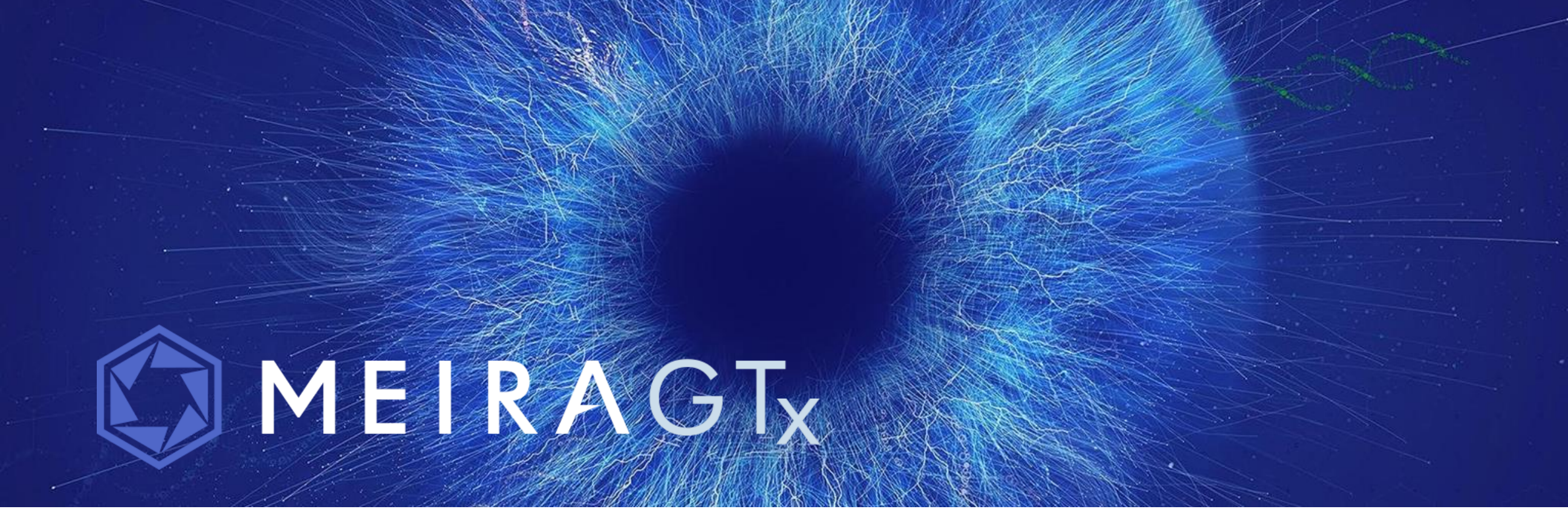


MeiraGTx | Gene therapy for Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) Preclinical efficacy of AAV-hUPF1 optimized for clinical translation with improved vector genome and novel CNS capsid.

ESGCT October 2023 | **T.D. Barbara Nguyen-Vu, MBA, PhD**

Disclosure

Employee of MeiraGTx

Amyotrophic Lateral Sclerosis (ALS)

Program Overview

- ALS is a severe neurodegenerative disease affecting motor neurons with mean survival of 2-5 years.
- Only 5-10% of ALS cases are inherited, familial ALS (fALS), whereas the rest are sporadic (sALS).
- MeiraGTx is developing a first-in-class gene therapy to potentially address **both** forms of ALS :

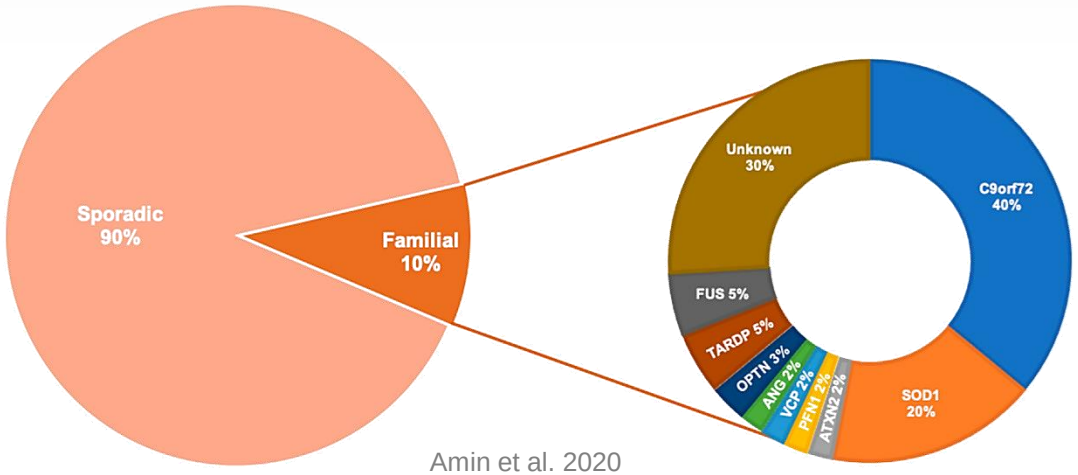
Therapeutic Target: AAV-hUPF1

» Targets underlying cellular defects associated with different forms of ALS

» Validated in multiple in-vivo and in-vitro models of ALS, in the context of AAV

TPD43 Proteinopathy is a Cellular Hallmark of ALS

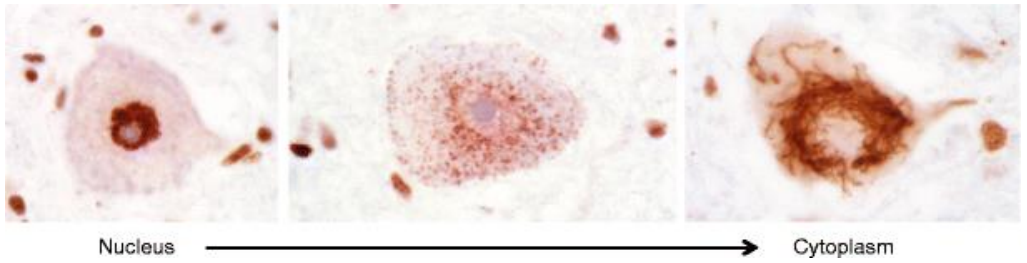
Only 5-10% of ALS is genetically driven



Amin et al. 2020

- Only 10% of ALS cases are inherited, familial ALS (fALS); remaining 90% of cases are sporadic (sALS)
- More than 20 genes were found to be associated with fALS

TDP43 cellular mislocalization and aggregation is present in 97% of ALS cases

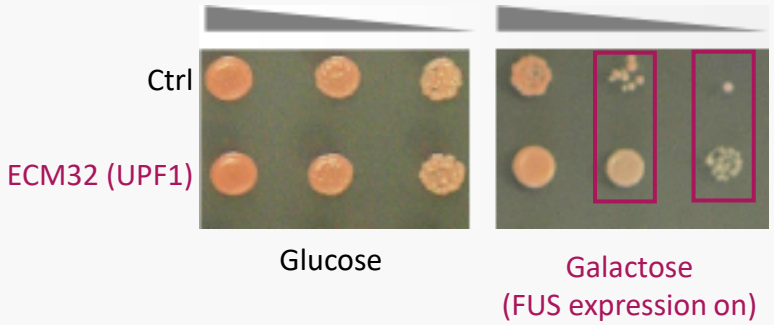


Feneberg et al. 2018

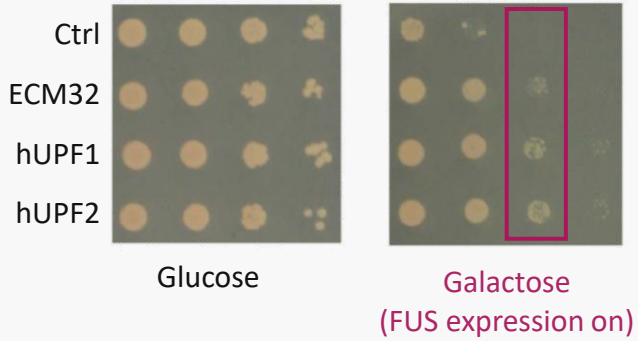
- TDP43 mis-localization and aggregation is a cellular hallmark of ALS (*except for SOD1-driven ALS*)
- TDP43 cytoplasmic granules are also observed in ~50% of frontotemporal dementia (FTD) patients

hUPF1 Identified as a Potential Therapeutic Target for ALS in a Yeast Genome-Wide Screen

ECM32, yeast homologue of UPF1, protects against FUS (and TDP43) cytotoxicity



Human UPF1 and UPF2 rescue FUS (and TDP43) cytotoxicity in yeast



- Overexpression of TDP43 or FUS recapitulates cellular hallmarks of ALS (i.e., mislocalization, aggregation and cytotoxicity)
- Only 5 out of 5,500 genes rescued - all were RNA binding proteins
- ECM32, a homologue of UPF1, was most potent
- human UPF1 (or UPF2) also rescued the toxic phenotype of FUS and TDP43 in yeast

