Identification of a novel disease mechanism and development of therapeutics for the recently identified neurodevelopmental disease "NEDAMSS"

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BACKGROUND

- The Interferon Regulatory Factor 2 Binding Protein Like (*IRF2BPL*) is an intronless gene that encodes a member of the *IRF2BP* family of transcriptional regulators and is ubiquitously expressed. The function of this protein is unknown.
- Recently, mutations in this gene were found to be associated with **NEDAMSS** disease:
  - Neurodevelopmental disorder with regression, abnormal movements, loss of speech, and seizure in adults and children (Marcogliese *et al* 2018).
  - Autosomal dominant inheritance
  - 25 people are known to be affected around the world so far.
- We received four patient fibroblast cell lines having heterozygous nonsense variants in the *IRF2BPL* gene resulting in the truncation of its RING finger domain (Figure 1).
- In this study, we reprogrammed patient fibroblasts to astrocytes and neurons to understand the disease mechanism and developed promising therapeutic strategies that might be able to slow or halt the progression of the disease.

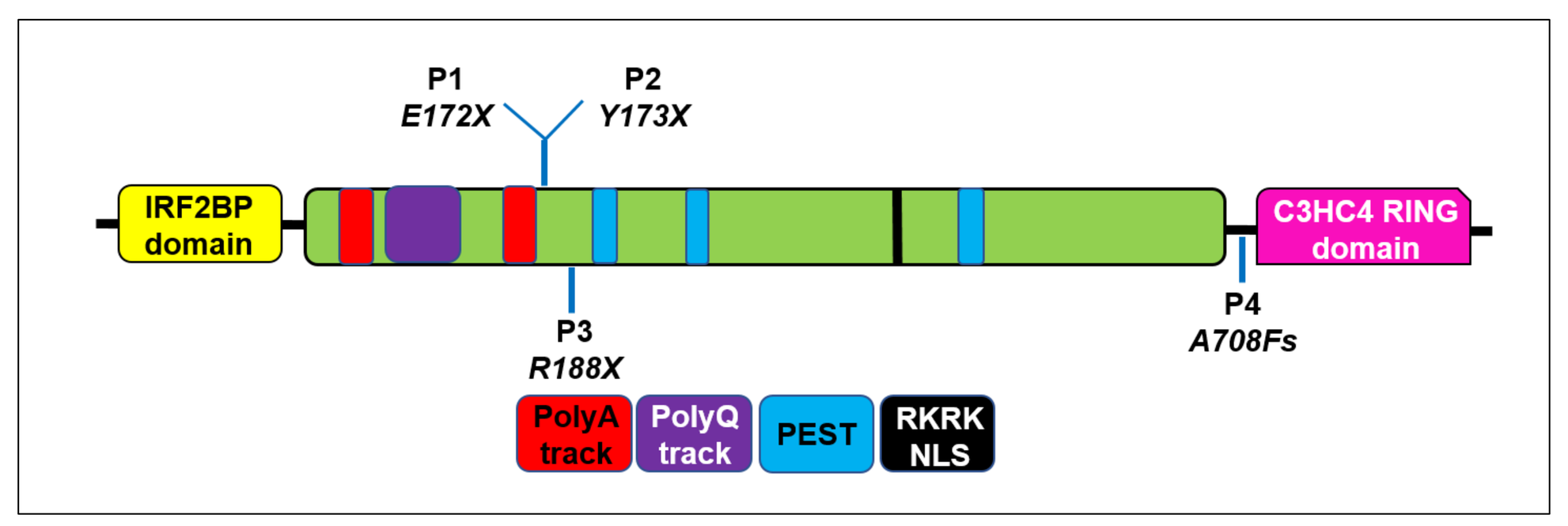
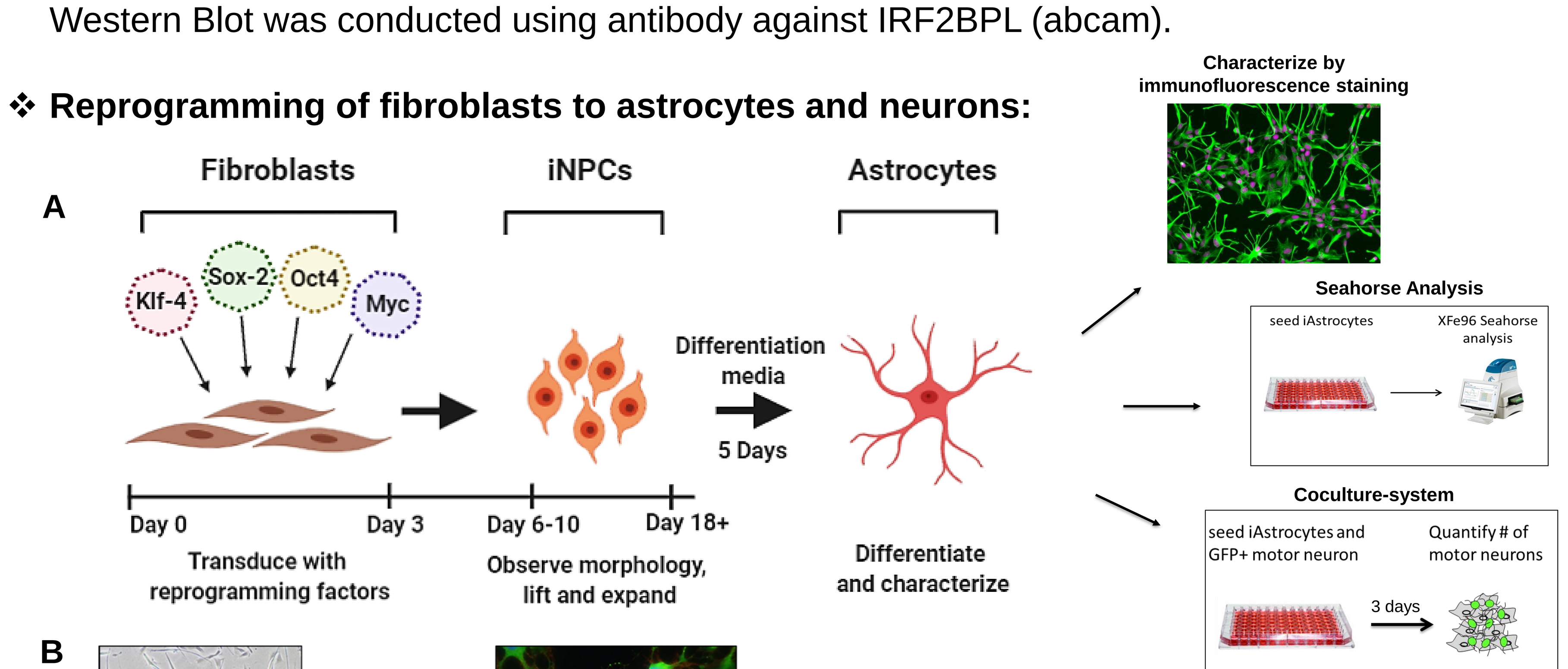


