



Metabolic constraints limit DNA repair following radiation exposure

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Abstract ID: 24091

Background

- Mutations in the gene Kelch-like ECH-associated protein 1 (KEAP1) are associated to resistance to radiotherapy resulting in greater local recurrence and poorer patient outcomes.
- Mutations in KEAP1 lead to Nuclear Factor (erythroid-derived) 2-like Factor (Nrf2) nuclear translocation where it acts as a transcription factor promoting the formation of antioxidant proteins.
- Tumors harboring KEAP1 mutations rely heavily on the amino acid glutamine, which is processed by the enzyme Glutaminase-1 (GLS1).
- Disrupting glutamine metabolism in KEAP1 mutant cells and tumors using small molecule inhibitors has shown promise as a radiosensitizing strategy.

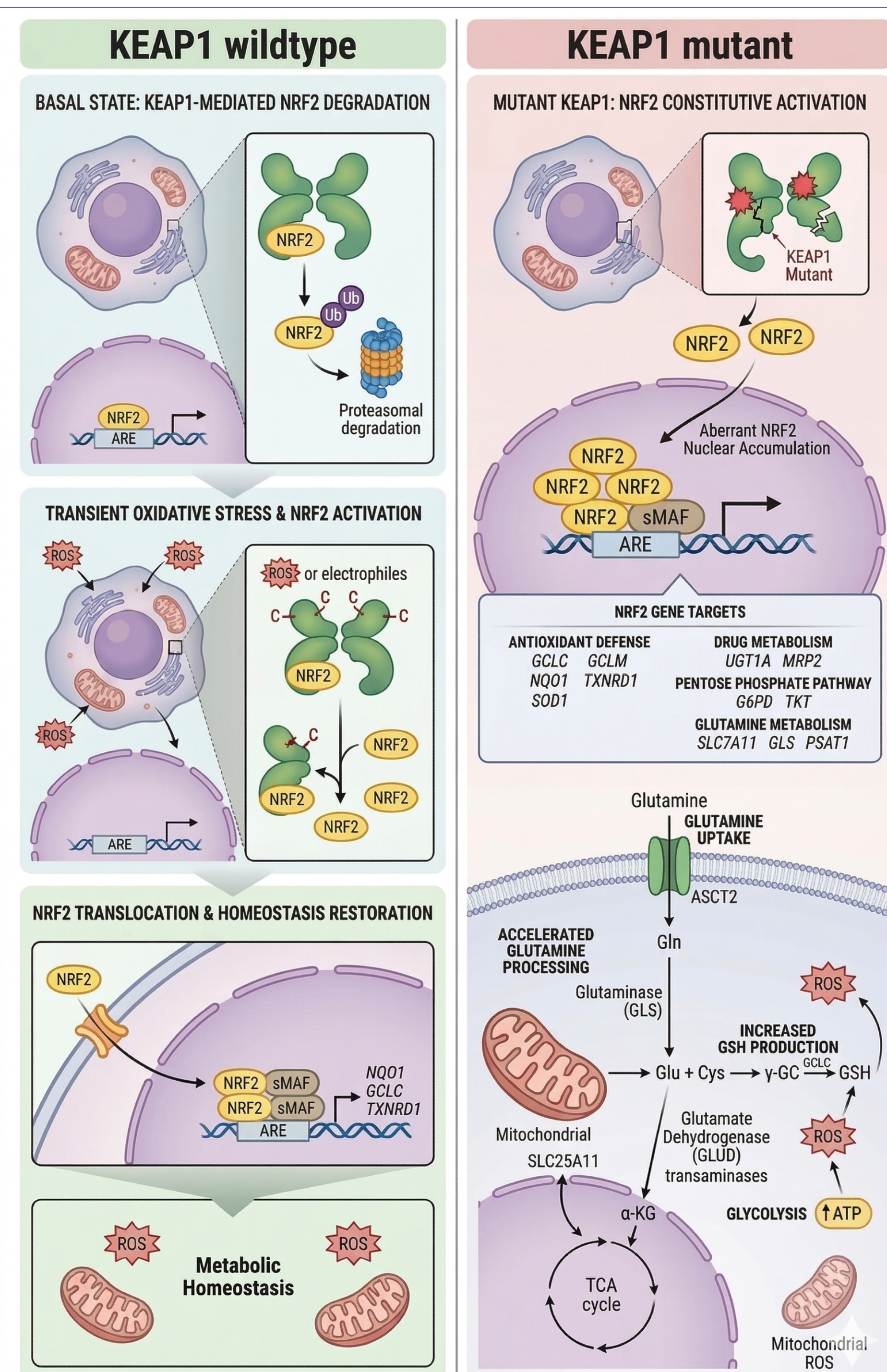


Fig 1: Cell metabolism in normal and KEAP1 mutant lung cancers. (Left) In KEAP1 wild type cells, NRF2 is bound by KEAP1 and targeted for proteasomal degradation. If the cell encounters oxidative stress, KEAP1 undergoes conformational change, releasing NRF2 where it promotes a transcriptional response to overcome oxidative stress. (Right) In KEAP1 mutant cells, NRF2 is constitutively activated, leading to a fundamentally altered metabolic state, where glutamine metabolism is increased to fuel antioxidant production and maintain TCA cycle anaplerosis.

Acknowledgments

Funding: Dept of Defense, MD Anderson IRG, AAPM seed grant, CPRIT and CPRIT student scholarships.

