Lysosomal enhancement prevents prion propagation

Robert C.C. Mercer[1], Nhat T.T. Le[1], Giada Lavigna[2], Nadia A. Mirza-Romero[1], Jenna Crowell[3], Douglas G. Fraser[4], Aravind Sundaravadivelu[1], Deepthi Yedlapudi[1], Isabel C. Orbe[1], Janelle S. Vultaggio[1], Aaron B. Beeler[4], Glenn C. Telling[3,5], Roberto Chiesa[2], and David A. Harris[1]

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Mechanisms of Neurodegeneration in Human Prion Diseases and Their Intersection with AD/ADRD
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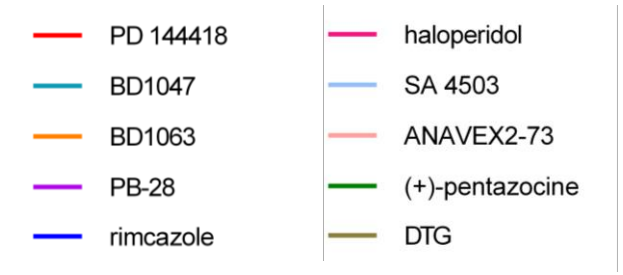
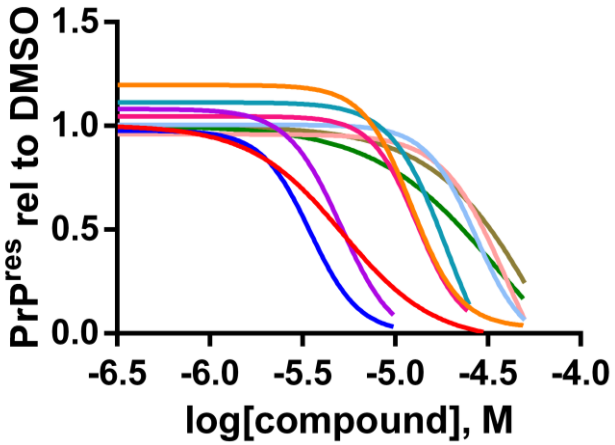
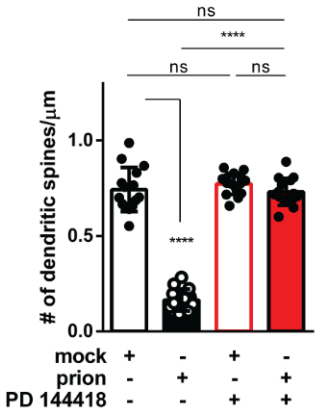
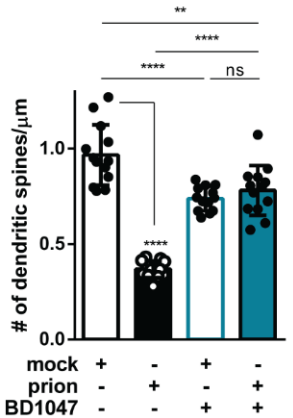
Sigma Receptor Ligands Are Potent Antiprion Compounds that Act Independently of Sigma Receptor Binding

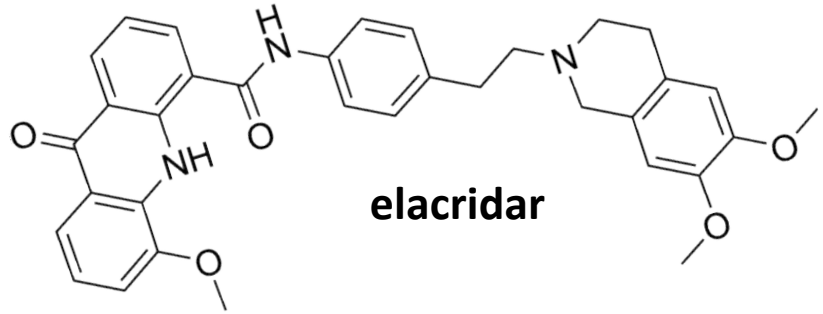
Robert C. C. Mercer, Nhat T. T. Le, Douglas G. Fraser, Mei C. Q. Houser, Aaron B. Beeler, and David A. Harris*

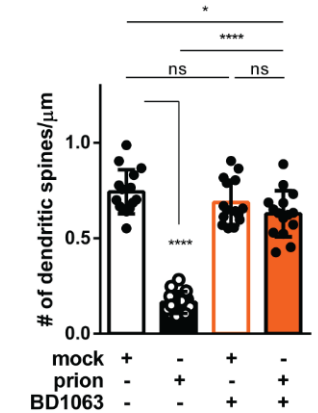


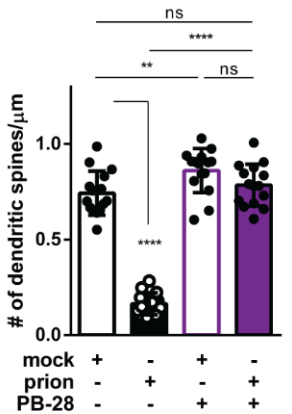












Elacridar is an inhibitor of the xenobiotic efflux pump MDR1 (*Abcb1a/b* in mice) and has been used in clinical trials to prevent chemotherapeutic efflux

Mercer *et al.*, 2024

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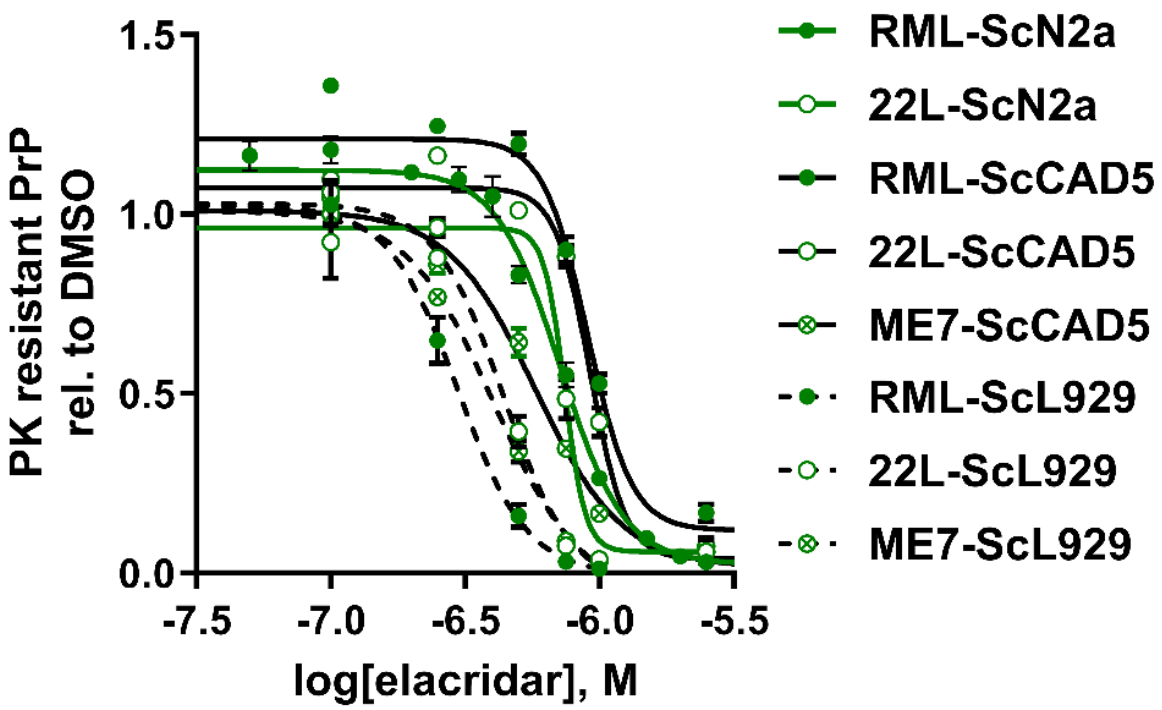
Elacridar is a potent anti-prion compound *in vitro*



