

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
<input type="checkbox"/>	<input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input checked="" type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input type="checkbox"/>	<input checked="" type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	For IP-MS purified peptide eluates were run through an Ultimate 3000 HPLC connected to an Orbitrap Ascend Tribrid instrument. For Bulk-RNA seq on untreated SCLC DFCI 393 and DFCI 402 PDX tumors that were used for the PDX studies, RNA-seq of experimental PDX, DFCI 393 tumors were treated with the orally bioavailable cyclin A/B RxL inhibitor CIRc-014 at 100 mg/kg PO TID or vehicle (30% PEG400, 20% Solutol HS15, 50% Phosal 53 MCT) and the NCI-H1048 cell line experiments done in 2 biological independent experiments, RNA was extracted using RNeasy mini kit (Qiagen #74106) including a DNase digestion step according to the manufacturer's instructions. Total RNA was submitted to Novogene Inc. The libraries were prepared using NEBNext Ultra II non-stranded kit. Paired end 150bp sequencing was performed on Novaseq6000 sequencer using S4 flow cell. Sequencing reads were mapped to the hg38 genome by STAR.
Data analysis	For CRISPR KO screen, raw Illumina reads were normalized between samples using: $\text{Log}_2[(\text{sgRNA reads}/\text{total reads for sample}) \times 1\text{e}6 + 1]$. Apron analysis, hypergeometric analysis, and STARS analysis were performed using the GPP web portal, https://portals.broadinstitute.org/gpp/screener/ . For CRISPR Base Editor screen, raw Illumina reads were normalized between samples using: $\text{Log}_2[(\text{sgRNA reads}/\text{total reads for sample}) \times 1\text{e}6 + 1]$. The Log Fold Change value was calculated between treatment arms and LFC value for each sgRNA was z-scored, using the following equation: $z\text{-score} = (x - \mu)/\sigma$, where <i>x</i> , <i>μ</i> , and <i>σ</i> correspond to LFC of an individual guide, the mean LFC of all negative control guides, and standard deviation of all control guides, respectively. Principal component analysis (PCA) on RNA-seq data was performed using the removeBatchEffect function in the limma package (version 3.58.1) and prcomp function of R software (version 4.3.3). Statistics for DEGs from bulk-RNA seq were calculated by DESeq2 (1.36.0). For HALLMARK pathway enrichment analysis, differentially expressed genes were tested if over-represented against the HALLMARK pathways from the MSigDB using R packages msigdb (7.5.1) and clusterProfiler (4.8.3). Heatmaps were generated by calculating z score using log transformed FPKM values. To calculate z score, a log transformed FPKM value was

subtracted from the mean and then divided by the standard deviation.

DIA-NN software (V8.1) was used to analyse the IP-MS data in library-free mode and using the recommended default settings.

Co-folding of proteins were done by AlphaFold 2 implemented in ColabFold. Default parameters were used to predict their relative positions. Specifically, those parameters are: msa_mode (MMseqs2_UniRef_Environmental), pair_mode (unpaired_paired), model_type (auto), number of cycles (3), recycle_early_stop_tolerance(auto), relax_max_iterations (200), and pairing_strategy (greedy). The resulting co-folding structure along with the PAE files were analyzed using ChimeraX software.

The Gene Set Variation Analysis (GSVA) method, utilizing the MSigDb Hallmark collection50 of RNA-seq data, was employed to calculate GSVA scores for E2F targets and G2/M checkpoint pathway using the GSVA Bioconductor package (version 1.50.5). The heatmap was constructed using the ComplexHeatmaps package (version 2.18.0) in R (version 4.3.2).

FACS analysis was performed on FlowJo 10.8.1. and FlowJo 10.9.0.

IF images were acquired using ZEN 2.3 SP1 software. Image analysis was performed Image J2 v2.9.0 software.

IHC images were analyzed using the HALO platform multiplex-IHC v3.2.5 algorithm (Indica lab).

Live cell images were analyzed using NIS Elements Viewer 4.2 (Nikon) and Incucyte Zoom / S3 live cell imagers (Essen Biosciences).

Schrodinger software suite (2024-3 version) was used to conduct modeling studies on cyclin B.

