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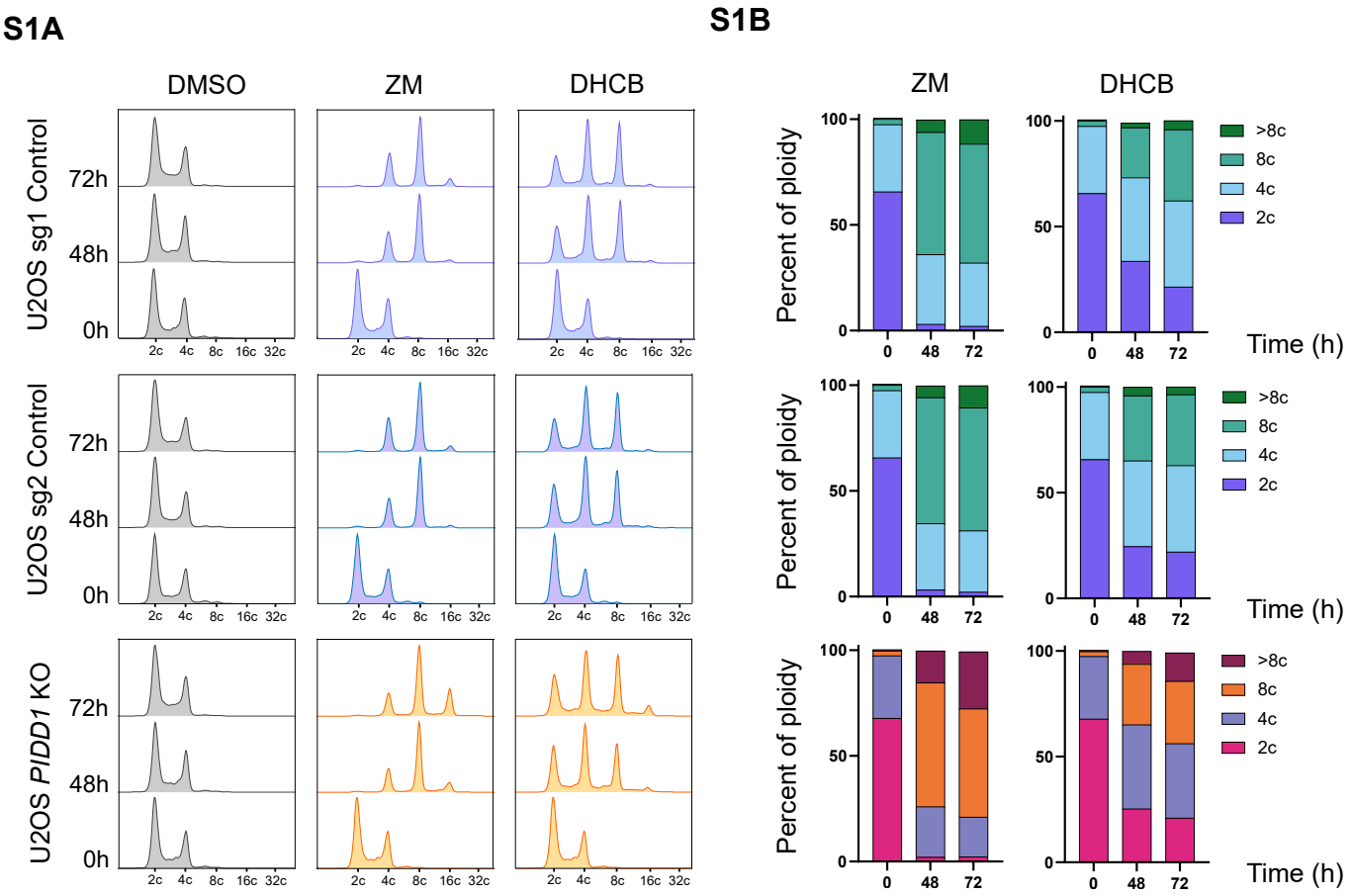
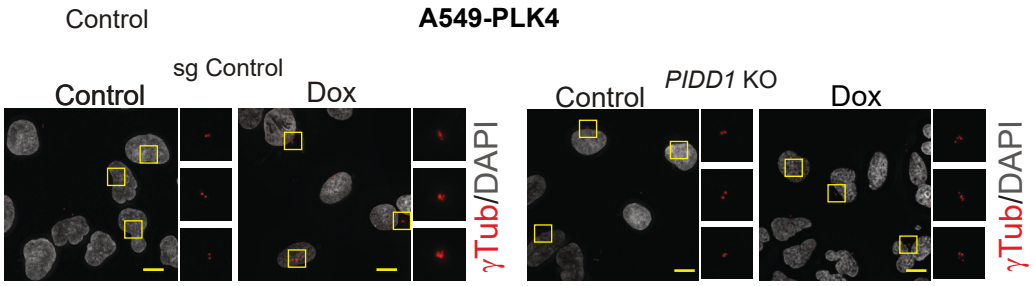


