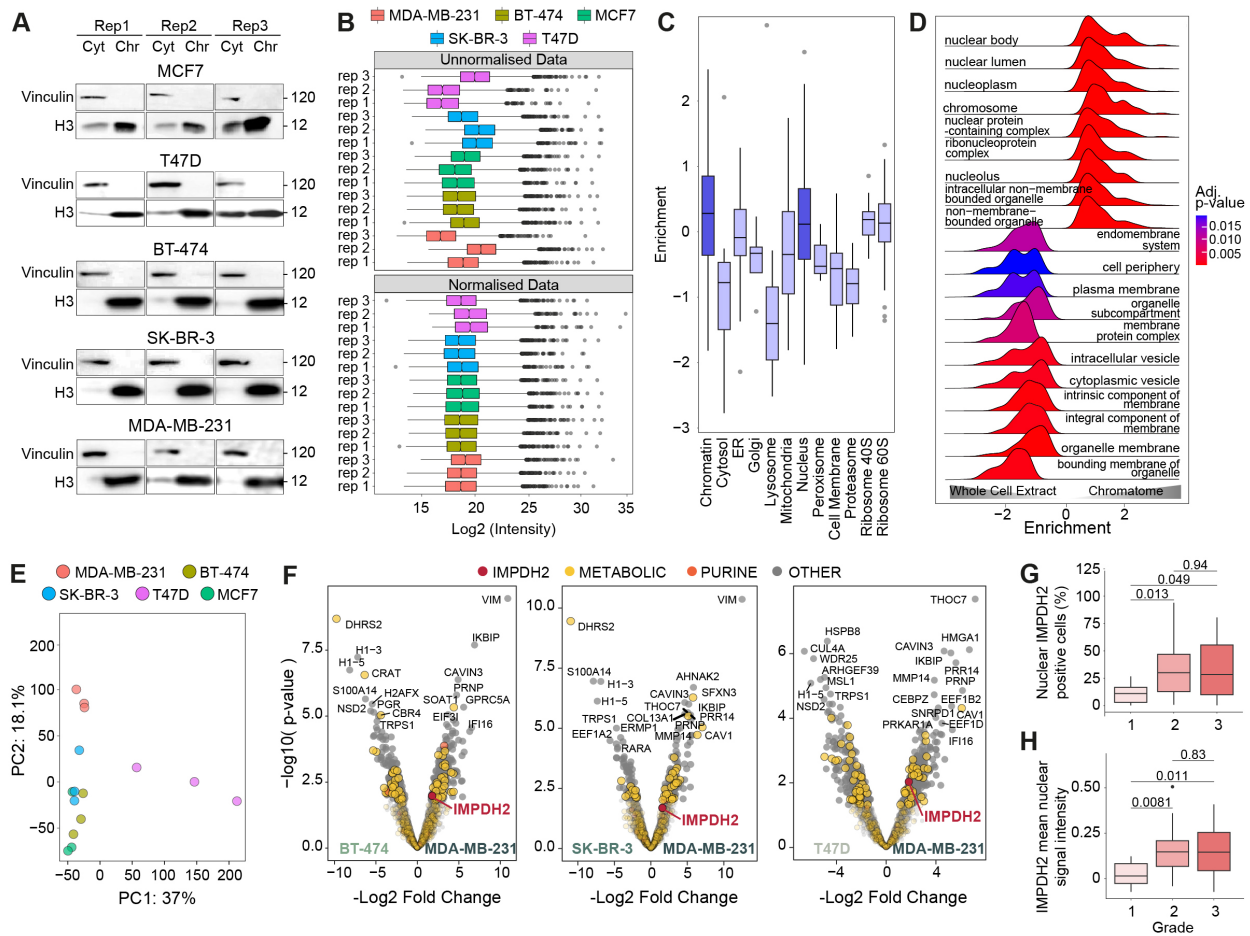
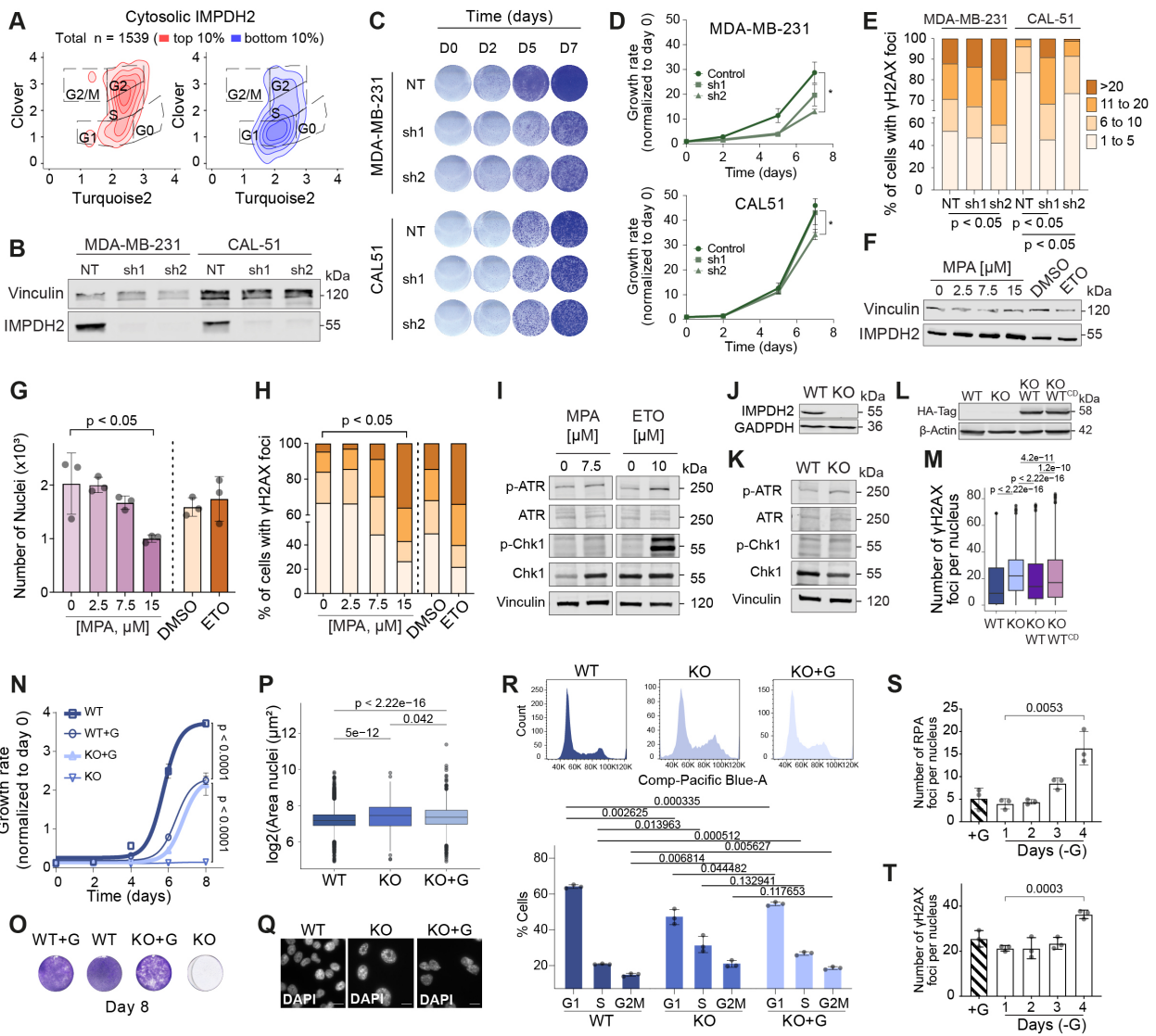