Elacridar lowers the levels of PK-resistant PrP in multiple cell types infected with multiple prion strains and permanently cures N2a cells of RML infection

Table 1: Anti-prion properties of elacridar in immortalized cells

Cell line	Tissue origin	EC_{50} (nM)			LC_{50} (nM)
		RML	22L	ME7	
N2a	mouse neuroblastoma	716	743	N/A	7300
CAD5	mouse catecholaminergic	904	926	499	>20,000
L929	mouse fibroblast	298	439	406	4500

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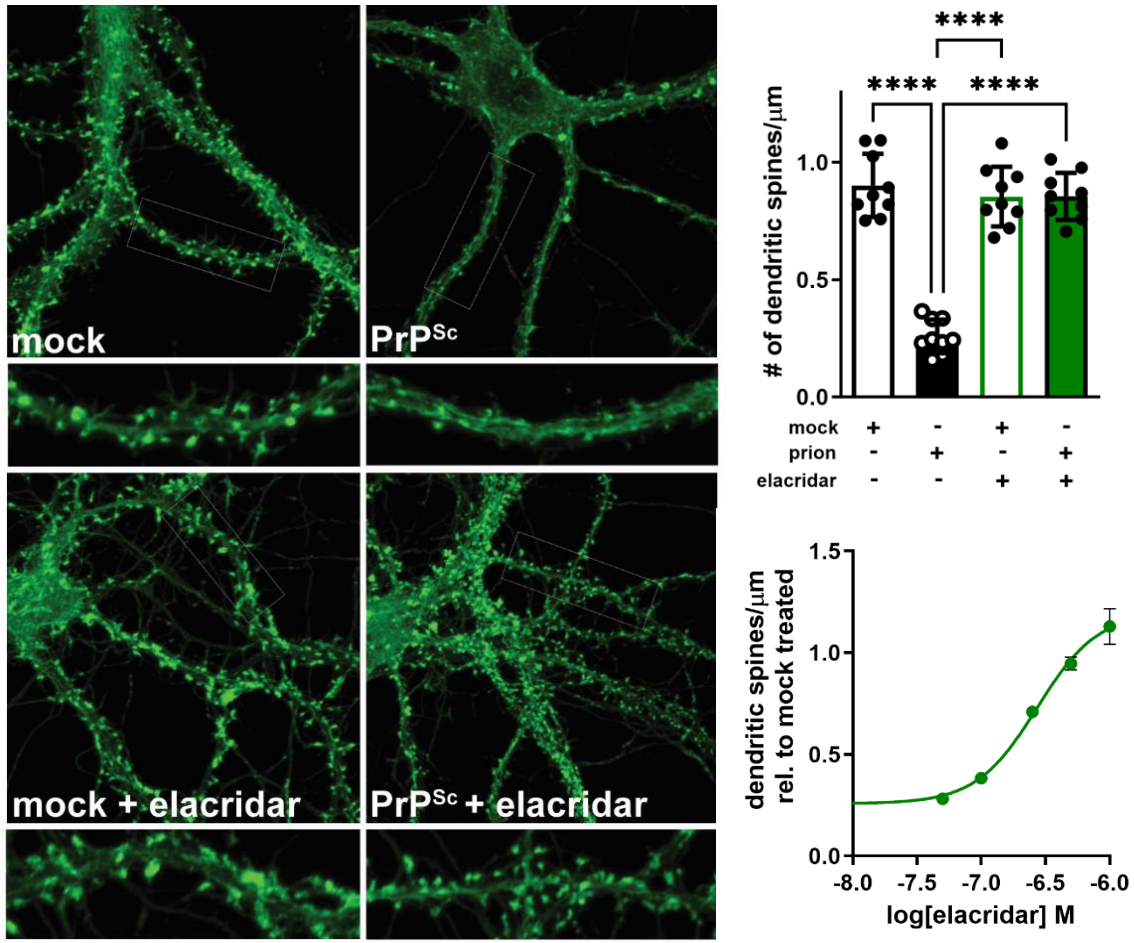
Elacridar is a potent anti-prion compound *in vitro*

Elacridar prevents hippocampal dendritic spine retraction following exposure to purified RML prions



Nhat T.T. Le; Fang *et al.*, 2016; 2018; Imberdis *et* al., 2016; Mercer *et al.,* 2024

