## Figure S3



## Figure S3



vehicle

🗖 2 mM

**5** mM

ironomycin [nM]

vehicle ■2.5 µM 📕 50 µM

Figure S3| (related to Figure 3). Metabolic remodelling to reduce glycolytic flux and mitochondrial respiration protects against ironomycin. A-B, Heatmaps showing the differential abundance of metabolites in the sgHK2 cell line (A) and the sgPGP cell line (B) compared to a non-targeting sgRNA cell line (sgNT). We treated cell lines with 500 nM ironomycin or DMSO for 24 hours and collected the samples to perform mass spectrometry analyses. Purple squares outline the decreased metabolites after ironomycin treatment. Black squares outline the increased metabolites after ironomycin treatment. Red squares outline the metabolites increased in the KO cells in comparison with the control cells. C, Proliferation curve showing cell viability assessed by flow cytometry (PI exclusion) of MV4;11 cells cultured with various glucose concentration in the absence of ironomycin treatment. A representative experiment is shown. D-E, Proportion of death in cells treated with ironomycin in combination with metformin (D) or phenformin (E). We performed FACS analysis of PI fluorescence in MV4;11 cell line treated with ironomycin for 48 hours after a 30 minutes pretreatment with metformin or phenformin (n=2 biological replicates, means  $\pm$  SD \*p < 0.05).