

# **Nanoparticle delivery of immunostimulatory oligonucleotides enhances response to checkpoint inhibitor therapeutics**

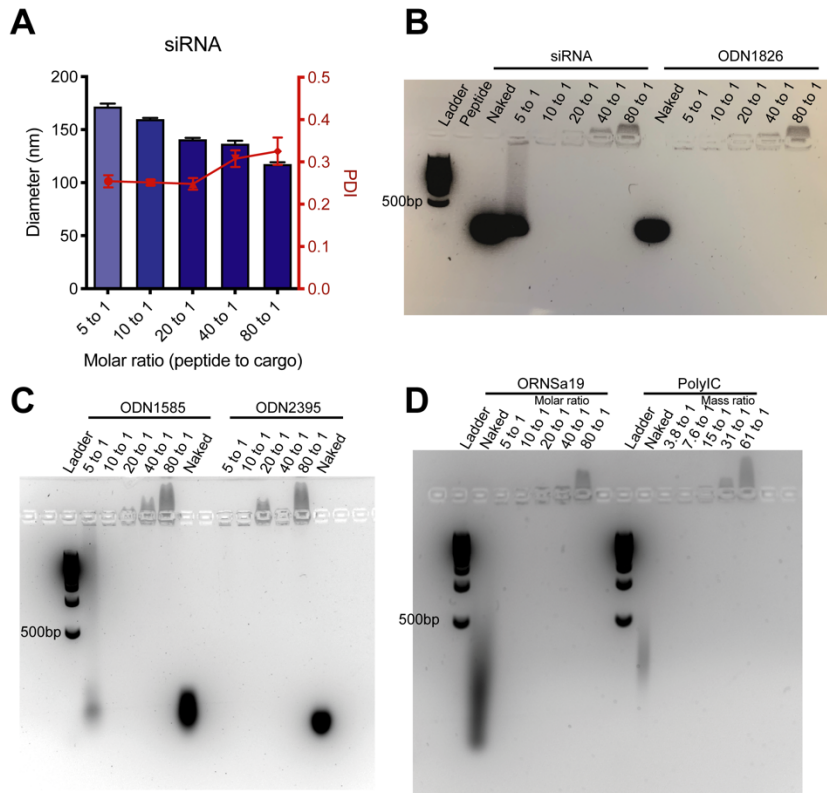
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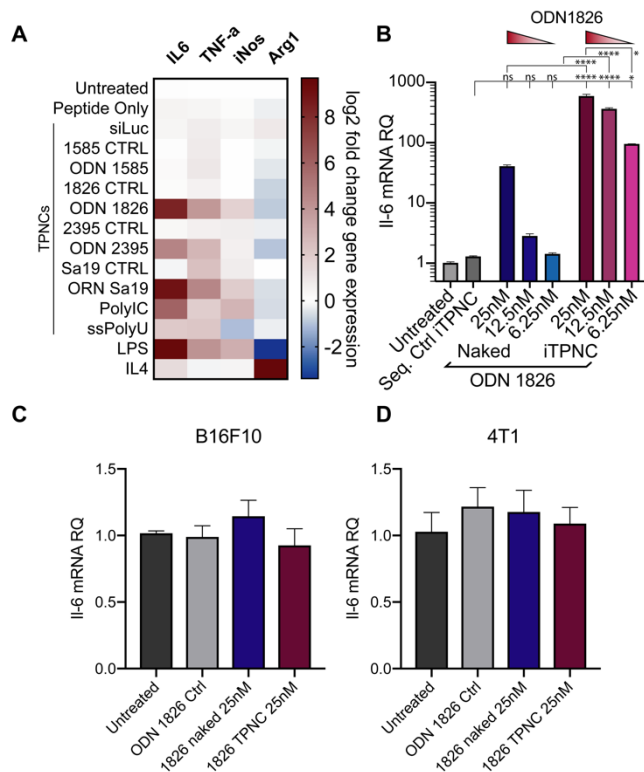
## **Supplementary Figures**



**Figure S1. Characterization of iTPNC encapsulation of immunostimulatory cargoes.**

**(A)** DLS measurements of hydrodynamic diameter (blue bars) and polydispersity index (red) of TPNCs formed with siLuc control cargo.

**(B – D)** Gel electrophoresis of naked cargoes or iTPNCs formed with siRNA **(B, left)**, ODN 1826 **(B, right)**, ODN 1585 **(C, left)**, ODN 2395 **(C, right)**, ORN Sa19 **(D, left)**, or poly(I:C) **(D, right)** at varying ratios of peptide to cargo (molar ratio except where indicated). Ladder lane shows 1 kb ladder, with 500bp band indicated.

