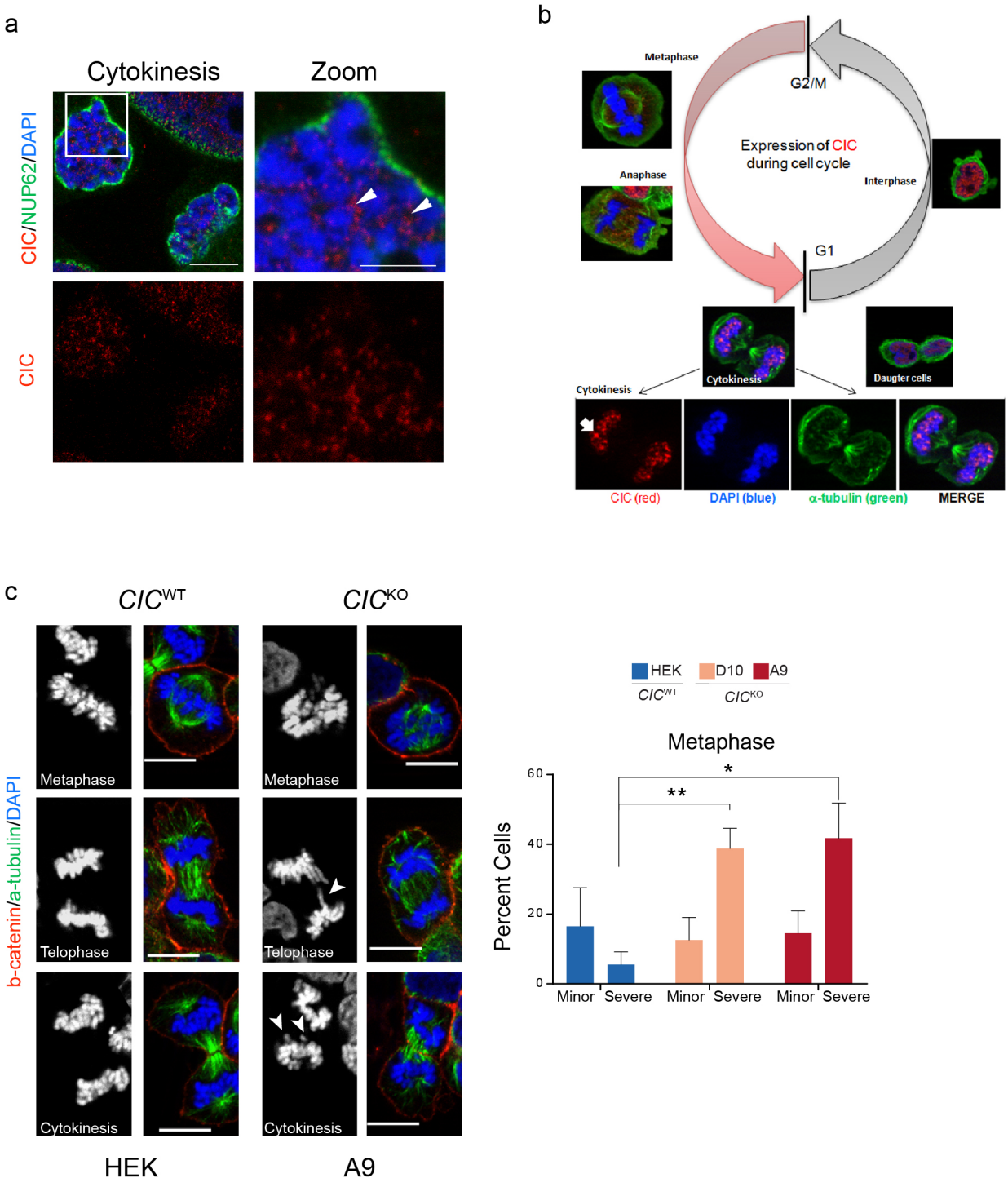
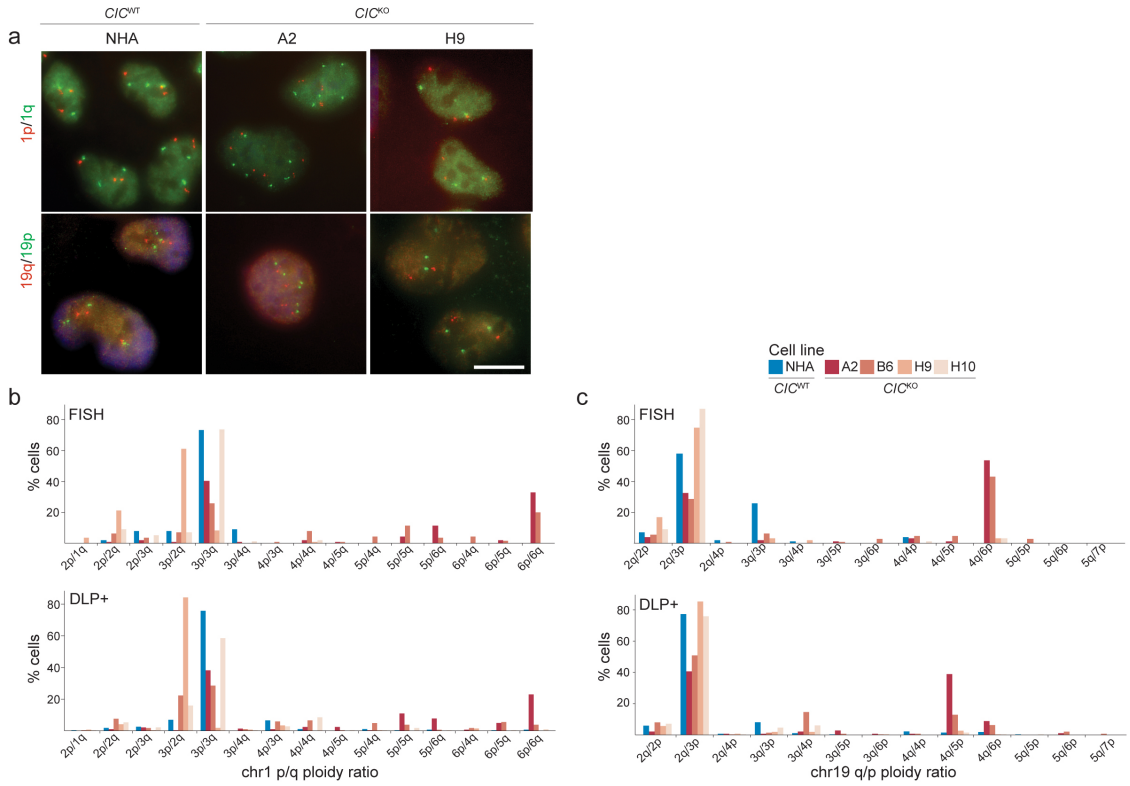


Supplementary Figure 1: CIC localization dynamics are consistent across different cell types.

a. Expression of endogenous CIC (red) and the nuclear envelope protein NUP62 (green) in the HOG line at cytokinesis. DNA was detected using DAPI staining (blue). Zoomed image of early cytokinesis (right) shows a punctate localization pattern for endogenous CIC throughout the decondensing nucleus (arrowheads). Scale bars: 10 µm and 5µm (zoomed image).

