



Fulcrum  
Therapeutics

# Characterizing microenvironmental changes to effectively treat muscle dysfunction

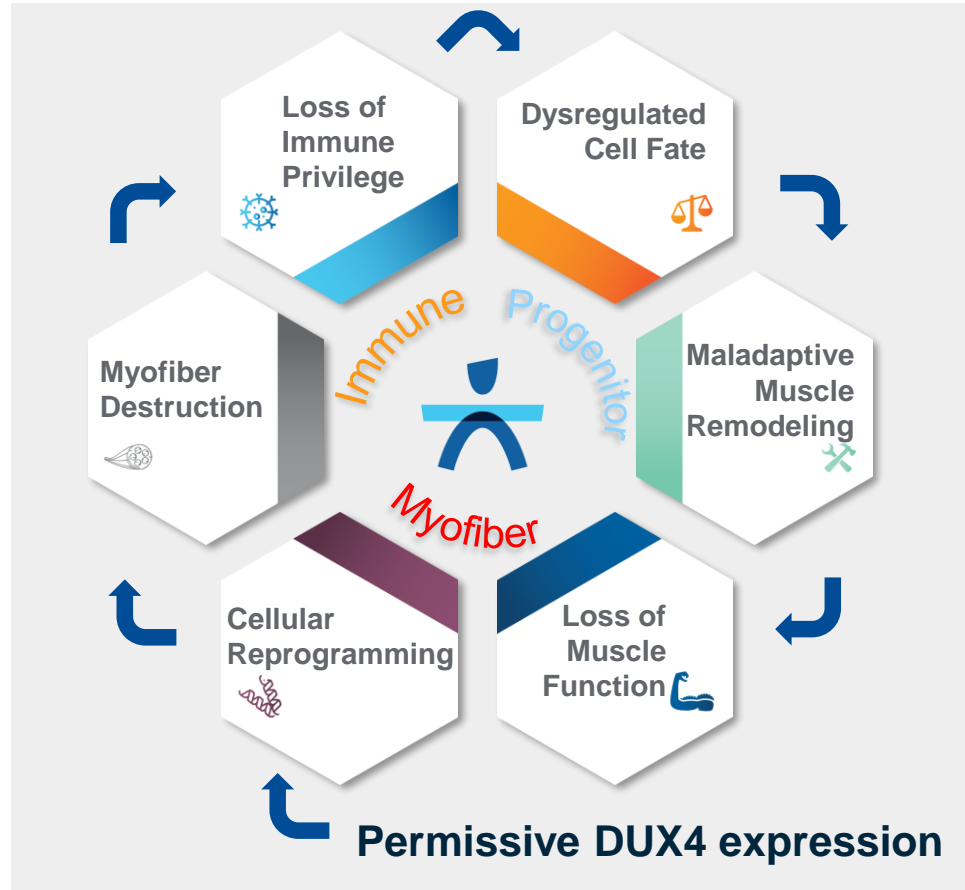
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June 24, 2021

