Supplemental Information

Eliminating hypoxic tumor cells improves response to PARP inhibitors in homologous recombination deficient cancer models

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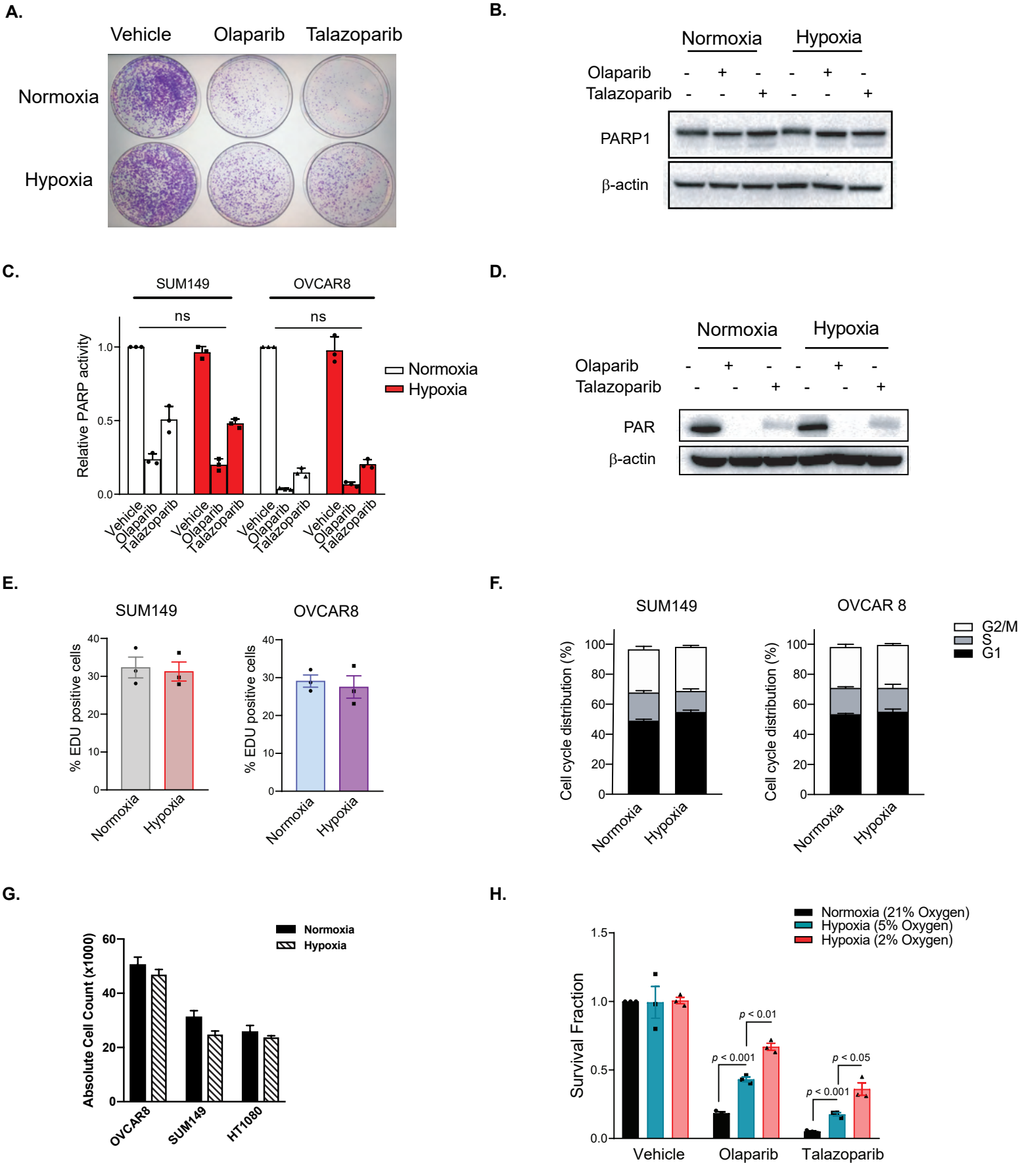
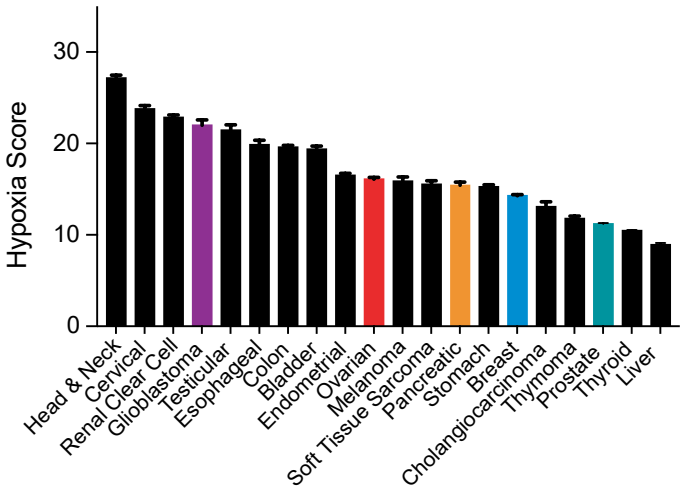