Figure 1: Illustration of the different domains in IRF2BPL protein and the location of nonsense variants in the four patient fibroblast cell lines.

METHODS

- **Cell culture and Western Blot:** Patient and healthy fibroblasts were maintained in DMEM media supplemented with 10% FBS. To quantify the expression of IRF2BPL protein in the fibroblasts, Western Blot was conducted using antibody against IRF2BPL (abcam).
- **Reprogramming of fibroblasts to astrocytes and neurons:**



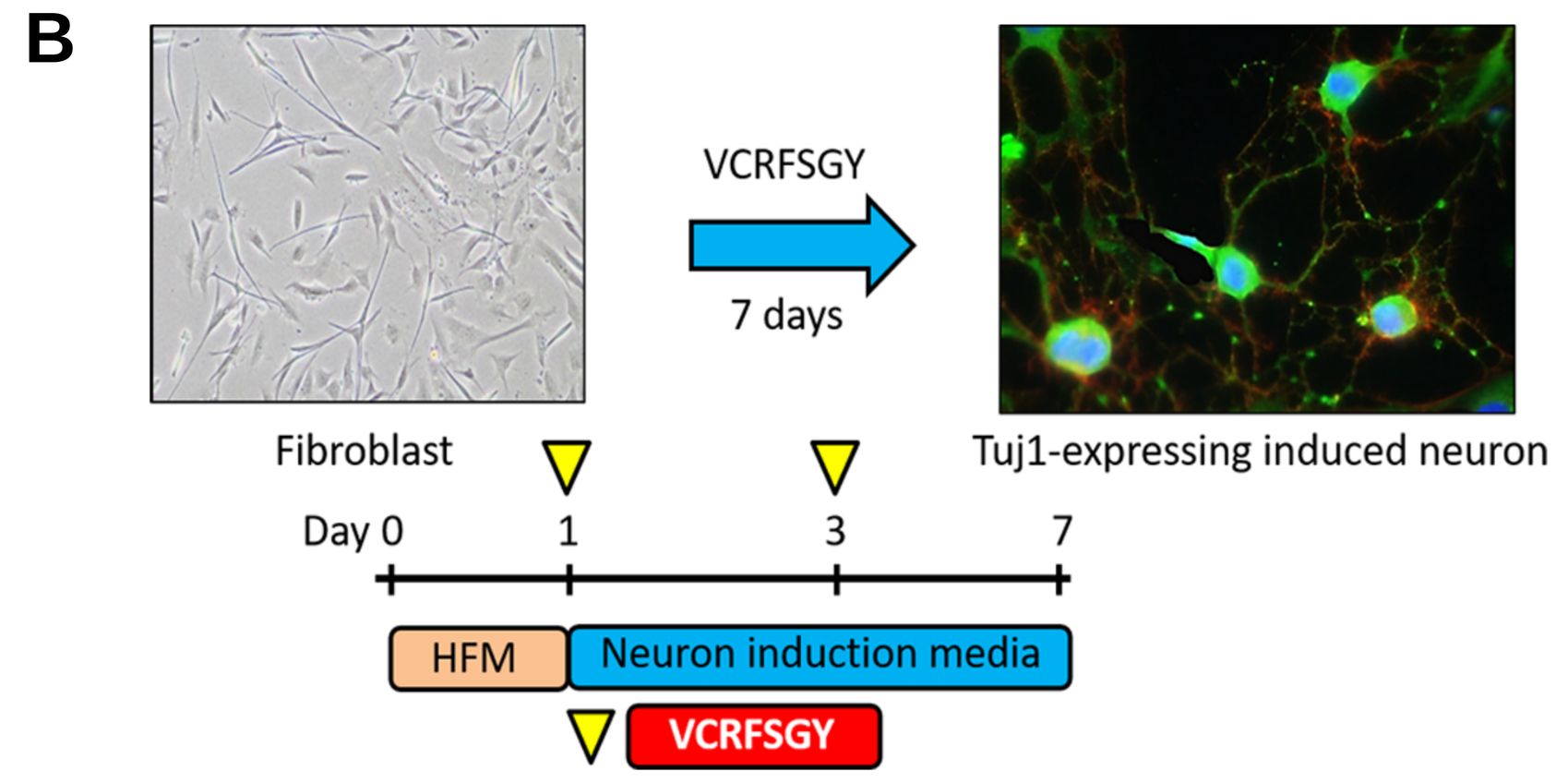


Figure 2: Direct reprograming of human skin cells can be used to model NEDAMSS. (A) Schematic representation of direct conversion of fibroblasts to neuronal progenitor cells (NPC), which were further differentiated into astrocytes (Meyer *et al* 2014). Following conversion, astrocytes were characterized, analyzed by Seahorse assay or co-cultured with stem cell derived GFP+ motor neurons. (B) Direct chemical conversion of fibroblasts to tuj1-expressing induced neurons using seven small molecules (Hu *et al* 2015).

