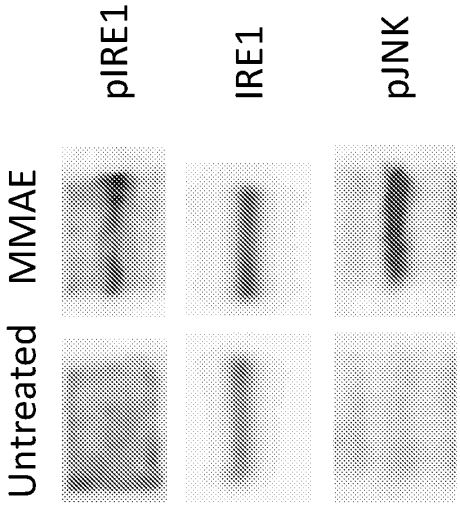


FIG. 1

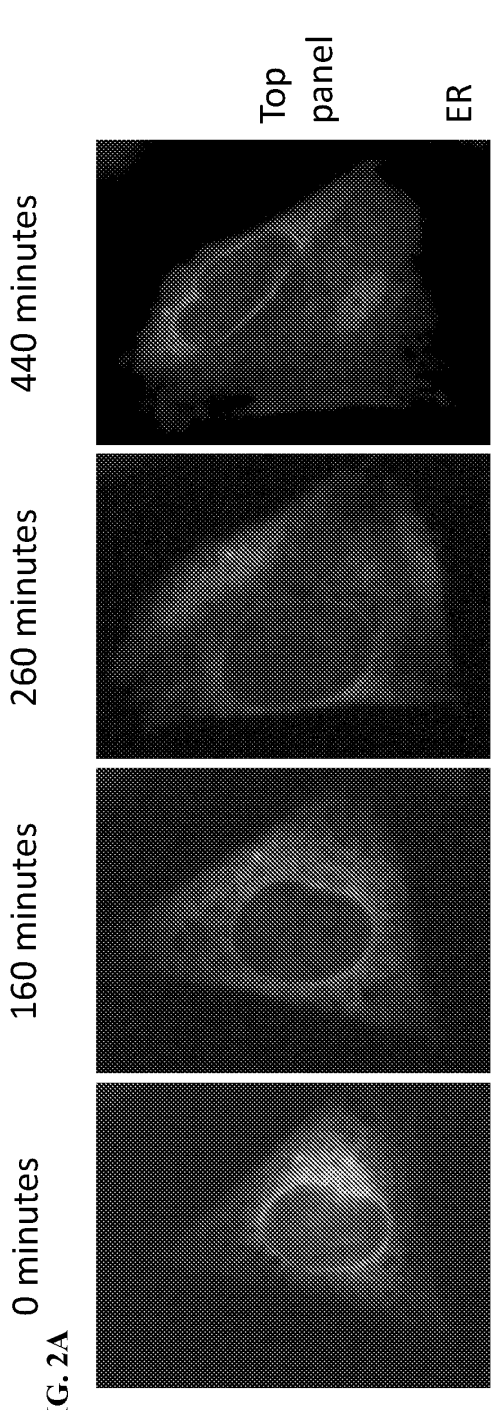

Top panel
ER
440 minutes
260 minutes
160 minutes
0 minutes

FIG. 2A

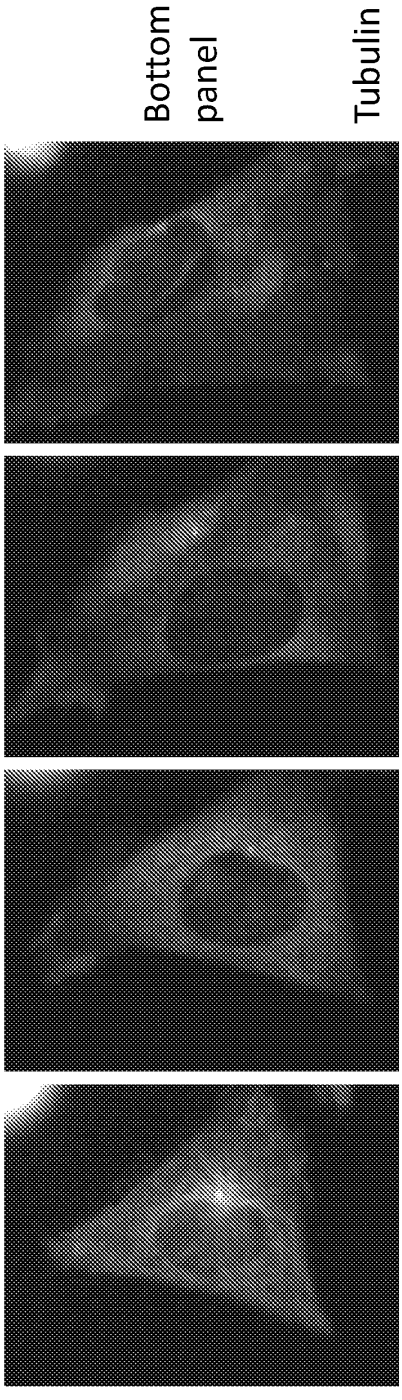

Bottom panel
Tubulin

FIG. 2B

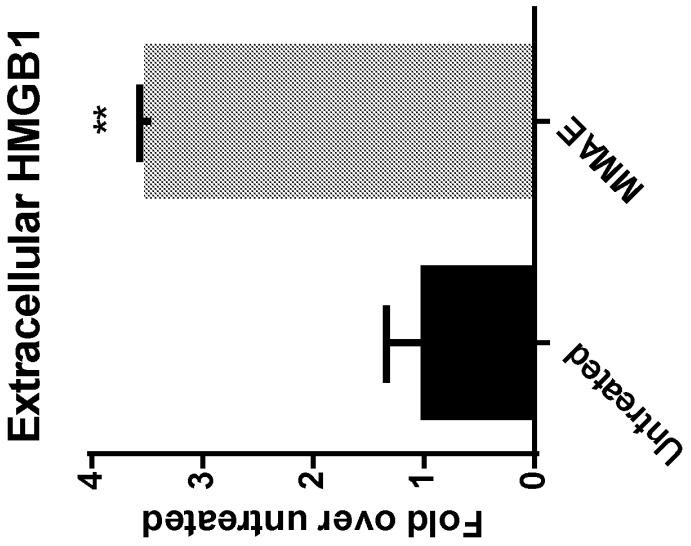

Extracellular HMGB1
**
Fold over untreated
0
1
2
3
4
Untreated
MMAE

FIG. 3B

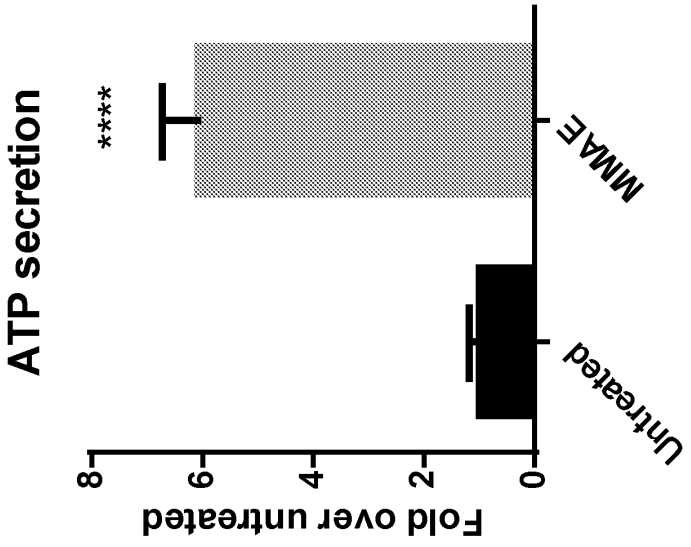

ATP secretion
****
Fold over untreated
0
2
4
6
8
