Supplementary Materials

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Fig. S7. Teniposide induced tumor infiltration of immune cells and potentiated anti-PD1 therapeutic efficacy on multiple mouse tumor models.
Figure S1 Design and verification of HMGB1-Gluc reporter system.

(A) HMGB1 with Gluc tag was cloned into a lentiviral backbone vector to create a HMGB1-Gluc reporter system. (B) LSV174T (HMGB1-Gluc) cells were treated with DMSO, TNFα+Z-VAD-FMK (T+Z), T+Z+Birinapant (T+Z+B), T+Z+LCL161 (T+Z+L) for 20 hrs, then HMGB1-Gluc luciferase activity was measured. (C) LSV174T (HMGB1-Gluc) and MC38 (HMGB1-Gluc) cells were treated with mitoxantrone (MTX) or DMSO for 20 hrs, then HMGB1-Gluc luciferase activity were measured. Right panel, Western blot verifying Gluc tag expression of HMGB1-Gluc-transfected cells described in (B-C). Actin was used as a loading control. Data in (B-C) represent mean ± SD of 3 independent experiments. ***P < 0.001, by one-way ANOVA with Bonferroni post-test.
Figure S2 Teniposide induced cell immunogenic cell death in different types of cancer cells.

(A) Western blot detection of Gluc tag expression of HMGB1 in different types of cancer cells. (B) PDAC (HMGB1-Gluc) cells were treated with increasing doses of teniposide for 20hrs, then the HMGB1-Gluc luciferase activity was measured. (C-E) PDAC and B16 cells were treated with teniposide or DMSO for 20 hrs, and the cells underwent apoptosis was detected by FACS (C) and LDH release (D); and surface expression of CRT (E) was detected by FACS. (F) Mice with established CT26 tumors were treated with teniposide or vehicle (n=3) on day 6 and 7,
then the tumor tissue were harvested on day 8, and the intratumor CRT expression was detected by IHC staining with anti-CRT antibody. Scale bar, 50 μm. Data in (B-E) represent mean ± SD of 3 independent experiments. ***$P < 0.001$, by one-way ANOVA with Bonferroni post-test (B, D), by unpaired Student’s $t$ test (C, E).
**Figure S3** Teniposide enhanced expression of antigen presenting machinery molecules on tumor cells.

(A) B16-OVA and MC38-OVA cells were treated with teniposide or DMSO for 20 hrs, then the surface expression of MHC-I-OVA-SIINFEKL complex was determined by FACS. (B) Mice with established CT26 tumors were treated with teniposide or vehicle (n=3) on day 6 and 7, and the tumor tissue were harvested on day 8. The expression of MHC-I and MHC-II on tumor cells were detected by FACS, and the expression of antigen presenting machinery genes were measured by qPCR. The data in A represents mean ± SD of 3 independent experiments. **P < 0.01, by unpaired Student’s t test.**
Figure S4 Teniposide-treated tumor cells induced T cell activation and dendritic cell maturation.
(A) B16-OVA cells were treated with teniposide or DMSO for 16 hrs, then co-cultured with BMDCs and B3Z cells for 18 hrs and treated with BFA for additional 6 hrs. Granzyme B (GZMB) and IFNγ expression in T cells were determined by intracellular immunostaining followed by FACS analysis. (B) B16-OVA cells were treated as in (A) except B3Z cells were replaced by OT-I cells. (C) MC38 cells were treated with DMSO or indicated concentration of teniposide for 16 hrs and then co-cultured with BMDCs for additional 24 hrs. The surface expression of CD86, CD80, MHC-I and MHC-II on CD11c+ DCs was determined by FACS. Data in A-B are representative of 3 independent experiments. Data in C represents mean ± SD of 3 independent experiments. **P < 0.01, ***P < 0.001, by one-way ANOVA with Bonferroni post-test.
Figure S5 Teniposide triggered IFN-I signaling activation through cGAS-STING axis in tumor cells.
(A) CT26 and MC38 cells were treated teniposide or DMSO for 24 hrs, then the expression of γH2AX was detected by immunofluorescence staining. Scale bar, 10 μm. (B) CT26 cells were treated with different topoisomerase inhibitors or DMSO for 20 hrs, then the cytosolic genomic DNA were detected by qPCR. (C) CT26 and PDAC cells were treated with increasing doses of teniposide for 24 hrs, then the expression of phosphorylated STAT1, total STAT1, cGAS and STING were measured by Western blot; actin was used as a loading control. (D) CT26 cells were treated as in (B), then the protein expression of cGAS and STING were detected by Western blot, actin was used as a loading control. (E) MC38 and CT26 cells were treated with teniposide or DMSO for 24 hrs, then the mRNA expression levels of IFNβ, CCL5 and CXCL10 genes were measured by qPCR. (F) MC38 Cells were treated as in (A), then the supernatant levels of CCL5 and CXCL10 were measured by ELISA. (G) IFNβ and CCL5 gene expression levels were measured in CT26 tumor (n=5) and MC38 tumor (n=5) after twice treatment with teniposide or vehicle. (H) MC38/WT and MC38/STING KO cells were treated with teniposide or DMSO for 24 hrs, then the supernatant levels of CCL5 and CXCL10 were measured by ELISA. (I) Western blot results verified the knockout effect of STING in B16 and MC38 cells. Data in (A, C-D, I) are representative of 3 independent experiments. Data in (B, E, F, H) represent mean ± SD of 3 independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001, by unpaired Student’s t test (B, E, F) or by one-way ANOVA with Bonferroni post-test (H).
Figure S6 NF-κB signaling activation is required for teniposide-induced ICD features.

