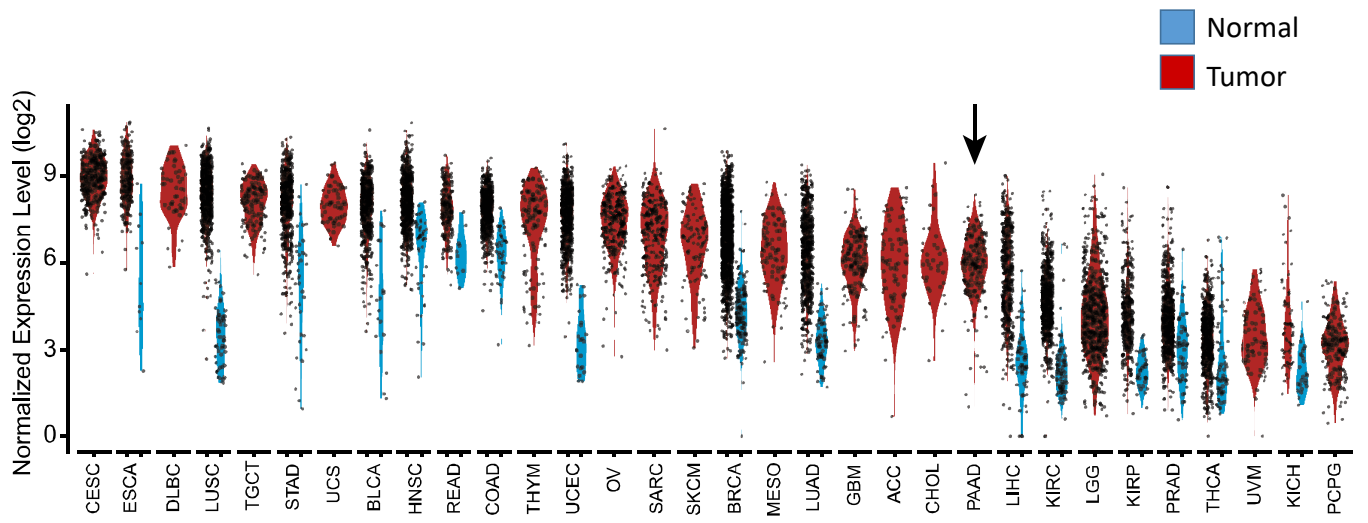
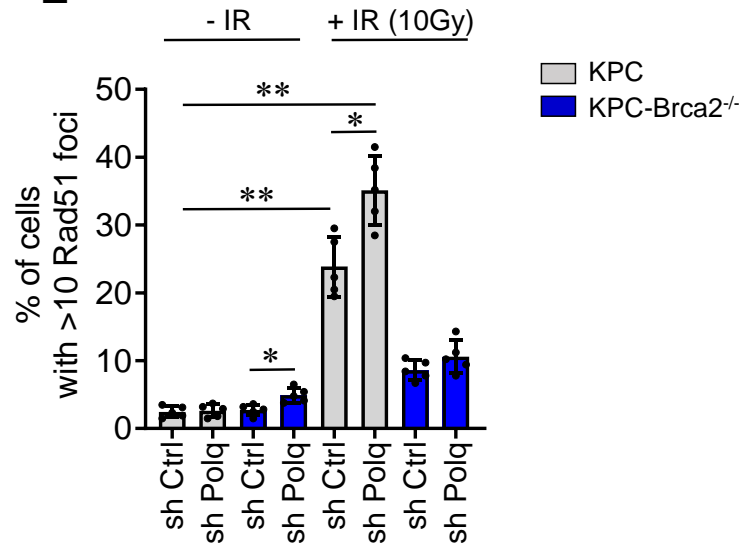


Supplemental Figure S1, related to figure 1

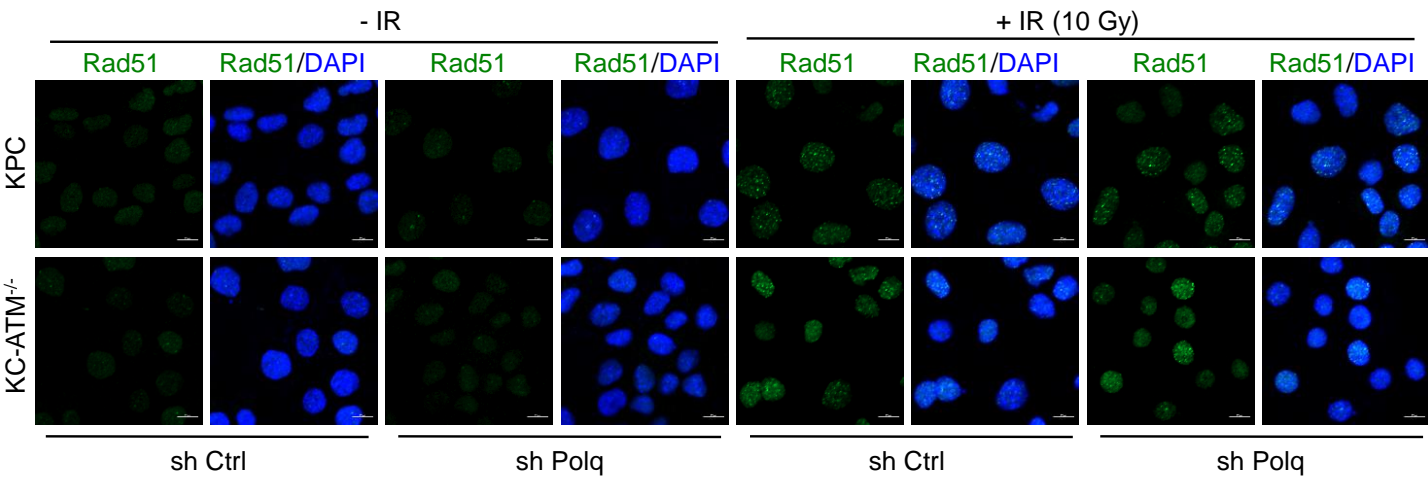


Supplemental Figure 1. *POLQ* expression is elevated in HR-deficient PDAC. Within the TCGA database, pancreatic adenocarcinoma (PAAD) (arrow) demonstrates elevated *POLQ* expression levels.

