

Single-cell transcriptomic analysis reveals genetic drivers of fast/slow motor neuron identity

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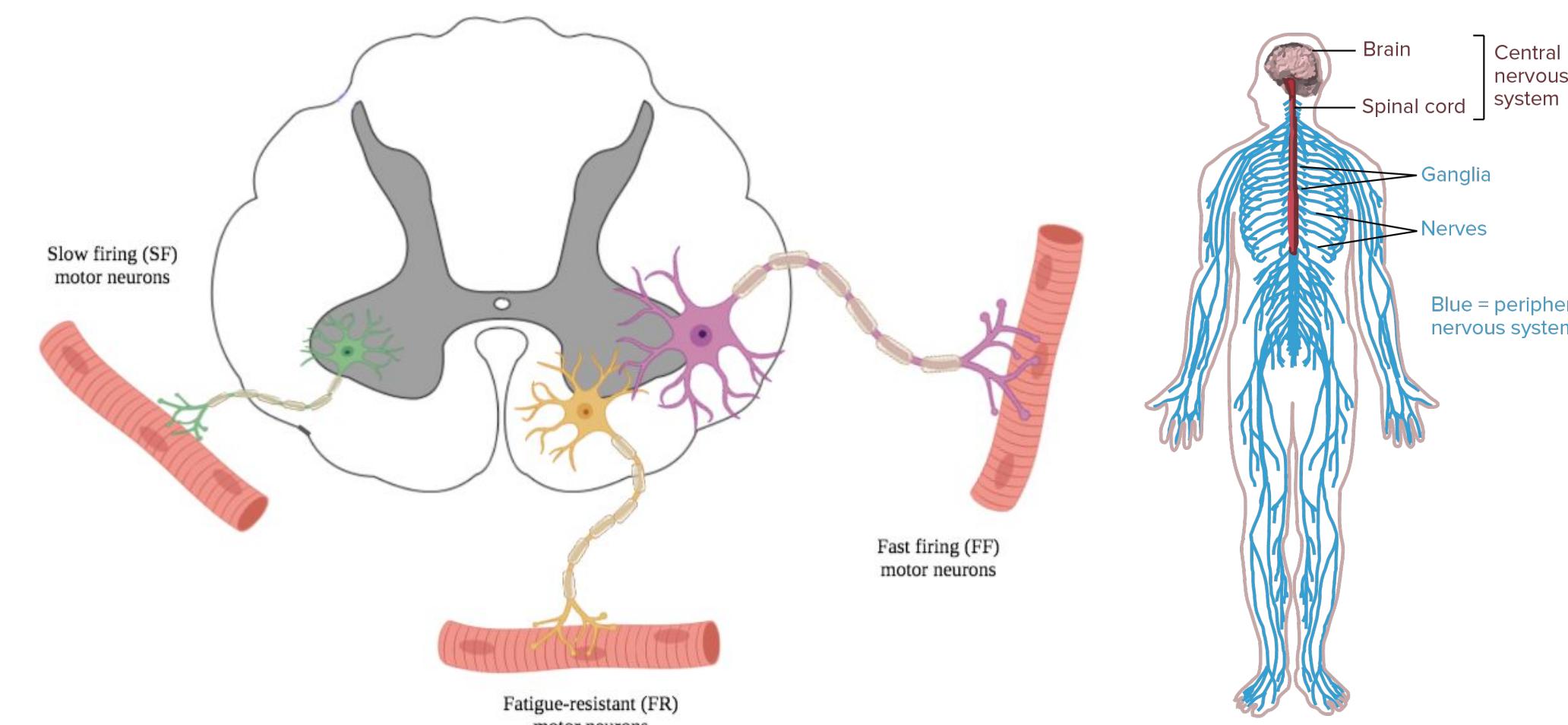
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ABSTRACT

Skeletal motor neurons play an indispensable role in translating our thoughts and needs into actions and response. To match the specific muscles they control, motor neurons must tune their functional properties to the unique demands of their environment. "Slow-firing" motor neurons innervate "slow-twitch" muscle fibers, and "fast-firing" motor neurons innervate "fast-twitch" fibers. Here, we analyze a previously published single-cell transcriptomic dataset to explore the gene expression differences that distinguish slow and fast motor neurons. Surprisingly, we find that the two subtypes do not transcriptionally cluster into separate, discrete populations. Rather, we find that *all motor neurons exist on a continuous spectrum that corresponds with electrophysiological identity*. We establish an individual per-neuron 'Fast Score', and we show that canonical markers of slow and fast motor neurons exist on opposite ends of a continuous spectrum. This finding challenges the conventional wisdom that fast and slow-firing motor neurons are discrete cell types, instead arguing for a newly-forming model of motor neuron identity. Using regression analysis, we explore the hypothesis that a distinct set of transcription factors are responsible for polarizing motor neurons along this continuous spectrum. We discover modules of genes controlling canonical properties of slow and fast motor neurons, like neuron projection/signaling pathways, affirming our hypothesis that gene expression differences encode functional motor neuron properties. Finally, we granularize our data into transcription factor networks, allowing us to resolve master regulators of cell identity and individual properties in slow and fast motor neurons. Understanding the regulatory logic that polarizes fast and slow-firing motor neurons holds the potential to unlock interconversion between these important and disease-relevant cell types. In the future, manipulation of these networks may present viable therapeutic strategies in neuromuscular diseases like amyotrophic lateral sclerosis (ALS) and inform our ability to generate motor neuron subtypes in induced pluripotent stem cell-derived models of disease.

