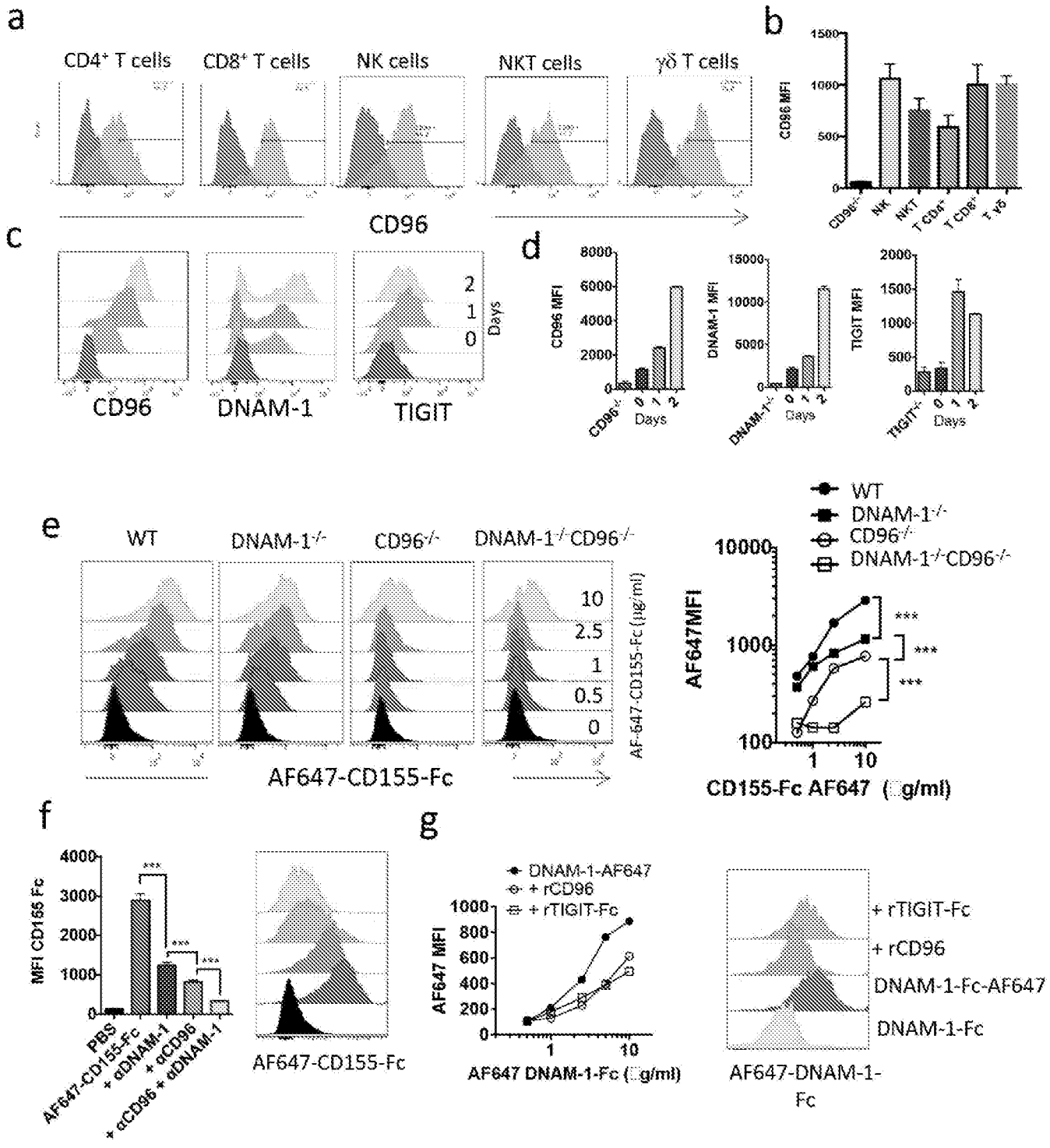

a
CD4+ T cells
CD8+ T cells
NK cells
NKT cells
γδ T cells
CD96
b
CD96 MFI
1500
1000
500
0
CD96-/-
NK
NKT
T CD4+
T CD8+
T γδ
c
CD96
DNAM-1
TIGIT
2
1
0
Days
d
CD96 MFI
8000
6000
4000
2000
0
CD96-/-
Days
DNAM-1 MFI
15000
10000
5000
0
DNAM-1-/-
Days
TIGIT MFI
2000
1500
1000
500
0
TIGIT-/-
Days
e
WT
DNAM-1-/-
CD96-/-
DNAM-1-/-CD96-/-
10
2.5
1
0.5
0
AF-647-CD155-Fc (μg/ml)
AF647-CD155-Fc
WT
DNAM-1-/-
CD96-/-
DNAM-1-/-CD96-/-
AF647MFI
10000
1000
100
1
10
***
***
***
CD155-Fc AF647 (μg/ml)
f
MFI CD155 Fc
4000
3000
2000
1000
0
***
***
***
PBS
AF647-CD155-Fc
+ αDNAM-1
+ αCD96
+ αCD96 + αDNAM-1
AF647-CD155-Fc
g
DNAM-1-AF647
+ rCD96
+ rTIGIT-Fc
AF647 MFI
1000
800
600
400
200
0
1
10
AF647 DNAM-1-Fc (μg/ml)
+ rTIGIT-Fc
+ rCD96
DNAM-1-Fc-AF647
DNAM-1-Fc
AF647-DNAM-1-Fc

Fig. 1

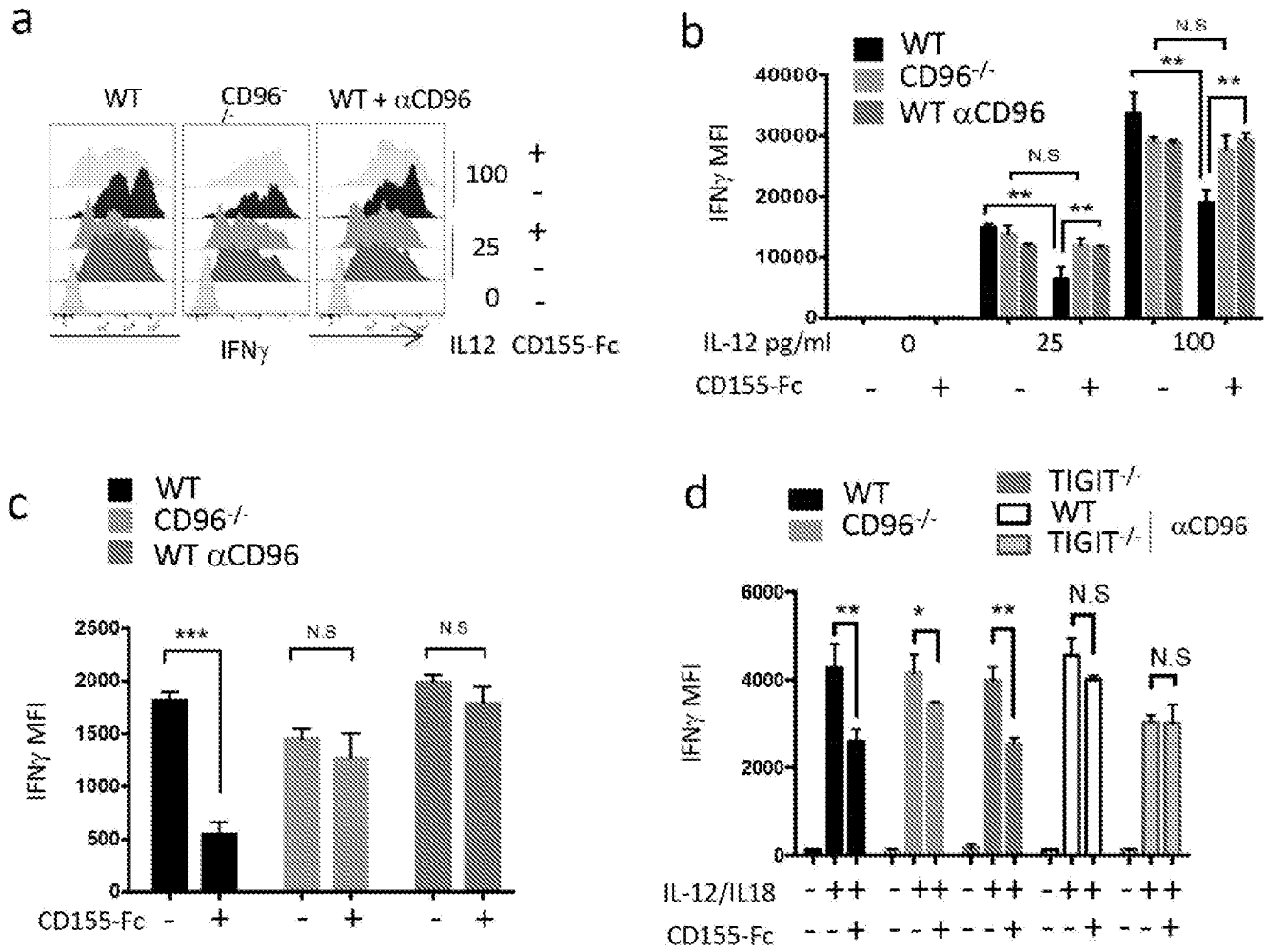

a
WT
CD96-/-
WT + αCD96
100
25
0
+
-
+
-
-
IFNγ
IL12
CD155-Fc
b
WT
CD96-/-
WT αCD96
IFNγ MFI
40000
30000
20000
10000
0
N.S
**
**
N.S
**
**
IL-12 pg/ml
0
25
100
CD155-Fc
- + - + - +
c
WT
CD96-/-
WT αCD96
IFNγ MFI
2500
2000
1500
1000
500
0
***
N.S
N.S
CD155-Fc
- + - + - +
d
WT
CD96-/-
TIGIT-/-
WT
TIGIT-/-
αCD96
IFNγ MFI
6000
4000
2000
0
**
*
**
N.S
N.S
IL-12/IL18
- ++ - ++ - ++ - ++ - ++
CD155-Fc
- - + - - + - - + - - + - - +

