CHK1 protects oncogenic KRAS-expressing cells from DNA damage and is a target for pancreatic cancer treatment

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**A**

| Cell line | KRAS | p53 | Other | ERU sensitivity |
|-----------|------|-----|-------|----------------|
| BxPC-3   | WT   |     |       |                |
| MIA PaCa-2 | G12C | R424W | S     |                |
| Pa14C    | G12D | T15P | R     |                |
| Pa16C    | G12D | L17P | S     |                |
| Pa20C    | G12D | D27N | S     |                |
| PANC-1   | G12D | R273H | R    |                |
| PATC-151 | WT   |     |       | ND             |

ND, not determined. Compiled from Hayes et al. (2016); S, sensitive (G12D < 4 µM, resistant (G12D = 4 µM)

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**B**

**Druggable genome CRISPR-Cas9 library** (2,500 genes, 5 sgRNA per gene)

- **Lentivirus packaged, pooled sgRNA druggable genome library infused at line**
  - **MOX**
  - **Genomic isolation**
  - **PCR amplification**
  - **RNA sequencing**
  - **Analyze for barcode depletion**

**C**

**Druggable genome siRNA library** (6,992 genes, 4 siRNAs per gene)

- **Hits defined as ≥ 2 hits with >5 Hits defined as ≥ 2 siRNAs per gene with >5**

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**D**

**CRISPR/ERK1 screen: top 50 hits (RSA)**

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**E**

**Essential lines in PDAC** (Depicted CRISPR vs Druggable genome)

**F**

**Essential lines in PDAC** (Depicted CRISPR vs Druggable genome)

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**G**

- **Pa01C**
  - **siRNA**
  - **xRNA**

- **Pa02C**
  - **siRNA**
  - **xRNA**

- **Pa14C**
  - **siRNA**

- **Pa16C**
  - **siRNA**

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**H**

- **siRNA (10 µM)**
  - **ATM**
  - **ATR**
  - **CHEK1**
  - **MYC**
  - **H3K4**
  - **Vinculin**

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**I**

- **ATM**
  - **ATR**
  - **CHEK1**
  - **CHEK2**

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**J**

- **ATM versus PFI (TCGA)**
  - **ATR versus PFI (TCGA)**
  - **CHEK1 versus PFI (TCGA)**
  - **CHEK2 versus PFI (TCGA)**
Figure S1. CHEK1 is an ERKi Sensitizers and Essential for PDAC growth, Related to Figure 1
(A) KRAS and TP53 mutation status, and SCH772984 ERK inhibitor sensitivity of PDAC cell lines used in this study.
(B) Categories of genes targeted by the CRISPR-Cas9 druggable genome library and experimental scheme for the screen.
(C) Categories of genes targeted by the siRNA druggable genome library and the experimental scheme for the screen.
(D) Heat map for the top 50 genes that increased ERKi sensitivity for each cell line and ERKi treatment time point. Shown is the median value of the replicates, scale bar represents the LogP (RSA).
(E) Comparison of kinase median dependency scores extracted from PDAC cells in DepMap and median log2 fold change from our druggable genome CRISPR screen.
(F) Comparison of kinase median dependency scores from the DepMap CRISPR and DepMap shRNA screens.
(G) PDAC cell growth following treatment with siRNAs targeting, non-silencing control (NS), KRAS, ATM, ATR, or CHEK1. Viable cells were quantitated at days 1 and 5 following siRNA treatment. Statistical significance was evaluated using one-way ANOVA and Dunnett’s multiple comparison test.
(H) Cell lysates were collected 72 h following siRNA treatment and evaluated for the indicated proteins by immunoblotting. A representative blot from two biological replicates is shown.
(I) TCGA pancreatic cancer patient data were analyzed for mRNA expression levels of the indicated DDR genes.
(J) Kaplan-Meier survival curves were determined from TCGA pancreatic cancer patient mRNA expression data and compared between the top and bottom quartiles based on expression of the indicated DDR gene.
Figure S2

A

| 0 | 2 | 4 | 8 | 16 | 32 | 0 | 2 | 4 | 8 | 16 | 32 | 0 | 2 | 4 | 8 | 16 | 32 |
|---|---|---|---|----|----|---|---|---|---|----|----|---|---|---|---|----|----|
| Pa02C | Pa14C | MIA PaCa-2 | PANC-1 |
| pCHK1 | CHK1 | γH2AX | MYC | β-actin |

