

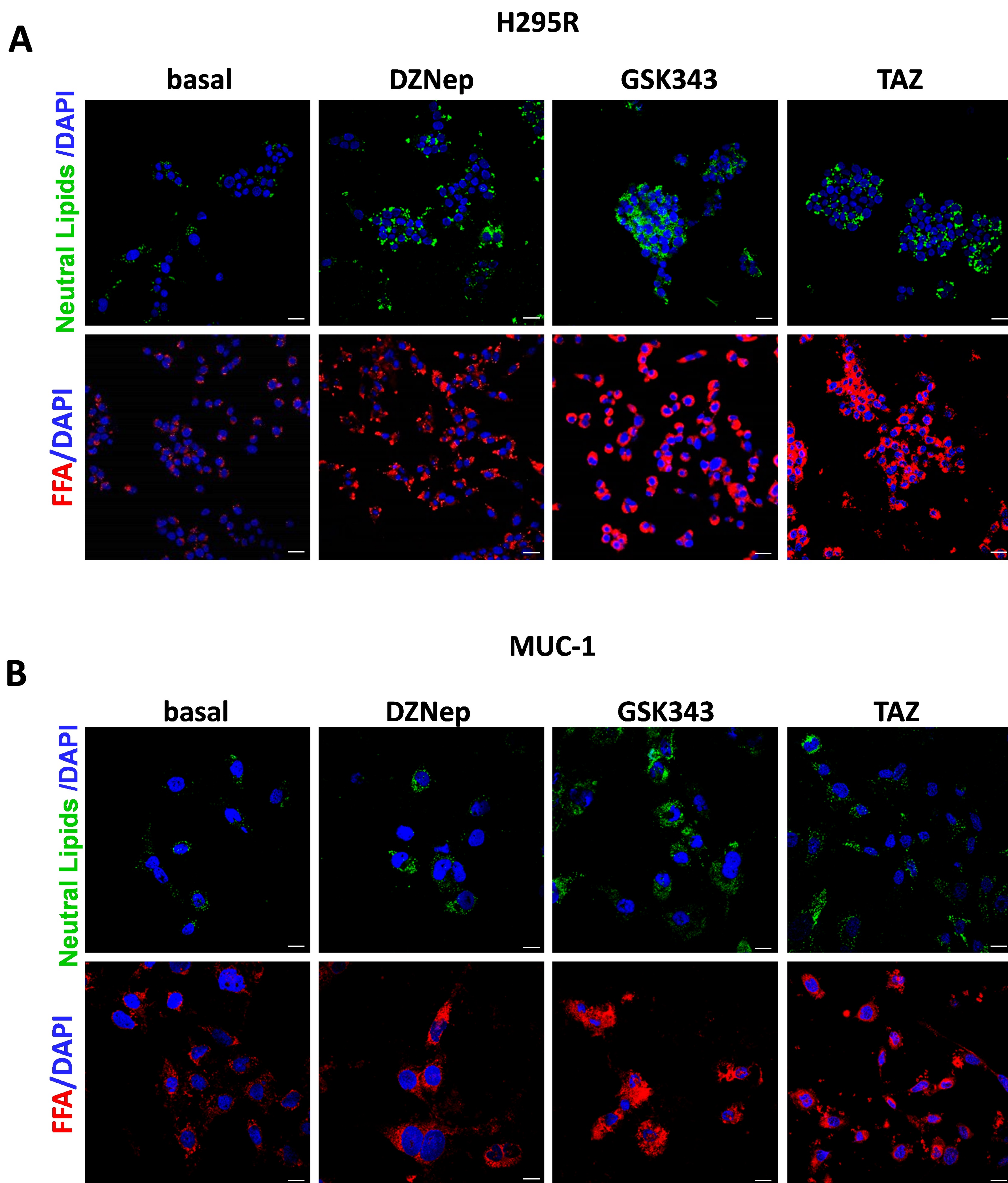


Figure S1. EZH2i affect lipid content in ACC cells.

A, B. Confocal images of LDs stained by BODIPY 493/503 fluorescent dye (Neutral lipids) and BODIPY™ 558/568 C12 (free fatty acids, FFA) in H295R (**A**) and MUC-1 (**B**) cells treated for 48h with DZNep (5 μ M), GSK343 (10 μ M) and Tazemetostat (TAZ, 5 μ M). Nuclei were stained by DAPI. (scale bar 50 μ m)

