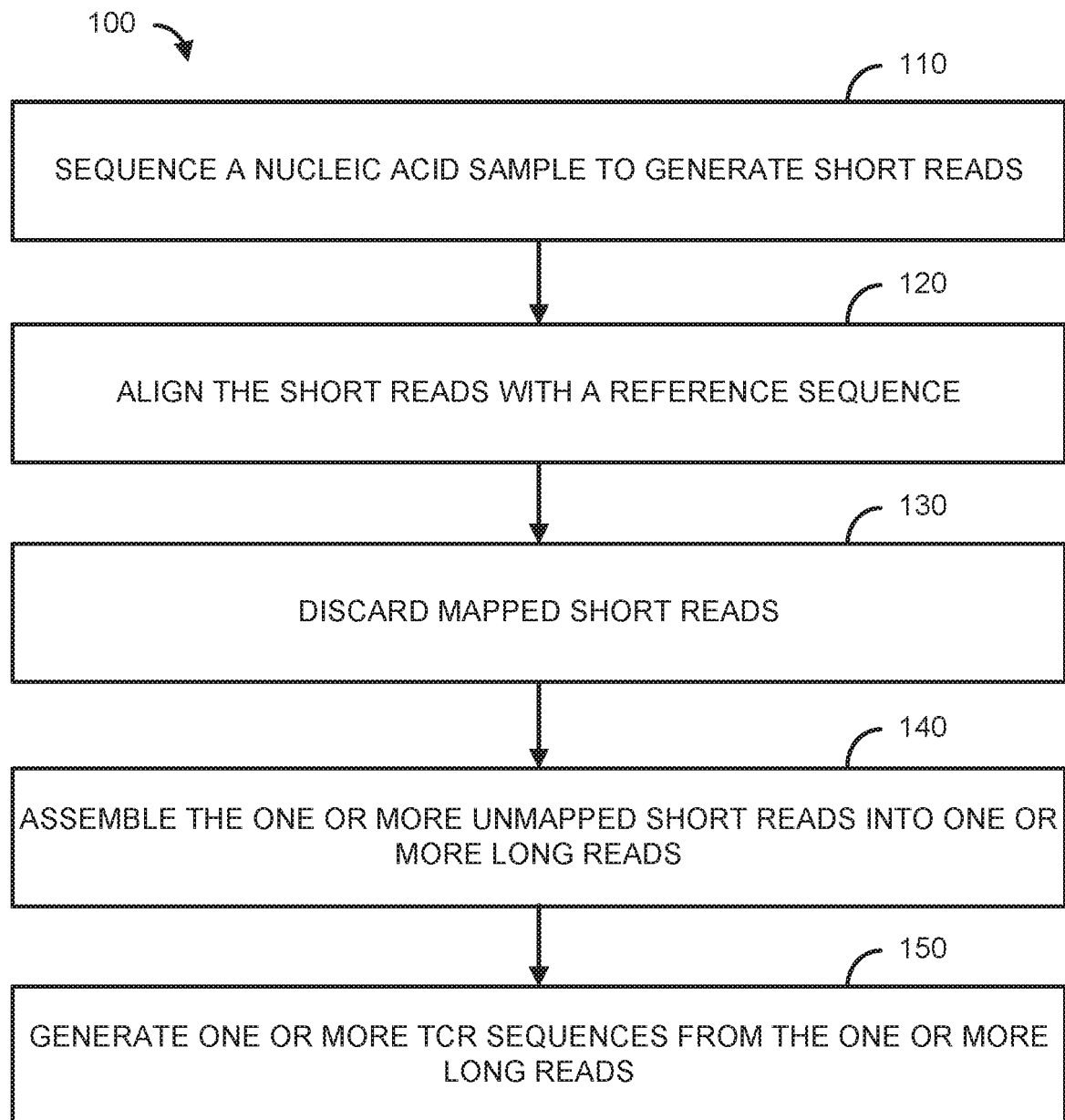


FIG. 1



CDR3	Tumor						Tumor bearing spleen					
	aGTR+aPD1		aPD1		aGTR		aGTR+aPD1		aGTR		aPD1	
	11 day	8 day	11 day	8 day	11 day	8 day	11 day	8 day	11 day	8 day	11 day	8 day
TCR Sequence 1	7	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 2	7	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 3	6	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 4	5	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 5	5	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 6	3	0	1	0	2	0	0	1	0	0	0	0
TCR Sequence 7	3	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 8	3	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 9	3	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 10	3	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 11	0	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 12	0	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 13	0	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 14	0	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 15	0	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 16	1	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 17	0	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 18	0	0	0	0	10	0	0	0	0	0	0	0
TCR Sequence 19	0	0	0	0	3	0	0	0	0	0	0	0
TCR Sequence 20	0	0	0	0	3	0	0	0	0	0	0	0
TCR Sequence 21	0	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 22	0	0	0	0	0	0	0	0	0	0	0	0
TCR Sequence 23	0	0	0	0	0	0	0	0	0	0	0	0
% of cells with the same clones (n>=3)	31.9% (45/141)	0% (0/81)	26.7% (32/120)	4.8% (3/63)	20.3% (16/79)	0% (0/127)	5.7% (6/105)	6.4% (3/47)	0% (0/72)	0% (0/86)	0% (0/42)	0% (0/31)

FIG. 2

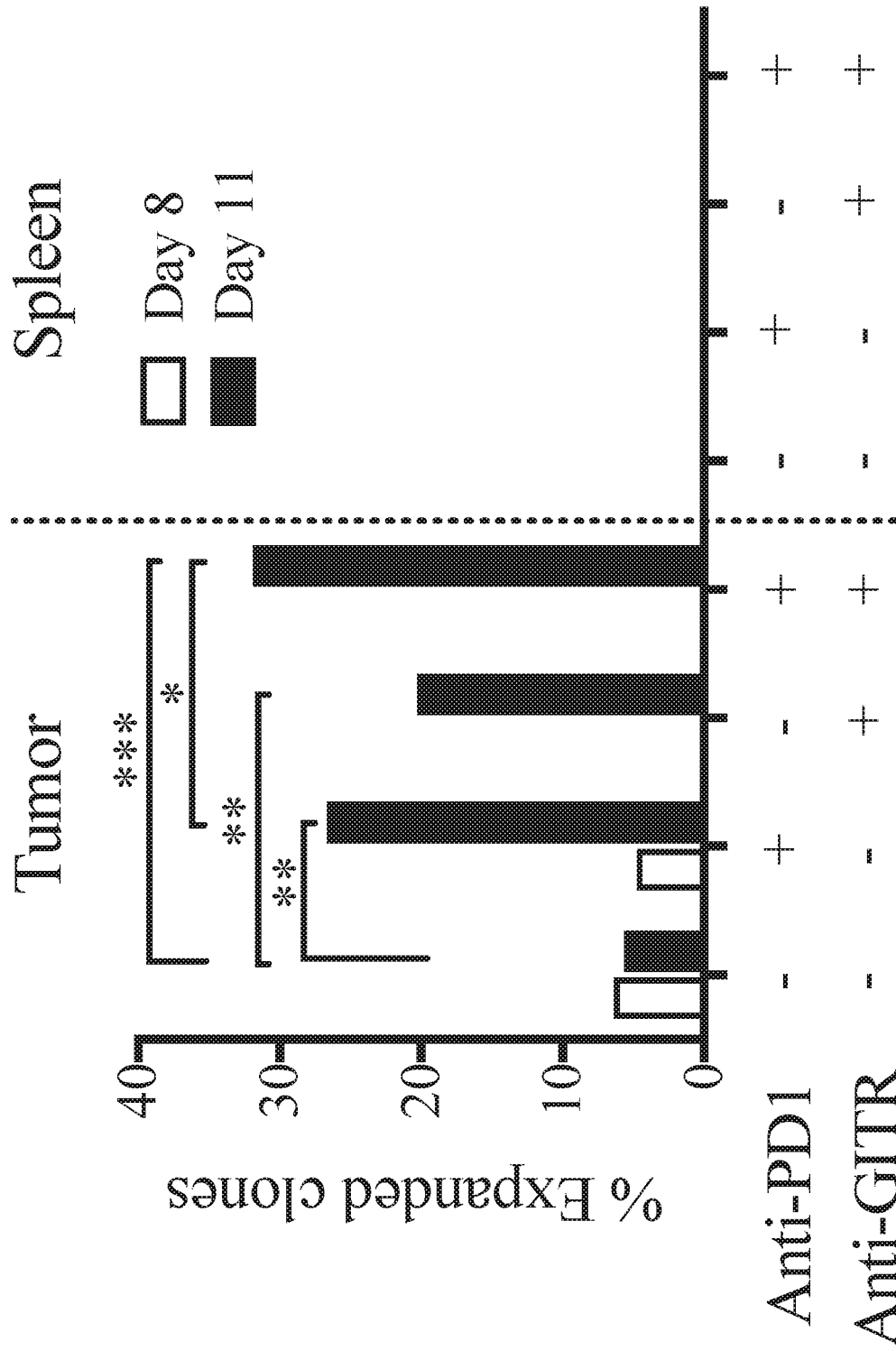


FIG. 3

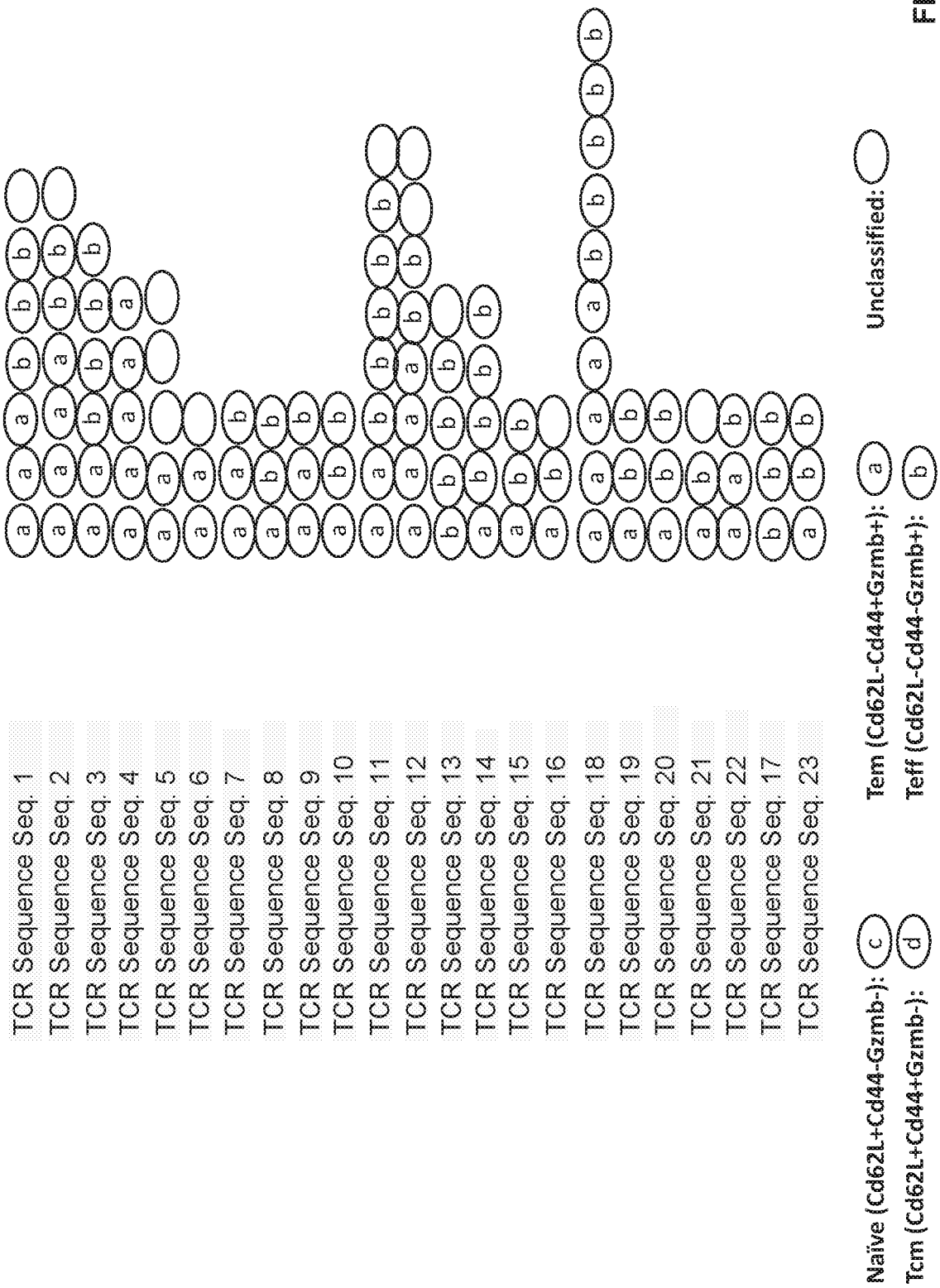


FIG. 4

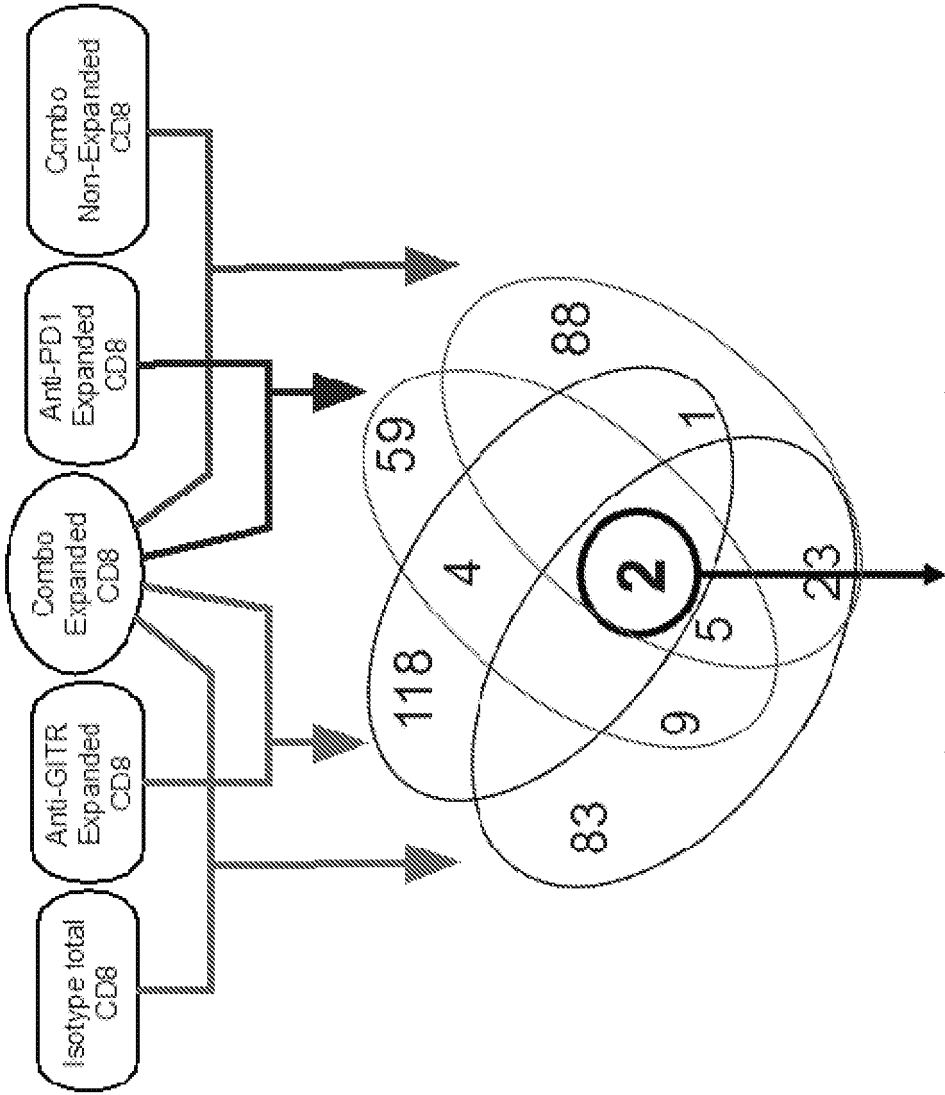
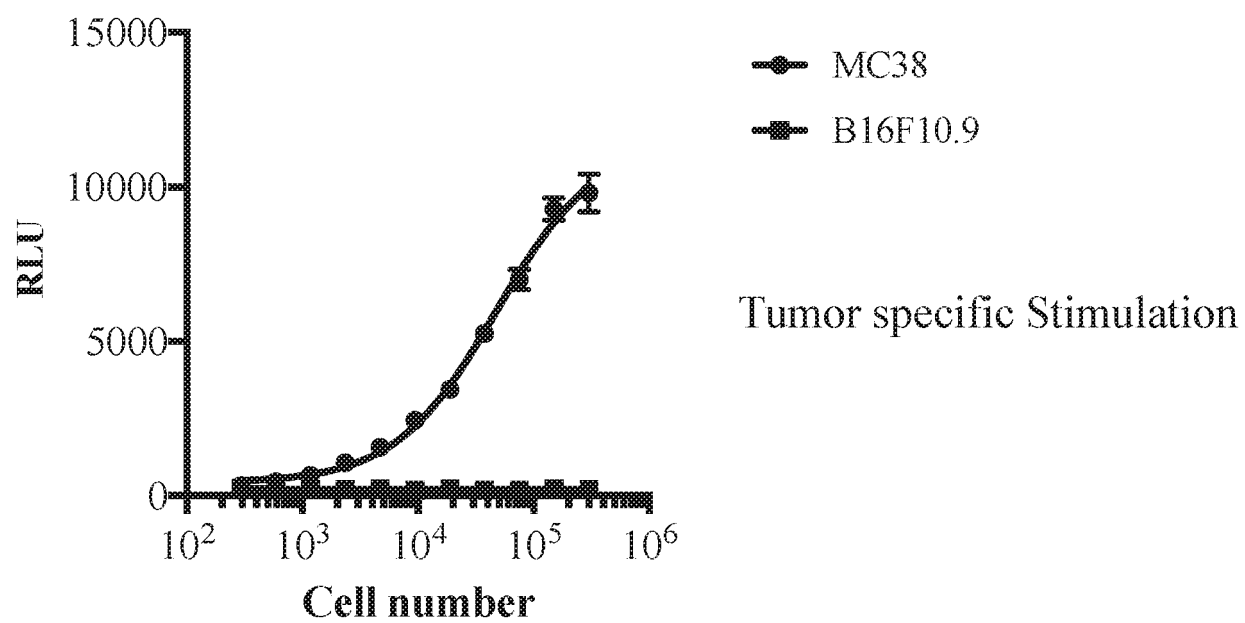


FIG. 5

	Combo expanded vs Isotype	Combo expanded vs Non-expanded	Combo expanded vs anti-GiTR expanded	Combo expanded vs anti-PD1 expanded
CD226	10.7	5.0	9.2	6.5
Pde4d	4.8	3.6	6.9	4.9

FIG. 6A



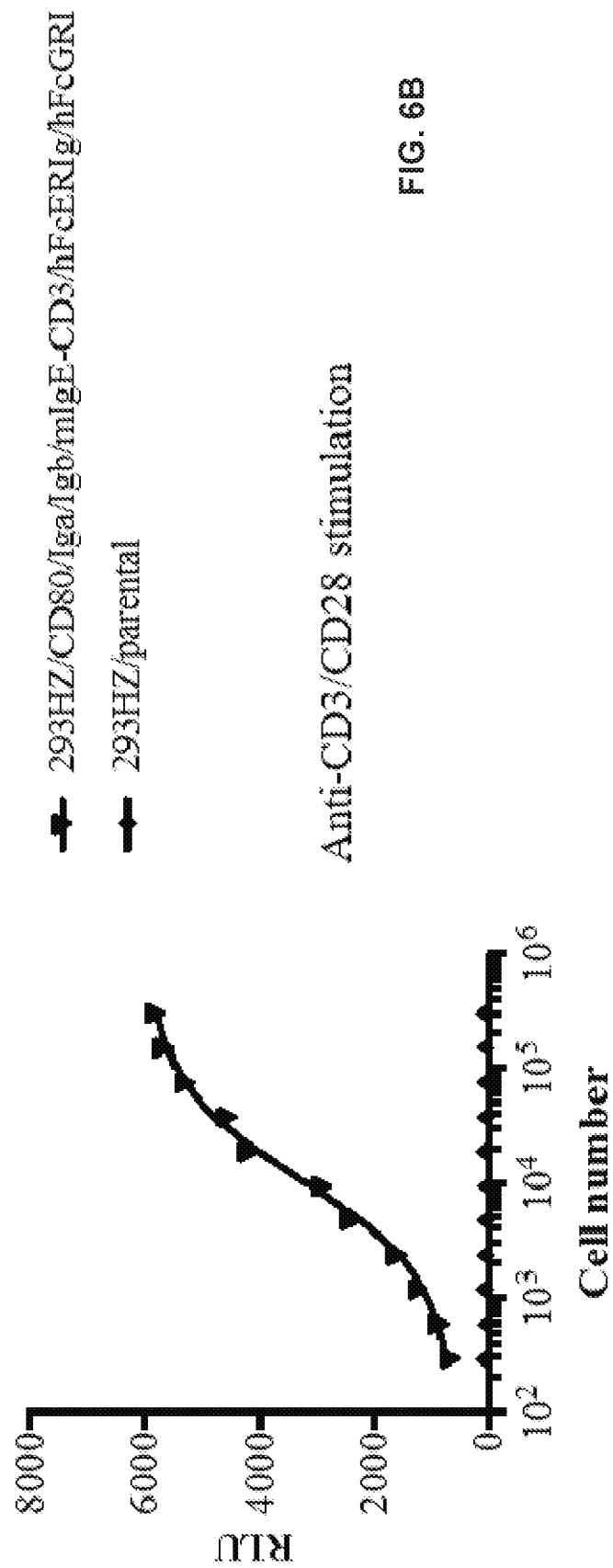
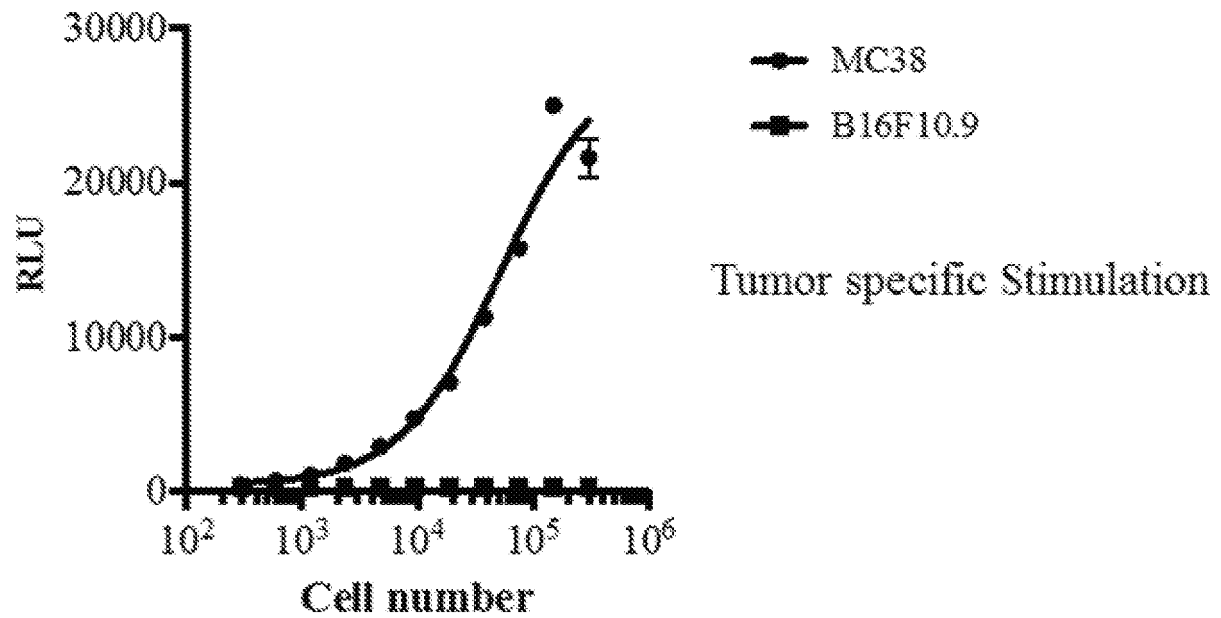


FIG. 6C



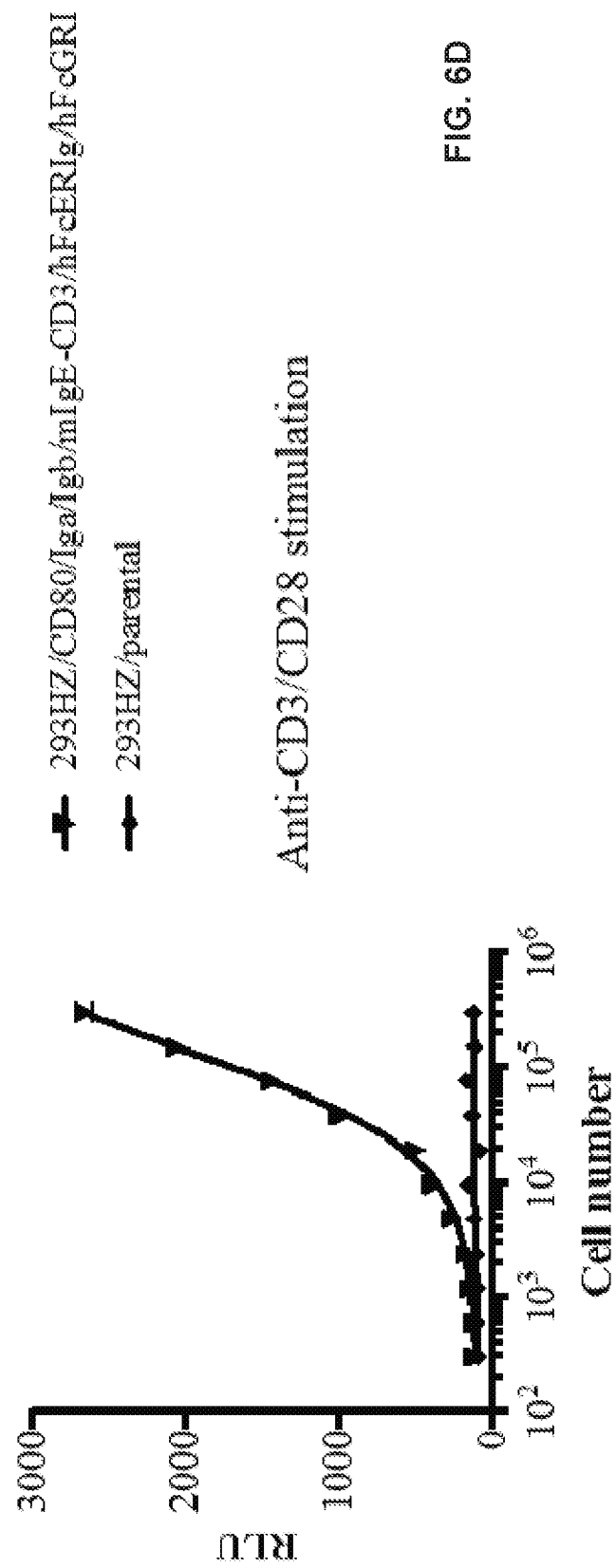
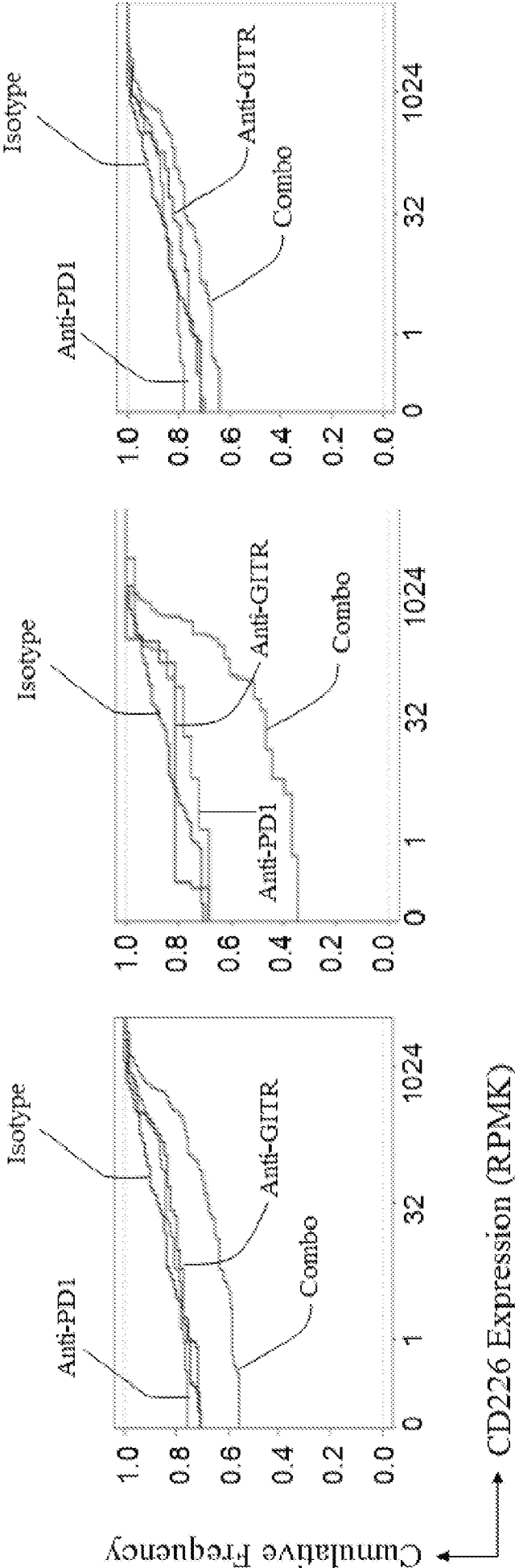


