

FIG. 1

	Colon adenocarcinoma	Ovarian	Lung Adenocarcinoma	Pancreatic Adenocarcinoma	Mesothelioma
Gene	TCGA-COADREAD- Xena-Median-FPKM (383 samples)	TCGA-OV-Xena- Median-FPKM (427 samples)	TCGA-LUAD-Xena- Median-FPKM (515 samples)	TCGA-PAAD-Xena- Median-FPKM (179 samples)	TCGA-MESO-Xena- Median-FPKM (87 samples)
VASN	1.7	5.1	3.5	3.7	5.1
TUSC3	1.8	5.0	4.8	4.9	5.3
VSIG4	1.8	3.8	4.5	4.0	5.0
CTXN1	0.3	6.6	2.1	2.1	5.0
NPR1	-0.4	5.2	1.2	1.3	5.5
HAS1	-3.0	-3.6	-2.7	-0.6	5.7
CD200	1.7	3.9	2.0	2.7	5.7
LRRN4*	-4.0	0.5	1.3	-1.8	5.0
UPK3B*	-1.5	6.3	0.7	0.6	9.2
PTGIS	-0.2	1.8	1.6	3.2	6.9
SCARA3	0.7	5.7	2.0	3.2	5.7
UPK1B	-10.0	2.4	-4.3	0.9	6.7
CLIC3	1.7	3.6	3.7	4.4	5.6

FIG. 2

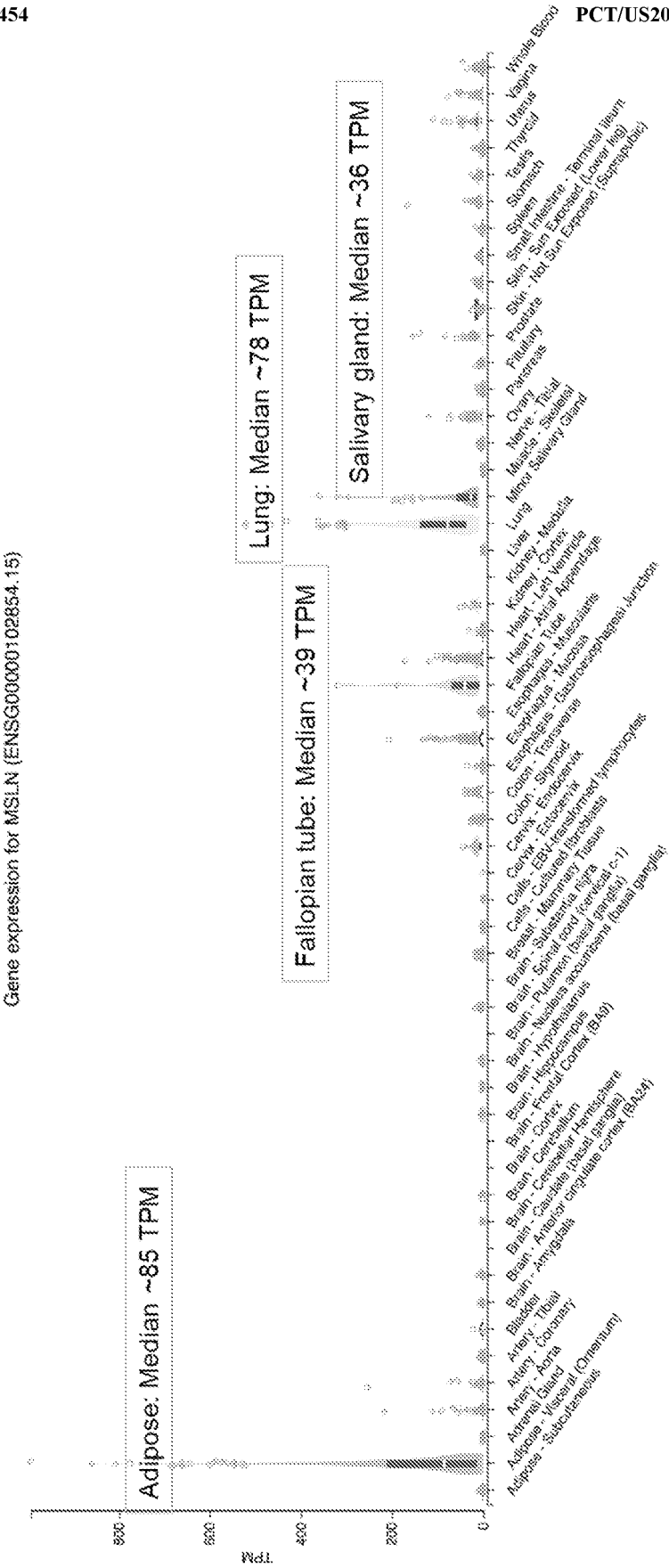


FIG. 3

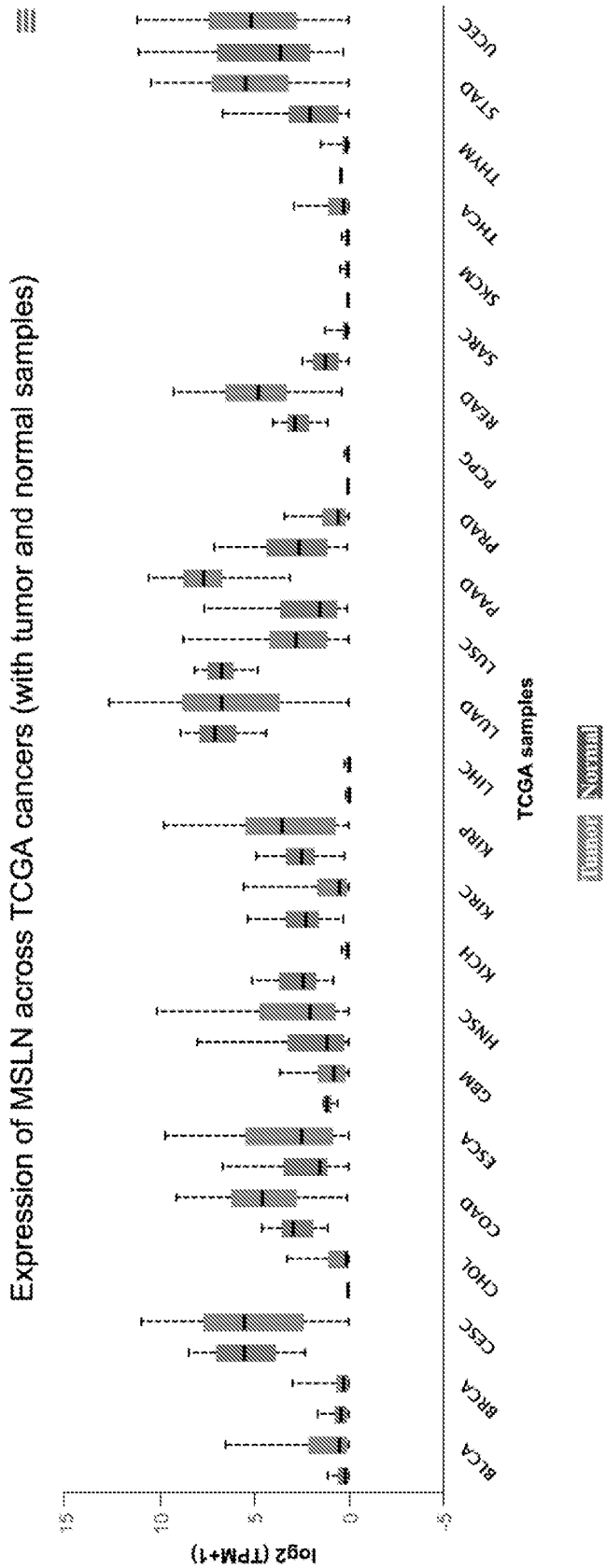


FIG. 4

mRNA expression (RNAseq): MSLN

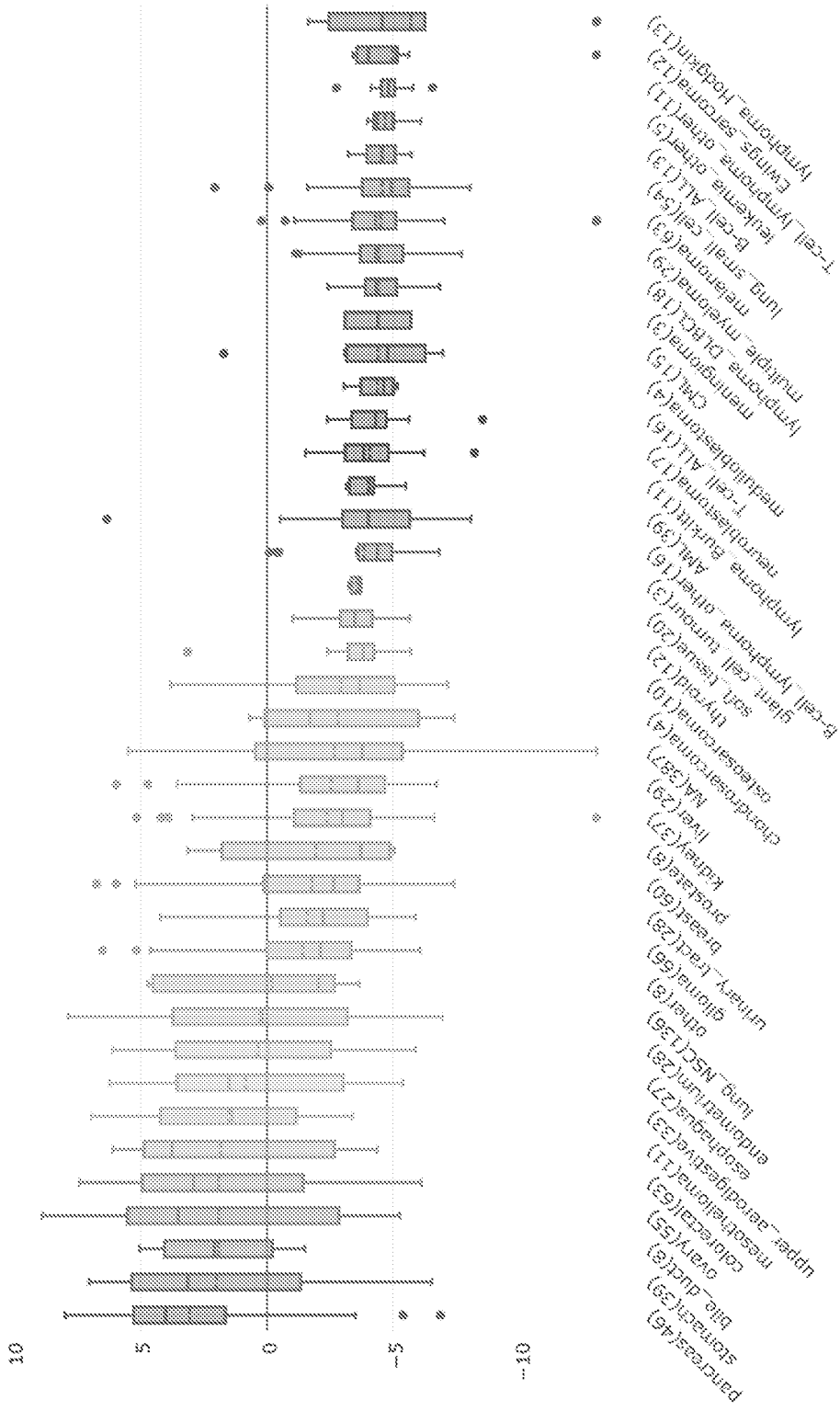


FIG. 5

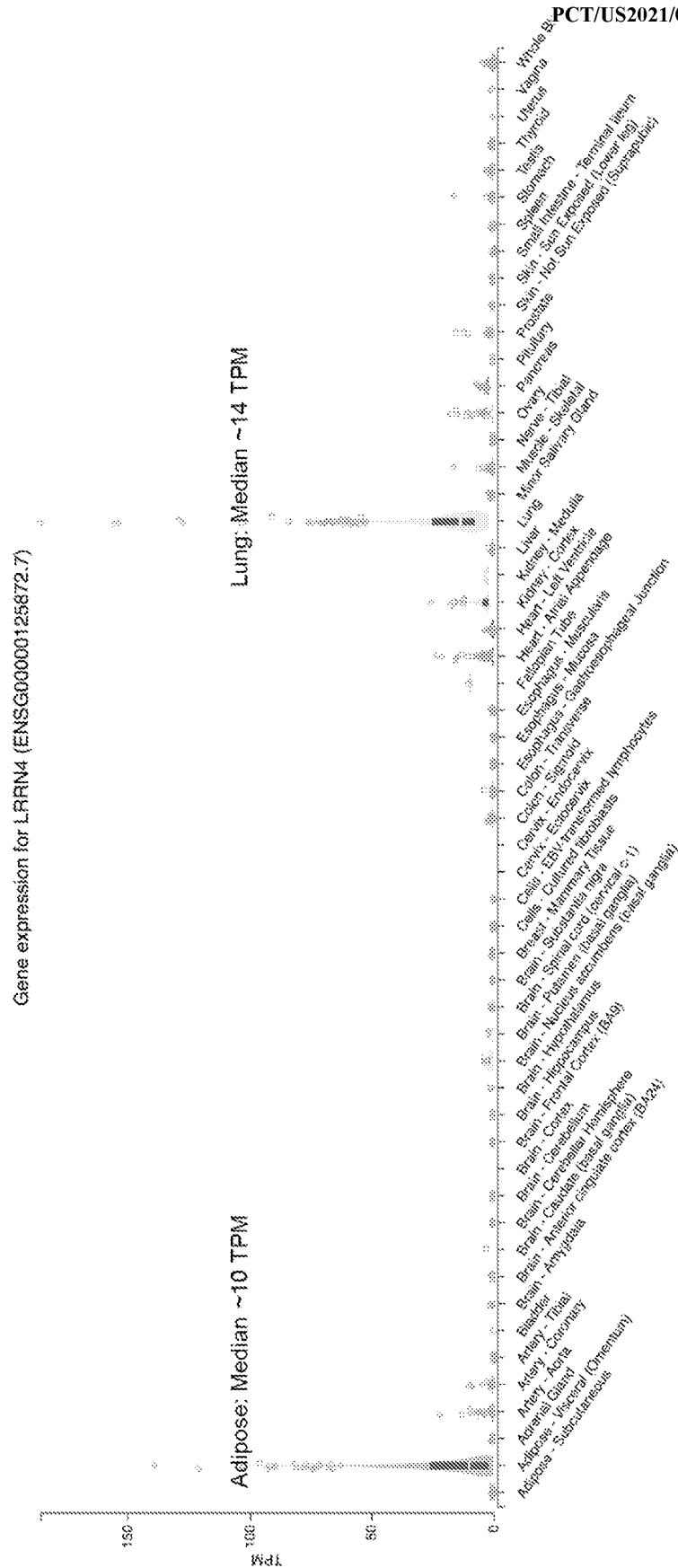


FIG. 6

Expression of LRRN4 across TCGA cancers (with tumor and normal samples)

