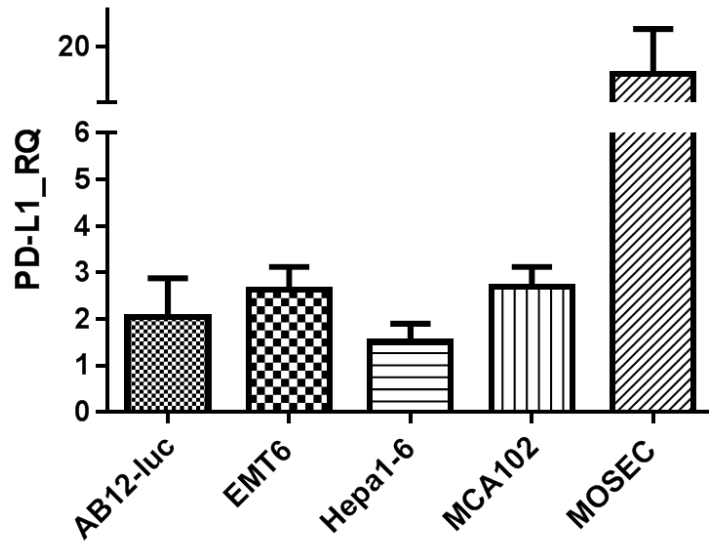
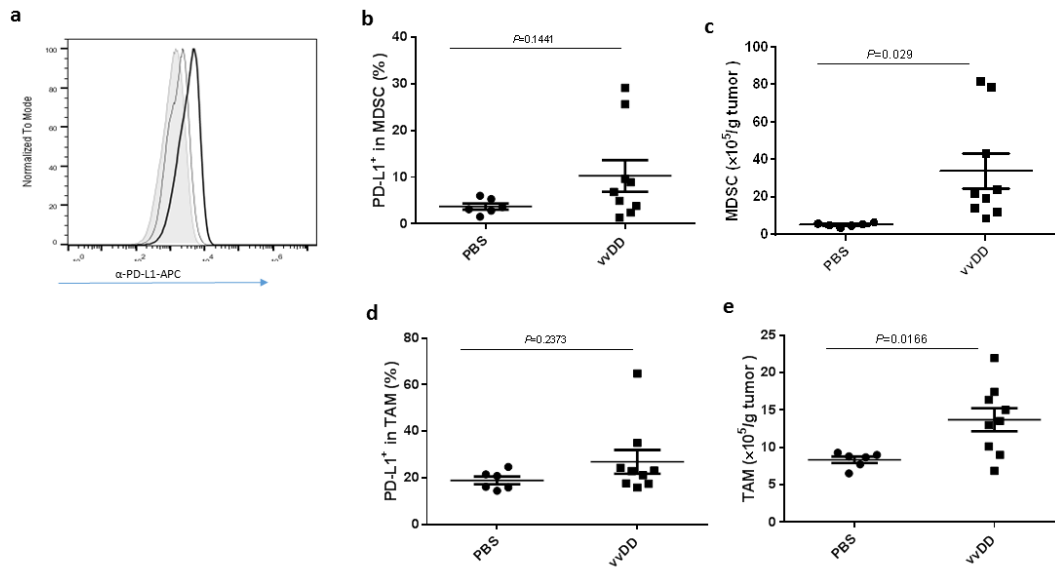


