**Supplemental Information**

**Supplemental Methods**

**Measurement of IC\textsubscript{50} Value in Tumor Cells**

Tumor cells were seeded in 96-well plates at a density of 2500/well and allowed to adhere overnight. Cells were then exposed to appropriate concentrations of therapeutic agents (or vehicle control) with continuous exposure for 72 h. Growth inhibition was measured by CellTiter-Glo® 2.0 Cell Viability Assay from Promega according to manufactory’s introduction. IC\textsubscript{50} values were calculated using non-linear regression model (logarithmic inhibitor vs. normalized response-variable slope) in Graphpad Prism 9.

**Flow Cytometry Analysis**

Tumors were chopped and digested in collagenase buffer as described previously \textsuperscript{1}. Single cell suspension of tumor and ascites were obtained by filtering through 70 um strainers and treated with 1x eBioscience RBC lysis buffer (Thermo Fisher) before staining. Single cell suspensions were incubated with LIVE/DEAD Fixable Aqua Dead Cell Stain (Life Technologies, Cat#L34965) for 30 min and then blocked with anti-CD16/32 (Biolegend, clone 93) for 20 min on ice. Samples were then incubated with appropriate antibodies for 30 min on ice. Foxp3 staining buffer set (eBioscience, Cat# 00-5523-00) was applied for intracellular markers staining. For the intracellular cytokine analysis, cells were stimulated with Leukocyte Activation Cocktail (BD Biosciences, Cat# 550583) at 37 °C for 4-6 hours prior to FACS staining. The following antibodies were used in this study (antibodies were purchased from BioLegend unless otherwise indicated): CD45 (clone 30-F11), CD3e (clone 145-2C11), CD4 (clone RM4-5), CD8 (clone 53-6.7), CD44 (clone IM7), CD62L (MEL-14), CD25 (PC61), IFN\textgamma (clone XMG1.2), TNF\textalpha (clone MP6-XT22), CD11b (clone M1/70), CD11c (clone BM8), F4/80 (clone BM8), Ly-6C (clone HK1.4), Ly-6G...
(clone 1A8), MHC-II (clone M5/114.15.2), CD80 (clone 16-10A1), CD86 (clone GL-1), MHC-I (clone KH114), FoxP3 (clone FJK-16 s; eBioscience), Phospho-IRF-3 (Ser396) (clone D6O1M, Cell signaling technology) and Phospho-TBK1/NAK (Ser172) (clone D52C2, Cell signaling technology). The following human antibodies were used in this study: cell surface markers includes CD45 (clone HI30), CD11b (clone M1/70), CD11c (clone Bu15), CD80 (clone 2D10), CD86 (clone IT2.2), CD14 (clone 63D3), CD15 (clone 30-F11), HLA-DR (clone L243) and HLA-A,B,C (clone W6/32); intracellular markers include CD163 (clone GHI/61), CD68 (clone Y1/82A) and CD206 (clone 15-2). Flow cytometry was performed on an LSRII (BD Biosciences) or Fortessa HTS (BD Biosciences) at DFCI Flow Cytometry Core, and all the data were analyzed using FlowJo software. Flow cytometry analysis was performed in a blinded fashion as the researchers conducting these assays were not aware of treatment groups until data gathering was complete.

Analysis of p-STAT3 and γ-H2AX (p-HA2X-Ser139) was performed according to a two-step protocol for intracellular phosphorylated signaling proteins (Thermo Fisher). Briefly, cells were incubated with LIVE/DEAD Fixable Aqua Dead Cell Stain for 30 min. After washing, cells were suspended in 100 µl PBS and then fixed by adding an equal volume of IC Fixation Buffer (CAT# 00-822-49, Thermo Fisher) directly to cells and incubated at room temperature for 20 min followed by fixing with ice-cold 90% methanol in PBS for 30 min. The fixed cells were blocked and stained with p-STAT3 or γ-H2AX antibodies for flow cytometry analysis as described above.

