# Supplementary Table

Table S1. Sequences of RT-PCR primers used in this study

| Gene | Sequence (5'to 3') | Length |
|---|---|---|
| *Pd-1*-S | ACCCTGGTCATTCACTTGGG | 20 |
| *Pd-1*-AS | CATTTGCTCCCTCTGACACTG | 21 |
| *Pd-l1*-S | GCTCCAAAGGACTTGTACGTG | 21 |
| *Pd-l1*-AS | TGATCTGAAGGGCAGCATTTC | 21 |
| *Pd-l2*-S | CTGCCGATACTGAACCTGAGC | 21 |
| *Pd-l2*-AS | GCGGTCAAAATCGCACTCC | 19 |
| *Lag3*-S | CTGGGACTGCTTTGGGAAG | 19 |
| *Lag3*-AS | GGTTGATGTTGCCAGATAACCC | 22 |
| *Tim3*-S | TCAGGTCTTACCCTCAACTGTG | 22 |
| *Tim3*-AS | GGCATTCTTACCAACCTCAAACA | 23 |
| *β-Actin*-S | GGCTGTATTCCCCTCCATCG | 20 |
| *β-Actin*-AS | CCAGTTGGTAACAATGCCATGT | 22 |

# Supplementary Figures

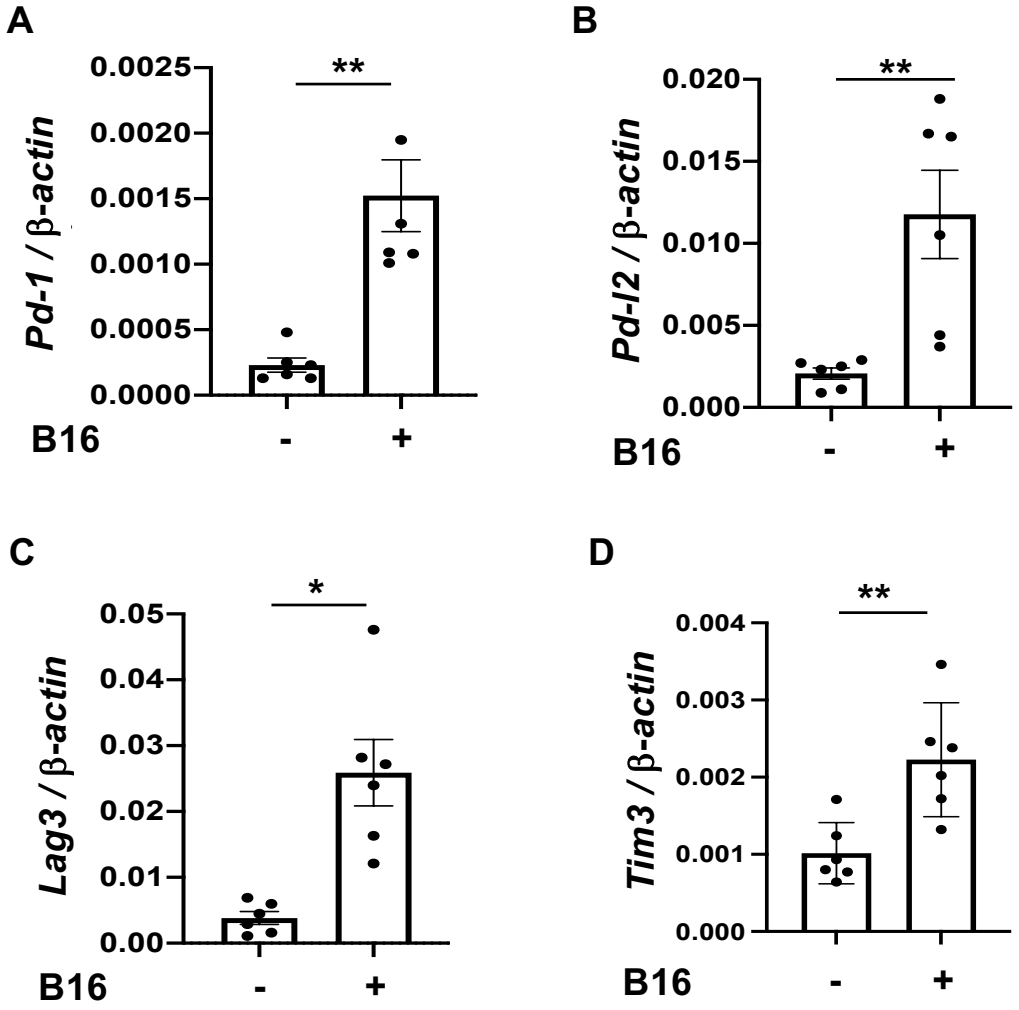


**Figure S1. Pulmonary melanoma metastasis stimulates PD-1, PD-L2, LAG3 and TIM3.** 8-week-old WT mice were given B16-F10 (B16) melanoma cells or control vehicle (PBS) and the expression of immune checkpoint molecules was evaluated 2 