Supplemental Data

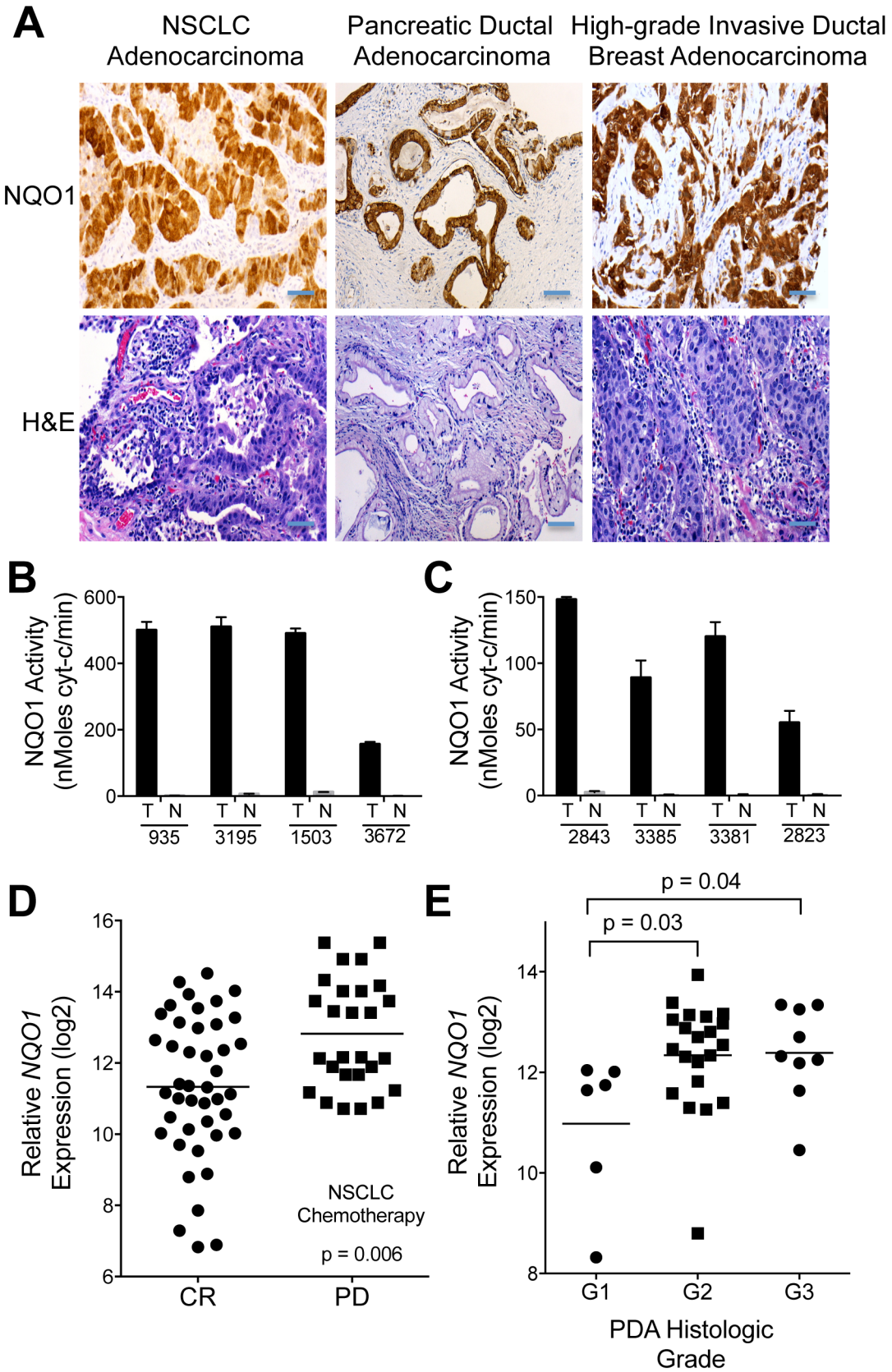


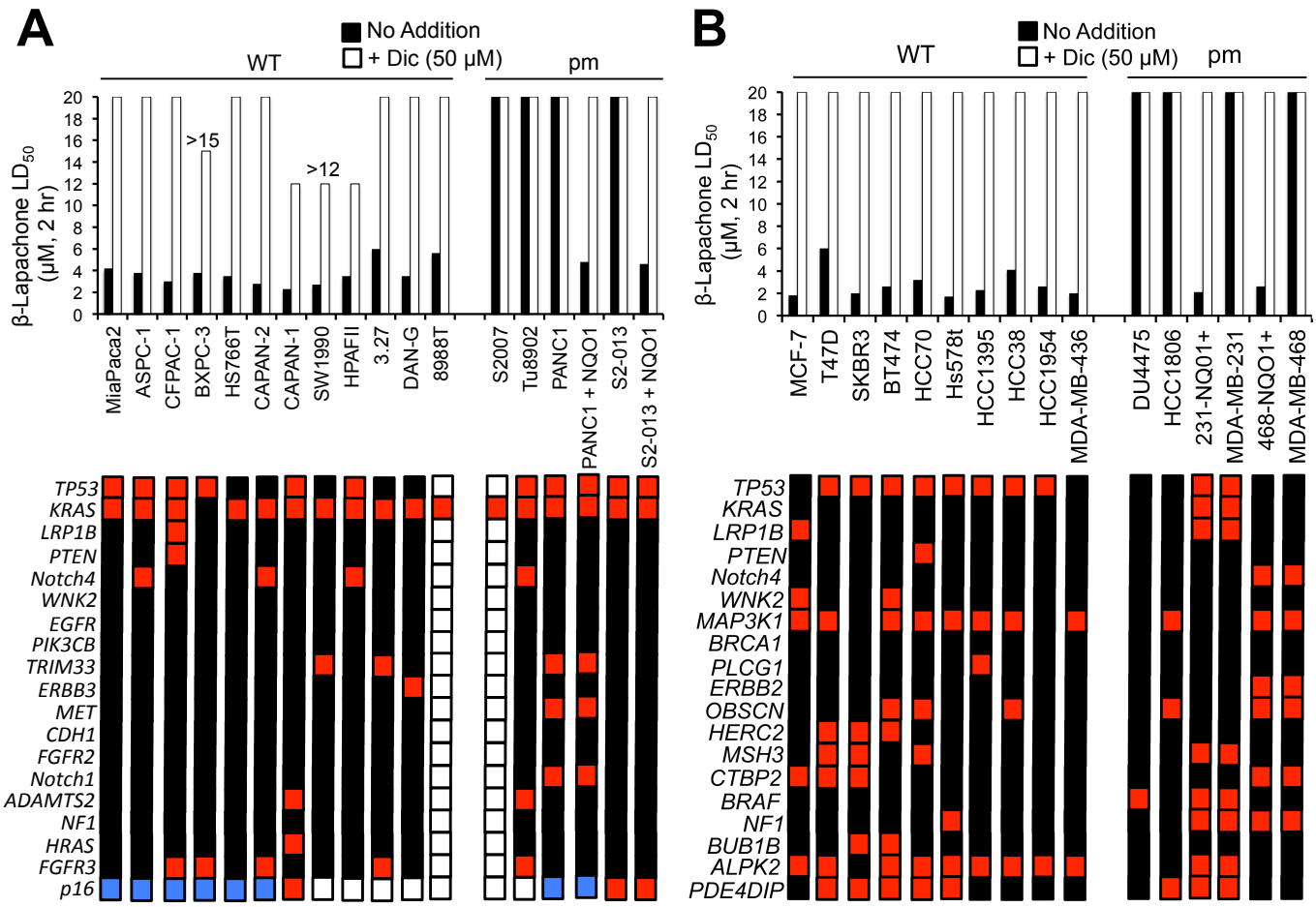
Figure S1, Related to Figure 1：NQO1 is elevated in recalcitrant non-small cell lung cancer (NSCLC), pancreatic ductal adenocarcinoma (PDA) and high grade invasive breast adenocarcinoma, correlates with resistance to therapy and is elevated in recalcitrant and high-grade tumors.

(A) Examples of immunohistochemical (IHC) staining of NQO1 levels and H&E in NSCLC, PDA, and high-grade breast adenocarcinomas. IHC staining, scale bar = 25 μm； H&E staining, scale bar =10 μm.

(B, C) NQO1 activities from NSCLC patient tumor (**T**) *versus* associated normal lung (**N**) tissues from the same de-identified patient samples in **Figure 1G**. Data represent means ± SEM of ten patient samples.

(D) *NQO1* mRNA expression data from NSCLC patients before chemotherapy were divided into two groups, those: who experienced a clinical response (CR) (i); and who exhibited progressive disease (PD), despite treatment (ii). Mean *NQO1* mRNA expression was higher in patients that did not respond to chemotherapy ($p \leq 0.006$). Data presented are individual patient samples with lines indicative of average values for data points, that give highest and lowest data per condition.

(E) *NQO1* expression in PDA groups based on histologic grade showing higher *NQO1* mRNA expression in grade 2 ($p \leq 0.03$) or grade 3 ($p \leq 0.04$) PanIN, compared to grade 1 lesions. Data presented are individual patient samples for G1, G2 and G3 PDA histological grades of pancreatic cancers, with lines indicative of average values for data points that give highest and lowest data per condition.