RESULTS

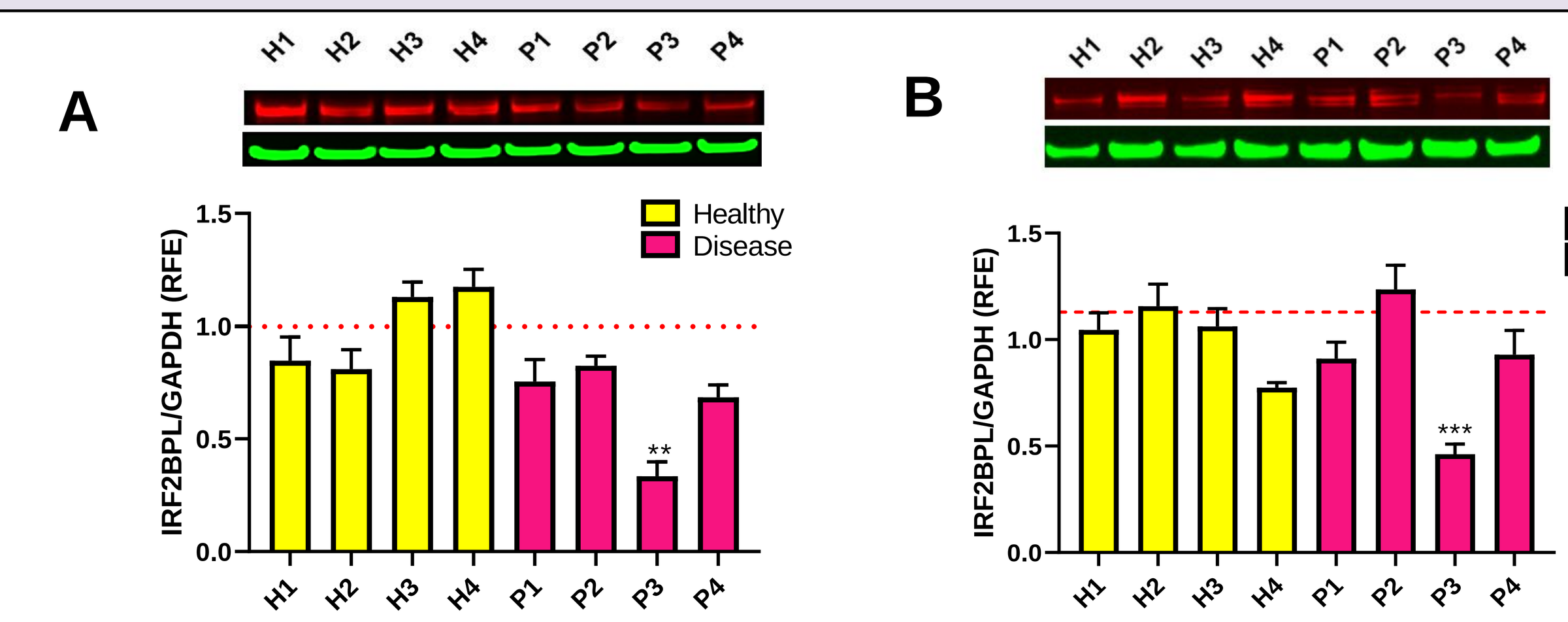


Figure 3: IRF2BPL protein levels is only reduced in one adult patient line. IRF2BPL protein expression in (A) fibroblasts and (B) astrocytes.

