



Mitochondrial Complex I Modulation: A New Therapeutic Paradigm for Alzheimer's Disease

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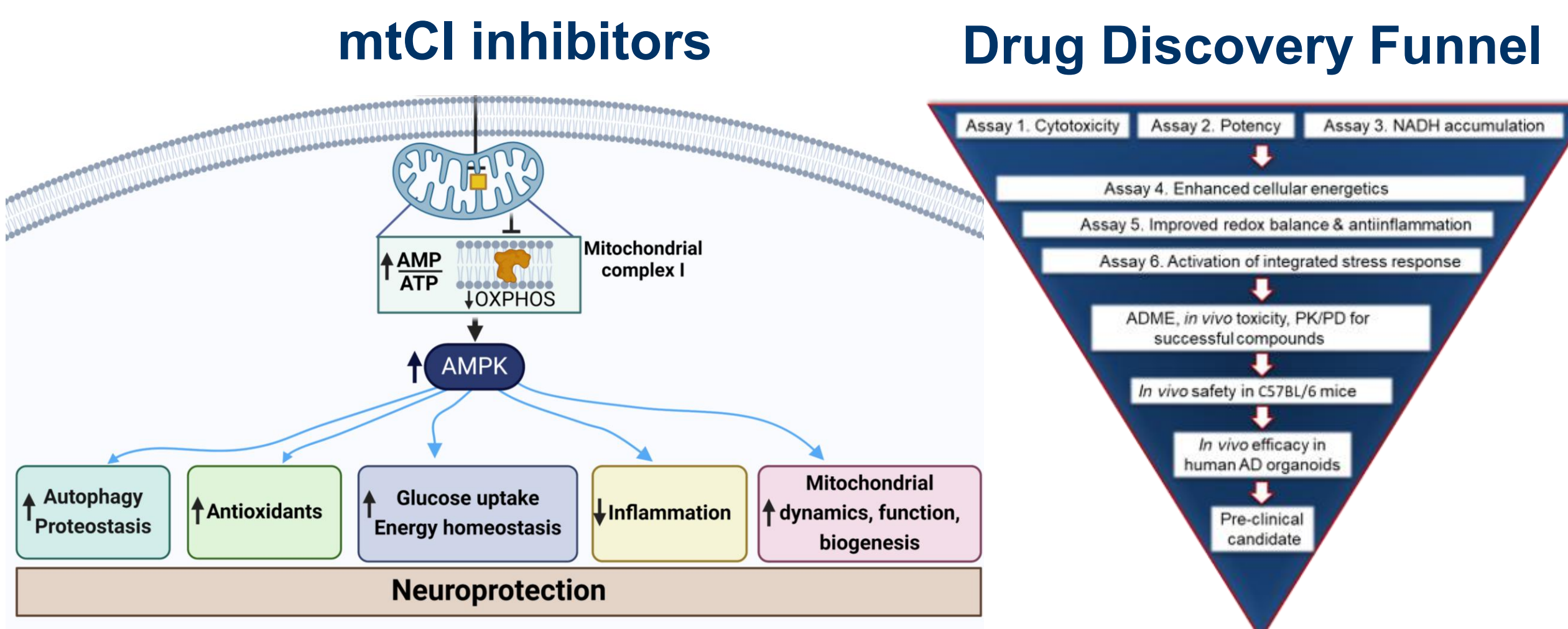
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Mitochondrial neurobiology and therapeutics laboratory

INTRODUCTION

- Despite recent approval of monoclonal antibodies that reduce amyloid accumulation, the development of disease-modifying strategies targeting the underlying mechanisms of Alzheimer's Disease (AD) are urgently needed. We identified mitochondrial complex I (mtCI) as a small-molecule druggable target for AD.
- Small molecules CP2 and C458 penetrate the blood brain barrier and accumulate in mitochondria where they weakly and specifically inhibit the activity of mtCI, activating the adaptive stress response and multiple neuroprotective mechanisms. Compounds restore mitochondrial function and energy homeostasis in brain and periphery.
- Since abnormal brain energy homeostasis underlies early stages of AD, this small molecule therapeutics could represent a disease-modifying approach.



OBJECTIVES

The objective was to develop and characterize a new generation of mtCI inhibitors derived from the tool compound CP2 with optimized ADME profiles and drug-like properties, and to define the molecular mechanisms through which mtCI inhibition activates neuroprotective signaling pathways in AD.

METHODS

Using CP2 as a tool compound, we performed rational drug design and extensive structure–activity relationship studies to develop a novel class of weak, reversible mtCI inhibitors with high dissociation rates, enhanced functional potency, and favorable drug-like properties. Target engagement, binding specificity for mtCI, selectivity, efficacy, and safety were evaluated using complementary biochemical, multi-omics, and functional assays. This culminated in the identification of the lead compound C273, which subsequently advanced to

CONCLUSIONS

- Rational drug design led to the development of a novel class of mtCI inhibitors with improved drug-like properties, including the lead candidate C273, which demonstrated robust efficacy, favorable pharmacokinetic properties, and an excellent preclinical safety profile.
- CP2 and C273 bind selectively to the ubiquinone (Q) site of mtCI, producing weak, reversible inhibition that activates AMPK-dependent neuroprotective signaling and protects against A β toxicity with nanomolar concentrations.
- AMPK activation enhanced glucose uptake, GLUT3/GLUT4 translocation, and mitochondrial bioenergetics while promoting pyruvate dehydrogenase activation, increased pyruvate utilization in the TCA cycle, and metabolic reprogramming.
- In cerebral organoids derived from patients with late-onset AD (LOAD), mtCI inhibitors activated adaptive stress responses and significantly reduced A β and pTau levels.

