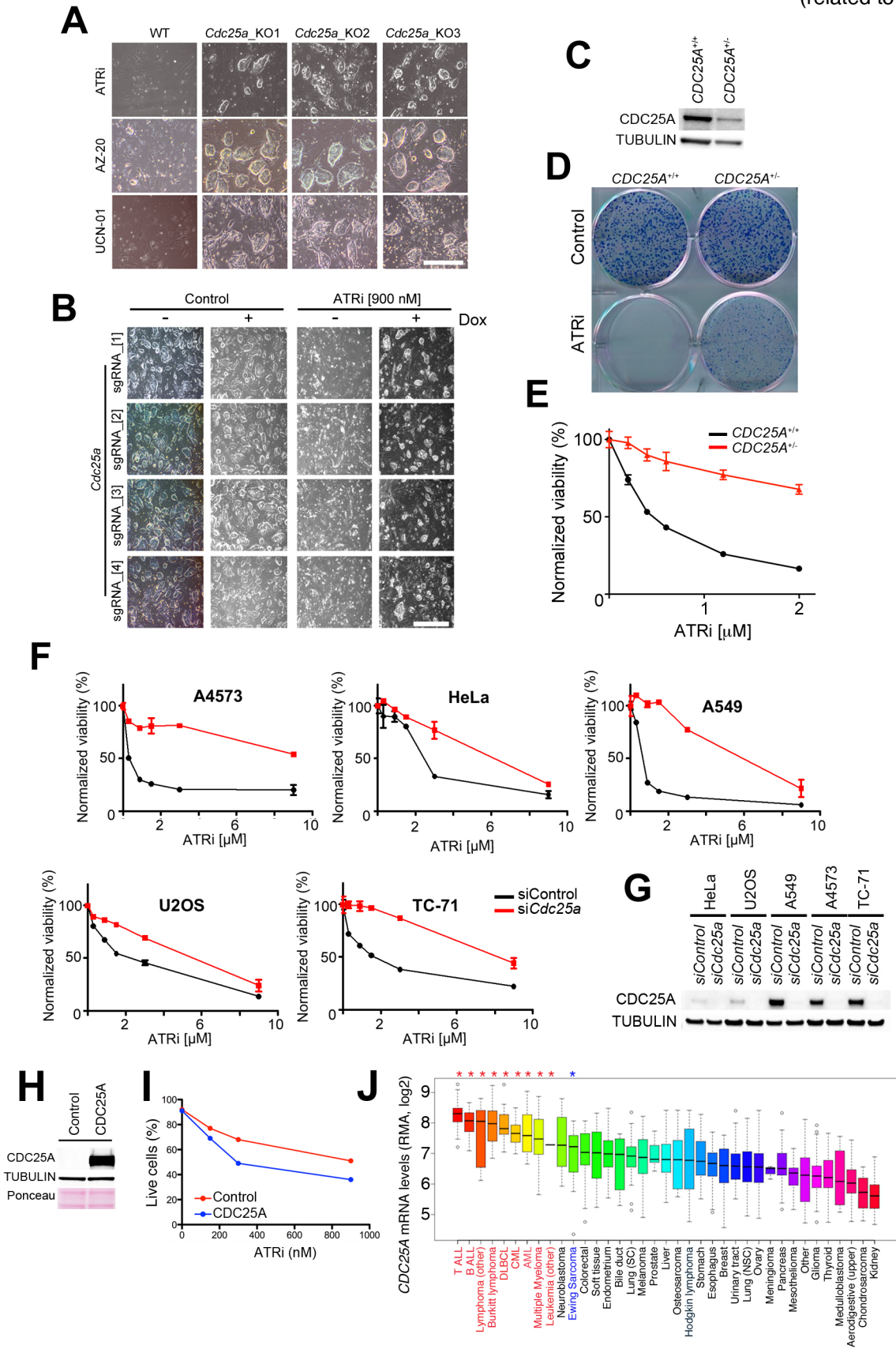


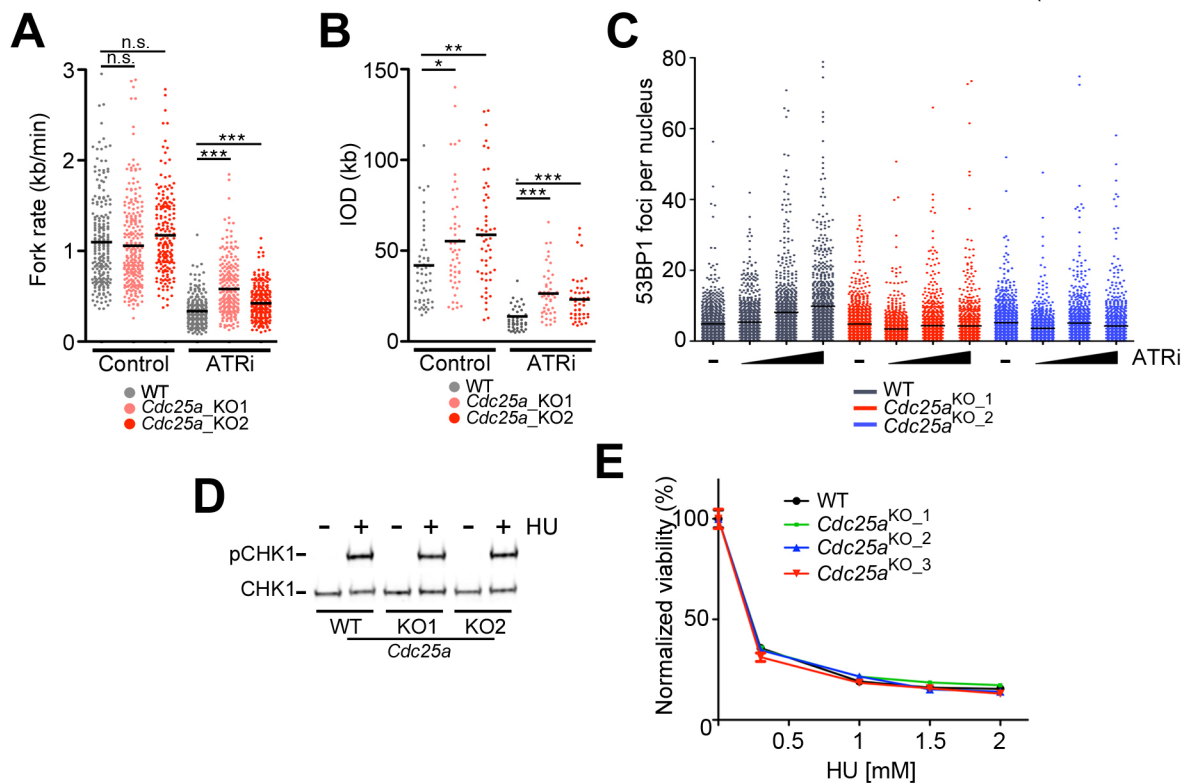
**Figure S1** (related to **Fig. 1**). (A,B) Levels of Cas9 mRNA evaluated by RT-PCR (normalized to levels of GAPDH mRNA) in response to Dox observed in the 2 clones of ES<sup>Cas9</sup> cells used in this study (0, 0.025, 0.05, 0.1, 0.25, 0.5, 1 and 2 µg/ml; 48 hr). The high stringency of the system prevents cleavage in the absence of Dox. In addition, the system is reversible as indicated by the rapid disappearance of Cas9 after Dox removal (2 µg/ml for 48 hr prior to the removal). Error bars indicate s.d. (n=3). (C) Scheme that illustrates the pipeline we have followed for our screenings. Briefly, ES<sup>Cas9</sup> cells were infected at a low (0.3) multiplicity of infection (MOI) with a mouse genomewide library of sgRNAs described before (Koike-Yusa et al., 2014). The library is contained in lentiviruses expressing BFP, which enabled sorting of the infected cells. Dox was added (2 µg/ml) for 10 consecutive days for the generation of mutant cells. 5 million cells from the library were used per screen (50X library coverage) and exposed to test compounds until resistant clones could be isolated and expanded. DNA fragments containing sgRNA sequences were amplified by PCR and identified by Sanger sequencing.