B

8 days

% Relative coverage

% Growth

C

5 days

% Growth

D

5 days

% Growth

E

5 days

% Growth

F

5 days

Average CHK1 GI50 (µM)

G

5 days

Average CHK1 GI50 (µM)

H

CHK1i (nM, 24 h): 0 8 32 0 8 32

pCHK1 | CHK1 | γH2AX | Vinculin

H

CellTox Green Cytotoxicity Assay (72 h)

I

Average fold change in cell tox (Log2)

J

Pa01C CHK1i Cell Cycle

K

3 days

% Apoptosis

DMSO | Paclitaxel | CHKi
**Figure S2: CHK1i Blocks PDAC Growth and Induces S-phase Arrest and Apoptosis, Related to Figure 2**

(A) The indicated cell lines were treated with CHK1i for 24 h and cell lysates were collected and probed for the indicated proteins.

(B) Quantification of mean % cell coverage and standard deviation from four biological replicates of clonogenic assays. The % coverage was quantified using masked images via FIJI. Example images from a representative replicate are shown in Figure 2B.

(C) Growth of CHK1-resistant PANC-1 cells was evaluated by live cell counting following CHK1i treatment for 5 days. Graph shows mean and standard deviation of biological triplicates; accompanies Figures 2C and 2D.

(D) Growth of PDAC cell lines was evaluated by live cell counting following treatment with additional CHK1i, AZD7762, for 5 days. The graph shows the mean and standard deviation of biological duplicates.

(E-F) Growth of KRAS wild-type PDAC cells following treatment with CHK1i, for 5 days.

(G) Growth of matched pairs of HPNE cells stably infected with empty vector or encoding KRAS$^{G12D}$ was evaluated by live cell counting following 5-day treatment with CHK1i. The graphs show the mean GI$_{50}$ for each cell line and standard deviation of biological triplicates. Statistical significance was evaluated by unpaired t-test analysis; ****p < 0.0001.

(H) HPNE and HPNE(KRAS$^{G12D}$) cells were treated with CHK1i for 24 h at the indicated doses. Cell lysates were collected and proteins were evaluated by immunoblot analyses.

(I) The average relative amounts of cell death were determined via CellTox Green staining and fluorescence levels at 72 h post CHK1i. Graphs represent the mean log$_{2}$ fold change in CellTox Green over DMSO from three to four biological replicates and the corresponding standard deviation.

(J) CHK1i causes S phase accumulation in sensitive cell lines. The schematic shows a representative cell cycle fit of Pa01C cells treated with DMSO, paclitaxel, and CHK1i (8 nM) for 24 h.

(K) Cells were treated with CHK1i for 3 days and apoptosis levels were evaluated via FITC labeled Annexin V and flow cytometry. Graphs represent the mean of three biological replicates. Statistical significance was evaluated using one-way ANOVA and Dunnett's multiple comparison test; **p < 0.01, ***p < 0.001, ****p < 0.0001.
**Figure S3**

A  

Pan  

Foci  

\[
\begin{array}{ccc}
\text{CHK1i (nM, 24 h)} & 8 & 32 \\
\text{\text{yH2AX}} & \text{\text{yH2AX/DAPI}} & \text{\text{\text{yH2AX}} & \text{\text{yH2AX/DAPI}} \\
\text{Pa01C} & \text{Pa16C} & \text{Not detected}
\end{array}
\]

B  

yH2AX  

\[
\begin{array}{c|c|c}
\text{\text{yH2AX}} & \text{\text{\text{Not detected}}}
\end{array}
\]

C  

\[
\begin{array}{cccc}
\text{DMSO} & 8 \text{ nM} & 32 \text{ nM} & \text{DMSO} & 8 \text{ nM} & 32 \text{ nM} \\
\text{53BP1} & \text{53BP1/DAPI} & \text{53BP1} & \text{53BP1/DAPI} & & \text{53BP1} & \text{53BP1/DAPI} \\
\text{Pa01C} & \text{Pa16C} & \text{Pa01C} & \text{Pa16C} & \text{Pa01C} & \text{Pa16C} \\
\text{NCS (1 h)} & & & & & &
\end{array}
\]

