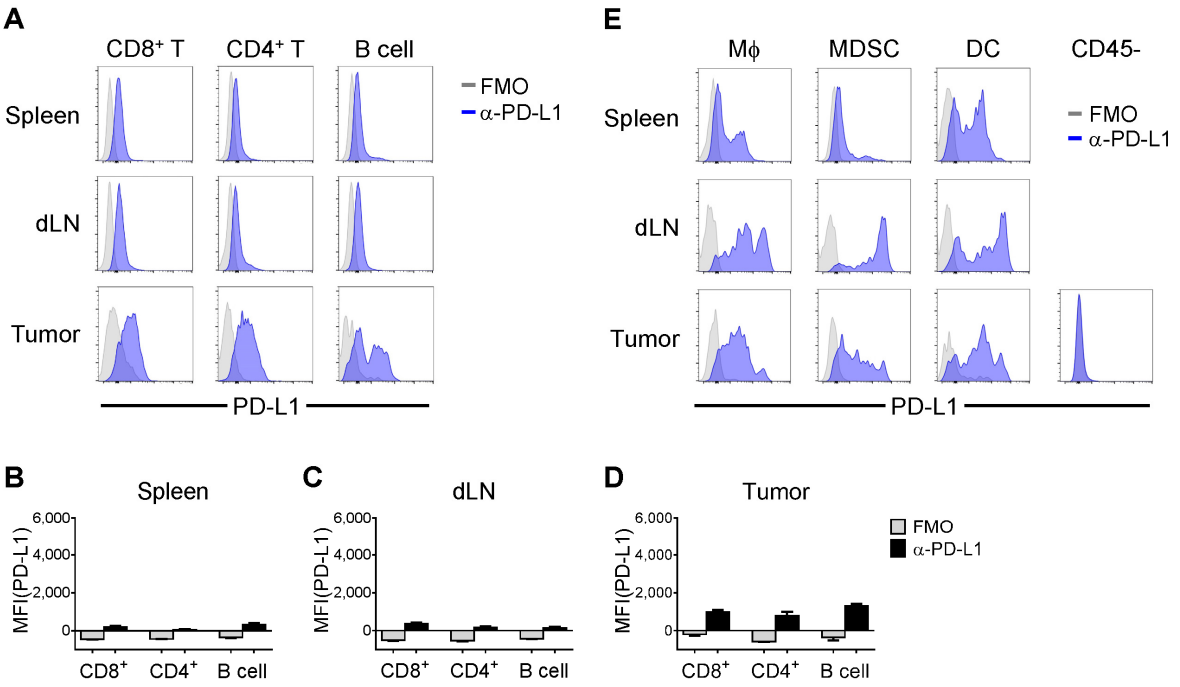
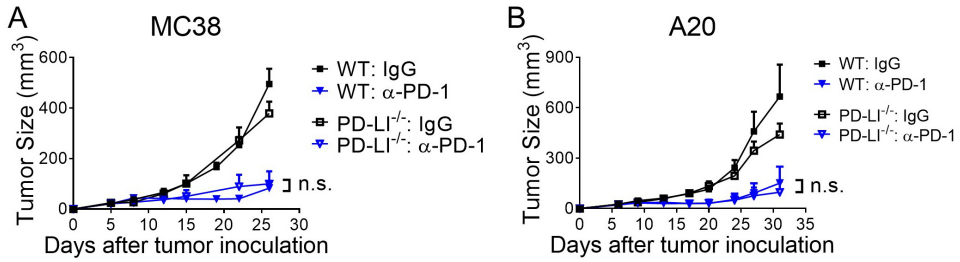