**Fig. S2**  
(related to Fig.2)



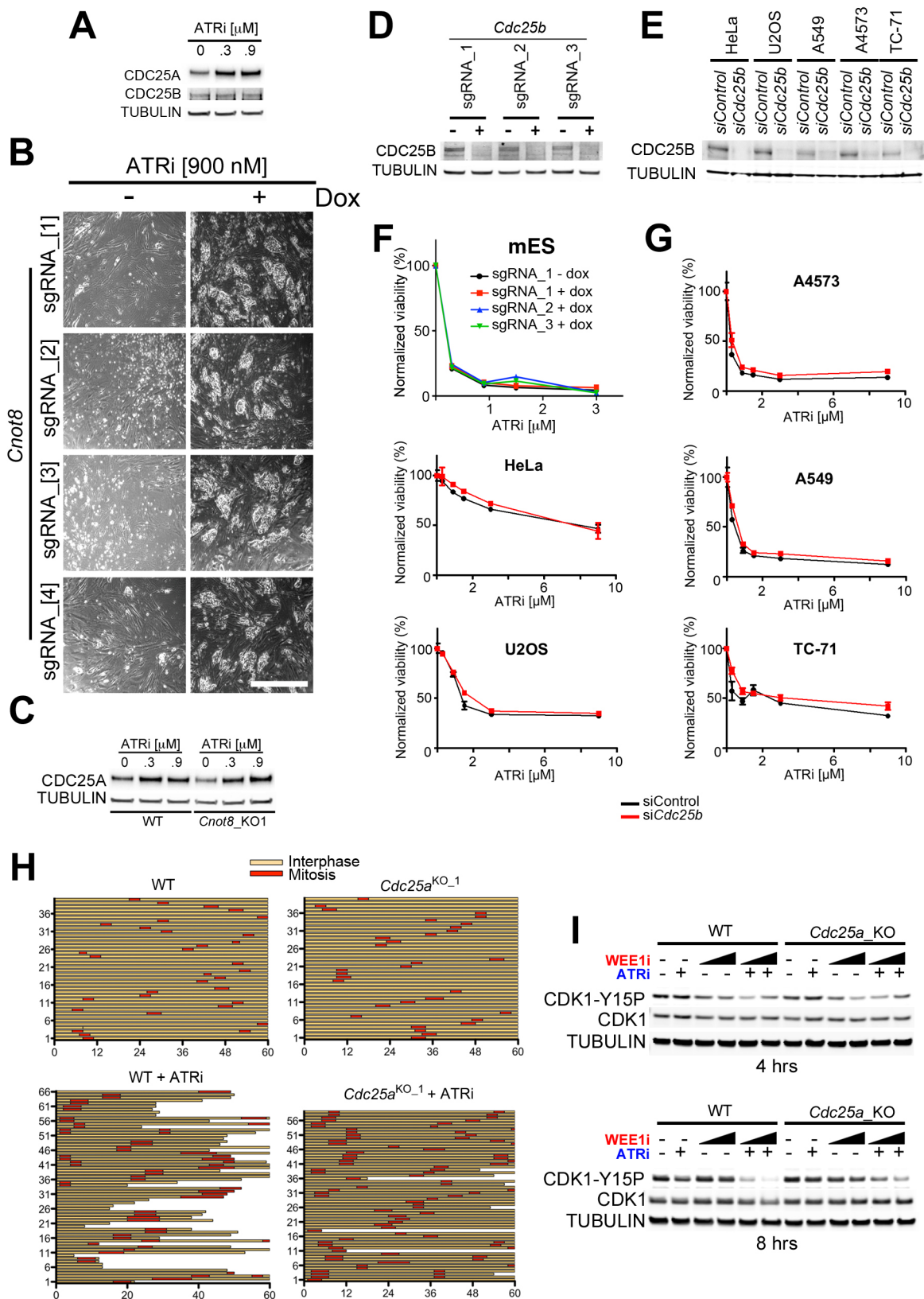
**Figure S2** (related to **Fig. 2**). (A) Representative pictures of the resistance to ATRi (1  $\mu$ M), AZ20 (2  $\mu$ M) and UCN-01 (800 nM) observed in CDC25A-deficient ES<sup>Cas9</sup> cells exposed to the compounds for 72 hr. ES cells are grown on top of a feeder layer of growth-arrested MEF, which are unaffected by the treatment. Scale bar (white) indicates 25  $\mu$ m. (B) Representative pictures of the resistance to ATRi (900 nM) observed in CDC25A-deficient ES<sup>Cas9</sup> cells exposed to the compound for 72 hr. Lentiviruses carrying 4 independent *Cdc25a*-targeting sgRNA sequences were used (primers provided in Supplementary Table 2). Note that resistance is only observed after cells are exposed to Dox (2  $\mu$ g/ml) for 5 days before being exposed to ATRi. Scale bar (white) indicates 25  $\mu$ m. (C) WB illustrating the reduction of CDC25A observed in *CDC25A*<sup>+/-</sup> HAP1 cells that were generated by CRISPR-Cas9. TUBULIN levels are shown as a loading control. (D) Representative picture of clonogenic cultures of WT and *CDC25A*<sup>+/-</sup> HAP1 cells exposed or not to ATRi (400 nM) for 3 days. Plates were stained with methylene blue to facilitate the visualization of colonies. (E) XTT viability assay in WT and *CDC25A*<sup>+/-</sup> HAP1 cells exposed to ATRi for 24 hr at the indicated doses. Data are representative of 3 independent experiments. Error bars indicate s.d. **NOTE:** HAP1 is an adherent derivative of the human near-haploid KBM7 cell line that was generated in an attempt to reprogram KBM7 cells to pluripotency (Carette et al., 2011). Consistent with a previous study that found CDC25A to be essential in HAP1 cells (Blomen et al., 2015), no nullzygous mutants of *CDC25A* could be obtained. In contrast, we were able to obtain *CDC25A*<sup>+/-</sup> clones that had spontaneously diploidized during the gene editing process. (F) CellTiter-Glo viability assay in A4573, HeLa, A549, U2OS and TC-71 human cancer cell lines transfected with control or *CDC25A*-targeting siRNAs and exposed to ATRi for 36 hr at the indicated doses. Data are representative of 3 independent experiments. Error bars indicate s.d. (G) WB illustrating the reduction of CDC25A levels observed in each cell line 72 hours after siRNA transfection. (H) WB illustrating the expression of CDC25A observed in NIH3T3 48 hrs after transfection with a plasmid coding for human CDC25A (pCMV-HA-CDC25A; kind gift of Jiri Lukas) or mock transfected (control). TUBULIN levels and the Ponceau image are shown as loading control. (I) Percentage of cells that remain alive (as determined through DAPI exclusion by FACS) 3 days after transfection with the plasmid coding for CDC25A defined above or mock transfected, and in the presence of increasing doses of ATRi (0, 150, 300 and 900 nM). The inhibitor was added 24 hr after transfection. (J) The figure illustrates *CDC25A* mRNA expression levels in various human cancers. *CDC25A* expression is highest on tumors, significantly hematopoietic, that have been already shown to be highly dependent on a proficient ATR/CHK1 pathway (red). Among solid tumors, we have also observed a high sensitivity to ATR inhibitors in Ewing Sarcoma (blue; unpublished observations). Data were obtained from the Cancer Cell Line Encyclopedia (Barretina et al., 2012) (<http://www.broadinstitute.org/ccle/home>).