Appendix Figure S1: DNA content analysis of U2OS cells after drug-treatment

A: Representative histograms of U2OS cells of the indicated genotypes before or after treatment with ZM (2 µM) or DHCB (4µM) for up to 48h. DNA content was analyzed after fixation and propidium iodide staining in a flow cytometer.

B: Quantification of different ploidy stages based on DNA profiles obtained in (A). Bars represent means of two independent experiments.

S2A



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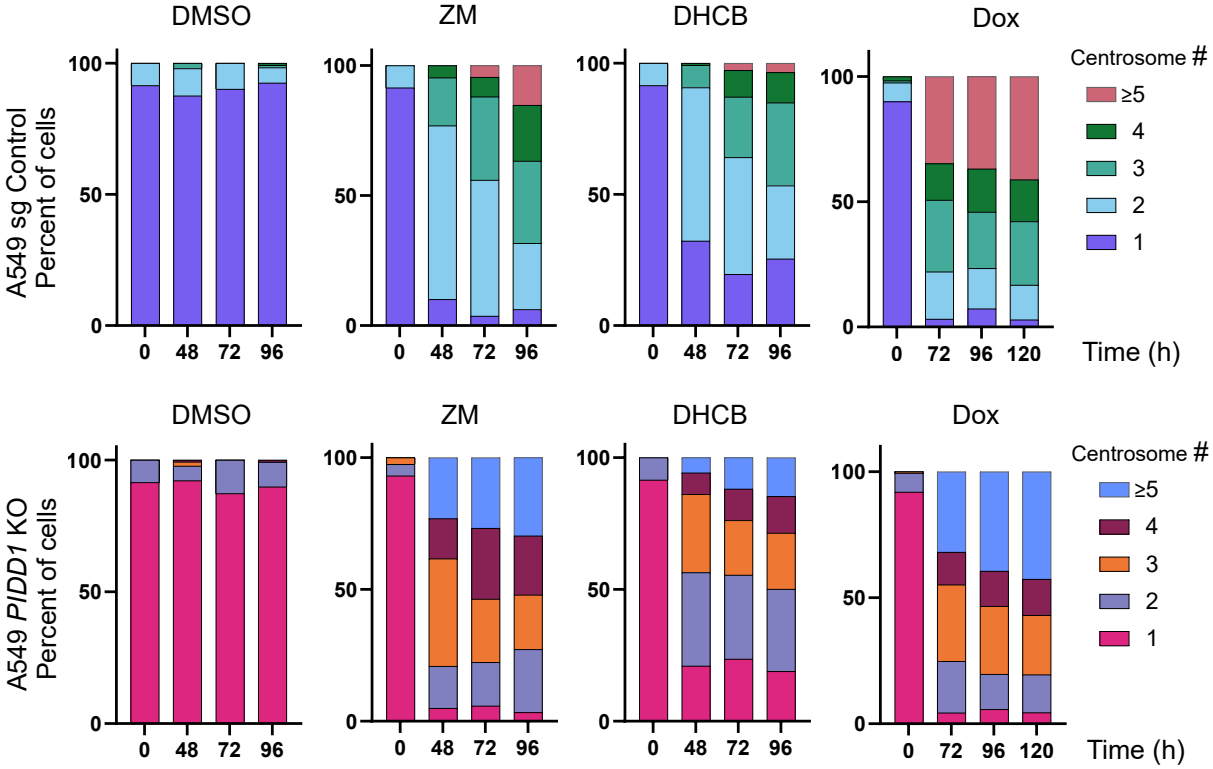

