Università
degli Studi
della Campania
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MUSE stem cells as a new cell-based model for the study of neural diseases

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Background

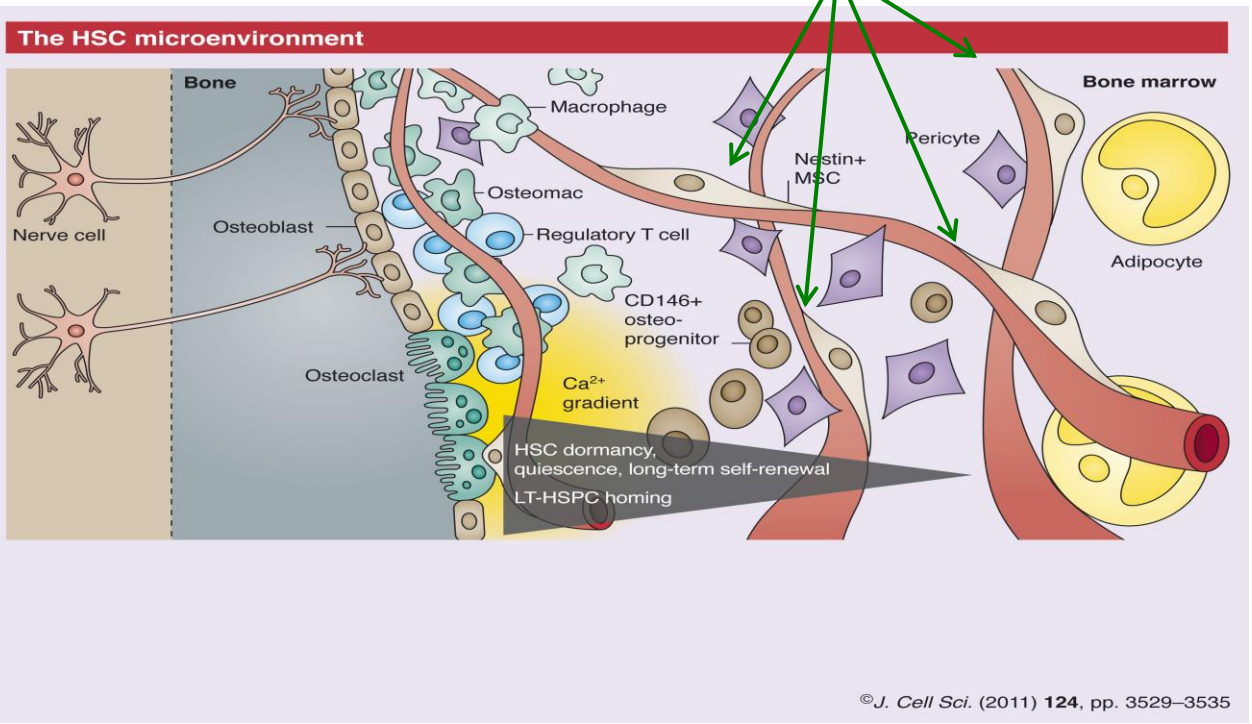
The study of biomolecular phenomena underlying human diseases is paramount to developing strategies for their treatment and/or prevention.

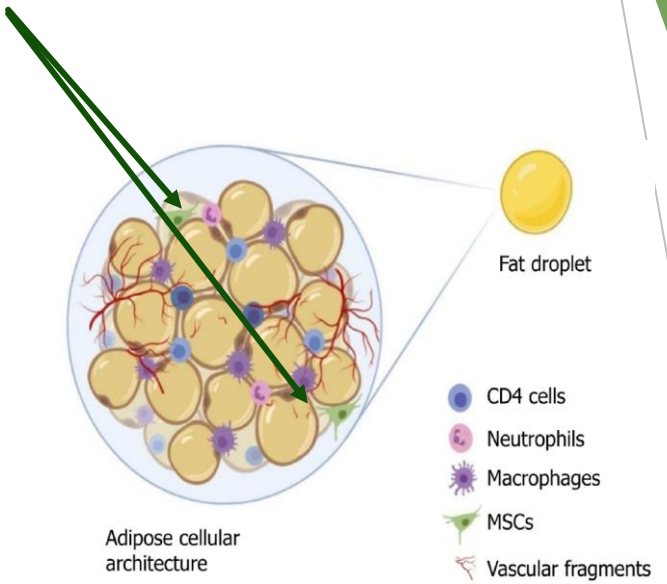
- Animal models
- Adult stem cells from patients' tissues which present the main pathological status
- Induced pluripotent stem cells from patients' fibroblasts

Aim of the study

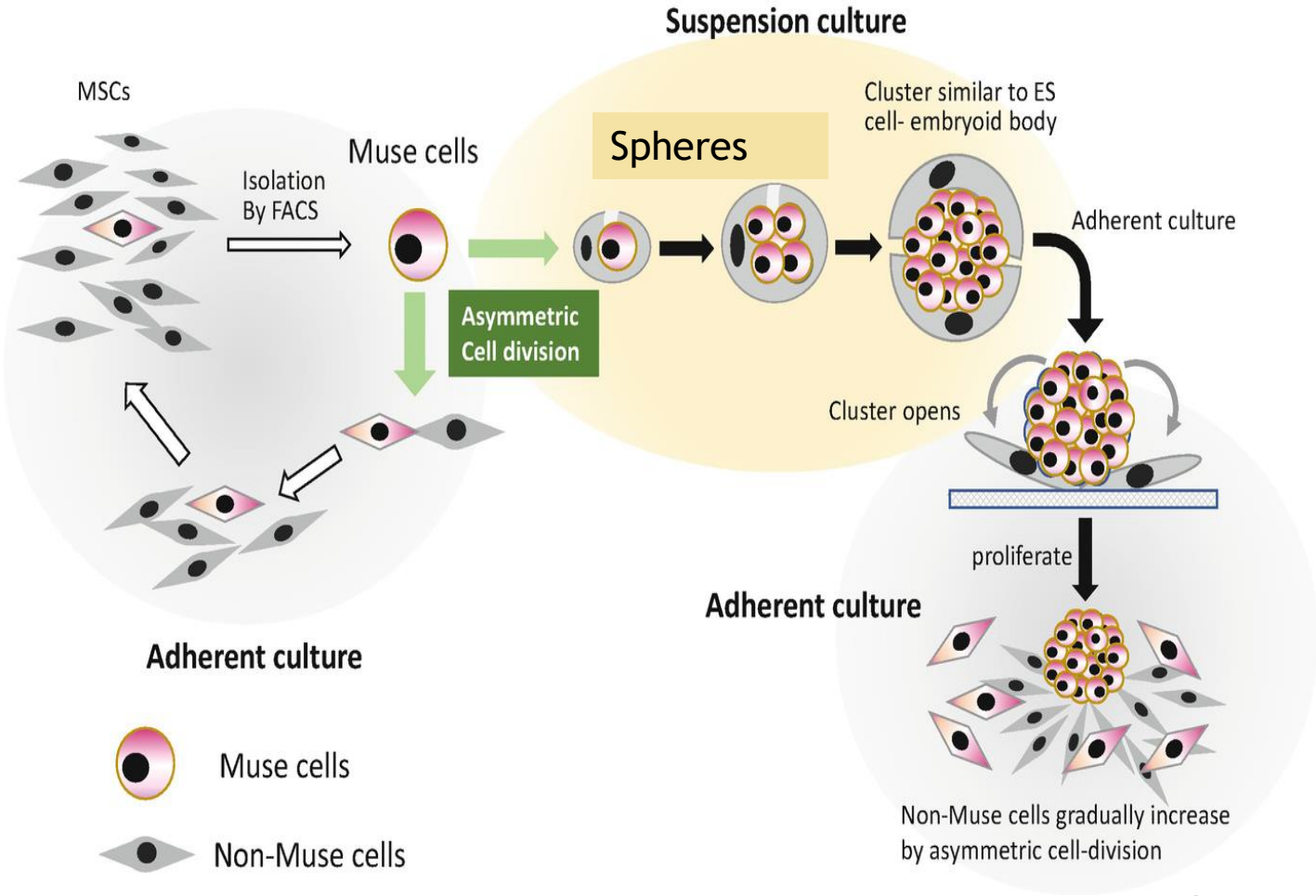
The aim of this study is to evaluate if MUSE stem cells may represent a valid cell-based model to study neural diseases

