IL-2, a key regulator of the immune system

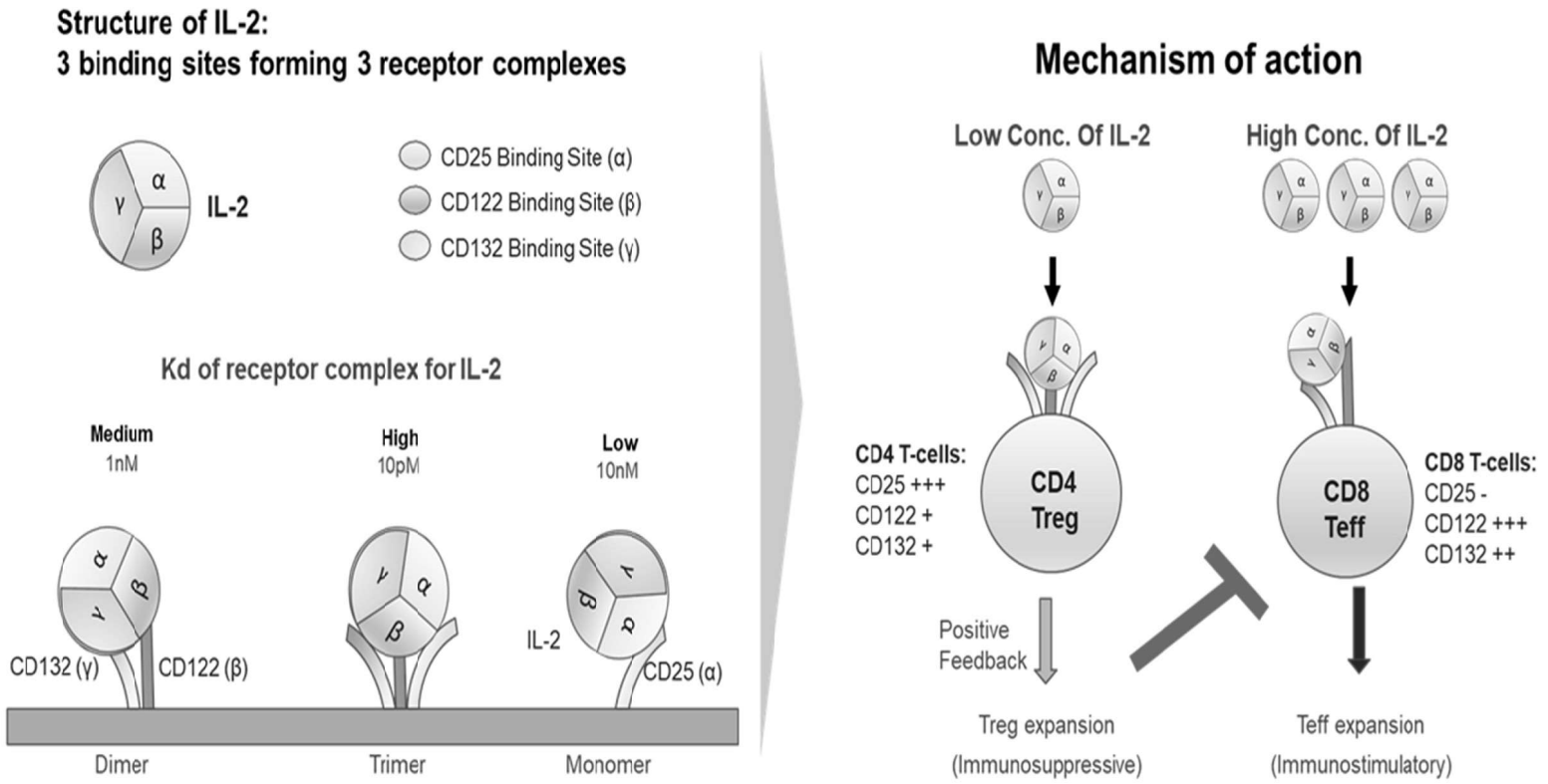


FIG. 1

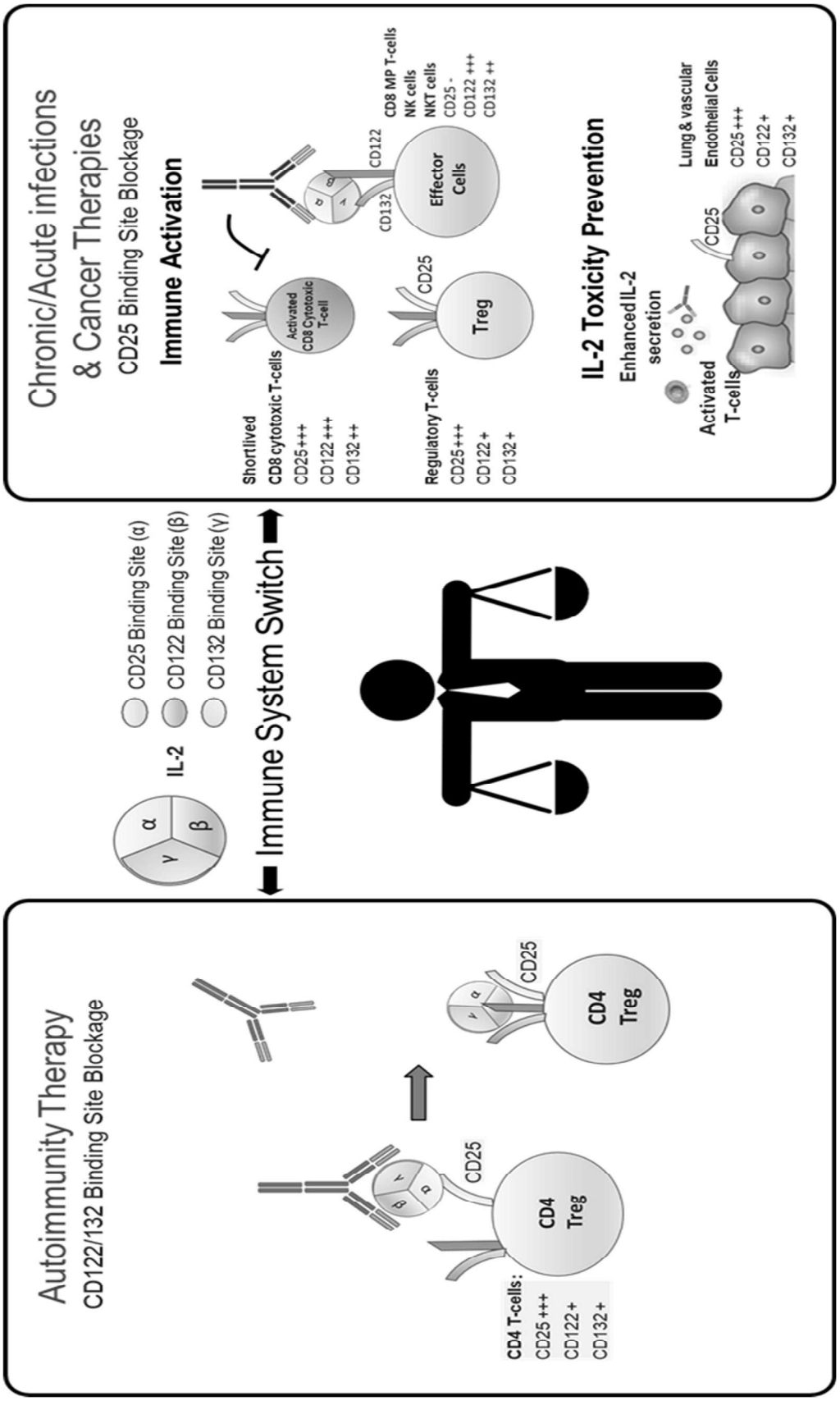

Autoimmunity Therapy
CD122/132 Binding Site Blockage
CD4 T-cells:
CD25 +++
CD122+
CD132+
CD25
CD4
Treg
CD4
Treg
CD25
IL-2
CD25 Binding Site (α)
CD122 Binding Site (β)
CD132 Binding Site (γ)
Immune System Switch
Chronic/Acute infections
& Cancer Therapies
CD25 Binding Site Blockage
Immune Activation
Shortlived
CD8 cytotoxic T-cells
CD25+++
CD122 +++
CD132 ++
Activated
CD8 Cytotoxic
T-cell
CD122
CD132
Effector
Cells
CD8 MP T-cells
NK cells
NKT cells
CD25 -
CD122 +++
CD132 ++
Regulatory T-cells
CD25+++
CD122+
CD132+
CD25
Treg
IL-2 Toxicity Prevention
Enhanced IL-2
secretion
Activated
T-cells
CD25
Lung & vascular
Endothelial Cells
CD25+++
CD122+
CD132+

FIG. 2

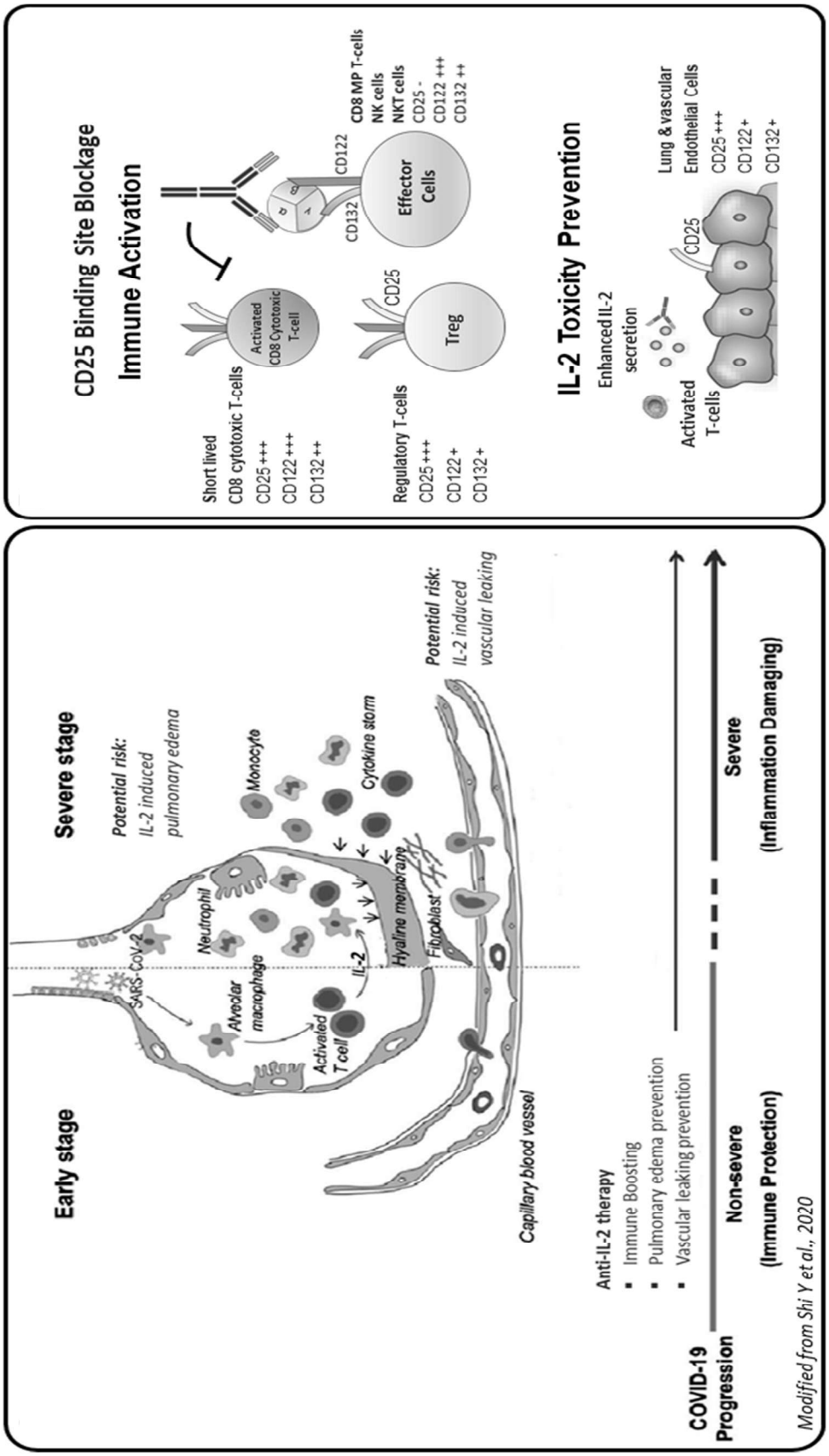

Early stage
Severe stage
Potential risk:
IL-2 induced
pulmonary edema
SARS-CoV-2
Neutrophil
Alveolar
macrophage
Monocyte
Activated
T cell
IL-2
Cytokine storm
Hyaline membrane
Fibroblast
Potential risk:
IL-2 induced
vascular leaking
Capillary blood vessel
Anti-IL-2 therapy
Immune Boosting
Pulmonary edema prevention
Vascular leaking prevention
COVID-19
Progression
Non-severe
(Immune Protection)
Severe
(Inflammation Damaging)
Modified from Shi Y et al., 2020
CD25 Binding Site Blockage
Immune Activation
Short lived
CD8 cytotoxic T-cells
CD25 +++
CD122 +++
CD132 ++
Activated
CD8 Cytotoxic
T-cell
CD122
CD132
CD8 MP T-cells
NK cells
NKT cells
CD25 -
CD122 +++
CD132 ++
Effector
Cells
Regulatory T-cells
CD25 +++
CD122 +
CD132 +
CD25
Treg
IL-2 Toxicity Prevention
Enhanced IL-2
secretion
Activated
T-cells
CD25
Lung & vascular
Endothelial Cells
CD25 +++
CD122 +
CD132 +

FIG. 3A

FIG. 3B

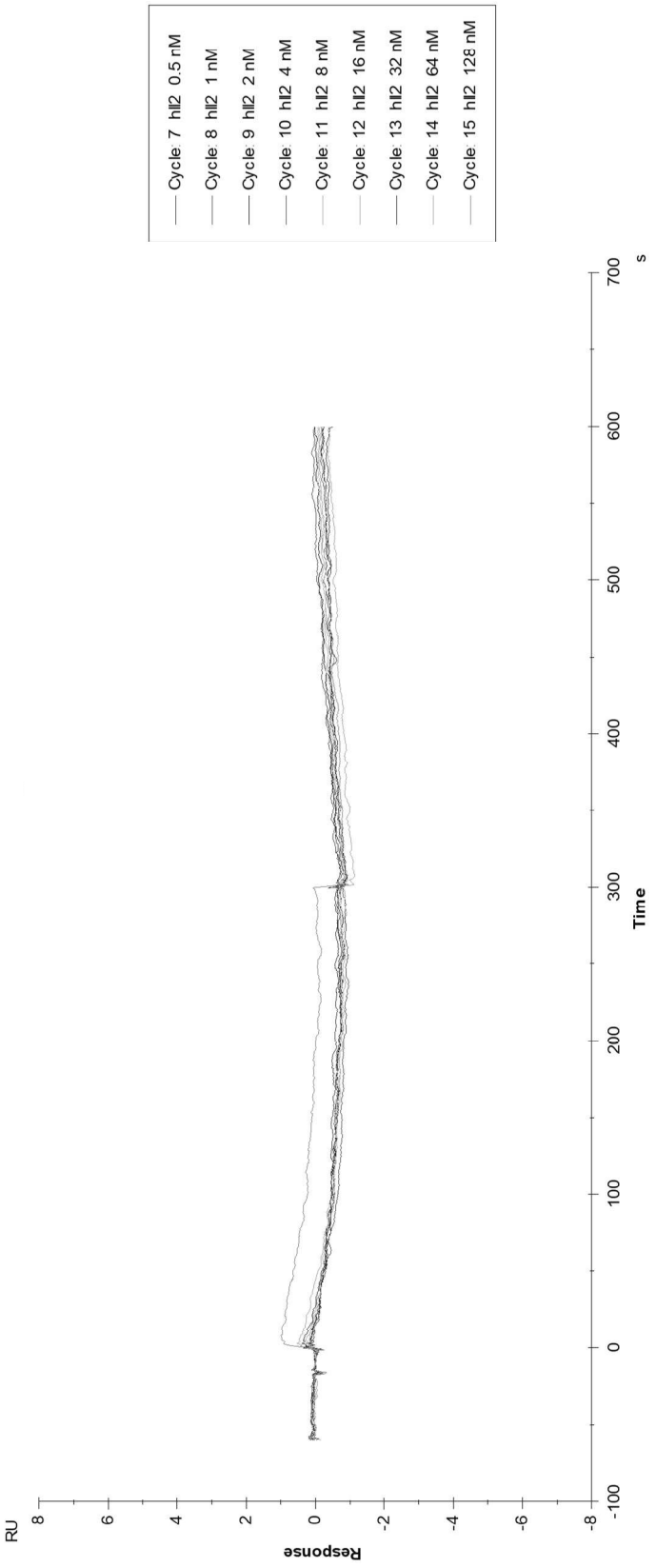

Cycle: 7 hIl2 0.5 nM
Cycle: 8 hIl2 1 nM
Cycle: 9 hIl2 2 nM
Cycle: 10 hIl2 4 nM
Cycle: 11 hIl2 8 nM
Cycle: 12 hIl2 16 nM
Cycle: 13 hIl2 32 nM
Cycle: 14 hIl2 64 nM
Cycle: 15 hIl2 128 nM
RU
Response
8
6
4
2
0
-2
-4
-6
-8
-100
0
100
200
300
400
500
600
700
Time
s

FIG. 4A

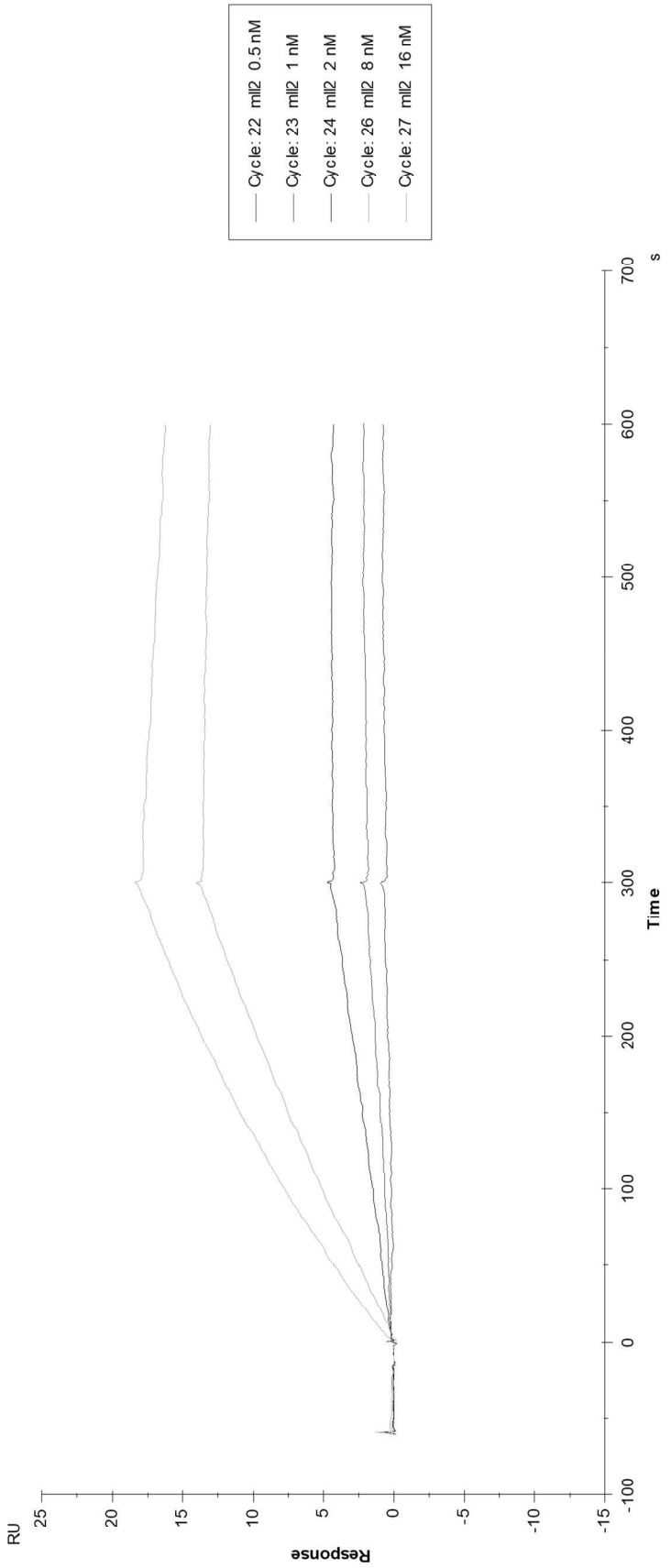

Cycle: 22 mll2 0.5 nM
Cycle: 23 mll2 1 nM
Cycle: 24 mll2 2 nM
Cycle: 26 mll2 8 nM
Cycle: 27 mll2 16 nM
RU
Response
25
20
15
10
5
0
-5
-10
-15
-100
0
100
200
300
400
500
600
700
Time
s

FIG. 4B

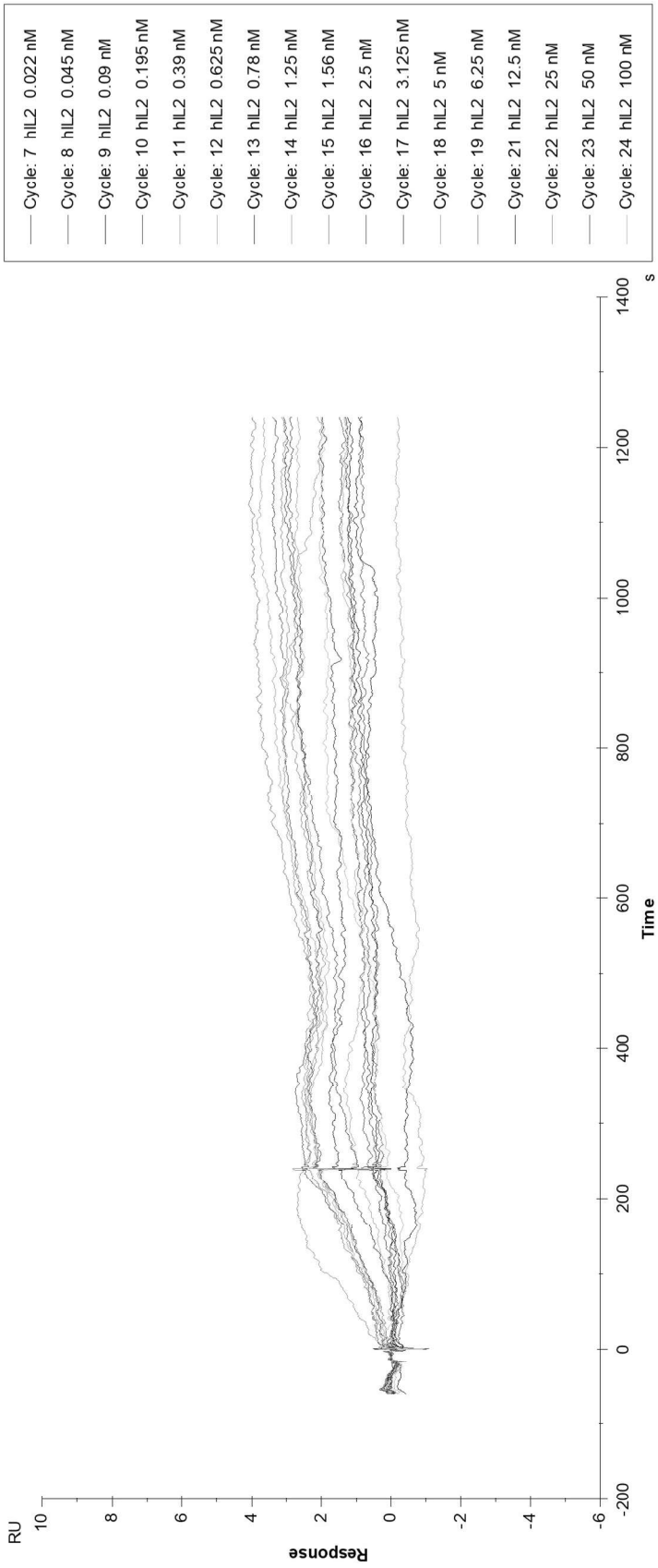

Cycle: 7 hIL2 0.022 nM
Cycle: 8 hIL2 0.045 nM
Cycle: 9 hIL2 0.09 nM
Cycle: 10 hIL2 0.195 nM
Cycle: 11 hIL2 0.39 nM
Cycle: 12 hIL2 0.625 nM
Cycle: 13 hIL2 0.78 nM
Cycle: 14 hIL2 1.25 nM
Cycle: 15 hIL2 1.56 nM
Cycle: 16 hIL2 2.5 nM
Cycle: 17 hIL2 3.125 nM
Cycle: 18 hIL2 5 nM
Cycle: 19 hIL2 6.25 nM
Cycle: 21 hIL2 12.5 nM
Cycle: 22 hIL2 25 nM
Cycle: 23 hIL2 50 nM
Cycle: 24 hIL2 100 nM
RU
Response
Time
s
10
8
6
4
2
0
-2
-4
-6
-200
0
200
400
600
800
1000
1200
1400

FIG. 4C

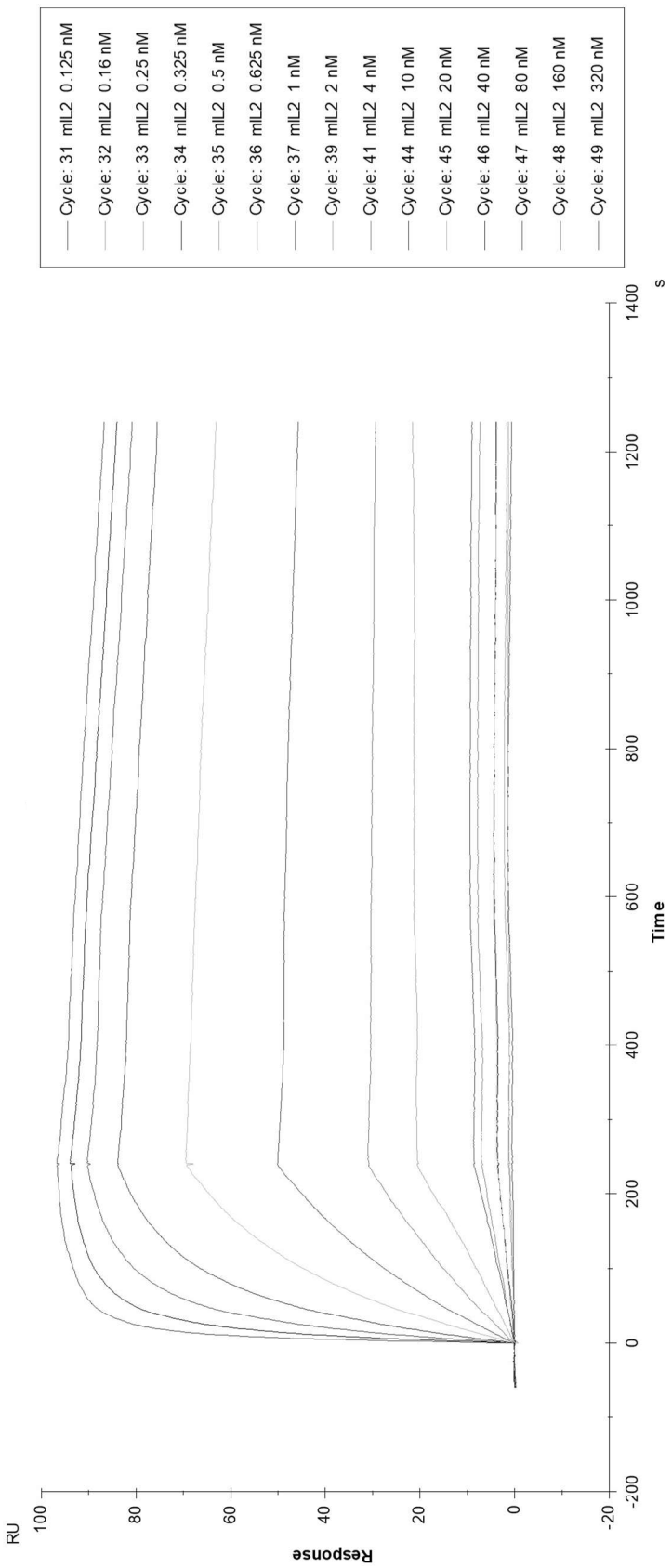

Cycle: 31 mIL2 0.125 nM
Cycle: 32 mIL2 0.16 nM
Cycle: 33 mIL2 0.25 nM
Cycle: 34 mIL2 0.325 nM
Cycle: 35 mIL2 0.5 nM
Cycle: 36 mIL2 0.625 nM
Cycle: 37 mIL2 1 nM
Cycle: 39 mIL2 2 nM
Cycle: 41 mIL2 4 nM
Cycle: 44 mIL2 10 nM
Cycle: 45 mIL2 20 nM
Cycle: 46 mIL2 40 nM
Cycle: 47 mIL2 80 nM
Cycle: 48 mIL2 160 nM
Cycle: 49 mIL2 320 nM
RU
Response
100
80
60
40
20
0
-20
-200
0
200
400
600
800
1000
1200
1400
Time
s

FIG. 4D

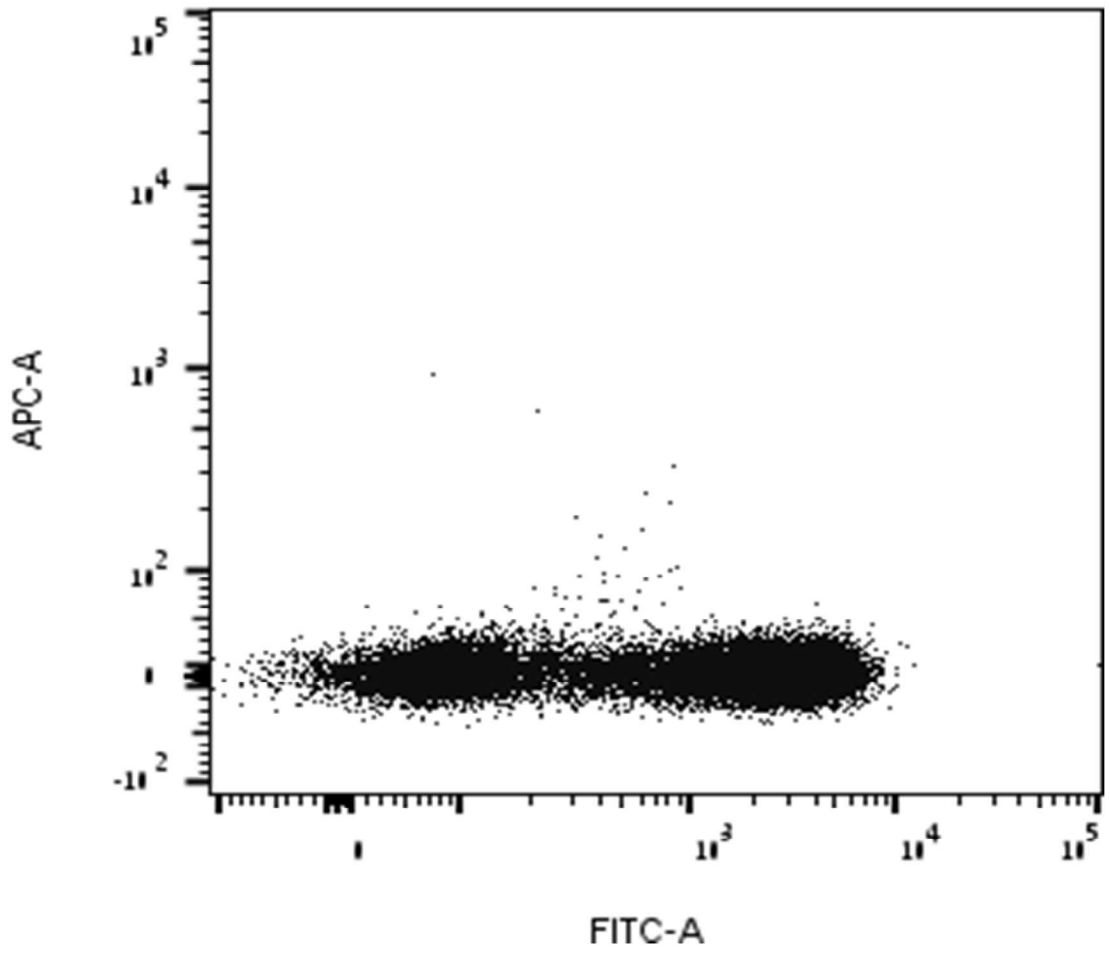


FIG. 5A

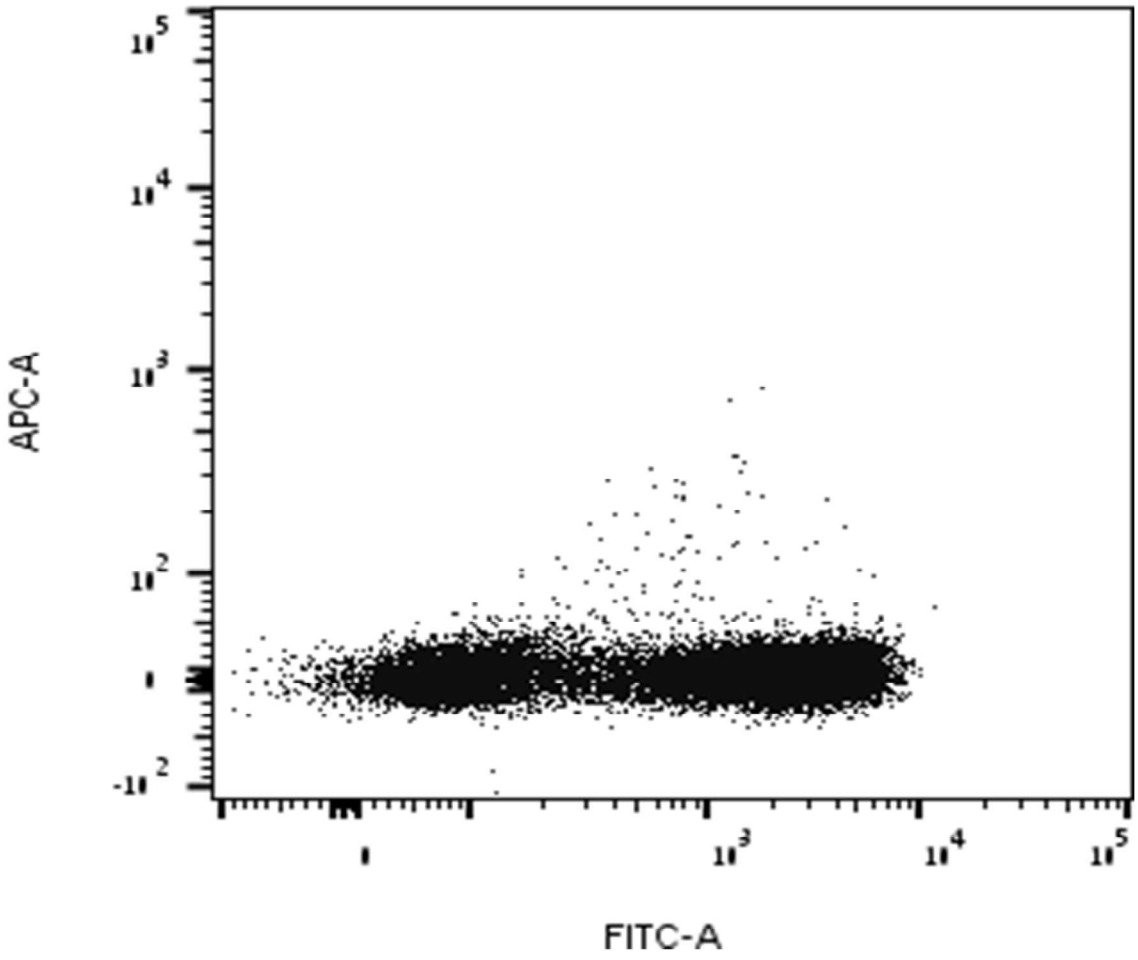


FIG. 5B

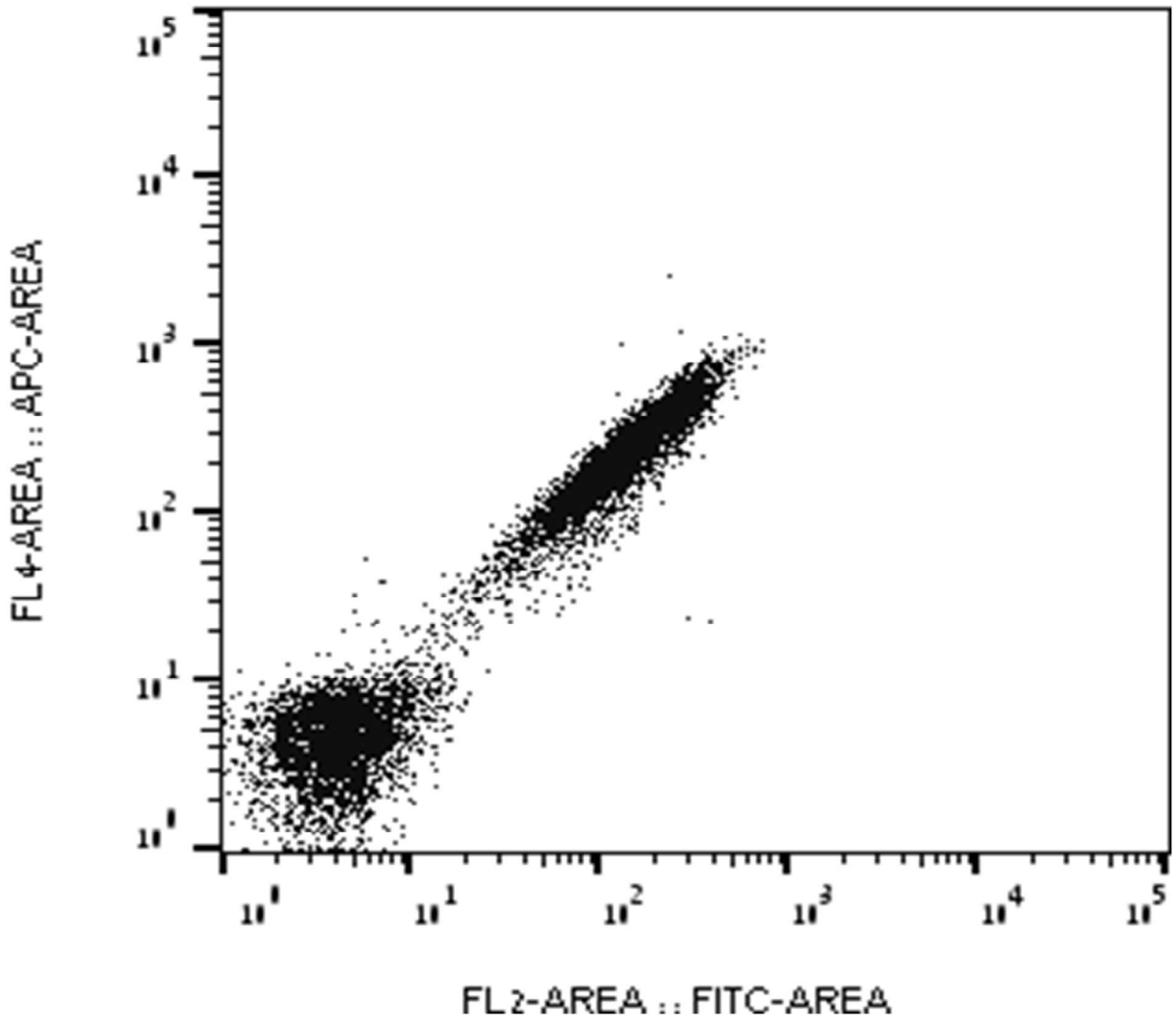


FIG. 5C

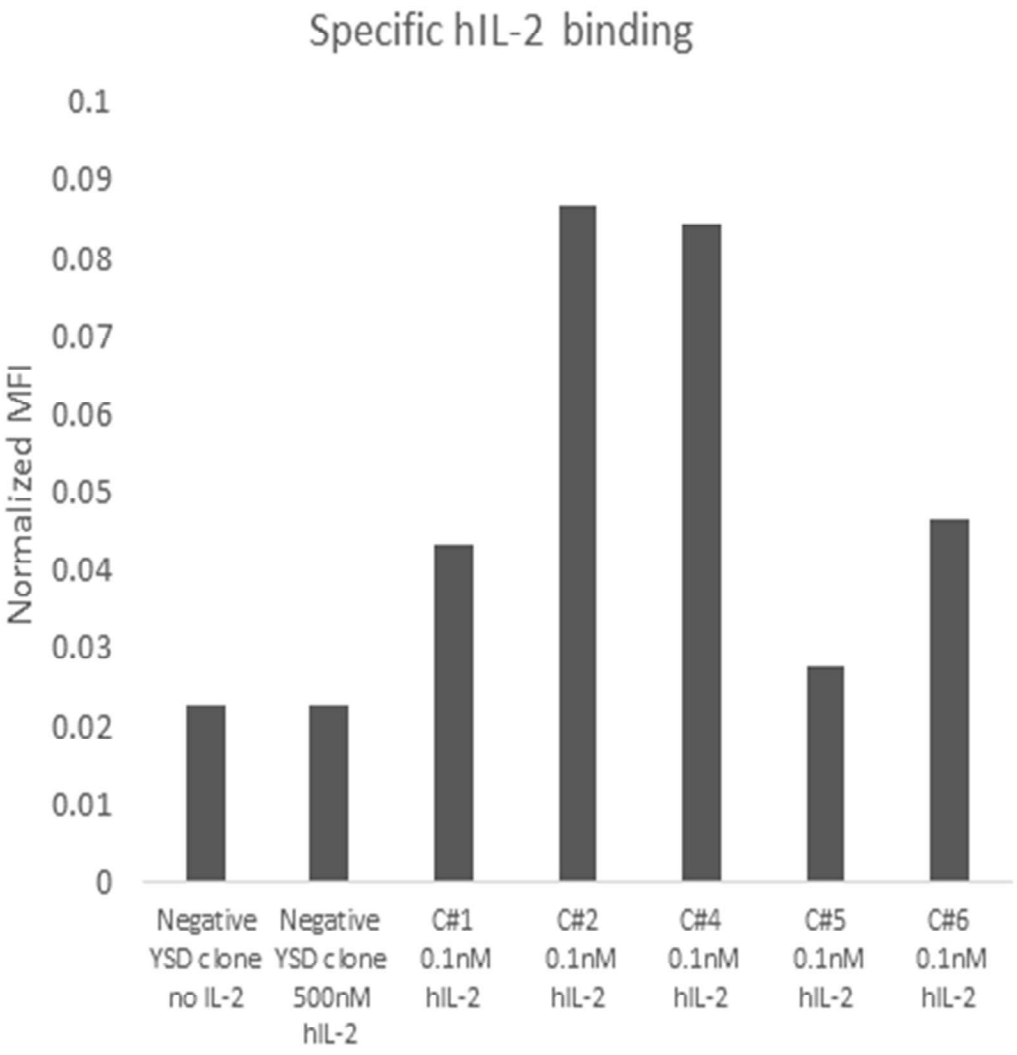

Specific hIL-2 binding
Normalized MFI
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Negative YSD clone no IL-2
Negative YSD clone 500nM hIL-2
C#1 0.1nM hIL-2
C#2 0.1nM hIL-2
C#4 0.1nM hIL-2
C#5 0.1nM hIL-2
C#6 0.1nM hIL-2

FIG. 6A

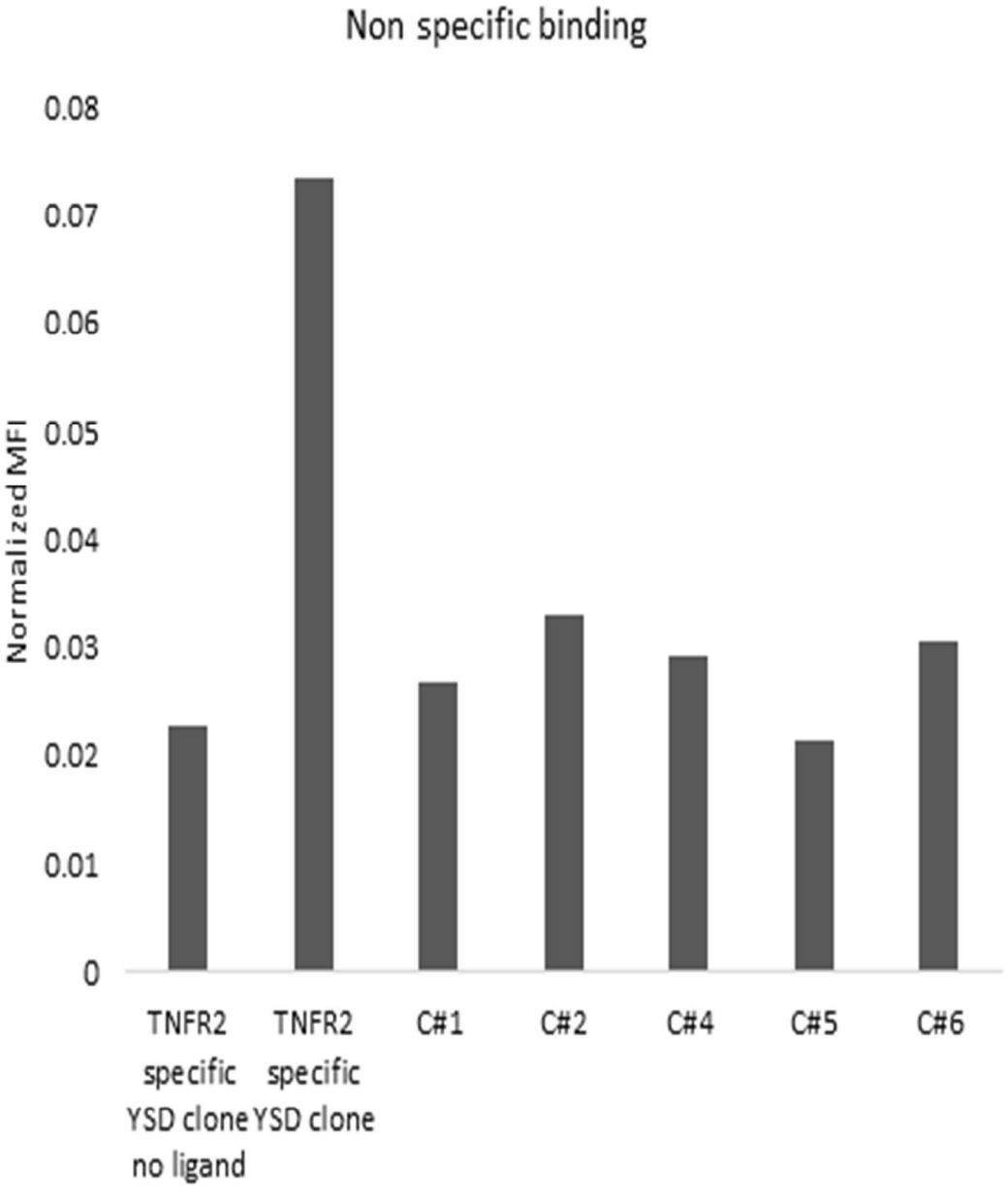

Non specific binding
Normalized MFI
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
TNFR2 specific YSD clone no ligand
TNFR2 specific YSD clone
C#1
C#2
C#4
C#5
C#6

FIG. 6B

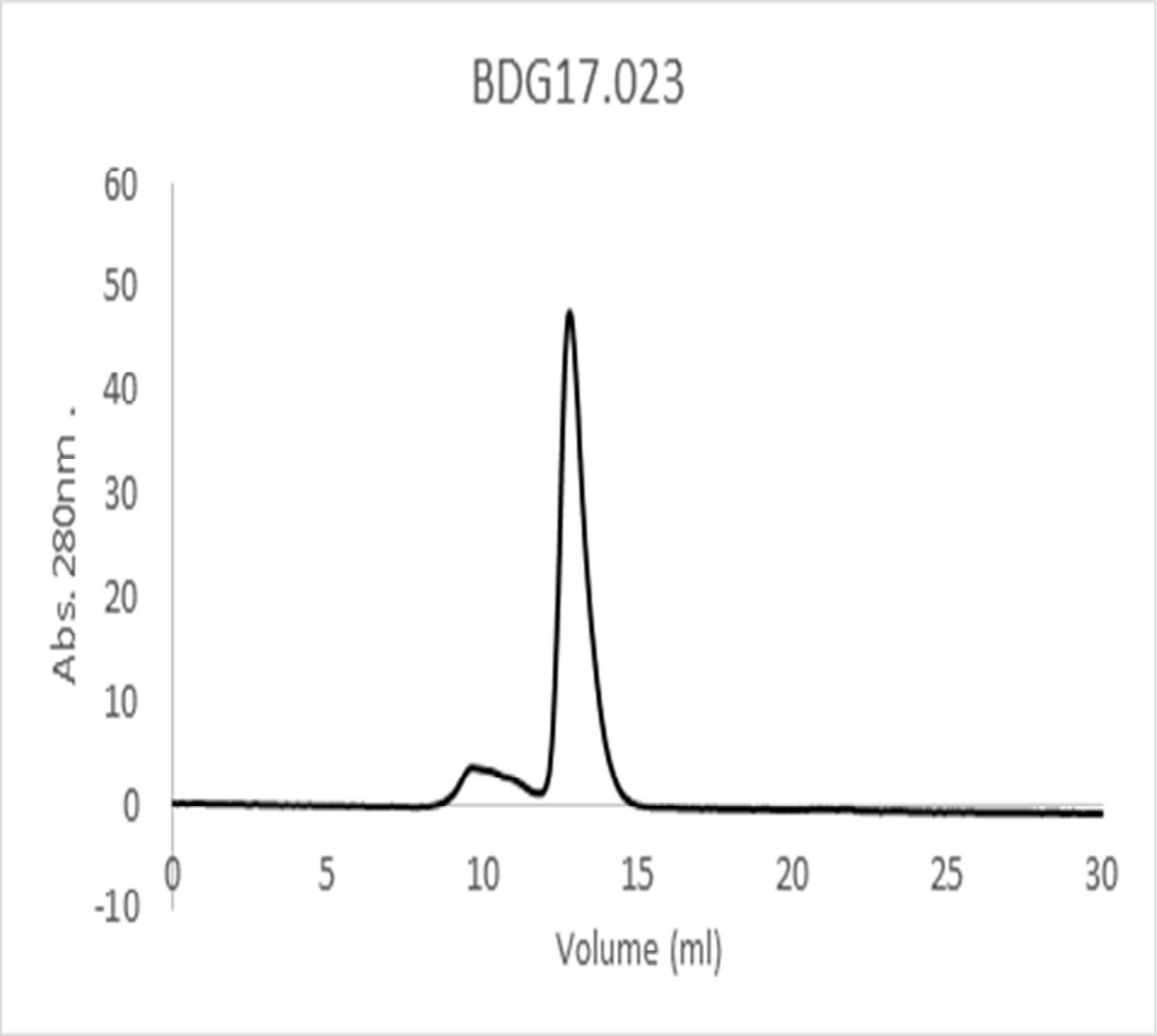

BDG17.023
Abs. 280nm .
60
50
40
30
20
10
0
-10
0
5
10
15
20
25
30
Volume (ml)

FIG. 7

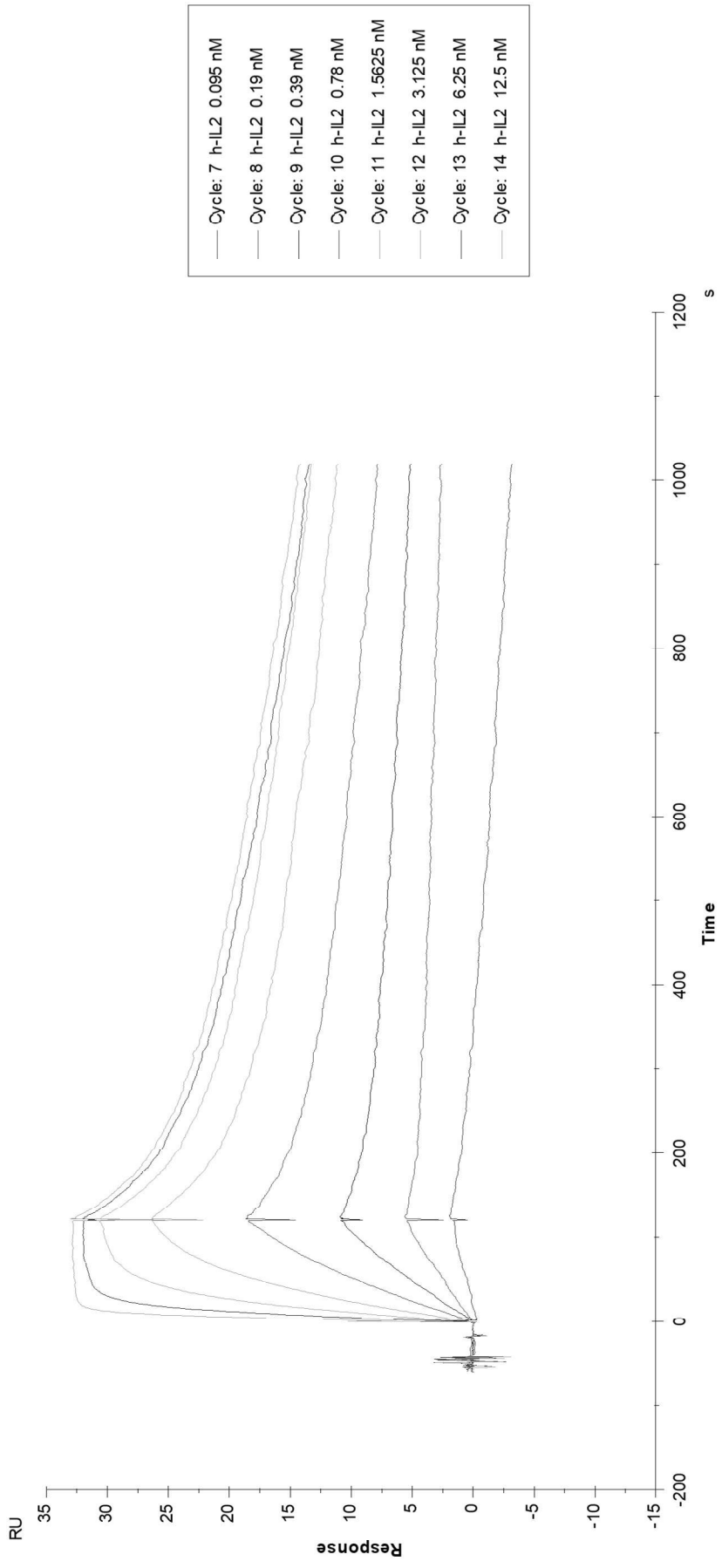

Cycle: 7 h-IL2 0.095 nM
Cycle: 8 h-IL2 0.19 nM
Cycle: 9 h-IL2 0.39 nM
Cycle: 10 h-IL2 0.78 nM
Cycle: 11 h-IL2 1.5625 nM
Cycle: 12 h-IL2 3.125 nM
Cycle: 13 h-IL2 6.25 nM
Cycle: 14 h-IL2 12.5 nM
RU
Response
35
30
25
20
15
10
5
0
-5
-10
-15
-200
0
200
400
600
800
1000
1200
Time
s

FIG. 8A

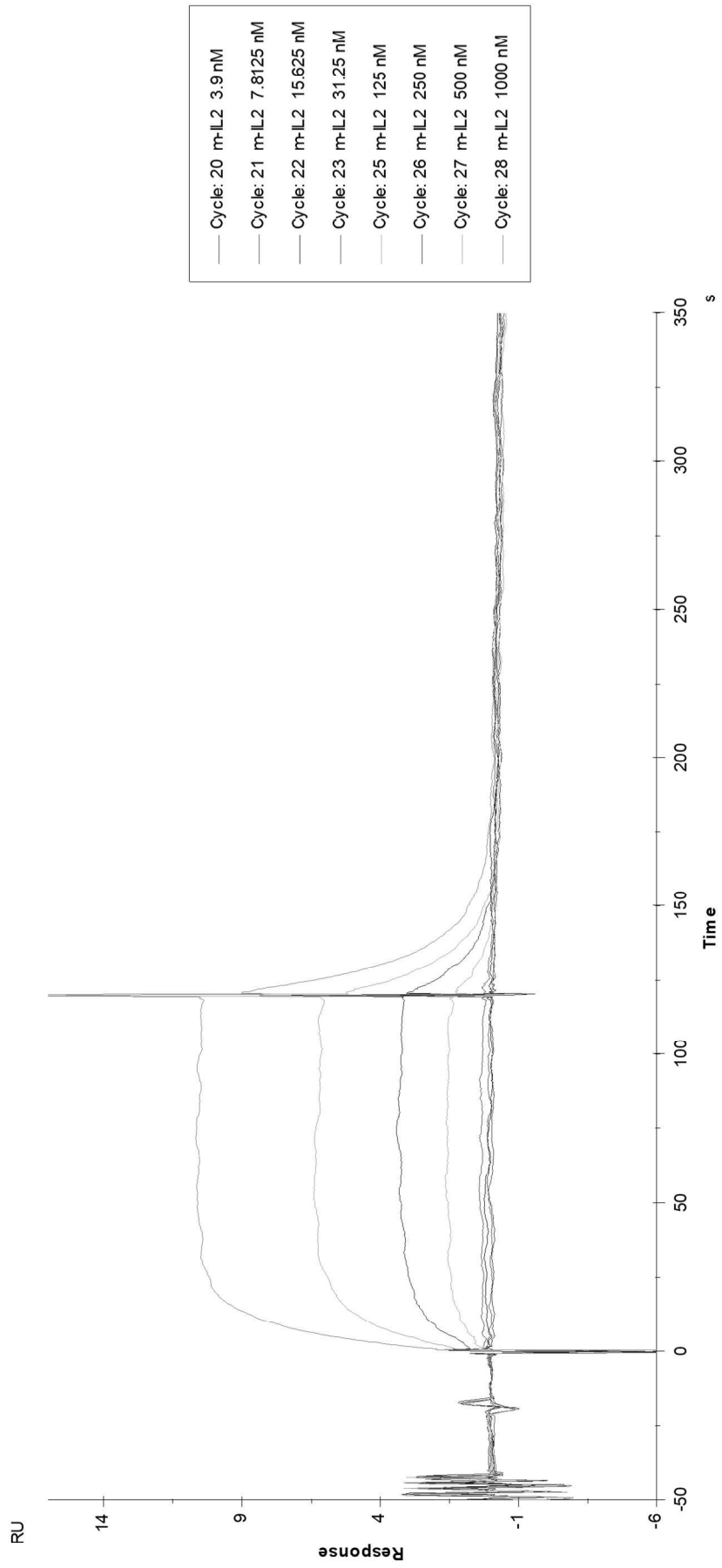

Cycle: 20 m-IL2 3.9 nM
Cycle: 21 m-IL2 7.8125 nM
Cycle: 22 m-IL2 15.625 nM
Cycle: 23 m-IL2 31.25 nM
Cycle: 25 m-IL2 125 nM
Cycle: 26 m-IL2 250 nM
Cycle: 27 m-IL2 500 nM
Cycle: 28 m-IL2 1000 nM
RU
Response
14
9
4
-1
-6
-50
0
50
100
150
200
250
300
350
Time
s

FIG. 8B

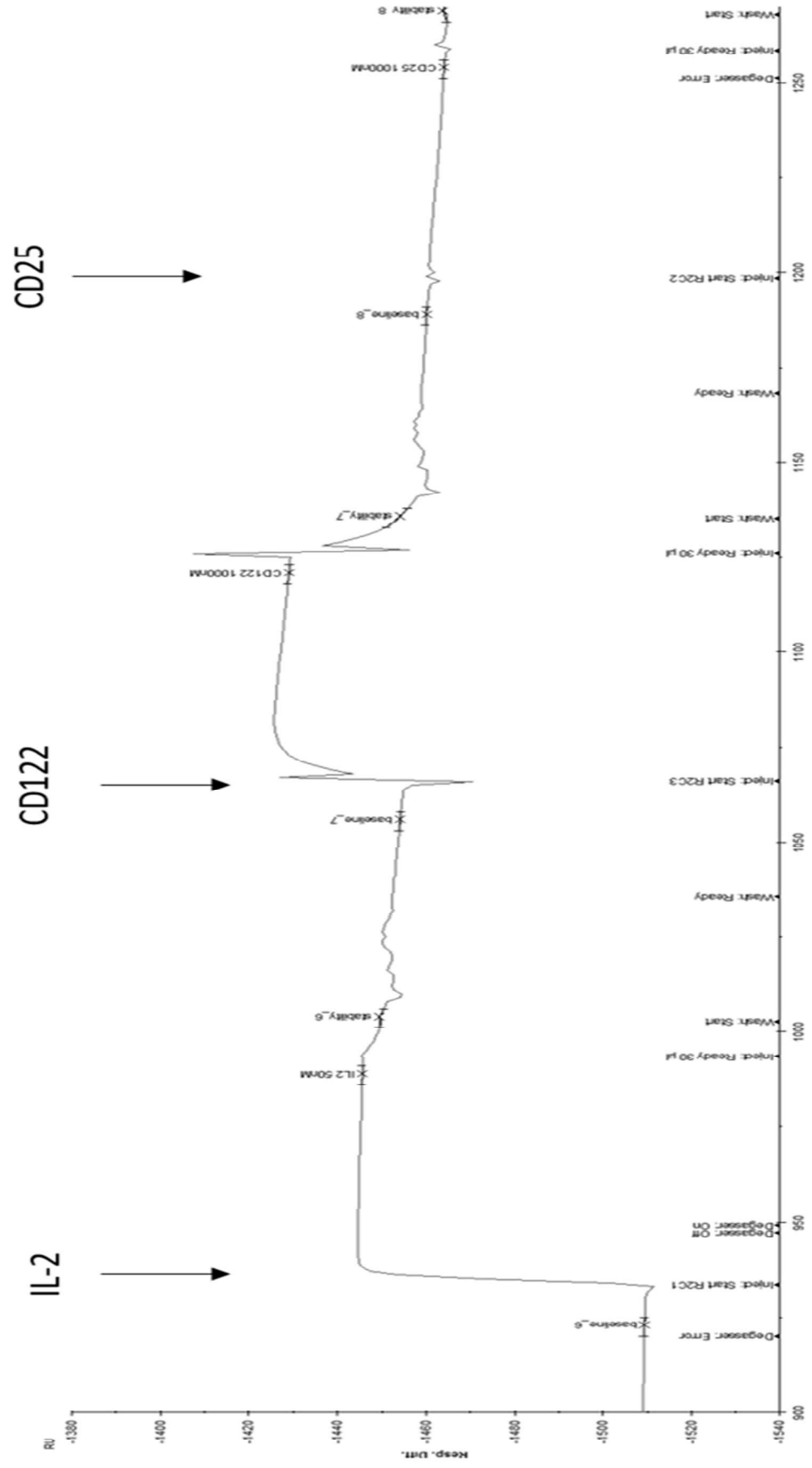

IL-2
CD122
CD25
RU
Resp. Diff.
-1380
-1400
-1420
-1440
-1460
-1480
-1500
-1520
-1540
900
950
1000
1050
1100
1150
1200
1250
Degasser: Error
Inlet: Start R2C1
Degasser: Off
Degasser: On
baseline_5
IL2 50nM
stability_6
baseline_7
Inlet: Start R2C3
Wash: Ready
Wash: Start
Inlet: Ready 30 µl
CD122 1000nM
stability_7
baseline_8
Inlet: Start R2C2
CD25 1000nM
stability 8

