SUPPLEMENTARY METHODS

Healthy donor blood collection and ascites-derived T cell isolation

The City of Hope Institutional Review Board authorized the procurement of healthy donor blood samples (IRB# 21173). We isolated PBMCs by density gradient centrifugation using Histopaque-1077® reagent (Sigma,10771) and cultured them in complete RPMI 1640 media supplemented with 10% FBS, 0.2% Plasmocin® (InvivoGen, ant-mpp), and 1% penicillin/streptomycin (RPMI+++ medium). We centrifuged ascites from ovarian cancer patients at 1500 rpm for 5 min, resuspended cell pellets in flow buffer (HBSS -/- with 2% FBS), and isolated T cells using a human T cell isolation kit (Biolegend, 480022). We then cultured T cells with Dynabeads™ Human T-Activator CD3/CD28 (Invitrogen, 11161D) in the presence of IL-2 for 14 days and measured the fold of T cell expansion.

Isolation of Mouse Immune Cells

We isolated mouse immune cells as previously described[1]. To enrich $CD19^+$ B cells, $CD4^+$, or $CD8^+$ cells from splenocytes, we utilized negative selection through magnetic bead separation using EasySep™ Mouse Immune Cell Isolation Kits (StemCell Technologies, 19854, 19852, and 19853, respectively) according to the manufacturer's instructions.

Chemicals and Antibodies

We purchased COH34 from MedChemExpress (HY-128760) and olaparib (AZD2281) from SelleckChem (S1060). We dissolved all inhibitors at 50 mM concentrations in DMSO. We carried out antibody conjugation chemistry as described previously[2]. Briefly, we synthesized a fully phosphorothioated (PS) single-stranded DNA (ssDNA) 20-mer with a 5′ extension featuring 4× C3 (propyl) spacers/linkers and a DBCO-TEG reactive group for antibody conjugation via click chemistry. We sourced antibodies, specifically mouse anti-PARG and mouse IgG1 isotype

control, from Santa Cruz Biotechnology and BioXCell, respectively. The rest of primary antibodies are listed below:

Clone	Reagent	Manufacturer	Cat. Number
	Primary antibodies (IB and IF)		
	anti-pY705-STAT3, rabbit monoclonal	Cell Signaling	9131
124H6	anti-STAT3, mouse monoclonal	Cell Signaling	9139
F-2	anti-STAT3, mouse monoclonal (for Co-IP)	Santa Cruz	sc-8019
	anti-PAR/pADPr, mouse monoclonal	Bio-Techne	4335-MC-100
	anti-PAX8, rabbit polyclonal	Proteintech	10336-I-AP
	anti-Bcl-xL, rabbit polyclonal	Cell Signaling	2762
6C5	anti-GAPDH, mouse monoclonal	Santa Cruz	sc-32233
H-1	anti-PARG, mouse monoclonal	Santa Cruz	sc-398563
71G4B7	anti-Survivin, rabbit monoclonal	Cell Signaling	2808
D4M2N	anti-MMP2, rabbit monoclonal	Cell Signaling	5453
G657	anti-MMP9, rabbit polyclonal	Cell Signaling	40994
DO-1	anti-p53, mouse monoclonal	Santa Cruz	sc-126
	Flow cytometry antibodies		
FJK-16s	anti-FoxP3, PE	BioLegend	12-5773-82
13A3-1	anti-pY705-STAT3, [Alexa Fluor® 647], mouse IgG1	BioLegend	651008
4S.B3	anti-IFNγ, APC/Cyanine 7, mouse IgG1	BioLegend	502530
QA16A02	anti-Granzyme B, FITC, mouse IgG1	BioLegend	372206
H1.2F3	anti-CD69, FITC	BioLegend	104506
GK1.5	anti-CD4, FITC	BioLegend	11-0041-85
30-F11	anti-CD45, FITC	BioLegend	11-0451-85
SK1	anti-CD8, APC/Cyanine 7, mouse IgG1	BioLegend	344714
53-6.7	anti-CD8, FITC, mouse IgG1	BioLegend	100706
H4A.3	anti-CD107, PE, mouse IgG1	BioLegend	328608
BM.8	anti-F4/80, PE	eBioscience	12-4801-82
	Secondary antibodies		
	anti-mouse IgG, HRP-linked	Cell Signaling	7076
	anti-rabbit IgG, HRP-linked	Cell Signaling	7074
	goat anti-mouse IgG, [Alexa Fluor® 555]	ThermoFischer	A-32727
	goat anti-rabbit IgG, [Alexa Fluor® 488]	ThermoFischer	A-11008

