



APR-246 induces ferroptosis and overcomes cisplatin resistance in ovarian cancer

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Background

- Ovarian cancer (OC)** is the fifth deadliest cancer in women and is predicted to cause nearly 13,000 deaths in the United States in 2022.
- p53** is a well-studied tumor suppressor gene responsible for regulating the cell cycle and inducing apoptosis. Mutations in p53 are therefore associated with increased cell proliferation and aggressive forms of cancer.
- 96% of high-grade serous ovarian cancers express mutations in p53.
- APR-246** is a novel small molecule thiol-reactive drug, thus restoring p53 activity by targeting C277 in the protein's core domain.
- APR-246 is also capable of suppressing the function of thiol-based antioxidants, such as **glutathione (GSH)**, suggesting an ability to induce cell death by increasing intracellular reactive oxygen species (ROS).
- Ferroptosis** is an iron-dependent mechanism of programmed cell death characterized by lipid peroxidation due to the accumulation of ROS which may be combatted with antioxidants, such as GSH.
- The mechanism by which APR-246 induces cell death in ovarian cancer is currently under study with recent data suggesting a link to ferroptosis and GSH deficiency. We have, therefore, examined this effect in p53-knockout cells to exclude p53-dependent apoptotic events.

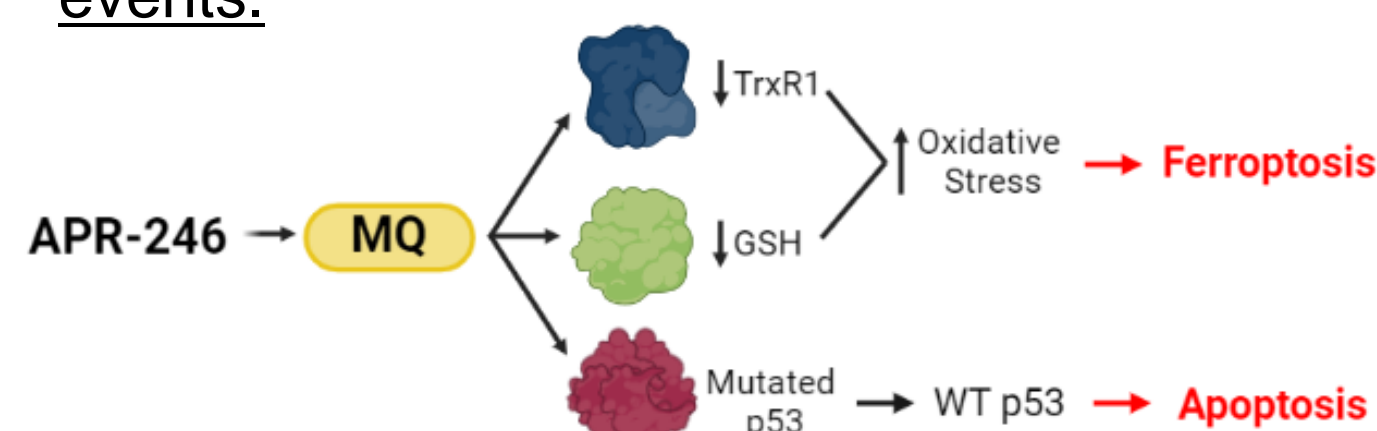
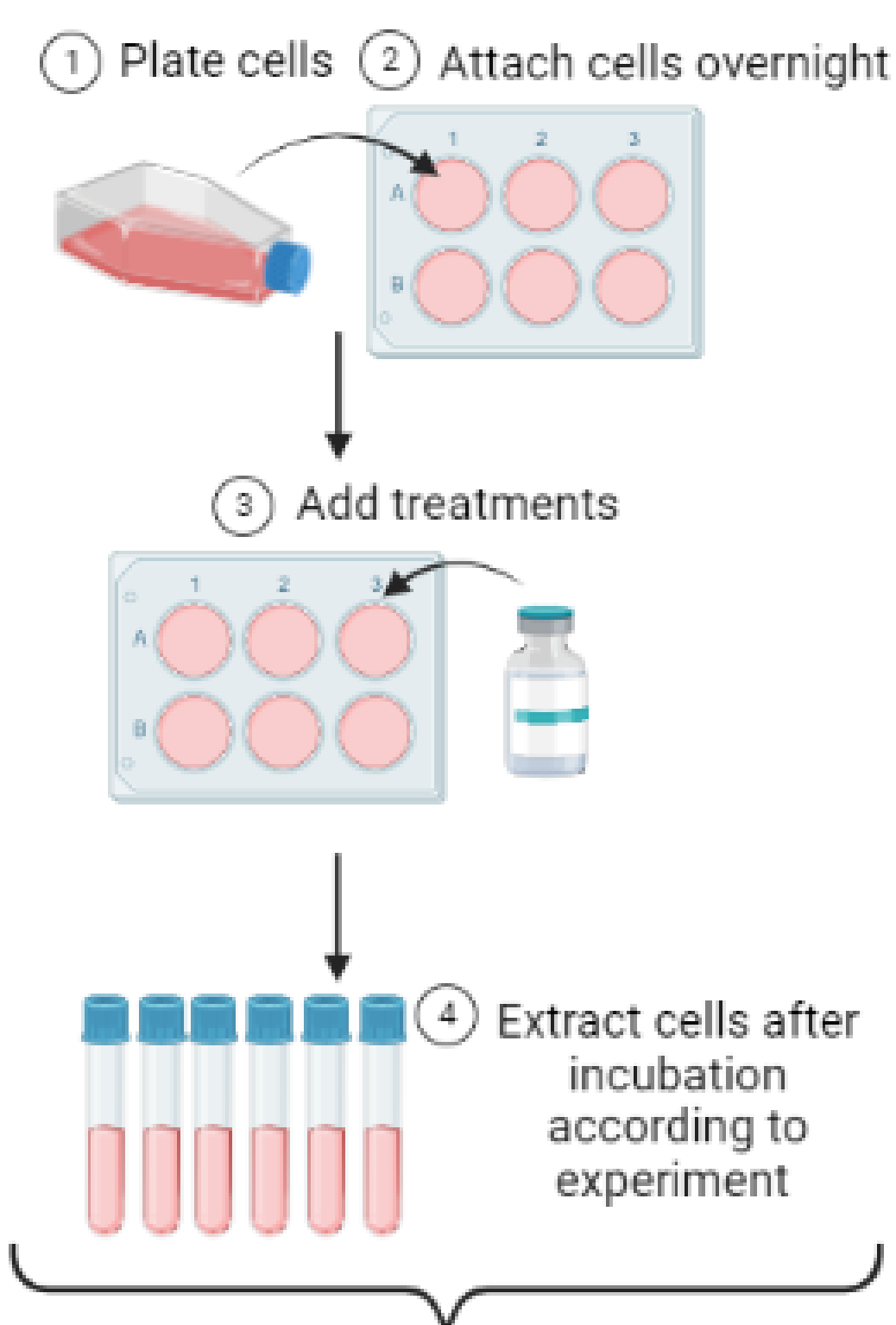