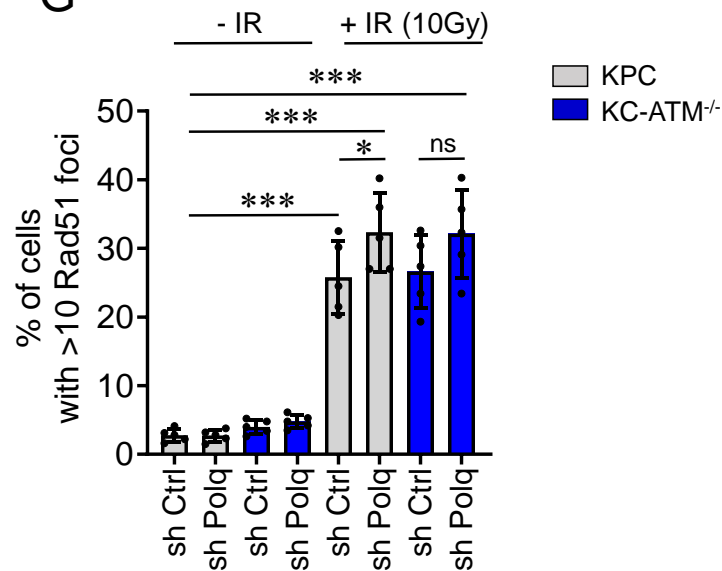
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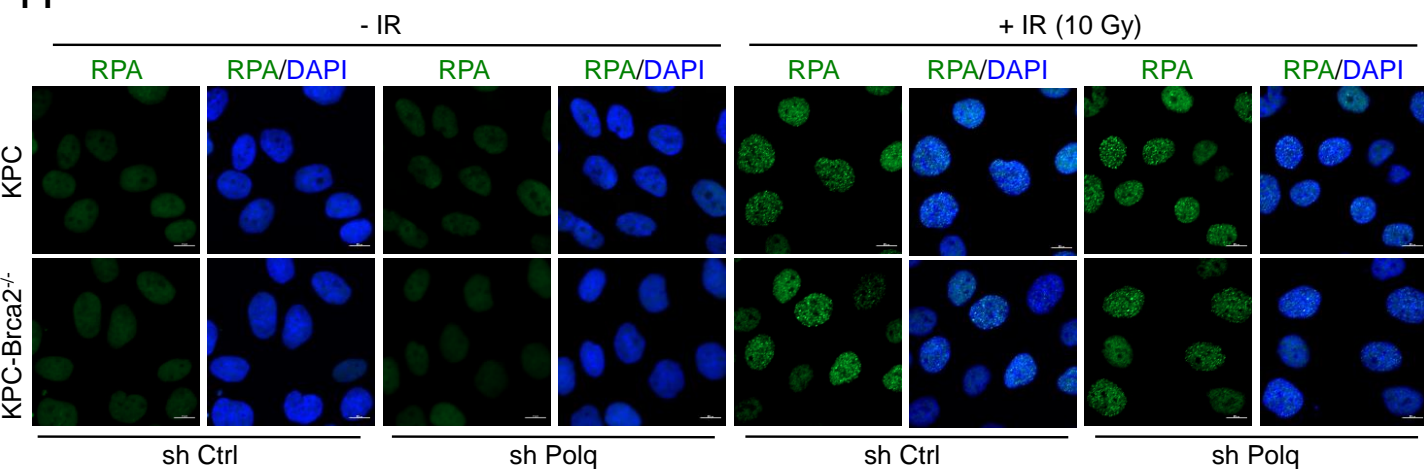
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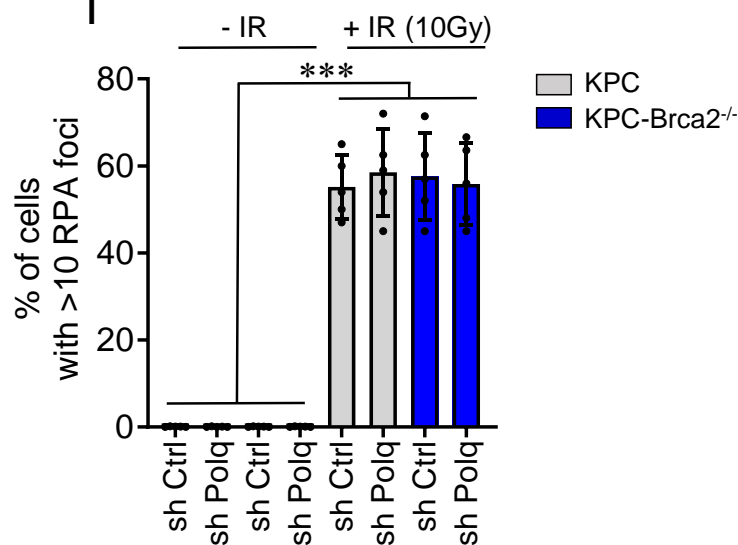
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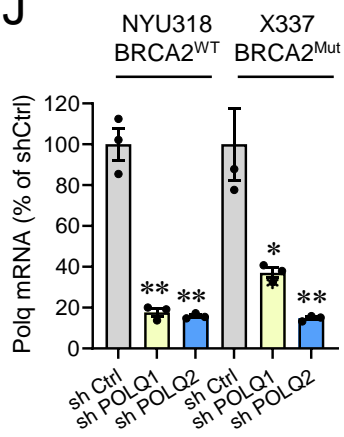
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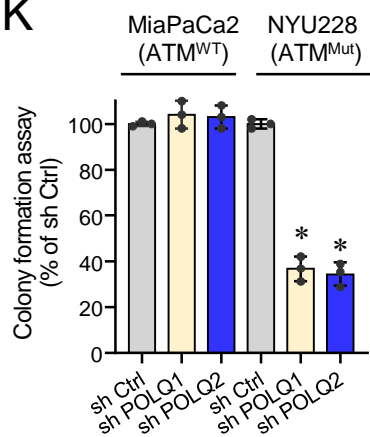
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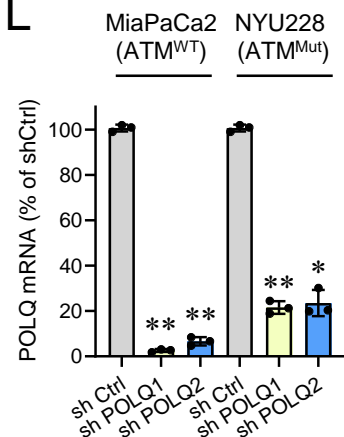
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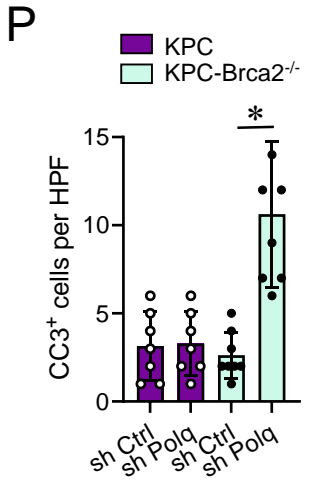
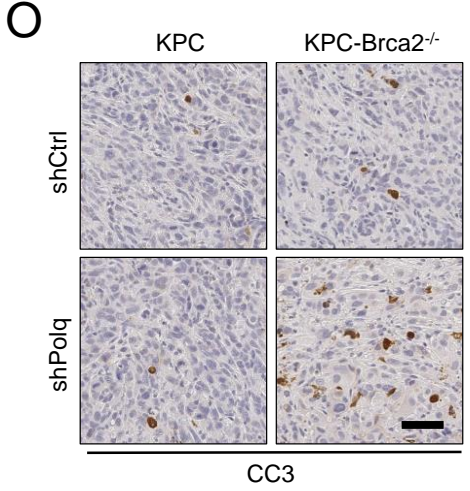
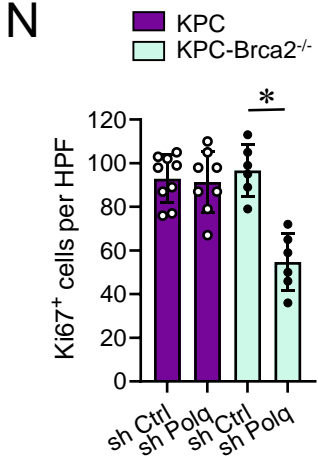
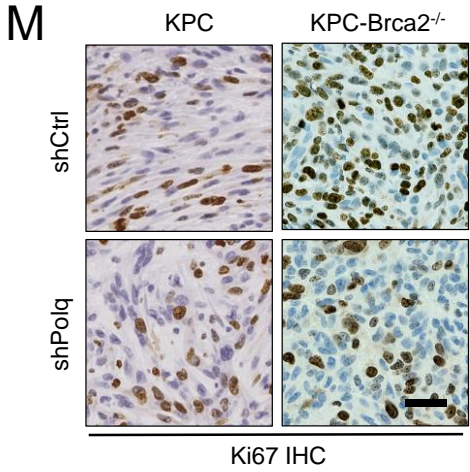


