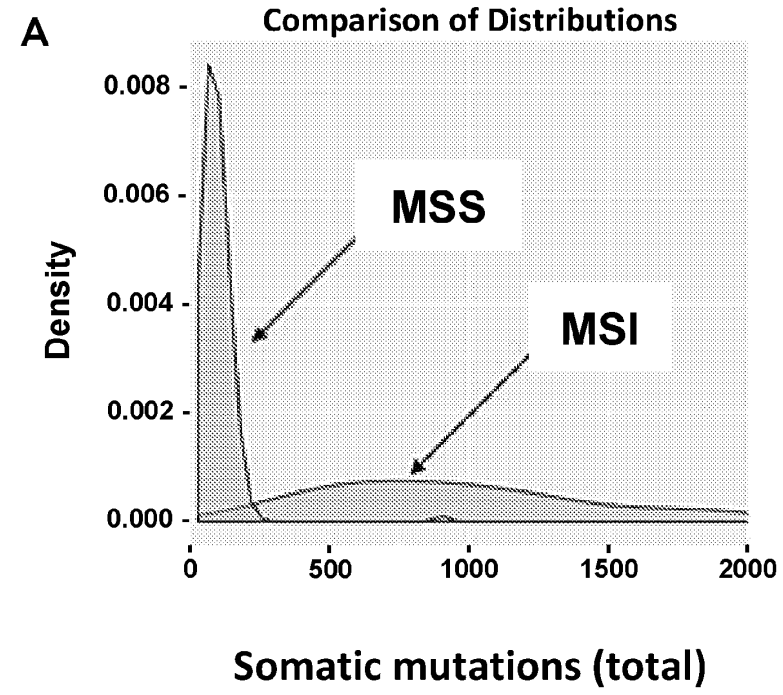

A
Comparison of Distributions
0.008
0.006
0.004
0.002
0.000
Density
MSS
MSI
0
500
1000
1500
2000
Somatic mutations (total)

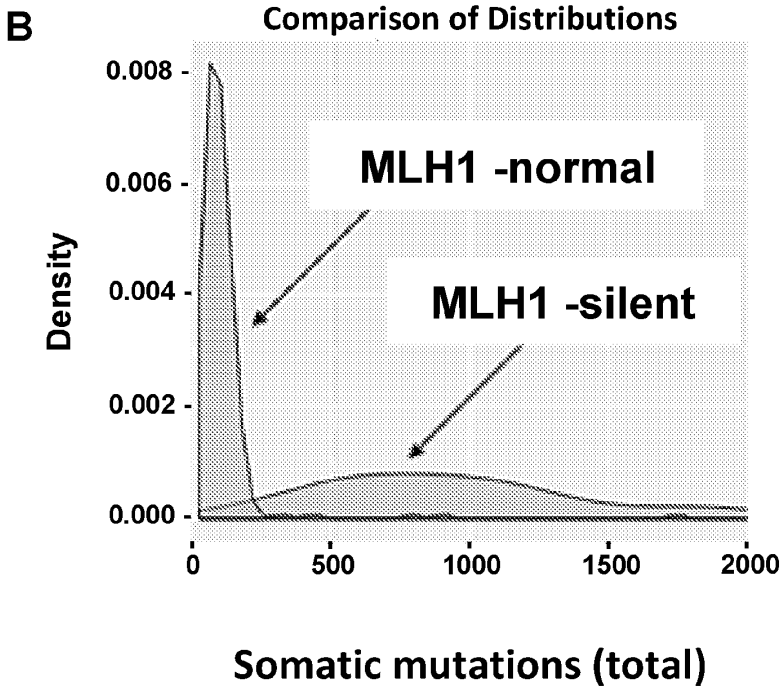

B
Comparison of Distributions
0.008
0.006
0.004
0.002
0.000
Density
MLH1 -normal
MLH1 -silent
0
500
1000
1500
2000
Somatic mutations (total)

Fig. 1

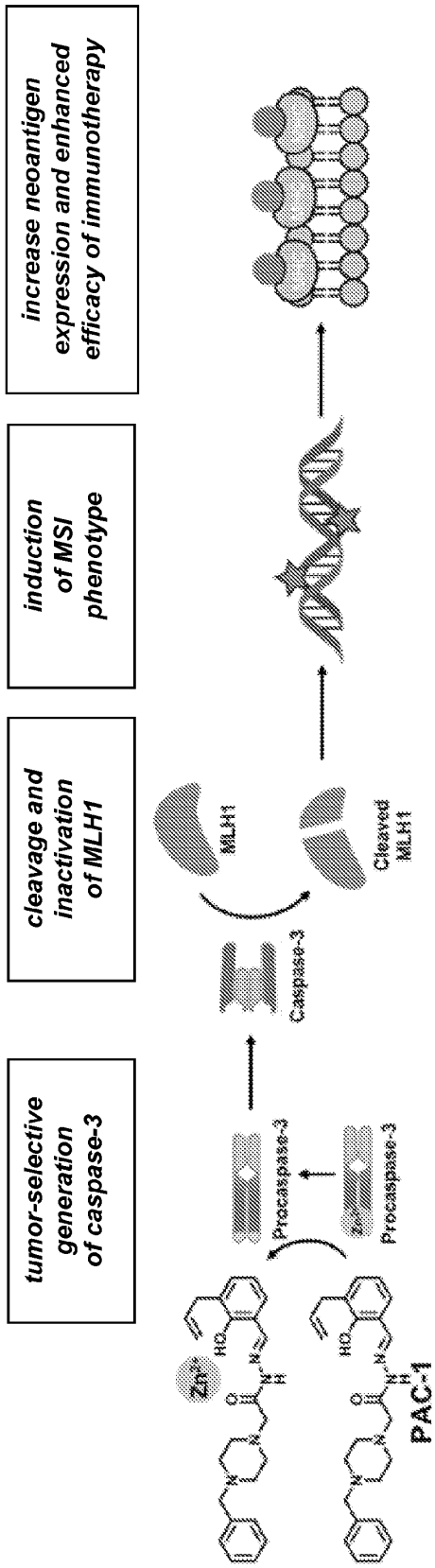

tumor-selective generation of caspase-3
cleavage and inactivation of MLH1
induction of MSI phenotype
increase neoantigen expression and enhanced efficacy of immunotherapy
Procaspase-3
Procaspase-3
Caspase-3
MLH1
Cleaved MLH1
PAC-1

Fig. 2

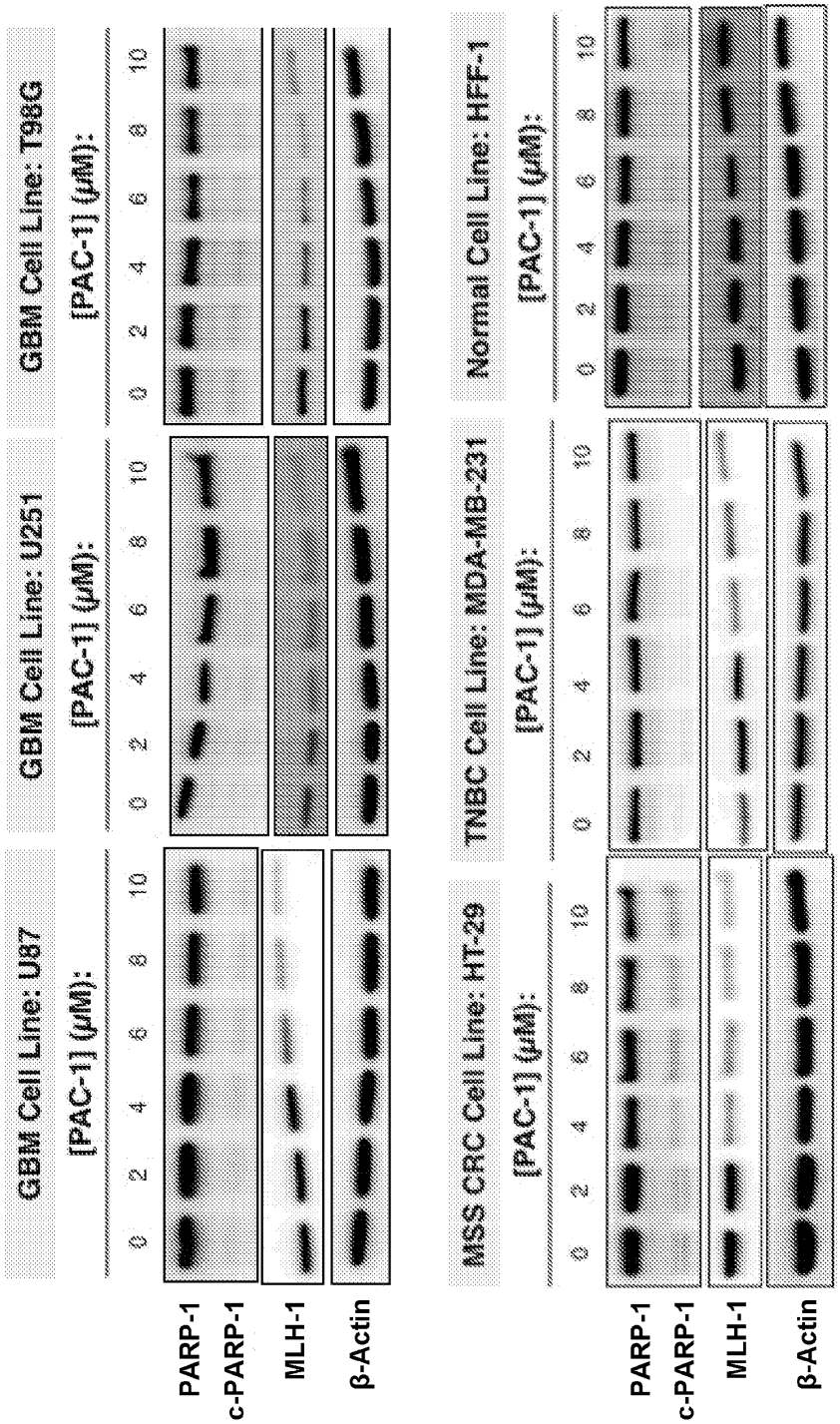

GBM Cell Line: U87
[PAC-1] (μM):
0 2 4 6 8 10
GBM Cell Line: U251
[PAC-1] (μM):
0 2 4 6 8 10
GBM Cell Line: T98G
[PAC-1] (μM):
0 2 4 6 8 10
PARP-1
c-PARP-1
MLH-1
β-Actin
MSS CRC Cell Line: HT-29
[PAC-1] (μM):
0 2 4 6 8 10
TNBC Cell Line: MDA-MB-231
[PAC-1] (μM):
0 2 4 6 8 10
Normal Cell Line: HFF-1
[PAC-1] (μM):
0 2 4 6 8 10
PARP-1
c-PARP-1
MLH-1
β-Actin