Figure 1. APR-246 is metabolized to methylene quinclidinone (MQ) inside the cell which targets thiol groups. Cell death is caused by ferroptosis due to an increase in oxidative stress and apoptosis due to reactivation of p53.

Methods

A2780 parental and CRISPR-induced p53-knockout (KO) cisplatin (CP)-resistant A2780 cell lines were used.



Cell viability assay via flow cytometry
Lipid peroxidation assay via BODIPY stain
Oxidative stress assay via CellROX probe
GSH concentration assay via luminometer

Results: Cell Death & p53

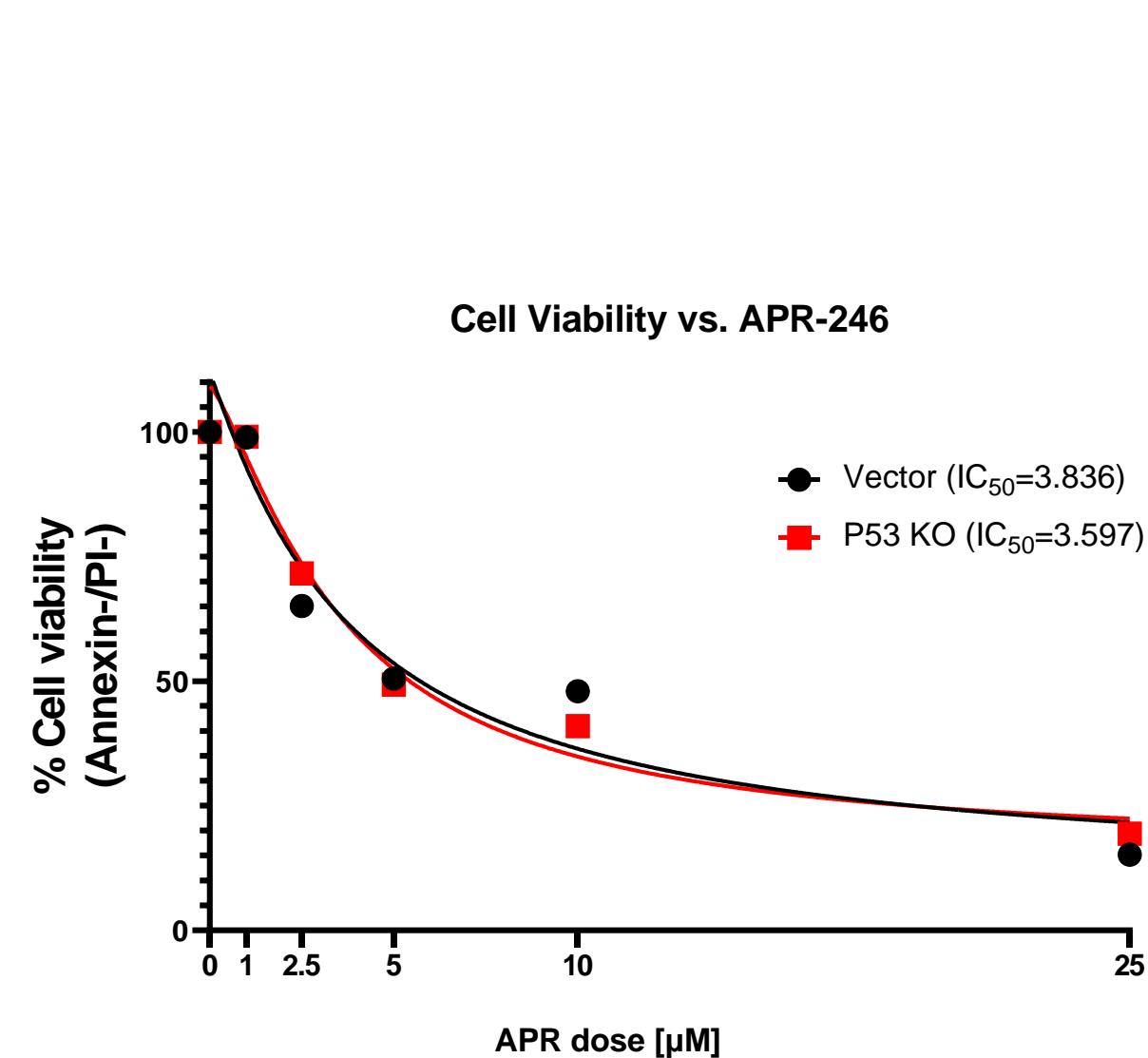


Figure 2. APR-246 induces cell death in ovarian cancer irrespective of p53 status.

