

## Supplementary figure legends

### Figure S1. Effect of anti-PD-L1 treatment on LLC volume in mice.

(A-C) WT, NSG, and Rag1<sup>-/-</sup> mice were inoculated with LLC tumor cells. The mice were treated from day 3 every 3 days with anti-PD-L1 or isotype control (rlgG1). Tumor volume was monitored. n = 5-7. Wilcoxon test was used for two-way comparisons.

### Figure S2. Expression and effect of tumor PD-L1 on tumor growth.

(A-B) WT, PD-L1<sup>-/-</sup>, and PD-1<sup>-/-</sup> mice were inoculated with MC38 (A), and ID8 (B) tumor cells. Tumor volume was monitored. n = 5-7. T-test was used for two-way comparisons (\*P < 0.05). (C-E) CRISPR PD-L1 homozygous PD-L1 knockout (PD-L1<sup>-/-</sup>) tumor cell clones were made for MC38 (C), ID8 (D), and B16-F10 (E). PD-L1<sup>-/-</sup> and control tumor cells were stimulated for 48 hours with or without IFN $\gamma$  (10ng/ml). PD-L1 expression was analyzed by FACS. One of 4 experiments is shown. (F-I) PD-L1<sup>-/-</sup> MC38 (F, G), PD-L1<sup>-/-</sup> B16-F10 (H, I) and wild type control tumor cells were inoculated into NSG and Rag1<sup>-/-</sup> mice. Tumor load was monitored. n=5 mice per group. (J) PD-L1<sup>-/-</sup> MC38 cells were inoculated into wild type mice. Single cells were prepared from tumor tissues on day 15. PD-L1 expression was analyzed by FACS on CD45<sup>+</sup> immune cells and CD45<sup>-</sup> tumor cells. One of 5 is shown. (K-L) PD-L1 overexpressed (PD-L1-OV) or scrambled MC38 cells were analyzed for PD-L1 expression by FACS (K). PD-L1<sup>-/-</sup> mice were inoculated with PD-L1-OV MC38 cells (L, n = 8) and treated with anti-PD-L1 or isotype control (rlgG1). Tumor volume was monitored. Wilcoxon test was used for two-way comparisons. (\*P < 0.05).

### Figure S3. Effect of anti-PD-L1 on T cell effector cytokine expression.

(A-G) T cell effector cytokines were analyzed with intracellular staining in MC38 tumor draining lymph nodes (TDLN) (A, B), MC38 tumor tissues (C-E), and ID8 tumor ascites (F, G) in PD-L1<sup>-/-</sup> and PD-1<sup>-/-</sup> mice. Data are expressed as the mean  $\pm$  SEM (n = 3-5 per group). Representative original flow cytometry data are shown (E). (H, I) T cell infiltration was analyzed with immunofluorescence staining for CD3 in tumors from PD-L1<sup>+/+</sup> and PD-L1<sup>-/-</sup> mice. Data are expressed as the mean  $\pm$  SEM (I, n = 10 per group). Representative staining images are shown (H). T-test was used for two-way comparisons (\*P < 0.05). (J, K) T cell effector cytokines were analyzed with intracellular staining in MC38 tumor in WT and PD-L1<sup>-/-</sup> mice, n = 5. T-test was used for two-way comparisons (\*P < 0.05).

### Figure S4. APC subsets in tumor tissues and tumor PD-L1 expression *in vivo*.

(A-C) PD-L1 expression in tumor infiltrating immune cells. (A) PD-L1 expression on CD45<sup>+</sup> immune cells in MC38 tumor tissues. (B) FACS gating on different immune cell subsets in MC38 tumor tissues. Representative flow cytometry data showed gates on CD45<sup>+</sup>CD90<sup>-</sup> cells, DCs, macrophages, and MDSCs. (C) The percentages of PD-L1 expression in each immune cell subset in MC38 and ID8 ascites. One of five replicates is shown. (D) PD-L1 expression on tumor cells *in vivo*. Tumor cells were isolated from wild type and PD-L1<sup>-/-</sup> mice. PD-L1 expression was detected by Western blots in tumor cells. Experiments were performed in triplicates; representative replicate is shown. (E) CD45.1<sup>+</sup> peritoneal APCs were injected into ID8 tumor bearing CD45.2<sup>+</sup> mice. 48 hours after APC injection, CD45.1<sup>+</sup> APCs were examined in ID8 tumor tissues and ascites by flow cytometry. n = 5. (F) MC38 tumor bearing PD-L1<sup>-/-</sup> mice were adoptively transferred with (black) or without (red) WT DCs. Mice were treated with anti-PD-L1 or isotype IgG1. Tumor volume was monitored. Wilcoxon test was used for two-way comparisons (n = 7, \*P < 0.05).

**Figure S5. PD-L1<sup>+</sup> APCs and tumor cells in patients with melanoma and ovarian cancer.**

(A-B) Percentages of patients with PD-L1<sup>+</sup> tumor cells or PD-L1<sup>+</sup> non-tumor cells were shown in melanoma (A) and ovarian cancer (B) tissues.