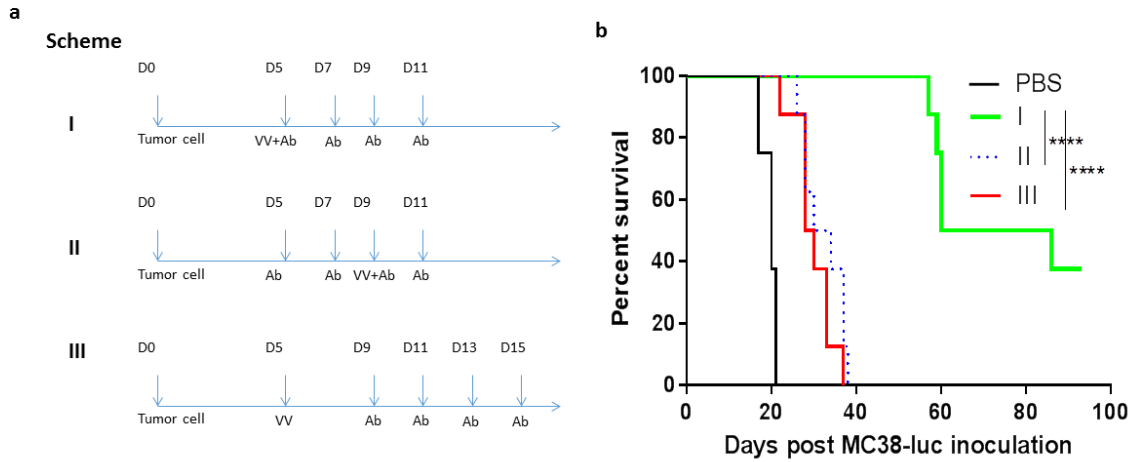
Supplementary Figures



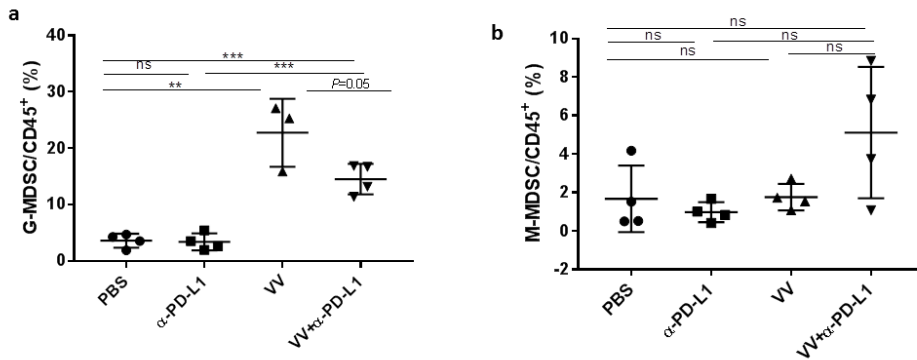
Supplementary Figure 1. PD-L1 is elevated post vvDD treatment in other murine cancer cells *in vitro*. Murine AB12-luc, EMT6, Hepa1-6, MCA102, and MOSEC cancer cells were infected with vvDD. The infected cells were harvested 24 h post infection to make RNA for RT-qPCR assay to determine relative PD-L1 expression.