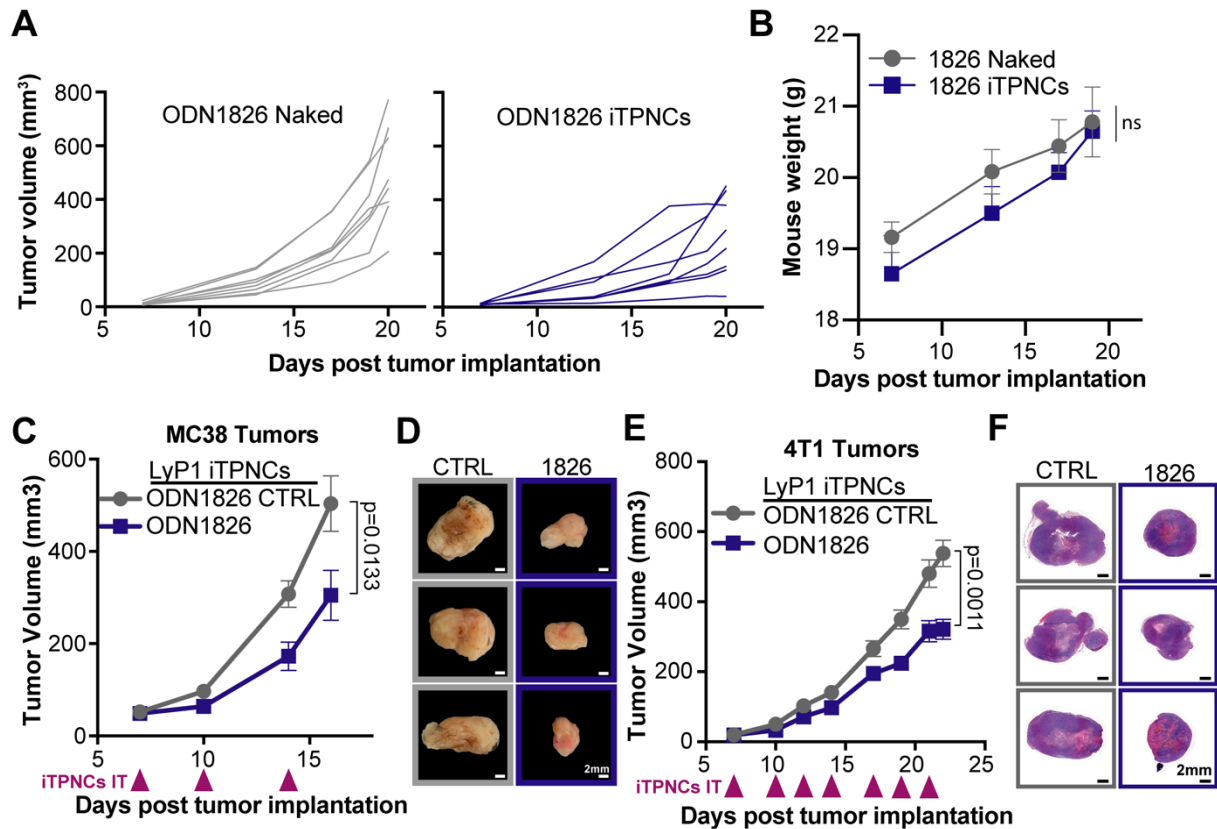


**Figure S2. iTPNC stimulation of inflammatory signaling is dependent upon cargo, dose, and cell type.**

**(A)** Heatmap of log<sub>2</sub> fold changes of gene expression in J774A.1 macrophages measured 6 hours after treatment with iTPNCs formed with various TLR ligand cargoes, control sequence cargoes, tandem peptide without cargo, LPS (10ng/mL), or IL-4 (20ng/mL) expressed relative to gene expression in untreated control cells.

**(B)** Expression of Il-6 in J774A.1 macrophages measured 18 hours after treatment with ODN1826 naked or encapsulated in iTPNCs at various concentrations, or treated with iTPNCs carrying sequence control for ODN1826. Expression values are shown relative to expression in untreated macrophages. (\*  $P < 0.05$ , \*\*\*\*  $P < 0.0001$ , one-way ANOVA)

**(C – D)** Expression of Il-6 in B16F10 **(C)** or 4T1 **(D)** murine cancer cells measured 6 hours after treatment with 25nM ODN1826 naked or encapsulated in iTPNCs, or 25nM of sequence control for ODN1826 in iTPNCs. Expression is shown relative to untreated cells.



**Figure S3. Effects of ODN 1826 iTPNCs after intratumoral administration.**

(A) Individual tumor growth curves for B16F10 tumors injected intratumorally with unencapsulated ODN 1826 (left, gray curves) or ODN 1826 iTPNCs (right, blue curves). Average final volume for these tumors is shown in Fig. 3B.

