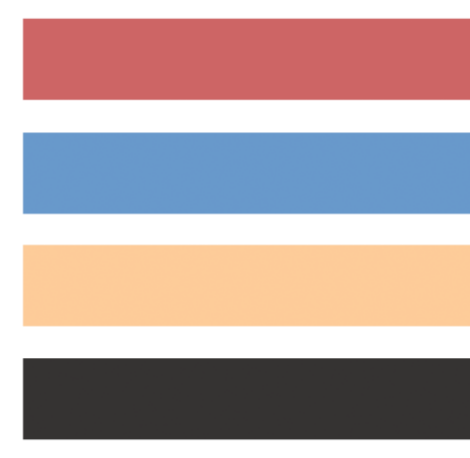




Identification of therapeutic vulnerabilities of *KEAP1*-mutated lung adenocarcinoma

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1. Introduction

KEAP1 is mutated in 15–20% of non-small cell lung cancer (NSCLC) cases and is associated with poor prognosis. There is an urgent clinical need to develop new therapeutic strategies for *KEAP1*-mutated NSCLC. This study aims to establish a cellular model system to investigate *KEAP1*-mutant tumors, characterize the genomic evolution of *KEAP1* wildtype (WT) and knockout (KO) cell lines under chemotherapy, and identify therapeutic strategies in *KEAP1* KO cells. We generated three syngeneic *KEAP1* WT/KO NSCLC cell line pairs and assessed their responses to oxidative stress, NRF2 pathway activity, cell viability, and proteomic profiles. We also screened an FDA-approved drug library and performed pilot studies with NRF2 and glutaminase inhibitors. Proteomic analyses revealed a ferroptosis-resistant landscape in *KEAP1* KO, while pharmacological studies identified NRF2 inhibition and specific drug classes as promising strategies to resensitize *KEAP1*-deficient cells to reactive oxygen species.