A
No Addition
+ Dic (50 μM)
WT
pm
β-Lapachone LD50 (μM, 2 hr)
>15
>12
MiaPaca2
ASPC-1
CFPAC-1
BXPC-3
HS766T
CAPAN-2
CAPAN-1
SW1990
HPAFII
3.27
DAN-G
8988T
S2007
Tu8902
PANC1
PANC1 + NQO1
S2-013
S2-013 + NQO1
TP53
KRAS
LRP1B
PTEN
Notch4
WNK2
EGFR
PIK3CB
TRIM33
ERBB3
MET
CDH1
FGFR2
Notch1
ADAMTS2
NF1
HRAS
FGFR3
p16
B
No Addition
+ Dic (50 μM)
WT
pm
β-Lapachone LD50 (μM, 2 hr)
MCF-7
T47D
SKBR3
BT474
HCC70
Hs578t
HCC1395
HCC38
HCC1954
MDA-MB-436
DU4475
HCC1806
231-NQ01+
MDA-MB-231
468-NQO1+
MDA-MB-468
TP53
KRAS
LRP1B
PTEN
Notch4
WNK2
MAP3K1
BRCA1
PLCG1
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OBSCN
HERC2
MSH3
CTBP2
BRAF
NF1
BUB1B
ALPK2
PDE4DIP

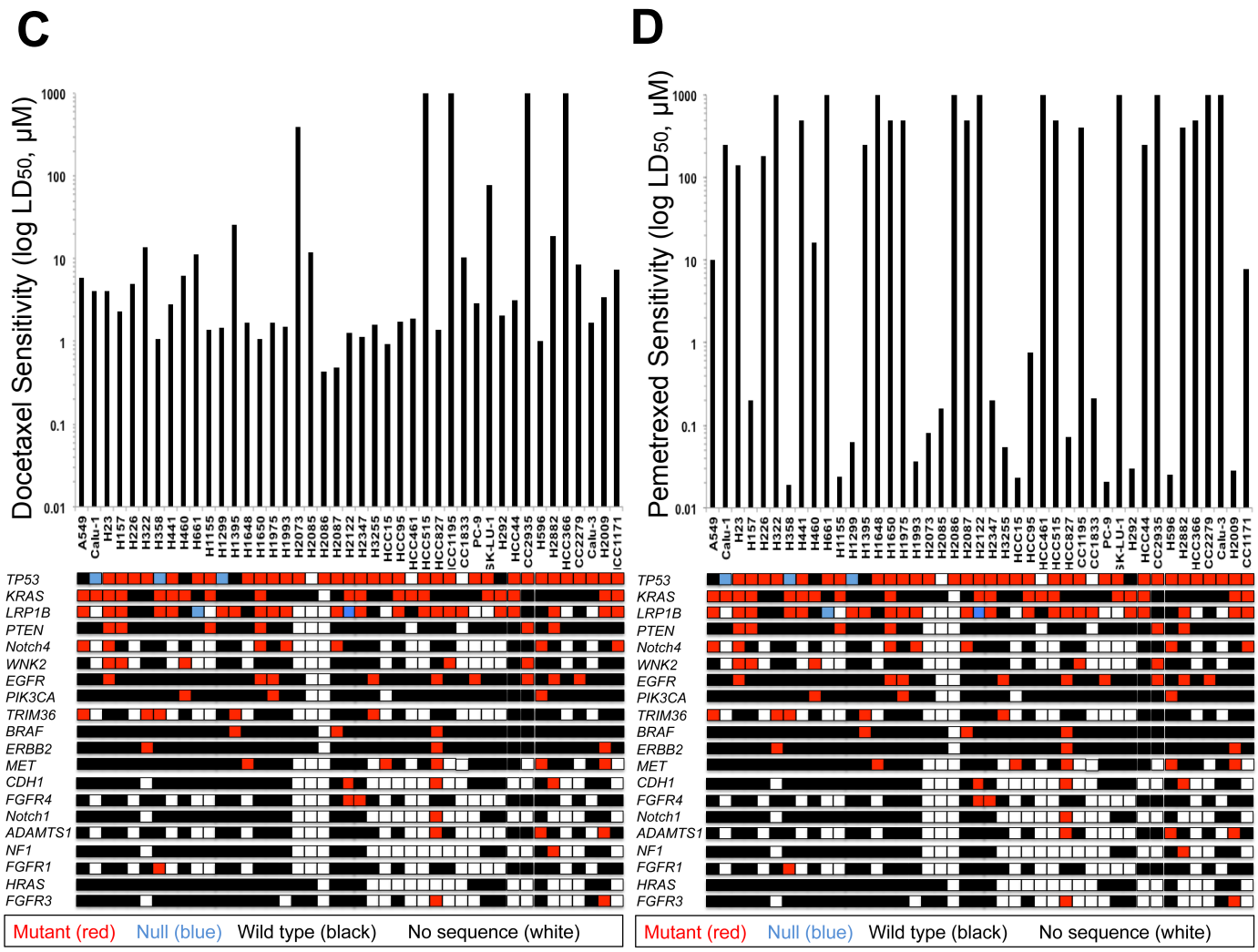


Figure S2, Related to Figure 2: β-Lap-induced lethality of human PDA and breast cancer cell lines is NQO1-dependent, independent of oncogenic driver or passenger mutations and differs from NSCLC cell line responses to docetaxel or pemetrexed.

(A,B) Human pancreatic adenocarcinoma (PDA **(A)** or breast cancer **(B)** cells were exposed to varying concentrations of β-lap (0-20 µM, or as indicated, closed bars), with or without dicoumarol (DIC, 50 µM, open bars) for 2 hr, and survival was determined by 7-day DNA content assays. In a double blind manner, *NQO1* polymorphism (pm) status was determined using assays specific for *2 or *3 *NQO1* SNPs as in **Figure 2**. Genomic sequencing was performed on all cell lines, revealing a limited number of mutations as illustrated in **Figure 2**. To obtain mutation information, 'maf files' were downloaded from http://www.broadinstitute.org/ccle/home. Processing was done using custom scripts run in Python v2.6.6. Only mutations flagged as "Start Codon", "Stop Codon", "Frame", "Missense" or "Nonsense" were considered for further analyses.

(C,D) Human NSCLC cell lines were exposed to continuous doses of docetaxel **(C)** or pemetrexed **(D)** as indicated. Survival was then determined and lethality expressed by reporting LD_{50} values (µM). Gene mutations reported are identical to those for β-lap responses (**Figure 2**). Note uniform LD_{50} values for β-lap against various NSCLC cells **(Figure 2)** vs docetaxel or pemetrexed (**Figures S2C, S2D**).

