An Oxidative Stress-Based Flow Cytometry Assay for *SELENON*-Congenital Myopathy Therapeutic Development

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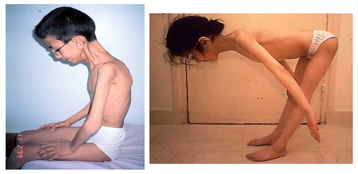
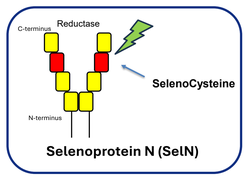
Introduction

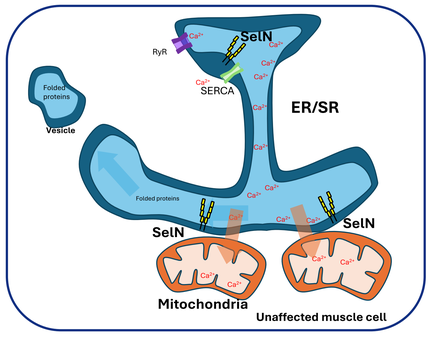


SELENON-Congenital myopathy is a rare condition that prevents Selenoprotein-N production, where patients present with muscle weakness, spinal rigidity, and respiratory insufficiency with no current non-palliative treatments.

Patients with *SELENON*-RM have rigid spines and necks. They succumb to respiratory insufficiency at a young age, but maintain ambulation throughout most of their lives (Silwal et al., 2020).

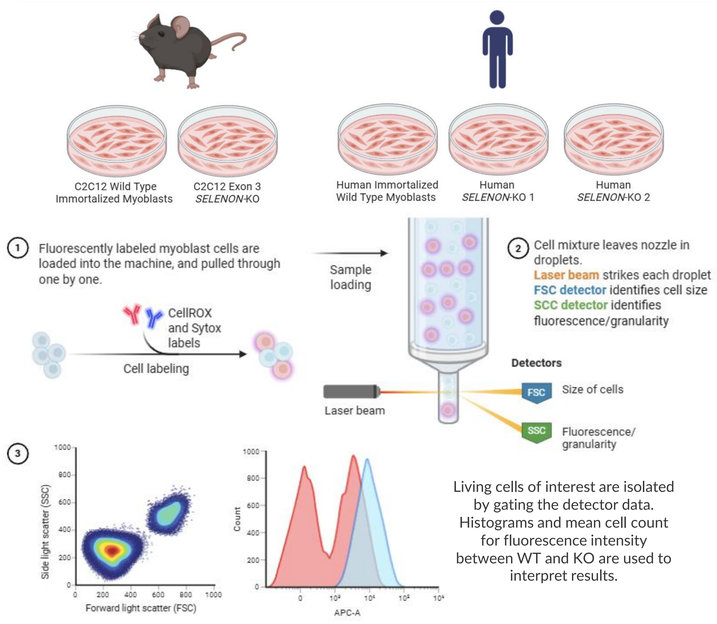


SelN, encoded by the *SELENON* gene, is an endoplasmic reticulum membrane reductase that regulates cellular redox homeostasis.



This project aims to develop a flow cytometry-based assay to measure oxidative stress at the single cell level to discriminate *SELENON*-deficient and Wild Type myoblasts.

Methods



Living cells of interest are isolated by gating the detector data. Histograms and mean cell count for fluorescence intensity between WT and KO are used to interpret results.

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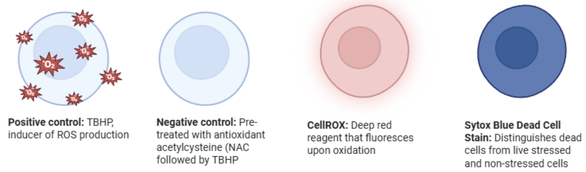
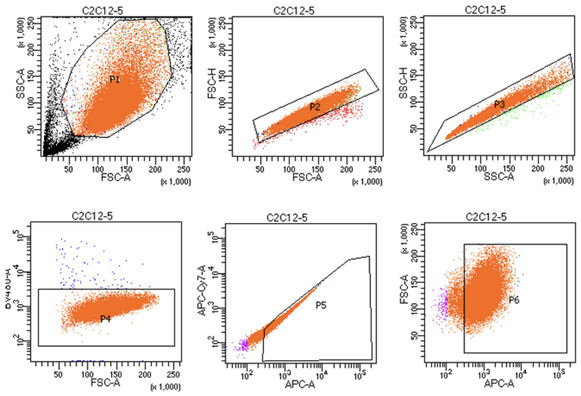
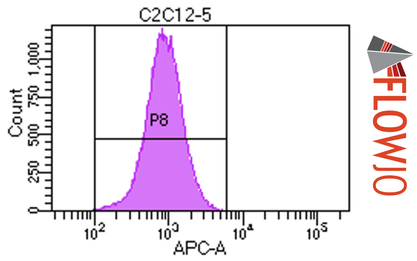
Results

