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Supplemental information

DARPin-fused T cell engager

for adenovirus-mediated cancer therapy

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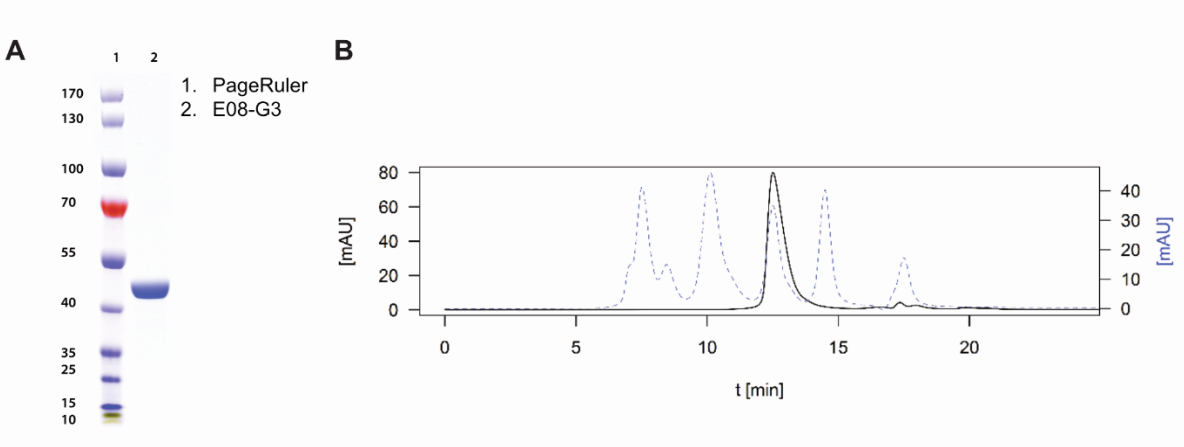

Figure S1. Quality control of recombinantly produced and purified DATE. (A) SDS-PAGE gel purity analysis of 5 µg DATE E08-G3. (B) Purity and monomeric behavior of DATE E08-G3 as measured by analytical SEC-HPLC.