Untreated
MMAE

FIG. 3A

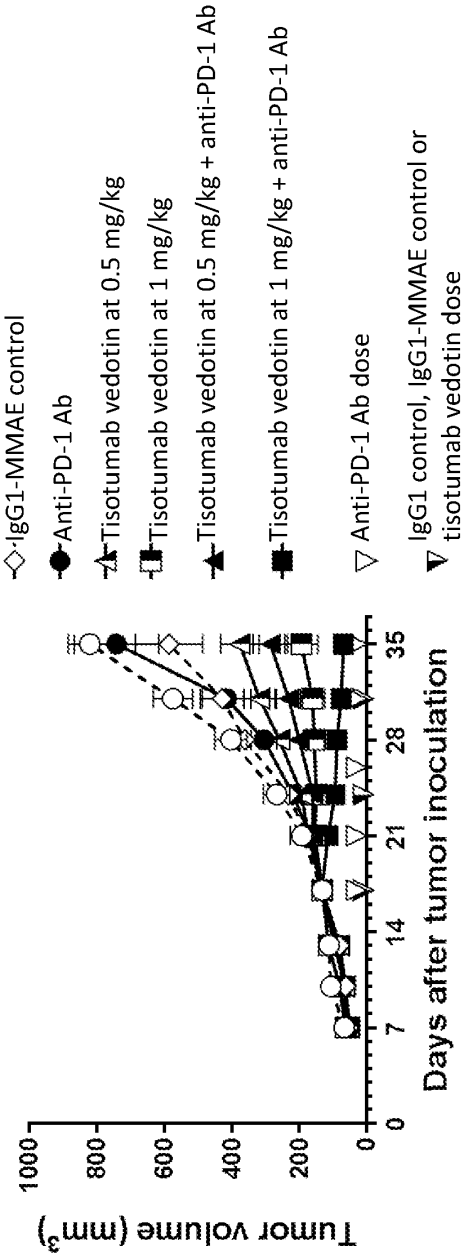

IgG1 control
IgG1-MMAE control
Anti-PD-1 Ab
Tisotumab vedotin at 0.5 mg/kg
Tisotumab vedotin at 1 mg/kg
Tisotumab vedotin at 0.5 mg/kg + anti-PD-1 Ab
Tisotumab vedotin at 1 mg/kg + anti-PD-1 Ab
Anti-PD-1 Ab dose
IgG1 control, IgG1-MMAE control or tisotumab vedotin dose
Tumor volume (mm3)
1000
800
600
400
200
0
0
7
14
21
28
35
Days after tumor inoculation

FIG. 4A

