## Figure 1 – Figure Supplement 1

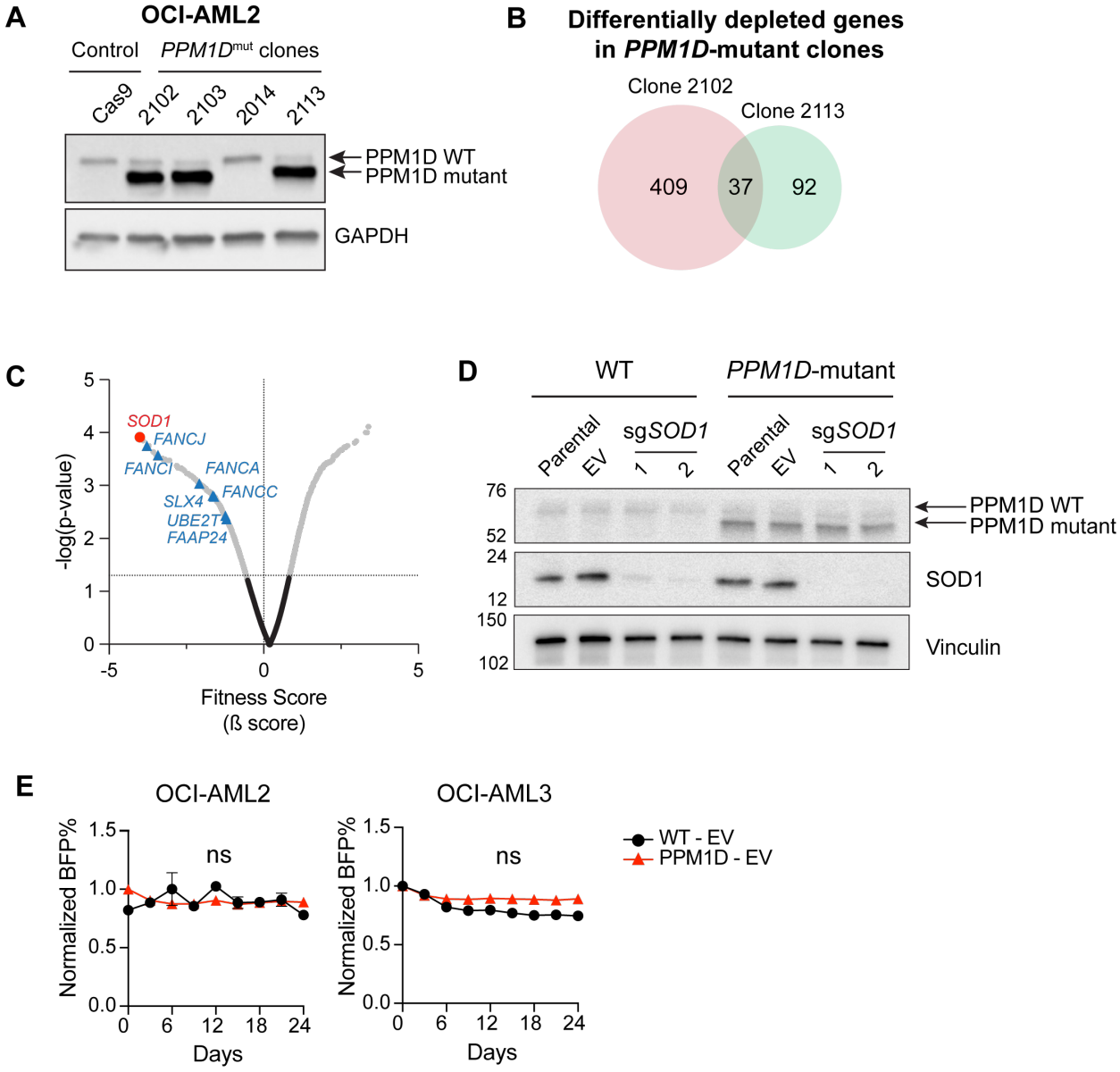


**Figure 1 – Figure Supplement 1. *SOD1* is a synthetic lethal vulnerability of *PPM1D*-mutant leukemia cells. (A)** Immunoblot validation of *PPM1D*-mutant Cas9-expressing OCI-AML2 cells generated and used for CRISPR screening. Blots were probed with anti-PPM1D (1:1000) and GAPDH (1:1000). Clones 2102 and 2113 were selected for the dropout screen. **(B)** Venn diagram of genes that were depleted from the two *PPM1D*-mutant clones (#2102, 2113) used in the dropout screen, but not depleted in the WT control lines. 37 genes were found to be depleted in both mutant clones. For a full list of genes, see Figure 1–source data 1. **(C)** Volcano plot of synthetic lethal hits ranked by fitness score with the Fanconi Anemia pathway genes highlighted in blue. **(D)** Immunoblot validation of *SOD1*-deletion. WT and *PPM1D*-mutant Cas9-OCI-AML2 cells were transduced with control (EV) or sg*SOD1* lentiviruses. Two sgRNAs targeting *SOD1* were tested. Three days post-transduction, the cells underwent puromycin selection (3 ug/mL) for three days after which they were harvested for western blot. Blots were probed with anti-PPM1D (1:1000), anti-SOD1 (1:500), and anti-vinculin (1:2500). **(E)** Cas9-OCI-AML2 and Cas9-OCI-AML3 WT or *PPM1D*-mutant cells were transduced with the empty vector control backbone tagged with a blue fluorescent protein (BFP) reporter. Cells were assayed by flow cytometry between 3- and 24-days post-transduction and normalized to the BFP percentage at day 3. Data shown are mean $\pm$ SD (n=2 per condition).