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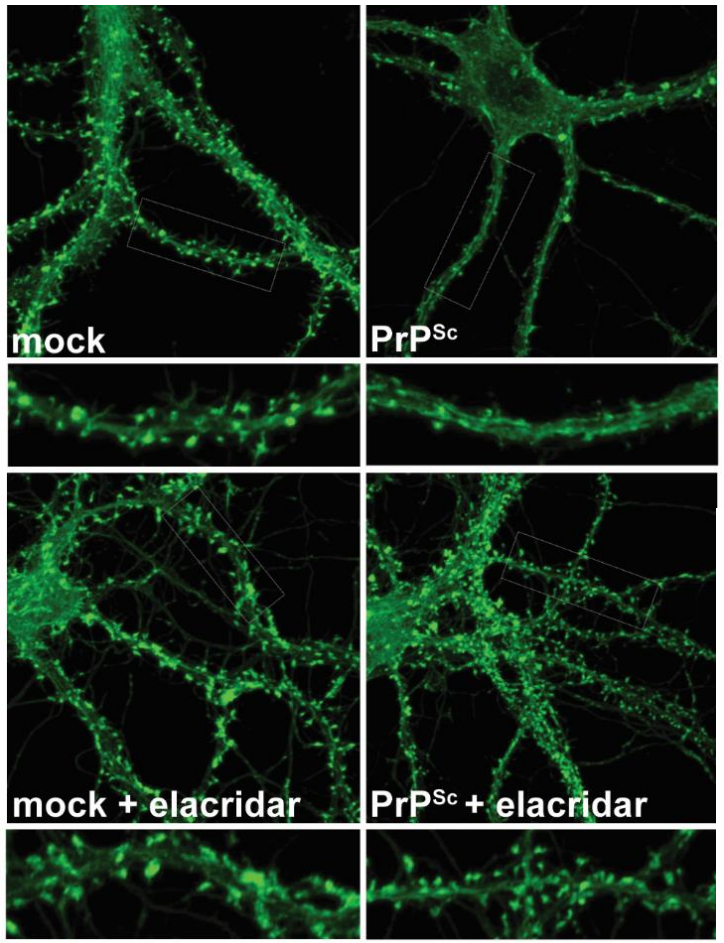
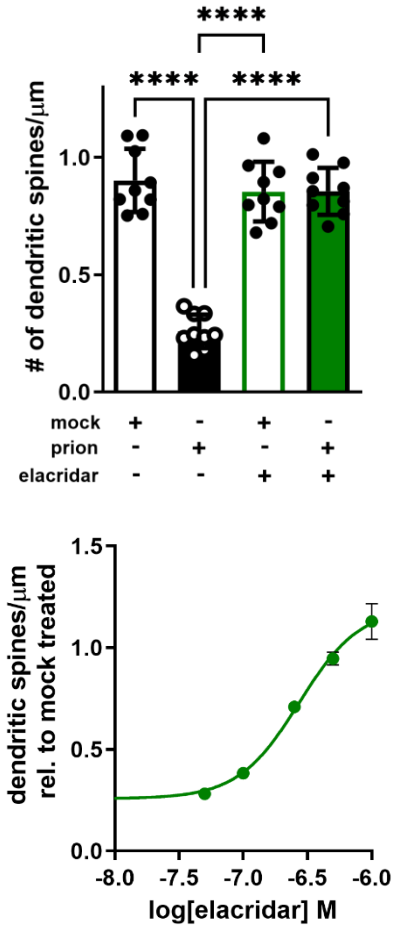
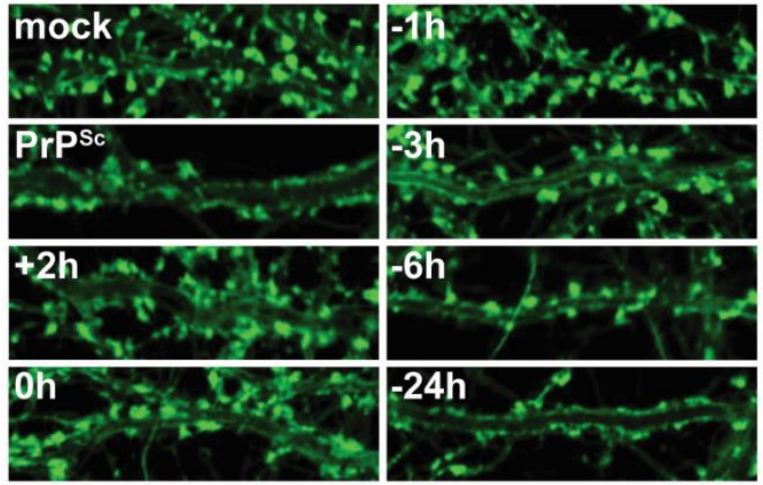
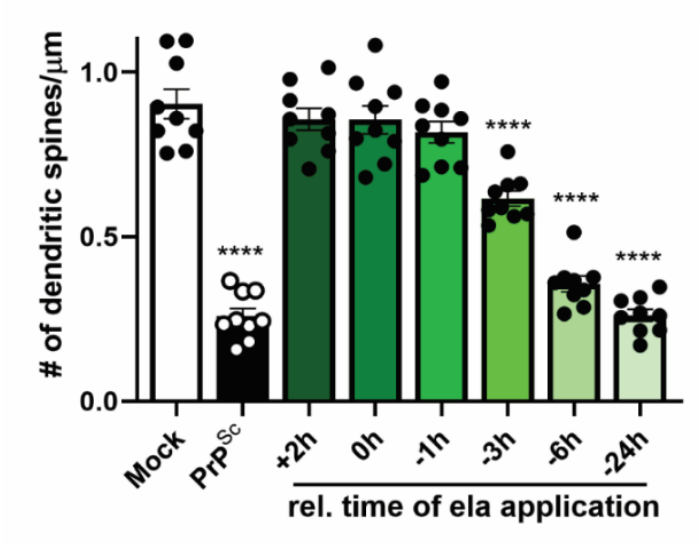
Elacridar is a potent anti-prion compound *in vitro*

Elacridar prevents hippocampal dendritic spine retraction following exposure to purified RML prions… if applied early in the infection process









Nhat T.T. Le; Fang *et al.*, 2016; 2018; Imberdis *et* al., 2016; Mercer *et al.,* 2024

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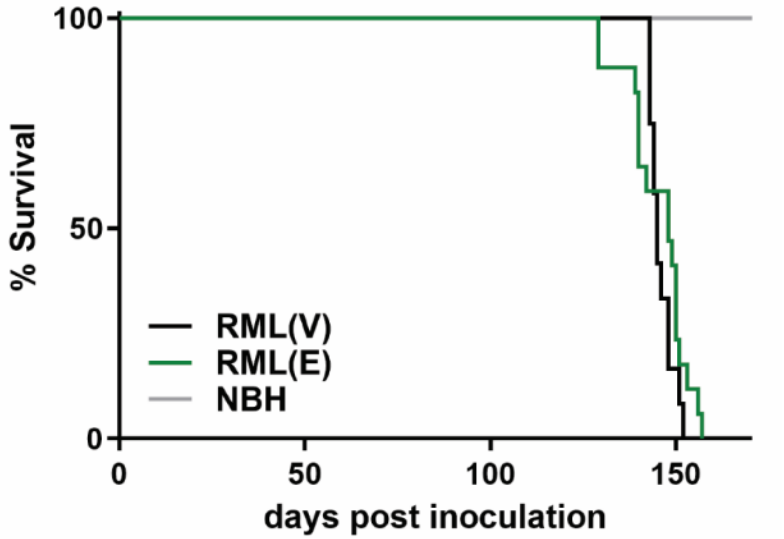
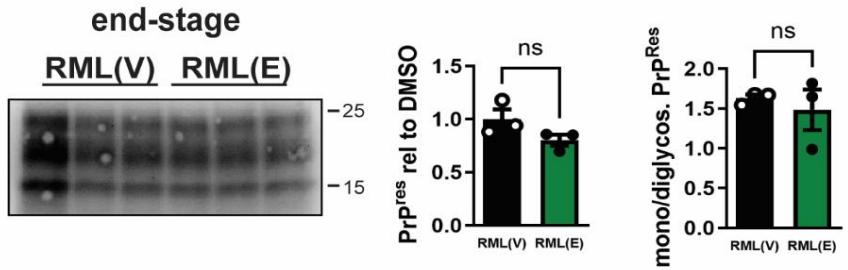
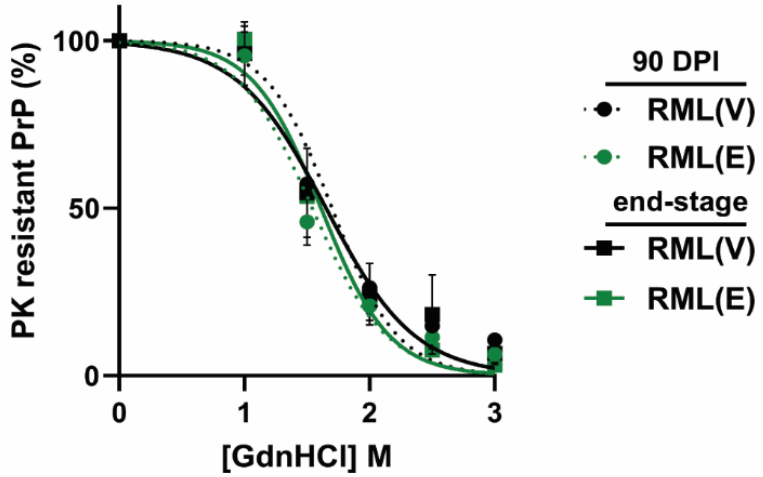
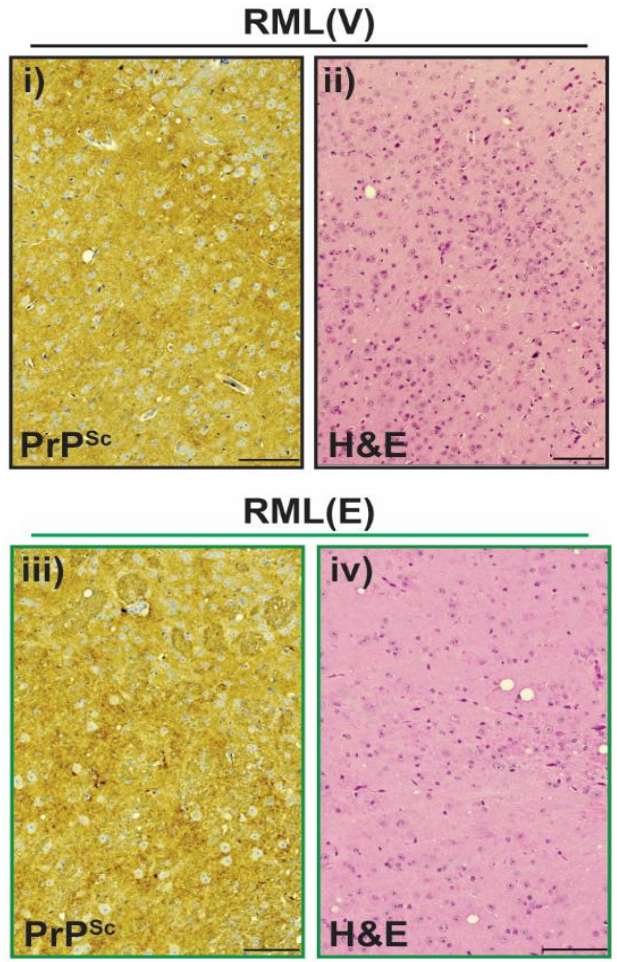
Elacridar is ineffective *in vivo*