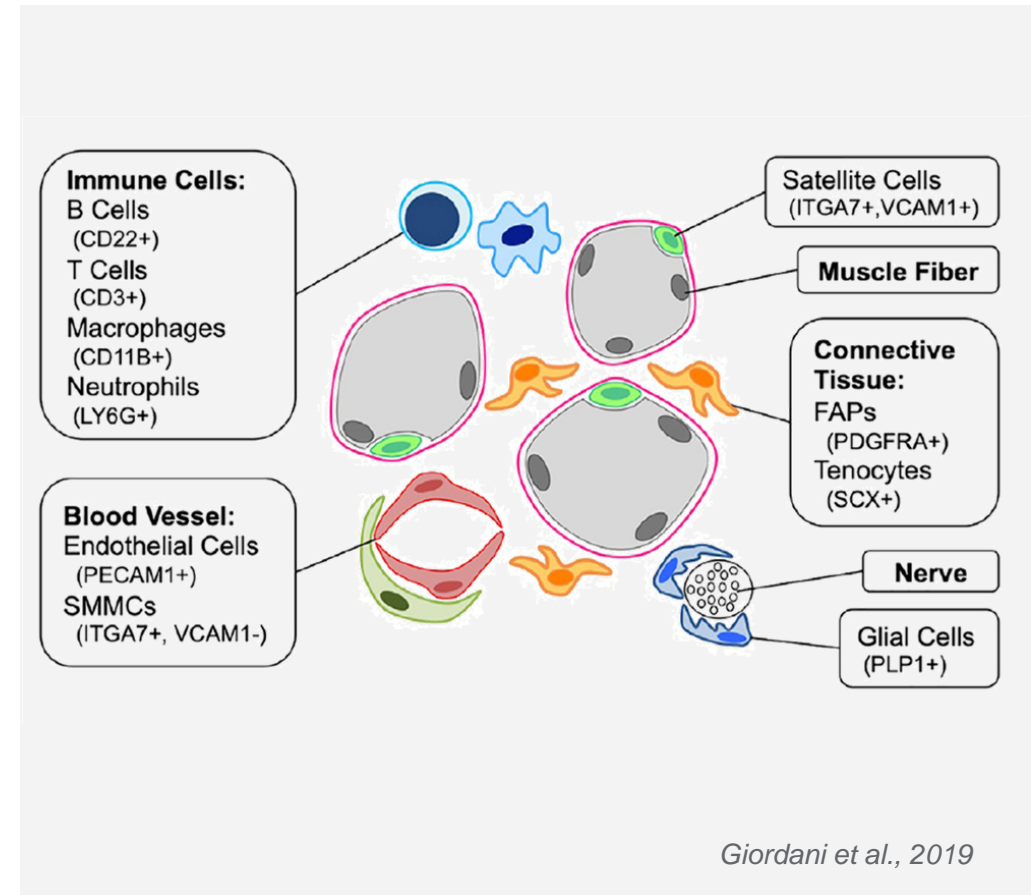


# Background: Hallmarks of FSHD

## Hallmarks of FSHD

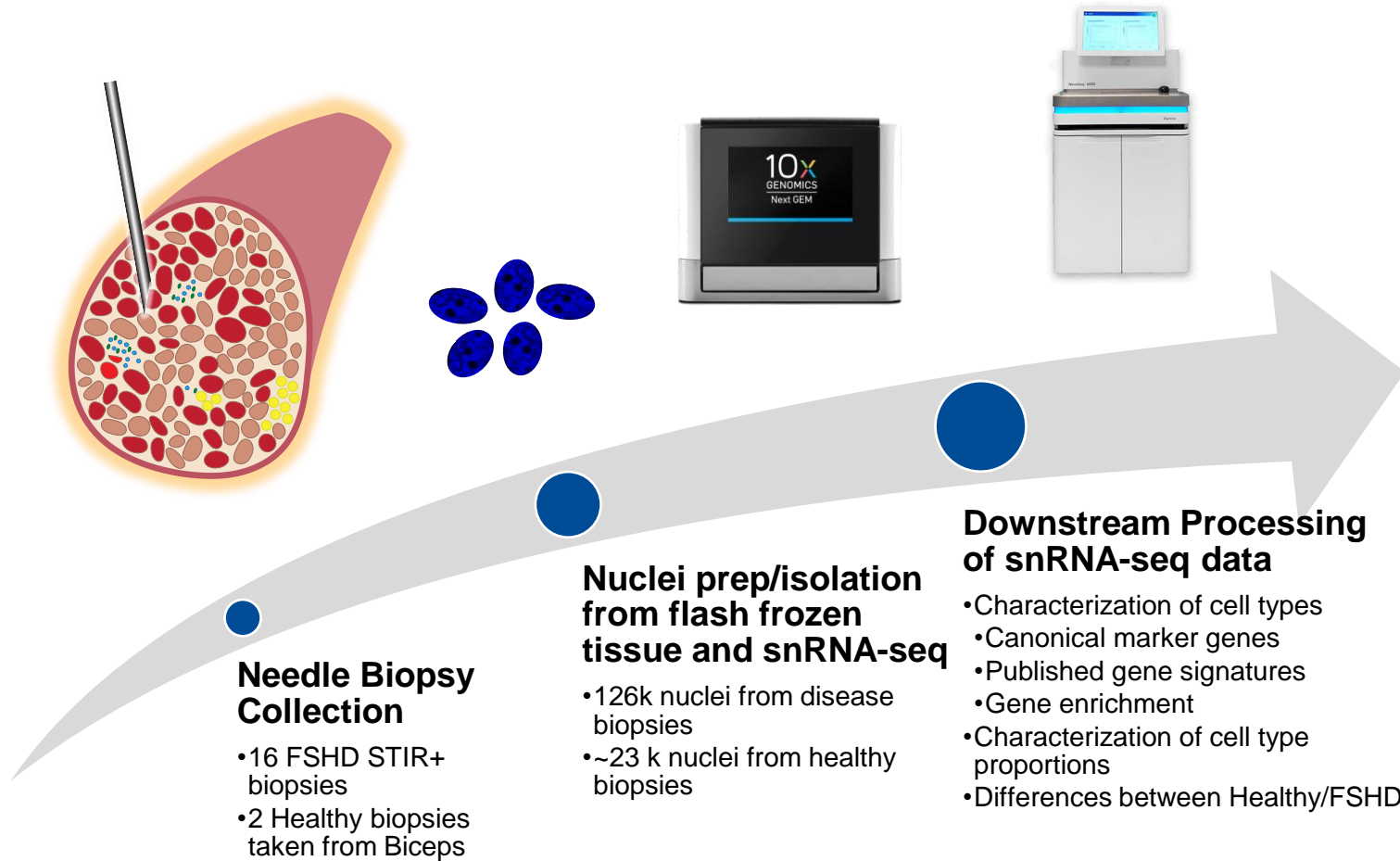


## Many known cell types in Muscle

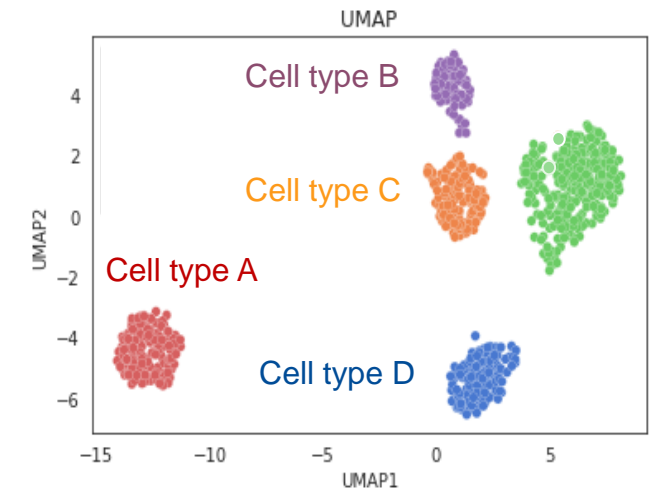


Characterization of muscle composition to understand the *micro-environment changes*, that drive muscle dysfunction.