## Figure 2 – Figure Supplement 1

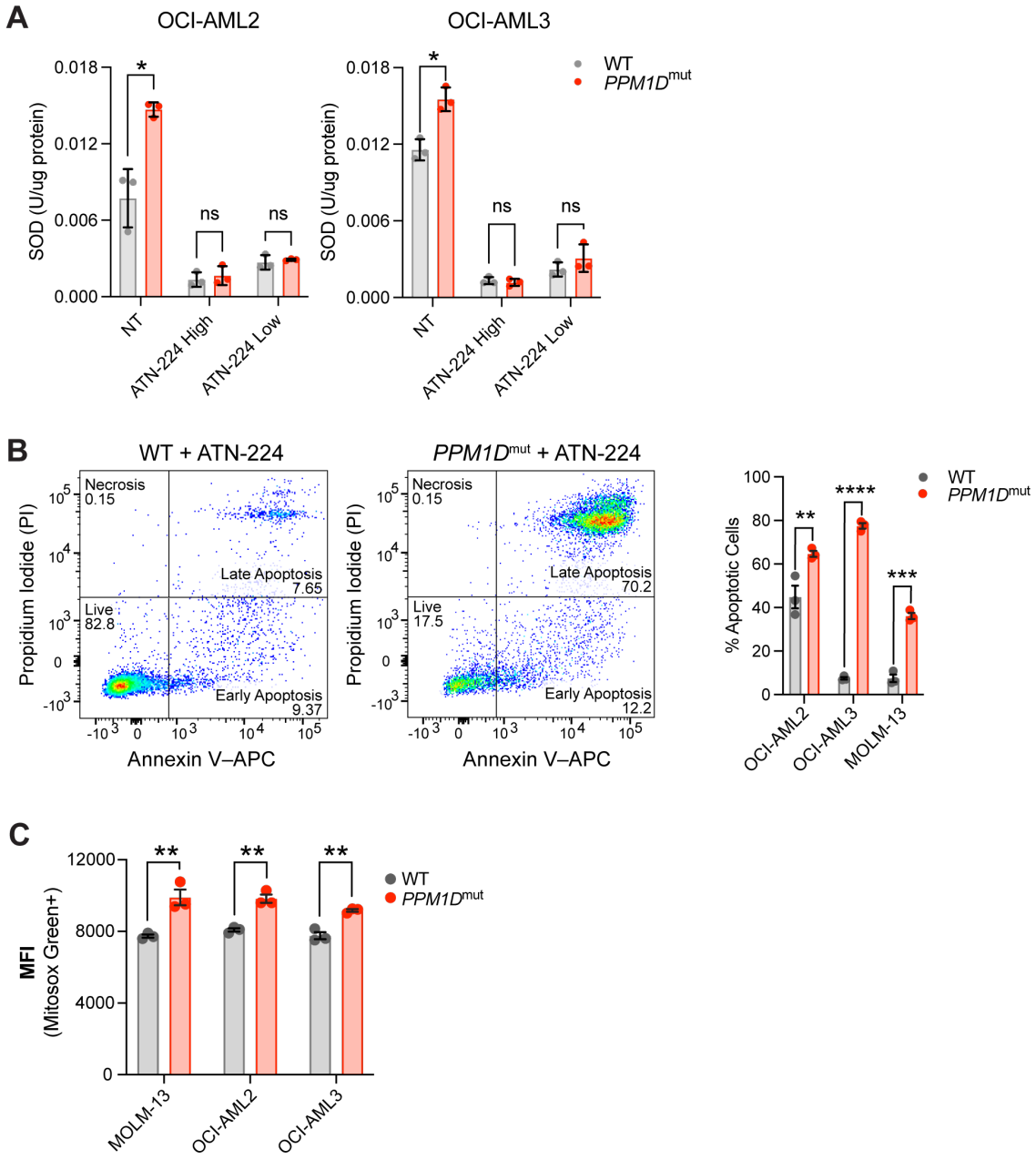



**Figure 2 – Figure Supplement 1. *PPM1D*-mutant cells have increased oxidative stress. (A)** SOD activity assays in OCI-AML2 and OCI-AML3 cells at baseline (NT), or treated with high (12.5 uM) or low (6.25 uM) doses of ATN-224 for 16 hours. **(B)** Left: Representative flow cytometry plots of WT and *PPM1D*-mutant cells treated with ATN-224 (25 uM for 24 hours) and stained for Annexin V-APC and PI for apoptosis; multiple unpaired t-tests, ns=non-significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. **(C)** Endogenous mitochondrial superoxide levels of WT and *PPM1D*-mutant leukemia cell lines were measured using MitoSox Green staining (1 uM). The mean fluorescence intensity (MFI) of MitoSox Green was measured by flow cytometry. Mean ± SD (n=3) is shown.