**Lentivirus-mediated knockdown of Stat3 in PBM-R tumor cells**

Control shRNA and Stat3 shRNAs plasmids (sh-Stat3-1: TRCN0000071456 and sh-Stat3-2: TRCN0000071453) were acquired from Sigma-Aldrich. The Stat3 shRNAs and control shRNA plasmids were co-transfected with pCMV-delta8.9 and pVSVG at the ratio of 2:2:1 into HEK293T.
cells by PEI (1 μg/μl) (4:1 to DNA). The medium was changed 24 hours after transfection and the viral supernatants were collected 48 hours later by filtering through a 0.45-μm filter. PBM-R cells were cultured in a 6-well plate and infected with Stat3-shRNA lentiviral particles, puromycin (3 μg/mL) was added to the culture for selection. Puromycin resistant cells were selected and expanded. Western blot analysis was performed to evaluate the silencing effect of lentiviral Stat3 in PBM-R tumor cells.

**Western Blot Analysis**

Tumor cells were harvested and lysed with ice-cold RIPA buffer supplemented with protease phoshatase inhibitor cocktail (Thermo Fisher). Protein concentration was determined using a Pierce BCA Protein Assay Kit (Thermo Fisher). About 50 μg protein extracts were loaded and separated by SDS-PAGE, and then transferred to polyvinylidene fluoride (PVDF) membranes. After blocking with 5% non-fat milk (Bio-Rad) in PBST (PBS plus 0.2% Tween 20) at room temperature for 1 hour, membranes were incubated with primary antibodies overnight at 4 °C. Fluorescently labeled anti-mouse IgG (Rockland Immunochemicals, # RL610-145-002) or anti-rabbit IgG (Molecular Probes, # A-21109) were used as second antibodies and the western blots were visualized on an Odyssey scanner (LI-COR).

**Cytokine Array Analysis**

The PBM tumor cells were cultured in 6-well plate for 24 hours and treated with olaparib or vehicle control. Drug was removed after 24 hours’ treatment, and cells were cultured in fresh medium for another 48 hours. Cell culture supernatants were obtained by centrifugation at 1,500g for 5 min at 4 °C to remove all the debris and cells then subjected to Cytokine array analysis (ARY028, R&D system) according to manufactory’s introduction. Briefly, the cell culture supernatants were mixed with a cocktail of biotinylated detection antibodies, and then incubated with the Mouse Cytokine
Array. The array was then incubated with streptavidin-horseradish peroxidase followed by chemiluminescent detection. Array images were analyzed using the Image J software.

**Immunofluorescent staining and analysis of patient and mouse samples**

Formalin-fixed paraffin-embedded 4 µm ovarian tumor sections from female ovarian cancer patients were obtained through a City of Hope Institutional Review Board approved protocol, and subsequently de-paraffinized and stained for immunofluorescence with specific primary and fluorophore-conjugated secondary antibodies as previously described. The following primary antibodies were used in this study: CD163 (clone 10D6, Leica Biosystems), CD68 (clone KP1, ThermoFisher Scientific), CD86 (clone E2G8P, Cell Signaling Technology), p-STAT3 (clone D3A7, Cell Signaling Technology), Pan-Cytokeratin (clone AE1-1/AE-3, Novus Biologicals). Secondary antibodies used in this study include MACH 2 Rabbit HPR-Polymer and MACH 2 mouse HPR-Polymer from Biocare Medical, OPAL 520 REAGENT, OPAL 570 REAGENT and OPAL 650 REAGENT from Akoya Biosciences. Confocal imaging was performed with a Zeiss LSM 880 confocal microscope and staining quantification was analyzed by ZEN 2.3 lite software and plotted in GraphPad Prism 9. Seven random images were quantified in each patient sample. Immunohistochemical (IHC) staining of p-STAT3 in mouse tumor samples was performed as described previously. Data were collected with SPOT advanced version 4.6 or version 5.6 (SPOT imaging). Quantification of p-STAT3 was conducted using the Image J software (version 1.53t). Three random images were quantified in each mouse tumor sample.

**Transcriptome Analysis**

**Transcriptome analysis of tumor cells:** total RNA was isolated by RNeasy Plus Mini Kit (QIAGEN) from bulk tumors collected from PBM and PBM-R tumor-bearing mice. PBM tumors were generated by orthotopic injection of PBM tumor cells into FVB/NJ mice. PBM-R are
refractory tumors generated in PBM tumor-bearing mice after long-term treatment with olaparib. The majority of these samples are tumor cells, immune cells account for less than 2%, which is confirmed by flow cytometry analyses. The RNA samples were sequenced on an Ion Torrent platform (Thermo Fisher) using an Ion AmpliSeq Custom Panel targeting 4,604 murine genes most relevant to our studies, as we have previously described. To generate read counts per gene, data were analyzed using Torrent Suite and AmpliSeqRNA analysis plugin (Thermo Fisher). Differential gene expression analyses were carried out using DESeq2 with default parameters to obtain log2 fold change (MAP) and adjusted p-values (Benjamini-Hochberg procedure). Genes were ranked by log2 fold change (MAP), and GSEA were carried out using the GSEA Preranked tool.