Fig. 2

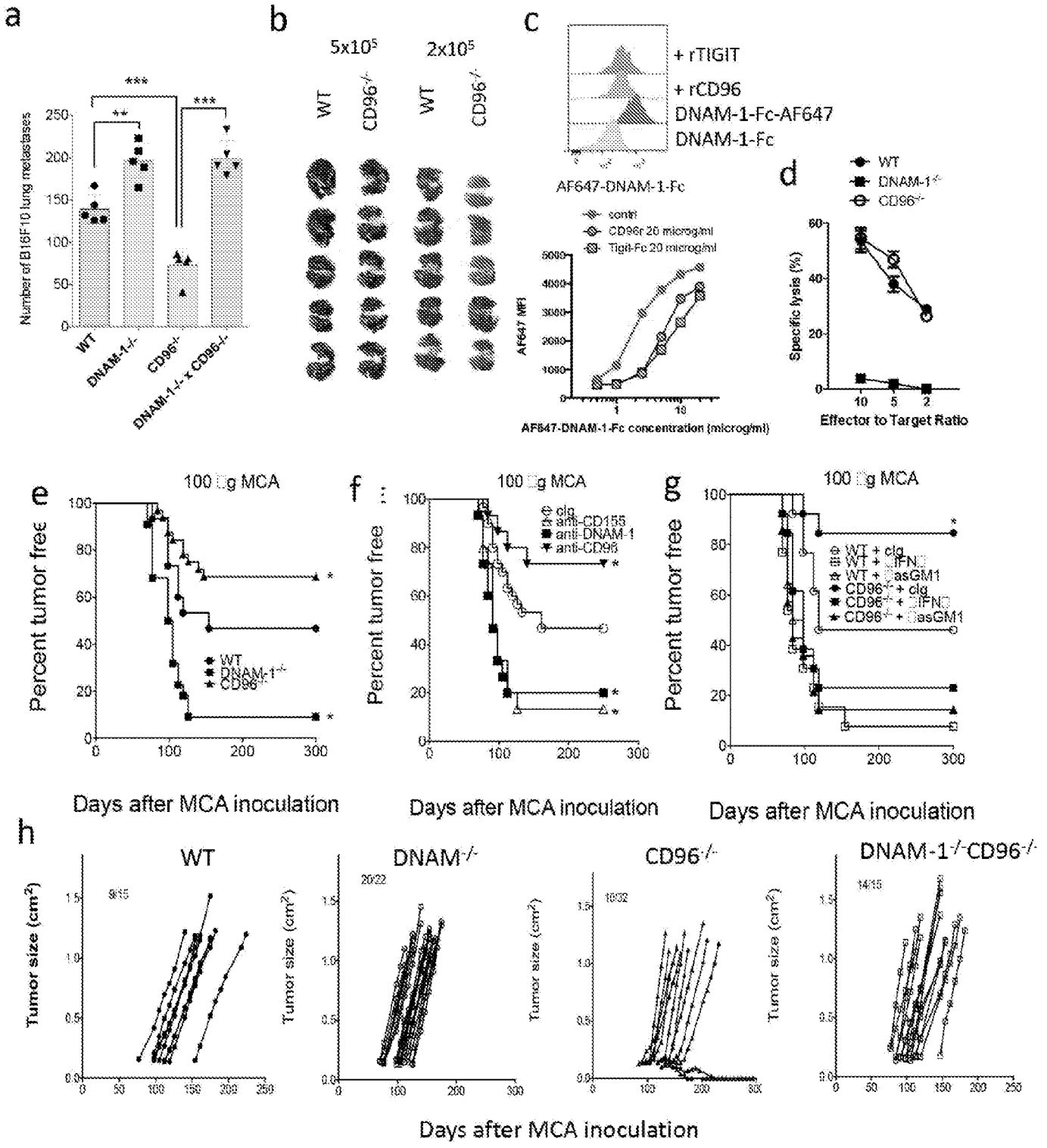

a
Number of B16F10 lung metastases
WT
DNAM-1-/-
CD96-/-
DNAM-1-/- x CD96-/-
b
5x10^5
2x10^5
WT
CD96-/-
c
+ rTIGIT
+ rCD96
DNAM-1-Fc-AF647
DNAM-1-Fc
AF647-DNAM-1-Fc
contr
CD96r 20 microg/ml
Tigit-Fc 20 microg/ml
AF647 MFI
AF647-DNAM-1-Fc concentration (microg/ml)
d
WT
DNAM-1-/-
CD96-/-
Specific lysis (%)
Effector to Target Ratio
e
100 µg MCA
Percent tumor free
WT
DNAM-1-/-
CD96-/-
Days after MCA inoculation
f
100 µg MCA
Percent tumor free
clg
anti-CD155
anti-DNAM-1
anti-CD96
Days after MCA inoculation
g
100 µg MCA
Percent tumor free
WT + clg
WT + IFN
WT + asGM1
CD96-/- + clg
CD96-/- + IFN
CD96-/- + asGM1
Days after MCA inoculation
h
WT
DNAM-/-
CD96-/-
DNAM-1-/-CD96-/-
Tumor size (cm2)
9/15
20/22
16/32
14/15
Days after MCA inoculation

Fig. 3

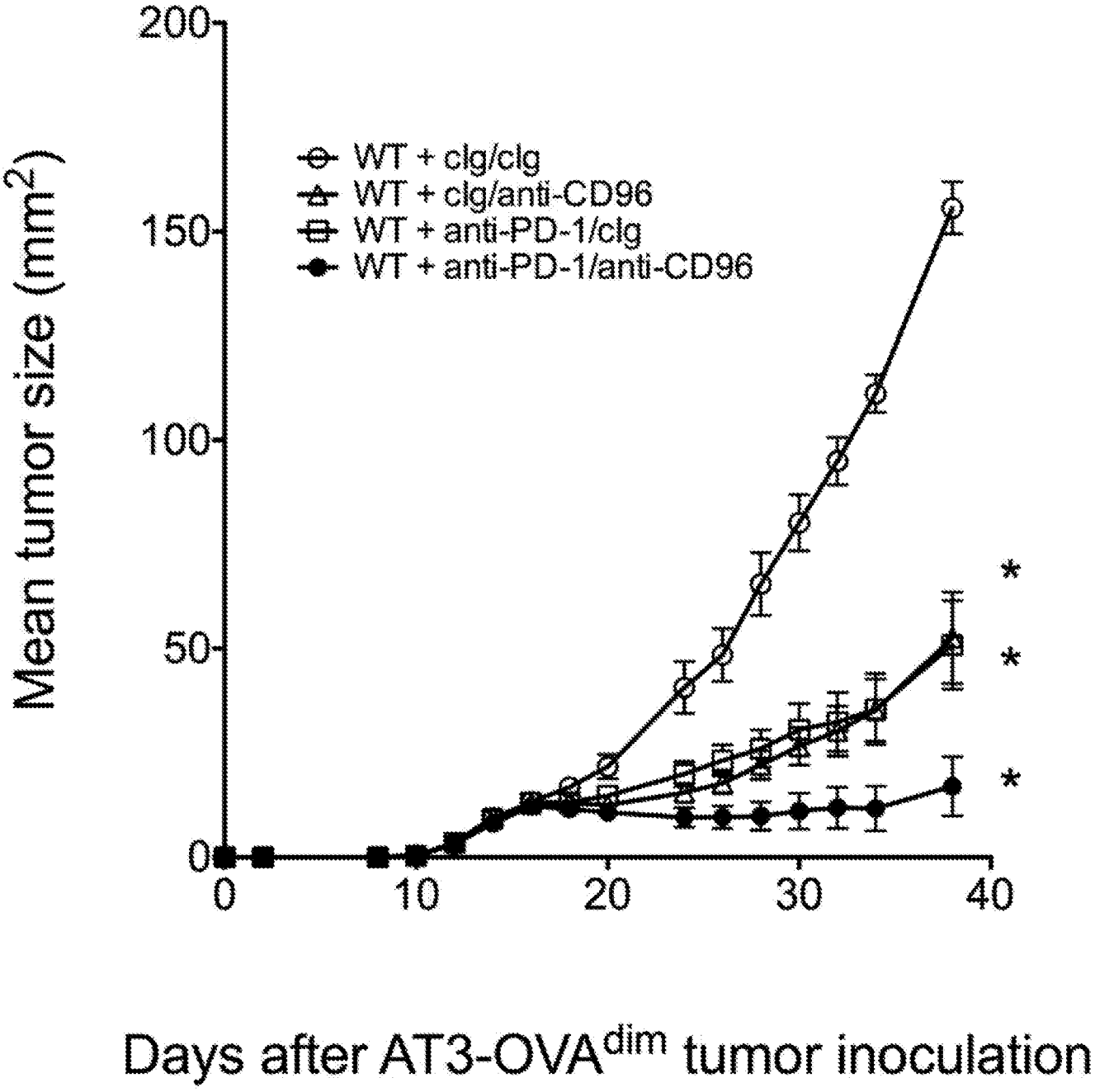

200
150
100
50
0
Mean tumor size (mm2)
WT + clg/clg
WT + clg/anti-CD96
WT + anti-PD-1/clg
WT + anti-PD-1/anti-CD96
*
*
*
0
10
20
30
40
Days after AT3-OVAdim tumor inoculation

