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Supplemental Information

Pre-existing Immunity to Oncolytic Virus Potentiates Its Immunotherapeutic Efficacy

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Supplementary Materials

Figure S1.

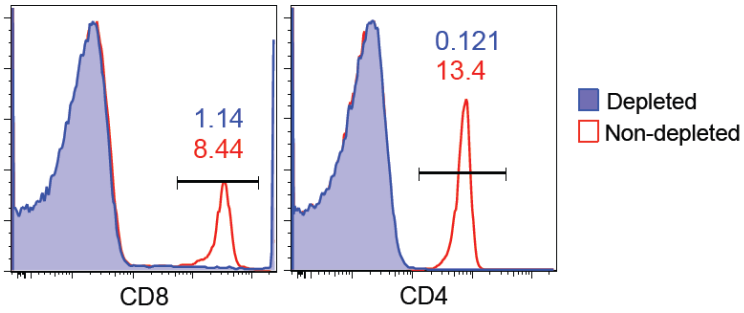

Figure S1. Antibodies to CD4 and CD8 lymphocytes deplete the cells of interest *in vivo*. Animals were treated as specified in Figure 1. Peripheral blood was collected 5 days after the initial injection and processed by flow cytometry for CD4+ and CD8+ cells with non-crossreactive antibodies. Gate percentages in red (bottom) and blue (top) represent the percentages of cells in non-depleted and depleted animals, respectively. Representative plots from 1 of 2 independent experiments with 5 mice per group are shown.

Figure S2

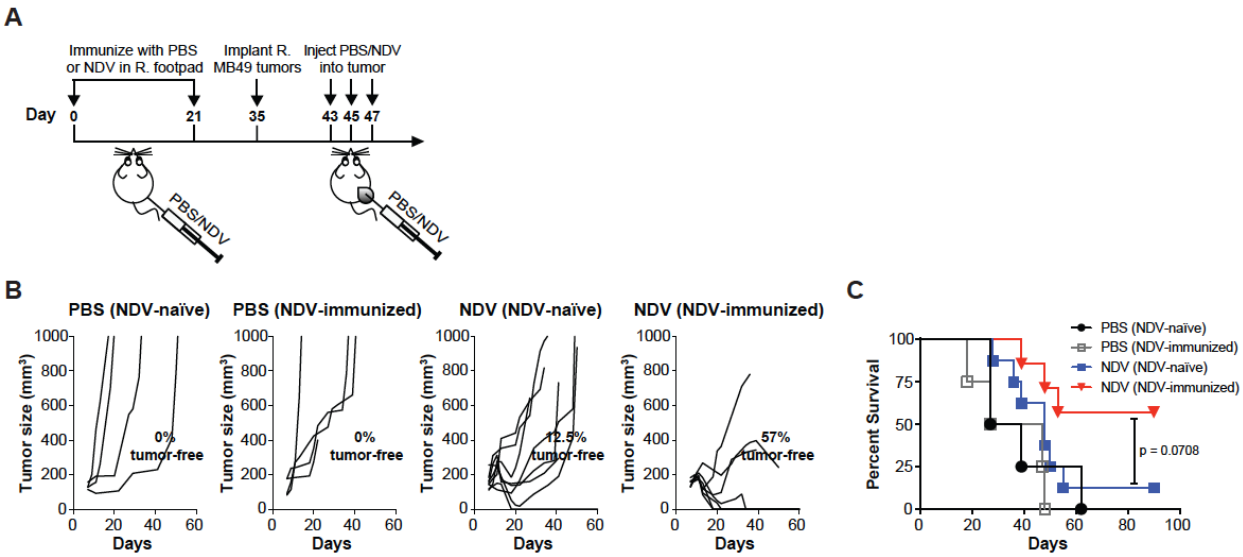

Figure S2. Effect of pre-existing immunity to NDV on anti-tumor efficacy in the MB49 bladder tumor model. (A) Treatment scheme. tumors were implanted by injection of 2 x 10^5 cells into the right or bilateral flanks on day 35 intradermally (100 µl). On days 43, 45 and 47, right tumors were injected with 100 µl PBS or NDV (1 x 10^7 pfu). (B) Growth of injected tumors. (C) Overall survival. Data represent results from one of two independent experiments with n = 4 (NDV-naïve PBS), n = 4 (NDV-immunized PBS), n = 8 (NDV-naïve NDV) and n = 7 (NDV-immunized NDV).

Figure S3.

