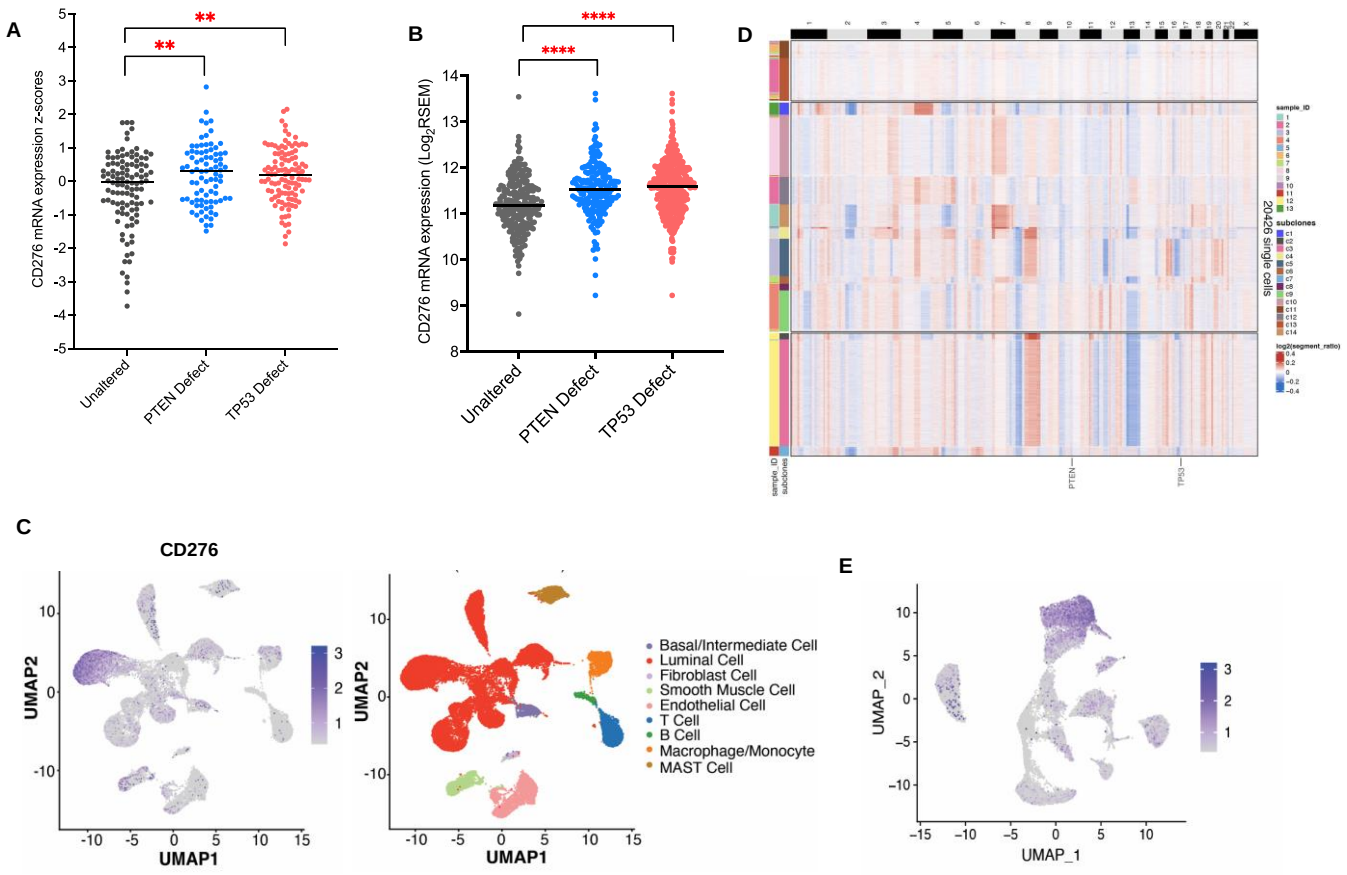

Figure S1. B7-H3(*CD276*) expression is elevated in tumors with *PTEN* and *TP53* defects
(**A,B**) *CD276* mRNA expression in metastatic prostate cancer (**A**, SU2C/PCF dataset) and invasive breast cancer (**B**, TCGA dataset) containing *PTEN* and/or *TP53* defects. One-way ANOVA with Tukey's post hoc tests was performed using GraphPad Prism version 9.2.0.. ** $P < 0.01$, **** $P < 0.0001$. (**C**) Single-cell transcriptional profiling showed the expression pattern of *CD276* in human prostate tumors (*Chen et al., 2021 (41); n=12 patients*). UMAP plots of *CD276* gene expression (left) and cell type annotation (right) are shown. (**D,E**) The CopyKat inferred copy number segmentation of luminal cells heatmap (**D**) and *CD276* gene expression pattern in 14 luminal cell subclones (**E**).