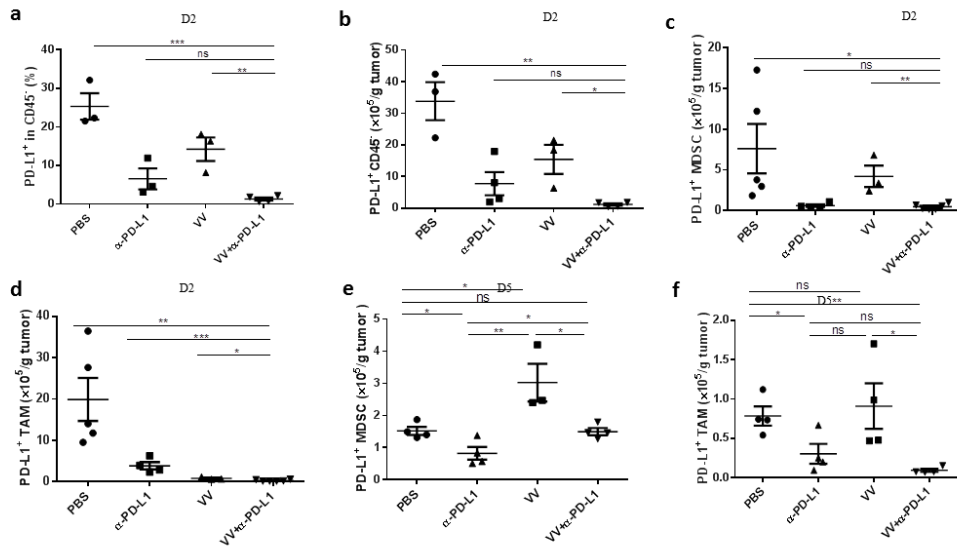
Supplementary Figure 2. PD-L1 is impacted in MDSC and TAM post vvDD treatment *in vivo*. B6 mice were subcutaneously inoculated with MC38-luc cells (4×10^5 cells). PBS or vvDD was intratumorally injected at 2×10^8 pfu/tumor when the s.c tumor area reached 5×5 mm². Tumor tissues were collected from PBS or virus-treated mice 4 days post the treatment. Collected tumor tissues were weighed and incubated in RPMI 1640 containing 2% FBS, 1 mg/ml collagenase, 0.1 mg hyaluronidase, and 200 U DNase I at 37°C for 1-2h to make single cells. The single cells were blocked with α -CD16/32 Ab and then stained with antibodies against CD45, CD11b, Gr1, PD-L1, F4/80 to determine PD-L1 expression on CD45⁺ cells, tinted: Ab isotype; (a). hairline: PBS-treated; normal: vvDD-treated; (b,c). MDSC (CD45⁺CD11b⁺Gr1⁺); (d, e); TAM (CD45⁺CD11b⁺F4/80⁺). Student's *t*-test was used to analyze the data.



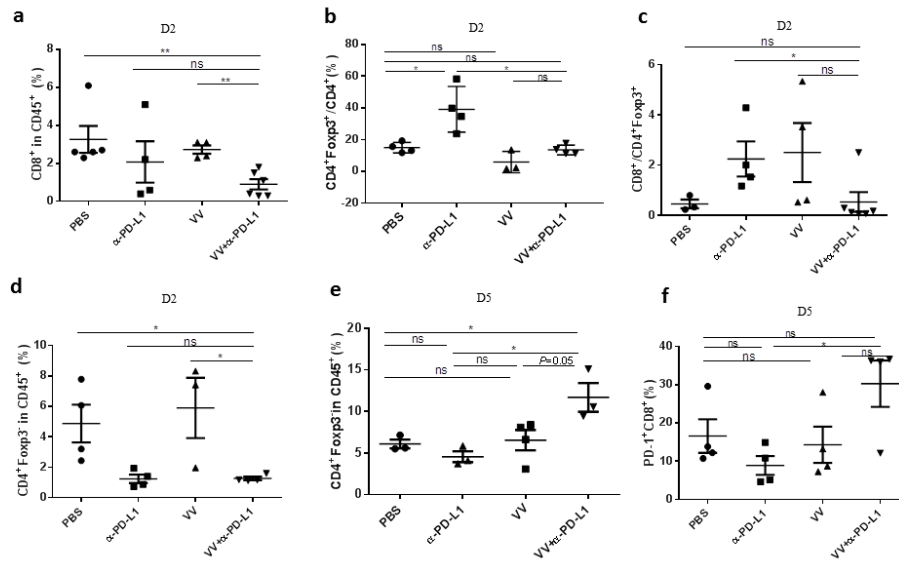
Supplementary Figure 3. Simultaneous treatment with VV-CXCL11 plus α -PD-L1 is more effective at early treatment time points. (a). B6 mice were intraperitoneally inoculated with 5×10^5 MC38-luc and treated with VV-CXCL11 + α -PD-L1 as per the timelines, and (b). The survival data was shown. The survival was monitored by Kaplan-Meier analysis and statistical analyses were performed with Log rank test. In all of the figures, the standard symbols for *P* values are, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and **** $P < 0.0001$; and ns: not significant.