weeks later. Real-time RT-PCR (RT-PCR) was used to quantitate the levels of mRNA encoding PD-1 (A), PD-L2 (B), LAG3 (C) and TIM3 (D) in the lungs from mice treated intravenously with PBS (B16 -) or B16 cells (B16 +). Each dot represents an evaluation in an individual animal. The plotted values represent the mean±SEM of the evaluations represented by the individual dots. *p<0.05, **p<0.01 (*t*-test).

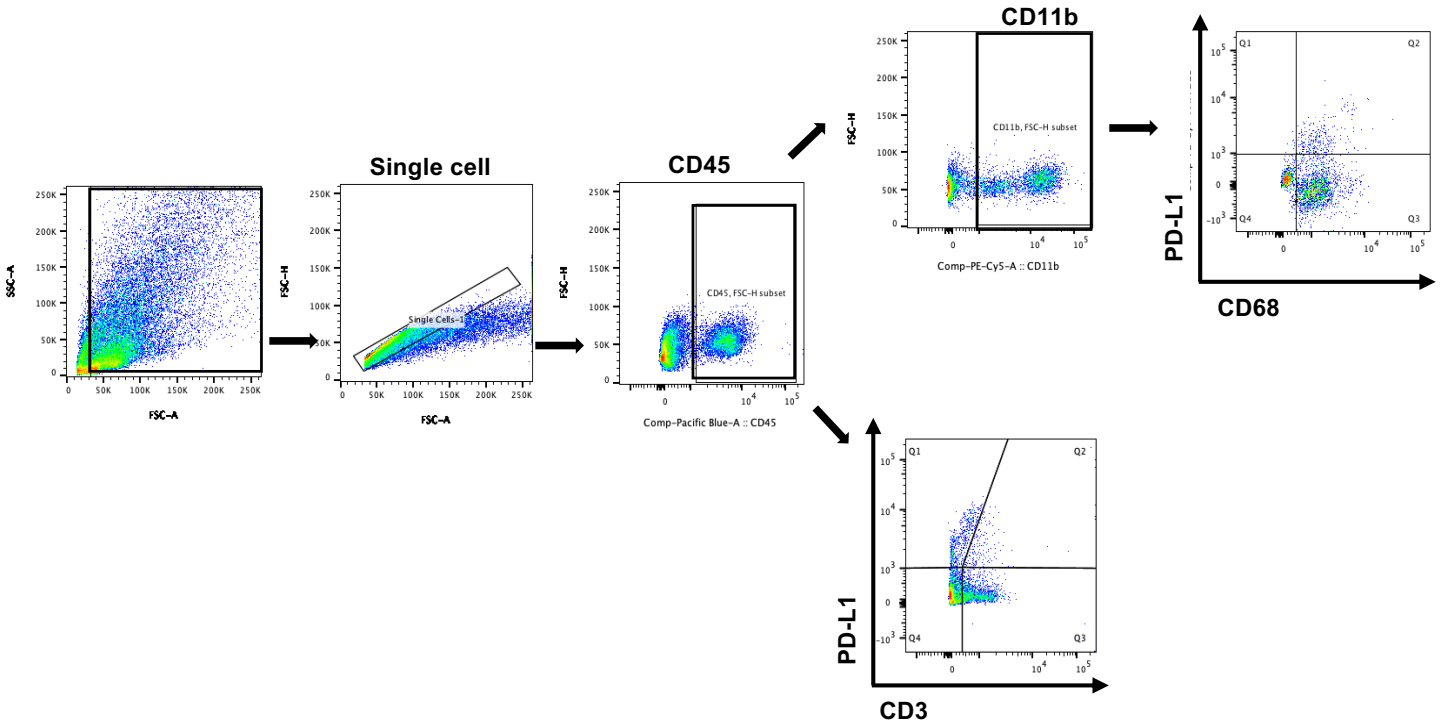


**Figure S2. Gating strategy of the FACS analysis used in the evaluation of macrophages and T cells in the lung of mice after melanoma cell challenge.** 8-week-old WT mice were challenged with vehicle (PBS) or B16-F10 melanoma cells, then the lungs were harvested on day 14 after melanoma cell injection and subjected to FACS evaluation. The inflammatory cells were first enriched with CD45 (+) then followed CD11b/CD68(+) for macrophages and CD3(+) T cells were subjected to the evaluation of PD-L1 expression.

