Development of rAAV-ADAM8/shRNA Mediated PrP-Lowering Gene Therapy for CJD

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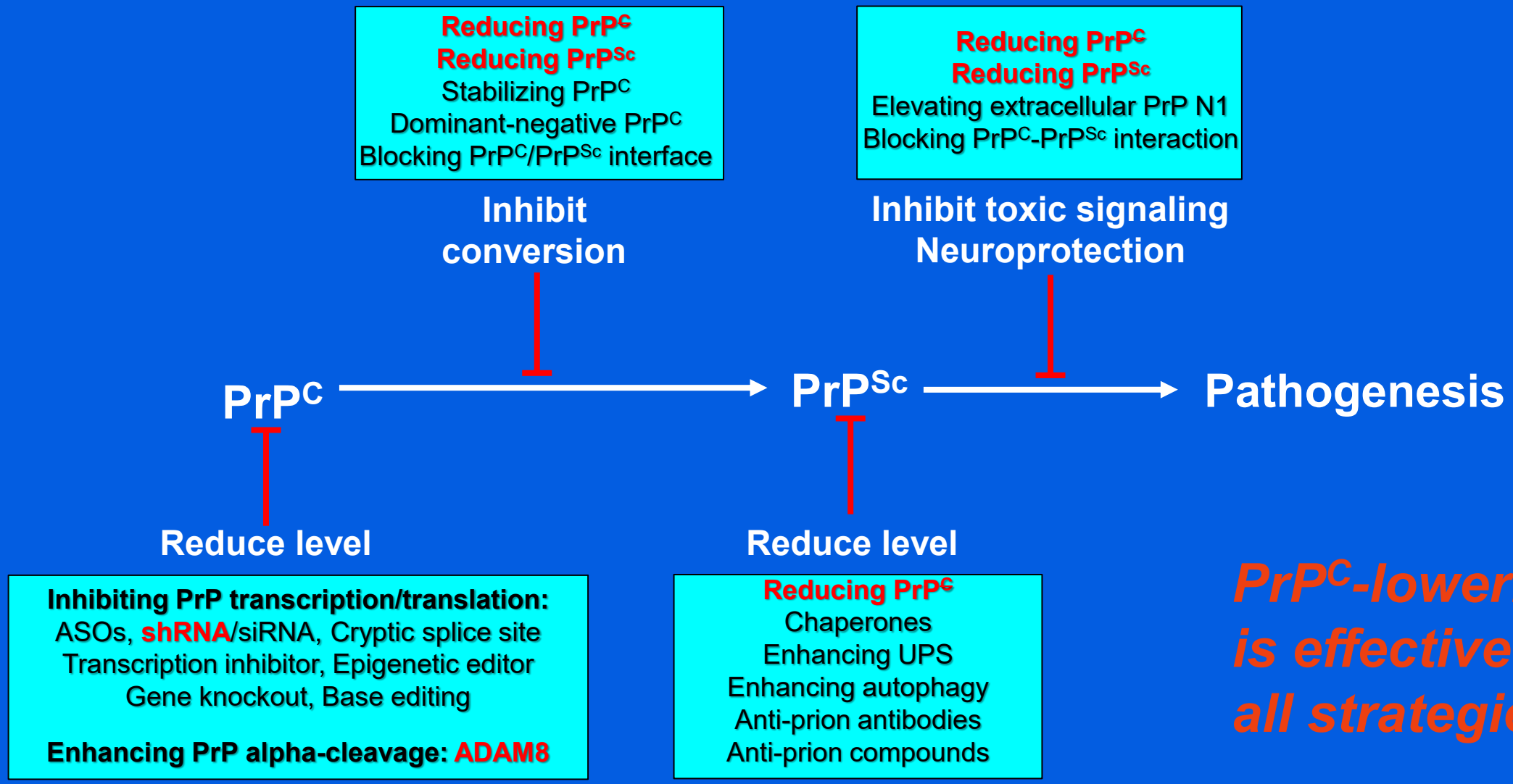
Prion Protein (PrP) and Prion Diseases

- Prion disease is always fatal and no treatment is available. Effective treatment is badly needed.

- The cellular PrP (PrP^C) is the central player in prion diseases, essential for both prion replication and prion pathogenesis, but it is dispensable for life, making it the ideal target for interventions.

- The PrP^C protein also plays a critical role in several more common neurodegenerative diseases by serving as a receptor for toxic oligomers of β-amyloid and/or tau in Alzheimer's disease and other tauopathies, α-synuclein oligomers in Parkinson's disease and other synucleinopathies.

Strategies for prion therapy development



PrP^C-lowering is effective in all strategies.

