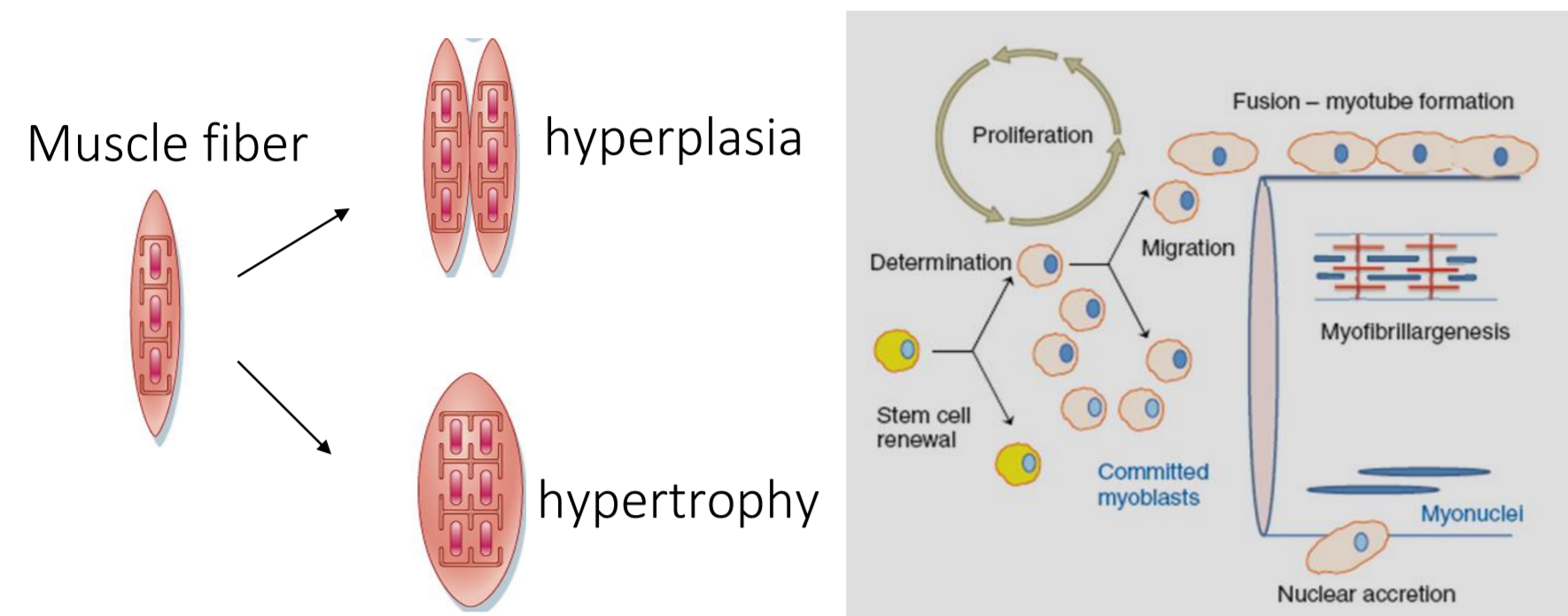


INTRODUCTION AND OBJECTIVES

Post-larval growth in salmonids results from intense muscle hyperplasia and hypertrophy^[1]. These processes are directly linked to the specific proliferation and differentiation behaviors of muscle stem cells, also known as satellite cells (SCs)^[2].