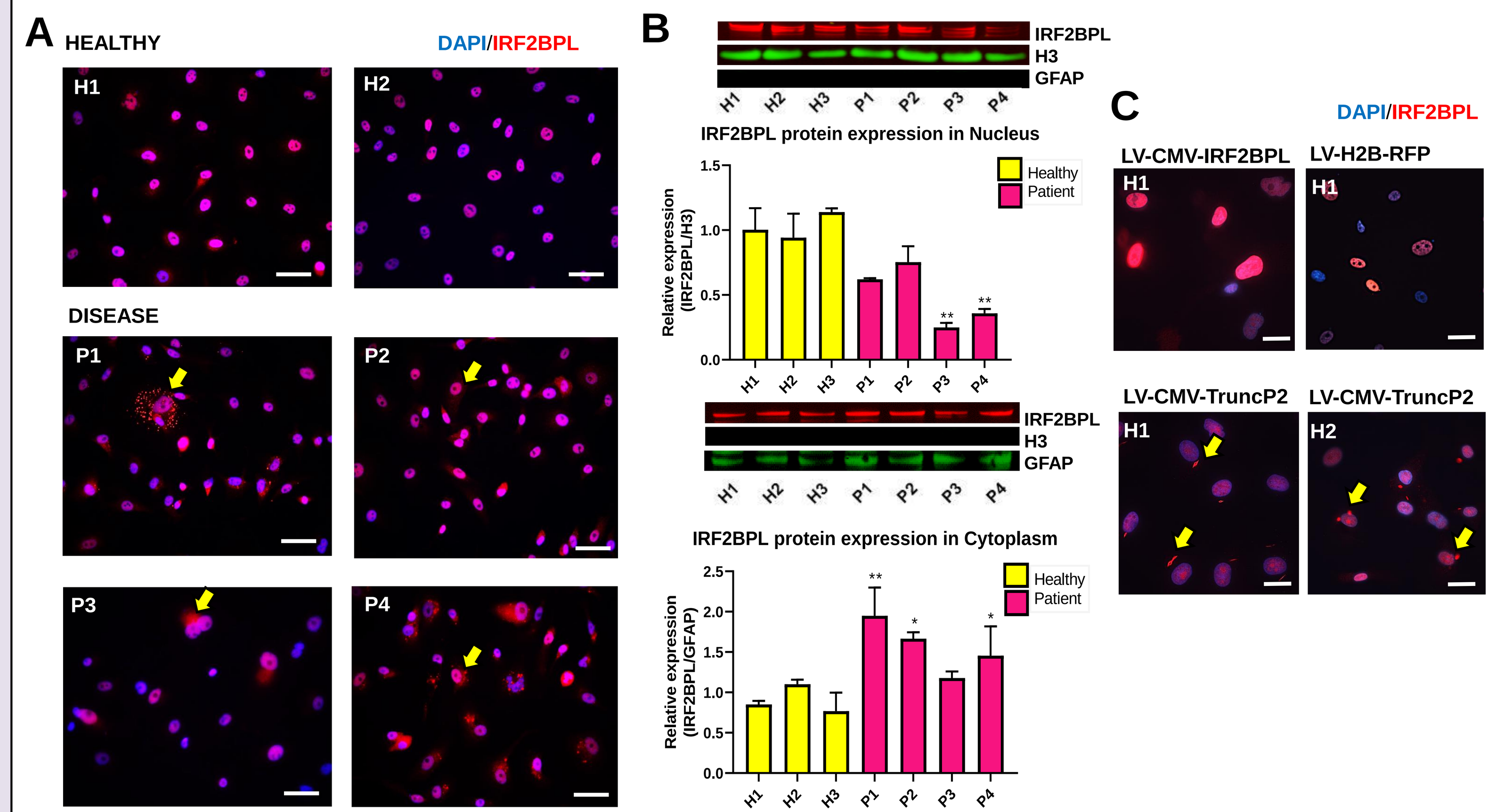


Figure 4: IRF2BPL mutations lead to mislocalization of the protein. (A) Immunofluorescence staining shows accumulation of the protein in the cytoplasm of patient astrocytes. **(B)** Western blot confirms the more accumulation of IRF2BPL in the cytoplasmic extract than in the nucleus. **(C)** Healthy astrocytes (H1 and H2) infected with lenti-virus expressing