Fig. 4

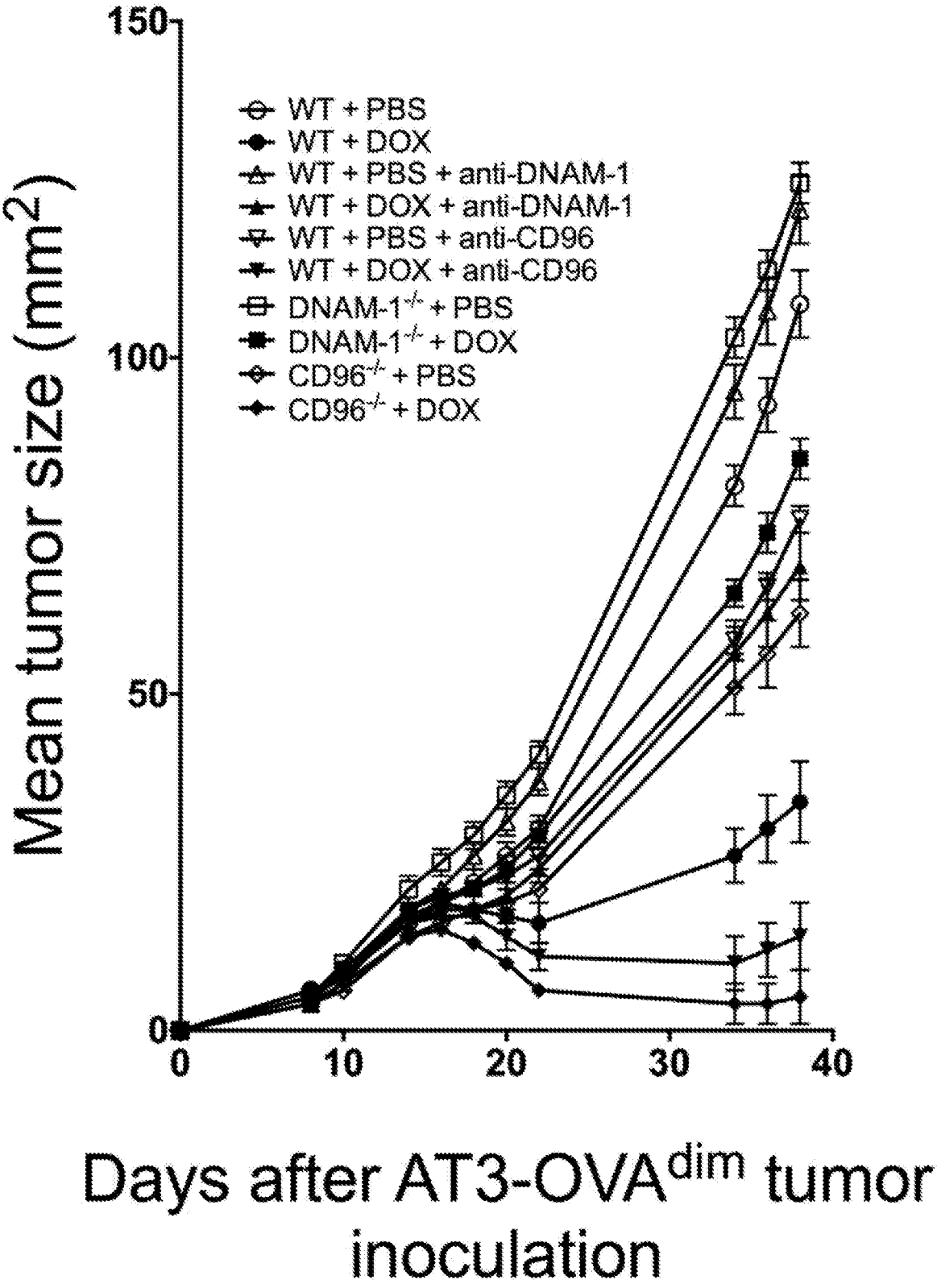

150
100
50
0
0
10
20
30
40
Mean tumor size (mm2)
WT + PBS
WT + DOX
WT + PBS + anti-DNAM-1
WT + DOX + anti-DNAM-1
WT + PBS + anti-CD96
WT + DOX + anti-CD96
DNAM-1-/- + PBS
DNAM-1-/- + DOX
CD96-/- + PBS
CD96-/- + DOX
Days after AT3-OVAdim tumor inoculation

Fig. 5

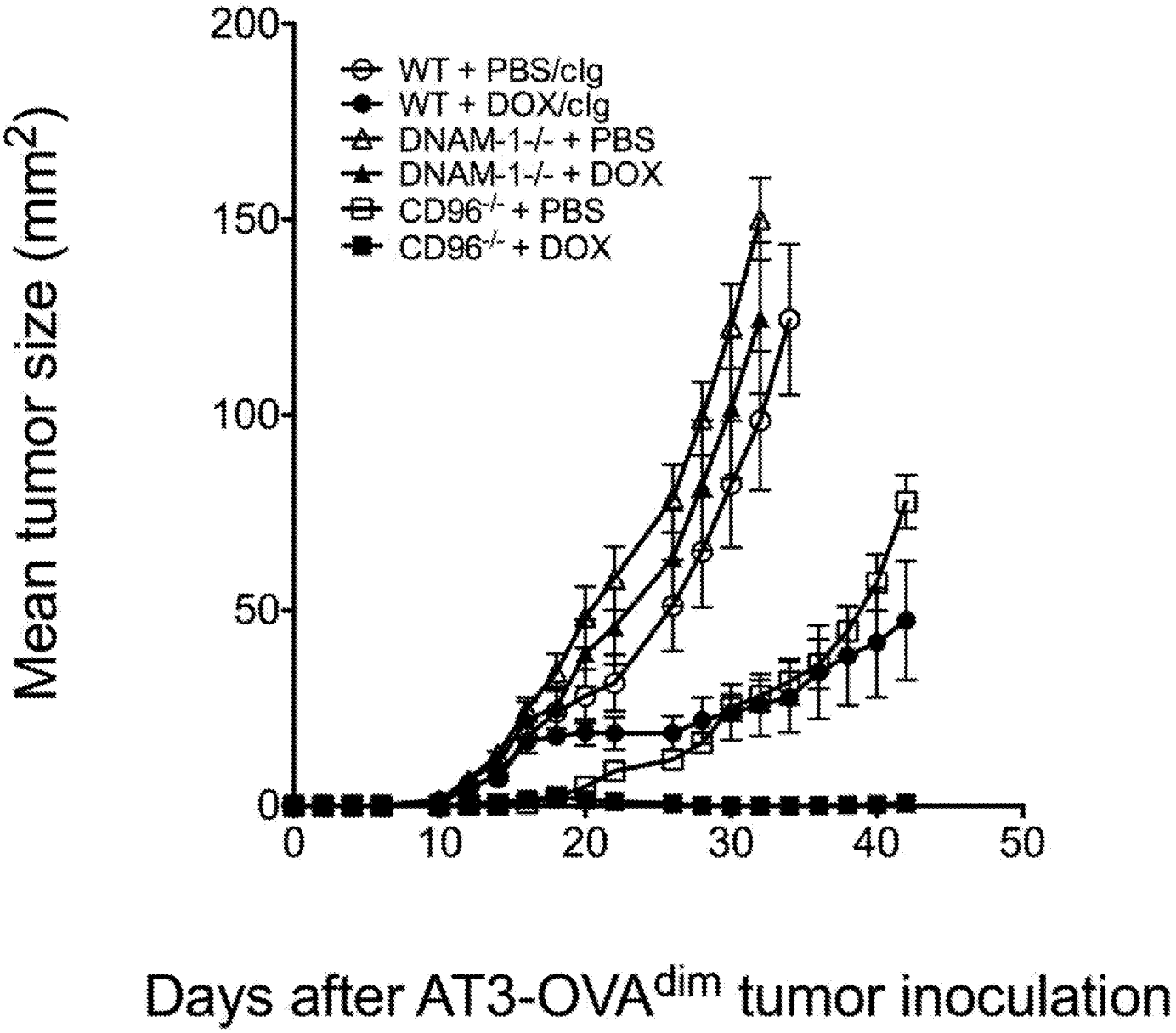

Mean tumor size (mm2)
200
150
100
50
0
0
10
20
30
40
50
WT + PBS/clg
WT + DOX/clg
DNAM-1-/- + PBS
DNAM-1-/- + DOX
CD96-/- + PBS
CD96-/- + DOX
Days after AT3-OVAdim tumor inoculation

Fig. 6

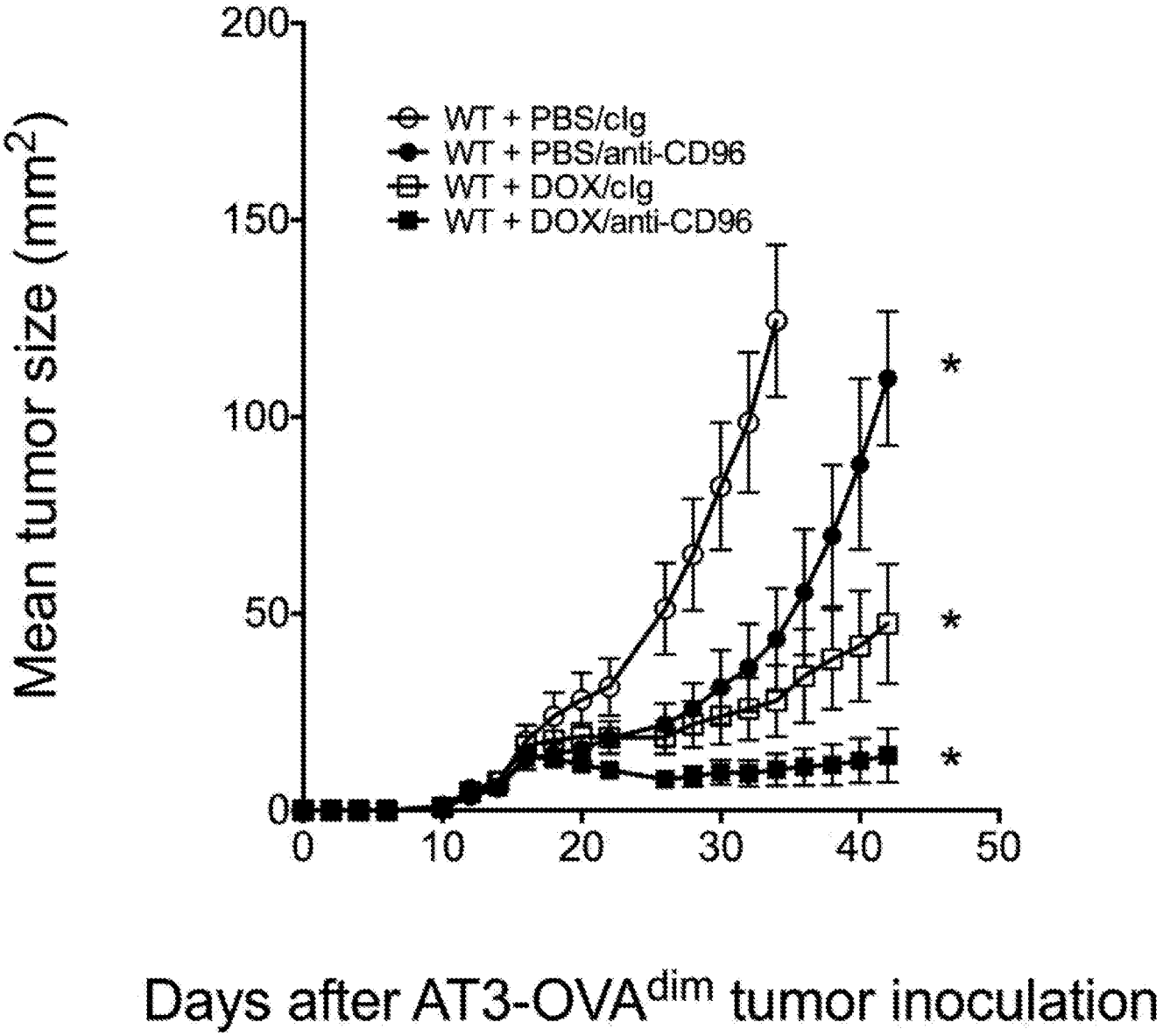

Mean tumor size (mm2)
200
150
100
50
0
WT + PBS/clg
WT + PBS/anti-CD96
WT + DOX/clg
WT + DOX/anti-CD96
*
*
*
0
10
20
30
40
50
Days after AT3-OVAdim tumor inoculation