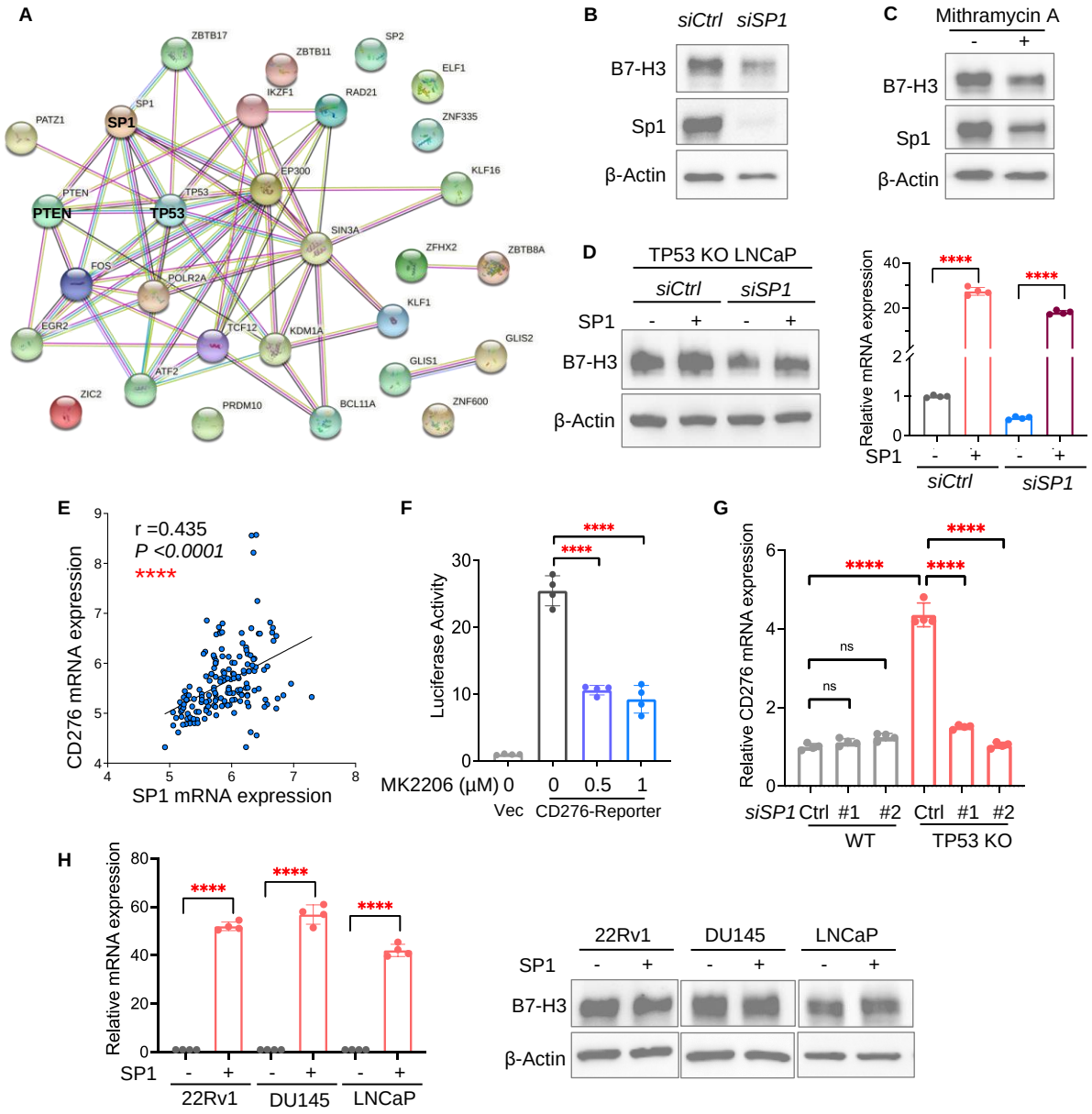

Figure S2. Loss of *PTEN* and *TP53* upregulates B7-H3(*CD276*) by activating Sp1

(**A**) 27 putative transcriptional regulators of B7-H3 were found in the GeneHancer database. Their protein-protein interaction network with PTEN and p53 was analyzed using STRING (Search Tool for the Retrieval of Interacting Genes/Proteins). (**B**) PTEN/p53-deficient LNCaP cells (PTEN-null; TP53 knockout (KO)) were transfected with siRNA targeting *SP1* or scrambled control, followed by western blot analysis. (**C**) PTEN/p53-deficient LNCaP cells were treated with 20 nM Sp1 inhibitor, mithramycin A, followed by western blot analysis. (**D**) PTEN/p53-deficient LNCaP cells were transfected with siRNA targeting *SP1* or scrambled control, followed by overexpressing SP1. B7-H3 protein was determined by western blot (left), and SP1 expression was determined by qPCR (right; *n* = 4 per group). (**E**) Pearson correlation between *SP1* and *CD276* mRNA in human

metastatic prostate tumors (*Brady et al., 2021[29]*). The Pearson correlation coefficient (r) and p-value are shown. (**F**) Dual luciferase assay of *CD276*-Reporter in LNCaP cells treated with MK2206 or DMSO (*n* = 4 per group). (**G**) Control or *TP53* knockout 22Rv1 cells were transfected with siRNA targeting *SP1* or scrambled control, followed by *CD276* mRNA expression analysis using qPCR (*n* = 4 per group). (**H**) Overexpression of Sp1 in LNCaP, DU145 and 22Rv1 cells, followed by determination of SP1 (left; *n* = 4 per group) and B7-H3 (right). Data represent the mean ± SD. One-way ANOVA with Tukey's post hoc tests (D, F, and G) and Student's t-tests (H) were performed using GraphPad Prism version 9.2.0.. $****$ $P < 0.0001$. ns, not significant.