A.
Vehicle
Olaparib
Talazoparib
Normoxia
Hypoxia
B.
Normoxia
Hypoxia
Olaparib - + - - + -
Talazoparib - - + - - +
PARP1
β-actin
C.
SUM149
OVCAR8
ns
ns
Relative PARP activity
1.0
0.5
0.0
Normoxia
Hypoxia
Vehicle
Olaparib
Talazoparib
D.
Normoxia
Hypoxia
Olaparib - + - - + -
Talazoparib - - + - - +
PAR
β-actin
E.
SUM149
OVCAR8
% EDU positive cells
40
30
20
10
0
Normoxia
Hypoxia
F.
SUM149
OVCAR 8
Cell cycle distribution (%)
100
80
60
40
20
0
G2/M
S
G1
Normoxia
Hypoxia
G.
Absolute Cell Count (x1000)
60
40
20
0
Normoxia
Hypoxia
OVCAR8
SUM149
HT1080
H.
Normoxia (21% Oxygen)
Hypoxia (5% Oxygen)
Hypoxia (2% Oxygen)
Survival Fraction
1.5
1.0
0.5
0.0
p < 0.01
p < 0.001
p < 0.05
p < 0.001
Vehicle
Olaparib
Talazoparib

Figure S1. Related to Figure 1:
(A) Representative images of clonogenic survival assays of SUM149 cells treated with Olaparib (0.1 μM) or Talazoparib (1 nM) for 7 days under normoxia or hypoxia.
(B) Representative Western Blot analysis of PARP1 shows the protein levels are not affected by incubation in hypoxia. SUM149 were treated with Olaparib and Talazoparib for 7 days, β-actin is a loading control.
(C) Anti-PAR ELISA confirms efficiency of inhibition of PAR formation after treatment with PARPi in both normoxia and hypoxia (*n*=3 per condition), two-way ANOVA, interaction *p* value (normoxia vs hypoxia for each treatment).
(D) PAR formation, as detected by WB, in OVCAR8 cells treated with PARPi for 7 days in both normoxia and hypoxia. β-actin is a loading control.
(E) Percentage of EDU positive cells after 7 days incubation in normoxia or hypoxia (*n*=3 per condition).
(F) Cell cycle profiles of OVCAR8 and SUM149, 7 days after incubation in normoxia or hypoxia (*n*=3 per condition).
(G) Number of OVCAR8, SUM149 and HT1080 cells after 7 days after incubation in normoxia or hypoxia (*n*=3).
(H) Clonogenic formation of HR deficient OVCAR 8 cells treated with indicated doses of Olaparib or Talazoparib for 7 days under normoxic or hypoxic conditions (2% oxygen and 5% oxygen), followed by 7-10 days culture in the absence of inhibitor Survival relative to vehicle-treated cells is plotted. (*n*=3 per condition, *p* values from two-way ANOVA).
Data represented as means ± SEM (represented by error bars).

A.


Hypoxia Score
30
20
10
0
Head & Neck
Cervical
Renal Clear Cell
Glioblastoma
Testicular
Esophageal
Colon
Bladder
Endometrial
Ovarian
Melanoma
Soft Tissue Sarcoma
Pancreatic
Stomach
Breast
Cholangiocarcinoma
Thymoma
Prostate
Thyroid
Liver

B.