patient P2 mutant form of IRF2BPL indicates mislocalization and formation of aggregates in the cytoplasm.

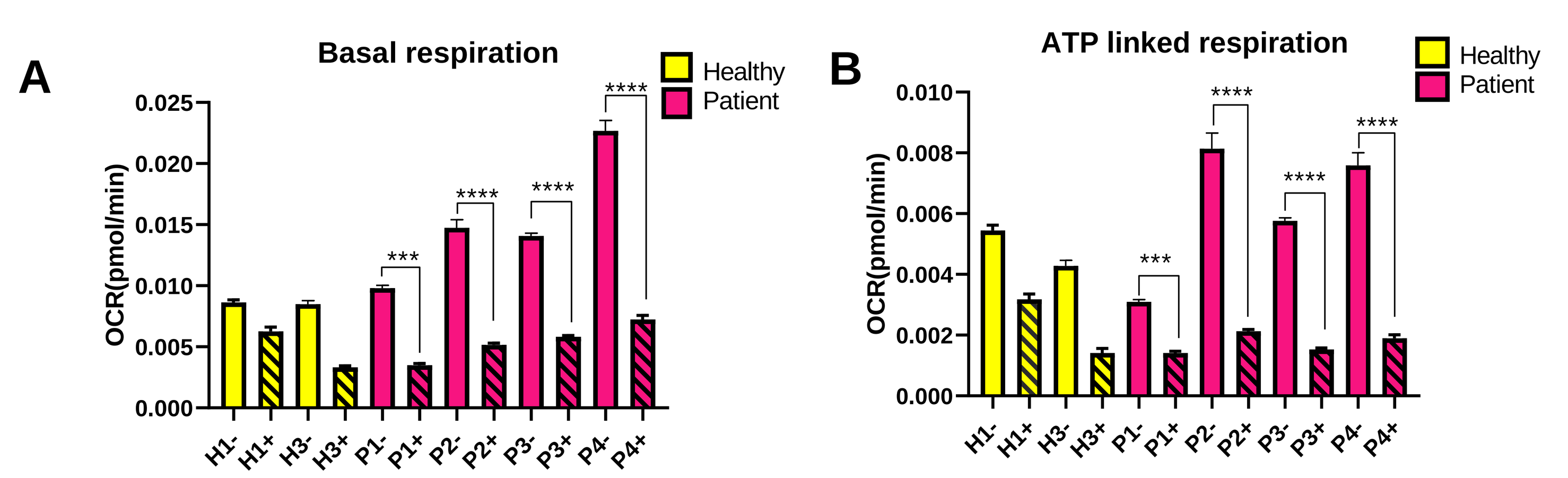


Figure 5: NEDAMSS astrocytes indicate elevated basal and ATP-linked respiration; CuATSM drug reduced the elevated levels to normal levels . (A). Basal respiration and (B) ATP–linked respiration of drug treated and untreated healthy and patient astrocytes.