## Figure 2 – Figure Supplement 2

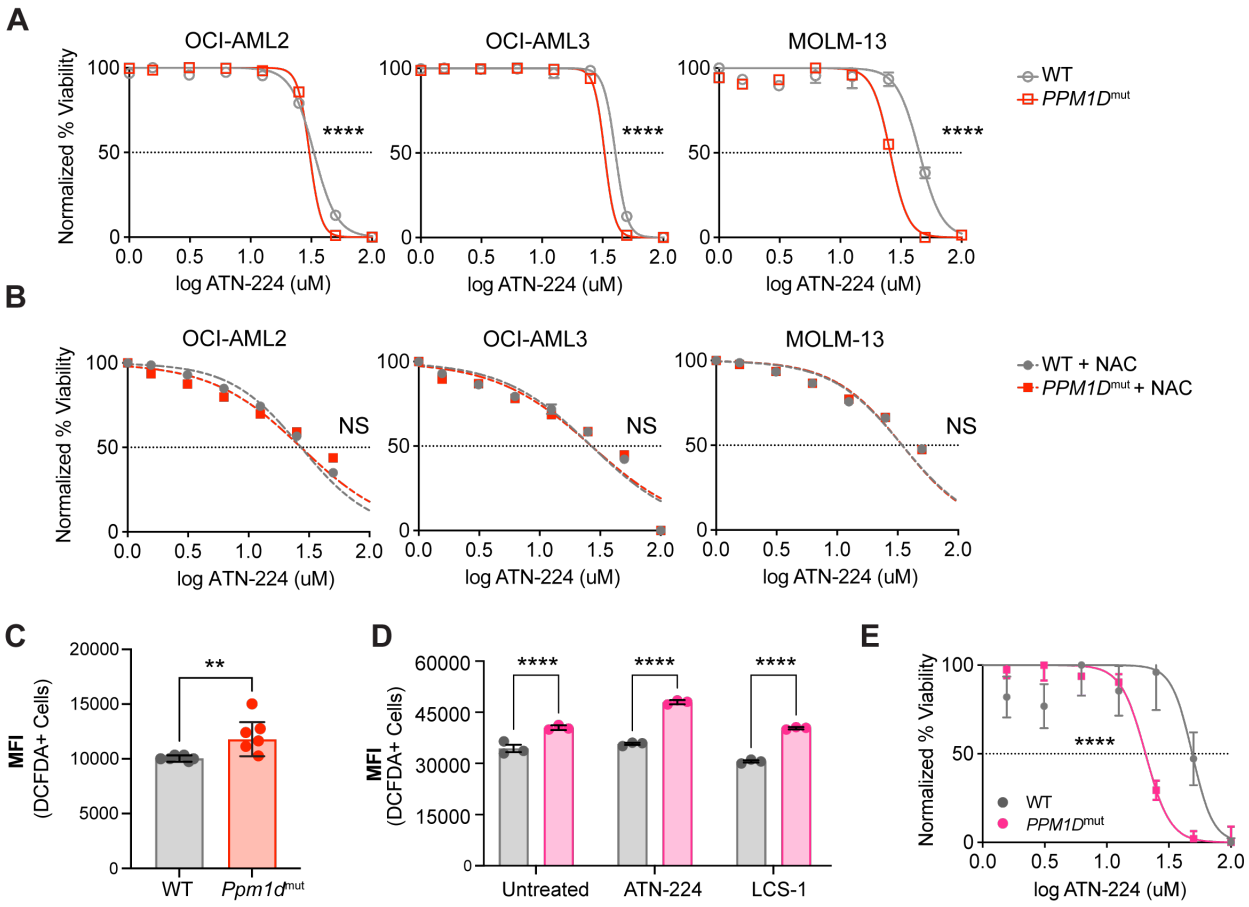



**Figure 2 – Figure Supplement 2. *PPM1D*-mutant cells have increased oxidative stress. (A-B)** Dose response curves for cell viability with SOD1-inhibitor (ATN-224) **(A)** or ATN-224 in combination with 0.25 uM NAC **(B)** in WT and *PPM1D*-mutant leukemia cell lines after 24-hours. Mean ± SD (n=3) is shown along with a non-linear regression curve. All values are normalized to the baseline cell viability with vehicle, as measured by MTT assay. **(C)** Total reactive oxygen species (ROS) of WT and *Ppm1d*-mutant MEFs measured by DCFDA (10 uM) staining. Mean fluorescence intensity (MFI) was determined by flow cytometry. n=6 biological replicates were used for each genotype. Data shown are the mean of each biological replicate; unpaired t-test. **(D)** Total ROS of WT GM12878 (grey) and *PPM1D*-mutant (pink) patient lymphoblastic cell lines (LCLs) at baseline, and after 24-hrs of SOD1 inhibition measured by DCFDA (10 uM) staining. MFI was determined by flow cytometry**;** multiple unpaired t-tests, **(E)** Dose response curve of WT and *PPM1D*-mutant LCLs after ATN-224 treatment. IC50s of WT and *PPM1D*-mutant LCLs were 48.8 uM and 20.51 uM, respectively as measured by MTT assay; non-linear regression analysis, ns=non-significant (p>0.05), **p<0.01, ***p<0.001, ****p<0.0001.

