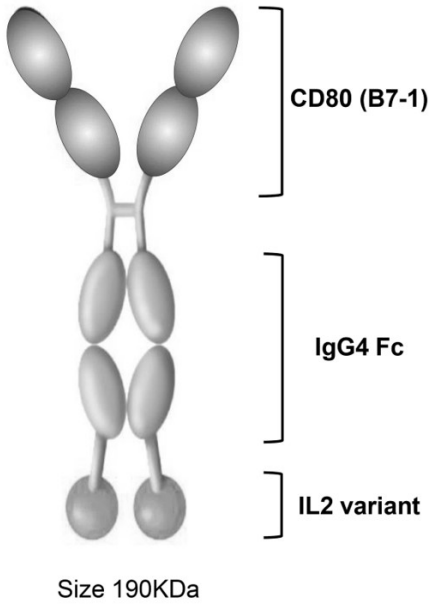
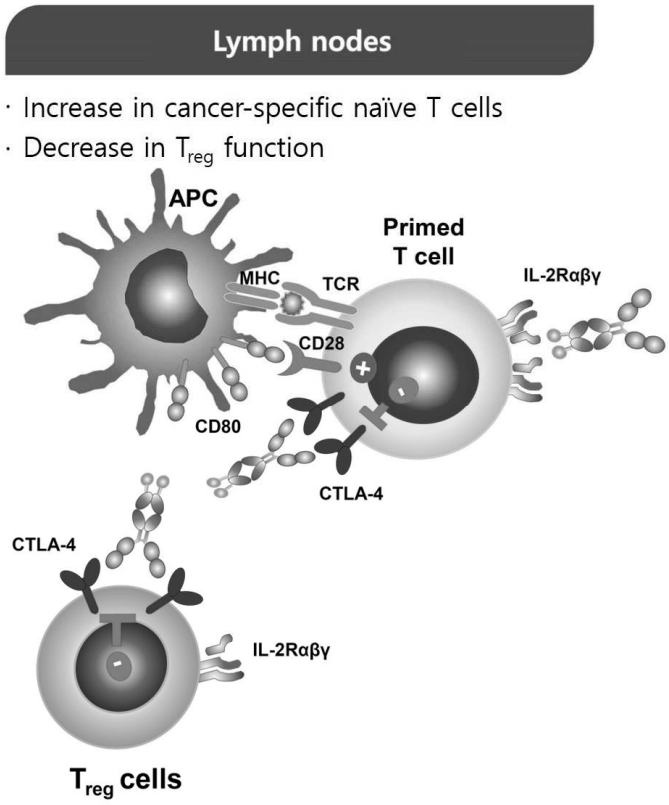
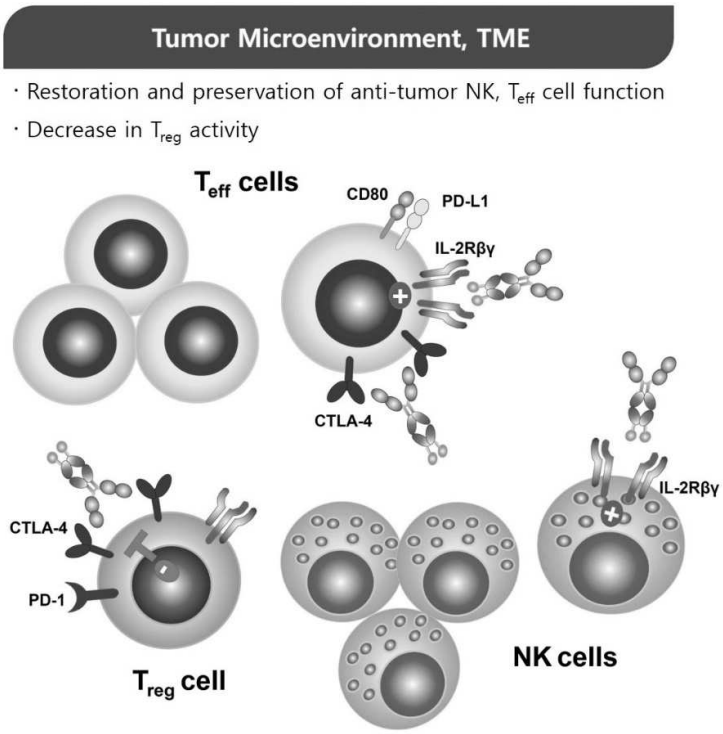
[Fig. 1]


CD80 (B7-1)
IgG4 Fc
IL2 variant
Size 190KDa

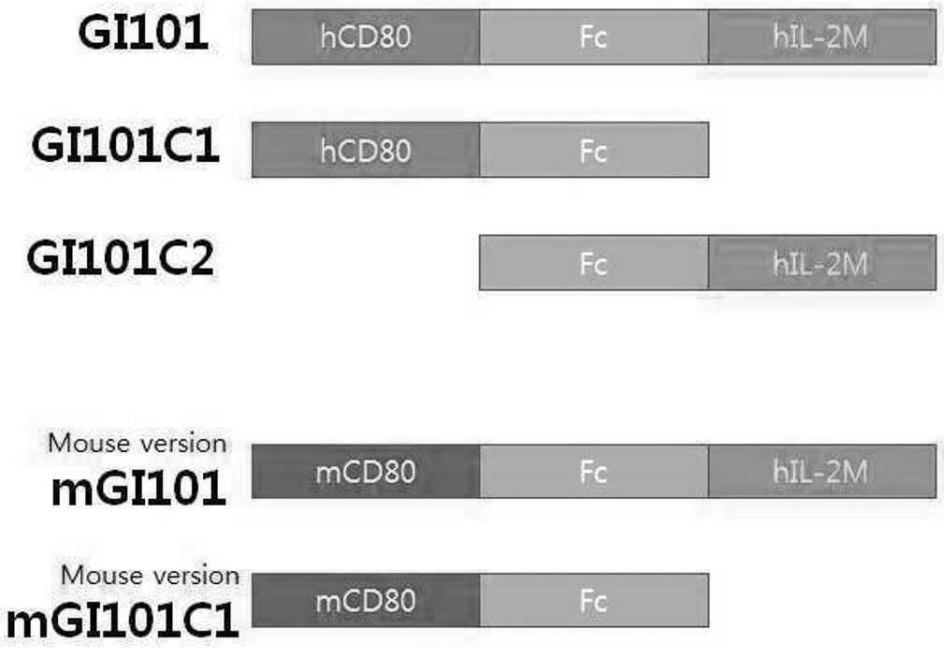
[Fig. 2]


Lymph nodes
· Increase in cancer-specific naïve T cells
· Decrease in T_{reg} function
APC
Primed
T cell
MHC
TCR
IL-2Rαβγ
CD28
CD80
CTLA-4
CTLA-4
IL-2Rαβγ
T_{reg} cells

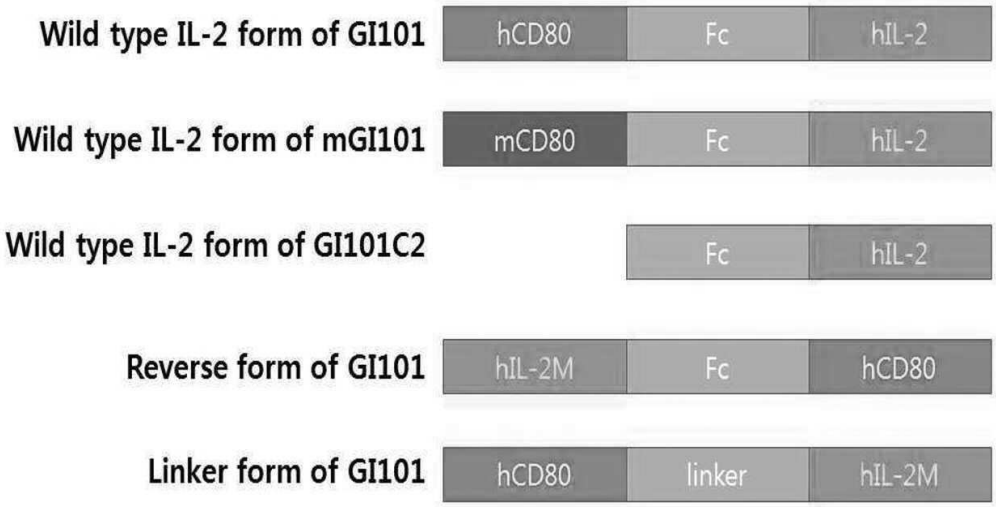
[Fig. 3]


Tumor Microenvironment, TME
· Restoration and preservation of anti-tumor NK, T_{eff} cell function
· Decrease in T_{reg} activity
T_{eff} cells
CD80
PD-L1
IL-2Rβγ
CTLA-4
CTLA-4
PD-1
IL-2Rβγ
T_{reg} cell
NK cells

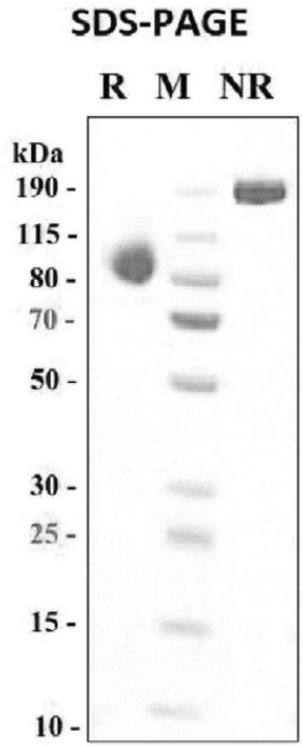
[Fig. 4]


GI101
hCD80
Fc
hIL-2M
GI101C1
hCD80
Fc
GI101C2
Fc
hIL-2M
Mouse version
mGI101
mCD80
Fc
hIL-2M
Mouse version
mGI101C1
mCD80
Fc

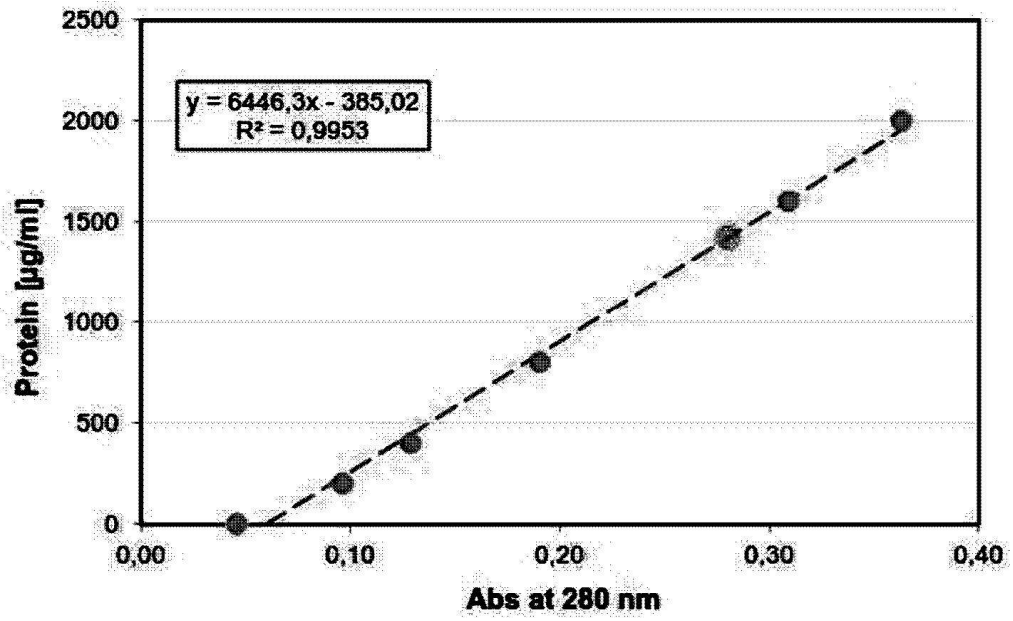
[Fig. 5]


Wild type IL-2 form of GI101
hCD80
Fc
hIL-2
Wild type IL-2 form of mGI101
mCD80
Fc
hIL-2
Wild type IL-2 form of GI101C2
Fc
hIL-2
Reverse form of GI101
hIL-2M
Fc
hCD80
Linker form of GI101
hCD80
linker
hIL-2M

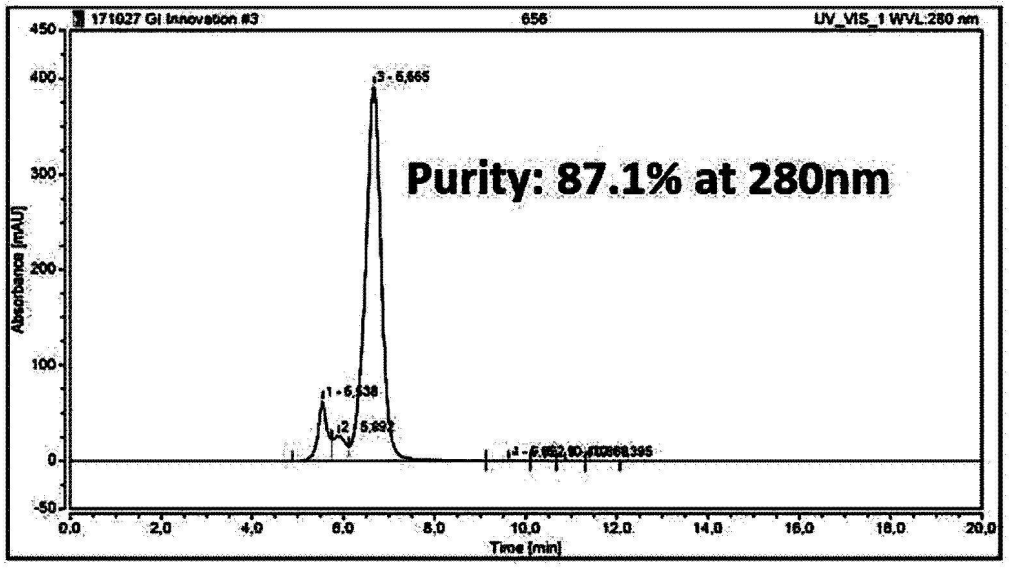
[Fig. 6]


SDS-PAGE
R
M
NR
kDa
190 -
115 -
80 -
70 -
50 -
30 -
25 -
15 -
10 -

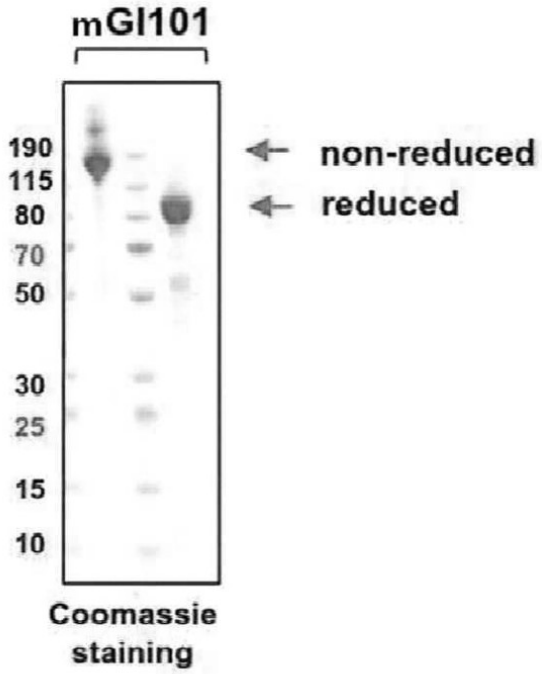
[Fig. 7]


y = 6446,3x - 385,02
R² = 0,9953
Protein [µg/ml]
Abs at 280 nm
2500
2000
1500
1000
500
0
0,00
0,10
0,20
0,30
0,40

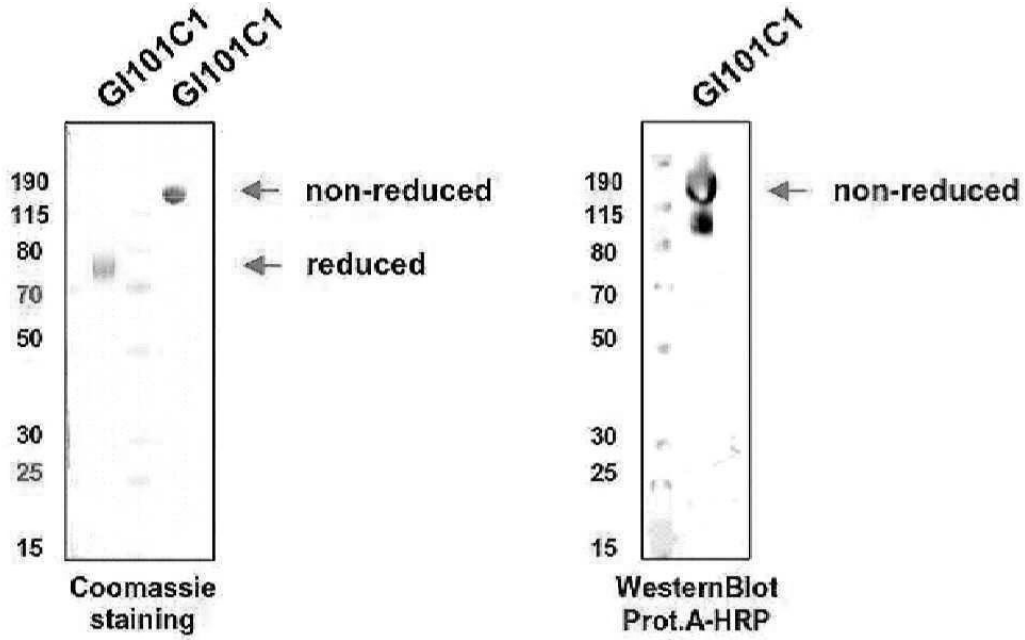
[Fig. 8]


Anlytical size exclusion chromatography (SEC)
171027 GI Innovation #3
656
UV_VIS_1 WVL:280 nm
Purity: 87.1% at 280nm
Absorbance [mAU]
Time [min]

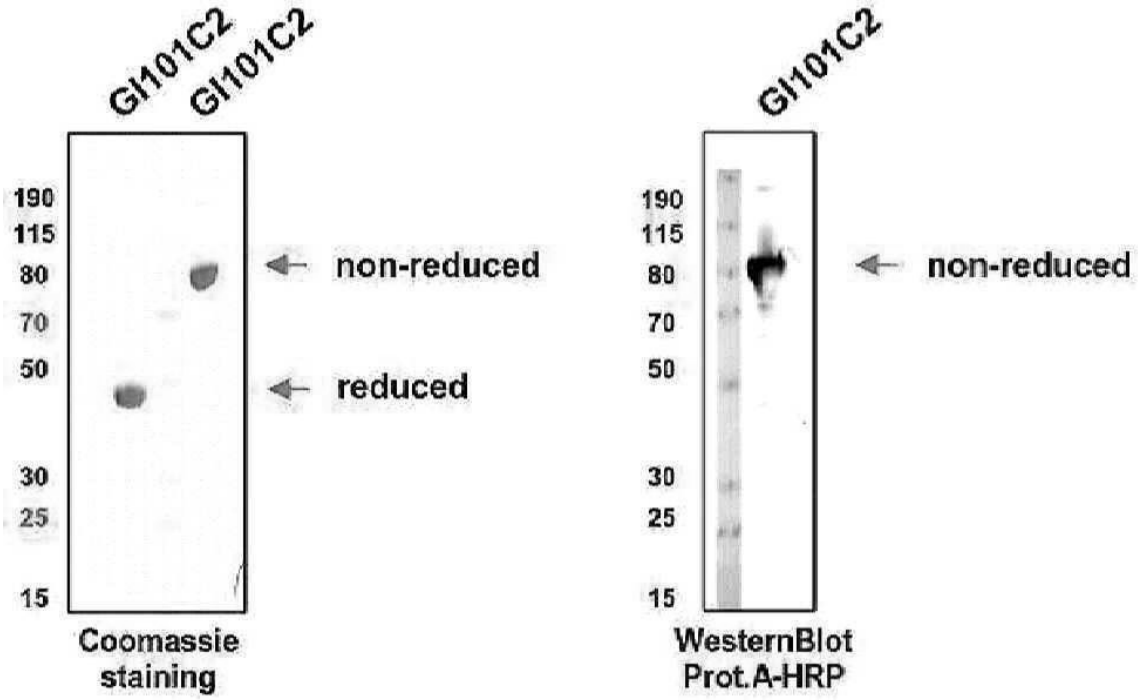
[Fig. 9]


mGI101
190
115
80
70
50
30
25
15
10
non-reduced
reduced
Coomassie
staining

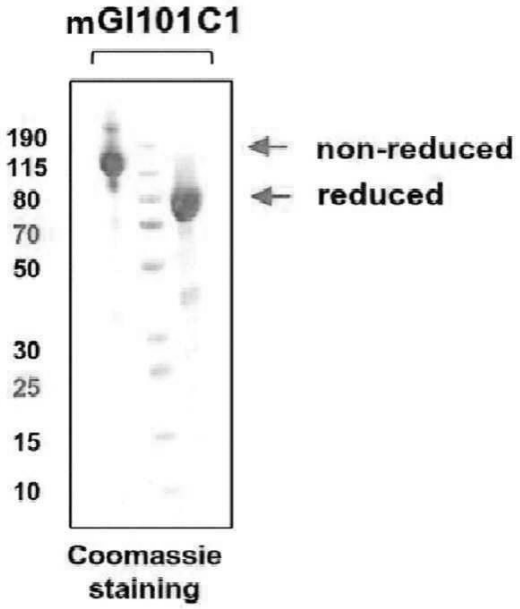
[Fig. 10]


GI101C1
GI101C1
190
115
80
70
50
30
25
15
non-reduced
reduced
Coomassie
staining
GI101C1
190
115
80
70
50
30
25
15
non-reduced
WesternBlot
Prot.A-HRP

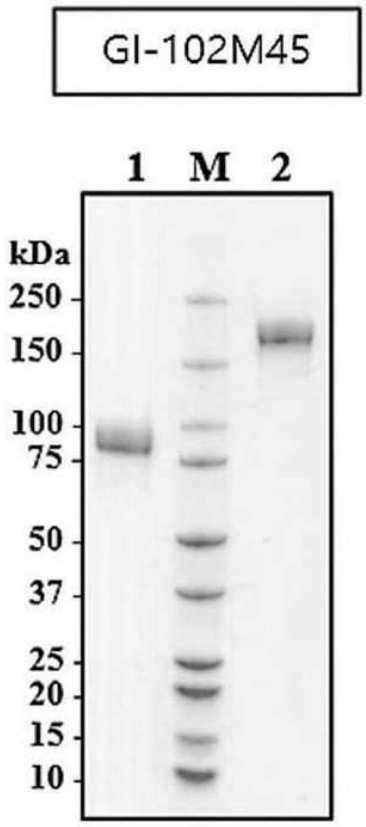
[Fig. 11]


GI101C2
GI101C2
190
115
80
70
50
30
25
15
non-reduced
reduced
Coomassie staining
GI101C2
190
115
80
70
50
30
25
15
non-reduced
WesternBlot Prot.A-HRP