Immunoblots were quantified using Fiji (Image J2 v2.9.0) or LICOR Odyssey built-in Image Studio Software (v5.2).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Data from the genome-wide CRISPR/Cas9 Resistance Screen with the Cyclin A/B RxL Inhibitor, the Cyclin A/B/E RxL Inhibitor, or the Cdk2 Inhibitor are included in Supplementary Table 3. The RNA-seq experiments of NCI-H1048 cells treated with cyclin A/B RxL inhibitors or the CDK2 inhibitor are included in Supplementary Table 4. Data from the CRISPR/Cas9 Base Editor Screens with the Cyclin A/B RxL Inhibitor in Figure 3 are included in Supplementary Table 5. Data from the Mass Spectrometry of the Cyclin B1 CIRC-004-Dependent Interactome in Figure 3 are included in Supplementary Table 6. FPKM values from the bulk RNA-sequencing experiments of SCLC PDX models DFCI-393 and DFCI-402 are included in Supplementary Table 7. The RNA-seq experiments of DFCI-393 PDXs treated with cyclin A/B RxL inhibitors are included in Supplementary Table 7. The raw proteomics data are available via ProteomeXchange with identifier PXD055112 via the PRIDE (PRoteomics IDentifications - <https://www.ebi.ac.uk/pride/>) partner repository. The raw RNA-seq data are available on Gene Expression Omnibus (GEO) accession number GSE291451. Any other data and materials can be requested from the corresponding author upon reasonable request.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For NCI-H1048 xenograft study, n=10 independent mice for vehicle and n=8 independent mice for CIRC-028 arm. For NCI-H69 xenograft, n=10 independent mice for vehicle and CIRC-028 arm. For DFCI 393 PDX in n=10 independent mice for vehicle and n=9 independent mice for CIRC-014 arm. For DFCI 402 PDX n=10 independent mice per arm.
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For immunofluorescence experiment, 15 blind images were taken per experiment and repeated in 3 biological independent experiments. For IP-MS data, samples from 3 independent biological experiments were collected and analyzed. For live-cell imaging experiment in Extended Data Fig. 6a-b, 2 biological experiments were performed and 50 blind cells total were analyzed. For live-cell imaging experiment in Extended Data Fig. 2l, n=6 wells per treatment condition per replicate from 2 independent experiments, 1 representative experiment is shown. For all other experiments, sample size was not predetermined and multiple biological replicates were performed (mostly 3, but exact numbers specified in figure legends) and repeated n times based on reproducibility and statistical significance. For all experiments, no statistical methods were used to predetermine sample sizes but our sample sizes are similar to those reported in previous publications.

Data exclusions	There were no data excluded in our study.
Replication	To ensure reproducibility, multiple independent biological replicates were performed as described above in sample size with specific numbers of biological independent experiments specified with exact numbers in each figure legend. All attempts at replication were successful for all experiments in this manuscript and there were no data excluded.
Randomization	Samples were randomly allocated to experimental groups. For in vivo studies in Figure 5 and Extended Data Fig. 11, all mice used were randomly assigned to receive either vehicle or treatment. For in vitro experiments, this means that the same cell lines from the same maintenance culture were infected with the indicated sgRNAs at the same time for each biological independent experiment and each biological independent experiment was performed independently on a different day.
Blinding	For the Bulk RNA-sequencing studies, the experiments were performed and analyzed by Novogene (RNA-seq) who were blinded to the purpose of the study and the study group. For imaging experiments, blind images were taken using counter-stained nuclei and cells were chosen randomly to analyze. Forward genetic screen by its virtue is an unbiased screen and did not require blinding. Blinding was not possible for the analysis portions of the CRISPR Knock-out screen, base editor screen, IP-MS but no data were excluded and therefore blinding was not relevant for analysis. For low throughput experiments, blinding was not possible as the scientist performing the experiments needed to know the conditions to perform the experiment, but the experiments were performed with the appropriate controls in multiple independent biological experiments, all data are included in this manuscript, and no data were excluded.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