Fig. 3

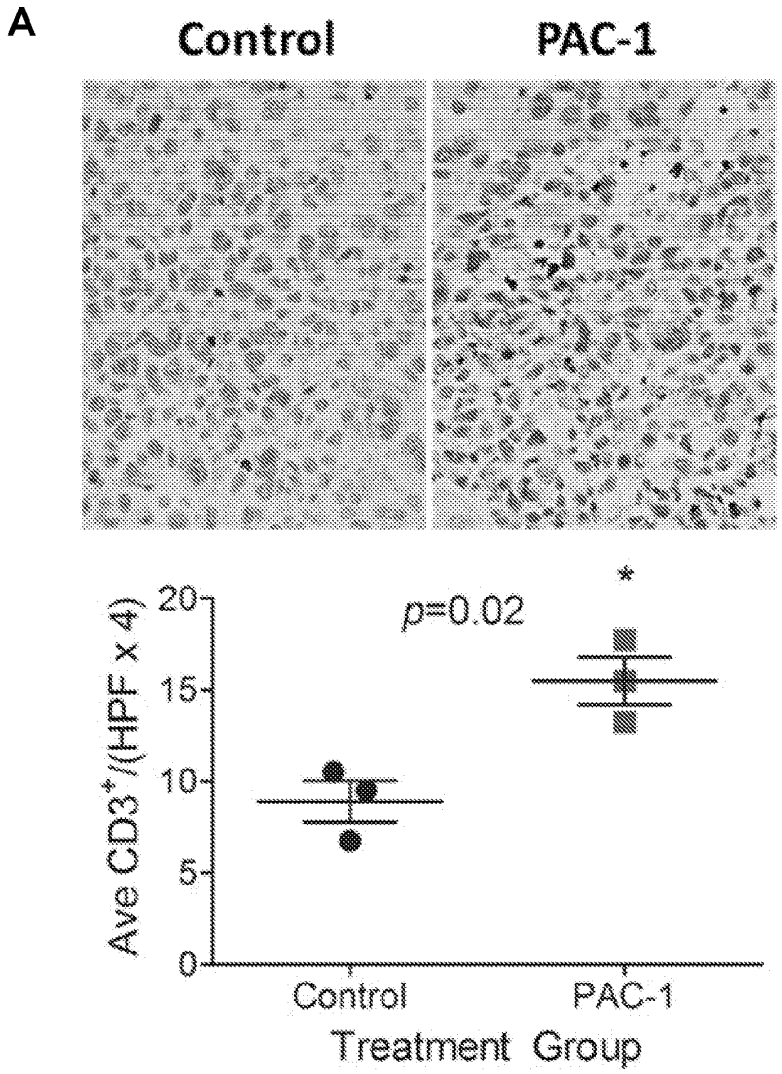

A
Control
PAC-1
p=0.02
*
Ave CD3+/(HPF x 4)
20
15
10
5
0
Control
PAC-1
Treatment Group

Fig. 4

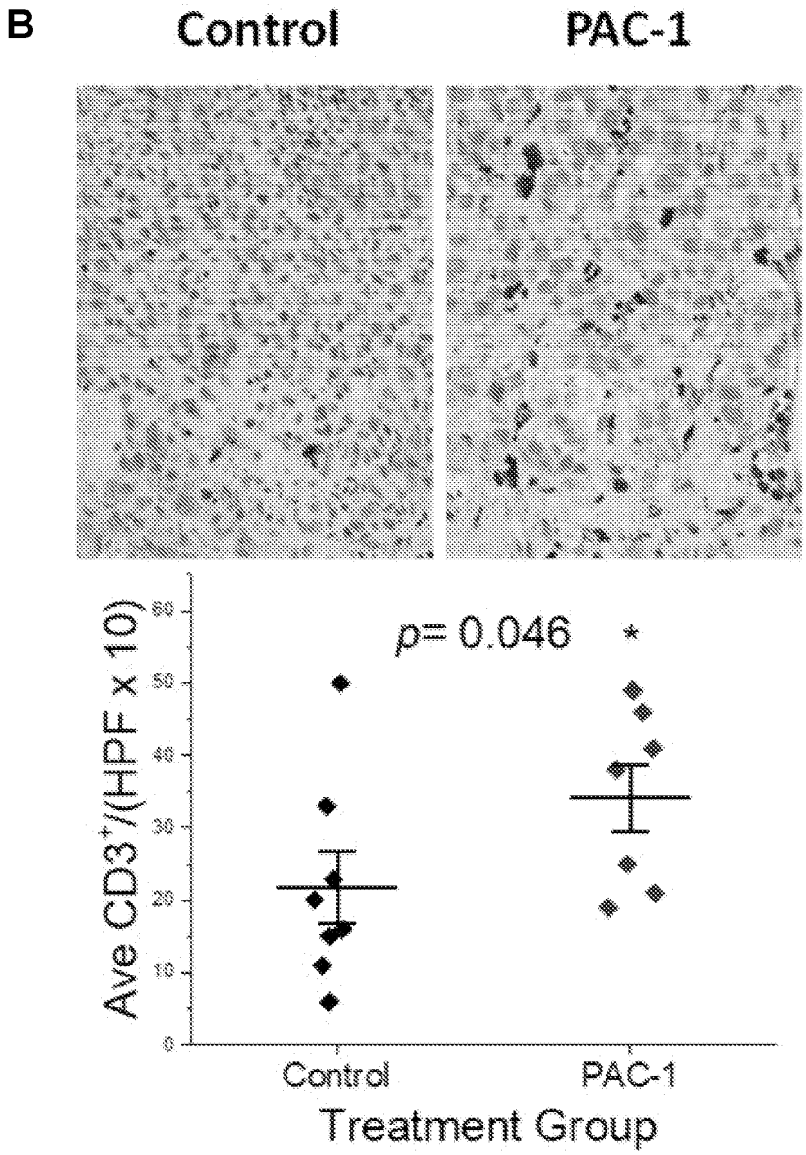

B
Control
PAC-1
p= 0.046
*
Ave CD3+/(HPF x 10)
0
10
20
30
40
50
60
Control
PAC-1
Treatment Group

Fig. 4 (cont.)

