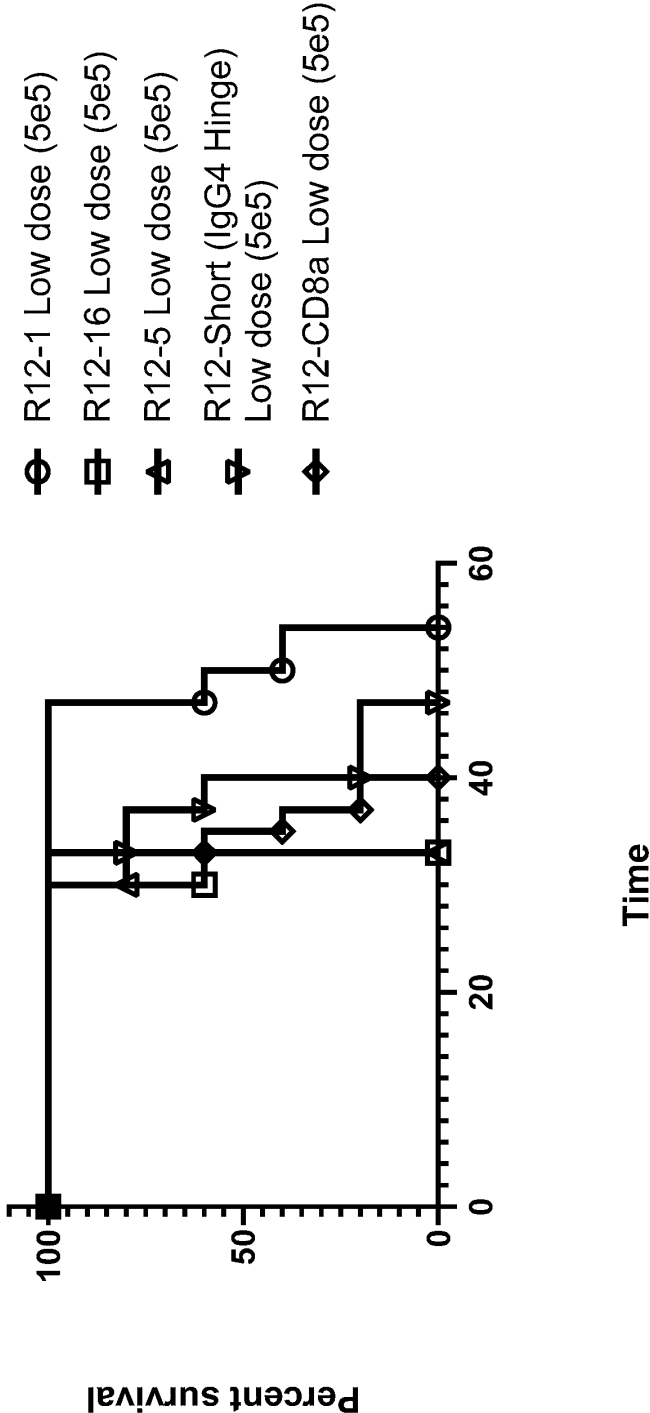


FIG. 81

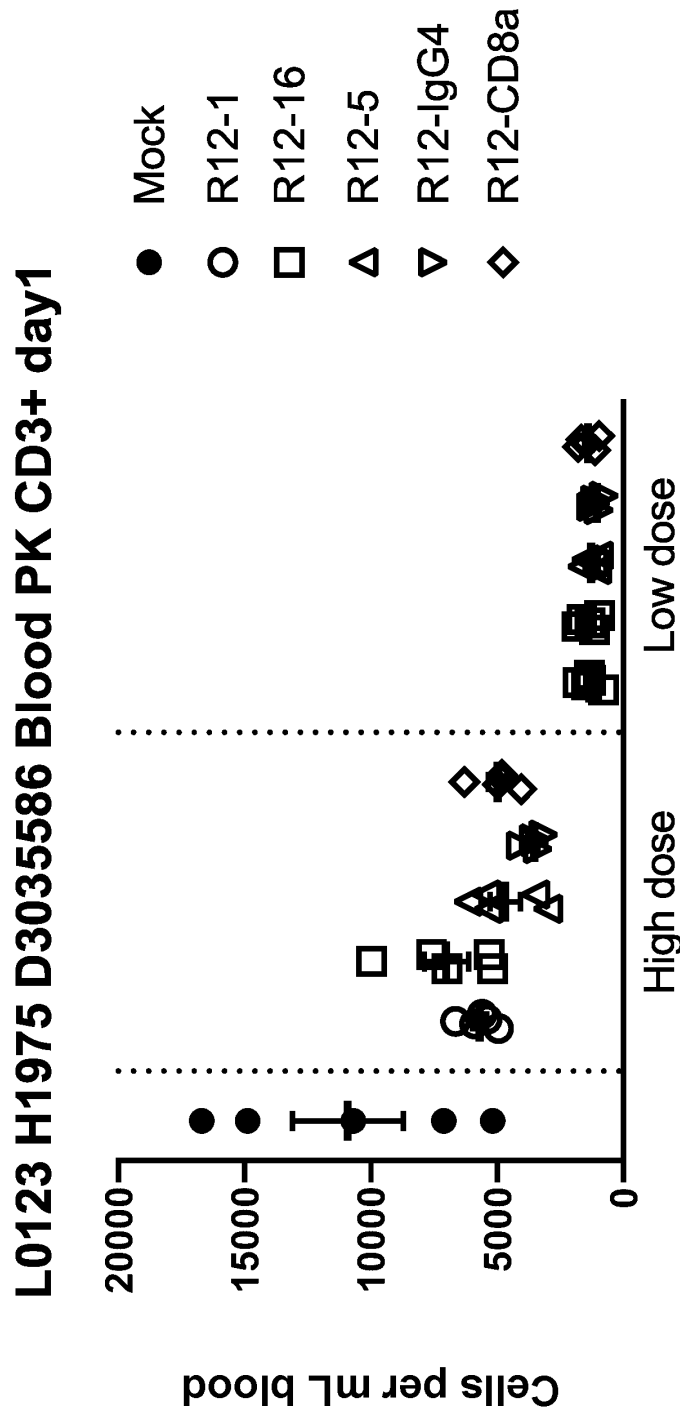


FIG. 82

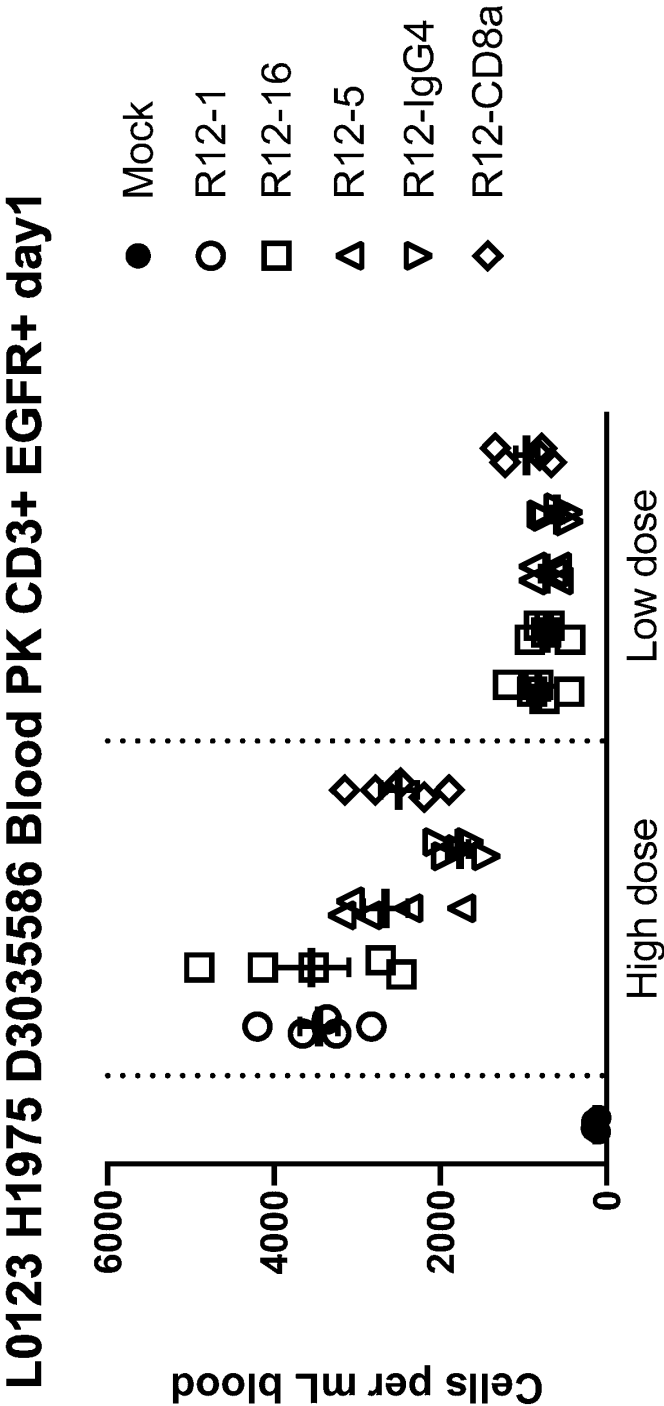


FIG. 83

