

Disrupted Lipid Homeostasis as a Pathogenic Mechanism in ABCA7-Associated Alzheimer's Disease Risk

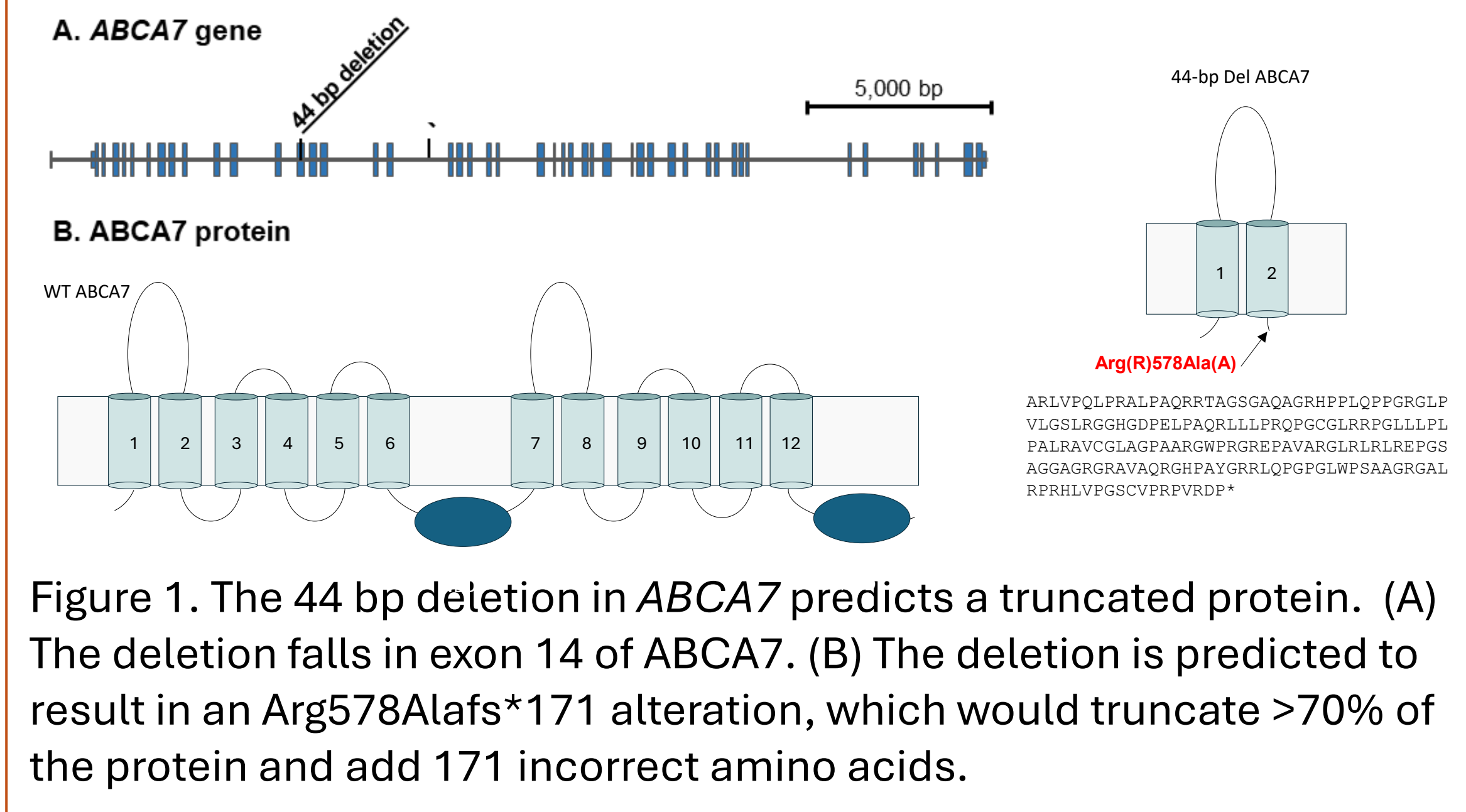
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INTRODUCTION

The *ABCA7* gene contributes to Alzheimer's disease (AD) risk in both European and African ancestry (AA) populations and plays a key role in lipid metabolism processes linked to AD pathology. *ABCA7* exerts a larger risk effect in AA populations, providing a unique opportunity to explore its functional impact on the AD phenotype. Previously, we identified a 44-base pair deletion in *ABCA7*, significantly associated with AD in AA individuals, which introduces a frameshift mutation predicted to produce a truncated protein (p.Arg578Alafs)^{1,2,3} (Figure 1). *ABCA7* normally transports lipids. While we have demonstrated that the mutant mRNA is expressed in neurons, it is still unclear whether it is translated into a stable protein. Further, the effect of this prematurely terminated *ABCA7* on lipid metabolism remains unknown. We are employing isogenic induced pluripotent stem cell (iPSC) lines to investigate these outstanding questions.