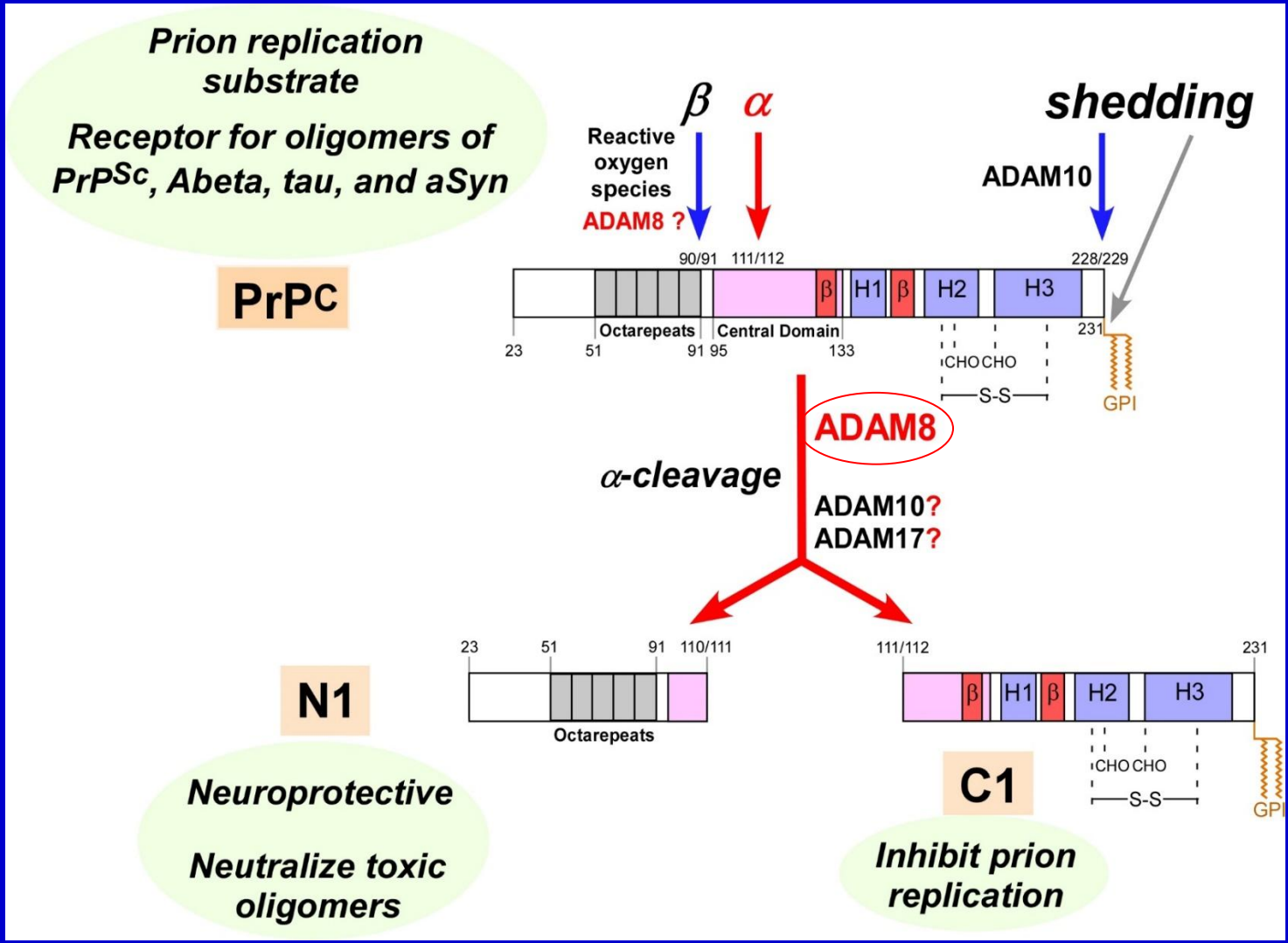
Previous prion therapy development efforts

- Small molecule anti-prion compounds, such as quinacrine, doxycycline, and pentosan polysulfate, worked well against rodent prions in rodent models, but they all failed against human prions in clinical trials.
- A small-scale clinical trial with a “humanized” anti-PrP monoclonal antibody (PRN100) is inconclusive and did not establish clear clinical benefits.
- Antisense oligonucleotides (ASOs)/siRNA targeting PrP mRNAs achieved markedly prolonged survival in mouse model studies using mouse-adapted prion strains.
- Sangamo’s AAV-based ZFR inhibition of PRNP gene transcription led to significant lowering of brain PrP levels and survival extension in mice and non-human primates.
- **David Liu and collaborators just published an exciting report demonstrating that prophylactic AAV-mediated base editing targeting human PrP at a dose of 1x 10^{14} vg/kg led to 50% reduction in brain PrP level and 52% survival extension in a humanized mouse model inoculated with genetic or sporadic CJD.**

Our strategy: Developing gene therapy for CJD using CJD-infected humanized mice

- Reducing brain PrP^C levels with rAAV-shRNAs (targeting human PrP mRNA) administered systemically at clinical onset.
- Enhancing the beneficial PrP^C α-cleavage in the brain with rAAV-ADAM8 administered systemically at clinical onset.
- rAAV capsids: Three BBB-penetrating AAV capsids (PHP.eB or eB, CAP.B10 or B10, and AAV.CPP16 or CPP16) used for rAAV packaging
- CJD mouse models: Tg40 and Tg40h mice overexpressing wild type human PrP (at 1.5x or 3.0x WT level, respectively) in the FVB strain background.