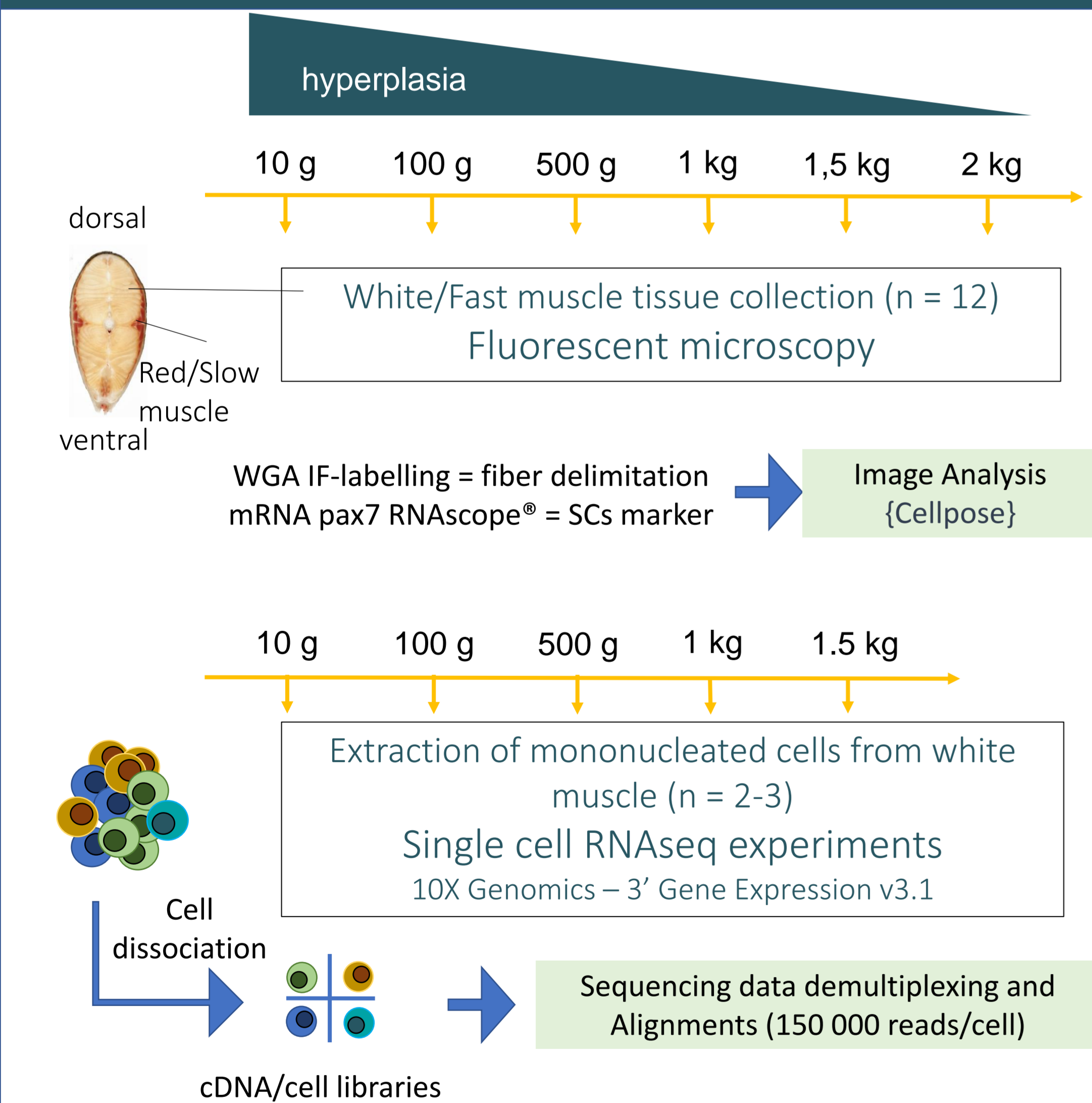


Hyperplasia allows exponential muscle growth during the post-larval phase but declines during the juvenile stage to cease afterwards. Thus, a 200-fold increase of muscle fiber number is observed between the hatching embryo and a 4 kg salmon^[3]. Therefore, the intensity and duration of post-larval hyperplastic growth are crucial for the potential development of fish muscle mass.

While evidences for the existence of a diversity of SC subtypes are clear in mammals^[4], similar data on fish are still lacking.

We aimed to elucidate the cellular and molecular dynamics of SCs during the decline of muscle hyperplasia in trout.