FIG. 6D

FIG. 7A



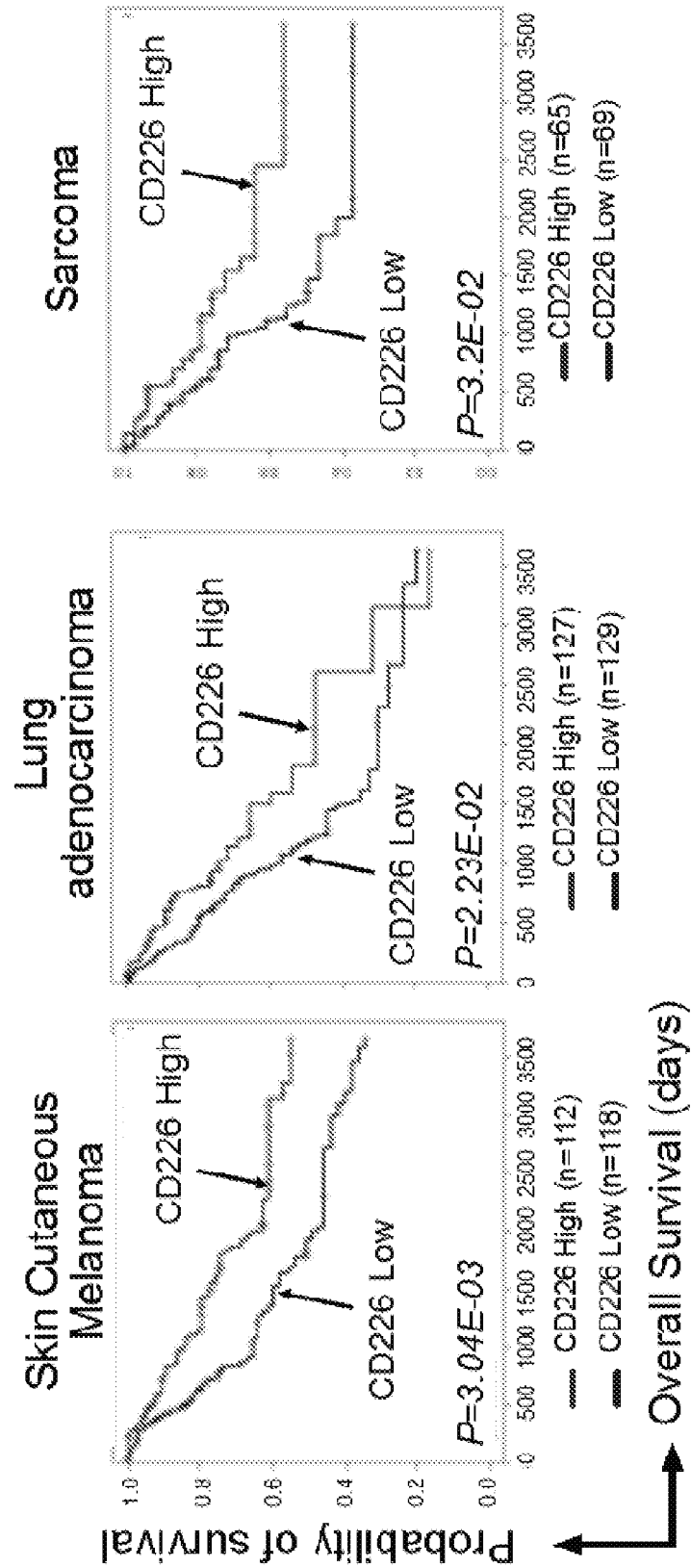


FIG. 7B

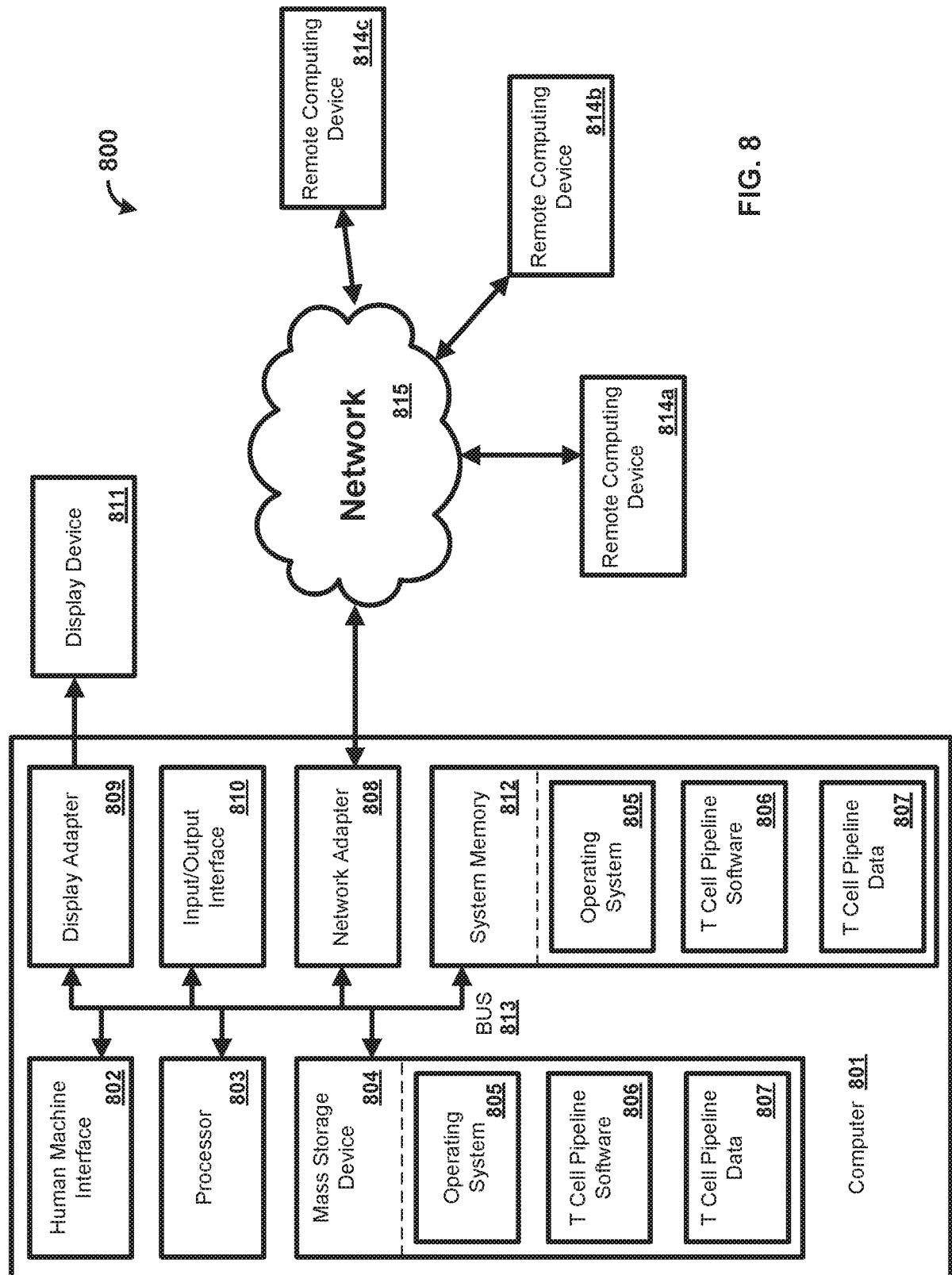
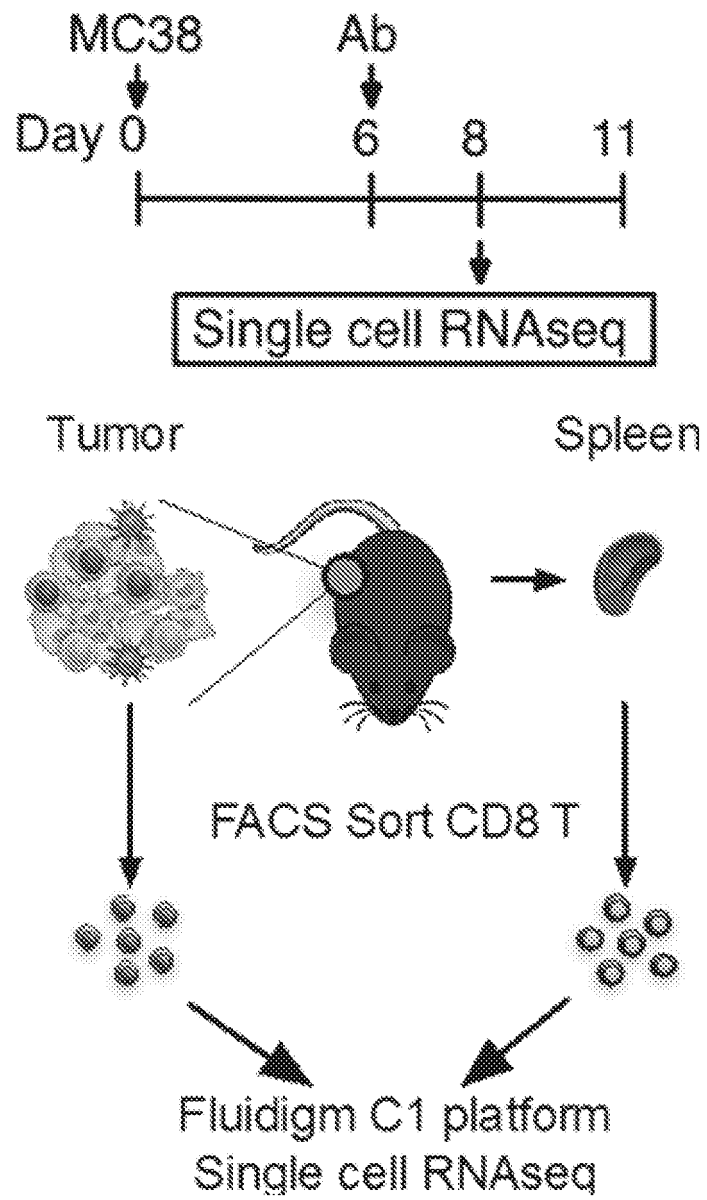


FIG. 8

FIG. 9A



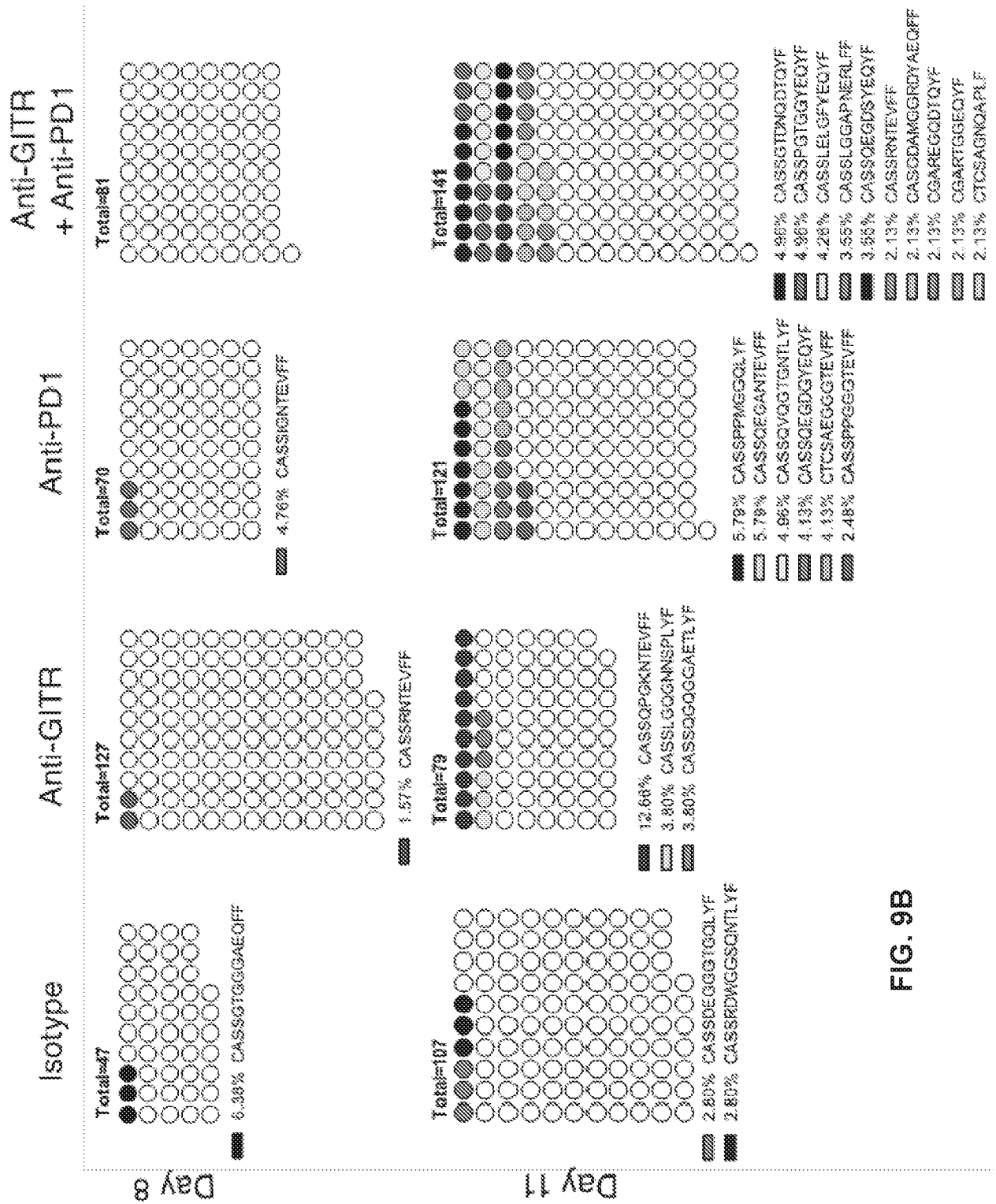


FIG. 9B

FIG. 9C

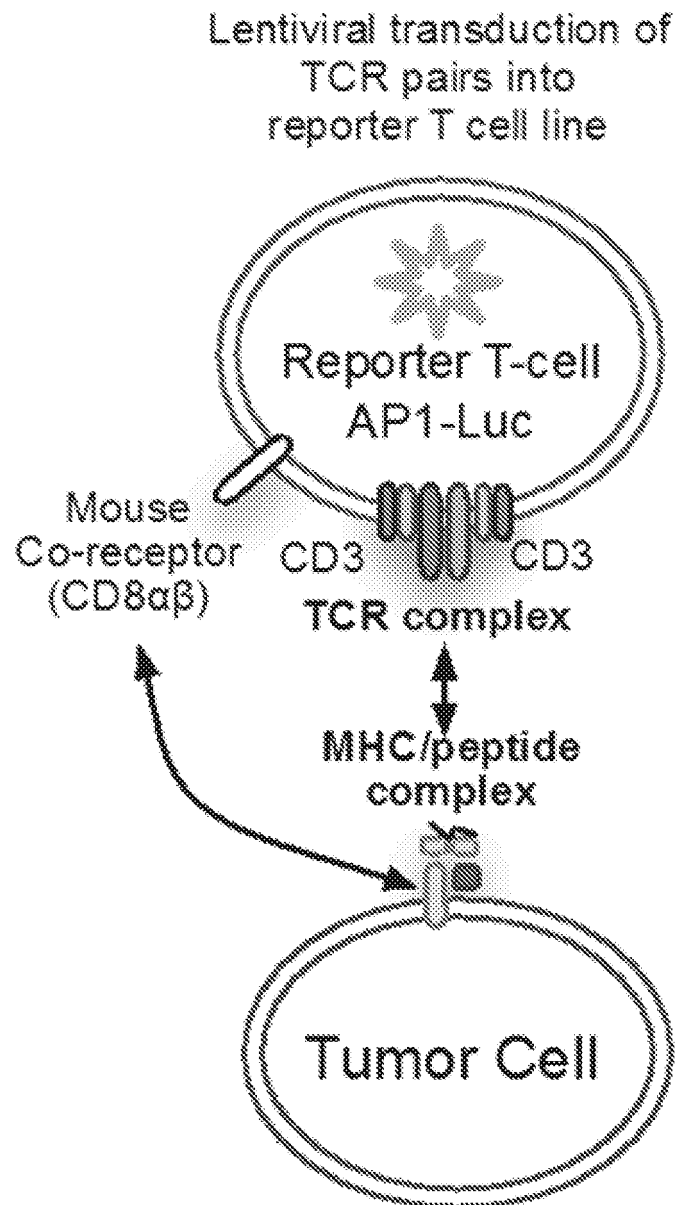


FIG. 9D

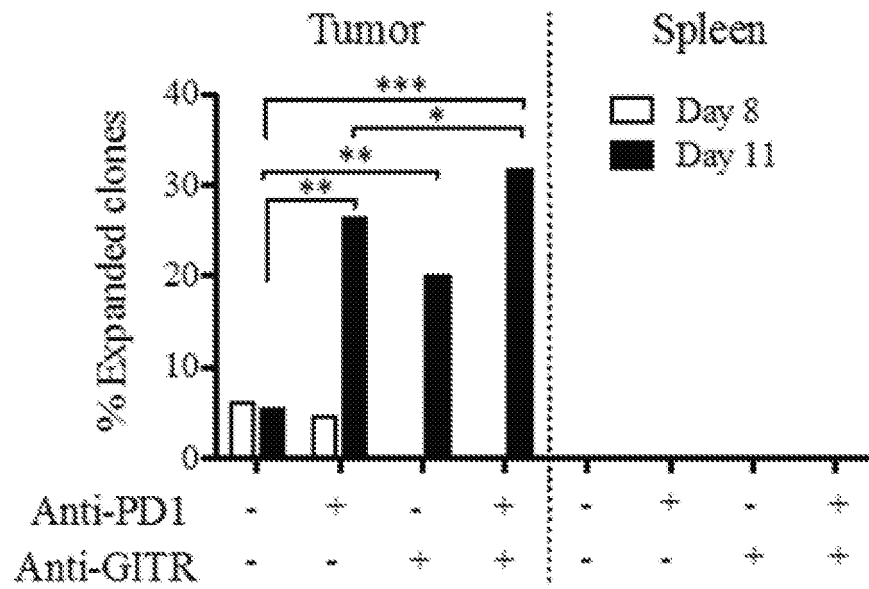
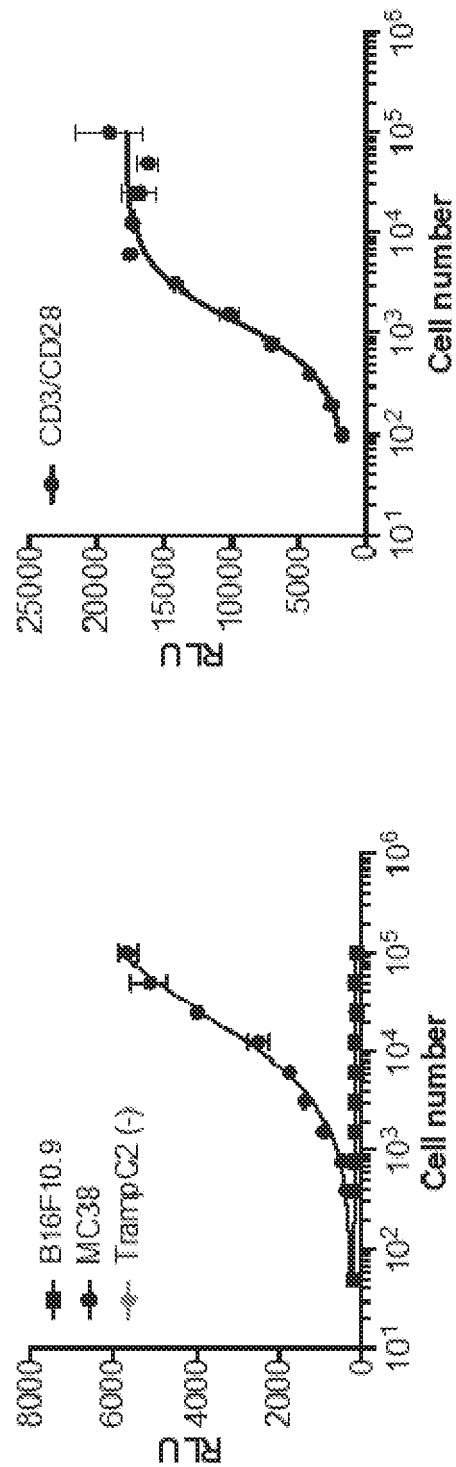
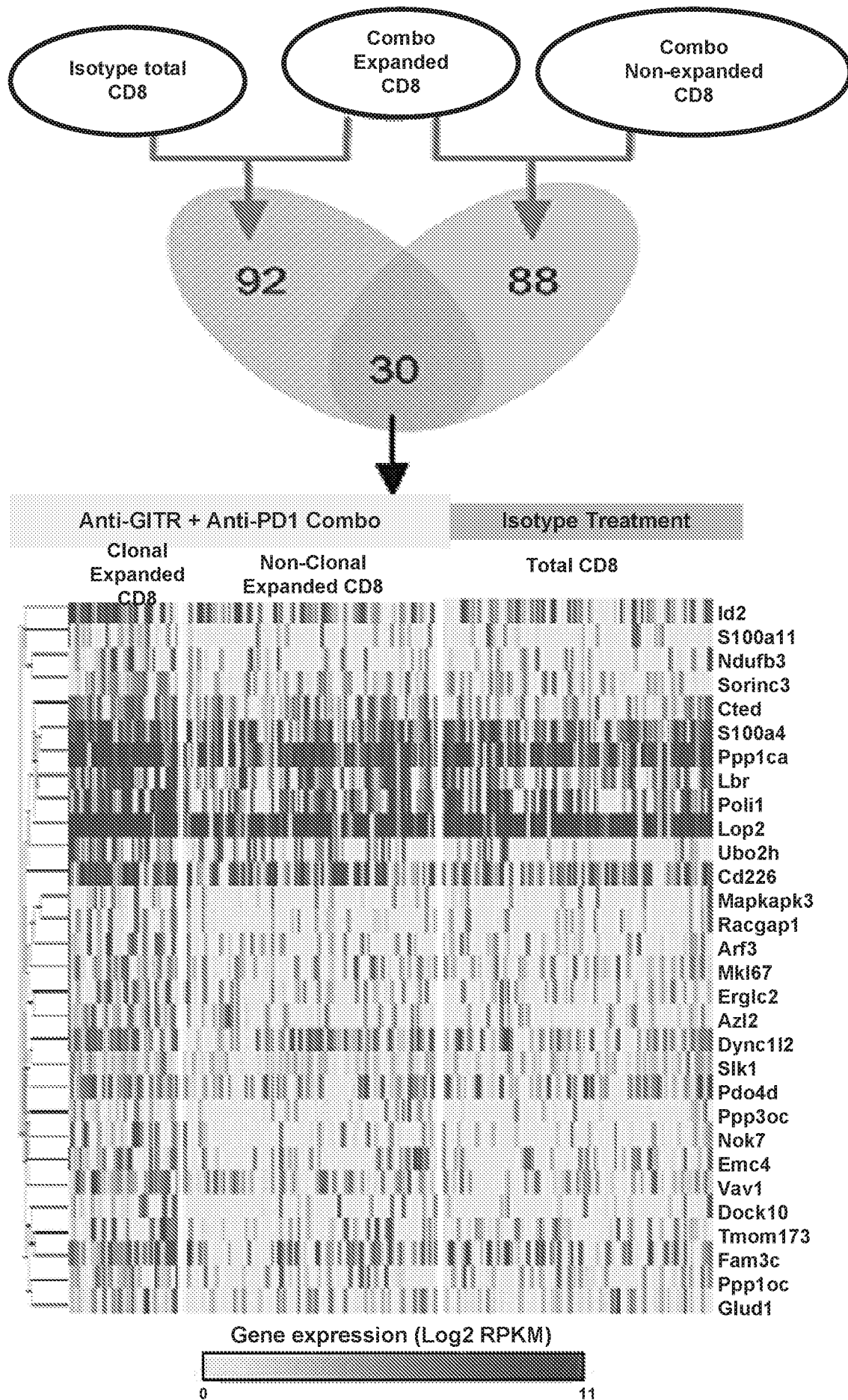


FIG. 9E





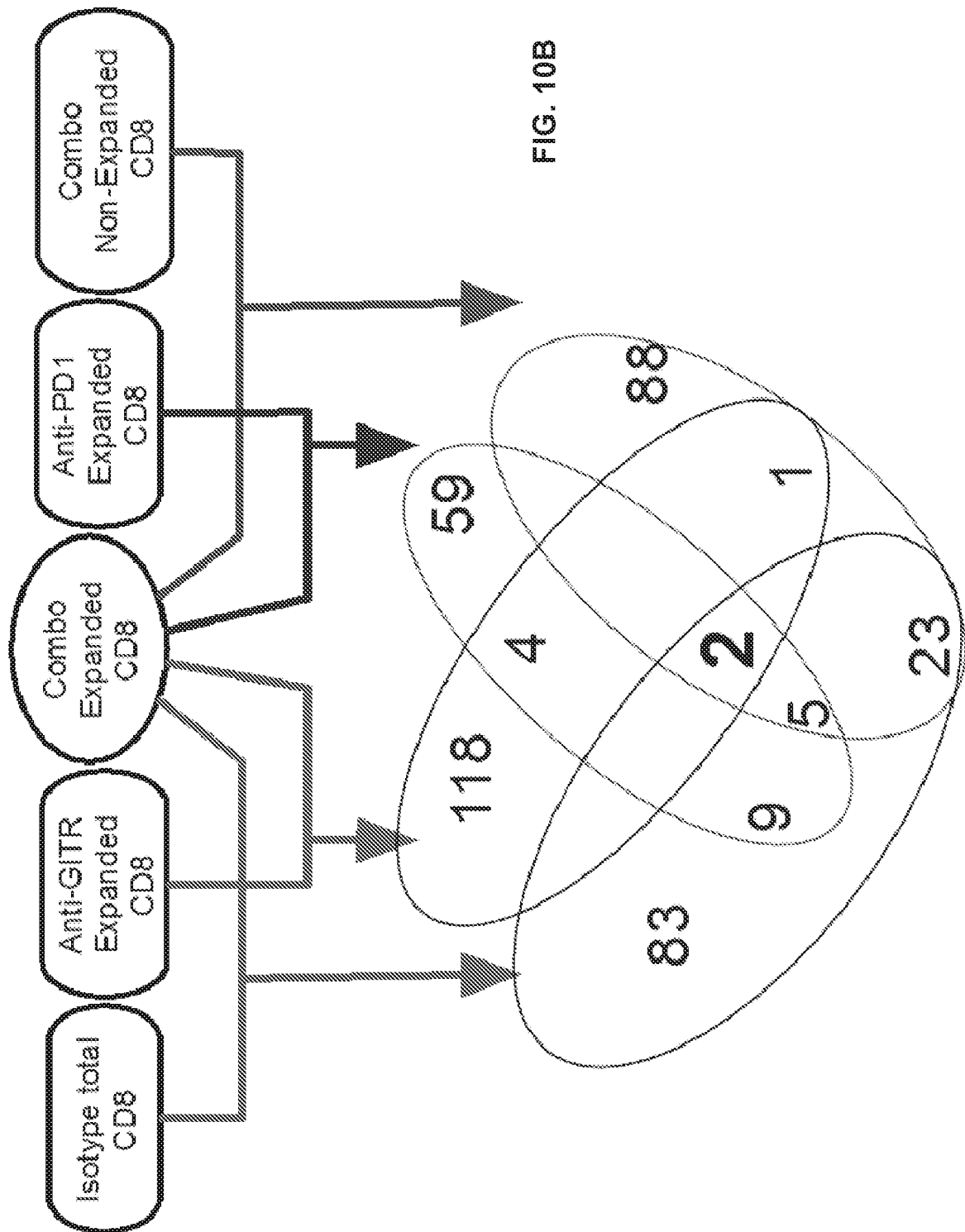
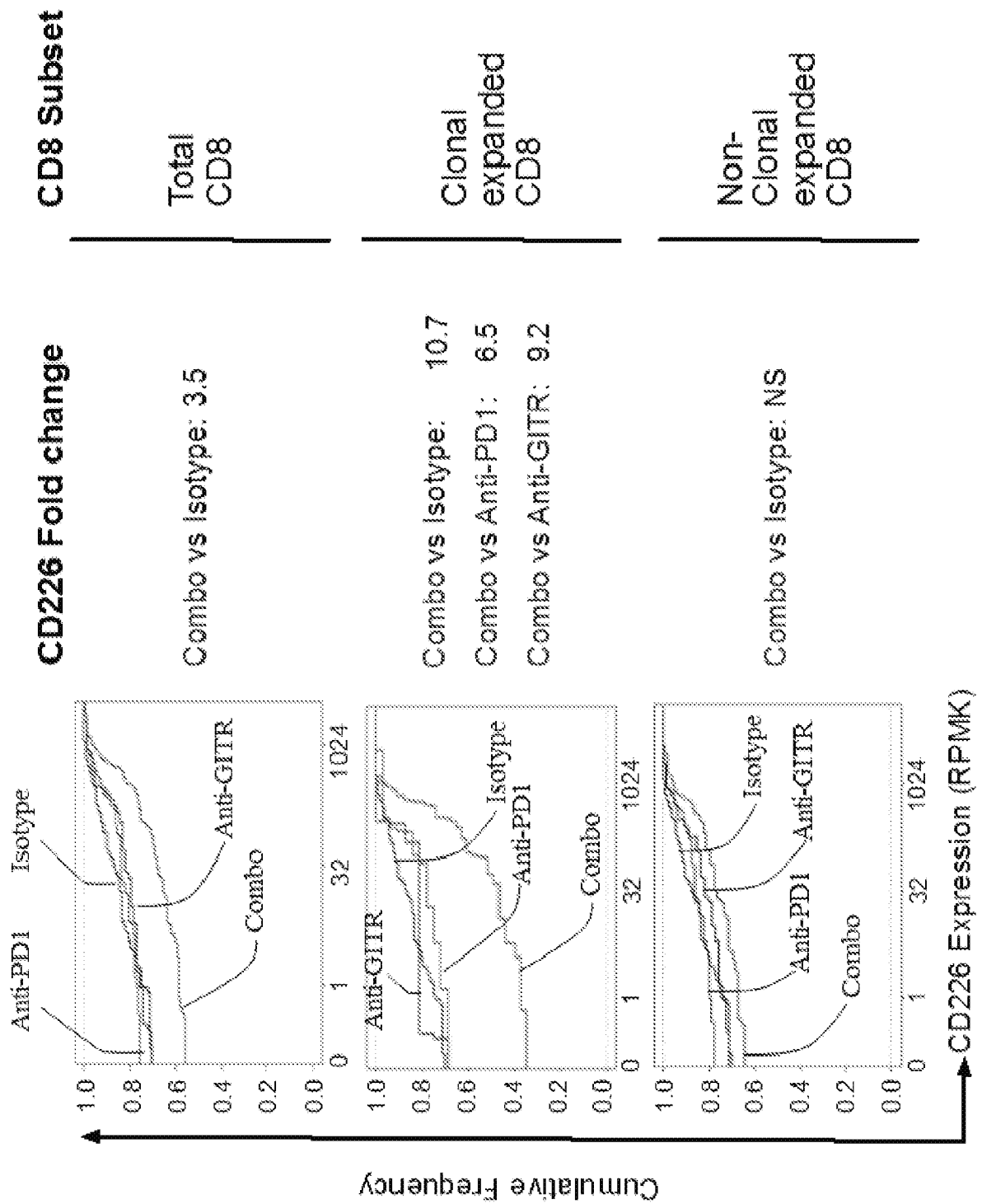


