

Blocking interaction between SHP2 and PD-1 denotes a novel opportunity for developing

PD-1 inhibitors

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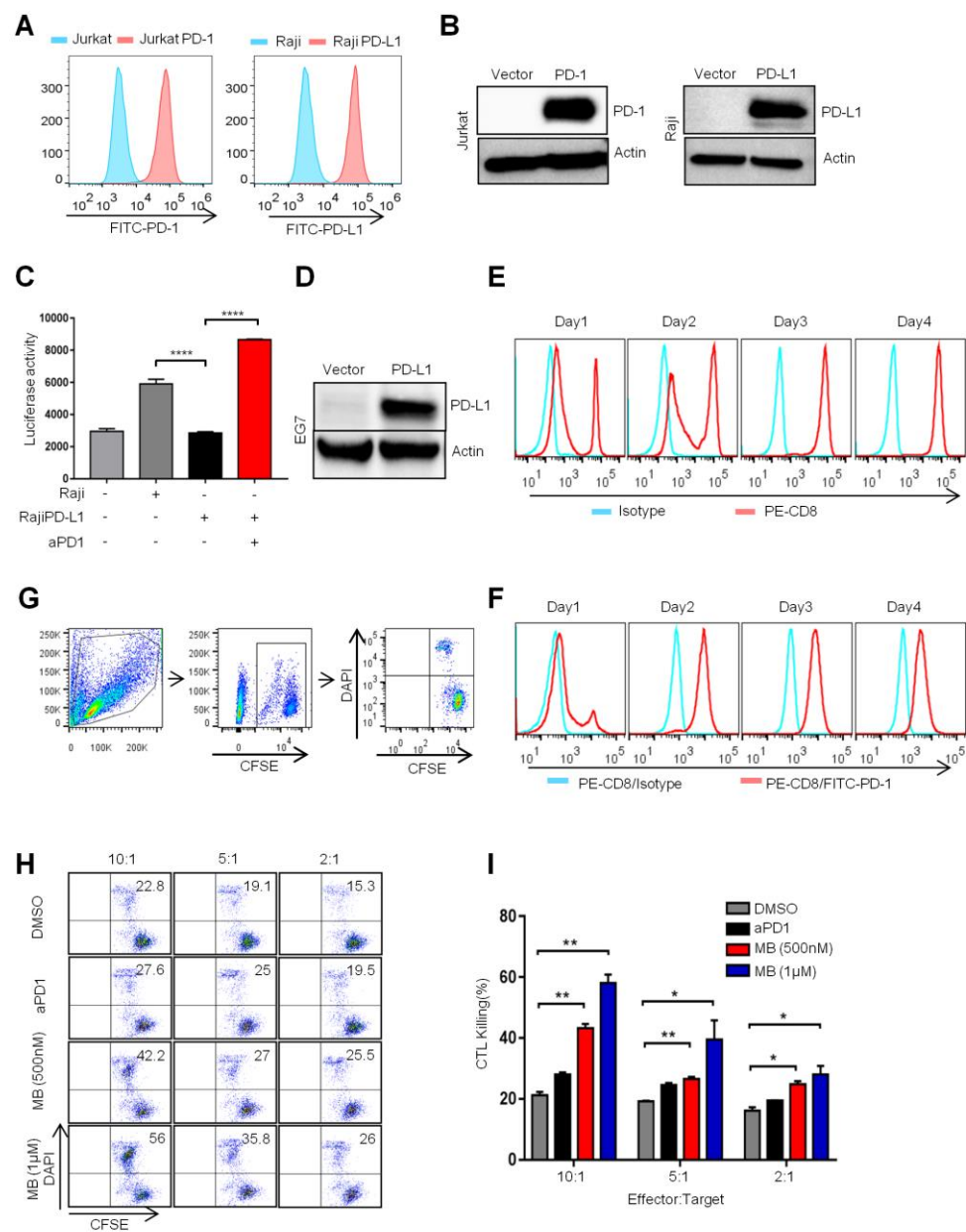
Appendix

Appendix Fig S1-S5 (Page 2-18)

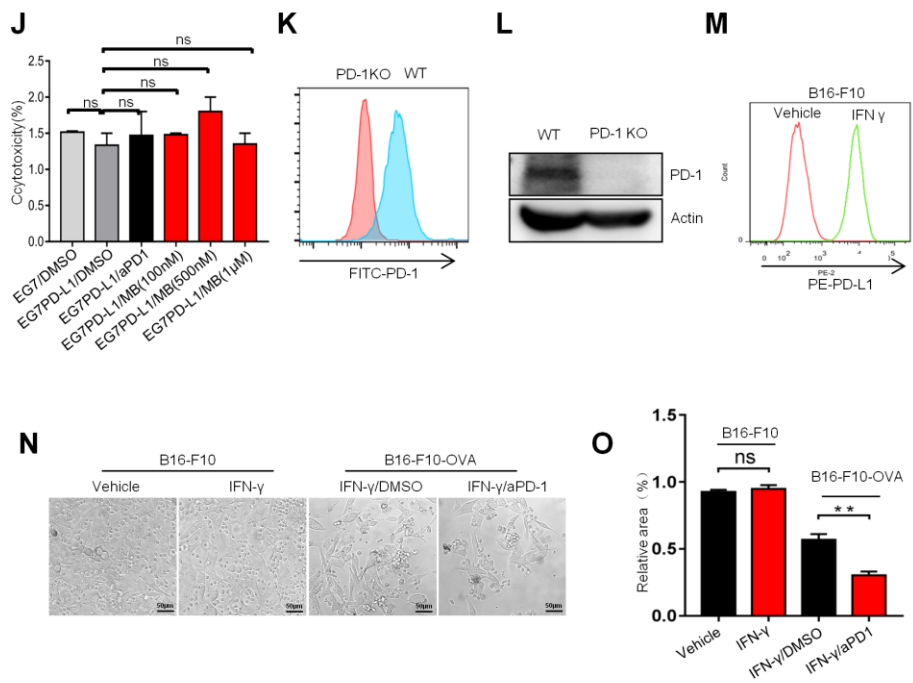
Appendix Table S1 (Page 19-30): Exact P-values and statistical tests.

Appendix Table S2 (Page 31-32): Antibody dilution list.

Appendix Fig S1



Appendix Fig S1



Appendix Fig S1. MB enhances cytotoxicity of activated CTL against PD-L1 expressing target cells

- A.** FACS histograms showing the expression of PD-1 in JP-luc stable cell line (left panel) and PD-L1 in Raji-L1 stable B cells (right panel). JP-luc: Jurkat cell harboring NFAT-luciferase transgene and overexpressing PD-1; Raji-L1: Raji overexpressing PD-L1.
- B.** Western blot analysis the expression of PD-1 in JP-luc stable cell line (left panel) and PD-L1 in Raji-L1 stable cell line (right panel).
- C.** Inhibition of PD1 resulted in activation of JP-luc cells. JP-luc cells were co-cultured with SEE-Loaded Raji or SEE-Loaded Raji-L1 cells for 6 hours in the presence of 10 µg/mL of PD-1 antibody.
- D.** Western blot analysis of PD-L1 expressing stable EG7 cell lines (referred to as EG7-L1).
- E.** FACS histograms analysis of CD8 population in splenocytes of OT-1 mice with media supplemented with 10 nM of SINFEEL peptide on the indicated days after stimulation.
- F.** Daily FACS histograms analysis of expression of PD-1 in splenocytes of OT-1 mice with media supplemented with 10 nM of SINFEEL peptide on the indicated days after stimulation.
- G.** FACS analysis method of (EV1C).
- H.** FACS analysis the cytotoxicity of OT-I CTLs against EG7-L1 in the presence of MB. aPD1 antibody served as positive control. Splenocytes from OT-I mice in culture were stimulated with 10 nM of SINFEEL peptide for 3 days to generate mature CTLs. CTLs were incubated with CFSE labeled EG7-L1 cells in the presence of MB at indicated concentrations. Cytotoxicity was determined by flow cytometry. Data are representative of three independent

experiments. (effector to target ratio = 10:1, 5:1, 2:1, unpaired t-test. killing time:4h).