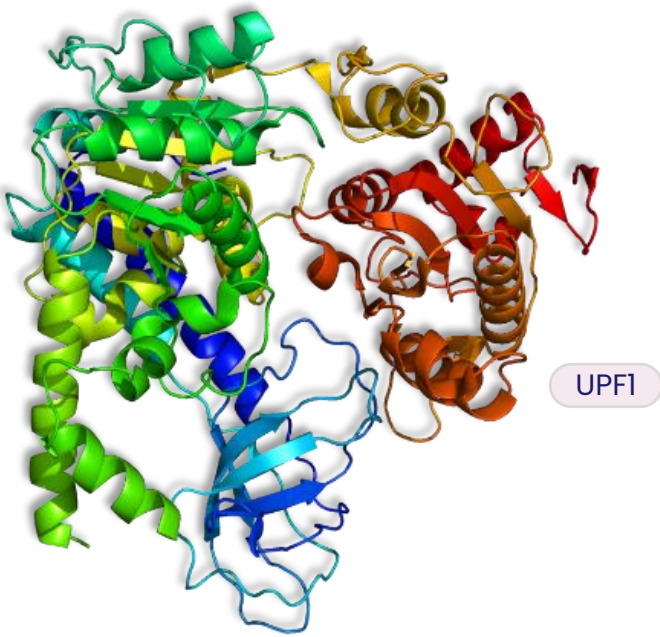
Ju S. et al. (2011) A Yeast Model of FUS/TLS-Dependent Cytotoxicity. PLOS Biology 9(4)

UPF1 is a Key Player in RNA Metabolism and Homeostasis Pathways Dysregulated in ALS

UPF1 plays a central role in RNA metabolism & quality control; mechanisms dysregulated in ALS:

- UPF1 is a core component of the Nonsense-Mediated Decay (NMD) pathway
- UPF1 has multiple roles beyond NMD, including targeting misfolded proteins to the 'aggresome' for clearance
- Dysregulation of RNA metabolism is known to contribute to ALS pathogenesis:
  - Mutations such as C9orf72, TDP43 and FUS, have been found to disrupt RNA processing
  - Aberrant RNA species & impaired clearance may disrupt cellular homeostasis and contribute to pathological protein aggregates, such as TDP43 - a cellular hallmark of ALS.

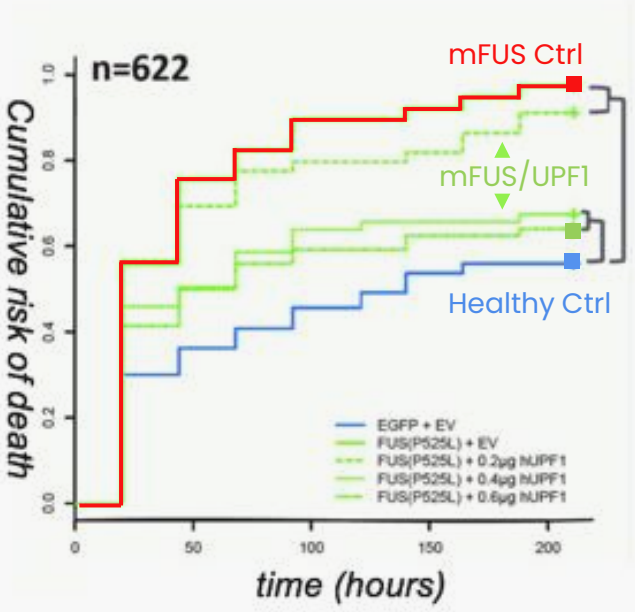
AAV-UPF1 potentially targets cellular defects underlying ALS, providing a way to address both familial and sporadic forms of the disease (>95% of patients)



UPF1 Decreases Risk of Neuronal Death by TDP-43 and FUS

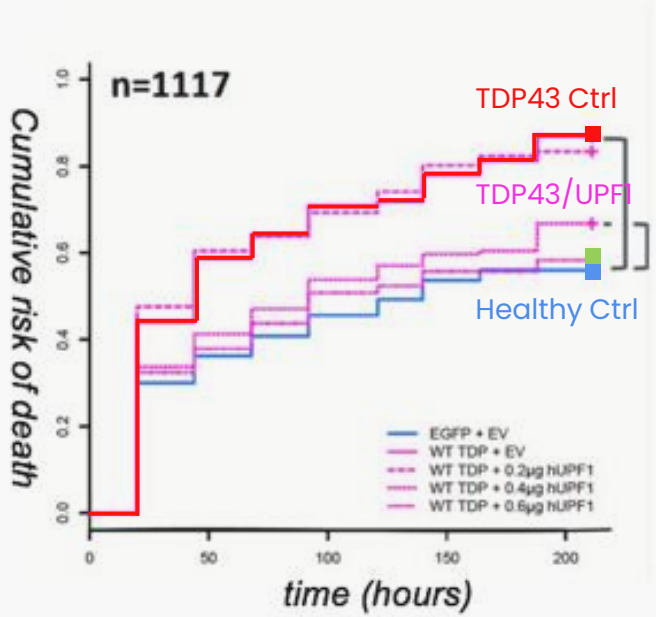
In primary rodent neurons, **hUPF1 expression rescues** FUS & TDP43 cytotoxicity in a dose-dependent manner

FUS disease allele



- mFUS + empty vector
- mFUS + UPF1 (0.6 ug DNA)
- Ctrl (EGFP + empty vector)

TDP43

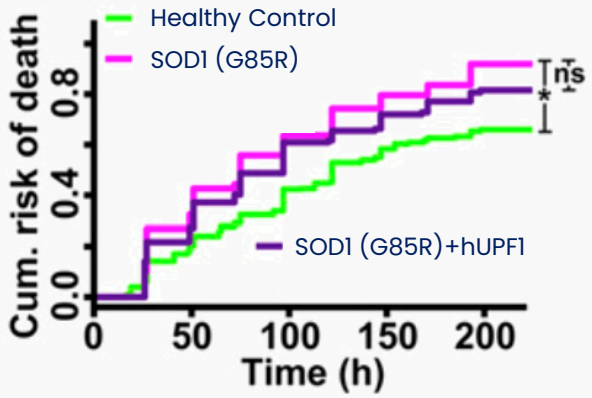


- TDP43 + empty vector
- TDP43 + UPF1 (0.6 ug DNA)
- Ctrl (EGFP + empty vector)

Protective Role of UPF1 is Specific to TDP43 proteinopathy

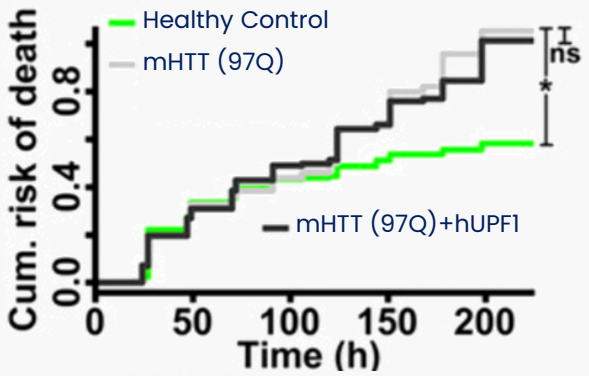
hUPF1 expression rescues **specific** to FTD and ALS associated with TDP43 proteinopathy (>95% of ALS cases)

SOD1 ALS



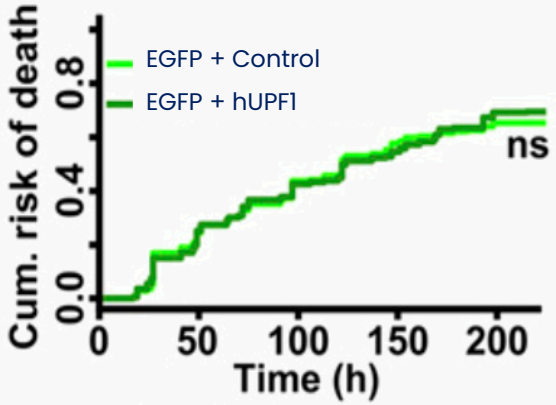
- UPF1 **unable** to rescue SOD1 ALS mutant cytotoxicity, a form of fALS which lacks cytoplasmic inclusions of TDP43

mHTT (Huntington's)



- UPF1 **unable** to rescue cytotoxicity induced by mutant Huntington (mHTT)