Elacridar did not effect:
-incubation time
-biochemical properties of PrP^{Sc}
-neuropathology









Jenna Crowell, Glenn C. Telling

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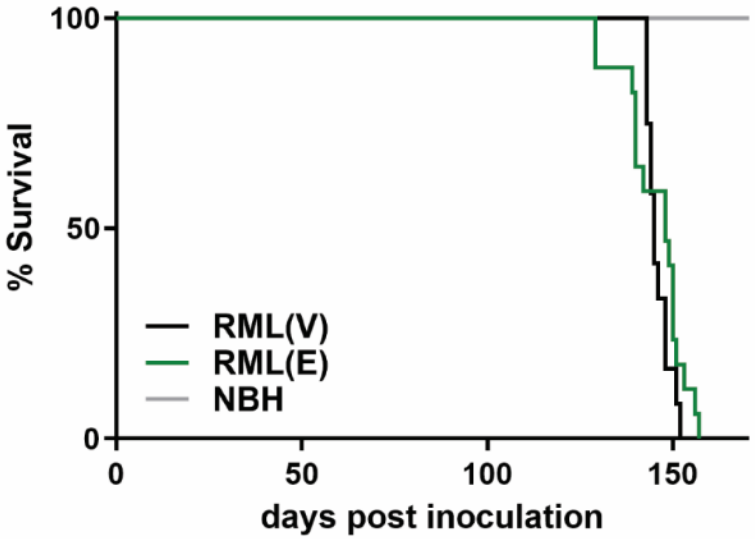

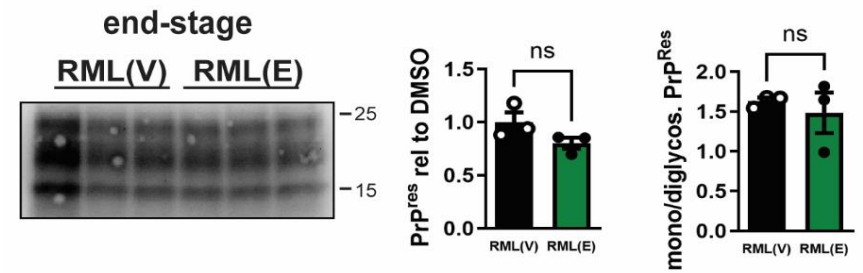

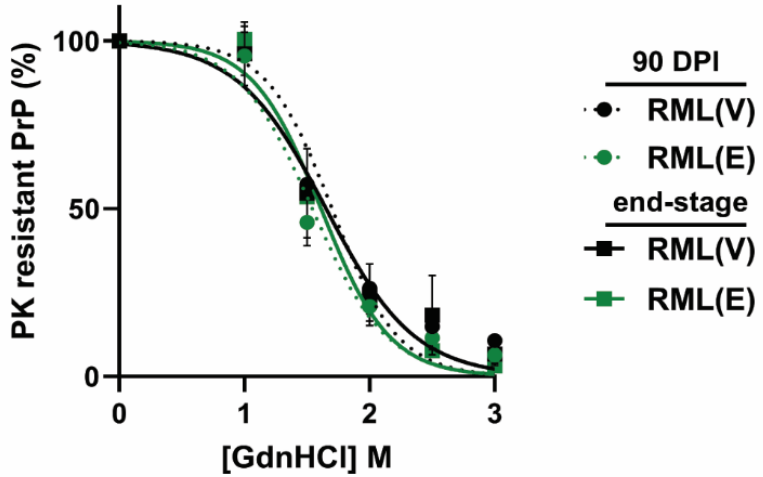

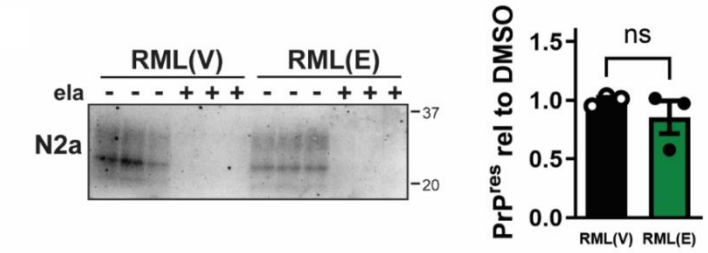

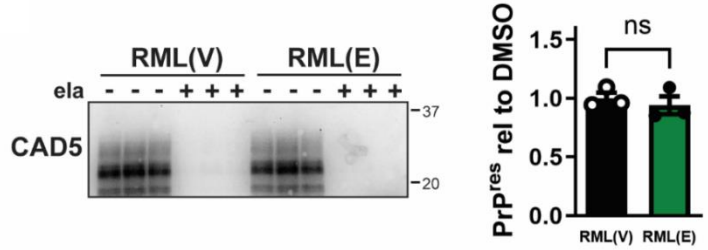

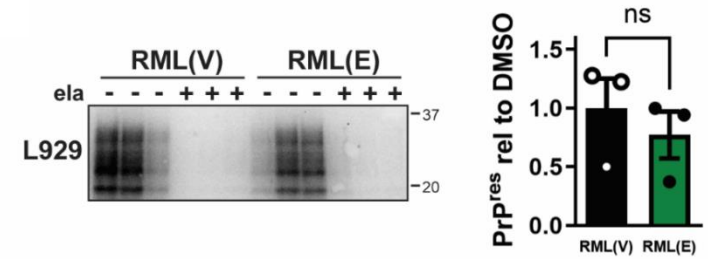

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Elacridar is ineffective *in vivo*

Why?

