Supplementary Materials

# Dual inhibition of H3K9me2 and H3K27me3 promotes tumor cell senescence without triggering the secretion of SASP

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**Supplementary Figures**

## Supplementary Figure Legends

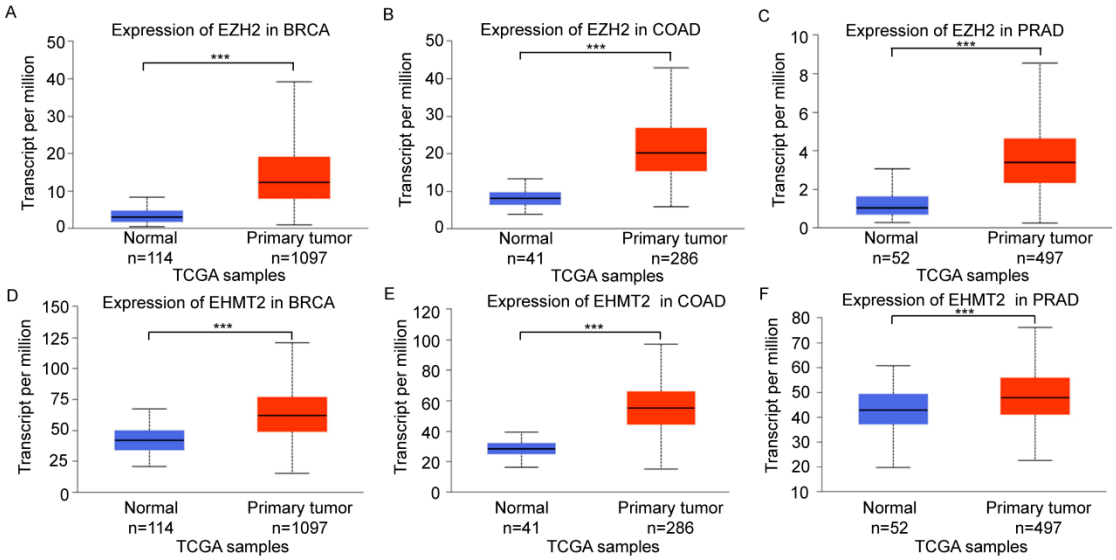


**Figure S1.** Expression of EZH2 and G9a in primary tumor (breast cancer, colon cancer and prostate cancer) samples compared with the normal samples, BRCA, breast invasive carcinoma; COAD, colon adenocarcinoma; PRAD, prostate adenocarcinoma. (**A-C**) Expression analysis of EZH2 in different tumor samples according to the TCGA database. (**D-E**) Expression analysis of EHMT2 in different tumor samples according to the TCGA database. $^{***}p < 0.001$.