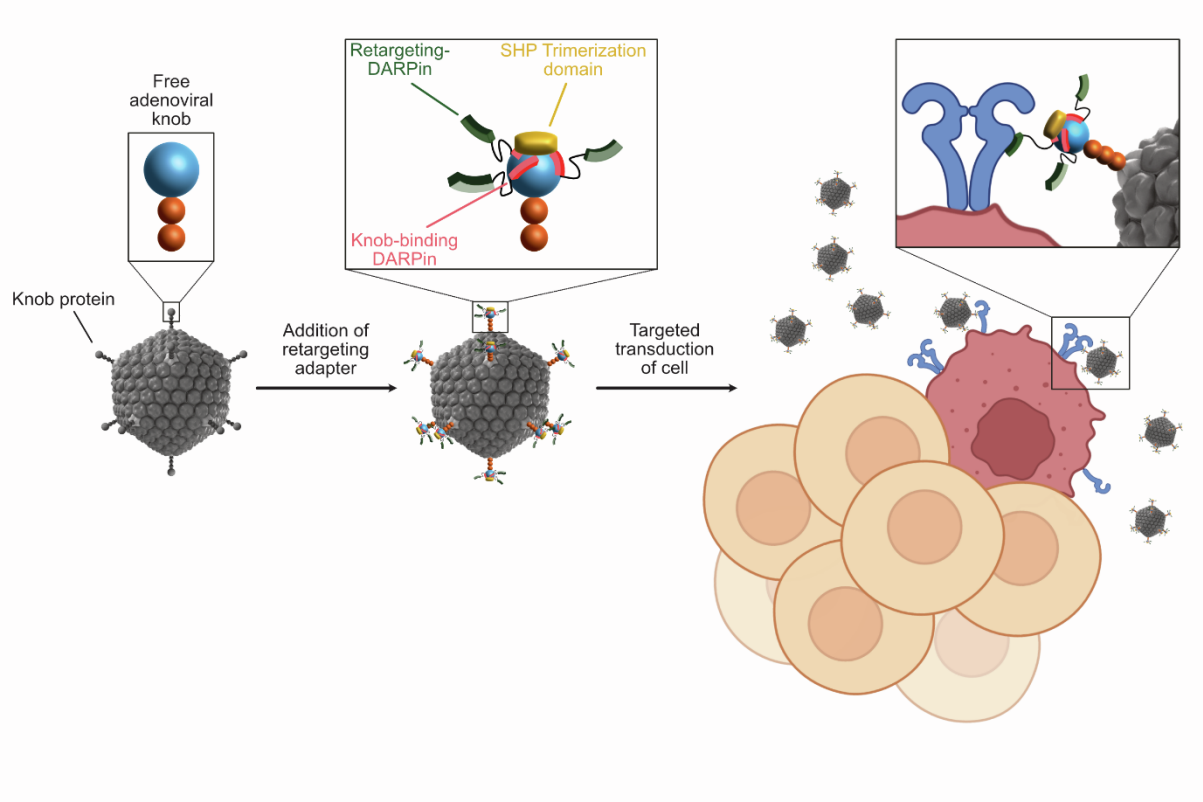

Figure S2. Schematic representation of a bispecific adapter for adenoviral retargeting. The retargeting adapter comprises a C-terminal SHP trimerization domain (yellow), fused to the Ad5 knob-binding DARPin 1D3 (light red) that is connected via a long flexible linker to an N-terminal retargeting DARPin (green) that binds to a cell surface molecule of choice (e.g. HER2). Upon trimerization of the SHP domain, the retargeting adapter binds quasi-covalently via the knob-binding DARPins to the adenoviral knob protein (light blue) and blocks the knob's natural cellular interactions, in this case to the coxsackie adenovirus receptor (CAR). The retargeting DARPin can bind to a suitable biomarker (e.g. HER2, depicted in dark blue) and thus the adenoviral tropism is redirected to the desired cell (e.g. a cancer cell, depicted in dark red).

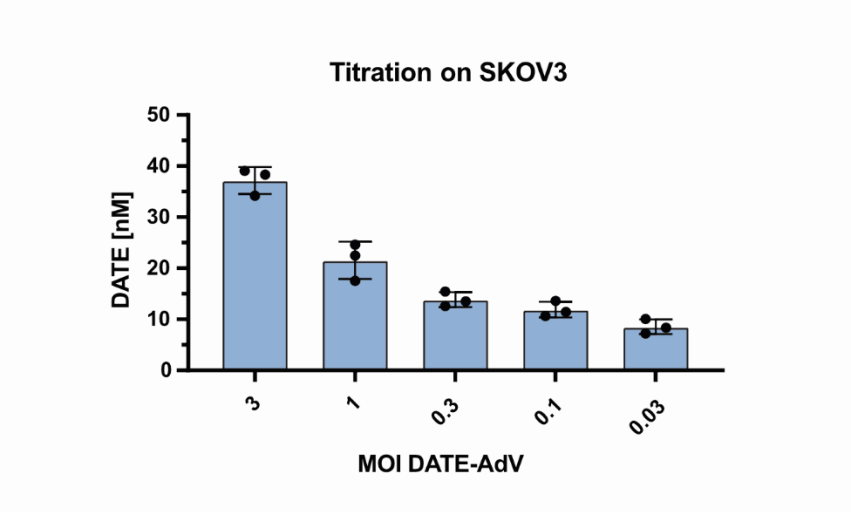

Figure S3. DATE expression by SKOV3 cells. ELISA quantification of DATE expression 72 h after transduction using decreasing MOIs of DATE-AdV. Bar graphs represent mean ± SD (n = 3).