# Background: *Experimental Design*



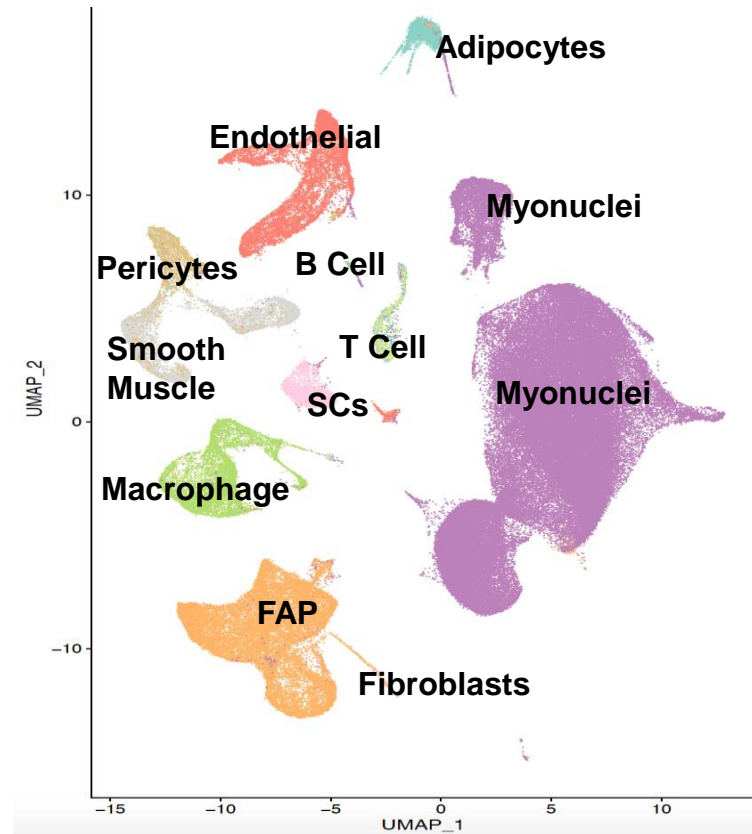
## Example single-cell profile



- Each point on this plot represents a cell
- Each cluster represents cells with a similar transcriptional profile
- Distances between and position of the clusters should not be interpreted

# Data: In-depth characterization of the diverse composition of cell types in the FSHD skeletal muscle

- Transcriptomic profiling at single cell resolution allows us to obtain a detailed map of the cell type composition in FSHD
- Myonuclei comprise ~60% of total nuclei captured from 18 flash-frozen muscle needle biopsies
- Other abundant cell-types associated with pathology previously described in the literature include: adipocytes, T/B cells, macrophages and fibroadipogenic progenitors (FAPs)



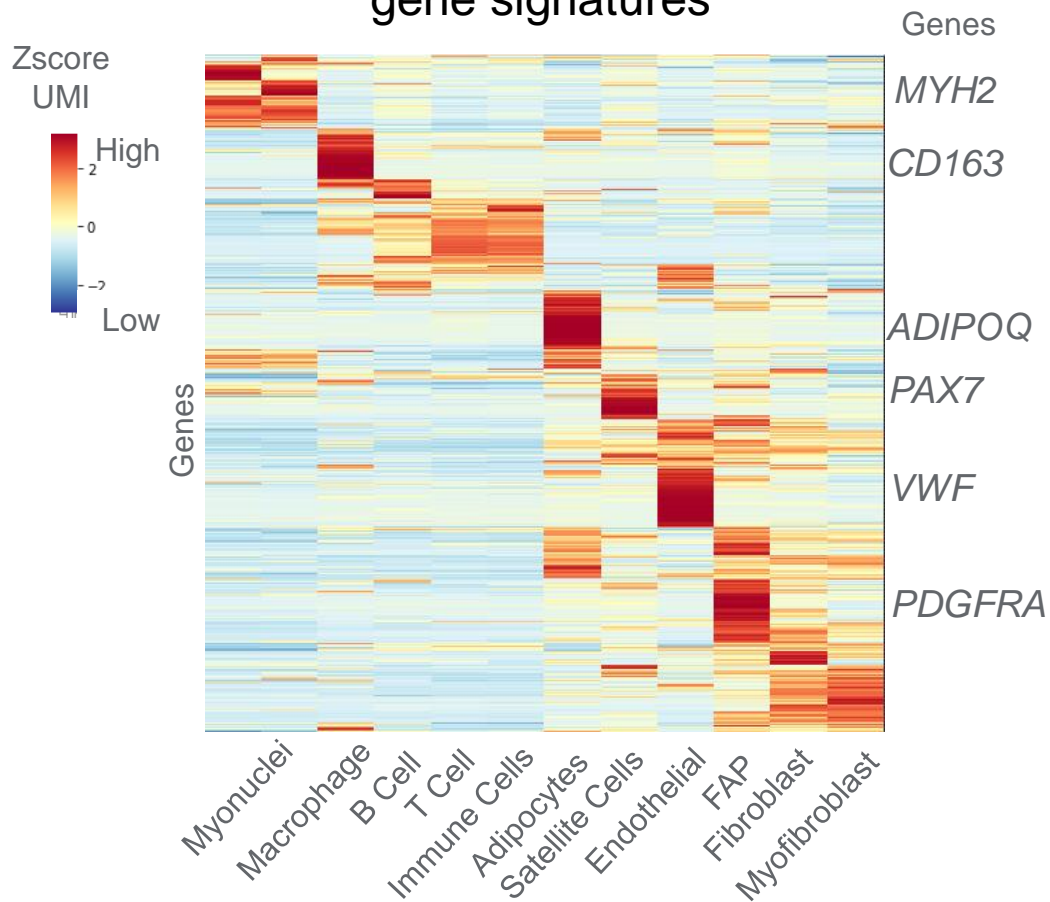
Cell type	# of Cells	% of Total
Adipocytes	4048	2.8
B Cell	265	0.2
Endothelial	9300	6.4
FAP	18941	13.1
Fibroblast	387	0.3
Macrophage	6994	4.8
Myonuclei	89018	61.7
Pericytes	3669	2.5
Satellite Cells	3620	2.5
Smooth Muscle	5790	4.0
T Cell	1410	1.0
Other Immune	898	0.6