FIG. 9

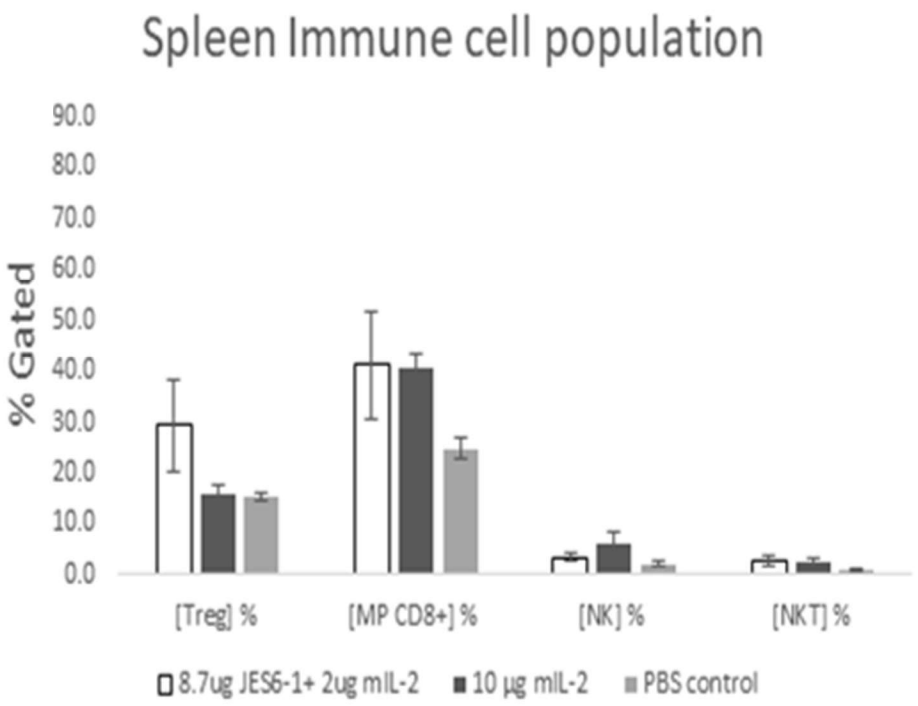


FIG. 10A

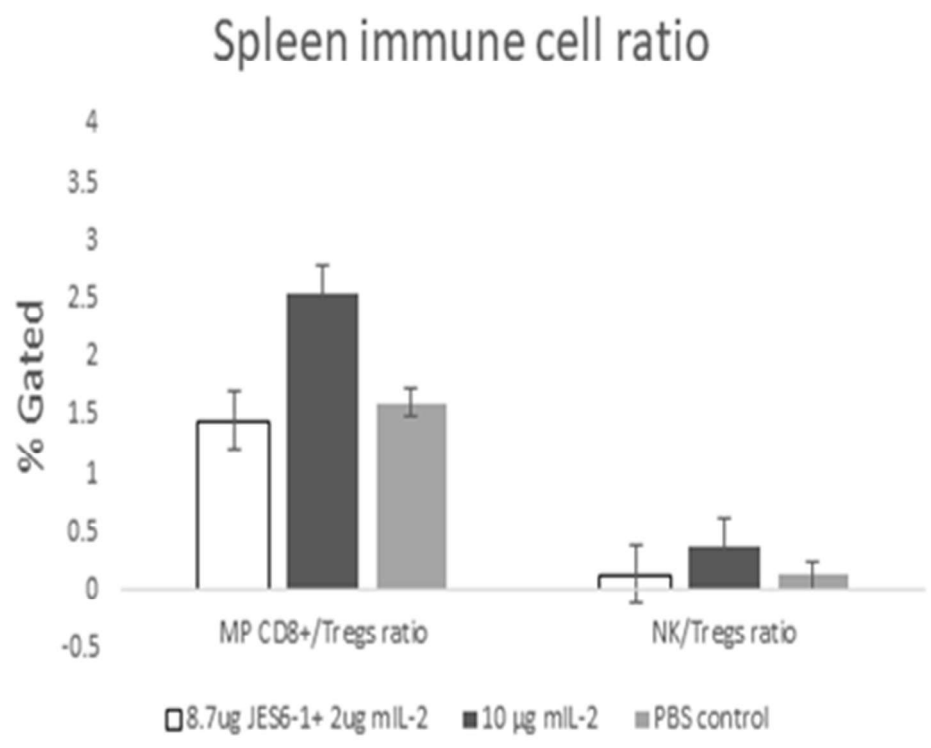


FIG. 10B

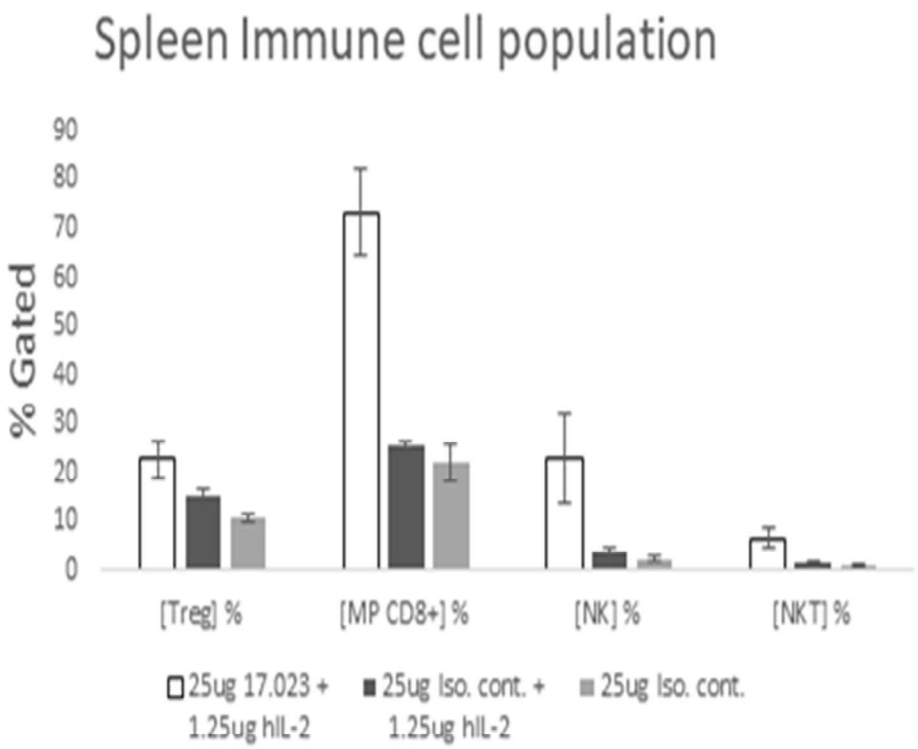


FIG. 10C

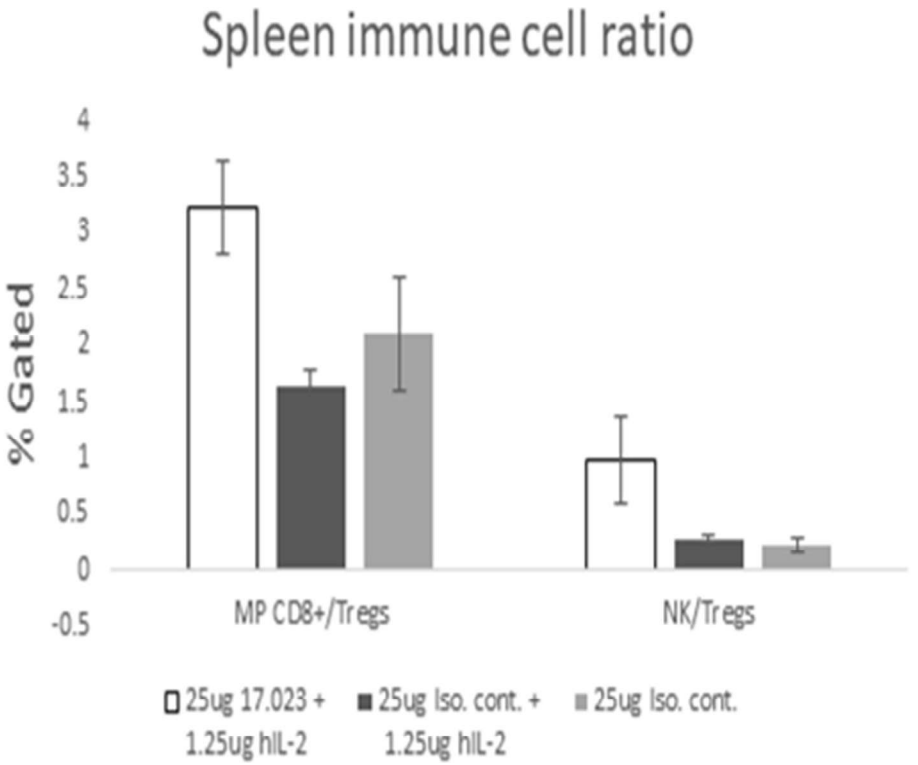
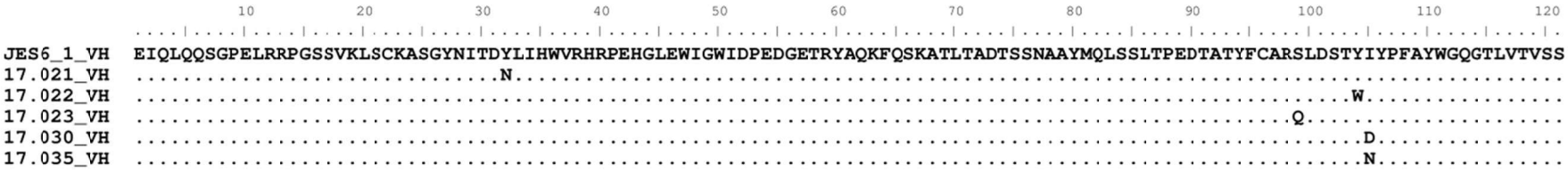
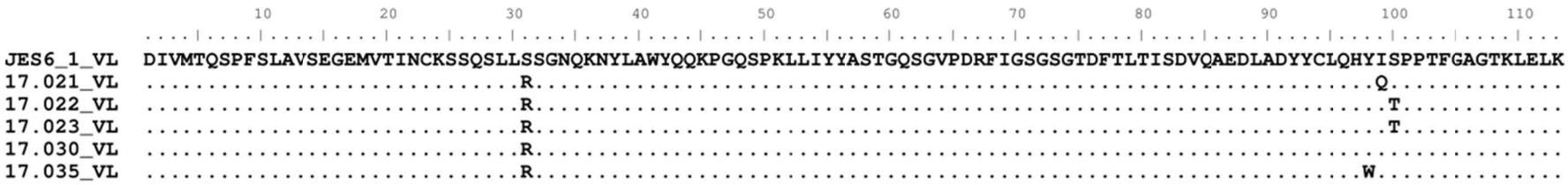


FIG. 10D



JES6_1_VH = SEQ ID NO: 6
17.021_VH = SEQ ID NO: 10
17.022_VH = SEQ ID NO: 12
17.023_VH = SEQ ID NO: 14
17.030_VH = SEQ ID NO: 16
17.035_VH = SEQ ID NO: 18

FIG. 11



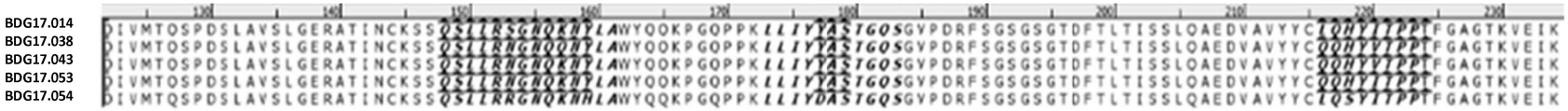
JES6_1_VL = SEQ ID NO: 7
17.021_VL = SEQ ID NO: 11
17.022_VL = SEQ ID NO: 13
17.023_VL = SEQ ID NO: 15
17.030_VL = SEQ ID NO: 17
17.035_VL = SEQ ID NO: 19

FIG. 12



BDG17.014= SEQ ID NO: 36
BDG17.038= SEQ ID NO: 20
BDG17.043= SEQ ID NO: 22
BDG17.053= SEQ ID NO: 24
BDG17.054= SEQ ID NO: 26

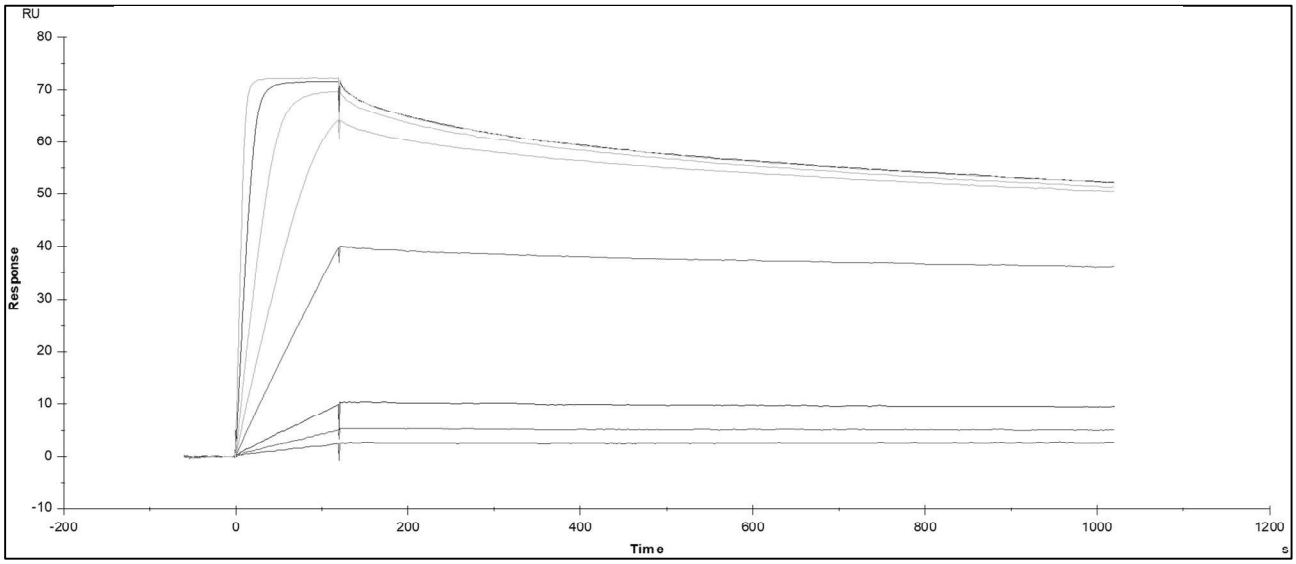
FIG. 13A



BDG17.014 = SEQ ID NO: 37
BDG17.038= SEQ ID NO: 21
BDG17.043= SEQ ID NO: 23
BDG17.053= SEQ ID NO: 25
BDG17.054= SEQ ID NO: 27

FIG. 13B

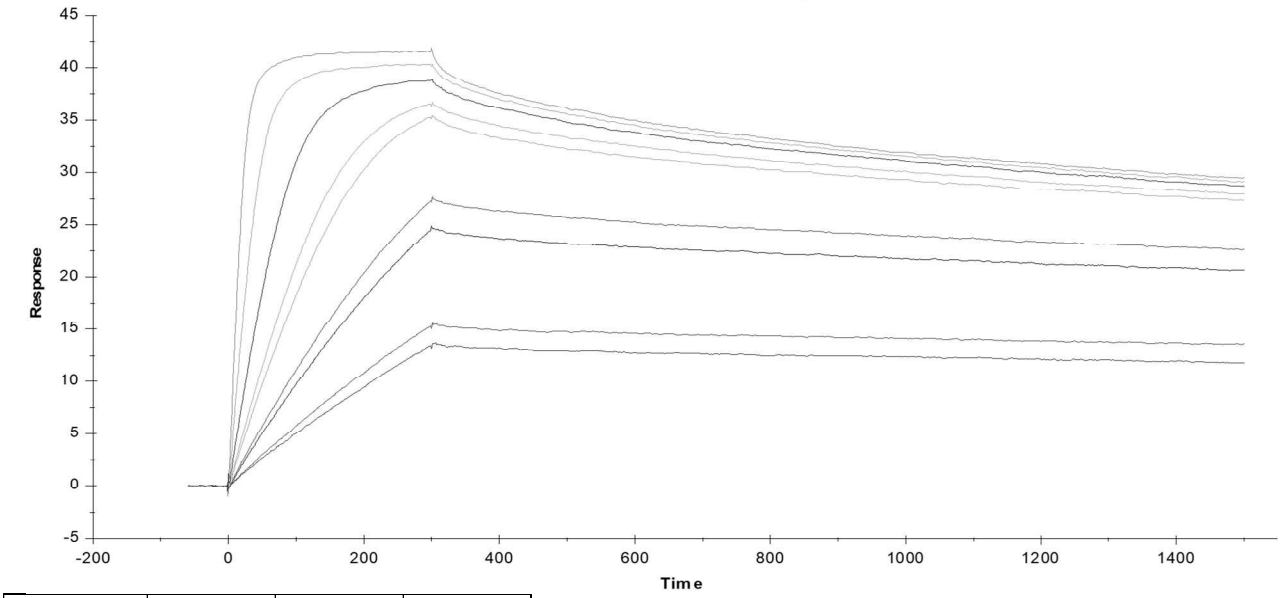
Binding to human IL-2 17.038:



ka (1/Ms)	kd (1/s)	KD (M)	Chi² (RU²)
1.41E+07	2.005E-04	2.02E-11	0.04

FIG. 14A

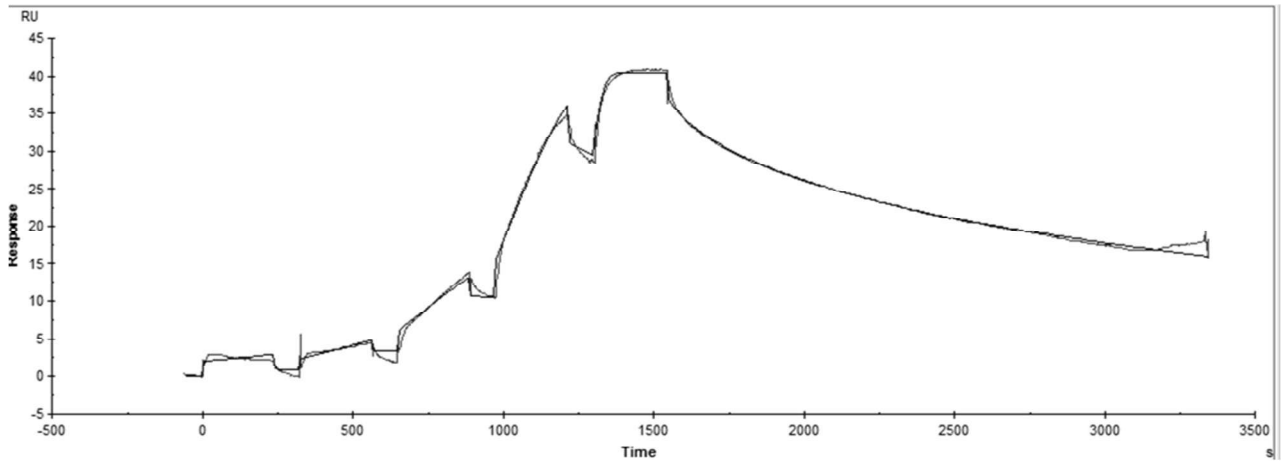
Binding to human IL-2 17.043:



ka (1/Ms)	kd (1/s)	KD (M)	Chi² (RU²)
3.39E+07	2.66E-04	47.85E-12	0.441

FIG. 14B

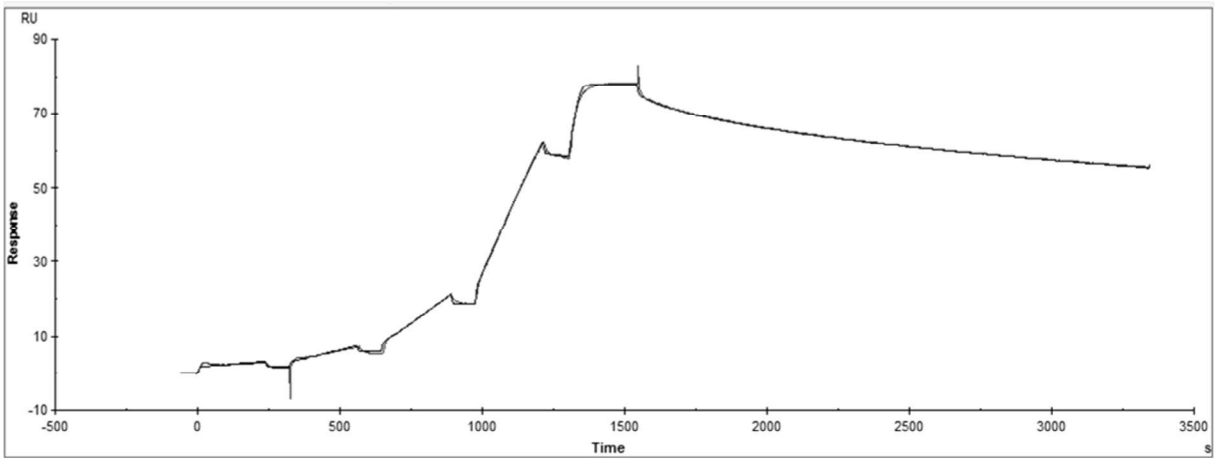
Binding to human IL-2 17.053:



ka (1/Ms)	kd (1/s)	KD (M)	Chi² (RU²)
3.39E+07	2.54E-03	6.43E-11	0.331

FIG. 14C

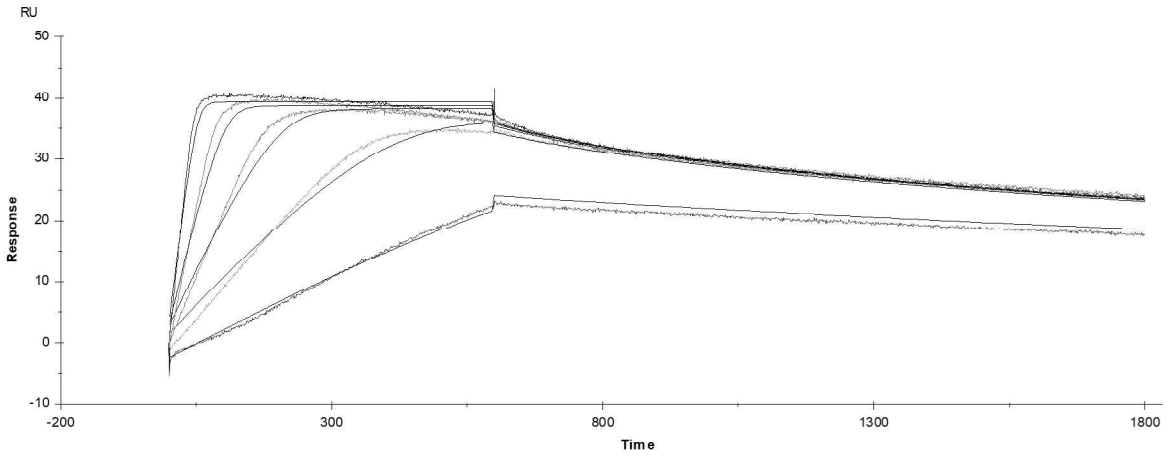
Binding to human IL-2 17.054:



ka (1/Ms)	kd (1/s)	KD (M)	Chi² (RU²)
5.50E+07	7.00E-04	1.27E-11	0.043

FIG. 14D

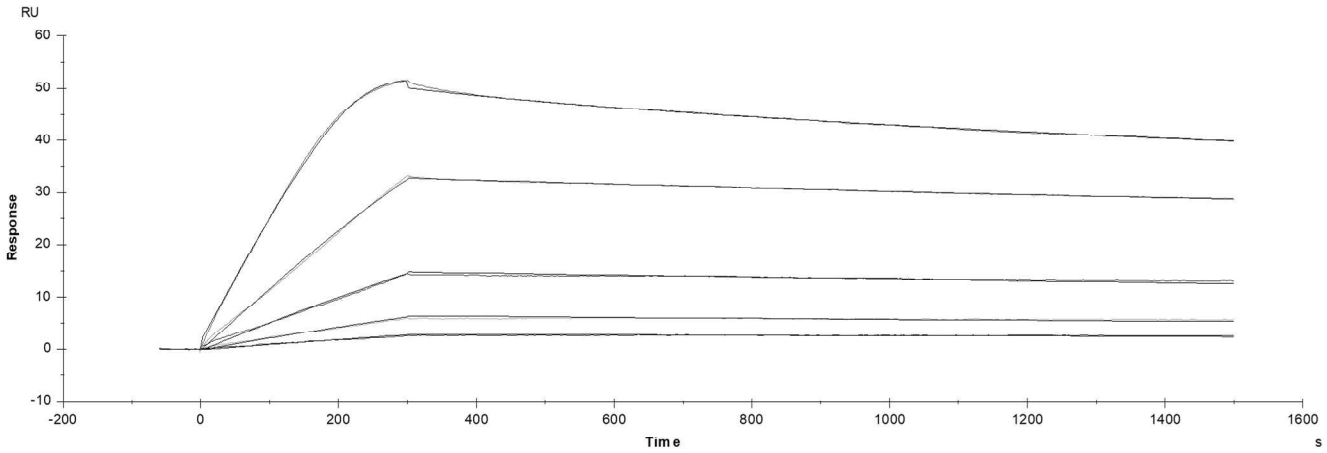
Binding to human IL-2 17.066:



ka (1/Ms)	kd (1/s)	KD (M)	Chi² (RU²)
1.52E+07	7.85E-04	5.15E-11	0.864

FIG. 14E

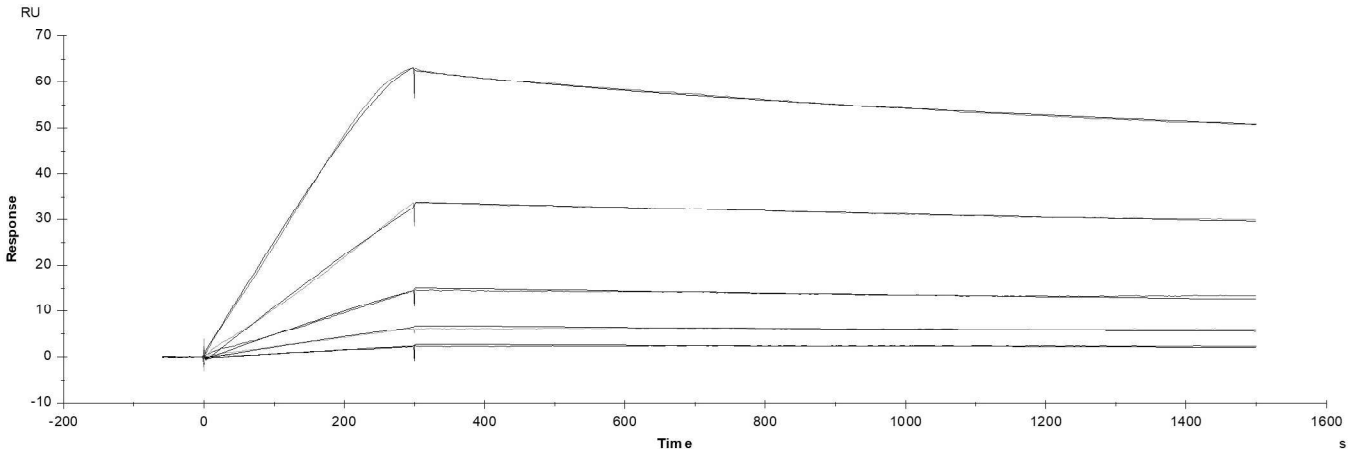
Binding to human IL-2 17.067:



ka (1/Ms)	kd (1/s)	KD (M)	Chi² (RU²)
3.37E+07	3.72E-04	1.10E-11	0.0513

FIG. 14F

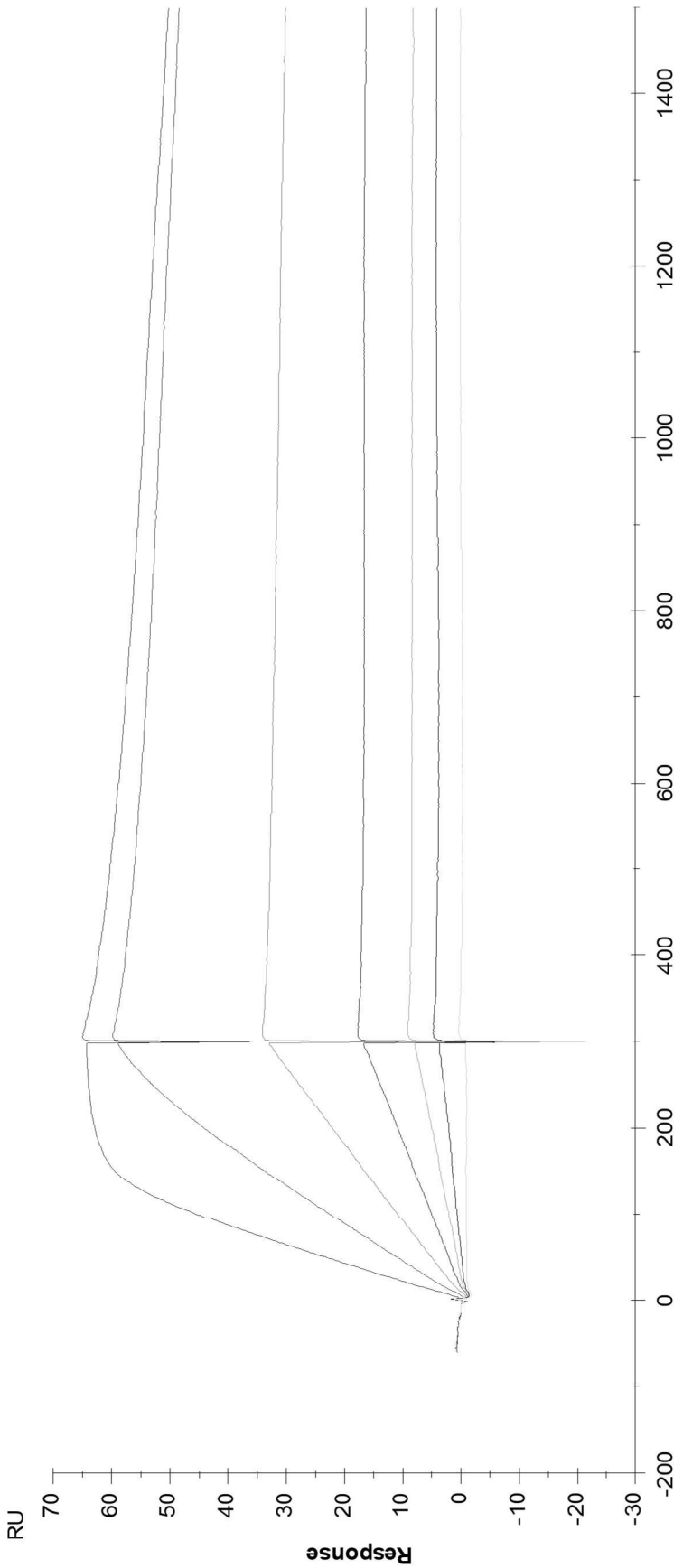
Binding to human IL-2 17.069:



ka (1/Ms)	kd (1/s)	KD (M)	Chi² (RU²)
4.50E+07	4.87E-04	1.08E-11	0.1

FIG. 14G

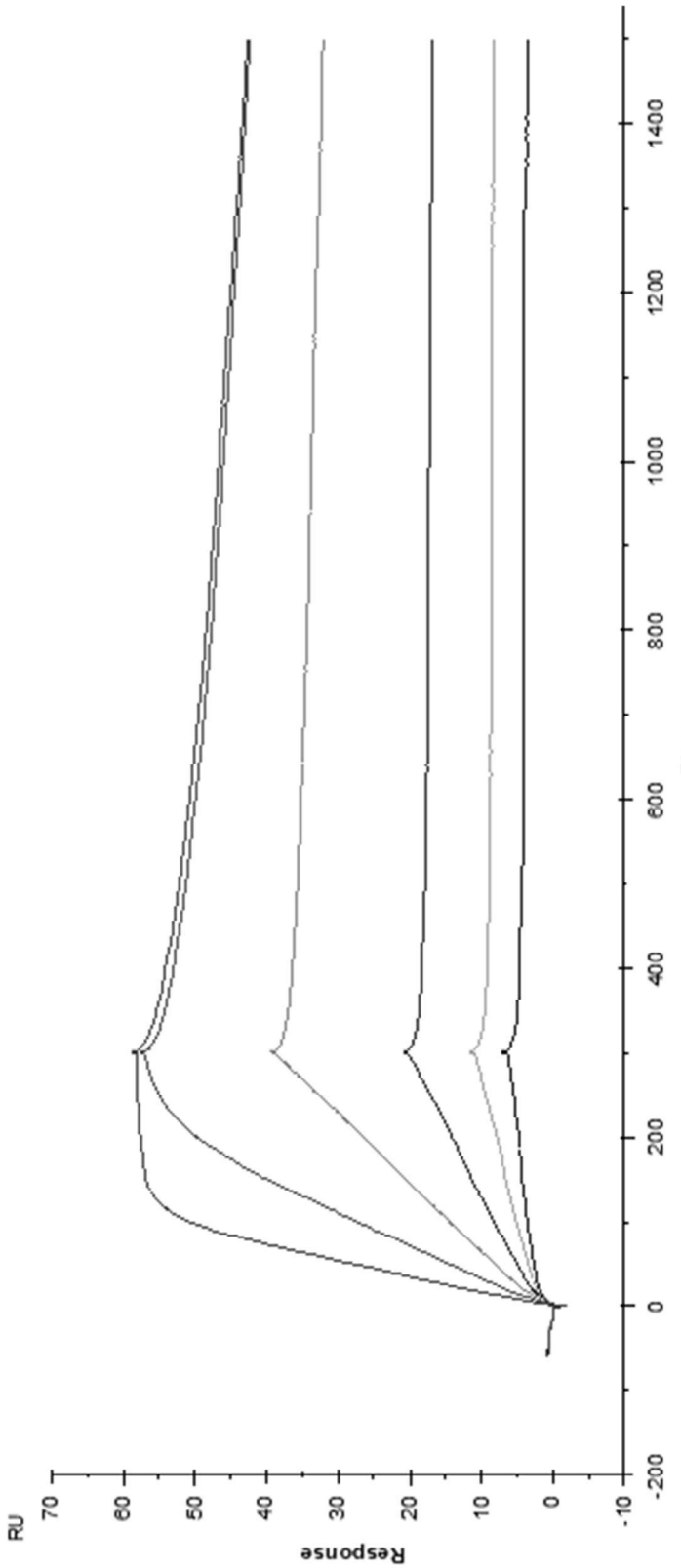
Binding to Cyno IL-2: 17.067



ka (1/Ms)	kd (1/s)	KD (M)	Chi² (RU²)
2.83E+07	3.98E-04	1.40E-11	0.0262

FIG. 15A

Binding to Cyno IL-2: 17.069



ka (1/Ms)	kd (1/s)	KD (M)	Chi² (RU²)
4.98E+07	5.84E-04	1.17E-11	0.102

FIG. 15B

17.038

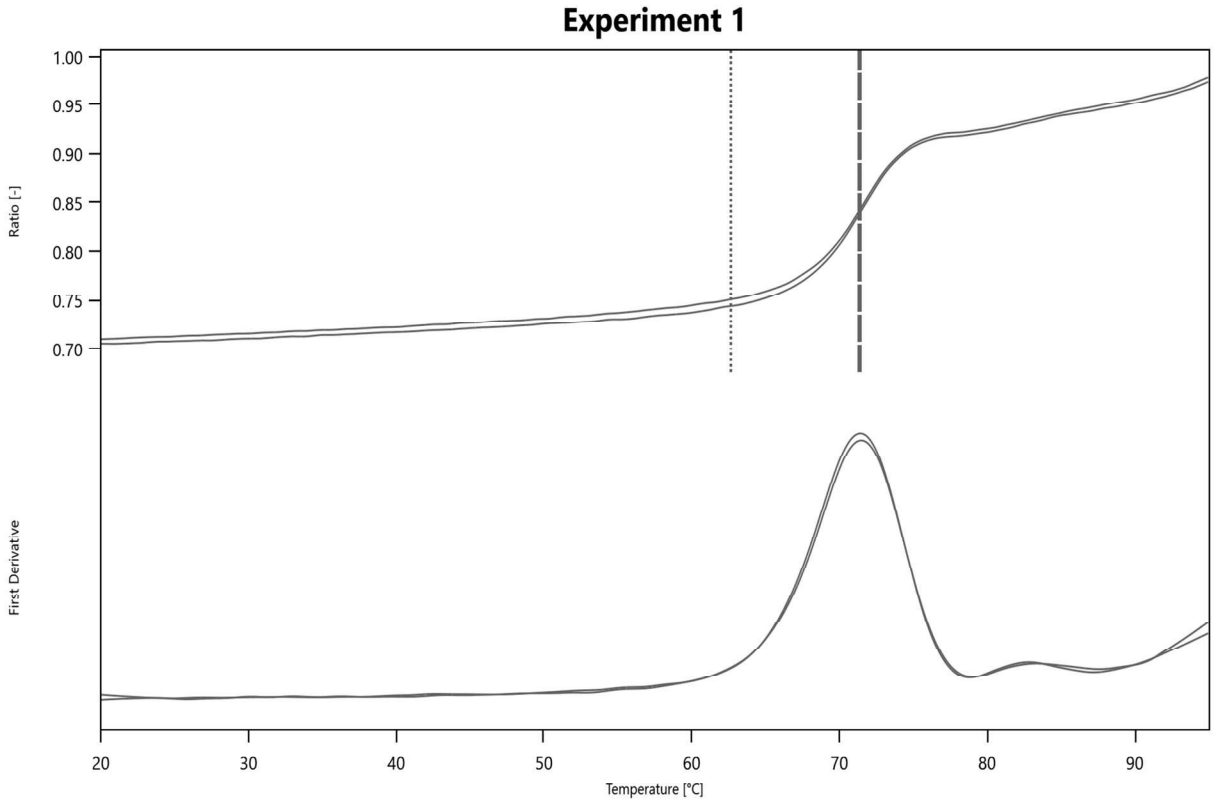


FIG. 16A

17.043

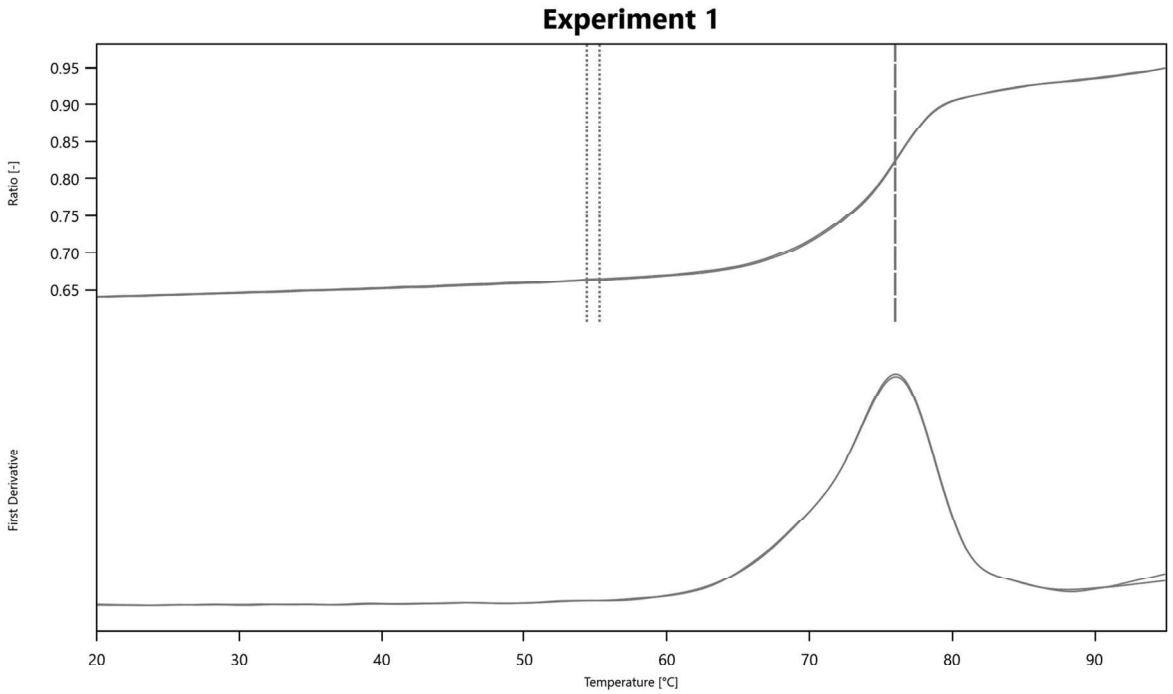


FIG. 16B

17.053

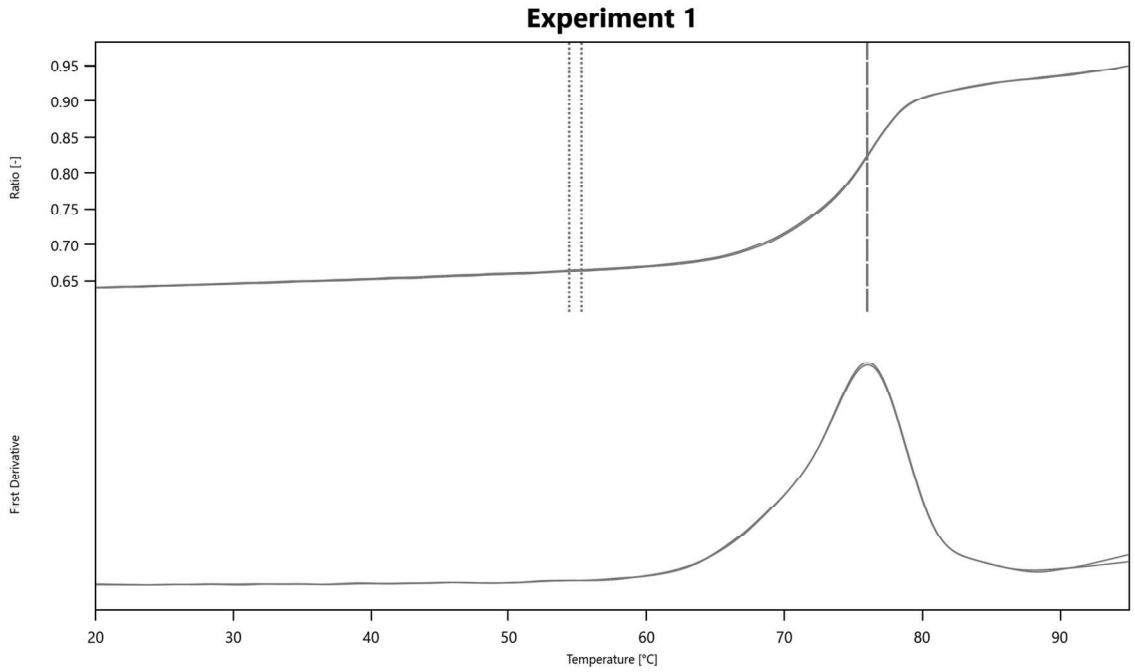


FIG. 16C

17.054

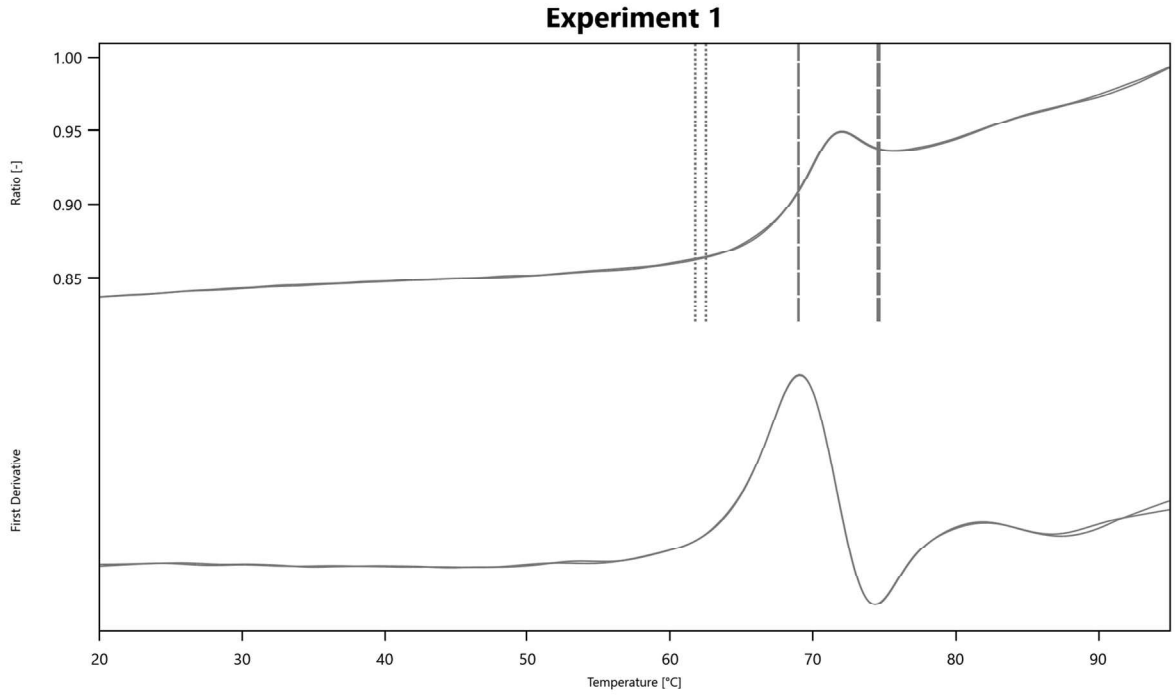


FIG. 16D

17.066

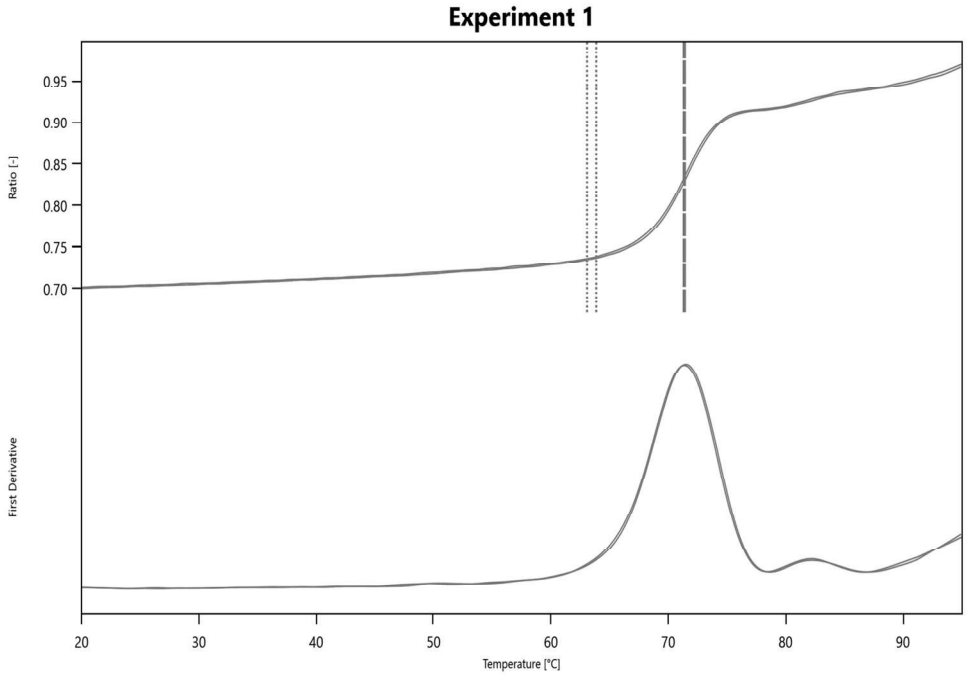


FIG. 16E

17.067

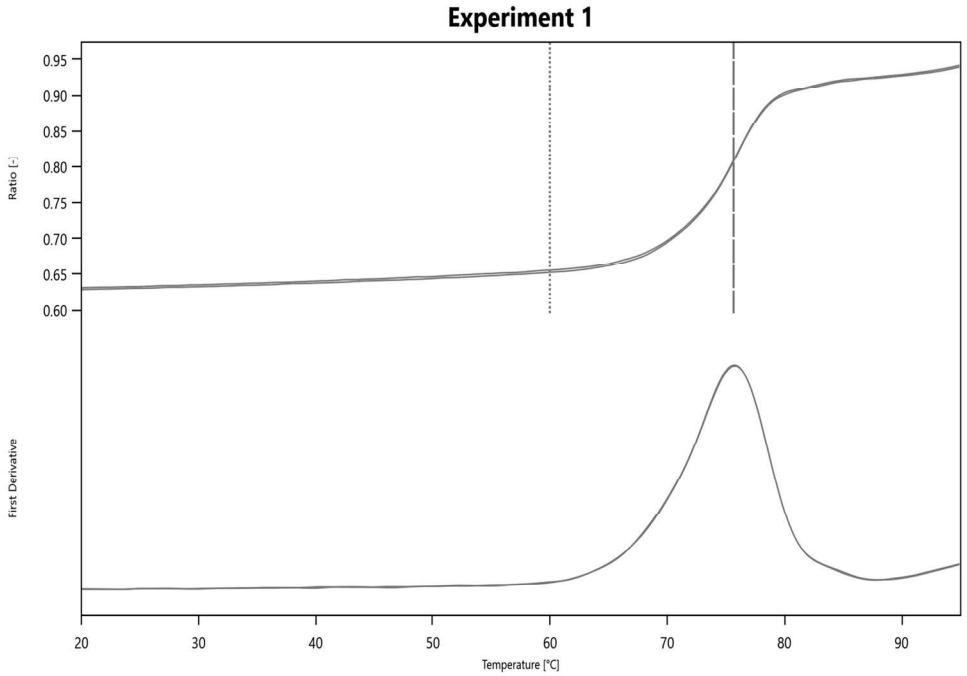


FIG. 16F

17.069

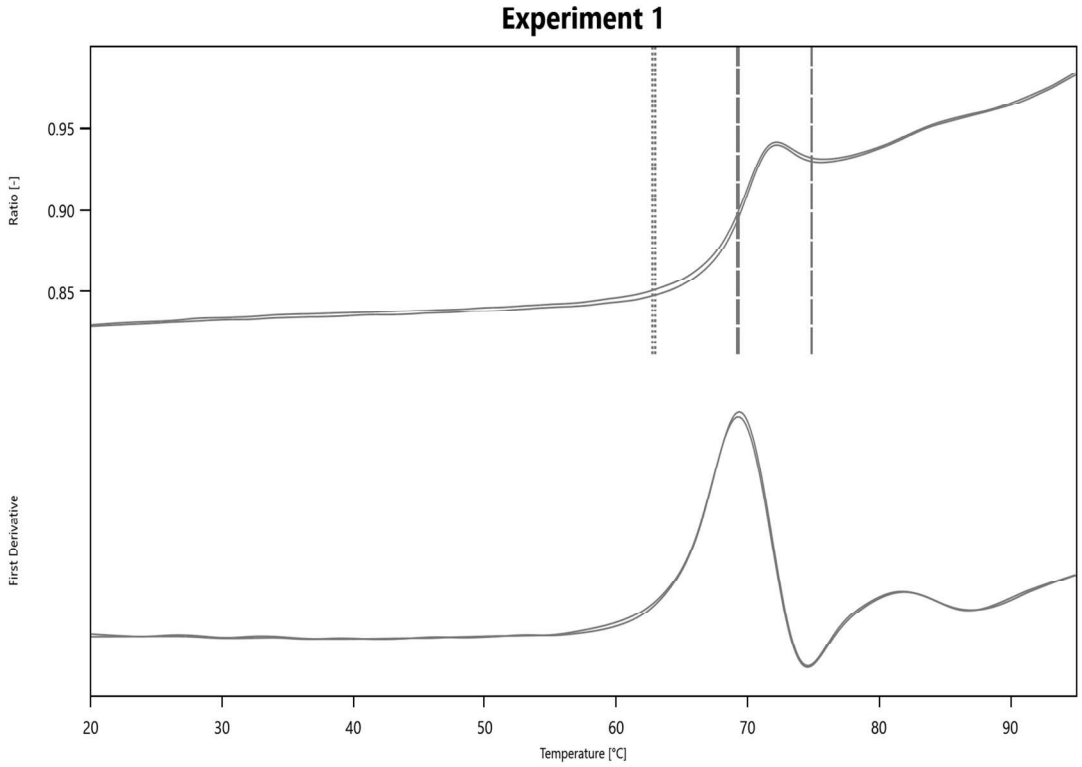


FIG. 16G

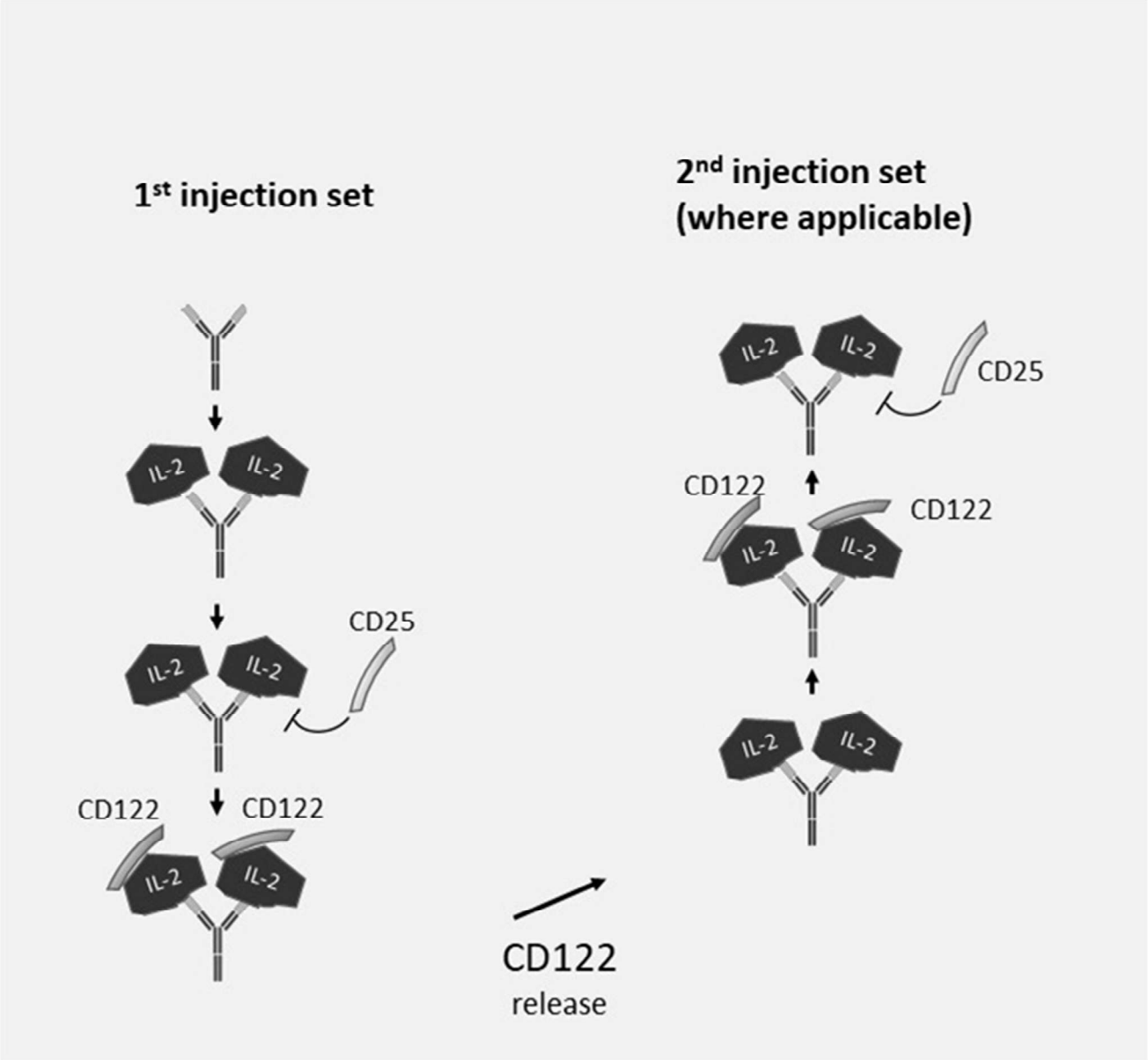

1st injection set
2nd injection set
(where applicable)
IL-2
IL-2
CD25
CD122
CD122
CD122
CD122
release

FIG. 17A

BDG17.038

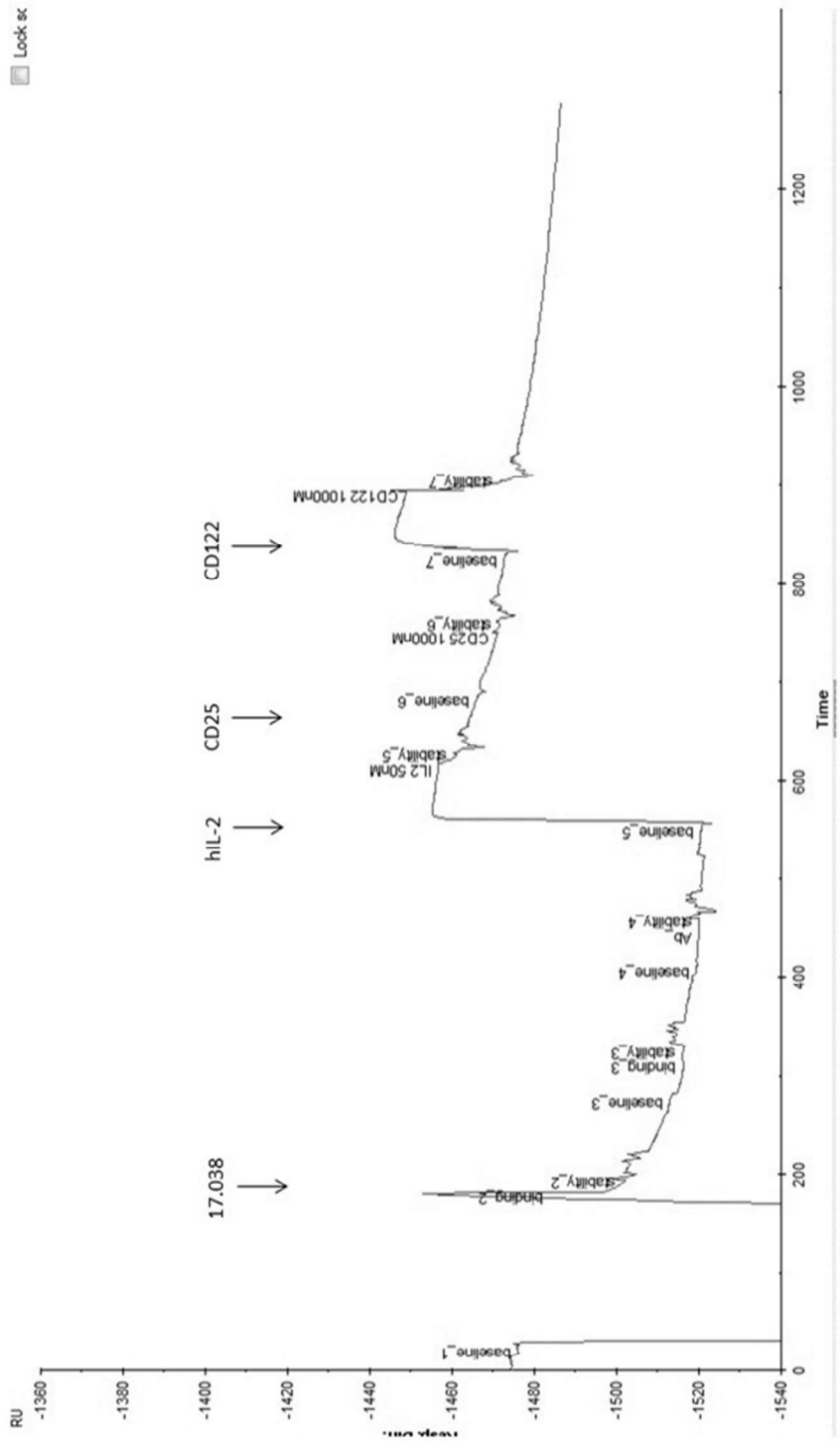


FIG. 17B

BGD17.043

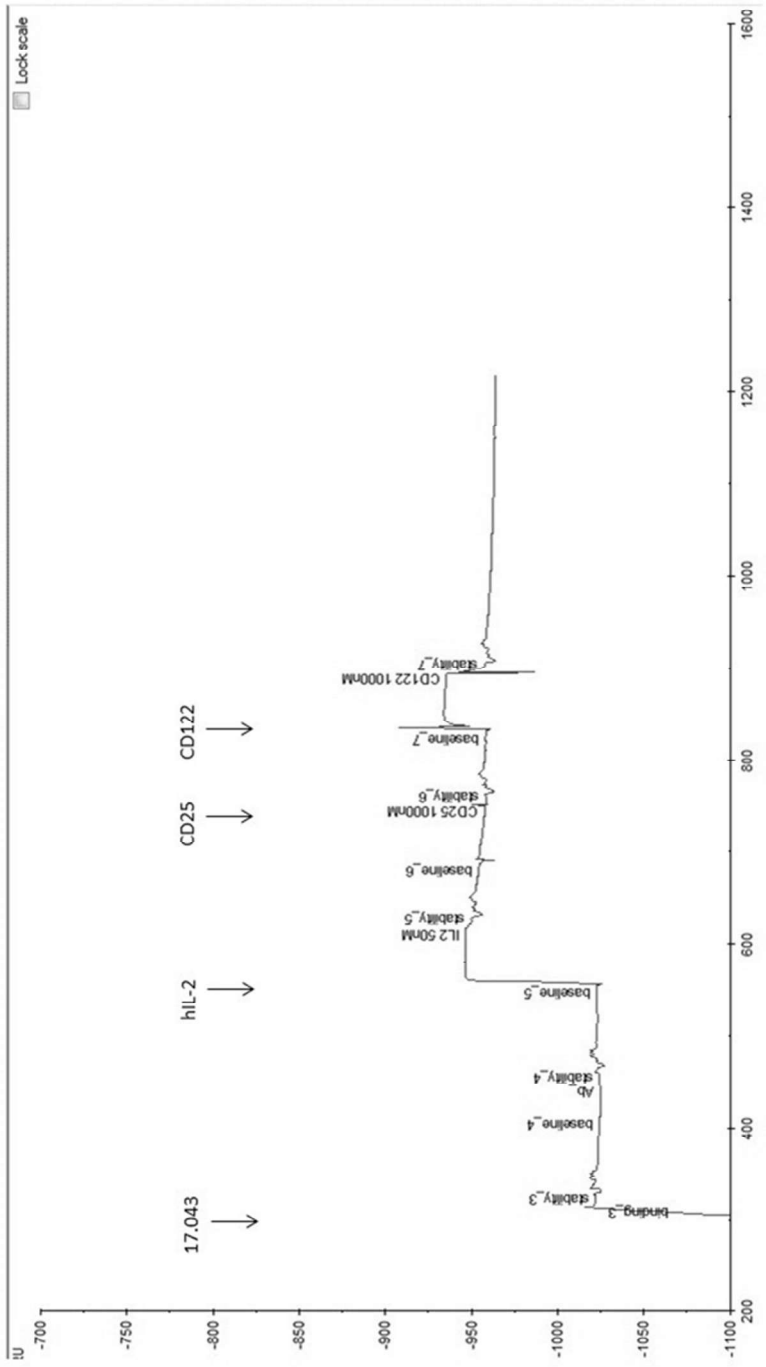


FIG. 17C

BGD17.054

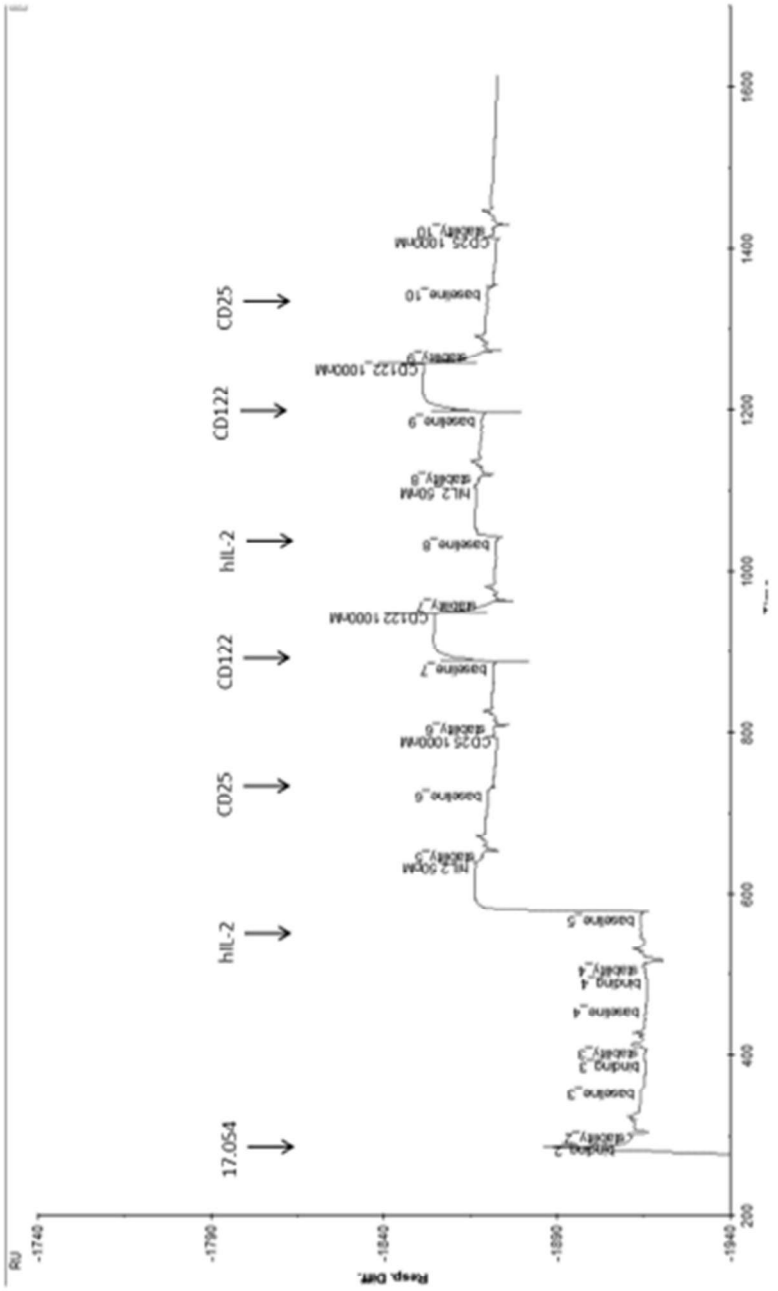


FIG. 17D

BDG17.066

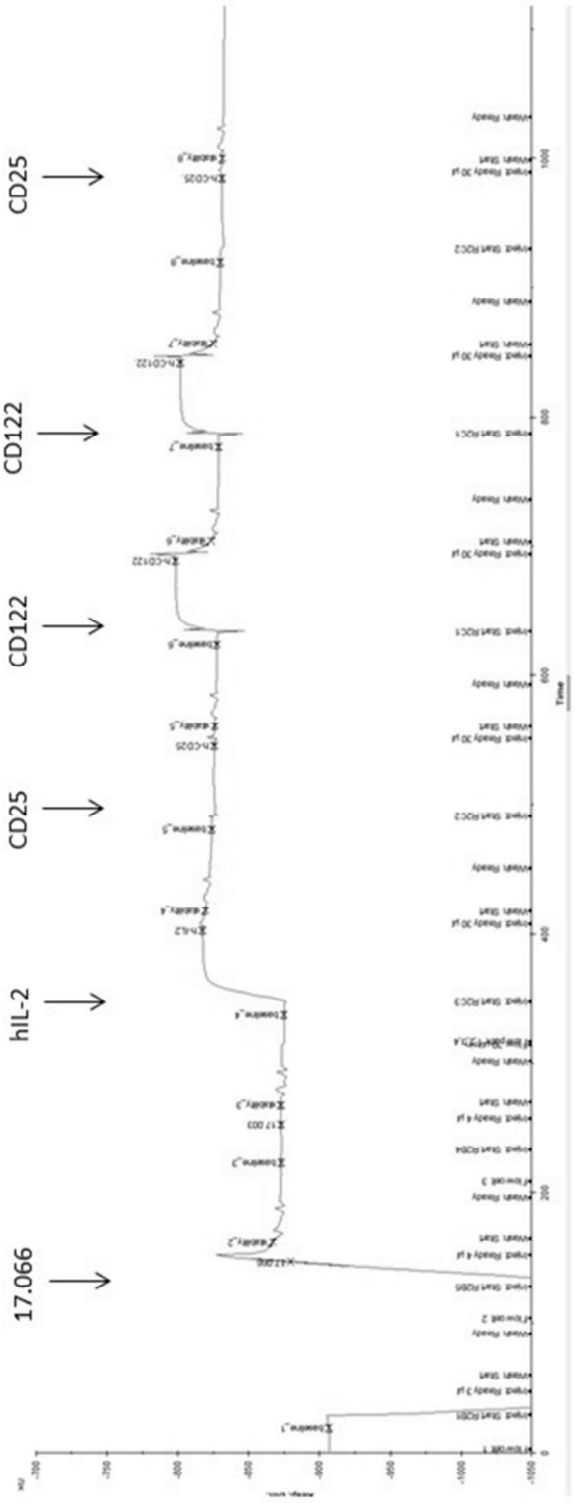


FIG. 17E

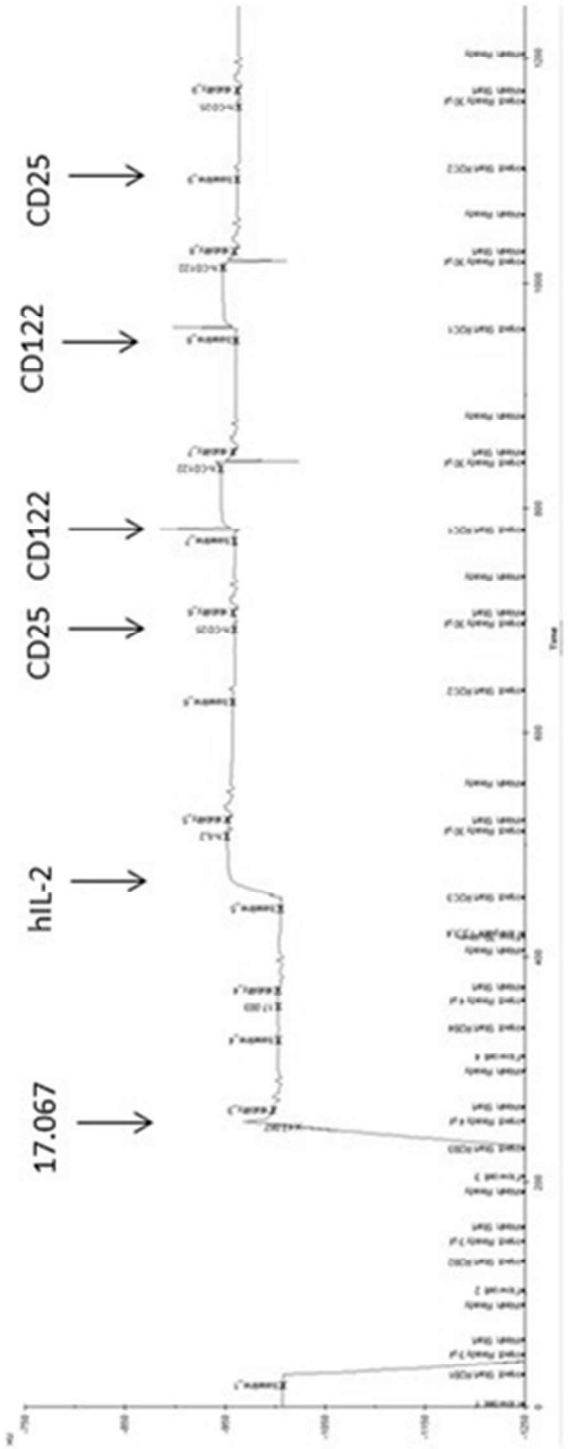


FIG. 17F

BDG17.069 (AU-007)

