ALS VAPB P56S Variant Impairs Recovery and Adaptability to External Stressors in human iPSC-derived Motor Neurons

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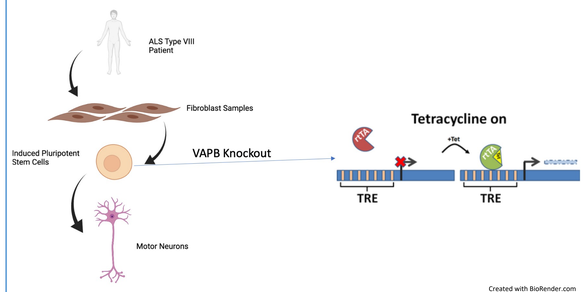
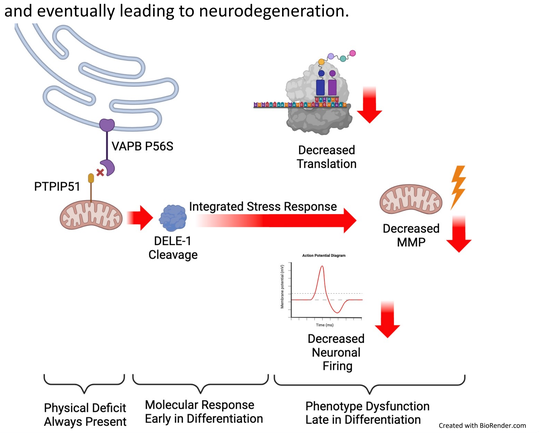
ABSTRACT

- Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease, is the most common adult–onset motor neuron (MN) disease. An autosomal dominant c.166C>T mutation in the *VAPB* gene leads to a change of a proline to a serine at amino acid position 56 (P56S) and is known to be the causative mutation of ALS8. However, the mechanism through which this mutation causes disease remains unknown.
- VAPB is an ER tethering protein, and previous literature has shown that the P56S mutation disrupts the binding to PTPIP51, a mitochondrial tethering protein.
- We obtained ALS Type VIII patient fibroblast samples, reprogrammed them into iPSC, and then used CRISPR Cas-9 to knockout the VAPB gene. We then inserted VAPB WT or VAPB P56S genes under the control of a tetracycline response element, creating doxycycline-inducible VAPB WT or VAPB P56S lines.

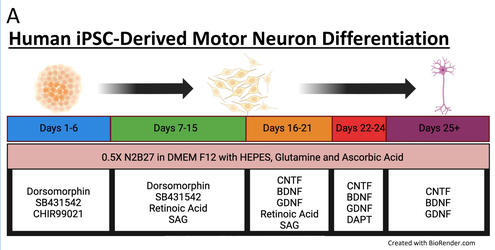


CONCLUSIONS

- The VAPB P56S mutation causes loss of PTPIP51 binding and a reduction in ER-mitochondrial contact in affected motor neurons.
- In addition, these cells exhibit the following phenotypes
  1. Decreased neuronal firing rate
  2. Decreased mitochondrial membrane potential (JC-1)
  3. Increased ER Sensitivity and Impaired Recovery
- Upon further investigation, it was shown that the VAPB P56S mutation constitutively activates the Integrated Stress Response via DELE-1 cleavage due to mitochondrial stress.
- The Integrated Stress Response Pathway was shown to be driving the phenotypes listed above, as treatment with ISRIB rescued them.
- In conclusion, it appears that the VAPB P56S mutation causes loss of PTPIP51 binding, resulting in a reduction of ER-Mitochondrial contact, leading to a buildup of mitochondrial stress, thereby activating the ISR and eventually leading to neurodegeneration.