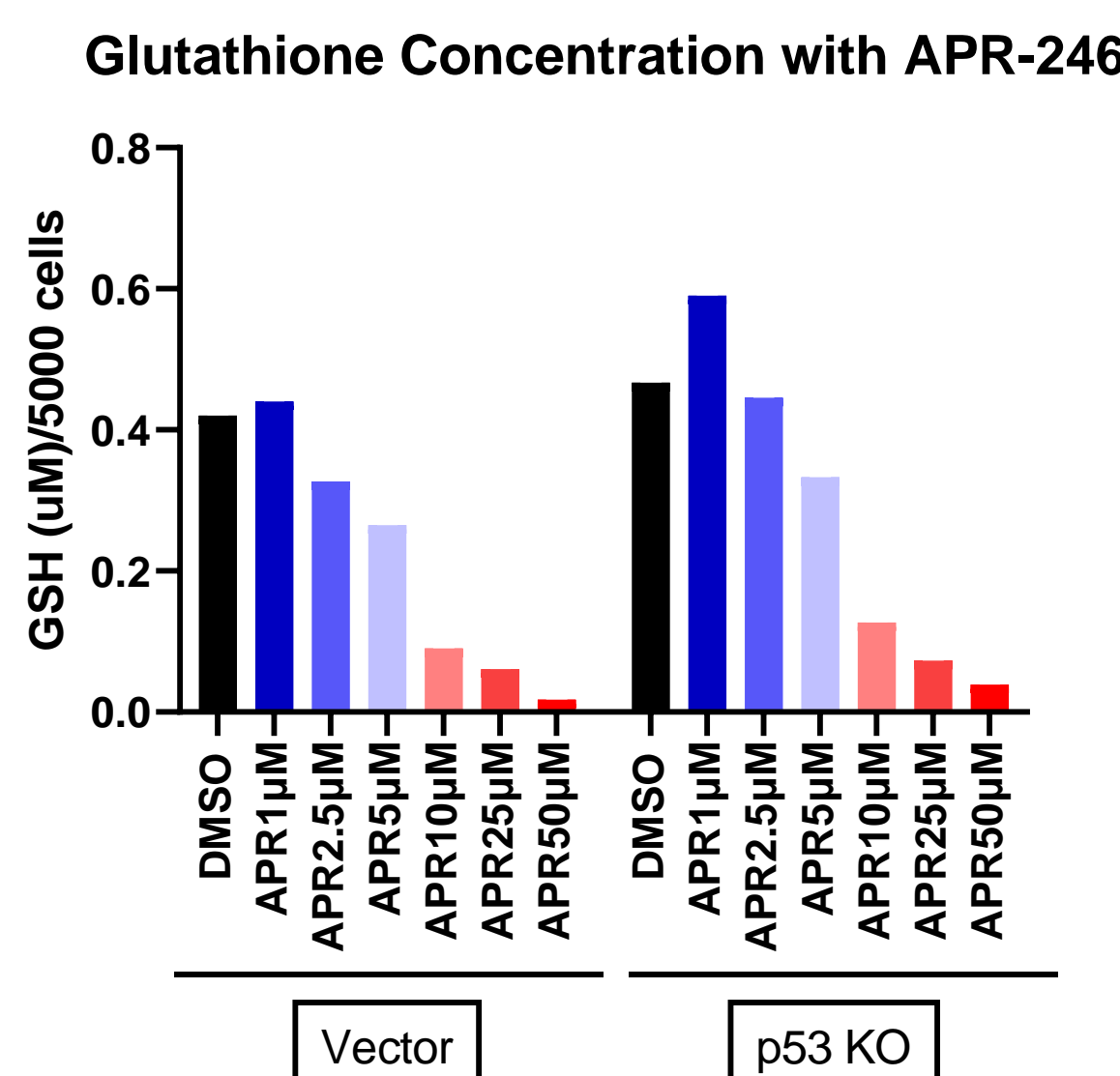


Figure 3. APR-246 results in dose-dependent glutathione depletion.