## Figure 3 – Figure Supplement 1

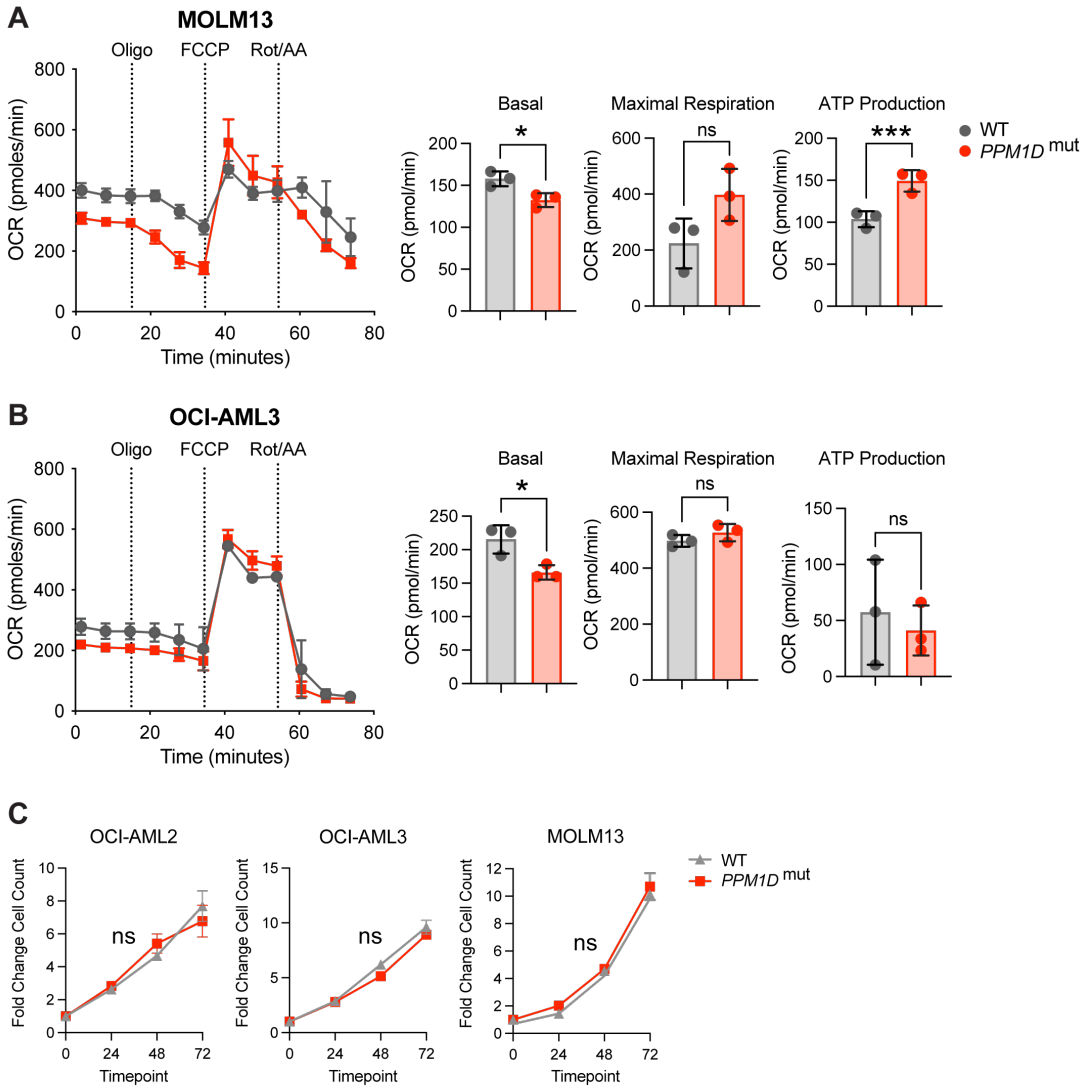



**Figure 3 – Figure Supplement 1. *PPM1D*-mutant cells have altered mitochondrial function.** **(A,B)** Measurement of mitochondrial oxygen consumption rate (OCR) by seahorse assay in WT vs. *PPM1D*-mutant MOLM-13 **(A)** and OCI-AML3 **(B)** cells after treatment with oligomycin (1.5 uM), FCCP (0.5 uM), and rot/AA (0.5 uM). Quantification of basal, maximal, and ATP-linked respiration shown. Each cell line was performed in technical triplicates, student's t-test. **(C)** Growth curves of WT and *PPM1D*-mutant leukemia cell lines at 24-, 48-, and 72-hours. Cell counts were normalized to day 0. ns=non-significant (p>0.05), *p<0.05, ***p<0.001.