**In-depth characterization of the diverse composition of cell types in the FSHD skeletal muscle**

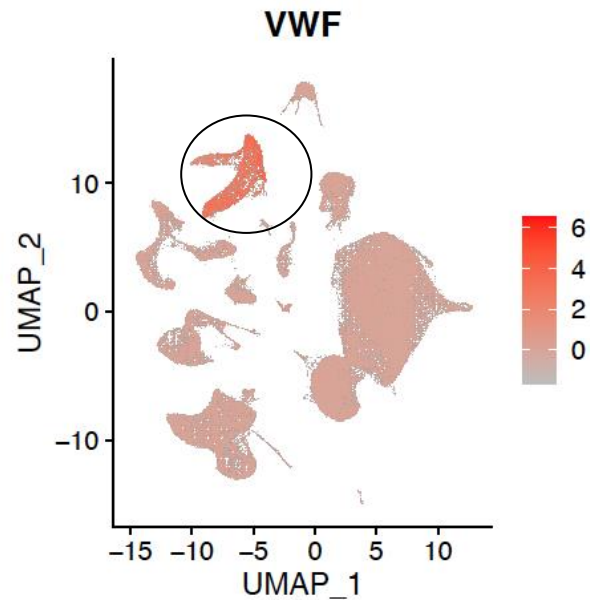
# Data: FSHD: snRNA-seq muscle profiles

## Identification of expected cell types characterized by cell type specific gene expression

Cell type specific gene signatures

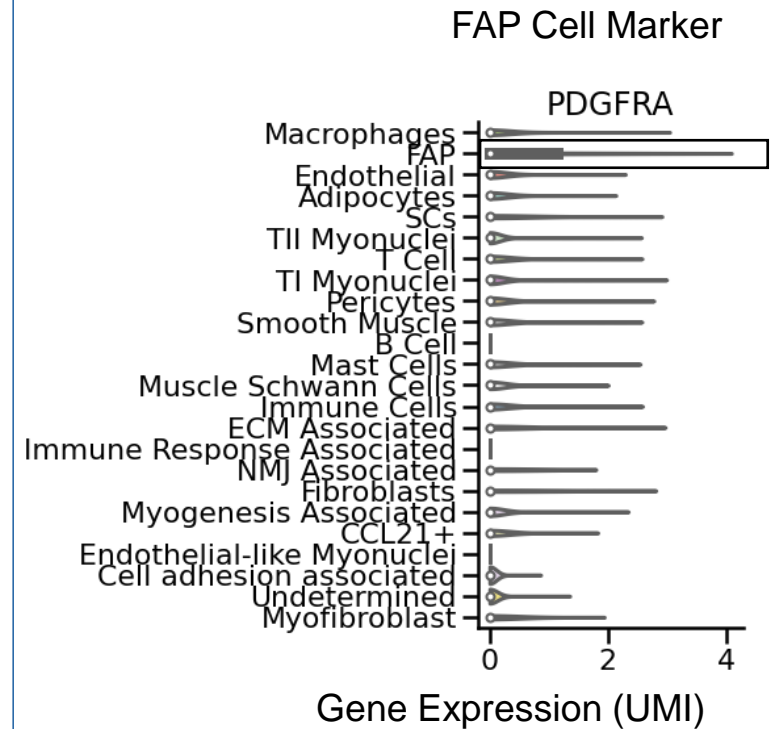


Canonical Marker Gene Expression

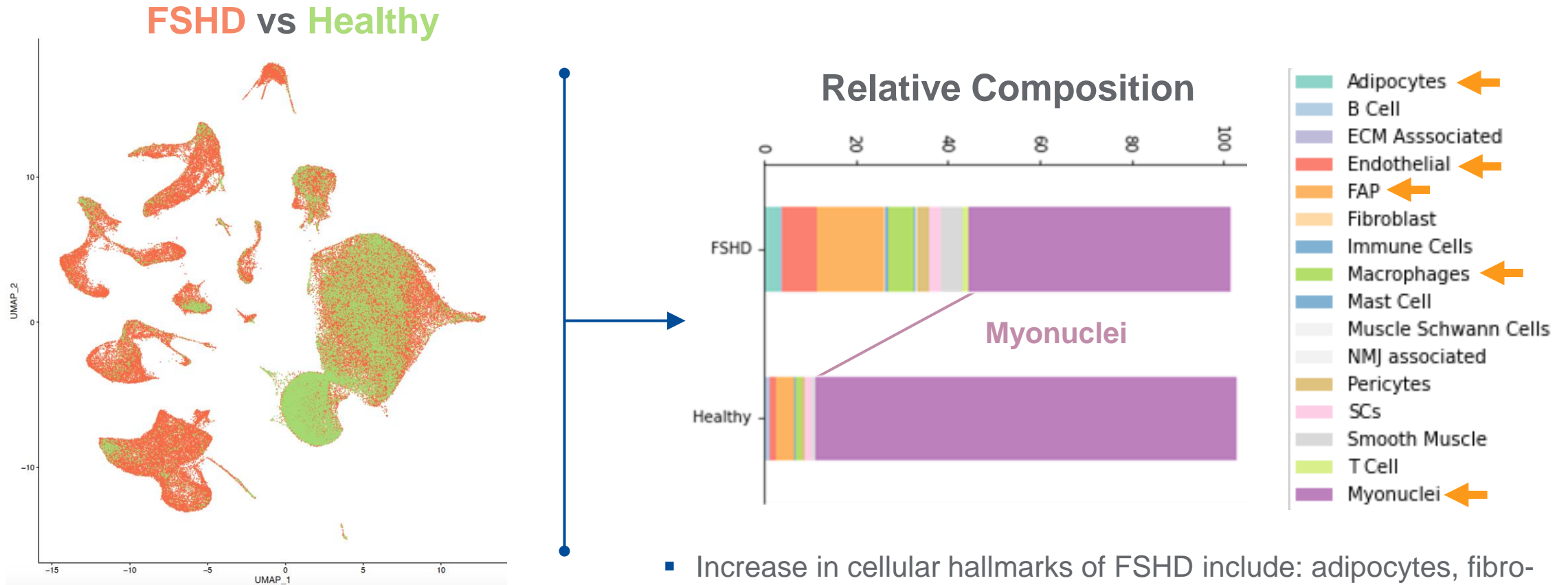


Scaled Gene Expression (UMI) represented on single-cell profiles

Canonical Marker Genes

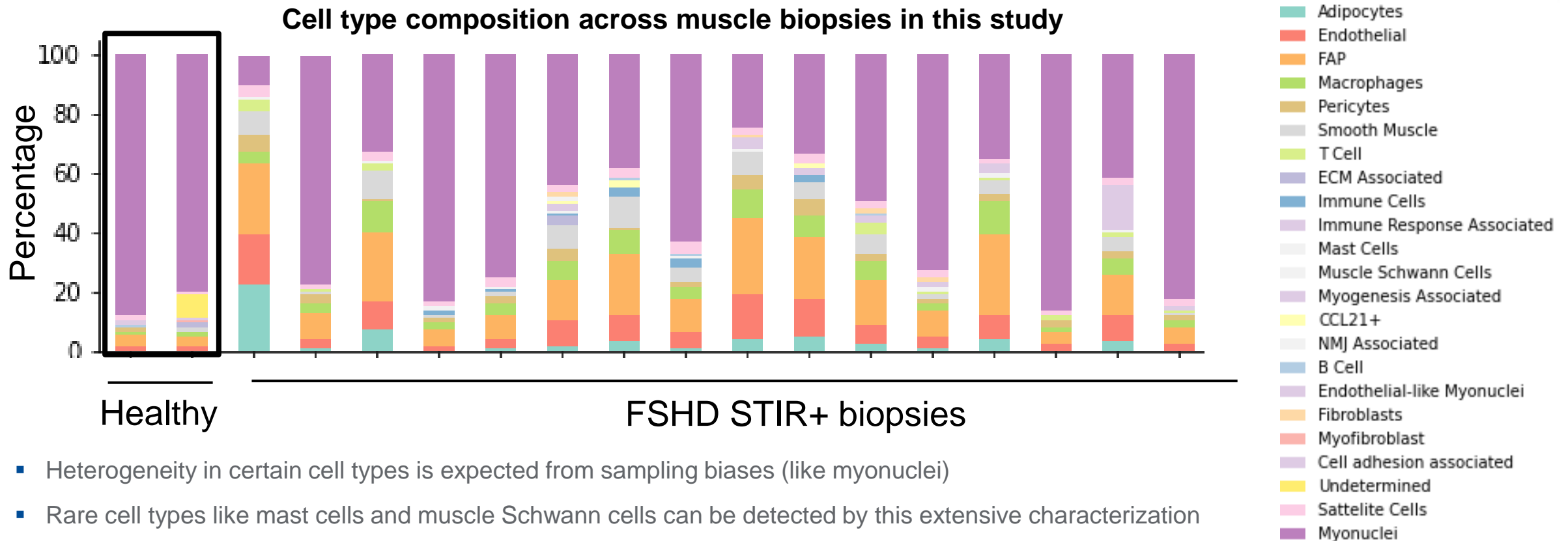