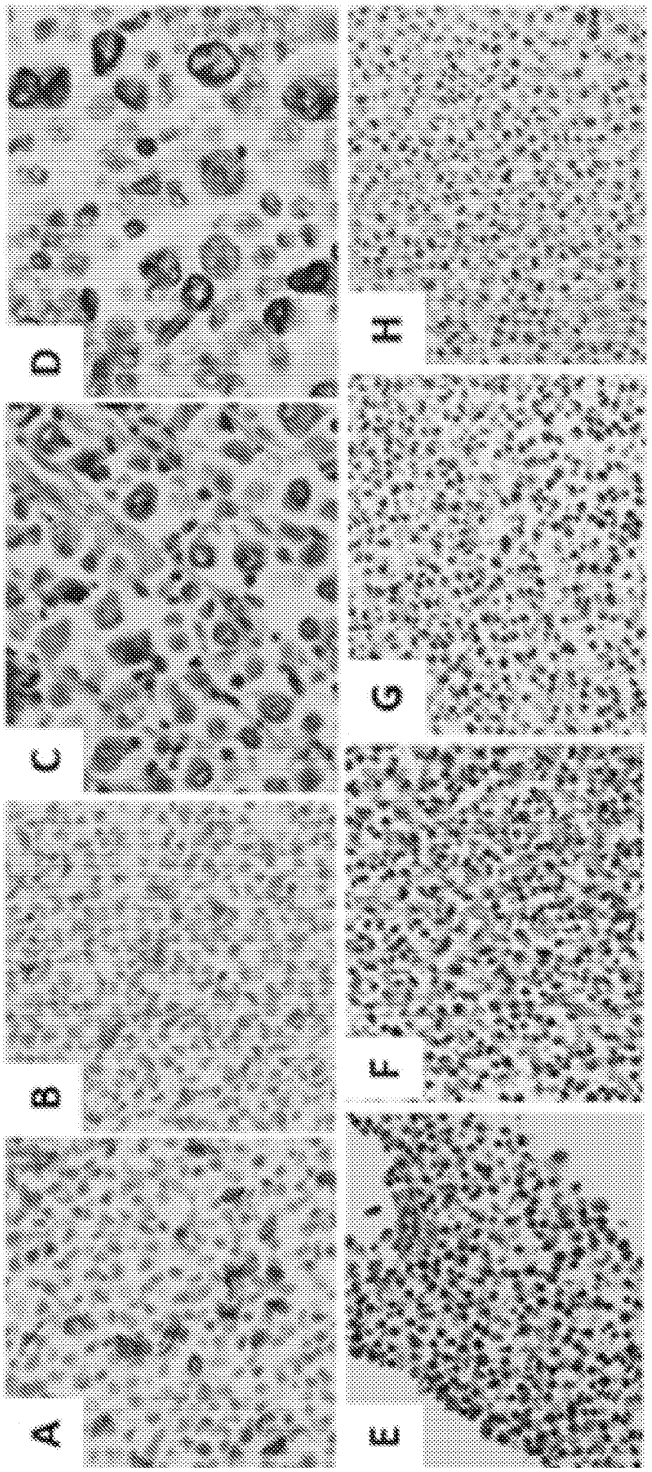


Fig. 5

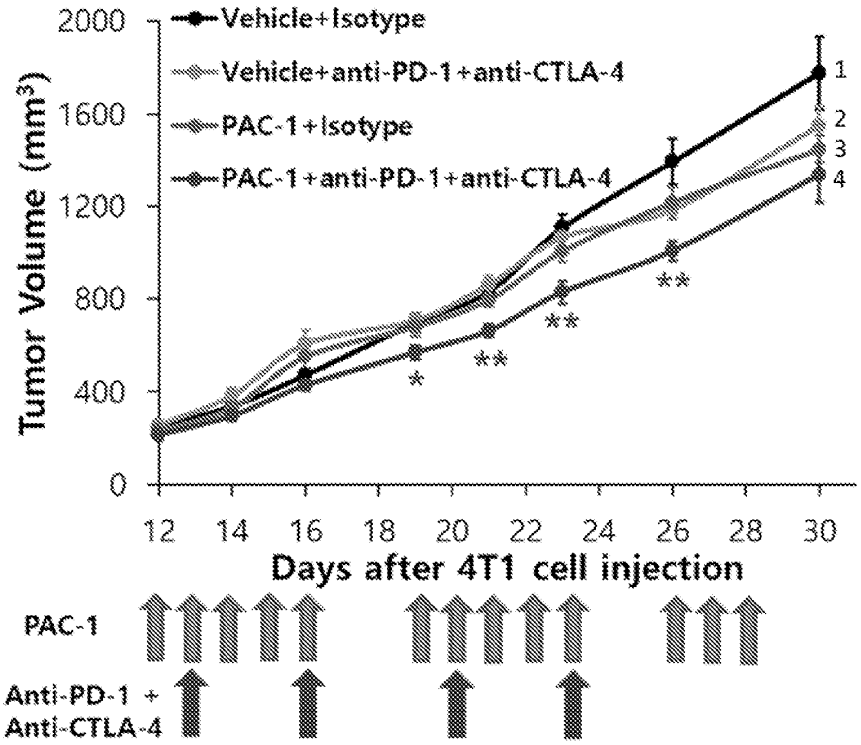
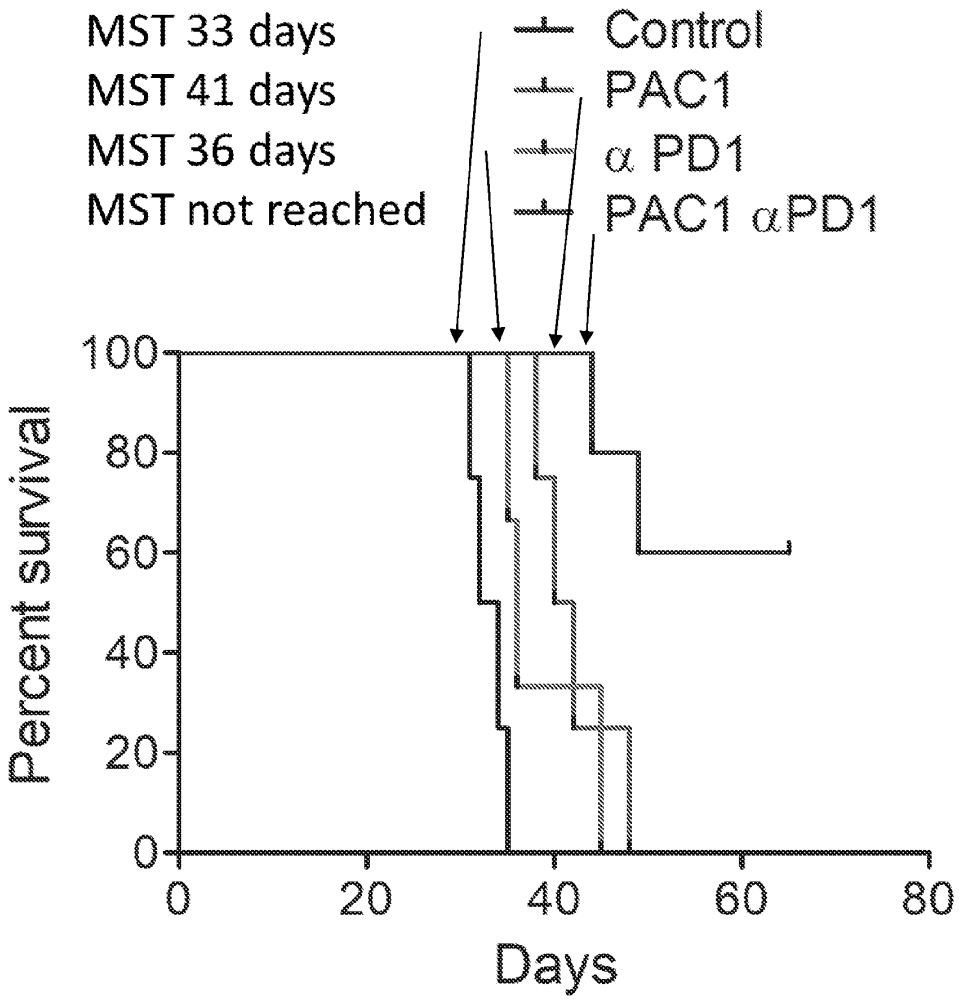

Vehicle+Isotype
Vehicle+anti-PD-1+anti-CTLA-4
PAC-1+Isotype
PAC-1+anti-PD-1+anti-CTLA-4
Tumor Volume (mm³)
2000
1600
1200
800
400
0
12
14
16
18
20
22
24
26
28
30
Days after 4T1 cell injection
1
2
3
4
*
**
**
**
PAC-1
Anti-PD-1 +
Anti-CTLA-4

Fig. 6



Day 1: K7M2 establish
Days 3-21: PAC-1 125 mg/kg PO
Days 14 & 18: 200µg αPD1 IP

Fig. 7

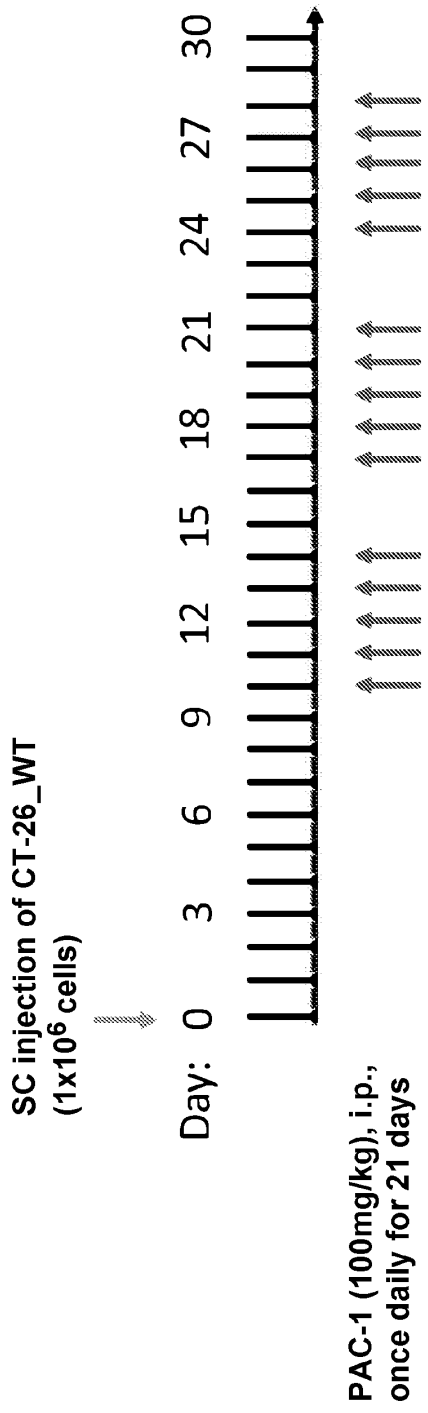

SC injection of CT-26_WT
(1x10^6 cells)
Day: 0 3 6 9 12 15 18 21 24 27 30
PAC-1 (100mg/kg), i.p.,
once daily for 21 days
Anti-PD1 antibody
(10mg/kg), i.p., 4 doses
Anti-PD1 antibody
(10mg/kg), i.p., 2 doses

Fig. 8

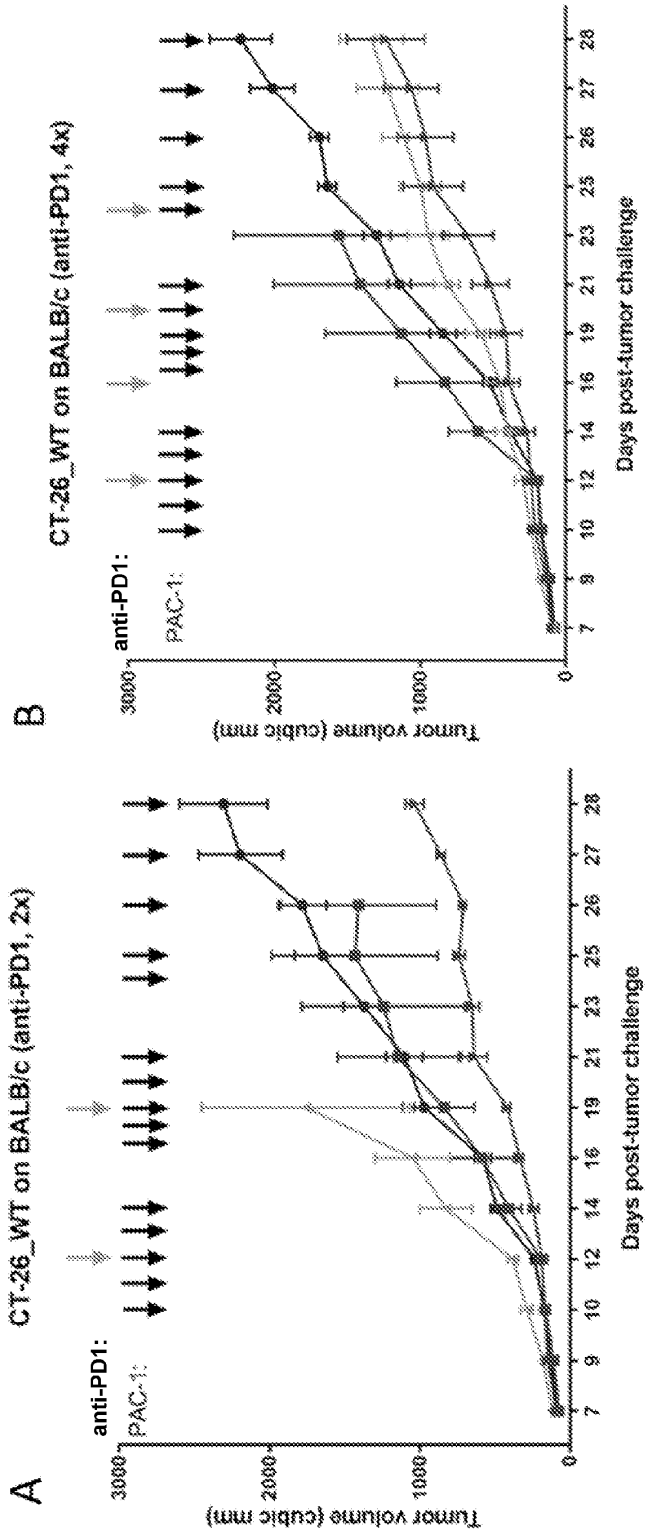
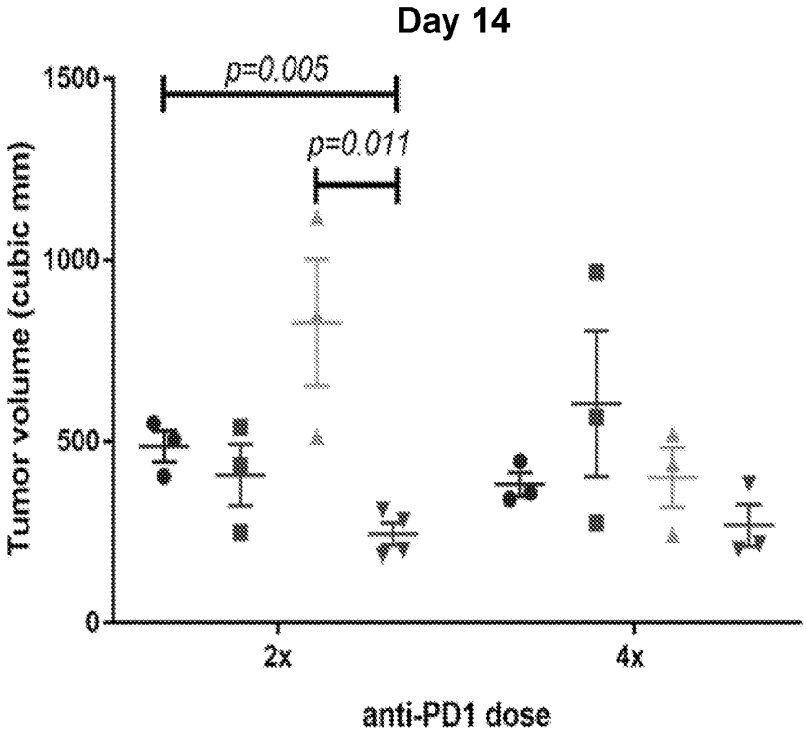