**Fig. S3**  
(related to Fig.3)



**Figure S3** (related to Fig. 3). (A,B) Replication fork rates (A) and inter-origin distances (B) analyzed by DNA fiber analyses (see Methods) measured in WT and CDC25A-deficient ES<sup>Cas9</sup> cells exposed to ATRi for 1 hr (900 nM). Note that whereas there is a partial reduction on the effects of ATRi both in origin firing and fork rates, this is likely reflecting the slightly reduced origin densities that are found on unchallenged CDC25A-deficient cells. (C) HTM analysis of the number of 53BP1 foci per individual nucleus in WT and CDC25A-deficient ES<sup>Cas9</sup> cells treated (or not) with ATRi (0.3, 0.9 and 2 μM; 4 hr). Data are representative of 3 independent experiments. (D) WB illustrating the levels of CHK1 phosphorylation (S345) and total CHK1 in WT and CDC25A-deficient ES<sup>Cas9</sup> cells exposed to HU for 2 hr (2 mM). (E) CellTiter-Glo viability assay in WT and CDC25A-deficient ES<sup>Cas9</sup> cells exposed to HU for 24 hr at the indicated doses. Data are representative of 3 independent experiments. Error bars indicate s.d.

**Fig. S4**  
(related to Fig.4)



**Figure S4** (related to **Fig. 4**). (A) WB illustrating the induction of CDC25A and CDC25B levels that occurs in ES<sup>Cas9</sup> cells in response to ATRi. TUBULIN levels are shown as a loading control. (B) Representative pictures of the resistance to ATRi (900 nM) observed in CNOT8-deficient ES<sup>Cas9</sup> cells exposed to the compound for 72 hr. Lentiviruses carrying 4 independent *Cnot8*-targeting sgRNA sequences were used. Note that resistance is only observed after cells are exposed to Dox (2  $\mu$ g/ml) for 5 days before being exposed to ATRi. Scale bar (white) indicates 25  $\mu$ m. (C) WB illustrating the induction of CDC25A levels that occurs in WT and CNOT8-deficient ES<sup>Cas9</sup> cells in response to ATRi at the indicated doses for 4 hr. TUBULIN levels are shown as a loading control. (D) WB illustrating the loss of CDC25B expression in independent pools of ES<sup>Cas9</sup> cells infected with lentiviruses encoding 3 independent sgRNAs targeting *Cdc25b*. Note that only ES<sup>Cas9</sup> cells treated with DOX expressed Cas9 which enabled *Cdc25b* editing and subsequent loss of protein expression. (E) WB illustrating the reduction of CDC25B levels observed in A4573, HeLa, A549, U2OS and TC-71 human cancer cell lines transfected with control or *Cdc25b* targeting-siRNAs 72 hours after siRNA transfection. (F) CellTiter-Glo viability assay in wild type and CDC25B-deficient ES<sup>Cas9</sup> cells exposed to ATRi for 24 hr at the indicated doses. Data are representative of 3 independent experiments. Error bars indicate s.d. (G) CellTiter-Glo viability assay in A4573, HeLa, A549, U2OS and TC-71 human cancer cell lines transfected with control or *Cdc25b* targeting-siRNAs and exposed to ATRi for 36 hr at the indicated doses. Data are representative of 3 independent experiments. Error bars indicate s.d. (H) Schematic representation showing the time spent in mitosis and interphase in individual asynchronously growing WT and CDC25A-deficient ES<sup>Cas9</sup> cells untreated or treated with 900nM ATRi and imaged every 15 minutes for a total of 15 hr. Time spent in mitosis was defined by the time between chromosome condensation and subsequent cytokinesis. Cells were infected with H2B-eGFP expressing lentiviruses to facilitate the monitoring of chromosome dynamics. Each row represents one cell, and the x-axis indicates the frame. A minimum of 40 individual cells was scored for each condition. (I) WB showing the levels of CDK1 and CDK1-Y15P in WT and CDC25A-deficient ES<sup>Cas9</sup> cells untreated or treated with ATRi (300 nM) and/or WEE1i (100 or 300 nM) for 4 hr or 8 hr.

