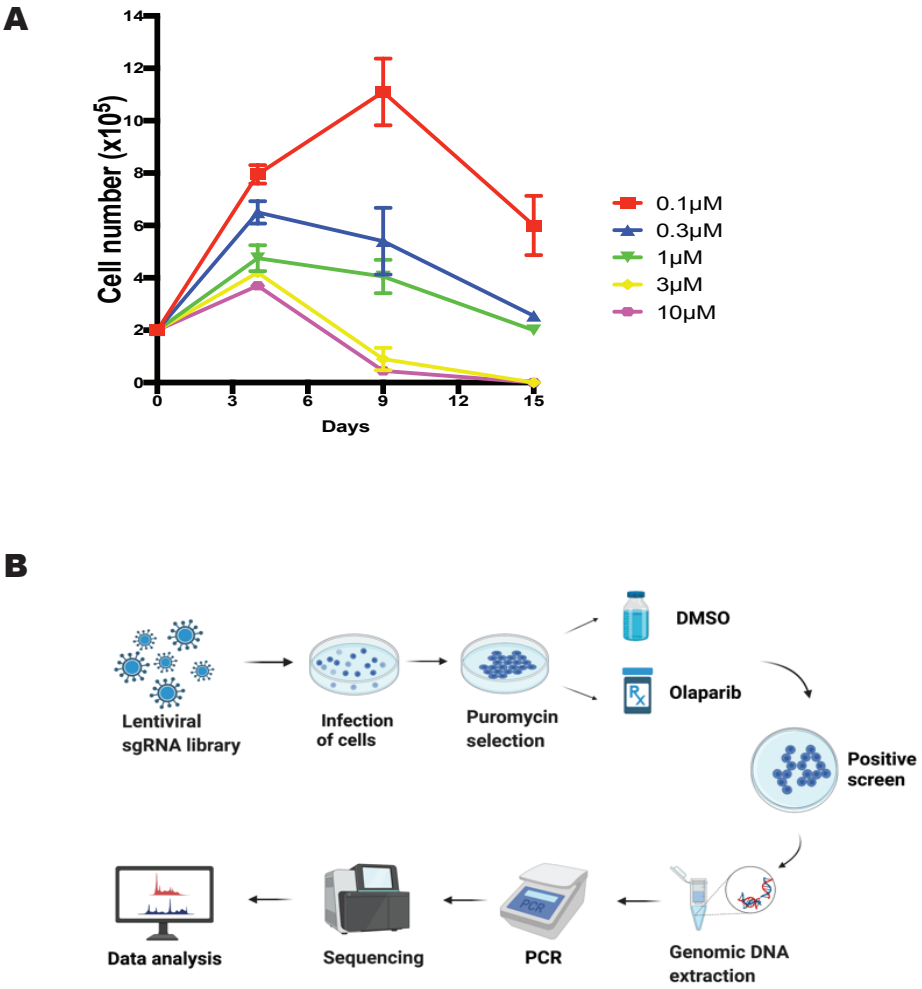
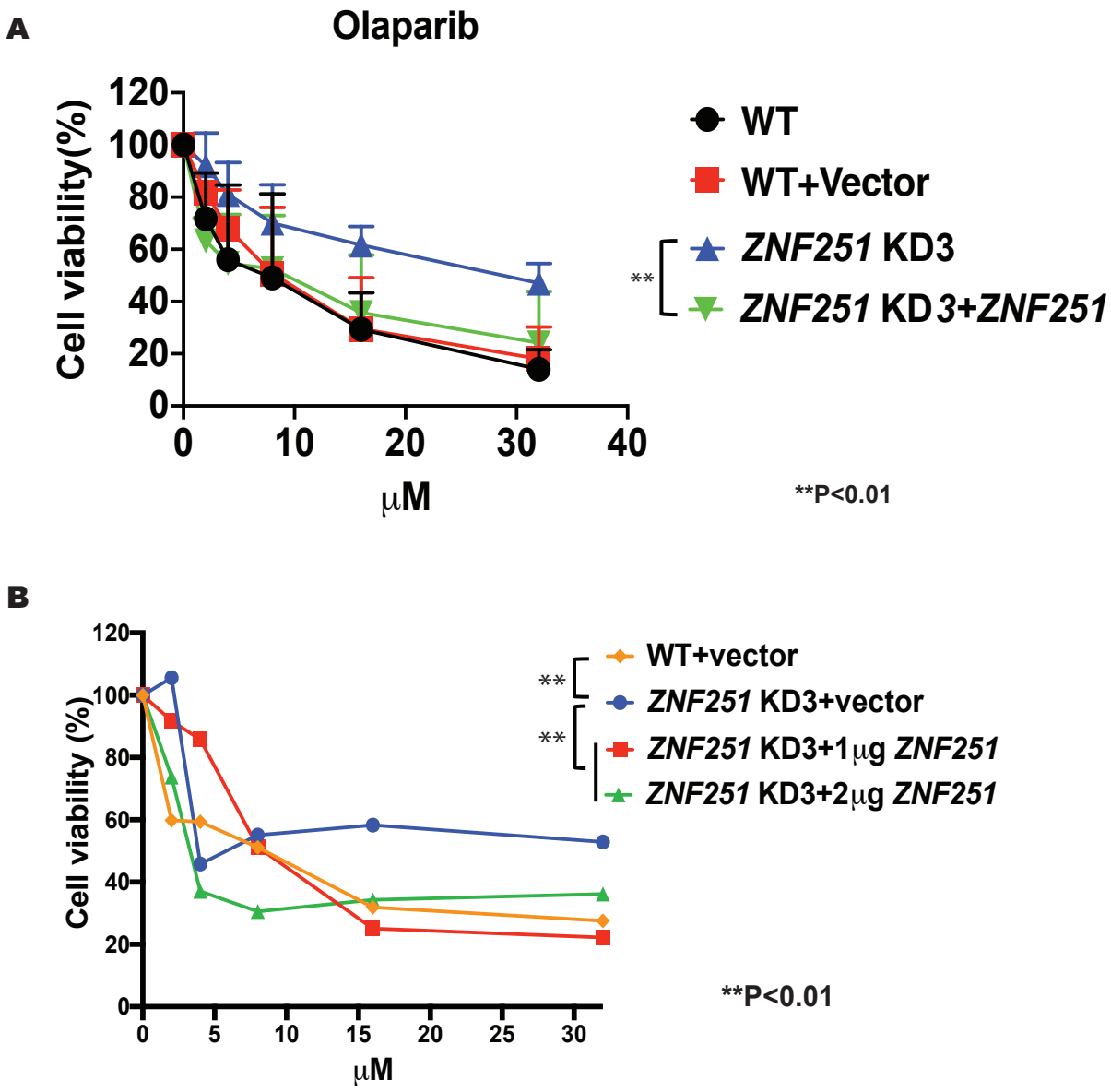
Supplementary Figure S1. The dose response of MDA-MB-436 breast cancer cells to olaparib and all three *ZNF251* KD clones showed reduced ZNF251 protein level.