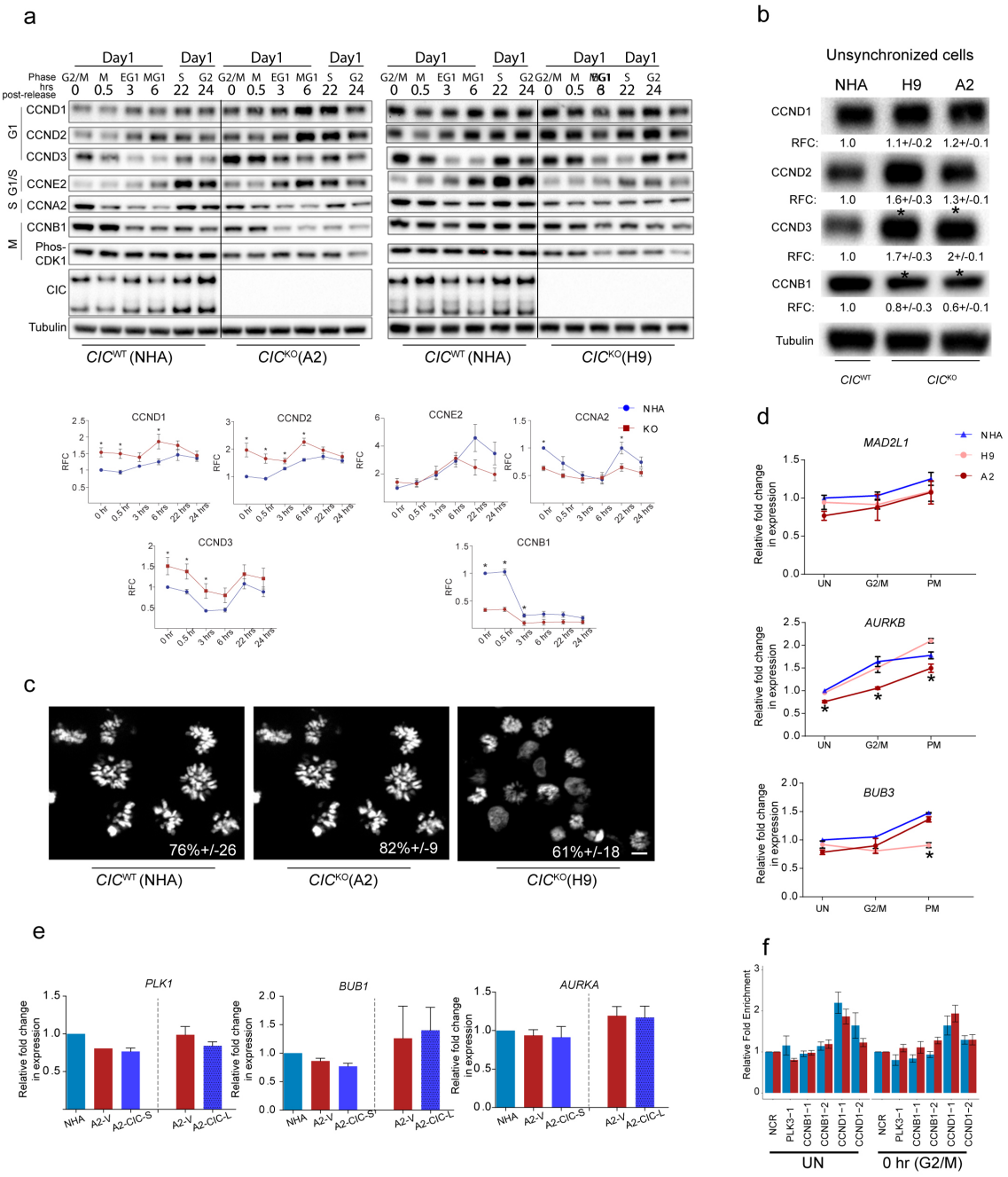
b. Expression of F-CIC-S (FLAG, red) and a-tubulin (green) in *CIC*^{KO} HEK cells stably expressing F-CIC-S (HEK$^{F\text{-}CIC\text{-}S}$) during different stages of the cell cycle. Foci of CIC protein can be observed adjacent to decondensing chromosomes at early cytokinesis (arrow, bottom panel).
c. Left: representative IF images of unsynchronized HEK (*CIC*^{WT}) and A9 (*CIC*^{KO}) cells at metaphase and telophase/cytokinesis. Microtubules (a-tubulin, green), DNA (DAPI, blue) and the cell membrane (b-catenin, red) were detected. DAPI staining alone is shown on the left of each image. *CIC*^{KO} cells (right) show defects in metaphase alignment and lagging chromosomes at telophase and cytokinesis (arrowheads). Scale bar: 10μm. Right: bar graphs show proportions of cells with defects at metaphase (top) and cytokinesis (bottom). Bars represent the mean of three independent experiments and error bars indicate s.e.m. *$p < 0.05$, **$p < 0.01$ (two-sided Student's *t*-test).



Supplementary Figure 2. Loss of CIC is associated with chromosomal instability and aneuploidy.

a. Representative interphase FISH images of *CIC*^{WT} (NHA) and *CIC*^{KO} (A2 and H9) cells. Red dots indicate marker (1p or 19q) and green dots indicate reference (1q, 19p; Methods).