**Table S1.** sgRNA sequences identified in the screenings

6-TG [20mM]		
<i>1<sup>st</sup> screening (ES<sup>Cas9</sup> clone #1)</i>		
Clone Number	Gene(s) Identified	sgRNA sequence
Clone 1	PDE2A	GCAGTTGACCATGGGACGTC
Clone 2	HPRT	GTAGAATGATCAGTCAACGG
Clone 3	HPRT	GTCATGGACTGATTATGGAC
Clone 4	HPRT	GTAGAATGATCAGTCAACGG
Clone 5	HPRT	GTAGAATGATCAGTCAACGG
<i>2<sup>nd</sup> screening (ES<sup>Cas9</sup> clone #2)</i>		
Clone 1	HPRT	GTCATGGACTGATTATGGAC
Clone 2	HPRT	GATTCCTCATGGACTGATTA
Clone 3	HPRT	GTAGAATGATCAGTCAACGG
Clone 4	HPRT	GATTCCTCATGGACTGATTA
Clone 5	HPRT	GATTCCTCATGGACTGATTA
Clone 6	HPRT	GATTCCTCATGGACTGATTA
ATRi [900 nM]		
<i>1<sup>st</sup> screening (ES<sup>Cas9</sup> clone #1)</i>		
Clone 1	TMEM123	GTAGTTTTTGGCAGTACGGA
Clone 2	-	-
Clone 3	CDC25a	GCCTCAGAATCGACCGATTC
Clone 4	CDC25a	GCCTCAGAATCGACCGATTC
Clone 5	CDC25a	GCCTCAGAATCGACCGATTC
Clone 6	CDC25a	GCCTCAGAATCGACCGATTC
Clone 7	CDC25a	GCCTCAGAATCGACCGATTC
<i>2<sup>nd</sup> screening (ES<sup>Cas9</sup> clone #2)</i>		
Clone 1	CEACAM11, CNOT8	GCATTTTCTTCAACCCTAGA, GATGTCATAAGCAGCTCTGC
Clone 2	CDC25a	GCCTCAGAATCGACCGATTC
Clone 3	CDC25a	GCCTCAGAATCGACCGATTC
Clone 4	PCDHB2, HOXB5	GGTCTTGAGCACGTTCTATT, GTGGCTGTCGCCGTTAGTGC
Clone 5	CNOT8	GCAGAGCTGCTTATGACATC
Clone 6	CDC25a	GCCTCAGAATCGACCGATTC
Clone 7	CDC25a	GCCTCAGAATCGACCGATTC
Clone 8	CDC25a	GCCTCAGAATCGACCGATTC
Clone 9	CDC25a	GCCTCAGAATCGACCGATTC
Clone 10	CNOT8	GCAGAGCTGCTTATGACATC
Clone 11	CLCA1, IL20	GGTTTTGGCCTCGTATATTC, TTCCTTTTGTATTGCCTGT
Clone 12	DGCR8,	GACTACAGTTCGGGTCTATG
Clone 13	OLFR877	GTATGGGATGGGAGTTTTTG
Clone 14	CNOT8	GCAGAGCTGCTTATGACATC
Clone 15	CNOT8	GCAGAGCTGCTTATGACATC
Clone 16	CNOT8	GCAGAGCTGCTTATGACATC

# Supplementary Experimental Procedures

## Cell culture

ES cells were grown on a feeder layer of inactivated mouse embryonic fibroblasts (MEF) with DMEM (high glucose) supplemented with 15% knockout serum replacement (Invitrogen), LIF (1000 U/ml), 0.1 mM non-essential amino acids, 1% glutamax and 55 mM β-mercaptoethanol. MEFs were obtained from 13.5 embryos by standard methods. MEFs and NIH-3T3 cells were cultured in DMEM (Invitrogen), 15%FBS and 0.1 mM non-essential amino acids in low-oxygen conditions. 293T, HeLa, U2OS, A549, A4573 and TC71 cells (ATCC) were grown in DMEM, 10% FBS and 0.1 mM non-essential amino acids. HAP1 cells (kind gift of T Brummelkamp) were grown in IMDM (Invitrogen) and 15%FBS.

## Plasmids

The Cas9 expression vector was acquired from Addgene (42230) and used to amplify the coding sequences of Cas9 by PCR. The PCR product was digested with EcoRI and subcloned into an EcoRI-linearized pBS31 plasmid to generate pBS31-Cas9. The lentiviral plasmid pKLV-U6gRNA-PGKpuro2ABFP (Addgene, 50946) was used to express sgRNAs in cells. The sequences of the sgRNAs used were designed and cloned as described by using the MIT CRISPR design tool (<http://www.genome-engineering.org/crispr/>). Only sgRNAs with the higher scores and lower probabilities of generating exonic off-target effects were selected. The genomewide mouse lentiviral CRISPR sgRNA library (Koike-Yusa et al., 2014) was acquired from Addgene (50947) and amplified following supplier's recommendations. pCMV-HA-CDC25A and pLenti-H2B-EGFP were kind gifts from Jiri Lukas and Marcos Malumbres, respectively. All generated constructs were sequenced to rule out the presence of mutations.