Validation of PS-PARG antibody

We verified the binding of cell-penetrating PS-PARG antibody to PARG protein by immunoprecipitation and western blotting after transfecting 10 µg/ml of PS-PARG antibody in a well of 6-well SKOV3 cells for 24 h. We used PS-IgG isotype control under the same conditions as a control. After a 24-h incubation, we harvested the cells and used a Dynabeads Protein A Immunoprecipitation Kit (Invitrogen, 10006D) to precipitate the PS-PARG antibody and PARG

protein complex according to the manufacturer's instructions. We then detected PARG protein using SDS-PAGE and Western blotting.

Cell lines

We obtained PEO1 and WT ID8 cell lines from Sigma. OVCAR8 and SKOV3 cell lines were generously provided by Dr. Edward Wang, City of Hope Comprehensive Cancer Center, Duarte, CA. Dr. Jean Zhao from Dana-Farber Cancer Institute at Harvard Medical School in Boston, MA, kindly provided *Brca1*-null ID8 cells[3]. Cells were cultured in complete DMEM medium supplemented with 10% fetal bovine serum, 5 ug/ml Plasmocin®, and 1% penicillin/streptomycin at 37 °C and 5% CO2. Upon reaching 80-90% confluency, cells were subcultured at a 1:10 ratio in T75 culture flasks (NEST Scientific, 708003). OVCAR8, WT ID8, and *Brca1*-null ID8 cells were subcultured every 3-4 days by seeding $5x10^5$ cells per flask. PEO1 and SKOV3 cells were subcultured every 6-7 days by seeding $3\text{-}4x10^5$ cells per flask. We regularly confirmed all cells to be mycoplasma-free using the MycoAlert® Detection Kit (Lonza, LT07-318). All cells used in the experiments were below 10 passages.

Collagen Gel Matrix (CGM) recipe

Prepare CGM by mixing 8 parts collagen, one part 10x medium (solution B) and one part reconstitution buffer (solution C) on ice (8:1:1):

Solution A: Cultrex® Rat Collagen I, (Trevigen)

Solution B: 10× concentrated sterile culture medium (Ham's F-12/DMEM F-12).

Solution C: sterile reconstitution buffer. Make up to 100 ml with sterile milli-Q water, 0.05 N NaOH, 200 mM HEPES, and 2.2 g NaHCO3. Adjust pH to 11, filter through a 0.22 µm Corning filter.

Ovarian organoid medium (OOM) recipe

Reagent	Volume (a total 200ml)	unit
DMEM/F12 containing 1x Glutamax, 10 mM HEPES, and Pen Strep	192ml	ml
Noggin (2µg/µl)	10	ul
Rspo1 (20µg/ml)	10	ul
B27 (50x)	4	ml
N-Acetylcysteine (500mM)	0.5	ml
Primocin	0.4	ml
Nicotinamide (1M)	2	ml
A83-01 (5mM)	20	ul
Fgf10 (100µg/ml)	20	ul
Heregulinβ-1 (75µg/ml)	100	ul
Y27632 (100mM)	10	ul
EGF (500µg/ml)	2	ul
Forskolin (10mM)	200	ul
Hydrocortisone (250µg/ml)	400	ul
β-Estradiol (100µM)	200	ul

