SUPPLEMENTARY METHODS

In vitro culture/expansion of B-ALL cells and HSPCs

Primary B-ALL blast were cultured in Stemspan medium (Stem Cell Technologies, Vancouver, Canada) supplemented with 20% FCS, the hematopoietic cytokines SCF (100 ng/mL), FLT3 ligand (100 ng/mL), IL3 (10 ng/mL) and IL7 (10 ng/mL) (all from PeproTech), Insulin-Transferrin Selenium (ITS) and antibiotics (Gibco). Nestin+ mesenchymal stem cells (MSCs) were derived from human fetal BM (BM-MSCs) and used for *ex vivo* expansion of primary B-ALL blasts. BM-MSCs were isolated, characterized and maintained as described by our group[1,2]. Cell lines were cultured in standard culture media supplemented with 10% fetal calf serum, for MHH-CALL2 1% β-mercaptoethanol, sodium pyruvate, ITS, 1% antibiotics and non-essential aminoacids were added to the media. Cell cultures were maintained at 37°C in 5% CO_2 in a humidified atmosphere.

Immunofluorescence on metaphase chromosome spreads

For immunofluorescence on metaphase chromosome spreads, cells were treated with 0.1µg/mL colcemid (Gibco) for 5 hours, then pelleted, counted with a hemocytometer and resuspended in pre-warmed 75mM KCl to a concentration of $2x10^6$ cells/mL. After 5 min incubation at 37°C, cells were spun on poly-L-lysine-coated glass coverslips (500g, 10 min), and subjected to immunofluorescence as indicated above.

Chromosome function assays

For chromosome biorientation assays, B-ALL cells were treated with 100mM of the spindle bipolarity inhibitor Monastrol (Sigma) for 16h, washed 3X with D-PBS and incubated with 10 µM of the proteasome inhibitor MG-132 (Sigma) for 5h. B-ALL cells were then stained with anti-α-tubulin, anti-pericentrin and anti-ACA (**Table S2**). The orientation of the metaphase plate was determined based on the pericentrin foci. Only cells with metaphase plates with two pericentrin foci properly localized in a perpendicular axis and on the same z-plane were analysed, thus preventing

misclassification of prometaphase cells with naturally occurring misaligned chromosomes. For the analysis of chromosome segregation defects in late mitosis, cells were treated for 1h with 50 μM of the cytokinesis inhibitor Blebbistatin (Tocris), and then subjected to immunofluorescence.

Standard cytogenetic and fluorescence *in situ* hybridization (FISH) analysis

Fixed (Carnoy solution) cytogenetic B-ALL pediatric samples were spread onto methanol-cleaned slides and kept at -20°C until processed. Primary B-ALL blasts and primografts were processed following standard cytogenetics procedures[3]. For metaphase chromosome analysis, samples were stained with Giemsa 1:10 diluted in distilled water (10min at RT). Slides were then rinsed with distilled water and dried at RT before microscope analysis. Giemsa-labeled samples were observed in a DMRB-Leica microscope, equipped with a wide field Leica DFC 450 camera. Acquisition of the bright field images was performed with the LAS v4.0 Leica software using 100xPL APO Oil Immersion Numerical Aperture 1.4. Modal karyotypes and PCS analysis were performed using Cell Counter plug-in in FIJI-Image J. FISH was performed following standard procedures[3].

Fluorescence quantification

For quantitative imaging, image datasets were all acquired with identical acquisition settings. Immunofluorescence signal quantification was performed using FIJI-Image J (Wayne Rasband, NIH, USA). Custom-made macros were programmed with instructions for the automated 3D image analysis pipelines for SMC2 and Kinetochore fluorescence signals. First, DAPI channel was background subtracted, converted to mask by intensity threshold (Mean auto-threshold method) and holes filled. 3D Object Counter was run in order to segment and analyze volume of connected DNA structures (volume preset in 3D Object Counter Options). Second, SMC2 or Kinetochore channels were mean filtered, converted to mask by intensity threshold (Moments auto-threshold method) and resulting fused particles were split by Watershed operation. Finally, SMC2 or

Kinetochore signals overlapping with DNA regions were selected by a logical “and” operation between DAPI Mask and SMC2/Kinetochore Mask. 3D segmentation and particle analysis of SMC2/Kinetochores was performed with 3D Object Counter. Objects of interest defining either SMC2 or Kinetochores signals were added to 3D Manager and used to quantify the number, intensity and volume parameters from the original SMC2 or Kinetochore images.

For Aurora B Kinase (AURBK) and Survivin fluorescence signal quantification, individual centromeres for each nucleus were identified by CENP-A staining, selected by Find Maxima and marked by a square 0.624μm^2 region-of-interest (ROI). The signal fluorescence intensity from AURBK and Survivin at the expanded centromere ROI (space between CENP-A sister pairs) was measured from the best focused Z-plan. For AURBK and Survivin, approximately 300 centromeres from 10 individual cells (n=3 HyperD and n=3 non-HyperD B-ALL patients) were analysed, and results reported as the average of fluorescence intensity at centromeres per individual cell.

The distance between individual kinetochore pairs (first identified in the same Z-plan) was measured in calibrated images using the line scan plug-in in FIJI-Image J. Inter-centromeric distances were measured under tension (MG132-treated cells) and without tension (Colcemid-treated cells) in 130 kinetochore pairs from HyperD (n=3) and non-HyperD (n=3) B-ALL samples.

Cell cycle and Phospho-H3S10 quantification

For cell cycle analysis, a total of $1x10^6$ B-ALL cells were fixed with 1mL of 70% ethanol at -20°C. Cells were resuspended in 200-500µl of staining solution (1xPBS, 50g/mL propidium iodide (PI), 0.1mg/mL RNaseA, 0.05% Triton X-100), and analysed by FACS at slow speed. PI fluorescence was analysed on a minimum of 25,000 cells, and cell cycle was analysed using ModFit (VeritySoftware). For phospho-H3S10 analysis, B-ALL cells were harvested and treated with

FIX&PERM kit (Nordic-MUbio) following manufacturer´s instructions. Cells were stained with mouse anti-H3S10-Phospho antibody (15min at RT, Cell-Signalling; 1:50), washed and stained with anti-mouse Alexa-488 secondary antibody (Invitrogen; 1:100). B-ALL primary blasts were co-stained with anti-CD19-FITC (cell cycle analysis) or anti-CD19-APC (SAC assays). The percentage of H3S10-phospho+ cells and the mean fluorescence intensity (MFI) were quantified on a FACS Canto-II equipped with FACSDiva software. At least 30.000 cells were analysed per experiment.

Real-Time PCR analysis

One µg of RNA from B-ALL primary blasts was reverse transcribed to cDNA using the SuperScript III Reverse Transcriptase (Invitrogen) following manufacturer's instructions. cDNA samples were used as templates for real-time PCR analysis using SYBR Green Mastermix (Invitrogen) on a BIO-RAD CFX™ Real-Time system (BIO-RAD) and the primers detailed in **Table S4**.