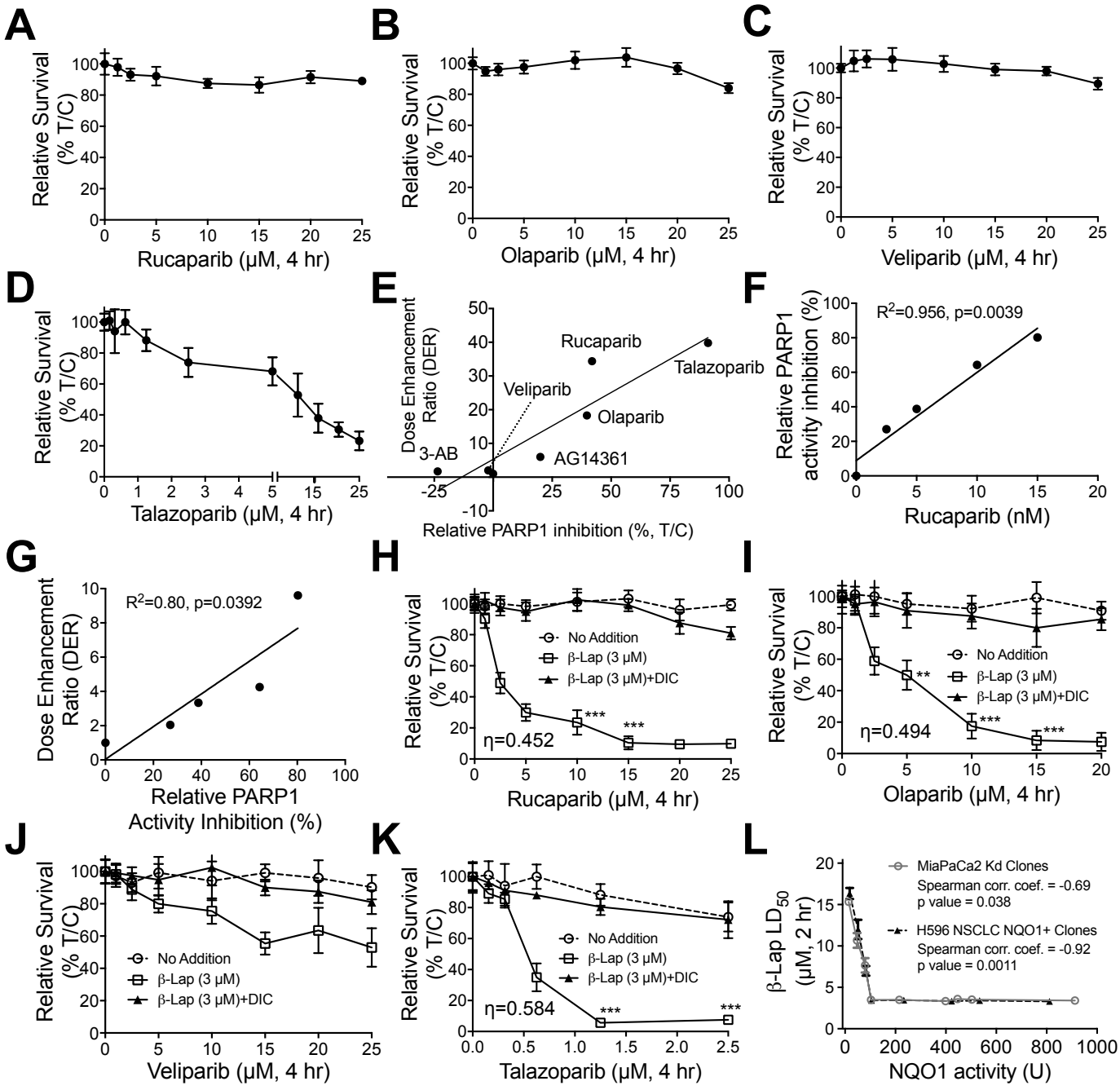
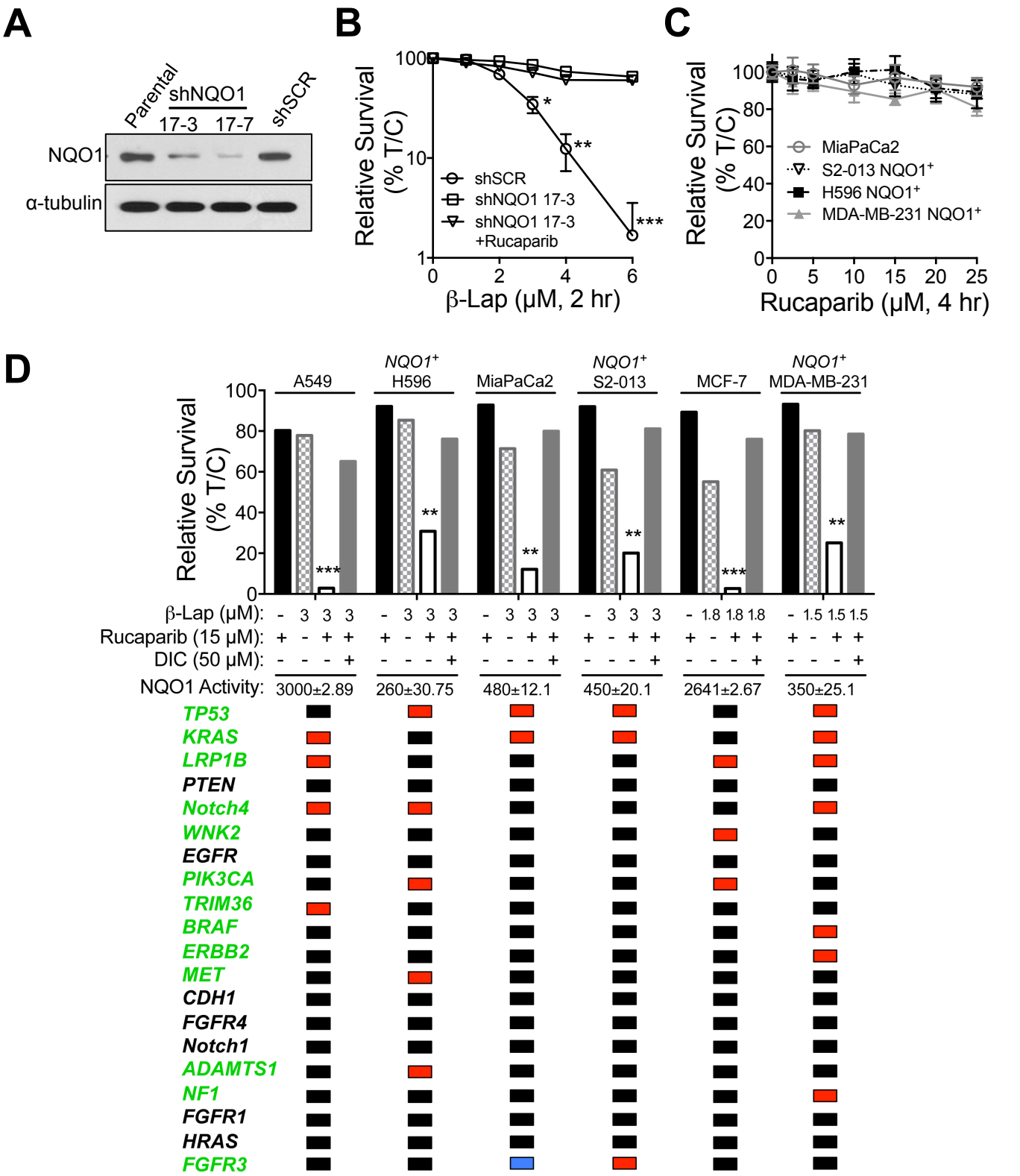


Figure S3, Related to Figure 3: Lethalities of the four most potent PARP inhibitors alone against A549 NSCLC cells, with PARP inhibitor dose-response studies in combination with the NQO1 bioactivatable drug, β-lapachone (β-lap).

(A-D) Log-phase A549 NSCLC cells were exposed to various concentrations (μM) of: Rucaparib **(A)**; Olaparib **(B)**; Veliparib **(C)**; or Talazoparib **(D)** for 4 hr (representing 2 hr pre-treatment and 2 hr co-treatments used in combination with β-lap).

(E) Survey of relative PARP1 activity inhibition potential of indicated PARP inhibitors (10 nM) using purified recombinant Flag-tagged PARP1. Inhibition potential of each PARP inhibitor directly correlated with their ability to enhance β-lap lethality (DER), p=0.004, R^2=0.83. See Supplemental methods for more details.

(F) Relative PARP1 inhibition was assessed for Rucaparib using recombinant PARP1 protein and standard PAR formation enzyme assays.
(G) Dose enhancement ratios (DERs) at indicated concentrations of Rucaparib correlate with Rucaparib PARP1 activity inhibition at different concentrations in a cell-free PARP1 activity assay. See Supplemental methods for more details.
(H-K) A549 NSCLC cells were pretreated with or without various doses (μM) of Rucaparib **(H)**; Olaparib **(I)** ; Veliparib **(J)**; and Talazoparib **(K),** as indicated for 2 hr. Cells were then exposed or not to each PARP inhibitor (μM) + a sublethal dose of β-lap (3 μM for A549 cells) for 2 hr. Survival was then assessed using 7-day relative survival DNA content assays.
(L) Endogenous *NQO1*$^{+}$ MiaPaCa2 cells were knocked down for NQO1 expression using stable shRNA-NQO1 retroviral expression Simultaneously, *2 homopolymorphic H596 NSCLC cells were transfected with a *CMV-NQO1* mammalian expression vector. *NQO1*$^{+}$ cells were *NQO1*$^{-}$ transfected with *CMV-NQO1* for expression.
All error bars are means ± SEM from three experiments. Student's t tests were performed. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.
Synergy values (eta, η) were reported based on multiple dose-responses or on comparative p values indicated.


A
Parental
shNQO1
17-3
17-7
shSCR
NQO1
α-tubulin
B
Relative Survival (% T/C)
100
10
1
shSCR
shNQO1 17-3
shNQO1 17-3 +Rucaparib
*
**
***
0
2
4
6
β-Lap (μM, 2 hr)
C
Relative Survival (% T/C)
100
80
60
40
20
0
MiaPaCa2
S2-013 NQO1+
H596 NQO1+
MDA-MB-231 NQO1+
0
5
10
15
20
25
Rucaparib (μM, 4 hr)
D
Relative Survival (% T/C)
A549
NQO1+ H596
MiaPaCa2
NQO1+ S2-013
MCF-7
NQO1+ MDA-MB-231
β-Lap (μM): - 3 3 3 - 3 3 3 - 3 3 3 - 3 3 3 - 1.8 1.8 1.8 - 1.5 1.5 1.5
Rucaparib (15 μM): + - + + + - + + + - + + + - + + + - + + + - + +
DIC (50 μM): - - - + - - - + - - - + - - - + - - - + - - - +
NQO1 Activity: 3000±2.89 260±30.75 480±12.1 450±20.1 2641±2.67 350±25.1
TP53
KRAS
LRP1B
PTEN
Notch4
WNK2
EGFR
PIK3CA
TRIM36
BRAF
ERBB2
MET
CDH1
FGFR4
Notch1
ADAMTS1
NF1
FGFR1
HRAS
FGFR3