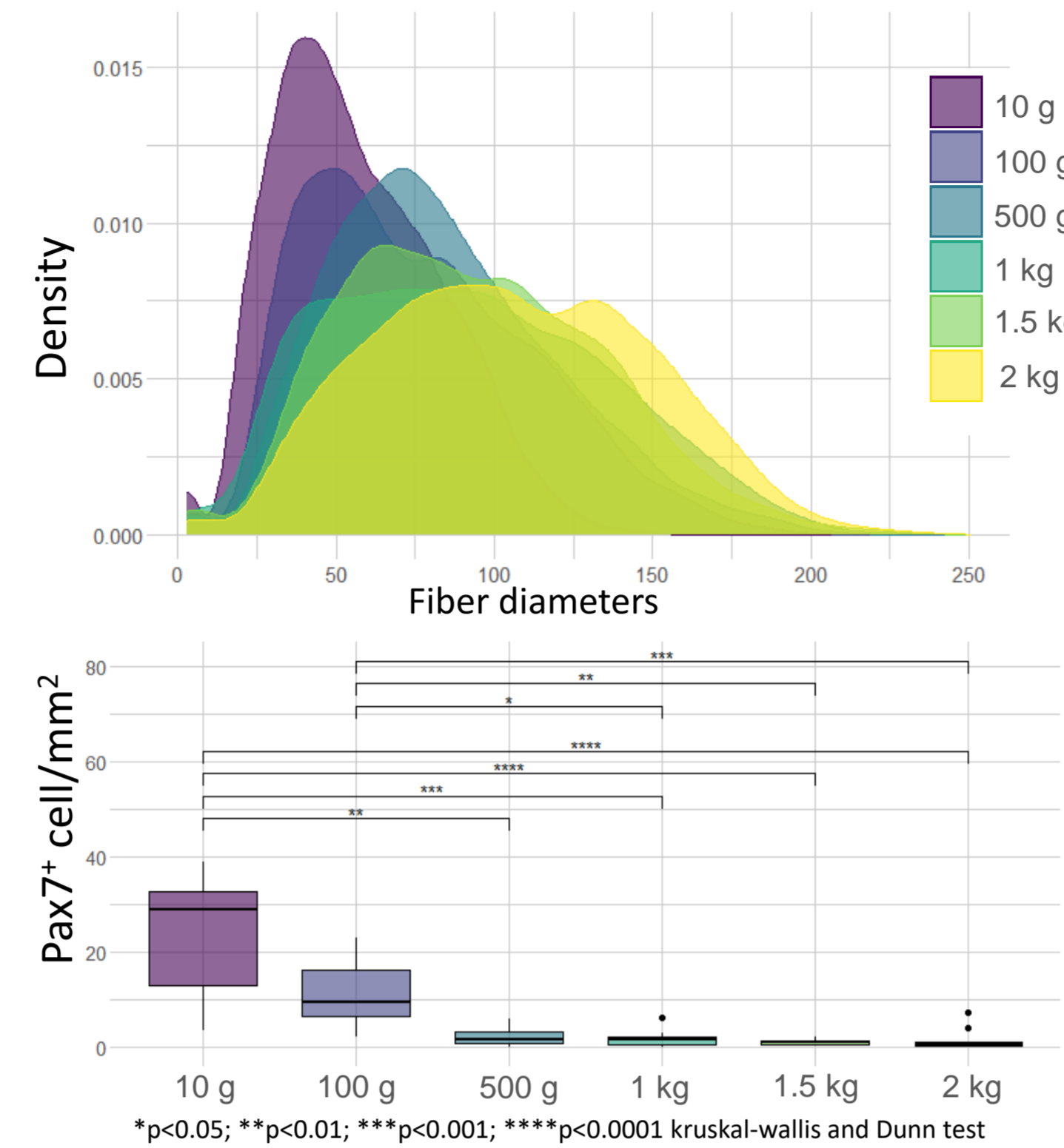
METHODS



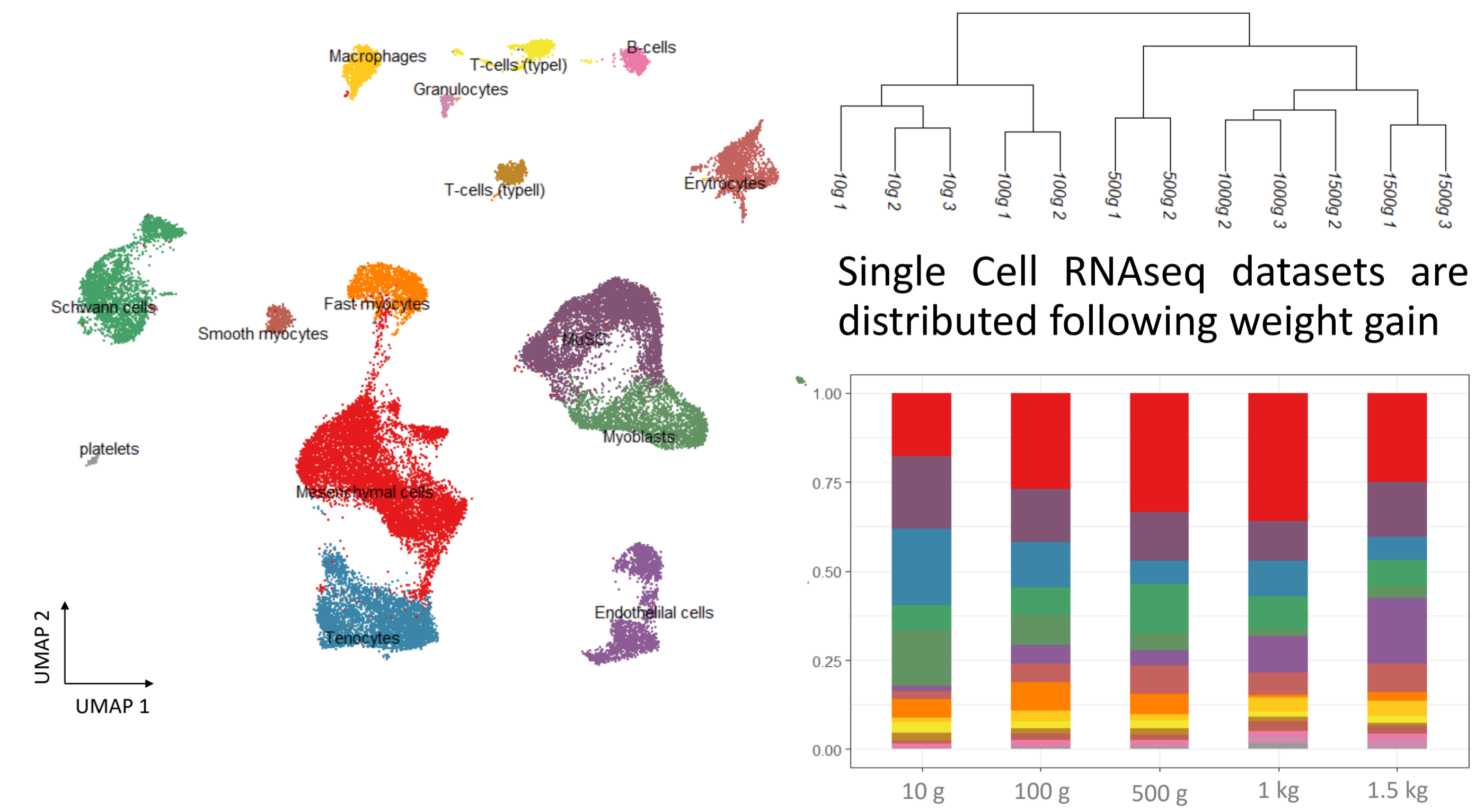
Preprocessing	Clustering	Data Exploration
Quality filters on cells and samples	Dimension reduction on the most variable genes	Trajectory inferences {scVelo – UniTVelo}
Normalization and Integration of datasets {FastMNN}	Clustering {Seurat v4 Louvain algorithm}	Differential analysis between conditions (weights) {DESeq2-LTR}
Doublet correction {DoubletFinder}	Gene marker identification {MAST test}	Pathway activity enrichment analysis {Hipathia}
	Cluster annotation	