The post-mitotic nature of neurons prompted us to explore the contribution of cell division to the observed anti-prion effects of elacridar

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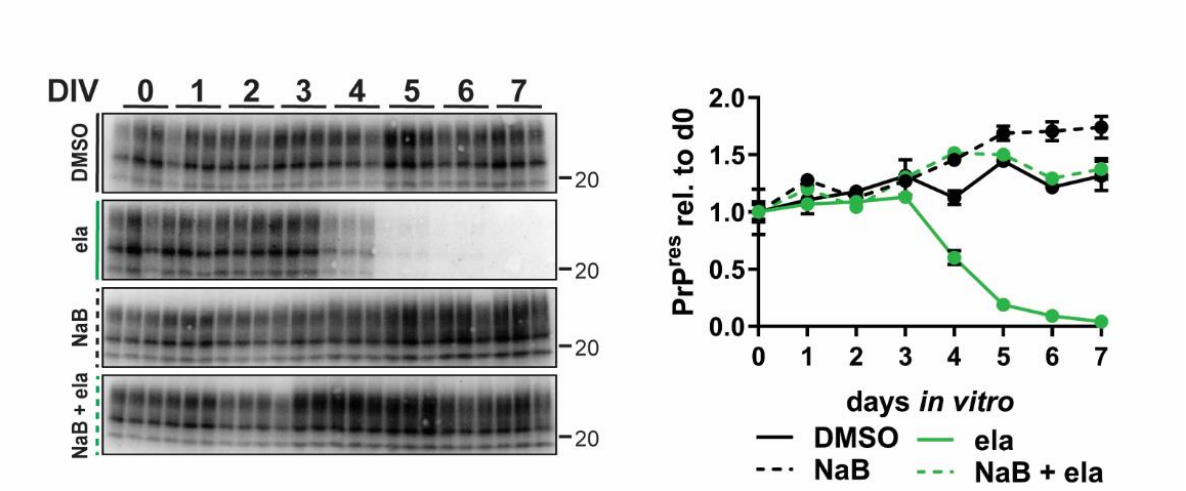
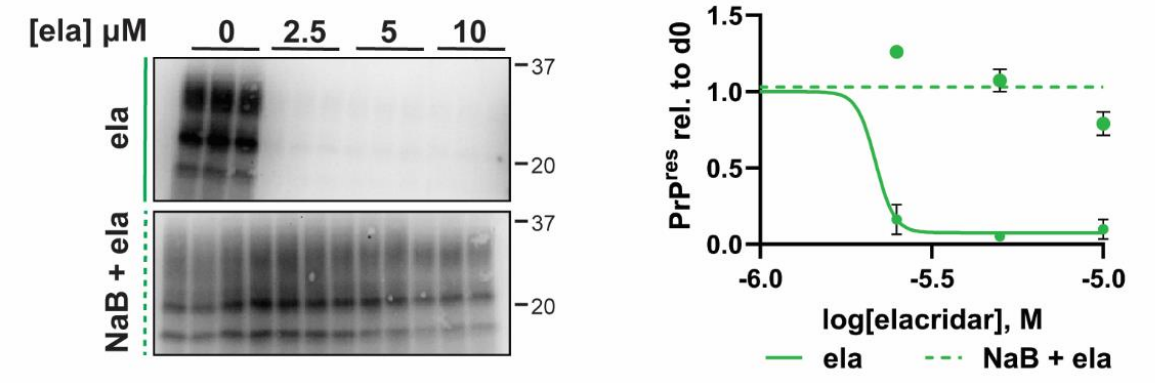
The efficacy of elacridar is diminished in division-arrested cells



When sodium butyrate (NaB) was used to inhibit cell division, 2.5 µM elacridar was ineffective over 7 days



Concentrations of elacridar up to 10 µM also had no effect on NaB treated cultures

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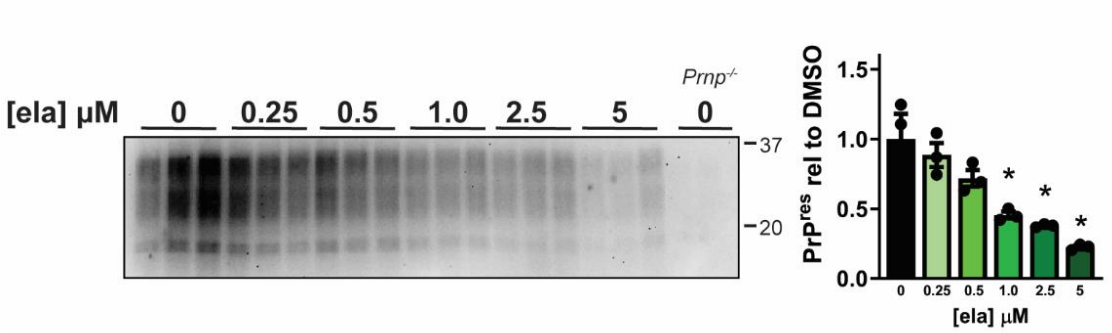
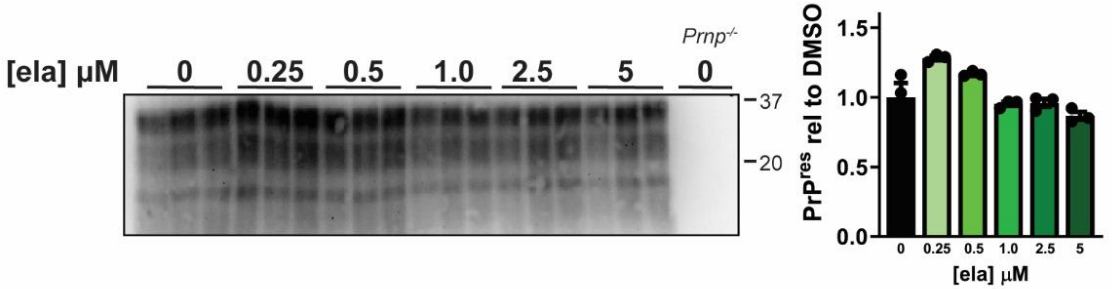
The efficacy of elacridar is diminished in division-arrested cells

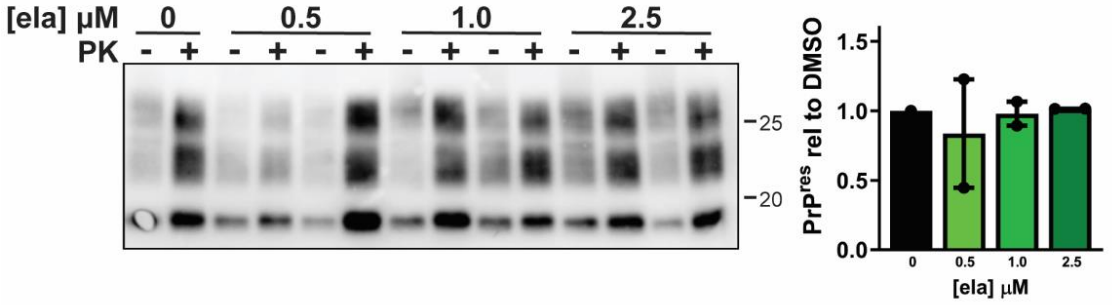
Proliferative astrocyte cultures infected with 22L are sensitive to the anti-prion effects of elacridar



Contact-inhibited astrocyte cultures infected with 22L are ***not*** sensitive to the anti-prion effects of elacridar