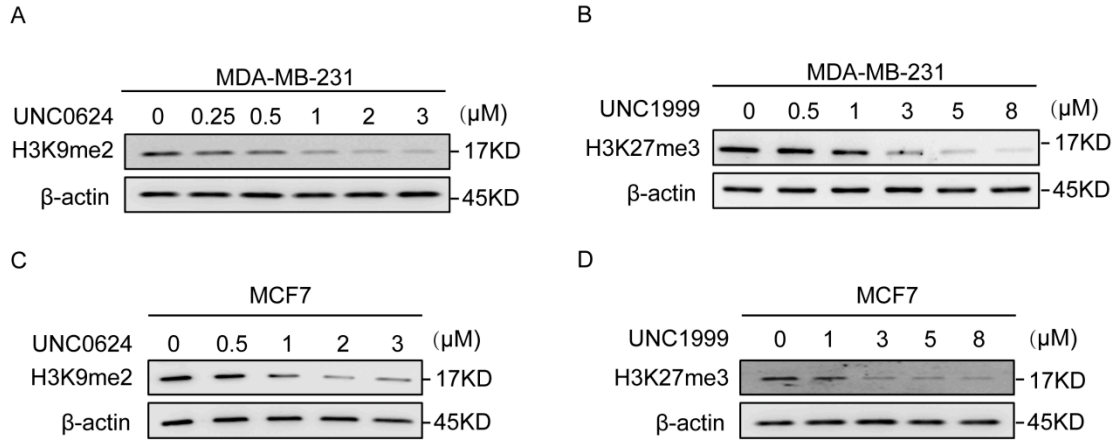


**Figure S2.** Determination of the optimum concentration of inhibitors. (**A**) Western blot analysis of expression of H3K9me2 in MDA-MB-231 cells treated with UNC0642 (0 μM, 0.25 μM, 0.5 μM, 1 μM, 2 μM, 3 μM) for 3 days. (**B**) Western blot analysis of expression of H3K27me3 in MDA-MB-231 cells treated with UNC1999 (0 μM, 0.5 μM, 1 μM, 3 μM, 5 μM, 8 μM) for 3 days. (**C**) Western blot analysis of expression of H3K9me2 in MCF7 cells treated with UNC0642 (0 μM, 0.5 μM, 1 μM, 2 μM, 3 μM) for 3 days. (**D**) Western blot analysis of expression of H3K27me3 in MCF7 cells treated with UNC1999 (0 μM, 1 μM, 3 μM, 5 μM, 8 μM) for 3 days.