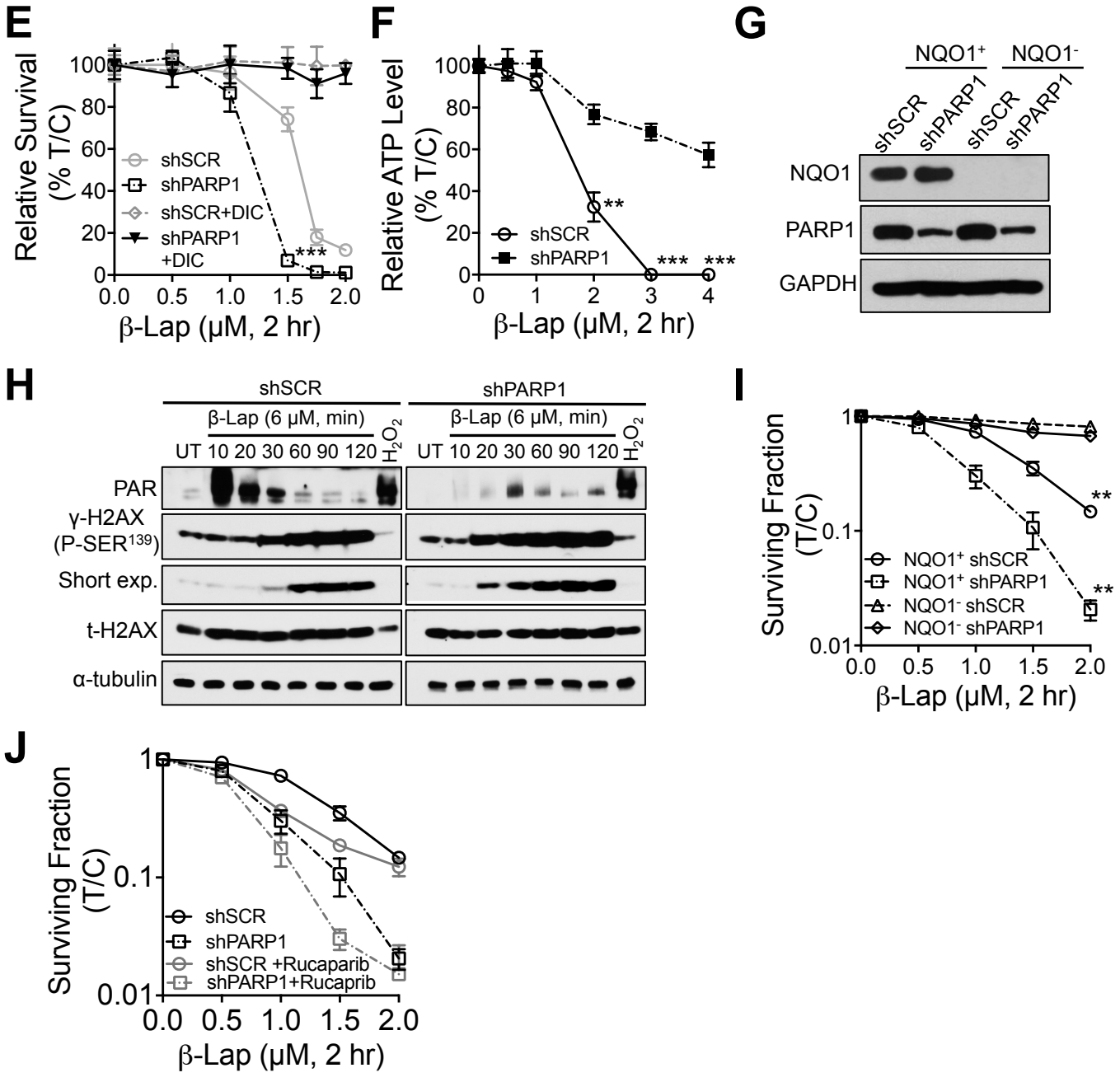

E
Relative Survival (% T/C)
shSCR
shPARP1
shSCR+DIC
shPARP1 +DIC
***
β-Lap (μM, 2 hr)
F
Relative ATP Level (% T/C)
shSCR
shPARP1
**
***
***
β-Lap (μM, 2 hr)
G
NQO1+
NQO1-
shSCR
shPARP1
shSCR
shPARP1
NQO1
PARP1
GAPDH
H
shSCR
shPARP1
β-Lap (6 μM, min)
UT 10 20 30 60 90 120 H2O2
PAR
γ-H2AX (P-SER139)
Short exp.
t-H2AX
α-tubulin
I
Surviving Fraction (T/C)
NQO1+ shSCR
NQO1+ shPARP1
NQO1- shSCR
NQO1- shPARP1
**
**
β-Lap (μM, 2 hr)
J
Surviving Fraction (T/C)
shSCR
shPARP1
shSCR +Rucaparib
shPARP1+Rucaprib
β-Lap (μM, 2 hr)

Figure S4, Related to Figures 4, 5: NQO1 expression loss results in resistance to β-lapachone, with or without PARP inhibitors and stable PARP1 knockdown sensitizes MDA-MB-231 breast cancer cells to β-lap in an NQO1-dependent manner.

(A) MiaPaCa2 cells were knocked down for *NQO1* by stable shRNA-NQO1 lentiviral infection. Stable MiaPaCa2 knockdown clones (17-3 and 17-7) were isolated and analyzed for NQO1 expression. α-Tubulin was used for loading.

(B) Stable shSCR or shPARP knockdown 17-3 MiaPaCa2 cells were pretreated or not with Rucaparib (15 μM, 2 hr) and then exposed to Rucaparib (15 μM) in combination with varying doses of β-lap (0-6 μM) for 2 hr as indicated. Survival was then determined by 7-day DNA content assays.

(C) NQO1 overexpressing MiaPaCa2 PDA, Suit-2 (S2-013) PDA, H596 NSCLC, and MDA-MB-231 breast cancer cells were treated for 4 hr with varying concentrations of Rucaparib (μM) and survival was assessed using 7-day relative survival DNA content assays.

(D) A summary of the synergistic responses of non-small cell lung (NSCLC) (A549, *NQO1*$^+$ H596), PDA (MiaPaCa2, *NQO1*$^+$ Suit2 (S2-013)) and breast (MCF-7, *NQO1*$^+$ MDA-MB-231) cancer cells following Rucaparib + β-lapachone therapy as described in **Figure 4**. Examination of sequenced mutations of major oncogenic driver or passenger mutations indicates a clear independence of synergistic lethal activity of Rucaparib + β-lap with respect to mutations in these genes (indicated in green).

(E) Stable shSCR vector alone or shPARP1 knockdown MCF-7 cells were treated with various concentrations of β-lap (μM) in the presence or absence of dicoumarol (DIC, 50 μM) for 2 hr. Relative survival was then assessed.

(F) Stable shSCR vector alone or shPARP1 knockdown MCF-7 cells were treated with or without various doses (0-4 μM) of β-lap for 2 hr and relative ATP levels were assessed at the end of this treatment.

(G) Stable MDA-MB-231 cells harboring shSCR vector and shRNA-NQO1 knockdown were generated and knockdown confirmed by Western blotting. GAPDH is for loading control.

(H) shSCR or shPARP1 knockdown MDA-MB-231 cells were exposed or not to β-lap (6 μM) and whole cell lysates prepared at times indicated. Samples were interrogated for PAR formation, γH2AX vs total-H2AX (t-H2AX) and α-tubulin for loading. Cells were also treated with 500 μM H_2O_2 for 15 min as the positive control.

(I) Stable shSCR vs shPARP1 knockdown MDA-MB-231 cells expressing or lacking NQO1 were exposed to β-lap at various concentrations (μM) for 2 hr and surviving fractions assessed by colony forming ability assays.

(J) MDA-MB-231-*NQO1*$^+$ Cells were pretreated with or without 15 μM Rucaparib for 2 hr, then exposed or not to Rucaparib (15 μM) + various β-lap concentrations (μM) as indicated for 2 hr. Cells were then assessed for survival using colony forming assays as in **(I)**.

All error bars are means ± SEM from three experiments. Student's t tests were performed. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.

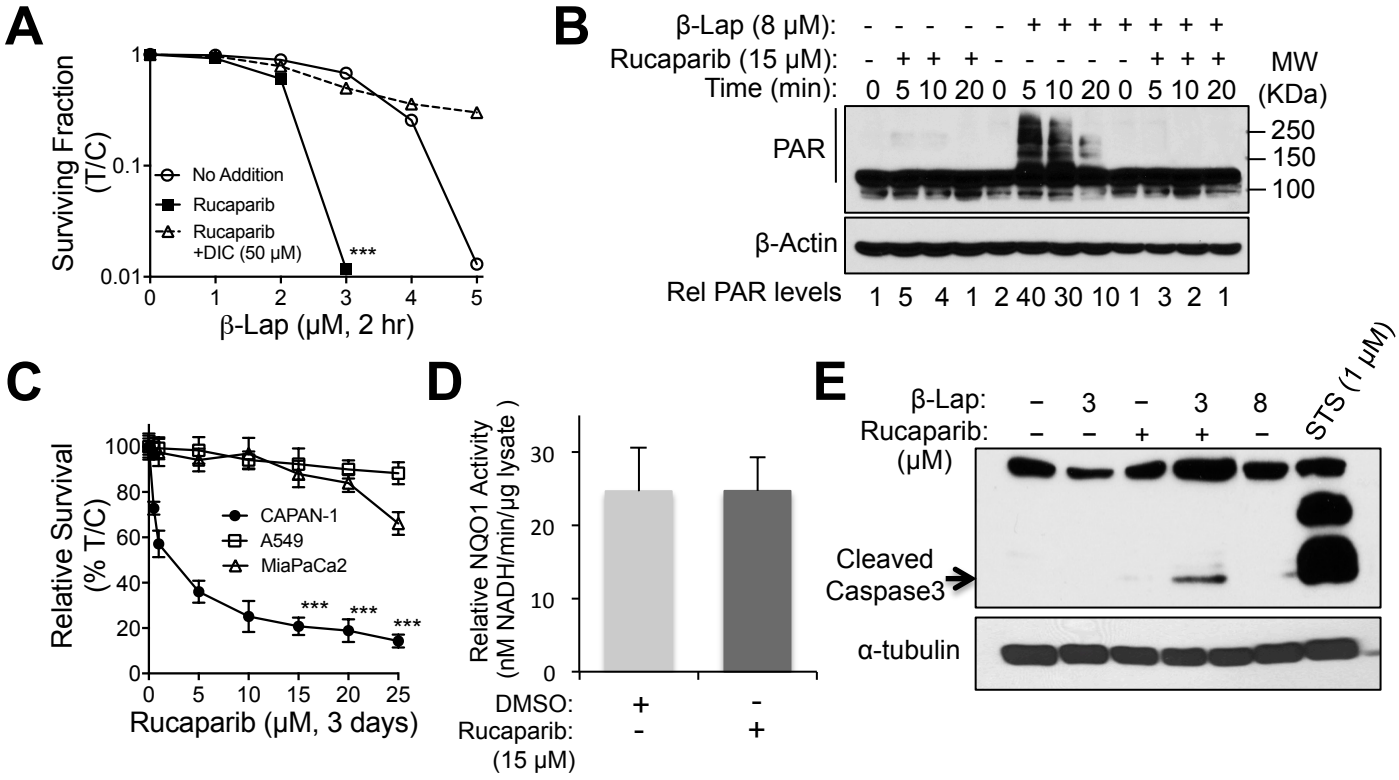


Figure S5, Related to Figures 6-7: β-Lap-induced PARP1 hyperactivation in NSCLC cells was blocked by PARP inhibition via Rucaparib.