FIG. 10B



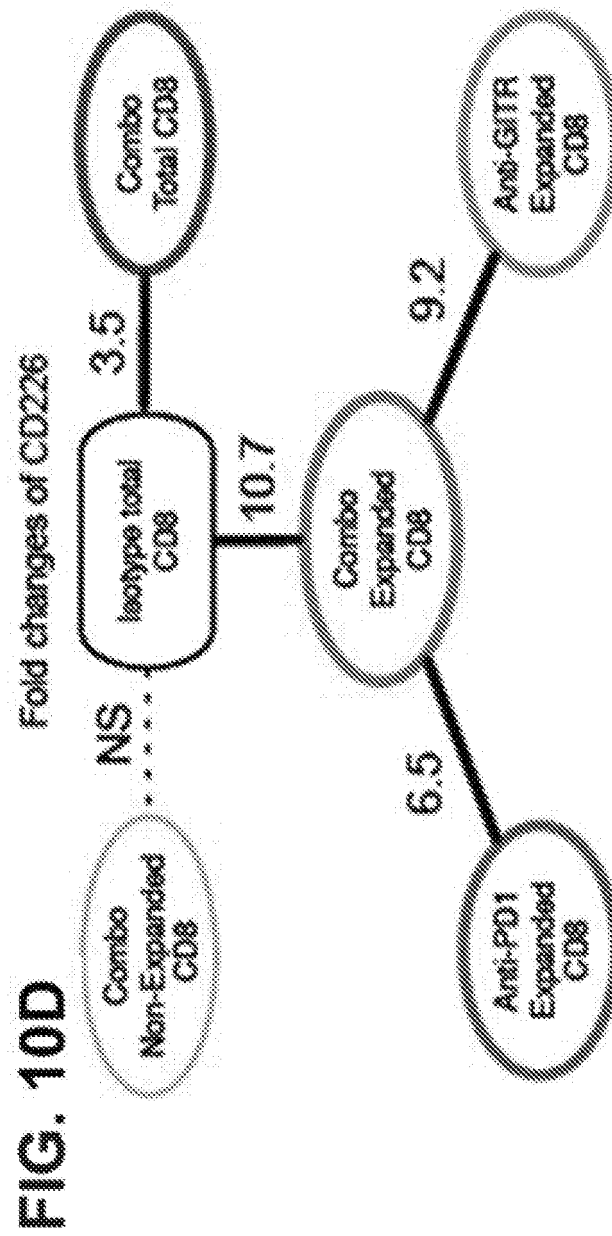


FIG. 11A

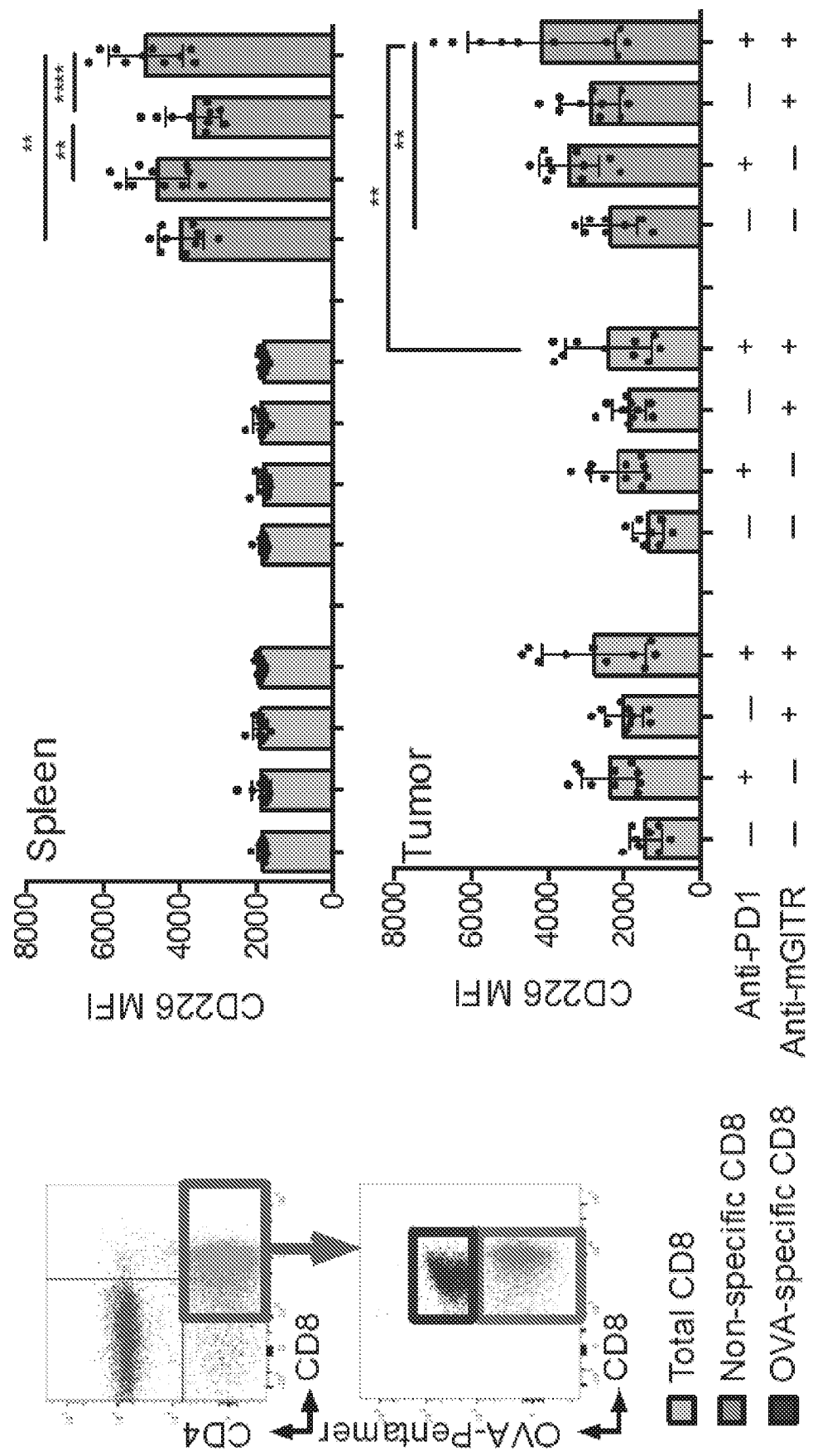


FIG. 11B

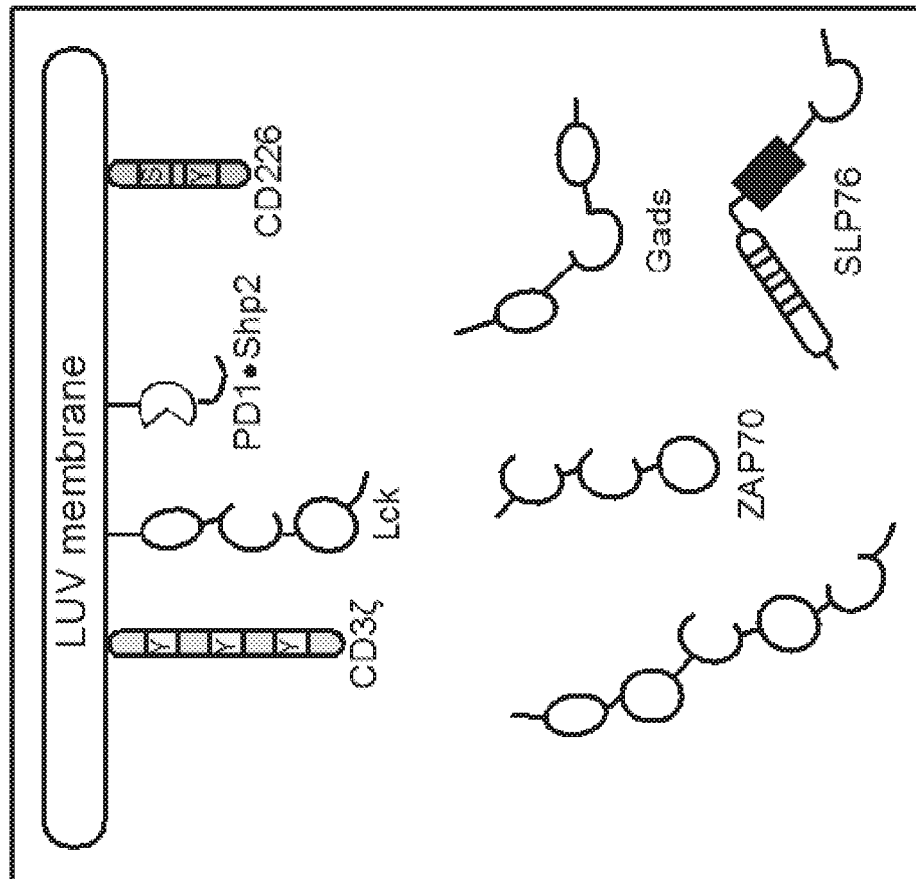
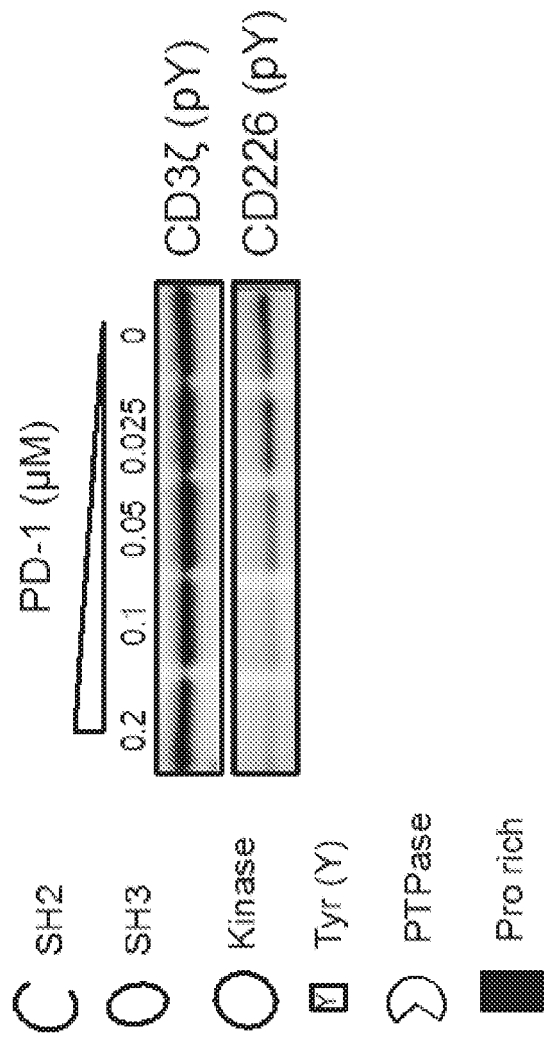


