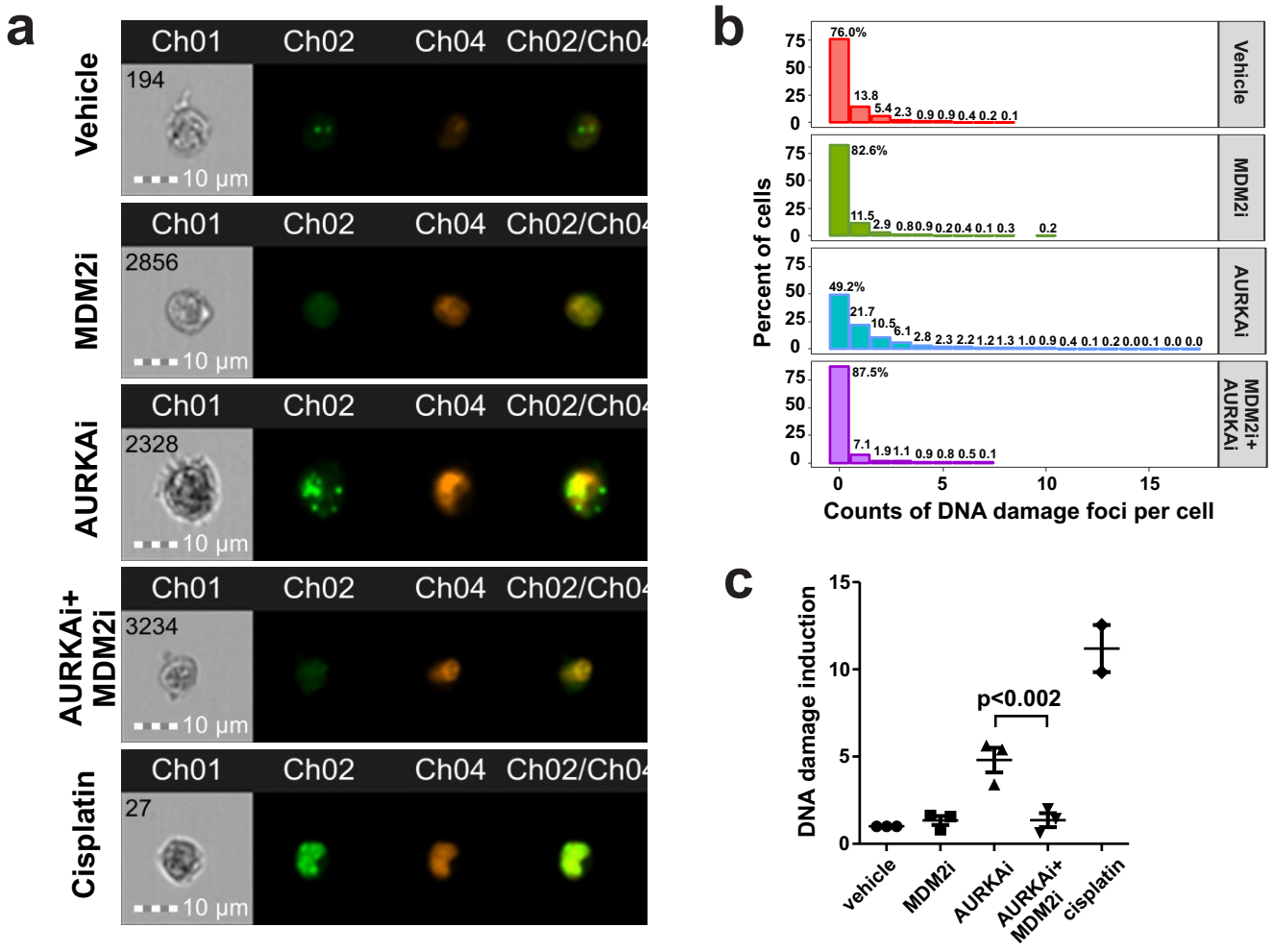


Figure S1. MDM2i abrogates formation of DNA damage foci in AURKAi-treated cells. Imaging flow cytometry analysis of Hs294T cells treated with 10µM Nutlin-3a (MDM2i) ± 1µM alisertib (AURKAi) for 3 days. **(a)** Representative images of cells. Ch01 – bright field, Ch02 – γH2AX, Ch04 – DNA. **(b)** Histogram shows the percentages of cells with indicated counts of foci per cell. **(c)** Statistical analysis based on 3 independent experiments. ANOVA test was used on average foci/per cell values that were log transformed and blocked for inter-experimental variability.