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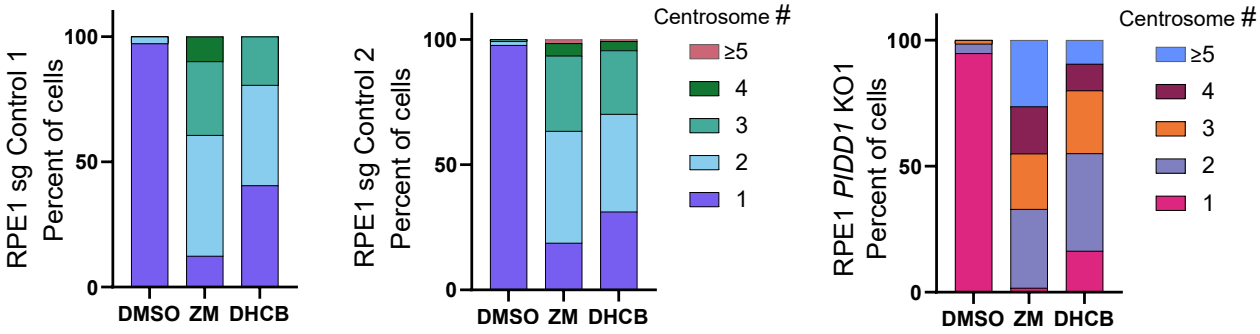


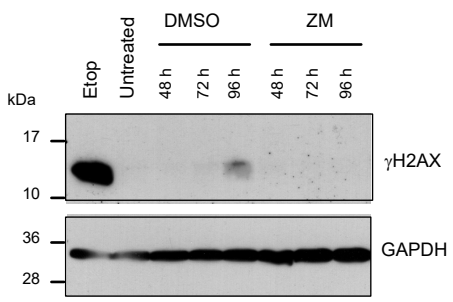
Appendix Figure S2: Evaluation of centrosome counts in A549-PLK4 and RPE1-PLK4 cells

A: Representative IF images of A549-PLK4 cells treated with DMSO (control) or Doxycycline (1µg/ml) for 72h. Cells were fixed and stained with an antibody specific for γ-Tubulin and DAPI.

B: Evaluation of centriole counts after treatment with either solvent (DMSO), ZM (2 µM), DHCB (4µM) or Doxycycline (1 µg/ml) for the indicated periods of time. A minimum of 50 cells per time point, condition and experiment were evaluated in a blinded manner. Bars represent means of two experiments.

C: RPE1 cells of the indicated genotypes were treated with ZM (2 µM) or DHCB (4µM) for 72h, or left untreated. A minimum of 50 cells per time point, condition and experiment were evaluated in a blinded manner.Bars represent means of two experiments.

S3A



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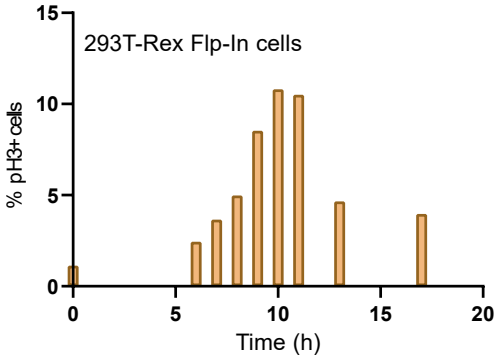
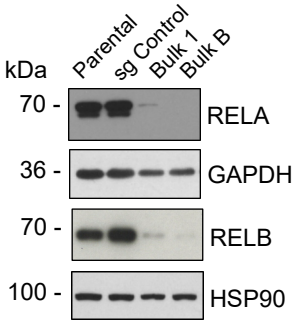