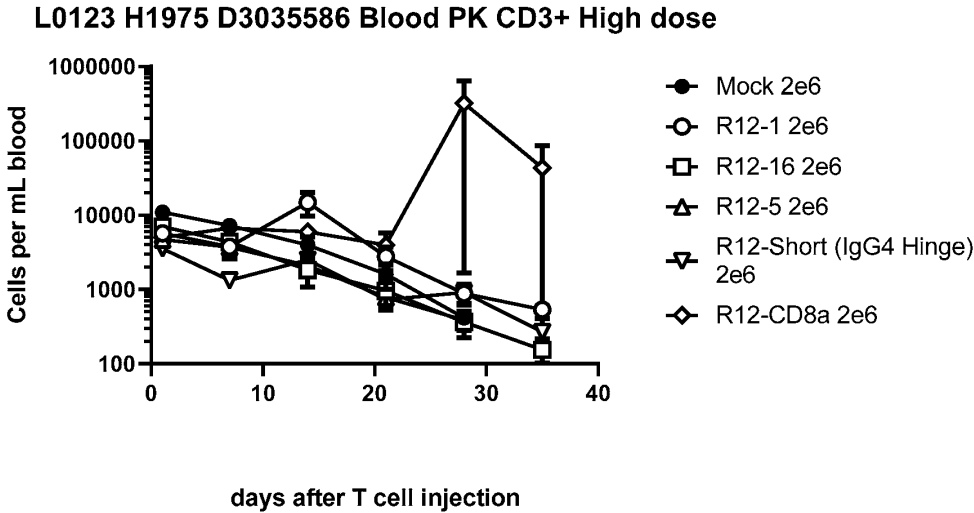


FIG. 84A

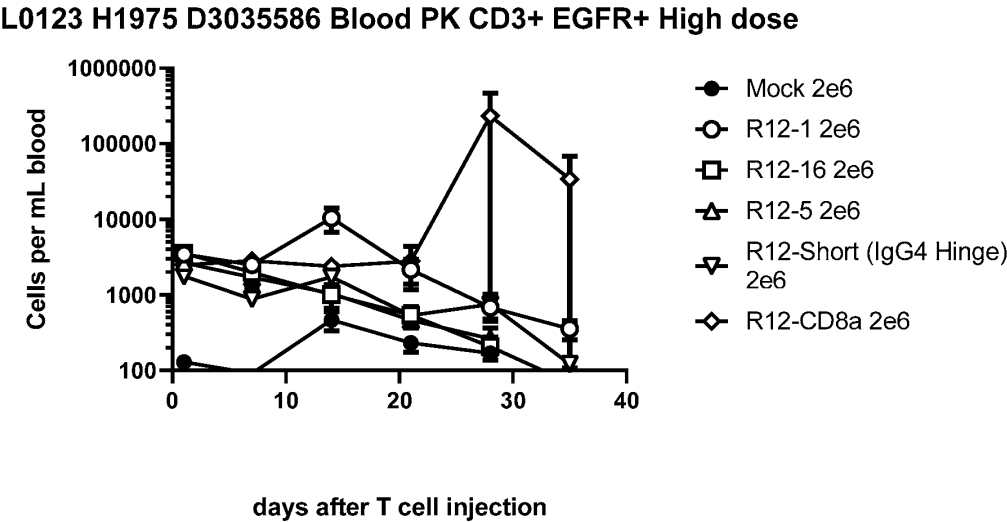
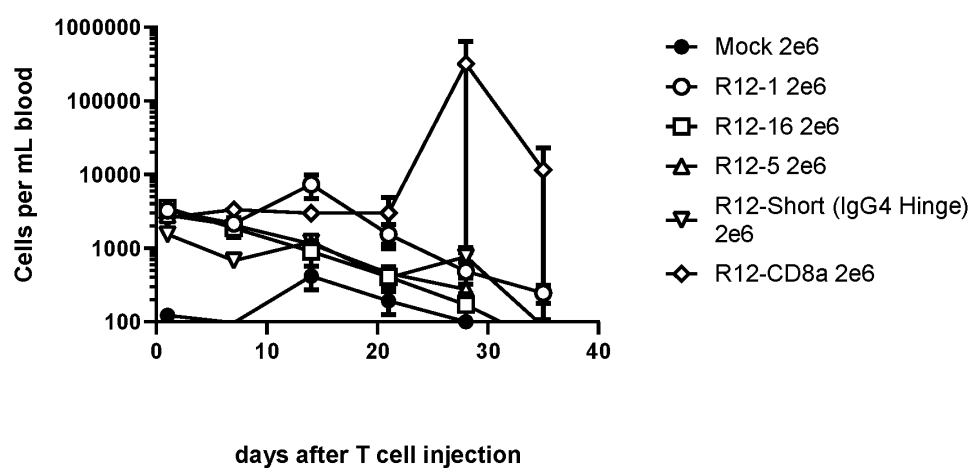