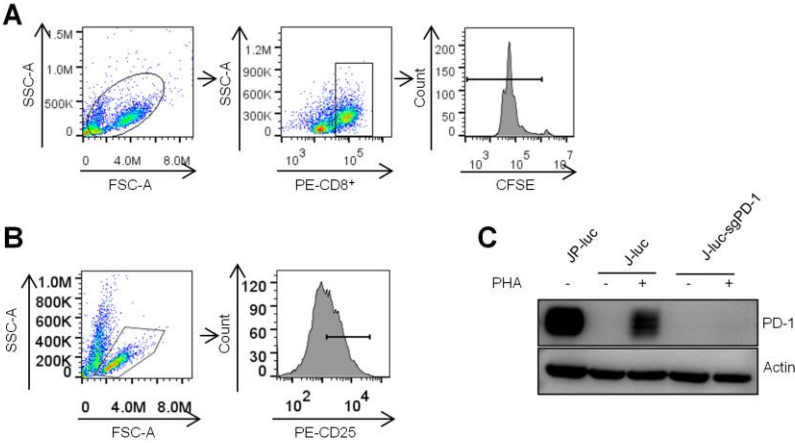
EG7-L1: EG7 overexpressing PD-L1.

- I.** Statistical results of **(H)**.
- J.** The Cytotoxicity of MB on EG7 or EG7-L1.
- K.** FACS analysis the expression of PD-1 in CTL from PD-1^{-/-} (PD-1KO) mice or WT mice.
- L.** Western blot analysis the expression of PD-1 in CTL from PD-1KO mice or WT mice.
- M.** FACS histograms analysis of expression of PD-L1 in IFN γ treated B16-F10 cells.
- N.** PD-1 antibody enhanced Cytotoxicity of OT-I CTLs against IFN γ -treated B16-F10-OVA
(scale bar = 50 μ m).
- O.** Statistical results of **(N)**.

Data information: Data are representative of three independent experiments, and were analyzed by unpaired t-test. Error bars denote s.e.m. *P < 0.05; **P < 0.01; ****P < 0.0001.

Source data are available online for this figure.

Appendix Fig S2



Appendix Fig S2. MB enhanced activation and effector function of CTL

A. FACS analysis of proliferation of OT-I CTLs. CTLs were labeled with 5 nM of CFSE.

Dilution of CFSE were shown as histograms on CD8⁺ T cells populations.

B. FACS analysis the surface expression of CD25.

C. Western blot analysis of PD-1 expression in JP-luc, J-luc and J-luc-sgPD-1 stable cell line

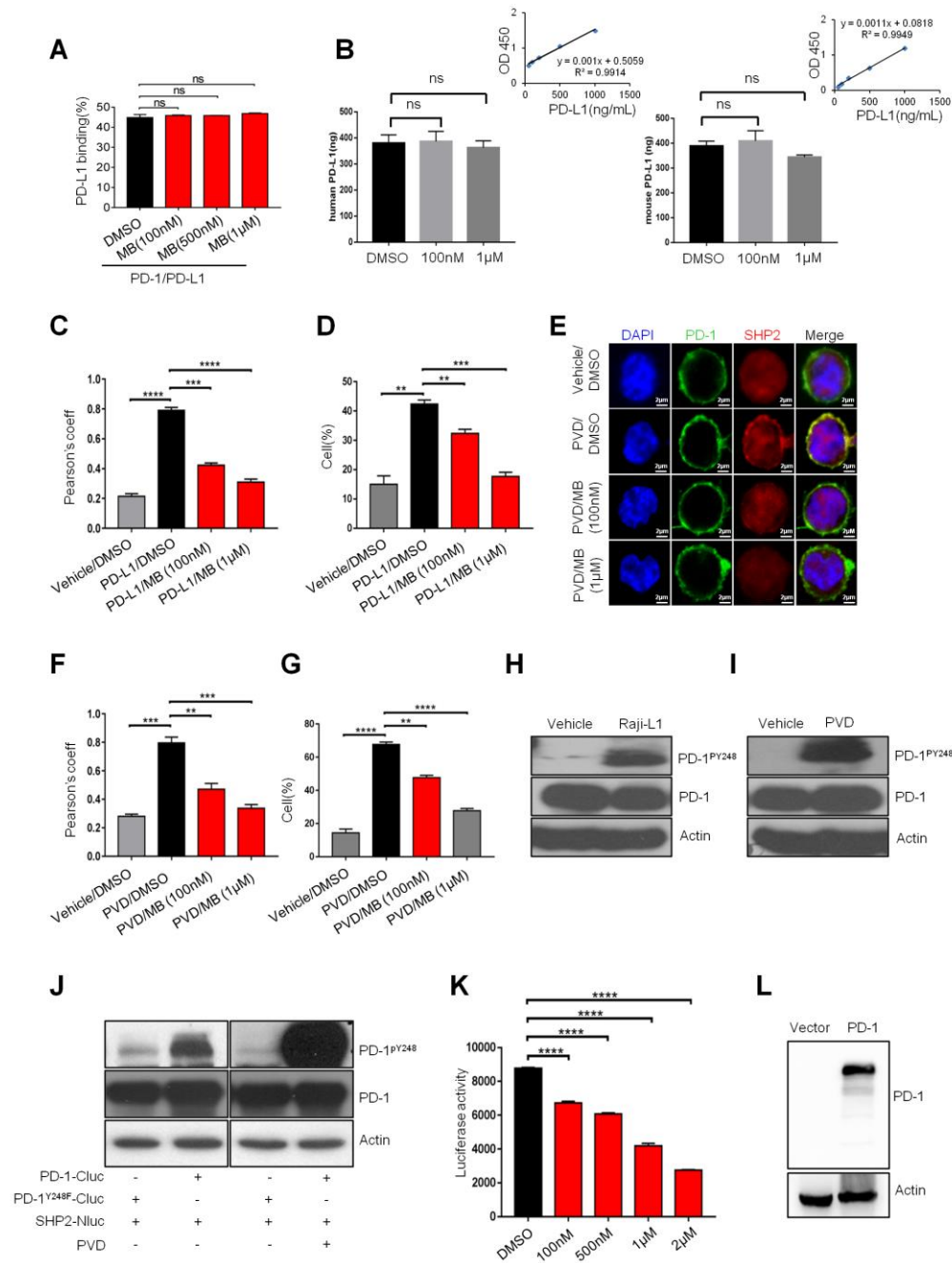
stimulate without or with 500 ng/mL of PHA for 2 days. J-luc: Jurkat cell harboring