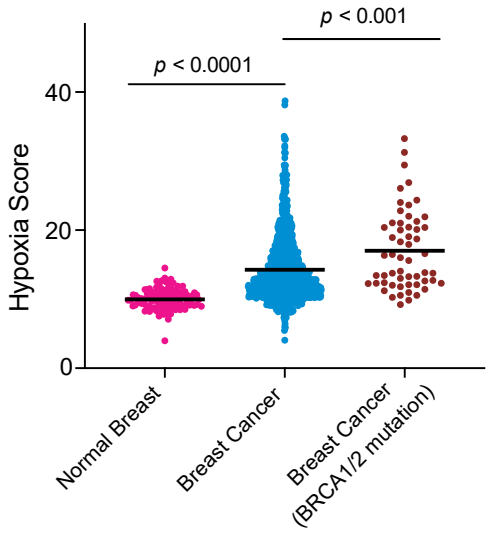

p < 0.001
p < 0.0001
Hypoxia Score
40
20
0
Normal Breast
Breast Cancer
Breast Cancer
(BRCA1/2 mutation)

Figure S2. Related to Figure 2:
(A) Hypoxia is a hallmark of cancer. Hypoxia score (Buffa) of cancers from TCGA cohort.
(B) Dot plot and mean values of hypoxia scores of normal breast tissue and breast cancer with and without BRCA1/2 deficiency. *p* Mann Whitney test.
Data represented as means ± SEM (represented by error bars).