(A) A549 NSCLC cells were pretreated with or without Rucaparib (15 μM, 2 hr), then exposed or not to β-lap at various doses, with or without dicoumarol (DIC, 50 μM) for 2 hr. Survival by colony forming assays were then assessed.

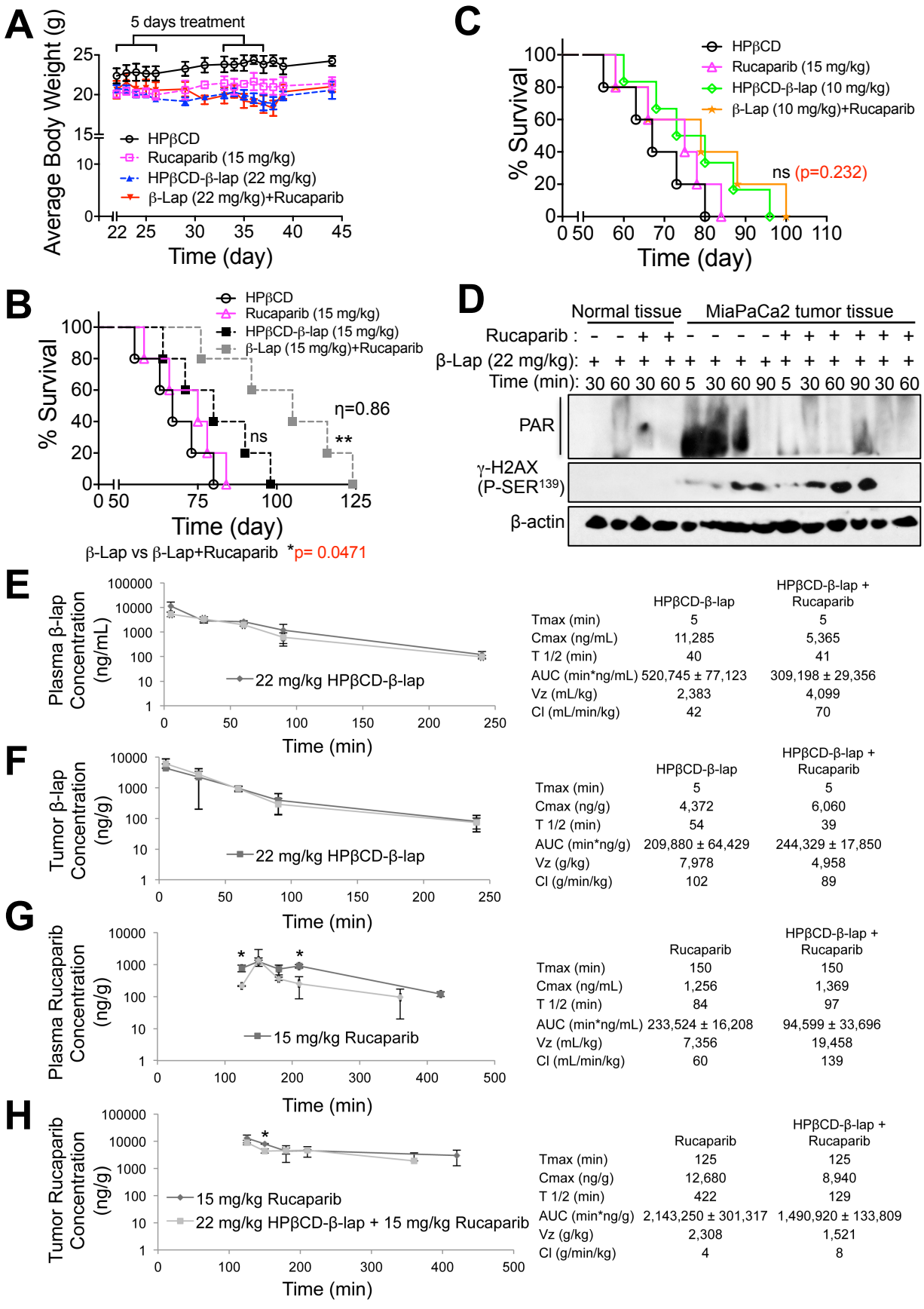
(B) A549 NSCLC cells were pretreated or not with Rucaparib (15 μM, 2 hr), then exposed to β-lap (8 μM) with or without Rucaparib (15 μM) and whole cell extracts prepared at various times (min) post-exposure as indicated. Extracts were then interrogated for PAR formation with β-actin as loading. Relative PAR-PARP1 protein levels were quantified using NIH Image J and normalized to β-actin are given.

(C) *BRCA2*-deficient CAPAN-1 cells were hypersensitive to Rucaparib. A549 NSCLC and MiaPaCa2 PDA cells, expressing wild-type *BRCA1/2* were resistant. ***, $p < 0.001$ for curve of CAPAN-1 with Rucaparib treatment vs A549 and MiaPaCa2 with Rucaparib treatment.

(D) NQO1 recycling assays were performed in which β-lap is substrate and cytochrome C is absent from the reaction.

(E) A549 NSCLC cells were pretreated with or without Rucaparib (15 μM, 2 hr) and then exposed or not to Rucaparib (15 μM) + β-lap (3 μM) or β-lap (8 μM) for 2 hr. Drugs were then removed and cells were then assayed for caspase 3 cleavage, a marker of apoptosis, 48 hr later. Staurosporine (STS, 1 μM), a universal apoptosis-inducer, served as a positive control for Caspase 3 activation, and α-tubulin as a loading control.

All error bars are means ± SEM from three experiments. Student's t tests were performed. ***, $p < 0.001$.



	HPβCD-β-lap	HPβCD-β-lap + Rucaparib
Tmax (min)	5	5
Cmax (ng/mL)	11,285	5,365
T 1/2 (min)	40	41
AUC (min*ng/mL)	520,745 ± 77,123	309,198 ± 29,356
Vz (mL/kg)	2,383	4,099
Cl (mL/min/kg)	42	70

	HPβCD-β-lap	HPβCD-β-lap + Rucaparib
Tmax (min)	5	5
Cmax (ng/g)	4,372	6,060
T 1/2 (min)	54	39
AUC (min*ng/g)	209,880 ± 64,429	244,329 ± 17,850
Vz (g/kg)	7,978	4,958
Cl (g/min/kg)	102	89

	Rucaparib	HPβCD-β-lap + Rucaparib
Tmax (min)	150	150
Cmax (ng/mL)	1,256	1,369
T 1/2 (min)	84	97
AUC (min*ng/mL)	233,524 ± 16,208	94,599 ± 33,696
Vz (mL/kg)	7,356	19,458
Cl (mL/min/kg)	60	139

	Rucaparib	HPβCD-β-lap + Rucaparib
Tmax (min)	125	125
Cmax (ng/g)	12,680	8,940
T 1/2 (min)	422	129
AUC (min*ng/g)	2,143,250 ± 301,317	1,490,920 ± 133,809
Vz (g/kg)	2,308	1,521
Cl (g/min/kg)	4	8

Figure S6, Related to Figure 8: Weight loss changes and antitumor activities of Rucaparib + β-lapachone treatments in PDA animal model and pharmacokinetics (PK) of HPβCD-β-lap and Rucaparib in plasma and MiaPaCa2 pancreatic tumors (PDA).

(A-D) MiaPaCa2 cells (1 X 10^6) were injected into the pancreatic tissue of NOD/SCID female 20-22 g mice and drug treatments began 21 days post-injection.