Mechanism of radiosensitization induced by GLS1 inhibition

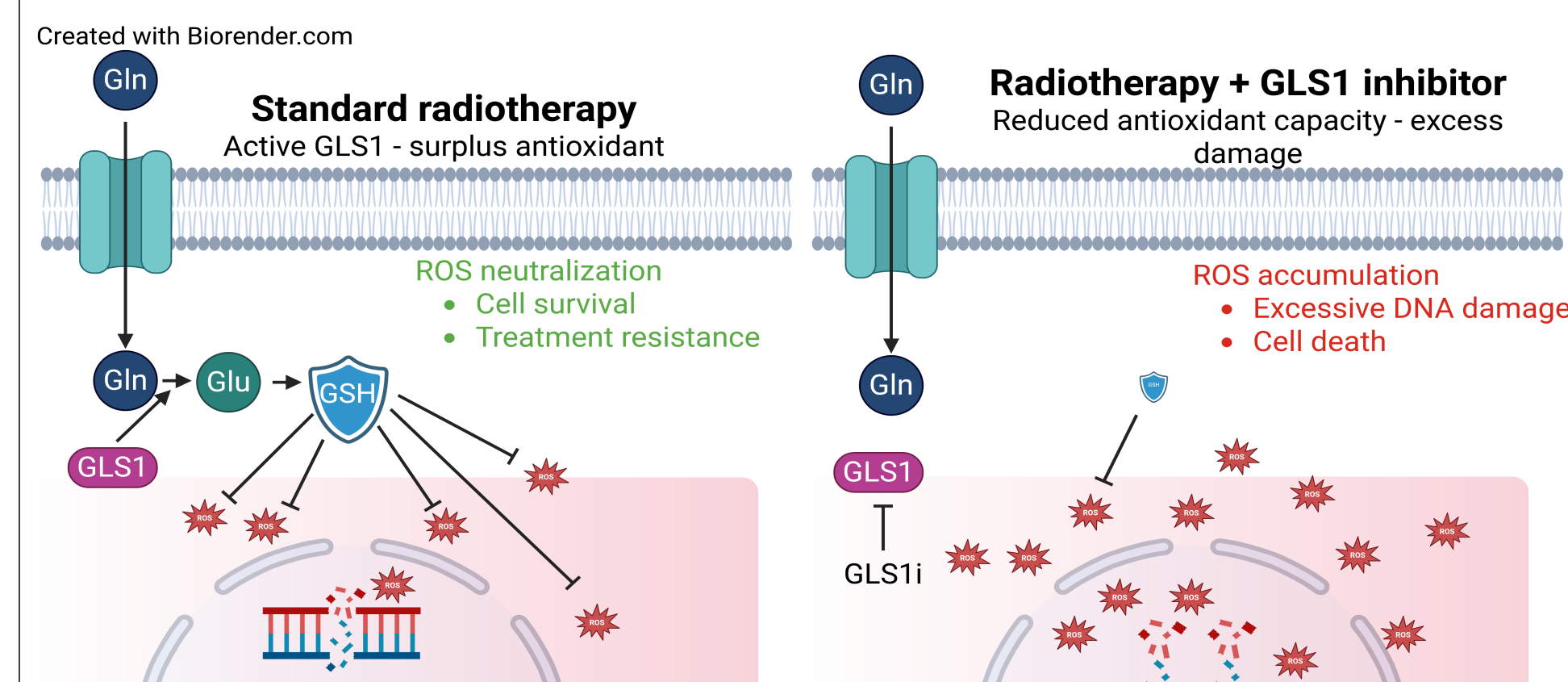


Fig 2: (Left) In the setting of mutant KEAP1 cells, glutamine metabolism is increased facilitating increased levels of the antioxidant glutathione (GSH) which neutralizes radiation induced ROS. (Right) In KEAP1 mutant, cells with blocked GLS1, glutamate is reduced leading to reduced GSH formation and the inability to neutralize radiation-induced ROS.

Results

Does GLS1 inhibition radiosensitize lung cancer cells?

Using the GLS1 inhibitor, IACS-6274, we performed clonogenic survival assays with lung cancer cell lines H460, A549 (KEAP1 mutants), H1299 and KEAP1 silenced H1299 (H1299-Keap1). We also performed long term proliferation assays (7 days) with various supplements to identify metabolites critical for cell survival. Our results show KEAP1 mutant cells can be radiosensitized using the GLS1 inhibitor IACS-6274. We also show that the supplements pyruvate and α -ketoglutarate (α KG) can overcome the effects of IACS-6274.