## Lentiviral production

Individual lentiviral vectors (pKLV-U6gRNA-PGKpuro2ABFP) or the genomewide mouse lentiviral CRISPR sgRNA library (Koike-Yusa et al., 2014) (Addgene 50947) were co-transfected with 3<sup>rd</sup> generation packaging vectors in 293T cells using Lipofectamine 2000 (Invitrogen) in order to generate viral supernatants as described (Ruiz et al., 2011). The lentiviral vector used contain a PGKpuro2ABFP cassette and thus, the library was titrated by using a serial dilution of the viral supernatant and evaluating the percentage of BFP-expressing cells 72 hours after infection.

## Western blot

Cell pellets were lysed in 50mM Tris, 150mM NaCl, 1% TritonX-100 or in 50mM Tris pH 7.9/8M Urea/1%Chaps and incubated with shaking at 4°C for at least 30 min. 25 μg of supernatants were run and transferred for detection by using the corresponding antibodies. Tubulin was used as control loading (see the Table below for further details about Antibodies).

### **High-throughput microscopy**

High Throughput Microscopy (HTM) analysis was performed as previously described (Toledo et al., 2008). Briefly, a total of 8,000-20,000 cells per well, depending on the cell type or experiment, were seeded on  $\mu$ CLEAR bottom 96-well plates (Greiner Bio-One). One day after seeding, cells were untreated or treated with ATRi and/or WEE1i at the indicated concentrations and incubation times.  $\gamma$ H2AX, 53BP1 and H3S10P immunofluorescences were performed using standard procedures. To measure ssDNA, cells were cultured in 10  $\mu$ M BrdU for 36 h before adding ATRi for 4h. Cells were fixed with methanol at  $-20^{\circ}\text{C}$ , blocked with PBS containing 5% of BSA and then incubated overnight at  $4^{\circ}\text{C}$  with an anti-BrdU antibody and a secondary antibody AlexaFluor-488 (1:250, Life Technologies). Images from each well were automatically acquired by an Opera High-Content Screening System (Perkin Elmer) at non-saturating settings. Images were segmented using the DAPI staining to generate masks matching cell nuclei from which the mean signals or number of foci were calculated. Data were represented with the Prism software (GraphPad Software). For Edu incorporation analyses, Edu was added to the media at a final concentration of 20 $\mu$ M during the last 45 minutes of incubation with the drug. Edu incorporation into DNA was detected using the Click-iT™ Edu Alexa Fluor® Imaging kit (Invitrogen/Molecular Probes).

### **Flow cytometry**

To analyse cell cycle profiles by flow cytometry, ES cells were incubated with ATRi for the indicated time and 20 $\mu$ M Edu was added during the last 45 minutes of incubation. Trypsinized cells were fixed with 4% paraformaldehyde for 10 minutes and Edu was detected by the Click-iT™ Edu Alexa Fluor® Imaging kit (Invitrogen/Molecular Probes). To analyse the percentage of H3S10P or  $\gamma$ H2AX-positive cells, ES cells were incubated with ATRi, trypsinized and fixed in suspension by the addition of cold 70% ethanol and maintained at  $-20^{\circ}\text{C}$  at least for 24h. Cells were suspended in 0.25% Triton X-100 in PBS, and incubated on ice for 15 min. After centrifugation, the cell pellets were suspended in PBS containing 1% BSA and antibodies recognizing H3S10P and mouse  $\gamma$ H2AX. Secondary antibodies conjugated with Alexa-647 or Alexa-488 (Life Technologies) were used at a dilution of 1:250. DNA content was visualized with either propidium iodide (PI) or Cell Cycle blue-405.

### **DNA fiber analyses**

Exponentially growing cells were pulse-labeled with 50  $\mu$ M CldU (20 min) followed by 250  $\mu$ M IdU (20 min). Labeled cells were collected and DNA fibers were spread in buffer containing 0.5% SDS, 200 mM Tris pH 7.4 and 50 mM EDTA. For immunodetection of labeled tracks, fibers were incubated with primary antibodies (for CldU, rat anti-BrdU; for IdU, mouse anti-BrdU) for 1 hour at room temperature and developed with the corresponding secondary antibodies for 30 minutes at room temperature. Mouse anti-ssDNA antibody was used to assess fiber integrity. Slides were examined with a Leica DM6000 B microscope, as described previously (Jacome et al., 2015). The

conversion factor used was  $1 \mu\text{m}=2.59 \text{ kb}$ . In each assay, >300 tracks were measured to estimate fork rate and >50 fibers containing two or more origins were analyzed to estimate inter-origin distance.

### **RNA isolation and real-time PCR**

Total RNA was isolated using the Absolutely RNA Microprep Kit (Stratagene) and cDNA was synthesized using the SuperScript III Reverse Transcriptase kit for RT-PCR (Invitrogen). Real-time PCR was performed using the SYBR-Select Master Mix (Applied Biosystems) in the QuantStudio 6K (Thermo Scientific). GAPDH expression level was used to normalize values of gene expression. Data are shown as fold change relative to the sample control and at least two independent experiments in triplicate were performed. See the Table below for further details about the sequences of the sgRNA primers used in this work.