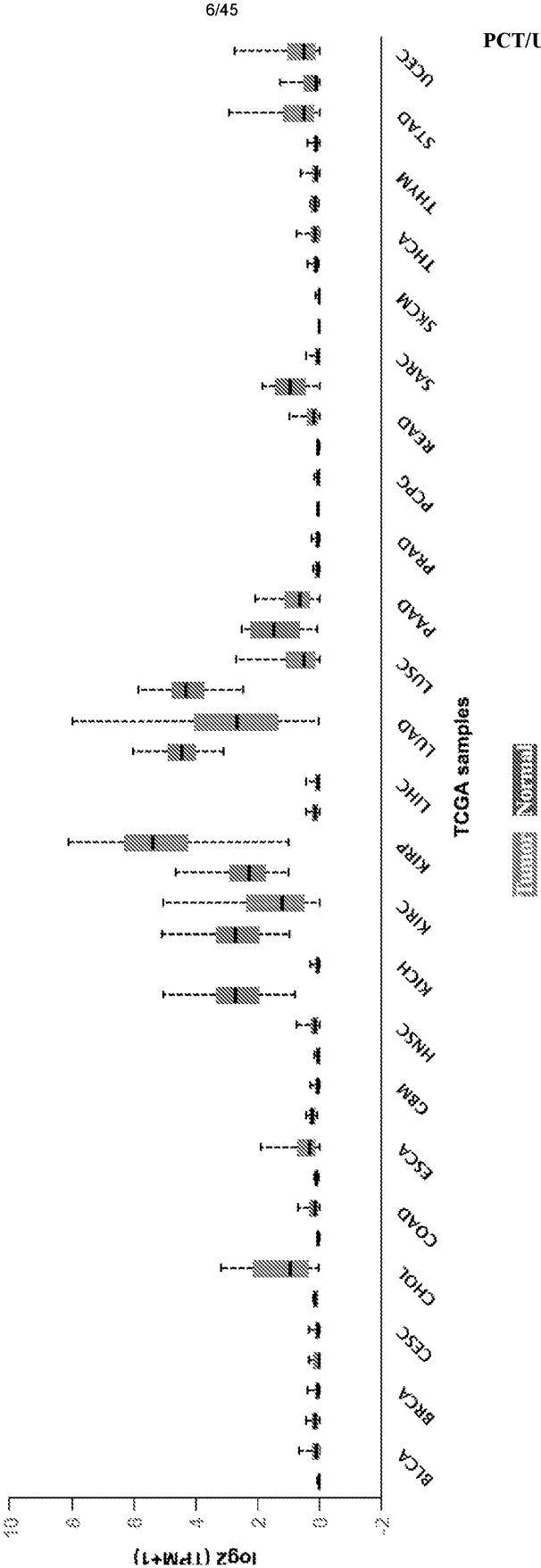
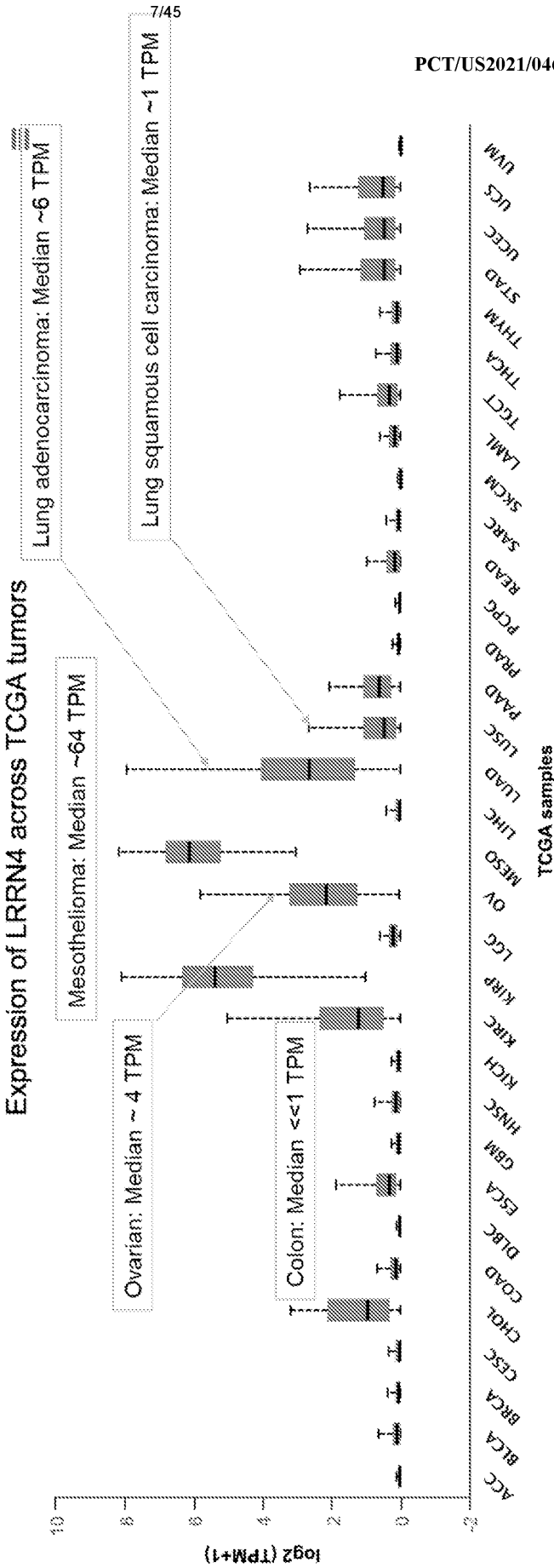


FIG. 7



mRNA expression (RNAseq): LRRN4

Mesothelioma (n=11)

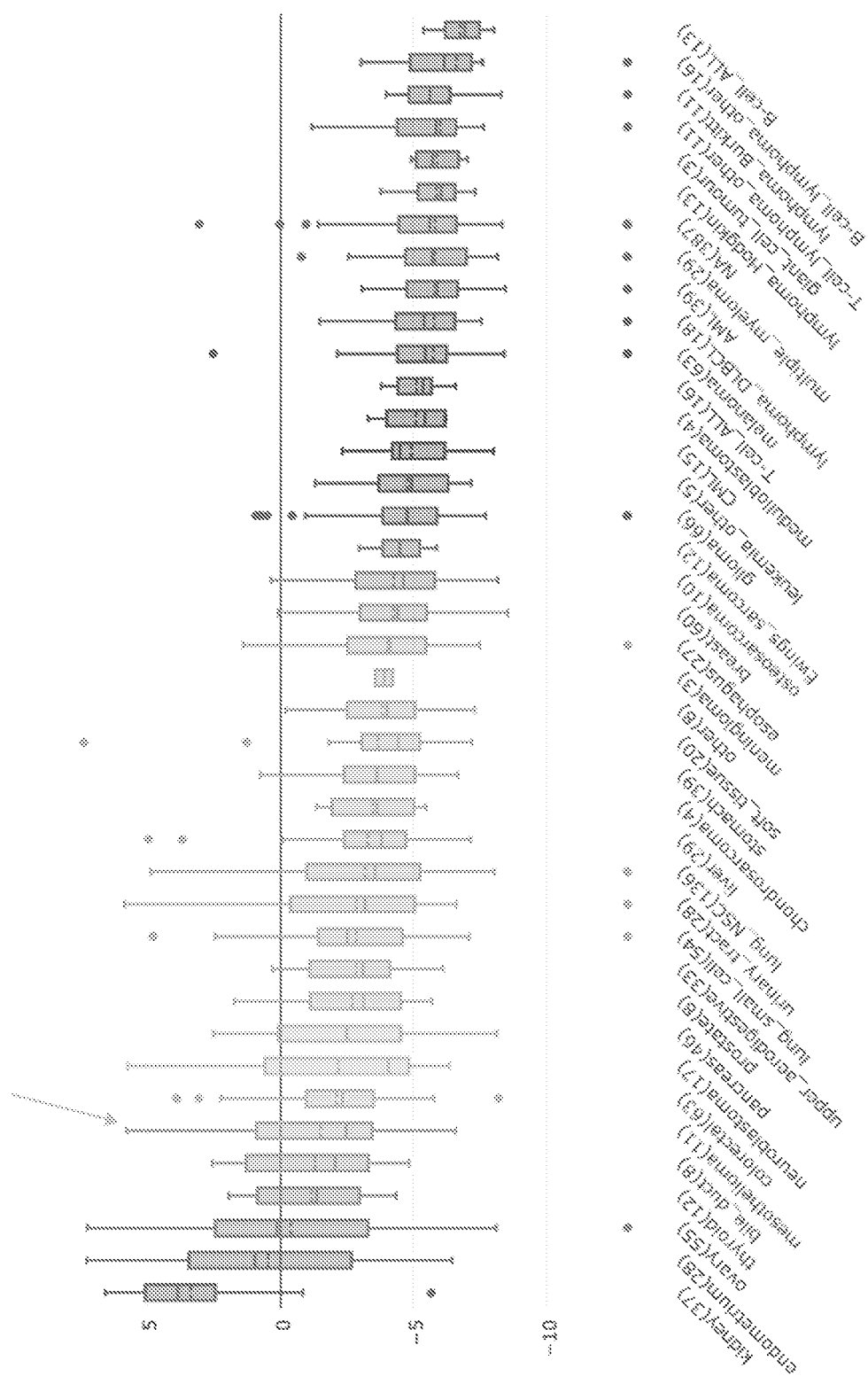


FIG. 9

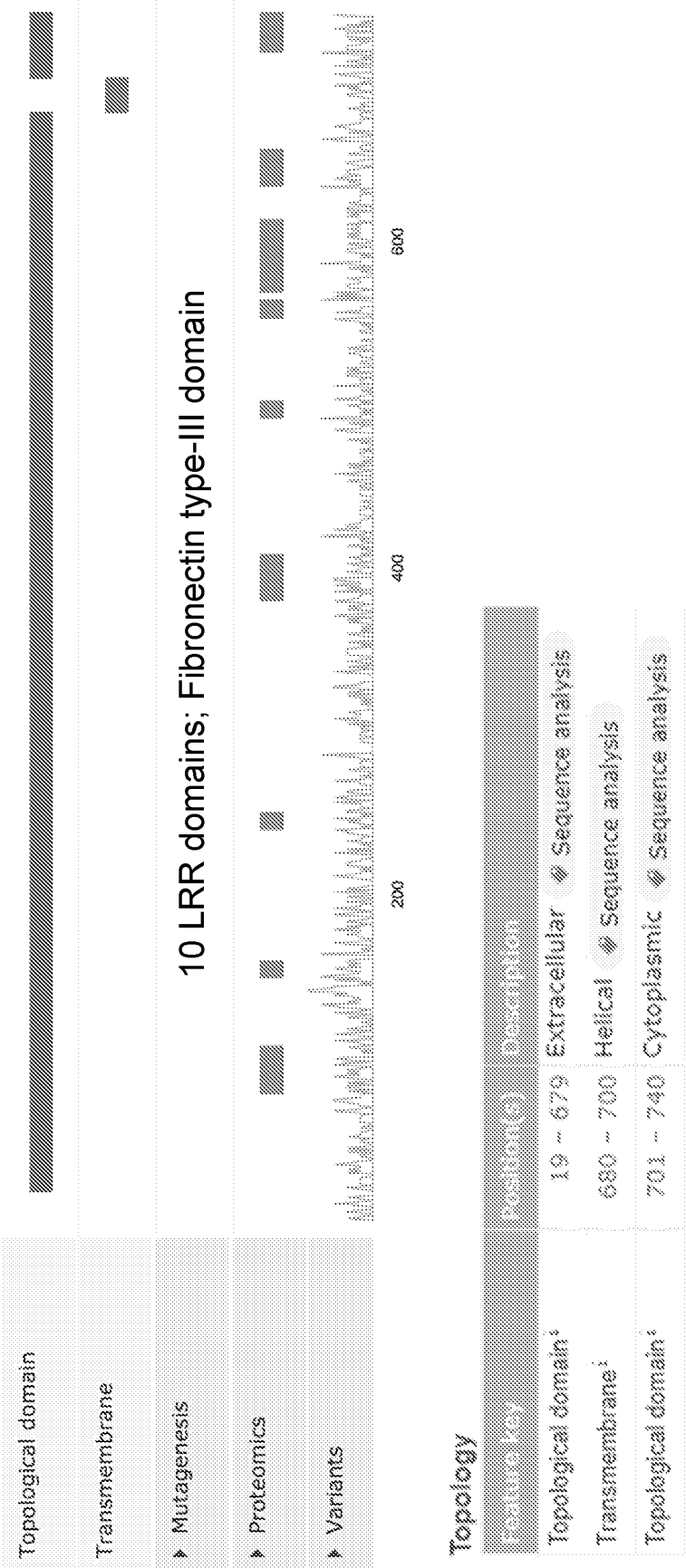


FIG. 10A

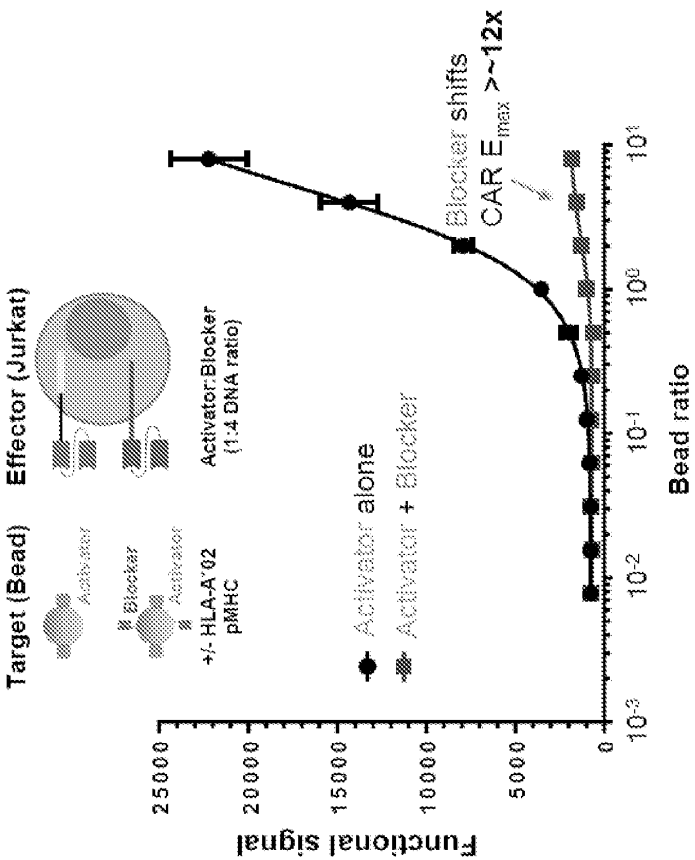
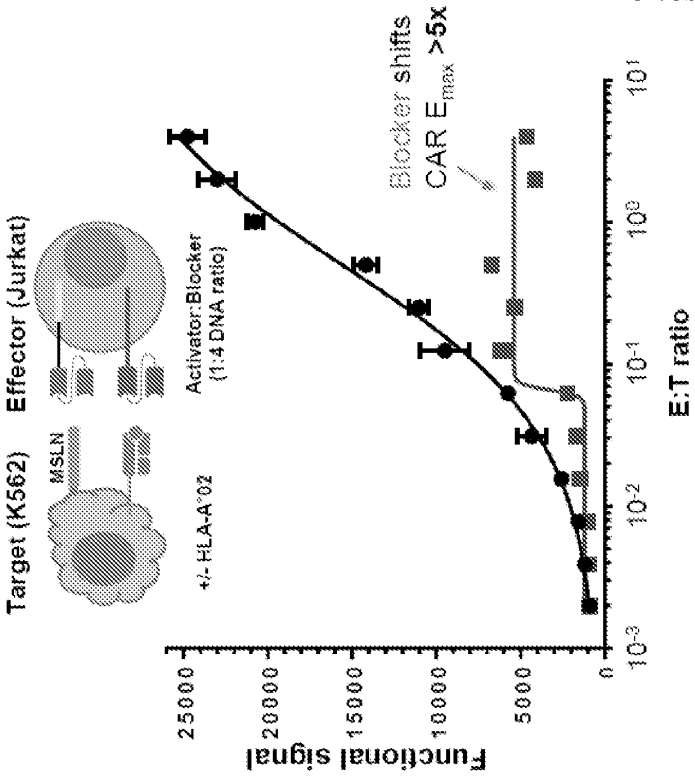
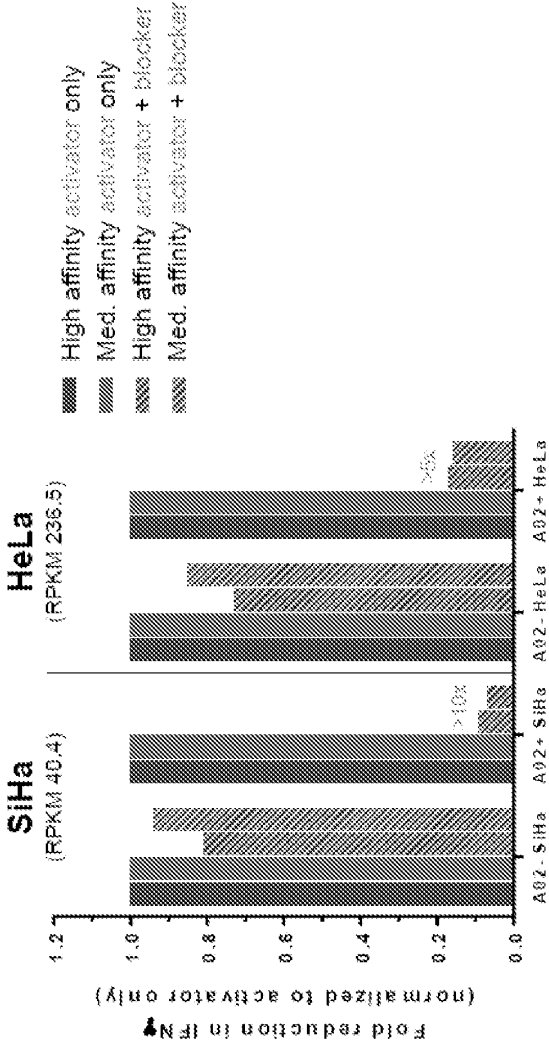


FIG. 10B



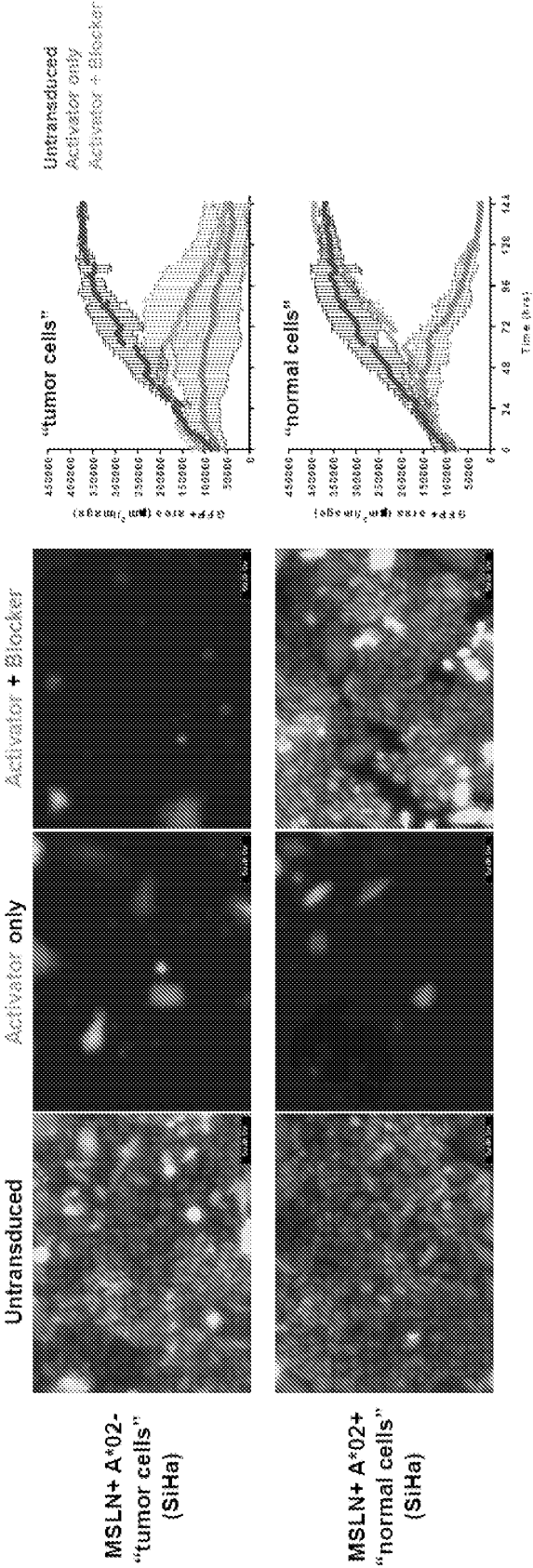
Activator = MSLN CAR; Blocker = A2-LIR-1