## Figure 4 – Figure supplement 1

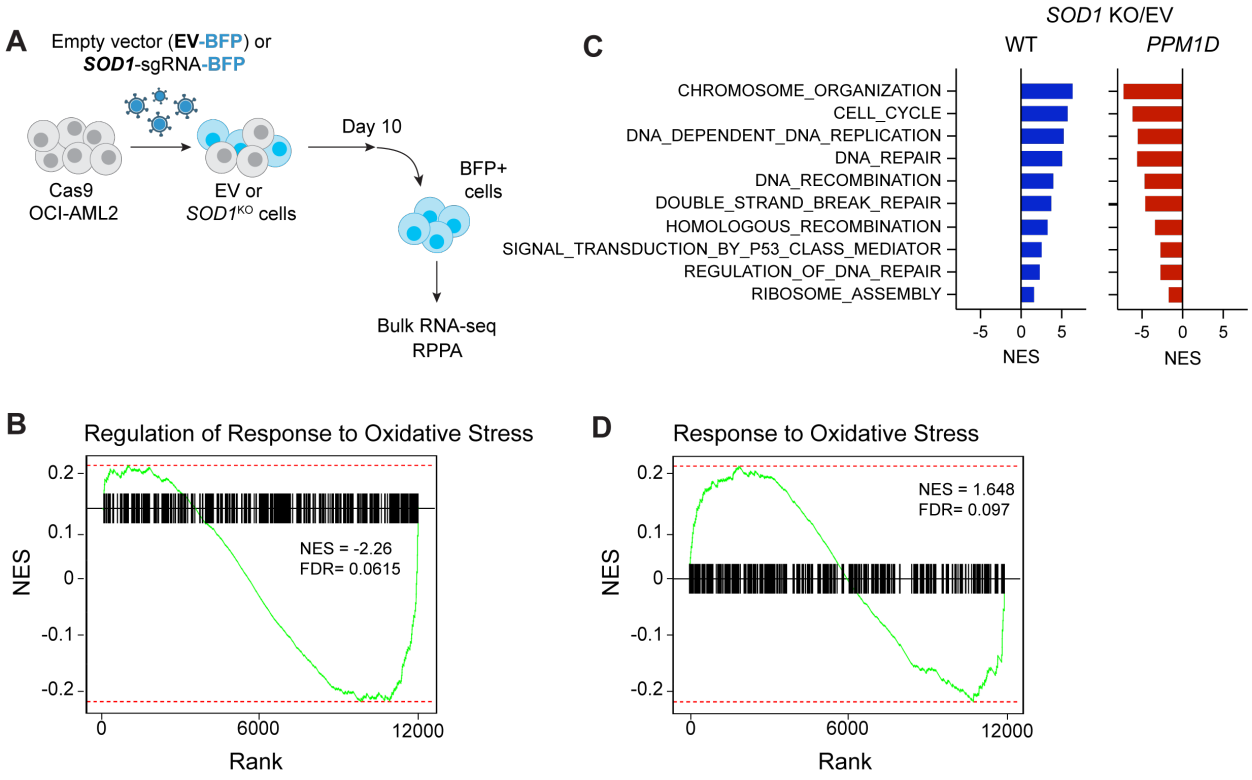



**Figure 4 – Figure Supplement 1. *PPM1D*-mutant cells have reduced oxidative stress response. (A)** Schematic of the experimental setup for the bulk RNA-sequencing and reverse-phase protein array. WT and *PPM1D*-mutant Cas9 OCI-AML2 cells were transduced with either empty vector (EV)-BFP or *SOD1*-sgRNA-BFP. Cells were passaged for ten days and then sorted for BFP expression for downstream analysis. **(B, D)** GSEA enrichment plots for *PPM1D*-mutant cells compared to WT after transduction with EV **(B)** or after *SOD1*-knockout **(D)** for the "Regulation of Response to Oxidative Stress" (GO:1902882) and "Response to Oxidative Stress" (GO:0006979). NES are shown with FDR<0.25. **(C)** GSEA analysis of RNA-sequencing of *SOD1*-deleted cells compared to EV control in WT and *PPM1D*-mutant cells. Blue and red bars indicate significantly up- and downregulated pathways, respectively. Normalized enrichment scores (NES) are indicated. All pathways filtered for FDR<0.25.