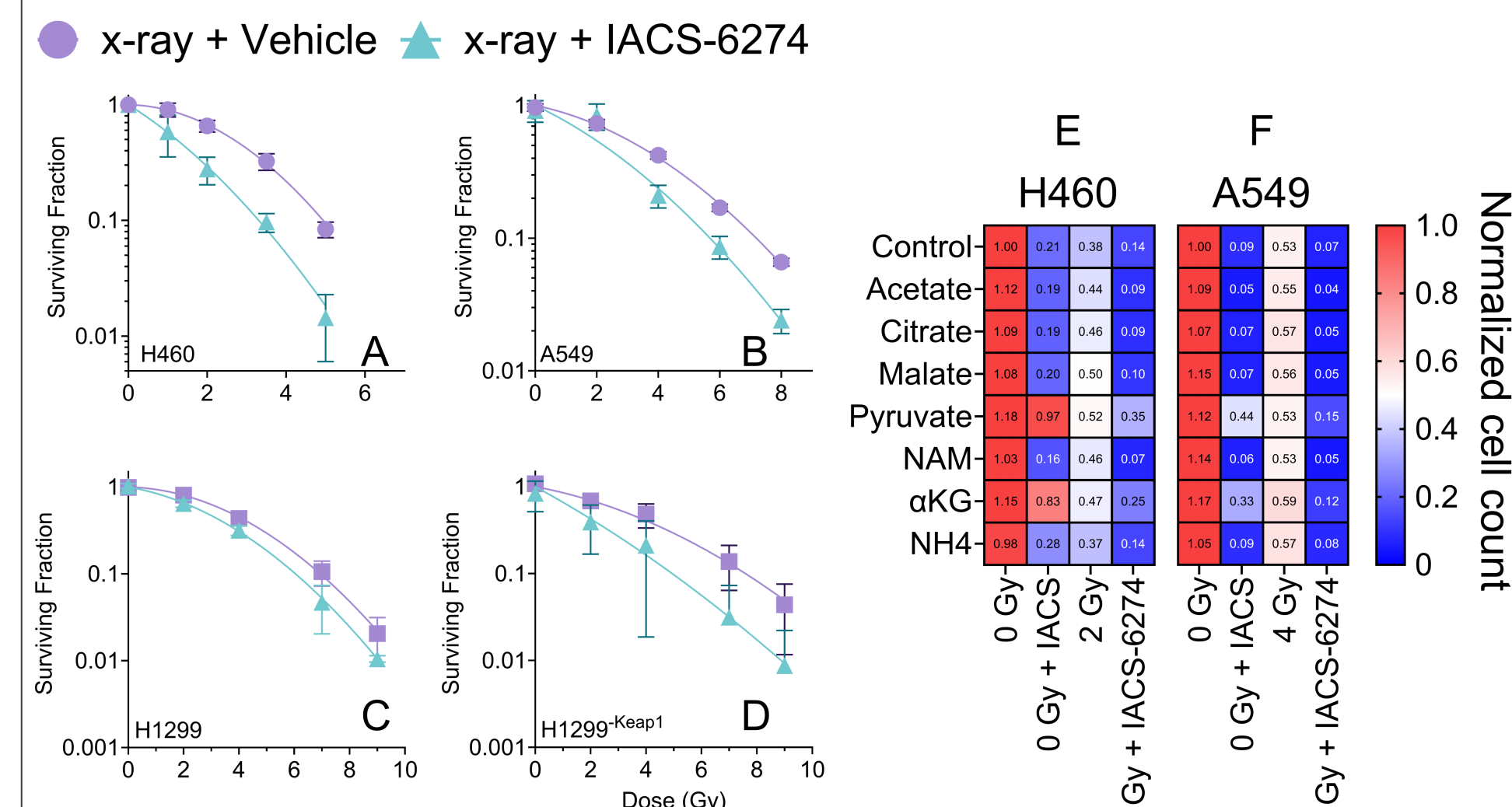


Fig 3: Clonogenic survival in (A) H460, (B) A549, (C) H1299, (D) H1299-Keap1. Long term viability in (E) H460 and (F) A549. Cells were treated with 1 μ M IACS-6274 6 hours prior to irradiation.

Does GLS1 inhibition, alone or in combination with radiation modulate mitochondrial status and function?

We assessed mitochondrial mass and membrane potential using MitoTracker Green™ (MTG) and Tetramethylrhodamine (TMRM), respectively. We quantified the ratio of mass to potential. Furthermore, we quantified intracellular and mitochondrial calcium using Fluo-3 AM (5 μ M) and Rhod-2 AM (1 μ M). Mitochondrial superoxide was measured using mitoSOX™. ATP was measured using ATP Determination Kit (Molecular Probes, A22066).

Cont. Does GLS1 inhibition, alone or in combination with radiation modulate mitochondrial status and function?