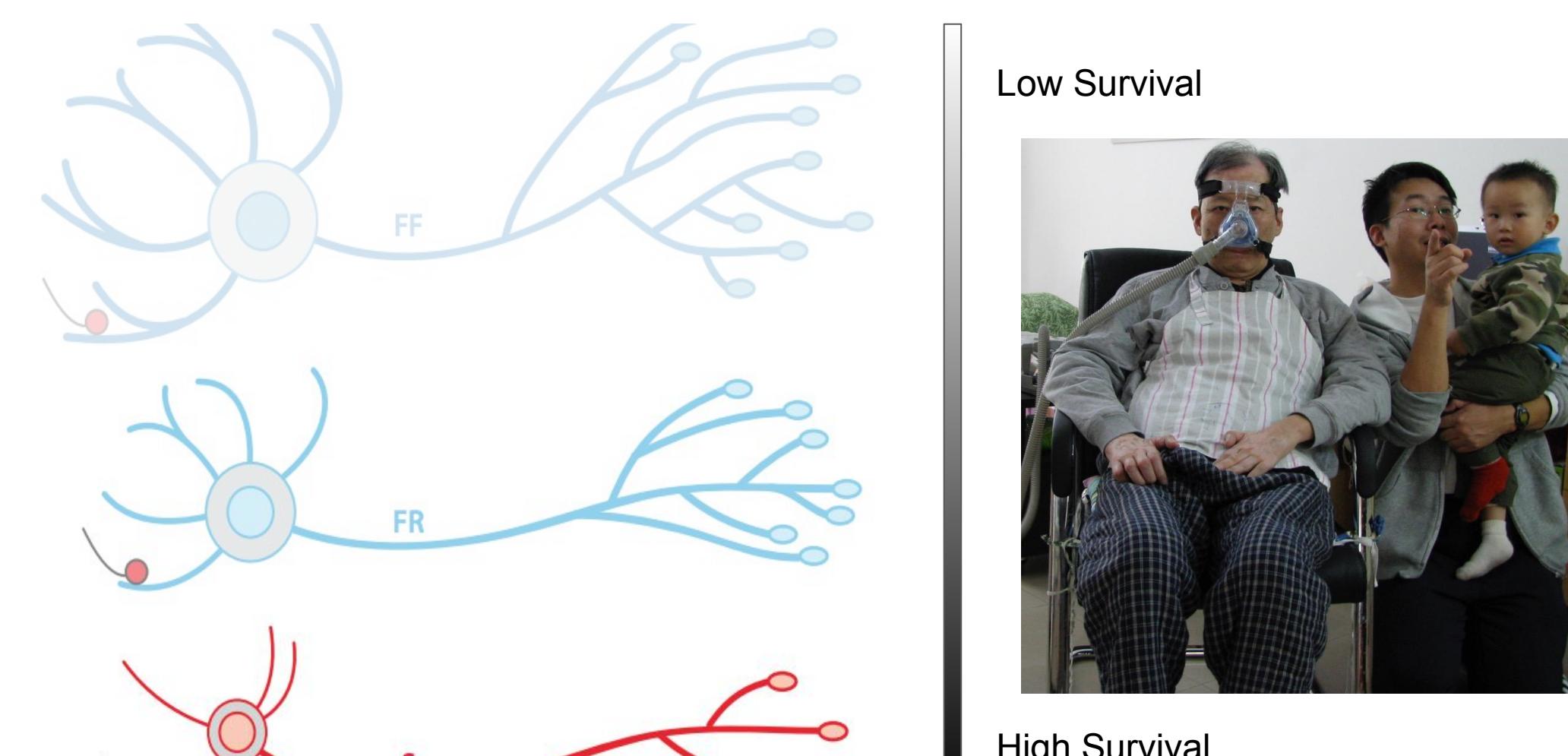
INTRODUCTION

Motor neurons are the essential cells connecting the brain and muscles. 3 subtypes control voluntary movement: SF, FR, FF.