(B) Weights over time of mice from (A) bearing B16F10 tumors injected intratumorally with ODN1826 naked (gray circles) or ODN 1826 iTPNCs (blue squares).

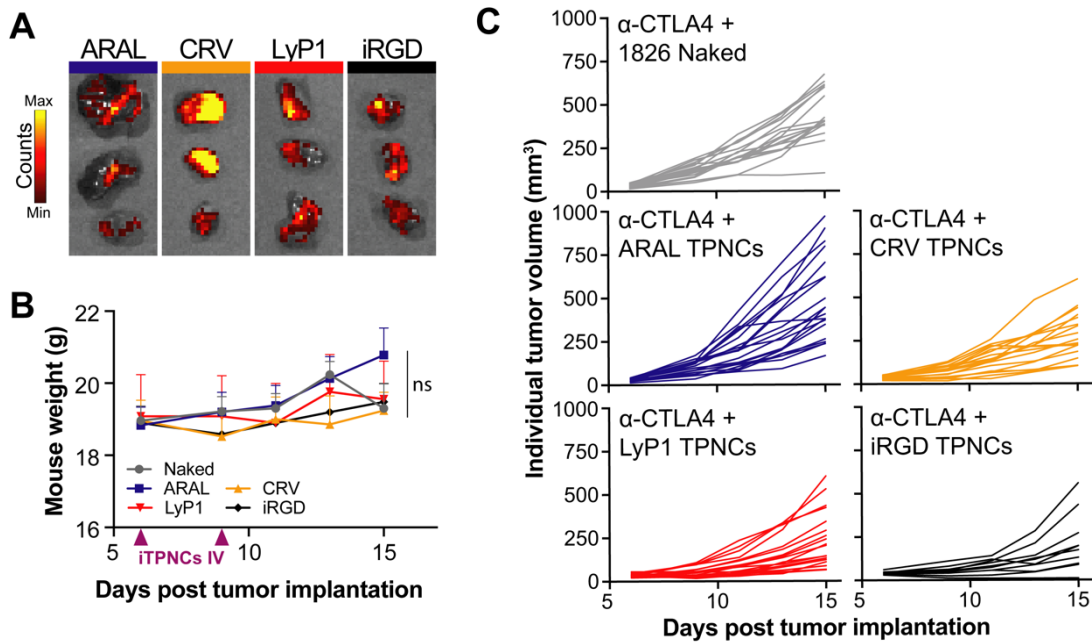
(C) Tumor volume over time of subcutaneous flank MC38 colon cancer tumors injected intratumorally with ODN 1826 iTPNCs (blue squares) or sequence control TPNCs (gray circles) (0.2nmol ODN per injection). (*P* value calculated by two-way ANOVA; *n* = 9 tumors per group, data are representative of three independent experiments)

(D) Photos of representative tumors from (C).

(E) Tumor volume over time of mammary fat pad 4T1 breast cancer tumors injected intratumorally with ODN 1826 iTPNCs (blue squares) or sequence control TPNCs (gray circles) (0.2nmol ODN per injection). (*P* value calculated by two-way ANOVA; *n* = 10 tumors per group, data are representative of three independent experiments)

(F) Images of H&E stained sections of representative tumors from (E).

Scale bars for (D) and (F) are 2mm. *P* values for B, C, E, and H were calculated using two-way ANOVA.

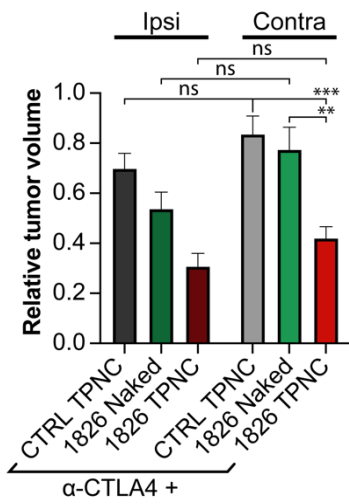


**Figure S4. Characterization of effects of iTPNC intravenous administration.**

**(A)** Nanoparticle fluorescence images, all captured simultaneously, of B16F10 tumors explanted from mice 30 minutes after intravenous injection of ODN 1826 iTPNCs synthesized with ARAL non-homing control peptide or one of CRV, LyP1, or iRGD homing peptides.

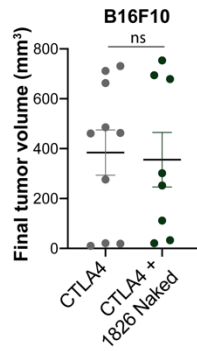
**(B)** Weights over time of mice from Fig. 3B treated intraperitoneally with anti-CTLA4 and intravenously with ODN 1826 unencapsulated ('naked') or formulated into iTPNCs with ARAL non-homing control peptide or one of CRV, LyP1, or iRGD homing peptides.