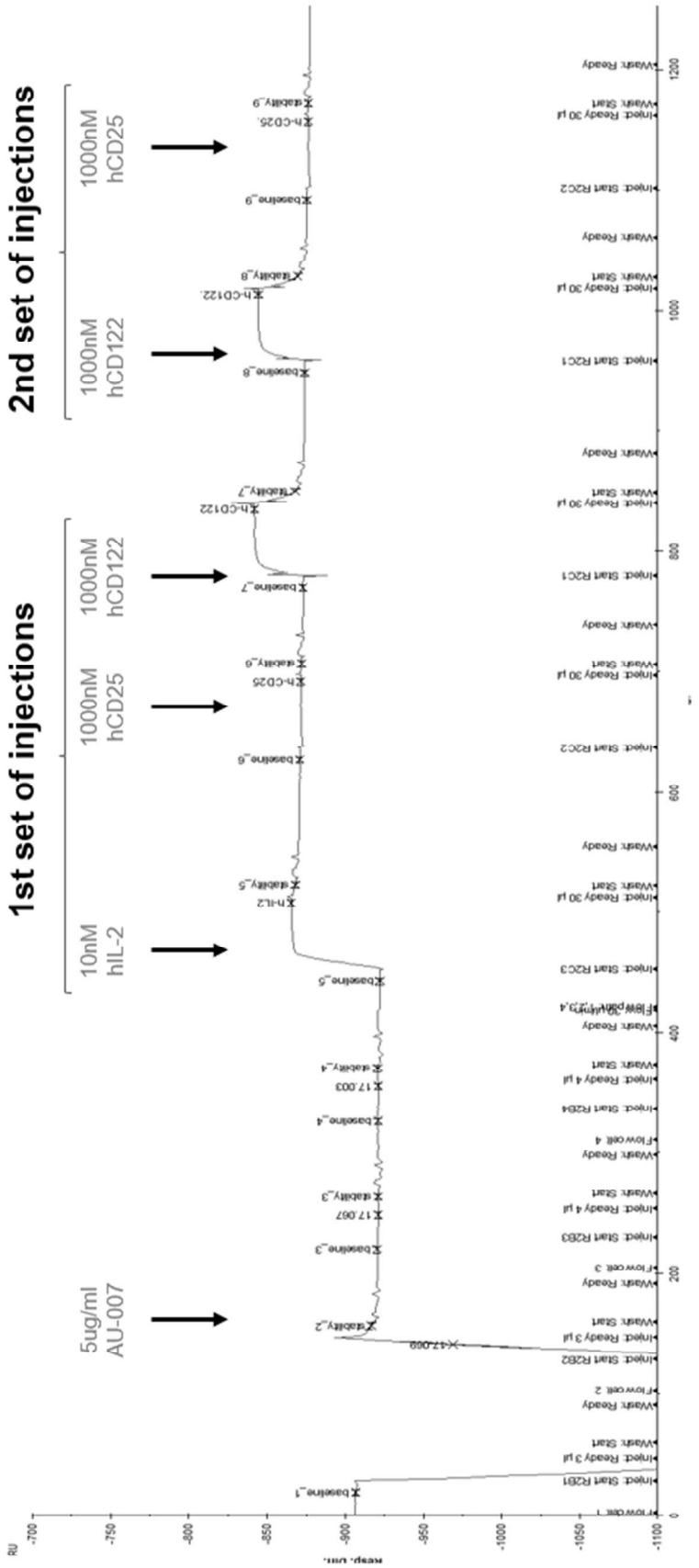


FIG. 17G

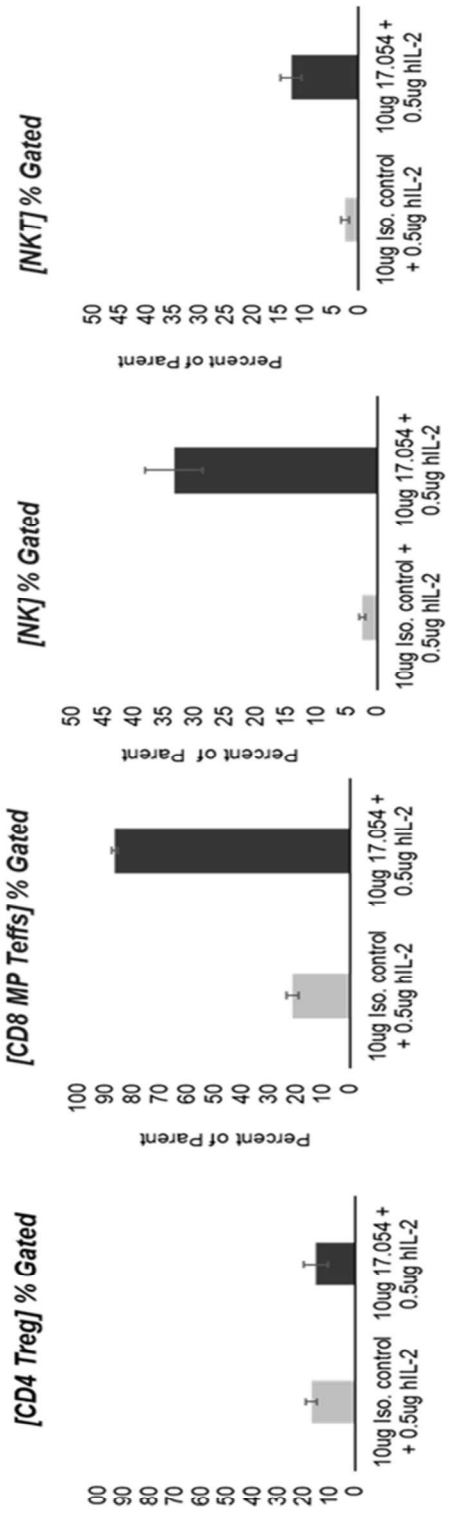


FIG. 18A

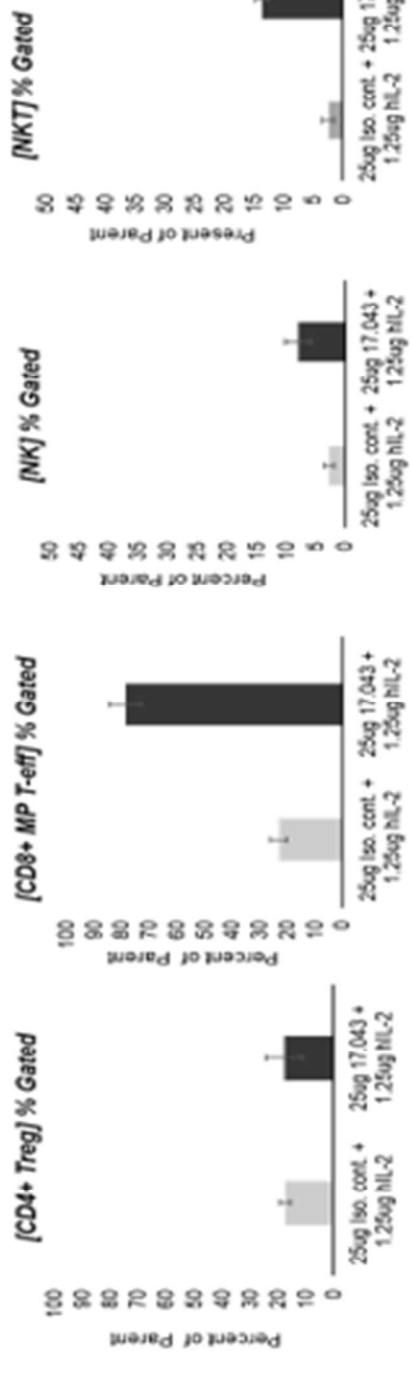


FIG. 18B

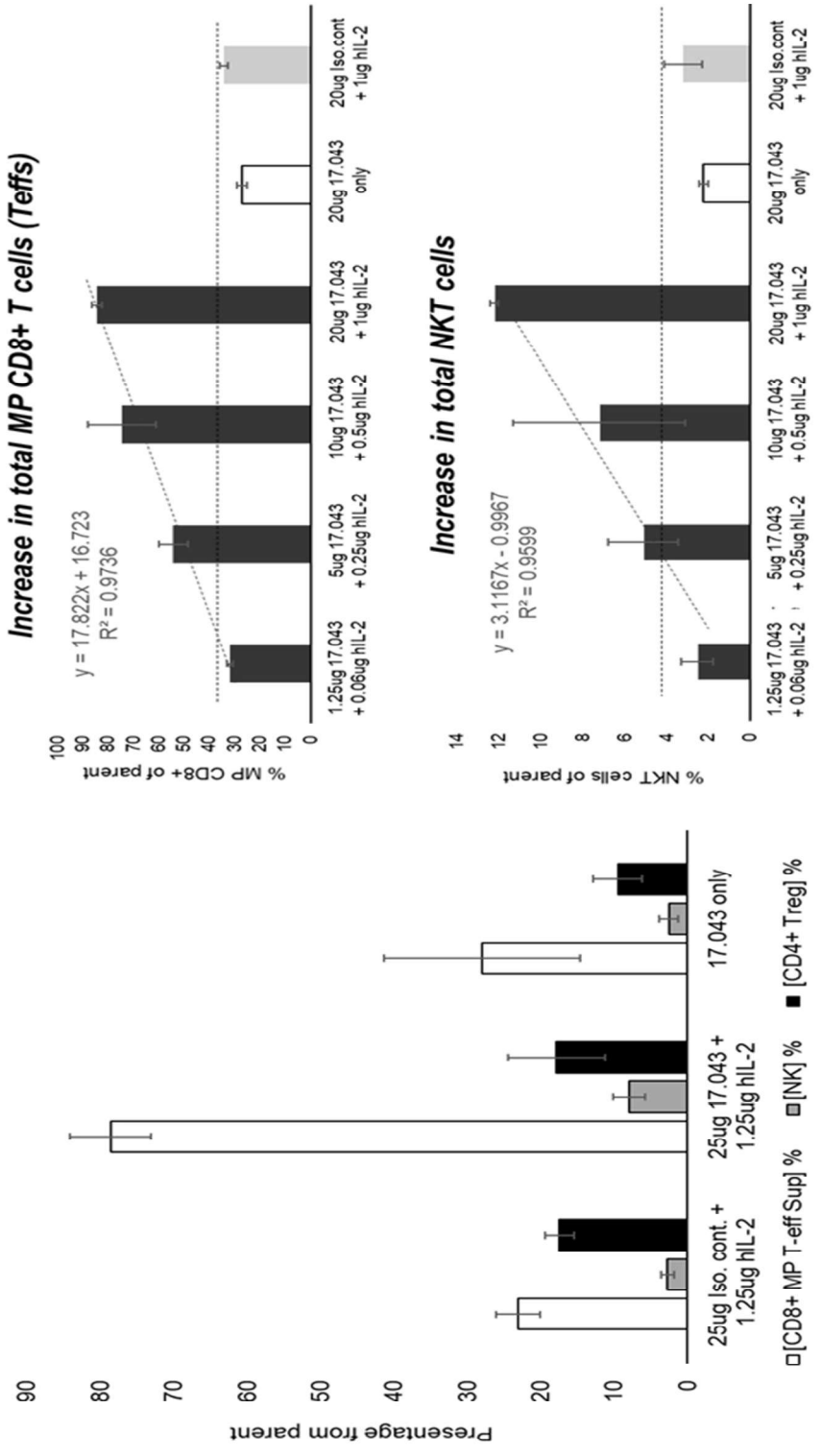


FIG. 19A

FIG. 19B

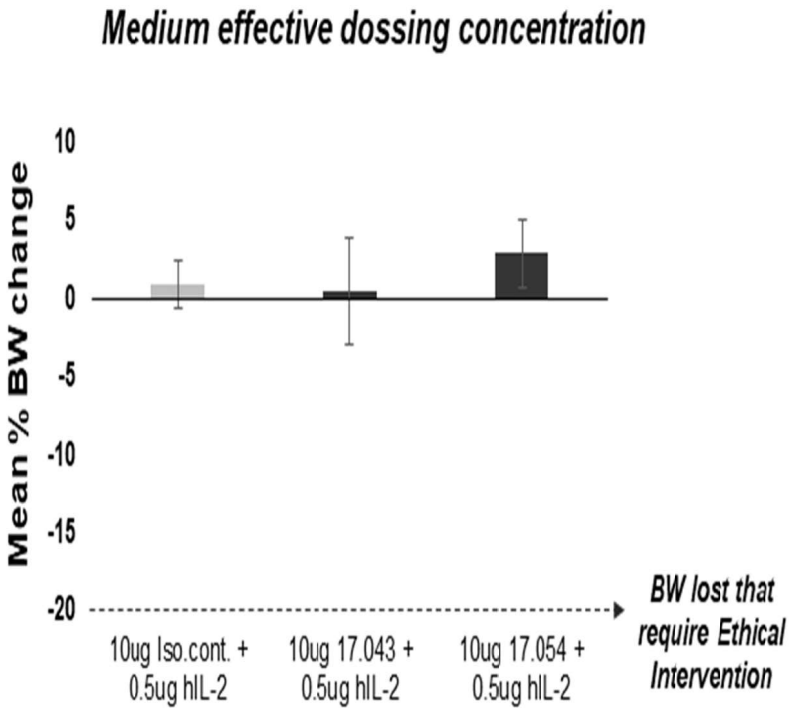


FIG. 20A

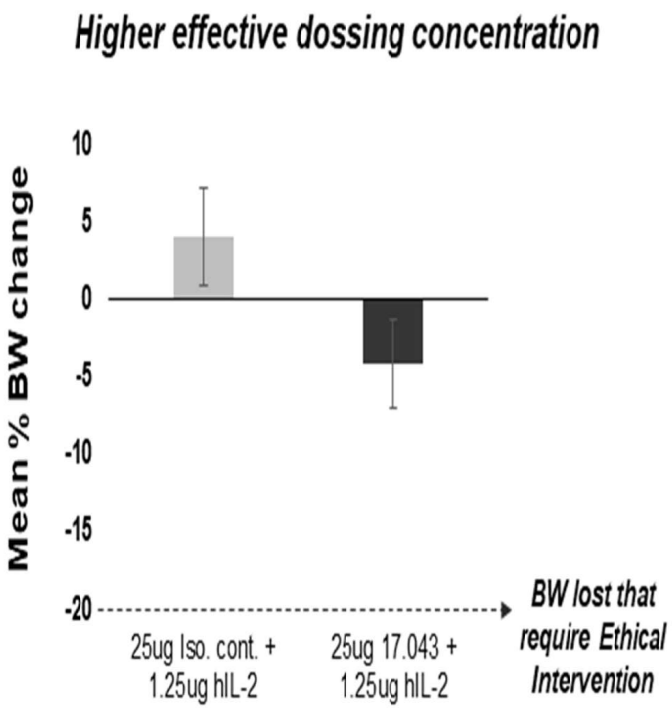


FIG. 20B

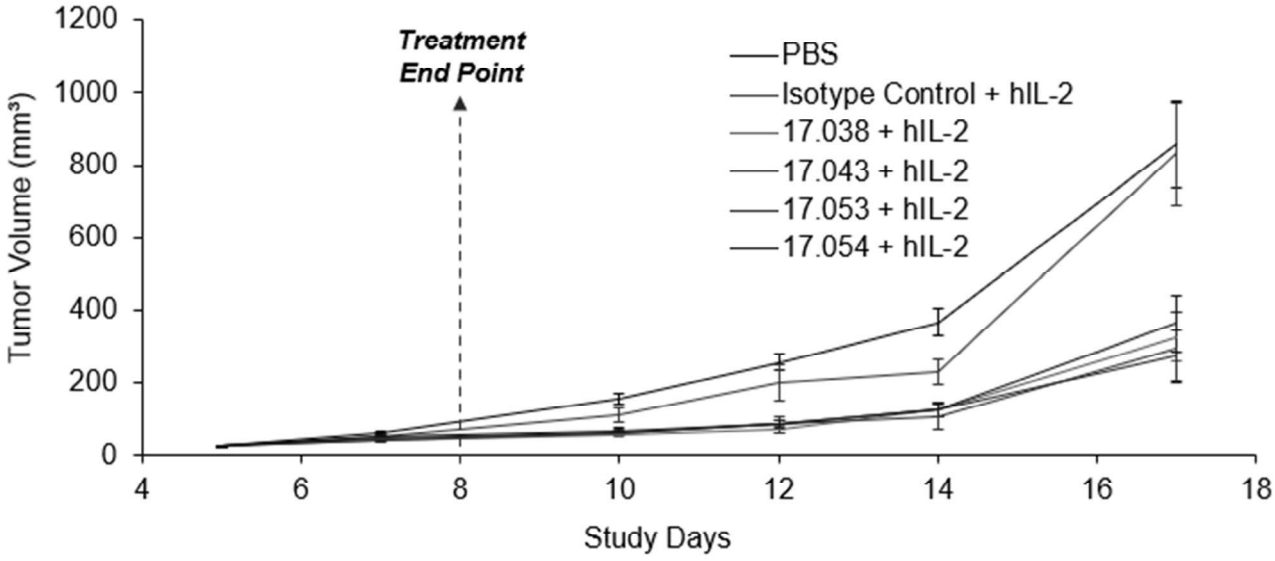


FIG. 21A

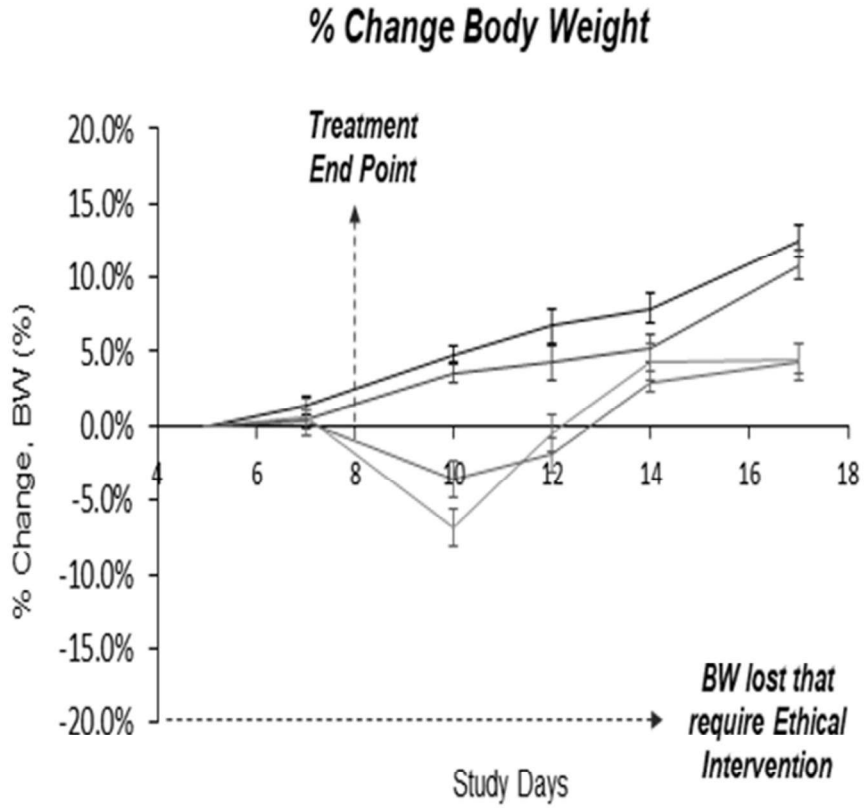


FIG. 21B

Candidates No.	Appearance	Protein concentration (mg/mL)	Osmolality (mOsmo/kg)	Tg' /℃	DSC				pH
					Tm onset (℃)	Tm1 (℃)	Tm2 (℃)	Tm3 (℃)	
F1	SY, SO,PF	31.1	313	-29.80	59.88	68.09	73.83	84.07	5.5
F2	SY, SO,PF	30.8	325	-29.52	63.77	73.5	86.04	NA	6.0
F3	SY, SO,PF	31.2	335	-29.88	63.47	73.88	85.32	NA	5.5
F4	SY, SO,PF	30.9	349	-30.11	60.11	67.4	74.04	83.03	5.5
SY (slightly yellow); SO (slightly opalescent liquid); FP (free of visible particle)									

FIG. 22A

Candidates No.	Appearance			pH			Protein concentration (mg/mL)		
	T0	40℃-1W	40℃-2W	T0	40℃-1W	40℃-2W	T0	40℃-1W	40℃-2W
F1	SY,SO,FP	SY,SO,FP	SY,SO,PO(5)	5.5	5.5	5.6	31.1	31.3	31.2
F2	SY,SO,FP	SY,SO,FP	SY,SO,FP	6.0	6.0	6.1	30.8	30.9	30.8
F3	SY,SO,FP	SY,SO,FP	SY,SO,FP	5.5	5.5	5.4	31.2	31.1	31.2
F4	SY,SO,FP	SY,SO,FP	SY,SO,FP	5.5	5.5	5.6	30.9	30.9	30.9
SY (slightly yellow); SO(slightly opalescent liquid); FP(free of visible particle), PO(particle observed)									

Candidates No.	MFI											
	T0				40℃-1W				40℃-2W			
	>=2 um	>=5 um	>=10 um	>=25 um	>=2 um	>=5 um	>=10 um	>=25 um	>=2 um	>=5 um	>=10 um	>=25 um
F1	1532	190	20	4	1781	187	15	0	3507	482	58	10
F2	561	76	23	2	3094	322	32	5	1717	118	19	0
F3	587	84	22	4	818	115	17	0	1591	210	32	4
F4	692	66	15	4	1560	171	10	0	2958	251	23	0

FIG. 22B

Candidates No.	SEC-UPLC % (MP/HMW/LMW)			Caliper-SDS-NR % (Purity)			
	T0	40℃-1W	40℃-2W	T0	40℃-1W	Normalization with T0 STD 40℃-1W	40℃-2W
F1	99.1/0.9/ND	98.2/1.6/0.2	95.8(↓3.3)/2.2(↑1.3)/2.0(↑2.0)	97.6	98.0	96.9	95.4(↓2.2)
F2	99.1/0.9/ND	98.0/1.8/0.2	95.6(↓3.5)/2.4(↑1.5)/2.0(↑2.0)	97.6	98.1	97.0	95.8(↓1.8)
F3	99.1/0.9/ND	98.0/1.7/0.2	95.8(↓3.3)/2.2(↑1.3)/2.1(↑2.1)	97.6	98.2	97.1	95.4(↓2.2)
F4	99.1/0.9/ND	98.2/1.6/0.2	95.9(↓3.2)/2.0(↑1.1)/2.1(↑2.1)	97.7	98.4	97.3	95.8(↓1.9)
NA				STD: 98.4	STD: 99.1	NA	STD: 98.3

Candidates No.	Caliper-SDS-R % (Purity/LC/HC)			cIEF pI			cIEF % Acidic/Main/Basic		
	T0	40℃-1W	40℃-2W	T0	40℃-1W	40℃-2W	T0	40℃-1W	40℃-2W
F1	99.2/30.4/68.8	99.3/31.5/67.8	98.4(↓0.8)/29.5/68.8	7.5	7.6	7.5	27.0/57.3/15.7	31.3/54.8/13.9	39.4(↑12.4)/48.2(↓9.1)/12.4(↓3.3)
F2	99.2/30.3/68.9	99.3/31.1/68.2	**98.8(↓0.4)/29.7/69.1**	7.6	7.6	7.5	28.3/57.0/14.7	32.5/54.5/13.0	40.7(↑12.4)/48.6(↓8.4)/10.7(↓4.0)
F3	98.9/30.6/68.3	99.2/30.6/68.6	98.3(↓0.6)/29.8/68.4	7.6	7.6	7.6	25.8/59.7/14.4	**37.6/50.1/12.2**	**47.1(↑21.3)/42.1(↓17.6)/10.7(↓3.7)**
F4	99.1/30.5/68.5	99.3/31.3/68.0	**98.8(↓0.4)/29.9/68.9**	7.6	7.6	7.6	25.5/57.9/16.6	32.2/53.9/13.9	38.7(↑13.2)/49.0(↓8.9)/12.3(↓4.3)

FIG. 22C

Candidates No.	Appearance			pH		Protein concentration (mg/mL)	
	T0	A-1 Day	A-3 Days	T0	A-3 Days	T0	A-3 Days
F1	SY,SO,FP	SY,SO,FP	SY,SO,FP	5.5	5.5	31.1	31.2
F2	SY,SO,FP	SY,SO,FP	SY,SO,PO+	6.0	6.1	30.8	30.8
F3	SY,SO,FP	SY,SO,FP	SY,SO,PO+	5.5	5.5	31.2	31.1
F4	SY,SO,FP	SY,SO,FP	SY,SO,FP	5.5	5.5	30.9	31.1

- SY (slightly yellow); SO(slightly opalescent liquid); FP(free of visible particle), PO(particles observed)/+(a few visible particles)
- Few visible particles were observed in F2/F3 samples for agitation 3 days.

Candidates No.	MFI							
	T0				A-3 Days			
	>=2 um	>=5 um	>=10 um	>=25 um	>=2 um	>=5 um	>=10 um	>=25 um
F1	1532	190	20	4	2077	110	12	2
F2	561	76	23	2	644	146	20	7
F3	587	84	22	4	313	55	9	2
F4	692	66	15	4	1856	77	4	0

FIG. 22D

Candidates No.	SEC-UPLC % (MP/HMW/LMW)		Caliper-SDS-NR % (Purity)		Caliper-SDS-R % (Purity/LC/HC)		pI		cIEF % Acidic/Main/Basic	
	T0	A-3 Days	T0	A-3 Days	T0	A-3 Days	T0	A-3 Days	T0	A-3 Days
F1	99.1/0.9/ND	99.0/1.0/ND	97.6	97.7	99.2/30.4/68.8	99.2/30.9/68.3	7.5	7.6	27.0/57.3/15.7	28.3/56.3/15.3
F2	99.1/0.9/ND	99.0/1.0/ND	97.6	97.6	99.2/30.3/68.9	99.2/31.1/68.1	7.6	7.6	28.3/57.0/14.7	27.2/57.4/15.4
F3	99.1/0.9/ND	99.0/1.0/ND	97.6	97.5	98.9/30.6/68.3	99.0/30.7/68.4	7.6	7.6	25.8/59.7/14.4	22.9/61.8/15.3
F4	99.1/0.9/ND	99.1/0.9/ND	97.7	97.5	99.1/30.5/68.5	99.2/30.8/68.4	7.6	7.6	25.5/57.9/16.6	24.5/60.1/15.5

FIG. 22E

Candidates No.	Appearance			pH		Protein concentration (mg/mL)	
	T0	FT-3C	FT-5C	T0	FT-5C	T0	FT-5C
F1	SY,SO,FP	SY,SO,FP	SY,SO,FP	5.5	5.6	31.1	31.3
F2	SY,SO,FP	SY,SO,FP	SY,SO,FP	6.0	6.1	30.8	30.9
F3	SY,SO,FP	SY,SO,FP	SY,SO,FP	5.5	5.6	31.2	31.1
F4	SY,SO,FP	SY,SO,FP	SY,SO,FP	5.5	5.6	30.9	31.0

SY (slightly yellow); SO(slightly opalescent liquid); FP(free of visible particle)

Candidates No.	MFI							
	T0				FT-5C			
	>=2 um	>=5 um	>=10 um	>=25 um	>=2 um	>=5 um	>=10 um	>=25 um
F1	1532	190	20	4	770	76	4	0
F2	561	76	23	2	469	46	4	0
F3	587	84	22	4	439	30	9	0
F4	692	66	15	4	670	44	14	2

FIG. 22F

Candidates No.	SEC-UPLC % (MP/HMW/LMW)		Caliper-SDS-NR % (Purity)		Caliper-SDS-R % (Purity/LC/HC)		pI		cIEF % Acidic/Main/Basic	
	T0	FT-5C	T0	FT-5C	T0	FT-5C	T0	FT-5C	T0	FT-5C
F1	99.1/0.9/ND	99.1/0.9/ND	97.6	97.7	99.2/30.4/68.8	99.2/30.4/68.8	7.5	7.6	27.0/57.3/15.7	22.3/60.5/17.2
F2	99.1/0.9/ND	99.1/0.9/ND	97.6	97.6	99.2/30.3/68.9	99.2/30.9/68.4	7.6	7.6	28.3/57.0/14.7	20.7/63.5/15.8
F3	99.1/0.9/ND	99.1/0.9/ND	97.6	97.5	98.9/30.6/68.3	99.2/30.6/68.6	7.6	7.6	25.8/59.7/14.4	21.8/62.8/15.4
F4	99.1/0.9/ND	99.1/0.9/ND	97.7	97.6	99.1/30.5/68.5	99.1/30.3/68.8	7.6	7.6	25.5/57.9/16.6	24.4/60.1/15.5

FIG. 22G

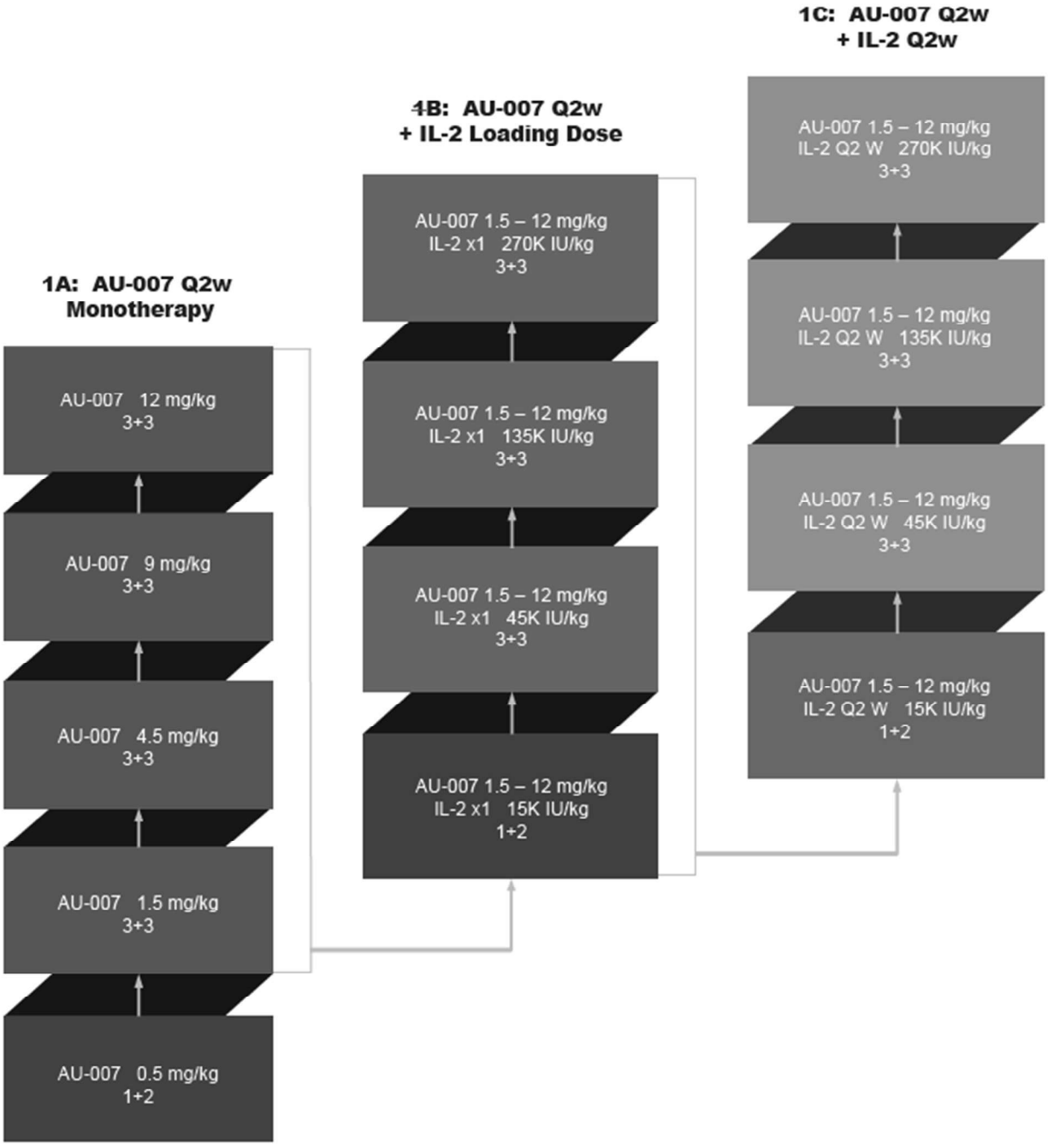

1A: AU-007 Q2w
Monotherapy
AU-007 12 mg/kg
3+3
AU-007 9 mg/kg
3+3
AU-007 4.5 mg/kg
3+3
AU-007 1.5 mg/kg
3+3
AU-007 0.5 mg/kg
1+2
1B: AU-007 Q2w
+ IL-2 Loading Dose
AU-007 1.5 – 12 mg/kg
IL-2 x1 270K IU/kg
3+3
AU-007 1.5 – 12 mg/kg
IL-2 x1 135K IU/kg
3+3
AU-007 1.5 – 12 mg/kg
IL-2 x1 45K IU/kg
3+3
AU-007 1.5 – 12 mg/kg
IL-2 x1 15K IU/kg
1+2
1C: AU-007 Q2w
+ IL-2 Q2w
AU-007 1.5 – 12 mg/kg
IL-2 Q2 W 270K IU/kg
3+3
AU-007 1.5 – 12 mg/kg
IL-2 Q2 W 135K IU/kg
3+3
AU-007 1.5 – 12 mg/kg
IL-2 Q2 W 45K IU/kg
3+3
AU-007 1.5 – 12 mg/kg
IL-2 Q2 W 15K IU/kg
1+2

FIG. 23

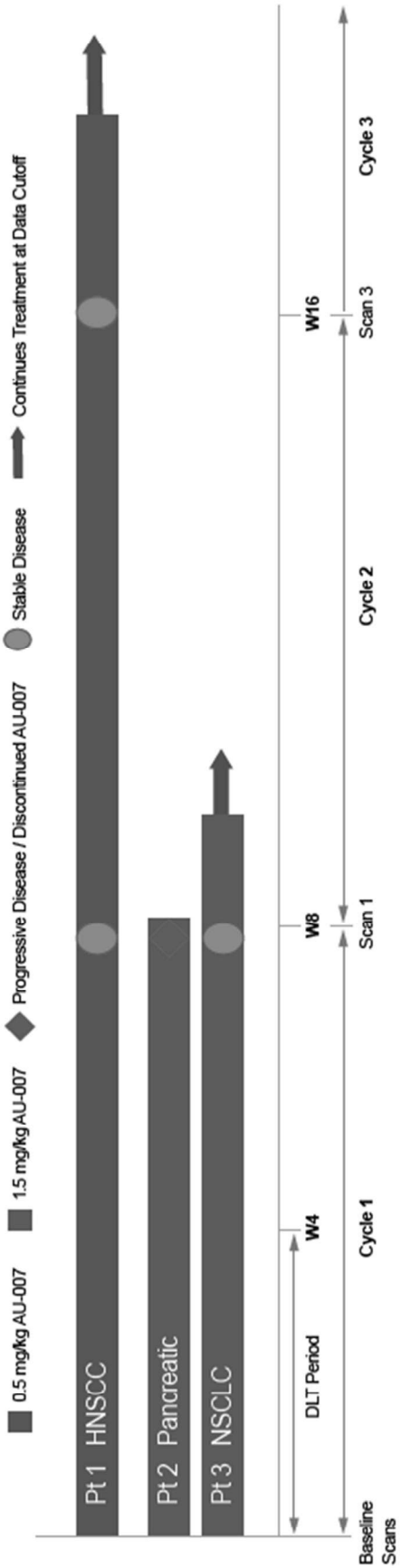

0.5 mg/kg AU-007
1.5 mg/kg AU-007
Progressive Disease / Discontinued AU-007
Stable Disease
Continues Treatment at Data Cutoff
Pt 1 HNSCC
Pt 2 Pancreatic
Pt 3 NSCLC
DLT Period
W4
W8
W16
Baseline Scans
Cycle 1
Scan 1
Cycle 2
Scan 3
Cycle 3

FIG. 24

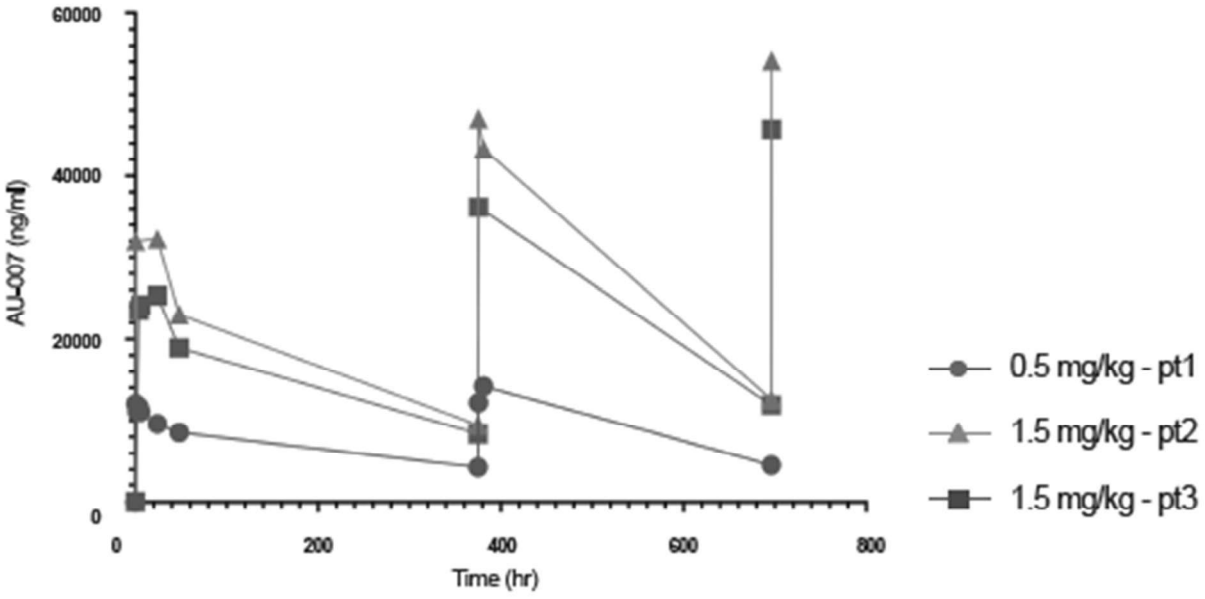


FIG. 25

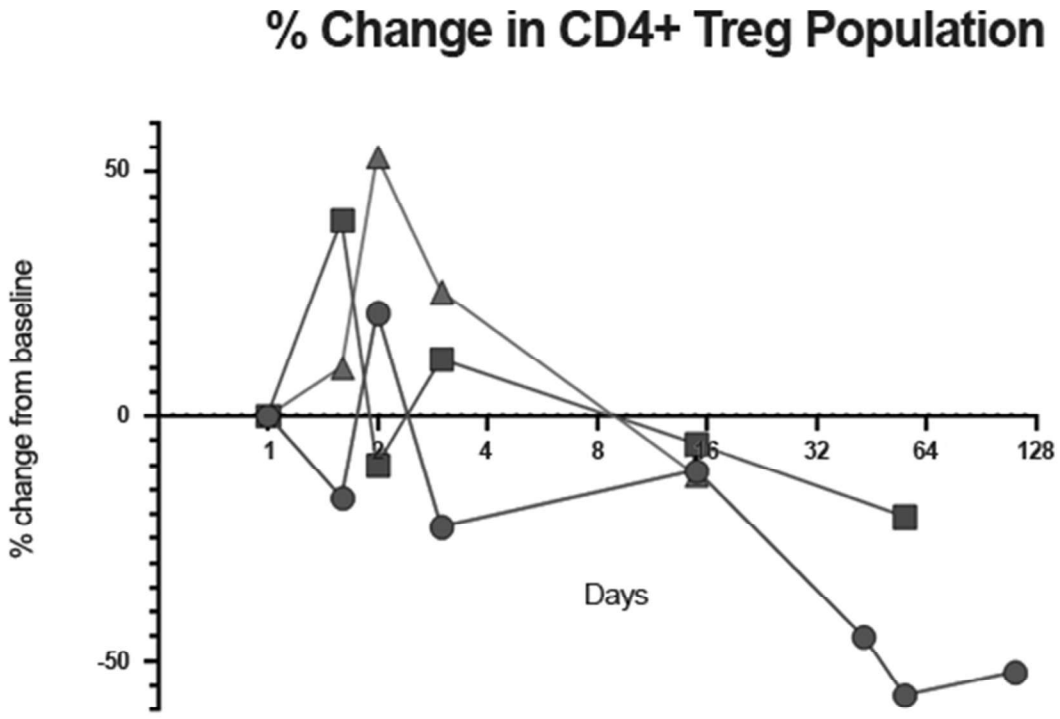


FIG. 26A

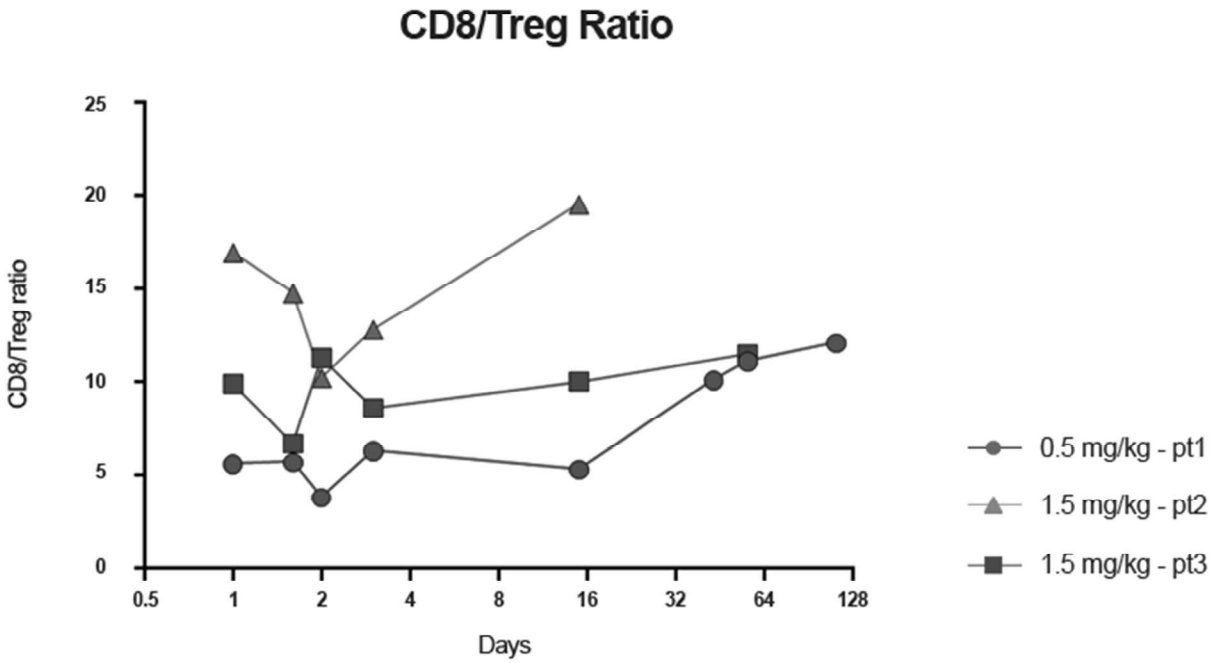


FIG. 26B

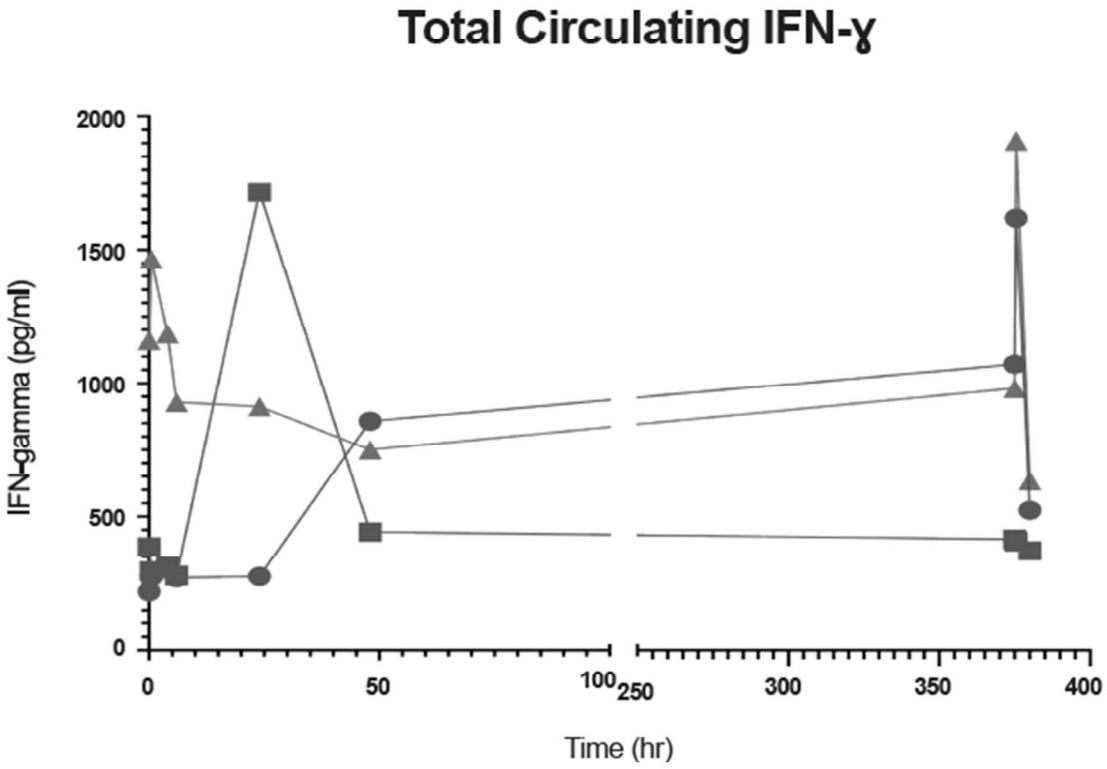


FIG. 26C

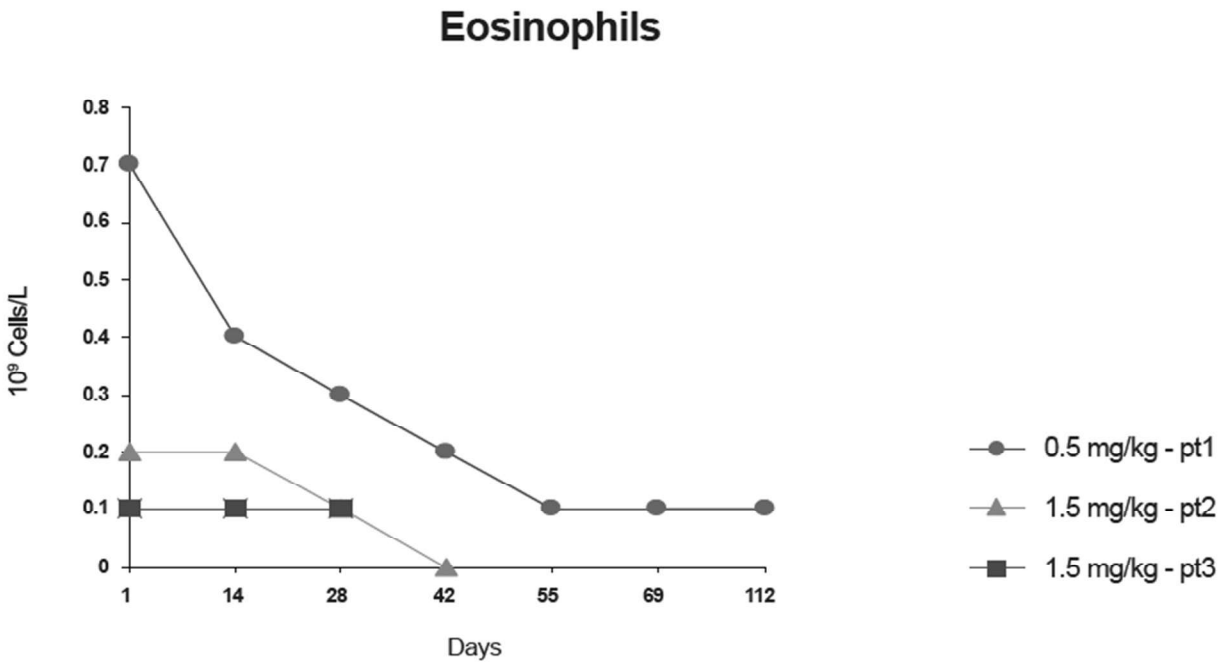
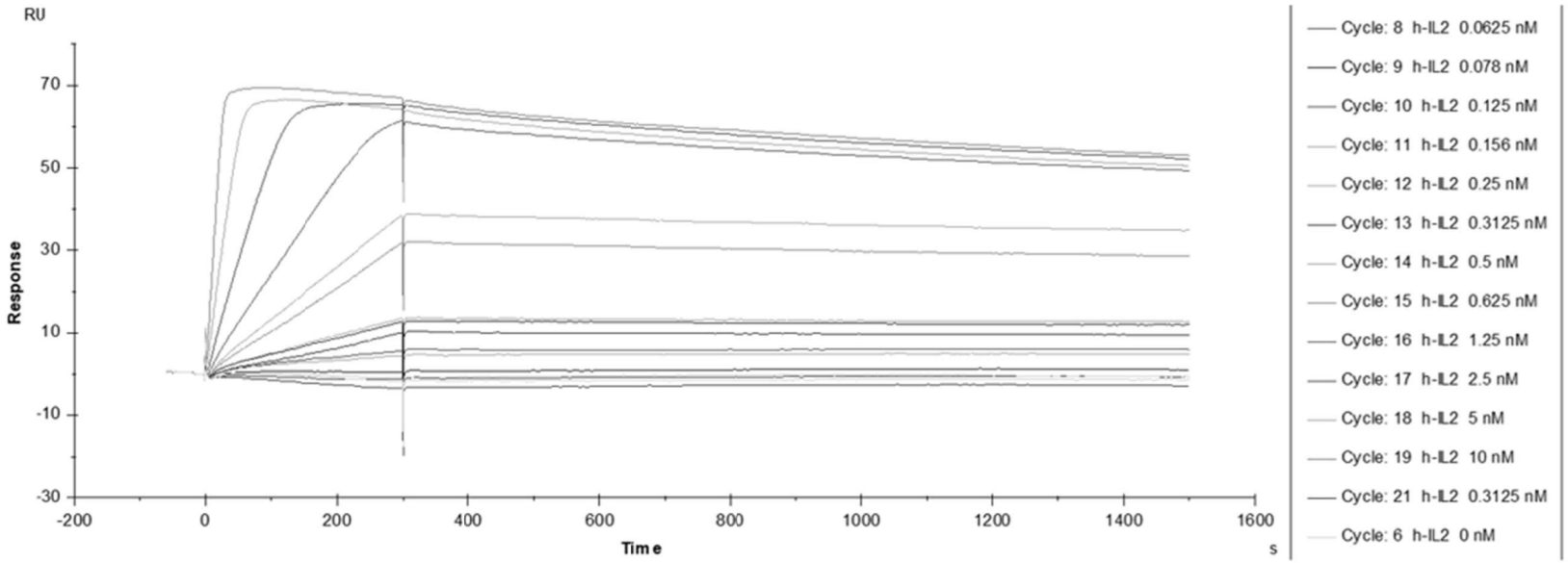
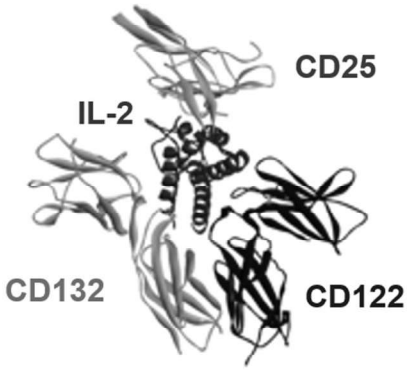

Eosinophils
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10⁹ Cells/L
1
14
28
42
55
69
112
Days
0.5 mg/kg - pt1
1.5 mg/kg - pt2
1.5 mg/kg - pt3

FIG. 26D

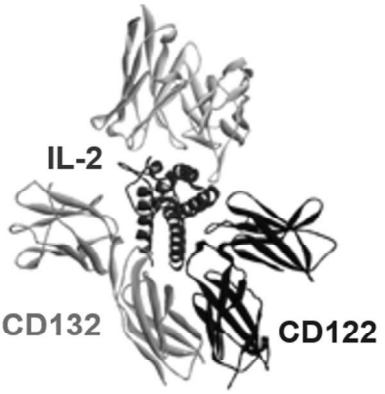


KD (M)	1.08E-11
Koff (1/s)	4.87E-04
ka (1/Ms)	4.50E+07
Chi² (RU²)	0.1

FIG. 27A



Human IL-2-Trimeric
Receptor Complex



Illustrative Model of AU-007 (yellow)
Blocking the Access of IL-2 to CD25

FIG. 27B

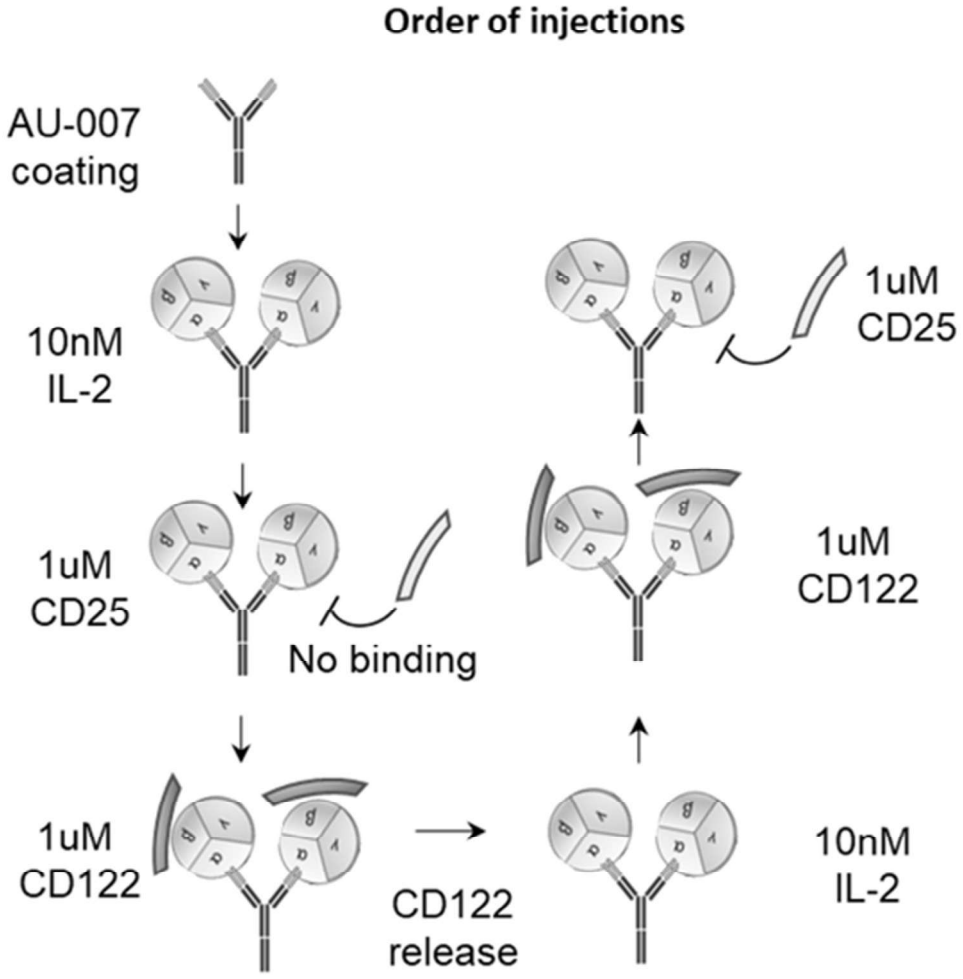


FIG. 27C

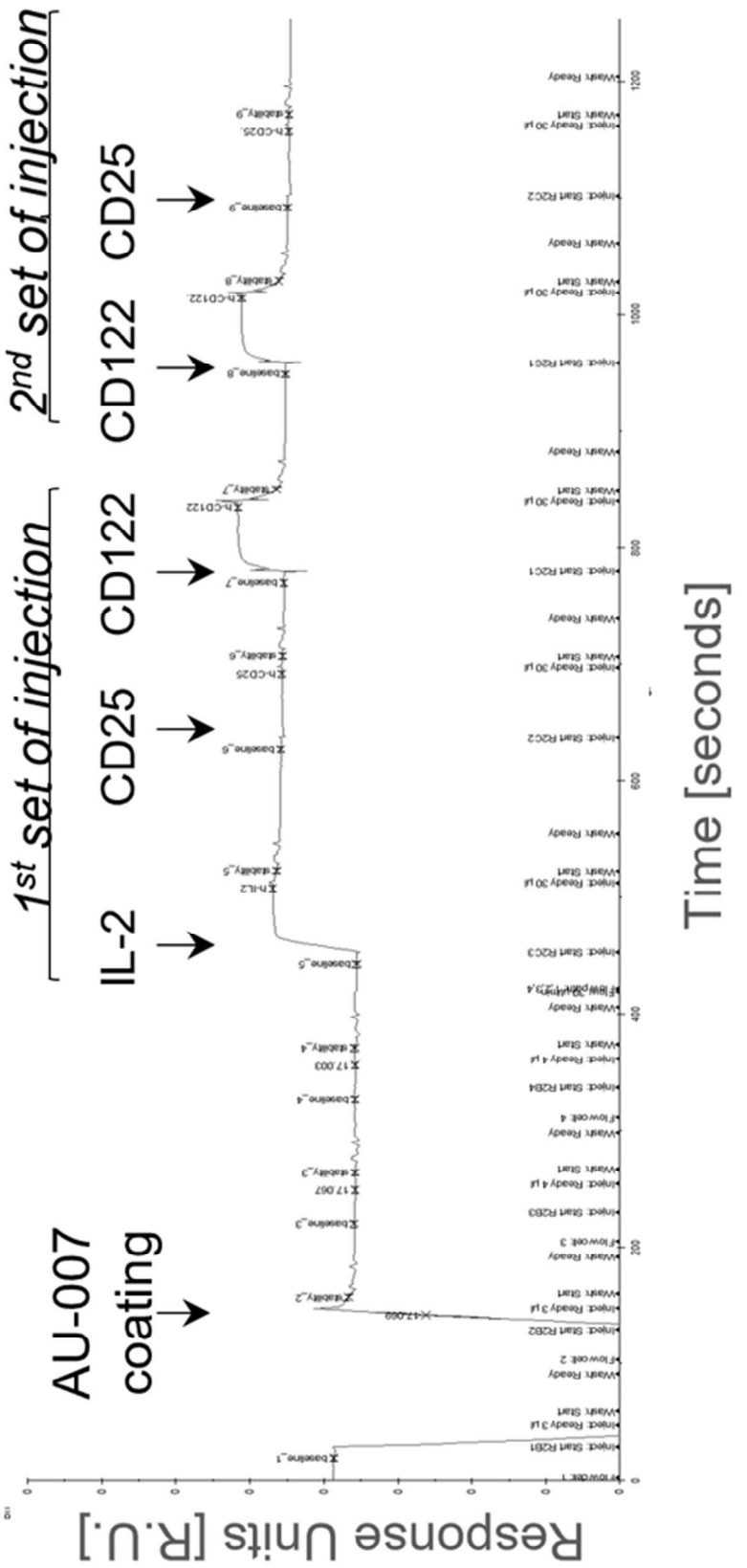

Response Units [R.U.]
Time [seconds]
AU-007 coating
1st set of injection
IL-2
CD25
CD122
2nd set of injection
CD122
CD25