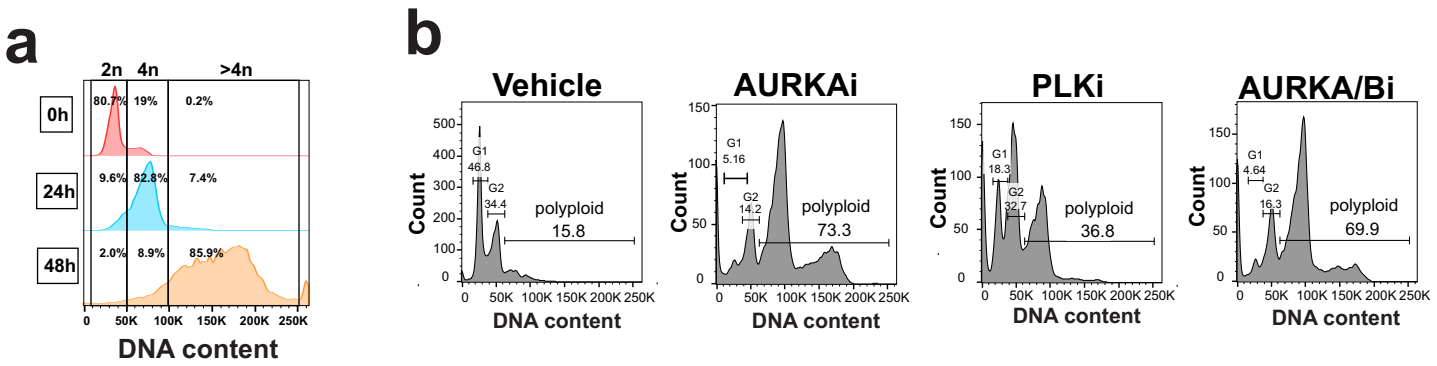


Figure S2. Induction of polyploidy by various inhibitors of mitotic kinases. (a) SK-Mel5 cells were treated with 1µM alisertib (AURKAi) for 0h, 24h, or 48h and DNA content was analyzed by flow cytometry. (b) Flow cytometric analysis of DNA content in SK-Mel5 cells after 3 days of treatment with 1µM alisertib (AURKAi), 5µM danusertib (AURKA/Bi) or 5nM volasertib (PLK1i).