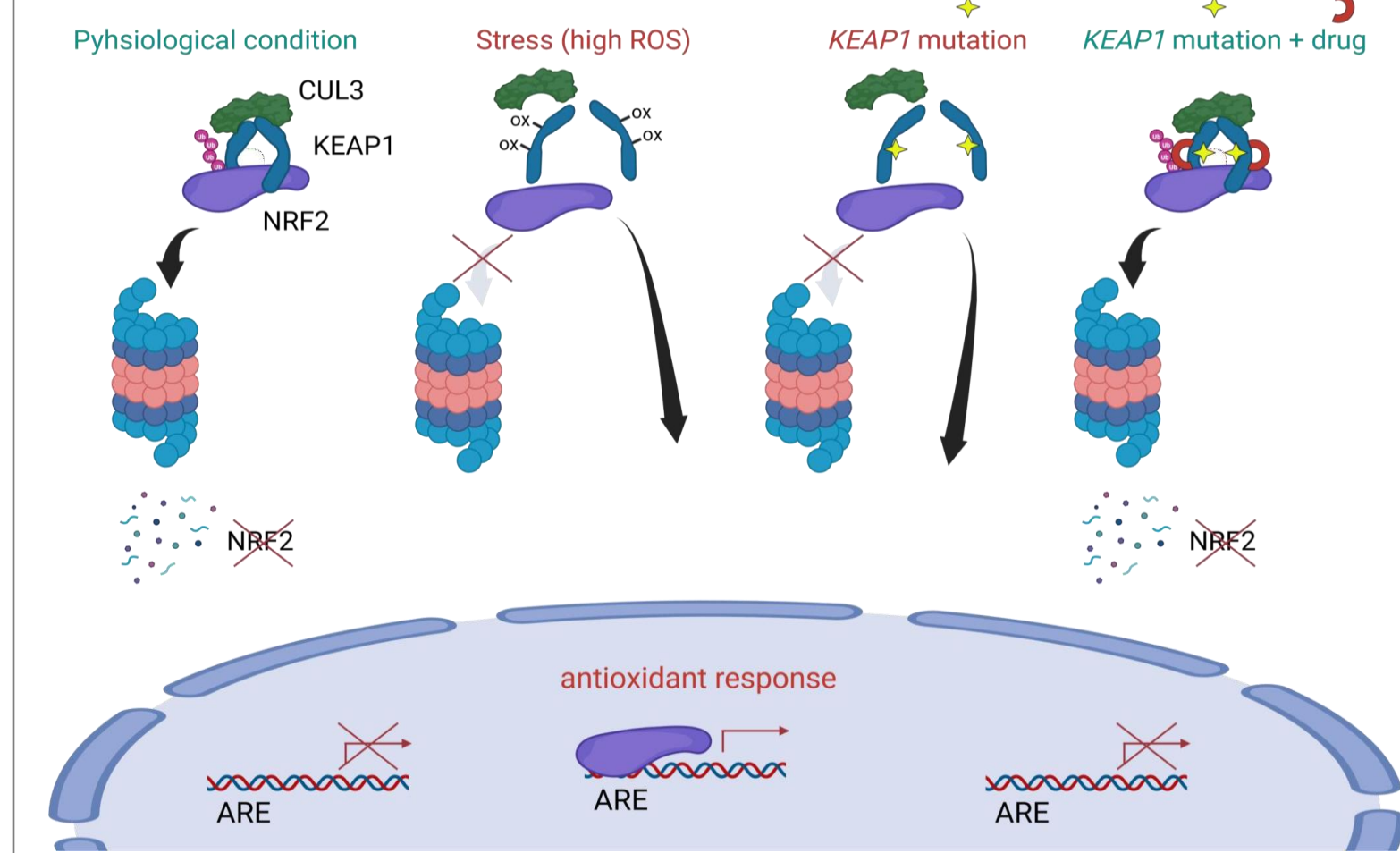
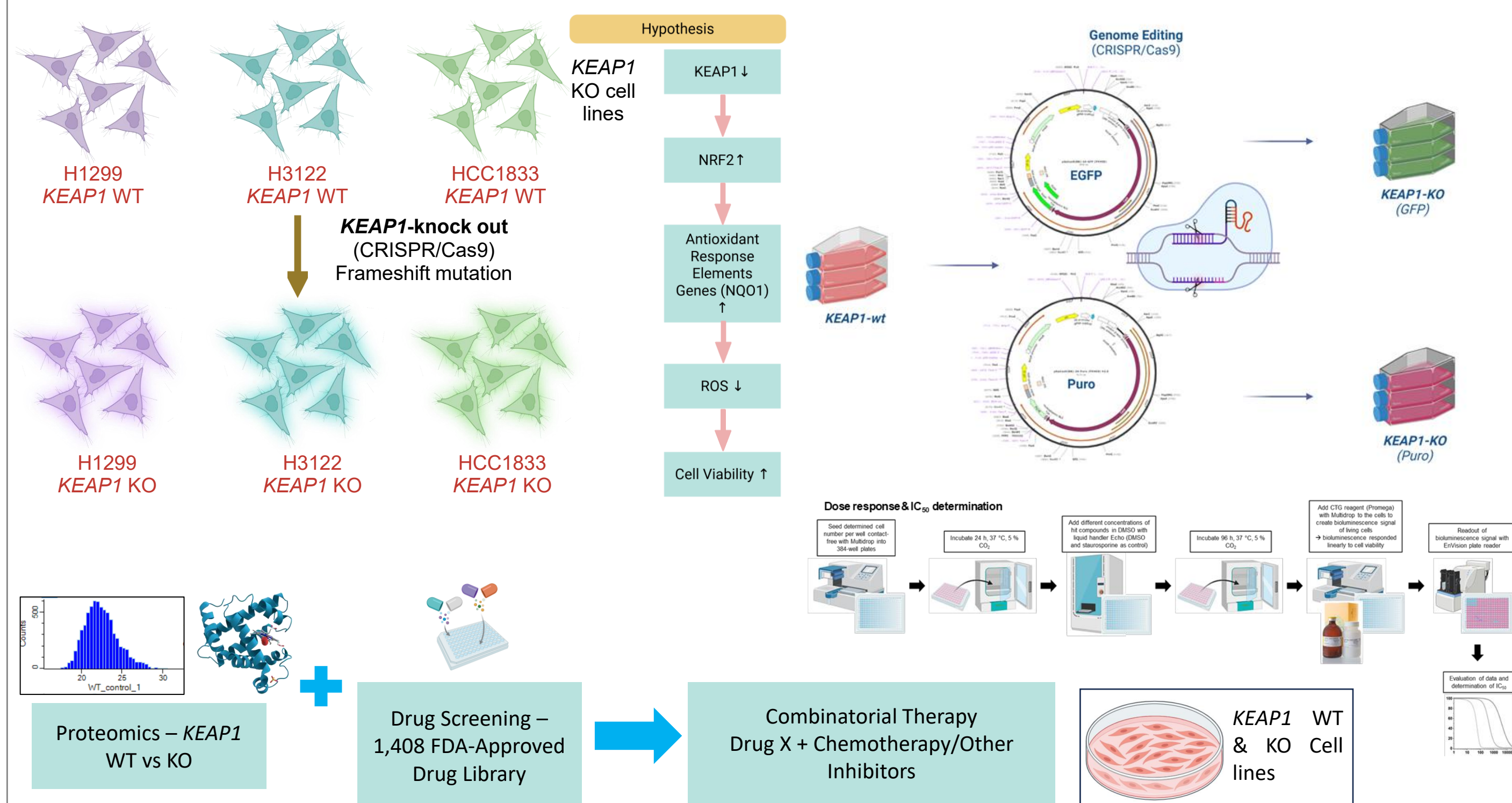


