Targeting GPX8 enhances the efficacy of chemotherapy and radiation in Colorectal cancer

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Introduction

- Colorectal cancer (CRC) is the second leading cause of cancer related deaths worldwide (1).
- 50% of metastatic CRC cases do not respond to the conventional therapies such as chemotherapy and radiotherapy (2).
- The need to discover biomarkers to identify patients who could benefit from chemotherapy and radiation is of utmost importance.
- To ensure survivability, CRC cells maintain equitable ROS levels by expressing antioxidant genes, such as the Glutathione peroxidase (GPX) family (3).
- GPX2 is a gut specific antioxidant which is frequently overexpressed in CRC however, 32% of CRC cases are observed to have low GPX2 expression (OTL, unpublished data).
- Interestingly, when GPX2 high and low CRC cells were subjected to escalating dosages of radiation, the low GPX2 cells did not exhibit a higher sensitivity to radiation dosages.
- **Therefore, we sought to investigate the compensators for GPX2 loss in the CRC landscape.**

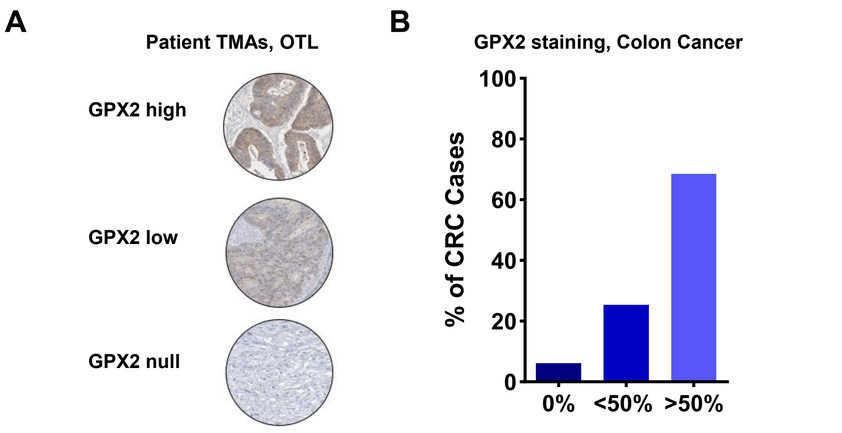

Figure 1. Characterization of GPX2 loss in stage III CRC cases and its impact on radiation response. (A-B) 197 stage III CRC cases were stained for GPX2 expression. 30% of CRC cases were observed to have lost GPX2. GPX2 **(C)** high and **(D)** low CRC cell lines were subjected to radiation. 72h following treatment apoptosis was determined by PI staining and FACS analysis.

Project aims

- **To investigate the compensators of GPX2 loss in CRC**
- **To determine the role of GPX8 in regulating CRC cell viability and basal ROS levels in CRC cells**
- **To investigate the impact of targeting the compensator in sensitizing CRC to the conventional treatments**

Identifying compensators of GPX2 loss in CRC

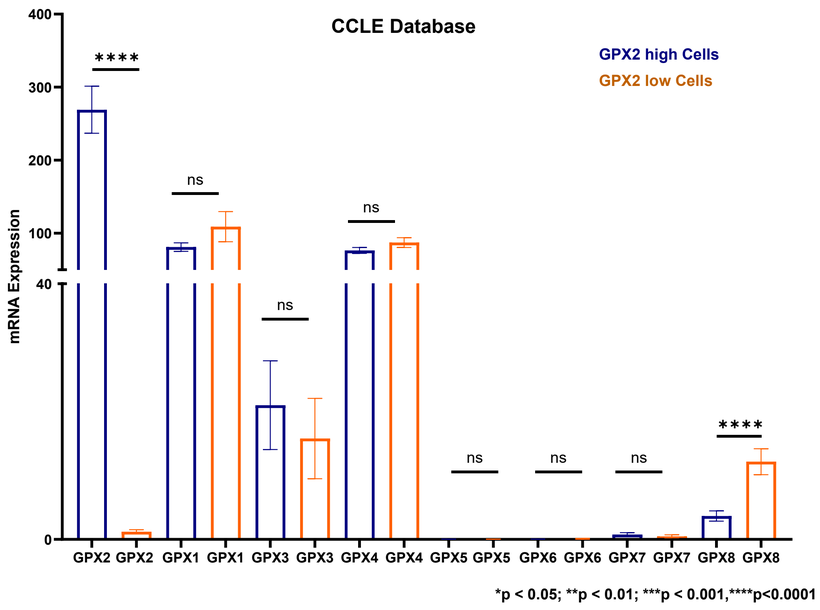

Figure 2. Bioinformatic analysis reveals upregulation of GPX8 in low GPX2 CRC cells. The CCLE database highlights increased expression of GPX8 in CRC cell lines with low GPX2 expression.

