Supporting Information

Programmed Cell Death Protein Ligand-1 (PD-L1) Silencing with Polyethylenimine-Dermatan Sulfate Complex for Dual Inhibition of Melanoma Growth

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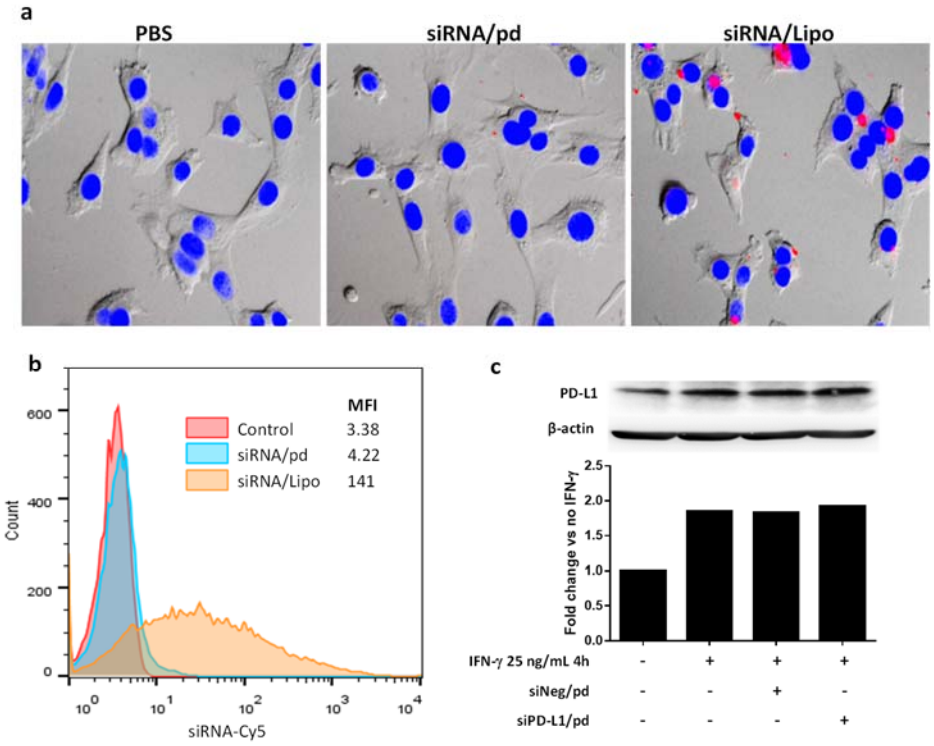
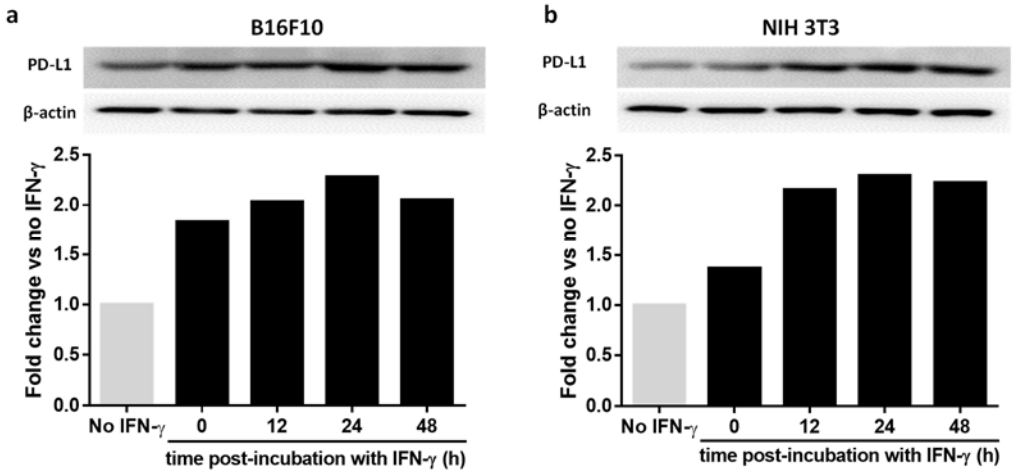
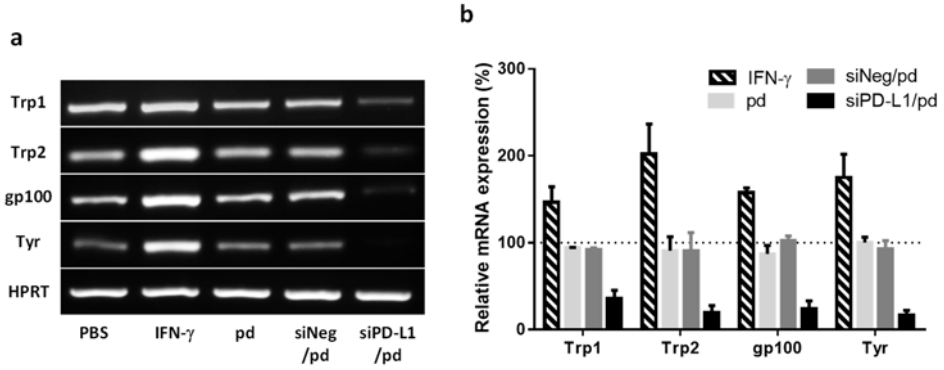