Elacridar is ineffective in RML-infected COCS cultures



Nadia A. Mirza-Romero, Giada Lavigna, Roberto Chiesa

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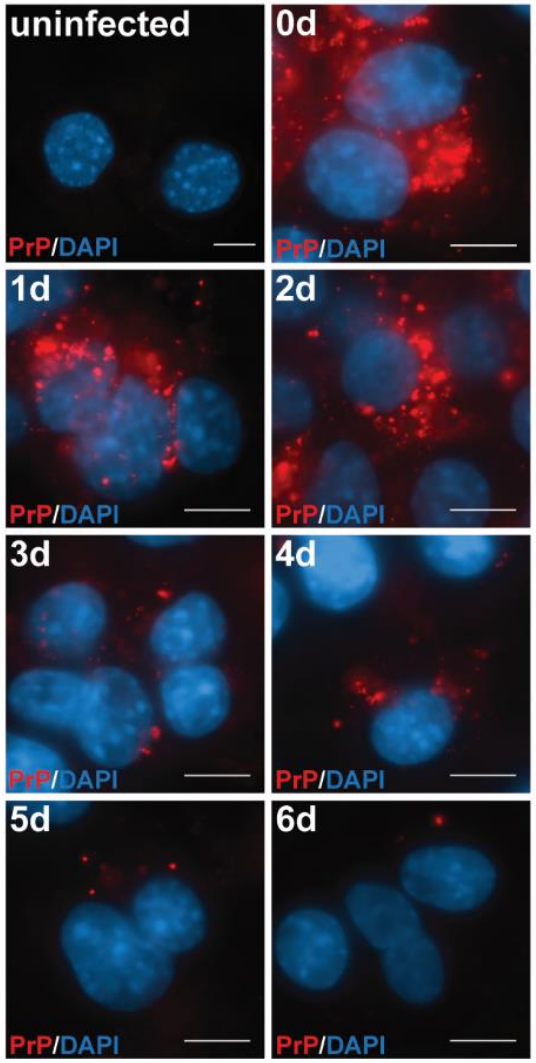
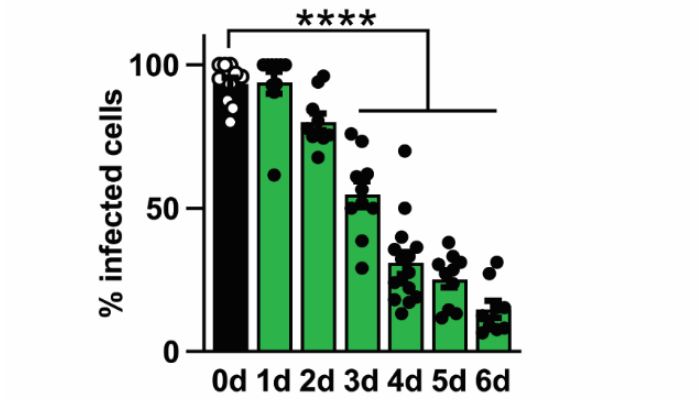
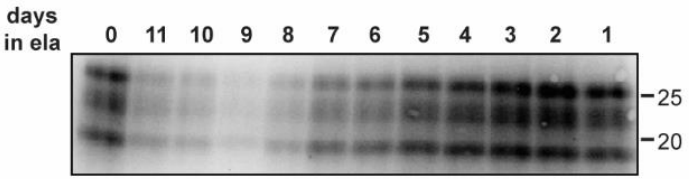
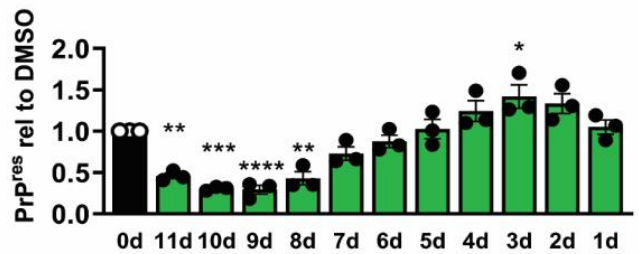
Elacridar prevents the infection of naïve cells



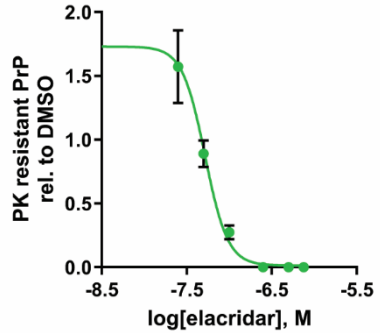






Following PIPLC-resistant PrP over the course of elacridar treatment reveals that the number of infected cells decreases over time

Post-mitotic C2C12 myotube cultures show that elacridar inhibits prion infection when added early in the infection process



Elacridar prevents the infection of N2a cells
EC_{50} = 52 nM

Herbst *et al.*, 2013

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Elacridar prevents the infection of naïve cells

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 279, No. 28, Issue of July 9, pp. 29218–29225, 2004
Printed in U.S.A.

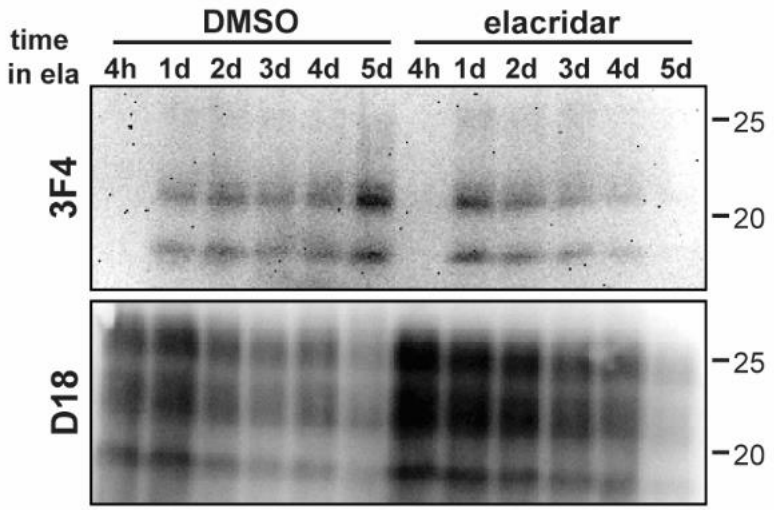
Acute Formation of Protease-resistant Prion Protein Does Not Always Lead to Persistent Scrapie Infection *in Vitro**

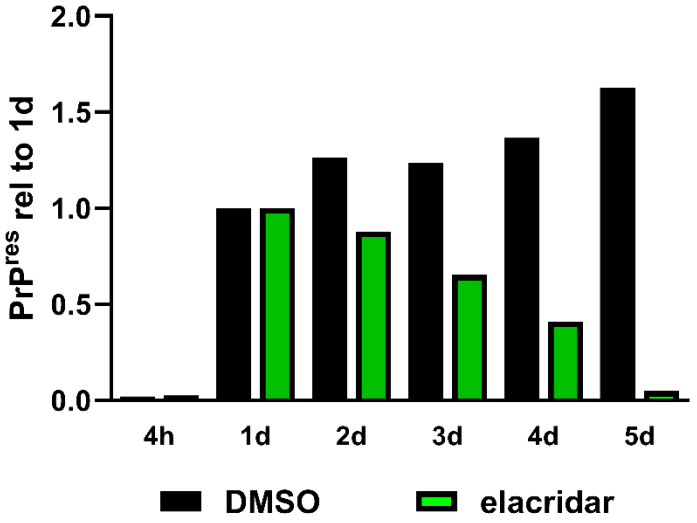
Ina Vorberg‡, Anne Raines, and Suzette A. Priola§