ACKNOWLEDGEMENTS

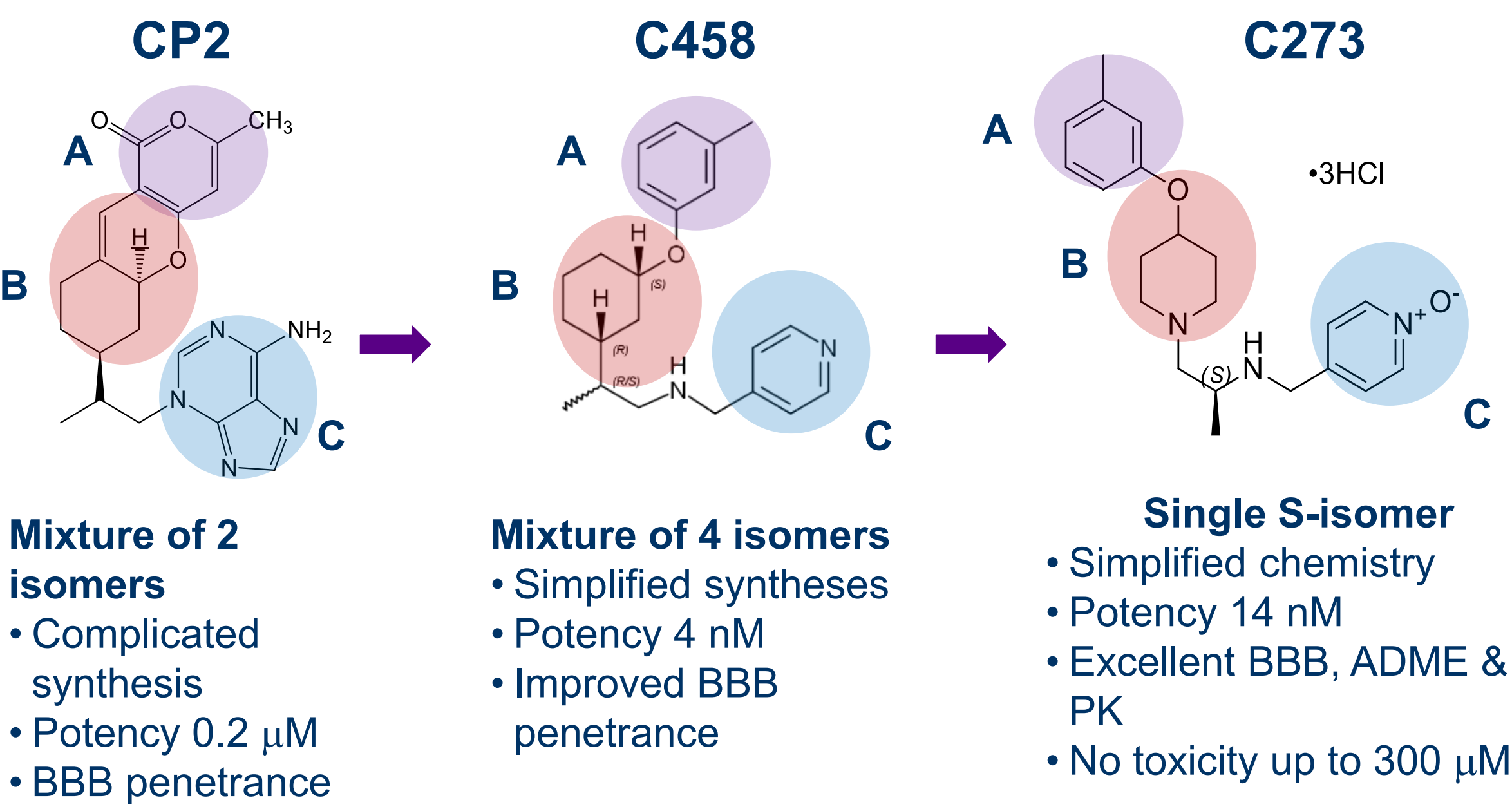
This research was supported by grants from NIH AG 5549-06, NS1 07265, AG 062135, UG3/UH3 NS 113776, and ADDF 291204 (all to ET). Dr. Nguyen is supported by the Alzheimer's Association Research Fellowship grant # 23AARF-1027342.