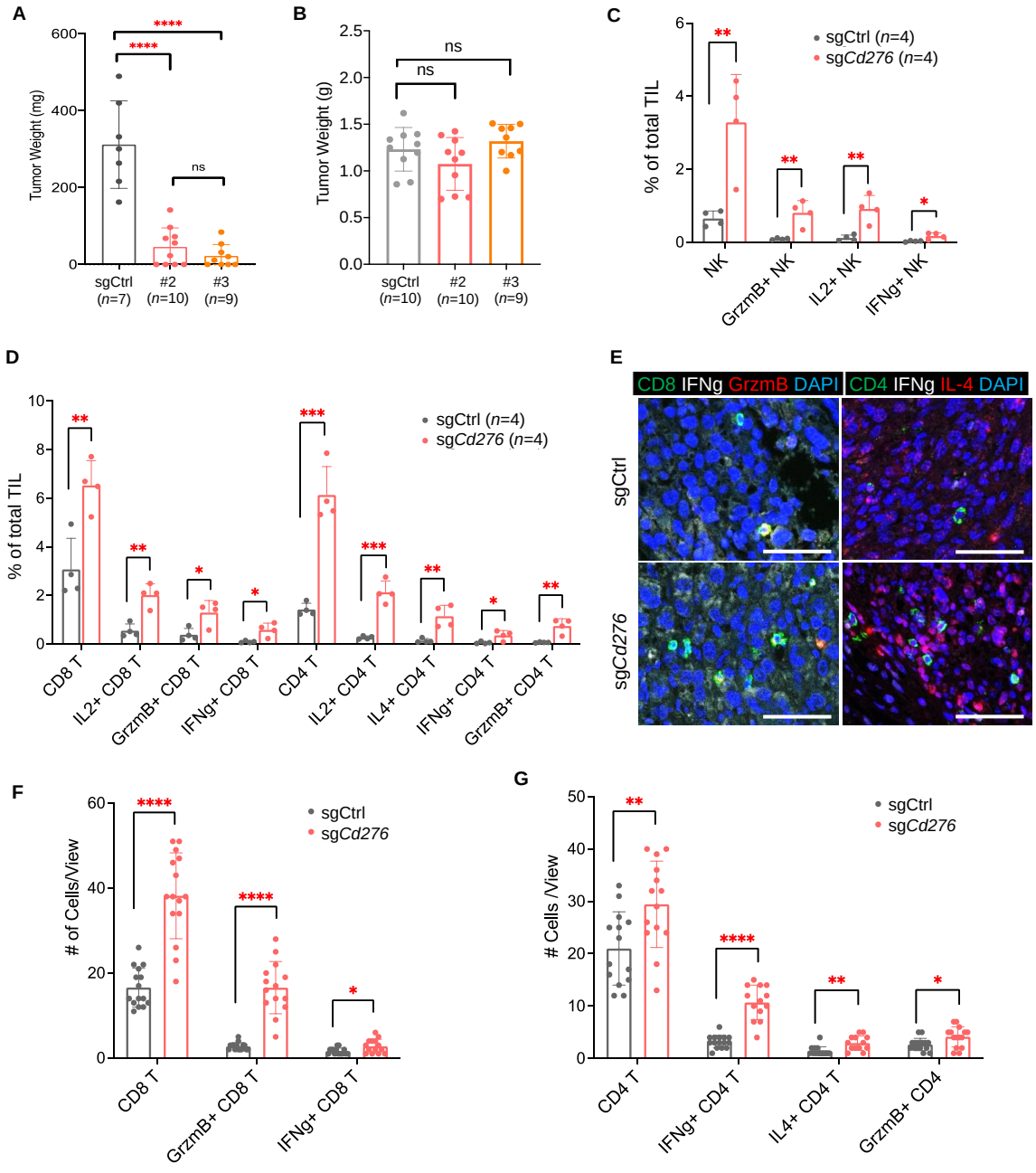

Figure S3. B7-H3 promotes PTEN/p53-deficient tumors and immunosuppression

(**A**) Tumor weight of control and B7-H3-depleted syngeneic tumors in immunocompetent C57BL/6 mice. (**B**) Weight of control and B7-H3-depleted tumors in immunodeficient NSG mice. (**C, D**) Analyses of cytokines in NK (**C**) and T (**D**) cells in control and B7-H3-depleted syngeneic tumors, determined by CyTOF. TIL: Tumor-Infiltrating Leukocytes. Data represent the mean ± SD. (**E, F, G**) Representative images of multiplex IHC staining (**E**) and quantifications of CD8+ T (**F**) and CD4+ T cells (**G**) expressing indicated cytokines in control and B7-H3-depleted syngeneic tumors (*n* >10 individual views from 3 mice per group). Scale bar = 50 µm. Left panel in **E**: Green, CD8a; White, IFNg; Red, GrzmB; Blue, DAPI. Right panel in **E**: Green, CD4; White, IFNg; Red, IL-4; Blue,

DAPI. Data represent the mean ± SD. One-way ANOVA with Tukey's post hoc tests was performed using GraphPad Prism version 9.2.0.. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$. ns, not significant.