Fig. 7

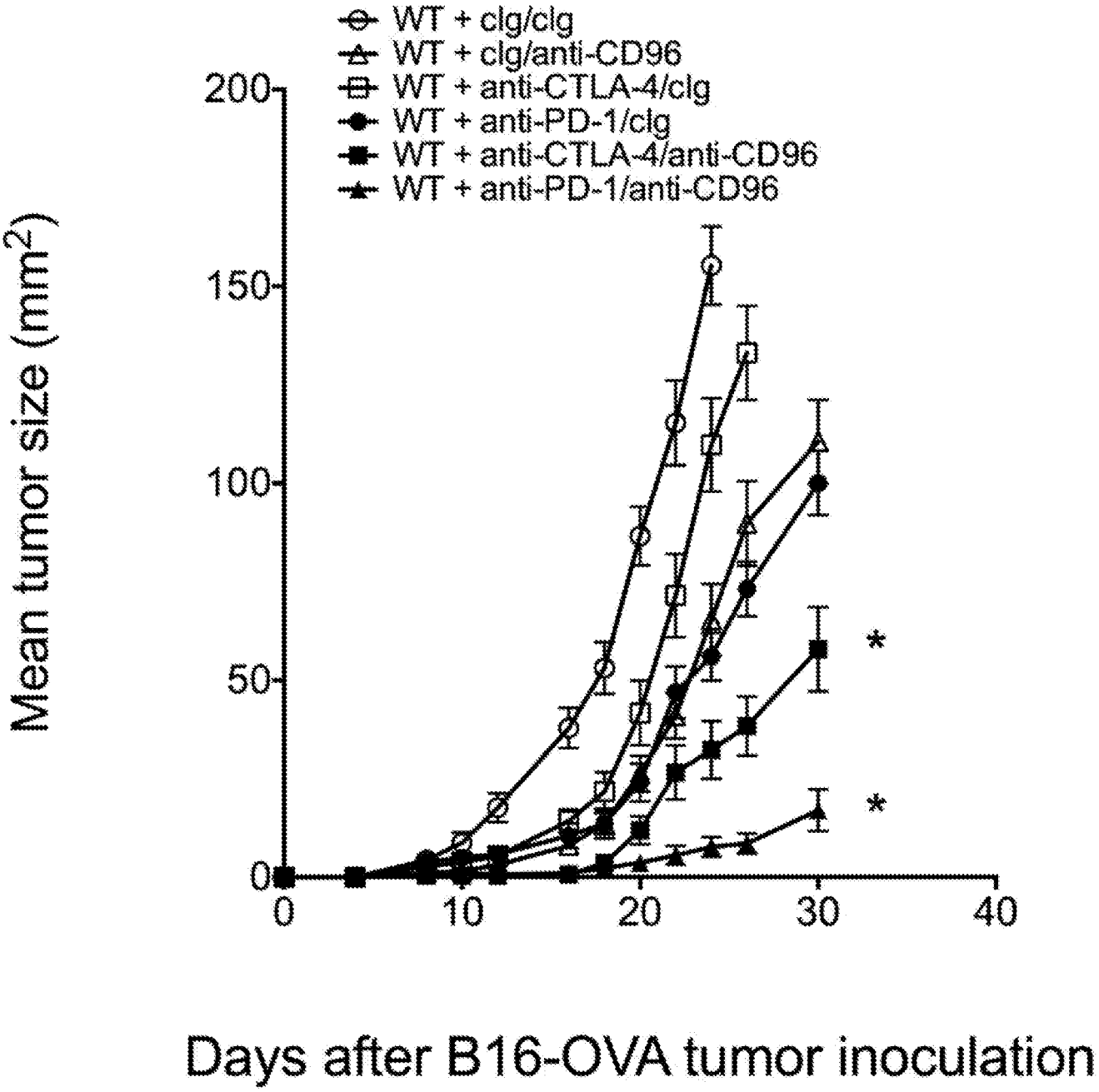

WT + clg/clg
WT + clg/anti-CD96
WT + anti-CTLA-4/clg
WT + anti-PD-1/clg
WT + anti-CTLA-4/anti-CD96
WT + anti-PD-1/anti-CD96
Mean tumor size (mm2)
200
150
100
50
0
0
10
20
30
40
*
*
Days after B16-OVA tumor inoculation

Fig. 8

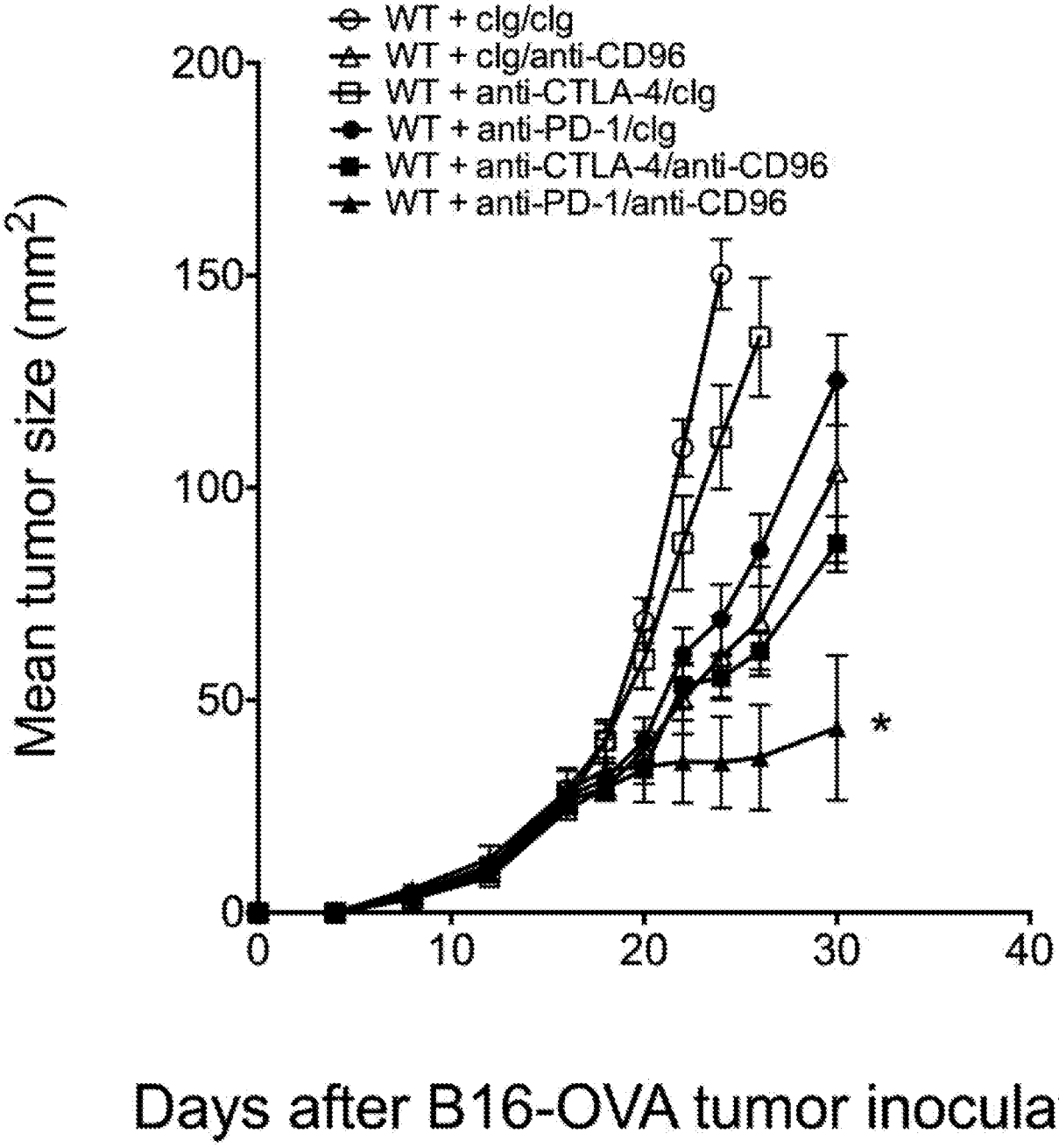

WT + clg/clg
WT + clg/anti-CD96
WT + anti-CTLA-4/clg
WT + anti-PD-1/clg
WT + anti-CTLA-4/anti-CD96
WT + anti-PD-1/anti-CD96
Mean tumor size (mm2)
200
150
100
50
0
0
10
20
30
40
*
Days after B16-OVA tumor inoculation

