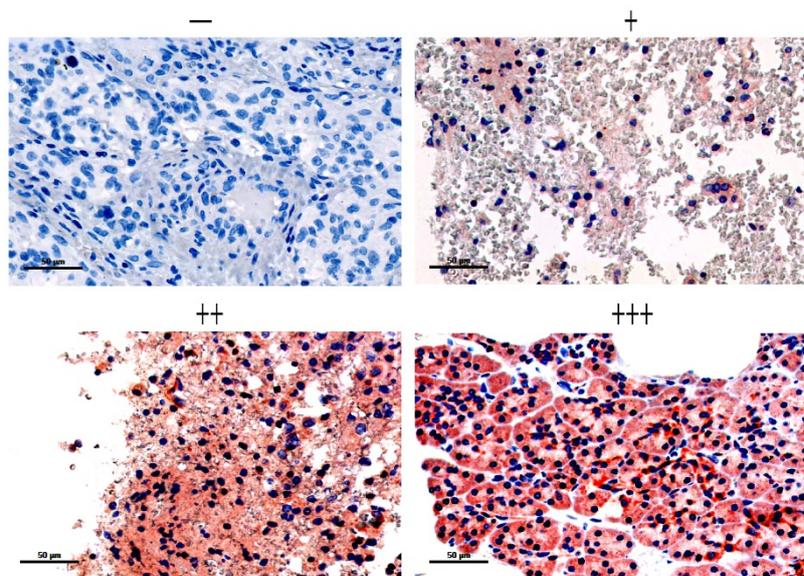


# Supplementary Information

## Gasdermin C sensitizes tumor cells to PARP inhibitor therapy in cancer models

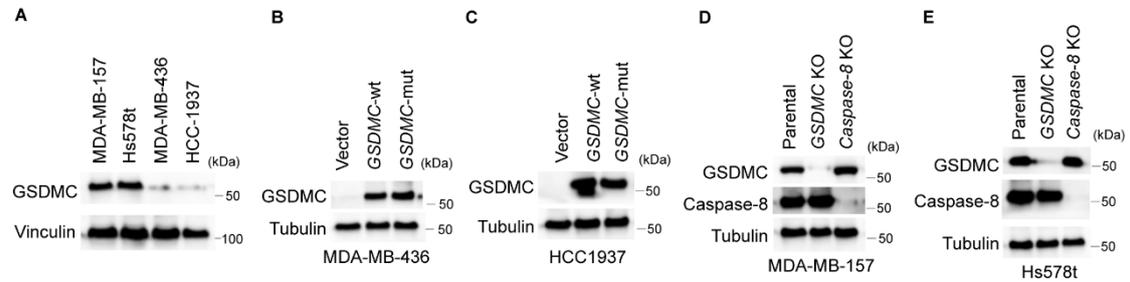
Shuanglian Wang, Chiung-Wen Chang, Juan Huang, Shan Zeng, Xin Zhang, Mien-Chie Hung, Junwei Hou

Supplemental Figure 1



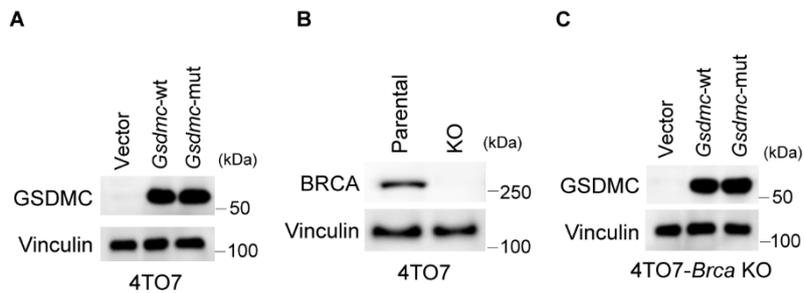
Supplemental Figure 1. Representative immunohistochemical (IHC) staining results for GSDMC in human TNBC tissues. Scale bar, 50 µm.