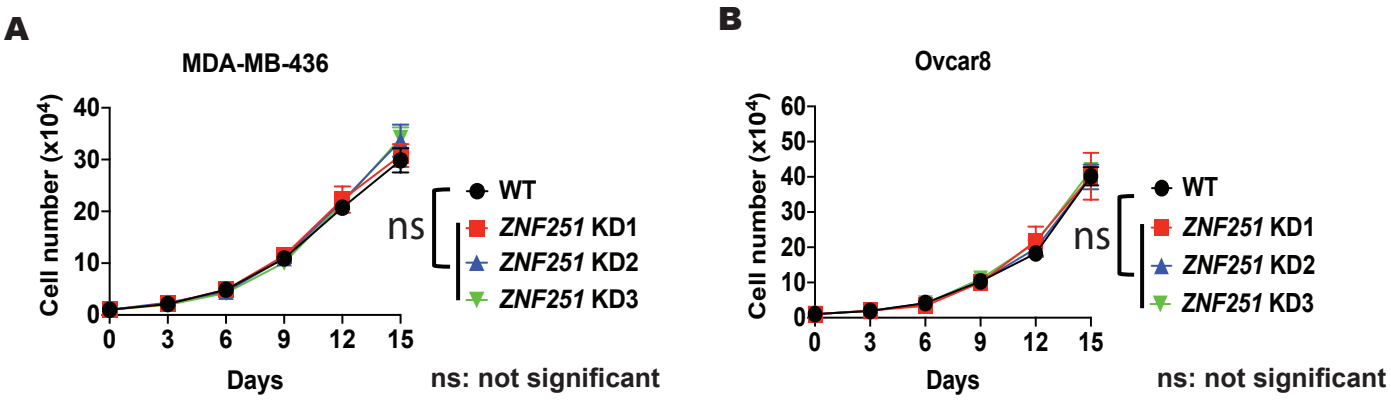
Supplementary Fig. S1. The dose response of MDA-MB-436 breast cancer cells to olaparib and schematic illustration of olaparib resisatance CRISPR screen. A. The growth curve of MDA-MB-436 breast cancer cells treated with various doses of olaparib. B. A simplified scheme of the olaparib resisatance CRISPR screen with MDA-MB-436 cells.

