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UPF1 delivered by novel expression-enhanced promoters protects cultured neurons in a genetic ALS model

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Abstract

Regulator of nonsense transcripts 1 (UPF1) is an RNA helicase important for nonsense mediated decay (NMD) of mRNA transcripts that contain premature stop codons. **Studies have demonstrated that UPF1 overexpression protects against neurodegeneration in animal models of ALS involving TDP-43 and FUS and C9orf72 toxicity.** We aimed to enhance the potency of our UPF1 expression vectors further while decreasing their size, since greater potency will allow lower MOIs of virus to be used clinically, while the smaller construct size improves AAV packaging and manufacturing efficiency. First, we enhanced the expression of UPF1 by rationally optimizing cis-regulatory elements such as the promoter. In three different mammalian cell lines, the top promoter construct, UPF1-11, drives greater UPF1 expression than the controls, including the myc-UPF1 construct used in the original mouse neuroprotection experiments. Next, we tested the function of the best candidates to rescue toxicity in a TDP-43 and C9orf72 cellular model of iPSC-induced neurons (iNeurons). The AAV vector containing UPF1-11 protected iNeurons at lower MOIs than the myc-UPF1 vector containing the original promoter, indicating a functional potency enhancement. At 4.9 kb in size, UPF1-11 is 1.5 kb smaller than the original construct, and thus offers AAV packaging and manufacturing benefits as well. **UPF1 delivered by MeiraGTx vectors shows promise in treating ALS with TDP-43-related proteinopathy, and animal studies are ongoing.**

UPF1 protects against toxic proteins in yeast and rats

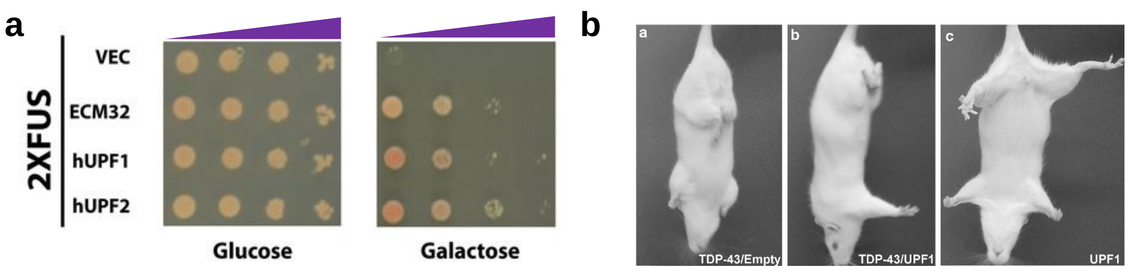

Figure 1. UPF1 protects against ALS-related toxic proteins in yeast and rats. (**a**) The yeast homolog of hUPF1, ECM32, was identified from a large yeast toxicity suppressor screen from Ju et al., *PLoS Biology*, 2011. Overexpression of toxic protein, FUS, was induced using galactose (no induction from glucose). (**b**) Rats injected with TDP-43/vehicle display hindlimb and forelimb clasping when tail suspended. By contrast, rats injected with TDP-43/UPF1 exhibit normal forelimb extension and escape reflex, a significant improvement in motor function. Figure from Jackson et al., *Gene Ther*., 2015 (Ron Klein lab).