FIG. 11C



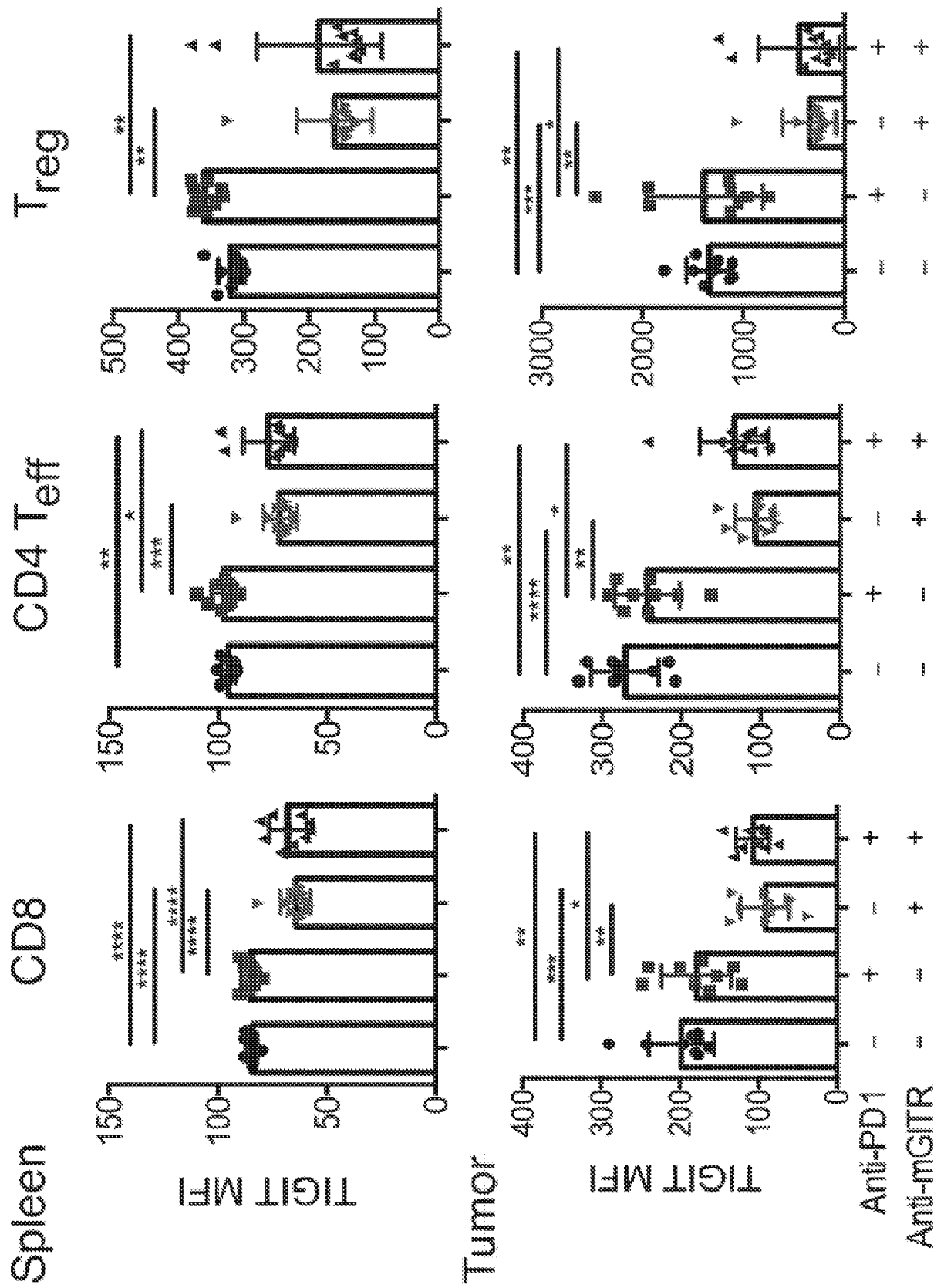


FIG. 11D

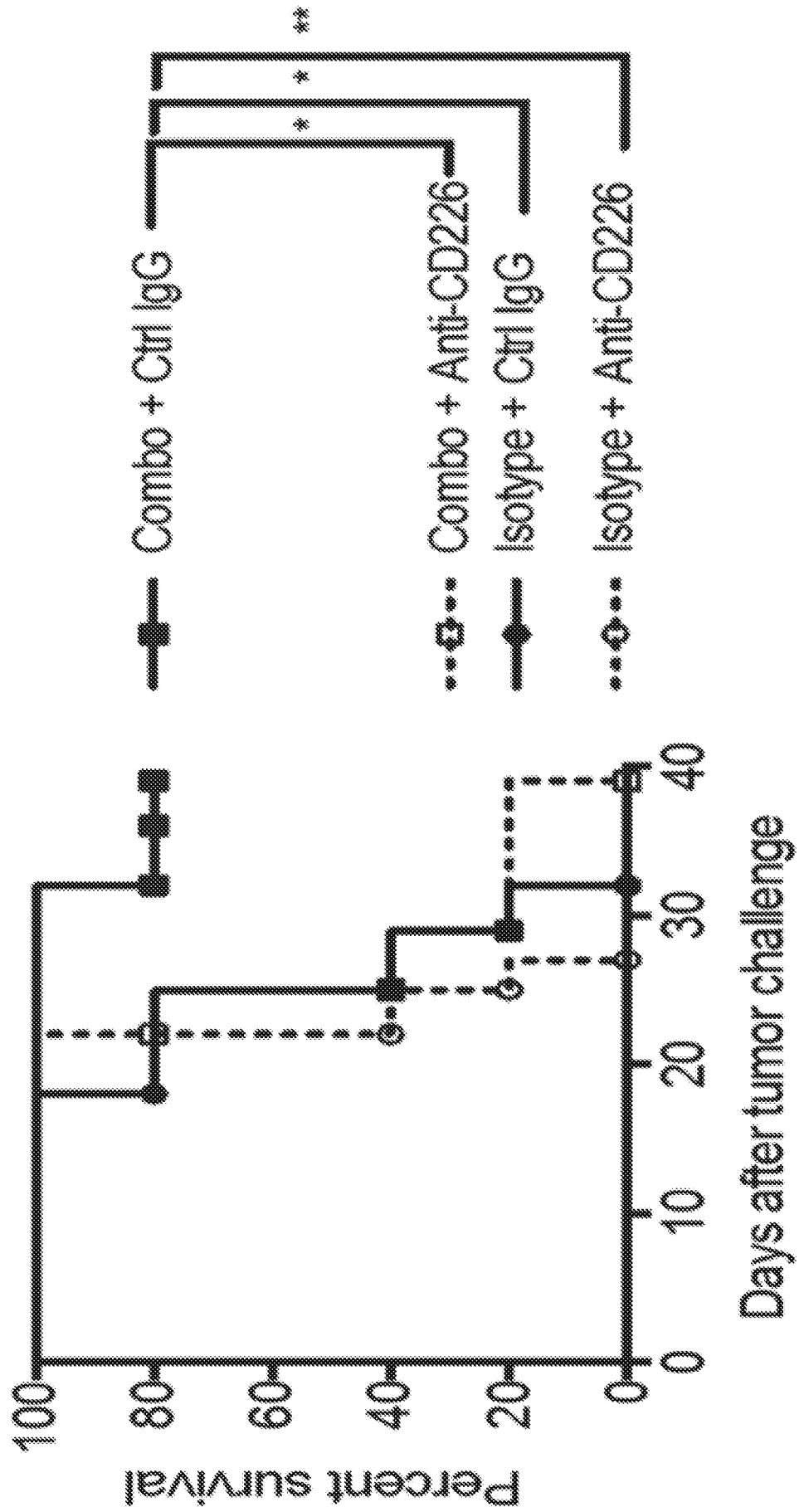


FIG. 12A

FIG. 12B

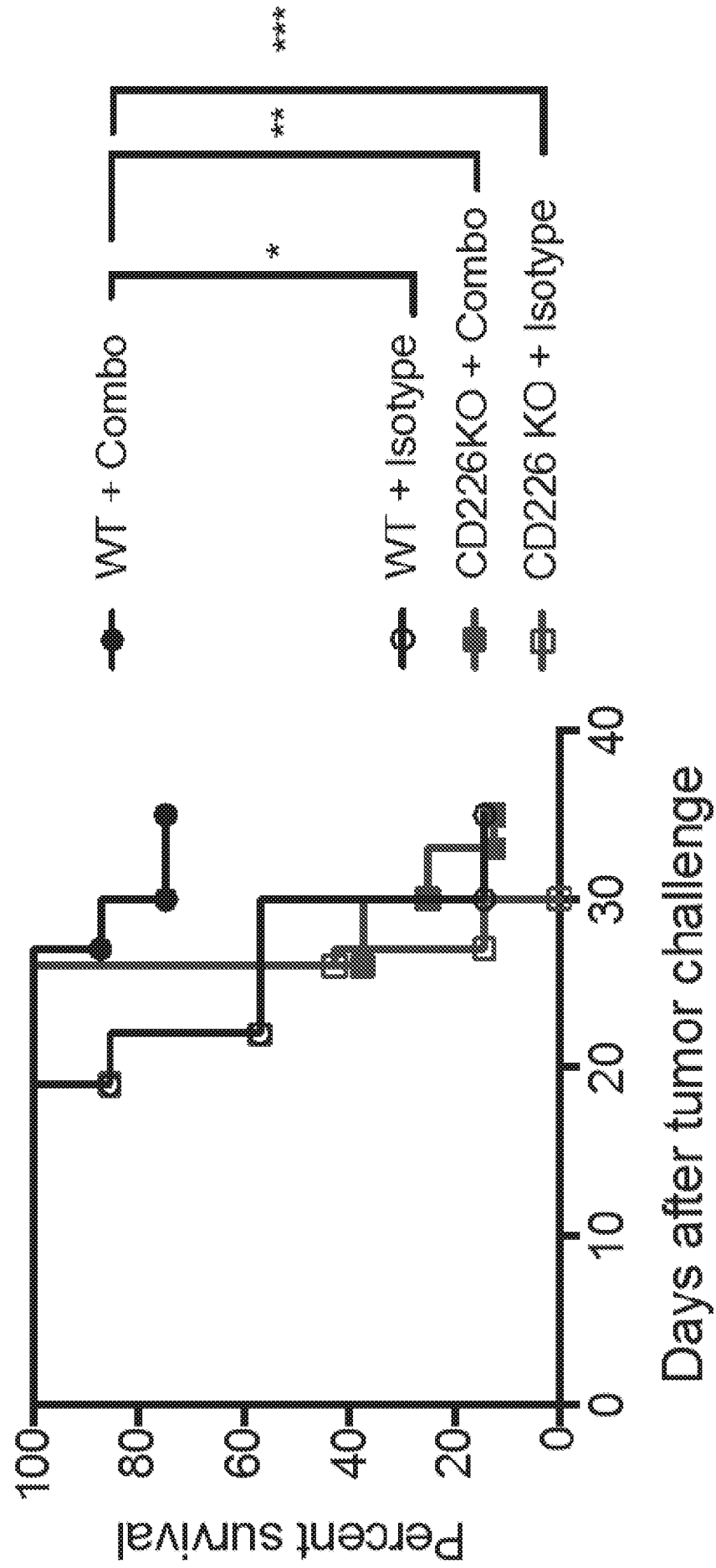


FIG. 12C

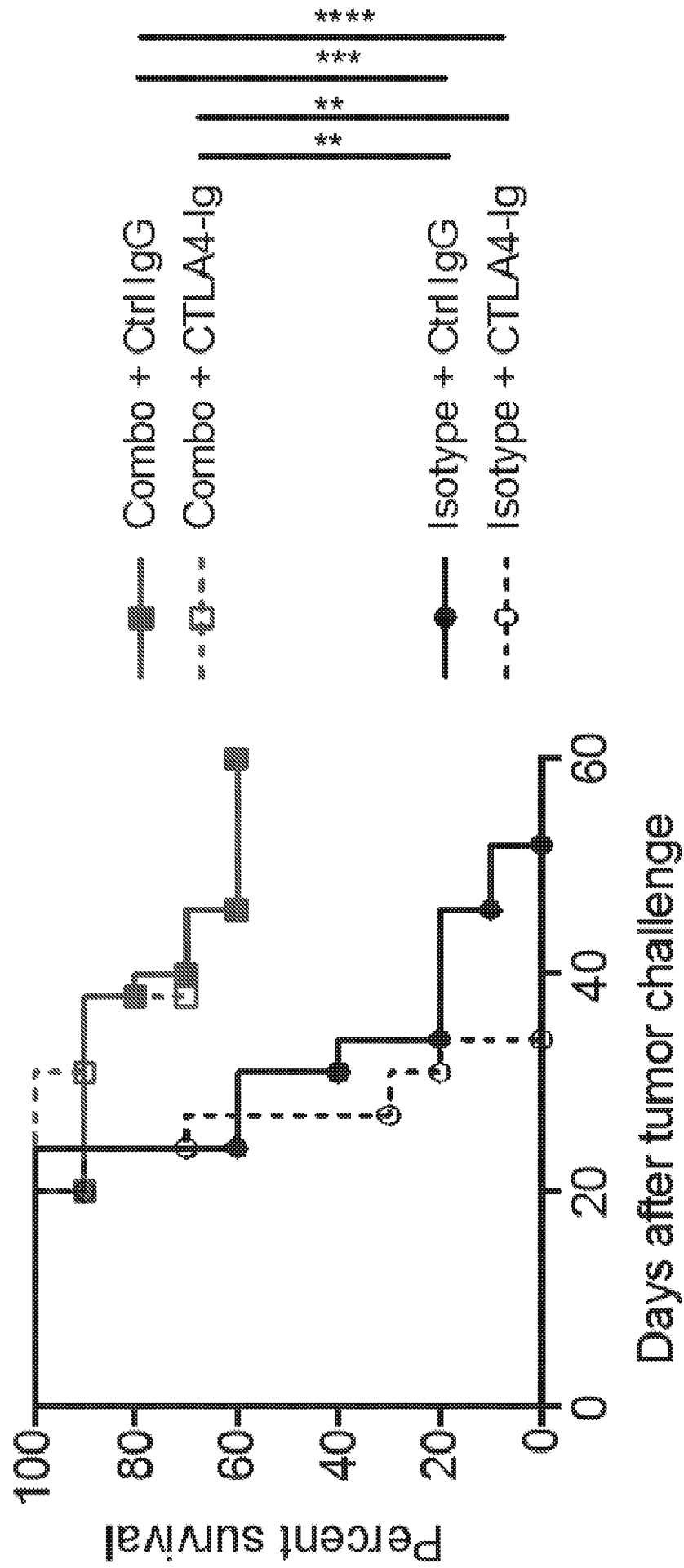


FIG. 12D

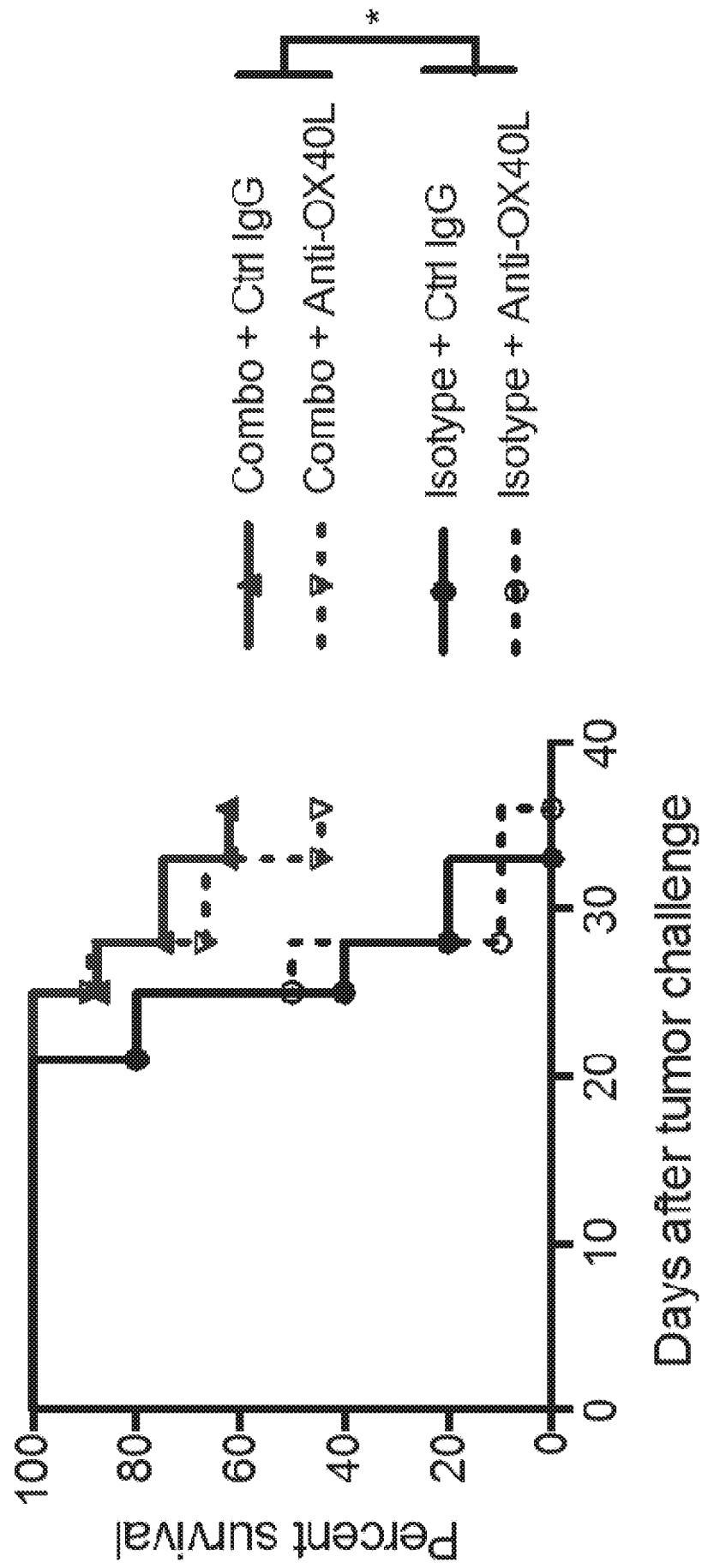


FIG. 12E

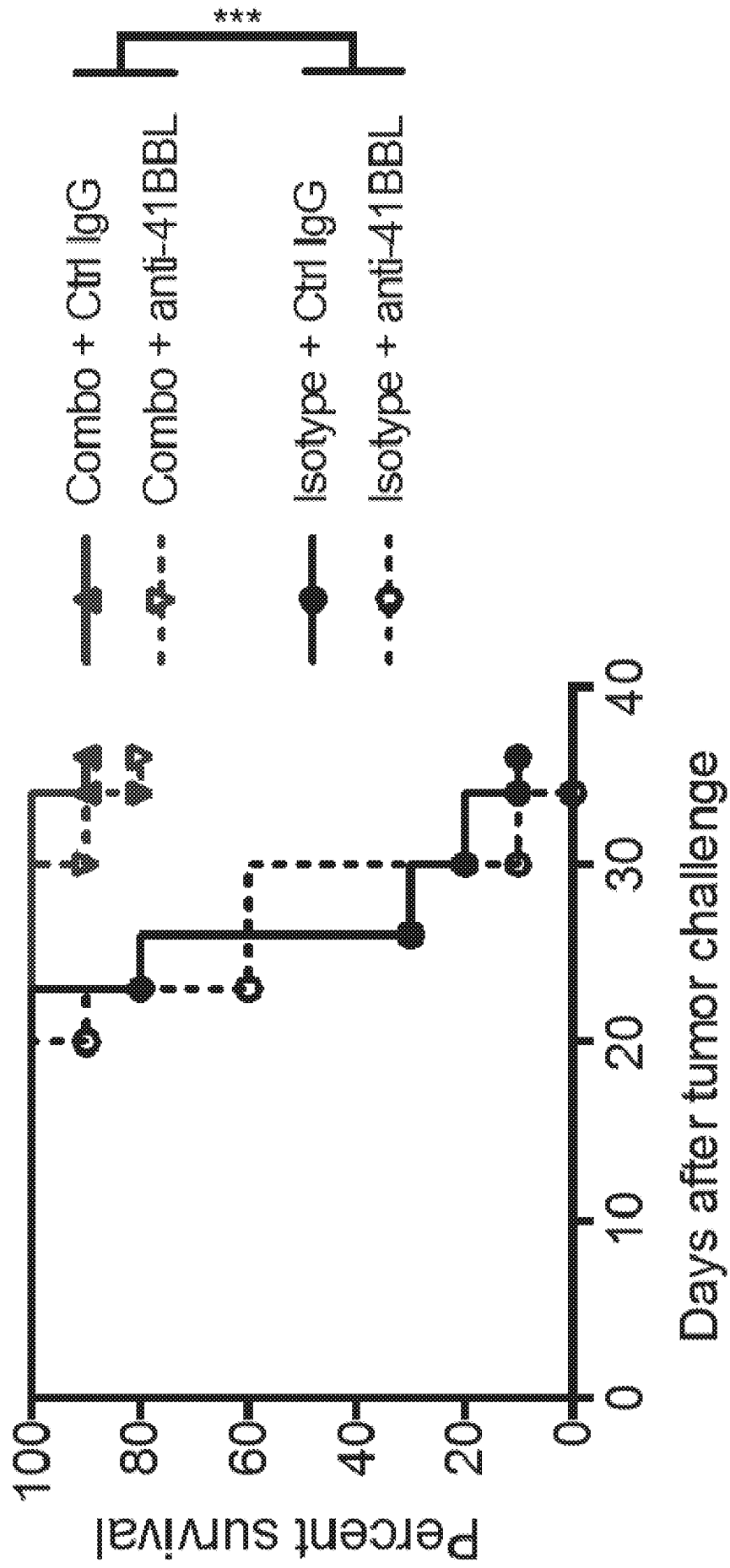


FIG. 12F

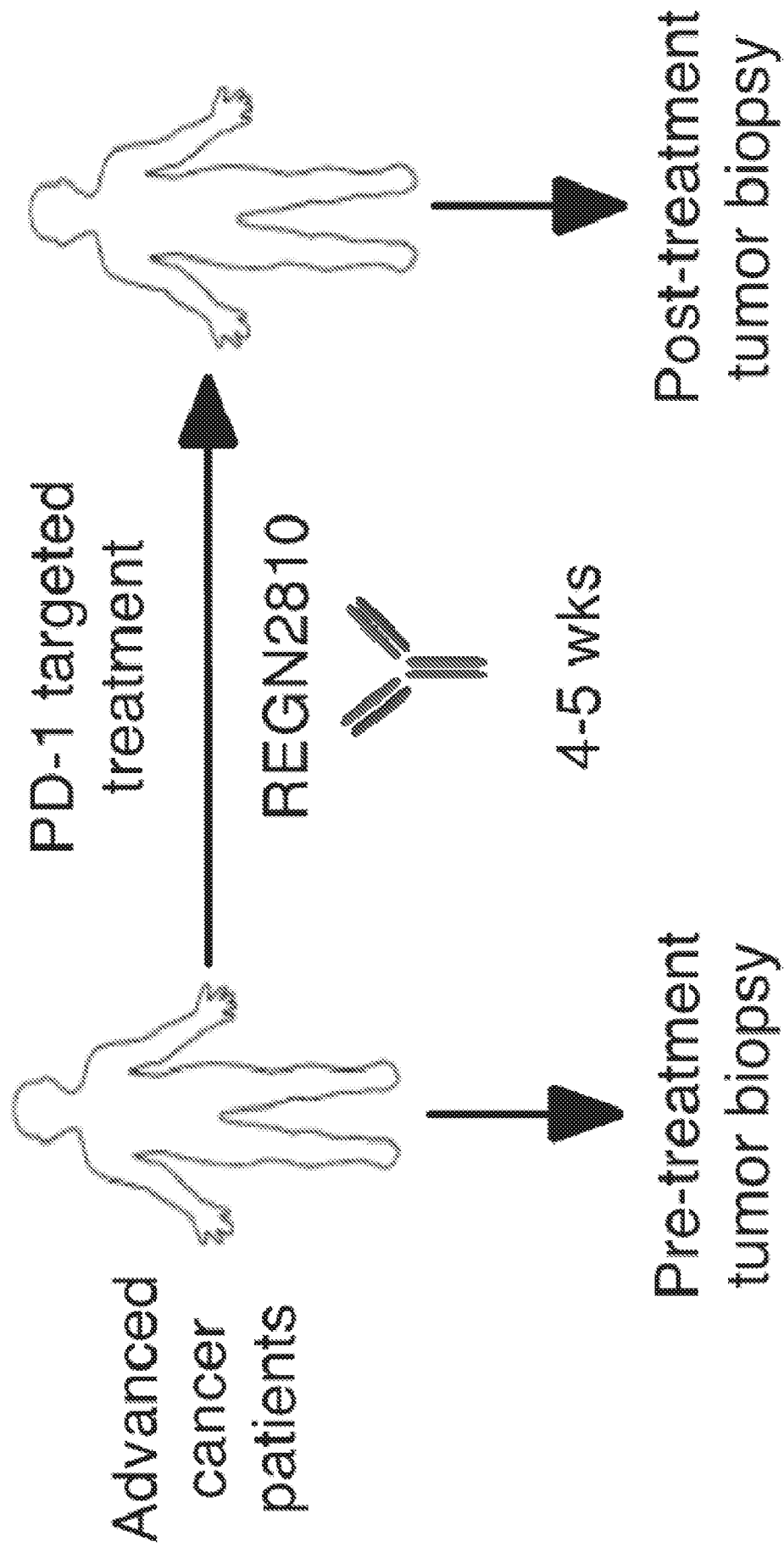


FIG. 12G

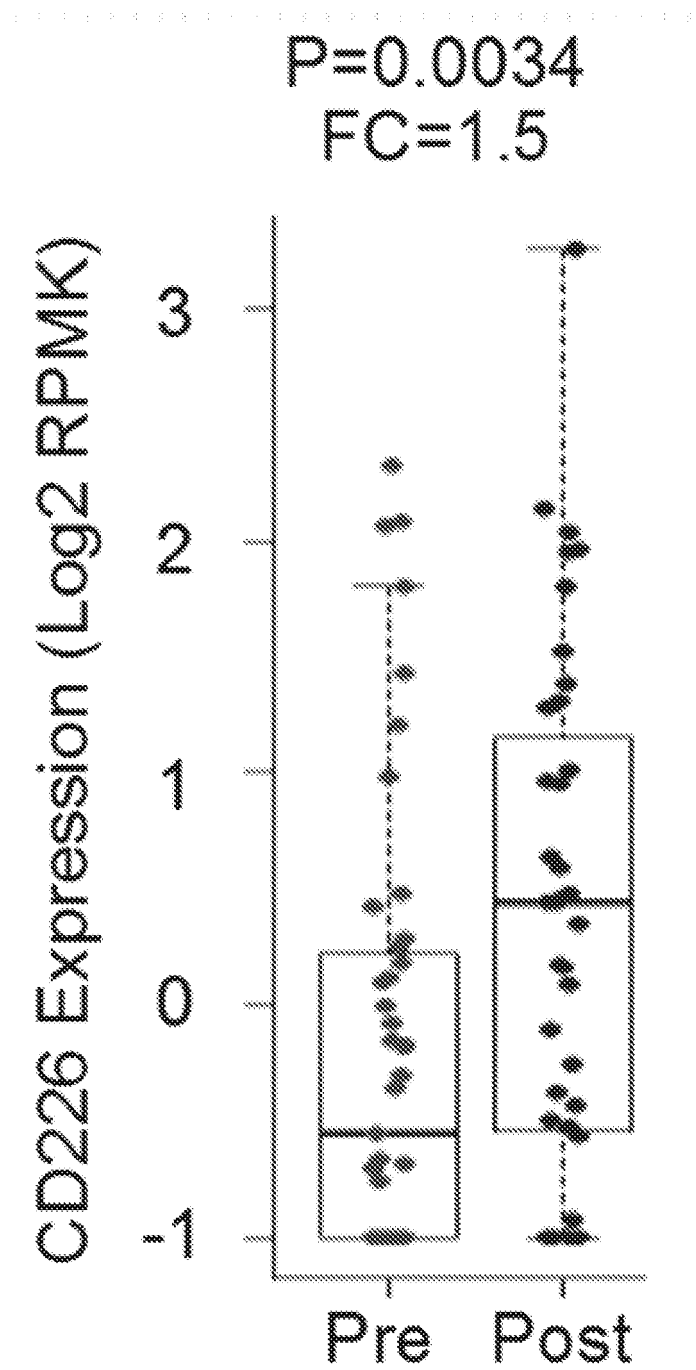


FIG. 12H

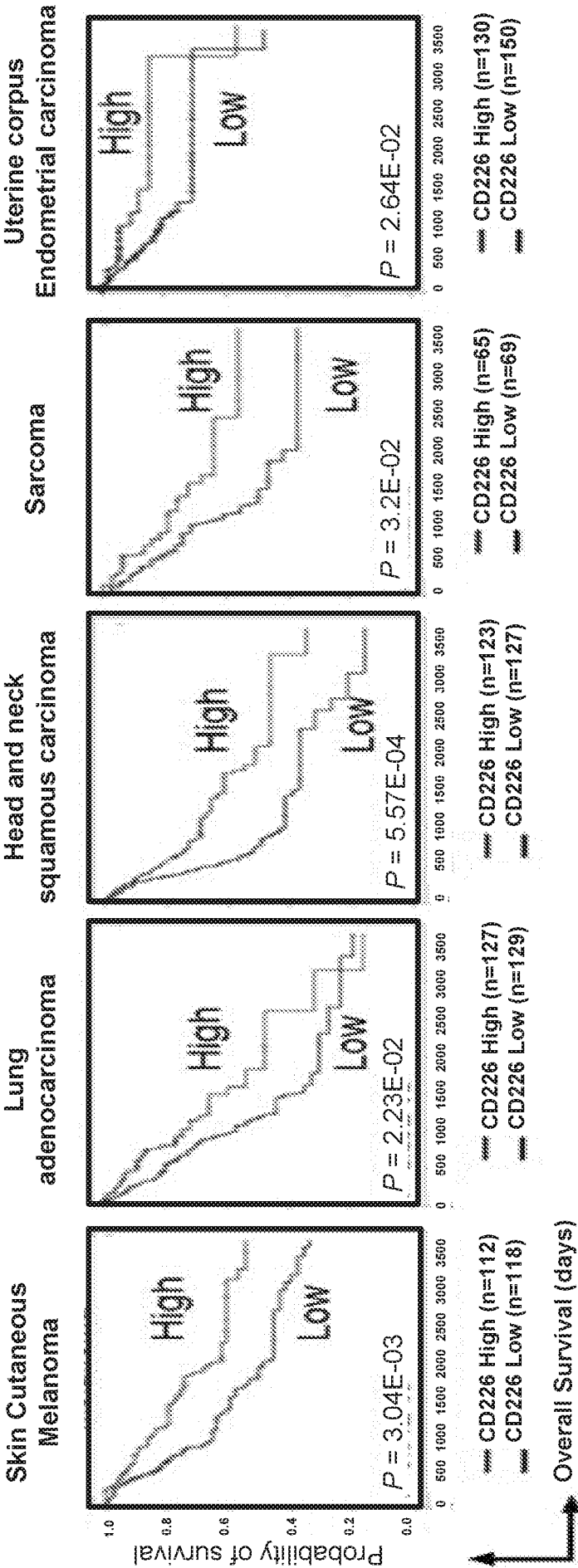


FIG. 13

Sample ID	Description	Species	Sample type	MiTCR	TCRklass	rpsTCR	Input reads (passed QC>=25)
Renca	kidney tumor cell line	mouse	negative control	23	0	0	44,906,248
B16F1	melanoma tumor cell line	mouse	negative control	76	0	0	39,305,839
Colon26	Colon tumor cell line	mouse	negative control	22	1	0	39,233,756
MC38	colon tumor cell line	mouse	negative control	99	1	0	48,661,267
T-ALL_neg1	Spleen from Rag1/2 KO mouse	mouse	negative control	17	1	0	39,068,155
T-ALL_neg2	Spleen from Rag1/2 KO mouse	mouse	negative control	51	0	0	39,469,692
NeuProgCell	Neural progenitor cell line	human	negative control	123	0	0	37,684,399
LHCN-M2	Skeletal myoblast cell line	human	negative control	77	0	0	36,639,557

FIG. 14

TCR type	Total	CDR3 detected by our pipeline	Detection rate of our pipeline	Detection rate reported by Han A. et al.
TRB-CDR3	1,379	1,186	86.0%	92%
TRA-CDR3	1,379	1,078	78.2%	87%
TRA&B-CDR3	1,379	1,009	73.1%	82%

FIG. 15

Treatment	Pathway	p Value
Anti-PD1 + Anti-GITR	Adaptive immune response	1.50E-08
	Cell cycle	5.80E-07
	Metabolism of lipids and lipoproteins	3.00E-04
Anti-PD1	Lymphocyte activation	2.30E-05
	Gene targets for miR-124u (T cell activation)	1.80E-05
	Jak-STAT signaling pathway	3.00E-04
Anti-GITR	Glucose metabolism	8.20E-10
	Protein metabolism	7.50E-08
	The citric acid cycle and respiratory electron transport	3.90E-06

FIG. 16A

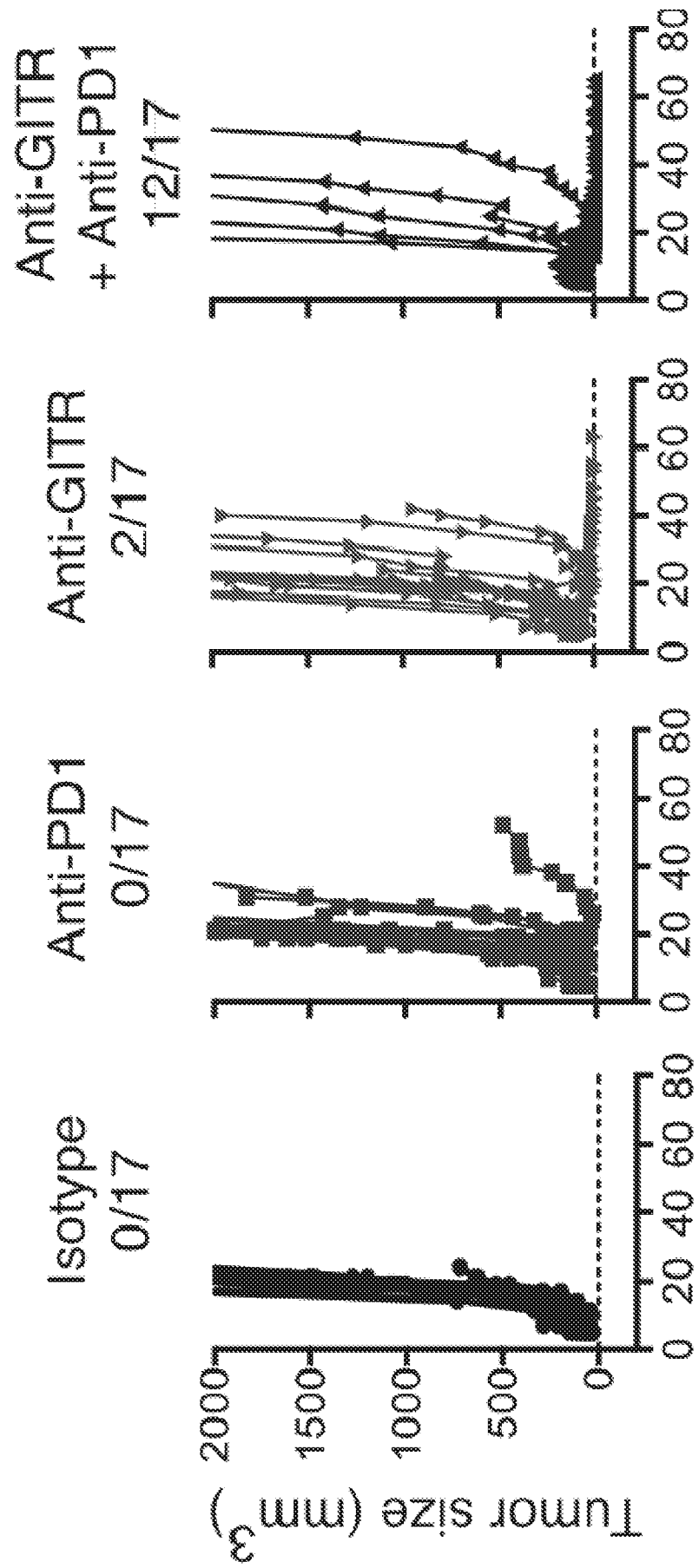
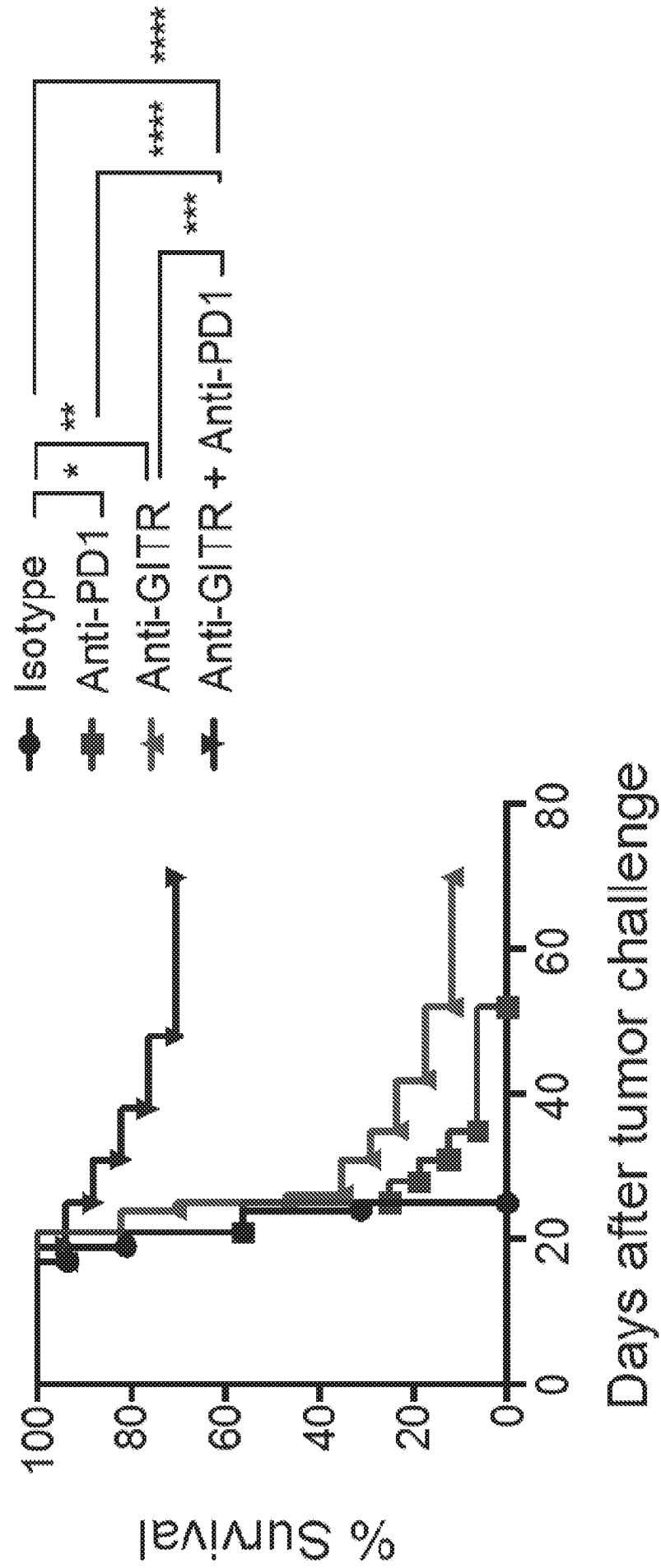