Cleavages of PrP Protein



Cellular Prion Protein Regulates Its Own α-Cleavage through ADAM8 in Skeletal Muscle*

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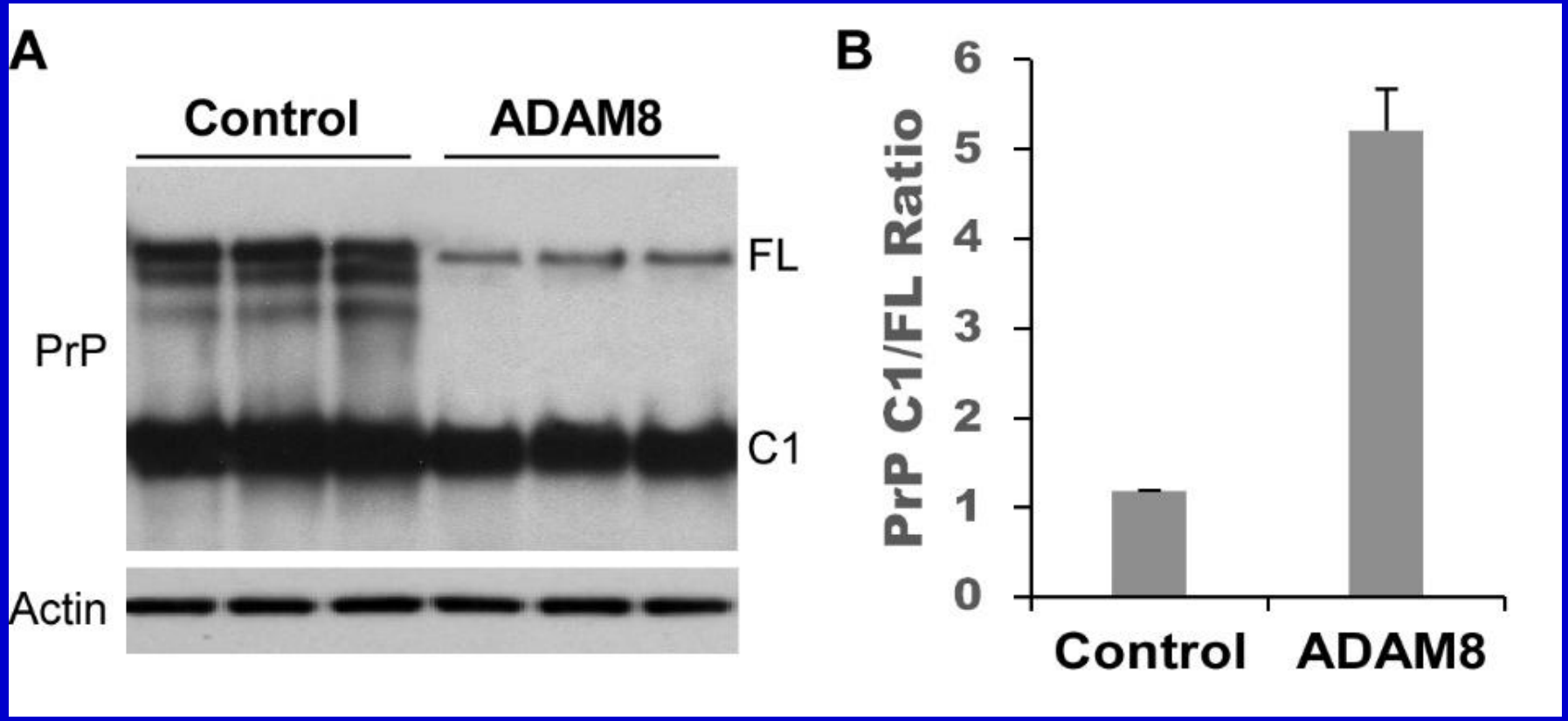
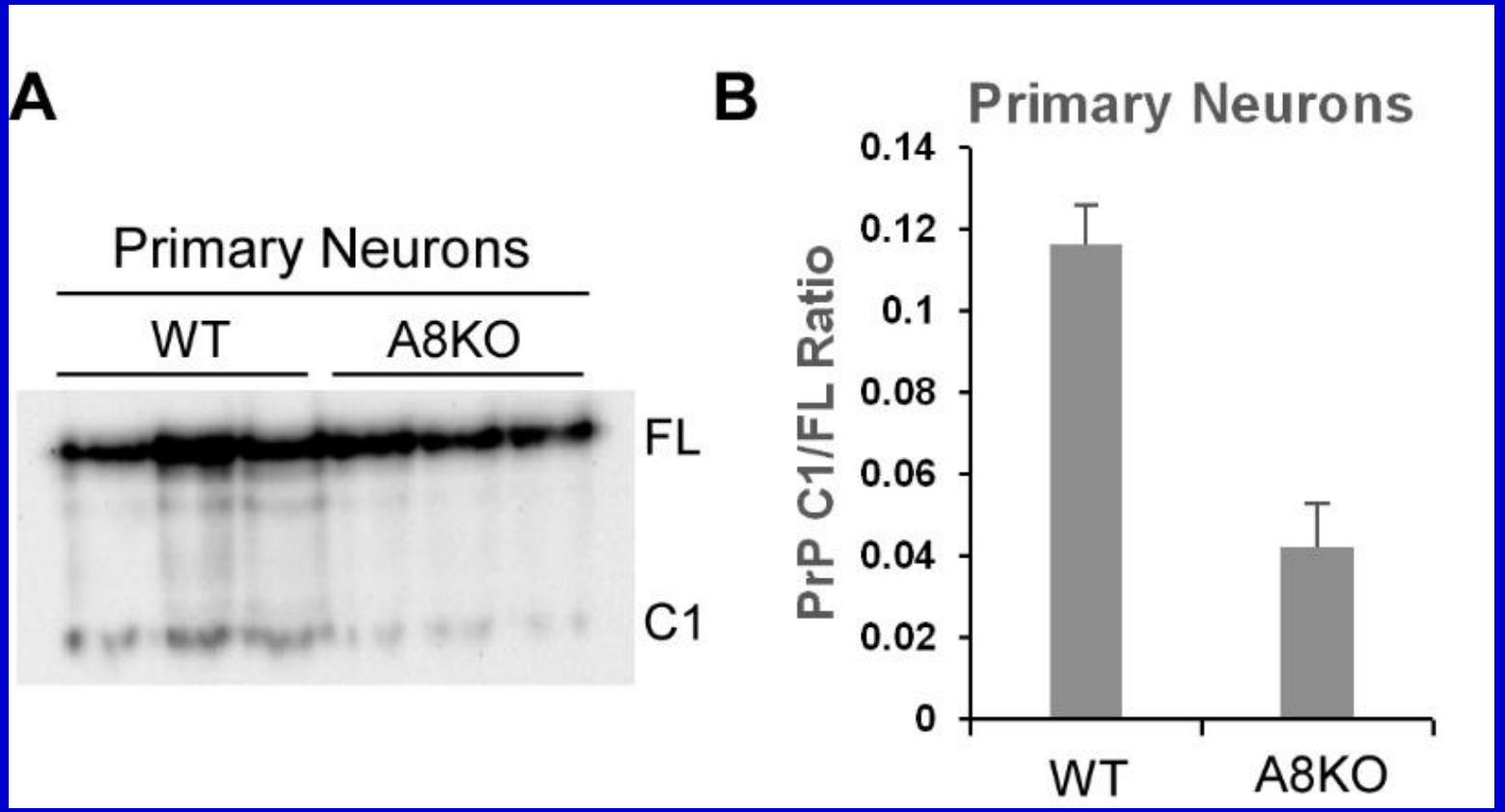
ADAM8: the primary alpha-cleavage enzyme for PrP^C in skeletal muscle