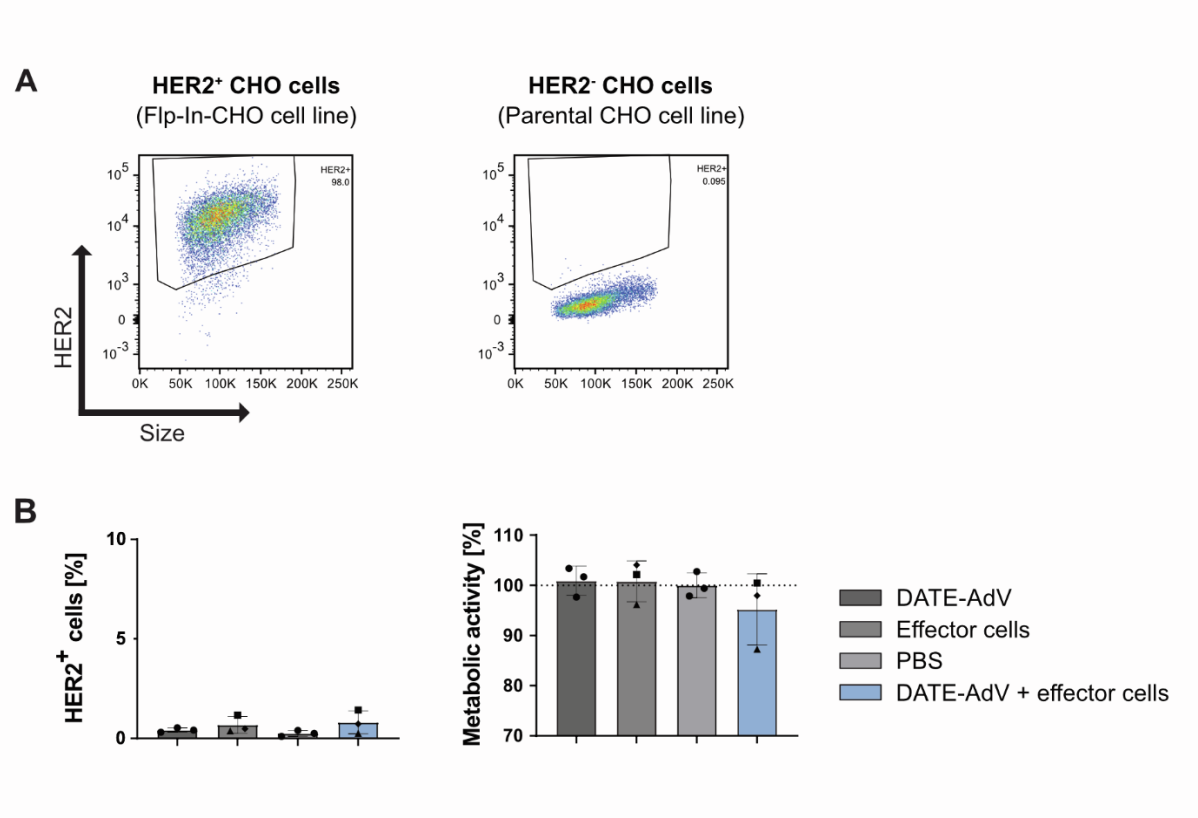

Figure S4. Characterization of HER2$^+$ and HER2$^-$ CHO cells. (A) Flow cytometry analysis of surface-bound HER2 levels of HER2$^+$ Flp-In-CHO cells (left graph) and of parental HER2$^-$ CHO cells (right graph). (B) Parental, HER2$^-$ CHO cells were either only transduced with DATE-AdV (MOI of 10), only co-cultured with purified PBMCs (E:T of 10), left untreated (PBS control) or were transduced with DATE-AdV (MOI of 10) while being co-cultured with effector cells (E:T of 10). For direct comparison to Figure 4A and Figure 4B, the level of HER2 was measured by flow cytometry to demonstrate that these cells indeed still express only negligible levels of HER2 (here, stated as cells that would fall in the positive window (right panel in A)), and the metabolic activity of CHO cells was assessed via XTT assays after these treatments. Individual symbols represent single donors. Bar graphs represent mean ± SD.