## Supplemental Figure 2



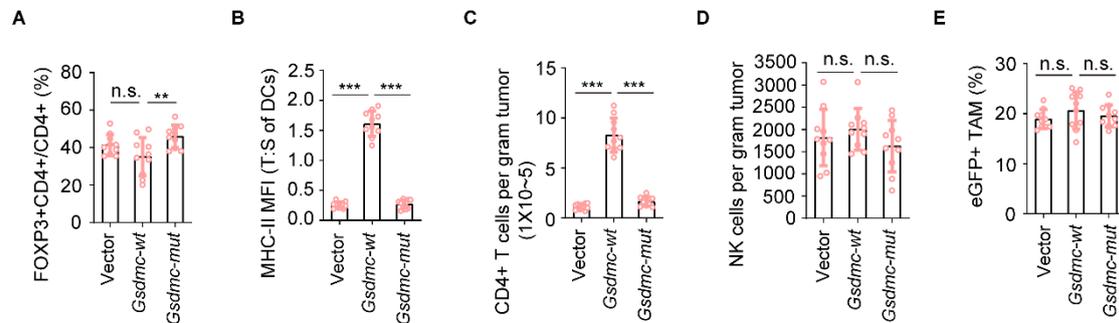
**Supplemental Figure 2. GSDMC and caspase-8 expression in TNBC cells.** (A) Endogenous GSDMC expression level in TNBC cells as indicated. (B and C) GSDMC expression level in MDA-MB-436 and HCC1937 stable cells harboring an empty vector (Vector) or expressing wild-type *GSDMC* (*GSDMC*-wt) or the D365A mutant (*GSDMC*-mut). (D and E) Establishment of MDA-MB-157 and Hs578t stable cells with deletion of *GSDMC* or *caspase-8*.

### Supplemental Figure 3



**Supplemental Figure 3. Establishment of 4TO7 stable cells.** (A) Enforced mouse *Gsdmc* expression in 4TO7 cells. Empty vector, vector; Wild-type mouse *Gsdmc*, *Gsdmc*-wt; The caspase-8 cleavage site D263A mutant, *Gsdmc*-mut. (B) Deletion of *Brca* gene in 4TO7 cells. KO, *Brca* knockout. (C) Ectopic expression of vector, *Gsdmc*-wt, and *Gsdmc*-mut in 4TO7-*Brca* KO cells.