A
CT-26_WT on BALB/c (anti-PD1, 2x)
anti-PD1:
PAC-1:
Tumor volume (cubic mm)
3000
2000
1000
0
7 9 10 12 14 16 19 21 23 25 26 27 28
Days post-tumor challenge
B
CT-26_WT on BALB/c (anti-PD1, 4x)
anti-PD1:
PAC-1:
Tumor volume (cubic mm)
3000
2000
1000
0
7 9 10 12 14 16 19 21 23 25 26 27 28
Days post-tumor challenge

Fig. 9


Day 14
p=0.005
p=0.011
1500
1000
500
0
Tumor volume (cubic mm)
2x
4x
anti-PD1 dose

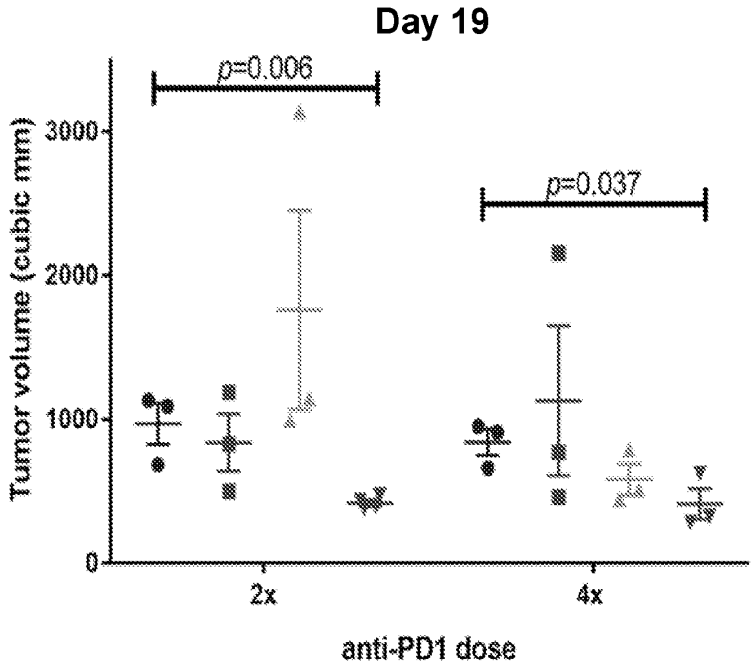

Day 19
p=0.006
p=0.037
3000
2000
1000
0
Tumor volume (cubic mm)
2x
4x
anti-PD1 dose

Fig. 10

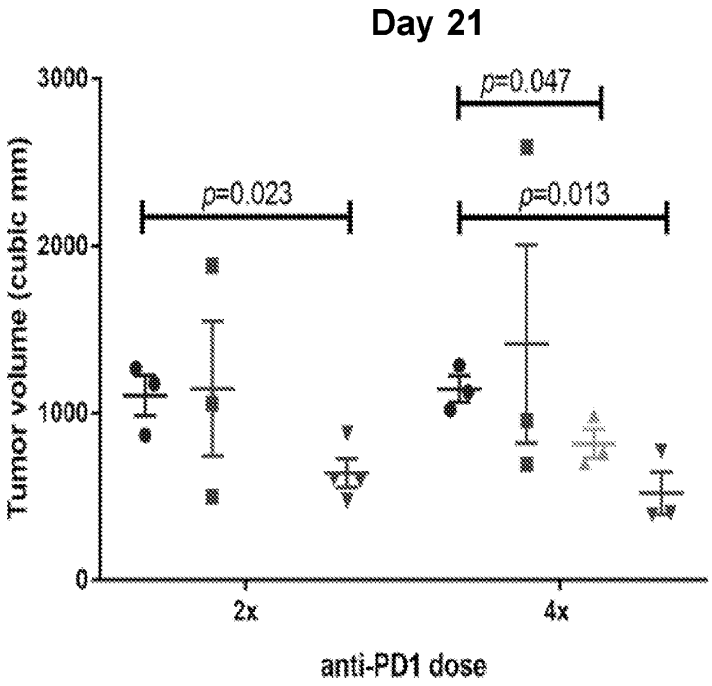


Fig. 10 (cont.)

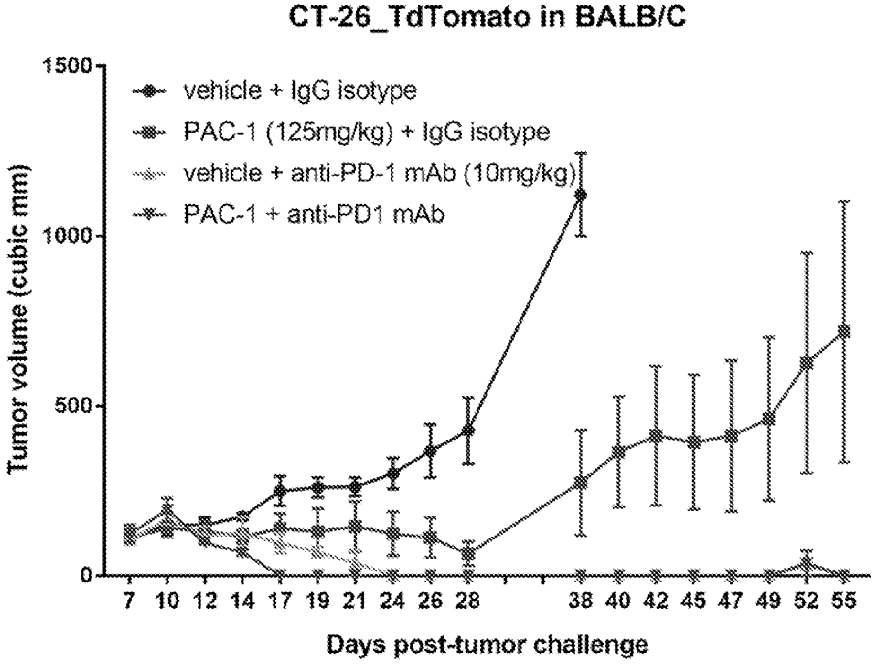


Fig. 11

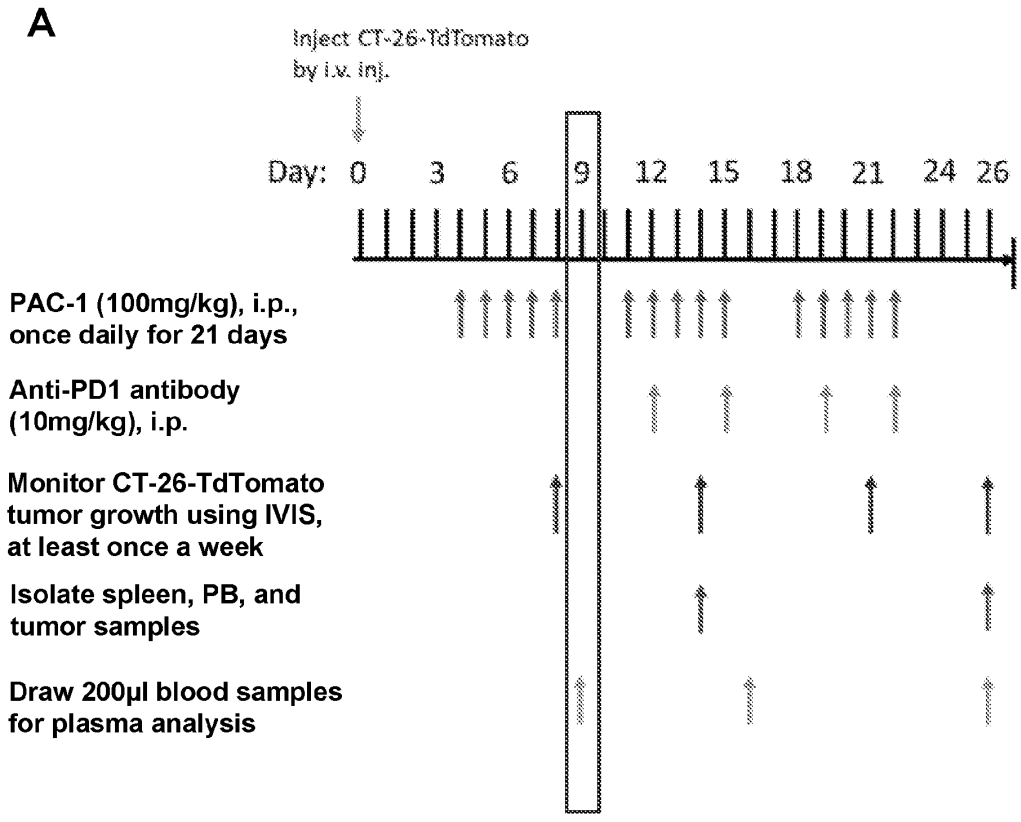

A
Inject CT-26-TdTomato
by i.v. inj.
Day: 0 3 6 9 12 15 18 21 24 26
PAC-1 (100mg/kg), i.p.,
once daily for 21 days
Anti-PD1 antibody
(10mg/kg), i.p.
Monitor CT-26-TdTomato
tumor growth using IVIS,
at least once a week
Isolate spleen, PB, and
tumor samples
Draw 200µl blood samples
for plasma analysis