**Transcriptome analysis of myeloid cells:** about 1 x10^6 PBM-R tumor cells were intraperitoneally injected to FVB/NJ mice. About 2-3 weeks after injection, mice were grouped and treated with control, olaparib, MSA-2 and MSA-2 in combination with MSA-2 for 24 hours. After treatment, myeloid cells (CD45^+CD11b^+) were isolated from the ascites of each mouse (n=3 per group) by EasySep Mouse CD11b positive Selection Kit II (STEMCELL Technology, #18970). Total RNAs was isolated and sequenced on the Ion Torrent platform (Thermo Fisher) that described above using the Ion AmpliSeq Transcriptome Mouse Gene Expression Panel. Gene ontology (GO) analysis of DEGs was performed using topGO package in R. GSEA analysis was performed as described above.

**Co-culture Experiments**

For *ex vivo* culture of bone marrow cells in ascites supernatants, bone marrow cells were isolated from FVB/NJ mice and cultured in the conditioned medium containing 50% of ascites supernatant and 50% of complete DMEM medium (90% DMEM and 10%FBS), supplied with 100 μg/ml
penicillin–streptomycin. The cells were incubated in conditioned medium for 5 days with media replacement on day 3. The components of the cells were analyzed by on flow cytometry analysis.

For ex vivo culture of mouse macrophages, bone marrow cells were isolated from FVB/NJ mice and cultured in DMEM containing 10% FBS, 55µM 2-Mercaptoethanol and 20 ng/ml M-CSF. BMDMs (bone marrow-derived macrophages) were harvested on day 7 and further cultured in 2.0 ml control medium (90% DMEM, 10% FBS, 55µM 2-Mercaptoethanol and 5 ng/ml M-CSF) described above or the medium containing 50% tumor cell conditioned medium for 72 hours before analysis. For the tumor cell conditioned medium, about 3x10^5 tumor cells were cultured in 6-well plate for 24 hours and then treated with DMSO or olaparib. After 24 hours incubation with DMSO or olaparib, the tumor cells were washed twice with PBS and cultured in DMEM containing 10%FBS for 48 hours. The tumor cell conditioned medium was collected by centrifugation at 1,500g for 5 min at 4 °C to remove all the debris and cells. For the cytokine blocking experiment, monoclonal antibodies that specific to each cytokine were added to the tumor cell conditioned medium before it was applied to culture macrophages.

For ex vivo culture of human macrophages, human BMDMs were generated from bone marrow mononuclear cells (BMMs) (CAT#70001) obtained from STEMCELL Technologies. Briefly, BMMs were cultured in DMEM containing 10% FBS, 55µM 2-Mercaptoethanol (CAT# 21985023, Thermo Fisher) and 50ng/ml M-CSF for 5-7 days with media replacement every 3 days to obtain matured macrophages (BMDMs). BMDMs were further cultured in the medium with or without addition of 50% conditioned medium obtained from human ovarian cancer cell lines for 72 hours. Flow cytometry analysis was performed to analyze the phenotypes of macrophages for both mouse and human BMDMs.
References


**Supplementary Figure S1**

Genetic variations of DNA repair genes detected in the *in vitro* resistant PBM-R3 line

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**Figure S1.** Copy number variations of DNA repair pathway genes detected in PBM-R3.
Figure S2. Analysis of immune cells in PBM and PBM-R tumor-bearing mice. Growth curve (A) and tumor burden (B) of PBM and PBM-R tumor-bearing mice. Flow cytometric analysis of tumor-infiltrating (C) CD11b+, (D) MDSCs, (E) CD4+, (F) CD8+ and (G) Treg cells in PBM and PBM-R tumors (n=6). (H) Flow cytometric analysis of CD11b+ cells in bone marrow cells (BMCs) cultured in 50% complete medium and 50% ascites supernatant harvested from PBM and PBM-R tumor-bearing mice for 5 days. (I) Gating strategies of immune cells (representative images show immune cells in ascites). Data are presented as mean ± SD. One-way analysis of variance (ANOVA). *** P < 0.001.
Supplementary Figure 3