CRC cells are functionally dependent on GPX8

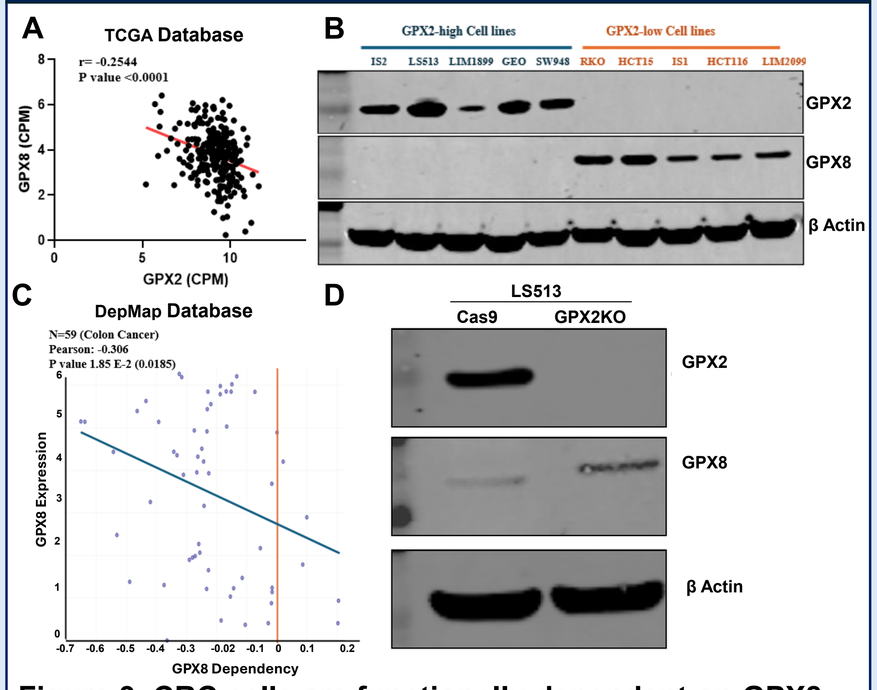

Figure 3. CRC cells are functionally dependent on GPX8. **(A)** Inverse correlation between GPX2 and GPX8 in patient tumours analysed by TCGA database. **(B)** Western blotting analysis on CRC cell lines supports the findings from the TCGA database**. (C)** The DepMap database highlights that CRC cells that express GPX8 are functionally dependent on it. **(D)** GPX2 KO in CRC cells upregulates GPX8 expression.

Investigating GPX2 and GPX8 expression location

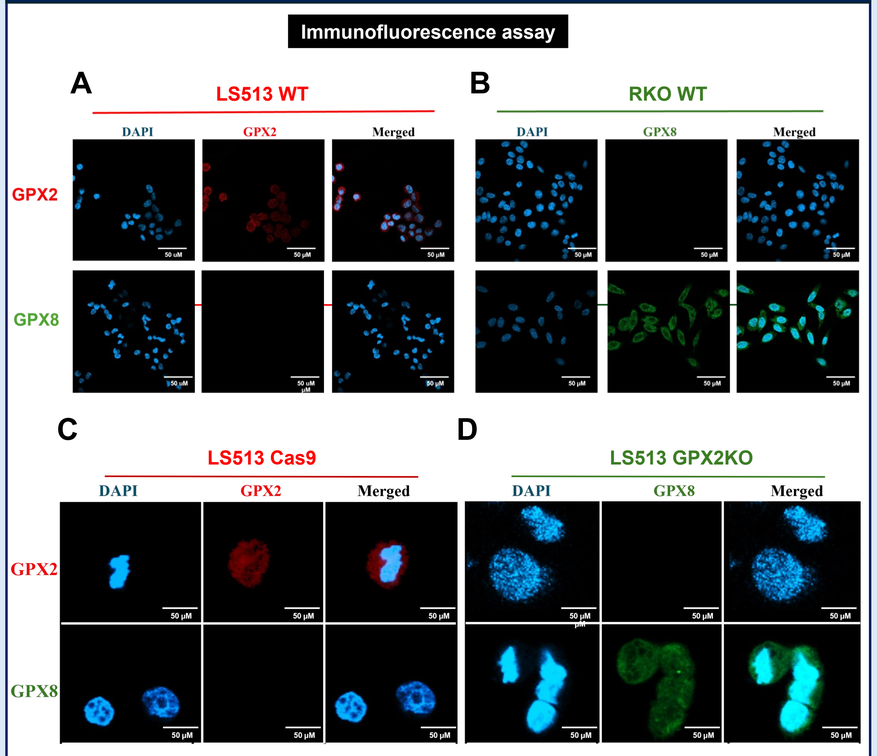

Figure 4. GPX2 and GPX8 have a cytosolic expression location. (A) LS513**, (B)** RKO, **(C)**, LS513 Cas9 and **(D)** LS513 GPX2KO cells were probed with GPX2 and GPX8 and subjected to confocal microscopy.