The primary antibodies and dilutions used were: Mouse Anti- β -actin AC-15 (Sigma #A3854, 1:25,000), mouse anti-Vinculin hVIN-1 (Sigma# V9131, 1:10000), rabbit anti-phospho CASC5/KNL1 Thr943/Thr1155 D8D4N (Cell Signaling #40758, 1:1000), rabbit anti-CASC5/KNL1 E4A5L (Cell Signaling #26687, 1:1000), rabbit anti-cyclin B1 D5C10 (Cell Signaling #12231, 1:1000), rabbit anti-E2F1 (Cell Signaling #3742, 1:1000), CDC20 (Cell Signaling #48235, 1:1000), FLAG (Sigma-Aldrich #A8592), rabbit anti-phospho-stathmin (S38) (Cell Signaling #3426S, 1:1000), rabbit anti-total stathmin (Abcam #ab52630), rabbit anti-phospho-Cyclin B (S126) (Abcam #ab3488, 1:1000), rabbit anti-phospho-nucleolin (T84) (Abcam #ab155977, 1:1000), rabbit anti-phospho-FOXO1(T600) (Cell Signaling #14655S, 1:500), rabbit anti-phospho-RPA2(S33) (Fortis Life Sciences #A300-246A, 1:2000), rabbit anti-tubulin-HRP (Cell Signaling #9099S 1:5000), rabbit anti-Histone 3 DH12 (Cell Signaling #4499, 1:5000), rabbit anti-phospho Histone H2A.X Ser 139 (Cell signaling #2577, 1:1000) and anti-phospho KAP1 antibody (Cell Signaling #4127, 1:1000). The secondary antibodies used and dilutions were: goat anti-rabbit-IgG-HRP (Cell Signaling #7074S, 1:5000), Goat Anti-Mouse (Jackson ImmunoResearch #115-035-003, 1:5000) and Goat anti-Rabbit (Jackson ImmunoResearch #111-035-003, 1:5000).

For immunoprecipitation the primary antibodies and dilutions used were: anti-Myt1 (Fortis Life Sciences A302-424A 1:1000), mouse anti-cyclin B (Cell Signaling #4135 1:1000), mouse anti-E2F1 (SCBT #sc-251, 1:1000), mouse anti-cyclin A (Cell Signaling #4656, 1:1000), mouse anti-CDK2 (Origene TA502935), rabbit anti-Cdc2 (Cell Signaling 28439). The secondary antibodies used and dilutions were: mouse anti-rabbit (confirmation specific)-IgG HRP (Cell Signaling #5127, 1:2000), goat anti-rabbit IgG-HRP (Cell Signaling #7074S, 1:5000), horse anti-mouse IgG-HRP (Cell Signaling #7076S, 1:5000), goat anti-mouse-IgE-HRP (Southern Biotech #1110-05, 1:5000), rabbit anti-Cyclin B1 D5C10 (Cell Signaling #12231, 1:1000), rabbit anti-Cdk2 E8J9T (Cell Signaling #18048, 1:1000) and mouse anti-HA. 11 epitope tag (BioLegend #901501, 1:1000). Anti-Wee1 antibody was used at 1:1000 (CST, Cat #4936). Anti-E2F3 antibody was used at 1:1000 (SCBT, cat #sc-56665).

For immunofluorescence, the primary antibodies used were rabbit anti-phospho Histone H2A.X Ser 139 (Cell Signaling #2577, 1:400); rabbit anti-phospho CASC5/KNL1 Thr943/Thr1155 D8D4N (Cell Signaling #40758, 1:400); ACA (Antibodies inc. #15-235, 1:500) and secondary antibodies used included Alexa Fluor 568 Goat anti-rabbit (Thermo Fisher Scientific #A11011, 1:300) and Alexa Fluor 647

Goat anti-human (Thermo Fisher Scientific #A21445, 1:300).

For FACS antibodies used included Pacific blue conjugated cleaved PARP antibody (Cell Signaling, Asp214, D64E10, #60068) and Alexa-647 conjugated phospho-histone H3 (Ser10) antibody (Cell Signaling, #3458)

Validation

All antibodies used for immunoblot analysis and IP experiments described above have been validated. This was done using different approaches. For cyclin B and Cdk2 antibodies, CRISPR inactivation of the target was performed followed by immunoblot analysis showing loss of target protein expression. CDC20, E2F1 and Flag were validated in cell lines engineered to overexpress Flag tagged CDC20 and E2F1. p-KNL1 antibody was validated by treating the cells with MPS1 inhibitor which has been validated previously to inhibit KNL1 phosphorylation. Gamma-H2AX antibody was validated by showing increase in DNA damage after chemotherapy treatment and H3 antibody is used as a loading control. P-cyclin B1, p-Stathmin and p-nucleolin were validated using selective Cdk1 and Cdk2 inhibitor treatments. Vinculin and b-actin are used in the lab as standard loading controls. Antibodies used for IF and IHC (p-KNL1) were validated in cell blocks after treatment with a cyclin A/B RxL inhibitor which was shown to increase phospho-KNL1 by immunoblot as described above. Antibodies used in FACS (p-H3, cleaved PARP) have been validated in a previously published paper where Aurora B Kinase inhibition induced mitotic arrest and apoptosis (PMID: 30373918). Anti-cdc2 and anti-CDK2 antibodies for WB were validated by co-IP western blot. Briefly, anti-cdc-2 or anti-CDK2 antibodies (cdc2 SCBT #SC-54; CDK2 SCBT #SC-6248) were used to immunoprecipitate CDK1 or CDK2 from cell lysates followed by western blot for CDK1 (CST #284395) or CDK2 (Origene TA502935). The anti-cdc2 antibody detected a band of the appropriate molecular weight in the CDK1 pulldown but no band was detected in the CDK2 pull-down or IgG control and vice versa for the anti-CDK2 antibody. For anti-cyclin A and Cyclin B (CST # 4135) immunoblots, shRNA knockdown of the target was performed followed by immunoblot analysis showing >75% reduction in target protein expression. The anti-Myt1 antibody was validated in a previously published paper where stable PKMYT1 knock-out cell lines were generated by CRISPR inactivation and immunoblot analysis demonstrated loss of target protein expression (PMID: 35444283). Anti-Wee1 antibody (CST, Cat #4936) was validated in a previously published paper where Wee1 was knocked-down by siRNA. (PMID: 33257507). Anti-E2F3 antibody was (SCBT cat #sc-56665) validated in a previously published paper where exogenous tagged-E2F3 was inducibly expressed by doxycycline treatment (PMID: 33264622). Anti-phospho KAP1 antibody has been validated in a previously published paper showing induction after treating cells with DNA damage causing agents like Etoposide or exposing to irradiation (PMID: 37541219).