# Data: Comparison FSHD vs healthy muscle needle biopsies



**Analysis at single cell resolution reveals altered muscle composition in FSHD**

# Data: *snRNA-seq analysis reveals high heterogeneity in the composition of biopsies from FSHD patients*

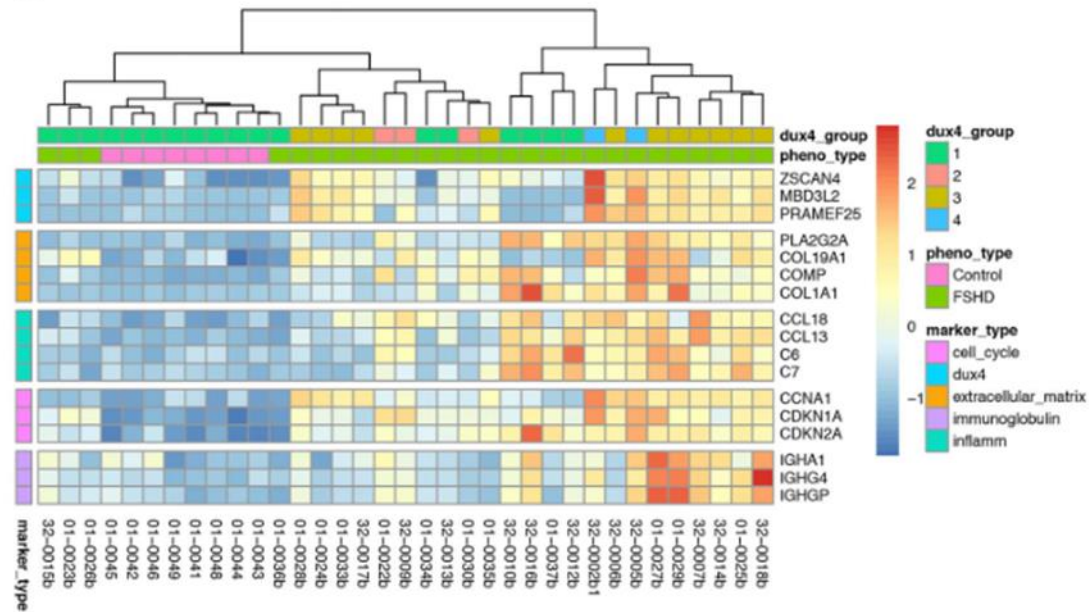


- Heterogeneity in certain cell types is expected from sampling biases (like myonuclei)
- Rare cell types like mast cells and muscle Schwann cells can be detected by this extensive characterization
- Some FSHD biopsies have profiles that resemble healthy muscle composition highlighting the heterogeneity of progression in this disease