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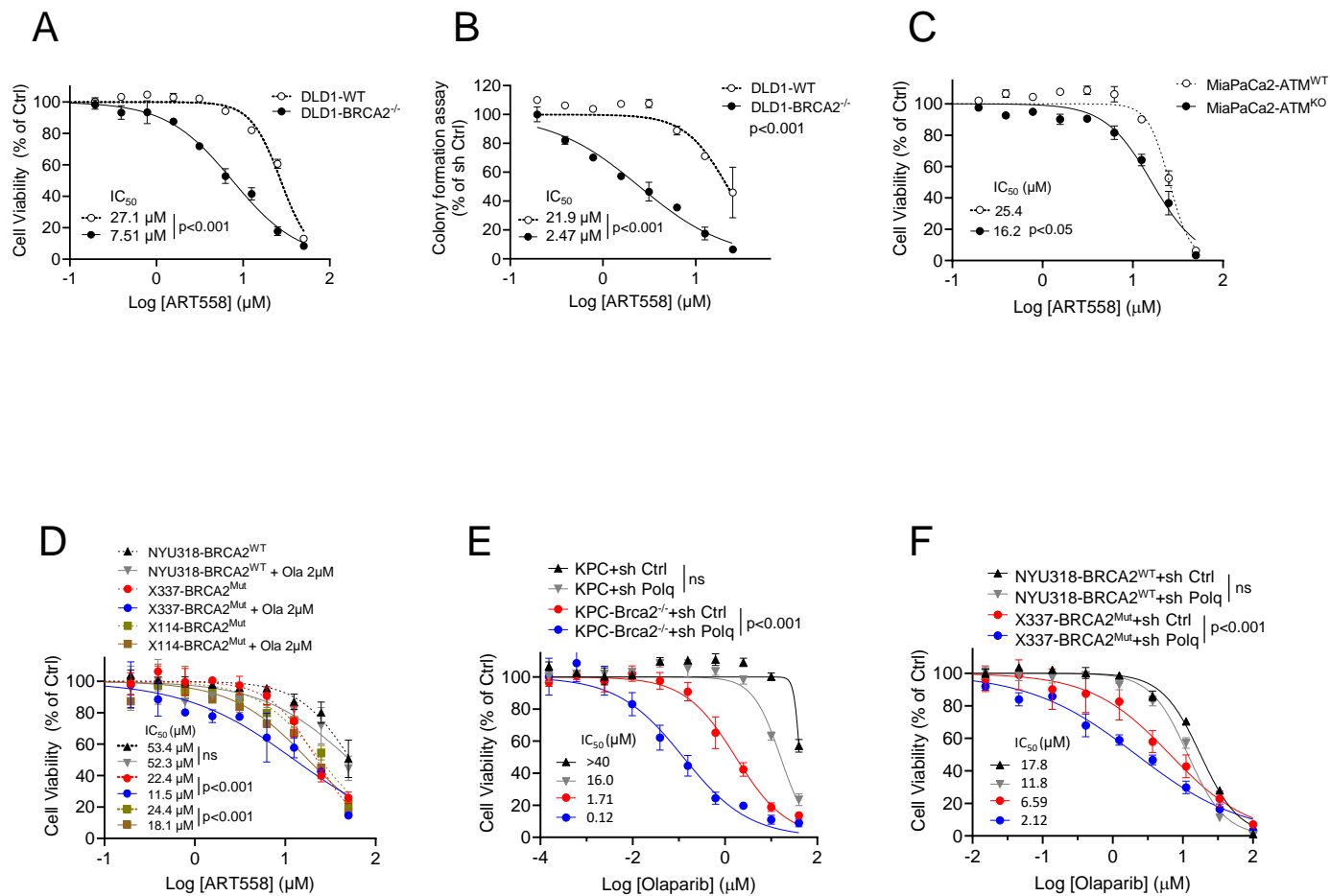
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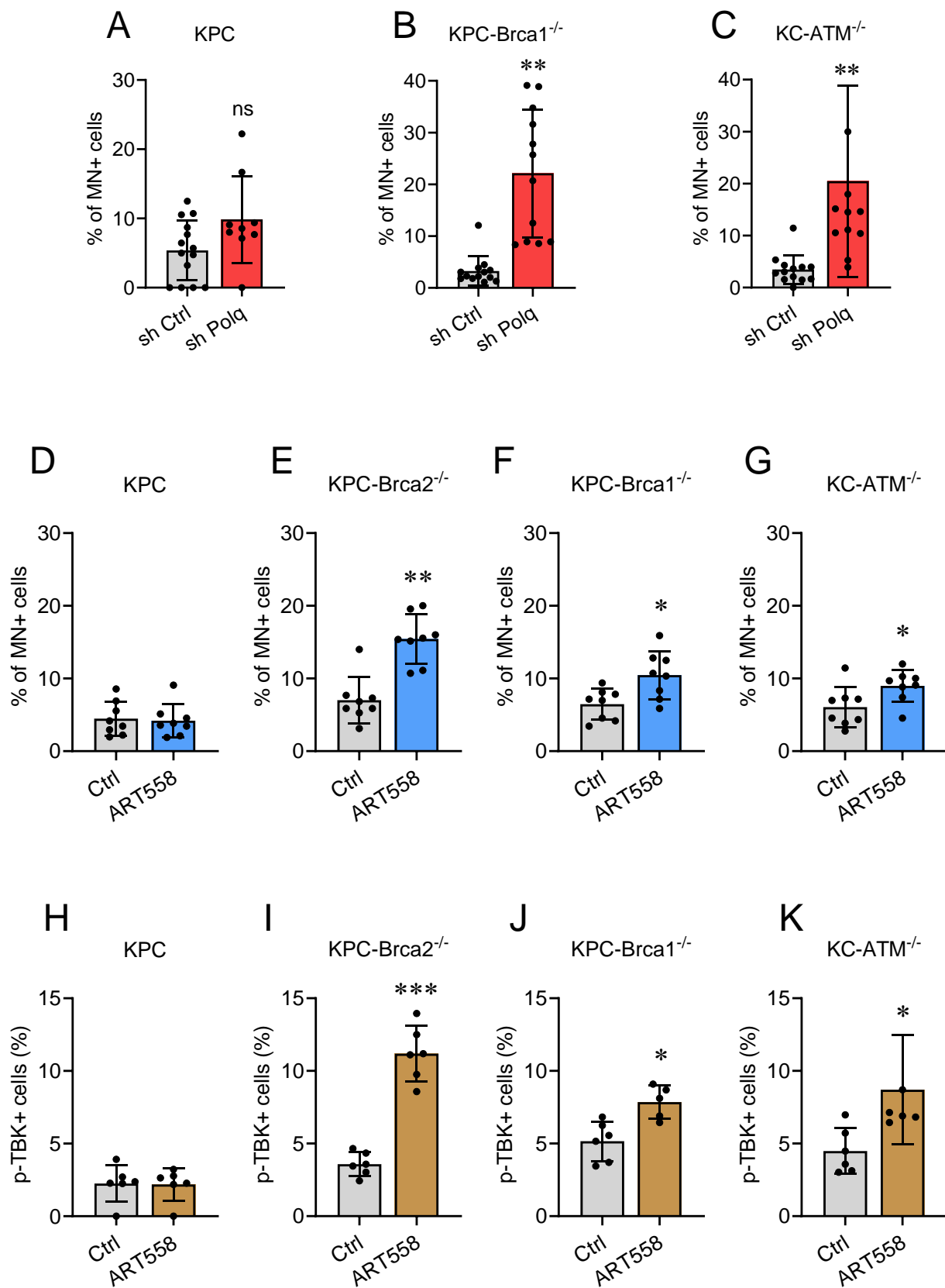