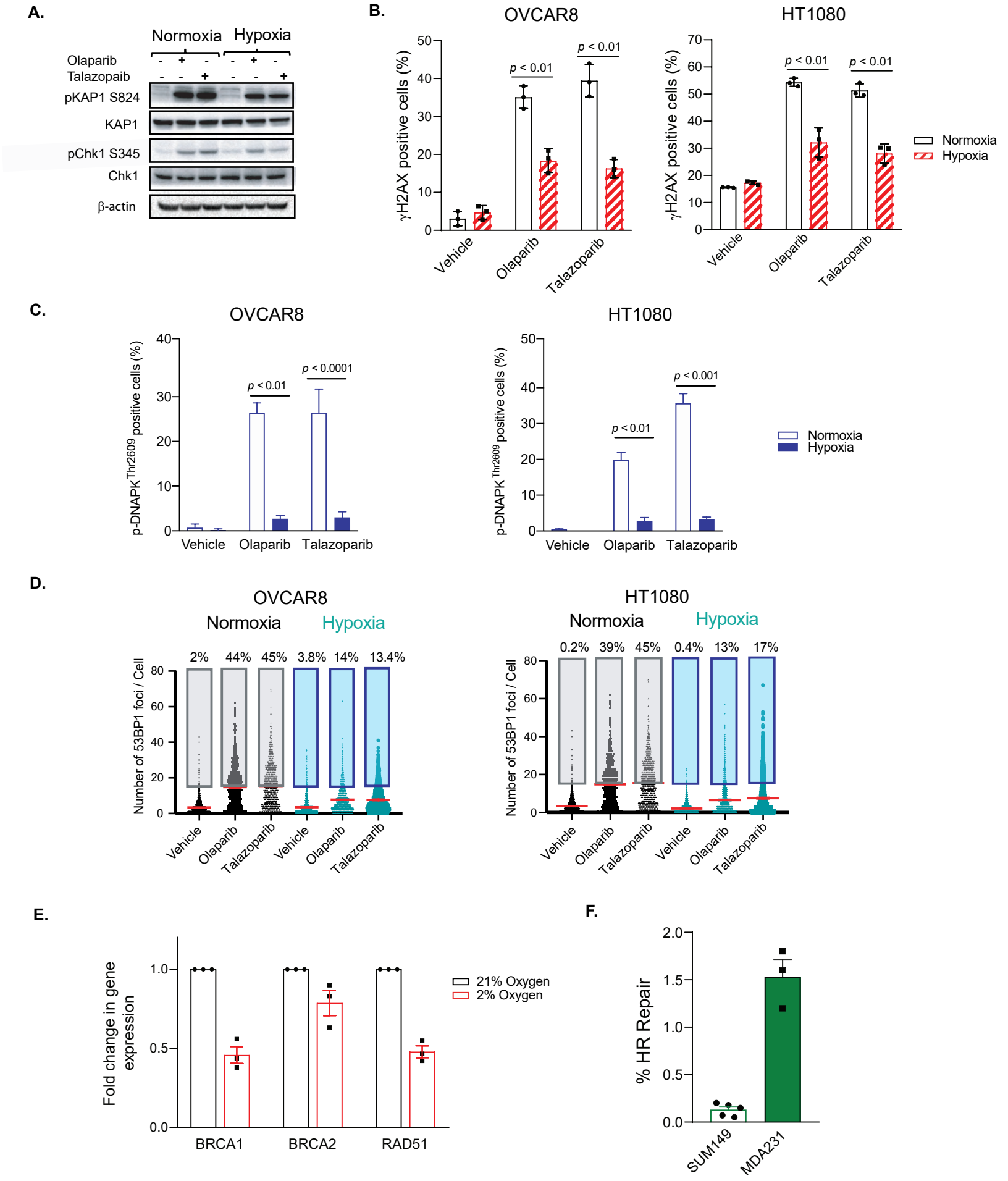

A.
Normoxia
Hypoxia
Olaparib
Talazopaib
pKAP1 S824
KAP1
pChk1 S345
Chk1
β-actin
B.
OVCAR8
HT1080
γH2AX positive cells (%)
p < 0.01
p < 0.01
p < 0.01
p < 0.01
Vehicle
Olaparib
Talazoparib
Normoxia
Hypoxia
C.
OVCAR8
HT1080
p-DNAPK Thr2609 positive cells (%)
p < 0.01
p < 0.0001
p < 0.01
p < 0.001
Vehicle
Olaparib
Talazoparib
Normoxia
Hypoxia
D.
OVCAR8
HT1080
Normoxia
Hypoxia
2%
44%
45%
3.8%
14%
13.4%
0.2%
39%
45%
0.4%
13%
17%
Number of 53BP1 foci / Cell
Vehicle
Olaparib
Talazoparib
E.
Fold change in gene expression
21% Oxygen
2% Oxygen
BRCA1
BRCA2
RAD51
F.
% HR Repair
SUM149
MDA231

Figure S3. Related to Figure 3.

(A) Immunoblots of DDR proteins in OVCAR8 cells treated with vehicle or PARPi for 48 h in normoxia or hypoxia.

(B) Percentage of OVCAR8 and HT1080 cells with more than 10 γH2AX foci per nucleus after 48 h of PARPi treatment in normoxic or hypoxic culture conditions. *p* value calculated by one-way ANOVA, drug treatments vs vehicle (*n*=3).

(C) Average number of pDNA-PKcs (T2609) foci per nucleus in OVCAR8 and HT1080 cells. *p* value calculated by one-way ANOVA, drug treatments vs vehicle (*n*=3).

(D) Number of total 53BP1 foci per nucleus after 48 h of PARPi treatment, average number of foci per cell (red line), percentage of cells with more than 15 foci (blue box).

(E) Hypoxia does not reverse HR status in HR deficient cells. Quantitative real-time PCR (qRT-PCR) measuring BRCA1, BRCA2 and RAD51 mRNA levels in SUM149 cells incubated under different oxygen tensions. Measurements were normalized to 18*S* mRNA levels and expressed as fold change compared to normoxia (*n*=3 per condition).

(F) Detection of HR activity in the HR proficient MDA231 cells (*n*=3) but not HR deficient SUM149 cells (*n*=5) as measured by the TLR system.

Data represented as means ± SEM.