UPF1 protects iNeurons against TDP-43 toxicity

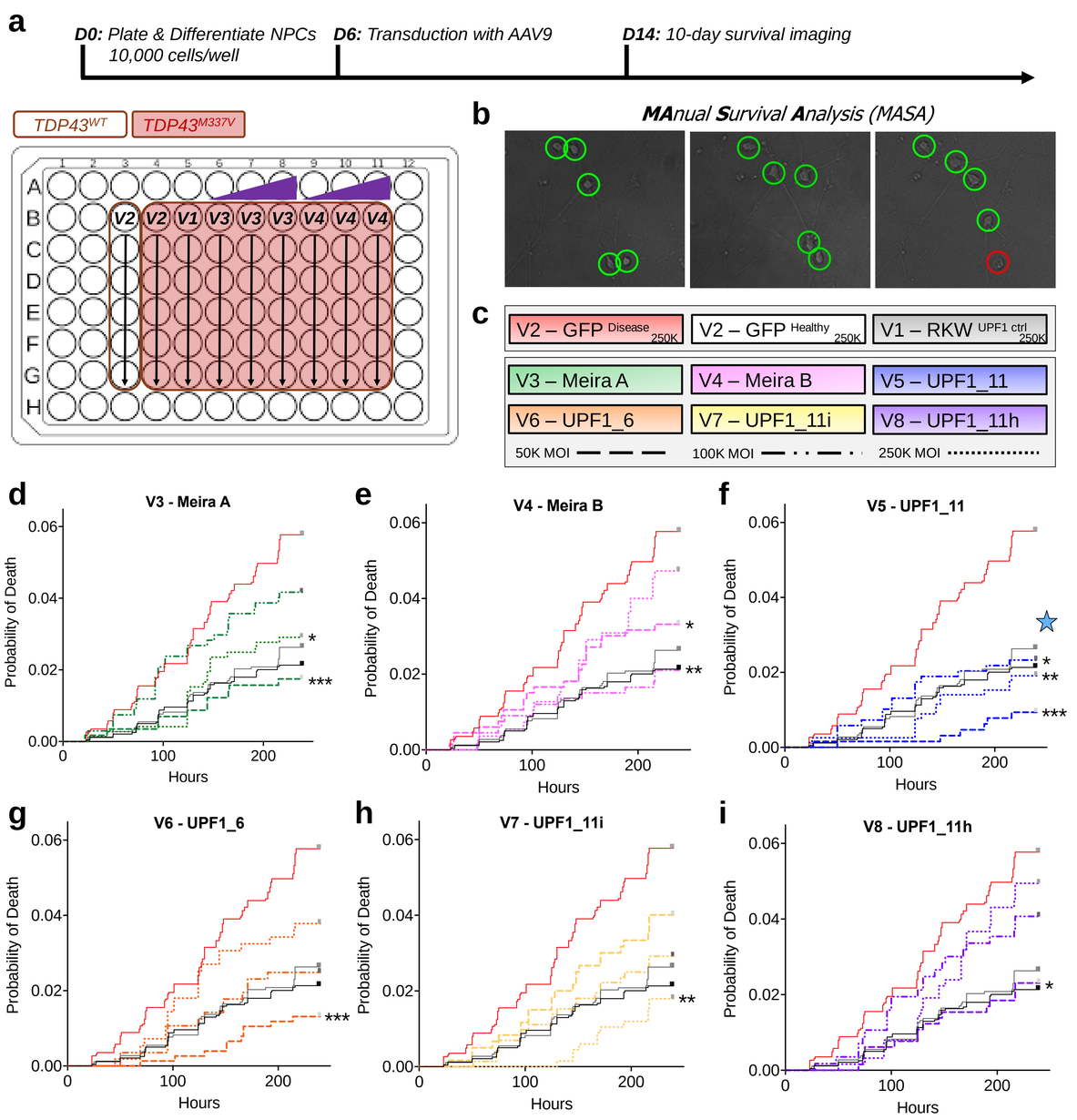

Figure 3. Expression of UPF1 variants protects against TDP-43 toxicity in iNeurons at different dose level. (**a**) Schematic showing UPF1 rescue of neuronal survival in the TDP43 iNeuron model of ALS. iPSC-derived neural progenitor cells (NPCs) with WT or isogenic disease mutation M337V for TDP43 were plated and differentiated into motor neurons and transduced with AAV9 containing optimized UPF1 constructs at different dose levels with MOIs of 50, 100 or 250K then monitored for survival. (**b**) Neuronal survival was monitored with sequential imaging across multiple days. The time to death for each cell is used to create cumulative hazard plots displaying the risk of death over time for neurons in each population in panel d-i. (**c**) Table of constructs tested. (**d-i**) V 2-GFP expression in the TDP43 mutant was the disease model and negative control (red), V2-GFP expression in WT was healthy control (black), and V1-RKW was UPF1 reference control (grey) because it is the same construct that showed rescue in yeast and rat of Figure 1. UPF1 expression by AAV9 transduction reduces the risk of death for all UPF1 variants at different dose levels. V5-UPF1_11 was the most potent vector that protected against TDP-43 toxicity at all dose levels, and showed the greatest survival at the lowest MOI of 50K. Each line summarizes experiments pooled from 6 technical replicates of at least 4 biological replicates. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 for the cumulative hazard ratio relative to V2-GFP in TDP43 mutant iNeurons.