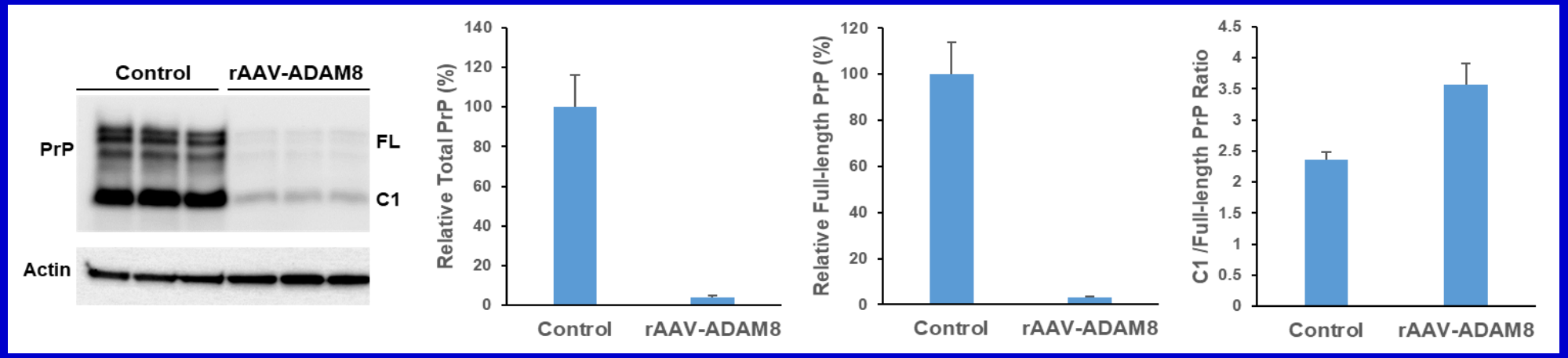
ADAM8 overexpression from a plasmid dramatically elevates PrP alpha-cleavage activity in human neuroblastoma (M17) cells



PrP alpha-cleavage activity drops by >60% in primary neurons from ADAM8-KO (A8KO) mice

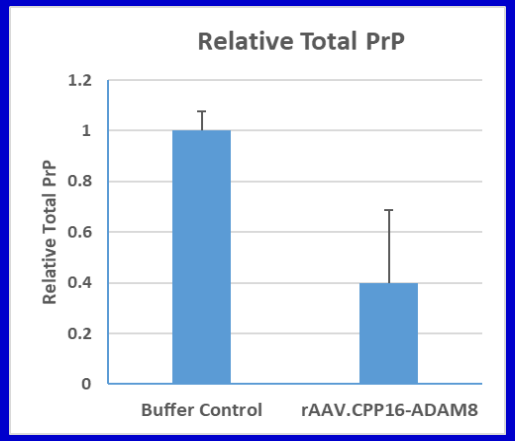


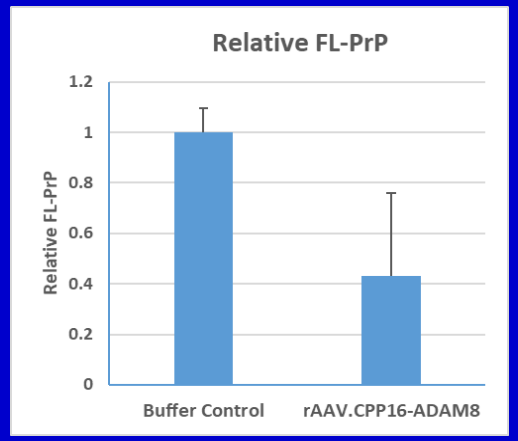
rAAV-mediated ADAM8 overexpression dramatically reduces PrP levels and elevates PrP α-cleavage in human neuroblastoma cells

