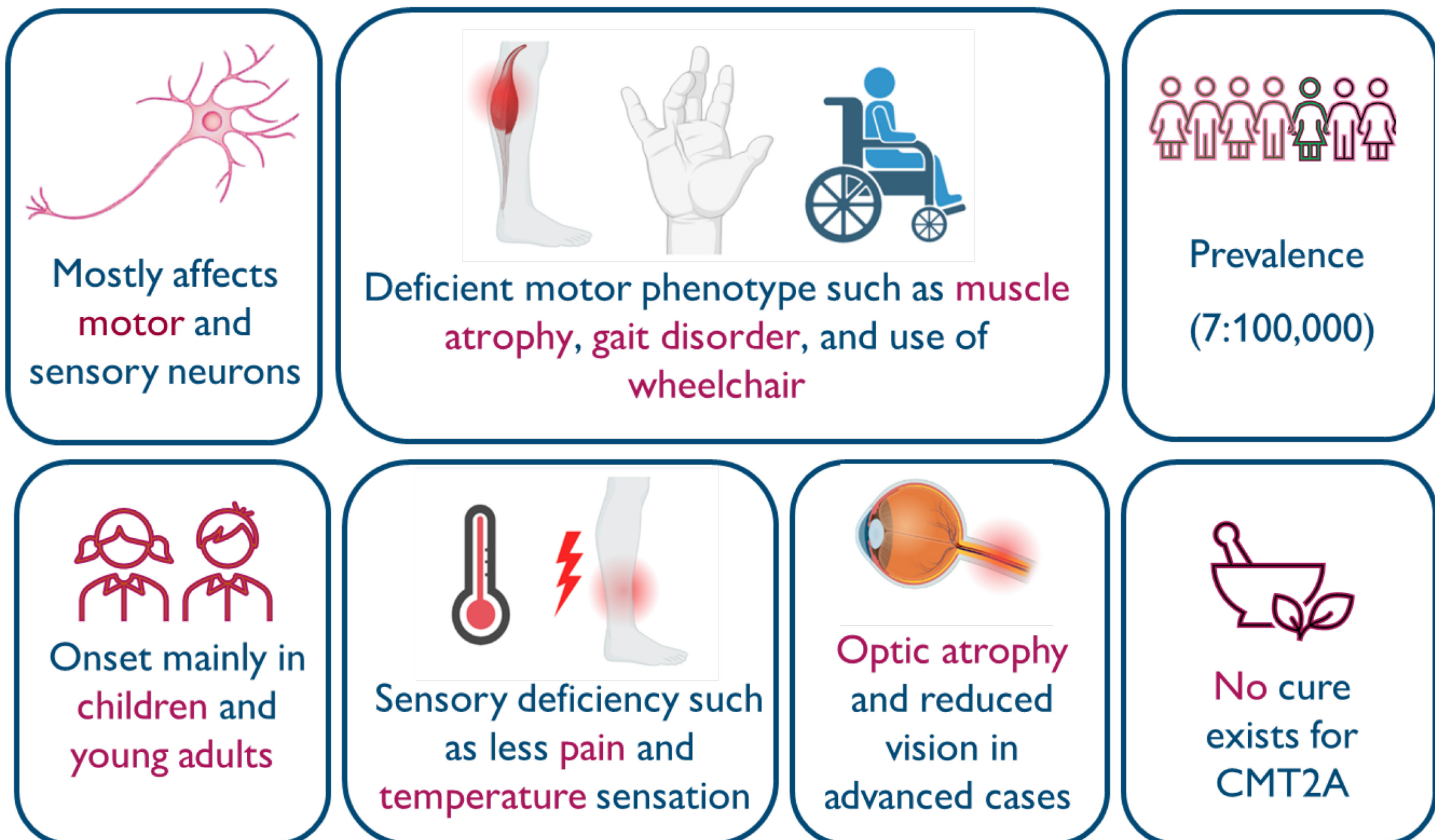


Zinc Finger Mediated Repression and Replacement of MFN2 Leads to the Rescue of Cellular Disease Phenotype in CMT2A Patient-Derived Cells.