FIG. 11



Activator = MSLN CAR; Blocker = A2-LIR-1

FIG. 12



Activator = MSLN CAR; Blocker = A2-LIR-1

FIG. 13

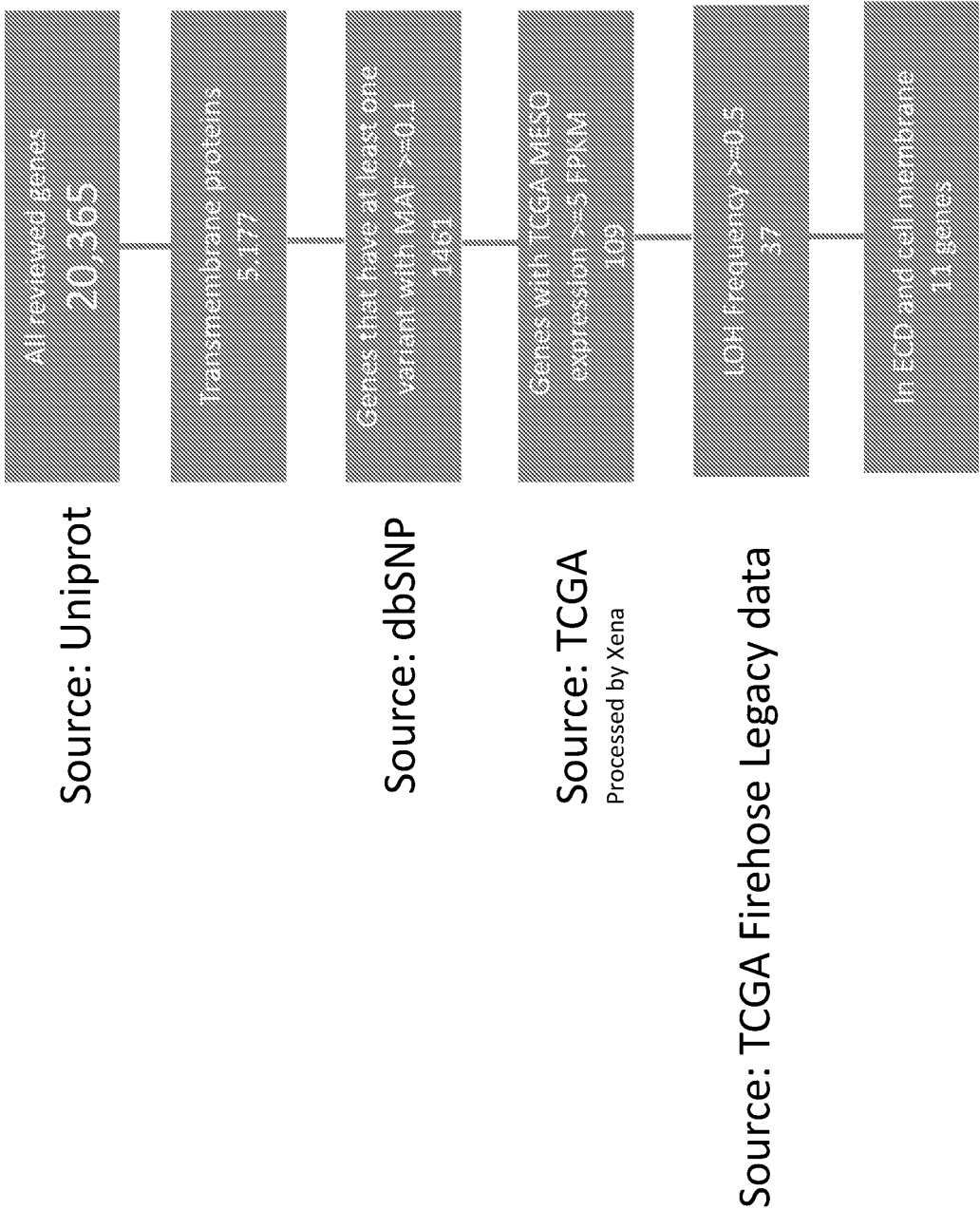
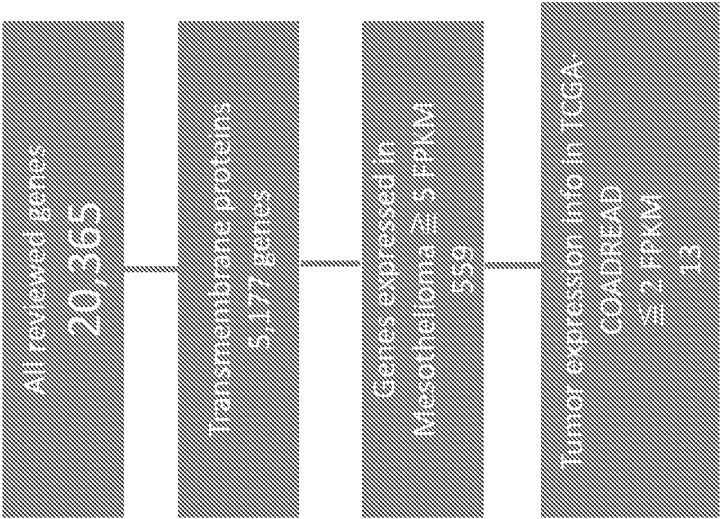