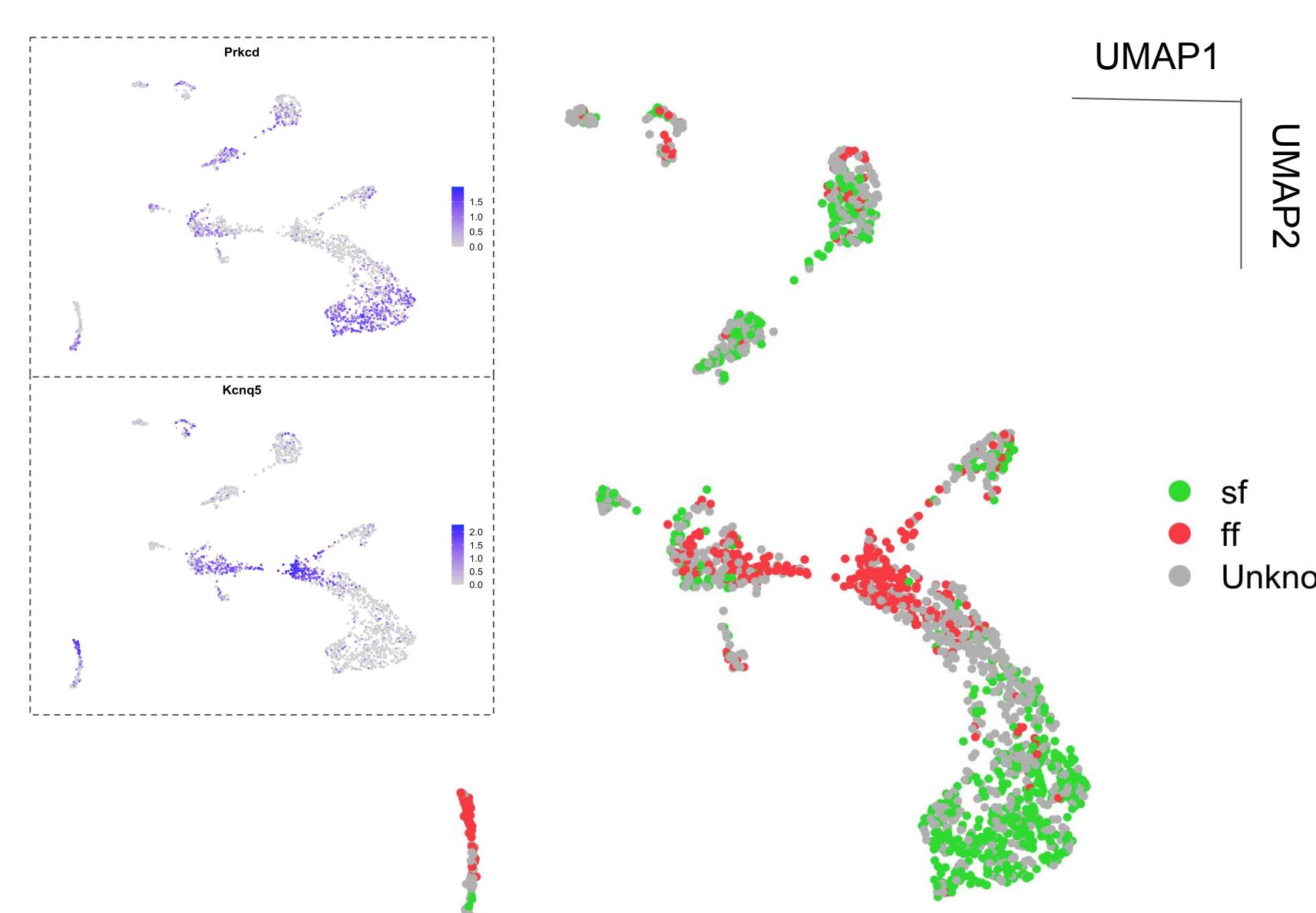
Given their physiological importance, it is unsurprising that motor neuron dysfunction underlies many neuromuscular diseases like ALS.

But motor neuron subtypes are affected differently in disease.