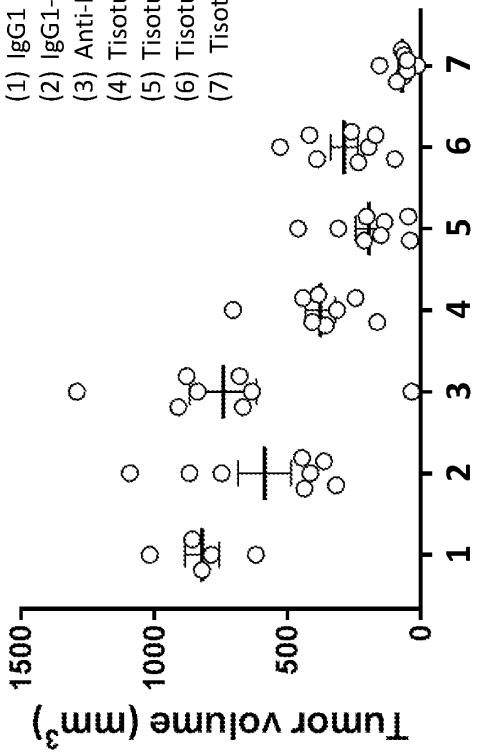

(1) IgG1 control
(2) IgG1-MMAE control
(3) Anti-PD-1 Ab
(4) Tisotumab vedotin at 0.5 mg/kg
(5) Tisotumab vedotin at 1 mg/kg
(6) Tisotumab vedotin at 0.5 mg/kg + anti-PD-1 Ab
(7) Tisotumab vedotin at 1 mg/kg + anti-PD-1 Ab
Tumor volume (mm3)
1500
1000
500
0
1
2
3
4
5
6
7

FIG. 4B

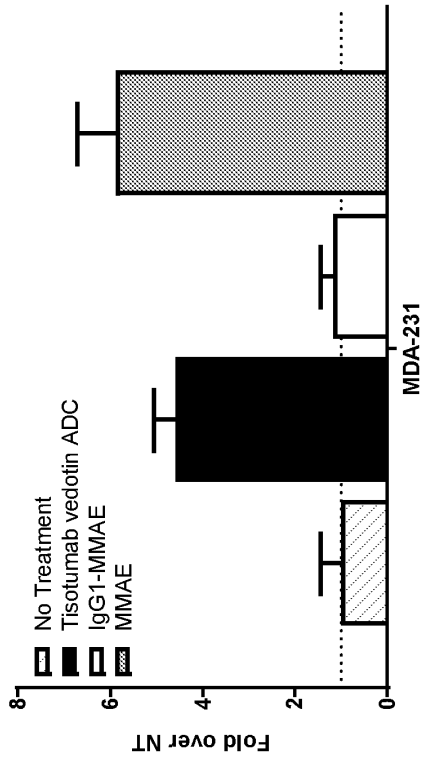

ATP Secretion
No Treatment
Tisotumab vedotin ADC
IgG1-MMAE
MMAE
Fold over NT
0
2
4
6
8
MDA-231