WT primary neurons

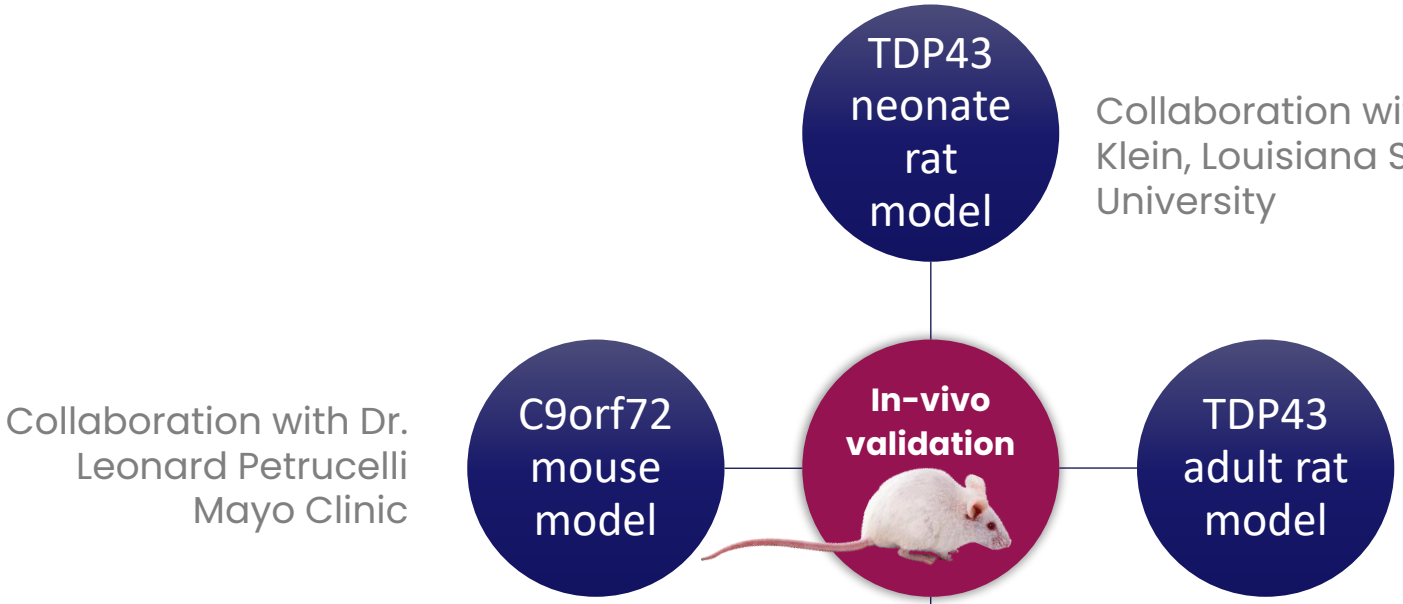


- **No effect in WT** primary neurons of UPF1 over-expression

Barmada, S. J. et al. (2015). Amelioration of toxicity in neuronal models of amyotrophic lateral sclerosis by hUPF1. Proc Natl Acad Sci, 112(26), 7821-7826.

UPF1 Validation in Multiple *in-vivo* Models of ALS

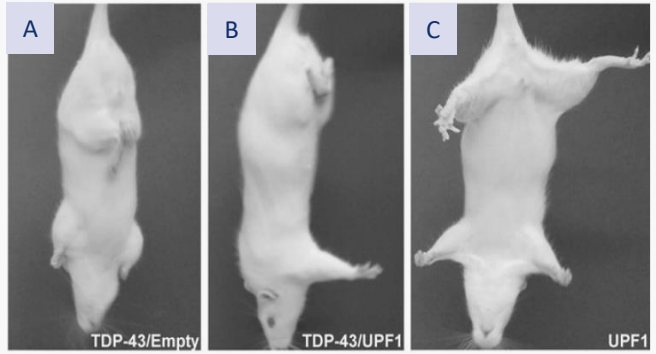
Collaborations with leading ALS researchers to validate UPF1 in multiple in-vivo models of ALS provide **strong evidence** for the **therapeutic potential of UPF1**

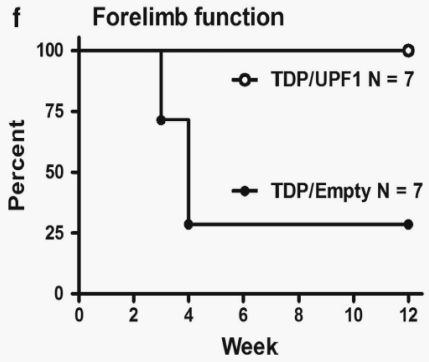


In-vivo validation: Functional Evidence for Therapeutic Potential

In-vivo model 1: TDP43 neonate rat model

AAV-UPF1 in **neonate rescues escape reflex** impairments induced by TDP43

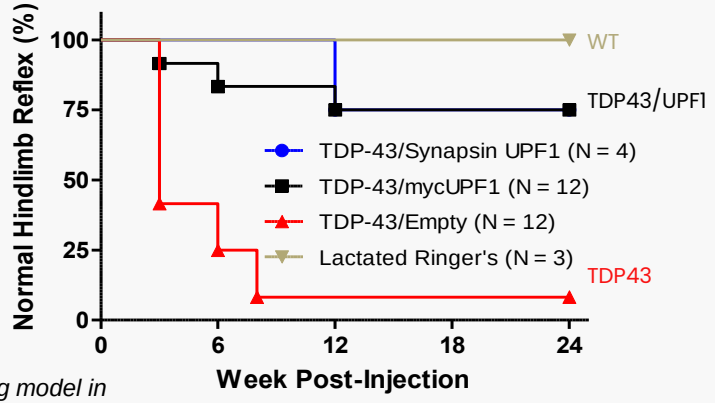




Jackson et al. Gene Ther. 2015

In-vivo model 2: TDP43 adult rat model

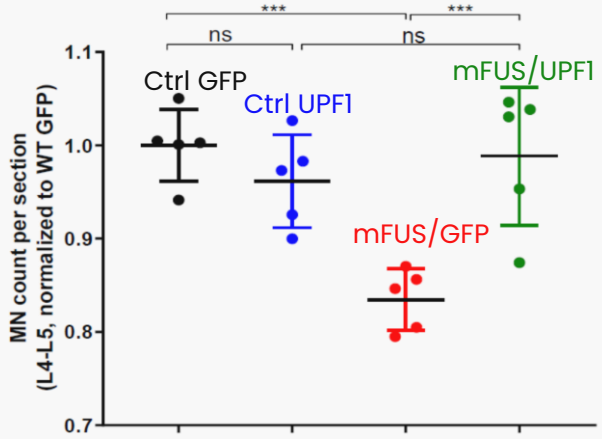
AAV-UPF1 in **adult rat rescues escape reflex** impairments induced by TDP43



Unpublished using model in
Jackson et al. Mol. Ther. 2015

In-vivo model 3: conditional FUS mouse model

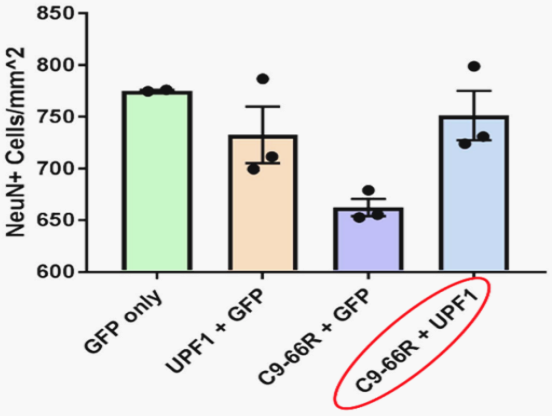
AAV-UPF1 in FUS mouse model **protects against motor neuron loss** & improves hindlimb grip strength



Unpublished using model in
Korobeynikov et al.Nat.Med. 2022

In-vivo model 4: mouse C9orf72 model

AAV-UPF1 in (G4C2)66 mouse model **protects against neuron loss** & improves locomotor activity

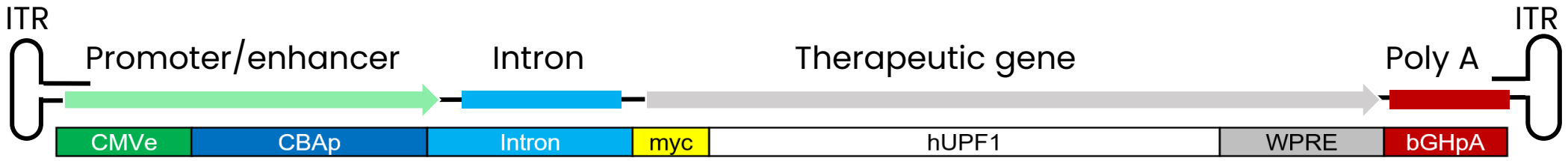


Unpublished using model in
Chew et al. Science 2015

UPF1 Construct Optimization for the Clinic

Optimized to **decrease size**, increase production yield, and **increase potency**

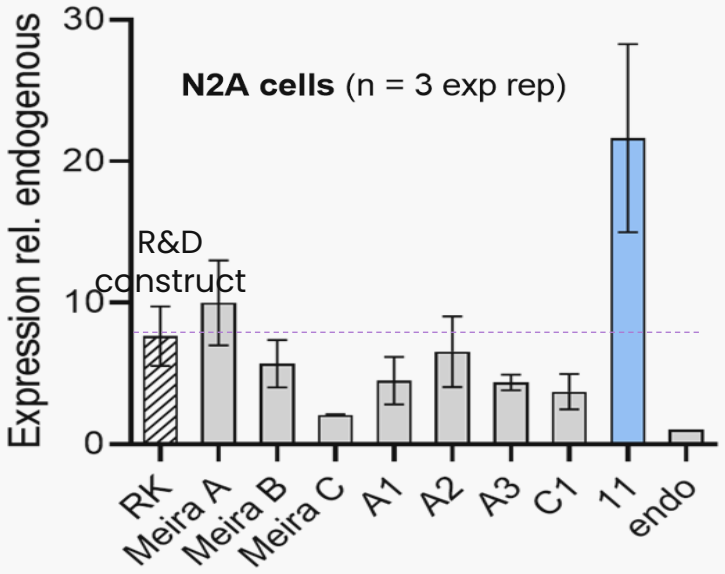
Original R&D Construct 6.5kb



Optimization Platform

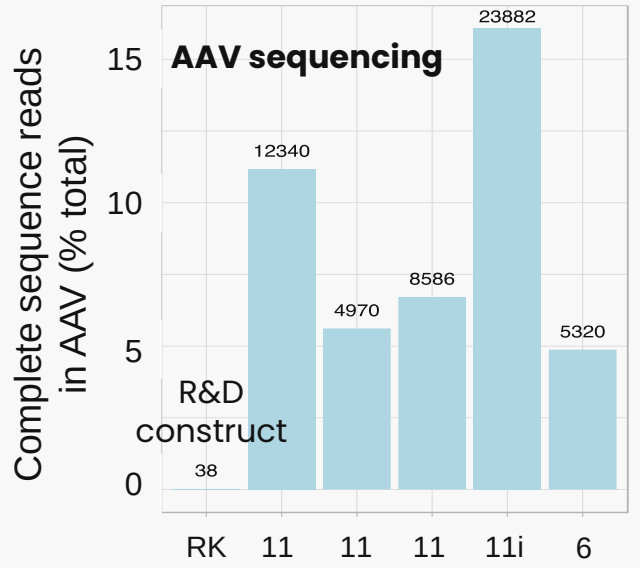
- MGTX proprietary mini promoter/enhancer
- Codon optimized sequence
- Optimized WPRE
- Optimized PolyA