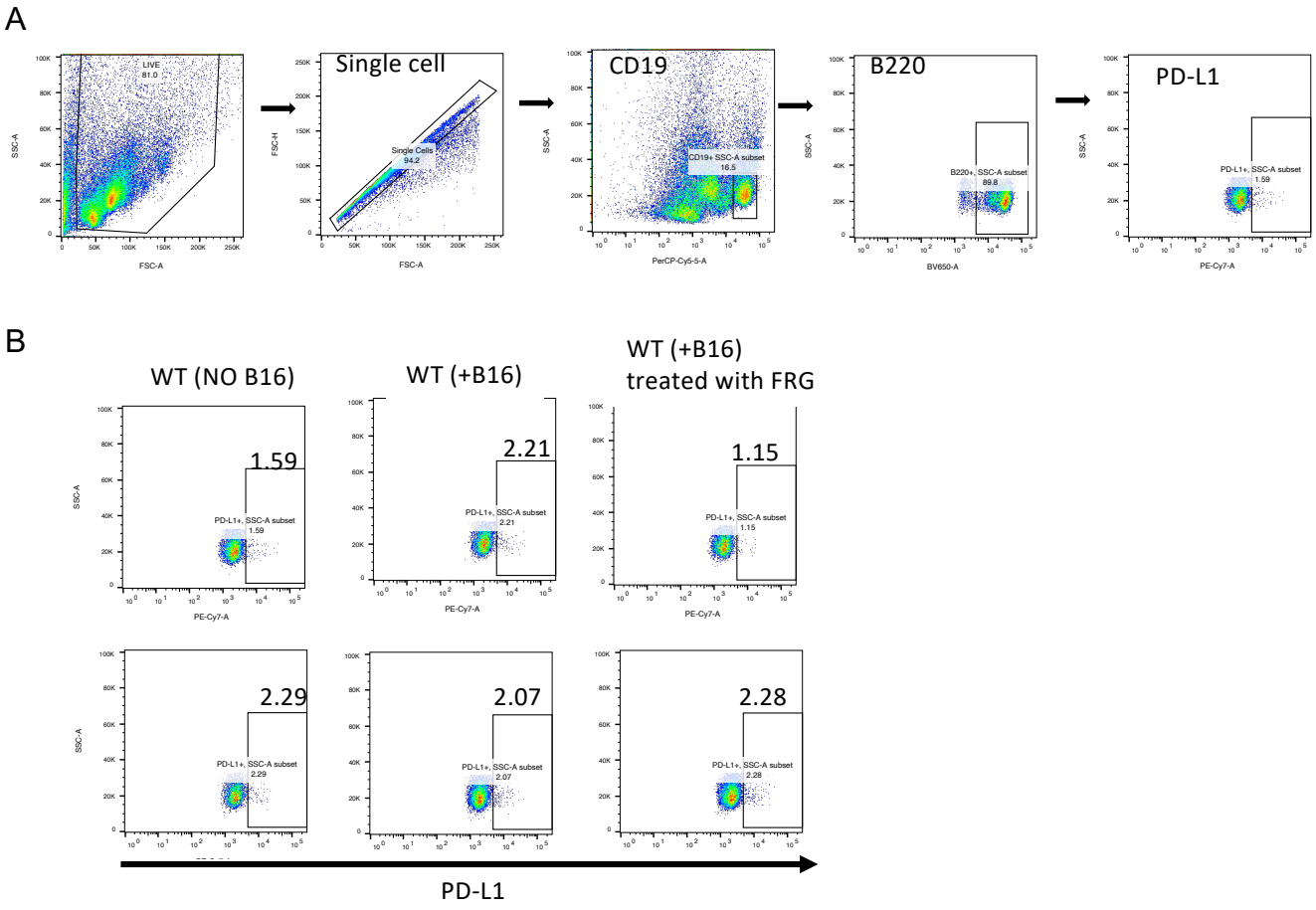


**Figure S3. PD-L1 expression in lung resident B cells with and without melanoma lung metastasis**. 8 weeks old WT mice were challenged with vehicle (PBS) or B16-F10 melanoma cells, then the lungs were harvested on day 14 after melanoma cell injection and subjected to FACS evaluation. (A) Gating strategy of lung resident B cells (CD19+/B220+) expressing PD-L1. (B) Representative FACS evaluations (two mice/group) on the PD-L1 expressing lung resident B cells.