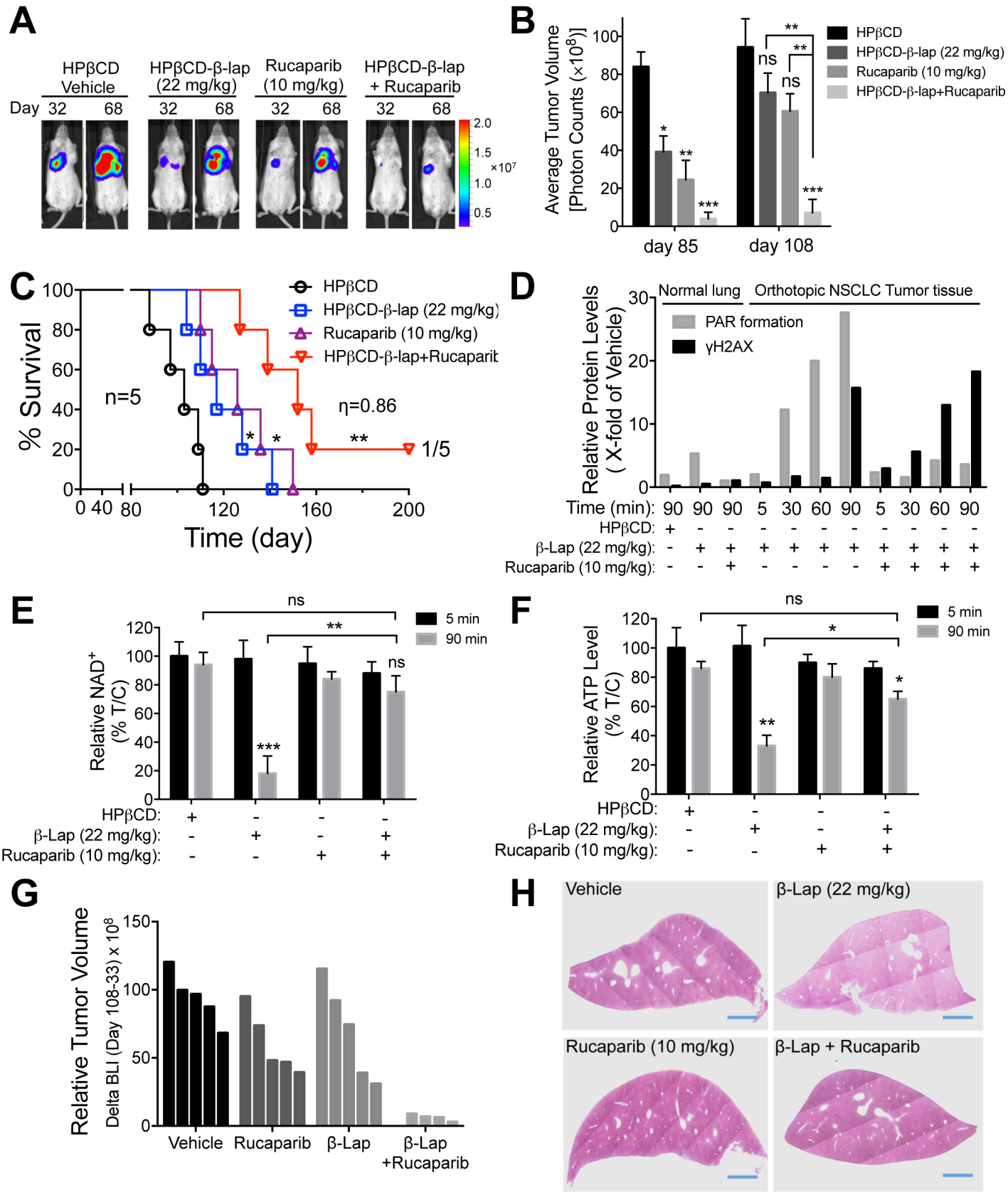
(A) Changes in weights were monitored over a 20 day period after exposure with HPβCD (vehicle), Rucaparib (15 mg/kg, ip), β-lapachone (22 mg/kg, iv), as well as 2 hr pretreatment with Rucaparib (15 mg/kg, ip), then with β-lapachone (β-lap, 22 mg/kg, iv) once every day for 5 injections. Mice were allowed seven days rest, then treated with 5 additional injections.

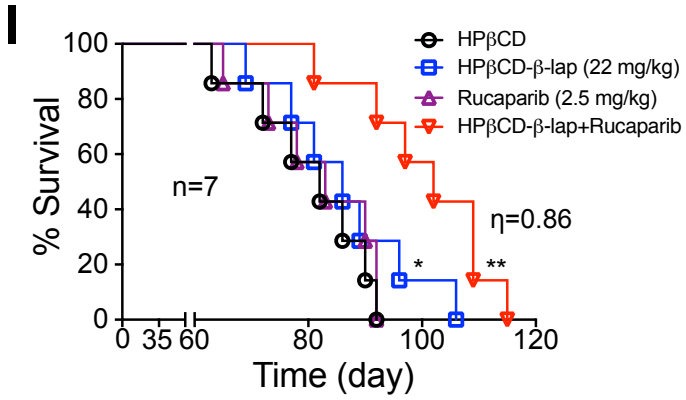
(B, C) Mice were pretreated 2 hr with 15 mg/kg Rucaparib (ip), then with 15 **(B)** or 10 **(C)** mg/kg β-lapachone (β-lap), iv. Overall survival was monitored. **(D)** Western blot analyses of tumor and normal tissue from orthotopic xenografts derived from MiaPaCa2 cells grown in female NOD/SCID mice treated with Rucaparib alone (15 mg/kg), β-lapachone alone (22 mg/kg) or the combination of Rucaparib + β-lapachone as in Figure 8D. Monitored are poly(ADP)-modified proteins (PAR), mostly PAR-PARP1, γH2AX as an indicator of DSB formation, and β-actin as a loading control.

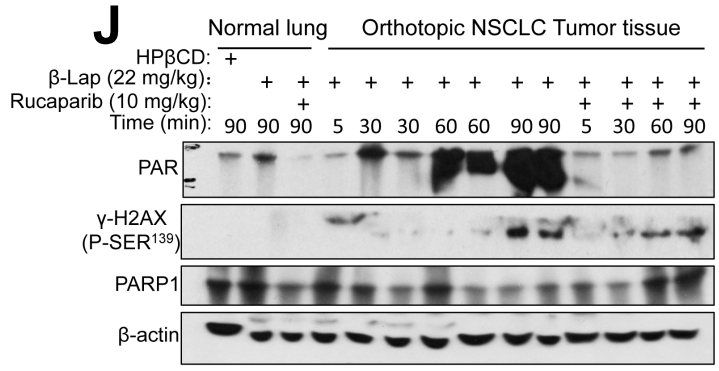
(E-H) Mice were dosed in combination with 15 mg/kg Rucaparib ip two hours prior to dosing with 22 mg/kg HPβCD-β-lap iv. At varying times after administration, the mice were sacrificed and whole blood and tumor tissues were harvested. Plasma was obtained from the whole blood by centrifugation and tumors were homogenized in PBS to generate a lysate. The plasma and tumor samples were then analyzed by LC-MS/MS. HPβCD-β-lap concentrations were measured in plasma **(E)** and tumor **(F)** over time for both types of treatment. Rucaparib concentrations were also measured in plasma **(G)** and tumor **(H)** for both types of treatment. Non-compartmental pharmacokinetic parameters are shown for maximum time (Tmax), maximum concentration (Cmax), half-life (T 1/2), area under the concentration time curve (AUC), volume of distribution (Vz), and clearance (Cl).

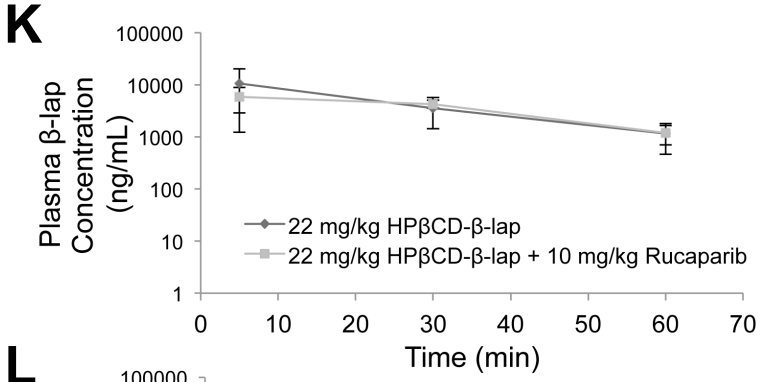
All error bars are means ± SD from three mice in each group. Student's t tests were performed. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

Synergy values (eta, η) were reported based on multiple dose-responses or on comparative p values indicated.

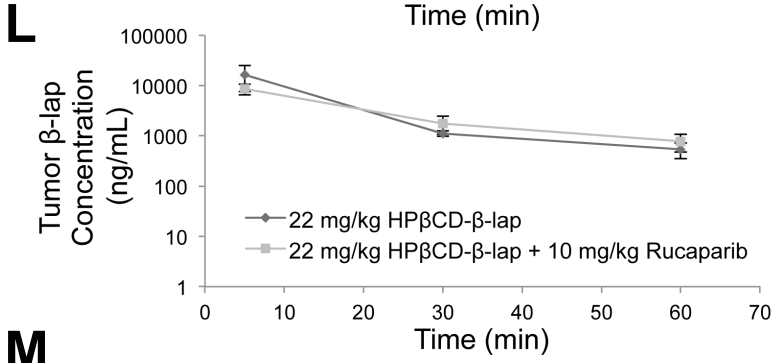

A
HPβCD Vehicle
HPβCD-β-lap (22 mg/kg)
Rucaparib (10 mg/kg)
HPβCD-β-lap + Rucaparib
Day 32 68
2.0
1.5
×10⁷
1.0
0.5
B
Average Tumor Volume [Photon Counts (×10⁸)]
HPβCD
HPβCD-β-lap (22 mg/kg)
Rucaparib (10 mg/kg)
HPβCD-β-lap+Rucaparib
ns
day 85
day 108
C
% Survival
HPβCD
HPβCD-β-lap (22 mg/kg)
Rucaparib (10 mg/kg)
HPβCD-β-lap+Rucaparib
n=5
η=0.86
1/5
Time (day)
D
Relative Protein Levels (X-fold of Vehicle)
Normal lung
Orthotopic NSCLC Tumor tissue
PAR formation
γH2AX
Time (min): 90 90 90 5 30 60 90 5 30 60 90
HPβCD:
β-Lap (22 mg/kg):
Rucaparib (10 mg/kg):
E
Relative NAD⁺ (% T/C)
5 min
90 min
ns
HPβCD:
β-Lap (22 mg/kg):
Rucaparib (10 mg/kg):
F
Relative ATP Level (% T/C)
5 min
90 min
ns
HPβCD:
β-Lap (22 mg/kg):
Rucaparib (10 mg/kg):
G
Relative Tumor Volume
Delta BLI (Day 108-33) x 10⁸
Vehicle
Rucaparib
β-Lap
β-Lap +Rucaparib
H
Vehicle
β-Lap (22 mg/kg)
Rucaparib (10 mg/kg)
β-Lap + Rucaparib