(A) B16 and MC38 cells were treated with teniposide or DMSO for 16 hrs, then the cellular protein level of phospho-p65 (p-p65) and p65 was determined by Western blot. (B) B16-OVA cells were treated with teniposide together with or without IKK inhibitor BAY-117082 for 16 hrs, then co-cultured with BMDC and B3Z for 24 hrs, supernatant IL-2 production was measured by ELISA. (C) B16-OVA cells transduced with IKKβ shRNA were treated with teniposide for
16hrs, then the cellular protein level of p-p65 were detected by Western Blot. (D) B16-OVA cells transduced with IKKβ shRNA were treated with teniposide or DMSO for 16hrs, then co-cultured with BMDC and B3Z for 24hrs, supernatant IL-2 were measured by ELISA. (E) B16-OVA cells were treated with teniposide for 20 hrs, then the cellular protein levels of phospho-IRF3 (p-IRF3) were determined by Western blot. (F) B16-OVA cells transduced with TBK1 or IRF3 shRNA were treated with teniposide or DMSO for 16hrs, then co-culture with BMDC and B3Z for 24hr, supernatant IL-2 were measured, and expression of TBK1 and IRF3 were detected by western blot. (G) B16-OVA cells were treated with teniposide together with different inhibitors, then co-cultured with BMDCs and B3Z for 24 hrs, supernatant IL-2 were measured by ELISA. Data in (A, C, E) are representative of 3 independent experiments. Data in (B, D, F, G) represent mean ± SD of 3 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001, and ns=not significant, by one-way ANOVA with Bonferroni post-test.
Figure S7 Teniposide induced tumor infiltration of immune cells and potentiated anti-PD1 therapeutic efficacy on multiple mouse tumor models.

(A) B6 mice were inoculated with B16 cells (1x10^6 cells/mouse), and followed by teniposide or vehicle treatment on Day 7 and 8 (10 mg/kg, i.p., n=3 per group). Tumors were isolated on day 12, weighed, and processed into single cells for flow cytometry analysis. (B) B6 mice with established MC38 or PDAC tumors were treated with teniposide or vehicle on day 6 and 7, and tumors were harvested on day 13. Tumor-infiltrating CD8+ T cells were detected by immunohistochemistry (n=3). Scale bar, 50 μm. (C) Mice with established B16 tumors were treated as in (A), and the surface expression of activation marker CD69 in CD4+ or CD8+ T cells were detected by FACS (n=3). (D) CT26, MC38 and PDAC cells were treated with teniposide or DMSO for 24 hrs, then the surface expression of PD-L1 were detected by FACS. (E) B6 mice were inoculated with MC38 or PDAC tumor cells. The mice were divided into 4 groups with 5 mice per group, and the treatment was administrated as arrows indicated. Teniposide (10 mg/kg) and anti-PD1 (100 μg/mouse) were both administrated by i.p. injection. (F) Western blot verified the knockdown effect of STING in CT26 cells by shRNA. *P < 0.05, **P < 0.01, ***P < 0.001, and ns=not significant by unpaired Student’s t test or two-way ANOVA with Bonferroni post-test (E). Data in (D, F) are representative of 3 independent experiments.