Supplemental Figure 2. POLQ inhibition induces synthetic lethality in BRCA2-deficient PDAC. (A) *POLQ* mRNA transcript levels by qRT-PCR in KPC and KPC-*Brca2*^{-/-} sh Ctrl and sh *POLQ* cells (n=3). (B) POLQ inhibition reduces colony formation in KPC, KPC-*Brca1*^{-/-} and KC-*Atm*^{-/-} cell lines (n=3). (C) *POLQ* mRNA transcript levels by qRT-PCR in KPC, KPC-*Brca1*^{-/-} and KC-*Atm*^{-/-}, cell lines (n=3). (D) Representative images of immunofluorescence staining for RAD51 (green) and DAPI (blue) in KPC and KPC-*Brca2*^{-/-} sh Ctrl and sh *POLQ* cells. Scale bar, 10 μ m. (E) Quantification of cells with more than 10 RAD51 foci from (D). Each point on the graph represents one visual field (n=5). (F) Representative images of immunofluorescence staining for RAD51 (green) and DAPI (blue) in KPC and KC-*Atm*^{-/-} sh Ctrl and sh *POLQ* cells. Scale bar, 10 μ m. (G) Quantification of cells with more than 10 RAD51 foci from (F). Each point on the graph represents one visual field (n=5). (H) Representative images of immunofluorescence staining for RPA (green) and DAPI (blue) in KPC and KPC-*Brca2*^{-/-} sh Ctrl and sh *POLQ* cells. Scale bar, 10 μ m. (I) Quantification of cells with more than 10 RPA foci from (H). Each point on the graph represents one visual field (n=5). (J) *POLQ* mRNA transcript levels by qRT-PCR in NYU318^{WT}, X337-*BRCA2*^{Mut} sh Ctrl and sh *POLQ* cells (n=3). (K) POLQ inhibition reduces colony formation in NYU 228 (*ATM*^{Mut}) cells but has minimal effect on colony formation in MIA PaCa-2 cells (n=3). (L) *POLQ* mRNA transcript levels by qRT-PCR in MIA PaCa-2 (*ATM* WT) and NYU 228 (*ATM*^{Mut}) sh Ctrl and sh *POLQ* cells (n=3). (M) Representative images of tumor sections stained for Ki67 by IHC. Scale bar, 20 μ m. (N) Quantification of Ki67+ cells per HPF on tumor sections stained for Ki67 by IHC (M). Each point on the graph represents one mouse (n=6-9 mice/group). (O) Representative images of tumor sections stained for CC3 by IHC. Scale bar, 50 μ m. (P) Quantification of CC3+ cells per HPF on tumor sections stained for CC3 by IHC (O) (n=7-8 mice/group). Data are representative of at least three independent experiments. (*) P < 0.05, (**) P < 0.01, (***) P < 0.005, (ns) not significant. Error bars=Mean \pm SEM.

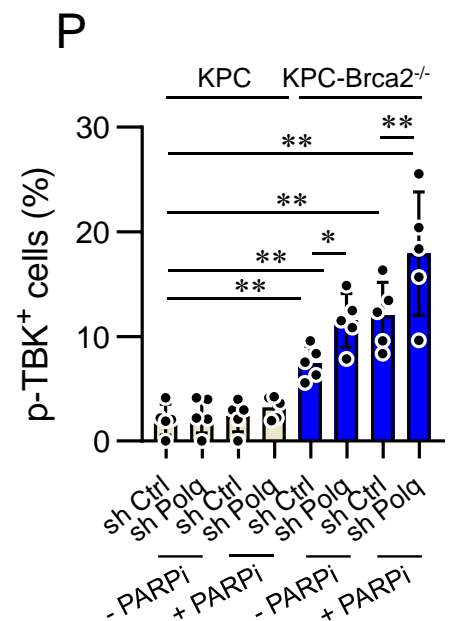
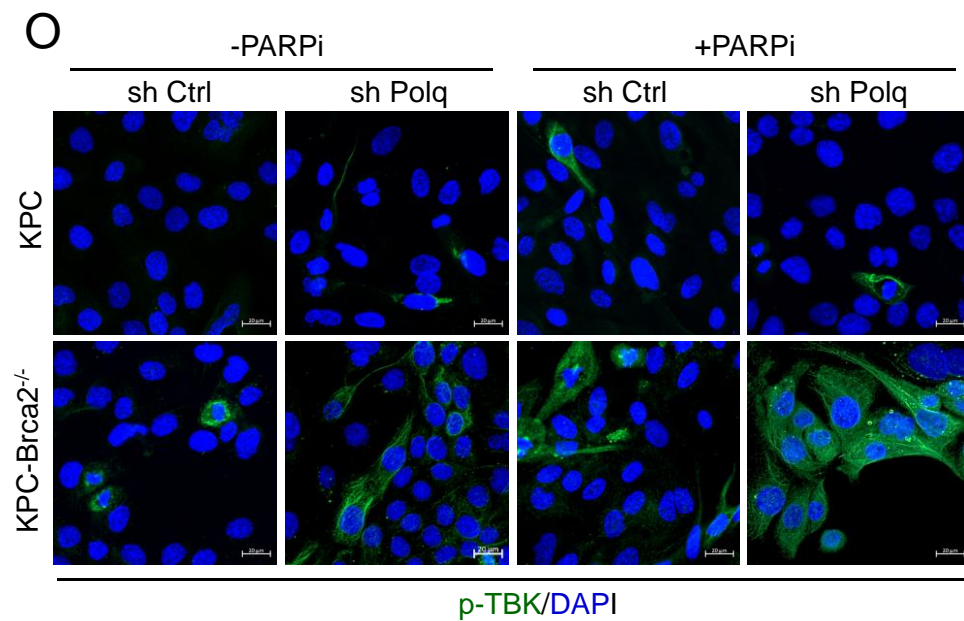
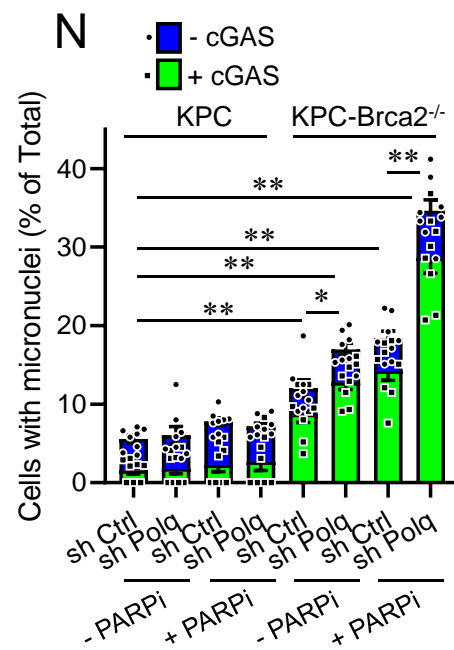
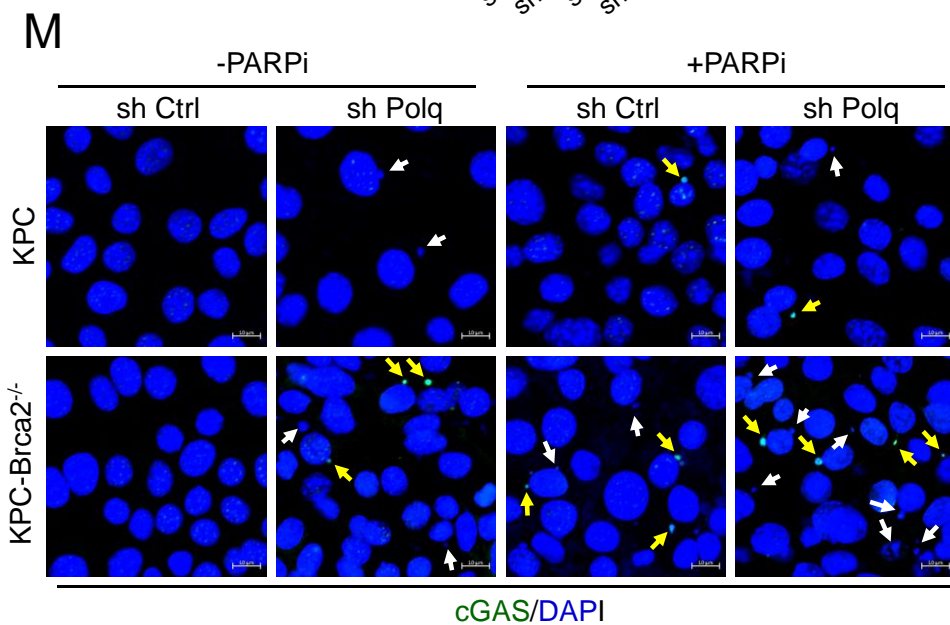
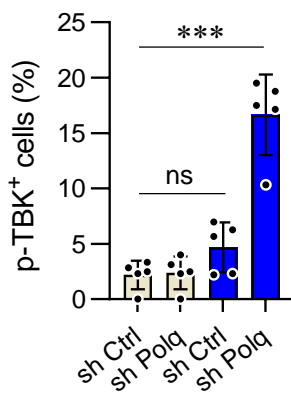
Supplemental Figure S3, related to figure 3