## Figure 4 – Figure supplement 2

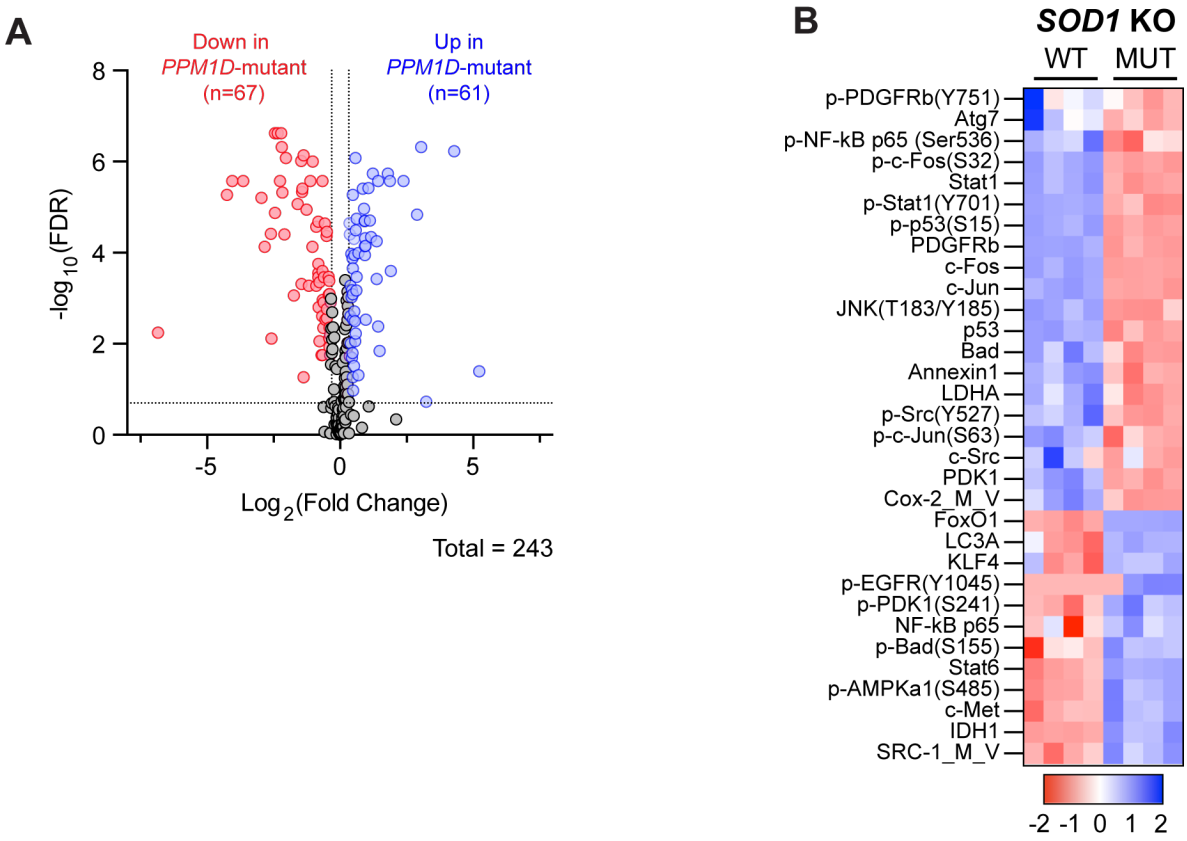
