Supplemental Material

Supplemental table

Table S1. List of antibodies used in the study

Purpose	Antibody	Conjugate	Host, isotype	Clone	Concentration/dilution	Company
Treatment	PD-1	—	Rat IgG2a	RMP1-14	2 mg/mL	BioXcell
	PD-L1	—	Rat IgG2b	10F.9G2	2 mg/mL	BioXcell
	PD-L1	—	Rat IgG2a	MIH6	2 mg/mL	BioLegend
	IgG2a	—	Rat IgG2a	2A3	2 mg/mL	BioXcell
	IgG2b	—	Rat IgG2b	LTF-2	2 mg/mL	BioXcell
	Gr-1	—	Rat IgG2b	RB6-8C5	2 mg/mL	BioXcell
FCM	CD45	BV 605	Rat	30-F11	2 µg/mL	BioLegend
	CD3	APC	Rat	17A2	2 µg/mL	BioLegend
	B220	PE	Rat	RA3-6B2	5 µg/mL	Thermo
	CD11b	BV 421	Rat	M1/70	0.05 µg/mL	BioLegend
	F4/80	PE	Rat	BM8	5 µg/mL	BioLegend
	Ly-6C	FITC	Rat	HK1.4	2.5 µg/mL	BioLegend
	Ly-6G	BV 650	Rat	1A8	2 µg/mL	BioLegend
	CD49b	PE-Cy/7	Hamster	HMα2	1 µg/mL	BioLegend
	FCεRIa	PE/Dazzle	Hamster	MAR-1	2 µg/mL	BioLegend
	CD117	APC-Cy/7	Rat	2B8	2 µg/mL	BioLegend
	CD138	BV421	Rat	281-2	2 µg/mL	BioLegend
	CD16/32	—	Rat	93	10 µg/mL	BioLegend
ELISA	anti-mouse IgG	HRP	Goat	poly4053	1:4000	BioLegend
	anti-mouse IgE	—	Rat	R35-72	2 mg/mL, 1:250	BD Pharmingen

FCM: flow cytometry, BV: brilliant violet, APC: Allophycocyanin, PE: Phycoerythrin, FITC: Fluorescein Isothiocyanate, PE-Cy7: phycoerythrin-cyanine 7, HRP: horseradish peroxidase.

Supplemental Figures

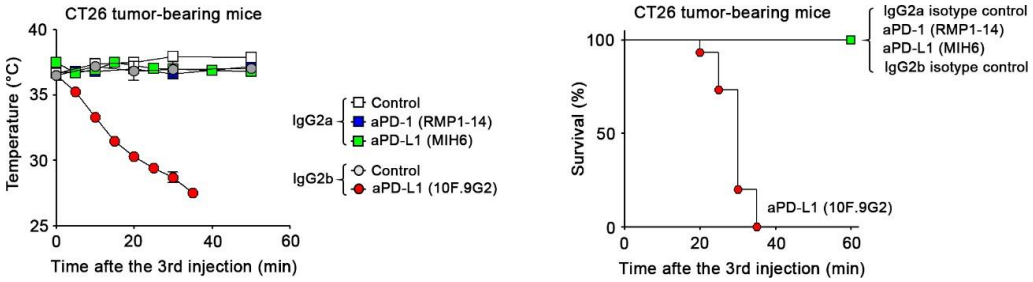

Figure S1 Induction of target- and clone-dependent anaphylaxis to PD-1/PD-L1 blockades.

A Body temperature of CT26 tumor-bearing mice was monitored after the third treatment with either control rat IgG2a (2A3) (n = 4), RMP1-14 (n = 4), MIH6 (n = 5), control rat IgG2b (LTF-2) (n = 4), or 10F.9G2 (n = 11). Lethal anaphylaxis was induced by 10F.9G2, but another aPD-L1 mAb (clone MIH6) and aPD-1 mAb (clone RMP1-14) treatment showed no anaphylactic symptoms. **B** The average tumor volumes of CT26 tumor-bearing mice treated with either RMP1-14 or 10F.9G2 at a dose of 200 μg/mouse on days 10, 13, and 17 post-inoculation.