D  

\[
\begin{array}{c|c|c|c|c|c|c}
\text{DMSO} & 8 \text{ nM} & \text{DMSO + NCS} & 8 \text{ nM + NCS} & 32 \text{ nM} & 32 \text{ nM + NCS} & \text{CHK1i (24 h)}
\end{array}
\]

\[
\begin{array}{c|c|c|c|c|c|c}
\text{DMSO} & 8 \text{ nM} & \text{DMSO + NCS} & 8 \text{ nM + NCS} & 32 \text{ nM} & 32 \text{ nM + NCS} & \text{CHK1i (24 h)}
\end{array}
\]
Figure S3: CHK1i promotes DNA damage and Loss of 53BP1 Mediated Repair, Related to Figure 3
(A-B) PDAC cells were treated with CHK1i at the indicated doses for 24 h, then fixed, stained for γH2AX and the nuclear marker DAPI, and imaged via wide-field microscopy, scale bar, 25 µm. (A) Representative images showing γH2AX pan-nuclear distribution and foci following CHK1i. (B) The mean of % nuclei for each γH2AX phenotype was calculated from three biological replicates. Error bars represent the standard deviation. Significance was evaluated using one-way ANOVA with Dunnett’s multiple comparison test; **p < 0.01, ***p < 0.001.

(C) Representative images showing 53BP1 distribution following CHK1i treatment (24 h) of control or neocarzinostatin (NCS) treated (1 h) cultures. Cells were then fixed and stained for endogenous 53BP1 and the fluorescent DNA stain DAPI to visualize nuclei, and imaged using wide-field microscopy. Scale bar, 25 µm.

(D) Quantification of 53BP1 foci per nuclei from cells treated as shown panel (C). The mean and standard deviation are shown. Each dot represents one nucleus (collected from 2 independent biological replicates). Statistical significance was evaluated using one-way ANOVA with Sidak’s multiple comparison test; ***p < 0.001, ****p < 0.0001.
Figure S4

A

|          | γH2AX | 53BP1 | γH2AX/53BP1 | DAPI |          | γH2AX | 53BP1 | γH2AX/53BP1 | DAPI |
|----------|-------|-------|-------------|------|----------|-------|-------|-------------|------|
| DMSO     |       |       |             |      |          |       |       |             |      |
| CHK1i (nM, 24 h) | 8     |       |             |      |          |       |       |             |      |
|           |       |       |             |      |          |       |       |             |      |
|          |       |       |             |      |          |       |       |             |      |
| Pa01C    |       |       |             |      |          |       |       |             |      |
| DMSO     |       |       |             |      |          |       |       |             |      |
| CHK1i (nM, 24 h) | 32    |       |             |      |          |       |       |             |      |
|           |       |       |             |      |          |       |       |             |      |
|          |       |       |             |      |          |       |       |             |      |
| Pa16C    |       |       |             |      |          |       |       |             |      |
| DMSO     |       |       |             |      |          |       |       |             |      |
| CHK1i (nM, 24 h) | 8     |       |             |      |          |       |       |             |      |
|           |       |       |             |      |          |       |       |             |      |
|          |       |       |             |      |          |       |       |             |      |

B

|          | γH2AX/RAD51 | γH2AX | RAD51 | DAPI |
|----------|-------------|-------|-------|------|
| DMSO     |             |       |       |      |
| CHK1i (nM, 24 h) | 8     |       |       |      |
|           |             |       |       |      |
|          |             |       |       |      |
| CHK1i (nM, 24 h) | 32    |       |       |      |
|           |             |       |       |      |
|          |             |       |       |      |
| DMSO     |             |       |       |      |
| CHK1i (nM, 24 h) | 8     |       |       |      |
|           |             |       |       |      |
|          |             |       |       |      |
| CHK1i (nM, 24 h) | 32    |       |       |      |

C

% Cells ≤3 foci/nucleus

CHK1i (24 h), NCS (100 ng/mL, 24 h)
Figure S4: CHK1i promotes DNA damage and Loss of 53BP1 Mediated Repair, Related to Figure 3

(A) PDAC cells were treated with CHK1i at the indicated doses for 24 h, and/or the irradiation mimic, neocarzinostatin (NCS) for 1 h at 100 ng/ml. then fixed, stained for endogenous 53BP1, γH2AX, and the nuclear marker DAPI, and imaged via confocal microscopy. Scale bar, 10 µm.

(B) Representative images showing RAD51 and γH2AX distribution Control or NCS-treated (100 ng/ml) PDAC cells were treated with CHK1i at the indicated concentrations for 24 h, then fixed and stained for endogenous RAD51, γH2AX, and nuclei (DAPI), and imaged via wide-field microscopy. Scale bar, 25 µm.