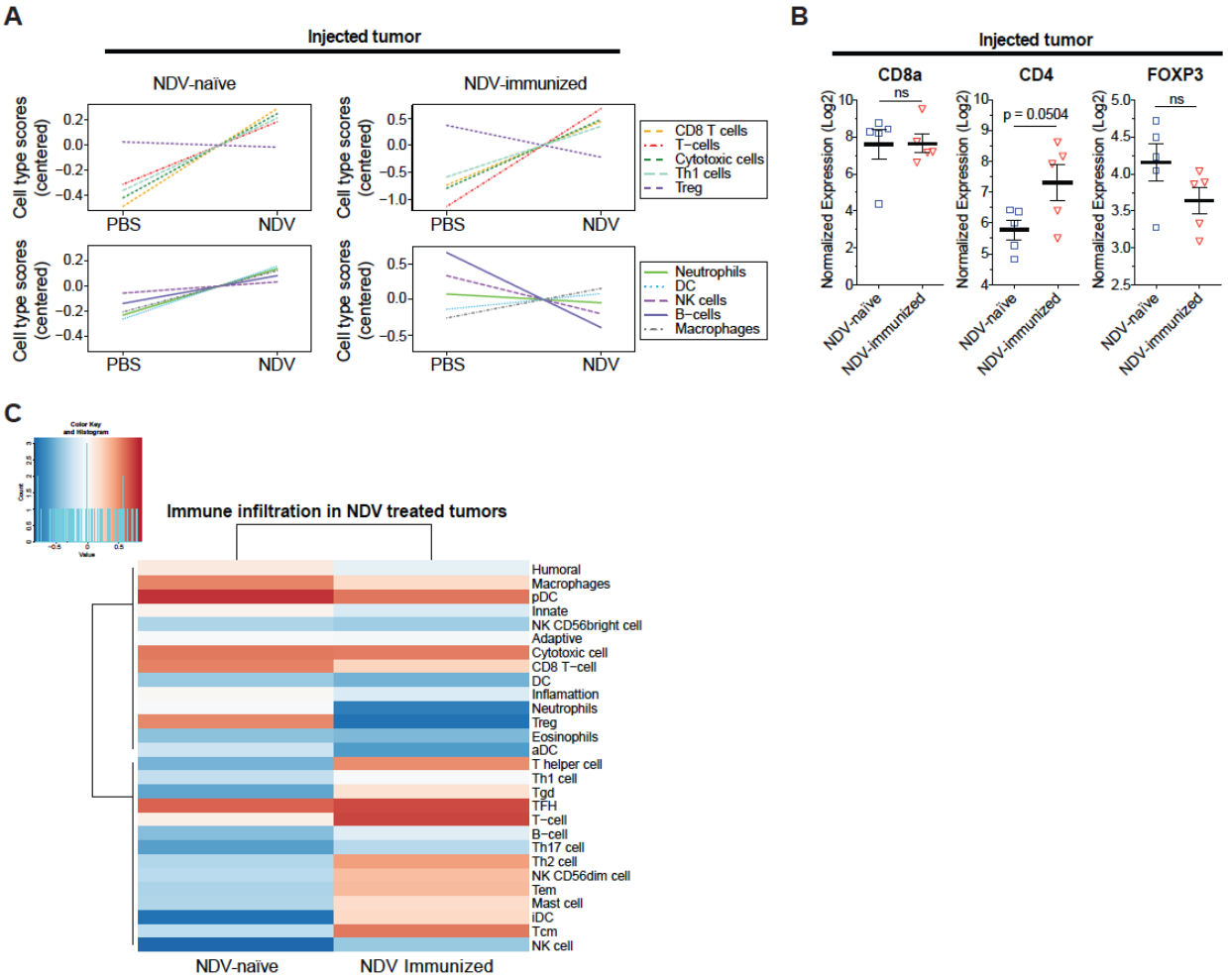

Figure S3. NDV induces inflammatory effects in the microenvironment of the treated tumors. Animals were treated according to the schema in Figure 2A. Gene expression analyses were performed using NanoString PanCancer immune profiling gene panel focusing on over 760 immune response-related genes. (A) Relative cell type gene signature scores in the virus-treated tumors compared to their respective controls. (B) Normalized gene expression for CD8a, CD4, and FoxP3 from the NDV-treated tumors of NDV-naïve and NDV-immunized mice. (C) Heat map displaying GSVA signature scores across immunological cell types for immunized and non-immunized NDV treated tumors. Data represent results from one experiment with n = 3 (NDV-naïve PBS), n = 3 (NDV-immunized PBS), n = 5 (NDV-naïve NDV) and n = 5 (NDV-immunized NDV). Mean ± SEM is shown. ns, not significant.

Figure S4.