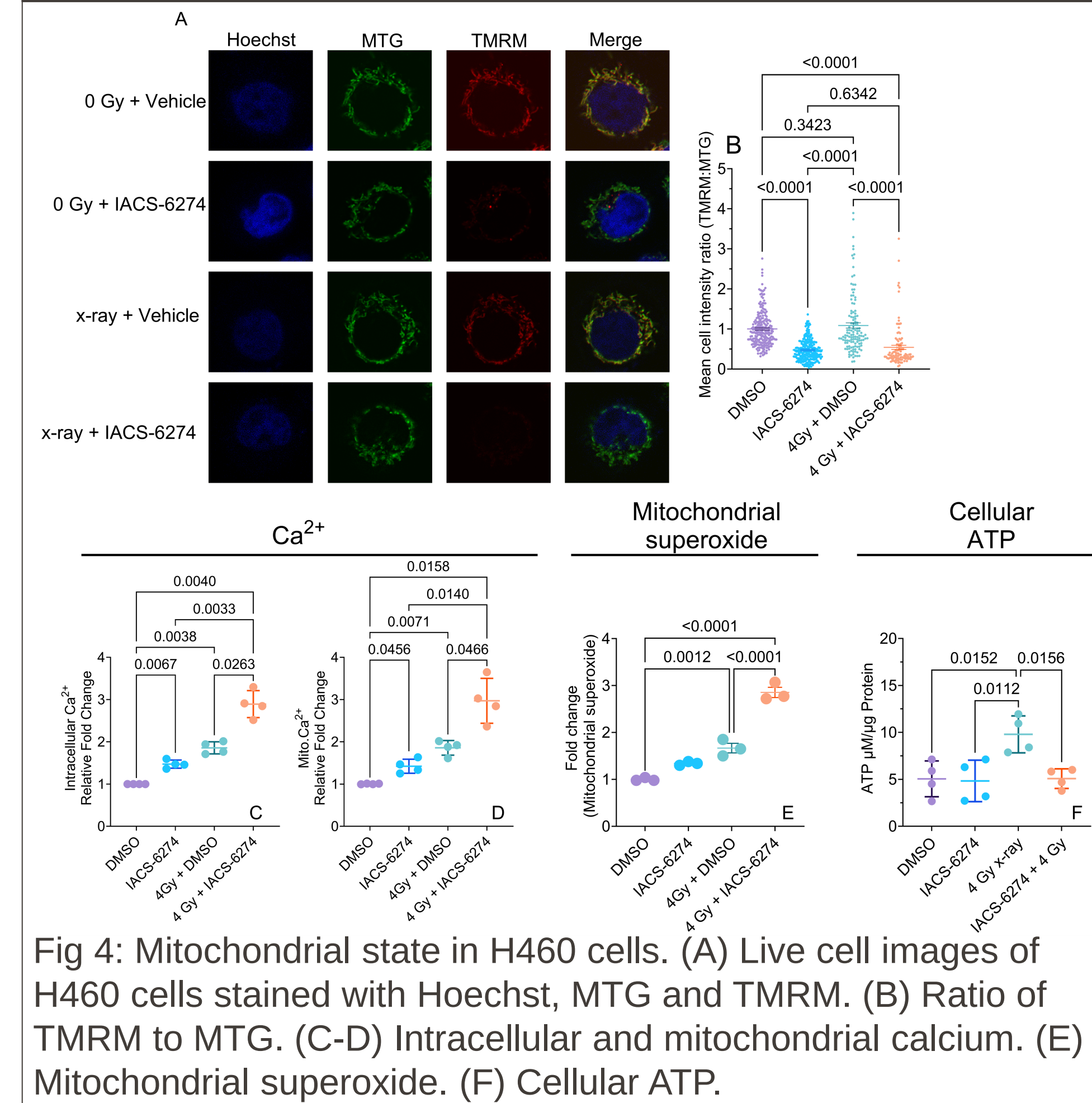


Fig 4: Mitochondrial state in H460 cells. (A) Live cell images of H460 cells stained with Hoechst, MTG and TMRM. (B) Ratio of TMRM to MTG. (C-D) Intracellular and mitochondrial calcium. (E) Mitochondrial superoxide. (F) Cellular ATP.

Does GLS1 inhibition increase radiation induced DNA damage?

We measured total DNA damage using the alkaline comet assay in H460 cells. Cells were treated with IACS-6274 (1 μ M) to inhibit GLS1. Cells were irradiated and analyzed immediately and 24 hours after irradiation. In IACS-6274 treated cells, DNA damage was greater after irradiation. DNA damage was still increased at 24 hours after irradiation.