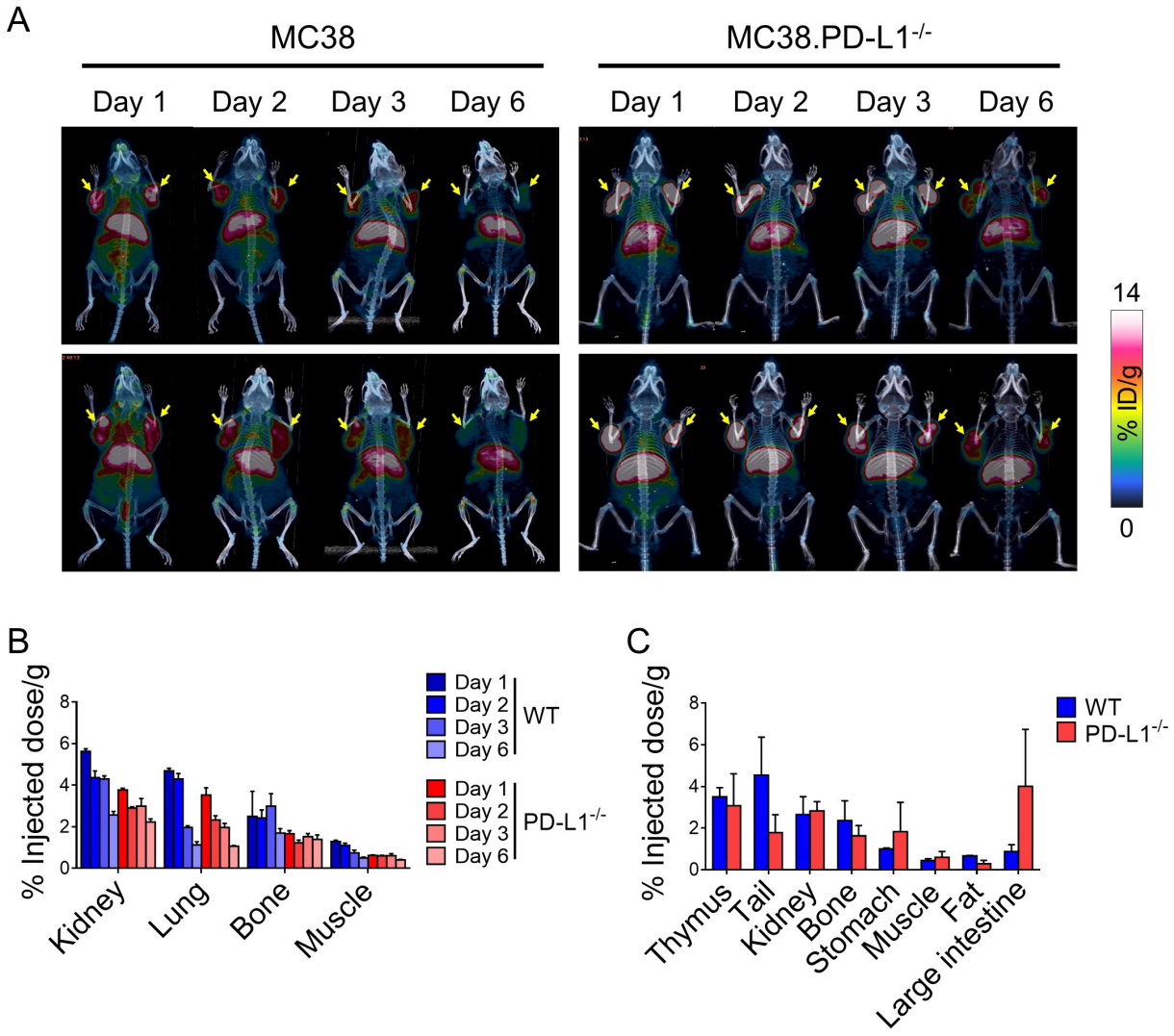
Supplemental data

