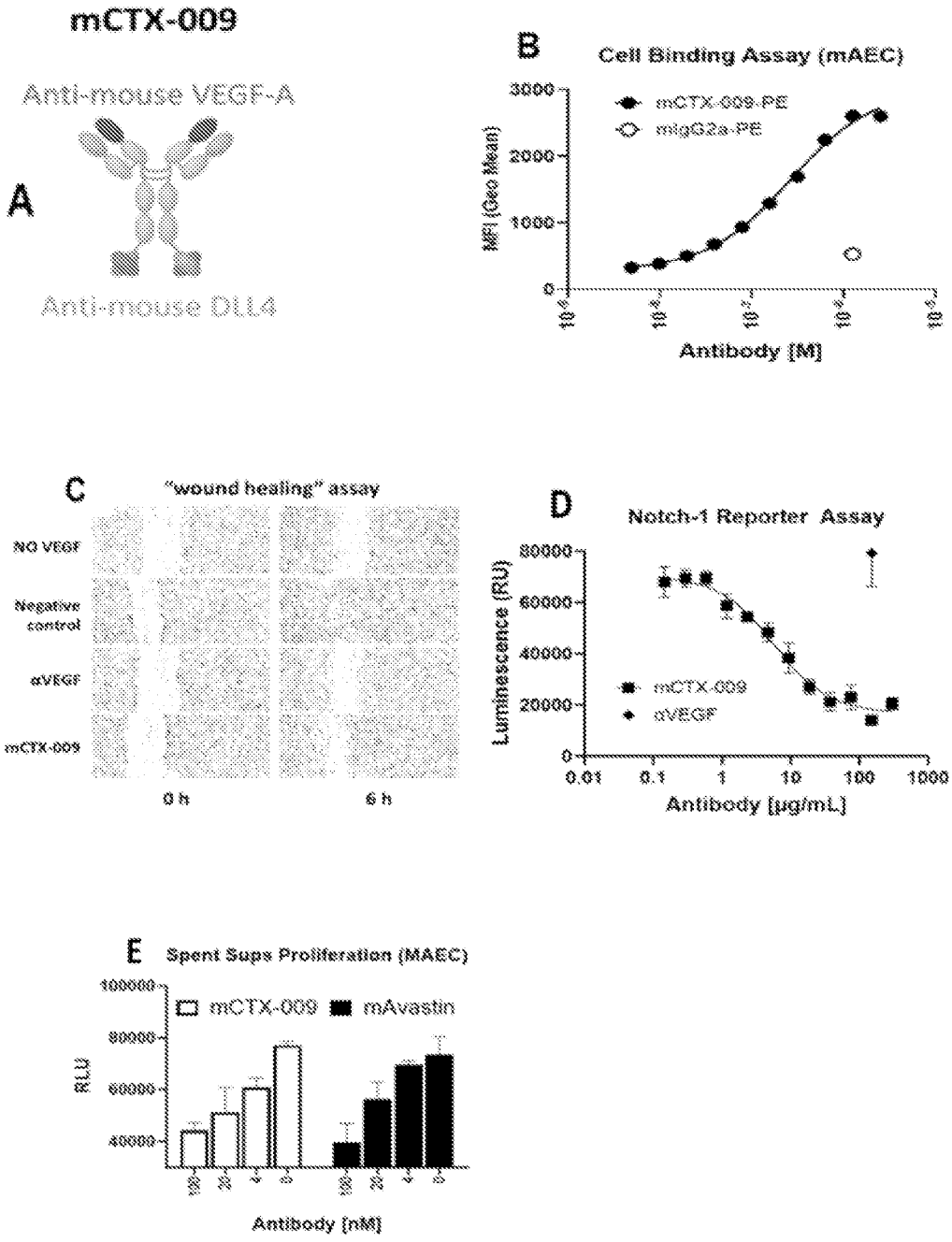

mCTX-009
Anti-mouse VEGF-A
A
Anti-mouse DLL4
B
Cell Binding Assay (mAEC)
mCTX-009-PE
mIgG2a-PE
MFI (Geo Mean)
Antibody [M]
C
"wound healing" assay
NO VEGF
Negative control
αVEGF
mCTX-009
0 h
6 h
D
Notch-1 Reporter Assay
Luminescence (RU)
mCTX-009
αVEGF
Antibody [μg/mL]
E
Spent Sups Proliferation (MAEC)
mCTX-009
mAvastin
RLU
Antibody [nM]