Fig. 12

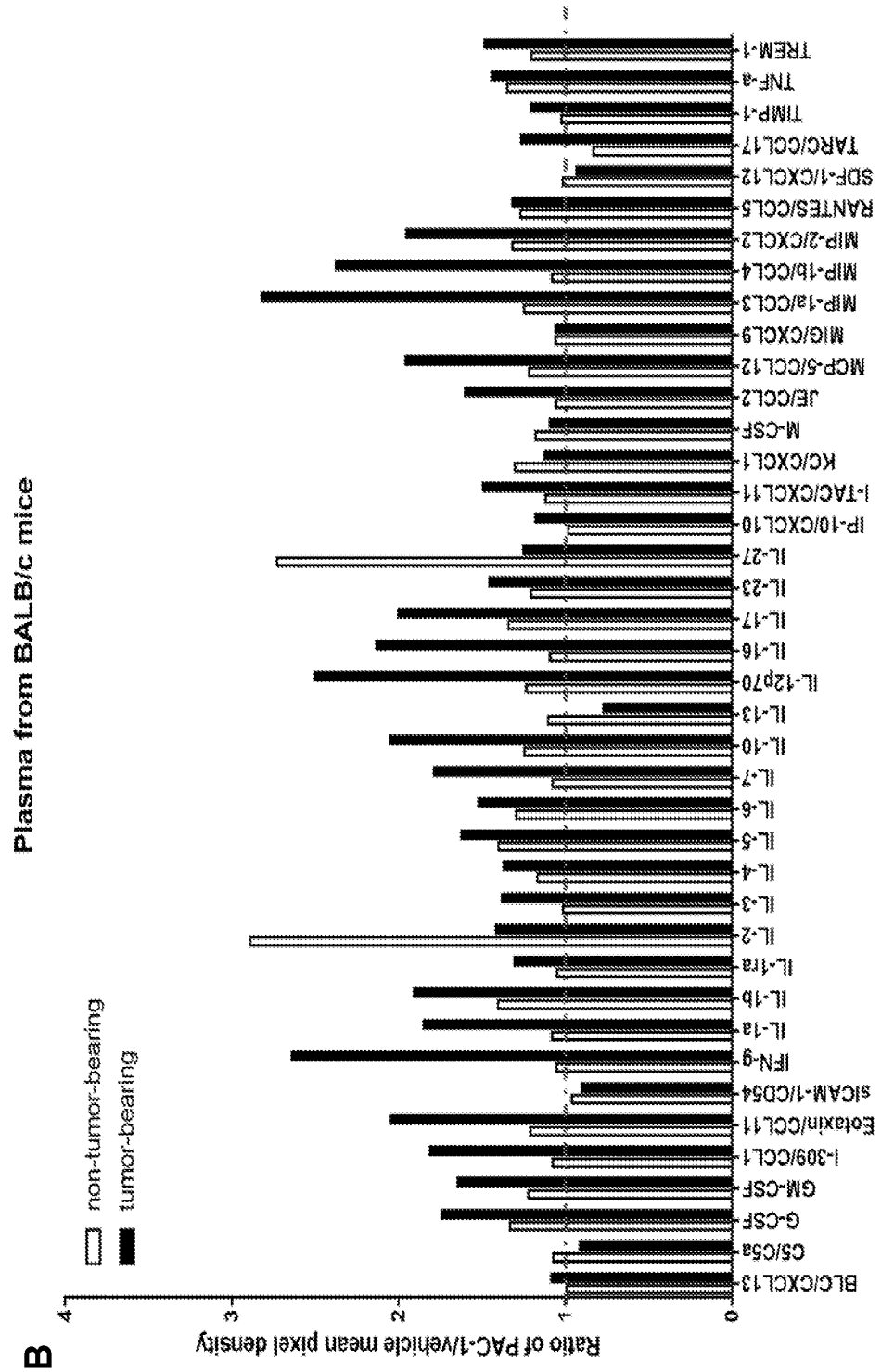

B
Plasma from BALB/c mice
non-tumor-bearing
tumor-bearing
Ratio of PAC-1/vehicle mean pixel density
0
1
2
3
4
BLC/CXCL13
C5/C5a
G-CSF
GM-CSF
I-309/CCL1
Eotaxin/CCL11
sICAM-1/CD54
IFN-g
IL-1a
IL-1b
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-10
IL-13
IL-12p70
IL-16
IL-17
IL-23
IL-27
IP-10/CXCL10
I-TAC/CXCL11
KC/CXCL1
M-CSF
JE/CCL2
MCP-5/CCL12
MIG/CXCL9
MIP-1a/CCL3
MIP-1b/CCL4
MIP-2/CXCL2
RANTES/CCL5
SDF-1/CXCL12
TARC/CCL17
TIMP-1
TNF-a
TREM-1

Fig. 12 (cont.)

Lungs

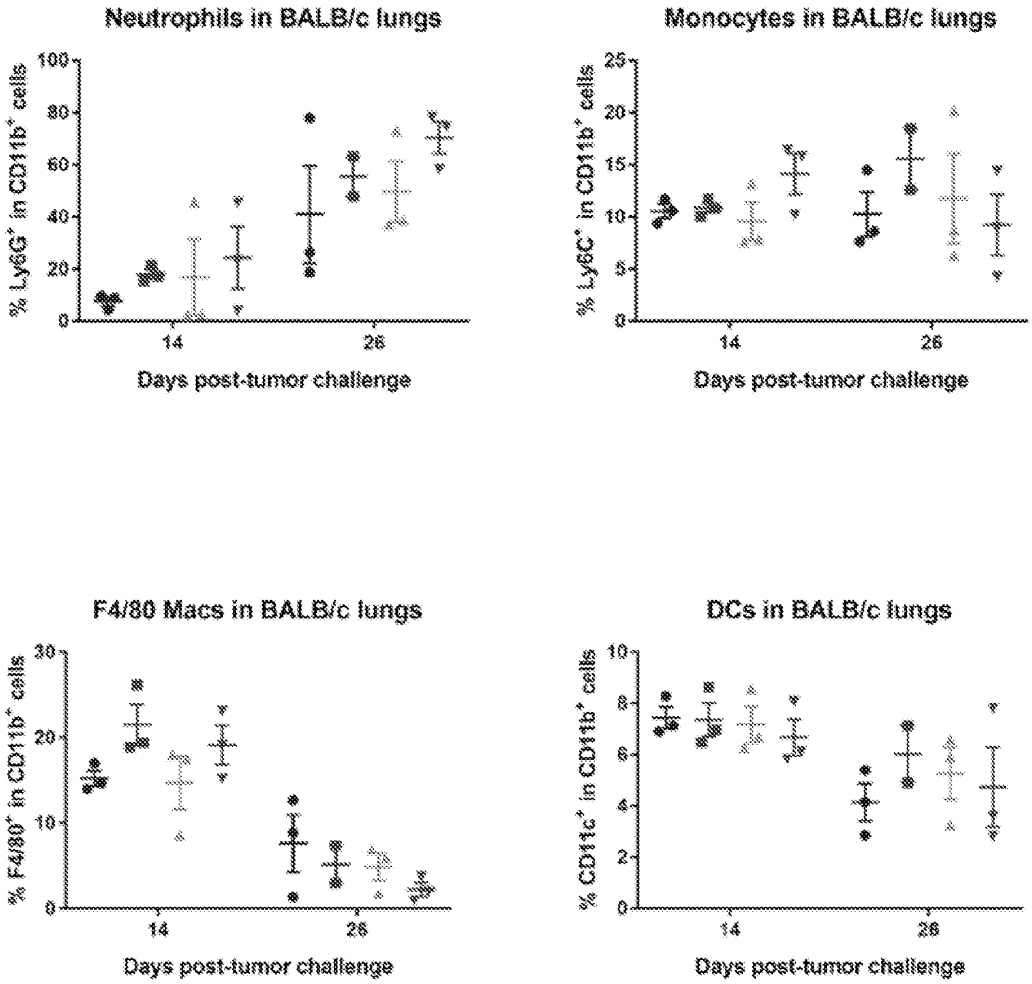

Neutrophils in BALB/c lungs
% Ly6G+ in CD11b+ cells
Days post-tumor challenge
Monocytes in BALB/c lungs
% Ly6C+ in CD11b+ cells
Days post-tumor challenge
F4/80 Macs in BALB/c lungs
% F4/80+ in CD11b+ cells
Days post-tumor challenge
DCs in BALB/c lungs
% CD11c+ in CD11b+ cells
Days post-tumor challenge