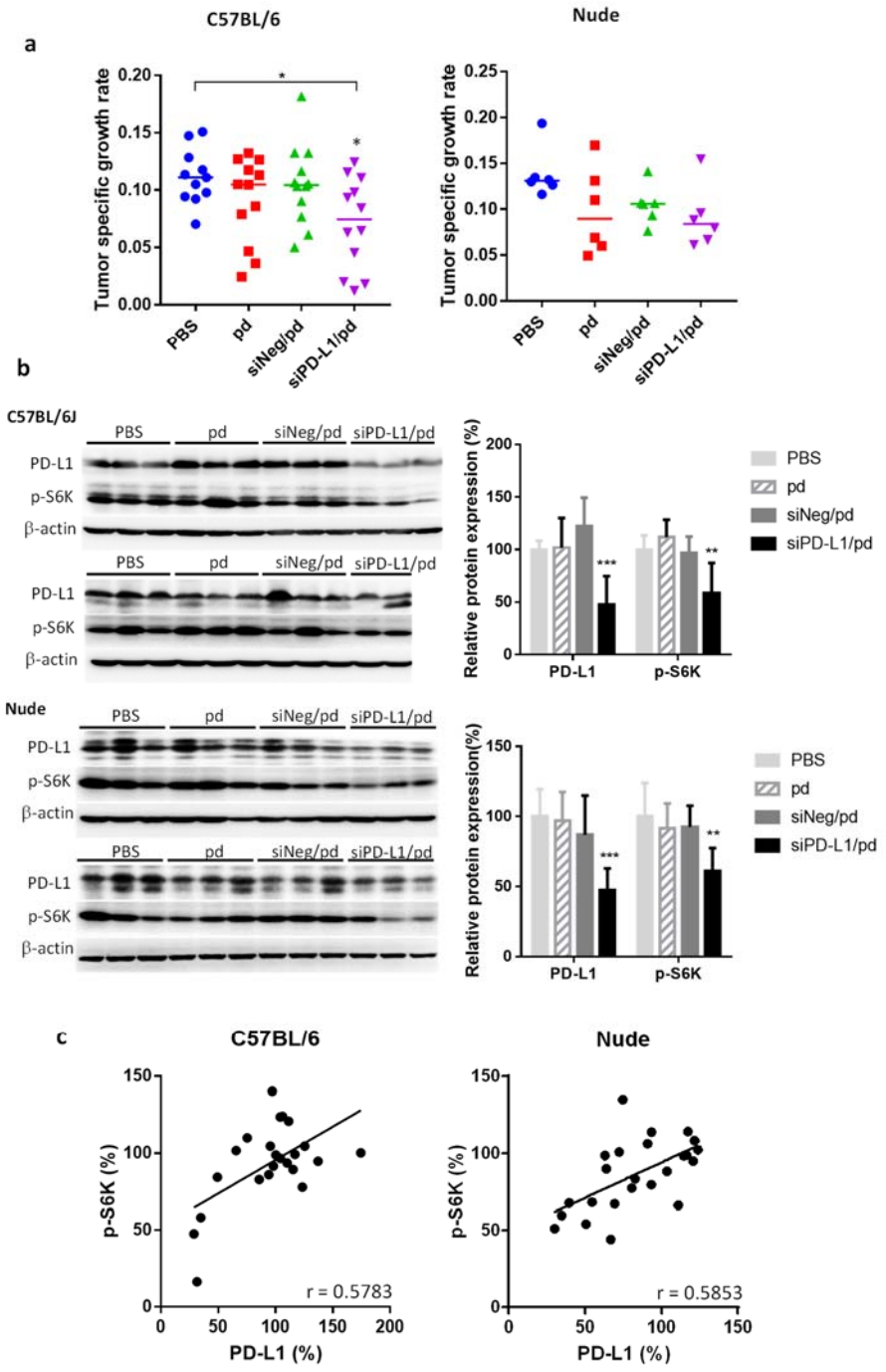
Supporting Fig. 1. Uptake of siRNA/pd complex by NIH 3T3 fibroblasts observed with (a) fluorescence microscopy (red: Cy 5-labeled siRNA; blue: nuclei) and (b) flow cytometry. (c) Western blotting image and quantitative representation of PD-L1 expression in IFN-γ–activated NIH 3T3 cells treated with PBS, siNeg/pd, and siPD-L1/pd complex.