Epigenetic analysis of the centromere by chromatin Immunoprecipitation (ChIP)

A total of $10x10^6$ primograft B-ALL cells were resuspended in PBS to a concentration of $1x10^6$ cells/mL and crosslinked with 0.8% formaldehyde solution for 5 min at RT, followed by quenching with 2.5M Glycine for 5 min at RT. Cells were then lysed for 10 min on ice in lysis buffer (10mM Tris pH=8.0; 10mM NaCl; 0.5% NP-40) containing protease inhibitors (1 µg/mL CLAP; 0.5 µg/mL Aprotinin; 1mM PMSF, all from Sigma). Nuclei were briefly washed in protease inhibitors-containing lysis buffer prepared in Dilution Buffer 1 (50 mM Tris pH=8.0; 2 mM EDTA; 0.2% SDS; 134 mM NaCl; 0.88% Triton X-100; 0.088% Na-deoxycholate), and the chromatin was sheared by sonication in a Bioruptor sonicator (Diagenode) for 9 cycles (30s ON-30s OFF) at 4°C. Sonication products were diluted with 300 µl of Dilution Buffer 1, 500 µl of Dilution Buffer 2 (50 mM Tris pH=8.0; 167 mM NaCl; 1.1% Triton X-100; and 0.1% Na-deoxycholate) and 500 µl of RIPA buffer containing 150 µl of NaCl (RIPA150) and protease inhibitors. Total chromatin-sheared products were pre-

cleaned by overnight rotating incubation with anti-mouse IgG Dynabeads (Invitrogen) pre-coated (overnight at 4°C in RIPA-150/0.5% BSA) with the following mouse monoclonal antibodies: anti-CENP-A (ab13939), anti-histone H3 (ab24834), anti-H3K9me3 (Diagenode C15200146), anti-H3K27me3 (Diagenode C15200181), and mouse IgG1 (ab18443) as isotype control. After washing the IgG Dynabeads twice with RIPA-150/0.5% BSA, 500 µl of sheared chromatin were incubated with the beads (4°C for 6h) in a rotation wheel, and the beads were afterwards washed with RIPA-150, RIPA buffer containing 500 mM of NaCl (RIPA-500; 3x) and a final wash with TE pH=8.0 in ice. Chromatin was released by incubating beads with elution buffer (100mM $NaHCO_3$/1%SDS in H_20) at 65°C with agitation. De-crosslinking of eluted chromatin was performed by overnight incubation at 65°C. Immunoprecipitated DNA was treated with 50 µg/mL Proteinase K (Invitrogen) and RNAseA (Roche) and then recovered using a PCR purification kit (Invitrogen). Purified DNA was used as template for RT-PCR using oligonucleotide primers for total human satellite, satellites of chromosomes 17 and 21, and actin as non-centromeric locus (**Table S4**). Percentage of IP was calculated using the formula $2^{-\Delta Ct}$ (dilution factor [IP] /dilution factor [input])x100). Results were normalized against canonical histone H3.

RNA-sequencing

Publicly available RNA-Seq data from 193 patients diagnosed with B-ALL were accessed from EGAS00001001795[4]. HyperD-ALL (n=58) and Non-HyperD (n=30) genetic subtypes of B-ALL (8 B-other, 9 MLLr, 1 BCR-ABL1, 6 ETV6-RUNX1 and 6 TCF3-PBX1) were selected for comparison. St Jude's RNA-Seq data (https://pecan.stjude.cloud/proteinpaint/study/PanALL) was also accessed for further validation. RNAseq computational analysis was performed as previously described by our group[5].

Western blotting (WB), Co-immunoprecipitation (Co-IP) and protein analysis

For WB analysis, whole cell lysates (WCL) were prepared with RIPA buffer (1% NP40, 0.5% Sodium deoxycolate, 0.1% SDS in 1xPBS) from $3x10^6$ PDX-expanded B-ALL primary blasts. WCL were incubated for 30 min in ice, and after centrifugation, 20-30 µg of protein/sample were subjected to SDS-PAGE. Primary antibodies used are listed in **Table S3**. Immunoblots were performed using the BM Chemiluminiscence Western Blotting Kit (Roche) following manufacturer's instructions.

For Co-IPs, Protein G agarose beads (Roche) were conjugated with anti-SMC2 antibody for 4 hours in a rotating wheel at 4°C, and subsequently washed once with cold 1XPBS. $10x10^6$ PDX-expanded B-ALL blasts were lysed in lysis buffer (1% Triton X-100, 0.1M NaCl, 10mM Tris-HCl and 5 mM EDTA) containing protease (cOmplete Mini, Roche) and phosphatase (PhosSTOP EASYpack, Roche) inhibitors for 30 min in ice. After centrifugation at 14000 rpm for 10 min at 4°C, protein lysates were mixed with the anti-SMC2-conjugated beads in a rotating wheel for 2 hours at 4°C. After 3 washes with cold 1XPBS, 30 µl of 2X loading buffer was added and headed at 95°C. Supernatant was recovered and used for either WB as previously described or processed for mass-spectrometry (MS), as below.

Analysis of post-translational modifications of Condensin by MS

For MS analyses, proteins were run for 0.5 cm in an SDS-PAGE gel, and fixed for 10 min with 40:10:50 methanol:acetic acid:water. Gels were then stained with comassie blue and bands containing total protein were excised. Protein-containing gel slabs were cut into small pieces of ~1 mm^3 and cleaned through three consecutive washes of 25%-40%-50% Acetonitrile (ACN) in 50 mM ammonium bicarbonate. Samples were finally dehydrated in 100% ACN and dried in a speedvac. Gel pieces were rehydrated in trypsin digestion buffer and digested overnight using modified trypsin (Roche). Peptides were recovered in four consecutive elutions of 200 µL of 50%-

40%-25%-15% ACN in 50 mM ammonium bicarbonate, and samples were then completely dried in speedvac. Samples were desalted using graphite (binds polar peptides) and C18 (binds less/non-polar peptides) solid-phase extraction (SPE) columns. Peptide pellets were dissolved in 5% ACN/0.1% FA and extracted in graphite columns according to manufacturer (Thermo-Pierce). The eluate and the first wash of each column were mixed and loaded onto a C18 column (Agilent) to recover the peptides not binding to graphite, according to manufacturer's instructions. Peptides were eluted from the columns using 0.1% Formic Acid (FA) in methanol (C18) or 0.1% FA in 50% ACN (Graphite). Both fractions were mixed and dried in a speedvac. Samples were reconstituted in 0.1% FA in 15% ACN for analyses.

The analysis of the samples by high performance liquid chromatography (HPLC)-electrospray source (ESI)-MS provided information on the acetylation and phosphorylation of HsCapE/SMC2 through the measurement of the peptides SQAASILTK and GPAASTQEK, respectively. The chromatographic separation of the target peptides (SQAASILTK and GPAASTQEK) and their acetylated and phosphorylated analogues was carried out using a HPLC system Agilent 1290 (Agilent Technologies, Santa Clara, CA) equipped with a reversed-phase Aeris Peptide XB-C18 RP column (2.1 x 100 mm,1.7 mm particle size, 100 Å pore size, Phenomenex). The HPLC system was connected to a triple quadrupole mass spectrometer Agilent 6460 equipped with an ESI with a jet stream, and a volume of 5µL was injected into the HPLC system. Mobile phases A and B were water and acetonitrile, respectively, both with 0.1% of formic acid. The chromatographic method held the initial mobile phase composition at 2% B for 7 min. Then, a linear gradient to 50% B for 7 min was applied and from 7 to 10 min the % B was increased to 80%. Finally, the initial conditions were reached and the column was equilibrated for 3 min. The HPLC system was connected to the triple quadrupole mass spectrometer through an ESI working in the positive ionization mode. The nozzle voltage was 1000 V and the nebulizer pressure was set at 40 psi. The flow rate and

temperature for the drying and sheath gases were 8.0 L min^{-1} and 325 ºC, and 11 L min^{-1} and 394 ºC, respectively.

The measurement of the samples were carried out in SIM mode by monitoring for each peptide the double charged ions $[M+2H]^{2+}$ and the single charged ions $[M+1H]^{+}$. The charge state of each ion was confirmed from their isotopic patterns measured in the SCAN mode. It was also confirmed for each peptide that both single and double charged ions eluted at the same retention time in the HPLC chromatograms. For the peptide SQAASILTK, the double- and single-charged ions were measured at m/z 459.8 and m/z 918.5, respectively. For its acetylated analogue, the double- and single-charged ions were measured at m/z 480.8 and m/z 960.5, respectively. For the peptide GPAASTQEK, only the monophoshorylated and the diphosphorylated analogues could be detected. For the monophosphorylated analogue of GPAASTQEK, the double- and single-charged ions were measured at m/z 484.7 and m/z 968.4, respectively. For the diphosphorylated analogue, the double- and single-charged ions were measured at m/z 524.7 and at m/z 1048.4, respectively.

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1 (Related to Figure 2): **Characterization of nestin+ fetal BM-MSCs for *ex vivo* expansion of B-ALL primary blasts. (a)** Representative FACS analysis of fetal hBM-MSCs using the indicated antibodies. Histogram overlay shows the intensity of Nestin in the fetal hBM-MSCs. **(b)** Representative IF showing nestin expression in the indicated cellular types.

