Discovery of ARP-4922, an orally bioavailable NRF2 degrader that leads to tumor growth regression in KEAP1-mutant NSCLC

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Abstract

Loss-of-function mutations in Kelch-like ECH-associated protein 1 (KEAP1) are common in non-small cell lung cancer (NSCLC) and result in particularly poor prognosis. Such mutations activate the transcription factor NRF2, leading to aberrant expression of chemoprotective genes and rendering cells resistant to chemotherapy. We generated a KEAP1 knockout cell line in NCI-H1975 (NCI-H1975 KEAP1 -/-) and used high-throughput screening to identify small molecules that reduces viability of NCI-H1975 KEAP1 -/- more than that of the parental NCI-H1975 cell line. Following early hit optimization, ARP-4922 appeared as the most selective for cell lines with endogenous KEAP1 alterations. ARP-4922 reduces NRF2 activity in an ARE reporter assay and downregulates the known NRF2 target genes *SLC7A11* and *NQO1* in KEAP1 mutant cell lines. Western blot analyses found that ARP-4922 leads to degradation of NRF2 in a proteasome-dependent manner even in the absence of functional KEAP1. Initial *in vivo* studies show that ARP-4922 is orally bioavailable and leads to sustained tumor regression in a NCI-H2122 mouse xenograft model at well tolerated doses. Efforts to identify the cellular target and mechanism of action of ARP-4922 are currently underway.

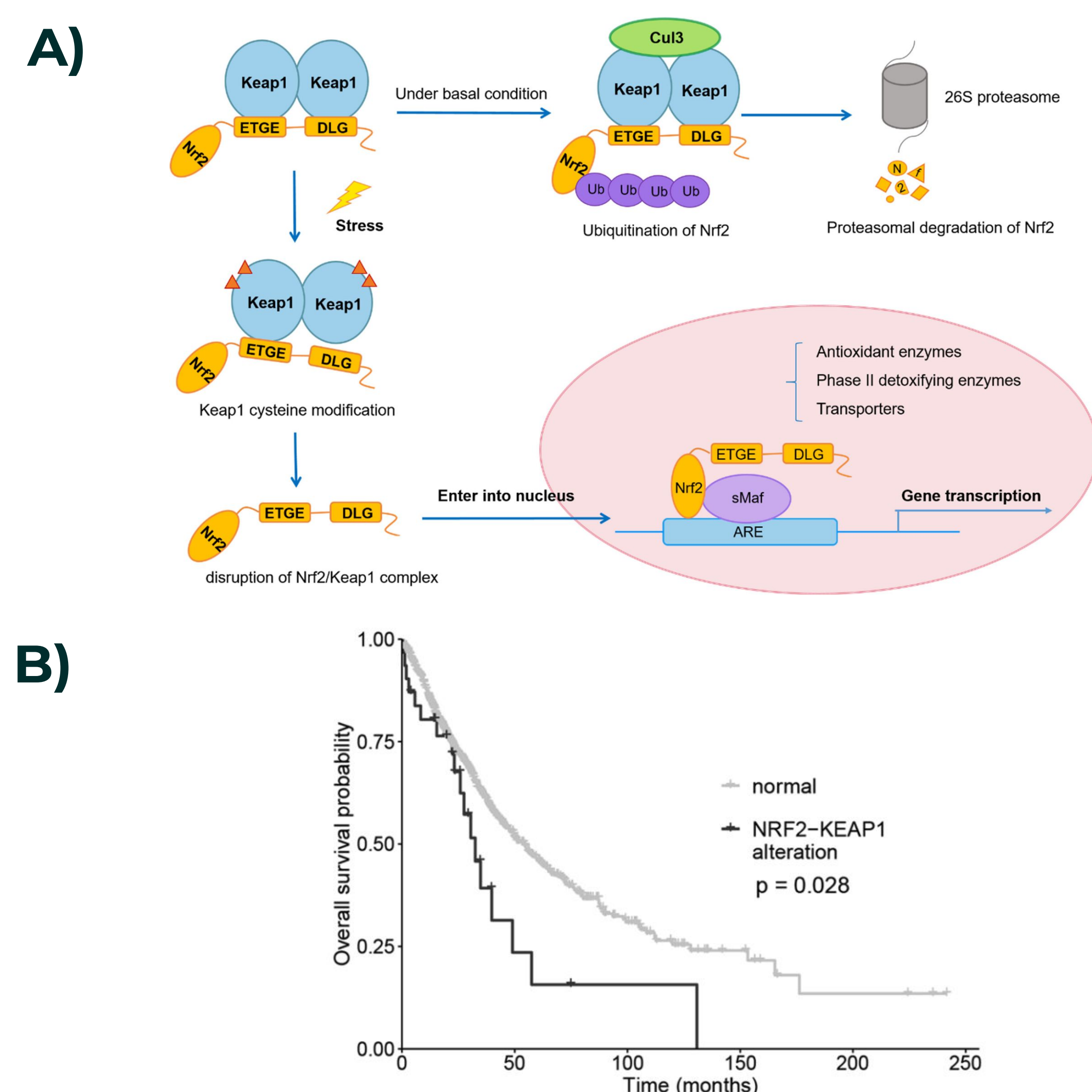## Background and Clinical Relevance

Figure 1. A) Overview of NRF2 signaling. In KEAP1 mutant NSCLC, NRF2 is no longer sequestered, leading to enhanced expression of pro-survival genes. Figure from Wu *et al, Cancer Med.* 2019; 8: 2252. **B)** Kaplan-Meier survival curves comparing overall survival of NSCLC patients with NRF2 and/or KEAP1 alterations to those with wild type NRF2 and KEAP1. Data from Silva *et al, Sci Rep.* 2019; 9: 17639.

