FIG. 1.

(1) Wild Type IL12B without signal (IL12B) Amino Acids (SEQ ID NO: 1):
IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEV
LSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTC
GAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK
PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSS
WSEWASVPCS

(2) Wild Type IL12B without signal (IL12B) Nucleic Acids (SEQ ID NO: 2):
ATATGGGAACTGAAGAAAGATGTTTATGTCGTAGAATTGGATTGGTATCCGGATGCCCCTGGAGAAATGGTGGT
CCTCACCTGTGACACCCCTGAAGAAGATGGTATCACCTGGACCTTGGACCAGAGCAGTGAGGTCTTAGGCTCTG
GCAAAACCCTGACCATCCAAGTCAAAGAGTTTGGAGATGCTGGCCAGTACACCTGTCACAAAGGAGGCGAGGTT
CTAAGCCATTCGCTCCTGCTCCTTCACAAAAAGGAAGATGGAATTTGGTCCACTGATATTTTAAAGGACCAGAA
AGAACCCAAAAATAAGACCTTTCTAAGATGCGAGGCCAAGAATTATTCTGGACGTTTCACCTGCTGGTGGCTGA
CGACAATCAGTACTGATTTGACATTCAGTGTCAAAAGCAGCAGAGGCTCTTCTGACCCCCAAGGGGTGACGTGC
GGAGCTGCTACACTCTCTGCAGAGAGAGTCAGAGGGGACAACAAGGAGTATGAGTACTCAGTGGAGTGCCAGGA
GGACAGTGCCTGCCCAGCTGCTGAGGAGAGTCTGCCCATTGAGGTCATGGTGGATGCCGTTCACAAGCTCAAGT
ATGAAAACTACACCAGCAGCTTCTTCATCAGGGACATCATCAAACCTGACCCACCCAAGAACTTGCAGCTGAAG
CCATTAAAGAATTCTCGGCAGGTGGAGGTCAGCTGGGAGTACCCTGACACCTGGAGTACTCCACATTCCTACTT
CTCCCTGACATTCTGCGTTCAGGTCCAGGGCAAGAGCAAGAGAGAAAAGAAAGATAGAGTCTTCACGGACAAGA
CCTCAGCCACGGTCATCTGCCGCAAAAATGCCAGCATTAGCGTGCGGGCCCAGGACCGCTACTATAGCTCATCT
TGGAGCGAATGGGCATCTGTGCCCTGCAGT

(3) Wild Type IL12A without signal peptide Amino acids (SEQ ID NO: 3):
RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESC
LNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQAL
NFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

(4) Wild Type IL12A without signal peptide Nucleic acids (SEQ ID NO: 4):
AGAAACCTCCCCGTGGCCACTCCAGACCCAGGAATGTTCCCATGCCTTCACCACTCCCAAAACCTGCTGAGGGCC
GTCAGCAACATGCTCCAGAAGGCCAGACAAACTCTAGAATTTTACCCTTGCACTTCTGAAGAGATTGATCATGAA
GATATCACAAAAGATAAAACCAGCACAGTGGAGGCCTGTTTACCATTGGAATTAACCAAGAATGAGAGTTGCCTA
AATTCCAGAGAGACCTCTTTCATAACTAATGGGAGTTGCCTGGCCTCCAGAAAGACCTCTTTTATGATGGCCCTG
TGCCTTAGTAGTATTTATGAAGACTTGAAGATGTACCAGGTGGAGTTCAAGACCATGAATGCAAAGCTTCTGATG
GATCCTAAGAGGCAGATCTTTCTAGATCAAAACATGCTGGCAGTTATTGATGAGCTGATGCAGGCCCTGAATTTC
AACAGTGAGACTGTGCCACAAAAATCCTCCCTTGAAGAACCGGATTTTTATAAAACTAAAATCAAGCTCTGCATA
CTTCTTCATGCTTTCAGAATTCGGGCAGTGACTATTGATAGAGTGATGAGCTATCTGAATGCTTCC

(5) Wild Type IL12B signal peptide Amino acids: (SEQ ID NO: 45)
MCHQQLVISWFSLVFLASPLVA

(6) Wild Type IL12B signal peptide Nucleic acids: (SEQ ID NO: 46)
ATGTGTCACCAGCAGTTGGTCATCTCTTGGTTTTCCCTGGTTTTTCTGGCATCTCCCCTCGTGGCC

FIG. 2A. IL12 Levels

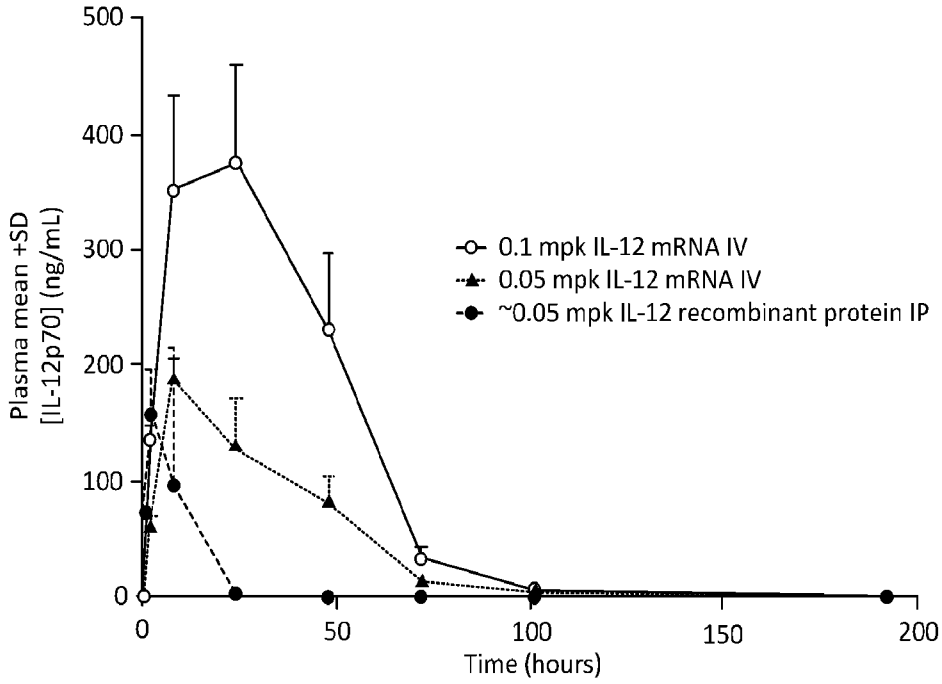


FIG. 2B. IFNγ Levels

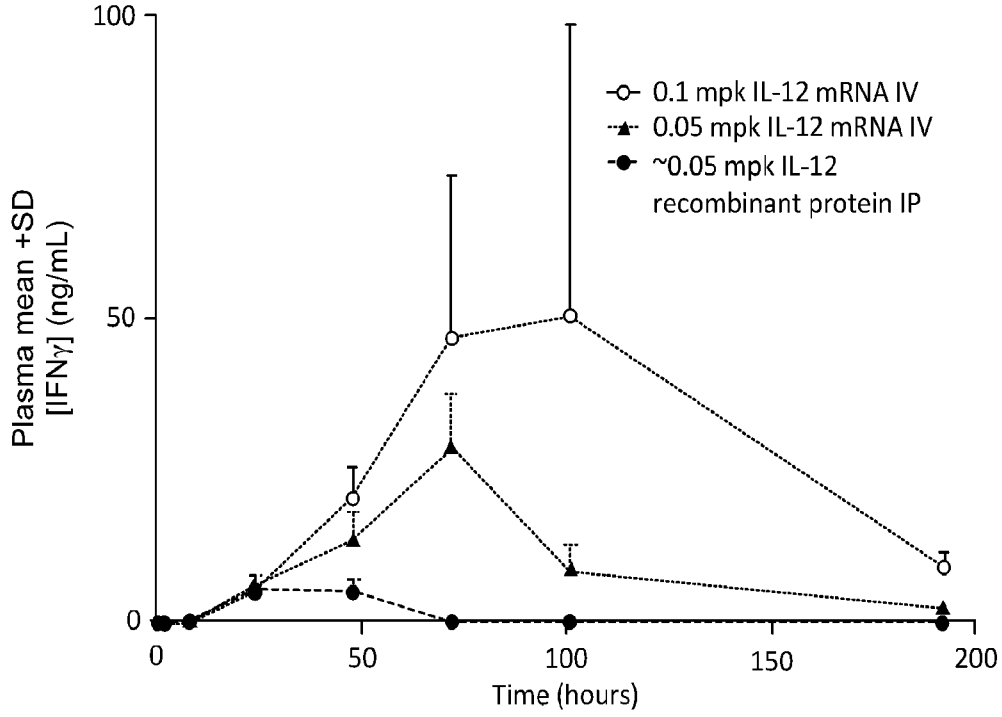


FIG. 3. A single IV dose of IL12 mRNA in LNP has robust efficacy

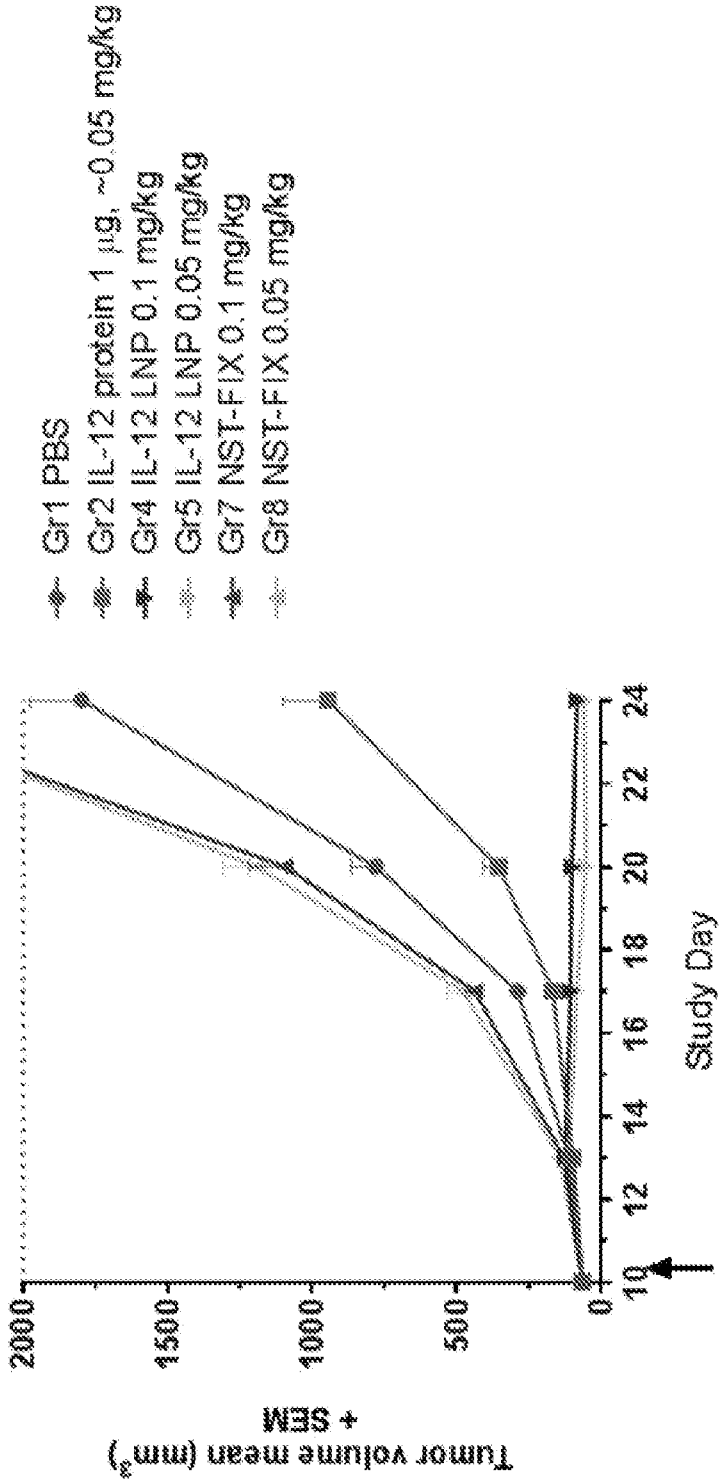


FIG. 4A

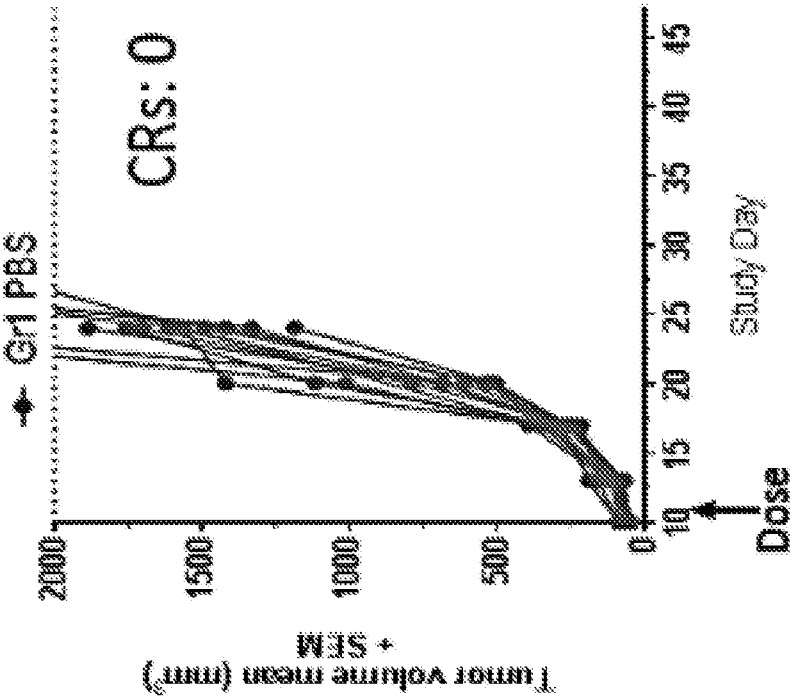

Gr1 PBS
CRs: 0
Tumor volume mean (mm³) + SEM
2000
1500
1000
500
0
10
15
20
25
30
35
40
45
Study Day
Dose

FIG. 4B

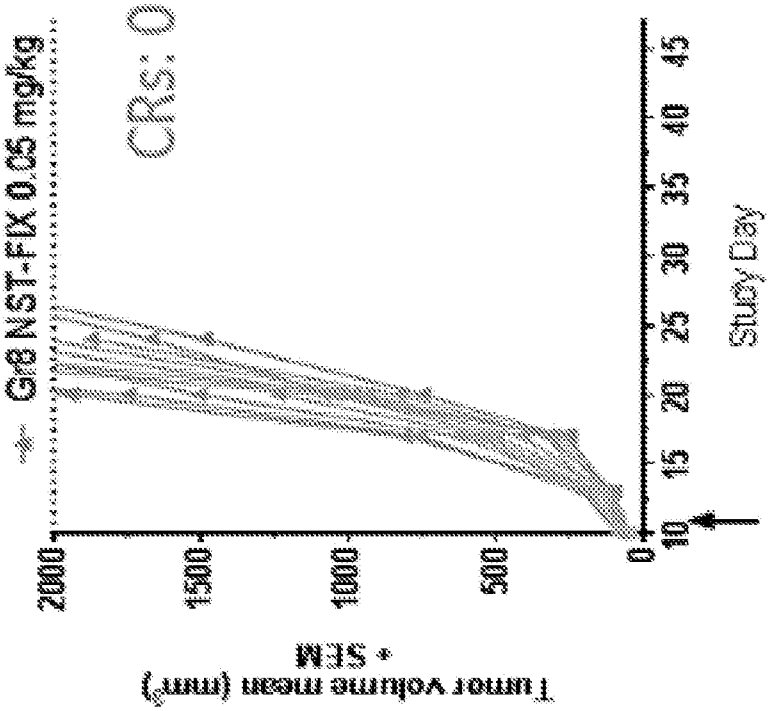

Gr8 NST-FIX 0.05 mg/kg
CRs: 0
Tumor volume mean (mm³) + SEM
2000
1500
1000
500
0
10
15
20
25
30
35
40
45
Study Day

FIG. 4D

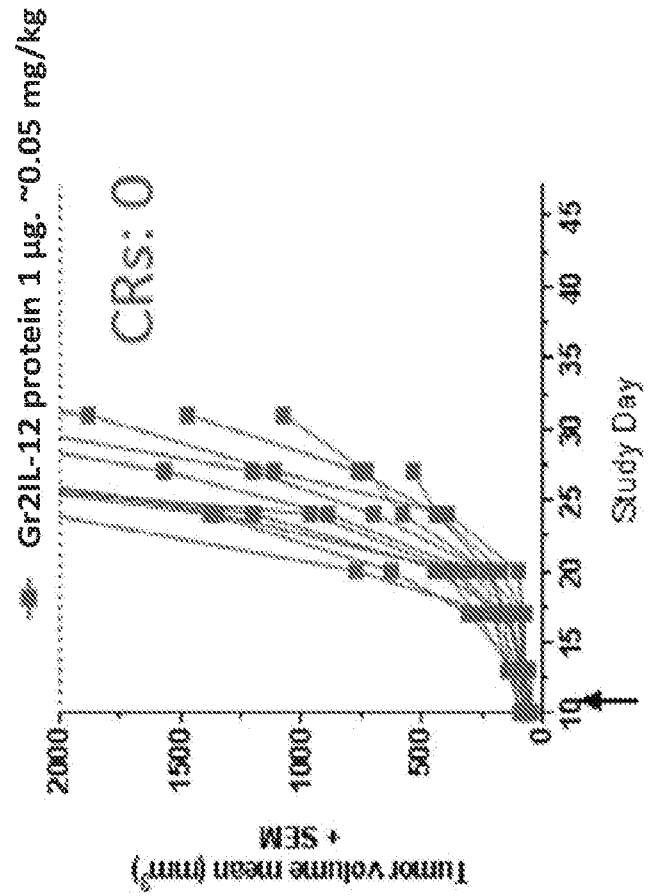

Gr2IL-12 protein 1 µg. ~0.05 mg/kg
CRS: 0
Tumor volume mean (mm³)
+ SEM
2000
1500
1000
500
0
10
15
20
25
30
35
40
45
Study Day

FIG. 4C

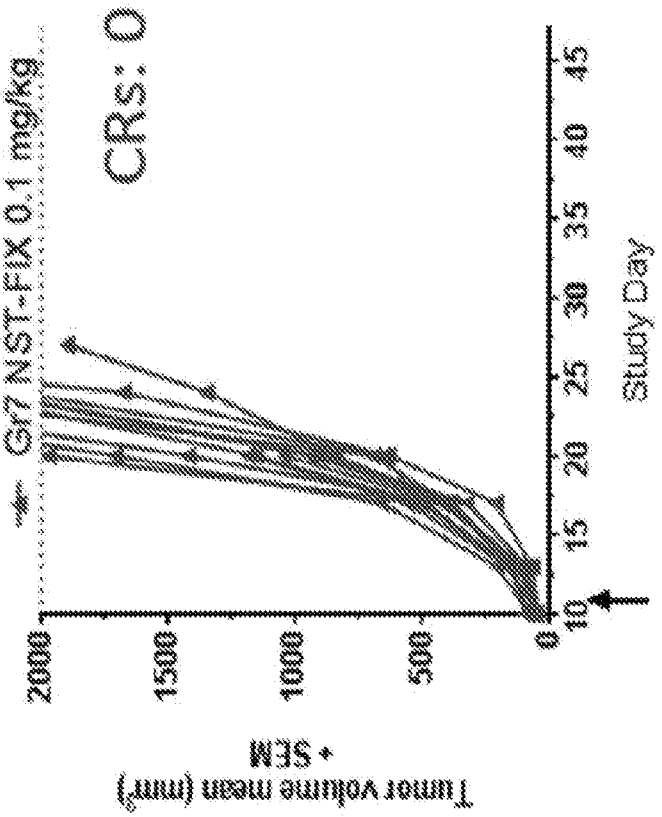

Gr7 NST-FIX 0.1 mg/kg
CRS: 0
Tumor volume mean (mm³)
+ SEM
2000
1500
1000
500
0
10
15
20
25
30
35
40
45
Study Day

FIG. 4F

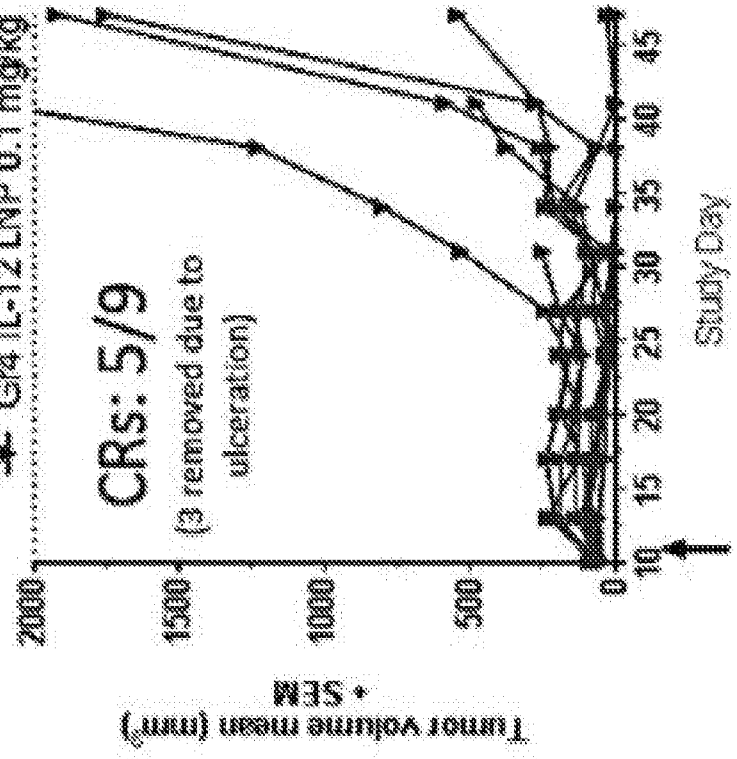

Gr4 IL-12 LNP 0.1 mg/kg
CRs: 5/9
(3 removed due to ulceration)
Tumor volume mean (mm³) + SEM
Study Day
2000
1500
1000
500
0
10
15
20
25
30
35
40
45

FIG. 4E

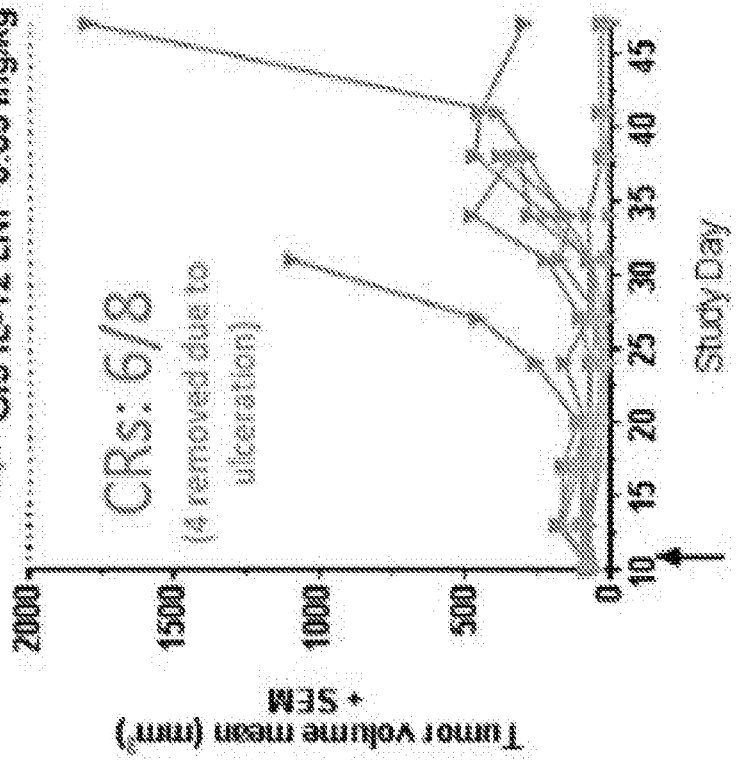

Gr5 IL-12 LNP 0.05 mg/kg
CRs: 6/8
(4 removed due to ulceration)
Tumor volume mean (mm³) + SEM
Study Day
2000
1500
1000
500
0
10
15
20
25
30
35
40
45

FIG. 5.

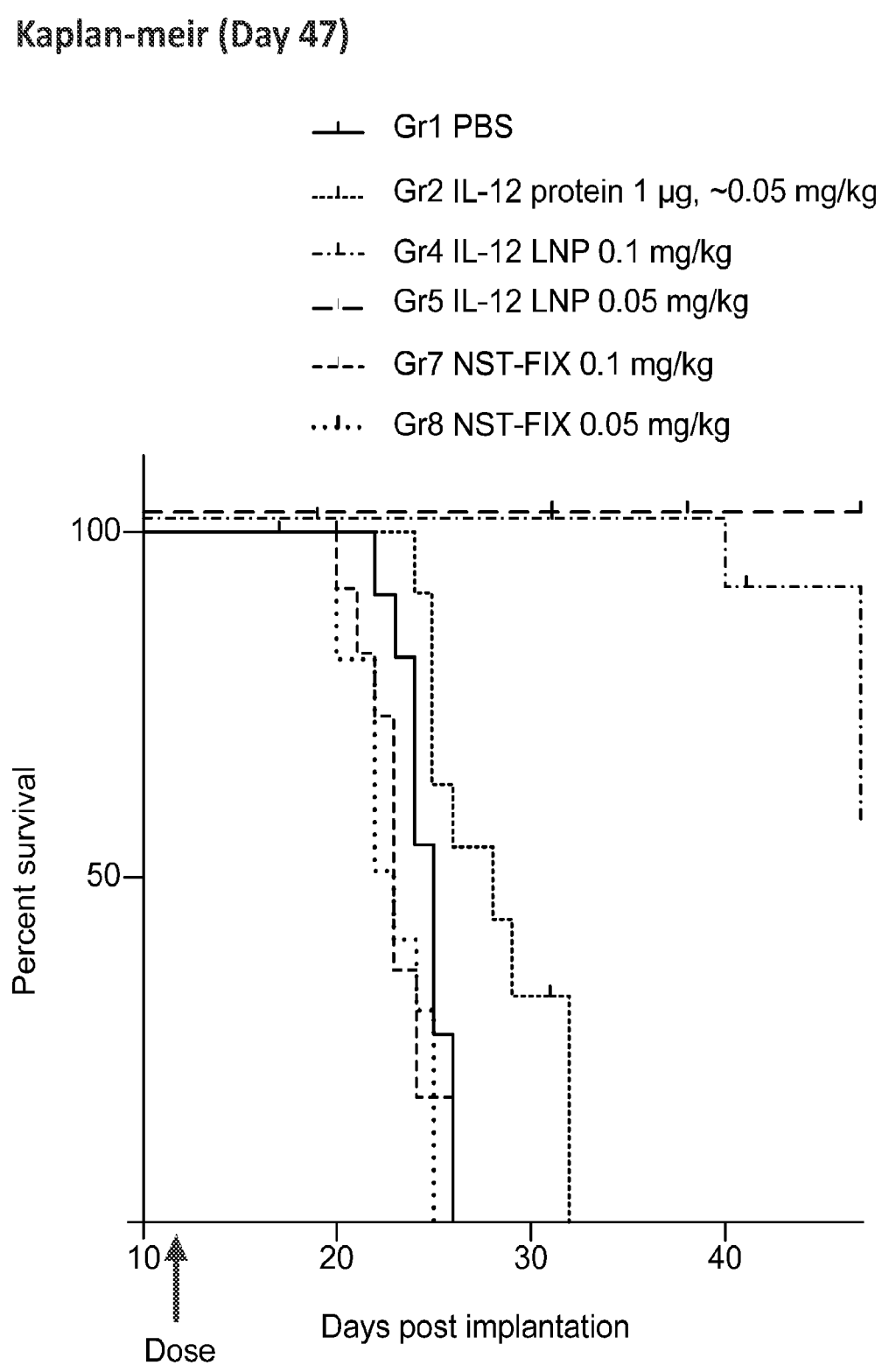

Kaplan-meir (Day 47)
Gr1 PBS
Gr2 IL-12 protein 1 µg, ~0.05 mg/kg
Gr4 IL-12 LNP 0.1 mg/kg
Gr5 IL-12 LNP 0.05 mg/kg
Gr7 NST-FIX 0.1 mg/kg
Gr8 NST-FIX 0.05 mg/kg
100
50
Percent survival
10
20
30
40
Dose
Days post implantation

FIG. 6A

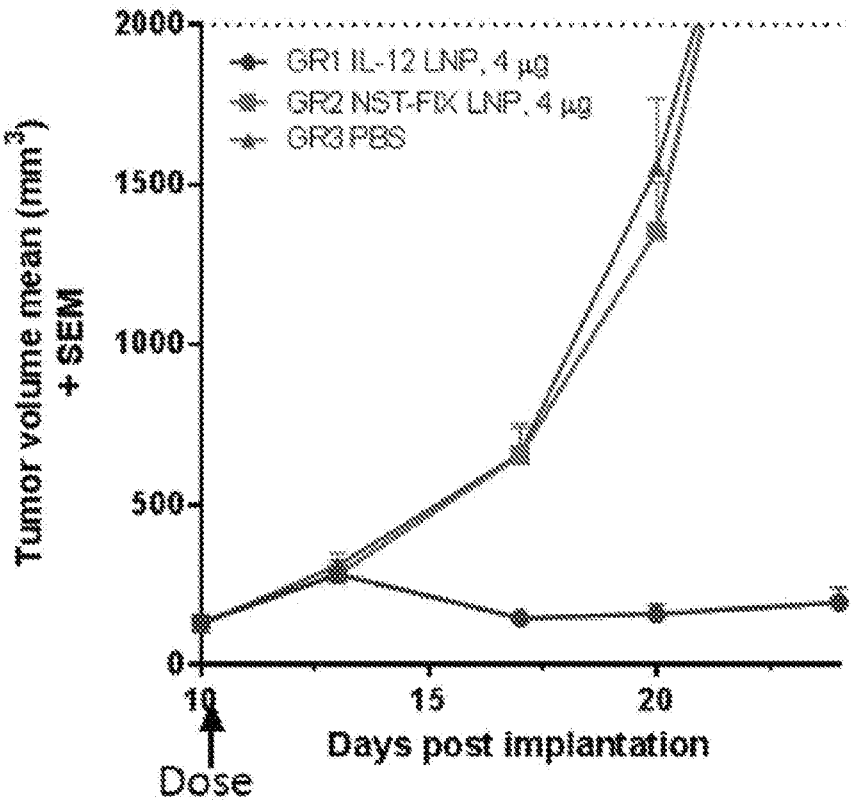

2000
1500
1000
500
0
GR1 IL-12 LNP, 4 µg
GR2 NST-FIX LNP, 4 µg
GR3 PBS
Tumor volume mean (mm3) + SEM
10
15
20
Days post implantation
Dose

FIG. 6B

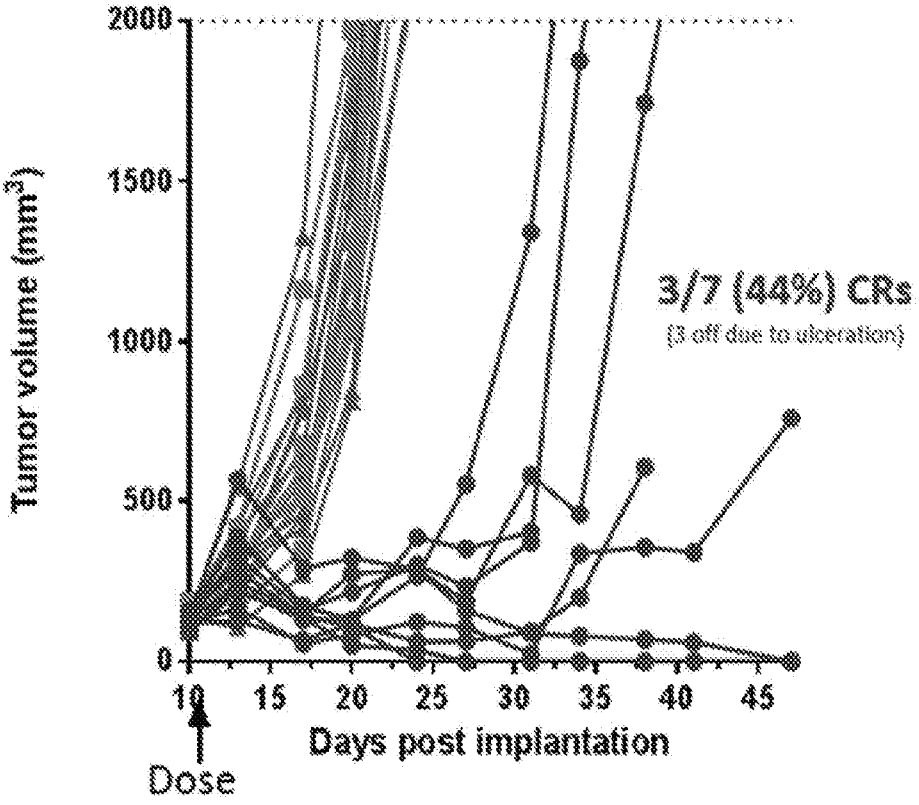

2000
1500
1000
500
0
Tumor volume (mm3)
3/7 (44%) CRs
(3 off due to ulceration)
10
15
20
25
30
35
40
45
Days post implantation
Dose