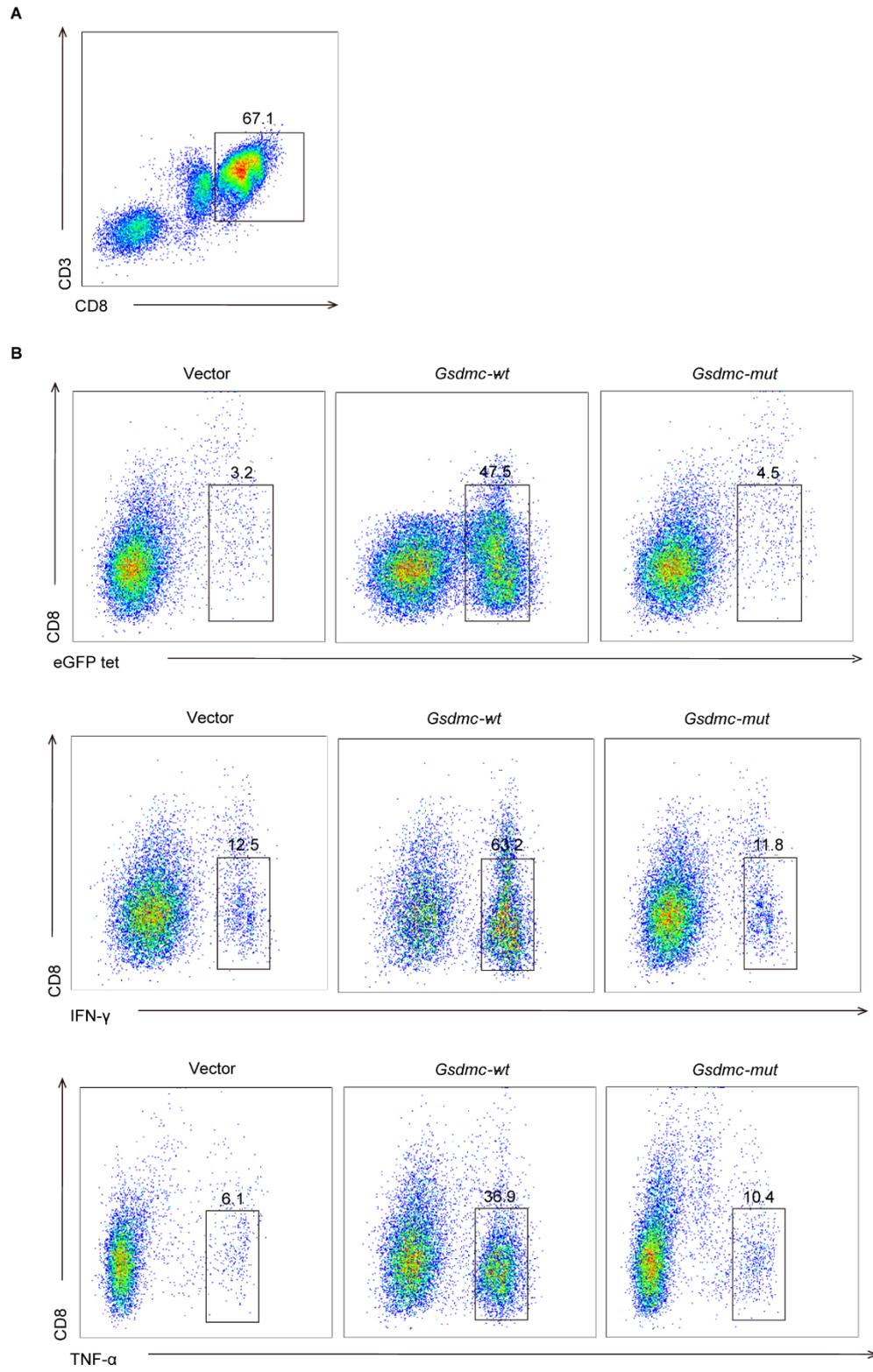
## Supplemental Figure 4



### Supplemental Figure 4. Infiltrating immune cells in 4TO7-*Brca* KO tumor. (A-D)

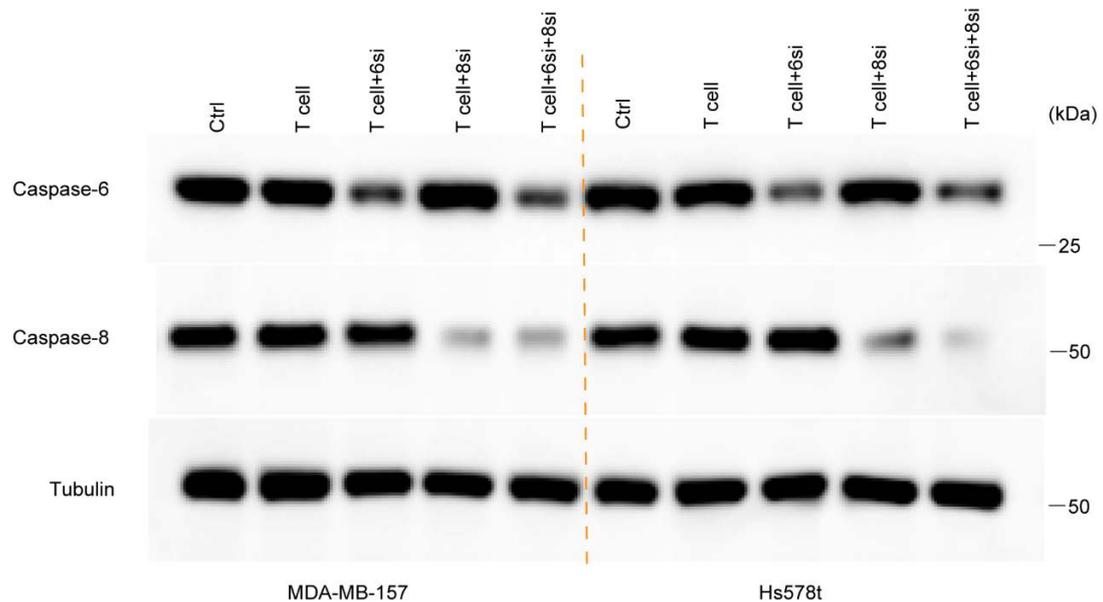
4TO7-*Brca* KO cells stably expressing an empty vector (vector) or wild-type mouse *Gsdmc* (*Gsdmc*-wt) or the D263A mutant (*Gsdmc*-mut) were inoculated into the mammary fat pad of immunocompetent BALB/c mice (n = 10). Mice were orally administered olaparib (50 mg/kg) five times per week for 18 days. Percentage of Treg cells (A). Percentage of activated DC cells (B). Mean numbers of CD4+ T cells/gram of tumor tissue (C). Mean numbers of NK cells/gram of tumor tissue (D). (E) Ectopic expression of *eGFP* in the stable cells as indicated in (A-D). Then cells were inoculated into the mammary fat pad of immunocompetent BALB/c mice (n = 10). Mice were orally administered olaparib (50 mg/kg) five times per week for 18 days. Percentage of eGFP+ macrophages that have engulfed tumor cells. Data represent mean  $\pm$  SD. 1-way ANOVA was used. \*\*p < 0.01, \*\*\*p < 0.001. n.s., no significance.