FIG. 14



Source: Uniprot

FIG. 15A

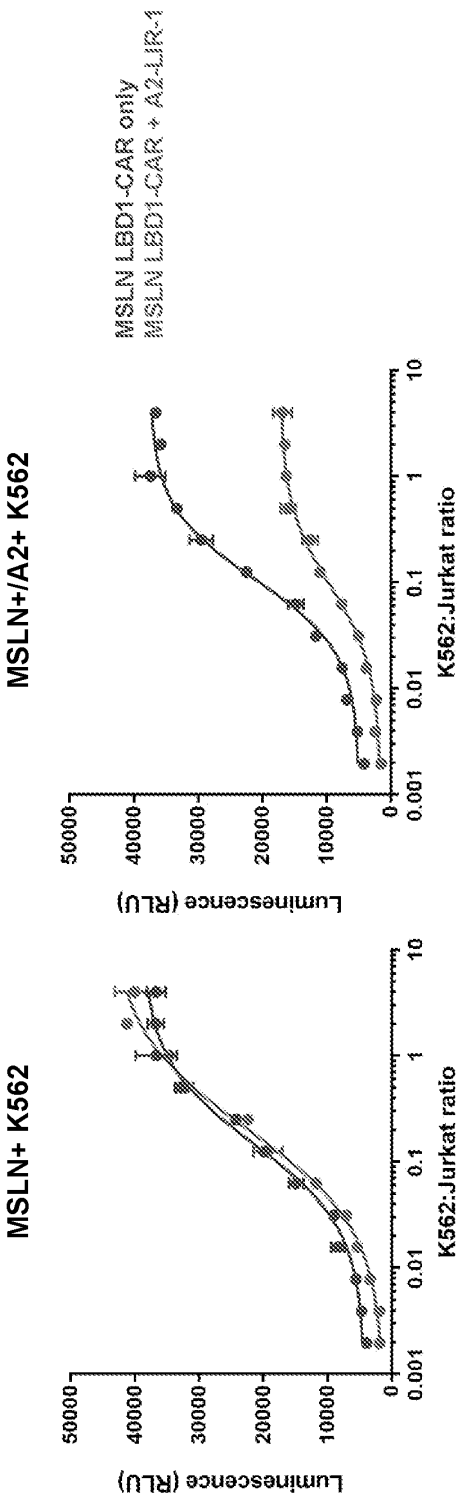


FIG. 15B

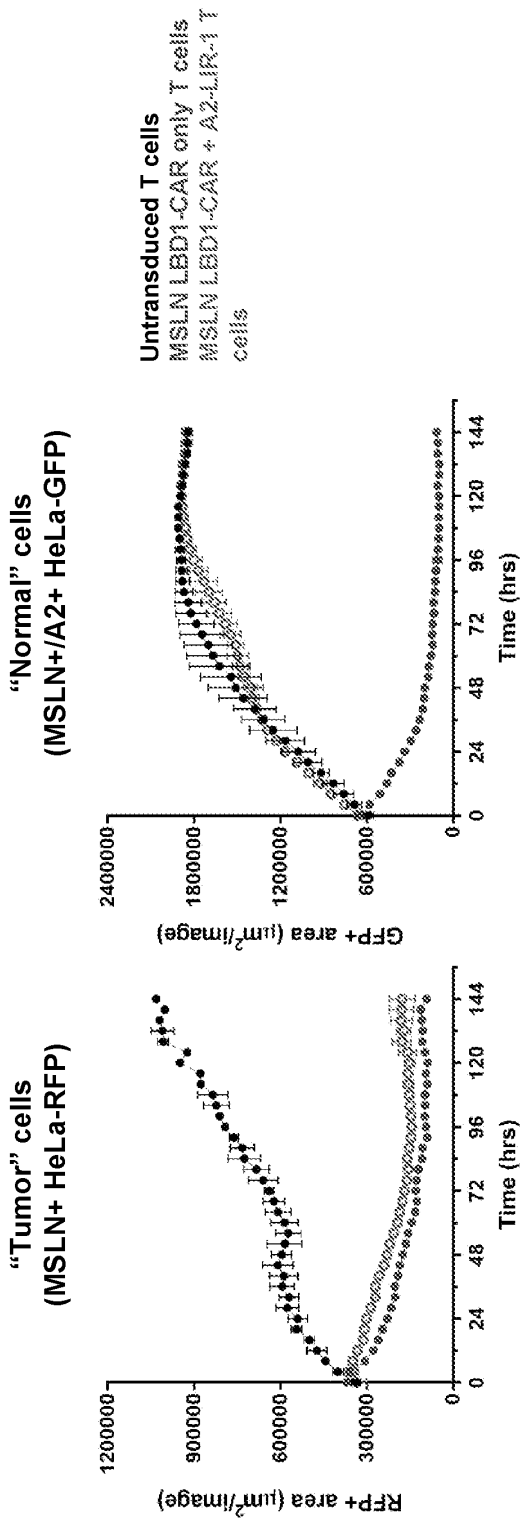


FIG. 15C

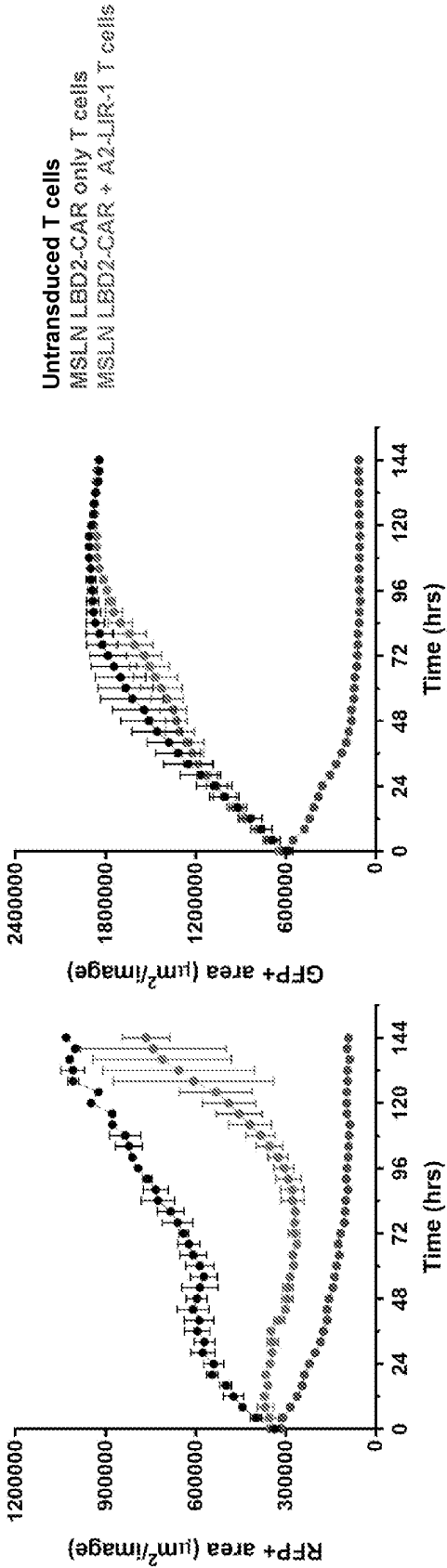


FIG. 16

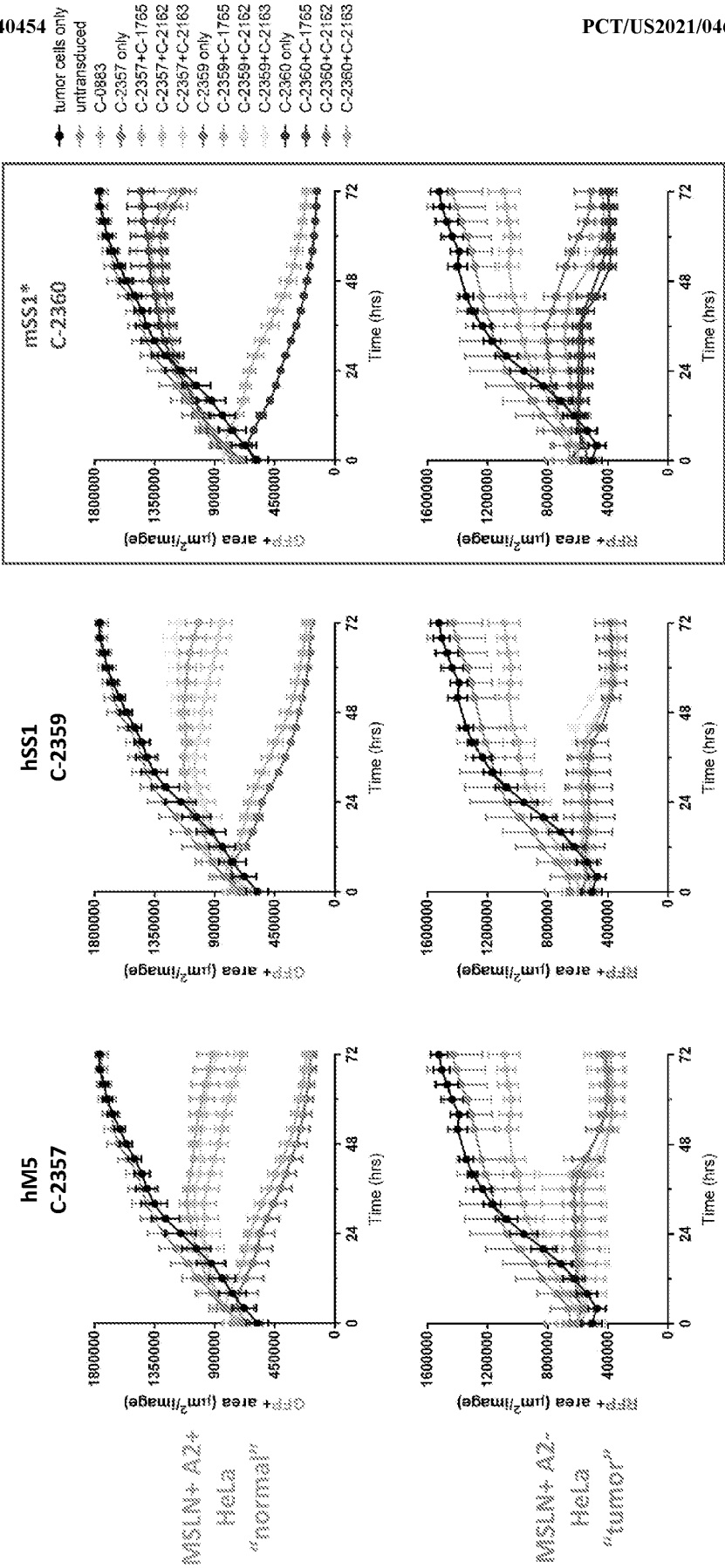


FIG. 17

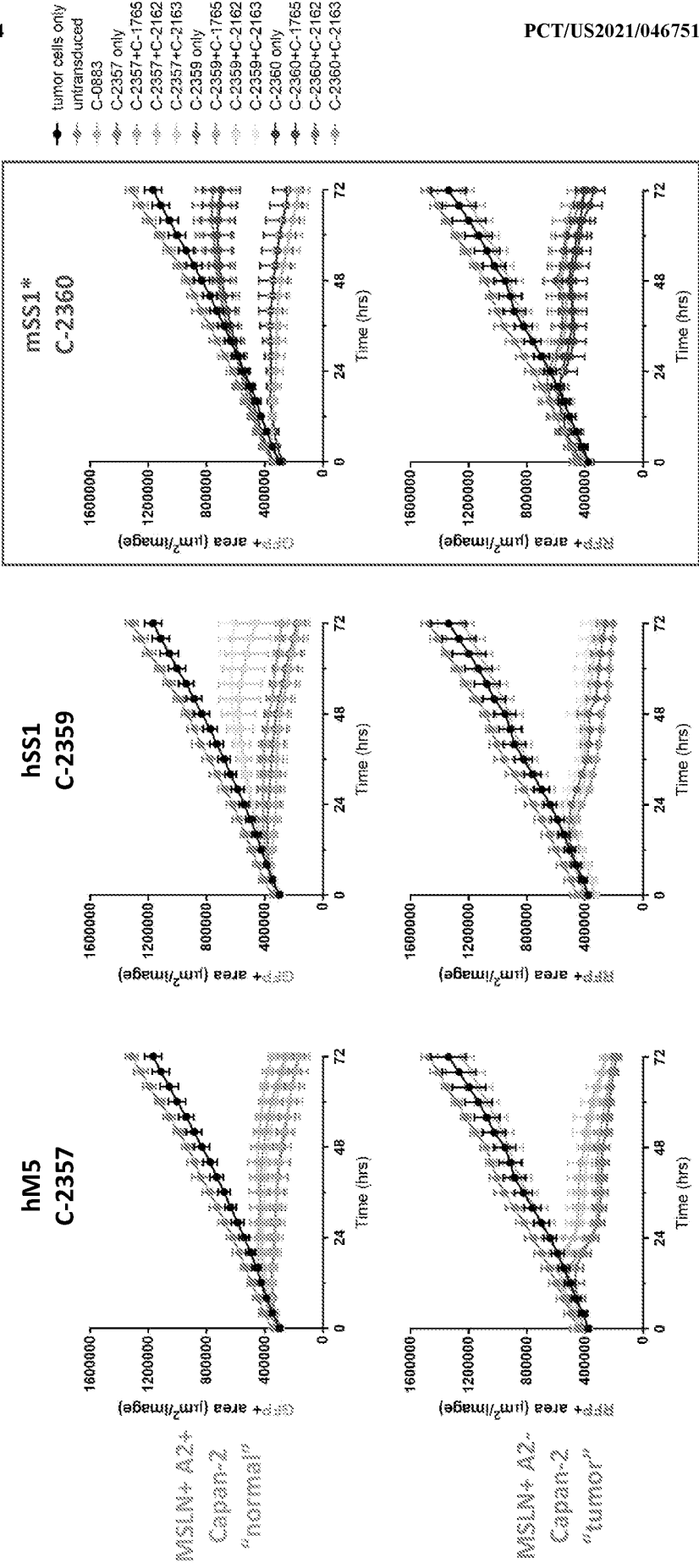


FIG. 18

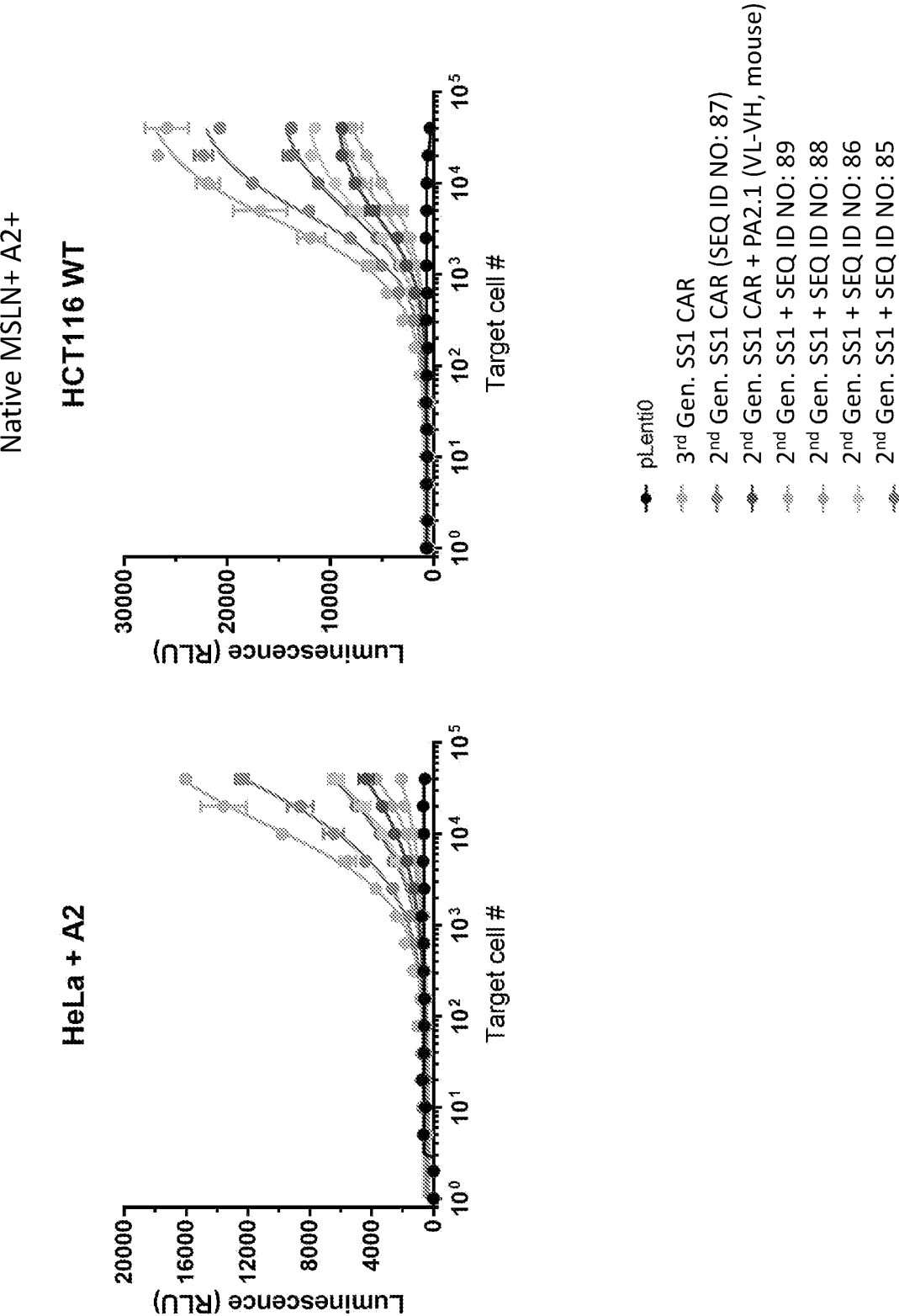


FIG. 19

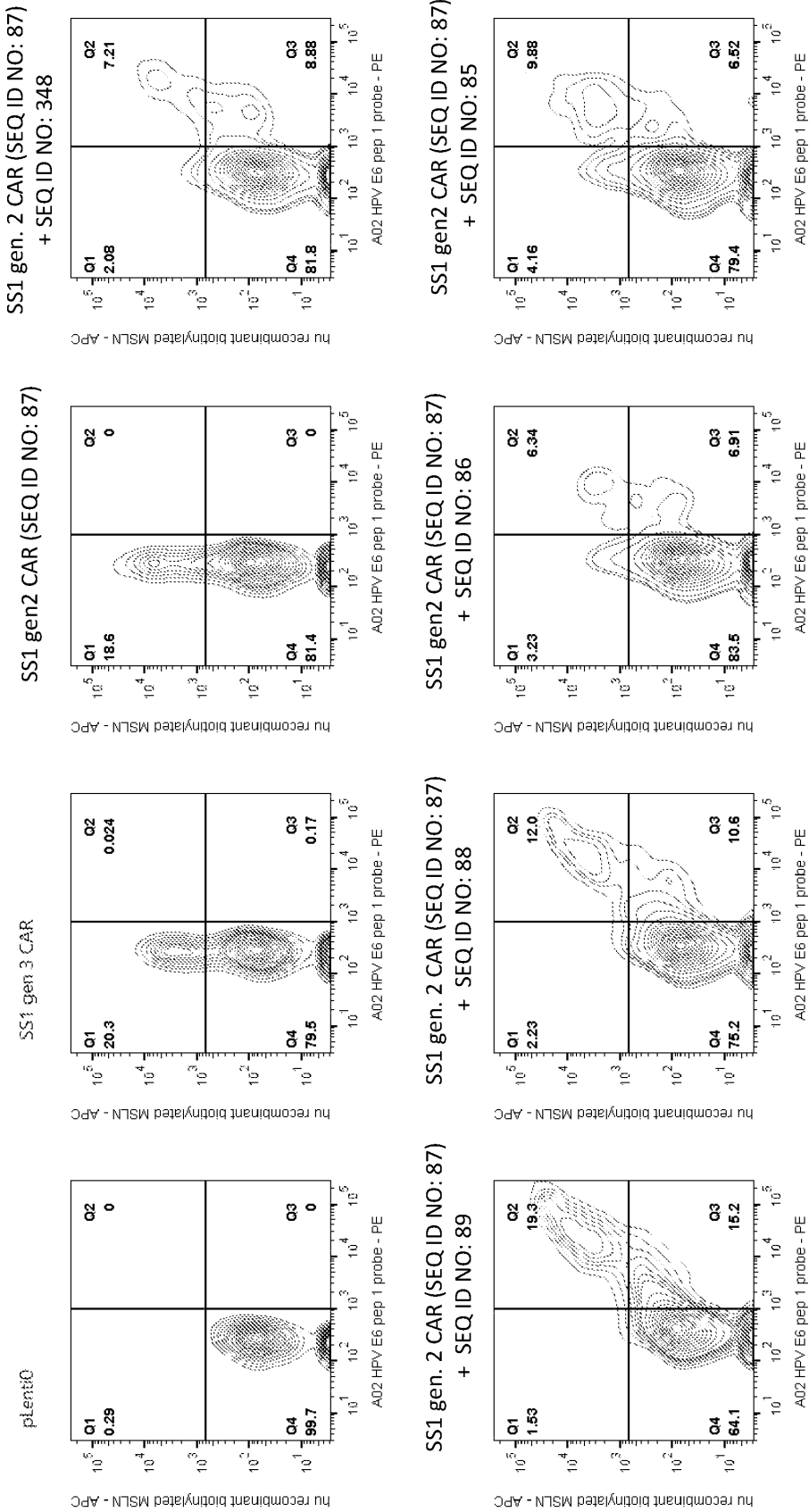


FIG. 20A

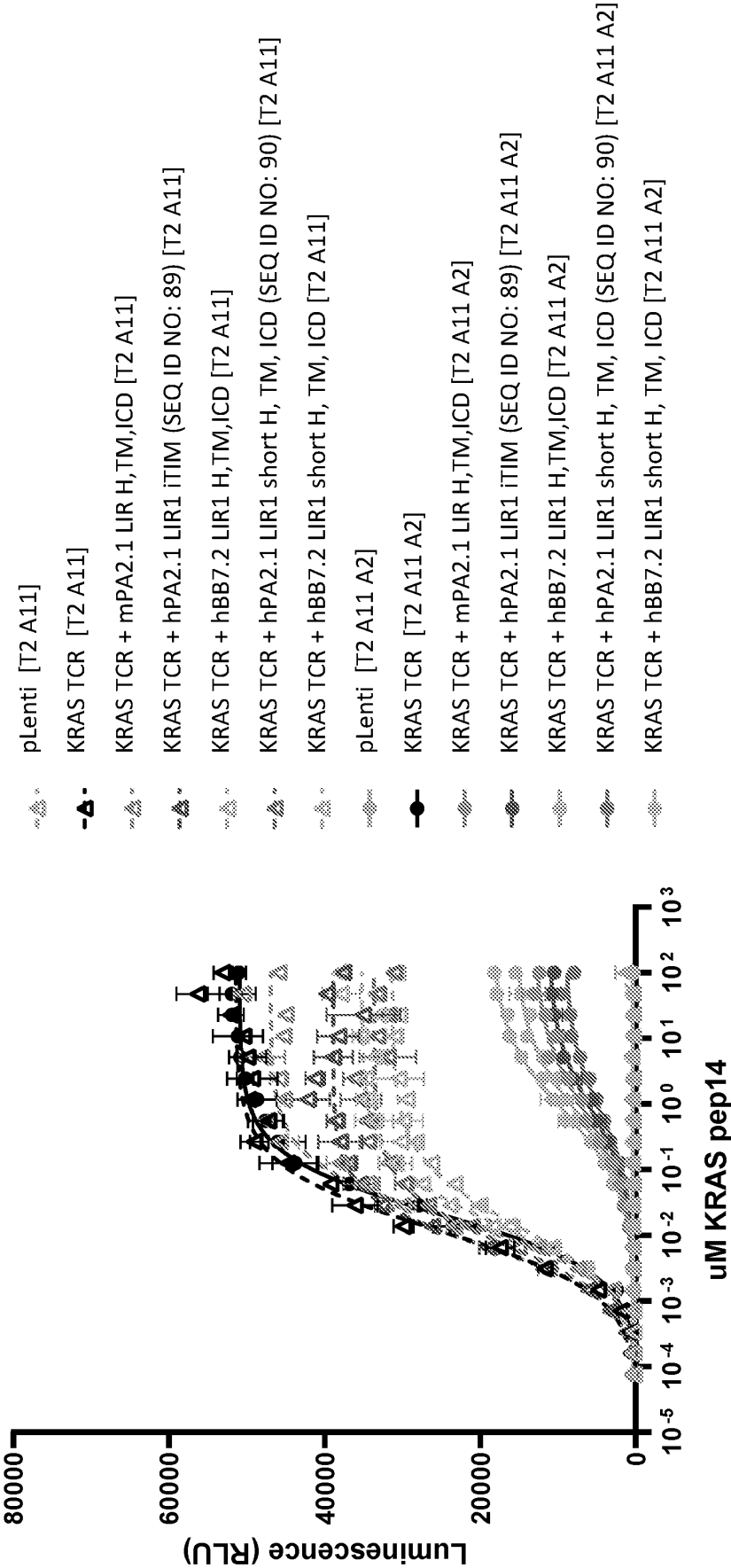
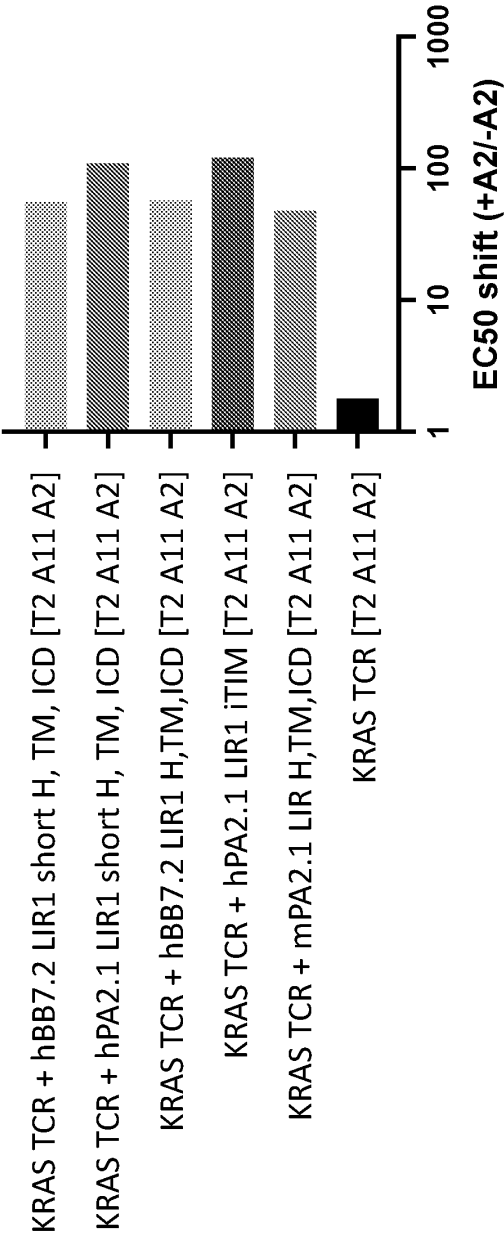