Fig. 1

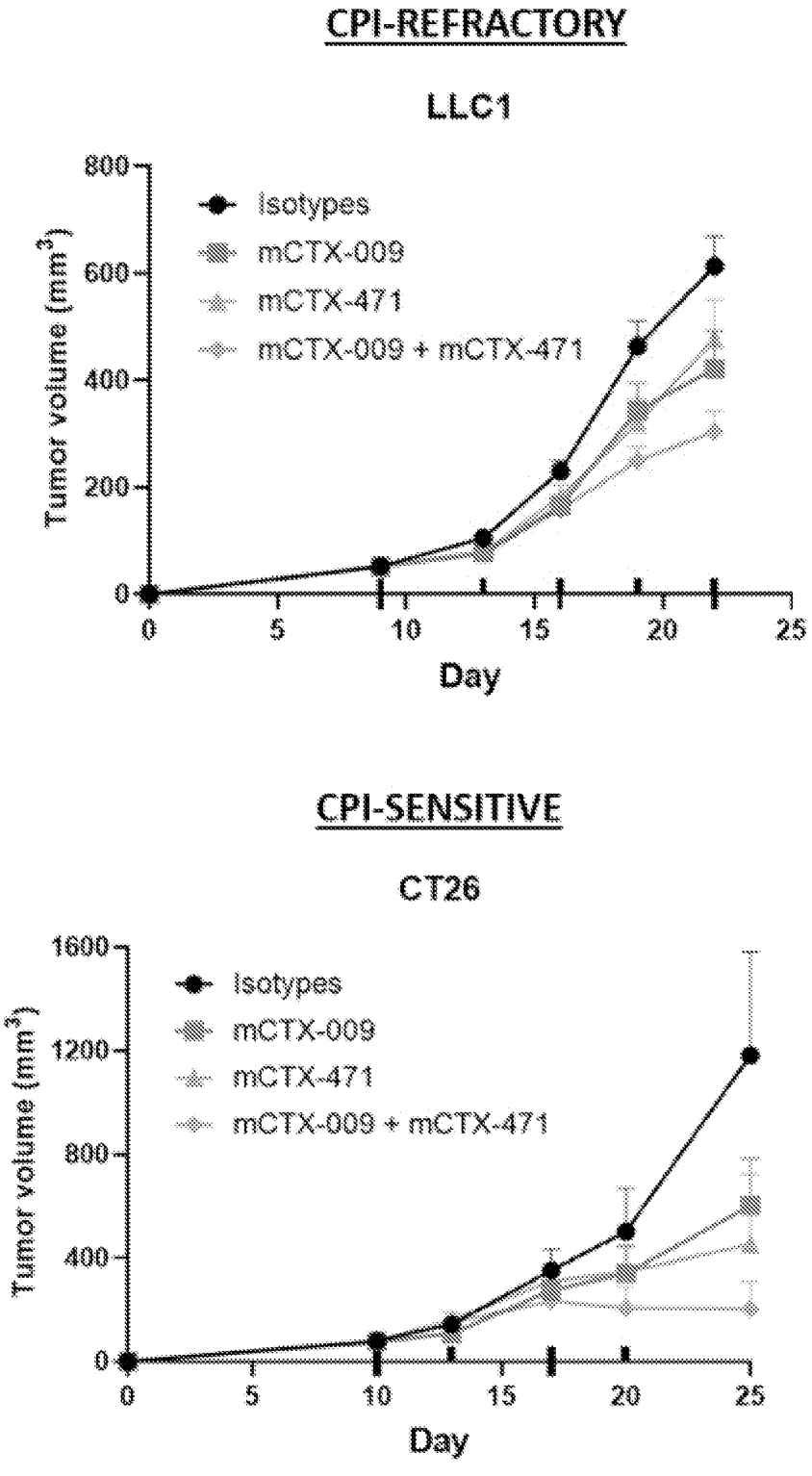

CPI-REFRACTORY
LLC1
Tumor volume (mm3)
800
600
400
200
0
Isotypes
mCTX-009
mCTX-471
mCTX-009 + mCTX-471
0
5
10
15
20
25
Day
CPI-SENSITIVE
CT26
Tumor volume (mm3)
1600
1200
800
400
0
Isotypes
mCTX-009
mCTX-471
mCTX-009 + mCTX-471
0
5
10
15
20
25
Day

Fig.2

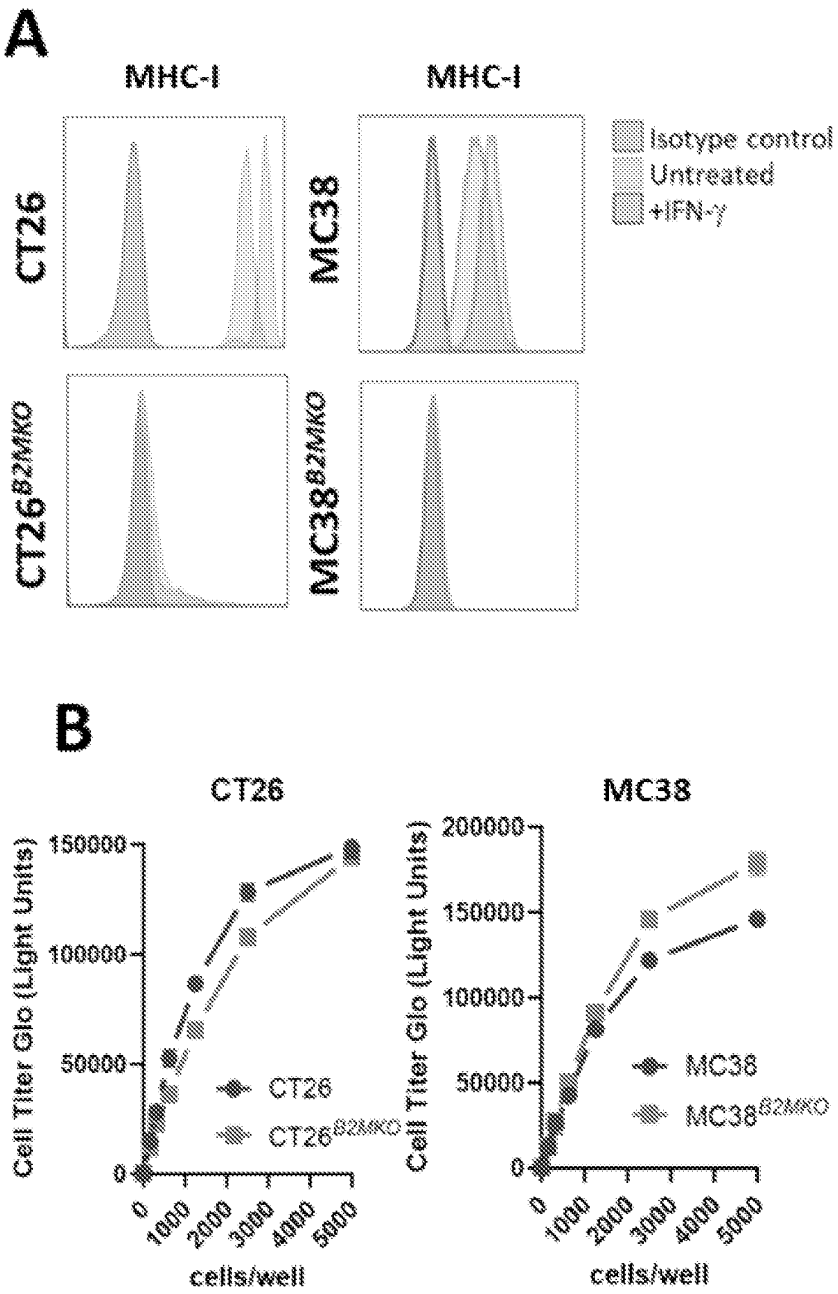

A
MHC-I
MHC-I
CT26
MC38
CT26β2MKO
MC38β2MKO
Isotype control
Untreated
+IFN-γ
B
CT26
MC38
Cell Titer Glo (Light Units)
150000
100000
50000
0
200000
CT26
CT26β2MKO
MC38
MC38β2MKO
0
1000
2000
3000
4000
5000
cells/well