Mesenchymal stromal cells (MSCs)

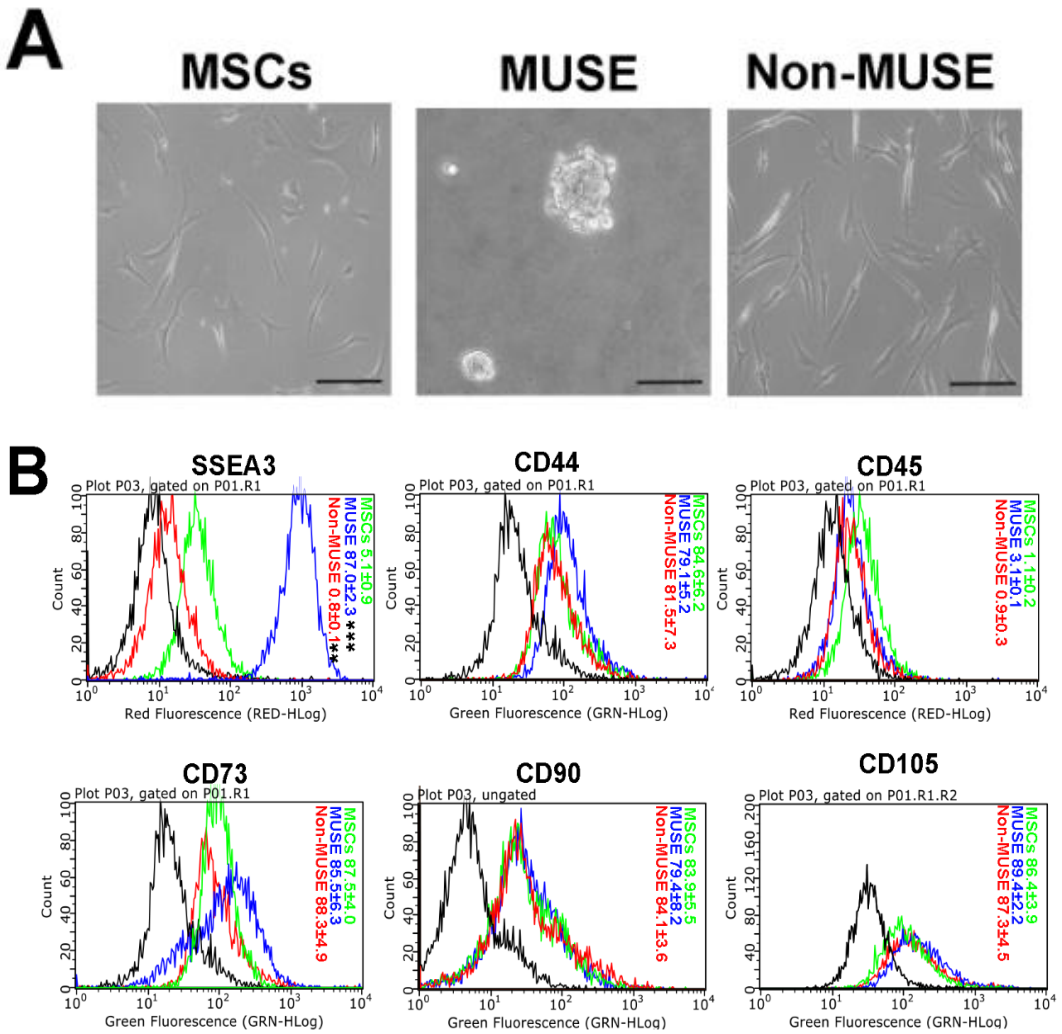




Within the population of MSCs, there is a cellular subpopulation that it is unique in that it shows the expression of SSEA-3: MUSE cells

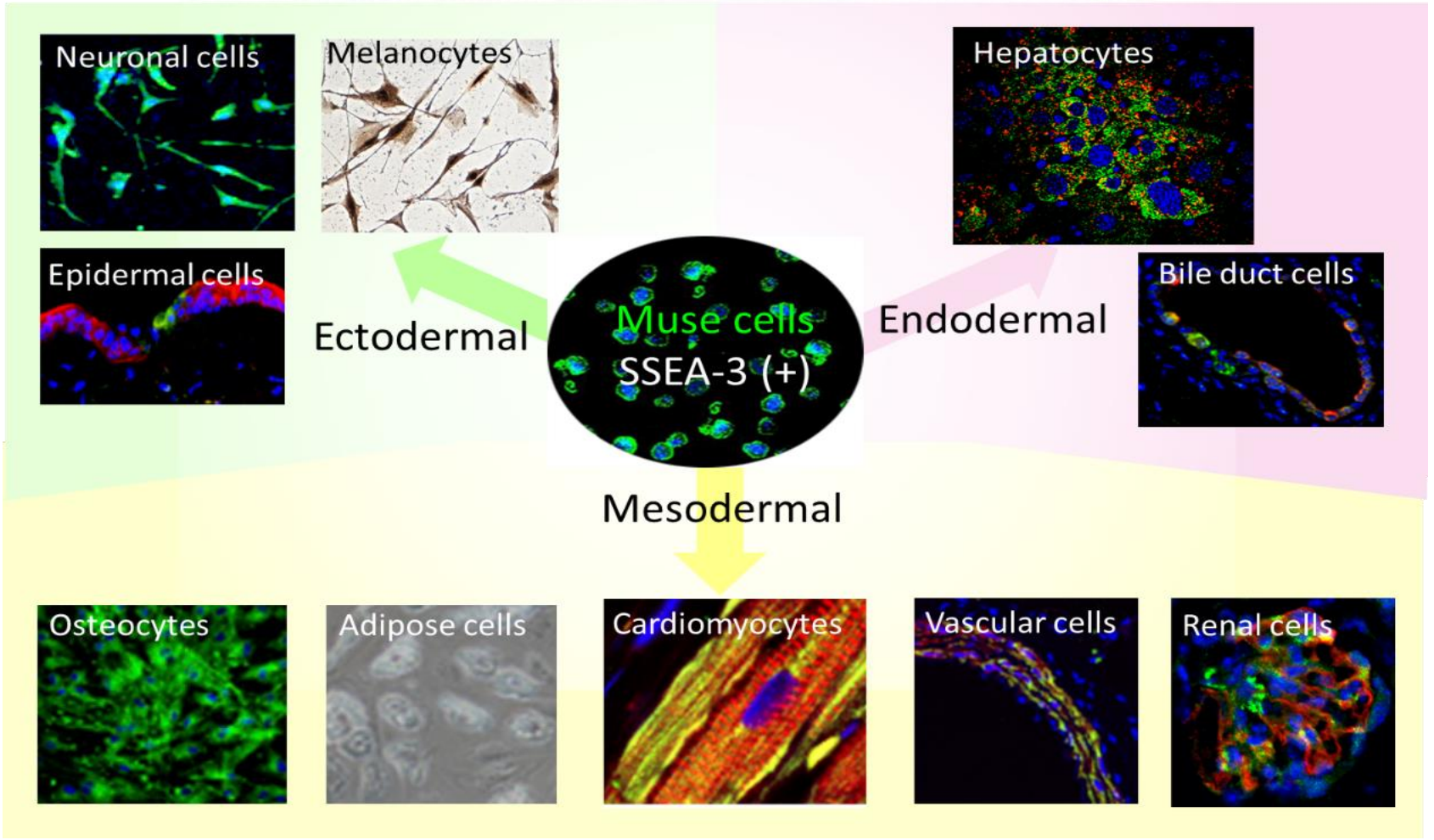


Within the population of MSCs, there is a cellular subpopulation that it is unique in that it shows the expression of SSEA-3: MUSE cells