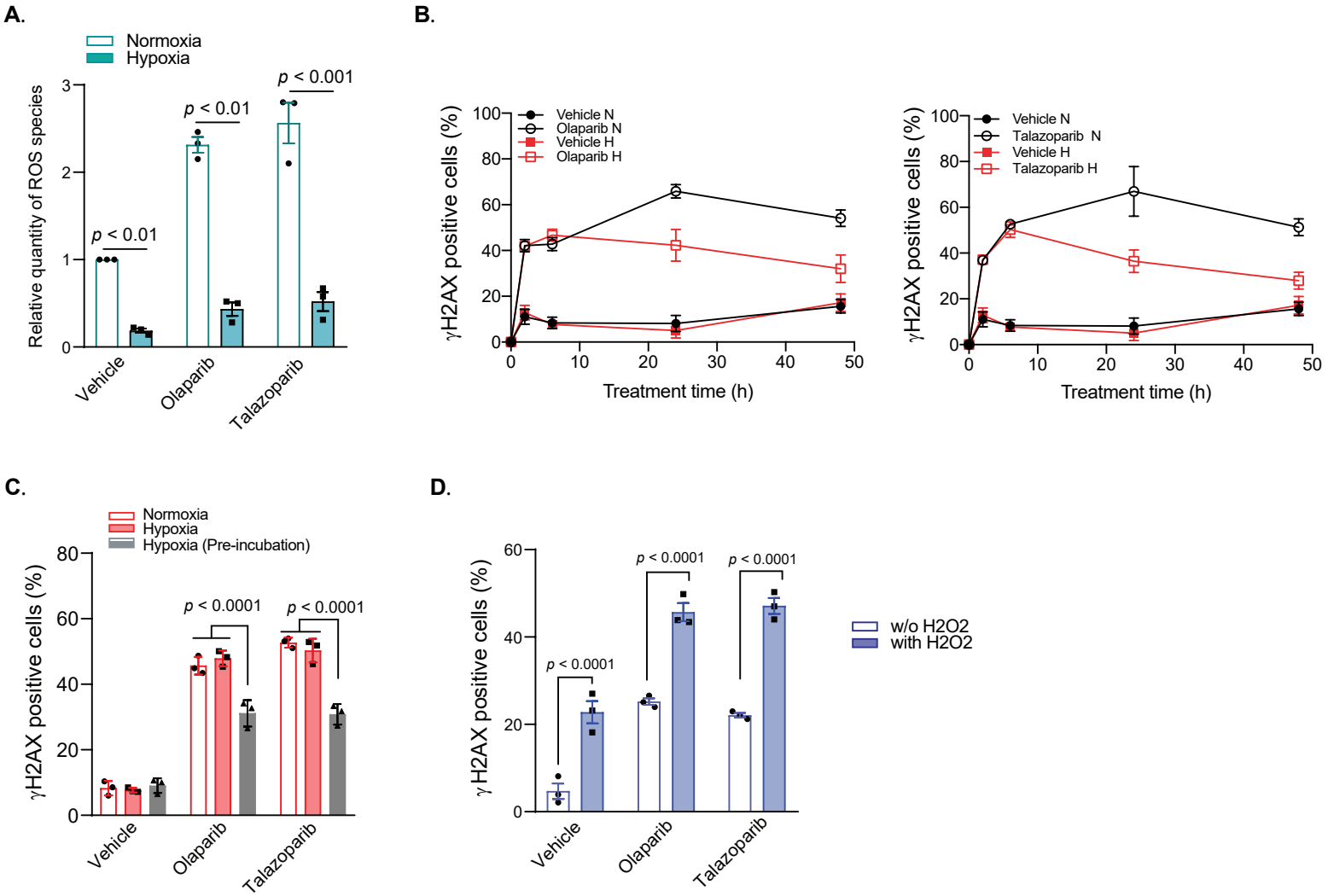

A.
Normoxia
Hypoxia
Relative quantity of ROS species
p < 0.01
p < 0.01
p < 0.001
Vehicle
Olaparib
Talazoparib
B.
γH2AX positive cells (%)
Vehicle N
Olaparib N
Vehicle H
Olaparib H
Treatment time (h)
γH2AX positive cells (%)
Vehicle N
Talazoparib N
Vehicle H
Talazoparib H
Treatment time (h)
C.
Normoxia
Hypoxia
Hypoxia (Pre-incubation)
γH2AX positive cells (%)
p < 0.0001
p < 0.0001
Vehicle
Olaparib
Talazoparib
D.
γH2AX positive cells (%)
p < 0.0001
p < 0.0001
p < 0.0001
w/o H2O2
with H2O2
Vehicle
Olaparib
Talazoparib

Figure S4. Related to Figure 4.

(A) Relative quantity of ROS species (detected by DCF) produced upon treatment of cells with PAPRi for 48h under normoxia or hypoxia. *p* value calculated by one-way ANOVA, *n*=3.

(B) Percentage of HT1080 cells with more than 10 γH2AX foci per nucleus after 2h, 6h, 24h and 48h of PARPi treatment in normoxic or hypoxic culture conditions, *n*=3 for each condition.

(C) Percentage of HT1080 cells with more than 10 γH2AX foci per nucleus after 6h PARPi treatment in normoxic, hypoxic culture conditions and after 48h preincubation in hypoxia showing decreased staining in cell preincubated in hypoxia. *p* value calculated by one-way ANOVA, *n*=3.

(D) Percentage of HT1080 cells with more than 10 γH2AX foci per nucleus after 48h of PARPi treatment in hypoxic culture conditions followed by 2h treatment with 50 μM H_2O_2. *p* values determined by two-way ANOVA, *n*=3.

Data represented as means ± SEM (represented by error bars).