NFAT-luciferase transgene; JP-luc: J-luc cell overexpressing PD-1; J-luc-sgPD-1: J-luc cells

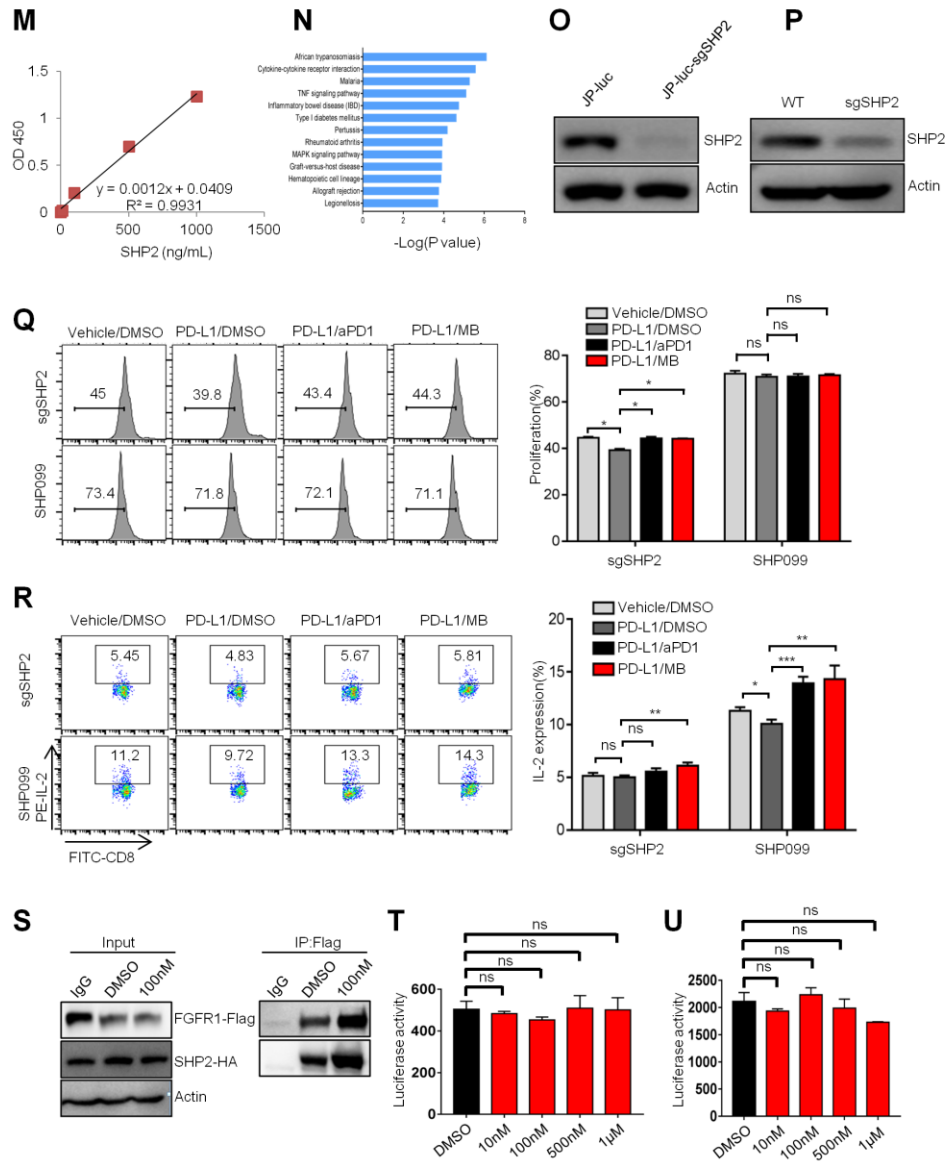
treated with lentivirus expressing sgPD-1/CAS9 simultaneously.

Source data are available online for this figure.

Appendix Fig S3



Appendix Fig S3



Appendix Fig S3. MB suppress PD-1 signaling through blocking SHP2 recruitment by PD-1

- A.** Bar graph of (EV3A).
- B.** Impact of MB on the interaction between PD-1 and PD-L1 protein revealed through ELISA analysis. 10 µg/mL of PD-1 protein coated on 96-well plate overnight. PD-L1-Fc fusion protein were incubated for 1 hour at RT. Plate-bound PD-L1 were then measured with horse-radish peroxidase conjugated anti-Fc secondary antibody.
- C.** Colocalization signals of PD-1-EGFP/SHP2-mCherry were quantitatively analyzed with LAS AF Lite software and displayed as Pearson's coefficient. Five horizons were randomly selected at each time (Pearson's coefficient between 0 and 1. 0 denotes no colocalization; 1 denotes complete colocalization).
- D.** Statistics of Jurkat cells showing colocalization of EGFP/mCherry. 4 fields were randomly chosen under by confocal microscope and percentage of cells showing colocalization of EGFP/mCherry were shown as mean value.
- E.** Jurkat cells showing EGFP-mCherry colocalization under confocal microscope analysis. Jurkat cells co-expressing PD-1-EGFP and SHP2-mCherry were incubated with MB for 1 hour with the media supplemented with 2 µM of PVD for the last 5min. Cells were then fixed with 4% Paraformaldehyde and stained with DAPI. (scale bar = 2 µm).
- F.** Pearson's coefficient determined in (E).
- G.** Percentage of Jurkat cells showing EGFP-mCherry colocalization were calculated against total cell population in (E).
- H.** Representative western blot showing the levels PD-1 pY248 phosphorylation from the lysates of the indicated Jurkat-PD-1 cells. Jurkat-PD-1 cells were cocultured with parental Raji cells

or Raji-L1. Cell lysate were subject to SDS-PAGE and total PD-1 and Y248 phosphorylated PD-1 were analyzed through western blot.

