# Supplemental Material

# Preferential Killing of Tetraploid Colon Cancer Cells by Targeting the Mitotic Kinase PLK1

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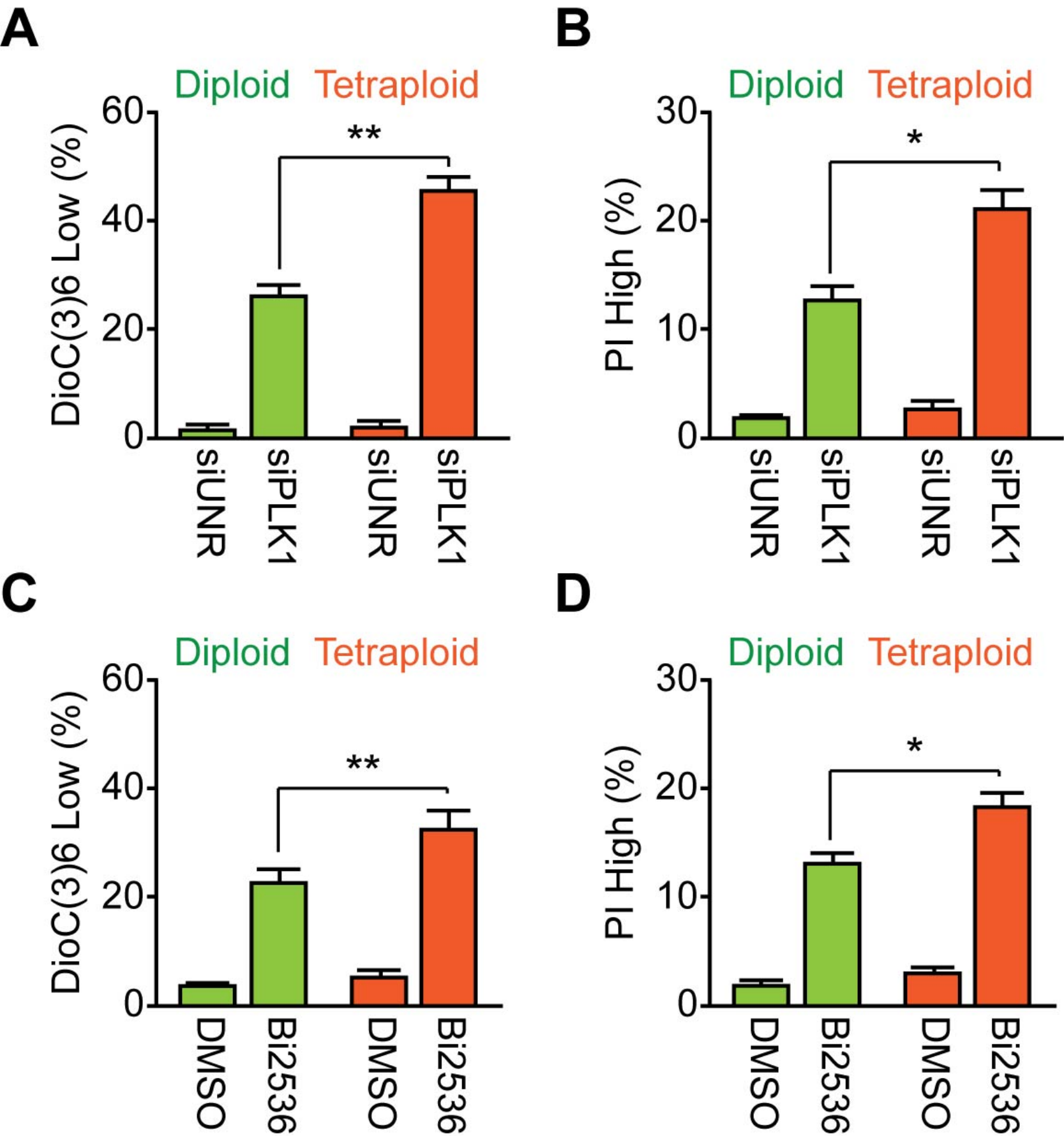
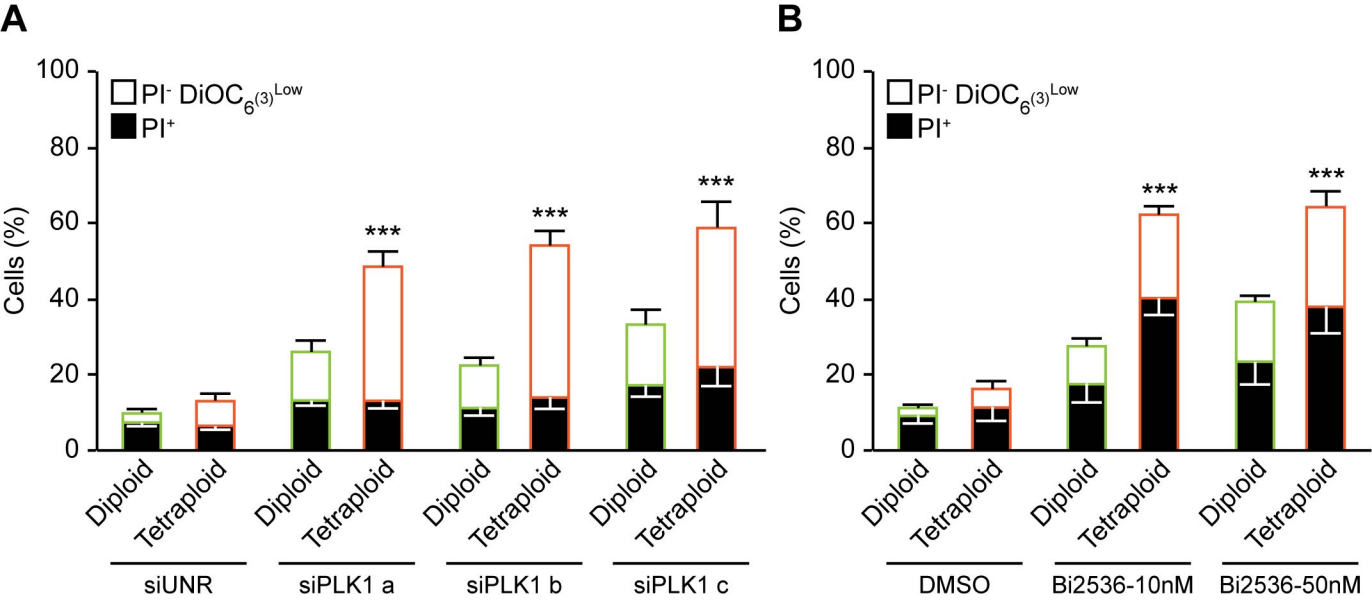
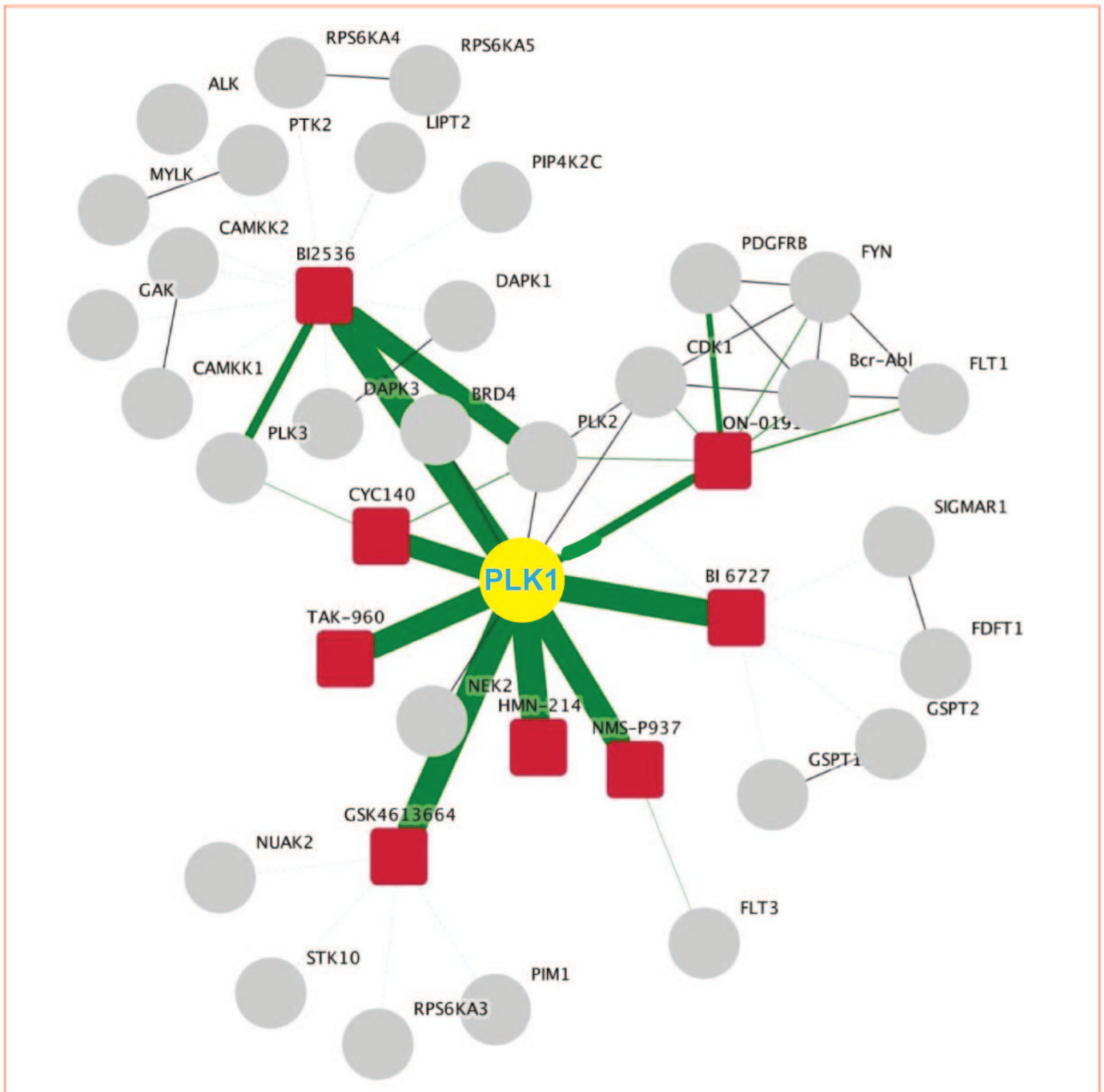