Figure 1

KEAP1 controls NRF2 pathway activity. Under physiological conditions, *KEAP1* binds to NRF2, mediating its degradation through the CUL3 E3 ubiquitin ligase. High levels of ROS or *KEAP1* mutations prevent KEAP1 binding to NRF2. NRF2 can accumulate and migrate to the nucleus, where it acts as a transcription factor and binds to antioxidant response elements (ARE), initiating an antioxidant transcription program.

2. Methods



3. Activation of NRF2 signaling in *KEAP1* KO cell lines

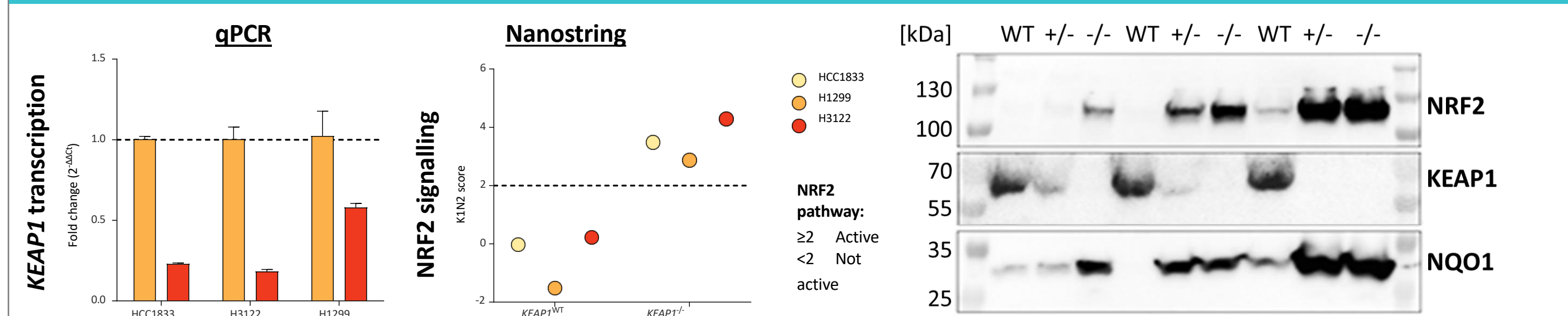


Figure 2
A) qPCR analysis demonstrates reduced *KEAP1* expression in *KEAP1* KO cells compared to *KEAP1* WT cells in all three cell lines. B) Western blot analysis shows decreased *KEAP1* protein expression in *KEAP1* KO cells, resulting in increased protein expression of NRF2 and its target NQO1.

4. *KEAP1* KO cell lines are more resistant to ROS induction

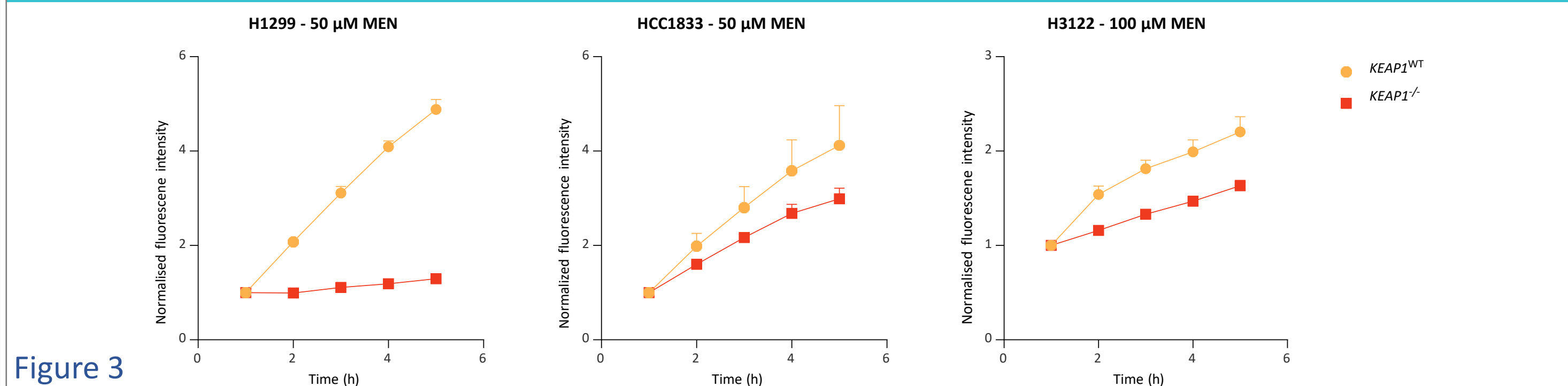


Figure 3

DCFDA ROS assay demonstrates reduced ROS levels in all three *KEAP1* KO cell lines compared with *KEAP1* WT following menadione-induced oxidative stress. This reduction is consistent with enhanced antioxidant capacity associated with *KEAP1* loss and NRF2 activation.