FIG. 7A

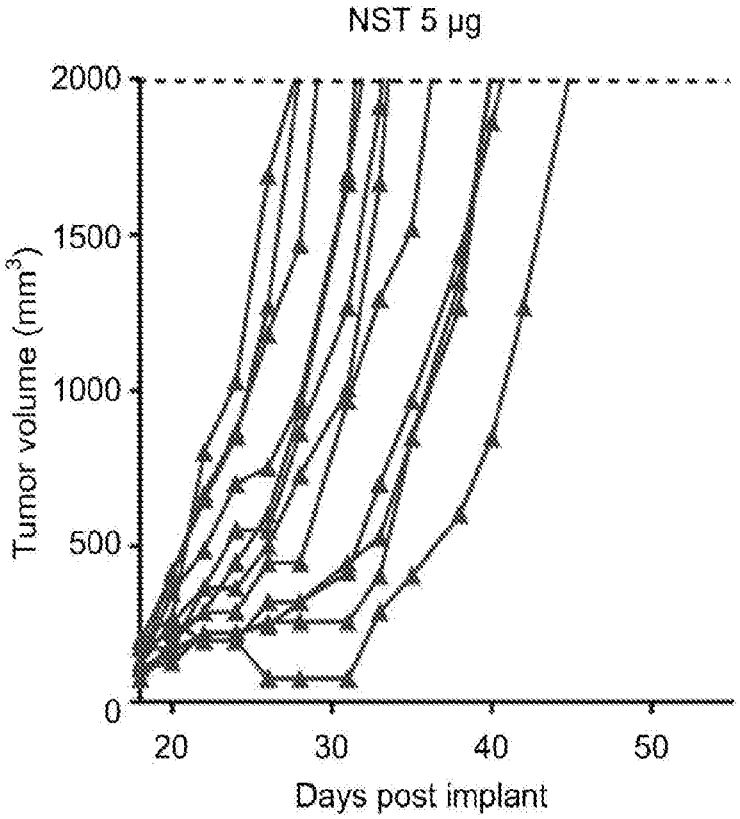

NST 5 µg
2000
1500
1000
500
0
Tumor volume (mm3)
20
30
40
50
Days post implant

FIG. 7B

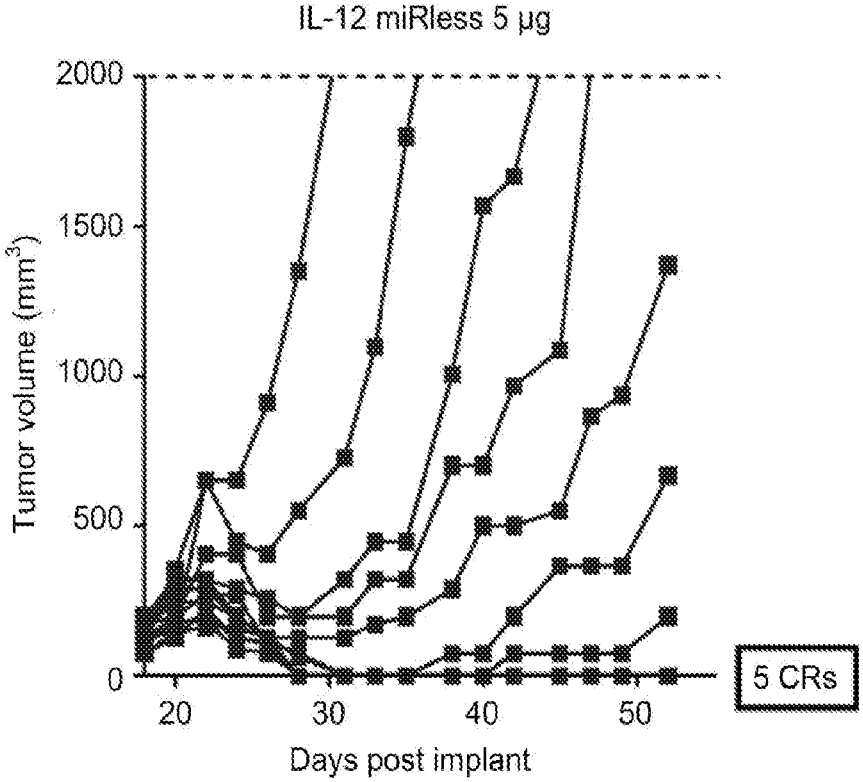

IL-12 miRless 5 µg
2000
1500
1000
500
0
Tumor volume (mm3)
20
30
40
50
Days post implant
5 CRs

FIG. 7C

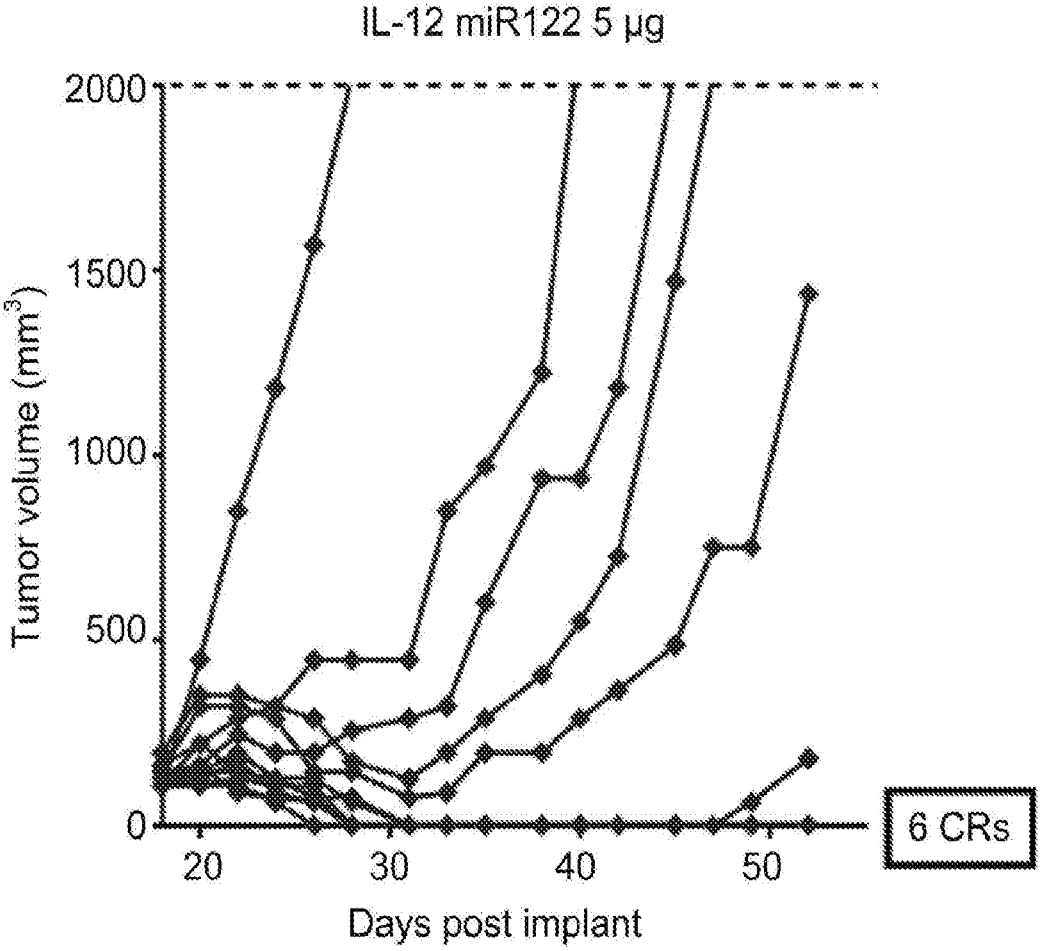

IL-12 miR122 5 µg
2000
1500
1000
500
0
Tumor volume (mm3)
20
30
40
50
Days post implant
6 CRs

FIG. 8A

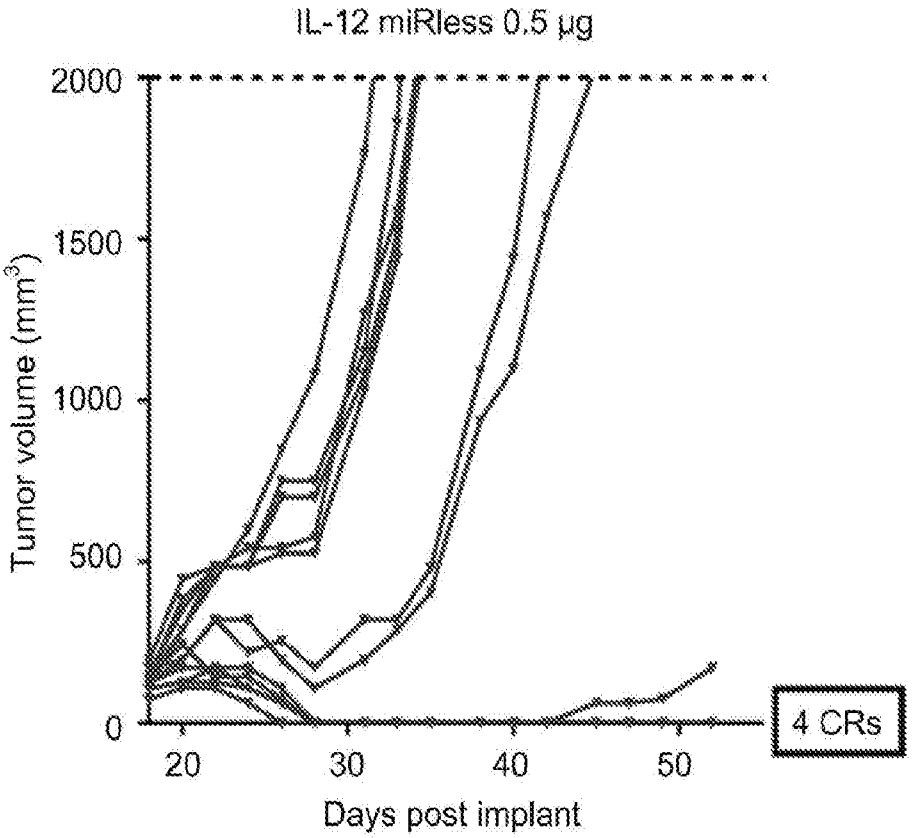

IL-12 miRless 0.5 µg
2000
1500
1000
500
0
Tumor volume (mm3)
20
30
40
50
Days post implant
4 CRs

FIG. 8B

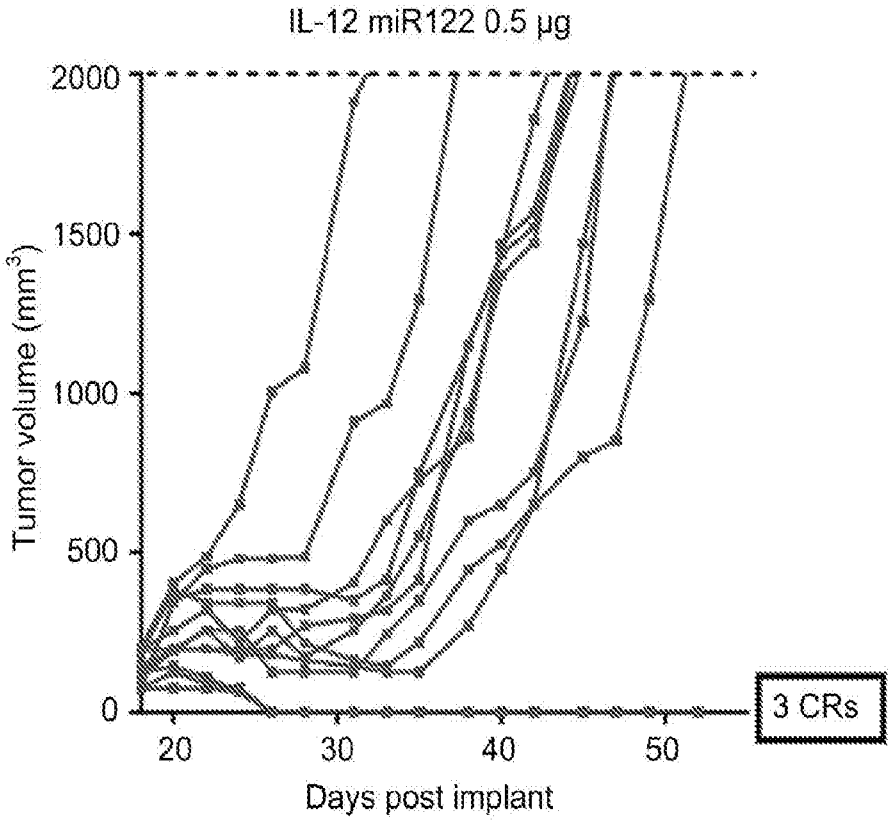

IL-12 miR122 0.5 µg
2000
1500
1000
500
0
Tumor volume (mm3)
20
30
40
50
Days post implant
3 CRs

FIG. 8C

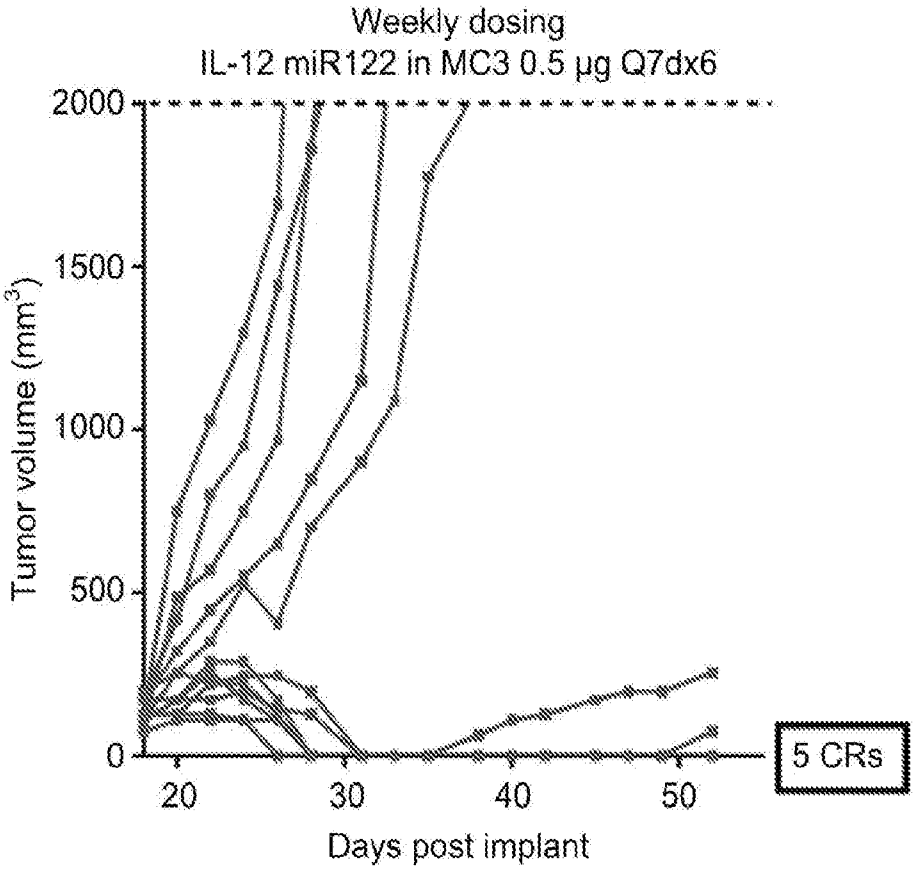

Weekly dosing
IL-12 miR122 in MC3 0.5 µg Q7dx6
Tumor volume (mm3)
2000
1500
1000
500
0
20
30
40
50
Days post implant
5 CRs

FIG. 8D

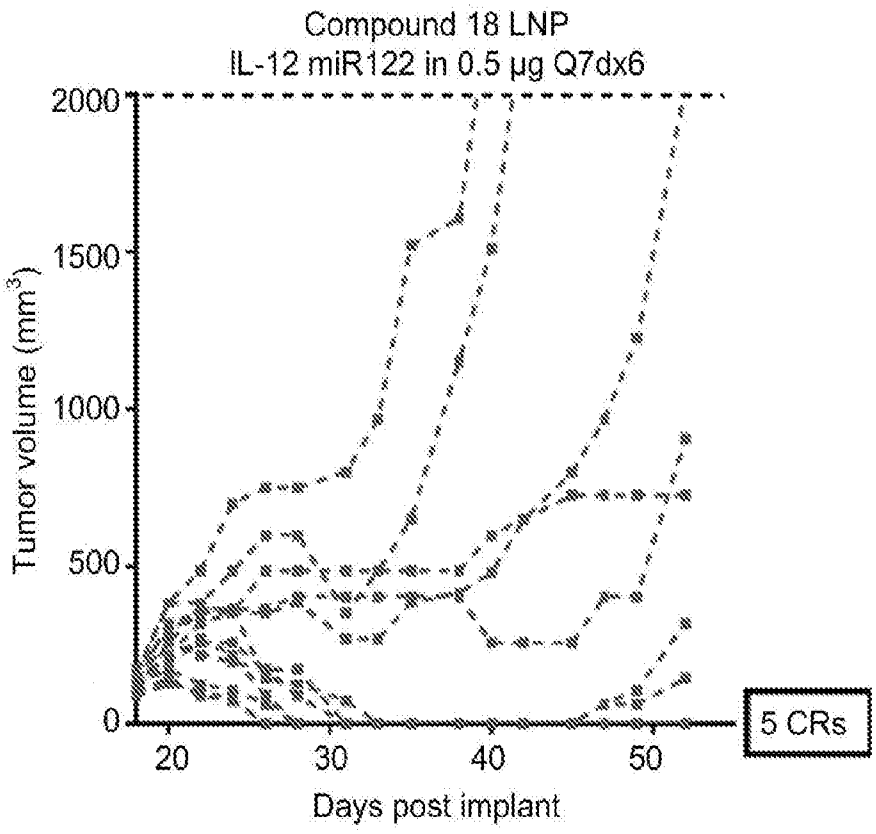

Compound 18 LNP
IL-12 miR122 in 0.5 µg Q7dx6
Tumor volume (mm3)
2000
1500
1000
500
0
20
30
40
50
Days post implant
5 CRs

FIG. 8E

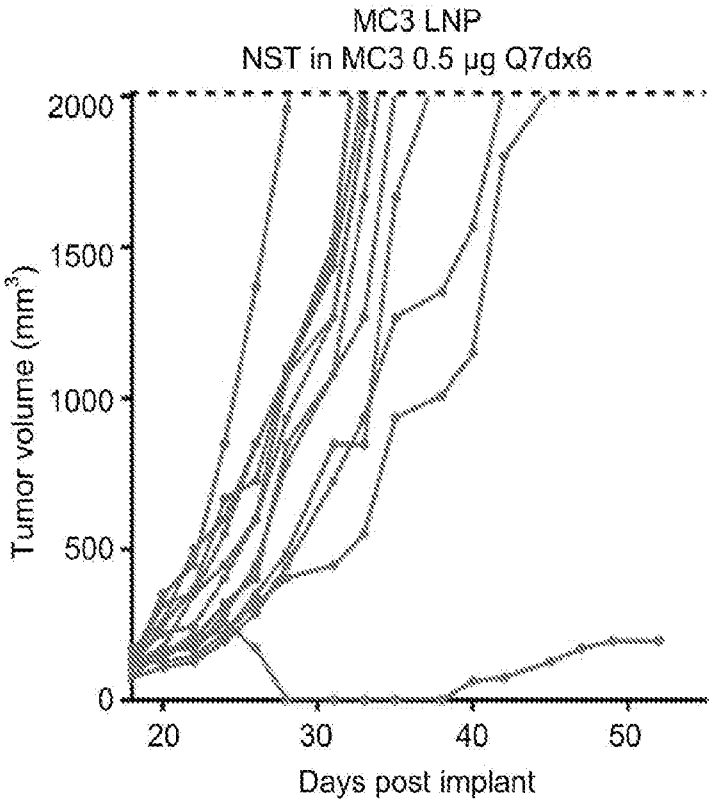

MC3 LNP
NST in MC3 0.5 µg Q7dx6
Tumor volume (mm3)
2000
1500
1000
500
0
20
30
40
50
Days post implant

FIG. 8F

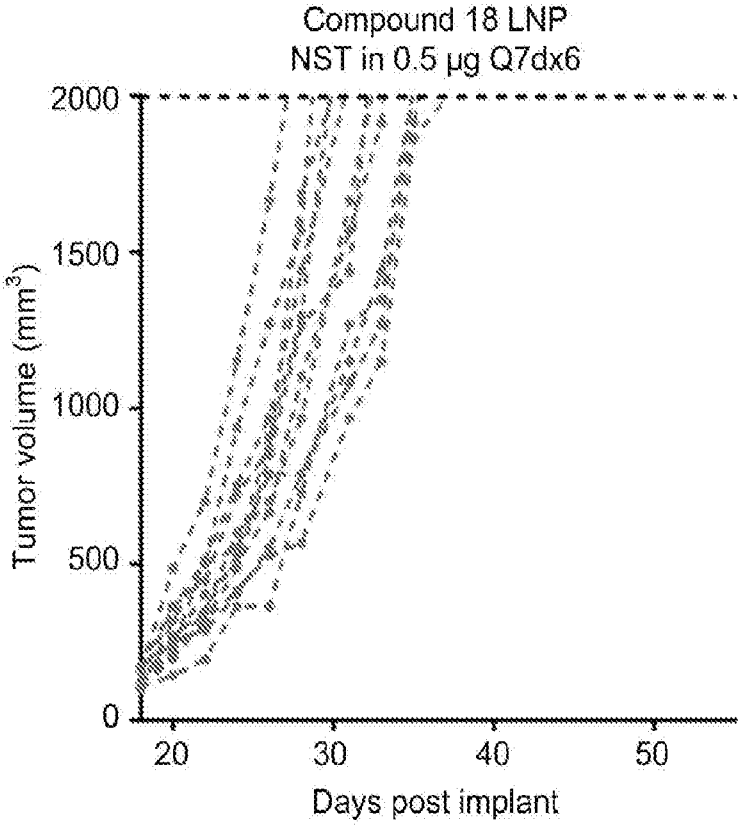

Compound 18 LNP
NST in 0.5 µg Q7dx6
Tumor volume (mm3)
2000
1500
1000
500
0
20
30
40
50
Days post implant

FIG. 9A

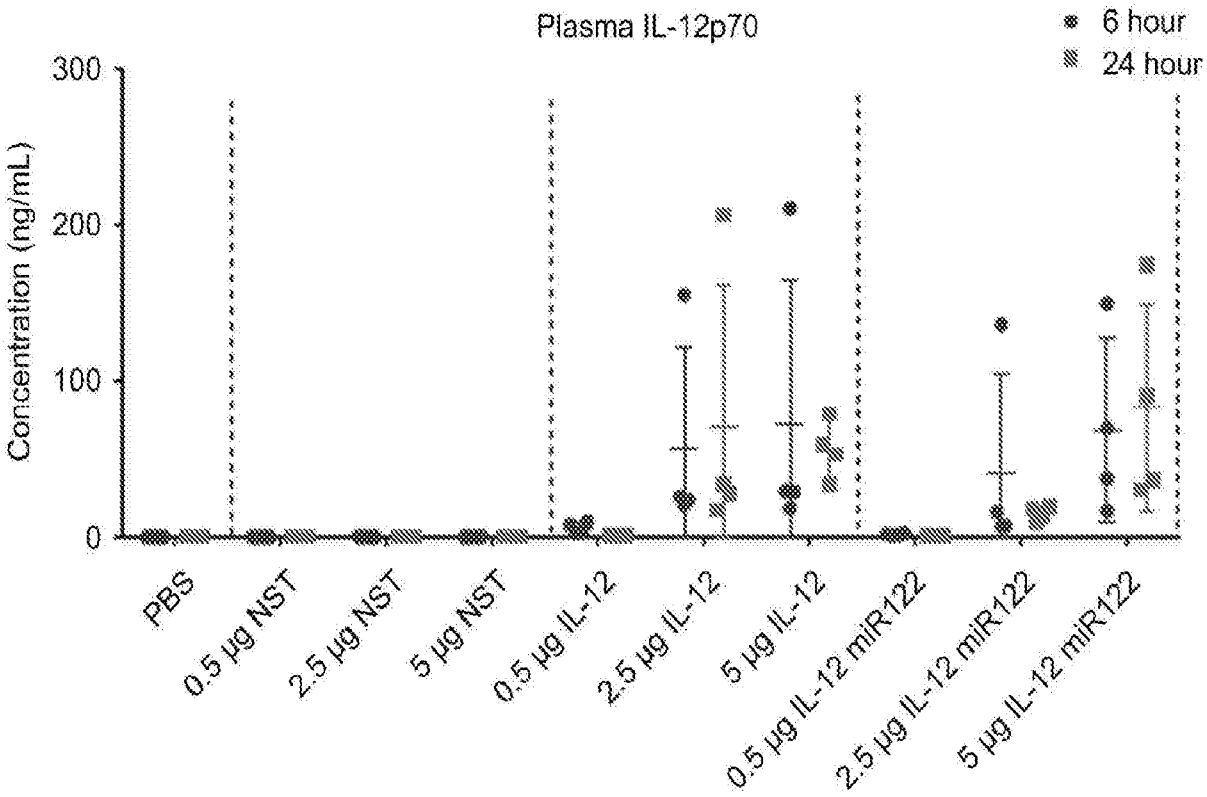

Plasma IL-12p70
6 hour
24 hour
Concentration (ng/mL)
300
200
100
0
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122

FIG. 9B

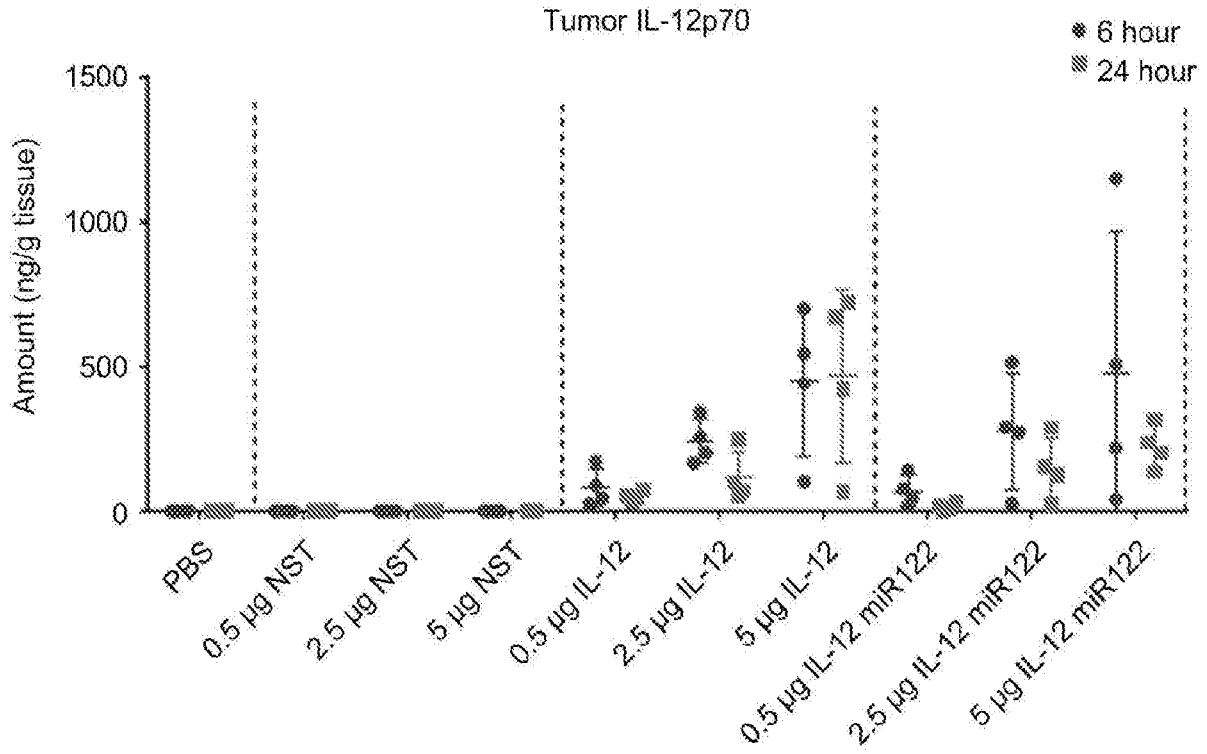

Tumor IL-12p70
6 hour
24 hour
Amount (ng/g tissue)
1500
1000
500
0
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122

FIG. 9C

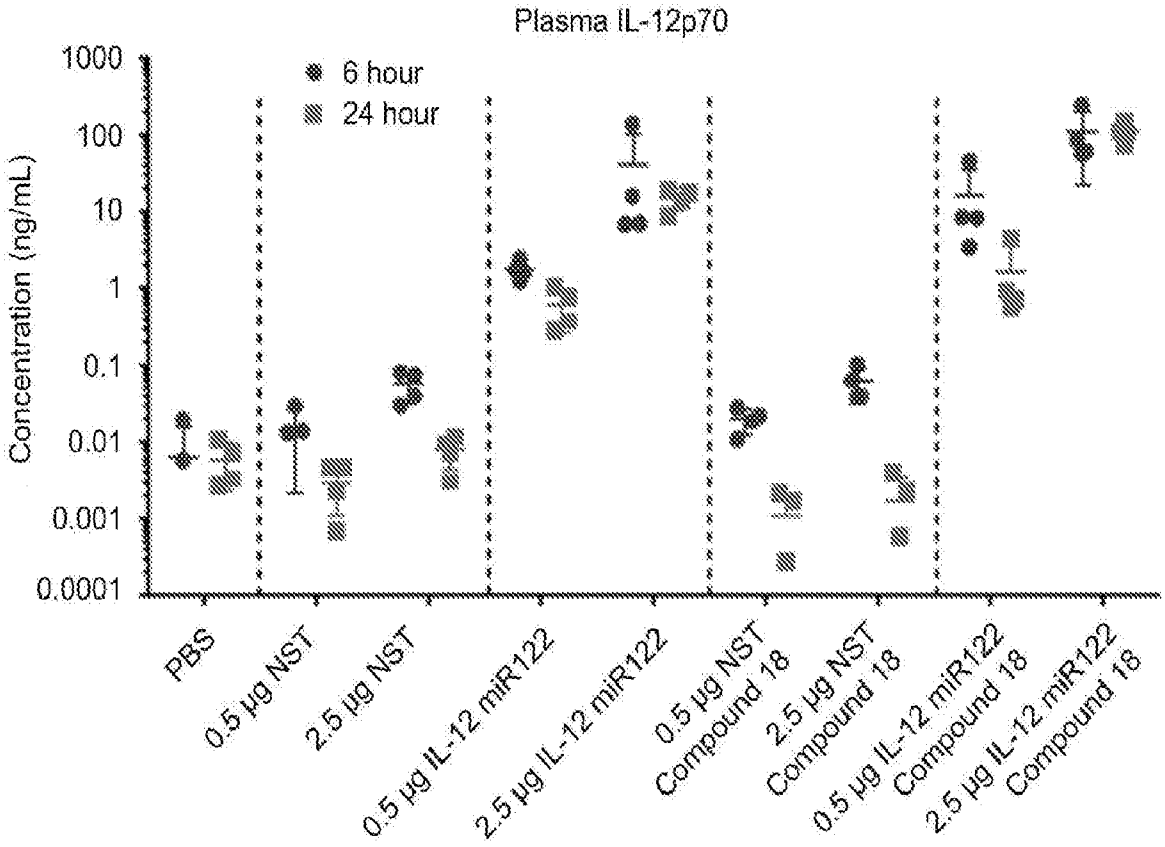

Plasma IL-12p70
6 hour
24 hour
Concentration (ng/mL)
1000
100
10
1
0.1
0.01
0.001
0.0001
PBS
0.5 µg NST
2.5 µg NST
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
0.5 µg NST Compound 18
2.5 µg NST Compound 18
0.5 µg IL-12 miR122 Compound 18
2.5 µg IL-12 miR122 Compound 18