### **Cell viability assays**

Cells were seeded at 10,000 cells per well in a 96-well tissue culture plate and treated with the indicated concentrations of ATRi or left untreated. 24-36 hours after the treatment, cell viability was measured using either a XTT colorimetric assay (Roche) or a luminescent system (CellTiter-Glo, Promega), according to the manufacturer's protocols. Viability is plotted as percentage of viability compared to untreated control.

### **Knockdown experiments**

Exponentially growing Hela, U2OS, A549, A4573 and TC71 cells (ATCC) were trypsinized and transfected in suspension with 75nM of human siRNAs targeting CDC25A or CDC25B (SMART pools, Dharmacon) by using Lipofectamine RNAiMAX reagent (Thermo Fisher Scientific). For each independent experiment, the efficiency of the knockdown on transfected cells was evaluated by WB.

### **Live cell imaging**

To evaluate mitosis entry and duration on ES cells, exponentially growing ES<sup>Cas9</sup> cells were infected with lentiviruses encoding the histone H2B-eGFP and seeded on 8 wells  $\mu$ -Slide (Ibidi, 80826) pre-treated with gelatin 0.1%. The day after, cells were untreated or treated with 900nM ATRi and imaged every 15 minutes for a total of 15 hours in a Leica DMI 6000 B system. Mitotic events were easily observed by chromatin condensation. At least 40 cells were followed for the duration of the recording in order to evaluate the time spent in mitosis and interphase for each individual cell.

### Table of oligos used in this work:

Primer name	Sequence 5'-3'
Cas9-qPCR-F	CCGAAGAGGTCGTGAAGAAG
Cas9-qPCR-F	GCCTTATCCAGTTCGCTCAG
pKLV-U6gRNA-EGFP-sgRNA1-F	CACCGGGCACGGGCAGCTTGCCGGGT
pKLV-U6gRNA-EGFP-sgRNA1-R	TAAAACCCGGCAAGCTGCCCGTGCCC
pKLV-U6gRNA-m-p53-sgRNA1-F	CACCGTCTACAGATGACTGCCATGGGT
pKLV-U6gRNA-m-p53-sgRNA1-R	TAAAACCCATGGCAGTCATCTGTAGAC
pKLV-U6gRNA-m-p53-sgRNA2-F	CACCGTCTCTACAGATGACTGCCAGT
pKLV-U6gRNA-m-p53-sgRNA2-R	TAAAACTGGCAGTCATCTGTAGAGAGC
pKLV-U6gRNA-m-p53-sgRNA3-F	CCACGAGTGAAGCCCTCCGAGTGTCGT
pKLV-U6gRNA-m-p53-sgRNA3-R	TAAAACGACACTCGGAGGGCTTCACTC
pKLV-U6gRNA-m-cdc25a-sgRNA1-F	CACCGCCTCAGAATCGACCGATTCTGT
pKLV-U6gRNA-m-cdc25a -sgRNA1-R	TAAAACGAATCGGTCTGATTCTGAGGC
pKLV-U6gRNA-m-cdc25a -sgRNA2-F	CACCGTGCAAGCGAAGAACAGGCGGGT
pKLV-U6gRNA-m-cdc25a -sgRNA2-R	TAAAACCCGCCTGTTCTTCGCTTGAC
pKLV-U6gRNA-m-cdc25a -sgRNA3-F	CACCGACGCCTGCGCCGAGCCACGT
pKLV-U6gRNA-m-cdc25a -sgRNA3-R	TAAAACGTGGGCTGCGGCGCAGGCGTC
pKLV-U6gRNA-m-cdc25a -sgRNA4-F	CACCGTTCCTCCGTTGGGCTGCGGCGCGT
pKLV-U6gRNA-m-cdc25a -sgRNA4-R	TAAAACGCGCCGAGCCACGGGGAAC
pKLV-U6gRNA-m-cdc25b-sgRNA1-F	CACCGATGCCACCCAGCACGCGGGCGT
pKLV-U6gRNA-m-cdc25b-sgRNA1-R	TAAAACGCCCCGCTGCTGGGTGGCATC
pKLV-U6gRNA-m-cdc25b-sgRNA2-F	CACCGTCTCAGTCCTGCCGCGTGCGT
pKLV-U6gRNA-m-cdc25b-sgRNA2-R	TAAAACGCACGCGGGCAGGACTGAGAC
pKLV-U6gRNA-m-cdc25b-sgRNA3-F	CACCGTCAGCGGCGCGCCACCTCTGT
pKLV-U6gRNA-m-cdc25b-sgRNA3-R	TAAAACAGAGGTGGCGCGGCGCTGAC
pKLV-U6gRNA-m-cdc25b-sgRNA4-F	CACCGCCAGCCTGTTCTGGAACGACGT
pKLV-U6gRNA-m-cdc25b-sgRNA4-R	TAAAACGTCGTTCCAGAACAGGCTGGC
pKLV-U6gRNA-h-cdc25a-sgRNA1-F	CACCGCCAAATAGCGCCTTCACGACGT
pKLV-U6gRNA-h-cdc25a -sgRNA1-R	TAAAACGTCGTGAAGGCGCTATTTGGC