FIG. 16B



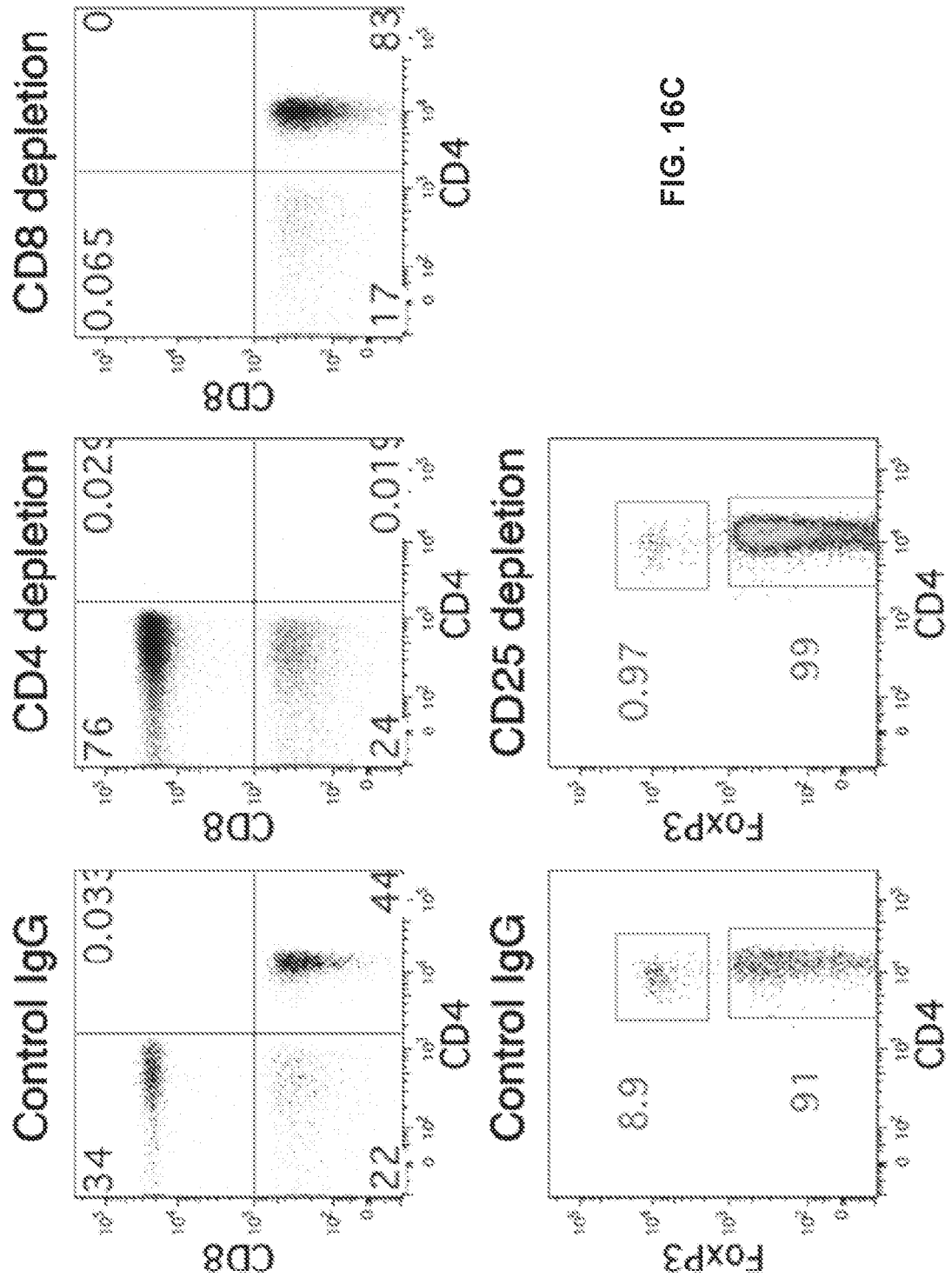


FIG. 16C

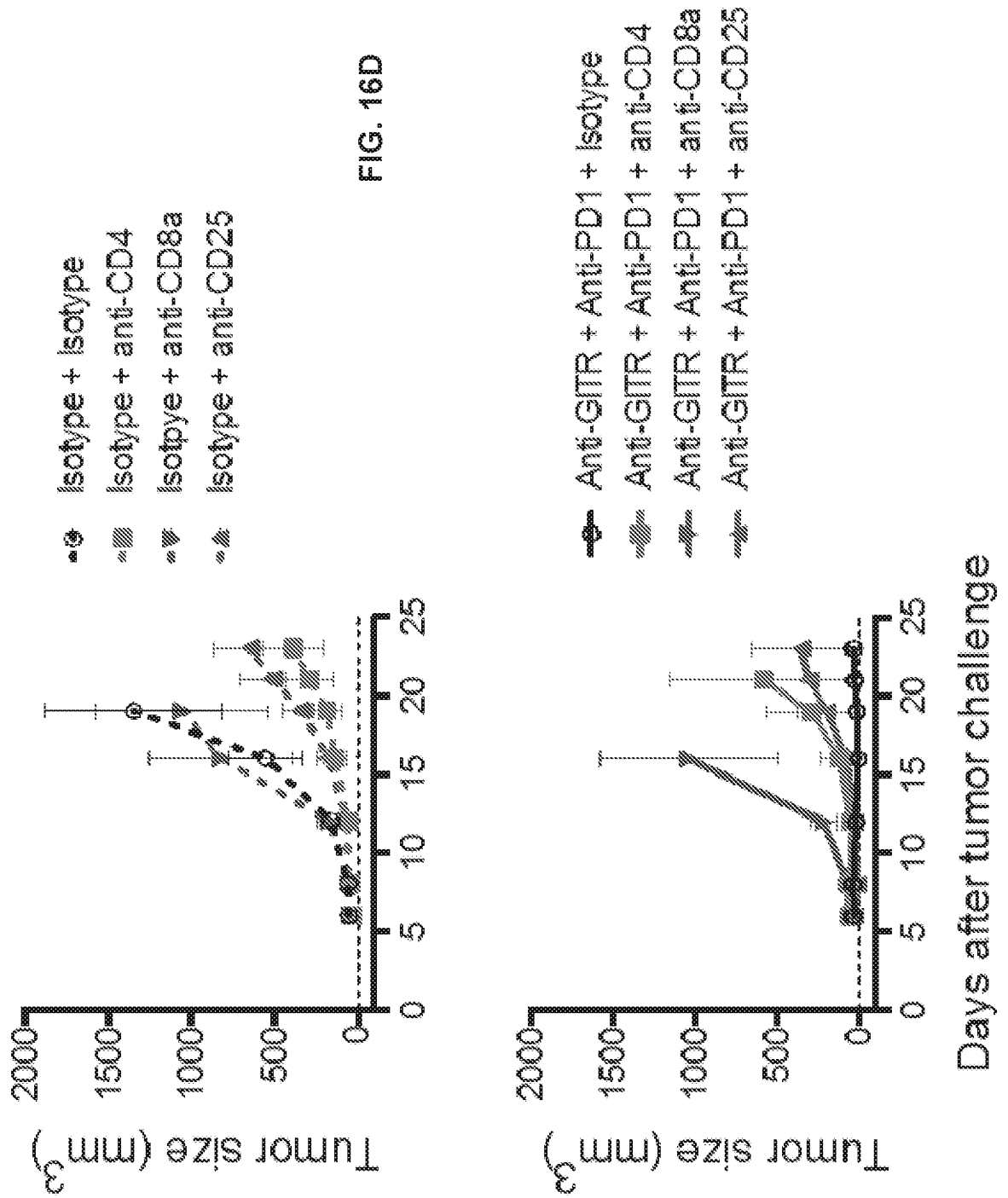


FIG. 16E

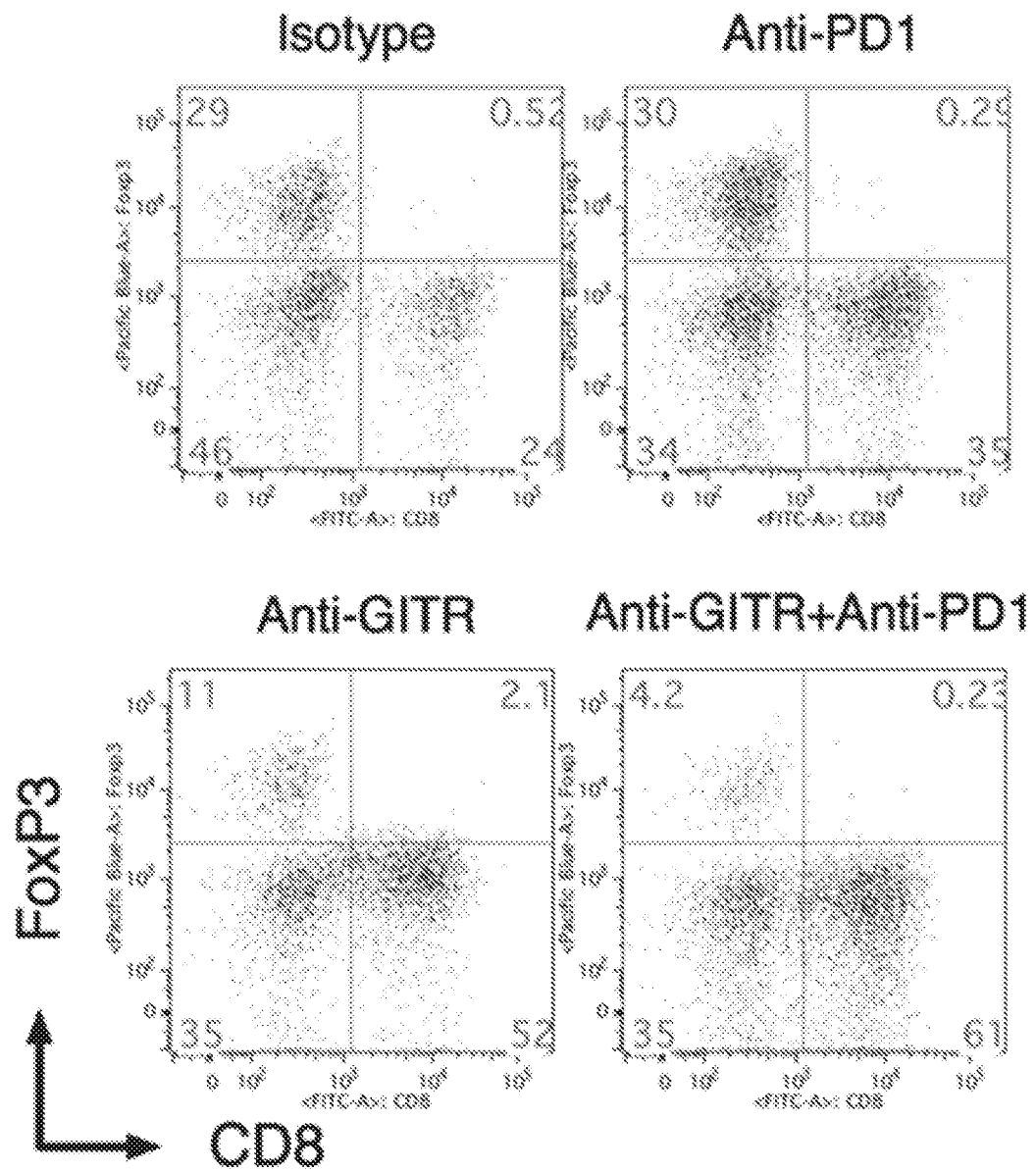


FIG. 16F

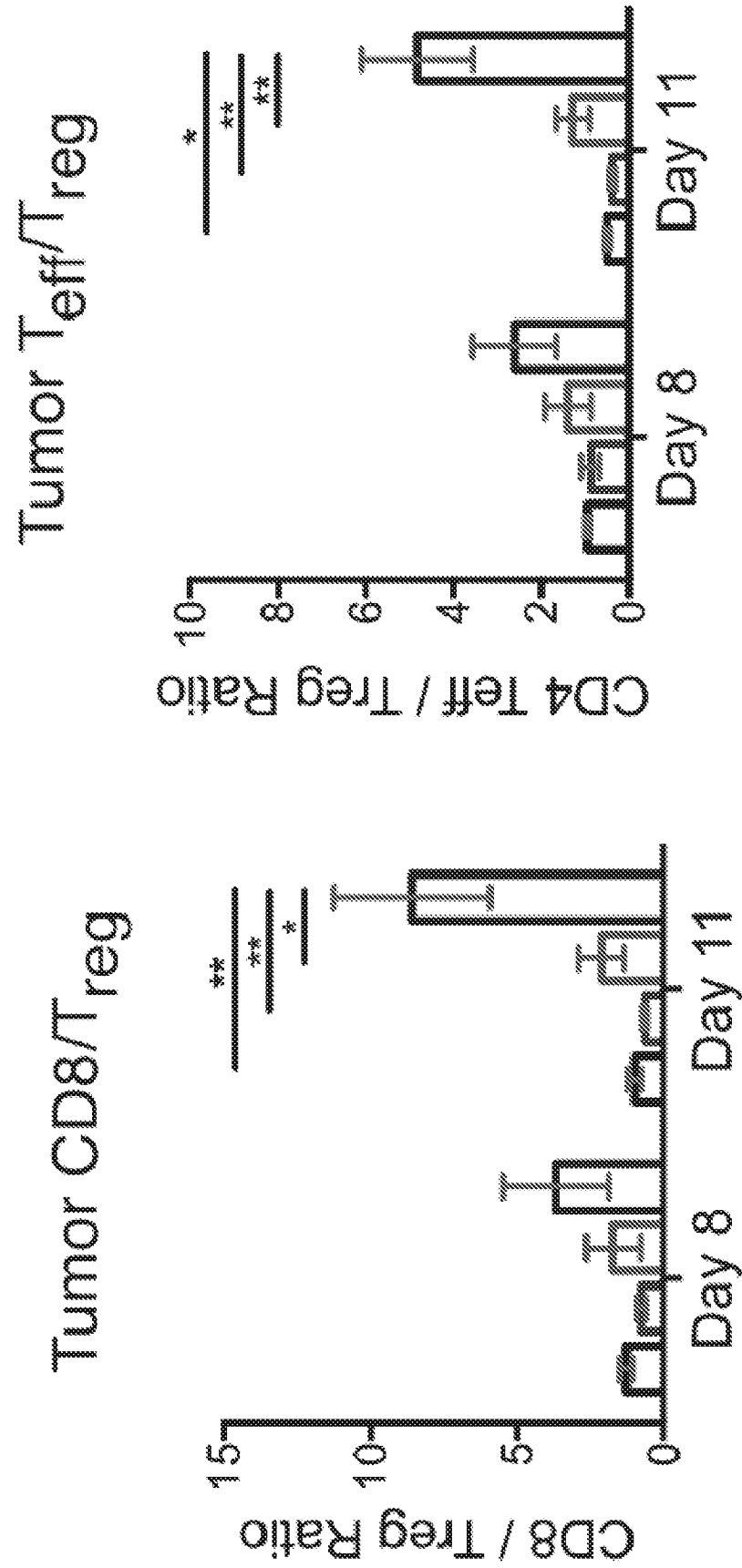


FIG. 16G

Ki67

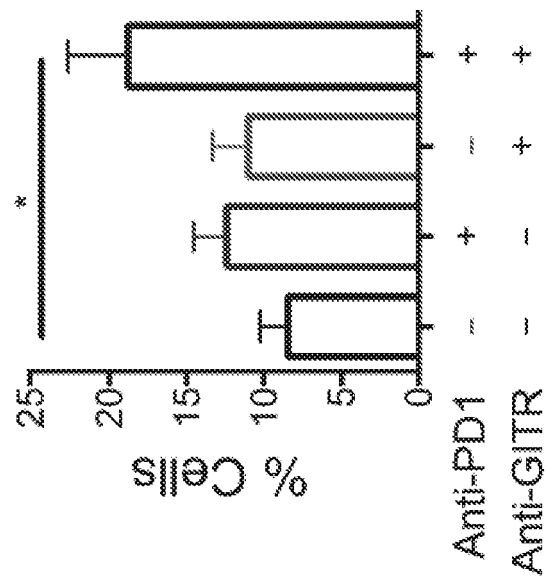


FIG. 16H

Granzyme A

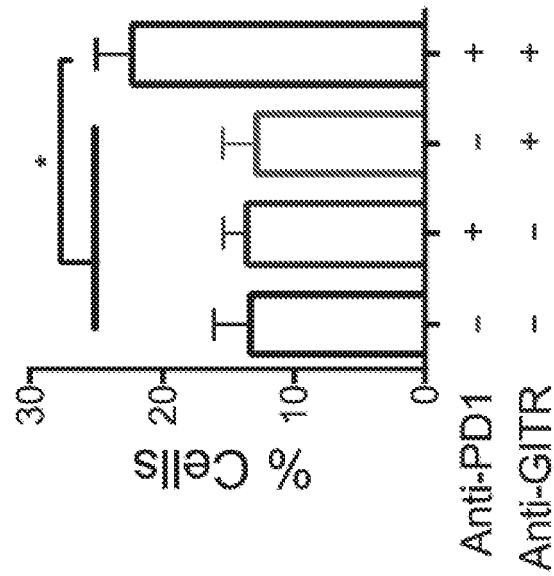


FIG. 16I

Granzyme B

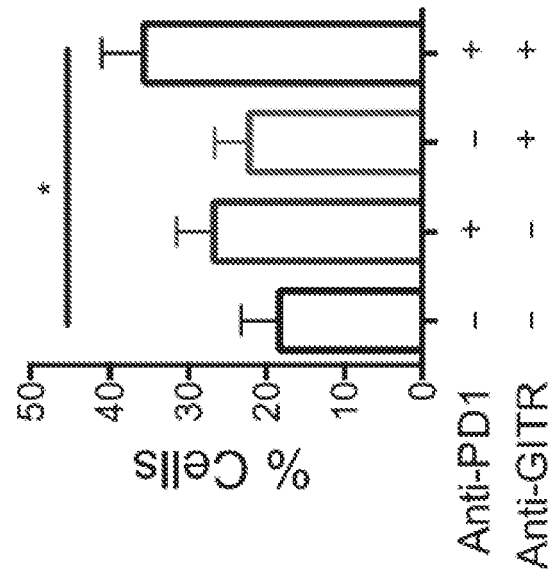


FIG. 16J

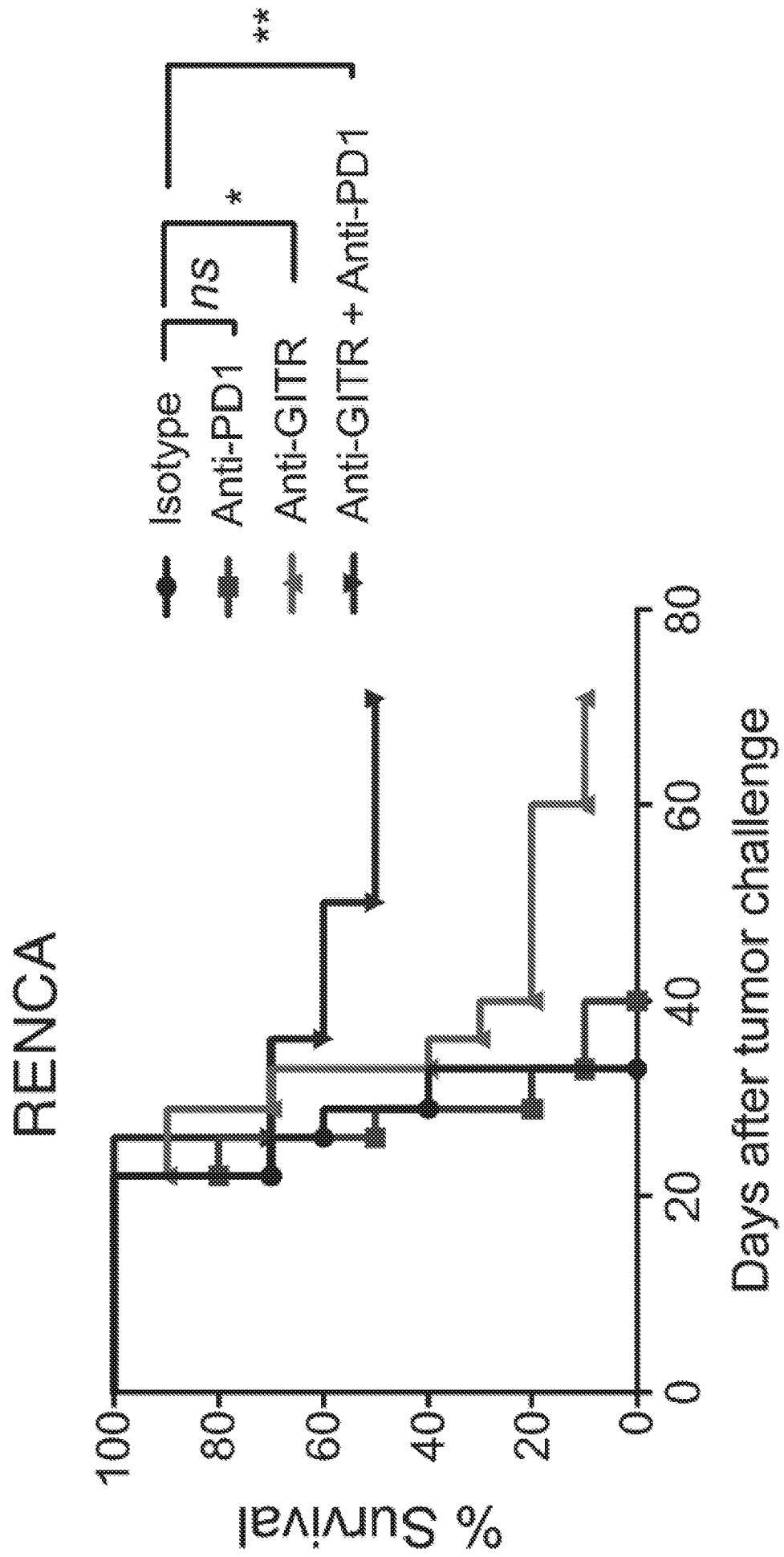
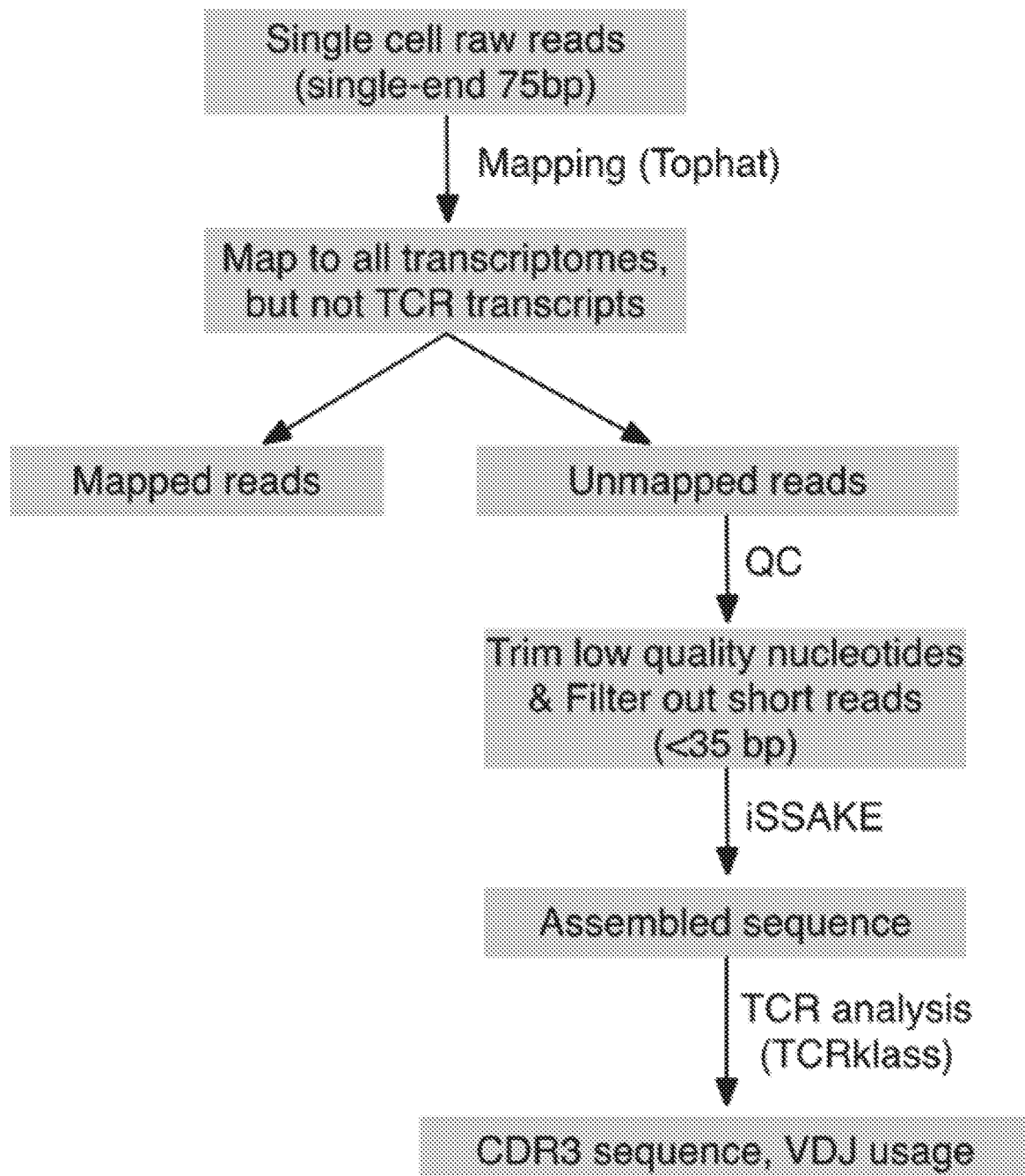


FIG. 17



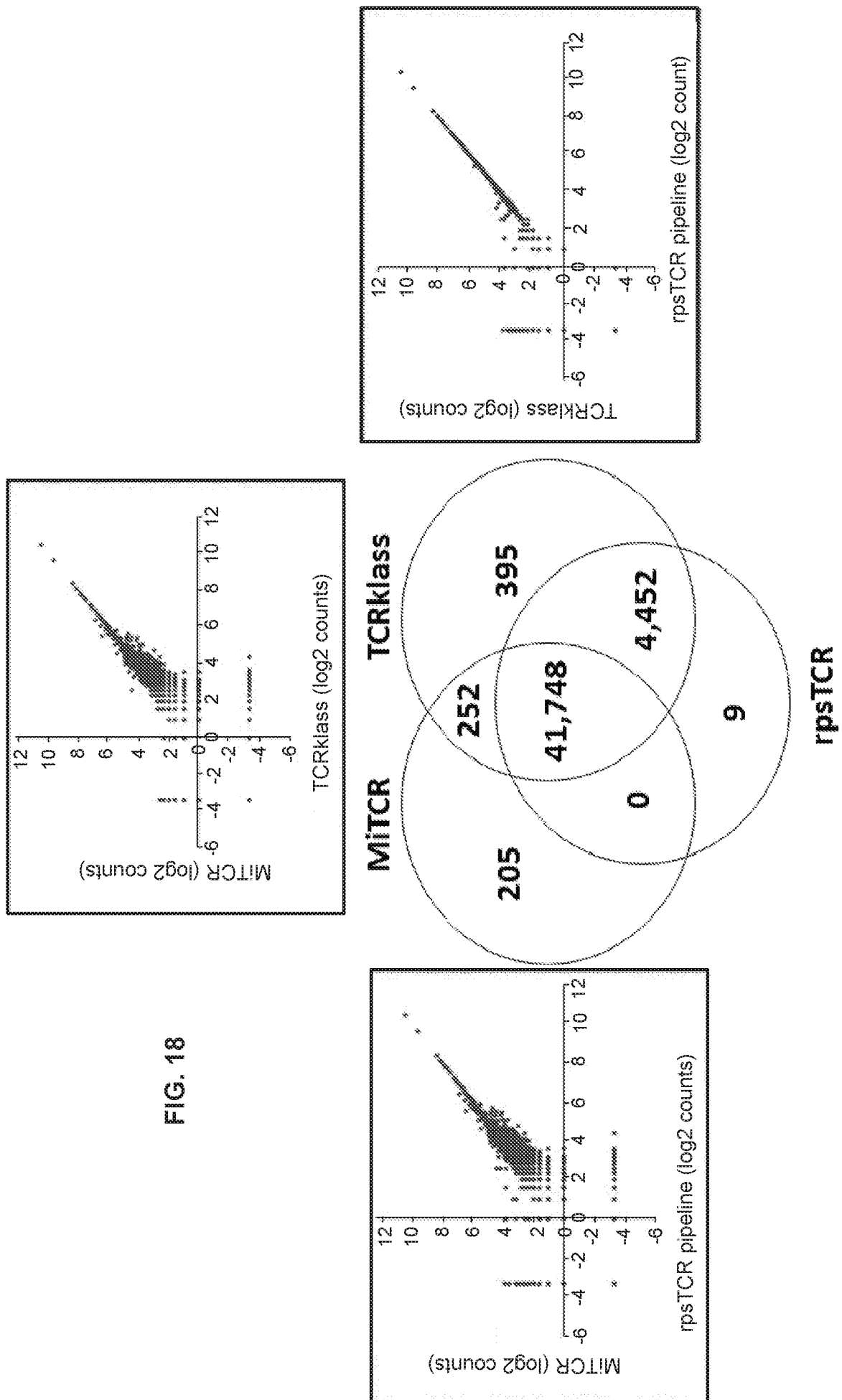
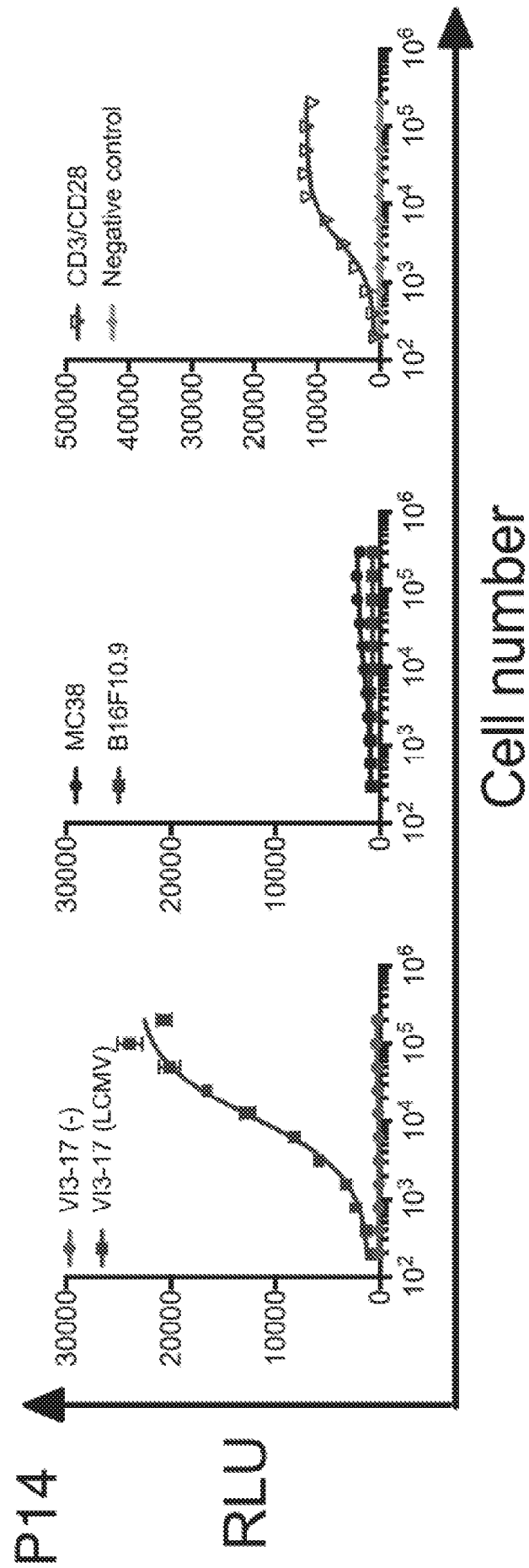