FIG. 5A

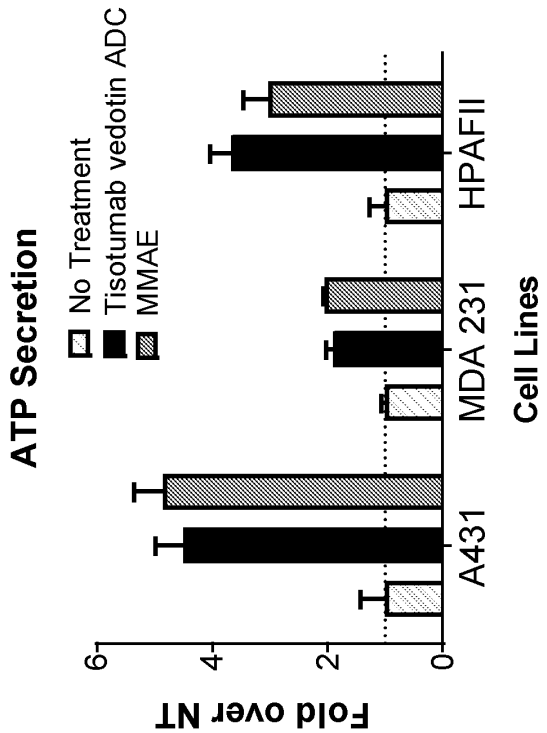

ATP Secretion
No Treatment
Tisotumab vedotin ADC
MMAE
Fold over NT
0
2
4
6
A431
MDA 231
HPAFII
Cell Lines