Figure S1



Supp. Fig. 1. Chromatin fractionation quality control. **a**, Western blot detection of vinculin (cytosolic marker) and Histone H3 (chromatin marker) on cytosolic and chromatin fractions of breast cancer cell lines representing the different subtypes of breast cancer. **b-c**, Sample normalization (**b**) and compartment enrichment (**c**) of the chromatome fraction. **d**, GO CC Enrichment of the whole cell extract and chromatome fractions. **e**, Principal component analysis (PCA) plot of the three chromatin samples fraction replicate from each breast cancer associated subtype. **f**, Volcano plots of changes in protein abundance on chromatin in MDA-MB-231 vs BT-474 (left panel), SK-BR-3 (middle panel) and T47D (right panel); IMPDH2 highlighted in red, purine synthesis and metabolic pathways colored in orange and yellow, respectively. **g-h**, Quantification of IMPDH2 nuclear positive cells (**g**), and IMPDH2 nuclear signal (**h**) detected by immunohistochemistry in breast cancer tissue microarray (grade 1, $n = 7$; grade 2, $n = 53$; grade 3, $n = 40$; unpaired two-tailed Wilcoxon test). Box plots indicate median value (central line), interquartile range IQR (box boundaries) and up to 1.5*IQR beyond the box boundaries (whiskers). Source data are provided as a Source Data file.

