SUPPLEMENTARY FIGURES & TABLES

Tumor-targeted silencing of the peptide transporter TAP induces potent antitumor immunity

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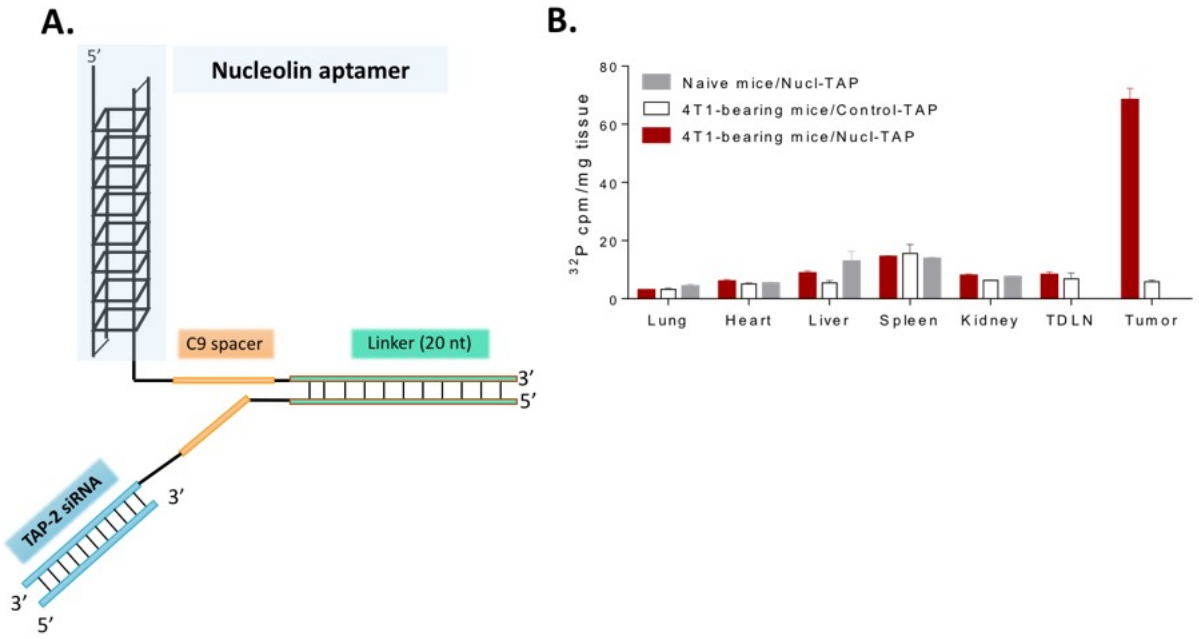
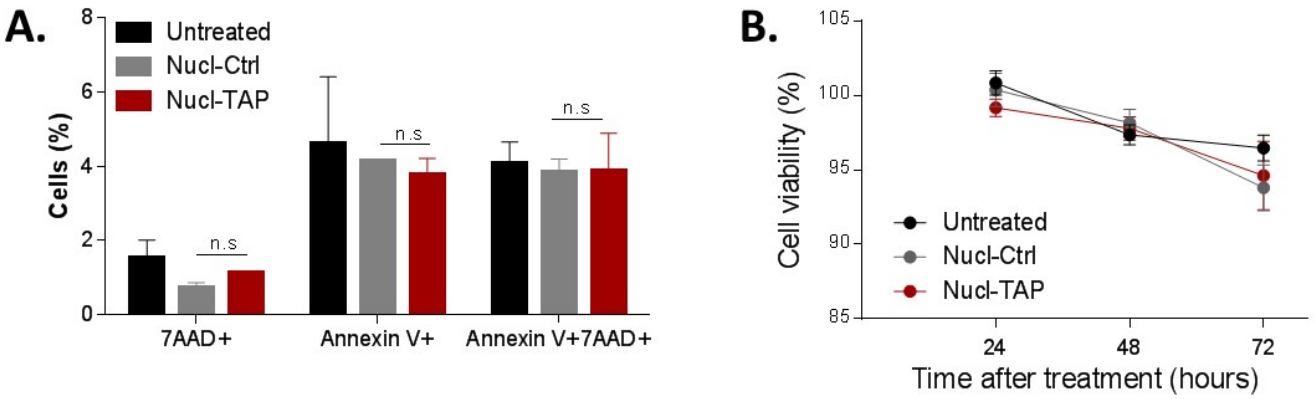
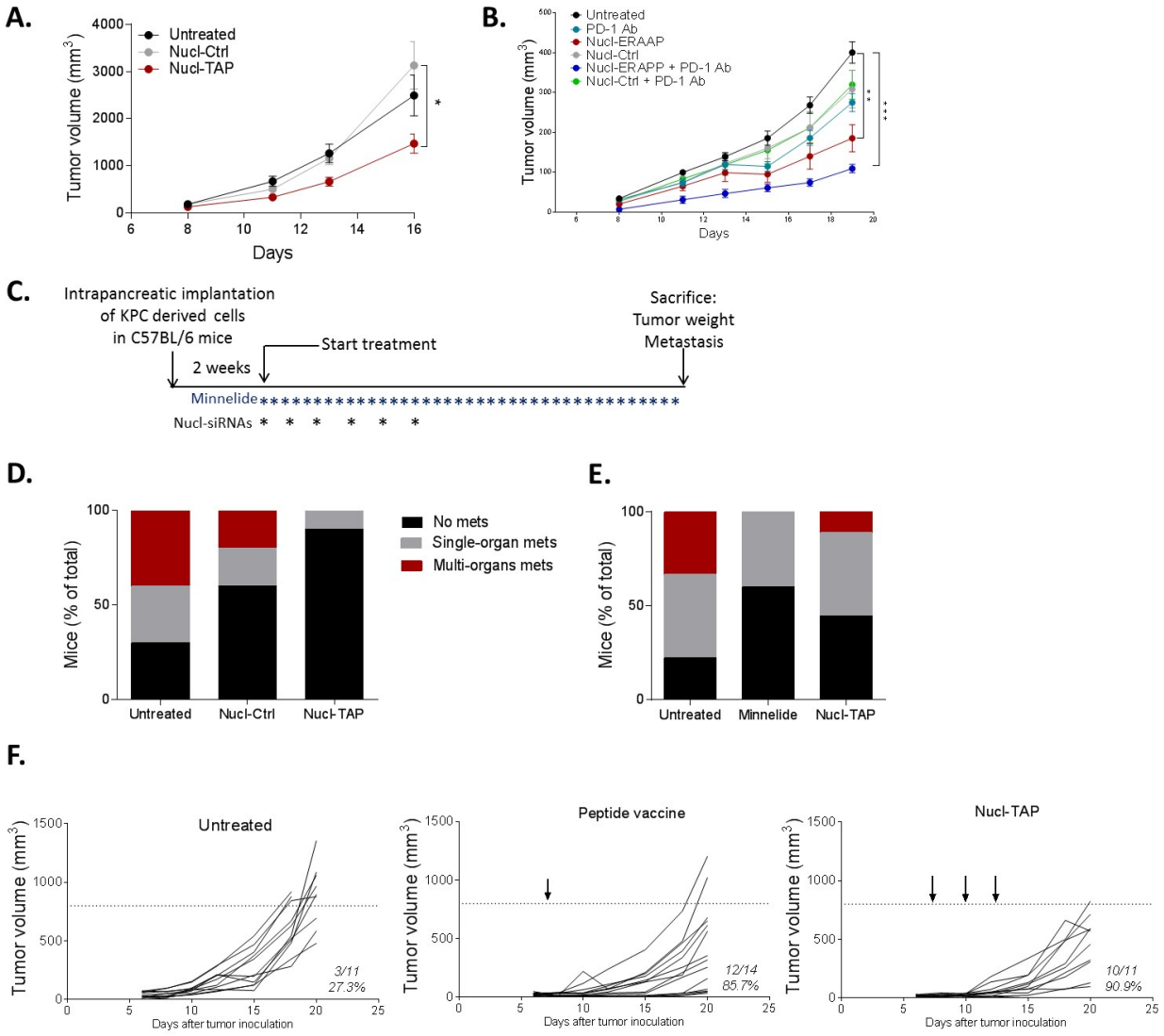