Supplemental Figure 3. POLQ inhibitor elicits synthetic lethality and synergizes with PARPi in HR-deficient PDAC cells. (A) Dose-dependent viability assays of DLD1-WT and DLD1-*BRCA2*^{-/-} cells exposed to ART558 at the indicated concentrations. Cell viability was measured by CellTiter-Glo after 6 days of drug exposure. Data are % of DMSO control (% of Ctrl)(n=3). (B) Clonogenic survival of DLD1-WT and DLD1-*BRCA2*^{-/-} cells with increasing concentrations of ART558. Survival is normalized to the values from the untreated sample (n=3). (C) Dose-dependent viability assays of MiaPaCa2-*ATM*^{WT} and MiaPaCa2-*ATM*^{KO} cells exposed to ART558 at the indicated concentrations. Cell viability was measured by CellTiter-Glo after 6 days of drug exposure. Data are % of DMSO control (% of Ctrl)(n=3). (D) Dose-dependent viability assays of NY318^{WT}, X337-*BRCA2*^{Mut}, and X114-*BRCA2*^{Mut} cells exposed to increasing concentration of ART558 alone or in combination with 2 μ M PARPi (Olaparib). Data are % of DMSO control (% of Ctrl). (E) Dose-dependent viability assays of KPC or KPC-*Brca2*^{-/-} cells with or without sh Control (Ctrl) or sh *Polq* exposed to increasing concentration of Olaparib. Data are % of DMSO control (% of Ctrl). (F) Dose-dependent viability assays of NYU318-*BRCA2*^{WT} or X337-*BRCA2*^{Mut} cells with or without sh Control (Ctrl) or sh *Polq* exposed to increasing concentration of Olaparib. A two-way ANOVA test was used to calculate p values. Data are representative of at least three independent experiments. (*) P < 0.05, (**) P < 0.01, (***) P < 0.005, (ns) not significant. Error bars=Mean \pm SEM.

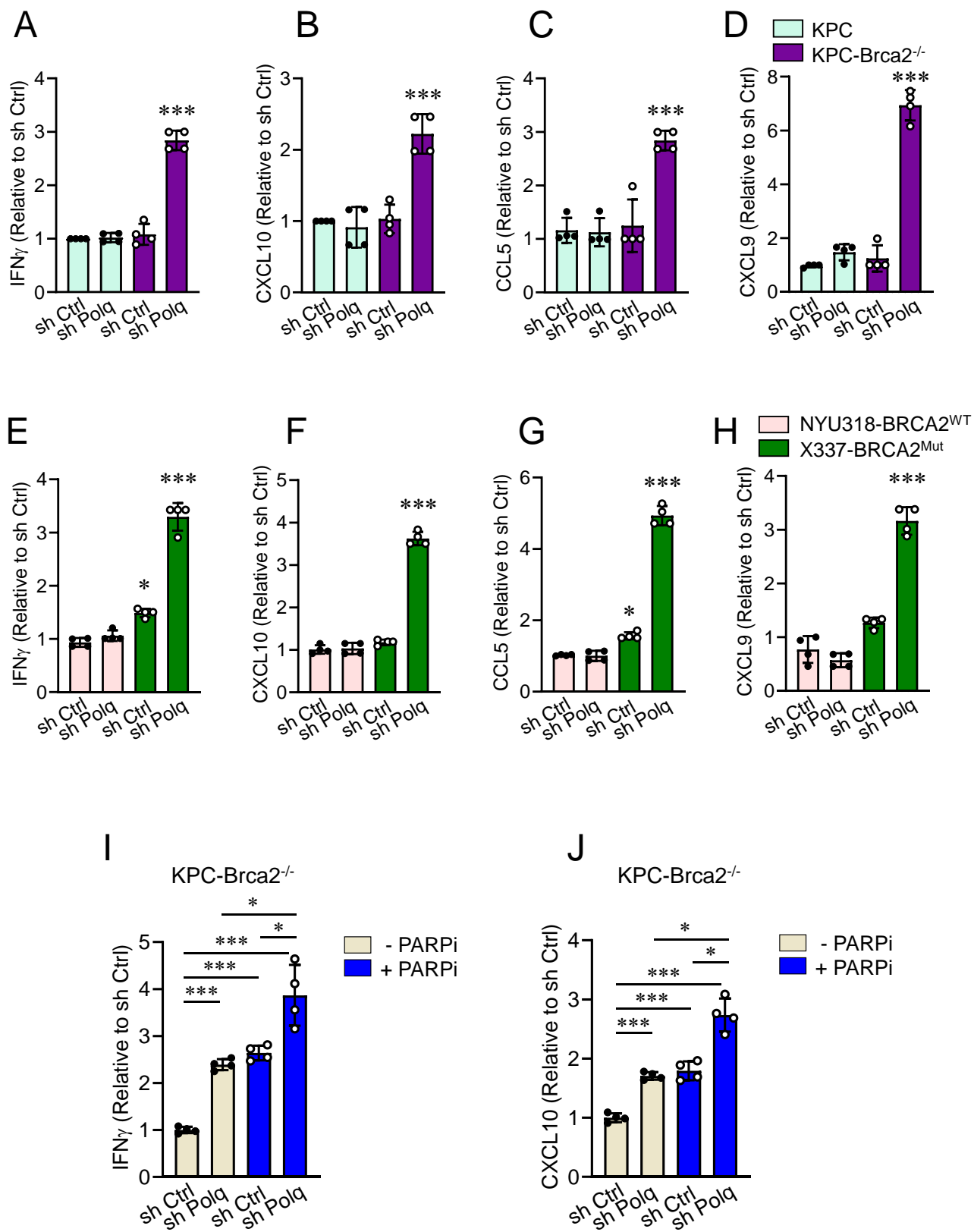


L NYU318-BRCA2^{WT}
 X337-BRCA2^{Mut}



Supplemental Figure 4. POLQ inhibition activates the cGAS-STING signaling pathway in BRCA2-deficient PDAC