5. *KEAP1* KO cell lines have higher cell viability under oxidative stress

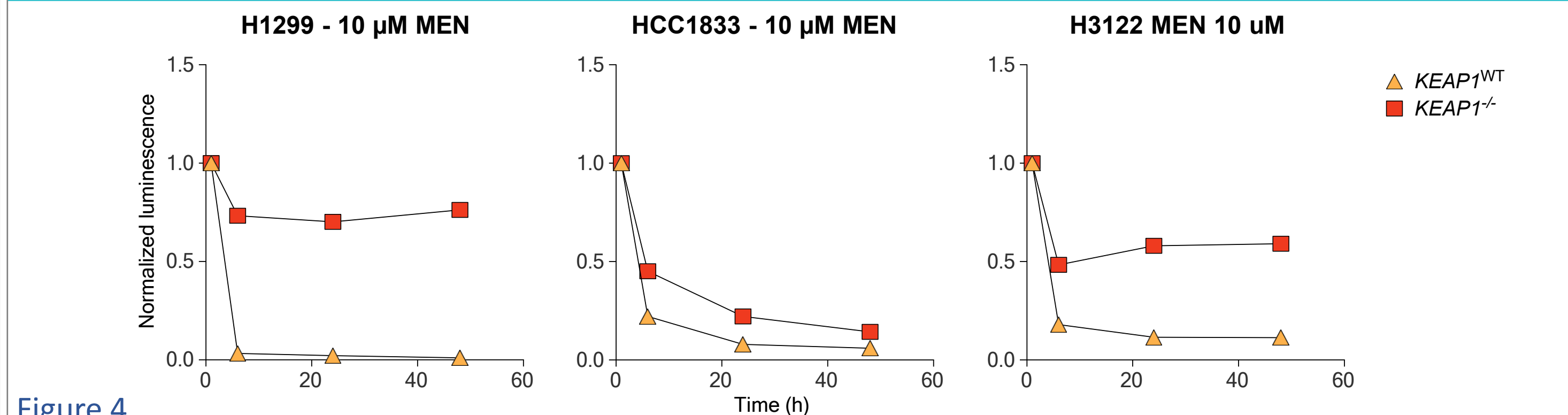


Figure 4

Cell viability measured using CellTiter-Glo demonstrates relatively higher viability in all three *KEAP1* KO cell lines compared with *KEAP1* WT following treatment with the ROS inducer menadione. These findings support a survival advantage associated with *KEAP1* loss and NRF2 activation.