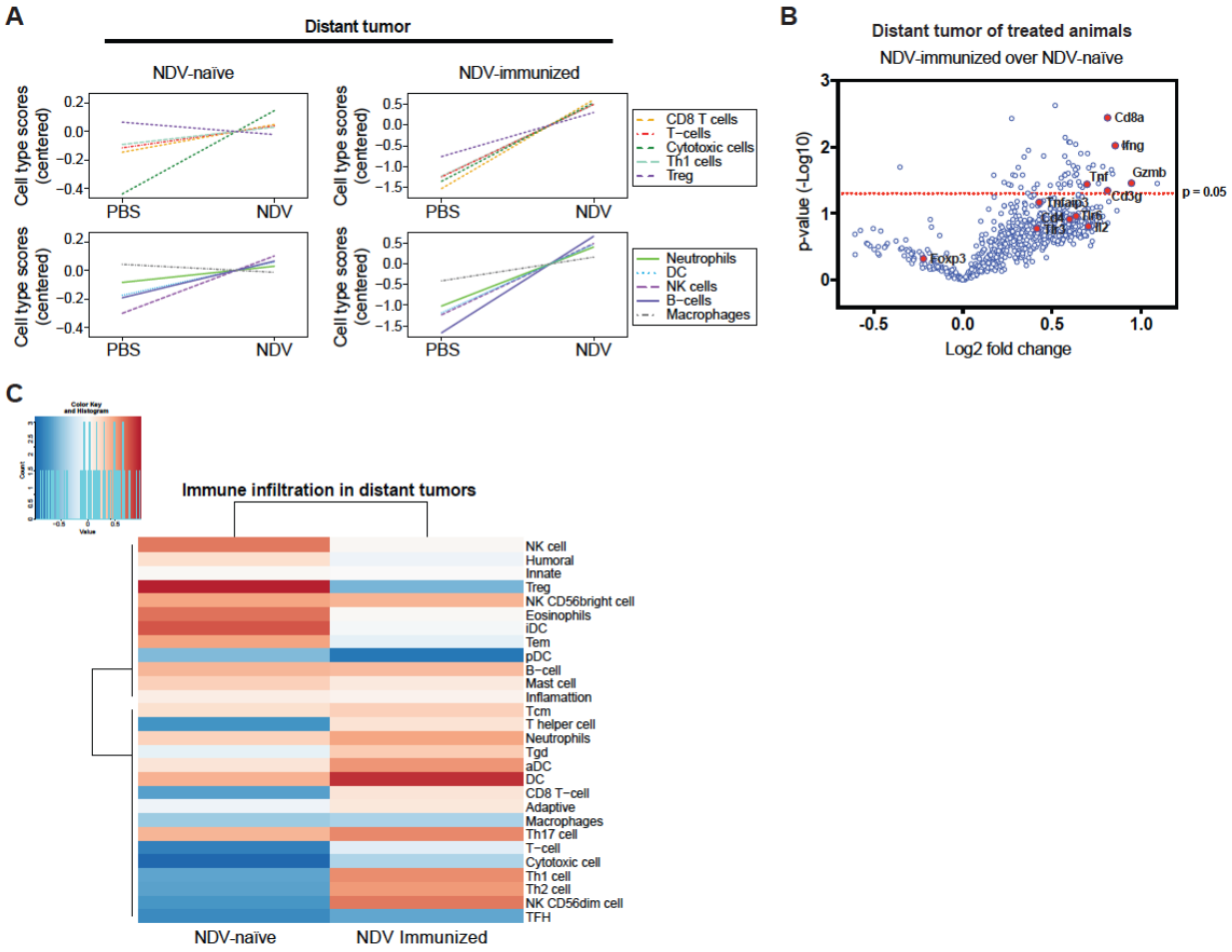

Figure S4. Anti-NDV immunity potentiates abscopal inflammatory effects. Animals were treated according to the schema in Figure 2A. Gene expression analyses were performed using NanoString PanCancer immune profiling gene panel focusing on over 760 immune response-related genes. (A) Relative cell type gene signature scores in the distant, non-injected tumors compared to their respective controls. (B) Global expression of immune-related genes in distant tumors of NDV-treated NDV-immunized vs. NDV-treated NDV-naïve animals. (C) Heat map displaying GSVA signature scores across immunological cell types for immunized and non-immunized NDV treated tumors. Data represent results from one experiment with n = 3 (NDV-naïve PBS), n = 3 (NDV-immunized PBS), n = 9 (NDV-naïve NDV) and n = 5 (NDV-immunized NDV).

Figure S5.