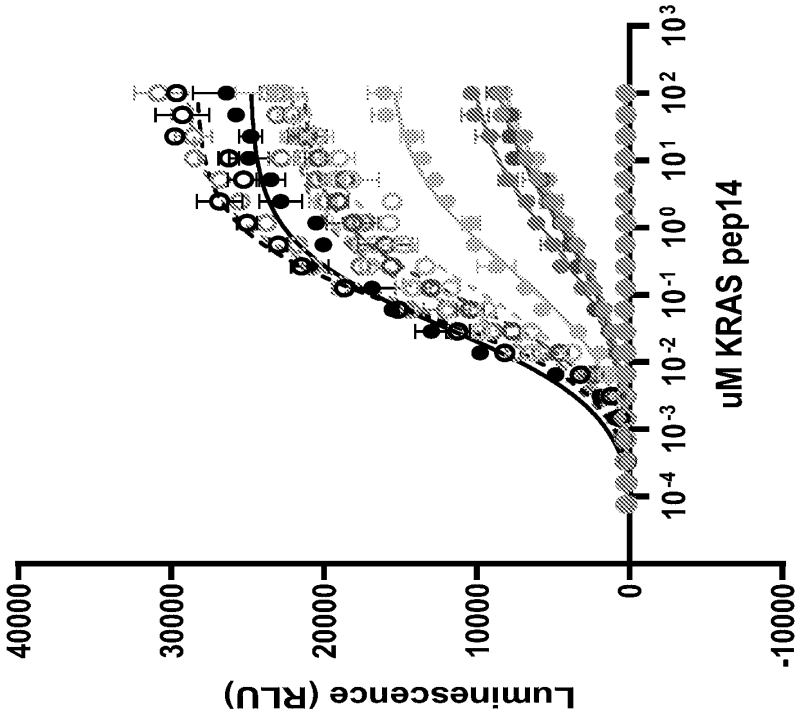
FIG. 20B



PA2.1 scFv LIR1 HTICD	Mouse PA2.1	YGSQSSKPYLLTHPSDPLEIVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGGLGRHLGV
BB7.2.6 scFv LIR1 HTICD	hPBB7.2.6	YGSQSSKPYLLTHPSDPLEIVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGGLGRHLGV
BB7.2.6 scFv LIR1 sHTICD	hPBB7.2.6	VVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGGLGRHLGV
PA2.1.14 scFv LIR1 iTIM	hPA2.1.14	YGSQSSKPYLLTHPSDPLEIVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGGLGRHLGV
PA2.1.14 scFv LIR1 sHTICD	hPA2.1.14	VVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGGLGRHLGV

FIG. 21A

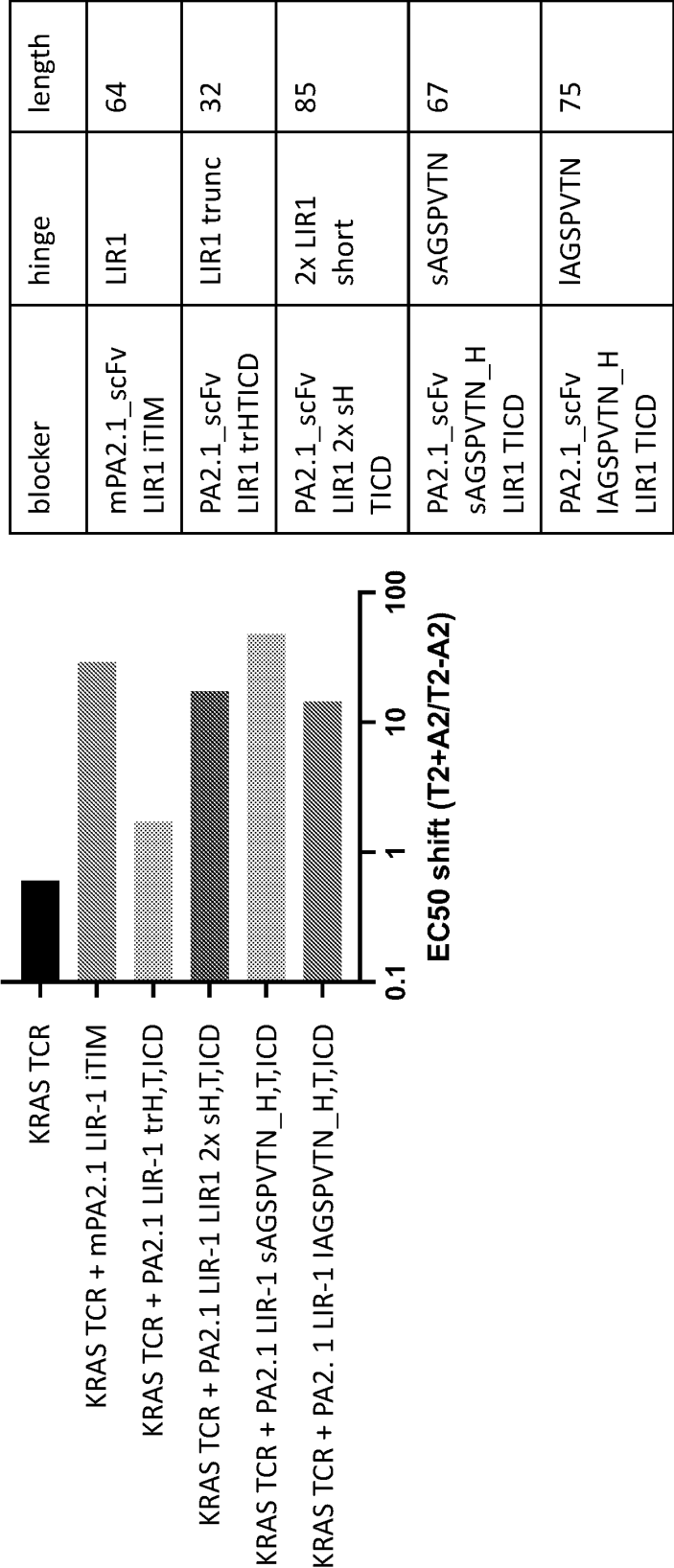
T2 A11



T2 A11 A2

- | | | | |
|---|---|---|---|
| ○ | pLenti | ○ | pLenti |
| ● | KRAS TCR | ● | KRAS TCR |
| ○ | KRAS TCR + mPA2.1 LIR-1 iTIM | ○ | KRAS TCR + mPA2.1 LIR-1 iTIM |
| ○ | KRAS TCR + PA2.1 LIR-1 trH,T,ICD | ○ | KRAS TCR + PA2.1 LIR-1 trH,T,ICD |
| ○ | KRAS TCR + PA2.1 LIR-1 LIR1 2x sH,T,ICD | ○ | KRAS TCR + PA2.1 LIR-1 LIR1 2x sH,T,ICD |
| ○ | KRAS TCR + PA2.1 LIR-1 sAGSPVTN_H,T,ICD | ○ | KRAS TCR + PA2.1 LIR-1 sAGSPVTN_H,T,ICD |
| ○ | KRAS TCR + PA2.1 LIR-1 IAGSPVTN_H,T,ICD | ○ | KRAS TCR + PA2.1 LIR-1 IAGSPVTN_H,T,ICD |

FIG. 21B



mPA2.1_scFv LIR1 iTIM	SSYGSSQSSKPYLLTHPSDPLELVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLRHLGVVIGIL
PA2.1_scFv LIR1 trHTICD	SSTTGPTSTSGPEDQPLTPTGSDPQSGLRHLGVVIGIL
PA2.1_scFv sAGSPVTN_H LIR1 TICD	SSAGSGGGGGGGSPVPSTPPTPSPSTPPTPSPSGGSGNSSGGSPVPSTPPTPSPSTPPTPSPSASVVIGIL
PA2.1_scFv IAGSPVTN_H LIR1 TICD	SSAGSGGGGGGGSPVPSTPPTNSSSTPPTPSPSPVPSTPPTNSSSTPPTPSPSPVPSTPPTNSSSTPPTPSPSASVVIGIL
PA2.1_scFv LIR1 2x sH TICD	SSVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLRHVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGR

FIG. 22A

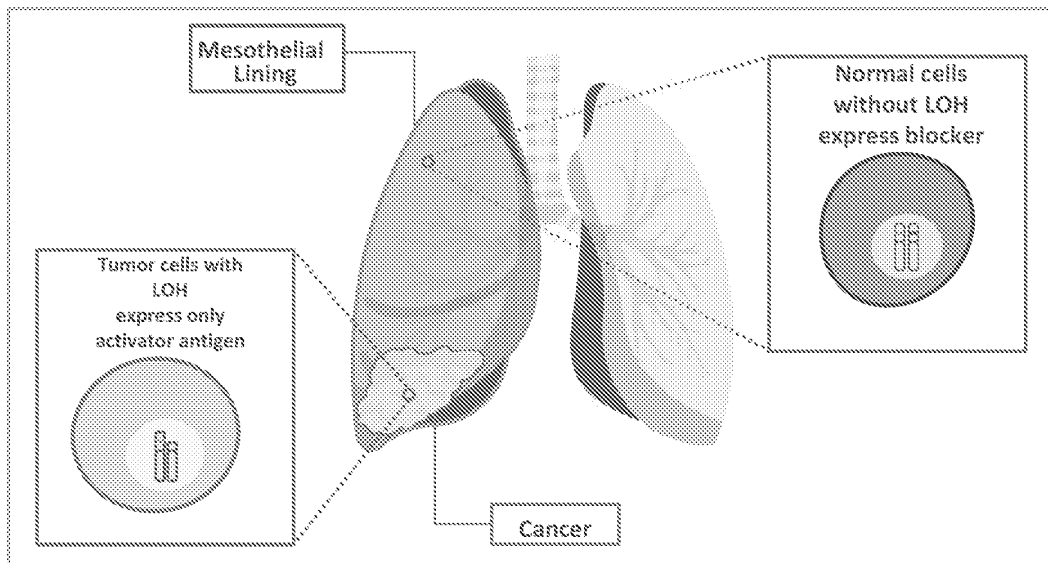


FIG. 22B

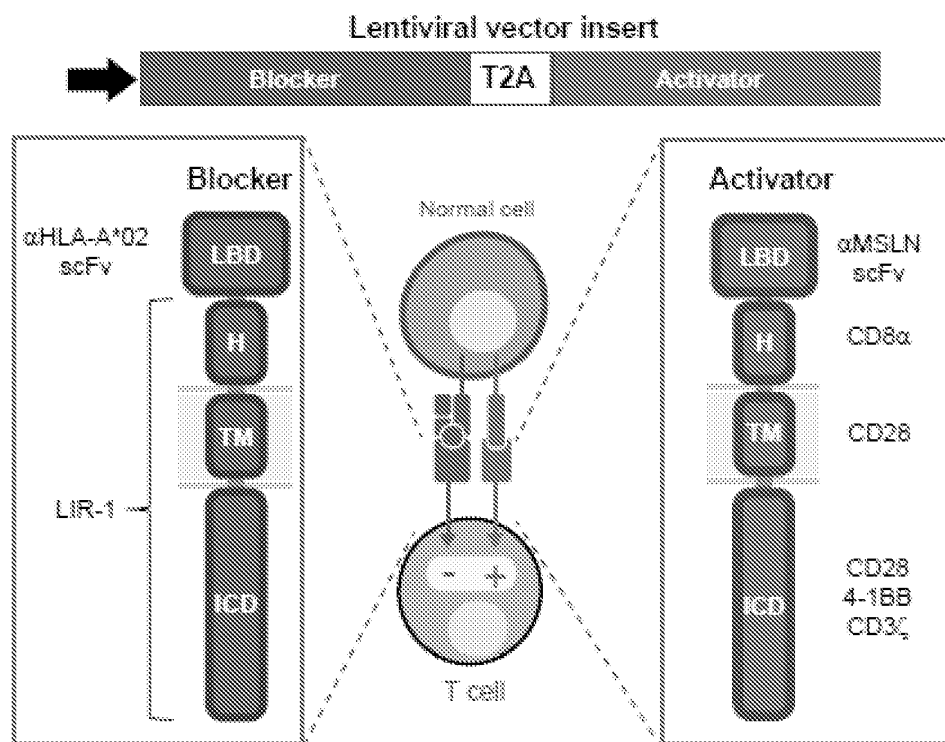


FIG. 23A

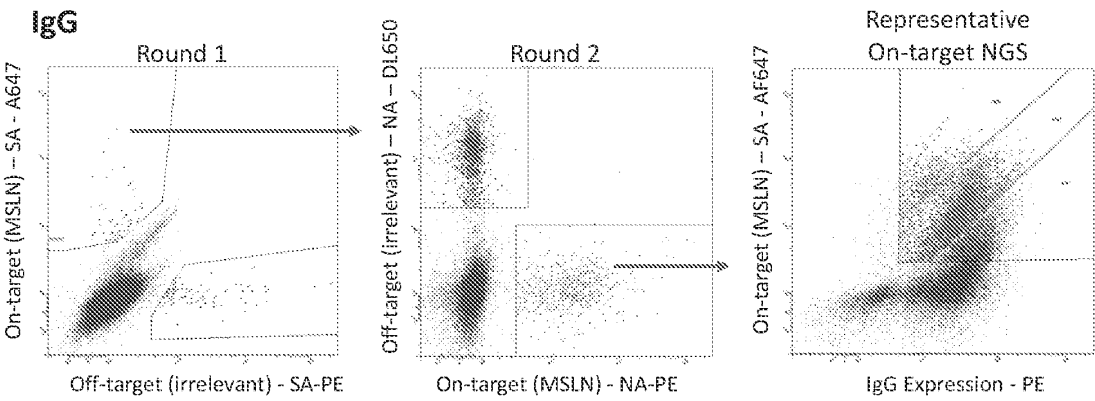


FIG. 23B

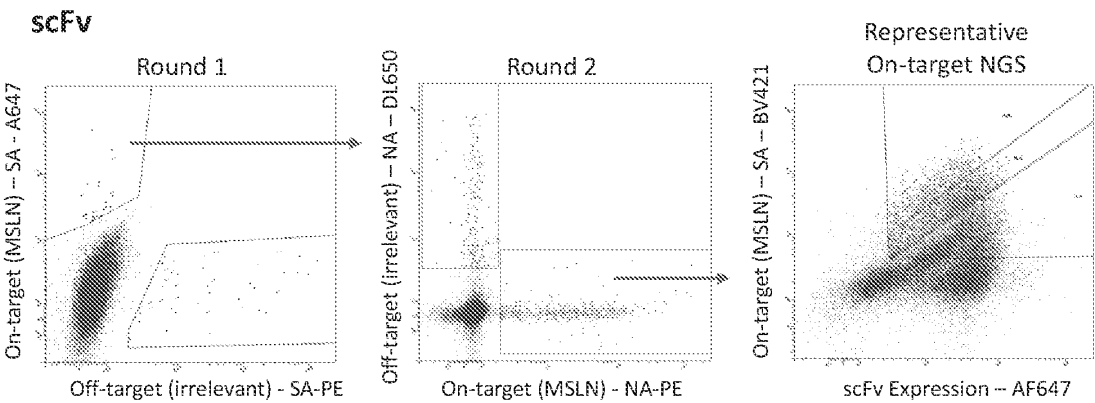


FIG. 23C

Surface expression of novel CAR binders and benchmarks in Jurkat cells

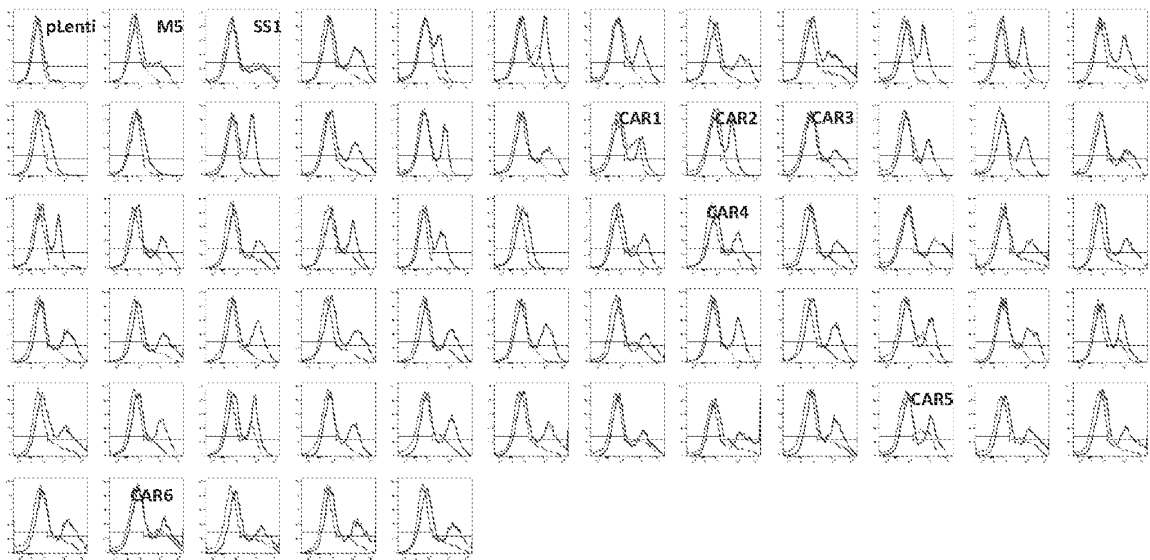
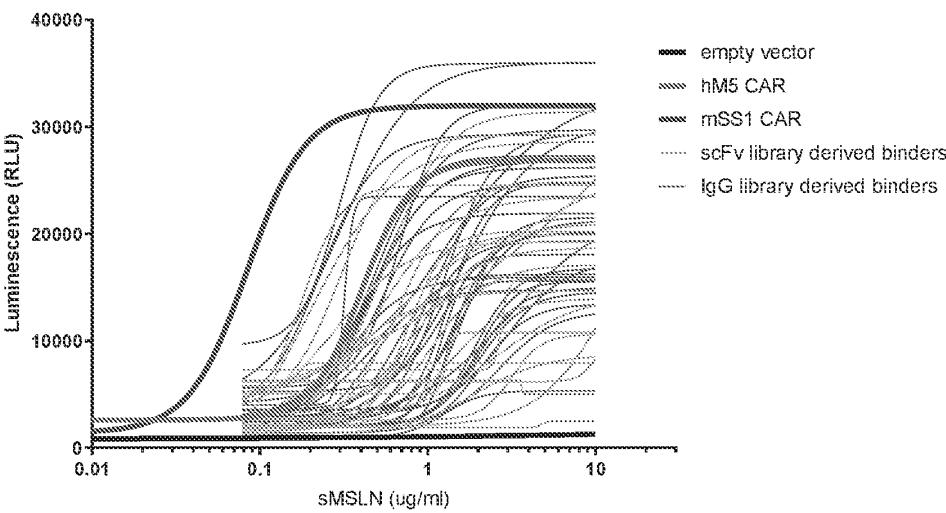
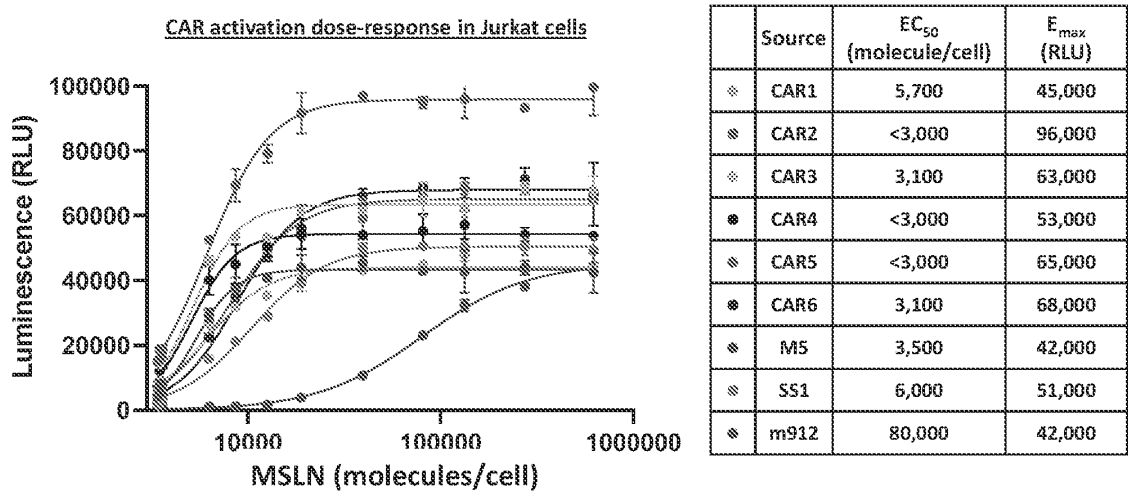