7. Proteomics confirms NRF2 pathway distortion and indicates ferroptosis resistance landscape

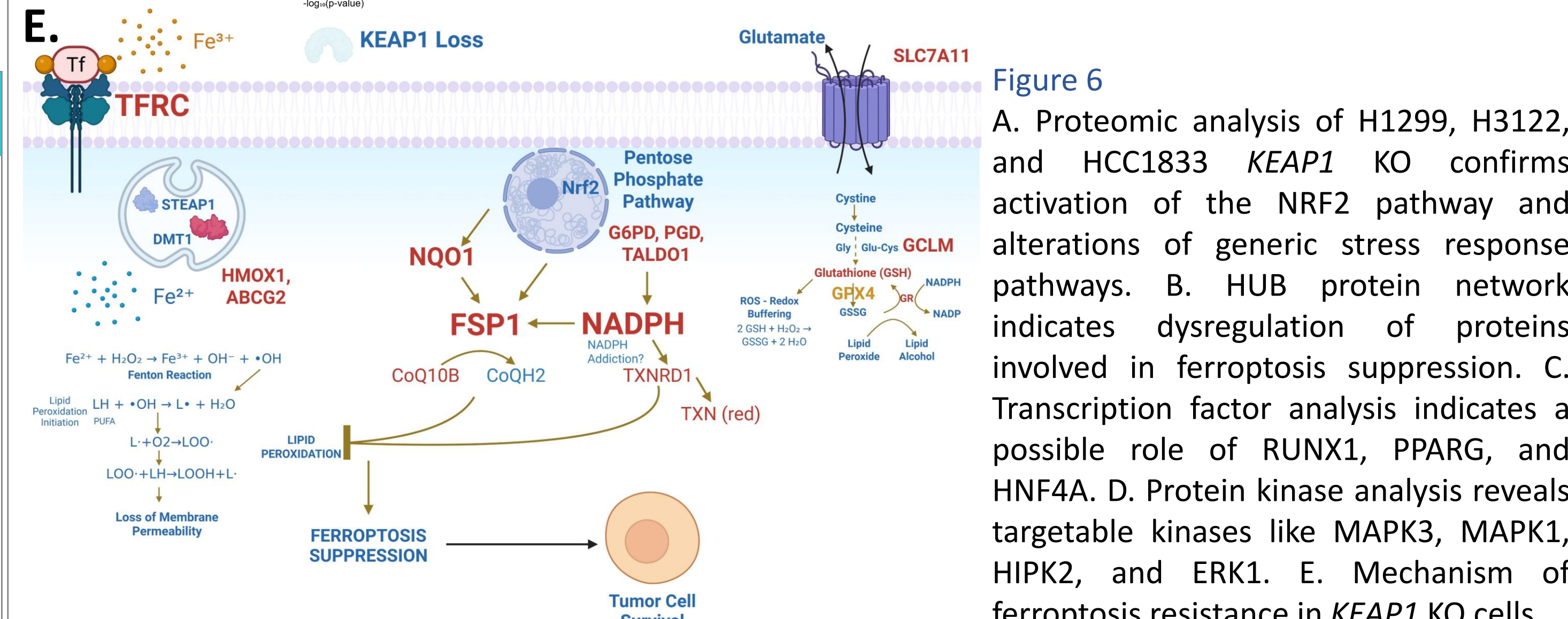
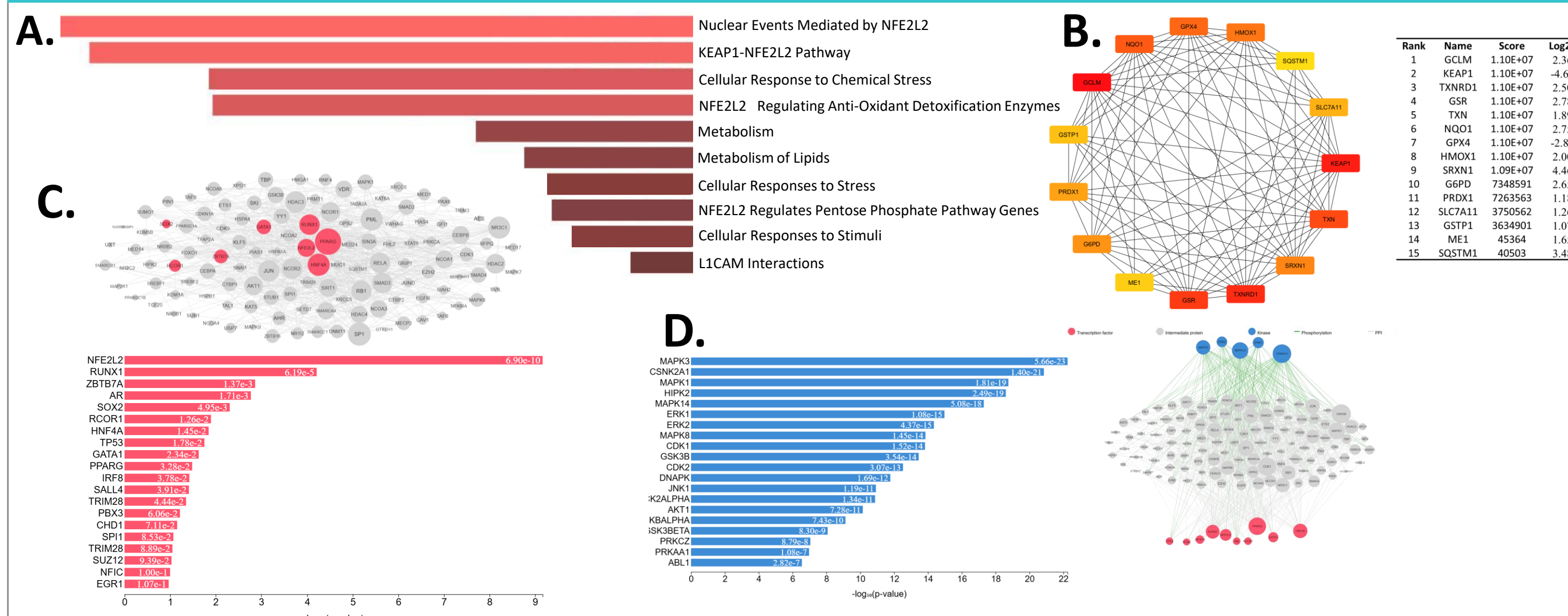