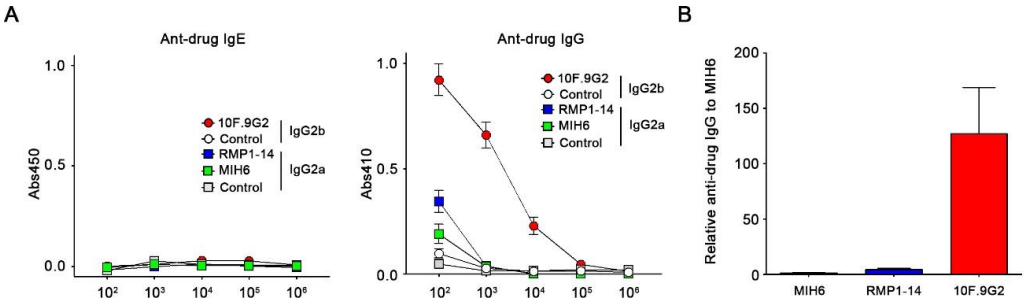

Figure S2 Evaluation of anti-drug antibodies against 10F.9G2, RMP1-14, and MIH6. **A** Concentrations of anti-drug IgE and IgG to 10F.9G2, RMP1-14, and MIH6 in serum were evaluated by ELISA. CT26 (n = 6) tumor-bearing mice were treated with rat 10F.9G2 (n = 6), RMP1-14 (n = 6), MIH6 (n = 4), control rat IgG2a (2A3, n = 4), and control rat IgG2b (LTF-2, n = 4) on days 10 and 13 post-inoculation, and serum was collected on day 17. The x-axis represents dilution of the serum sample, and the y-axis indicates the absorbance (A450 or A410). **B** Relative anti-drug IgG levels in the serum. Serum dilution ratios that resulted in an Abs 410 of 0.2 for anti-drug IgGs against 10F.9G2, RMP1-14, and MIH6 were determined. Data are represented as the mean ± S.E.