FIG. 5B

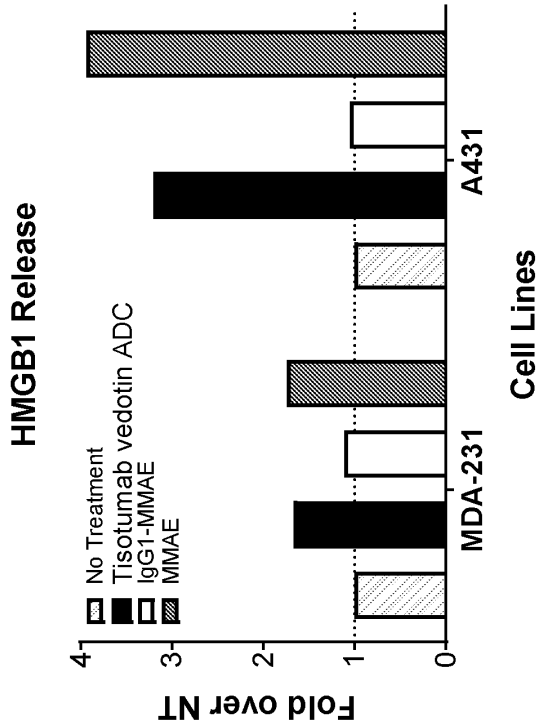
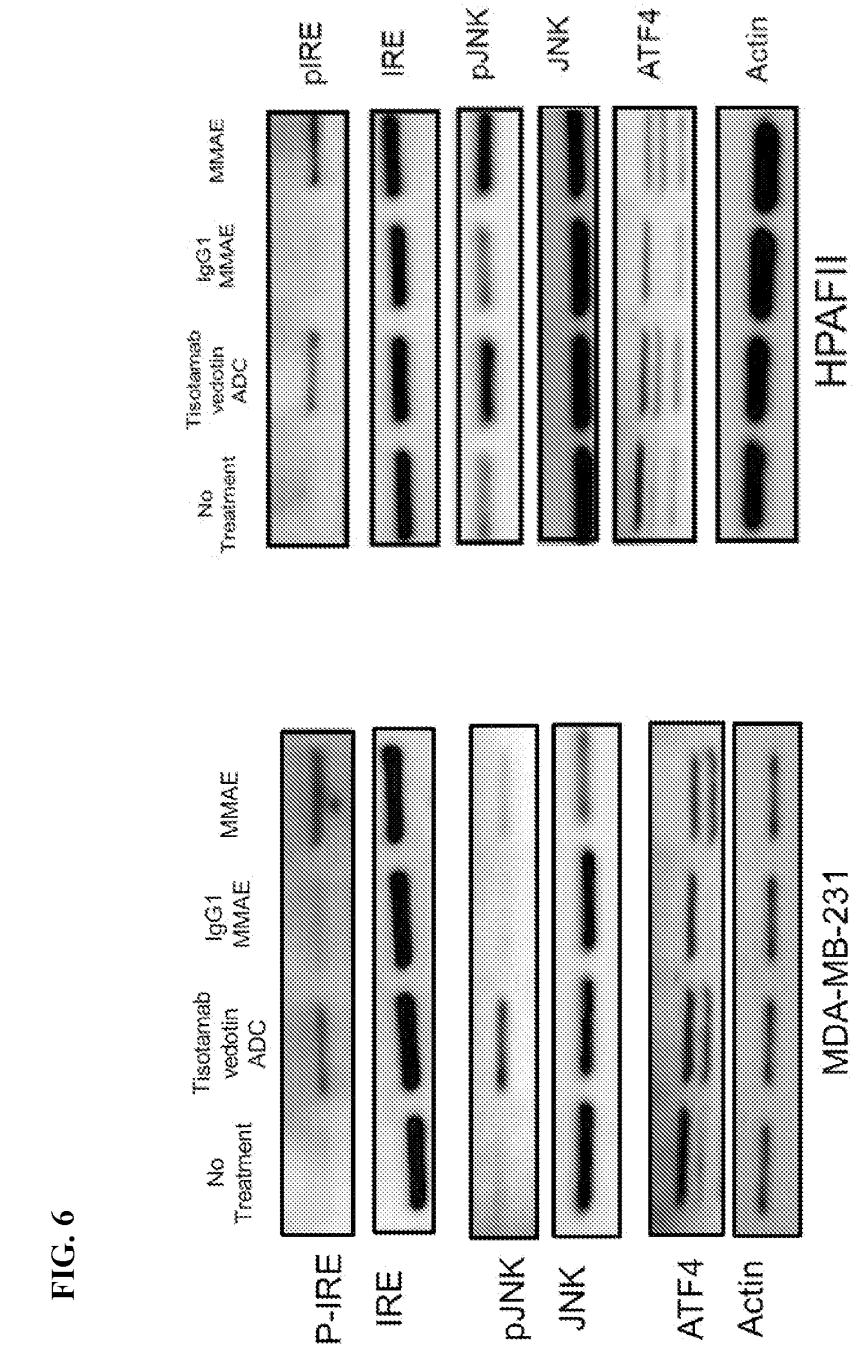

HMGB1 Release
No Treatment
Tisotumab vedotin ADC
IgG1-MMAE
MMAE
Fold over NT
0
1
2
3
4
MDA-231
A431
Cell Lines

FIG. 5C

FIG. 6


No Treatment
Tisotamab vedotin ADC
IgG1 MMAE
MMAE
P-IRE
IRE
pJNK
JNK
ATF4
Actin
MDA-MB-231
pIRE
IRE
pJNK
JNK
ATF4
Actin
HPAFII