**snRNA-seq analysis reveals high heterogeneity in the composition of biopsies from FSHD patients**

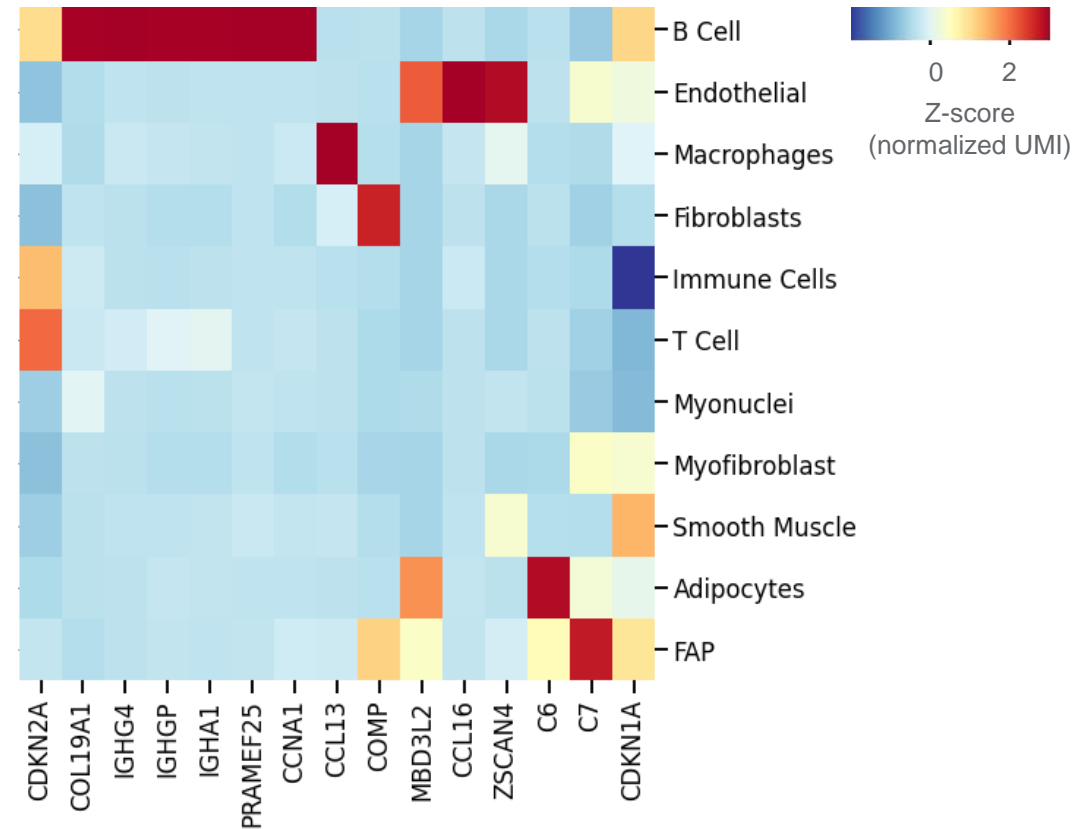
# Data: What cell types drive known signatures present in FSHD

Previously described\* gene signatures found in biopsies with higher expression of *DUX4/DUX4* associated genes



\*Wong, C.-J., Wang, L.H., Friedman, S.D., Shaw, D., Campbell, A.E., Budech, C.B., Lewis, L.M., JFL Lemmers, R., Statland, J.M., van der Maarel, S.M., et al. (2020) *Longitudinal measures of RNA expression and disease activity in FSHD muscle biopsies*. Hum. Mol. Genet., 0

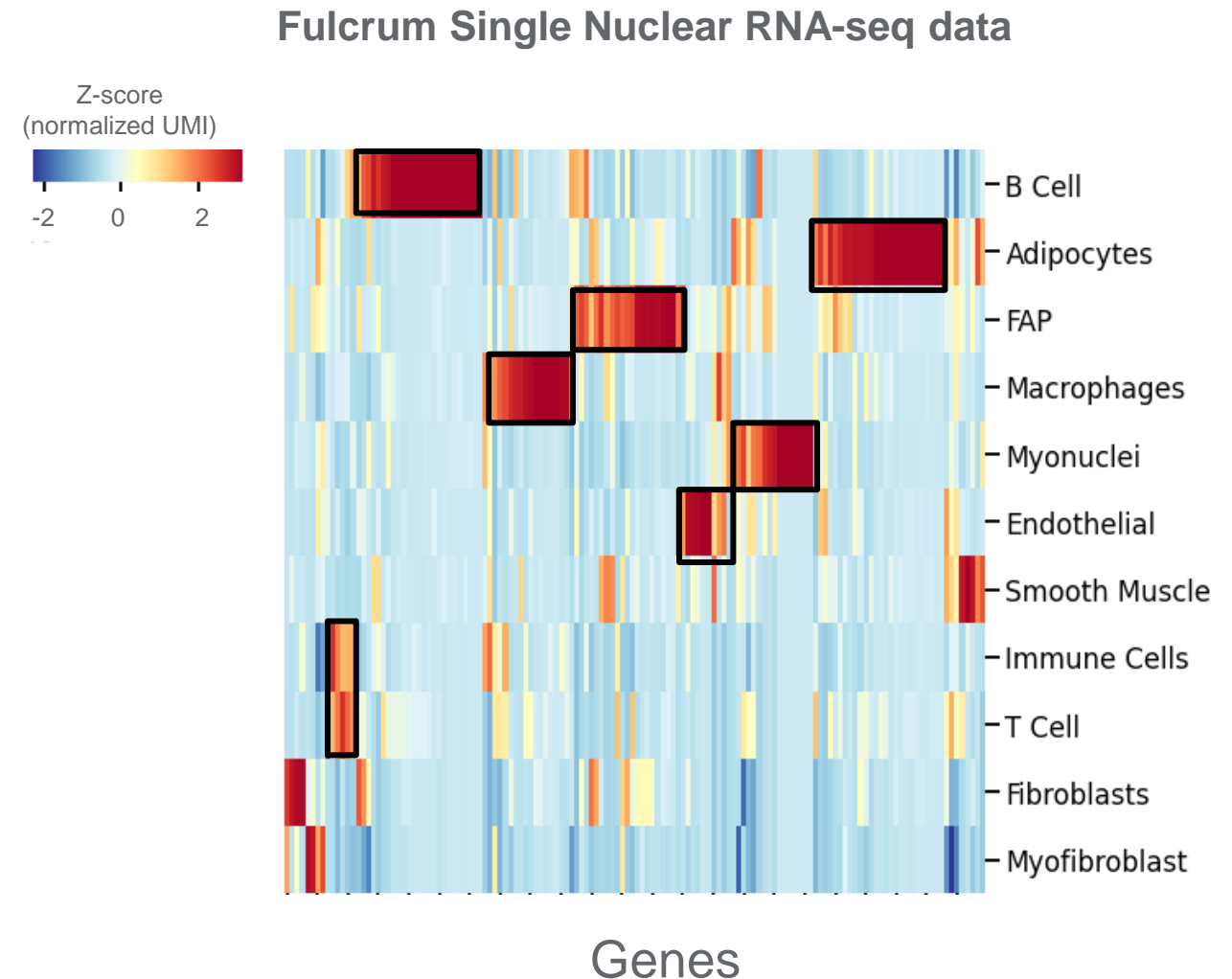
## Fulcrum Single Nuclear RNA-seq data



snRNA-seq analysis allow us to identify specific cell types responsible for previously identify signatures