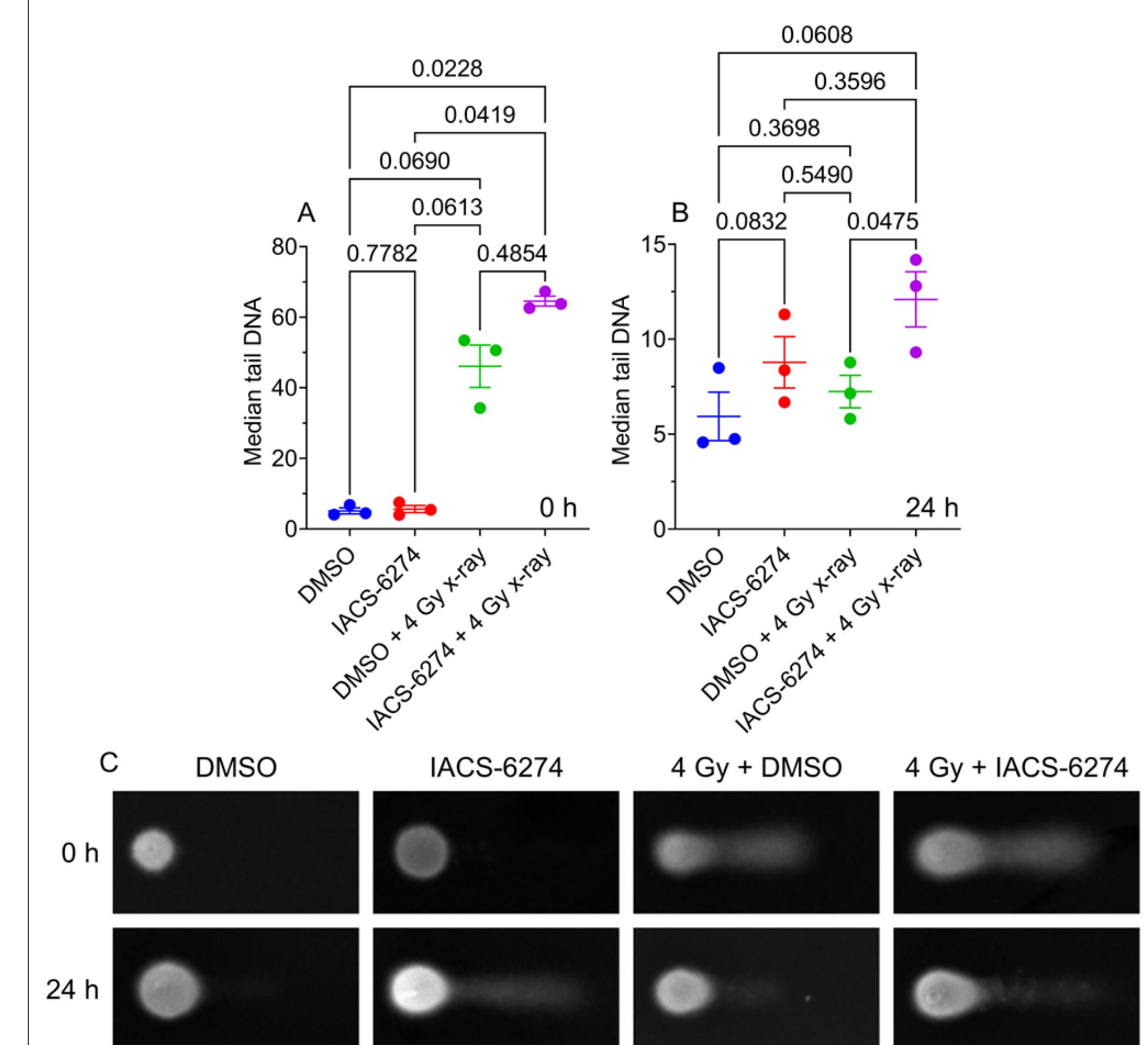