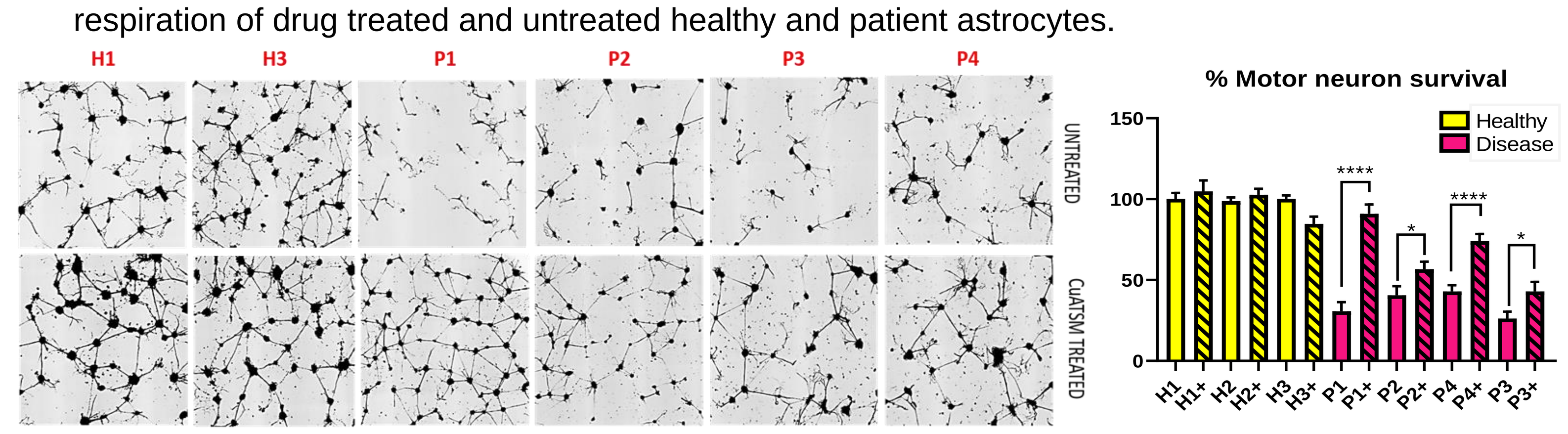
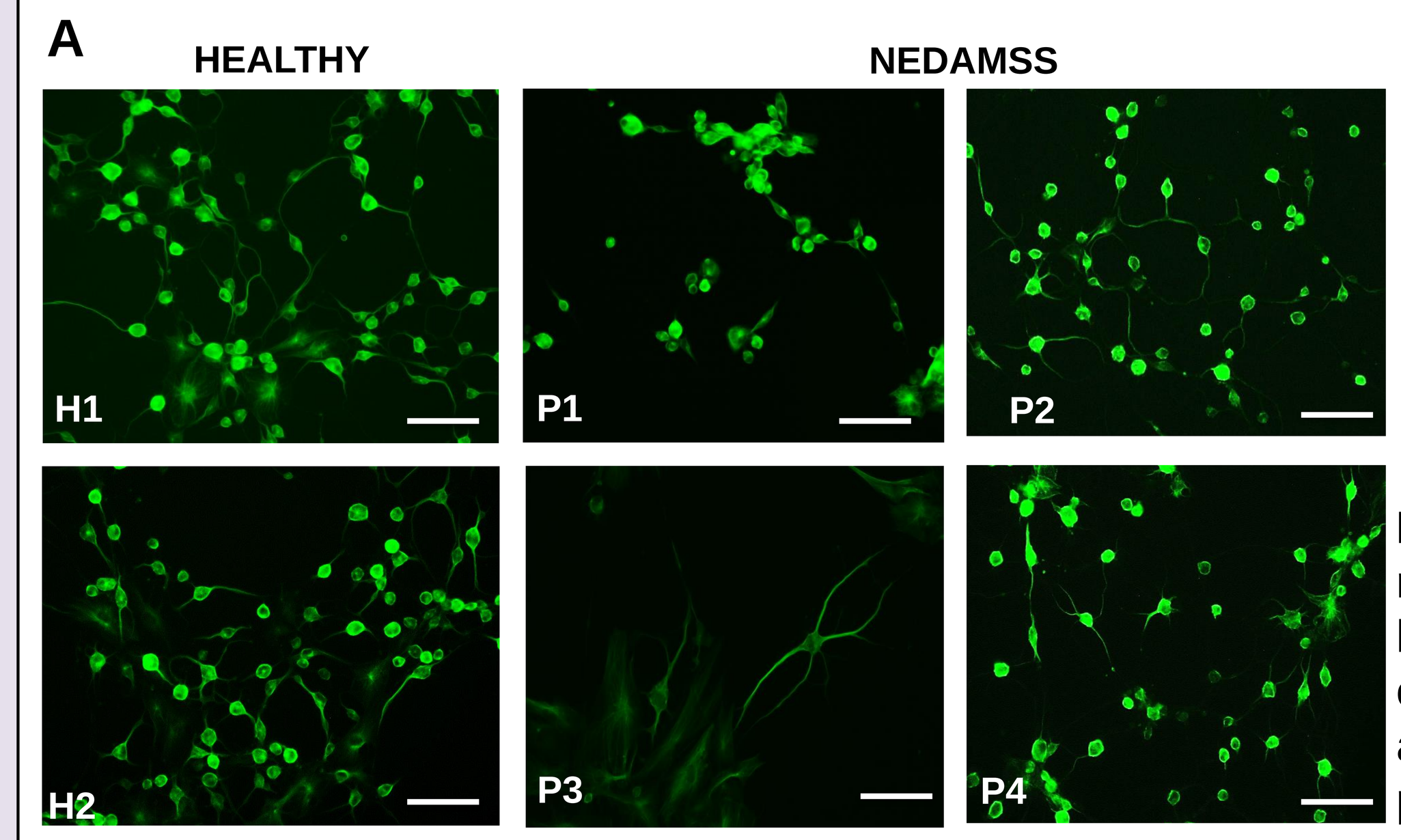