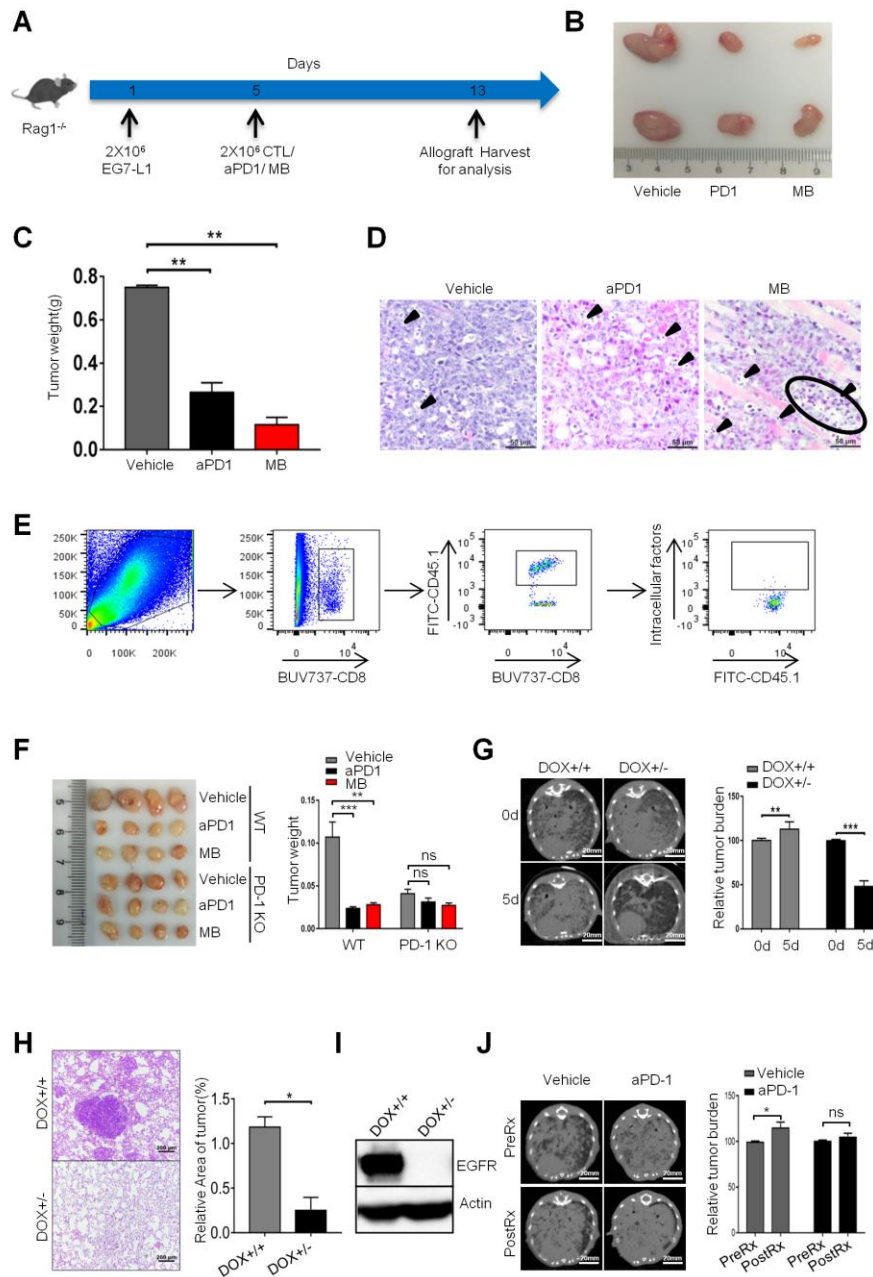
- I.** Representative western blot showing the levels PD-1 pY248 phosphorylation from the lysates of the indicated Jurkat-PD-1 cells. Jurkat-PD-1 cells were cocultured with PVD. Cell lysate were subject to SDS-PAGE and total PD-1 and Y248 phosphorylated PD-1 were analyzed through western blot.
- J.** Representative western blot showing the levels PD-1 pY248 phosphorylation from the lysates of the indicated 293T cells. Stable 293T cells clones stably co-expressing PD-1-C-luc or PD-1 Y248F-C-luc and SHP2-N-luc, treated w/o PVD for 5 minutes.
- K.** Luciferase complementation analysis of the effect of MB on the interaction between PD-1 with SHP2. 293T cells stably co-expressing PD-1-C-luc and SHP2-N-luc were treated with MB at indicated concentration for 6 hours in the presence of PVD for the last 5 minutes. Luciferase activity was measured as readout for PD-1/SHP2 interaction.
- L.** PD-1 protein expression in 293T. 293T cells were transfect with PD-1 expressing plasmid or control vector for 48h. Cell lysate were subject to SDS-PAGE for western blot analysis with anti-PD-1 antibody.
- M.** Standard Curve of figure **3D**.
- N.** Alteration of signaling pathways of OT-1 CTL at transcriptomic level when treated with MB. OT-1 CTLs were treated with 10 ng/mL of mouse PD-L1 protein and w/o 100nM MB for 24 hours. mRNAs were harvested for RNA-sequencing. Genes upregulated in MB treated cells were analyzed by KEGG. Top 13 pathways were graphed based on -log value of statistical significance.

- O.** Western blot analysis of SHP2 expression in JP-luc and JP-luc-sgSHP2. JP-luc: J-luc cell overexpressing PD-1; J-luc-sgPD-1: J-luc cells treated with lentivirus expressing sgSHP2/CAS9 simultaneously.
- P.** Western blot analysis of SHP2 expression in CTL infected with sgSHP2/CAS9 lentivirus or control (WT) cells.
- Q.** Effect of MB on the proliferation of sgSHP2 CTLs and 10 μ M of SHP2099.
- R.** FACS analysis of IL-2 expression by CTLs of indicated genetic backgrounds and treatment.
- S.** Co-IP analysis of impact of MB on interaction between SHP2 and FGFR1.
- T.** Fluorescence complementation analysis of the effect of MB on the interaction between SHP2 and EGFR.
- U.** Fluorescence complementation analysis of the effect of MB on the interaction between SHP2 and FGFR1.

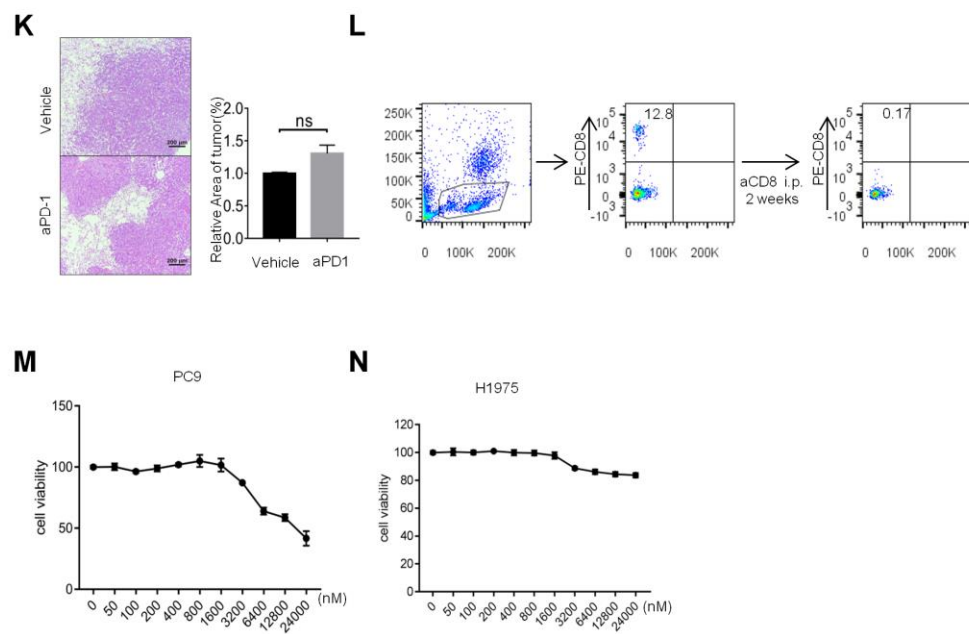
Data information: Data are representative of three independent experiments, and were analyzed by unpaired t-test. **P < 0.01; ***P < 0.001; ****P < 0.0001.

Source data are available online for this figure.