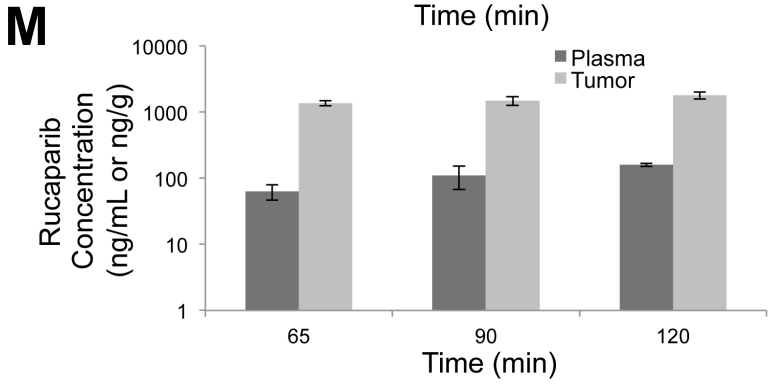




	HPβCD-β-lap	HPβCD-β-lap + Rucaparib
Tmax (min)	5	5
Cmax (ng/mL)	10,693	5,933
T 1/2 (min)	17	23
AUC (min*ng/mL)	308,414 ± 88,717	241,365 ± 29,493
Vz (mL/kg)	1,614	2,629
Cl (mL/min/kg)	65.3	78.2



	HPβCD-β-lap	HPβCD-β-lap + Rucaparib
Tmax (min)	5	5
Cmax (ng/g)	16,440	8,540
T 1/2 (min)	11.4	16.1
AUC (min*ng/g)	355,812 ± 77,242	217,310 ± 20,656
Vz (g/kg)	991	2,176
Cl (g/min/kg)	60.3	93.4



	Plasma	Tumor
Tmax (min)	120	120
Cmax (ng/mL or ng/g)	161	1,821
AUC (min*ng/mL or min*ng/g)	74,167 ±11,358	129,975 ± 5,195

Figure S7, Related to Figure 8: PARP inhibitors synergize with β-lap against orthotopic A549 NSCLC xenografts, PARP inhibition by Rucaparib results in super-additive antitumor activity with β-lapachone (β-lap) against A549 NSCLC xenografts in NOD/SCID female mice, and Pharmacokinetics of HPβCD-β-lap and Rucaparib in plasma and A549 NSCLC tumors.

(A, B) Orthotopic A549 NSCLC tumors expressing luciferase were established in 18-20 g female NOD/SCID mice by injecting ~1 X 10^6 cells, iv, tail vein. After 6 days, mice were treated or not with Rucaparib (10 mg/kg, ip). Rucaparib was given daily for 11 days. On day 7(t=0, cell injection), mice were injected with vehicle (HPβCD, iv) alone, or HPβCD-β-lap (22 mg/kg, iv, tail vein) every other day for a total of 5 injections over 10 days. Bioluminescence imaging (BLI) was used to monitor relative tumor volumes. Representative BLI images are shown at days 32 and 68 **(A)**. Average tumor volumes were calculated at days 85 and 108 post-treatment **(B)**.

(C) Kaplan-Meier survival curves of animals treated in **(A)**. **, $p < 0.01$; *, $p < 0.05$, β-lap (22 mg/kg) or combined treatment versus HPβCD alone treatment (log-rank test).

(D) Orthotopic A549 NSCLC tumor-bearing female NOD/SCID mice (3/group) were treated as in **(A)** and sacrificed at indicated times (min). Blood, tumor and various normal tissues (including associated normal lung tissue) were extracted and analyzed for PAR-PARP1, γH2AX levels and loading using β-actin (**Figure S7J**). Relative levels of each biomarker were calculated using loading controls. Experiments were repeated at least three times.

(E, F) Tumor tissues from **(D)** were assessed for relative NAD^+ **(E)** or ATP **(F)** levels at indicated times (5 and 90 min).

(G) Mice (5/group) were treated as described in **Figure S7A-S7F**. Tumor volumes were estimated using BLI imaging (means ± SEM, **Figure S7B**) and individual tumors in mice graphed as a waterfall plot as the differences in volumes from days 33 to 108. Change in BLI values were divided by 1 X 10^8 to fit values on scale.

(H) Mice (3/group) were treated as described in **Figure S7A-S7F**, and day 45 following the last of the 5 treatments, mice were sacrificed and all organs examined for long-term toxicities to normal tissue. 10% formalin fixed and examined for toxicities. H&E staining for liver, scale bar = 0.5 cm.

(I) Female NOD/SCID mice bearing A549 NSCLC lung cancer xenografts were exposed to vehicle or Rucaparib (2.5 mg/kg, ip) 1 hr, and then injected with or without β-lap (22 mg/kg, iv). Each group had 7 mice. Rucaparib was injected daily and β-lap injected every other day for 5 days over an 11-day period. A Kaplan-Meier survival curve was then constructed.

(J) Mice were treated as described in **Figure S7D,** tumor and various normal tissues (including associated normal lung tissue) were extracted and analyzed for PAR-PARP1, γH2AX levels and loading using β-actin. Relative levels of PAR and γH2AX were calculated and graphed in **Figure S7D**.

All error bars are means ± SEM from three mice in each group. Student's t tests were performed. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

(K-M) Mice were dosed with 10 mg/kg Rucaparib ip 1 hr prior to dosing with 22 mg/kg HPβCD-β-lap iv or 22 mg/kg HPβCD-β-lap was given alone. At varying times after administration of HPβCD-β-lap, mice were sacrificed and whole blood and tumor tissues harvested. Plasma was obtained from the whole blood by centrifugation and tumors were homogenized in PBS to generate a lysate. Plasma and tumor samples were then analyzed by LC-MS/MS. HPβCD-β-lap concentrations were measured in plasma **(K)** and tumor **(L)** over time for both types of treatment. Rucaparib concentrations were also determined for both plasma and tumor **(M)**. Error bars on the graphs represent standard deviation between samples. Non-compartmental pharmacokinetic parameters are shown for maximum time (Tmax), maximum concentration (Cmax), half-life (T 1/2), area under the concentration time curve (AUC), volume of distribution (Vz), and clearance (Cl). Errors for the AUC values are represented by the standard error of the mean.

Synergy values (eta, η) were reported based on multiple dose-responses or on comparative p values indicated.

Supplemental Experimental Procedures

Cell Culture

A majority of breast and PDA cancer cells were obtained from the American Tissue Culture Collection (ATCC, Manasas, VA), and all lung cancer cell lines were generated by the UT Southwestern-MD Anderson SPORE in lung cancer, or from the ATCC. All cells were cultured under 5% CO_2-95% air atmosphere at 37 °C, generally in DMEM or RPMI (Life Technologies, Carlsbad, CA) containing 10% or 5% fetal bovine serum (Hyclone, Thermo Sci., Logan UT), as required. All cells were mycoplasma-free and MAP tested.

NQO1 Enzyme Activities and Protein Levels

NQO1 enzyme levels were monitoring cytochrome C (Sigma-Aldrich, St. Louis, MO) reduction as the terminal electron acceptor and menadione as the quinone electron donor as substrate (Sigma-Aldrich). Reactions were initiated with NADH addition. NQO1 activity to recycle β-lap was also assessed as described (Li et al., 2011; Pink et al., 2000) Tumor and associated normal de-identified patient samples were obtained from two IRBs: (i) the UT Southwestern Medical Center Tissue Resource (UTSTR), IRB# STU 102010-051 (PI: A Witkiewicz), where fresh frozen tissues for enzyme assays were obtained; and (ii) IRB STU 0142011-005 (PI: DE Gerber), 'A Phase I dose-escalation study of ARQ 761 (β-lapachone) in adult patients with advanced solid tumors', from which 10% formalin-fixed sections were stained for NQO1 expression and counterstained for H&E.

PARP1 Enzymatic Assays and Dose Enhancement Ratio (DER) Calculations

Cell-Free Purified PARP1 Enzymatic Assays. PARP1 activity was assessed in vitro using recombinant flag-tagged PARP1 enzyme. In a reaction buffer containing Tris-HCl (10 mM, pH 8.0) and MgCl2 (10 mM) was added purified Flag-PARP1 (10 nM) and purified DNA substrate containing blunt-ends and an internal synthetic abasic site (1000 nM, Sigma-Aldrich), which are known to bind and activate PARP1 (D'Silva et al., 1999; Khodyreva et al., 2010). After 15s incubation at room temperature, the reaction was initiated by addition of NAD+ (100 μM) with or without the indicated PARP inhibitors (10 nM) in **Figure S3E** or at varying concentrations of Rucaparib (0, 2.5, 5.0, 10, 15 nM) in **Figures S3F, S3G**. Enzymatic reactions were incubated at room temperature for 1 min and immediately quenched with ice-cold trichloroacetic acid (TCA, 20% w/v solution). Equal aliquots of each reaction were then loaded on to a nitrocellulose membrane using a dot blot apparatus. Membrane were then blocked with casein blocking solution (Sigma-Aldrich) for 1 hr at room temperature and processed using standard Western blot procedures: PAR antibody (1:500 dilution, Trevigen) at 4°C overnight; secondary anti-mouse antibody (1:1000, Sta. Cruz) for 1 hr at room temperature. PAR formation densitometry was measured via Image J, and PARP1 activity inhibition was calculated relative to no PARP inhibitor control. Linear regression correlation was calculated via Prism 7 software.

Dose Enhancement Ratio (DER) Calculations. Dose enhancement ratio (DER) calculations in **Figure S3E** were obtained as described in the equation shown below:

$$DER = \frac{(\beta - lap)_{3\mu M,\ \%CellSurvival}}{[(\beta - lap)_{3\mu M} + (PARPi)_{LD10}]_{\%CellSurvival}}$$

or the combination [β-lap (3 μM) + PARP inhibitors (~LD_{10})] were obtained via DNA assay as previously described. These values were then used in the equation described above to calculate the DER. Linear regression correlation was calculated via Prism 7 software. (See text for DER calculation for **Figures S3F, S3G**.