Supplementary Figure 2 (Related to Figure 2): **Mitotic analysis of PDX-expanded B-ALL primary blasts. (a)** Representative FACS analysis depicting human B-ALL engraftment (HLA-ABC+CD45+CD19+) in PDX mice. **(b)** Frequency of mitotic phases in each individual non-hyperD and HyperD-ALL PDX-expanded leukemia.

Supplementary Figure 3 (Related to Figure 2 and 3): **CALL-2 cells show chromosome alignment and segregation defects. (a)** Quantification of metaphase cells showing misaligned chromosomes in B-ALL cells lines (n=3 independent experiments). **(b)** Frequency of late mitosis from the indicated B-ALL cell lines with lagging and bridge chromosomes, n=50 mitosis in 3 independent experiments. **(c)** Comparison of modal karyotypes for the indicated B-ALL cell lines, n=50 metaphases/cell line from 3 independent experiments. Graphs represent the mean and error bars represent the s.e.m. *p<0.05, **p<0.01 (two-way ANOVA).

Supplementary Figure 4 (Related to Figure 3)**: Modal karyotypes for each individual non-hyperD and HyperD-ALL primary samples.** Graphs show the frequency of metaphases with different chromosome numbers at the indicated primary samples, n=50 metaphases per sample.

Supplementary Figure 5 (Related to Figure 4): **HyperD-ALL blasts show chromosome condensation defects. (a)** Frequency of metaphases with hypocondensed chromosomes for the indicated B-ALL primary samples (n=50 metaphases/sample). **(b)** Intra-patient frequency of metaphases with hypocondensed chromosomes within the diploid normal hematopoietic cells and within the HyperD blasts, n=92 metaphases analysed for pt# LLA26. **(c)** Frequency of metaphases with hypocondensed chromosomes in the indicated B-ALL cell lines. n=150 metaphases from 3 independent experiments. **(d)** Individual analysis of the chromosome arm width for the indicated B-ALL patients. **(e)** Individual analysis of the SMC2 total volume in metaphase chromosomes in the indicated patients. **(f)** qRT-PCR analysis of the indicated condensin complex members in B-ALL primary samples, n=7 non-HyperD and 6 HyperD-ALL. **(g)** Co-IPs with anti-SMC2 of protein lysates from PDX-expanded B-ALL samples. *Left*, WB with the indicated antibodies. *Right*, WB quantification normalized to SMC2 levels. Graphs represent the mean and error bars represent the s.e.m. *p<0.05, **p<0.01; ***p<0.001; **** p<0.0001 (two-way ANOVA in *c,d*; t-Student in *e*).

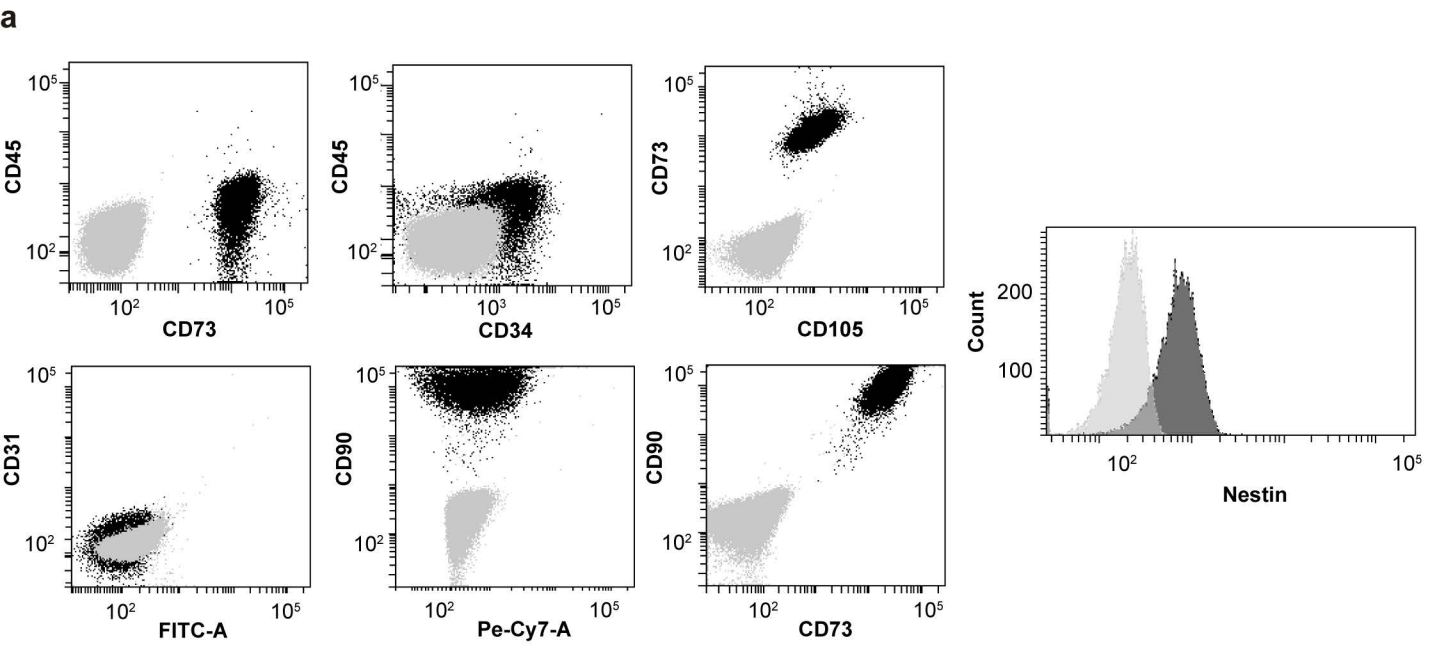
Supplementary Figure 6: Kinetochores are normal in HyperD-ALL blasts. **(a)** ChIP analysis in B-ALL primary samples for CENP-A, H3K9me3 and H3K27me3 at total human satellite normalized to histone H3. Actin was used as non-centromeric control, n=3 non-HyperD and 3 HyperD-ALLs. **(b)** Representative IF staining of NUF2 in the indicated B-ALL primary samples. **(c)** Quantification of NUF-2 levels in mitotic B-ALL primary blasts. Dots represent the average levels of kinetochore fluorescent signals in each cell, n=30 cells from 3 non-HyperD and n=30 cells from 3 HyperD-ALLs. Graphs represent the mean and error bars represent the s.em (two-way ANOVA in *a* or t-Student in *c*). Scale bar=10 μm.

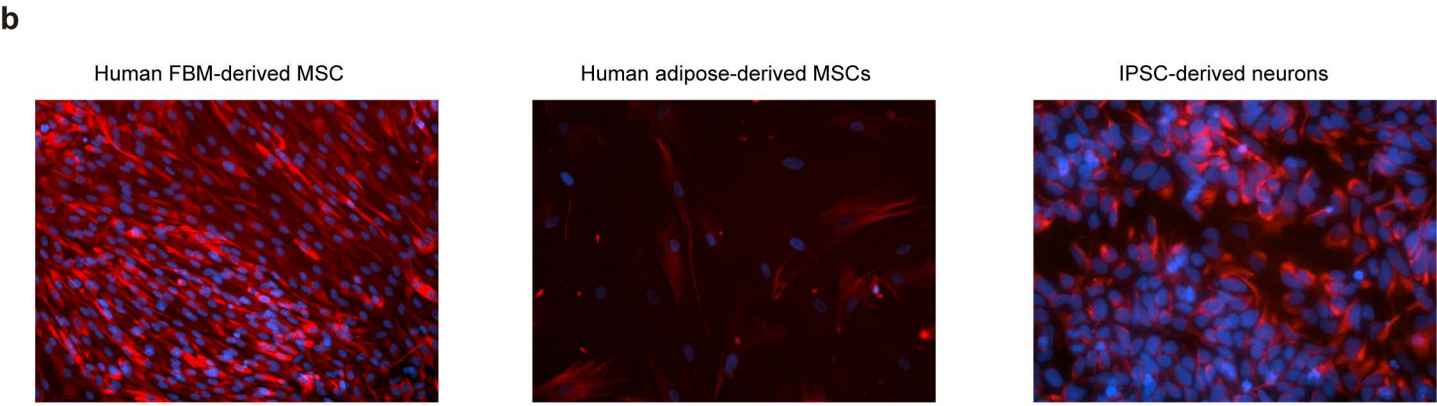
Supplementary Figure 7 (Related to Figure 5): **HyperD-ALL cells show CPC mis-slocalization from the inner-centromeres**. (**a,b**) Quantification of AURBK **(a)** (n=904 centromeres, n=3