Autologous Organoid/PBMC Co-culture

We thawed patient-matched PBMCs (pPBMCs) in pre-warmed (37°C) RPMI ++ medium and incubated them for 15 minutes with CTL Anti-Aggregate Wash™ Supplement (Sanvitra, CTL-AA-001) at 37°C, one day before co-culture. After washing, we resuspended cells at 2-3 x 10^6 cells/mL in RPMI+++ medium supplemented with 150 U/mL IL-2 and cultured them overnight at 37°C. The next day, we resuspended pPBMCs in OOM medium containing the indicated drugs or PS-conjugated antibodies and added them to the outer layer of ALI-PDTOs. After four days of co-culture and treatment, we collected outer media containing pPBMCs and centrifuged them for subsequent ELISA and immunoblotting analyses of supernatant and pPBMCs pellet, respectively. We dissociated ALI-PDTOs with 200 U/mL collagenase IV (Worthington) at 37°C for 30-60 min, followed by three 5-min washes with 100% fetal bovine serum (FBS) and two washes with HBSS. We subjected the resulting pellet to protein lysis and immunoblotting.

Non-autologous Organoid/PBMC Co-culture

We generated tumor-reactive T cells by co-culturing peripheral blood immune cells with tumor organoids using a previously described method[4]. Before co-culture, we stimulated ALI-PDTOs overnight with 200 ng/mL human recombinant IFNγ (Peprotech). We then dissociated ALI-PDTOs into single cells using collagenase IV and TrypLE Express and resuspended them in RPMI+++. We washed anti-CD28-coated plates with phosphate-buffered saline (PBS) and seeded non-autologous PBMCs from healthy donors (10^5 cells/well), stimulating them with dissociated organoids at a 20:1 effector:target ratio. Co-cultures included 200 U/mL IL-2 and 20 µg/mL anti-PD-1-blocking antibody. We refreshed the medium, IL-2, and anti-PD-1 two to three times per week. After one week, we collected PBMCs, counted them, replated them (10^5 cells/well), and re-stimulated them with fresh tumor organoids. After two weeks of co-culture, we exposed PBMCs to either DMSO, COH34, PS-IgG, or PS-PARG for four days and assessed immune cell tumor reactivity using flow cytometry.

Ovarian cancer cell line/PBMC co-culture

We stimulated OVCAR8 cells overnight with 200 ng/mL human recombinant IFNγ (Biolegend) and irradiated them with a single dose of 120 Gy. We then co-cultured OVCAR8 tumor cells with healthy donor PBMCs in the presence of 150 U/mL IL-2 and 20 ng/mL anti-PD-1 blocking antibody in anti-CD28-coated 96-well plates. After seven days of co-culture, we harvested the suspended co-cultured PBMCs and re-stimulated them with OVCAR8 cells for further analysis.

Mouse ovarian cancer cell line/$CD8^+$ T cell co-culture

We isolated mouse $CD8^+$ T cells from the spleen and stimulated them using plate-bound anti-CD3 and soluble anti-CD28 antibodies for two days. We then irradiated wild-type (WT) ID8 tumor cells with a single dose of 70 Gy. After activation, we co-cultured mouse $CD8^+$ T cells with the irradiated ID8 cells in either DMSO or COH34 for an additional three days to assess the impact on the interaction between T cells and tumor cells.

Immunoblotting and Co-Immunoprecipitation

We performed protein extraction on whole cell lysates or tissue homogenates using radioimmunoprecipitation assay (RIPA) lysis buffer with a protease and phosphatase inhibitor cocktail from Cell Signaling. We determined protein concentration using a bicinchoninic acid (BCA) protein assay (Thermo Fisher Scientific). After normalization, we subjected proteins to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred them to polyvinylidene fluoride (PVDF) membranes, and visualized them through western blotting using a Chemiluminescent Detection Kit from Thermo Fisher Scientific and a ChemiDoc MP Imaging System (BioRad). We used various primary antibodies (see Supp. Methods 3) and horseradish peroxidase (HRP)-conjugated antibodies from Cell Signaling Technology as secondary antibodies. Protein levels of phosphorylated STAT3 (pSTAT3), total STAT3, PAR, Bcl-xL, Survivin, MMP2, and MMP9 were quantified and normalized to GAPDH using ImageJ (NIH) software.