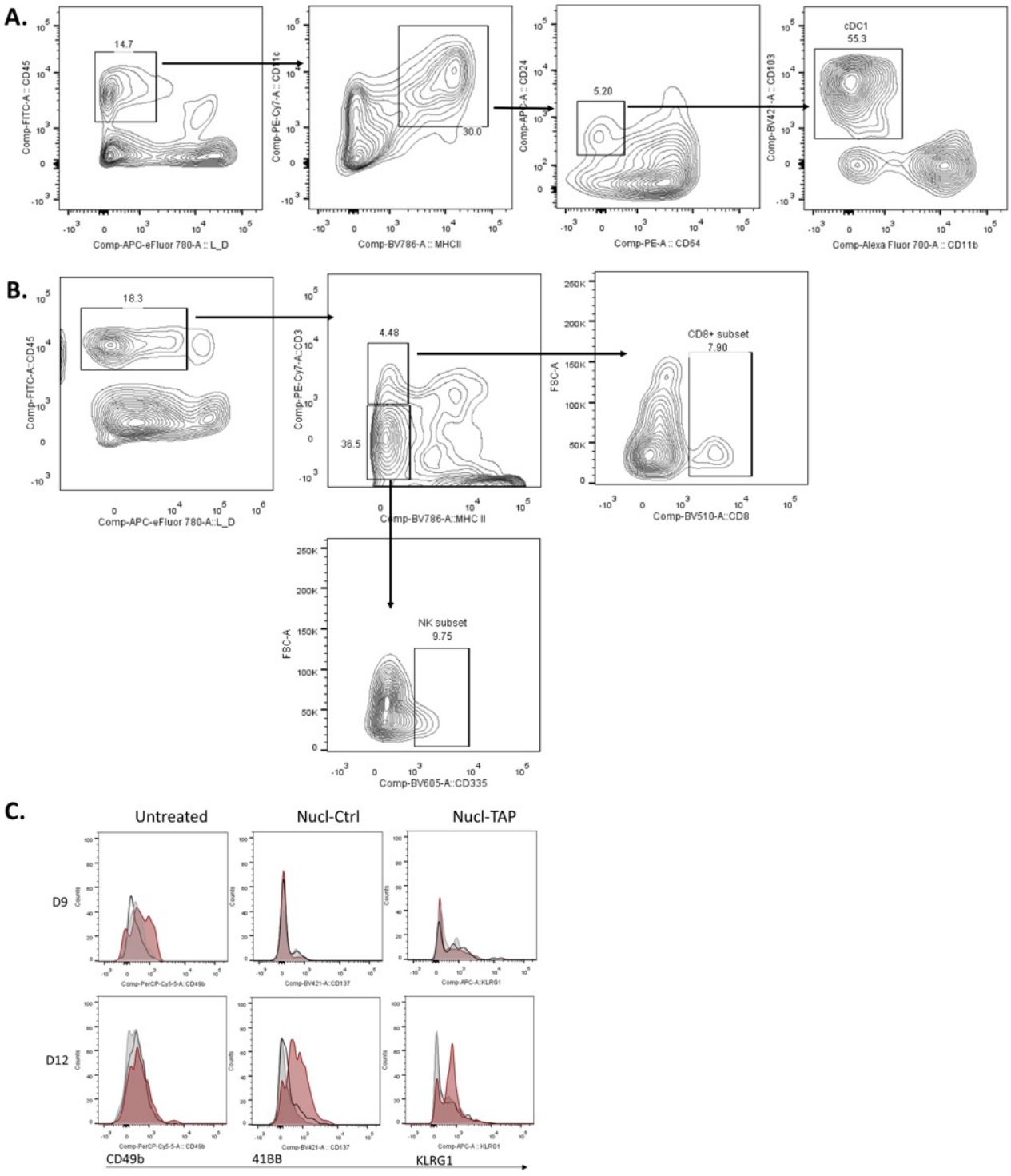
Supplementary Fig.1. Accumulation of systemically administered nucleolin aptamer-shRNA conjugates in tumors. **A.** Structure of a 26 nt G-rich G-quartet forming nucleolin aptamer[17] conjugated to a TAP siRNA. **B.** ^{32}P-labeled nucleolin or a non-binding aptamer conjugated to TAP-shRNA were administered via intraperitoneal to subcutaneously implanted 4T1 tumor bearing mice or the nucleolin aptamer was administered to tumor-free mice, and 24 h later tumor and indicated organs were excised and radioactivity was measured using a scintillation counter. Data represent means ± SEM (4 mice/group).