Survival Assays

Relative survival assays based on 7 day DNA content assessments were performed as described (Huang et al., 2012; Pink et al., 2000). Briefly, cells (1 X 10^4) were seeded onto 48-well plates, pretreated with PARP inhibitors (Selleck Chemicals, Houston, TX; Cayman Chemical, Ann Arbor, MI) or vehicle for 2 hr, and then co-treated with Rucaparab (15 μM) + β-lap (synthesized by us) at various doses, with or without dicoumarol (DIC, 50 μM) (Sigma-Aldrich) in 6 replicates per dose. After ~7 days growth, or once vehicle-treated cells reached 90% confluence, media were removed, cells washed, lysed by freeze-thaw, DNA stained by Hoechst 33258 dye (Sigma-Aldrich, 14530) and quantified by fluorescence (460 nm) in a Victor X3 (Perkin-Elmer, Waltham, MA) plate reader. For colony forming ability assays, cells were treated as described above at 500-1000 cells per 60 mm plates. After ~7 days, colonies were fixed in methanol and stained with crystal violet (C6158, Sigma-Aldrich). Colonies of >50 healthy appearing cells were counted and total colonies normalized to vehicle-treated cells.

Western Blotting

Lysates were prepared from cancer cells or homogenized tumor tissue in RIPA-containing buffer with protease (S8820, Sigma-Aldrich) and phosphatase inhibitors (sc45044, sc45045, Santa Cruz). Proteins were separated by 8% SDS-PAGE gels, transferred to PVDF membranes (IPVH00010, Millipore), blocked in casein buffer (B6429, Sigma-Aldrich) and incubated with primary antibodies overnight at 4 °C. Secondary antibody incubation was performed for 1 hr at room temperature with anti-mouse or anti-rabbit HRP conjugated antibodies (Santa Cruz). ECL chemiluminescent detection was then performed (Thermo Scientific, 34077) and density analyses were performed in NIH ImageJ with intensity normalization as indicated.

Immunofluorescence

Cells were grown on glass coverslips and treated with Rucaparib, β-lap or the combination as described in "Survival Assays". Cells were fixed, washed, blocked in 1% BSA, (30 min, rm. temp.) in PBS with 5% normal goat serum (NGS, Jackson ImmunoResearch, West Grove, PA), incubated

with primary antibody in PBS/NGS overnight at 4 ^{o}C, and incubated with Alexa-Fluor conjugated secondary antibody (Life Technologies) for 1 hr at room temperature. Cells were then imaged on a Leica DM5500 fluorescent microscope and gH2AX foci/nuclei quantified.

Pharmacokinetic Analyses of HPβCD-β-lap and Rucaparib in mice bearing A549 NSCLC Xenografts

Pharmacokinetic studies were performed in NOD/SCID mice bearing orthotopic A549 lung cancer cells. Rucaparib was injected at 10 mg/kg via the intraperitoneal (ip) route. One hour after Rucaparib treatment, the mice were injected with HPβCD-β-lap (22 mg/kg) via tail vein (iv). Animals were sacrificed at different time points (from 5 to 60 min) after HPβCD-β-lap treatment. Whole blood and tumor tissue was harvested at the same time. The blood sample was separated by centrifugation for 10 min at 9600 x g and the plasma supernatant was saved. Tumor tissues were homogenized in PBS For standards, blank commercial plasma (Bioreclamation, Westbury, NY) or untreated tumor tissue homogenate was spiked with varying concentrations of compound. Standards and samples were mixed with a 2X volume of 100% acetonitrile containing 0.15% formic acid, vortexed, and then spun 5 min at 16,100 x g. The supernatant was removed and spun again and the resulting second supernatant was put into an HPLC vial with an insert and then analyzed by LC-MS/MS using an AB Sciex 3200 QTRAP® coupled to a Shimadzu Prominence LC. β-lap and Rucaparib were detected with the mass spectrometer in MRM (multiple reaction monitoring) mode by following the precursor to fragment ion transition 243.1 → 187.2 and 324.1 → 293.2, respectively. An Agilent Zorbax XDB-C18 column (50 x 4.6 mm, 5 micron packing) was used for chromatography with the following conditions: Buffer A: dH_20 + 0.1% formic acid, Buffer B: MeOH + 0.1% formic acid, 1.5 mL/min flow rate, 0-1.5min 3%B, 1.5-2.5 min gradient to 100% B, 2.5-3.5 min 100% B, 3.5-3.6 min gradient to 3% B, 3.6-4.5 min 3% B. In general, back-calculation of standard curve and quality control samples were accurate to within 20% for 70% of these samples at concentrations ranging from 5 ng/ml to 10,000 ng/ml. Pharmacokinetic parameters from 0-1 hr for β-lap and 0-2 hr for Rucaparib were calculated using the non-compartmental analysis tool of Phoenix WinNonlin (Certara Corporation, Princeton, NJ). An unpaired *t*-test (GraphPad QuickCalcs, San Diego, CA) was used to test for significant differences in β-lap concentrations in the different treatment groups.

Pharmacokinetic Analyses of HPβCD-β-lap and Rucaparib in mice bearing orthotopic MiaPaCa2 xenografts

Pharmacokinetic studies were performed in NOD/SCID mice bearing orthotopic MiaPaCa2 pancreatic cancer cells. Rucaparib was injected at 15 mg/kg via the intraperitoneal (ip) route. Two hours after Rucaparib treatment, some of the mice were injected with HPβCD-β-lap (22 mg/kg) via tail vein (iv). Animals were sacrificed at different time points (from 5 to 240 min) after potential HPβCD-β-lap treatment. Whole blood and tumor tissue was harvested at the same time. Processing and analysis followed the same procedure outlined previously for A549 tumor bearing

mice except tolbutamide (transition 271.2 → 91.2) was included as an internal standard and β-lapachone levels were measured with an AB Sciex 4000 QTRAP®. Pharmacokinetic parameters from 0-4 hr for β-lap and 0-7 hr for Rucaparib were calculated using the non-compartmental analysis tool of Phoenix WinNonlin (Certara Corporation, Princeton, NJ). An unpaired *t*-test (GraphPad QuickCalcs, San Diego, CA) was used to test for significant differences in β-lap and Rucaparib concentrations in the different treatment groups.

Microarray Expression Data, Processing and Analyses

Gene expression data series were retrieved from the Gene Expression Omnibus (GEO) database on September 30, 2011 subject to the following criteria: Study included NSCLC tumor or cell lines of NSCLC origin, more than 50 samples in the full study, processed using the GeneChip Human Genome U133 Plus 2.0 expression array. A total of 8 series met these criteria and were included in the cohort for analysis: GSE2109, GSE8332, GSE10445, GSE10843, GSE14315, GSE18842, GSE19804, GSE31546. The assembled cohort included 327 NSCLC tumor samples, 105 normal lung and 128 NSCLC cell line specimens, for a total of 560 specimens. Within this assembled cohort were 105 matched-pair specimens from two independent studies, in which biopsies were taken from both tumor and adjacent normal lung for each NSCLC patient studied. The 560 specimen data files included in the cohort were downloaded as raw CEL files for post-processing together, following the standard gene expression data preparation workflow (Irizarry et al., 2003). We used the R package aroma.affymetrix, which uses persistent memory to allow analyses of very large datasets. Data were processed using the linear model from RMA, then fit robustly using probe level models as described (Robinson and Speed, 2007). Probe level models were fit to RMA-background corrected and quantile normalized data to obtain gene-level summaries. Gene-level summarization used the standard CDF provided by Affymetrix. The Welch's t-test for unequal variance was used to compute p values for the difference in the means. All analyses were performed in R. Statistical tests were performed using base R statistical functions, graphics were generated using the ggplot2 graphics package (Wickham, 2009).

Response analyses in NSCLC patients

We accessed *NQO1* expression levels from RNAseq data in 467 lung adenocarcinoma (LUAD) cases from The Cancer Genome Atlas (TCGA: https://tcga-data.nci.nih.gov/tcga). From this dataset, we only included cases that: (1) were treated with standard of care chemotherapy (cisplatin and/or a taxol-based agent) and (2) contained information on patient response outcomes. Next, we separated cases into either clinical response (CR) or progressive disease (PD) groups as per Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (Therasse et al., 2000), resulting in 71 total LUAD cases: 44 cases in the CR group and 27 cases in the PD group. Statistical significance was measured using a two-tailed Student's t-test.

Histologic grade analyses in PDA patients

We accessed *NQO1* expression levels from RNAseq data in 128 pancreatic adenocarcinoma (PDA) cases from TCGA. Next, we analyzed cases that contained PDA histologic grade between Grade 1 and Grade 3 (G1-G3) (Kloppel et al., 1985). This filter resulted in 36 total PDA cases: 6 cases in G1 group, 21 cases in G2 group and 9 cases in G3 group. Statistical significance was measured using a two-tailed Student's t-test.

Statistical considerations in evaluation the combined effects of PARP inhibitors + β-lap therapies

The effects of PARP inhibitors or β-lap alone, or combined treatment of PARP inhibitors + β-lap were examined using one-way ANOVA followed by two-group comparisons for continuous measures of log-rank tests for survival outcome measures in vivo. Synergistic effects in vitro and in vivo were easily established with multiple doses examined. Evaluations of 'synergistic effects' in vivo between PARP inhibitors and β-lapachone on tumor volumes and/or survival measurements in vivo were completed as dose-response curves (containing three doses) (Loewe, 1953; Chou and Talalay, 1984; Lee et al., 2007; and Tallarida, 2011).

Supplemental References

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