Figure 6: IRF2BPL astrocytes are toxic to motor neurons; CuATSM drug significantly alleviates the toxicity (A) Representative image and **(B)** percent motor neuron survival following three days co-culture of drug treated or untreated patient or healthy astrocytes with GFP+ motor neurons (in black), n=3.

RESULTS



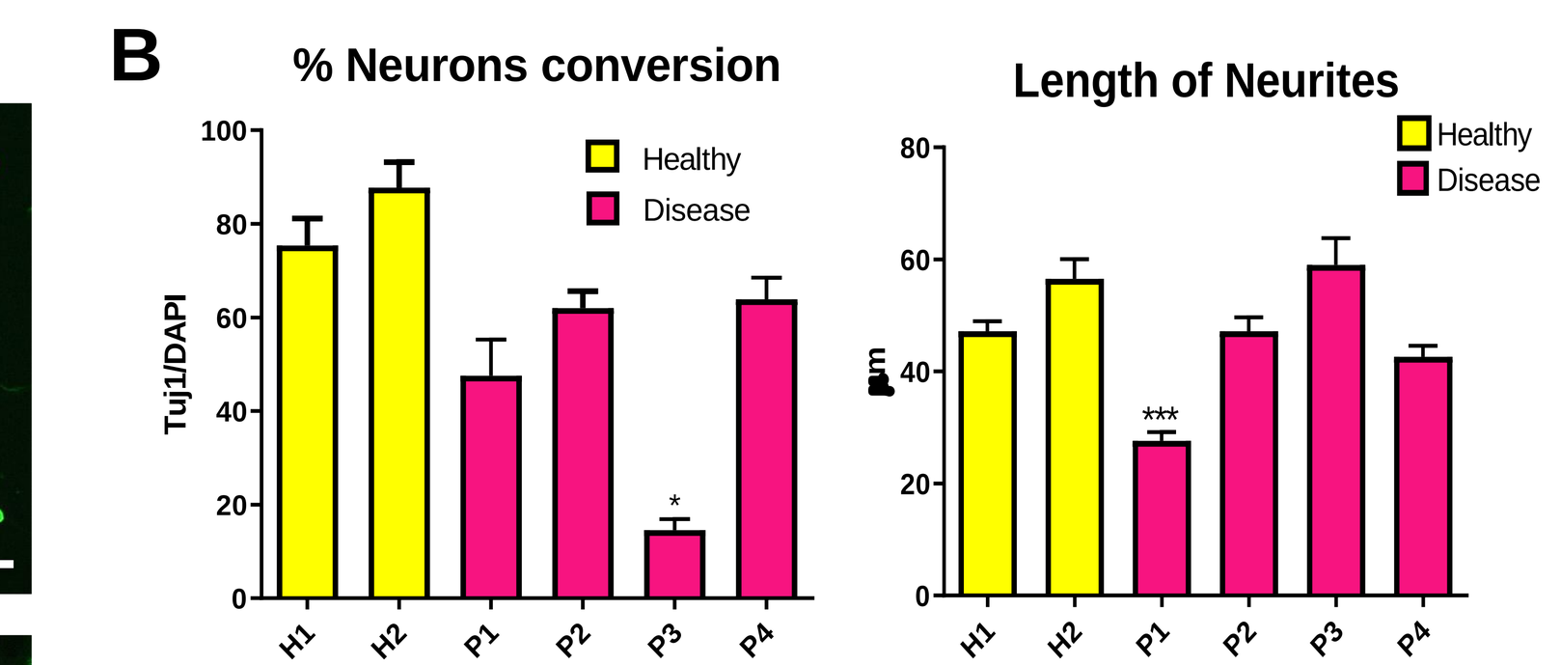


Figure 7: Two NEDAMSS patients indicate reduced number of neuron converted than healthy controls. (A) Immunofluorescence staining of day 7 chemically-induced neurons with tuj1 antibody. (B) Neuron analysis indicates variable phenotype in converted neurons from NEDAMSS patients and significantly shorter neurite length for P1.

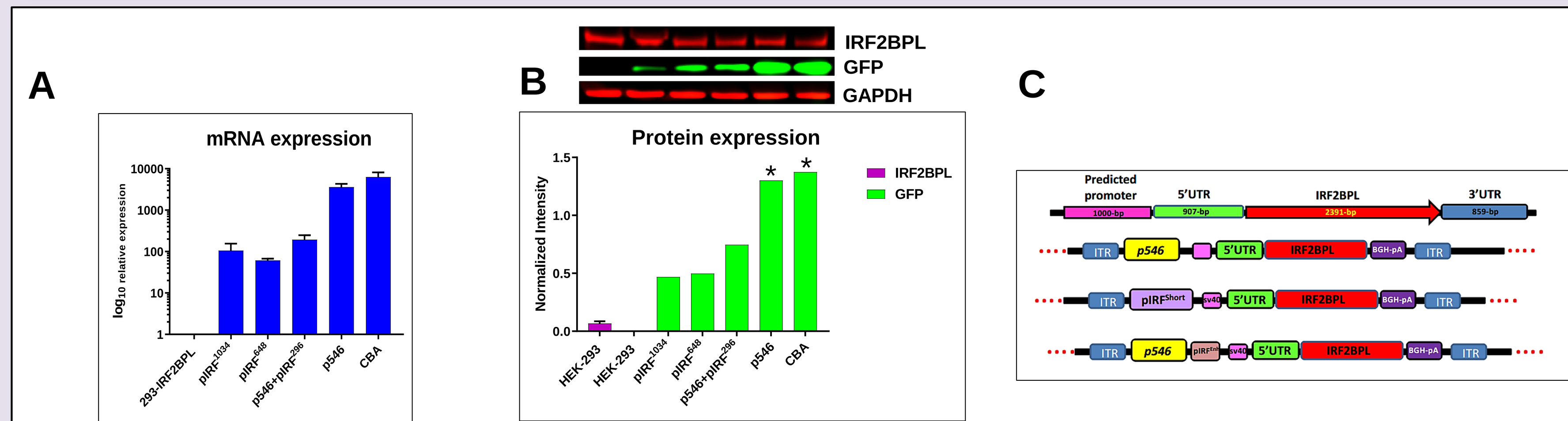


Figure 8: AAV9 vectors with truncated variants of IRF2BPL endogenous promoter expresses GFP at more regulated levels compared to traditionally used gene therapy promoters. (A) GFP mRNA expression and (B) GFP protein expression after transfection of HEK293s with the five designed AAV9 vectors (C) AAV9 gene therapy constructs expressing full length IRF2BPL under three selected promoters will be tested as a potential treatment strategy.

CONCLUSION

- Although Marcogliese *et al.* suggested a loss of function mechanism for the disease, only adult patient 1911 had significantly reduced IRF2BPL expression in both fibroblasts and astrocytes compared to child patients and healthy controls. This may be explained by the healthy allele compensating the expression of IRF2BPL in younger patients.
- IRF2BPL mutations accumulate in the cytoplasm of astrocytes and could be one cause for neurodegeneration.
- NEDAMSS patient astrocytes were found to have elevated levels of mitochondrial respiration and are toxic to motor neurons in co-culture assays. **Treatment with CuATSM significantly improved the respiration levels and motor neuron survival in all patients.**
- Development of gene therapy approach using AAV9 as discussed above, to see if it ameliorates the disease phenotype *in vitro* and potentially *in vivo*.

FUNDING: We are very grateful to Stand By Eli Foundation (through Giving Back Fund) for funding our research on this rare neurodegenerative disease. Special thanks also to Families of SCN2A for sponsoring the Seahorse equipment.

REFERENCES

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