Summary and Conclusions

- KEAP1-mutant NSCLC poses a challenge in the clinic, as overactive NRF2 leads to increased expression of cytoprotective genes
- We used HTS transcriptomics to identify and optimize a molecule that inhibits NRF2 signaling, ARP-4922
- ARP-4922 reduces expression of NRF2 target genes and leads to the proteasome-mediated degradation of NRF2 in KEAP1 mutant NSCLC cells
- ARP-4922 is orally bioavailable with favorable PK attributes and leads to tumor regression in a NSCLC mouse xenograft model

High-throughput transcriptomics and viability screening identifies an NRF2 pathway inhibitor
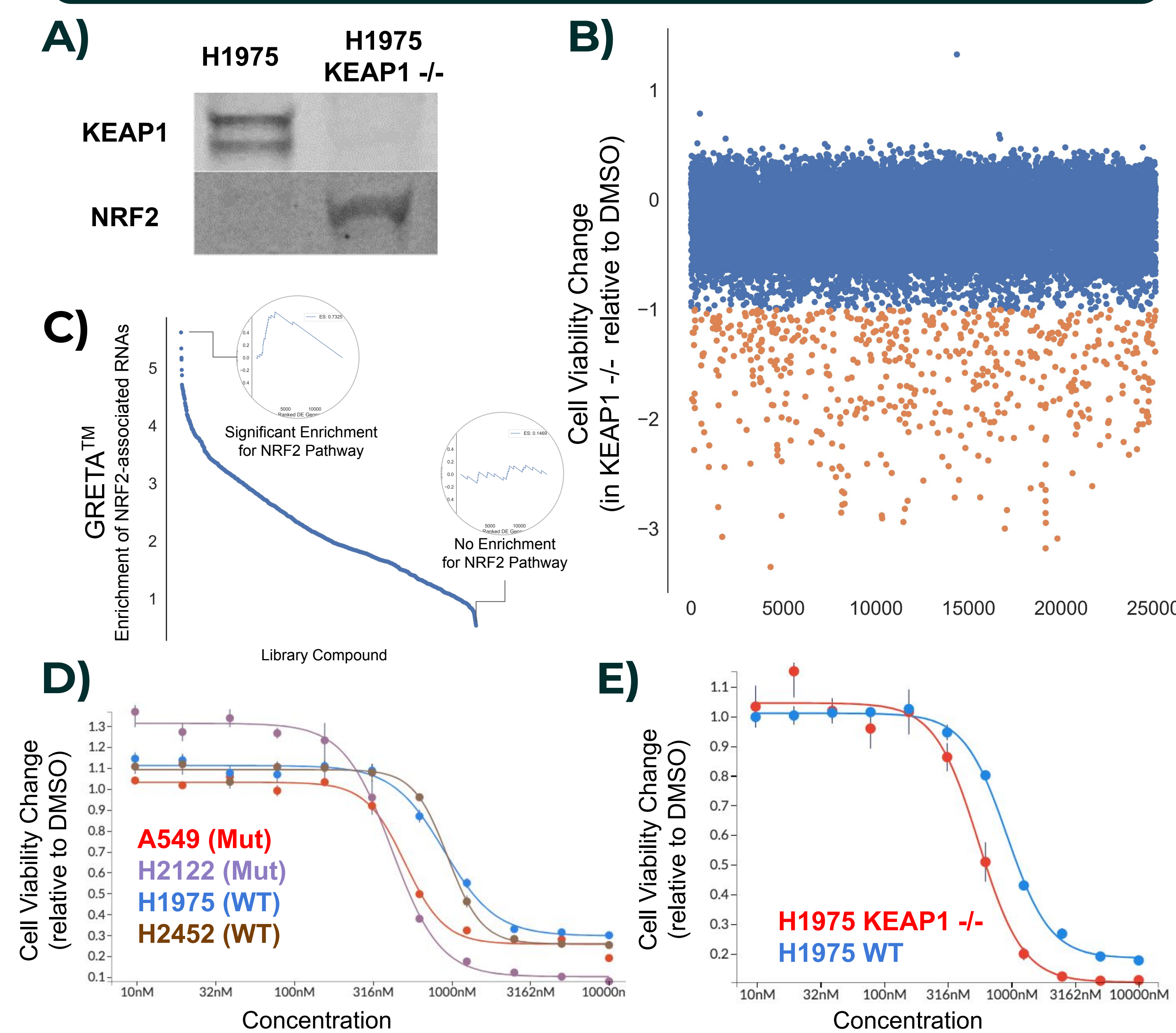

Figure 2 . A) Western blot comparing KEAP1 and NRF2 protein levels in H1975 WT and H1975 KEAP1 -/- cells. **B)** Cell viability (-log2FoldChange) of H1975 KEAP1 -/- cells treated with compound. Viability was measured using CellTiter-Glo after 5 days of compound treatment and normalized to DMSO. **C)** Profiling enrichment of NRF2-associated genes using a global reporter on transcript abundance (GRETA) in H1975 KEAP1 -/- cells after treatment with a subset of library of compounds. **D)** Relative cell viability (normalized to DMSO) of KEAP1 mutant (A549, H2122) and KEAP1 WT (H1975, H2452) after treatment with ARP-4922. **E)** Relative cell viability (normalized to DMSO) of H1975 KEAP1 -/- and H1975 WT cells after treatment with hit compound, ARP-4922.