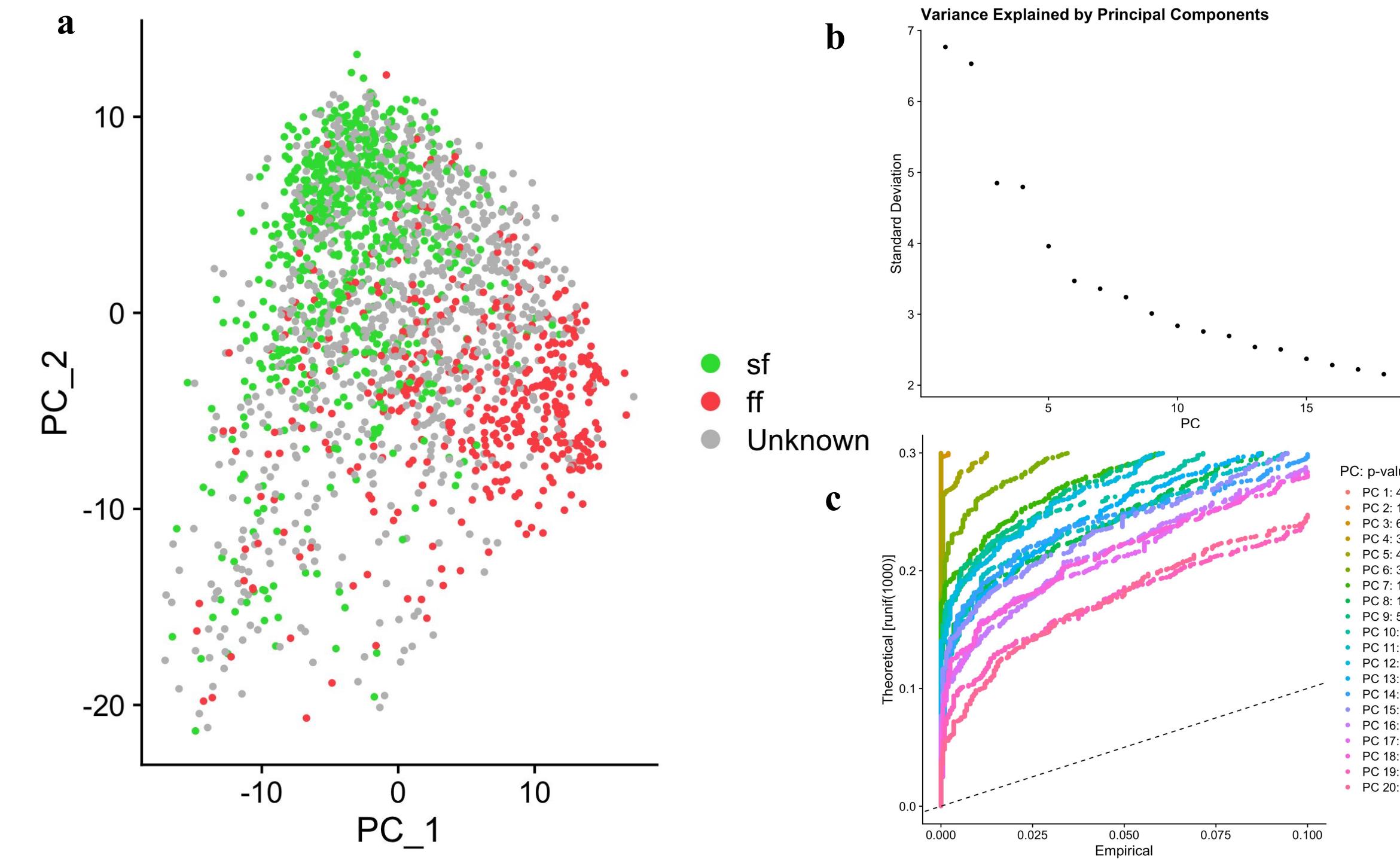
We can explore the transcriptional basis of this difference using single cell RNA sequencing.

Subtypes can be distinguished by expression of several established SF and FF marker genes –though no exclusive markers exist for FR motor neurons.