REFERENCES

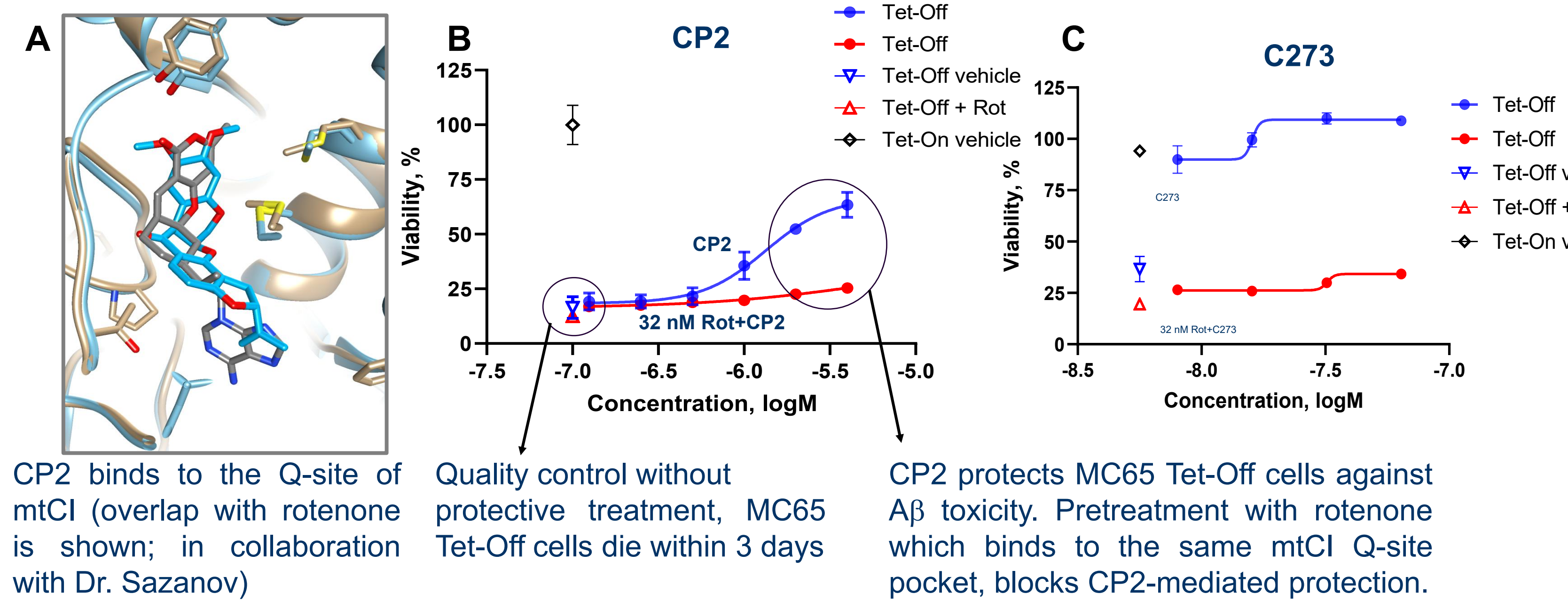
- Trushina E, Nguyen TKO, Trushin S. Modulation of Mitochondrial Function as a Therapeutic Strategy for Neurodegenerative Diseases. J Prev Alzheimers Dis. 2023;10:675-85.
- Trushin, S., Nguyen TKO, et al., Discovery and Preclinical Validation of a Clinically Optimized Mitochondrial Complex I Modulator for Alzheimer's Disease. BioRxiv, 2026, <https://doi.org/10.64898/2026.04.10.717554>
- Trushin S, Nguyen T, et al. Therapeutic assessment of a novel mitochondrial complex I inhibitor in *in vitro* and *in vivo* models of Alzheimer's disease. eBioMedicine. 2025; 120.
- Stojakovic, A., et al., Partial Inhibition of Mitochondrial Complex I Reduces Tau Pathology and Improves Energy Homeostasis and Synaptic Function in 3xTg-AD Mice. J Alzheimers Dis. 2020.
- Stojakovic A, ...Trushina E. Partial inhibition of mitochondrial complex I ameliorates Alzheimer's disease pathology and cognition in APP/PS1 female mice. Commun Biol. 2021;4:61.