Fig. 13

PBMC

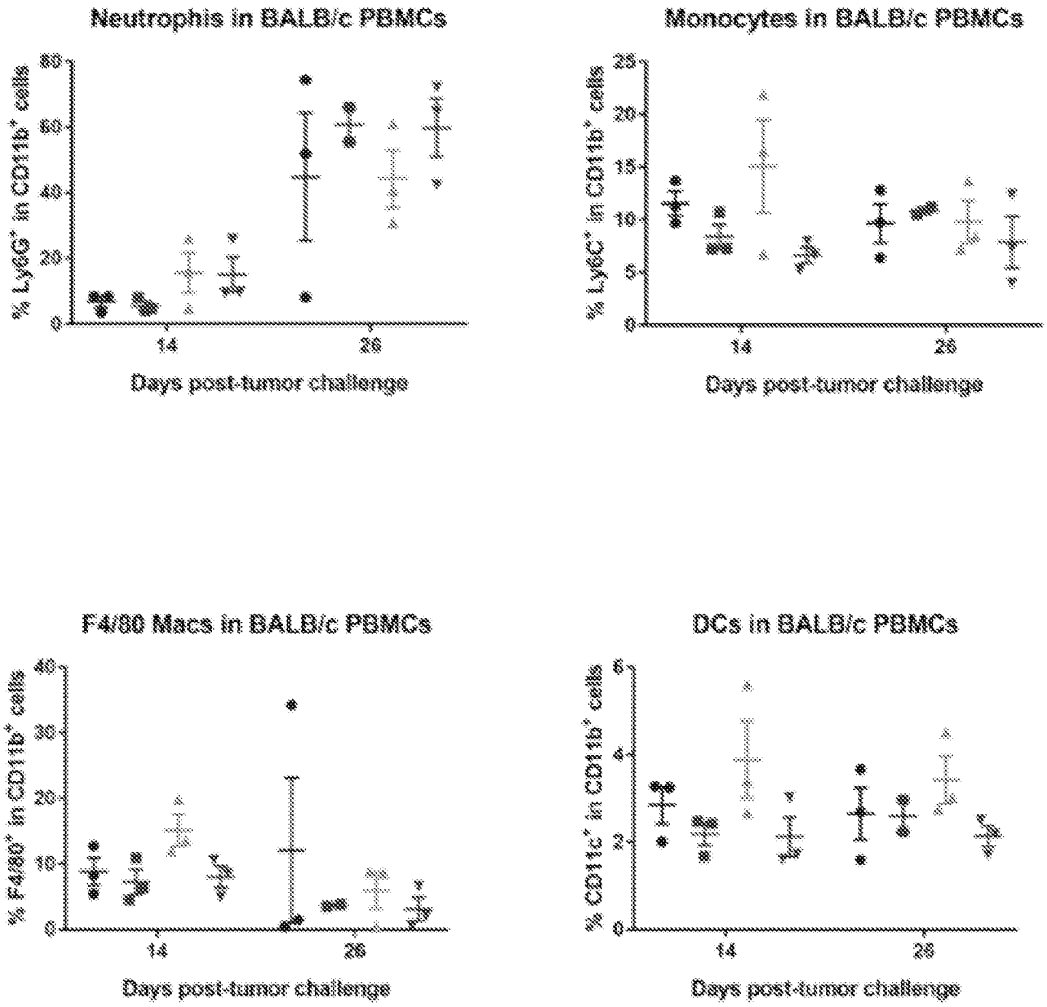


Fig. 13 (cont.)

Spleen

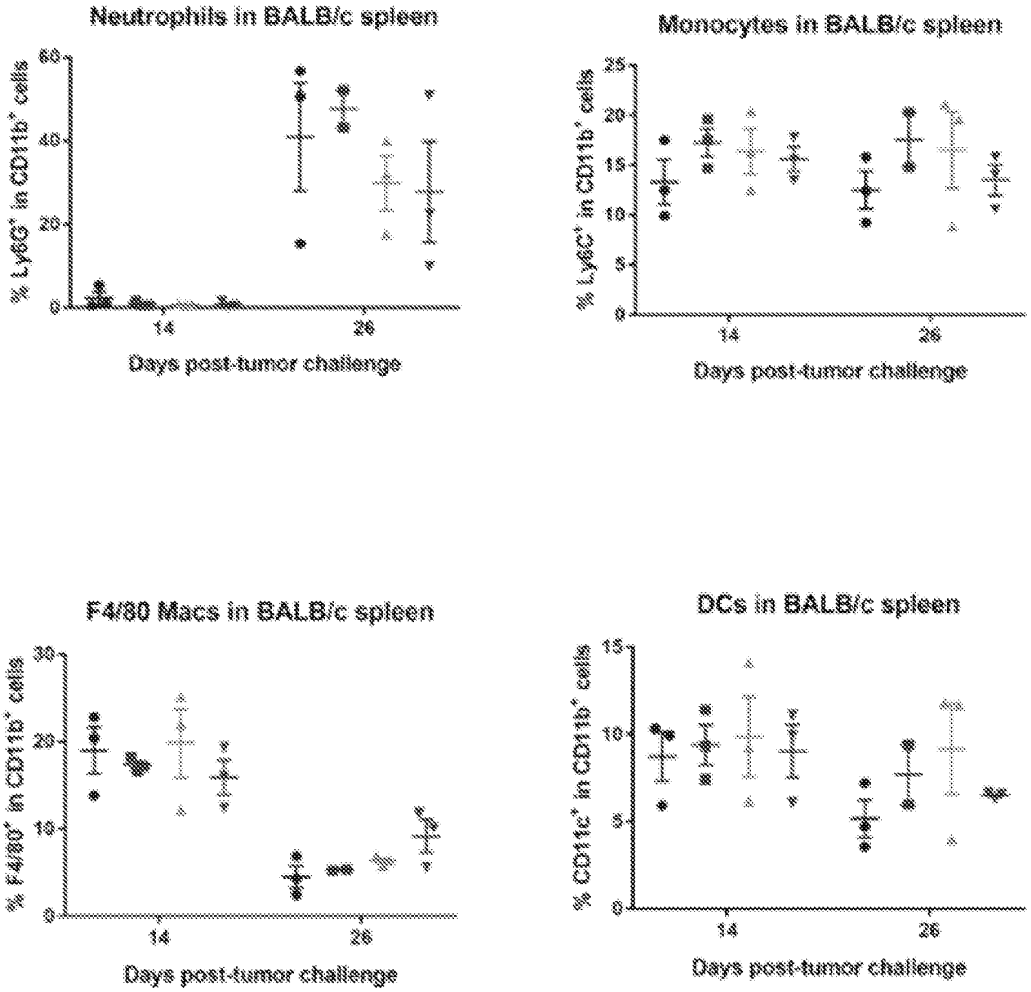


Fig. 13 (cont.)

Lungs

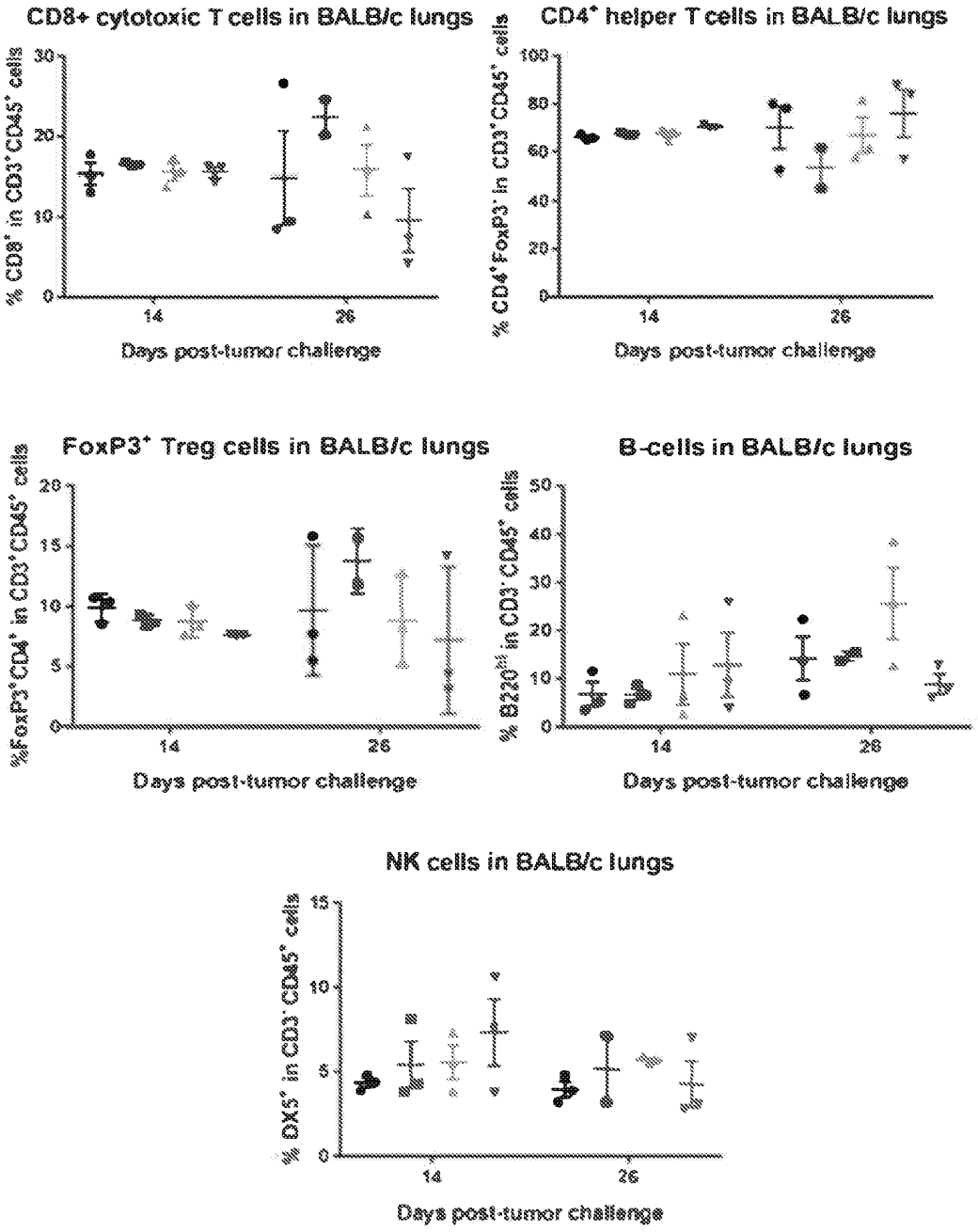

CD8+ cytotoxic T cells in BALB/c lungs
% CD8+ in CD3+CD45+ cells
Days post-tumor challenge
CD4+ helper T cells in BALB/c lungs
% CD4+FoxP3- in CD3+CD45+ cells
Days post-tumor challenge
FoxP3+ Treg cells in BALB/c lungs
%FoxP3+CD4+ in CD3+CD45+ cells
Days post-tumor challenge
B-cells in BALB/c lungs
% B220hi in CD3-CD45+ cells
Days post-tumor challenge
NK cells in BALB/c lungs
% DX5+ in CD3-CD45+ cells
Days post-tumor challenge