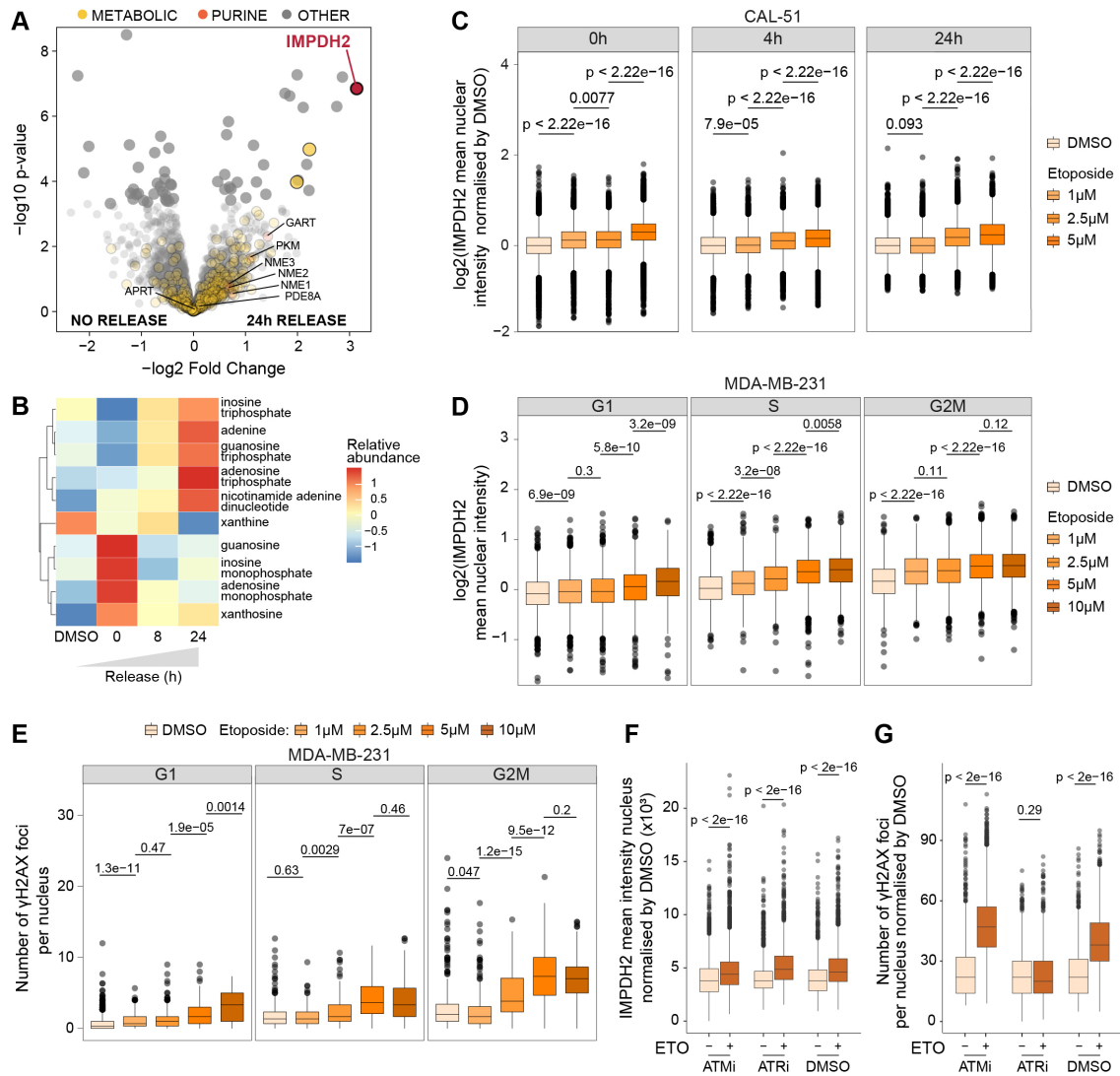
Figure S2



Supp. Fig. 2. Pharmacological or genetic inhibition of IMPDH2 leads to γ H2AX accumulation and proliferative defects. **a**, Cell cycle profile of cytosolic IMPDH2 expression in U2OS cells using the adapted FUCCI system. **b-e**, IMPDH2 levels (**b**), representative pictures ($n=3$) (**c**) from growth curves (**d**) and distribution of γ H2AX foci-positive populations (**e**) in NT control or IMPDH2 knock-down MDA-MB-231 and CAL-51 cells. **f-h**, IMPDH2 protein levels (**f**), number of DAPI-stained nuclei (**g**), and distribution of γ H2AX foci-positive populations (**h**) in MDA-MB-231 cells treated with increasing amounts of MPA (72 h) or etoposide (3h); ($n=3$). **i**, Western blot detection of DDR markers in MDA-MB231 cells treated with MPA 7.5 μ M (72h) or etoposide 10 μ M (24h); vinculin used as loading control. **j**, Western blot of MDA-MB-231 WT or KO cells; GADPDH used as loading control. **k**, Western blot detection of DDR markers in MDA-MB-231 WT or KO cells without guanosine supplementation for 96h; vinculin used as loading control. **l-m**, HA-Tagged IMPDH2 levels (**l**) and quantification of γ H2AX foci (**m**) in IMPDH2 reconstituted cells without guanosine supplementation for 96h (WT, $n = 9254$; KO, $n = 2014$; KO-WT, $n = 4588$; KO-WT^{CD}, $n = 3806$; outliers removed, 3SD; unpaired two-tailed Wilcoxon test). **n-o**, growth curves (**n**) with representative pictures (**o**) of MDA-MB-231 KO and WT cells in the presence (+G) or absence of guanosine supplementation ($n = 3$, unpaired two-tailed t -test). **p-q**, nuclei area distribution (WT, $n = 8215$; KO, $n = 465$; KO+G, $n = 1372$; unpaired two-tailed Wilcoxon test) (**p**), and representative