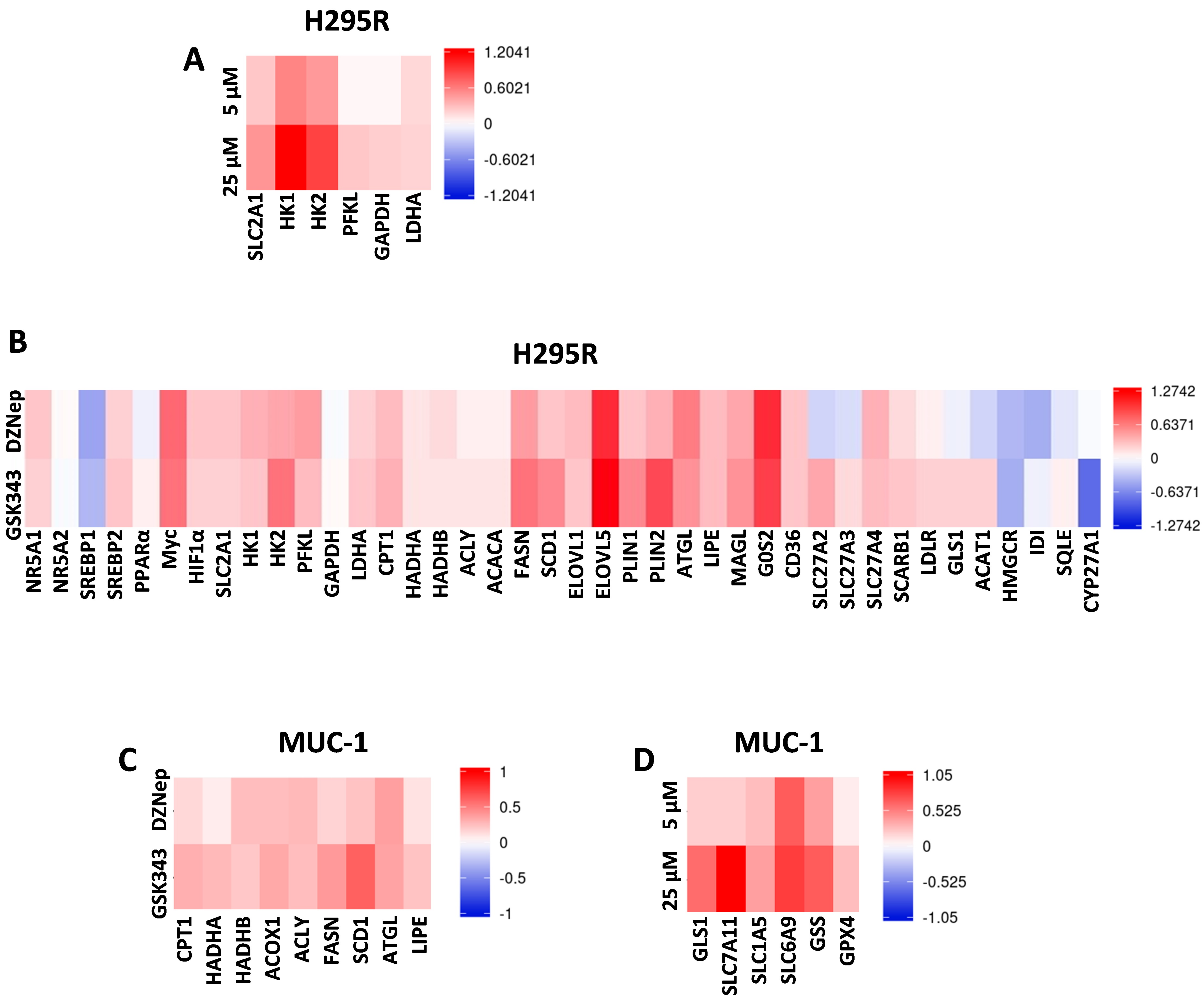
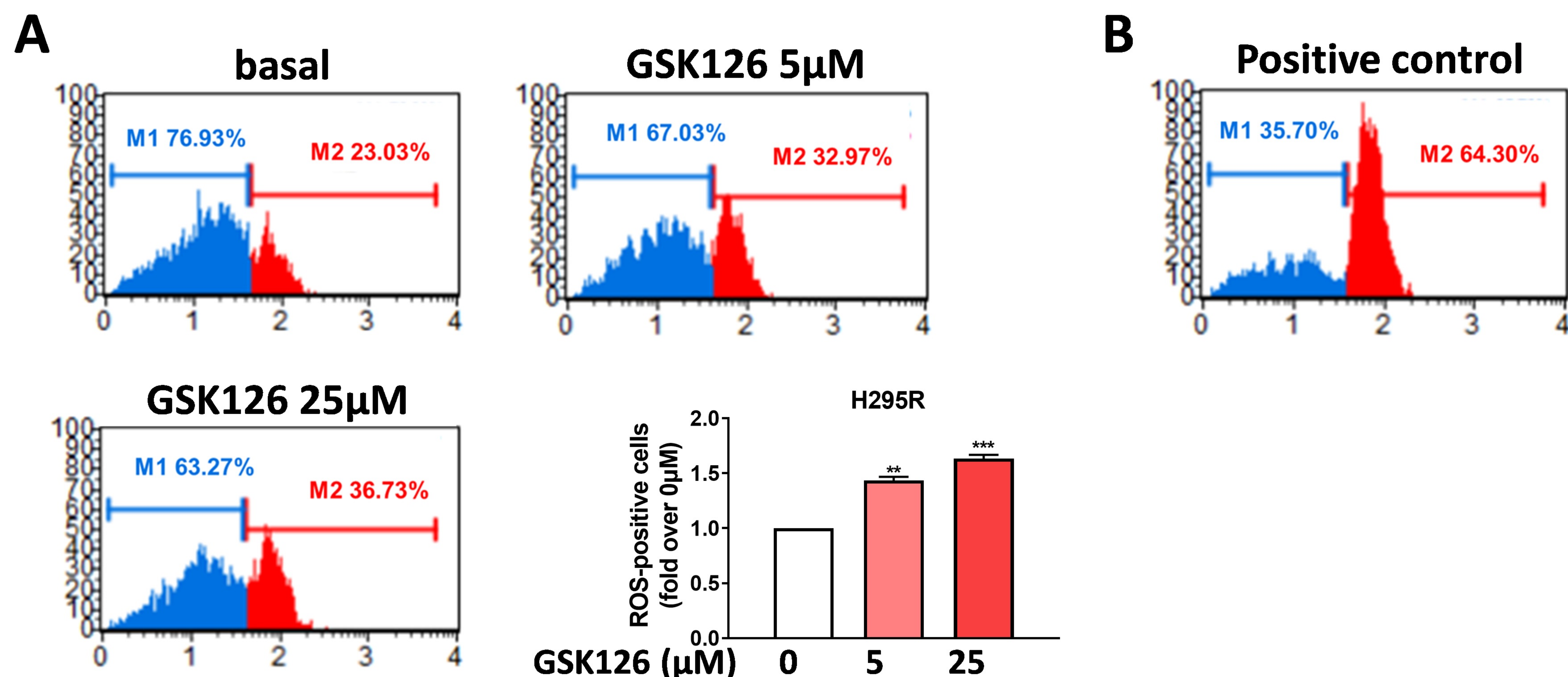


Figure S2. Effects of EZH2i on the expression of metabolic genes.

A-D. Heatmaps of mRNA expression of metabolic genes in ACC cells evaluated by real-time RT-PCR after 48 h treatment with GSK126 (5 and 25 μ M) (**A**, **D**), DZNep (5 μ M) and GSK343 (10 μ M) (**B**, **C**). Data are the values from 3 separate RNA samples presented as log₁₀ of fold-change.