Supplementary Figure S2. Transfection of wild type *ZNF251* on a plasmid reversed olaparib resistance of *ZNF251* KD breast cancer cells



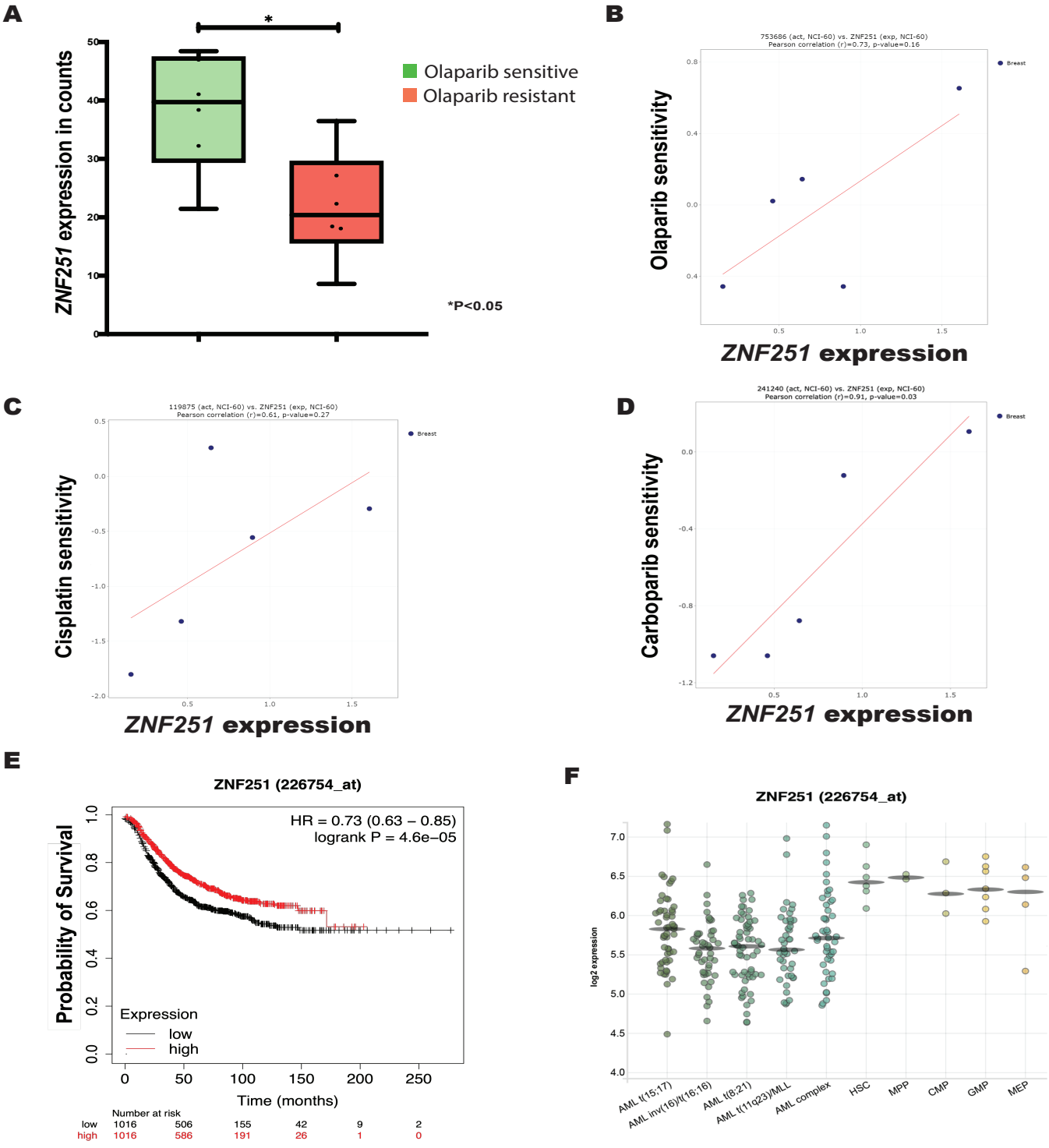
Supplementary Fig. S2 A. Transfection of wild type *ZNF251* on a plasmid reversed olaparib resistance of *ZNF251* KD breast cancer cells. Wildtype (WT), WT cells transfected with PC DNA3.1 vector, *ZNF251* KD and *ZNF251* KD transfected with *ZNF251* cDNA on PC DNA3.1 were treated with olaparib for 72 hours and cell viability was measured. B. Transfection of wild type *ZNF251* on a plasmid reversed olaparib resistance of *ZNF251* KD breast cancer cells. Wildtype (WT), WT cells transfected with PC DNA3.1 vector, *ZNF251* KD and *ZNF251* KD transfected with PC DNA3.1 vector or *ZNF251* cDNA on PC DNA3.1(1μg and 2μg) then were treated with olaparib for 72 hours and cell viability was measured.