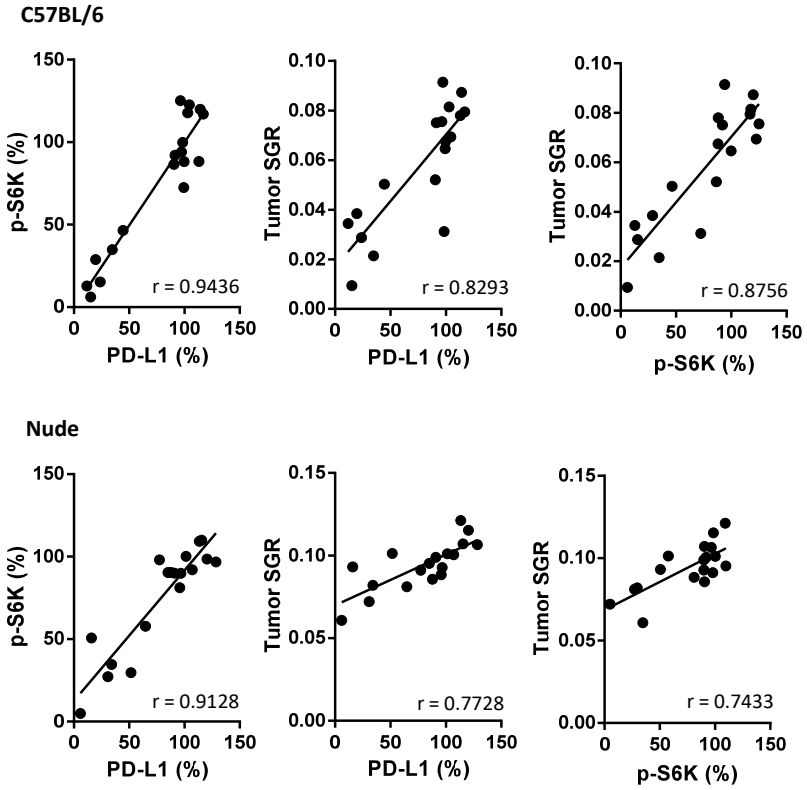
Supporting Fig. 2. PD-L1 expression in (a) B16F10 and (b) NIH 3T3 cells after 4 h incubation with IFN-γ (25 ng/mL). X-axis indicates the incubation time post-incubation with IFN-γ.