H295R



MUC-1

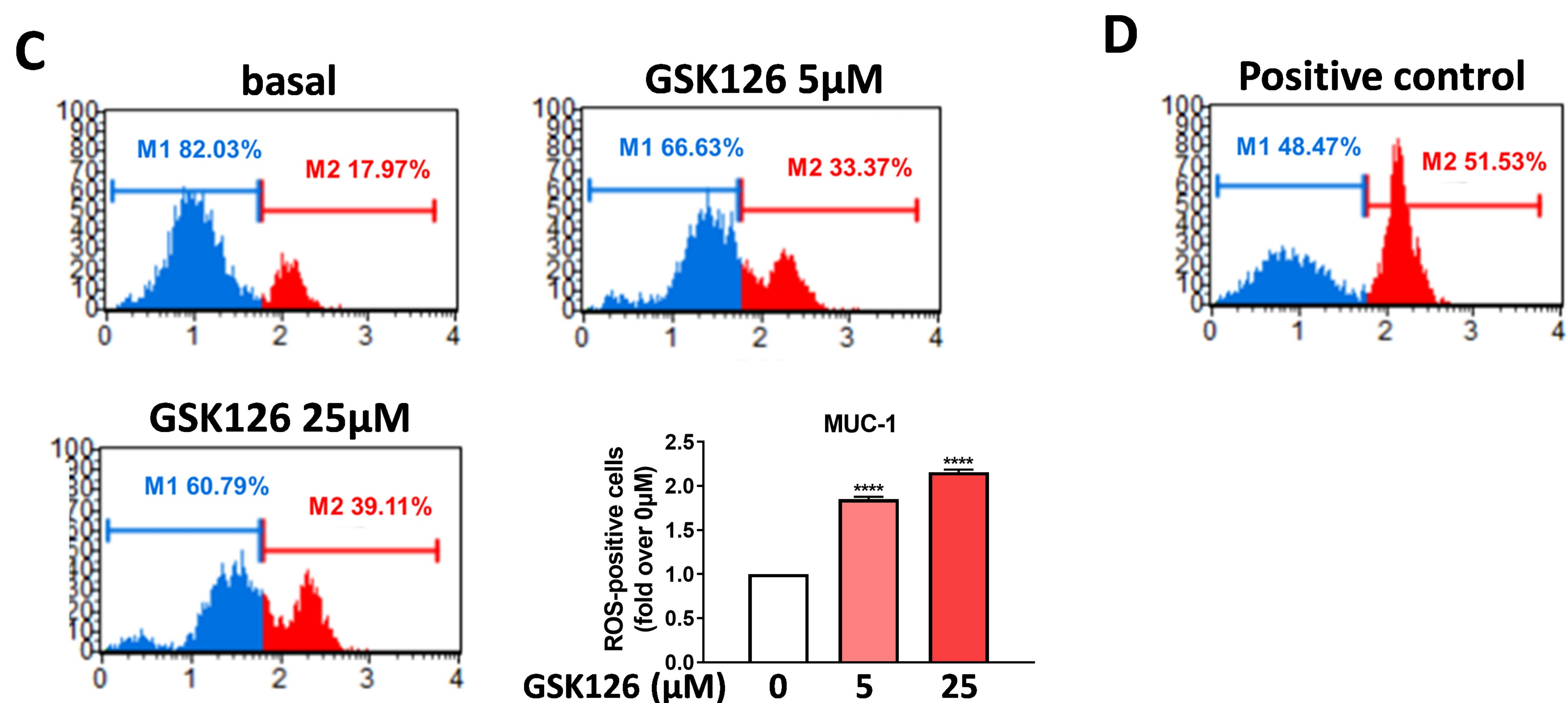
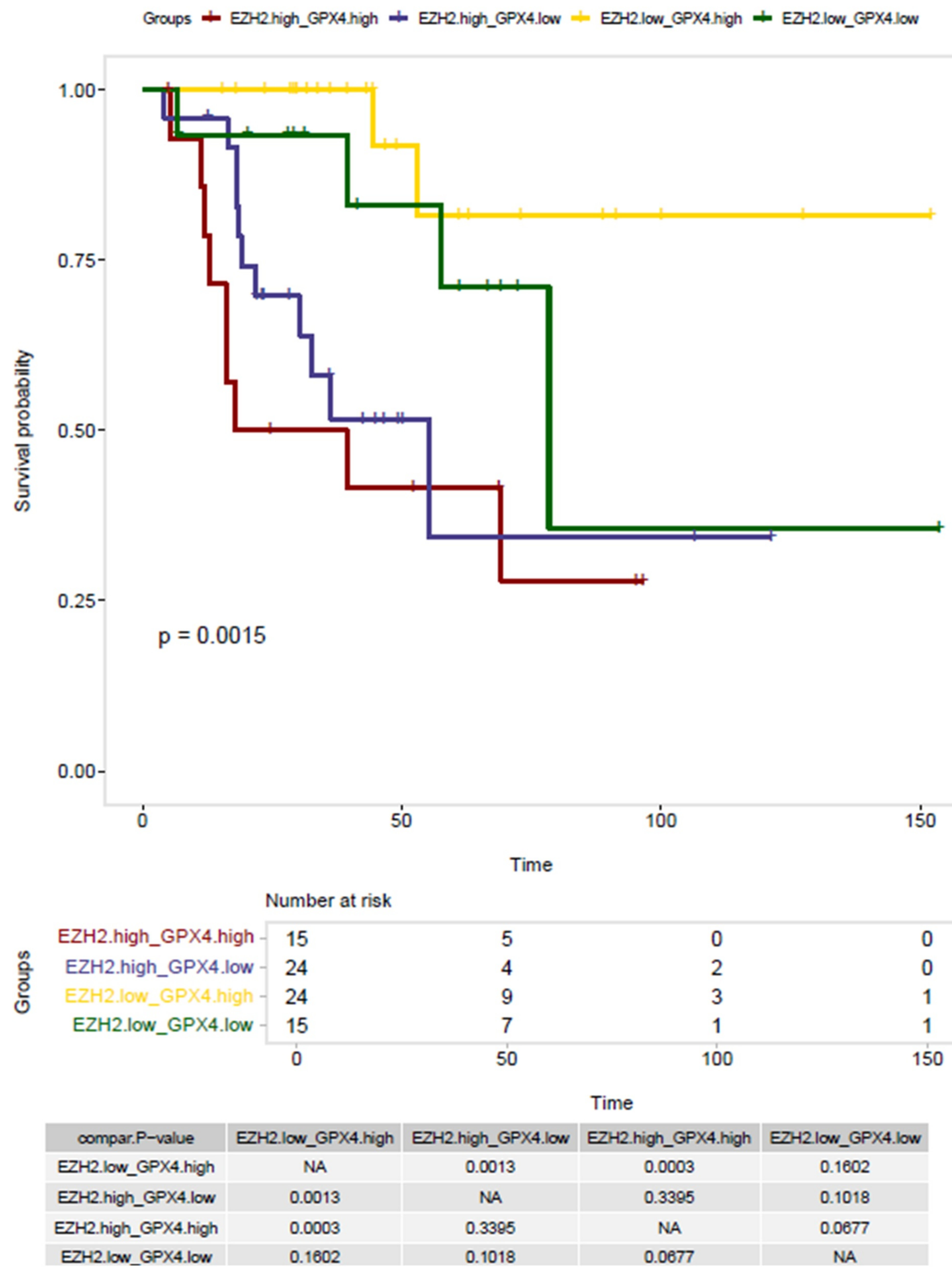