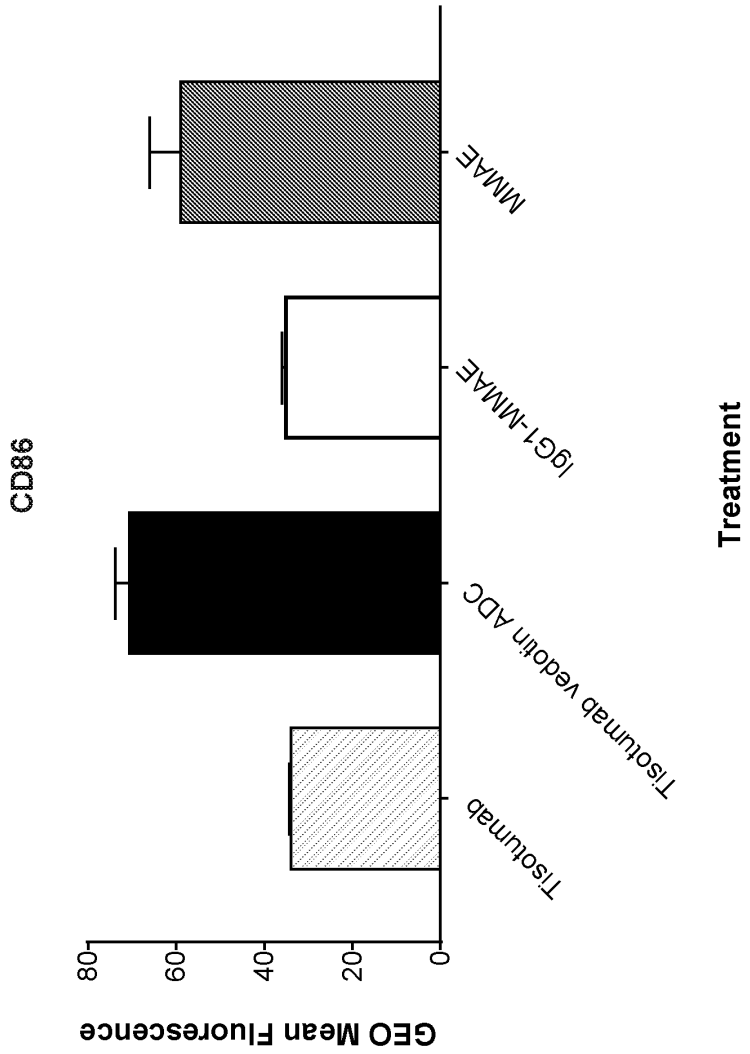

CD86
GEO Mean Fluorescence
0
20
40
60
80
Tisotumab
Tisotumab vedotin ADC
IgG1-MMAE
MMAE
Treatment

FIG. 7A

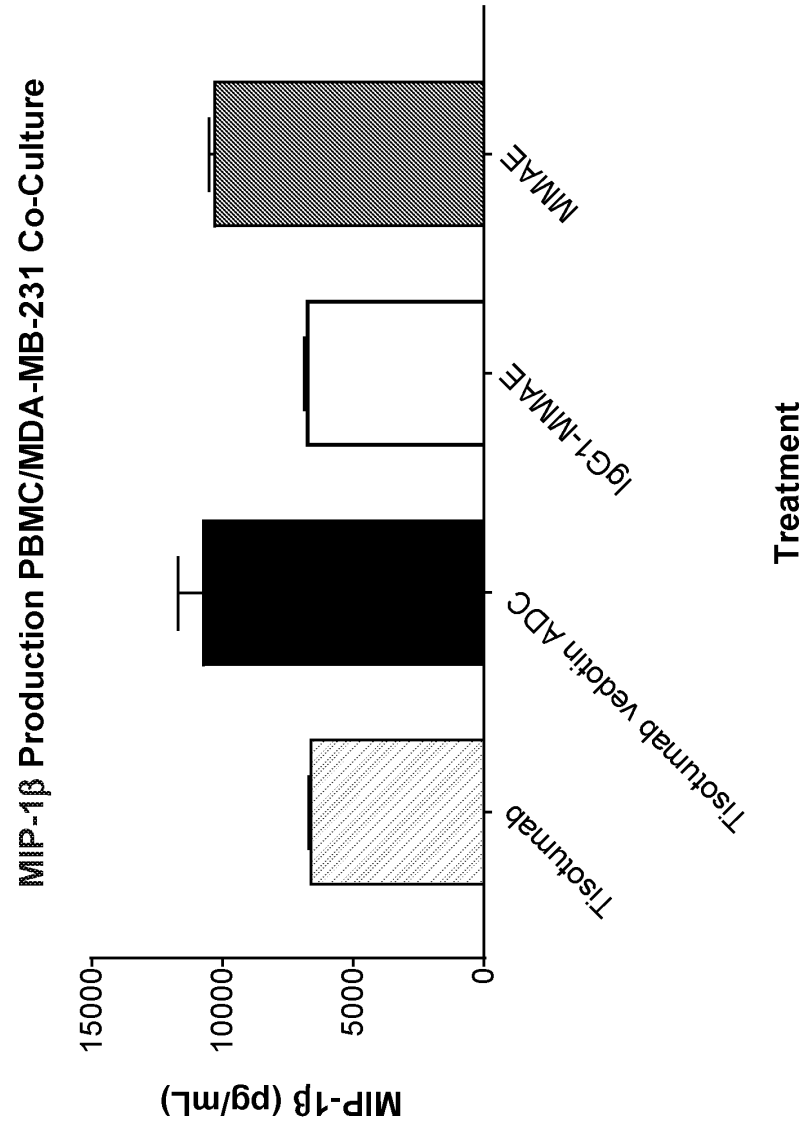

MIP-1β Production PBMC/MDA-MB-231 Co-Culture
MIP-1β (pg/mL)
15000
10000
5000
0
Tisotumab
Tisotumab vedotin ADC
IgG1-MMAE
MMAE
Treatment