RESULTS

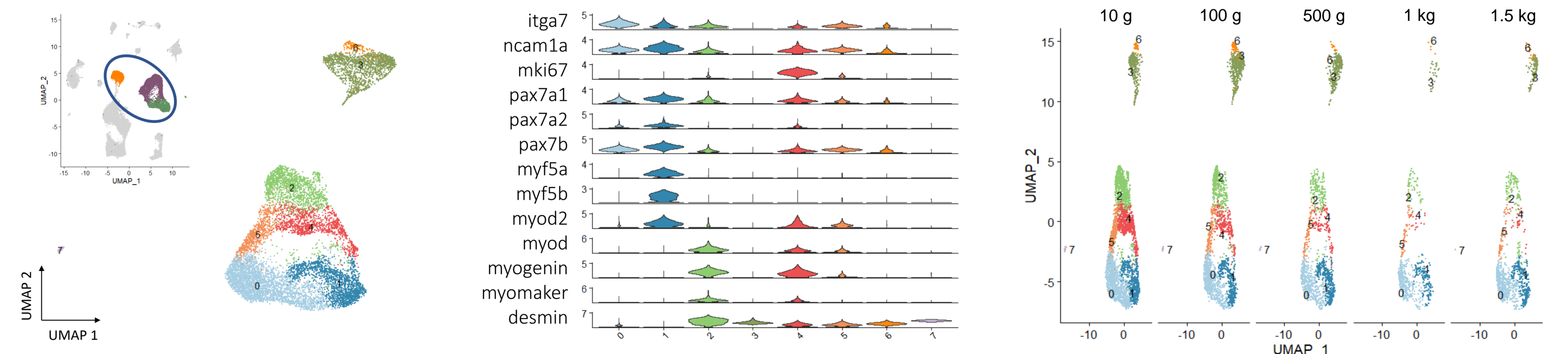
1) Hyperplasia and pax7⁺ SCs decrease with trout weight



2) Identification of 16 cell types in trout white muscles and their distinct evolution with the decline of hyperplasia