# Supplemental Figure 5



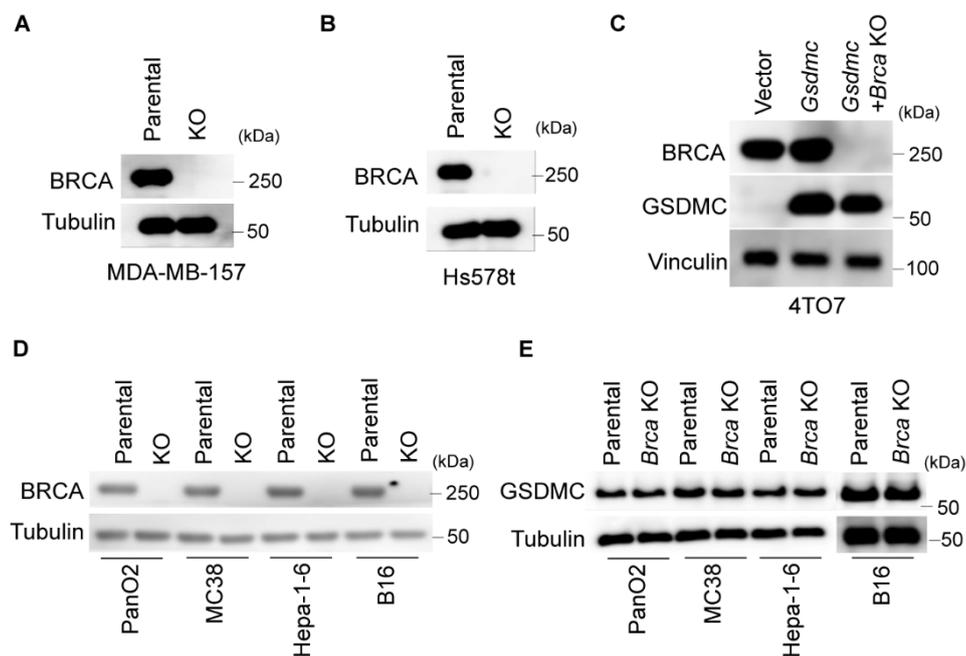
**Supplemental Figure 5. Gating strategy and representative flow-cytometry plots for assessing tumour-infiltrating CD8<sup>+</sup> T cells (A) or IFN- $\gamma$ /TNF- $\alpha$ /eGFP tet-positive CD8<sup>+</sup> T cells (B).**