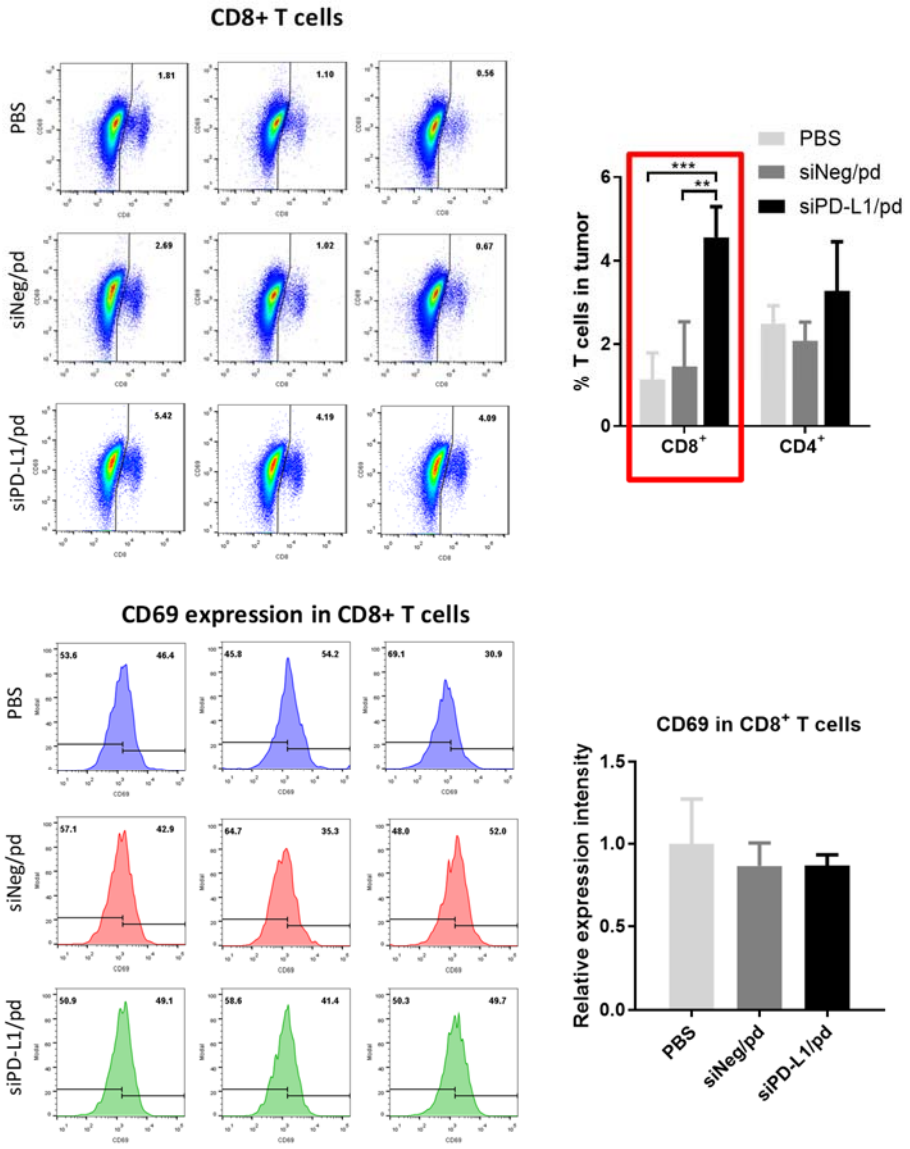
Supporting Fig. 3. (a) RT-PCR demonstrating melanoma specific gene expression in B16F10 cells treated with IFN-γ, pd, siNeg/pd, and siPD-L1/pd. (b) Quantitative presentation of mRNA expression relative to PBS-treated cells. n = 3 separate experiments, mean ± s.d. In all genes, pd vs. siNeg/pd: n.s.; all other pairs: $p < 0.001$ by Tukey's multiple comparisons test.