[Fig. 12]


mGI101C1
190
115
80
70
50
30
25
15
10
non-reduced
reduced
Coomassie staining

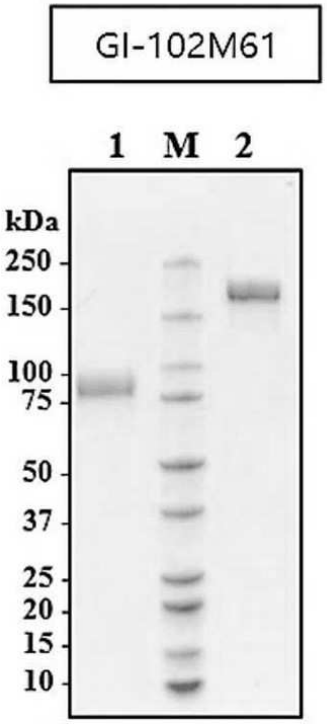
[Fig. 13]



Lane	Sample name	Loading amount
M	Size Marker	5 uL
1	Reduced	3 ug
2	Non-reduced	3 ug

BIO-RAD 4-15% TGX™ gel
200 V, 30 min running, 1X TGS buffer

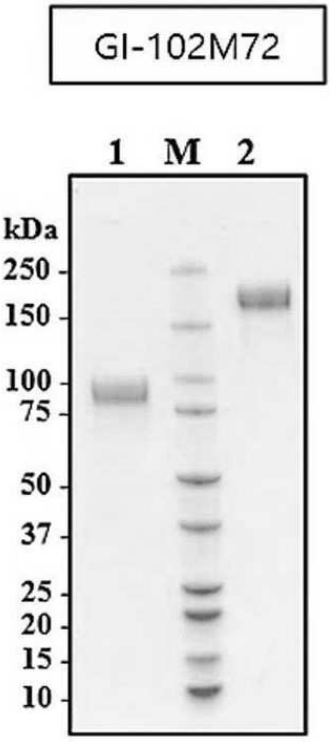
[Fig. 14]



Lane	Sample name	Loading amount
M	Size Marker	5 uL
1	Reduced	3 ug
2	Non-reduced	3 ug

BIO-RAD 4-15% TGX™ gel
200 V, 30 min running, 1X TGS buffer

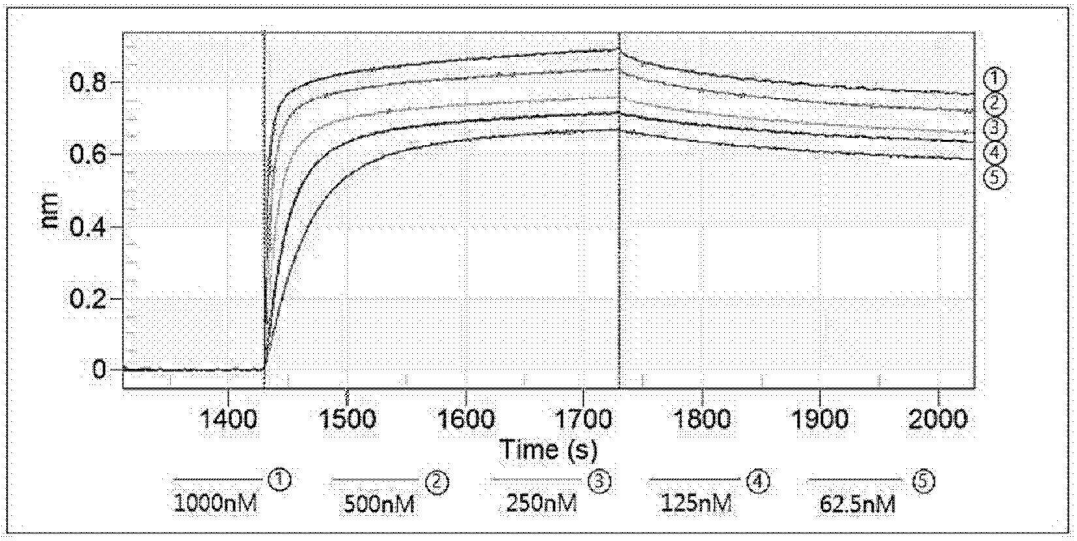
[Fig. 15]



Lane	Sample name	Loading amount
M	Size Marker	5 uL
1	Reduced	3 ug
2	Non-reduced	3 ug

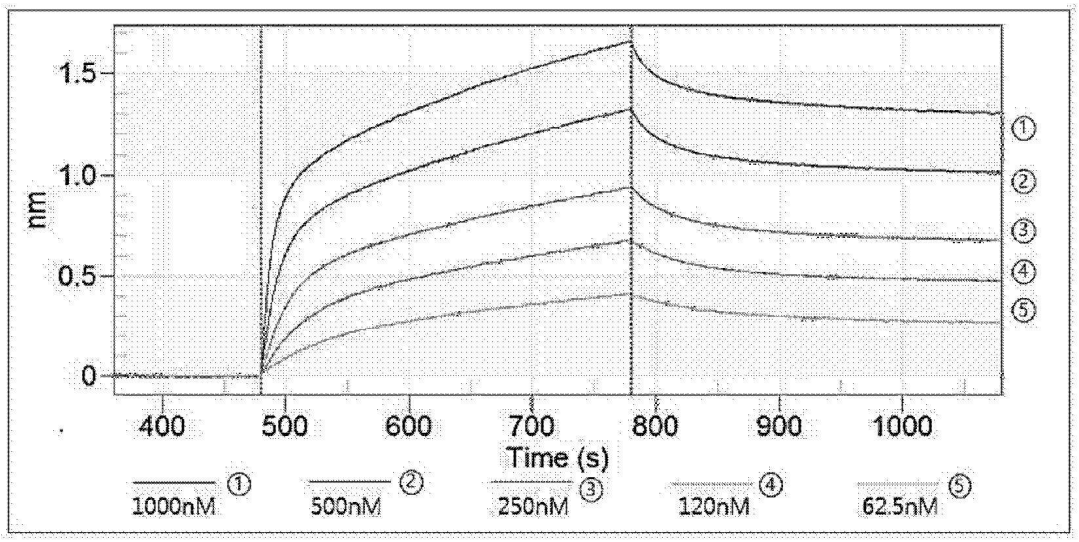
BIO-RAD 4-15% TGX™ gel
200 V, 30 min running, 1X TGS buffer

[Fig. 16]



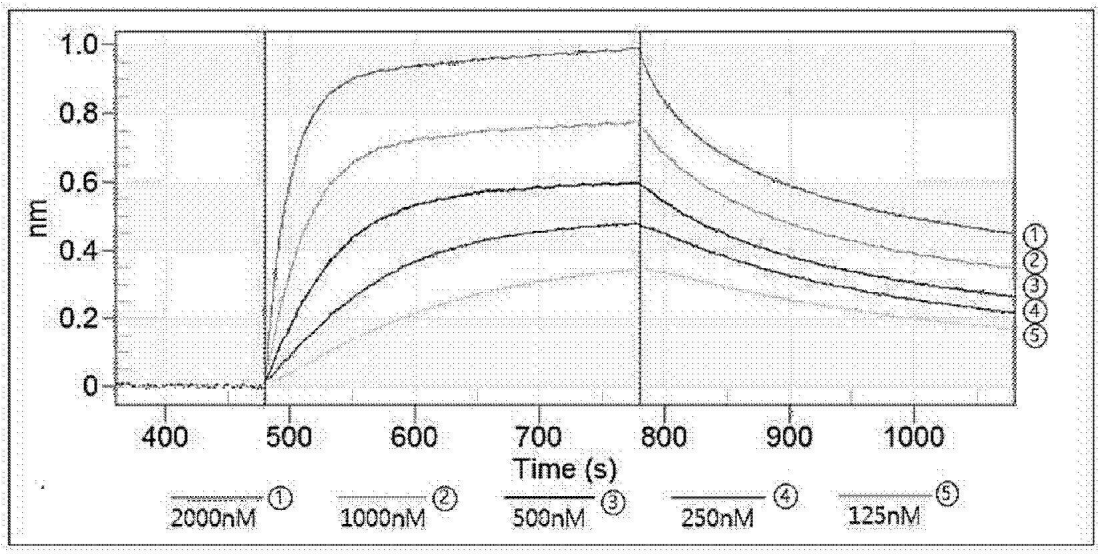
Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
GI101	1.07E-09	3.86E+05	4.14E-04

[Fig. 17]



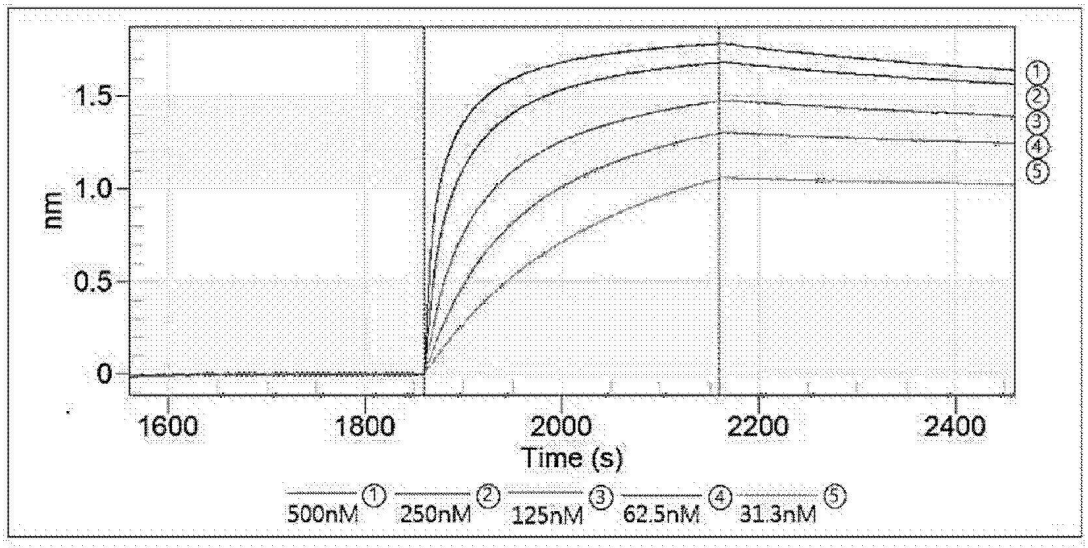
Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
GI101	3.46E-08	7.0E+04	2.42E-03

[Fig. 18]



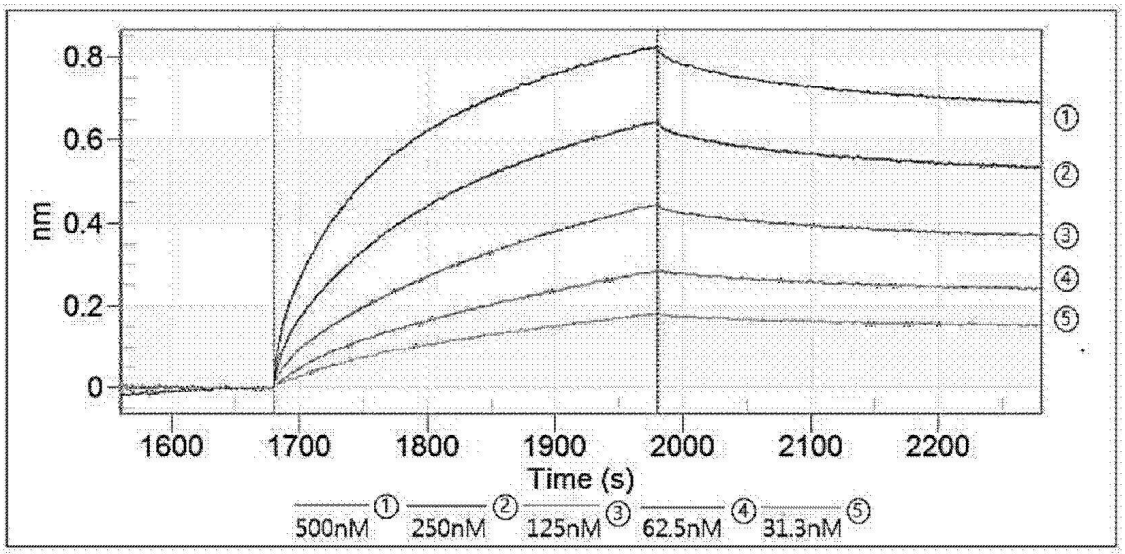
Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
PD-1	1.13E-07	2.72E+04	3.07E-03

[Fig. 19]



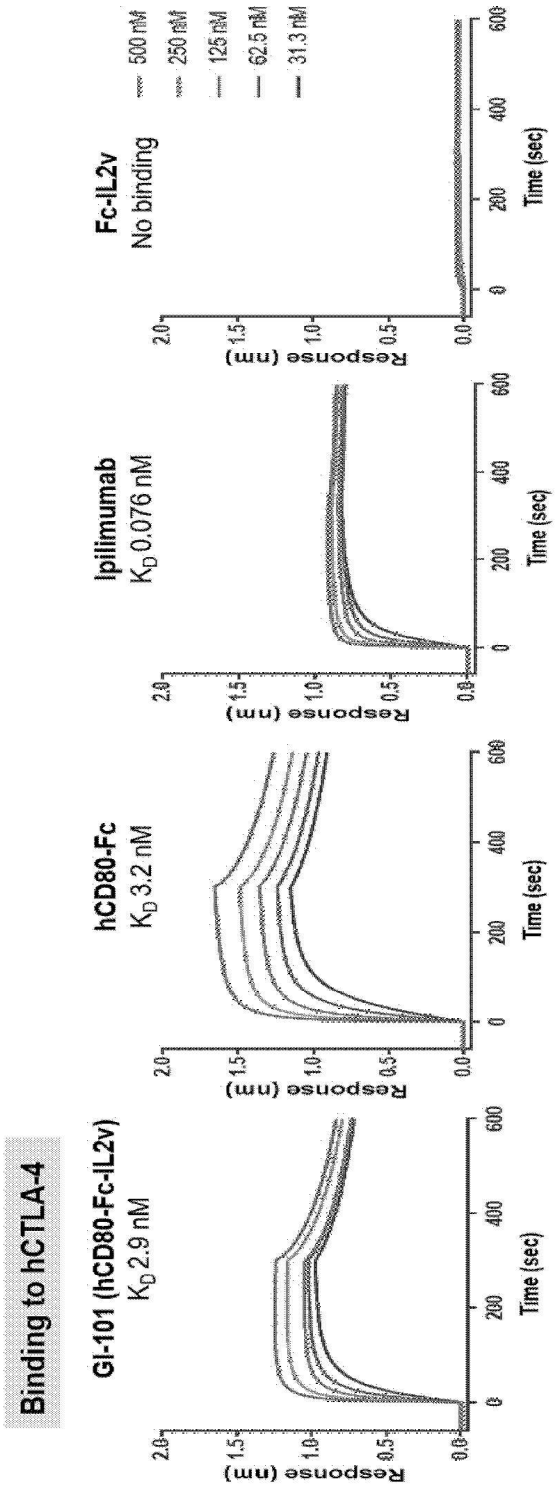
Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
mGI101	2.58E-08	3.05E+05	7.88E-04

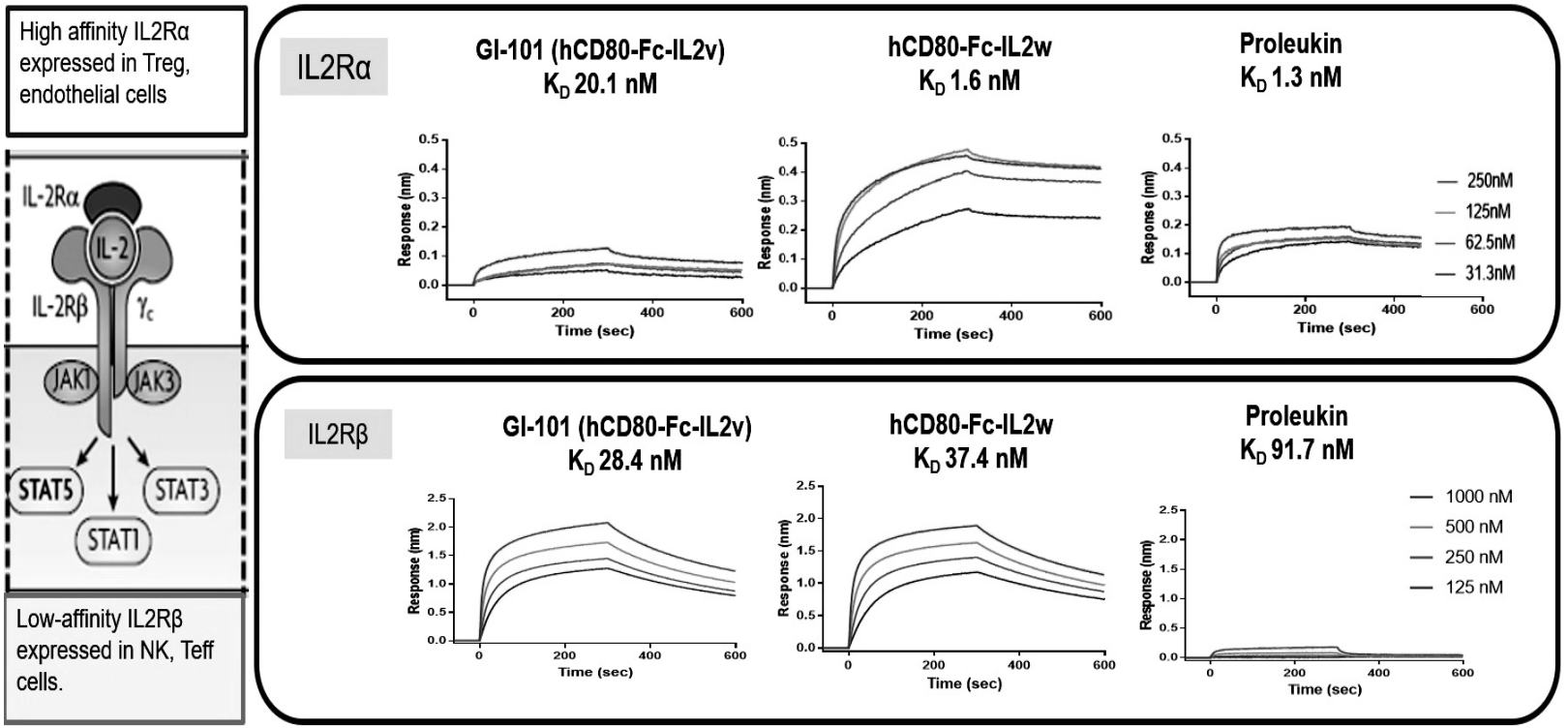
[Fig. 20]



Analyte	K_D (M)	K_{on} (1/Ms)	K_{dis} (1/s)
mGI101	9.91E-09	8.12E+05	8.04E-03

[Fig. 21]

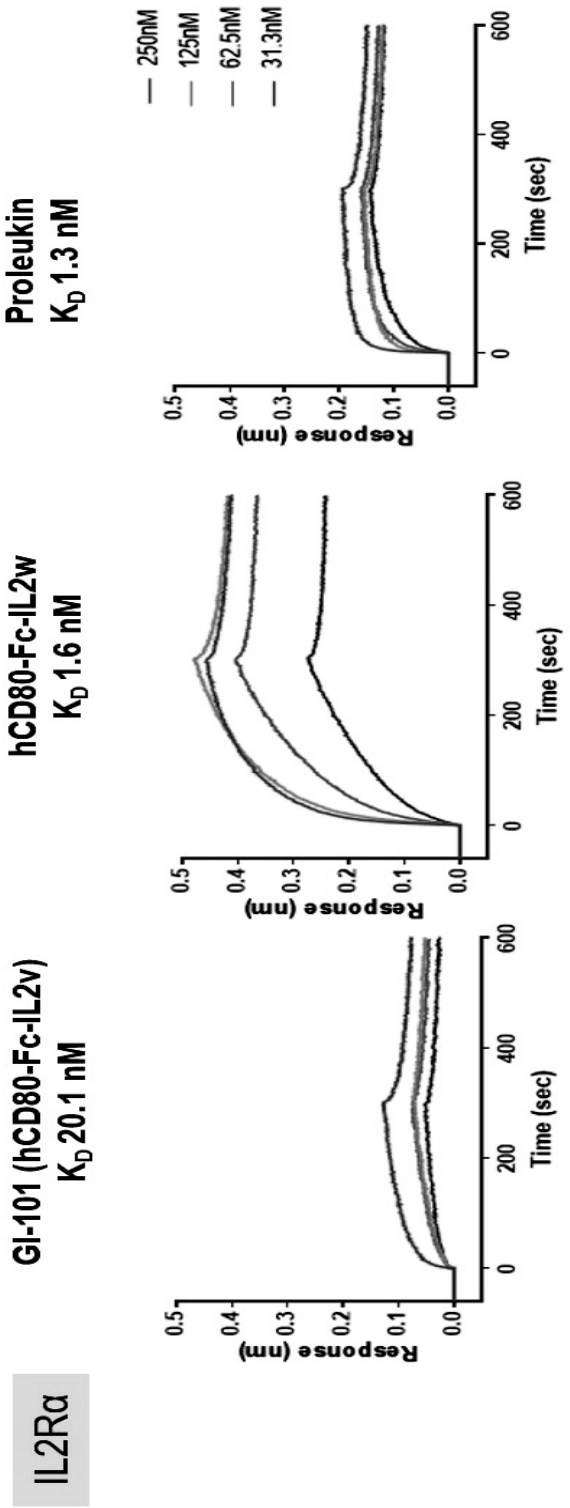

Binding to hCTLA-4
GI-101 (hCD80-Fc-IL2v)
K_D 2.9 nM
hCD80-Fc
K_D 3.2 nM
Ipilimumab
K_D 0.076 nM
Fc-IL2v
No binding
500 nM
250 nM
125 nM
62.5 nM
31.3 nM
Response (nm)
Time (sec)



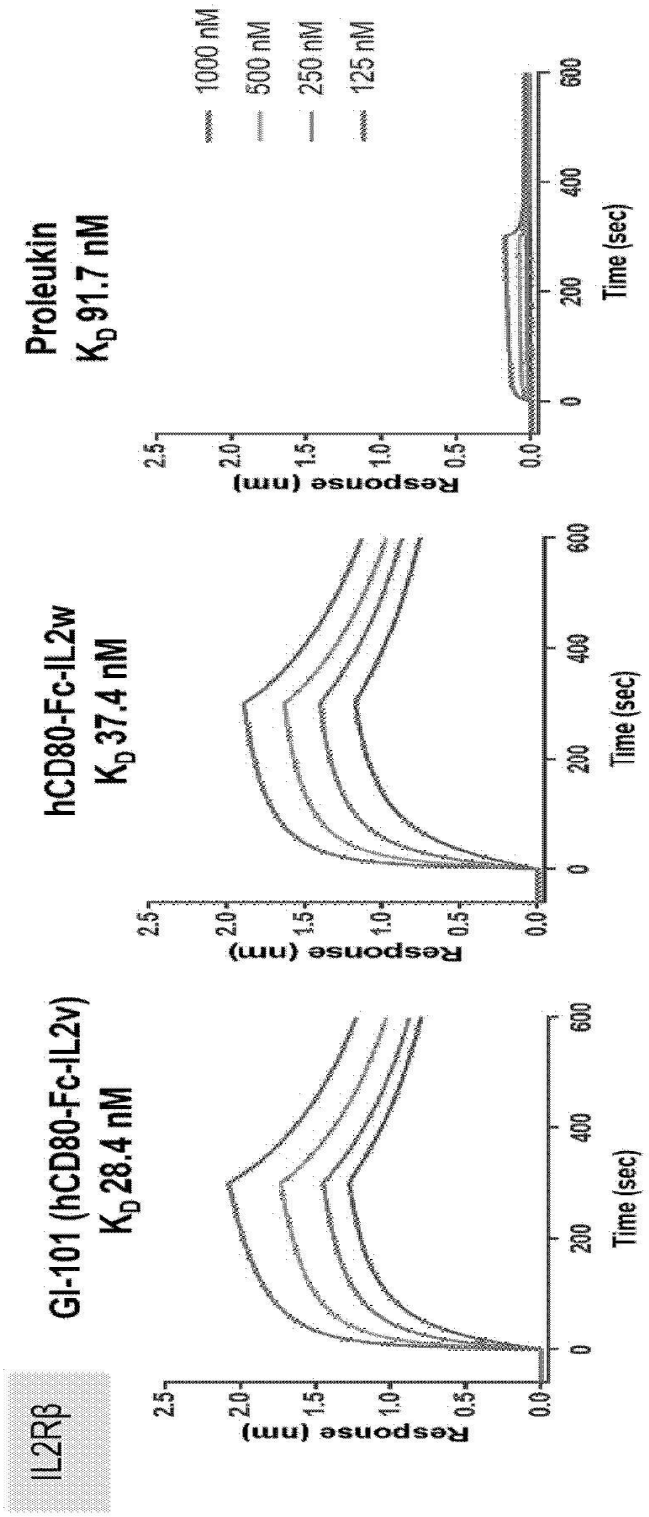
References	Kd (nM)			
Receptor	*hIL2w	**NKTR-214 (IL2)	**NKTR-214 (1-PEG-IL2)	**NKTR-214 (2-PEG-IL2)
hIL2Rα	10 (x14)	8.6 (x28)	189.7 (x9)	486.6 (x8)
hIL2Rβ	144	238.9	1769.5	3951.8