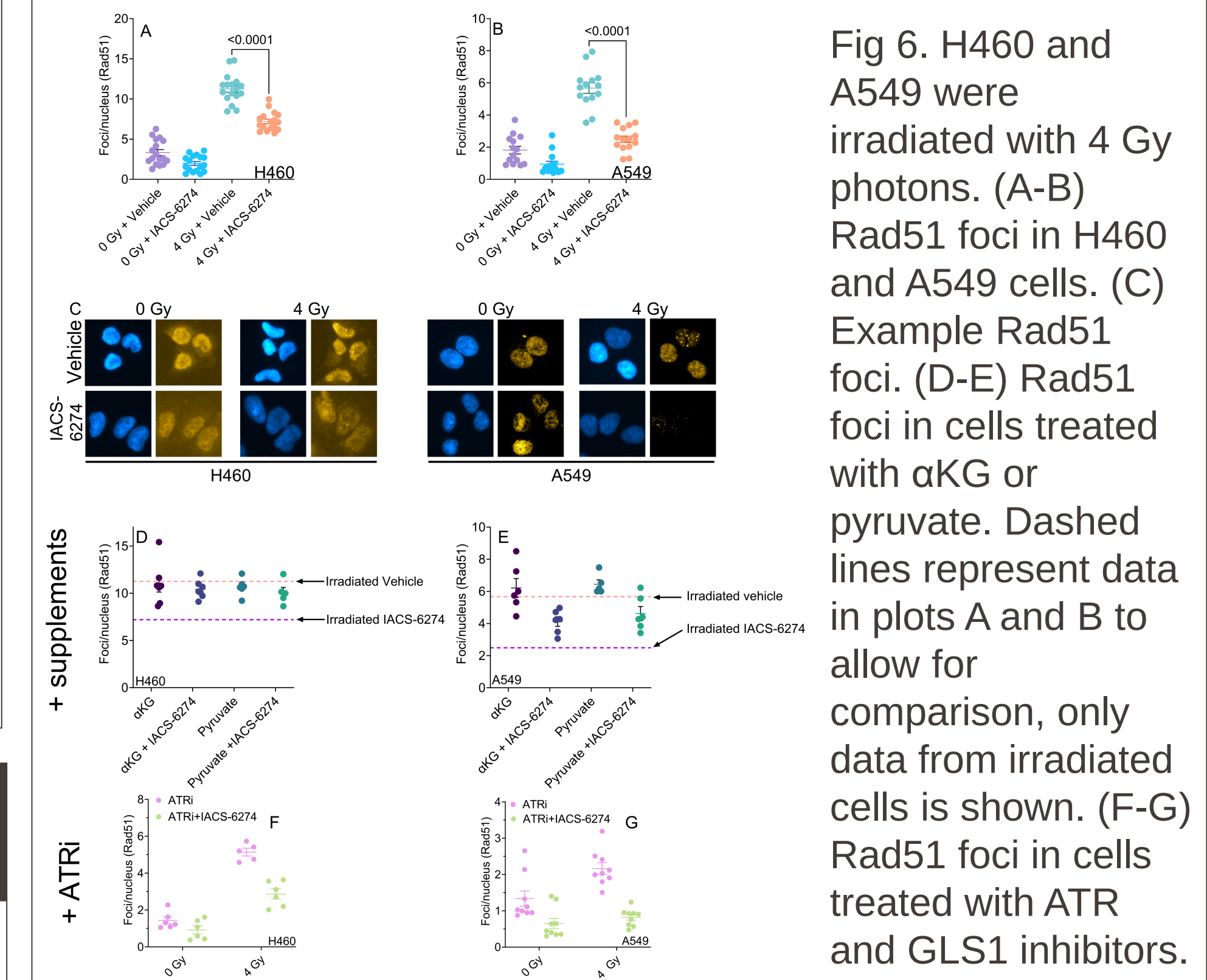


