Chromatin immunoprecipitation (ChIP) in human B cells

To evaluate Myc binding to human *AURKA* and *AURKB* the human B cell line, P493-6, was cultured with tetracycline (0.1 ug/ml) for 72 hours (+Tet) and released from tetracycline for 8 hours (-Tet). Chromatin from 1.3×10^7 cells/ condition was cross-linked, isolated, sonicated and reverse cross linked (Lee, Johnstone et al. 2006). Myc protein was immunoprecipitated from sonicated chromatin using a rabbit polyclonal antibody (Santa Cruz) and magnetic protein G beads (Active Motif, Carlsbad, CA). 1:100 volume of chromatin was processed without antibody and used as total input control for qRT- PCR. qRT-PCR was run on immunoprecipitated and control chromatin using primers designed to detect E-boxes in the human *AURKA* and *AURKB* genes. Primers to detect the known Myc target gene *CCND2* were run as positive controls for the human Myc ChIP (Fernandez, Frank et al. 2003). Percent total chromatin immunoprecipitated was calculated using the equation % total = $2^{\text{Ct input} - \text{Ct ChIP}}$ X % input used for ChIP.

RNA preparation and analyses

Expression profiling was performed using data previously accumulated (Keller, Nilsson et al. 2005; Nilsson, Keller et al. 2005). Briefly, RNA was prepared using the RNeasy kit (Qiagen, Valencia, CA). cRNA was synthesized using the One-Cycle Target Labeling and Control Reagent package (Affymetrix Inc., Santa Clara, CA) and the reaction was probed to the 430A mouse Affymetrix chip (Affymetrix Inc.). The scanned data output was imported into the Spotfire software (TIBCO Spotfire, Somerville, MA). Following normalization, selected probe sets of the genes indicated in Supplemental Figure S1 were clustered using the Hierarchical Clustering function of Spotfire (TIBCO Spotfire).

Fluorescence **in situ** *hybridization (FISH)*

FISH was performed using dual-colour *AURKA* and *AURKB* probes (Supplemental Table S1) labeled with PlatinumBright550 and PlatinumBright495 (Kreatech Diagnostics, Amsterdam, Netherlands). 8-µm paraffin sections were deparaffinized and pretreated with 0.1% proteinase K (Invitrogen) for 15 – 18 min at 37°C. Samples were aged in 0.1% NP-40/2×SSC and DNA was denatured by treatment in 70% formamide/2×SSC. After dehydration slides were hybridized with 8µl of the probes overnight at 37°C and washed. Nuclei were counterstained with 4,6-diamino-2-phenylindole dihydrochloride (DAPI, Sigma-Aldrich, Heidelberg, Germany). FISH analysis was performed using a fluorescence microscope equipped with the tripple bandpass filter set. Signals were scored manually (×100 oil immersion objective) in morphologically intact and non-overlapping nuclei. 100 cells were scored for each case (n=5 human Burkitt lymphomas) by two independent operators.

Table S1. Sequences of primers used for real-time PCR and cloning. List of antibodies used for immunoblotting, immunohistochemistry and ChIP assay. Probes used for FISH analyses. qRT-PCR data analysis was performed by comparing ΔΔCt values with a control sample set as 1 (Livak and Schmittgen 2001).

Figure S1. Hierarchical clustering of selected mitosis-associated genes and some control genes. Gene expression analysis was performed using RNA prepared from $B220^+$ splenic B cells from five weanling-age wild type mice (wt), from five Eμ-*Myc* transgenic mice (Eμ-*Myc*), and from thirteen Eμ-*Myc* lymphomas. Probe set signals were normalized to the mean across mice, and values of each individual case are represented by a colour, with green corresponding to expression below, and red corresponding to expression above the mean.