Isolation and characterization of MUSE cells

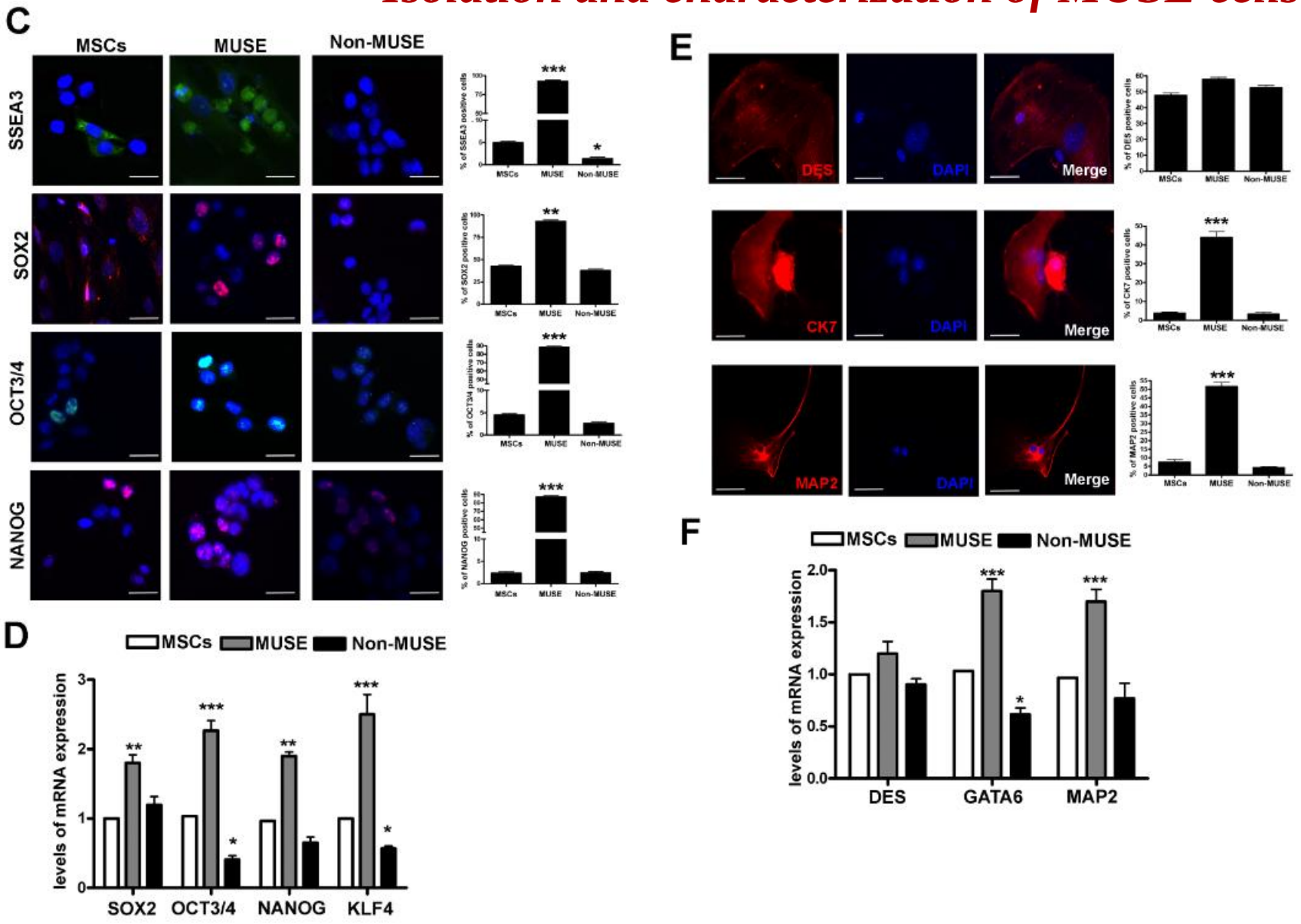
Multilineage differentiating Stress Enduring (MUSE) cells



MUSE cells are identified as **endogenous**, **stress-resistant stem cells**, expressing several **pluripotency master genes** and able to differentiate in mature cells of the **three embryonic sheets**.

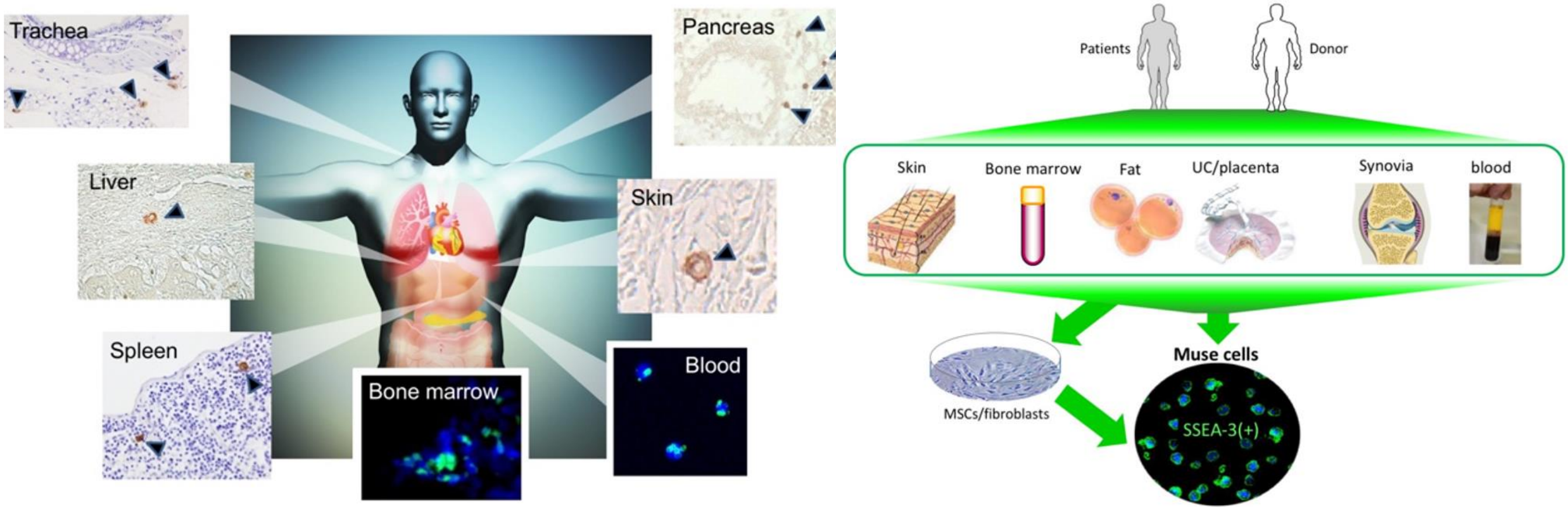
adapted from Wakao S et al., 2018

Isolation and characterization of MUSE cells



The isolated MUSE cell population displayed the characteristics of pluripotent stem cells: expression of stem cell markers and trilineage differentiation capacity.

Multilineage differentiating Stress Enduring (MUSE) cells



MUSE cells are identified as **SSEA-3 (+) cells** in various tissues and can be collected from various sources by positive selection for this marker.

adapted from Wakao S et al., 2018

Muse Stem Cells as Neurological disease model

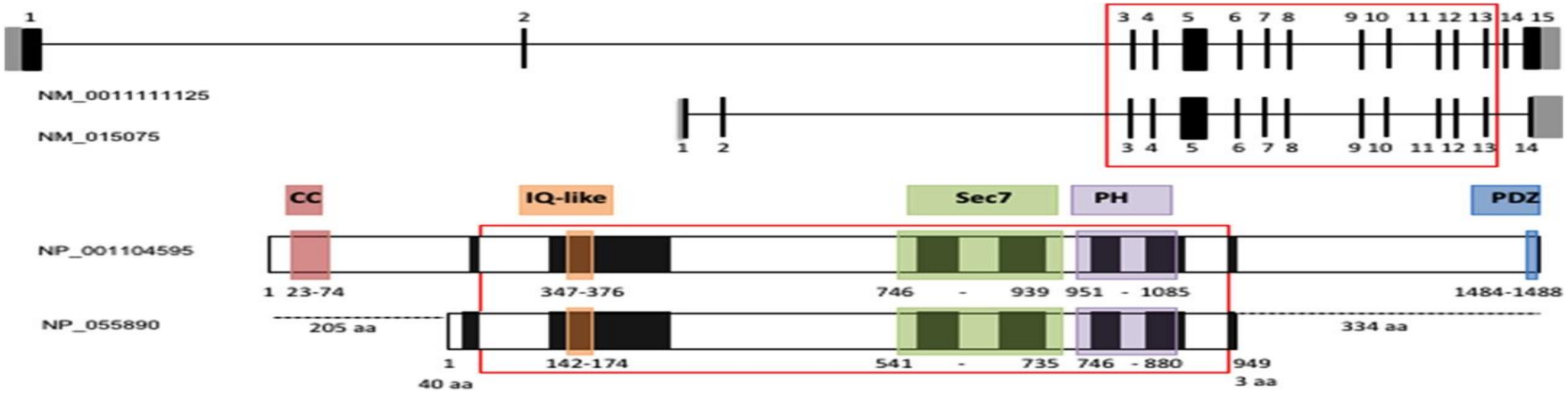
Mutations in the coding region of ***IQSEC2* gene** have been identified in patients with intellectual disability (ID), epilepsy, and autism since 2008.