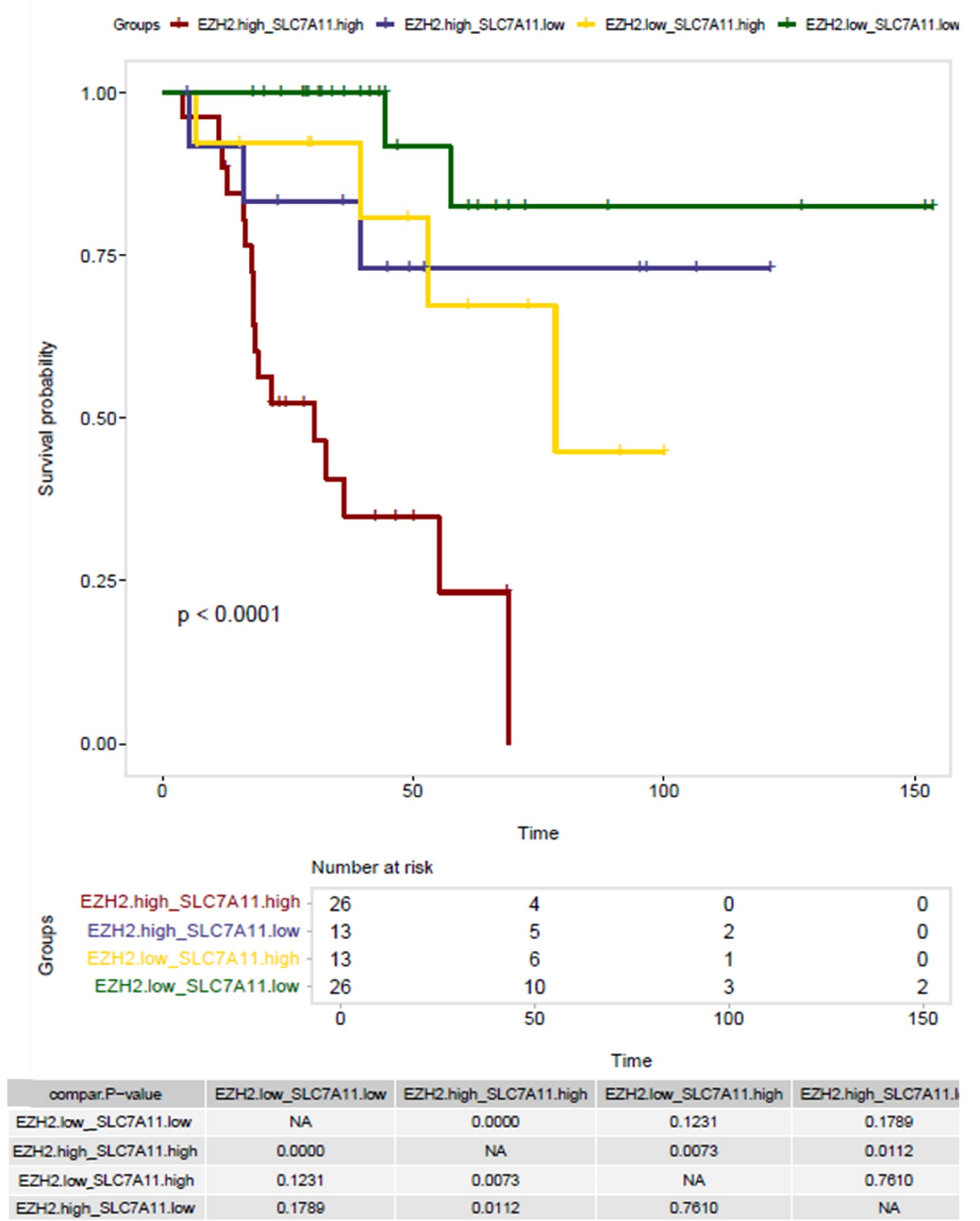
Figure S3. EZH2i increase ROS in ACC cells.

A-D. Flow cytometry analysis of reactive oxygen species (ROS) in ACC cells treated with GSK126 for 48 h. Cells treated with H₂O₂ for 2 h were used as positive control (**B**, **D**). ROS-positive cells are shown in red. Histograms represent the fold change in the number of ROS-positive cells. Data are expressed as mean $\pm$ SEM. n=3 independent experiments. ** p < 0.01, *** p < 0.001, **** p < 0.0001.