Clinical Candidates **4.5-4.9kb**



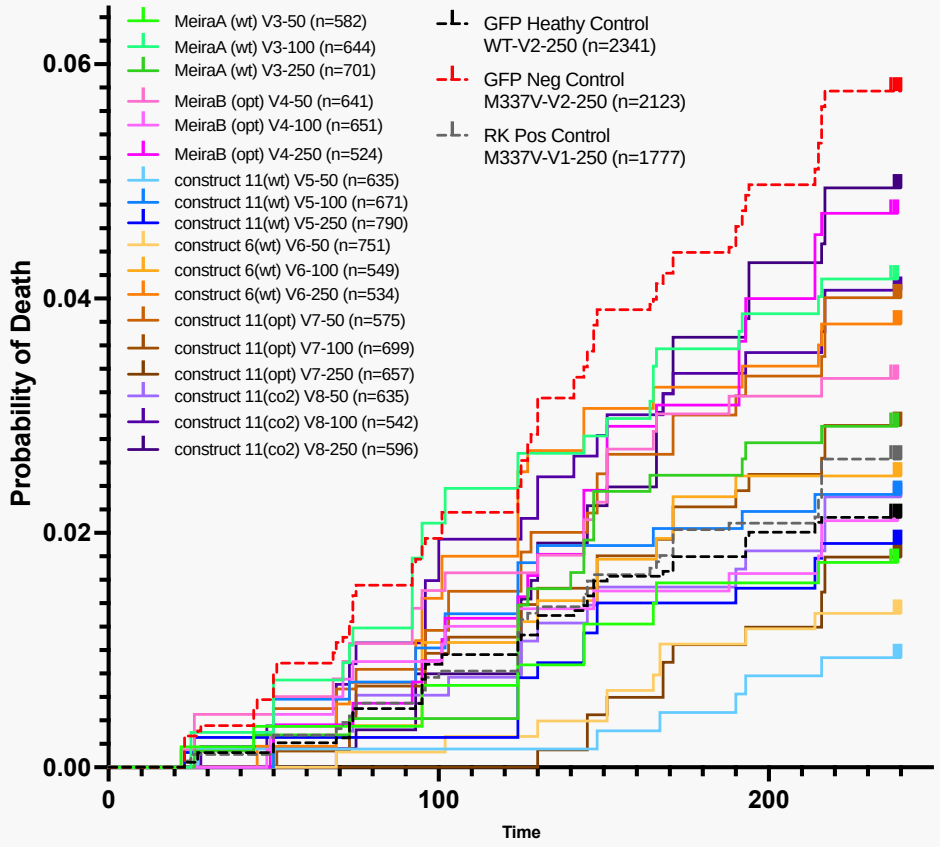
Increased expression

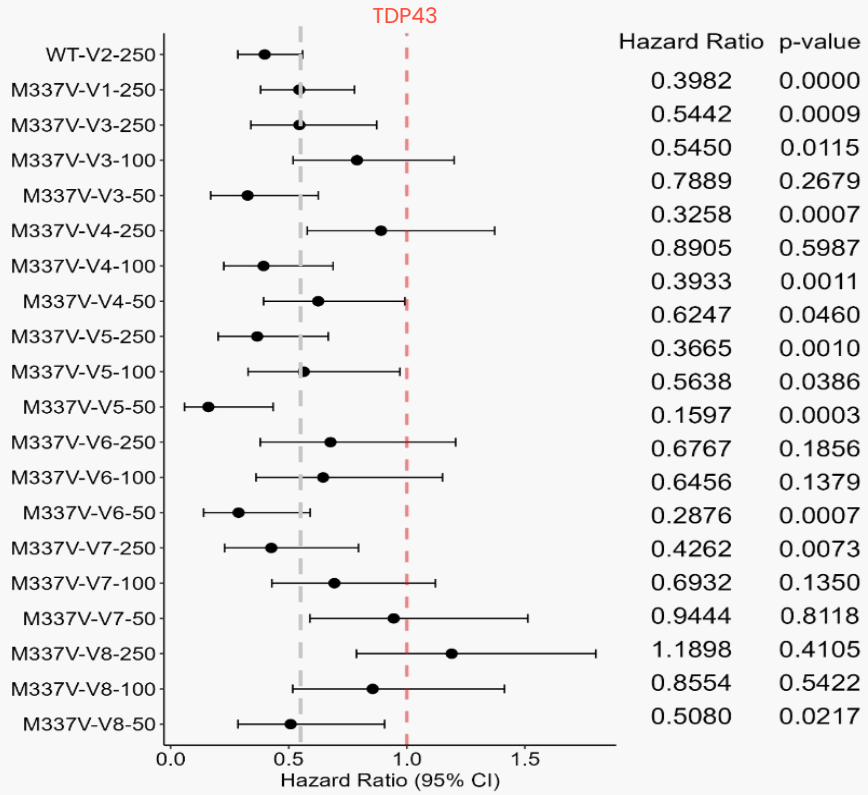
Improved AAV packaging efficiency



Optimized hUPF1 Constructs Validated in iNeuron Models of ALS

All optimized AAV-UPF1 **constructs rescued,** in parallel testing, of optimized hUPF1 constructs in human iNeuron TDP-43 Disease Model



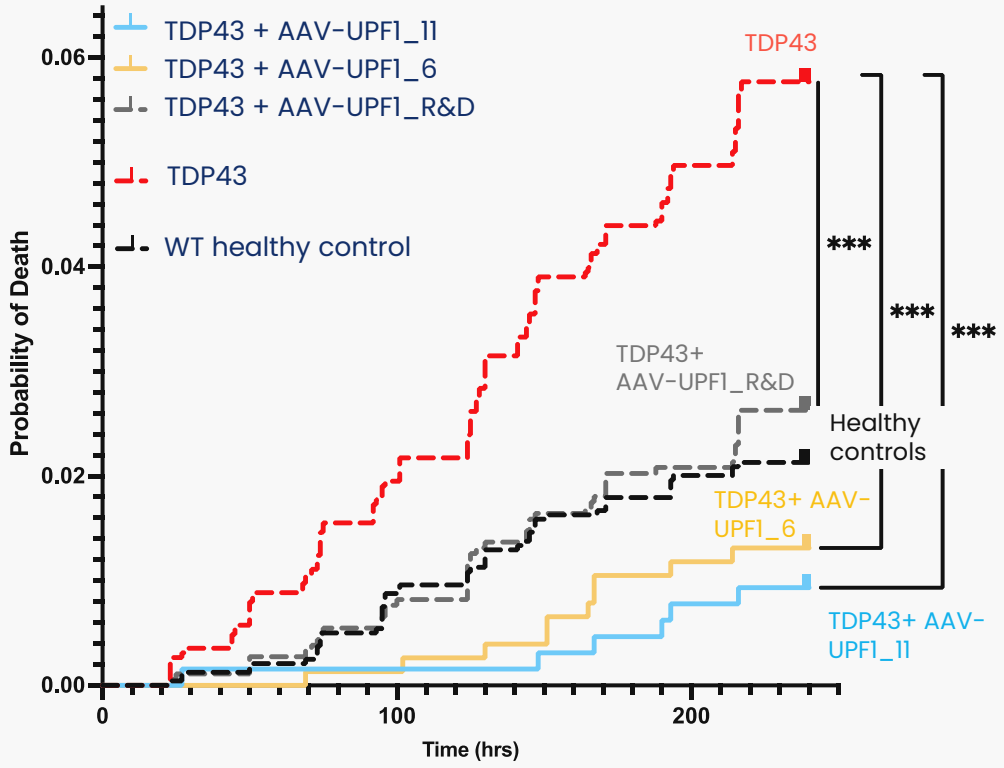


	Hazard Ratio	p-value
WT-V2-250	0.3982	0.0000
M337V-V1-250	0.5442	0.0009
M337V-V3-250	0.5450	0.0115
M337V-V3-100	0.7889	0.2679
M337V-V3-50	0.3258	0.0007
M337V-V4-250	0.8905	0.5987
M337V-V4-100	0.3933	0.0011
M337V-V4-50	0.6247	0.0460
M337V-V5-250	0.3665	0.0010
M337V-V5-100	0.5638	0.0386
M337V-V5-50	0.1597	0.0003
M337V-V6-250	0.6767	0.1856
M337V-V6-100	0.6456	0.1379
M337V-V6-50	0.2876	0.0007
M337V-V7-250	0.4262	0.0073
M337V-V7-100	0.6932	0.1350
M337V-V7-50	0.9444	0.8118
M337V-V8-250	1.1898	0.4105
M337V-V8-100	0.8554	0.5422
M337V-V8-50	0.5080	0.0217