RESULTS

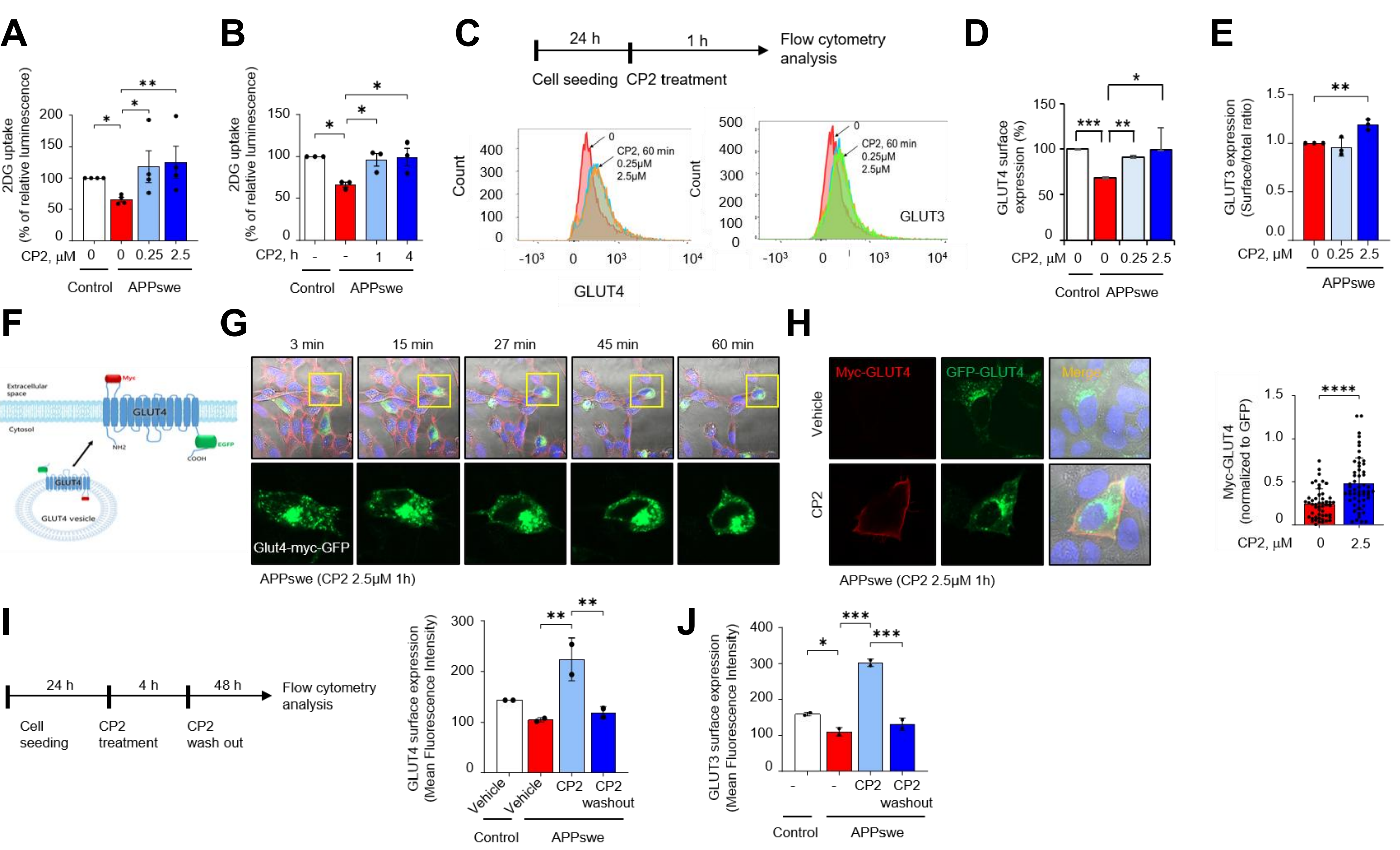
1. Rational design and synthesis of C273



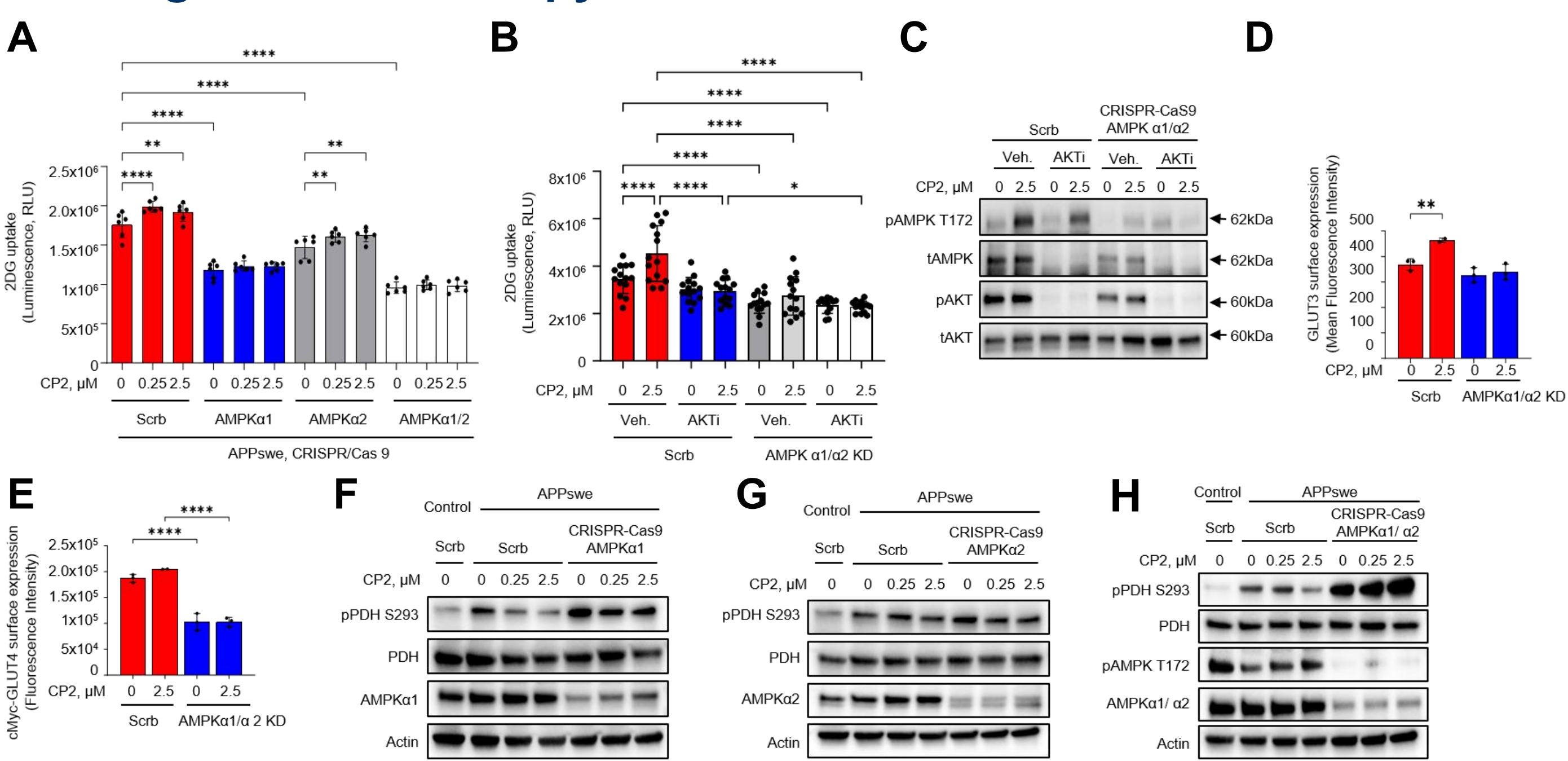
3. mtCI Q-Site Engagement Is Required for Protection Against A β Toxicity in MC65 Cells