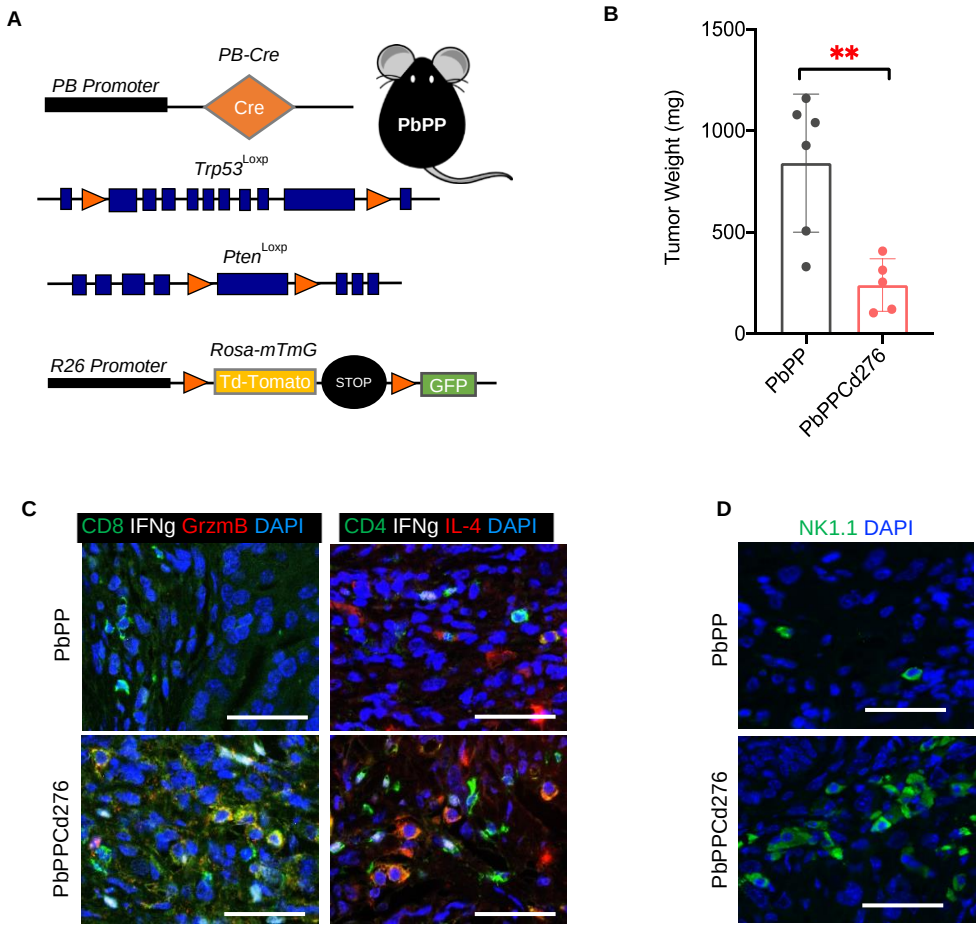

Figure S4. Prostate-specific Cd276 deletion GEMM model revealed B7-H3's impact on cancer progression

(**A**) GEMM model design: conditional knockout (KO) alleles of *Pten*^{Loxp}, and *Trp53*^{Loxp} were crossed with prostate-specific *PB-Cre* and *Rosa-mTmG* to establish *PB-Cre; Pten*$^{L/L}$*; Trp53*$^{L/L}$ (PbPP) GEMM model. (**B**) Weight of prostate tumors from PbPP (n = 6) and PbPPCd276 (n = 5) mice at five months of age. $**P < 0.01$ (**C**) Multiplex IHC staining and representative images of CD8 T, CD4 T cells, and cytokines in prostate tumors from PbPP and PbPPCd276 mice. Scale bar = 50 µm. Left panel: Green, CD8a; White, IFNg; Red, GrzmB; Blue, DAPI. Right panel: Green, CD4; White, IFNg; Red, IL-4; Blue, DAPI. (**D**) IF staining and representative images of NK cells in PbPP and PbPPCd276 tumors. Scale bar = 50 µm. Green, NK1.1+; Blue, DAPI Student's t-tests were performed using GraphPad Prism version 9.2.0. $** P < 0.01$.