For immunoprecipitation, we incubated anti-STAT3 antibodies with precleared cell lysates overnight and pulled down the antibody-antigen complex using recombinant protein G agarose beads from ThermoFisher Scientific. We then subjected the samples to SDS-PAGE followed by immunoblotting. PAR quantification by ImageJ from immunoprecipitation immunoblots was normalized to total STAT3.

Immunofluorescence and confocal microscopy

Cells were seeded onto Falcon® 8-well Culture slides (Falcon, 354108) and washed twice with HBSS++ before fixation in 4% paraformaldehyde for 15 minutes at room temperature. Cells were then permeabilized using ice-cold methanol for 20 minutes at −20°C and HBSS++ containing 0.1% Triton X-100 for 5 minutes at room temperature. They were quenched with 50 mM NH_4Cl in HBSS++ and blocked with 5% BSA in HBSS++ for 1 hour. Subsequently, cells

were incubated overnight with primary mouse anti-human STAT3 antibody, followed by a 90-minute incubation with Alexa Fluor® 555 anti-mouse secondary antibody. Nuclei were stained using Hoechst 33342 (Thermo Fisher, H3570) for 15 minutes. After the final washing step, cells were mounted with VectaMount® (Vector Laboratories, H-5000) and analyzed.

Ovarian tumor tissue and corresponding organoids were fixed in 4% paraformaldehyde, permeabilized with ice-cold methanol, blocked with 10% goat serum in HBSS++, and incubated overnight at 4°C with rabbit anti-human PAX8 and mouse anti-human p53 primary antibodies. The next day, slides were washed and secondary antibodies were applied for 90 minutes, including Alexa Fluor 488 goat anti-rabbit and Alexa Fluor 555 goat anti-mouse. Afterward, tissue samples were incubated with Hoechst 33342 for nuclear staining, resuspended in VectaMount®, and mounted on Superfrost Plus® (Thermo Fischer, 1255015) microscope slides.

Confocal imaging was performed with a Zeiss LSM 880 confocal microscope (Zeiss) using 20x and 40x objectives. Alexa Fluor® 555 fluorescence was excited using a 561 nm laser, and emission was detected in the 562–650 nm range. Alexa Fluor® 488 signal was excited using an argon 488 nm laser, and emission was detected in the 493–550 nm range. Hoechst 33342 nuclear staining was detected using a 405 nm laser and a 426-500 nm detection range. Mean fluorescent intensities of the nuclear A555-stained STAT3 fluorescence were calculated using the profile function of the ZEN software (Zeiss, Jena, Germany), plotted using GraphPad Prism 9.5.1 software, and statistically evaluated by the Student's t-test.

Plasmids and Transfection

The DNA plasmids used in this study are pRc/CMV-STAT3C-Flag (a generous gift from Jim Darnell [RRID: Addgene_8722; http://n2t.net/addgene:8722]) and pRc/CMV (V75020, Invitrogen) as a control for DNA transfection. STAT3C[5] or control expression vector transfection

was performed in OVCAR8, PEO1, and *Brca1*-null ID8 ovarian tumor cells using Lipofectamine 2000 (#11668027, Invitrogen) according to the manufacturer's instructions. Following transfection, cells were selected in a medium containing 1 mg/ml G418.