FIG. 23D

Solid-state dose-response in Jurkat cells



FIGs. 24A



FIGs. 24B

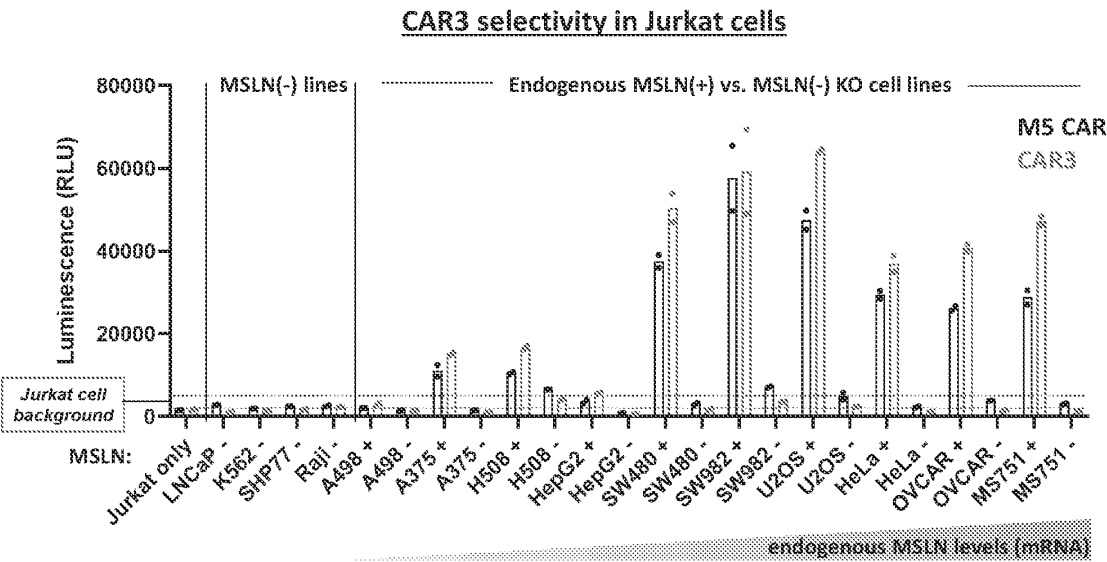


FIG. 25A

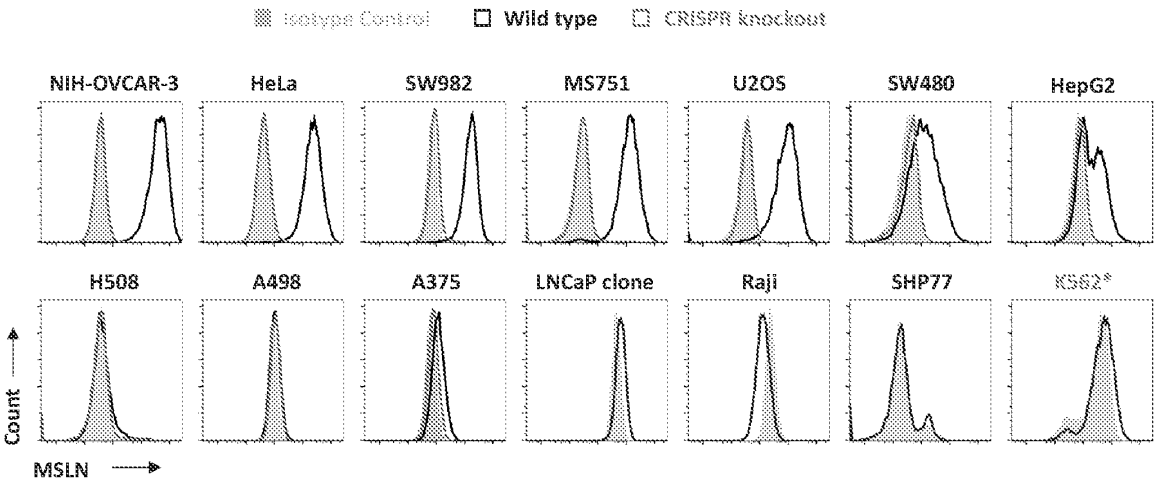


FIG. 25B

mRNA vs. protein correlation

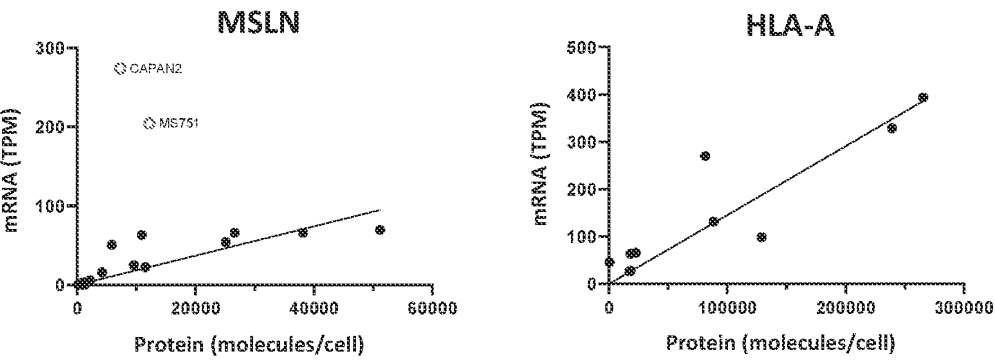


FIG. 26A

	mRNA			Protein		
Tumor cell line	MSLN (TPM)	HLA-A (TPM)	A*02:MSLN ratio	MSLN (#mol/cell)	A*02 (#mol/cell)	A*02:MSLN ratio
MS751 (cervix; A*02:01, A*24:02)	205	156	0.4	12,000	28,000	2.3
MS751 (transduced with A*02)	205	438	2.1	12,000	301,000	25
HeLa (cervix; A*68:02, A*68:02; transduced with A*02)	66	85	na	27,000	740,000	27
NIH-OVCAR-3 (ovary; A*02:01, A*29:02)	70	81	0.6	51,000	25,000	0.5
U2OS (bone; A*02:01, A*32:01)	25	108	2.2	9,600	18,000	1.9
SW982 (synovium; A*02:01, A*02:01)	22	533	24	12,000	240,000	20
Tumor	MSLN (TPM)	A*02 (TPM)	A*02:MSLN ratio			
Lung adenocarcinoma	371	LOH	na			
Mesothelioma	784	LOH	na			
Ovarian	511	LOH	na			
Normal tissue (GTex)	MSLN (TPM)	HLA-A (TPM)	A*02:MSLN ratio			
Lung	88	1934	22			

FIG. 26B

MSLN expression (molecules/cell) in cell lines used in study

	Cell line	Avg	Stan dev
1	NIH-OVCAR-3	51,000	12,000
2	HeLa	27,000	1,900
3	SW982	12,000	730
4	MS751	12,000	1,700
5	U2OS	9,600	2,800
6	SW480	2,200	1,200
7	HEPG2	1,900	120
8	H508	1,000	400
9	LnCAP cloneFGC	930	220
10	A498	820	39
11	A375	630	170
12	Raji	180	5
13	SHP77	180	-
14	K562	n/a	n/a

FIG. 27A

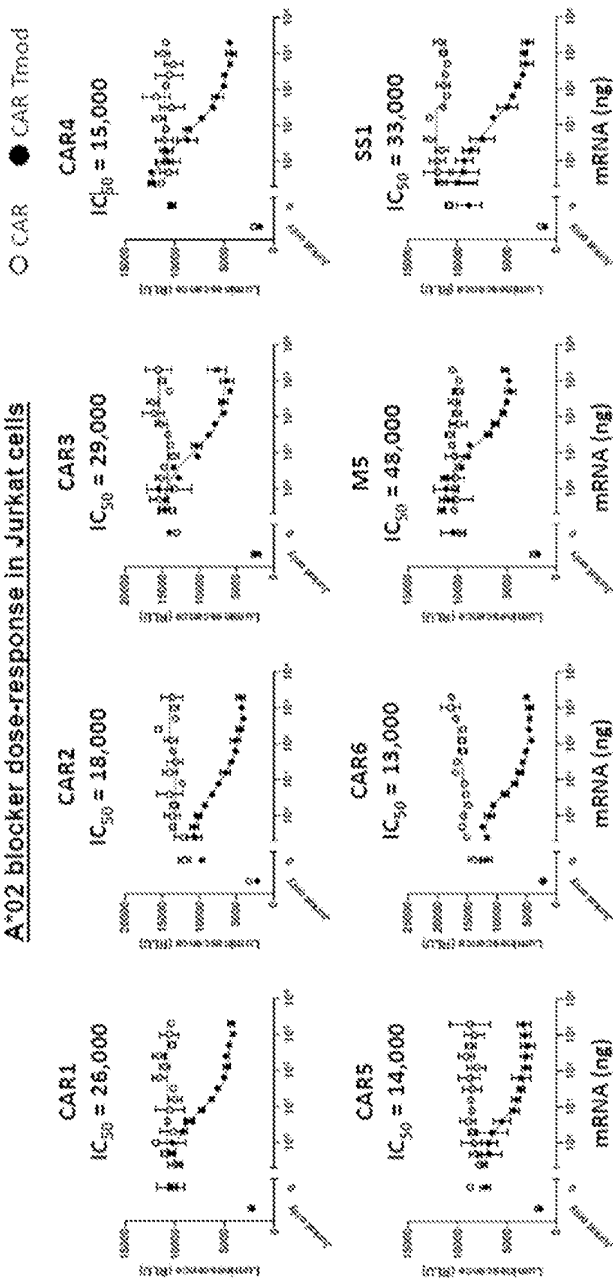


FIG. 27B

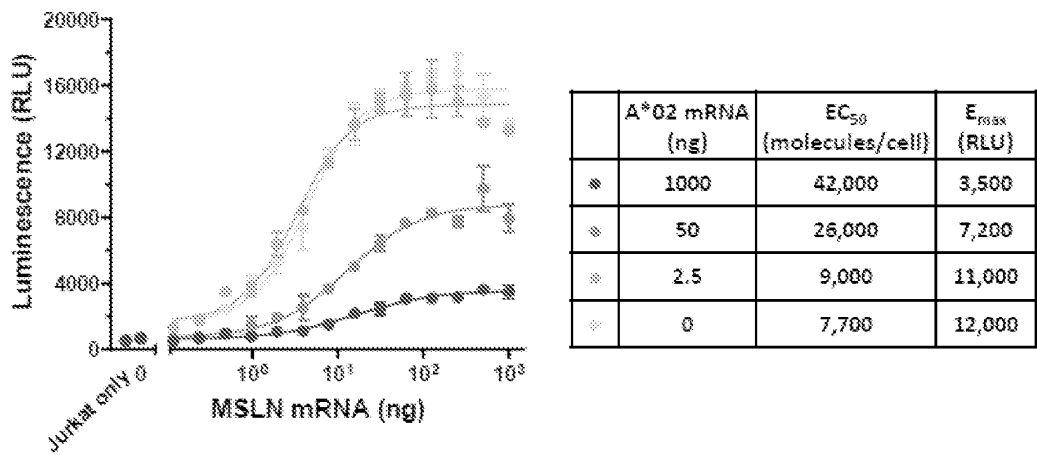


FIG. 27C

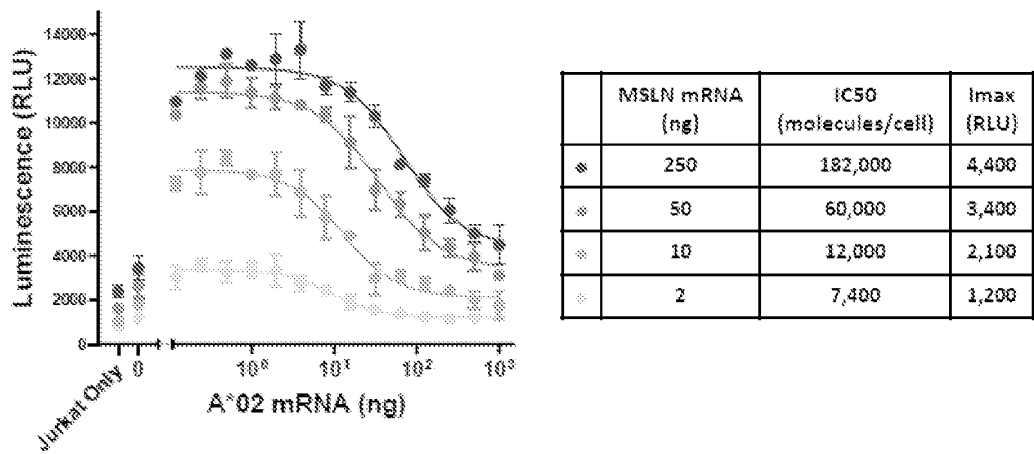


FIG. 28A

MSLN Tmod cell sensitivity mapped on MSLN- vs. A*02-antigen expression

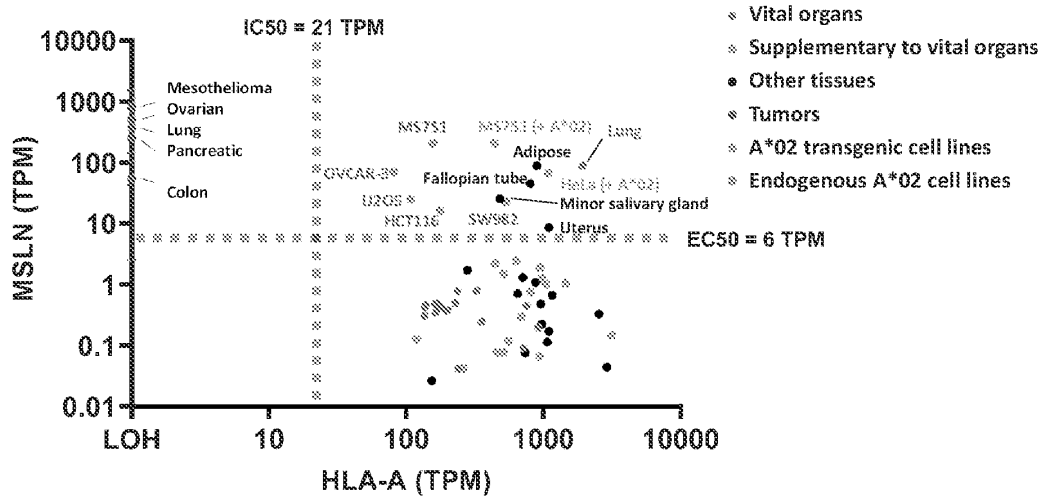
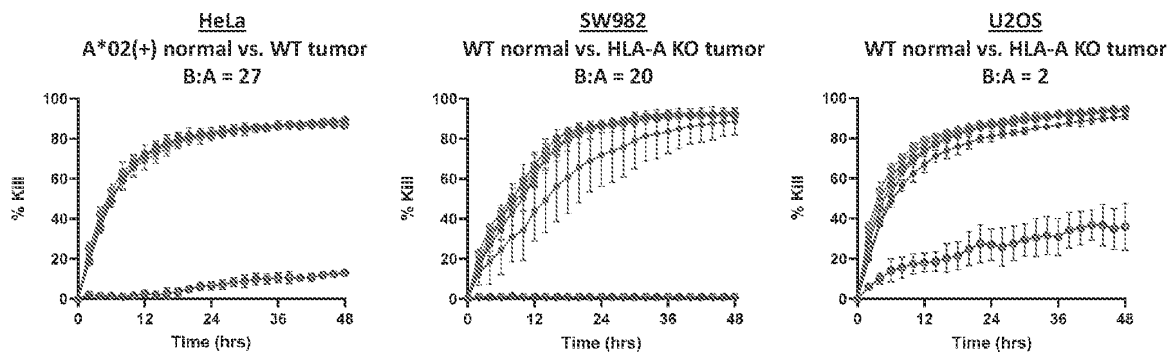