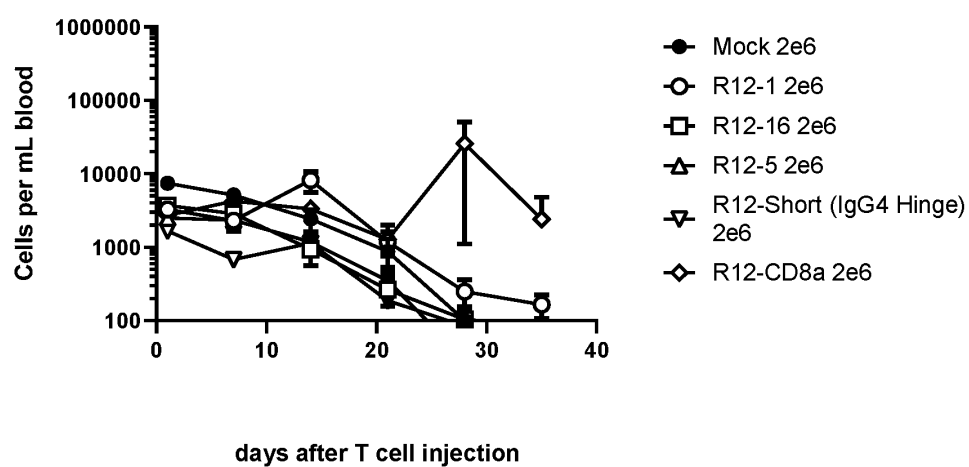


FIG. 84B

L0123 H1975 D3035586 Blood PK CD3+ CAR+ High dose**FIG. 84C****L0123 H1975 D3035586 Blood PK CD8+ High dose****FIG. 84D**