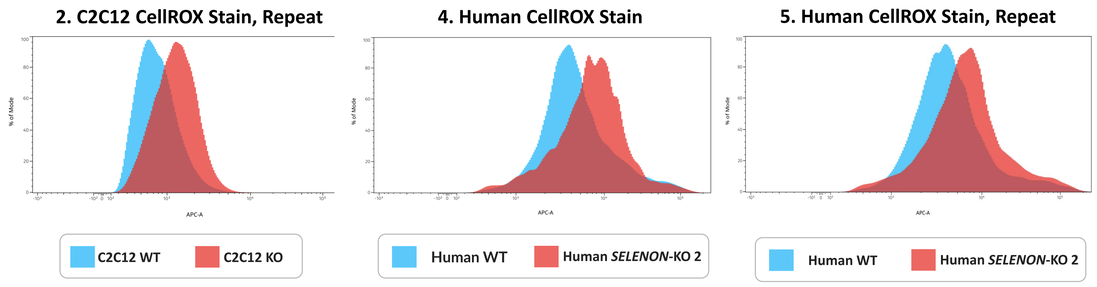


Flow Run	Results
1. C2C12 CellROX Stain	C2C12 KO exhibits a rightward shift in CellROX intensity from WT. Dye controls show CellROX and Sytox do not bleed into each other.
2. C2C12 CellROX Stain, Repeat	Positive control shows an increase in ROS compared to negative control. Experimental WT has higher CellROX than experimental KO. We hypothesize that the WT was dying.
3. Human MitoSOX stain	MitoSOX stain had overall low levels of fluorescence, overshadowed by the Mitotracker stain.
4. Human CellROX Stain + NAC Curve	Human KO-2 exhibits a rightward shift in CellROX intensity from WT. Created NAC curve from 0-800 µM.
5. Human CellROX Stain+ NAC Curve, Repeat	Human KO-2 exhibits a rightward shift in CellROX intensity from WT, Human KO-1 more similar to WT. Performed NAC curve from 0-200 µM.





Flow cytometry histograms show the absolute number of cells detected at each specific fluorescence intensity level. FlowJo was used to filter viable and CellROX positive cells.



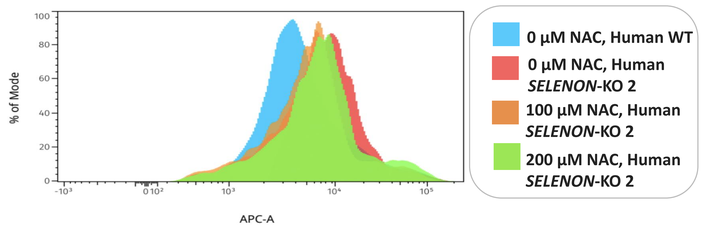
Across experiments, there is a consistent separation in CellROX expression, or ROS, between WT and *SELENON* knockouts in both immortalized mice and human myoblasts.

Conclusion

- Flow cytometry can be used as a tool to quantify changes in oxidative stress in myoblast cells.
- *SELENON*-knockout mouse and human myoblasts show higher CellROX fluorescence compared to Wild Type, producing two population peaks.
- Treatment with NAC reduced CellROX fluorescence levels in human myoblast KO compared to no treatment.

Next Steps

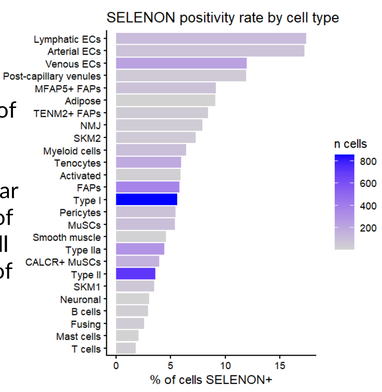
- Repeat CellROX staining and NAC dose curve for reproducibility.
- Test with MitoSOX stain to separate mitochondrial from total cellular ROS.
- Use other similar antioxidants like NAC to use as positive control.

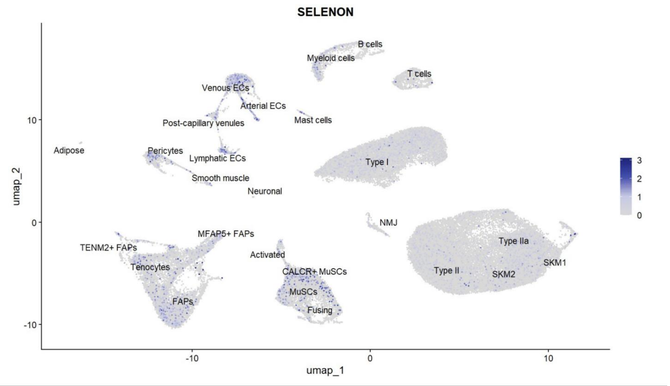


Increasing doses of the antioxidant NAC progressively shift *SELENON*-KO myoblasts toward lower CellROX fluorescence, approaching the WT baseline. This suggests that oxidative stress is reversible in *SELENON*-KO myoblasts. Further tests are needed to confirm these findings.

Future Direction

To further understand the role that *SELENON* takes in healthy human muscle, single-nuclei RNA-seq from muscle biopsies of 16 healthy pediatric donors are being analyzed using Seurat package in R. We generated a bar graph showing the percentage of *SELENON*-positive cells each cell type. A comprehensive UMAP of the 16 samples demonstrated cell type labeled by *SELENON* expression levels.





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