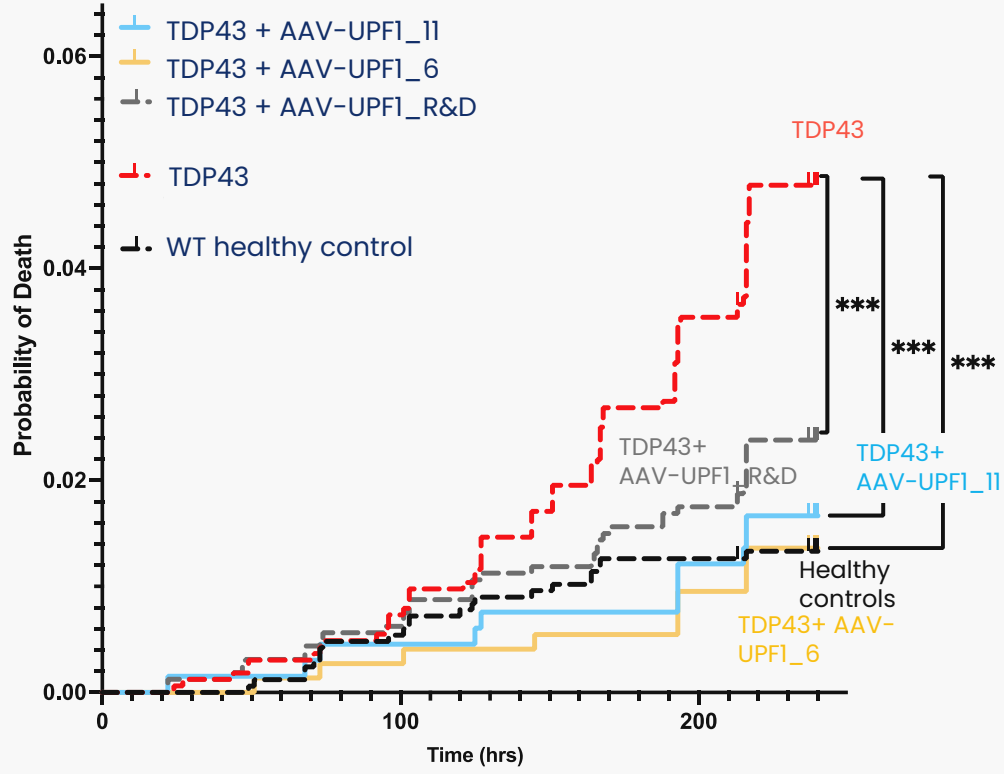
Optimized hUPF1 Decrease Risk of Death in iNeuron Models of ALS

hUPF1 expression **promotes survival** of human iPSC-derived neurons (iNeurons) with isogenic mutations of **TDP43 or C9orf72**

TDP43 (M337V) iNeuron

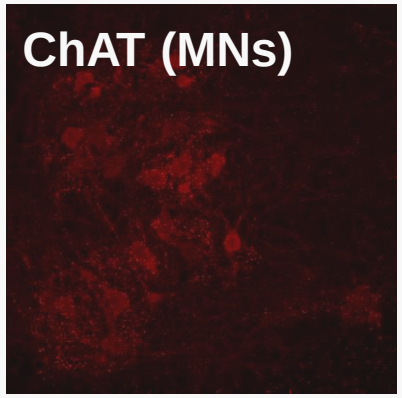


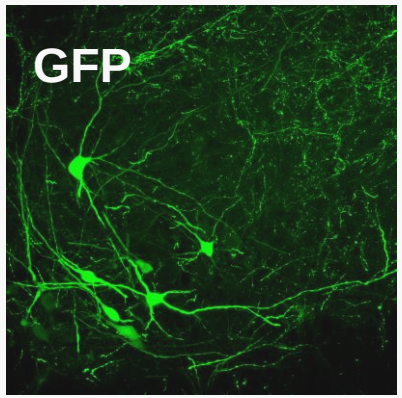
C9 iNeuron

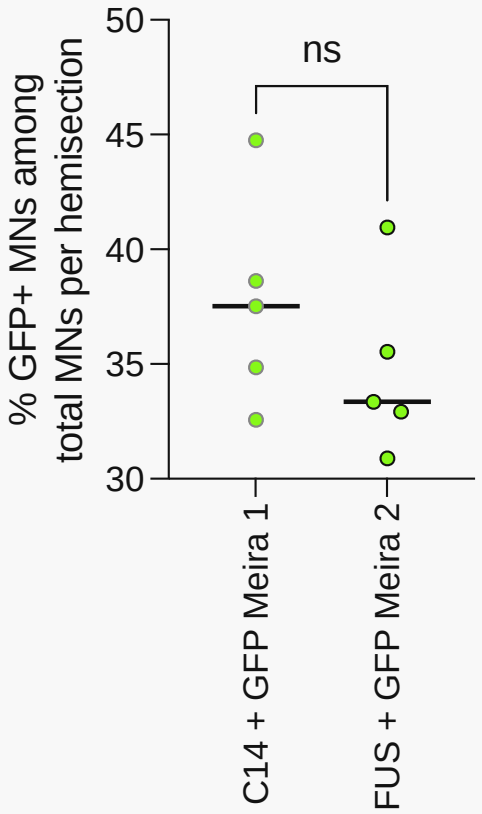


Optimized hUPF1 Promotes Neuronal Survival In-Vivo in FUS mouse

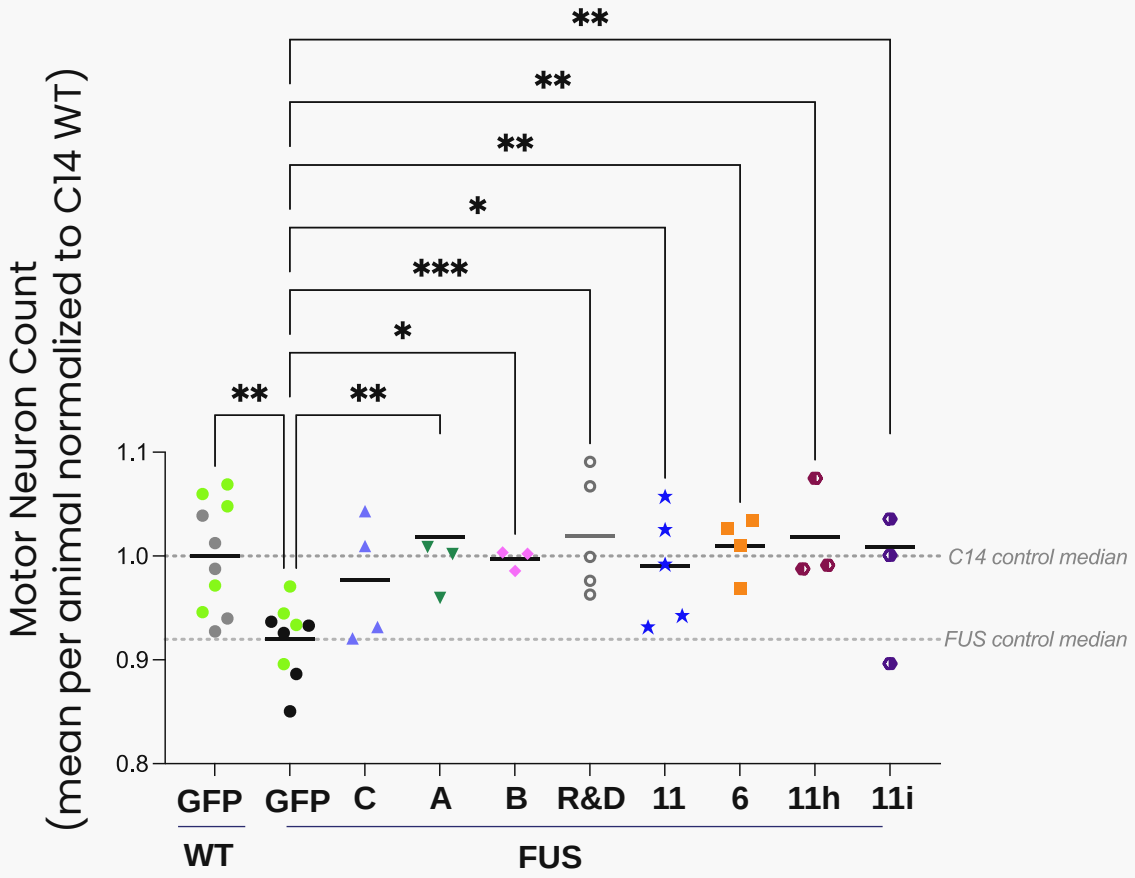
Optimized AAV-UPF1 constructs **rescued loss of motor neurons in-vivo** in FUS mouse model