Supplemental Figure 6



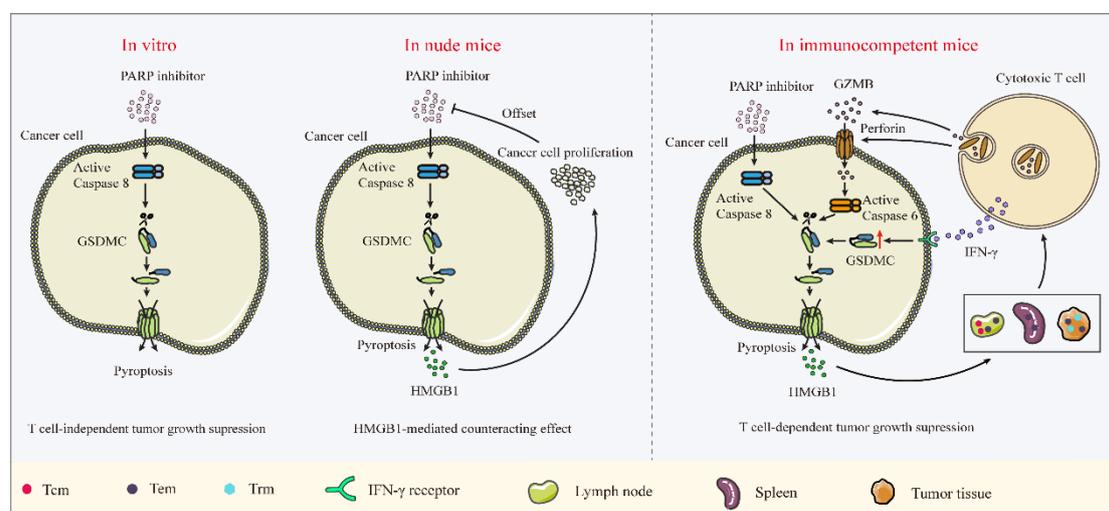
**Supplemental Figure 6. Knockdown of caspase-6 and caspase-8 in MDA-MB-157 and Hs578t cells. Ctrl, control. 6si, caspase-6 siRNA treatment. 8si, caspase-8 siRNA treatment.**

Supplemental Figure 7



**Supplemental Figure 7. Establishment of *BRCA* knockout and/or *GSDMC* overexpressing stable cells. (A and B) Knockout of *BRCA* gene in MDA-MB-157 (A) and Hs578t (B) cells. (C) Ectopic expression of *Gsdmc* and/or deletion of *Brca* in 4TO7 cells. (D) Knockout of *Brca* gene in mouse cell lines as indicated. (E) Ectopic expression of *Gsdmc* in stable cells established in (D).**