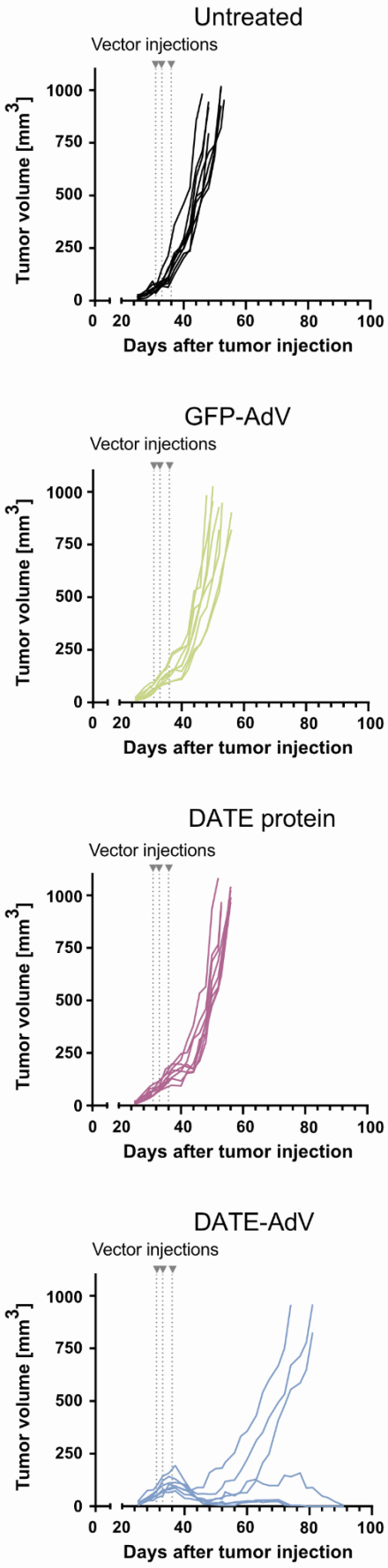

Figure S5. DATE-AdV treatment in tumor-bearing NSG mice potently inhibits tumor growth shown for individual mice. Subcutaneous SKOV3 tumor-bearing mice were treated with three intratumoral injections of indicated constructs (untreated, GFP-AdV, recombinant DATE as protein or DATE-AdV) and a single, intravenous injection of 7×10^6 human T cells one day after the first virus administration. T cells were derived from 2 different healthy donors. Treatment with recombinant DATE protein results in limited tumor growth delay, whereas intratumoral application of DATE-AdV (bottom graph) reduces tumor growth in a most pronounced way and results in relapse-free survival in half of the cohort.