FIG. 27D

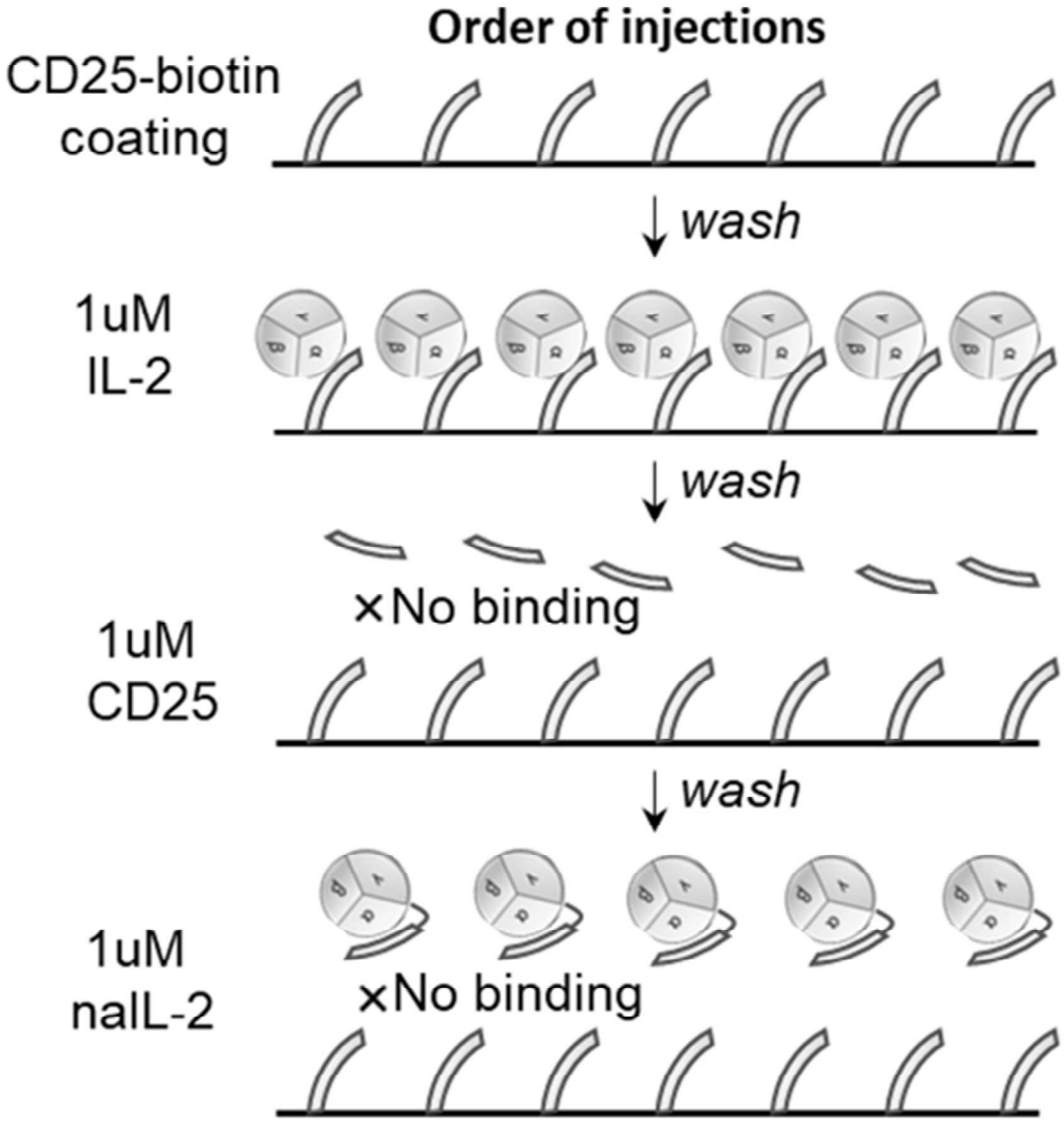

Order of injections
CD25-biotin coating
wash
1uM IL-2
wash
×No binding
1uM CD25
wash
1uM nalL-2
×No binding

FIG. 27E

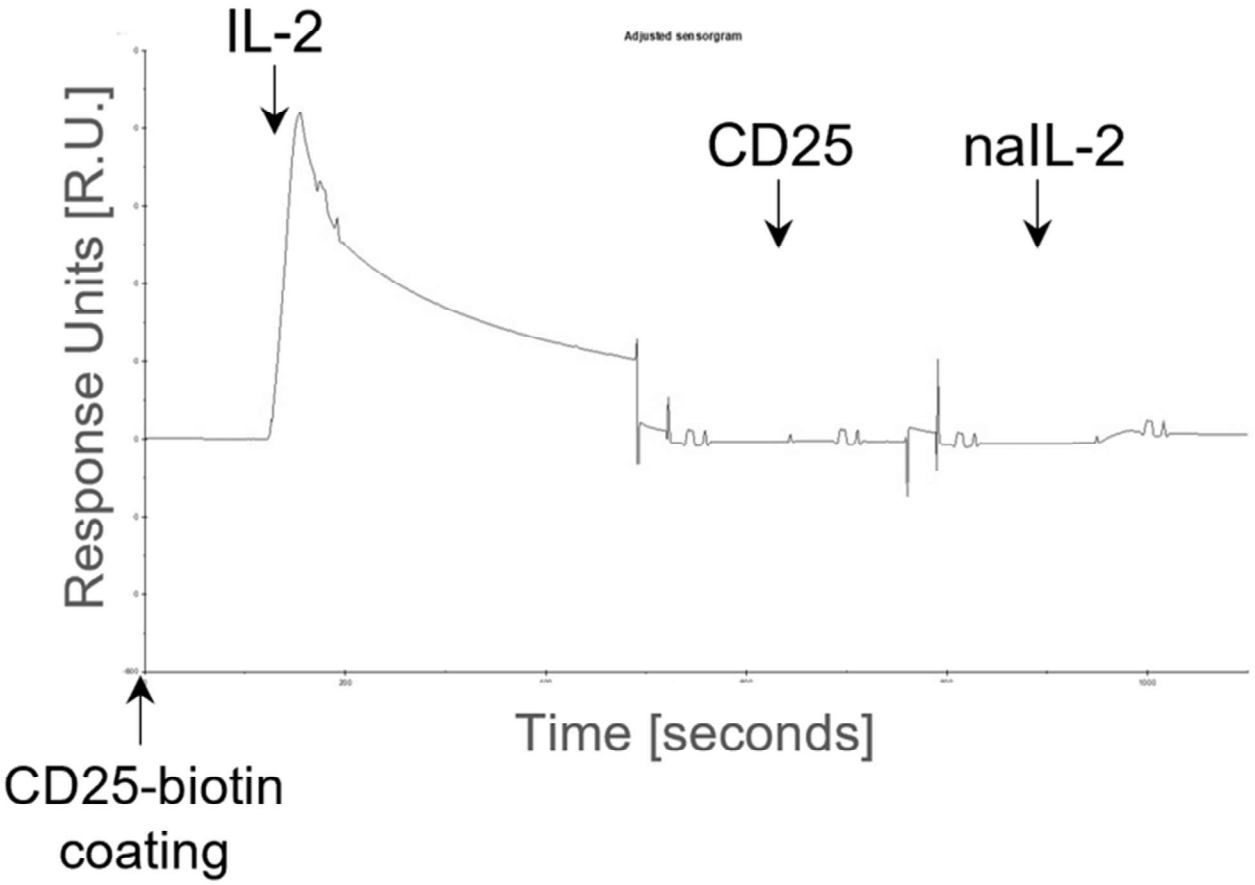

IL-2
Adjusted sensorgram
CD25
naIL-2
Response Units [R.U.]
Time [seconds]
CD25-biotin
coating

FIG. 27F

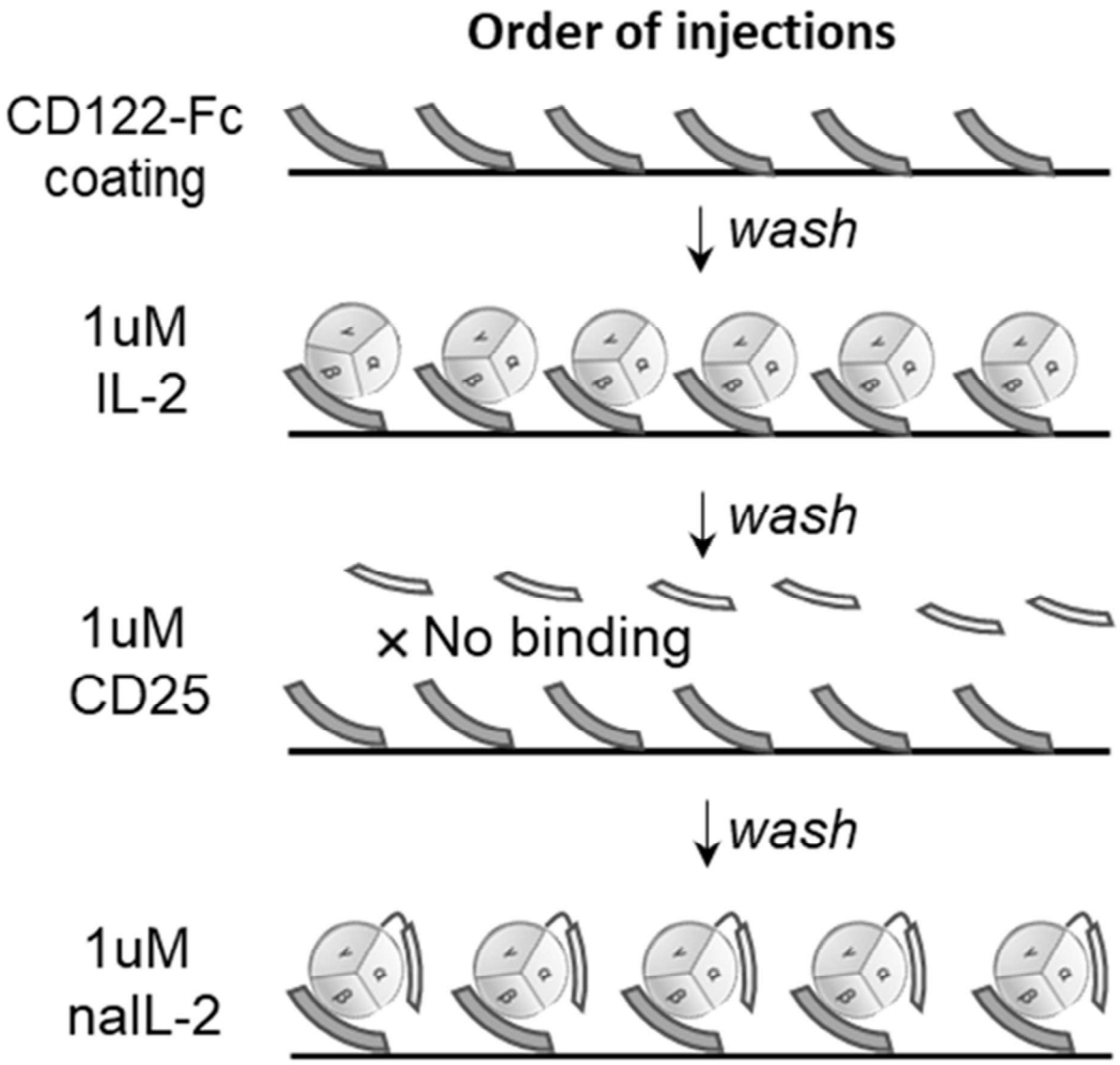

Order of injections
CD122-Fc coating
wash
1uM IL-2
wash
1uM CD25
× No binding
wash
1uM nalL-2

FIG. 27G

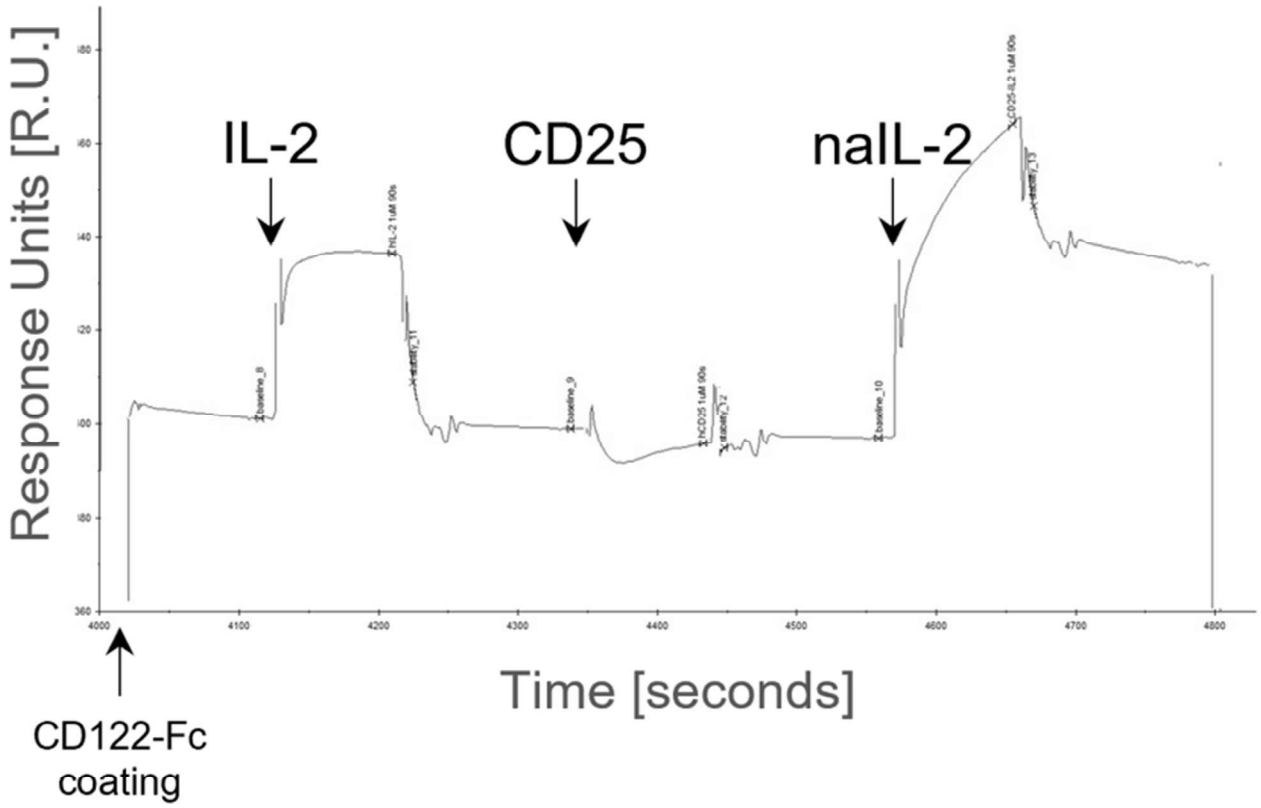

IL-2
CD25
naIL-2
Response Units [R.U.]
Time [seconds]
CD122-Fc
coating

FIG. 27H

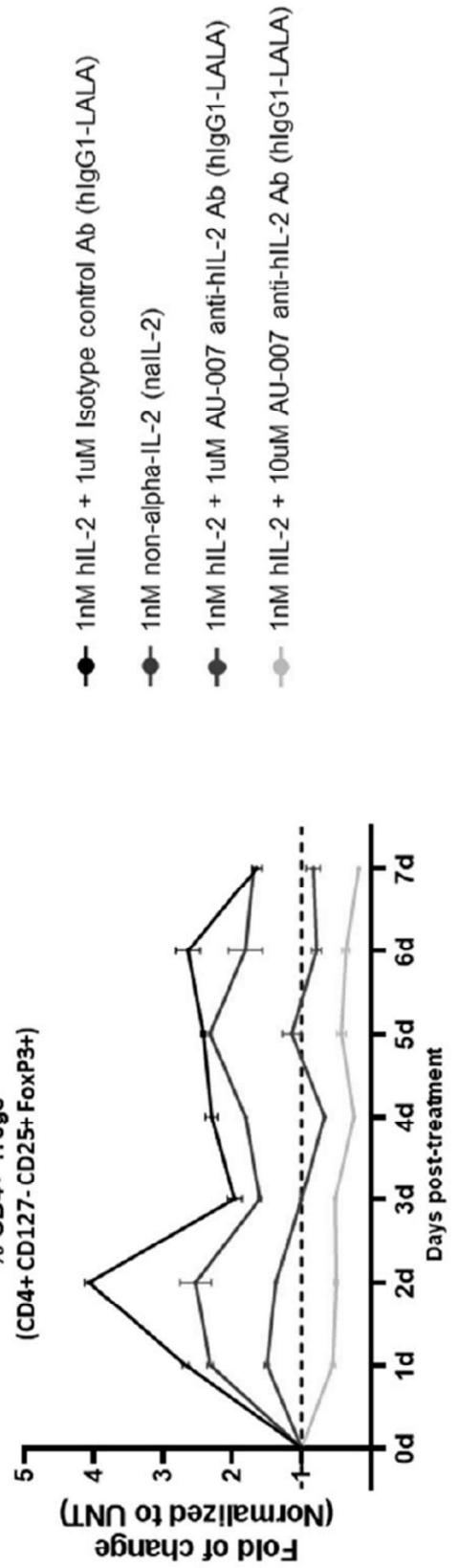

1nM hIL-2 + 1uM Isotype control Ab (hIgG1-LALA)
1nM non-alpha-IL-2 (nalL-2)
1nM hIL-2 + 1uM AU-007 anti-hIL-2 Ab (hIgG1-LALA)
1nM hIL-2 + 10uM AU-007 anti-hIL-2 Ab (hIgG1-LALA)
% CD4+ Tregs
(CD4+ CD127- CD25+ FoxP3+)
Fold of change
(Normalized to UNT)
5
4
3
2
1
0d
1d
2d
3d
4d
5d
6d
7d
Days post-treatment

FIG. 28A

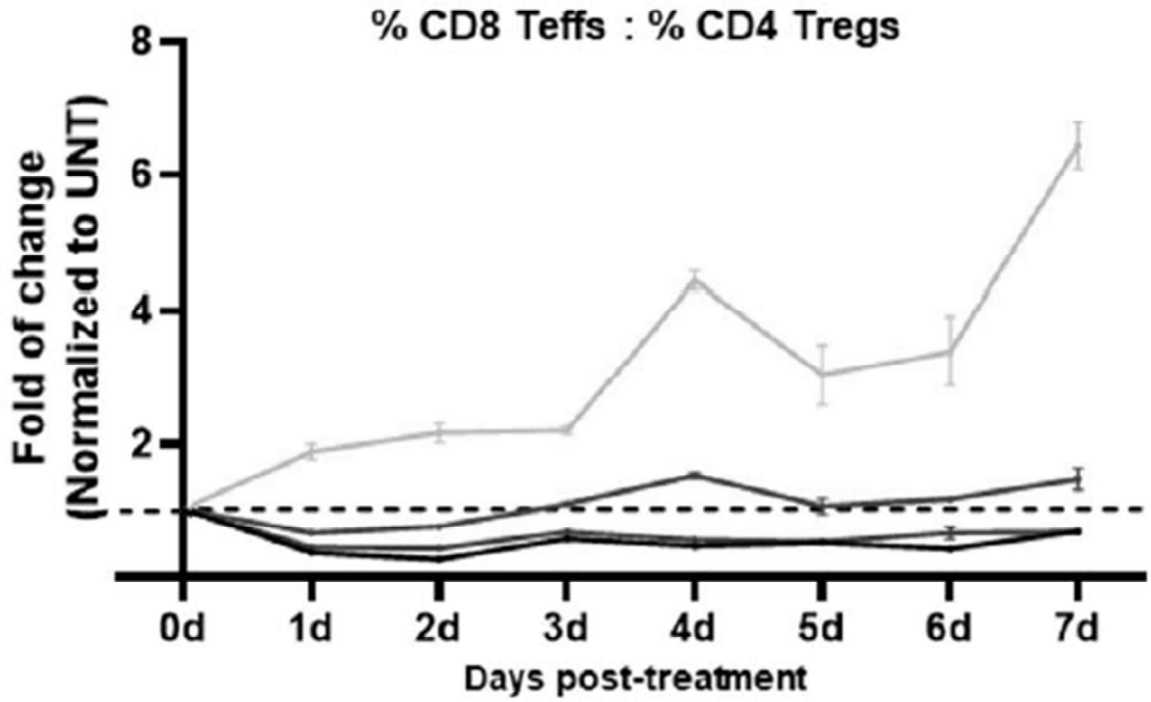


FIG. 28B

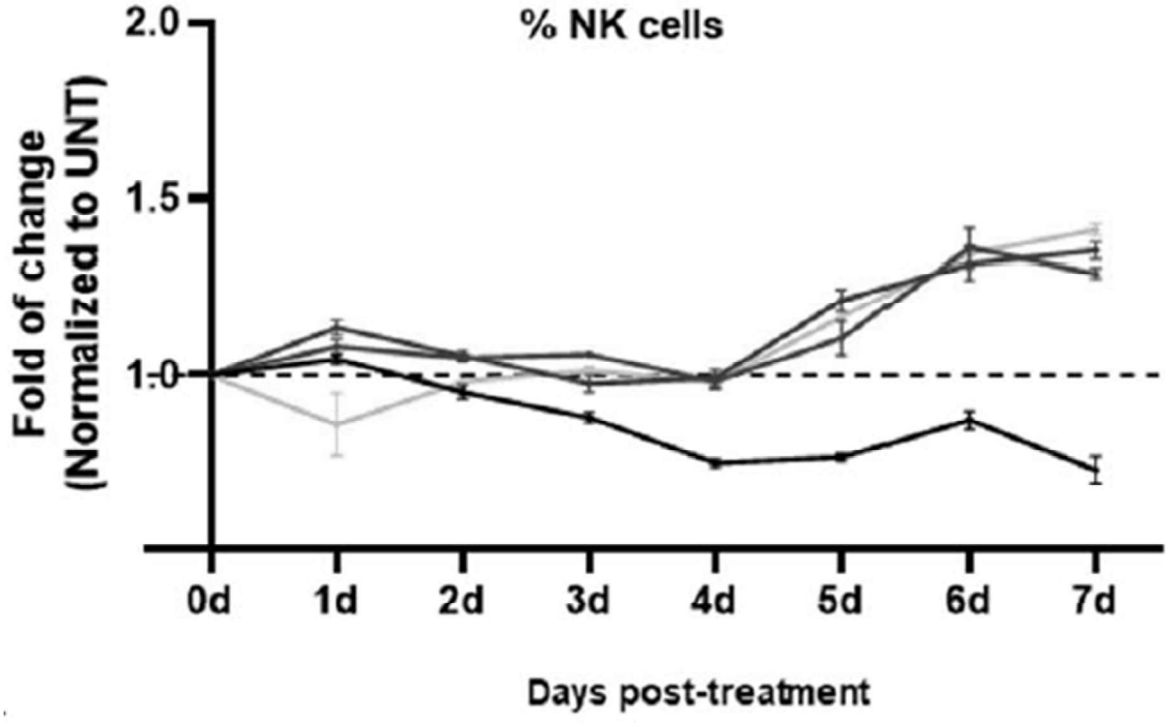


FIG. 28C

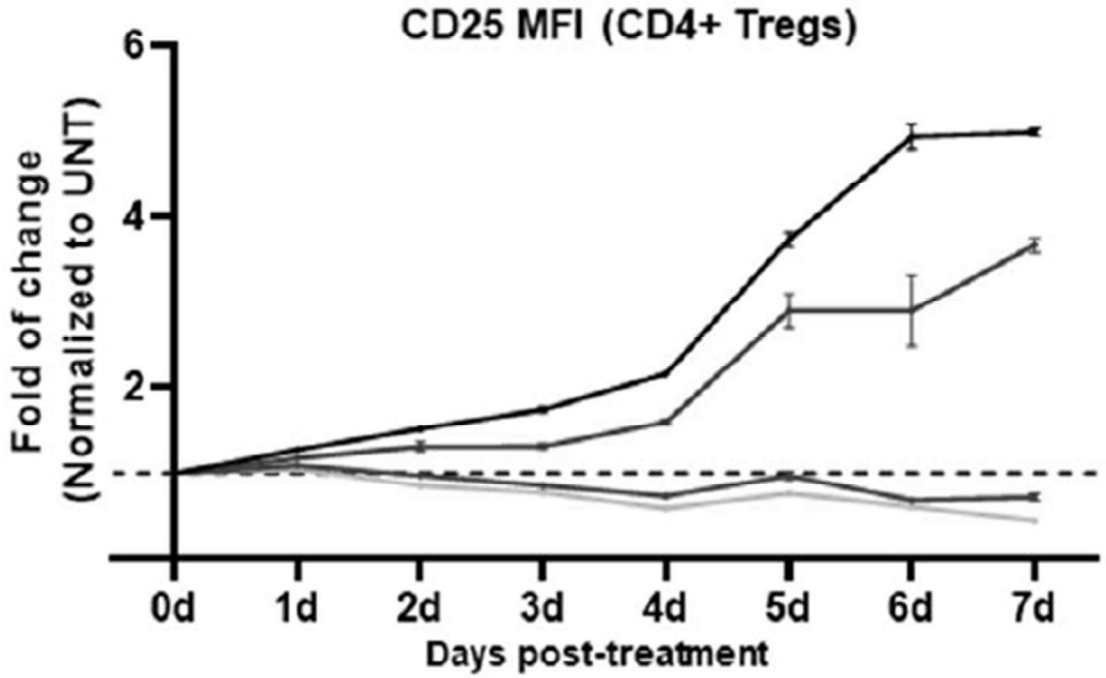


FIG. 28D

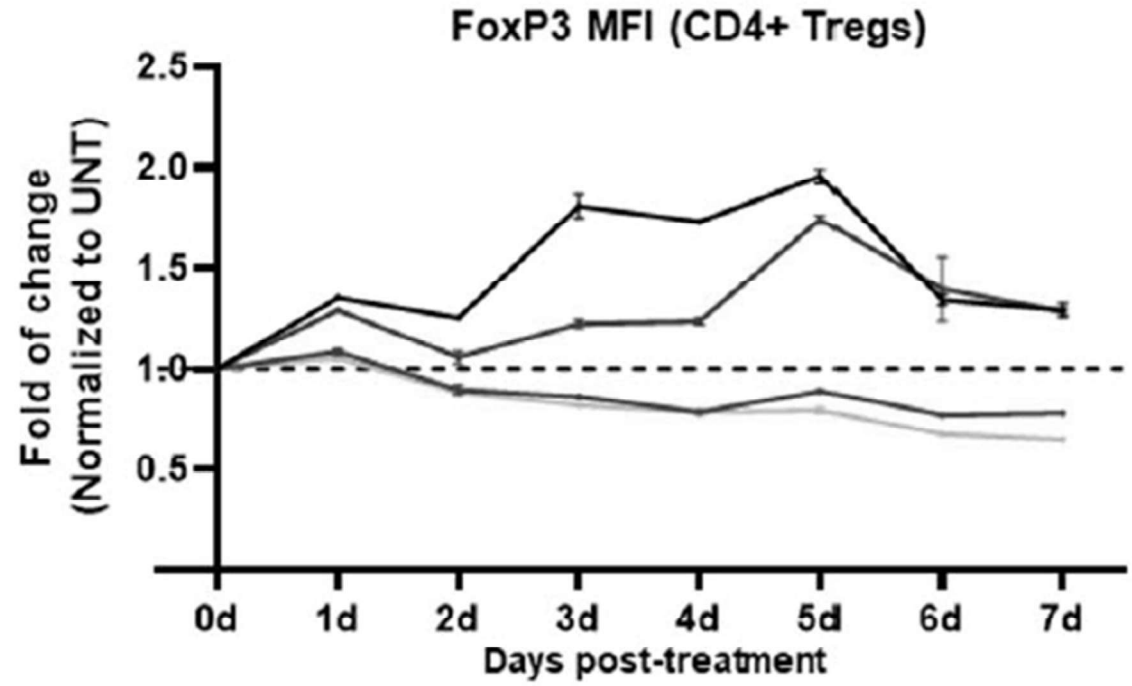


FIG. 28E

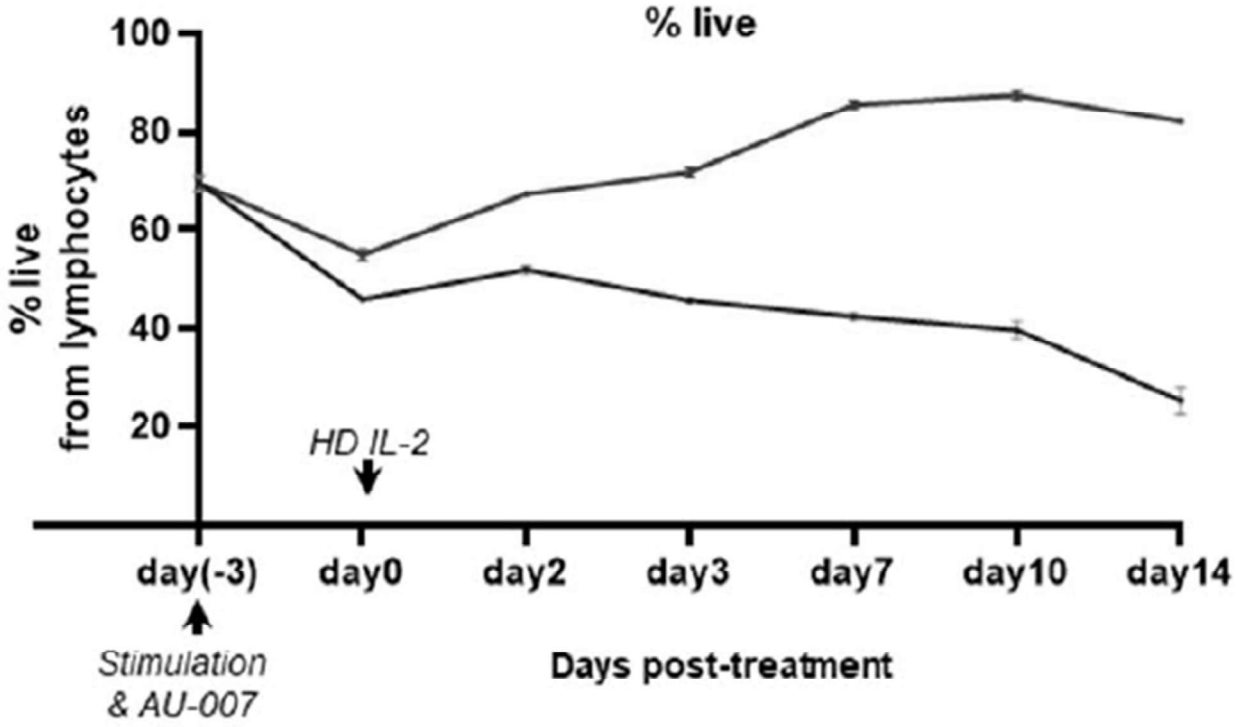


FIG. 28F

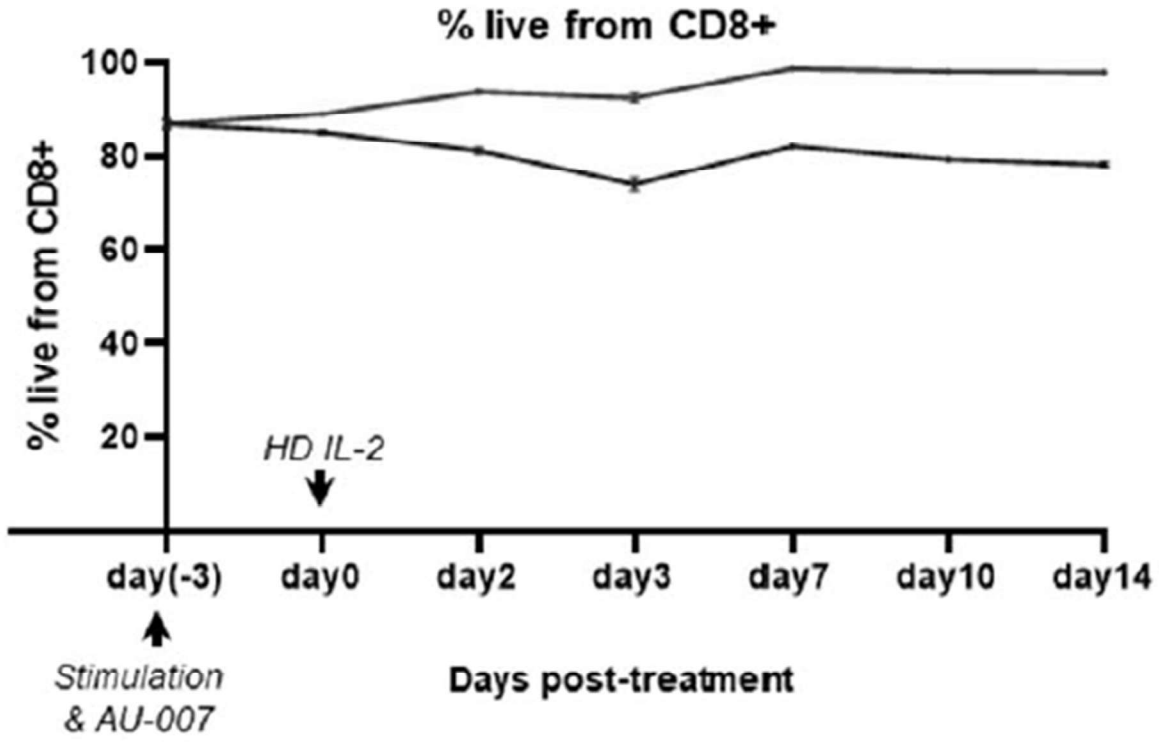


FIG. 28G

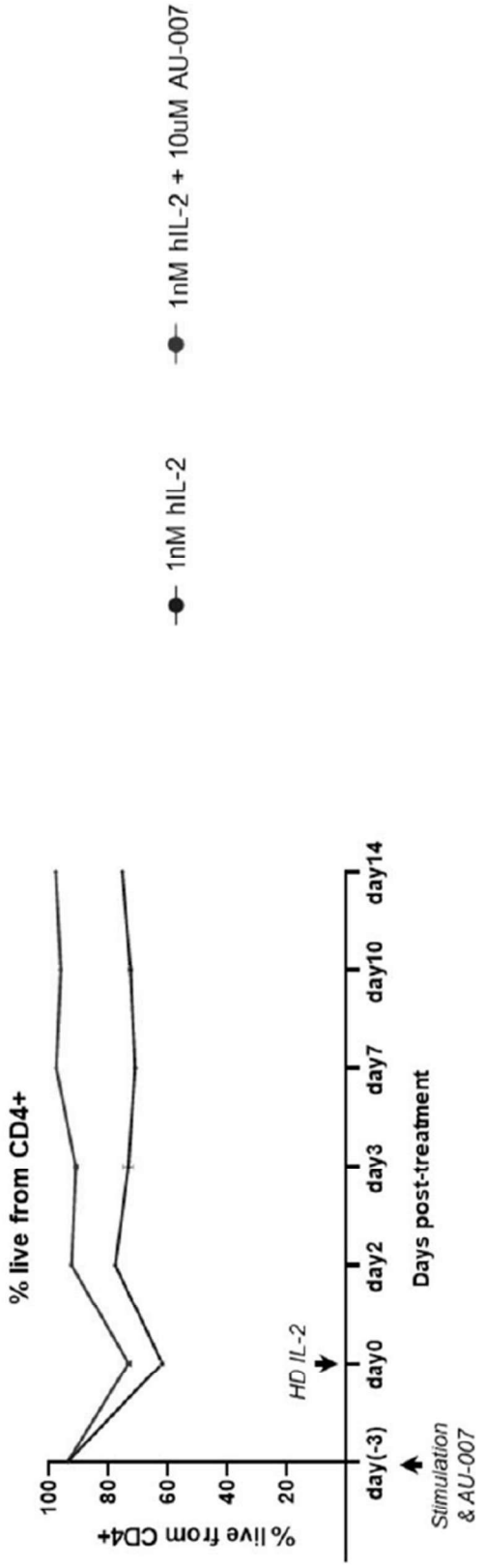

% live from CD4+
100
80
60
40
20
% live from CD4+
HD IL-2
day(-3)
day0
day2
day3
day7
day10
day14
Days post-treatment
Stimulation & AU-007
1nM hIL-2
1nM hIL-2 + 10uM AU-007

FIG. 28H

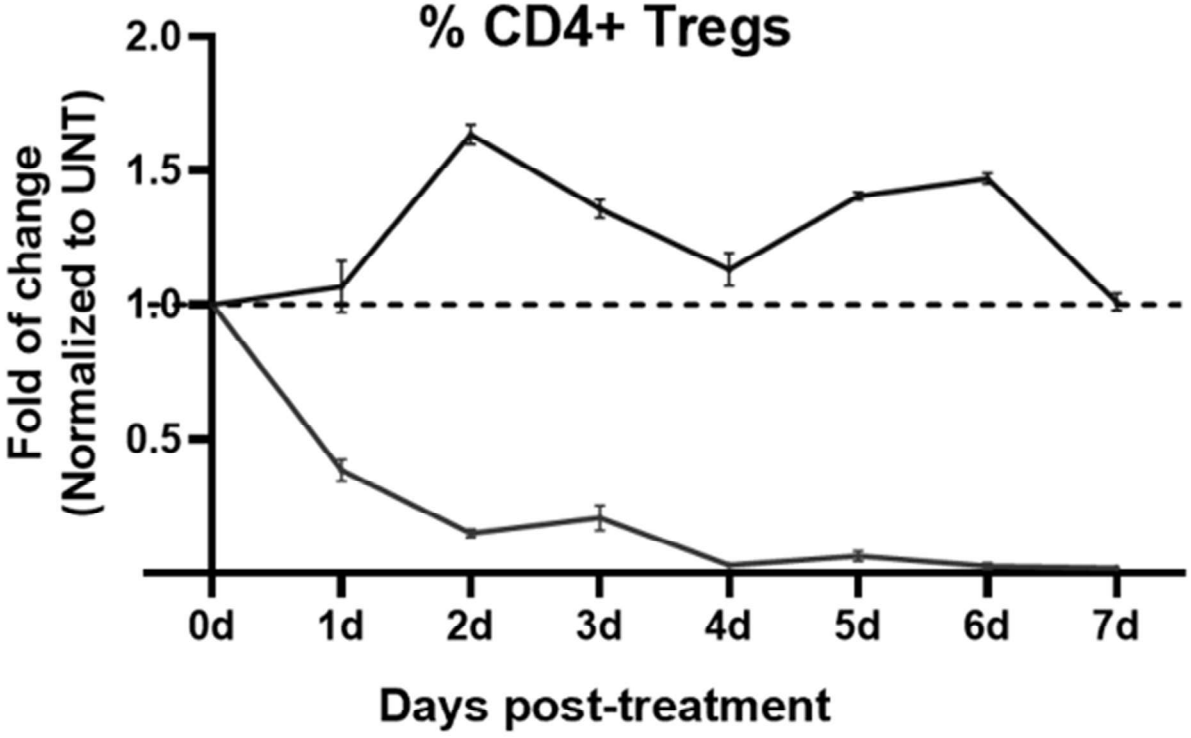


FIG. 29A

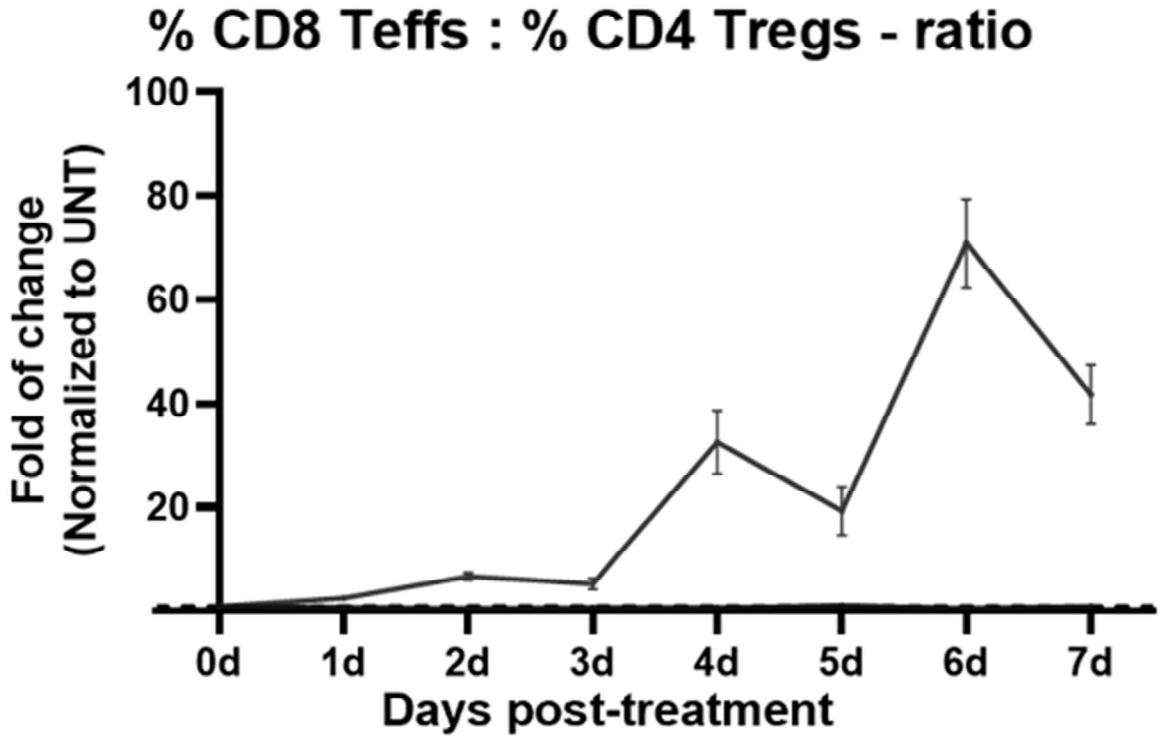


FIG. 29B

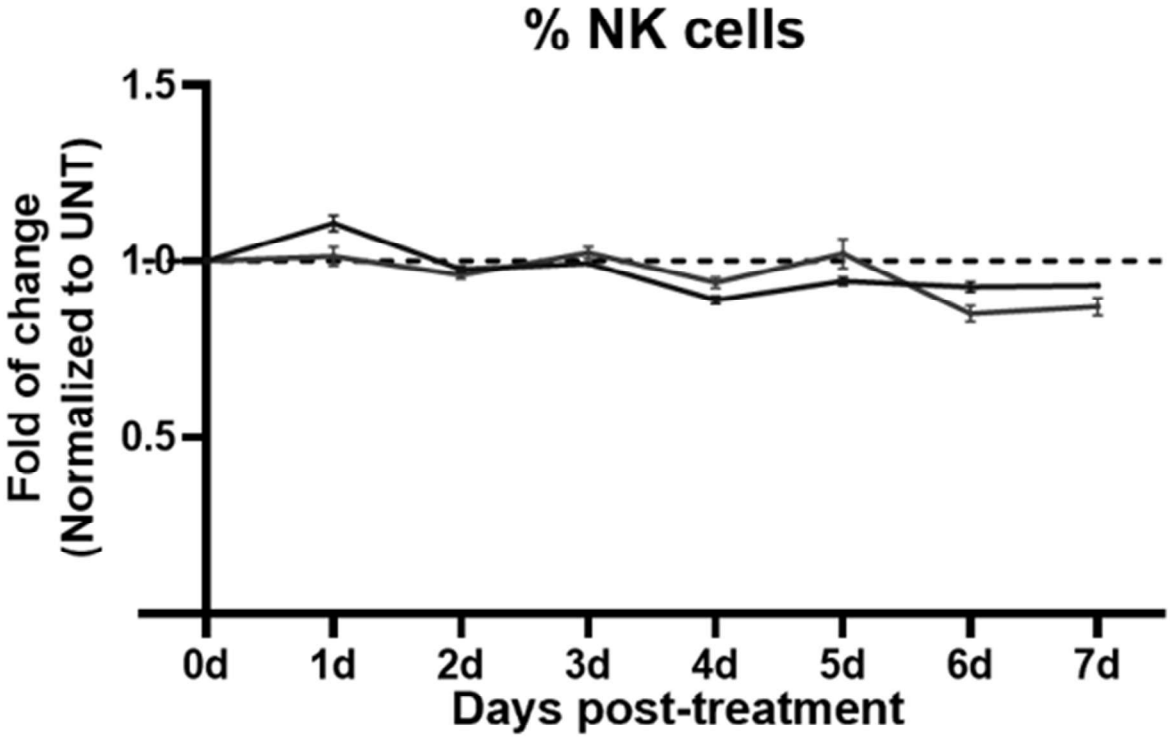


FIG. 29C

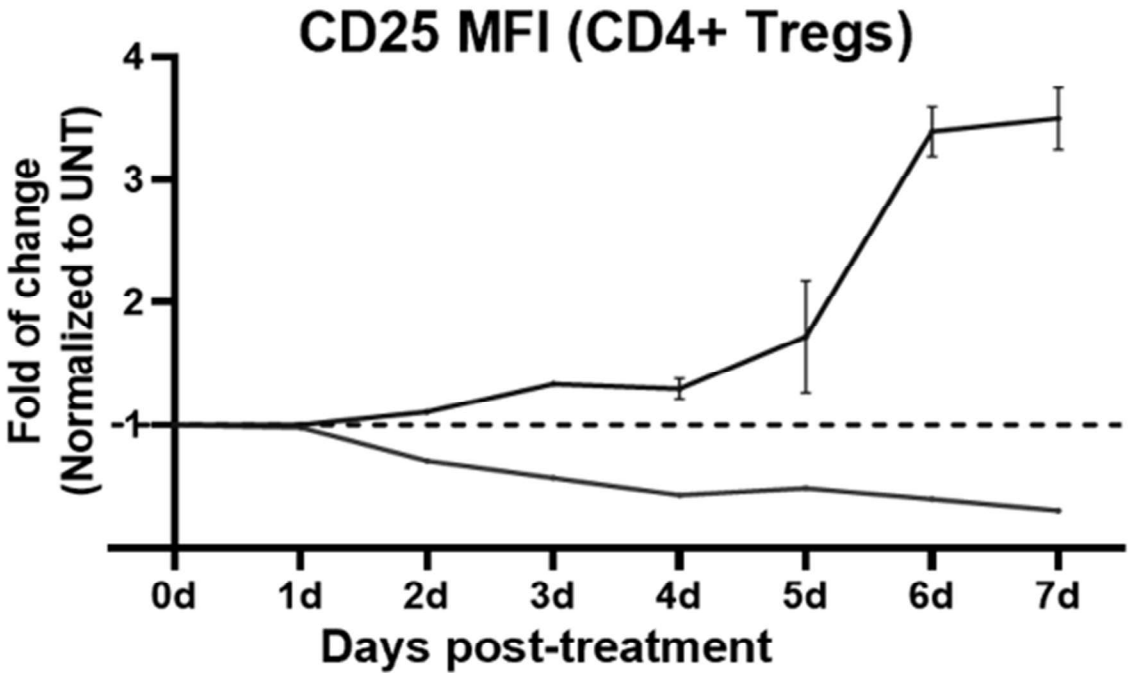


FIG. 29D

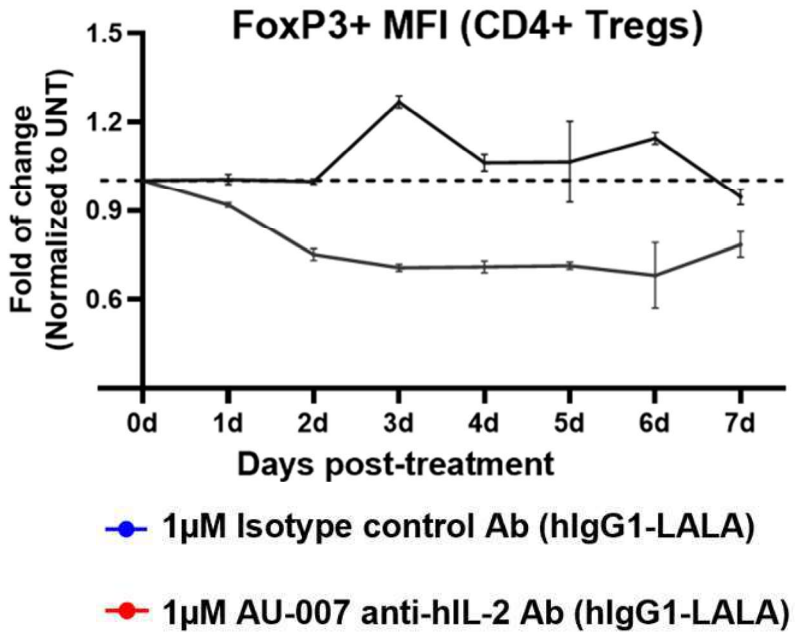


FIG. 29E

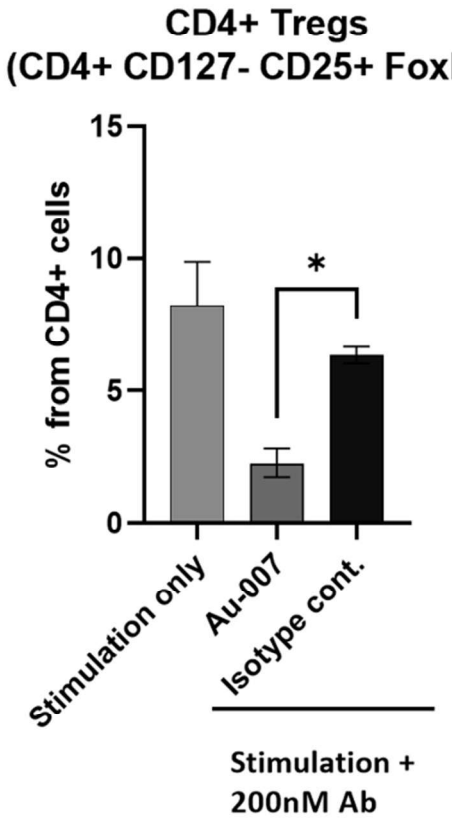


FIG. 29F

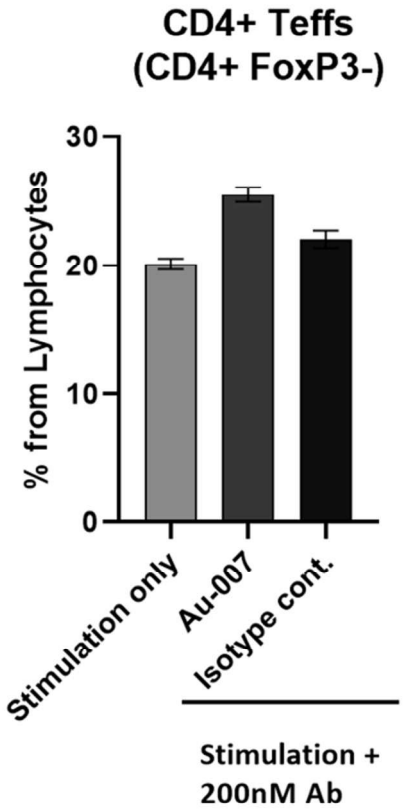


FIG. 29G

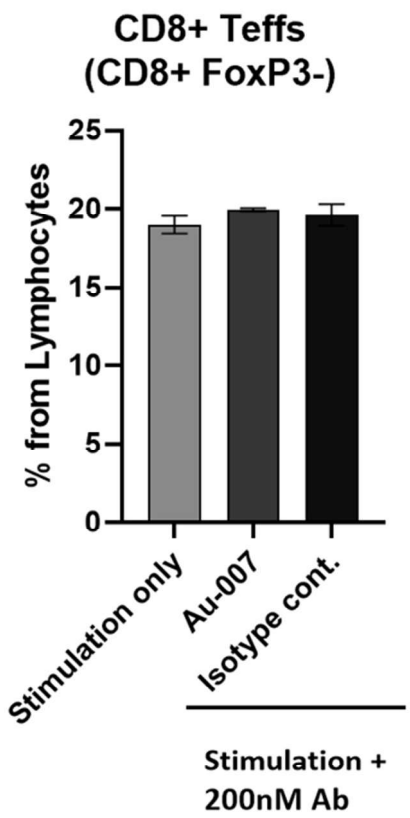


FIG. 29H

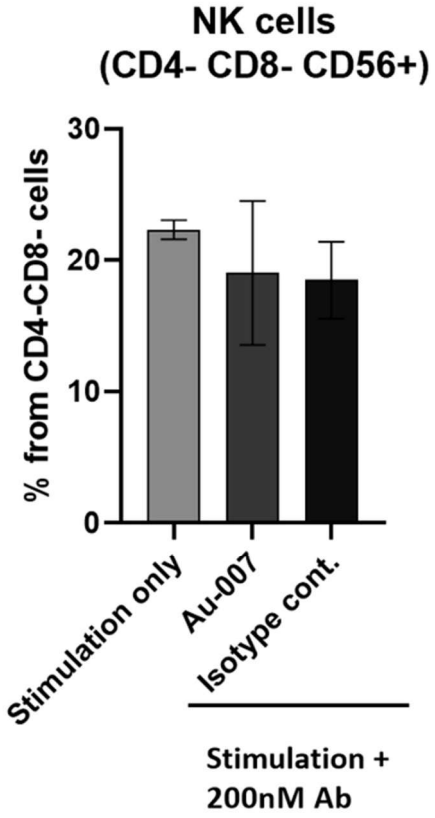


FIG. 29I

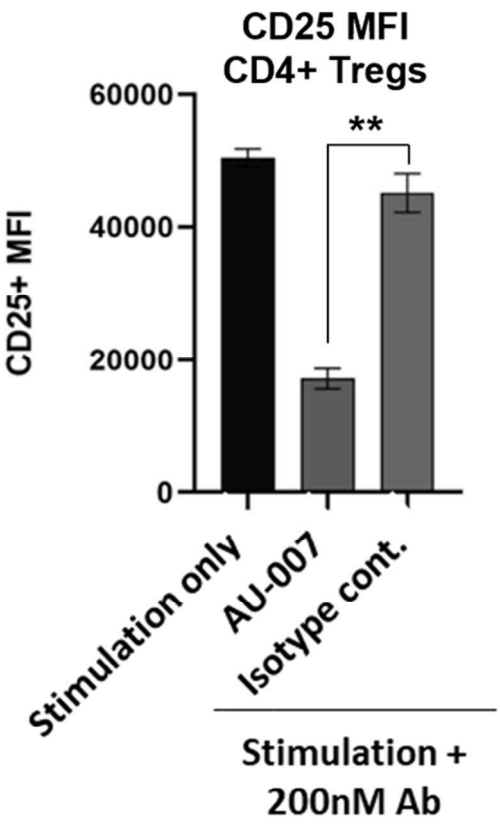


FIG. 29J

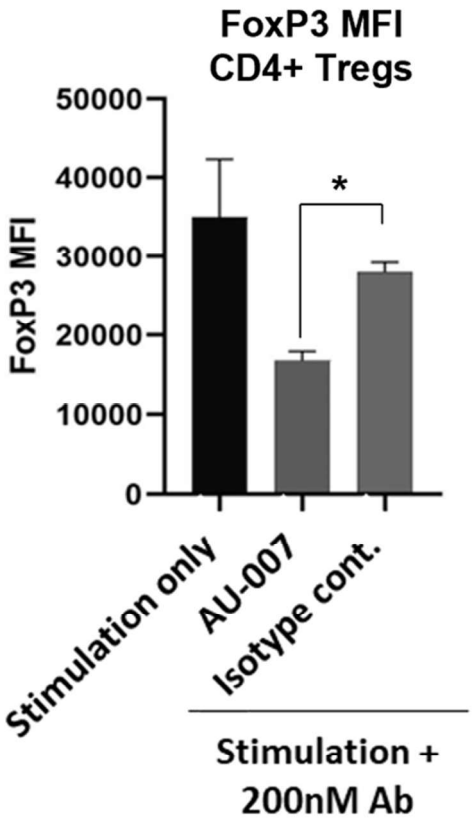


FIG. 29K

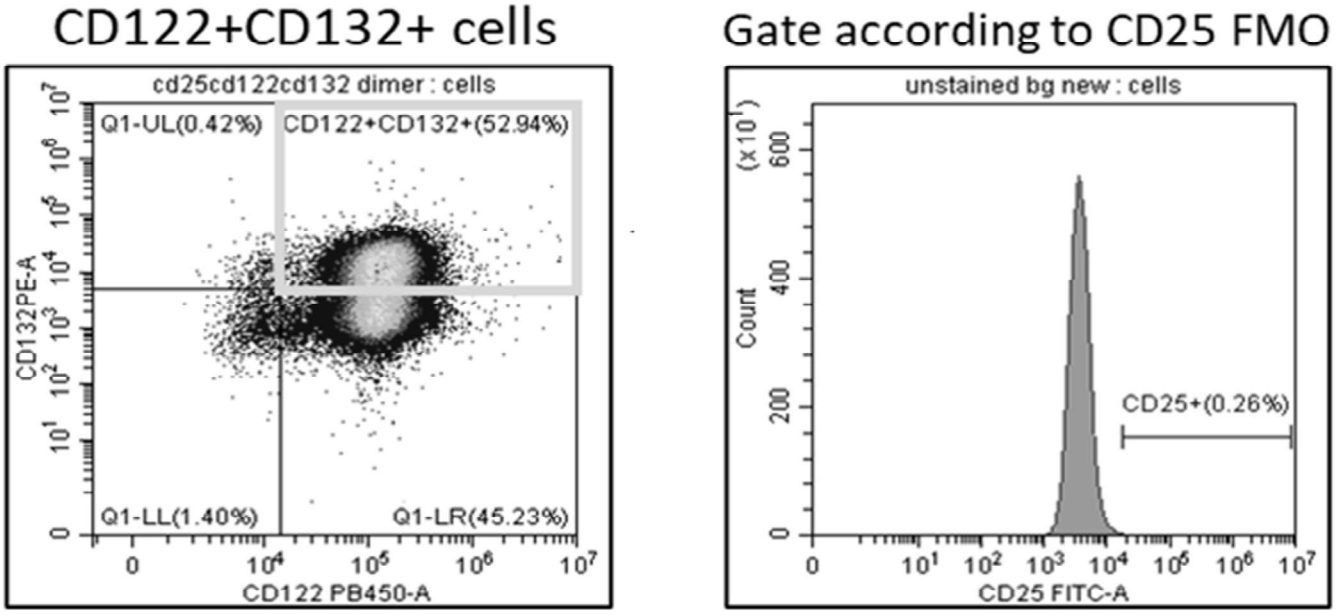


FIG. 30A

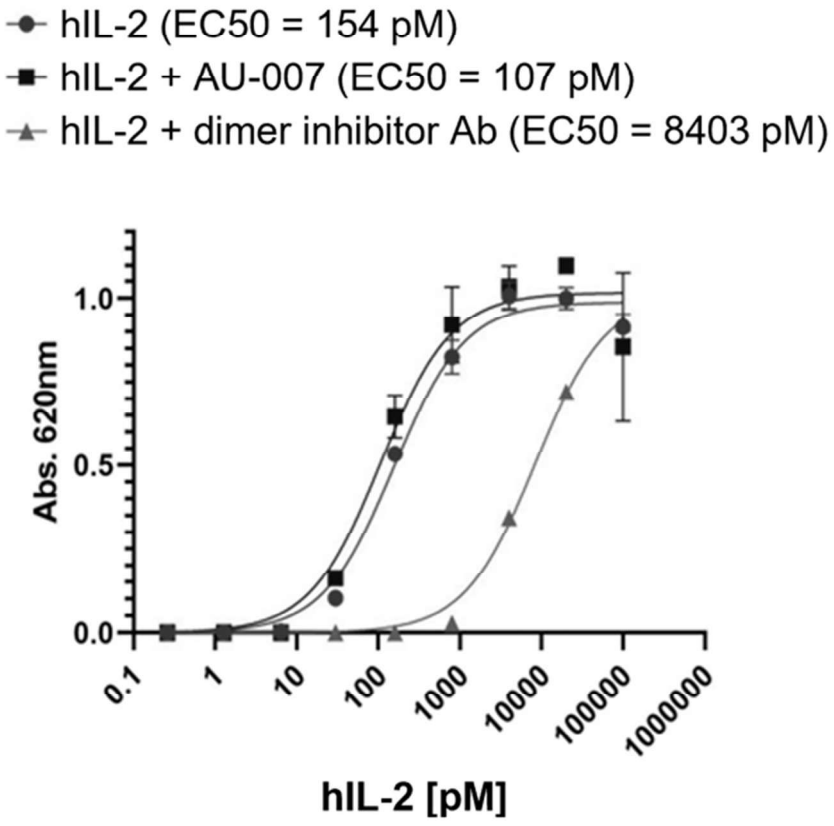


FIG. 30B

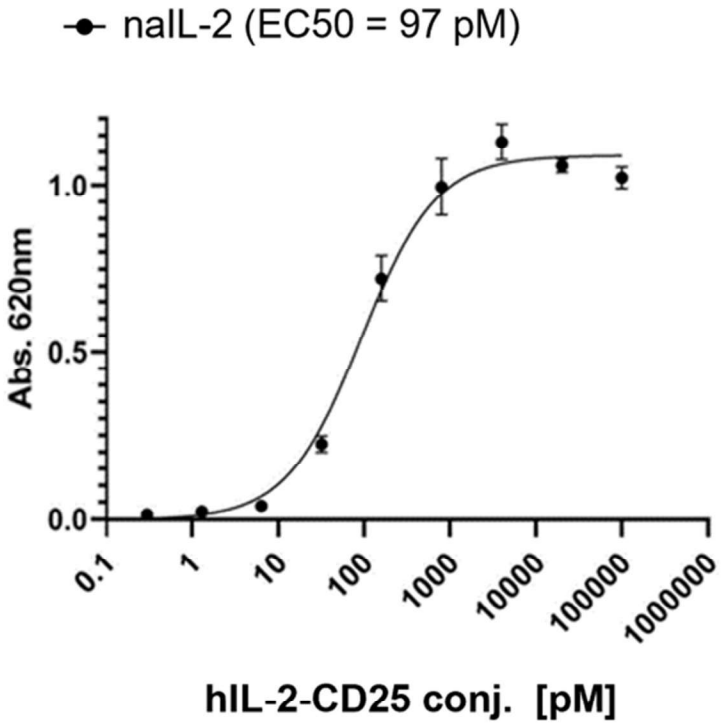


FIG. 30C

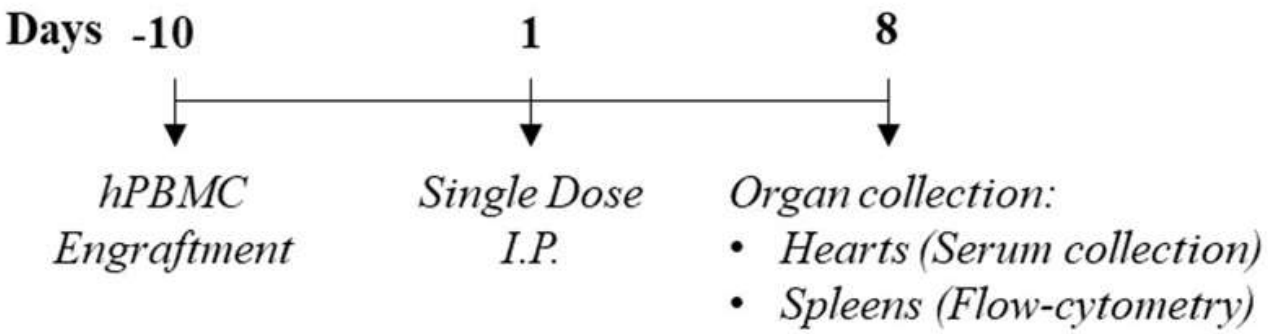


FIG. 31A

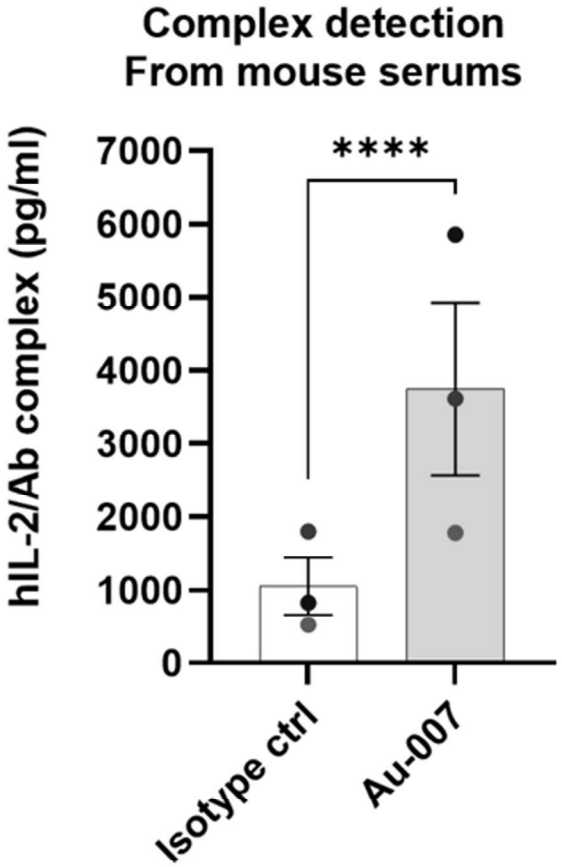


FIG. 31B

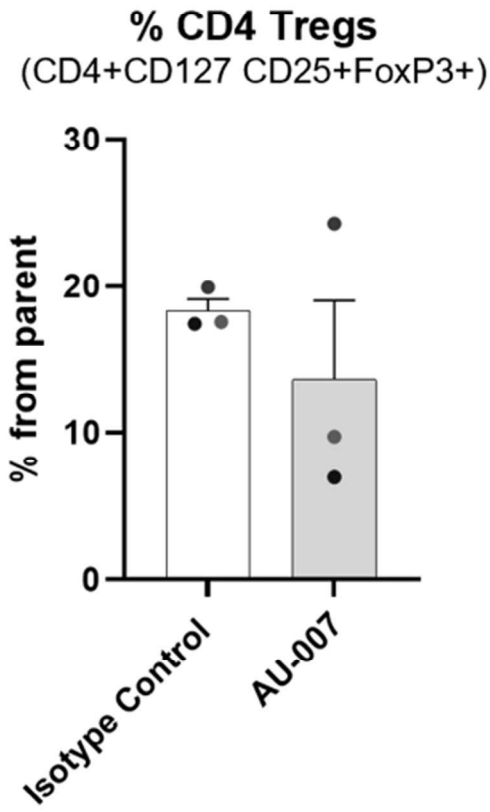


FIG. 31C

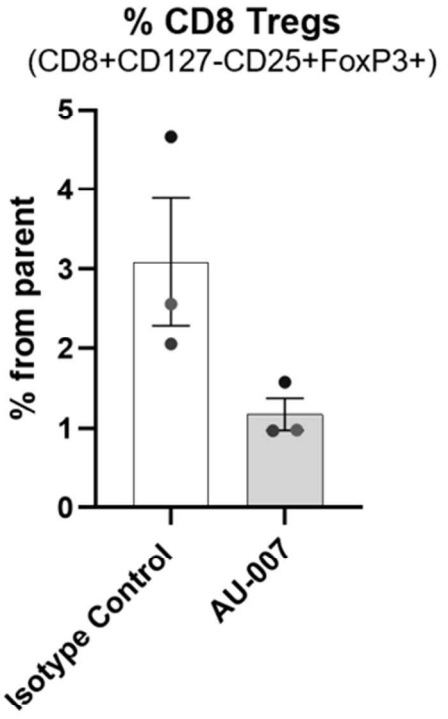


FIG. 31D

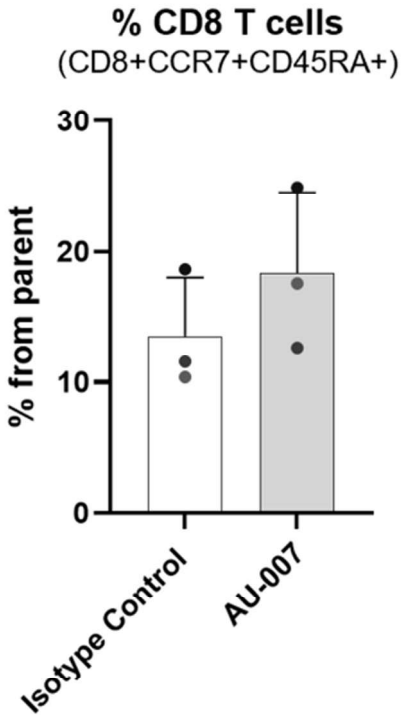


FIG. 31E

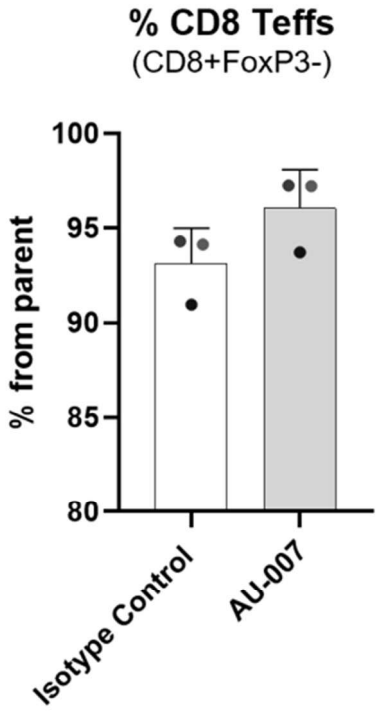


FIG. 31F

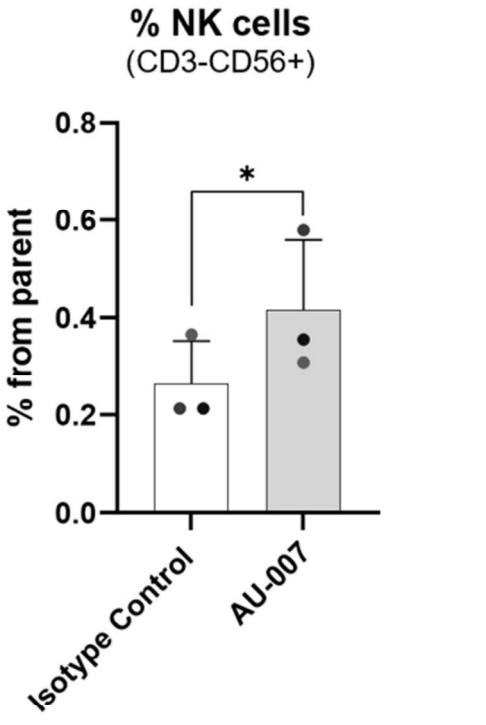




FIG. 31G

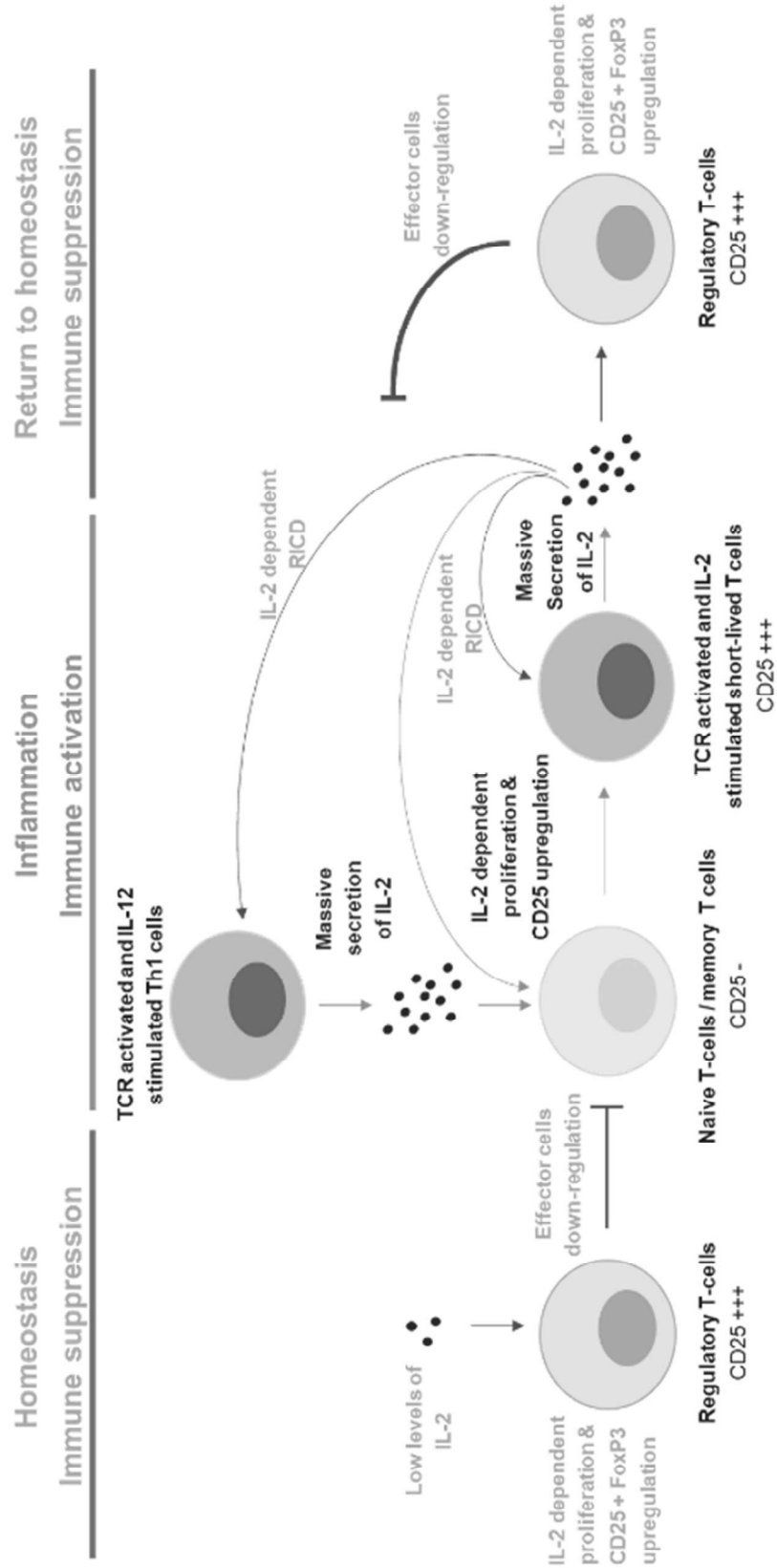

Homeostasis
Immune suppression
Inflammation
Immune activation
Return to homeostasis
Immune suppression
TCR activated and IL-12 stimulated Th1 cells
Massive secretion of IL-2
IL-2 dependent RICD
IL-2 dependent proliferation & CD25 upregulation
IL-2 dependent RICD
Massive Secretion of IL-2
TCR activated and IL-2 stimulated short-lived T cells
CD25 +++
Naive T-cells / memory T cells
CD25 -
Effector cells down-regulation
Regulatory T-cells
CD25 +++
IL-2 dependent proliferation & CD25 + FoxP3 upregulation
Low levels of IL-2
Effector cells down-regulation
Regulatory T-cells
CD25 +++
IL-2 dependent proliferation & CD25 + FoxP3 upregulation