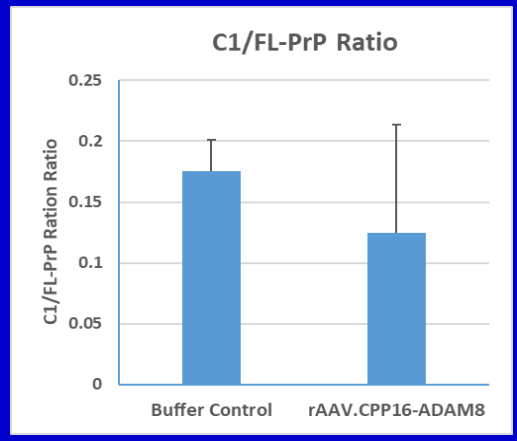


Total PrP: ↓95.8% *FL-PrP: ↓96.9%* *C1/FL-PrP ratio: ↑52.3%*

Systemic delivery of rAAV.CPP16-ADAM8 significantly reduces PrP levels in the brains of transgenic mice expressing human PrP

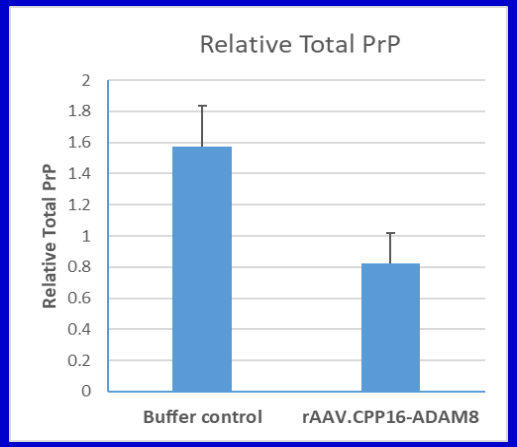


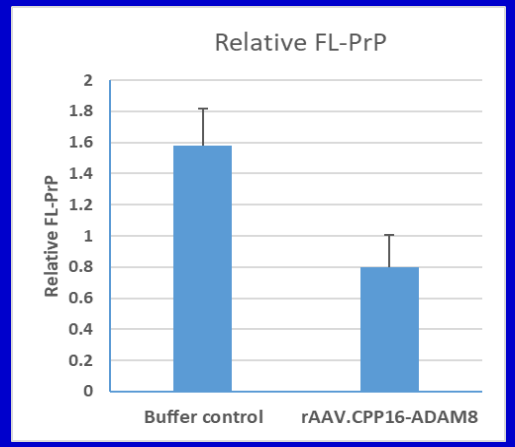