Investigating basal ROS levels following GPX8 KD

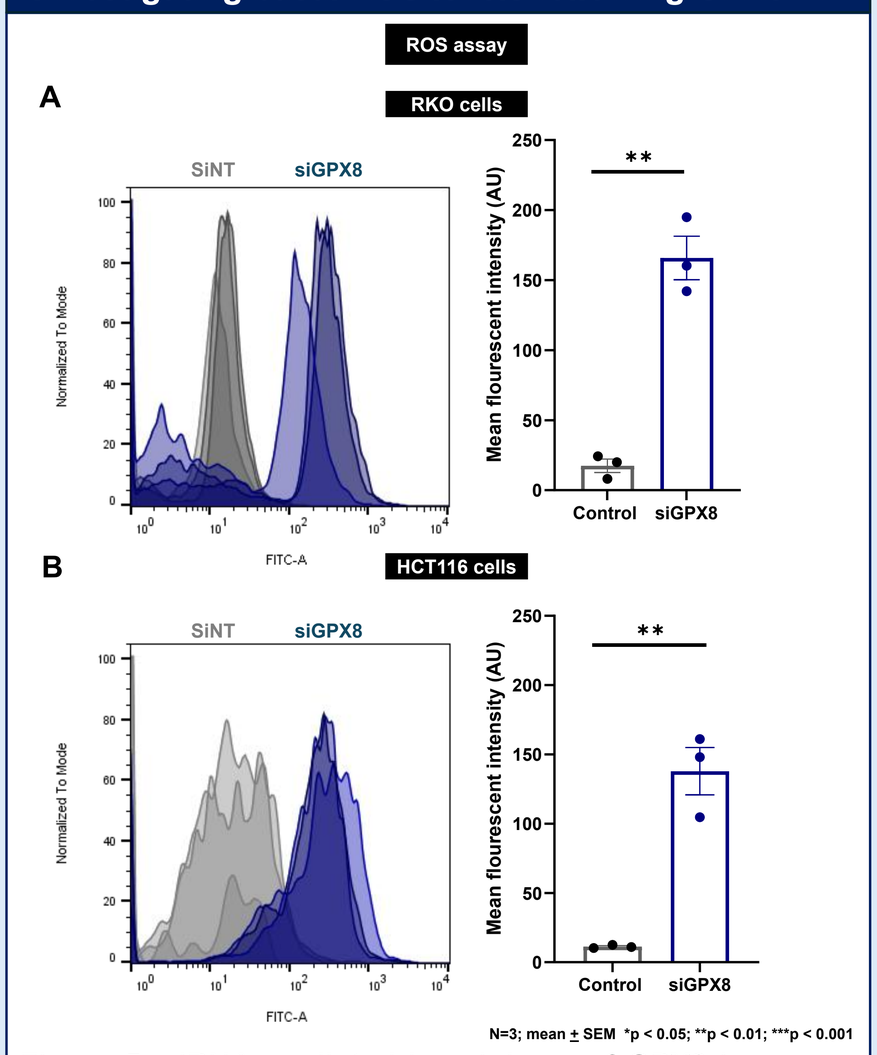

Figure 5. siRNA mediated knockdown of GPX8 increases basal ROS levels in CRC cells. (A) RKO and **(B)** HCT116 cells were treated with siNT or GPX8 siRNAs for 72h and stained with 20μM DCFDA for 30 min. Following this, Basal ROS levels was determined by flow cytometry.

GPX8 loss reduces clonogenicity of CRC cells

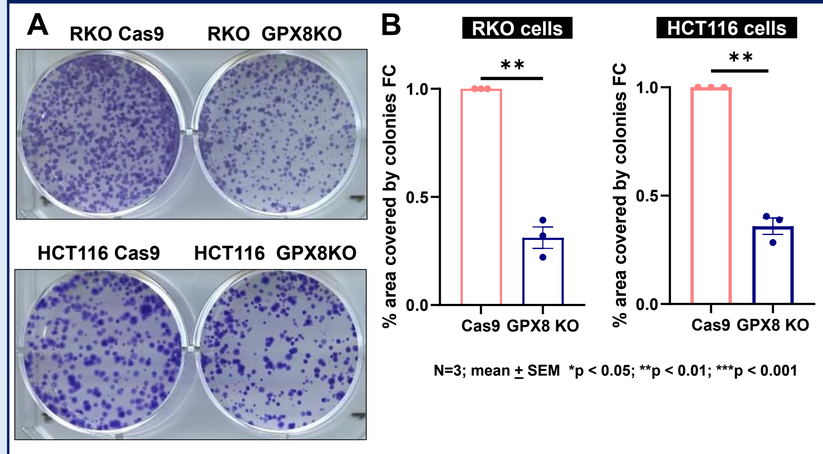

Figure 6. GPX8KO decreases clonogenicity of CRC cells. (A-B) Colony formation assay of RKO and HCT116 Cas9 and GPX8 CRISPR KO cells showed decreased clonogenicity after 14 days.