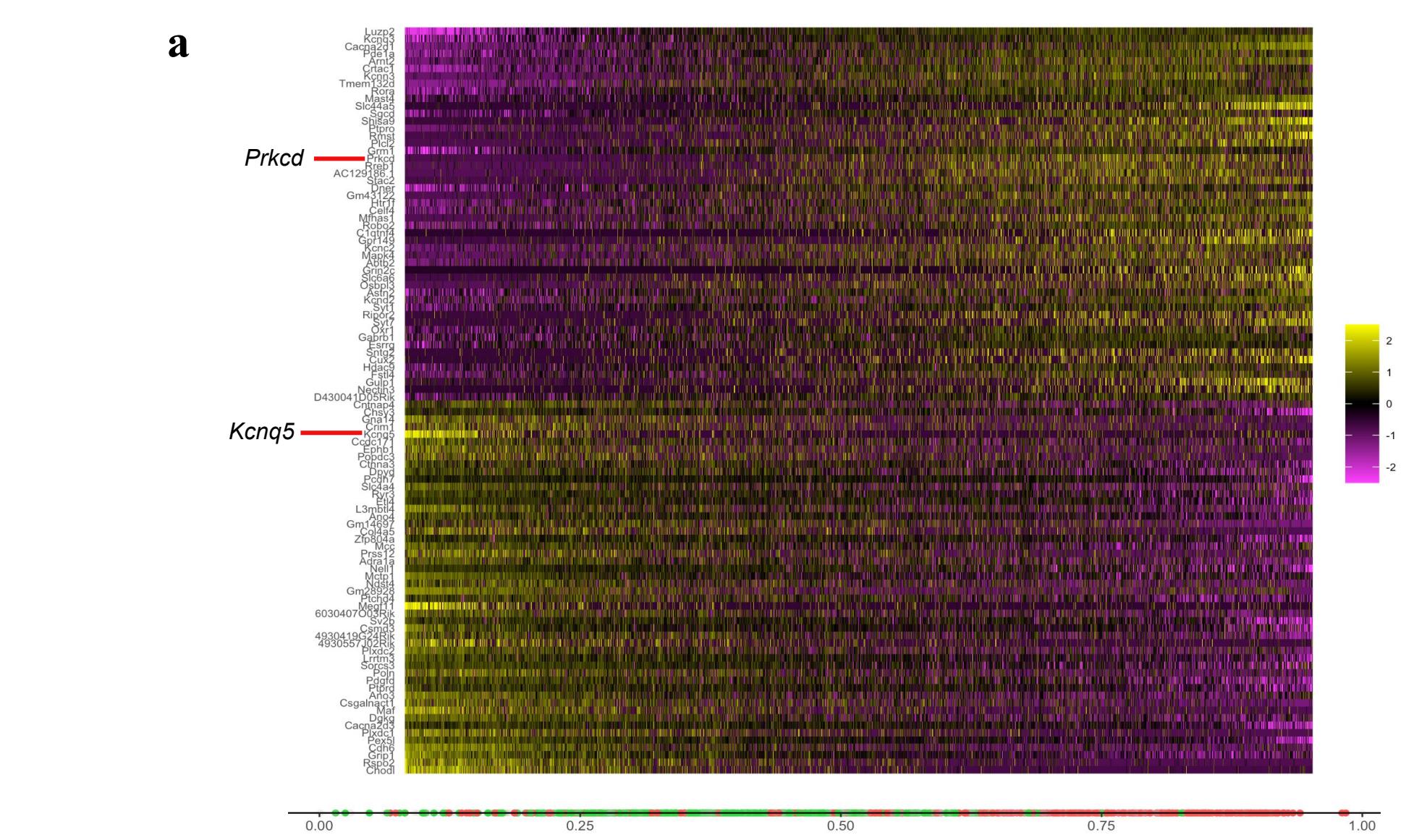


RESULTS

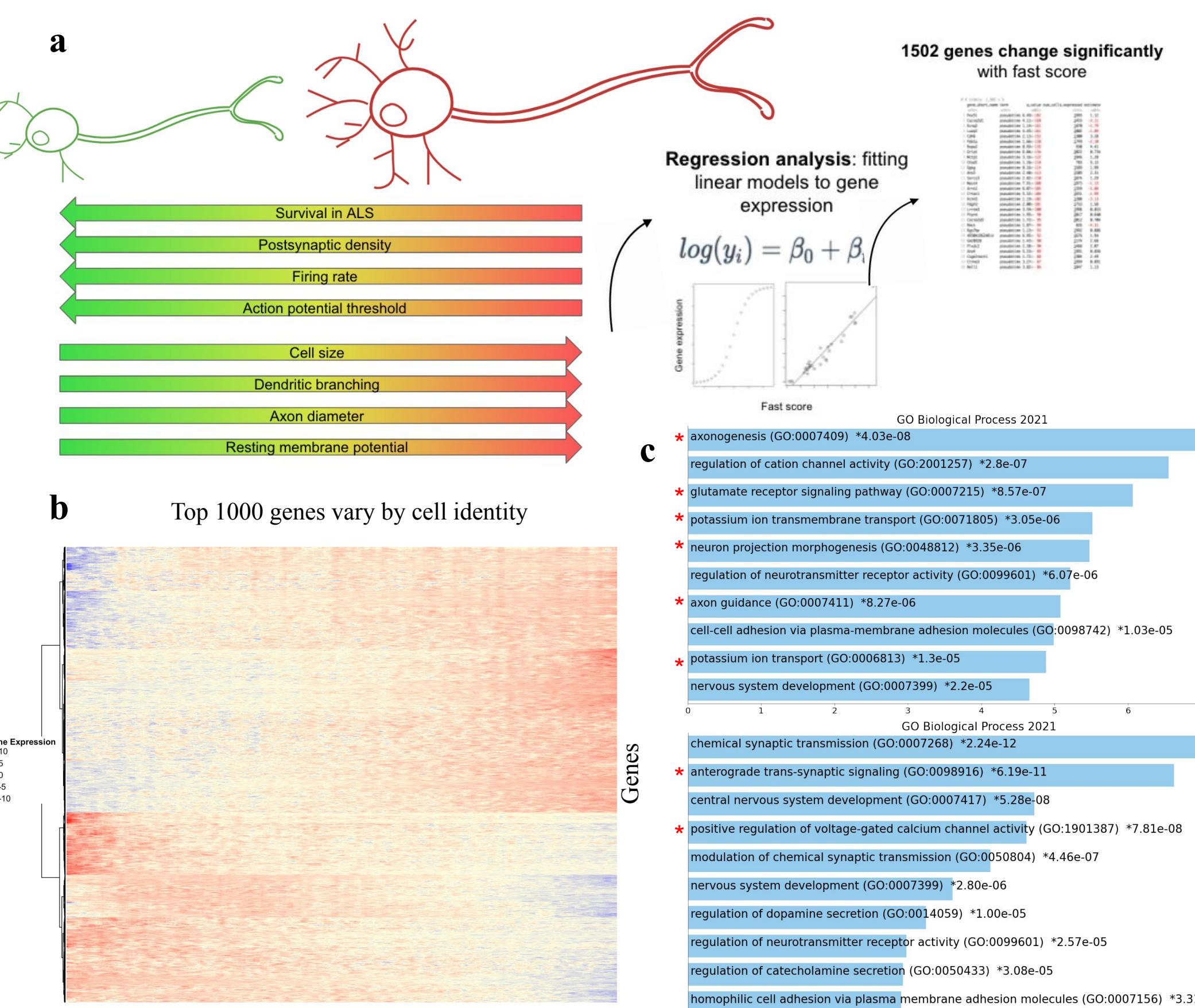
Principal Component Analysis reveals a **continuous** distribution of motor neurons along PC1 rather than a discrete one. Additional plots demonstrate the first two PCs are highly significant and capture the vast majority of variance in the data.