FIG. 7B

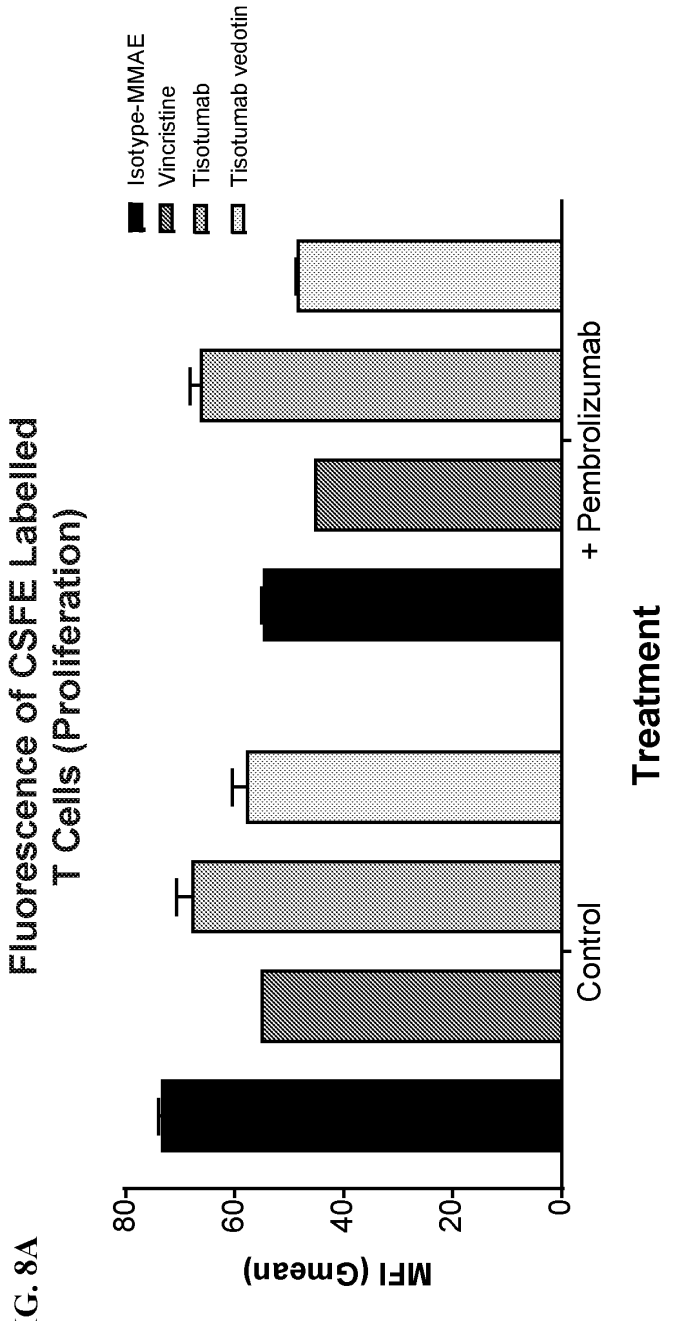

Fluorescence of CSFE Labelled
T Cells (Proliferation)
Isotype-MMAE
Vincristine
Tisotumab
Tisotumab vedotin
MFI (Gmean)
0
20
40
60
80
Control
+ Pembrolizumab
Treatment