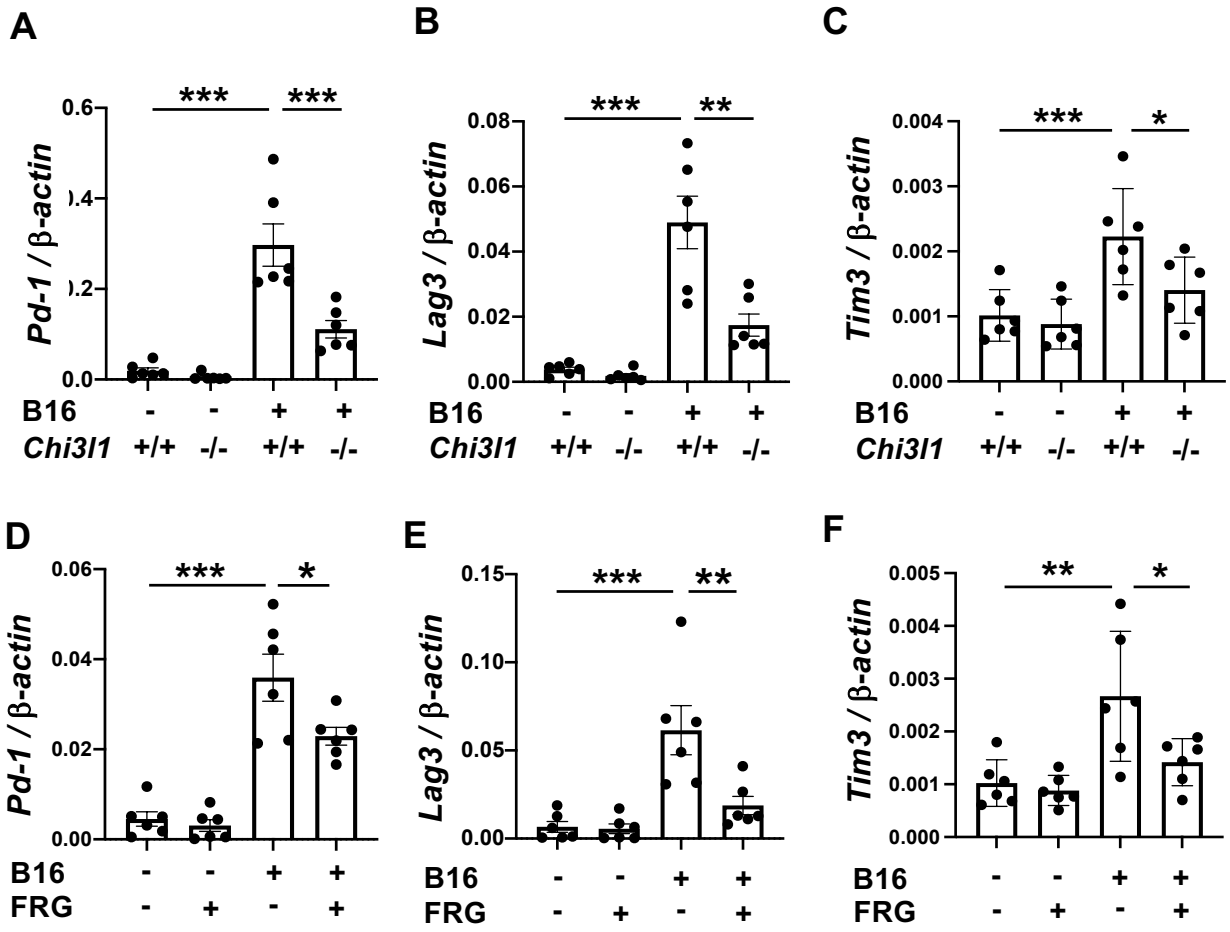


**Figure S4. Chi3l1 plays a critical role in B16 melanoma stimulation of pulmonary PD-1, LAG3 and TIM3.** 8-week-old WT (+/+) and Chi3l1 null (-/-) (*Chi3l1*$^{-/-}$) mice were given B16 melanoma cells or vehicle control. They were then treated with an anti-Chi3l1 antibody (FRG) or isotype control antibodies and the expression of PD-1, LAG3 and TIM3 was evaluated 2 weeks later. (A-C) RT-PCR was used to quantitate the levels of mRNA encoding PD-1, LAG3 and TIM3 in the lungs from wild type (WT) (*Chi3l1* +/+) and Chi3l1 null (*Chi3l1* -/-) mice given PBS vehicle (B16 -) or B16 cells (B16 +). (D-F) RT-PCR evaluation of the levels of mRNA encoding PD-1, LAG-3 and TIM3 in the lungs from WT mice treated with PBS vehicle (B16 -) or B16 cells (B16 +) that were treated with FRG (FRG+) or vehicle (FRG-). Each dot represents the