METHODS

1. *ABCA7* transcripts from noncarriers and carriers of *ABCA7* 44-del were amplified by RTPCR and identified.
2. To determine if the truncated protein is expressed and determine its intracellular localization, we performed immunocytochemistry (ICC) and western blot analysis in HEK-293 cells transiently transfected with Flag-tagged versions of *ABCA7*.
3. To explore a defect in lipid metabolism by *ABCA7* 44-del, we overexpressed it in HepG2 cells and exposed to oleic acid. Lipid droplet formation was measured by staining with LipidSpot.
4. We generated CRISPR-isogenic iPSC carrying or lacking the *ABCA7* p.Arg578Alafs variant and differentiated them into mature (28 day-old) and aged (60 days-old) neurons.
5. The iPSC derived neurons were expressed to oleic acid and lipid droplet formation was measured, and we performed a lipidomics analyses of cell pellet and conditioned media.
6. To explore AD-associated phenotypes associated to the *ABCA7* deletion, we measured the mitochondria active potential.

RESULTS

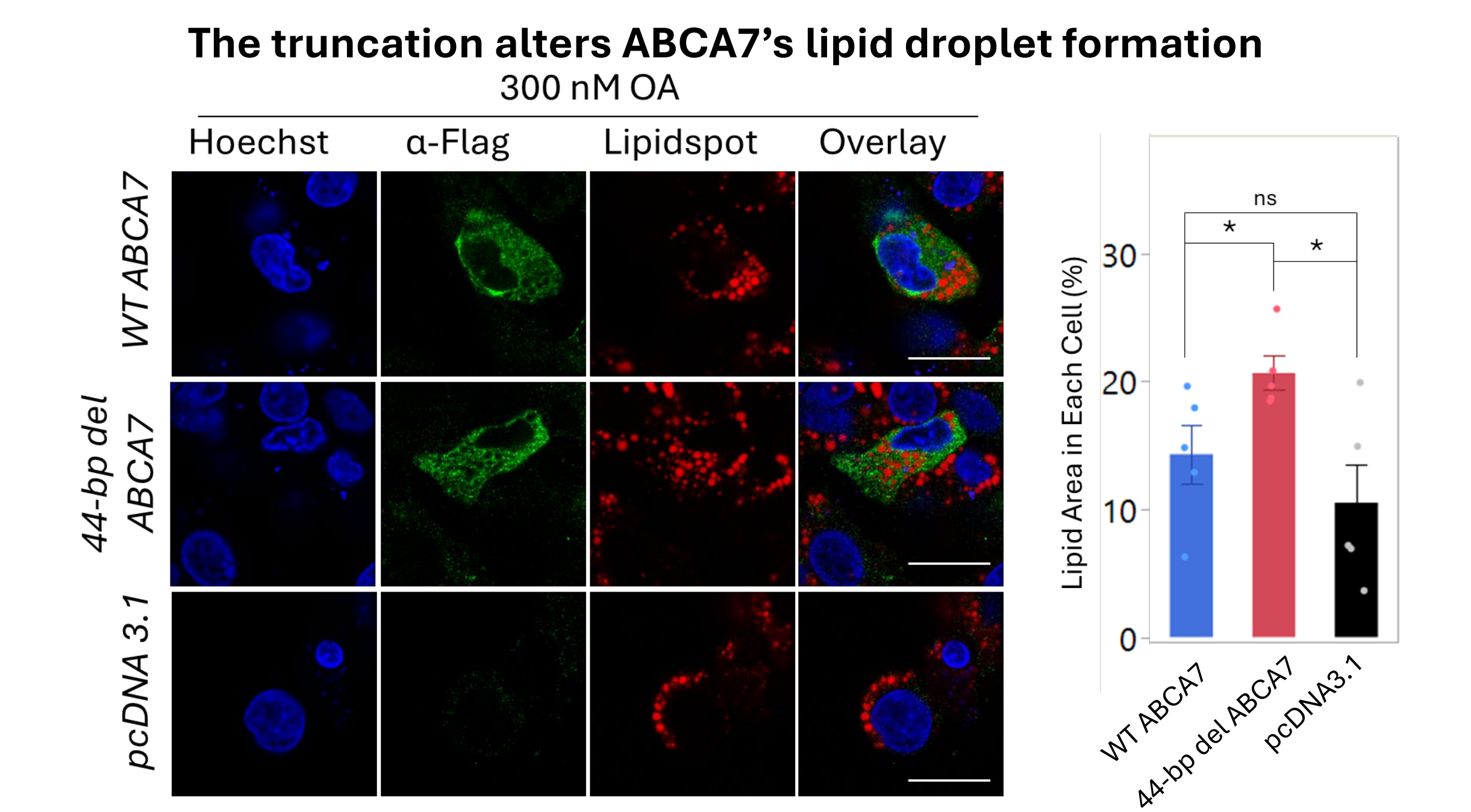
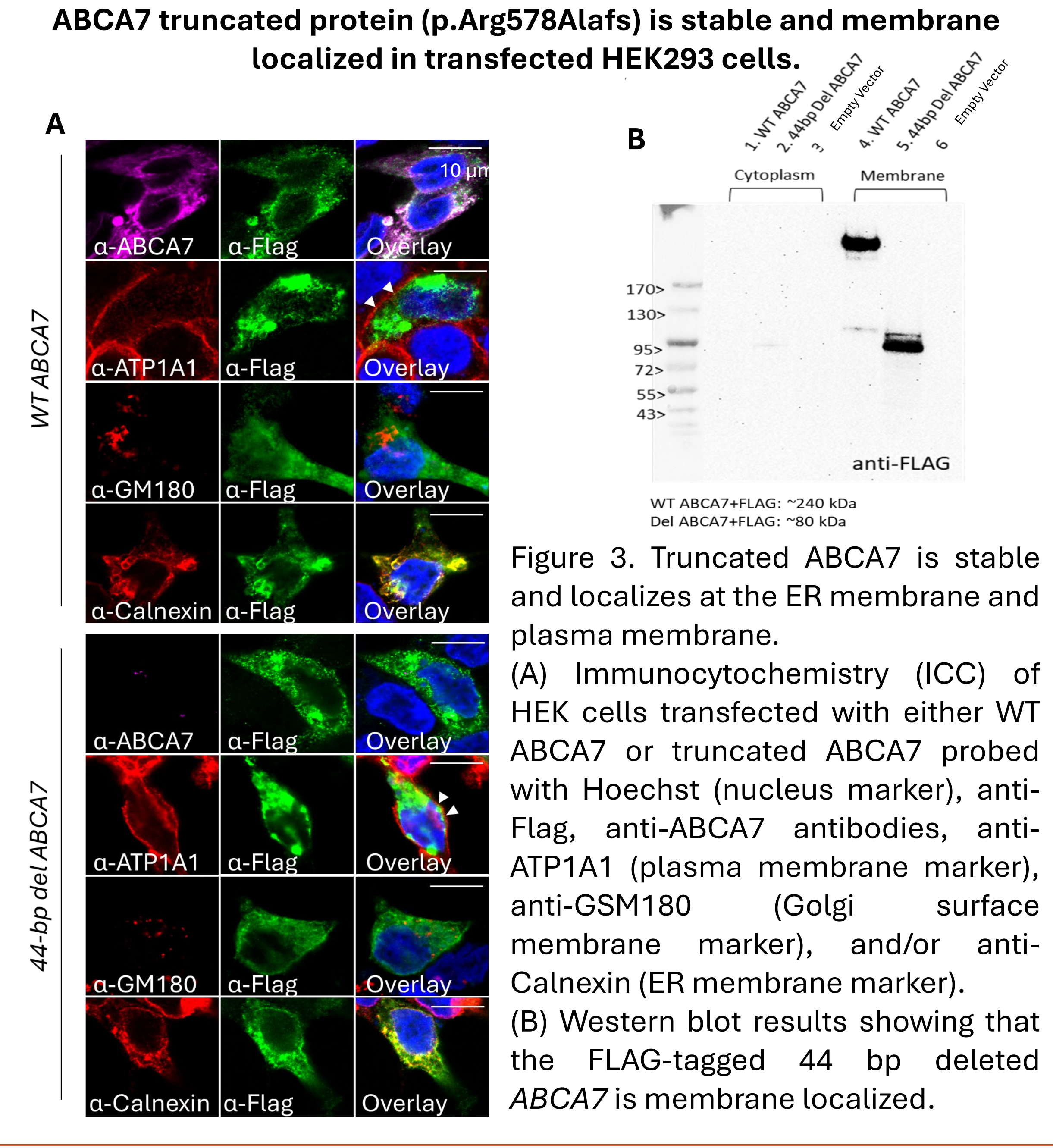
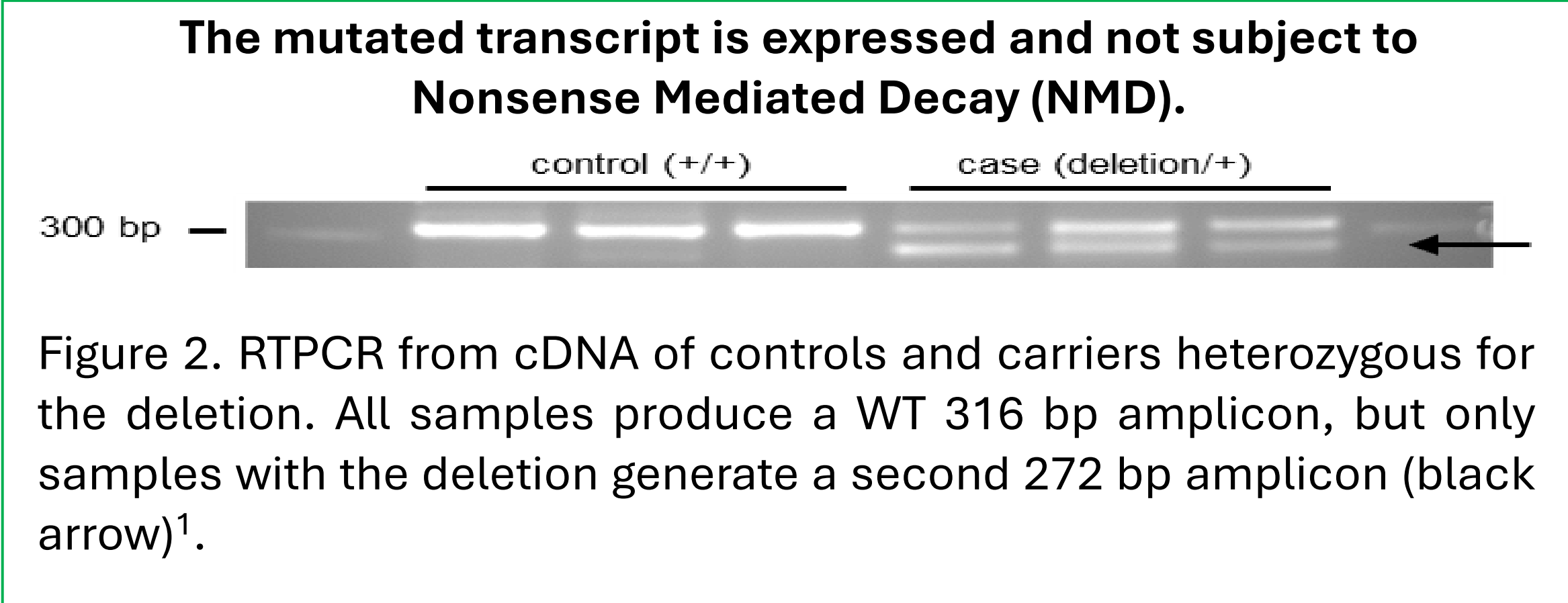
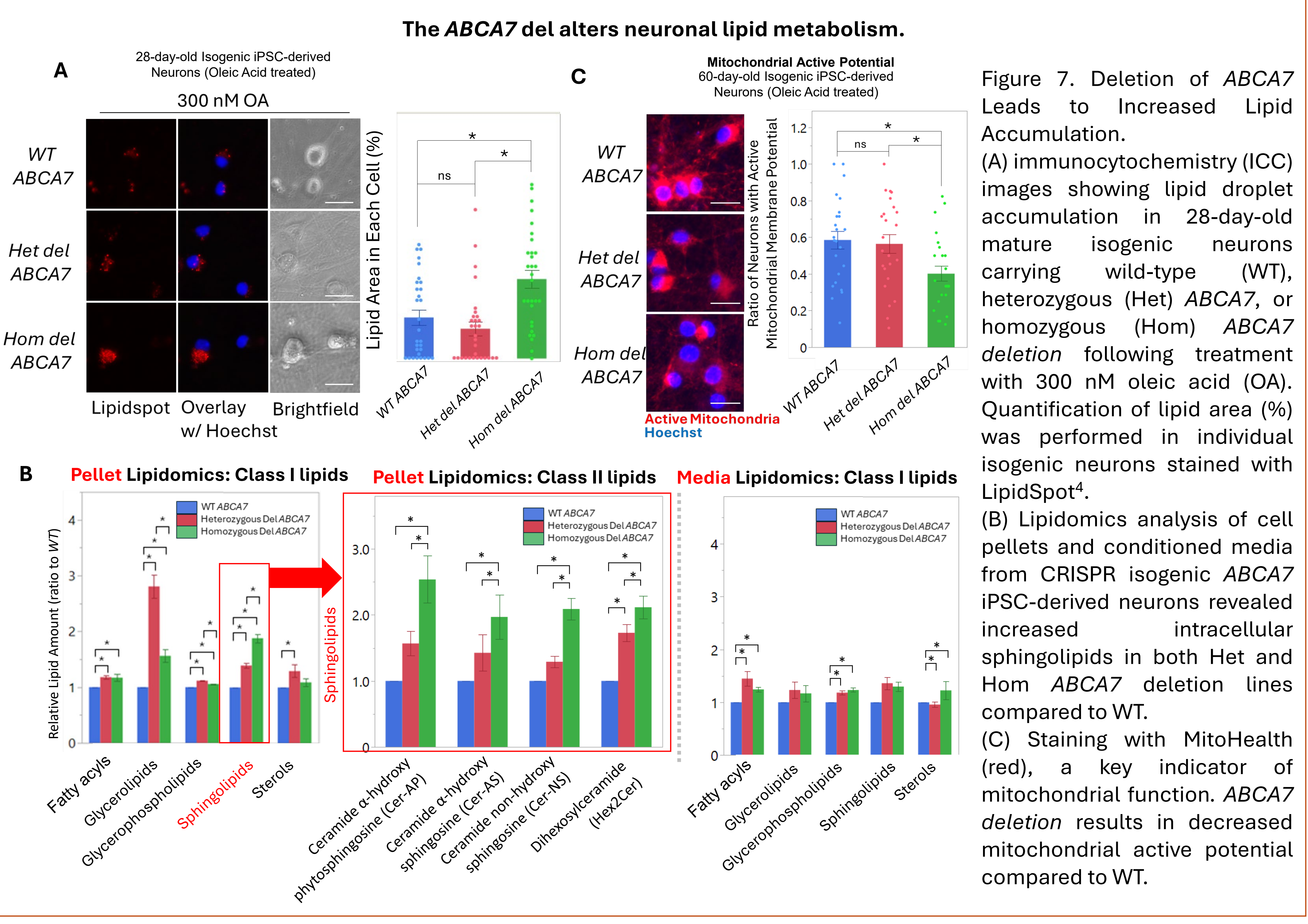
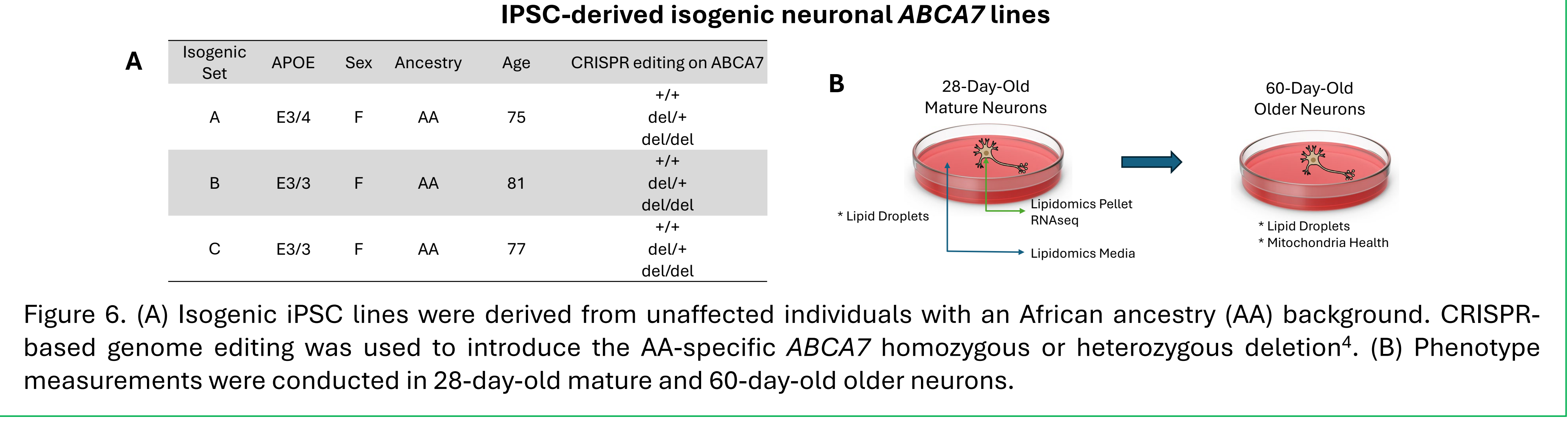