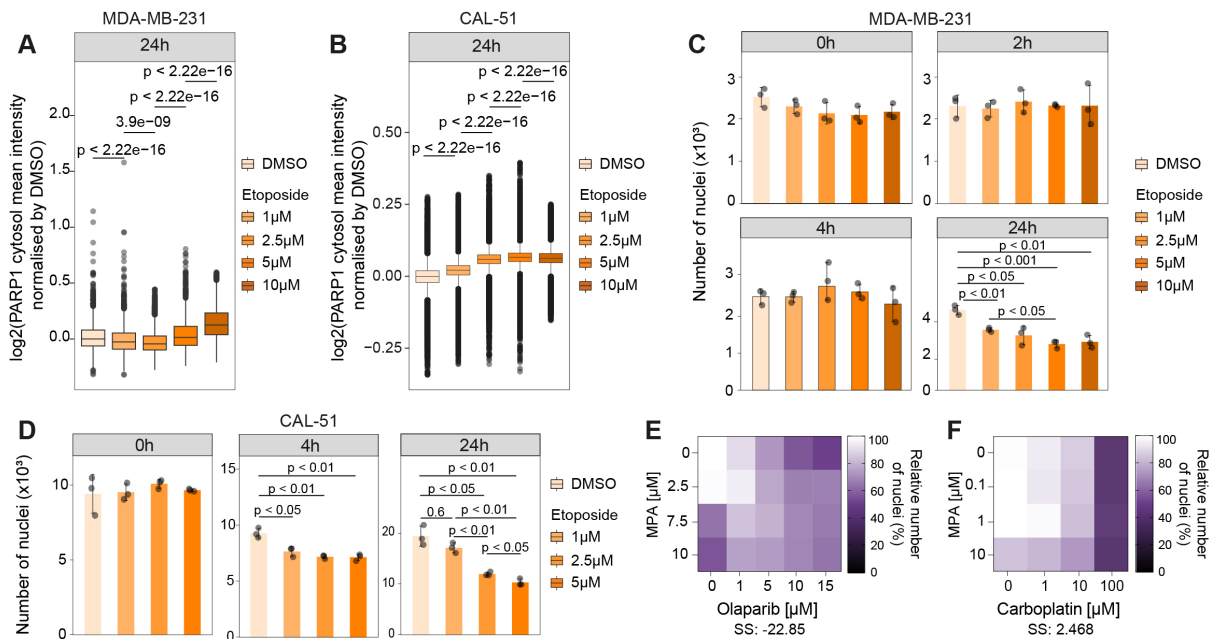
pictures (DAPI, grey; scale bar 15 μm) (q) of WT and KO cells cultured for 96h in the presence (+G) or absence of guanosine supplementation. **r**, Representative profile (top) and percentage of cells (bottom) in different cell cycle phases ($n=3$, unpaired two-tailed t -test). **s-t**, Quantification of number of RPA70 foci (s) and γH2AX foci (t) in KO cells with (+G) or without (-G) guanosine supplementation ($n=3$, unpaired two-tailed t -test). Bar charts represent mean values \pm SD. Box plots indicate median value (central line), interquartile range IQR (box boundaries) and up to 1.5*IQR beyond box boundaries (whiskers). Source data are provided as a Source Data file.