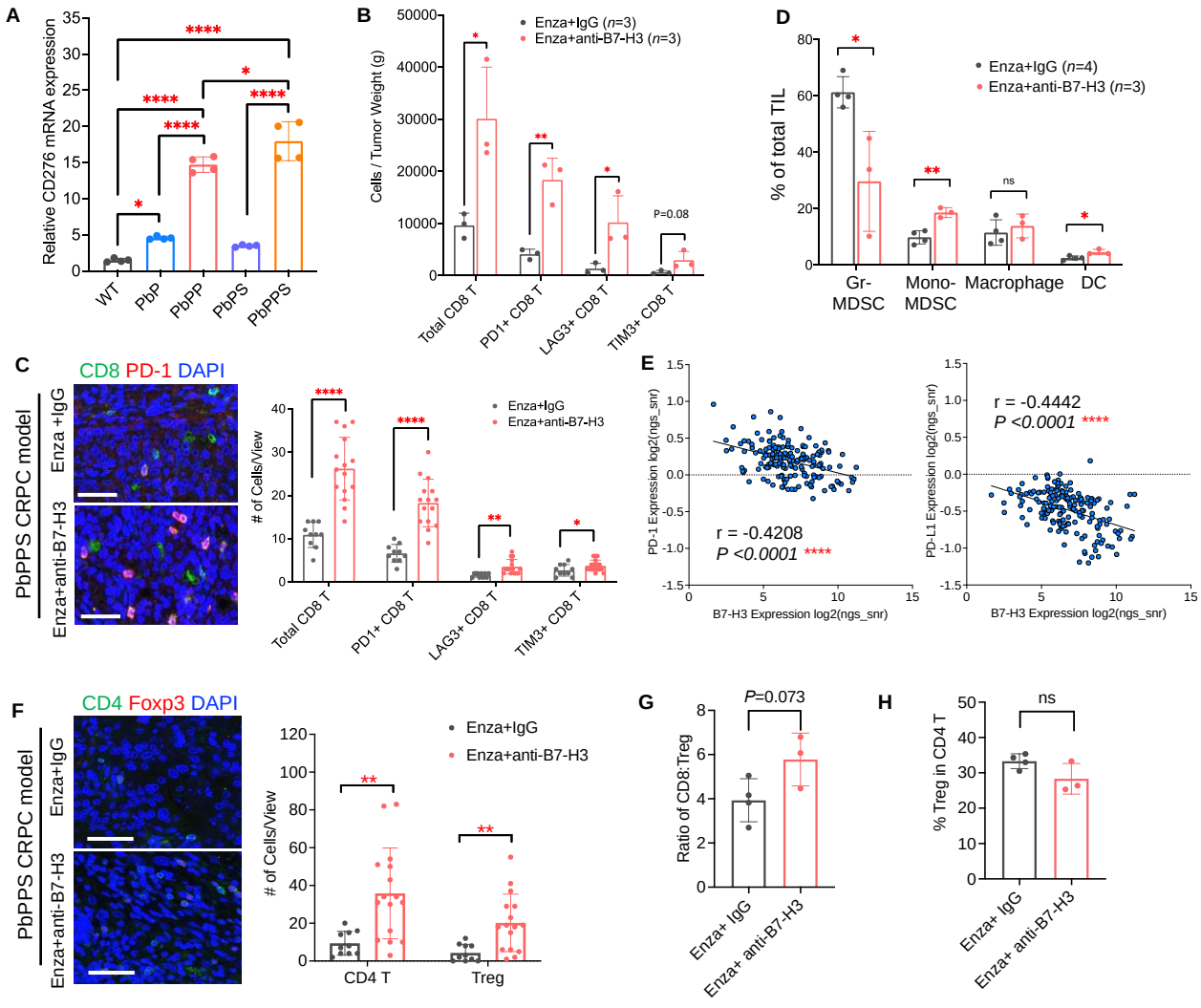

Figure S5. Effects of B7-H3-blocking mAb in preclinical models of PTEN/p53-deficient CRPC