**Stauber, PNAS 2006, 103 (8), **Charych, PLOS ONE July 2017*

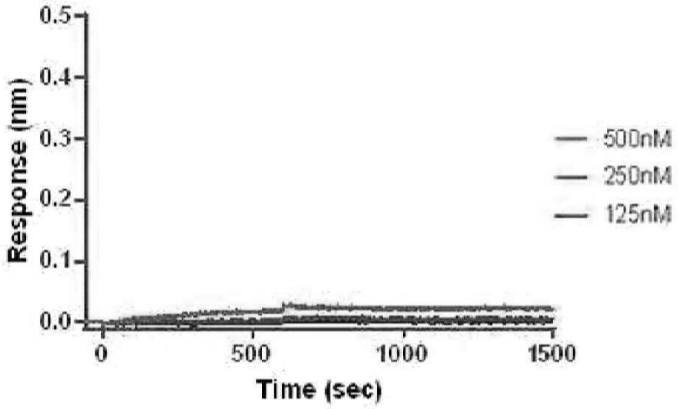
[Fig. 23]


IL2Rα
GI-101 (hCD80-Fc-IL2v)
K_D 20.1 nM
hCD80-Fc-IL2w
K_D 1.6 nM
Proleukin
K_D 1.3 nM
250nM
125nM
62.5nM
31.3nM
Response (nm)
Time (sec)
0.0
0.1
0.2
0.3
0.4
0.5
0
200
400
600

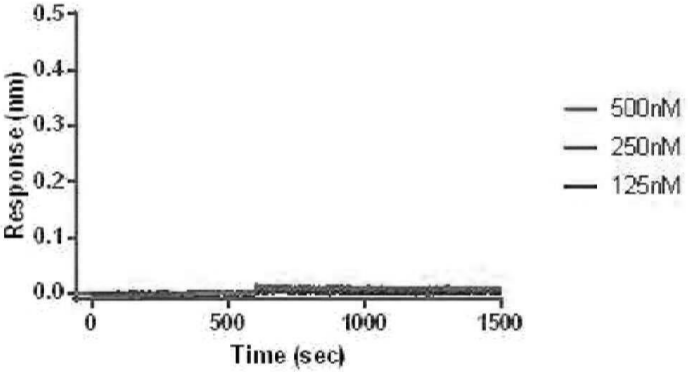
[Fig. 24]


IL2Rβ
GI-101 (hCD80-Fc-IL2v)
K_D 28.4 nM
hCD80-Fc-IL2w
K_D 37.4 nM
Proleukin
K_D 91.7 nM
1000 nM
500 nM
250 nM
125 nM
Response (nm)
Time (sec)
0.0
0.5
1.0
1.5
2.0
2.5
0
200
400
600

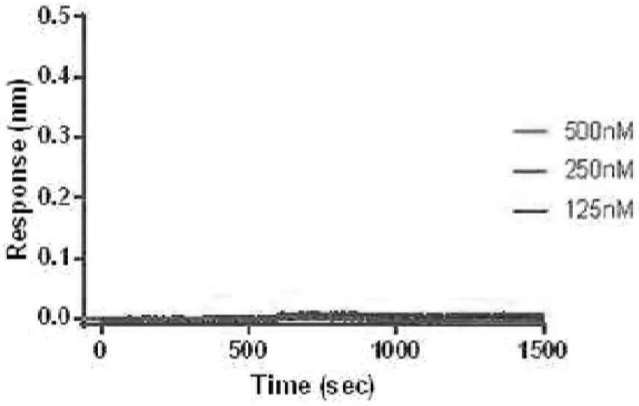
[Fig. 25]



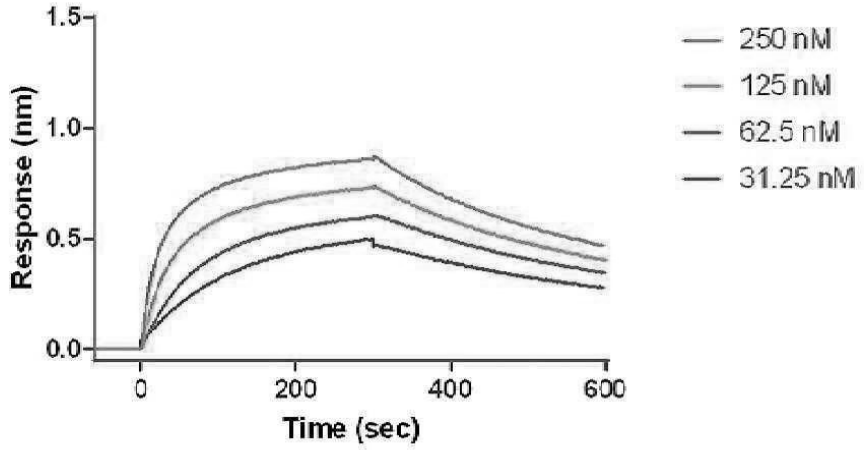
[Fig. 26]



[Fig. 27]

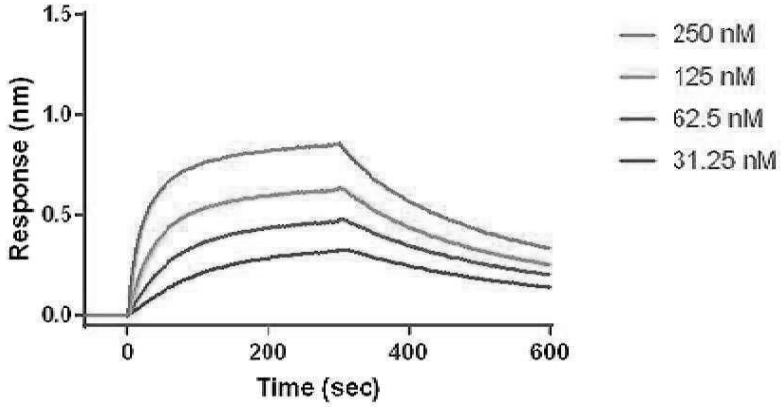


[Fig. 28]



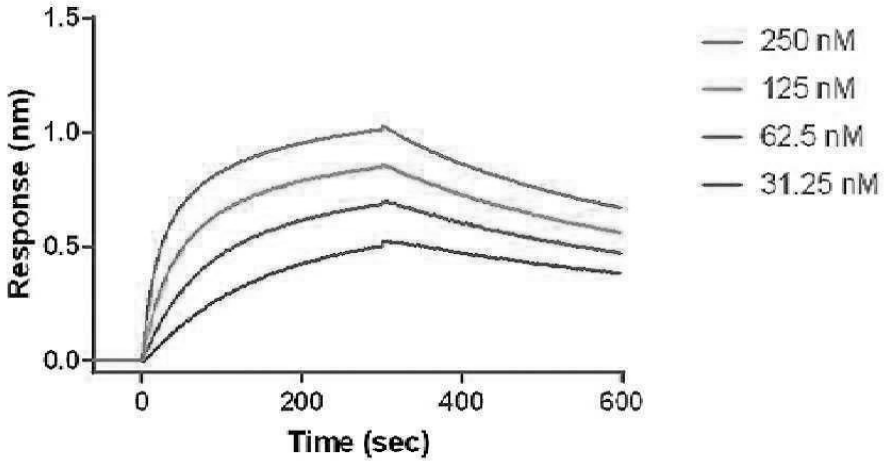
Kon	Koff	Kd
1.30X105	2.01X10-3	1.55X10-8

[Fig. 29]



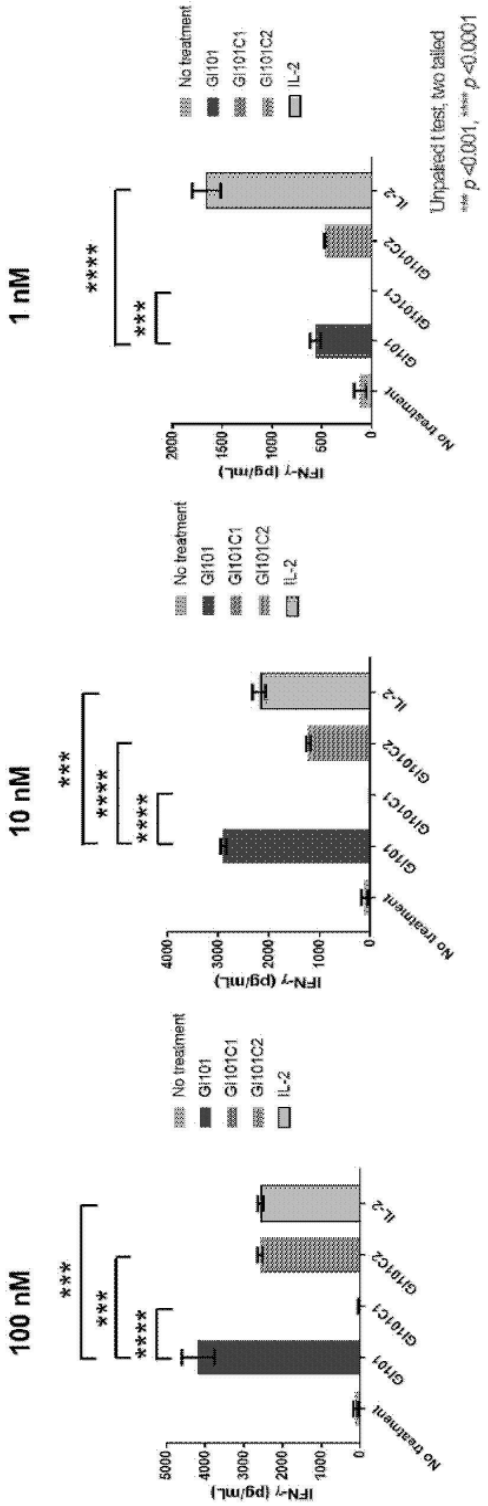
Kon	Koff	Kd
1.32X105	3.11X10-3	2.36X10-8

[Fig. 30]

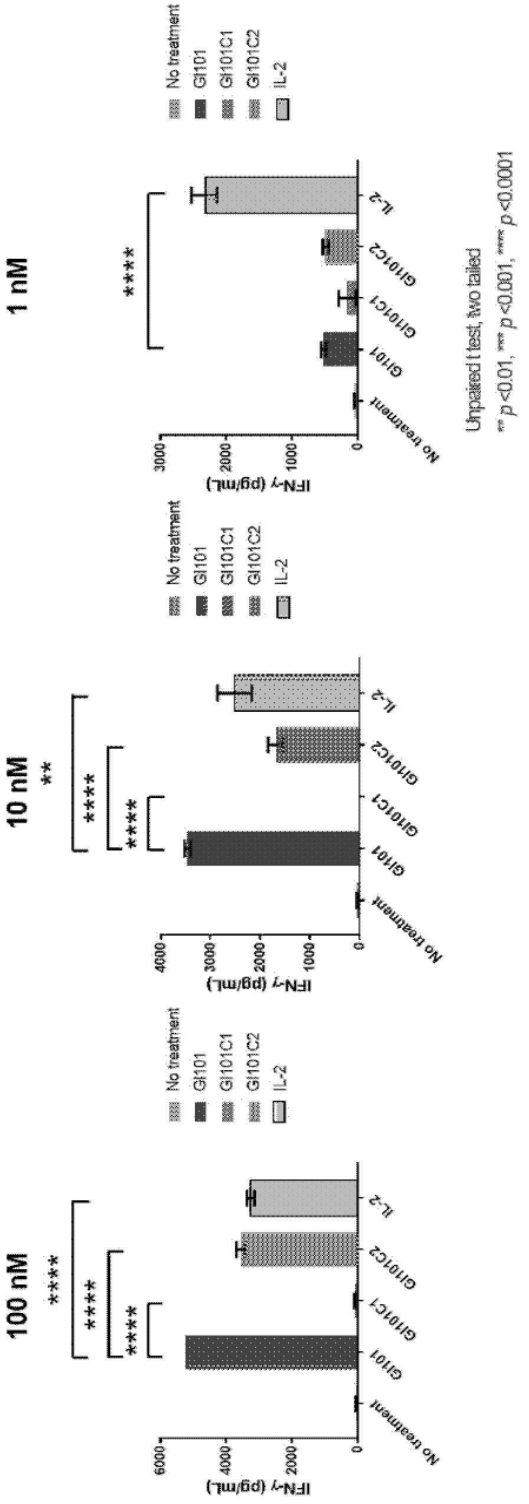


Kon	Koff	Kd
1.10X105	1.27X10-3	1.15X10-8

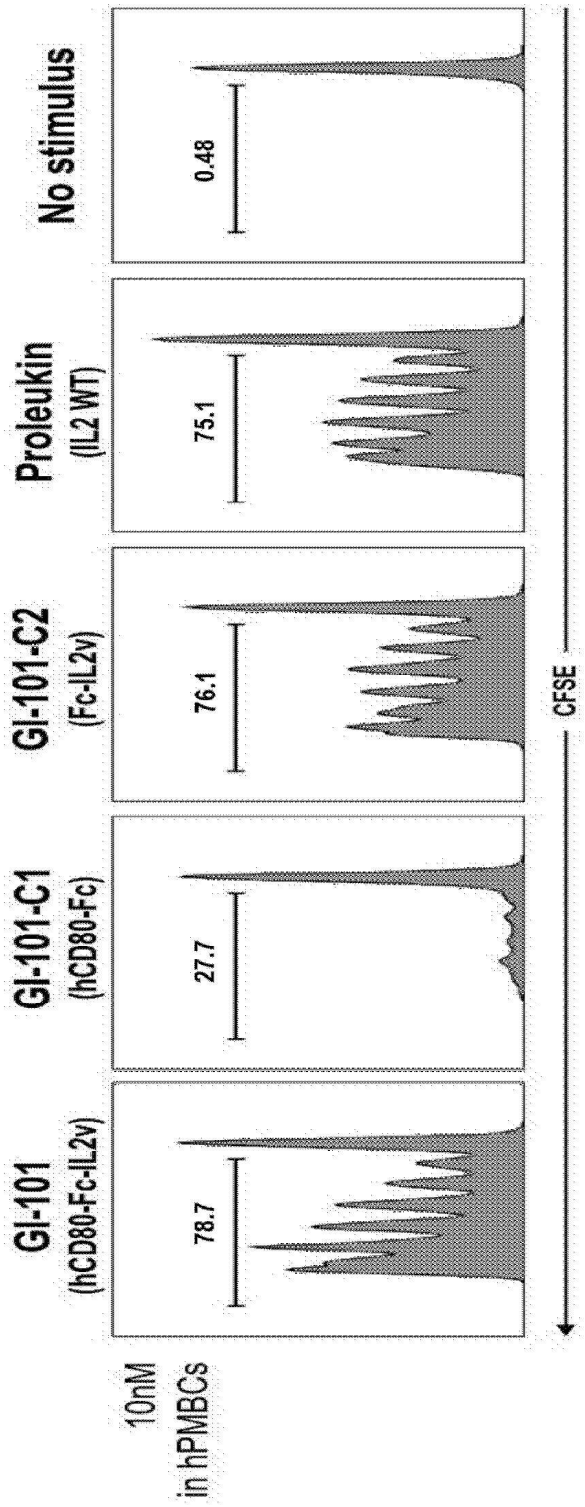
[Fig. 31]


100 nM
10 nM
1 nM
IFN-γ (pg/mL)
No treatment
GI101
GI101C1
GI101C2
IL-2
Unpaired t test, two tailed
** p <0.001, **** p <0.0001

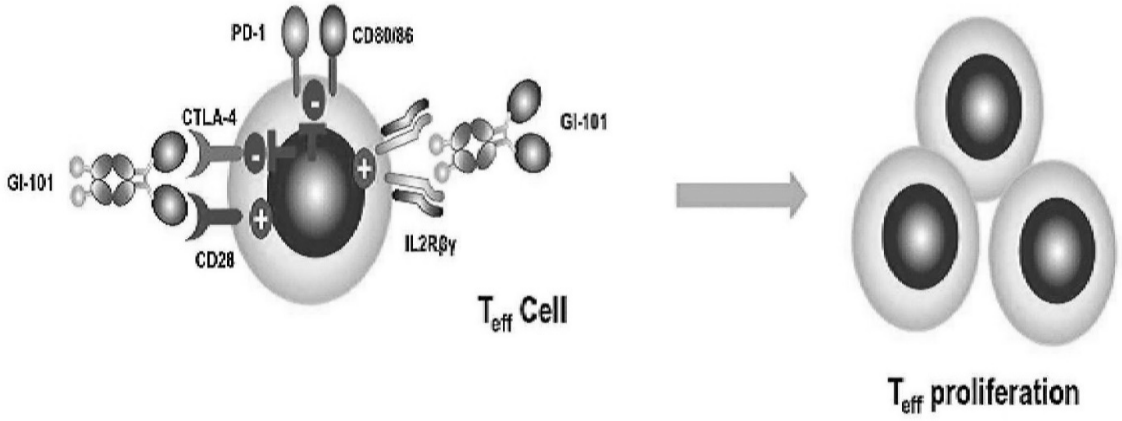
[Fig. 32]


100 nM
10 nM
1 nM
IFN-γ (pg/mL)
No treatment
GI101
GI101C1
GI101C2
IL-2
Unpaired t test, two tailed
** p <0.01, *** p <0.001, **** p <0.0001

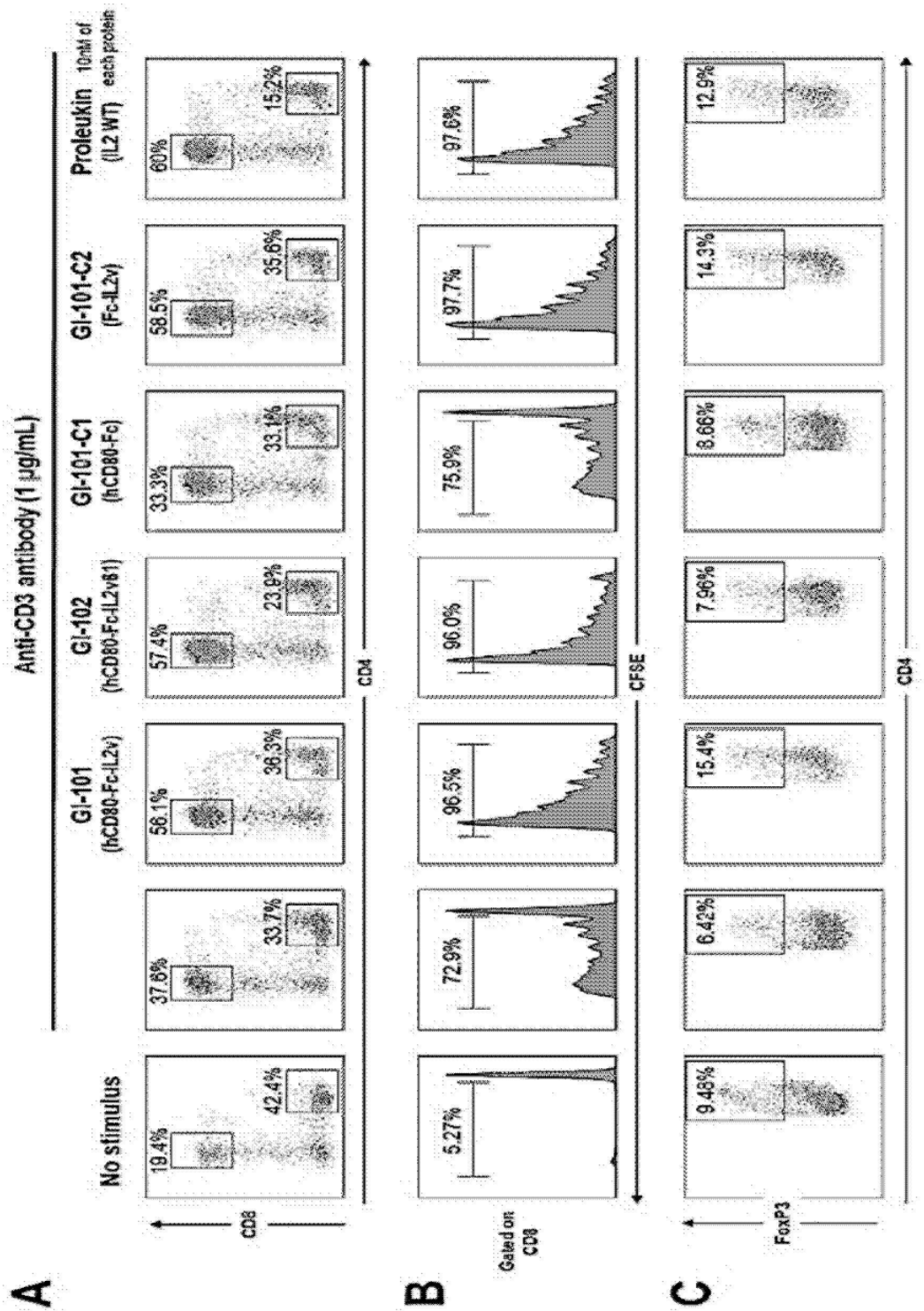
[Fig. 33]


GI-101
(hCD80-Fc-IL2v)
GI-101-C1
(hCD80-Fc)
GI-101-C2
(Fc-IL2v)
Proleukin
(IL2 WT)
No stimulus
78.7
27.7
76.1
75.1
0.48
CFSE
10nM
in hPMBCs