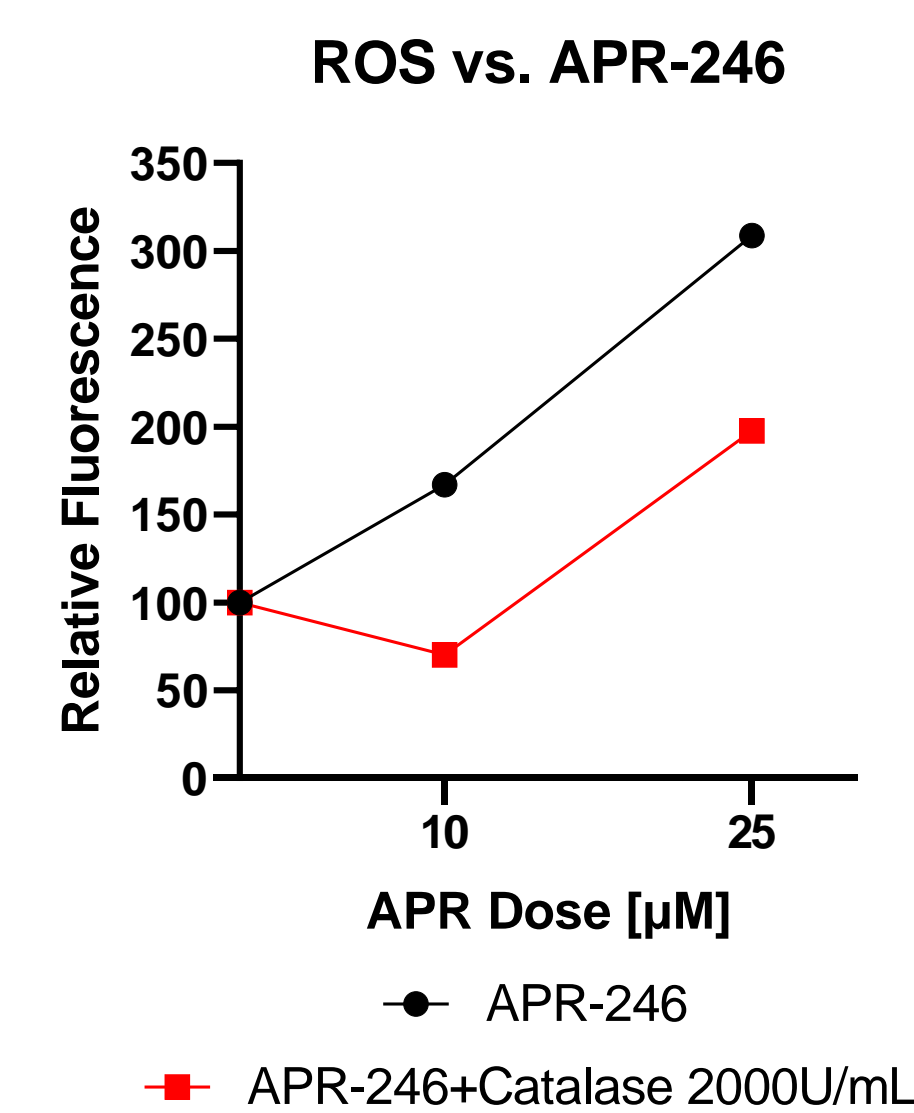


Figure 4. APR-246 increases ROS which was partially rescued by catalase.