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Poster #637

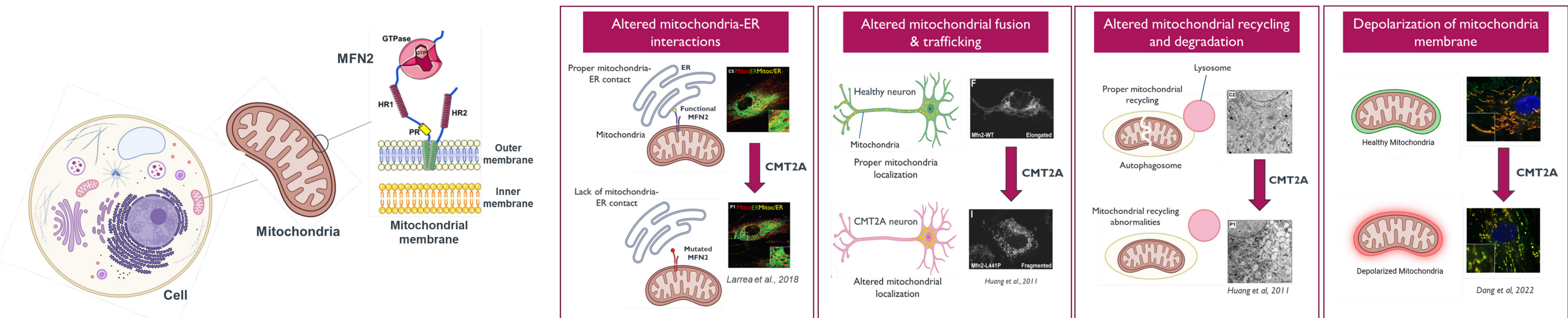
CMT2A disease Introduction



- CMT2A is a hereditary neuropathy disorder of the peripheral motor systems (90% autosomal dominant)
- Peripheral motor neurons are primarily affected
- There is currently no cure or treatments available for CMT2A

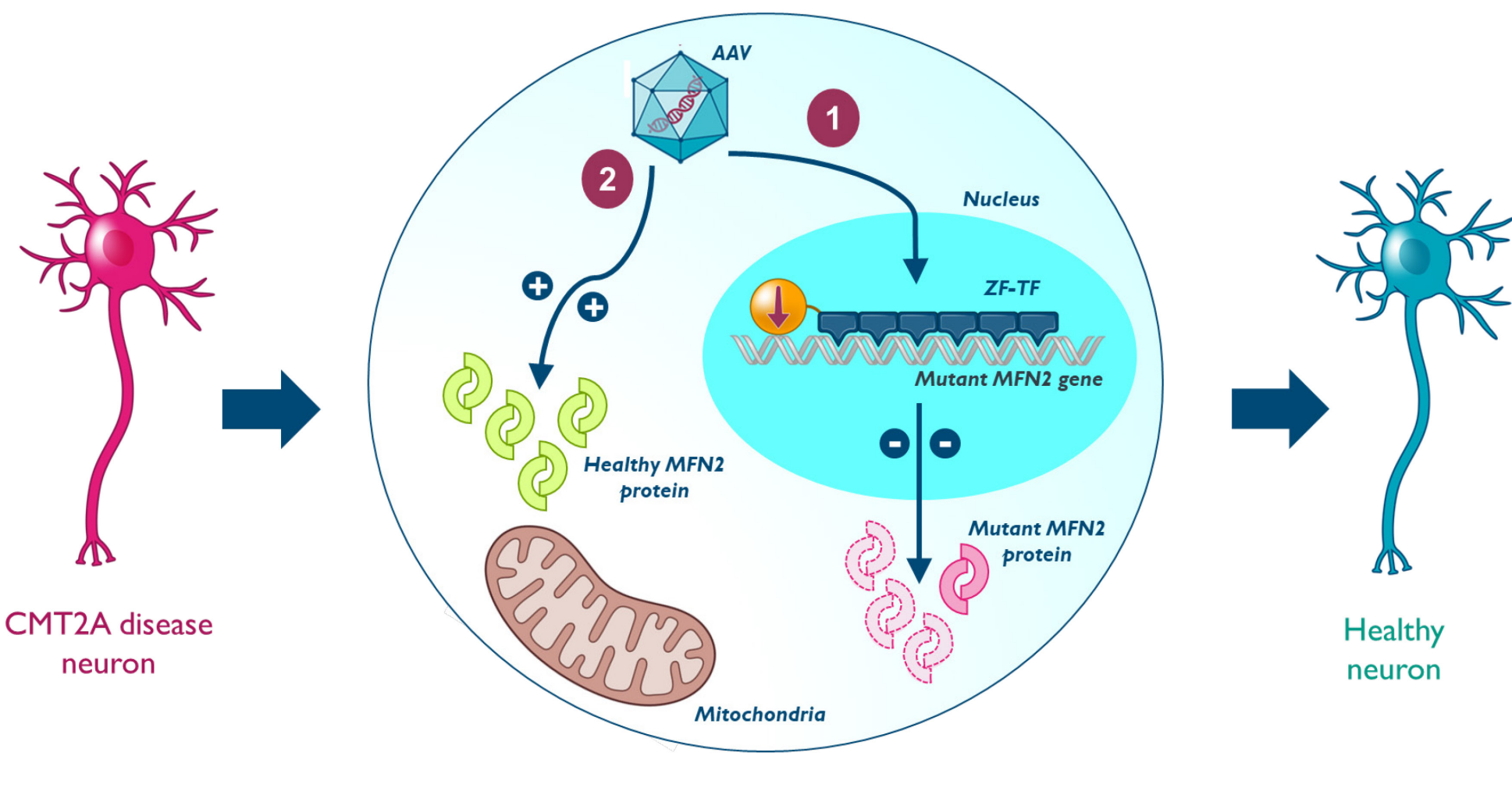
Mutations in the Mitofusion 2 (MFN2) gene underpin CMT2A pathology