FIG. 9D

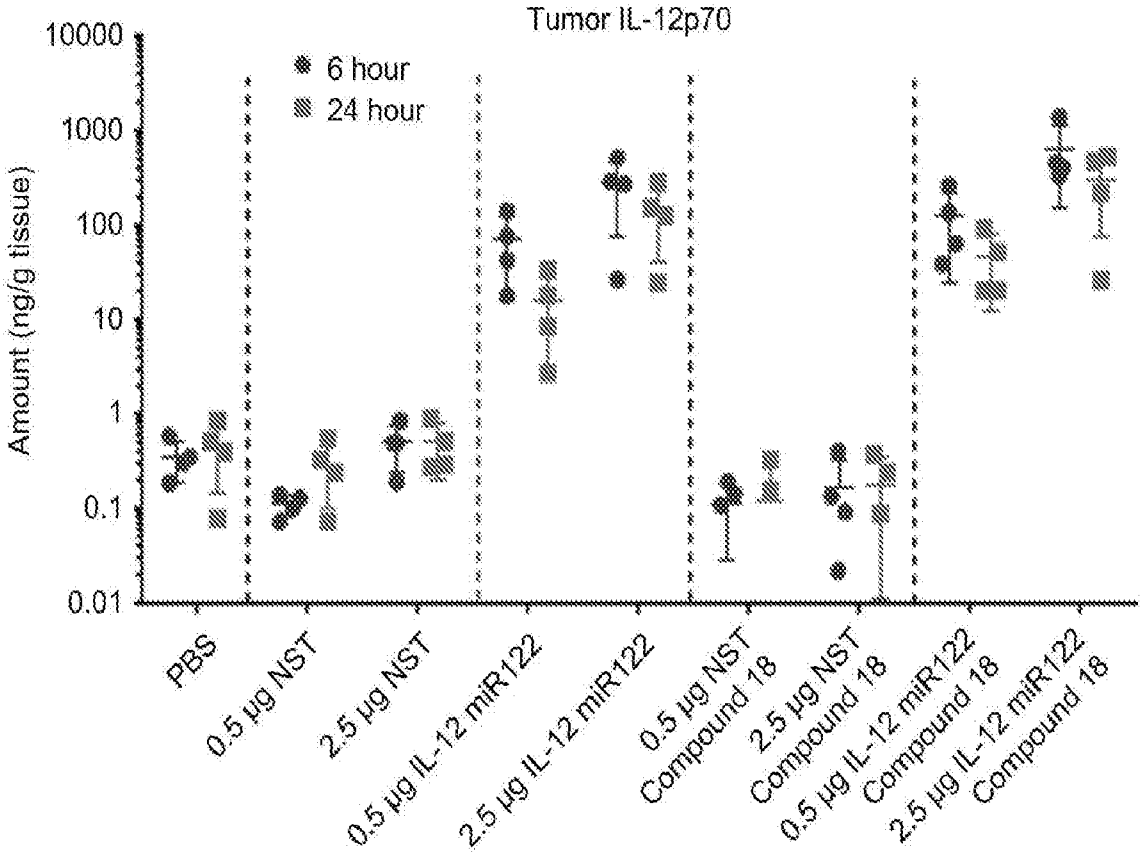

Tumor IL-12p70
6 hour
24 hour
Amount (ng/g tissue)
10000
1000
100
10
1
0.1
0.01
PBS
0.5 µg NST
2.5 µg NST
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
0.5 µg NST Compound 18
2.5 µg NST Compound 18
0.5 µg IL-12 miR122 Compound 18
2.5 µg IL-12 miR122 Compound 18

FIG. 9E

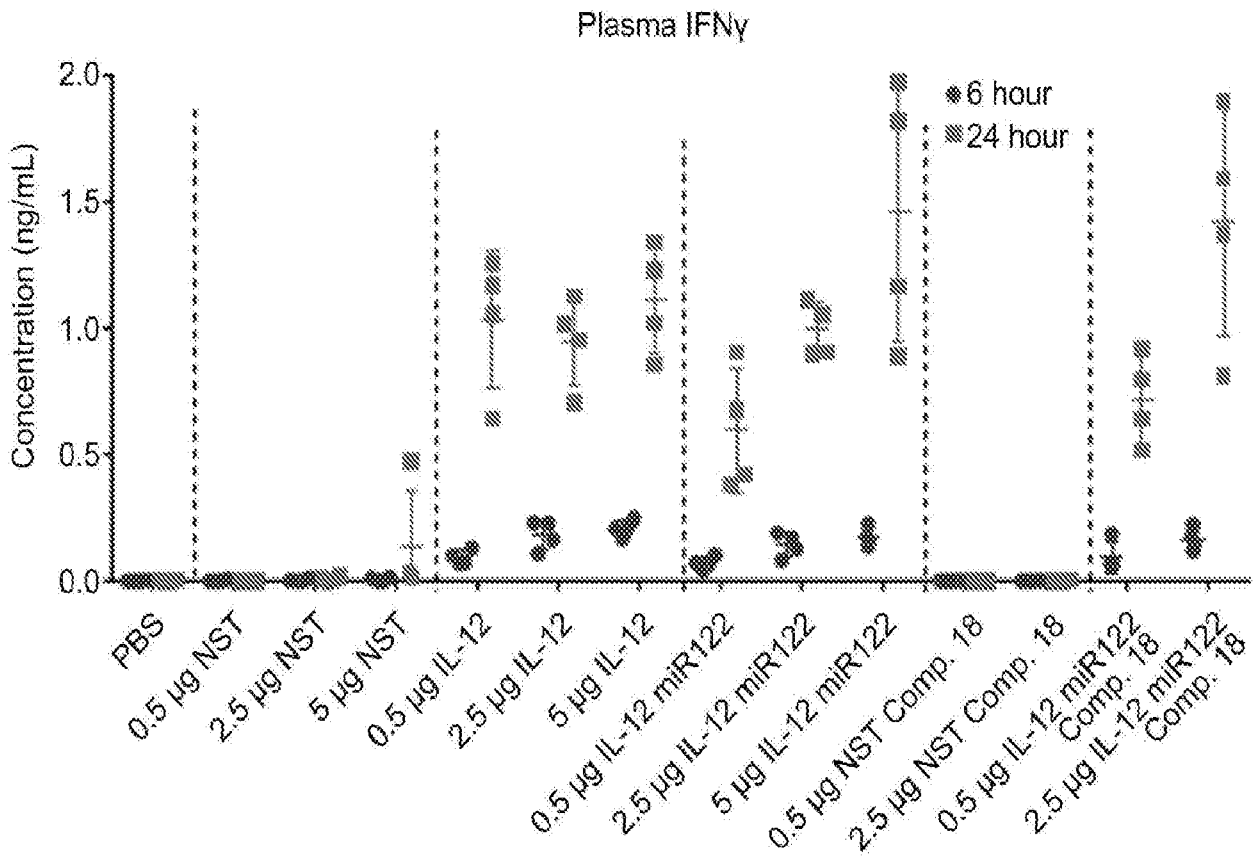

Plasma IFNγ
Concentration (ng/mL)
2.0
1.5
1.0
0.5
0.0
6 hour
24 hour
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9F

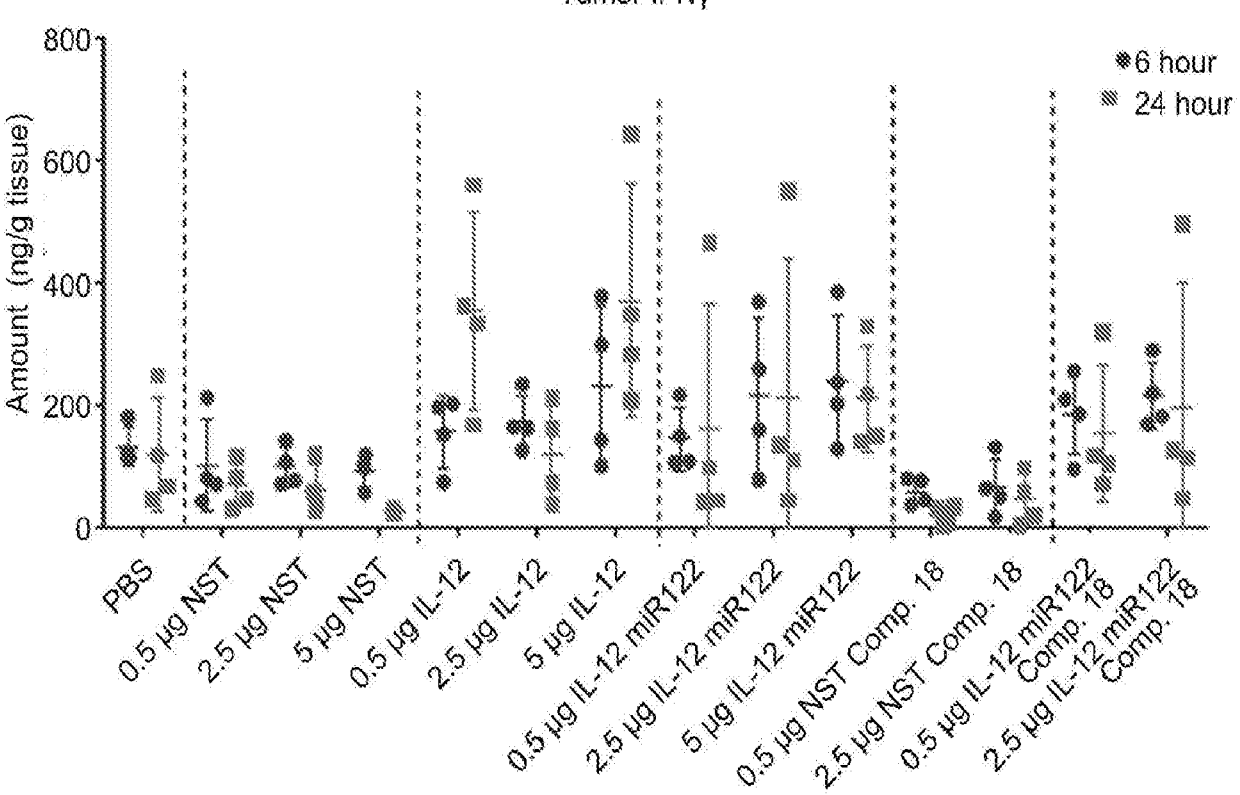

Tumor IFNγ
Amount (ng/g tissue)
800
600
400
200
0
6 hour
24 hour
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9G

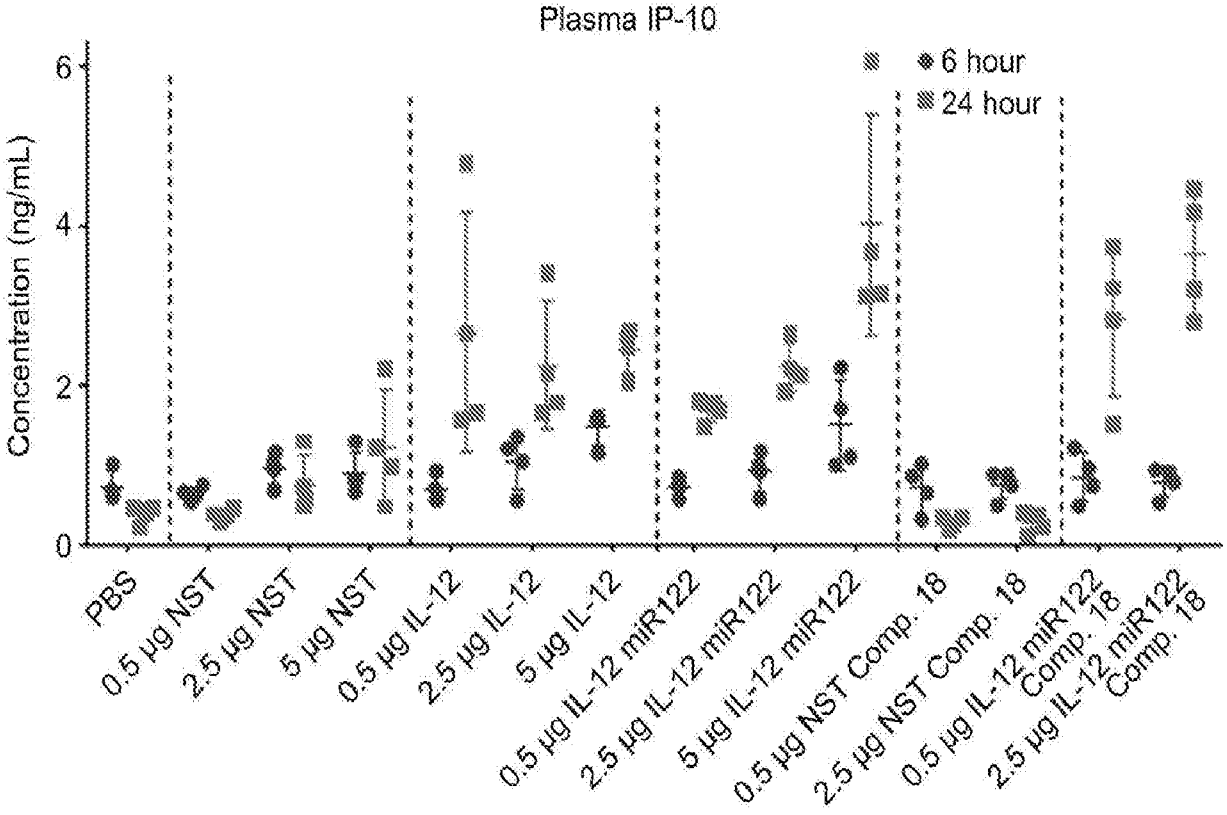

Plasma IP-10
6 hour
24 hour
Concentration (ng/mL)
0
2
4
6
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9H

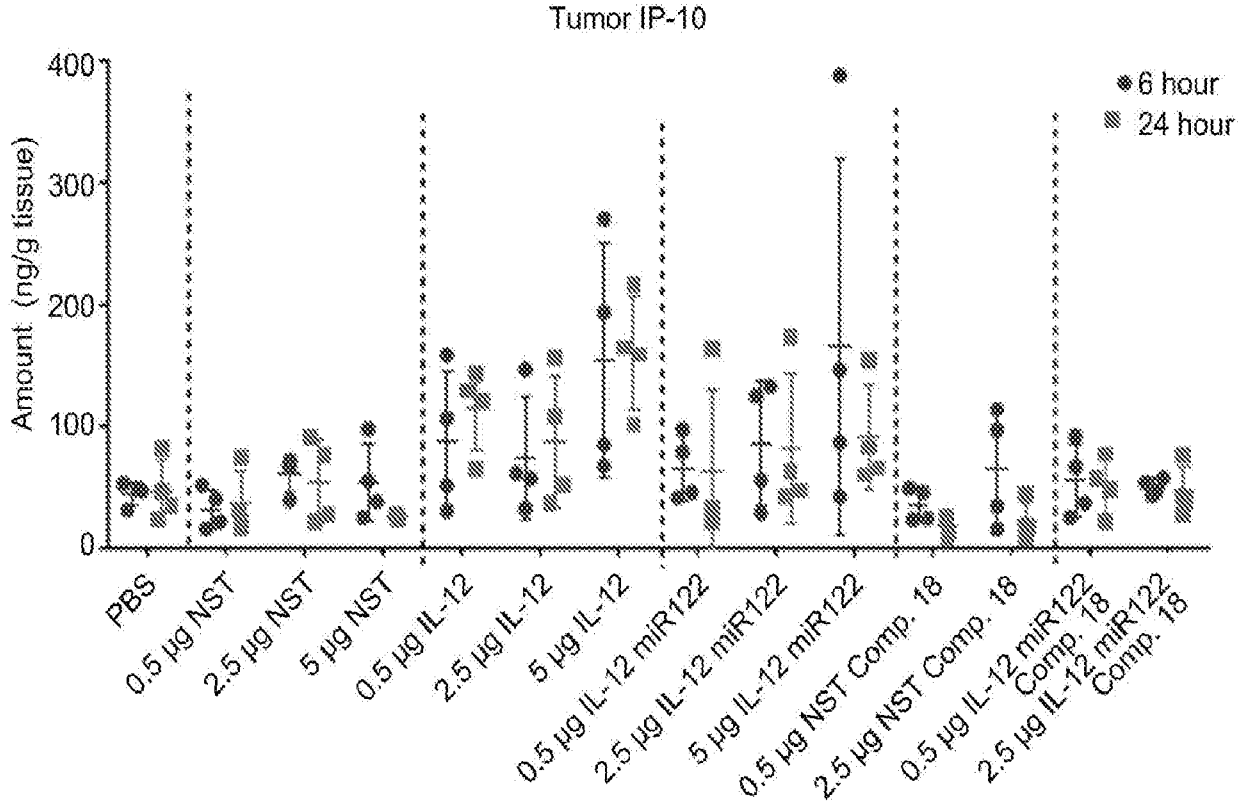

Tumor IP-10
6 hour
24 hour
Amount (ng/g tissue)
0
100
200
300
400
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9I

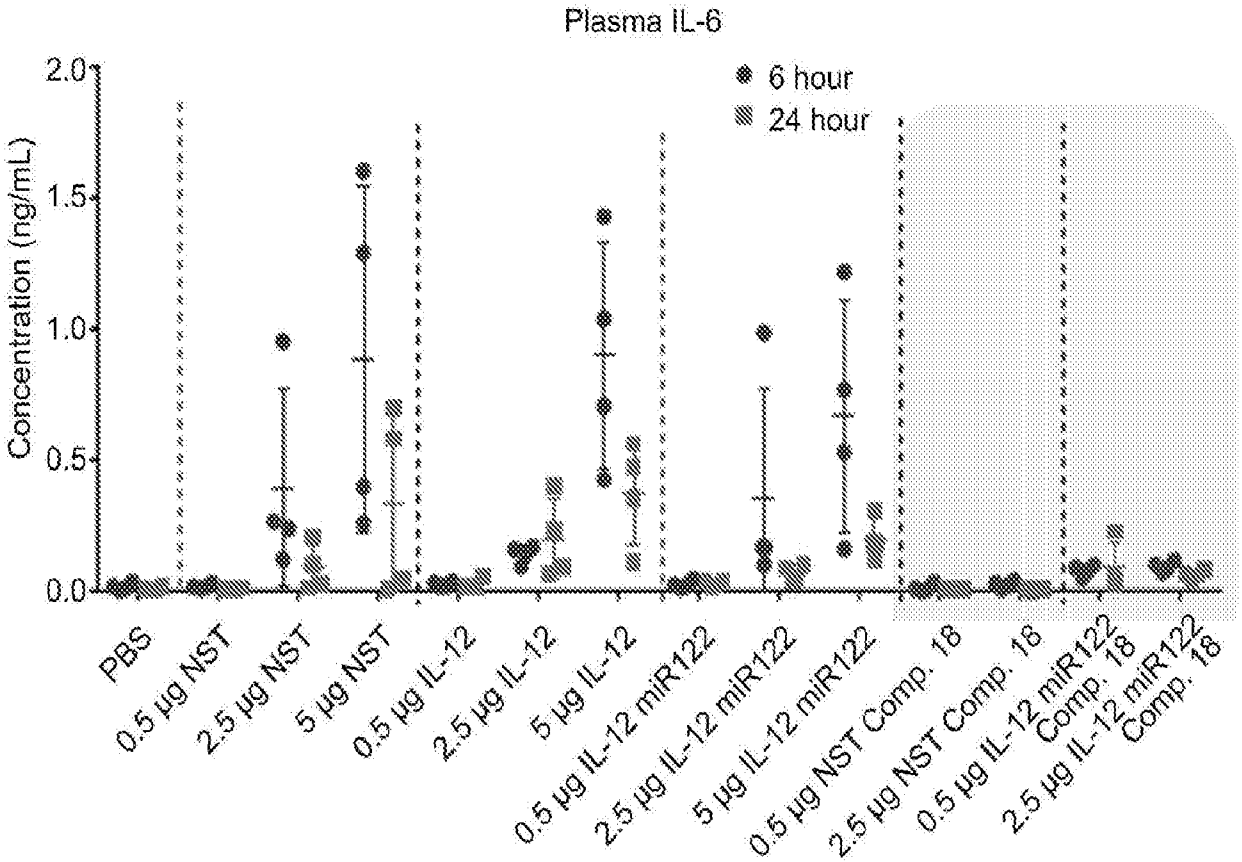

Plasma IL-6
6 hour
24 hour
Concentration (ng/mL)
2.0
1.5
1.0
0.5
0.0
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9J

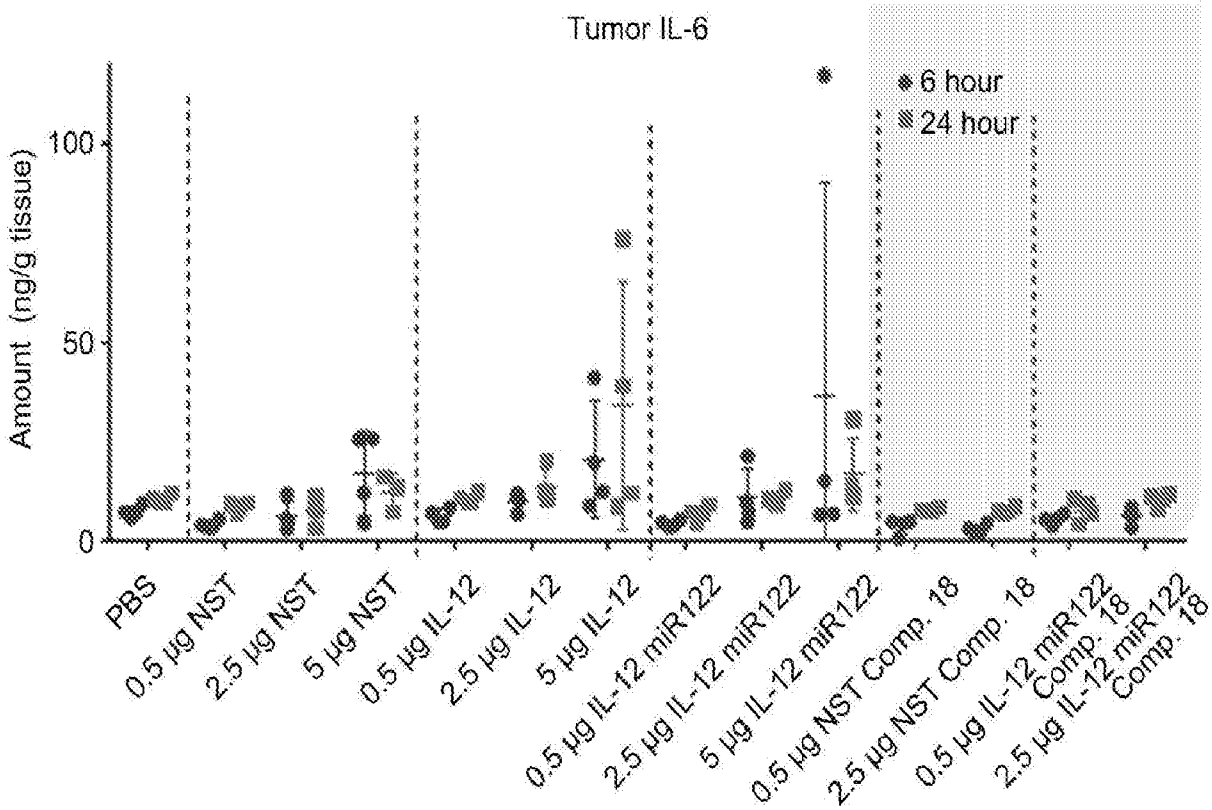

Tumor IL-6
6 hour
24 hour
Amount (ng/g tissue)
100
50
0
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9K

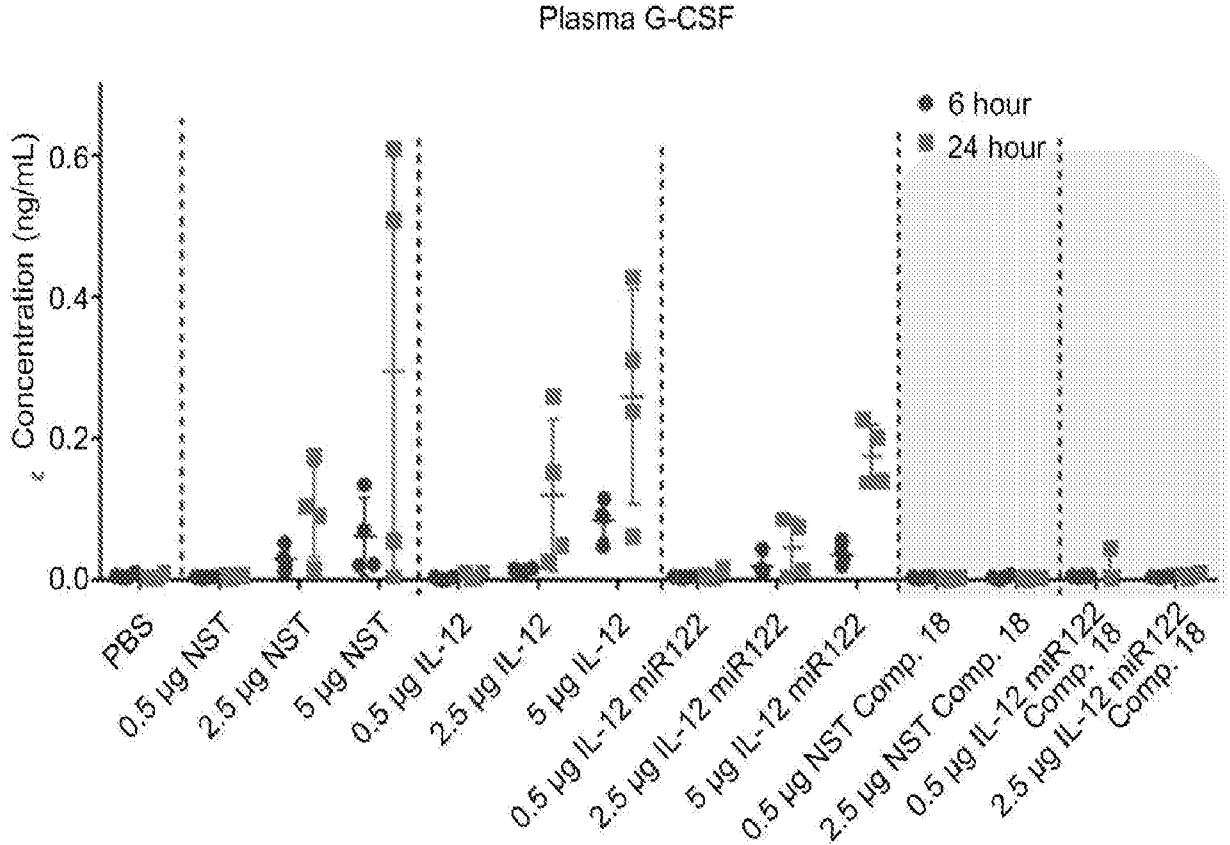

Plasma G-CSF
6 hour
24 hour
Concentration (ng/mL)
0.0
0.2
0.4
0.6
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9L

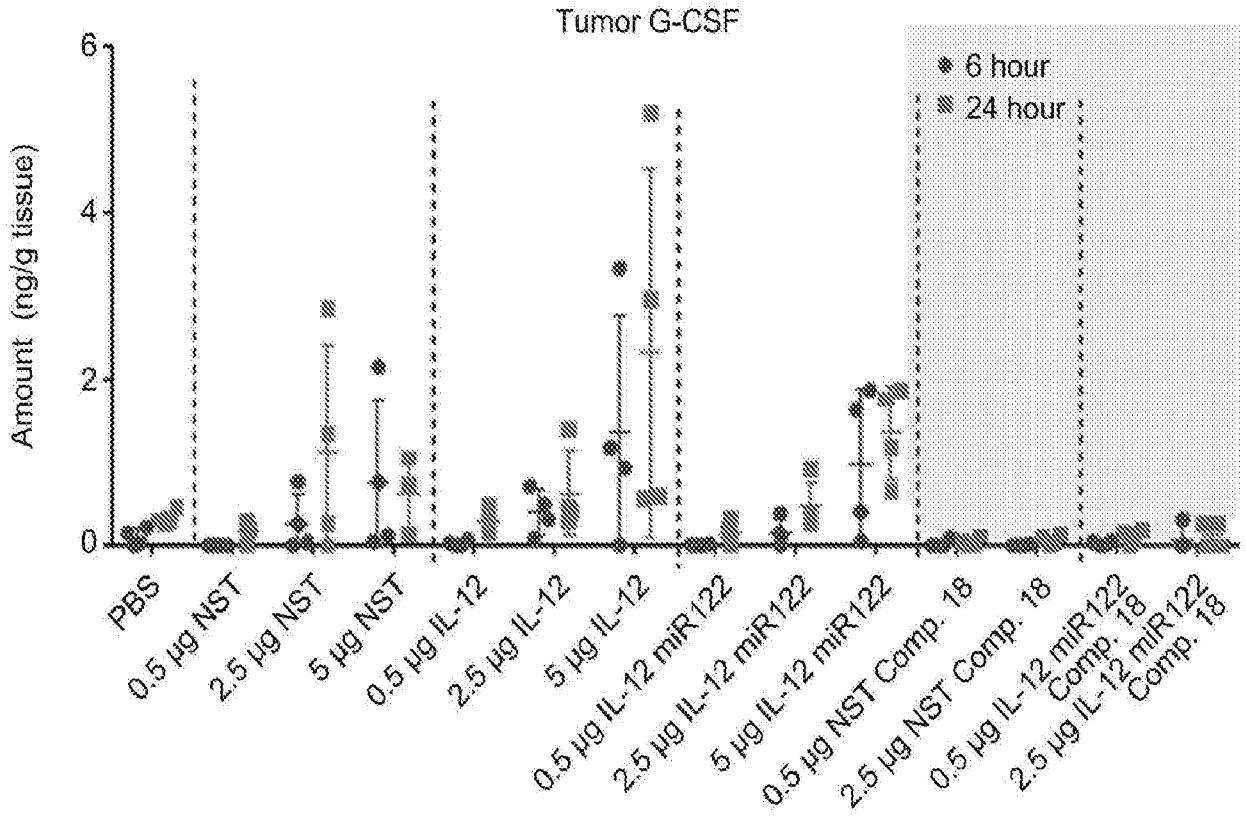

Tumor G-CSF
6 hour
24 hour
Amount (ng/g tissue)
0
2
4
6
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9M

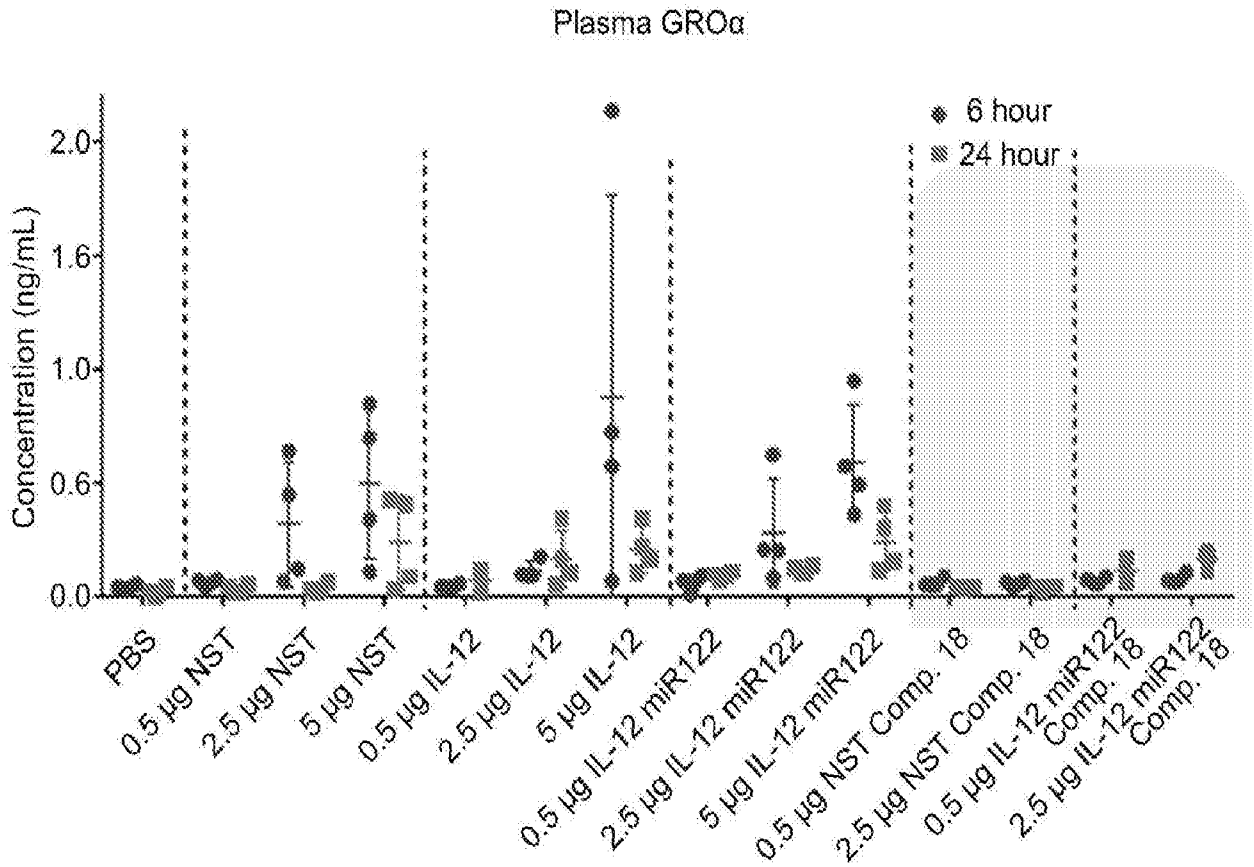

Plasma GROα
6 hour
24 hour
Concentration (ng/mL)
2.0
1.6
1.0
0.6
0.0
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9N