Results: Rescue Experiments

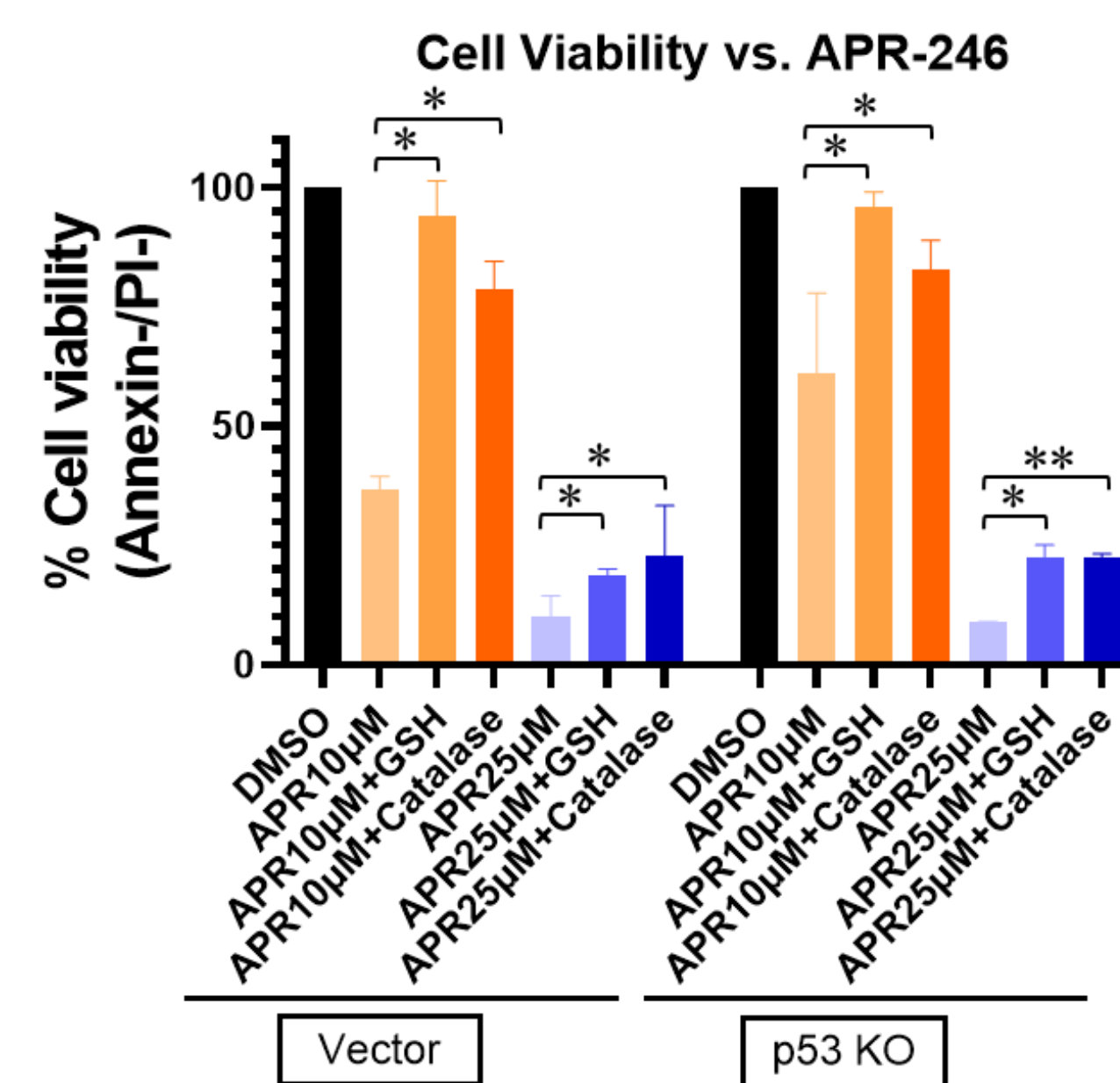


Figure 5. Antioxidants rescue cell death induced by APR-246. n=2, p<0.05*, p<0.01**

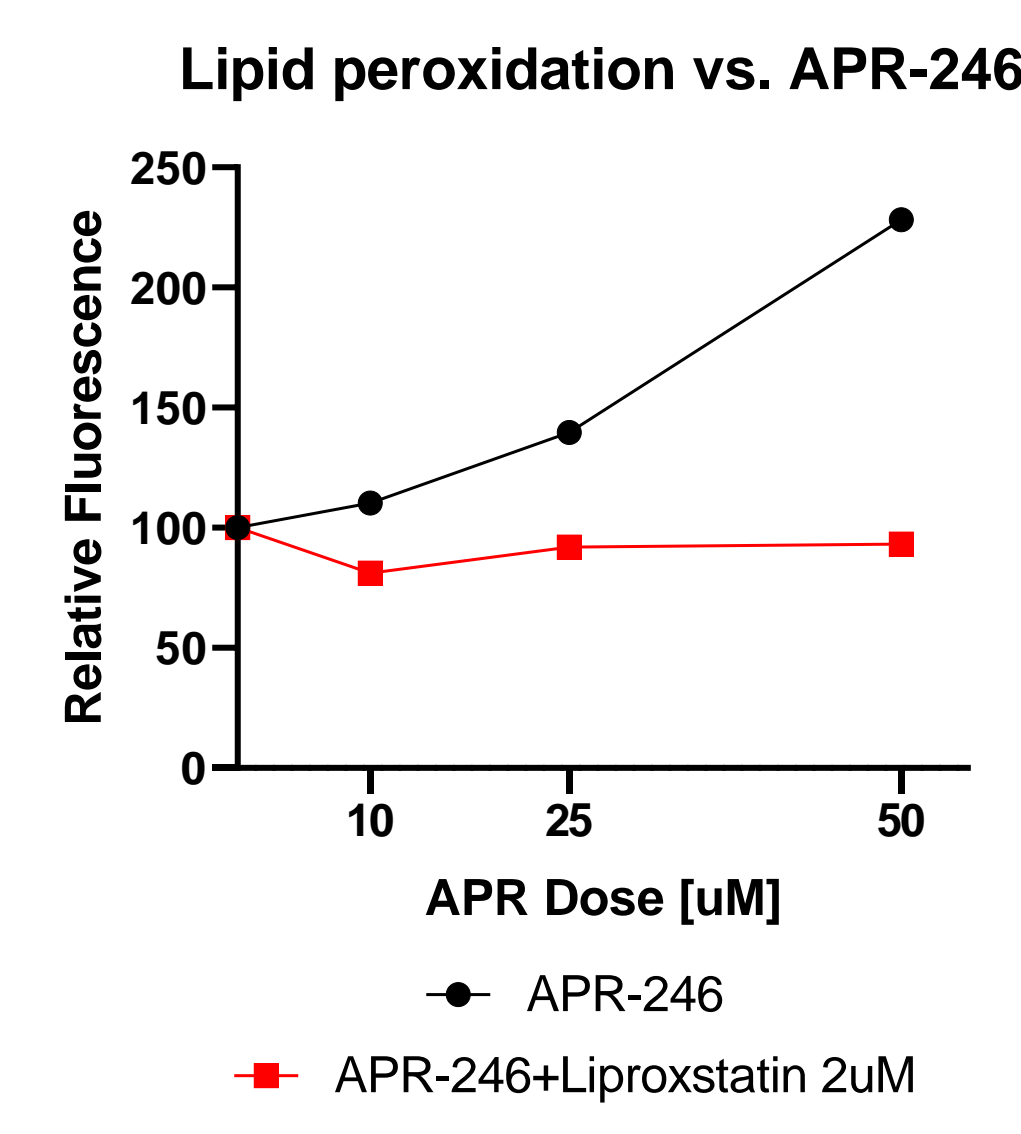


Figure 6. APR-246 increases lipid peroxidation which was completely rescued by liproxstatin.