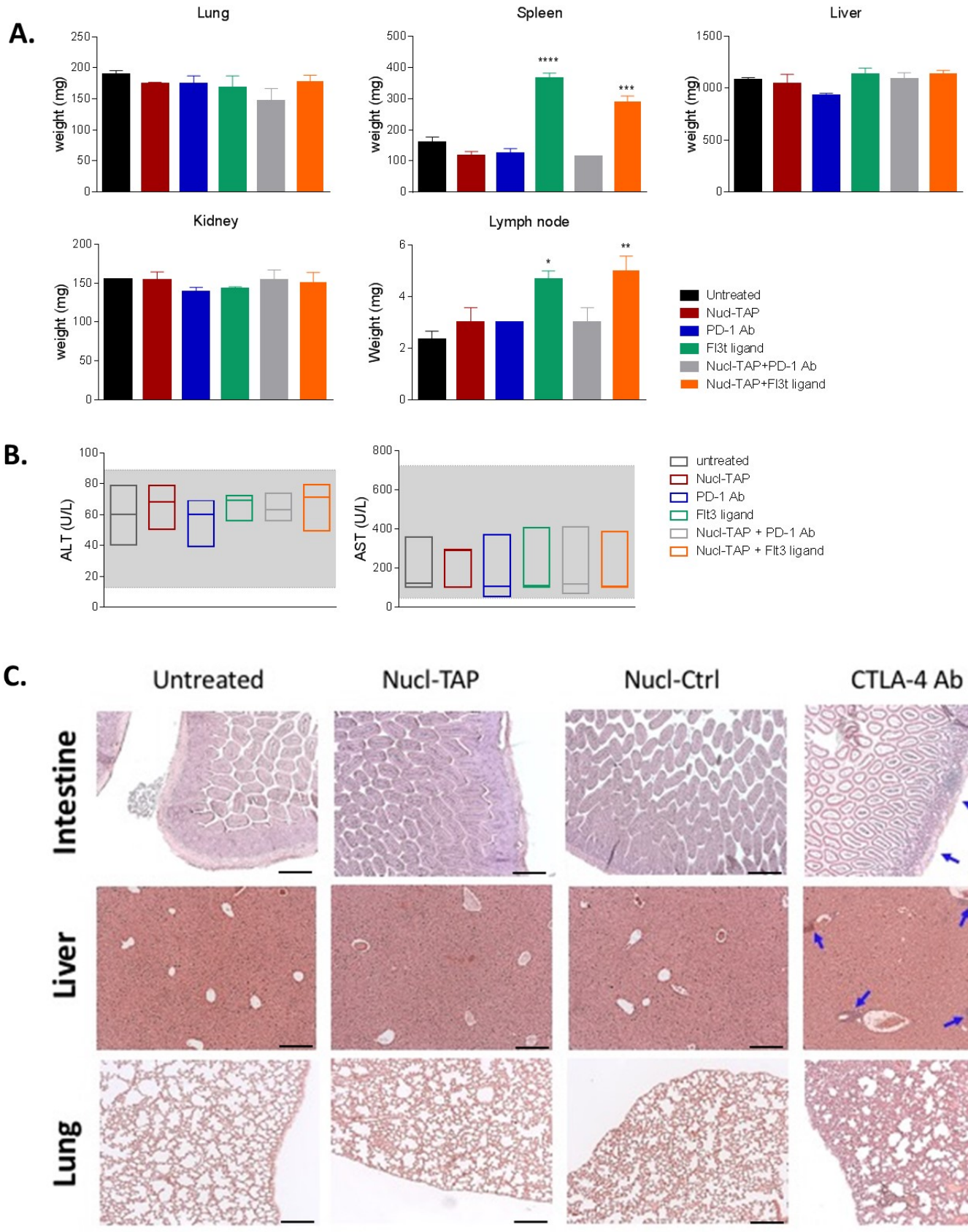
Supplementary Fig. 2. Toxicity of Nucl-TAP or Nucl-Ctrl siRNAs in cultured tumor cells. 4T1 tumor cells were cultured in the presence of 0.5 μM aptamer conjugates (n=2). **A.** Apoptosis. After 24 h was determined by staining with 7AAD and Annexin-V, and analyzed by flow cytometry. **B.** Cell viability was determined by MTT assay 1, 2 and 3 days after aptamer-siRNA conjugate treatment. Data represent means ± SEM of triplicate wells.