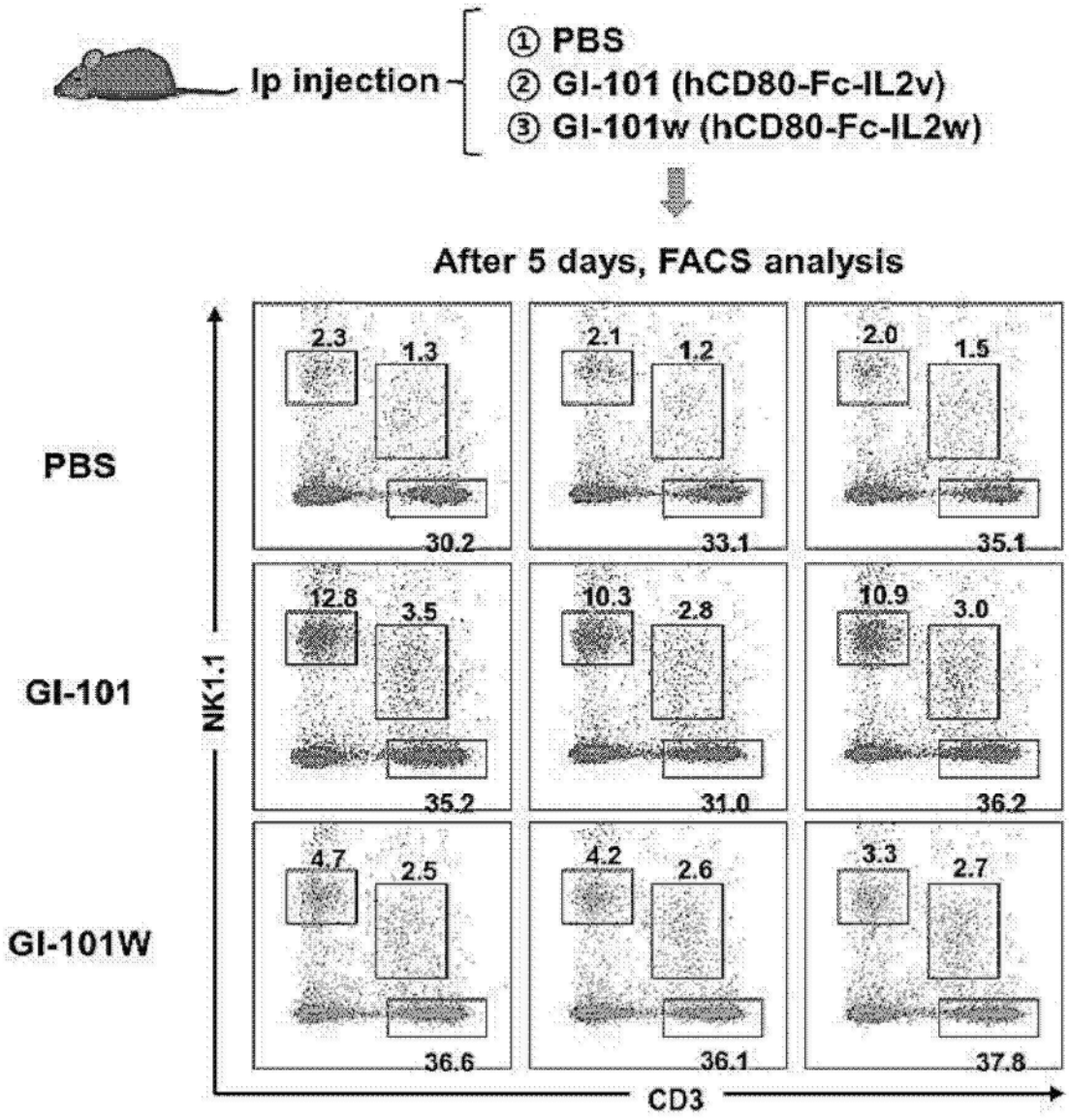
[Fig. 34]


PD-1
CD80/86
CTLA-4
GI-101
GI-101
CD28
IL2Rβγ
T_{eff} Cell
T_{eff} proliferation

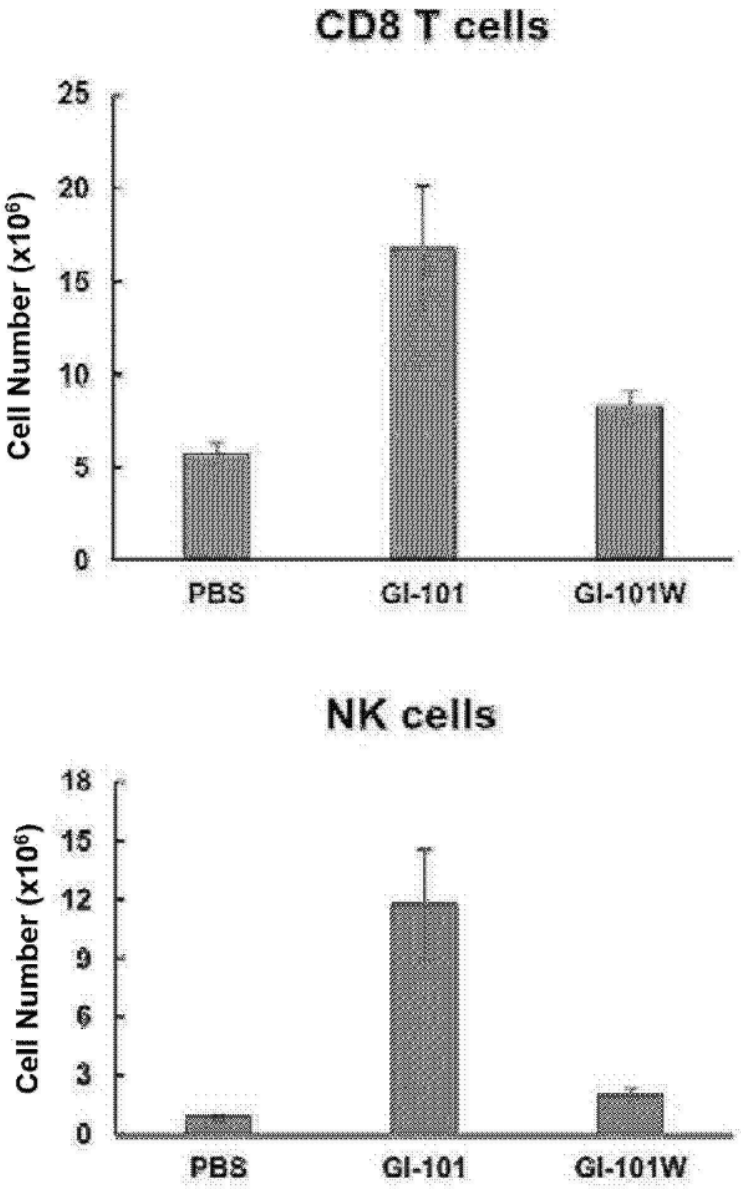
[Fig. 35]


A
Anti-CD3 antibody (1 μg/mL)
No stimulus
GI-101 (hCD80-Fc-IL2v)
GI-102 (hCD80-Fc-IL2v(61)
GI-101-C1 (hCD80-Fc)
GI-101-C2 (Fc-IL2v)
Proleukin 10nM of each protein (IL2 WT)
19.4%
42.4%
37.8%
33.7%
56.1%
36.3%
57.4%
23.9%
33.3%
33.1%
58.5%
35.6%
60%
15.2%
CD8
CD4
B
Gated on CD8
5.27%
72.9%
96.5%
96.0%
75.9%
97.7%
97.6%
CFSE
C
FoxP3
9.48%
6.42%
15.4%
7.96%
8.66%
14.3%
12.9%
CD4

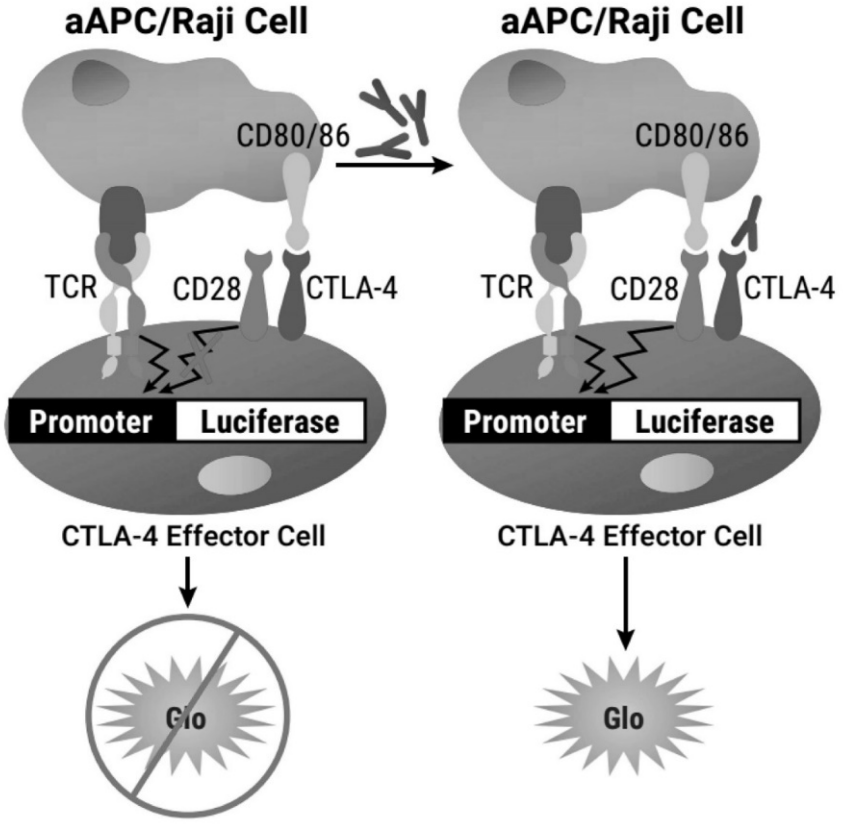
[Fig. 36]


Ip injection
① PBS
② GI-101 (hCD80-Fc-IL2v)
③ GI-101w (hCD80-Fc-IL2w)
After 5 days, FACS analysis
PBS
GI-101
GI-101W
NK1.1
CD3
2.3
1.3
30.2
2.1
1.2
33.1
2.0
1.5
35.1
12.8
3.5
35.2
10.3
2.8
31.0
10.9
3.0
36.2
4.7
2.5
36.6
4.2
2.6
36.1
3.3
2.7
37.8

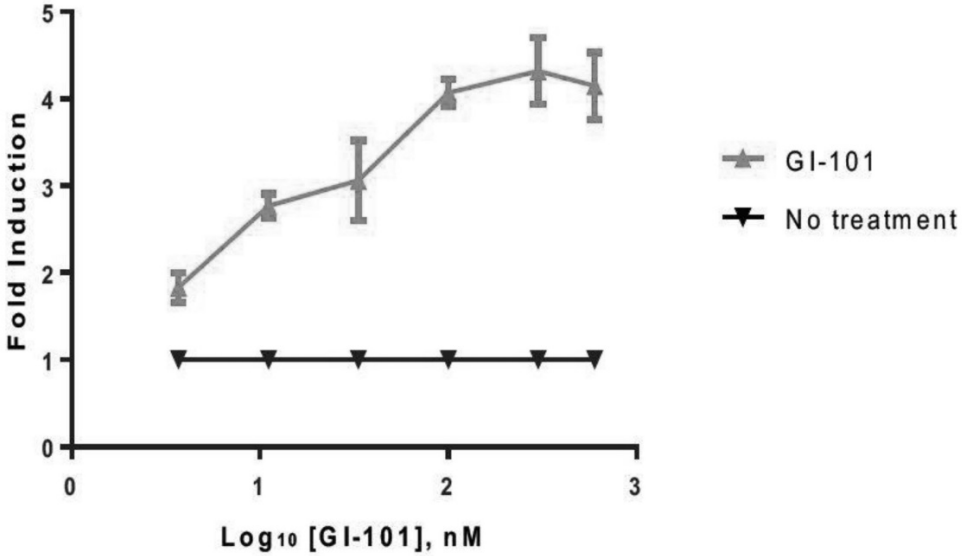
[Fig. 37]


CD8 T cells
Cell Number (x10⁶)
25
20
15
10
5
0
PBS
GI-101
GI-101W
NK cells
Cell Number (x10⁶)
18
15
12
9
6
3
0
PBS
GI-101
GI-101W

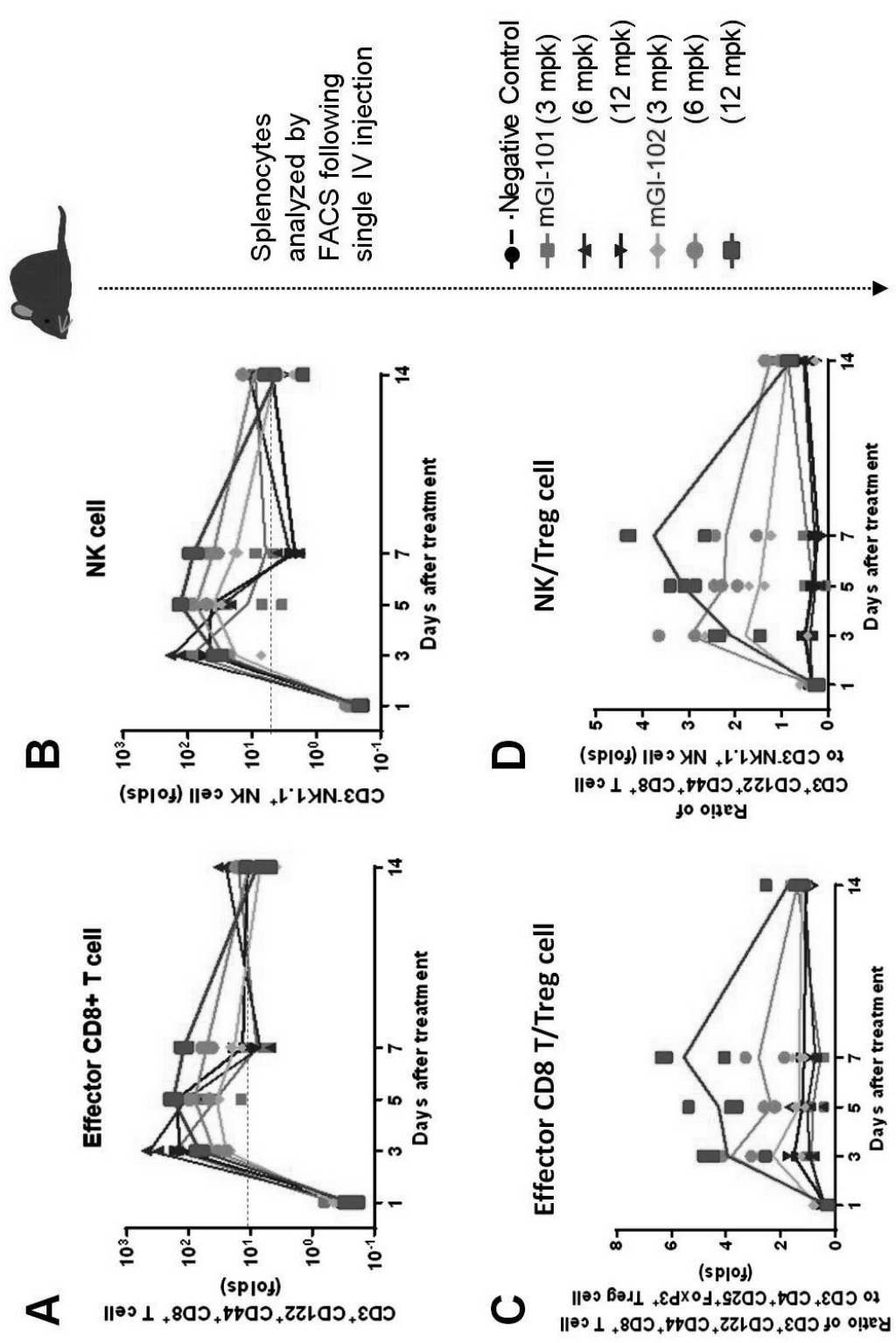
[Fig. 38]


aAPC/Raji Cell
aAPC/Raji Cell
CD80/86
CD80/86
TCR
CD28
CTLA-4
TCR
CD28
CTLA-4
Promoter
Luciferase
Promoter
Luciferase
CTLA-4 Effector Cell
CTLA-4 Effector Cell
Glo
Glo

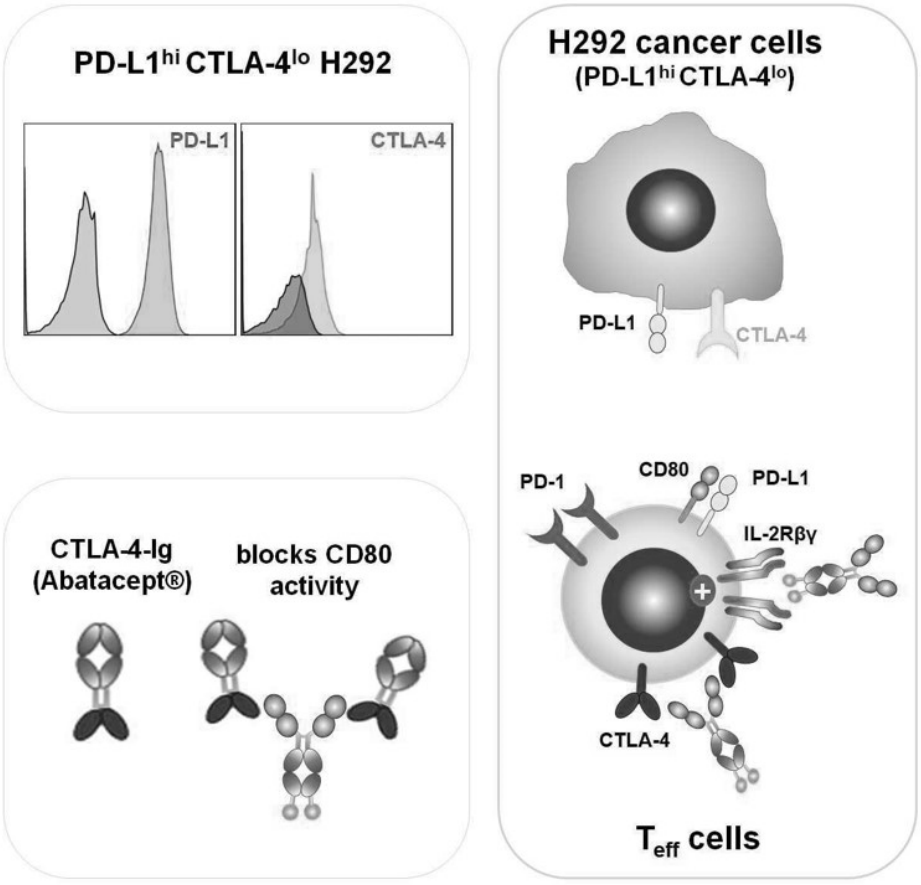
[Fig. 39]


Fold Induction
0
1
2
3
4
5
0
1
2
3
Log10 [GI-101], nM
GI-101
No treatment

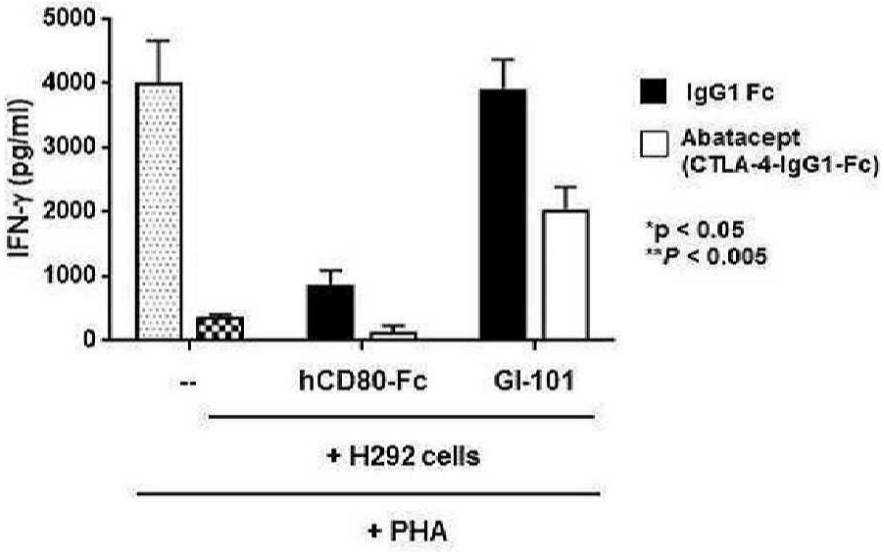
[Fig. 40]


A
Effector CD8+ T cell
CD3+CD122+CD44+CD8+ T cell (folds)
Days after treatment
B
NK cell
CD3-NK1.1+ NK cell (folds)
Days after treatment
Splenocytes analyzed by FACS following single IV injection
Negative Control
mGI-101 (3 mpk)
(6 mpk)
(12 mpk)
mGI-102 (3 mpk)
(6 mpk)
(12 mpk)
C
Effector CD8 T/Treg cell
Ratio of CD3+CD122+CD44+CD8+ T cell to CD3+CD4+CD25+FoxP3+ Treg cell (folds)
Days after treatment
D
NK/Treg cell
Ratio of CD3+CD122+CD44+CD8+ T cell to CD3-NK1.1+ NK cell (folds)
Days after treatment

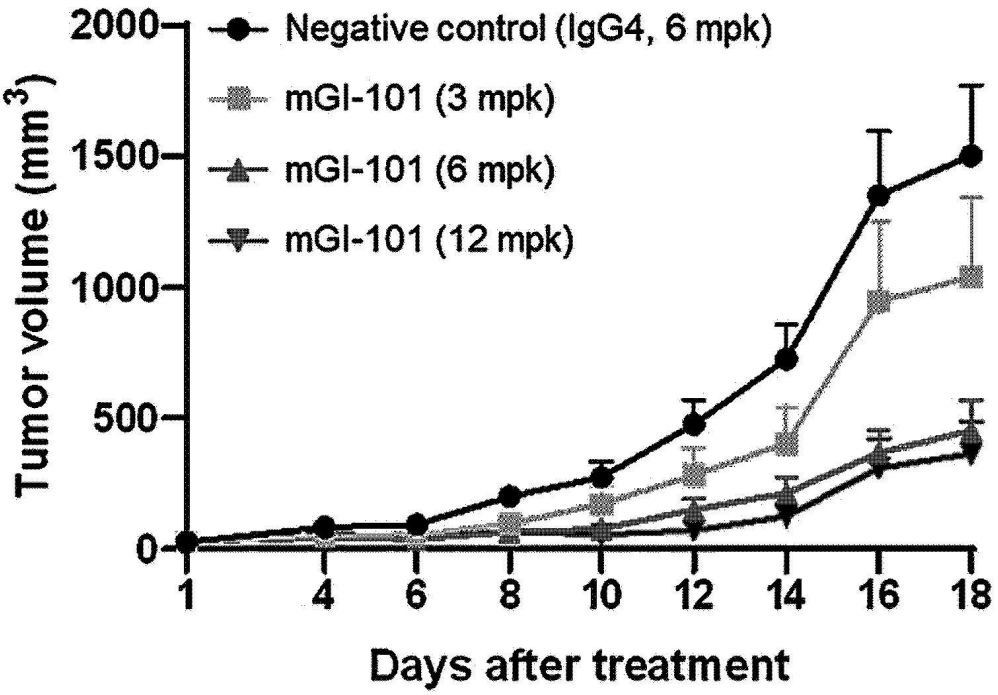
[Fig. 41]