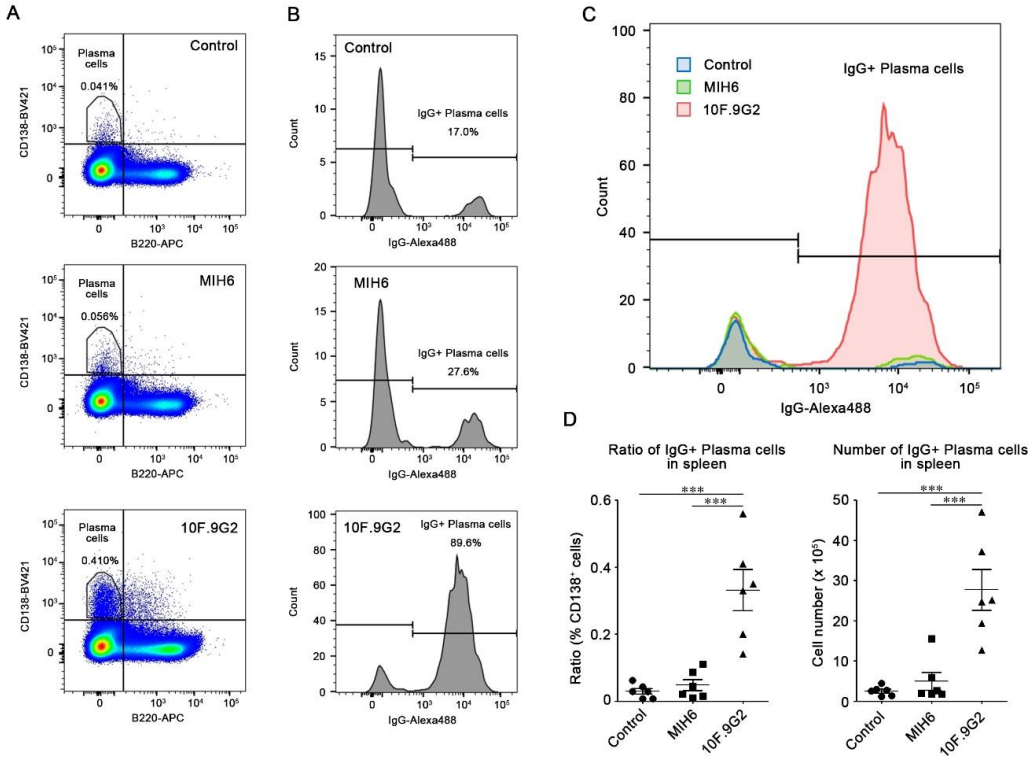

Figure S3 Flow cytometry analysis of IgG-expressing CD138$^+$ plasma cells in the spleen.

CT26 tumor-bearing mice were treated with either MIH6 or 10F.9G2 on days 10 and 13 post-inoculation. Untreated mice were used as a control. At day 17, splenocytes were collected, and surface CD138 and B220 were immunostained, followed by permeabilization and staining of IgG. Cells were analyzed using a flow cytometer as follows: a singlet gate was used to exclude doublets by gating the forward scatter-height (FSC-H) as a function of the forward scatter-area (FSC-A), followed by a viability gate to exclude dead cells. **A** CD138$^+$B220$^-$ plasma cells were gated from the living cells. **B** IgG expression was quantified in CD138$^+$B220$^-$ plasma cells. **C** Overlay histogram of IgG expression level in plasma cells from the untreated or treated mice with MIH6 and 10F.9G2. **D** Ratio and cell number of IgG-expressing plasma cells (n=6 per group). Data represent mean ± S.E. ***P < 0.001 by one-way ANOVA followed by Tukey test.

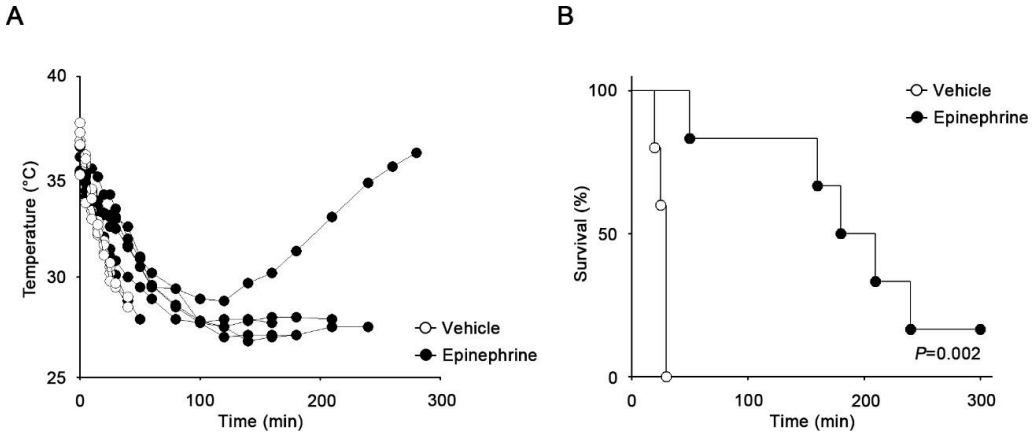

Figure S4 Treatment with epinephrine for anaphylaxis caused by 10F.9G2.

CT26 tumor-bearing mice were treated with 10F.9G2 on days 10 and 13 post-inoculation. Epinephrine (6 mg/kg, PBS 100 µl/mouse, n = 6) or vehicle (PBS 100 µl/mouse, n = 5) were i.p. injected 10 min before the 3rd treatment with 10F.9G2. **A** and **B** Body temperature and survival (right) of CT26 tumor-bearing mice treated with either vehicle (n = 5) or epinephrine (n = 6).