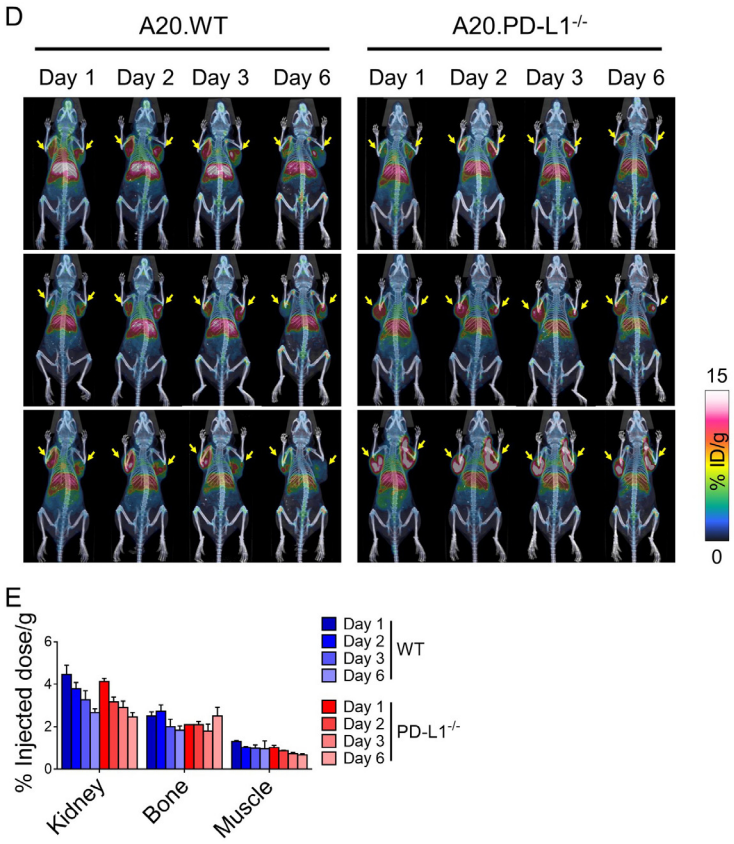


Supplementary Figure 1. PD-L1 is less abundant in lymphocytes. C57BL/6 mice were inoculated with 1×10^6 MC38 cells. Spleen, dLN, and tumor tissues were collected on day 22. PD-L1 expression in lymphocytes was measured by flow cytometry. (**A**) Representative flow cytometry histograms are shown. (**B-D**) MFIs of PD-L1 staining in spleen (**B**), dLN (**C**), and tumor (**D**) are shown (n = 3). (**E**) C57BL/6 mice were inoculated with MC38.PD-L1$^{-/-}$ cells. Spleen, dLN, and tumor tissues were collected on day 22. PD-L1 expression was measured by flow cytometry. Data indicate mean ± SEM and are representative of two independent experiments. Statistical analysis was performed using an unpaired Student's two-tailed t test.

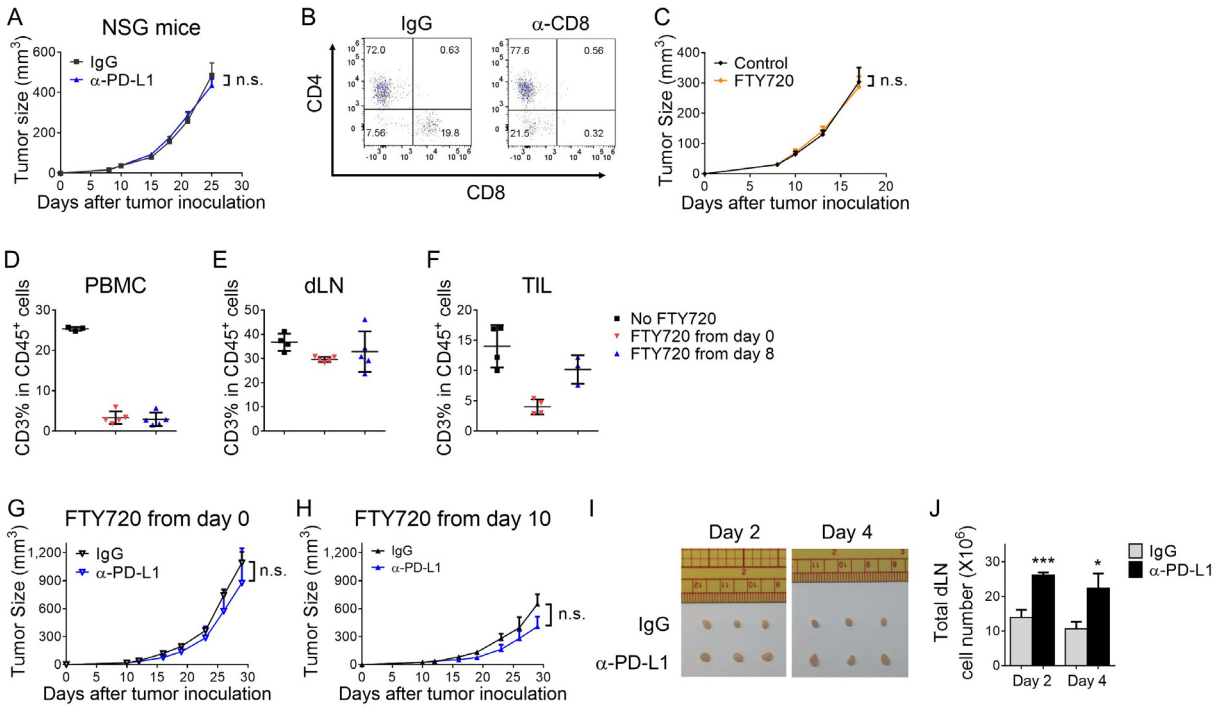


Supplementary Figure 2. Tumor expressed-PD-L1 is dispensable for the responses to anti-PD-1. (**A**) C57BL/6 mice (n = 5) were inoculated with 1×10^6 MC38.WT or MC38.PD-L1$^{-/-}$ cells. After tumors established, mice were treated with 200 μg anti-PD-1 on days 5, 8, and 10. Tumor growth was measured twice a week. (**B**) Balb/c mice (n = 5) were inoculated with 3×10^6 A20.WT or A20.PD-L1$^{-/-}$ cells. Mice were treated with 200 μg anti-PD-1 on days 6, 9, and 13. n.s., not significant. Data indicate mean ± SEM and are representative of two independent experiments. Statistical analysis was performed using an unpaired Student's two-tailed t test.