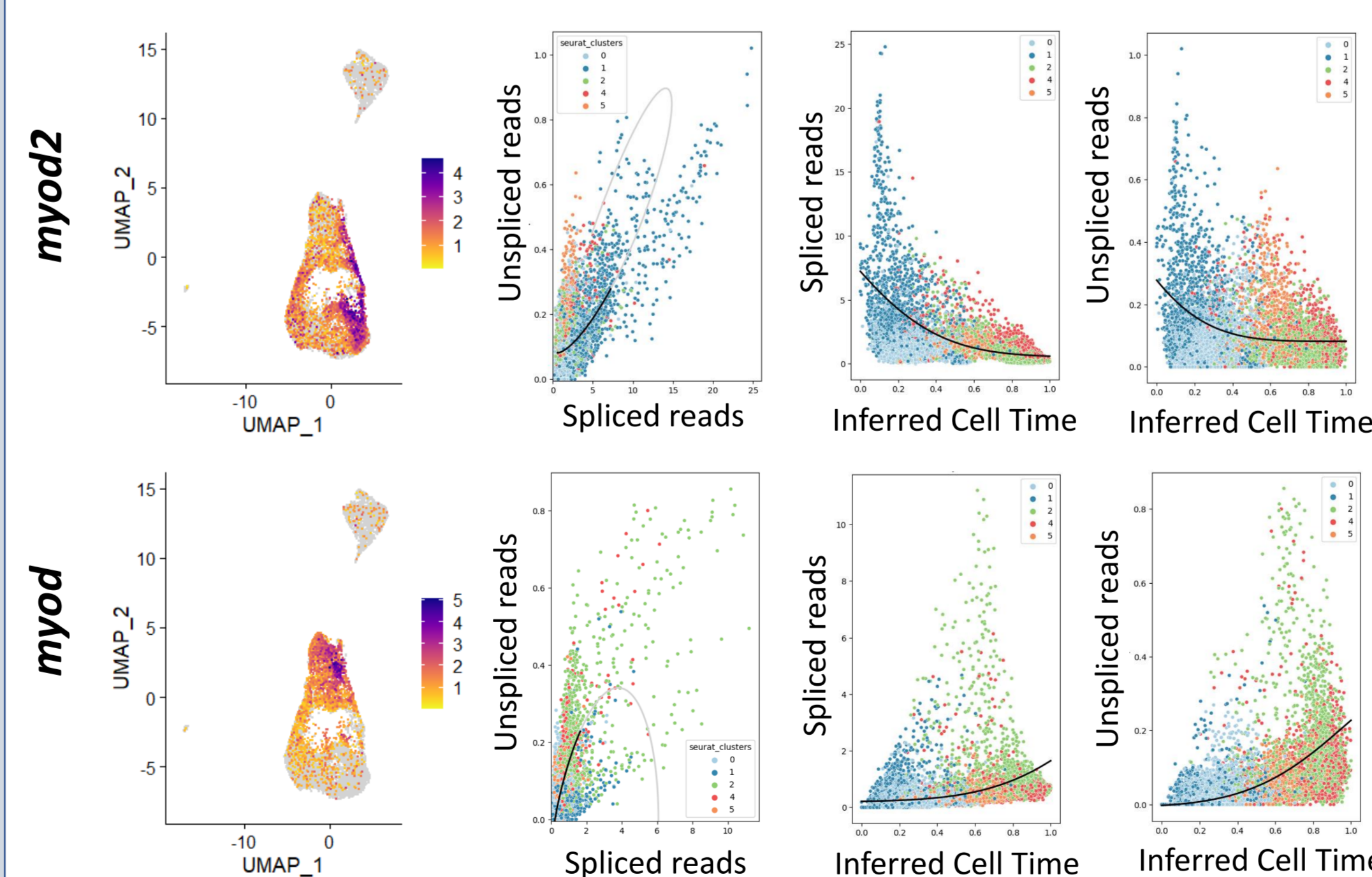


3) Eight clusters belonging to myogenic lineage are identified at various stages between quiescent SCs to myocytes