FIG. 8A

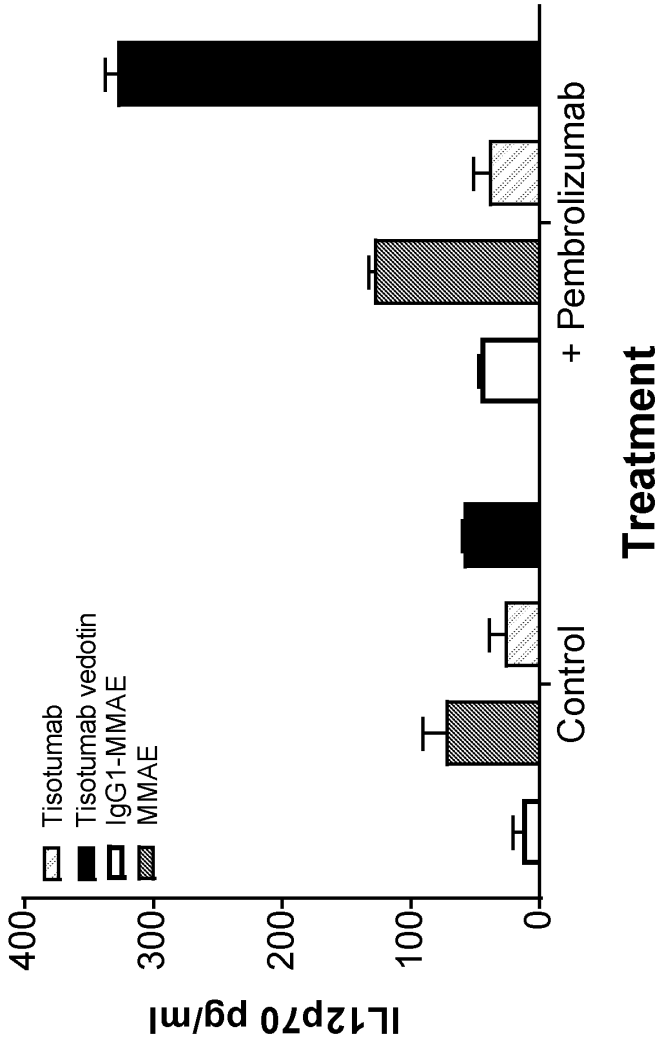

IL12p70 Production PBMC/MDA-MB-231 Co-Culture
Tisotumab
Tisotumab vedotin
IgG1-MMAE
MMAE
IL12p70 pg/ml
400
300
200
100
0
Control
+ Pembrolizumab
Treatment

FIG. 8B

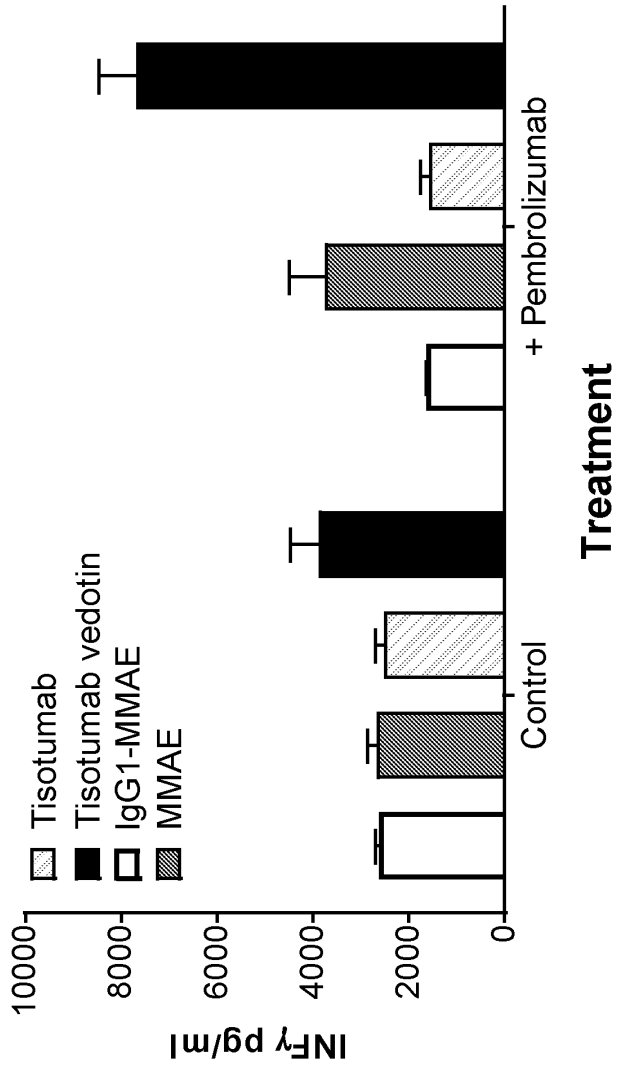

IFNγ Production PBMC/MDA-MB-231 Co-Culture
Tisotumab
Tisotumab vedotin
IgG1-MMAE
MMAE
INFγ pg/ml
10000
8000
6000
4000
2000
0
Control
+ Pembrolizumab
Treatment

FIG. 8C