Figure S2. Analysis of Myc binding to human *AURKA* and *AURKB* assessed by chromatin immunopreciptiation. (A) Map of the human *AURKA* and *AURKB* gene regions analyzed for Myc binding. A1.1, A1.2 and A1.3 indicate the PCR amplicons for *AURKA*, B1.3 indicates the PCR amplicon for *AURKB*. (B) P493-6 cells were cultured in the presence of tetracycline (Myc-off) for 72 hours. Tetracycline was then washed off and cells were left untreated (Myc-on; red columns: ChIP-tet) or again cultured with tetracycline (Myc-off; blue columns: ChIP+tet) for 8 hours. Genomic DNA was crosslinked, sheared by sonification and incubated with isotype control antibody or anti-Myc antibody and subsequently analyzed by quantitative real-time PCR for Myc-bound DNA with the primers pairs indicated in (A). The left panel shows the increase in % of total (= [2Ct input – Ct ChIP] x % chromatin used) (Frank, Schroeder et al. 2001), the right panel shows the calculated –fold increase in Myc-bound genomic DNA upon tetracycline withdrawal. Shown is one respresentative experiment of 2 experiments each performed in duplicate. No significant binding of Myc to the analyzed regions of human *AURKA* and *AURKB* was detected.

Figure S3. Fluorescence in Situ Hybridization to assess *AURKA* and *AURKB* gene copy number in human BL. 5 human BL samples were analyzed for *AURKA* and *AURKB* gene amplification by Fluorescence *In Situ* Hybridization (FISH). A representative case is shown. *AURKA* analysis: red colour shows the probe for chromosome 20 and green colour indicates the specific *AURKA* probe. *AURKB* analysis: red colour shows the specific probe for chromosome 17 and green colour indicates the specific *AURKB* probe. FISH analysis did not reveal *AURKA* and *AURKB* gene amplification in n=5 analyzed human Burkitt lymphoma samples.

Figure S4. Quantification of DNA content upon treatment with the Aurora kinase inhibitor AS703569. Eμ-*Myc* lymphomas cultured ex vivo were treated with vehicle only (untreated) or treated with 25nM AS703569 (AKI) for 6h, 24h or 48h. The PI-stained cells were analyzed using the FL2 channel in a linear scale. Apoptosis measurements (Sub-G1) were based on the number of cells which carried less than diploid DNA content (sub-G1) in the FL3 channel in a logarithmic scale. The bars represent the mean ± SEM from three independently performed experiments. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$.

Figure S5. Aurora kinase inhibition by chemical means triggers transient mitotic arrest, polyploidization and apoptosis of Eμ-*Myc* lymphoma cells. (A) Eμ-*Myc* lymphomas cultured ex vivo were treated with 25nM AS703569 for 24h and then collected by cytospin. The Giemsa

and May-Gruenwald staining shows one cell arrested in mitosis and one which has undergone endoreduplication (arrows). A representative image is shown. (B) Ex vivo cultured Eμ-*Myc* lymphoma cells were assessed for DNA content by flow cytometry analysis of propidium iodide (PI) stained cells. Cells were treated with vehicle only (untreated) or with AS703569 25nM (AKI) ± pretreatment with the pan-caspase inhibitor Q-VD-OPH 10μM for the indicated time. The PI-stained cells were analyzed using the FL2 channel in a linear scale. Apoptosis measurements were based on the number of cells which carried less than diploid DNA content (Sub-G1) in the FL3 channel in a logarithmic scale. The upper panel shows representative histograms from one experiment. The lower panel represents the results from three independently performed experiments with bars representing the mean ± SEM. * indicates statistically significant differences ($p<0.05$).