Fig. 3

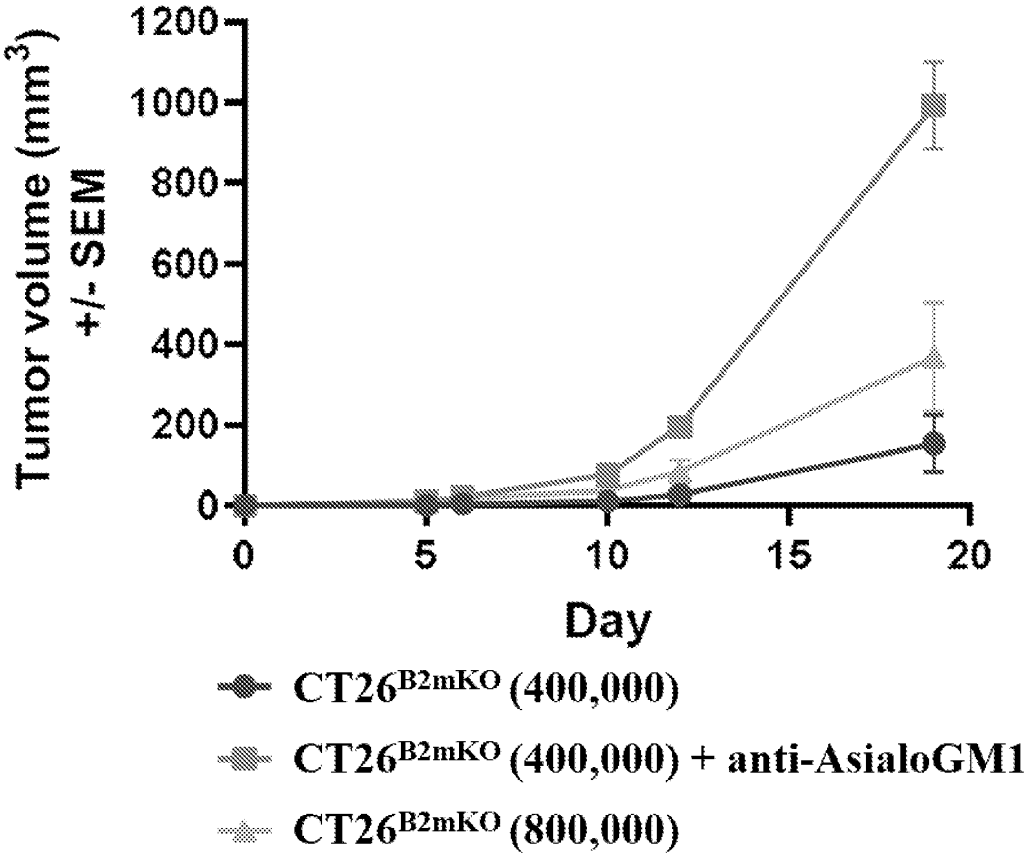

Tumor volume (mm3)
+/- SEM
1200
1000
800
600
400
200
0
0
5
10
15
20
Day
CT26B2mKO (400,000)
CT26B2mKO (400,000) + anti-AsialoGM1
CT26B2mKO (800,000)

Fig. 4

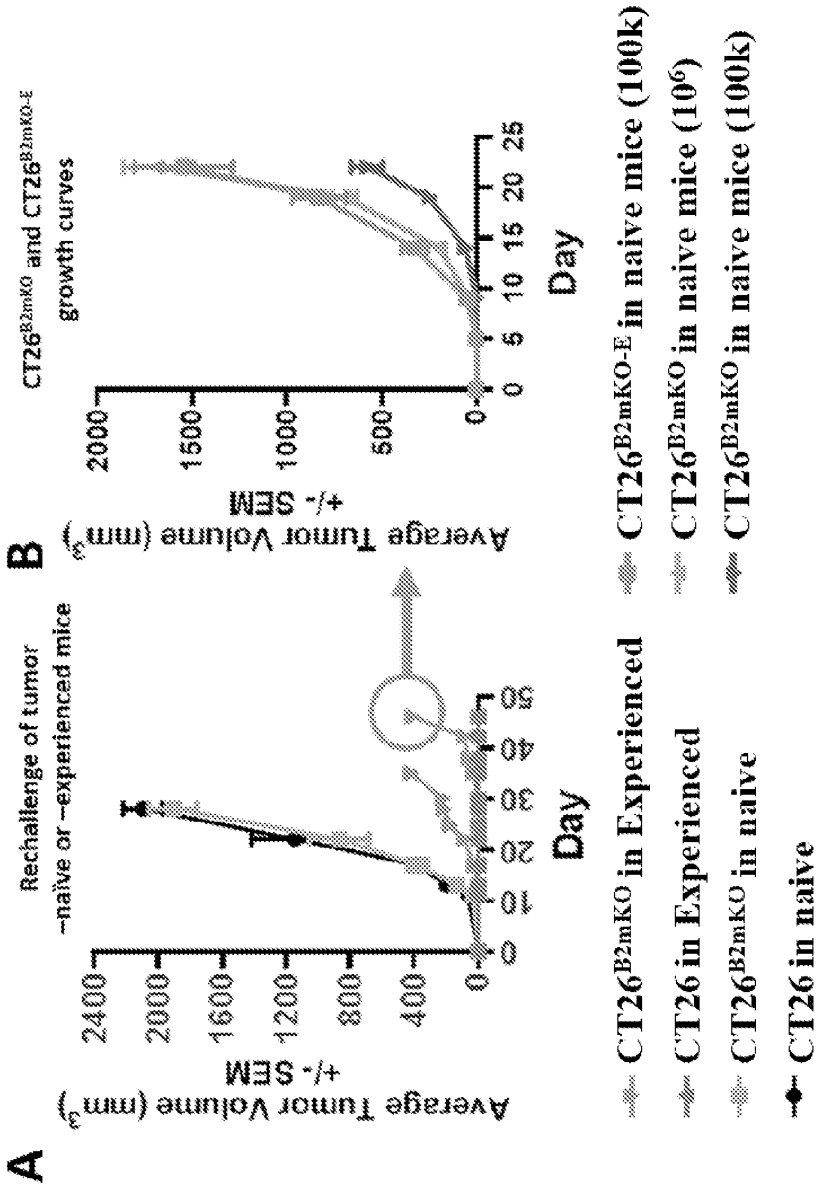

A
Rechallenge of tumor
–naïve or –experienced mice
Average Tumor Volume (mm³)
+/- SEM
Day
B
CT26B2mKO and CT26B2mKO-E
growth curves
Average Tumor Volume (mm³)
+/- SEM
Day
CT26B2mKO in Experienced
CT26 in Experienced
CT26B2mKO in naive
CT26 in naive
CT26B2mKO-E in naive mice (100k)
CT26B2mKO in naive mice (10⁶)
CT26B2mKO in naive mice (100k)