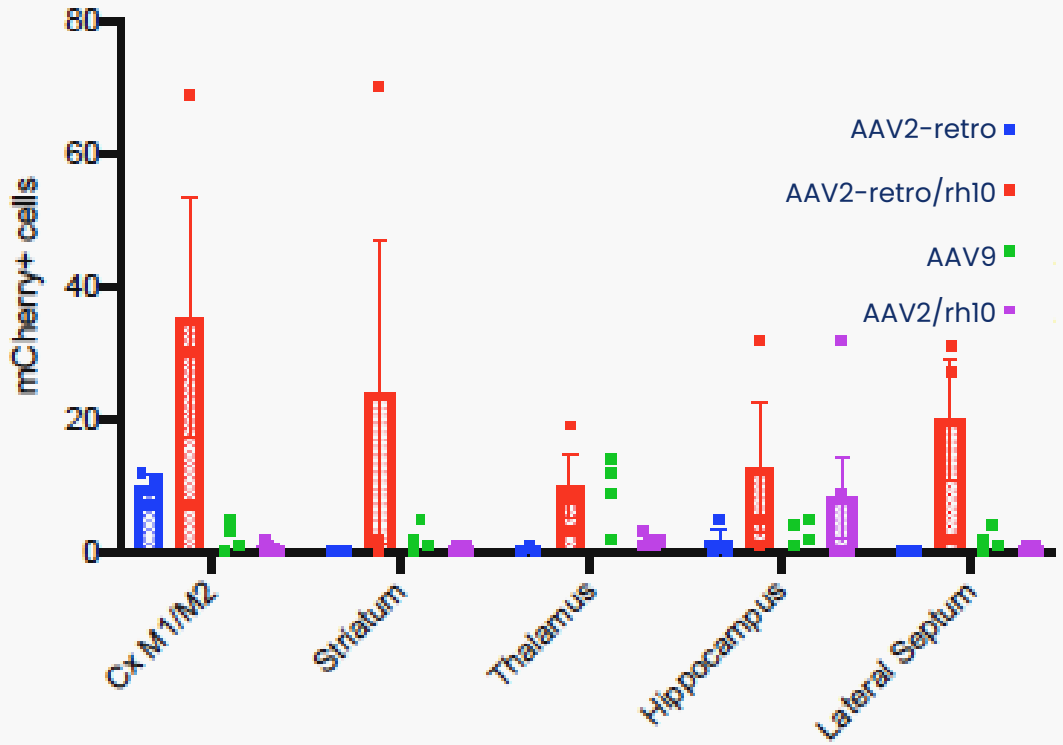
- 36% AAV transduction efficiency of motor neuron via neonate ICV was sufficient for in-vivo rescue



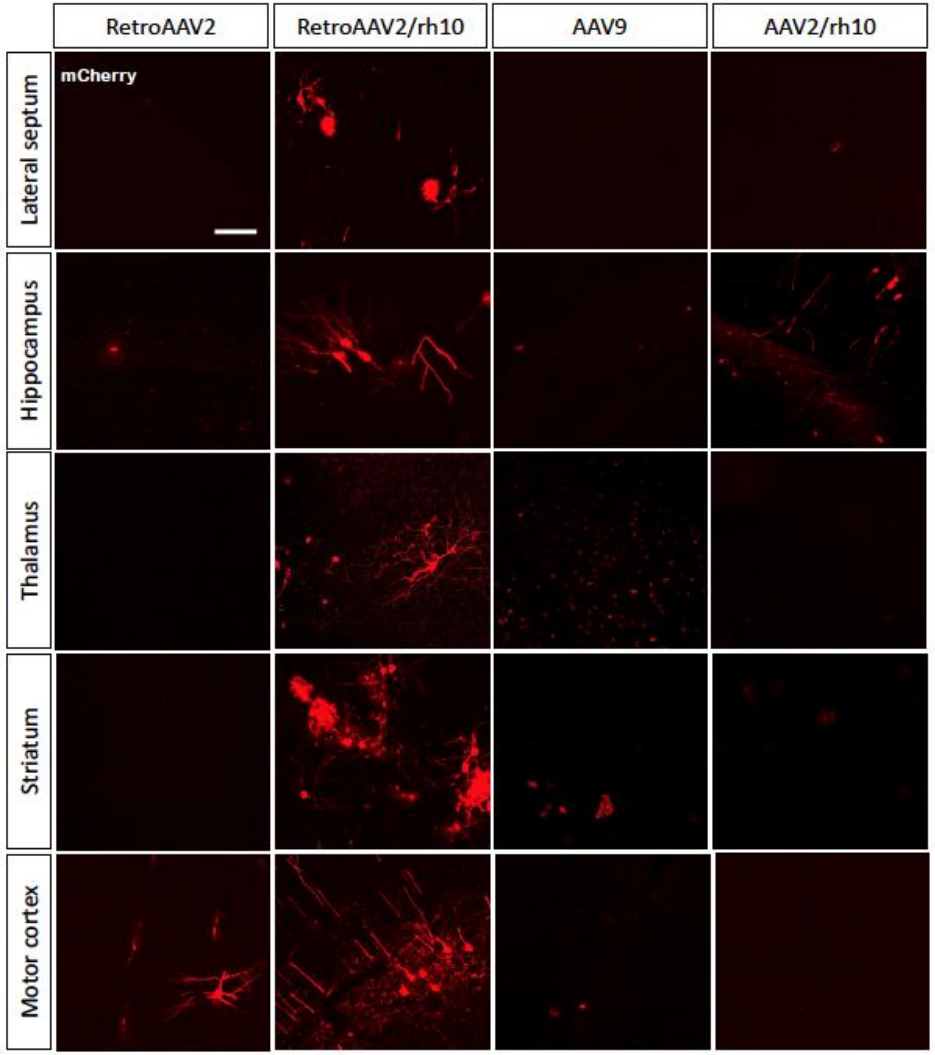
- AAV-hUFP1 gene therapy protective against motor neuron loss in FUS mouse model

Novel AAV Capsids for Broad CNS Transduction

Novel Capsid: AAV2-retro & variants



- AAV2-retro variants transduced both upper and lower motor neurons by ICM injection in mice

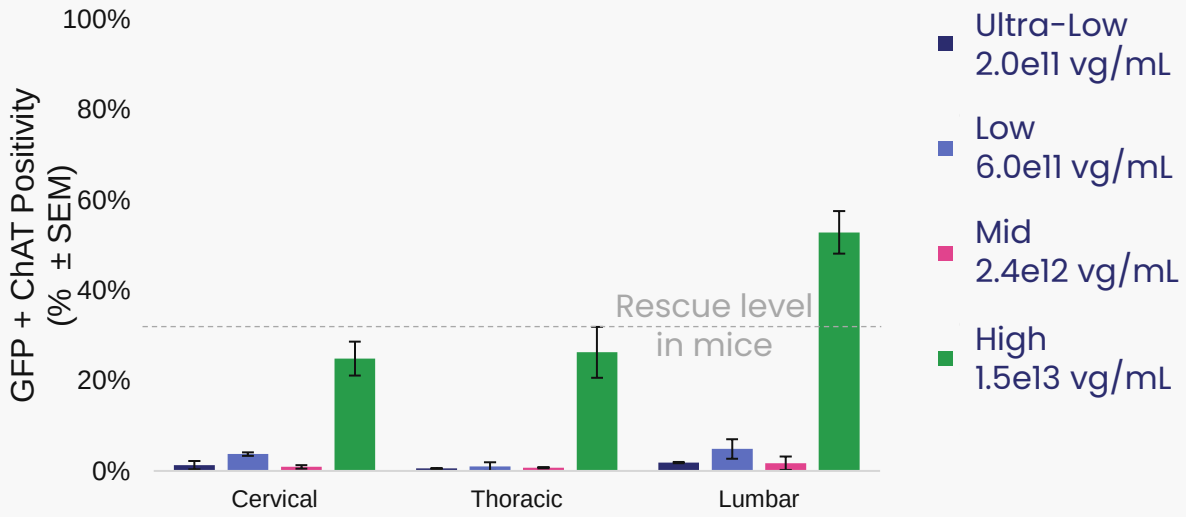


Novel AAV Capsid Transduces Spinal Motor Neurons in NHP

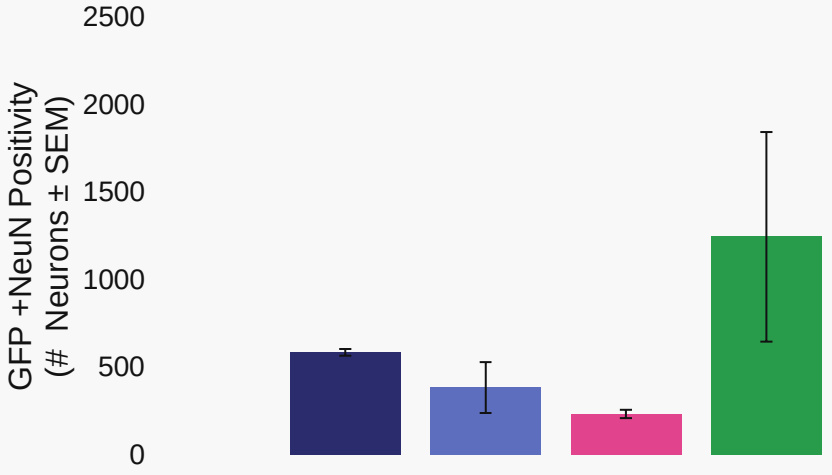
AAV2-retro/rh10 mosaic produced broad CNS **transduction** of neurons **in both brain and spinal cord** of NHPs via intra-cisterna magna (ICM) administration