Figure S3



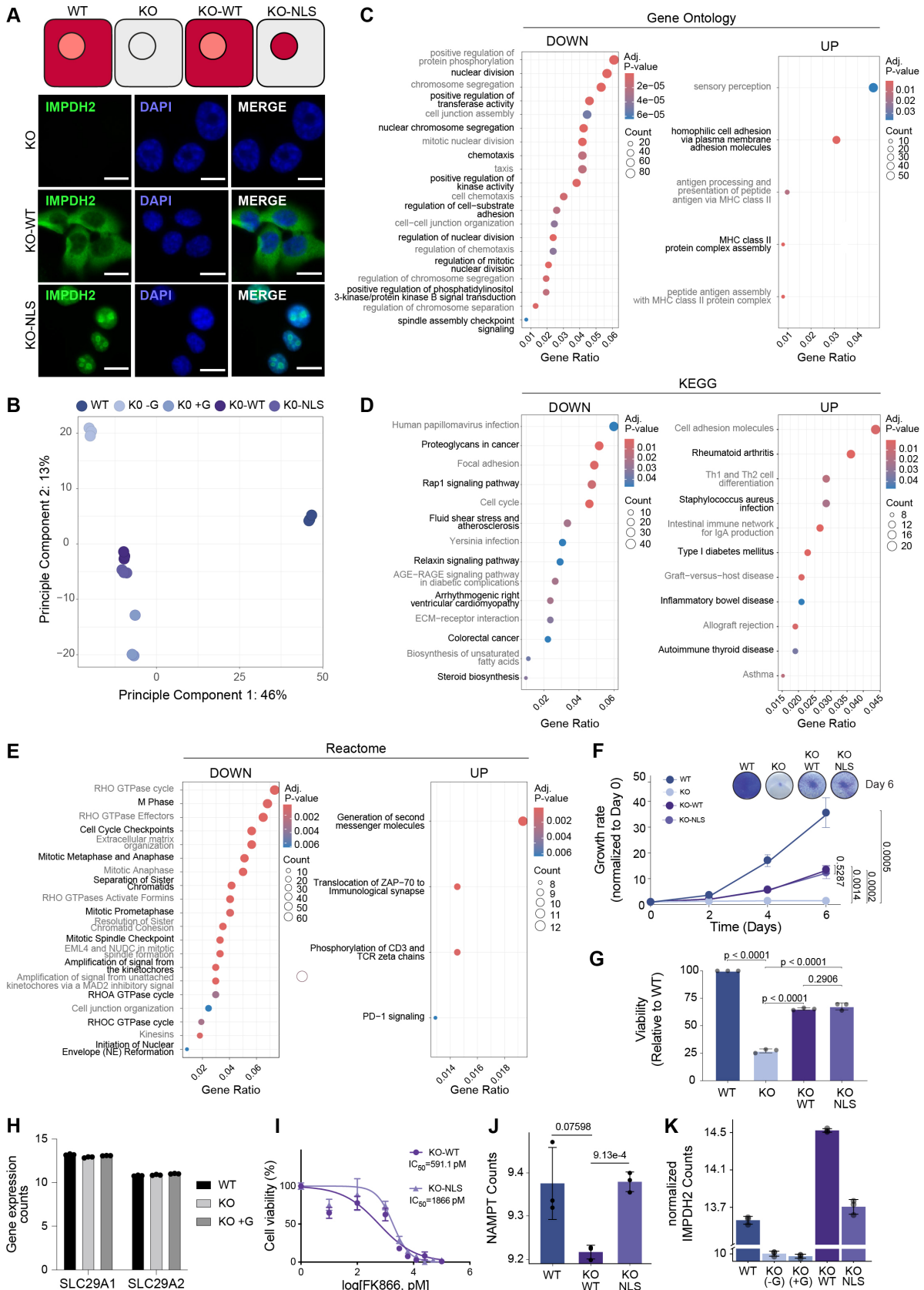
Supp. Fig. 3. IMPDH2 and other purine-pathway enzymes increase on chromatin upon DNA damage. **a**, Volcano plot showing IMPDH2 enrichment on chromatin after 3 hours of 1µM etoposide treatment and 24 hours release in U2OS cells. IMPDH2 depicted in red. Proteins related to metabolic or purine processes depicted in yellow and orange, respectively. **b**, Targeted metabolomics profiling after treatment of U2OS cells with DMSO or etoposide (1µM, 3h) and release for 0h, 8h, and 24h. **c**, IMPDH2 nuclear mean intensity normalized by DMSO upon 3h etoposide treatment (DMSO control, 1, 2.5, and 5µM) in CAL-51 cells after, 4 and 24 hours post release. (DMSO, n0h = 2642; n4h = 2592; n24h = 5798; 1µM, n0h = 2673; n4h = 2133; n24h = 5109; 2.5µM, n0h = 2831; n4h = 1998; n24h = 3572; 5µM, n0h = 2714; n4h = 1987; n24h = 3073). Unpaired two-tailed Wilcoxon test, ($n=3$, minimum of 2 technical replicates per condition). **d**, Distribution across the different phases of the cell cycle of the 24h data represented in Fig. 3c. **e**, Distribution across the different phases of the cell cycle of the 24h data represented in Fig. 3f. **f-g**, Quantification of nuclear IMPDH2 signal intensity (f) and γH2AX foci (g, constant added to improve data visualization) normalized to median of respective DMSO condition in MDA-MB-231 WT cells in the presence or absence of ATM or ATR inhibitors (10 µM) an treated with DMSO or etoposide 10 µM followed by a 24 hours release period (with maintenance of ATM or ATR inhibition) (DMSO: nDMSO= 1485, nATMi= 1948 nATRi= 1903; Etoposide: nDMSO= 2228, nATMi= 3751, nATRi= 1991). Unpaired two-tailed Wilcoxon test, ($n=3$). Box plots indicate median value (central line), interquartile range IQR (box boundaries) and up to 1.5*IQR beyond the box boundaries (whiskers). Source data are provided as a Source Data file.