NonhyperD; n=743 centromeres, n=3 HyperD-ALL) and Survivin **(b)** (n=859 centromeres, n=3 NonhyperD; n=932 centromeres, n=3 HyperD-ALL) levels in individual centromeres for the indicated PDX-expanded B-ALL samples. **(c)** Representative IF staining for Survivin and CENP-A for the indicated colcemid-treated B-ALL cell lines. **(d)** Representative IF staining for AURBK (top), Survivin (bottom) and CENP-A for the indicated B-ALL cell lines (not colcemid-treated). **(e)** WB showing CAPD2 knock-down in B-ALL cell lines transfected with shRNA for CAPD2. A scramble shRNA was used as a control. (**f**) Representative IF images for AURBK and CENP-A for the scramble- and CAPD2-KD SEM and REH lines generated in *e*. Scale bar=10 μm.

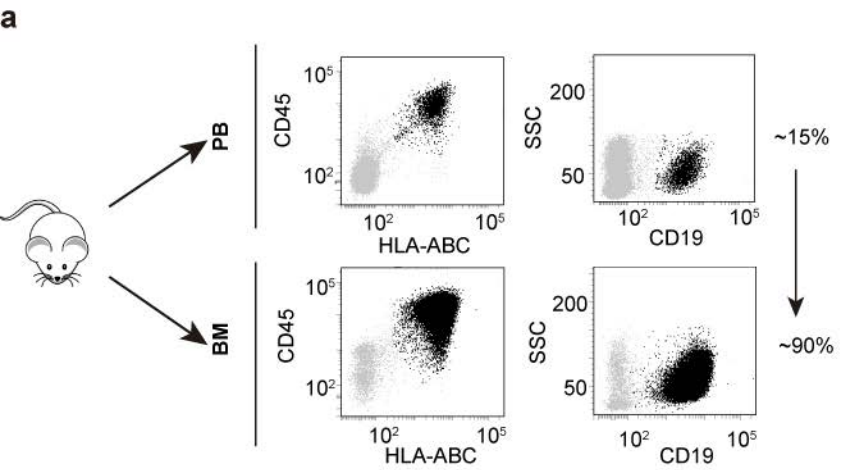
Supplementary Figure 8 (Related to Figure 5): **HyperD-ALL cells show PCS and AURKB activity defects. (a,b)** Frequency of metaphases showing the indicated number of chromosomes with PCS for the indicated B-ALL primary samples **(a)** and cell lines **(b)** (n=50 metaphases per experiment). **(c)** Percentage of H3S10P+ mitotic blasts in DMSO- or Nocodazol-treated B-ALL cell lines, n=3 independent experiments. **(d)** Percentage of SubG0/SubG1 apoptotic nocodazol-treated B-ALL primary blasts (n=3 non-HyperD and n=3 HyperD-ALLs).

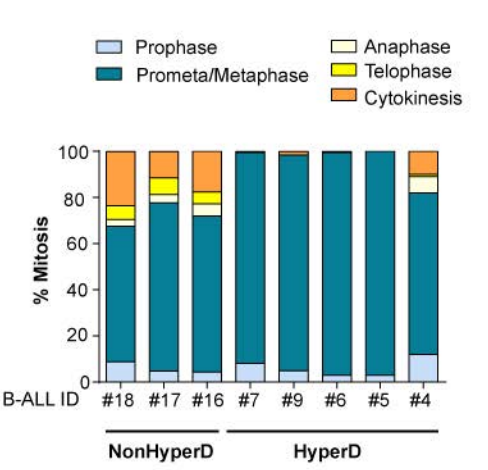
Supplementary Figure 9 (Related to Figure 6): **ZM447439 treatment inhibits the activity of AURKB in CD34+ HSPCs. (a)** H3S10P FACS quantification of CD34+ HSPCs treated with the indicated inhibitors. **(b, c)** Representative images of G-band karyotypes of CD34+ HSPCs treated with either ZM447439 **(b)** or 0.5 μM Reversine (reduced concentration) **(c)**. DMSO-treated CD34+ cells were used as controls.