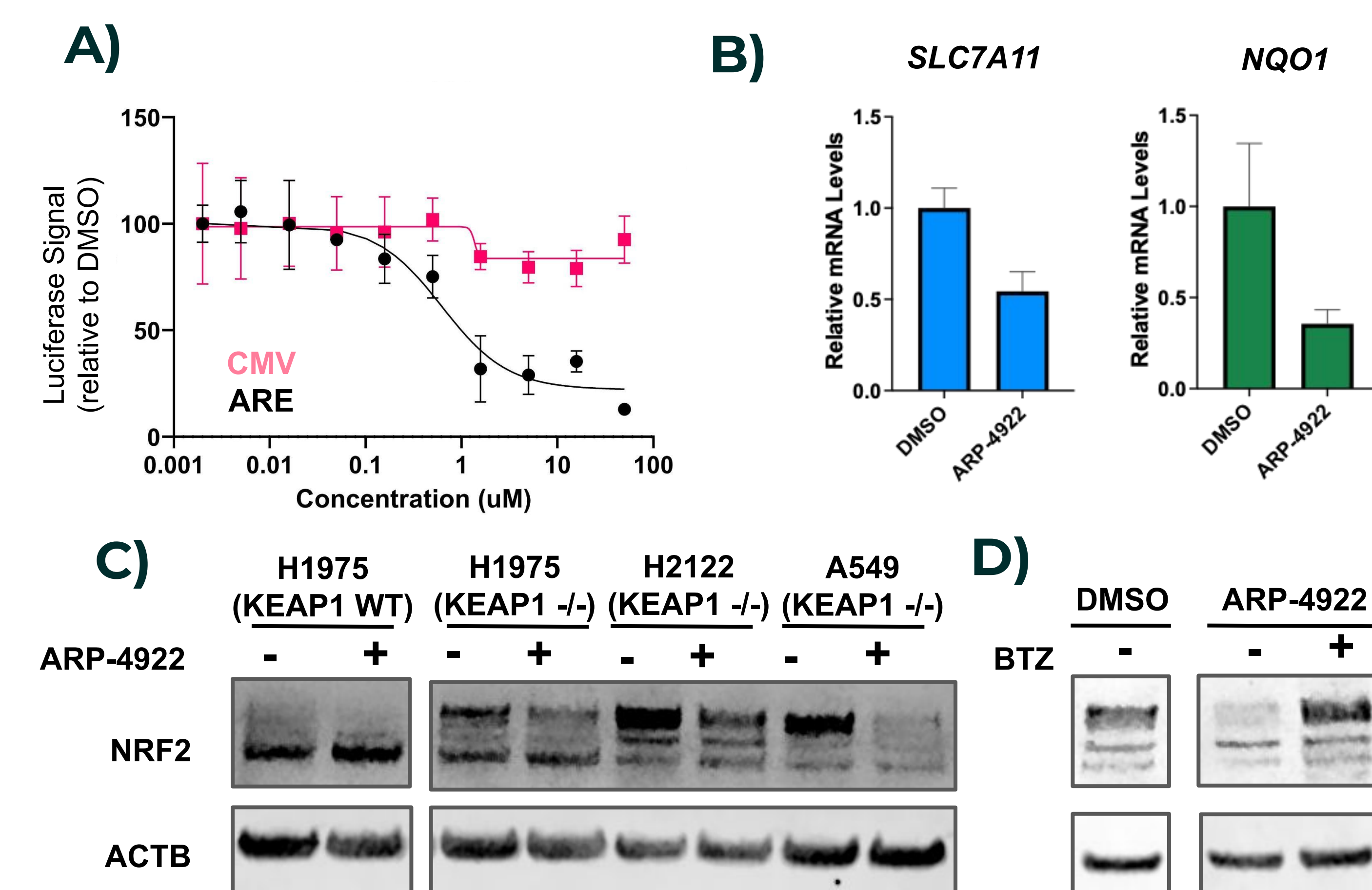## ARP-4922 inhibits NRF2 signaling through KEAP1-independent NRF2 degradation

Figure 3. A) HepG2 cells expressing either an antioxidant response element (ARE) or a CMV luciferase reporter treated with ARP-4922. **B)** RT-qPCR of H2122 cells treated with either DMSO or ARP-4922 for 72hrs. **C)** Western blot displaying degradation of Nrf2 across KEAP1 mutant cell lines with ARP-4922 treatment. **D)** Western blot displaying proteasome-mediated degradation of Nrf2 in H2122 cells. BTZ = Bortezomib.