Supplementary Fig. 3. Nucleolin aptamer-targeted TAP siRNA mediated inhibition of tumor growth in mice. **A.** RMA T lymphoma model. Subcutaneously implanted palpable day 7 RMA tumor bearing C57BL/6 mice were administered i.p. with Nucl-TAP or Nucl-Ctrl, repeated two additional times 3 days apart, and tumor growth was monitored. (8-9 mice/group) (n=4). **B.** Nucleolin aptamer-targeted ERAAP siRNA mediated inhibition of tumor growth in mice – synergy with anti-PD-1 antibody treatment. Subcutaneously implanted palpable day 8 4T1 tumor bearing Balb/c mice were administered intraperitoneally with Nucl-ERAAP or Nucl-Ctrl followed by PD-1 antibody a day later, repeated two additional times 3 days apart, and tumor growth was monitored. (7-13 mice/group) (n=2). Data are represented as mean ± SEM. **C-E**. Nucl-TAP mediated inhibition of metastasis in the KPC-derived pancreatic cancer model. Each column represents the metastatic burden per group. **C**. Experimental protocol. See Methods & details in Fig. 3D & E. **F**. Nucl-TAP mediated inhibition of tumor growth in the MC38 tumor model. Experiments as shown in Fig. 3G, except that data are presented for individual mice as shown in reference[21]. Arrows indicate treatment frequency for each group.