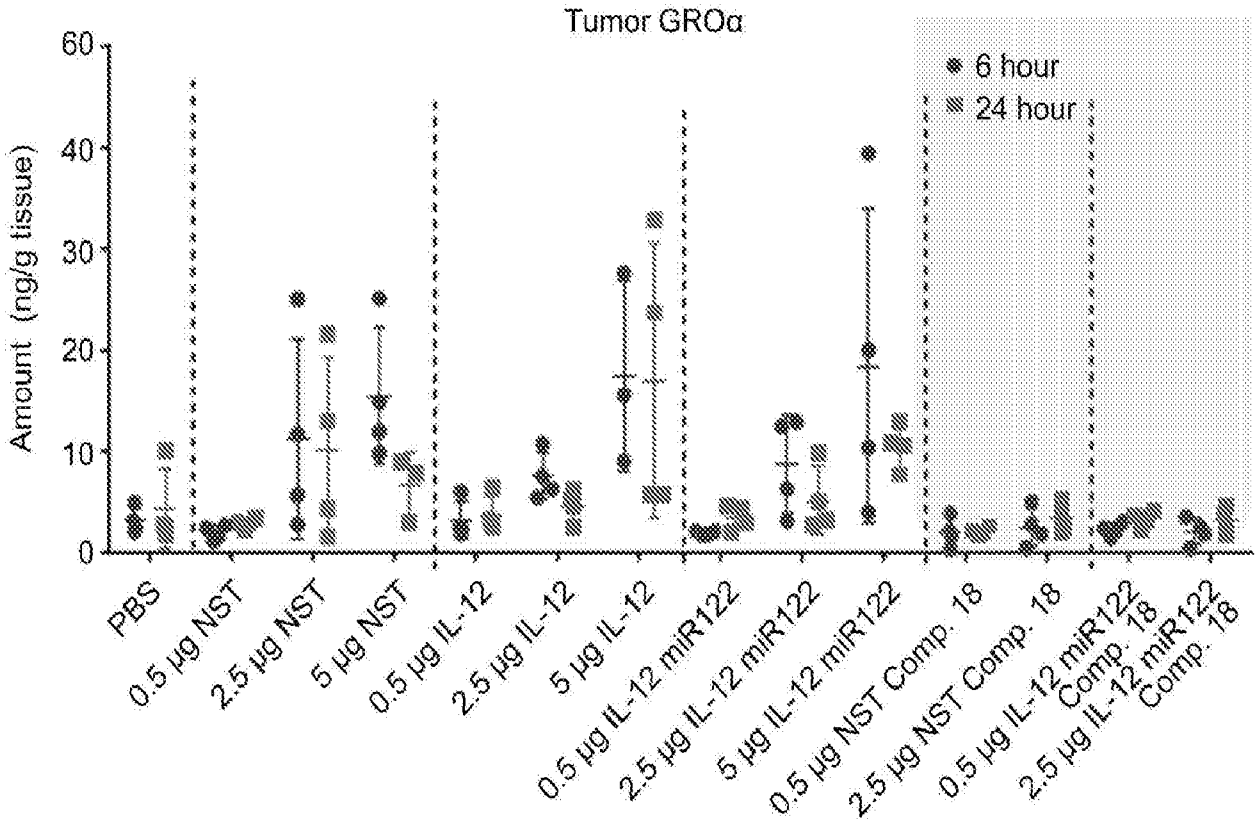

Tumor GROα
6 hour
24 hour
Amount (ng/g tissue)
60
40
30
20
10
0
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 10B

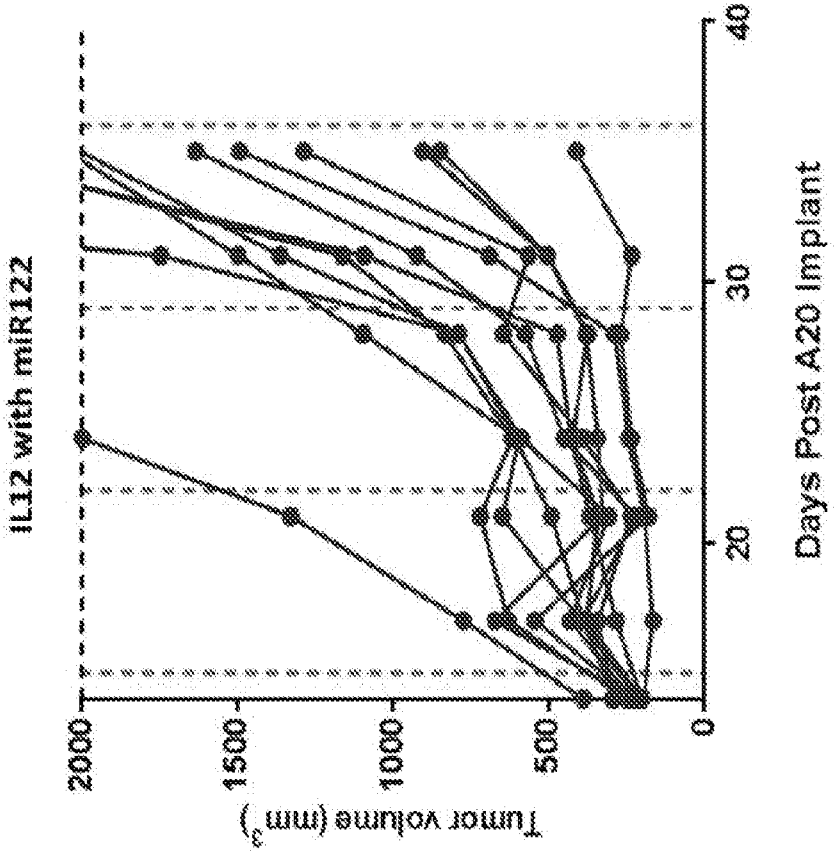


FIG.10A

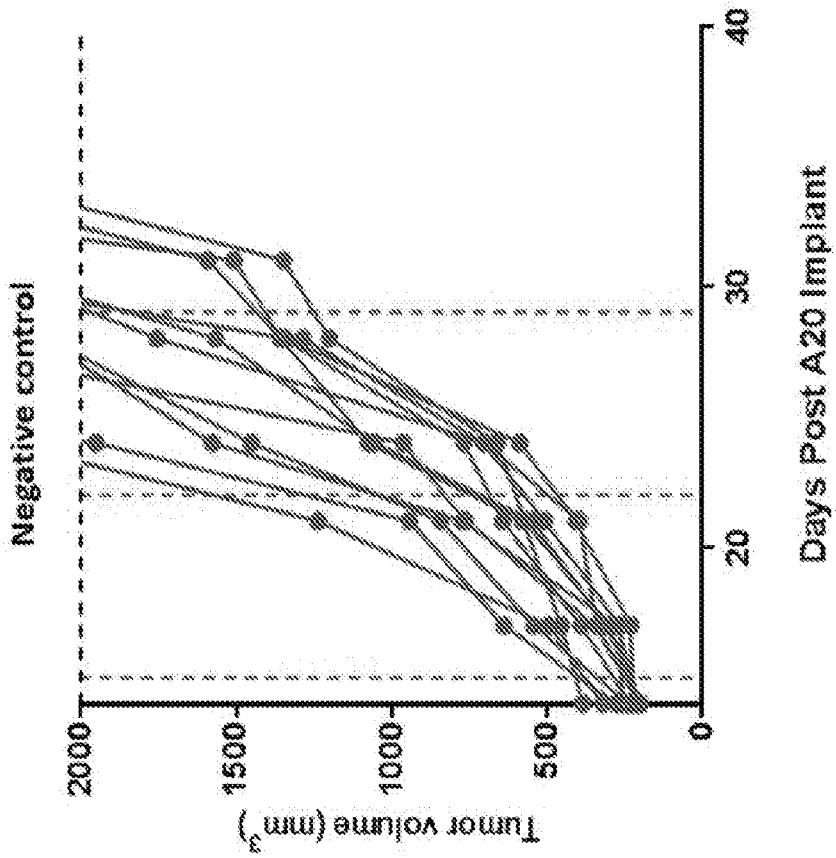


FIG.10D

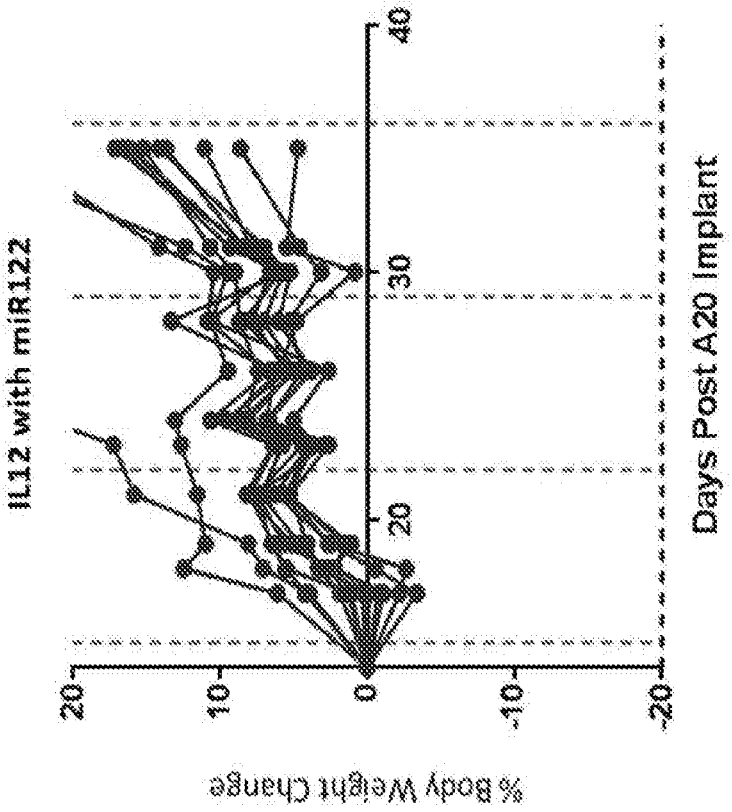


FIG.10C

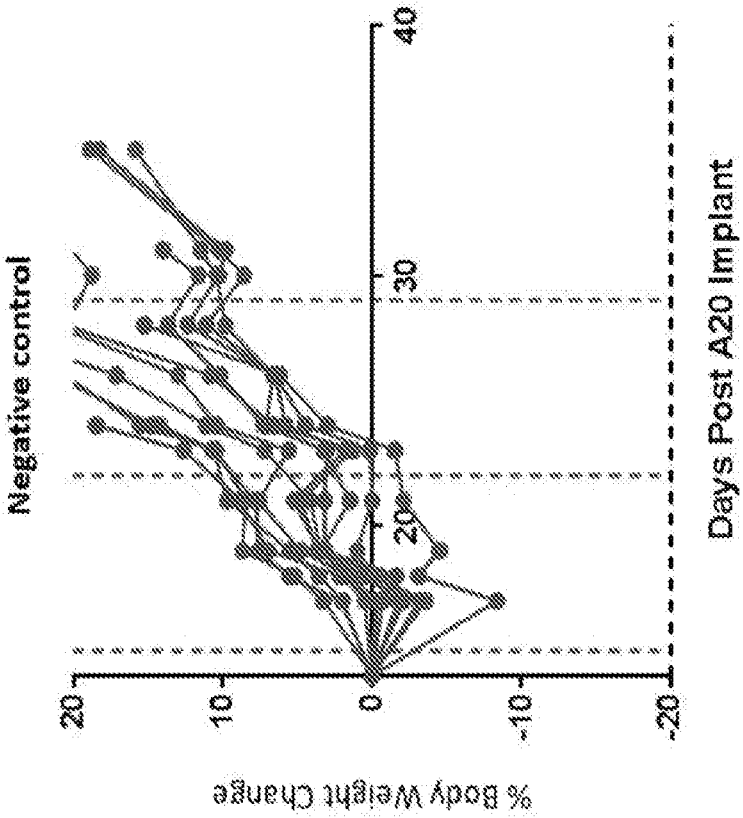


FIG. 11A

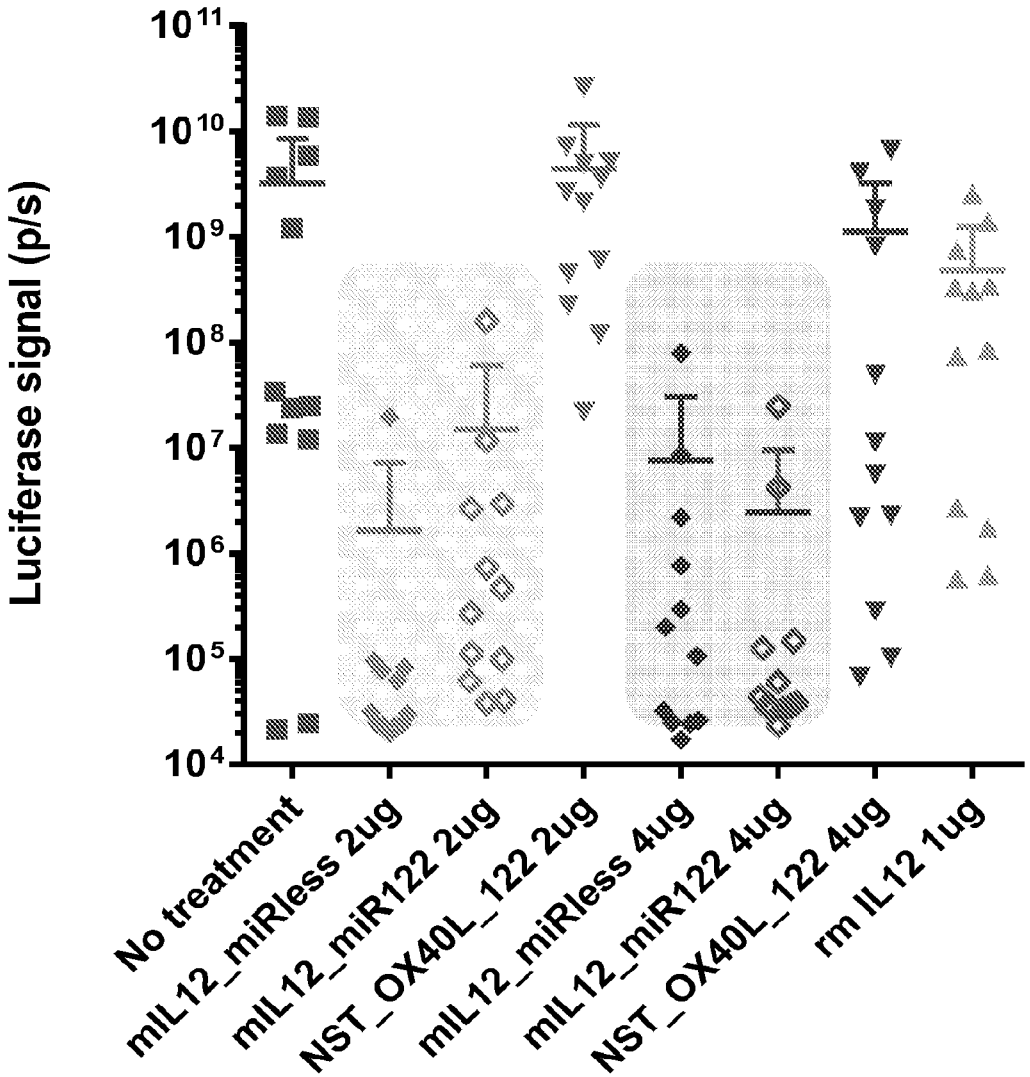
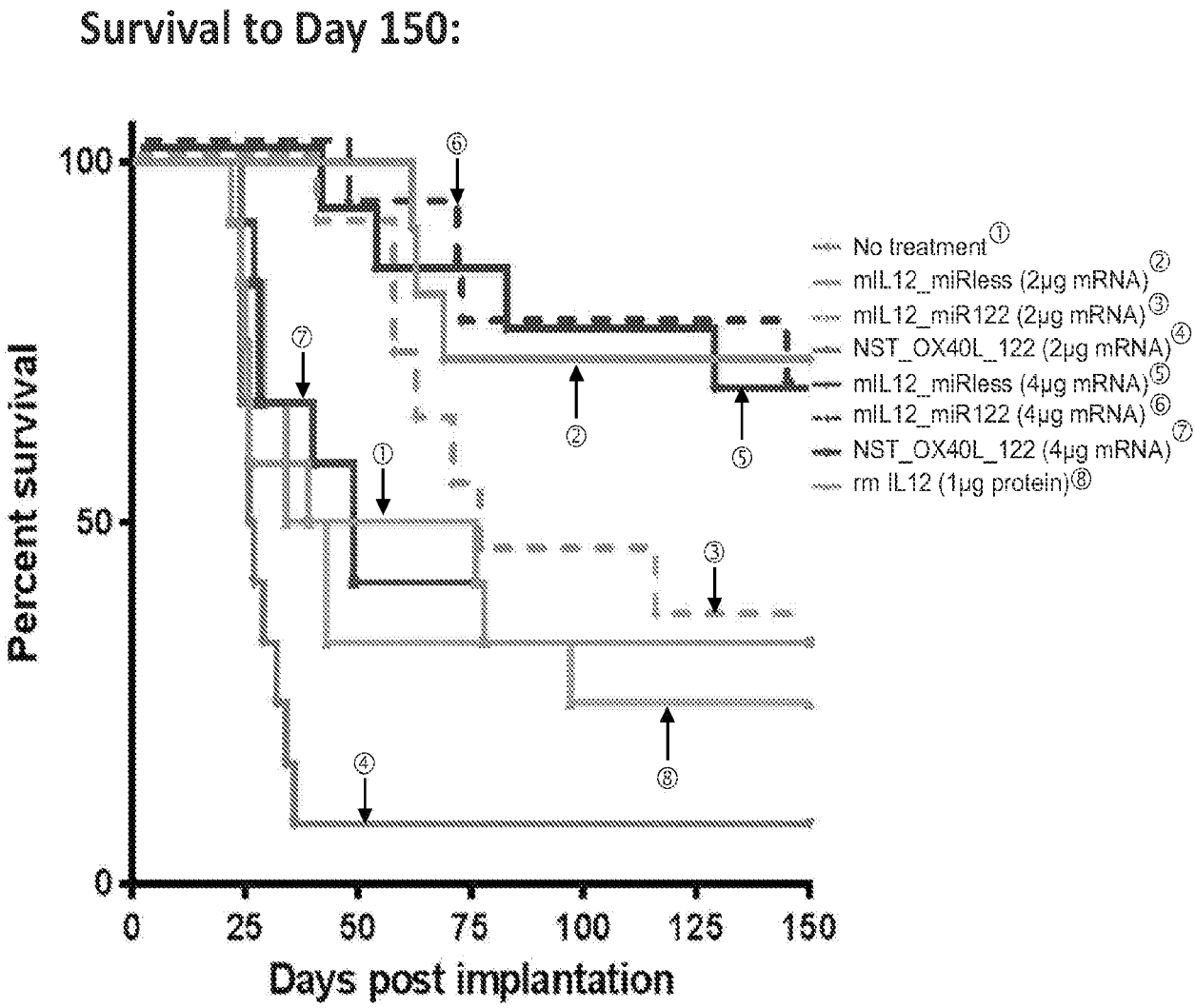

Luciferase signal (p/s)
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
No treatment
mIL12_miRless 2ug
mIL12_miR122 2ug
NST_OX40L_122 2ug
mIL12_miRless 4ug
mIL12_miR122 4ug
NST_OX40L_122 4ug
rm IL12 1ug

FIG. 11B


Survival to Day 150:
Percent survival
100
50
0
0
25
50
75
100
125
150
Days post implantation
No treatment ①
mIL12_miRless (2µg mRNA) ②
mIL12_miR122 (2µg mRNA) ③
NST_OX40L_122 (2µg mRNA) ④
mIL12_miRless (4µg mRNA) ⑤
mIL12_miR122 (4µg mRNA) ⑥
NST_OX40L_122 (4µg mRNA) ⑦
rm IL12 (1µg protein) ⑧

Protein	Length	Theoretical Minimum U (%)	Theoretical Minimum U (abs)			
IL12B WT	306	10.78%	99			
Nucleic Acid	Length	U Content (abs)	U Content (%)	U Content v WT (%)		U Content v Theoretical Minimum (%)
IL12B WT	918	195	21.24%	100.00%		196.97%
	MAX	275	29.96%	141.03%		277.78%
	MIN	109	11.87%	55.90%		110.10%
	MEAN	173.25	18.87%	88.85%		175.00%
	MEDIAN	176	19.17%	90.26%		177.78%
	STD DEV	44.19	4.81%	22.66%		44.63%

FIG. 12A

Protein	Length	Theoretical Maximum G (%)	Theoretical Maximum G (abs)			
IL12B WT	306	40.74%	374			
Nucleic Acid	Length	G Content(abs)	G Content(%)	G Content v WT(%)		G Content v Theoretical Maximum (%)
IL12B WT	918	243	26.47%	100.00%		64.97%
	MAX	374	40.74%	153.91%		100.00%
	MIN	219	23.86%	90.12%		58.56%
	MEAN	260.55	28.38%	107.22%		69.67%
	MEDIAN	249.5	27.18%	102.67%		66.71%
	STD DEV	35.31	3.85%	14.53%		9.44%

FIG. 12B

Protein	Length	Theoretical Maximum C (%)	Theoretical Maximum C (abs)		
IL12B WT	306	41.61%	382		
Nucleic Acid	Length	C Content (abs)	C Content (%)	C Content v WT (%)	C Content v Theoretical Maximum (%)
IL12B WT	918	223	24.29%	100.00%	58.38%
	MAX	279	30.39%	125.11%	73.04%
	MIN	213	23.20%	95.52%	55.76%
	MEAN	238.05	25.93%	106.75%	62.32%
	MEDIAN	238.5	25.98%	106.96%	62.43%
	STD DEV	18.41	2.01%	8.26%	4.82%

FIG. 12C

Protein	Length	Theoretical Maximum GC (%)	Theoretical Maximum GC (abs)		
IL12B WT	306	65.25%	599		
Nucleic Acid	Length	GC Content (abs)	GC Content (%)	GC Content v WT (%)	GC Content v Theoretical Minimum (%)
IL12B WT	918	466	50.76%	100.00%	77.80%
	MAX	587	63.94%	125.97%	98.00%
	MIN	449	48.91%	96.35%	74.96%
	MEAN	498.6	54.31%	107.00%	83.24%
	MEDIAN	489.5	53.32%	105.04%	81.72%
	STD DEV	43.23	4.71%	9.28%	7.22%

FIG. 12D

Protein	Length	Theoretical Minimum U (%)	Theoretical Minimum U (abs)		
IL12B WT	306	10.78%	99		
Nucleic Acid	Length	U Content (abs)	U Content (%)	U Content v WT (%)	U Content v Theoretical Minimum (%)
IL12B WT	918	195	21.24%	100.00%	196.97%
	MAX	149	16.23%	76.41%	150.51%
	MIN	131	14.27%	67.18%	132.32%
	MEAN	141.1	15.37%	72.36%	142.53%
	MEDIAN	141	15.36%	72.31%	142.42%
	STD DEV	5.37	0.58%	2.75%	5.42%

FIG. 13A

Protein	Length	Theoretical Maximum G (%)	Theoretical Maximum G (abs)		
IL12B WT	306	40.74%	374		
Nucleic Acid	Length	G Content (abs)	G Content (%)	G Content v WT (%)	G Content v Theoretical Minimum (%)
IL12B WT	918	243	26.47%	100.00%	64.97%
	MAX	294	32.03%	120.99%	78.61%
	MIN	279	30.39%	114.81%	74.60%
	MEAN	287.35	31.30%	118.25%	76.83%
	MEDIAN	288	31.37%	118.52%	77.01%
	STD DEV	4.63	0.50%	1.90%	1.24%

FIG. 13B

Protein	Length	Theoretical Maximum C (%)	Theoretical Maximum C (abs)		
IL12B WT	306	41.61%	382		
Nucleic Acid	Length	C Content (abs)	C Content (%)	C Content v WT (%)	C Content v Theoretical Maximum (%)
IL12B WT	918	223	24.29%	100.00%	58.38%
	MAX	279	30.39%	125.11%	73.04%
	MIN	262	28.54%	117.49%	68.59%
	MEAN	270.15	29.43%	121.14%	70.72%
	MEDIAN	270	29.41%	121.08%	70.68%
	STD DEV	4.76	0.52%	2.13%	1.25%

FIG. 13C

Protein	Length	Theoretical Maximum GC (%)	Theoretical Maximum GC (abs)		
IL12B WT	306	65.25%	599		
Nucleic Acid	Length	GC Content (abs)	GC Content (%)	GC Content v WT (%)	GC Content v Theoretical Maximum (%)
IL12B WT	918	466	50.76%	100.00%	77.80%
	MAX	565	61.55%	121.24%	94.32%
	MIN	547	59.59%	117.38%	91.32%
	MEAN	557.5	60.73%	119.64%	93.07%
	MEDIAN	557	60.68%	119.53%	92.99%
	STD DEV	4.67	0.51%	1.00%	0.78%

FIG. 13D

Protein	Length	Theoretical Minimum U (%)	Theoretical Minimum U (abs)		
IL12A WT	197	12.86%	76		
Nucleic Acid	Length	U Content (abs)	U Content (%)	U Content v WT (%)	U Content v Theoretical Minimum (%)
IL12A WT	591	151	25.55%	100.00%	198.68%
	MAX	189	31.98%	125.17%	248.68%
	MIN	83	14.04%	54.97%	109.21%
	MEAN	126.3	21.37%	83.64%	166.18%
	MEDIAN	125	21.15%	82.78%	164.47%
	STD DEV	28.03	4.74%	18.56%	36.88%

FIG. 14A

Protein	Length	Theoretical Maximum G (%)	Theoretical Maximum G (abs)		
IL12A WT	197	34.69%	205		
Nucleic Acid	Length	G Content (abs)	G Content (%)	G Content v WT (%)	G Content v Theoretical Maximum (%)
IL12A WT	591	123	20.81%	100.00%	60.00%
	MAX	205	34.69%	166.67%	100.00%
	MIN	108	18.27%	87.80%	52.68%
	MEAN	134.7	22.79%	109.51%	65.71%
	MEDIAN	131	22.17%	106.50%	63.90%
	STD DEV	22.88	3.87%	18.60%	11.16%

FIG. 14B

Protein	Length	Theoretical Maximum C (%)	Theoretical Maximum C (abs)		
IL12A WT	197	43.82%	259		
Nucleic Acid	Length	C Content (abs)	C Content (%)	C Content v WT (%)	C Content v Theoretical Maximum (%)
IL12A WT	591	138	23.35%	100.00%	53.28%
MAX		191	32.32%	138.41%	73.75%
MIN		140	23.69%	101.45%	54.05%
MEAN		164.8	27.88%	119.42%	63.63%
MEDIAN		164	27.75%	118.84%	63.32%
STD DEV		12.22	2.07%	8.85%	4.72%

FIG. 14C

Protein	Length	Theoretical Maximum GC (%)	Theoretical Maximum GC (abs)		
IL12A WT	197	62.27%	368		
Nucleic Acid	Length	GC Content (abs)	GC Content (%)	GC Content v WT (%)	GC Content v Theoretical Maximum (%)
IL12A WT	591	261	44.16%	100.00%	70.92%
MAX		359	60.74%	137.55%	97.55%
MIN		265	44.84%	101.53%	72.01%
MEAN		299.5	50.68%	114.75%	81.39%
MEDIAN		293	49.58%	112.26%	79.62%
STD DEV		29.23	4.95%	11.20%	7.94%

FIG. 14D

Protein	Length	Theoretical Minimum U (%)	Theoretical Minimum U (abs)		
IL12A WT	197	12.86%	76		
Nucleic Acid	Length	U Content (abs)	U Content (%)	U Content v WT (%)	U Content v Theoretical Maximum (%)
IL12A WT	591	151	25.55%	100.00%	198.68%
	MAX	109	18.44%	72.19%	143.42%
	MIN	95	16.07%	62.91%	125.00%
	MEAN	101.9	17.24%	67.48%	134.08%
	MEDIAN	102	17.26%	67.55%	134.21%
	STD DEV	4.27	0.72%	2.83%	5.61%

FIG. 15A

Protein	Length	Theoretical Maximum G (%)	Theoretical Maximum G (abs)		
IL12A WT	197	34.69%	205		
Nucleic Acid	Length	G Content (abs)	G Content (%)	G Content v WT (%)	G Content v Theoretical Maximum (%)
IL12A WT	591	123	20.81%	100.00%	60.00%
	MAX	160	27.07%	130.08%	78.05%
	MIN	147	24.87%	119.51%	71.71%
	MEAN	153.45	25.96%	124.76%	74.85%
	MEDIAN	153.5	25.97%	124.80%	74.88%
	STD DEV	3.38	0.57%	2.75%	1.65%

FIG. 15B

Protein	Length	Theoretical Maximum C (%)	Theoretical Maximum C (abs)		
IL12A WT	197	43.82%	259		
Nucleic Acid	Length	C Content (abs)	C Content (%)	C Content v WT (%)	C Content v Theoretical Maximum (%)
IL12A WT	591	138	23.35%	100.00%	53.28%
	MAX	195	32.99%	141.30%	75.29%
	MIN	179	30.29%	129.71%	69.11%
	MEAN	187.8	31.78%	136.09%	72.51%
	MEDIAN	187.5	31.73%	135.87%	72.39%
	STD DEV	4.05	0.68%	2.93%	1.56%

FIG. 15C

Protein	Length	Theoretical Maximum GC (%)	Theoretical Maximum GC (abs)		
IL12A WT	197	62.27%	368		
Nucleic Acid	Length	GC Content (abs)	GC Content (%)	GC Content v WT (%)	GC Content v Theoretical Maximum (%)
IL12A WT	591	261	44.16%	100.00%	70.92%
	MAX	350	59.22%	134.10%	95.11%
	MIN	337	57.02%	129.12%	91.58%
	MEAN	341.25	57.74%	130.75%	92.73%
	MEDIAN	340	57.53%	130.27%	92.39%
	STD DEV	4.08	0.69%	1.56%	1.11%

FIG. 15D

IL12B (w/o SP) G5 SEQUENCES				
Sequence	GC	GC 1st	GC 2nd	GC 3rd
IL12B_WT	**50.76**	**48.04**	**43.14**	**61.11**
MAX	63.94	50.65	43.14	100
MIN	48.91	48.04	43.14	54.25
MEAN	54.31	49.46	43.14	70.34
MEDIAN	53.325	49.35	43.14	66.34
STD DEV	4.71	0.80	0.00	14.42

FIG. 16A

IL12B (W/O SP) G6 SEQUENCES				
Sequence	GC	GC 1st	GC 2nd	GC 3rd
IL12B_WT	**50.76**	**48.04**	**43.14**	**61.11**
MAX	61.55	51.63	43.14	91.83
MIN	59.59	49.35	43.14	85.29
MEAN	60.73	50.33	43.14	88.73
MEDIAN	60.68	50.33	43.14	88.725
STD DEV	0.51	0.61	0.00	1.60

FIG. 16B

IL12A (W/O SP) G5 SEQUENCES				
Sequence	GC	GC 1st	GC 2nd	GC 3rd
IL12A_WT	**44.16**	**45.18**	**35.53**	**51.78**
MAX	60.74	48.73	35.53	100
MIN	44.84	43.65	35.53	52.28
MEAN	50.68	46.35	35.53	70.15
MEDIAN	49.58	46.7	35.53	67.765
STD DEV	4.94	1.44	0.00	14.35

FIG. 16C

IL12B (W/O SP) G6 SEQUENCES				
Sequence	GC	GC 1st	GC 2nd	GC 3rd
IL12A_WT	**44.16**	**45.18**	**35.53**	**51.78**
MAX	59.22	51.27	35.53	93.91
MIN	57.02	46.7	35.53	85.79
MEAN	57.74	48.45	35.53	89.24
MEDIAN	57.53	48.22	35.53	88.83
STD DEV	0.69	1.02	0.00	1.80