A
MC38
MC38.PD-L1-/-
Day 1
Day 2
Day 3
Day 6
14
% ID/g
0
B
% Injected dose/g
Day 1
Day 2
Day 3
Day 6
WT
PD-L1-/-
Kidney
Lung
Bone
Muscle
C
% Injected dose/g
WT
PD-L1-/-
Thymus
Tail
Kidney
Bone
Stomach
Muscle
Fat
Large intestine

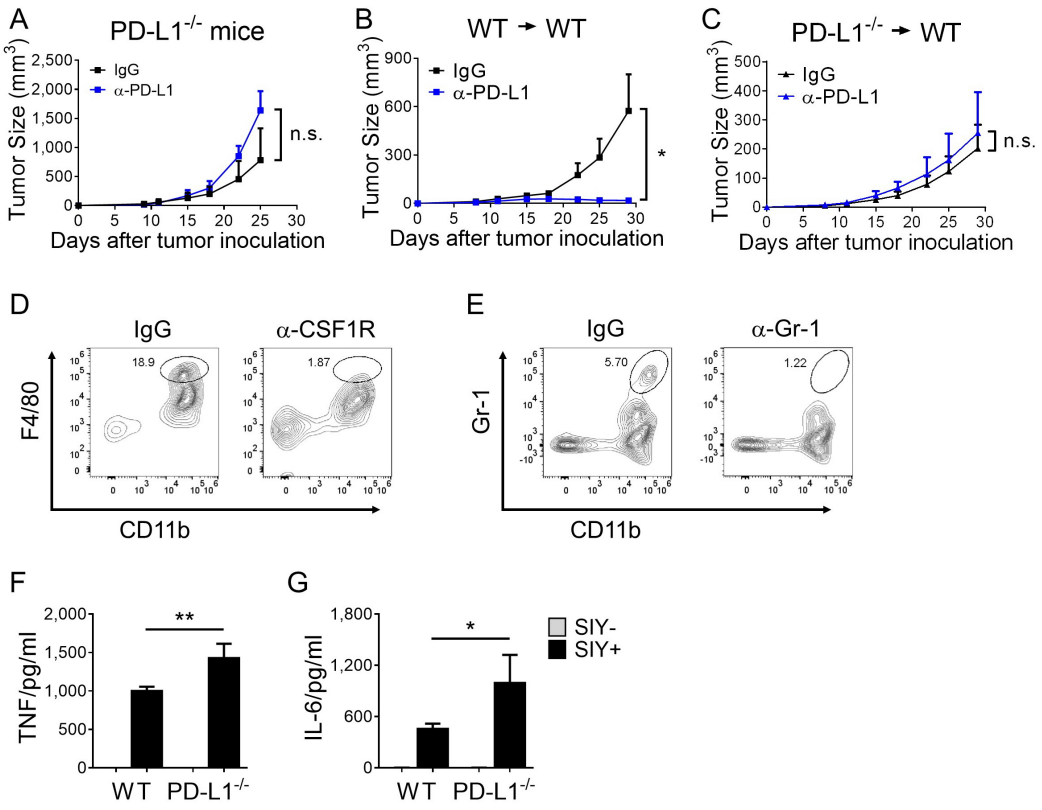


Supplementary Figure 3. Imaging data show the distribution of anti-PD-L1 antibody in tumor-bearing mice. (**A-C**) Mice were treated as in Figure 2A. (**A**) Antibody distribution was measured by PET/CT (n = 3). Two representative mice were shown. (**B**) Quantitation of the uptake of ^{89}Zr-anti-PD-L1 antibody on days 1, 2, 3, and 6 post injection. (**C**) *Ex vivo* biodistribution of ^{89}Zr-anti-PD-L1 antibody uptake on day 6 post injection is shown. (**D**) A20.WT or A20.PD-L1$^{-/-}$ tumor-bearing mice were injected with 50 µCi of ^{89}Zr radiolabeled deferoxamine conjugated anti-PD-L1 (^{89}Zr-anti-PD-L1) antibody (n = 3 per group). Antibody distribution was imaged by PET/CT on 1, 2, 3, and 6 days post injection. (**E**) The uptake of ^{89}Zr-anti-PD-L1 antibody in A20 tumors was measured by PET/CT and quantitated in various organs on days 1, 2, 3, and 6 post injection. Yellow arrows indicate tumors. Statistical analysis was performed using an unpaired Student's two-tailed t test.