6. Modulation of mtCI activity promotes translocation of GLUT3/ GLUT4 to the plasma membrane and glucose uptake in SH-SY5Y APPSwe cells

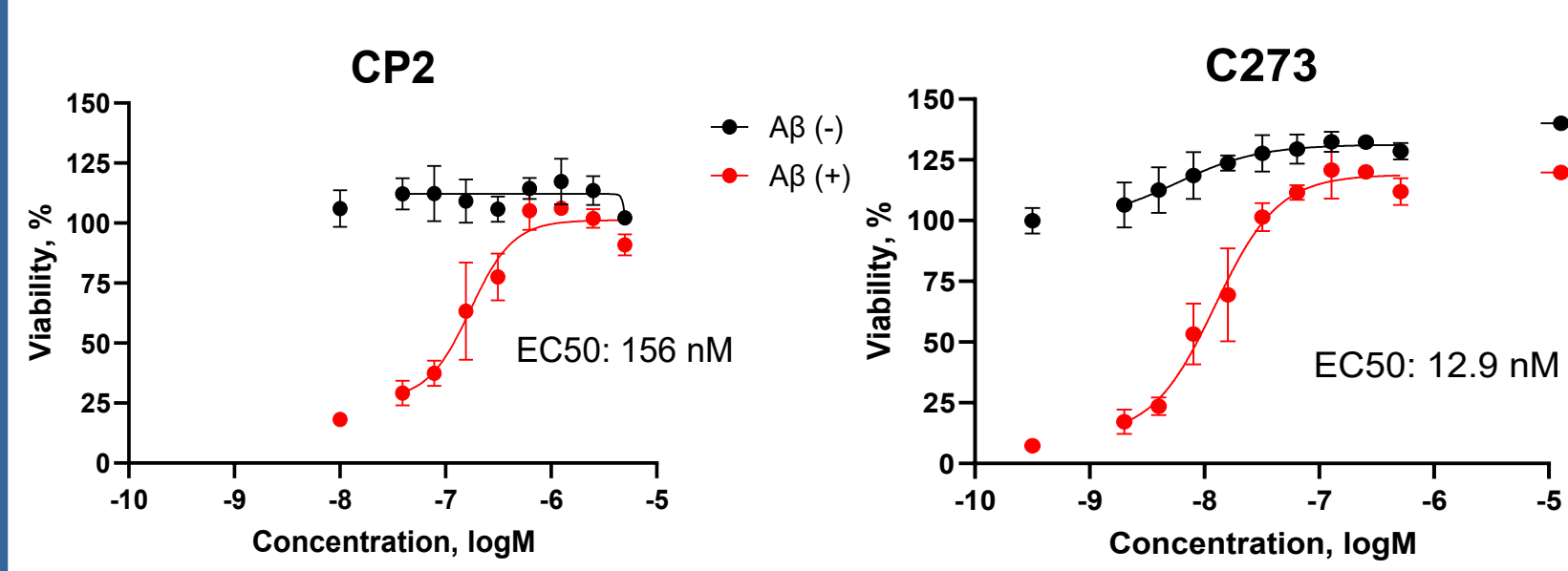


7. AMPKα1 and AMPKα2 are critical for the glucose