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

NCI-H446, NCI-H69 cells, NCI-H1048, NCI-H526, WI-38, NCI-H82, RPE-1 and 293T cells were originally obtained from American Type Culture Collection (ATCC). A549, HCC4006, and NCI-H1299 cells were a kind gift from Dr. Pasi Jänne's laboratory at DFCl. NCI-H1048, Jurkat, NCI-H82 and MDA-MB-231 cells were originally obtained from ATCC and early passage cell lines were genetically modified to stably express a DOX-ON inducible E2F1 expression and were a kind gift from Dr. William G. Kaelin's lab. G-CSF mobilized human PB CD34+ cells were obtained from Stemcell Technologies (#700060.1 Lot:230472503C).

Authentication

Human cell lines as described above were originally obtained from ATCC where they were authenticated and then early passage cell lines were frozen and used for all experiments within 4 months of thawing.

Mycoplasma contamination

Early passage cells of all the cell lines listed above were tested for mycoplasma (Lonza #LT07-218) and then were frozen using Bambanker's freezing medium (Bulldog Bio) and maintained in culture no more than 4 months where early passage vials were thawed.

Commonly misidentified lines (See [ICLAC](#) register)

There were no commonly misidentified cell lines used.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

SCLC xenograft studies were performed in 7-8 week old female Nude mice (Envigo, Hsd:Athymic Nude-Foxn1nu). SCLC PDX studies were performed in 7-8 week-old female NSG (Jackson Laboratories, NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice.

Wild animals

There are no wild animals used in this study.

Reporting on sex

All mice used in the animal studies were 7-8 week old females.

Field-collected samples

This study does not include field-collected samples.

Ethics oversight

All experiments herein comply with all ethical regulations. Specifically, all mouse experiments complied with National Institutes of Health guidelines and were approved by Dana-Farber Cancer Institute Animal Care and Use Committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

For PI cell cycle analysis, early passage cells were washed once in ice-cold PBS and then fixed in ice-cold 80% ethanol (added dropwise) for at least 2 hours at -20 degrees celsius. The cells were then centrifuged at 206 x g (Eppendorf centrifuge 5804 R, A-4-44 rotor, 2000 rpm) for 5 mins, washed once in PBS, centrifuged again at 206 x g, and then washed again in PBS containing 0.5% BSA. Finally, cells were stained with Propidium Iodide (PI) (BD # 550825) for 15 minutes at room temperature.

For p-H3 and cleaved PARP FACS analysis early passage cells were treated with the drug for appropriate time and then washed once in room temperature PBS and then fixed in 4% paraformaldehyde for 15 minutes at room temperature. The cells were then centrifuged at 206 x g (Eppendorf centrifuge 5804 R, A-4-44 rotor, 2000 rpm) for 5 mins at room temperature, washed once in PBS, centrifuged again at 206 x g, and then permeabilized with ice cold methanol at 4 degrees celsius for 30 minutes. The cells were then washed again in PBS and then incubated with Alexa-647 conjugated phospho-histone H3 (Ser10) antibody (Cell Signaling, #3458) or Pacific blue conjugated cleaved PARP antibody (Cell Signaling, Asp214, D64E10, #60068) for 1 hour at room temperature, then washed once in PBS containing 0.5% BSA, centrifuged at 400 x g. FACS analysis was then performed to determine the % positive stained cells.