Fig. 9

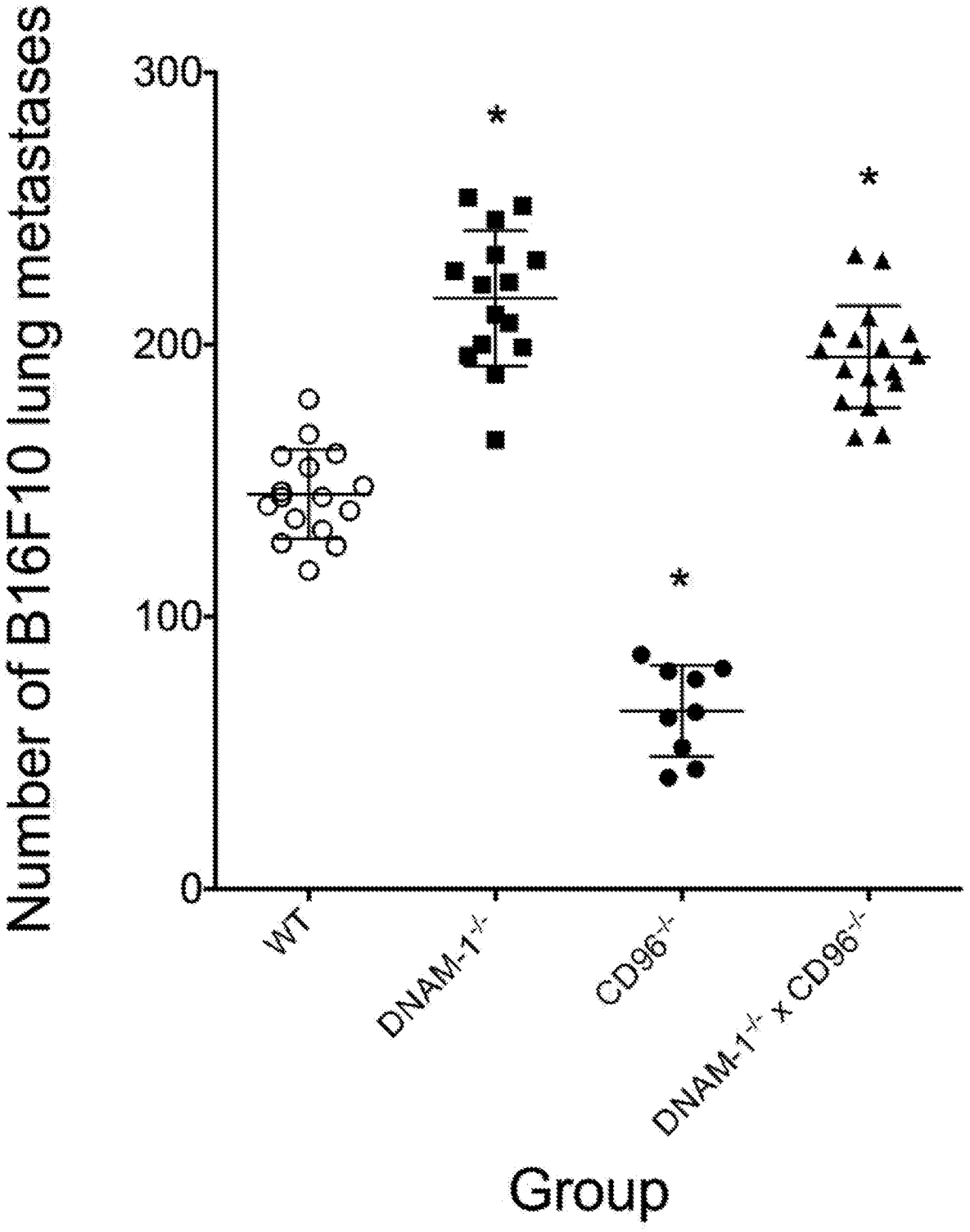

Number of B16F10 lung metastases
300
200
100
0
*
*
*
WT
DNAM-1-/-
CD96-/-
DNAM-1-/- x CD96-/-
Group

Fig. 10

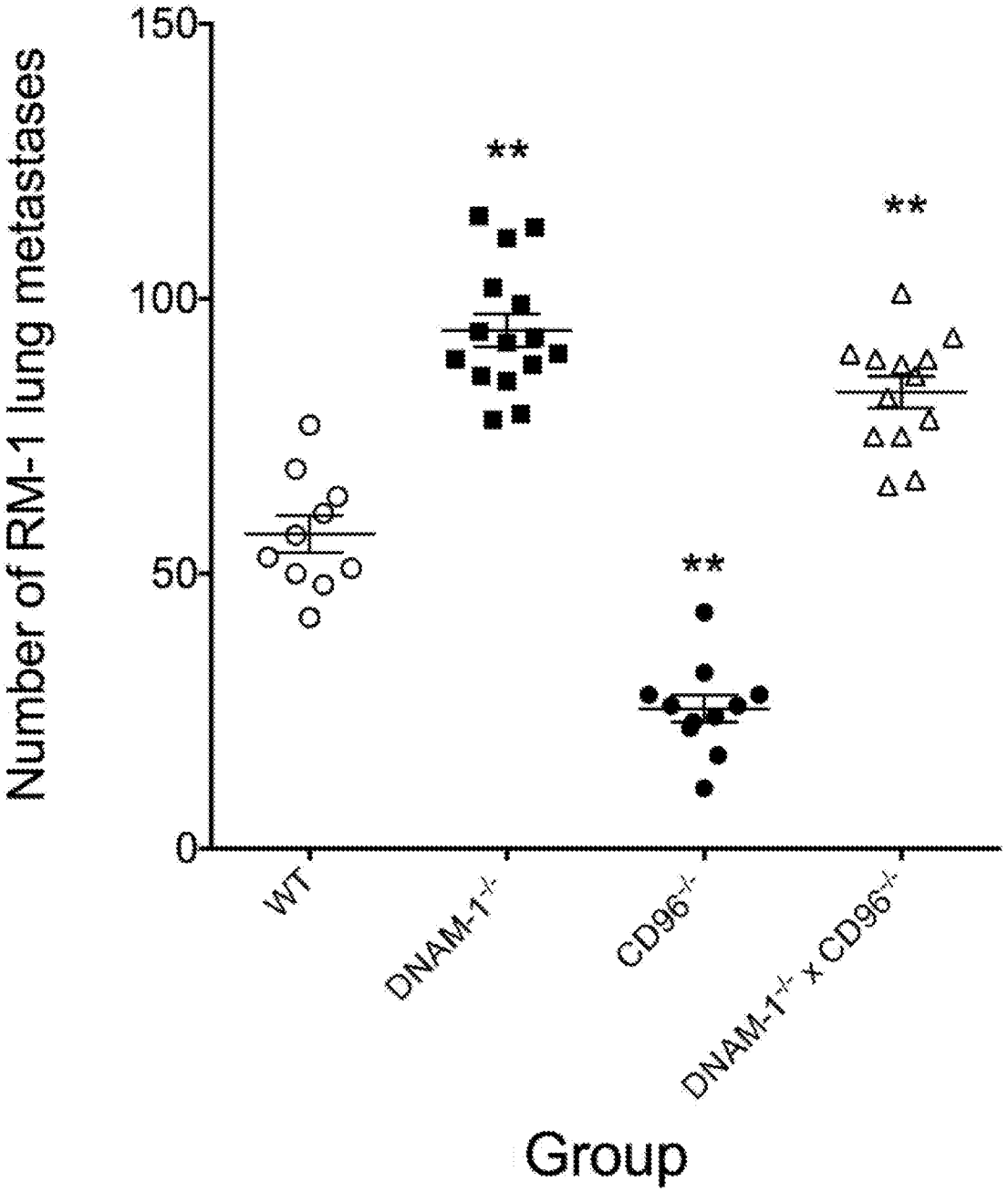

Number of RM-1 lung metastases
150
100
50
0
**
**
**
WT
DNAM-1-/-
CD96-/-
DNAM-1-/- x CD96-/-
Group

Fig. 11

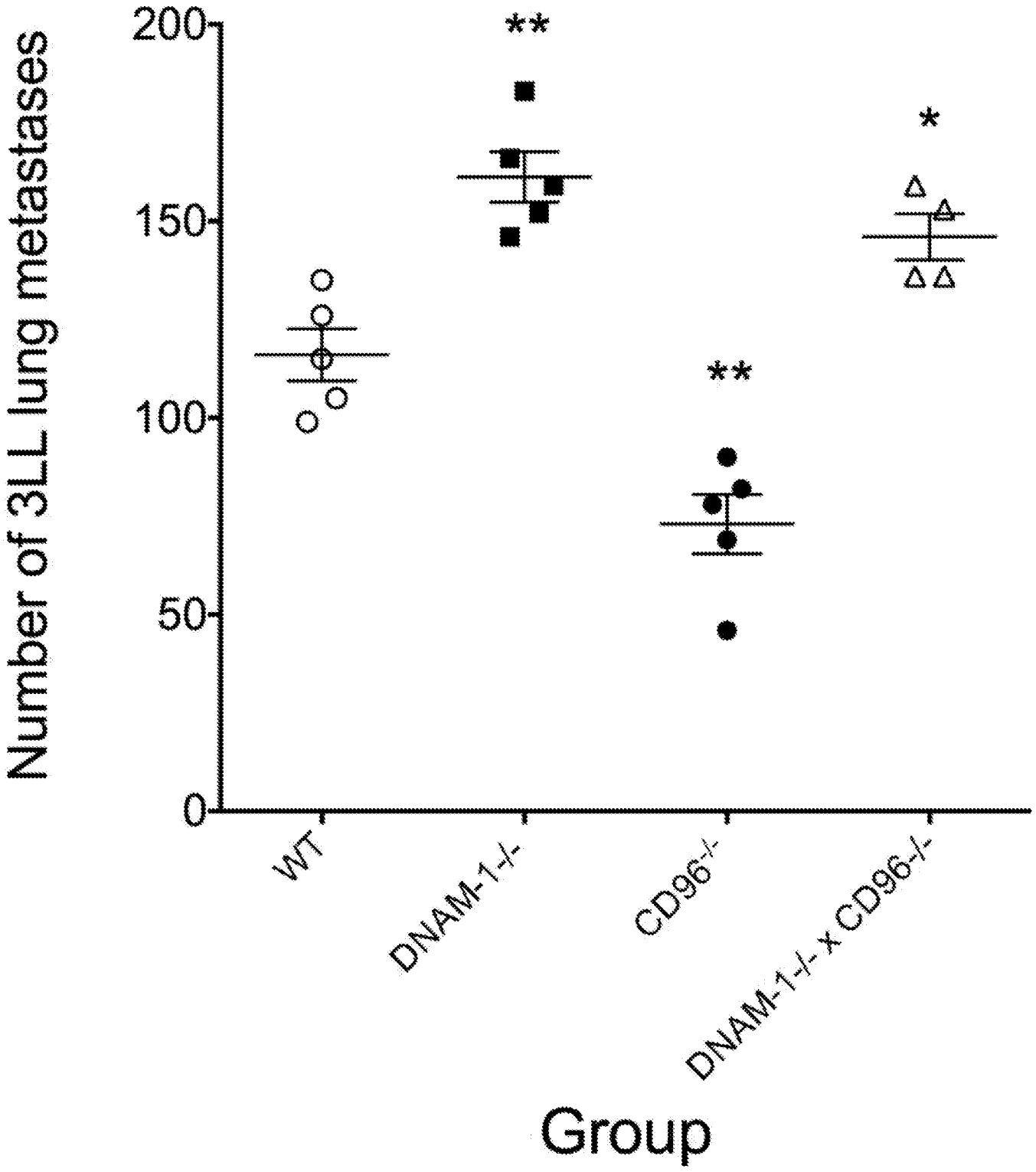

Number of 3LL lung metastases
200
150
100
50
0
**
**
*
WT
DNAM-1-/-
CD96-/-
DNAM-1-/- x CD96-/-
Group