Appendix Fig S4



Appendix Fig S4



Appendix Fig S4. MB shrinks tumor *in vivo* through enhancing cytotoxic function of CTL

- A.** Schematic of the allograft mouse model for MB treatment. EG7-L1 cells were inoculated (s.c.) into the right flank of Rag1^{-/-} mice on day 1 and OT1 CTLs were injected (i.v.) on day 5. Mice were randomized into 3 groups (n=2) and treated with vehicle, aPD1 (10 mg/kg, every other day, i.p.) or MB (i.g. 20mg/kg/day). Tumor size was measured by caliper every other day. EG7-L1: EG7 overexpressing PD-L1.
- B.** Representative image of the tumors on day 13 in (A).
- C.** Tumor weight on day 13 (n = 2) in (A).
- D.** Haematoxylin and Eosin staining for figure 4C. Apoptotic bodies are pointed with triangle. The area of dense apoptotic bodies in MB treated tumors are highlighted with solid circle.
- E.** FACS analysis of the expression of effector molecules by CD8⁺ CD45.1⁺ tumor-infiltrating lymphocytes (TILs).
- F.** Image of the tumors and bar graph of tumor weight (n=4).
- G.** Characterization of TetO-EGFR L858R/CC10rtTA mice (referred to as EC mice). EC mice (n = 3) were fed with doxycycline diet for 2 weeks and tumor burdens were recorded through Computed tomography scanning. The mice were fed with normal diet (withdrawing Doxycycline) for 5 days and CT imaged again. Left panel showing lung tumors continued growing in the mice fed with doxycycline diet (DOX^{+/+}). Right panel showing the lung tumor regression when withdrawing doxycycline for 5 days (DOX^{+/-}).
- H.** Haematoxylin and Eosin staining (lung section = 3) for (G).
- I.** Western Blot analysis of EGFR expression in lung of mice shown in (G).
- J.** PD-1 antibody treatment is ineffective to shrink EC tumors (n=3). Computed tomography for

EC mice were shown before and after treatment with aPD1 antibody.

K. Haematoxylin and Eosin staining result of lung section of EC mice (n = 3, lung section = 3) treated with aPD1 antibody.

L. Depleting CD8⁺ T cells in EC mice. EC mice were treated with aCD8 antibody (200 µg per mouse, every other day, i.p.) to deplete CD8⁺ T cells. Peripheral CD8⁺ T cells were monitored through FACS analysis. Result indicated the CD8⁺ T cells were almost completely depleted after treatment with aCD8 antibody for 2 weeks.

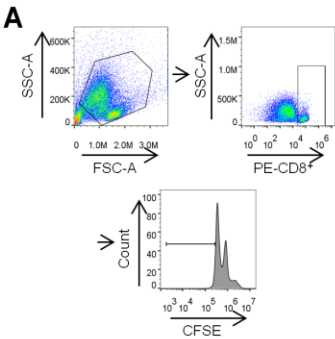
M. Effects of MB on cell viability. PC9 cells were treated with MB at indicated concentrations for 48 h. CCK-8 assay was performed to measure cell viability.

N. Effects of MB on cell viability. H1975 cells were treated with MB at indicated concentrations for 48 h. CCK-8 assay was performed to measure cell viability.

Data information: Data are representative of three independent experiments, and were analyzed by unpaired t-test. Error bars denote s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001.

Source data are available online for this figure.

Appendix Fig S5



Appendix Fig S5. MB is effective to activate human CD8⁺ T cells

A. CFSE dilution of CD8⁺ T cells through FACS analysis. CFSE (5nM) labeled human peripheral blood mononuclear cells (PBMC) were pre-incubated with DMSO, MB, 25 µg/ml of pembrolizumab or 20 µg/ml of Nivolumab for 1 h and then seeded in the 96-well plate precoated with 10 µg/ml of aCD3/aCD28 in the presence of 10 µg/ml human PD-L1 Protein. Cell proliferation was analyzed through FACS analysis, Dilution of CFSE are shown as histograms on CD8⁺ T cells populations.

Appendix Table S1: Exact P-values and statistical tests

Fig1C	P-value	Test
EG7/DMSO vs EG7PD-L1/DMSO	<0.0001	unpaired t-test
EG7PD-L1/DMSO vs EG7PD-L1/aPD1	<0.0001	unpaired t-test
EG7PD-L1/DMSO vs EG7PD-L1/100nM	0.0010	unpaired t-test
EG7PD-L1/DMSO vs EG7PD-L1/500nM	0.0005	unpaired t-test
EG7PD-L1/DMSO vs EG7PD-L1/1 μ M	0.0004	unpaired t-test

Fig1D		P-value	Test
10:1	DMSO vs aPD1	0.0039	unpaired t-test
	DMSO vs MB	0.0017	unpaired t-test
5:1	DMSO vs aPD1	0.0006	unpaired t-test
	DMSO vs MB	0.0001	unpaired t-test
2:1	DMSO vs aPD1	0.0007	unpaired t-test
	DMSO vs MB	0.0004	unpaired t-test

Fig1F	P-value	Test
EG7/DMSO vs EG7PD-L1/DMSO	0.1150	unpaired t-test
EG7PD-L1/DMSO vs EG7PD-L1/aPD1	0.3060	unpaired t-test
EG7PD-L1/DMSO vs EG7PD-L1/MB	0.4319	unpaired t-test

Fig1H	P-value	Test
DMSO vs MB	0.1483	unpaired t-test
IFN- γ +DMSO vs IFN- γ +aPD1	<0.0001	unpaired t-test
IFN- γ +DMSO vs IFN- γ +MB	<0.0001	unpaired t-test

Fig2B		P-value	Test
WT	CON/DMSO vs PD-L1/DMSO	<0.0001	unpaired t-test
	PD-L1/DMSO vs PD-L1/aPD1	<0.0001	unpaired t-test
	PD-L1/DMSO vs PD-L1/MB	<0.0001	unpaired t-test

PD-1 KO	PD-L1/DMSO vs PD-L1/DMSO	0.7110	unpaired t-test
	PD-L1/DMSO vs PD-L1/aPD1	0.5461	unpaired t-test
	PD-L1/DMSO vs PD-L1/MB	0.5543	unpaired t-test

Fig2D		P-value	Test
CD25	CON/DMSO vs PD-L1/DMSO	<0.0001	unpaired t-test
	PD-L1/DMSO vs PD-L1/aPD1	<0.0001	unpaired t-test
	PD-L1/DMSO vs PD-L1/MB	<0.0001	unpaired t-test
CD69	PD-L1/DMSO vs PD-L1/DMSO	0.0007	unpaired t-test
	PD-L1/DMSO vs PD-L1/aPD1	<0.0001	unpaired t-test
	PD-L1/DMSO vs PD-L1/MB	<0.0001	unpaired t-test

Fig2F		P-value	Test
JP-luc	Raji vs Raji PD-L1	0.0012	unpaired t-test
	Raji PD-L1 vs Raji PD-L1+aPD1	0.0045	unpaired t-test
	Raji PD-L1 vs Raji PD-L1+MB	0.0017	unpaired t-test
J-luc-sgPD-1	Raji vs Raji PD-L1	0.1776	unpaired t-test
	Raji PD-L1 vs Raji PD-L1+aPD1	0.3206	unpaired t-test
	Raji PD-L1 vs Raji PD-L1+MB	0.5050	unpaired t-test

Fig2G	P-value	Test
DMSO vs 100nM	0.0093	unpaired t-test
DMSO vs 1μM	0.0027	unpaired t-test