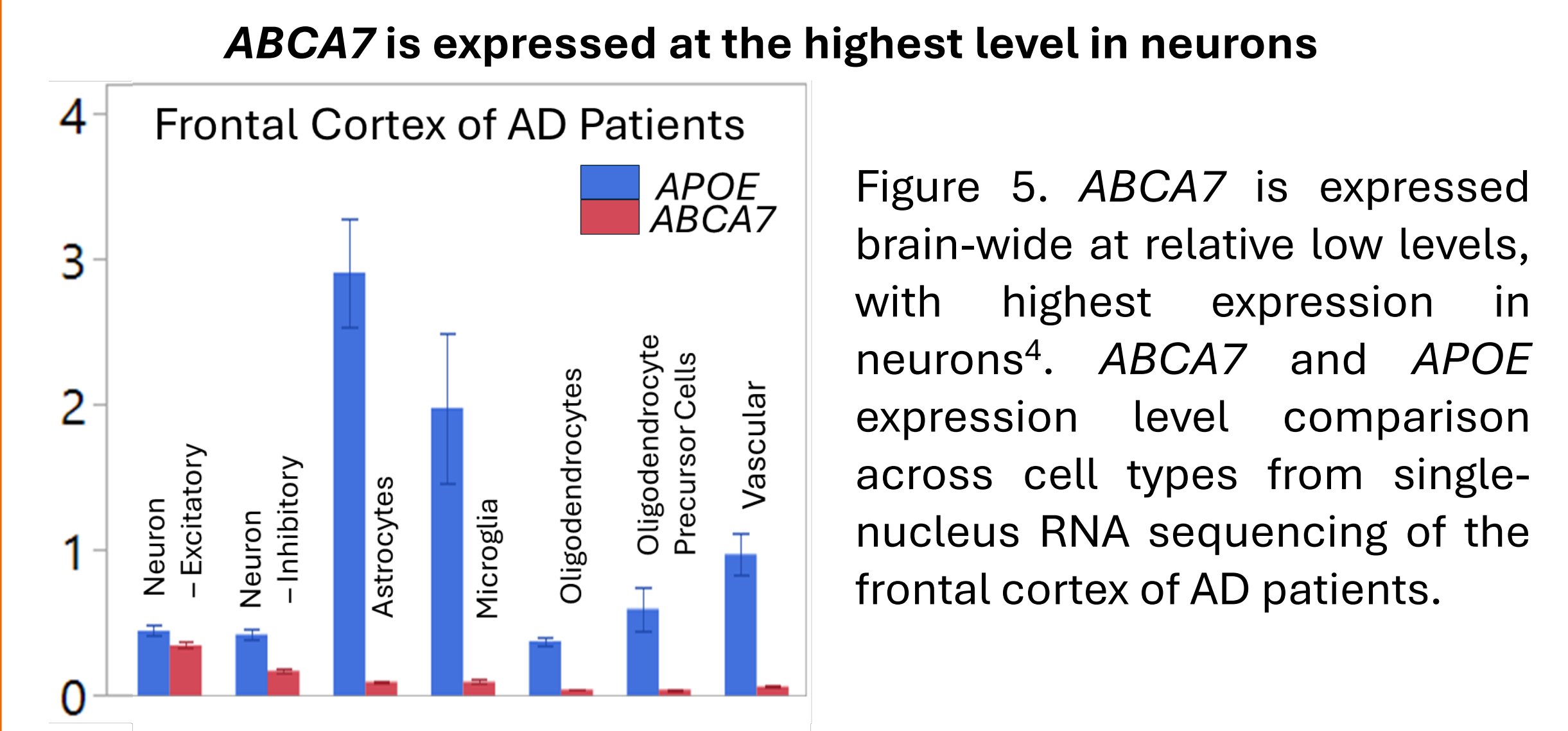


Figure 4. Functional effect in lipid droplet metabolism of truncated *ABCA7* in transfected HepG2 cells. Images of HepG2 cells transfected with WT *ABCA7*, truncated *ABCA7*, pcDNA3.1 (empty vector, transfection control), stained for the detection of Flag and LDs in the presence of 300 nM BSA. Quantitation of LD area (%) per transfected cell in the presence of 300 nM OA shows that the effect of the *ABCA7* truncated protein is different than the WT.



CONCLUSIONS

- A. The *ABCA7* deletion produces a truncated protein that is stably expressed and membrane localized in the HEK293 transfection model despite missing most transmembrane domains.
- B. The *ABCA7* deletion leads to a functional defect in lipid processing, resulting in an increase in intracellular lipid droplets.
- C. The *ABCA7* deletion in neurons leads to an increased amount of lipid species, especially sphingolipids. Further, it associates with decreased mitochondria potential.
- D. Our data suggest that pathogenesis of *ABCA7* deletion involves sphingolipids increases promoting mitochondrial permeabilization, loss of $\Delta\Psi_m$ (mitochondrial membrane potential).

FUNDING & CONTACT

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