From the Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, NIAID, National Institutes of Health, Hamilton, Montana 59840

3F4 tagged PrP can be used to follow the earliest PrP conversion events, ignoring exogenous prions

PK-resistant PrP forms in the presence of elacridar and disappears by d5





Vorberg *et al.*, 2004

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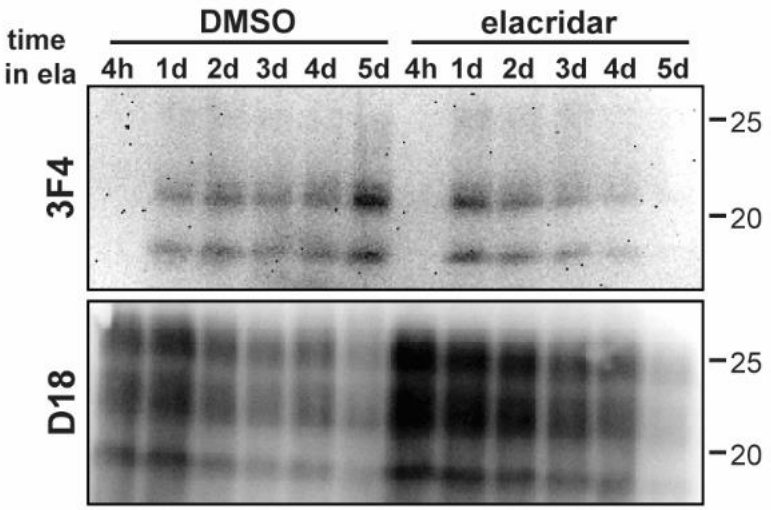

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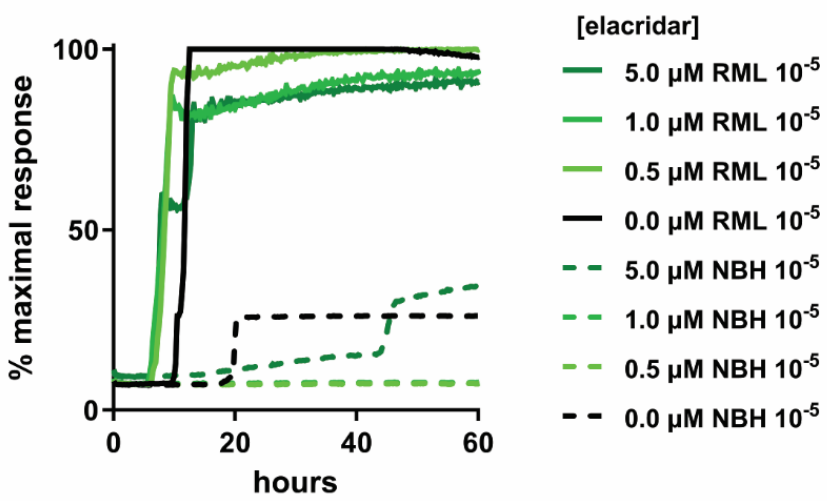
Elacridar does not inhibit the seeding activity of RML prions by RT-QuIC



Vorberg *et al.*, 2004

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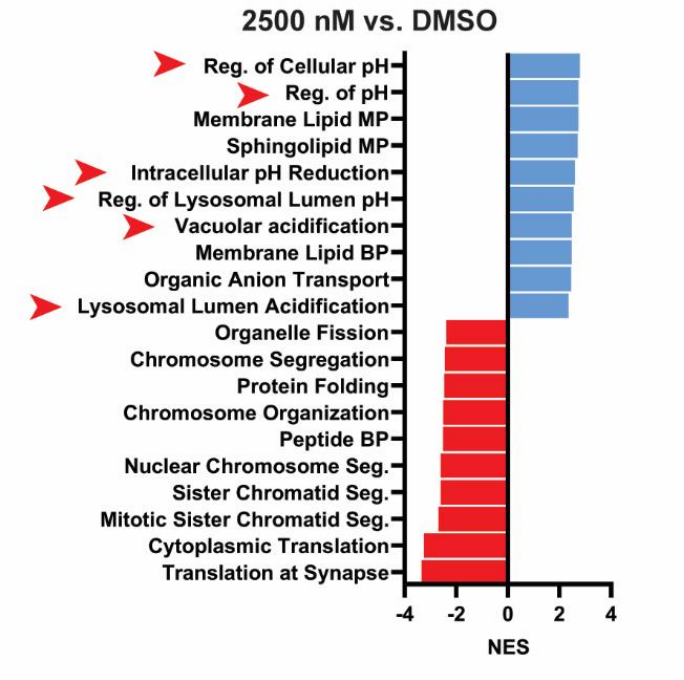
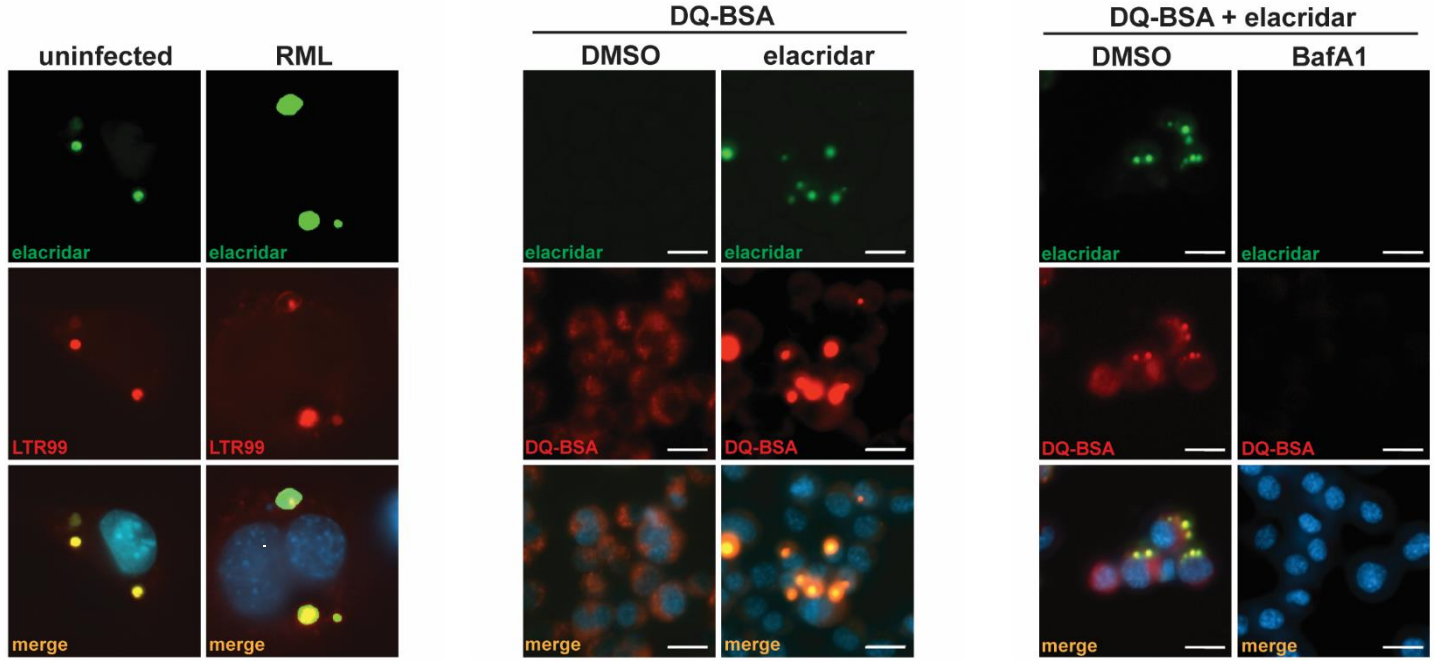
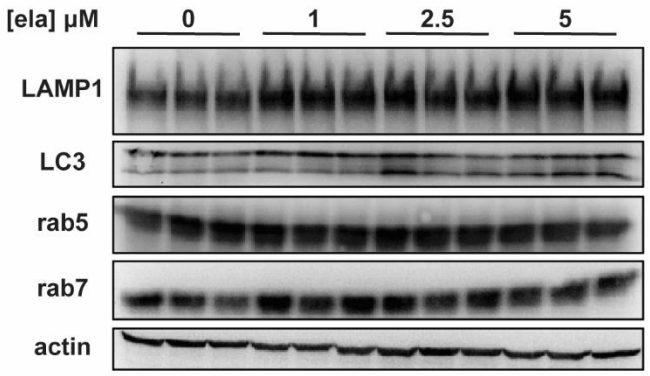
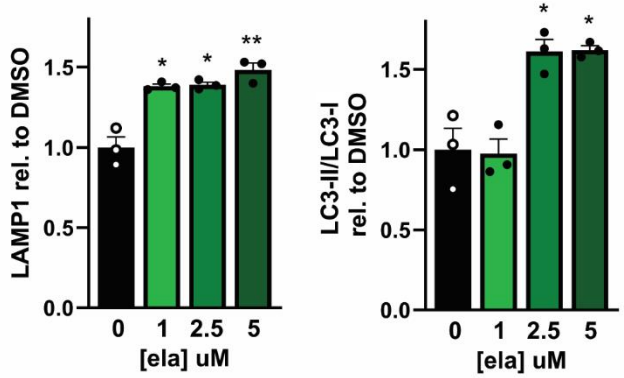
Elacridar concentrates in lysosomes, increases their activity, and activates autophagy