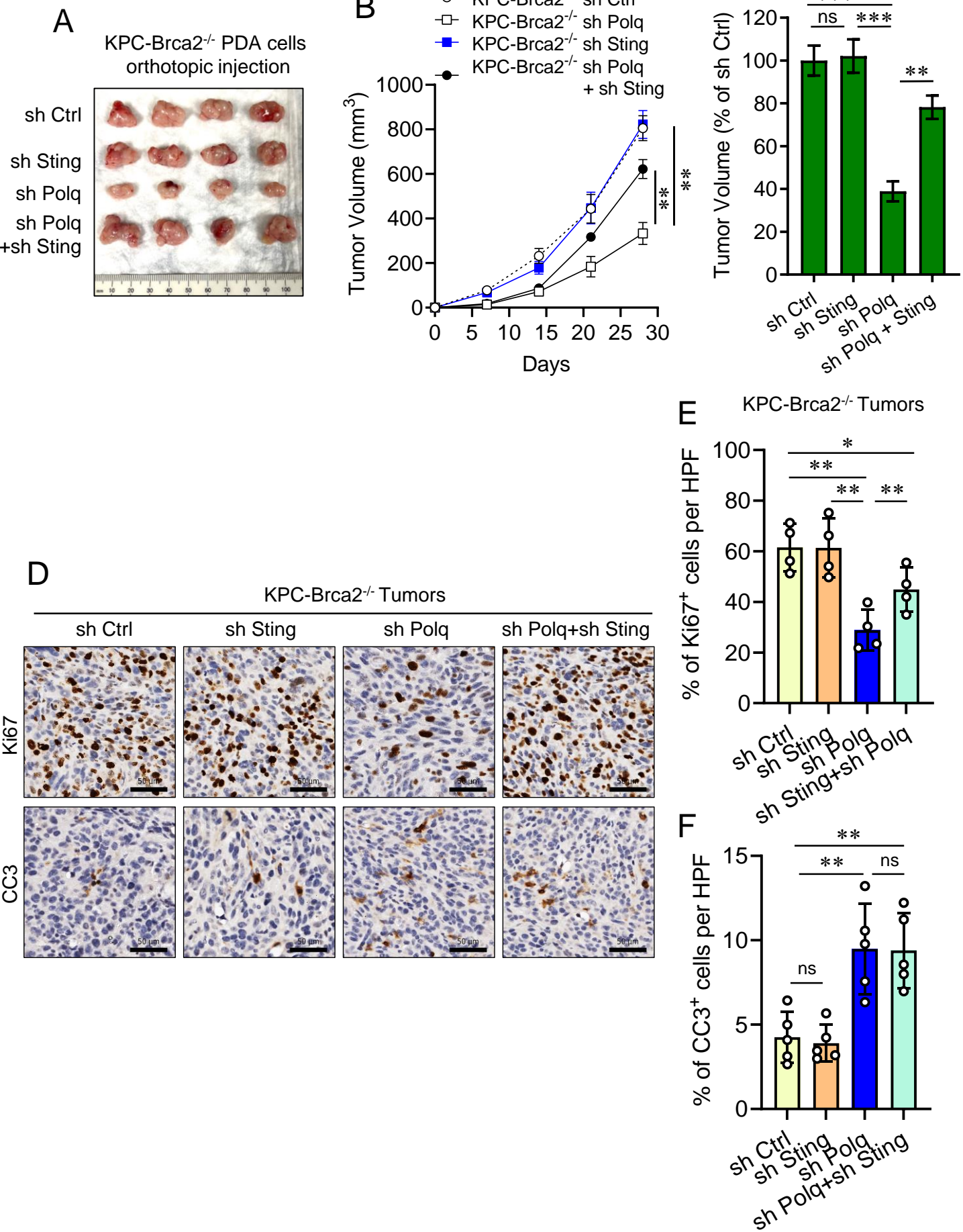
(A-C) Quantification of cells with micronuclei (MN) in KPC, KPC-*Brca1*^{-/-}, KC-*Atm*^{-/-}, and KPC-*Palb2*^{-/-} cells with or without sh Polq expression. Each point on the graph represents one visual field. (D-G) Quantification of cells with micronuclei (MN) in KPC (D), KPC-*Brca2*^{-/-} (E), KPC-*Brca1*^{-/-} (F), and KC-*Atm*^{-/-} (G) cells with or without POLQ inhibitor ART558 treatment. Each point on the graph represents one visual field. (H-K) Quantification of cells that are p-TBK+ in KPC (H), KPC-*Brca2*^{-/-} (I), KPC-*Brca1*^{-/-} (J), and KC-*Atm*^{-/-} (K) cells with or without POLQ inhibitor ART558 treatment. Each point on the graph represents one visual field. (L) Quantification of cells that are p-TBK+ from (Figure 4G). (M) Representative image of immunofluorescence staining for cGAS (green) and DAPI (blue) in KPC and KPC-*Brca2*^{-/-} cells with or without sh POLQ in combination with 2 μM PARPi (Olaparib). Scale bar, 10 μm. White arrows indicate cGAS cGAS-negative micronuclei. Yellow arrows indicated cGAS-positive micronuclei. (N) Quantification of cells with micronuclei from (M). (O) Representative image of immunofluorescence staining for p-TBK (green) and DAPI (blue) in KPC and KPC-*Brca2*^{-/-} cells with or without sh POLQ in combination with 2 μM PARPi (Olaparib). Scale bar, 20 μm. (P) Quantification of cells that are p-TBK+ from (O). Data are representative of at least three independent experiments. (*) P < 0.05, (**) P < 0.01, (***) P < 0.005, (ns) not significant. Error bars=Mean ± SEM.



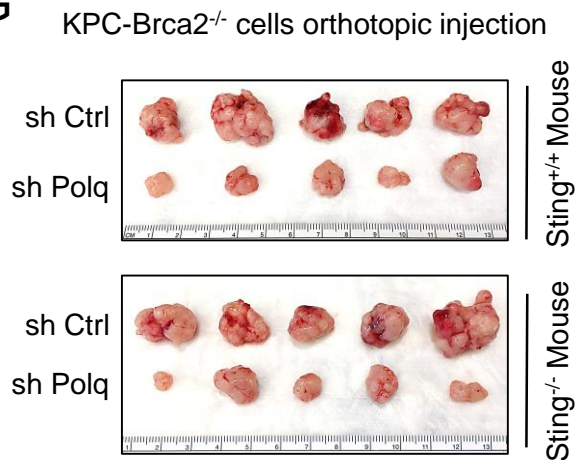
Supplemental Figure 5. POLQ inhibition enhances immune infiltration in BRCA2-deficient PDAC

(A-D) Normalized levels of immune activity markers INF γ (A), CXCL10 (B), CCL5 (C), and CXCL9 (D) in KPC and KPC-*Brca2*^{-/-} sh Ctrl and sh POLQ cells as measured by cytokine array (n=4). (E-H) Normalized levels of immune activity markers INF γ (E), CXCL10 (F), CCL5 (G), and CXCL9 (H) in NYU318-*BRCA2*^{WT} and X337-*BRCA2*^{Mut} sh Ctrl and sh POLQ cells as measured by cytokine array (n=4). (I, J) Normalized levels of immune activity markers INF γ (I) and CXCL10 (J) in KPC and KPC-*Brca2*^{-/-} cells with or without sh POLQ in combination with 2 μ M PARPi (Olaparib) as measured by cytokine array (n=4). (* P < 0.05, (**) P < 0.01, (***) P < 0.005), (ns) not significant. Error bars=Mean \pm SEM.

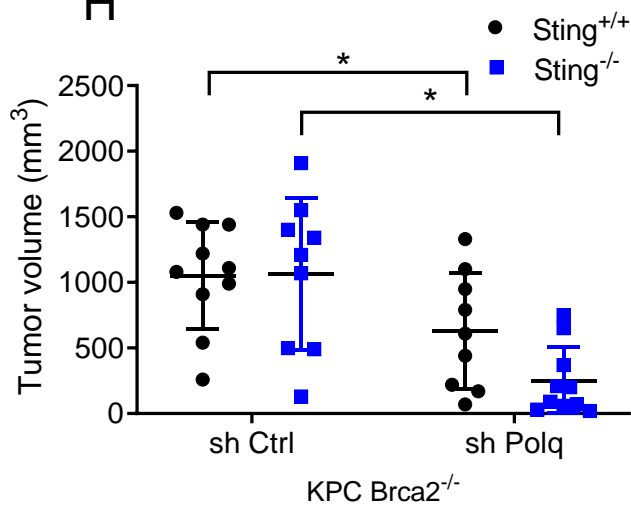
Supplemental Figure S6, related to figure 6