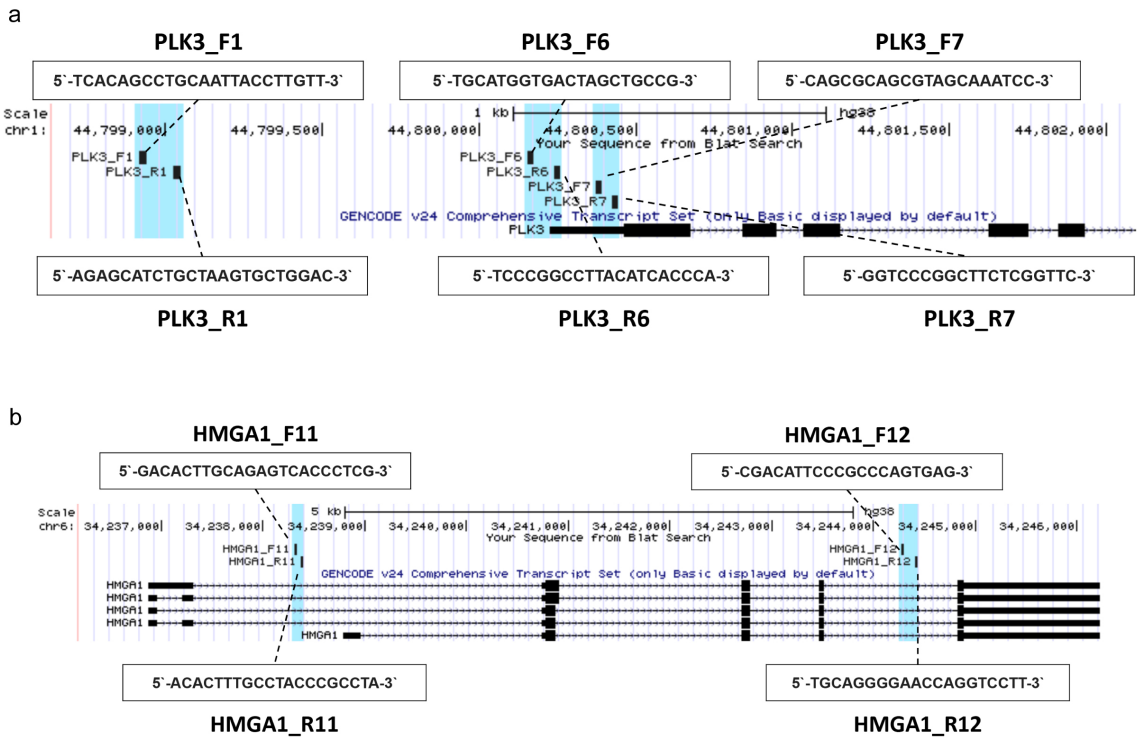
b-c. Proportion of cells with indicated p/q ploidy ratios for chromosome 1 (b) and q/p ploidy ratios for chromosome 19 (c). Top: ratios obtained using interphase FISH analysis (100 cells per cell line). Bottom: ratios obtained using DLP+ (range 266-287 cells per cell line).



Supplementary Figure 3. Transcript and protein expression of CIC and cell cycle regulators.

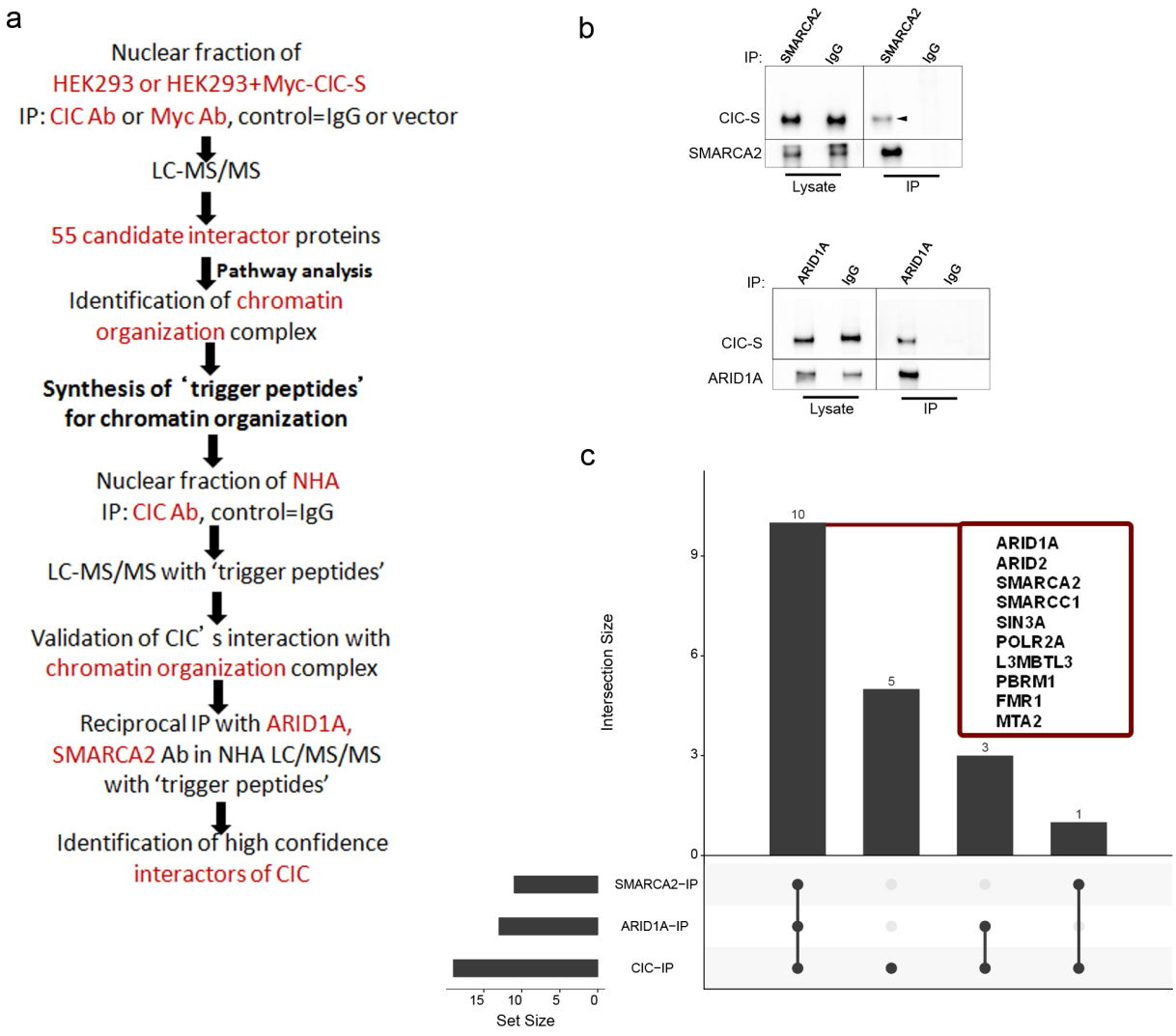
a. Top: representative western blots showing expression of cell cycle proteins in synchronized *CIC*^{WT} (NHA) and *CIC*^{KO} (A2 and H9) cells collected over the course of cell cycle phases at indicated time points post-RO-3306 release (shown below phase labels). Tubulin was used as a loading control. Bottom: western blot quantifications for *CIC*^{WT} (NHA) and *CIC*^{KO} (A2 and H9 combined) cells at indicated time point post-release. Data were obtained from at least three biological replicates. *p < 0.05 (two-sided student *t*-test comparing WT vs KO at each time point).