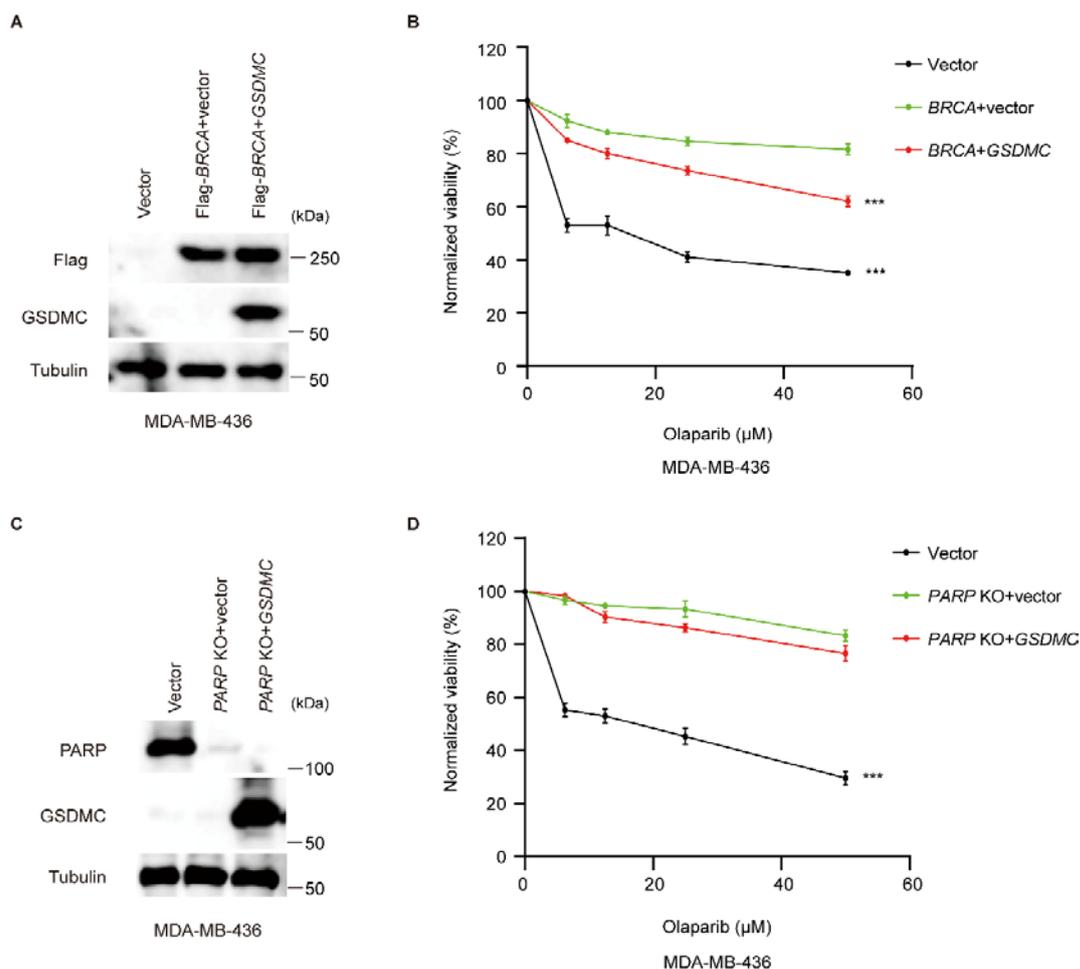
## Supplemental Figure 8



### Supplemental Figure 8. A proposed model of GSDMC-mediated pyroptosis increasing PARPi sensitivity in cancers by enhancing antitumor immunity.

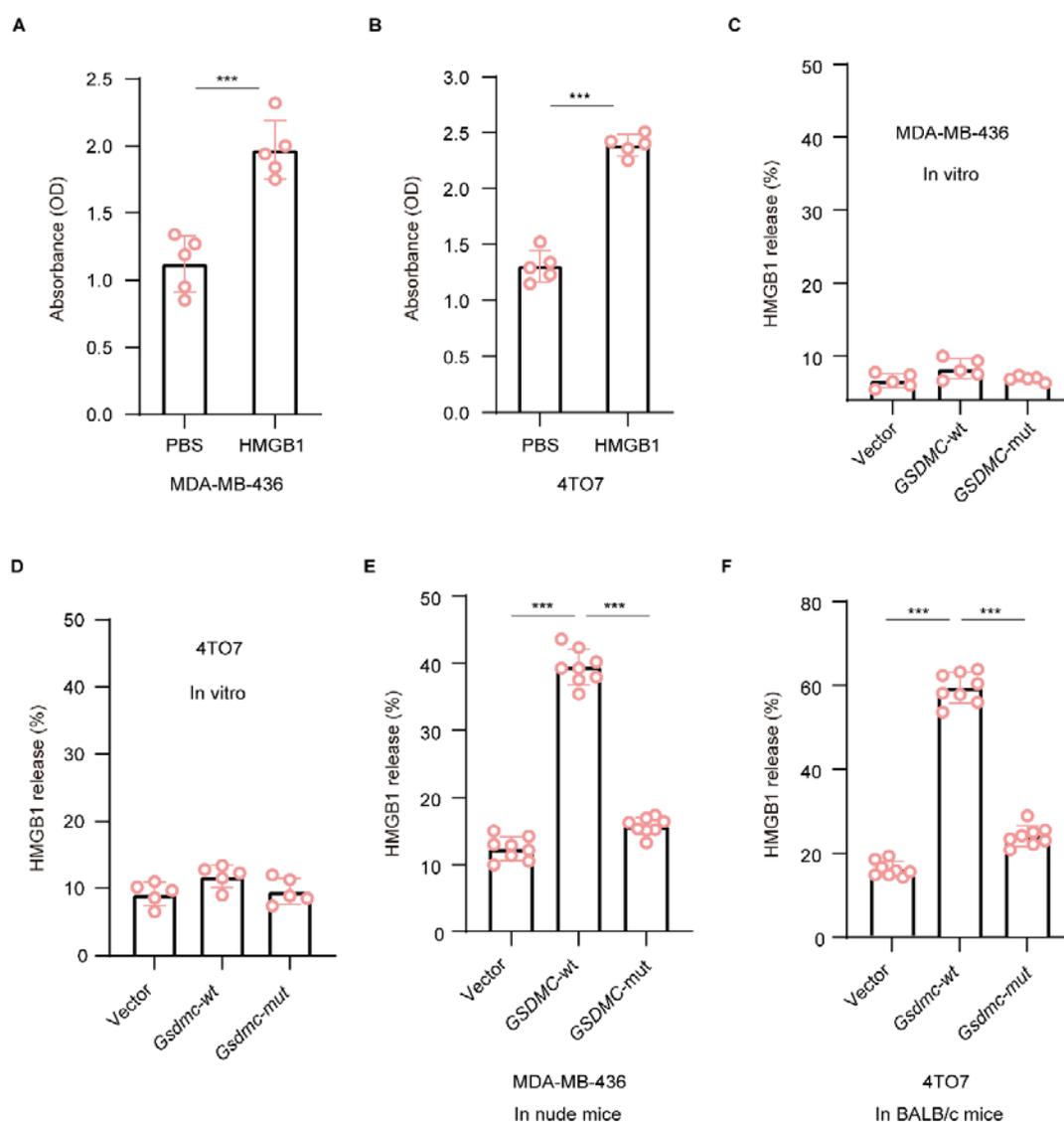
PARPi induces caspase-8 cleavage of GSDMC to trigger CCP. The killing effect of PARPi is offset by pyroptotic cell-released HMGB1 through promoting cancer cell proliferation in nude mice. However, GSDMC-mediated CCP activates Tcm and Tem in lymph node, Tem in spleen, Tem and Trm in tumor microenvironment, and thus increases tumor infiltration and cytotoxicity of T cell. T cell-derived GZMB further triggers CCP by caspase-6 cleavage of GSDMC. Interestingly, IFN- $\gamma$  that is generated from CCP-activated cytotoxic T cell upregulates GSDMC expression in cancer cells, which in turn enhances the cytotoxicity of PARPi and T cell. Taken together, GSDMC-mediated CCP kills tumor cells and augments antitumor immunity in an exacerbating feedback manner in response to PARPi treatment.