Fig. 12

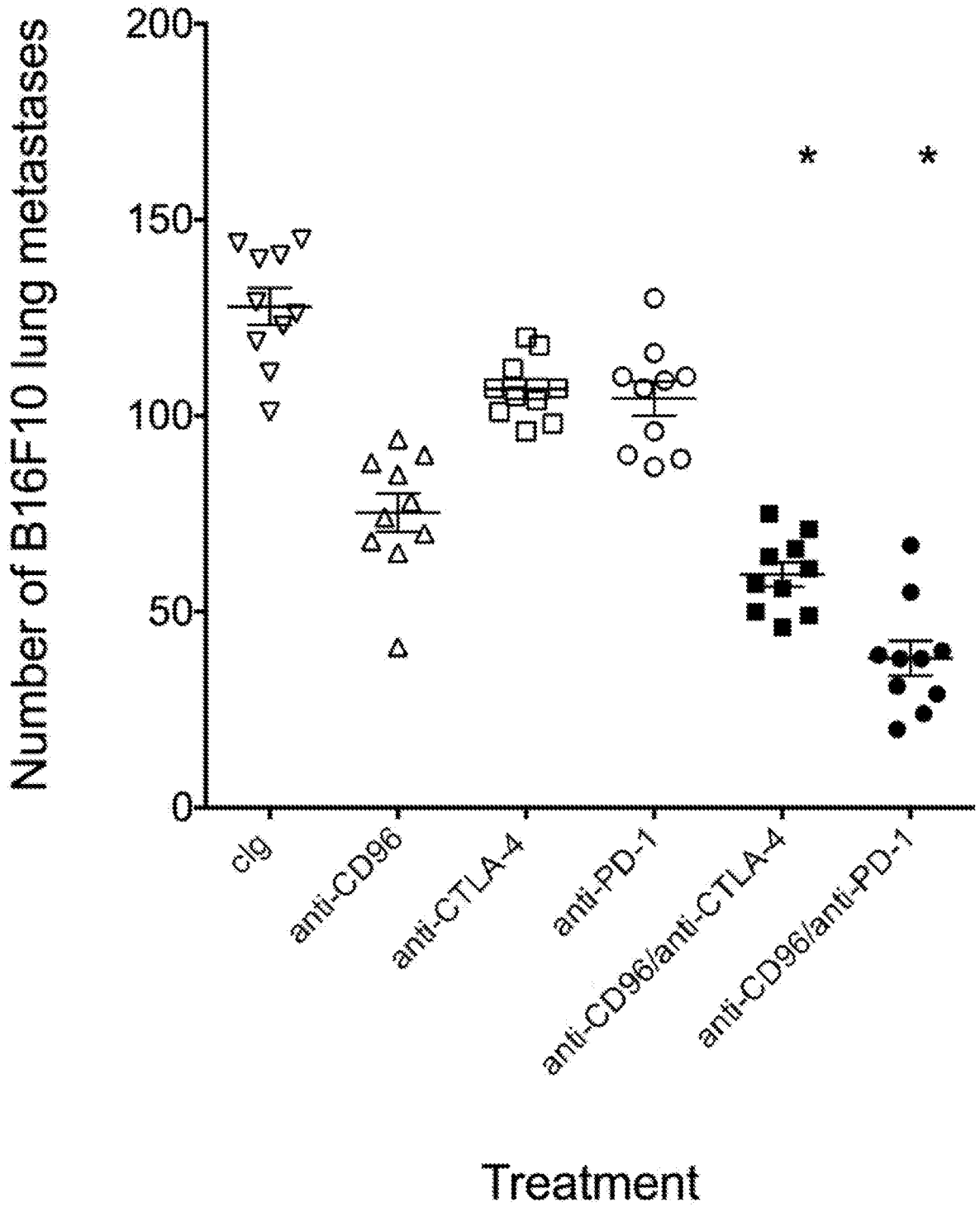

Number of B16F10 lung metastases
200
150
100
50
0
*
*
clg
anti-CD96
anti-CTLA-4
anti-PD-1
anti-CD96/anti-CTLA-4
anti-CD96/anti-PD-1
Treatment

Fig. 13

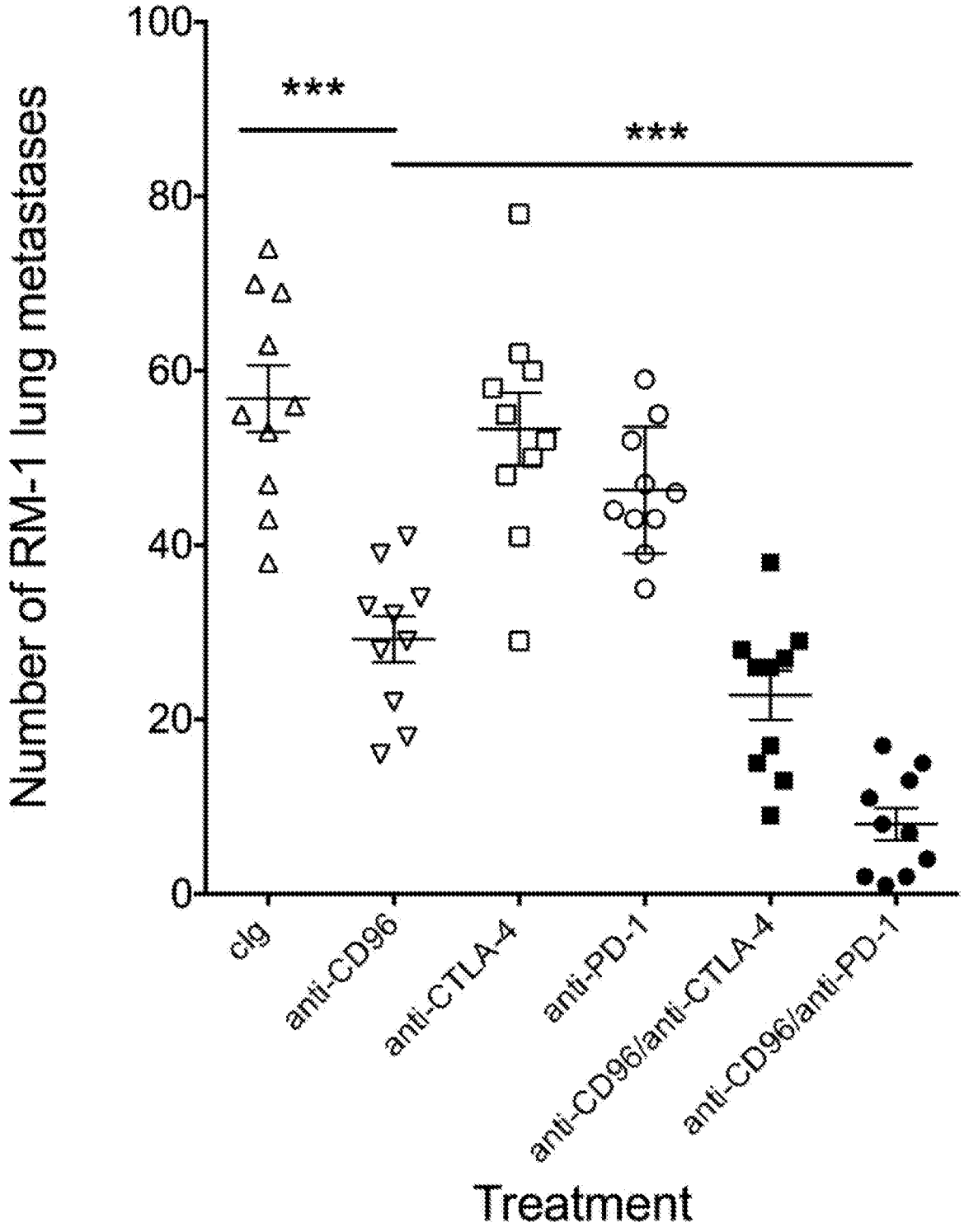

Number of RM-1 lung metastases
100
80
60
40
20
0
***
***
cIg
anti-CD96
anti-CTLA-4
anti-PD-1
anti-CD96/anti-CTLA-4
anti-CD96/anti-PD-1
Treatment