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Fernandez, P. C., S. R. Frank, et al. (2003). "Genomic targets of the human c-Myc protein." Genes Dev **17**(9): 1115-1129.
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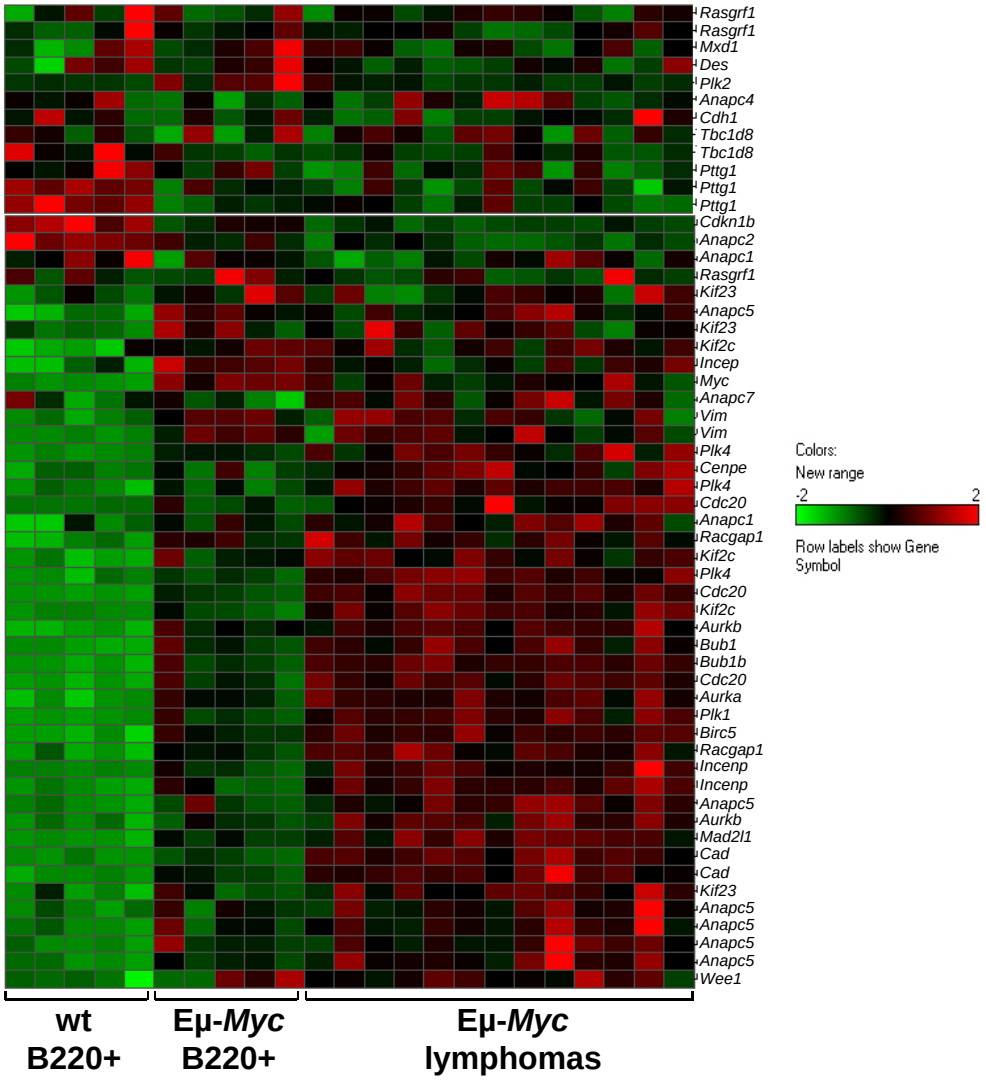
Nilsson, J. A., U. B. Keller, et al. (2005). "Targeting ornithine decarboxylase in Myc-induced lymphomagenesis prevents tumor formation." Cancer Cell **7**(5): 433-444.