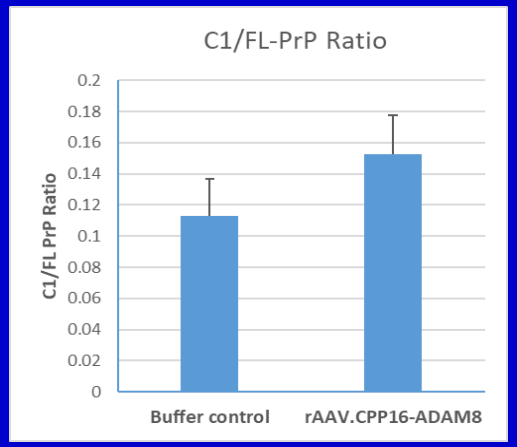


Mice	PrP Level
Tg40:	1.5x WT

Total PrP: ↓60.1% ***FL-PrP: ↓57.0%*** ***C1/FL PrP ratio: ↓28.8%***



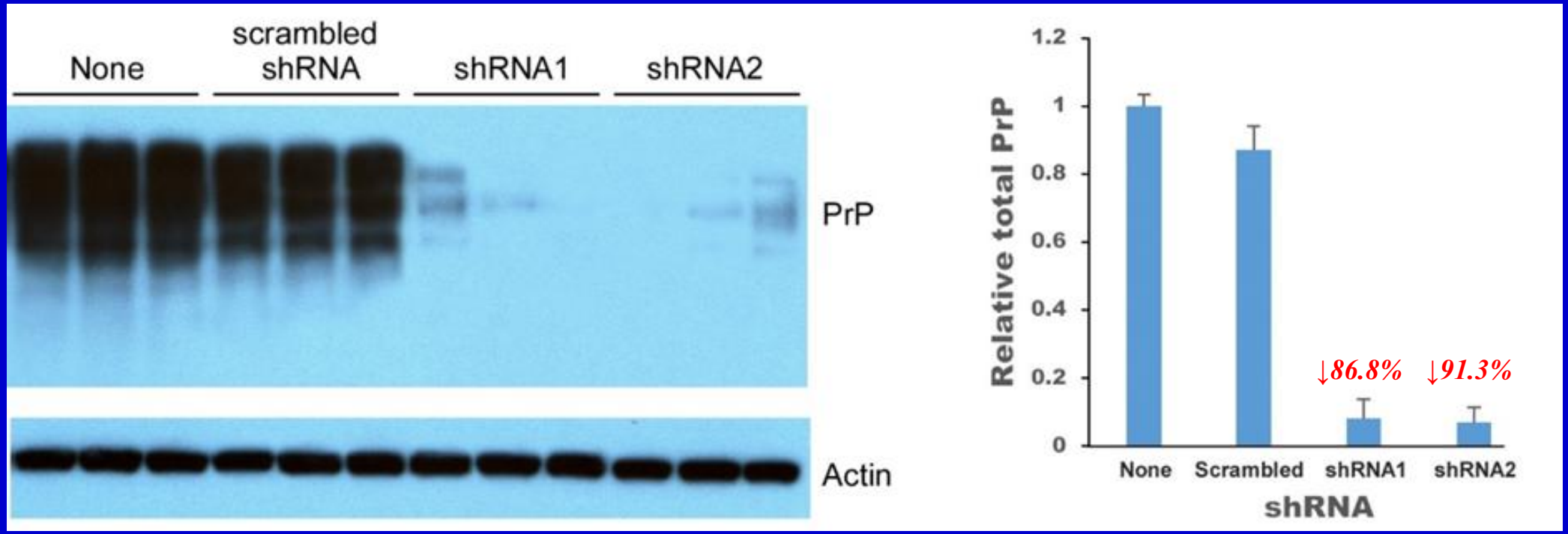




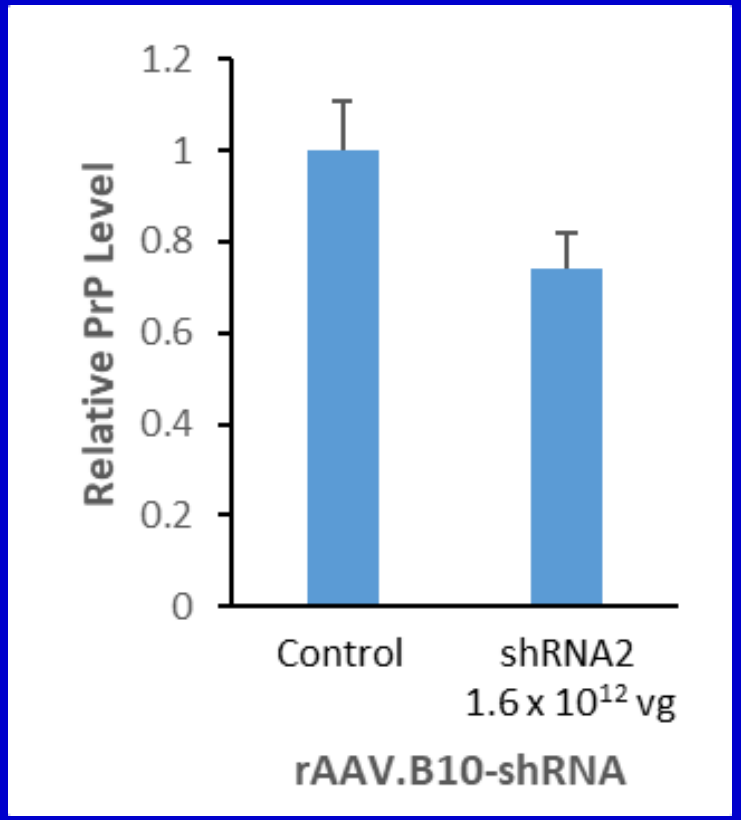
Tg40h:	3x WT

Total PrP: ↓47.8% ***FL-PrP: ↓49.3%*** ***C1/FL PrP ratio: ↑35.5%***