Fig. 14

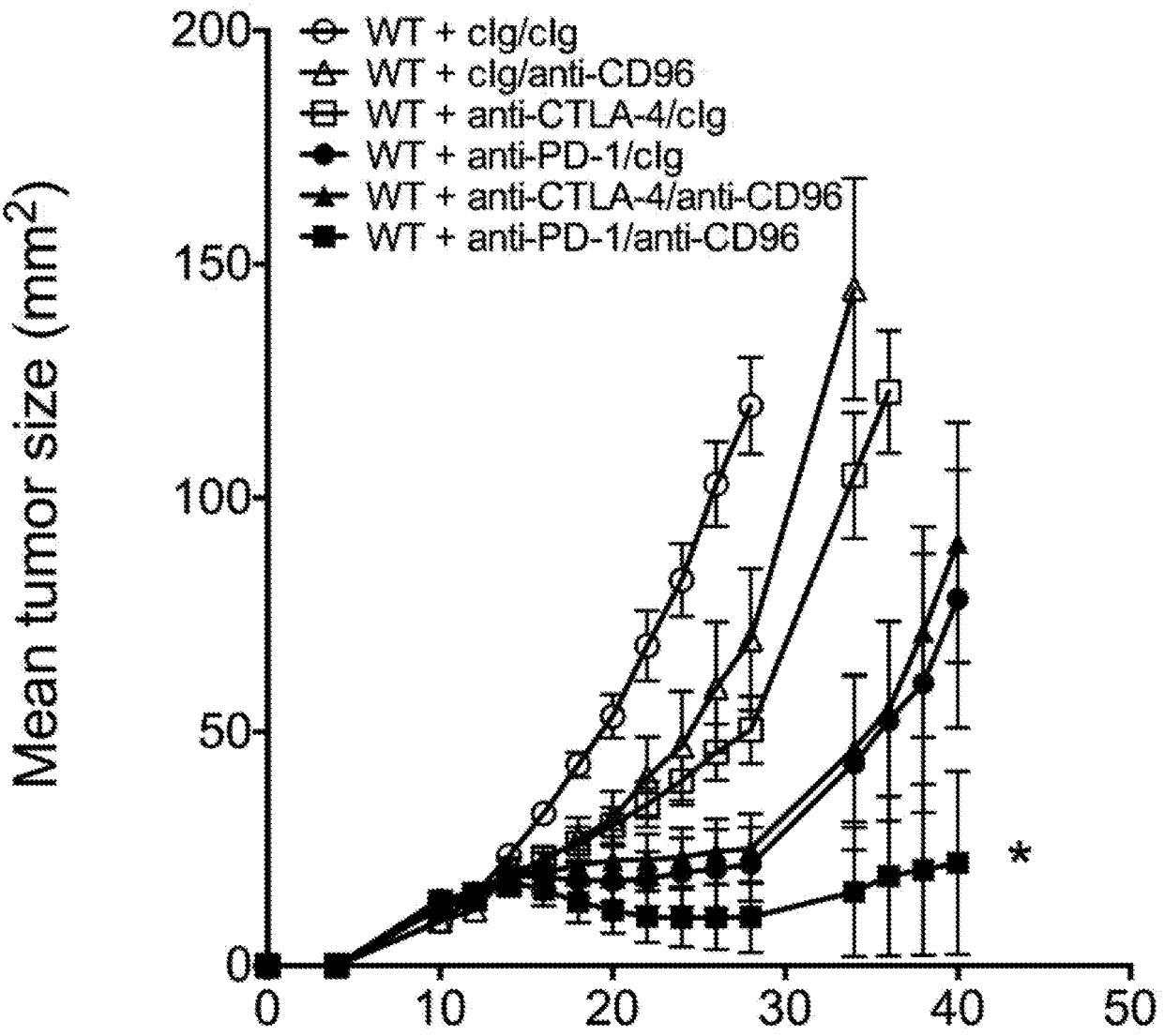
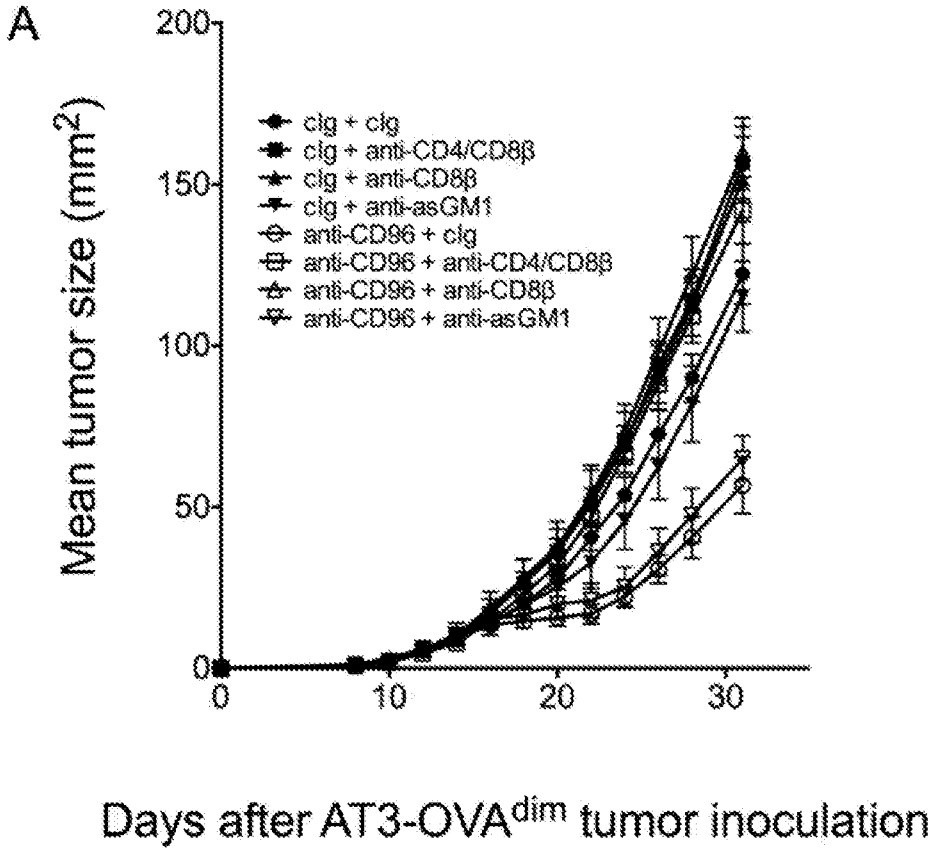

200
150
100
50
0
0
10
20
30
40
50
Mean tumor size (mm2)
WT + clg/clg
WT + clg/anti-CD96
WT + anti-CTLA-4/clg
WT + anti-PD-1/clg
WT + anti-CTLA-4/anti-CD96
WT + anti-PD-1/anti-CD96
*
Days after MC38-OVAdim tumor inoculation

Fig. 15


A
Mean tumor size (mm2)
200
150
100
50
0
0
10
20
30
clg + clg
clg + anti-CD4/CD8β
clg + anti-CD8β
clg + anti-asGM1
anti-CD96 + clg
anti-CD96 + anti-CD4/CD8β
anti-CD96 + anti-CD8β
anti-CD96 + anti-asGM1
Days after AT3-OVAdim tumor inoculation

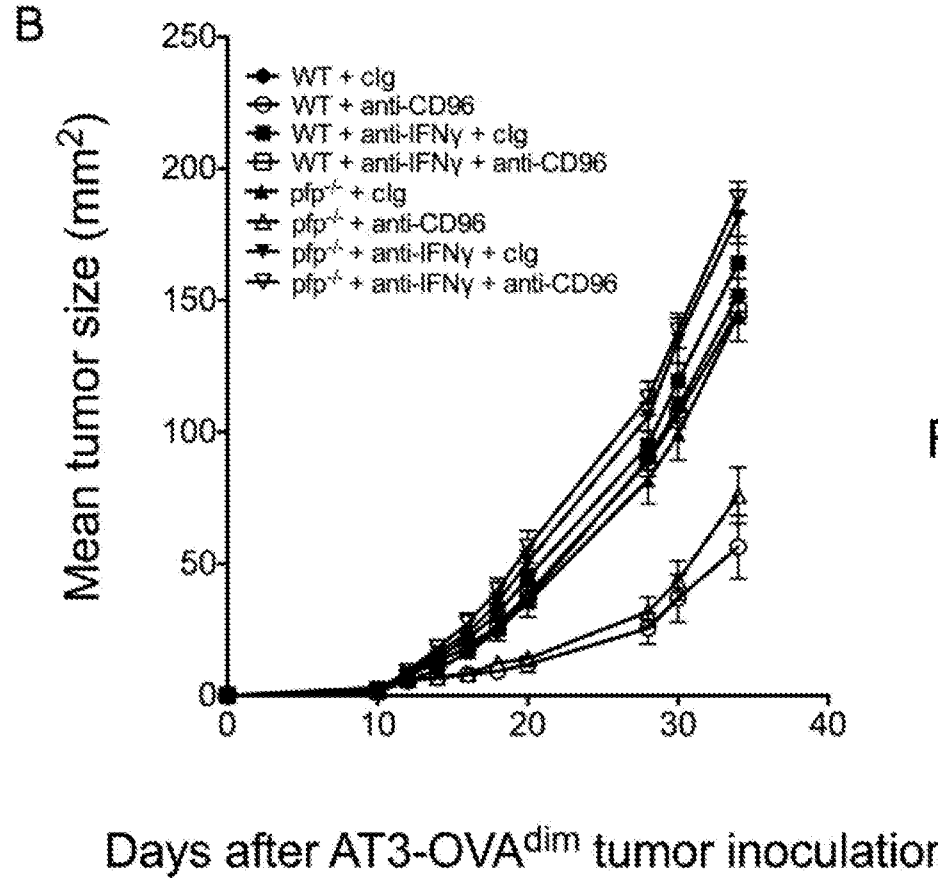

B
Mean tumor size (mm2)
250
200
150
100
50
0
0
10
20
30
40
WT + clg
WT + anti-CD96
WT + anti-IFNγ + clg
WT + anti-IFNγ + anti-CD96
pfp-/- + clg
pfp-/- + anti-CD96
pfp-/- + anti-IFNγ + clg
pfp-/- + anti-IFNγ + anti-CD96
Days after AT3-OVAdim tumor inoculation