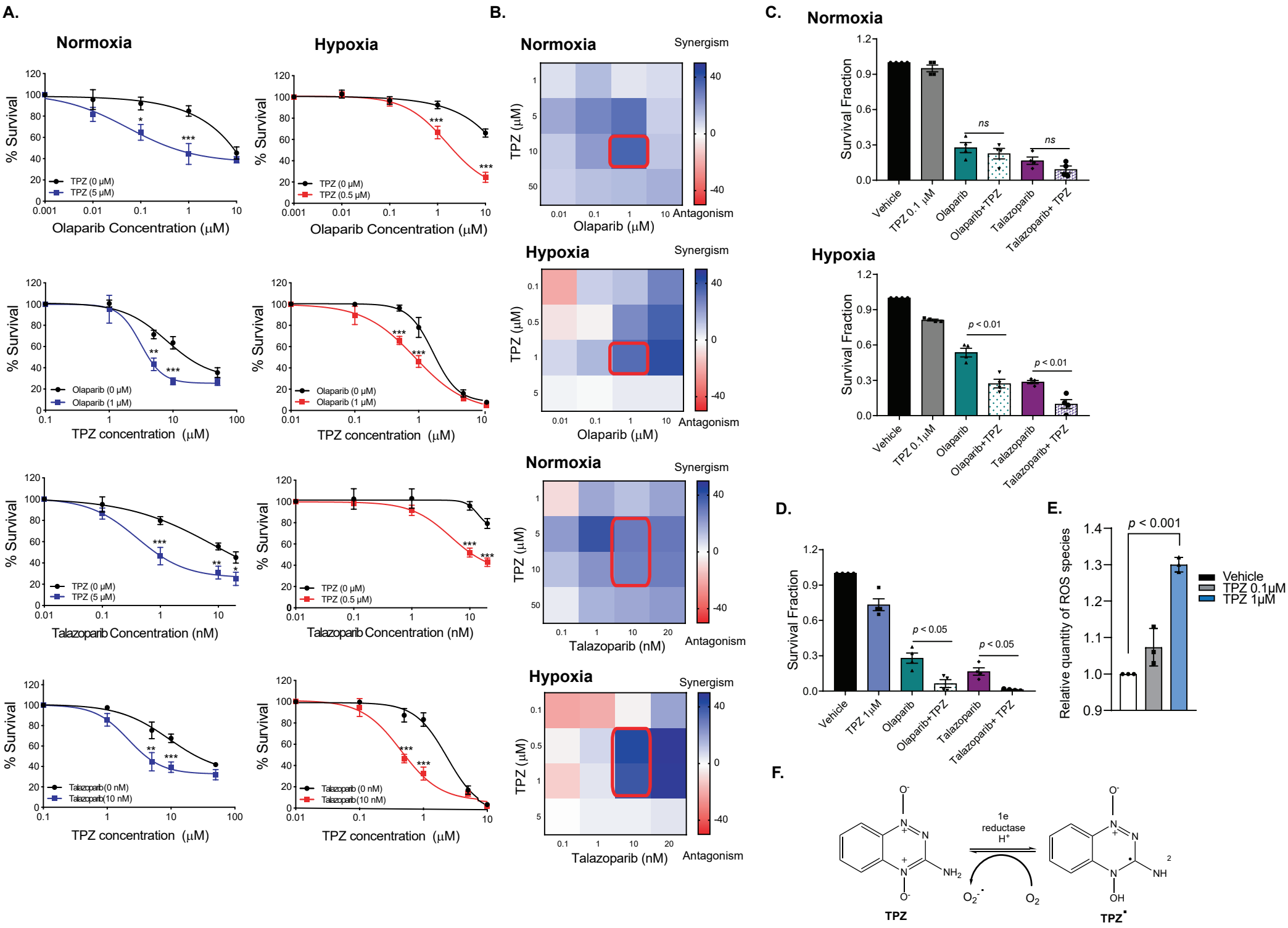

A.
Normoxia
Hypoxia
% Survival
TPZ (0 μM)
TPZ (5 μM)
TPZ (0.5 μM)
Olaparib Concentration (μM)
Olaparib (0 μM)
Olaparib (1 μM)
TPZ concentration (μM)
Talazoparib (0 nM)
Talazoparib (10 nM)
Talazoparib Concentration (nM)
B.
Normoxia
Hypoxia
Synergism
Antagonism
TPZ (μM)
Olaparib (μM)
Talazoparib (nM)
C.
Normoxia
Hypoxia
Survival Fraction
Vehicle
TPZ 0.1 μM
Olaparib
Olaparib+TPZ
Talazoparib
Talazoparib+ TPZ
ns
p < 0.01
D.
Survival Fraction
TPZ 1μM
p < 0.05
E.
Relative quantity of ROS species
p < 0.001
Vehicle
TPZ 0.1μM
TPZ 1μM
F.
1e
reductase
H+
TPZ
TPZ•

Figure S5. Related to Figures 6 & 7.