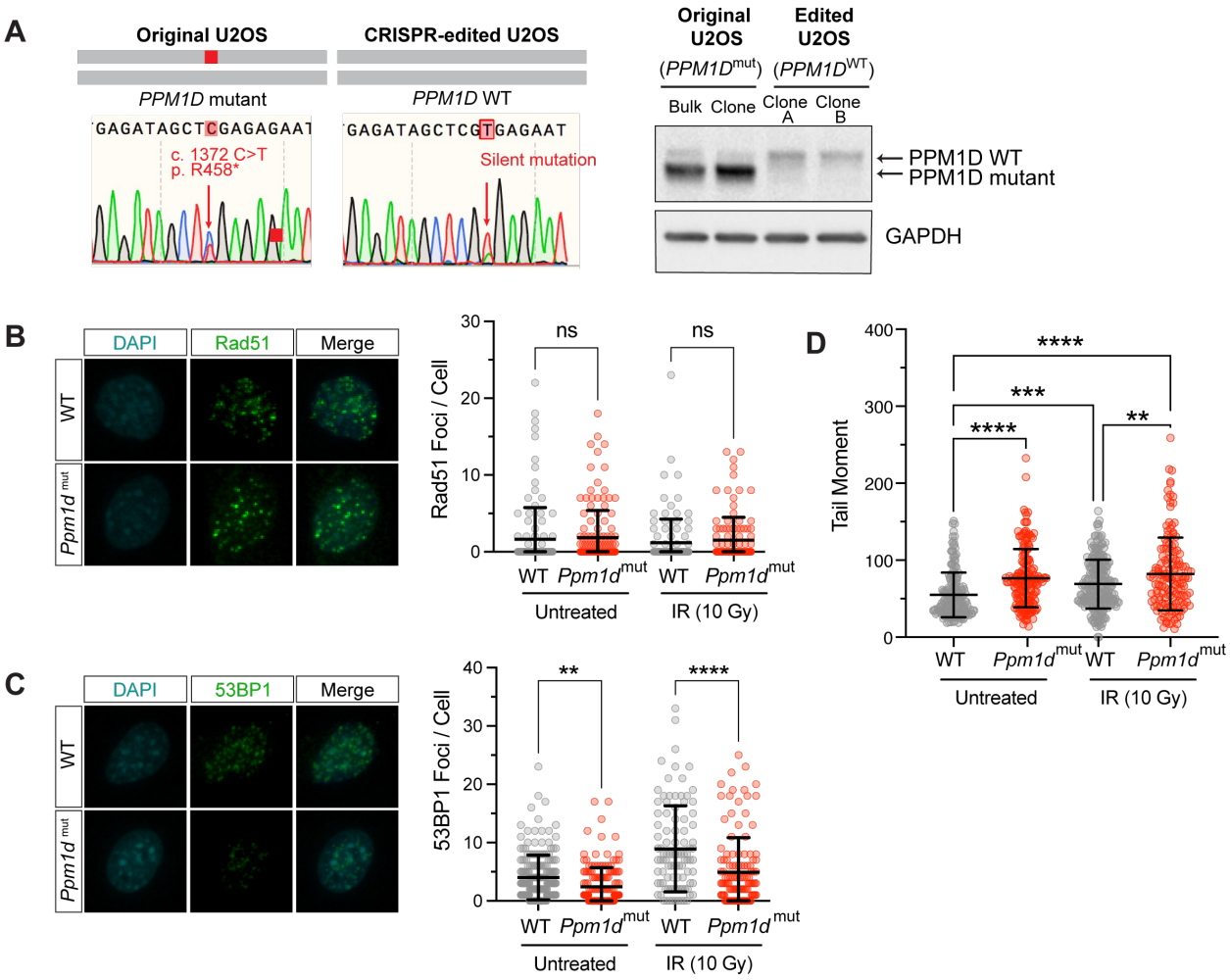