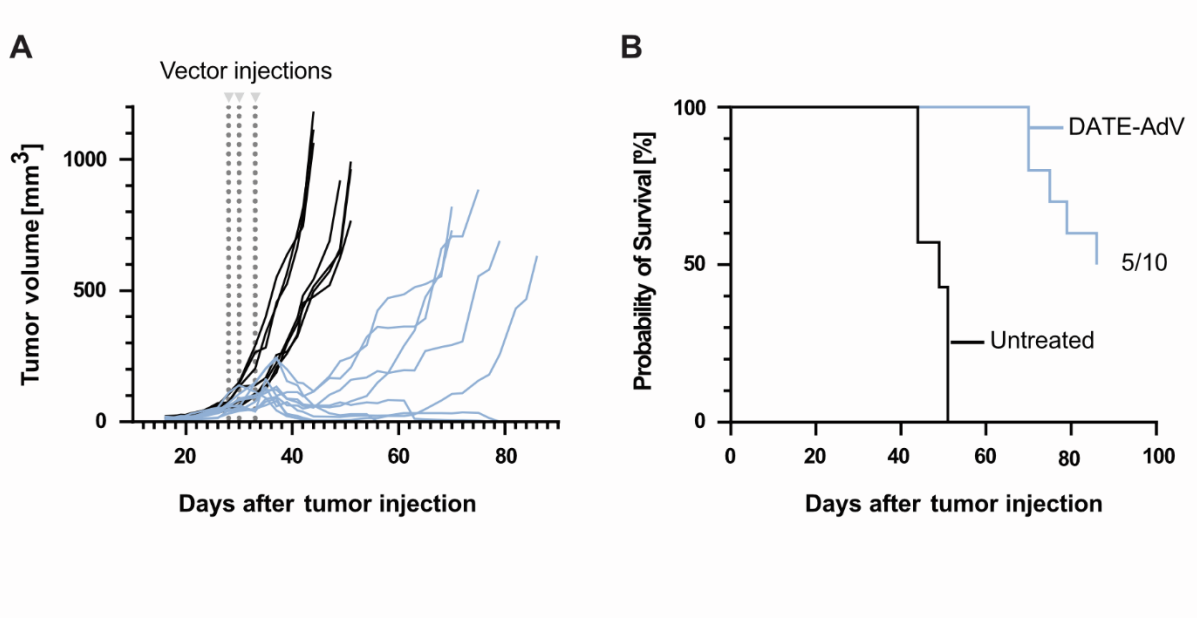

Figure S6. Complete remission in 50 % of DATE-AdV treated tumor-bearing mice in pilot study. (A) Treatment with DATE-AdV (light blue) results in relapse-free remission in 50 % of human T cell reconstituted mice for 86 days after tumor injection compared to untreated, reconstituted mice (black). Injections of DATE-AdV are indicated by dotted lines. (B) Intratumoral injections of DATE-AdV (light blue) greatly improved overall survival and 50 % of animals show complete tumor regression compared to untreated animals (black).