AAV vector engineering and screening

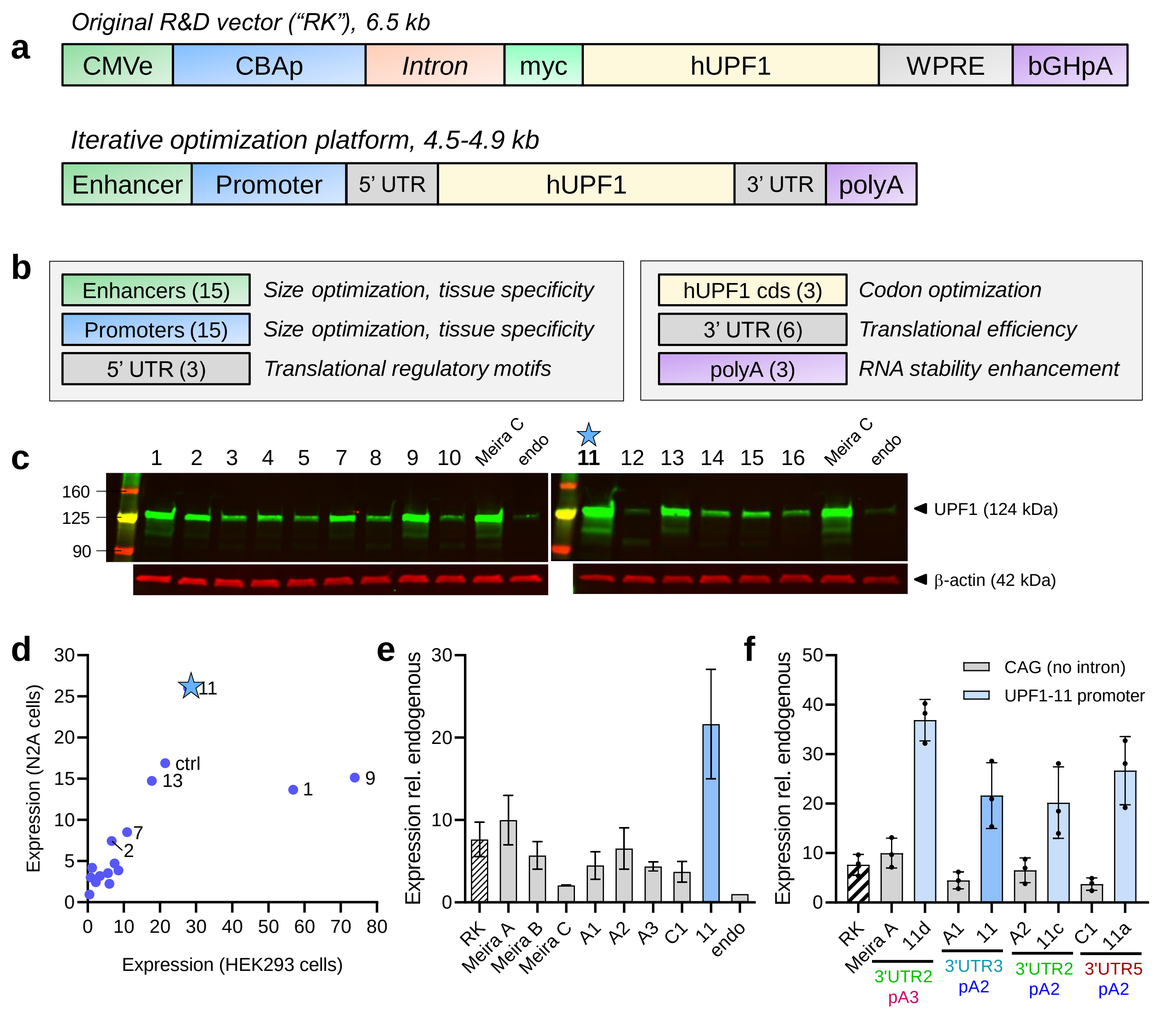

Figure 2. AAV vector engineering and screening. (**a**) Vectors reported here are at least 1.6 kb smaller than the original myc-UPF1 vector used in the Fig. 1b experiment. (**b**) Summary of promoter and *cis*-regulatory elements used to design the new vectors. Parentheses: number of unique variants. (**c**) Western blot of 16 new UPF1 vectors and the Meira C vector control after transfection in Neuro2A (N2A) cells. Expression level of over 40 constructs were screened iteratively this way. The star indicates UPF1-11. Endo: endogenous UPF1 level rel. mCherry-transfected well. (**d**) Protein expression levels quantified from Western blotting in HEK293T and N2A cells shown in **c**. (**e**) UPF1-11 was still the most potent vector after three rounds of optimization. (**f**) The UPF1-11 promoter was more potent than CAG even in the context of other *cis*-regulatory elements.