Fig. 5

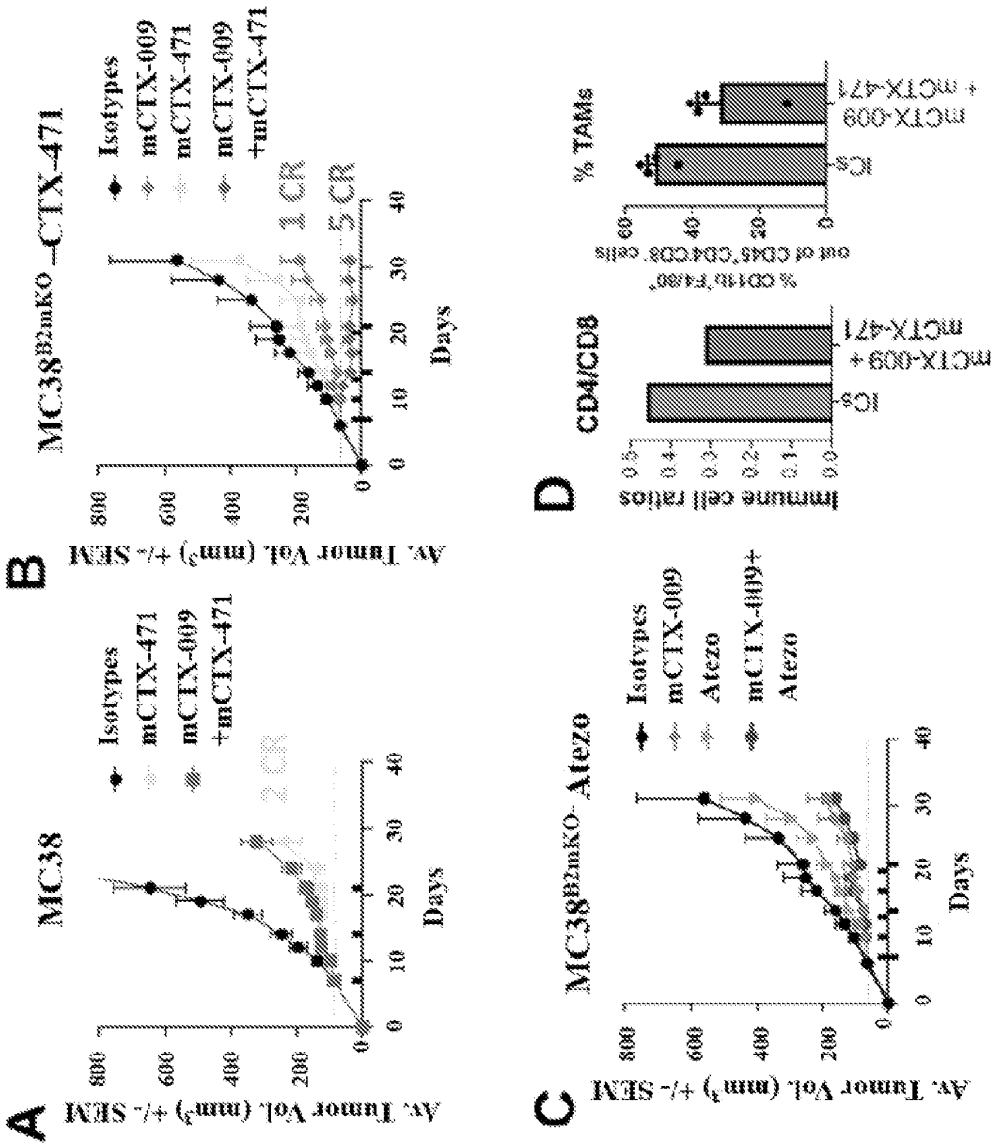

A
MC38
Isotypes
mCTX-471
mCTX-009
+mCTX-471
2 CR
Av. Tumor Vol. (mm³) +/- SEM
Days
B
MC38B2mKO –CTX-471
Isotypes
mCTX-009
mCTX-471
mCTX-009
+mCTX-471
1 CR
5 CR
Av. Tumor Vol. (mm³) +/- SEM
Days
C
MC38B2mKO- Atezo
Isotypes
mCTX-009
Atezo
mCTX-009+
Atezo
Av. Tumor Vol. (mm³) +/- SEM
Days
D
CD4/CD8
Immune cell ratios
IC8
mCTX-009 +
mCTX-471
% TAMs
% CD11b+F4/80+
out of CD45+CD4-CD8- cells
IC8
mCTX-009
+ mCTX-471

Fig. 6

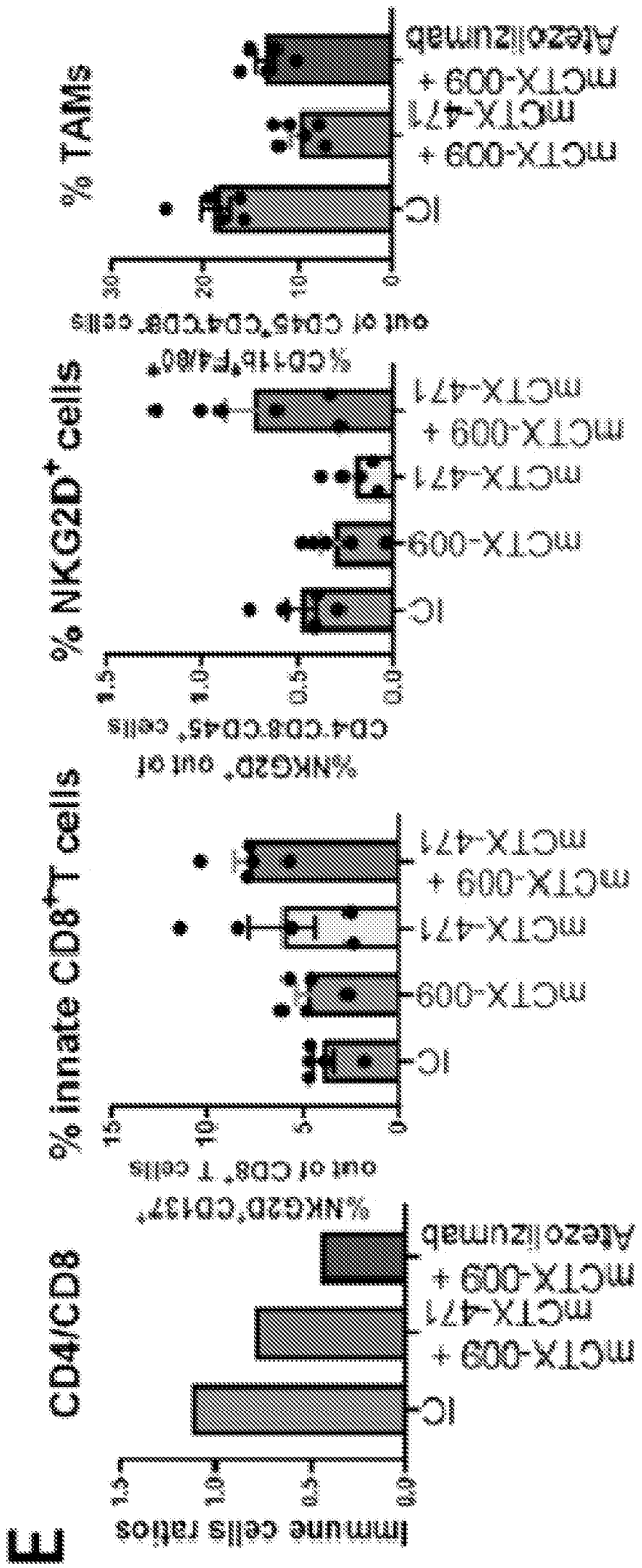

E
immune cells ratios
CD4/CD8
%NKG2D+CD137+
out of CD8+ T cells
% innate CD8+T cells
%NKG2D+ out of
CD4+CD8-CD45+ cells
% NKG2D+ cells
%CD11b+F4/80+
out of CD45+CD4+CD8- cells
% TAMs
IC
mCTX-009
mCTX-471
mCTX-009 +
mCTX-471
Atezolizumab

Fig. 6 cont.