uptake and the flux of glucose-derived pyruvate into the mitochondria

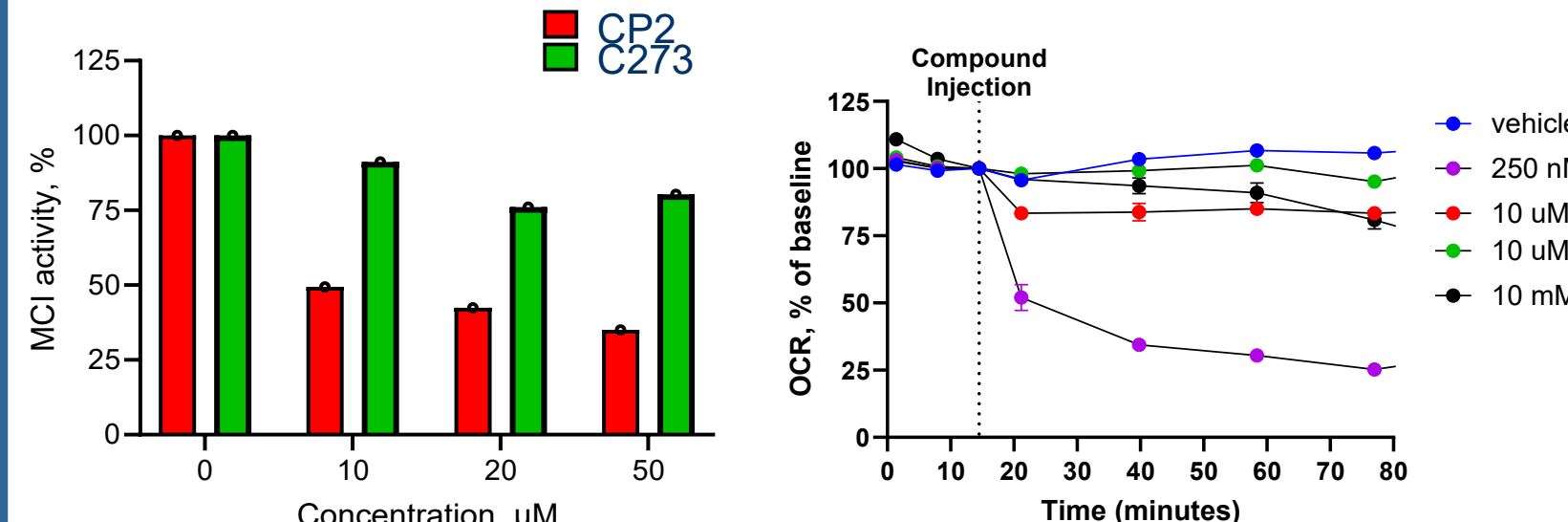


2. *In vitro* primary screens

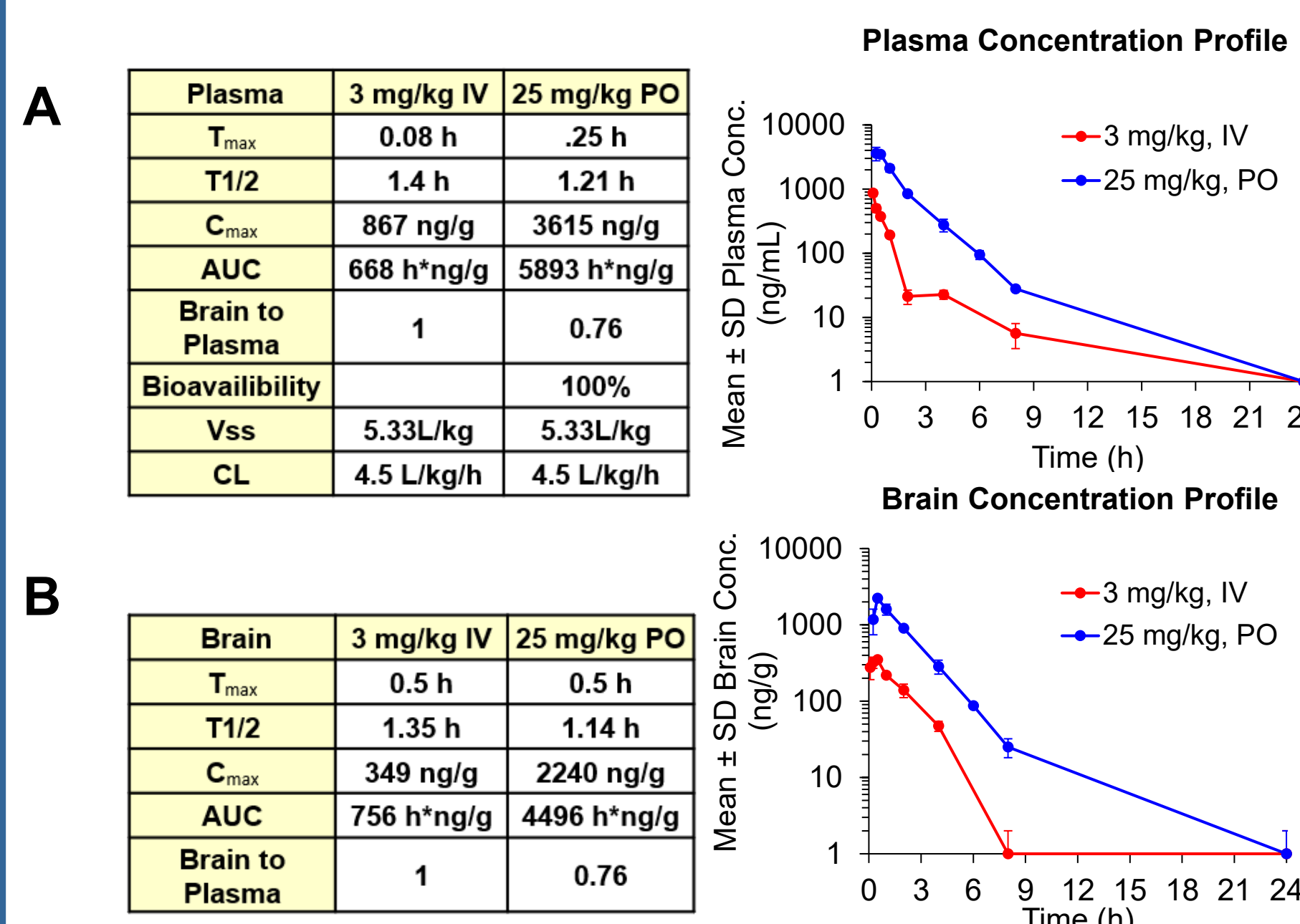
A Evaluation of Cytotoxicity and Potency