evaluation in an individual animal. The plotted values represent the mean ± SEM of the noted evaluations represented by the individual dots. **P*<0.05, ***P*<0.01, ***P<0.001 (One-way ANOVA with Turkey post hoc test).

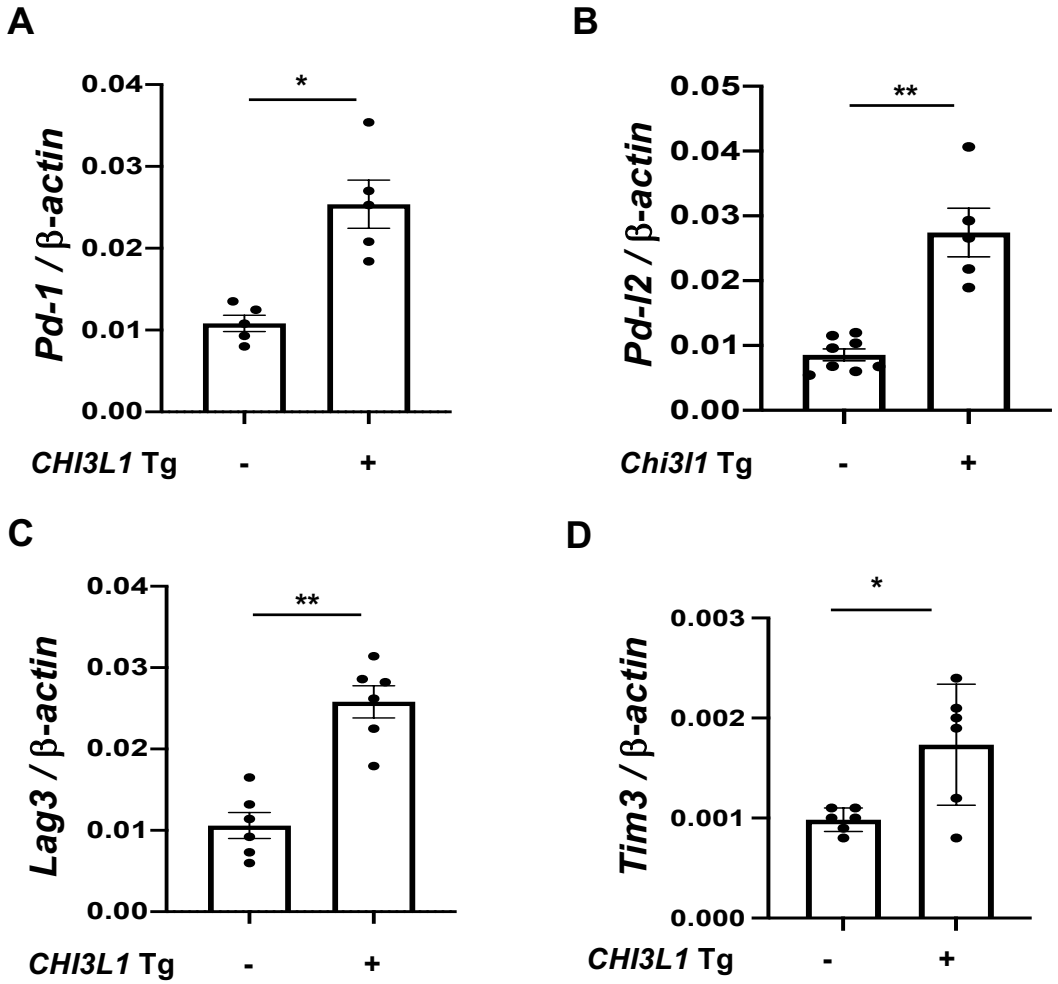


**Figure S5. Transgenic Chi3l1 stimulates PD-1, PD-L2, LAG3 and TIM3 in the normal lung.** 8-week-old WT (-), *CHI3L1* Tg (+) mice were used to evaluate the levels of mRNA encoding PD-1 (A), PD-L2 (B), LAG3 (C), and TIM3 (D) in the lung. RT-PCR was used to quantitate the levels of mRNA in the lungs from WT mice (CHI3L1 Tg -) and mice in which CHI3L1 was overexpressed in the lung in a transgenic manner (*CHI3L1* Tg +). Each dot represents the evaluation in an individual animal. The plotted values represent the mean ± SEM of the noted evaluations represented by the individual dots. *p<0.05,**p<0.01 *(t* test).