PD-L1hi CTLA-4lo H292
PD-L1
CTLA-4
CTLA-4-Ig
(Abatacept®)
blocks CD80
activity
H292 cancer cells
(PD-L1hi CTLA-4lo)
PD-L1
CTLA-4
PD-1
CD80
PD-L1
IL-2Rβγ
CTLA-4
Teff cells

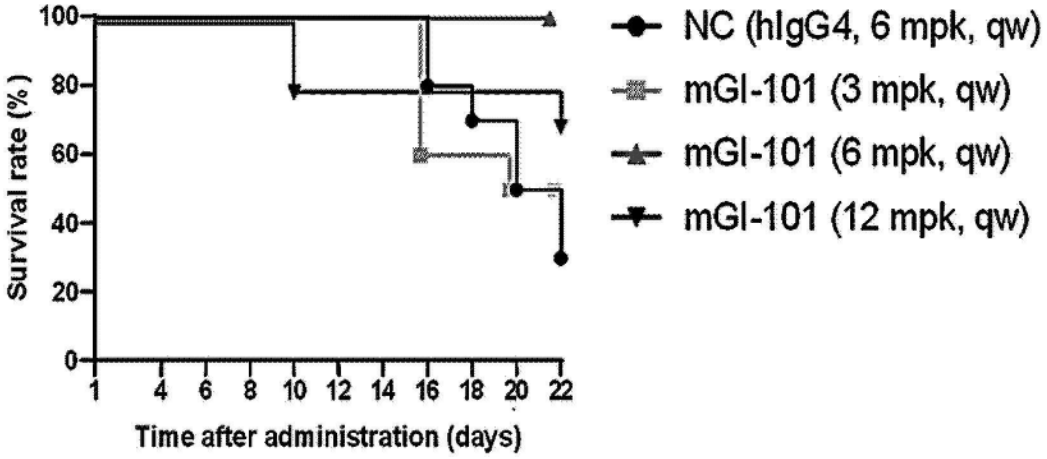
[Fig. 42]


5000
4000
3000
2000
1000
0
IFN-γ (pg/ml)
--
hCD80-Fc
GI-101
+ H292 cells
+ PHA
IgG1 Fc
Abatacept
(CTLA-4-IgG1-Fc)
*p < 0.05
**P < 0.005

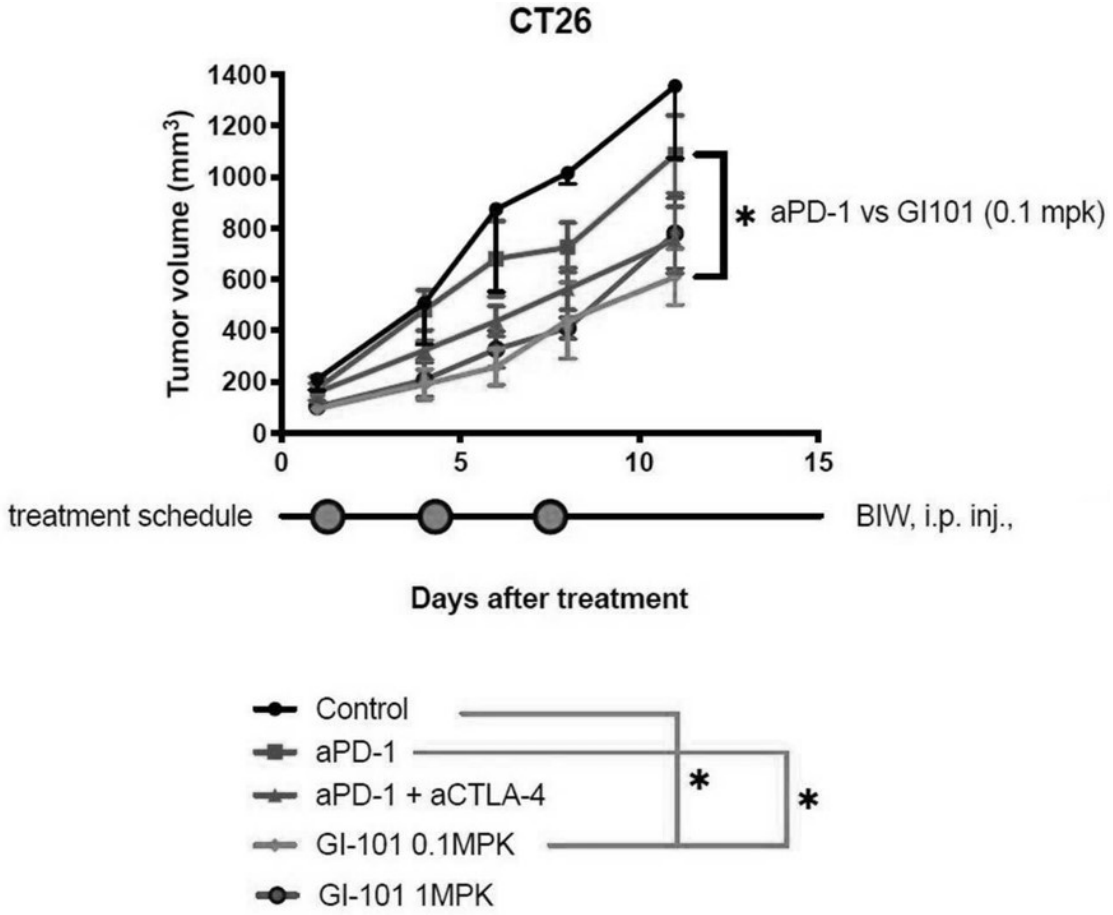
[Fig. 43]


Negative control (IgG4, 6 mpk)
mGI-101 (3 mpk)
mGI-101 (6 mpk)
mGI-101 (12 mpk)
Tumor volume (mm3)
2000
1500
1000
500
0
1
4
6
8
10
12
14
16
18
Days after treatment

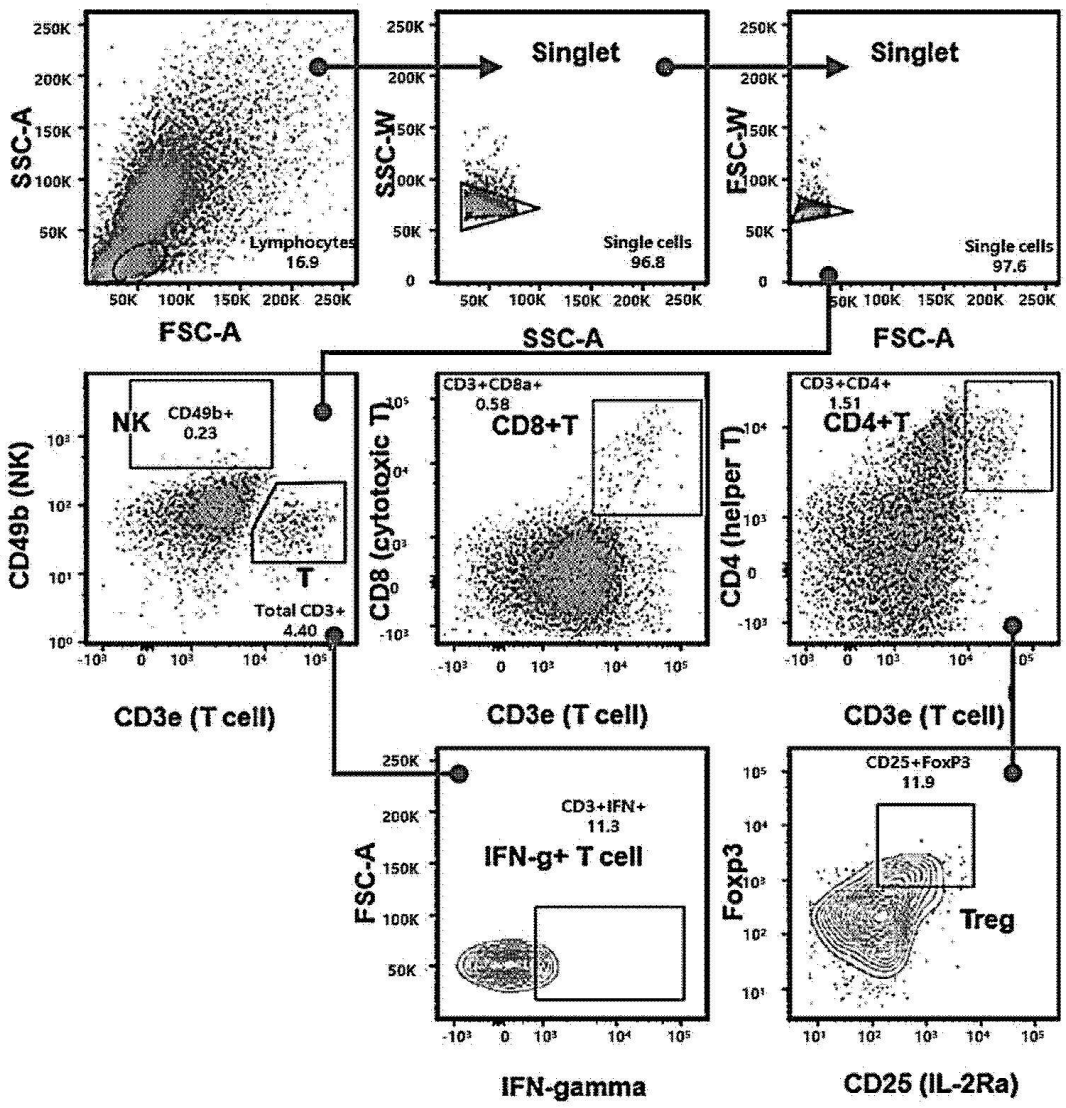
[Fig. 44]


NC (hIgG4, 6 mpk, qw)
mGI-101 (3 mpk, qw)
mGI-101 (6 mpk, qw)
mGI-101 (12 mpk, qw)
Survival rate (%)
100
80
60
40
20
0
1
4
6
8
10
12
14
16
18
20
22
Time after administration (days)

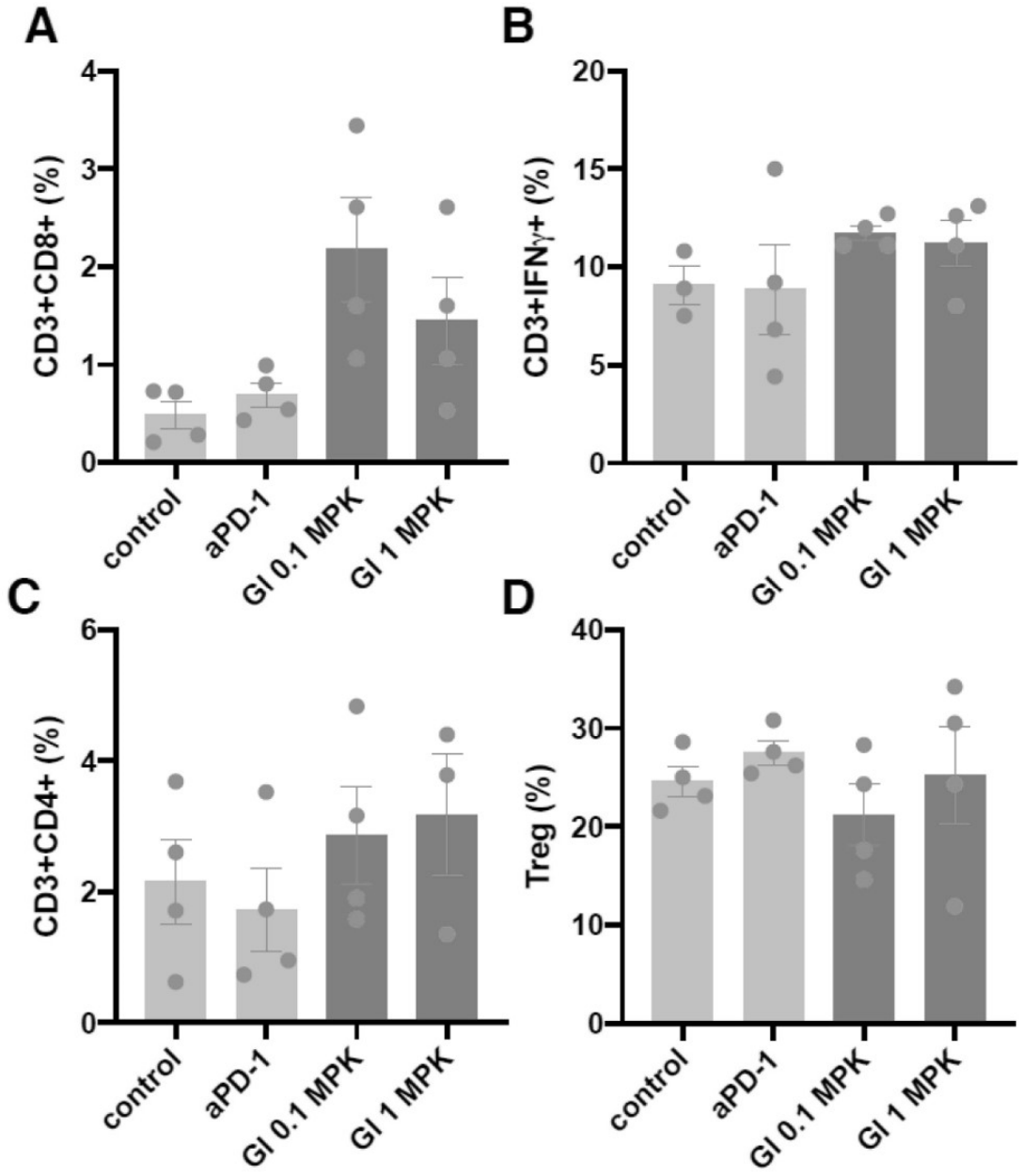
[Fig. 45]


CT26
Tumor volume (mm3)
1400
1200
1000
800
600
400
200
0
0
5
10
15
* aPD-1 vs GI101 (0.1 mpk)
treatment schedule
BIW, i.p. inj.,
Days after treatment
Control
aPD-1
aPD-1 + aCTLA-4
GI-101 0.1MPK
GI-101 1MPK
*
*

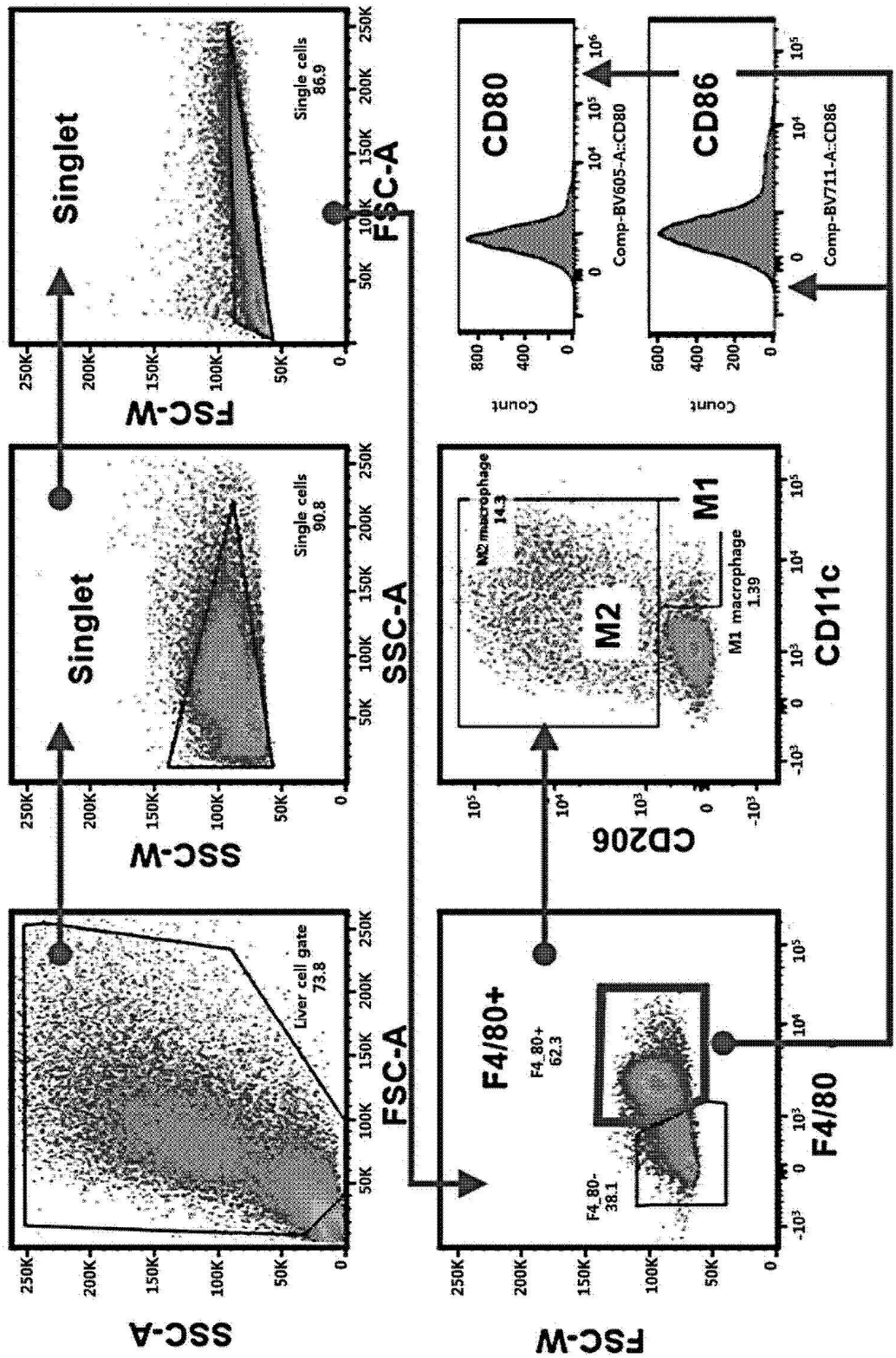
[Fig. 46]


SSC-A
FSC-A
Lymphocytes
16.9
Singlet
SSC-W
SSC-A
Single cells
96.8
Singlet
FSC-W
FSC-A
Single cells
97.6
CD49b (NK)
CD3e (T cell)
NK
CD49b+
0.23
T
Total CD3+
4.40
CD8 (cytotoxic T)
CD3e (T cell)
CD3+CD8a+
0.58
CD8+T
CD4 (helper T)
CD3e (T cell)
CD3+CD4+
1.51
CD4+T
FSC-A
IFN-gamma
CD3+IFN+
11.3
IFN-g+ T cell
Foxp3
CD25 (IL-2Ra)
CD25+FoxP3
11.9
Treg

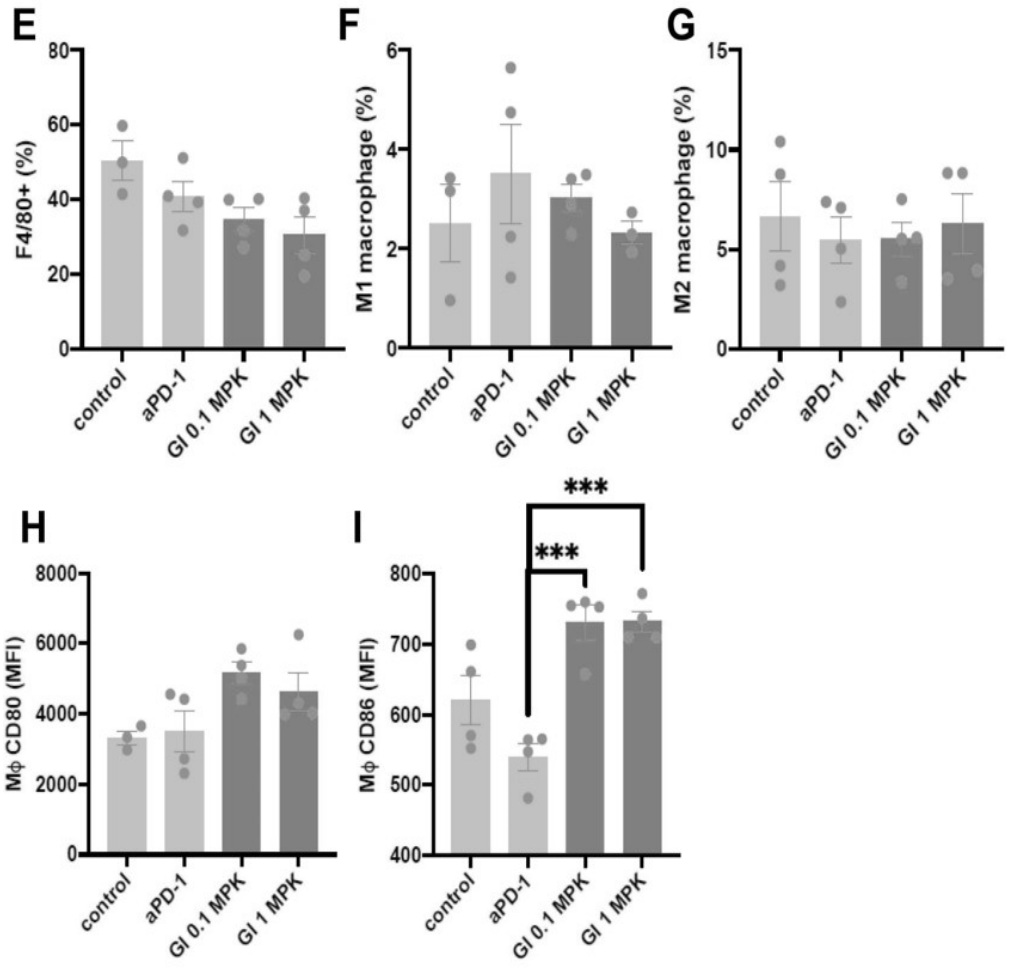
[Fig. 47]


A
CD3+CD8+ (%)
0
1
2
3
4
control
aPD-1
GI 0.1 MPK
GI 1 MPK
B
CD3+IFNγ+ (%)
0
5
10
15
20
control
aPD-1
GI 0.1 MPK
GI 1 MPK
C
CD3+CD4+ (%)
0
2
4
6
control
aPD-1
GI 0.1 MPK
GI 1 MPK
D
Treg (%)
0
10
20
30
40
control
aPD-1
GI 0.1 MPK
GI 1 MPK