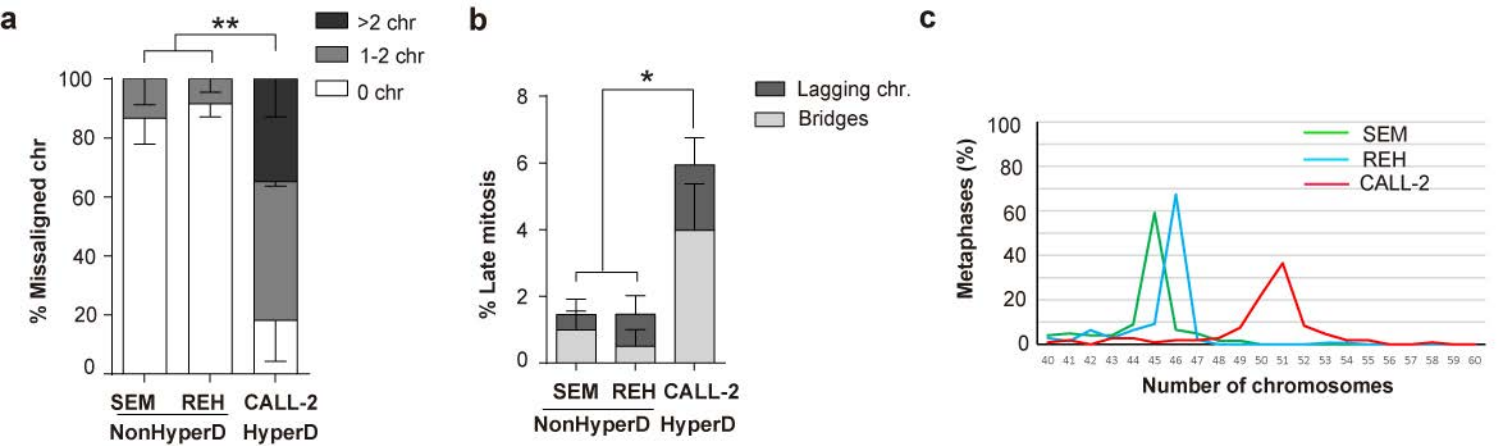


Supplementary FIGURE 1

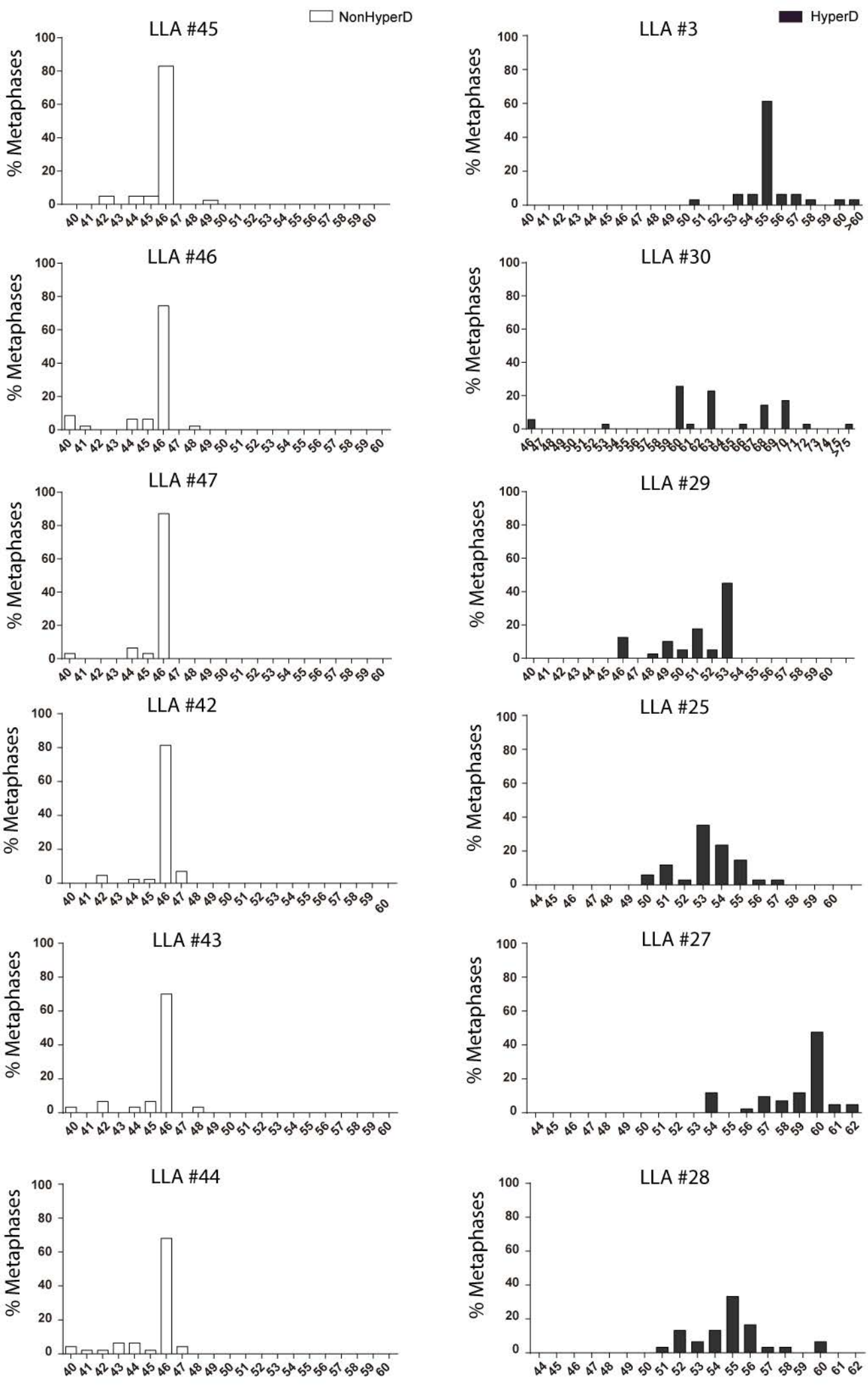




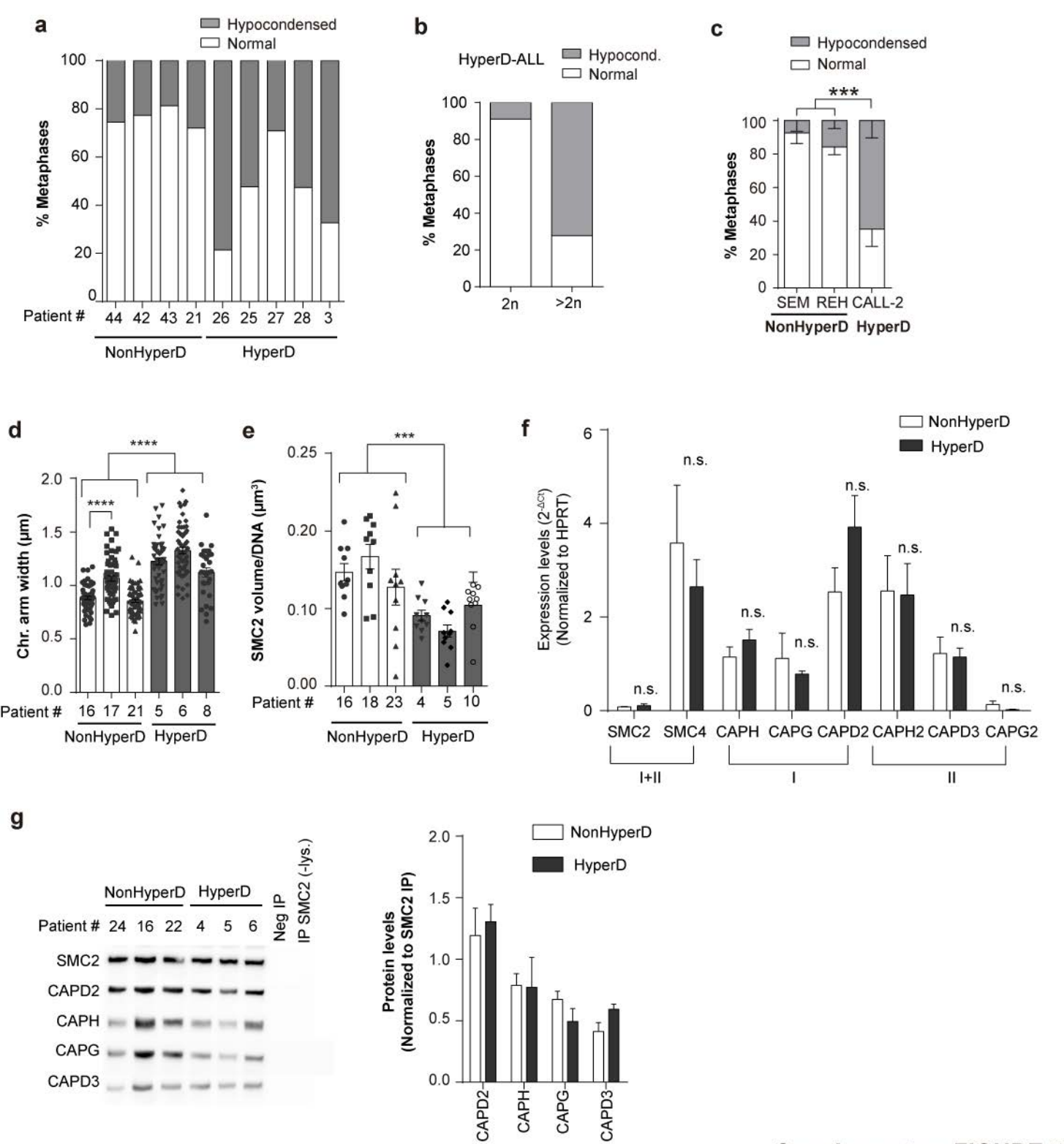
Supplementary FIGURE 2



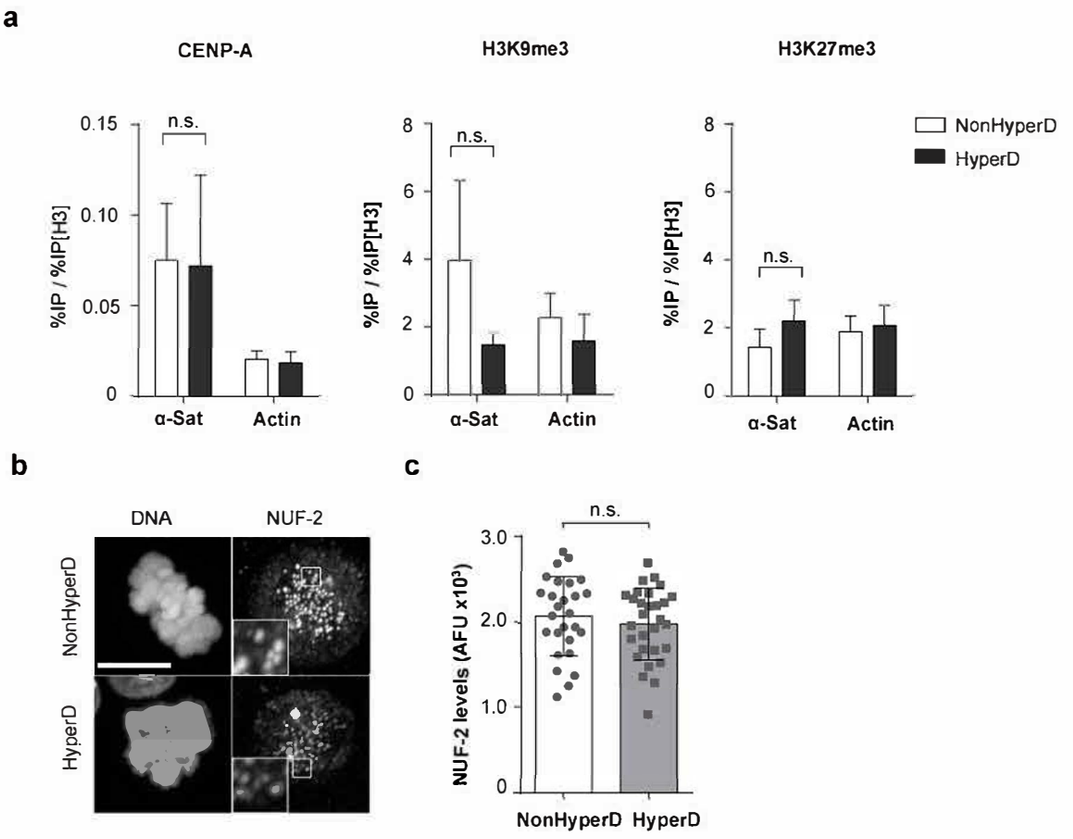
Supplementary FIGURE 3



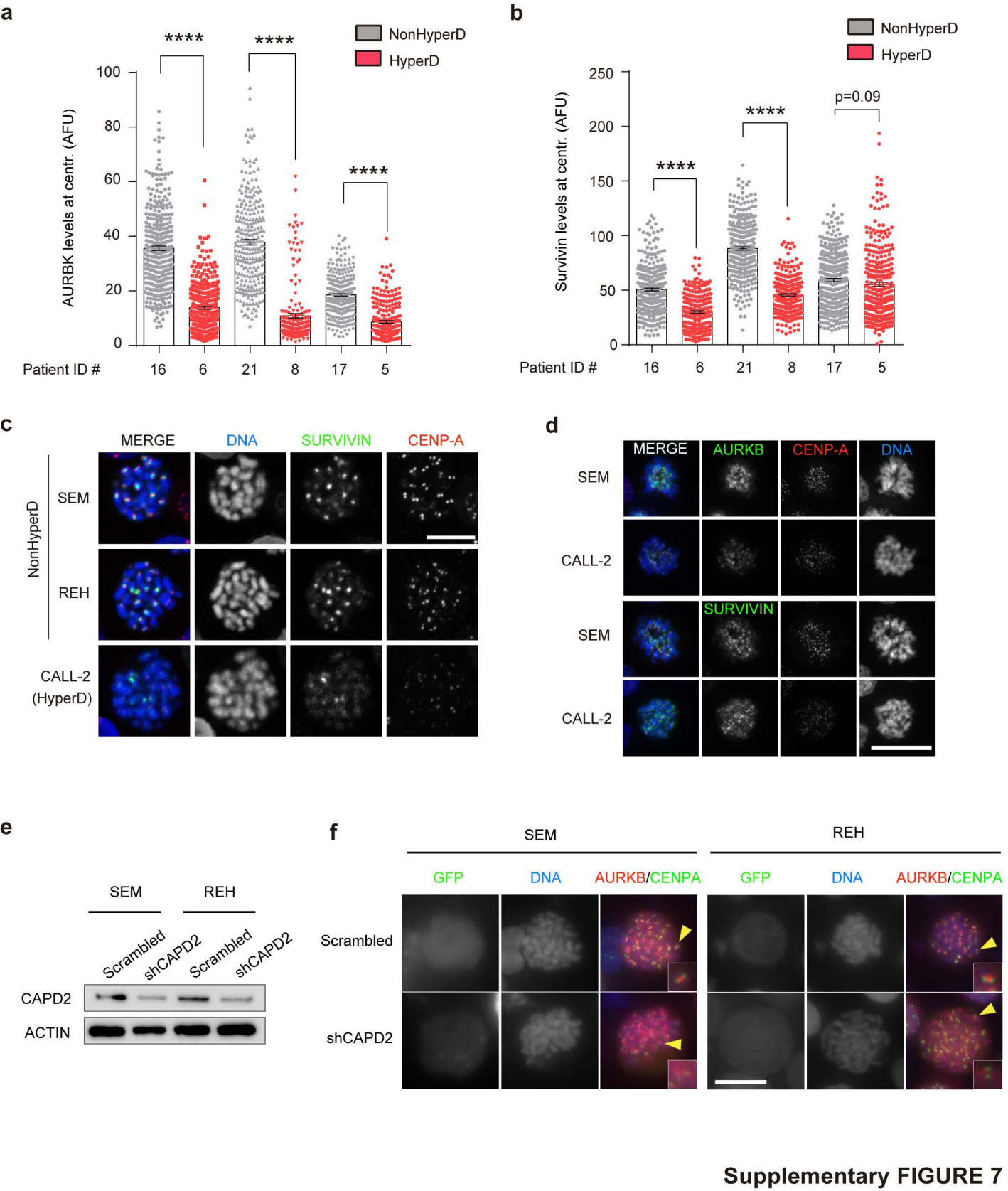
Supplementary FIGURE 4



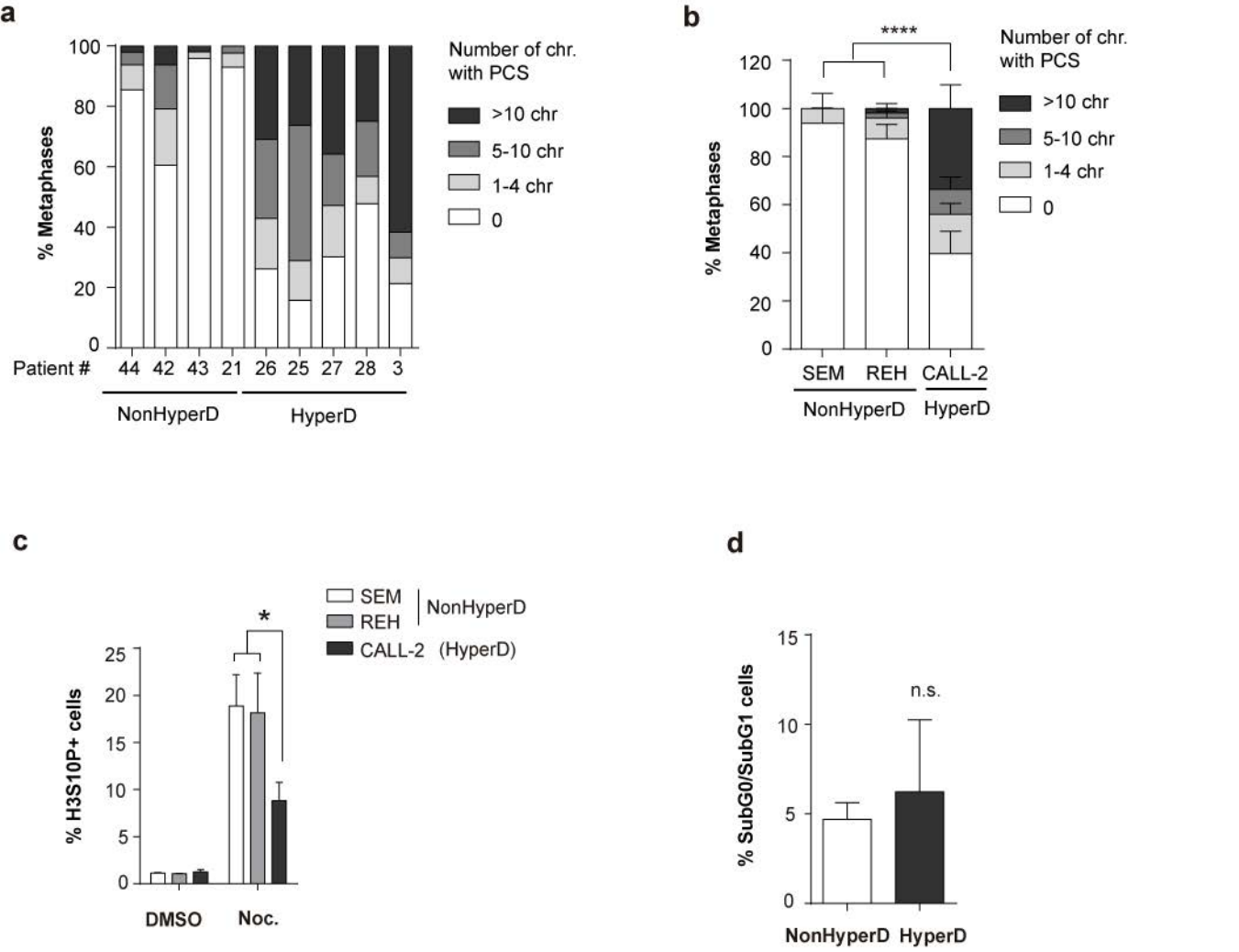
Supplementary FIGURE 5



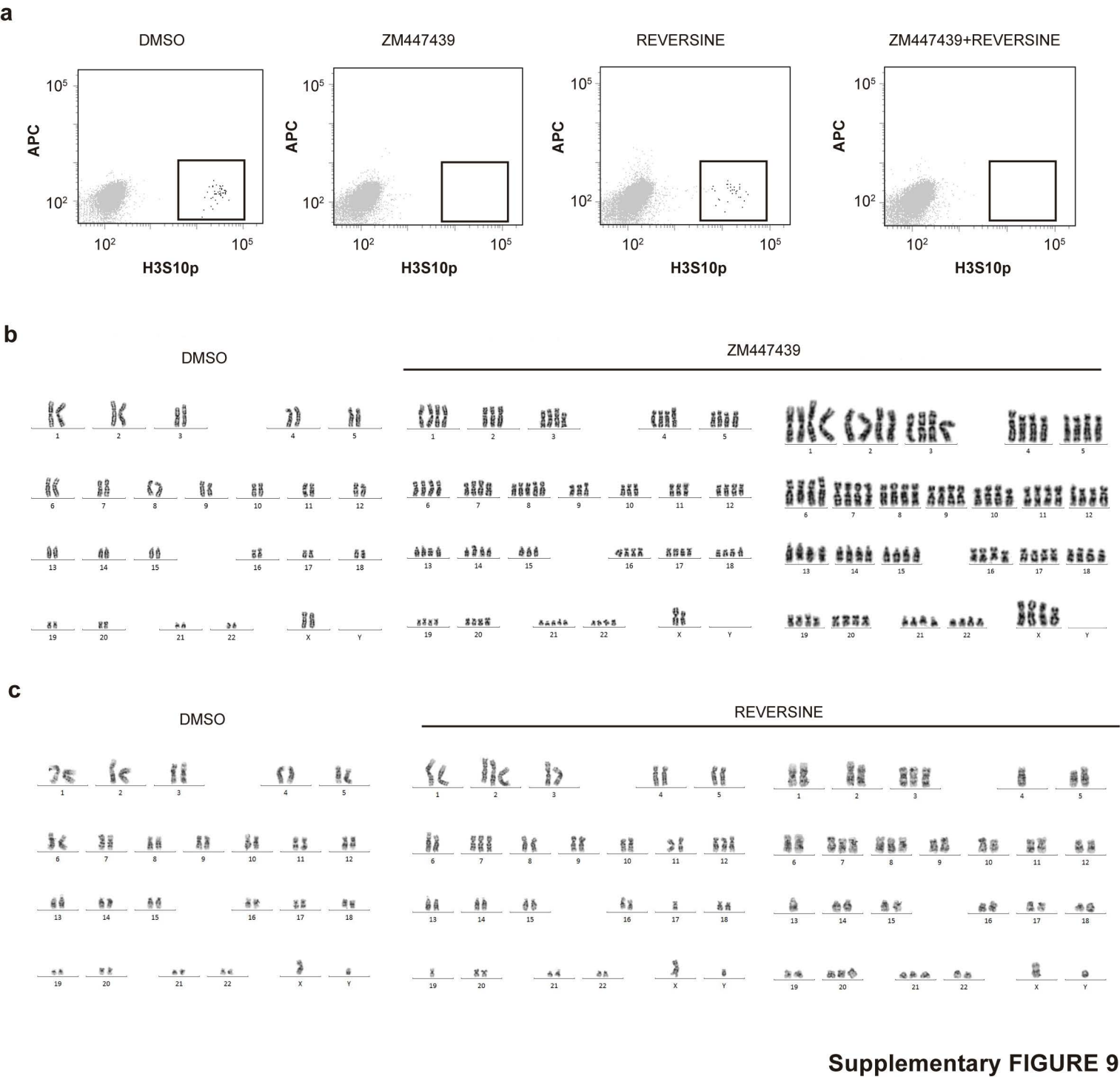
Supplementary FIGURE 6



Supplementary FIGURE 7



Supplementary FIGURE 8



Supplementary FIGURE 9

• B-ALL primary samples used for functional in vitro and in vivo assays

Patient ID	B-ALL subtype	Cytogenetics	Gender	% blasts at dx
#1	HyperD	57,X,+X,+X,-Y,dup(1)(q11~12q31),+4,+6,+7,+8,+10,+14,+17,+18,+21,+21[27]/46,XY[6]	M	96
#2	HyperD	FISH: +4, +10, +17, +21	M	86
#3	HyperD	Hyperdiploid by DNA index	N.A.	N.A.
#4	HyperD	51-55,XY,X,+6,+10,+14,+17,+18,+19,+21, +21,+22[cp19]/46,XY[2]	M	97*
#5	HyperD	57-58 XX, +X +X, +3 ,+ 6, +8, +8, +10, +10, +13, +14,+14, +17, +18,+18, +21, +21 [cp13] /46, XX [6]	F	90*
#6	HyperD	56-59, XX + X,+4,+5,+6,+7,+8,+10,+14,+14,+14,+15,+17,+18, +21,+21,+22 [cp6]	F	93*