For FACS base EDU incorporation assay, early passage cells were pulsed for 1h with 10 micromolar EdU (Click-It Plus EdU Flow Cytometry Assay kit, Invitrogen #C10632) prior to collection. Following incubation, cells were washed once with ice-cold PBS and fixed by incubation in ice-cold 80% ethanol for at least 2 hours at -20 degrees celsius. EdU was fluorescently tagged with Alexa FluorTM 488 by click reaction (Click-it Plus EdU flow cytometry assay kit, Invitrogen, #C10632). DNA content was monitored by FxCycle Violet stain (Invitrogen, #F10347) or DAPI (Cell Signaling, #4083).

For HSPC cell cycle and apoptosis analysis, Cell cycle profile and apoptosis detection: For FACS based EDU incorporation assay and apoptosis detection, HSPCs were pulsed for 1h with 10 micromolar EdU (Click-It Plus EdU Flow Cytometry Assay kit, Invitrogen #C10634) prior to collection. Following incubation, cells were washed once with PBS-BSA and then stained with an anti-CD34-Per-CP antibody. Next cells were fixed by incubation in 4% PFA. EdU was fluorescently tagged with Alexa FluorTM 647 by click reaction (Click-it Plus EdU flow cytometry assay kit, Invitrogen, #C10634). After EdU labeling, cells were stained with FITC-mouse anti-cleaved PARP (Asp214). DNA content was monitored by FxCycle Violet stain (Invitrogen, #F10347).

Instrument

All independent biological replicates of PI, p-H3 and cleaved PARP FACS experiments were analyzed on a LSR Fortessa flow cytometer (Becton Dickinson, Franklin Lakes, NJ) using the same Fortessa at the Dana-Farber Hematology Neoplasia flow cytometry core.

For EDU incorporation FACS, the cytometry analysis for EdU and DNA content was performed by full spectral flow cytometry on a Northern Lights cytometer (Cytek Biosciences)

Software

For all FACS experiments data was analyzed on FlowJo 10.8.1. and FlowJo 10.9.0.

Cell population abundance

Given these were healthy cell lines, the majority of cells (>80%) were live and all live cells were used for analysis of PI, p-H3 and EDU.

For cleaved PARP FACS all cell population was considered including dead cells to analyze apoptosis.

Gating strategy

Live cells were first gated on by forward scatter/side scatter, doublets were excluded, and then percent positive Alexa-647 conjugated phospho-histone H3 (Ser10) antibody (Cell Signaling, #3458), Propidium Iodide (BD # 550825) and Alexa Fluor 488 by click reaction (Click-it Plus EdU flow cytometry assay kit, Invitrogen, #C10632) was compared to a matched negative control.

For cleaved PARP analysis, all populations were gated on side scatter versus Pacific blue conjugated cleaved PARP (Cell

Signaling, Asp214, D64E10, #60068) to identify apoptotic cells.

To detect cells with EdU+ 4C DNA content, initial gating was performed to exclude cell debris by plotting SSC-H vs FSC-H.

From this population, single cells were then selected by plotting FxCycle-Violet-H vs Fx-Cycle Violet-A and a gate was drawn to exclude doublets (cells with 2:1 Fx-Cycle-Violet area). Within the single cell population gate, EdU+ cells were identified by plotting EdU-AlexaFluor488 vs FxCycle-Violet. The EdU+ 4C DNA content population was identified as cells with AF488 signal above background (DMSO, no EdU control) with 4C DNA content.

HSPC cells were first gated by FSC-H vs SSC-H. From this population, single cells were then selected by plotting FxCycle-Violet-H vs Fx-Cycle Violet-A and a gate was drawn to exclude doublets (cells with 2:1 Fx-Cycle-Violet area). Within the single cell population gate, CD34+ cells were identified as cells with Per-CP signal above background (DMSO, no Per-CP antibody control). From the CD34+ population, EdU+ (S phase population) cells were identified as cells with AF647 signal above background (DMSO, no EdU control). G0-G1 population cells were identified as cells with background level EdU and 2N DNA content. G2-M phase cells were identified as cells with background level EdU and 4N DNA content. Cells with cleaved PARP were identified within the single cell gate as cells with FITC signal above background (DMSO, no anti-cPARP-FITC antibody control). Staurosporine was used as a positive control to identify cells with cleaved-PARP signal.

A gating strategy figure for all FACS analysis is included in supplementary figure 3 of the supplementary Information section.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.