Fig. 14

PBMC

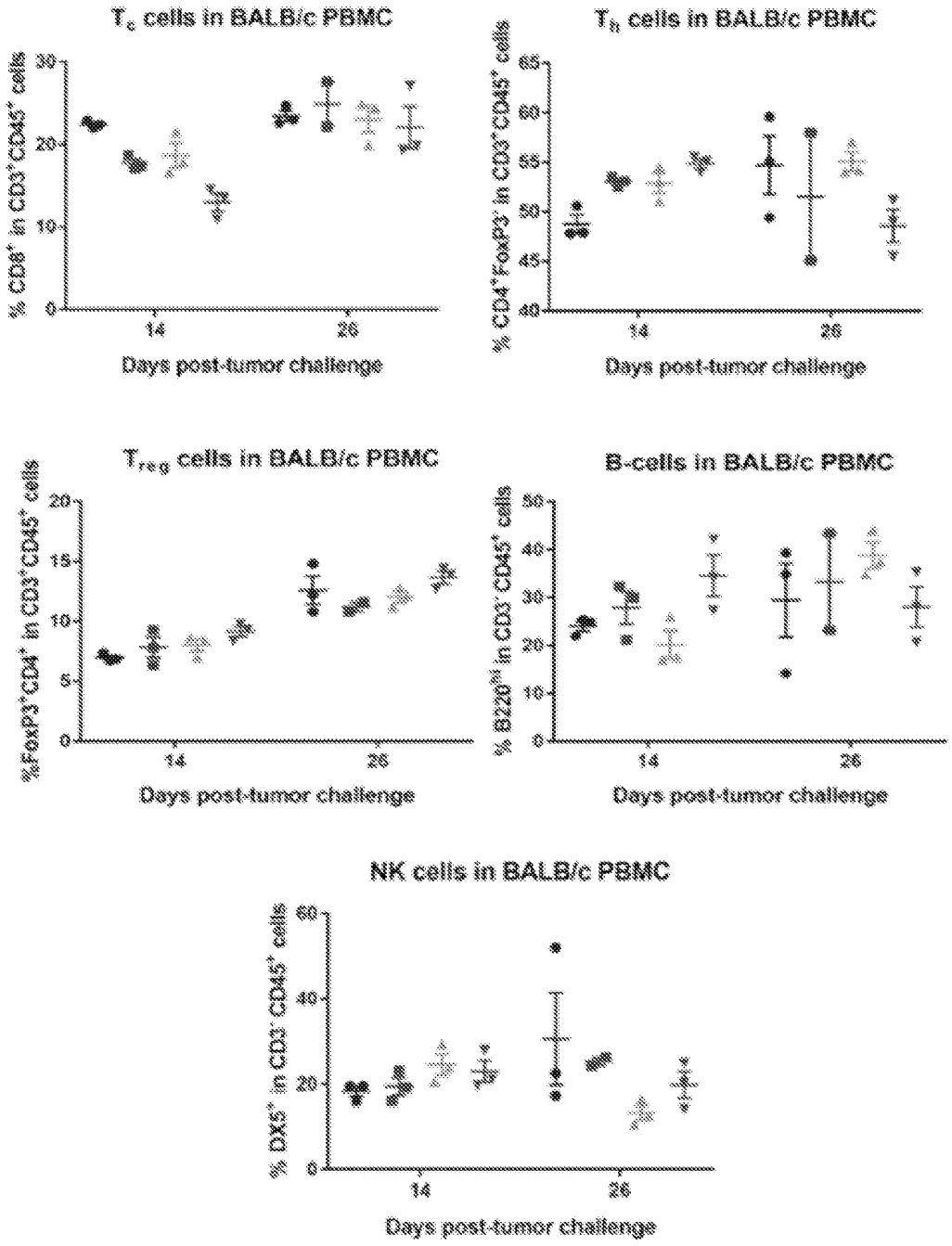


Fig. 14 (cont.)

Spleen

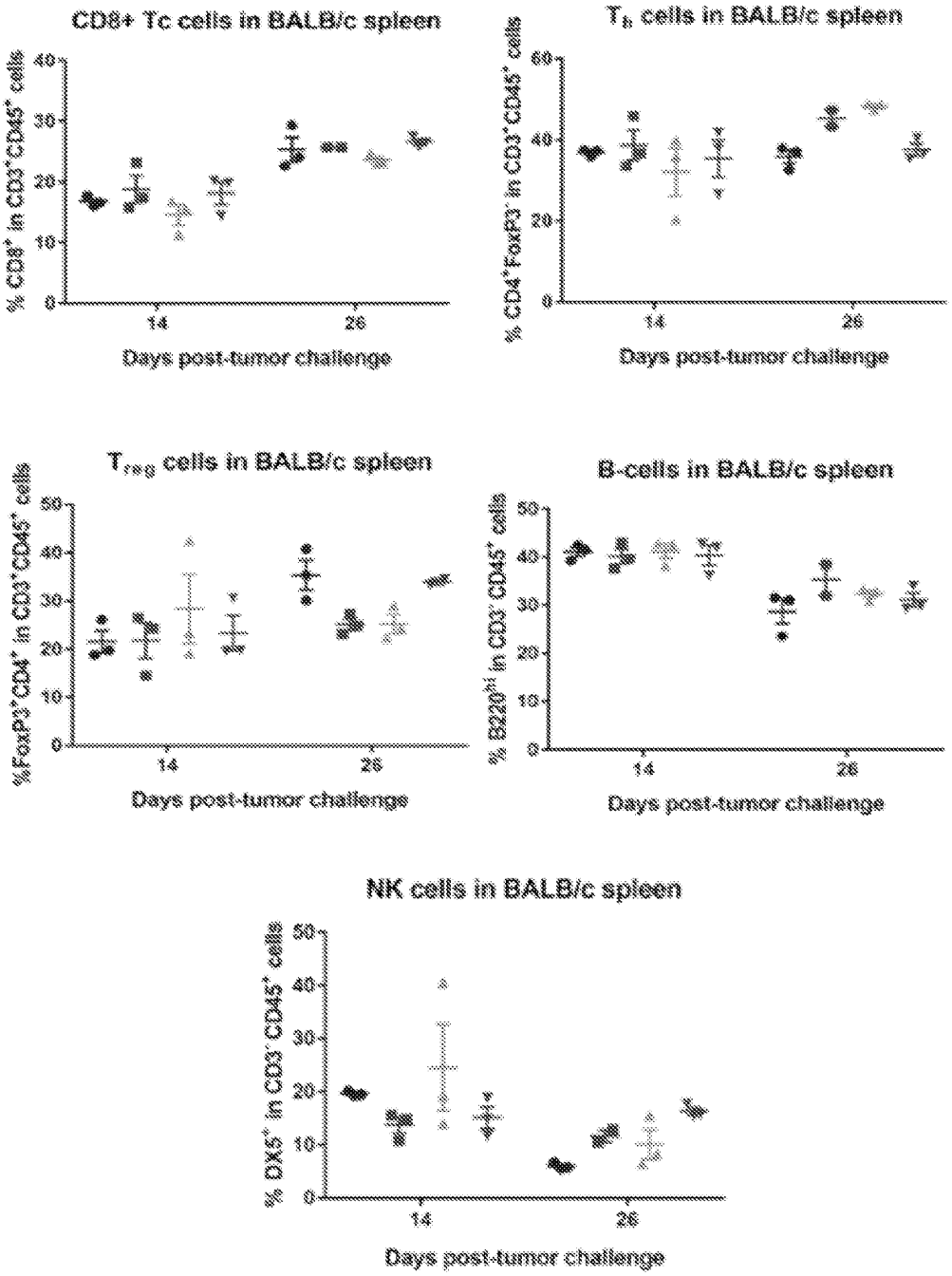


Fig. 14 (cont.)

Lungs

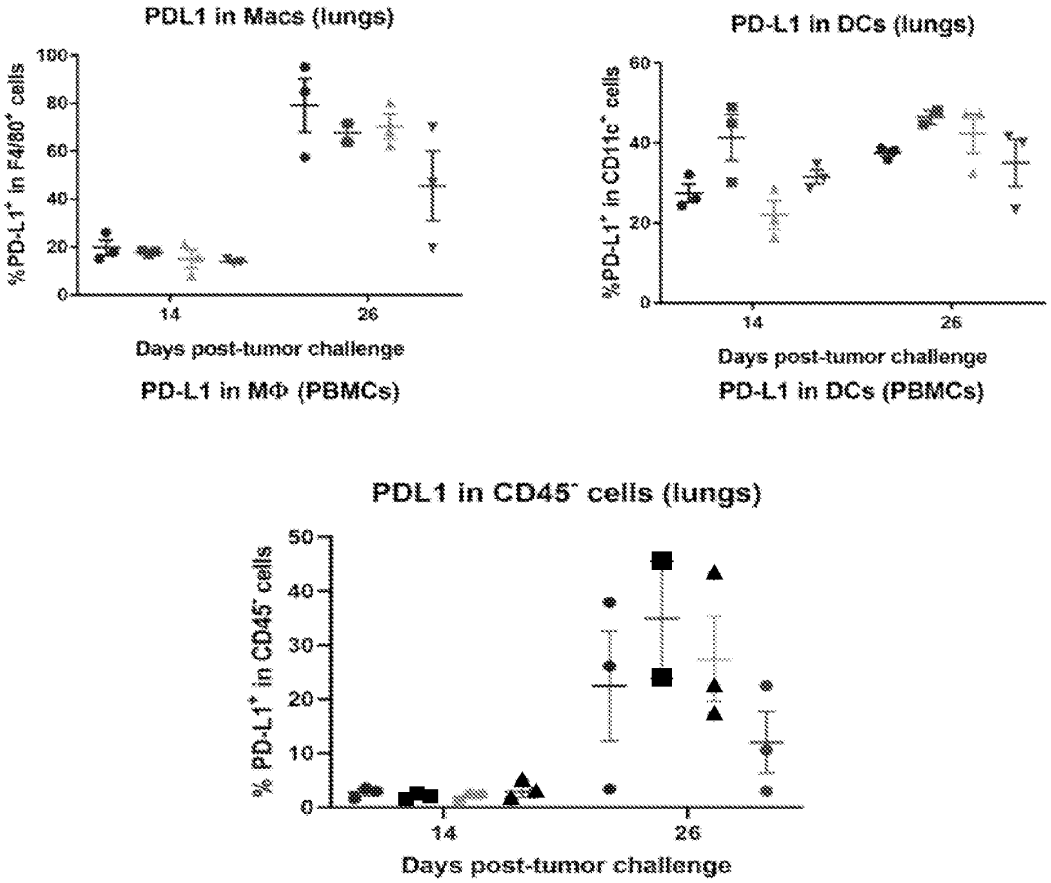
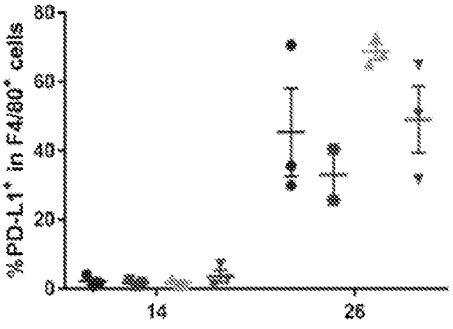
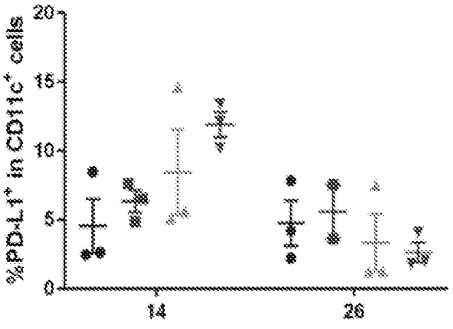