FIG. 28B



MS CAR; CAR3; CAR3 Tmod; Tumor target (solid); Normal target (open)

FIG. 29A

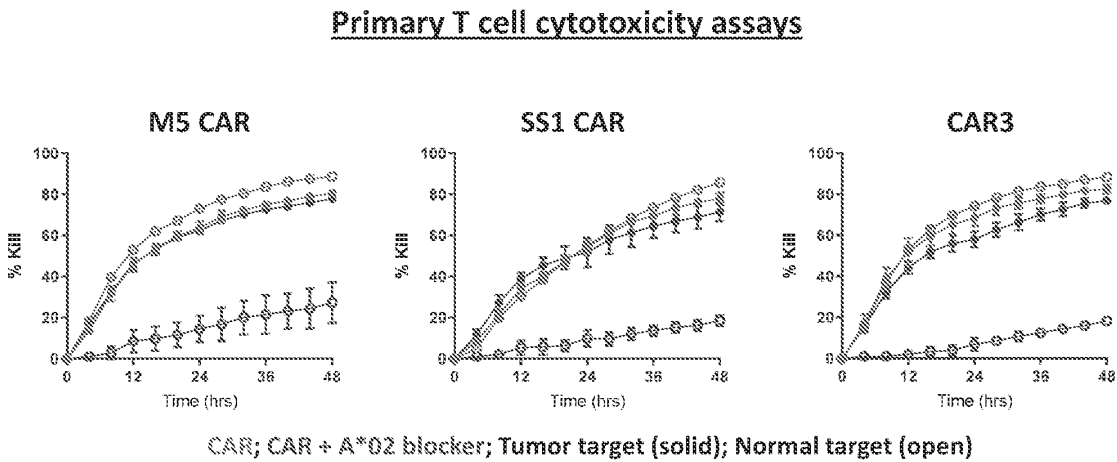


FIG. 29B

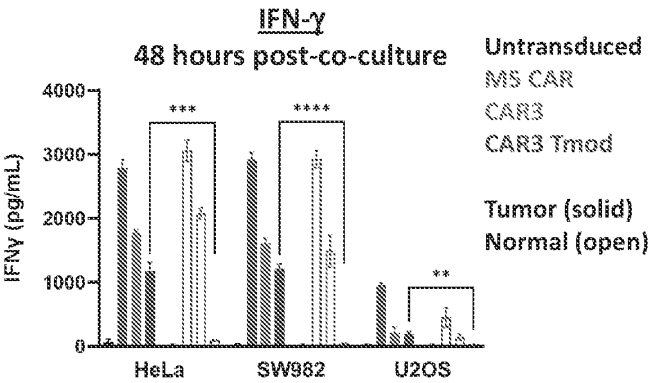


FIG. 30A

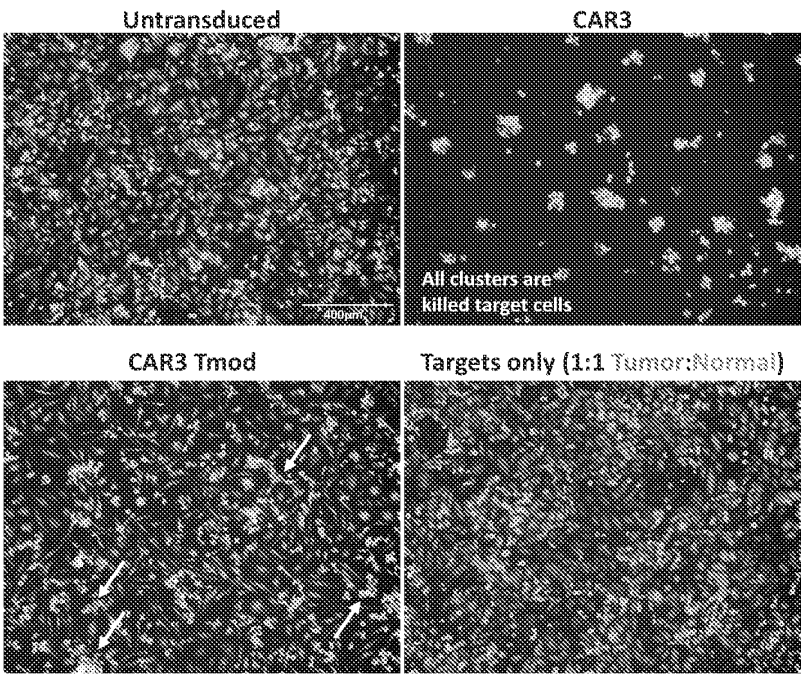


FIG. 30B

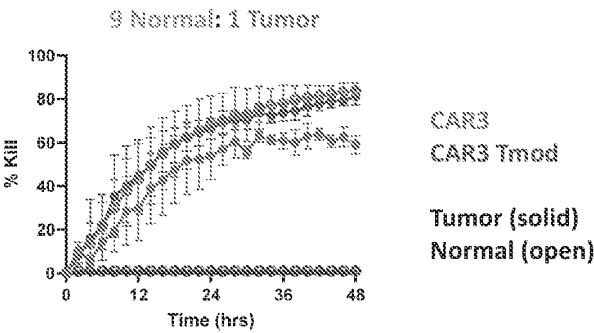


FIG. 31A

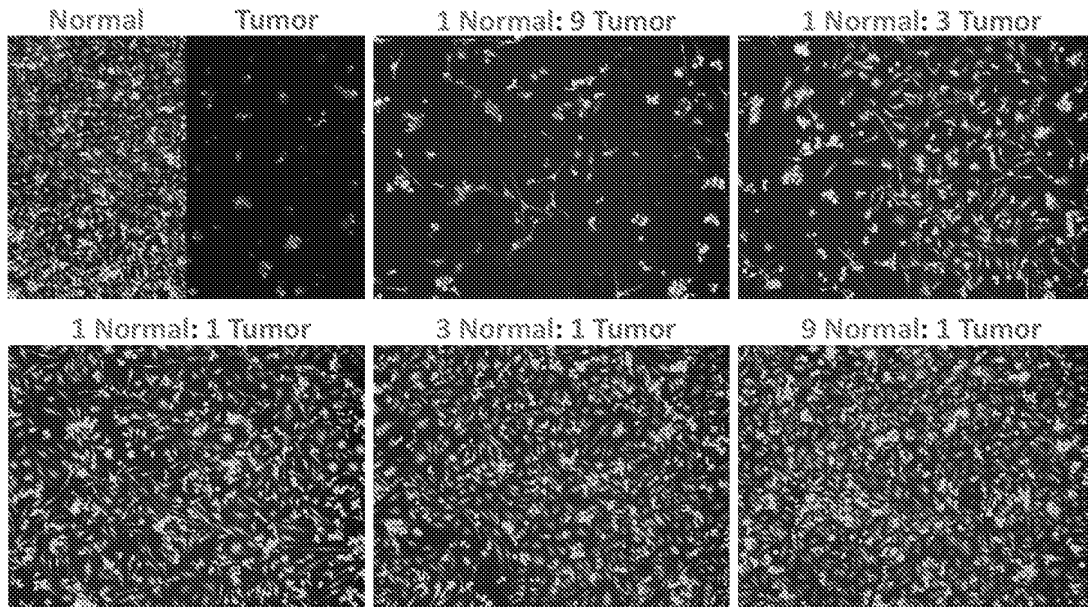


FIG. 31B

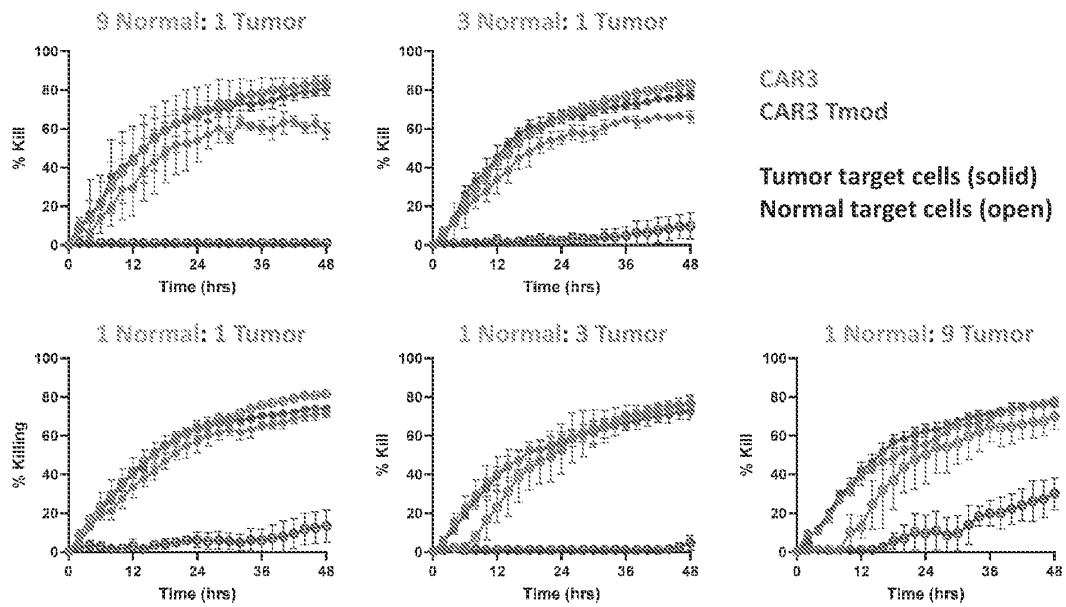


FIG. 32A

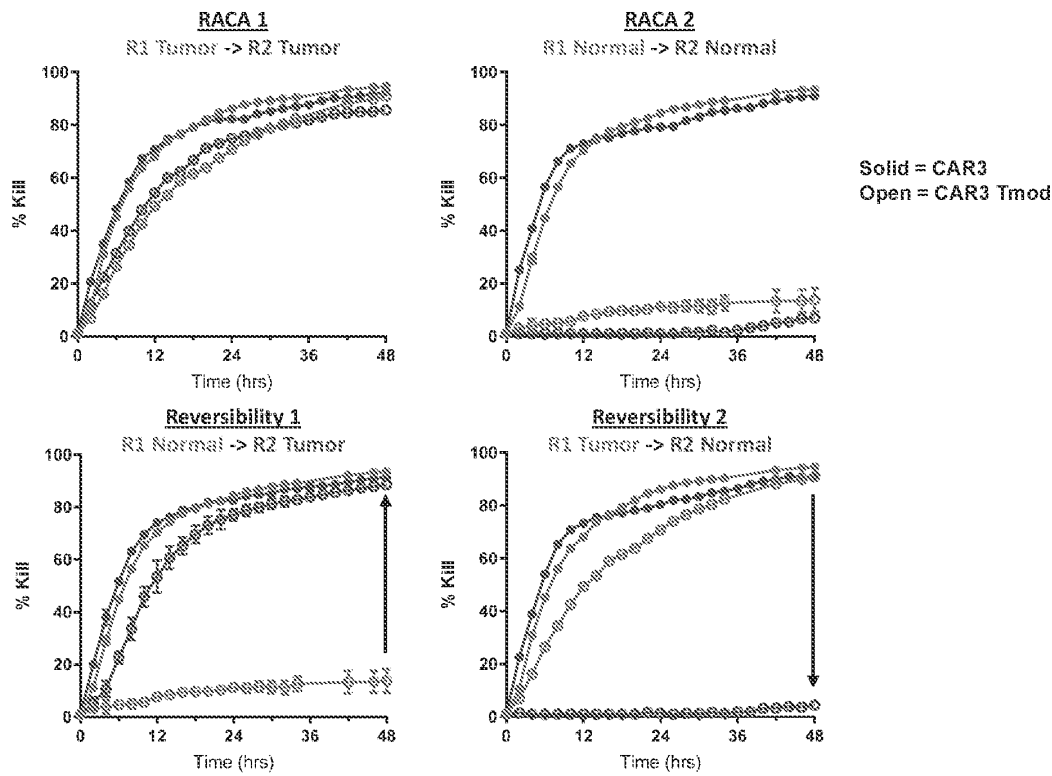


FIG. 32B

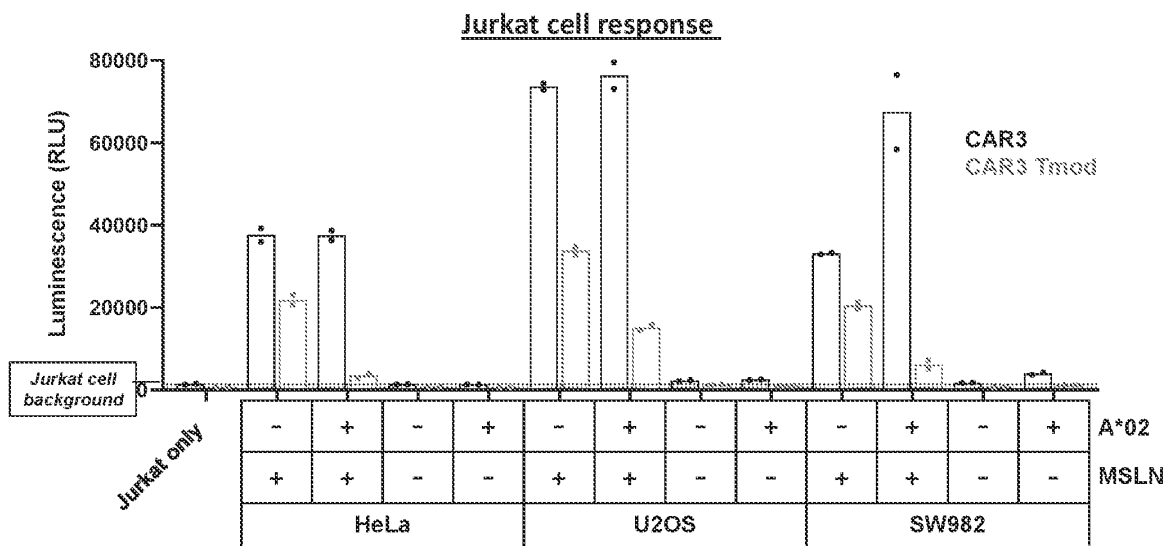


FIG. 33A

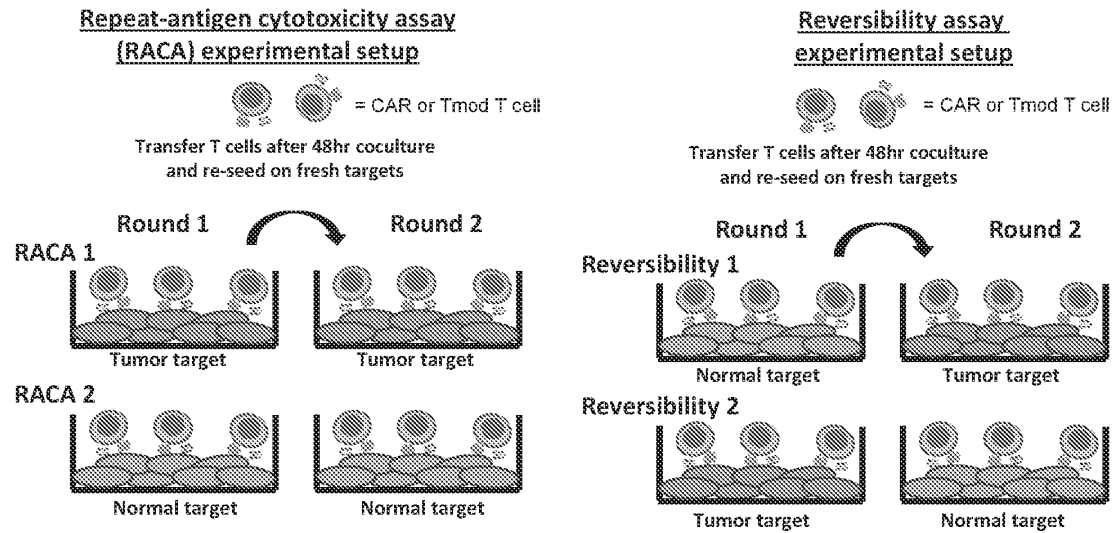


FIG. 33B

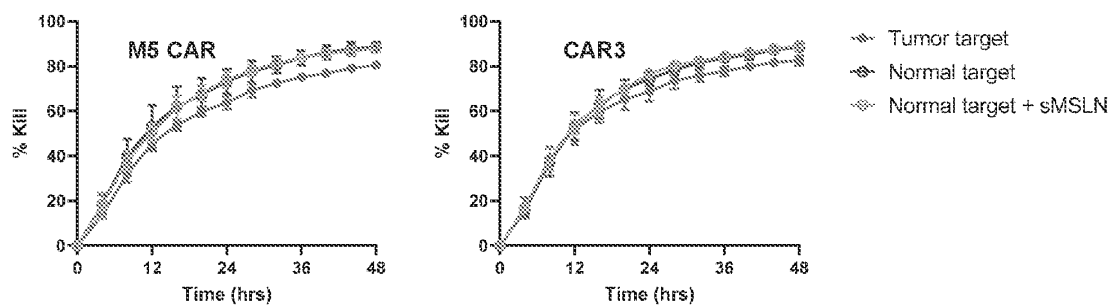
Cytotoxicity assays +/- sMSLN

FIG. 33C

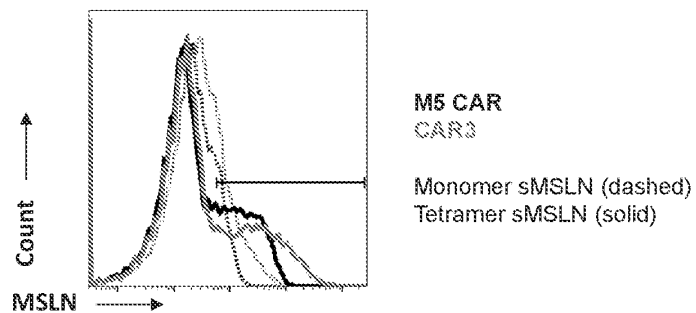
sMSLN-stained CAR(+) Jurkat cells

FIG. 34A