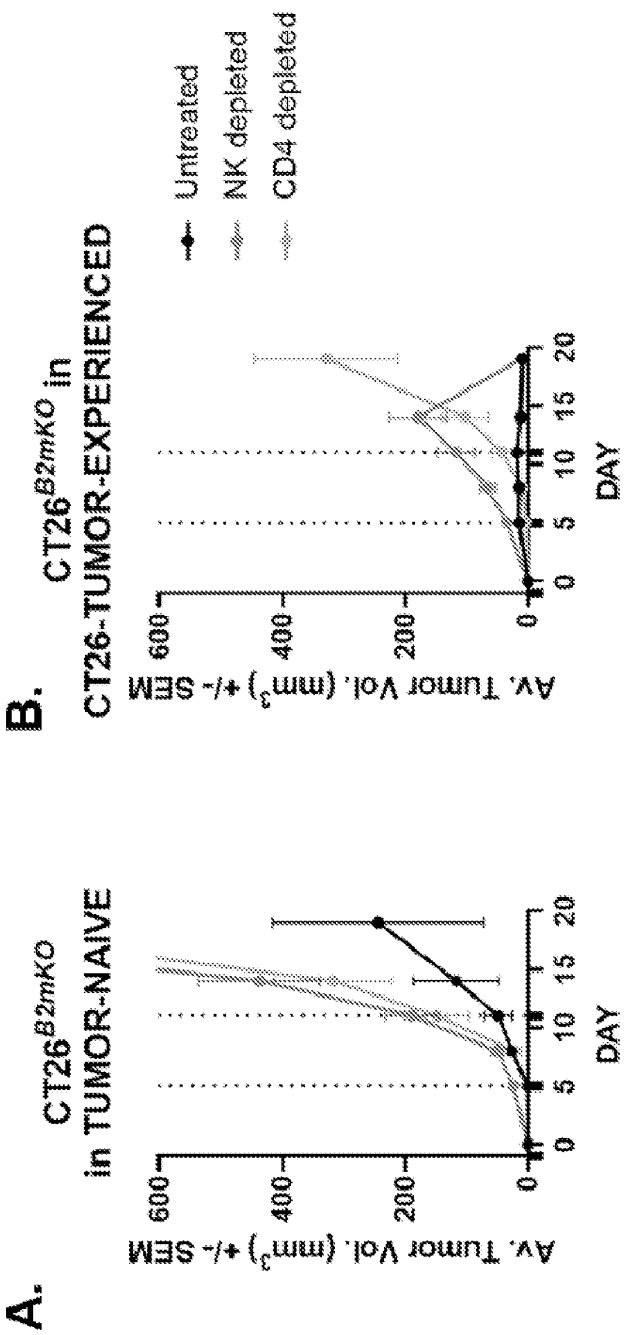

A.
CT26B2mKO
in TUMOR-NAIVE
Av. Tumor Vol. (mm3) +/- SEM
600
400
200
0
0
5
10
15
20
DAY
B.
CT26B2mKO in
CT26-TUMOR-EXPERIENCED
Untreated
NK depleted
CD4 depleted
Av. Tumor Vol. (mm3) +/- SEM
600
400
200
0
0
5
10
15
20
DAY

Fig.7

Summary of *in vivo* activity

Cell Line Name	Strain	Notes	Inoculum *	Monotherapy Activity				Combo Activity**	
				mCTX-471	mCTX-009	αPD-L1	mCTX-471+ CTX-009	PD-L1+ CTX-009
MC38	C57BL/6		0.25	++++	+	++++	No	No
MC38$^{H-2k-/-}$	C57BL/6	CRISPR KO H-2k1	0.25	++++	++	++	Yes	No
CT26	BALB/c		0.5	++++	+++	++++	Yes	Yes
CT26$^{B2m-/-}$	BALB/c	CRISPR KO B2m	1	+	+	-	Yes	No
CT26$^{B2m-/-E}$	BALB/c	Passaging of CT26$^{B2m-/-}$ in cured mice	0.1	++	++	-	Yes	Yes

*Cells implanted subcutaneously in millions.

**Refers to whether combination produces better tumor growth inhibition than monotherapies.

Fig.8

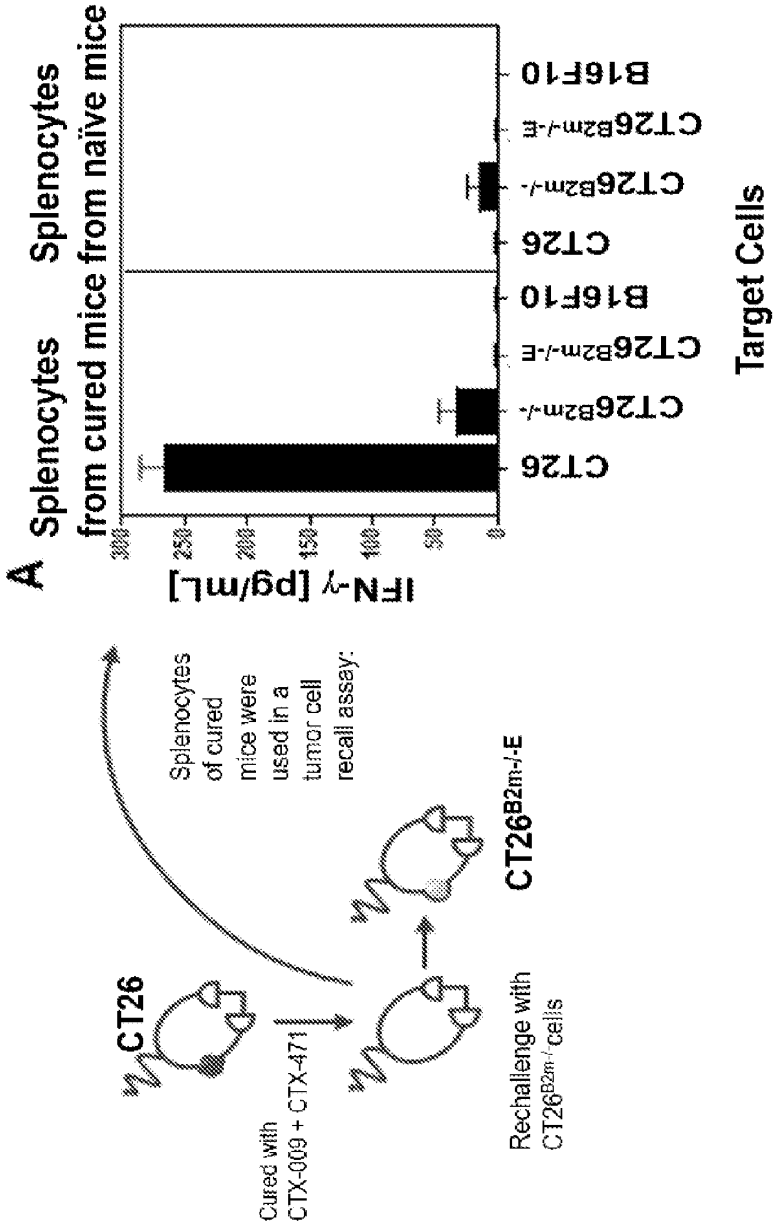

A
Splenocytes from cured mice from naïve mice
Splenocytes
IFN-γ [pg/mL]
300
250
200
150
100
50
0
CT26
CT26B2m-/-
CT26B2m-/-E
B16F10
CT26
CT26B2m-/-
CT26B2m-/-E
B16F10
Target Cells
CT26
Cured with CTX-009 + CTX-471
Splenocytes of cured mice were used in a tumor cell recall assay:
Rechallenge with CT26B2m-/- cells
CT26B2m-/-E