UPF1 protects iNeurons against C9orf72 toxicity

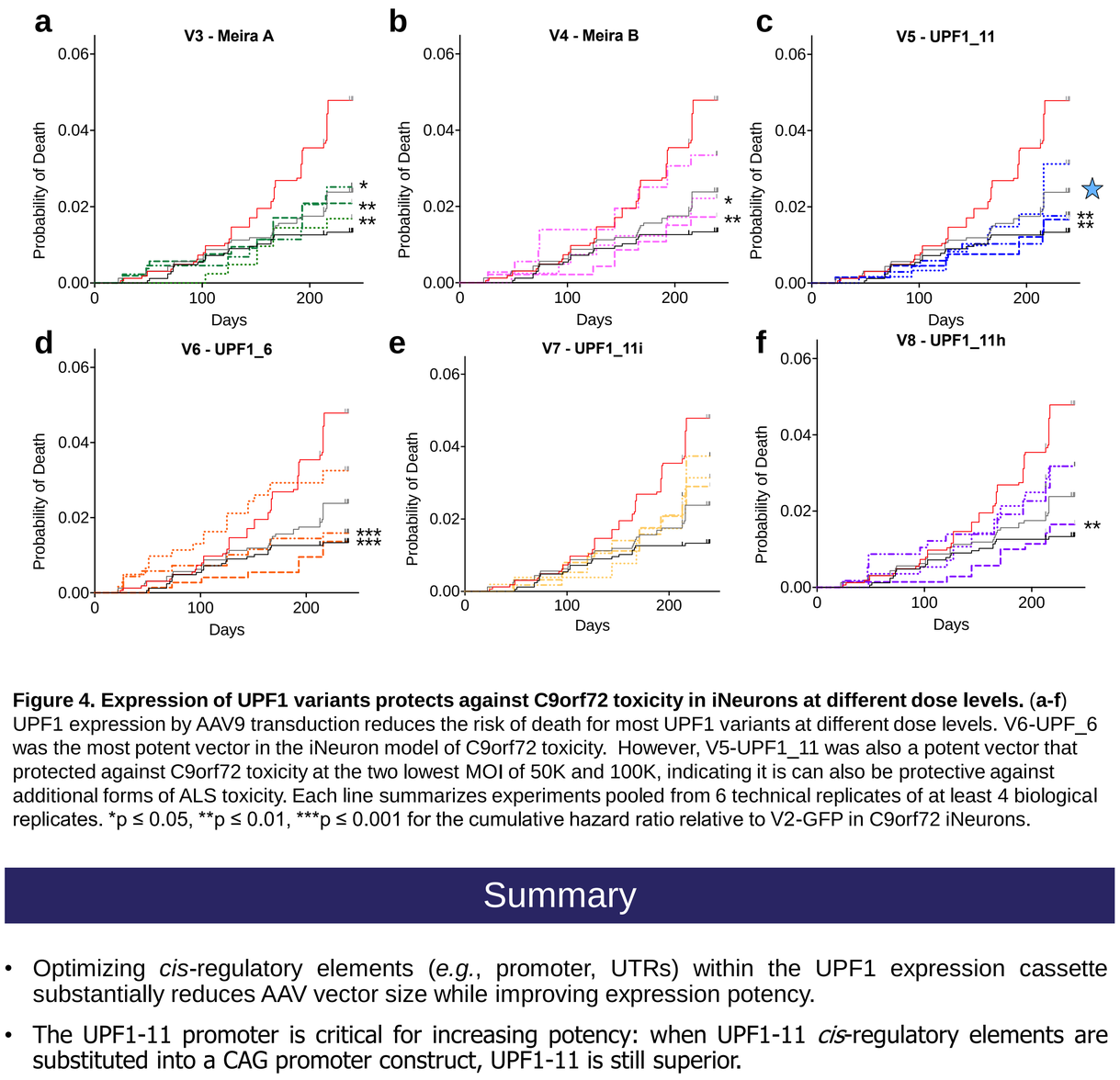

Figure 4. Expression of UPF1 variants protects against C9orf72 toxicity in iNeurons at different dose levels. (**a-f**) UPF1 expression by AAV9 transduction reduces the risk of death for most UPF1 variants at different dose levels. V6-UPF_6 was the most potent vector in the iNeuron model of C9orf72 toxicity. However, V5-UPF1_11 was also a potent vector that protected against C9orf72 toxicity at the two lowest MOI of 50K and 100K, indicating it is can also be protective against additional forms of ALS toxicity. Each line summarizes experiments pooled from 6 technical replicates of at least 4 biological replicates. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 for the cumulative hazard ratio relative to V2-GFP in C9orf72 iNeurons.

Summary

- Optimizing *cis*-regulatory elements (*e.g.*, promoter, UTRs) within the UPF1 expression cassette substantially reduces AAV vector size while improving expression potency.
- The UPF1-11 promoter is critical for increasing potency: when UPF1-11 *cis*-regulatory elements are substituted into a CAG promoter construct, UPF1-11 is still superior.
- UPF1 expression is protective in multiple models of ALS including TDP-43, FUS, and C9orf72-related toxicity.
- The optimized construct, UPF1-11, offers the greatest iNeuron survival improvement at the lowest AAV dosage, suggesting that it will have clinical advantages.

References

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