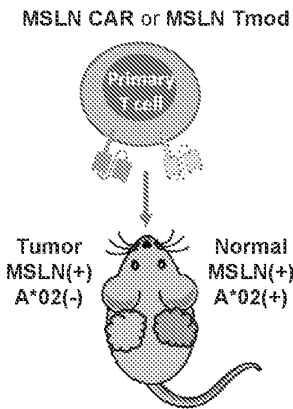


FIG. 34B

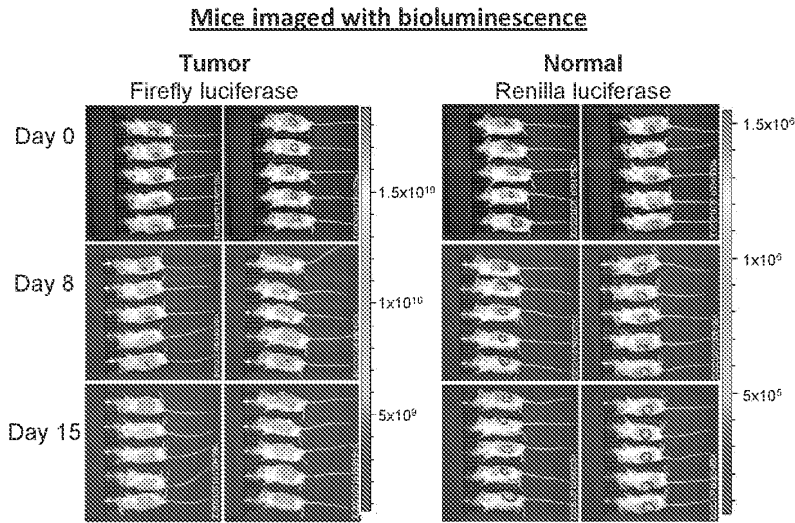


FIG. 34C

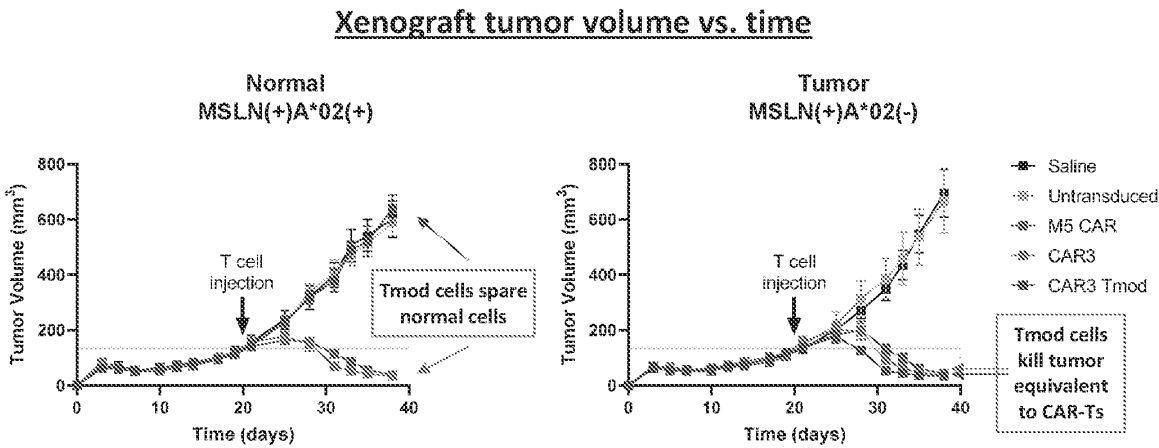


FIG. 35A

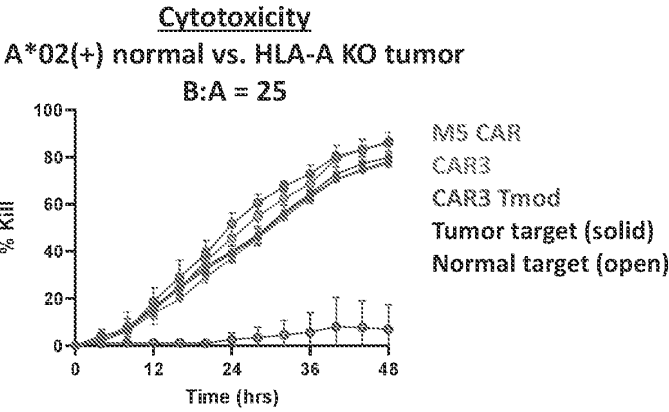


FIG. 35B

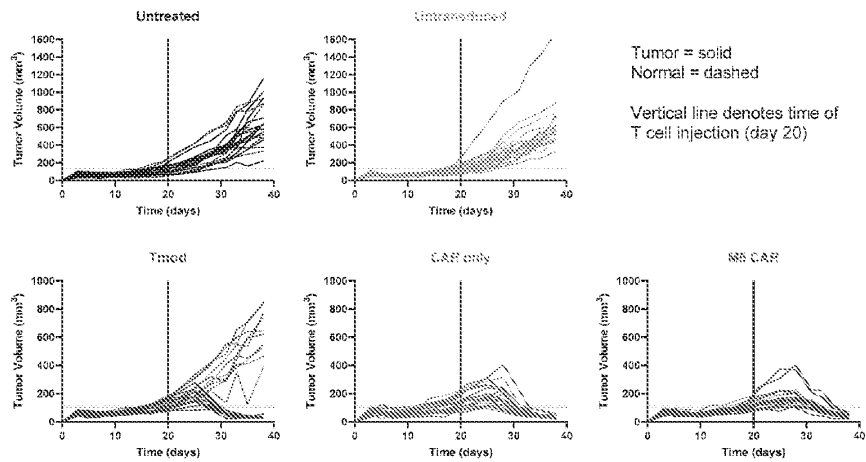


FIG. 35C

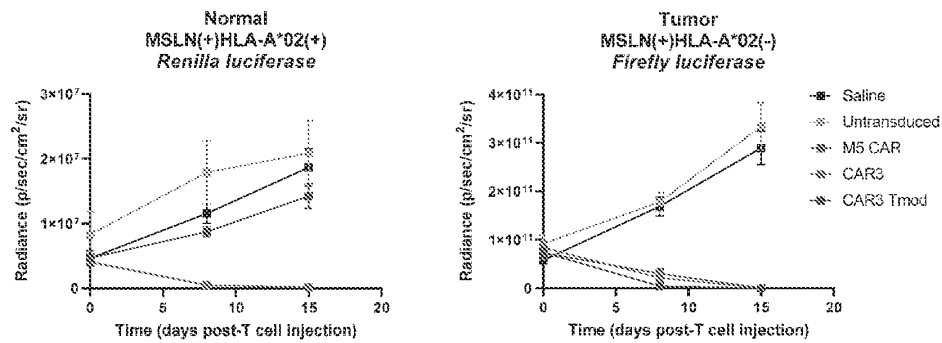


FIG. 36

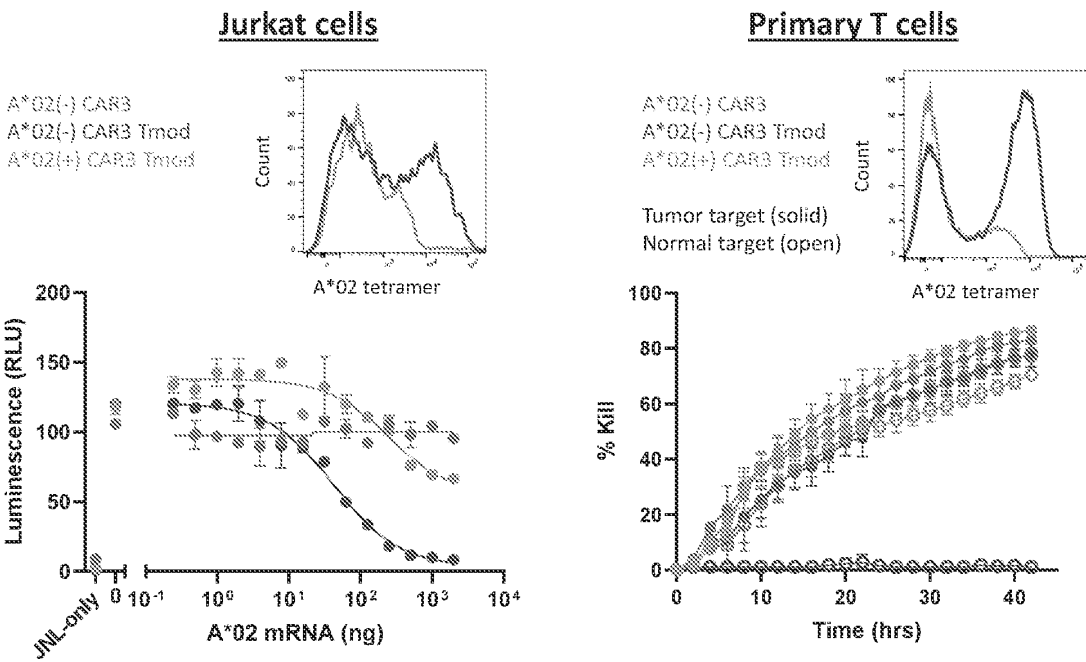


FIG. 37A

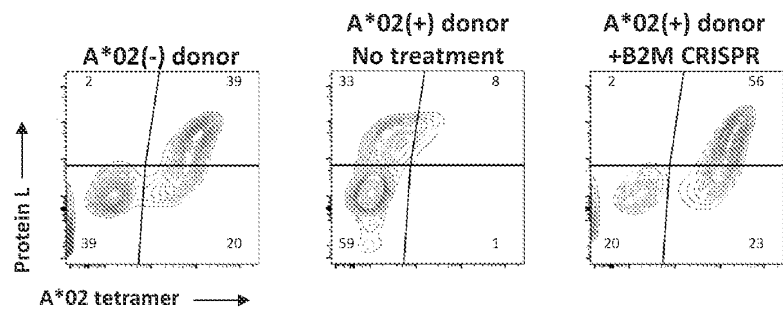


FIG. 37B

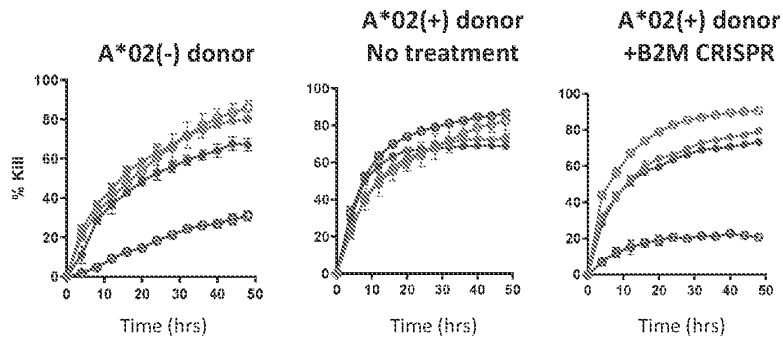


FIG. 37C

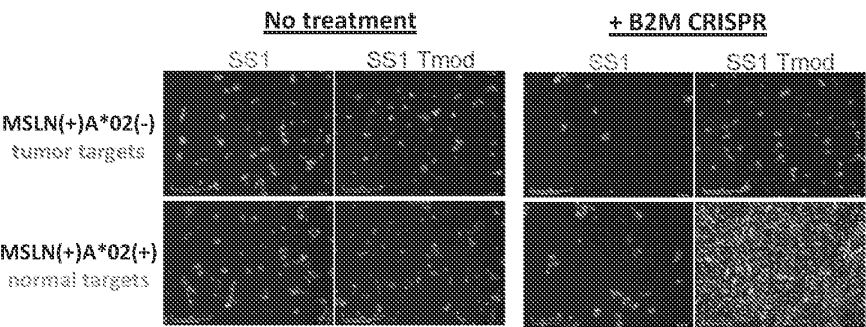


FIG. 38A

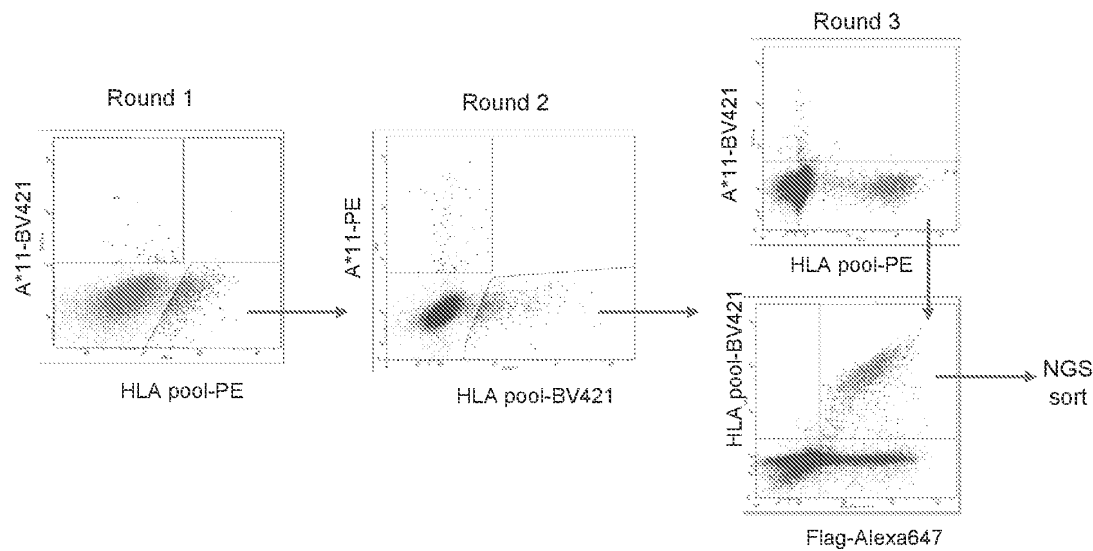


FIG. 38B

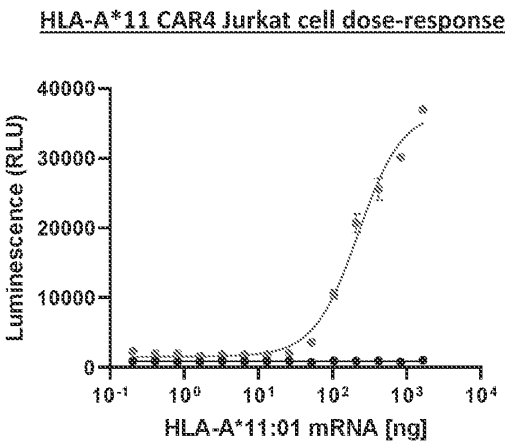


FIG. 39A

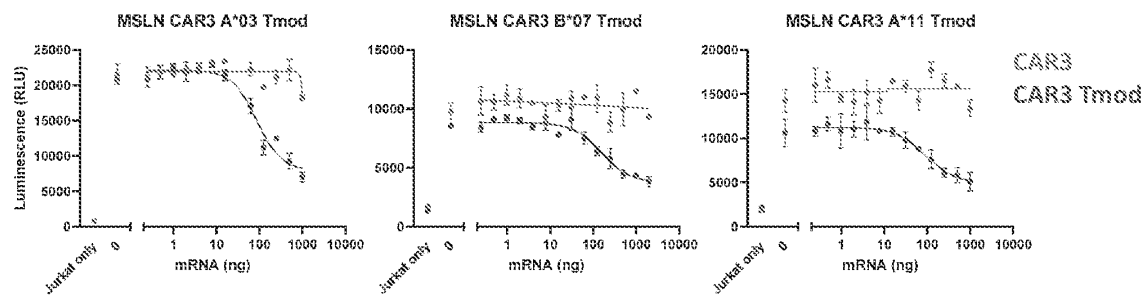


FIG. 39B

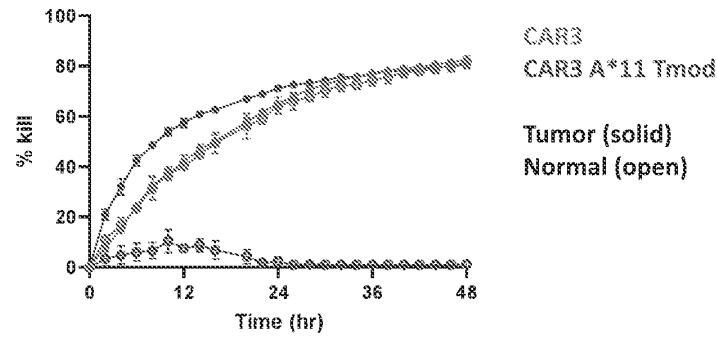


FIG. 39C