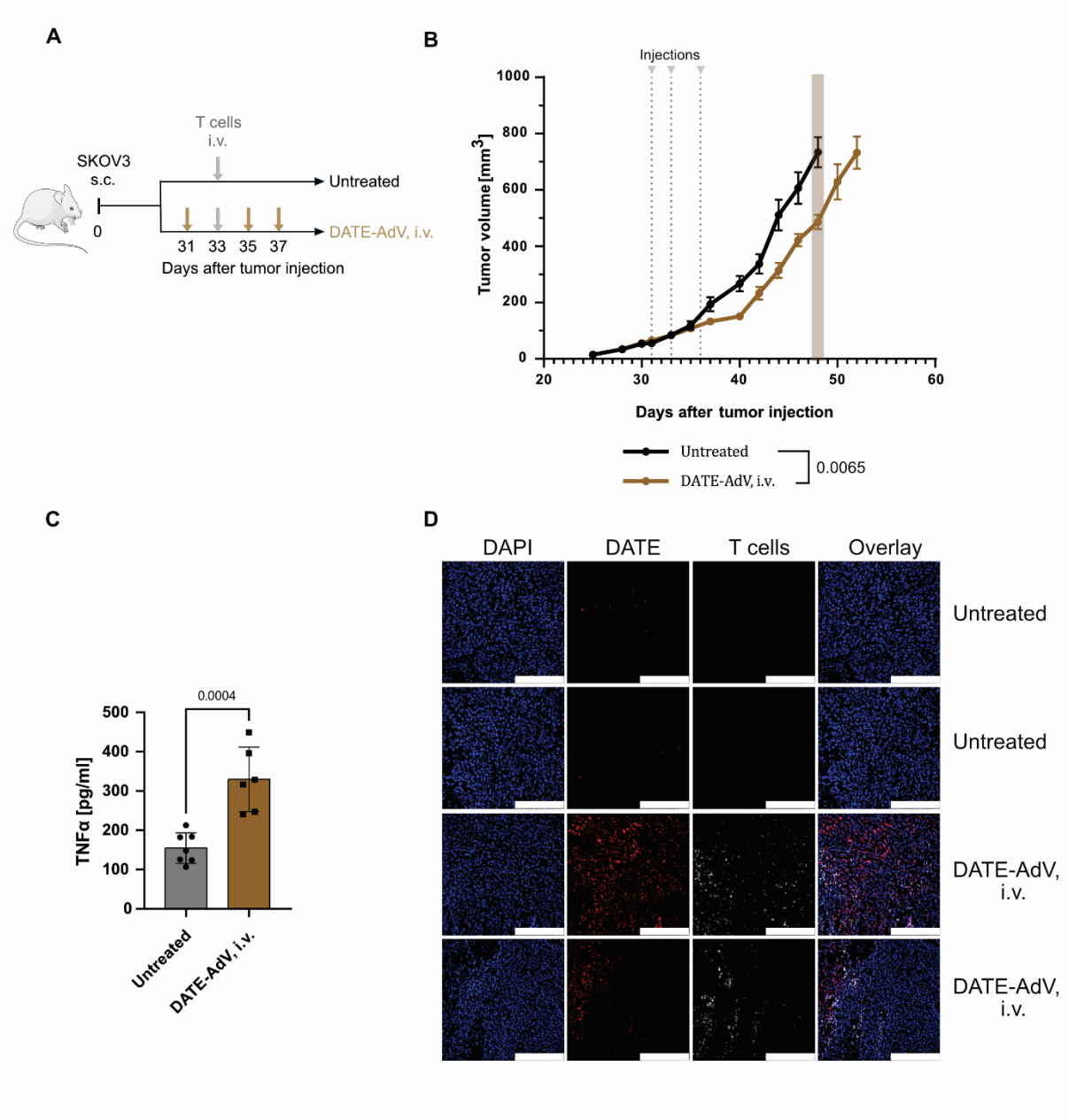

Figure S7. Intravenous DATE-AdV treatment of tumor-bearing NSG mice reconstituted with human T cells. Subcutaneous SKOV3 tumor-bearing mice were treated with three intravenous injections of DATE-AdV and a single, intravenous injection of 7×10^6 human T cells. T cells were derived from 2 different healthy donors. (A) Depiction of treatment scheme. NSG mice were subcutaneously injected with human ovarian HER-2-expressing SKOV-3 cancer cells and treated intravenously with 3 doses (indicated by brown arrows) of DATE-AdV (each 1.7×10^8 transducing units) or were left untreated once the tumors reached a tumor volume of 30-100 mm^3. Additionally, mice were reconstituted with human T cells two days after the first injection of therapeutics (grey arrow). (B) Intravenous administration of DATE-AdV results in significant reduction of tumor growth compared to untreated control

group at day 46 (indicated by brown bar). Statistical analysis was performed by a mixed-effect model multiple with Dunnett's multiple comparison test (n = 6 and 7 animals per group, dots represent mean ± SD). (C) TNFα concentrations detected in lysed tumor tissue. Statistical analysis was performed by an unpaired two tailed t-test (n = 6 and 7, bar graphs represent mean ± SD). (D) Representative immunofluorescence images of tumor tissues stained for DATE (red) or T cells (light grey) and counter-stained with DAPI (blue) for nuclei staining. Scale bar = 250 μm.