L0123 H1975 D3035586 Blood PK CD8+ EGFR+ High dose

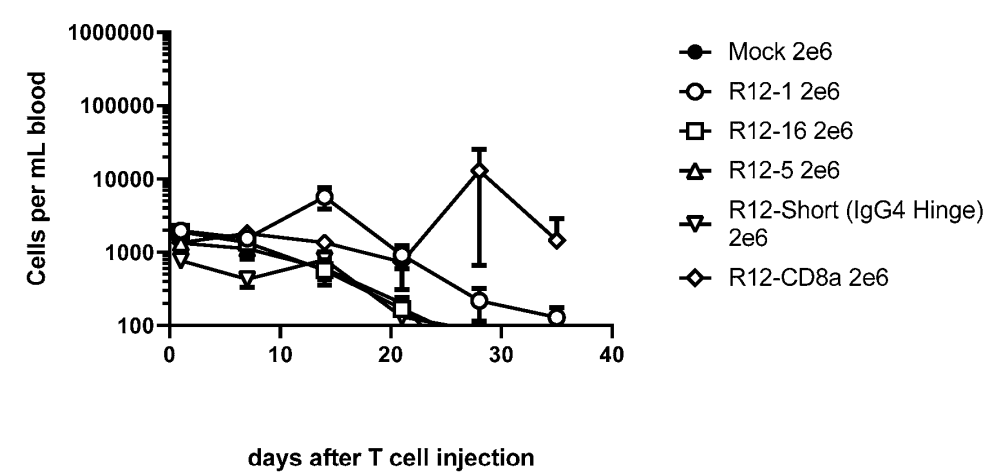


FIG. 84E

L0123 H1975 D3035586 Blood PK CD8+ CAR+ High dose

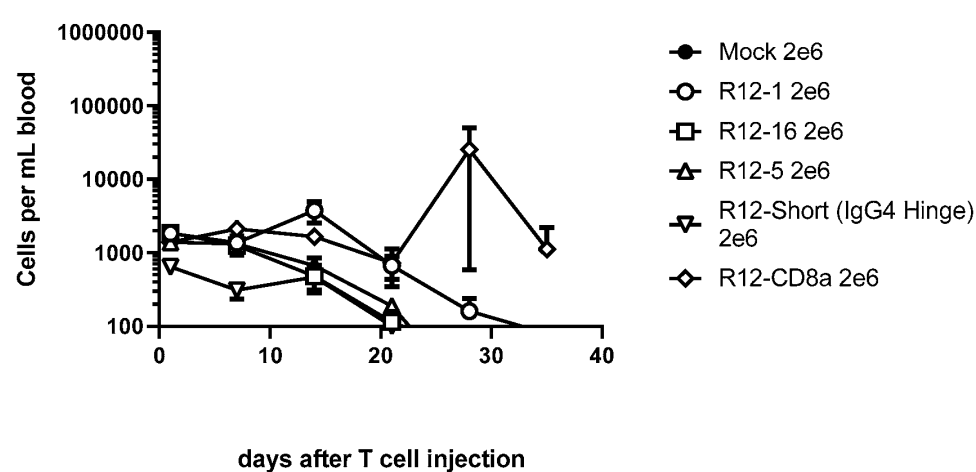


FIG. 84F

L0123 H1975 D3035586 Blood PK CD8+CD4+ High dose

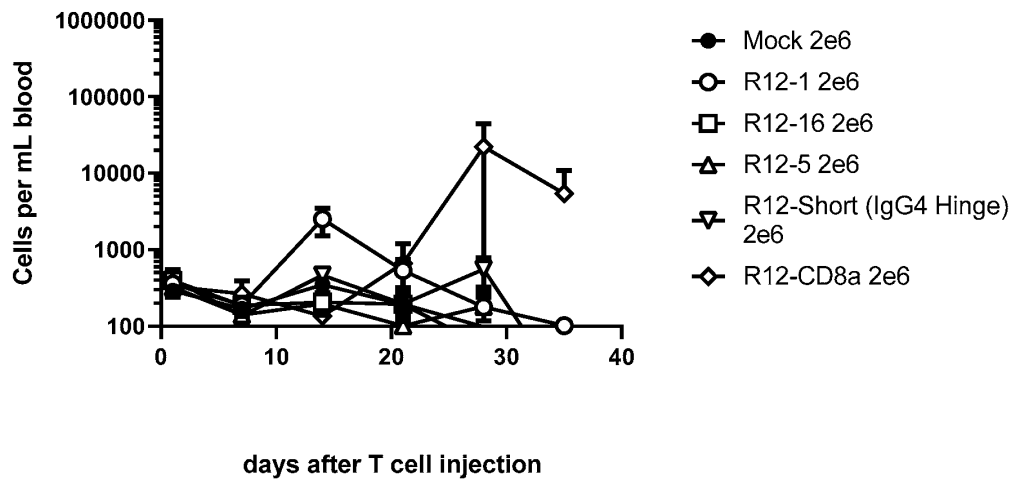


FIG. 84G

L0123 H1975 D3035586 Blood PK CD8+CD4+ EGFR+ High dose