A) Cell survival of OVCAR8 cells treated with Olaparib (0.001-10 μM) and TPZ (0.1-50 μM) or Talazoparib (0.01-20nM) and TPZ (0.1-50 μM). Cells were treated for 4 days with the indicated drug combinations in normoxia or hypoxia, followed by SRB assay to determine cytotoxicity, *p* determined by *t*-tests, combination treatments vs single treatments, *n*=3 per condition.

(B) HSA synergism analysis of OVCAR8 cells treated with varying doses of TPZ and Olaparib or TPZ and Talazoparib.

(C) Colony formation of OVCAR8 cells. Cells were treated for 96 hours with TPZ (0.1 μM) and Olaparib (1 μM) or Talazoparib (10 nM) in normoxia or hypoxia. Results expressed as survival ratio relative to vehicle treated groups. *p* value calculated by one-way ANOVA, drug treatments vs vehicle (*n*=4).

(D) Colony formation of OVCAR8 cells treated for 96 hours with with TPZ (1 μM) and Olaparib (1 μM) or Talazoparib (10 nM) in normoxia or hypoxia, *p* value calculated by *t*-tests, combination treatments vs single treatments (*n*=4).

(E) Relative levels of ROS species produced upon treatment of cells with 0.1 μM and 1 μM of TPZ for 96 h, *p* value calculated by one-way ANOVA, TPZ treatment versus vehicle (*n*=3).

(F) Schematic diagram of the mechanism of action of Tirapazamine (Adapted from Siim et al., 2004).

Data represented as means ± SEM (represented by error bars).