- MFN2 is a GTPase dynamin-like protein localizing to the outer membrane of mitochondria
- Dysfunctional MFN2 disrupts mitochondrial function in cells

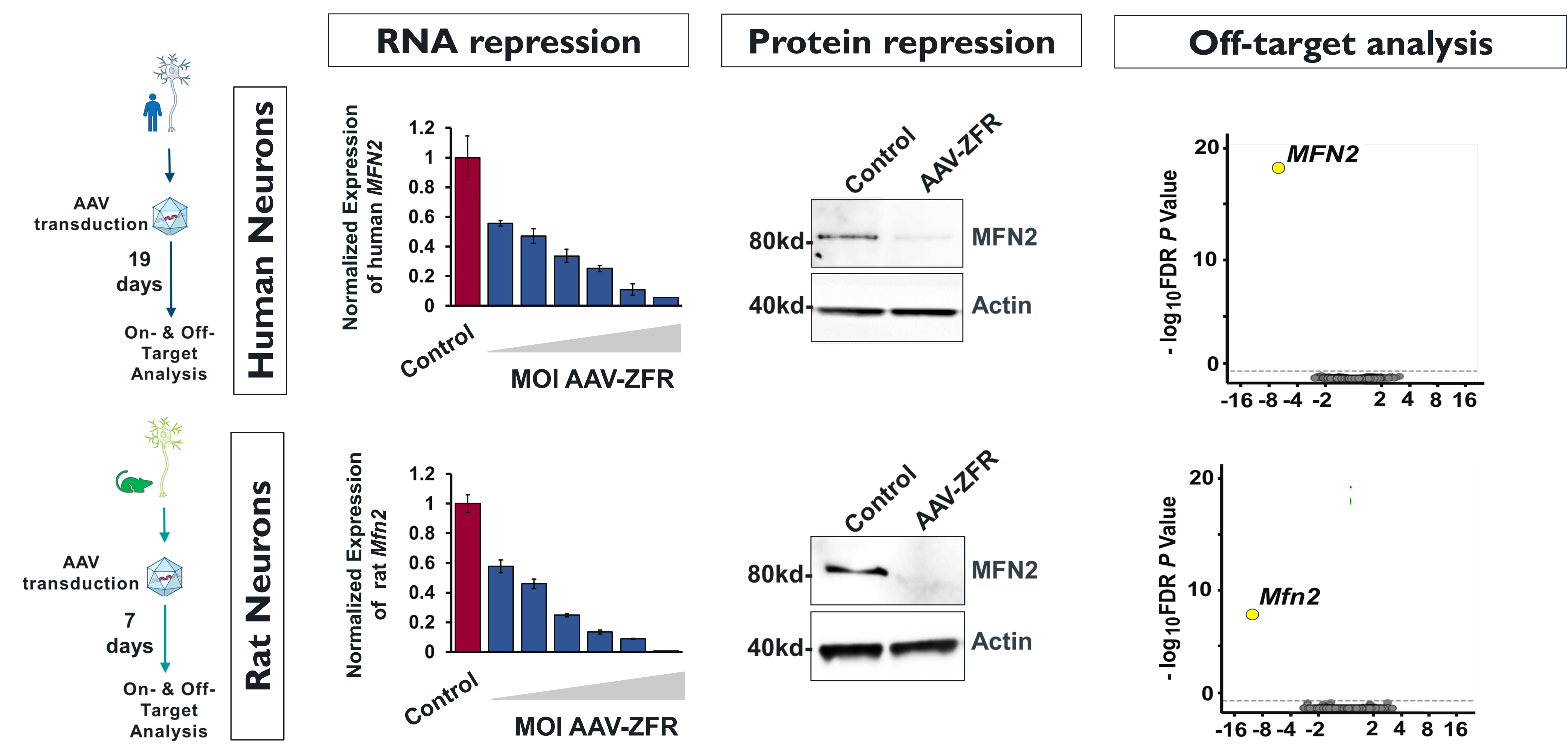


Repress and Replace (R&R) strategy as a therapeutic strategy for CMT2A disease

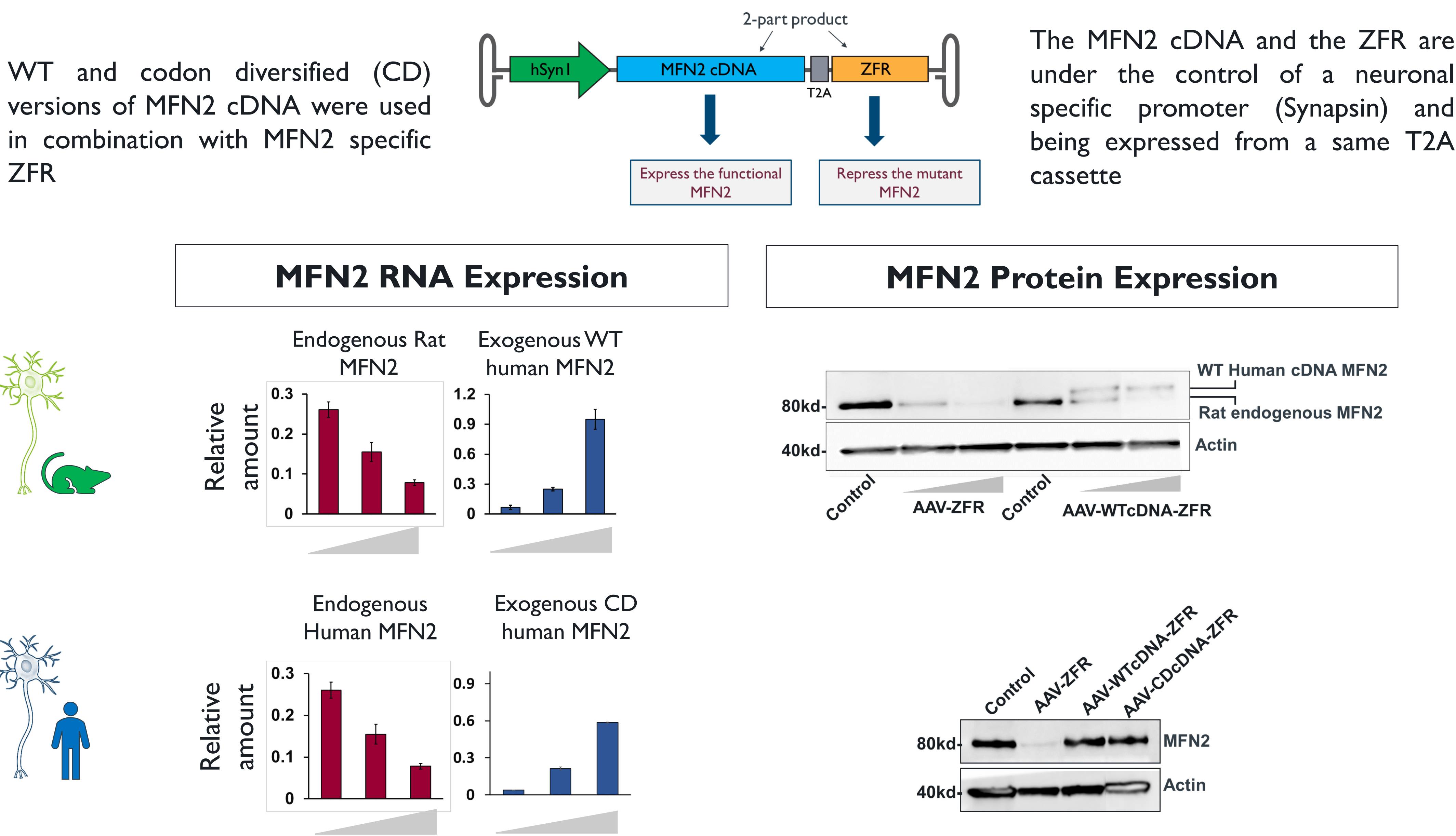
- The functionality of healthy MFN2 protein is essential for cell survival
- Biallelic repression of a mutated MFN2 is predicted to reduce its toxicity, but will result in a loss-of-function phenotype due to the lack of a healthy protein
- With R&R approach, the transduction of disease motor neurons by a single AAV vector will lead to the expression of a zinc finger repressor (ZFR) specifically repressing mutant MFN2 gene and thus reducing the disease burden in affected motor neurons. Concurrently, the expression of healthy MFN2 cDNA will lead to the production of healthy MFN2 protein (replacing) that is necessary for motor neuron survival.