Supplementary Figure 1




Supplementary Figure 2

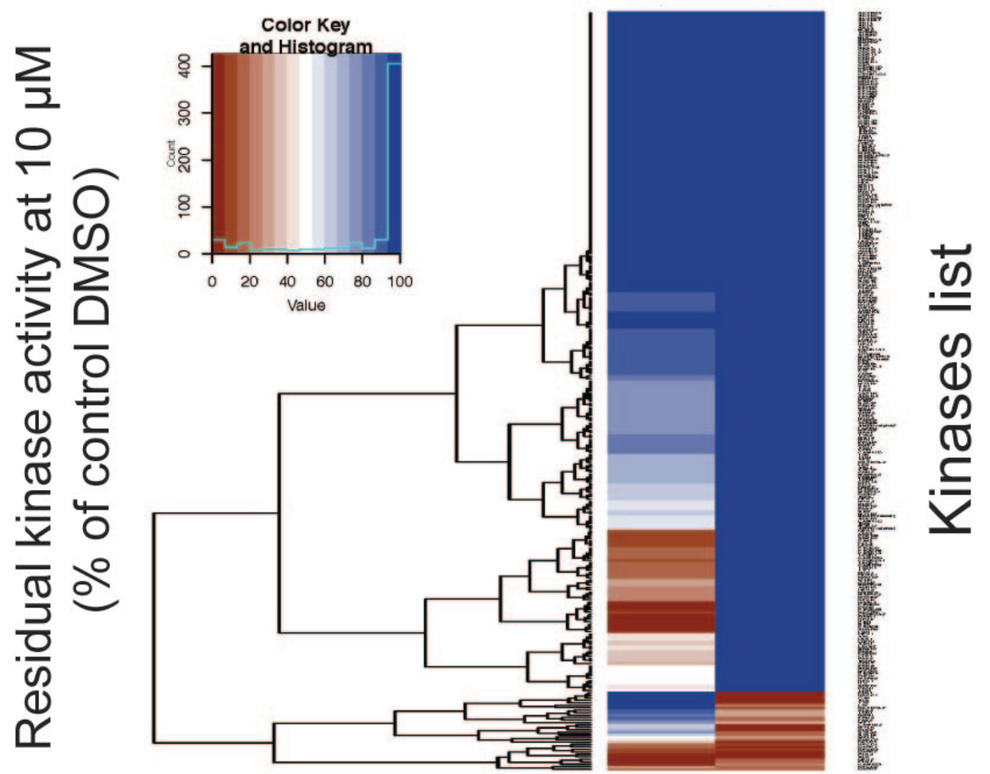
A



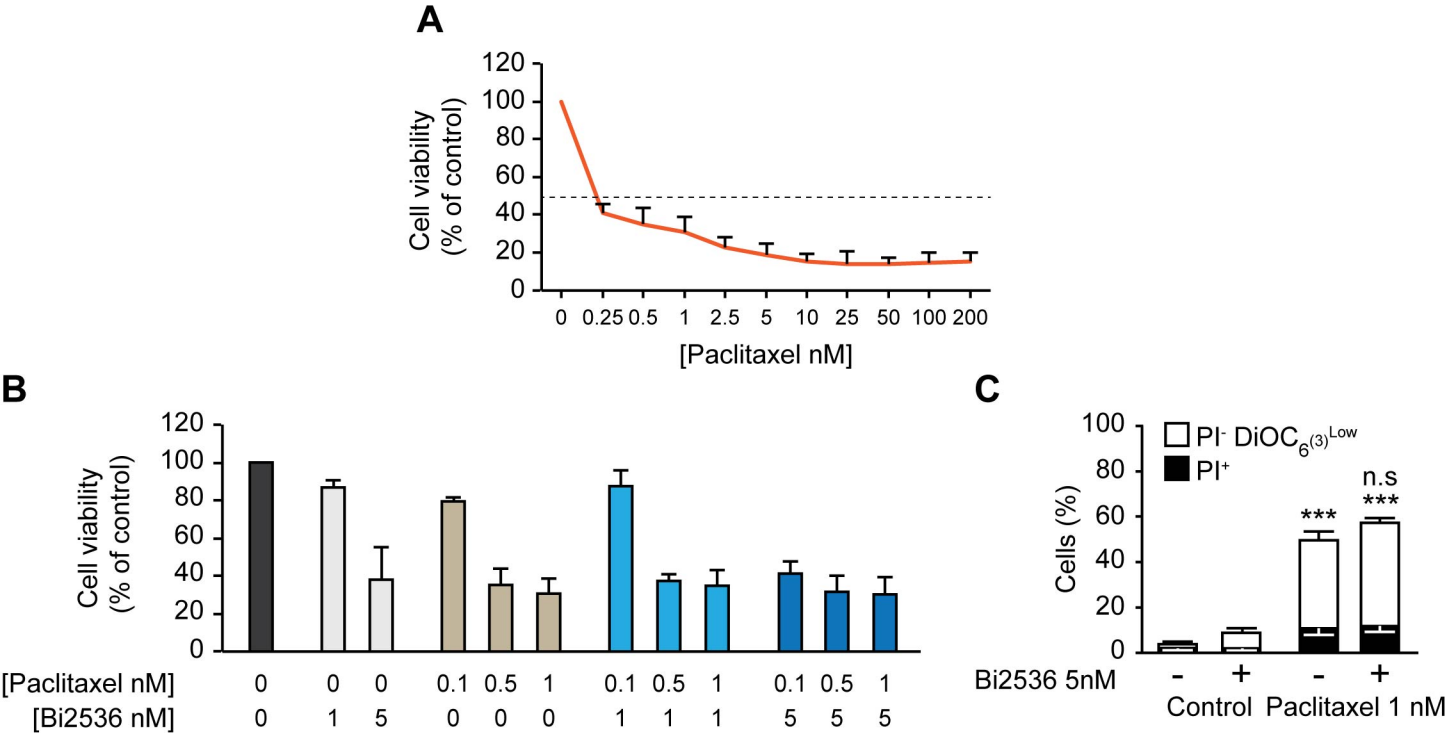

B

| Compounds | $IC_{50}$ *in vitro* |
|---|---|
| BI 6727 | 0.87 nM |
| ON-01910 | 9 nM |
| BI 2536 | 0.8 nM |
| GSK461364 | 0.5 nM |
| NMS-P937 | 2 nM |
| TAK-960 | 2 nM |
| HMN-214 | 118 nM |
| CYC140 | 2.95 nM |

C




Supplementary Figure 3




Supplementary Figure 4

Figure S1. Preferential killing of tetraploid HCT 116 cells after PLK1 knockdown or inhibition.