Figure S3. PARP inhibition upregulates STAT3 signaling in Brca1-deficient ovarian tumors. (A) Western blot analysis of total and phosphorylated STAT3 in PBM cells treated with indicated concentrations of olaparib or vehicle control for 24 hours. (B) Flow cytometric analysis of BMDMs cultured in control medium treated with indicated concentrations of olaparib (n=3). (C) Western blot analysis of total and phosphorylated-Stat3 in PBM and PBM-R cells stably expressing control shRNA or shRNAs targeting Stat3. (D) Evaluation of IC\textsubscript{50} values in the control and Stat3-silenced PBM-R cells after olaparib treatment (n=5). (E) GSEA analysis of STAT3 signaling pathway (Nanostring) and the ratio of M2/M1 (tCyCIF) in tumor tissues of ovarian cancer patients in TAPOCIO clinical trial.
**Figure S4.** **STING agonists modulate myeloid cells in the TME of ovarian tumors.** (A-C) Flow cytometric analysis of TAMs (CD11b<sup>+</sup>F4/80<sup>+</sup>), myeloid DCs (CD11b<sup>+</sup>CD11c<sup>+</sup>) (B) and phosphorylated TBK-1 (p-TBK-1) (C) in myeloid DCs in the ascites of PBM-R tumor-bearing mice treated with control, olaparib, MSA-2 and olaparib in combination with MSA-2 for 24 hours (n=4 or 5). Data are presented as median with quartiles. One-way analysis of variance (ANOVA). * P < 0.05, ** P < 0.01, *** P < 0.001.
Supplementary Figure S5

A. CD45<sup>+</sup> Cells

B. CD11c<sup>+</sup> MHC-II<sup>+</sup> CD68<sup>+</sup> Cells

C. CD3<sup>+</sup> Cells

D. CD11c<sup>+</sup> MHC-II<sup>+</sup> CD68<sup>-</sup> Cells

E. TAMs (CD11<sup>+</sup>F4/80<sup>+</sup>)

F. M1-like TAMs

G. M2-like TAMs

H. Tumor and Ascites

Figure S5. Treatment of PBM-R tumors with a STING agonist enhances antitumor immunity in tumor-infiltrating immune cells and blood immune cells in PBM-R tumor-bearing mice. (A-C) Flow cytometric analysis of tumor-infiltrating immune cells in PBM-R tumor-bearing mice treated with control, olaparib, MSA-2 and olaparib in combination with MSA-2 for 14 days (n=7). (A) CD45⁺; (B) activated DCs (CD11c⁺ MHC-II⁺CD80⁺); (C) CD3⁺. (D) Flow cytometric analysis of immune cells in the blood of PBM-R tumor-bearing mice as described above: left, activated DCs (CD11c⁺ MHC-II⁺CD86⁺); right, MHC-I⁺ myeloid DCs (CD11b⁺ CD11c⁺). (E-F) Flow cytometric analysis of tumor-infiltrating immune cells in PBM-R3 tumor-bearing mice treated with control, olaparib, MSA-2 and olaparib in combination with MSA-2 for 14 days (n= 6 or 7): (E) TAMs (CD11b⁺F4/80⁺); (F) M1-like macrophages (CD206⁻ MHC-II⁺high) and M2-like macrophages (CD206⁺ MHC-II⁺low). (G-H) Analyses of total and phosphorylated STAT3 in the tumors (G) and ascites (H) of PBM-R tumor-bearing mice treated with MSA-2 or vehicle control. Data are presented as median with quartiles. One-way analysis of variance (ANOVA). ns, not significant; * P < 0.05, ** P <0.01, *** P < 0.001, **** P < 0.0001.
Supplementary Figure S6
Figure S6. Combined treatment of a STING agonist and PARPi in ovarian PDXs. (A) Evaluation of IC\textsubscript{50} values in human ovarian cancer cell lines and PDXs. (B-D) Analysis of human immune cells (CD45\textsuperscript{+}), TAMs (CD11b\textsuperscript{+} CD68\textsuperscript{+}) and M2-like TAMs (CD163\textsuperscript{+} HLA-DR\textsuperscript{low}) in the ascites of DF86 and DF101 PDX-bearing mice as described in Figure 6H. (E) Gating strategies of immune cells in ascites of PDX-bearing mice. Data are presented as median with quartiles. One-way analysis of variance (ANOVA), * P < 0.05, ** P < 0.01.