(**A**) B7-H3 expression in normal prostate (WT) and prostate tumors from PbP, PbPP, *PB-Cre; Pten$^{L/L}$; Smad4$^{L/L}$* (PbPS), and *PB-Cre; Pten$^{L/L}$; Trp53$^{L/L}$; Smad4$^{L/L}$* (PbPPS) mice (*n* = 4 per group), as determined by qPCR. (**B**) Flow cytometry analysis of CD8 T and exhaustion markers in syngeneic tumors treated with Enza in combination with IgG or anti-B7-H3 (*n* = 3 per group). (**C**) Representative multiplex IHC staining and quantification of total CD8 T (CD8a+) and exhausted CD8 T cells (PD1+/LAG3+/TIM3+) in PbPPS CRPC tumors after combination treatment. Green, CD8; Red, PD-1; Blue, DAPI. Scale bar = 50 µm. *n* >10 individual views from 3 mice per group. Data represent the mean ± SD. (**D**) Quantification of tumor-infiltrating myeloid cells in syngeneic tumors treated with Enza in combination with IgG or anti-B7-H3, determined by CyTOF. TIL: Tumor-Infiltrating Leukocytes. (**E**) Pearson correlations of B7-H3 expression with PD-1 (left) or PD-L1 (right) expression in human metastatic prostate tumors (Brady dataset, *[29]*). Pearson correlation coefficient (r) and *P* values are shown. (**F**) IF staining and quantification of CD4 T

(CD4+) and Treg (CD4+/Foxp3+) cells in PbPPS CRPC tumors after combination treatment. Green, CD4; Red, Foxp3; Blue, DAPI. Scale bar = 50 μm. Data represent the mean ± SD of >10 individual views. (**G,H**) The ratio of CD8 T to Treg (**G**) and the percentage of Treg (**H**) in total CD4 T in syngeneic tumors treated with Enza in combination with IgG (n = 4) or anti-B7-H3 (n = 3), determined by CyTOF. Data represent the mean ± SD. One-way ANOVA with Tukey's post hoc tests (A) and student's t-tests (B, C, D, F, G, and H) were performed using GraphPad Prism version 9.2.0. $*P < 0.05$, $**$ $P < 0.01$, $****$ $P < 0.0001$. ns, not significant.