FIG. 32A

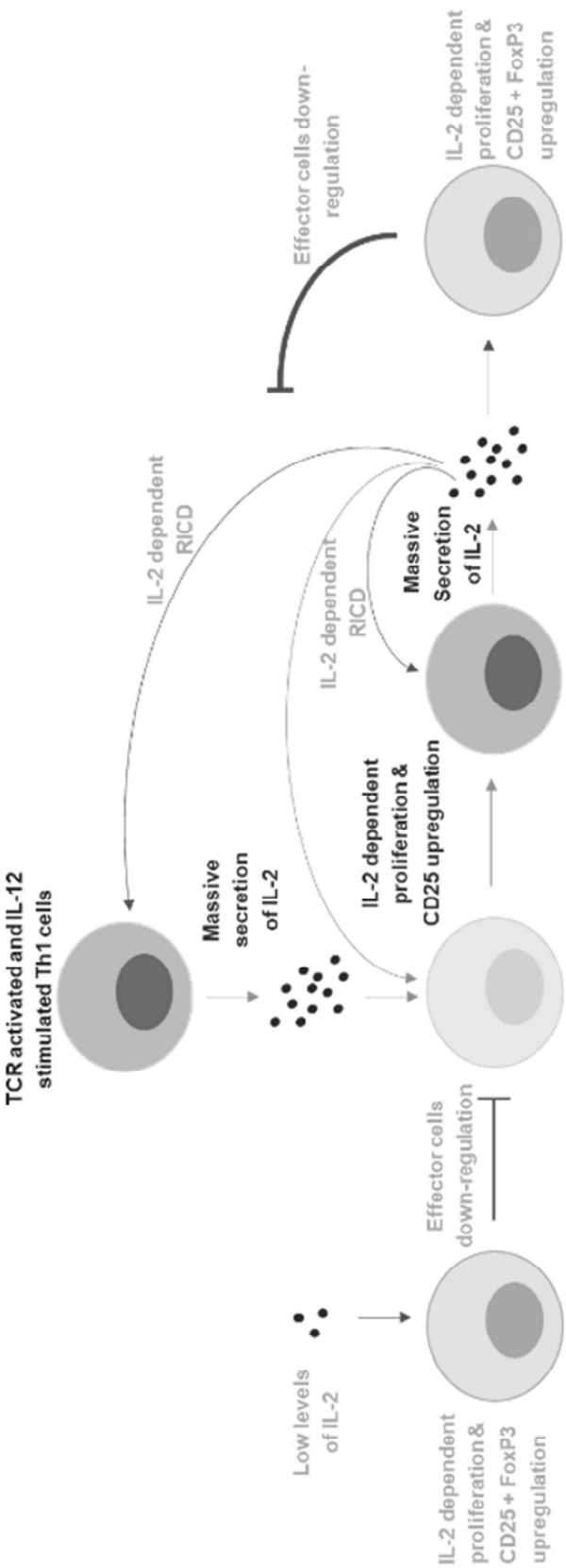

TCR activated and IL-12 stimulated Th1 cells
Massive secretion of IL-2
IL-2 dependent RICD
IL-2 dependent proliferation & CD25 upregulation
IL-2 dependent RICD
Massive Secretion of IL-2
TCR activated and IL-2 stimulated short-lived T cells
CD25 +++
Effector cells down-regulation
IL-2 dependent proliferation & CD25 + FoxP3 upregulation
Regulatory T-cells
CD25 +++
Low levels of IL-2
IL-2 dependent proliferation & CD25 + FoxP3 upregulation
Regulatory T-cells
CD25 +++
Effector cells down-regulation
Naive T-cells / memory T cells
CD25 -
Enhancement of dimer expressing cells expansion
Modified IL-2 with dimer selectivity (CD25-)

FIG. 32B

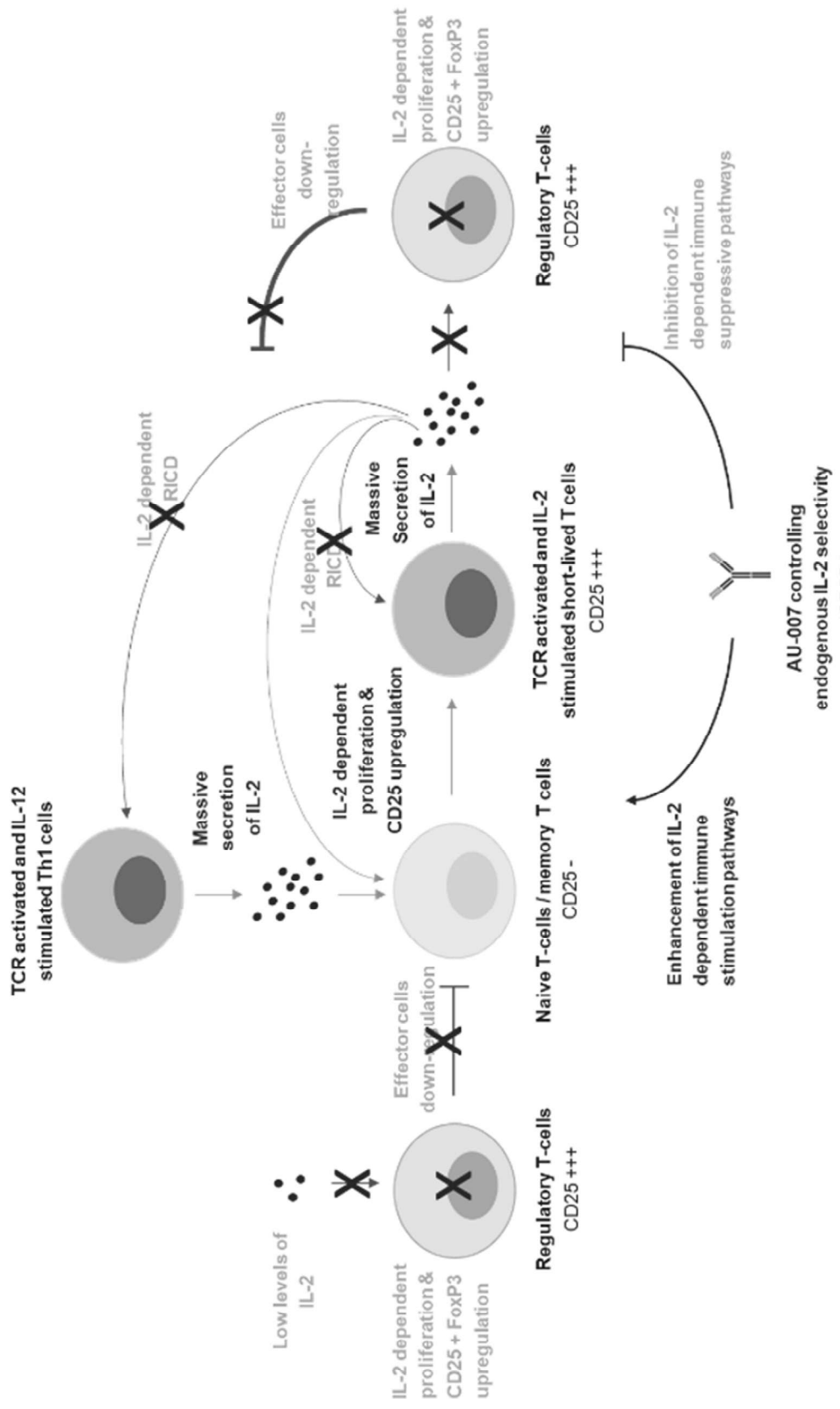
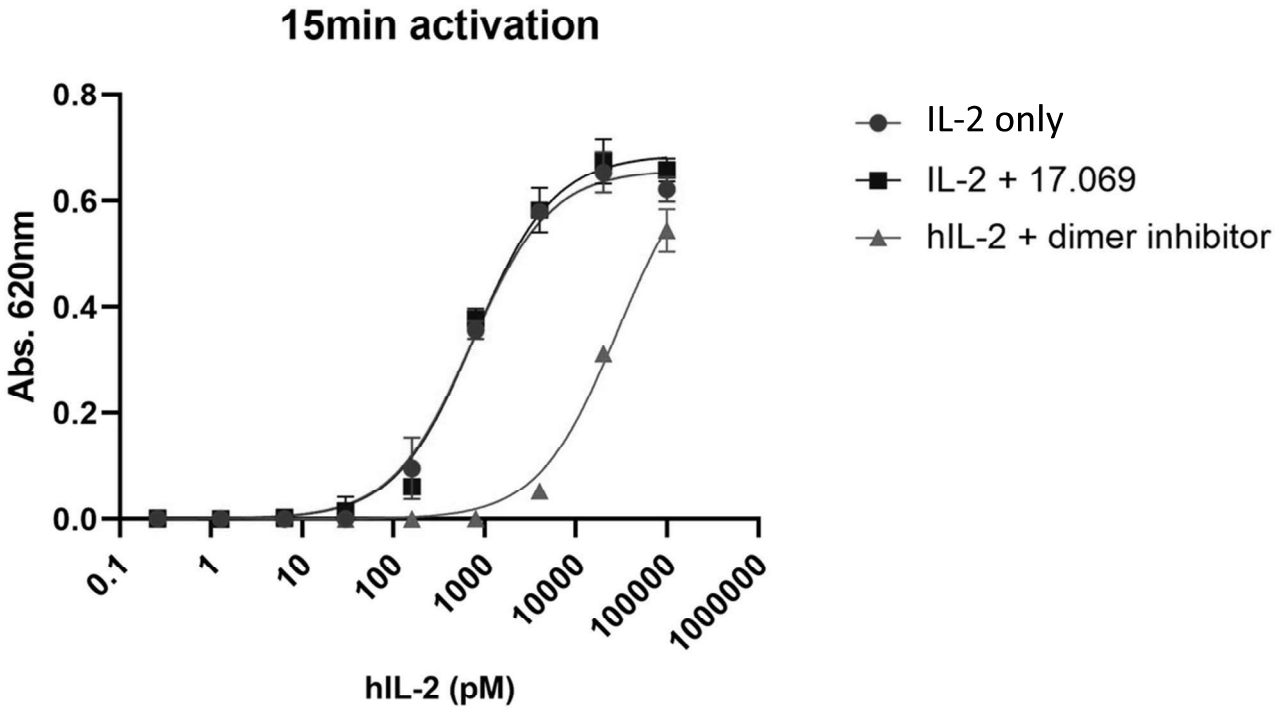
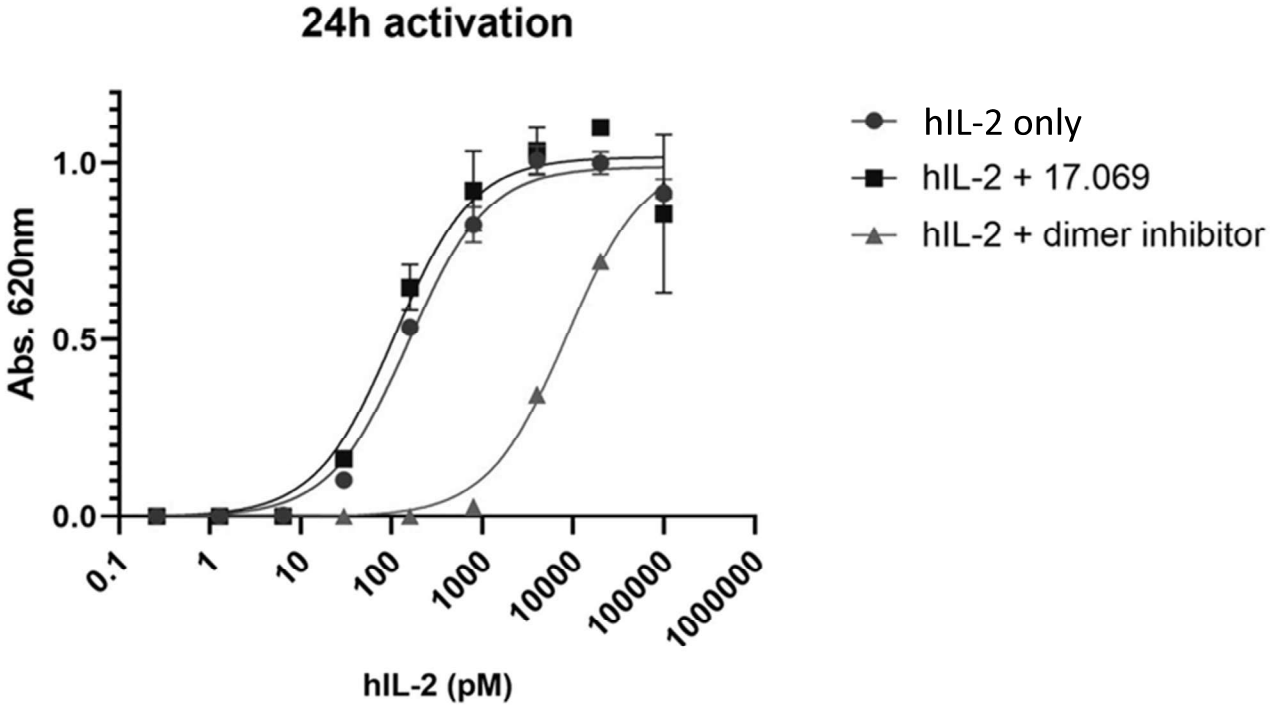

TCR activated and IL-12 stimulated Th1 cells
Massive secretion of IL-2
IL-2 dependent RICD
IL-2 dependent proliferation & CD25 upregulation
Naive T-cells / memory T cells
CD25 -
TCR activated and IL-2 stimulated short-lived T cells
CD25 +++
Massive Secretion of IL-2
IL-2 dependent RICD
Effector cells down-regulation
Regulatory T-cells
CD25 +++
IL-2 dependent proliferation & CD25 + FoxP3 upregulation
Effector cells down-regulation
Regulatory T-cells
CD25 +++
IL-2 dependent proliferation & CD25 + FoxP3 upregulation
Low levels of IL-2
Enhancement of IL-2 dependent immune stimulation pathways
Inhibition of IL-2 dependent immune suppressive pathways
AU-007 controlling endogenous IL-2 selectivity (CD25-)

FIG. 32C



IL-2 only EC50= 0.7099
IL-2 + 17.069 EC50= 0.7737
IL-2 + dimer inhibitor EC50= 28.45

FIG. 33A



IL-2 only EC50= 154.7 pM
IL-2 + 17.069 EC50= 107.3 pM
IL-2 + dimer inhibitor EC50= 8403 pM

FIG. 33B

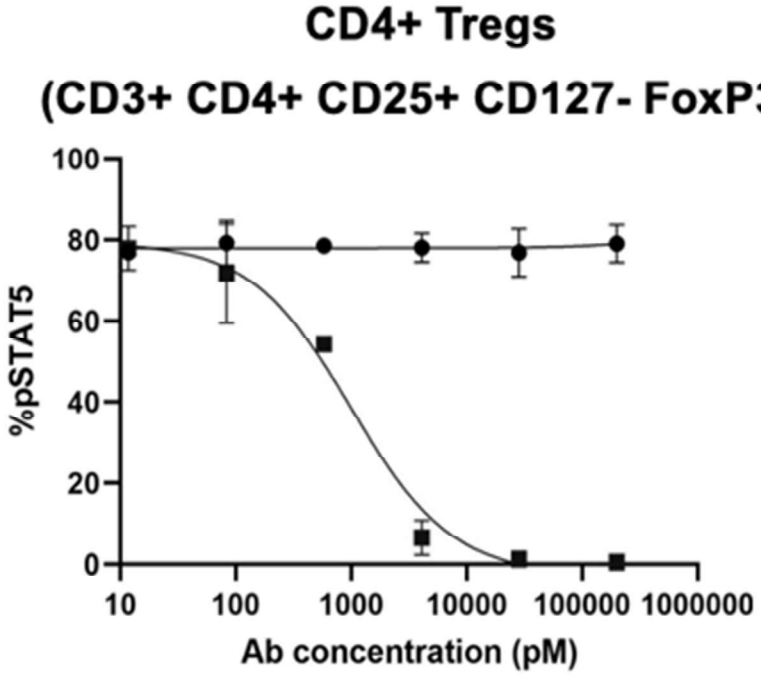


FIG. 34A

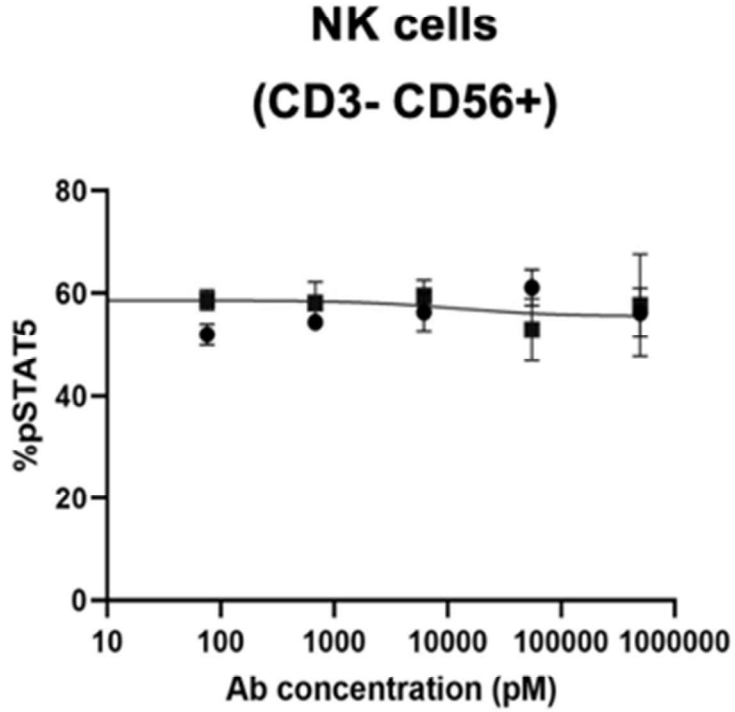


FIG. 34B

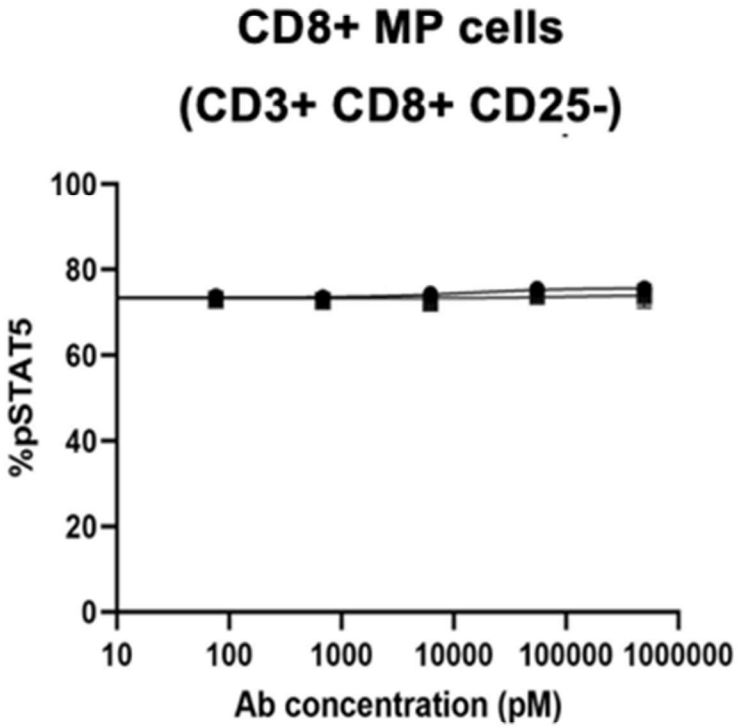


FIG. 34C

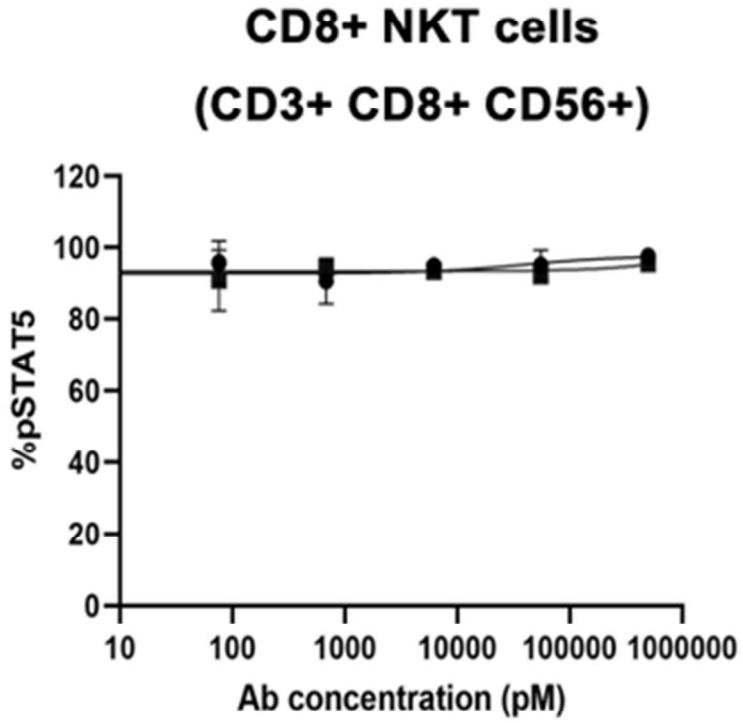


FIG. 34D

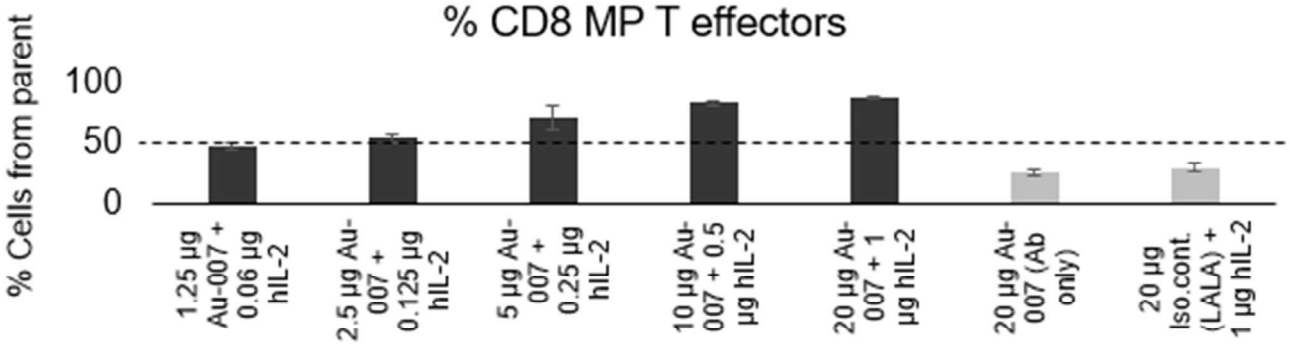


FIG. 35A

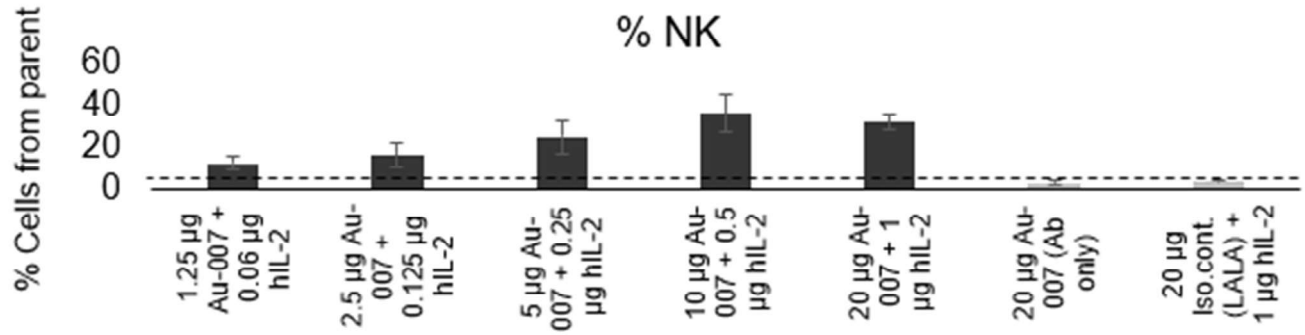


FIG. 35B

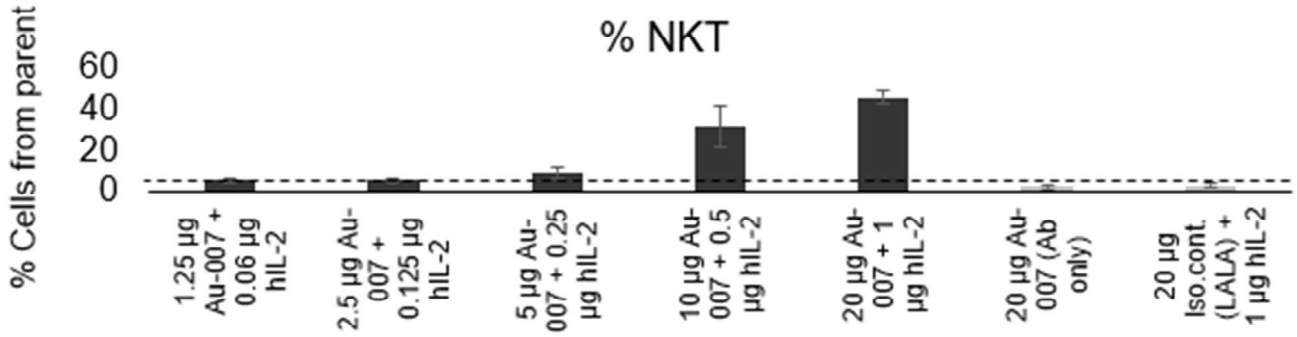


FIG. 35C

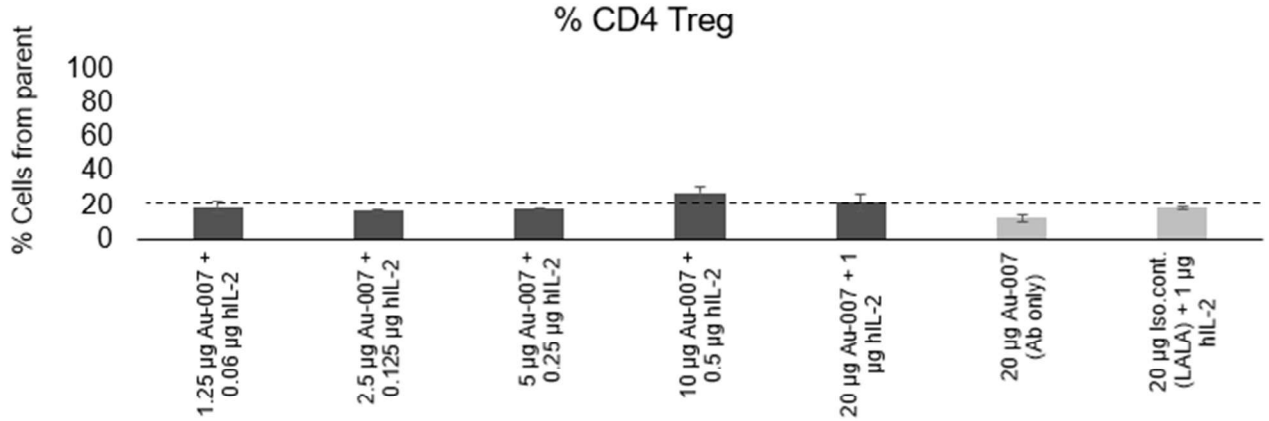


FIG. 35D

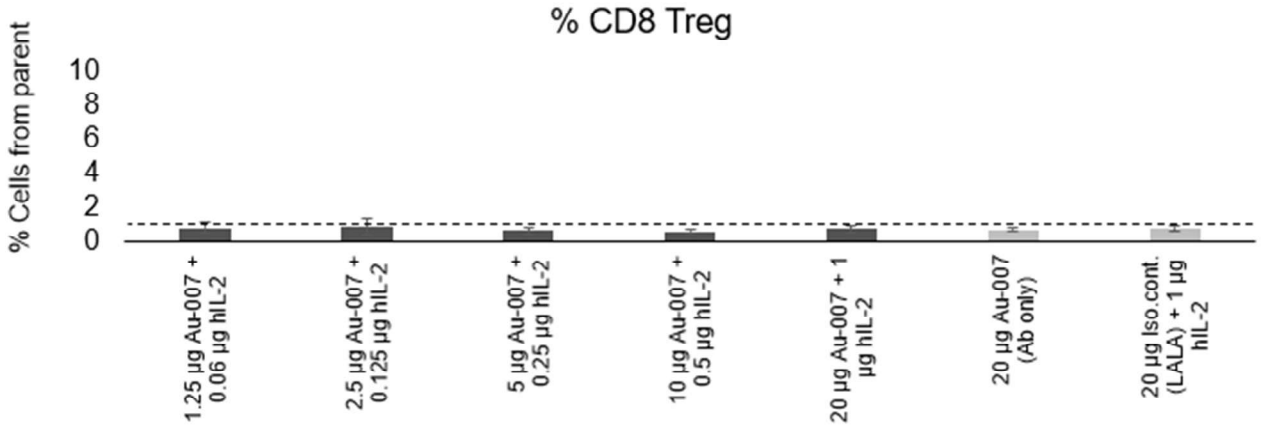


FIG. 35E

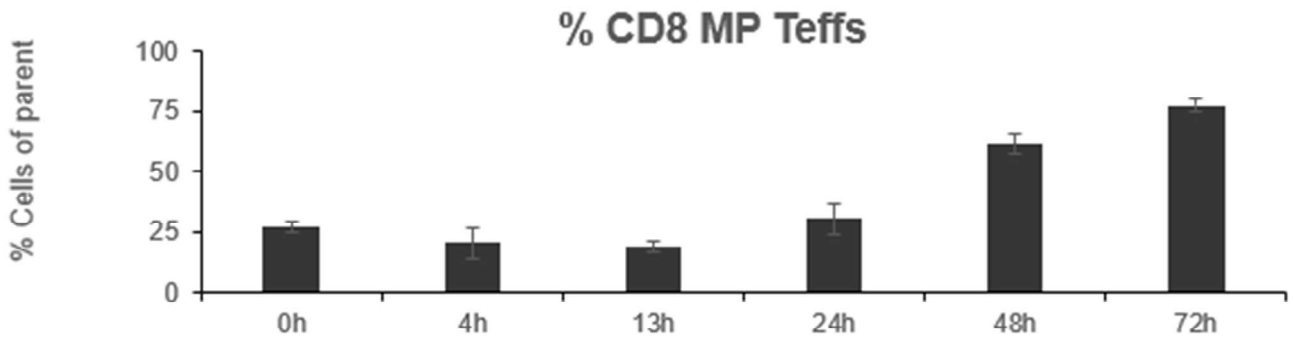


FIG. 36A

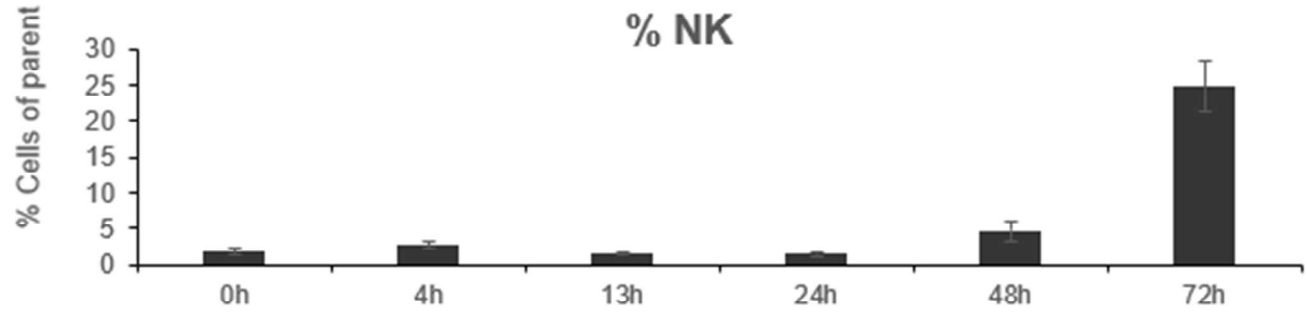


FIG. 36B

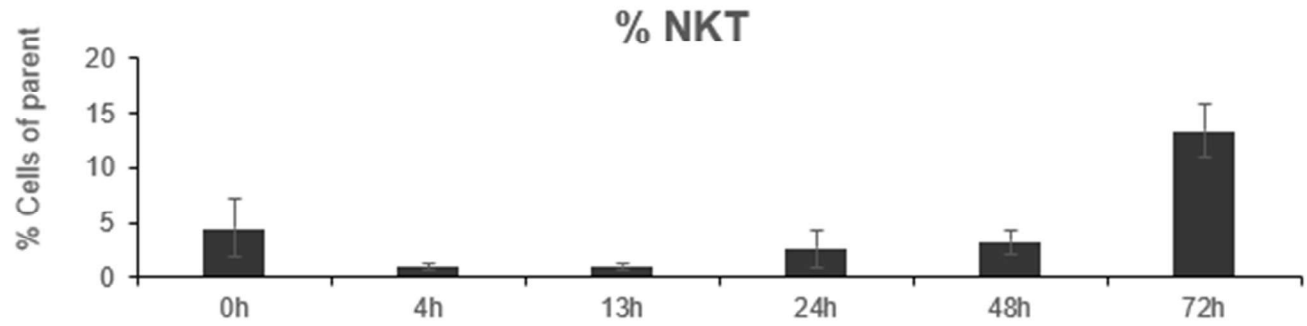


FIG. 36C

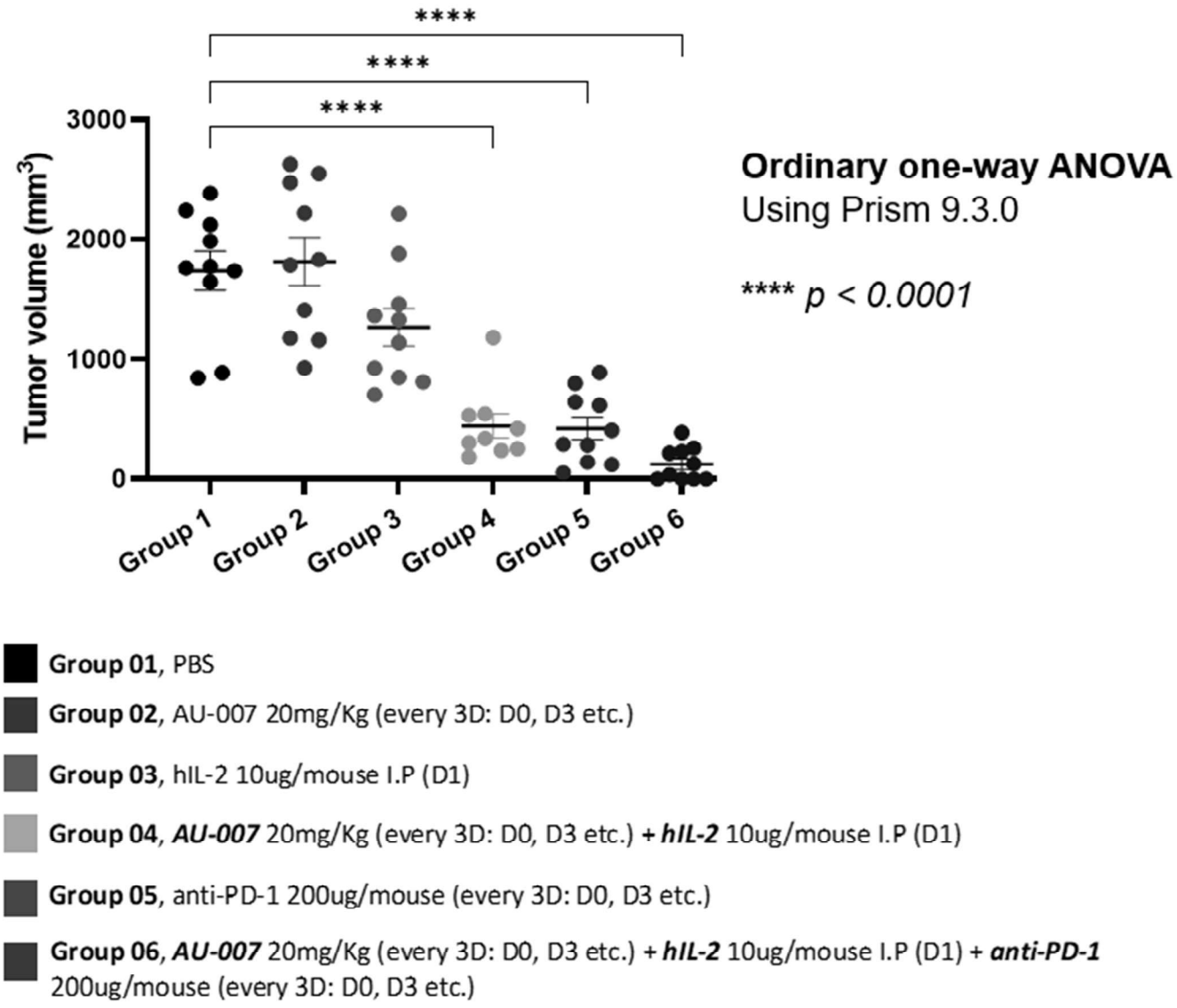
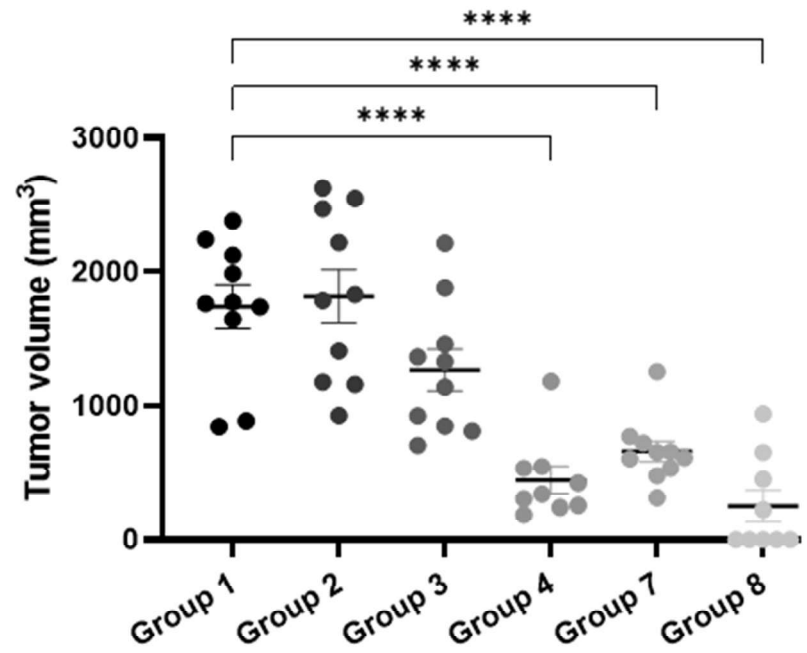

****
****
****
3000
2000
1000
0
Tumor volume (mm3)
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Ordinary one-way ANOVA
Using Prism 9.3.0
**** p < 0.0001
Group 01, PBS
Group 02, AU-007 20mg/Kg (every 3D: D0, D3 etc.)
Group 03, hIL-2 10ug/mouse I.P (D1)
Group 04, AU-007 20mg/Kg (every 3D: D0, D3 etc.) + hIL-2 10ug/mouse I.P (D1)
Group 05, anti-PD-1 200ug/mouse (every 3D: D0, D3 etc.)
Group 06, AU-007 20mg/Kg (every 3D: D0, D3 etc.) + hIL-2 10ug/mouse I.P (D1) + anti-PD-1 200ug/mouse (every 3D: D0, D3 etc.)

FIG. 37A



Ordinary one-way ANOVA
Using Prism 9.3.0

**** $p < 0.0001$

Group 01, PBS

Group 02, AU-007 20mg/Kg (every 3D: D0, D3 etc.)

Group 03, hIL-2 10ug/mouse I.P (D1)

Group 04, ***AU-007*** 20mg/Kg (every 3D: D0, D3 etc.) + ***hIL-2*** 10ug/mouse I.P (D1)

Group 07, anti-PD-L1 200ug/mouse (every 3D: D0, D3 etc.)

Group 08, ***AU-007*** 20mg/Kg (every 3D: D0, D3 etc.) + ***hIL-2*** 10ug/mouse I.P (D1) + ***anti-PD-L1*** 200ug/mouse (every 3D: D0, D3 etc.)

FIG. 37B

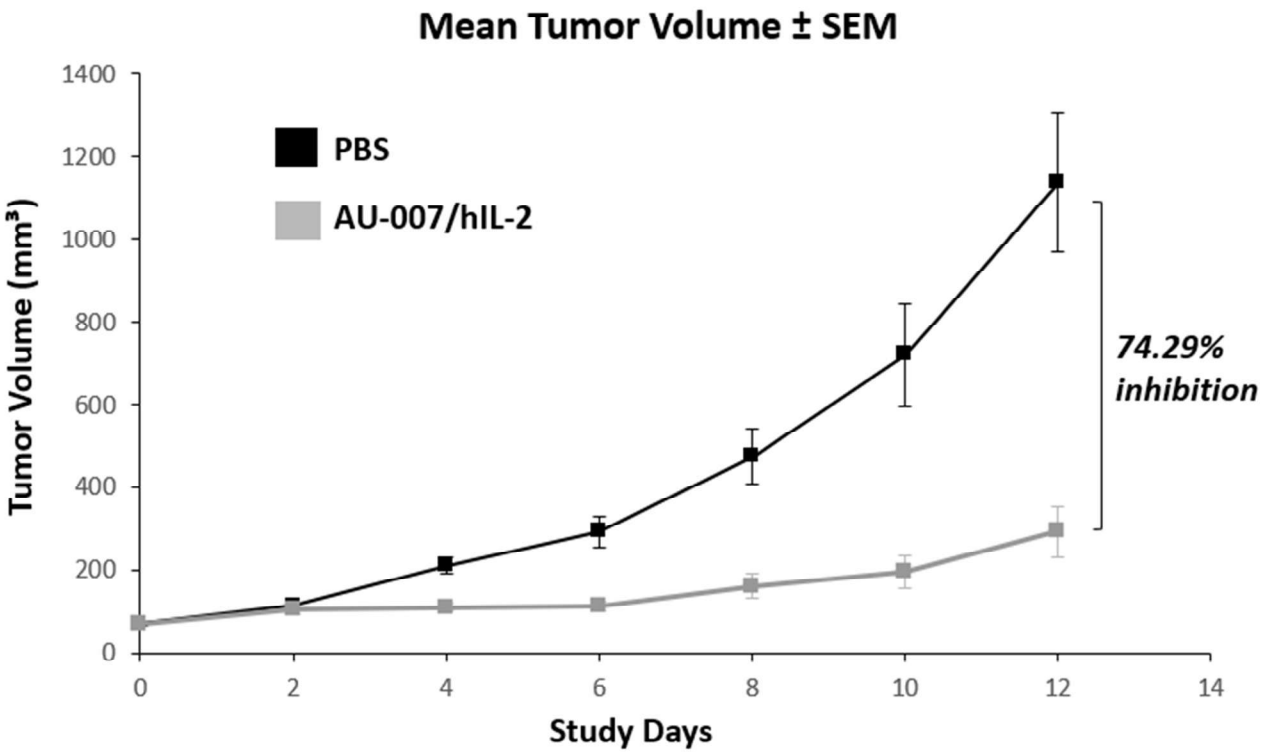


FIG. 38A

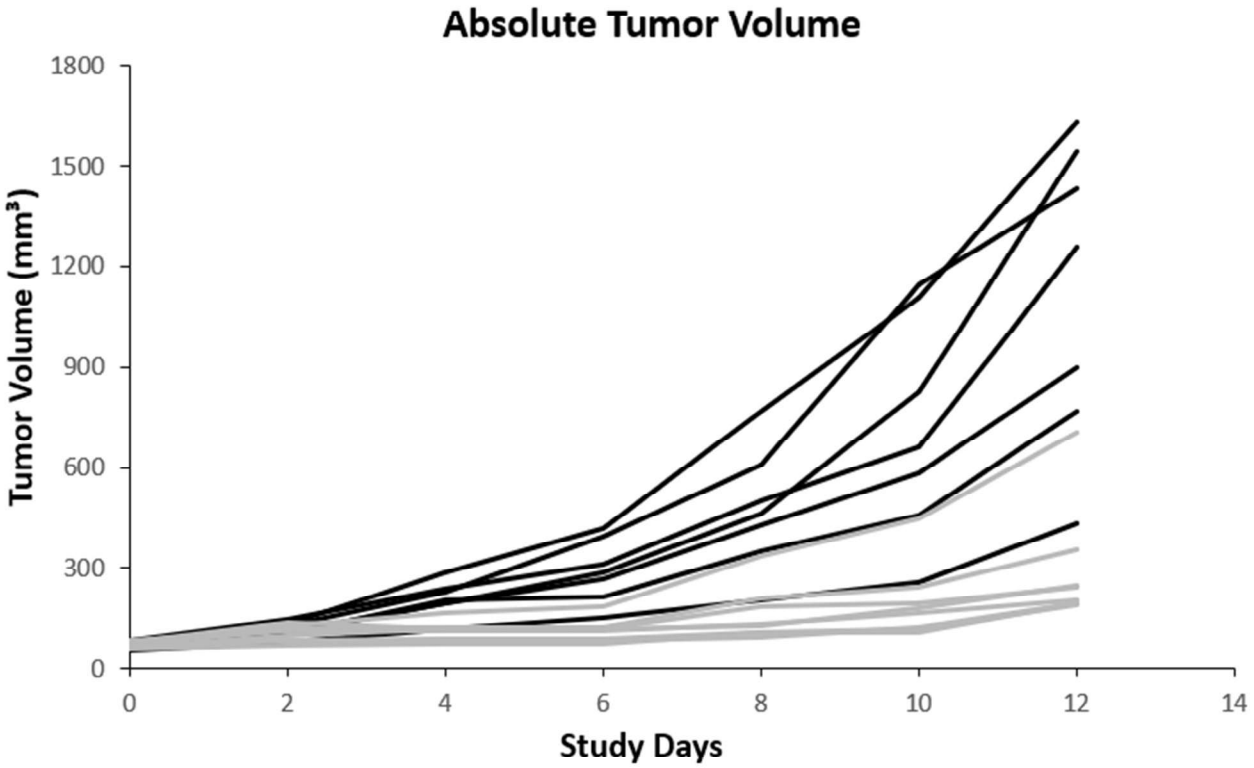


FIG. 38B

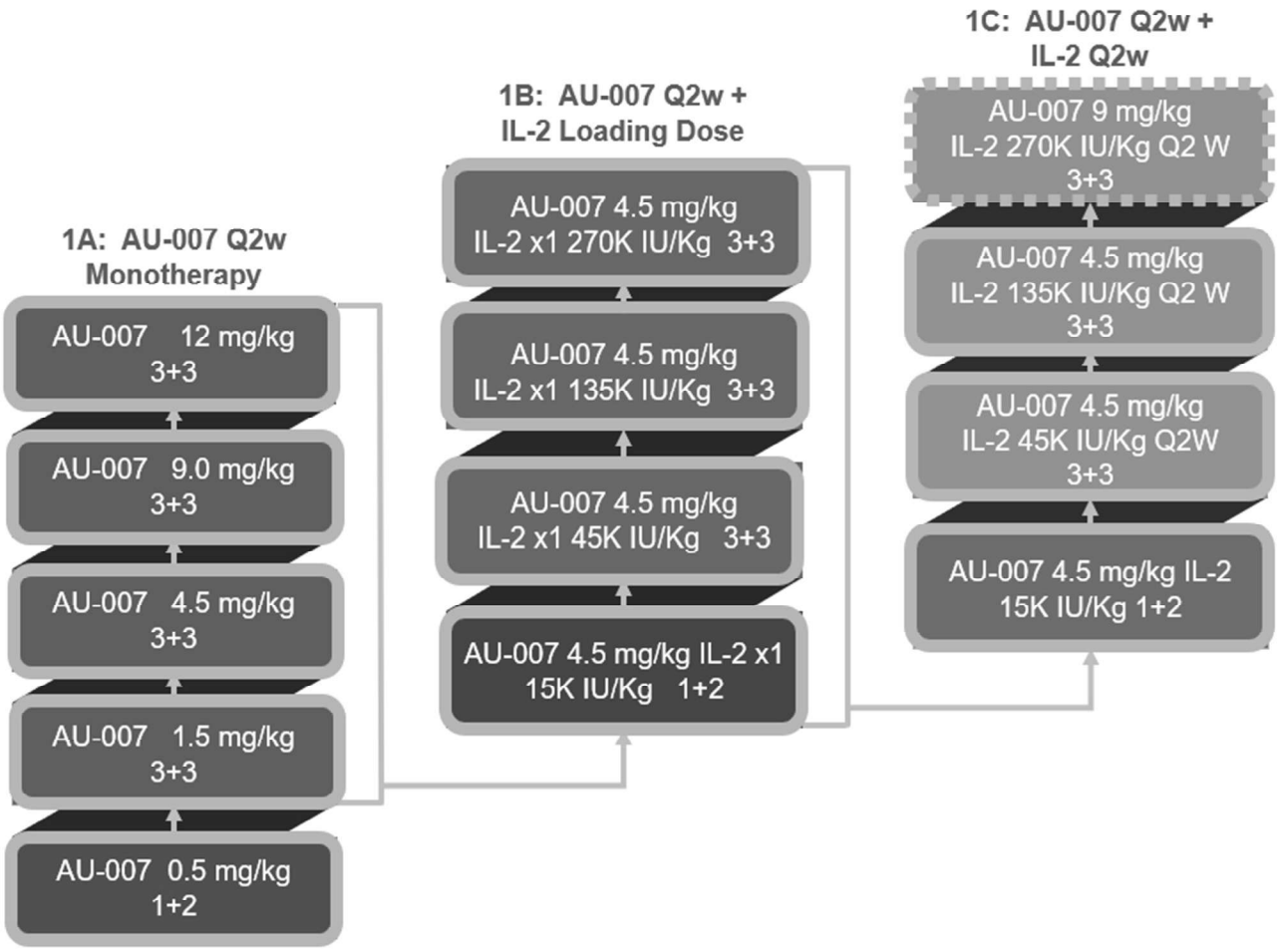

1A: AU-007 Q2w Monotherapy
AU-007 12 mg/kg 3+3
AU-007 9.0 mg/kg 3+3
AU-007 4.5 mg/kg 3+3
AU-007 1.5 mg/kg 3+3
AU-007 0.5 mg/kg 1+2
1B: AU-007 Q2w + IL-2 Loading Dose
AU-007 4.5 mg/kg IL-2 x1 270K IU/Kg 3+3
AU-007 4.5 mg/kg IL-2 x1 135K IU/Kg 3+3
AU-007 4.5 mg/kg IL-2 x1 45K IU/Kg 3+3
AU-007 4.5 mg/kg IL-2 x1 15K IU/Kg 1+2
1C: AU-007 Q2w + IL-2 Q2w
AU-007 9 mg/kg IL-2 270K IU/Kg Q2 W 3+3
AU-007 4.5 mg/kg IL-2 135K IU/Kg Q2 W 3+3
AU-007 4.5 mg/kg IL-2 45K IU/Kg Q2W 3+3
AU-007 4.5 mg/kg IL-2 15K IU/Kg 1+2

FIG. 39

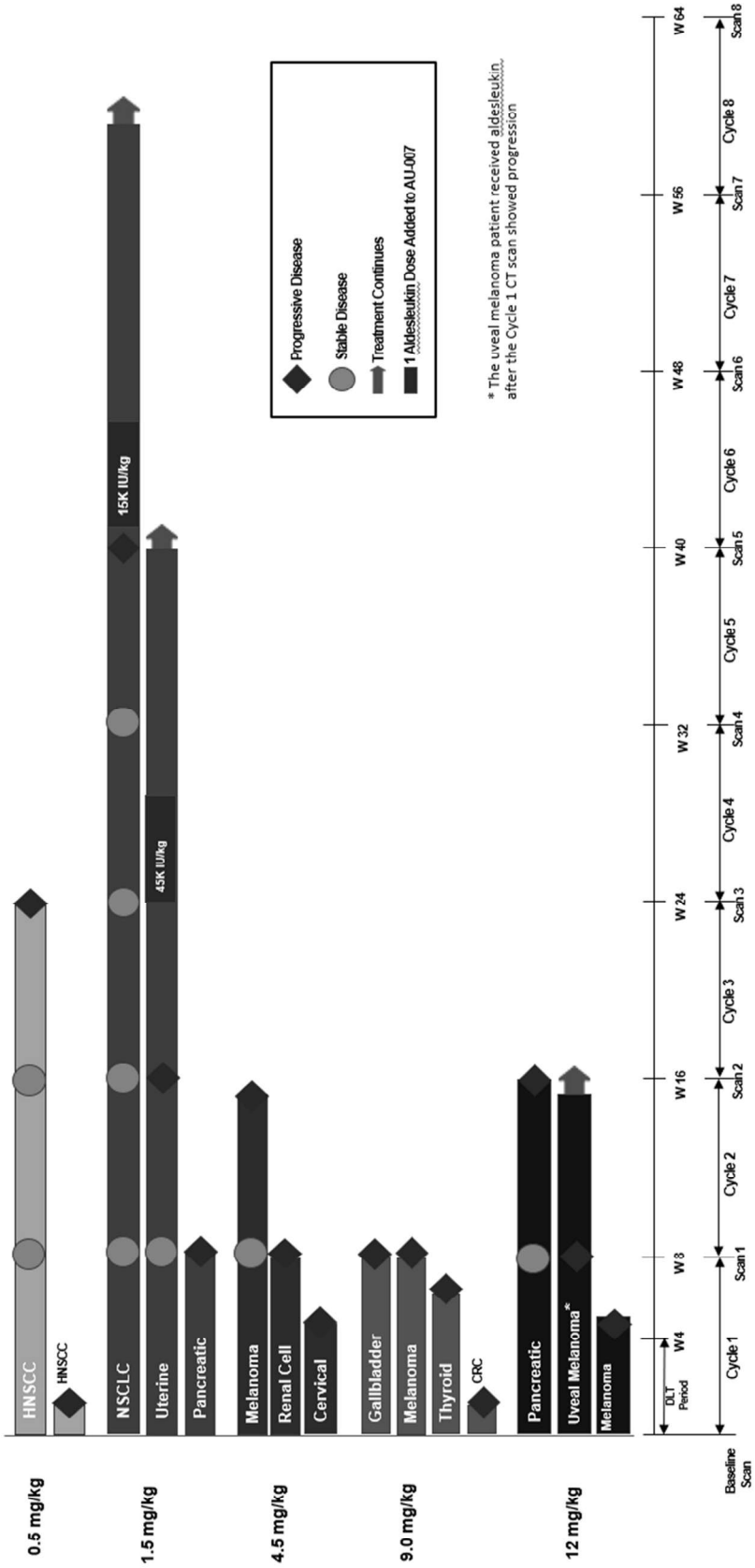

0.5 mg/kg
HNSCC
HNSCC
1.5 mg/kg
NSCLC
Uterine
Pancreatic
4.5 mg/kg
Melanoma
Renal Cell
Cervical
9.0 mg/kg
Gallbladder
Melanoma
Thyroid
CRC
12 mg/kg
Pancreatic
Uveal Melanoma*
Melanoma
15K IU/kg
45K IU/kg
Progressive Disease
Stable Disease
Treatment Continues
1 Aldesleukin Dose Added to AU-007
* The uveal melanoma patient received aldesleukin after the Cycle 1 CT scan showed progression
Baseline Scan
DLT Period
W 4
W 8
W 16
W 24
W 32
W 40
W 48
W 56
W 64
Cycle 1
Scan 1
Cycle 2
Scan 2
Cycle 3
Scan 3
Cycle 4
Scan 4
Cycle 5
Scan 5
Cycle 6
Scan 6
Cycle 7
Scan 7
Cycle 8
Scan 8