Supplementary Figure S3. ***ZNF251*** **KD doesn't affect cell growth in the absence of PARPi treatment**



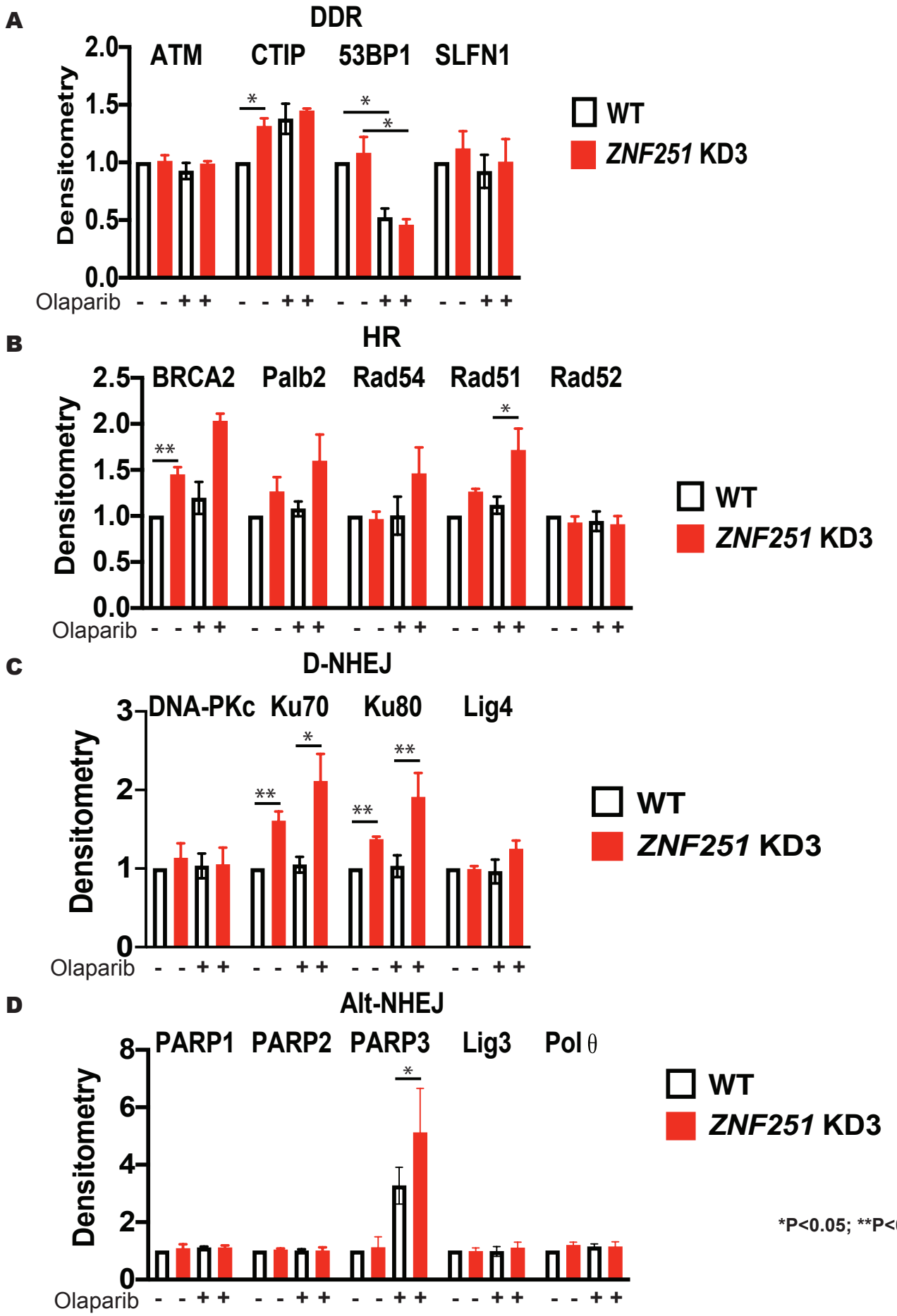
Supplementary Fig. S3. *ZNF251* KD doesn't affect cell growth in the absence of PARPi treatment. A. Cell growth of WT, *ZNF251* KD1, *ZNF251* KD2 and *ZNF251* KD3 MDA-MB-436 breast cancer cells was measured. B. Cell growth of WT, *ZNF251* KD1, *ZNF251* KD2 and *ZNF251* KD3 Ovcar8 ovarian cancer cells was measured.