FIG. 16D

FIG. 17A

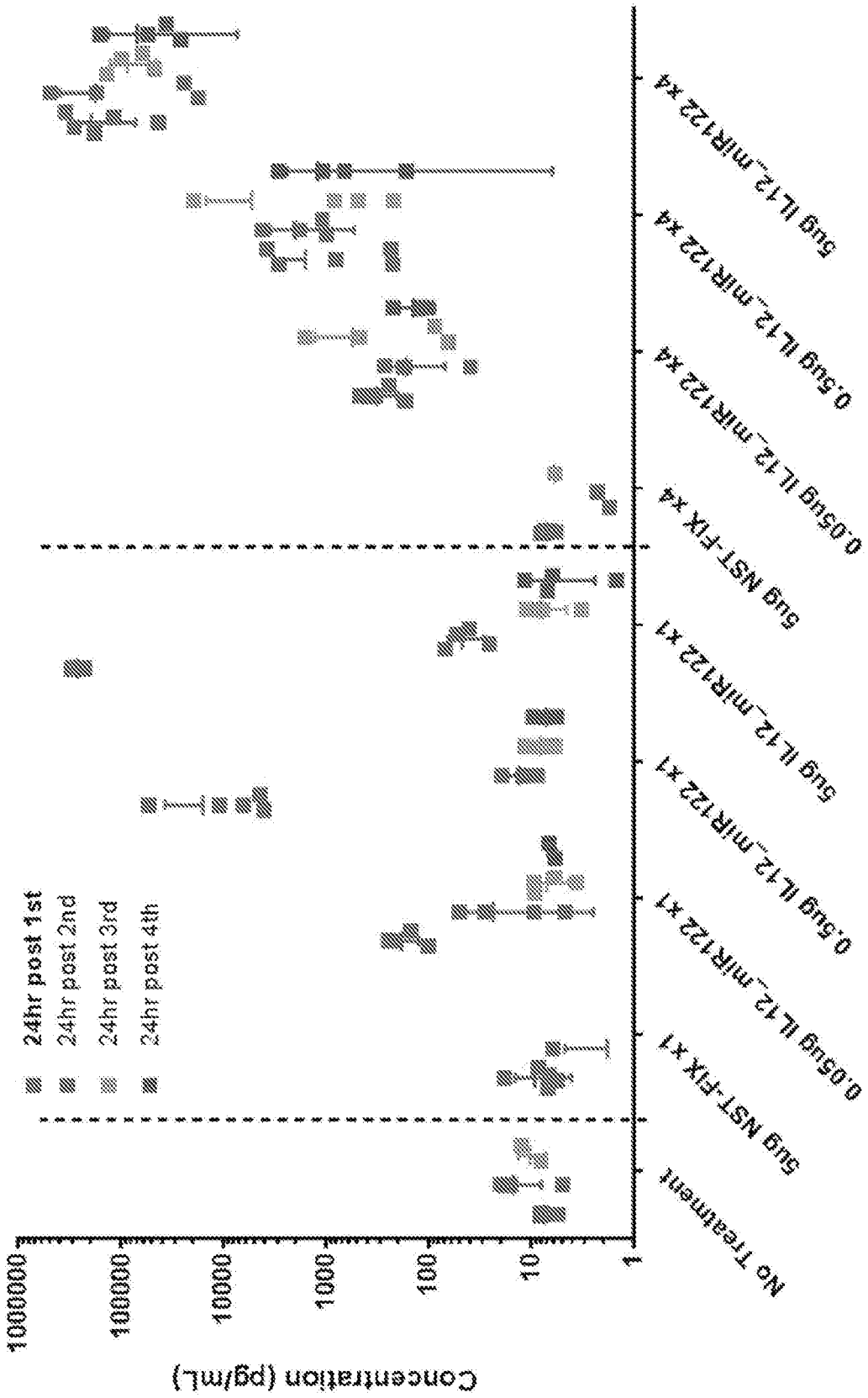

24hr post 1st
24hr post 2nd
24hr post 3rd
24hr post 4th
Concentration (pg/mL)
1000000
100000
10000
1000
100
10
1
No Treatment
5ug NST-FIX x1
0.05ug IL12_mIR122 x1
0.5ug IL12_mIR122 x1
5ug IL12_mIR122 x1
5ug NST-FIX x4
0.05ug IL12_mIR122 x4
0.5ug IL12_mIR122 x4
5ug IL12_mIR122 x4

FIG. 17B

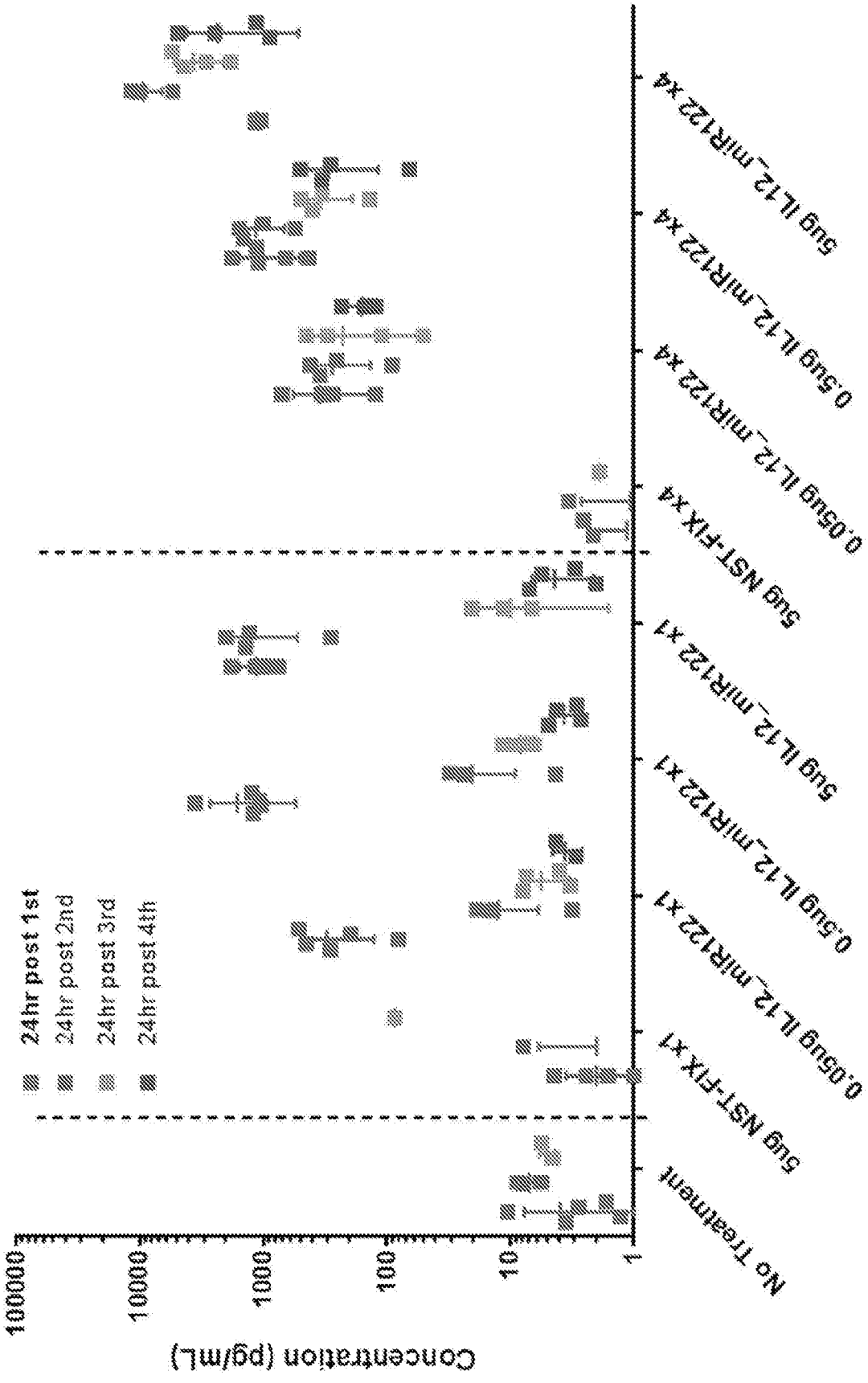

24hr post 1st
24hr post 2nd
24hr post 3rd
24hr post 4th
Concentration (pg/mL)
100000
10000
1000
100
10
1
No Treatment
5ug NST-FIX x1
0.05ug IL-12_mIR122 x1
0.5ug IL-12_mIR122 x1
5ug IL-12_mIR122 x1
5ug NST-FIX x4
0.05ug IL-12_mIR122 x4
0.5ug IL-12_mIR122 x4
5ug IL-12_mIR122 x4

FIG. 18A

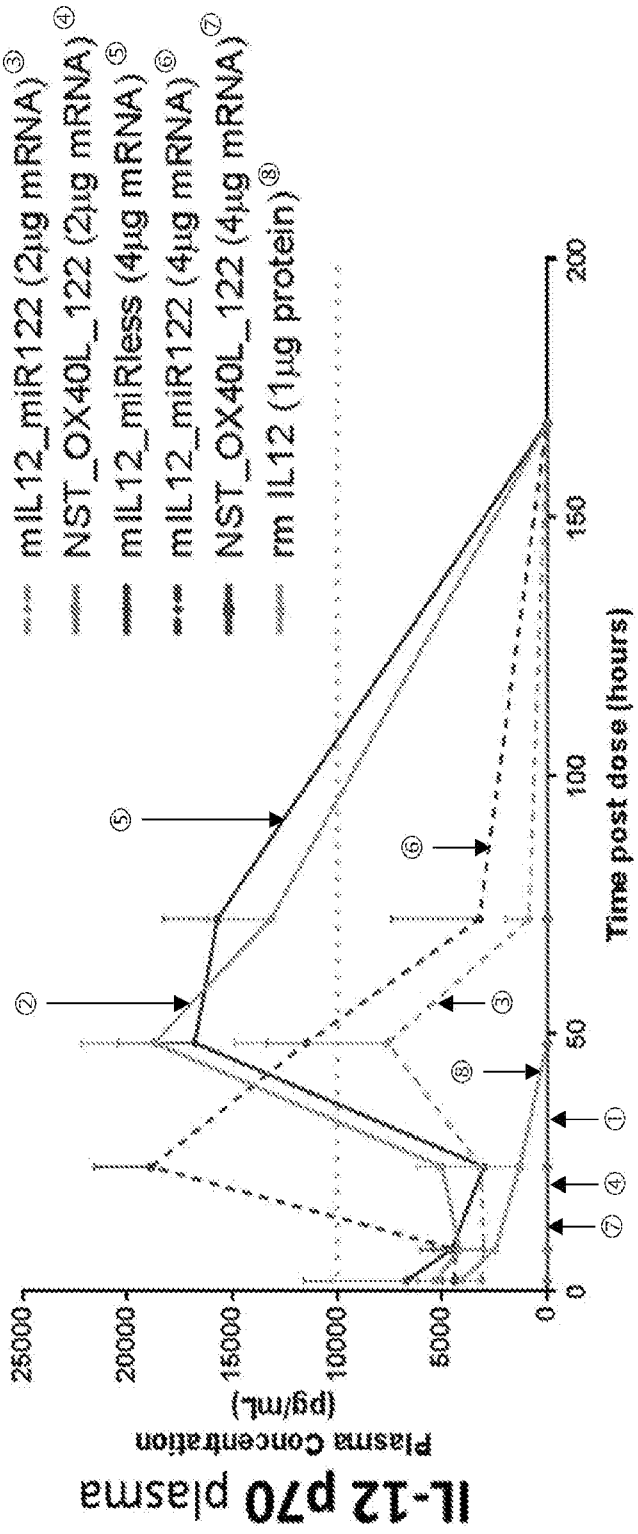

IL-12 p70 plasma
Plasma Concentration
(pg/mL)
25000
20000
15000
10000
5000
0
0
50
100
150
200
Time post dose (hours)
① No treatment
② mIL12_miRless (2μg mRNA)
③ mIL12_miR122 (2μg mRNA)
④ NST_OX40L_122 (2μg mRNA)
⑤ mIL12_miRless (4μg mRNA)
⑥ mIL12_miR122 (4μg mRNA)
⑦ NST_OX40L_122 (4μg mRNA)
⑧ rm IL12 (1μg protein)

FIG. 18B

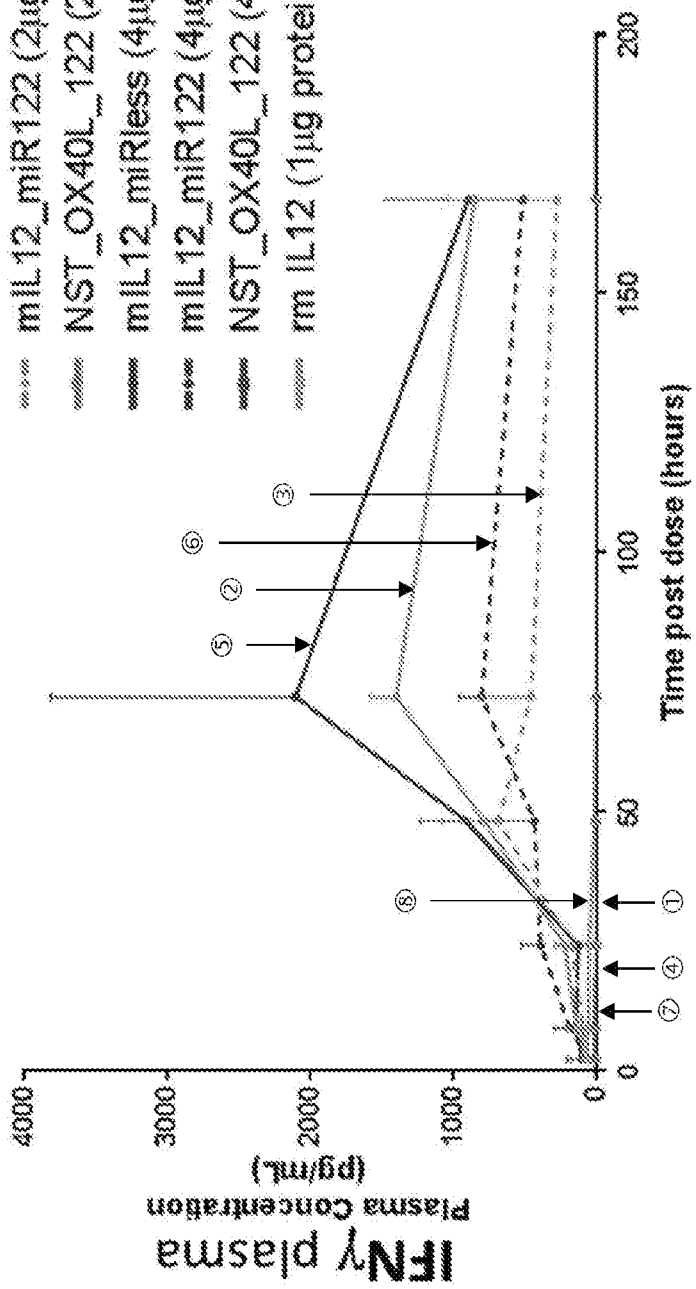

IFNγ plasma
Plasma concentration
(pg/mL)
4000
3000
2000
1000
0
0
50
100
150
200
Time post dose (hours)
① No treatment
② mIL12_miRless (2μg mRNA)
③ mIL12_miR122 (2μg mRNA)
④ NST_OX40L_122 (2μg mRNA)
⑤ mIL12_miRless (4μg mRNA)
⑥ mIL12_miR122 (4μg mRNA)
⑦ NST_OX40L_122 (4μg mRNA)
⑧ rm IL12 (1μg protein)

FIG. 19B

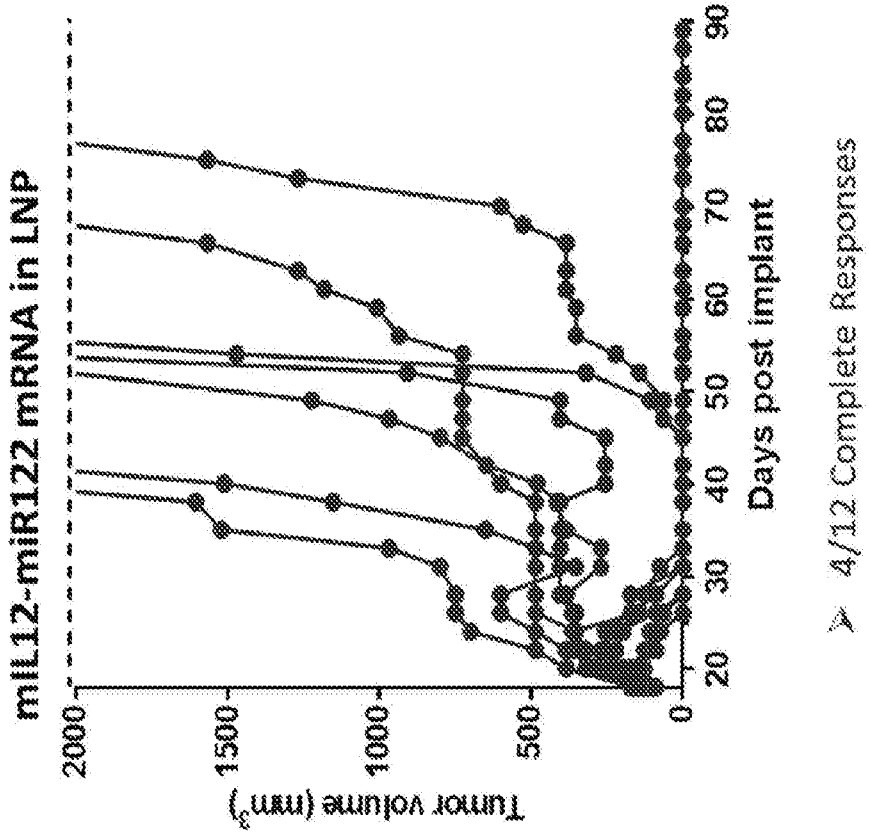


FIG. 19A

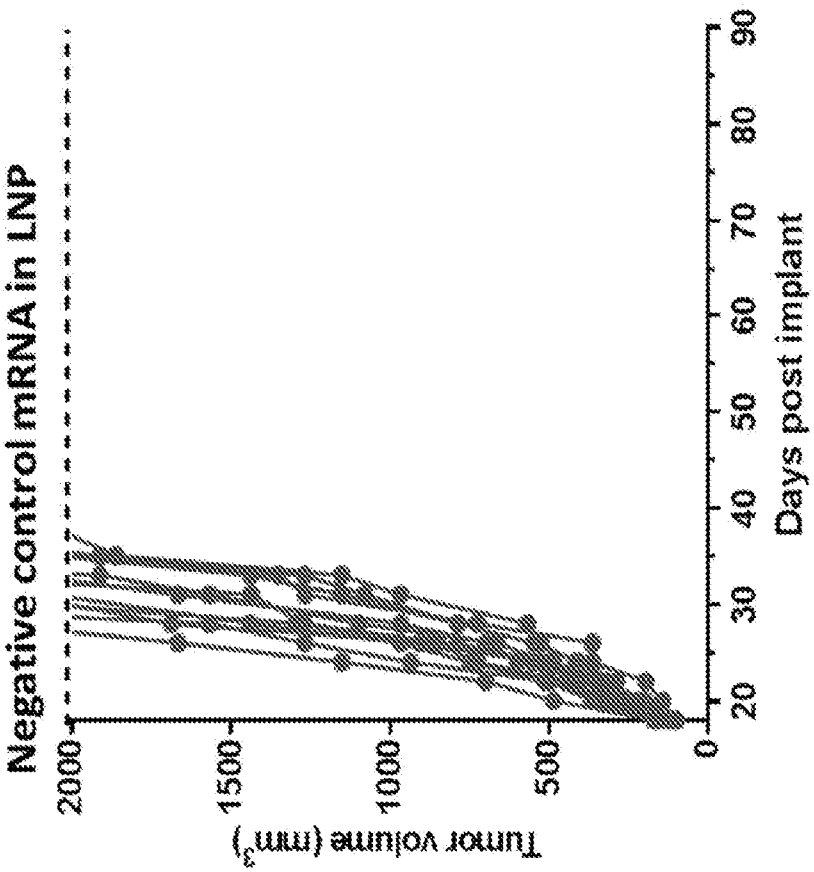


FIG. 19C

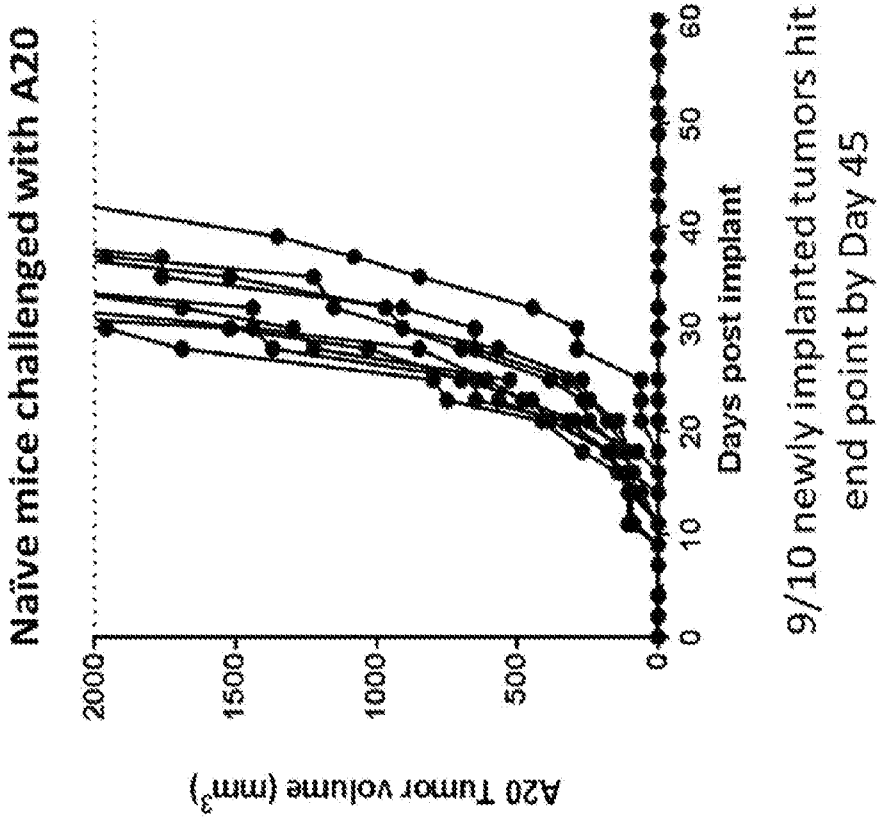
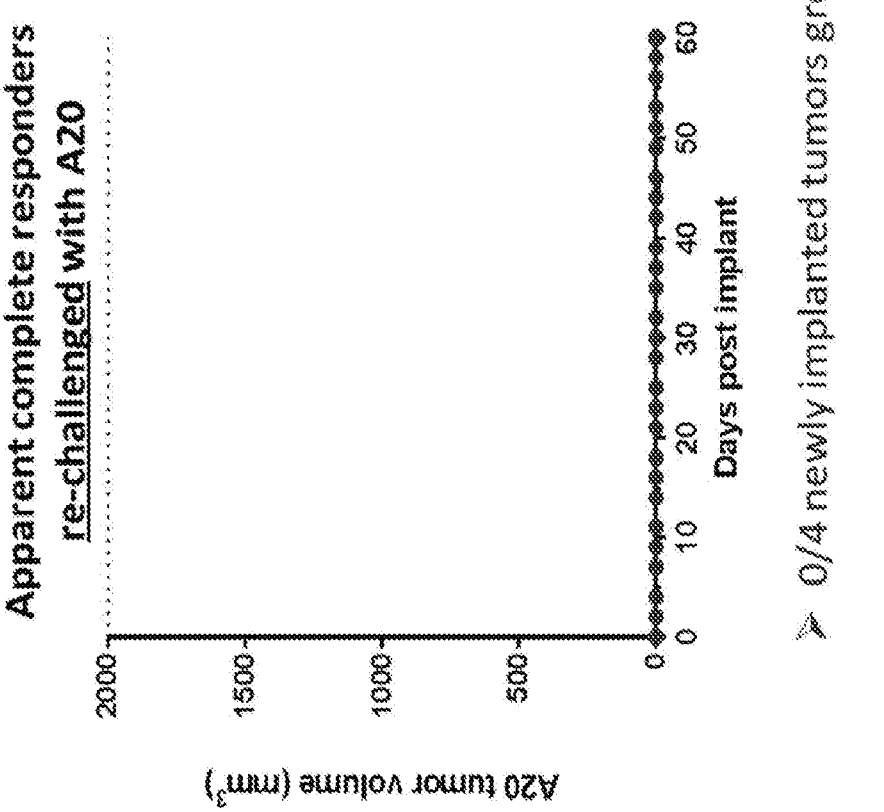


FIG. 19D



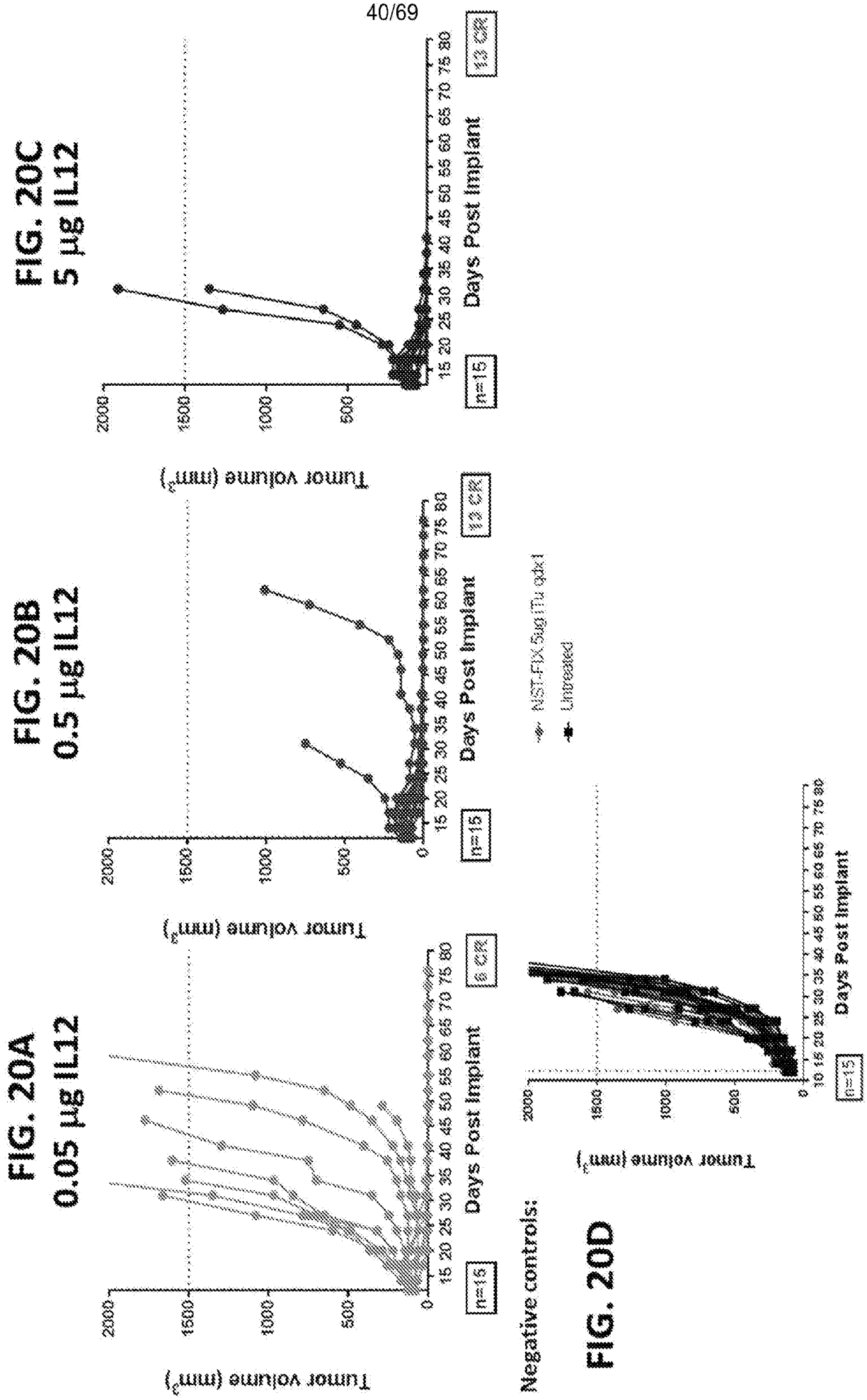


FIG. 20A
0.05 µg IL12
FIG. 20B
0.5 µg IL12
FIG. 20C
5 µg IL12
Tumor volume (mm³)
Days Post Implant
n=15
Negative controls:
NST-FIX 5ug iTu qdx1
Untreated

FIG. 20D

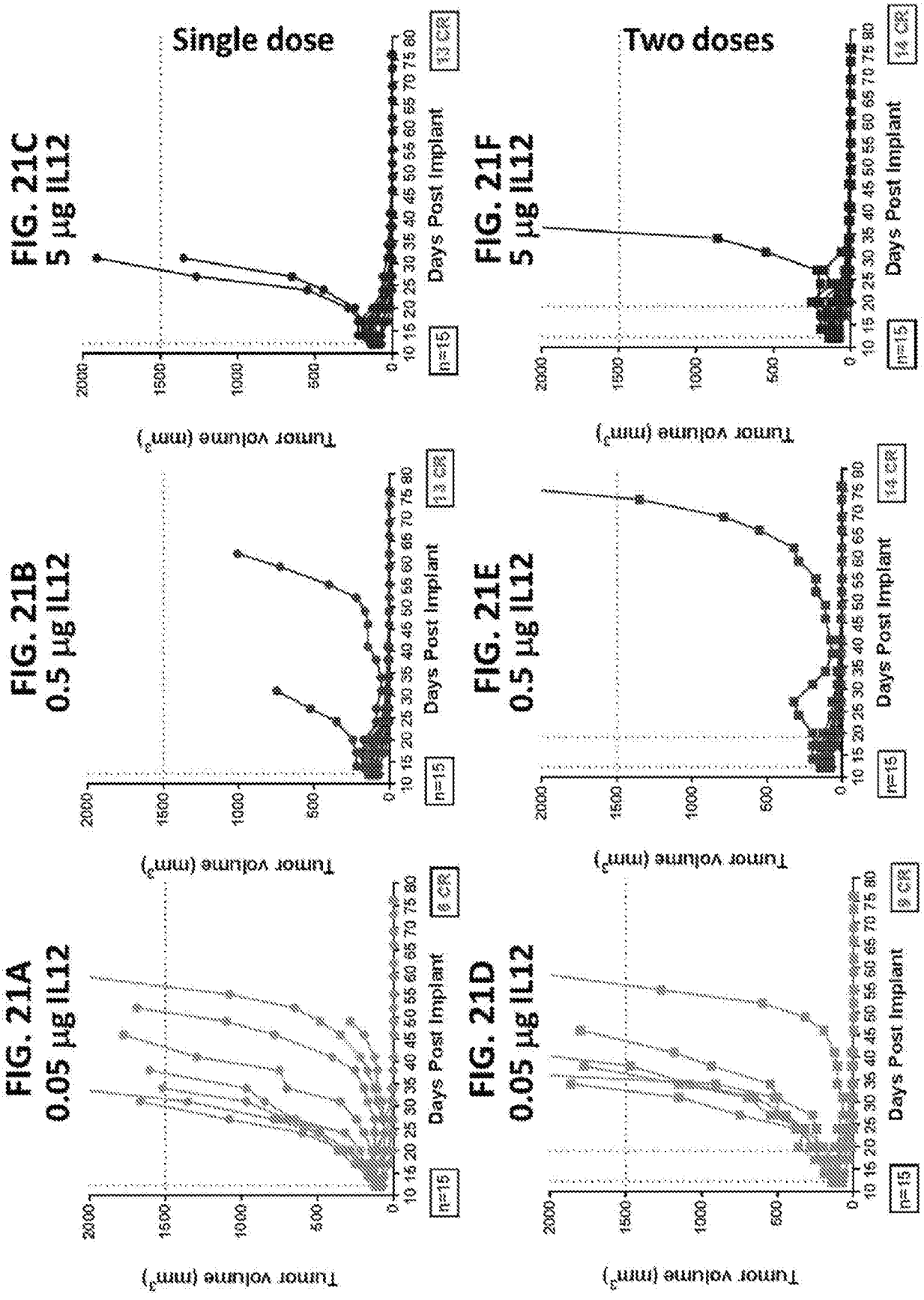
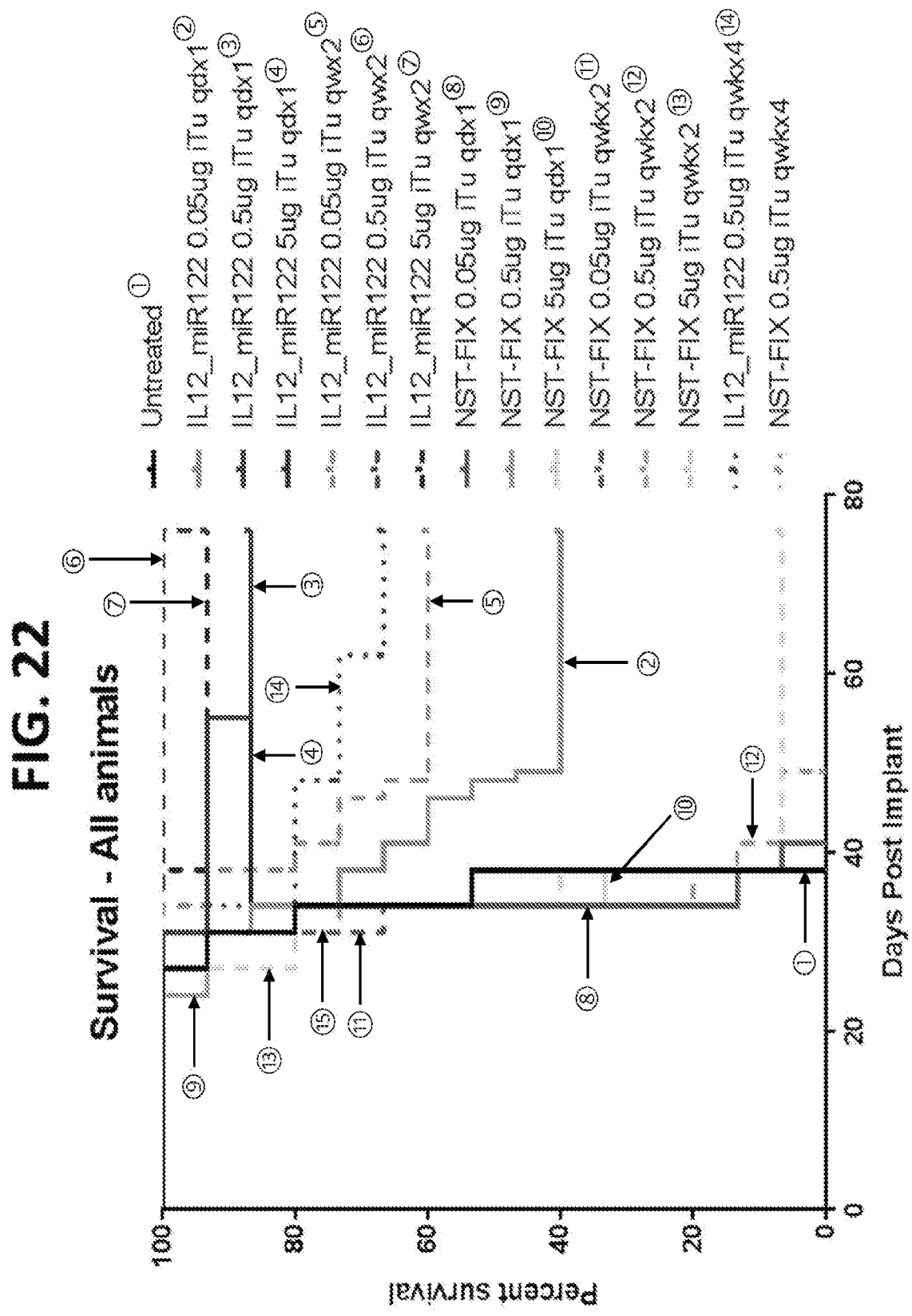