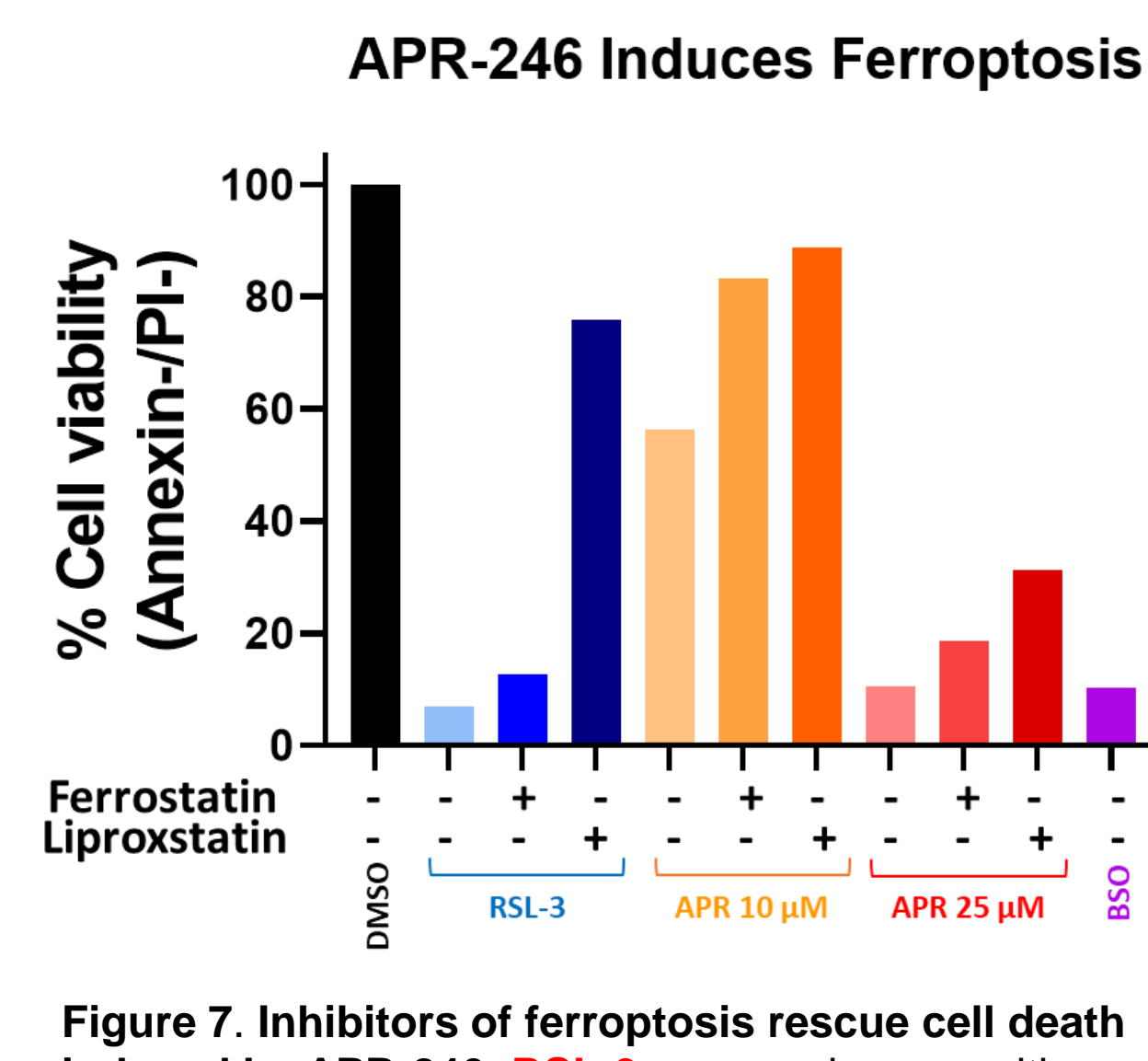


Figure 7. Inhibitors of ferroptosis rescue cell death induced by APR-246. RSL-3 was used as a positive control (ferroptosis inducer). Buthionine sulfoximine (BSO) inhibits GSH synthesis by targeting γ-glutamylcysteine synthetase.

Results: APR-246 & Cisplatin

15-30% of patients with OC develop resistance to platinating agents, making re-sensitization imperative. To study the effects of platinum, we used parental and cisplatin resistant A2780 cells.