Novel Capsid: AAVmosaic

Transduction in NHP Spinal Motor Neurons



Transduction in NHP Neurons in Brain



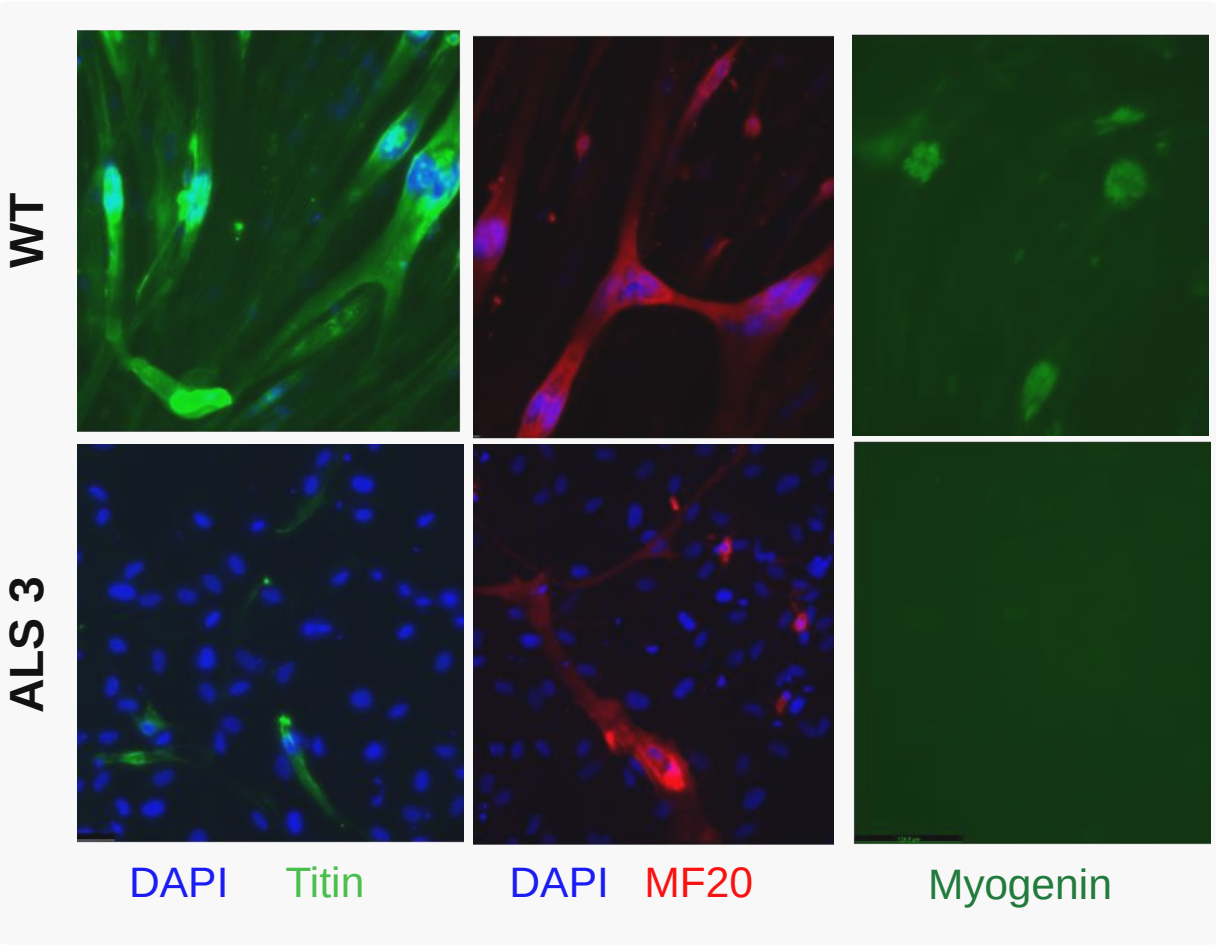
- Transduction in spinal motor neurons at levels sufficient to drive in-vivo rescue in mice
- Confirmed transduction in cortical neurons via ICM route of administration

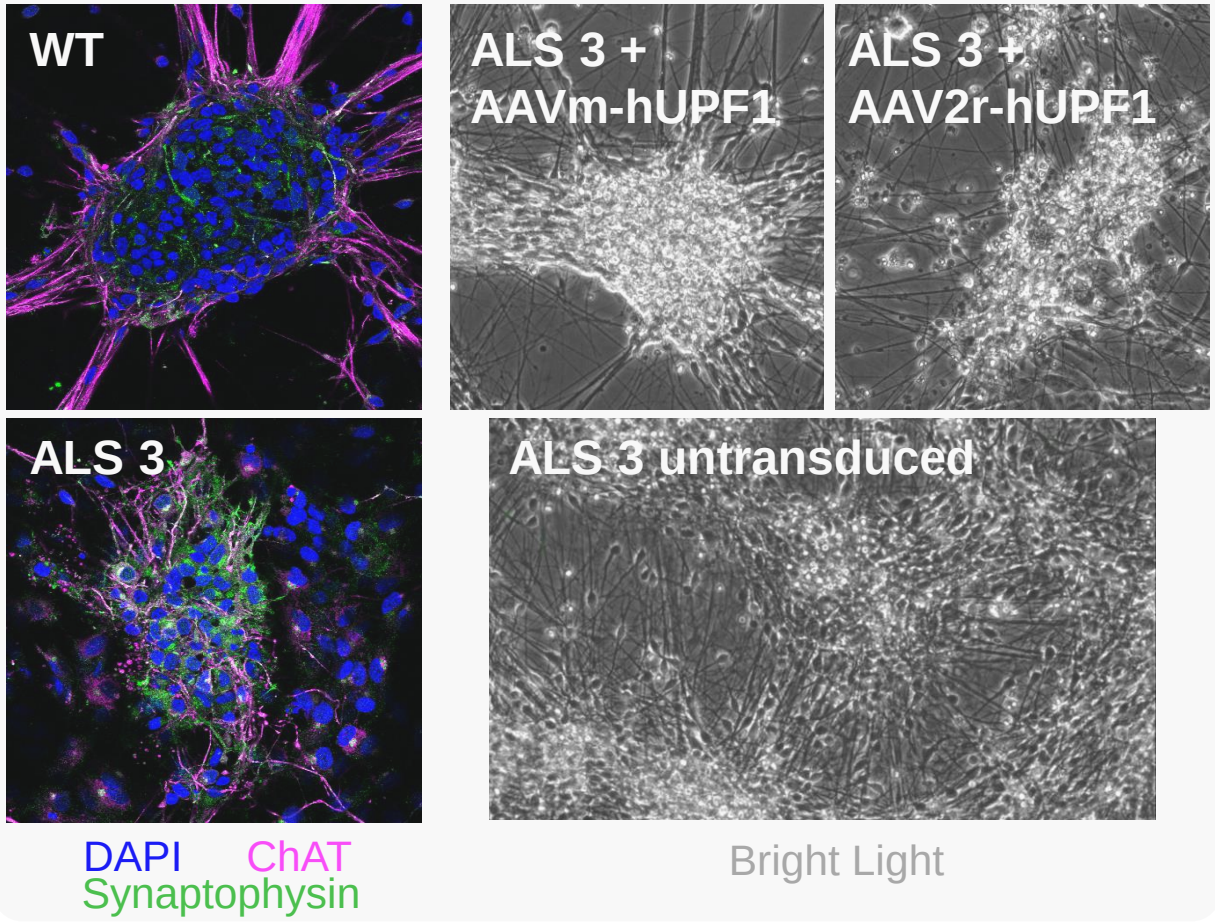
Clinical AAV-hUPF1 Validated in ALS Patient-Derived Neurons