b. Representative western blot showing expression of select cyclins in unsynchronized *CIC*^{WT} (NHA) and *CIC*^{KO} (A2 and H9) cells. Relative fold-changes (RFC, using NHA as a reference) of densitometry quantifications of select cyclins, calculated as a ratio relative to the respective tubulin band, are shown below the western blots. Data were obtained from three independent biological replicates and the standard deviation (+/-) is also indicated. *$p < 0.05$ (two-sided Student's *t*-test relative to NHA).
c. Representative image showing *CIC*^{WT} (NHA) and *CIC*^{KO} (A2 and H9) cells isolated by mitotic shake-off (Methods) and stained with DAPI. Percentages indicate the fraction of cells in pro-mitosis (mean from three biological replicates +/- standard deviation; n = at least 142 cells for each replicate). Scale bar: 10 μm
d. mRNA expression of select mitotic kinases in unsynchronized cells (UN), synchronized cells at G2/M, and synchronized PM cells isolated by mitotic shake-off, as measured by RT-qPCR. Error bars indicate s.e.m. *$p < 0.05$ (one-way ANOVA with Holm-Sidak's multiple comparison for each time point).
e. Gene expression in unsynchronized *CIC*^{KO} (A2) cells expressing F-CIC-S or F-CIC-L cells, as measured by RT-qPCR. Bars represent the mean from three independent experiments and error bars indicate s.e.m.
f. Enrichment of CIC binding to select sites relative to the NCR, as measured by ChIP-qPCR against CIC in unsychronized (UN) and G2/M *CIC*^{WT} (NHA) and *CIC*^{KO} (A2) cells. Bars represent the mean over three independent replicates and error bars represent s.e.m.

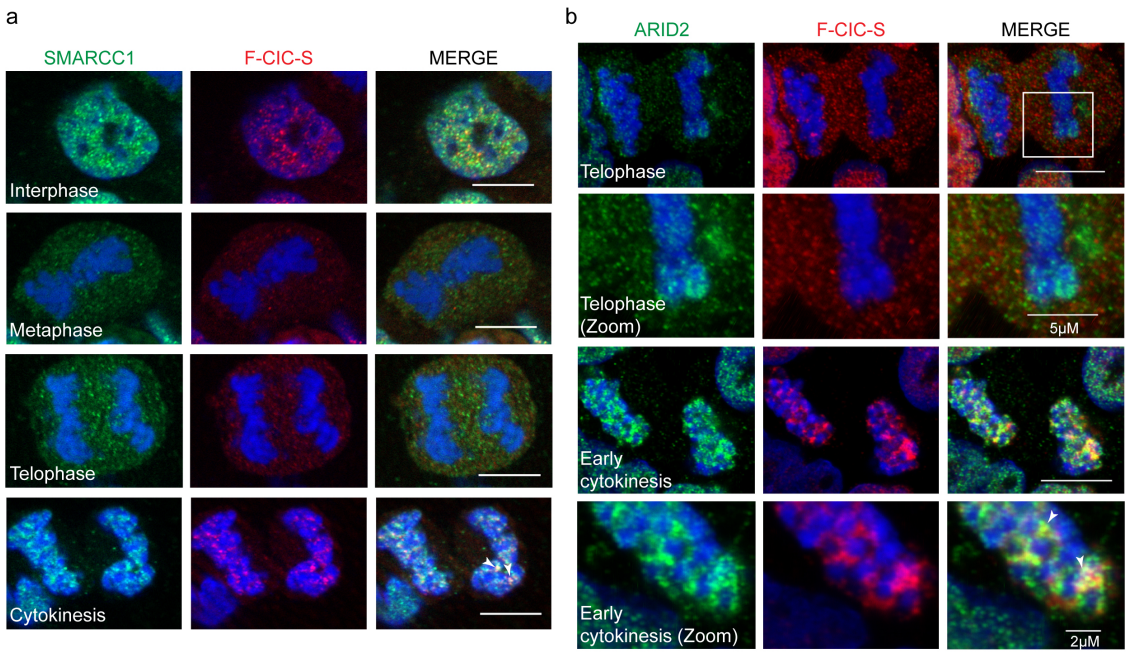


Supplementary Figure 4. Locations of forward and reverse primers used to assess CIC binding at PLK3 and HMGA1 by ChIP-qPCR.

a-b. PLK3 (a) and HMGA1 (b) loci as shown on the UCSC genome browser. The regions tested for enrichment are indicated by black bars, and forward and reverse primer sequences are shown.



Supplementary Figure 5. IP-MS assays identify candidate CIC interactors.
a. Schematic showing the workflow for IP-MS assays used to identify candidate CIC interactors (see Methods section).
b. Western blots showing reciprocal IPs confirming the interaction between F-CIC-S (detected using a FLAG antibody) and SMARCA2 (top) and ARID1A (bottom) in HEKD10$^{F\text{-}CIC\text{-}S}$ cells.
c. An UpSet plot showing common interactors identified from IP-MS experiments against CIC, SMARCA2, and ARID1A.



Supplementary Figure 6. CIC co-localizes with SMARCC1 and ARID2.
a-b. IF images showing localization of F-CIC-S (FLAG, red) and SMARCC1 (a) or ARID2 (b, both green) at indicated phases of the cell cycle. DNA was stained with DAPI (blue). Arrowheads indicate co-localization of CIC and the relevant interactor at early cytokinesis (yellow foci). Scale bars: 10μm or as indicated for zoomed images (b).

Materials and Methods

Cell culture

The HEK293A cell line was obtained from Dr Gregg Morin (Canada's Michael Smith Genome Sciences Centre, Vancouver, BC, Canada) and authenticated by Genetica DNA Laboratories (Cincinnati, OH, USA). The HOG cell line was obtained from Dr G. Dawson (The University of Chicago, Illinois, USA), and the IDH1-stable NHA cell line was obtained from Applied Biological Materials (ABM) Inc (T3022; Richmond, BC, Canada). The HEK293A, HOG and NHA cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) heat-inactivated (HI) FBS (Invitrogen). Stable cell lines expressing F-CIC-S or F-CIC-F derived from HEK293A (D10) or NHA (A2) were cultured in DMEM+10% HI FBS with Zeocin (Invitrogen) selection. Cell culture was performed in a humidified, 37°C, 5% CO_2 incubator.

CRISPR-Cas9 and knockout cell line generation and CIC expression constructs

CRISPR-Cas9 sgRNA sequences were designed to target exon 2 of the *CIC* gene and CIC^{KO} lines were established from NHA (A2, H9, B6, and H10) and HEK293A (A9 and D10) cells as described previously[27]. *CIC* knockout status was verified by western blot and Sanger sequencing. N-terminal FLAG-CIC-short form (FLAG-CIC-S) and long form (FLAG-CIC-L) plasmids were prepared as described previously[38].