FIG. 40

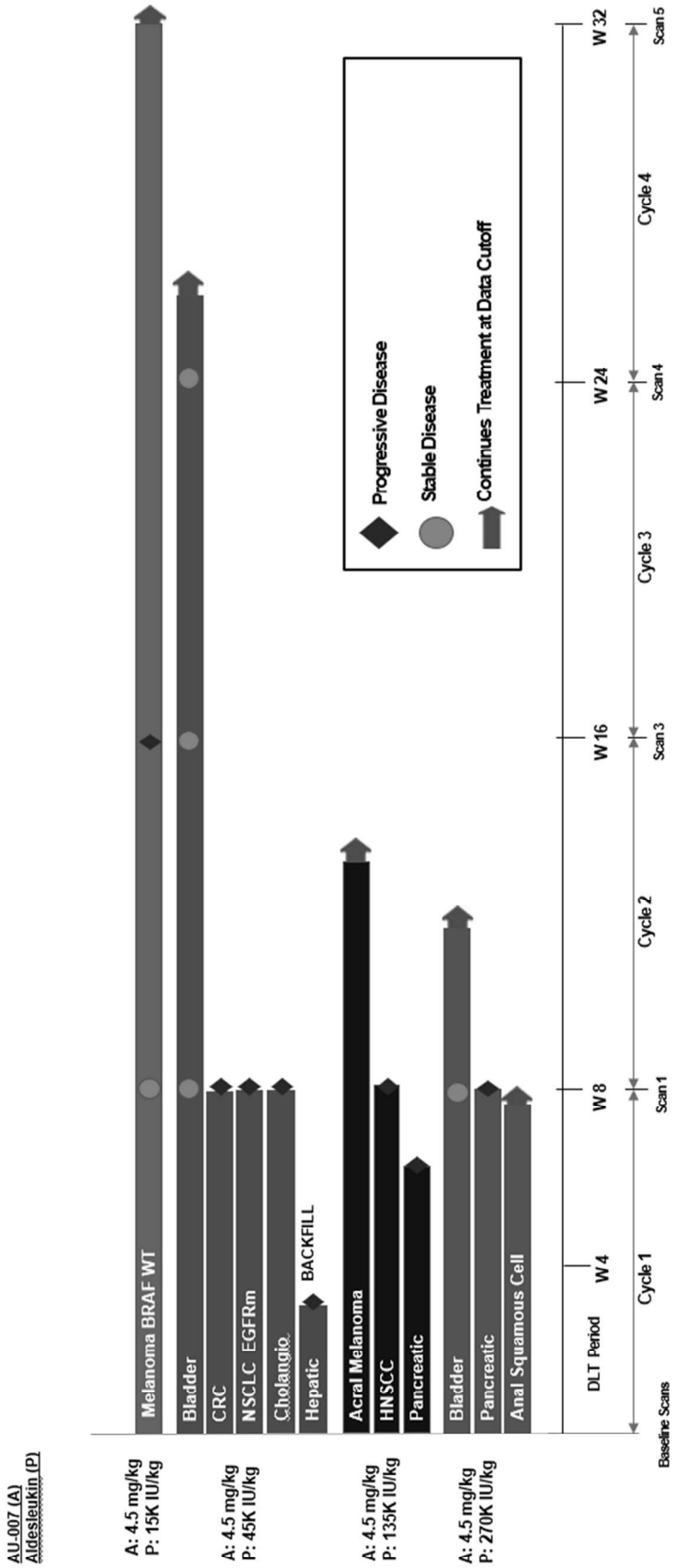

AU-007 (A)
Aldesleukin (P)
A: 4.5 mg/kg
P: 15K IU/kg
A: 4.5 mg/kg
P: 45K IU/kg
A: 4.5 mg/kg
P: 135K IU/kg
A: 4.5 mg/kg
P: 270K IU/kg
Melanoma BRAF WT
Bladder
CRC
NSCLC EGFRm
Cholangio.
Hepatic
BACKFILL
Acral Melanoma
HNSCC
Pancreatic
Bladder
Pancreatic
Anal Squamous Cell
Progressive Disease
Stable Disease
Continues Treatment at Data Cutoff
Baseline Scans
DLT Period
W 4
W 8
W 16
W 24
W 32
Cycle 1
Cycle 2
Cycle 3
Cycle 4
Scan 1
Scan 3
Scan 4
Scan 5

FIG. 41A

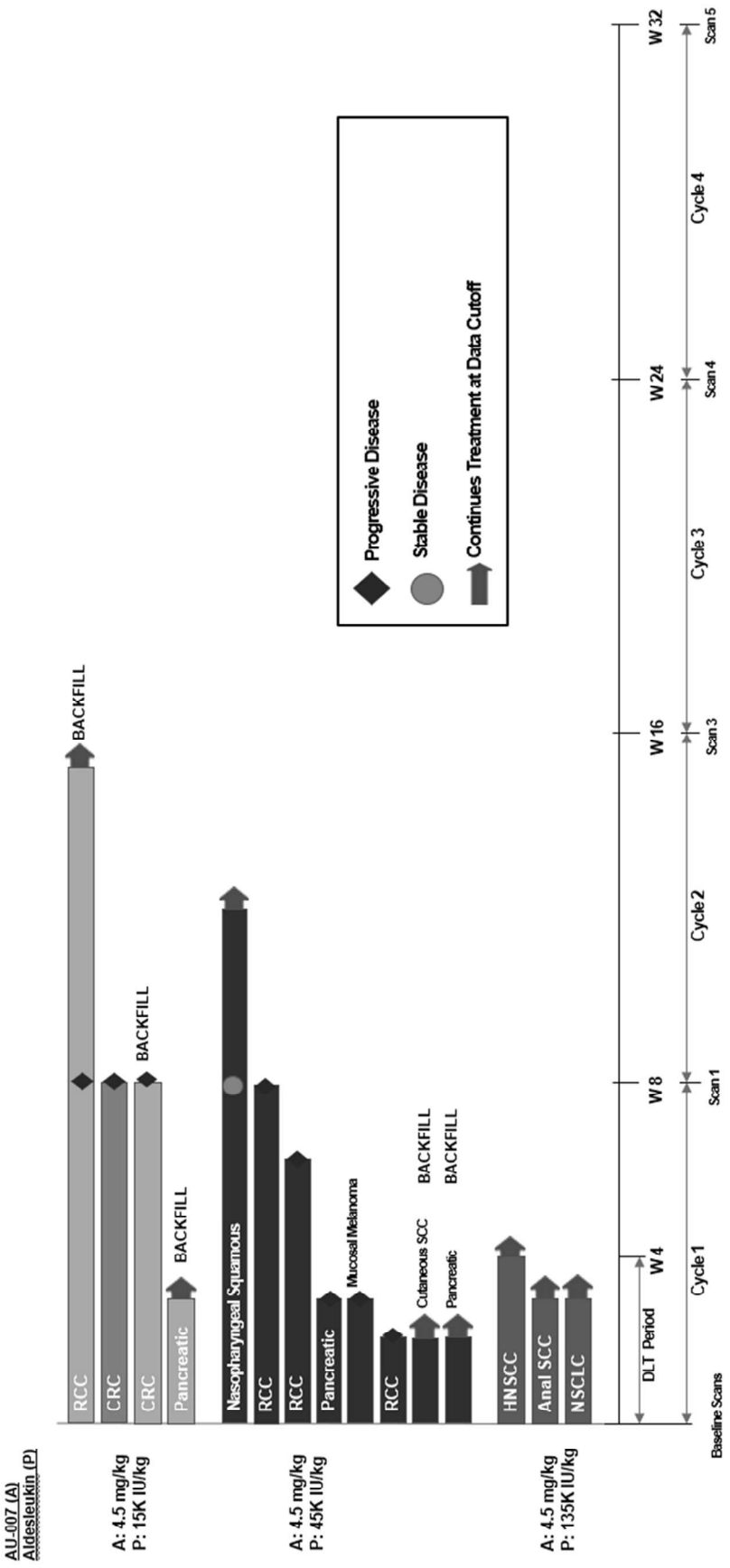

AU-007 (A)
Aldesleukin (P)
A: 4.5 mg/kg
P: 15K IU/kg
A: 4.5 mg/kg
P: 45K IU/kg
A: 4.5 mg/kg
P: 135K IU/kg
RCC
CRC
CRC
Pancreatic
BACKFILL
BACKFILL
BACKFILL
Nasopharyngeal Squamous
RCC
RCC
Pancreatic
Mucosal Melanoma
RCC
Cutaneous SCC
Pancreatic
BACKFILL
BACKFILL
HNSCC
Anal SCC
NSCLC
Progressive Disease
Stable Disease
Continues Treatment at Data Cutoff
Baseline Scans
DLT Period
W 4
W 8
W 16
W 24
W 32
Cycle 1
Cycle 2
Cycle 3
Cycle 4
Scan 1
Scan 3
Scan 4
Scan 5

FIG. 41B

Drug Related AEs

AU-007	Patient's Tumor Type	AE	Grade
0.5 mg/kg	HNSCC	Diarrhea	G1
1.5 mg/Kg	NSCLC	Fatigue	G1
		Pruritus	G1
		Postural Dizziness	G1
4.5 mg/kg	Melanoma	Nausea	G1
		Gastroesophageal Reflux	G1
		Fatigue	G1
		Loss of Appetite	G1
		Tumor Pain	G1
		Rash	G1
		Constipation	G1
9.0 mg/kg	Gall Bladder	Flushing	G1

FIG. 42A

	Safety Population (Received ≥ 1 Dose of Study Drug)			
	All Grades n (%)	Grade 1/2 n (%)	Grade 3 n (%)	Grade 4 n (%)
TEAEs n = 42	35 (83)	34 (81)	21 (50)	4 (10)
All drug-related AEs	20 (48)	20 (48)	1* (2)	2* (5)
Drug-related AEs AU-007 monotherapy n = 15	4 (27)	4 (27)	0	0
Drug-related AEs AU-007 + aldesleukin n = 27	16 (59)	16 (59)	1* (2)	2* (5)
SAEs	18 (43)			
Drug-related SAEs	1** (2)			
Drug-related AEs leading to discontinuation	0			

FIG. 42B

	Safety Population (Received ≥ 1 Dose of Study Drug)			
	All Grades n (%)	Grade 1/2 n (%)	Grade 3 n (%)	Grade 4 n (%)
TEAEs n = 42	35 (83)	34 (81)	21 (50)	4 (10)
All drug-related AEs	20 (48)	20 (48)	1* (2)	2* (5)
Drug-related AEs AU-007 monotherapy n = 15	4 (27)	4 (27)	0	0
Drug-related AEs AU-007 + aldesleukin n = 27	16 (59)	16 (59)	1* (2)	2* (5)
SAEs	18 (43)			
Drug-related SAEs	1** (2)			
Drug-related AEs leading to discontinuation	0			

FIG. 42C

Drug Related AEs: AU-007 + 1 Proleukin Loading Dose

AU-007	Proleukin	Tumor Type	AE	Attribution	Grade
4.5 mg/kg	15K IU/kg	Melanoma	Nausea	AU-007	G2
4.5 mg/kg	45K IU/Kg	Colon	Chills	Proleukin	G1
			Headache	AU-007	G1

FIG. 43A

Drug Related AEs: AU-007 + Q2W Proleukin Dosing

AU-007	Proleukin	Tumor Type	AE	Attribution	Grade
4.5 mg/kg	15K IU/kg	Colon dMMR*	Infusion Site Reaction	Proleukin	G1

* dMMR: mismatch repair deficient

FIG. 43B

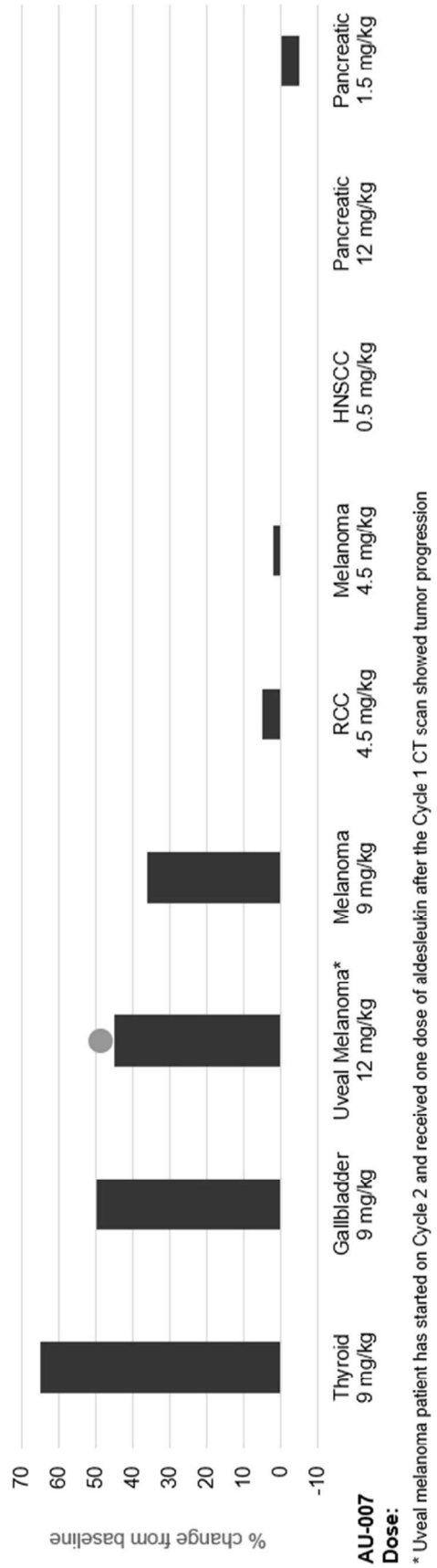

70
60
50
40
30
20
10
0
-10
% change from baseline
AU-007 Dose:
Thyroid 9 mg/kg
Gallbladder 9 mg/kg
Uveal Melanoma* 12 mg/kg
Melanoma 9 mg/kg
RCC 4.5 mg/kg
Melanoma 4.5 mg/kg
HNSCC 0.5 mg/kg
Pancreatic 12 mg/kg
Pancreatic 1.5 mg/kg
* Uveal melanoma patient has started on Cycle 2 and received one dose of aldesleukin after the Cycle 1 CT scan showed tumor progression

FIG. 44A

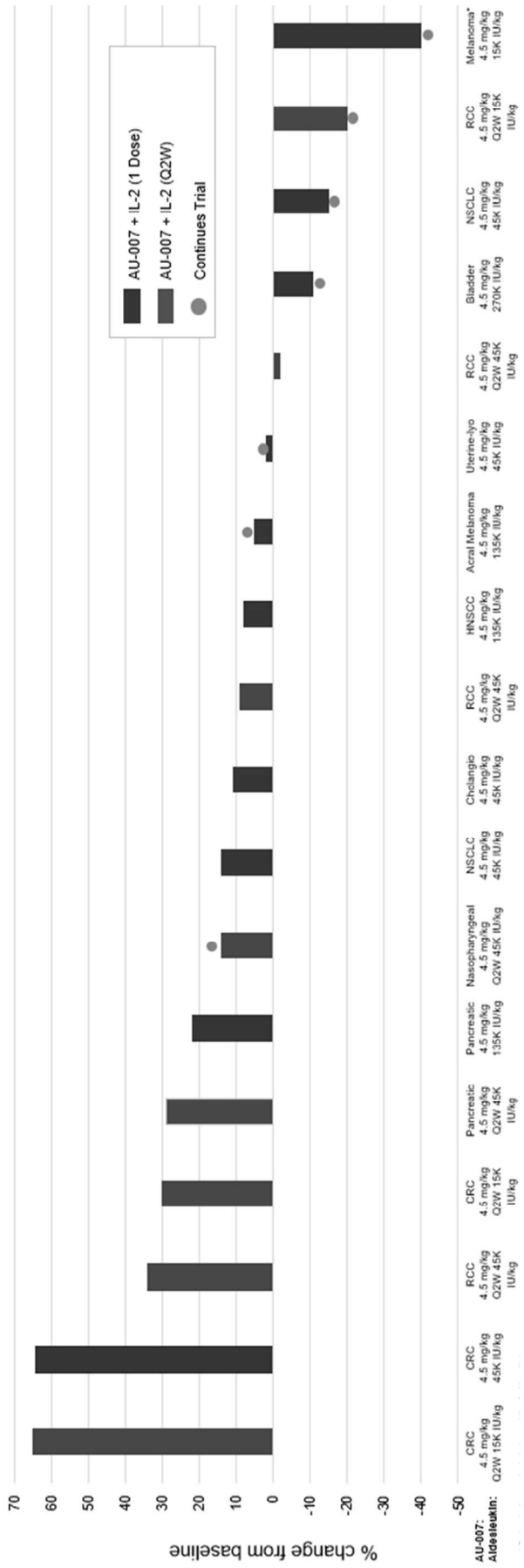

AU-007 + IL-2 (1 Dose)
AU-007 + IL-2 (Q2W)
Continues Trial
% change from baseline
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
AU-007 + Aldesleukin:
CRC 4.5 mg/kg Q2W 15K IU/kg
CRC 4.5 mg/kg 45K IU/kg
RCC 4.5 mg/kg Q2W 45K IU/kg
CRC 4.5 mg/kg Q2W 15K IU/kg
Pancreatic 4.5 mg/kg Q2W 45K IU/kg
Pancreatic 4.5 mg/kg 135K IU/kg
Nasopharyngeal 4.5 mg/kg Q2W 45K IU/kg
NSCLC 4.5 mg/kg 45K IU/kg
Cholangio 4.5 mg/kg 45K IU/kg
RCC 4.5 mg/kg Q2W 45K IU/kg
HNSCC 4.5 mg/kg 135K IU/kg
Acral Melanoma 4.5 mg/kg 135K IU/kg
Uterine-lyo 4.5 mg/kg 45K IU/kg
RCC 4.5 mg/kg Q2W 45K IU/kg
Bladder 4.5 mg/kg 270K IU/kg
NSCLC 4.5 mg/kg 45K IU/kg
RCC 4.5 mg/kg Q2W 15K IU/kg
Melanoma* 4.5 mg/kg 15K IU/kg

FIG. 44B

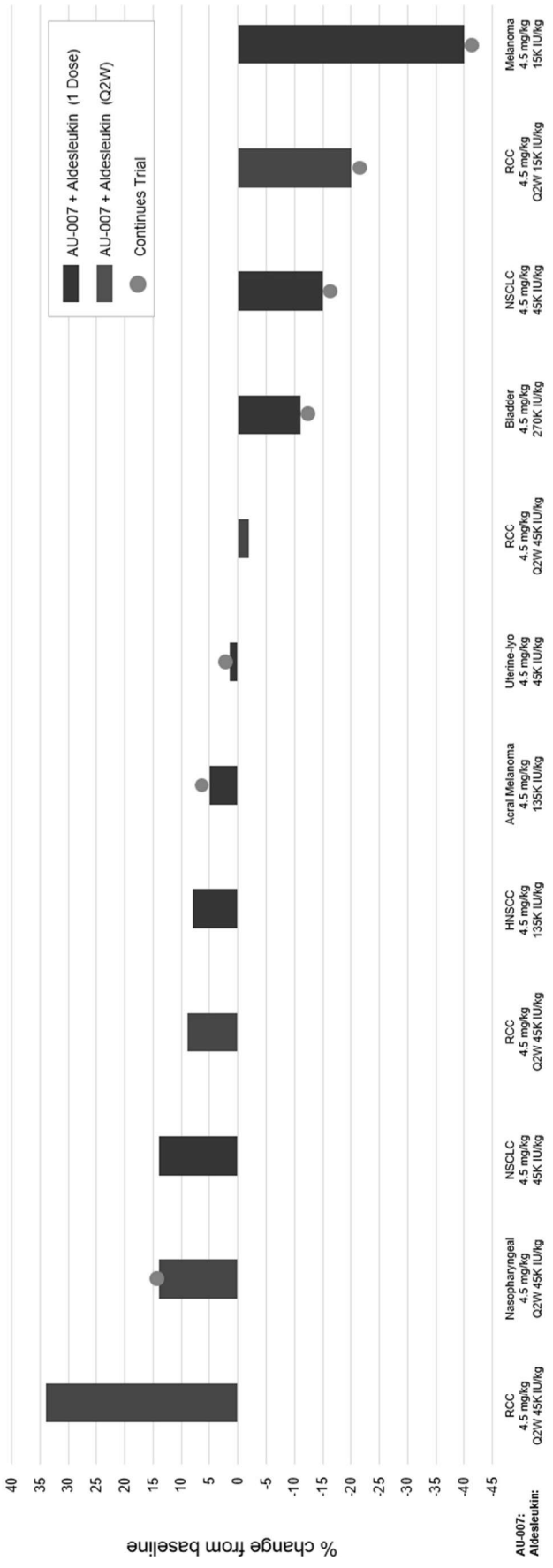

AU-007 + Aldesleukin (1 Dose)
AU-007 + Aldesleukin (Q2W)
Continues Trial
% change from baseline
40
35
30
25
20
15
10
5
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
AU-007:
Aldesleukin:
RCC 4.5 mg/kg Q2W 45K IU/kg
Nasopharyngeal 4.5 mg/kg Q2W 45K IU/kg
NSCLC 4.5 mg/kg 45K IU/kg
RCC 4.5 mg/kg Q2W 45K IU/kg
HNSCC 4.5 mg/kg 135K IU/kg
Acral Melanoma 4.5 mg/kg 135K IU/kg
Uterine-lyo 4.5 mg/kg 45K IU/kg
RCC 4.5 mg/kg Q2W 45K IU/kg
Bladder 4.5 mg/kg 270K IU/kg
NSCLC 4.5 mg/kg 45K IU/kg
RCC 4.5 mg/kg Q2W 15K IU/kg
Melanoma 4.5 mg/kg 15K IU/kg

FIG. 44C

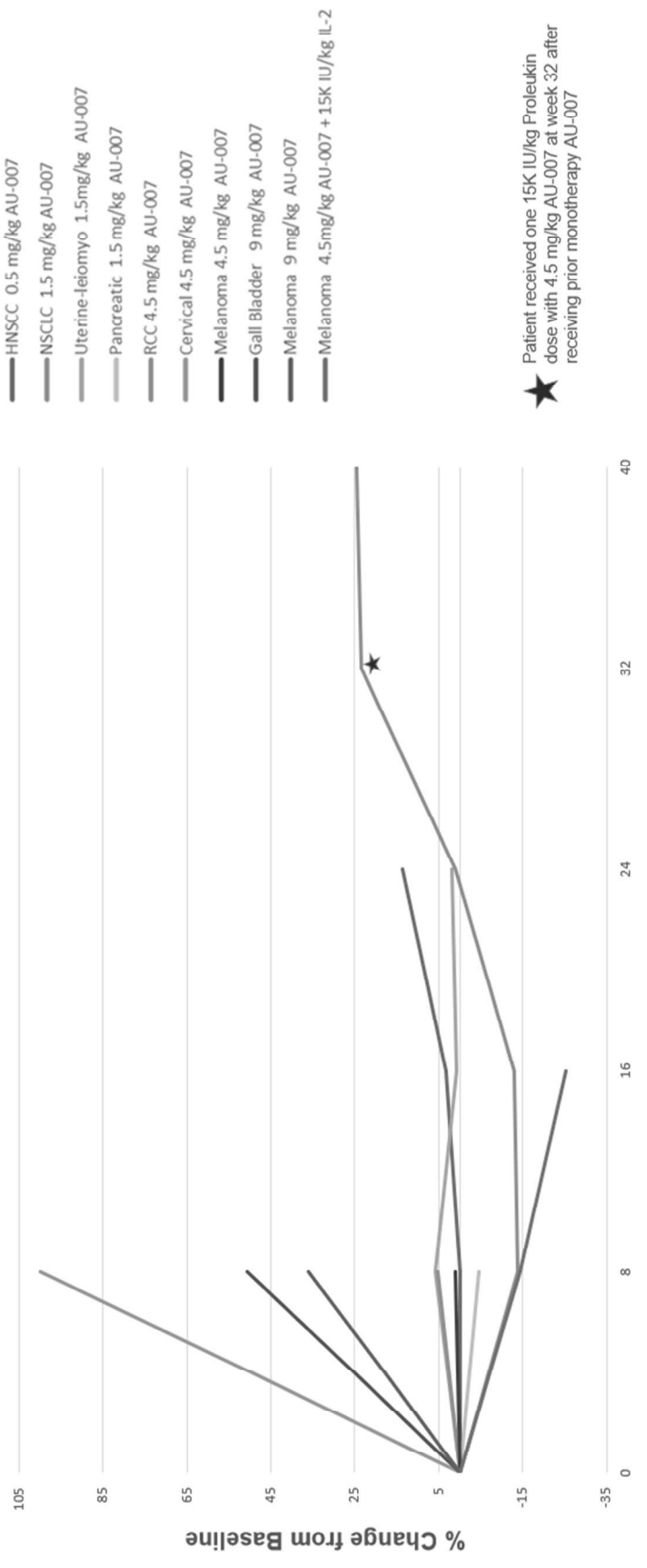

HNSCC 0.5 mg/kg AU-007
NSCLC 1.5 mg/kg AU-007
Uterine-leiomyo 1.5mg/kg AU-007
Pancreatic 1.5 mg/kg AU-007
RCC 4.5 mg/kg AU-007
Cervical 4.5 mg/kg AU-007
Melanoma 4.5 mg/kg AU-007
Gall Bladder 9 mg/kg AU-007
Melanoma 9 mg/kg AU-007
Melanoma 4.5mg/kg AU-007 + 15K IU/kg IL-2
Patient received one 15K IU/kg Proleukin dose with 4.5 mg/kg AU-007 at week 32 after receiving prior monotherapy AU-007
% Change from Baseline
105
85
65
45
25
5
-15
-35
0
8
16
24
32
40
Weeks from Baseline

FIG. 45A

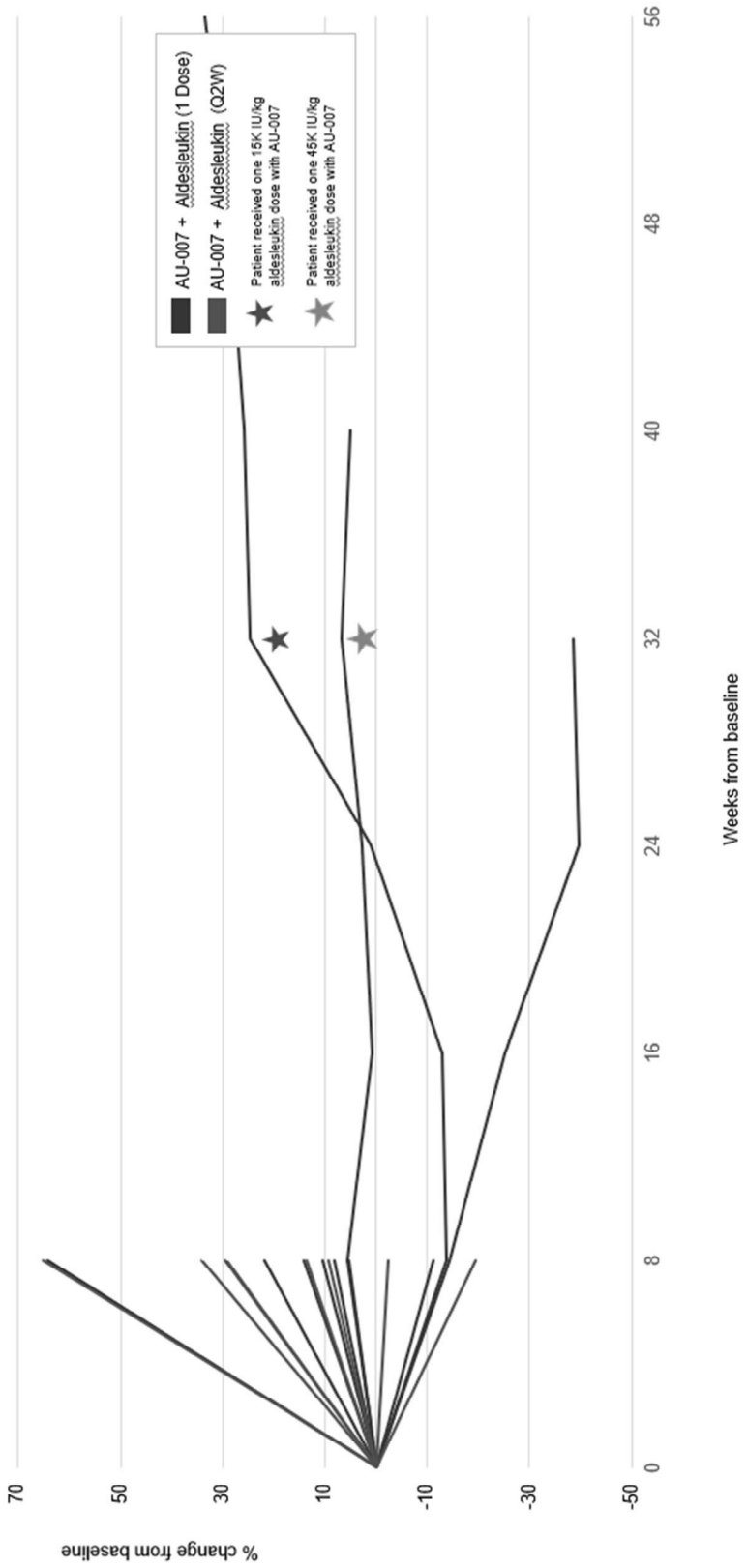

AU-007 + Aldesleukin (1 Dose)
AU-007 + Aldesleukin (Q2W)
Patient received one 15K IU/kg aldesleukin dose with AU-007
Patient received one 45K IU/kg aldesleukin dose with AU-007
% change from baseline
70
50
30
10
-10
-30
-50
0
8
16
24
32
40
48
56
Weeks from baseline

FIG. 45B

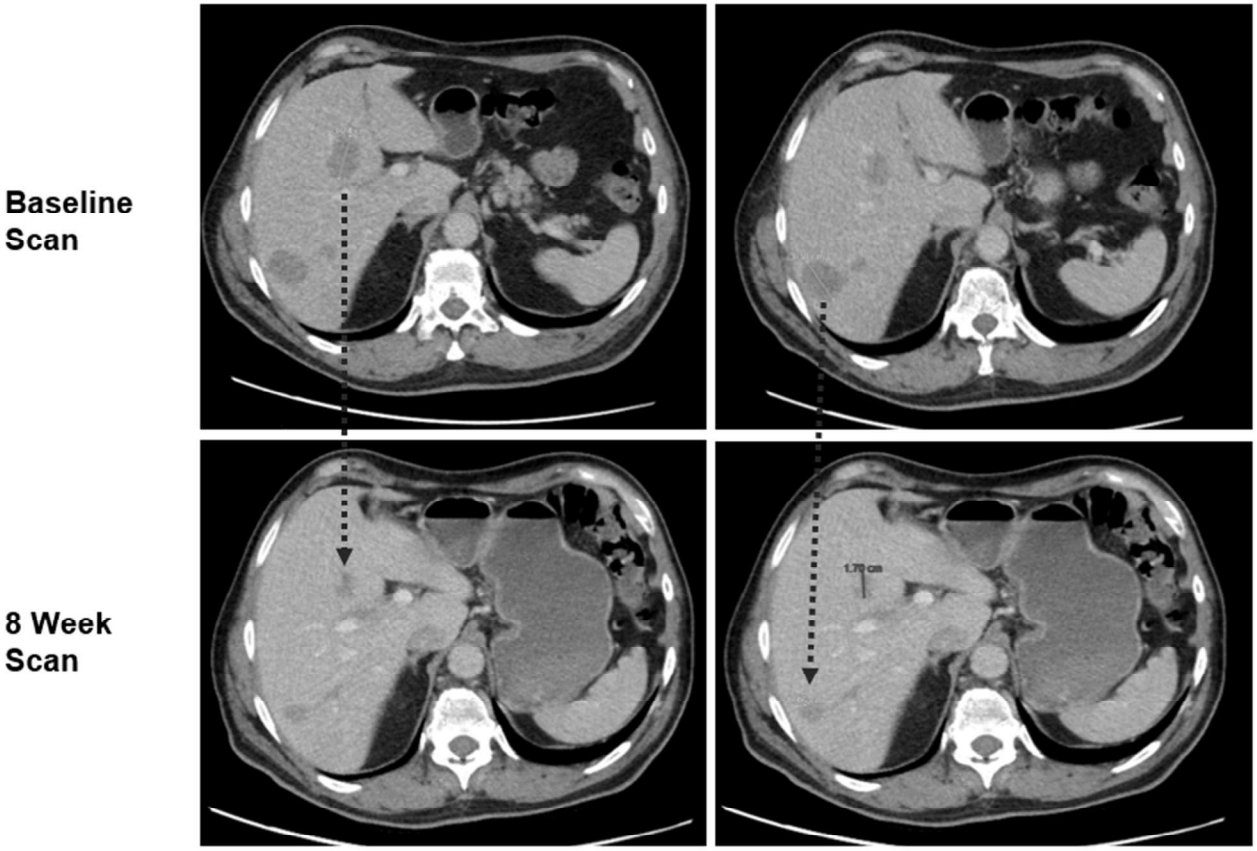


FIG. 46A

FIG. 46B

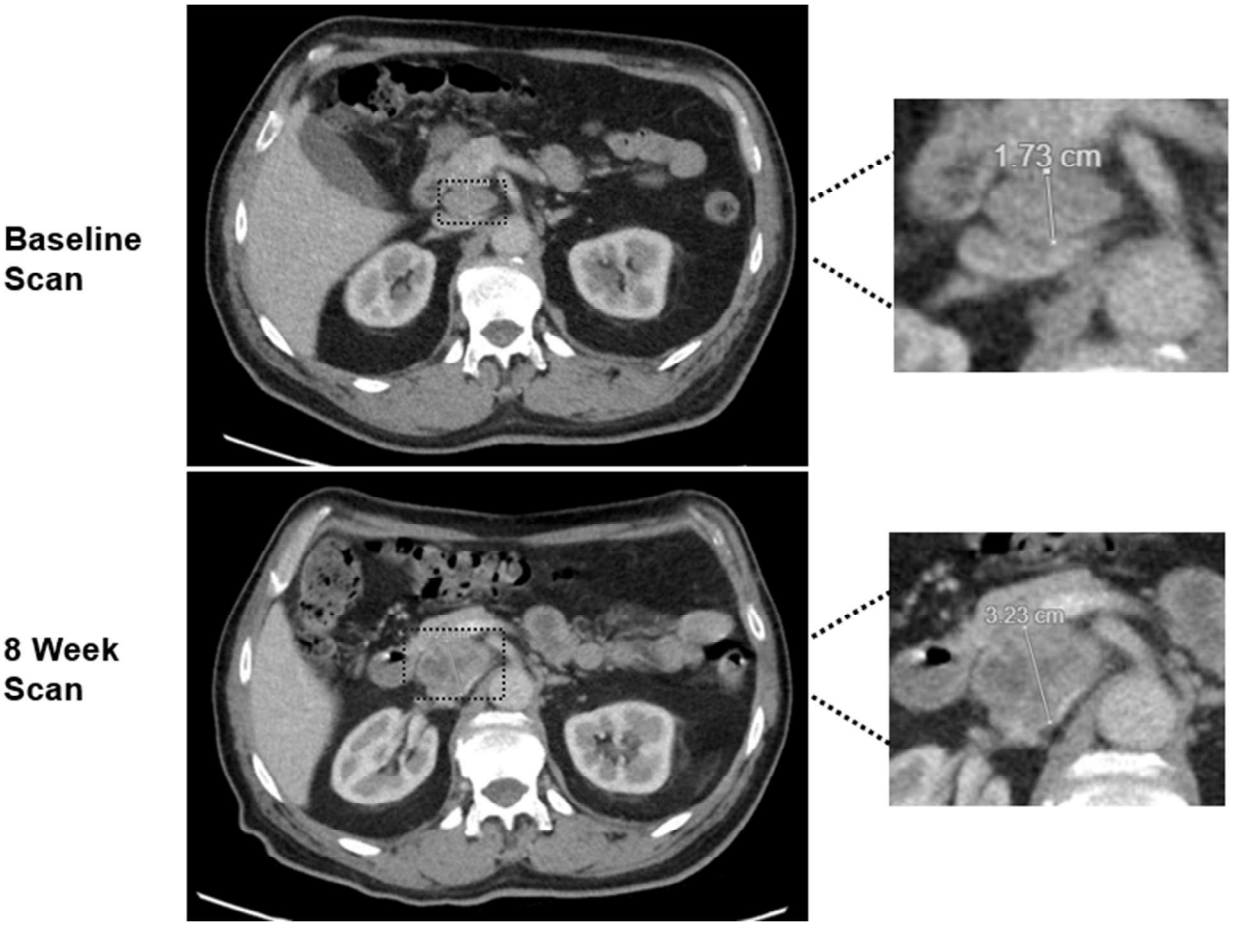


FIG. 46C

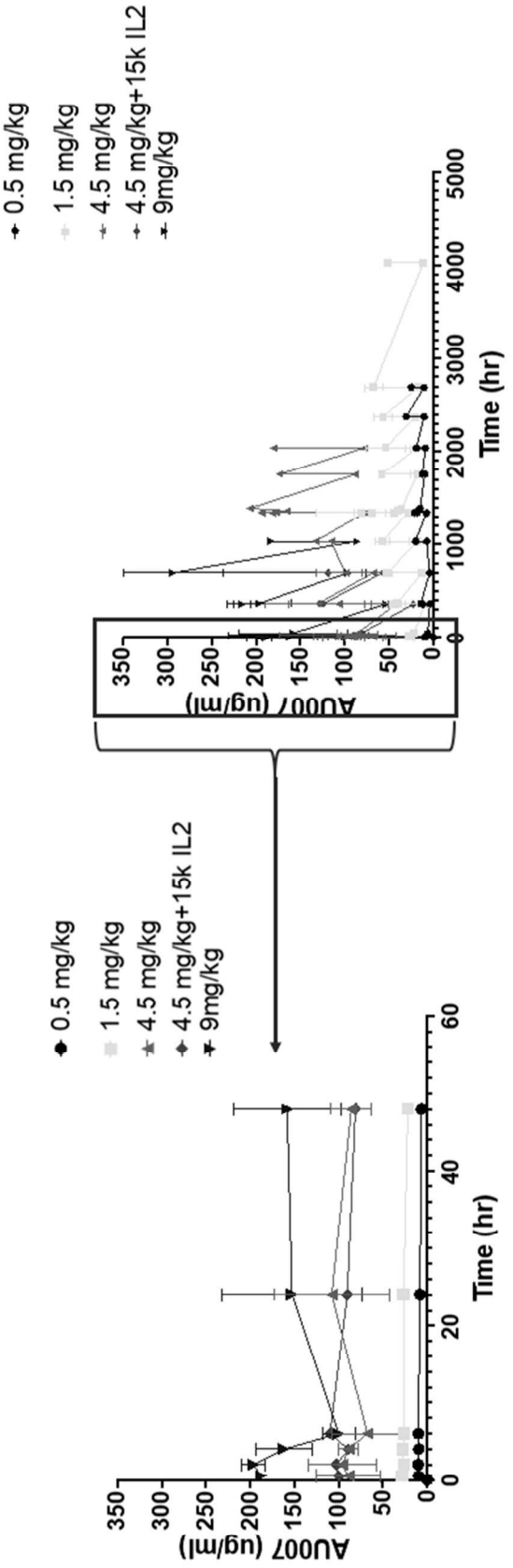

0.5 mg/kg
1.5 mg/kg
4.5 mg/kg
4.5 mg/kg+15k IL2
9mg/kg
AU007 (ug/ml)
Time (hr)
0
50
100
150
200
250
300
350
0
20
40
60
0
1000
2000
3000
4000
5000

FIG. 47

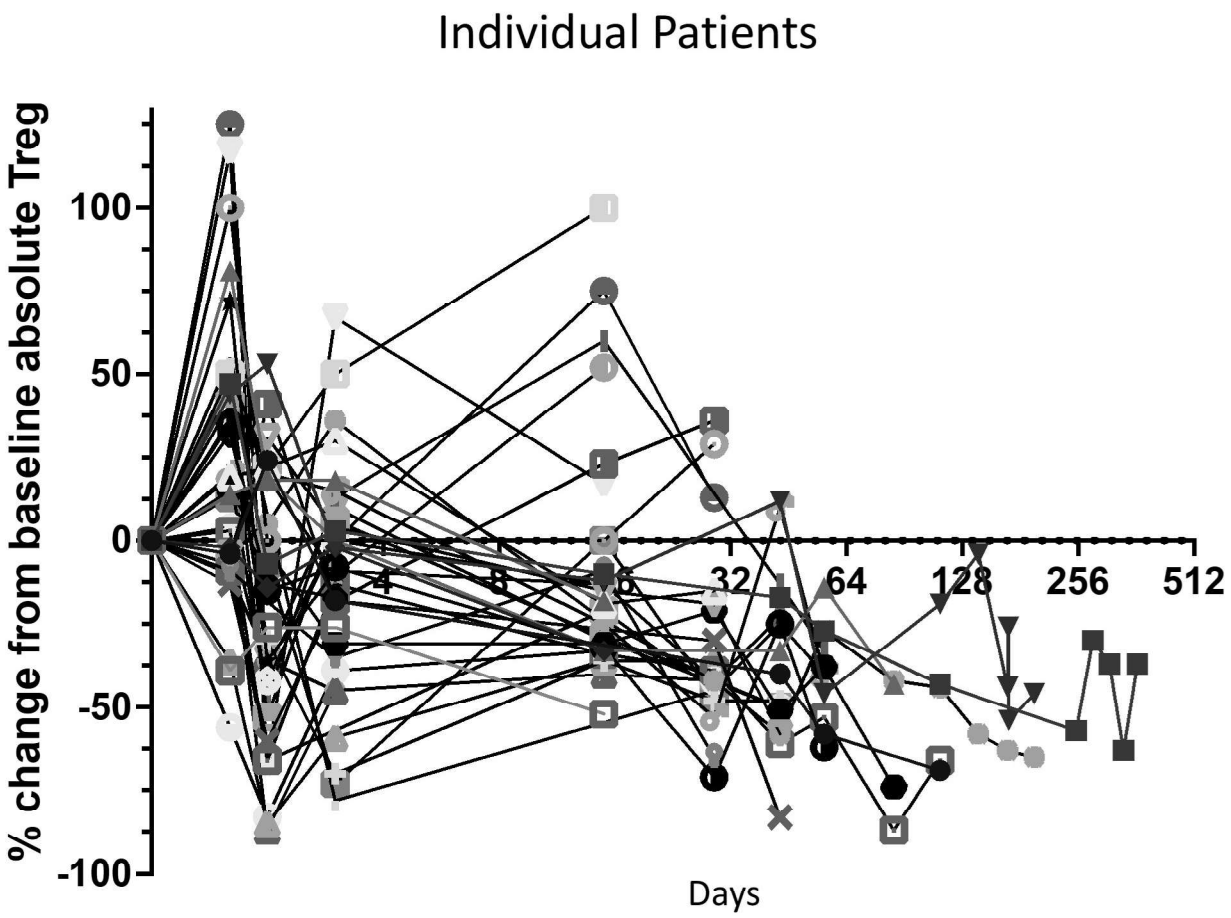

Individual Patients
% change from baseline absolute Treg
100
50
0
-50
-100
4
8
16
32
64
128
256
512
Days

FIG. 48A

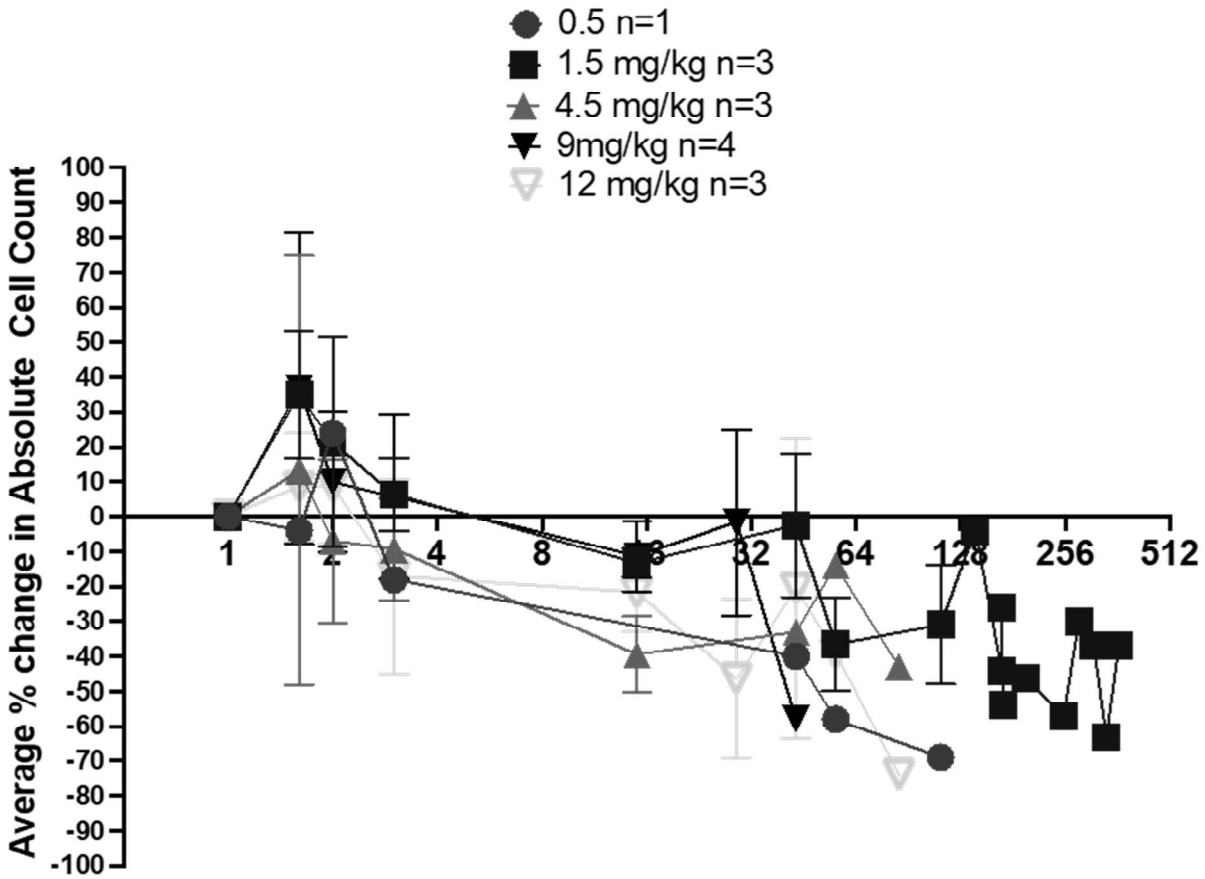

0.5 n=1
1.5 mg/kg n=3
4.5 mg/kg n=3
9mg/kg n=4
12 mg/kg n=3
Average % change in Absolute Cell Count
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
1
4
8
32
64
256
512

FIG. 48B

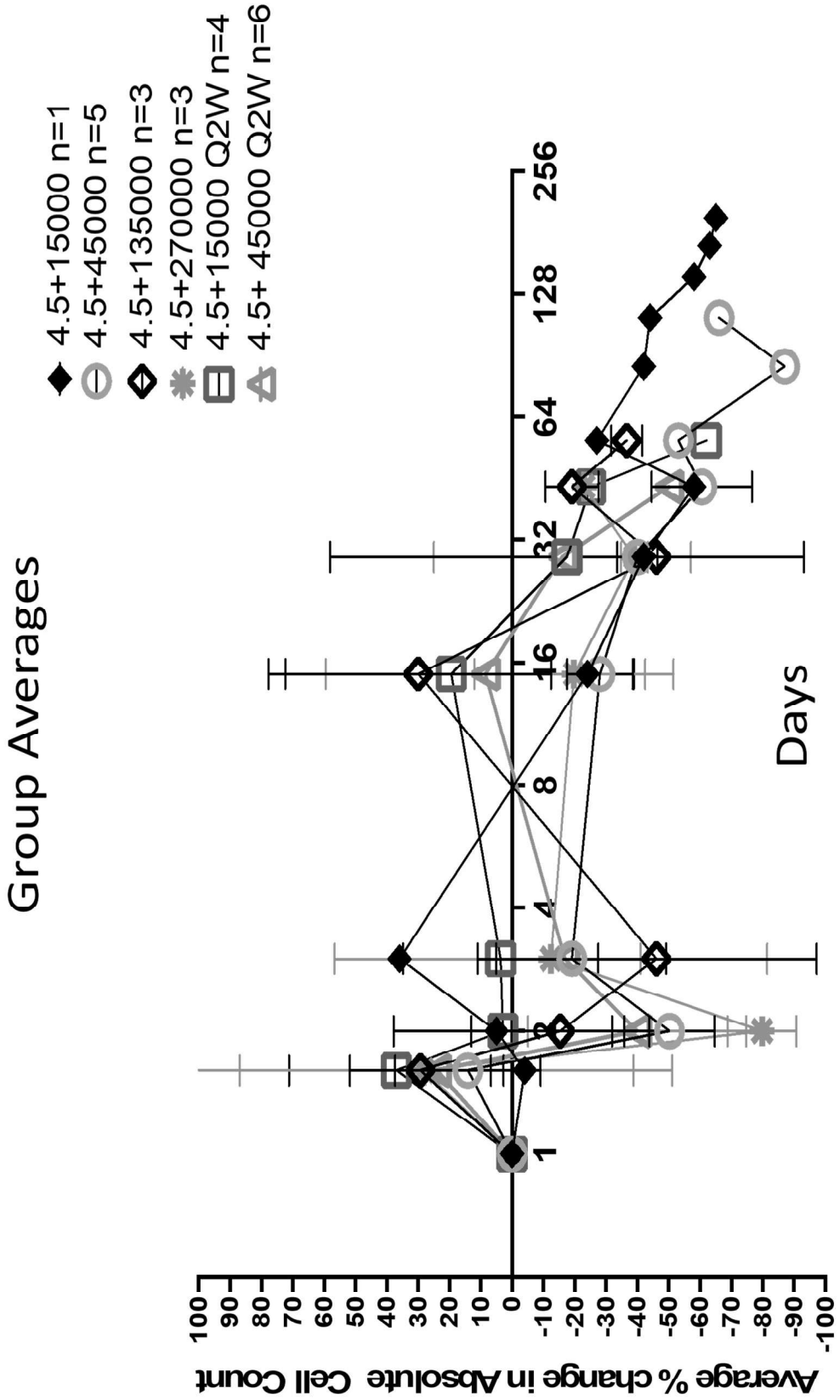

Group Averages
4.5+15000 n=1
4.5+45000 n=5
4.5+135000 n=3
4.5+270000 n=3
4.5+15000 Q2W n=4
4.5+ 45000 Q2W n=6
Average % change in Absolute Cell Count
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
1
2
4
8
16
32
64
128
256
Days

FIG. 49

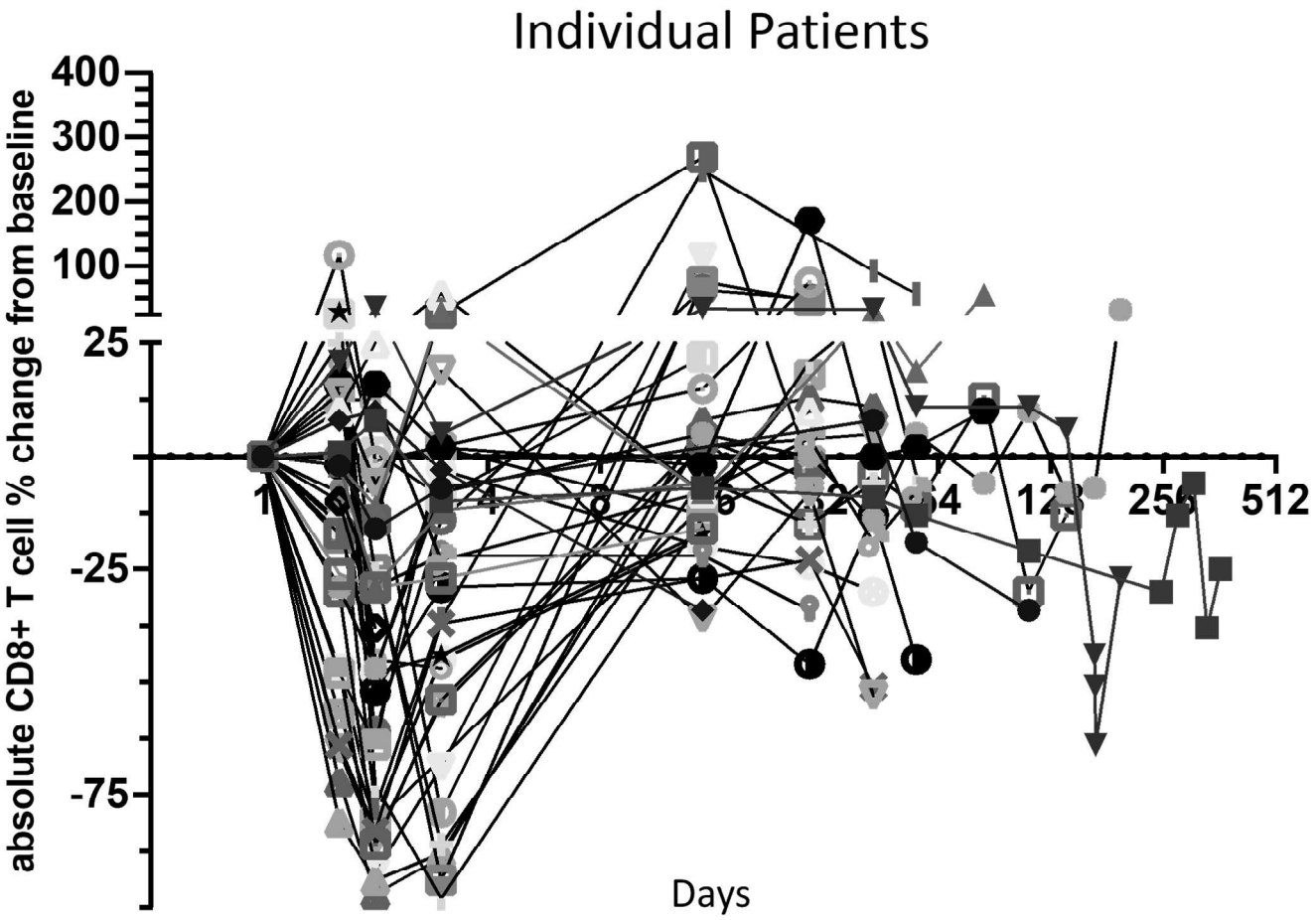

Individual Patients
absolute CD8+ T cell % change from baseline
400
300
200
100
25
-25
-75
512
Days

FIG. 50A

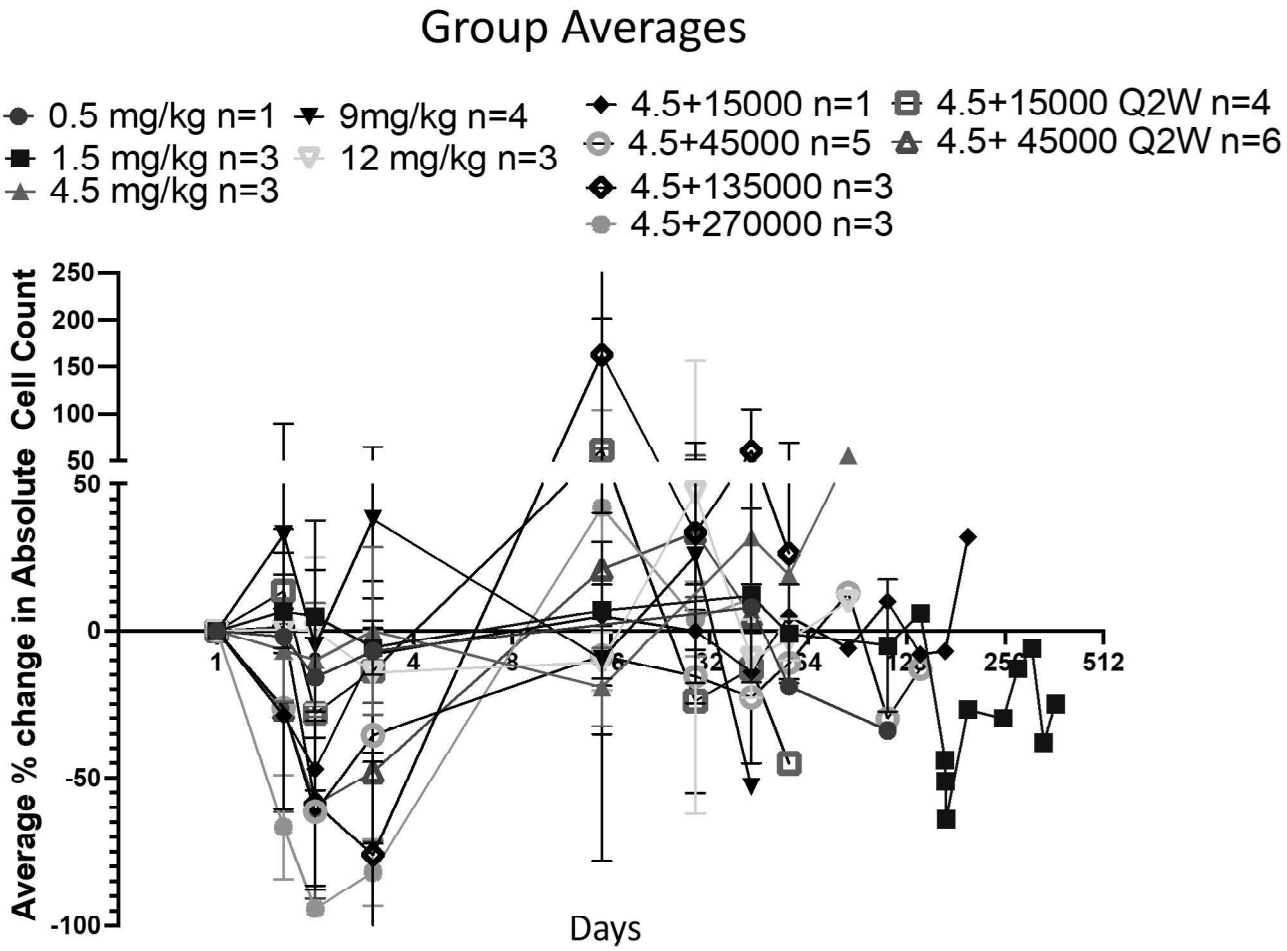

Group Averages
0.5 mg/kg n=1
9mg/kg n=4
1.5 mg/kg n=3
12 mg/kg n=3
4.5 mg/kg n=3
4.5+15000 n=1
4.5+15000 Q2W n=4
4.5+45000 n=5
4.5+ 45000 Q2W n=6
4.5+135000 n=3
4.5+270000 n=3
Average % change in Absolute Cell Count
250
200
150
100
50
50
0
-50
-100
1
4
8
16
32
64
128
256
512
Days

FIG. 50B

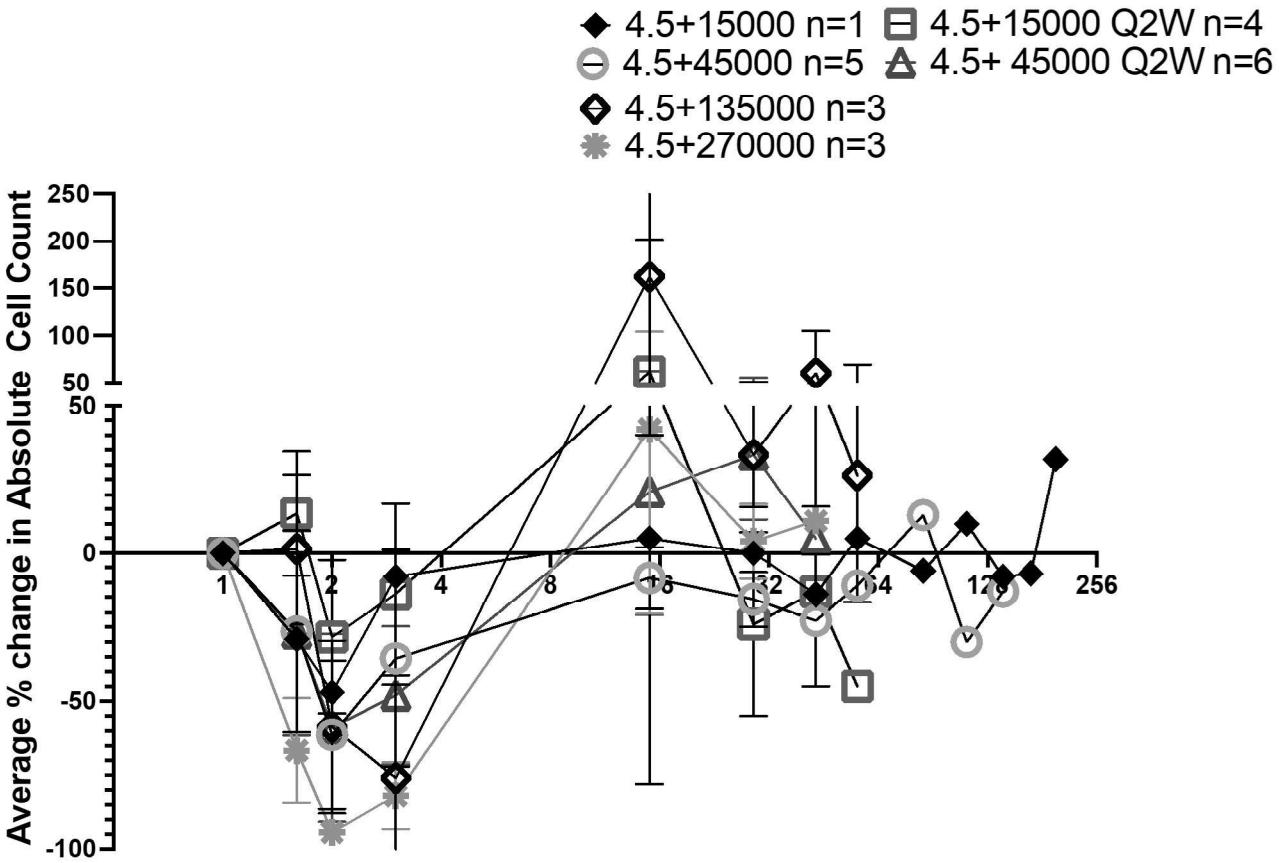

Group averages
4.5+15000 n=1
4.5+15000 Q2W n=4
4.5+45000 n=5
4.5+ 45000 Q2W n=6
4.5+135000 n=3
4.5+270000 n=3
Average % change in Absolute Cell Count
250
200
150
100
50
50
0
-50
-100
1
2
4
8
256

FIG. 51

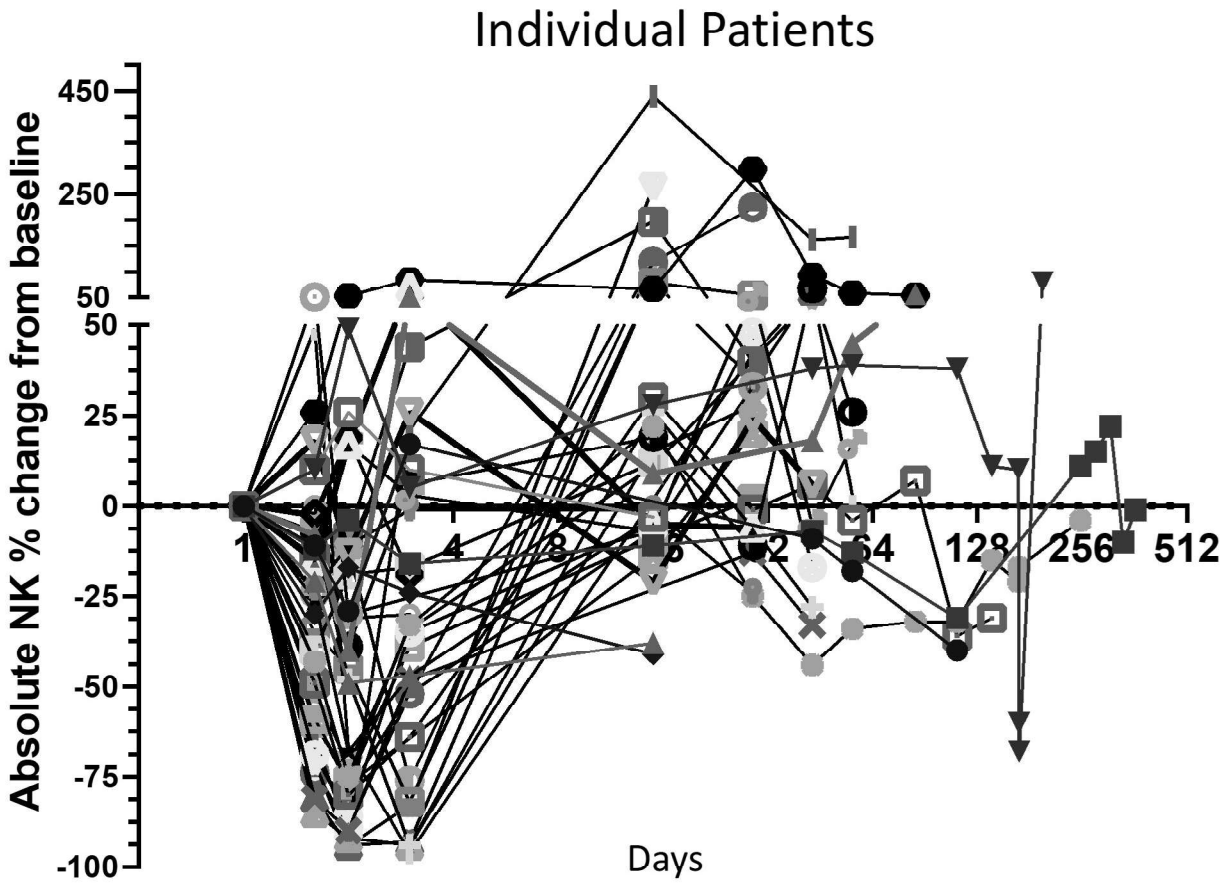

Individual Patients
Absolute NK % change from baseline
450
250
50
50
25
0
-25
-50
-75
-100
1
4
128
256
512
Days