(C) Quantification of percent nuclei containing RAD51 foci ≥ 3 per nucleus in Pa16C cells following each treatment shown in panel (F). Each dot represents one field of view representing > 400 nuclei and imaged from biological duplicates. Mean and standard deviation are shown. Statistical significance was evaluated using one-way ANOVA with Sidak’s multiple comparison test; ***p < 0.001, ****p < 0.0001.
Figure S5

RPPA: ERKi (200 nM)/CHK1i (16 nM)
Figure S5: CHK1i promotes DNA damage and Loss of 53BP1 Mediated Repair, Related to Figure 3
Cells were treated with either CHK1i, ERKi, or ERKi+CHK1i for the indicated times. Cell lysates were collected and analyzed via RPPA using 162 antibodies. A complete list of antibodies is in Supplemental Table 1. The heatmap shows the relative median change upon inhibitor treatment compared to DMSO control for each antibody.
Figure S6

A. Top UP Regulated Proteins

B. Top DOWN Regulated Proteins

C. yH2AX

D. Top CHK1 up 24 h

E. MG132 (4 h)

F. MYC

UP at all time points

UP and DOWN at different time points

DOWN at all time points
Figure S6: CHK1i promotes DNA damage and Loss of 53BP1 Mediated Repair, Related to Figure 3

(A) Top protein changes up and down following CHK1i, extracted from panel C. Changes listed in red represent the top upregulated proteins at all time points, blue reflects the top down-regulated changes at all times, and purple indicates proteins that switched from up to down or from down to up.

(B) The top five protein changes up and down following CHK1i, extracted from panel C. Each cell line point is the mean of four biological replicates analyzed by RPPA. Significance was evaluated using one-way ANOVA with Dunnett's multiple comparison test; *p < 0.05, **p < 0.01.

(C) Selected protein changes for each cell line at each time point relative to the respective DMSO treated control.

(D) Immunoblot analysis of MYC protein levels following CHK1i treatment with or without MG132. γH2AX was blotted to confirm CHK1i activity and vinculin served as a loading control.

(E) The mean relative expression of MYC protein quantified from three biological replicates, of which data in panel (E) is a representative example. Relative expression compares CHK1i treatment to vehicle (DMSO) control.

(F) qRT-PCR was used to determine MYC levels following CHK1i treatment with or without cycloheximide. Graph represents the mean from three biological replicates and their standard deviations.
Figure S7

A

| CHK1i (nM) | Vehicle | Pa01C | Pa14C | Pa16C |
|-----------|---------|-------|-------|-------|
|           | 15.6    | 31.2  | 62.5  | 125   | 250   |
| ERKi (nM) |         |       |       |       |       |

B

| CHK1i (nM) | Vehicle | Pa01C | Pa14C | Pa16C |
|-----------|---------|-------|-------|-------|
|           | 15.6    | 31.2  | 62.5  | 125   | 250   |
| ERKi (nM) |         |       |       |       |       |

C

| Vehicle | Pa01C | Pa14C | PANC-1 |
|---------|-------|-------|--------|
| ERKi (Log10 μM) |       |       |        |

D

| Vehicle | Pa01C | Pa14C | MIA PaCa-2 | PANC-1 |
|---------|-------|-------|------------|--------|
| ERKi (Log10 μM) |       |       |            |        |

E

| Vehicle | Pa01C | Pa14C | MIA PaCa-2 | PANC-1 |
|---------|-------|-------|------------|--------|
| ERKi (Log10 μM) |       |       |            |        |

F

| Vehicle | Pa01C | Pa14C | MIA PaCa-2 | PANC-1 |
|---------|-------|-------|------------|--------|
| ERKi (Log10 μM) |       |       |            |        |

G

| Vehicle | Pa01C | Pa14C | MIA PaCa-2 | PANC-1 |
|---------|-------|-------|------------|--------|
| ERKi (Log10 μM) |       |       |            |        |

H

| Vehicle | Pa01C | Pa14C | MIA PaCa-2 | PANC-1 |
|---------|-------|-------|------------|--------|
| ERKi (Log10 μM) |       |       |            |        |

I

| Vehicle | Pa01C | Pa14C | MIA PaCa-2 | PANC-1 |
|---------|-------|-------|------------|--------|
| ERKi (Log10 μM) |       |       |            |        |

J

| Vehicle | Pa01C | Pa14C | MIA PaCa-2 | PANC-1 |
|---------|-------|-------|------------|--------|
| ERKi (Log10 μM) |       |       |            |        |
Figure S7: Concurrent CHK1i Treatment Enhances ERKi-mediated Growth Suppression and Apoptosis, Related to Figure 4

(A-B) Clonogenic growth assay to monitor growth suppression of PDAC cell lines treated for 8 days with the indicated inhibitors (A). Quantification of average % coverage and standard deviation from four biological replicates (B).