#7	HyperD	N.A.	M	N.A.*
#8	HyperD	53-55, XY, +X,+3,+6,+9,+14,+17,+18,+18,+21[cp8]	M	98*
#9	HyperD	67 chromosomes	M	100
#10	HyperD	53,XY,+X,+4,+6,+14,+17,+21,+21[24]/46,XY[1]	M	97
#11				
#12				
#13	B-other	-	M	93
#14	B-other	-	F	97
#15	11q23/MLLr	46,XX,t(4;11)(q21;q23)[6]/46,XX[4]		N.A.*
#16	B-other	-	M	34
#17	11q23/MLLr	FISH: MLLr		N.A.*
#18	TCF3/PBX1	46,XX,t(1;19)	F	N.A.*
#19	11q23/MLLr	FISH: MLLr	F	90*
#20	11q23/MLLr	46,XX,t(9;11)(p21;q23)	F	N.A.*
#21	B-Other	-	F	N.A
#22	B-Other	-	M	54
#23	11q23/MLLr	46,XX,t(9;11)(p21;q23)	F	90*
#24	Ph +	t(9;22)(q34;q11)		90*

• B-ALL patient samples used for cytogenetics and gene expression analysis

Patient ID	B-ALL subtype	Cytogenetics	Sample type
#25	HyperD	55, XXY, +4, +6, +9, +13, +17, +18, +21, +21/46, XY	Carnoy fixation
#26	HyperD	57,XX, +X, +4, +6, +10, +14, +14, +17, +18, +18, +21, +21/46, XX	Carnoy fixation
#27	HyperD	60, XY, +X, +4, +6, +7, +8, +9, +14, +17, +18, +21, +21, +3mar/46,XY	Carnoy fixation
#28	HyperD	56, XX, +4, +6, +7, +8, +14, +17, +18, +21, +21, +22	Carnoy fixation