Supplementary Figure S4. Bioinformatic analysis showed that *ZNF251* expression is correlated with PARPi and platinum drugs sensitivity as well as prognosis of breast cancer patients



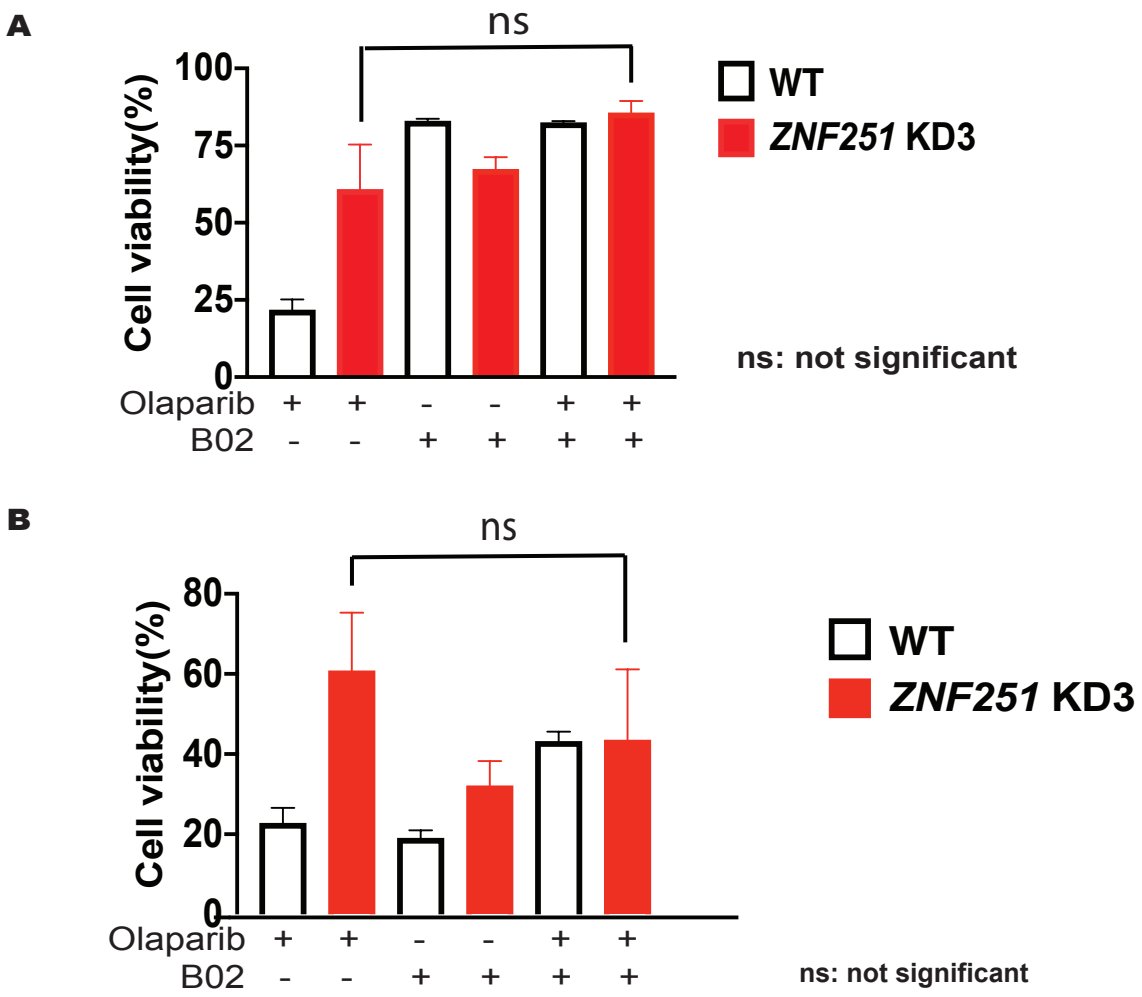
Supplementary Fig. S4. Bioinformatic analysis showed that *ZNF251* expression is corelated with PARPi and platinum drugs sensitivity as well as prognosis of breast cancer patients. A. *ZNF251* expression was correlated with olaparib resistance of breast cancer cells. Box-whisker plots indicating the *ZNF251* expression values of olaparib sensitive and resistant cells collected from the Gene Expression Omnibus (GEO) database datasets. Green color plot represents sensitivity towards PARPi whereas red color plot represents resistance towards PARPi. Box-whisker plot for olaparib sensitive and resistant cells was generated using the GEO dataset GSE165914. Y-axis represents the expression of *ZNF251* in the respective cells. Statistical analysis was performed using the 2-tailed Student's t test. *p-value<0.05. B-D. *ZNF251* expression is positively correlated with sensitivity to olaparib, cisplatin and carboplatin of the breast cancer cells by CellMiner database analysis. E. Low *ZNF251* expression is correlated with worse survival for breast cancer patients using online Kaplan-Meier plotter, which integrates gene expression and clinical data from 2,032 patients. F. Expression of ZNF251 in AMLs displaying the indicated karyotype and in normal hematopoietic cells (HSC = hematopoietic stem cells, MPP = multipotent progenitors, CMP = common myeloid progenitors, GMP = granulocyte-monocyte progenitors, MEP = megakaryocyte-erythrocyte progenitors according to Bloodspot database. https://servers.binf.ku.dk/bloodspot/?gene=ZNF251&dataset=normal_human_v2_with_AMLs.

Supplementary Figure S5. Quantification of western analysis of wildtype (WT) and *ZNF251* KD breast cancer cells treated with vehicle or olaparib



Supplementary Fig. S5. Quantification of western analysis of vehicle-treated or olaparib-treated wildtype (WT) and *ZNF251* KD breast cancer cells. A-D. Densitometry was measured for western analysis of key factors involved in DDR, HR, D-NHEJ, alt-NHEJ pathways.

Supplementary Figure S6. Treating *ZNF251* KD breast cancer cells with Rad51 inhibitor B02 at 4μM or 16μM cannot reverse olaparib resistance.



Supplementary Fig. S6. Treating *ZNF251* KD3 breast cancer cells with Rad51 inhibitor B02 at 4μM (A) or16μM (B) cannot reverse olaparib resistance.