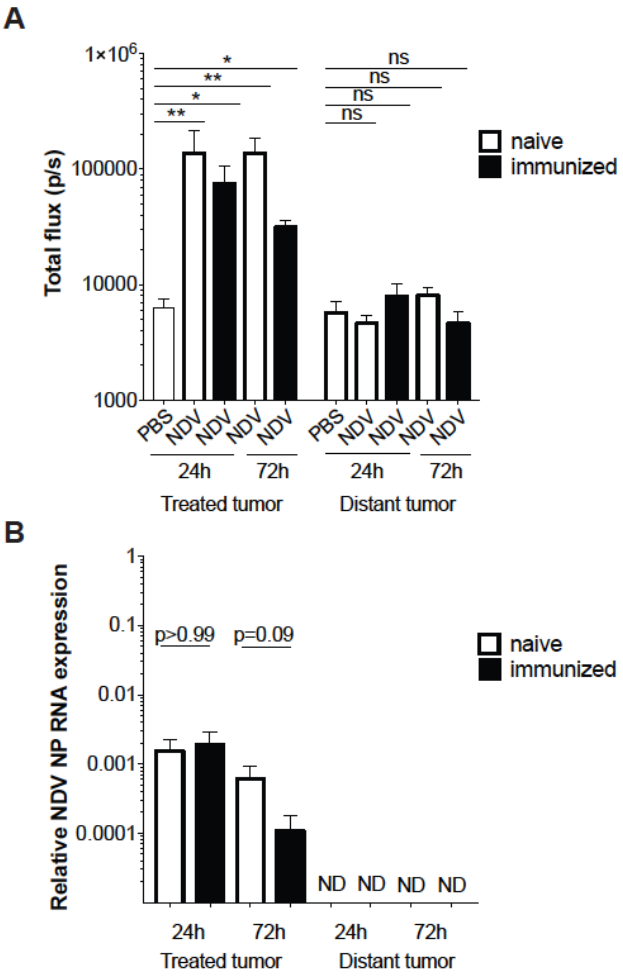

Figure S5. Pre-existing immunity to NDV does not enhance viral spread to distant tumors. Bilateral flank B16-F10 melanoma-bearing animals were treated with NDV expressing luciferase administered to a single flank tumor. A) Luminescence measured from the virus-treated and distant tumors. B) Levels of NDV NP RNA detected in the virus-treated and distant tumors measured by quantitative RT-PCR. ns, not significant; *p < 0.05; **p < 0.01

Figure S6.

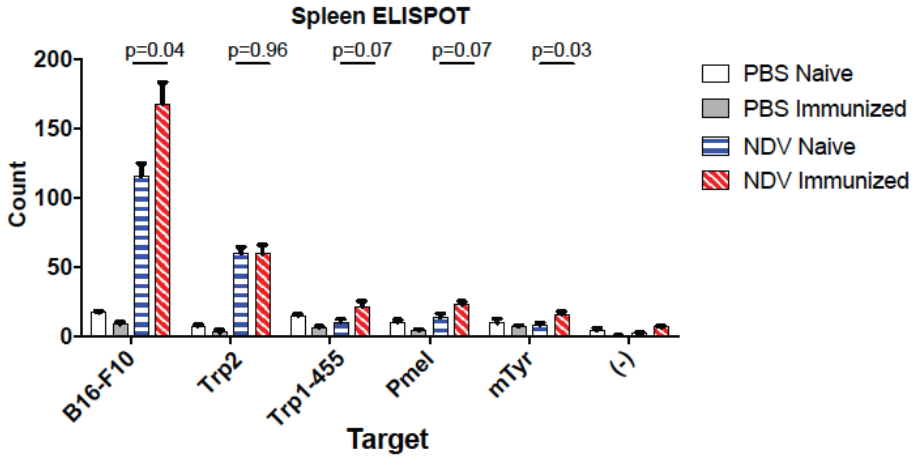

Figure S6. Pre-existing immunity to NDV potentiates CD8 response to B16-F10 melanoma antigens. Animals were treated as in Figure 3A, and splenic CD8+ lymphocytes were isolated and co-cultured with stimulator CD11b+ APCs loaded with the indicated peptides or irradiated B16-F10 cells at 1:1 ratio. IFNγ production was assessed at 24 hours by ELISPOT assay.

Figure S7.

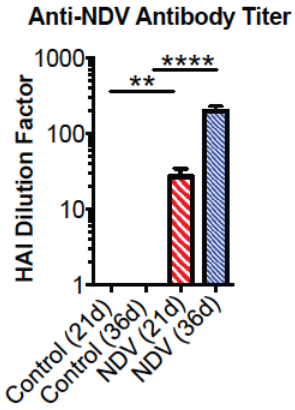

Figure S7. Intratumoral NDV therapy leads to neutralizing anti-NDV antibodies. Anti-NDV antibody serum titers from tumor-bearing animals after initial treatment and day 21 boost with NDV (n = 9) or PBS (n = 8) were determined by hemagglutination inhibition (HAI) of the serum samples collected at the specified time points. **$p < 0.01$; ****$p < 0.0001$.