Cell cycle synchronization and mitotic shake off

For immunofluorescence, we plated 1.3 x 10^5 cells/well in a 6-well plate with sterilized cover slips (CA48380-068, VWR). For western blot analyses, we plated 1.2 x 10^6 cells in 10 cm

tissue culture dishes. For RT-qPCR analyses, including mitotic shake off experiments, 1.2 x 10^6 cells were plated in 3x T75 flasks (Falcon). After 48 h, freshly prepared 9μM RO-3306 (SML0569, Sigma) in DMEM + 10% (v/v) HI FBS was added to initiate synchronization. After 19 h, cells were washed with 1X Hank's Balanced Salt Solution (HBSS, Invitrogen) three times and were either released from treatment by addition of fresh media or terminated by pelleting or fixing with 4% paraformaldehyde (PFA). For mitotic-shake off experiments, mitotic cells were gently tapped and collected into a falcon tube 0.5 h post-release. Cell pellets were collected by centrifugation at 1000 rpm for 5 min.

Immunofluorescence assays

For cell lines, fixed cells were permeabilized using 0.2% TritonX-100/PBS for 5 min, blocked with 2.5% goat serum (G6767, Sigma) in PBS for 1 h on a rocker, and stained with appropriate primary antibodies (Supplementary Table 6) in 0.25% goat serum in PBS overnight at 4°C. Alexa Fluor® 488 and/or 568 were used as secondary antibodies (Supplementary Table 6), followed by mounting with DAPI (D3571, Invitrogen) and SlowFade™ Diamond Antifade Mountant (36967, Invitrogen).

For mouse tissue, embryonic (E13.5) mouse brain section slides were quickly pre-washed in 1X TBST (0.1% Tween) followed by a 15 min permeabilization in TBST (0.25% Tween) and an additional 5 min wash in TBST (0.1% Tween) with gentle shaking. Slides were blocked with 3% goat serum in 1X PBST (0.1% Tween), covered with parafilm, for 30 min in a humidified staining chamber. α-Phospho Histone H3 (Cell Signaling) and α-Rabbit Alexa Fluor® 568 antibodies were applied in blocking solution overnight at 4°C and for 1 h at room temperature, respectively, with three 5 min TBST (0.1% Tween) washes performed in between. Slides were

then mounted for imaging and quantification. All steps were performed at room temperature unless indicated otherwise.

Quantification of cell cycle phases and chromosomal defects

To quantify cells at different cell cycle phases, 7-8 images were randomly captured per replicate at each time point with 20x magnification. Cells at interphase/prophase (pro-metaphase), metaphase, and telophase/cytokinesis were counted and the proportion of cells at each phase relative to the total number of cells was calculated for each image, at each time point. Full cell counts can be found in Supplementary Table 7.

For metaphase defects, 20-25 images per replicate were captured with 40x magnification. Normal, minor (a few alignment defects), and moderate-severe defective (severe alignments and multipolar alignments) phenotypes were observed, and the ratio of cells with a defective phenotype (number of cells with defective phenotypes in metaphase/total number of cells in metaphase) was calculated for each image. Metaphase cell counts for the NHA lines (Figure 1d): n = 146 (NHA), n = 104 (H9), and n = 99 (A2). Similarly, for cytokinesis defects, 50 images per replicate were captured with 63x (oil) magnification and the ratio of cells with a defective phenotype (number of cells with defective phenotypes in cytokinesis/ total number of cells in cytokinesis) was calculated for each image. Cytokinesis cell counts for the NHA lines: n = 122 (NHA), n = 89 (H9), and n = 94 (A2). Cell counts for Figure 6b: n = 81 (NHA scr), n = 82 (A2 scr), n = 70 (NHA *ARID1A*^{KD}), and n = 75 (A2 *ARID1A*^{KD}). Metaphase cell counts for the HEK line (Supplementary Figure 1c): n = 59 (HEK), n = 56 (D10), and n = 61 (A9). All images were captured with a Zeiss Axio Observer with Apotome.2 fluorescence microscope.

Mouse chromosomal defect counts

E13.5 mouse brain[29] section slides were mounted on glass slides and stored at −80°C until analysis. Each slide contained four consecutive sections each from two mice, for a total of eight sections per slide. α-tubulin (abcam) and DAPI DNA stain were used to quantify chromosomal defects and α-phospho-Histone H3 (Cell Signaling) was used to visualize mitotically active cells. For each brain section, images from 10 regions with cells in anaphase and telophase were captured at 63x (oil) magnification using an Axioplan microscope. The ratio of cells with defective anaphase or telophase to the total number of cells at anaphase or telophase, respectively, was used for quantifications.

Cell lysis preparations

Cell pellets were re-suspended in 4x packed cell volume of RIPA lysis buffer freshly supplemented with 1% sodium orthovanadate (100mM), 1% PMSF (200mM), 2% (v/v) protease inhibitor (sc-24948, Santa Cruz), and PhosSTOP (04906837001, Roche, following the manufacturer's recommendations). Cell pellets were sonicated briefly and mixed for 1 h at 4°C on a rotator. Cellular debris was removed using centrifugation at 13000 rpm for 10 min at 4°C. Total protein was quantified using the Pierce™ BCA Protein Assay Kit (Thermo Fisher).

Western blots

Samples were subjected to gel electrophoresis on NuPage® 3-8% Tris Acetate or 10% Bis-Tris pre-cast mini-gels with 1x NuPage® MOPS buffer (Invitrogen). Separated proteins were transferred onto a methanol-activated PVDF membrane (162-0177, Bio-Rad) in 1x NuPage® Transfer buffer (Invitrogen) + 20% (v/v) methanol. Membranes were blocked with either 2% or

5% (w/v) skim milk in PBST or ReliaBLOT BLOCK (WB 120, Bethyl) in TBST for 1 h at room temperature prior to incubation with primary antibodies (Supplementary Table 6) at 4°C overnight. For protein detection, membranes were incubated with HRP-IgG goat α-mouse or α -rabbit (1:5000, Santa Cruz), or ReliaBLOT α -Rabbit HRP (1:5000, Bethyl) for 1 h at room temperature followed by three PBST washes before application of ECL substrate (Bio-Rad) or SuperSignal West Femto substrate (Thermo Scientific). Images were captured using a ChemiDoc™ MP Imager and processed with Protein Image Lab 5.1 (Bio-Rad).

Single-cell whole-genome sequencing (DLP+)

DLP+ was performed as described[33]. Specifically, cells were harvested using 0.05% Trypsin-EDTA and passed through a 35µm filter (Falcon) to generate a single-cell suspension. Cells were then stained with CellTrace CFSE and LIVE/DEAD Fixable Red Dead stains (Thermo Fisher), washed with PBS (Gibco), and brought to a standard concentration of 220,000 cells/mL. For each cell line, 300 single cells were spotted into individual wells of Wafergen Seq-Ready TE FLEX chips (Takara) using a sciFLEXARRAYER S3 (Scienion) with the CellenONE software add-on. Ten no-cell negative control wells were also spotted from each cell suspension, and 10 additional wells were designated as no-template negative controls. After spotting, chips were imaged on a Nikon Ti-E Eclipse fluorescent microscope, and wells containing a single live cell were identified using in-house software. Cells were lysed in DirectPCR Lysis Reagent - Cell (Viagen) supplemented with QIAGEN Protease (Qiagen), and then the protease was heat-inactivated. Twelve ng of HL60 genomic DNA was added to 10 wells as a positive control for lysis. Sequencing libraries were constructed using the Nextera DNA Library Prep kit (Illumina), with sequential addition and incubation of the reactions with Nextera tagmentation mix,

QIAGEN protease to degrade the tagmentation enzyme, and Nextera PCR mix. Eight cycles of PCR were performed using dual-indexing Nextera primers preloaded into chip wells prior to the experiment. Well contents for each cell line were recovered and pooled for cleanup with 1.8 volumes of PCRClean DX magnetic beads (Aline) and sequencing on the HiSeq X platform (Illumina).