Supplementary Figure 4. FTY720 treatment abrogates antitumor effects of checkpoint blockade. (**A**) NSG mice (n = 5) were inoculated with 1×10^6 MC38 cells. After tumors established, mice were treated with 200 μg anti-PD-L1 on days 11 and 14. Tumor growth was measured twice a week. (**B**) MC38 tumor-bearing mice were treated with 200 μg control IgG or anti-CD8 antibody. Two days later, tumor tissues were collected and tumor-infiltrating $CD3^+$ T cells were analyzed by flow cytometry. (**C**) NSG mice (n = 5) were inoculated with 1×10^6 MC38 cells. FTY720 were injected from day 0. Tumor growth curve is shown. (**D-F**) MC38 tumor-bearing mice were treated with FTY720 from day 0 or day 8. Tissues were collected on day 11. Percentages of $CD3^+$ T cells among $CD45^+$ cells in PBMC (**D**), draining lymph node (**E**), and tumor tissues (**F**) were measured by flow cytometry. (**G-H**) A20 tumor-bearing mice (n = 5) were treated with 200 μg IgG or anti-PD-L1 on days 10 and 13. Mice were also treated with FTY720 from day 0 (**G**) or day 10 (**H**) after tumor inoculation. (**I**) MC38 tumor-bearing mice were treated with 200 μg IgG or anti-PD-L1 on day 8. Draining LNs were collected 2 or 4 days after treatment (n = 3). (**J**) Total cell numbers in

dLN were shown. Data indicate mean ± SEM and are representative of at least two independent experiments. Statistical analysis was performed using an unpaired Student's two-tailed t test.



Supplementary Figure 5. Antitumor effects of anti-PD-L1 depend on PD-L1 from hematopoietic cells. (**A**) PD-L1$^{-/-}$ mice were inoculated with 1×10^6 E.G7 cells. Mice were treated with 200 μg IgG or anti-PD-L1 on days 9 and 12 (n = 4). Tumor growth was measured twice a week. (**B-C**) C57BL/6 mice were reconstituted with bone marrow cells from WT (**B**) or PD-L1$^{-/-}$ (**C**) mice. Ten weeks after reconstitution, mice were inoculated with 1×10^6 E.G7 cells and treated with 200 μg anti-PD-L1 on days 12 and 15 (n = 5). (**D-E**) Mice were treated with depletion antibodies. Depletion of macrophages (**D**) or MDSCs (**E**) in tumor tissues were evaluated by flow cytometry. (**F-G**) BMDMs from WT or PD-L1$^{-/-}$ mice were loaded with SIY peptide, then co-cultured with 2C T cells for 3 days. TNF (**F**) and IL-6 (**G**) levels in culture supernatants were measured by CBA. Statistical analysis was performed using an unpaired Student's two-tailed t test. *, $p<0.05$. **, $p<0.01$.

Supplementary Table 1. List of antibodies used.

Antibody	Supplier	Catalog number	Dilution	Application
7-AAD	BioLegend	420404	1:100	Flow cytometry
APC anti-mouse CD11c	BioLegend	117310	1:500	Flow cytometry
APC anti-mouse PD-L1	BioLegend	124312	1:200	Flow cytometry
APC/Cy7 anti-mouse CD8a	BioLegend	100714	1:500	Flow cytometry
APC/Cy7 anti-mouse Gr-1	BioLegend	108424	1:200	Flow cytometry
BV421 anti-mouse CD45	BioLegend	103133	1:500	Flow cytometry
FITC anti-mouse B220	BioLegend	103206	1:200	Flow cytometry
FITC anti-mouse CD69	BioLegend	104506	1:200	Flow cytometry
FITC anti-mouse F4/80	BioLegend	123108	1:200	Flow cytometry
PE anti-mouse CD11c	BioLegend	117308	1:500	Flow cytometry
PE anti-mouse CD3e	BioLegend	100308	1:500	Flow cytometry
PE/Cy7 anti-mouse CD4	BioLegend	100422	1:1000	Flow cytometry
APC anti-mouse CD3e	eBioscience	17-0031-82	1:500	Flow cytometry
APC anti-mouse CD8a	eBioscience	17-0081-82	1:500	Flow cytometry
PE anti-mouse PD-L1	eBioscience	12-5982-83	1:200	Flow cytometry
PE/Cy7 anti-mouse CD11b	eBioscience	25-0112-82	1:1000	Flow cytometry