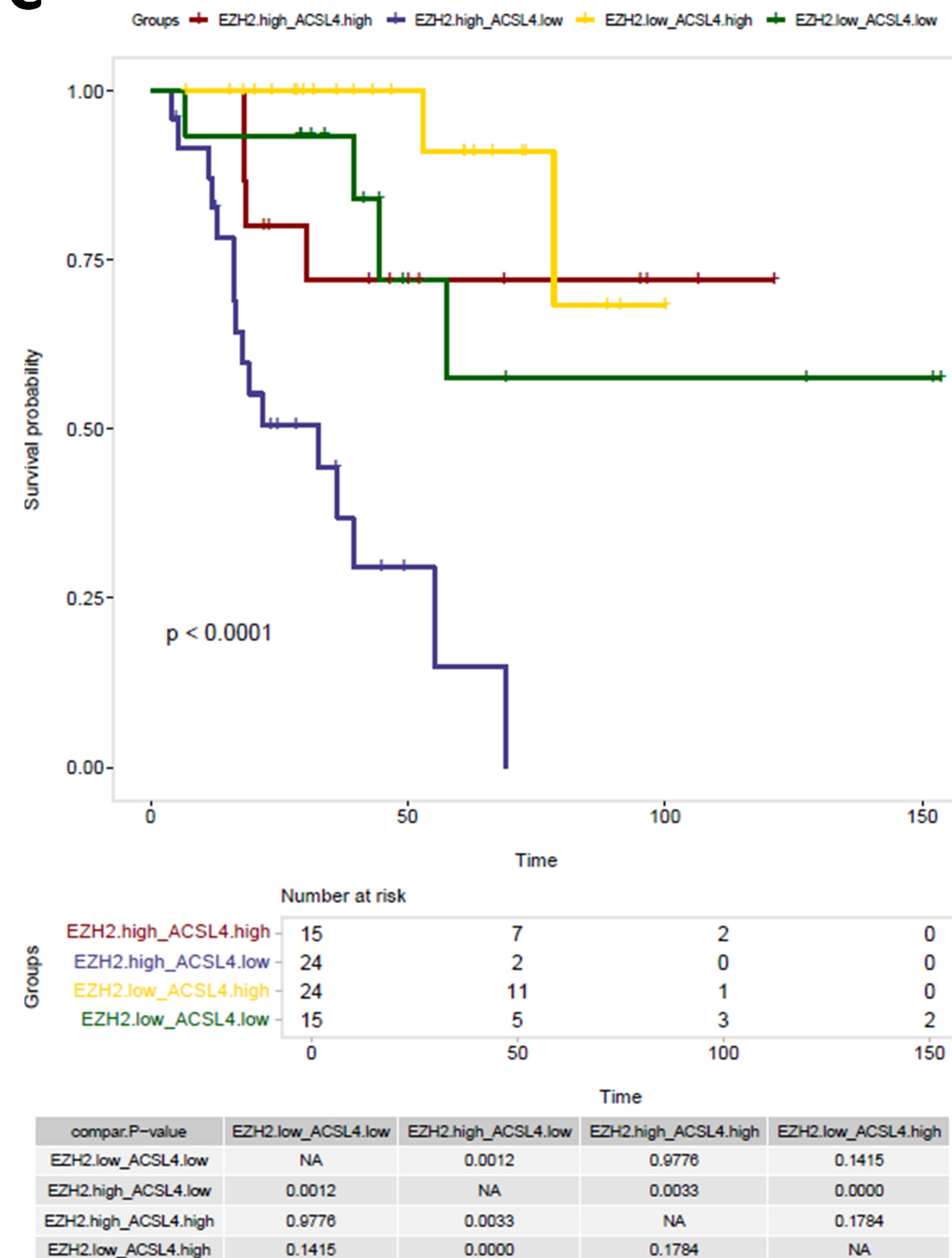
A



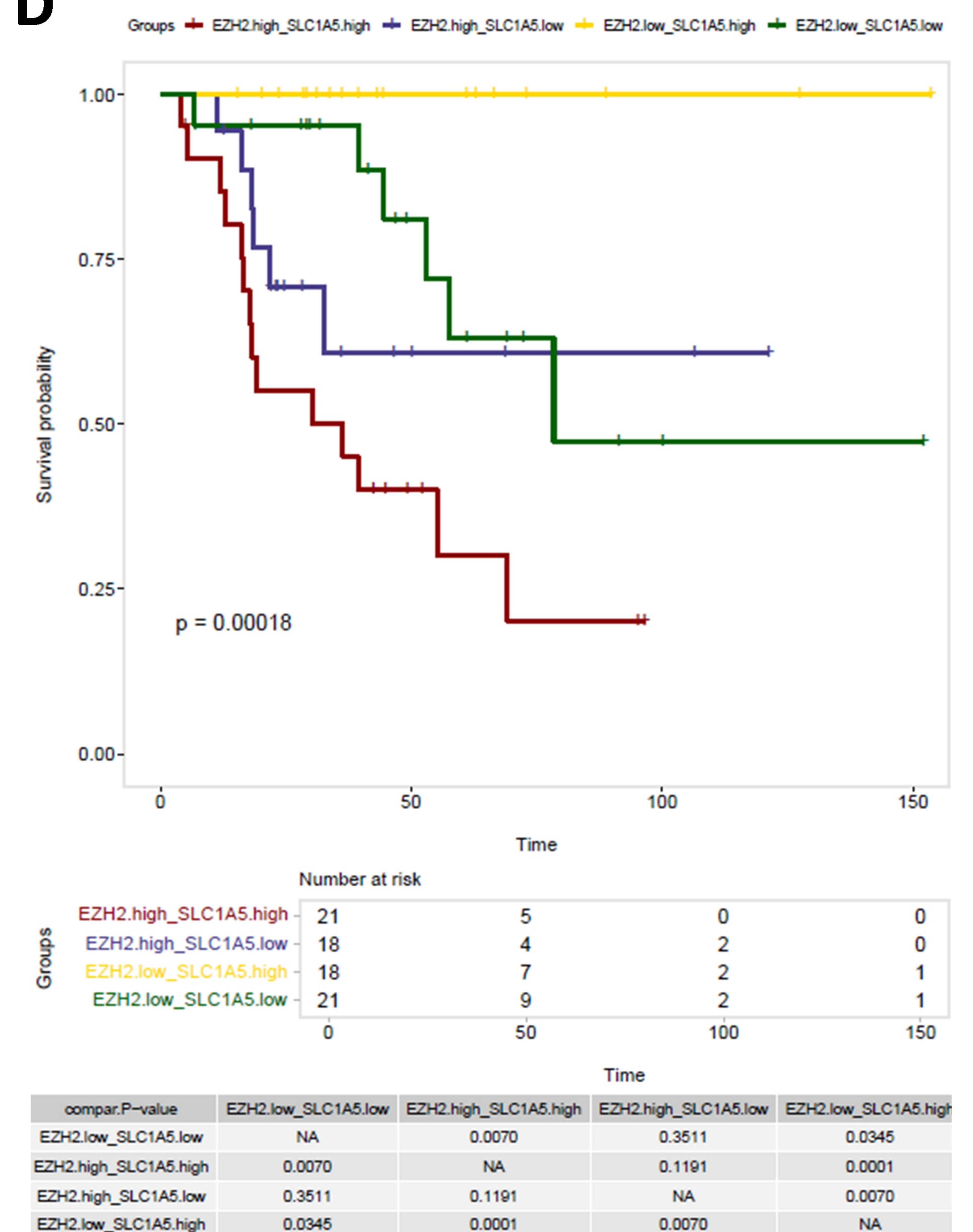
B



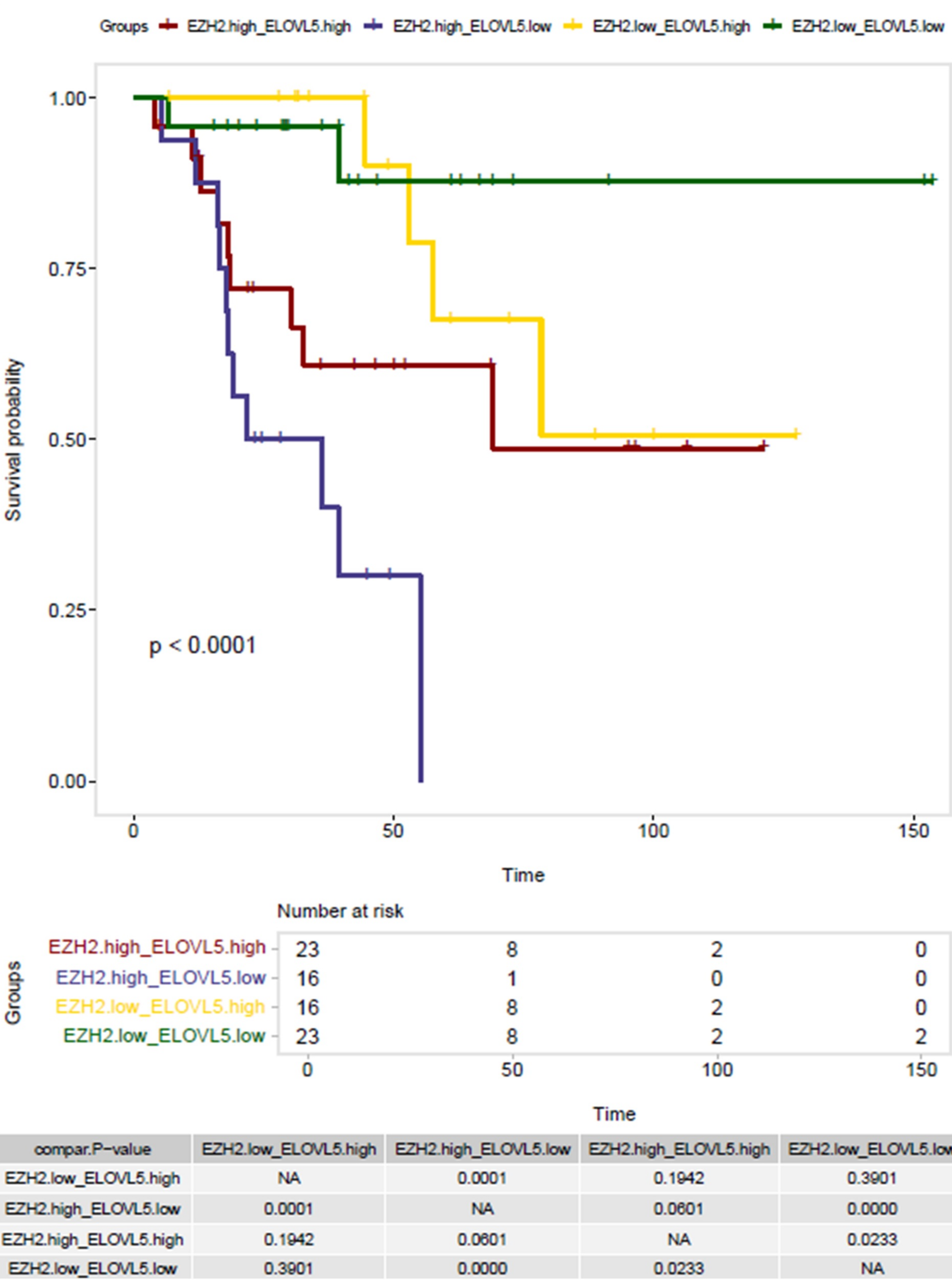
C



D



E



F

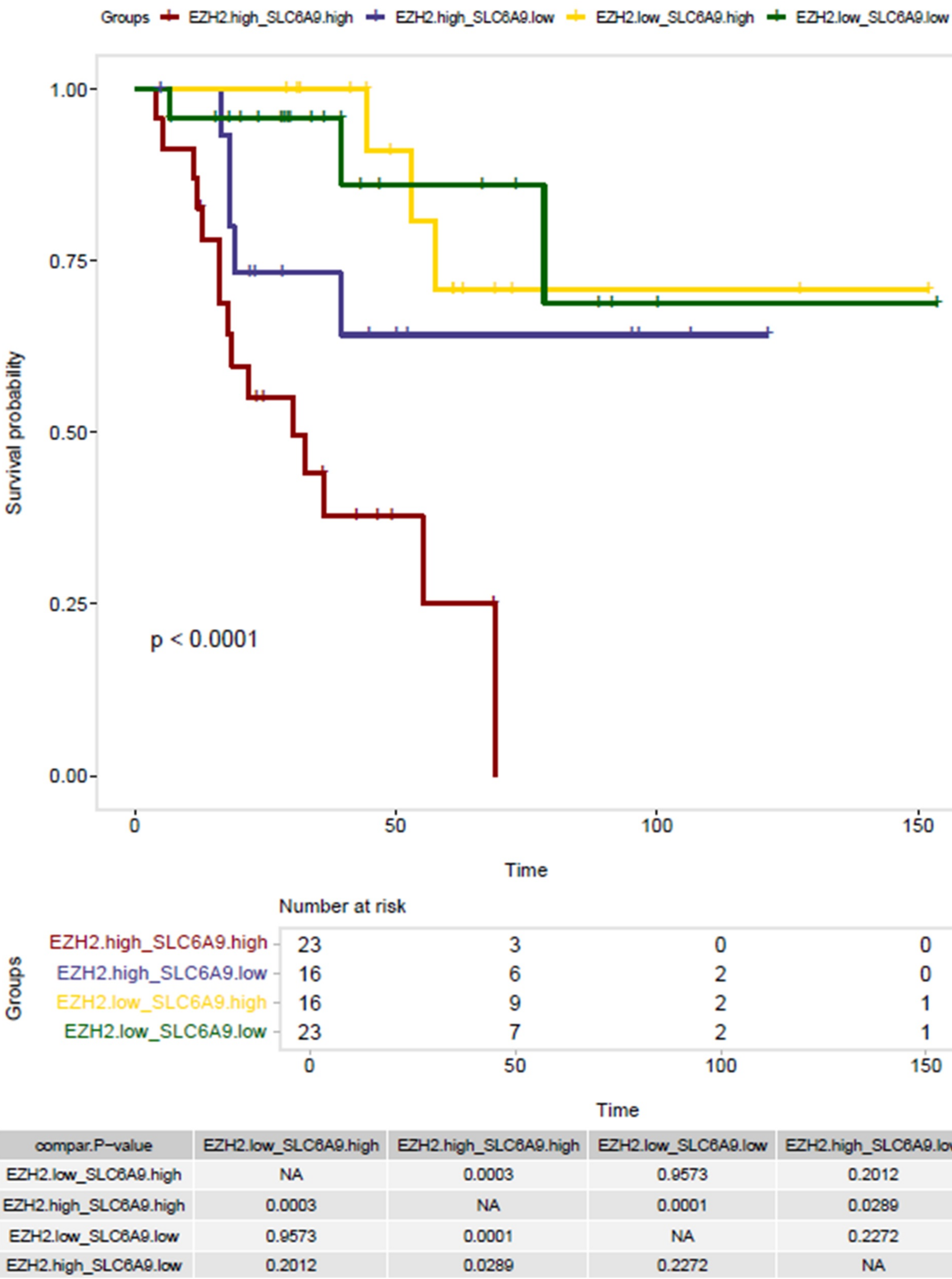


Figure S4. Risk stratification according to the expression of EZH2 and ferroptosis-related genes in ACC patients. A-F. Kaplan-Meier curves showing overall survival (in months) of subgroups of patients stratified according to the median expressions of EZH2 and other ferroptosis-related genes. The total number of patients in each subgroup is shown in the middle panel. The p-value on top of the plot represents the overall result of the log-rank test including all groups. Tables at the bottom panels of each figure indicate the p-values of pairwise log-rank tests between each subgroup.