ARP-4922 is an orally bioavailable NRF2 inhibitor that leads to tumor growth regression in a KEAP1-mutant mouse xenograft model (NCI-H2122)
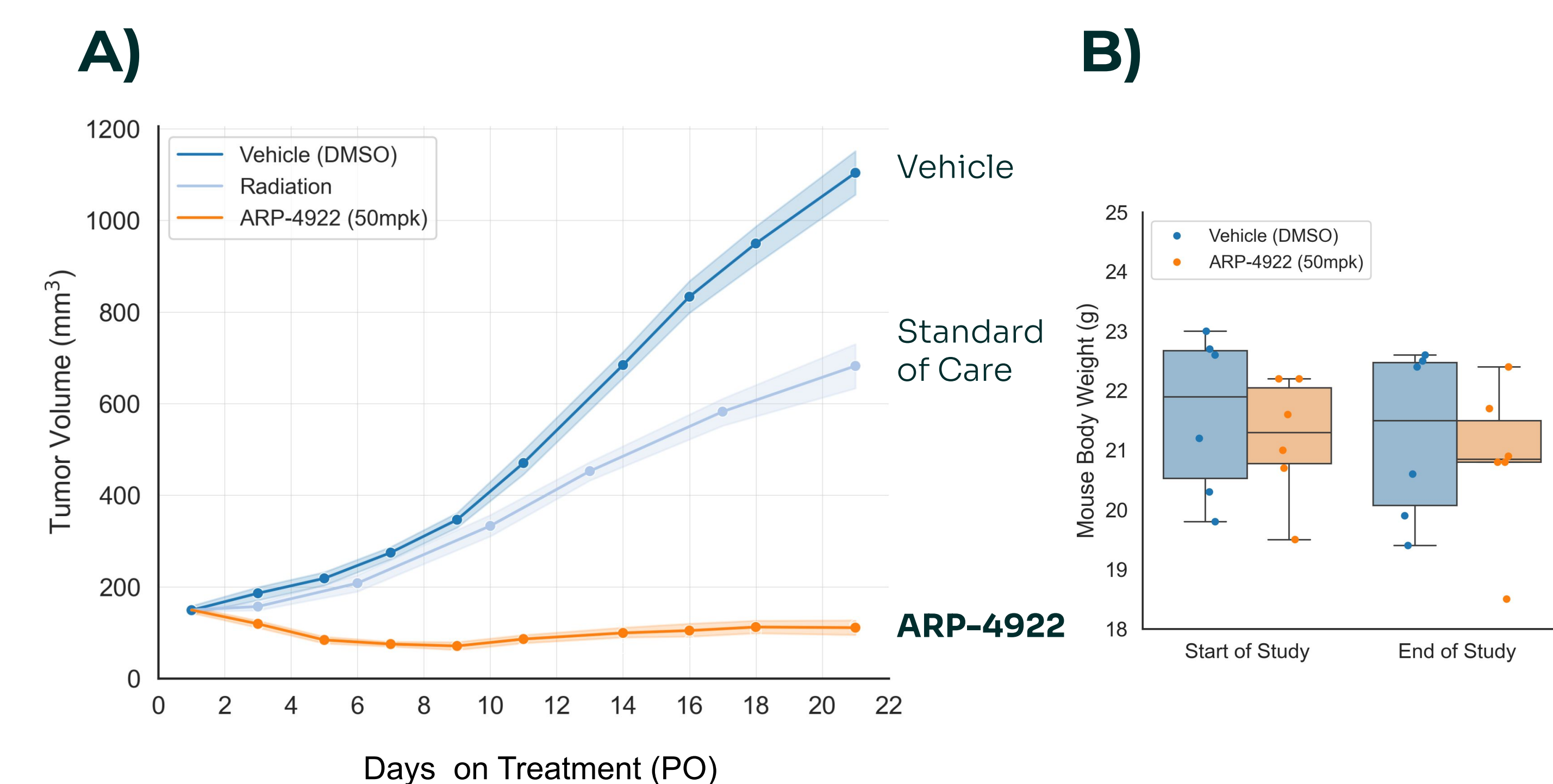

Figure 4. A) Absolute change in H2122 tumor volume after dosing with ARP-4922 or vehicle alone. Each treatment arm contains 6 mice. Radiation data is estimated from CRO data (X-Ray, 2 Gy BIW, 6 mice) in a H2122 xenograft model; >100% TGI & p-value < 10^{-4}. **B)** Body weight data for mice in TGI study at the start of the study (Day 0) and the end of the study (Day 21).

Inducing an immunogenic mode of ferroptotic cell death in difficult-to-treat cancers with an orally-bioavailable GPX4 inhibitor

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Abstract

Recent advancements in immunotherapy have transformed cancer treatment, resulting in extended survival times for some patients. However, despite these advancements, many patients do not benefit from immunotherapy because of an immunosuppressive tumor microenvironment. Consequently, there is a need for new strategies to improve the effectiveness of immunotherapy by stimulating inflammatory immunogenic cell death in tumors. Ferroptosis is a recently-described form of non-apoptotic cell death that induces a uniquely inflammatory immunogenic cell death. Glutathione Peroxidase 4 (GPX4) is the cell's major defense system against ferroptosis as it is the only known enzyme capable of reducing toxic lipid hydroperoxides to inert lipid alcohols. Here we describe a series of potent and selective small molecule GPX4 inhibitors that result in significant tumor growth inhibition in several aggressive tumor CDX models. We verified induction of ferroptosis in tumors using known biomarkers of ferroptosis such as HMOX1 mRNA induction as well as with 4-HNE staining. GPX4 target engagement and degradation was confirmed by mass spectrometry and western blot in tumors. GPX4 inhibition, when combined with PDL-1 blockade in a syngeneic sarcoma model, resulted in sustained significant tumor growth inhibition throughout the course of a 28-day study at well-tolerated doses when compared to either monotherapy alone. Current studies are underway to more carefully define the unique immunogenic cell death imparted by GPX4 inhibition and resulting ferroptosis.

Global Reporter on Transcript Abundance (GRETA) discovers novel ferroptosis-inducing small molecules

Figure 1: A) Diagram describing the mechanism of ferroptosis (adapted from Kathman et. al.) B) UMAP analysis of high-throughput bulk RNA sequencing (GRETA) identifies compounds that induce a similar transcriptional response to that of a known GPX4 inhibitor, RSL3, in HepG2 cells. C) Cell Titer Glo displaying ferrostatin-1 rescue of ARP-002-mediated toxicity in NCI-H522 cells. D) qRT-PCR displaying induction of a ferroptosis biomarker, HMOX1. E) Bodipy-C11 staining showing ARP-002-mediated lipid peroxidation in NCI-H522 cells.