(C) Growth of PDAC cell lines was evaluated by live cell counting following CHK1i and/or ERKi treatment(s) for 5 days. Graphs show average and standard deviation of biological triplicates; accompanies Figure 4D.

(D) Cell growth was evaluated via live cell counting following CHK1 inhibition (AZD7762) and/or ERKi treatment(s) for 5 days. The graphs show average and standard deviation of biological duplicates.

(E) Cell lysates were collected following treatment with the indicated inhibitors, doses and treatment times are shown, and analyzed via immunoblotting.

(F-I) Pa02C cells stably expressing either WT-MYC or vector control were generated. (F) Cell lysates collected at 24 h post CHK1i and/or ERKi and analyzed for the indicated proteins via immunoblotting. (G-I) Cell growth was evaluated via live cell counting following ERKi (G), CHK1i (H), or CHK1i+ERKi (I). The graphs show the average and standard deviation of biological duplicates.

(J) Cell growth was evaluated via live cell counting following ATR inhibition (AZD6738) and/or ERKi treatment(s) for 5 days. The graphs show average and standard deviation of biological triplicates.
Figure S8

A

ATR

ATRIP

CHEK1

B

ERK 24 h DDR

ERK (200 nM)

ERK 72 h DDR

C

Relative protein expression

ERKi (nM, 4 h)

D

pERK
Total ERK
pCHK1
Total CHK1
Vinculin

E

siRNA (72 h)

NS, K1, K2

CHK1
KRAS
MYC
b-actin

Pa01C Pa02C Pa16C

F

Relative protein expression

Pa01C Pa02C Pa16C

G

KRAS

CHEK1

H

Relative expression (2-ΔΔCt)

\% Phase of cell cycle

NS, K1, K2

Pa01C Pa02C Pa16C

I

Relative protein expression

CDK4/6 (nM, 72 h)

Pa01C Pa02C Pa16C
Figure S8: ERK Inhibition Decreases CHEK1 Gene and Protein Expression by Causing G1 Cell Cycle Arrest, Related to Figure 5

(A-B) Cells were treated with 1 µM ERKi for the indicated amount of time. Statistical significance was determined via dispersion corrected, moderated t-tests as implemented in limma. RNA was collected and analyzed via RNA-seq. Each dot represents a cell line and red indicates a significant decrease in the average of the seven cell lines evaluated; *p < 0.05, **p < 0.01, ****p < 0.0001.

(B) Reverse Phase Protein Array (RPPA) was used to evaluate protein changes following ERKi (200 nM) in six different PDAC cell lines at 24 and 72 h. Graphs show the mean of the six PDAC cell lines for the selected protein changes. Significance was determined via a one-way ANOVA and Dunnett’s multiple comparison test; *p < 0.05, **p < 0.01.

(C) Quantification of CHK1 protein levels following 24 h of ERKi. Representative images are shown in Figure 5C. The mean and standard deviation were calculated from three biological replicates. Significance was determined using one-way ANOVA and Dunnett’s multiple comparison test; *p < 0.05, **p < 0.01, ****p < 0.0001.

(D) Cell lysates were collected after 4 h of ERKi and evaluated for the indicated proteins by immunoblotting. A representative blot from two biological replicates is shown.

(E-G) KRAS was depleted in cells using two independent siRNAs or non-targeted control siRNA (NS) for 72 h. Cell lysates (E-F) and RNA (G) were collected. Protein changes were evaluated via immunoblotting (E-F) and RNA levels via qRT-PCR (G). Graphs show the mean and standard deviations from biological triplicates. Significance was determined using one-way ANOVA and Dunnett’s multiple comparison test; ****p < 0.0001. (H) Following KRAS depletion for 72 h, cell cycle status was evaluated using propidium iodide and flow cytometry. Graph shows the mean and standard deviations from biological triplicates.

