**SUPPLEMENTARY MATERIALS AND METHODS**

**Antibodies**

For immunostaining and immunoblotting, primary antibodies were from Cell

Signaling unless otherwise stated: ATM S1981 (Rockland), CHK1, CHK1 S317,

CHK1 S345, CHK1 S296, CHK2, CHK2 T68 (Abcam), c-caspase 3, c-PARP, CDK1

(Abcam), CDK1 Y15, CDK2, CDK2 Y15 (Abcam), H2AX, H2AX S139 (Milipore),

HH3, HH3 S10, RPA (Abcam), RPA32 S4/8 (Bethyl Laboratories), alpha-tubulin

(Abcam), beta-actin (Abcam). For secondary antibodies, Alexa 488 and Alexa 647

from Cell Signaling were used in immunostaining. IRDye800CW- and IR680CWconjugated

antibodies from LI-COR were used in immunoblotting.

**Cytotoxicity agent combination assay and synergy calculation**

Cells were seeded for 24 hours in 96-well plates and then treated with a serial

dilution of each agent in an 8 X 8 concentration format. After 72 hours, cells were

fixed with trichloroacetic acid and stained with sulforhodamine B. Fluorescent

readout was obtained using the Infinite 200 PRO microplate reader (Tecan) at

excitation and emission wavelengths of 488nm and 585nm, respectively. To quantify

synergy, an in-house integrated software that could analyze numerical readout

based on the Bliss Independence, Loewe or Highest Single Agent models was

employed (1, 2). First, single-agent inhibition values were used to assign an additive

value to each concentration ratio. The calculated value was then subtracted from the

measured values, yielding a difference value. In the final synergy surface, a positive

value indicates synergistic effects while a negative value indicates antagonistic

effects. To date, many combination assays use the combination-index isobologram

method, which is based on the median effect principle. This permits only the analysis

of fixed dose ratios of the two agents in question. The key feature of the integrated

software was its flexibility in determining the expected interaction for any

combination of agent concentrations, allowing more exhaustive quantification of

combined effects from the generation of a 3D interaction surface.

**Acquisition, processing and analysis of live-cell time-lapse sequences**

Cells were kept in a humidified chamber under cell culture conditions. Images were

taken on three fields of view per well, every seven minutes over forty-eight hours,

using a Nikon Eclipse TE2000-E microscope with 10X objective. An equalization of

intensities over time was then performed to each channel using the NIS-Elements

software (Nikon). A shading correction and a background subtraction were also

applied to each image in each channel. Image analysis was carried out using the

TrackMate plugin available on Fiji (IMageJ, http://fiji.sc/Fiji). This plugin follows an

operator-defined scheme, which allows automation of spot segmentation and

particle-tracking over time. Detection of cells was performed based on the Laplacian

of Gaussian filtering, and particle-tracking was achieved frame-to-frame using the

Linear Assignment Problem tracker.

**Quantitative fluorescence-based microscopy**

Cells were immobilized on chambered coverslip (ibidi) and immunostained. Images

were acquired using the iCys laser scanning cytometer (CompuCyte) (40X objective)

equipped with a motorized Olympus IX71 inverted fluorescence microscope, three

lasers (405nm violet diode laser, 488nm argon laser, 633nm helium-neon laser) and

three optical filter sets (blue 450/40, green 530/30, far-red 650LP) coupled to

photomultipliers (PMT). A digital image was created for each PMT on a pixel-to-pixel

basis, and objects from thousands of measurement were identified based on predetermined

signal thresholds. The in-built iCys software was then used to generate a

number of datasets per object such as integral fluorescence and maximal intensity,

as well as mean values of these parameters. The advantage of this approach was

that the precise position of every object was recorded alongside corresponding

fluorescence data, allowing direct visualization of each object and verification on

whether it was a single cell, a doublet or an artefact. Furthermore, it enabled

simultaneous assessment of the fluorescence readout with cell cycle profile. An

example of this capability is illustrated in Supplementary Fig. S4C, where RPA32

S4/8-positive cells were found to be predominantly in S-phase.

**SUPPLEMENTARY REFERENCES**

1. Lin Y, Richards FM, Krippendorff BF, Bramhall JL, Harrington JA, Bapiro TE,

et al. Paclitaxel and CYC3, an aurora kinase A inhibitor, synergise in pancreatic

cancer cells but not bone marrow precursor cells. British journal of cancer.

2012;107:1692-701.

2. Fitzgerald JB, Schoeberl B, Nielsen UB, Sorger PK. Systems biology and

combination therapy in the quest for clinical efficacy. Nature chemical biology.

2006;2:458-66.