FIG. 21A
0.05 µg IL12
FIG. 21B
0.5 µg IL12
FIG. 21C
5 µg IL12
FIG. 21D
0.05 µg IL12
FIG. 21E
0.5 µg IL12
FIG. 21F
5 µg IL12
Single dose
Two doses
Tumor volume (mm³)
Days Post Implant
n=15
8 CR
13 CR
14 CR

FIG. 22


Survival - All animals
Percent survival
Days Post Implant
0
20
40
60
80
100
Untreated ①
IL12_miR122 0.05ug iTu qdx1 ②
IL12_miR122 0.5ug iTu qdx1 ③
IL12_miR122 5ug iTu qdx1 ④
IL12_miR122 0.05ug iTu qwx2 ⑤
IL12_miR122 0.5ug iTu qwx2 ⑥
IL12_miR122 5ug iTu qwx2 ⑦
NST-FIX 0.05ug iTu qdx1 ⑧
NST-FIX 0.5ug iTu qdx1 ⑨
NST-FIX 5ug iTu qdx1 ⑩
NST-FIX 0.05ug iTu qwkx2 ⑪
NST-FIX 0.5ug iTu qwkx2 ⑫
NST-FIX 5ug iTu qwkx2 ⑬
IL12_miR122 0.5ug iTu qwkx4 ⑭
NST-FIX 0.5ug iTu qwkx4

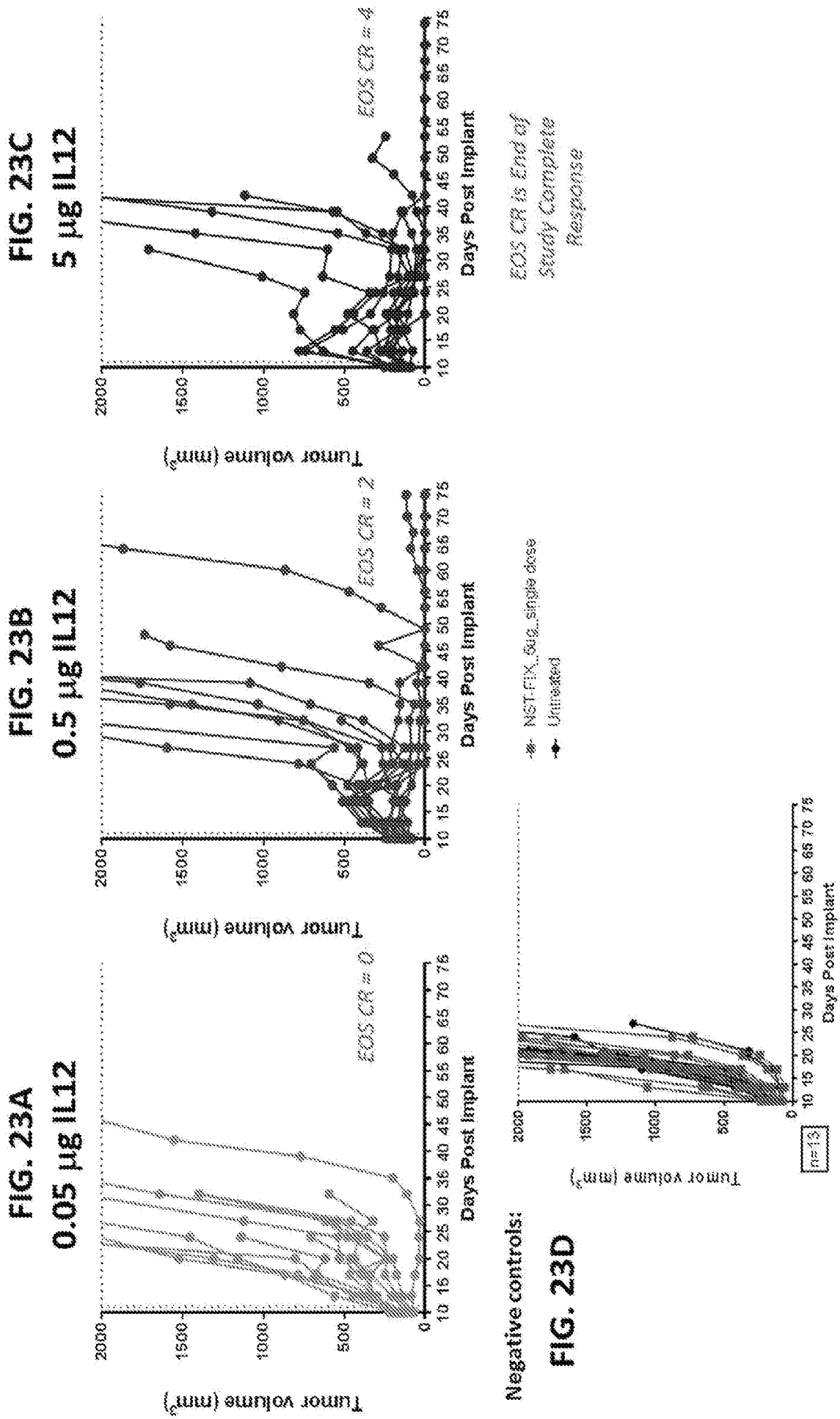


FIG. 23A
0.05 µg IL12
EOS CR = 0
FIG. 23B
0.5 µg IL12
EOS CR = 2
FIG. 23C
5 µg IL12
EOS CR = 4
Tumor volume (mm³)
Days Post Implant
EOS CR is End of Study Complete Response
Negative controls:
NST-FIX_5ug_single dose
Untreated
n=13
FIG. 23D

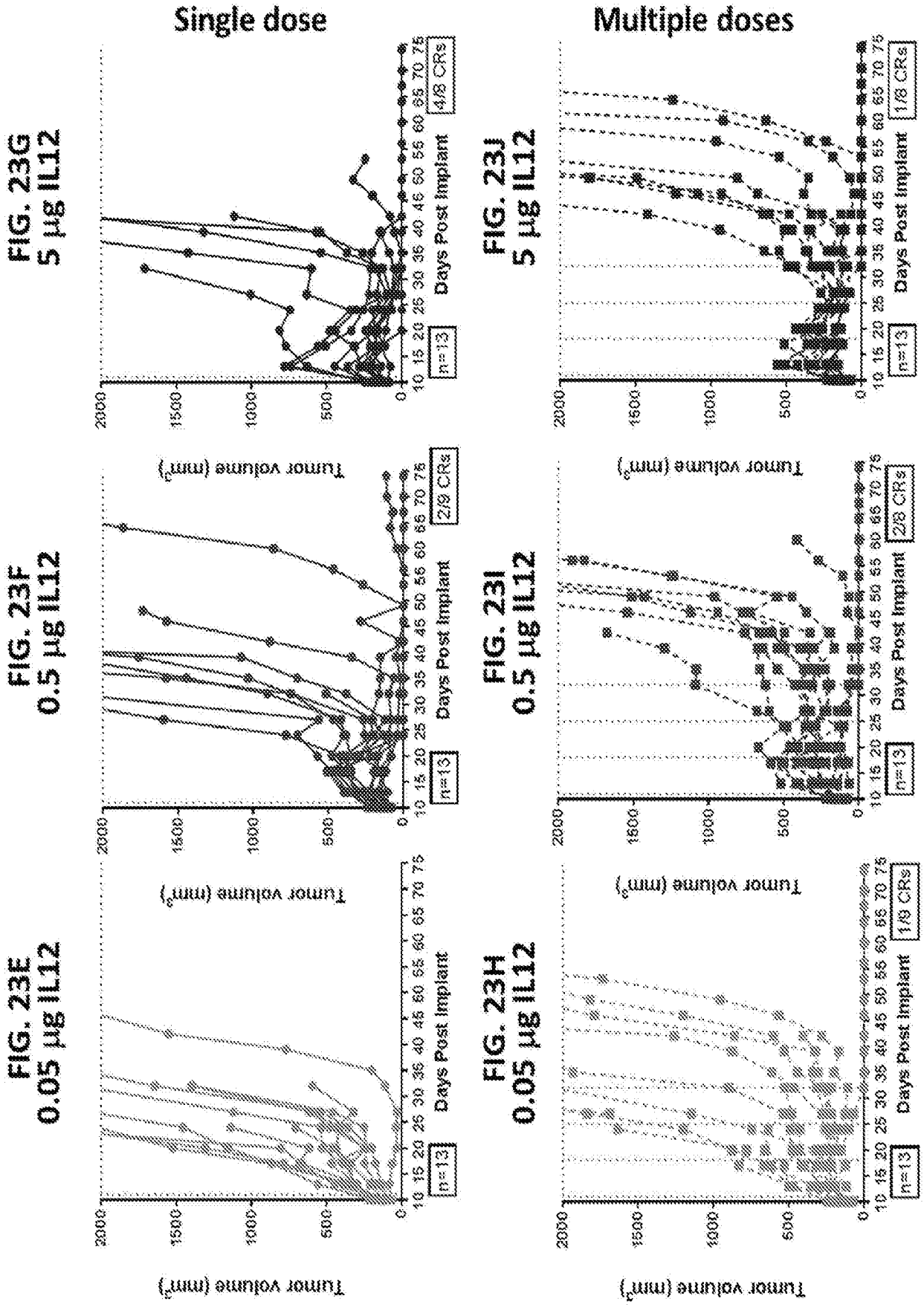

Single dose
Multiple doses
FIG. 23G
5 µg IL12
FIG. 23J
5 µg IL12
FIG. 23F
0.5 µg IL12
FIG. 23I
0.5 µg IL12
FIG. 23E
0.05 µg IL12
FIG. 23H
0.05 µg IL12
Tumor volume (mm³)
Days Post Implant
n=13
4/8 CRs
1/8 CRs
2/8 CRs
1/8 CRs

FIG. 24

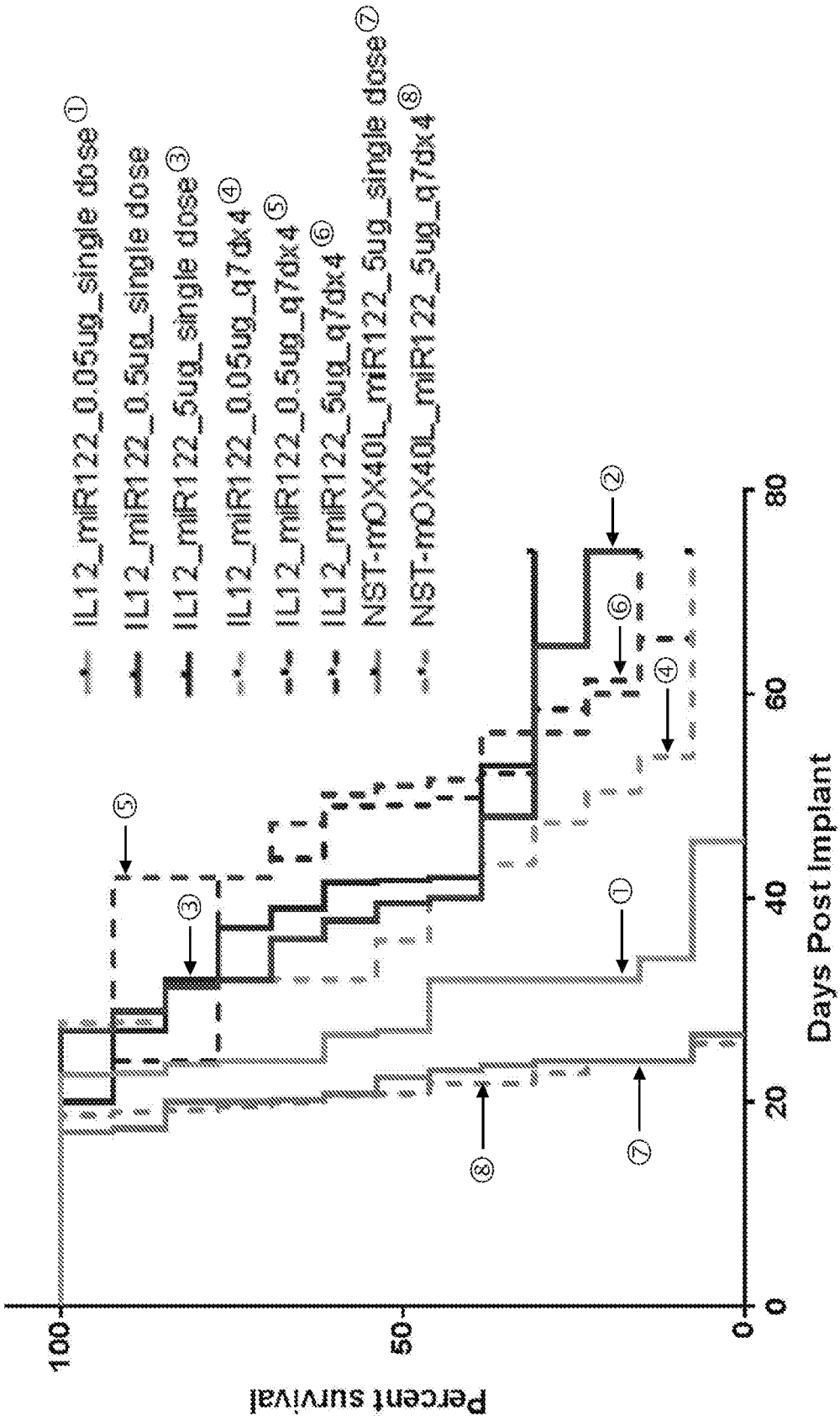

IL12_miR122_0.05ug_single dose ①
IL12_miR122_0.5ug_single dose ②
IL12_miR122_5ug_single dose ③
IL12_miR122_0.05ug_q7dx4 ④
IL12_miR122_0.5ug_q7dx4 ⑤
IL12_miR122_5ug_q7dx4 ⑥
NST-mOX40L_miR122_5ug_single dose ⑦
NST-mOX40L_miR122_5ug_q7dx4 ⑧
Percent survival
100
50
0
0
20
40
60
80
Days Post Implant

FIG. 25

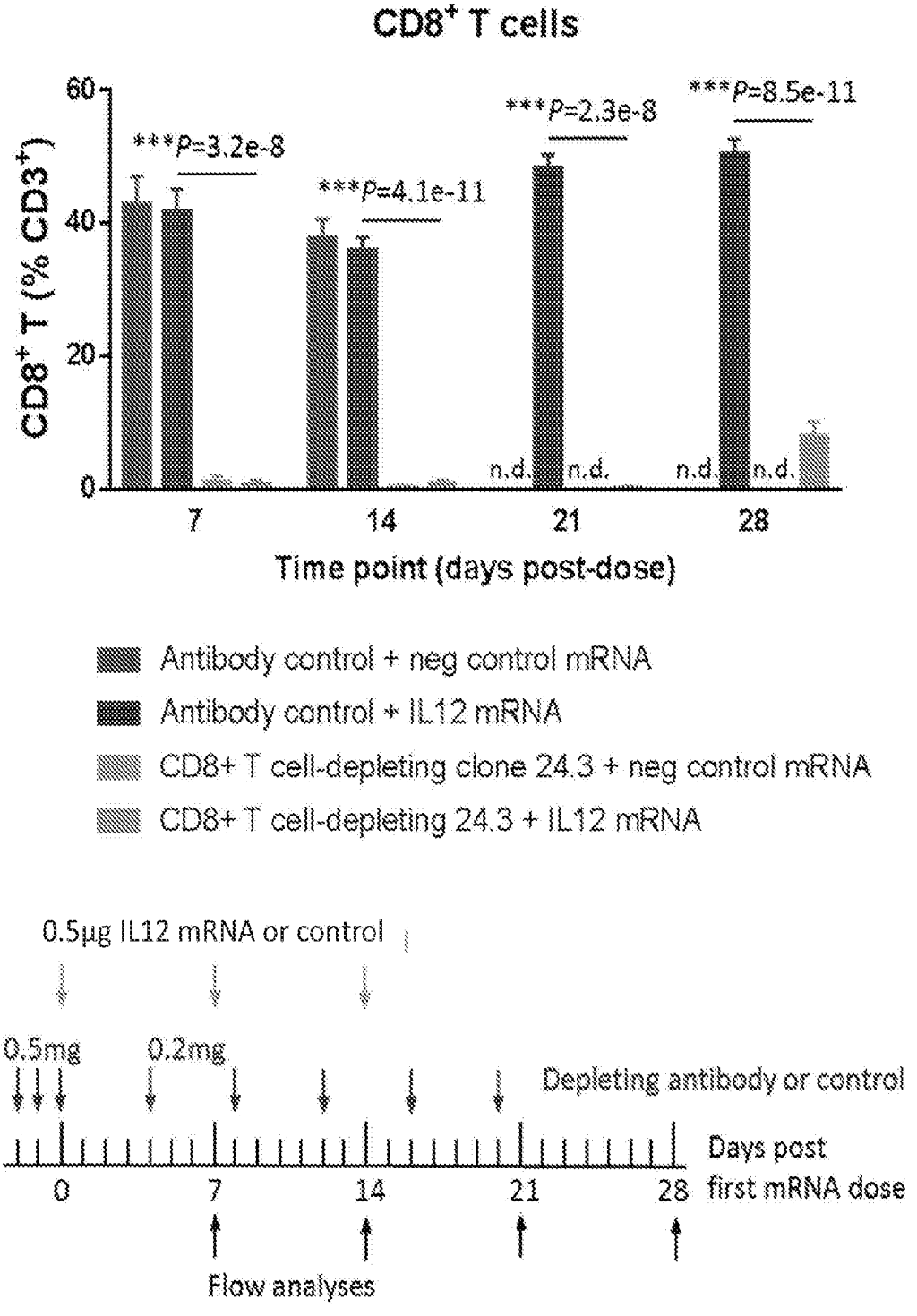

CD8+ T cells
CD8+ T (% CD3+)
60
40
20
0
***P=3.2e-8
***P=4.1e-11
***P=2.3e-8
***P=8.5e-11
n.d.
n.d.
n.d.
n.d.
7
14
21
28
Time point (days post-dose)
Antibody control + neg control mRNA
Antibody control + IL12 mRNA
CD8+ T cell-depleting clone 24.3 + neg control mRNA
CD8+ T cell-depleting 24.3 + IL12 mRNA
0.5µg IL12 mRNA or control
0.5mg
0.2mg
Depleting antibody or control
Days post
first mRNA dose
0
7
14
21
28
Flow analyses

FIG. 26A

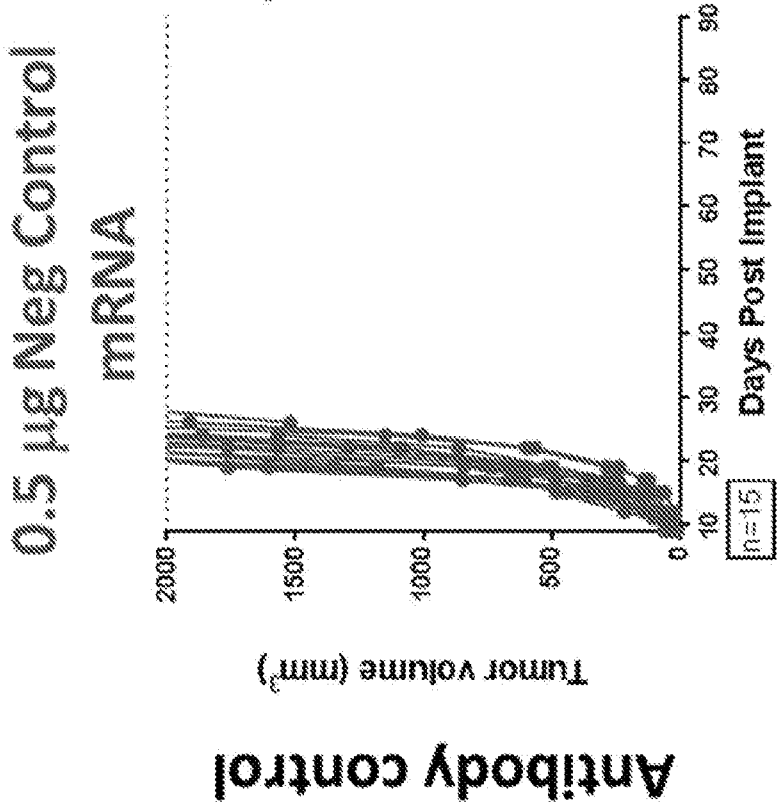
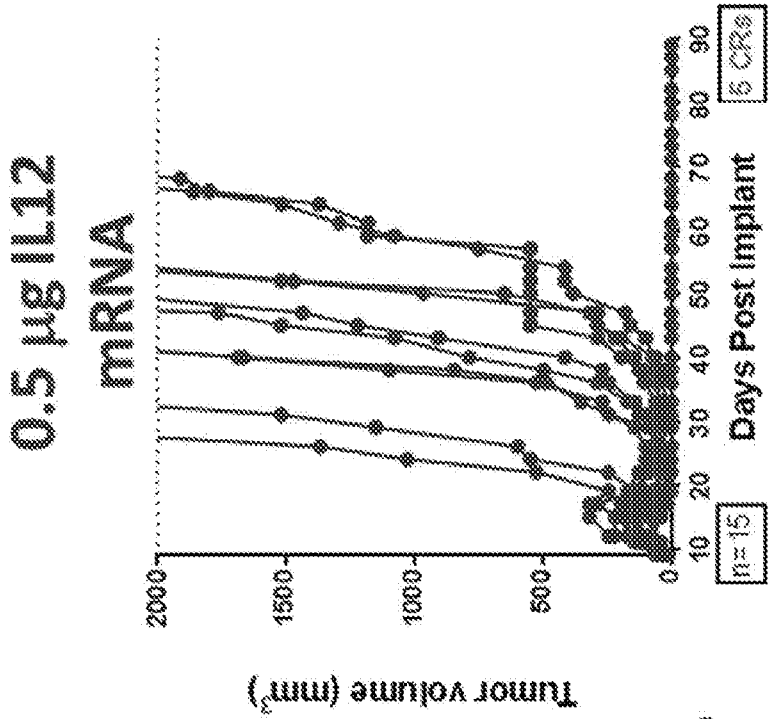


FIG. 26B



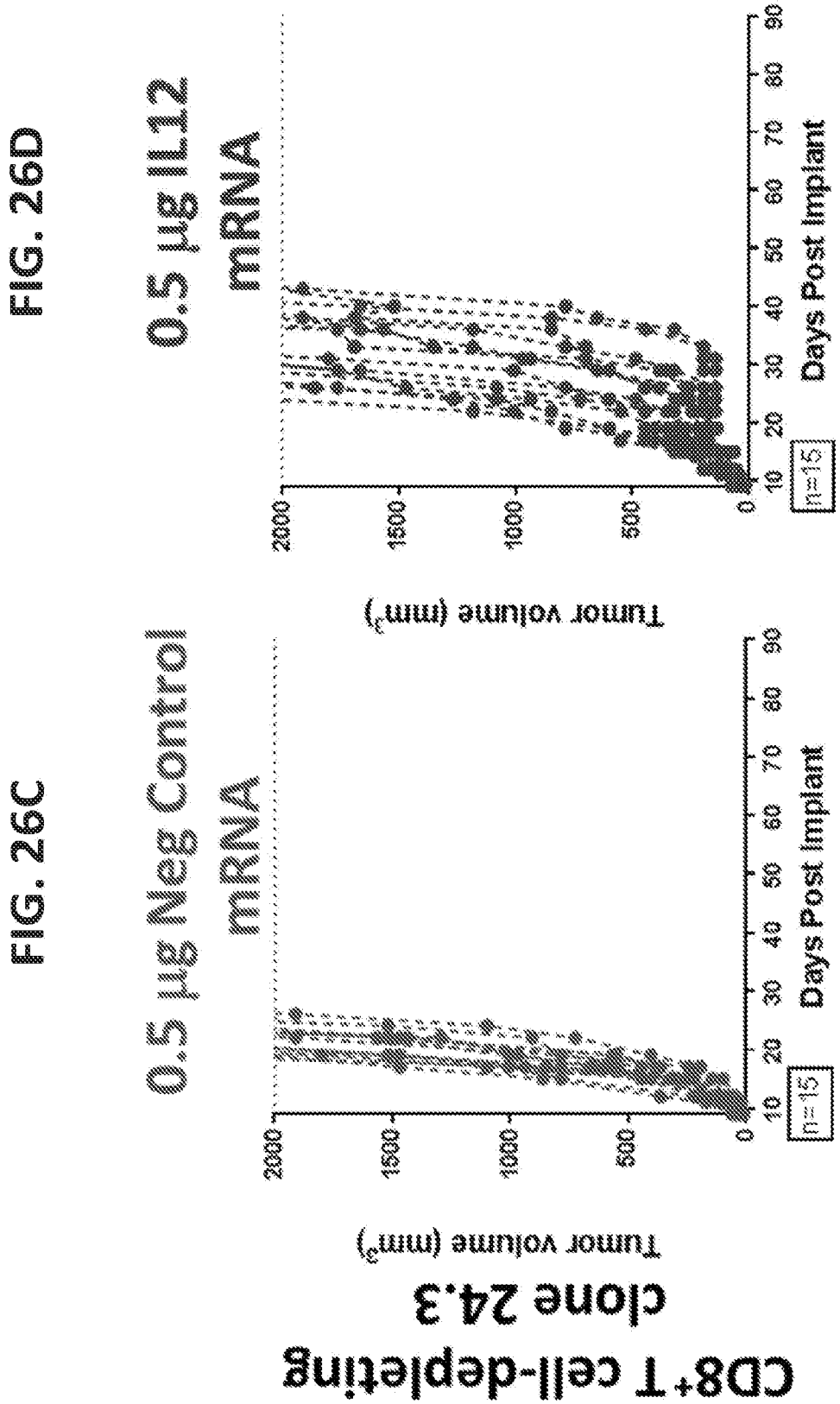


FIG. 26C

FIG. 26D

FIG. 26E

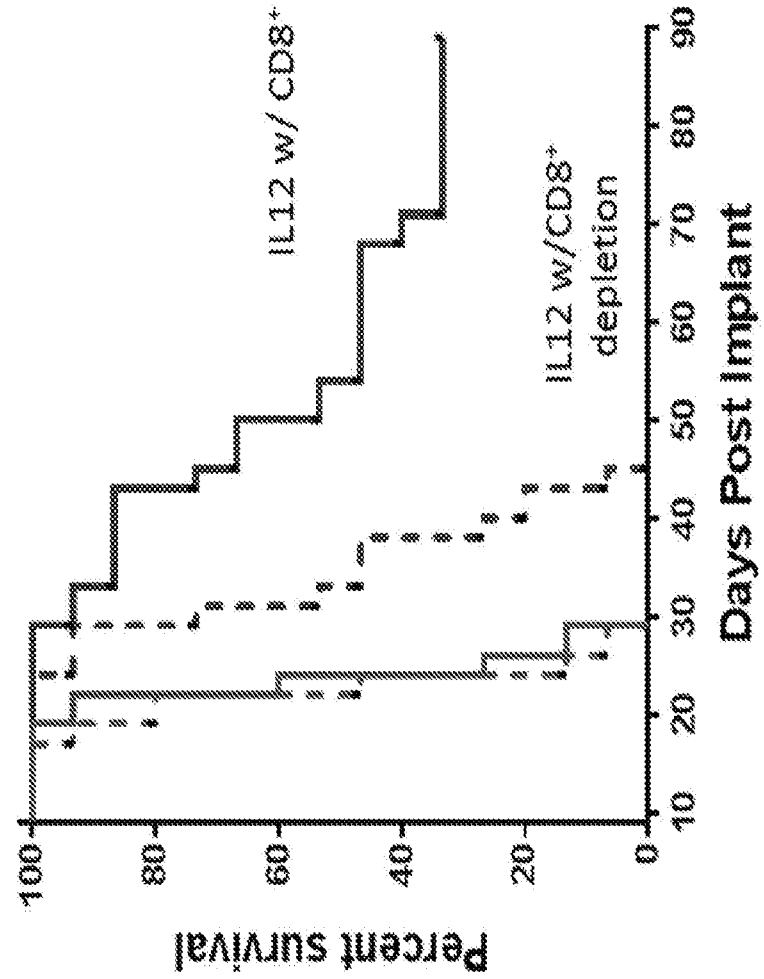

Kaplan-Meier:
Percent survival
Days Post Implant
IL12 w/ CD8+
IL12 w/CD8+ depletion
100
80
60
40
20
0
10
20
30
40
50
60
70
80
90