ARP-002 and analogs are GPX4 inhibitors that lead to proteasome-dependent GPX4 degradation
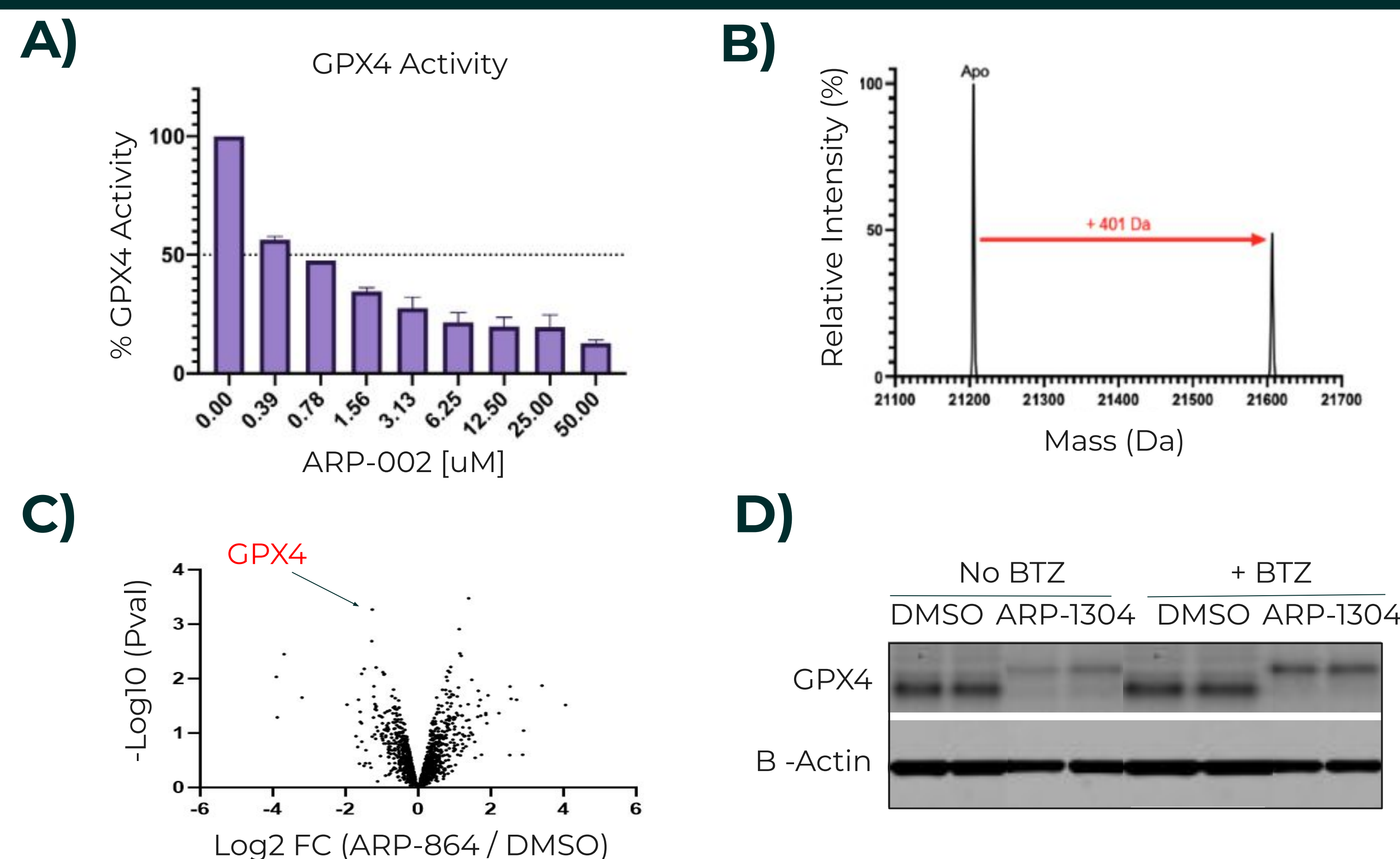

Figure 2: A) ARP-002 inhibits GPX4 in a biochemical enzymatic activity assay. B) ARP-002 binds to GPX4 in an *in vitro* intact protein mass spectrometry assay. C) Activity-based protein profiling (ABPP) identifies GPX4 as the most highly ARP-864-occupied protein in NCI-H522 cells. D) Western blot confirms GPX4 binding by ARP-1304 which results in proteasome-dependent degradation of GPX4.

ARP-864 displays selectivity for pancreatic cancer and sarcoma cell lines across a panel of 160 human tumor models

Figure 3: A) Cell Line viability panel reveals pancreatic and sarcoma cell lines as particularly vulnerable to ARP-0864-mediated GPX4 inhibition. B) ARP-0864 cell line specificity is most correlated with depmap GPX4 knockout effect across 160 cell lines. C) ARP-0864 cell line specificity is most correlated with other GPX4 inhibitors (ML162, RSL3, ML210) across the Cancer Therapeutics Response Portal (CTRP).