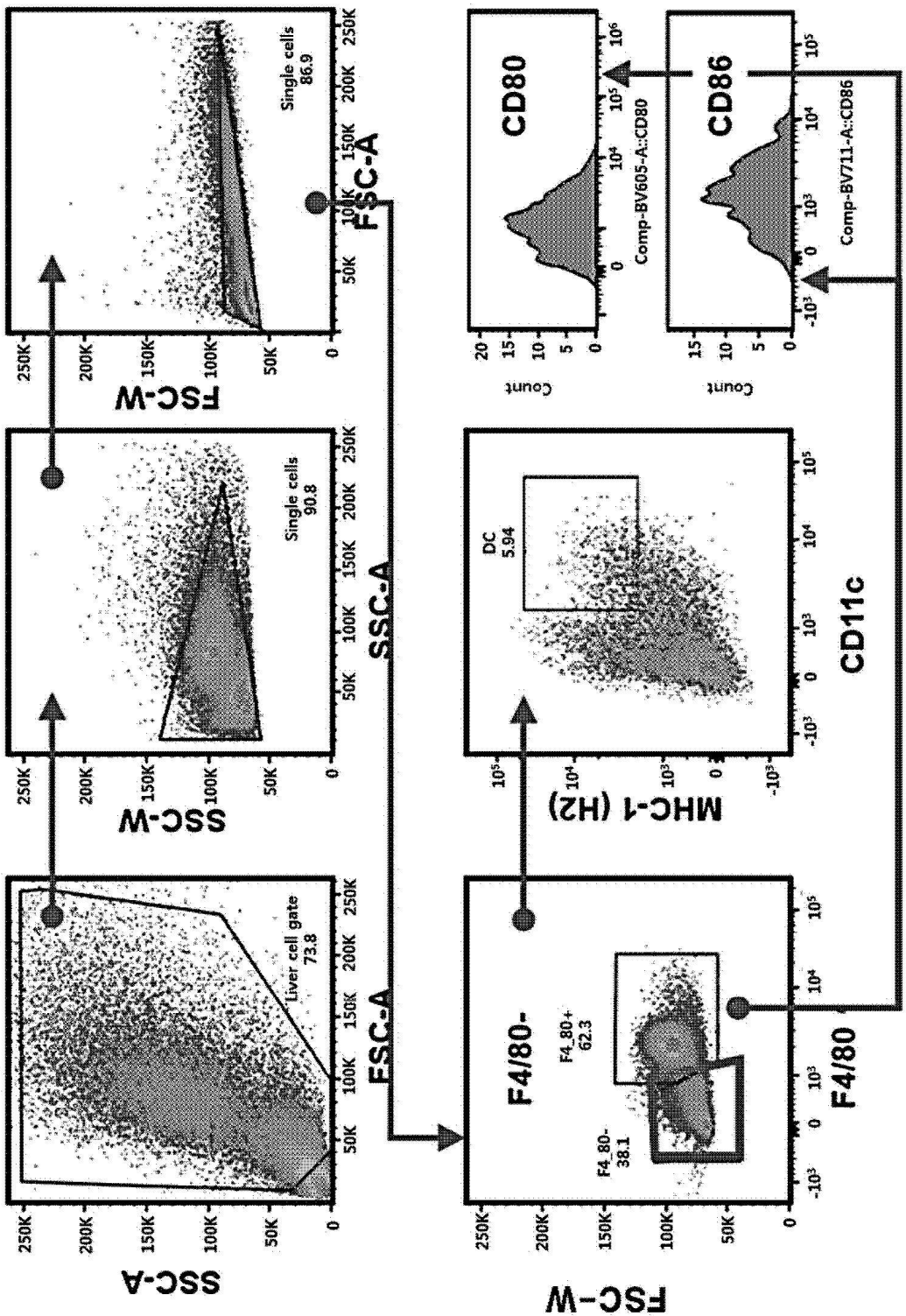
[Fig. 48]


SSC-A
FSC-A
Liver cell gate
73.8
FSC-W
SSC-A
Singlet
Single cells
90.8
SSC-W
FSC-A
Singlet
Single cells
86.9
FSC-W
F4/80
F4/80+
F4_80+
62.3
F4_80-
38.1
CD206
CD11c
M2 macrophage
14.3
M2
M1
M1 macrophage
1.39
Count
CD80
Comp-BV605-A::CD80
Count
CD86
Comp-BV711-A::CD86

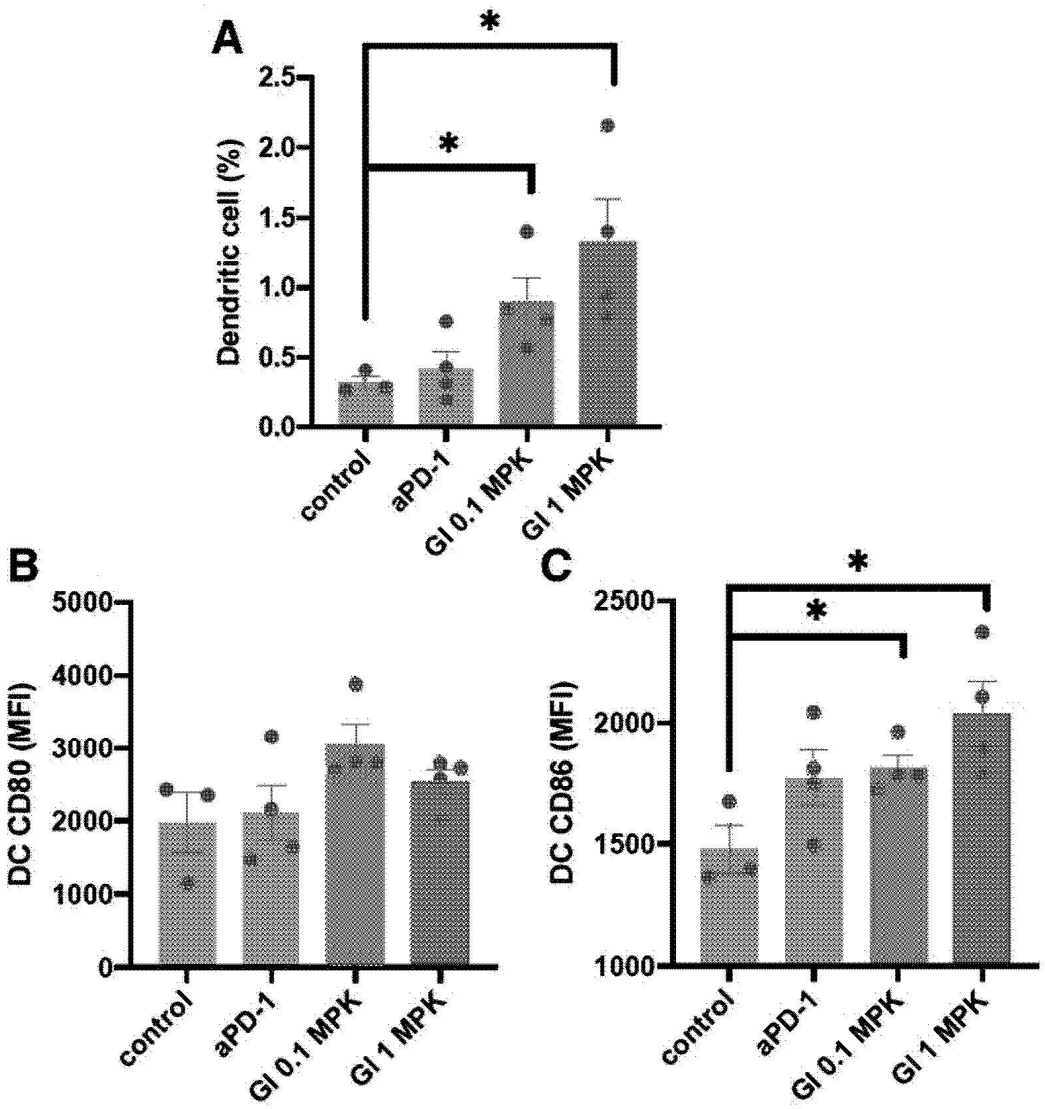
[Fig. 49]


E
F4/80+ (%)
80
60
40
20
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
F
M1 macrophage (%)
6
4
2
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
G
M2 macrophage (%)
15
10
5
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
H
Mϕ CD80 (MFI)
8000
6000
4000
2000
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
I
Mϕ CD86 (MFI)
800
700
600
500
400
***
***
control
aPD-1
GI 0.1 MPK
GI 1 MPK

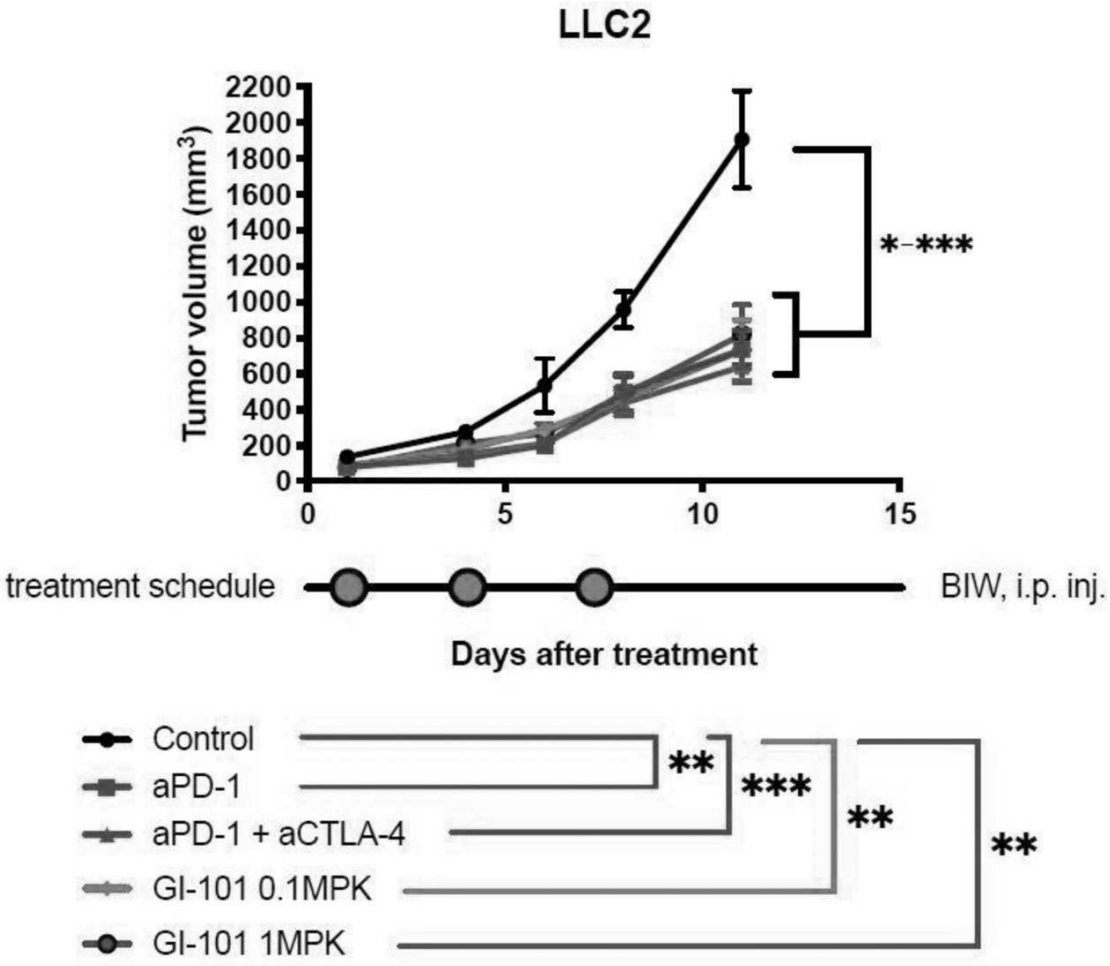
[Fig. 50]


SSC-A
Liver cell gate
73.8
FSC-A
SSC-W
Single cells
90.8
SSC-A
FSC-W
Single cells
86.9
FSC-A
FSC-W
F4/80-
F4_80+
62.3
F4_80-
38.1
F4/80
MHC-1 (H2)
DC
5.94
CD11c
Count
CD80
Comp-BV605-A::CD80
Count
CD86
Comp-BV711-A::CD86

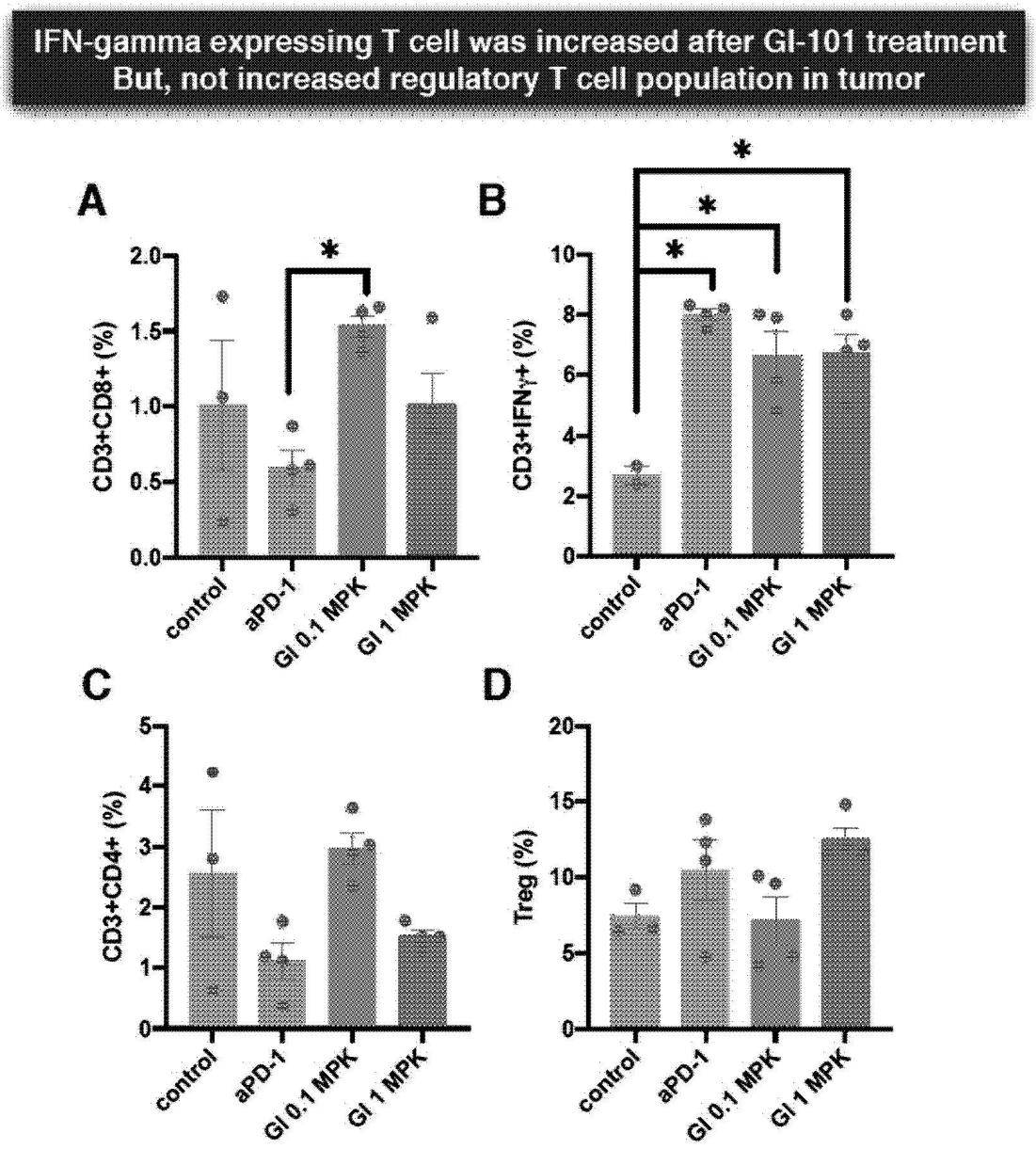
[Fig. 51]


A
Dendritic cell (%)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
control
aPD-1
GI 0.1 MPK
GI 1 MPK
B
DC CD80 (MFI)
5000
4000
3000
2000
1000
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
C
DC CD86 (MFI)
2500
2000
1500
1000
*
*
control
aPD-1
GI 0.1 MPK
GI 1 MPK

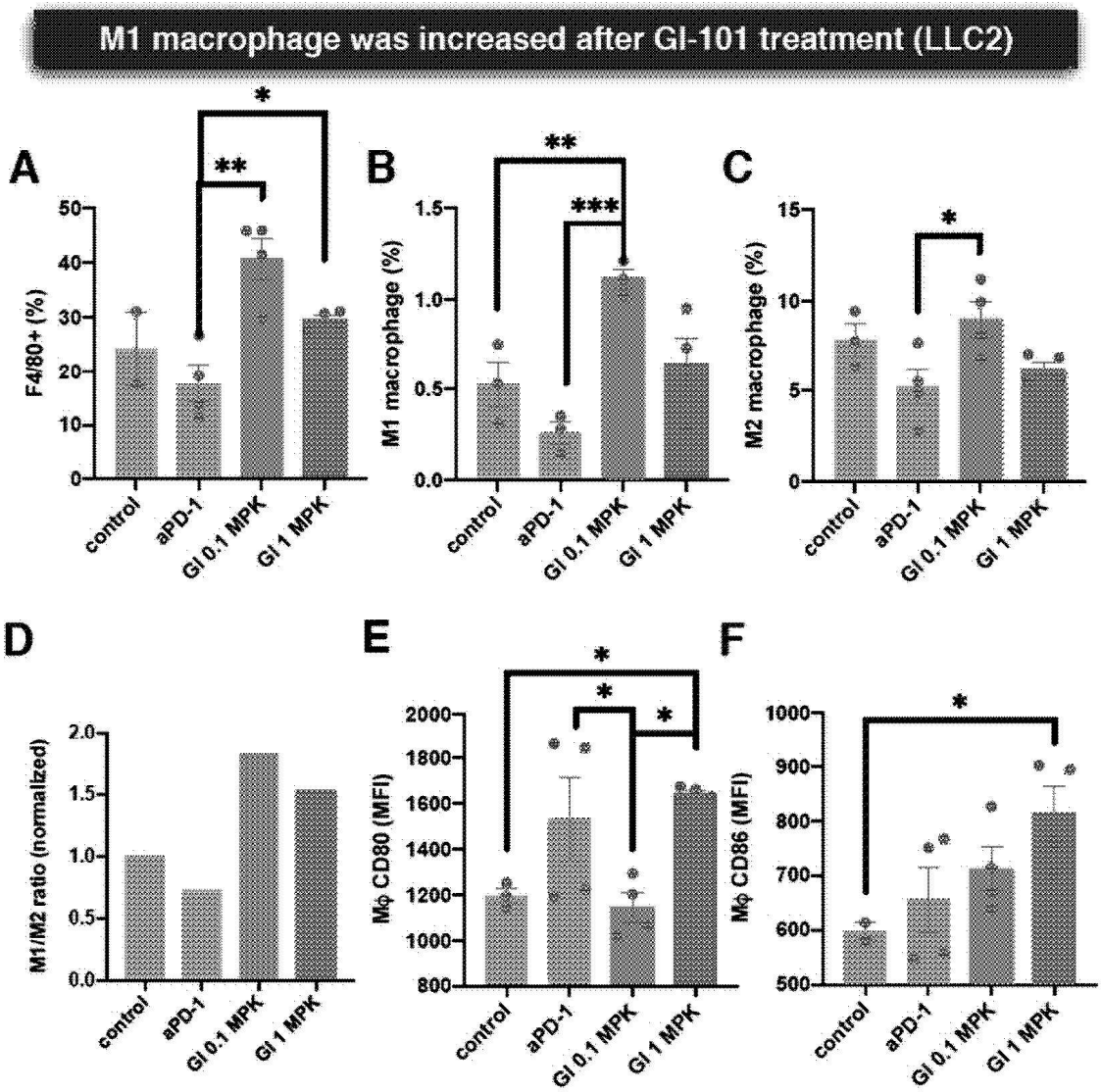
[Fig. 52]


LLC2
Tumor volume (mm3)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
*-***
treatment schedule
BIW, i.p. inj.
Days after treatment
Control
aPD-1
aPD-1 + aCTLA-4
GI-101 0.1MPK
GI-101 1MPK
**
***
**
**

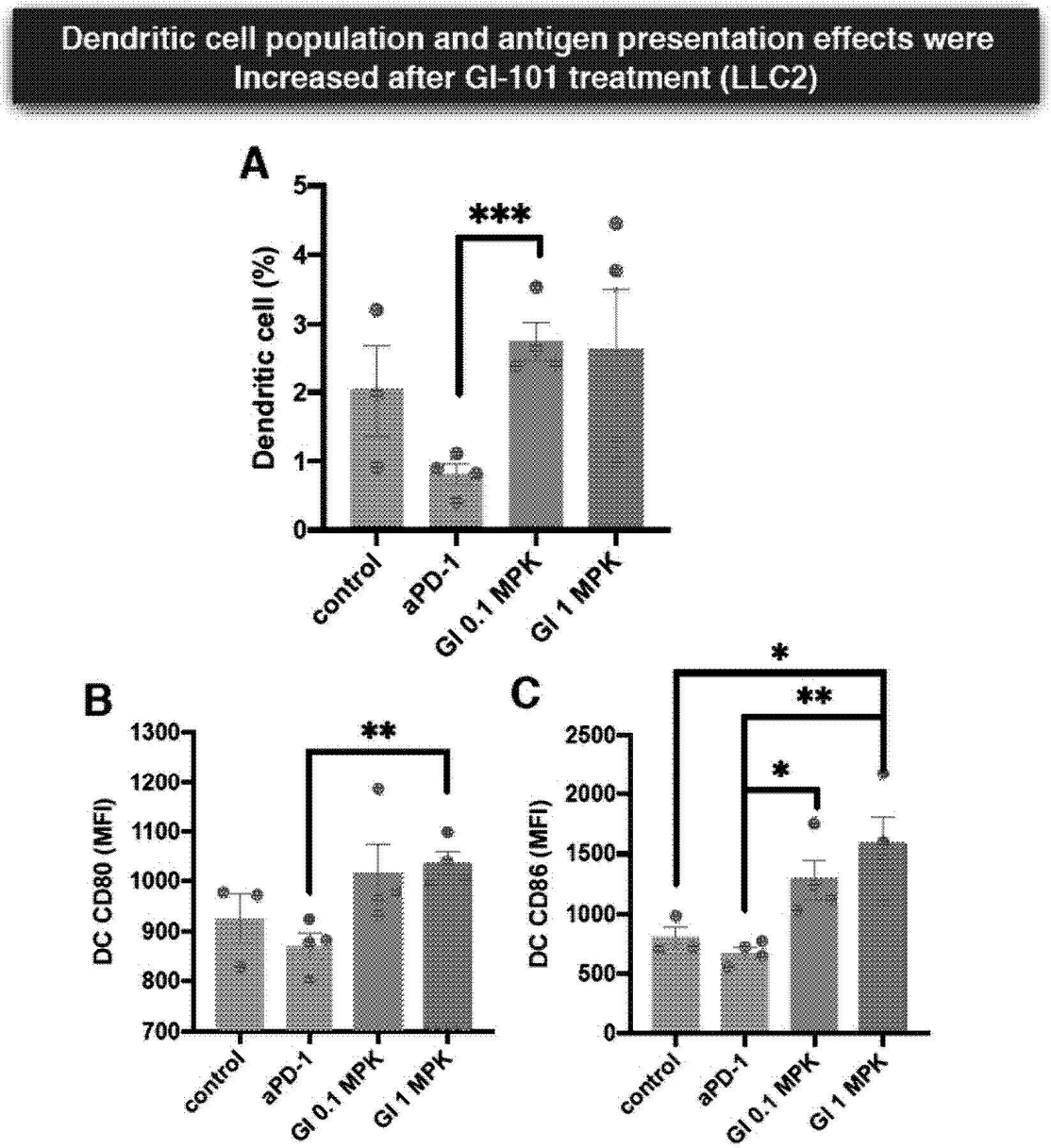
[Fig. 53]


IFN-gamma expressing T cell was increased after GI-101 treatment
But, not increased regulatory T cell population in tumor
A
CD3+CD8+ (%)
2.0
1.5
1.0
0.5
0.0
*
control
aPD-1
GI 0.1 MPK
GI 1 MPK
B
CD3+IFNγ+ (%)
10
8
6
4
2
0
*
*
*
control
aPD-1
GI 0.1 MPK
GI 1 MPK
C
CD3+CD4+ (%)
5
4
3
2
1
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK
D
Treg (%)
20
15
10
5
0
control
aPD-1
GI 0.1 MPK
GI 1 MPK