Knock-down of human PrP by pscAAV-shRNAs in human neuroblastoma cells

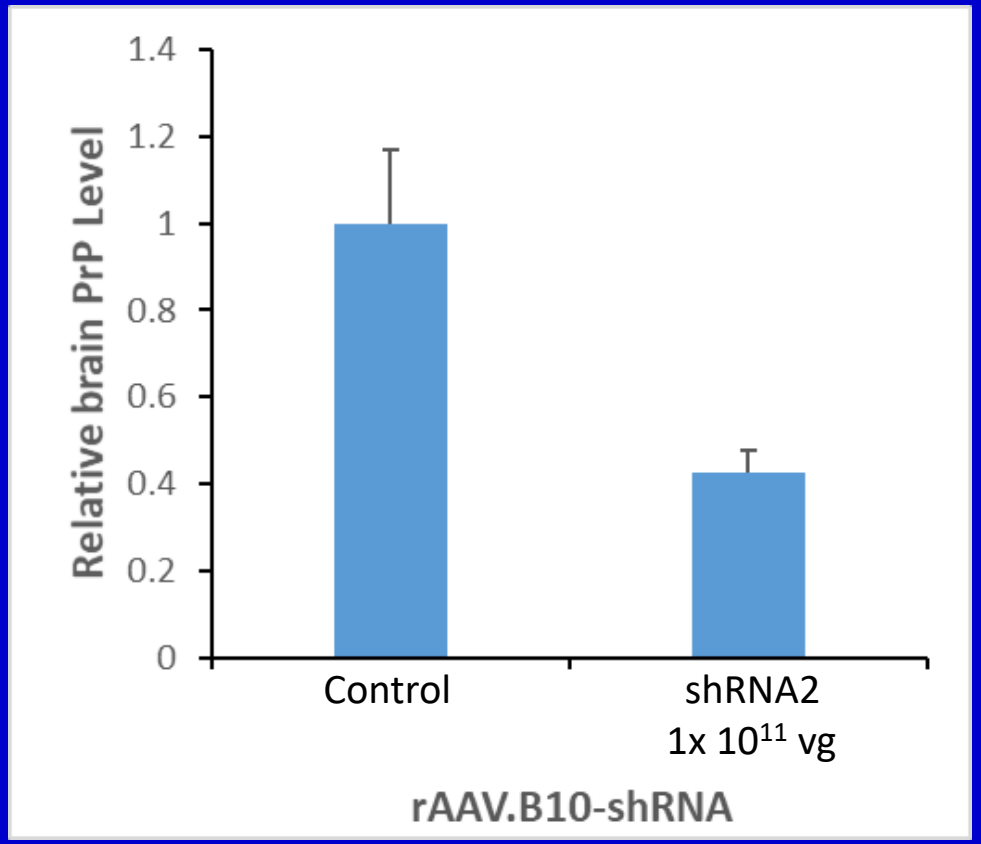


Knock-down of human PrP protein level by rAAV.B10-shRNA2 in the Tg40h mouse brain



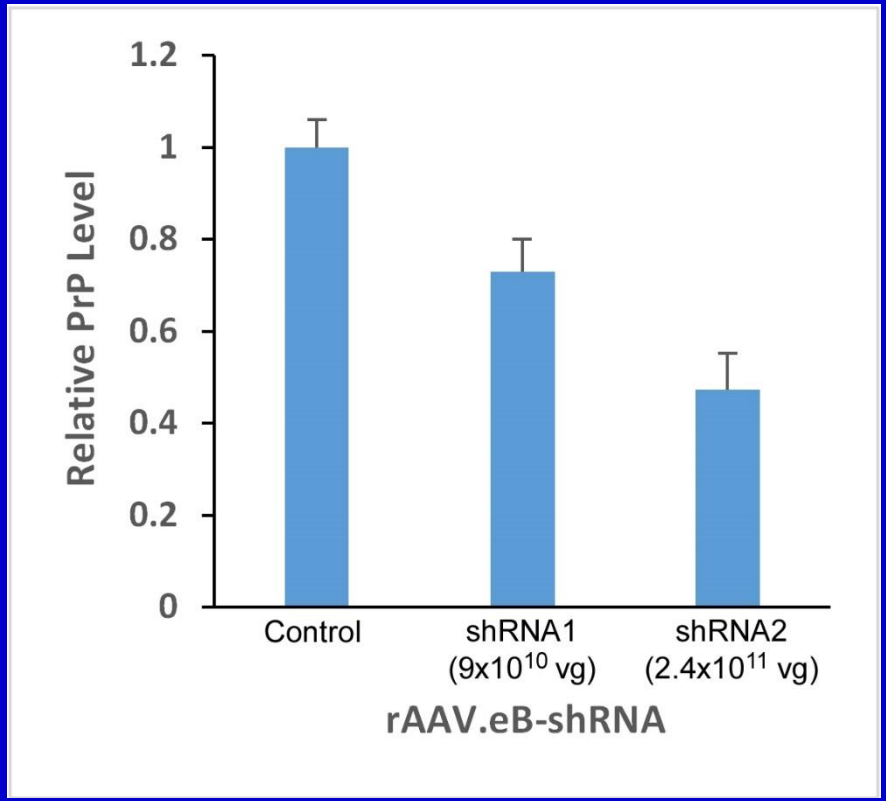
Brain PrP decrease by rAAV.B10-shRNA2 ($6x10^{13}$ vg/kg*) in Tg40h: 25.8%