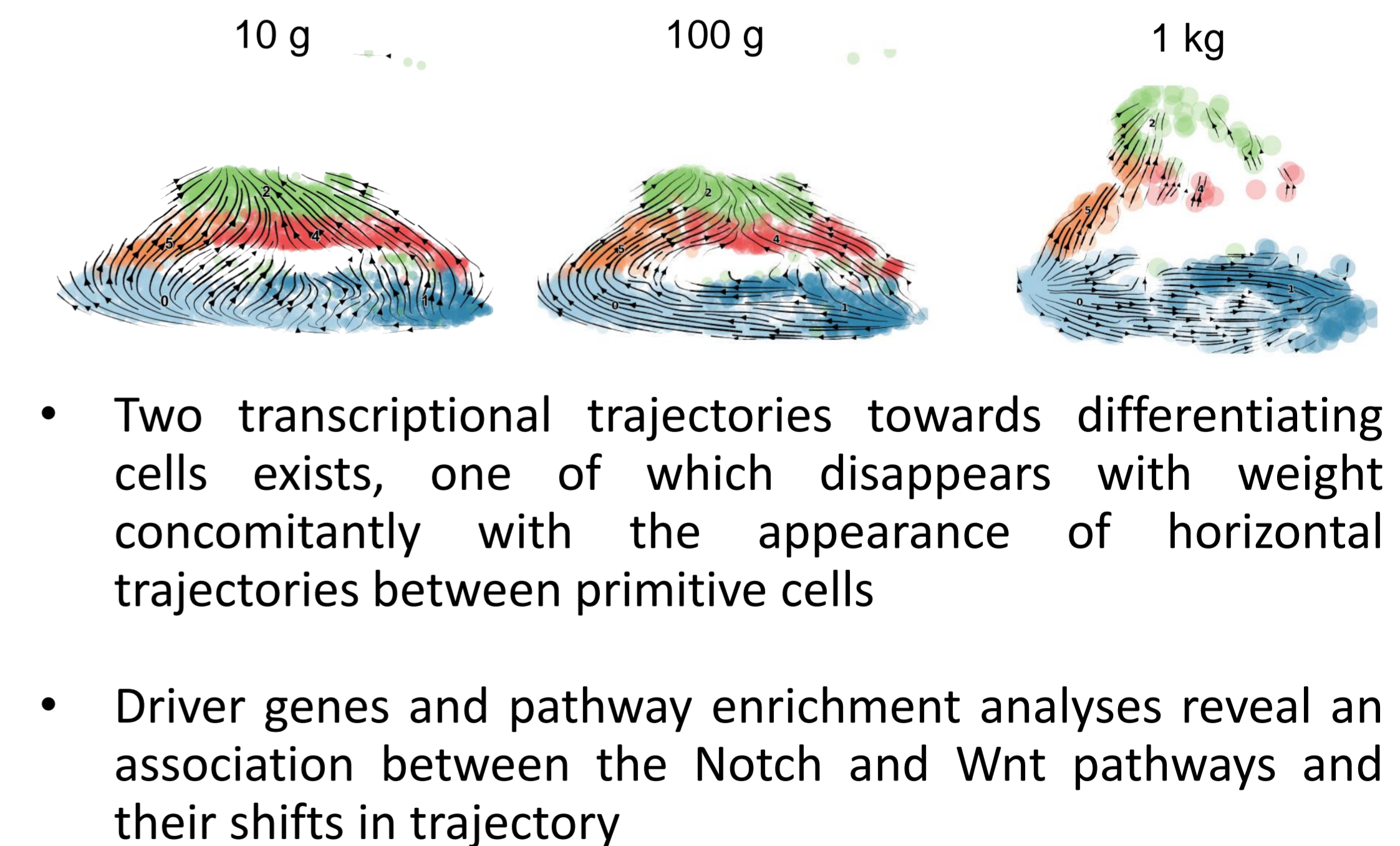


Along with weight gain, the proportion of primitive cells increases while the most committed myogenic precursors into the differentiation program decreases

4) myod paralogs shows differential expression across cell subtypes and stages in the pseudotime analysis



5) Differential transcriptional trajectories associated with weight determined by velocity analysis



- Two transcriptional trajectories towards differentiating cells exists, one of which disappears with weight concomitantly with the appearance of horizontal trajectories between primitive cells
- Driver genes and pathway enrichment analyses reveal an association between the Notch and Wnt pathways and their shifts in trajectory

CONCLUSIONS

We show that decline of hyperplasia is associated with a weight-related decrease of SC number in trout. We found that duplicated *myod2* and *myod* genes provide molecular markers of myogenic cell lineage heterogeneity. Additionally, our results show the loss of generation of new committed myogenic precursors associated with the hyperplasia arrest, although the most primitive SCs are preserved, revealing a shift of the latter towards symmetrical division modalities. Importantly, our data reveal that these primitive SCs exist in 2 subtypes that differentially expressed *pax7* paralogs and describe clearly distinct trajectories with the decline of hyperplasia. Overall, our findings underline specific behaviors in growth that suggest a modified founder cell/fusion competent myoblast model operates in fish.

REFERENCES

- [1] B. M. Koeppen and B. A. Stanton, *Berne & Levy Physiology*, 6th ed. Elsevier Health Sciences, 2009.
- [2] I. A. Johnston, N. I. Bower, and D. J. Macqueen, "Growth and the regulation of myotomal muscle mass in teleost fish," *J. Exp. Biol.*, vol. 214, no. 10, pp. 1617–1628, 2011, doi: 10.1242/jeb.038620.
- [3] I. A. Johnston, "Muscle development and growth: Potential implications for flesh quality in fish," *Aquaculture*, vol. 177, no. 1–4, pp. 99–115, 1999, doi: 10.1016/S0044-8486(99)00072-1.
- [4] S. Dell'Orso *et al.*, "Single cell analysis of adult mouse skeletal muscle stem cells in homeostatic and regenerative conditions," *Dev.*, vol. 146, no. 12, Jun. 2019, doi: 10.1242/dev.174177.