GPX8 loss sensitises CRC cells to Radiation

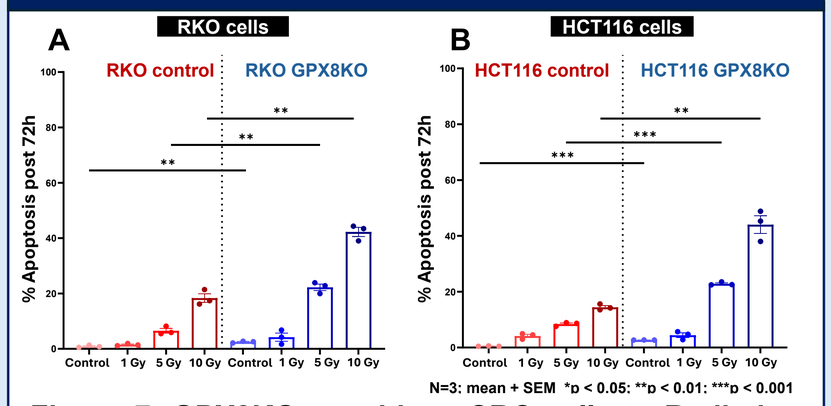

Figure 7. GPX8KO sensitises CRC cells to Radiation treatment. (A) RKO and **(B)** HCT116 GPX8 KO and control cells subjected to 1 Gy or 5 Gy or 10 Gy dosages of radiation. 72h following treatment apoptosis was determined by PI staining and FACS analysis.

GPX8 loss sensitises CRC cells to Chemotherapy

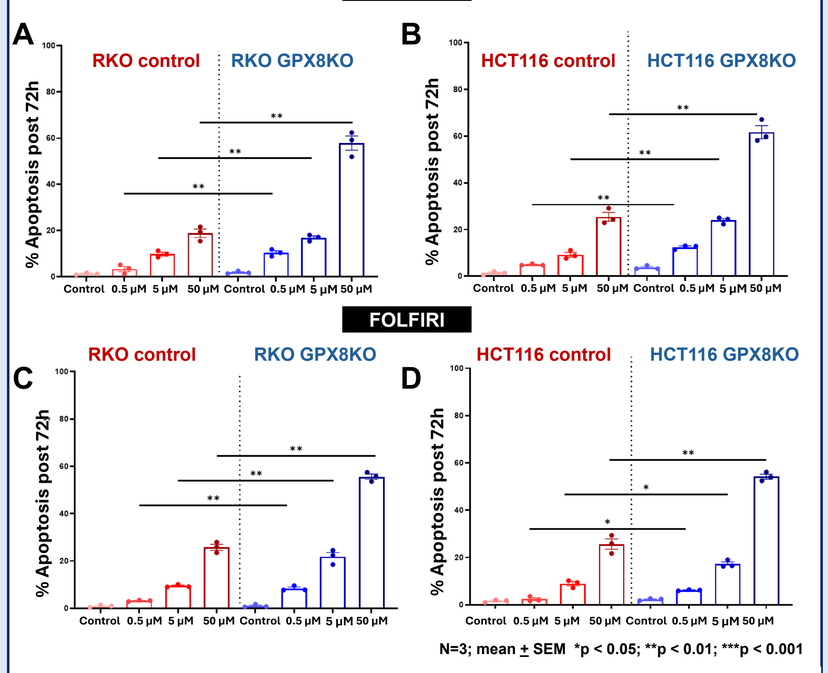

Figure 8. GPX8KO sensitises CRC cells to chemotherapy regimens. RKO and HCT116 GPX8KO and control cells were treated with 0.5 μM or 5 μM or 50 μM of **(A,B)** FOLFOX and **(C,D)** FOLFIRI. 72h following treatment apoptosis was determined by PI staining and FACS analysis.

Conclusion and Future directions

Conclusion:

- GPX8 compensates for the loss of GPX2 in CRC cells.
- Targeting GPX8 increases basal ROS levels in CRC cells.
- Targeting GPX8 sensitises CRC cells to Chemotherapy and radiation therapy *in vitro*.

Future directions:

- To perform *in vivo* experiments to validate the chemotherapy and radiation sensitivity of GPX8KO cells.

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References

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4. All graphs made using Prism software under paid licence