**(C)** Individual tumor growth curves for curves in Fig. 3B.



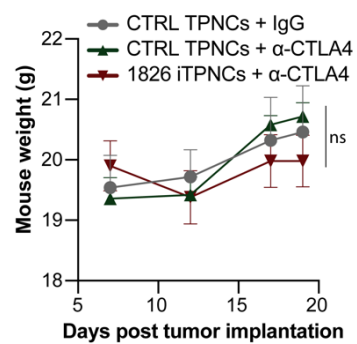
**Figure S5. iTPNCs suppress growth of MC38 tumors via an abscopal effect**

Final MC38 tumor volumes shown relative to IgG-treated mice injected intratumorally with sequence control TPNCs. Treatment was initiated when average tumor volume was 49 mm<sup>3</sup>. Mice were treated in the method described in Fig. 5A. (\*\* P < 0.01, \*\*\* P < 0.001, one-way ANOVA; data are average of three independent experiments, error bars  $\pm$ SEM)



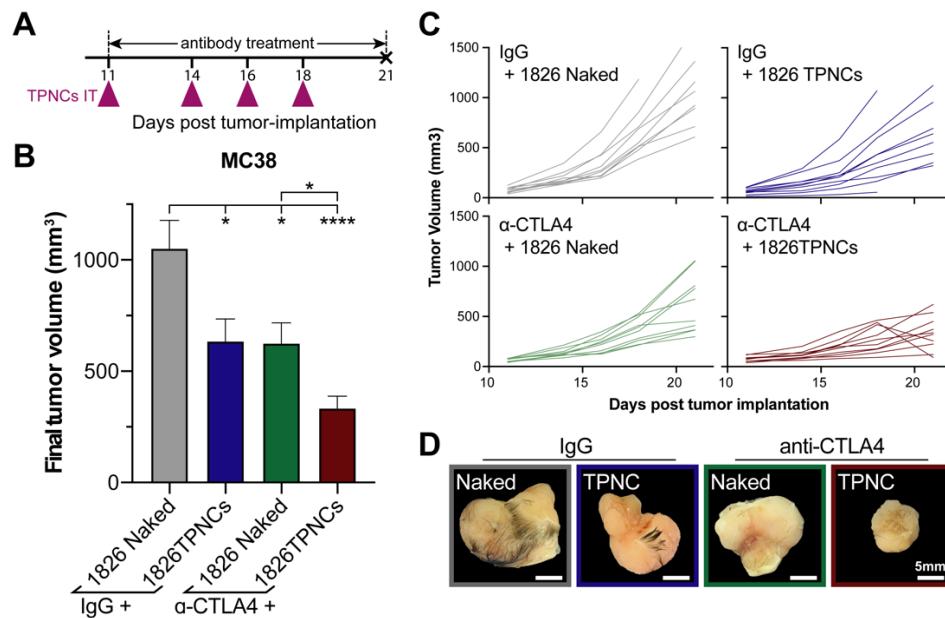
**Figure S6. Effects of unencapsulated ODN 1826 or iTPNCs in combination with anti-CTLA4.**

Final volumes of B16F10 tumors from mice treated with anti-CTLA4 antibody alone (grey) or in combination with intratumoral injections of unencapsulated ODN 1826 (green). Treatments were started when tumors were ~20mm<sup>3</sup>. Anti-CTLA4 was administered intraperitoneally at a dose of 200ug per week. ODN 1826 was injected intratumorally three times per week at a dose of 0.2nmol per injection. ( $P = 0.9162$  by Mann-Whitney test, error bars  $\pm$ SEM)



**Figure S7.** Weights over time of mice from Fig. 6 bearing B16F10 tumors injected intratumorally with sequence control TPNCs or ODN 1826 iTPNCs and treated systemically with anti-CTLA4 or IgG isotype control antibody.





**Figure S8.**

(A) Timeline of treatment. iTPNCs were injected intratumorally on days marked with a pink arrowhead, and antibody treatment was continued for the duration of the study.

(B) Average final tumor volume for sub-cutaneous MC38 tumors treated intratumorally with unencapsulated ODN 1826 ('naked') or ODN 1826 iTPNCs in mice treated intraperitoneally with anti-CTLA4 or IgG isotype control. Treatment was initiated when the average tumor volume was 70 mm<sup>3</sup>. (\*P < 0.05, \*\*\*\*P < 0.0001, Mann-Whitney test; n = 8-10 tumors per group, error bars ±SEM; data are representative of three independent experiments)

(C) Individual tumor growth curves for the groups shown in (B).

(D) Photos of representative tumors for each of the groups shown in (B–C). Scale bars are 5mm.