Figure 6

A. Proteomic analysis of H1299, H3122, and HCC1833 *KEAP1* KO confirms activation of the NRF2 pathway and alterations of generic stress response pathways. B. HUB protein network indicates dysregulation of proteins involved in ferroptosis suppression. C. Transcription factor analysis indicates a possible role of RUNX1, PPARG, and HNF4A. D. Protein kinase analysis reveals targetable kinases like MAPK3, MAPK1, HIPK2, and ERK1. E. Mechanism of ferroptosis resistance in *KEAP1* KO cells.

6. NRF2 & Glutaminase inhibitors resensitize *KEAP1* KO cells to oxidative stress

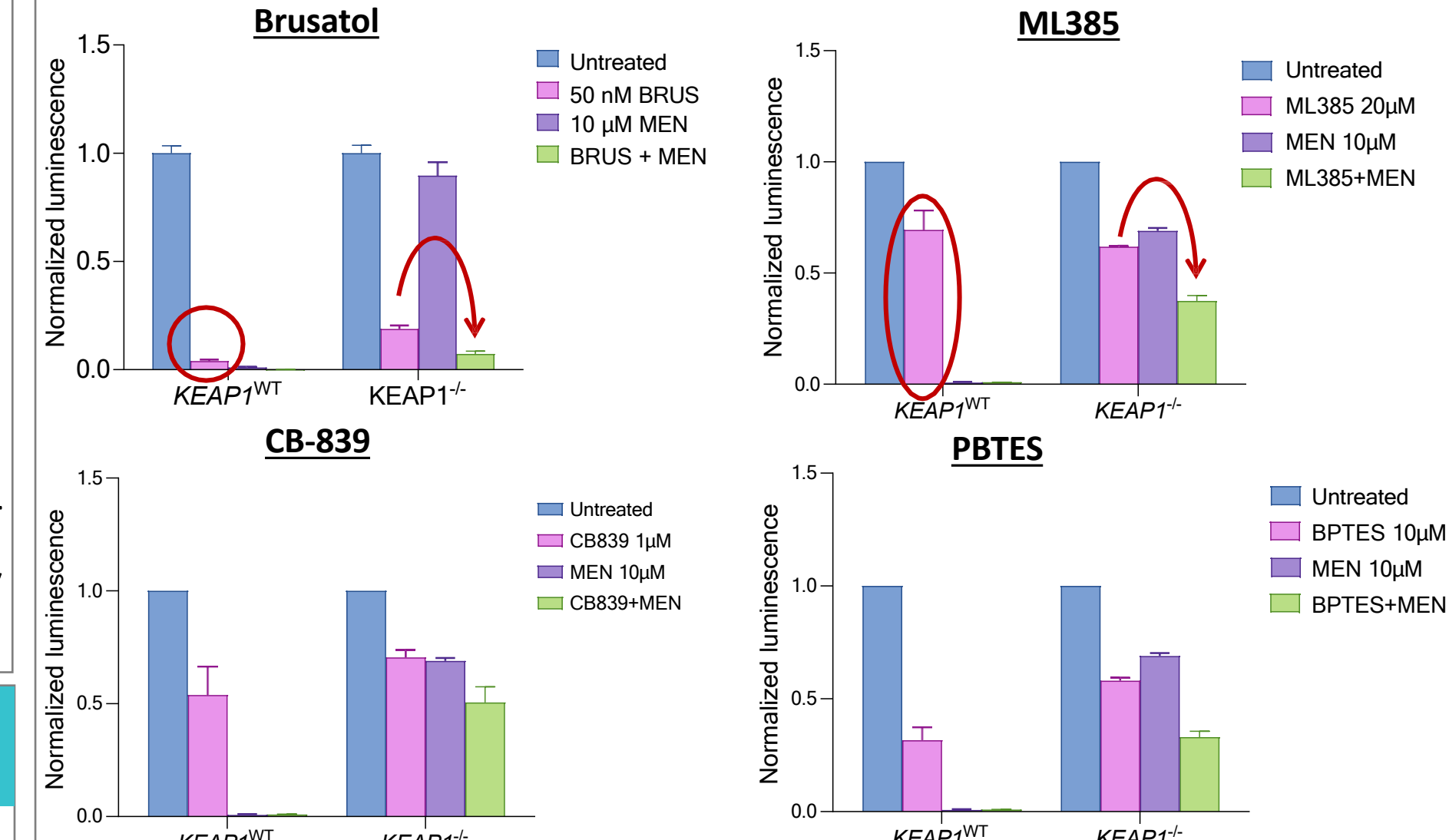


Figure 5

While *KEAP1* KO cell lines exhibit higher viability than *KEAP1* WT following menadione treatment, inhibition of NRF2 with Brusatol or ML385 in H1299 cells reduces this survival advantage, highlighting the critical role of NRF2 in stress resistance. Similarly, treatment with the glutaminase inhibitors CB-839 and PBTES reduces cell viability, supporting glutamine dependency in *KEAP1*-deficient cells and highlighting glutamine metabolism as a potential therapeutic target.

8. Screen of 1,408 FDA-approved drugs with *KEAP1* altered LUAD cell lines reveals antifolates as potential therapeutics

		[IC50 in nM]									
		KEAP1 altered									
		(KRAS, KEAP1)	(TP53, SMARCA4)	(KEAP1, TP53, MET)	(TP53, STK11)	(TP53, STK11)	(TP53, MET)	(TP53, MET)	ALK-fusion	H3122 WT	ALK-fusion
		A549	H1299_KO	H1299_KO	H1299_KO	H1299_KO	H1299_KO	H1299_KO	H1299_KO	H1299_KO	H1299_KO
		Average	Average	Average	Average	Average	Average	Average	Average	Average	Average
Carfilzomib	proteasome inhibitor	27.4	29.0	74.1	2.5	2.2	6.1	8.0	45.2	24.3	24.3
Bortezomib	proteasome inhibitor	23.7	33.6	128.8	8.1	9.4	10.4	20.8	164.6	49.9	49.9
Gracimicin	antimicrobial	38.1	51.6	131.9	48.0	46.0	29.5	37.9	94.4	59.7	59.7
Izaxomib citrate	proteasome inhibitor	86.8	159.7	586.2	39.7	49.8	46.1	172.8	863.5	250.6	250.6
Izaxomib	proteasome inhibitor	129.0	203.2	856.7	42.4	53.0	64.1	104.6	1,428.2	360.2	360.2
Mitoxantrone (dihydrochloride)	chemotherapeutic	60.0	581.4	399.1	338.1	302.5	276.1	461.6	791.6	393.8	393.8
MLN9708	proteasome inhibitor	159.5	372.6	1,212.6	77.8	70.1	112.1	159.1	1,950.4	519.5	519.5