#29	HyperD	53, XY,+X,+4,+6,-13,+14,+21,+mar1,+mar2 [10]/53,idem,-6,+der(6?)t(6?;?)(q?;?) [5]	Carnoy fixation
#30	HyperD	62-70, X,-XX,+der(1)t(1;?)(p?;?)x2,+2x2,+3,+4,+5,+6x2,+8,+10,+11x2,+12x2,+13x2,+14,+17,+18, +19x2,+20,+21x2,+22x2,+2-4mar inc[cp16]	Carnoy fixation
#31	HyperD	61,XXY,der(1),+5,+6,+8,+9,+10,+14,+15+16+17+18+19+20+21+22 [20]	RNA
#32	HyperD	53-56,XXYY,+4,+6,+10,+17,+21[6]/46,XY[14]	RNA
#33	HyperD	50,XXY,+8,+10,+21[18]/46,XY[2]	RNA
#34	HyperD	54,XX,+X,dup(1)(q21;q32)?,+4,+6,+10,+14,+17,+18,+21[20]/46,XX[7]	RNA
#35	HyperD	FISH: High hyperdiploid	RNA
#36	HyperD	FISH: High hyperdiploid	RNA
#37	HyperD	FISH: High hyperdiploid	RNA
#38	HyperD	FISH: High hyperdiploid	RNA
#39	HyperD	FISH: High hyperdiploid	RNA
#40	HyperD	54,X,+X,Y,+6,+10,+14,+17,+18,+21,+mar[30]	RNA
#41	HyperD	59,XXYY,der(1)(q?),+4,+5,+6,+8,+10,+11,+18,+18,+21,+22,mar [9]/46,XY [41]	RNA
#42	B-other	-	Carnoy fixation
#43	B-other	-	Carnoy fixation
#44	B-other	-	Carnoy fixation
#45	B-other	-	Carnoy fixation
#46	B-other	-	Carnoy fixation
#47	B-other	-	Carnoy fixation
#48	B-other	-	RNA
#49	B-other	-	RNA