FIG. 19



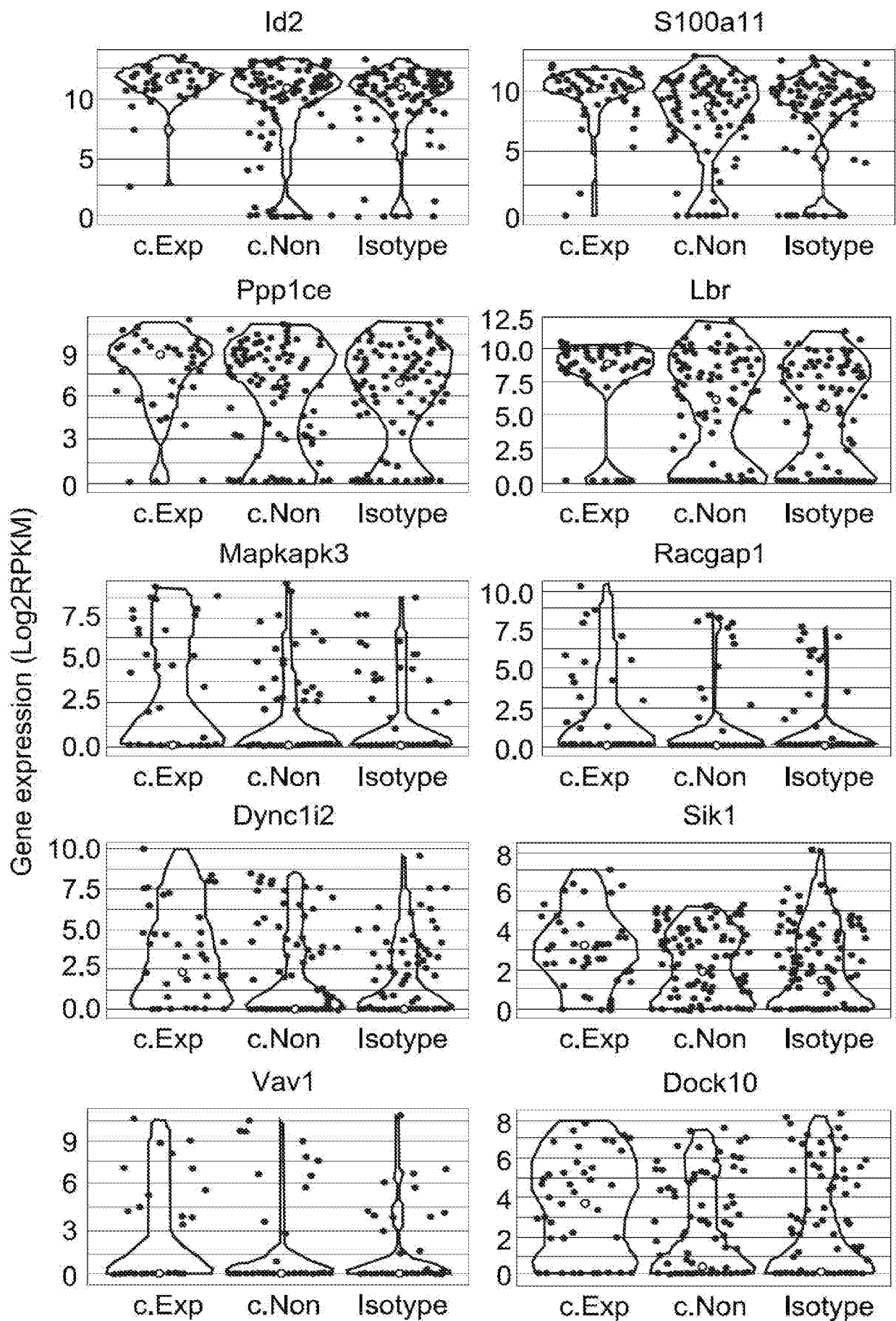


FIG. 20

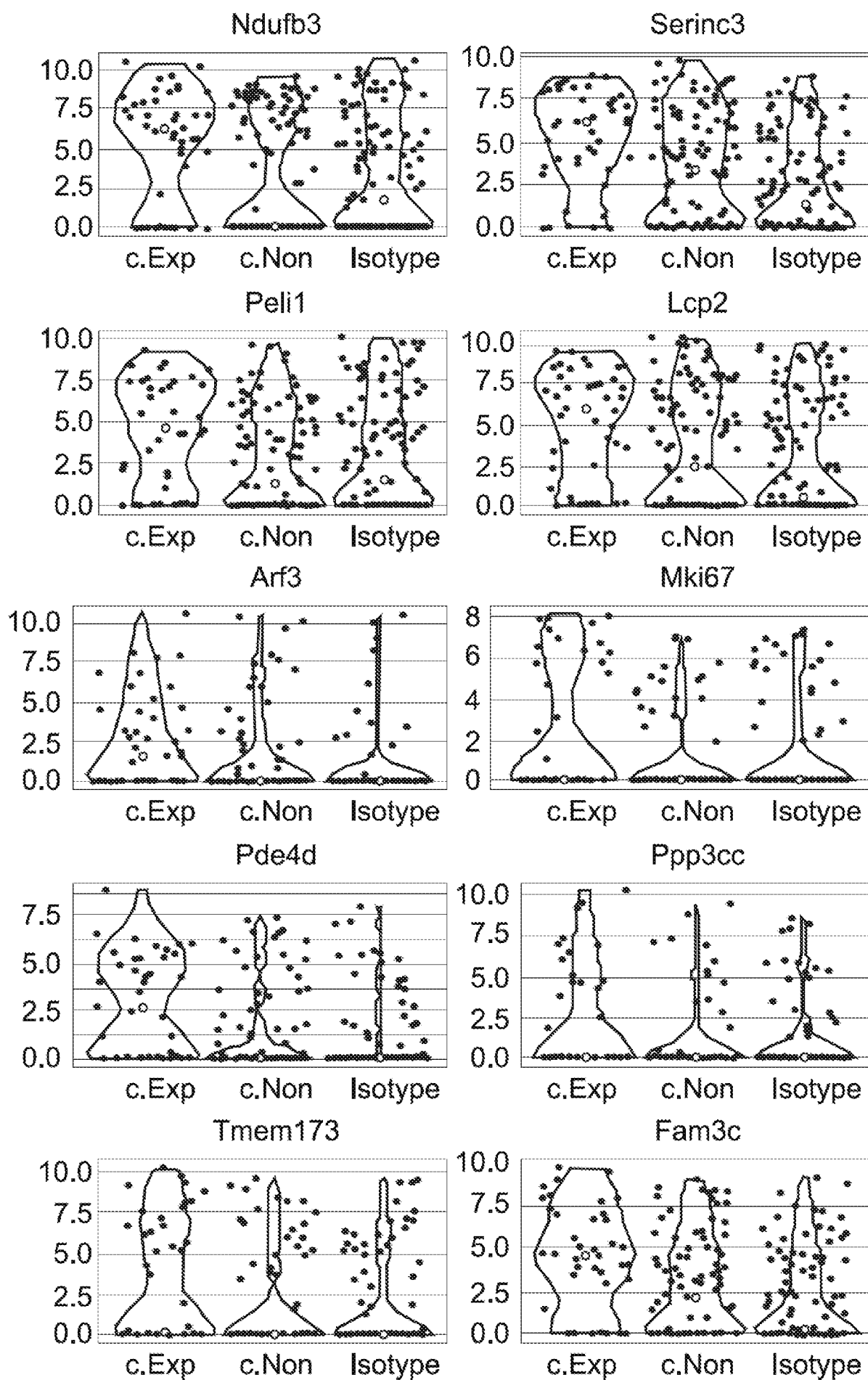


FIG. 20 Cont'd

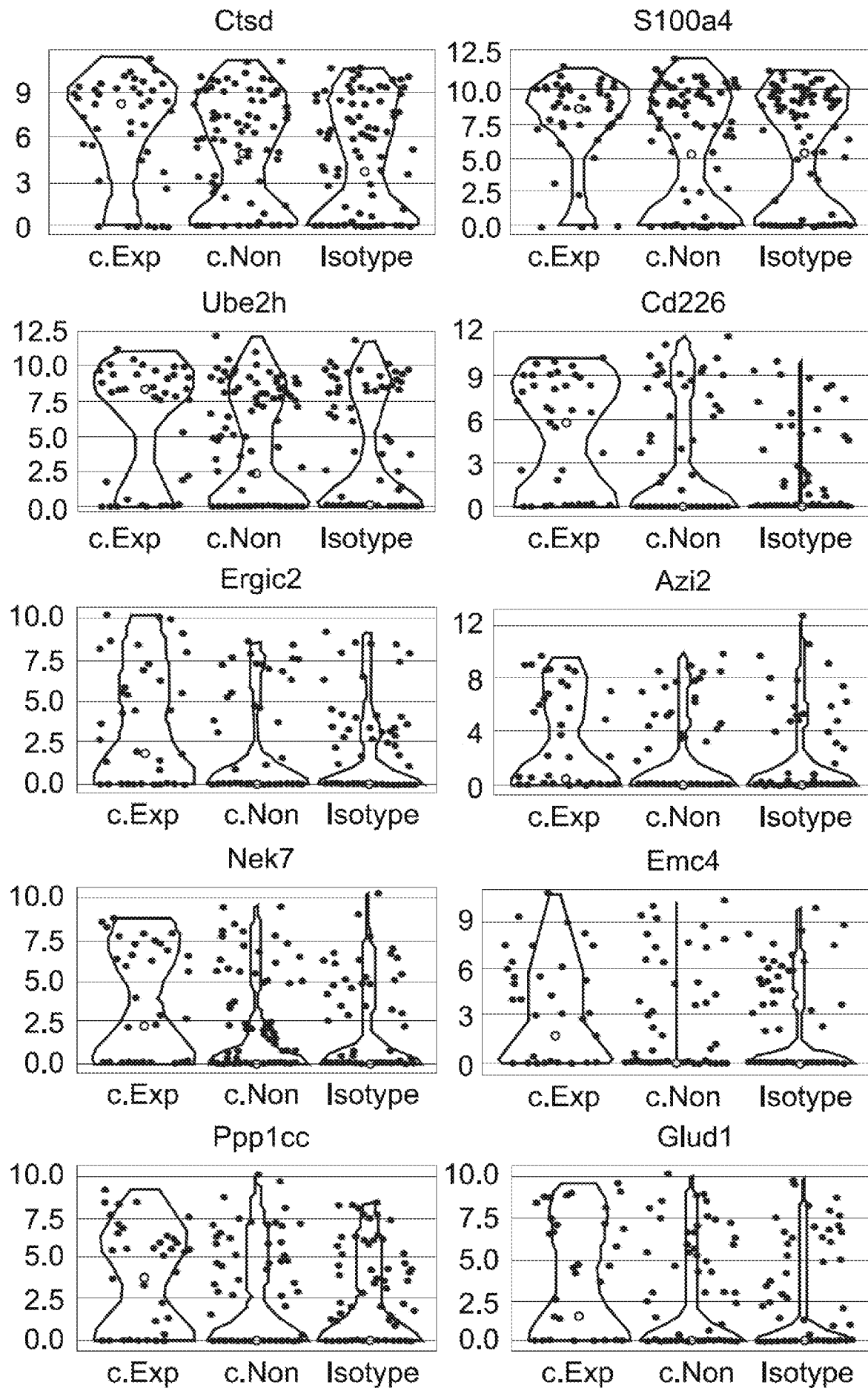
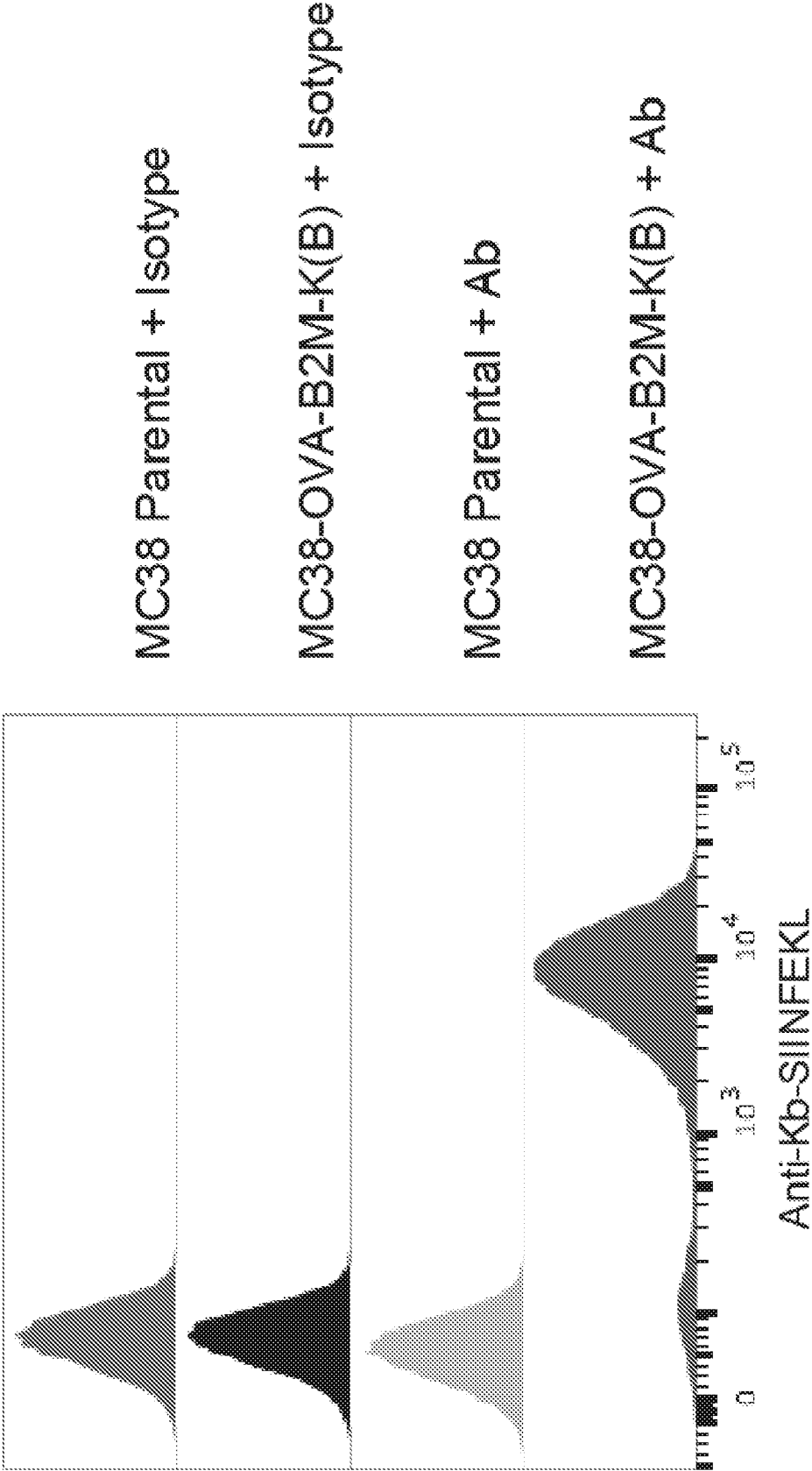


FIG. 20 Cont'd

FIG. 21A



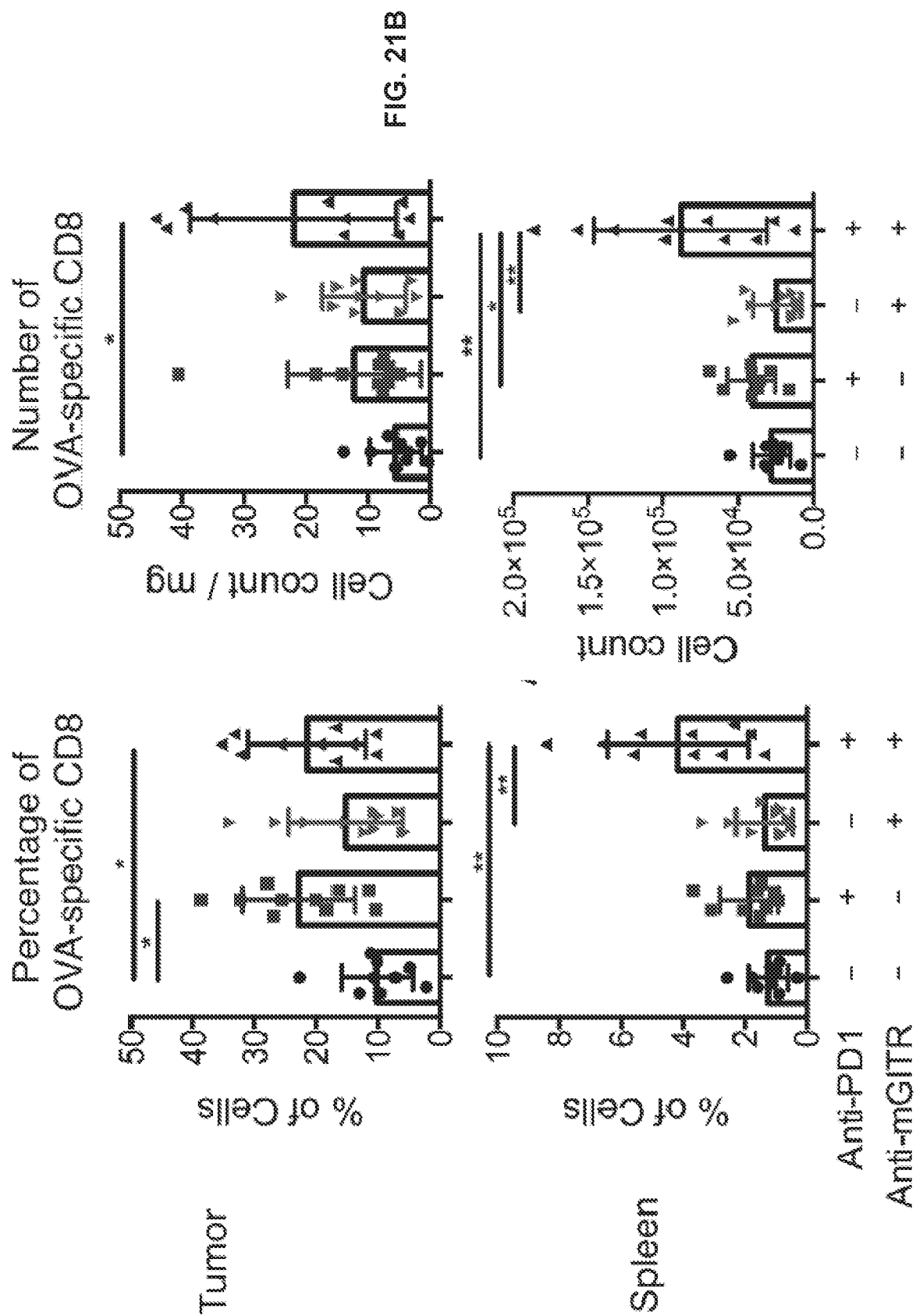


FIG. 21C

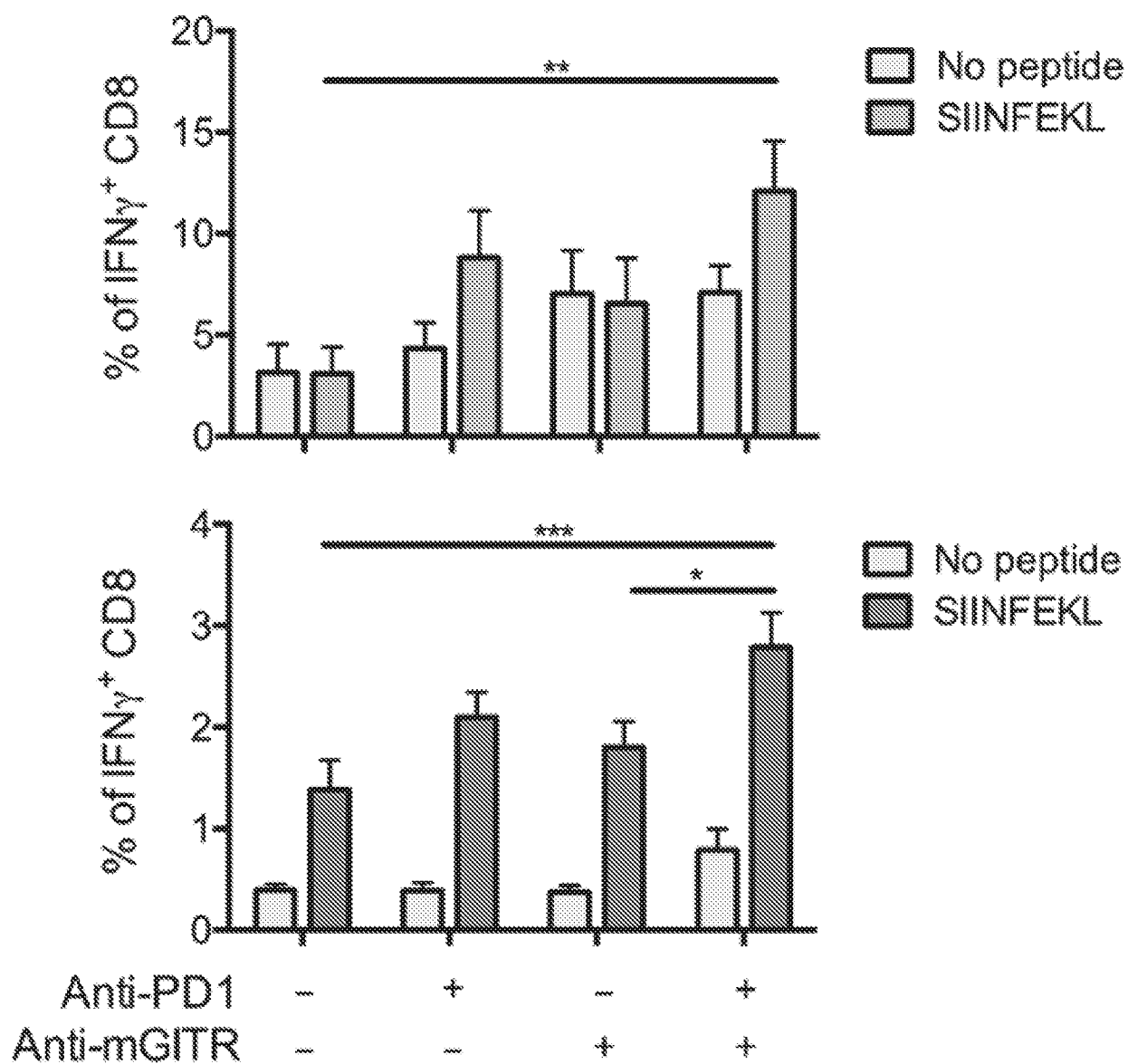


FIG. 22A

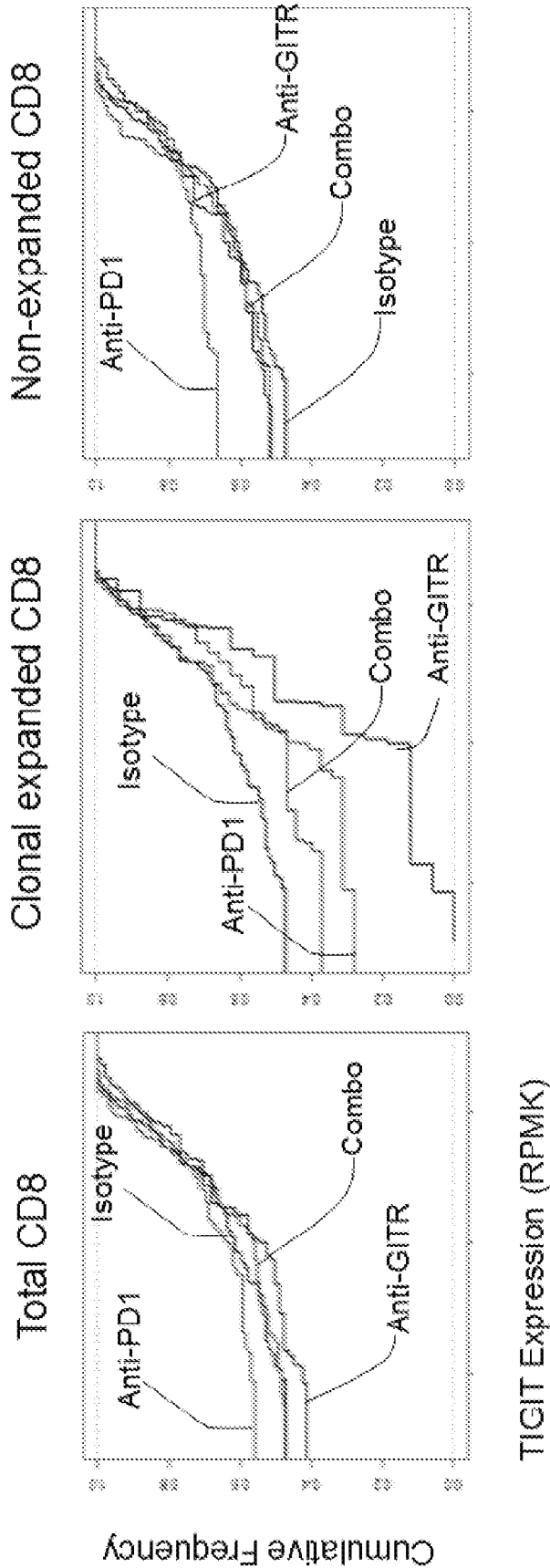


FIG. 22B

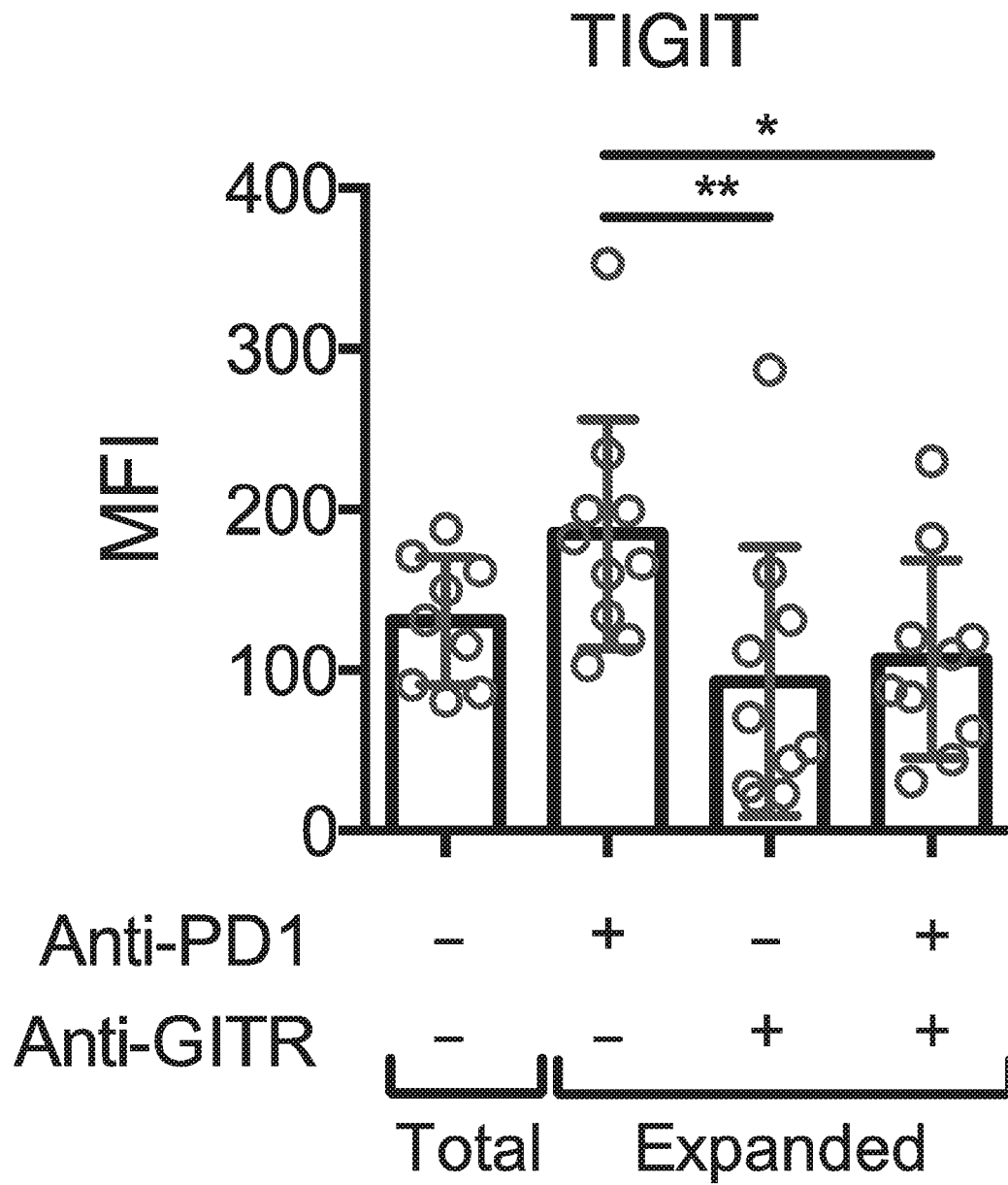


FIG. 22C

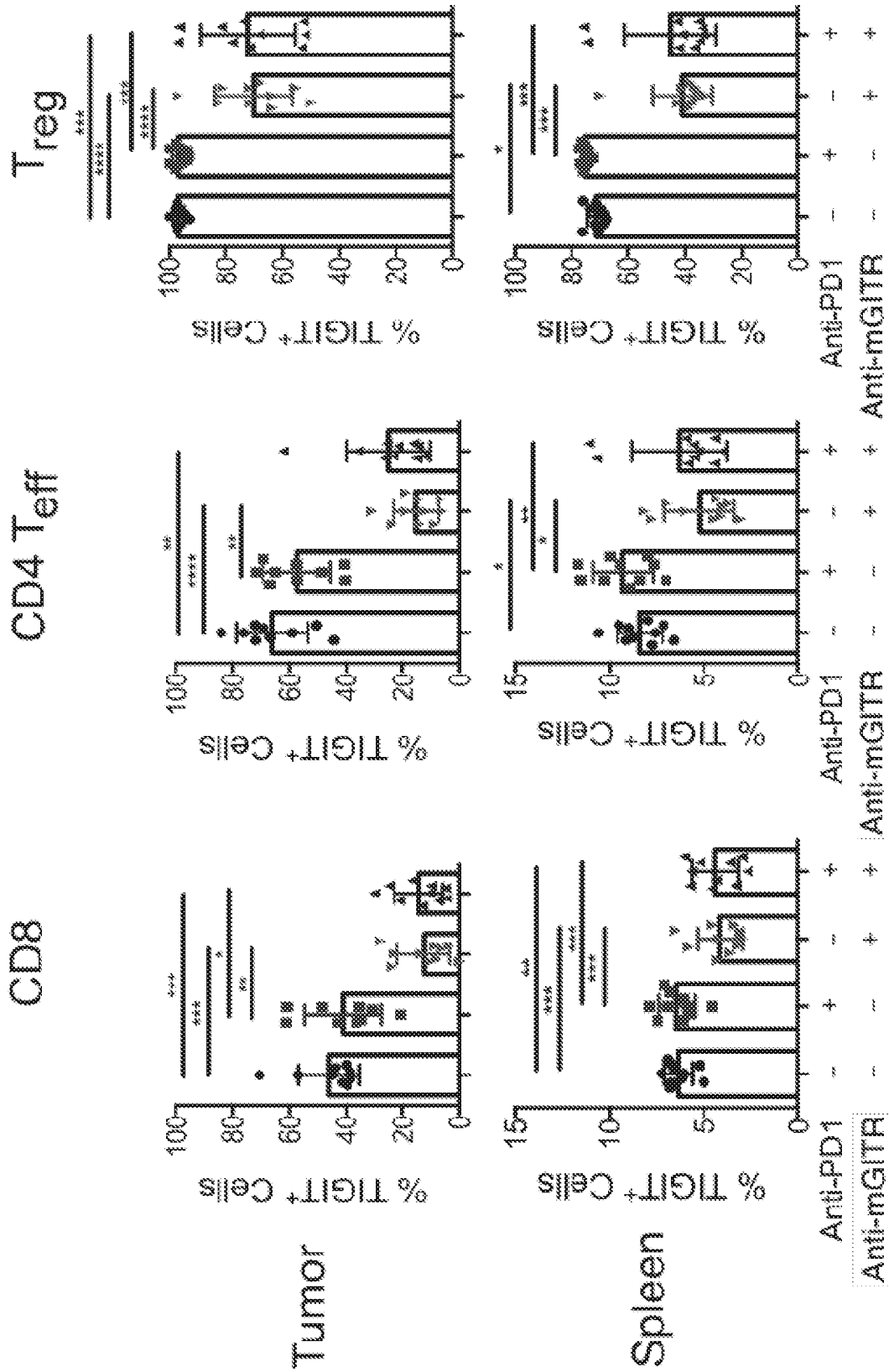
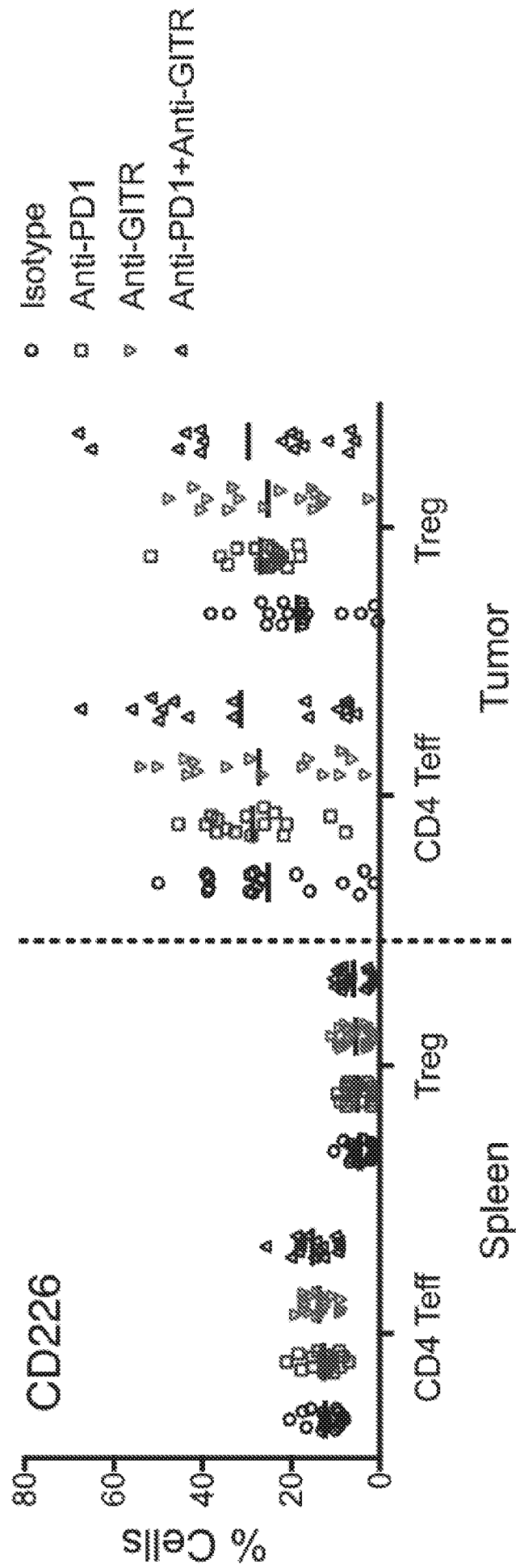