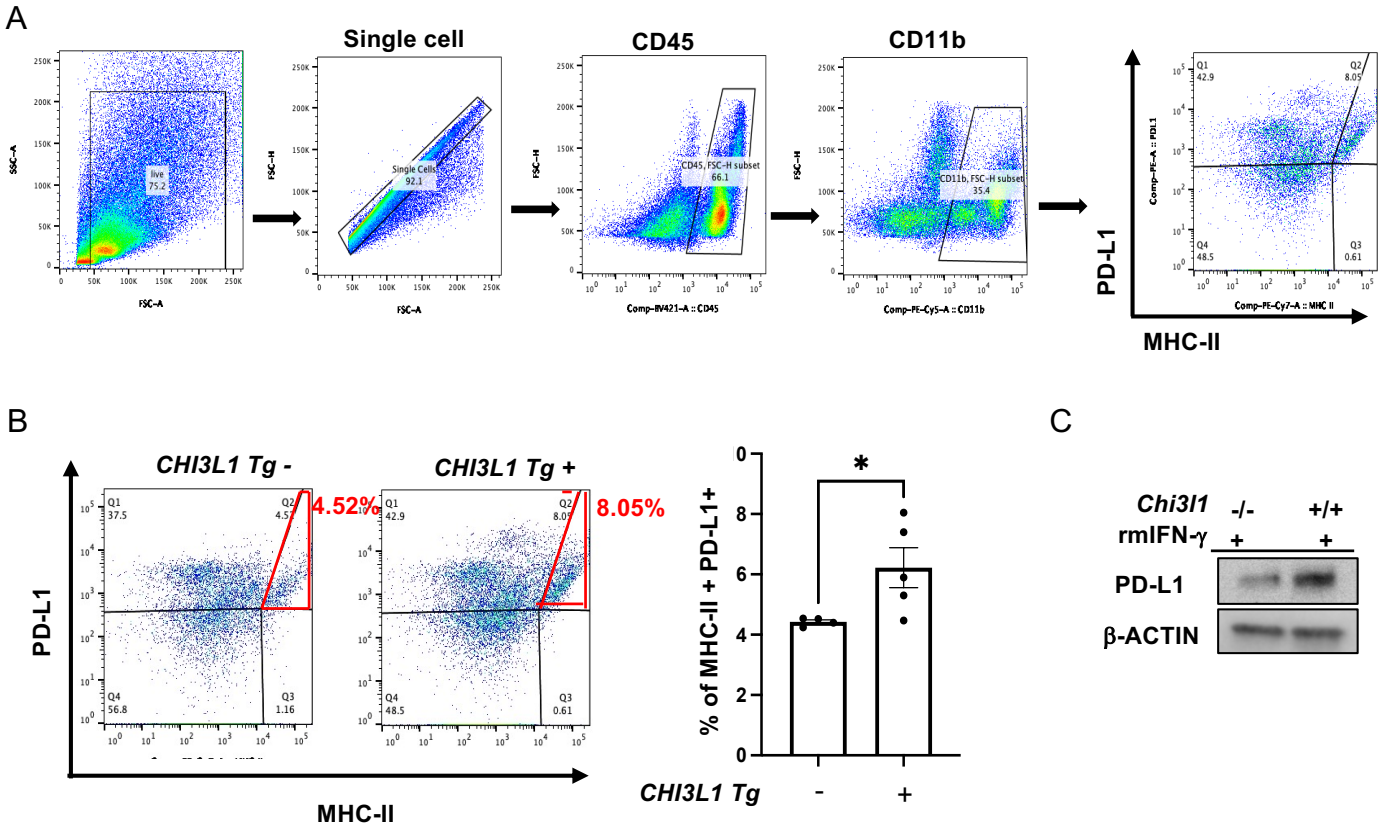


**Figure S6. Chi3l1 mediates rIFN-γ-stimulated expression of PD-L1 in lung resident macrophages.** (A) Gating strategy used for the evaluation of PD-L1 in lung resident macrophages (CD45+/CD11b(+)/MHCII(+)/PD-L1(+) cells). (B) FACS evaluations on PD-L1 expression in lung resident macrophages from WT (*CHI3L1*-) and CHI3L1 Tg mice. (C) Western blot evaluation to test the ability of rIFN-γ to stimulate PD-L1 in lung resident macrophages from WT and Chi3l1$^{-/-}$ mice. The values in bar graph of panel B represent the mean±SEM of the noted evaluations. *p<0.05 (*t* test).