RESULTS



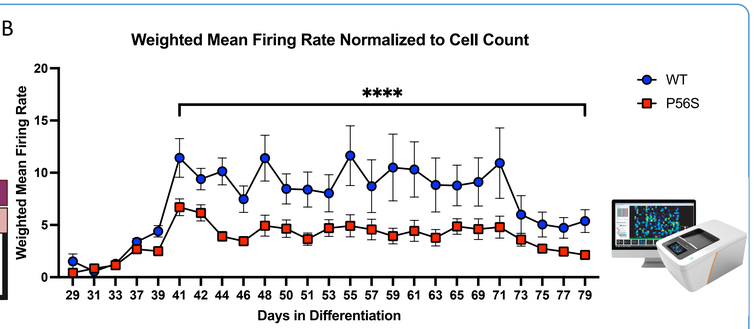
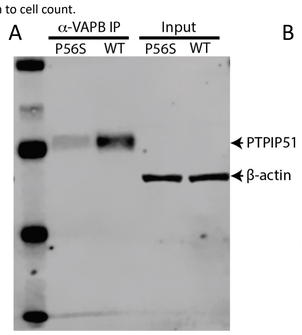
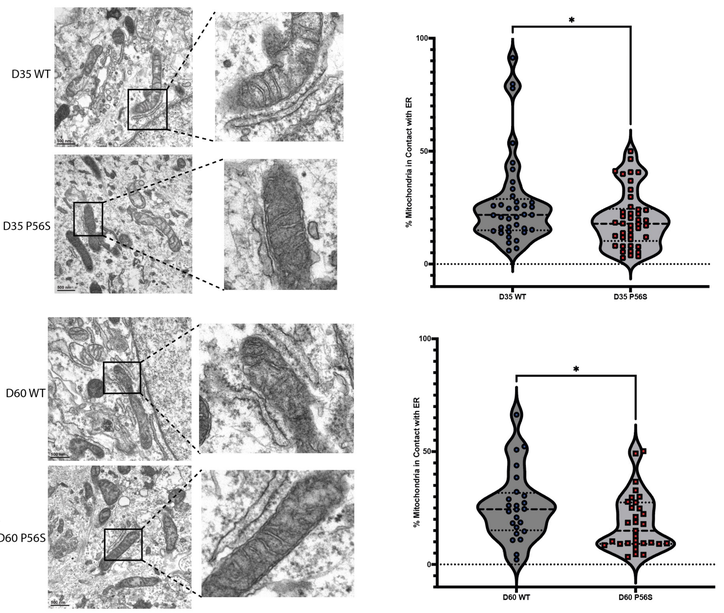
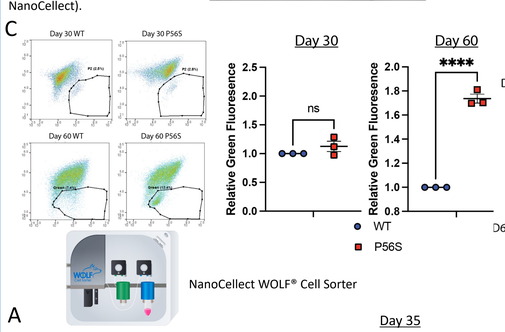


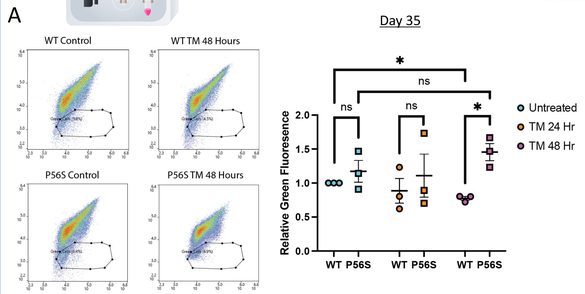
Figure 1: VAPB P56S Causes a Decrease in Neuronal Firing. Doxycycline inducible patient iPSC-derived motor neurons were differentiated using dual-SMAD inhibition and then plated on a 48-well MEA plate (1x10^5 cells/well) and electrophysiology recordings were taken approximately every other day on the Maestro Pro MEA system (Axion Biosystems). The P56S line shows a significant decrease in firing compared to WT beginning on day 41 of differentiation, after normalization to cell count.

Figure 2: VAPB P56S Results in a Loss of ER-Mitochondrial Contact. A coimmunoprecipitation and proteomic identification of VAPB interactors performed on VAPB WT and P56S lines indicated disruption of mitochondrial binding partners due to the P56S mutation, including PTPIP51, an ER-Mitochondrial tethering protein. Electron Microscopy of day 30 and day 60 neurons reveled the P56S lines have a decrease in ER-Mitochondrial contact sites, and exhibit decreased mitochondrial membrane potential (MMP) on day 60 (assayed using JC-1 dye, using the WOLF® cell sorter from NanoCellect).









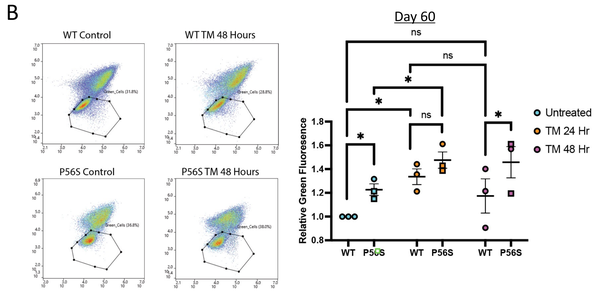
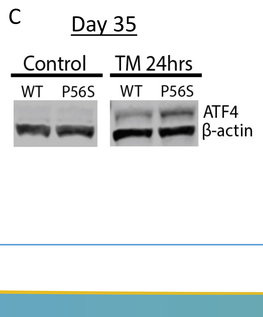
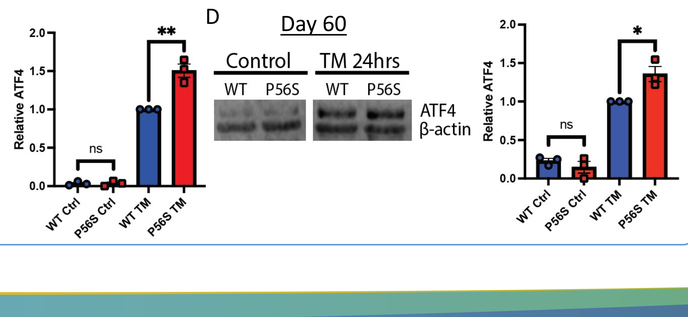