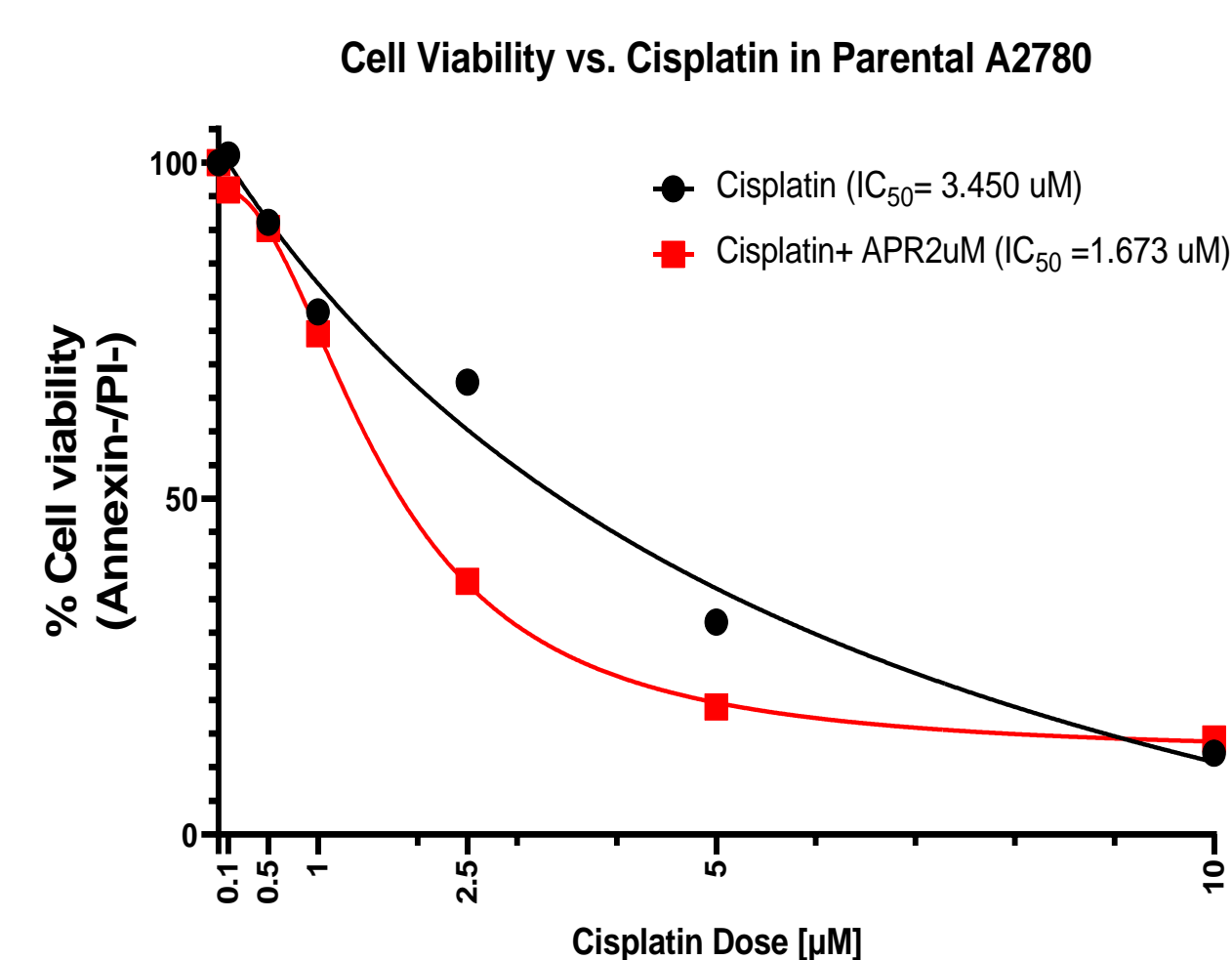


Figure 8. APR-246 and cisplatin synergistically increase cell death in the A2780 parental cell line.

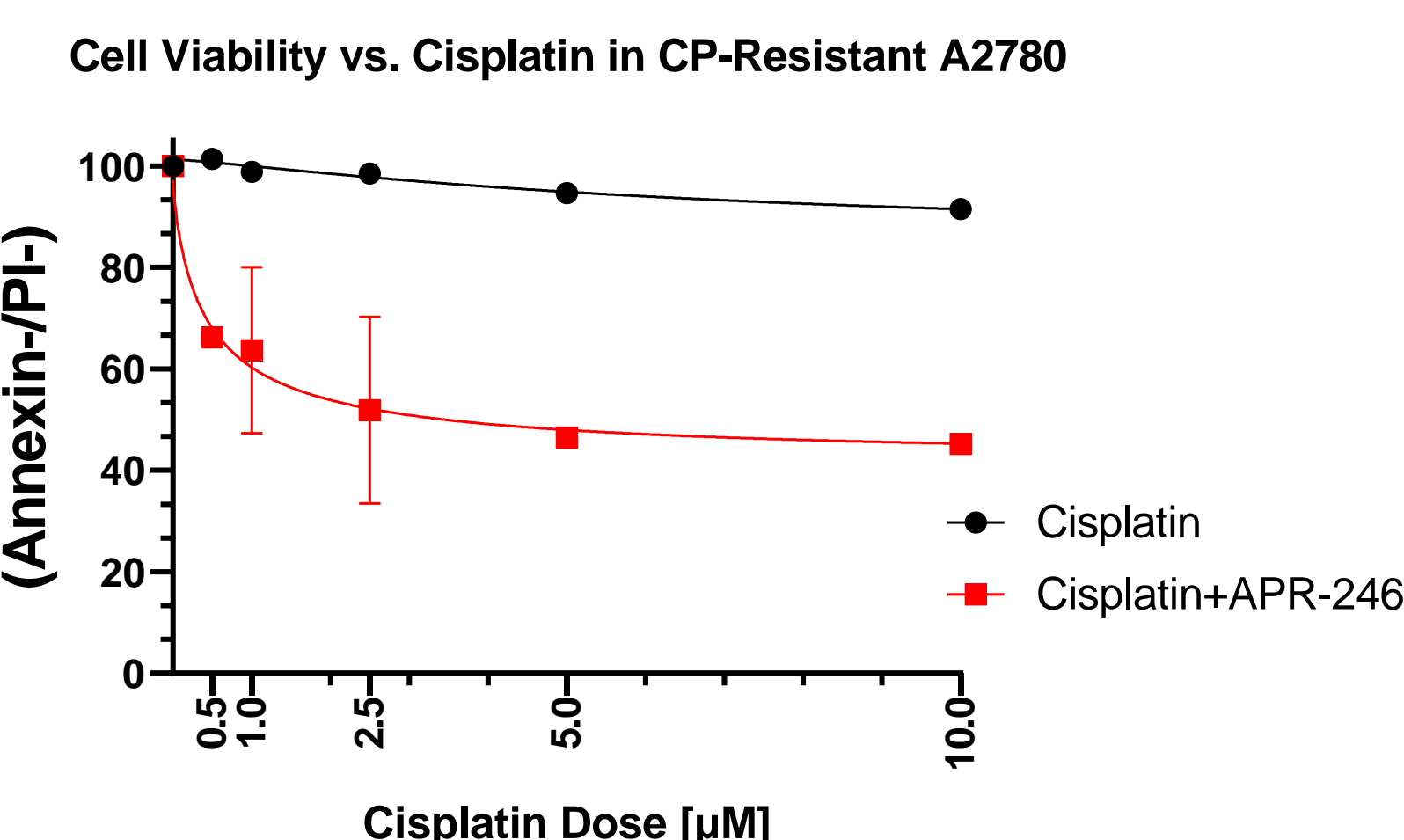


Figure 9. APR-246 overcomes cisplatin resistance in the A2780 cisplatin-resistant cell line. n=2, p=0.0052.