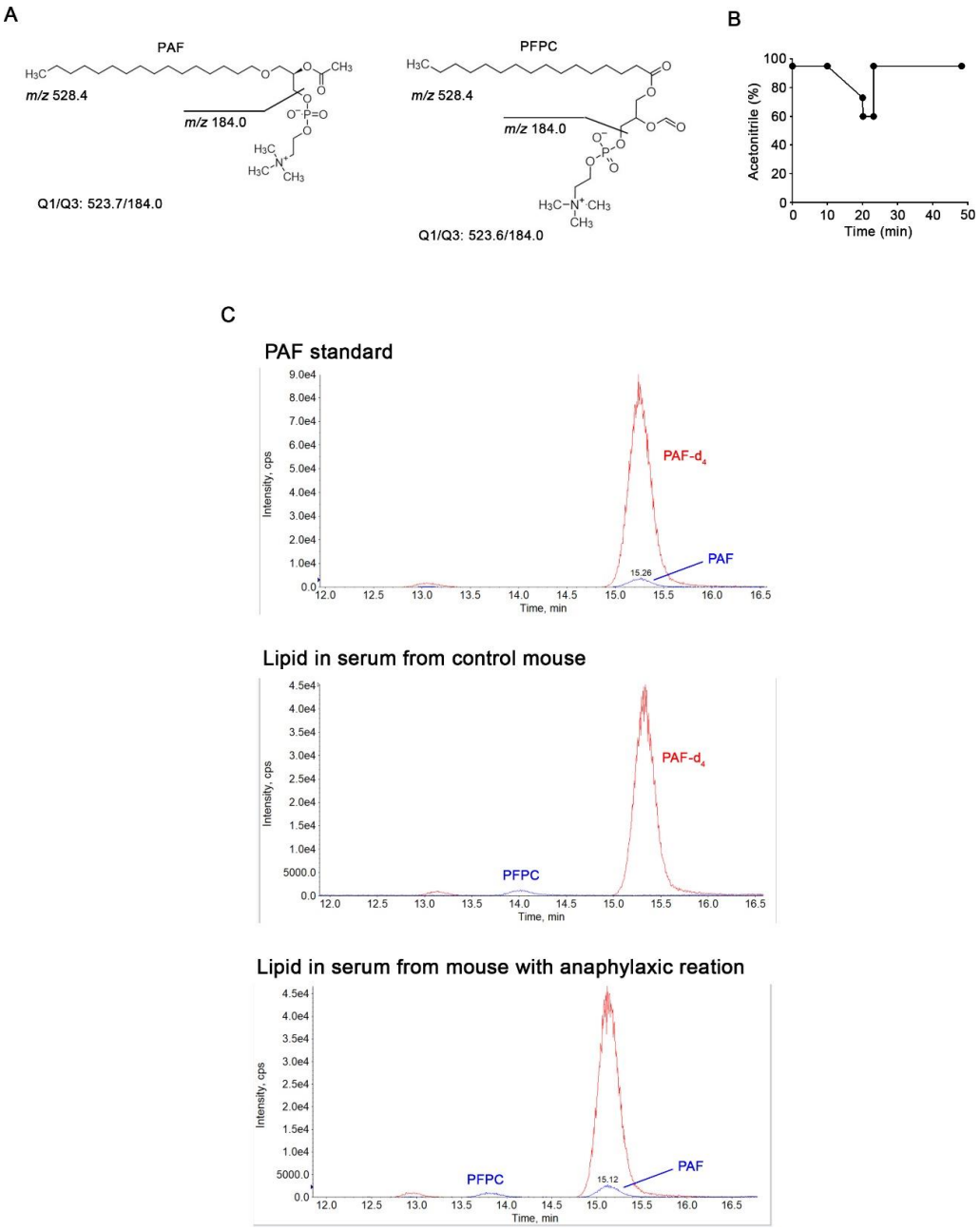

Figure S5 Quantification of platelet-activating factor (PAF) by LC-MS/MS.