(I) Cells were treated with palbociclib (CDK4/6i, 72 h) and cell lysates were collected and evaluated via immunoblotting. Representative blots are shown in Figure 5H. Graph represents the mean and standard deviation of biological triplicates. Significance was determined using one-way ANOVA and Dunnett’s multiple comparison test; ****p < 0.0001.
Figure S9

A

Log2 (FC)

Pa16C

B

RIF1
γH2AX
Composite/DAPI

Pa01C

C

% Nuclei RIF1 foci positive

Pa01C

D

CHK1i (nM)

Pa16C

ERKi (nM)

Pa16C

53BP1-mApple Biosensor

E

Pa16C

F

Pa16C

C-HK1 (nM)

Pa16C

G

Pa16C

53BP1-mApple Biosensor

53BP1-mApple Biosensor

53BP1-mApple Biosensor

53BP1-mApple Biosensor

53BP1-mApple Biosensor
Figure S9: Loss of RIF1 Increases Sensitivity to CHK1i, Related to Figure 6

(A) Identification of RIF1 via a CRISPR/Cas9 loss of function screen for CHK1i sensitizers among known/putative ERK substrates performed in Pa16C. The log2 fold change for all genes evaluated is shown, with RIF1 and other top hits highlighted.

(B-C) Representative images of Pa01C cells treated with CHK1i for 24 h and evaluated for RIF1 (green) and γH2AX (red) via immunofluorescence. DAPI (white) identified cell nuclei; arrow marks a nucleus containing RIF foci. Scale bar, 25 µm.

(C) Quantification of percent nuclei containing RIF1 foci in Pa01C cells following each treatment. Each dot represents one field of view and imaged from biological duplicates. Mean and standard deviation are shown; treatments were compared via an unpaired t-test; **p < 0.01, ****p < 0.0001.

(D) Representative images of 53BP1-mApple-positive foci in Pa16C cells treated with ERKi and/or CHK1i for 24 h. Scale bar, 25 µm.

(E) Quantification of 53BP1-mApple-positive foci per nucleus from Pa16C cells treated as shown in (D). The mean and standard deviation are shown. Each dot represents one nucleus. Statistical significance was evaluated using two-way ANOVA with Tukey's multiple comparison test; **p < 0.01, ****p < 0.0001.

(F) Representative images of 53BP1 foci following reverse transfection with NS or two different siRNAs targeting RIF1 for 72 h, with or without CHK1i for 24 h, performed in Pa16C cells. Scale bar, 25 µm.

(G) Quantification of trun53BP1-mApple foci per nuclei from cells treated as described in (F). The mean and standard deviation are shown. Each dot represents one nucleus. Statistical significance was evaluated using two-way ANOVA with Tukey's multiple comparison test.
Figure S10: CHK1 Inhibition Induces Autophagy, Related to Figure 7

(A) Cell lines stably expressing the mCherry-EGFP-LC3B autophagic flux biosensor were imaged following treatment with CHK1i (24 h). Representative images for each cell line and treatment are shown. Scale bar, 25 µm.

(B) Cell growth was evaluated via live cell counting following treatment with CHK1i and CQ (5 days). Graph shows the mean and standard deviation from three to four biological replicates. Significant shifts are shown in red. One-way ANOVA with Dunnett's multiple comparison test was used to determine significance; *p < 0.05, **p < 0.01.

(C) The mean GI50 for the combination of CHK1i with CQ was determined from the growth assays performed in (B). Significant shifts are shown in red. One-way ANOVA with Dunnett's multiple comparison test was used to determine significance; *p < 0.05, **p < 0.01.

(D) Cell growth was evaluated by live cell counting following 5-day treatment with ATRi and CQ at the indicated concentrations. Graph shows the mean and standard deviation from three biological replicates. Significant shifts are shown in red. One-way ANOVA with Dunnett's multiple comparison test was used to determine significance; *p < 0.05, **p < 0.01.

(E-G) Cells were treated with the indicated concentrations of ERKi, CHK1i and/or chloroquine (CQ) for five days. (E) Bliss scores were determined based on the mean value of three biological replicates over a range of doses for each inhibitor. Synergy is indicated by red, antagonism by blue, and white reflects additivity. (F) Graph shows growth inhibition of cells treated with ERKi alone or with CQ and/or CHK1i. (G) Comparison 5-day growth inhibition of Pa16C and NIH/3T3 cells treated with CHK1i, ERKi and CQ at the indicated concentrations. (H) Triple combinations were evaluated in patient-derived KRAS-mutant PDAC organoids. Organoids were treated (10 days) with the indicated concentrations of ERKi and CHK1i with or without CQ (3.125 µM). The median of three biological replicates for each treatment is shown. A shift from blue to red indicates reduction in organoid growth.