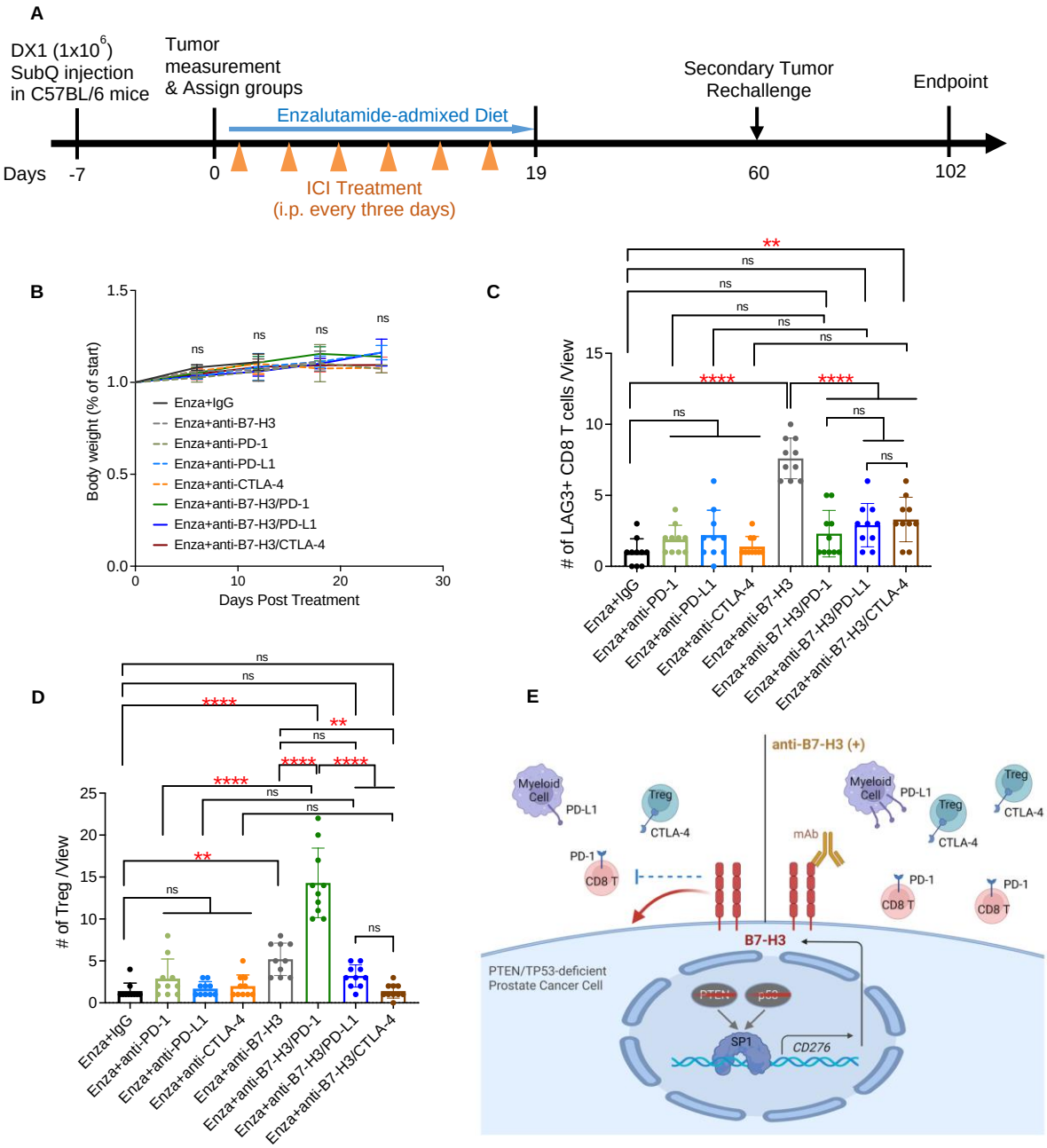

Figure S6. B7-H3 inhibition combined with PD-L1 or CTLA-4 blockade achieves durable anti- effects in PTEN/p53-deficient CRPC

(**A**) Schematics of the treatment design using the syngeneic CRPC model. Enzalutamide-resistant DX1 (1x10^6) cells were subcutaneously injected into one flank of C57BL/6 male mice. One week later, tumor volumes were measured by calipers, and mice were randomly assigned to treatment groups: Enza in combination with IgG, anti-B7-H3, anti-PD-1, anti-PD-L1, anti-CTLA-4, anti-B7-

H3/PD-1, anti-B7-H3/PD-L1, and anti-B7-H3/CTLA-4. For secondary tumor rechallenge, DX1 ($1x10^6$) cells were subcutaneously injected into another flank of tumor-free mice on Day 60. ICI: immune checkpoint inhibitors. The blue line indicates the duration of Enza treatment; orange triangles indicate drug administrations. (**B**) Changes in body weight of tumor-bearing mice during treatment (*n* = 4 per group). (**C,D**) $LAG3^+$ CD8 T (**C**) and Treg (**D**) cells in tumors treated with single agents or combinations, determined by multiplex IHC. Data represent the mean ± SD of 10 individual views. One-way ANOVA with Tukey's post hoc tests was performed using GraphPad Prism version 9.2.0. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. ns, not significant. (**E**) Schematic model: B7-H3, encoded by the *Cd276* gene, is a promising therapeutic target in PTEN/TP53-deficient prostate cancer. PTEN and p53 pathways negatively regulate B7-H3 expression by suppressing transcriptional factor Sp1. In PTEN/TP53-deficient tumors, Sp1 is activated and induces transcription of the *CD276* gene (bottom). The elevated B7-H3 promotes tumor progression and causes the suppression of cytotoxic T cells (CD8 T) in the tumor microenvironment (left). Inhibition of B7-H3 with monoclonal antibody (anti-B7-H3 mAb) increases the infiltration of cytotoxic T cells, but its anti-tumor effect is hindered by the enriched regulatory T cells (Treg) and upregulated PD-L1 expression in myeloid cells in the tumor microenvironment (right). Combining B7-H3 inhibition with the blockade of PD-L1 or CTLA-4 can achieve durable anti-tumor effects in PTEN/TP53-deficient prostate cancer. The schematic figure was generated by BioRender.