Table S1

Primers			
Protein	Gene	Sense primer	Antisense primer
Human:			
c-Myc	*MYC*	5′-TCAGAGTCTGGATCACCTTCTGCT-3′	5′-TGCGTAGTTGTGCTGATGTGTGGA-3′
Aurora kinase A	*AURKA*	5′-TCCTGAGGAGGAACTGGCATCAAA-3′	5′-TACCCAGAGGGCGACCAATTTCAA-3′
Aurora kinase B	*AURKB*	5′-ATCAGCTGCGCAGAGAGATCGAAA-3′	5′-CTGCTCGTCAAATGTGCAGCTCTT-3′
Ubiquitin	*UB*	5′-ACCTGACCAGCAGCGTCTGATATT-3′	5′-TCGCAGTTGTATTTCTGGGCAAGC-3′
Mouse:			
c-Myc	*Myc*	5′-CAAATCCTGTACCTCGTCCGATTC-3′	5′-CTTCTTGCTCTTCTTCAGAGTCGC-3′
Aurora kinase A	*Aurka*	5′-CACACGTACCAGGAGACTTACAGA-3′	5′-AGTCTTGAAATGAGGTCCCTGGCT-3′
Lactate DehydrogenaseA	*Ldha*	5′-AGGTTACACATCCTGGGCCATT-3′	5′-TCAGGAGTCAGTGTCACCTTCACA-3′
Aurora kinase B	*Aurkb*	5′-GCCCCTCGCGGGGAACTCTA-3′	5′-GGCATGCACCGACCAGCCAA-3′
Ubiquitin	*Ub*	5′-TGGCTATTAATTATTCGGTCTGCAT-3′	5′GCAAGTGGCTAGAGTGCAGAGTAA-3′
Mouse *Aurk* genomic			
Promoter cloning			
Aurka		5′-CAATGCTTGCTGACAGACACCCC-3′	5′-CACAGGCAGGAACCTGCTCC-3′
Aurkb		5′-ACGCGTAAATGCACACAAGCCCTGTCTTCC-3′	5′-AGATCTAGCTGGACAGAGAGGGAAACAACA-3′
ChIP-mouse			
A1.1		5′-ACCGAGGCCCTTTGAGAG-3′	5′-TAAGGAAGCGGGCTAGTGTG-3′
A1.2		5′-CCTAGCCAACCACACTAGCC-3′	5′-ACGATTCGCTCCTCCATTTT-3′
A1.3		5′-AAAATGGAGGAGCGAATCGT-3′	5′-GTTCGGAGACGGTGAATGAG-3′
A2.1		5′-CTGTGGCATGTGCAACTTTC-3′	5′-GGAGGAGTCCAAGTCCTTCA-3′
A2.2		5′-GGAGTGTGAGGTAGCTGCAA-3′	5′-CACCAAATGACCTTGATGGTT-3′
A3.2		5′-TGCACACTAGAAGAGGGCATT-3′	5′-ATGGCTCAGCGATGAAGAGT-3′
A control		5′-CAGACCCGCTAAAGCTCAG-3′	5′-GGCTGGTTTTTCGGACCTAC-3′
B1.1		5′-CTCTGGCTGACCAGGAACTC-3′	5′-ATGTGCACGCTCCTTTTTCT-3′
B1.2		5′-GTGCACATCTTGGGACACAG-3′	5′-CTTTCGAATCTCCCGCTCTA-3′
B1.3		5′-GAGCGCCTAGTGGCGTAG-3′	5′-AGAAGGAAGCGAGTCAGCAC-3′
B2.1		5′-GCCATCCTGGAACTCAGTCT-3′	5′-ACGGTCTGCCTGCCTAGC-3′
B2.2		5′-TGGCTGCCAGTTGTTTCTTA-3′	5′-TATGCTACAAGGCGCATTTC-3′
B control		5′-TACATGATGGGATCCTGTGG-3′	5′-ACTGCACGCTTGTCAGTTTG-3′
CAD		5′-CACTACGCTTAGGGCTCTGG-3′	5′-GGAGCTGAGACAGGGCAAG-3′
ChIP-human			
A1.1		5′-TCCCTCCCTCATTAGCTTCA-3′	5′-AGTGCTGTTGCTCGATAGGC-3′
A1.2		5′-GTGTGTTGTGGAGGCCTTTT-3′	5′-ACCTTAACAGGTCCTGAAATGC-3′
A1.3		5′-GCTTTGTTCAAATTAAAGGTTCTT-3′	5′-CCCGACAAGACCAACCTC-3′
A control		5′-CTGGATGTACTCGGCTAGGG-3′	5′-AAGGATTTGCCCAGGATCTC-3′
B1.3		5′-ACCTGGGAGGTGGAGGTT-3′	5′-GGACACTAAGAGCTACCCTGATG-3′
B control		5′-TCCTAAACTGGAAGCCAAGC-3′	5′-TGGCCAAGGACTTTTCAAAT-3′
CCND2		5′-CCTTGACTCAAGGATGCGTTAGA-3′	5′-GAGCCGACTGCGGTGAAGT-3′
Antibodies		**Company**	**Catalogue number**
Anti-c-Myc		Santa Cruz Biotechnology, Santa Cruz, CA USA	sc-764
Anti-Aurka		Sigma-Aldrich, Saint Louis, MIS USA	A5102
Anti-Aurkb		Sigma-Aldrich, Saint Louis, MIS USA	A1231
Anti-pS10-HH3		Upstate, Lake Placid, NY USA	#06-570
Anti-Casp3 p17		Cell Signaling, Danvers, MA USA	#9661
Anti-p27 ^{Kip1}		BD Pharminogen, San Jose, CA USA	554002
Anti-ER		Santa Cruz Biotechnology, Santa Cruz, CA USA	sc-542
Anti-B220		BD Pharmingen, Heidelberg, Germany	550286
Ki-67		DCS Innovative Diagnostik-Systeme, Hamburg, Germany	KI681R06
Anti-Actin		Sigma-Aldrich, Saint Louis, MIS USA	A2066
Probes	**Gene**	**Company**	**Catalogue number**
Aurora kinase A	*AURKA*	Kreatech Diagnostics, Amsterdam, Netherlands	KBI-10721
Aurora kinase B	*AURKB*	Kreatech Diagnostics, Amsterdam, Netherlands	KBI-10722