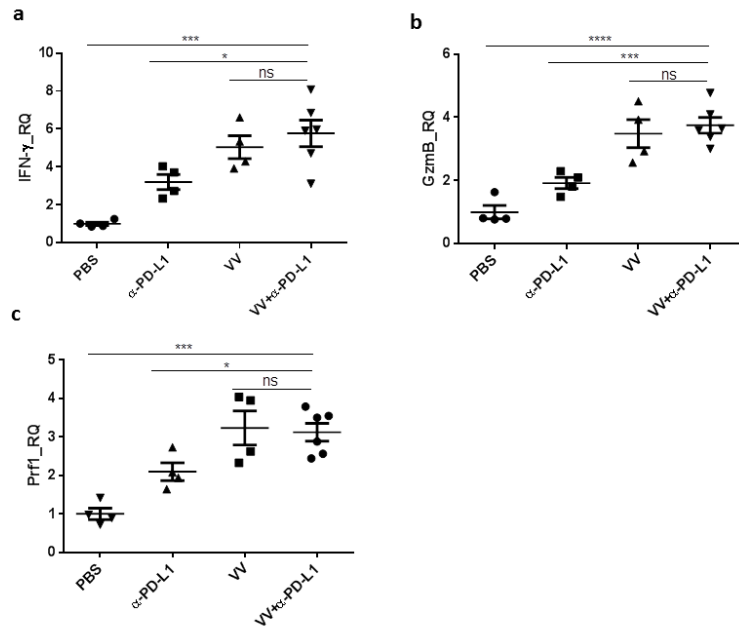
Supplementary Figure 4. Vaccinia virus treatment elevates G-MDSC in TME. B6 mice were intraperitoneally inoculated with 5×10^5 MC38-luc cells and treated with VV (short-named for vvDD-CXCL11) and/or α -PD-L1 as described. Tumor-bearing mice were sacrificed at day 5 post first treatment and primary tumors were collected and analyzed as Figure 5 for G-MDSC and M-MDSC in TME. Student's *t*-test was used to analyze the significance of the differences.



Supplementary Figure 5. The α -PD-L1 treatment reduces the PD-L1⁺ cells in TME. C57BL/6 mice were intraperitoneally inoculated with 5×10^5 MC38-luc and treated with VV-CXCL11 and/or α -PD-L1 as described. Tumor-bearing mice were sacrificed at day 2 or day 5 post first treatment and primary tumors were collected and analyzed as Figure 2 to determine the PD-L1⁺ tumor cells (a,b), PD-L1⁺ MDSC (c, e) PD-L1⁺ TAM (d, f) in the TME. Student's *t*-test was used to analyze the significance of the differences.



Supplementary Figure 6. The combination of VV plus α -PD-L1 treatment impacts tumor-infiltrating effector T cells in TME. B6 mice were intraperitoneally inoculated with 5×10^5 MC38-luc and treated with VV and/or α -PD-L1 as described. Single cells were made from primary tumors collected from tumor-bearing mice at day 2 or day 5 post first treatment as Figure 2, blocked with α -CD16/32 Ab and then stained with antibodies against CD45, CD8, CD4 and Foxp3 to determine the CD8⁺ T cells (a), CD4⁺Foxp3⁺ (b), CD8⁺/CD4⁺Foxp3⁺ (c), CD4⁺Foxp3⁻ (d, e) and PD-1⁺ CD8⁺ (f) in the TME. Student's *t*-test was used to analyze the significance of the differences.



Supplementary Figure 7. The combination therapy of VV plus α -PD-L1 increases immune-favorable molecules in the TME. B6 mice were intraperitoneally inoculated with 5×10^5 MC38-luc and treated with VV-CXCL11 and/or α -PD-L1 as described. RNA extracted from tumor tissues collected from tumor-bearing mice at day 2 post first treatment was used for RT-qPCR assay to determine IFN- γ (a), Granzyme B (b) and perforin (c) in the TME. Student's *t*-test was used to analyze the significance of the differences. The standard symbols for *P* values are, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and **** $P < 0.0001$.