Figure S4



Supp. Fig. 4. Cytoplasmic PARP1 translocation increases upon DNA damage. **a-b**, PARP1 cytosolic mean intensity normalized by DMSO control condition after 3 hours of treatment with increasing etoposide concentrations (1, 2.5, 5 and 10 μM) after 24h release in MDA-MB-231 cells, (DMSO, n=3741; n1 μM=3155; n2.5 μM=3742; n5 μM=2865; n10 μM=3676) (a) and CAL-51 cells (DMSO, n=59750; n1 μM=53085; n2.5 μM=37261; n5 μM=32280; n10 μM=24410); outliers removed (3SD); unpaired two-tailed Wilcoxon test, (n=3, minimum of 2 technical replicates per condition) (b). **c-d**, Number of nuclei upon 3h etoposide treatment (DMSO control, 1, 2.5, 5 and 10 μM), and 2, 4 and 24h release in MDA-MB-231 (c) and CAL-51 cells, (DMSO control, 1, 2.5, 5 μM) after the treatment, 4, and 24h of release (d). Unpaired two-tailed *t*-test, (n=3, minimum of 2 technical replicates per condition). **e-f**, Synergy matrixes showing cell viability measured with crystal violet assay. MDA-MB-231 cells were treated with MPA and Olaparib (n=2) (e) or MPA and Carboplatin (f) alone or in combination at the indicated concentrations for 96h (n=3). SS indicates the synergy score. (A synergy score lower than -10 indicates that the effect of both drugs is likely to be antagonistic, and from -10 to 10: the interaction between the two drugs is likely to be additive) The synergy score was calculated using the Synergy Finder 3.0 <https://doi.org/10.1093/nar/gkac382>. Box plots indicate median value (central line), interquartile range IQR (box boundaries) and up to 1.5*IQR beyond the box boundaries (whiskers). Source data are provided as a Source Data file.

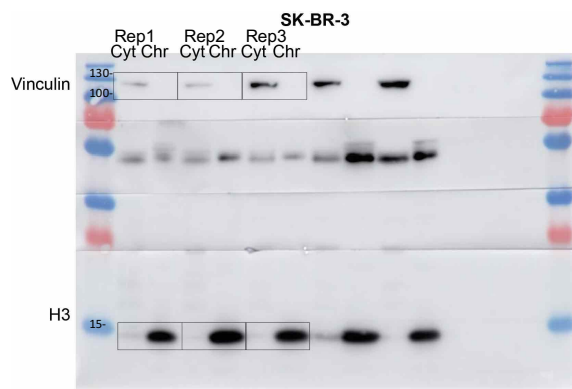
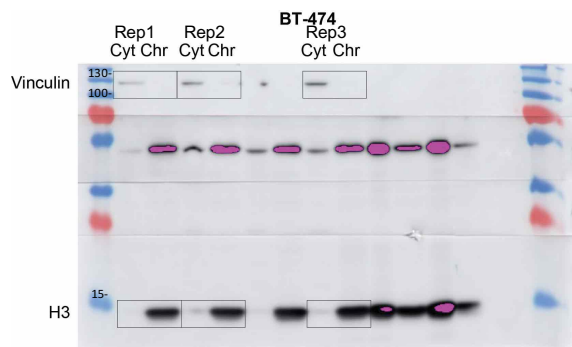
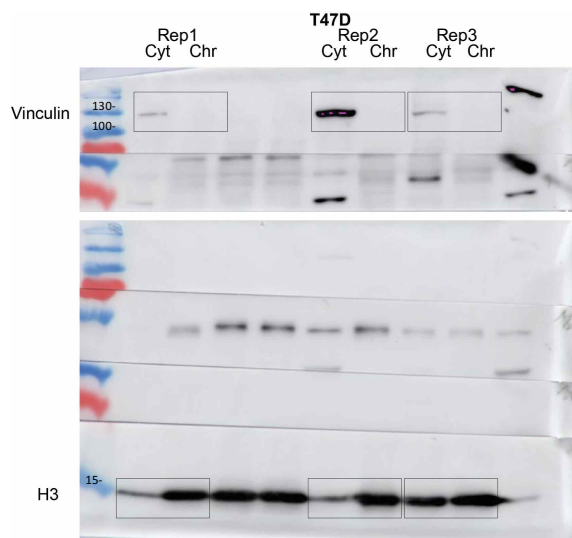
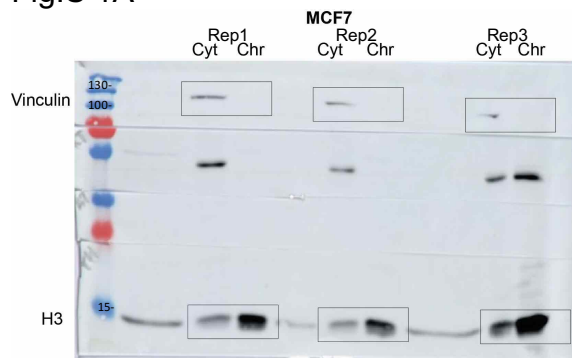
Figure S5