DLP+ analysis

Raw paired-end FASTQ files were trimmed with Skewer[58] to remove any adapter sequence prior to alignment to the hg19 human reference with BWA mem (version 0.7.13)[59]. Aligned reads were sorted and duplicates marked using Sambamba[60] (version 0.6.5) to provide a single BAM file for each sequenced cell. Only cells identified as live were used for downstream analyses (n = 273 NHA cells, 283 A2 cells, 287 B6 cells, 266 H9 cells, and 282 H10 cells). BAM files were converted to individual BED files and copy number and ploidy analysis was performed using Ginkgo[61] with the following parameters: variable bin size of 500 kb, 150 bp simulated reads aligned with BWA, independent segmentation, and using the "mask bad bins" option. Ginkgo was run independently for each cell line on all libraries from wells identified as containing a live cell. The remaining analyses, including statistical tests, were performed in R. For calculation of chromosomal arm ratios, centrosome regions were obtained from the UCSC Genome Browser (hg19 gaps; https://genome.ucsc.edu/) and the median copy number across bins from each chromosomal arm was calculated. For segment quantifications, the number and median length of segments (*i.e.* regions of continuous copy number) was calculated for each copy number state (neutral, gain, or loss) for each cell. Copy number states were defined according to each cell's ploidy as determined by Ginkgo: neutral segments were those with the

same copy number as the ploidy of that cell, and gain and loss segments were those with higher or lower copy number, respectively. Wilcoxon tests were used to evaluate differences in the distribution of segment numbers or lengths for each copy number status, using the parental NHA line as a reference. Adjusted p-values were obtained by applying a Bonferroni correction to p-values obtained within each copy number status.

Fluorescence in-situ hybridization (FISH) analysis

Freshly pelleted cell cultures were fixed and re-suspended in a 3:1 ratio of methanol:acetic acid solution. For each culture, 20 µL of suspension was dropped onto each of 2 slides and allowed to air dry for at least 30 min. Slides were then dehydrated through an alcohol series (70%, 80%, and 100%) and allowed to air dry at room temperature. Five µL of either 1p36/1q25 or 19q13/19p13 Vysis dual-colored FISH probe (Abbott Molecular Inc.) was added to the corresponding slide. A coverslip was applied to each slide and sealed with rubber cement. Slides and probes were co-denatured at 73°C for 5 min and allowed to hybridize for 18 h at 37°C in a ThermoBrite System (Abbott Molecular Inc.). After hybridization, slides were soaked in 0.4X SSC/0.3% NP-40 solution to remove the coverslip, washed in 0.4X SSC/0.3% NP-40 solution at 73°C for 2 min and rinsed in 2X SSC/0.1% NP-40 solution for 1 min at room temperature. Slides were then allowed to dry upright and protected from light. Once dry, 10µL of DAPI counterstain (Abbott Molecular Inc.) was applied to each slide and overlaid with a coverslip.

Slides were visualized and probe signals enumerated using a Metafer Slide Scanning Platform (MetaSystems) at 63X magnification. Spectrum orange-labeled target probe and spectrum green-

labeled control probe signals were counted in a minimum of 100 nuclei for each slide to determine the 1p/1q or 19q/19p (orange/green) ratio.

RNA extraction and RT-qPCR

Total RNA was extracted with the RNeasy Plus Mini Kit (74136, QIAGEN) following the manufacturer's protocol and was quantified using an 8-sample spectrophotometer (NanoDrop). RT-qPCR was performed with 20 ng of input RNA using the Power SYBR® Green RNA-to-Ct1™ 1-Step Kit (4389986, Applied Biosystems) following the manufacturer's recommendations. A QuantStudio 6 Flex Real-Time PCR system and the QuantStudio™ Real-Time PCR Software v1.3 (Applied Biosystems) were used to obtain C_T values. Relative mRNA expression levels were calculated using the $2^{\Delta\Delta ct}$ method with *GAPDH* as an internal control. Student's *t*-tests were used to calculate *p*-values using Microsoft Excel. RT-qPCR primer sequences are provided in Supplementary Table 8.

Targeted ChIP-qPCR analysis

Each replicate consisted of three ~70-80% confluent 15 cm plates. Cells were crosslinked using 1% formaldehyde (Sigma) in PBS for 10 min with gentle rocking, and then treated with 0.125M glycine (Sigma) for 5 min. Crosslinked cells were collected by scraping, pooled, and then pelleted by centrifugation (1200 rpm for 5 min at 4°C). Cell pellets were resuspended in ChIP cytoplasmic lysis buffer (50mM HEPES-KOH pH7.5, 140mM NaCl, 1mM EDTA pH 8.0, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100, 1X Complete EDTA-free Protease Inhibitor cocktail [PIC, Roche], and 1X PhosSTOP [Roche]), homogenized using a 20-gauge needle (10 passages) and lysed at 4°C for 1 h with end-to-end rotation. Lysed cells were pelleted at 5000

rpm for 5 min at 4°C, and washed twice with MNase lysis buffer (0.1% Triton X-100, 0.1% deoxycholate, 1X EDTA-free PIC, 1X PhosSTOP) to remove any cytoplasmic content. Nuclei were resuspended with 3x pellet volume MNase lysis buffer, to which 4x pellet volume of MNase Master Mix (1mM DTT, 2x MNase buffer [NEB]) was then added along with MNase (NEB, 1/100 final volume). MNase treatment was performed for 7 min at room temperature. To stop the reaction, 0.5M EGTA was added to a final concentration of 20 mM. MNase-digested nuclei were transferred to 1 mL milliTUBEs with AFA fiber (D-Mark Biosciences) with a 30-gauge needle and then briefly sonicated to disrupt the nuclear membrane using a Covaris S2 sonicator with the following settings: 20% duty, 4 intensity, 200 burst, 20 seconds, 4-8 °C). All subsequent steps, including chromatin quantification, pre-clearing, immunoprecipitation and qPCR were performed as described previously[27].