The genes that contribute to PC1 include all known marker genes of SF-FF motor identity (*Prkcd*, *Kcnn3*, *Sv2a* — *Kcnq5*, *Chodl*). Collectively, these data strongly support the hypothesis that PC1 corresponds with the SF-FF identity of motor neurons, subsequently referred to as the 'Fast Score.' Many motor neurons express different percentages of 'fast' and 'slow' signatures simultaneously, constituting the middle of this continuous distribution from slow to fast.

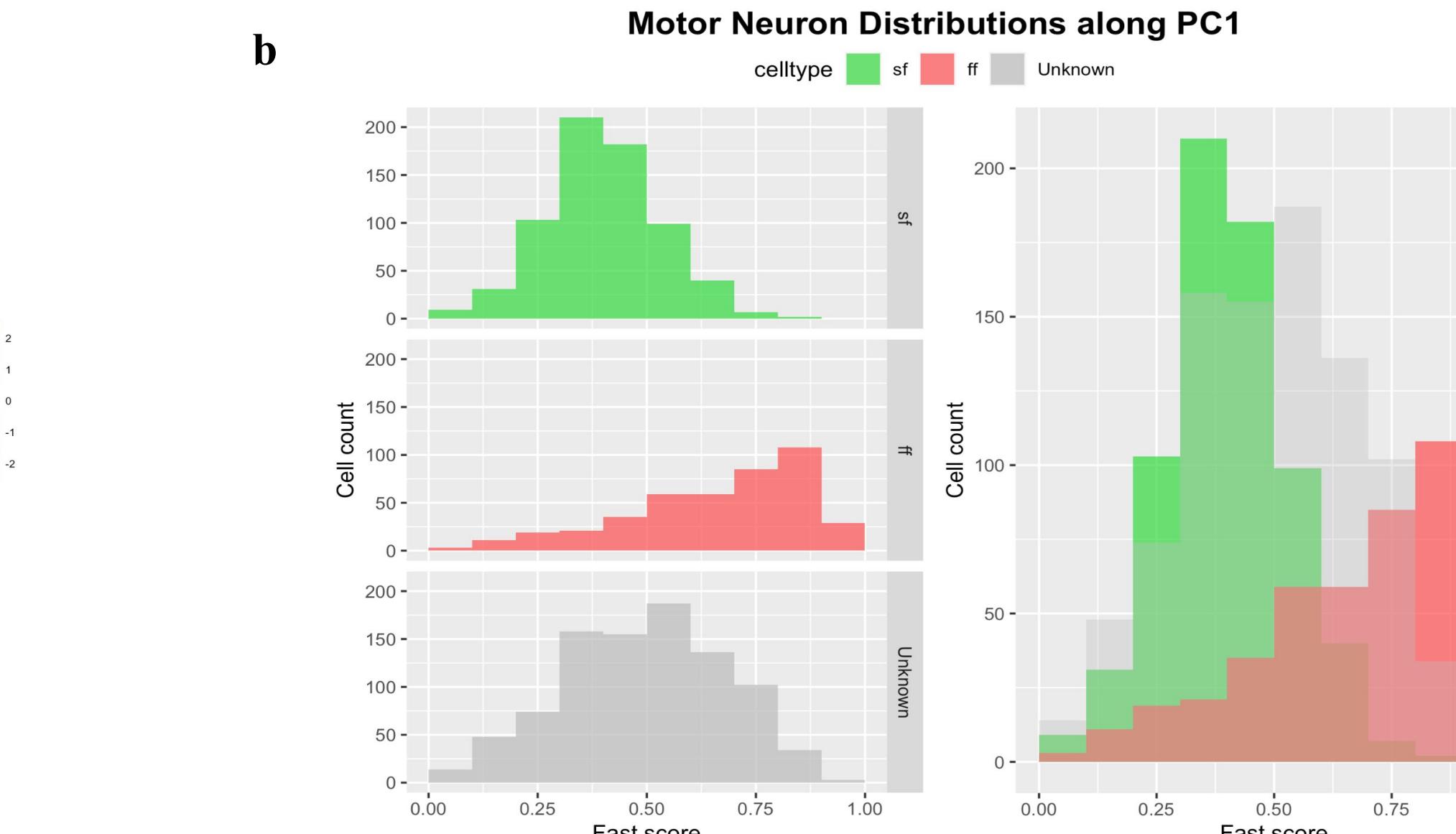


We hypothesize that specific gene expression differences underlie the distinct electrophysiological properties between slow-fast motor neurons. Regression analysis identifies 1502 genes changing significantly.