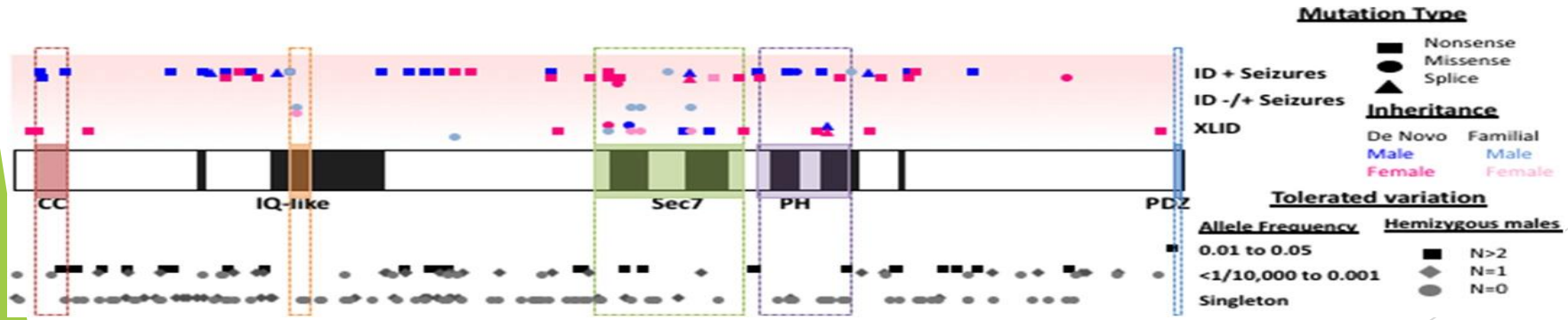
Wide phenotypic diversity has been described for *IQSEC2*-related ID, as is also the case for Rett syndrome (RTT). Indeed, several patients with *IQSEC2* mutations show clinical symptoms similar to RTT, and some meet all of the criteria for canonical RTT.

We aimed to study the possibility to isolate Muse stem cells from subjects with IQSEC2 gene mutation. The Muse stem cells of patients would be differentiated into neural lineage for establishing *in vitro* neural-disease model.

IQSEC2 Gene and protein

- The *IQSEC2* gene (i.e., *BRAG1* gene) encodes for several protein isoforms that vary at the N- and C-termini
- IQSEC2 proteins, meanwhile, are guanine nucleotide exchange factors for the RAS superfamily GTPase, including ARF6, which IQSEC2 isoforms activate by exchanging their GDP for GTP via the Sec7 domain





IQSEC 2 gene Mutations Sites for the Three Patients

Homo sapiens IQ motif and Sec7 domain ArfGEF 2 (IQSEC2), transcript variant 1, mRNA

NCBI Reference Sequence: NM_001111125.3

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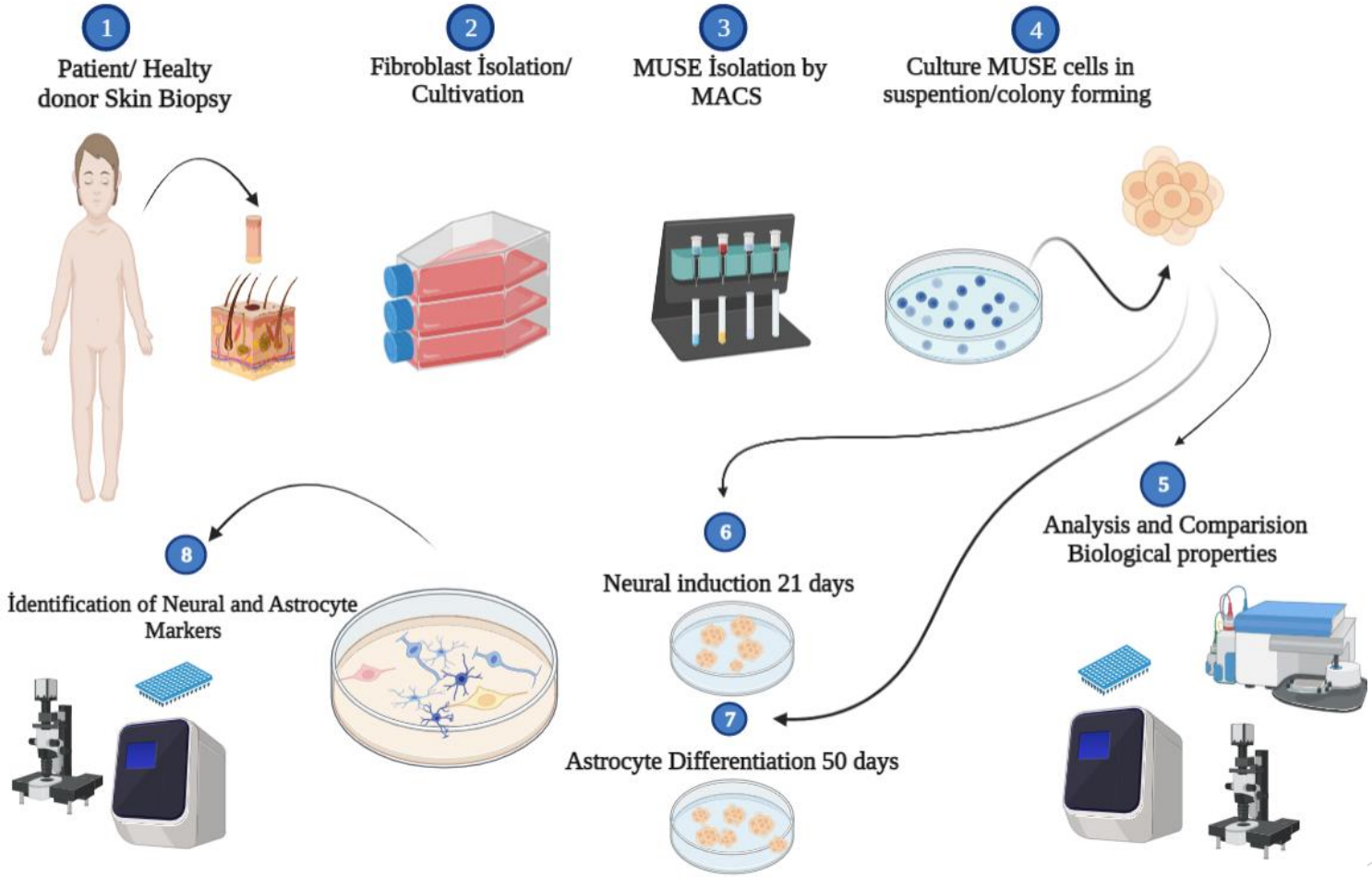
Thanks to Prof. Alessandra Renieri team at Medical Genetic Unit of Siena University