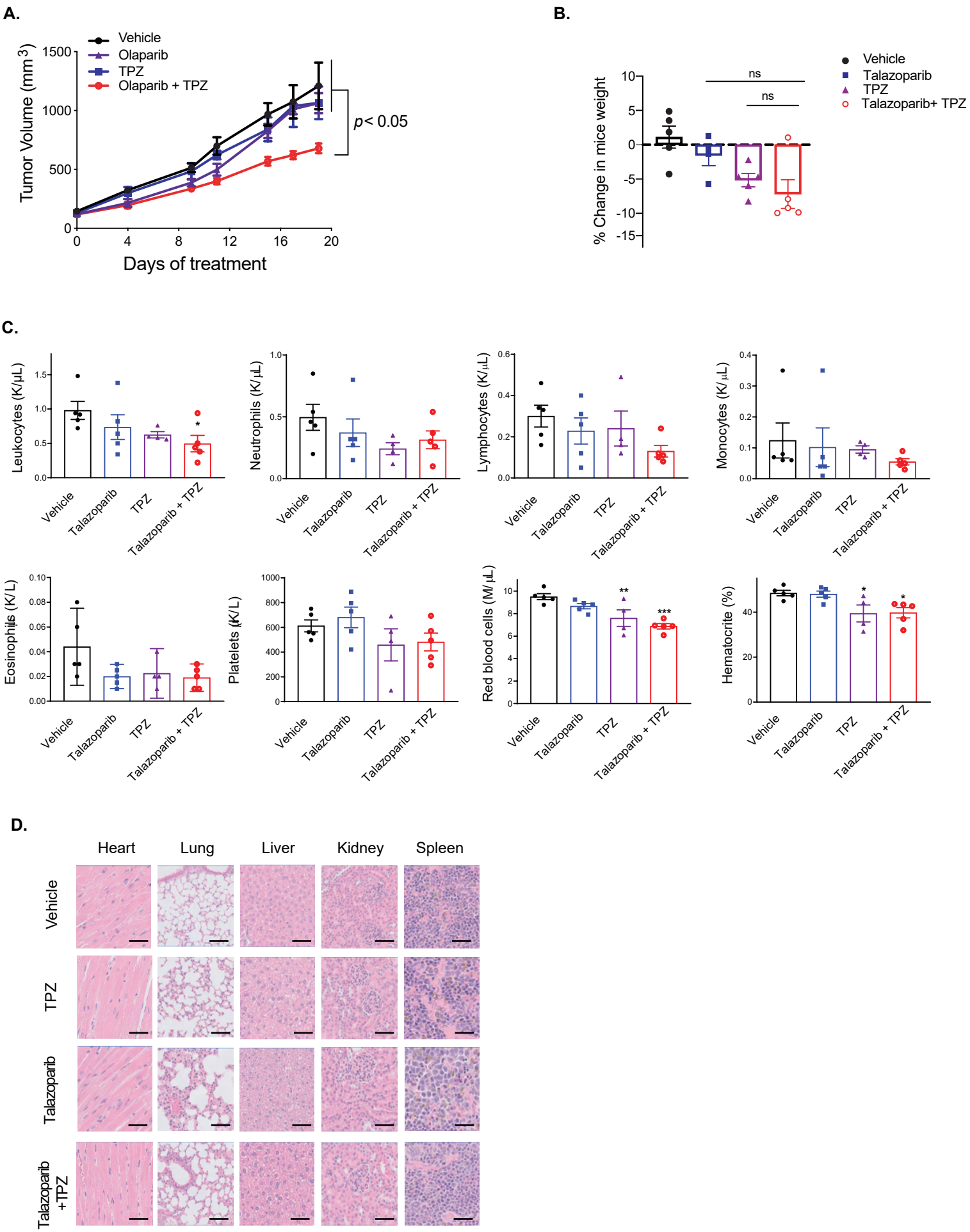

A.
Vehicle
Olaparib
TPZ
Olaparib + TPZ
Tumor Volume (mm3)
Days of treatment
p< 0.05
B.
Vehicle
Talazoparib
TPZ
Talazoparib+ TPZ
% Change in mice weight
ns
ns
C.
Leukocytes (K/µL)
Neutrophils (K/µL)
Lymphocytes (K/µL)
Monocytes (K/µL)
Eosinophils (K/L)
Platelets (K/L)
Red blood cells (M/µL)
Hematocrite (%)
Vehicle
Talazoparib
TPZ
Talazoparib + TPZ
D.
Heart
Lung
Liver
Kidney
Spleen
Vehicle
TPZ
Talazoparib
Talazoparib +TPZ

Figure S6. Related to Figure 8.

(A) **HT1080 xenograft model**. Growth curves of HT1080 xenograft tumors in control mice and mice treated with Olaparib 50mg/kg and/or TPZ 20mg/kg (left panel), (two-way ANOVA: vehicle vs combination interaction p <0.0001, Olaparib vs combination interaction p <0.0001; TPZ vs combination interaction p = 0.04, n = 5 mice per group)). Data are presented as mean ± SEM.

(B) Percentage weight loss in mice treated with vehicle, BMN673, TPZ or combination of TPZ and BMN673 (n = 5 mice per group). Data are presented as mean ± SEM.

(C) Plot of Leukocytes, white blood cell (WBC), red blood cell (RBC), platelet count, and hematocrit levels in mice with SUM149 allograft treated with vehicle, BMN673 0.1mg/kg, TPZ 20 mg/kg, or combination of BMN673 and TPZ (n = 5 mice in each group). *p < 0.05, by one-way ANOVA, drug treatment vs vehicle.

Data are presented as mean ± SEM across all panels.

(D) Multiple organs were analyzed for histological signs of toxicity. Representative images are shown from each treatment group. Scale bars 50 μm.

Table S1

Primers for QPCR

Gene		Sequence (5' –> 3')
BRCA1	Forward Primer	GAAACCGTGCCAAAAGACTTC
	Reverse Primer	CCAAGGTTAGAGAGTTGGACAC
BRCA2	Forward Primer	CACCCACCCTTAGTTCTACTGT
	Reverse Primer	CCAATGTGGTCTTTGCAGCTAT
RAD51	Forward Primer	CAACCCATTTCACGGTTAGAGC
	Reverse Primer	TTCTTTGGCGCATAGGCAACA
Polθ	Forward Primer	CTTGGCGGCAACTTCTACTC
	Reverse Primer	AGCTGCGAACAGGCTTTAGA

Target sequences for shRNAs and Cas9-CRISPER

Gene	Target Sequence
HIF1α	GTCTAGAGATGCAGCAAGA
HIF2α	AGGTGGAGCTAACAGGACATA
HIFβ	AAATAAACCATCTGACTTCTC
Polθ Target	GATTCGTTCTCGGGAAGCGG