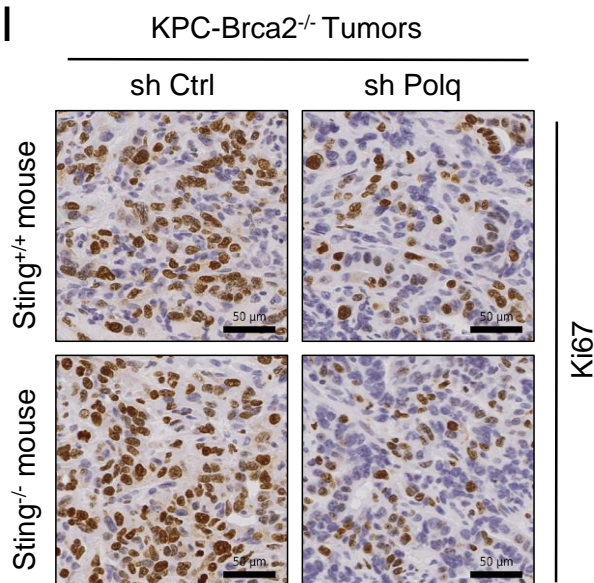
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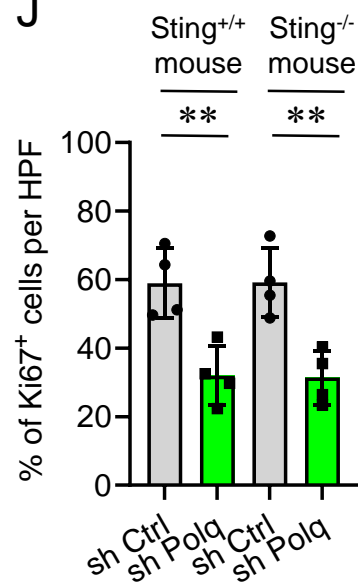
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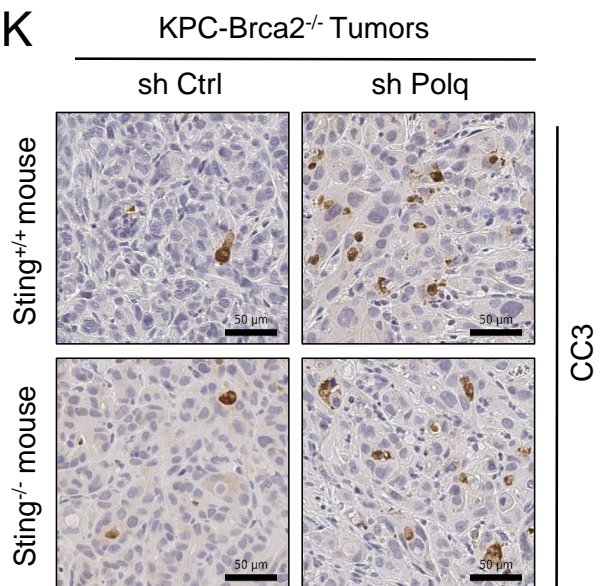
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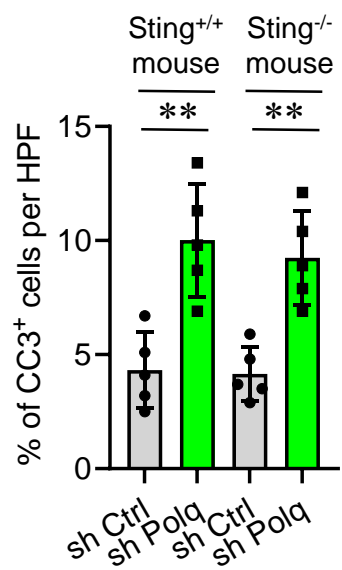
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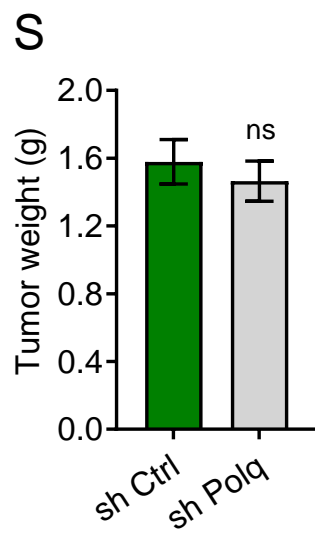
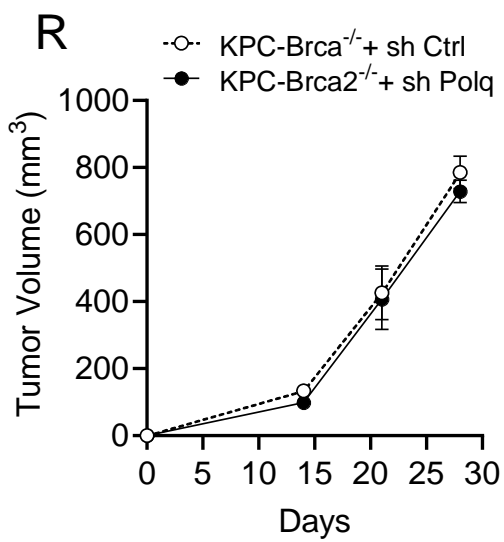
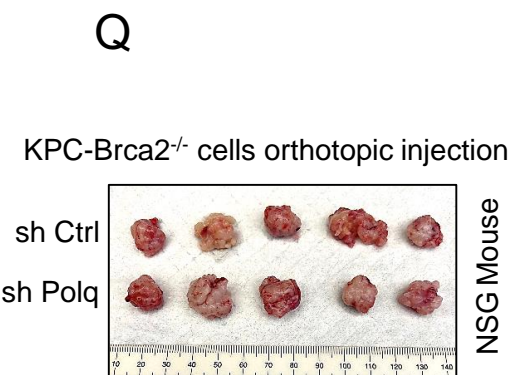
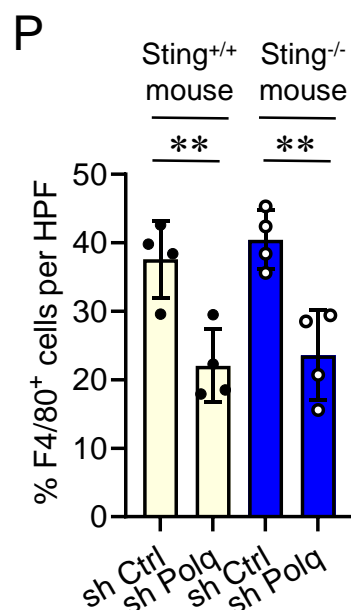
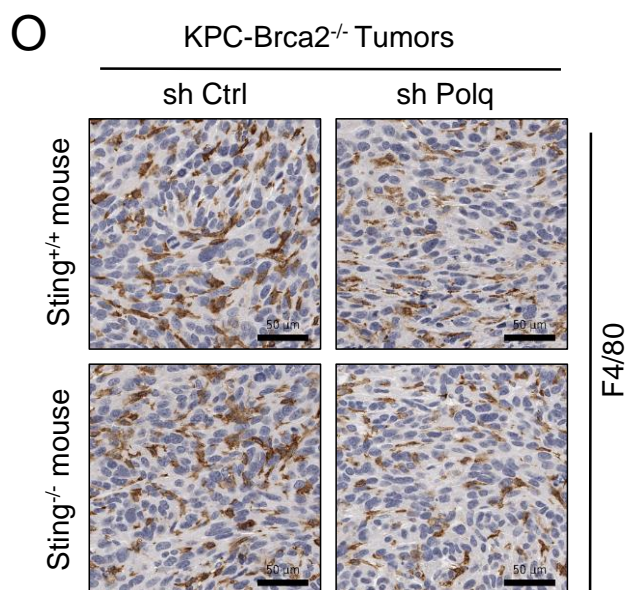
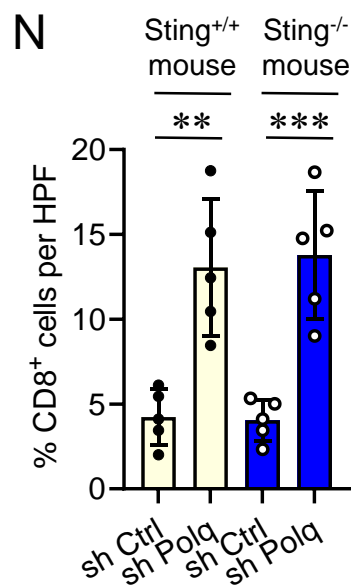
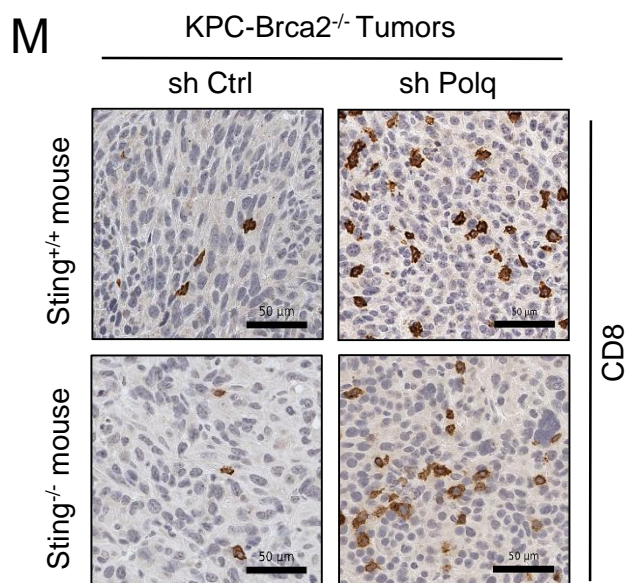