**Figure 4 – Figure Supplement 2. *PPM1D*-mutant cells have reduced oxidative stress response. (A)** Volcano plot of the differentially expressed proteins from the RPPA in *PPM1D*-mutant OCI-AML2 cells compared to WT. Red and blue dots indicate significantly up- or downregulated proteins, respectively, with a cutoff FDR<0.2 and linear fold change >|1.2|. **(B)** RPPA profiling of WT and *PPM1D*-mutant cells after *SOD1* deletion. Proteins from the "Response to Oxidative Stress" pathway have been selected for the heatmap. Each column represents a technical replicate. See Figure 4-source data 2 for the raw data.

## Figure 5 – Figure supplement 1




**Supplementary Figure 5. *PPM1D*-mutations increase genomic instability and impairs non-homologous end-joining repair. (A)** Left: Sanger sequencing traces of the parental U2OS cell line harboring a c.1372 C>T mutation in *PPM1D* and the CRISPR-edited U2OS cell line with mutation corrected to WT PPM1D. Right: Immunoblot validation of these clones are shown. Lysates were probe with anti-PPM1D (1:1000) and anti-GAPDH (1:1000). **(B,C)** Left: Representative images of Rad51 and 53BP1 immunofluorescence microscopy. Mouse embryonic fibroblasts were treated with 10 Gy irradiation, harvested 1-hour post-irradiation and stained for the indicated markers. Right: Quantification of the number of foci per cell is shown. Analysis was performed using CellProfiler. n>100 cells for each condition; students t-test. **(D)** Comet assay quantification of mouse embryonic fibroblasts at baseline and after 1-hour post-irradiation (10 Gy). Quantification and analyses of tail moments were performed using the Comet IV software. n≥150 comets were scored per experimental group; 2way ANOVA, ns=non-significant (p>0.05), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.