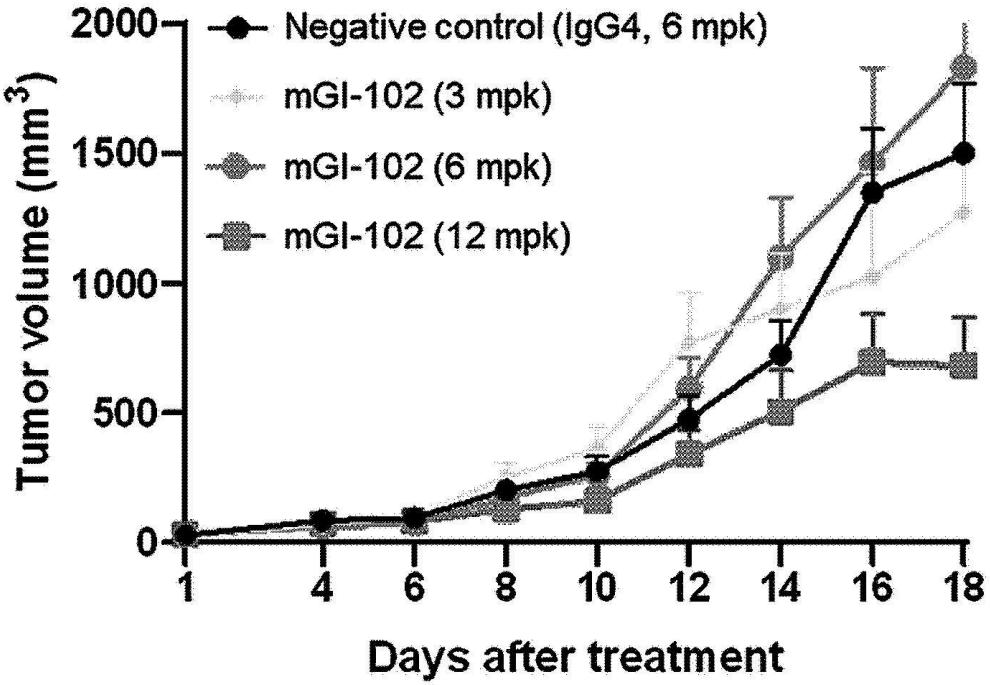
[Fig. 54]


M1 macrophage was increased after GI-101 treatment (LLC2)
A
F4/80+ (%)
B
M1 macrophage (%)
C
M2 macrophage (%)
D
M1/M2 ratio (normalized)
E
Mφ CD80 (MFI)
F
Mφ CD86 (MFI)
control
aPD-1
GI 0.1 MPK
GI 1 MPK

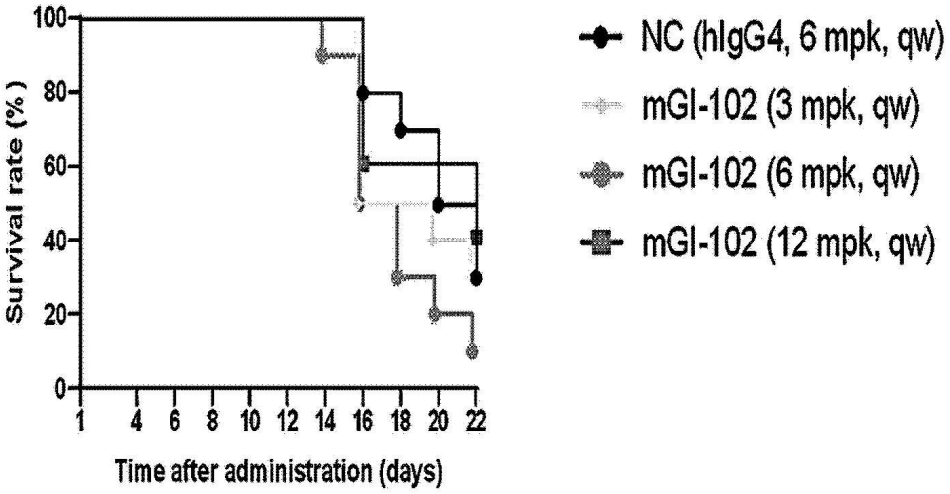
[Fig. 55]


Dendritic cell population and antigen presentation effects were Increased after GI-101 treatment (LLC2)
A
Dendritic cell (%)
***
control
aPD-1
GI 0.1 MPK
GI 1 MPK
B
DC CD80 (MFI)
**
C
DC CD86 (MFI)
*
**
*

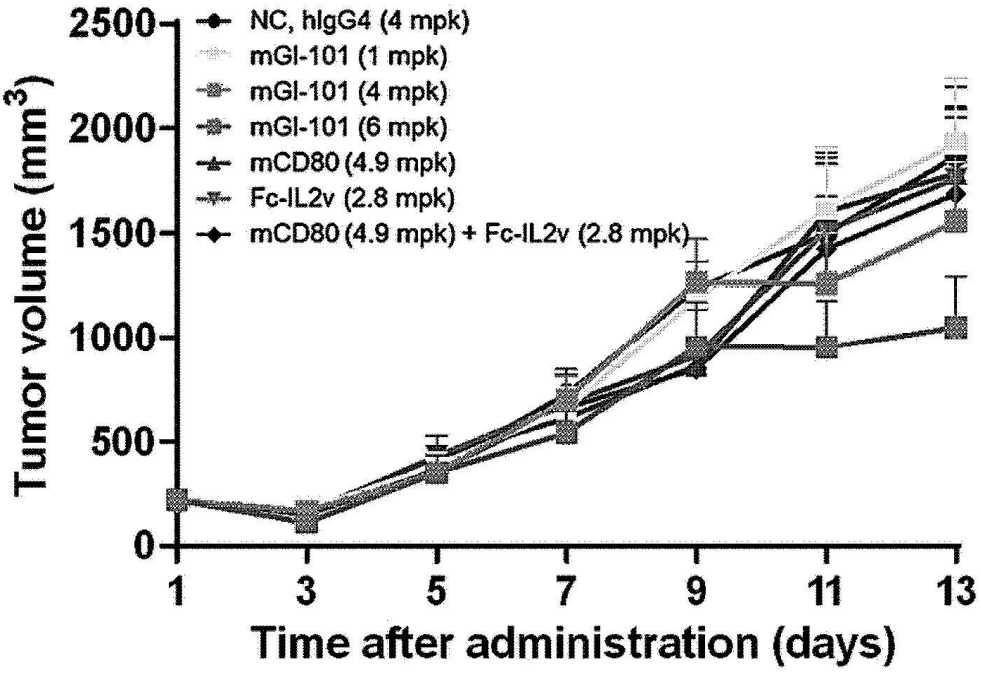
[Fig. 56]


Negative control (IgG4, 6 mpk)
mGI-102 (3 mpk)
mGI-102 (6 mpk)
mGI-102 (12 mpk)
Tumor volume (mm3)
2000
1500
1000
500
0
1
4
6
8
10
12
14
16
18
Days after treatment

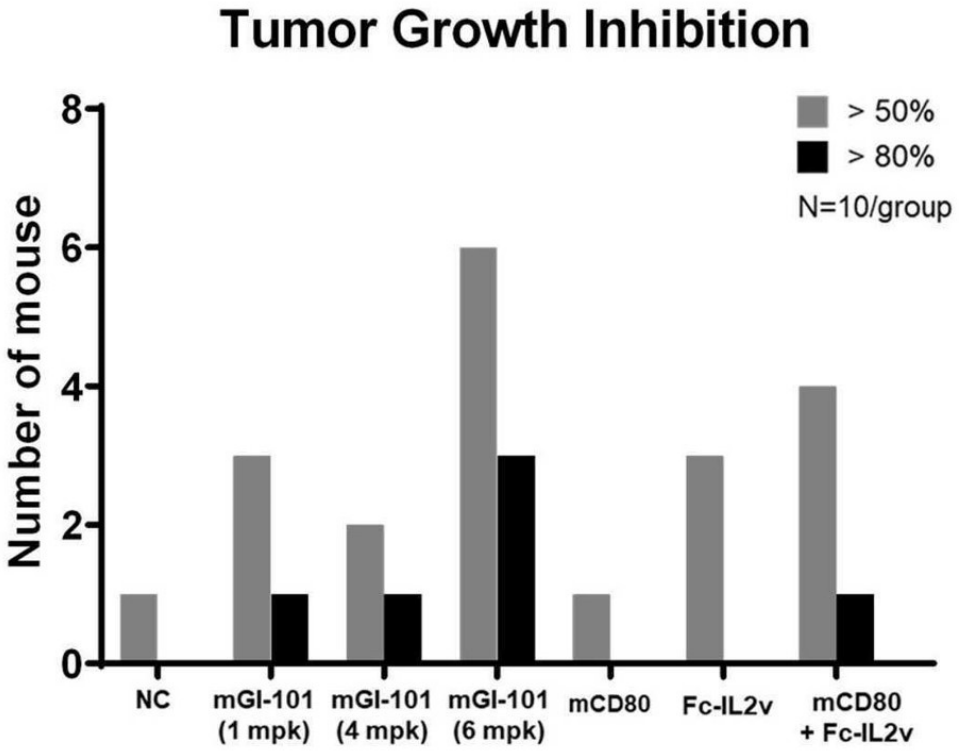
[Fig. 57]


NC (hIgG4, 6 mpk, qw)
mGI-102 (3 mpk, qw)
mGI-102 (6 mpk, qw)
mGI-102 (12 mpk, qw)
Survival rate (%)
100
80
60
40
20
0
1
4
6
8
10
12
14
16
18
20
22
Time after administration (days)

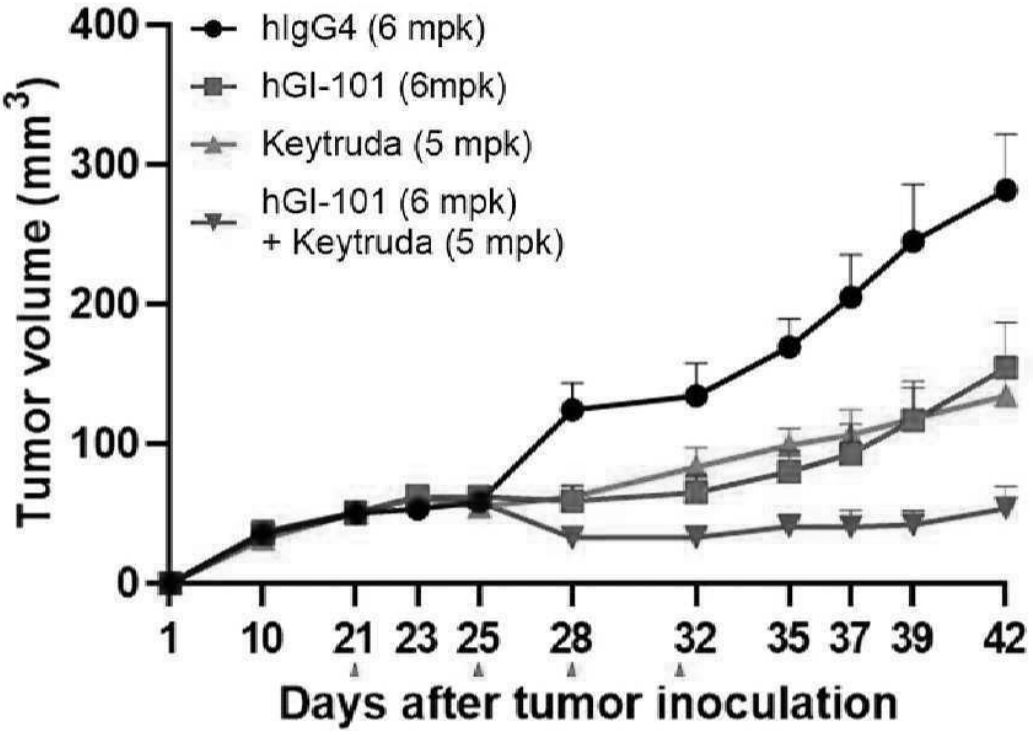
[Fig. 58]


NC, hIgG4 (4 mpk)
mGI-101 (1 mpk)
mGI-101 (4 mpk)
mGI-101 (6 mpk)
mCD80 (4.9 mpk)
Fc-IL2v (2.8 mpk)
mCD80 (4.9 mpk) + Fc-IL2v (2.8 mpk)
Tumor volume (mm3)
2500
2000
1500
1000
500
0
1
3
5
7
9
11
13
Time after administration (days)

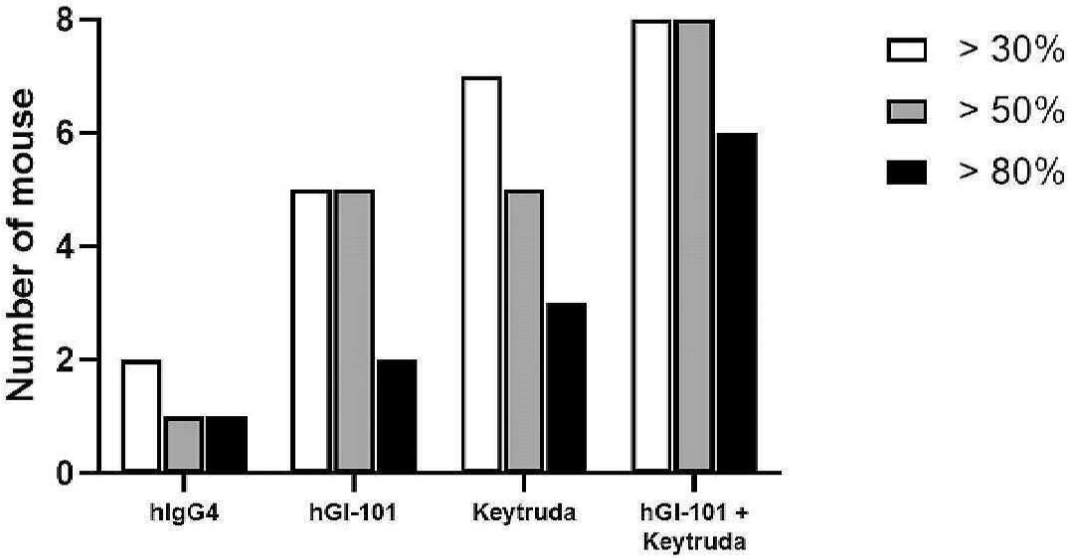
[Fig. 59]


Tumor Growth Inhibition
> 50%
> 80%
N=10/group
Number of mouse
8
6
4
2
0
NC
mGI-101 (1 mpk)
mGI-101 (4 mpk)
mGI-101 (6 mpk)
mCD80
Fc-IL2v
mCD80 + Fc-IL2v

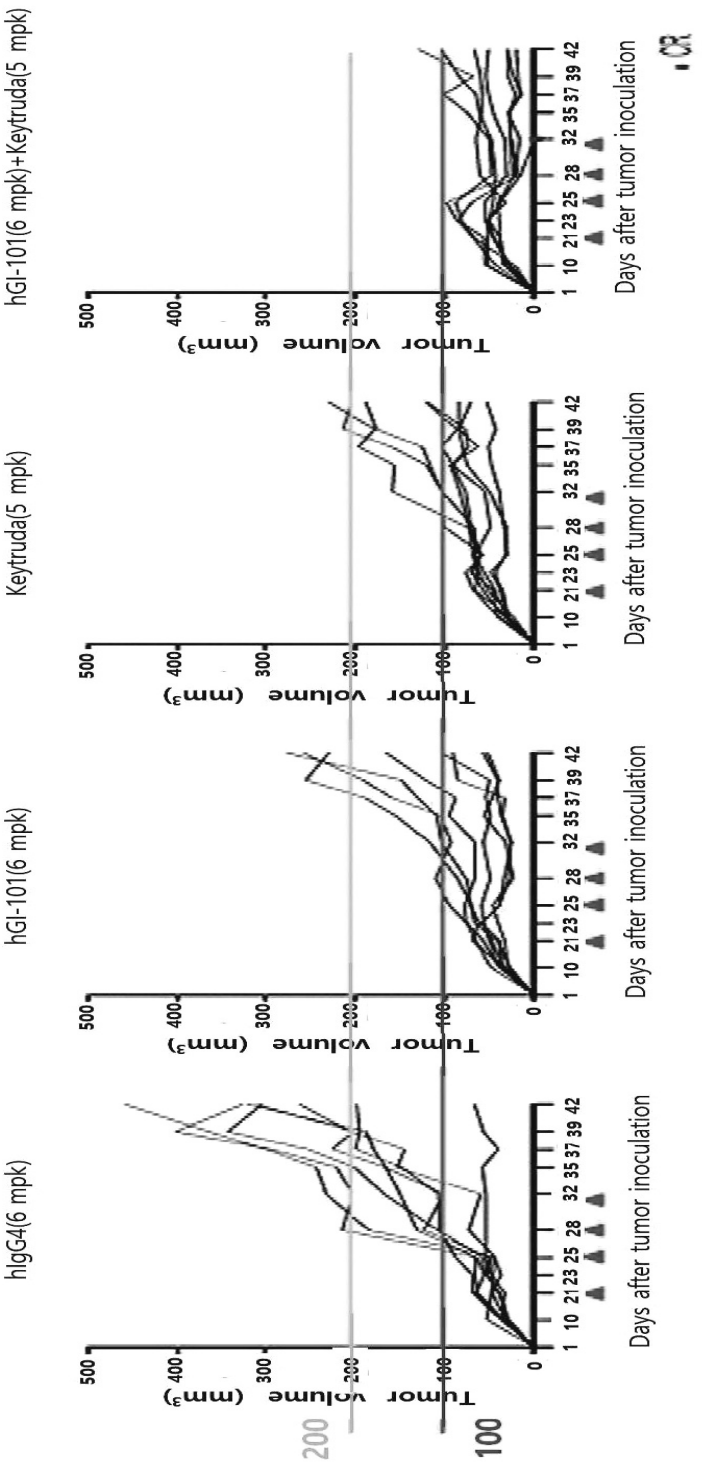
[Fig. 60]


hIgG4 (6 mpk)
hGI-101 (6mpk)
Keytruda (5 mpk)
hGI-101 (6 mpk)
+ Keytruda (5 mpk)
Tumor volume (mm3)
400
300
200
100
0
1
10
21
23
25
28
32
35
37
39
42
Days after tumor inoculation

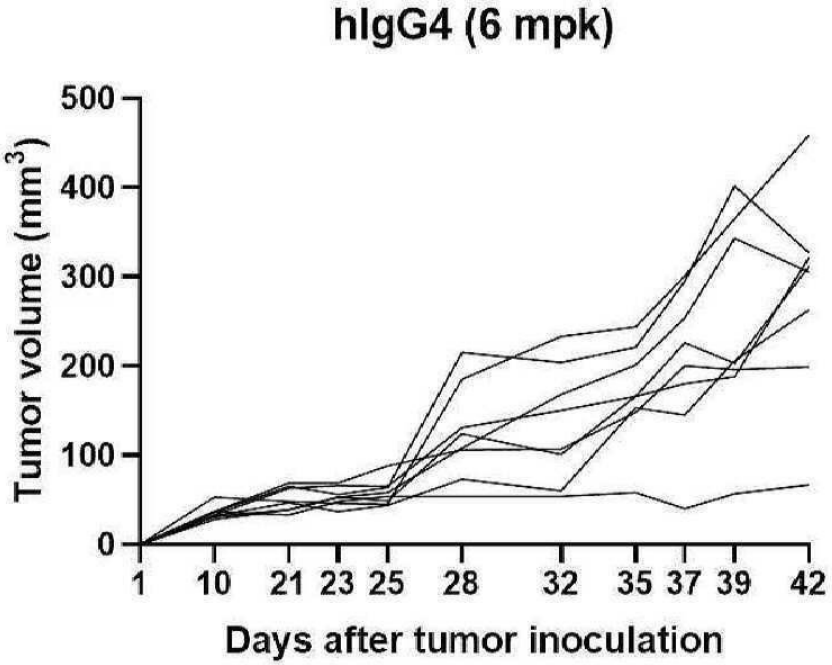
[Fig. 61]


Number of mouse
8
6
4
2
0
hIgG4
hGI-101
Keytruda
hGI-101 +
Keytruda
> 30%
> 50%
> 80%

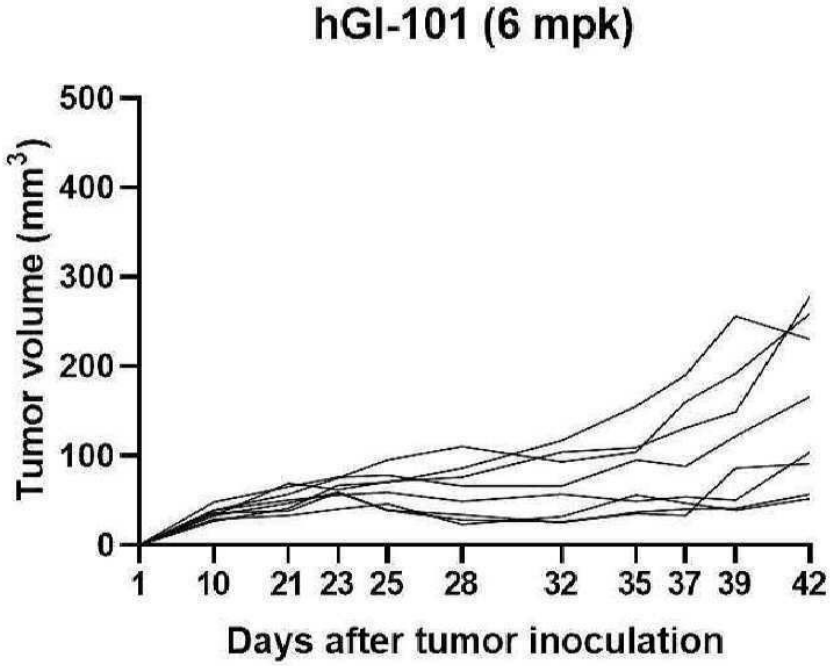
[Fig. 62]


hIgG4(6 mpk)
hGI-101(6 mpk)
Keytruda(5 mpk)
hGI-101(6 mpk)+Keytruda(5 mpk)
Tumor volume (mm3)
0
100
200
300
400
500
1 10 21 23 25 28 32 35 37 39 42
Days after tumor inoculation

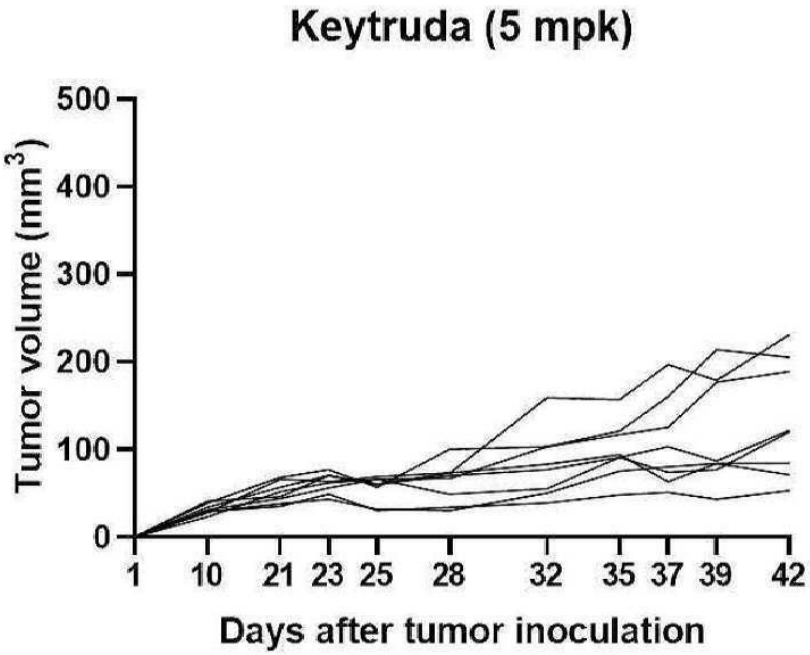
[Fig. 63]


hIgG4 (6 mpk)
Tumor volume (mm3)
500
400
300
200
100
0
1
10
21
23
25
28
32
35
37
39
42
Days after tumor inoculation