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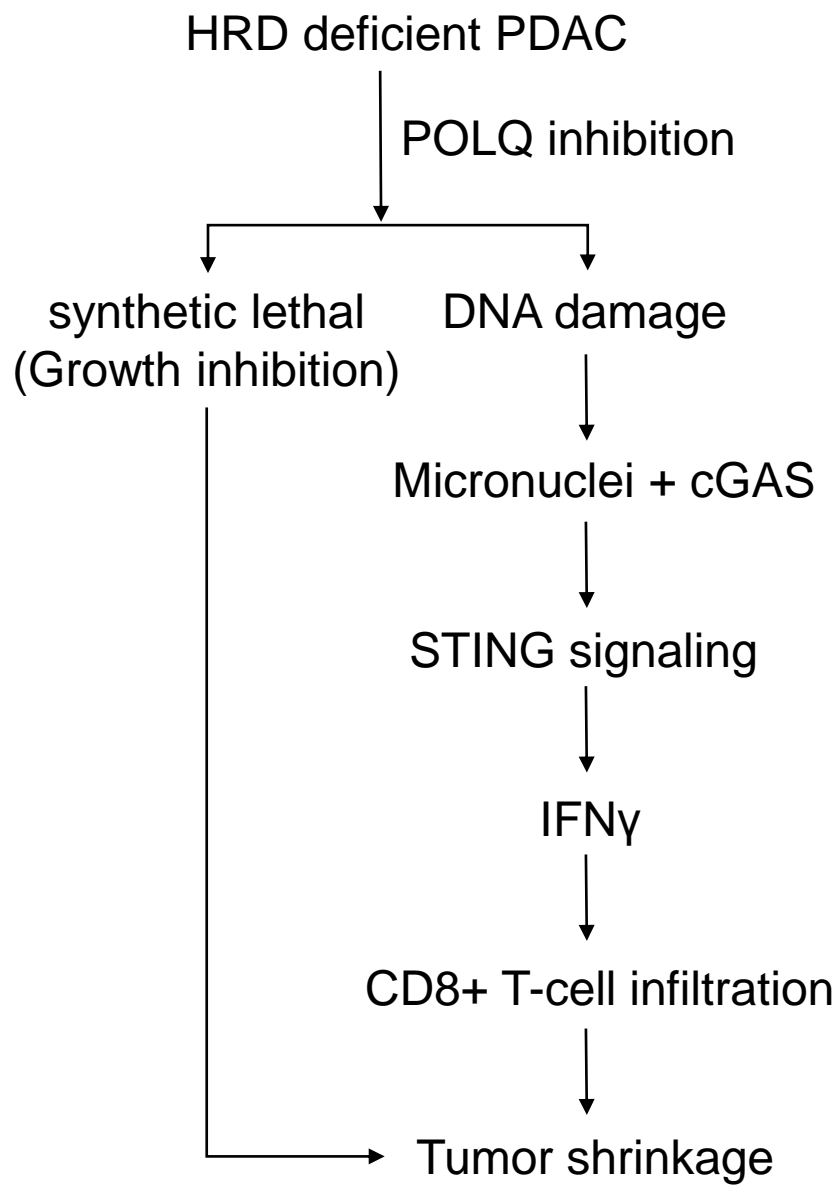


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Supplemental Figure 6. STING activation is essential for the POLQ inhibition-induced antitumor effect in BRCA2-deficient PDAC. (A) Representative image of KPC-*Brca2*^{-/-} sh Ctrl, sh *STING*, sh*POLQ*, and sh *POLQ* + sh *STING* tumors collected 4 weeks after orthotopic implantation into immunocompetent mice. (B) The growth curve of KPC and KPC-*Brca2*^{-/-} tumors with or without sh Ctrl, sh *Polq*, sh *Sting*, or sh *Polq* + sh *Sting* (n=10/group). (C) % of Volume for KPC-*Brca2*^{-/-} sh Ctrl, sh *STING*, sh *POLQ*, and sh *POLQ* + sh *STING* tumors vs sh Ctrl at 4 weeks after orthotopic implantation (n=6-8 mice/group). (D) Representative images of KPC-*Brca2*^{-/-} sh Ctrl, sh *STING*, sh *POLQ*, and sh *POLQ* + sh *STING* tumors, stained for Ki67 and CC3 expression by IHC. Scale bar, 50 μ m. (E) Quantification of Ki67 expression from (D). Each point on the graph represents one visual field. (F) Quantification of CC3 expression from (D). (G) Representative image of KPC-*Brca2*^{-/-} sh Ctrl and sh *POLQ* tumors, collected 3 weeks after orthotopic implantation into *Sting*^{+/+} and *Sting*^{-/-} mice. (H) Tumor volume was measured by ultrasound in mice 3 weeks after orthotopic implantation of cells from (G) (n=9-10 mice/group). (I) Representative images of KPC-*Brca2*^{-/-} sh Ctrl and sh *POLQ* tumors in *Sting*^{+/+} and *Sting*^{-/-} mice stained for Ki67 by IHC. Scale bar, 50 μ m. (J) Quantification of Ki67⁺ cells per HPF from (I). (K) Representative images of KPC-*Brca2*^{-/-} sh Ctrl and sh *POLQ* tumors in *Sting*^{+/+} and *Sting*^{-/-} mice stained for CC3 by IHC. Scale bar, 50 μ m. (L) Quantification of CC3⁺ cells per HPF from (K). (M) Representative images of KPC-*Brca2*^{-/-} sh Ctrl and sh *POLQ* tumors in *Sting*^{+/+} and *Sting*^{-/-} mice stained for CD8⁺ cells by IHC. Scale bar, 50 μ m. (N) Quantification of CD8⁺ cells per HPF from (M). (O) Representative images of KPC-*Brca2*^{-/-} sh Ctrl and sh *POLQ* tumors in *Sting*^{+/+} and *Sting*^{-/-} mice stained for F4/80⁺ cells by IHC. Scale bar, 50 μ m. (P) Quantification of F4/80⁺ cells per HPF from (O). (Q) Representative image of KPC-*Brca2*^{-/-} sh Ctrl and sh *POLQ* tumors, collected 3 weeks after orthotopic implantation into immunodeficient NSG mice. (R) The growth curve of KPC and KPC-*Brca2*^{-/-} tumors with or without sh Ctrl or sh *Polq* in NSG mice (n=6-8/group). (S) Tumor volume was measured by ultrasound in C57BL/6 mice orthotopically implanted with KPC and KPC-*Brca2*^{-/-} sh Ctrl and sh *POLQ* cells (n=6-8 mice/group). (T) POLQ inhibition in HR-deficient PDAC promotes tumor cell death by 1) synthetic lethal interaction and 2) recruitment of immune cells through the cGAS-STING pathway.

Data are representative of at least three independent experiments. (*) P < 0.05, (**) P < 0.01, (***) P < 0.005, (ns) not significant. Bars are the mean \pm SEM.