FIG. 27A

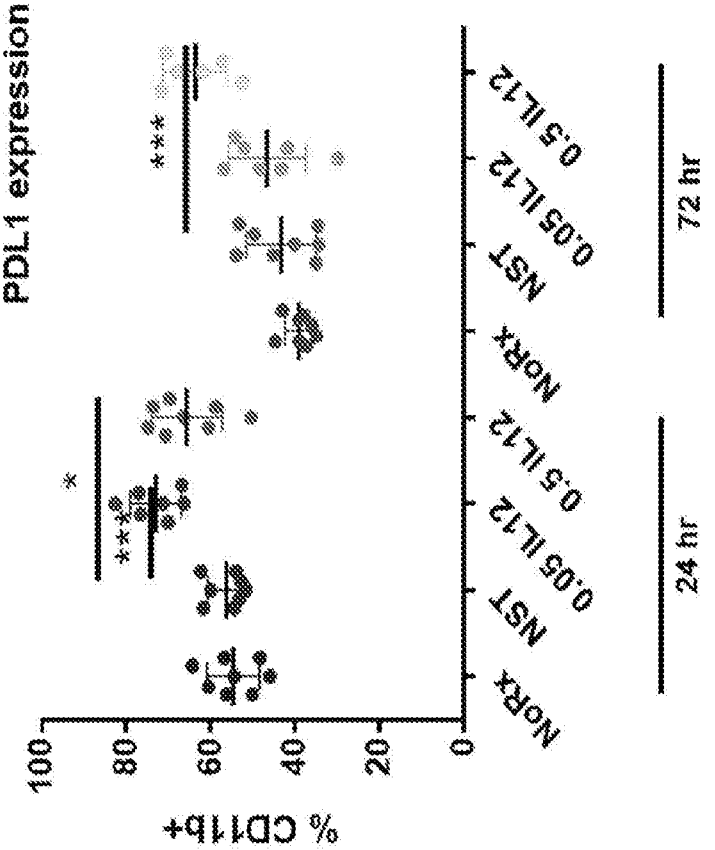


FIG. 27B

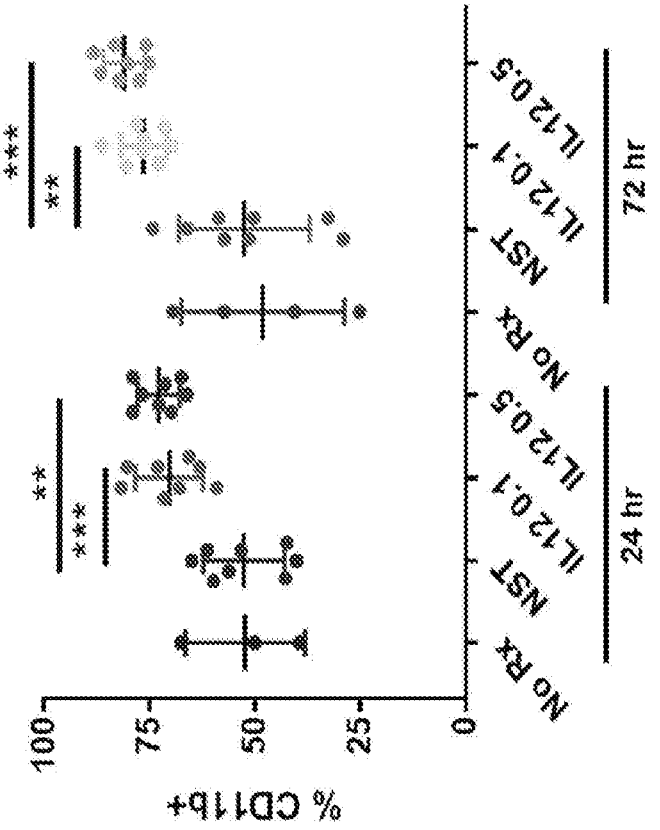


FIG. 28A

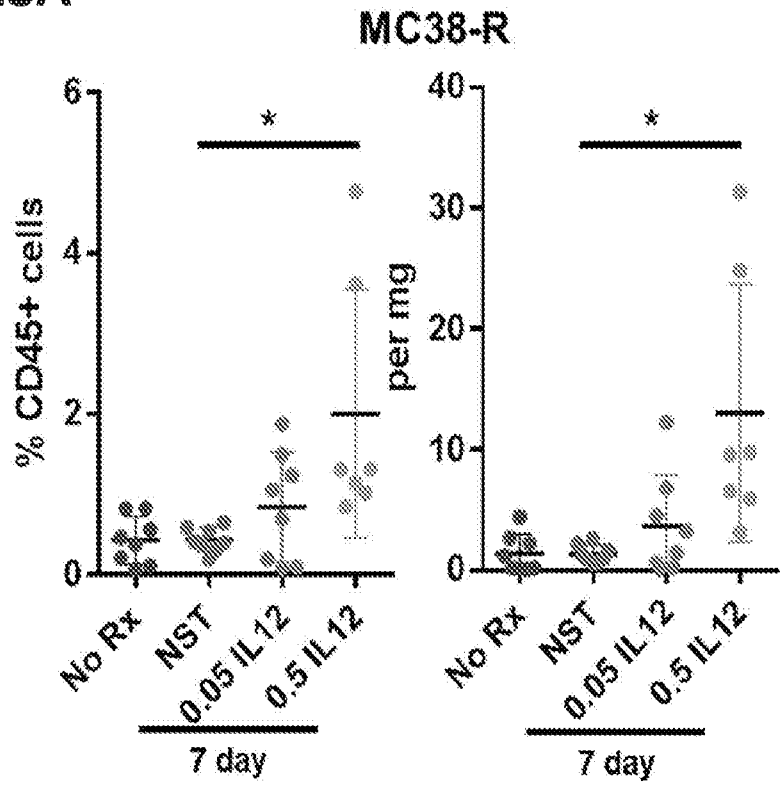


FIG. 28B

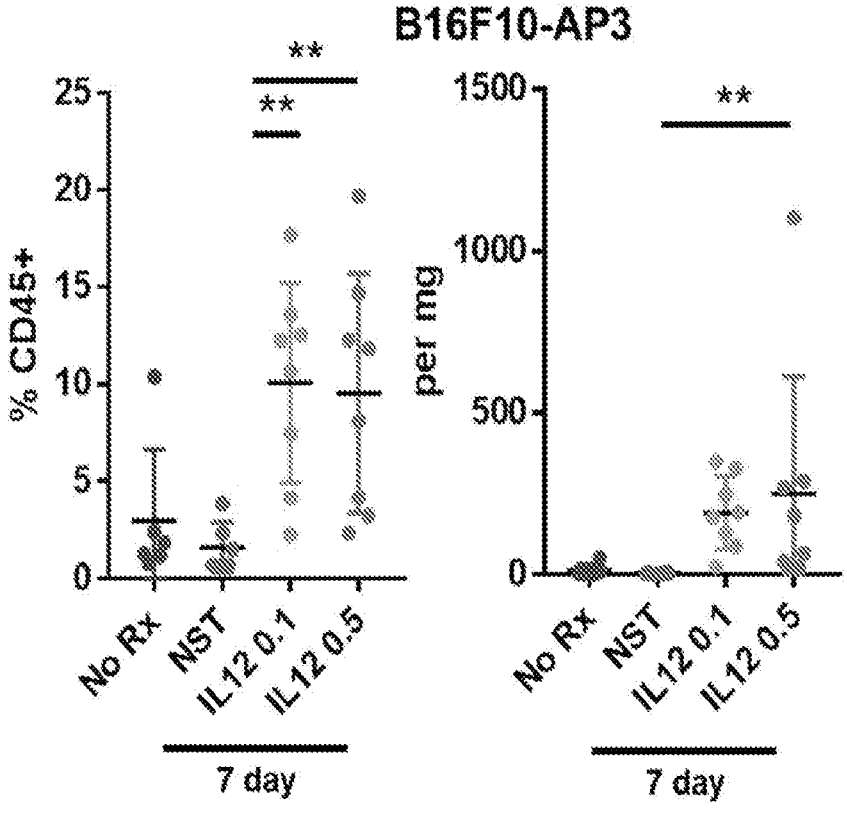


FIG. 29A

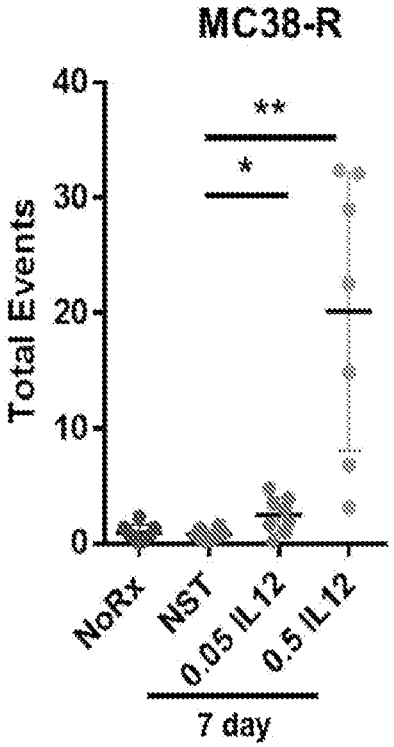


FIG. 29B

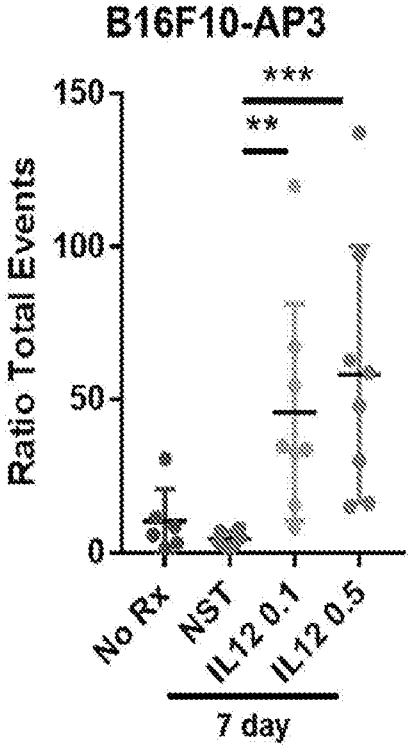


FIG. 30A

MC38-R

FIG. 30B

B16F10-AP3

CD69 early activation marker expression on CD8+ T cells

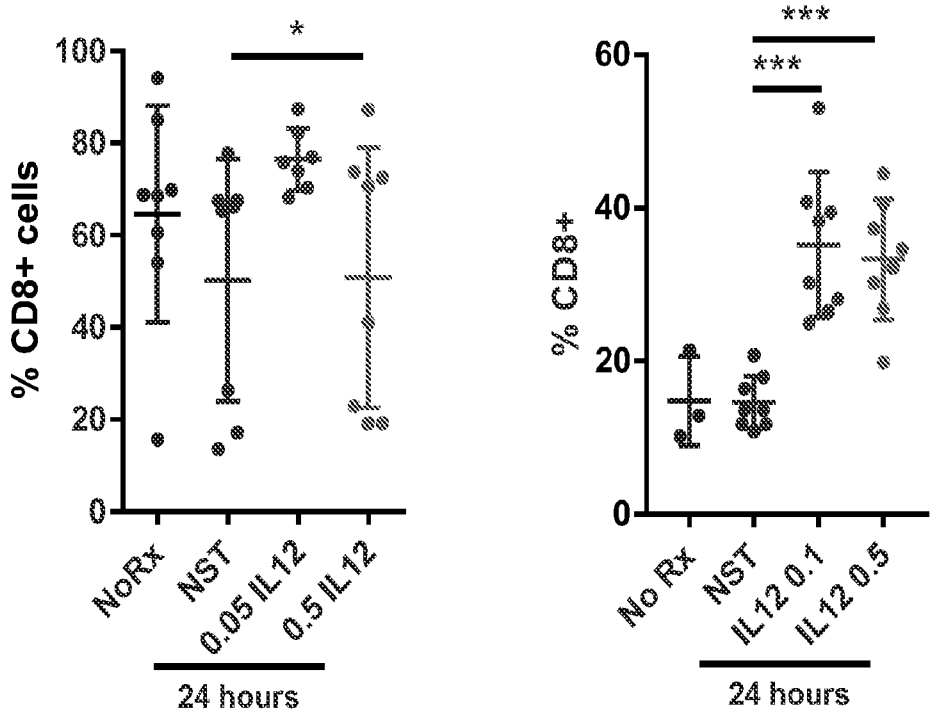


FIG. 31A

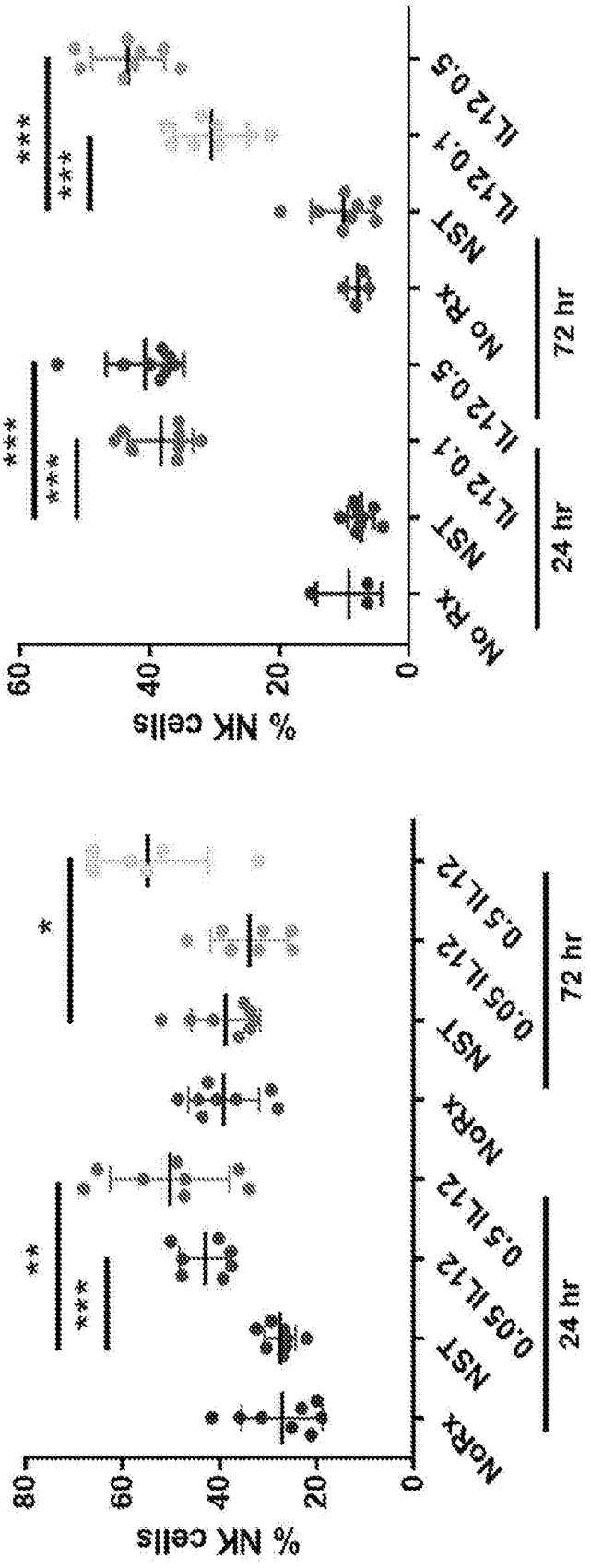


FIG. 31B

FIG. 32A

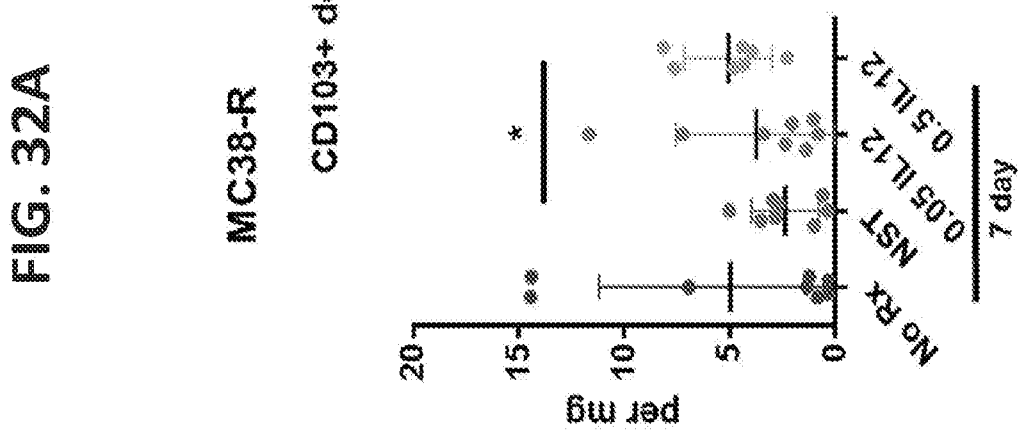


FIG. 32B

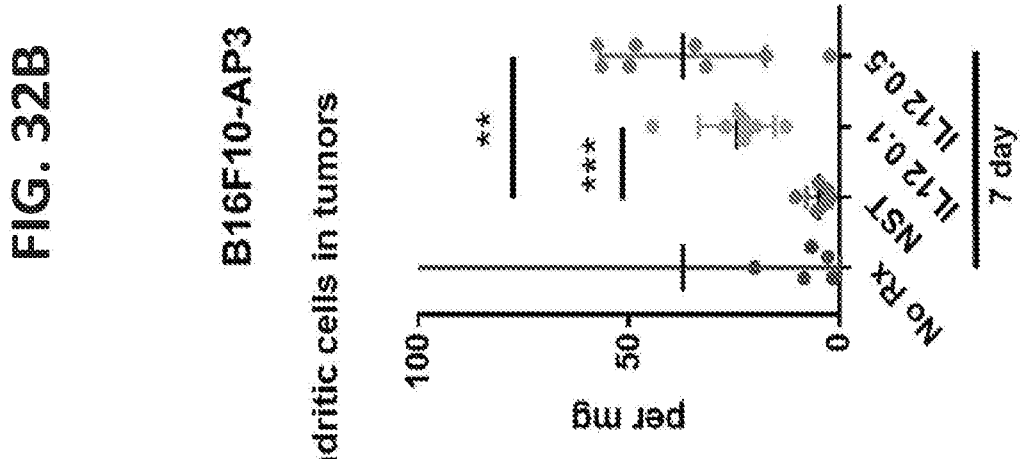


FIG. 32C

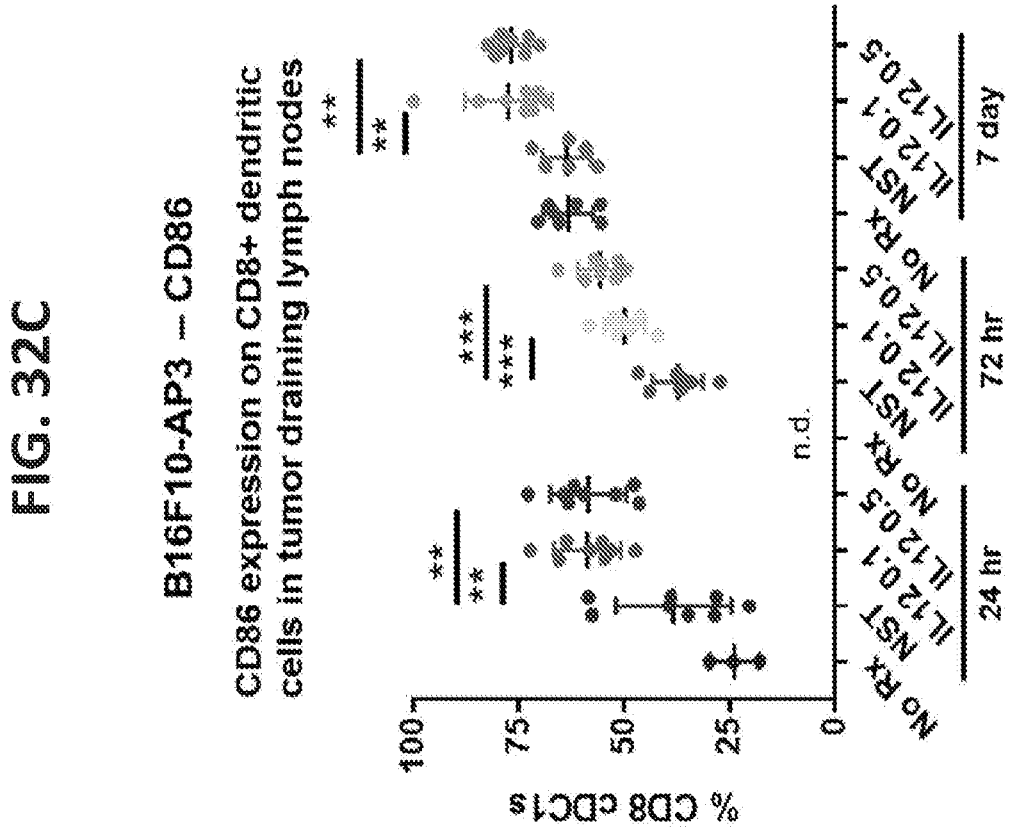


FIG. 33A

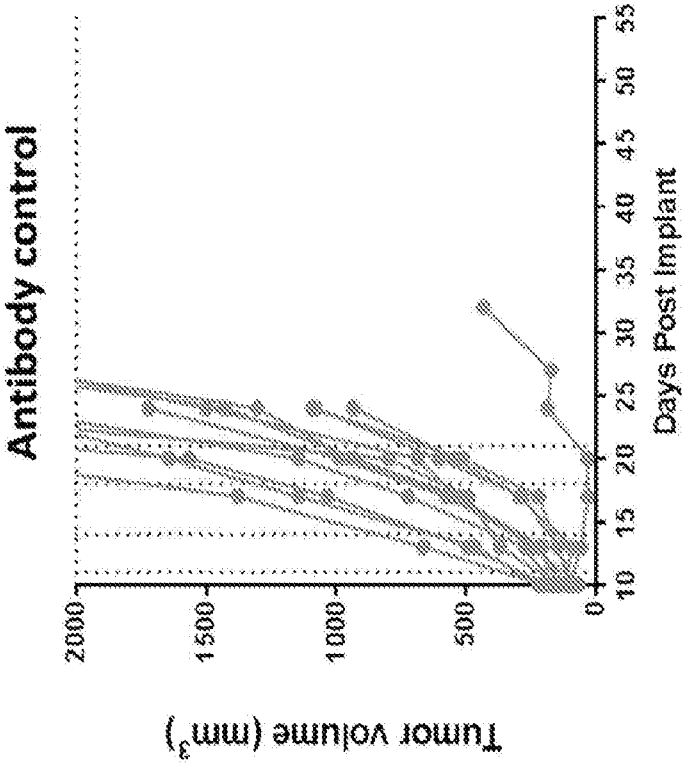


FIG. 33B

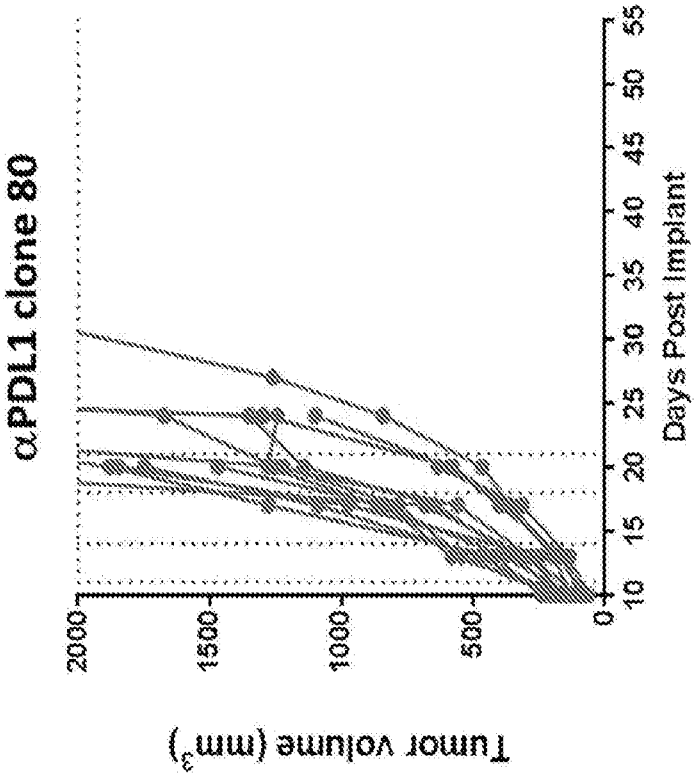


FIG. 33C

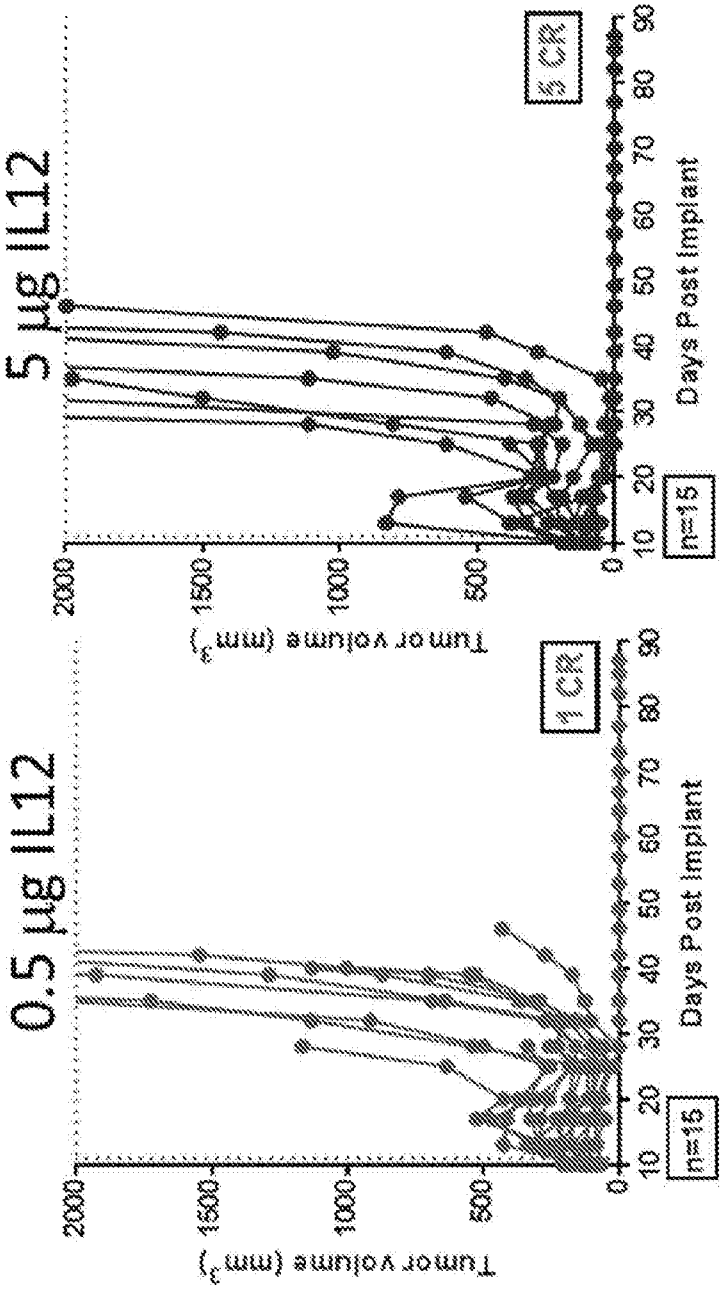


FIG. 33D

FIG. 33F

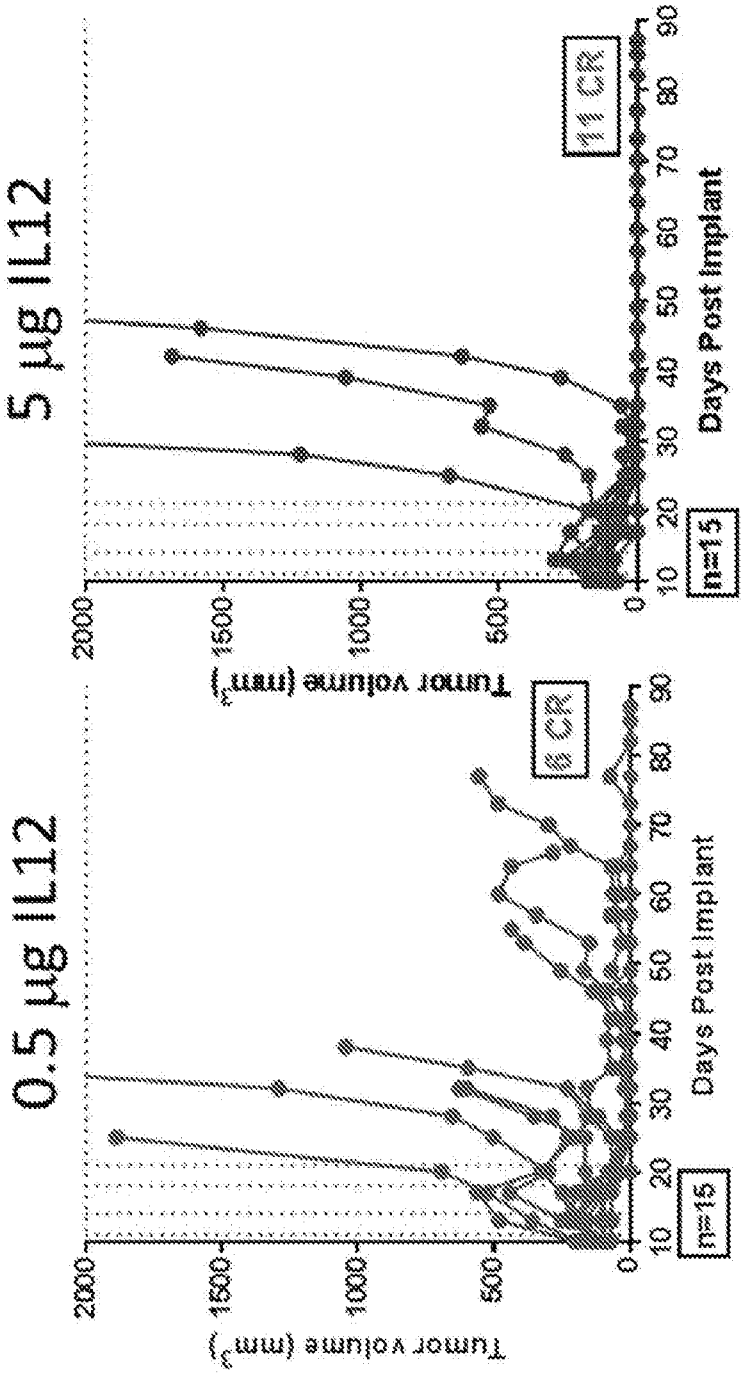


FIG. 33E

FIG. 33G

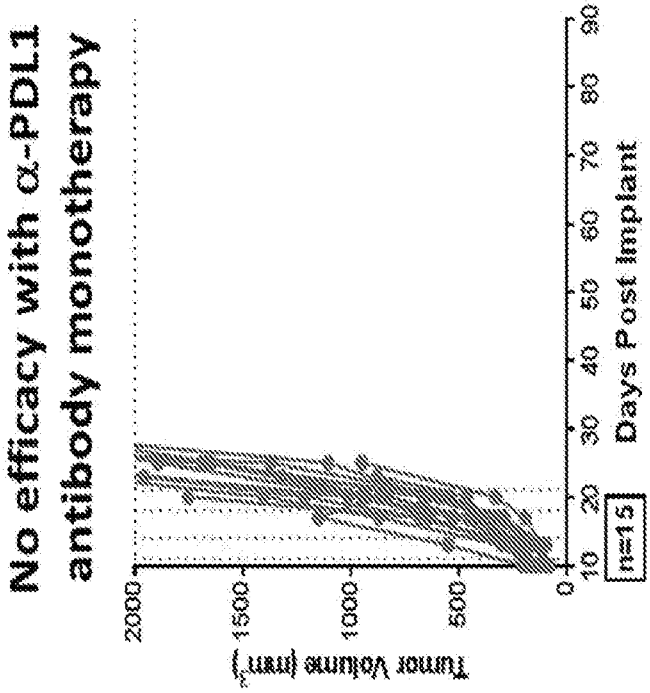

No efficacy with α-PDL1 antibody monotherapy
Tumor Volume (mm³)
2000
1500
1000
500
0
10
20
30
40
50
60
70
80
90
Days Post Implant
n=15