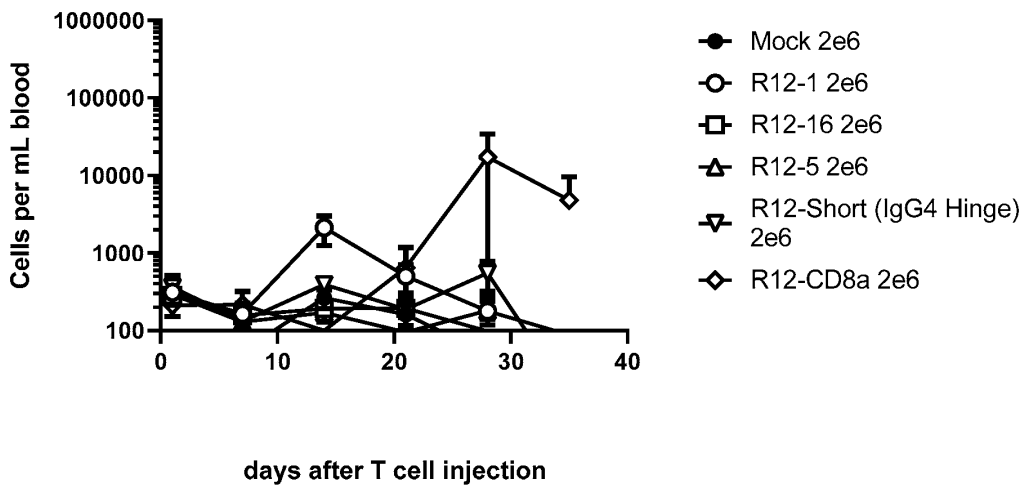


FIG. 84H

L0123 H1975 D3035586 Blood PK CD8+CD4+ CAR+ High dose

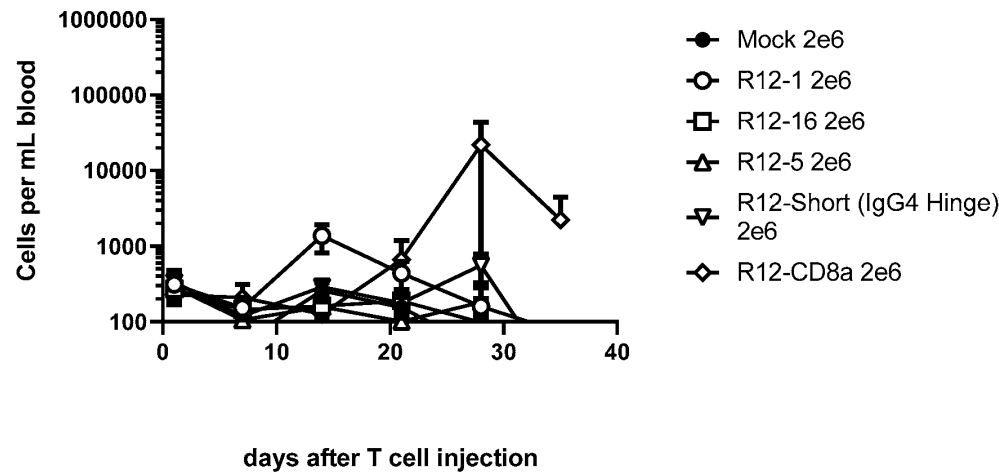


FIG. 84I

L0123 H1975 D3035586 Blood PK CD4+ High dose

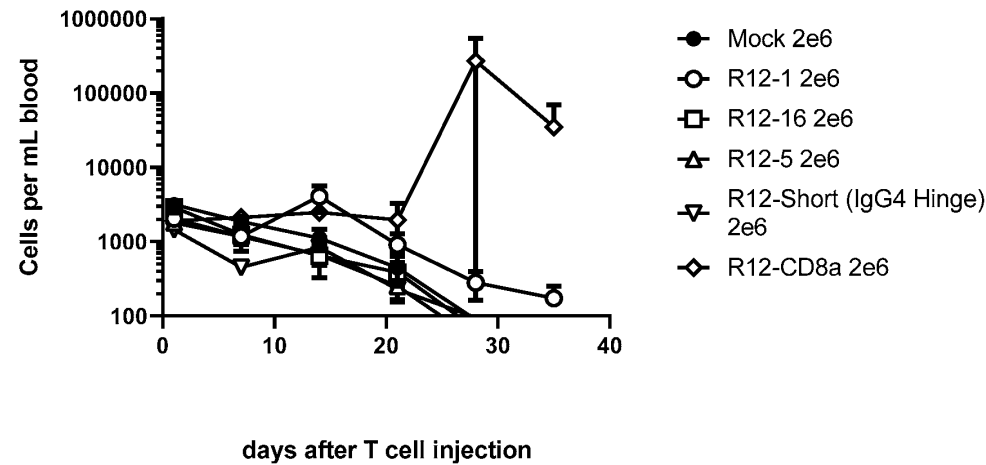


FIG. 84J

L0123 H1975 D3035586 Blood PK CD4+ EGFR+ High dose

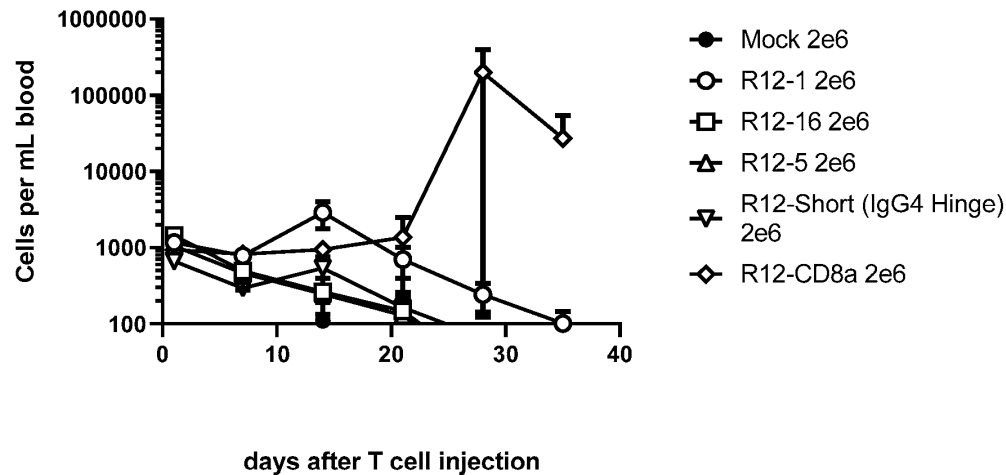