Fig2H	P-value	Test
Raji vs Raji PD-L1	0.0049	unpaired t-test
Raji PD-L1 vs Raji PD-L1+aPD1	0.0089	unpaired t-test
Raji PD-L1 vs Raji PD-L1+MB	<0.0001	unpaired t-test

Fig2J		P-value	Test
PE-IL-2	DMSO vs aPD1	0.0240	unpaired t-test
	DMSO vs 100nM	0.0034	unpaired t-test
	DMSO vs 1μM	0.0018	unpaired t-test
APC-Perforin	DMSO vs aPD1	0.0019	unpaired t-test
	DMSO vs 100nM	0.0011	unpaired t-test
	DMSO vs 1μM	0.0004	unpaired t-test

Fig3B		P-value	Test
Resting	PD-1 ^{Y248} -Cluc vs PD-1-Cluc	0.0016	unpaired t-test
Active/DMSO	PD-1 ^{Y248} -Cluc vs PD-1-Cluc	<0.0001	unpaired t-test
Active/PD-L1	PD-1 ^{Y248} -Cluc vs PD-1-Cluc	<0.0001	unpaired t-test
Active/PD-L1/MB	PD-1 ^{Y248} -Cluc vs PD-1-Cluc	0.0413	unpaired t-test
	Active/PD-L1vs Active/PD-L1/MB	<0.0001	unpaired t-test

Fig3D		P-value	Test
SHP2-FC Binding	DMSO vs 100nM MB	0.04909	unpaired t-test
	DMSO vs 500nM MB	0.0209	unpaired t-test
	DMSO vs 1μM MB	0.0096	unpaired t-test

Fig3H		P-value	Test
CD28-Cluc	Jurkat vs Jurkat PD-1	<0.0001	unpaired t-test
CD28 ^{Y191F} -Cluc	CD28-Cluc vs CD28 ^{Y191F} -Cluc	<0.0001	unpaired t-test
PD-L1	CD28-Cluc vs PD-L1	<0.0001	unpaired t-test
MB	PD-L1 vs v PD-L1 MB	<0.0001	unpaired t-test

Fig4B	Days after tumor injection	P-value	Test
Vehicle vs aPD1	7	0.0061	unpaired t-test
	10	0.0001	unpaired t-test
Vehicle vs MB	7	0.0476	unpaired t-test
	10	0.009	unpaired t-test

Fig4D	P-value	Test
Vehicle vs aPD1	0.0009	unpaired t-test
Vehicle vs MB	0.0010	unpaired t-test

Fig4F		P-value	Test
CD8	Vehicle vs aPD1	0.0411	unpaired t-test
	Vehicle vs MB	0.0020	unpaired t-test
Ki-67	Vehicle vs aPD1	0.0115	unpaired t-test
	Vehicle vs MB	0.0011	unpaired t-test

Fig4H		P-value	Test
Perforin	Vehicle vs aPD1	0.0227	unpaired t-test
	Vehicle vs MB	0.0025	unpaired t-test
GZMB	Vehicle vs aPD1	0.0208	unpaired t-test
	Vehicle vs MB	0.0050	unpaired t-test
IL-2	Vehicle vs aPD1	0.0063	unpaired t-test
	Vehicle vs MB	0.0043	unpaired t-test

Fig4K		P-value	Test
Vehicle	PreRx vs PostRx	0.0073	unpaired t-test
MB	PreRx vs PostRx	0.0001	unpaired t-test
aCD8/MB	PreRx vs PostRx	0.0065	unpaired t-test

Fig4M	P-value	Test
Vehicle vs MB	<0.0001	unpaired t-test
Vehicle vs aCD8/MB	0.0572	unpaired t-test

Fig5C	P-value	Test
Raji vs Raji PD-L1	<0.0001	unpaired t-test
Raji PD-L1 vs Raji PD-L1+ Niv	0.0011	unpaired t-test
Raji PD-L1 vs Raji PD-L1+ Pem	0.0024	unpaired t-test
Raji PD-L1 vs Raji PD-L1+0.1µM	0.0022	unpaired t-test
Raji PD-L1 vs Raji PD-L1+0.5µM	0.0012	unpaired t-test

Raji PD-L1+1 μ M	0.0013	unpaired t-test
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Fig EV1D	P-value	Test
Raji vs Raji PD-L1	<0.0001	unpaired t-test
Raji PD-L1 vs Raji PD-L1+ aPD1	<0.0001	unpaired t-test

Fig EV1K		P-value	Test
10:1	EG7/Vehicle vs EG7PD-L1/Vehicle	0.0001	unpaired t-test
	EG7PD-L1/Vehicle vs EG7PD-L1/aPD1	0.0004	unpaired t-test
5:1	EG7/Vehicle vs EG7PD-L1/Vehicle	<0.0001	unpaired t-test
	EG7PD-L1/Vehicle vs EG7PD-L1/aPD1	0.0002	unpaired t-test
2:1	EG7/Vehicle vs EG7PD-L1/Vehicle	<0.0001	unpaired t-test
	EG7PD-L1/Vehicle vs EG7PD-L1/aPD1	0.0001	unpaired t-test

Fig EV1M		P-value	Test
10:1	DMSO vs 500nM MB	0.0032	unpaired t-test
	DMSO vs 1 μ M MB	0.0034	unpaired t-test
5:1	DMSO vs 500nM MB	0.0048	unpaired t-test
	DMSO vs 1 μ M MB	0.0458	unpaired t-test
2:1	DMSO vs 500nM MB	0.0157	unpaired t-test
	DMSO vs 1 μ M MB	0.0312	unpaired t-test

Fig EV1N	P-value	Test
EG7/DMSO vs EG7PD-L1/DMSO	0.3908	unpaired t-test
EG7PD-L1/DMSO vs EG7PD-L1/aPD1	0.7495	unpaired t-test
EG7PD-L1/DMSO vs EG7PD-L1/100nM	0.4750	unpaired t-test
EG7PD-L1/DMSO vs EG7PD-L1/500nM	0.2148	unpaired t-test
EG7PD-L1/DMSO vs EG7PD-L1/1 μ M	0.9525	unpaired t-test

Fig EV1S	P-value	Test
CON vs IFN- γ	0.5345	unpaired t-test

IFN- γ /DMSO vs IFN- γ /aPD1	0.0071	unpaired t-test
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Fig EV1U	P-value	Test
DMSO vs 100nM	0.0455	unpaired t-test
DMSO vs 500nM	0.0426	unpaired t-test
DMSO vs 1 μ M	0.0078	unpaired t-test

Fig EV2B		P-value	Test
24h	PD-L1/Vehicle vs CON/Vehicle	0.2929	unpaired t-test
	PD-L1/Vehicle vs PD-L1/aPD1	0.0389	unpaired t-test
48h	PD-L1/Vehicle vs CON/Vehicle	0.0229	unpaired t-test
	PD-L1/Vehicle vs PD-L1/aPD1	0.0005	unpaired t-test
72h	PD-L1/Vehicle vs CON/Vehicle	0.0369	unpaired t-test
	PD-L1/Vehicle vs PD-L1/aPD1	0.0007	unpaired t-test

Fig EV2G	P-value	Test
DMSO vs aPD1	0.0042	unpaired t-test
DMSO vs 100nM	0.0220	unpaired t-test
DMSO vs 1 μ M	0.0012	unpaired t-test