sgRNA1-R	
pKLV-U6gRNA-h-cdc25a - sgRNA2-F	CACCGTTCACGACGGGCTGCGACGCGT
pKLV-U6gRNA-h-cdc25a - sgRNA2-R	TAAAACGCGTCGCAGCCCGTCGTGAAC
pKLV-U6gRNA-h-cdc25a - sgRNA3-F	CACCGCGCGTCGCAGCCCGTCGTGAGT
pKLV-U6gRNA-h-cdc25a - sgRNA3-R	TAAAACTCACGACGGGCTGCGACGCGC
pKLV-U6gRNA-h-cdc25a - sgRNA4-F	CACCGTATTTGGCGCTTCAGCCGCCGT
pKLV-U6gRNA-h-cdc25a - sgRNA4-R	TAAAACGGCGGCTGAAGCGCCAAATAC
mGAPDH-F	TTCACCACCATGGAGAAGGC
mGAPDH-R	CCCTTTTGGCTCCACCCT

**Table of antibodies used in this work:**

<b>Antibody</b>	<b>Use</b>	<b>Dilution</b>	<b>Reference</b>
cdc25a	Western blot	1:500	Santa Cruz sc7389
cdc25a	Western blot	1:1000	Cell Signaling, #3652
cdc25b	Western blot	1:1000	Santa Cruz, sc326
CDK1	Western blot	1:500	(Santa Cruz, sc54)
p-CDK1	Western blot	1:1000	Santa Cruz, sc7989
RPA	Western blot	1:1000	Cell Signaling, #2208
p-RPA	Western blot	1:750	Bethyl, A300-245A
H3S10P	Western blot	1:1000	Millipore, 06-570
CHK1	Western blot	1:500	Novocastra
p-CHK1	Western blot	1:500	Cell Signaling, #2348S
p-CDK substrates	Western blot	1:1000	Cell Signaling, #9477
$\gamma$ H2AX	Western blot	1:500	Millipore 05-636
p53	Western blot	1:1000	Cell Signaling, #2524
Tubulin	Western blot	1:50000	Sigma, #T9026
H3S10P	Immunofluorescence	1:500	Millipore, 06-570
$\gamma$ H2AX	Immunofluorescence	1:1000	Millipore 05-636
BrdU	Immunofluorescence	1:100	GE Healthcare Life Sciences
53BP1	Immunofluorescence	1:2000	(Novus 100-304A2)
H3S10P	FACS	1:50	Millipore, 06-570
$\gamma$ H2AX	FACS	1:500	Millipore 05-636
CldU	Fiber analysis	1:100	Abcam ab6326
IdU	Fiber analysis	1:150	BD Biosciences 347580
ssDNA	Fiber analysis	1:200	Millipore MAB3034

## Supplementary References

- Barretina, J., Caponigro, G., Stransky, N., Venkatesan, K., Margolin, A.A., Kim, S., Wilson, C.J., Lehár, J., Kryukov, G.V., Sonkin, D., *et al.* (2012). The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* **483**, 603-607.
- Blomen, V.A., Majek, P., Jae, L.T., Bigenzahn, J.W., Nieuwenhuis, J., Staring, J., Sacco, R., van Diemen, F.R., Olk, N., Stukalov, A., *et al.* (2015). Gene essentiality and synthetic lethality in haploid human cells. *Science*.
- Carrette, J.E., Guimaraes, C.P., Wuethrich, I., Blomen, V.A., Varadarajan, M., Sun, C., Bell, G., Yuan, B., Muellner, M.K., Nijman, S.M., *et al.* (2011). Global gene disruption in human cells to assign genes to phenotypes by deep sequencing. *Nat Biotechnol* **29**, 542-546.
- Jacome, A., Gutierrez-Martinez, P., Schiavoni, F., Tenaglia, E., Martinez, P., Rodriguez-Acebes, S., Lecona, E., Murga, M., Mendez, J., Blasco, M.A., *et al.* (2015). NSMCE2 suppresses cancer and aging in mice independently of its SUMO ligase activity. *EMBO J*.
- Koike-Yusa, H., Li, Y., Tan, E.P., Velasco-Herrera Mdel, C., and Yusa, K. (2014). Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library. *Nat Biotechnol* **32**, 267-273.
- Ruiz, S., Panopoulos, A.D., Herrerias, A., Bissig, K.D., Lutz, M., Berggren, W.T., Verma, I.M., and Izpisua Belmonte, J.C. (2011). A high proliferation rate is required for cell reprogramming and maintenance of human embryonic stem cell identity. *Curr Biol* **21**, 45-52.
- Toledo, L.I., Murga, M., Gutierrez-Martinez, P., Soria, R., and Fernandez-Capetillo, O. (2008). ATR signaling can drive cells into senescence in the absence of DNA breaks. *Genes Dev* **22**, 297-302.