FIG. 84K

L0123 H1975 D3035586 Blood PK CD4+ CAR+ High dose

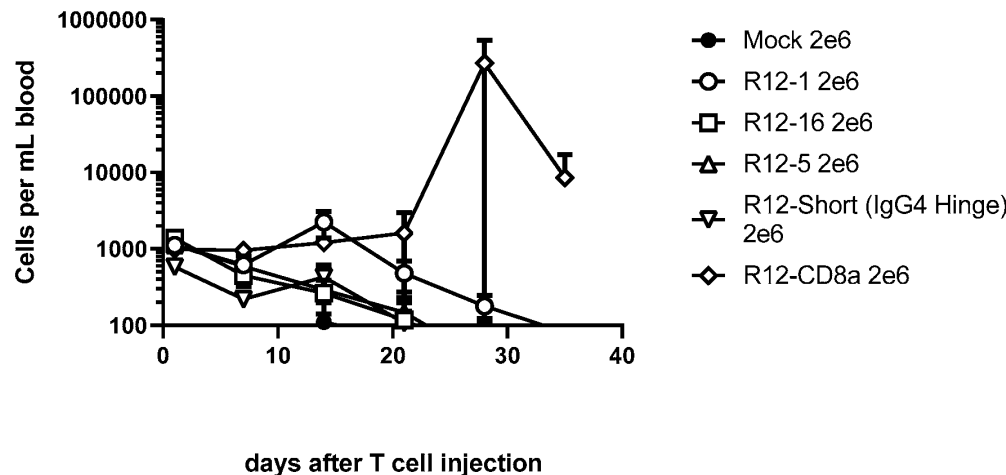


FIG. 84L

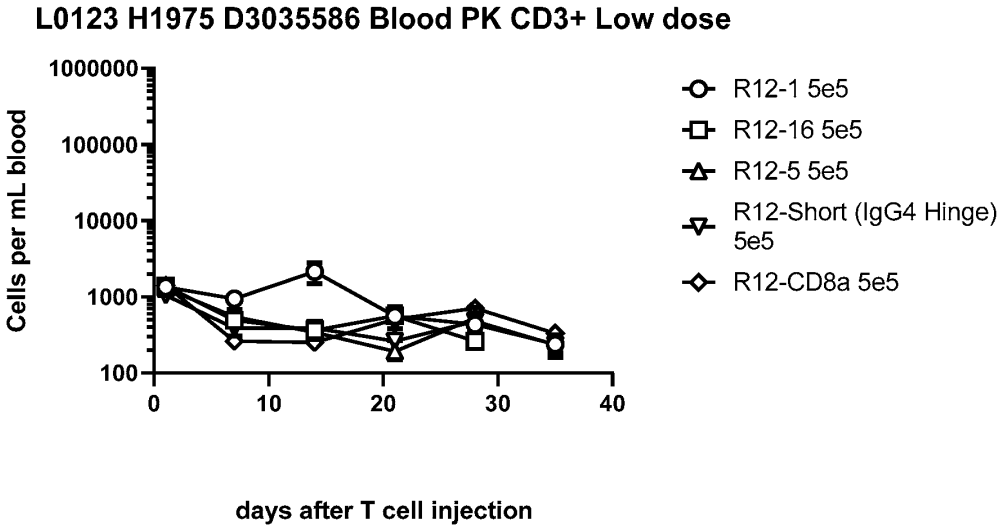


FIG. 85A

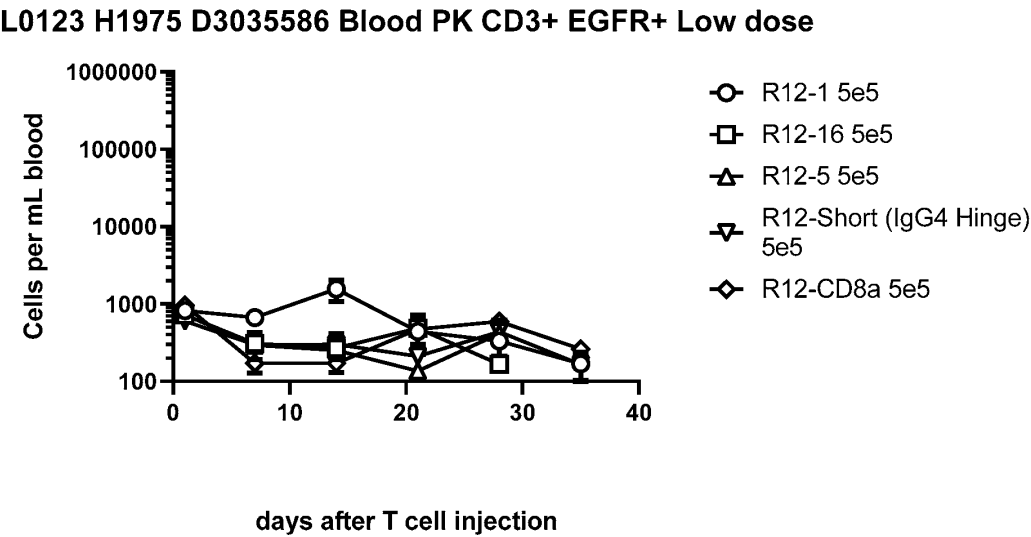


FIG. 85B

L0123 H1975 D3035586 Blood PK CD3+ CAR+ Low dose

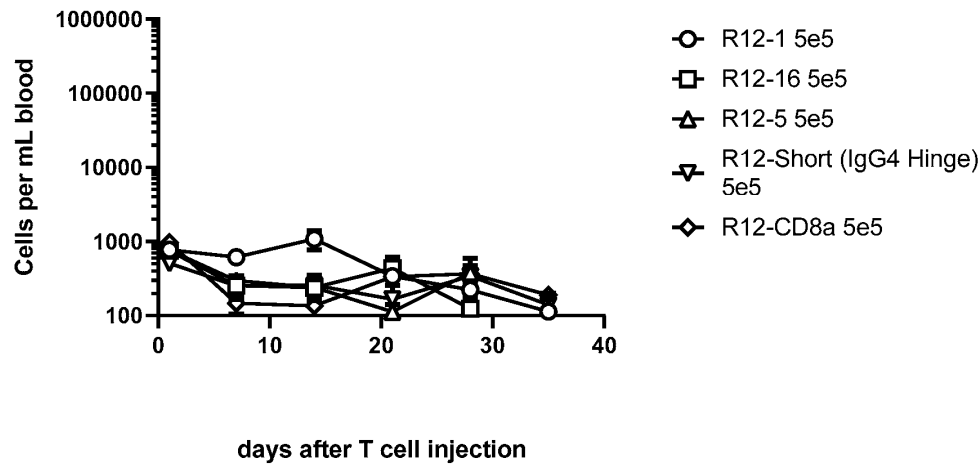


FIG. 85C

L0123 H1975 D3035586 Blood PK CD8+ Low dose