Supp. Fig. 5. Transcriptomics and functional characterization of IMPDH2 KO cells reconstituted with wild-type or a nuclear-specific version of IMPDH2. **a**, Schematic representation of IMPDH2 reconstituted cells (top) (WT: MDA-MB-231 wild-type cells reconstituted with empty vector; KO: IMPDH2 KO reconstituted with empty vector; KO-WT: IMPDH2 KO reconstituted with wild-type IMPDH2; KO-NLS: IMPDH2 KO reconstituted with a nuclear-specific version of IMPDH2) and IMPDH2 location (bottom) detected by anti HA-Tag immunofluorescence (HA-Tag green, DAPI blue; scale bar 15 μ m). **b**, Principal Component Analysis (PCA) of RNA-seq data ($n = 3$ per condition). **c-e**, Enrichment analysis for Gene Ontology Biological Process (c), KEGG (d) and Reactome (e) of differentially down- or upregulated genes in KO-G vs WT condition. **f-g**, growth curves ($n = 3$, unpaired two-tailed t -test) with representative crystal violet stained cell pictures (f) and MTT viability assay (g, time point 72 h) ($n = 3$, unpaired two-tailed t -test) of MDA-MB-231 WT, KO, KO-WT and KO-NLS cells cultured in the absence of guanosine supplementation. **h**, expression levels of nucleoside transporters SLC29A1 and SLC29A2 in WT, KO, and KO+G conditions determined by RNA-seq analysis ($n = 3$). **i**, IC50 determination for NAMPT Inhibitor FK866 in KO-WT and KO-NLS cells ($n = 3$). **j**, expression levels of NAMPT in WT, KO-WT and KO-NLS cells determined by RNA-seq analysis ($n = 3$). **k**, IMPDH2 counts in WT, KO(-G), KO (+G), KO-WT and KO-NLS cells determined by RNA-seq analysis ($n = 3$). Bar charts are presented as mean values \pm SD. Box plots indicate median value (central line), interquartile range IQR (box boundaries) and up to 1.5*IQR beyond the box boundaries (whiskers). Source data are provided as a Source Data file.