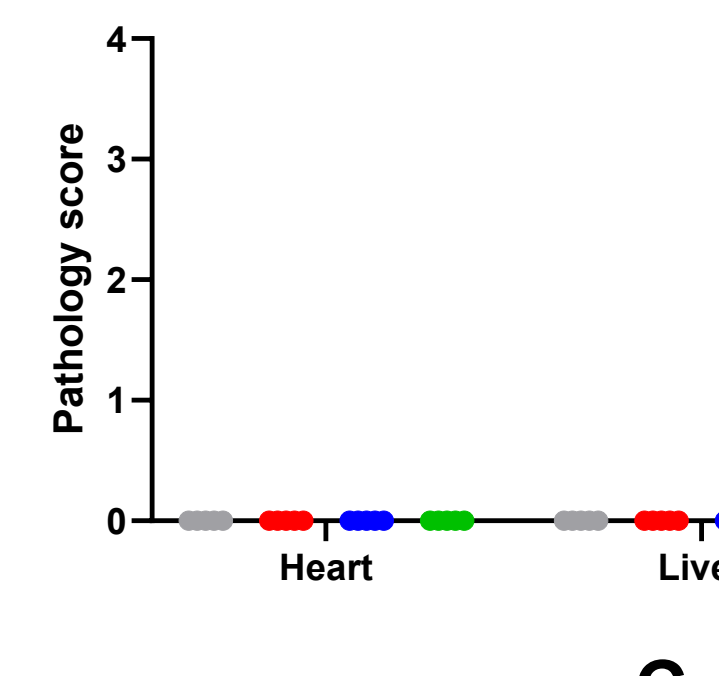
B CP2 and C273 weakly Inhibit mtCI



4. Single-Dose Plasma and Brain Pharmacokinetics of C273 in Mice



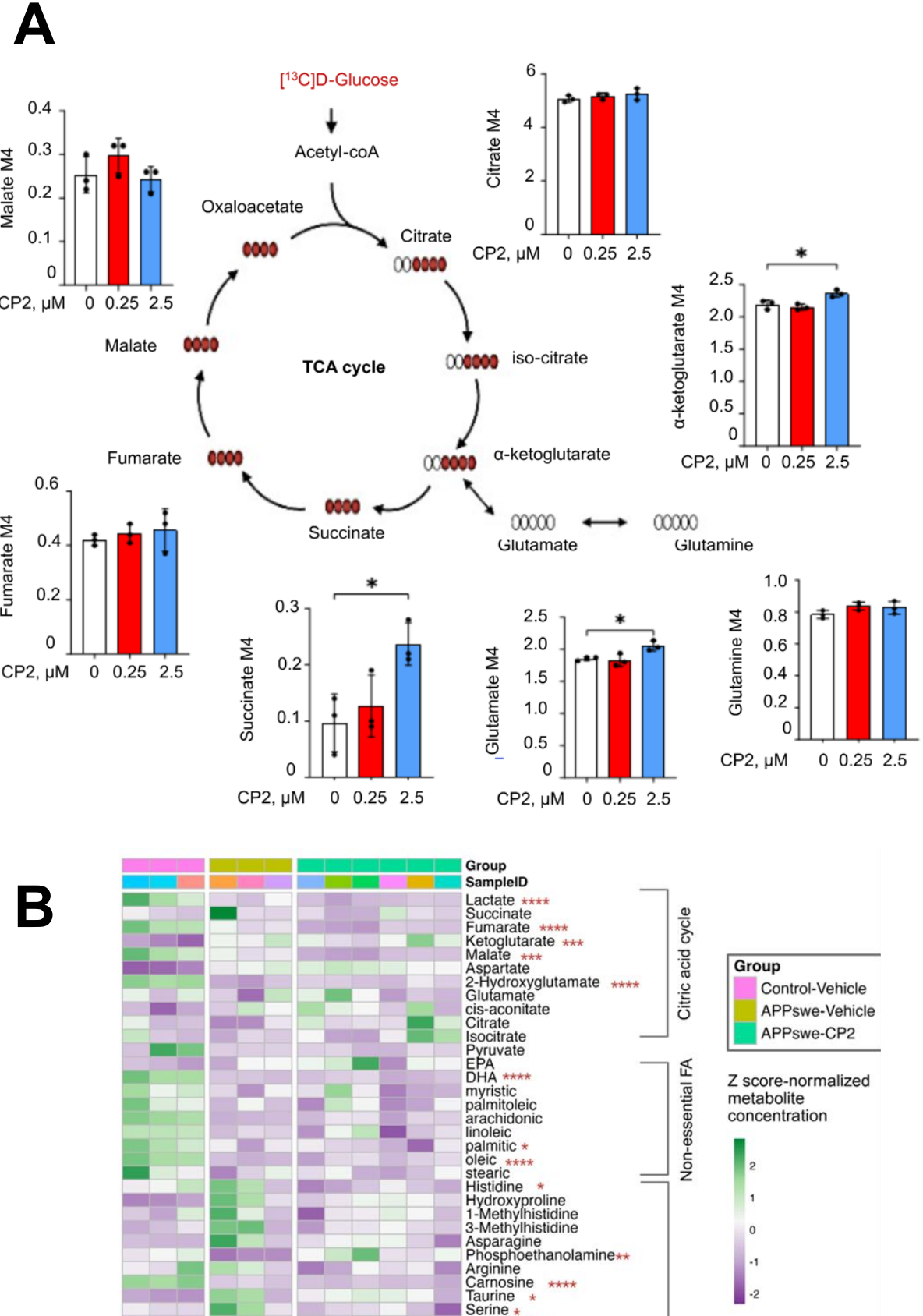
A Pathology Results



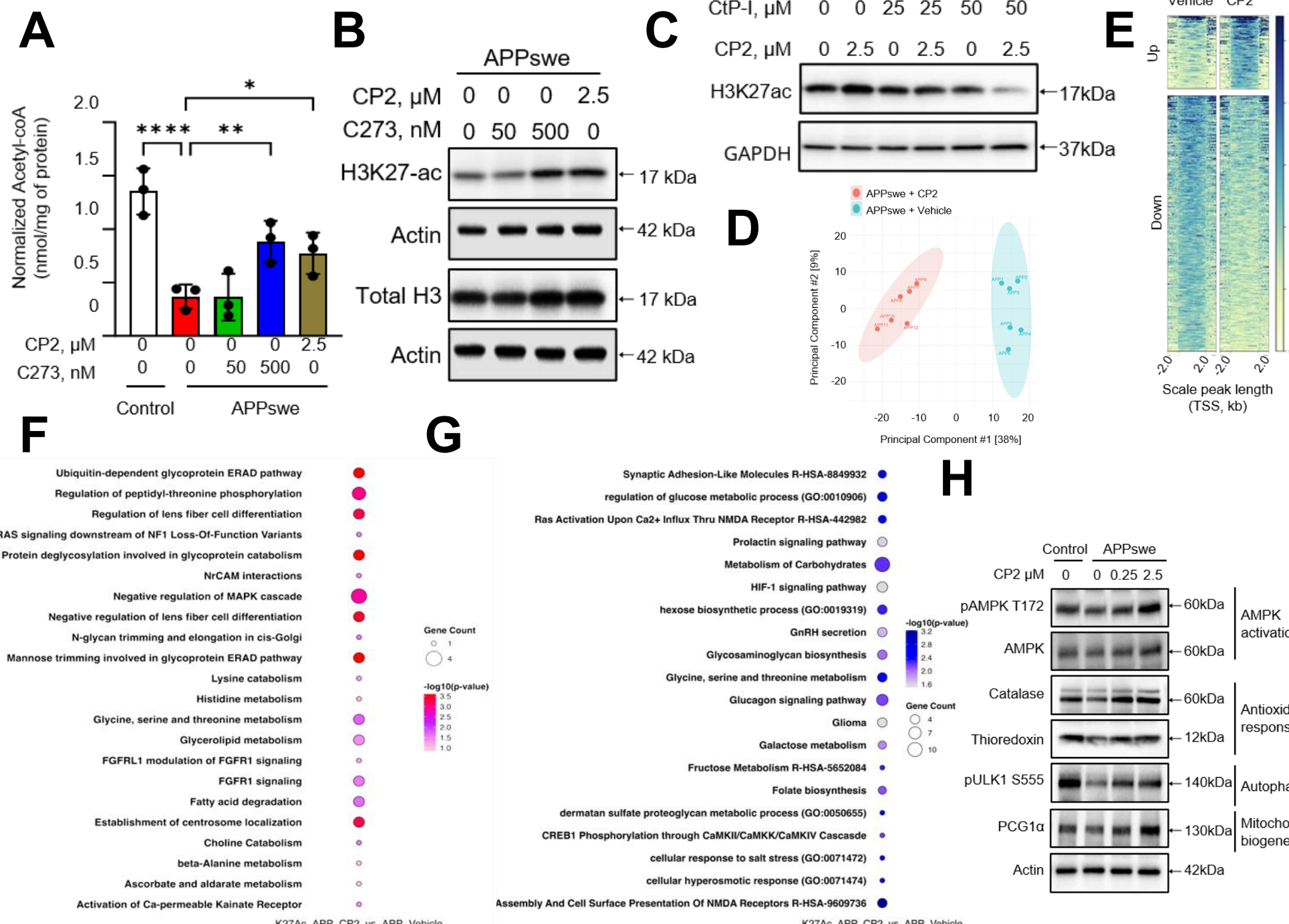
5. Preliminary safety and tolerability of C273



8. mtCI inhibitors promote adaptive mitochondrial metabolic rewiring



9. mtCI Inhibitors Increase Acetyl-CoA and Histone Acetylation, Linking Metabolic Remodeling to Gene Regulation



10. mtCI inhibitors activate AMPK-dependent neuroprotective cascade, reducing A β and pTau (T181) in iPSC-derived organoids from LOAD patients