Fig. 9

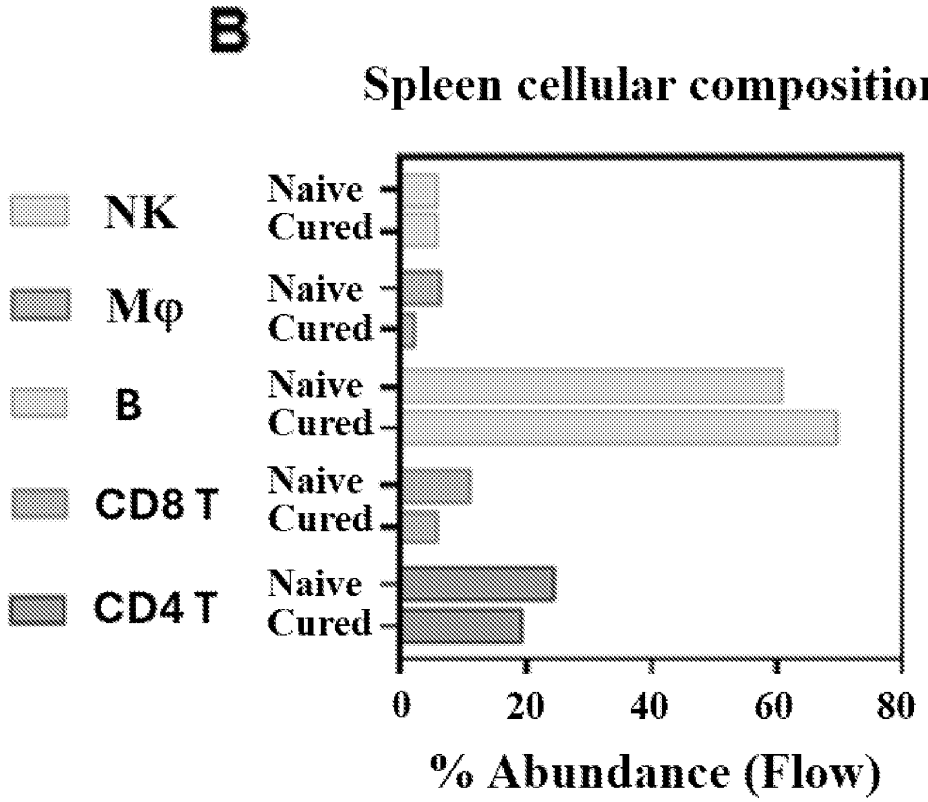

B
Spleen cellular composition
NK
Mφ
B
CD8 T
CD4 T
Naive
Cured
Naive
Cured
Naive
Cured
Naive
Cured
Naive
Cured
0
20
40
60
80
% Abundance (Flow)

Fig. 9 cont.

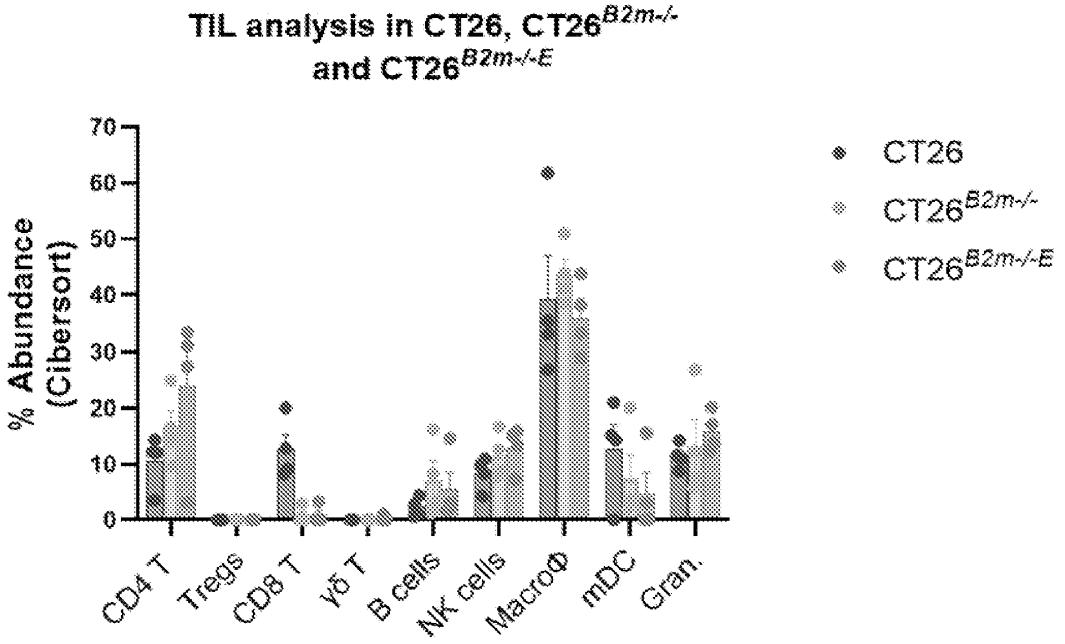

TIL analysis in CT26, CT26B2m-/- and CT26B2m-/-E
% Abundance (Cibersort)
70
60
50
40
30
20
10
0
CD4 T
Tregs
CD8 T
γδ T
B cells
NK cells
MacroΦ
mDC
Gran.
CT26
CT26B2m-/-
CT26B2m-/-E