Results: Nucleotide Pool Analyses

Using PCA analyses, we were able to quantify the nucleotide concentration in all four A2780 cell lines according to two different doses of APR-246.

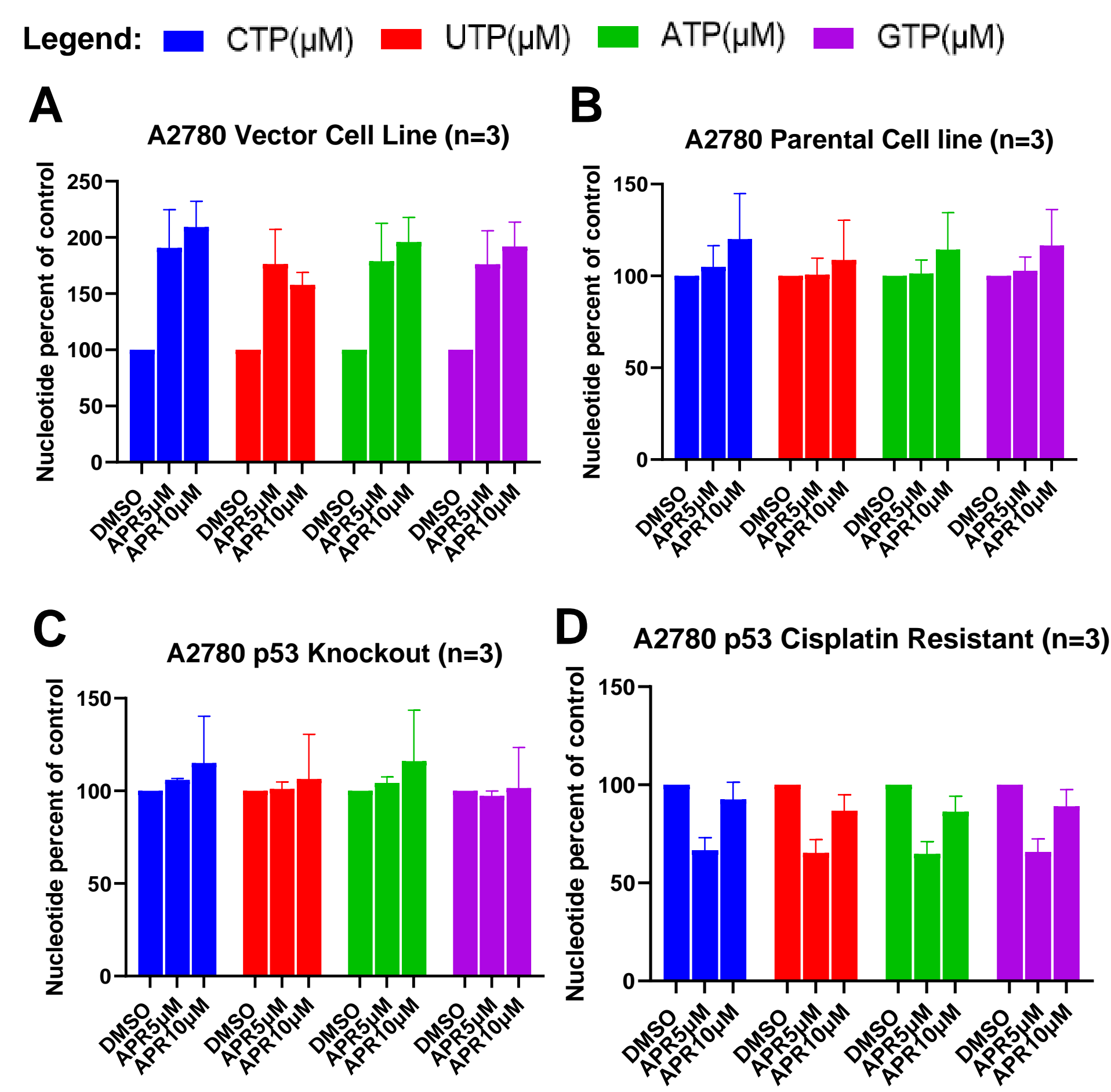


Figure 10. There are no detectable changes in nucleotide pool across all A2780 cell lines after treatment with APR-246. Interestingly, (C) the p53 KO and (D) cisplatin resistant cells lines have a higher nucleotide pool compared to the (A) vector and (B) parental cell lines, respectively.

Conclusions

- APR-246 induces GSH depletion associated with ferroptosis in ovarian cancer cell line A2780 as evidenced by increased lipid peroxidation and oxidative stress, which is rescued by ferroptosis inhibitors and antioxidants, respectively.
- Catalase can rescue ferroptosis by mitigating the oxidative stress necessary for lipid peroxidation which is induced by APR-246.
- APR-246 has a synergistic effect on the A2780 parental cell line when combined with cisplatin, causing a 52% decrease in IC₅₀.
- APR-246 sensitizes the A2780 cisplatin resistant cell line to cisplatin as evidenced by an increase in cell death.
- Future experiments will aim to replicate and further confirm the occurrence of ferroptosis with the use of iron chelators.

Acknowledgements

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References

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