S3C



Appendix Figure S3: Assessment of DNA damage and mitotic timing

A: U2OS-PLK4 cells were exposed to DMSO or ZM (2µg/ml) for the indicated times. Etoposide treatment (10µg/ml) for 24h was used as a postive control. Cell extracts were analyzed by western blot analysis using antibodies specific for γH2AX or GAPDH to control for protein loading.

B: 293T-Rex Flp-In cells were exposed to a single thymidine block and released into fresh medium. Cells were fixed at the indicated times after release and subjected to immunofluorescence analysis using an anti-phosho histone H3 specific antibody to evaluate the percentage of cells in mitosis over time.

C: Westerm blot analysis of A549 RELA/B double mutants and controls confirming lack of RELA and RELB expression.

Appendix Table S1: cloning and CRISR primers

primer name	seqence
PIDD_BamHI_Exon_DD_R	CCCCTCGGACCCTCGGGATCCCGGCAGCTTGATGG
F_hPIDD_G876S	GCAGTCTTGGAGCTCAGCCGCCGCAAGTACC
R_hPIDD_G876S	GGTACTTGCGG GGCTGAGCTCCAGACTGC
F_hPIDD_R815W	CTGGGGGTGTCCTACTGGAGGTGCAGCGCA
R_hPIDD_R815W	TGCGCTGCACCTCCCAGTAGGACACCCCCAG
hPIDD_flag_Q863stop_F	GAGCAGAGTGACCGGGATTACAAGGATGACGATGACAAATAAGTCTTGGAGCTCGG
hPIDD_flag_Q863stop_R	CCGAGCTCCAAGACTTATTTGTCATCGTCATCCTTGTAATCCCGGTCACTCTGCTC
PIDD1_C_fwd1	AACTTAAGCTTGCCACCATGTCCTGGTTCCTTGTGG
PIDD1_CC_fwd1	AACTTAAGCTTGCCACCATGTCCTGGTACTGGCTCTGG
PIDD1_S588A_frw1	CCAGGTCACACACTTCGCCTGGTACTGGC
PIDD1_S588A_rev1	GCCAGTACCAGGCGAAGTGTGTGACCTGG
PIDDC_S446A_F	GGTGCCCCACTTCGCCTGGTTCCTTGTGG
PIDDC_S446A_R	CCACAAGGAACCAGGCGAAGTGGGGCACC
rtTA-pInd fw	CTGGAACTAATCATATGTGG
IRES-pInd rv	GGTGGACCGGTAAGCTTATC
HygR_pInduc21 fw	GCTTACCGGTCCACCATGAAAAAGCCTGAACTCAC
HygR_pInduc21 rv	TCGCCGTCGGGCATGCTATTCCTTTGCCCTCGGAC
HA-PIDD1-N	AACTTAAGCTTGCCACCATGTACCCATACGACGTACCAGATTACGCTGAATTCGGATCCATGGCTGCA

Name	**gRNA sequence**
PIDD1 1	GCCGATAGCGGATGGTGATG
PIDD1 2	GGCCCGGCGCTGCCGTGAAG
CASP 2	TGGTGAGCAACATATCCTCC
RAIDD	CCAGCTCCAGGCGAAGTGAG
RIPK1	TAGTCTGACGGATAAACACC
NEMO	ACGCCGCCGTCCGAGAGACG
SCLT1	GGGCCTCAGTCATATGTTCC
RELA	ACTACGACCTGAATGCTGTG
RELB	GAAAGCACAGGATCCATC
sg Control 1	TATCTAATCGCGGAGTCGTA
sg Control 2	GCTGGGTGAGTCGATTATCC