Figure S1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Cdkn1b
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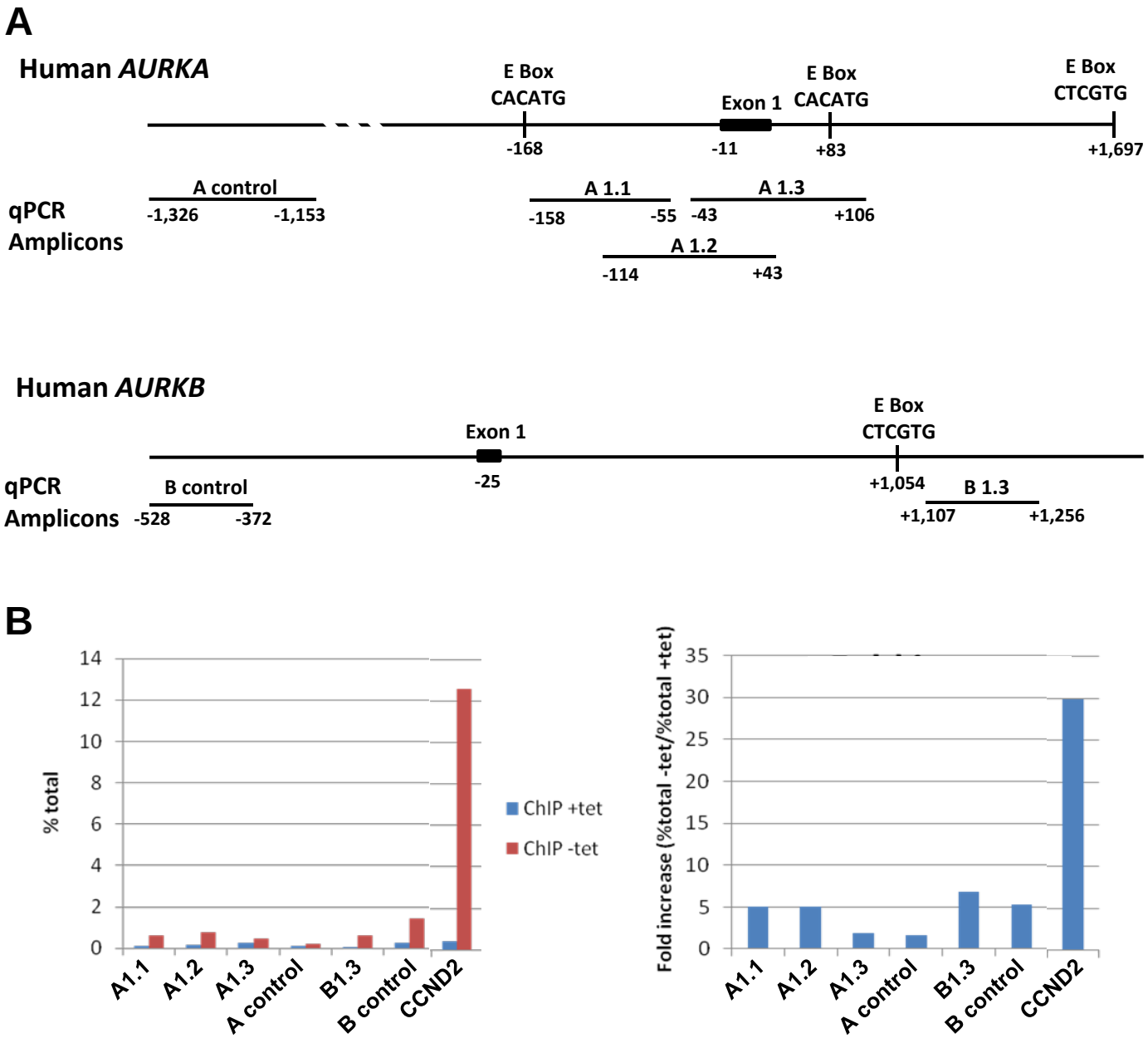
Eμ-Myc lymphomas