Cell Viability Assay

Ovarian cancer cells were seeded in quadruplicate on 96-well plates at a density of 0.5-1x10^4 cells per well and incubated overnight. Subsequently, cells were treated with compounds as specified in the figure legends, and cell viability was assessed using the CellTiter-Glo® Luminescent Cell Viability Assay (#G7570, Promega) according to the manufacturer's protocol. Luminescence measurements were obtained using a Cytation 5 Cell Imaging Multi-Mode Reader (BioTek).

Colony formation assay

Cells transfected with either pRc/CMV-STAT3C-Flag or pRc/CMV control vector were seeded into 96-well/6-well plates at 100-500 cells/well. Starting from the second day, cells were treated with indicated COH34 concentrations for 7-14 days and then fixed with ice-cold methanol, stained with crystal violet, washed with water, and photographed using a ChemiDoc MP Imaging System. For signal quantification, the colonies from three independent experiments were measured using ImageJ software and plotted using GraphPad Prism 8 software.

RT-PCR

Total RNA was extracted from cultured cells using the RNeasy kit (Qiagen) per the manufacturer's instructions. cDNA was synthesized using the iScript cDNA Synthesis kit (Bio-Rad) and analyzed with specific primers for *Ifng* (VMPS-3028) using real-time PCR with the CFX96 Real-time PCR Detector (Bio-Rad) and iQ SYBR Green Supermix (Bio-Rad). The gene expression levels were normalized using the mouse *Actb* (VMPS-3028) housekeeping gene as an internal control. The primers were ordered from RealTimePrimers.com and validated with a

single melting peak in a standard curve across the dynamic range of interest. The real-time PCR was performed in triplicate, and the relative fold change was measured by the ΔΔ(CT) method.

ELISA

Secreted IFN levels in mouse tumor and immune cell co-cultures were quantified using a sandwich ELISA with a mouse IFN kit (#430604, BioLegend). In addition, cytokines in supernatants from ovarian cancer patient ascites-derived T cells and ALI-PDTO medium were assessed using human IL-10 (#430604, BioLegend), human IFN, and human Granzyme B (#430504, BioLegend) ELISA kits, following the manufacturer's guidelines. Absorbance was measured at 450 nm with a Cytation 5 Cell Imaging Multi-Mode Reader (BioTek).

Intracellular Staining and Flow Cytometry

Flow cytometry preparation and analyses were performed as described previously[1]. Briefly, for intracellular staining, single-cell suspensions were stimulated for 4-5 hours with Cell Activation Cocktail (with Brefeldin A, 500x, Biolegend), blocked with CD16/CD32, and incubated for 30 minutes on ice with FITC-, PE-, Alexa Fluor® 647, and APC-Cy7-conjugated antibodies (1:100, see Antibody list) purchased from Biolegend. Aqua LIVE/DEAD was used for cell viability (purchased from Invitrogen). Cells were washed twice before analysis on the BD LSR Fortessa flow cytometer (Beckman Coulter Genomics).

REFERENCES

1 Zhang, C. *et al.* STAT3 Activation-Induced Fatty Acid Oxidation in CD8(+) T Effector Cells Is Critical for Obesity-Promoted Breast Tumor Growth. *Cell Metab* **31**, 148-161 e145, doi:10.1016/j.cmet.2019.10.013 (2020).
2 Herrmann, A. *et al.* An effective cell-penetrating antibody delivery platform. *JCI Insight* **4**, doi:10.1172/jci.insight.127474 (2019).
3 Ding, L. *et al.* PARP Inhibition Elicits STING-Dependent Antitumor Immunity in Brca1-Deficient Ovarian Cancer. *Cell Rep* **25**, 2972-2980.e2975, doi:10.1016/j.celrep.2018.11.054 (2018).
4 Dijkstra, K. K. *et al.* Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids. *Cell* **174**, 1586-1598.e1512, doi:10.1016/j.cell.2018.07.009 (2018).
5 Bromberg, J. F. *et al.* Stat3 as an oncogene. *Cell* **98**, 295-303, doi:10.1016/s0092-8674(00)81959-5 (1999).