## Supplemental Figure 9



**Supplemental Figure 9. The impact of *BRCA* reverse mutation and *PARP1* loss on GSDMC-mediated CCP.** (A) Enforced expression of reverse-mutated *BRCA1* (<sub>4065</sub>TCAAGAAGA<sub>4073</sub> deletion in *BRCA1* gene) in MDA-MB-436 cells with *GSDMC* overexpression. (B) Cells in A were treated with the indicated concentrations of olaparib for 72 h and subjected to a cell viability assay (n = 5). (C) Deletion of *PARP* in MDA-MB-436 cells with *GSDMC* overexpression. KO, knockout. (D) Cells in C were treated with the indicated concentrations of olaparib for 72 h and subjected to a cell viability assay (n = 5). Data represent mean ± SD. 1-way ANOVA was used. \*\*\*p < 0.001.

## Supplemental Figure 10



**Supplemental Figure 10. The release of HMGB1 protein in vitro and in vivo in response to PARPi treatment. (A and B)** CCK-8 assay to determine the proliferation of MDA-MB-436 (n = 5) (A) and 4TO7 (n = 5) (B) cells treated with HMGB1 protein (0.5  $\mu$ g/mL). (C and D) HMGB1 release from MDA-MB-436 (C) and 4TO7 (D) stable cells as indicated under PARPi treatment in vitro (n = 5). (E and F) HMGB1 release in tumor slurry of MDA-MB-436 tumors as indicated in nude mice (n = 8) (E)

and of 4TO7 tumors as indicated in immunocompetent BALB/c mice (n = 8) (**F**).

Data represent mean  $\pm$  SD. Unpaired 2-tailed t test for **A**, **B**. 1-way ANOVA for **C-F**.

\*\*\*p < 0.001.