Fig.10

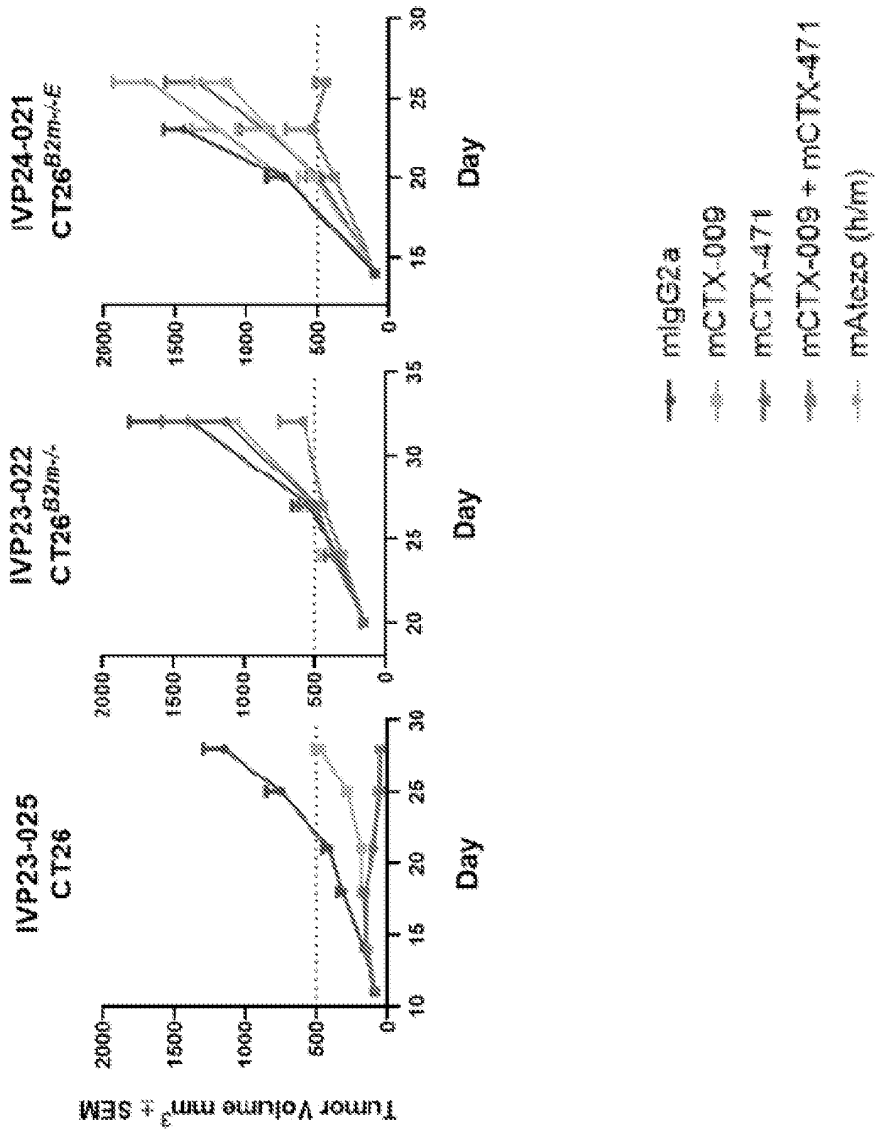

IVP23-025
CT26
IVP23-022
CT26B2m-/-
IVP24-021
CT26B2m-/-E
Tumor Volume mm3 ± SEM
Day
mIgG2a
mCTX-009
mCTX-471
mCTX-009 + mCTX-471
mAtezo (h/m)

Fig. 11

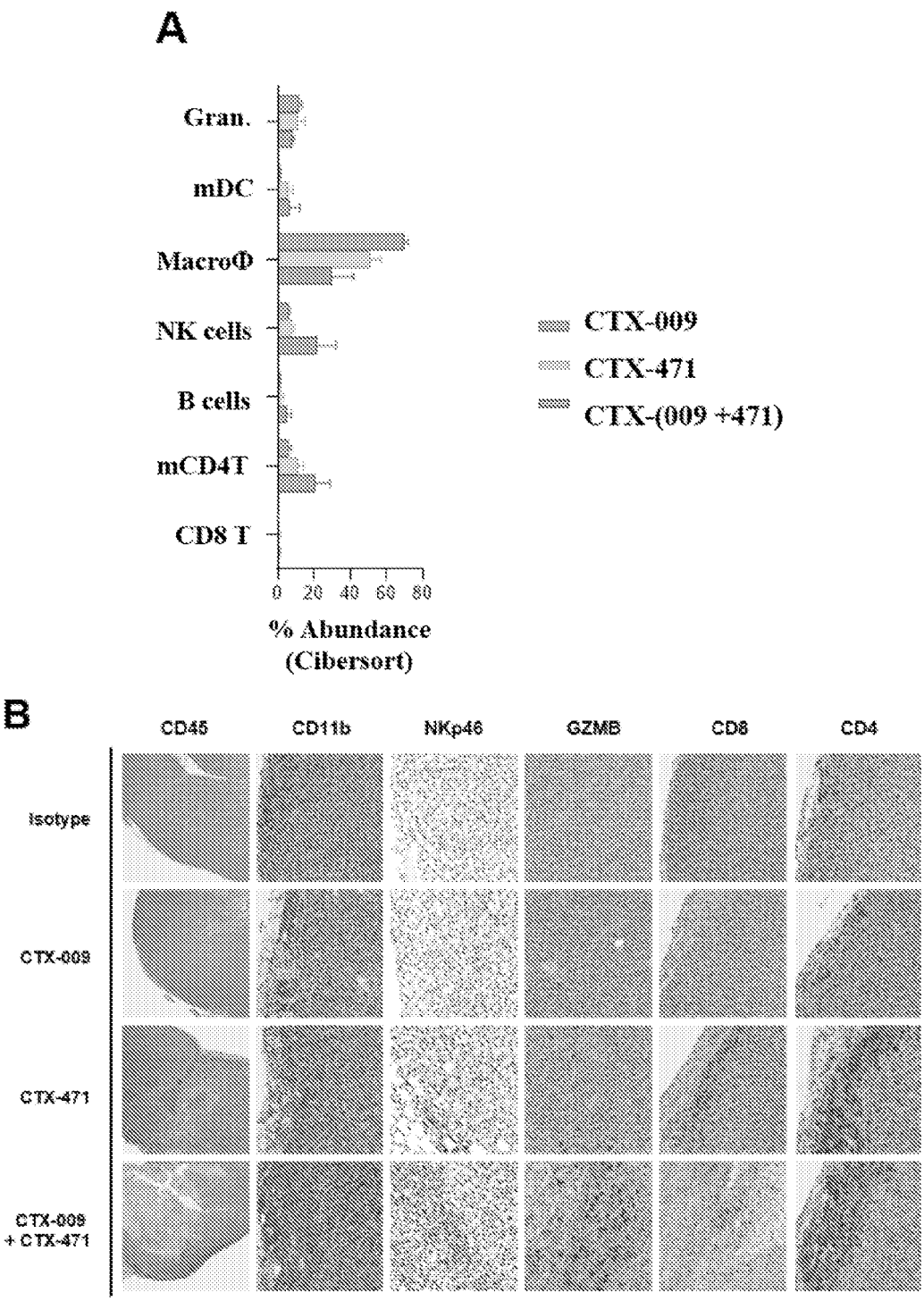
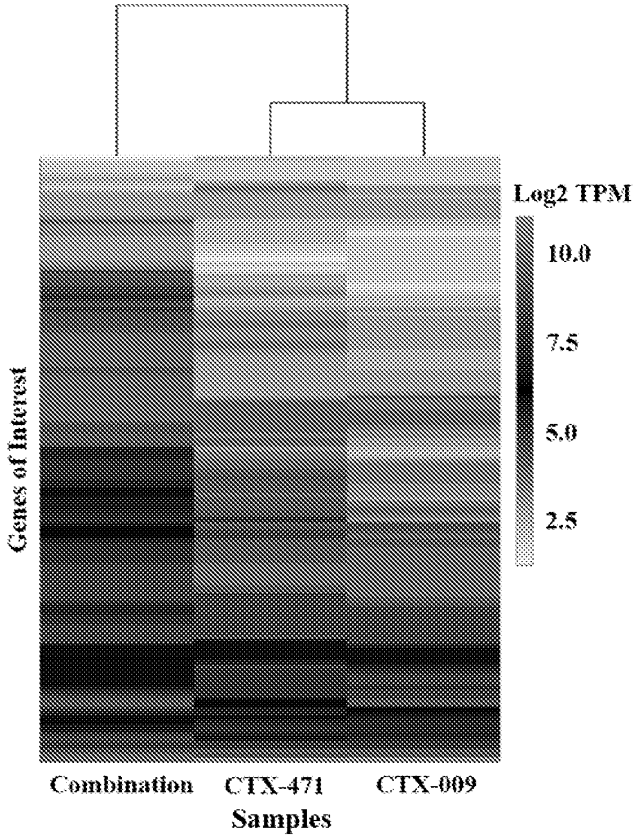