Crystall Violet	dye	1,445.0	1,286.9	2,012.1	894.9	605.8	961.1	1,119.9	1,540.1	1,233.2	1,233.2
Cinacalcet	transcription modulator	2,632.4	2,552.1	1,866.4	1,567.1	626.7	1,089.9	1,191.0	805.8	1,541.4	1,541.4
Ceritinib	TKI	2,530.4	3,819.2	2,872.8	576.4	242.2	2,365.2	33.0	223.6	1,582.9	1,582.9
Ponatinib	TKI	1,493.8	1,587.7	3,372.7	1,008.9	834.3	824.1	3,298.8	2,894.4	1,914.3	1,914.3
Paroxetene	neuroactive	2,813.5	3,107.5	2,468.9	1,729.9	1,833.6	1,409.8	1,689.4	1,744.3	2,099.6	2,099.6
Auranofin	transcription modulator	4,516.7	3,260.0	3,592.9	475.8	822.0	808.1	2,469.1	3,942.7	2,459.8	2,459.8
Entrectinib	TKI	3,038.2	3,771.3	3,564.7	2,654.6	2,678.6	3,911.7	431.4	718.6	2,597.4	2,597.4
Thronzonium (bromide)	antimicrobial	2,965.4	4,447.8	3,601.2	1,755.0	1,294.8	3,818.6	1,270.2	3,895.3	2,891.3	2,891.3
Methylene blue	dye	1,512.7	2,414.9	8,902.9	2,690.2	2,675.7	2,508.9	1,251.0	1,174.5	2,891.3	2,891.3
Crizotinib	TKI	3,151.5	3,235.7	6,311.9	3,145.4	3,054.0	2,700.6	994.1	1,416.1	3,001.2	3,001.2
Fedratrinb hydrochloride hydrate	TKI	1,791.1	1,667.1	4,887.6	1,604.4	1,420.8	1,087.6	7,287.9	5,441.1	3,148.5	3,148.5
Dacomitinib	TKI	1,447.7	3,920.7	4,935.1	2,917.1	2,202.8	3,281.2	4,332.5	3,637.1	3,334.3	3,334.3
Netersudil Dihydrochloride	TKI	3,437.4	6,834.0	3,796.7	2,751.0	2,033.9	4,555.8	3,699.0	4,298.1	3,925.7	3,925.7
Fedratrinb	TKI	2,273.6	1,883.8	7,217.6	2,143.4	1,859.4	1,438.9	8,157.9	6,993.4	3,596.0	3,596.0
Afatinib	TKI	1,102.8	3,415.4	12,690.5	3,201.9	3,462.2	3,126.9	1,683.7	3,759.8	4,055.4	4,055.4
Methylenedione (Hydrochloride)	cardiovascular	3,681.4	4,905.0	6,144.1	3,590.5	2,428.7	4,877.5	4,542.4	4,485.5	4,331.9	4,331.9
Methylene blue (trihydrate)	dye	2,935.2	6,512.2	11,204.1	6,323.2	4,484.9	4,304.3	2,625.8	1,869.0	5,032.3	5,032.3
Pimavanserin	neuroactive	4,114.8	7,769.6	4,561.1	3,520.2	3,370.7	5,897.7	6,540.9	4,363.6	5,042.3	5,042.3
Lumefantrine	cardiovascular	2,869.1	4,181.4	2,975.8	4,461.2	15,000.0	3,864.8	3,290.5	4,062.4	5,088.2	5,088.2
Dronedarsone	cardiovascular	9,388.1	5,179.6	6,301.3	3,438.5	2,016.3	4,304.0	8,626.4	4,884.2	5,593.2	5,593.2
Pralatrexate	chemotherapeutic	5.8	6.4	10,213.6	15,000.0	15,000.0	5.8	15,000.0	144.3	6,922.2	6,922.2