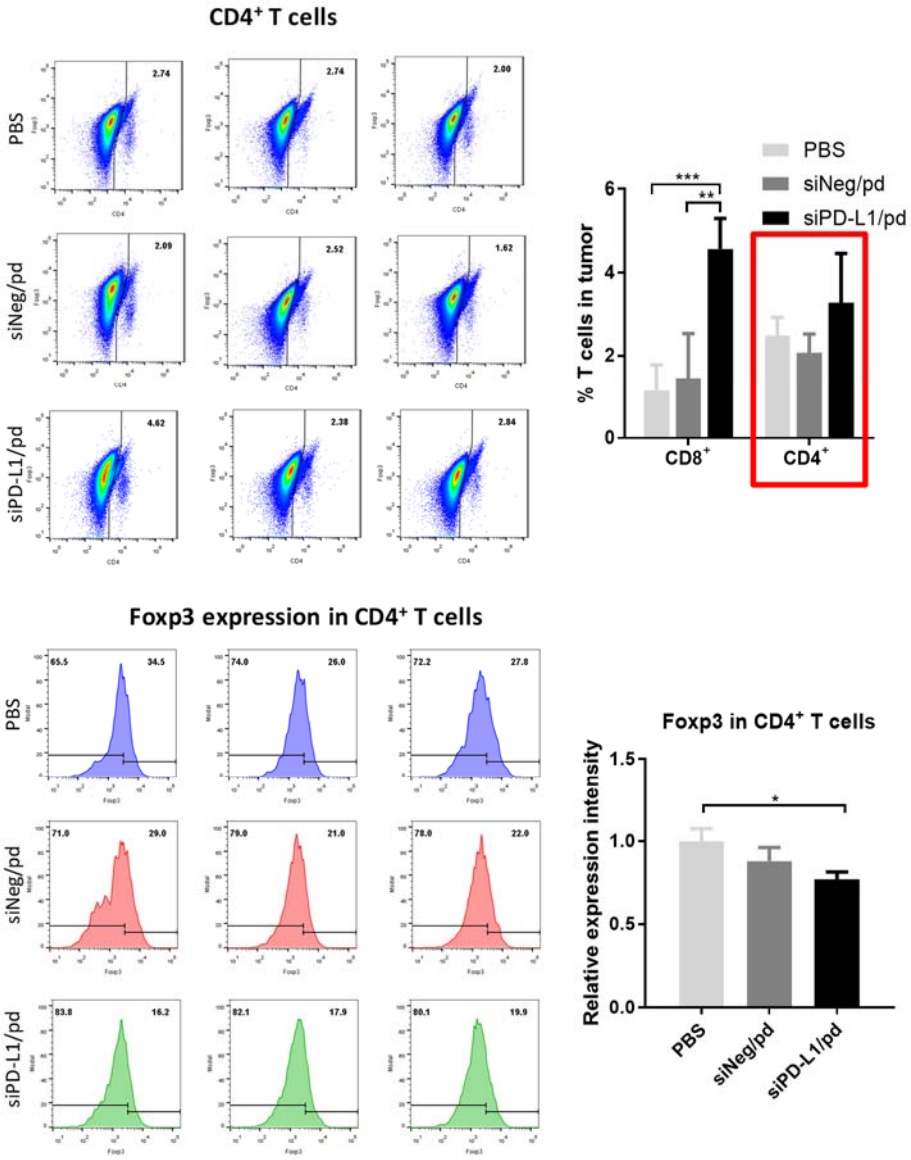
Supporting Fig. 4. (a) Tumor specific growth rate of B16F10 subcutaneous tumors in immune-competent C57BL/6 and immune-compromised Balb/c nude mice. 0.75 mg siRNA per kg, q5d × 3. n=11-12 mice per group for C57BL/6 mice; n = 6 mice per group for Balb/c nude mice, mean ± s.d. *: $p < 0.05$ by Tukey's test. (b) Expression of PD-L1 and p-S6K in B16F10 tumors in C57BL/6 and Balb/c nude mice. n = 5 for siPDL1/pd in C57BL/6, n = 6 for all other groups, mean ± s.d. **: $p < 0.01$; ***: $p < 0.001$ vs. PBS group by Dunnett's test. (c) Correlation between p-S6k vs. PD-L1 expression.