Fig. 16

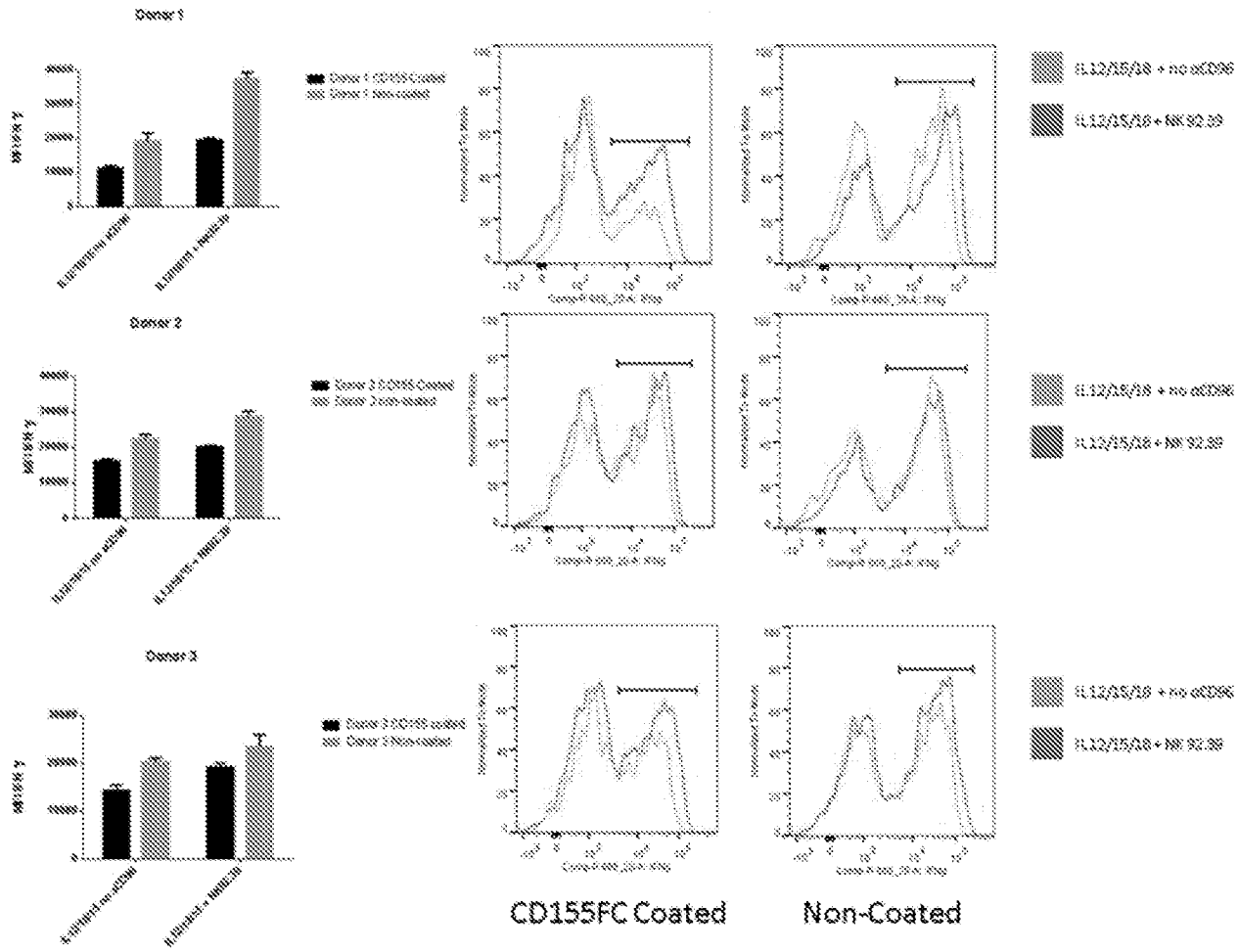


FIG. 17A

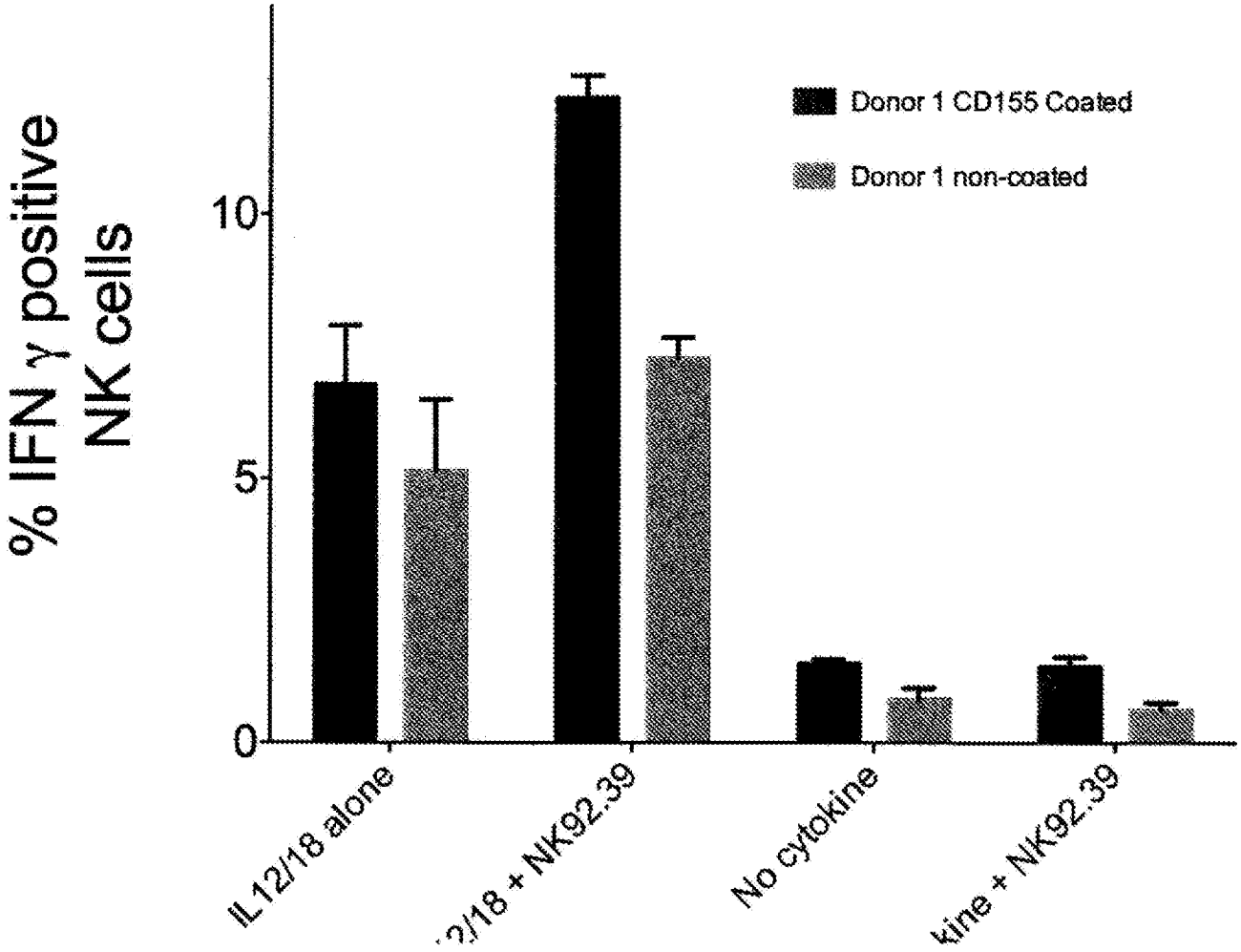
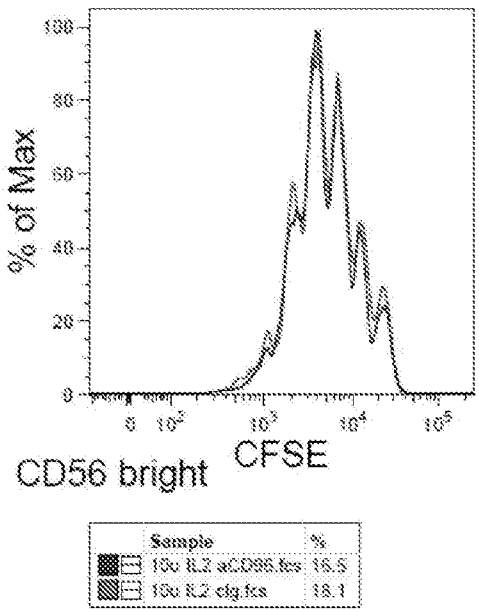
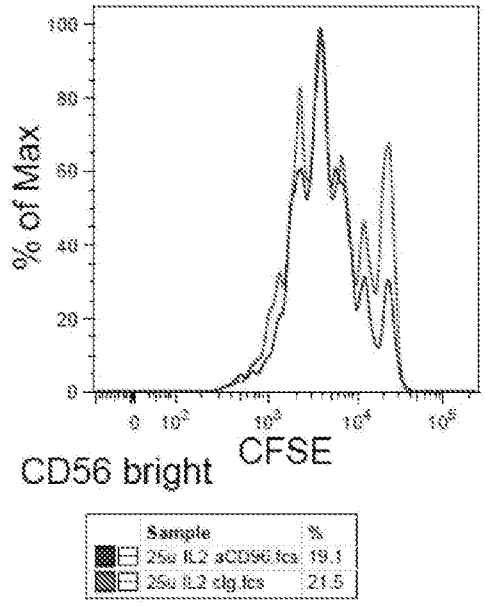

% IFN γ positive NK cells
10
5
0
Donor 1 CD155 Coated
Donor 1 non-coated
IL12/18 alone
No cytokine

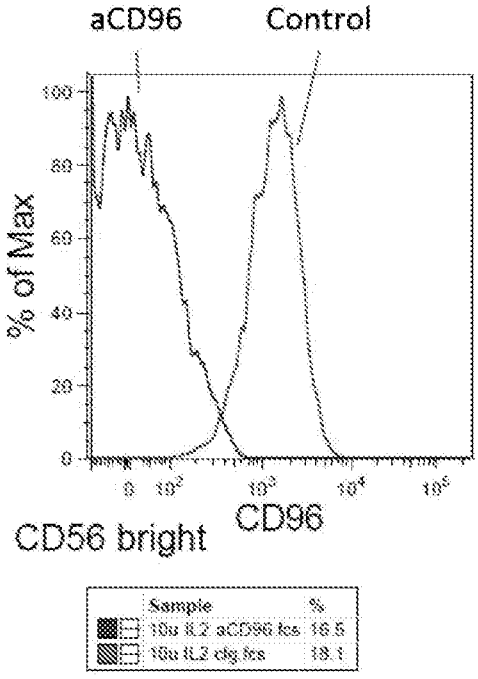
FIG. 17B



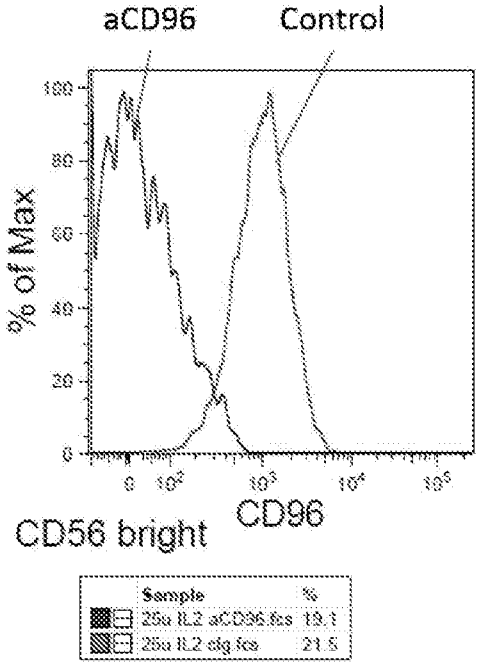
10unit/ml
IL-2



25unit/ml
IL-2



10unit/ml
IL-2



25unit/ml
IL-2

Fig. 18