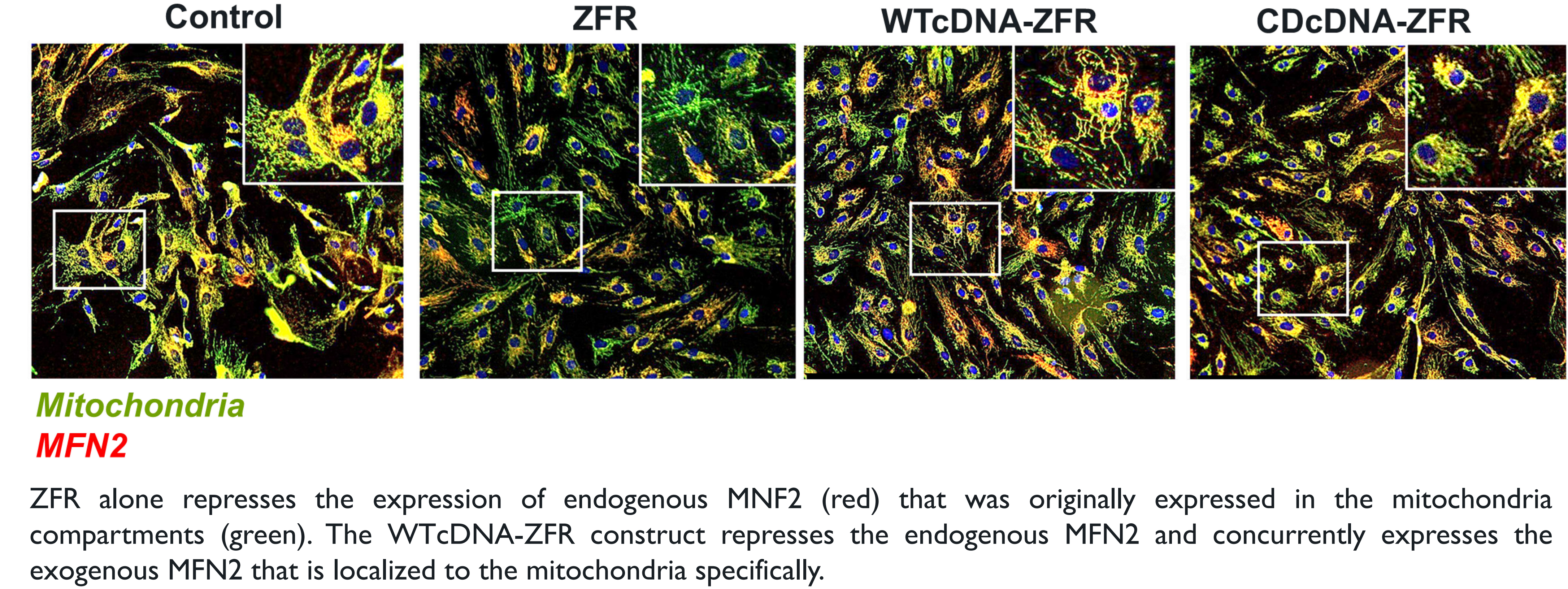
Identification of a potent and specific ZFR leading to the repression of MFN2 gene in rat and human neurons