A
Gran.
mDC
MacroΦ
NK cells
B cells
mCD4T
CD8 T
0 20 40 60 80
% Abundance
(Cibersort)
CTX-009
CTX-471
CTX-(009 +471)
B
CD45
CD11b
NKp46
GZMB
CD8
CD4
Isotype
CTX-009
CTX-471
CTX-009
+ CTX-471

Fig.12



GO biological process complete	fold Enrichment	raw P-value	FDR
positive regulation of AIM2 inflammasome complex assembly (GO:0140973)	> 100	2.93E-07	4.75E-05
MDA-5 signaling pathway (GO:0039530)	> 100	4.44E-05	4.55E-03
ISG15-protein conjugation (GO:0032020)	89.74	2.90E-06	3.78E-04
cytolysis in another organism (GO:0051715)	85.46	6.61E-08	1.34E-05
positive regulation of type II interferon pathway (GO:0060335)	74.78	1.31E-07	2.42E-05
immune response to tumor cell (GO:0002418)	74.78	2.64E-04	2.10E-02
positive regulation of pyroptotic inflammatory response (GO:0140639)	74.78	1.31E-07	2.36E-05
MHC class I protein complex assembly (GO:0002397)	59.82	4.38E-04	3.33E-02
interleukin-27-mediated signaling pathway (GO:0070106)	59.82	3.84E-10	9.60E-08

Fig. 13

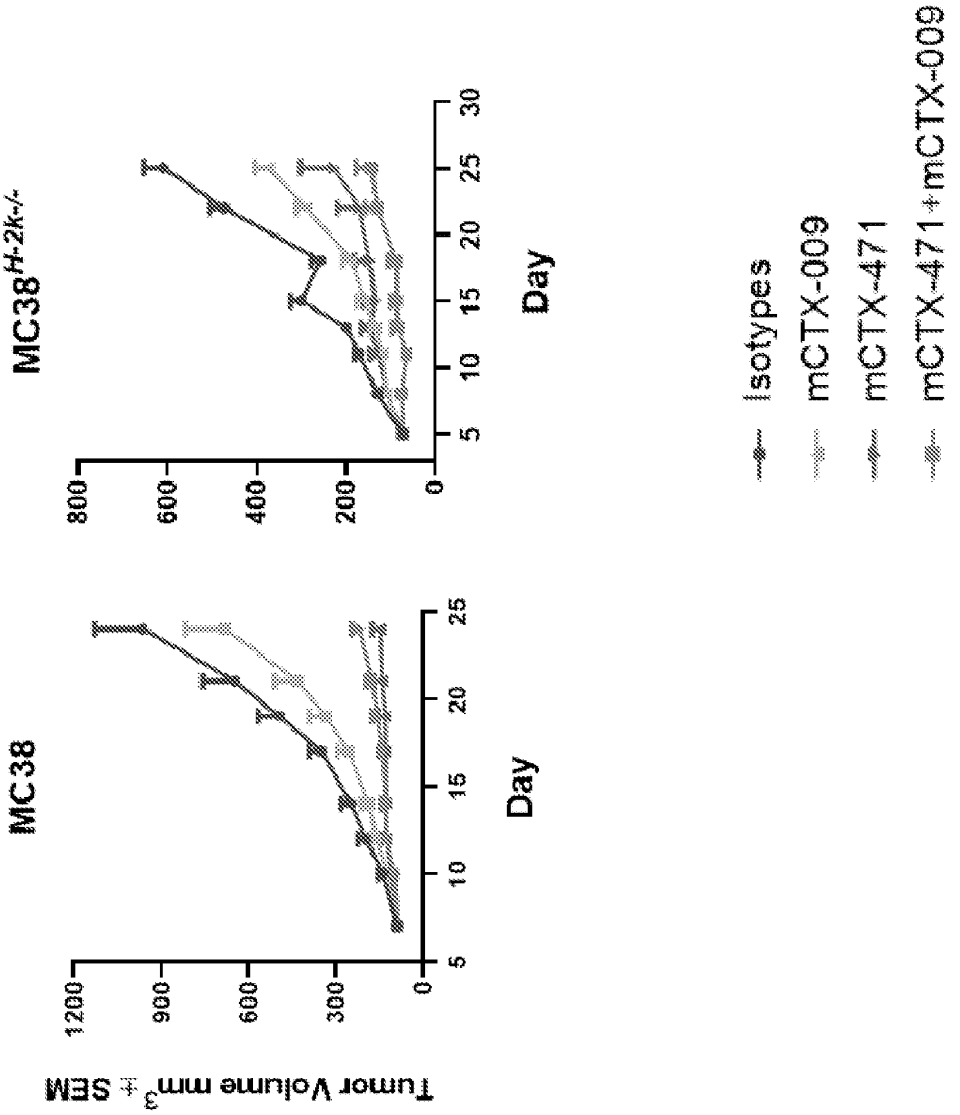
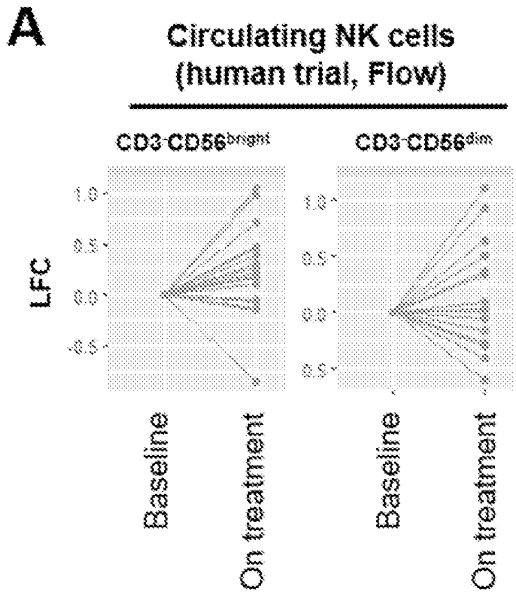

MC38
MC38H-2k-/-
Tumor Volume mm3 ± SEM
Day
Isotypes
mCTX-009
mCTX-471
mCTX-471+mCTX-009

Fig.14


A
Circulating NK cells
(human trial, Flow)
CD3-CD56bright
CD3-CD56dim
LFC
1.0
0.5
0.0
-0.5
Baseline
On treatment
Baseline
On treatment

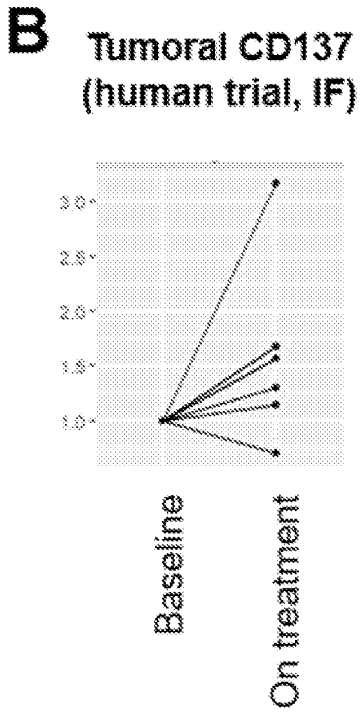

B
Tumoral CD137
(human trial, IF)
3.0
2.5
2.0
1.5
1.0
Baseline
On treatment

Fig.15