ARP-1304 leads to significant induction of Ferroptosis in a CDX model of pancreatic cancer (KP4)
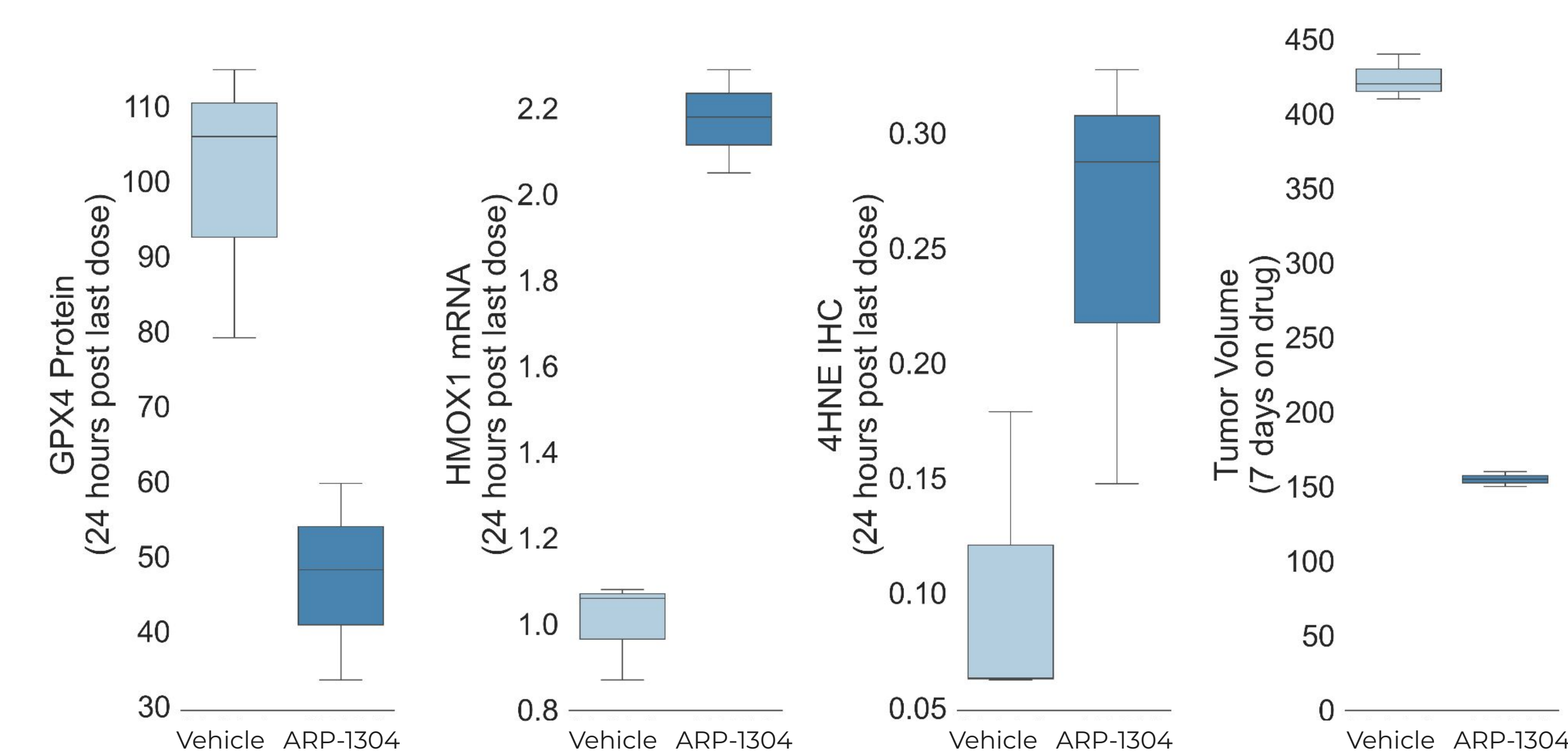

Figure 4: Pharmacodynamics study of ARP-1304 showing decreased GPX4 in tumors by western blot, Increased HMOX1 mRNA level in tumors by qRT-PCR, increased lipid peroxidation by 4-HNE by IHC, and reduced tumor volume following oral administration of ARP-1304 in pancreatic xenograft model (KP4).