uncropped Western Blots from Supplementary Figures

Fig.S 1A



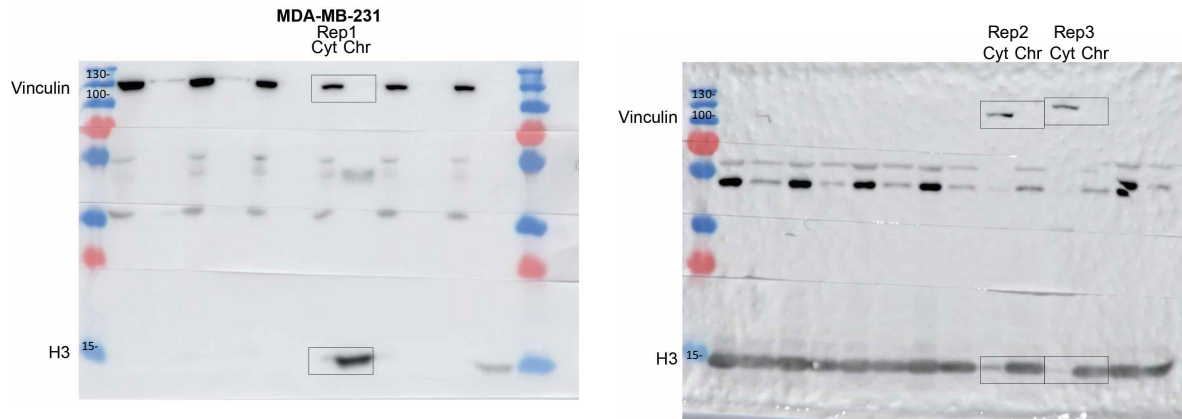


Fig. S2B

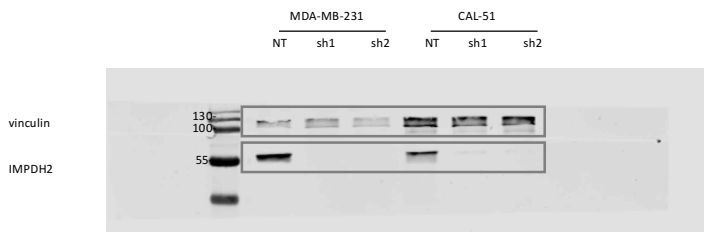


Fig. S2F

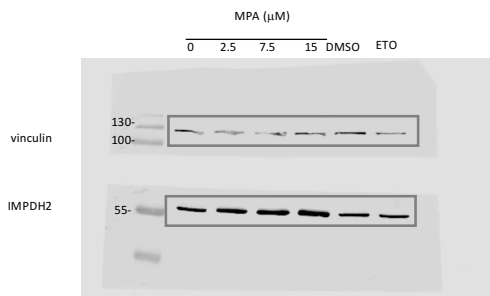


Fig S2I,K

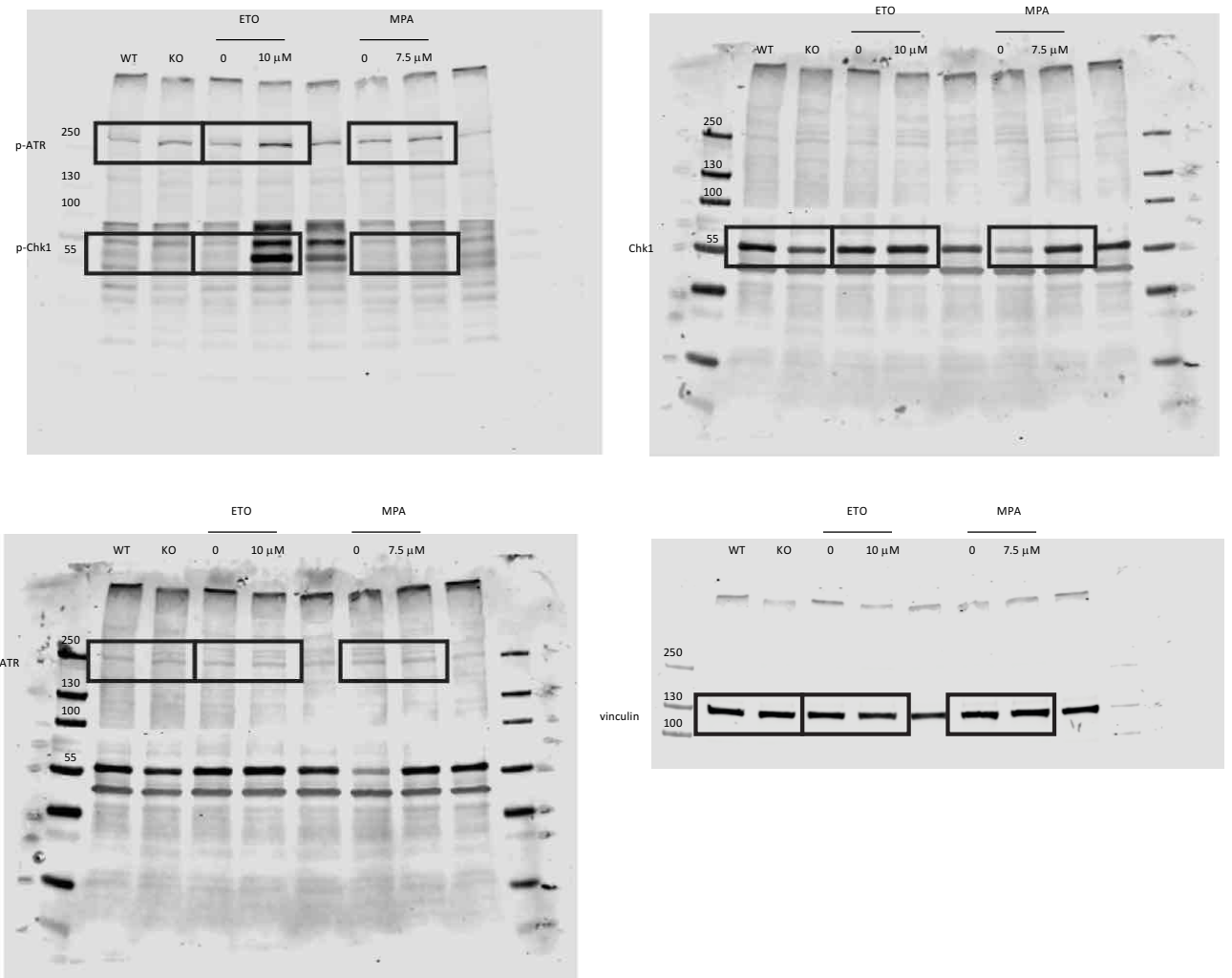


Fig. S2J

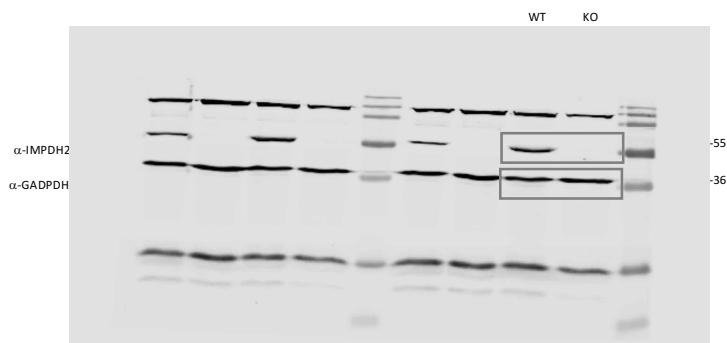


Fig.S2L