Cell fractionations of nuclear proteins and immunoprecipitation

HEK IPs were Cells were grown for 48 h and harvested at ~80% confluency. Fresh cell pellets were gently resuspended in 5x packed cell volume of cytoplasmic lysis buffer (10mM Tris-HCl pH7.2, 10mM NaCl, 2mM $MgCl_2$, 1mM EDTA, 0.05% NP-40, with 1X complete protease inhibitor and 1X PhosSTOP from Roche). Lysates were incubated on ice for 10 min before centrifugation at 300g for 5 min to collect nuclear pellets. After 3 to 4 washes with wash buffer (10mM Tris-HCl pH7.2, 10mM NaCl, 2mM $MgCl_2$, 1mM EDTA with 1X cOmplete protease inhibitor and 1X PhosSTOP from Roche), nuclear pellets were resuspended in 3x packed cell volume of nuclear lysis buffer (250mM NaCl, 20mM sodium phosphate pH7.0, 30mM sodium pyrophosphate, 5mM EDTA, 10mM sodium fluoride, 10% glycerol, 1% NP-40, 1mM DTT, with 1X complete protease inhibitor and 1X PhosSTOP from Roche) and then

homogenized using a 21-gauge needle. Cellular debris was removed by centrifugation at 13,000 rpm for 30 min at 4°C and then nuclear protein was quantified using the Pierce™ BCA Protein Assay Kit (Thermo Fisher). Before proceeding with immunoprecipitation, nuclear lysates were treated with 0.1X Benzonase (EMD Millipore) for 30 min at 4°C. Antibody-bound magnetic beads were prepared by incubating anti-CIC antibody or normal rabbit-IgG (Supplementary Table 6) with Protein G Dynabeads® (Thermo Fisher) in PBST (0.1% v/v) at 4°C for 30 min, and then rinsed three times with wash buffer (1X PBS, 1mM EDTA, 0.5% NP40, with 1X complete protease inhibitor and 1X PhosSTOP from Roche). Nuclear lysates were incubated with the prepared anti-CIC or rabbit IgG (control) Protein-G beads at 4ºC overnight. Protein and protein complexes were released by boiling the magnetic beads in elution buffer (2X Nupage LDS buffer [Invitrogen], 200mM DTT) at 98^{o}C for 10 min.

Reciprocal immunoprecipitation

Whole-cell extracts were prepared by lysing fresh cell pellets with 2x packed cell volume of lysis buffer (25mM Tris-HCl pH7.4, 150mM NaCl, 1% NP-40, 5% glycerol, with 1X complete protease inhibitor and 1X PhosSTOP from Roche), followed by homogenization from 10 passes through a 21-gauge needle. After 30 a min incubation on ice, lysis preparations were cleared of cellular debris by centrifugation at 13,000 rpm for 30 min at 4°C and resuspended in X. Whole-cell lysates were incubated with anti-ARID1A, anti-SMARCA2, anti-SMARCC2, anti-POLR2A, or mouse/rabbit-IgG antibodies (Supplementary Table 6) at 4ºC overnight. Proteins and complexes were captured by incubating antibody-lysate mixtures with Protein G Dynabeads® (ThermoFisher) for 1 h at 4°C. For elution, magnetic beads were boiled in elution buffer (2X Nupage LDS buffer (Invitrogen), 200mM DTT) at 98^{o}C for 10 min.

LC-MS/MS

The initial IP-MS analysis of nuclear fractions from HEK293A cells using an endogenous CIC antibody or anti-Myc for N-Myc-CIC-S (Supplementary Table 2) was performed as described previously[38]. Three replicates were performed against endogenous CIC, and one experiment was performed against N-terminal MYC-fused CIC stably expressed in HEK293 cells. The IP-MS validation experiments performed in nuclear fractions of the NHA cell line were also performed in triplicate, as were the matched IgG controls. See below for methodological details.

Protein elution, clean-up with SP3, and protease digestion

Proteins in SDS loading buffer were purified using the SP3 method, as described previously[62,63]. A total of 200 μg of protein was prepared in a final volume of 100 μL in a standard 1.5 mL tube. Twenty μL (400 μg) of a 1:1 combination of two different types of carboxylate-functionalized beads, both with a hydrophilic surface (Sera-Mag Speed Beads, GE Life Sciences, CAT#45152105050350 and CAT#65152105050350), was added to the lysate, with the beads rinsed in water prior to addition to the lysate. To promote binding to the beads, 100 μL of 90% ethanol was added to achieve a final concentration of 50% by volume (*i.e.* 45% ethanol final concentration). Tubes were mixed on a ThermoMixer unit (Eppendorf) at 1000rpm for 10 min at room temperature, then placed in a magnetic rack and incubated for 2 min. The supernatant was discarded, and the beads rinsed 3x with 180 μL of 90% ethanol by removing the tubes from the magnetic rack and gently re-suspending the beads using a brief sonication (30 s), followed by pipette mixing. For elution, the tubes were removed from the magnetic rack and

beads were re-suspended in 100 µL of 50 mM HEPES, pH 8 containing an appropriate amount of trypsin/rLysC mix (1:25 enzyme to protein concentration, Promega, CAT#V5071) and incubated for 14 h at 37ºC in a ThermoMixer with mixing at 1000 rpm. After incubation, the tubes were sonicated briefly (30 s) in a bath sonicator, placed on a magnetic rack, and the supernatant was recovered for further processing.

Synthetic peptide mix preparation

The set of standard peptides was taken as a subset from the collection analyzed in the ProteomeTools initiative[64]. Peptides were selected for a panel of 51 genes, resulting in a set of 254 total candidates that were synthesized in a 'SpikeMix' format (JPT Peptide Technologies). Upon delivery, dried peptides were reconstituted in 100 µL of DMSO, vortexed briefly (~15 s), and sonicated in a water bath for 5 min. Reconstituted peptides were measured in a dilution series to determine the concentration that represented the limit of detection for the majority of the pool. Reconstituted peptides were spiked into interactome samples at a concentration 10% above the determined limit of detection. In this way, the synthetic spikes would not negatively impact the resulting quantification of detected peptides due to a large difference in dynamic range in comparison to the IP samples.

Tandem mass tag labeling of peptides

TMT 6-plex labelling kits were obtained from Pierce. Each TMT label (5 mg per vial) was reconstituted in 500 µL of acetonitrile and refrozen. A maximum of 50 µg of combined peptide was present in any single channel. Labelling reactions were carried out through addition of 200 µg of TMT label in two volumetrically equal steps of 10 µL (100 µg per addition), 30 min

apart. IP samples were labelled using TMT126 – TMT131 labels. The synthetic peptide spikes were labelled using the TMT131C reagent (from the TMT 11-plex reagent set) to ensure no overlap with the samples. Reactions were quenched through addition of 10 µL of glycine (1M stock solution, Sigma). Labelled peptides were concentrated on a SpeedVac centrifuge (Thermo Scientific) to remove excess acetonitrile, acidified to 1% (v/v) trifluoroacetic acid (TFA), and purified with a C18 StageTips.