FIG. 52A

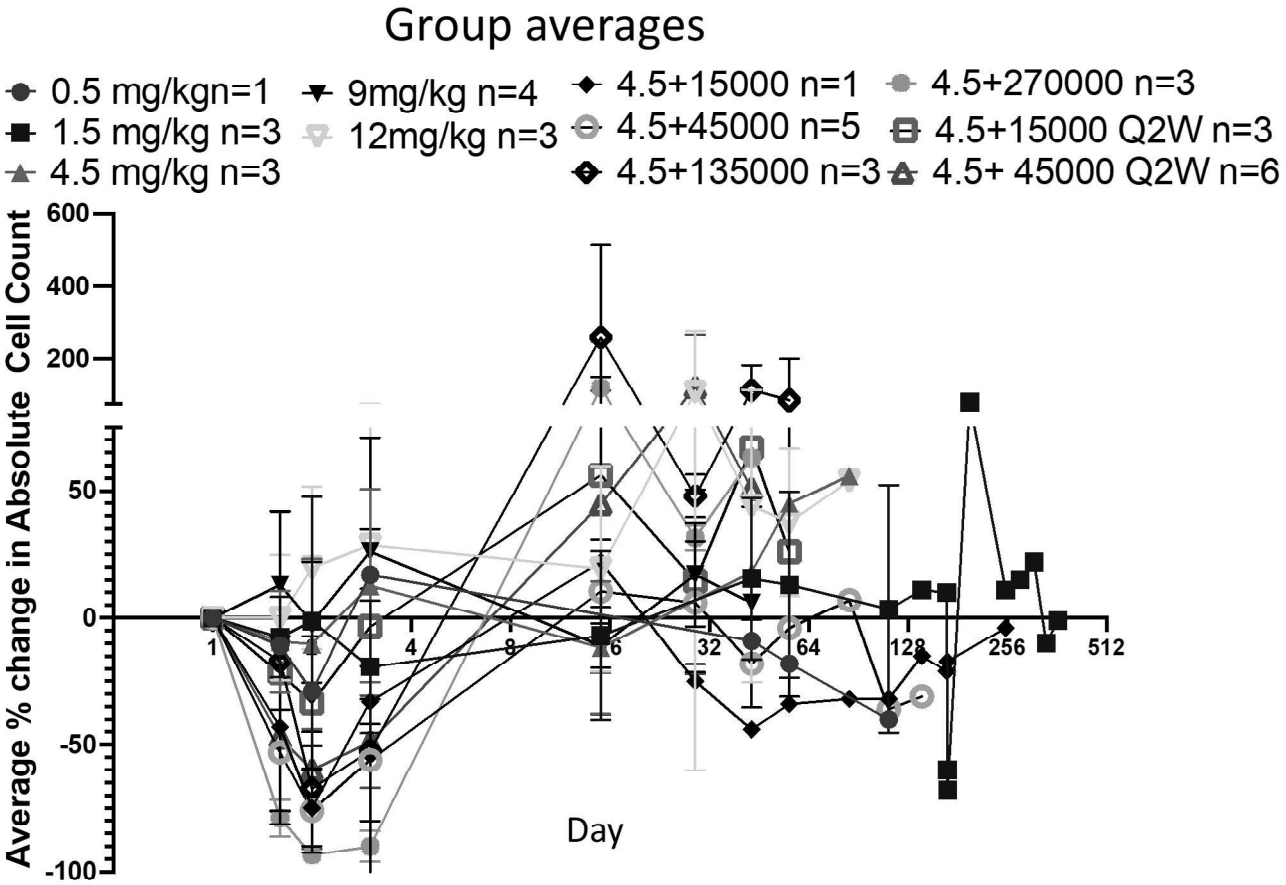

Group averages
0.5 mg/kgn=1
1.5 mg/kg n=3
4.5 mg/kg n=3
9mg/kg n=4
12mg/kg n=3
4.5+15000 n=1
4.5+45000 n=5
4.5+135000 n=3
4.5+270000 n=3
4.5+15000 Q2W n=3
4.5+ 45000 Q2W n=6
Average % change in Absolute Cell Count
600
400
200
50
0
-50
-100
1
4
8
16
32
64
128
256
512
Day

FIG. 52B

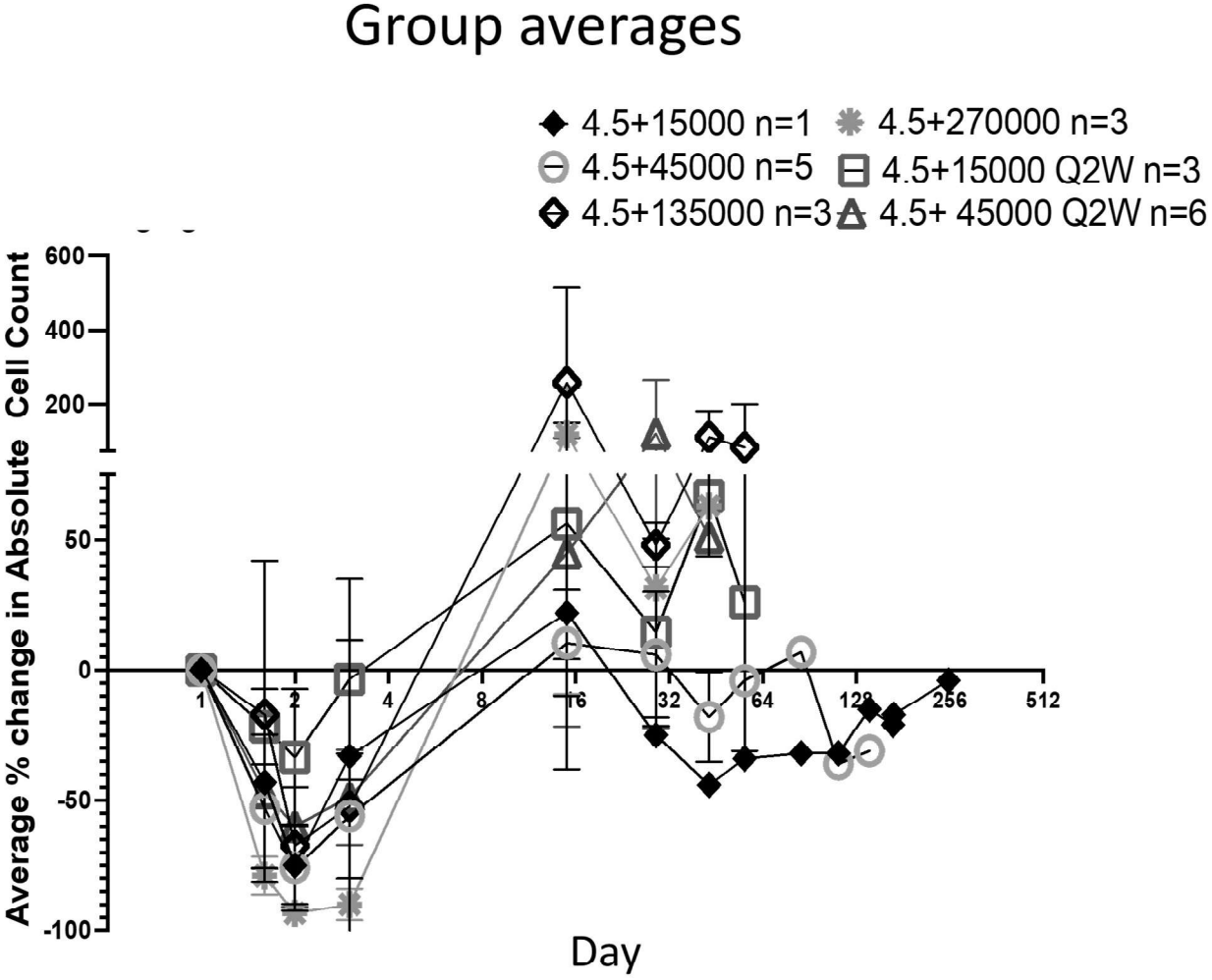

Group averages
4.5+15000 n=1
4.5+270000 n=3
4.5+45000 n=5
4.5+15000 Q2W n=3
4.5+135000 n=3
4.5+ 45000 Q2W n=6
Average % change in Absolute Cell Count
600
400
200
50
0
-50
-100
1
2
4
8
16
32
64
128
256
512
Day

FIG. 53

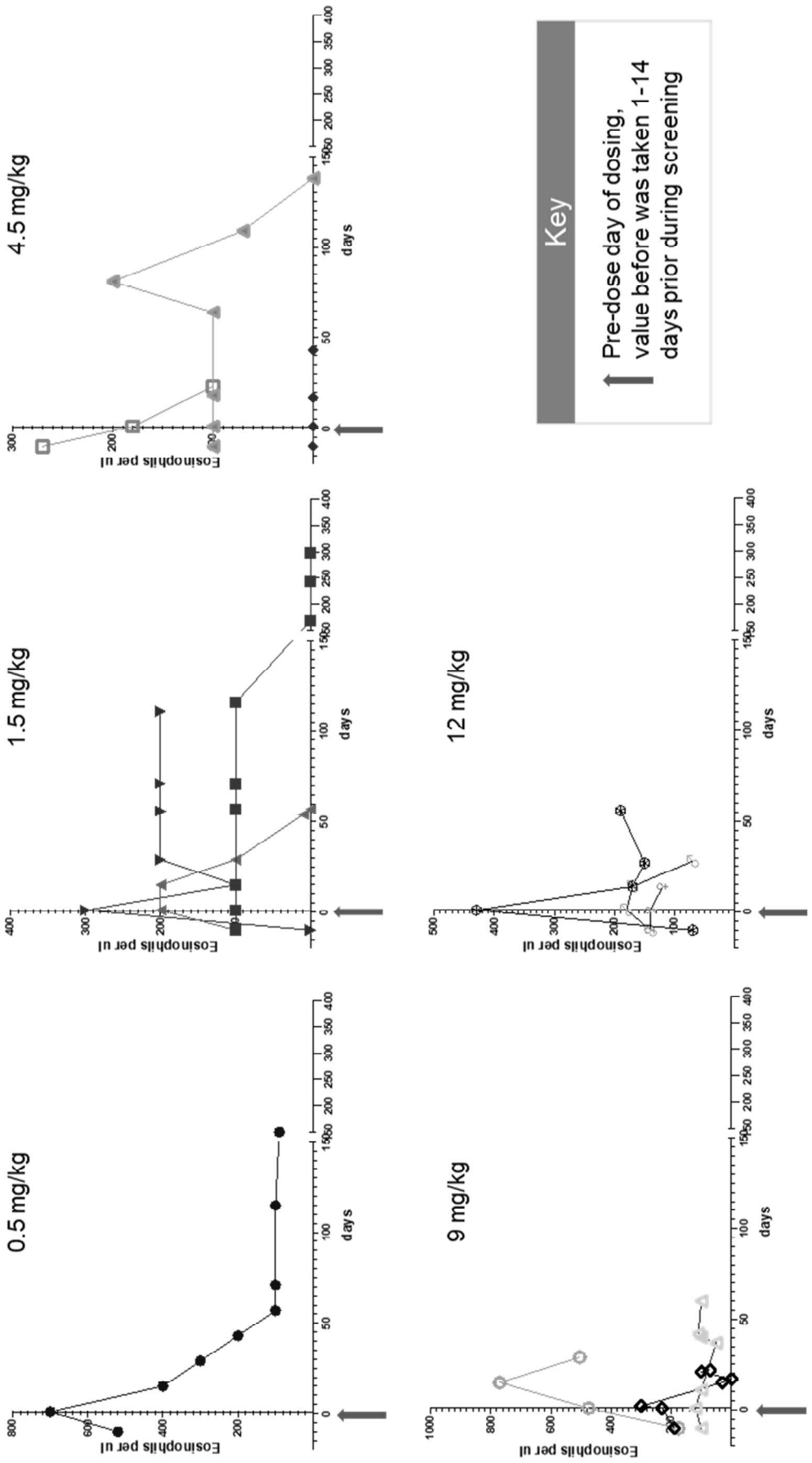

0.5 mg/kg
1.5 mg/kg
4.5 mg/kg
9 mg/kg
12 mg/kg
Eosinophils per ul
days
Key
Pre-dose day of dosing, value before was taken 1-14 days prior during screening

FIG. 54A

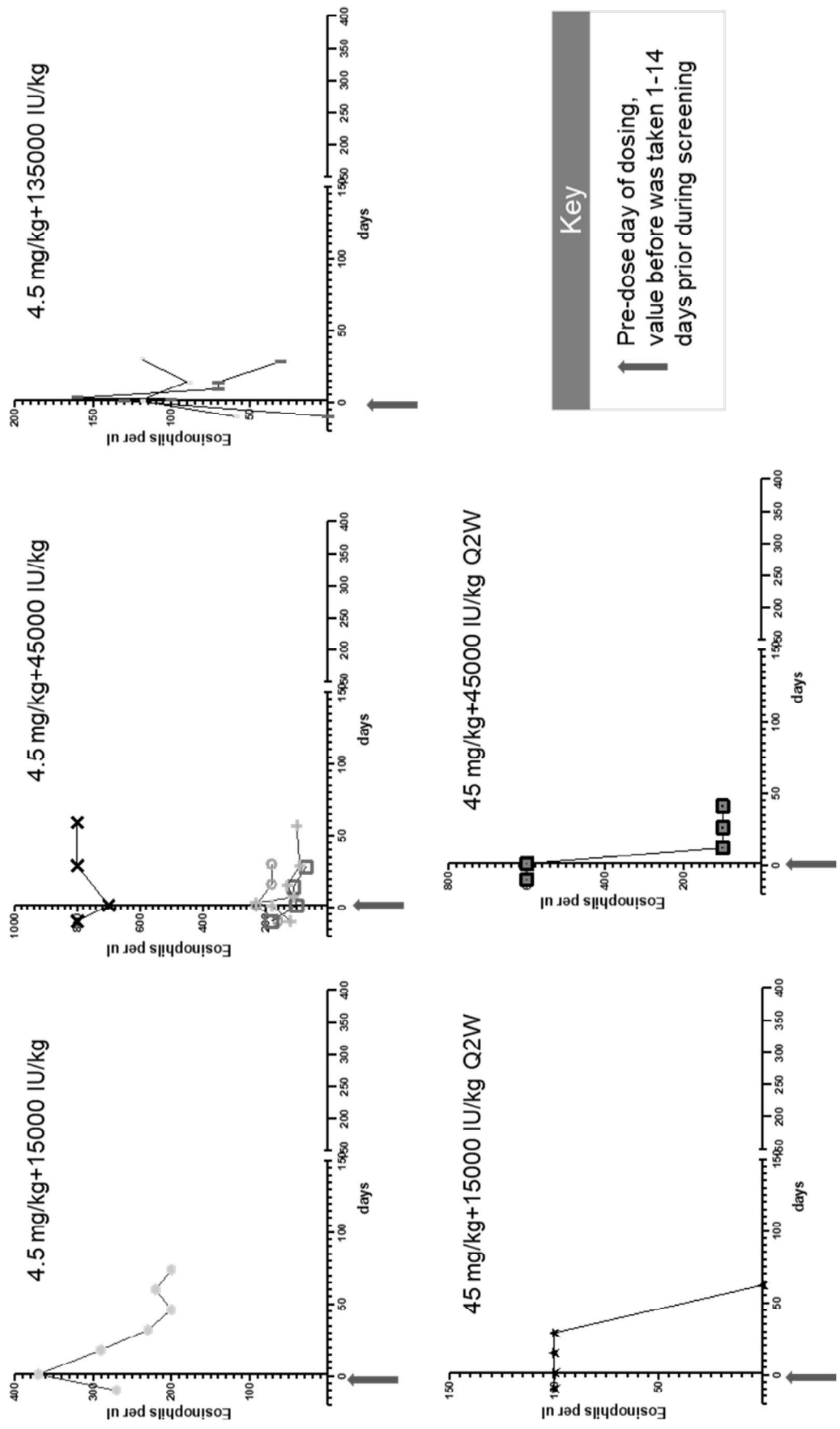

4.5 mg/kg+135000 IU/kg
4.5 mg/kg+45000 IU/kg
4.5 mg/kg+15000 IU/kg
45 mg/kg+45000 IU/kg Q2W
45 mg/kg+15000 IU/kg Q2W
Eosinophils per ul
days
Key
Pre-dose day of dosing, value before was taken 1-14 days prior during screening

FIG. 54B

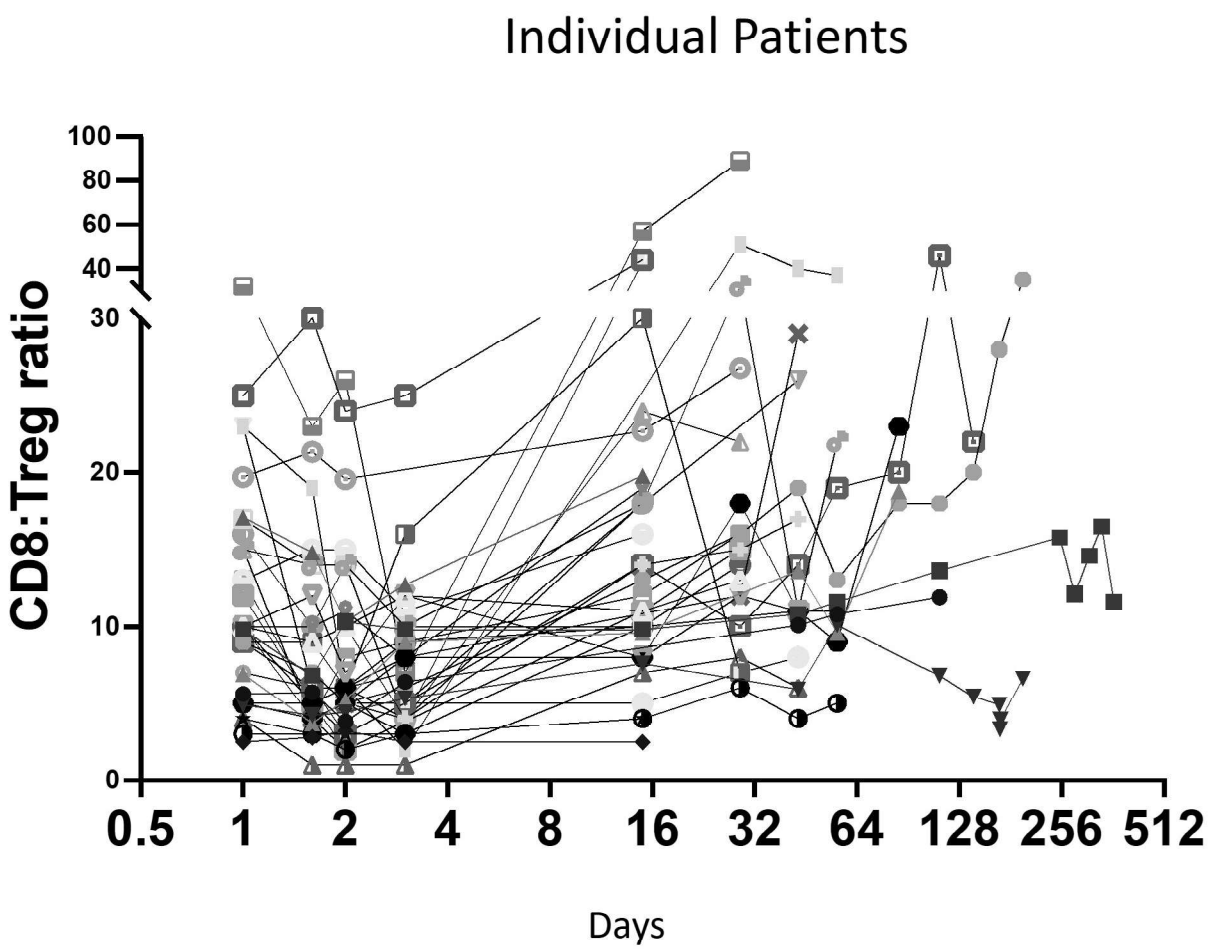

Individual Patients
CD8:Treg ratio
100
80
60
40
30
20
10
0
0.5
1
2
4
8
16
32
64
128
256
512
Days

FIG. 55A

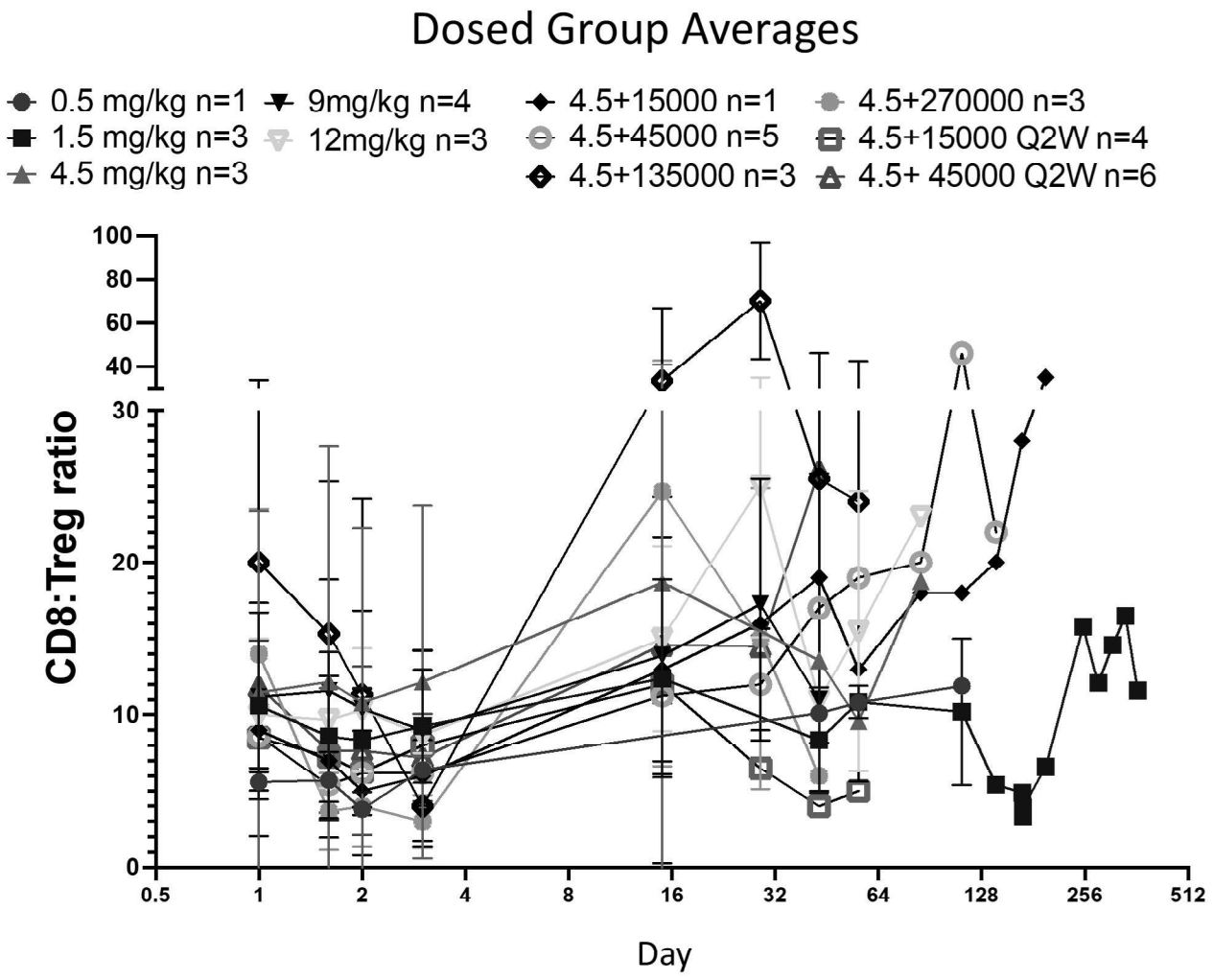

Dosed Group Averages
0.5 mg/kg n=1
1.5 mg/kg n=3
4.5 mg/kg n=3
9mg/kg n=4
12mg/kg n=3
4.5+15000 n=1
4.5+45000 n=5
4.5+135000 n=3
4.5+270000 n=3
4.5+15000 Q2W n=4
4.5+ 45000 Q2W n=6
CD8:Treg ratio
100
80
60
40
30
20
10
0
0.5
1
2
4
8
16
32
64
128
256
512
Day

FIG. 55B

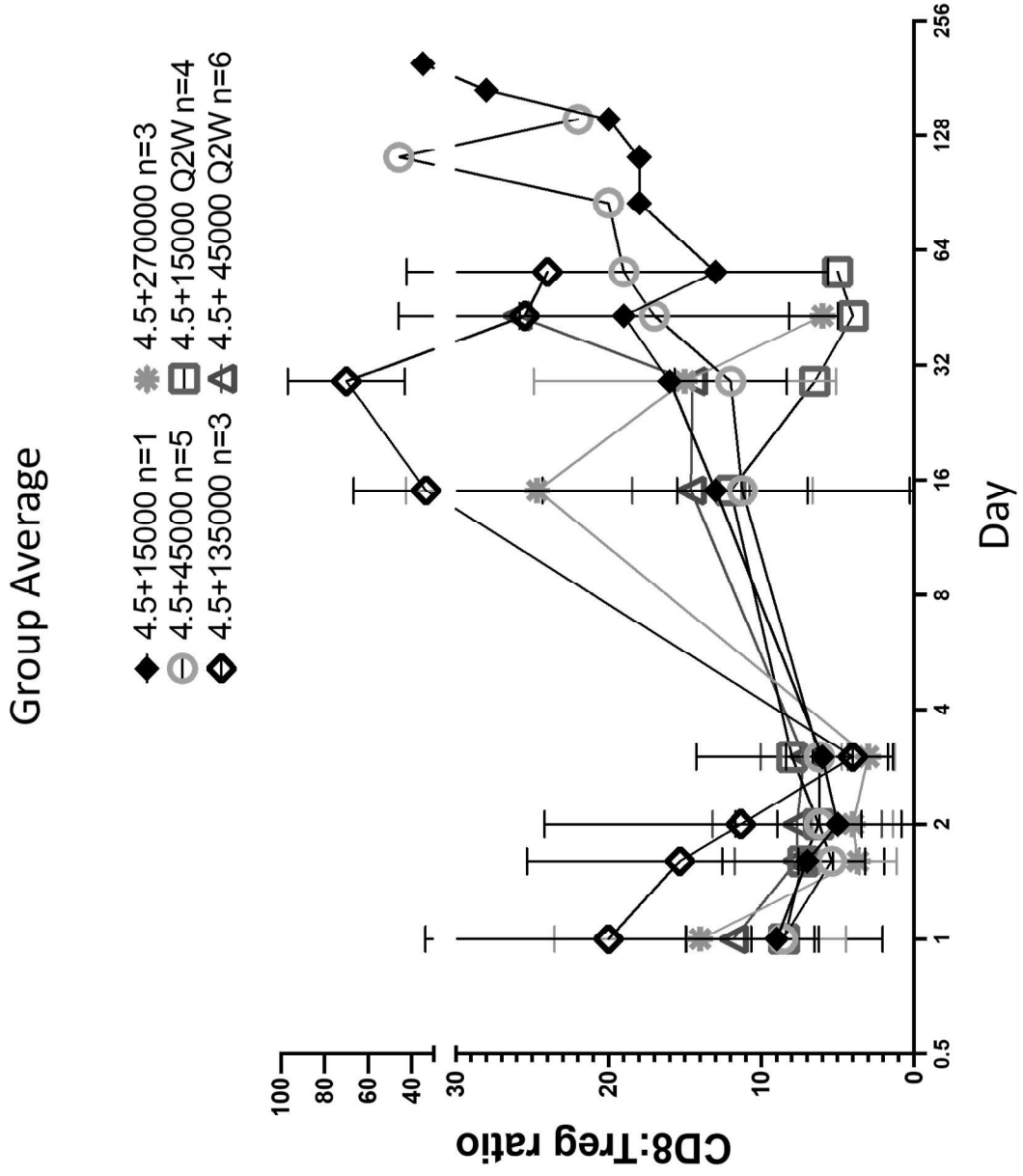

Group Average
4.5+15000 n=1
4.5+45000 n=5
4.5+135000 n=3
4.5+270000 n=3
4.5+15000 Q2W n=4
4.5+ 45000 Q2W n=6
CD8:Treg ratio
Day
0
10
20
30
40
60
80
100
0.5
1
2
4
8
16
32
64
128
256

FIG. 55C

Individual fold changes over time in IFN-γ

1.2-2
2-5
>5

Time (hr)	A3-02/0.5 HN	A1-05/1.5 NSC	A3-03/1.5 Pan	A3-07/1.5 UT	A1-08/4.5 ROC	A8-09/4.5 Mel	A5-010/4.5 Cerv	A3-011/9 GBla	A6-012/9 Mel	U1-13/9 CRC	u3-18/9 thy	u4-27/12 pan	U4-29/12 U.Mel
0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	1.24838475	0.94447807	1.28209552	0.89106909	0.9178304	0.88285204	0.97566917	1.18593525	0.73330366	1.18872017	0.85432099	0.86536323	1.01104653
6	1.38147238	1.23024241	1.02121024	1.1996059	1.12993321	0.86851354	0.90014332	1.03075439	0.72341846	1.29211858	0.79423868	0.7104954	1.04900697
24	1.23555374	0.8898162	0.80048417	0.81953617	NS	1.12680816	0.80774829	0.9436295	6.33811829	4.22559653	0.80246914	0.60536147	1.21941034
48	1.25666576	5.46395693	0.78688101	0.80556939	0.89732645	1.02618093	0.96062612	0.99517061	0.79057475	2.24005785	2.86419753	0.28983268	0.99516955
360	3.9037219	1.40837129	0.64726281	0.99653537	0.76209269	1.06060268		0.52937188	0.5596172		0.93415638	0.55094244	1.07214888
361	4.87464737	1.29197592	0.84501533	1.00012992	0.73360656	1.01740614		0.86244152	0.50753309		0.81069959	0.44987247	1.07668104
366	7.35940486	1.32252413	1.64488783	1.03310885	0.90356203	1.70884573		1.13197568	0.4535136		0.92592593	0.38931516	1.37629422
696	2.38329238	1.18593317	0.53123815	1.01190966	0.81181947	1.0035147		0.56578949	0.61592389			0.39032057	1.92448328
697	NS	1.18297073	0.54670216	1.06071761		0.97747723		0.56303445	0.63574992			0.41218555	1.91787268
1032	2.1020566	0.97031185		1.40871787		1.18208698		18.6805762	4.64327973			0.68755072	2.65382395
1033	1.7987988	0.92202083		1.39726294		1.0601484		16.2059535	3.88415957			0.74760025	2.35986875
1344	2.14773865	0.98821393		0.80297092		0.6789907						0.30449047	2.5114603
1341	2.12990283	0.82234893		0.86254283		0.6741291						0.27008435	2.14415342
1704	3.09177359	1.82792342		1.21608454		0.94229834						0.58078718	
1705	2.63508964	1.43391839		NS		0.86307811						0.62204066	
2376	5.03555101	0.83400758		0.93447522		6.73451659							
2377	3.42114842	0.75277928		0.97262944		5.45934185							
2688	2.94699245	0.82059695		0.82265434									
2689	2.18918919	0.85446482		0.85050129									
4031		2.71184659		1.18903878									
4032		2.85391648		1.33927373									
4034		NS		1.28178255									
4038		NS		1.33017908									
4056		NS		2.35707326									
5376		13.0602364											
5377		19.5118976											
6720		1.4174816											
6721		1.15334629											
8064		2.19262256											
8065		2.04762208											

NS=no sample available at analysis

FIG. 56A

Individual fold changes over time in IFN-γ

1.2-2
2-5
>5

Time (hr)	A6-01/4.5-15 Mel	U1-16/4.5-45 CRC	A1-17/4.5-45 NSC	U3-38/4.5-45 Mel	U3-21/4.5-45 Chol	A1-38/4.5-45 renal Sq	U4-35/4.5-45 HCC	U1-30/4.5_135 A Mel	A1-36/4.5-270 Mel	U4-48/4.5-270 Panc	U4-42/4.5-270 Anal SCC	A1-15/4.5+15 Q2W CRC	U3-33.4.5-15 RCC Q2W	A3-19/4.5-45 Q2W RCC	U3-22/4.5-45 Q2W Rec	A3-02/04.5-45 Q2W ccRCC	A6-31/4.5-45 Q2W Mac Mel	U1-23/4.5-45 Q2W Pan
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	1.00327576	1.28267668	0.8668781	0.93170581	1.01215508	0.94442943	0.9457513	0.85668611	0.82493651	1.02637866	0.82713221	0.98433097	0.95056651	1.07943882	1.03597933	1.89044532	0.62999533	0.999133317
5	1.01419159	1.28858725	2.8646732	2.43318226	1.88649389	2.2756872	1.53364751	0.72610935	11.9965868	0	28.6729321	1.25284747	1.3364825	2.82557878	1.4714794	4.22317248	0.42737372	1.194683262
24	2.01663315	1.16799059	3.6477464	6.0774622	1.61144767	3.03305092	2.03879316	0.61866625	24.8691818	27.6096606	0	1.84617793	2.49506586	4.65591291	2.94770246	3.84667485	1.76901513	1.362677958
48	0.9906634	21.8355551	1.61567879	7.44416076	1.78531759	1.4044842	1.37493244	13.0962716	23.6541772	17.6162826	6.04618184	0.57483316	2.0997667	2.21388451	0.4495627	4.02479333	1.35956576	6.166342195
360	16.1207643	0.94552345	NS	1.73156484	2.87593977	1.29576865	1.49171933	0.99096529	9.87483976	1.55141249		1.833379	3.38956885	1.13261962		0	0.49340492	0.733481627
361	15.4072296	0.97147409	NS	1.69973218	2.88873365	1.16942752	1.51324568	1.0552061	9.53253326	1.52866485		1.48514662	3.2356231	1.33788631		0	0.3891668	0.741139651
366	1.35212425	1.45499892	NS	1.82110674	3.62278199		2.05877186	1.27004613	3.2883432	1.45077586		2.24631501	2.82799792	3.43262524		0	0.33290362	0.84888852
696	9.85215533	0.7591099	0.71961621	1.61382365	1.87361136		0.89693212					1.78171357	2.09415585	1.46338429		1.30892787		
697	6.47445882	0.75251389	0.69177589	1.85857025	1.92022881							1.59782895	1.80017582	1.37646747		0.71018923		
1032	18.5086664	0.86889993	0.22910444	3.50384143	1.68097817							3.68615019						
1033	19.7541321	0.85347184	0.19790045	3.47366433	0.88449309							2.82338306						
1344	10.7736468			1.33666142														
1341	10.4573765			1.16766628														
1704	16.1474649			1.8215641														
1705	13.5512644			1.54291993														
2376	1.61536491			1.01555418														
2377	1.57243389			1.00666619														
2688	1.36147486			27.4402188														
2689	1.10067633			24.6645732														
4031																		
4032																		
4034																		
4036																		
4056																		
5376																		
5377																		
6720																		
6721																		
8064																		
8065																		

NS=no sample available at analysis

FIG. 56B

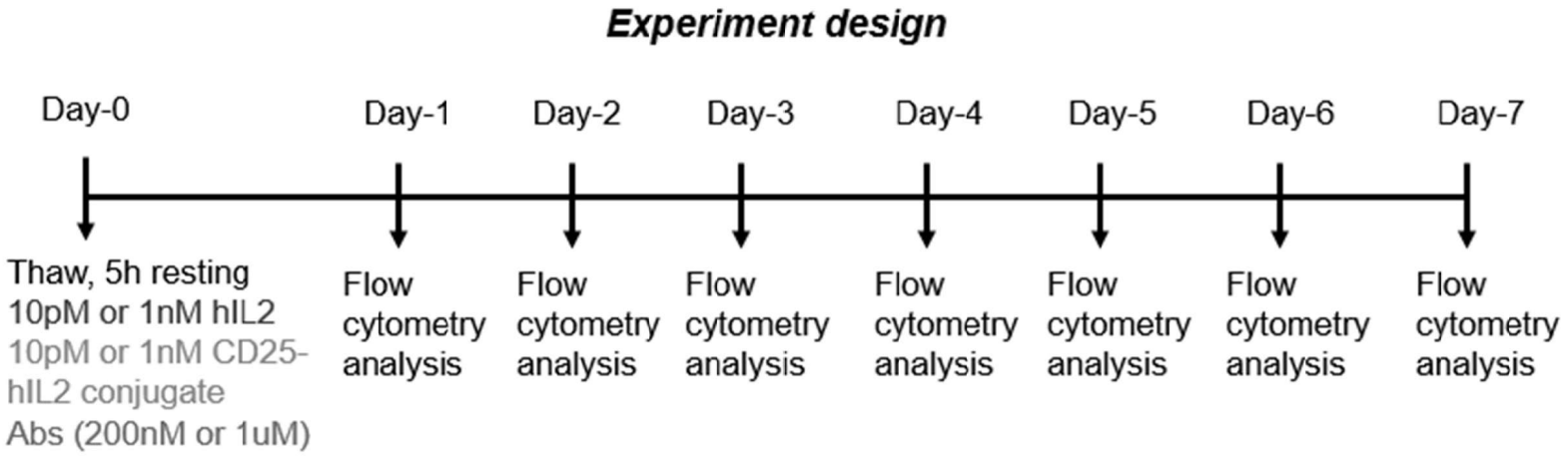

Experiment design
Day-0
Day-1
Day-2
Day-3
Day-4
Day-5
Day-6
Day-7
Thaw, 5h resting
10pM or 1nM hIL2
10pM or 1nM CD25-hIL2 conjugate
Abs (200nM or 1uM)
Flow cytometry analysis
Flow cytometry analysis
Flow cytometry analysis
Flow cytometry analysis
Flow cytometry analysis
Flow cytometry analysis
Flow cytometry analysis

FIG. 57

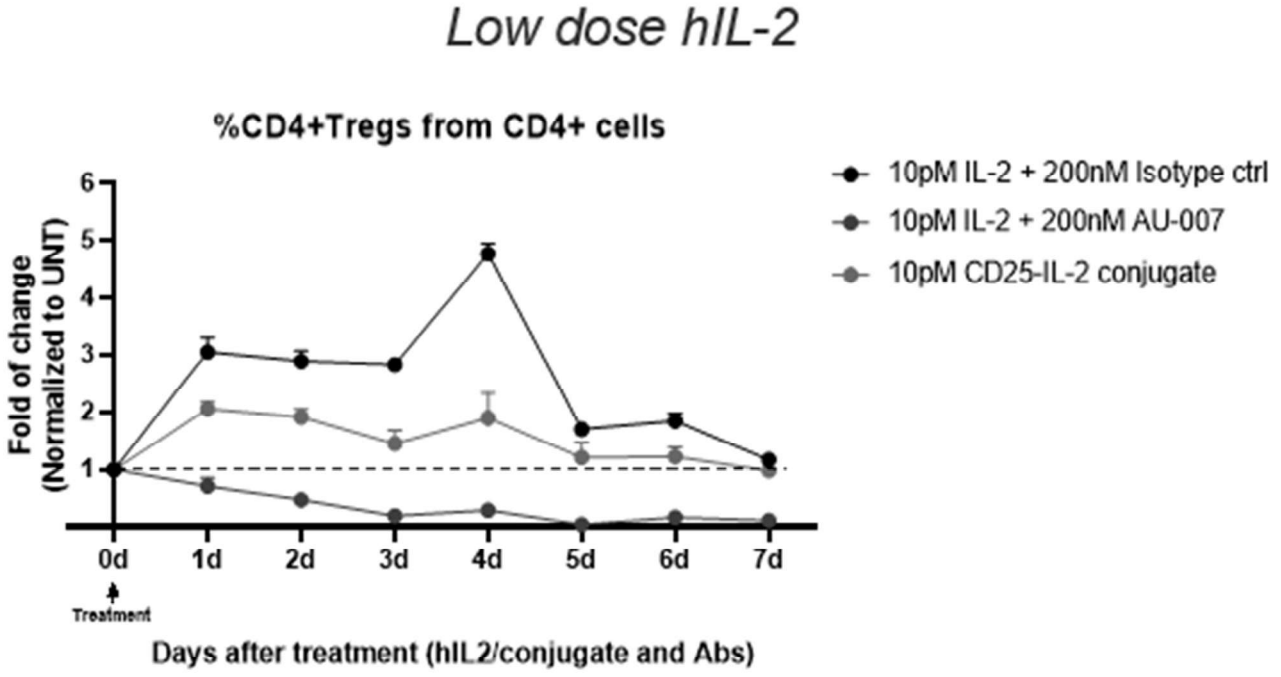


FIG. 58A

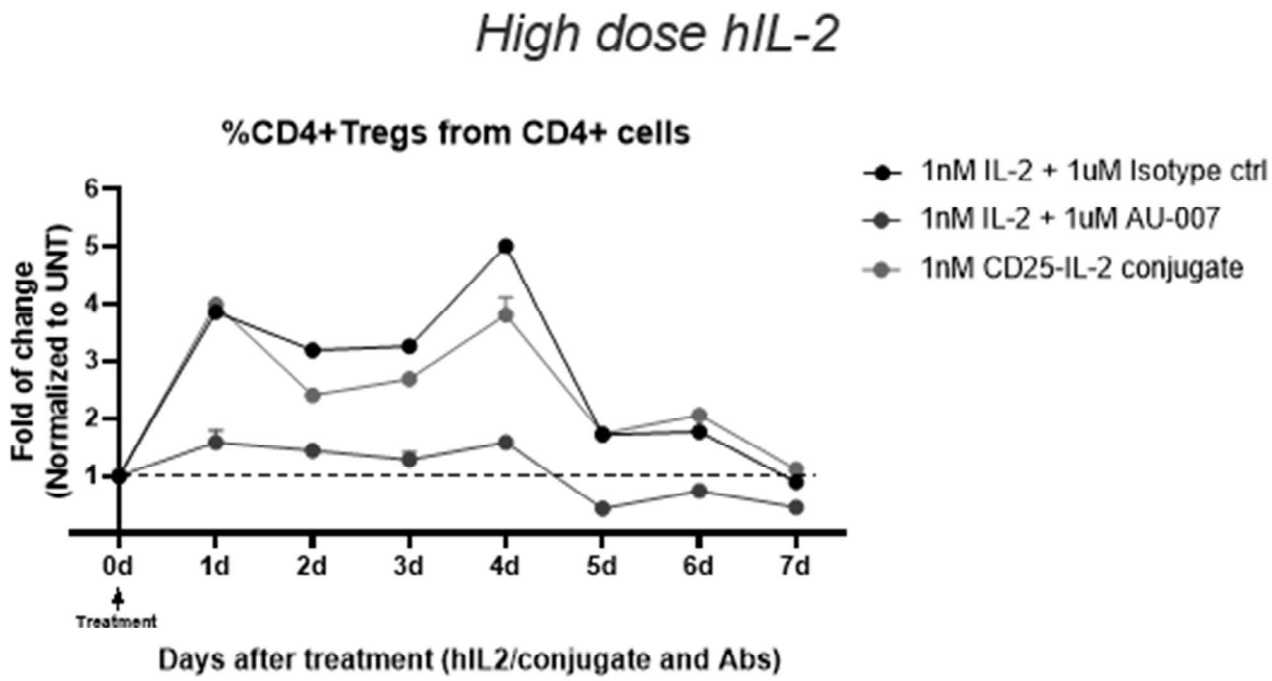


FIG. 58B

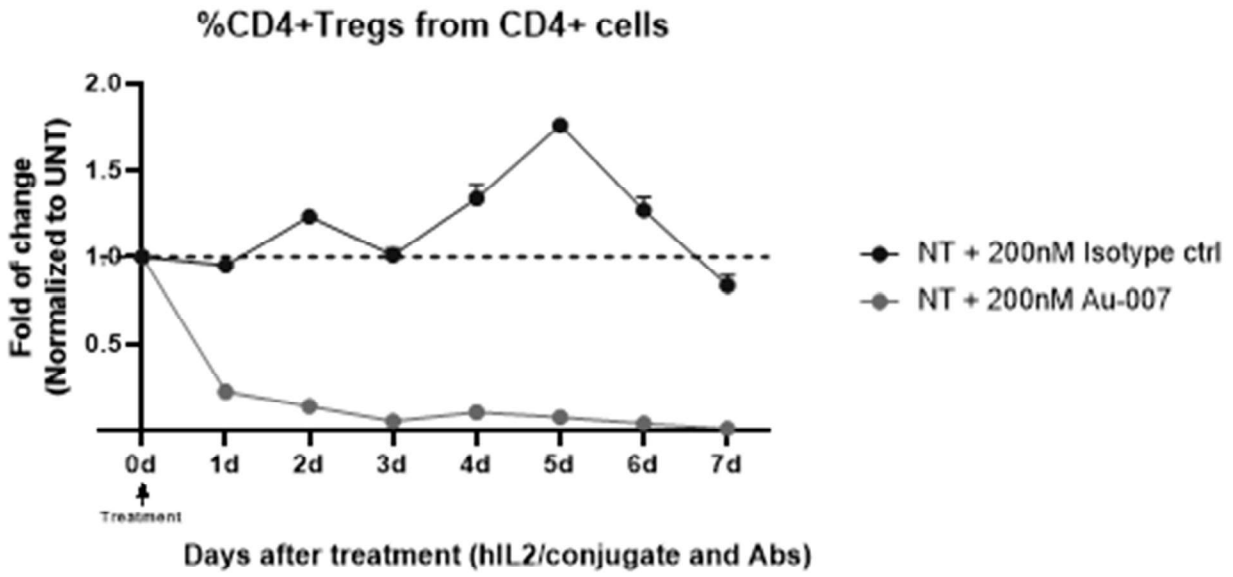


FIG. 59A

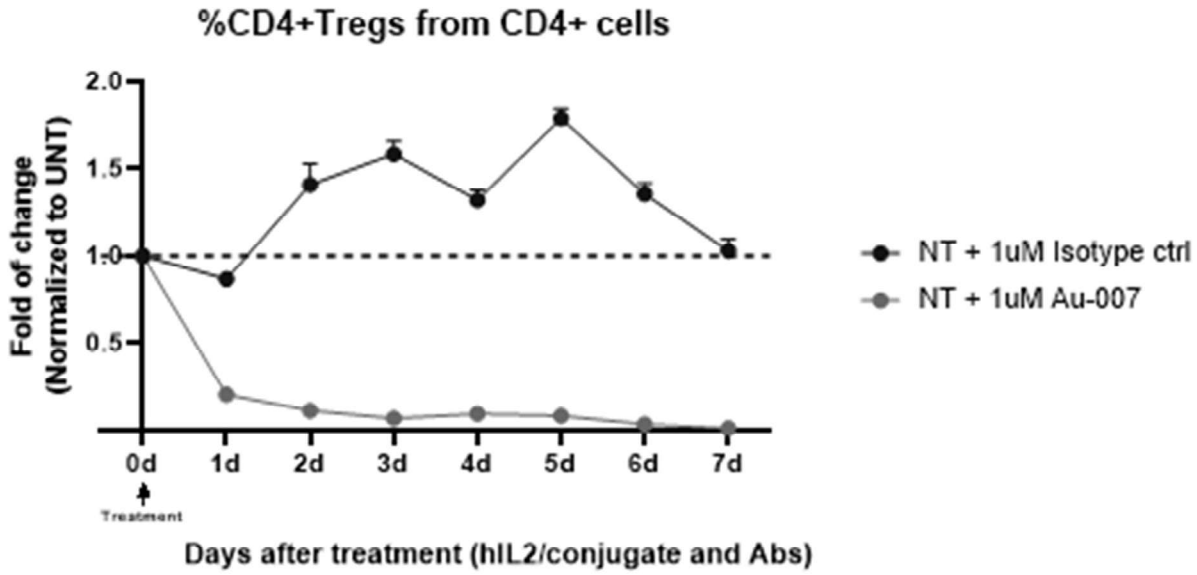


FIG. 59B

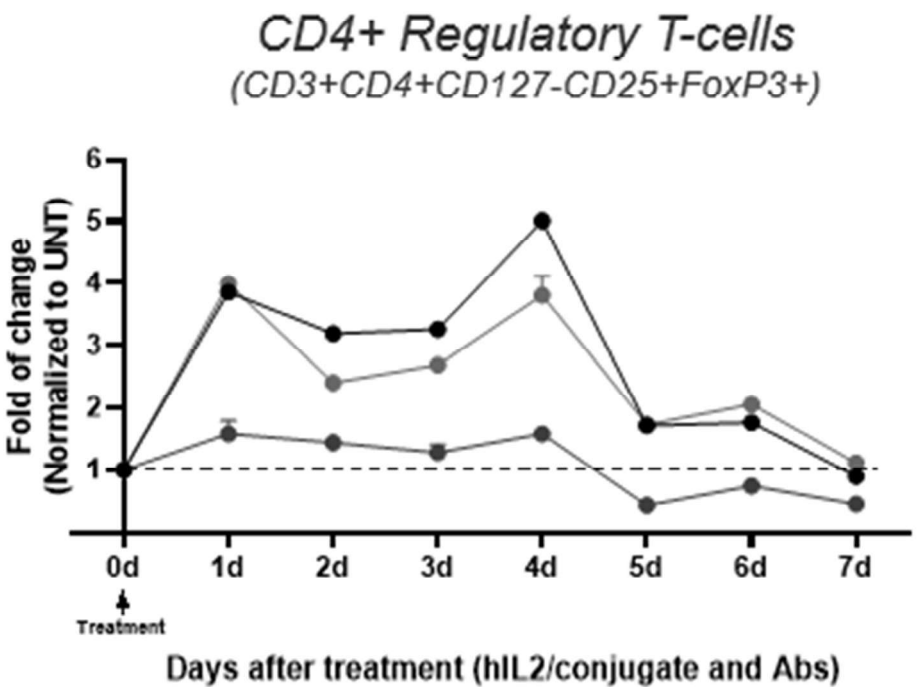


FIG. 60A

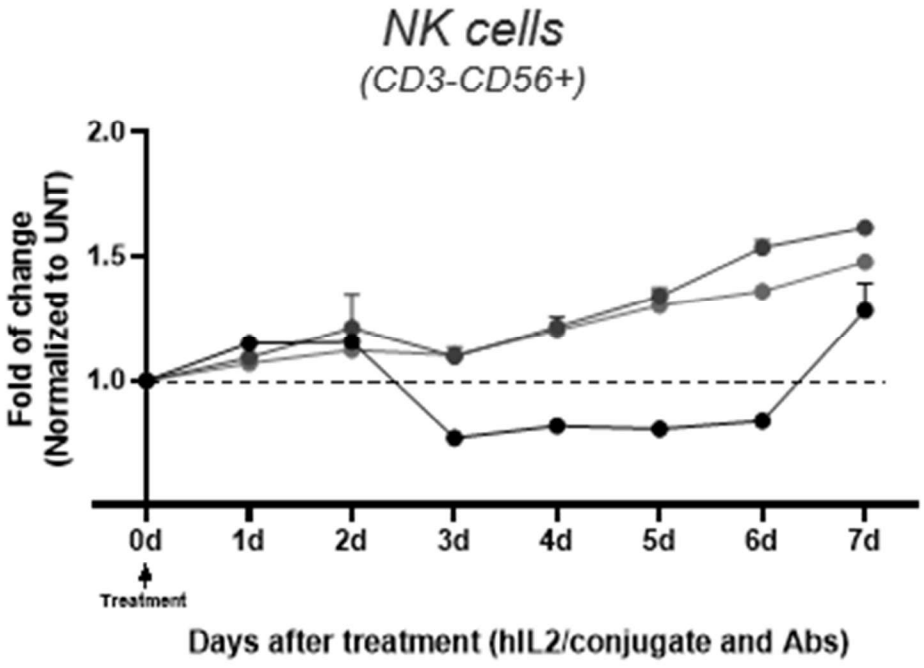


FIG. 60B

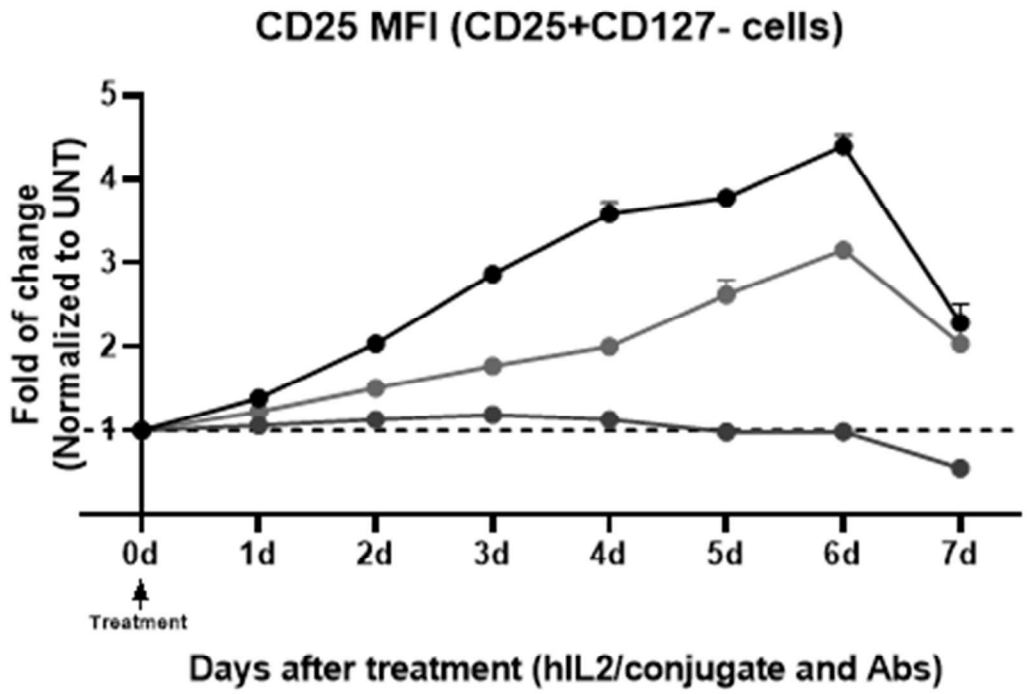


FIG. 61A

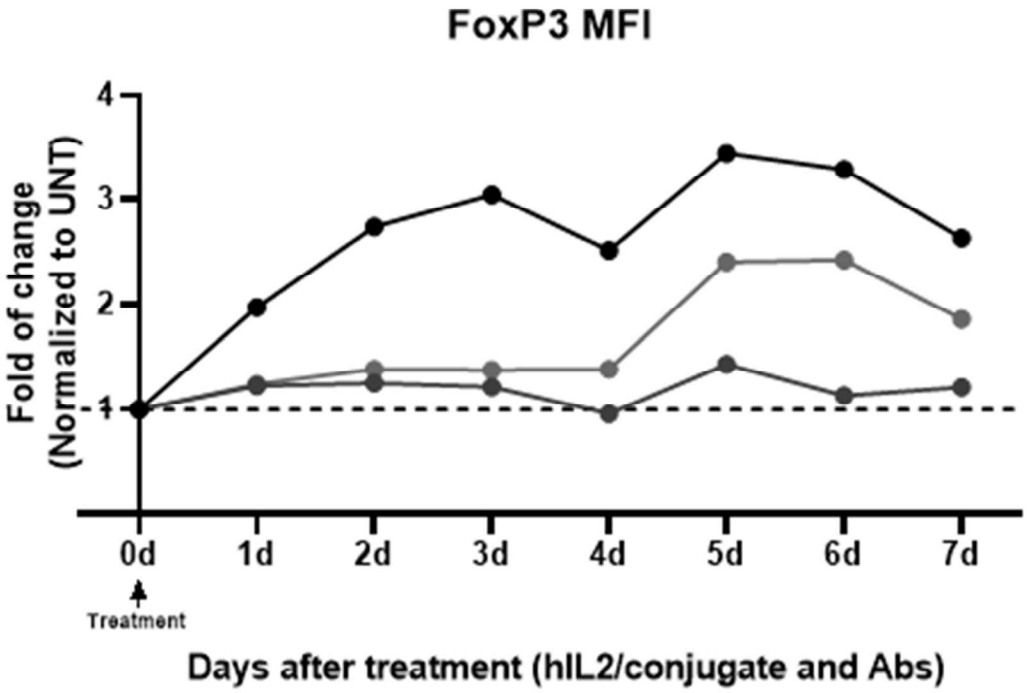


FIG. 61B

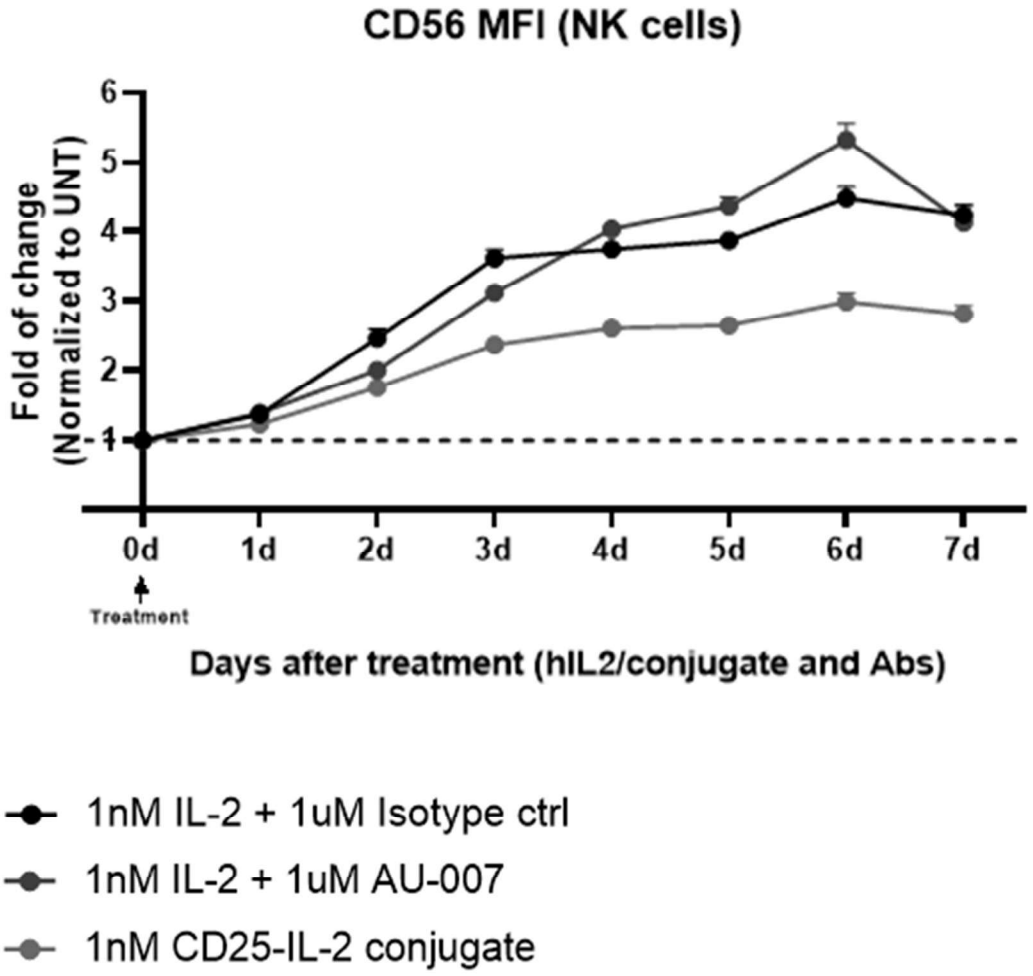

CD56 MFI (NK cells)
Fold of change (Normalized to UNT)
6
5
4
3
2
1
0d
1d
2d
3d
4d
5d
6d
7d
Treatment
Days after treatment (hIL2/conjugate and Abs)
1nM IL-2 + 1uM Isotype ctrl
1nM IL-2 + 1uM AU-007
1nM CD25-IL-2 conjugate

FIG. 61C

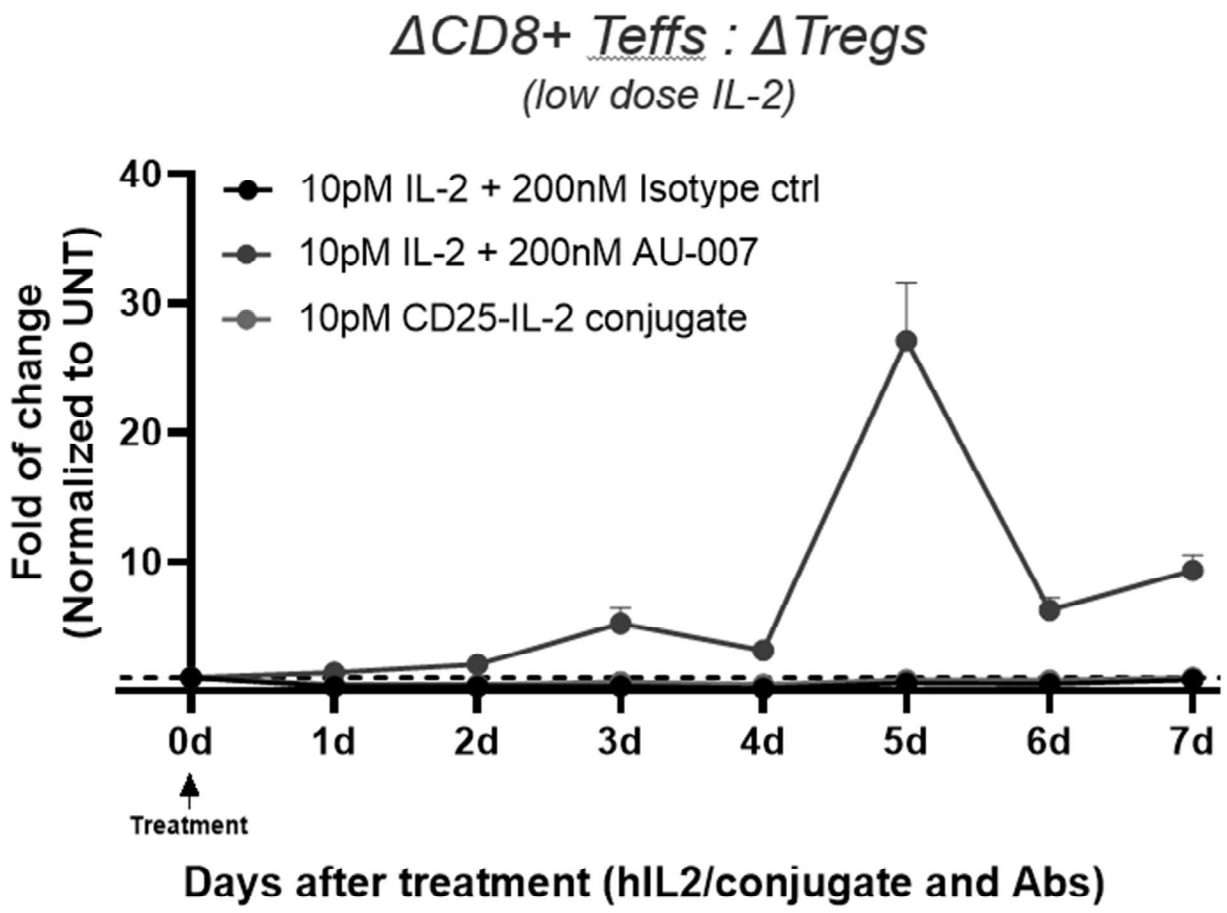

ΔCD8+ Teffs : ΔTregs
(low dose IL-2)
10pM IL-2 + 200nM Isotype ctrl
10pM IL-2 + 200nM AU-007
10pM CD25-IL-2 conjugate
Fold of change
(Normalized to UNT)
40
30
20
10
0d
1d
2d
3d
4d
5d
6d
7d
Treatment
Days after treatment (hIL2/conjugate and Abs)