PDL1 in Macs (lungs)
% PD-L1+ in F4/80+ cells
100
80
60
40
20
0
14
26
Days post-tumor challenge
PD-L1 in MΦ (PBMCs)
PD-L1 in DCs (lungs)
%PD-L1+ in CD11c+ cells
60
40
20
0
14
26
Days post-tumor challenge
PD-L1 in DCs (PBMCs)
PDL1 in CD45- cells (lungs)
% PD-L1+ in CD45- cells
50
40
30
20
10
0
14
26
Days post-tumor challenge

Fig. 15

PBMC

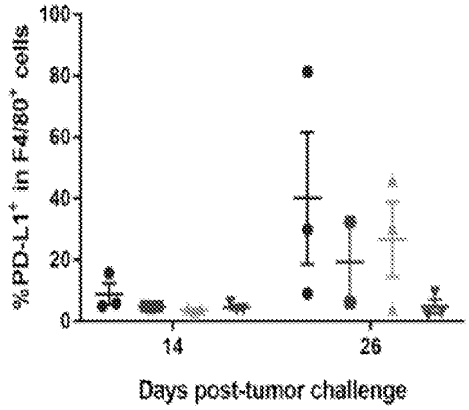


PDL1 in MΦ (spleen)



PD-L1 in DCs (spleen)

Spleen



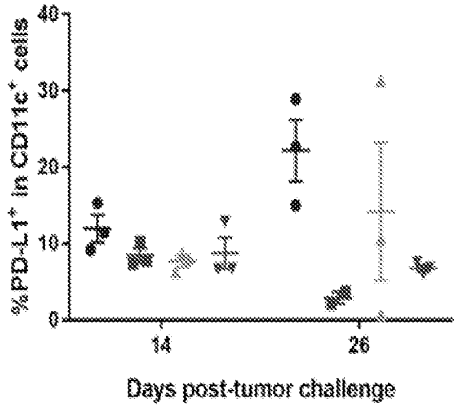


Fig. 15 (cont.)

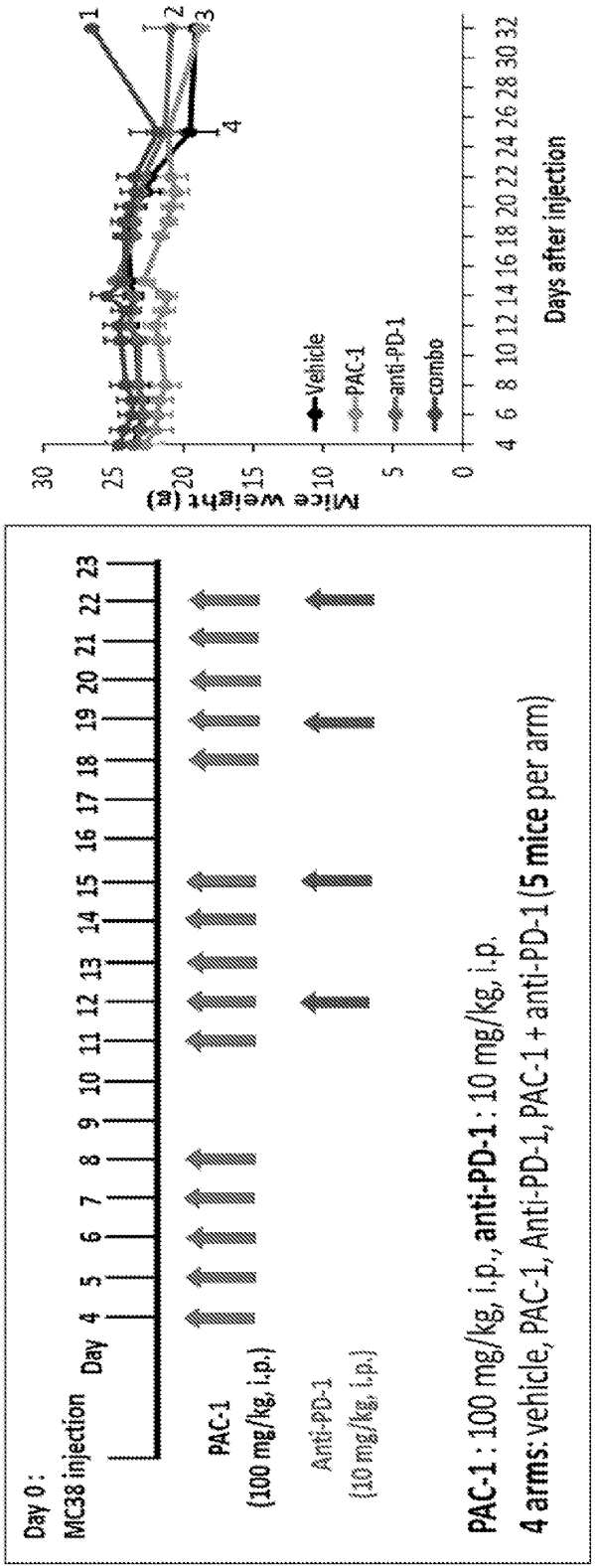

Day 0 :
MC38 injection
Day 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
PAC-1
(100 mg/kg, i.p.)
Anti-PD-1
(10 mg/kg, i.p.)
PAC-1 : 100 mg/kg, i.p., anti-PD-1 : 10 mg/kg, i.p.
4 arms: vehicle, PAC-1, Anti-PD-1, PAC-1 + anti-PD-1 (5 mice per arm)
Mice weight (g)
30 25 20 15 10 5 0
4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Days after injection
Vehicle
PAC-1
anti-PD-1
combo
1
2
3
4

Fig. 16

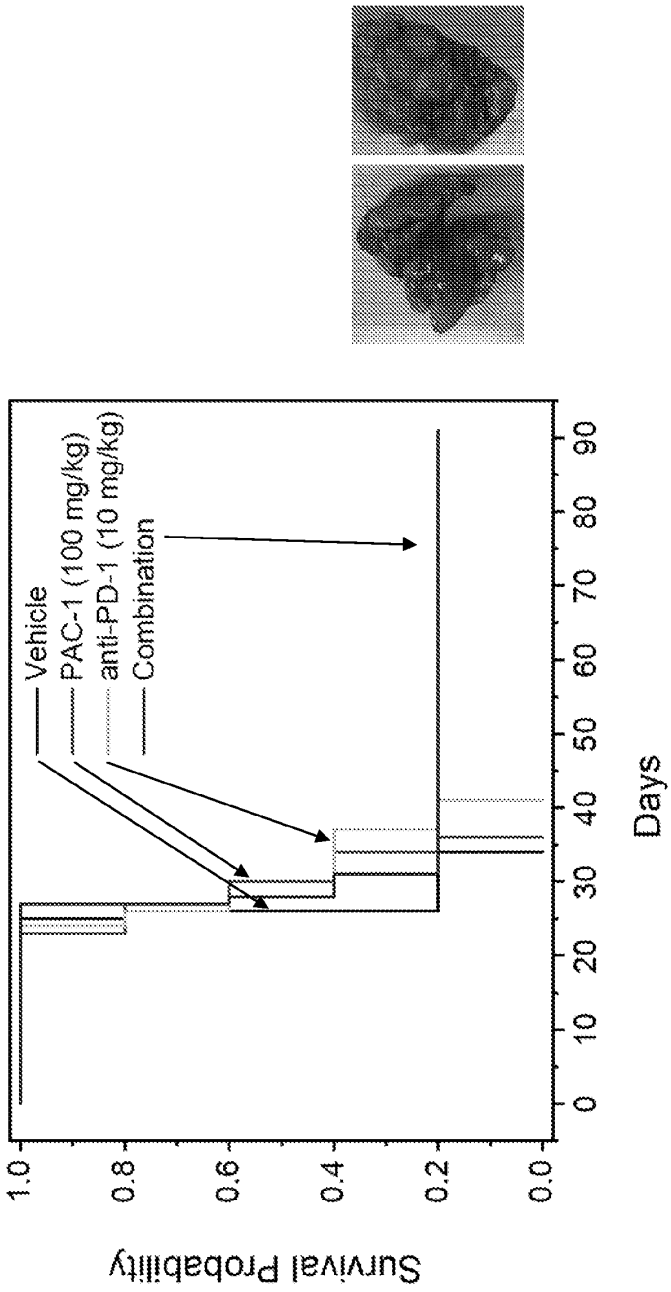

Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
70
80
90
Days
Vehicle
PAC-1 (100 mg/kg)
anti-PD-1 (10 mg/kg)
Combination

Fig. 17