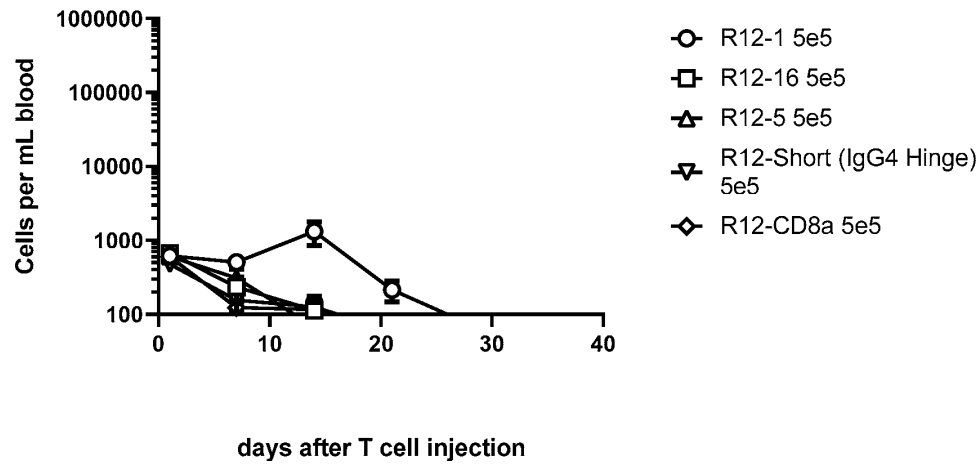


FIG. 85D

L0123 H1975 D3035586 Blood PK CD8+ EGFR+ Low dose

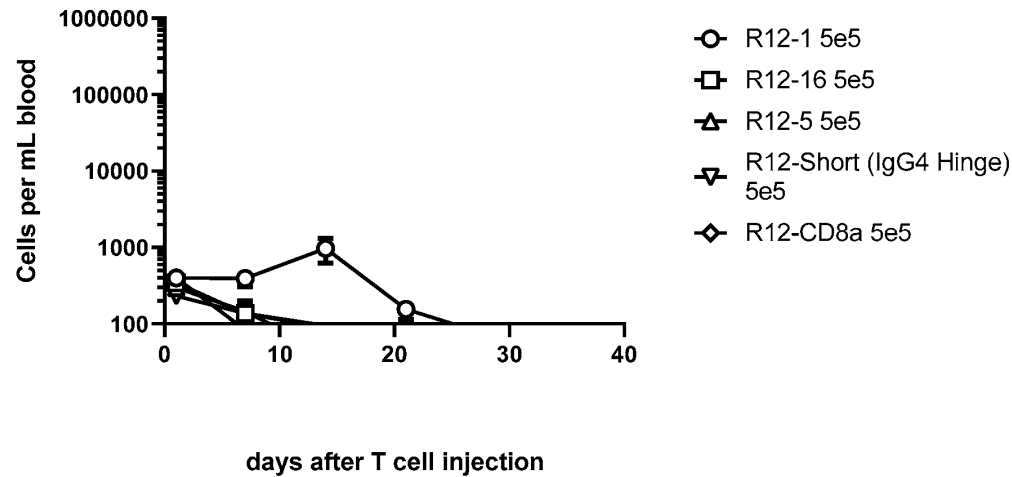


FIG. 85E

L0123 H1975 D3035586 Blood PK CD8+ CAR+ Low dose

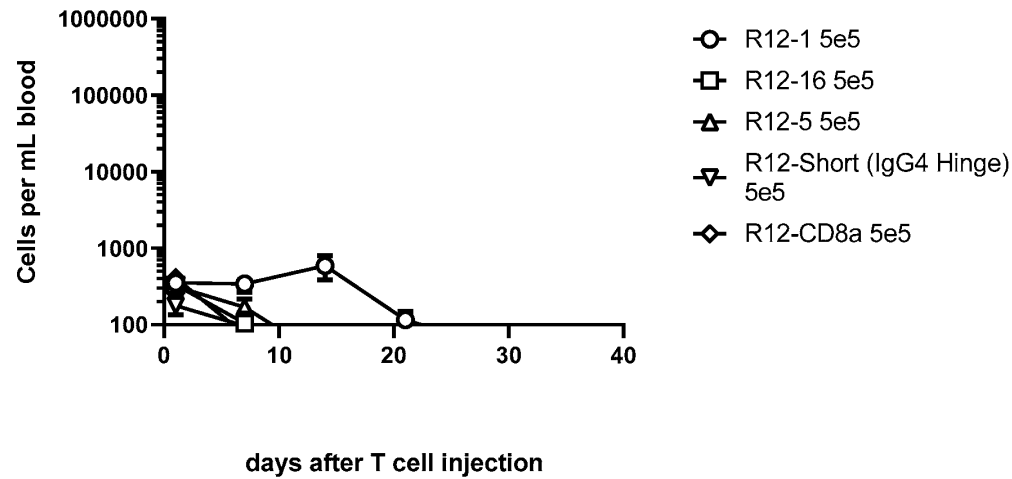


FIG. 85F

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L0123 H1975 D3035586 Blood PK CD8+CD4+ Low dose