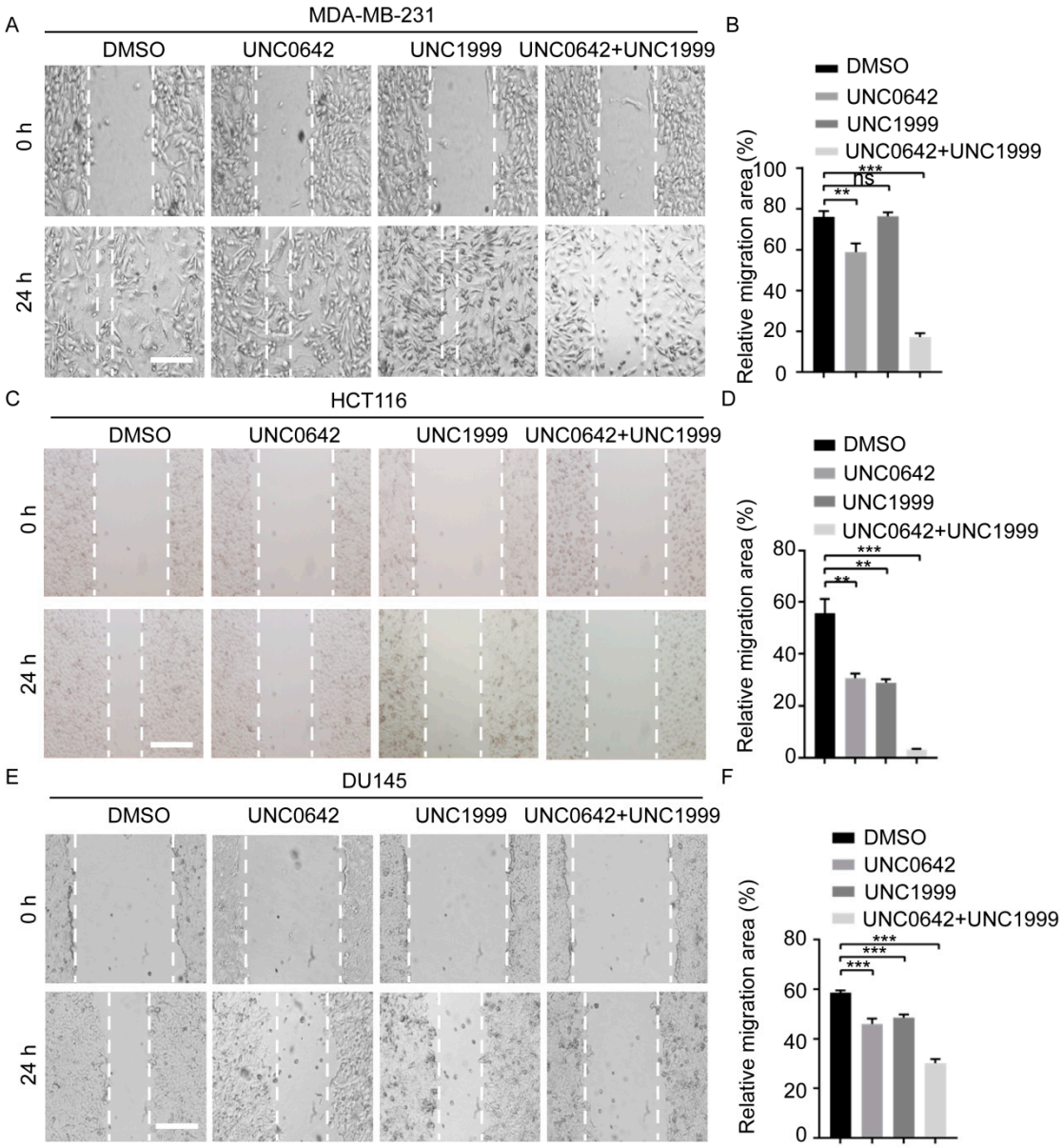


**Figure S3.** A combination of UNC0642 and UNC1999 blocks migration of tumor cells significantly. (**A-B**) Wound-healing assay in MDA-MB-231 and DU145 cells treated with indicated treatments. Representative micrographs of the wound-healing assay are shown (**A**) and data represent the relative migration area of cells per field, Scale bar: 100 μm (**B**). (**C-D**) Wound-healing assay in HCT116 cells treated with indicated treatments. Representative micrographs of the wound-healing assay are shown (**C**) and data represent the relative migration area of cells per field, Scale bar: 100 nm (**D**). (**E-F**) Wound-healing assay in DU145 cells treated with indicated treatments. Representative micrographs of the wound-healing assay are shown (**E**) and data represent the relative migration area of cells per field, Scale bar: 100 μm (**F**). Each experiment was repeated at least three times. Error bars, mean ± SD, ns: no significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