Fig EV2H	P-value	Test
DMSO vs aPD1	0.0398	unpaired t-test
DMSO vs 100nM	0.0216	unpaired t-test
DMSO vs 1 μ M	0.0083	unpaired t-test

Fig EV3B	P-value	Test
DMSO vs 100nM	0.5869	unpaired t-test
DMSO vs 500nM	0.5805	unpaired t-test
DMSO vs 1 μ M	0.3185	unpaired t-test

Fig EV3C		P-value	Test
Human PD-L1	DMSO vs 100nM	0.9005	unpaired t-test
	DMSO vs 1 μ M	0.7643	unpaired t-test
Mouse PD-L1	DMSO vs 100nM	0.6581	unpaired t-test
	DMSO vs 1 μ M	0.3118	unpaired t-test

Fig EV3D	P-value	Test
PD-L1/DMSO vs CON/DMSO	<0.0001	unpaired t-test
PD-L1/DMSO vs PD-L1/100nM MB	0.0001	unpaired t-test
PD-L1/DMSO vs PD-L1/1 μ M MB	<0.0001	unpaired t-test

Fig EV3E	P-value	Test
PD-L1/DMSO vs CON/DMSO	0.0011	unpaired t-test
PD-L1/DMSO vs PD-L1/100nM MB	0.0082	unpaired t-test
PD-L1/DMSO vs PD-L1/1 μ M MB	0.0003	unpaired t-test

Fig EV3G	P-value	Test
PVD/DMSO vs CON/DMSO	0.0003	unpaired t-test
PVD/DMSO vs PVD/100nM MB	0.0047	unpaired t-test
PVD /DMSO vs PVD /1 μ M MB	0.0006	unpaired t-test

Fig EV3H	P-value	Test
PVD/DMSO vs CON/DMSO	<0.0001	unpaired t-test
PVD/DMSO vs PVD/100nM MB	0.0006	unpaired t-test
PVD /DMSO vs PVD /1 μ M MB	<0.0001	unpaired t-test

Fig EV3L	P-value	Test
PD-1-Cluc-Y248F vs PD-1-Cluc	0.0002	unpaired t-test

Fig EV3M	P-value	Test
DMSO vs 100nM	<0.0001	unpaired t-test
DMSO vs 500nM	<0.0001	unpaired t-test
DMSO vs 1 μ M	<0.0001	unpaired t-test
DMSO vs 2 μ M	<0.0001	unpaired t-test

Fig EV3R		P-value	Test
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JP-luc sgSHP2	Raji PD-L1 vs Raji	0.5529	unpaired t-test
	Raji PD-L1 vs Raji PD-L1+aPD1	0.7102	unpaired t-test
	Raji PD-L1 vs Raji PD-L1+MB	0.2098	unpaired t-test
JP-luc SHP099	Raji PD-L1 vs Raji	0.1446	unpaired t-test
	Raji PD-L1 vs Raji PD-L1+aPD1	0.6664	unpaired t-test
	Raji PD-L1 vs Raji PD-L1+MB	0.1929	unpaired t-test

Fig EV3T		P-value	Test
sgSHP2	PD-L1/DMSO vs CON/DMSO	0.0227	unpaired t-test
	PD-L1/DMSO vs PD-L1/aPD1	0.0392	unpaired t-test
	PD-L1/DMSO vs PD-L1/MB	0.0173	unpaired t-test
SHP099	PD-L1/DMSO vs CON/DMSO	0.4775	unpaired t-test
	PD-L1/DMSO vs PD-L1/aPD1	0.9235	unpaired t-test
	PD-L1/DMSO vs PD-L1/MB	0.6190	unpaired t-test

Fig EV3U		P-value	Test
sgSHP2	PD-L1/DMSO vs CON/DMSO	0.5505	unpaired t-test
	PD-L1/DMSO vs PD-L1/aPD1	0.0542	unpaired t-test
	PD-L1/DMSO vs PD-L1/MB	0.0074	unpaired t-test
SHP099	PD-L1/DMSO vs CON/DMSO	0.0165	unpaired t-test
	PD-L1/DMSO vs PD-L1/aPD1	0.0008	unpaired t-test
	PD-L1/DMSO vs PD-L1/MB	0.0057	unpaired t-test

Fig EV3V		P-value	Test
sgSHP2	EG7 PD-L1/DMSO vs EG7/DMSO	0.2444	unpaired t-test
	EG7 PD-L1/DMSO	0.1917	unpaired t-test

	vs EG7 PD-L1/aPD1		
	EG7 PD-L1/DMSO vs EG7 PD-L1/MB	0.5053	unpaired t-test
SHP099	EG7 PD-L1/DMSO vs EG7/DMSO	0.2155	unpaired t-test
	EG7 PD-L1/DMSO vs EG7 PD-L1/aPD1	0.4226	unpaired t-test
	EG7 PD-L1/DMSO vs EG7 PD-L1/MB	0.0356	unpaired t-test

Fig EV3Y	P-value	Test
DMSO vs 10nM	0.6296	unpaired t-test
DMSO vs 100nM	0.2871	unpaired t-test
DMSO vs 500nM	0.9479	unpaired t-test
DMSO vs 1μM	0.9715	unpaired t-test

Fig EV3Z	P-value	Test
DMSO vs 10nM	0.3721	unpaired t-test
DMSO vs 100nM	0.5735	unpaired t-test
DMSO vs 500nM	0.6380	unpaired t-test
DMSO vs 1μM	0.0871	unpaired t-test

Fig EV4A	Days after tumor injection	P-value	Test
Vehicle vs 10mg/kgMB	11	0.0082	unpaired t-test
Vehicle vs 20mg/kgMB	11	0.0073	unpaired t-test
Vehicle vs 30mg/kgMB	11	0.0041	unpaired t-test

Fig EV4C	Days after tumor injection	P-value	Test
Vehicle vs aPD1	11	0.0122	unpaired t-test
	13	0.0439	unpaired t-test
Vehicle vs MB	11	0.0072	unpaired t-test
	13	0.0456	unpaired t-test

Fig EV4E	P-value	Test
Vehicle vs aPD1	0.0089	unpaired t-test
Vehicle vs MB	0.0033	unpaired t-test

Fig EV4F	Days after tumor injection	P-value	Test
Vehicle vs 10mg/kg MB	13	0.04	unpaired t-test
Vehicle vs 20mg/kg MB	13	0.029	unpaired t-test
Vehicle vs 30mg/kg MB	13	0.024	unpaired t-test

Fig EV4I	Days after tumor injection	P-value	Test
WT CTL Vehicle vs WT CTL aPD1	9	0.0040	unpaired t-test
	11	0.0031	unpaired t-test
WT CTL Vehicle vs WT CTL MB	9	0.0043	unpaired t-test
	11	0.0035	unpaired t-test
PD-1 KO CTL Vehicle vs PD-1 KO CTL aPD1	9	0.8711	unpaired t-test
	11	0.2096	unpaired t-test
PD-1 KO CTL Vehicle vs PD-1 KO CTL MB	9	0.1734	unpaired t-test
	11	0.2486	unpaired t-test

Fig EV4K		P-value	Test
WT CTL	Vehicle vs aPD1	0.0010	unpaired t-test
	Vehicle vs MB	0.0012	unpaired t-test
PD-1 KO CTL	Vehicle vs aPD1	0.2058	unpaired t-test
	Vehicle vs MB	0.0570	unpaired t-test