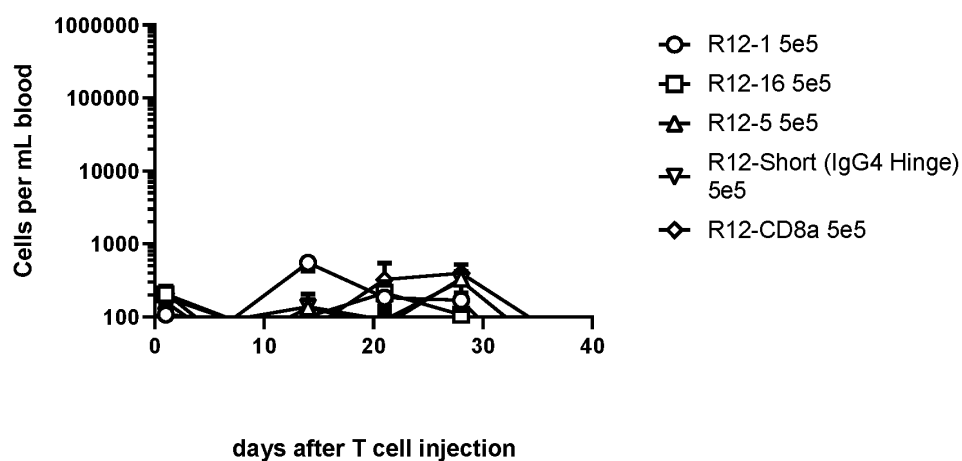


FIG. 85G

L0123 H1975 D3035586 Blood PK CD8+CD4+ EGFR+ Low dose

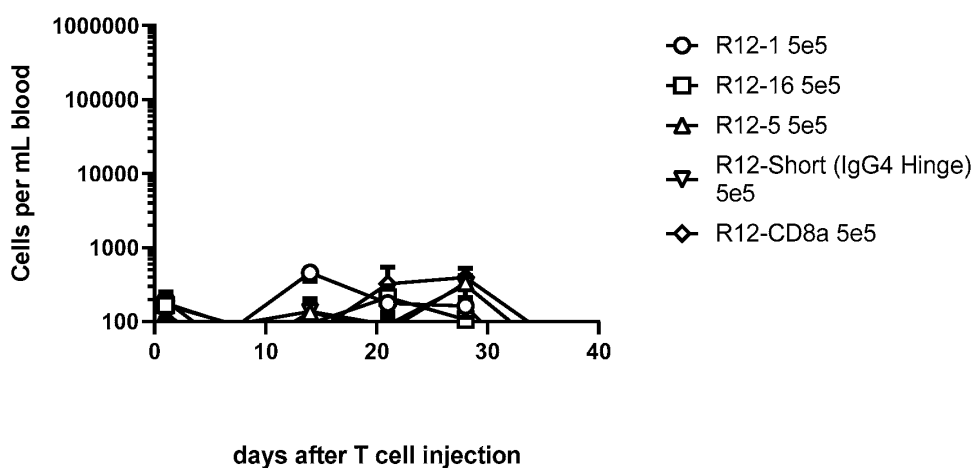


FIG. 85H

L0123 H1975 D3035586 Blood PK CD8+CD4+ CAR+ Low dose

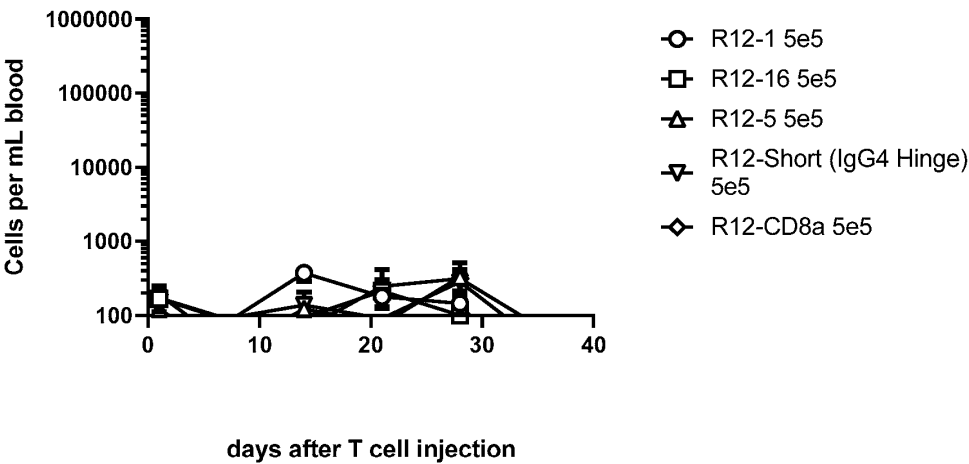


FIG. 85I

L0123 H1975 D3035586 Blood PK CD4+ Low dose

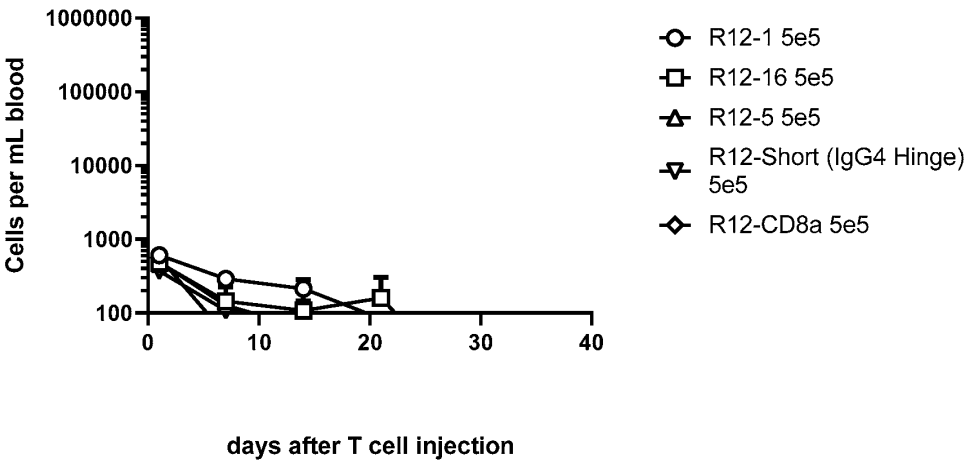


FIG. 85J

L0123 H1975 D3035586 Blood PK CD4+ EGFR+ Low dose

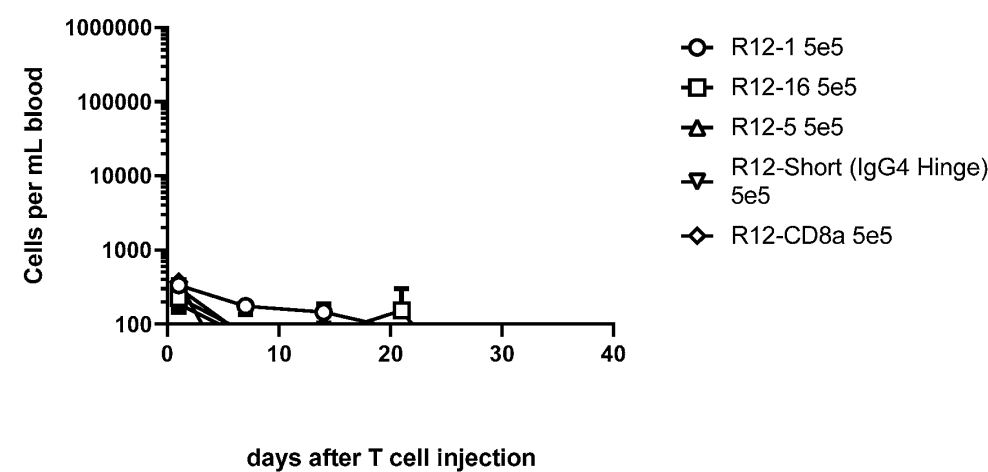


FIG. 85K

L0123 H1975 D3035586 Blood PK CD4+ CAR+ Low dose

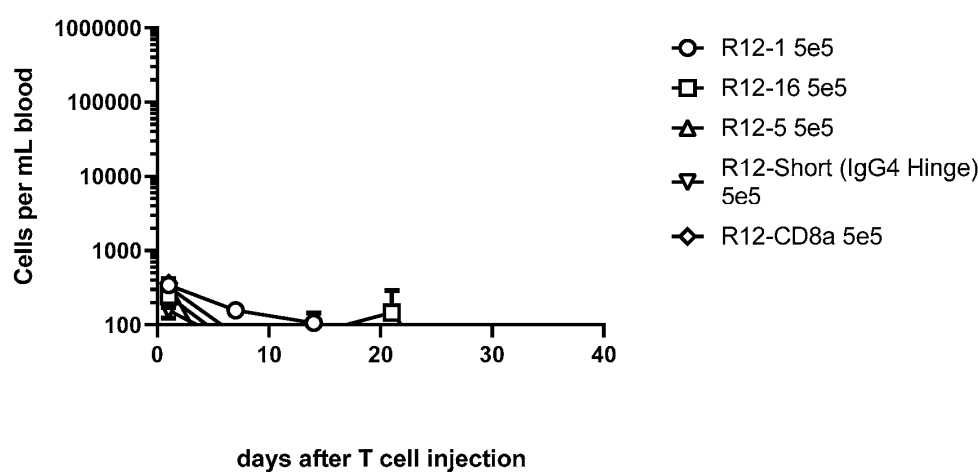


FIG. 85L