#50	B-other	-	RNA
#51	B-other	-	RNA
#52	B-other	-	RNA
#53	B-other	-	RNA
#54	B-other	-	RNA

B-other: absence of main B-ALL classifying alterations. Absence of both recurrent chromosomal translocations/fusion oncogenes (aneuploidy, 11q23/MLL, t(9;22)/BCR-ABL1, t(12;21)/ETV6-RUNX1, t(1;19)/TCF3-PBX1) and abnormalities affecting genes recurrently mutated/fused in B-cell ALL including *RAS, DUX4, PAX5, IKZF1-3, CDKN1/2, ERG*. *Primograft samples

Table S1: Biological and cytogenetic characterization of childhood B-ALL samples used in the study

Antibody	Host	Source	Identifiers	Fixation method	Buffer	Dilution
anti-α-Tubulin	Mouse	Sigma	Cat#DM1A	2-4% formaldehyde 10min,37°C	PEM	1:1000
anti-α-Tubulin	Rat	Abcam	Cat#ab6160	2-4% formaldehyde 10min,37°C	PEM	1:1000
anti-pericentrin	Rabbit	Abcam	Cat#ab4448	2-4% formaldehyde 10min,37°C	PEM	1:1000
ACA*	Human	Antibodies Incorporated	Cat#15-234	2-4% formaldehyde 10min,37°C	PEM	1:50
anti-SMC2	Rabbit	A. Losada Lab (CNIO, Spain)	Kind gift	4% formaldehyde/0.02% Triton X100 10min,37°C	PEM	1:1000
anti-CENP-A	Mouse	Abcam	Cat#ab13939	Methanol 10 min, -20°C	PBS	1:300
anti-CENP-A	Mouse	Antibodies Incorporated	Cat#ADI-KAM CC006-E	2-4% formaldehyde 8min (37°C), methanol 2min (-20°C)	PBS	1:300
anti-NUF2	Rabbit	Abcam	Cat#ab122962	Methanol 10 min, -20°C	PBS	1:200
anti-AURKB	Rabbit	Abcam	Cat#ab70238	2-4% formaldehyde 8min (37°C), methanol 2min (-20°C)	PBS	1:200
anti-Survivin	Rabbit	Novus Biologicals	Cat#NB500-201	2-4% formaldehyde 8min (37°C), methanol 2min (-20°C)	PBS	1:500

*ACA=anti-centromeres antibody.

Table S2: Antibodies and conditions used for immunofluorescence experiments.

Antibody	Host	Source of reference	Identifiers	Dilution
anti-SMC2	Rabbit	A. Losada Lab (CNIO, Spain)	-	1:1000
anti-CAPH	Rabbit	A. Losada Lab (CNIO, Spain)	-	1:1000
anti-CAPD3	Rabbit	A. Losada Lab (CNIO, Spain)	-	1:1000
anti-CAPD2	Rabbit	Bethyl Laboratories	A300-601A	1:500
anti-CAPG	Rabbit	Bethyl Laboratories	A300-602A	1:1000
anti-β-Actin	Mouse	Sigma	A5441	1:1000

Table S3: Antibodies and conditions used for Western-blot experiments.

Name Oligonucleotide	Sequence (5' - 3')	Lenght
α-satellite -Fw	CTCAGAAACTTCTTTGTGATGTGT	24
α-satellite-Rv	TATTCCCTTTTGGAACGAAGGC	22
Actin-Fw	GCCGGGACCTGACTGACTAC	20
Actin-Rv	AGGCTGGAAGAGTGCCTCAG	20
MAD2L2-Fw	CCTGGTGCACACGAGAGA	18
MAD2L2-Rv	GGATCCAGGGGAAATCCTT	19
MAD2L1-Fw	GAAATCGTGGCCGAGTTCTT	20
MAD2L1-Rv	GGATATATGCCACGCTGATA	20
CDC20-Fw	CTGTCTGAGTGCCGTGGAT	19
CDC20-Rv	TCCTTGTAATGGGGAGACCA	20
PLK1-Fw	AACGACTTCGTGTTCGTGGT	20
PLK1-Rv	AGGGCTTTCCTCCTCTTGTG	20
HASPIN-Fw	CAAGTGGTGCTCCGTCCT	18
HASPIN-Rv	GAGTGTTTAAGGGGCTTAGCAA	22
CYCB-Fw	CCCTGCTGCAACCTCCAA	18
CYCB-Rv	TGTTCACTGACTTTGTTACCA	21
AURKA-Fw	CATCTTCCAGGAGGACCACT	20
AURKA-Rv	TCCGACCTTCAATCATTTCA	20
CDK1-Fw	CCTAGTACTGCAATTCGGGAAATT	24
CDK1-Rv	TCCTGCATAAGCACATCCTGAA	22
AURKB-Fw	CCCATCTGCACTTGTCCTCA	20
AURKB-Rv	TGTCCCACTGCTATTCTCCA	20
ESPL1-Fw	GAGTTCCTGTCCAACCCTCC	20
ESPL1-Rv	AGGATGGCATCACAAGCTTG	20
BUB1B-Fw	TACTCTTCAGCAGCAGAAACG	21
BUB1B-Rv	CCAGAGGGTCATTTCCAGTG	20
RPL19-Fw	AGCGAGCTCTTTCCTTTCG	19
RPL19-Rv	GAGCCTCTTCTGAAGCCTGA	20
SMC2-Fw	AAATGACTTCCCCTCCTCCCG	21
SMC2-Rv	CAAACCAGTTTTGTCTGTAAGTTCC	25
SMC4-Fw	TTGAACAGCATTCCTCCTCCC	21
SMC4-Rv	GGAAAAGCGCTTATGGAAAGGT	22
CAPD2-Fw	ACTACAGTGGTGCAGGAGGT	20
CAPD2-Rv	TTGGCGGTAGGCATTAAGCA	20
CAPH-Fw	GGCACCCCAGTCCTCGAA	18
CAPH-Rv	GCTGAAATGTCAATACTCCTGCT	23
CAPG-Fw	TCCACATAGAGAAGAATGATGCTGA	25
CAPG-Rv	TGGTTGCACTTAAGCCTGTTG	21
CAPD3-Fw	CCCCGAGAAGAGCATCAGTG	20
CAPD3-Rv	CCTTGACTCCGGCCTTCAAT	20
CAPH2-Fw	CTGCGTGAAGGAGACTCCAGAC	22
CAPH2-Rv	CCTTCCTCTTGCGCTTCTGTC	21
CAPG2-Fw	AGCCCAGTACCAGACCTCCT	20
CAPG2-Rv	GGCTTGTACAAACGTCTCACG	21
HPRT-Fw	CCTGGCGTCGTGATTAGTGAT	21
HPRT-Rv	AGACGTTCAGTCCTGTCCATAA	22

Table S4: Oligonucleotides used for qPCR experiments (Fw: Forward, Rv: Reverse).