FIG. 22D



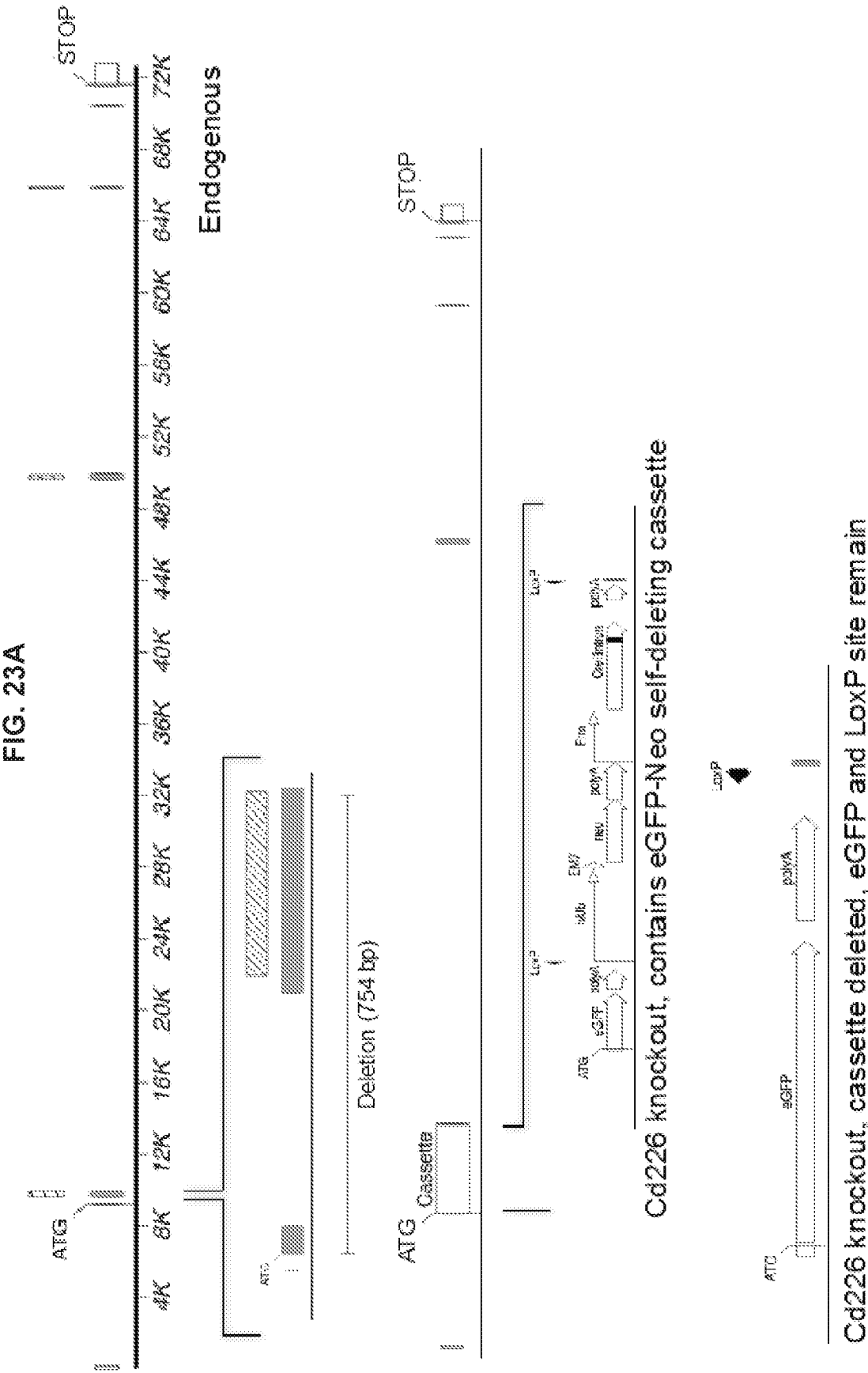


FIG. 23B

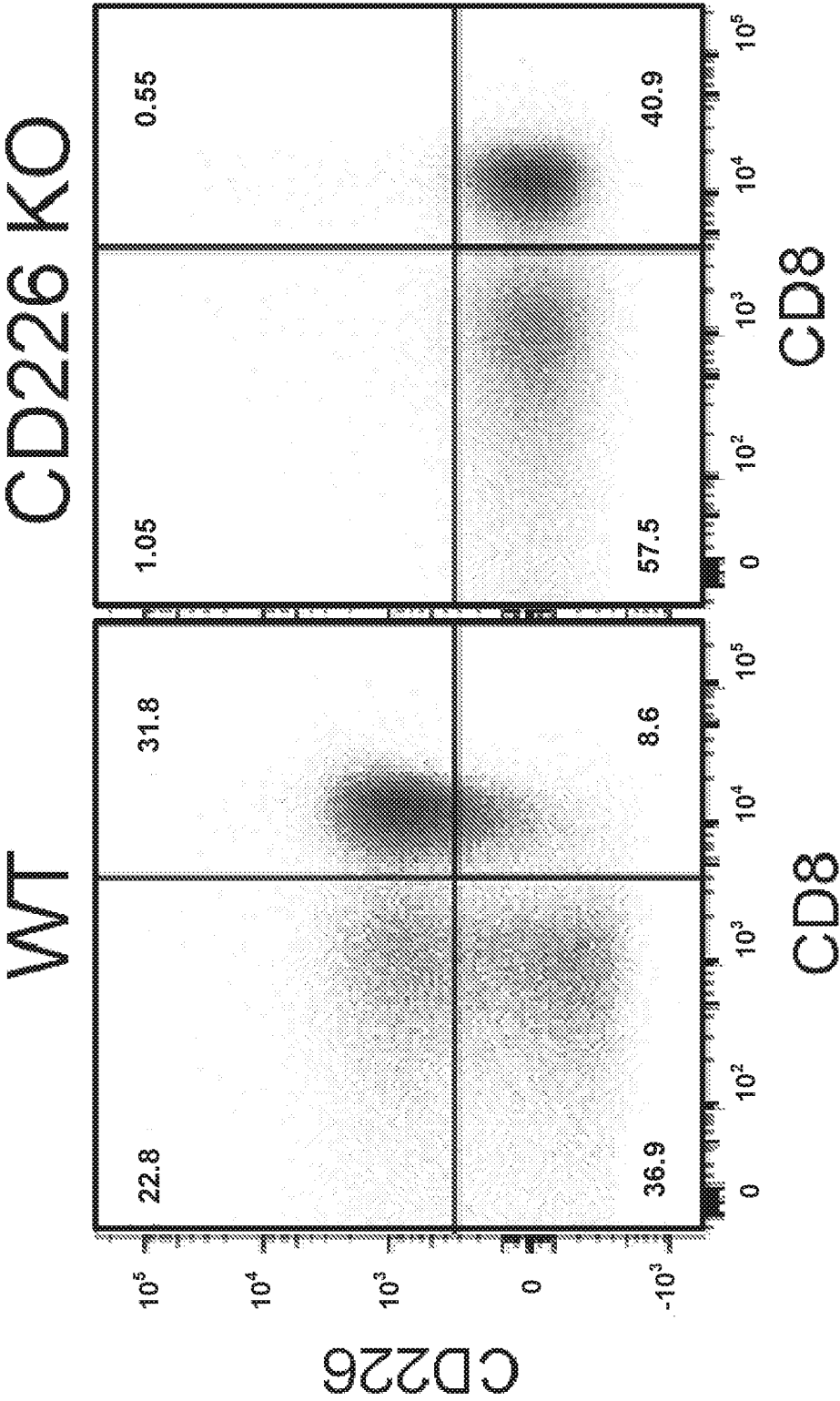
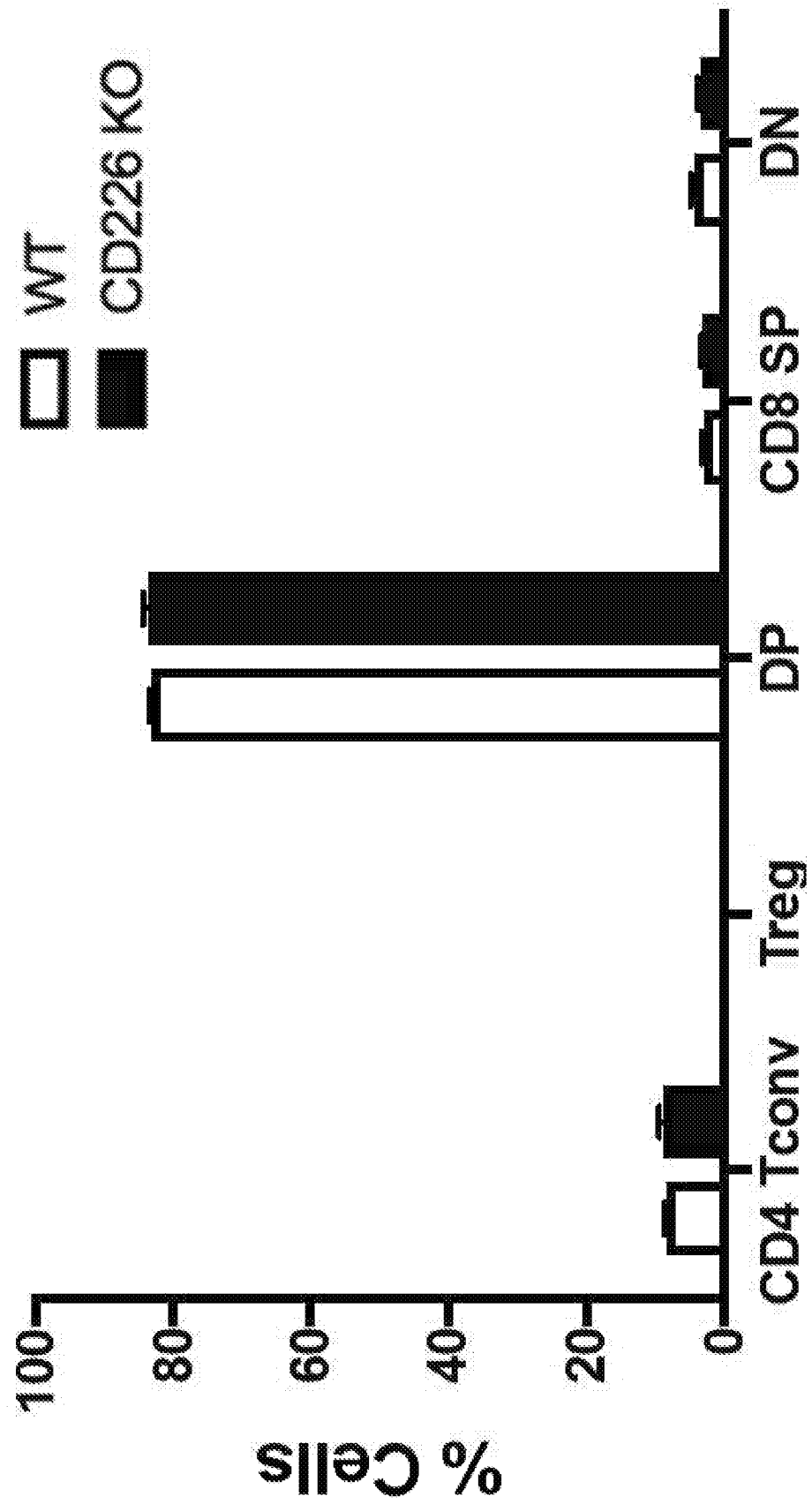


FIG. 23C



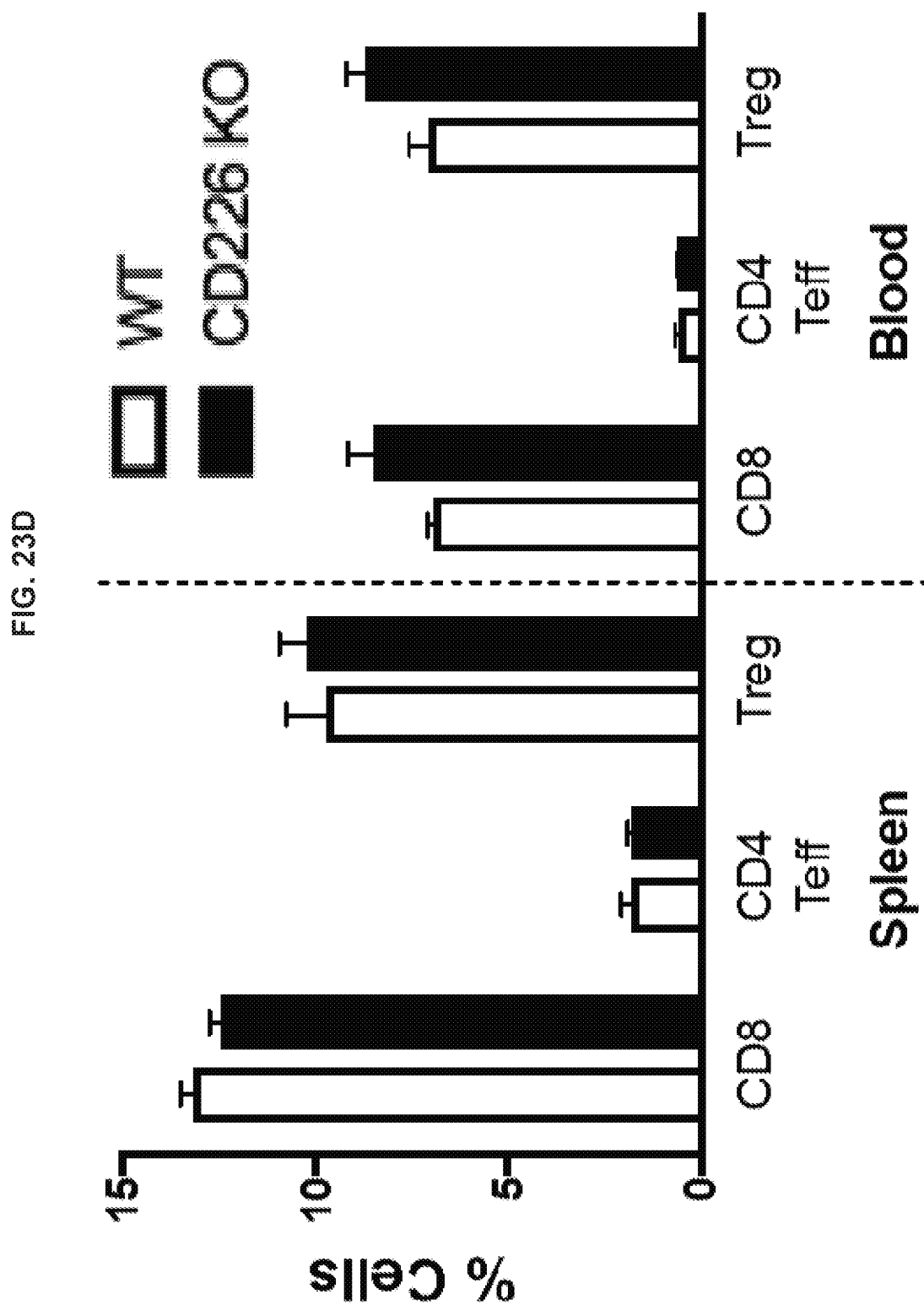


FIG. 23E

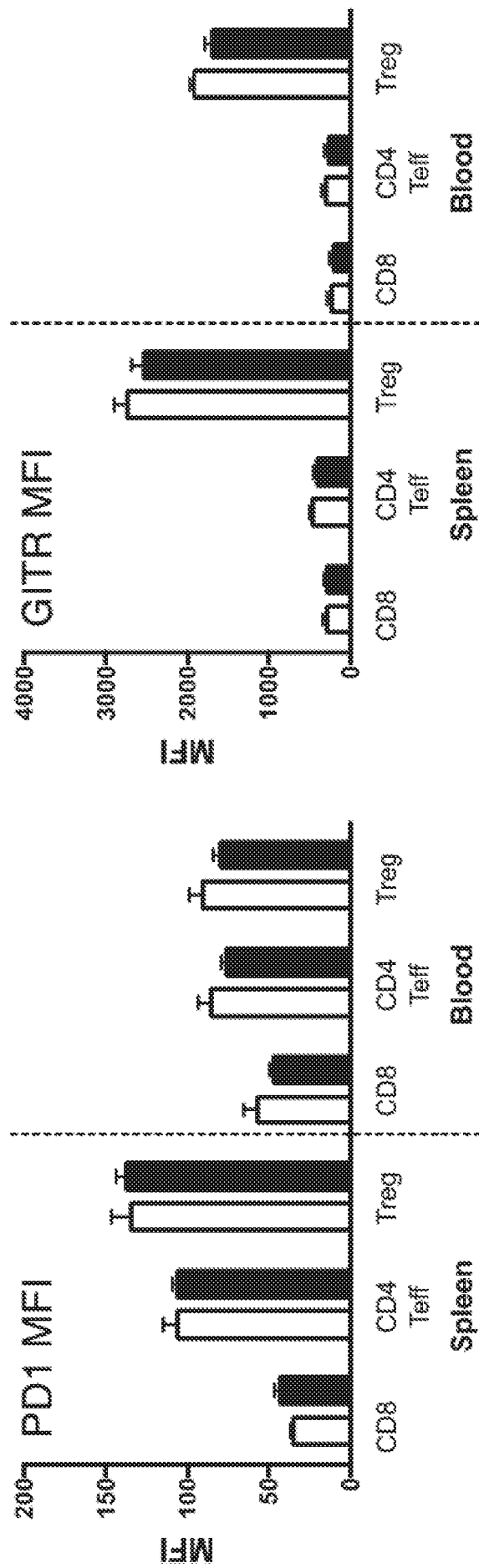


FIG. 23F

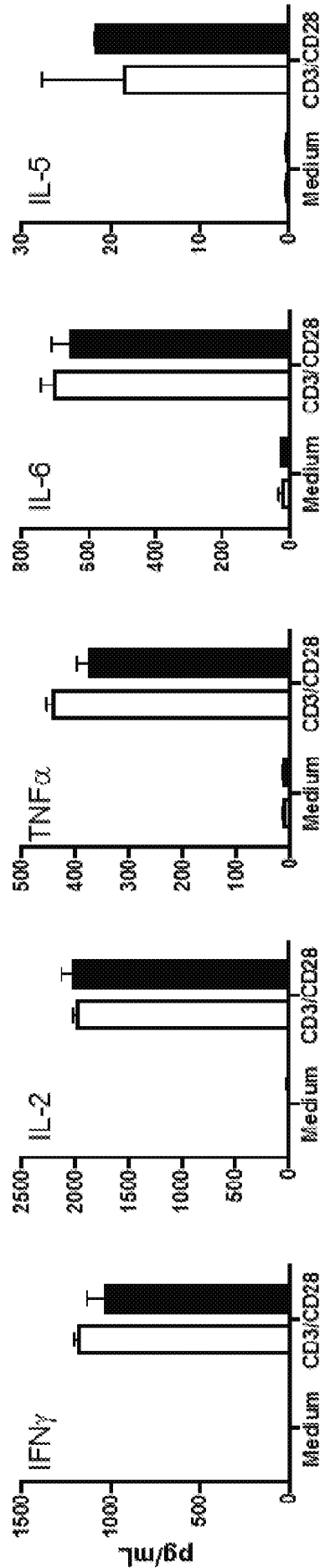


FIG. 24A

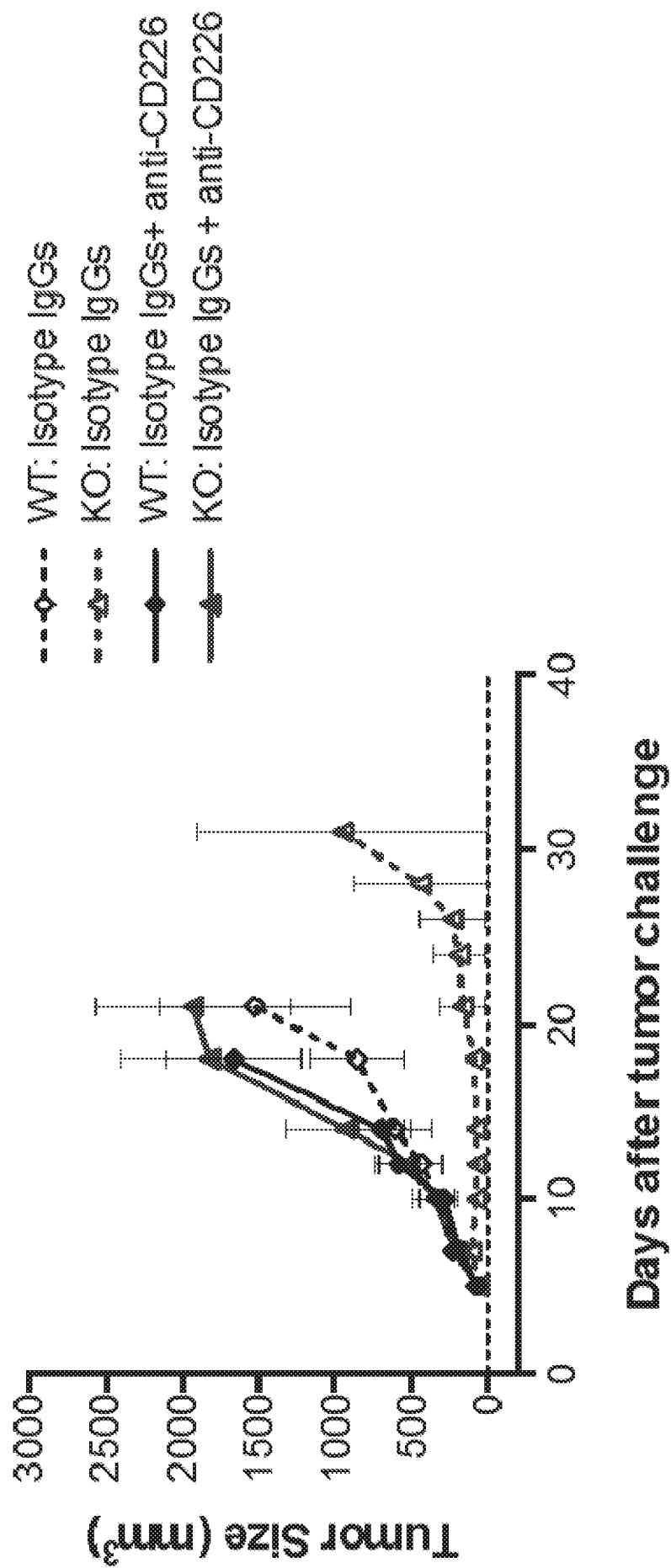
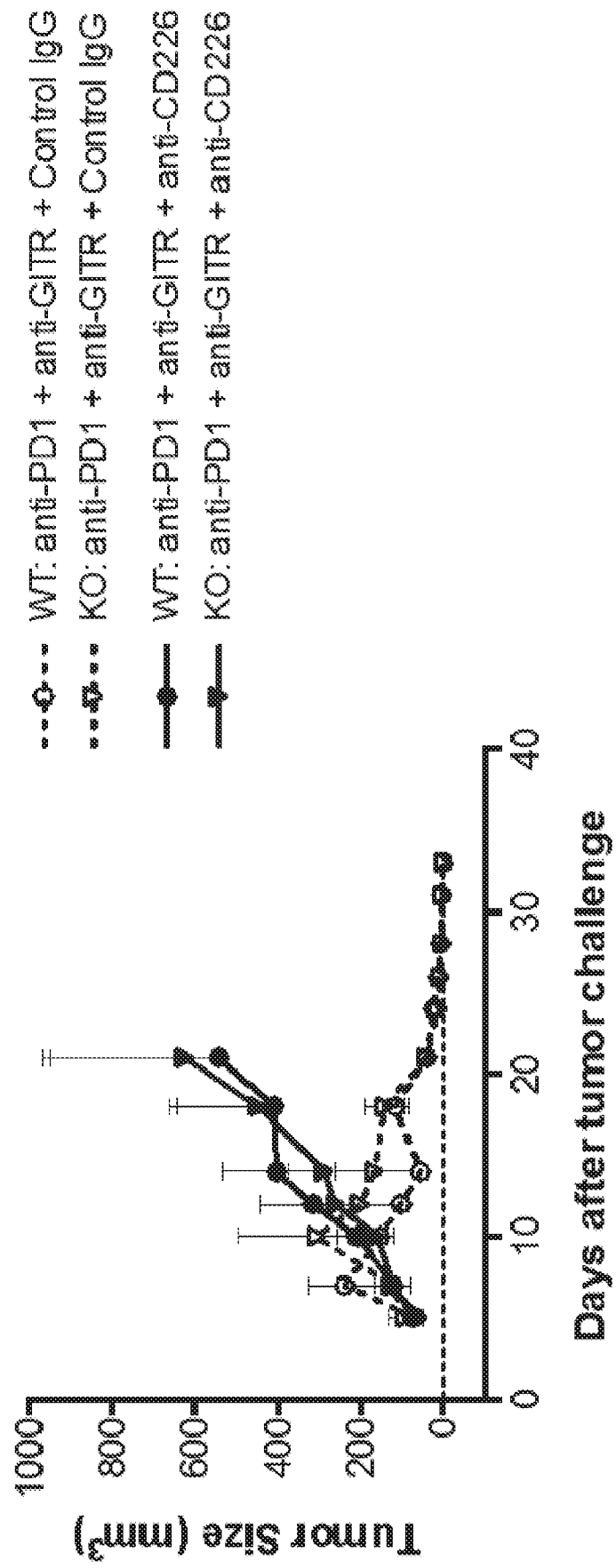


FIG. 24B



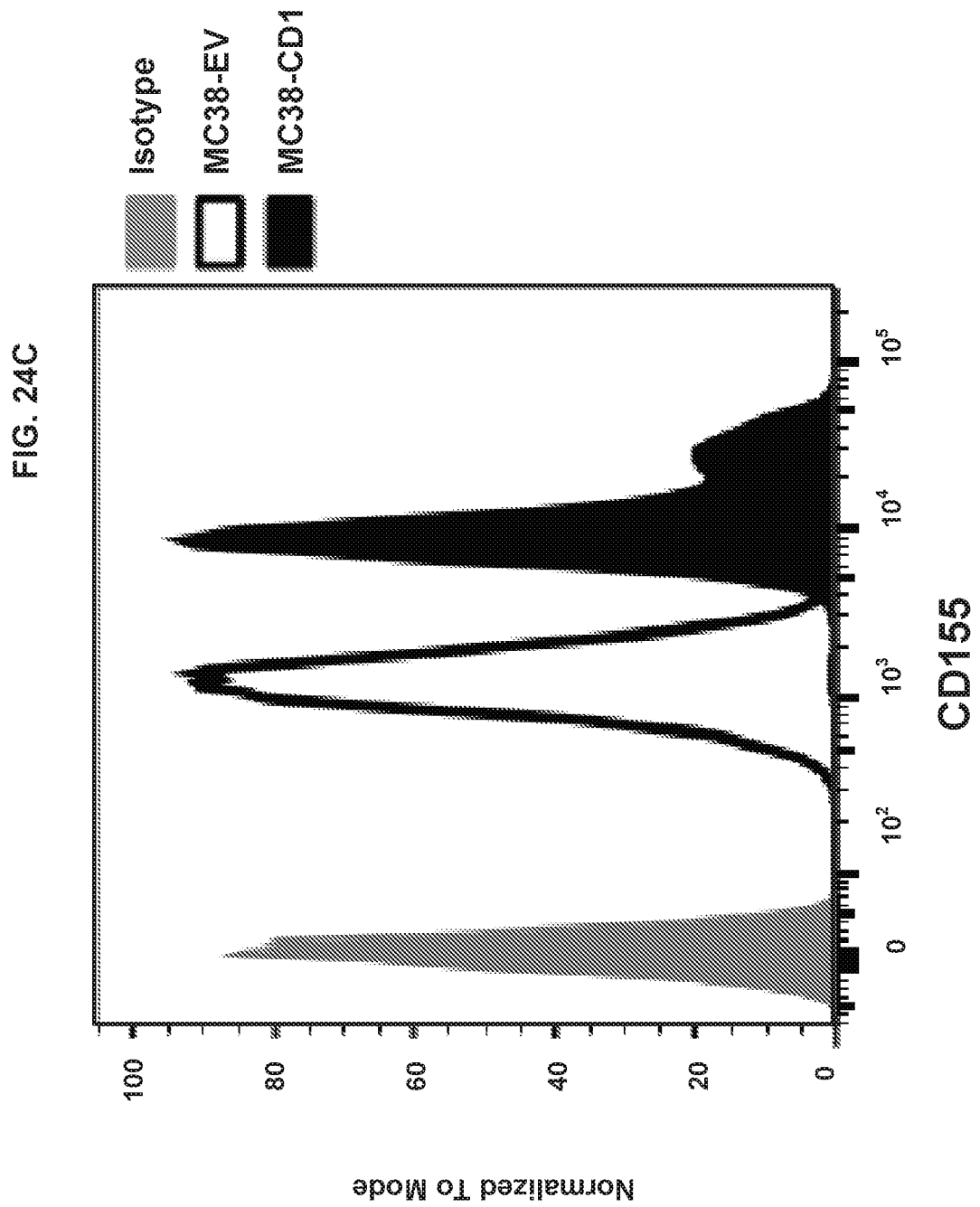
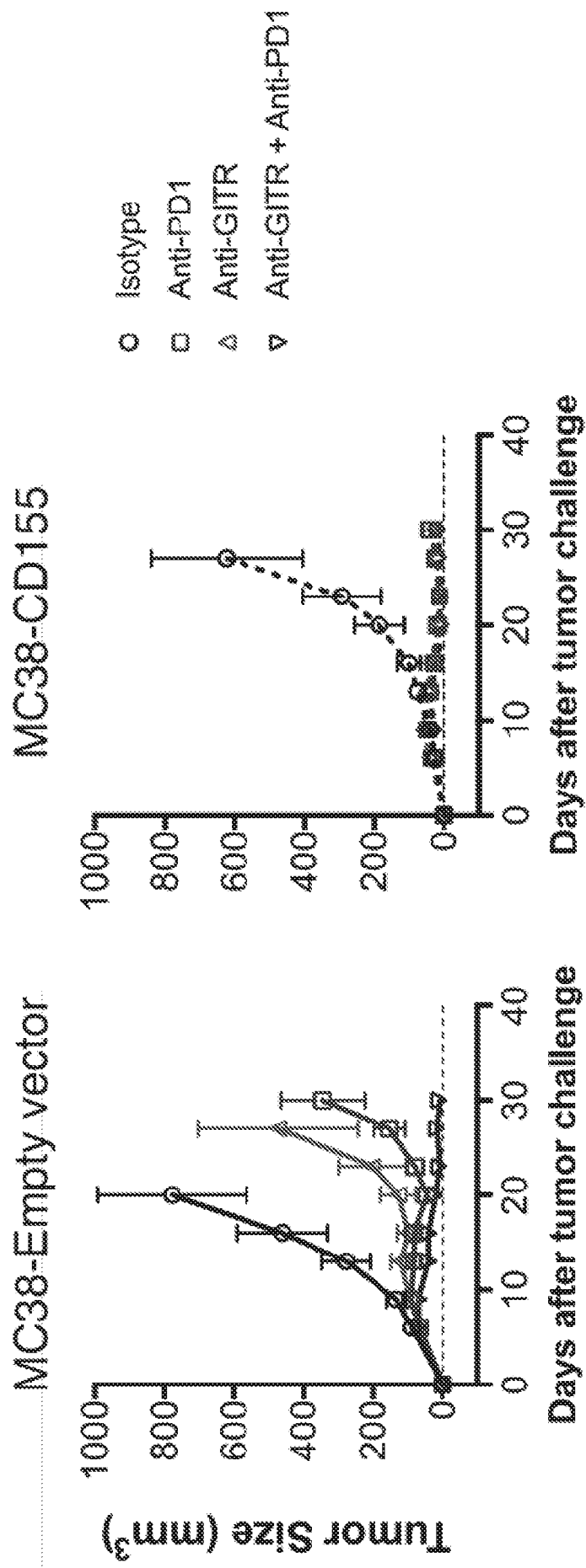