Figure 3: VAPB P56S Cells Have Increased ER Sensitivity and Decreased Recovery from Stressors. Dosing the VAPB P56S and WT motor neurons with tunicamycin (7.5ug/mL) for 24 and 48 hours, followed by assay with the JC-1 dye showed a decrease in MMP at 48 hours in the P56S lines on day 35, while on day 60 both lines exhibited a decrease in MMP at 24 hours, however by 48 hours the WT were able to recover while the P56S were not. Given the nature of tunicamycin as an ER stressor, we wanted to see if a classical effector of ER stress response (ATF-4) was also elevated, and it was significantly higher expressed in the P56S lines at both timepoints.





RESULTS

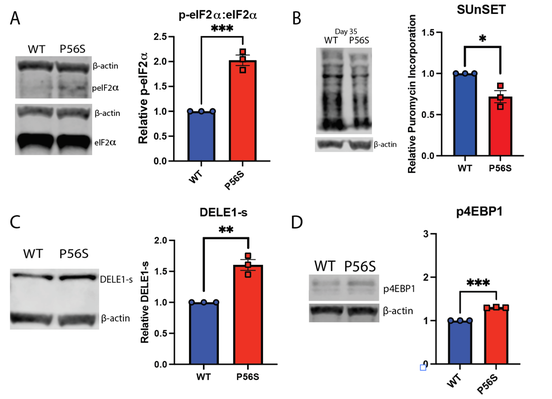
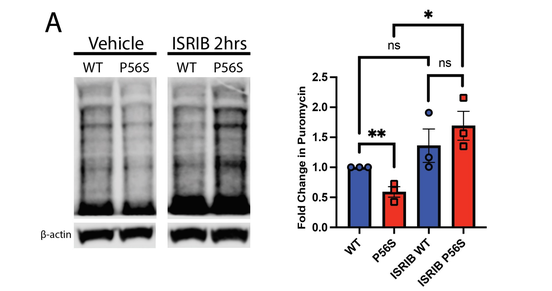
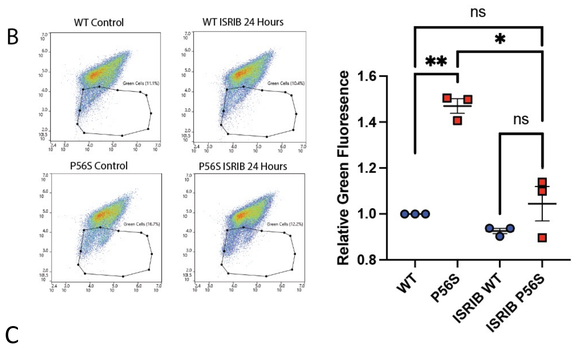


Figure 4: The Integrated Stress Response is Activated Through DELE1 in P56S Lines. After observing the increase in ATF-4 after tunicamycin dosing, we decided to examine aspects of the ISR, using the SUnSET assay to observe decreased mRNA translation, and increased p-eIF2α, indicative of ISR activation. DELE1 can activate the ISR and is itself activated through mitochondrial stress and was elevated in the P56S samples. Given the drastic nature of the hypotranslation we also examined p4EBP1 and found it decreased in the P56S lines, indicating mTOR involvement as well.





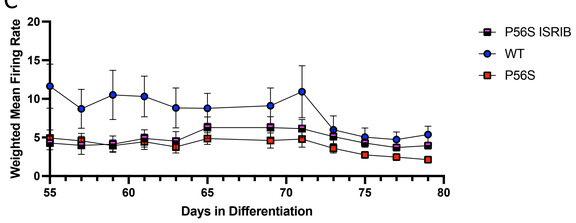


Figure 5:ISRIB Rescues Hypotranslation, Decreased MMP, and Decreased Neuronal Firing. Cells were dosed with 5ug/mL of ISRIB (Integrated Stress Response inhibitor) and we observed rescue of the hypotranslation phenotype, the decreased MMP phenotype and the neuronal firing phenotype, indicating the cause of these phenotypes to be overactivation of the ISR in the VAPB P56S lines.

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