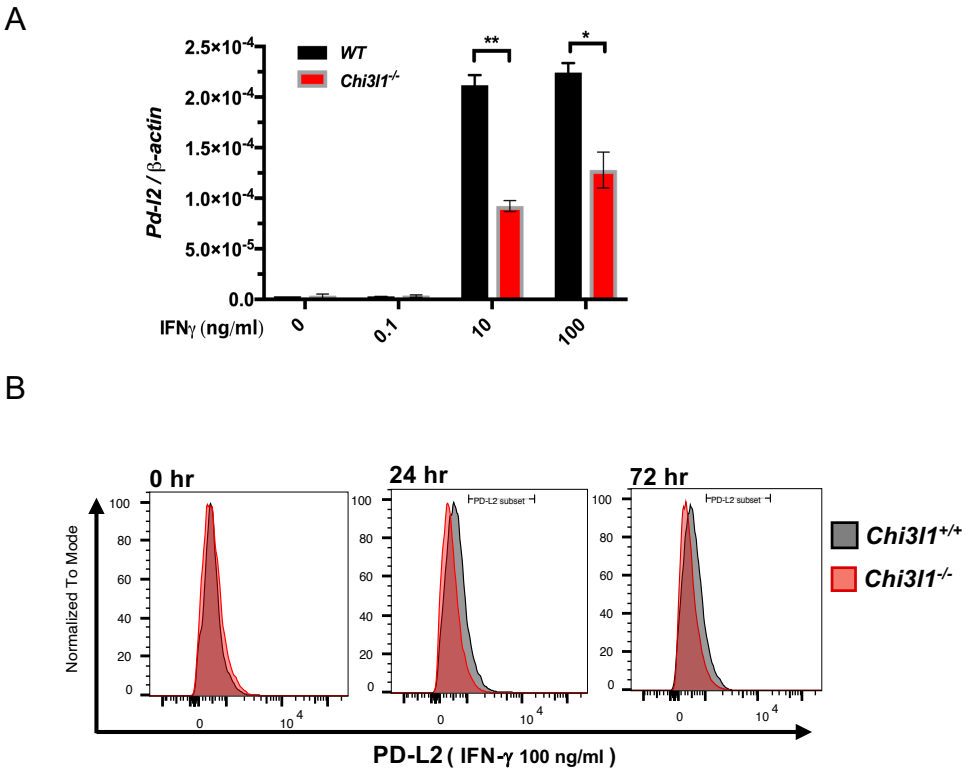


**Figure S7. IFN-γ-stimulates macrophage PD-L2 via a Chi3l1-dependent mechanism.** Bone marrow derived macrophages (BMDM) prepared from 6-8 weeks old male WT and Chi3l1$^{-/-}$ mice and were used to evaluate the importance of Chi3l1 in rIFN-γ stimulation of PD-L2. (A) rIFN-γ-stimulation of PD-L2 mRNA expression in BMDM from WT and Chi3l1$^{-/-}$ mice. (B) FACS evaluations of the ability of rIFN-γ to stimulate PD-L1 in BMDM prepared from WT and Chi3l1$^{-/-}$ mice. The values in panel A represent the mean±SEM of the noted evaluations (n=5 mice/each). *p<0.05,**p<0.01 (*t* test). Panel B is a representative of a minimum of 2 separate evaluations.