ALS Patient-derived iPSC Model: Myotubes & Neurons

Myotubes: ALS3 C9 patient

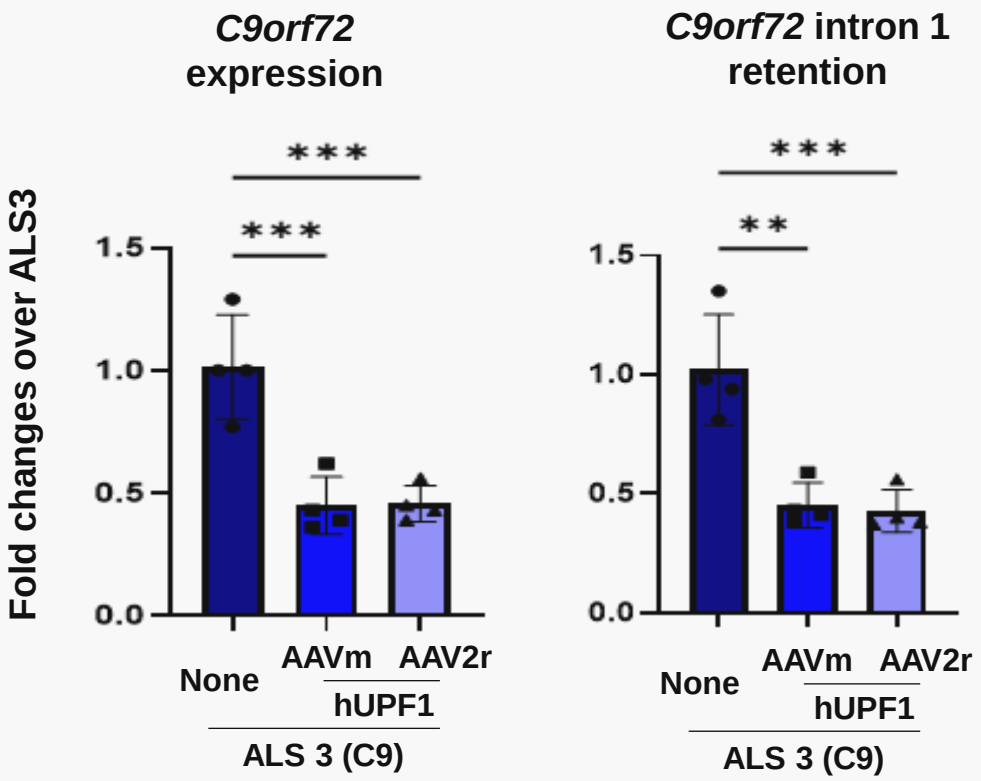
Neurons: ALS3 C9 patient



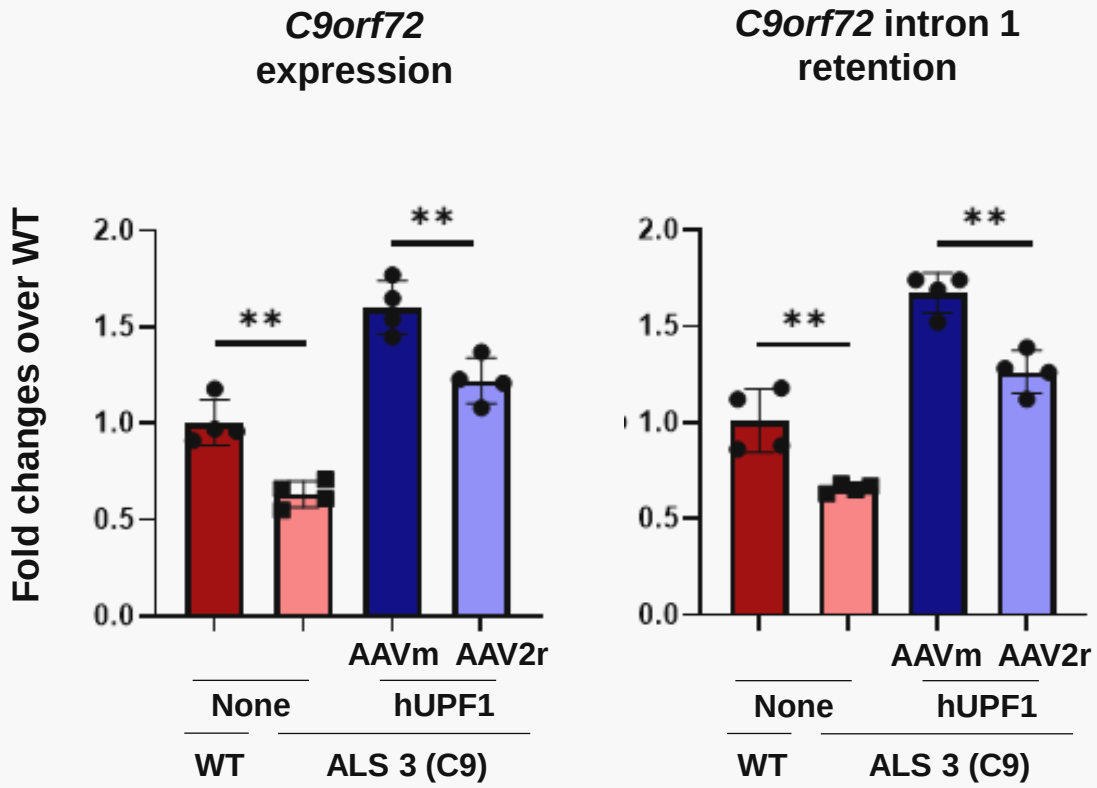


Efficacy of AAV-hUPF1 in C9 ALS Patient-Derived Neurons

Clinical AAV-hUPF1 candidates demonstrate **reproducible target engagement in C9** ALS patient-derived neurons



- Exp. 1 : 2 weeks after AAV transduction

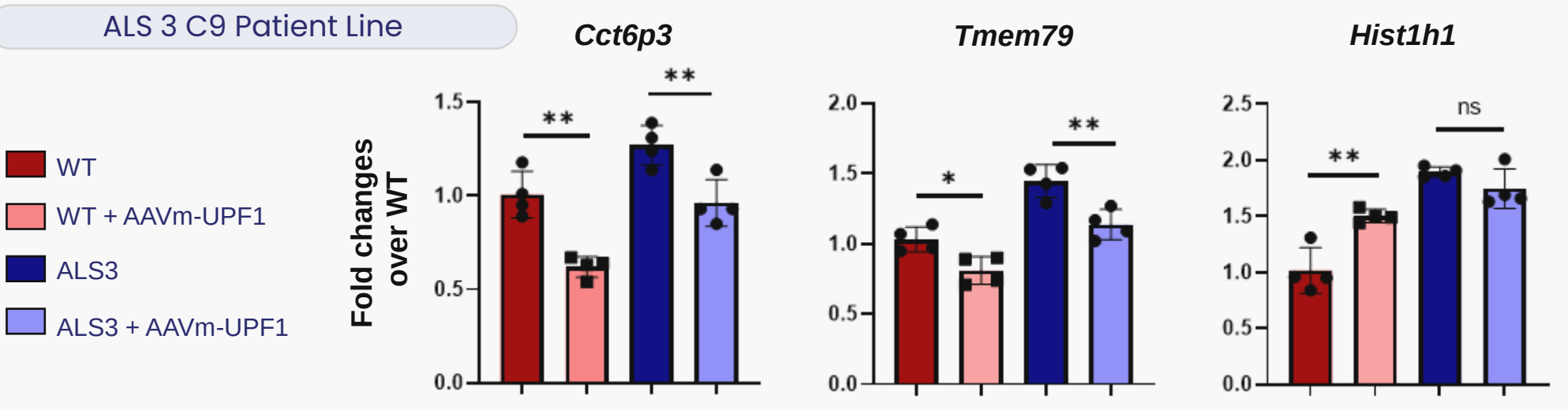


- Exp. 2 : 1.5 weeks after AAV transduction

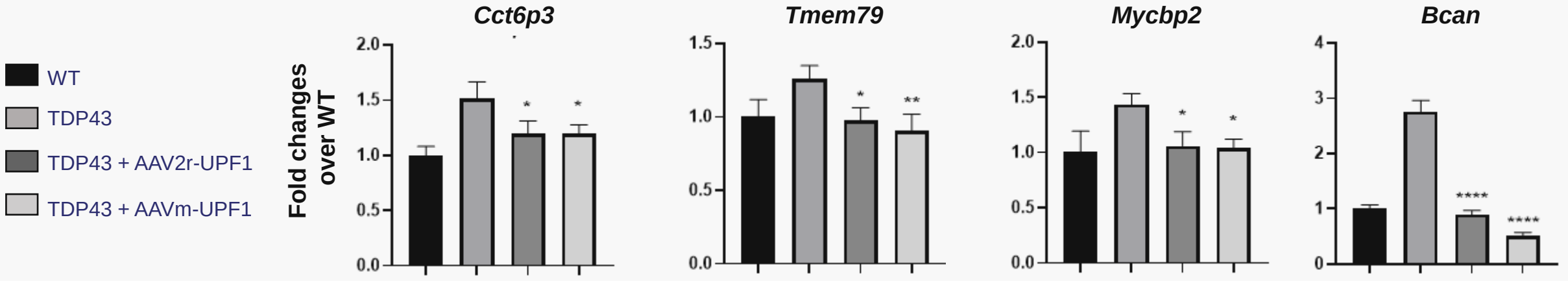
AAV-hUPF1 Rescued in Multiple ALS Patient-Derived Lines

AAV-hUPF1 **efficacy** in ALS patient–derived lines of **different genetic context**: C9 & TDP43

ALS 3 C9 Patient Line



TDP43 (A382T) Patient Line



MEIRAGTx

AAV-UPF1 Program **HIGHLIGHTS**

AAV-UPF1 is a novel gene therapy for ALS with the potential to treat both familial and sporadic ALD (>95% of patients) and FTD:

- UPF1 is a novel therapeutic target for ALS discovered in an unbiased genetic screen in yeast and validated in multiple in-vitro and in-vivo models of ALS
- AAV-UPF1 targets an underlying cellular defect of ALS– RNA metabolism and homeostasis. UPF1 is known to play a central role in RNA regulation, including in Nonsense-Mediated Decay (NMD).
- In-vivo studies in multiple models of ALS have demonstrated the ability of AAV-UPF1 to reduce neuronal death and ameliorate ALS symptoms related to limb strength and mobility
- A proprietary capsid, AAV2-retro, demonstrates favorable transduction of upper and lower motor neurons

Status:

- **Vector optimization** – reduced size for enhanced packaging efficiency, and improved potency over original academic construct
- Initiation of IND-enabling studies planned for 2024

Acknowledgements

MeiraGTx US:

UPF1 Construct Optimization & In-vitro Validation

Jeroen Bastiaans

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Ann Lettko

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Enrico Mossotto

Josepha M. Sullivan

Ce Feng Liu

T.D. Barbara Nguyen-Vu

Alexandria Forbes

MeiraGTx UK:

Patient-derived ALS Cell Models

Patricia Gordon

Tijmen Vermeule

Arifa Naeem

Tassos Georgiadis

MeiraGTx NL:

AAV production

Mostafa Zamatkesh

Cornell University:

Novel AAV Capsid in Mouse

Santiago Unda

Michael Kaplitt

Columbia University:

FUS Mouse Model

Vlad Korobeynikov

Emily R. Lowry

Neil A. Shneider

University of Michigan:

iNeuron Models

Michael Bekier

Sami Barmada

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