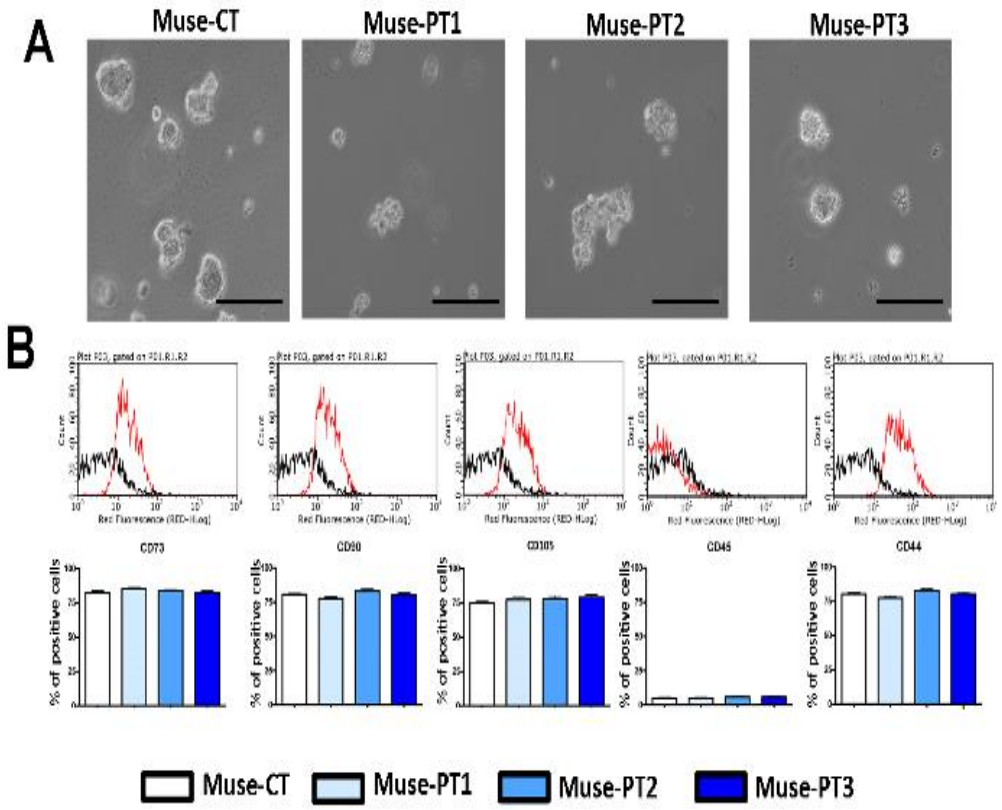
Methods



Soubannier, V., Maussion, G., Chaineau, M., Sigutova, V., Rouleau, G., Durcan, T.M. and Stifani, S., 2020. Characterization of human iPSC-derived astrocytes with potential for disease modeling and drug discovery. *Neuroscience Letters, 731*, p.135028

Kuroda, Y., Wakao, S., Kitada, M., Murakami, T., Nojima, M. and Dezawa, M., 2013. Isolation, culture and evaluation of multilineage-differentiating stress-enduring (Muse) cells. Nature protocols, 8(7), pp.1391-1415.

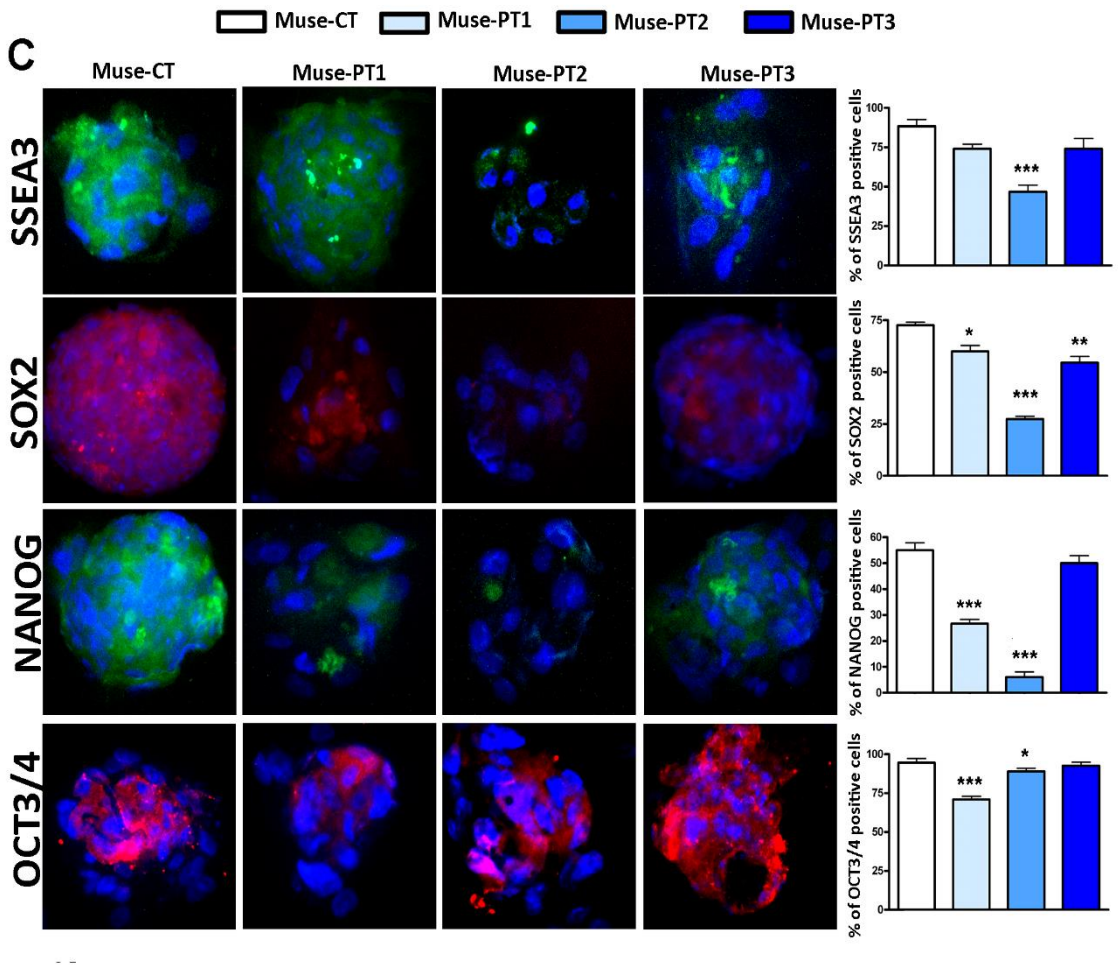
Muse cells Characterization



(A) Muse Cells were successfully isolated from control-healthy fibroblast (Muse-CT) and IQSEC2 fibroblast patients that we labelled as Muse-PT1, Muse-PT2 and Muse-PT3. The cells isolation was performed after achieving the 5th *in vitro* passage.

(B) The cells were positive for CD73, CD90, CD105, CD44, that are typical marker of mesenchymal stromal cells, and negative for CD45 hematopoietic marker.

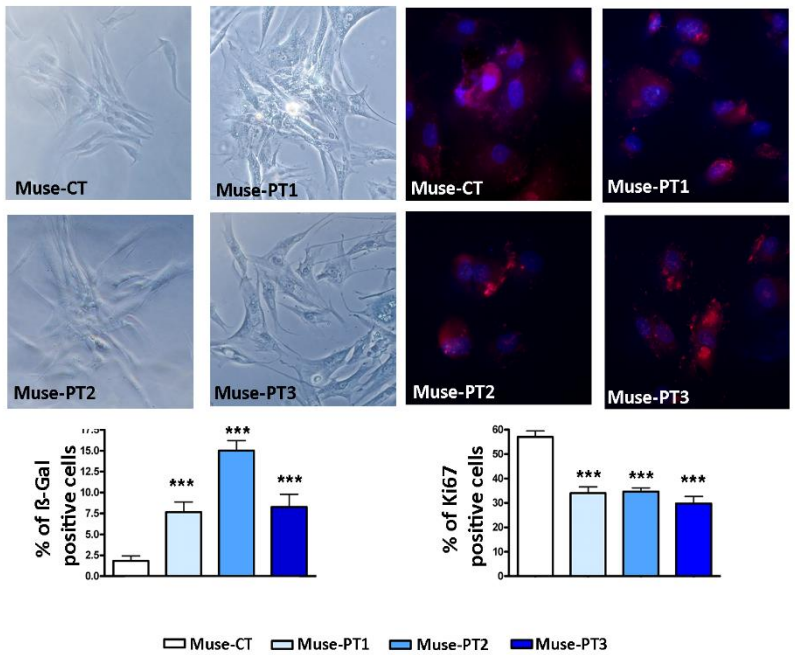
Stemness Markers Evaluation



- More than 70% of Muse-CT expressed the SOX2 stemness marker, which was less expressed in patients Muse cells.
- Muse-PT1 and Muse-PT2 showed a reduction in the percentage of cells positive for the NANOG marker compared with Muse-CT and Muse-PT3.
- OCT3/4 stemness marker was less expressed in patients Muse cells compared with Muse-CT, which in turn was positive for almost all cells.