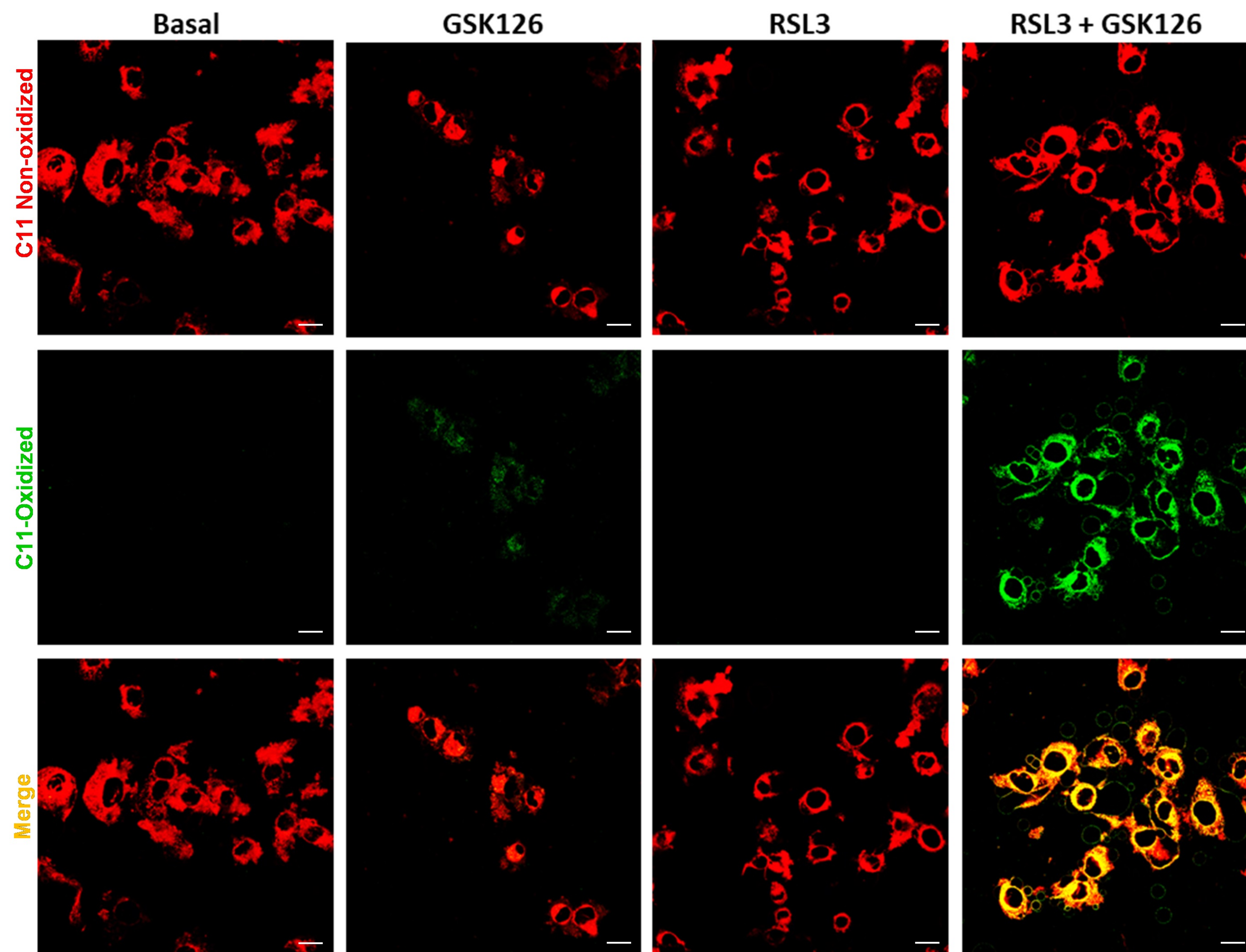


Figure S5. EZH2i sensitize MUC-1 cells to ferroptosis inducers.

A. Confocal images of lipid peroxidation detected by BODIPY C11 fluorescent dye in MUC-1 cells treated for 48 h with GSK126 (5 μ M) in the presence or absence of RSL3 (1 μ M, for the last 4h) (scale bar 50 μ m).