FIG. 62A

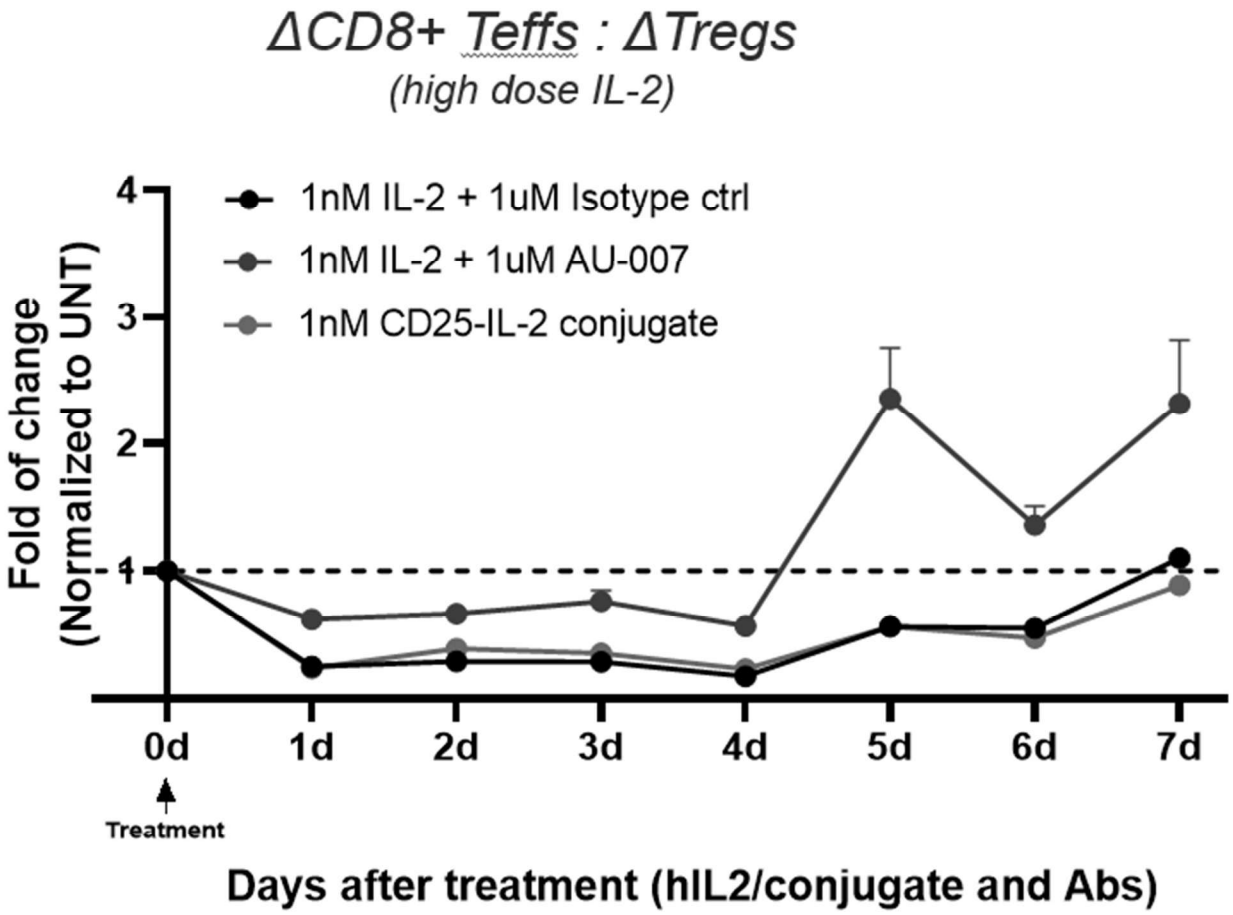


FIG. 62B

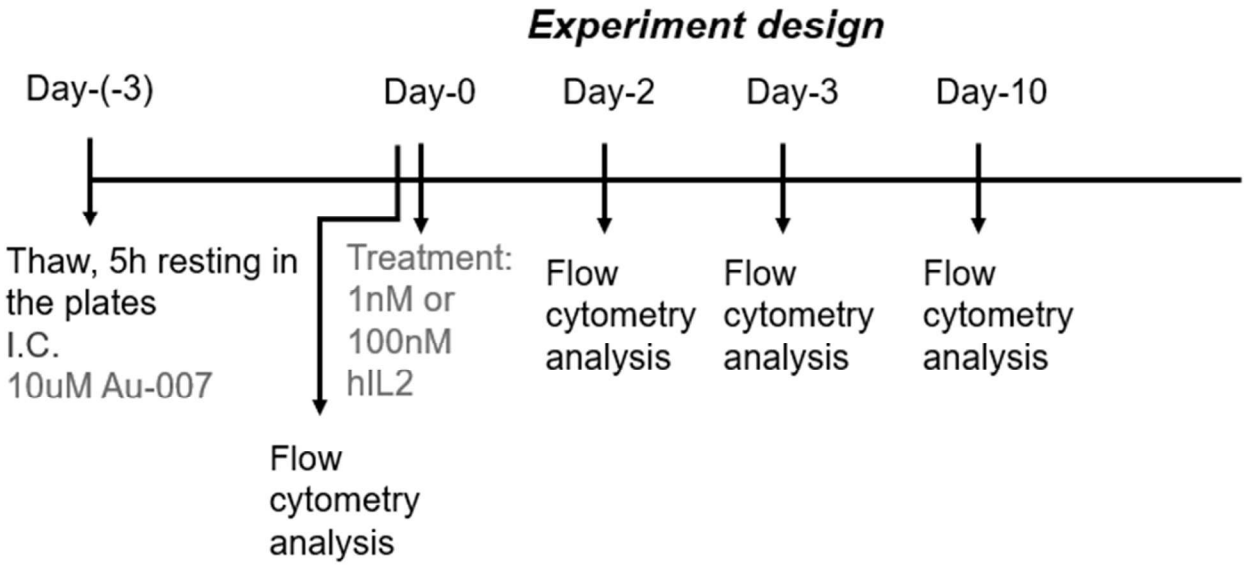


FIG. 63

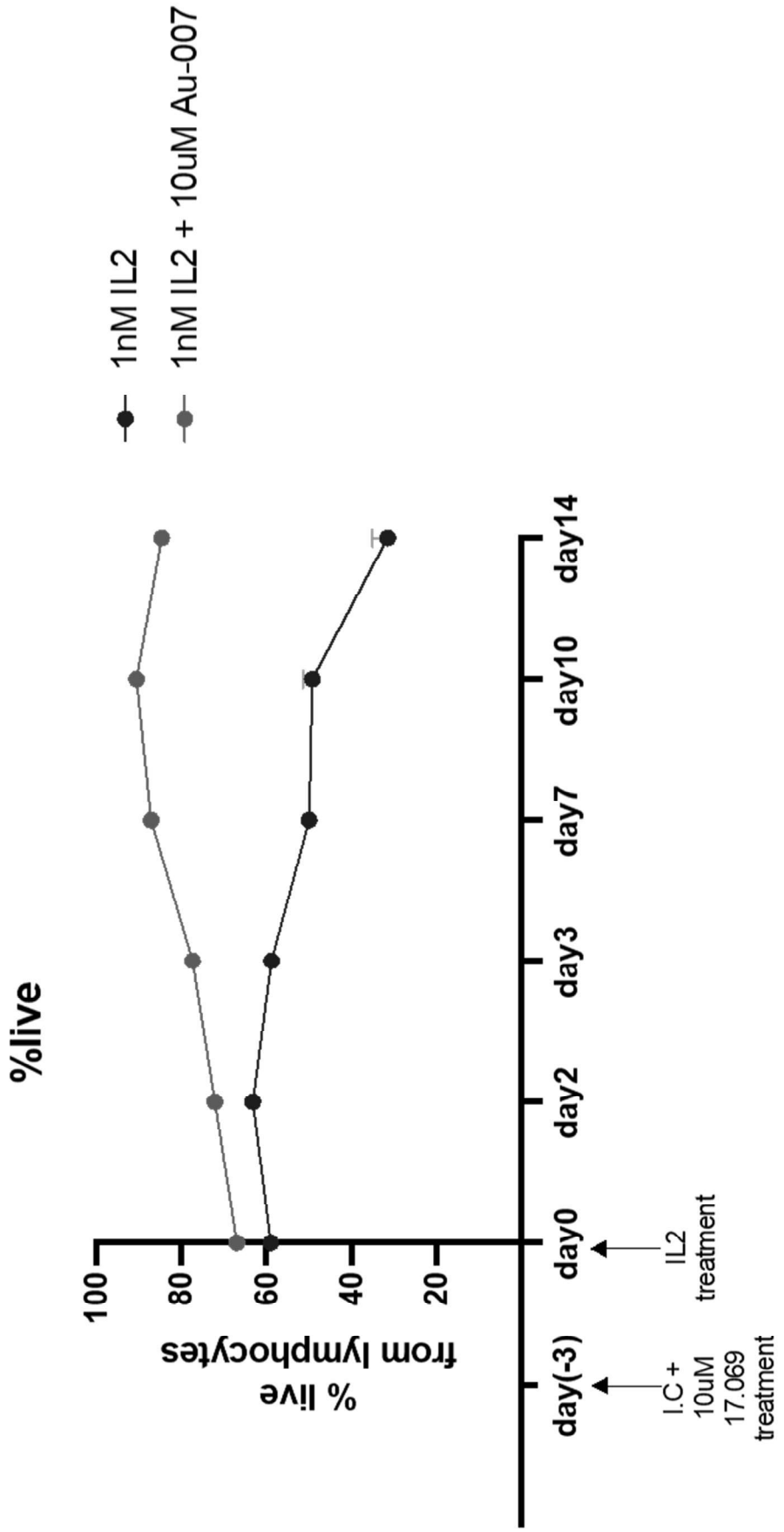

%live
% live from lymphocytes
100
80
60
40
20
day(-3)
day0
day2
day3
day7
day10
day14
I.C + 10uM 17.069 treatment
IL2 treatment
1nM IL2
1nM IL2 + 10uM Au-007

FIG. 64

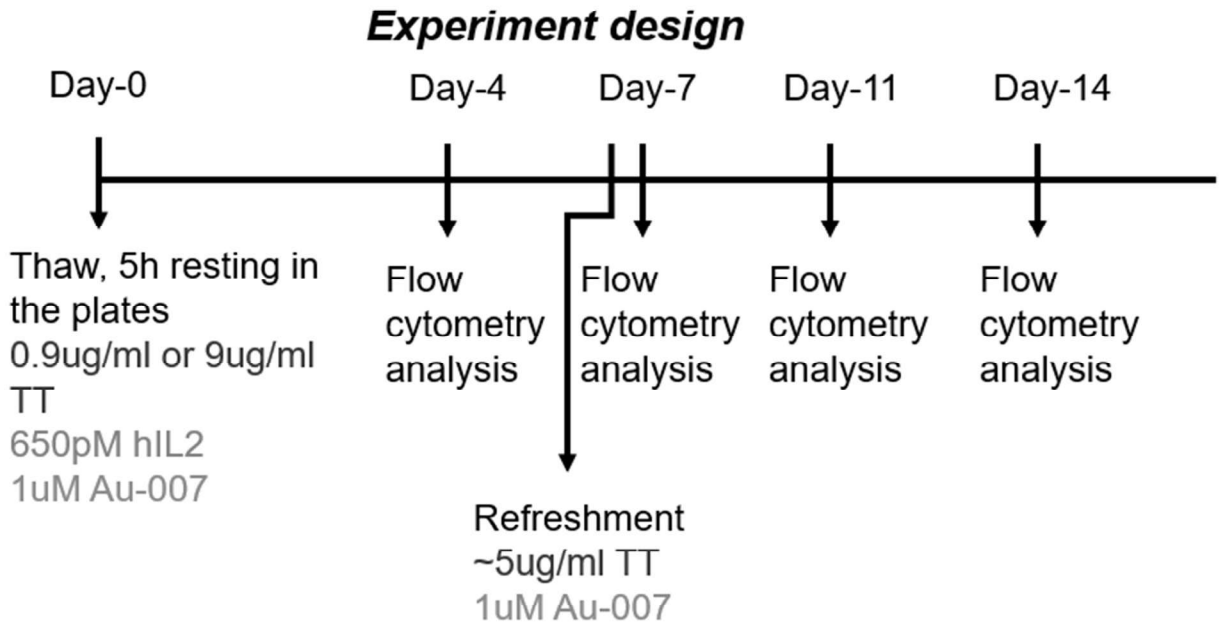

Experiment design
Day-0
Day-4
Day-7
Day-11
Day-14
Thaw, 5h resting in the plates
0.9ug/ml or 9ug/ml TT
650pM hIL2
1uM Au-007
Flow cytometry analysis
Flow cytometry analysis
Flow cytometry analysis
Flow cytometry analysis
Refreshment
~5ug/ml TT
1uM Au-007

FIG. 65

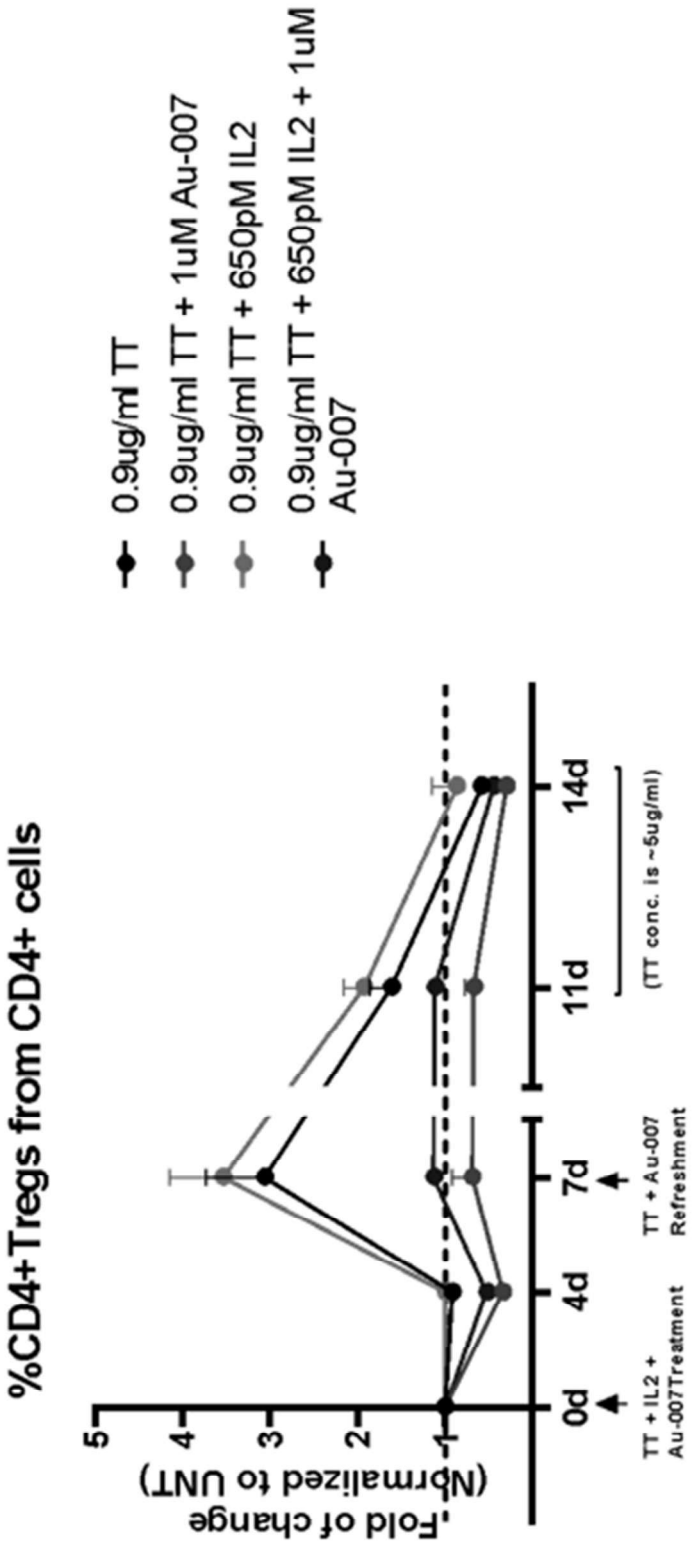

%CD4+Tregs from CD4+ cells
Fold of change (Normalized to UNT)
5
4
3
2
1
0d
4d
7d
11d
14d
TT + IL2 + Au-007Treatment
TT + Au-007 Refreshment
(TT conc. is ~5ug/ml)
Days after treatment
0.9ug/ml TT
0.9ug/ml TT + 1uM Au-007
0.9ug/ml TT + 650pM IL2
0.9ug/ml TT + 650pM IL2 + 1uM Au-007

FIG. 66A

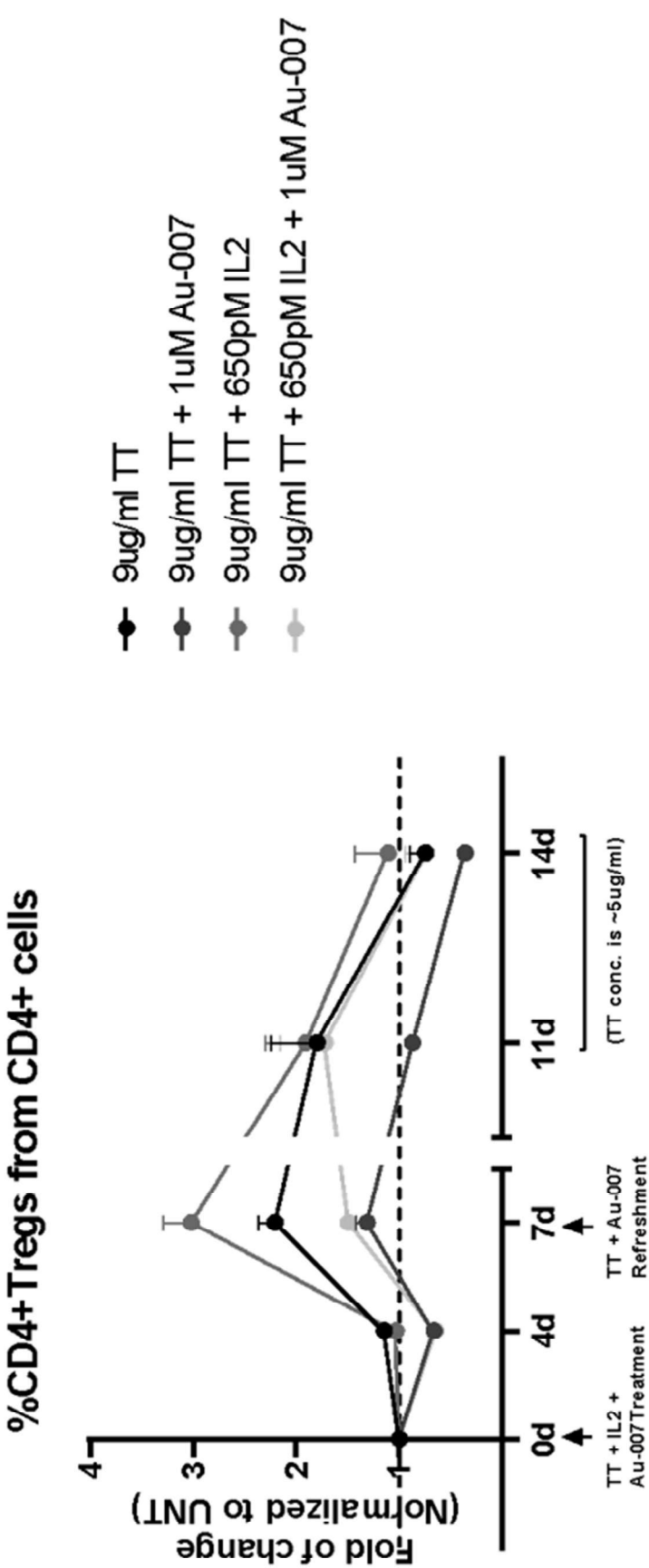

%CD4+Tregs from CD4+ cells
9ug/ml TT
9ug/ml TT + 1uM Au-007
9ug/ml TT + 650pM IL2
9ug/ml TT + 650pM IL2 + 1uM Au-007
Fold of change (Normalized to UNT)
4
3
2
1
0d
4d
7d
11d
14d
TT + IL2 + Au-007 Treatment
TT + Au-007 Refreshment
(TT conc. is ~5ug/ml)
Days after treatment

FIG. 66B

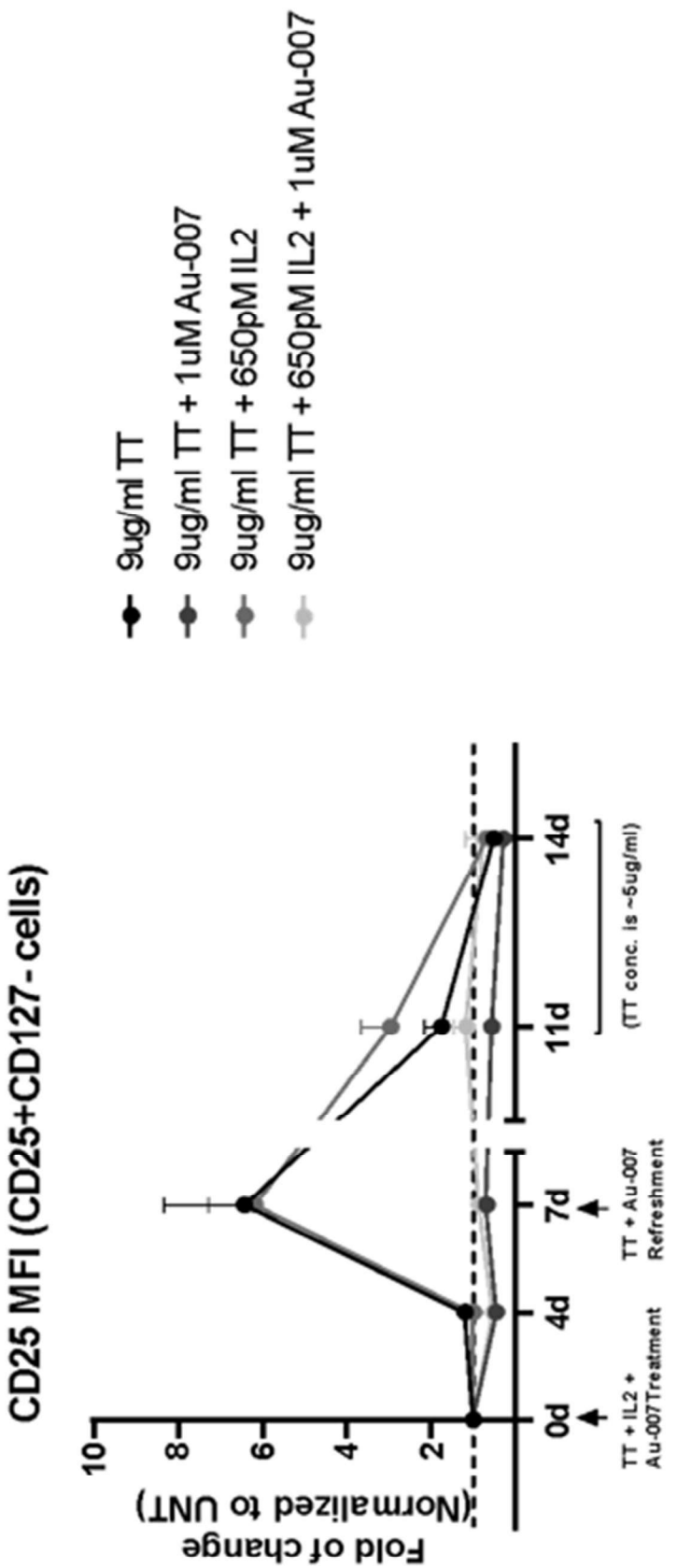

CD25 MFI (CD25+CD127- cells)
Fold of change (Normalized to UNT)
10
8
6
4
2
0d
4d
7d
11d
14d
TT + IL2 + Au-007 Treatment
TT + Au-007 Refreshment
(TT conc. is ~5ug/ml)
Days after treatment
9ug/ml TT
9ug/ml TT + 1uM Au-007
9ug/ml TT + 650pM IL2
9ug/ml TT + 650pM IL2 + 1uM Au-007

FIG. 66C

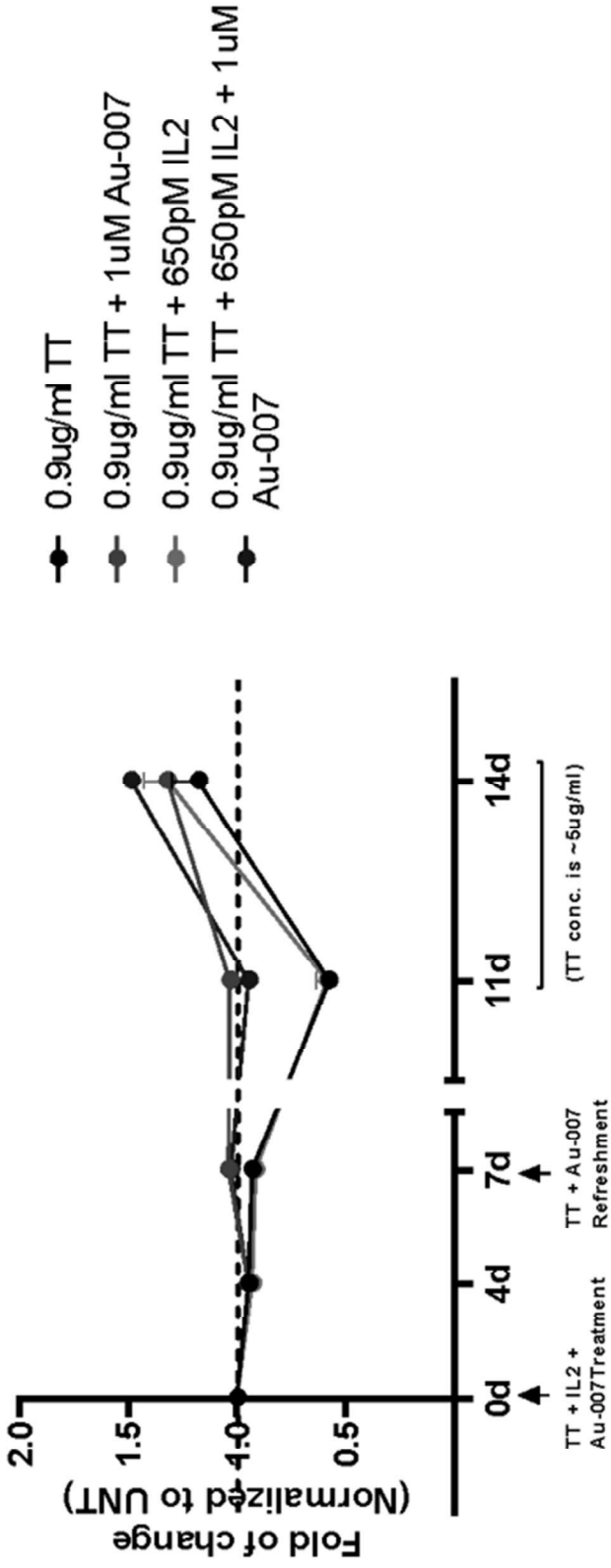

%CD8+
Fold of change (Normalized to UNT)
2.0
1.5
1.0
0.5
0d
4d
7d
11d
14d
TT + IL2 + Au-007Treatment
TT + Au-007 Refreshment
(TT conc. is ~5ug/ml)
Days after treatment
0.9ug/ml TT
0.9ug/ml TT + 1uM Au-007
0.9ug/ml TT + 650pM IL2
0.9ug/ml TT + 650pM IL2 + 1uM Au-007

FIG. 67A

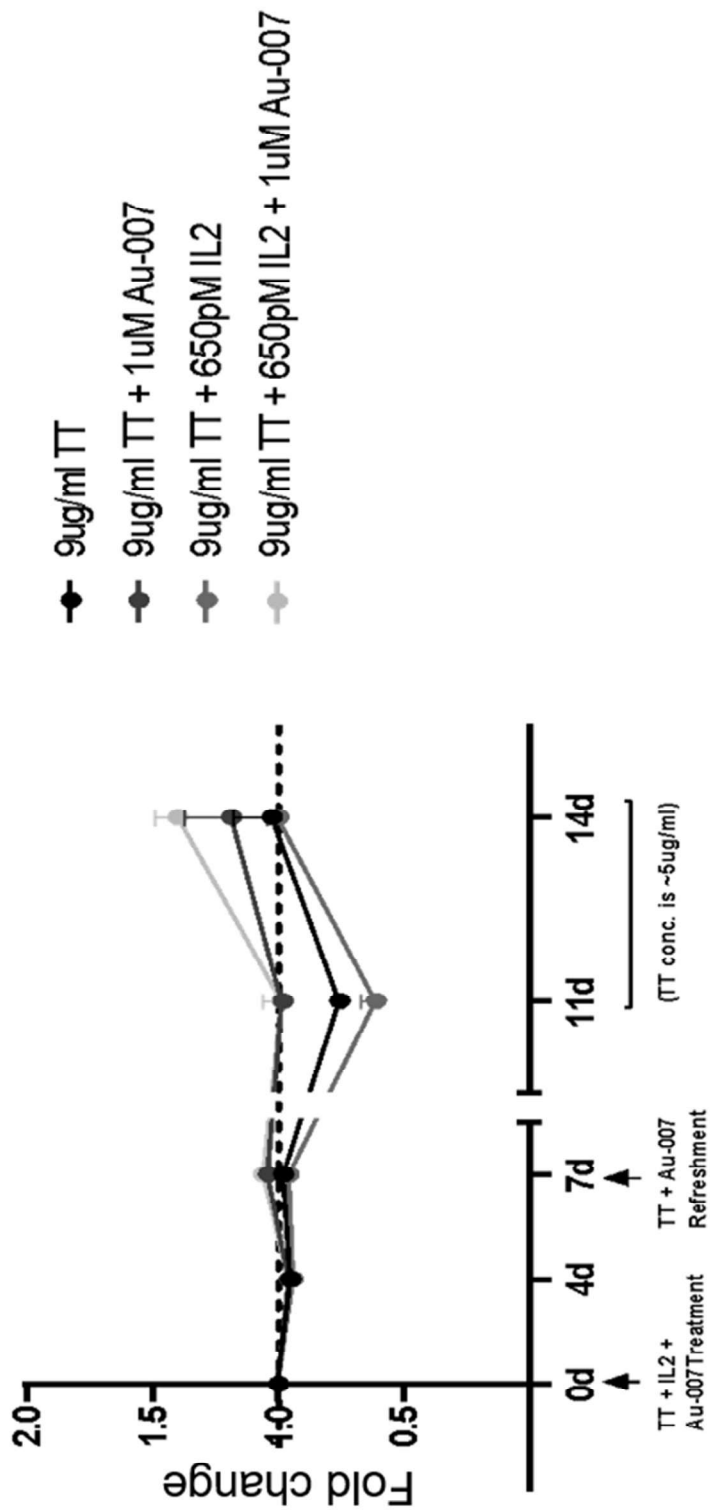

%CD8+
9ug/ml TT
9ug/ml TT + 1uM Au-007
9ug/ml TT + 650pM IL2
9ug/ml TT + 650pM IL2 + 1uM Au-007
Fold change
2.0
1.5
1.0
0.5
0d
4d
7d
11d
14d
TT + IL2 + Au-007 Treatment
TT + Au-007 Refreshment
(TT conc. is ~5ug/ml)
Days after treatment

FIG. 67B

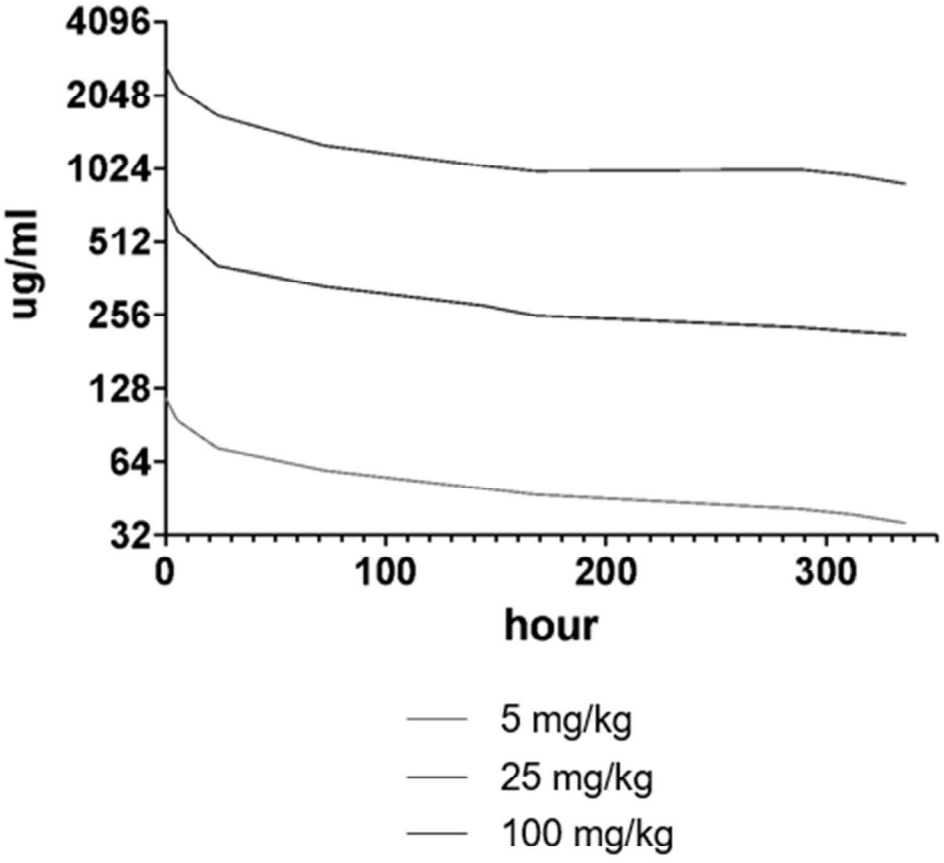

4096
2048
1024
512
256
128
64
32
ug/ml
0
100
200
300
hour
5 mg/kg
25 mg/kg
100 mg/kg

FIG. 68

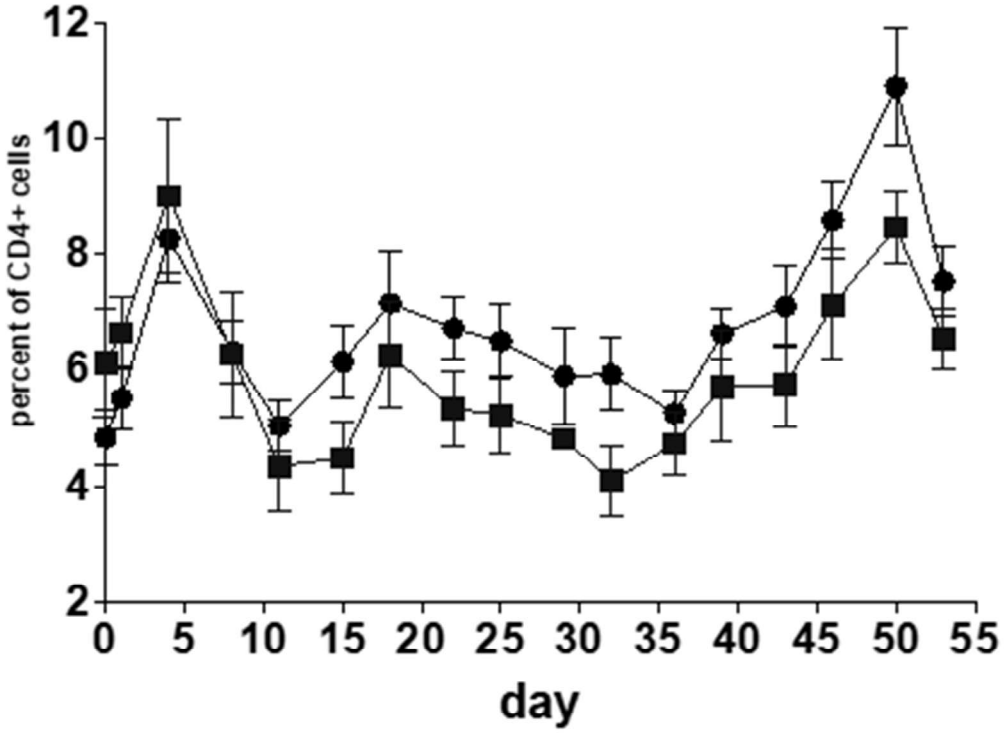


FIG. 69A

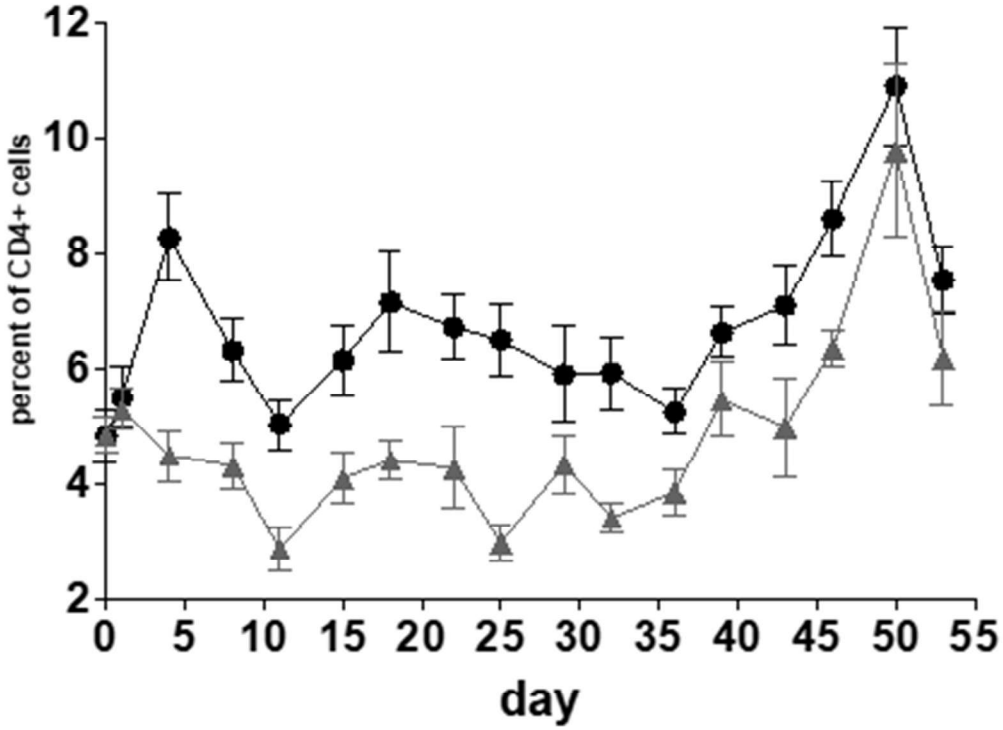


FIG. 69B

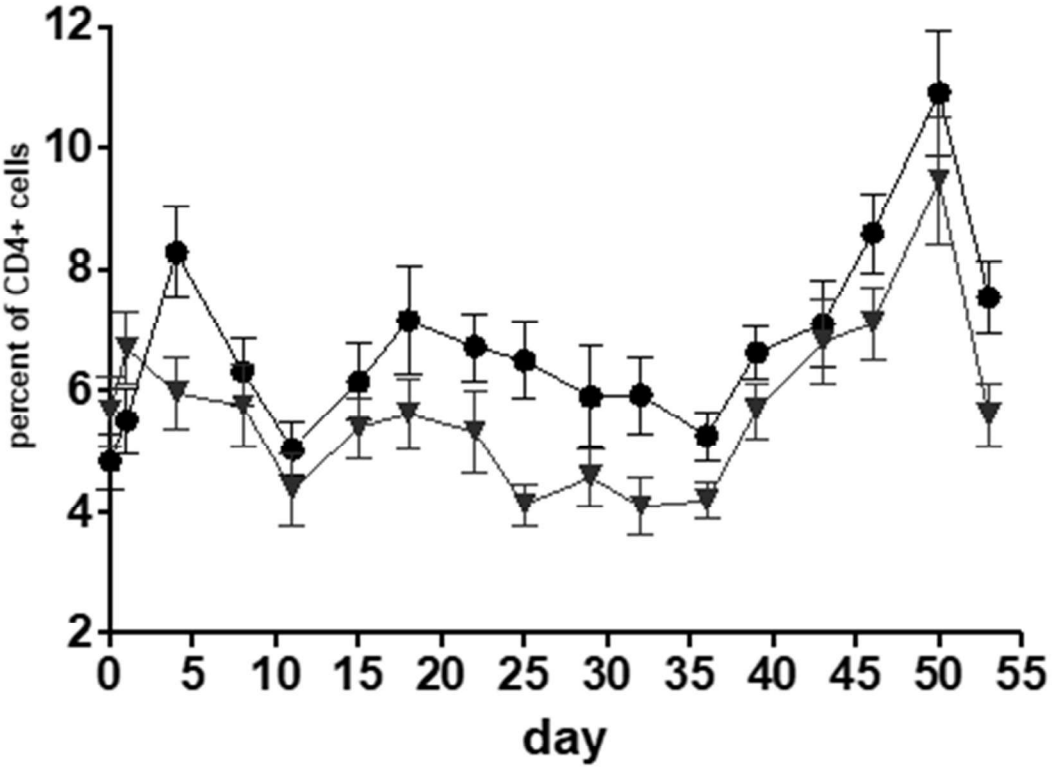


FIG. 69C

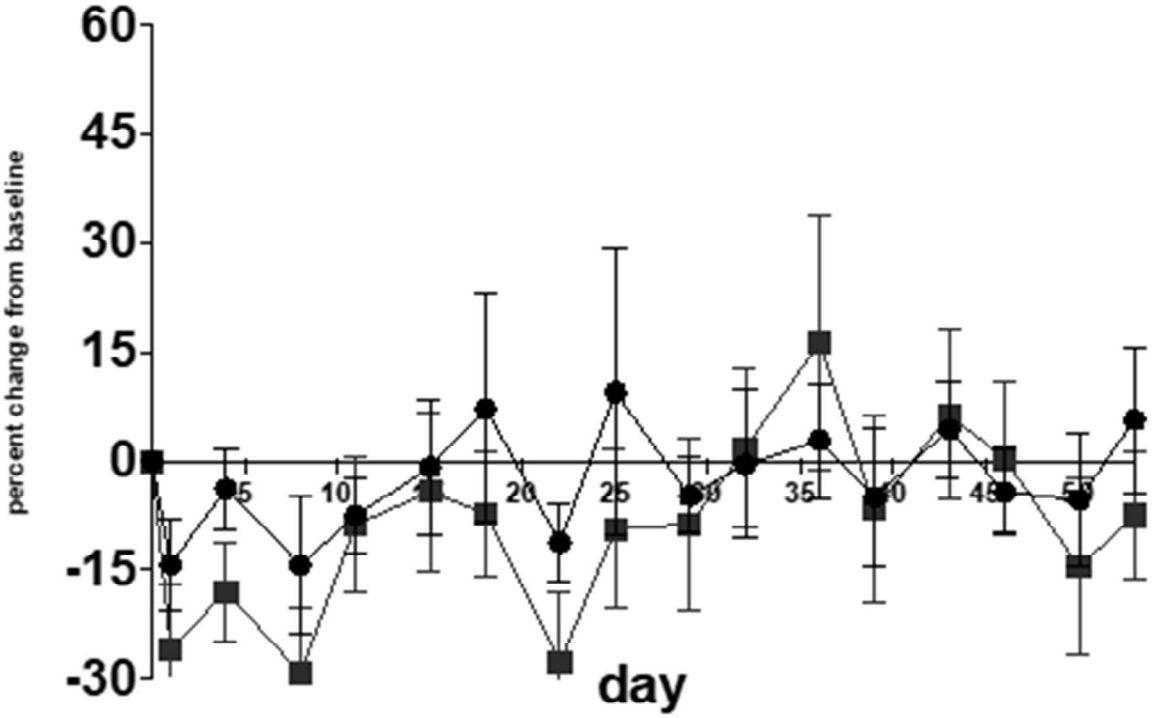


FIG. 70A

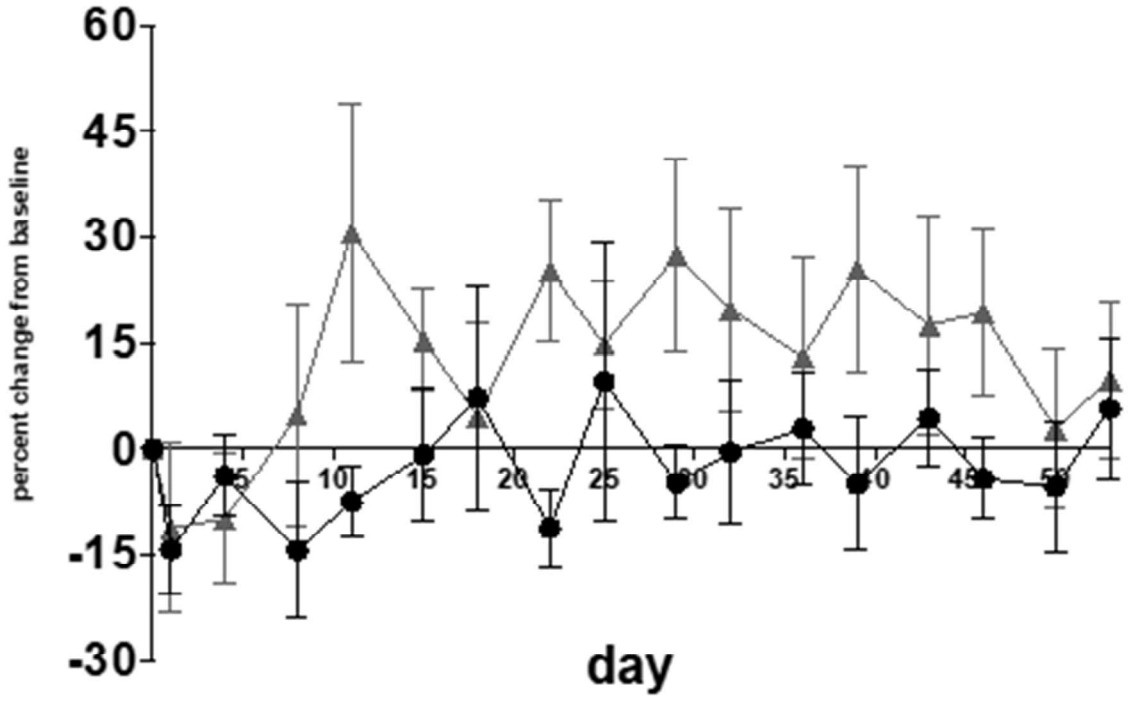


FIG. 70B

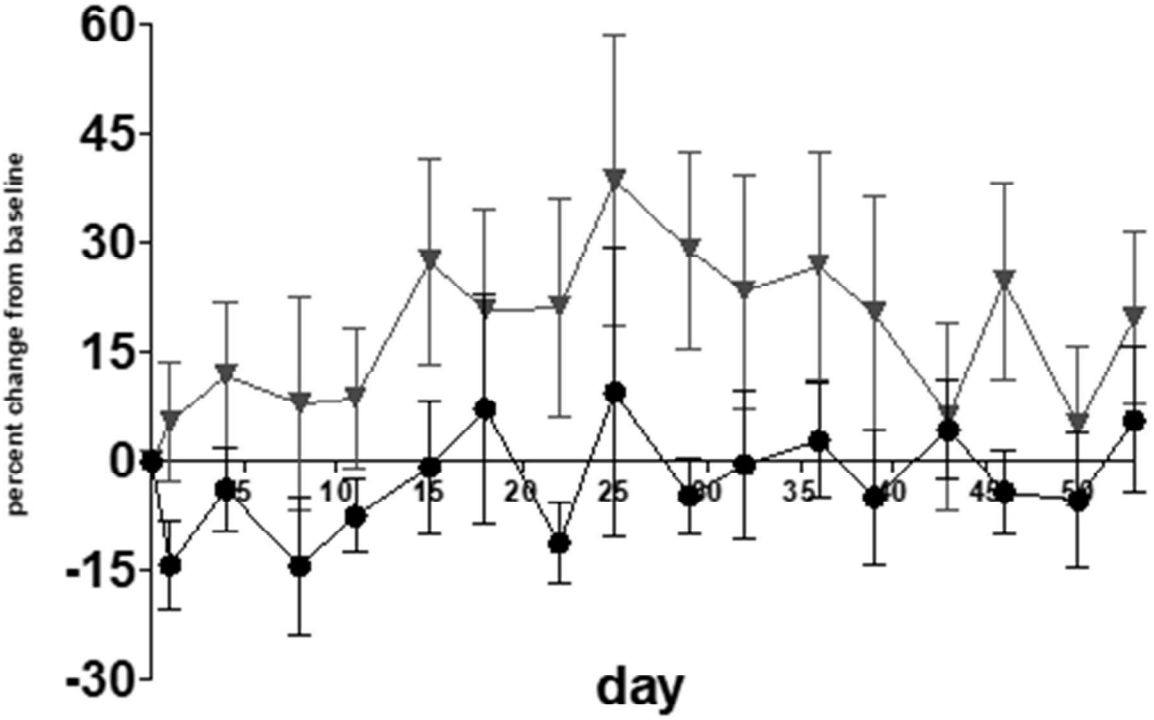


FIG. 70C

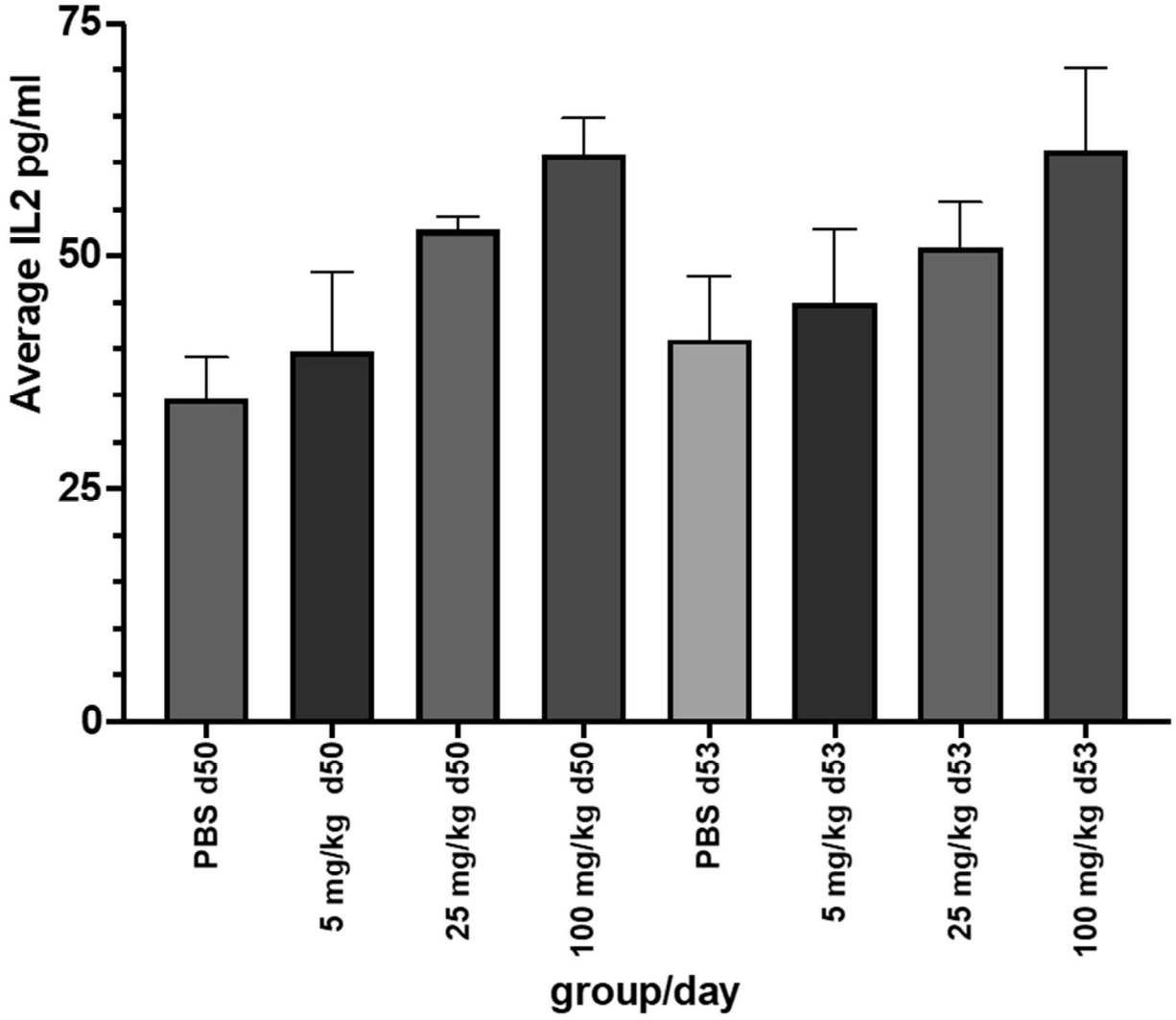

75
50
25
0
Average IL2 pg/ml
PBS d50
5 mg/kg d50
25 mg/kg d50
100 mg/kg d50
PBS d53
5 mg/kg d53
25 mg/kg d53
100 mg/kg d53
group/day

FIG. 71

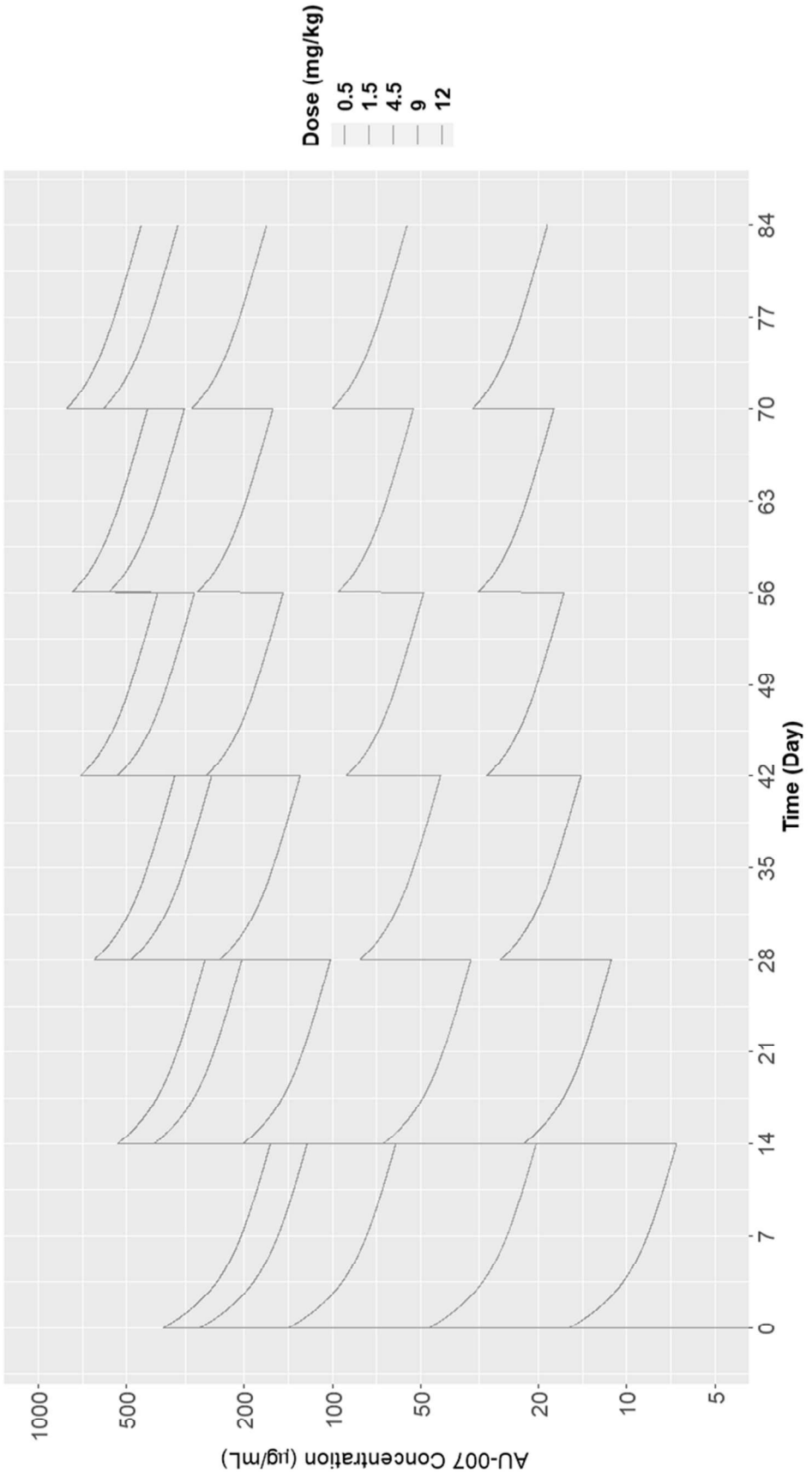

Dose (mg/kg)
0.5
1.5
4.5
9
12
AU-007 Concentration (µg/mL)
1000
500
200
100
50
20
10
5
0
7
14
21
28
35
42
49
56
63
70
77
84
Time (Day)

FIG. 72

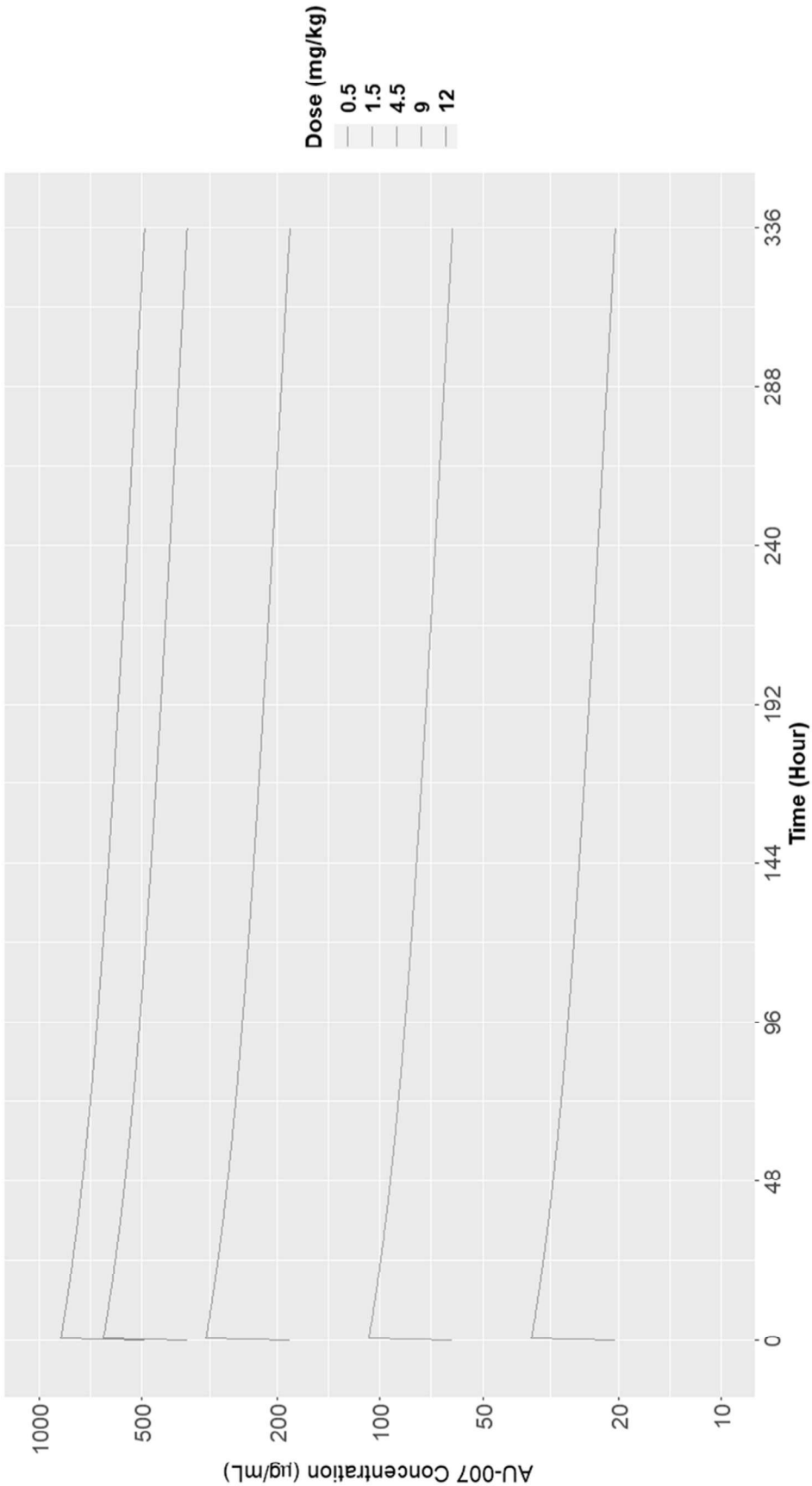

Dose (mg/kg)
0.5
1.5
4.5
9
12
AU-007 Concentration (µg/mL)
1000
500
200
100
50
20
10
0
48
96
144
192
240
288
336
Time (Hour)

FIG. 73

Dose Escalation Cohort (1A)	AU-007 Dose (mg/kg)	1st dose Trough (µg/cc)	IL-2 Covered[a] (IU/cc)	1 X HD IL-2[b]	Steady State Trough[c] (µg/cc)	IL-2 Covered[a] (IU/cc)	1 X HD IL-2[b]	2 X HD IL-2[d]
-1	0.15	-	-	-	-	-	-	
1	0.5	6.7	10.8K	1.1 x	20	32.4K	3.4 x	6.8 x
2	1.5	20	32.4K	3.4 x	60	97.2K	10 x	20 x
3	4.5	60	97.2K	10 x	180	291.6K	30 x	60 x
4	9.0	120	194.4K	20 x	360	583.2K	60 x	120 x
5	12	160	259.2K	27 x	480	777.6K	80 x	160 x

FIG. 74

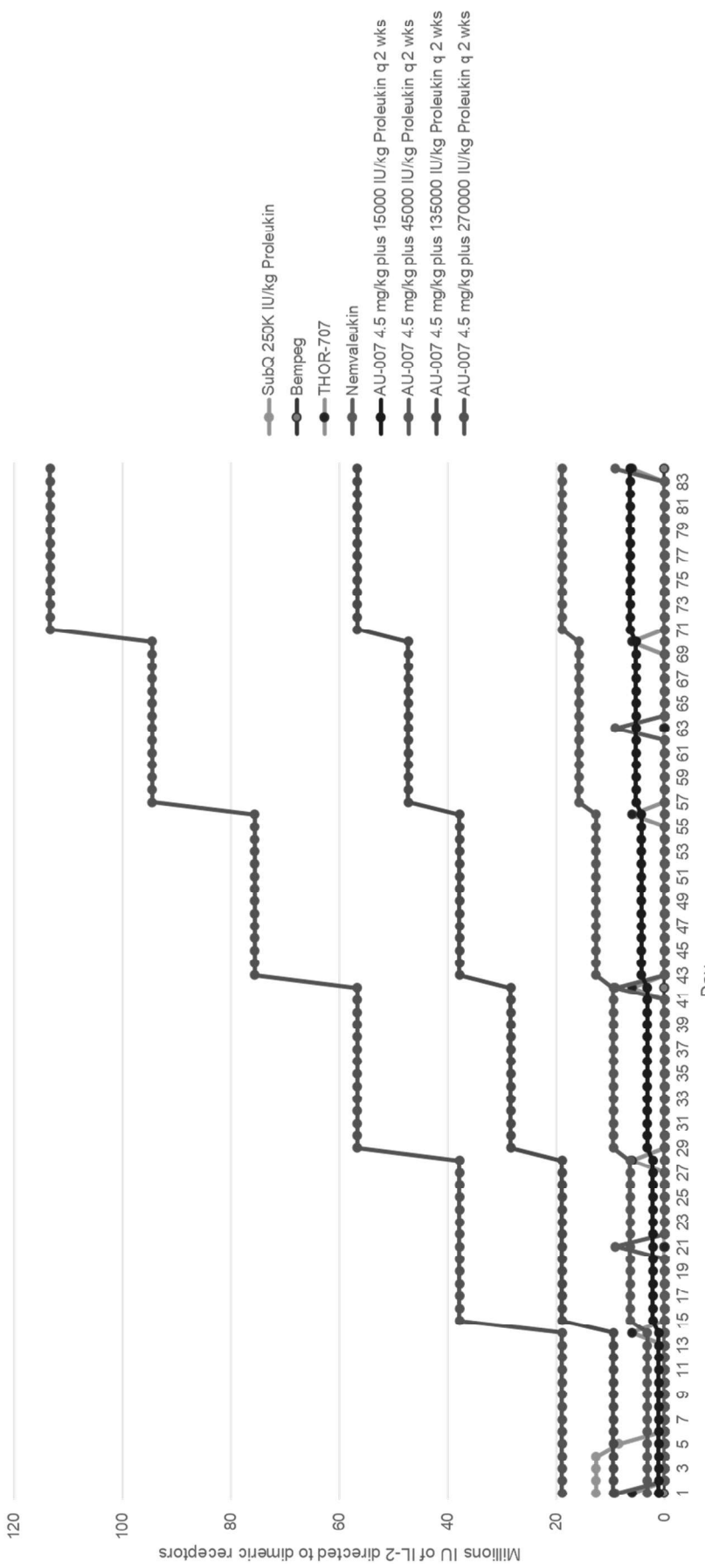

AU-007 will send much more daily IL-2 to dimeric receptors on effectors than competitor products achieve
SubQ 250K IU/kg Proleukin
Bempeg
THOR-707
Nemvaleukin
AU-007 4.5 mg/kg plus 15000 IU/kg Proleukin q 2 wks
AU-007 4.5 mg/kg plus 45000 IU/kg Proleukin q 2 wks
AU-007 4.5 mg/kg plus 135000 IU/kg Proleukin q 2 wks
AU-007 4.5 mg/kg plus 270000 IU/kg Proleukin q 2 wks
Millions IU of IL-2 directed to dimeric receptors
120
100
80
60
40
20
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63 65 67 69 71 73 75 77 79 81 83
Day

FIG. 75