Fig EV4L		P-value	Test
DOX+/+	0d vs 5d	0.0035	unpaired t-test
DOX+/-	0d vs 5d	0.0004	unpaired t-test

Fig EV4M	P-value	Test
DOX+/+ vs DOX+/-	0.0238	unpaired t-test

Fig EV4O		P-value	Test
Vehicle	PreRx vs PostRx	0.0178	unpaired t-test
aPD1	PreRx vs PostRx	0.1435	unpaired t-test

Fig EV4P	P-value	Test
Vehicle vs aPD1	0.2771	unpaired t-test

Fig EV4S		P-value	Test
IL-2	Vehicle vs aPD1	<0.0001	unpaired t-test
Perforin	Vehicle vs aPD1	0.0005	unpaired t-test
GZMB	Vehicle vs aPD1	0.0002	unpaired t-test

Fig EV5B		P-value	Test
24h	PD-L1/DMSO vs CON/DMSO	0.0001	unpaired t-test
	PD-L1/DMSO vs PD-L1/Pem	0.0099	unpaired t-test
	PD-L1/DMSO vs PD-L1/Niv	0.0289	unpaired t-test
	PD-L1/DMSO vs PD-L1/MB	0.0043	unpaired t-test
48h	PD-L1/DMSO vs CON/DMSO	0.0025	unpaired t-test
	PD-L1/DMSO vs PD-L1/Pem	0.0035	unpaired t-test
	PD-L1/DMSO vs PD-L1/Niv	0.0154	unpaired t-test
	PD-L1/DMSO vs PD-L1/MB	0.0068	unpaired t-test

Fig EV5C	P-value	Test
PD-L1/DMSO vs CON/DMSO	0.0045	unpaired t-test
PD-L1/DMSO vs PD-L1/Pem	0.0009	unpaired t-test
PD-L1/DMSO vs PD-L1/Niv	0.0211	unpaired t-test
PD-L1/DMSO vs PD-L1/100nM MB	0.0023	unpaired t-test
PD-L1/DMSO vs PD-L1/1μM MB	0.0006	unpaired t-test

Fig EV5D	P-value	Test
PD-L1/DMSO vs CON/DMSO	0.0010	unpaired t-test
PD-L1/DMSO vs PD-L1/Pem	0.0005	unpaired t-test
PD-L1/DMSO vs PD-L1/Niv	0.0333	unpaired t-test
PD-L1/DMSO vs PD-L1/100nM MB	0.0044	unpaired t-test
PD-L1/DMSO vs PD-L1/1 μ M MB	0.0005	unpaired t-test

Fig EV5E	P-value	Test
PD-L1/DMSO vs CON/DMSO	0.0009	unpaired t-test
PD-L1/DMSO vs PD-L1/Pem	0.0021	unpaired t-test
PD-L1/DMSO vs PD-L1/Niv	0.0009	unpaired t-test
PD-L1/DMSO vs PD-L1/100nM MB	0.0032	unpaired t-test
PD-L1/DMSO vs PD-L1/1 μ M MB	0.0005	unpaired t-test

Fig EV5F	P-value	Test
PD-L1/DMSO vs CON/DMSO	0.0076	unpaired t-test
PD-L1/DMSO vs PD-L1/Pem	0.0043	unpaired t-test
PD-L1/DMSO vs PD-L1/Niv	0.0046	unpaired t-test
PD-L1/DMSO vs PD-L1/100nM MB	0.0035	unpaired t-test
PD-L1/DMSO vs PD-L1/1 μ M MB	0.0002	unpaired t-test

*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001

Appendix Table S2: Antibody dilution list

Antibody	Company	Catalog Number	Dilution
CD8-PE anti-mouse	eBioscience	12-0081-81	1/100
IFN γ -PE-Cy7 anti-mouse	eBioscience	25-7311-82	1/100
Perforin-APC anti-mouse	eBioscience	17-9392-80	1/100
CD8-FITC anti-mouse	eBioscience	11-0081-82	1/100
IL-2-PE PE anti-mouse	eBioscience	12-7021-82	1/100
CD25-PE anti-mouse	eBioscience	12-0691-81	1/100
CD69-PE anti- mouse	eBioscience	12-0251-81	1/100
PD-1-FITC anti-mouse	eBioscience	11-9985-85	1/100
PD-L1-PE anti-mouse	eBioscience	12-5982-81	1/100
CD8-BUV737 anti-mouse	BD Horizon	564297	1/100
CD8-PE anti-human	eBioscience	MHCD0804	1/100
GZMB anti-mouse/human	Biolegend	515405	1/100
IL-2-PE anti-human	Biolegend	500306	1/100
IFN γ -PE anti-human	Biolegend	502508	1/100
Perforin-PE anti-human	Biolegend	353303	1/100
CD25-PE anti-human	Biolegend	356103	1/100
CD69-PE anti-human	Biolegend	310905	1/100
CD8-FITC anti-human	Biolegend	300906	1/100
CD45.1-FITC anti-mouse	Biolegend	110705	1/100
CD45.2-APC anti-mouse	Biolegend	109813	1/100
PD-1-FITC anti-human	Biolegend	329904	1/100
PD-1-PE anti-human	Biolegend	329905	1/100
PD-L1-PE anti-human	Biolegend	329706	1/100
His Tag-PE	Biolegend	362603	1/100
His-HRP	Biolegend	652504	1/5000
FC-HRP	Sino biological	SSA001	1/5000
CD28 anti-human	ebioscience	16-0289	2ug/mL
CD3 anti-human	ebioscience	16-0037	2ug/mL
CD28 anti-mouse	ebioscience	16-0281	2ug/mL
CD3 anti-mouse	ebioscience	16-0031	2ug/mL
Phospho-Zap-70	Cell Signaling Technology	2717	1/1000
ZAP70	Cell Signaling Technology	3165	1/1000
Phospho-PKC θ	Cell Signaling Technology	9377	1/1000
PKC θ	Cell Signaling Technology	13643	1/1000
Phospho-PLC γ 1	Cell Signaling Technology	14008	1/1000
PLC γ 1	Cell Signaling Technology	5690	1/1000
Phospho-AKT	Cell Signaling Technology	4060S	1/1000
AKT	Cell Signaling Technology	9272S	1/1000
PD-1 human	Cell Signaling Technology	86163	1/1000
PD-1 mouse	Cell Signaling Technology	84651	1/1000

PD-L1 mouse	Cell Signaling Technology	13684	1/1000
SHP2	Cell Signaling Technology	3397	1/1000
Phospho-Tyrosine Mouse mAb (P-Tyr-102)	Cell Signaling Technology	9416	1/1000
CD28	Cell Signaling Technology	38774	1/1000
CD8 α Mouse Specific	Cell Signaling Technology	98941	1/50
ki-67	Abcam	ab15580	1/500
β -Actin	sigma	A5316	1/5000
EGFR	Abcam	E234	1/1000
HA	Santa Cruz	C1091	1/1000
Flag	sigma	F1804	1/1000
Mouse PD-1 antibody	BioXcell	BP0033-2	10mg/kg
Mouse CD8 antibody	BioXcell	BE0061	10mg/kg
Anti-mouse IgG	sigma	A0168	1/2000
Anti-Rabbit IgG	sigma	A6154	1/2000