A-B. Diploid (labelled in green) and tetraploid (labelled in orange) human colorectal carcinoma HCT116 cells were transfected with an unrelated small interfering RNA (siRNA) or a SMARTpool siPLK1. After 72 h, the cells were stained with the mitochondrial membrane potential (Δψm)-sensing dye $DiOC_6(3)$ (shown in A) and the vital dye propidium iodide (PI; shown in B) to evaluate cell-death-associated parameters by cytofluorometry.

C-D. Diploid (labelled in green) and tetraploid (labelled in orange) human colorectal carcinoma HCT116 cells were treated with a control dose of DMSO or 10 nM of Bi2536. After 72 h, the cells were stained with the mitochondrial membrane potential (Δψm)-sensing dye $DiOC_6(3)$ (shown in C) and the vital dye PI (shown in D) to evaluate cell-death-associated parameters by cytofluorometry.

Data are reported as means ± SD ($n \geq 3$). $^{**}p < 0.01$, $^{*}p < 0.05$ (Mann–Whitney test), as compared to diploid subjected to the same treatment condition.

Figure S2. Preferential killing of tetraploid sarcoma cells after PLK1 knockdown or inhibition

A. Diploid (labelled in green) and tetraploid (labelled in orange) sarcoma MFH152 cells were transfected with siUNR and three different siPLK1 and then co-stained after 72 h with the vital dye propidium iodide (PI) and the mitochondrial membrane potential (Δψm)-sensing dye DiOC6(3) to evaluate cell-death-associated parameters by cytofluorometry. The white and black columns depict the percentage of dying (PI−DiOC6(3)low) and dead (PI+) cells, respectively.

B. Diploid (labelled in green) and tetraploid (labelled in orange) sarcoma MFH152 cells were treated with DMSO as a control or 10 or 50 nM Bi2536 and then co-stained after 72 h with the vital dye PI and the mitochondrial membrane potential (Δψm)-sensing dye $DiOC_6(3)$ for the evaluation of cell death–associated parameters by cytofluorometry. The white and black columns depict the percentage of dying (PI−$DiOC_6(3)$low) and dead (PI+) cells, respectively.

Data are reported as means ± SD ($n \geq 3$). $^{***}p < 0.001$ (Mann–Whitney test), as compared to diploid subjected to the same treatment condition.

Figure S3. Kinome interactome and half maximal inhibitory concentration ($IC_{50}$) for PLK1 inhibitors

A. A schematic of the PLK1-centred kinome interactome: drugs (red rectangular nodes) are connected to target protein (circular grey nodes, only PLK1 node is labelled in yellow), as reported in the STITCH database. The drug-target protein networks are merged together.

B. The table presents the $IC_{50}$ PLK1 values for different drugs collected from the STITCH and CHEMBL databases.

C. The heatmap shows the specificity of the PLK1 inhibitors Bi2536 and GSK461364. Drug binding efficiency at 10 μM was measured against a panel of 309 kinases. The values are between 0 (red) and 100% (blue), where 100% indicates no inhibition of kinase binding to the ligand and 0% indicates the strongest inhibition.

Figure S4. Paclitaxel and Bi2536 co-treatment do not engender synergistic lethality in tetraploid RKO cells

A. Tetraploid RKO cells were exposed to different doses of paclitaxel and cell survival was calculated after 72 h using a crystal violet assay.

B. Tetraploid RKO cells were treated with different concentrations of Bi2536 and paclitaxel to evaluate the synergetic effect of the combination treatment.

C. Tetraploid RKO cells were treated with 5 nM Bi2536 and 1 nM paclitaxel alone or in combination followed by $DiOC_6(3)$/PI co-staining. Quantitative data are represented. White and black columns depict the percentage of dying (PI−$DiOC_6(3)$ low) and dead (PI+) cells, respectively.

Data are reported as means ± SD ($n \geq 3$). ***$p < 0.001$ (Mann–Whitney test), as compared to the control.