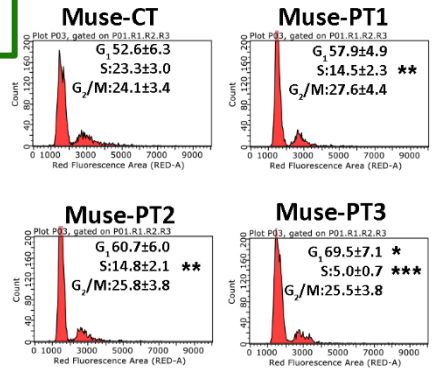
Evaluation of Biological properties

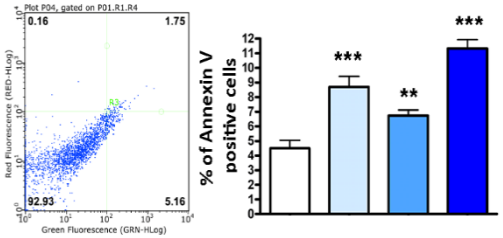
Senescence



- The cell cycle profile of Muse cells of the three different patients showed a reduction in S-phase cells compared with healthy-control Muse cells. This result is in line with reduction in proliferate or Cycling (Ki67+) cells with increase senescence cells in all patients Muse cells.
- In Muse-pt1 and Muse-Pt3 cell populations we detected a significant increase in apoptotic cells.