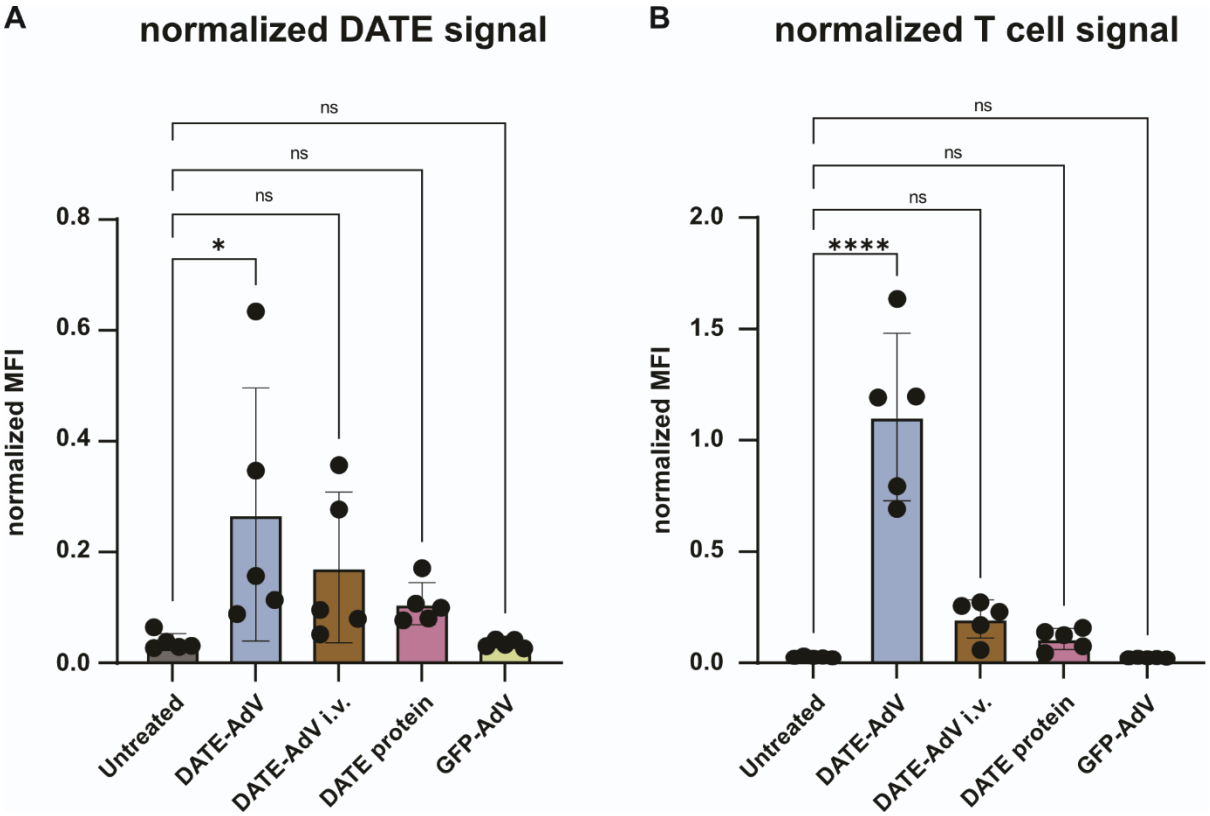

Figure S8. Quantification of DATE expression and T cell number within the tumor at endpoint. Representative immunofluorescent images of tumor tissue IHC analysis (Fig. 6 and S7) were converted to 8-bit grayscale and signal intensity was quantified using Fiji. (A) Mean fluorescence intensity (MFI) of representative tumor tissue slices was quantified after immunofluorescent detection of DATE proteins and signal was normalized to MFI derived from nuclei staining using DAPI. (B) T cells in representative tumor tissue slices were detected via immunostaining and the derived MFI was normalized to the MFI obtained upon nuclei staining using DAPI. Bar graphs represent mean ± SD and symbols show MFI ratios of individual tumor tissue slices. Not significant (ns) $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$. Statistical analysis was performed by a one-way ANOVA with Dunnett's multiple comparisons test, n = 5 tissue slices from 2-3 different animals.

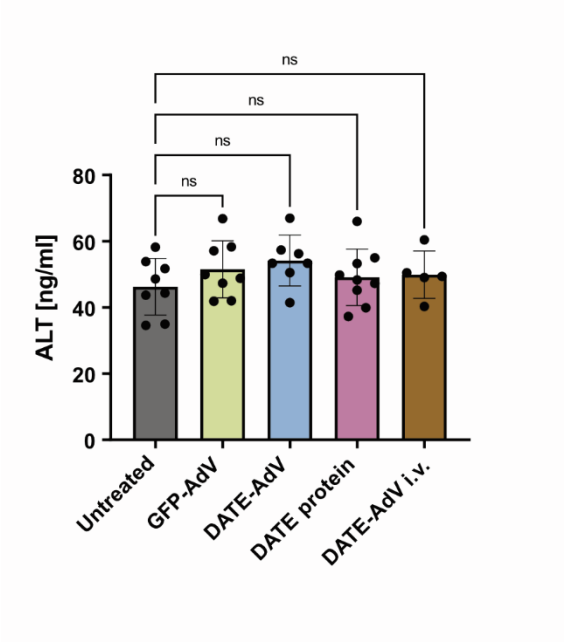

Figure S9. Alanine aminotransferase (ALT) concentrations in the serum of mice. ELISA measurements of serum samples taken at day 44 after tumor injection (8 days after the last vector injection) from mice shown in Fig. 5 and S7. Bar graphs represent mean ± SD and symbols show individual mice. Statistical analysis was performed by a one-way ANOVA with Dunnett's multiple comparisons test.