# Data: What cell types contribute to FSHD signatures derived from bulk RNA-seq?

- Fulcrum has previously characterized muscle biopsies from >15 patients using bulk RNA-seq
- Highly differential expressed genes in this set, identified ~170 DE genes
  - > 10 fold change between FSHD vs Healthy muscle biopsies
- Analysis of this gene set in the snRNA-seq profiles provides **insight about the dysregulated cell types present in the FSHD muscle**, including FAPs, adipocytes and multiple immune-related cell types



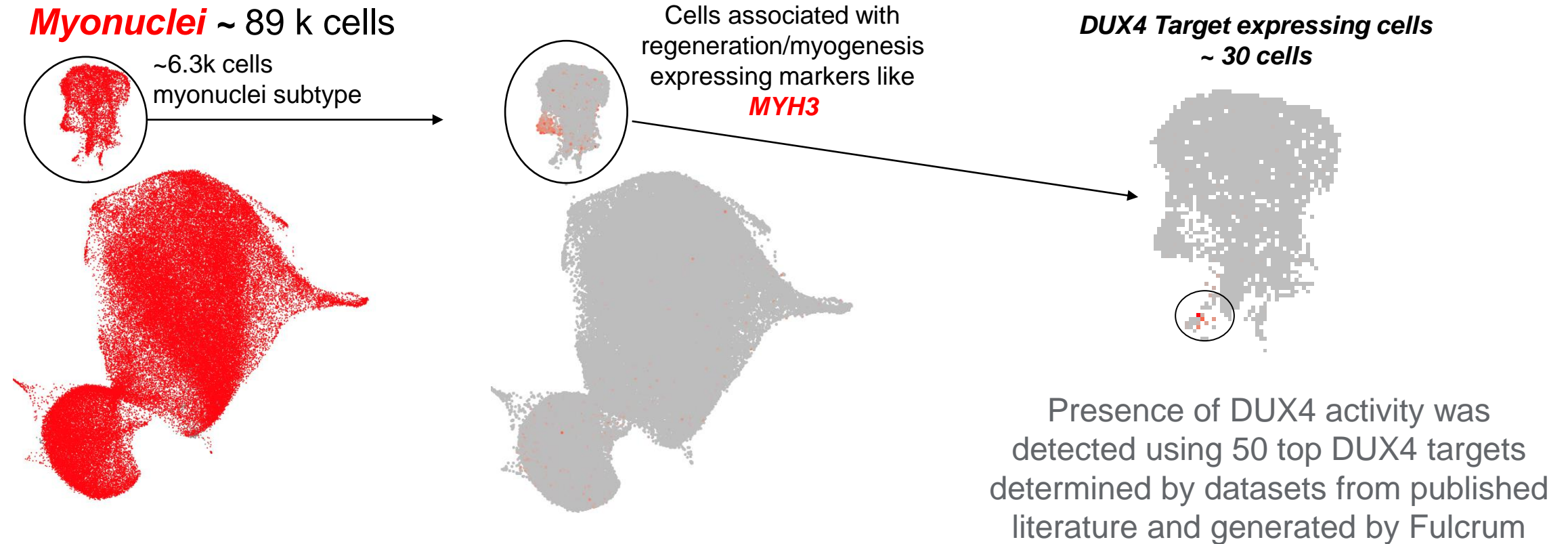
# Summary: *Key findings about cell type composition*

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- **Cell type composition of patient biopsies is heterogeneous, though we can still identify trends in the data**
  - Decreases in relative proportion of Myonuclei in FSHD vs healthy biopsies
  - Increase (Expansion) in relative proportion of FAP, Endothelial, Macrophages
  - Some biopsies have a cell type composition that is more similar to healthy tissue
- **Genes from signatures derived from bulk RNA-seq analysis are detected in single nuclear profiling, and are sometimes cell-type specific**
  - Observing that cell type specific genes are differentially expressed in bulk data, likely represents a difference in relative cell type proportion between FSHD and healthy muscle for the corresponding cell type
    - These cells can help us understand which hallmarks to further focus in on to study disease progression (ie: FAP: Macrophage; Endothelial)