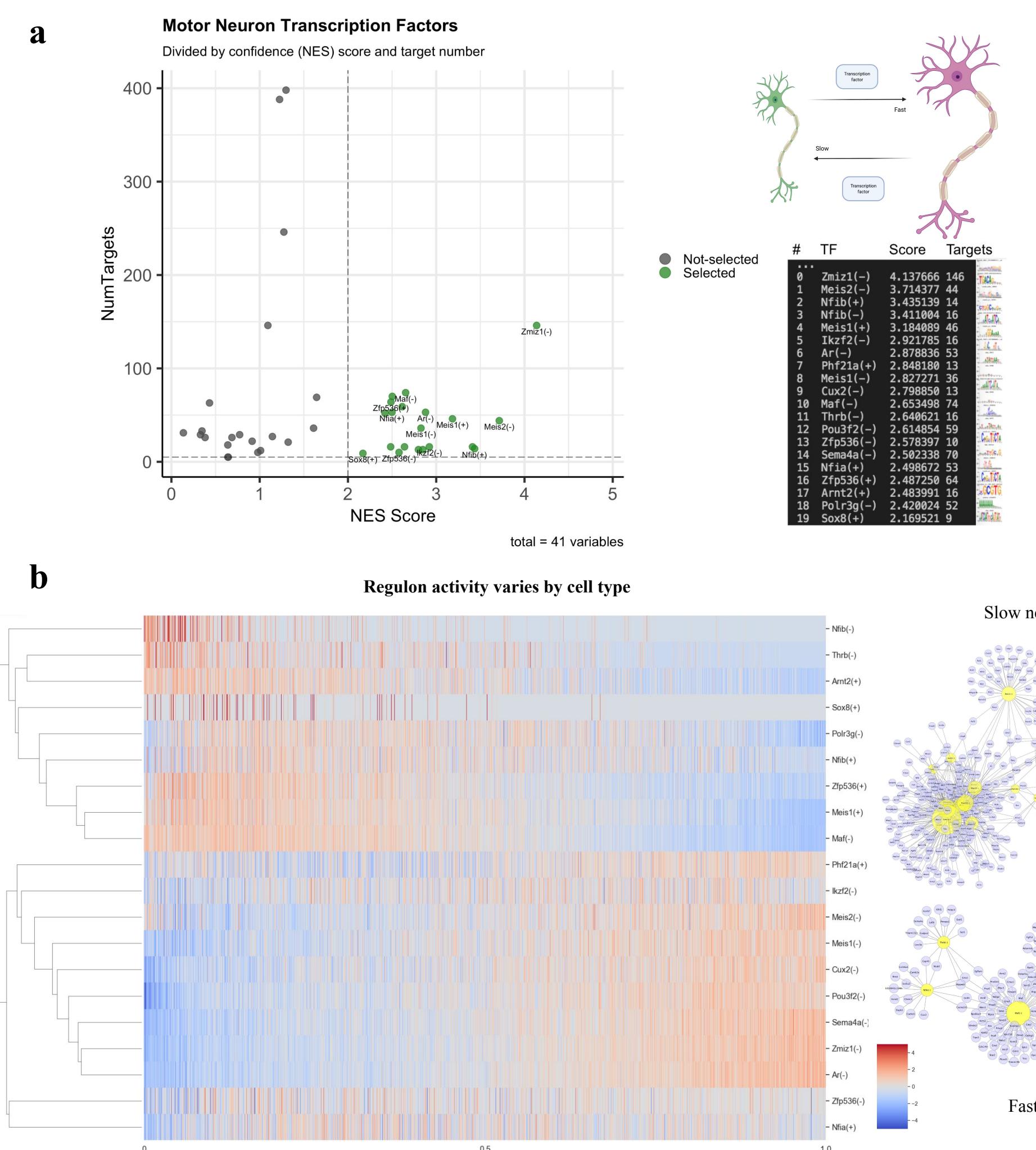


Interesting enrichments

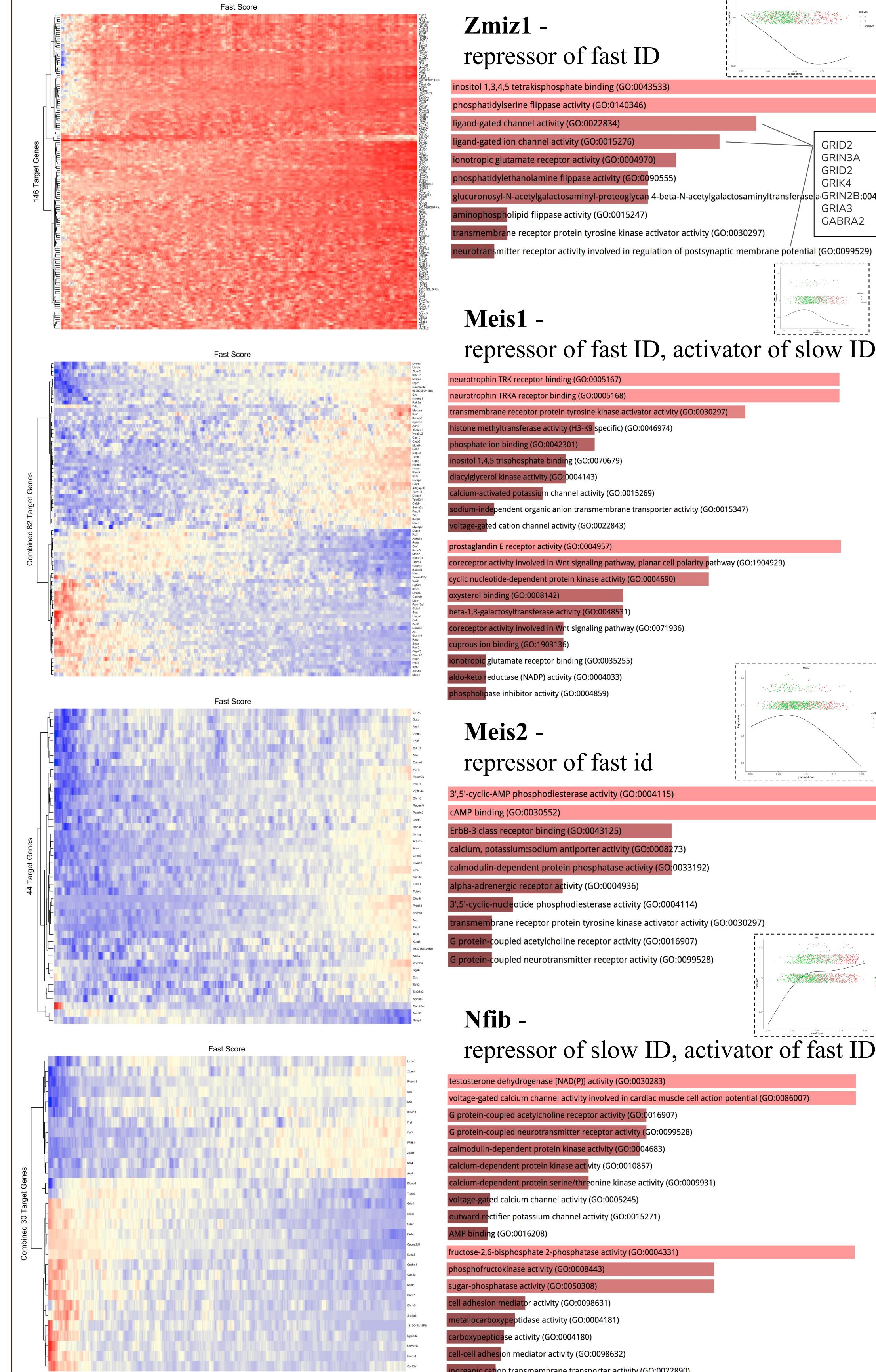
- FF: more axonogenesis, potassium channels, glutamate signaling → all found in FF
- SF: positive regulation of calcium channels → protective mechanism in ALS?



We discover the transcriptional networks driving gene expression differences. Out of 41 transcription factors found, 20 are selected.



Zmiz1, Meis1, Meis2, and Nfib represent the top 4 transcription factors with relevant molecular functions.



Other interesting enrichments

- Zmiz1: neg regulation of cell volume (BP), regulation of sodium channels (BP)
- Meis1: transcription factors (BP)
- Meis2: neg reg of collateral sprouting (BP), pos reg of neuron projection (BP)
- Nfib: too few targets to conclude relevant pathways

Zmiz1 is a coregulator of Ar, which regulates Meis1. Meis1 and Meis2 function in motor neuron differentiation in utero, are also implicated in GWAS of disease RLS.

DISCUSSION

1. New theory proposed: motor neuron identity is **continuous**
2. Knowledge of transcription factors
 - a. Enables **interconversion** between cell types → protective in ALS and other neuromuscular diseases?
 - b. Enables development of iPSC **motor neuron models** → more specific than our current models, allows further studies into fast v. slow differences and protective mechanisms in disease