Figure S2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8
6
4
2
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A1.3
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B control
CCND2
ChIP +tet
ChIP -tet
Fold increase (%total -tet/%total +tet)
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B control
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Figure S3

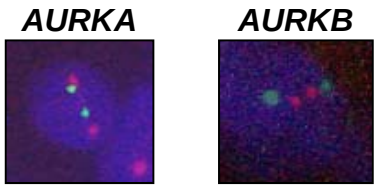
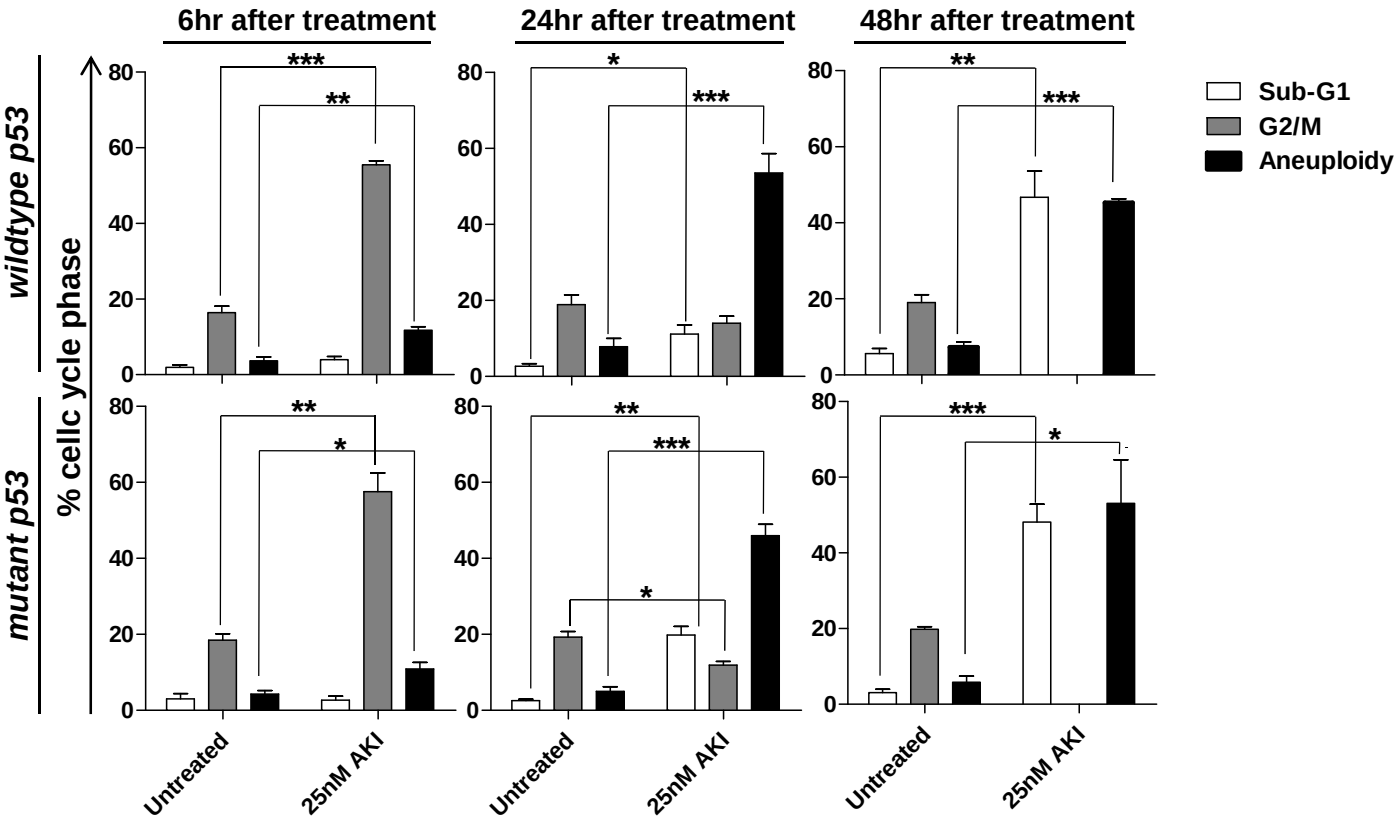