Validation of R&R strategy in human and rat neurons

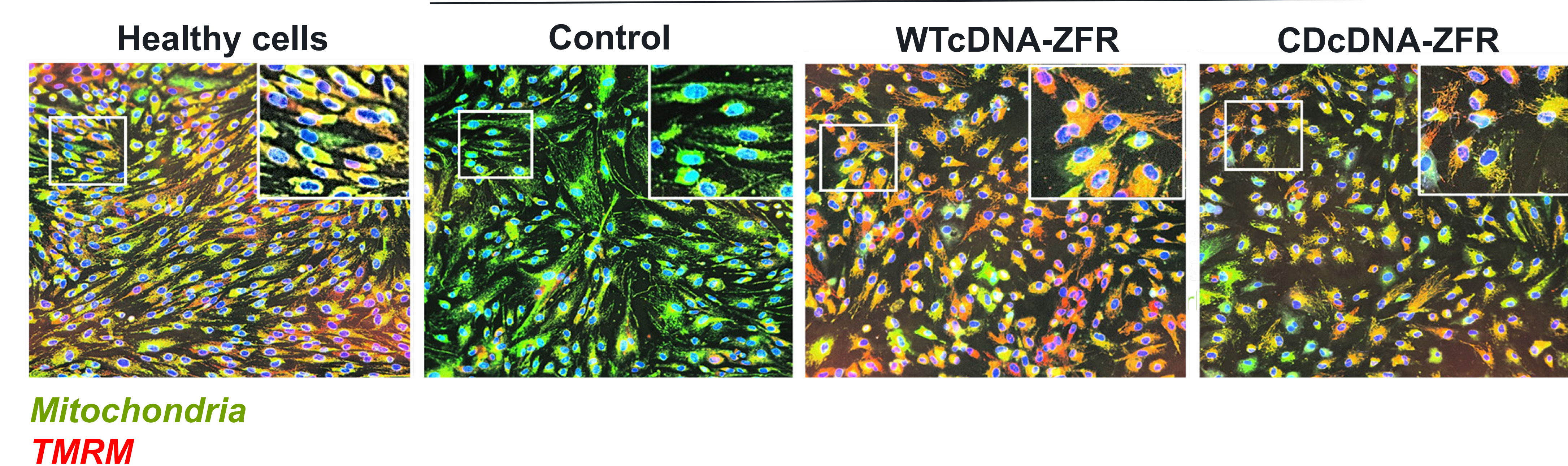


The repression of endogenous MFN2 and replacement by WT or CD cDNA in cells derived from CMT2A patients



R&R strategy rescues the disease phenotype in cells derived from CMT2A patients

- The polarization status of the mitochondria can be measured by charged dyes such as TMRM
- Polarized mitochondria (healthy) retains TMRM while depolarized or unhealthy mitochondria can not maintain TMRM
- Repolarization of the mitochondria in disease cells can be used as a readout for the functionality of the T2A constructs and R&R strategy



Conclusion

- Several ZFRs were identified able to potently and specifically repress the expression of MFN2 in rodents, NHP and human
- Repress and replace strategy was designed to repress the expression of mutated MFN2 and concurrently express the healthy MFN2 in neurons
- The expression of a single construct containing the MFN2 cDNA and MFN2 specific ZFR was able to rescue the disease phenotype in patient cells
- Taken together, these results support the further development of R&R strategy as a therapeutic avenue for CMT2A