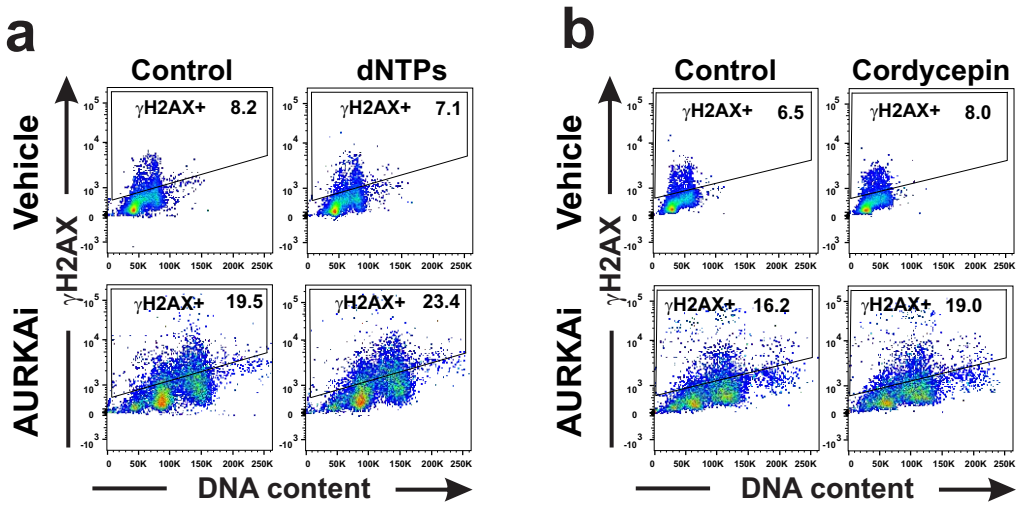
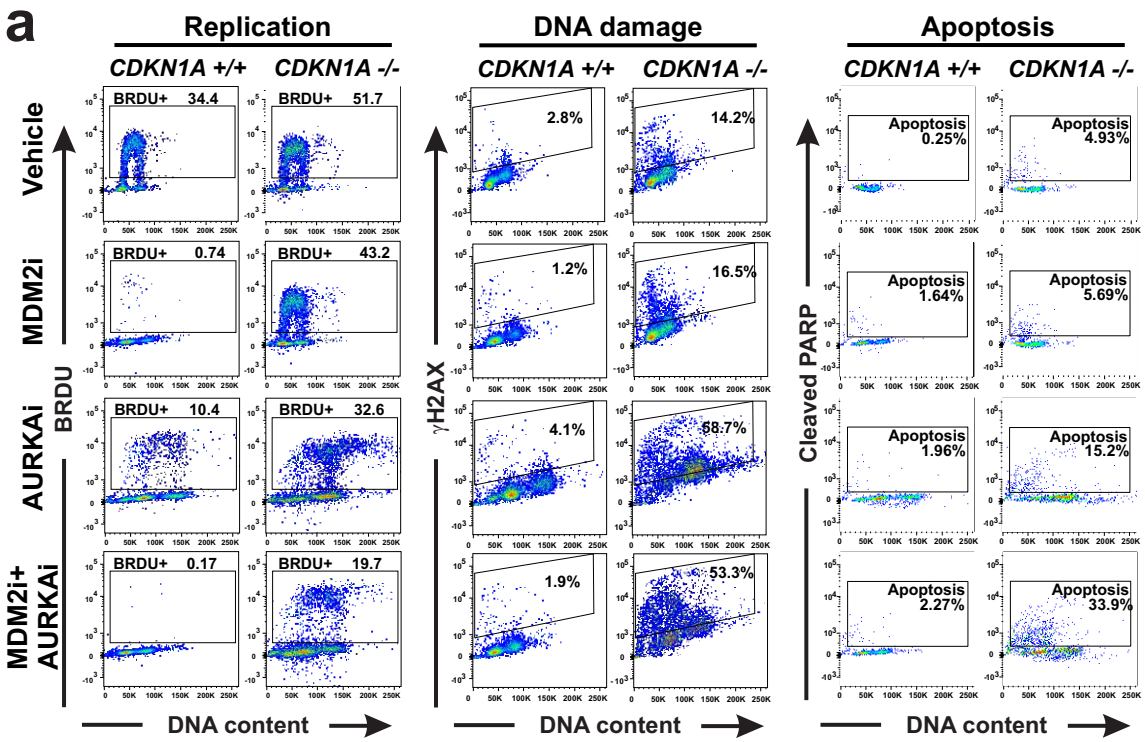


Figure S3. AURKAi-induced DNA damage is not associated with nucleotide depletion or interference with transcription. **(a)** SK-Mel5 cells were treated with vehicle or 1μM alisertib (AURKAi) for 2 days with and without supplementation of culture media with 250 nM of mixed dNTPs: adenosine, guanosine, thymidine, andcytosine. **(b)** SK-Mel5 cells were treated with vehicle or 1μM alisertib (AURKAi) for 2 days and then cultured 100 minutes with or without RNA transcription inhibitor cordycepin (50 μM).



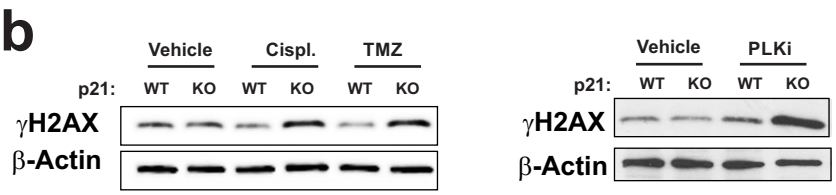


Figure S4. p21 knockout sensitizes cells to MDM2i and AURKAi treatment. **(a)** Isogenic HCT116 cells with or without *CDKN1A* gene (p21) knockout were treated with 10μM Nutlin-3a (MDM2i) ± 1μM alisertib (AURKAi) for 3 days and pulsed with BRDU for 2 hours. Representative histograms of flow cytometric analysis of BRDU, γH2AX and cleaved PARP. Ten thousand events were collected for each sample. **(b)** Wild type HCT116 cells (WT) and isogenic p21 knockout cells (KO) were treated with 5 μM cisplatin (Cispl.), 100 μM temozolomide (TMZ), or 20nM volasertib (PLK1i) ± 10 μM nutlin-3a (MDM2i) for 2 days. Levels of γH2AX were analyzed by western blot. Results are representative of two experiments.