Knock-down of human PrP protein level by rAAV.B10-shRNA2 in C57BL/6 mouse brain



Brain PrP decreased by rAAV.B10-shRNA2 (6x10^{13} vg/kg*) in C57BL/6: 57.2%

Knock-down of human PrP protein level by rAAV.eB-shRNAs in the Tg40h mouse brain



PrP decreased by rAAV.eB-shRNA1 (3.6x 10^{12} vg/kg) in Tg40h: 27.1%

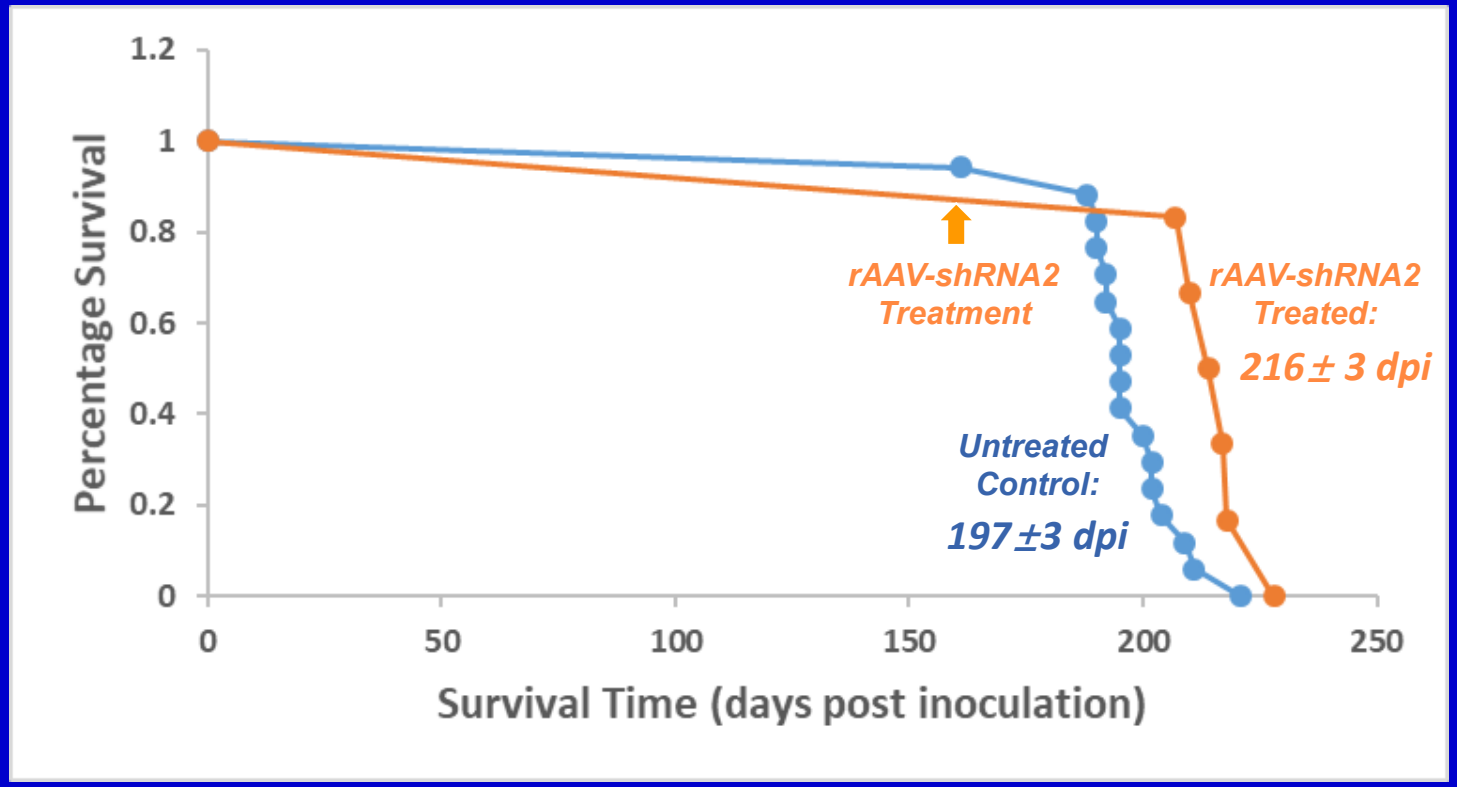
PrP decreased by rAAV.eB.shRNA2 (9.6x 10^{12} vg/kg) in Tg40h: 52.7%

Effect of systemic rAAV-ADAM8/shRNA treatment at clinical onset in sCJDMM1-infected humanized mice (Tg40h or Tg40)

Mice	Prion Inoculum	**rAAV Treatment** (retro-orbital)	Survival time
Tg40h (n=8)	sCJDMM1	None	204 ± 5 dpi
Tg40h (n=7)	sCJDMM1	rAAV.CPP16-ADAM8 *($4x10^{12}$ vg/kg)*	205 ± 4 dpi
Tg40 (n=6)	sCJDMM1	None	290 ± 22 dpi
Tg40 (n=9)	sCJDMM1	rAAV.CPP16-ADAM8 *($4x10^{12}$ vg/kg)*	281 ± 8 dpi
Tg40 (n=4)	sCJDMM1	None	280 ± 6 dpi
Tg40 (n=4)	sCJDMM1	rAAV.eB-ADAM8 *($4x10^{12}$ vg/kg)* rAAV.eB-shRNA1 *($4x10^{12}$ vg/kg)*	289 ± 11 dpi
Tg40 (n=4)	sCJDMM1	rAAV.eB-shRNA1 *($8x10^{12}$ vg/kg)*	> 327 ± 32 dpi
Tg40h (n=6)	sCJDMM1	None	197 ± 3 dpi
Tg40h (n=17)	sCJDMM1	rAAV.B10-shRNA2 *($6x10^{13}$ vg/kg*)*	216 ± 3 dpi

rAAV.B10-shRNA2 treatment at clinical onset extends survival of sCJDMM1-infected humanized mice (Tg40h)

rAAV-shRNA2 (6 x10^{13} vg/kg*) injected retro-orbitally at 157 dpi



Survival extension: 9.6% *(p<0.0005)*

Summary and Conclusions

- Intravenous administration of one low dose of rAAV-ADAM8 at symptom onset failed to prolong survival in CJD-infected humanized mice, although the same treatment significantly reduced prion protein level in the brains of uninfected mice with no apparent side effects, suggesting ADAM8 toxicity for CJD-infected mice.

- Intravenous administration of one low dose of rAAV-shRNA at symptom onset led to significant extension of survival in CJD-infected humanized mice, suggesting high potential for effective treatment at early stage of CJD.

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