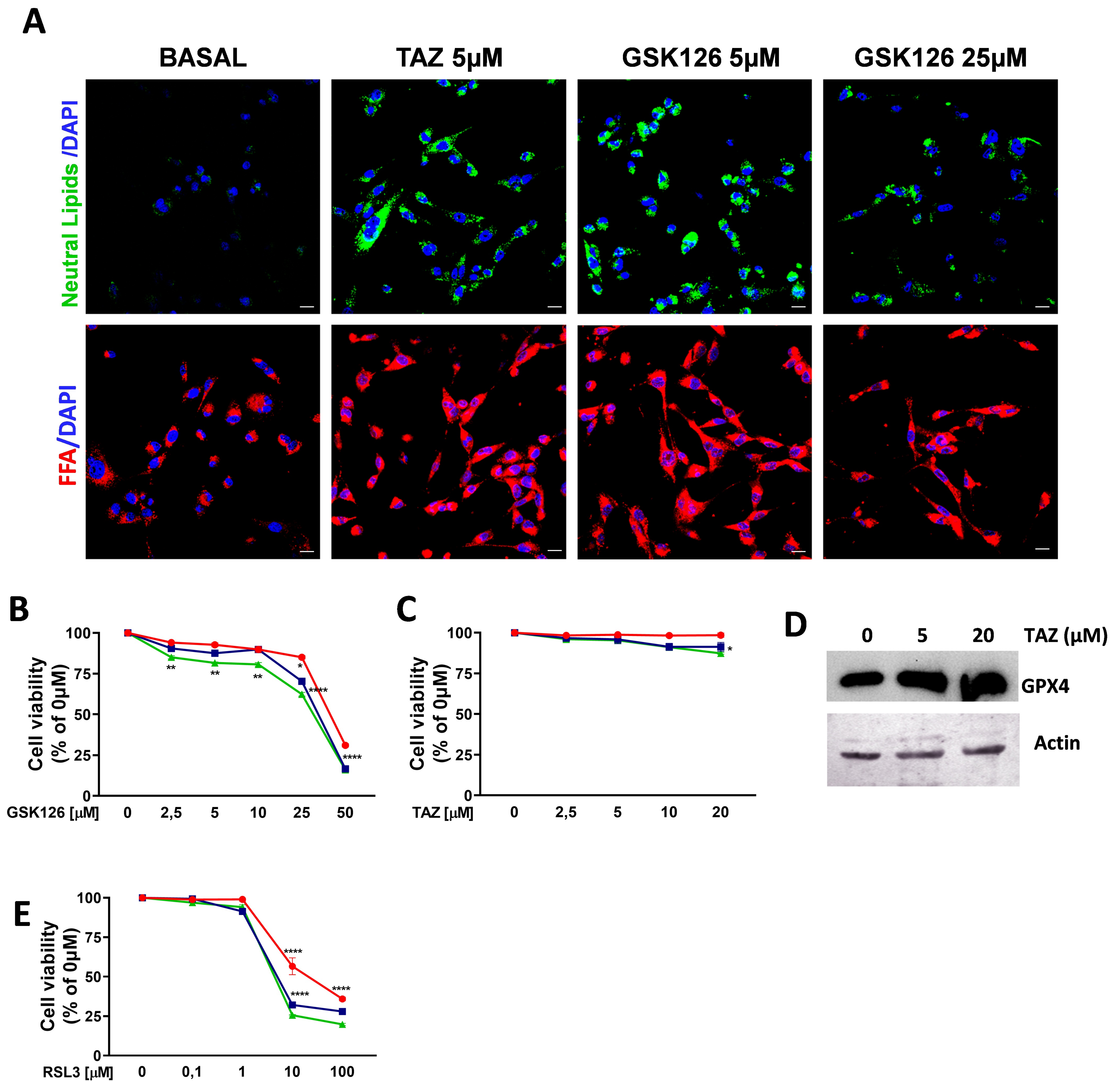


Figure S6. EZH2i increase lipid content and GPX4 expression in a cell model of TNBC.

A. Confocal images of lipid droplets stained by BODIPY 493/503 fluorescent dye (Neutral lipids) and BODIPY™ 558/568 C12 (free fatty acids, FFA) in MDA-MB231 cells treated for 48 h with TAZ (5 μ M) and GSK126 (5 μ M and 25 μ M). Nuclei were stained by DAPI. Scale bar 50 μ m. **B, C, E.** Cell viability by MTT assay of MDA-MB231 cells treated for 24, 48 and 72 h with the indicated doses of GSK126, TAZ and RSL3. n=3 independent experiments. **D.** Western blot of GPX4 in MDA-MB231 after 48 h of TAZ treatment. Actin was used as a loading control. Data are expressed as means $\pm$ SEM. * p < 0.05; ** p < 0.01 **** p < 0.0001.

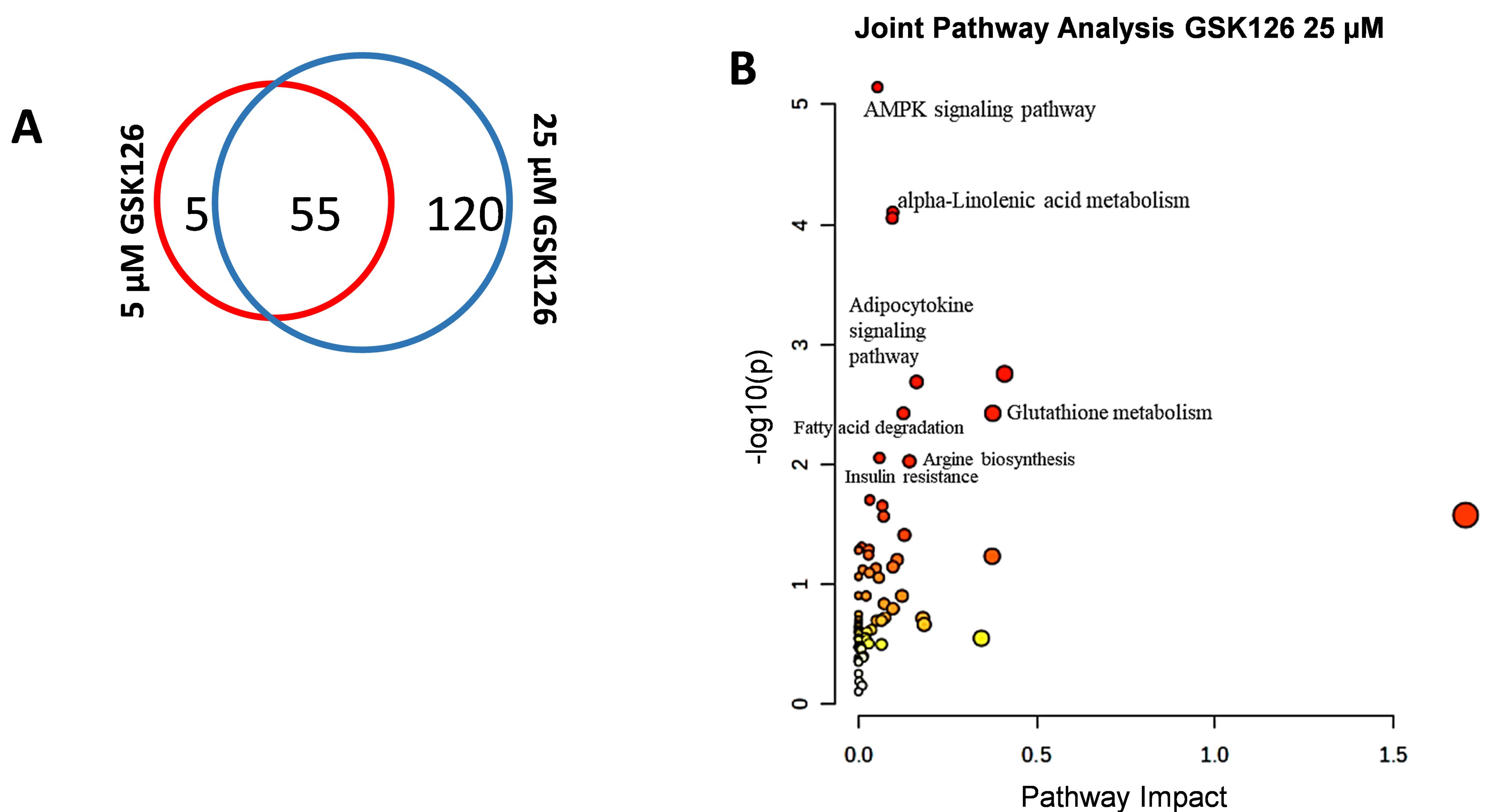


Figure S7. GSK126-treated TNBC cells are enriched in lipid and antioxidant genes and metabolites.

A. Untargeted metabolomics analysis was performed on MDA-MB231 cells treated with GSK126 (5 μ M and 25 μ M) for 48 h. Venn diagram represents statistically significant differentially abundant metabolites across 5 and 25 μ M GSK126-treated cells. **B.** Overview of the pathway enrichment analysis based on both transcripts and metabolites with statistically significant difference in 25 μ M GSK126-treated MDA-MB231 cells. Only pathways with $-\log_{10}(p) > 2$ are shown.