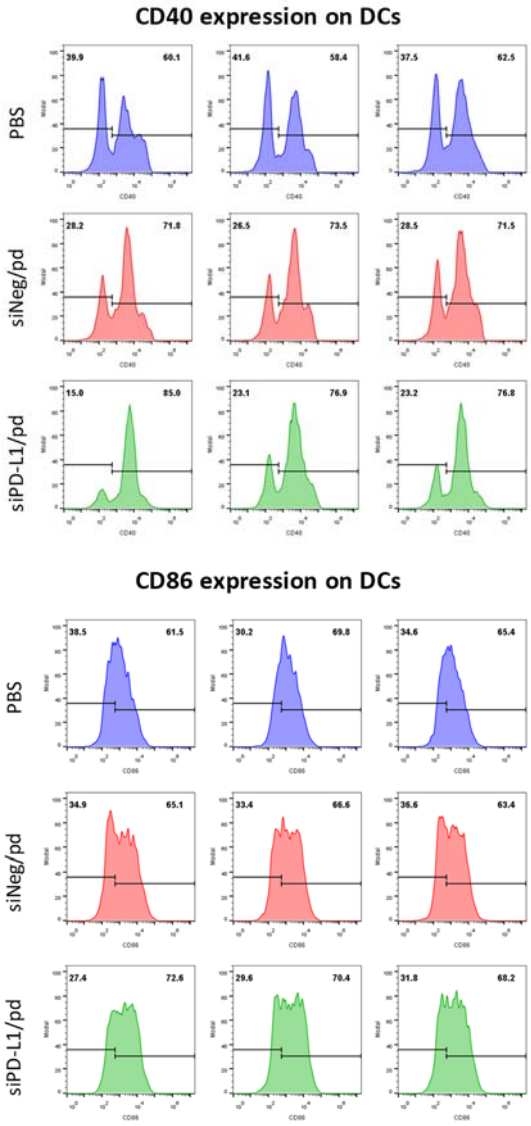
Supporting Fig. 5. Correlation between p-S6K vs. PD-L1 expression, Tumor SGR vs. PD-L1 expression and tumor SGR vs. p-S6K expression following 1.5 mg siRNA per kg, q3d × 5.



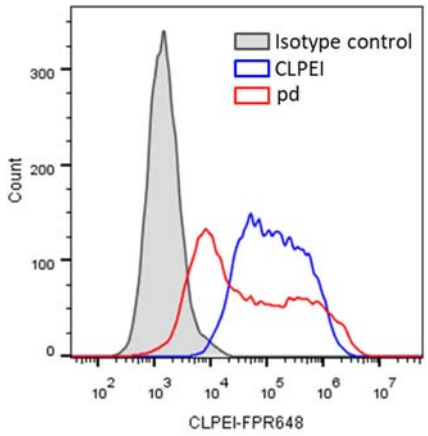
Supporting Fig. 6. (Top) Representative dot plots demonstrating the population of $CD8^+$ T cells in B16F10-OVA tumors of C57BL/6 mice receiving different treatments. (Bottom) Histograms demonstrating the intensity of CD69 expression on $CD8^+$ T cells. n = 3 mice per group.



Supporting Fig. 7. (Top) Representative dot plots demonstrating the population of $CD4^+$ T cells in B16F10-OVA tumors of C57BL/6 mice receiving different treatments. (Bottom) Histograms demonstrating the intensity of Foxp3 expression on $CD4^+$ T cells. n = 3 mice per group.



Supporting Fig. 8. Histograms demonstrating the intensity of CD40 (Top) and CD86 (Bottom) expression on DCs in DLNs of C57BL/6 mice with B16F10-OVA tumors receiving different treatments. n =3 mice per group.



Supporting Fig. 9. Fluorescence intensity of $CD11^{c+}$ BMDCs treated with FPR648-labeled CLPEI or pd complex containing FPR648-labeled for 6h. Both treatments contained 3 μg/mL of FPR648-labeled CLPEI.