FIG. 34A

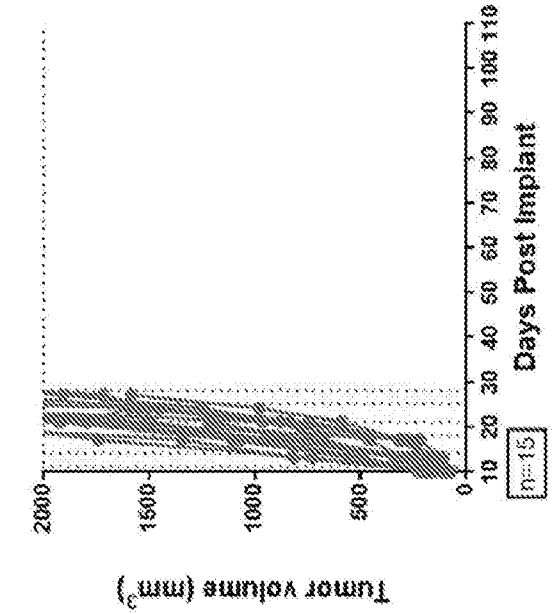


FIG. 34B

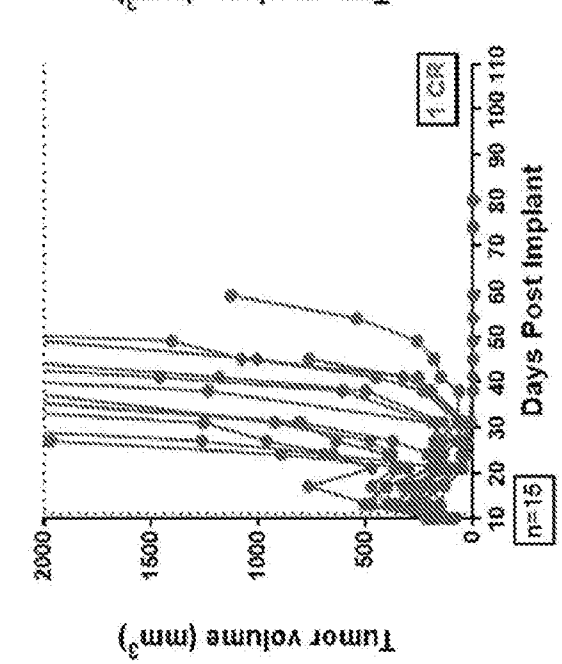


FIG. 34C

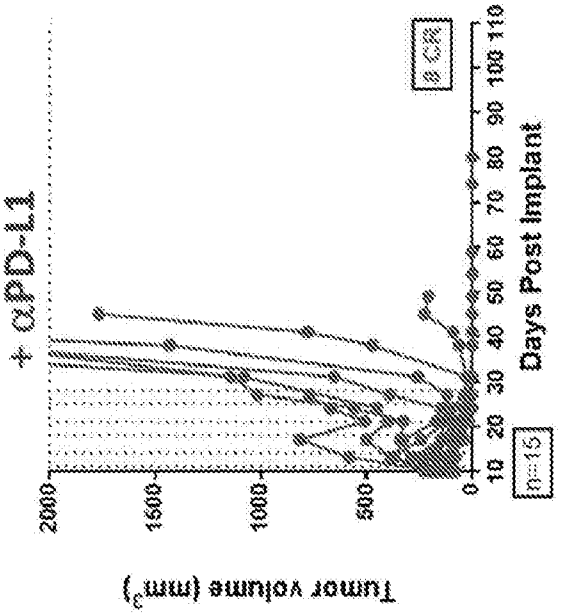


FIG. 35A

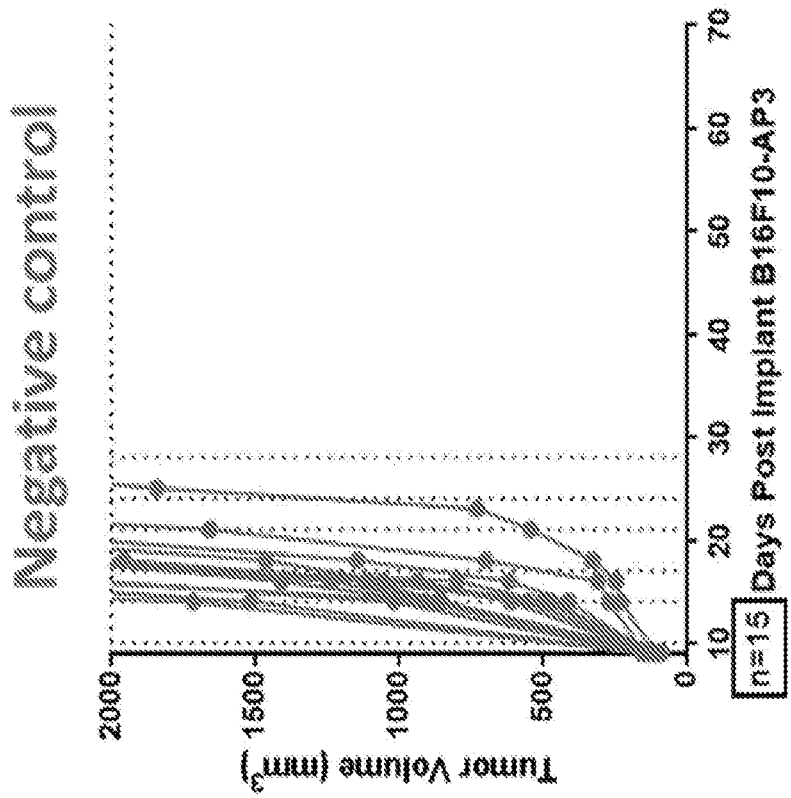


FIG. 35B

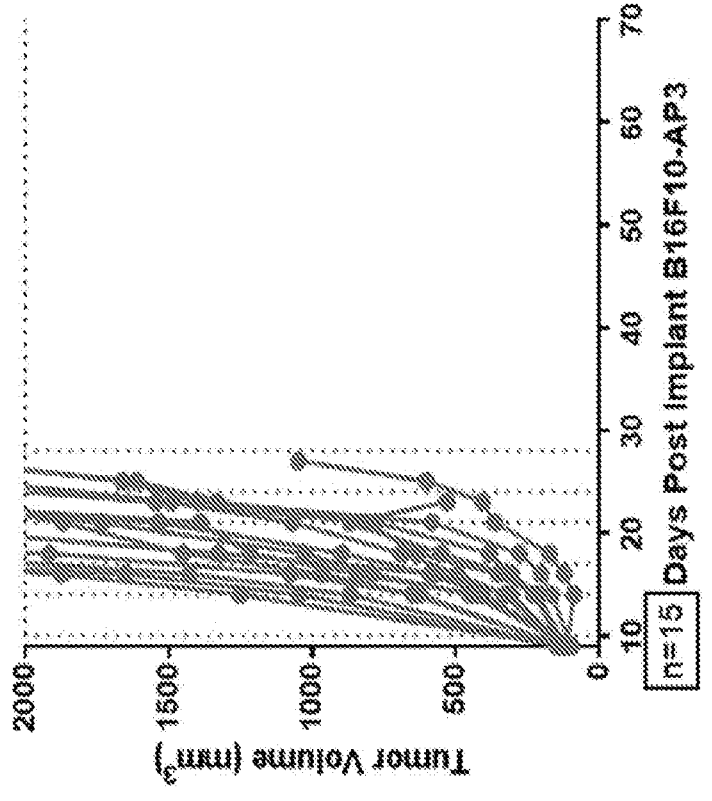


FIG. 35C

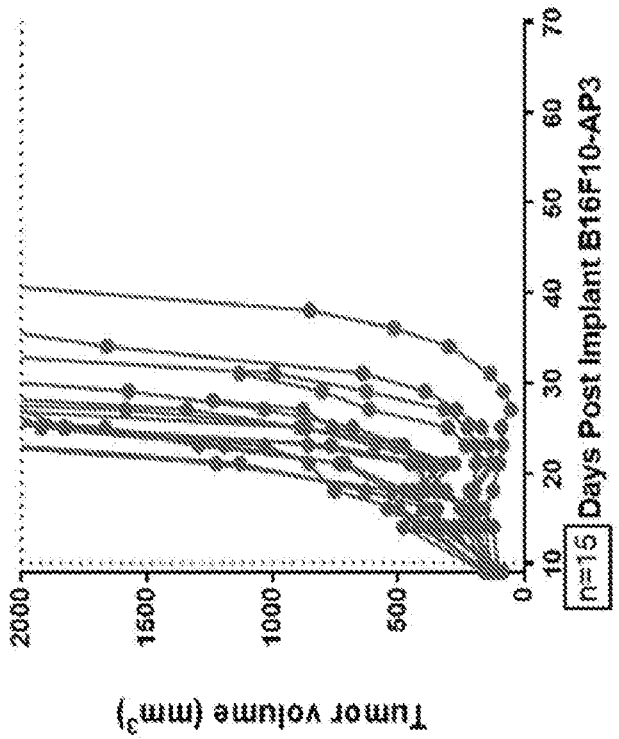


FIG. 35D

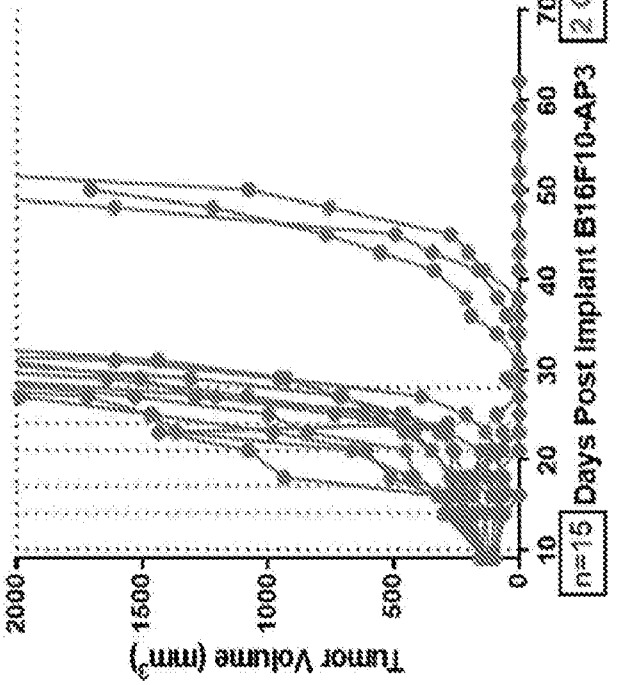


FIG. 36A

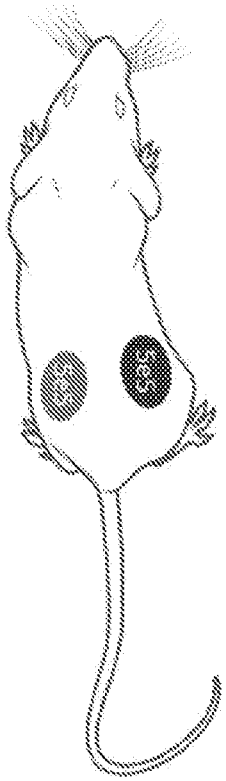


FIG. 36B

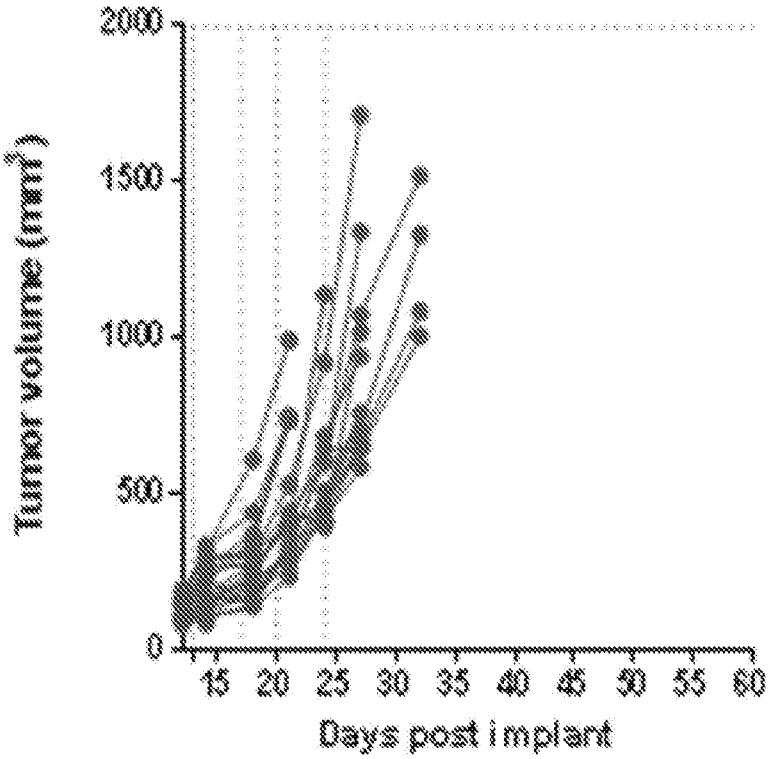


FIG. 36C

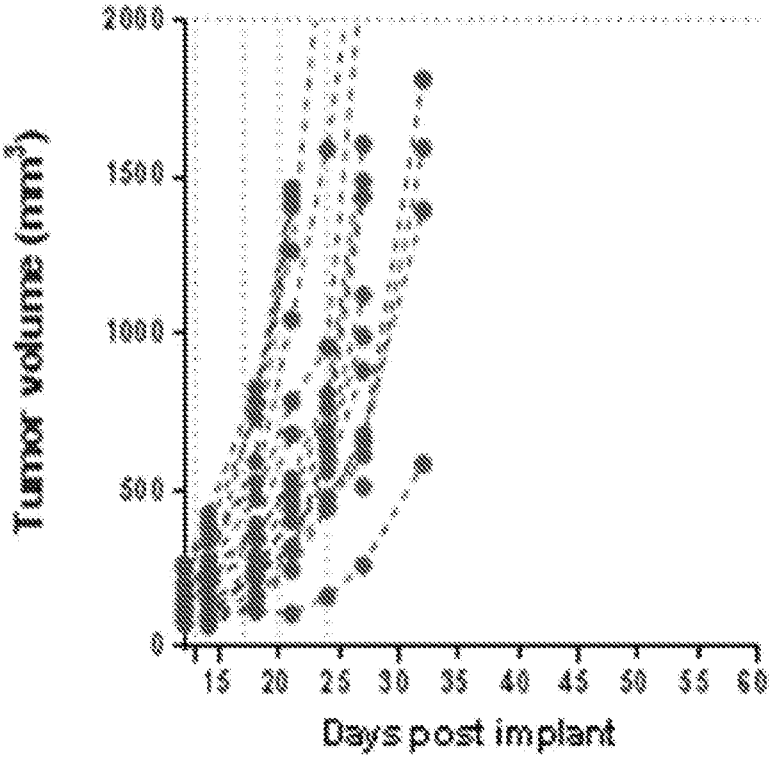
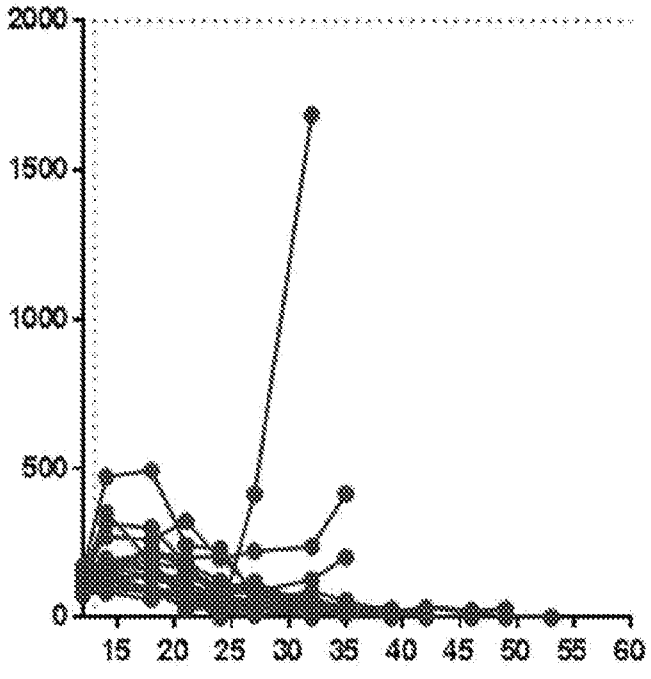


FIG. 36D



~3/20 CRs

FIG. 36E

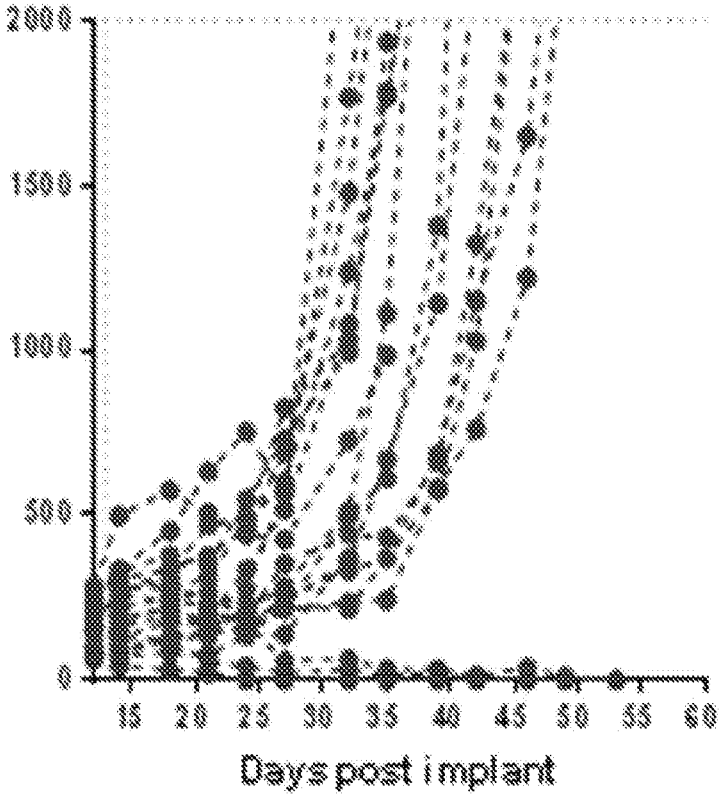
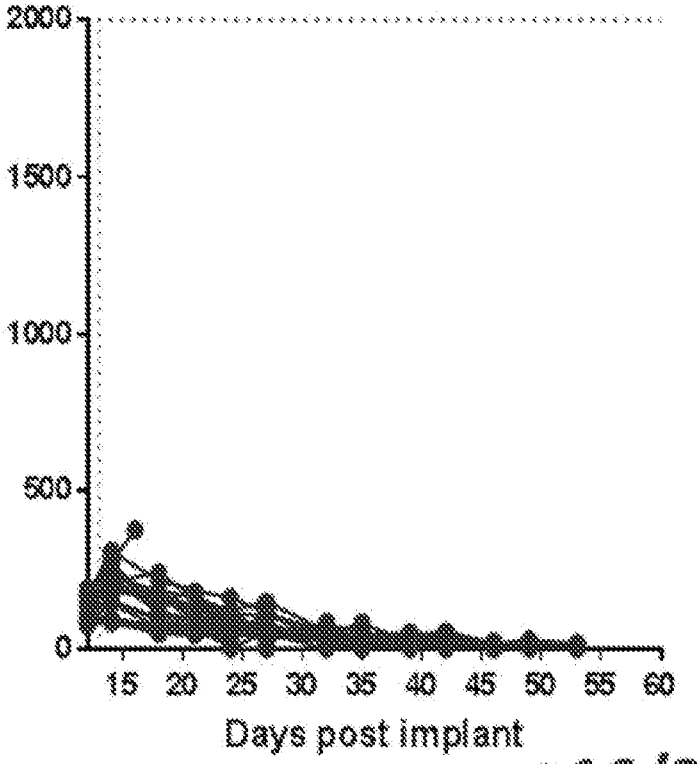


FIG. 36F



~16/20 CRs

FIG. 36G

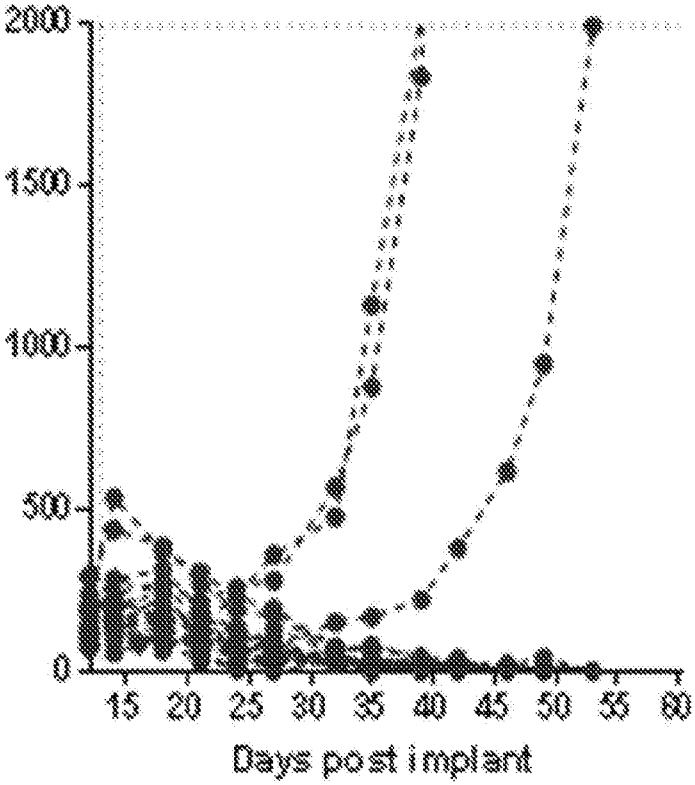


FIG. 37A

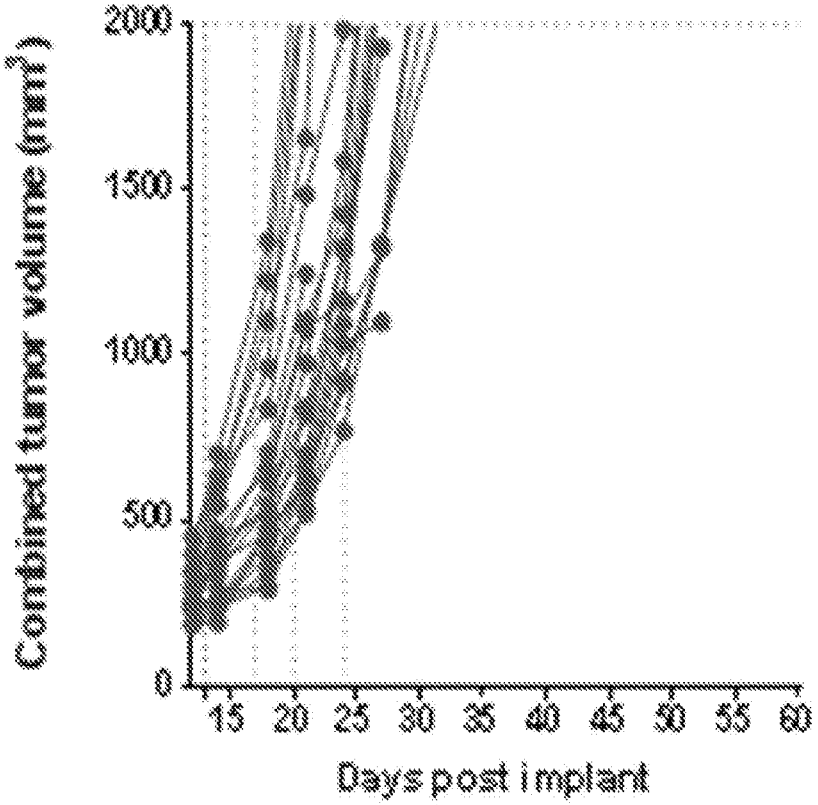


FIG. 37B

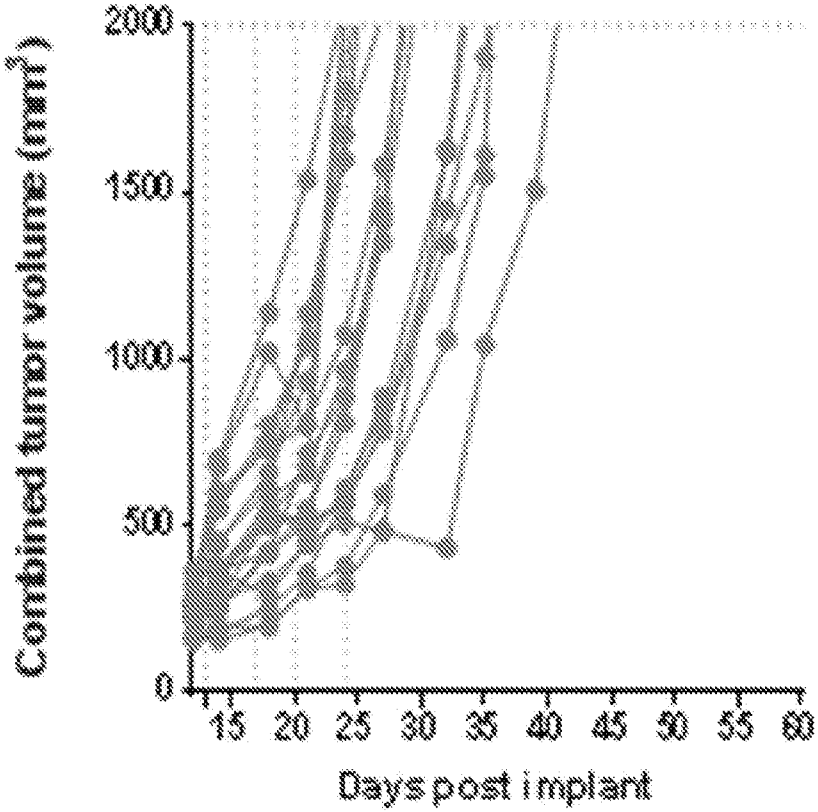


FIG. 37C

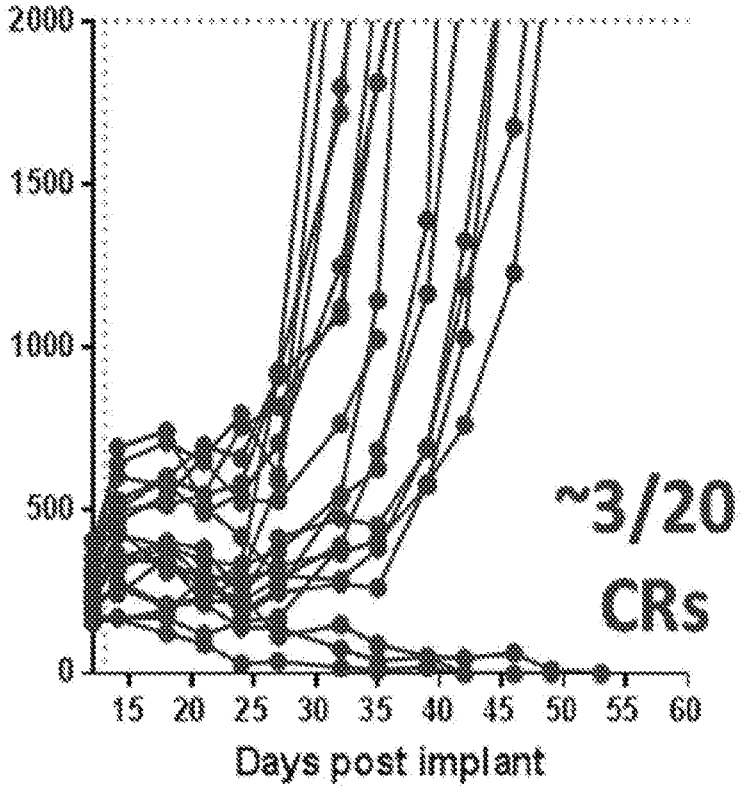


FIG. 37D

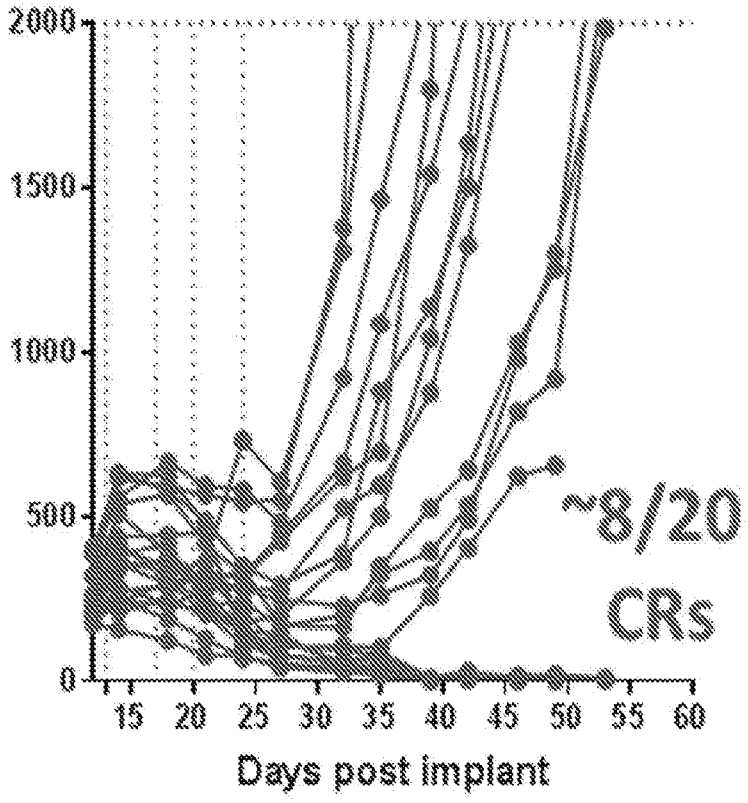


FIG. 37E

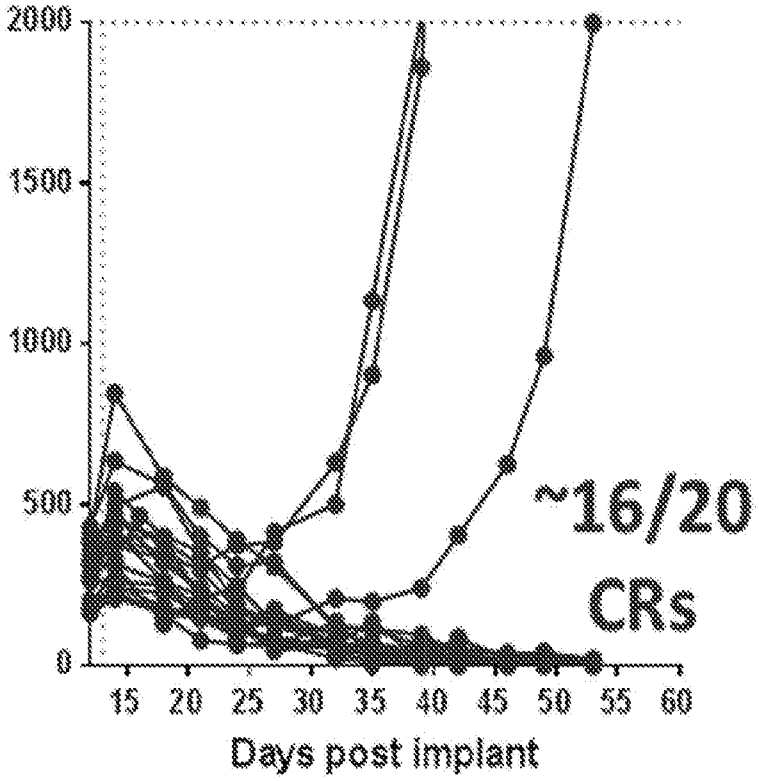


FIG. 37F

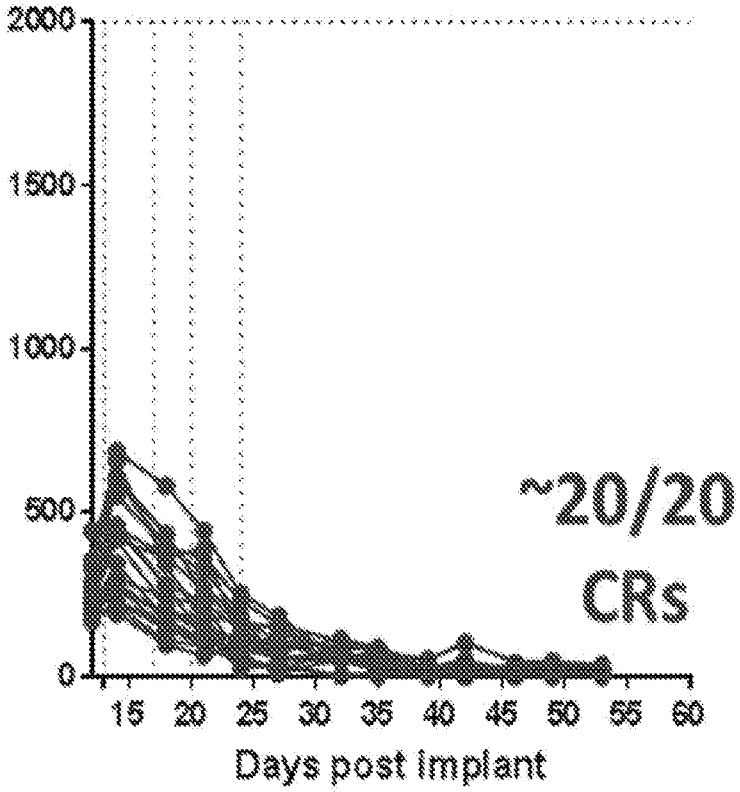
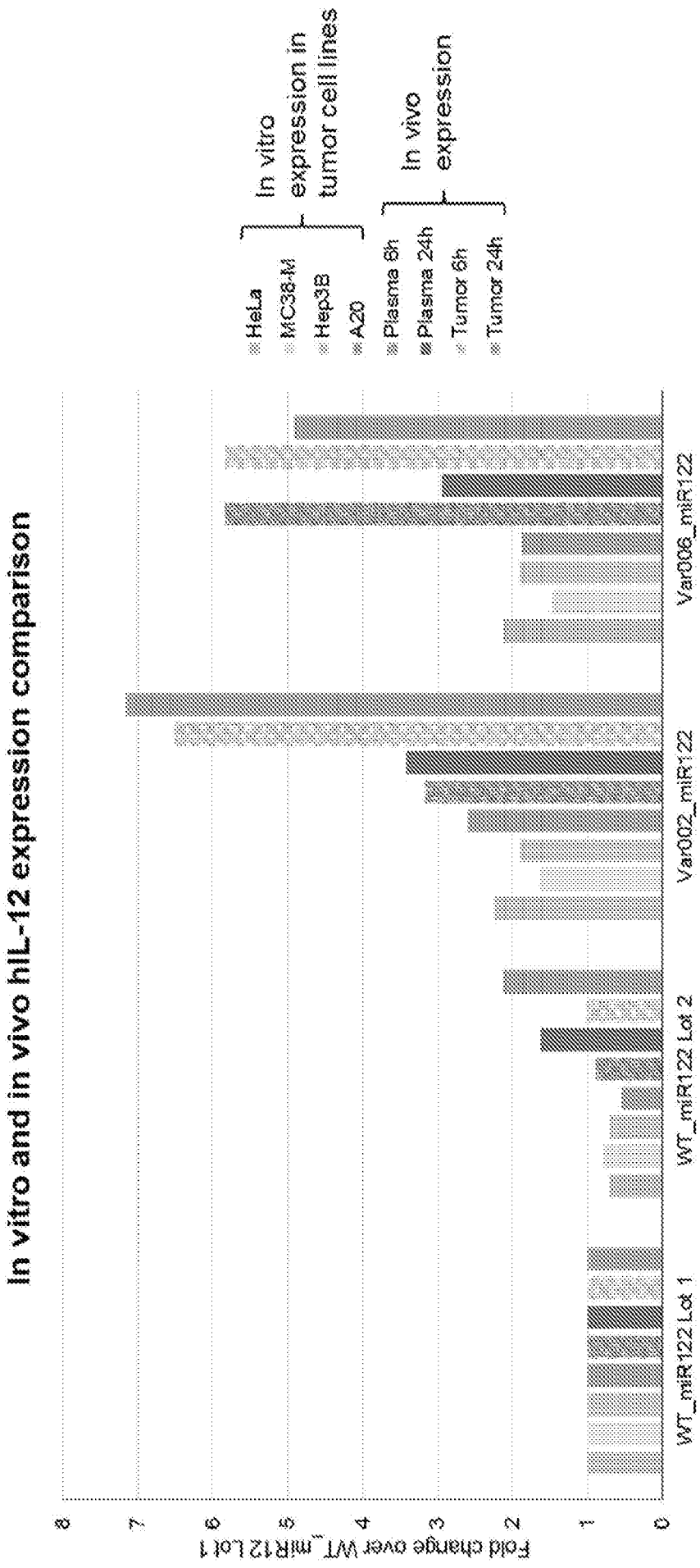


FIG. 38


In vitro and in vivo hIL-12 expression comparison
Fold change over WT_miR12 Lot 1
0
1
2
3
4
5
6
7
8
WT_miR122 Lot 1
WT_miR122 Lot 2
Var002_miR122
Var006_miR122
In vitro expression in tumor cell lines
In vivo expression
HeLa
MC38-M
Hep3B
A20
Plasma 6h
Plasma 24h
Tumor 6h
Tumor 24h