Supplementary Fig. 4. NK, CD8+ T cells and cDC1 accumulate in the TME of Nucl-TAP treated tumor bearing mice. 4T1-bearing mice were injected i.p. with Nucl-siRNA conjugates as described in Fig.3A and infiltrating immune cells were analyzed 2 days after the first and second dose by flow cytometry. Gating strategy to identify intratumoral NK and CD8+T cells **(A)** or cDC1 **(B)**. Numbers represent % cells within depicted gate. **C.** Analysis of intratumoral NK cells for maturation and activation markers, representative tumor.


A.
Lung
Spleen
Liver
Kidney
Lymph node
weight (mg)
Weight (mg)
****
***
*
**
Untreated
Nucl-TAP
PD-1 Ab
Flt3t ligand
Nucl-TAP+PD-1 Ab
Nucl-TAP+Flt3t ligand
B.
ALT (U/L)
AST (U/L)
untreated
Nucl-TAP
PD-1 Ab
Flt3 ligand
Nucl-TAP + PD-1 Ab
Nucl-TAP + Flt3 ligand
C.
Untreated
Nucl-TAP
Nucl-Ctrl
CTLA-4 Ab
Intestine
Liver
Lung

Supplementary Fig. 5. Toxicity in mice treated with Nucl-TAP. Balb/c mice (3 mice/group) were administered with Nucl-TAP, Nucl-Ctrl, PD-1 Ab, or Flt3 ligand as indicated using doses and regimen used in the immunotherapy studies. Two days post last administration mice were sacrificed and analyzed (see also Fig. 5F). **A.** Organ weight. Data are represented as means ± SEM. **B.** Liver enzymes AST and ALT in the serum. Shaded area represents normal levels of ALT or AST in Balb/c mice (from The Jackson Laboratories (MPD, http://phenome.jax.org/). Data represent box plot analysis. No statistical differences were found between groups using the Kruskal-Wallis test with Dunn posttest. **C.** Inflammatory responses in tissue sections stained with hematoxylin and eosin and visualized by light microscopy at 10X magnification (Scale bar: 100 μm). One group of mice was also treated with 200 μg of CTLA-4 antibody that elicits a comparable antitumor effect[27,28]. Arrows indicate inflammatory foci in mice.

Variable	Treatment groups (4 mice/group)		
	Untreated	Nucl-Ctrl	Nucl-TAP
White Blood Cell Count (WBC) (10^3 cells/µl)	4.22 ± 0.26	4.87 ± 0.50	3.65 ± 0.17
Red Blood Cell Count (RBC) (10^3 cells/µl)	9.36 ± 0.30	9.32 ± 0.20	9.95 ± 0.61
Hemoglobin (g/DL)	14.12 ±0.33	13.72 ± 0.28	14.4 ± 0.52
Hematocrit (%)	56.00 ± 3.16	54.00 ± 1.41	55.5 ± 1.91
Mean Cell Volume (MCV) (fL)	59.75 ± 2.06	57.75 ± 1.70	56.5 ± 1.00
Mean Cell Hemoglobin (MCH) (pg)	15.00 ± 0.00	14.50 ± 0.57	14.50 ± 0.57
Mean Cell Hemoglobin Concentration (MCHC) (%)	25.50 ± 1.00	25.25 ± 0.95	26.00 ± 1.41
Segmented Neutrophils (10^3/µl)	19.00 ± 4.54	15.50 ± 1.29	17.50 ± 0.57
Lymphocytes (10^3/µl)	72.75 ± 2.87	75.75 ± 2.21	72.00 ± 1.70
Monocytes (10^3/µl)	8.25 ± 2.21	8.5 ± 1.29	10.00 ± 1.50
RBC Morphology	Mild Polychromasia	Mild Polychromasia	Mild Polychromasia
Platelet Morphology	Normal	Normal	Normal
WBC Morphology	Normal	Normal	Normal

Supplementary Table 1. Complete blood count analysis in Bab/c mice treated with Nucl-siRNA conjugates. Untreated mice were used as internal control (normal values for Balb/c mice from The Jackson Laboratory: http://phenome.jax.org/). Values are represented as mean ± SD. No statistical differences were found comparing Nucl-siRNA conjugates treated mice to controls using the Kruskal–Wallis test with Dunn posttest.