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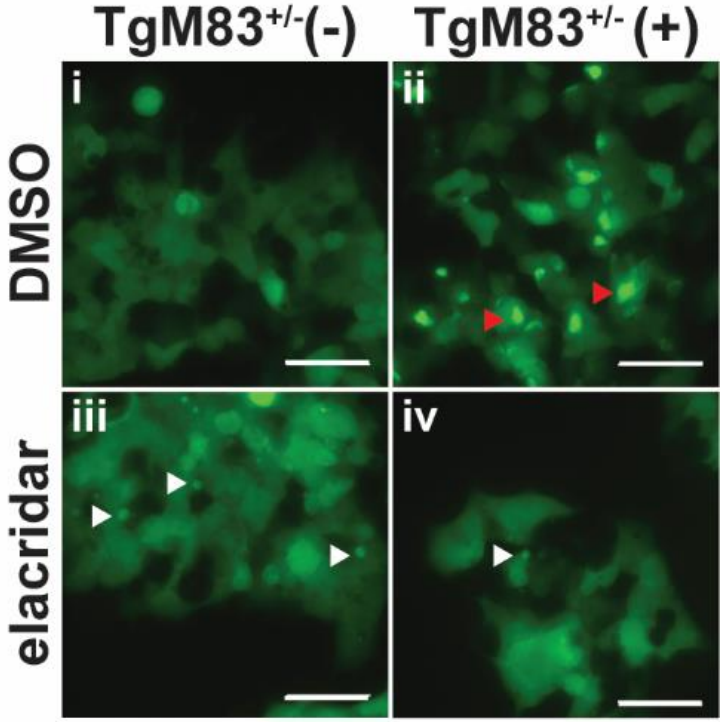
Elacridar inhibits the templated misfolding of α-syn and tau

These effects of elacridar prompted us to investigate its impact upon the transmission of other pathological protein assemblies

Elacridar prevents the misfolding of α-synuclein-YFP and tauRD(LM)-YFP biosensors by exogenous PTA precipitates of pathologically folded α-synuclein and tau



Giasson *et* al., 2002; Allen *et* al., 2002; Watts *et* al., 2013; Woerman *et al.*, 2015; Prusiner *et al.*, 2015

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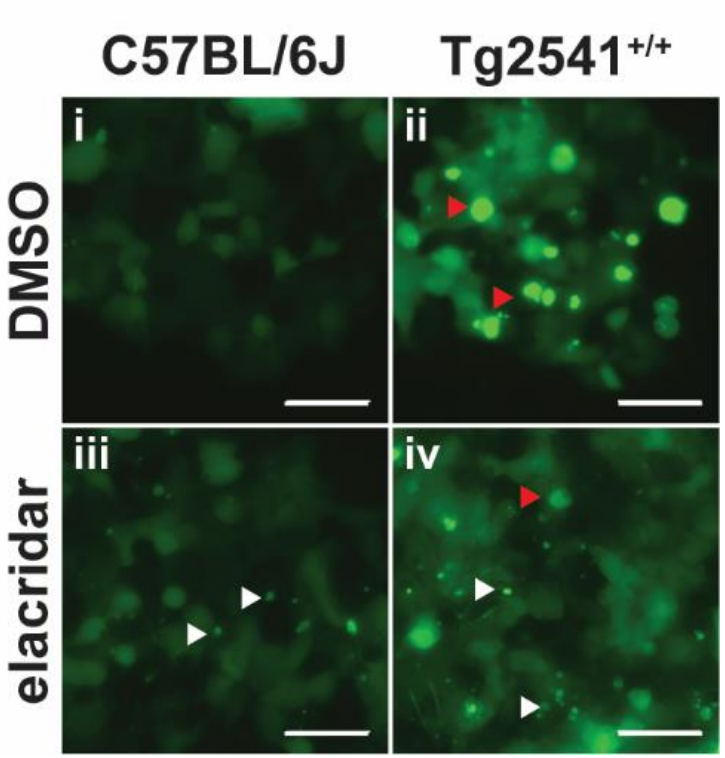

Giasson *et* al., 2002; Allen *et* al., 2002; Watts *et* al., 2013; Woerman *et al.*, 2015; Prusiner *et al.*, 2015

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Summary & Conclusions

1. **Elacridar prevents the prion infection of naïve cells**

   Dividing vs non-dividing cultures

2. **Elacridar activates lysosomes and/or autophagy**

   Current efforts are directed at identifying the relevant target of elacridar

3. **Elacridar prevents the spread of α-synuclein and tau prions**

   Suggests common mechanisms to be exploited for therapeutic intervention

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Aaron B Beeler
Doug G. Fraser

Colorado State University
Glenn C. Telling
Jenna Crowell
Amanda L. Woerman

Istituto di Ricerche Farmacologiche Mario Negri
Roberto Chiesa
Giada Lavigna

DZNE Bonn
Ina Vorberg

University of Toronto
Joel C. Watts

Rocky Mountain Laboratories
Brent Race
Byron Caughey

NIH National Institute of Neurological Disorders and Stroke

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CJD FOUNDATION

CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.

Supporting Families Affected by Prion Disease