FIG. 24D



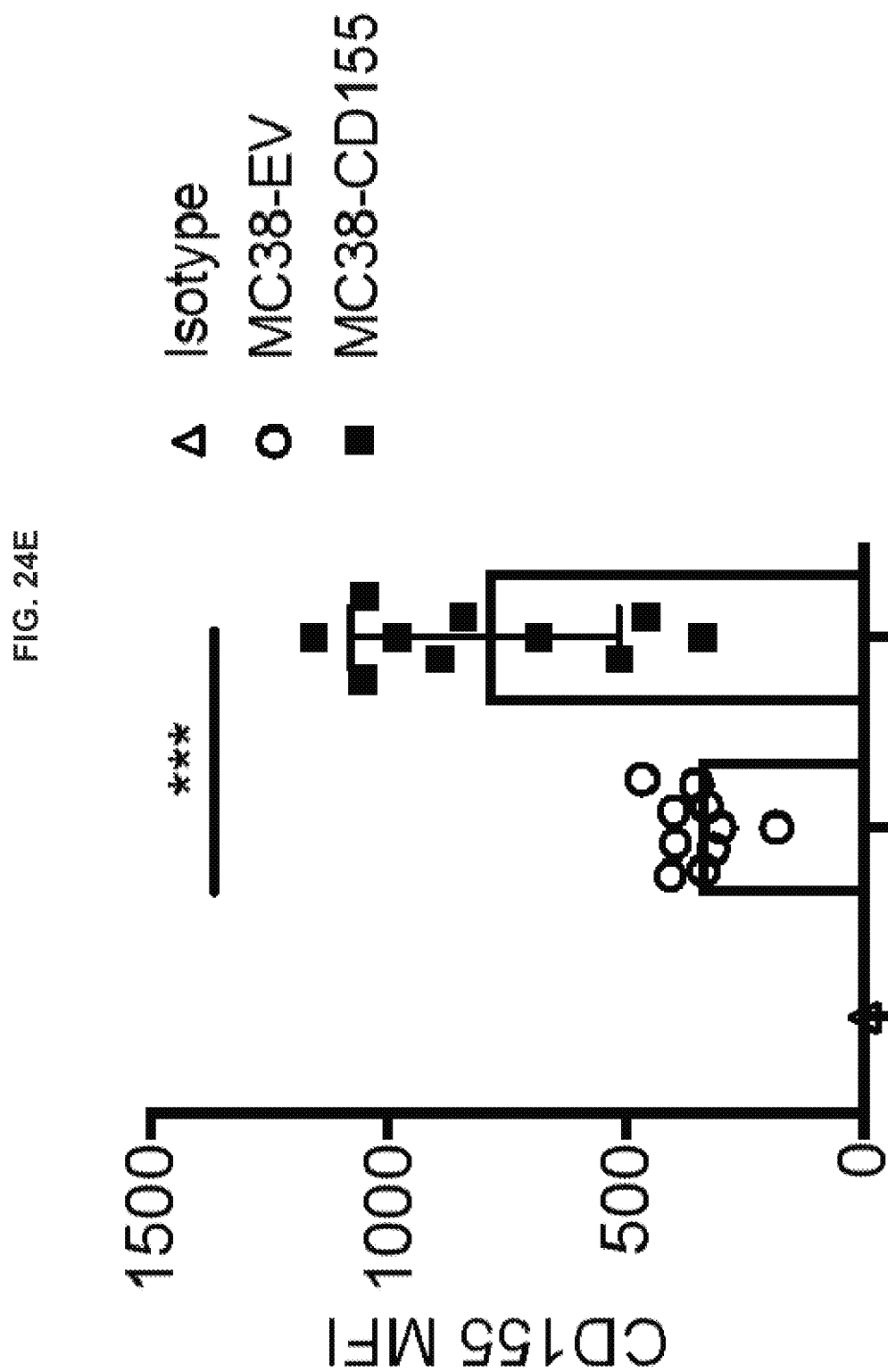


FIG. 24F

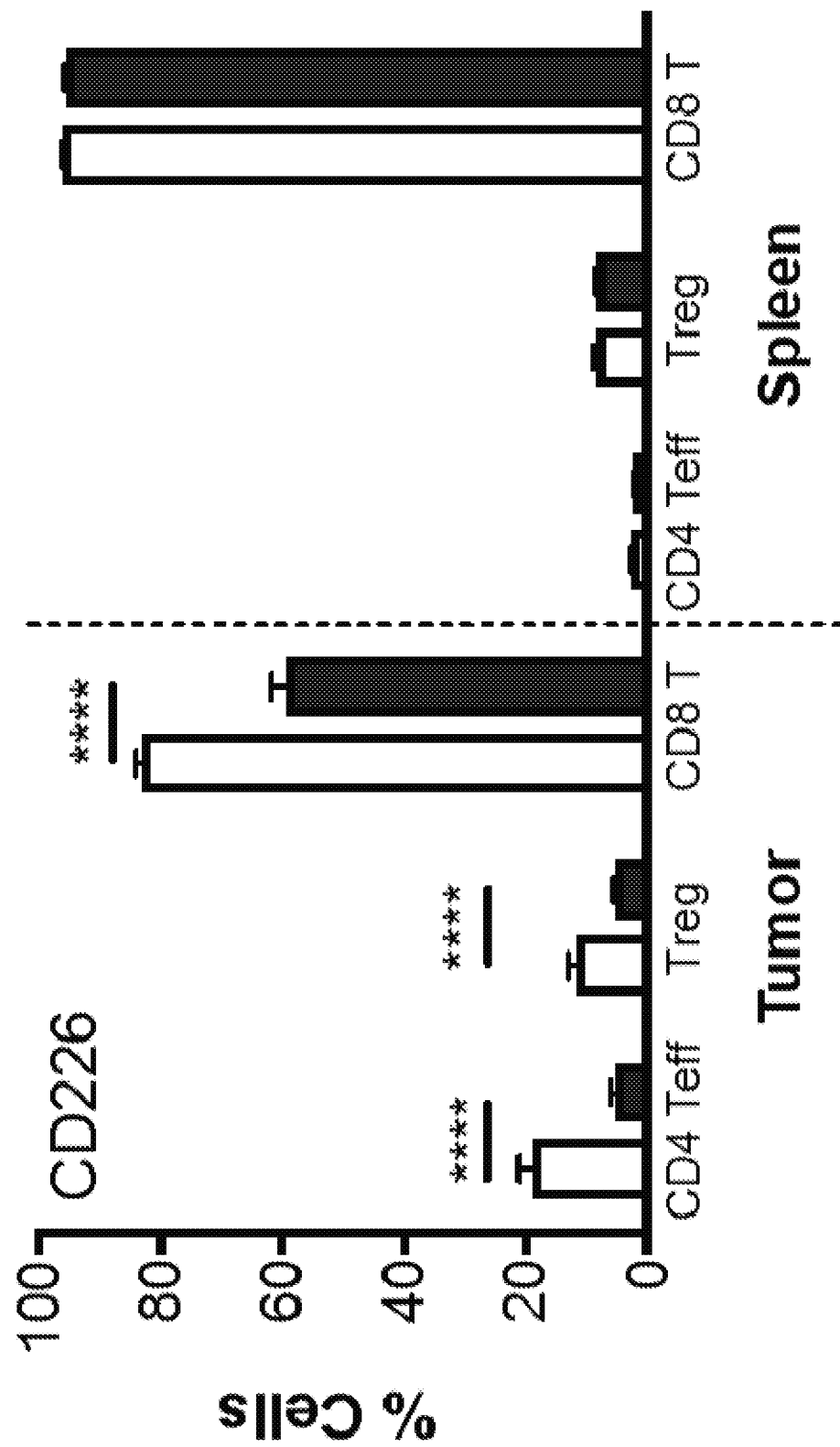
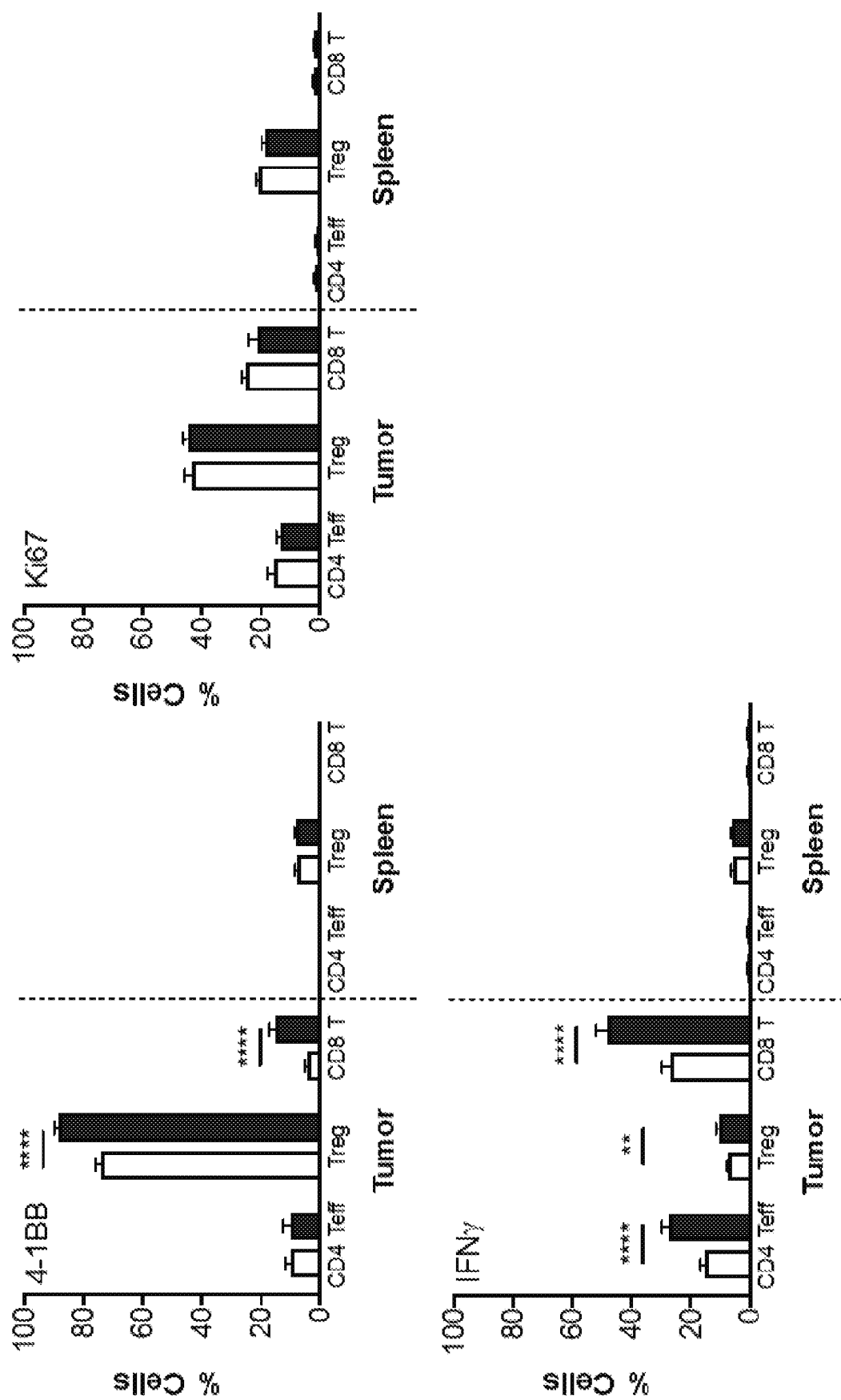


FIG. 24G



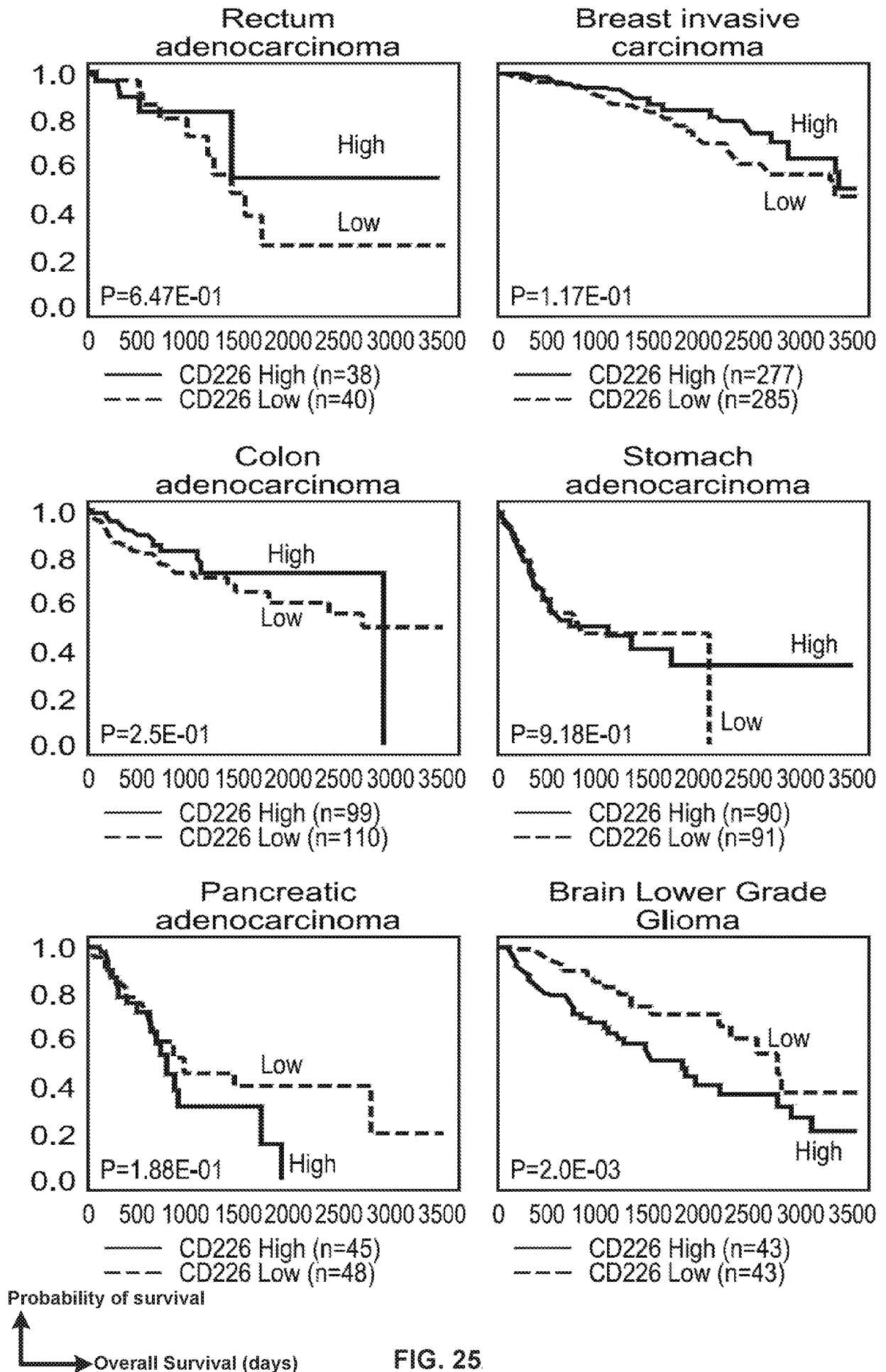


FIG. 25

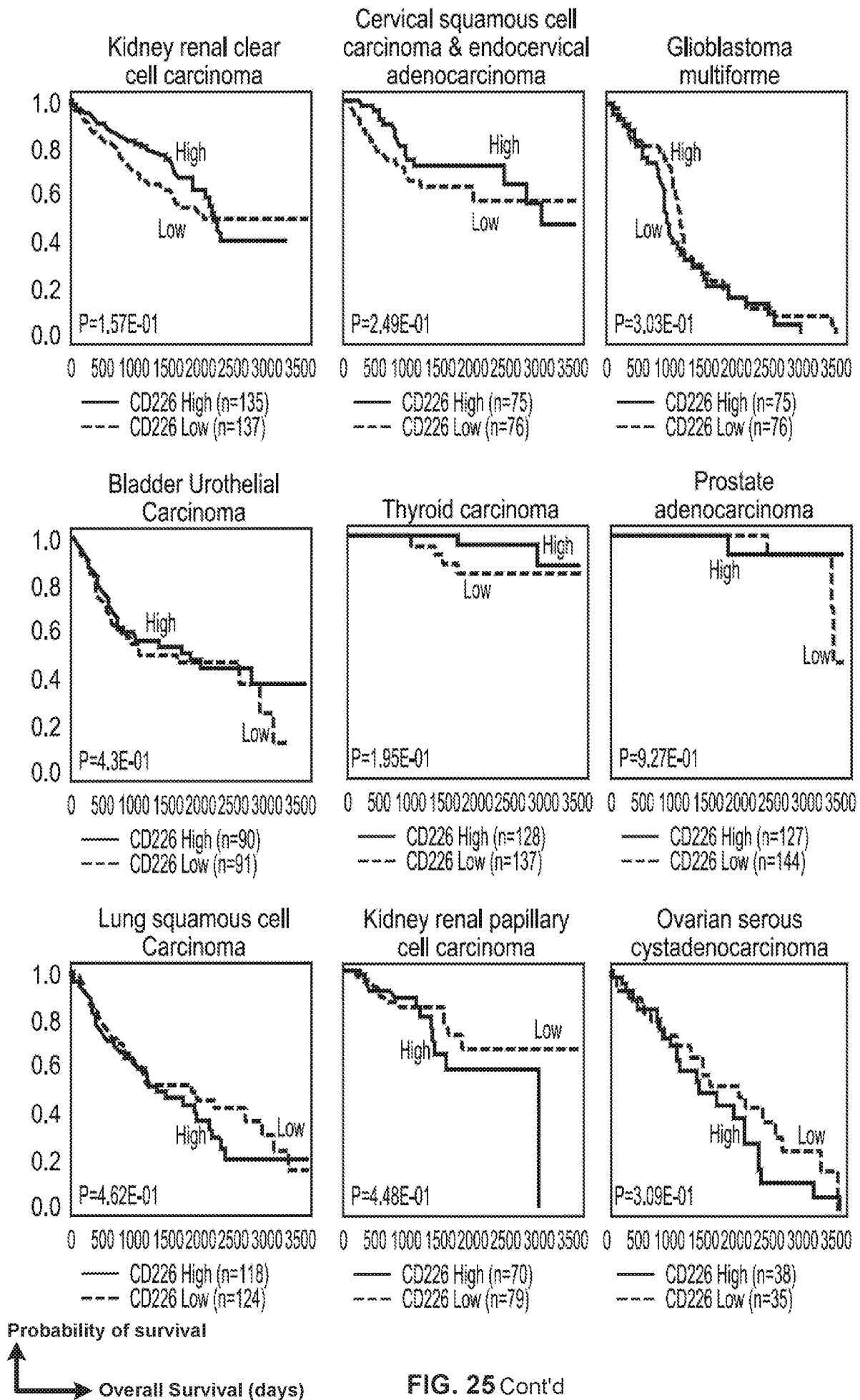


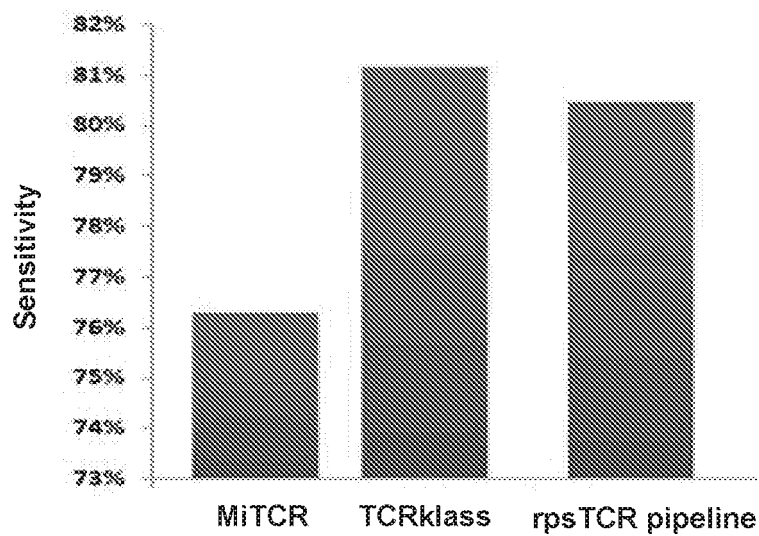
FIG. 25 Cont'd

RT primers	TCR α	5' - GCAGGTGAAGCTTGTCTGGTTGCT - 3' (SEQ ID NO:25)
	TCR β	5' - CGAGGGTAGCCCTTTTGTGTTTGC - 3' (SEQ ID NO:26)
1 st round PCR primers	TCR α	5' - ACACTCTTTCCCTACACGACGCTCTTCCGATCT TCAAAGTCGGTGAACAGGCAGAG - 3' (SEQ ID NO:27)
	TCR β	5' - ACACTCTTTCCCTACACGACGCTCTTCCGATCT GACCTTGGGTGGAGTCACATTCTC - 3' (SEQ ID NO:28)
	PE2-PIIA	5' - GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT AAGCAGTGGTATCAACGCAGAGT - 3' (SEQ ID NO:29)
	Forward	5' - AATGATACGGCGACCAACCGAGATCTACACXXXXXX ACACTCTTTCCCTACACGACGCTCTTCCGATCT- 3' (SEQ ID NO:30)
2 nd round PCR primers	Reverse	5' - CAAGCAGAAGACGGCATACGAGATXXXXXX GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT- 3' (SEQ ID NO:31)

FIG. 26

FIG. 27

Sample ID	Species	Sample type	MiTCR	TCRklass	rpsTCR pipeline	Input reads (passed QC>=25)
M620343	mouse	negative control	23	0	0	44,906,248
M620295	mouse	negative control	76	0	0	39,305,839
M620279	mouse	negative control	22	1	0	39,233,756
M620274	mouse	negative control	99	1	0	48,661,267
M620272	mouse	negative control	5	0	0	32,426,335
M620270	mouse	negative control	22	0	0	46,609,234
T-ALL_neg1-40M	mouse	negative control	17	1	0	39,068,155
T-ALL_neg2-40M	mouse	negative control	51	0	0	39,469,692
NeuProgCell-40M	human	negative control	123	0	0	37,684,399
LHCN-M2-40M	human	negative control	77	0	0	36,639,557
VI-next-mouse-T cell	mouse	positive control	128,956	137,156	135,980	169,009



Top CDR3s found by MiTCR

CDR3	Vi-next			MP-50bp-500M		
	MiTCR	TCRklass	rpsTCR	MiTCR	TCRklass	rpsTCR
CATSNYLGYF	0	0	0	63	0	0
CARHYF	0	0	0	63	0	0
CAFFYWLF	0	0	0	62	0	0
CGAGSFQHF	0	0	0	39	0	0
CARDRSIF	0	0	0	21	0	0
CARGRRITIF	0	0	0	15	0	0
CARHDLYYF	0	0	0	15	0	0
CARHGIF	0	0	0	15	0	0
CAKDRGTIF	0	0	0	14	0	0
CARGPRGIF	0	0	0	13	0	0

Top CDR3s found by TCRklass

CDR3	Vi-next			MP-50bp-500M		
	MiTCR	TCRklass	rpsTCR	MiTCR	TCRklass	rpsTCR
CASSPDADTQYF	0	0	0	3	3	0
CGAGTTETLYF	27	27	27	3	2	2
CASSDVRDTEVFF	178	174	174	2	2	0
CASSDLGEDTQYF	39	44	44	2	2	1
CTCSASAETLYF	35	30	29	2	2	1
CASSQEARNYAEQFF	21	21	21	2	2	0
CASSLGNYAEQFF	8	8	8	2	2	1
CASGDRYAEQFF	7	7	7	2	2	0
CASGDGGGNTLYF	5	5	5	2	2	2
CASSVGGQDTQYF	2	0	2	2	2	0

Top CDR3s found by rps TCR pipeline

CDR3	Vi-next			MP-50bp-500M		
	MiTCR	TCRklass	rpsTCR	MiTCR	TCRklass	rpsTCR
CASSSPGQGAREQYF	1423	1383	1374	3	0	21
CTCSAGQKAETLYF	805	788	773	1	1	9
CASSQDWGGDEQYF	287	277	274	1	1	7
CASSPGQISNERLFF	0	0	0	0	0	5
CTCSADWGAETLYF	266	265	260	2	1	4
CASSPGQLQNTLYF	103	102	102	0	0	4
CASSGRDRGLGNTLYF	77	76	75	0	0	4
CAKTENSDYTF	18	18	18	4	1	4
CASSVTGEGNTLYF	18	18	18	0	0	4
CASRRHLSYEQYF	8	8	8	3	0	4

FIG. 29

CDR3	VI-Next data (301bp)			
	Rank	MITCR	TCRklass	rps TCR pipeline
CASSSPGQGAREQYF	1	1423	1383	1374
CTCSAGQKAETLYF	2	805	788	773
CTCSAERASAETLYF	3	337	330	326
CASSRDWGASAETLYF	4	329	323	323
CASSQDWGGDEQYF	5	287	277	274
CTCSADWGAETLYF	6	266	265	260
CASGDAPGTNSDYFT	7	237	231	231
CASSDGTGDYAEQFF	8	224	217	217
CASSDGGKANERLFF	9	215	211	211
CTCSADGRGNYAEQFF	10	190	189	188

CDR3	MP-50BP-MITCR				
	10M	50m	100m	200m	500m
CASSSPGQGAREQYF	0	0	0	1	3(rank 15)
CTCSAGQKAETLYF	0	0	0	0	1
CTCSAERASAETLYF	0	0	0	0	1
CASSRDWGASAETLYF	0	0	0	0	0
CASSQDWGGDEQYF	0	0	0	1	1
CTCSADWGAETLYF	0	0	0	0	2
CASGDAPGTNSDYFT	0	0	0	0	0
CASSDGTGDYAEQFF	0	0	0	1	1
CASSDGGKANERLFF	0	0	0	0	0
CTCSADGRGNYAEQFF	0	0	0	0	0

CDR3	MP-50bp-TCRklass				
	10M	50m	100m	200m	500m
CASSSPGQGAREQYF	0	0	0	0	0
CTCSAGQKAETLYF	0	0	0	0	1
CTCSAERASAETLYF	0	0	0	0	0
CASSRDWGASAETLYF	0	0	0	0	0
CASSQDWGGDEQYF	0	0	0	1	1
CTCSADWGAETLYF	0	0	0	0	1
CASGDAPGTNSDYFT	0	0	0	0	0
CASSDGTGDYAEQFF	0	0	0	1	1
CASSDGGKANERLFF	0	0	0	0	0
CTCSADGRGNYAEQFF	0	0	0	0	0

CDR3	MP-50bp-rpsTCR pipeline				
	10M	50m	100m	200m	500m
CASSSPGQGAREQYF	1	2	6(rank 1)	9(rank 1)	21(rank 1)
CTCSAGQKAETLYF	0	0	3(rank 2)	4(rank 2)	9(rank 2)
CTCSAERASAETLYF	0	0	0	1	1
CASSRDWGASAETLYF	0	0	0	0	1
CASSQDWGGDEQYF	0	0	0	2	7(rank 3)
CTCSADWGAETLYF	0	0	1	1	4(rank 5)
CASGDAPGTNSDYFT	0	0	0	2	2
CASSDGTGDYAEQFF	0	0	0	0	2
CASSDGGKANERLFF	0	0	0	0	0
CTCSADGRGNYAEQFF	0	0	0	0	0

Top CDR3s found by MiTCR

CDR3	Vi-next			MP-100bp-200M		
	MiTCR	TCRklass	rpsTCR	MiTCR	TCRklass	rpsTCR
CATSNYLG YF	0	0	0	106	0	0
CARHYF	0	0	0	85	0	0
CAFFYWLF	0	0	0	83	0	0
CGAGSFQHF	0	0	0	60	0	0
CARGRRITIF	0	0	0	49	0	0
CAKDGGVTIF	0	0	0	33	0	0
CARDRSIF	0	0	0	31	0	0
CARGPRGIF	0	0	0	31	0	0
CARHDLYYF	0	0	0	25	0	0
CASSFKDKRMQDVNF	0	0	0	24	0	0

Top CDR3s found by TCRklass

CDR3	Vi-next			MP-100bp-200M		
	MiTCR	TCRklass	rpsTCR	MiTCR	TCRklass	rpsTCR
CASLQYF	0	0	0	0	99	0
CASGGKKTSTAFF	0	0	0	0	97	0
CASSSPGQGAREQYF	1423	1383	1374	23	18	13
CASRRGIGNTL YF	87	86	86	9	8	8
CTCSAGQKAETLYF	805	788	773	8	5	7
CASRRHLSYE QYF	8	8	8	6	5	6
CASGDAPGTNSDYTF	237	231	231	6	4	4
CASSQDWGGDEQYF	287	277	274	4	4	4
CASSGRDRGLGNTLYF	77	76	75	4	4	4
CGARDRGS GNTLYF	1	1	1	4	4	7

Top CDR3s found by rps TCR pipeline

CDR3	Vi-next			MP-100bp-200M		
	MiTCR	TCRklass	rpsTCR	MiTCR	TCRklass	rpsTCR
CASSSPGQGAREQYF	1423	1383	1374	23	18	13
CASRRGIGNTL YF	87	86	86	9	8	8
CTCSAGQKAETLYF	805	788	773	8	5	7
CGARDRGS GNTLYF	1	1	1	4	4	7
CASRRHLSYE QYF	8	8	8	6	5	6
CASSQDPWGV EQYF	2	2	2	3	3	5
CASGDAPGTNSDYTF	237	231	231	6	4	4
CASSQDWGGDEQYF	287	277	274	4	4	4
CASSGRDRGLGNTLYF	77	76	75	4	4	4
CASSDHSSAETLYF	78	77	77	3	3	4

FIG. 31

CDs	V-band data (2013ep)		MP-1000p-MITC8					MP-1000p-TCSdata					MP-1000p- rps1Cr pipeline						
	Rank	MITC8	TCSdata	Wendy	120M	30M	100M	200M	300M	120M	30M	100M	200M	300M	120M	30M	100M	200M	300M
CASSPQCGAREQYF	1	1423	1383	1376	3 (rank 2)	9 (rank 6)	14 (rank 8)	23 (rank 11)	35 (rank 12)	3 (rank 3)	6 (rank 3)	10 (rank 3)	13 (rank 3)	42 (rank 3)	3 (rank 3)	3 (rank 3)	3 (rank 3)	7 (rank 3)	13 (rank 3)
CTCSAGQKAE11YF	2	805	785	772	0 (rank 11)	4 (rank 13)	8 (rank 13)	8 (rank 20)	22 (rank 26)	0	0	2 (rank 4)	3 (rank 4)	16 (rank 4)	0	0	0	3 (rank 3)	7 (rank 3)
CTCSAEPASAE11YF	3	337	320	306	0	0	1 (rank 23)	3 (rank 23)	9 (rank 35)	0	0	0	1 (rank 7)	6 (rank 8)	0	0	0	0	0
CASS8QW6ASAE11YF	4	329	323	320	0	0	1	2 (rank 35)	5 (rank 35)	0	0	0	1	3 (rank 11)	0	0	0	1	1
CASS8QW6G8QYF	5	287	277	274	0	0	1	4 (rank 26)	9 (rank 35)	0	0	0	1	4 (rank 6)	0	0	0	1	1
CTCSADW6AE11YF	6	366	363	366	0	0	0	2 (rank 37)	7 (rank 37)	0	0	0	1	3 (rank 10)	0	0	0	0	0
CASS8QW6G8QYF	7	337	331	333	0	0	0	2 (rank 23)	6 (rank 38)	0	0	0	1	4 (rank 10)	0	0	0	3 (rank 3)	4 (rank 3)
CASS8QW6G8QYF	8	224	217	217	0	0	2 (rank 26)	3 (rank 38)	9 (rank 38)	0	0	1	1	2 (rank 7)	0	0	2	2	2
CASS8QW6G8QYF	9	215	211	213	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CTCSADW6AE11YF	10	190	189	186	0	0	0	0	1	1	0	0	0	1	1	0	0	0	1

FIG. 32

CDR3	# of cells	Single cell sequencing (75bp)			Bulk RNA sequencing (80bp)		
		MITCR	TCRklass	rpsTCR	MITCR	TCRklass	rpsTCR
CASSPTGYNSPLYF	4	495	465	982	242	302	512
CASSQVQGS AETLYF	9	142	138	646	200	183	394
CASSGTGGNQDTQYF	1	495	408	595	0	0	0
CASGDAGTGN YAEQFF	1	282	247	399	32	26	29
CASSLRTGYNSPLYF	3	206	202	294	123	141	114
CASRLGGDQNTLYF	3	292	151	291	82	53	131
CASKTGGYEQYF	1	297	140	285	154	93	75
CASSEGD TLYF	1	370	307	229	46	38	18
CASSPGTFNQDTQYF	3	113	78	195	45	23	36
CASASWTGDEQYF	1	110	49	144	0	0	0
CASSLPQSQNTLYF	1	98	61	140	0	0	0
CASSRDWAQDTQYF	2	127	81	128	141	125	169
CASSDNWGAGEQYF	1	161	107	99	5	5	2
CASSSGTASDTQYF	1	41	30	86	0	0	0
CASSQTRDWG YEQYF	1	33	17	75	28	18	70
CTCSGGLGLEQYF	1	66	34	74	10	6	9
CASSLGTGGIEQYF	1	56	32	54	4	4	5
CASSLSDSNQDTQYF	1	52	47	50	0	0	0
CASSERGG RDTQYF	1	49	34	46	0	0	0
CTCSAVREGNSPLYF	1	41	34	46	6	5	4
CASSLTGVSNERLFF	1	74	63	42	0	0	0
CASSRQLNSDYTF	2	50	46	35	129	114	96
CASSLRQGSNTEVFF	1	34	32	34	40	28	20
CASSQNRDISAETLYF	1	22	23	34	77	76	117
CASSWTANTEVFF	1	72	42	30	0	0	0
CASSLRDWGQDTQYF	1	26	19	28	39	27	39
CASSHWGGTTGQLYF	1	28	27	21	0	0	0
CASSYSKGS AETLYF	1	17	17	17	0	0	0
CAVSMINYNVLYF	1	0	1	17	0	6	15
CASSDGQYEQYF	1	10	7	14	0	0	0
CASSQRTGQNTLYF	1	12	9	6	0	0	0
CASSQEGPGQLYF	1	8	7	5	39	35	27
CASTGQGYNSPLYF	1	0	1	1	0	0	0
CGANSQIQLAVFF	1	5	0	0	0	0	0
CSFIYF	1	4	0	0	5	0	0
CAARGYNQGKLIF	1	5	0	0	0	0	0