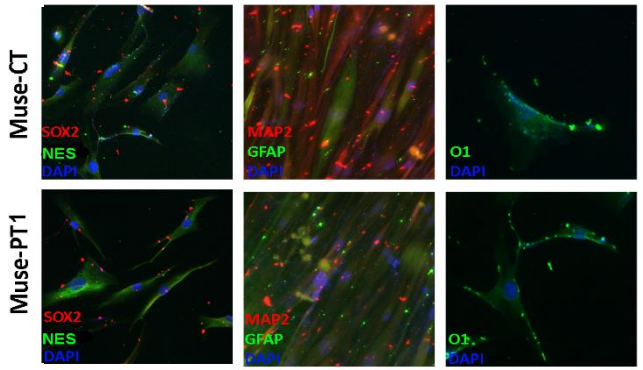
Cell Cycle

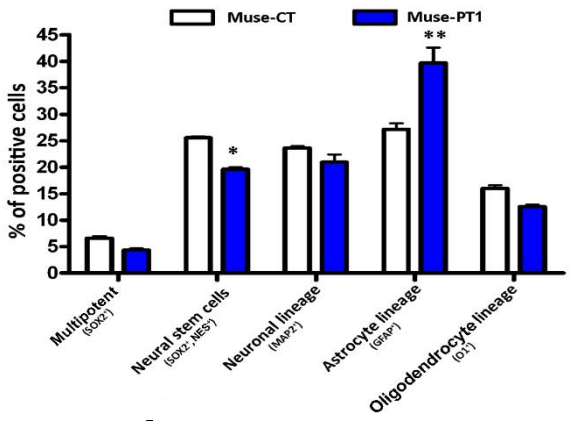


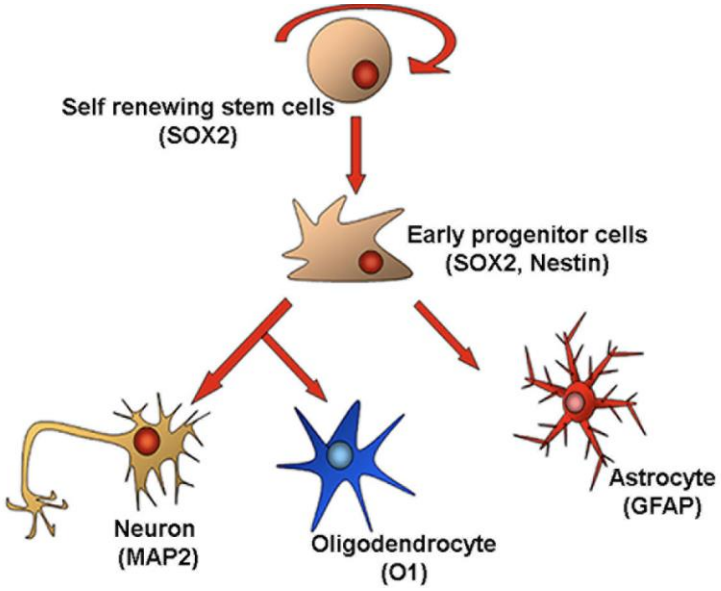


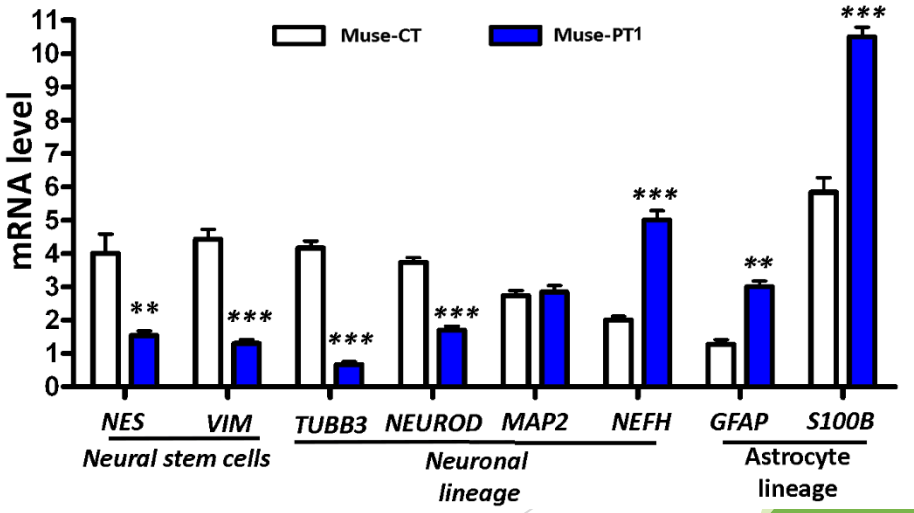
Apoptosis

Neural lineage Induction

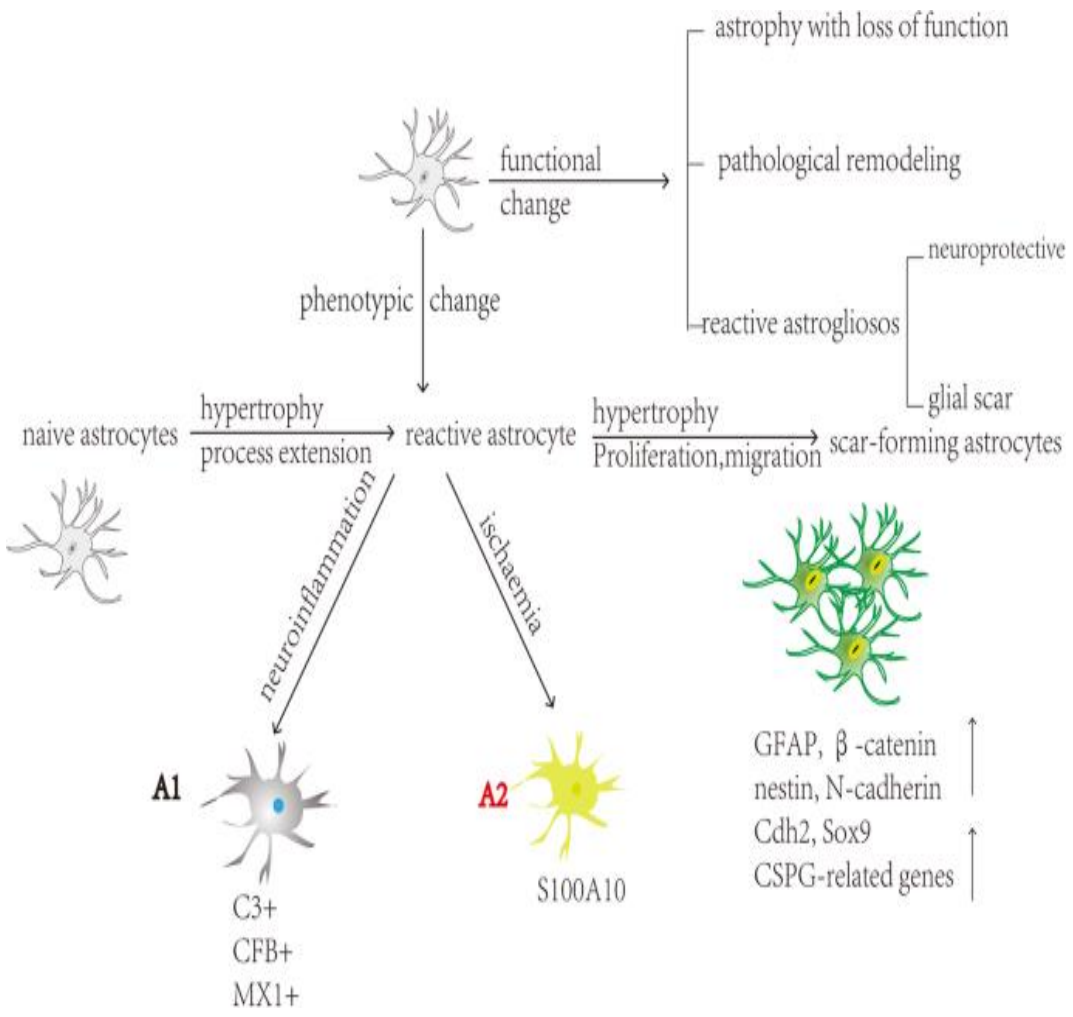


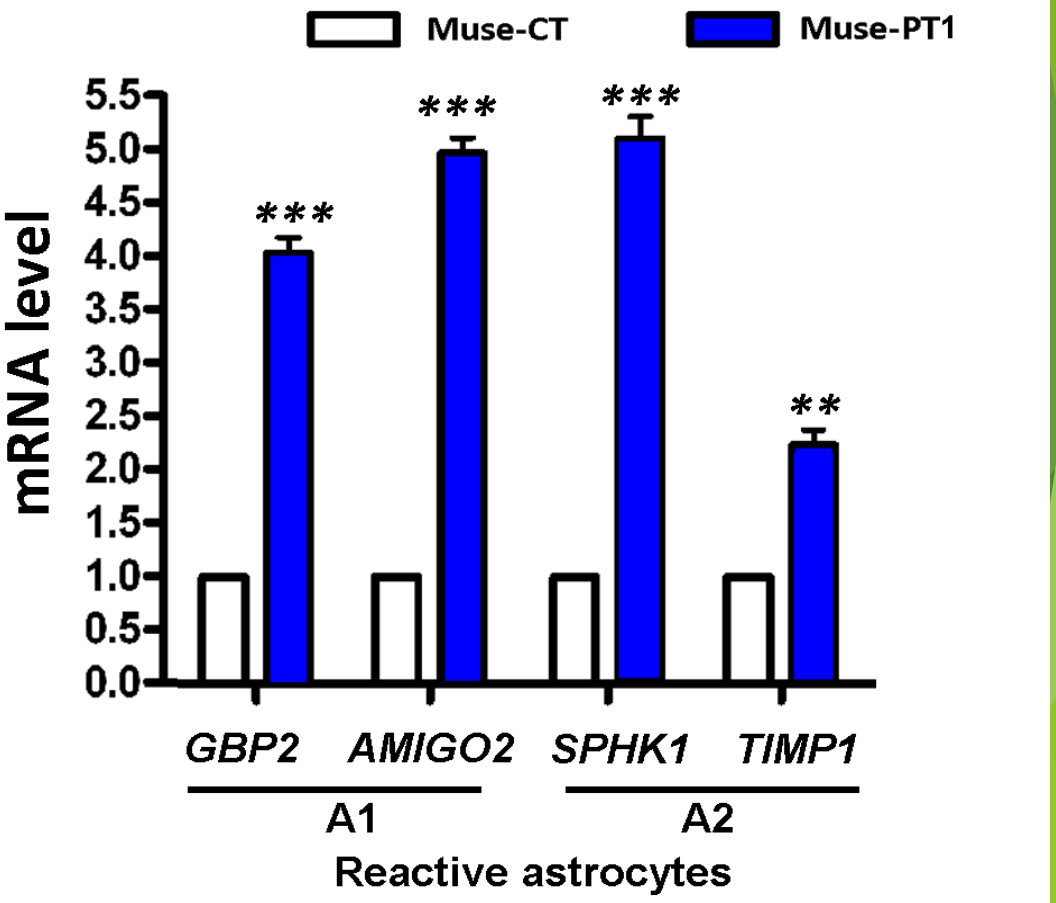




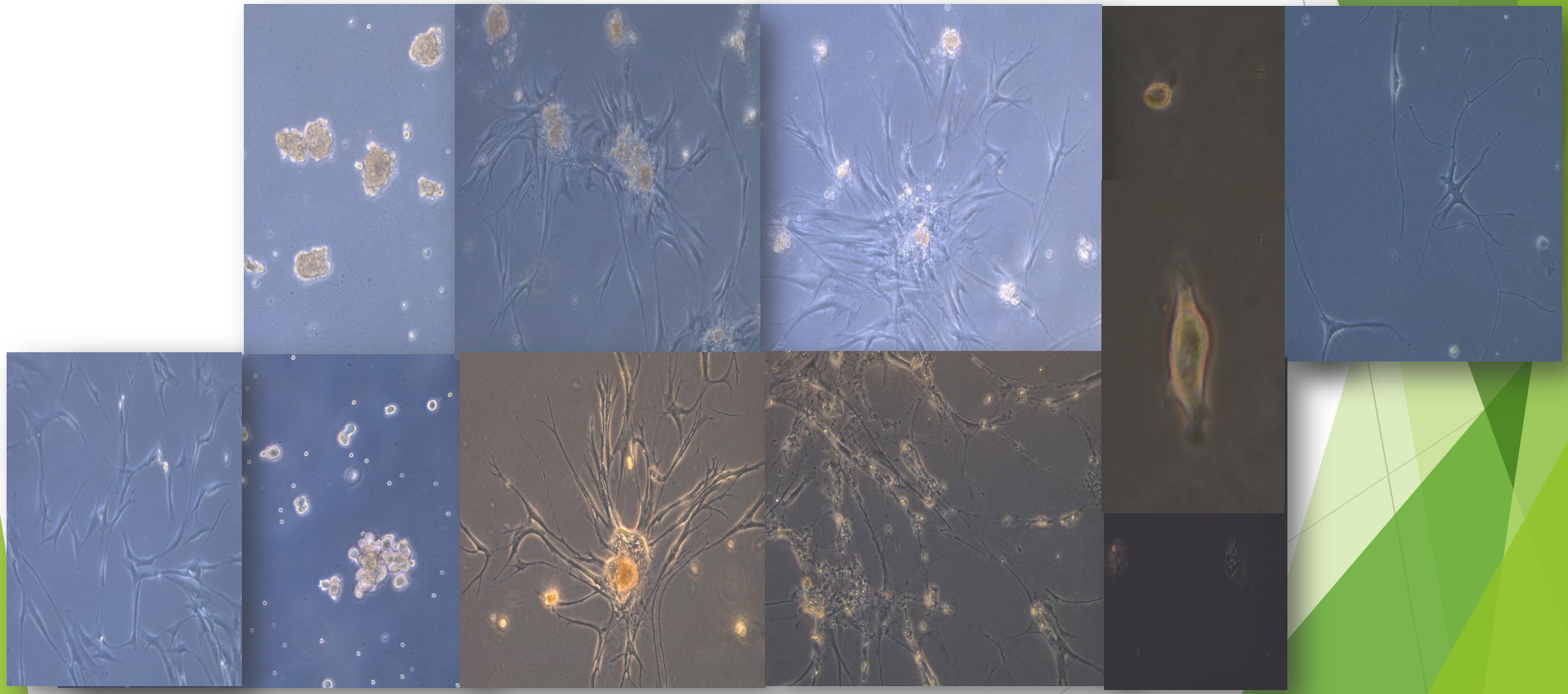


Reactive Astrocytes

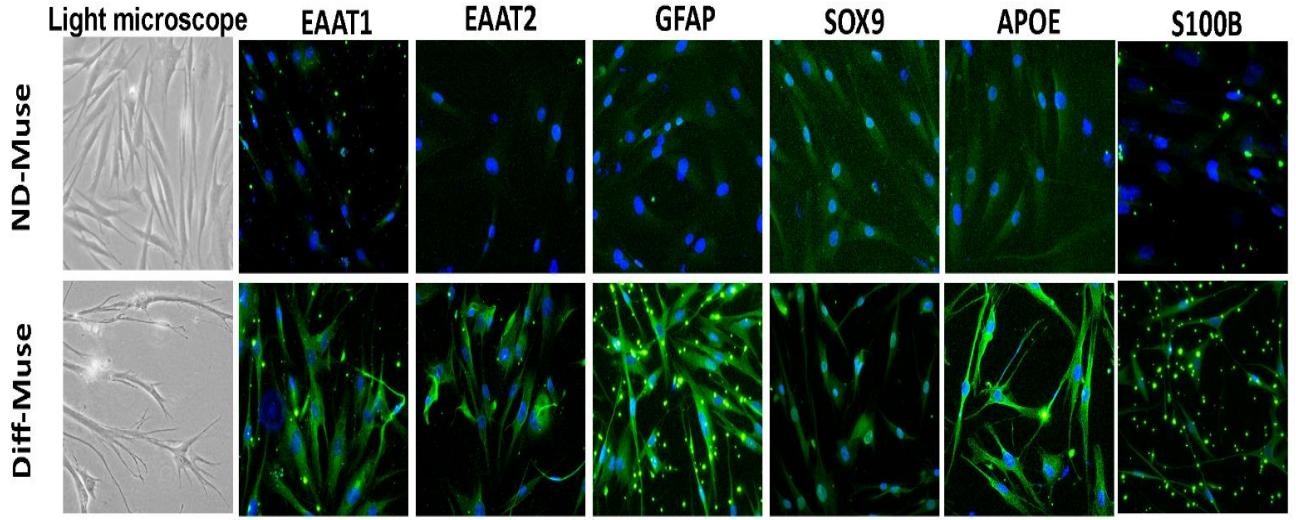


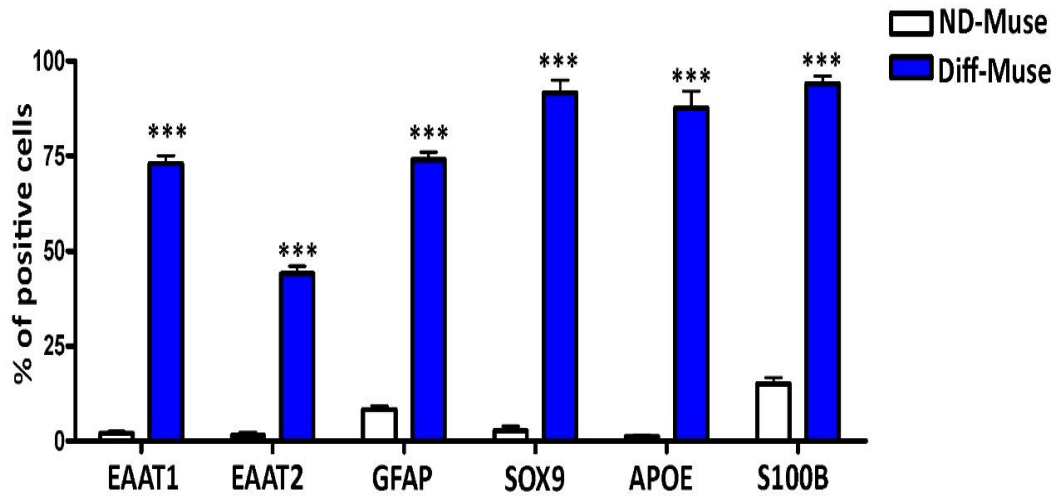