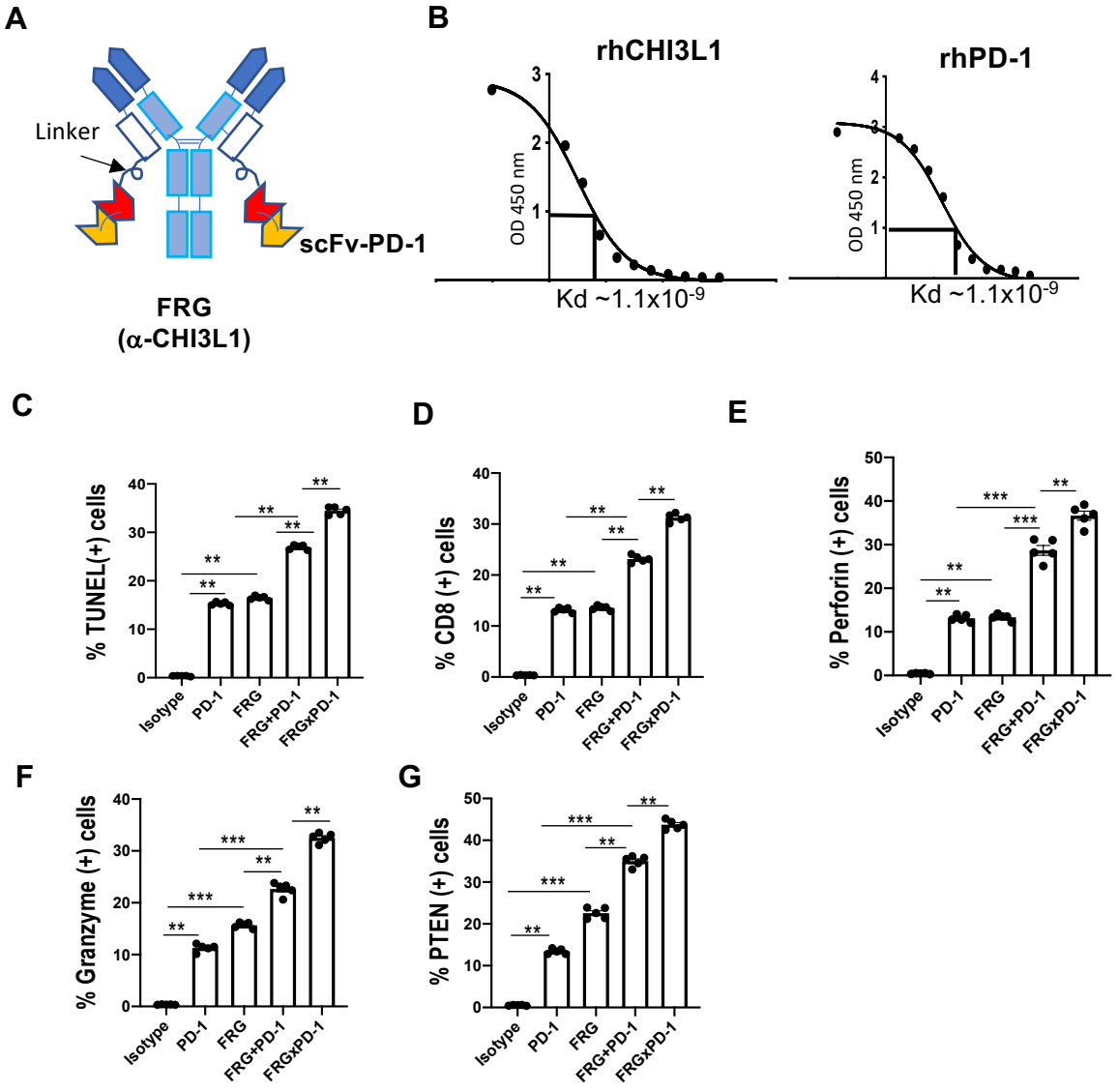


**Figure S8. The structure and the binding affinity of FRGxPD1 bispecific antibody and synergistic CTL-mediated tumor cell response and PTEN accumulation**. (A) Schematic illustration of the structure of the bispecific antibody FRGxPD-1 in which anti-PD-1 is linked to FRG via its light chain. (B) The affinity of FRGxPD1 antibody was evaluated by competitive ELISA against recombinant human (rh) CHI3L1 and rhPD-1. (C-G) Bispecific antibodies that simultaneously target Chi3l1 and PD-1 induce synergistic CTL-mediated tumor cell death responses and tumor cell PTEN accumulation. The antitumor effects of FRGxPD-1 bispecific antibody was tested in co-culture of system of Jurkat cells and B16-F10 murine melanoma cells as described in the Materials and Methods. (C) Quantitation of apoptotic tumor cell death using in situ cell death detection kit-fluorescein dUTP. (D-G) Quantification of T cell CD8+ (D), perforin (E) and granzyme (F) accumulation. (G) quantification of tumor cell PTEN accumulation in co-cultures. These evaluations were undertaken using fluorescent microscopy (x20 of original magnification). In these quantifications, 10 randomly selected fields were evaluated. The values in in these panels are the mean ± SEM. **p<0.01, ***<0.001 (One-way ANOVA with Turkey post hoc test).