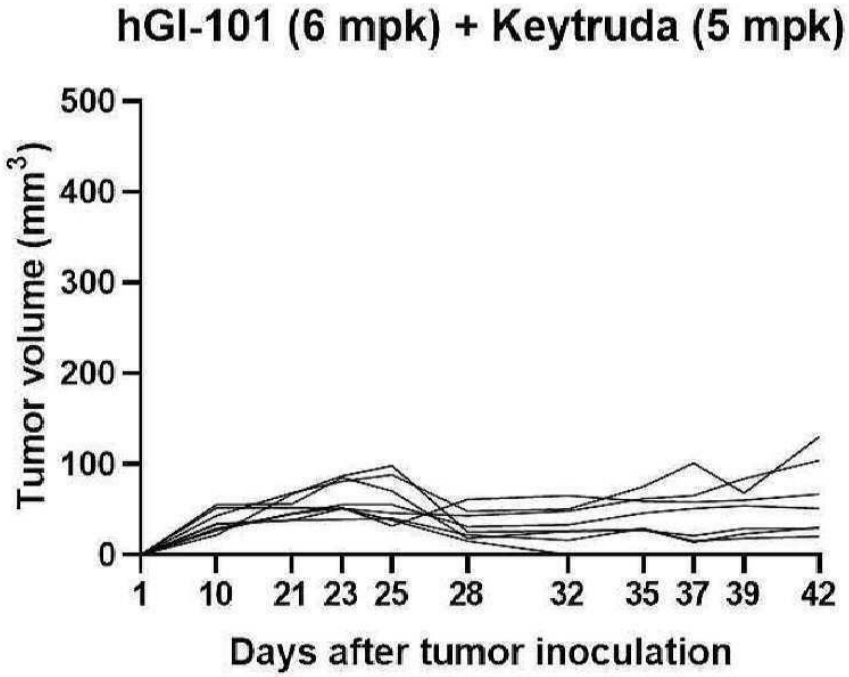
[Fig. 64]


hGI-101 (6 mpk)
Tumor volume (mm3)
500
400
300
200
100
0
1
10
21
23
25
28
32
35
37
39
42
Days after tumor inoculation

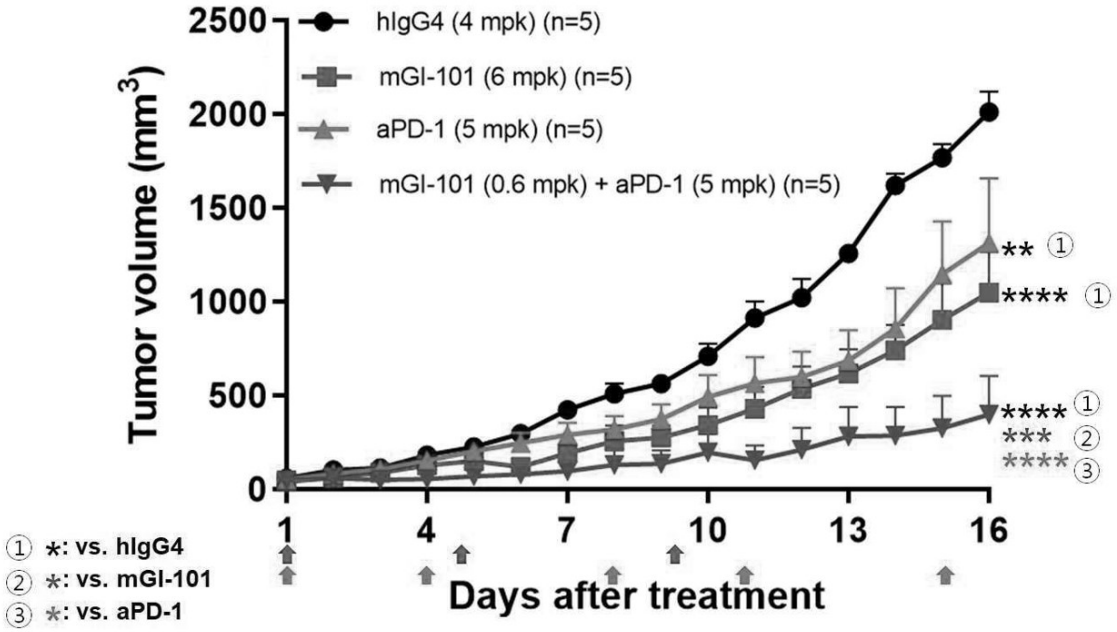
[Fig. 65]


Keytruda (5 mpk)
Tumor volume (mm3)
500
400
300
200
100
0
1
10
21
23
25
28
32
35
37
39
42
Days after tumor inoculation

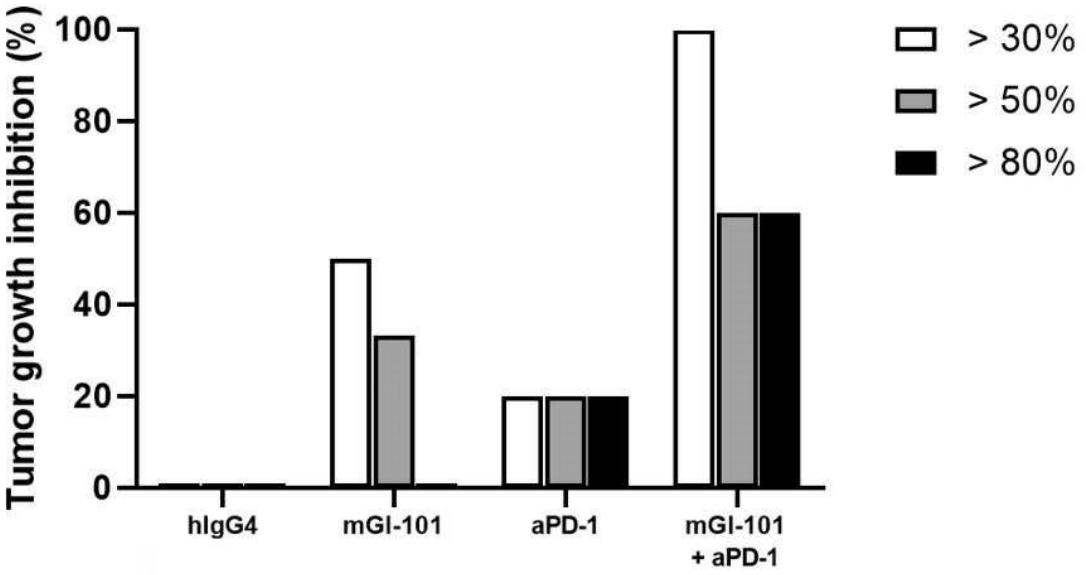
[Fig. 66]


hGI-101 (6 mpk) + Keytruda (5 mpk)
Tumor volume (mm3)
500
400
300
200
100
0
1
10
21
23
25
28
32
35
37
39
42
Days after tumor inoculation

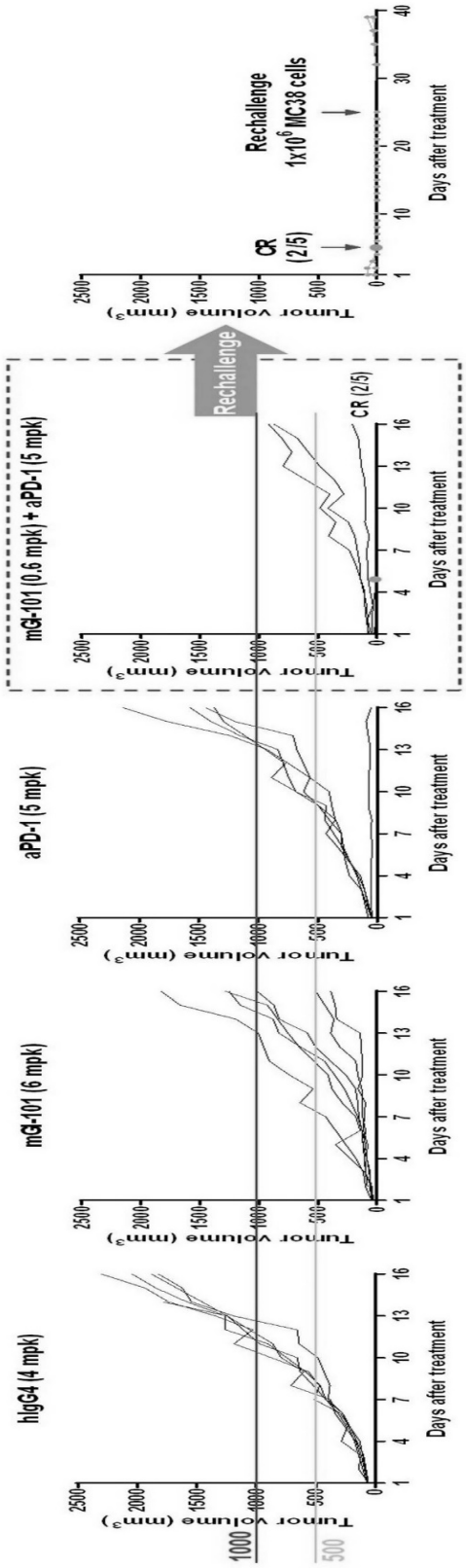
[Fig. 67]


hIgG4 (4 mpk) (n=5)
mGI-101 (6 mpk) (n=5)
aPD-1 (5 mpk) (n=5)
mGI-101 (0.6 mpk) + aPD-1 (5 mpk) (n=5)
Tumor volume (mm3)
2500
2000
1500
1000
500
0
1
4
7
10
13
16
Days after treatment
** ①
**** ①
**** ①
*** ②
**** ③
① *: vs. hIgG4
② *: vs. mGI-101
③ *: vs. aPD-1

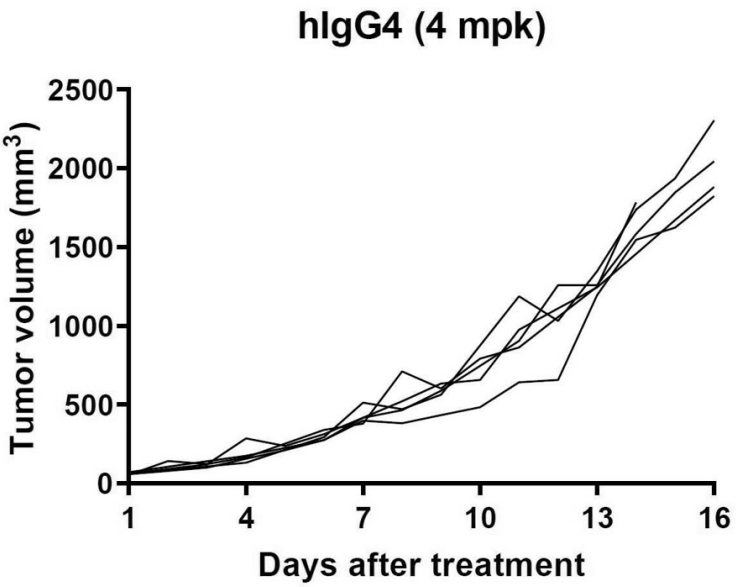
[Fig. 68]


Tumor growth inhibition (%)
100
80
60
40
20
0
hIgG4
mGI-101
aPD-1
mGI-101
+ aPD-1
> 30%
> 50%
> 80%

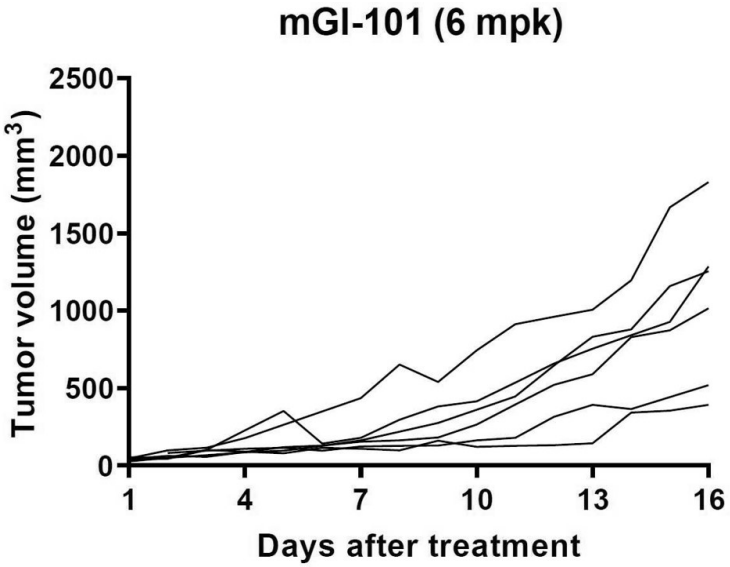
[Fig. 69]


hIgG4 (4 mpk)
mG-101 (6 mpk)
aPD-1 (5 mpk)
mG-101 (0.6 mpk) + aPD-1 (5 mpk)
Rechallenge
CR (2/5)
Rechallenge
1x10^6 MC38 cells
Tumor volume (mm^3)
Days after treatment
1000
500

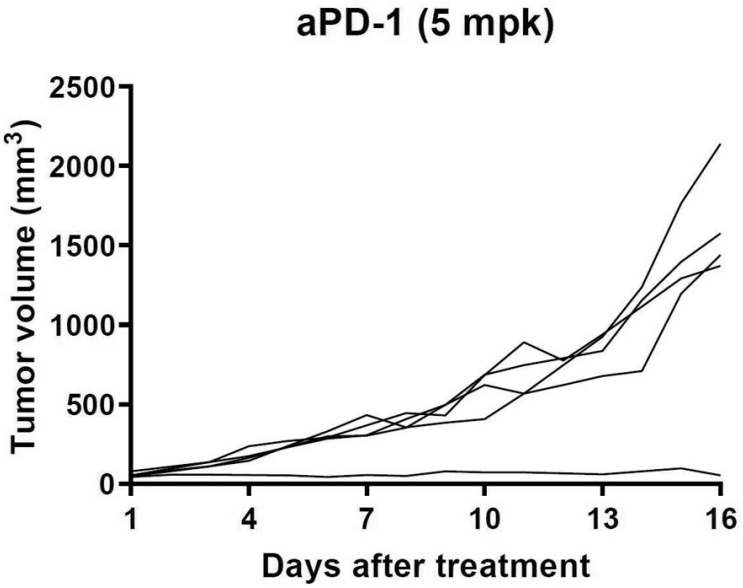
[Fig. 70]


hIgG4 (4 mpk)
Tumor volume (mm3)
2500
2000
1500
1000
500
0
1
4
7
10
13
16
Days after treatment

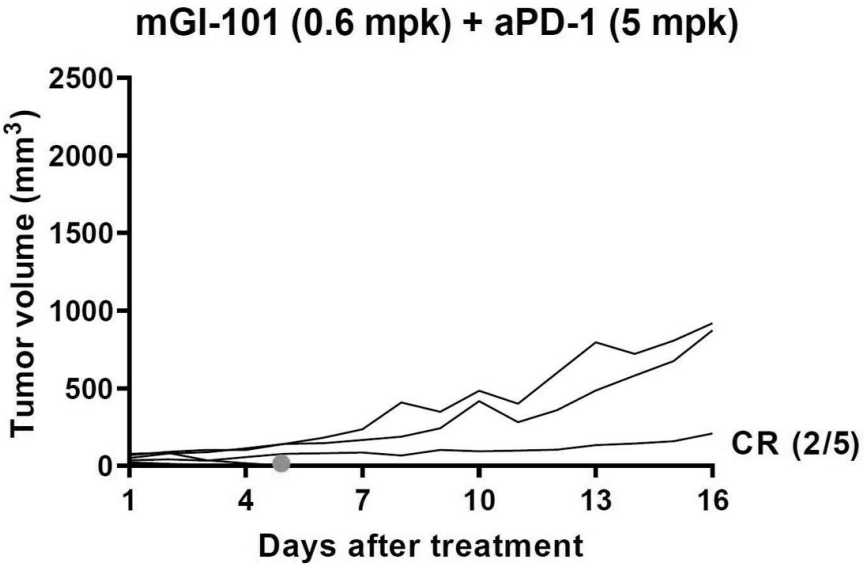
[Fig. 71]


mGI-101 (6 mpk)
Tumor volume (mm3)
2500
2000
1500
1000
500
0
1
4
7
10
13
16
Days after treatment

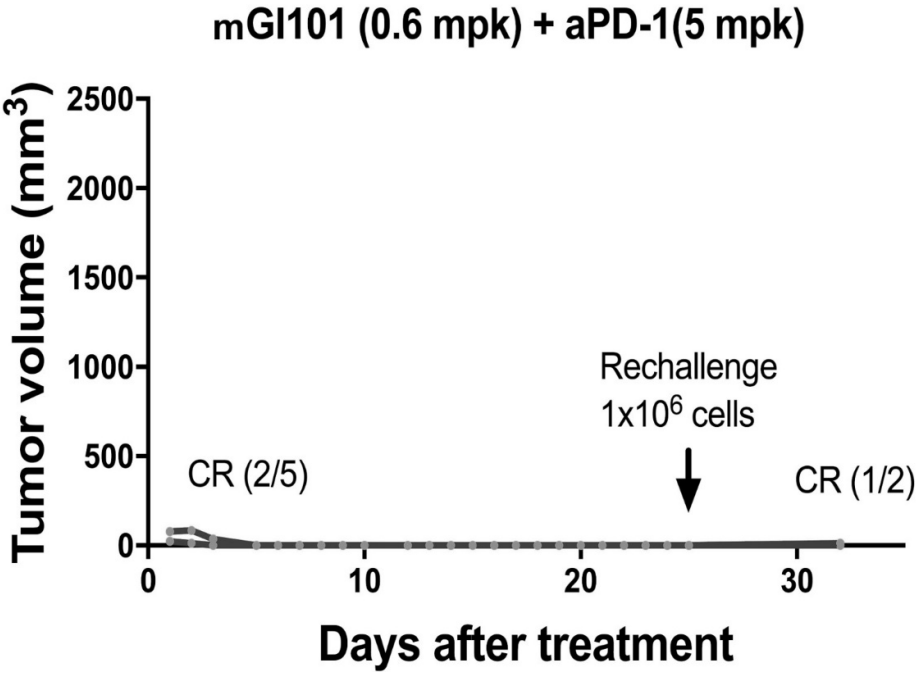
[Fig. 72]


aPD-1 (5 mpk)
Tumor volume (mm3)
2500
2000
1500
1000
500
0
1
4
7
10
13
16
Days after treatment

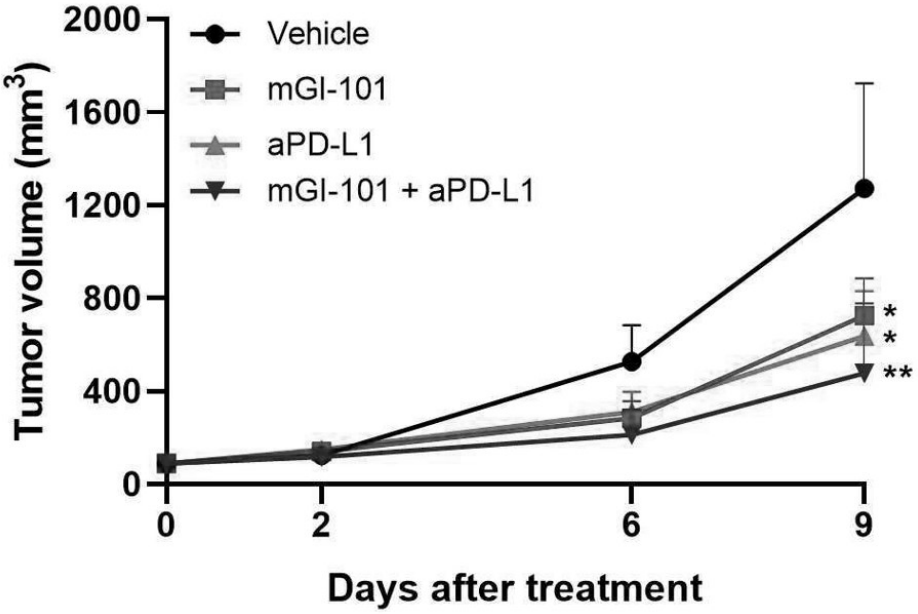
[Fig. 73]


mGI-101 (0.6 mpk) + aPD-1 (5 mpk)
Tumor volume (mm3)
2500
2000
1500
1000
500
0
1
4
7
10
13
16
Days after treatment
CR (2/5)

[Fig. 74]



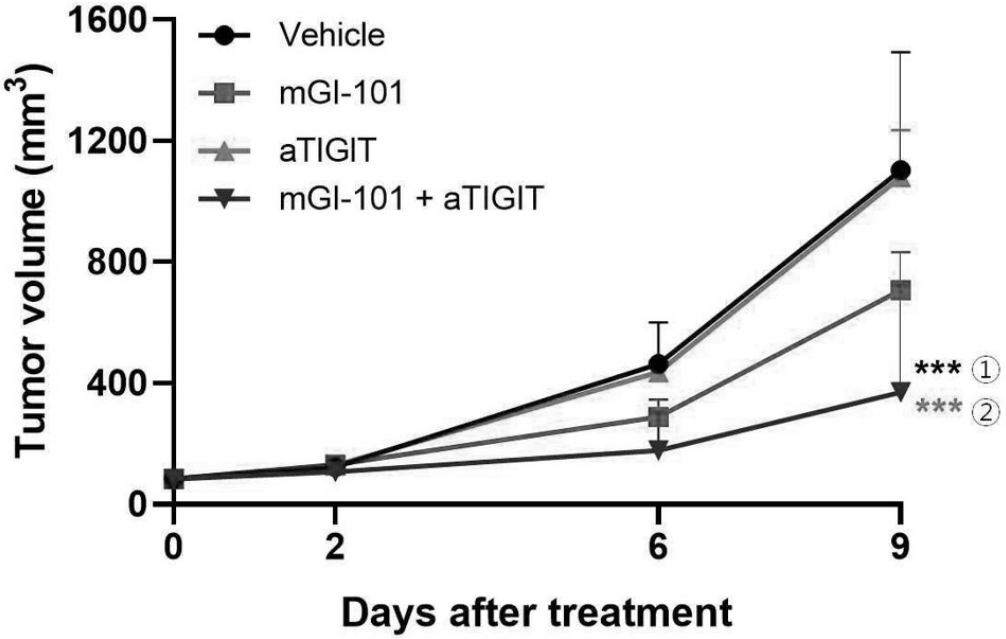
[Fig. 75]



*; $p < 0.05$ vs. Vehicle

**; $p < 0.01$ vs. Vehicle

[Fig. 76]



① ***; $p < 0.001$ vs. Vehicle

② ***; $p < 0.001$ vs. aTIGIT