Differentiation journey from fibroblasts to Astrocytes

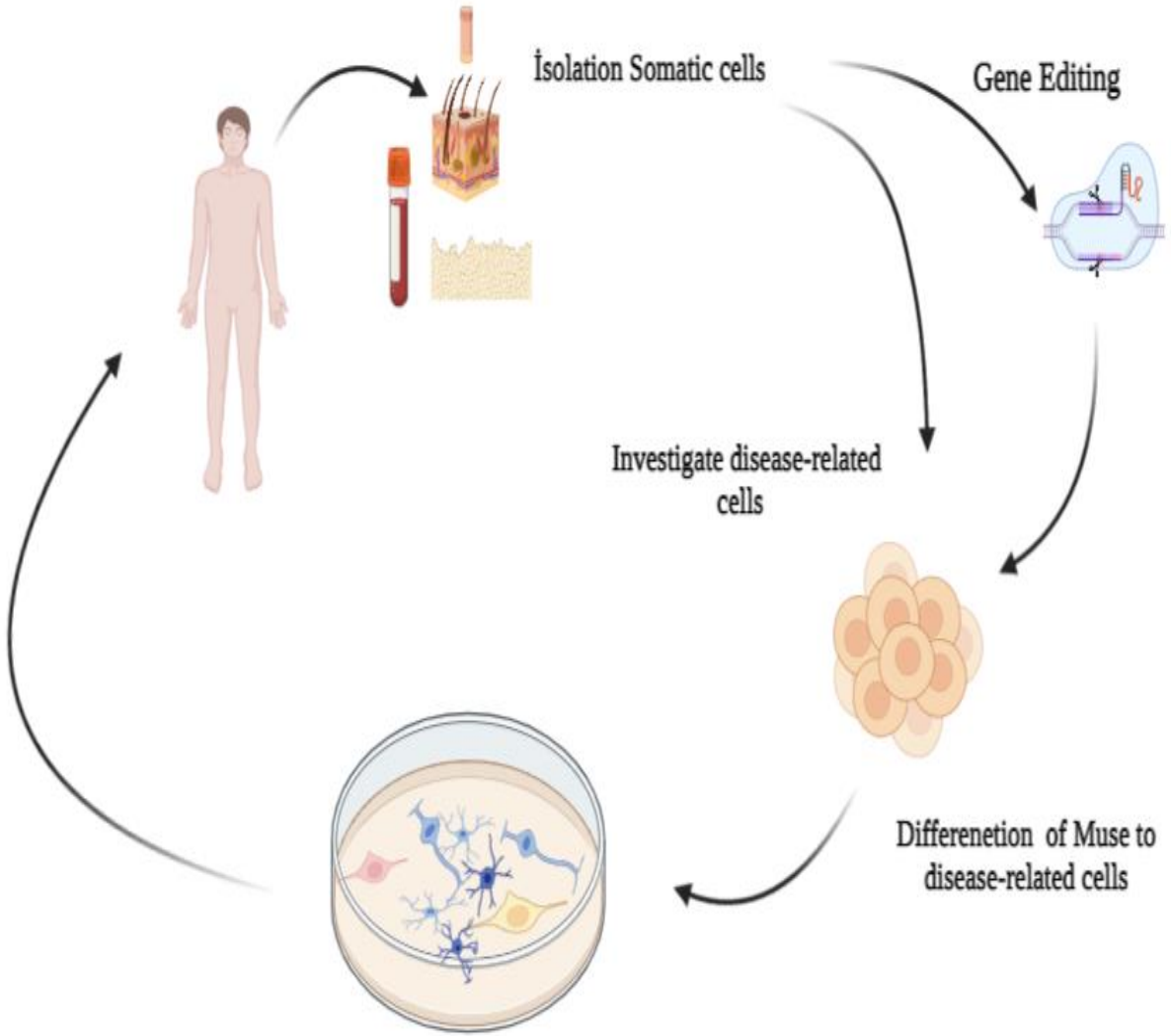


Astrocyte Differentiation





Take Home Message



Muse stem cells, which are multipotent stem cells and are naturally present in human body, could be an effective in vitro disease model.

Thanks for your attention

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- *Dr. Yesuf Siraj*
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