Figure S4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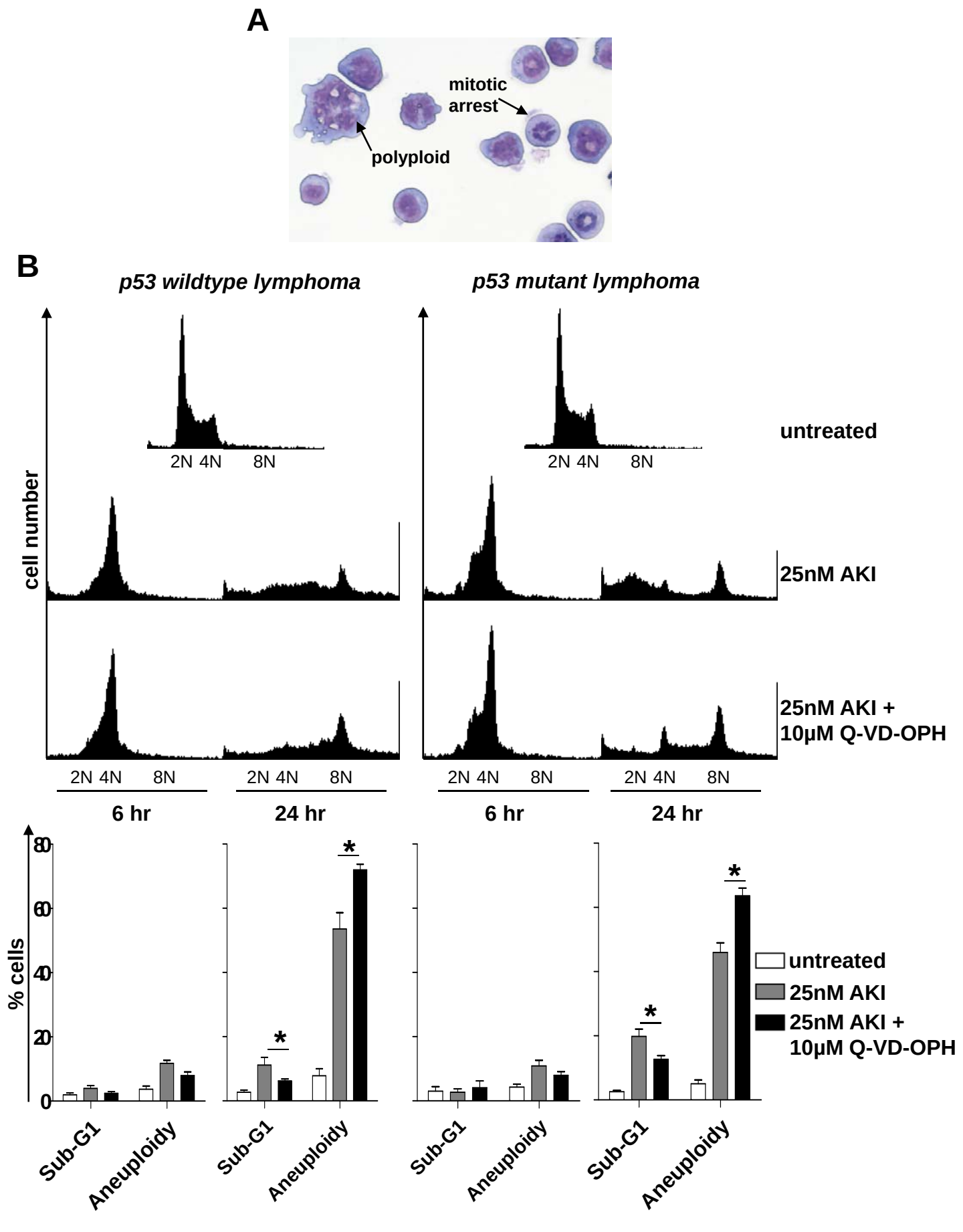
20
40
60
80
*
**
***

Figure S5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Sub-G1
Aneuploidy
untreated
25nM AKI
25nM AKI + 10μM Q-VD-OPH