A Molecular structure of PAF and palmitoyl-formyl-glycerophosphocholine (PFPC), and the positive ion MS/MS spectrum. **B** Gradient conditions used for LC separation. **C** LC-MS/MS chromatograms for PAF using hydrophilic interaction liquid chromatography (HILIC). PFPC and PAF were eluted at 14.1 and 15.2 min, respectively.

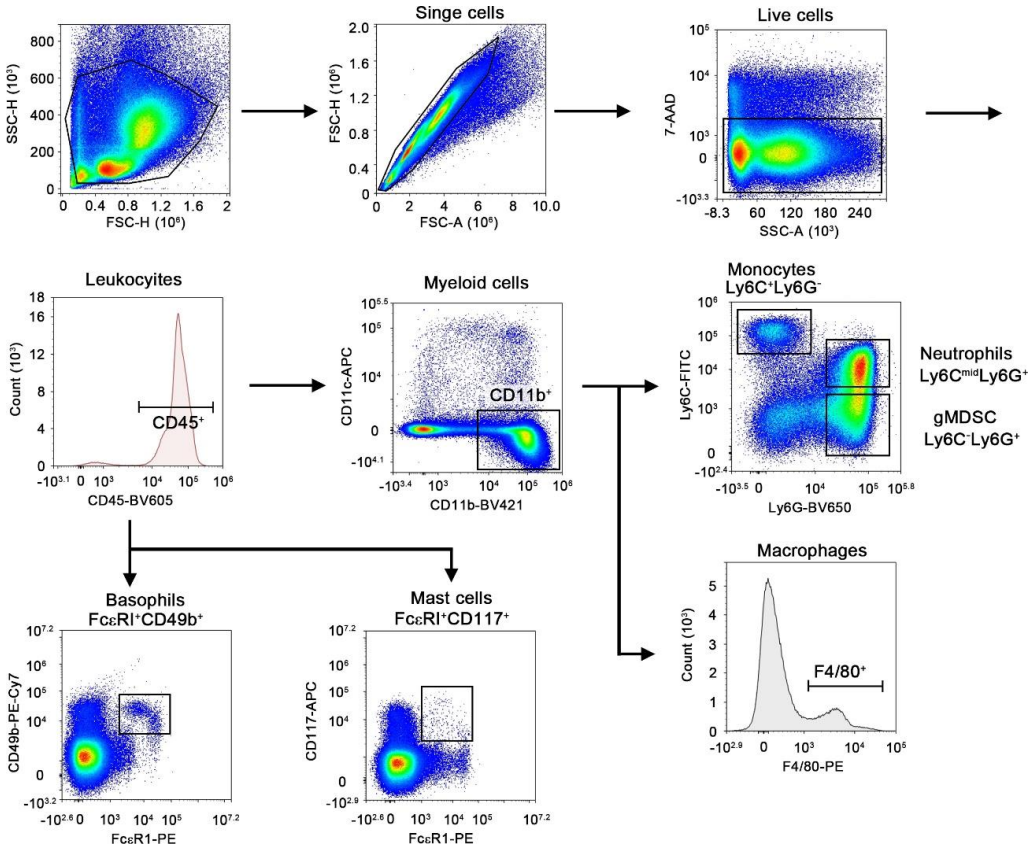

Figure S6 Flow cytometry analysis gating strategy for splenocytes.

A singlet gate was used to exclude doublets by gating the forward scatter-height (FSC-H) as a function of the forward scatter-area (FSC-A), followed by a viability gate to exclude dead cells. $CD11b^+$ myeloid cells were gated from $CD45^+$ leukocytes. $Ly6C^+Ly6G^-$ monocytes, $Ly6G^+Ly6C^{mid}$ neutrophils, $Ly6G^+Ly6C^-$ gMDSC, and $F4/80^+$ macrophages were gated from the $CD11b^+$ myeloid cell population. $FceRI^+CD49b^+$ basophils and $FceRI^+CD117^+$ mast cells were gated from the $CD45^+$ leukocyte population.