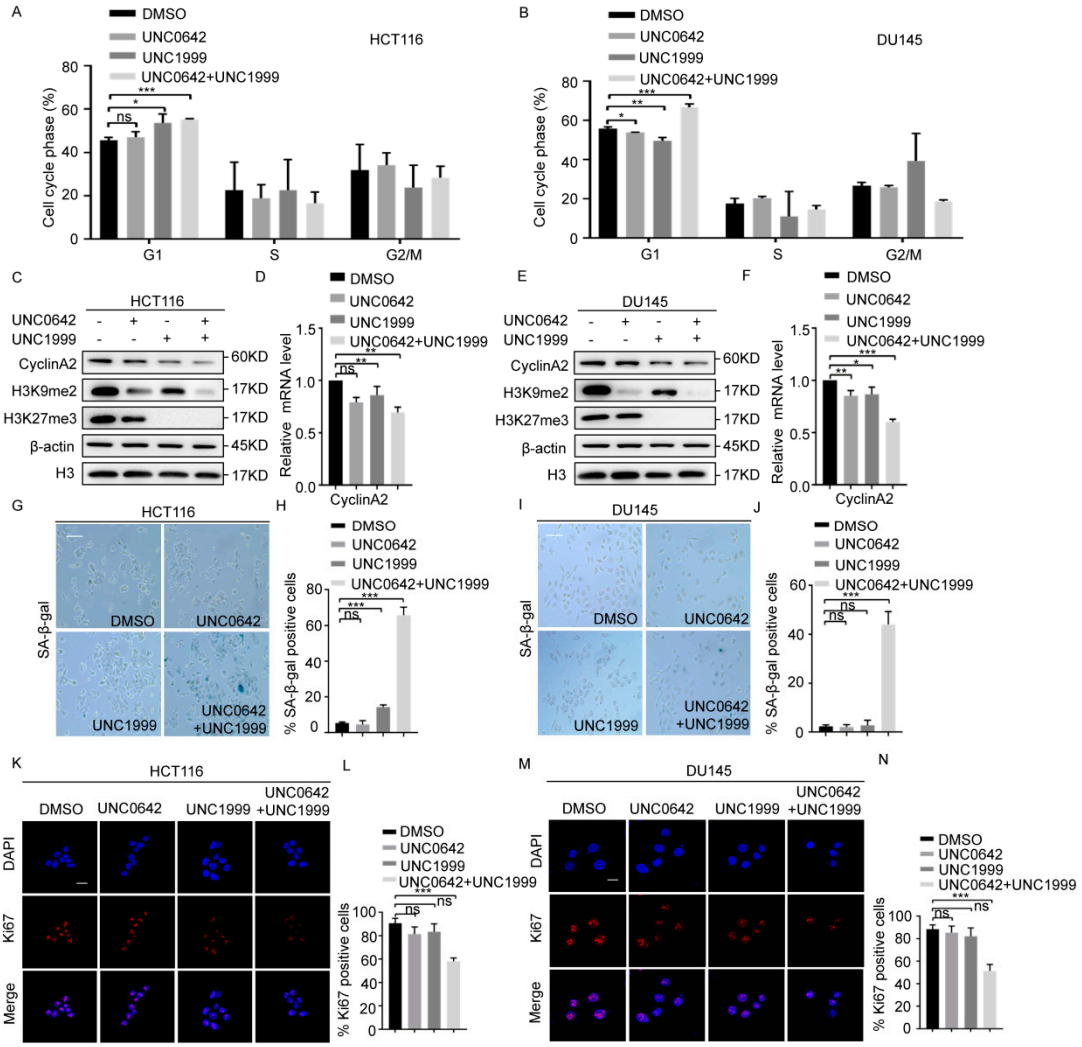


**Figure S4.** A combination of UNC0642 and UNC1999 induces HCT116 and DU145 cellular senescence. (**A-B**) Cell cycle analysis in HCT116 (**A**) and DU145 (**B**) cells treated with indicated treatments. (**C**) Western blot analysis of expression of CyclinA2, H3K9me2 and H3K27me3 in HCT116 cells treated with indicated treatments. (**D**) qRT-PCR analysis of the expression of CyclinA2 in HCT116 cells treated with indicated treatments. (**E**) Western blot analysis of expression of CyclinA2, H3K9me2 and H3K27me3 in DU145 cells treated with indicated treatments. (**F**) qRT-PCR analysis of the expression of CyclinA2 in HCT116 cells treated with indicated treatments. (**G-J**) SA-β-Gal staining in HCT116 and DU145 cells treated with indicated treatments. Representative images of SA-β-Gal positive cells were showed (**G** and **I**) and the percentage of the SA-β-Gal positive cells was calculated, Scale bar: 100 μm (**H** and **J**). (**K-N**) Immunofluorescence analysis of Ki67 in HCT116 and DU145 cells with the indicated treatments. Representative images of Ki67 positive cells were showed (**K** and **M**) and the percentage of the Ki67 positive cells was calculated, Scale bar: 20 μm (**L** and **N**). Each experiment was repeated at least three times. Error bars, mean ± SD, ns: no significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

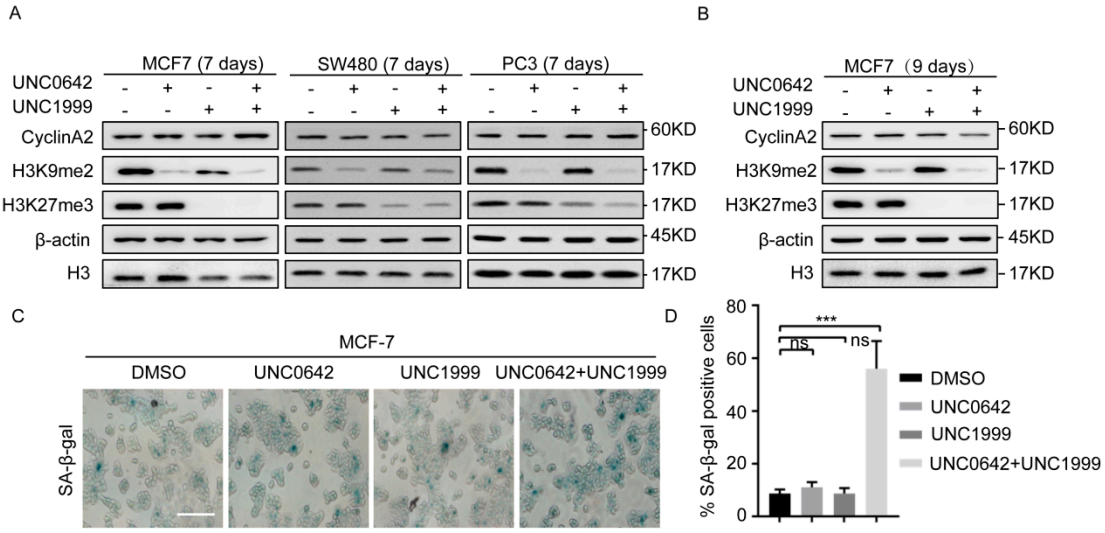


**Figure S5.** Drug sensitivity is related to tumor malignancy. (**A**) Western blot analysis of expression of CyclinA2, H3K9me2 and H3K27me3 in MCF7 cells, SW480 cells and PC3 cells treated with indicated treatments for 7 days. (**B**) Western blot analysis of expression of CyclinA2, H3K9me2 and H3K27me3 in MCF7 cells with indicated treatments for 9 days. (**C-D**) SA-β-Gal staining in MCF7 cells treated with indicated treatments for 9 days. Representative images of SA-β-Gal positive cells were showed (**C**) and the percentage of the SA-β-Gal positive cells was calculated, Scale bar: 100 nm (**D**). Each experiment was repeated at least three times. Error bars, mean ± SD, ns: no significant, $^{***}p < 0.001$.