Primaquine diphosphate	DNA binding	7,113.0	9,692.9	15,000.0	4,056.5	3,72.6	9,018.2	11,161.4	5,148.4	8,145.4	8,145.4
Pyrimethamine	chemotherapeutic	1,278.6	1,315.2	15,000.0	15,000.0	15,000.0	1,723.2	15,000.0	6,173.7	8,811.1	8,811.1
Methotrexate	chemotherapeutic	40.6	138.1	12,954.6	15,000.0	15,000.0	45.1	15,000.0	15,000.0	9,147.3	9,147.3
(-)-Parthenolide	transcription modulator	11,675.5	4,690.3	20,521.0	3,994.7	4,612.9	2,582.0	9,772.7	21,609.3	9,526.6	9,526.6
6-Mercaptopurine hydrate	antimetabolite	4,416.7	5,362.3	15,000.0	15,000.0	15,000.0	3,497.0	15,000.0	15,000.0	11,034.5	11,034.5
Disulfiram	chemotherapeutic	15,000.0	15,000.0	15,000.0	188.3	180.3	15,000.0	15,000.0	15,000.0	11,296.1	11,296.1
Sulfamerazine	antimicrobial	15,000.0	15,000.0	15,000.0	15,000.0	15,000.0	15,000.0	15,000.0	15,000.0	15,000.0	15,000.0
Digoxin	cardiovascular	30,000.0	30,000.0	30,000.0	30,000.0	30,000.0	126.5	185.2	139.9	18,806.5	18,806.5
Trimetazene	cardiovascular	6,437.5	4,236.8	30,000.0	30,000.0	30,000.0	6,915.1	30,000.0	15,464.3	19,631.7	19,631.7
R(-) Apomorphine hydrochloride hemihydrate	neuroactive	30,000.0	21,502.3	30,000.0	15,083.1	30,000.0	12,677.5	24,051.3	30,000.0	24,168.3	24,168.3

Figure 7

Dose response analysis of the 38 drugs in LUAD cell lines A549, H1623, HCC1833 *KEAP1* KO, H1299 *KEAP1* KO, and H3122 *KEAP1* KO. Drugs were selected based on a screen of 1,408 FDA-approved drugs in LUAD cell lines H1299WT and H1299 *KEAP1* KO under normal and high ROS conditions. Values represent IC50 in nM.

9. Conclusion & Future Directions

- ✓ We generated three syngeneic cell line pairs with a *KEAP1* WT and KO genotype and validated the activation of NRF2 pathway, increased cell viability and high tolerance to ROS in *KEAP1* KO cells compared to *KEAP1* WT cells.
- ✓ Proteomics analysis of the cell line pairs indicate ferroptosis resistance as a potential target.
- ✓ A screen of FDA-approved drugs with the syngeneic cell line pairs indicate antifolates as potential therapeutics.
- ✓ We are currently studying the genomic evolution of these cell lines under chemotherapy and conducting further drug screens against NRF2 activated tumors.