**Can we detect and characterize DUX4 expressing nuclei in this dataset?**

# Data: Can we learn about the DUX4 program with snRNA-seq?

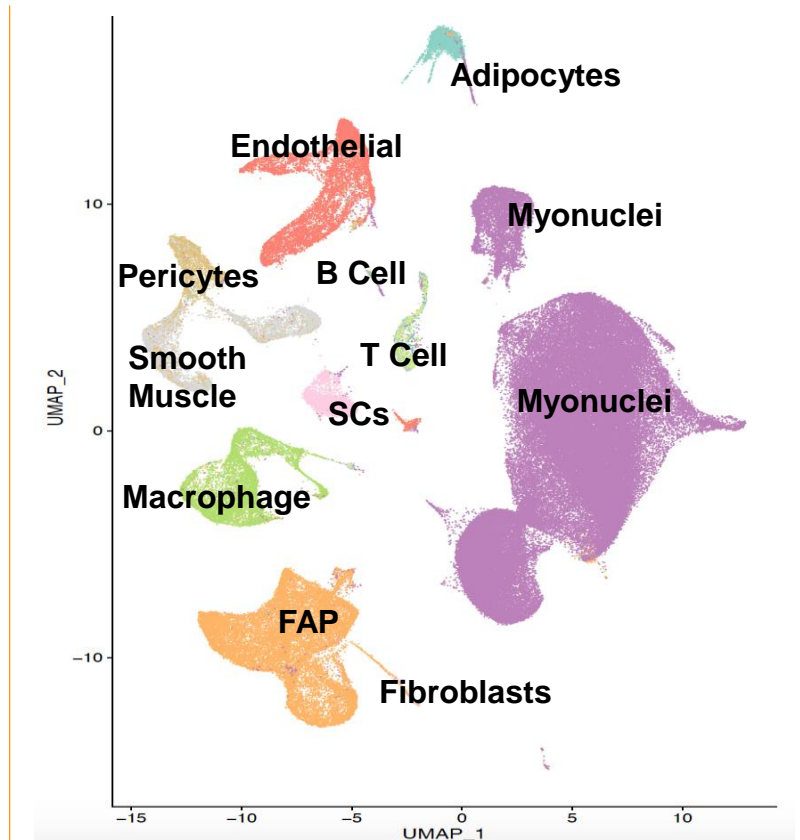


**1 in 3000 myonuclei have detectable DUX4 activity using snRNA-seq profiling**

# Conclusions

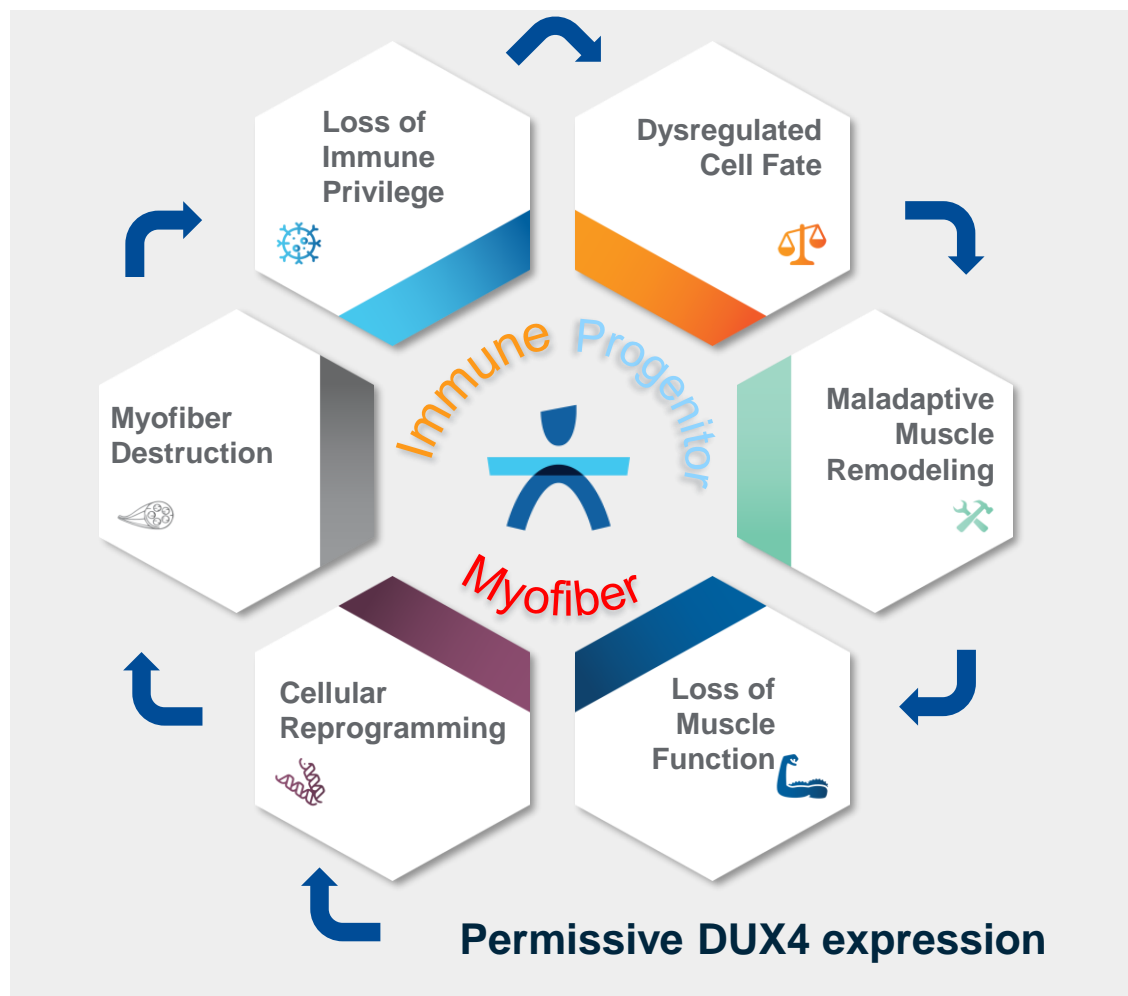
## Over 125,000 nuclei comprise the first single-cell resolution map of cell type composition in skeletal muscle of people living with FSHD

- Building on previous studies, this dataset demonstrates the **large amount of heterogeneity present in FSHD muscle**
- **DUX4+ nuclei are very rare** and DUX4 activity is only present in nuclei of cells undergoing early stages of myogenesis
- Expanded cell types in FSHD biopsies can be further characterized to understand their role in pathogenesis and serve as a novel therapeutic target identification approaches
  - Characterization of Ligand: Receptor interactions between cell types
  - Markers identified for cell type subpopulations to inform histological characterization



# Future Directions

## Hallmarks of FSHD



- Cell populations expanded in disease (FAP, Endothelial, Macrophage) are implicated in sub-pathways of FSHD hallmarks
- Profiling more healthy tissue in order to contrast differences in these cell-types between disease and healthy muscle will allow us to develop novel hypotheses around these hallmarks
- Ultimately novel hypotheses will lead to novel medicines for FSHD patients

# Acknowledgments

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