Fig 5: Median percentage tail DNA in H460 cells. (A) H460 cells analyzed 0 h hours after irradiation. (B) H460 cells analyzed 24 hours after irradiation. (C) Example comet images.

Does GLS1 inhibition modulate radiation-induced DNA damage repair?

We quantified DNA damage repair foci, specifically Rad51 foci which is a surrogate marker for the homologous recombination (HR) repair pathway. GLS1 inhibition with IACS-6274 led to significant reductions in Rad51 foci numbers. Supplementation with TCA metabolites such as α KG and pyruvate could restore foci formation, especially in H460 cells. Finally, we quantified the effect of combining ATR inhibition with GLS1 inhibition to further disrupt HR. Combined treatment led to further reductions in Rad51 foci in irradiated cells.



Does GLS1 inhibition synergize with DNA repair inhibitors because of its effects on HR?

We performed viability assays in H460 cells using CellTiter-Glo™ according to manufactures instructions. After 72 hours, viability was measured in cells with various concentrations of inhibitors of either PARP (Olaparib), ATR (AZD6738) or ATM (AZD1390). Synergy was seen with all combinations (>10) with ATR being the most effective combination. Data were analyzed with Synergyfinder.org.

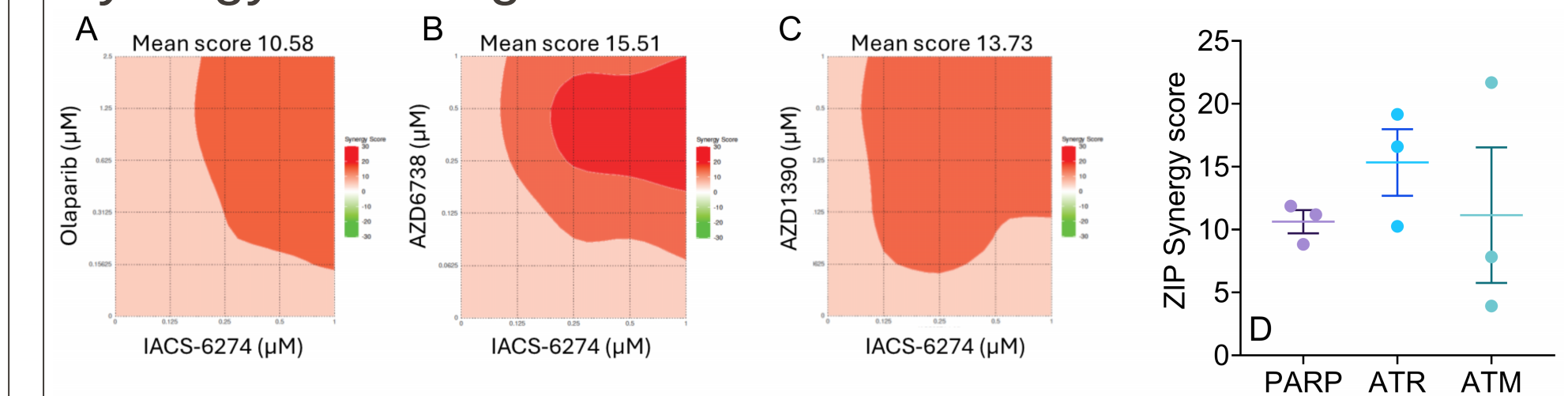


Fig 7: Synergy with (A) PARP, (B) AZD6738 and (C) ATM combined with IACS6274. D. Mean synergy score.

Discussion

- KEAP1 mutant cells are sensitized to radiation by GLS1 inhibition.
- IACS-6274 + radiation disrupts mitochondrial integrity (increased superoxide + Ca^{2+} and ATP response).
- Total DNA damage is increased with IACS-6274 and radiation.
- GLS1 inhibition compromises HR, that can be rescued with supplementation of TCA metabolites.
- Combining GLS1i with DNA repair inhibitors or in patients with defects in DNA repair proteins could be a therapeutic opportunity.