Peptide clean-up procedures

Peptides were desalted and concentrated using StageTip treatment as described previously[65]. For StageTip clean-up, 3-discs of C18 Empore material (Sigma, CAT#66883-U) packed in 200µL pipette tips were rinsed twice with 100µL of acetonitrile with 0.1% TFA. Cartridges were then rinsed twice with 100 µL of water with 0.1% TFA prior to sample loading. Loaded samples were rinsed twice with 0.1% formic acid (100 µL per rinse) and eluted with 100 µL of 80% acetonitrile containing 0.1% formic acid. All TopTip- or StageTip-processed samples were concentrated in a SpeedVac centrifuge (Thermo Scientific) and subsequently reconstituted in 1% formic acid with 1% DMSO in water.

MS analysis of peptide samples on the Orbitrap Fusion

Analysis of TMT-labelled peptide pools was carried out on an Orbitrap Fusion Tribrid MS platform (Thermo Scientific). Samples were introduced using an Easy-nLC 1000 system (Thermo Scientific). Columns used for trapping and separations were packed in-house. Trapping columns were packed in 100 µm internal diameter capillaries to a length of 25 mm with C18 beads (Reprosil-Pur, Dr. Maisch, 3 µm particle size). Trapping was carried out for a total volume

of 10 µL at a pressure of 400 bar. After trapping, gradient elution of peptides was performed on a C18 (Reprosil-Pur, Dr. Maisch, 1.9µm particle size) column packed in-house to a length of 20 cm in 100 µm internal diameter capillaries with a laser-pulled electrospray tip and heated to 50°C using AgileSLEEVE column ovens (Analytical Sales & Service). Elution was performed with a gradient of mobile phase A (water and 0.1% formic acid) to 8% B (acetonitrile and 0.1% formic acid) over 5 min, to 30% B over 88 min, and to 40% B over 19 min, with final elution (80% B) using a further 8 min at a flow rate of 350 nL/min.

Data acquisition on the Orbitrap Fusion was carried out using a data-dependent method with multi-notch synchronous precursor selection MS3 scanning for TMT tags. Survey scans covering the mass range of 350 – 1500 were acquired at a resolution of 120,000 (at m/z 200), with quadrupole isolation enabled, an S-Lens RF Level of 60%, a maximum fill time of 50 ms, and an automatic gain control (AGC) target value of 5e5. For MS2 scan triggering, monoisotopic precursor selection was enabled, charge state filtering was limited to 2 – 4, an intensity threshold of 5e3 was employed, and dynamic exclusion of previously selected masses was enabled for 60 s with a tolerance of 20 ppm. MS2 scans were acquired in the ion trap in Rapid mode after CID fragmentation with a maximum fill time of 150 ms, quadrupole isolation, an isolation window of 1.6 m/z (0.2 m/z offset), collision energy of 30%, activation Q of 0.25, injection for all available parallelizable time turned OFF, and an AGC target value of 4e3. Fragment ions were selected for MS3 scans based on a precursor selection range of 400-1600 m/z, ion exclusion of 20 m/z low and 5 m/z high, and isobaric tag loss exclusion for TMT. The top 10 precursors were selected for MS3 scans that were acquired in the Orbitrap after HCD fragmentation (NCE 60%) with a maximum fill time of 150 ms, 50,000 resolution, 120-750 m/z scan range, ion injection for all

parallelizable time turned OFF, and an AGC target value of 1e5. The total allowable cycle time was set to 4 s. MS1 and MS3 scans were acquired in profile mode, and MS2 in centroid format.

Mass spectrometry data analysis

Data from the Orbitrap Fusion were processed using the Proteome Discoverer Software (v2.1.1.21). MS2 spectra were searched using Sequest HT against a combined UniProt human proteome database appended to a list of common contaminants (24,624 total sequences). Sequest HT parameters were specified as: trypsin enzyme, 2 missed cleavages allowed, minimum peptide length of 6, precursor mass tolerance of 20 ppm, and a fragment mass tolerance of 0.6. Oxidation of methionine, and TMT 6-plex at lysine and peptide N-termini were set as variable modifications. Carbamidomethylation of cysteine was set as a fixed modification. Peptide spectral match error rates were determined using the target-decoy strategy coupled to Percolator modeling of positive and false matches[66,67]. Data were filtered at the peptide spectral match-level to control for false discoveries using a q-value cut off of 0.01 as determined by Percolator. Contaminant and decoy proteins were removed from all data sets prior to downstream analysis. Perseus (v1.5.2.6) was used to perform two-sided *t*-tests to identify proteins that were significantly enriched in each IP experiment compared to their respective IgG controls (FDR < 0.05).

Proximity ligation assays (PLAs)

Cells were plated on an 8-well CC_2 chamber slide with cover (12-565-1, Fisher) in growth media. After 19 h, cells were fixed with 4% PFA. PLAs were performed using the Duolink® In Situ Red Starter Kit Mouse/Rabbit (DUO92101, Sigma), following the

manufacturer's protocol. High-resolution images were obtained with an Eclipse Ti inverted confocal microscope equipped with A1 si laser (Nikon) and Rolera EM-C^{2TM} (QImaging) camera. Images used for quantifications were obtained with an Axio Observer inverted fluoresce microscope (Zeiss), equipped with Apotom.2 and AxioCam MRm camera. Images were obtained at 40x or 63x (oil) magnification using the Zen software (2.3, blue edition; Zeiss). PLA dots were counted using Blob-Finder software (http://www.cb.uu.se/~amin/BlobFinder/). Nuclei counts (Figure 5a): n = 732 for ARID1A/CIC, 583 for ARID1A/vector, 564 for SMARCA2/CIC, 533 for SMARCA2/vector, 563 for SMARCC1/CIC, 532 for SMARCC1/vector, 564 for ARID2/CIC, and 588 for ARID2/vector. Metaphase cell counts (Figure 5b): n = 25 for ARID1A/vector and n = 26 for ARID1A/CIC. Early cytokinesis cell counts: n = 40 for ARID1A/vector and n = 32 or ARID1A/CIC.

Microscopy imaging

Microscope information is included in relevant sections. Raw images were further processed in Photoshop (Adobe).

Crystal violet cell proliferation assays and siRNA transfections

Crystal violet assays were performed as previously described[38]. For siRNA experiments, cells were transected with 7nM of *ARID1A* siRNA (hs.Ri.ARID1A.13.2) or Scrambled Negative Control DsiRNA (51-01-19-08, IDT) using Opti-MEM™ and Lipofectamine™ RNAiMAX (Invitrogen), as previously described[27].

Statistical analysis

Statistical analysis was performed using Prism GraphPad unless otherwise indicated. Statistical significance was calculated using a two-sided Student's *t*-test, Welch's *t*-test, one-way ANOVA with Holm-Sidak multiple comparisons, or Wilcoxon test as described in the figure legends.

Code availability

Code is available upon request.

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