ARP-1304 induces immunogenic cell death in a sarcoma syngeneic mouse model (Sarcoma-180)
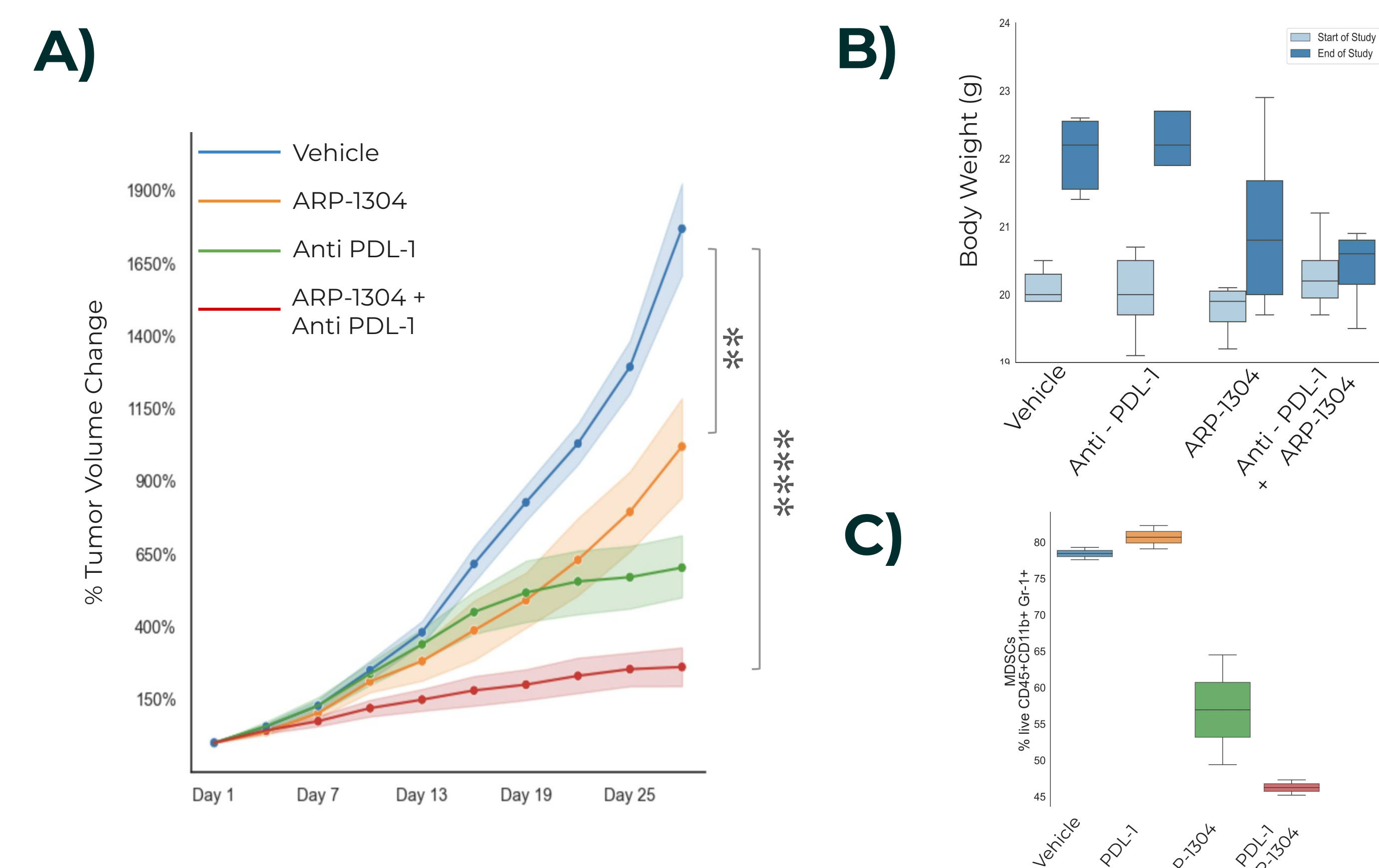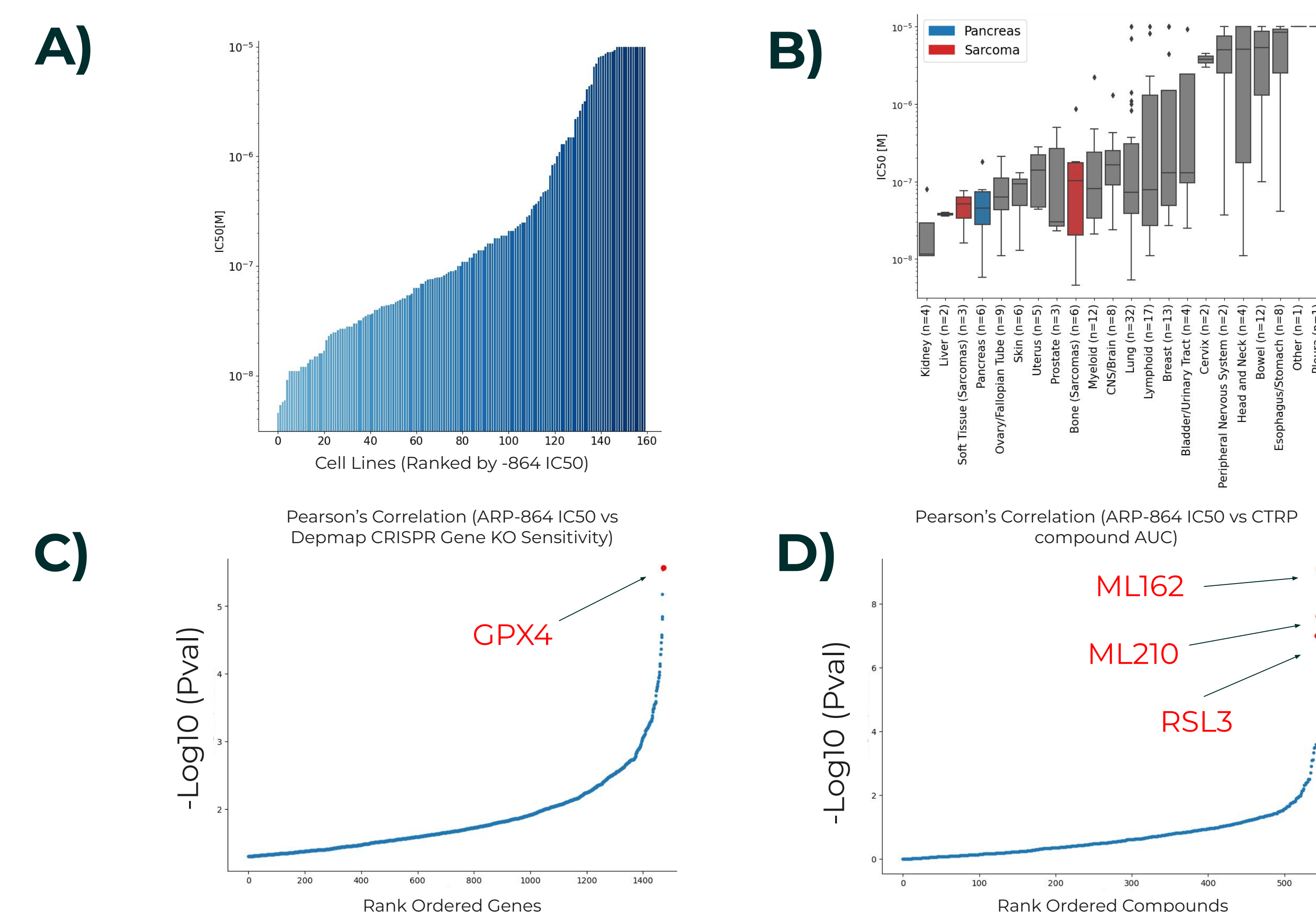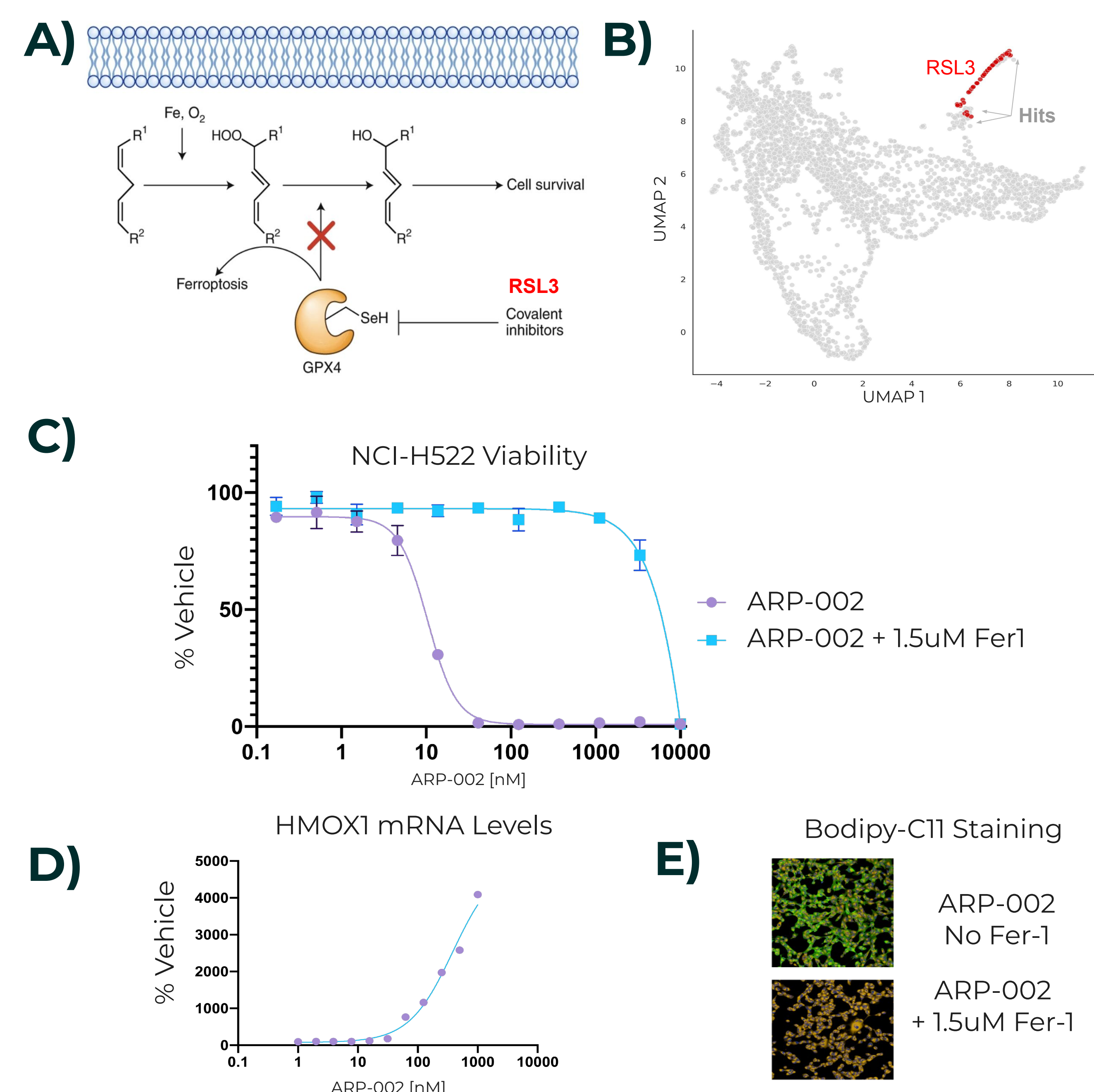

Figure 5: A) ARP-1304 improves the efficacy of PDL-1 blockade in a Sarcoma-180 syngeneic mouse model (**=Pval<.01, ****=Pval<.0001. B) ARP-1304 is well-tolerated in a 28 day study when dosed PO daily. C) Immunophenotyping of tumors shows a marked reduction in myeloid derived suppressor cells when used in combination with PDL-1 blockade.

We used our high throughput transcriptomics platform, GRETA, to identify small molecule inducers of ferroptosis. Follow up studies identified ARP-1304 as a potent and selective GPX4 inhibitor that leads to tumor growth inhibition in CDX models of sarcoma and pancreatic cancer at well-tolerated doses when dosed orally. Importantly, ARP-1304 increases the sensitivity of tumors to PDL-1 blockade *in vivo* as revealed through a syngeneic mouse model.
More information can be found at arpeggiobio.com